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NEW SYNTHETIC ROUTES TO THE STEREOSELECTIVE ASSEMBLY AND THE ELABORATION OF BIOACTIVE COMPOUNDS

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TABLE OF CONTENTS

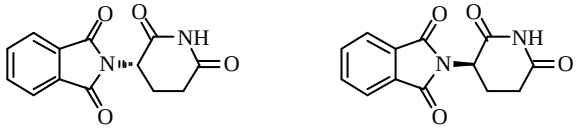
PREFACE

The property of an object to be distinguishable from its mirror image, the chirality, has intrigued scientists, and distinctively chemists, since the earlier studies of Pasteur on the tartrate crystals. The term "*chirality"* is derived from the Greek, χειρ, "hand", the most notorious chiral object.

In nature many biologically active molecules are chiral, and often form homochiral groups. A system is homochiral if all the constituent molecules feature the same chiral form. In biology, homochirality is commonly observed in aminoacids, that present L- chirality, and sugars, that are mostly D-enantiomers. Consequently, nucleosides, the building blocks of DNA and RNA, that are composed by D-ribose and D-deoxyribose, are homochiral. The origin of this phenomenon, that is the breaking mirror-symmetry, is not clearly understood and is subject of much debate. Most scientists believe that Earth life's "choice" of chirality was purely random, and that possible carbon-based life forms elsewhere in the universe would exist with both the chiral forms. However, there is some suggestion that early aminoacids would have formed in comet dust. In this case, circularly polarized radiation might be the cause for the selective development of one chirality of aminoacids, leading to a selection bias, which ultimately resulted in all life on Earth being homochiral.

Generally, biochemical pathways involve just one enantiomer of a chiral molecule. In particular, in living systems just one of the two enantiomers take part in metabolic cycles, while the other is ignored and may even be dangerous. This is true especially for drugs. One of the most famous cases of toxicity is given by thalidomide (**Figure 1**), a racemic antiemetic approved and marketed in 1956. While (*R*)-enantiomer is a sedative, the (*S*)-enantiomer has teratogenic effects. In the early sixties, the use of medications during pregnancy was not strictly controlled, and drugs were not thoroughly tested for potential harm to the fetus[1]. Thousands of pregnant women took the drug to relieve their symptoms. At the time of the drug's development, scientists did not believe any drug taken by a pregnant woman could pass across the placental barrier and harm the developing fetus. Indeed, soon appeared reports on abnormalities in children born form mothers who assumed thalidomide during pregnancy. Hence, while initially considered safe, the drug proved to be responsible for teratogenic deformities in children, if assumed by the mother prior to the third trimester of pregnancy. In November 1961, thalidomide was taken off the market due to

massive pressure from the press and public opinion. Experts estimate that the drug thalidomide led to the death of approximately 2,000 children and serious birth defects in more than 10,000 children.



(*S*)-thalidomide (*D*)-thalidomide

Figure 1: Enantiomers of thalidomide.

These side effects observed in thalidomide therapy are associated with its interaction with the cellular biological systems. Indeed, at the molecular level, chirality often has a profound impact on recognition and interaction events and has thus a relevant role in biochemistry and pharmacology. In fact, the biomolecular recognition is mostly based on the conformation, structural order and chirality of molecules. For example, madangamine A (**1, Figure 2**), a chiral bioactive marine sponge alkaloid isolated for the first time by Andersen and co-workers in 1990[2], with his unique structure and conformation has shown significant *in vitro* cytotoxic activity against a large number of tumour cell lines[3].

Enzymes, which are chiral structures, often distinguish the two enantiomeric forms of a chiral substrate. For example, L-forms of aminoacids tend to be tasteless, whereas D-forms tend to taste sweet. Spearmint leaves contain the L-enantiomer of the chemical carvone or *R*-(–)-carvone and caraway seeds contain the D-enantiomer or *S*-(+)-carvone[4]. These smell dissimilar to most people because our olfactory receptors also contain chiral molecules that respond differently in the presence of different enantiomers.

Distinction between different enantiomers is not a general rule in biological processes, as witnessed by some important drugs targeting chiral biological macromolecules.

For example, 3TC (lamivudine, **2**) and FTC (emtricitabine, **3**), two chiral antiviral nucleosidic agents approved by FDA in drug therapies against AIDS, are recognized and phosphorylated by the human deoxycytidine kinase independently from their chirality. The picture changes when looking at the deoxycytidine deaminase catalysed deamination. Indeed, this strictly enantioselective enzyme[5] does recognize 3TC and FTC (both L- enantiomers) but is inactive toward their D-enantiomers (**Figure 2**).

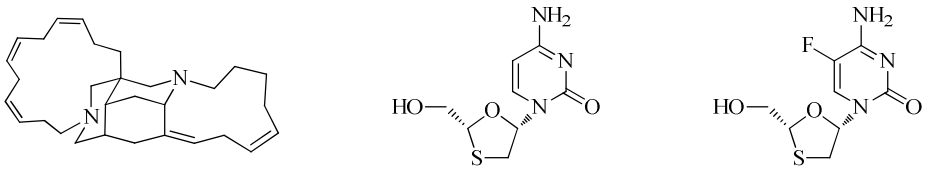


Figure 2: Bioactive chiral drugs.

The enzyme substrate interaction is even more complicated and may result in a better fitting of the active sites of the enzymes by those enantiomers that are not the natural substrates. For example, although glycosides belonging to D-series are the natural substrates for all glycosidases (except L-fucosidases), an L-enantiomer could own a structure that fits better the active site.

The iminosugars are an important class of glycosidase inhibitors and are witnessing a great interest as potential therapeutic agents. An example is given by deoxy-L-allonojirimycin (**4**), a much better inhibitor of α-D-mannosidase than deoxy-D-mannojirimycin[6] (**Figure 3**).

Figure 3: Glycosidase iminosugar inhibitors.

In light of these findings, in the last decades the development of new methodologies for the enantioselective synthesis of chiral compounds has experienced a huge evolution, carrying this topic from the status of a curiosity to a central issue in academia and in the life science industry.

In chemical synthesis, much effort has been directed towards developing asymmetric synthesis strategies that yield product molecules with a significant excess of either the left-handed or the right-handed enantiomer. This is usually achieved by making use of chiral auxiliaries or catalysts that influence the course of a reaction, with the enantiomeric excess (ee) of the product linearly related to the ee of the auxiliary or catalyst used. In recent years, however, an increasing number of asymmetric reactions have been documented where this relationship is nonlinear[7], suggesting the existence of effects that can lead to "asymmetric amplification". Theoretical models[8] have long suggested that autocatalytic processes can result in kinetically controlled "asymmetric amplification", a prediction that has now been verified experimentally[9] and rationalized mechanistically[10] in the case of autocatalytic alkylation reaction. It was observed that such

"asymmetric amplification" could explain the development of high enantiomeric excess in biomolecules from a presumably racemic prebiotic world.

In this context, this thesis is focused on the study of the central role of chirality in different kind of molecular structures; in particular, new synthetic pathways have been developed to improve the synthesis of already existing drugs, exploring at the same time the relationship between the chirality and the pharmacological properties of these compounds. Moreover, new enantiomeric systems have been synthetized and their pharmacological properties will be tested to verify the potential bioactivity and use as drugs.

The thesis is organized in three major sections focusing on the different molecule typology investigated (nucleosides, iminosugars, alkaloids). The studies on nucleosides and iminosugars have been carried out at the department of Chemical Sciences of University of Naples “Federico II”, while the synthesis of alkaloids has been accomplished at the department of Organic Chemistry of the Faculty of Pharmacy of the University of Barcelona.

REFERENCES

1. Franks, M.E.; Macpherson, G.R.; Figg, W.D. *Lancet.* **2004**, *363*, 1802.

2. Kong, F.; Andersen, R.J.; Allen, T.M. *J. Am. Chem. Soc.* **1994**, *116*, 6007.

3. Yoshimura, Y.; Inoue, J.; Yamazaki, N.; Aoyagi, S.; Kibayashi, C. *Tetrahedron Lett.* **2006**, *47*, 3489.

4. Leitereg, T.J.; Guadagni, D.J.; Harris, J.; Mon, T.R.; Teranishi, R. *J. Agric. Food Chem.* **1971**, *19*, 785.

5. (a) Shafiee, M.; Griffon, J.F.; Gosselin, G.; Cambi, A.; Vincenzetti, S.; Vita, A.; Eriksson, S.; Imbach, J.L.; Maury, G. *Biochem. Pharmacol*. **1998**, *56*, 1237. (b) Chang, C.N.; Doong, S.L.; Zhou, J.H.; Beach, J.W.; Jeong, L.S.; Chu, C.K.; Tsai, C.H.; Cheng, Y.C.; Liotta, D.; Schinazi, R. *J. Biol. Chem.* **1992**, *267*, 13938.

6. Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036.

7. Girard, C.; Kagan, H.B. *Angew. Chem. Int. Edn*. **1998**, *37*, 2923.

8. Frank, F.C. *Biochim. Biophys. Acta.* **1953**, *11*, 459.

9. (a) Shibata, T.; Morioka, H.; Hayase, T.; Choji, K.; Soai, K. *J. Am. Chem. Soc.* **1996**, *118*, 471. (b) Shibata, T.; Choji, K.; Hayase, T.; Aizu, Y.; Soai, K. *Chem. Commun.* **1996**, 1235. (c) Soai, K.; Shibata, T.; Morioka, H.; Choji, K. *Nature.* **1995**, *378*, 767.

10. (a) Blackmond, D.G.; McMillan, C.R.; Ramdeehul, S.; Schorm, A.; Brown, J.M. *J. Am. Chem. Soc.* **2001**, *123*, 10103. (b) Blackmond, D.G. *Adv. Synth. Catal.* **2002**, *344*, 156. (c) Buono, F.G.; Blackmond, D.G. *J. Am. Chem. Soc.* **2003**, *125*, 8978. (d) Buono, F.G.; Iwamura, H.; Blackmond, D.G. *Angew. Chem. Int. Edn.* **2004**, *43*, 2099. (e) Blackmond, D.G. *Proc. Natl. Acad. Sci. USA.* **2004**, *101*, 5732. (f) Gridnev, I.D.; Brown, J.M. *Proc. Natl. Acad. Sci. USA.* **2004**, *101*, 5727 (g) Gridnev, I.D.; Serafimov, J.M.; Brown, J.M. *Angew. Chem. Int. Edn.* **2004**, *43*, 4884. (h) Gridnev, I.D.; Serafimov, J.M.; Quiney, H.; Brown, J.M. *Org. Biomol. Chem.* **2003**, *1*, 3811.

Section 1

1. INTRODUCTION

Development of antiviral drugs entails combined competences in chemistry and biology. Nucleoside analogues (NAs) can play the role of antiviral and anticancer prodrugs owing to their ability of mimicking natural nucleosides in structural and functional terms. Consequently, they can interact with viral and/or cellular enzymes and inhibit critical processes in the metabolism of nucleic acids, such as the binding to host human cells[1], the transcription of genomic RNA[2] or the gene expression[3].

For this reason, NAs have been the most representative antivirals therapeutically adopted over the last decades and a large plethora of strategies for their synthesis has been surveyed. They play a prominent role among the drugs currently adopted against acquired immunodeficiency syndrome (AIDS). In particular, they work by interacting with human immunodeficiency virus type-1 reverse transcriptase (HIV-1 RT)[4], the viral RNA-dependent DNA polymerase. The inhibitory action towards HIV-1 replication is dependent on the following stages: 1) the cellular uptake of the analogue, 2) its phosphorylation mediated by cellular kinases, 3) the extent of competitive inhibition achieved, and 4) its binding to RT and subsequent incorporation into elongating viral DNA[5]. For this reason, the design and the synthesis of these active NAs has always been a challenge for chemist and biologist, also in view of the virus ability to develop easily a drug resistance.

1.1. HUMAN IMMUNODEFICIENCY VIRUS AND ACQUIRED IMMUNODEFICIENCY SYNDROME (HIV/AIDS)

Over the last decades new therapies for acquired immunodeficiency syndrome (AIDS) have been developed, providing much improved tools in the treatment of this pandemic disease. Unfortunately, even nowadays there is not a definitive therapeutic protocol and in 2014 AIDS still caused an estimated 1.2 million fatalities with about 36.9 million people infected by human immunodeficiency virus (HIV) worldwide[6]. At present, there are more drugs approved for the

treatment of HIV than for all other viral infections collected together. In absence of an effective vaccine, these drugs are the only therapeutic tools available for the treatment of human immunodeficiency virus type 1 (HIV-1) infections. Unfortunately, these drugs cannot heal HIV infections, so that drug therapy, once initiated for a patient, must be continued all life long. This represents a demanding limitation, and anti-HIV drugs are thereby required to be relatively nontoxic so to be used in a long-term therapy[7].

AIDS is caused by human immunodeficiency virus, a highly mutable lentivirus roughly spherical of 120 nm, a member of retrovirus family Retroviriadae, whose genome is encoded in RNA (**Figure 1.1**).

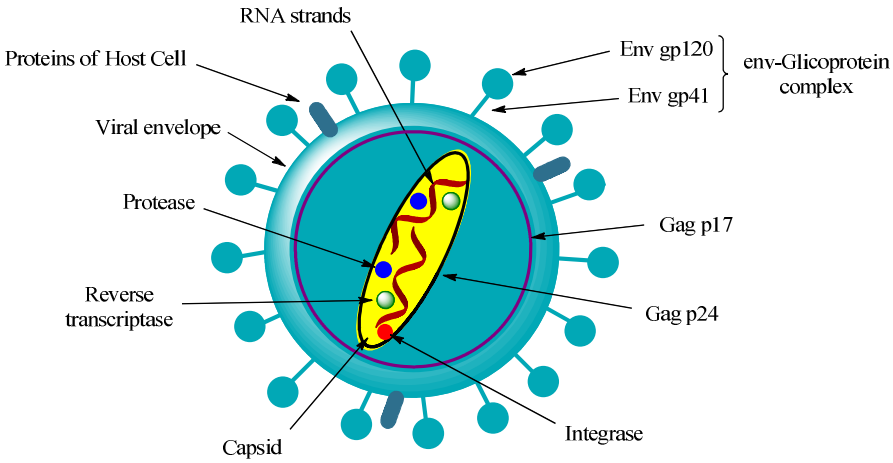


Figure 1.1: HIV virus.

Two copies of positive single-stranded RNA are present in the virus; they encode nine genes of the virus and are coated by a conical capsid made of 2,000 copies of the viral protein p24. The RNA strands are bound to nucleocapsid proteins, p7, and some enzymes necessary for the of the virion development: reverse transcriptase (RT), ribonuclease (RNase), proteases (PR) and integrase (IN). The reverse transcriptase, an enzyme found just in retroviruses, is the most important therapeutic target in AIDS treatment. RT transcribes retroviral RNA into DNA, which is then integrated into the host genome and expressed by the infected host cell under the control of viral genes. The viral RNase catalyzes the degradation of RNA into smaller fragments after the transcription into viral DNA mediated by RT. PR is essential for maturation and infectivity of the virus. Indeed, budded, immature viral particles possess catalytically inactive protease, and this immature form is not infective[8]. IN is instead involved at the stage in which viral DNA is inserted

into the host chromosomal DNA, an essential event for HIV replication. This is an irreversible step causing the cell to be a permanent carrier of the viral genome (provirus).

HIV has the 5'-gag-pol-env-3' gene organization typical of other retroviruses[9]. Gag, pol and env are three major genes encoding major structural proteins and essential enzymes: gag encodes proteins of the virion interior; pol (pol, polymerase) encodes viral enzymes; env (envelope) encodes the virion glycoproteins. The integrity of the virion particle is ensured by a matrix composed of the viral protein p17 surrounding the capsid. The matrix is in turn surrounded by the viral envelope, featuring a lipid bilayer (taken from the membrane of the host cell), proteins from the host cell, and a few copies of HIV Envelope protein. In particular, outside of the membrane are exposed several caps composed of three copies of a glycoprotein (gp) known as gp120, anchored to the envelope through a stem composed of three gp41 copies. The Envelop protein is responsible for the viral attack to the target cells, the fusion of the viral envelope with the cell membrane, and the release of the viral content into the invaded cell. Overall, these events represent the start of the infectious cycle, and not casually the Envelope protein is a major target for vaccine efforts addressed against HIV.

HIV is highly capable of both evading the host immune system and developing resistance to all available drugs[10]. This is a consequence of the error amenability of HIV replication combined with the rapidity of this latter process. Consequently, the only way to stop the development of resistance is to block completely viral replication; this, in turn, stops the evolution of resistance.

The currently approved anti-HIV drugs can be divided into seven groups: nucleoside reverse transcriptase inhibitors (NRTIs), nucleotide reverse transcriptase inhibitors (NtRTIs), non-nucleoside reverse transcriptase inhibitors (NNRTIs), protease inhibitors (PIs), fusion inhibitors (FIs), co-receptor inhibitors (CRIs), and integrase inhibitors (INIs). Current treatments are typically involving a combination of three drugs, and nowadays AIDS can be regarded as a chronic, manageable disease[11] after a long period in which a high mortality rate was sadly associated with the diagnosis of this disease. Of the approved drugs, most target the reverse transcriptase and the protease.

The earliest drugs developed in the fight against AIDS were NRTIs; specifically, they terminate further insertion of nucleotides into the elongating viral DNA chain carried out by the enzyme reverse transcriptase. The favourable effect of these drugs in AIDS patients has confirmed the importance of inhibiting RT, and led to the development and clinical use of another class of RT inhibitors that are not nucleosides, the NNRTIs. NNRTIs inhibit RT by binding to an allosteric site of the enzyme. Finally, the intense effort by scientists covering a wide spectrum of disciplines

has produced the other distinct mechanistic class of drugs in the fight against AIDS. These drugs have been designed to block the action of the PR, and their synergy with RT inhibitors has brought to the market place new and efficacious combination therapies for the treatment of this disease. Successful development of PR inhibitors as drugs was based on the early comprehension of its pivotal role in viral maturation as well as its structure and function. Indeed, many reviews have been published on the protease and the discovery and development of its inhibitors[12]. Furthermore, a new drug, raltegravir, which targets the IN, has recently been approved and shows good efficacy for resistant HIV infections[13].

However, the use of these drugs has been relatively limited by their toxicity[14], drug resistance insurgence[15], and more worryingly, the inherent resistance of some newly HIV-infected patients even to the currently approved AIDS treatments[16]. Combination of these issues with undesired side effects, and a possible poor tolerability of the drugs strongly spurs the research for new anti-HIV agemts with an acceptable toxicity and resistance profile and, more importantly, with novel mechanisms of action.

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1.1.1. HIV LIFE CYCLE

Before explaining the mechanism of action of these antiviral drugs, the HIV life cycle have to be outlined in few crucial steps, as summarized in **Figure 1.2**.

The first step is the specific interaction between the virion glycoprotein gp120 on the outer membrane and the CD4 (cluster of differentiation 4) receptor on the host cell surface. This reaction cause a change of conformation, allowing the interaction of gp120 with the chemokine coreceptor CXCR4 or CCR5. This is then followed by further conformational changes that expose a fusogenic peptide (gp41), which anchors into the host cell membrane. Once the viral envelope and cell membrane have fused, the virion is decapsidated releasing the viral RNA (the virion has two copies of a single-stranded RNA genome and viral proteins) into the host cell's cytoplasm.

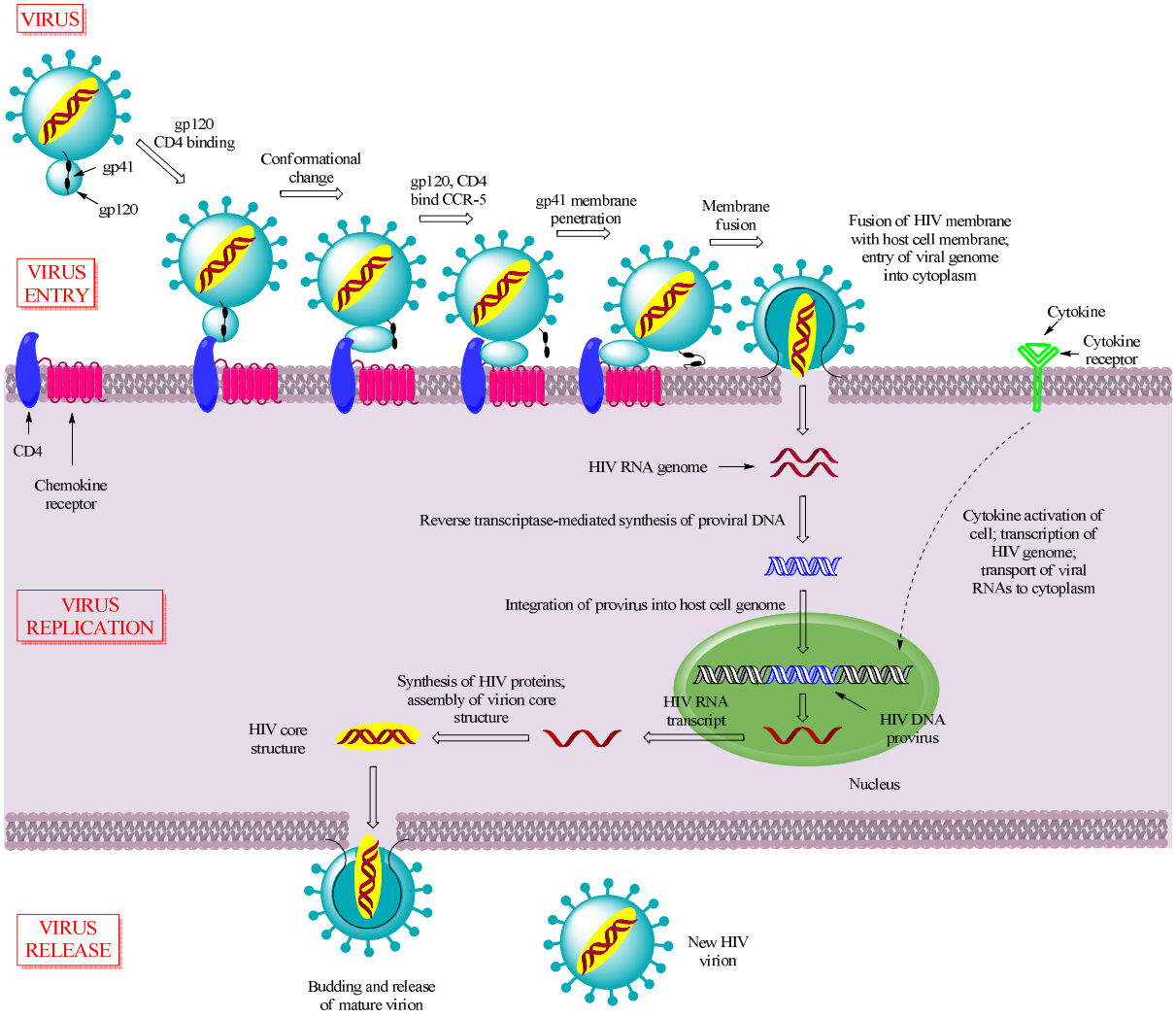


Figure 1.2: HIV life cycle.

The viral RNA is transcribed to viral double-stranded DNA with the reverse transcription. This process is catalysed by the reverse transcriptase, a RNA-dependent DNA polymerase, which is encoded by the viral genome. RT exhibits enzymatic activity in two ways: 1) it can act as a DNA polymerase, copying either a DNA or an RNA template; 2) it can serve as a RNase H, cleaving RNA when this latter is incorporated into an RNA/DNA duplex. Both these activities bring to the conversion of RNA into a double stranded linear DNA, a process occurring in the cytoplasm of the infected cell. As DNA synthesis proceeds, RNase H degrades the RNA strand. Upon completion of DNA synthesis, the produced linear double-stranded viral DNA is translocated to the nucleus where it is inserted into the host genome by integrase (IN). This inserted DNA copy, called a provirus, is the source of both viral genomic and viral messenger RNAs, which are generated by the host DNA-dependent RNA polymerase. RT is endowed with all enzymatic activity the necessary for the conversion of viral RNA into DNA; nonetheless it can be helped for

the goal by other viral proteins (notably the nucleic acid chaperone nucleocapsid, and perhaps IN), and probably some cellular factors. After transcription (helped by regulatory proteins tat and rev, which are themselves viral gene products) and translation into viral proteins using the cells' apparatus, the assembly of the gag and gag-pol polyproteins occurs near the cell membrane[17,18]. During viral assembly, two copies of single-stranded viral RNA are incorporated into the virion, which then buds off from the cell, taking with it part of the host cell membrane. Soon after budding, viral protease cleaves the gag-pol polyprotein to generate a mature, functional virion[18].

A question arises, why virions contain two copies of the viral RNA genome instead of one, if in theory, the reactions could be carried out with only one copy of the viral RNA genome The answer is that, with only one copy of the viral RNA in a virion, a single break in the RNA would be fatal, as the synthesis of a complete DNA copy would be prevented. Indeed, if there is a second copy of the RNA, and minus-strand DNA synthesis is blocked by a break in the RNA template, synthesis can be continued if minus-strand DNA synthesis is transferred to the second RNA genome. In fact, with two copies of genomic RNA present, a complete DNA copy can be made even when both RNA genomes are extensively nicked, unless both RNA copies are nicked at the same site. If a virion is generated by a cell bearing only one integrated DNA genome, the two RNA copies will be identical (unless RNA polymerase makes an error), and the fact that, during minus-strand DNA synthesis, RT often shifts back and forth between the two RNA templates will have no significant consequence. However, if a host cell contains two different integrated viral DNA genomes, then virions can be produced that contain two related RNA genomes that have different sequences. This sets the stage for the generation of viral recombinants. If RT makes a double-stranded viral DNA by copying from two different RNA genomes, the resulting DNA will contain sequences that are derived from both of the parental genomes. In HIV-1, recombination during reverse transcription is a routine, rather than unusual, process. This can have important consequences. For example, recombination can lead to the generation of viruses that are resistant to multiple drugs from parental viruses that are each resistant to only a single drug.

Generally, antiviral drugs could be targeted at either viral proteins or cellular proteins. The former strategy provides more specific and less toxic compounds, albeit with a limited spectrum of activity and a higher amenability to induce viral drug resistance. The latter strategy, instead, affords anti-HIV drugs with a broader activity spectrum and a lower resistance, but a higher probability of toxicity[17].

1.2. NUCLEOSIDE REVERSE TRANSCRIPTASE INHIBITORS (NRTIS)

As seen above, reverse transcriptase is essential in the replicative cycle of retroviruses, because it synthesizes the proviral DNA, which will then be integrated into the host cell genome and passed on to all of the progeny cells. The substrate (dNTP) binding site of HIV reverse transcriptase turned out to be an attractive target for nucleosidic HIV inhibitors. In fact, the first anti-HIV drug that was approved in 1987 for the treatment of AIDS was the nucleoside reverse transcriptase inhibitor 3'-azido-2',3'-dideoxythymidine[19] (zidovudine, ZDV or AZT, **I**), which is a nucleoside analogue that inhibits the HIV reverse transcriptase. In the following years the research has been focused on the synthesis of other nucleoside-based compounds with good inhibitory activities of RT. For this reason, six more NRTIs, 2',3'-dideoxynucleosides, have been identified and approved by the Food and Drug Administration (FDA) for treating HIV patients: 2′,3′-didehydro-2′,3′-dideoxythymidine[20] (stavudine, d4T, **II**), 2′,3′-dideoxycytidine[20] (zalcitabine, ddc, **III**), 2′,3′-dideoxyinosine[21] (didanosine, ddI, **IV**), (1*S*,4*R*)-4-[2-amino-6-(cyclopropylamino)-9*H*-purin-9-yl]-2cyclopentene-1-methanol[22] (abacavir, ABC, **V**), (-)-2′,3′-dideoxy-5-fluoro-3'-thiacytidine[23] (emtricitabine, FTC, **VI**), and (-)-2′,3′-dideoxy-3′-thiacytidine[24] (lamivudine, 3TC, **VII**) (**Figure 1.3**).

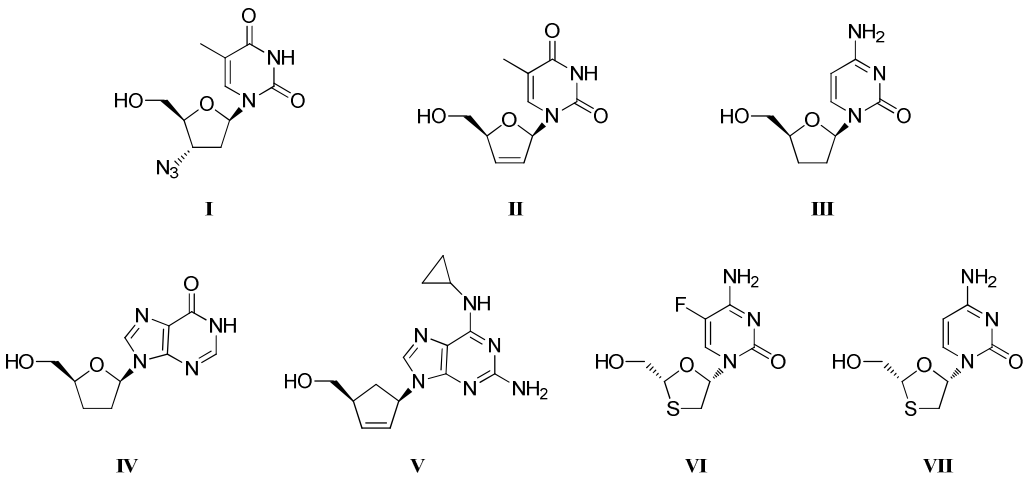


Figure 1.3: Zidovudine (**I**), stavudine (**II**), zalcitabine (**III**), didanosine (**IV**), abacavir (**V**), emtricitabine (**VI**), lamivudine (**VII**).

To overcome insurgence of virus drug resistance, they were also used in combination of two or three, but unfortunately even in combination they proved unable for a long term suppression of

viral activity and patients inevitably died[25]. To distinguish new advanced therapeutic approaches from this early anti-retroviral therapy (ART), the term highly active anti-retroviral therapy (HAART) was coined. The HAART relies on the use of multiple drugs acting on different viral targets. In 1996, sequential publications in *The New England Journal of Medicine* by Hammer and colleagues[26] and Gulick and coinvestigators[27] pointed out the beneficial effect of the combination of two NRTIs with the protease inhibitors. This concept of three-drug therapy quickly entered the clinical practice and proved successful within short times, with a 60% to 80% decline in rates of AIDS, death, and hospitalization[28]

These nucleosides are classified according to the structural differences with the natural substrates. In particular, they are divided in three different groups featuring: 1) modifications in the sugar moiety, 2) modifications in the nucleic base moiety and 3) modifications in both the sugar and base moieties. Generally, the main modifications are the configuration inversion of carbinol sites, their elimination (leading to dideoxy- or dideoxy-didehydro-nucleosides), their substitution or refunctionalization, or cleavage of the sugar ring (leading to acyclic nucleosides). Other structural modifications have also been attempted such as replacement of the endocyclic oxygen with a methylene group or a sulfur atom, transposition of the endocyclic heteroatom and/or additional insertion of a second heteroatom in the sugar moiety.

NRTIs are prodrugs; in order to serve as chain terminators in the reverse transcription (RNA→DNA), they need to be phosphorylated inside the host cell to their triphosphate derivatives[29] under the agency of three kinases. As all NRTIs follow the same inhibition pathway against HIV, an example of inhibitory mechanism is illustrated only for **VII** (**Figure 1.4**)[17] .

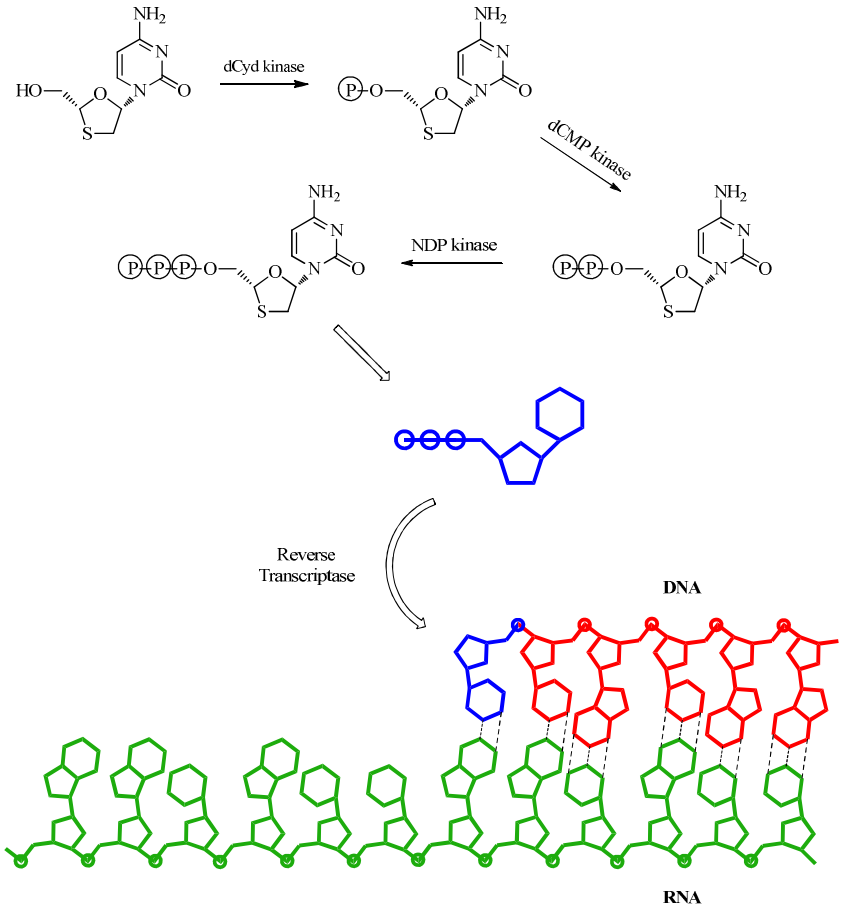


Figure 1.4: Mechanism of phosphorylation for lamivudine (**VII**).

Lamivudine (**VII**) is sequentially phosphorylated by deoxycytidine kinase, deoxycytidine monophosphate kinase and nucleoside diphosphate kinase to form the monophosphate, diphosphate, and the active triphosphate derivative of **VII**, respectively (for more informations on kinases, an interesting review has been published by *Cellular and Molecular Life Sciences*[30]).

All NRTIs are analogs of normal nucleosides, but not always NRTIs prove to be good substrates for the cellular kinases[31]. For example, low amounts of AZTTP are accessible because of the limited addition of the second and third phosphate[32]. However, for other NRTIs, the addition of the first phosphate is the limiting step in the overall metabolic pathway leading to the active 5′-triphosphate metabolites. Poor phosphorylation at the nucleoside-kinase level can account for the low anti-HIV activity displayed by some dideoxynucleoside analogues (for example, 2′,3′-dideoxyuridine) under conditions in which other derivatives are active. This issue has spurred the search of 2′,3′-dideoxynucleoside-5′-monophosphate pro-drugs prone to be elaborated in the host cell to the corresponding 5′-di- and 5′-triphosphate derivatives, in a strategy aimed at overcoming the initial nucleoside-kinase dependency.

The active triphosphate moiety is then incorporated into the growing DNA by cellular DNA polymerases and it causes the termination of the elongation of the growing DNA double strand. This happens because all NRTIs lack the 3′-hydroxyl group; therefore, they prevent the incorporation of the incoming nucleotide. A possible mechanism underlying the viral resistance is likely based on a pyrophosphorolysis repair reaction, removing the chain-terminating residue through a process that is substantially the reverse of the transcriptase-catalysed elongation[17].

Besides the seven NRTIs approved so far (**Figure 1.3**), further six derivatives advanced to either phase II or phase III clinical trials (**Figure 1.5**): apricitabine[33] (**VIII**), racivir[34] (**IX**), amdoxovir[35] (**X**), elvucitabine[36] (**XI**), reverset[37] (**XII**) and alovudine[4] (**XIII**).

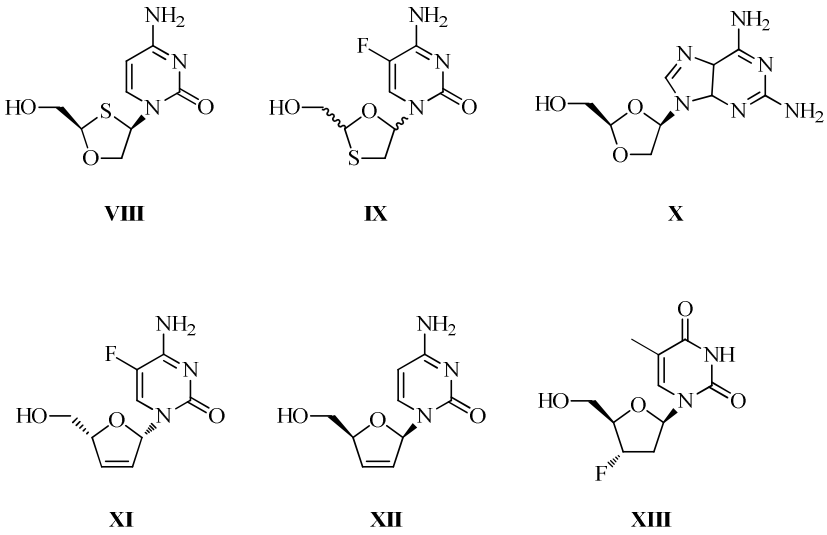


Figure 1.5: NRTIs currently undergoing either phase II or phase III of clinical trials.

Compound **VIII** is the most advanced in clinical trials, showing potent anti-HIV activity both *in vivo*[38] and *in vitro*[39], and probably FDA will approve it for clinical use soon. Racivir (**IX**) is a 50:50 mixture of **VI** and its enantiomer, and possesses a high anti-HIV activity *in vivo*[34] and *in vitro*[40]. Others compounds are showing an interesting potent activity in vitro, alone or in combination with other NRTIs, also against wild-type and HIV resistant to several other nucleoside analogues[41].

1.3. L-NUCLEOSIDES: LAMIVUDINE AND EMTRICITABINE

Enzymes are enantiomerically pure catalysts largely responsible for the conservation of chirality in living organisms. Enzyme-catalyzed reactions are generally highly stereoselective and often their kinetic outcome is strongly dependent by the chirality of the substrates: for instance, many enzymes are selective towards D-nucleosides and L-aminoacids. The enantioselectivity in enzymatic catalysis has been exploited in organic synthesis, for example in kinetic resolution of racemic mixtures as well as in the synthesis of chiral building blocks from achiral precursors. However, it was probed that the selectivity of enzymes used in synthesis with large series of substrate is not so strict, depending on the structure of the candidate substrate[42]. For this reason, the conviction that enzymes catalyze the transformation, for example, of just D-nucleosides and their analogues is absolutely wrong.

L- and D-nucleosides are (of course) displaying identical physical and chemical properties with the exception of optical rotation. However, the pharmacological properties in chiral environment can be differentiated for each isomer. The origin of these differences may be rooted in: 1) delivery of nucleosides into the cells, 2) anabolic and catabolic enzymes that act on these compounds, 3) interaction with the viral and/or cellular target enzymes.

L-nucleosides have an inverted configuration at all chiral centers, preserving the β-orientation (cis) of the nucleobase and the 4′-hydroxymethyl group. Atom at the position X, R1 and R2 may be oxygen, methylene group, sulfur or another single heteroatom (**Figure 1.6**).

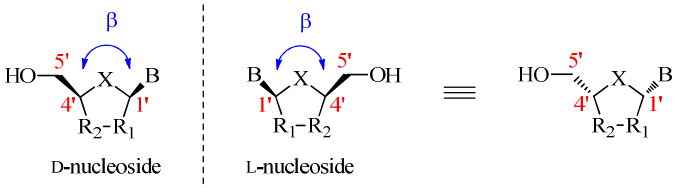


Figure 1.6: D- and L-nucleosides.

Although the first synthesis of L-nucleoside was reported in the 1960s[43], a little interest has been addressed to L-nucleosides until the emergence of 3TC. Belleau *et al*. first described the synthesis and anti-HIV activity of an unusual nucleoside analog, (±)-2,3′-dideoxy-3′-thiacytidine (BCH-189, **XIV**)[44], a compound in which the 3′-carbon with the hydroxyl function of the furanose

sugar moiety is replaced by a sulfur atom. Subsequently, it was found rather surprisingly that the L-isomer of BCH-189 (3TC, **VII**) was more powerful against HIV-1 and less toxic than its D-isomer (**XV**)[24,45] (**Figure 1.7**). 3TC and its D-enantiomer were found to possess comparable anti HIV-2, activities. 3TC displays a poor cytotoxicity, whereas (+)-BCH-189 proved to cytotoxic (IC_{50} = 2.7 μM) against human PBM cells.

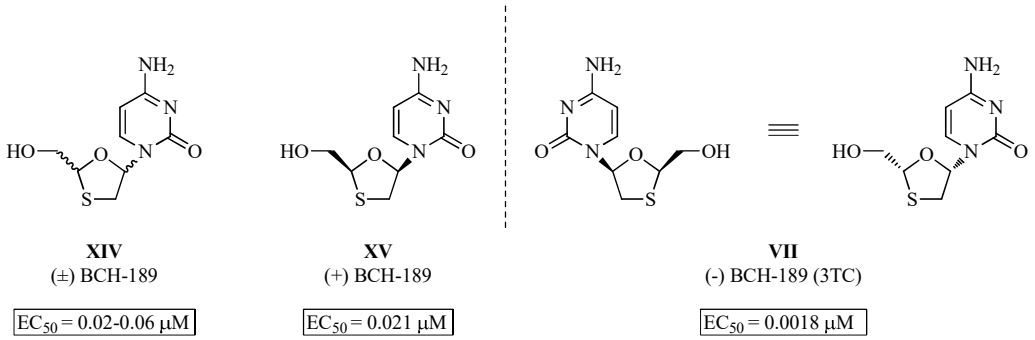


Figure 1.7: Isomers of BCH-189.

Since then, the synthesis and the biological evaluation of a large array of L-nucleosides analogs highlighted the importance of chirality and its influence on antiviral activity of the L-nucleosides. These properties are not always predictable: L-AZT, the L-isomer of **I** was synthesized using L-arabinose as a starting material[46]. The anti-HIV activity of L -AZT was reported to be about 10000 times lower than that of AZT.

However, the most surprising aspect in the related subject lies in some instances of cellular kinases capable of phosphorylating the L-nucleosides to their triphosphates, and thereby endowing them with various biological activities. For example, it was found that deoxycytidine kinase is the enzyme responsible for the monophosphorylation of 3TC[47] and that 3TC is a better substrate for this enzyme than the D-enantiomer[48]. Deoxycytidylate kinase and nucleoside diphosphate kinases are the enzymes that phosphorylate the monophosphate of 3TC to its di- and triphosphate, respectively. 3TC is resistant to deamination by deoxycytidine deaminase, whereas the D-enantiomer is deaminated to 2′-deoxy-3′-thiauridine by the same enzyme[49]. The half-life of 3TC triphosphate was 12–15.5 h in mock-infected cells, and 10.5–13 h in HIV-1-infected cells, whereas a significantly shorter half-life was recorded with the D-enantiomer (3.5 h in mock-infected cells and 5–7 h in HIV-1 infected cells)[50]. The better substrate specificity for deoxycytidine kinase, the resistance to deamination as well as the longer half-life of the triphosphate of 3TC may explain the observation that 3TC is more potent than its D-enantiomer against HIV-1 in vitro.

(−)-2′,3′-dideoxy-5-fluoro-3′-thiacytidine (FTC, **VI**), a 5-fluoro analog of 3TC, also showed potent antiretroviral activity against HIV- 1 and HIV-2[34] (**Figure 1.8**).

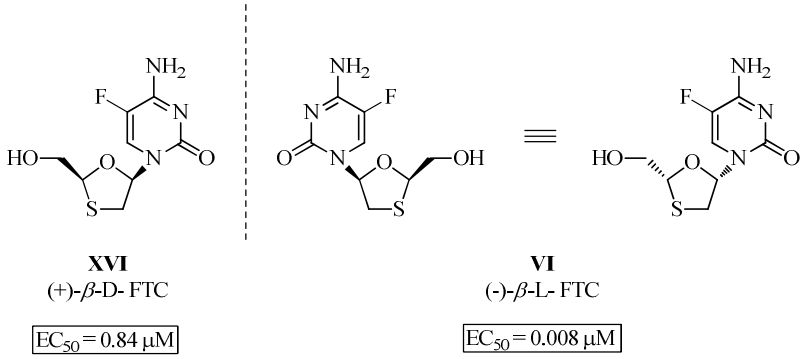


Figure 1.8: Isomers of FTC and their activity.

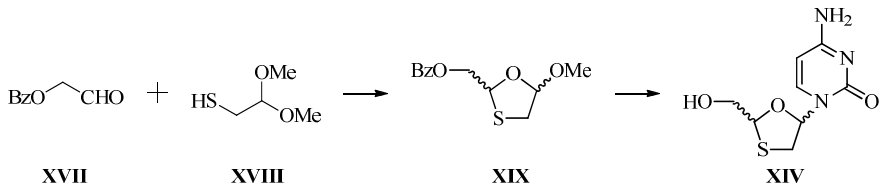
Like 3TC, (−)-β-L-FTC exhibits more potent anti-HIV-1 and HIV-2 activity than its D-enantiomer respectively in PBM cells and *in vitro*. It is also significantly less toxic than D-FTC, and both enantiomers are less cytotoxic than both BCH-189[51]: in fact, when the triphosphate form of emtricitabine (E-TP) was compared with 3TC triphosphate, the activity against mitochondrial DNA polymerases (pol γ) of E-TP was 24 times lower than that of 3TC[52]. As with 3TC, (+)-FTC was found to be a suitable substrate for deoxycytidine deaminase, whereas the enantiomer, (−)-FTC, was resistant to deamination mediated by the enzyme[53].

Biological studies suggested that like 3TC, deoxycytidine kinase, deoxycytidylate kinase and possibly nucleoside diphosphate kinases are the enzymes that phosphorylate L-FTC to emtricitabine 5′-triphosphate. E-TP is incorporated by RT into the elongating proviral DNA chain, causing termination of DNA synthesis because of the impossible incorporation of the next nucleotide at the E-TP 3′ position (**Figure 1.4**)[48,54]. Both D- and L-enantiomers of FTC triphosphates were equipotent inhibitors for RT, but L-FTC proved to be anabolized more efficiently and to be a better substrate for the deoxycytidine kinase than the corresponding D-enantiomer[55]. Intracellular concentrations of the D- and L-FTC triphosphates were compared following incubation with racemic FTC and there were 30 times more L-FTC triphosphate than the corresponding D-FTC triphosphate. Therefore, the potent anti-HIV activity of L-FTC and 3TC is caused by their selective phosphorylation steps together with the stereoselective deaminations of the D- enantiomers[56]. However, 1:1 racemic FTC (racivir, **IX**), is in phase II clinical trial and probably will be soon approved by FDA for its anti-HIV-1 activity.

Unfortunately, both 3TC and FTC could develop rapidly drug resistance. After few weeks of initiation of monotherapy, they cause a single point mutation at position 184 of RT[57]. The point mutation causes a replace of methionine by valine or isoleucine (M184V, M184I mutations). As expected, this mutation confers cross-resistance to 3TC, but other nucleoside agents retain their activity[58], so the combination of different antivirals of the HAART can avoid the problem[4].

1.3.1. PREVIOUS SYNTHETIC APPROACHES TO 3TC AND FTC

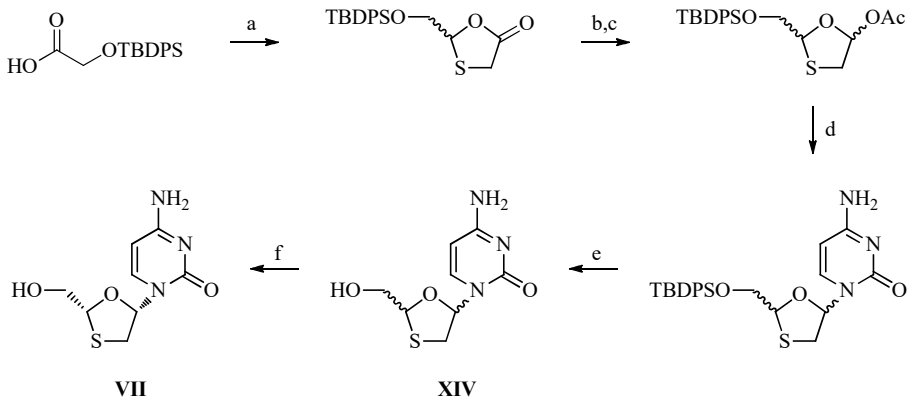
The first synthetic approach to (±)-BCH-189 reported by Belleau *et al*.[44] utilized an oxathiolane intermediate obtained as a 1:1 mixture of anomers by reaction of benzoyl oxyacetaldehyde (**XVII**) with protected mercaptoacetaldehyde **XVIII**. The intermediate **XIX** was then coupled with silylated cytosine by $TiCl_4$ promoted reaction to give a 1:1 mixture of α and β nucleosides. After separation by chromatography and following deprotection, (±)-BCH-189 (**XIV**) was obtained (**Scheme 1.1**).



XVII **XVIII** **XIX** **XIV**

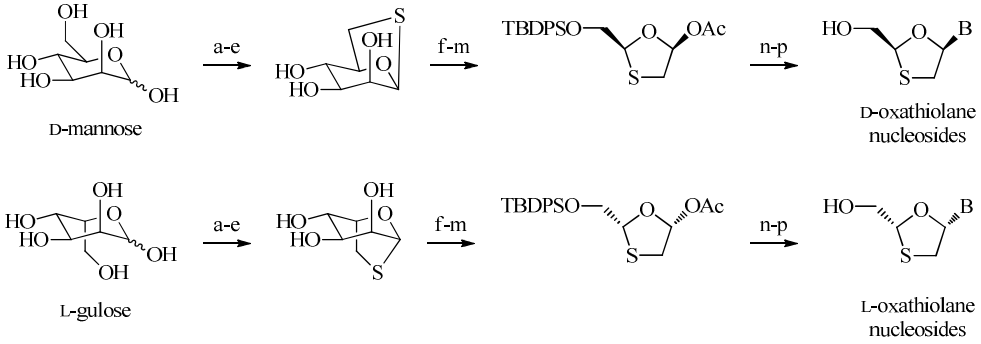
Scheme 1.1: First synthesis of racemic BCH-189.

Various approaches for the synthesis of 3TC have been reported, especially in nineties. Liotta *et al*. first reported the synthesis of 3TC using $SnCl_4$ as promoter obtaining the β-anomer as the major product (selectivity >300:1), and following the 3TC after enzyme resolution[59] (**Scheme 1.2**).



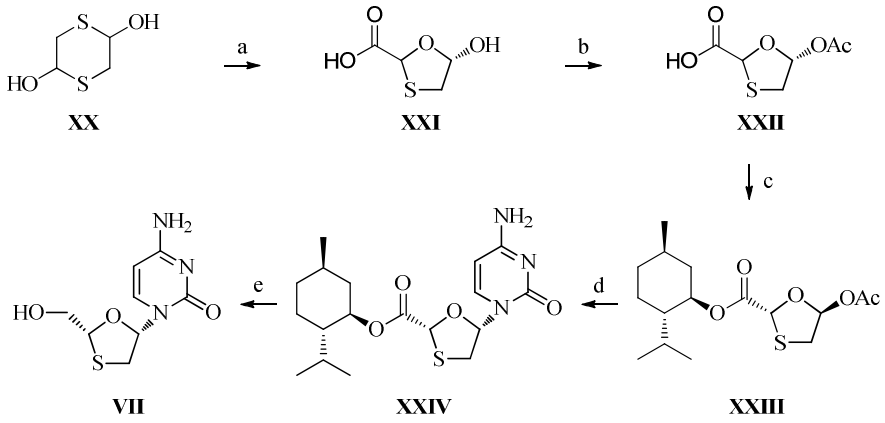
Scheme 1.2: (a) HSCH2CO2H; (b) DIBAL-H; (c) Ac_2O; (d) TMS-cytosine/$SnCl_4$ (e) TBAF; (f) enzyme.

Chu and co-workers reported an asymmetric synthesis of the enantiomeric pure isomers of D-oxathiolane nucleosides (enantiomers of 3TC and FTC included) starting from D-mannose and D-galactose[60] or L-gulose to finally get L-oxathiolane nucleosides (3TC and FTC included) [61,62] using TMSOTf (with a low ratio $\alpha:\beta$ = 1:2) and $SnCl_4$ (**Scheme 1.3**).



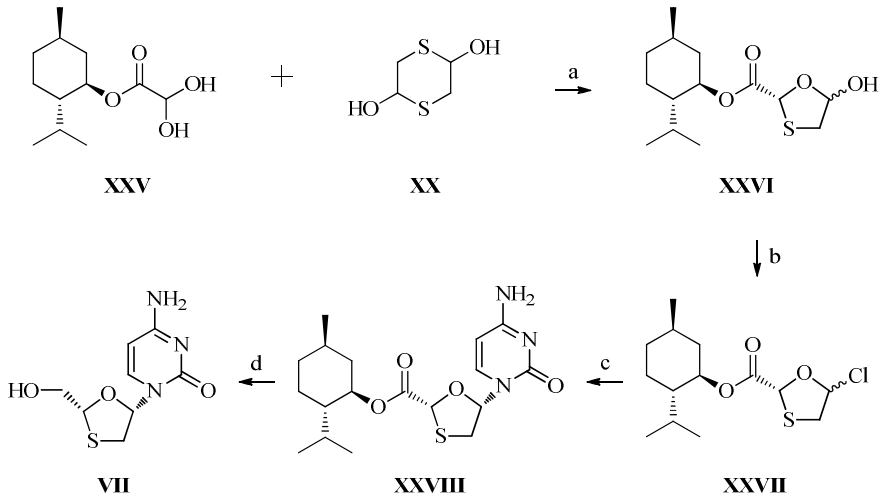
Scheme 1.3: (a) TsCl; (b) Ac_2O; (c) HBr/AcOH; (d) KEX; (e) NH_4OH/MeOH; (f) $NaIO_4$; (g) $NaBH_4$; (h) *p*-TsOH; (i) TBDPSCl; (j) *p*-TsOH/MeOH (k) $Pb(OAc)_4$; (l) PDC; (m) $Pb(OAc)_4$; (n) TMS-cytosine/$SnCl_4$ or TMSOTf; (o) NH_3/MeOH; (p) TBAF.

Goodyear *et al.*[63] in 1995 did a breakthrough in the synthesis of these oxathiolane derivatives, using the menthyl group as inductor of chirality and the TMSI as promoter of the *N*-glycosylation, obtaining a good $\alpha:\beta$ selectivity of 1:23 (**Scheme 1.4**).



Scheme 1.4: (a) glyoxylic acid hydrate/*t*-BuOMe; (b) (i) $Ac_2O/MeSO_3H$; (ii) recrystallization; (c) (i) (-)-L-menthol/DCC/DMAP; (ii) recrystallization; (d) $(TBDMS)_2$-cytosine/TMSI; (e) $LiAlH_4$.

After then, several papers and patents then described the synthesis of 3TC and FTC[64], but another substantial contribution in this field came from the Glaxo-Wellcome group[65] in the synthesis of the single enantiomer lamivudine. Indeed, condensation of L-menthyl glyoxate hydrate **XXV** with 1,4-dithiane-2,5-diol **XX** yielded an intermediate (**XXVI**) that was chlorinated with $SOCl_2$. The addition of silylated nucleobase in presence of TMSOTf gave the 3TC precursor **XXVII** (**Scheme 1.5**).

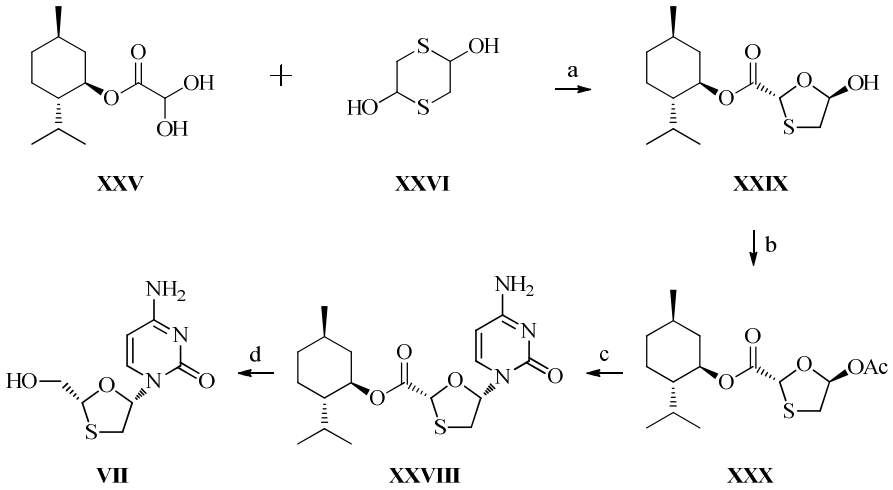


Scheme 1.5: (a) AcOH; (b) $SOCl_2$; (c) TMS-cytosine/TMSOTf; (d) (i) $K_2HPO_4/NaBH_4$ (ii) recrystallization.

Other chiral precursors[66] and chromatographic processing[67] have been described. For example, 5-fluorocytosine can be obtained via direct fluorination of cytosine using trifluoromethyl

hypofluorite and fluorotrichloromethane as solvent, or alternatively from fluoroacetic acid[68], but its production is the step with the largest price.

A last patent has been published in 2013 by Roy *et al.*[69], where the synthetic strategy is really similar to the Glaxo-Wellcome one except for the *N*-glycosylation, that starts from an acetylated intermediate and is promoted by $ZrCl_4$. The obtained β-product is >97% (**Scheme 1.6**).



Scheme 1.6: (a) AcOH; (b) Ac_2O; (c) TMS-cytosine/$ZrCl_4$; (d) (i) $K_2HPO_4/NaBH_4$ (ii) recrystallization.

Every explored strategy presents common drawbacks, in particular in the *N*-glycosylation step. The reaction is promoted by unwieldy and unstable promoters, which involves little reproducibility of the reaction, both in terms of yield and stereoselectivity. These two factors are very important for the large production of 3TC and FTC and for minimizing their contamination with undesired cytotoxic isomers.

1.4. CONCLUSIONS AND FUTURE PERSPECTIVES

In spite of all the worldwide efforts, AIDS remains a pandemic disease because the rapid evolution of the HIV virus and the development of drug resistance. However, a large number of antivirals has been licensed, most of them NRTIs, which can turn in most of cases this disease from mortal to chronical. In particular, L-nucleosides represent a good compromise between activity and toxicity, for their almost exclusive interaction with RT rather than human DNA

polymerase. Within this class of nucleosides, 3TC and FTC are the most representative drugs approved by FDA, showing a potent antiviral activity and a low cytotoxicity *in vitro* and *in vivo*. Their preparation still constitute a challenge for scientist for the difficult of obtaining at the same time good yields and stereoselectivity, enclosed to a practical, cheap and safe synthesis. Indeed, a new synthetic approach is requested, to avoid the use of unwieldy and unstable promoters of *N*-glycosylation and to obtain a higher selectivity.

More importantly, new anti-HIV agents featuring novel mechanisms of action and capable of minimizing the insurgence of resistance are still needed.

2. OBJECTIVES AND SYNTHETIC STRATEGY

This section of the thesis is addressed to the development of a convenient, cheap, safe and stereoselective synthesis of 3TC and FTC, using a stable promoter system in the key *N*-glycosylation step. Furthermore, on pursuing the search for new anti-HIV agents able to avoid the evolution of resistance, new derivatives of 3TC have been synthesized to be tested for their potential biological activity.

The stepwise strategy can be outlined in few critical points:

- The construction of new 3TC derivatives corresponding to the diastereoisomeric sulfoxides obtained from a racemic mixture of lamivudine and its D enantiomer via a kinetic resolution (**Figure 2.1**).

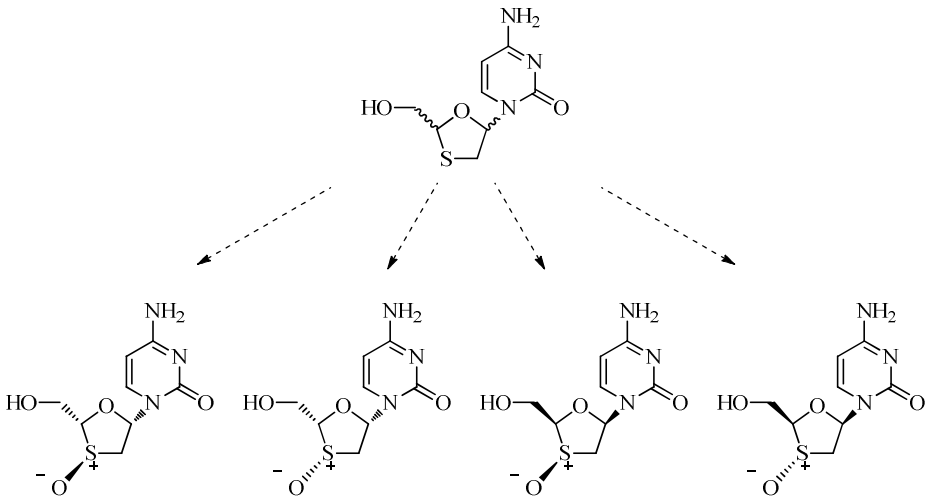


Figure 2.1: Construction of sulfoxides of 3TC and its enantiomer.

- The construction of 3TC sulfoxides starting from commercial Zeffix®, in case of an unsuccessful kinetic resolution (**Figure 2.2**).

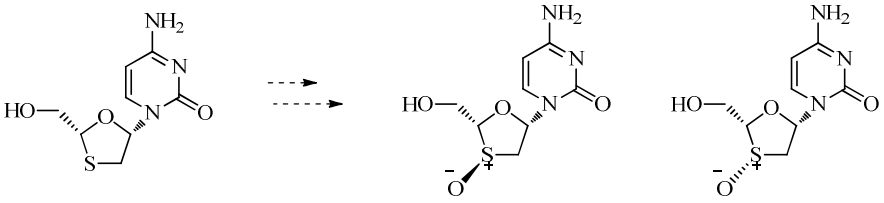


Figure 2.2: Construction of sulfoxides of 3TC starting from commercial drug.

- The synthesis of D enantiomers of 3TC and FTC with the silane/iodine combined reagent as the promoter of the *N*-glycosylation reaction (**Figure 2.3**).

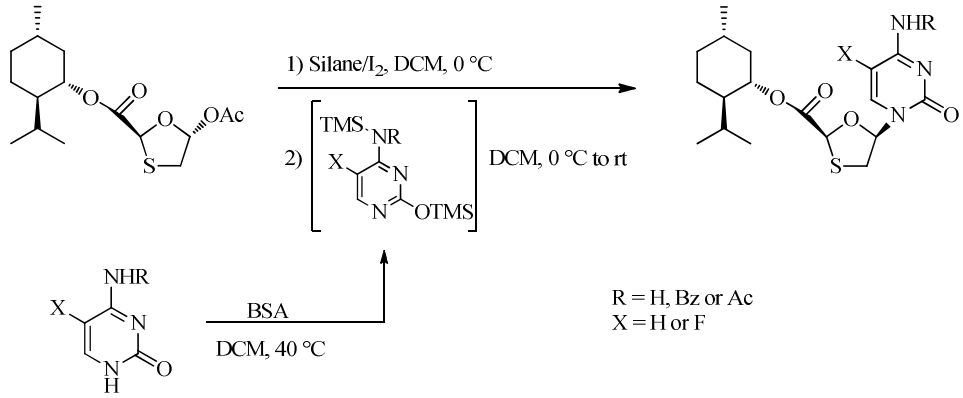


Figure 2.3: Employment of the silane/iodine system in *N*-glycosylation.

- The construction of D-sulfoxides from the synthetized (+)-BCH-189 (D-isomer of 3TC) (**Figure 2.4**).

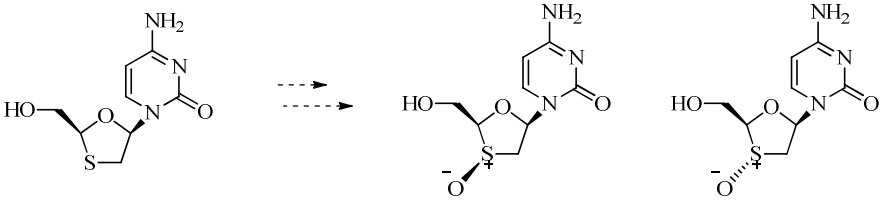
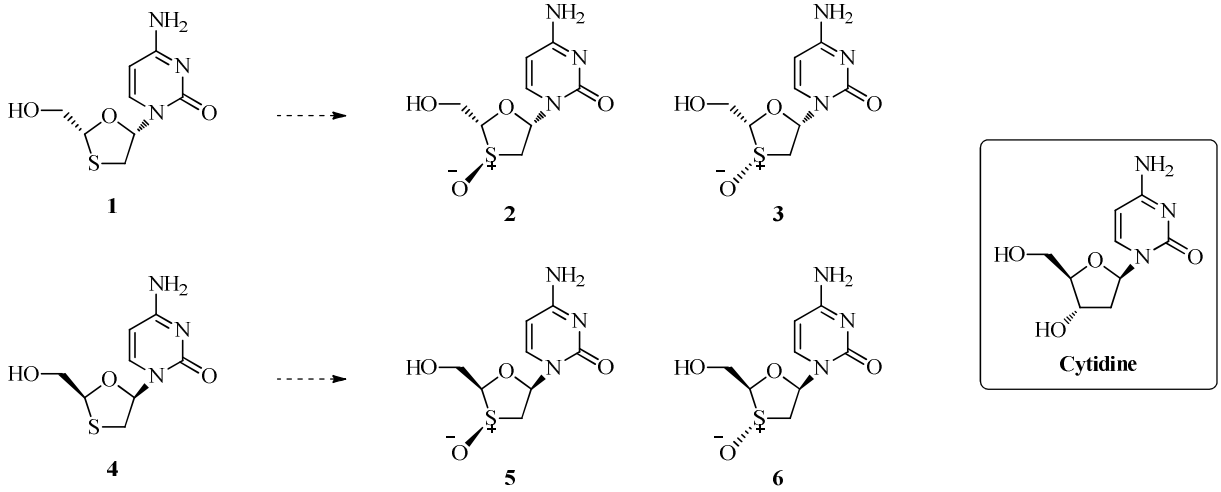


Figure 2.4: Construction of D-sulfoxides.

3. RESULTS AND DISCUSSION

3.1. CONSTRUCTION OF 3TC DERIVATIVES

As described in the introduction, the NRTIs feature modification in the sugar and/or nucleic base moieties to interact with RT and operate as chain terminators in the viral reverse transcription reaction (**Figure 1.4**)[17]. 3TC (**1**), in spite of its L-configuration, exhibits potent anti-HIV-1 and HIV-2 activity, that is expected to be amplified with structural modifications making it more similar to the cytidine (**Scheme 3.1**).



Scheme 3.1: Project of construction of 3TC sulfoxides.

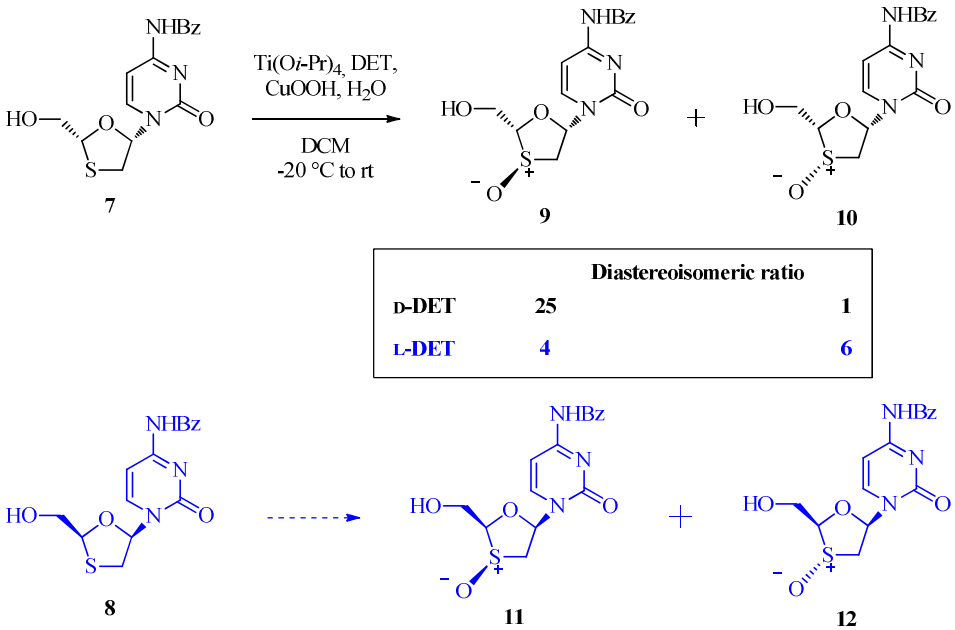
The introduction of an oxygen linked to the sulfur mimics the hydroxyl group attached to cytosine C-3′, and could better deceive the RT. At the same time, the impossible condensation reaction between the sulfoxide and the phosphate group of other triphosphate nucleotides prevents the elongation of the viral DNA chain. The preparation of both the sulfoxides starting from 3TC and its enantiomer was planned, considering that the biological activity and the toxicity of molecules is not easily predictable and every isomer could be a potential antiviral drug.

3.1.1. KINETIC RESOLUTION OF SULFOXIDE RACEMIC MIXTURE

In a previous work of the research group where this section of the thesis was carried out, the commercially 3TC (Zeffix®) was oxidized after a protection of the exocyclic amine group of the cytosine, to permit the chromatographic separation of the two isomeric sulfoxides.

Among the numerous existing methods[70] of sulfoxidation, the group selected the Kagan[71] and the Modena[72] oxidations. Both methods contemplate the use of diethyl tartrate (DET) and titanium(IV) isopropoxide (Ti(O*i*-Pr)$_4$) for the asymmetric induction and a hydroperoxide as oxidizing agent (in particular, the research group used the cumene hydroperoxide, CuOOH). The Kagan method involves a 1:2:1 of Ti(O*i*-Pr)$_4$/DET/H_2O ratio, with the surprising presence of the water that give a strong increase in the optical yield; the Modena method instead contemplates a 1:4 ratio of Ti(O*i*-Pr)$_4$/DET alone, but both the sulfoxidations shows similar results.

Protected 3TC **7** was oxidized with both D and L-DET under conditions of Kagan and Modena trying to mimic the different reactivity of the two enantiomers **7** and **8** with the only D-DET in the formation of four stereoisomeric sulfoxides **9**, **10**, **11**, and **12** (**Scheme 3.2**).



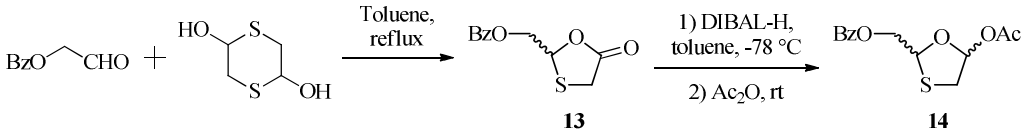
	Diastereoisomeric ratio	
D-DET	25	1
L-DET	4	6

Scheme 3.2: Previous work of the research group: stereoselective sulfoxidation of 3TC under Kagan conditions.

Analysis of these results indicated the potential applicability of a kinetic resolution approach starting from a racemic mixture of **7** and **8**. Indeed, this strategy was supported by combining the following observations: 1) the low diastereoisomeric ratio (dr) obtained by treatment of **7** with L-

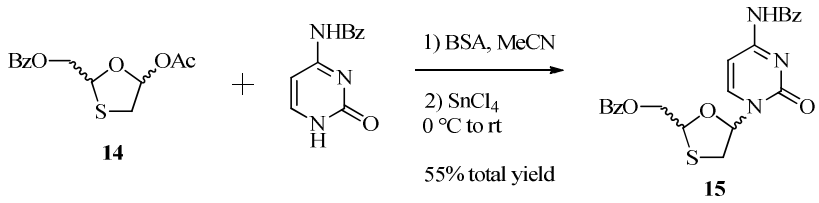
DET (4:6) corresponds to that obtainable on exposure of **8** to D-DET; 2) oxidation of 7 with D-DET is not only more diastereoselective, but is also faster than with L-DET.

The synthesis of racemic mixture of 3TC and its enantiomer started from compound **14**, previously obtained by the condensation of benzoyl oxyacetaldehyde with 1,4-dithiane-2,5-diol (dimeric mercaptoacetaldehyde), in a synthetic route similar to that described by Belleau *et al.*[44] (**Scheme 3.3**).



Scheme 3.3: Condensation and protection leading to compound **14**.

Next step has been the glycosylation of **14** with a protected cytosine (as seen above, the exocyclic amine group of the cytosine needs a benzoyl to permit the chromatographic separation of isomeric sulfoxides). In the first step of the reaction *N*,*O*-bis(trimethylsilyl)acetamide was added to the reaction mixture to silylate the nucleic base in order to minimize the undesired *O*-glycosylation process competing with desired *N*-functionalization. Then $SnCl_4$ was added into the flask via cannula to serve as the glycosylation promoter (**Scheme 3.4**).

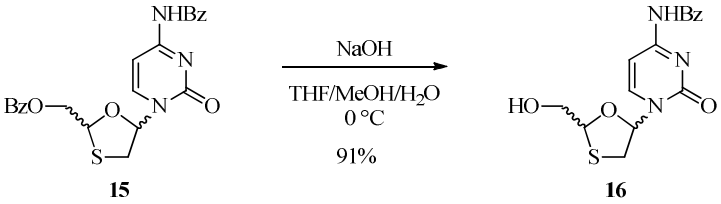


Scheme 3.4: *N*-glycosylation promoted by $SnCl_4$.

Use of an aged commercial sample of $SnCl_4$, probably for the presence of HCl, led to the formation of **15** as a 1:1 α/β mixture of anomers (α refers to the 2,5-*trans* isomers and β to the 2,5-*cis* isomers) in an overall 55% yield (28% for the β-anomer alone). Furthermore, the chromatographical separation did not allow a complete separation of the anomeric mixture, and just a low percentage of β isomers was recovered, underlining the need of a stable promoter system.

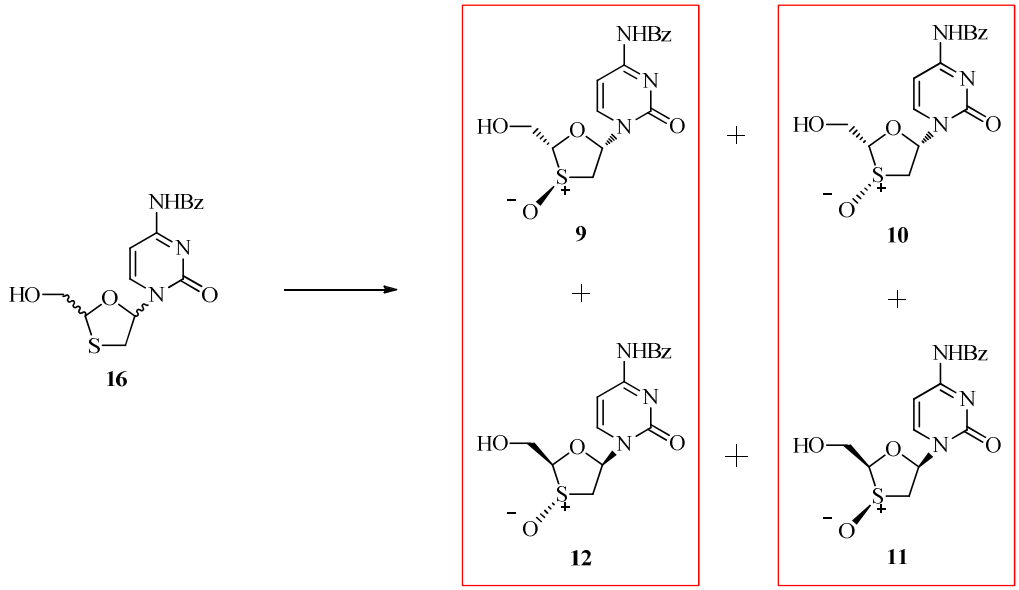
A further attempt was carried out with $ZrCl_4$ as reported by Roy *et al.*[69] but only the starting product was recovered, in spite of application of extended reaction times and high temperatures.

The deprotection of the hydroxyl moiety was easily achieved in presence of NaOH, affording the starting product for the sulfoxidation **16**, namely a mixture of nucleosides **7** and **8** (**Scheme 3.5**).



Scheme 3.5: Basic hydrolysis of benzoyloxy moiety of nucleosides **15**.

A large number of sulfoxidation experiments was carried out on **16** (**Table 3.1**) testing both Kagan and Modena methods under a variety of experimental conditions, but unfortunately the kinetic resolution gave disappointing results. In fact, the sulfoxides were always recovered as two racemic couples (in red in **Scheme 3.6**), also when the reaction was stopped after a few hours (entry 2). The recovered starting product never showed a significant enrichment of either enantiomer **7** or **8**.



Scheme 3.6: Sulfoxidation of racemic mixture **16**.

Entries	Ti(O*i*-Pr)$_4$ equivalents	D-DET equivalents	L-DET equivalents	H_2O equivalents	CuOOH equivalents	Temperature (°C)	Time
1	1	2		1	2	-10 to 0	24 h
2	1	1	1		2	0 to rt	4 h
3	1	2		1	2 + 2	-10 to rt	8 days

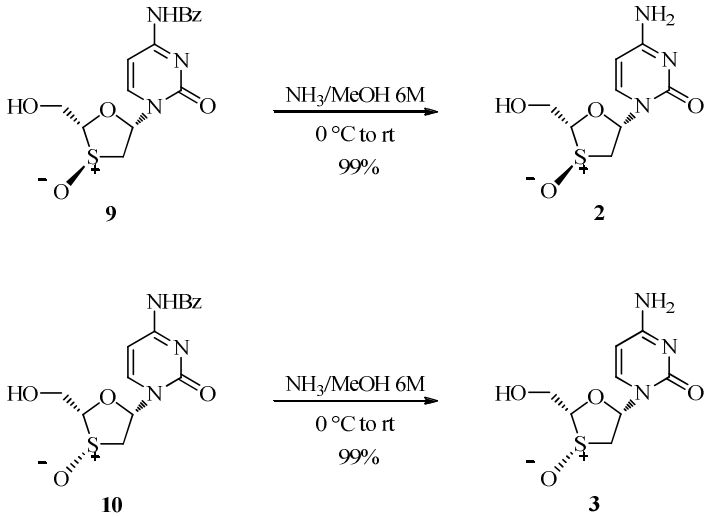
4	1 + 1	4			2	-20	24 h
5	1	4			2	-20 to 0	6 days
6	0.5	2			1	-20 to 0	3 days
7	1		4		2	-20 to 0	2 days

Table 3.1: Tested conditions for the attempted kinetic resolution via sulfoxidation.

The enantiomeric pairs of sulfoxides were hard to separate and for this reason unusable. A different strategy was then developed to obtain the desired product.

3.1.2. SYNTHESIS OF L-SULFOXIDES

The sulfoxides obtained by the research group during the evaluation of the model system previously described belong to the L- steric series, because were obtained from the commercial 3TC. For this reason, a difficult chromatographic separation was accomplished on about one gram of mixture, recovering 50 mg of each isomer, and the sulfoxides **9** and **10** were deprotected in presence of a solution of ammonia and methanol 6 M (**Scheme 3.7**).



Scheme 3.7: Deprotection of L-sulfoxides.

Crystallization of the products permitted to obtain 10 mg of each isomer, that will be tested *in vitro* for their antiviral activity.

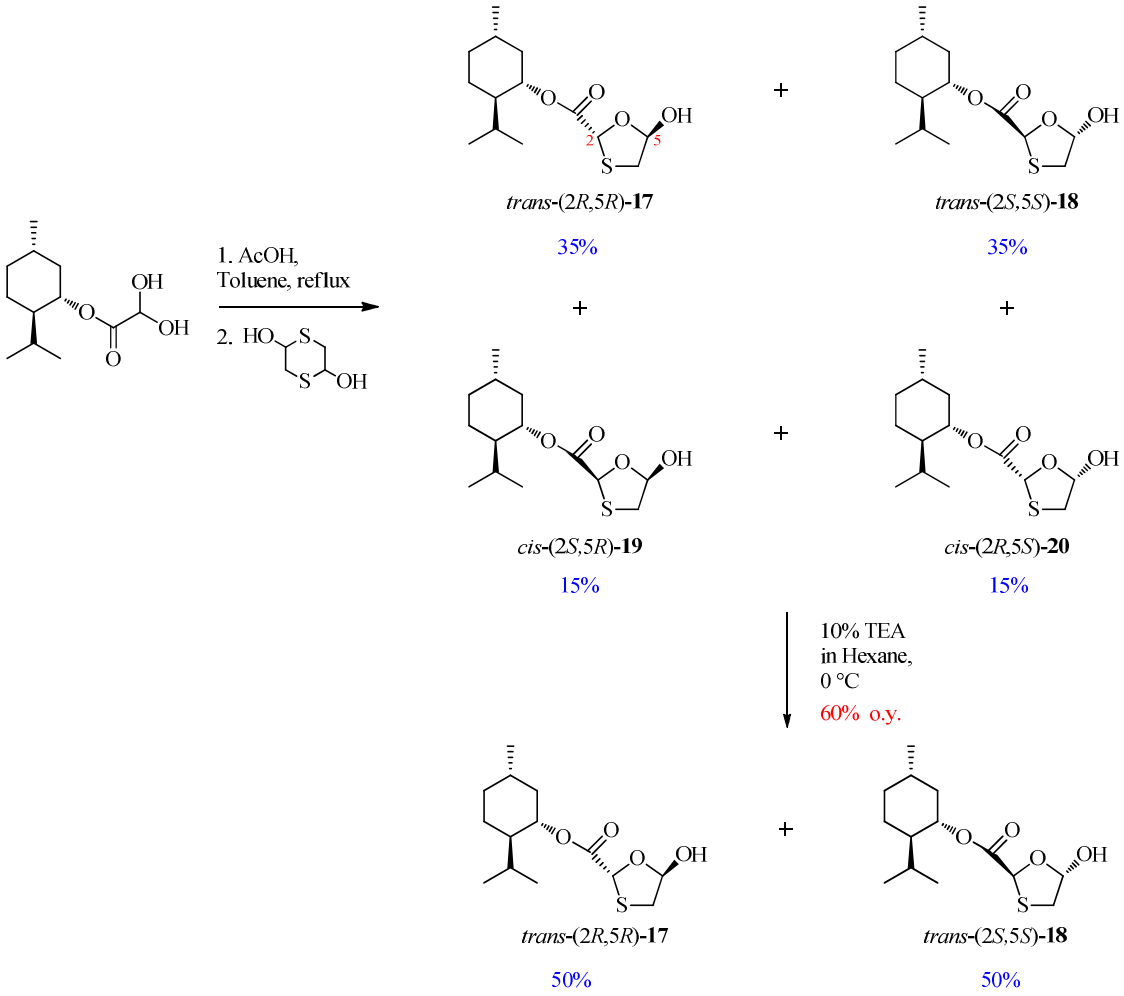
To obtain the D-sulfoxides a total synthesis of (+)-BCH-189 (D-isomer of 3TC) was accomplished, with a previous research of a better promoter system for the *N*-glycosylation. This promoter was also tested in the synthesis of D-FTC

3.2. SYNTHESIS OF (+)-BCH-189 AND D-FTC: THE EMPLOYMENT OF SILANE/IODINE SYSTEM AS A NEW *N*-GLYCOSYLATION PROMOTER

As seen in the introduction, the most recent and employed methods for the synthesis of 3TC and FTC (Glaxo-Wellcome group[65], Roy *et al.*[69]) involve the use of the menthyl ester group as chiral inductor. The new developed synthetic route, unlike the previous ones, takes advantage of (+)-menthol instead of the (-)-menthol to obtain the desired enantiomers of the commercial drugs.

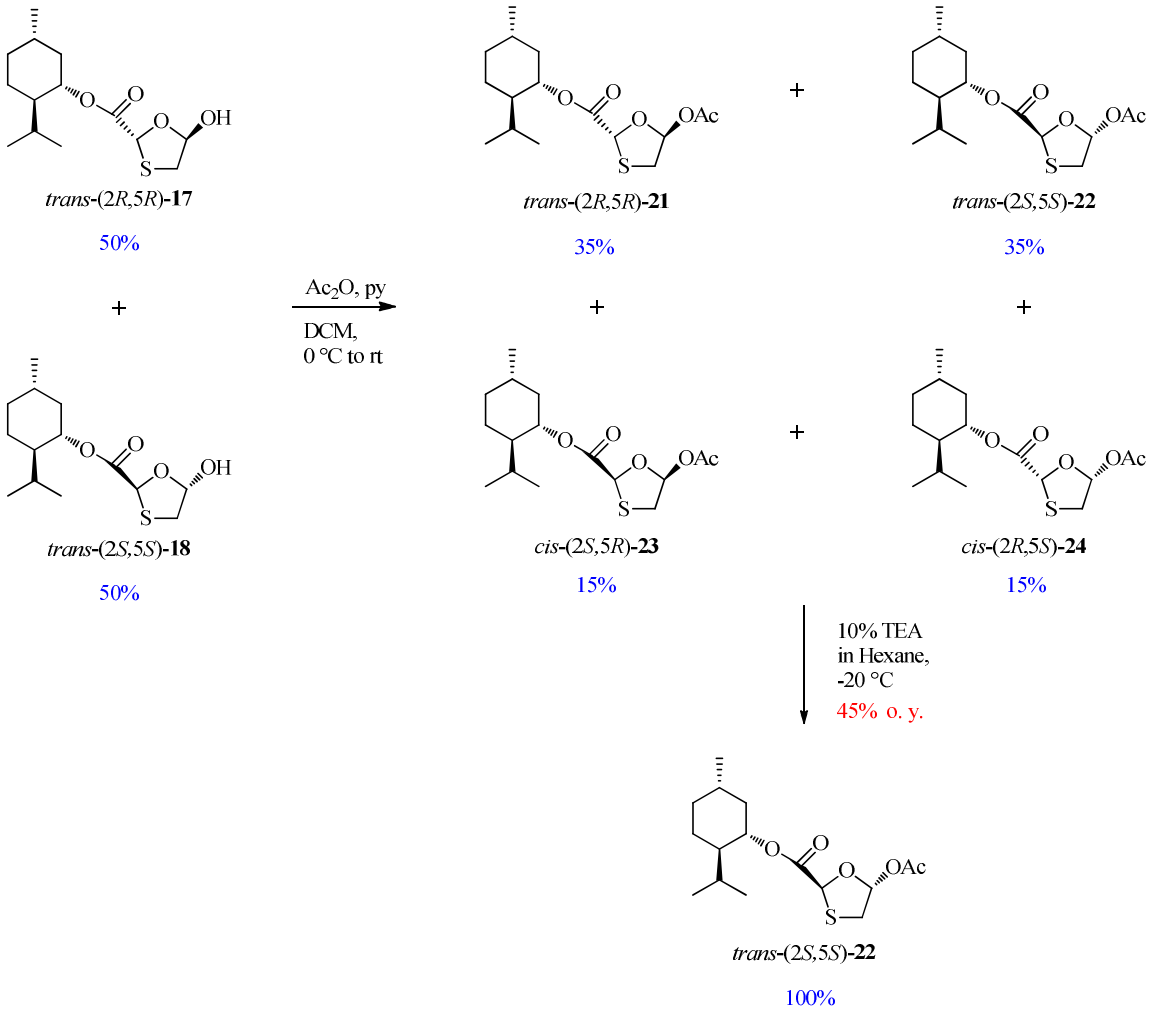
3.2.1. SYNTHESIS OF ACETYLATED STARTING PRODUCT FOR *N*-GLYCOSYLATION

The first step of the route is already reported in literature[65,69,73] for the synthesis of 3TC and FTC, and consists of a condensation between D-menthylglyoxylate and 1,4-dithiane-2,5-diol. The reaction was carried out with L-menthylglyoxylate to obtain the enantiomeric products (**Scheme 3.8**).



Scheme 3.8: Construction of oxathiolane ring.

The condensation lead to the formation of four diastereoisomers (**17**-**20**) because the creation of two chiral centers, in ratio 35:35:15:15 (where the two most abundant are the trans isomers, as determined by NMR). Crystallization in hexane at 0 °C in presence of triethylamine, that permits the opening of the new-formed oxathiolane rings and the rapid interconversion of the stereocenters, afforded pure hemiacetals **17** and **18** in a 60% overall yield without the need of further purifications. These results are in contrast to what described in literature[73], where the generation of the four diastereoisomers is reported to occur with a different ratio (hemiacetals with *R* configuration at C-2 are present with a 35% rate) and crystallization with hexane and triethylamine led to the formation of the **18** alone. This discrepancy has proved to be of little importance, because the subsequent acetylation was carried out in presence of pyridine, that promoted a second interconversion of the chiral centers and the formation of the four acetylated products in the same 35:35:15:15 ratio (**Scheme 3.9**).



Scheme 3.9: Acetylation of hemiacetals **17**-**18**.

Crystallization in hexane and triethylamine, carried out at -20 °C for three days, led to the formation of the sole desired *trans* product **22**, used as the starting product in the subsequent *N*-glycosylation studies.

3.2.2. STUDIES OF *N*-GLYCOSYLATION

On the basis of previous literature, the *N*-glycosylation system that gave the best results was relying on the unstable and expensive TMSI[63]; on pursuing the search for a cheaper, more stable and easier to handle promoter, the efforts in this thesis have been focused on a silane/iodine combined system. In particular, two different silanes proved useful for this purpose, triethylsilane (Et_3SiH) and polymethylhydrosiloxane (PMHS).

The combination of Et_3SiH and I_2, largely explored and used in organic synthesis (especially in carbohydrate chemistry), generates in DCM the species Et_3SiI, that presents lower but still significant reactivity in comparison with TMSI, and HI, otherwise difficult to be obtained in a anhydrous form. This system claims a large plethora of applications, including anomeric iodination, a reaction that often precedes the introduction of a large number of functional groups[74], the regioselective debenzylation of densely benzylated polyols, when the removal of the protecting group leads to a steric relief[75], *O*-glycosylation, where the combined reagent can activate haloacetimidate leaving groups[76], or allylic rearrangement of glycals, where the promoter entails a catalytic amount of iodine in combination with an excess of silane[77] (**Figure 3.1**).

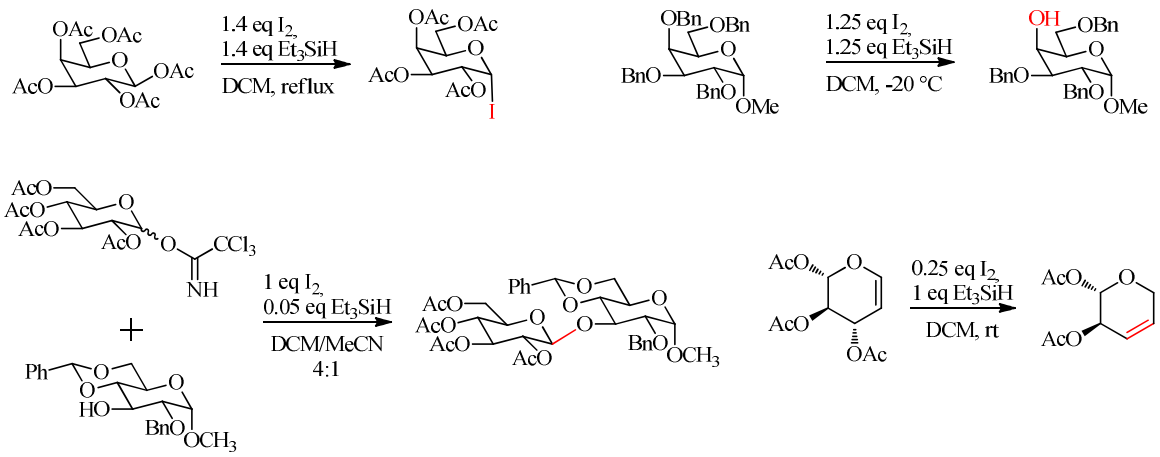


Figure 3.1: Examples of applications of the Et_3SiH/I_2 system.

In this study a particular interest was focused on the possible replacement of Et_3SiH with PMHS in the reagent system. This latter is a polymer synthetized for the first time in 1946, but employed in organic synthesis only recently; this polysilane is convenient for large-scale application, because is less expensive and more stable than other silanes and can be stored for larger periods. In combination with iodine, PMHS has been used in a large number of anomeric iodinations, where it showed a reactivity similar to Et_3SiH. Resulting glycosyl iodides can be quickly converted into alternative derivatives without an intermediate chromatographical purification[78]. In addition, PMHS can be employed in reductive processes for its ability to serve as stable hydride donor under acidic conditions, avoiding the use of boranes and aluminum hydrides that could cause problems of selectivity and the generation of toxic salts as byproducts[79]. Examples of these applications are the reductive dihydroxylation of benzylic alcohols, in combination with Lewis acids of iron[80] and one-pot reductive amination, in combination with TFA, allowing the alkylation of amines with aldehydes and ketones[81] (**Figure 3.2**).

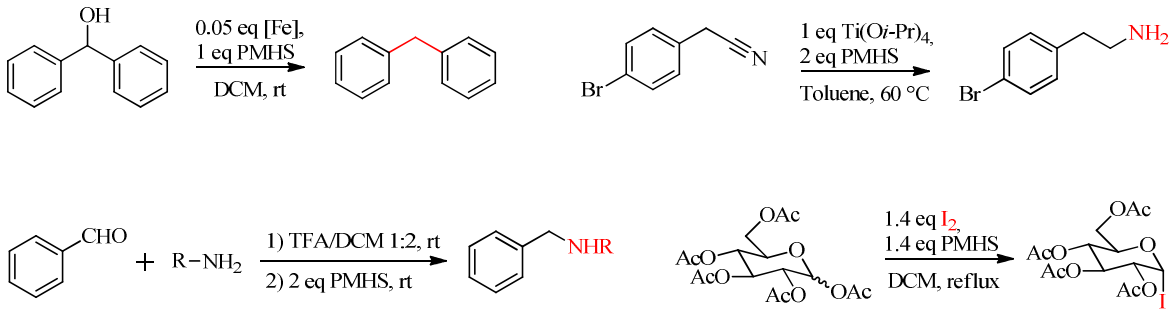
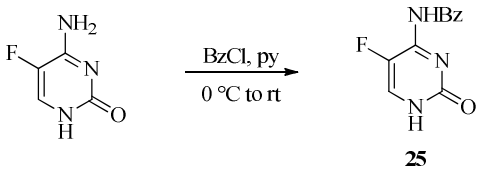


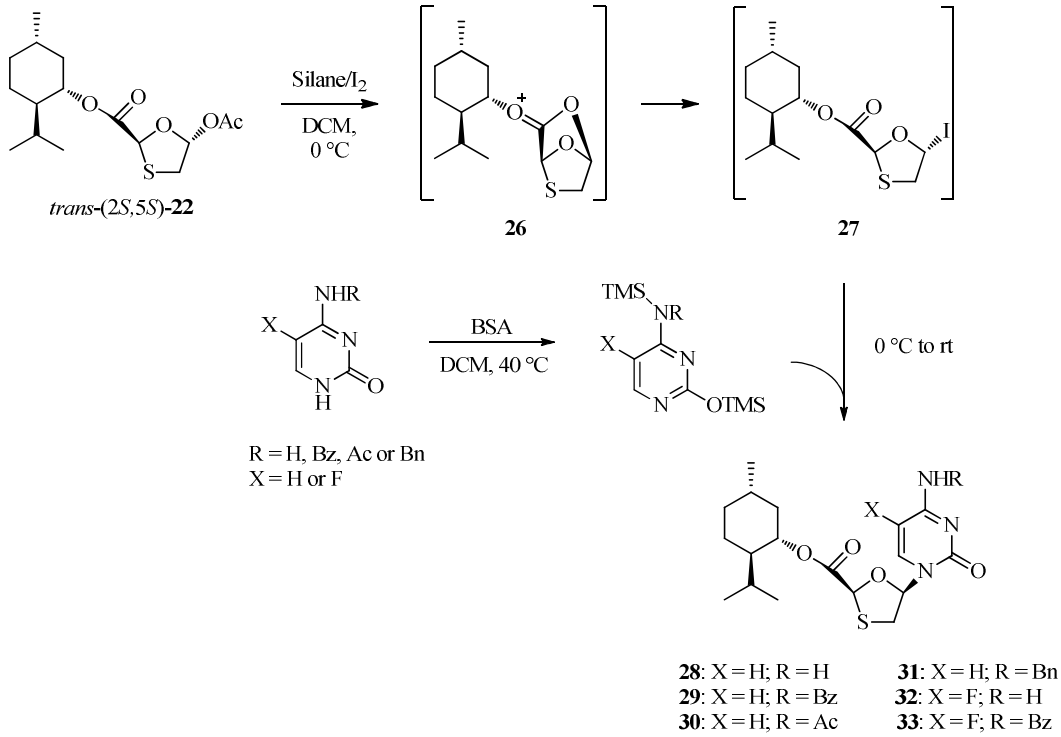
Figure 3.2: Examples of synthetic applications of the PMHS.

The *N*-glycosylation studies also explored the effect of the nucleobases protecting group on yields and stereoselectivity. For this reason the commercial cytosine, 5-fluorocytosine, acetylcytosine and benzoylcytosine have been employed, together with benzylcytosine, synthetized with a procedure reported in literature[82], and benzoyl 5-fluorocytosine, synthetized by an *N*-benzoylation with BzCl (**Scheme 3.10**).



Scheme 3.10: Synthesis of benzoyl 5-fluorocytosine.

N-glycosylation reaction consists of the iodination of starting acetyl oxathiolane **22** and its coupling with a presilylated nucleobase (**Scheme 3.11**).



Scheme 3.11: *N*-glycosylation reaction.

As detailed below, the explored *N*-glycosylation methodology generally gave a satisfying β-selectivity which may be accounted for by the following mechanistic picture. Treatment of starting **22** with a pre-mixed solution of silane and iodine leads to the formation of an oxonium ion **26**, stabilized through anchimeric assistance by the menthyl ester function, as confirmed in literature[73]. Iodination then occurs in the most accessible α-face, affording the *trans* iodinated product **27**. In a separate flask the nucleobase was silylated with BSA to avoid the participation of either the oxygen or the exocyclic amine in the reaction with the oxathiolane ring. Attack of this protected base via an S_N2 reaction gives the β nucleoside (*cis*) as final product (**28**-**33**) with a small amount of the inseparable α (*trans*) isomer.

Results of the experiments with the different nucleobases are summarized in **Table 3.2**.

Entry	Silane	R base	X base	Product	Yield (%)	Selectivity[a,b] (de,%)
1	Et_3SiH	H	H	**28**	98	88
2	PMHS	H	H	**28**	86	88
3	Et_3SiH[c]	Bz	H	**29**	72	95
4	PMHS[c]	Bz	H	**29**	73	95
5	Et_3SiH[d]	Bz	H	**29**	87	98

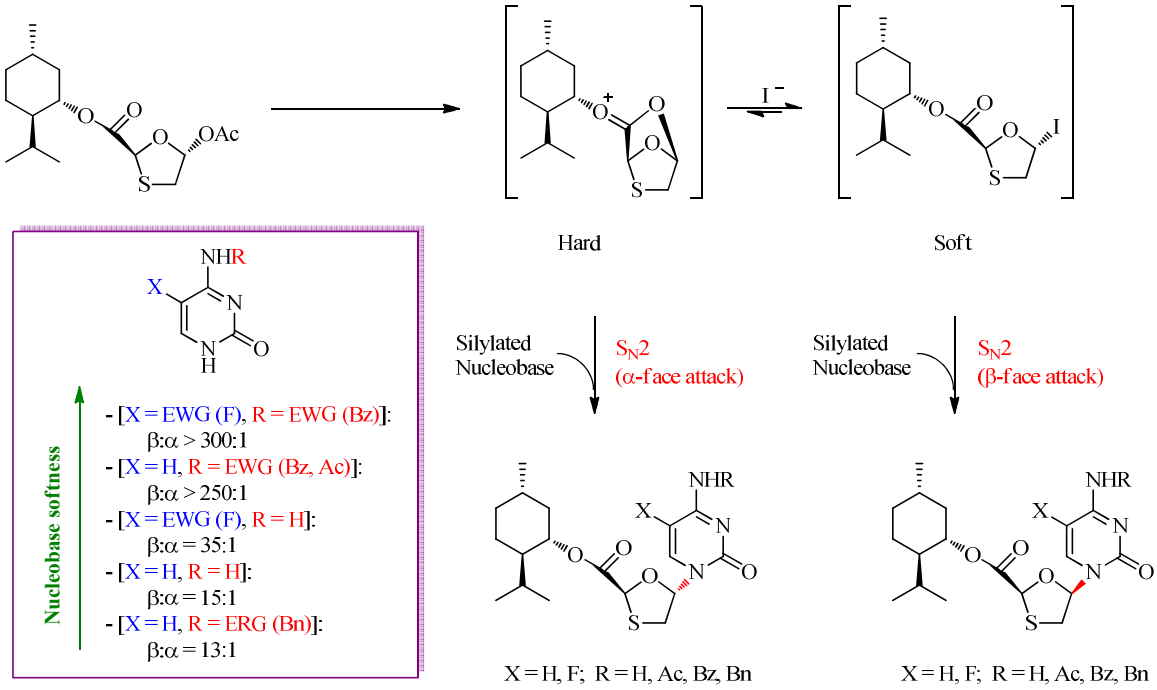
6	PMHS[d]	Bz	H	**29**	94	99
7	Et_3SiH	Ac	H	**30**	95	97
8	PMHS	Ac	H	**30**	80	98
9	Et_3SiH[d]	Bn	H	**31**	62[c]	86
10	Et_3SiH	H	F	**32**	91	94
11	PMHS	H	F	**32**	84	94
12	Et_3SiH	Bz	F	**33**	85	98
13	PMHS	Bz	F	**33**	90	99

Table 3.2: *N*-glycosylation reaction study.

[a]Determined by ^1H NMR analysis (400−600 MHz). [b]The structure of β- and α-nucleosides was determined by reduction of the menthyl ester group and NMR comparison with literature data[61]. [c]Silane/I_2 addition to the reaction mixture immediately after premixing of the two reagents. [d]Silane/I_2 addition to the reaction mixture carried out 15 min after premixing of the two reagents. [e]Mixture of regioisomeric N^1 and N^3 nucleosides (from NMR analysis).

Application of standard conditions to unprotected cytosine (entries 1-2) and 5-fluorocytosine (entries 10-11) gave satisfying yields and anomeric selectivity with both Et_3SiH and PMHS, although lower than with other described results. Conversely, when the cytosine was protected with benzoyl group (entries 3-6) a remarkable improvement of selectivity was reported, in particular when the addition of the silane/iodine system was carried out after 15 minutes after premixing of the two reagents. The trend was confirmed by reaction with acetylcytosine and benzoyl 5-fluorocytosine, that displayed the best results in terms of selectivity. PMHS gave generally higher selectivity for all the substrates, while Et_3SiH afforded better yields except for benzoyl cytosines (entries 6 and 13). When cytosine was protected with a benzyl group (entry 9), a dramatic decrease of yield and selectivity was reported, with the formation of an inseparable mixture of N^1 and N^3 nucleosides as products.

Rationalization of these results can be supported by the HSAB theory (**Scheme 3.12**).

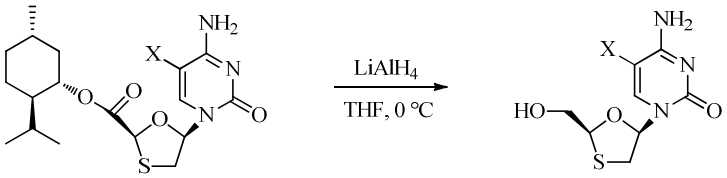


Scheme 3.12: HSAB theory applied to the *N*-glycosylation reaction.

Considering that an equilibrium is established between the oxonium ion and the iodinated oxathiolane, the latter being reasonably the prevalent intermediate, the silylated nucleobase can attack both the species through an S_N2 pathway, leading to the formation of two anomers. Indeed, an α nucleoside is obtained starting from the oxonium ion, whereas a β nucleoside derives from the nucleobase attack to the β face of the iodinated intermediate. The oxonium ion is a harder species than the iodide. As to nucleobases, if an electron withdrawing group (EWG) is present at the N-4 position, the N-1 will be electron poorer and, for this reason, softer. These bases will have a preference for an attack to the soft iodinated intermediate from the β-face, leading to the desired *cis* nucleoside. Conversely, an electron releasing group (ERG) like the benzyl group makes the N-1 position harder, leading to a lower β selectivity, as well as a reduced yield. Furthermore, the presence of the fluorine atom at C-5 makes the N-1 position even softer for inductive effect, resulting in the highest β:α ratio in the attack.

3.2.3. REDUCTION OF MENTHYL ESTER GROUP

The last step to obtain (+)-BCH-189 and D-FTC was the reduction of the menthyl group. This reaction was easily accomplished with nucleosides bearing an unprotected cytosine, following the Goodyear *et al.*[63] procedure, which contemplates the use of $LiAlH_4$ in THF (**Scheme 3.13**).

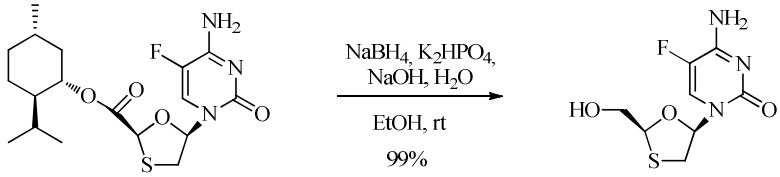


28: X = H
32: X = F

4: X = H 83%
34: X = F 60%

Scheme 3.13: Reduction of menthyl group with $LiAlH_4$.

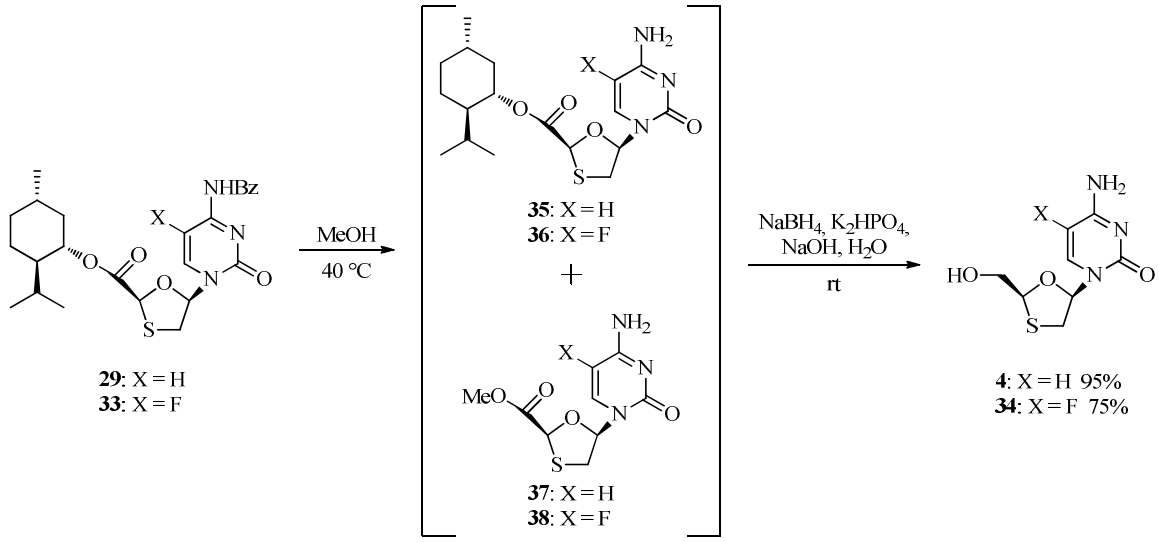
The reduction of nucleoside bearing the 5-fluorocytosine did not show satisfying yields, and consequently the reaction was accomplished with $NaBH_4$ as described in latest procedures[63,65,69], obtaining excellent results (**Scheme 3.14**).



32 **34**

Scheme 3.14: Reduction of menthyl group with $NaBH_4$.

When the reduction reactions were carried out on nucleosides with protected cytosines, the most interesting substrates, the final products were recovered in low (58% from acetylcytosine) or even negligible amounts (<10% from benzoylcytosine). In the light of these results, a new route was explored, involving the superheated methanol[83] to remove in a first step the protecting group bonded with an amide function to cytosine, and then a reduction of the menthyl ester group in presence of $LiAlH_4$ or $NaBH_4$ (**Scheme 3.15**).

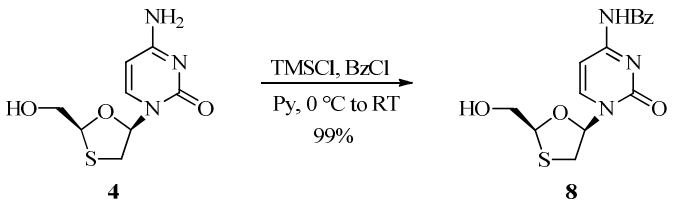


Scheme 3.15: Reduction of menthyl group with a two-steps reaction involving MeOH and $NaBH_4$.

Following the procedures described by Robins *et al.*[83] that involves MeOH at 100 °C, a large amount of epimerization products at C2 was recovered, along with transesterification products **37** and **38** (where a methoxy group replaces the menthyloxy group). Lowering the temperature down to 40 °C and extending the reaction time from one hour to two days, the epimerization and the transesterification were reduced (epimerization: at 100 °C **35**:**37**=2.2:1, at 50 °C **35**:**37**=24:1, at 40 °C **35**:**37**=60:1). The presence of the transesterification products did not affect the final yield, because the methyl ester is reduced under the same condition as the menthyloxy moiety. Addition to the mixture of $LiAlH_4$ or $NaBH_4$ led to the formation of desired products in a good anomeric selectivity (**4**: β:α > 50:1, **34**: β:α > 40:1).

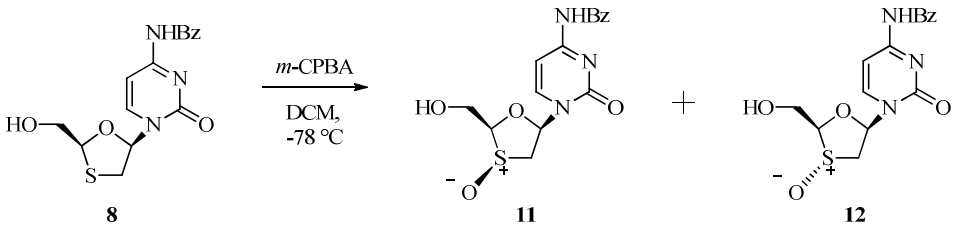
3.3. COMING BACK TO THE CONSTRUCTION OF SULFOXIDES

With (+)-BCH-189 in hand, the first step on the route to sulfoxide has been an easy selective benzoylation of the exocyclic nitrogen, that was carried out with a temporary protection of the oxygen with a silyl group, removed during the work-up of the reaction (**Scheme 3.16**).



Scheme 3.16: Protection of the exocyclic amine with a benzoyl group.

Oxidation of **8** was carried out with *m*-CPBA, being avoided in this case the problem of the dynamic resolution of products (**Scheme 3.17**).



Scheme 3.17: Construction of protected sulfoxides.

Unfortunately, the separation of the sulfoxides was not accomplished in spite of many and different attempts (flash chromatography, preparative TLC and HPLC were tried). To obtain the same results as L-sulfoxides (10 mg of final products), one gram of the mixture of sulfoxides is needed, which means that 30 g of menthyl glyoxylate should be employed in the first reaction. For this reason this strategy was discarded.

3.4. CONCLUSIONS AND FUTURE PERSPECTIVES

The synthesis of the sulfoxide derivatives of 3TC was accomplished and the products will be tested for their biological activity. Nevertheless, the high cost of the production of these substrates probably will not permit a large scale production, discouraged by the laborious synthesis and low yielding isolation observed for the D-enantiomers; on the other hand, in case of especially favourable activities and low toxicity, the reported approaches may represent a useful methodological reference for designing more effective pathways.

.The silane/iodine system proved to be an optimal *N*-glycosylation promoter, and in particular the PMHS, for its low cost, stability, reproducibility and easy of handling, has been the reagent of choice. This strategy opened a new route to antiviral drugs 3TC and FTC that ensure high stereoselectivity and yields.

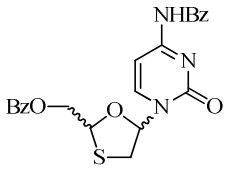
Furthermore, the critical role played by nucleobase protecting groups in influencing yields and selectivity of the reactions was unveiled, and a rationalization based on the HSAB theory was put forward.

In addition, an efficient one-pot reduction was developed, allowing the removal under mild conditions of both the cytosine protecting group and the menthyl ester moiety, by minimizing the epimerization of the reducing chiral centre. Overall, this study sets new perspectives for the synthesis of β nucleosides, pointing to the suitable combination of an effective activation system (the silane/iodine system), the nature of nucleobase protecting groups and the anchimeric assistance provided by the menthyl ester group, linked to the saccharide moiety.

4. EXPERIMENTAL SECTION

All air sensitive manipulations were carried out under dry nitrogen atmosphere. Solvents and all standard reagents were purchased from Sigma Aldrich, Alfa Aesar of VWR and were used without further purification.

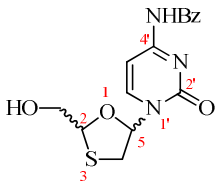
Analytical thin-layer chromatography was performed on SiO_2 (Merck silica gel 60 F_{254}), and the spots were located with ultraviolet radiation, iodine vapor and chromic mixture. Chromatography refers to flash chromatography and was carried out on SiO_2 (Merck Kieselgel 60, 230-400 mesh). Drying of organic extracts during workup of reactions was performed over anhydrous Na_2SO_4. Evaporation of solvent was accomplished with a rotary evaporator. NMR spectra were recorded at 400 MHz (^{1}H) and 100.6 MHz (^{13}C) (Bruker DRX, Bruker Avance), at 500 MHz (^{1}H) and 125.7 MHz (^{13}C) (Varian Inova equipped with a VnmrJ 4.0 software) and at 600 MHz (^{1}H) (Bruker DRX equipped with a CryoProbe), and chemical shifts are reported in δvalues downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, integrated intensity, multiplicity, coupling constant (*J*) in Hertz (Hz), and assignment (when possible). The determination of β/α ratios in the *N*-glycosylation reactions was performed by integration of ^{1}H NMR anomeric signals (H-5) of each pair of diastereoisomers at the given spectrometer (detection limit: down to 10^{-6} g). Combustion analyses were performed using a CHNS analyzer. Melting points are uncorrected and were determined with a capillary apparatus. Optical rotations were measured at 25 ± in the stated solvent. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.



2-(Benzoyloxymethyl)-5-(4-benzamido-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (15).

Oxathiolane derivative **14** (2.101 g, 7.450 mmol) was charged in a flask with benzoyl cytosine (2.085 g, 9.688 mmol) and the products were suspended in MeCN (95 ml) under nitrogen atmosphere. BSA (8.379 ml, 49.506 mmol) was then added, and the mixture was heated to 60 °C for 90 minutes. The reaction was cooled to 0 °C and $SnCl_4$ (1.828 ml, 15.648 mmol), suspended in MeCN (25 ml), was cannulated in the flask. The temperature was allowed to raise to rt and after two hours the reaction was quenched with pyridine and concentrated under reduced pressure. Flash chromatography (9:1 EtOAc-hexane) of the residue afforded α and β anomers of product **15** (55% of total conversion, 137 mg of anomers α, 211 mg of anomers β (**15**) and 1.454 g of mixture of α/β anomers).

NMR data not avaible.



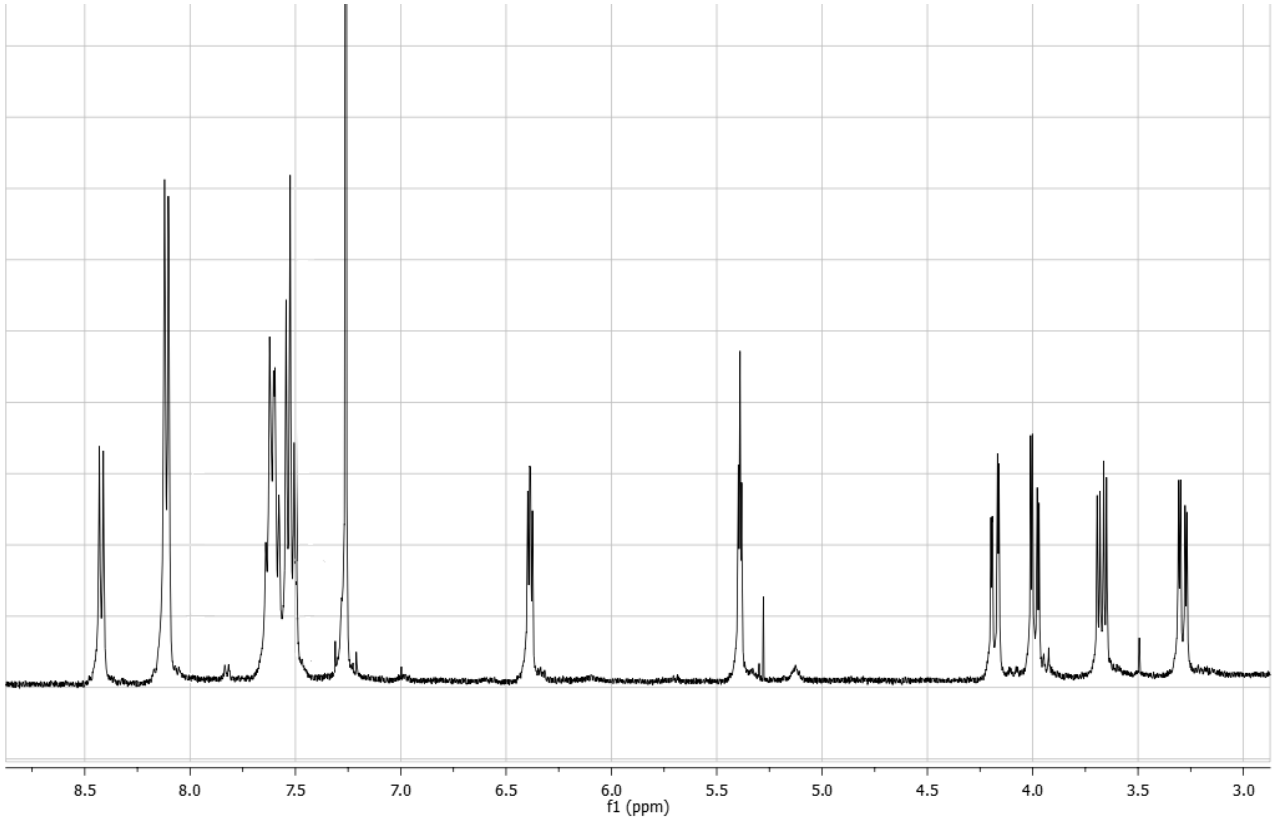
2-(Hydroxymethyl)-5-(4-benzamido-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (16).

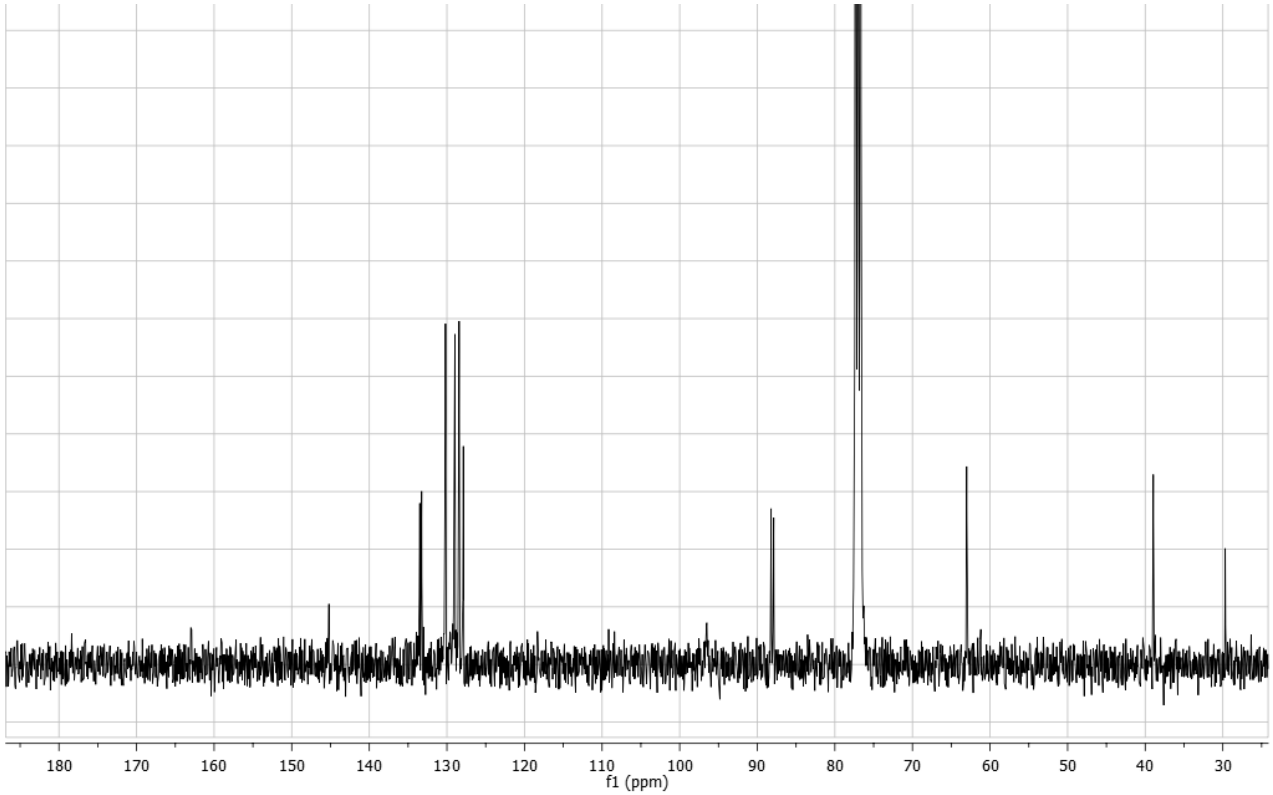
Protected compounds **15** (104 mg, 0.238 mmol) were dissolved in a mixture of THF/MeOH/H_2O (1.6 ml/0.7 ml/0.1 ml) and cooled to 0 °C. A solution 2 N of NaOH (2.4 ml) was added, and after stirring for one hour the reaction was neutralized with HCl and concentrated under reduced

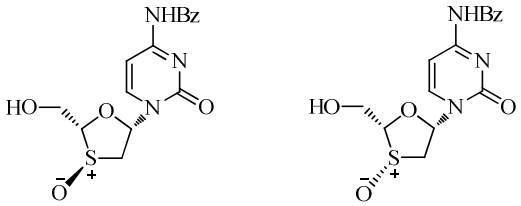
pressure. The crude was chromatographed (DCM to 7:3 DCM-MeOH) affording products **16** (72 mg, 91%).

^{1}H NMR (400 MHz, $CDCl_3$): δ3.29 (dd, *J* = 12.4, 3.6 Hz, 1H, H-4), 3.67 (dd, *J* = 12.4, 5.3 Hz, 1H, H-4), 3.99 (dd, *J* = 12.7, 3.5 Hz, 1H, CH_2OH), 4.18 (dd, *J* = 12.6, 2.6 Hz, 1H, CH_2OH), 5.39 (t, *J* = 3.2 Hz, 1H, H-2), 6.39 (dd, *J* = 5.2, 3.6 Hz, 1H, H-5), 7.52 (t, *J* = 7.9 Hz, 2H, H-*m* Ar), 7.58-7.64 (m, 2H, H-5', H-*p* Ar), 8.11 d, *J* = 7.2 Hz, 2H, H-*o* Ar), 8.42 (d, *J* = 7.5 Hz, 1H, H-6').

^{13}C NMR (100.6 MHz, $CDCl_3$): δ29.7 (C-4), 39.0 (CH_2OH), 87.9 (C-2), 88.2 (C-5), 127.9 (C-5'), 128.4 (C-*m* or *o* Ar), 129.0 (C-*m* or *o* Ar), 130.2 (C-*p* Ar), 133.2 (C-6'), 133.5 (C-*i* Ar), 145.2 (C-4'), 163.0 (C-2'), 178.4 (C=O benzoyl).







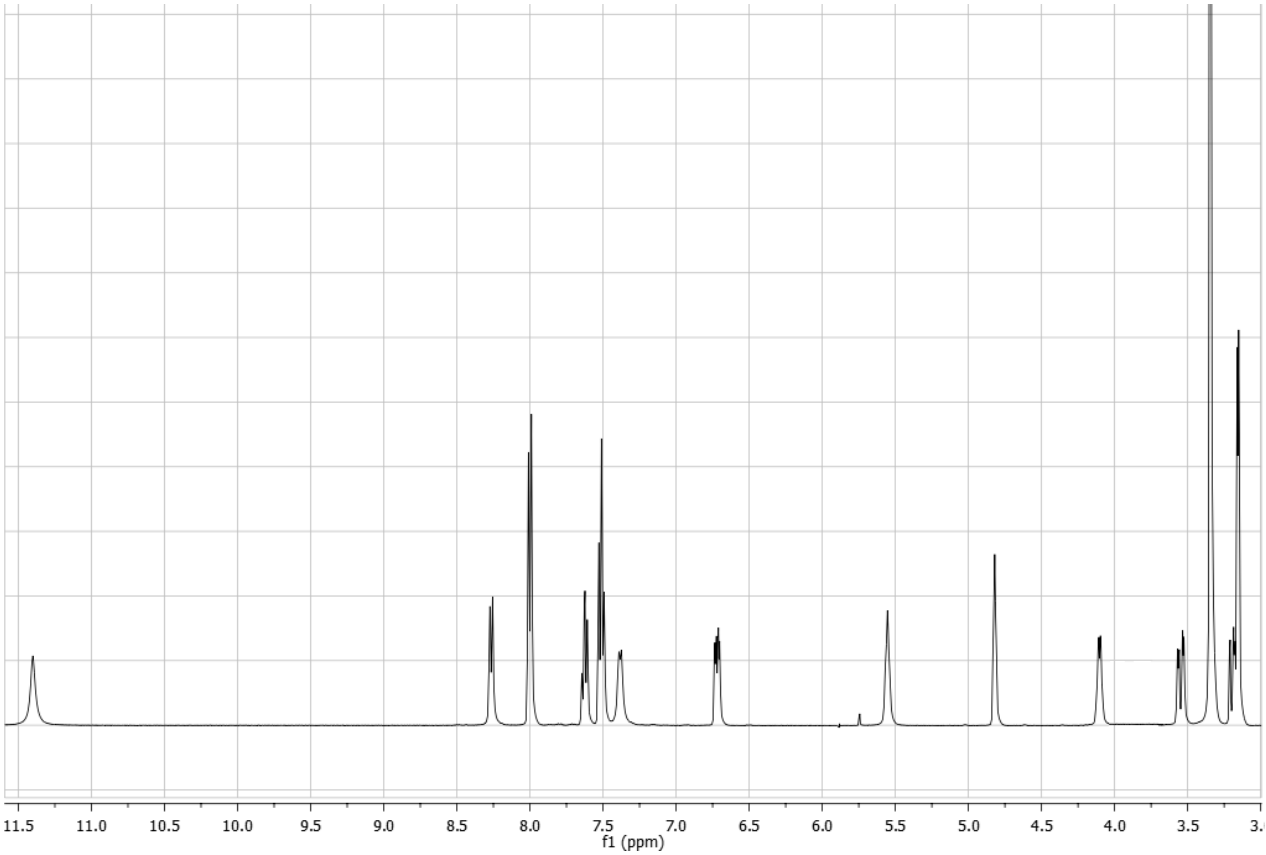
2(*R*)-(Hydroxymethyl)-5(*S*)-(4-benzamido-2-oxo-1,2-dihydropyrimidine)-1'-yl-3-oxido-1,3-oxathiolane (9 and 10).

Nucleosides **9** and **10** were obtained via flash chromatography (98:2 DCM-MeOH to 96:4 DCM-MeOH) from studies of sulfoxidation carried out under Kagan[71] and Modena[72] conditions. 1 g of crude afforded 53 mg of sulfoxide with higher R_f, 60 mg of sulfoxide with lower R_f and 825 mg of mixture**.**

Compound with higher R_f:

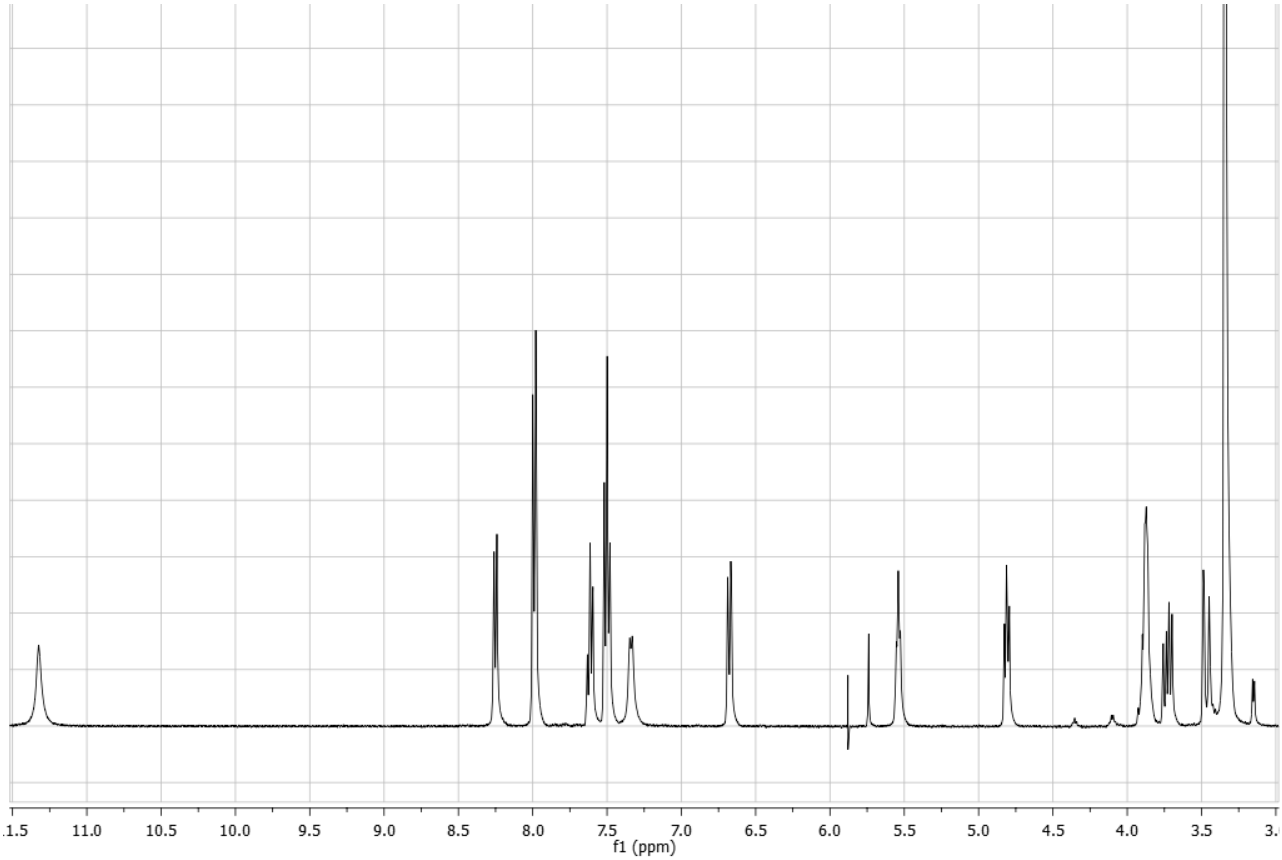
^{1}H NMR (400 MHz, MeOD): δ 3.20 (d, J = 3.5 Hz, 2H, H-2'), 3.23-3.26 (m, 1H, H-4), 3.60 (dd, J = 13.7, 3.3 Hz, 1H, H-4), 3.84-3.87 (m, 1H, H-4 other isomer), 4.03 (br d, J = 12.4 Hz, 1H, H-4

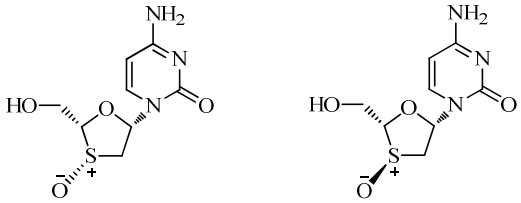
other isomer), 4.16 (app d, *J* = 4.1 Hz, 1H, CH_2OH), 4.87 (br s, 1H, CH_2OH), 5.60 (br s, 1H, H-2), 6.77 (dd, *J* = 9.6, 4.4 Hz, 1H, H-5), 7.43 (br d, *J* = 6.4 Hz, 1H, H-5'), 7.56 (t, *J* = 7.0 Hz, 2H, H-*m* Ar), 7.67 (app t, *J* = 8.4 Hz, 1H, H-*p* Ar), 8.05 (d, *J* = 8.0 Hz, 2H, H-*o* Ar), 8.31 (d, *J* = 6.4 Hz, 1H, H-6'), 11.45 (s, 1H, NH).



Compound with lower R_f:

^{1}H NMR (400 MHz, MeOD): δ3.47 (d, *J* = 15.3 Hz, 1H, H-4), 3.73 (dd, *J* = 15.4, 8.4 Hz, 1H, H-4), 3.86-3.90 (m, 1H, CH_2OH), 4.81 (app t, *J* = 5.6 Hz, 1H, CH_2OH), 5.54 (t, *J* = 5.0 Hz, 1H, H-2), 6.68 (app d, *J* = 8.4 Hz, 1H, H-5), 7.34 (br d, *J* = 7.6 Hz, 1H, H-5'), 7.50 (t, *J* = 7.8 Hz, 2H, H-*m* Ar), 7.61 (t, *J* = 7.3 Hz, 1H, H-*p* Ar), 7.99 (d, *J* = 8.0 Hz, 2H, H-*o* Ar), 8.25 (d, *J* = 7.6 Hz, 1H, H-6'), 11.33 (s, 1H, NH).

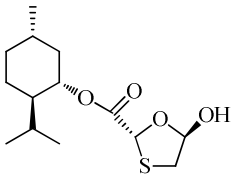
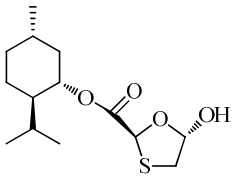




2(*R*)-(Hydroxymethyl)-5(*S*)-(4-amino-2-oxo-1,2-dihydropyrimidine)-1'-yl-3-oxido-1,3-oxathiolane (2 and 3).

5 ml of NH_3/MeOH solution (6 M) was added at 0 °C to starting products **9** and **10** (50 mg, 0.143 mmol), and the resulting mixture was stirred at rt overnight. Then, the crude was concentrated under reduced pressure. Flash chromatography (9:1 to 8:2 DCM-MeOH) of the residue afforded **2** and **3** (35 mg, 99% yield).

NMR data not avaible.

(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*R*,5*R*)-5-hydroxy-1,3-oxathiolane-2-carboxylate (17).

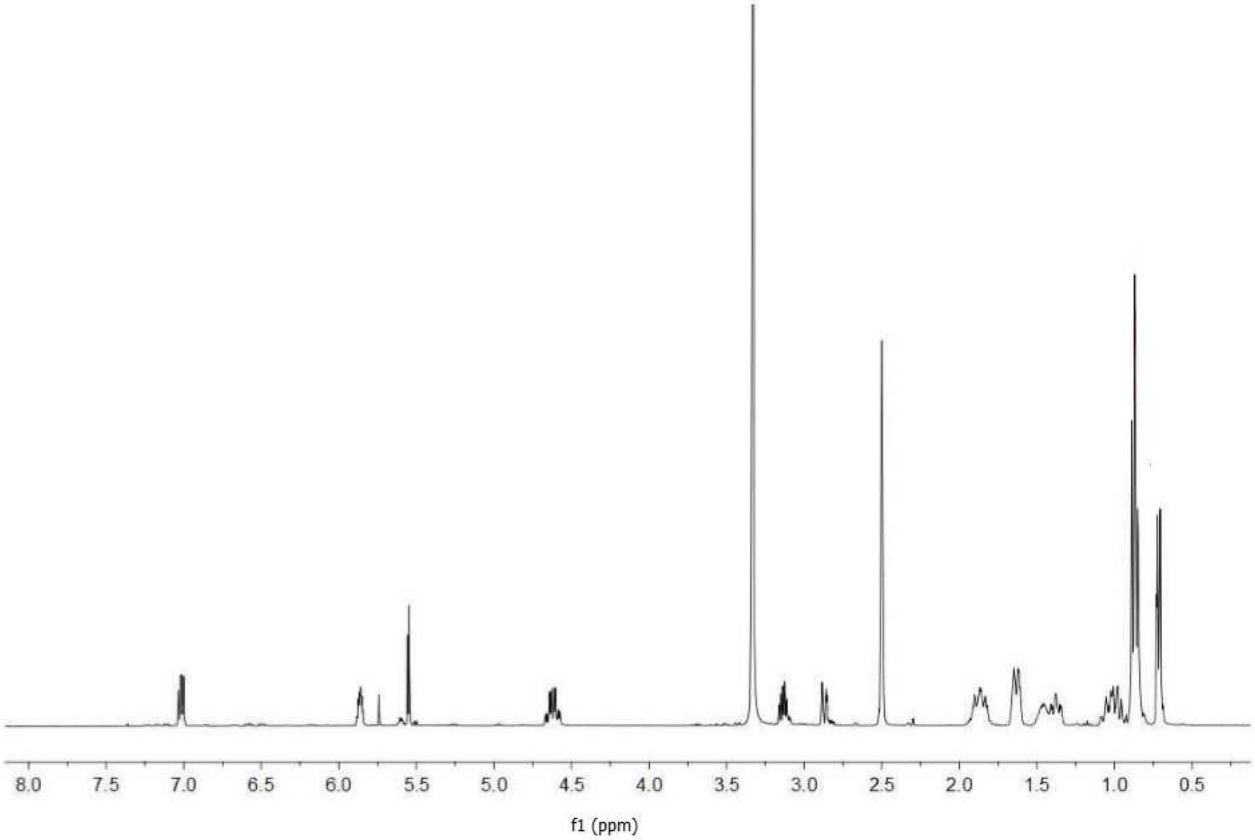
(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*S*)-5-hydroxy-1,3-oxathiolane-2-carboxylate (18).

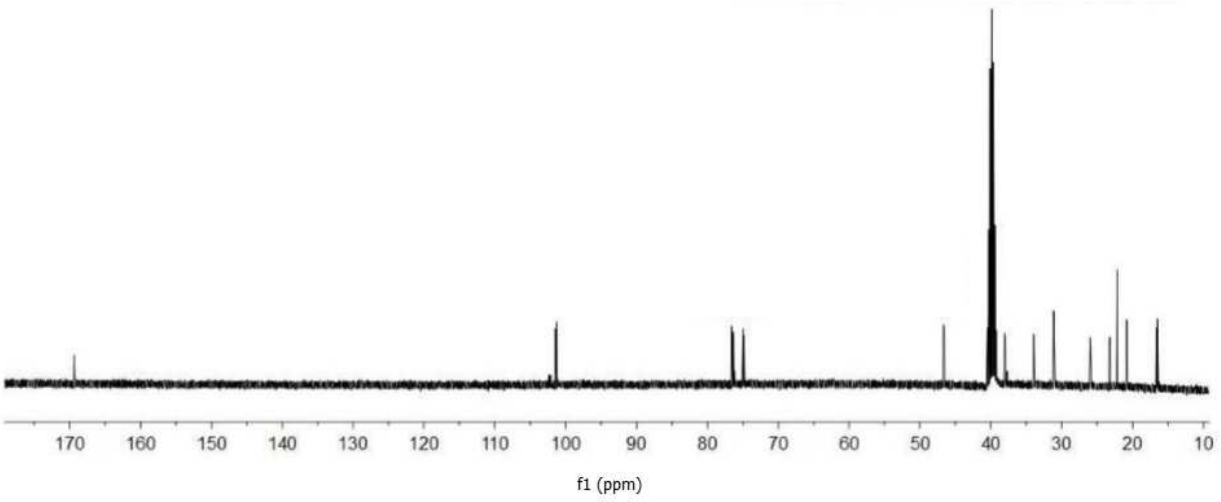
L-Menthyl glyoxylate monohydrate (2.5 g, 10.86 mmol), toluene (12.5 mL) and acetic acid (0.25 mL) were mixed under stirring and the resulting mixture was heated to 120 °C, removing water azeotropically with a Dean-Stark trap. The resulting solution was concentrated to collect 5 mL of distillate, and cooled to rt; then 1,4-dithiane-2,5-diol (826 mg, 5.43 mmol) was added. The reaction mixture was refluxed for 4 h; then it was cooled to 80 °C and clarified. The filtrate was cooled to 0 °C, and a solution of triethylamine (150 μL) in *n*-hexane (15 mL) was added dropwise. The mixture was stirred at 0 °C for 16 h, observing the formation of a precipitate. The isolated solid was filtered, washed with a mixture of toluene and *n*-hexane (1:3 v/v) and dried to give the hemiacetals **17** and **18** (1.86 g, 60% o.y.) as a mixture of two trans stereoisomers.

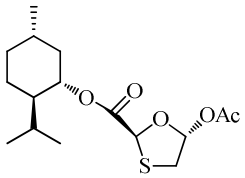
^{1}H NMR (400 MHz, DMSO, mixture of two isomers): δ0.69 (d, J = 6.9 Hz, 3H, CH_3 menthyl **17**), 0.70 (d, J = 6.9 Hz, 3H, CH_3 menthyl **18**), 0.76-0.90 (m, 14H, H menthyl **17** and **18**), 0.90-1.09 (m, 4H, H menthyl **17** and **18**), 1.31-1.52 (m, 4H, H menthyl **17** and **18**), 1.57-1.65 (m, 4H, H menthyl **17** and **18**), 1.77-1.94 (m, 4H, H menthyl **17** and **18**), 2.85 (br d, J = 10.6 Hz, 2H, H-4 **17** and **18**), 3.11 (dd, J = 10.0, 4.5, 1H, H-4 **17**), 3.12 (dd, J = 10.0, 4.7 Hz, 1H, H-4 **18**), 4.59 (dt, J = 11.1, 4.2 Hz, 1H, H-1' **17**), 4.62 (dt, J = 11.1, 4.2 Hz, 1H, H-1' **18**), 5.54 (s, 1H, H-2 **17**), 5.55 (s, 1H, H-2 **18**), 5.82-5.87 (m, 2H, H-5 **17**, H-5 **18**), 7.02 (d, J = 8.4, 1H, OH **17**), 7.03 (d, J = 8.4, 1H, OH **18**).

^{13}C NMR (100.6 MHz, DMSO, mixture of two isomers): δ16.5, 16.6, 20.8, 20.9, 22.2, 23.2, 23.3, 26.0, 26.1, 31.1, 31.2, 33.4 (C menthyl), 38.0 (C-4 **17**), 38.1 (C-4 **18**), 40.6, 46.6, 46.7 (C menthyl),

74.9 (C-1’ **17**), 75.0 (C-1’ **18**), 76.4 (C-2 **17**), 76.6 (C-2 **18**), 101.2 (C-5 **17**), 101.5 (C-5 **18**), 169.4 (*C*OO **17**), 169.5 (*C*OO **18**).







(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*S*)-5-methylcarbonyloxy-1,3-oxathiolane-2-carboxylate (22).

A solution of **17** and **18** (1.86 g, 6.52 mmol), acetic anhydride (3.75 mL, 39.75 mmol) and dichloromethane (10 mL) were mixed under nitrogen atmosphere. The solution was cooled to 0 °C and pyridine (0.95 mL, 18.20 mmol) was added dropwise under stirring. The reaction mixture was warmed to room temperature and stirred for 4 h; after completion, it was quenched by addition of water at 0 °C. The organic layer was washed with diluted HCl, dried over Na_2SO_4 and concentrated under reduced pressure. Flash chromatography of the crude residue (95:5 hexane-EtOAc) gave a mixture of four *cis* and *trans* diastereoisomers (2.34 g, 91% yield). A sample of 1.2 g was dissolved in 40 mL of hexane with 200 µL of TEA, and after 72 h at -20 °C the only desired trans-(2*S*,5*S*) stereoisomer **22** precipitated as white crystal (0.50 g, 42% of the acetylated product).

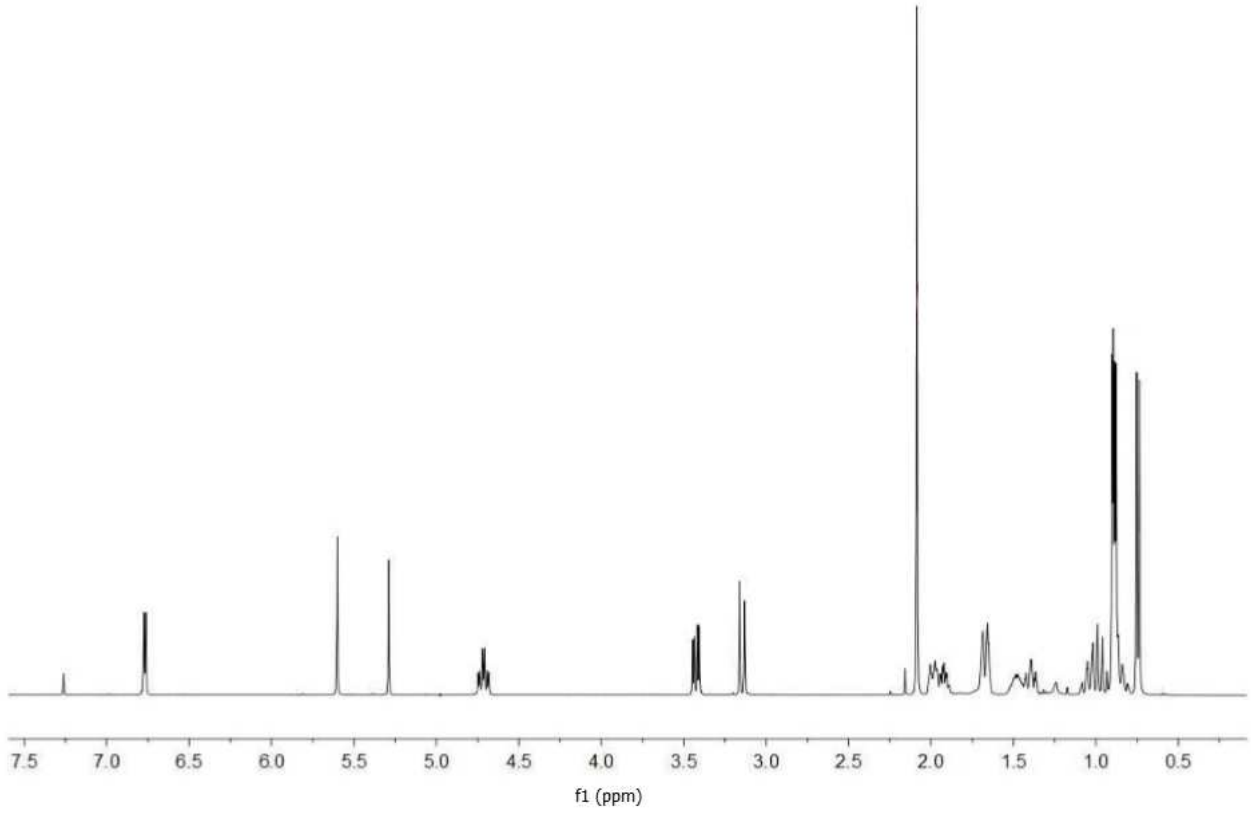
$[\alpha]^{25}_{D}$ = –56.0 (*c* 3.1, $CHCl_3$).

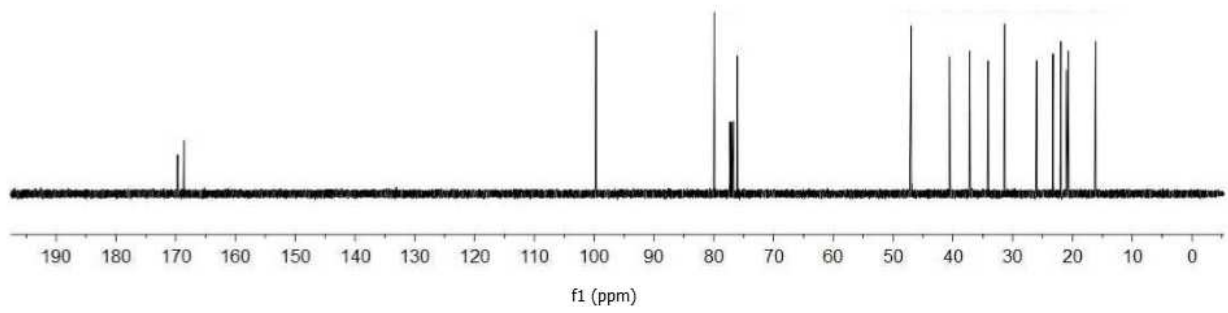
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.74 (d, *J* = 7.0 Hz, 3H, CH_3 menthyl), 0.81-0.92 (m, 7H, H menthyl), 0.92-1.10 (m, 2H, H menthyl), 1.39 (br t, *J* = 11.3 Hz, 1H, H menthyl), 1.44-1.55 (m, 1H, H menthyl), 1.67 (br d, *J* = 11.4 Hz, 2H, H menthyl), 1.87-1.95 (m, 1H, H menthyl), 1.99 (br d, *J* = 11.7 Hz, 1H, H menthyl), 2.09 (s, 3H, OC=OCH_3), 3.15 (d, *J* = 11.7 Hz, 1H, H-4), 3.43 (dd, *J* = 11.7, 4.2 Hz, 1H, H-4), 4.71 (dt, *J* = 10.9, 4.4 Hz, 1H, H-1'), 5.60 (s, 1H, H-2), 6.77 (d, *J* = 4.2 Hz, 1H, H-5).

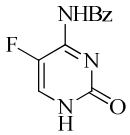
^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.0, 20.6, 21.0 (C menthyl), 21.9 (O-C=O-*C*H_3), 23.2, 26.0, 31.3, 34.0 (C menthyl), 37.1 (C-4), 40.5, 47.0 (C menthyl), 76.0 (C-1'), 79.8 (C-2), 99.7 (C-5), 168.5 (O-*C*=O-CH_3), 169.6 (*C*=OO-menthyl).

m.p. 99-103 °C.

Elemental analysis calcd (%) for [$C_{16}H_{26}O_5S$]: C 58.16, H 7.93, S, 9.70. Found: C 58.01, H 7.96, S, 9.74.





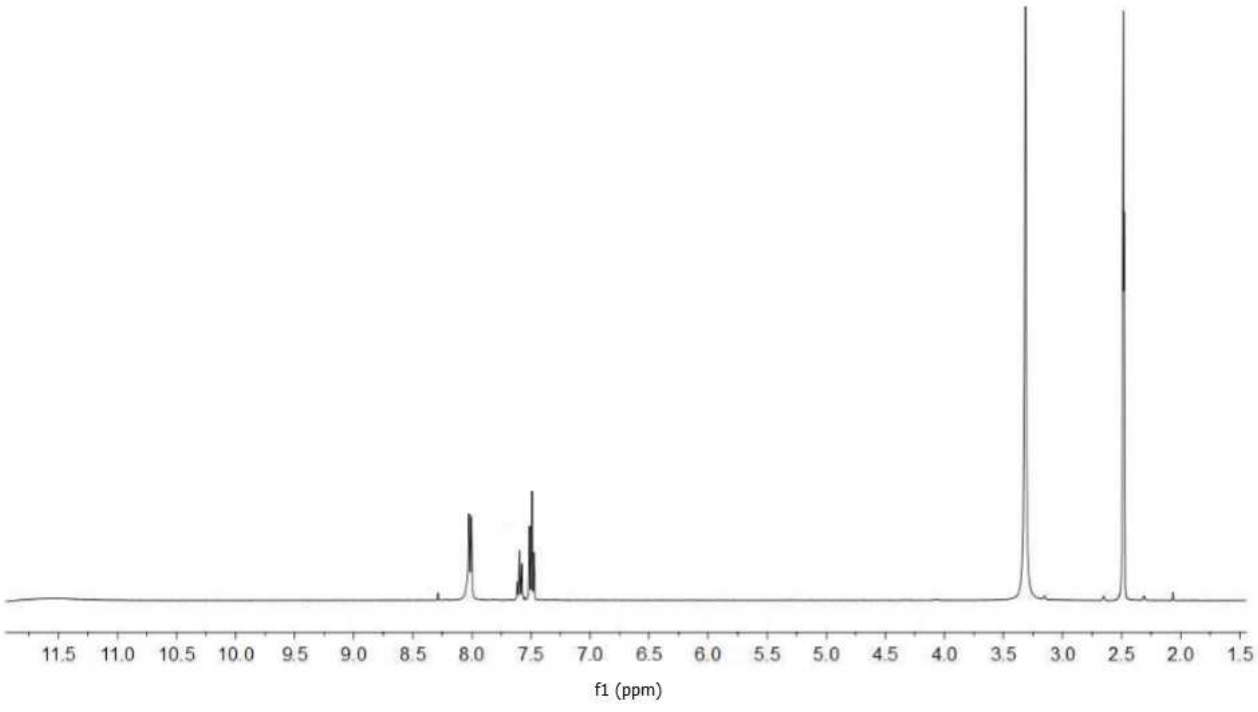


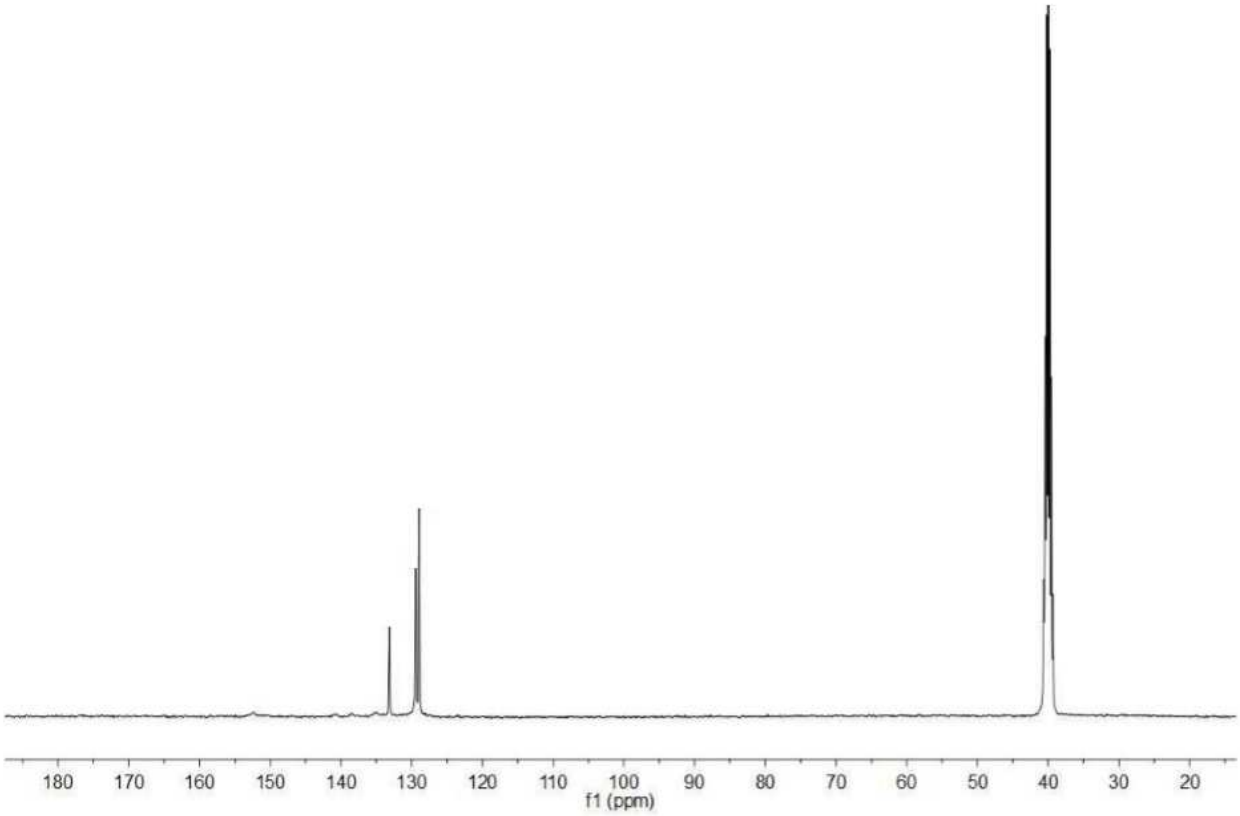
5-Fluoro-2-oxo-4-(benzamido)-1,2-dihydropyrimidine (25).

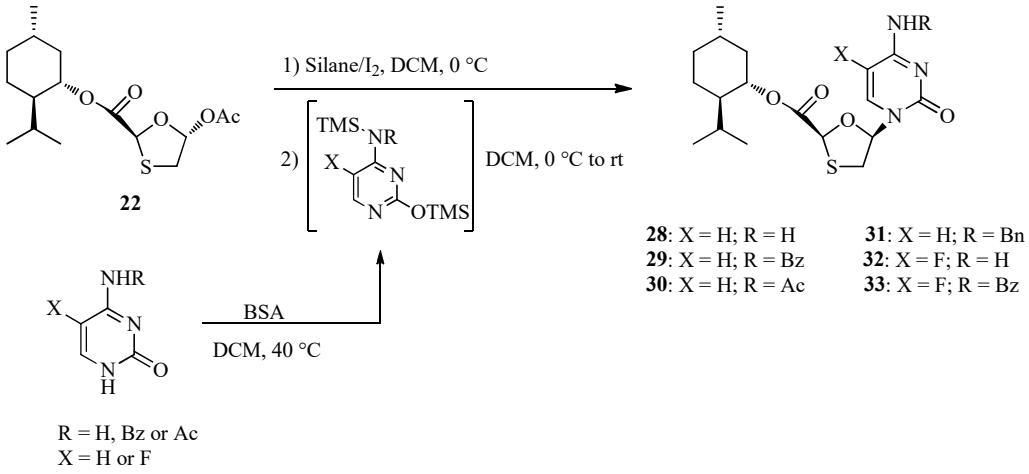
5-Fluorocytosine (0.2 g, 1.5 mmol) was suspended in pyridine (10 mL) and the resulting mixture was cooled to 0 °C. Benzoyl chloride (1.04 mL, 9 mmol) was added dropwise and the suspension was stirred at rt for 16 h. The reaction mixture was cooled with an ice bath, treated with NH_4OH (1 mL) for 90 min and concentrated under reduced pressure. Flash chromatography of the crude residue over silica gel (85:15 DCM-MeOH) provided the pure nucleobase **25** (0.36 g, 99%).

^{1}H NMR (400 MHz, DMSO): δ7.49 (t, *J* = 7.7 Hz, 2H, H-*m* benzoyl), 7.60 (t, *J* = 7.4 Hz, 1H, H-*p* benzoyl), 8.01 (br d, *J* = 7.4 Hz, 3H, H-6, H-*o* benzoyl).

^{13}C NMR (100.6 MHz, DMSO): δ128.9 (C-*o* benzoyl), 129.4 (C-*m* benzoyl), 133.2 (C-*p* benzoyl), 135.0 (C-*i* benzoyl), 139.5, (d, *J* = 225.9 Hz, C-5), 152.3 (d, *J* = 21.2 Hz, C-6), 162.0 (C-4), 164.9 (C-2), 168.5 (*C*=O benzoyl).



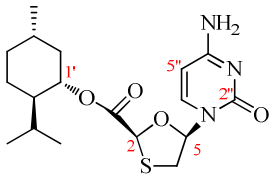




***N*-glycosylation: general procedure.**

First step: Iodine (1.2 eq) was suspended in anhydrous dichloromethane (7.50 mL) under nitrogen atmosphere and the silane (1.2 eq) was added. After 15 min, the resulting mixture was cooled to 0 °C and cannulated dropwise into a solution of oxathiolane **22** (1 mmol) in anhydrous dichloromethane (0.75 mL), using additional dichloromethane (7.50 mL) for rinsing. The reaction was then stirred at the same temperature for 1 h. (b) BSA (3.25 eq when reacting with cytosine or

5-fluorocytosine; 2 eq when reacting with benzoylcytosine, benzylcytosine or **25**) was added to a suspension of the nucleobase (1.3 eq) in anhydrous dichloromethane (7.50 mL) under nitrogen atmosphere. The resulting mixture was warmed to 40 °C until a clear solution was observed. The mixture (a) was cannulated dropwise into solution (b) under stirring at 0 °C, using additional dichloromethane (7.50 mL) for rinsing. The reaction mixture was then warmed to rt and stirred for 1 h. Afterwards, the reaction was quenched with few drops of saturated $NaHCO_3$ solution. The emulsion was washed with a 1 N $Na_2S_2O_3$ solution and brine and extracted with dichloromethane. The organic layer was dried (Na_2SO_4) and concentrated under reduced pressure. Flash chromatography of the crude residue over silica gel (**28**, **32**: 95:5 DCM-MeOH; **29**, **30**, **33**: 1:1 hexane-EtOAc) provided the pure nucleoside (**28**: 86-98%; **29**: 87-94%; **30**: 80-95%; **32**: 84-91%; **33**: 85-91% with Et_3SiH and PMHS respectively).



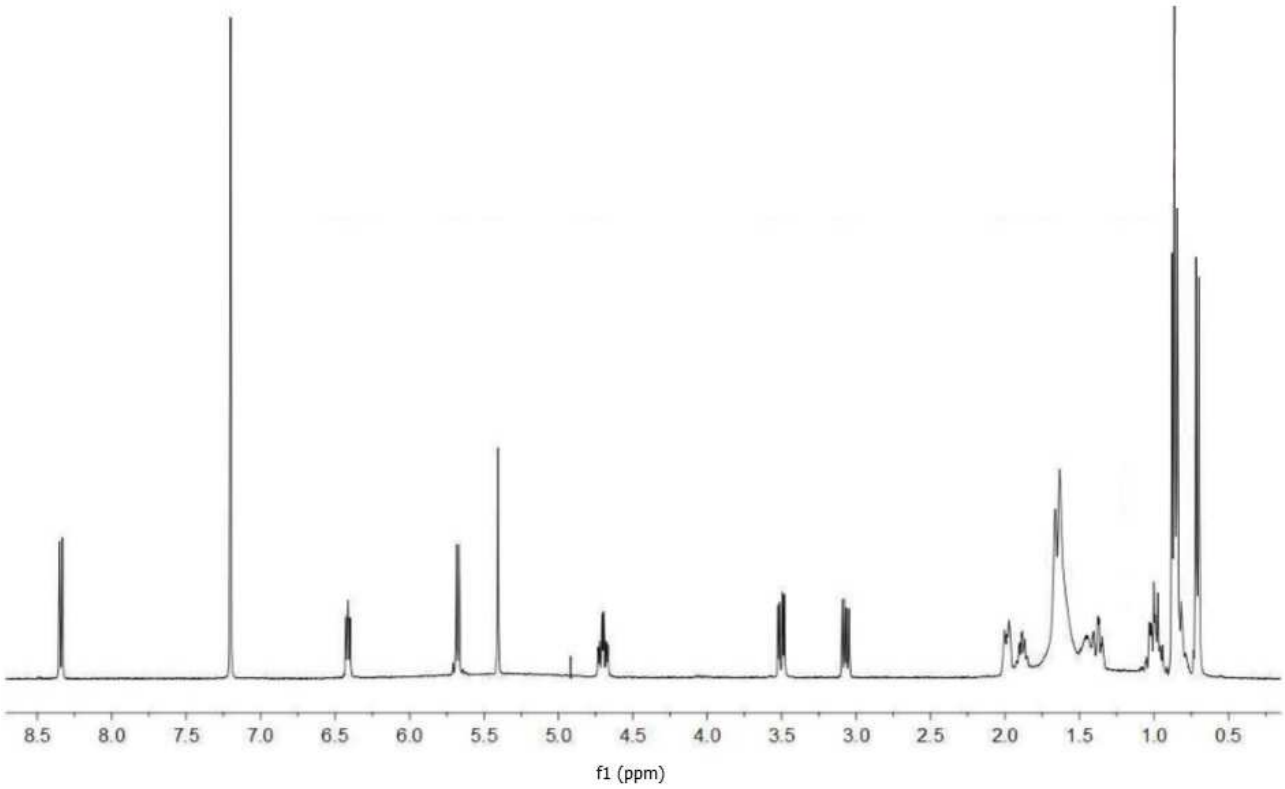
(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*R*)-5-(4-amido-2-oxo-1,2-dihydropyrimidine)-1"-yl-1,3-oxathiolane (28).

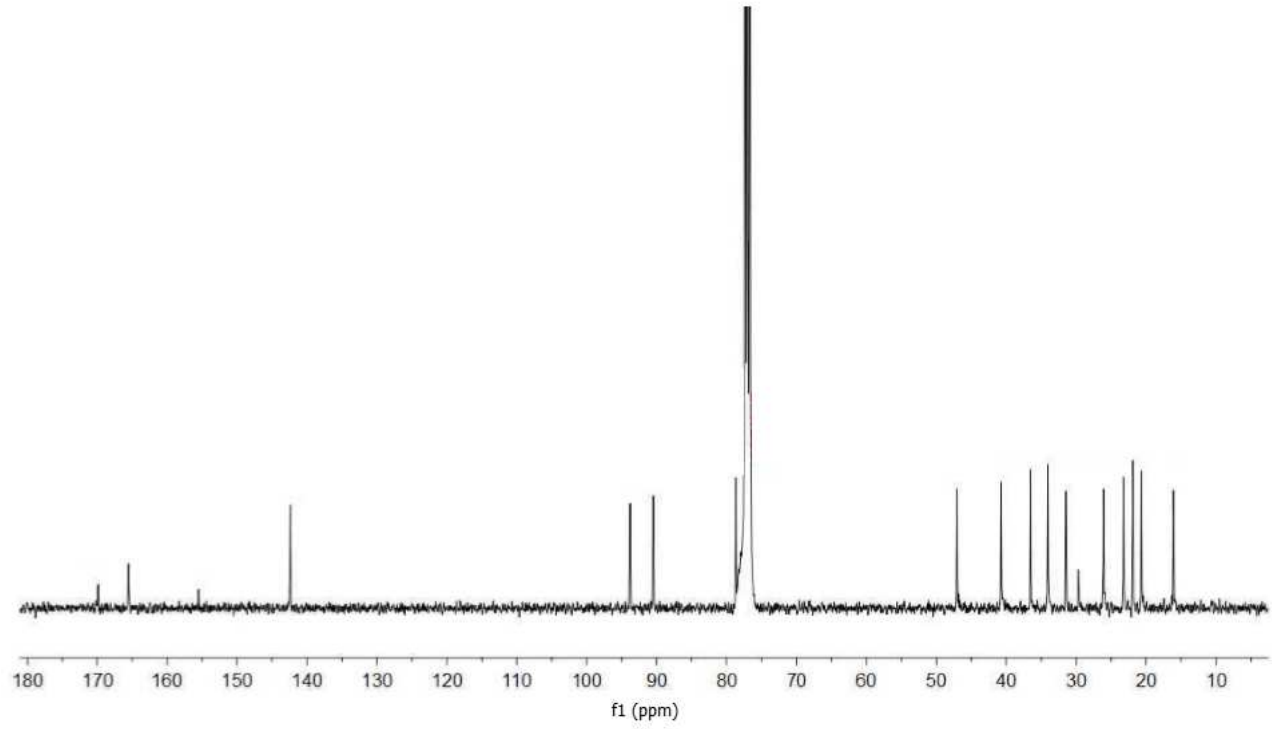
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.77 (d, *J* = 7.0 Hz, 3H, CH_3 menthyl), 0.84-0.96 (m, 7H, H menthyl), 0.98-1.11 (m, 2H, H menthyl), 1.43 (br t, *J* = 12.0 Hz, 1H, H menthyl), 1.48-1.57 (m, 1H, H menthyl), 1.71 (br d, *J* = 11.3 Hz, 2H, H menthyl), 1.88-1.99 (m, 1H, H menthyl), 2.01-2.09 (m, 1H, H menthyl), 3.13 (dd, *J* = 12.1, 6.6 Hz, 1H, H-4), 3.56 (dd, *J* = 12.1, 4.7 Hz, 1H, H-4), 4.76 (td, *J* = 11.1, 4.4 Hz, 1H, H-1'), 5.46 (s, 1H, H-2), 5.73 (d, *J* = 7.5 Hz, 1H, H-5"), 6.47 (dd, *J* = 6.5, 4.7 Hz, 1H, H-5), 8.40 (d, *J* = 7.5 Hz, 1H, H-6").

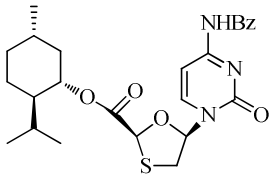
^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.1, 20.7, 21.9, 23.2, 26.1, 31.5, 34.1 (C menthyl), 36.6 (C-4), 40.8, 47.1 (C menthyl), 76.7 (C-1'), 78.6 (C-2), 90.4 (C-5"), 93.8 (C-5), 142.4 (C-6"), 155.5 (C-2"), 165.5 (C-4"), 169.8 (*C*=OO-menthyl).

.

Elemental analysis calcd (%) for [$C_{18}H_{27}N_3O_4S$]: C 56.67; H 7.13; N 11.01; S 8.41. Found: C 56.56; H 7.16; N 11.05; S 8.44.





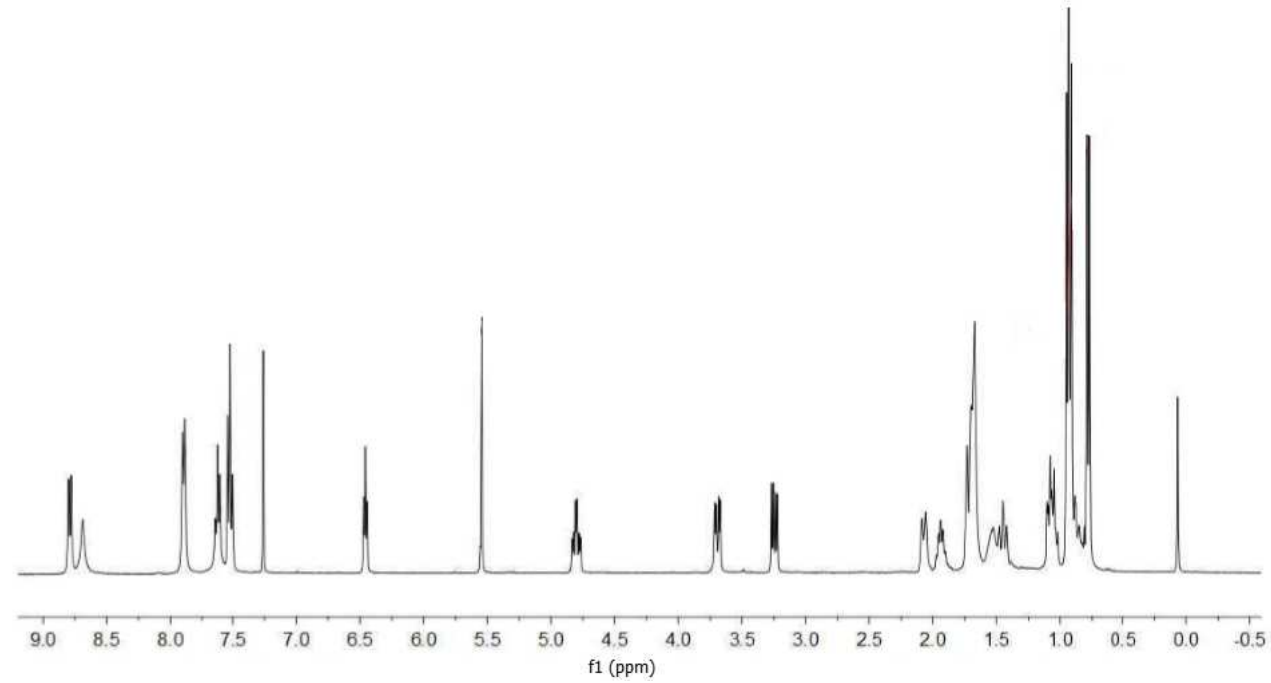


(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*R*)-5-(4-benzamido-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (29).

^{1}H NMR (600 MHz, $CDCl_3$):δ 0.78 (d, *J* = 6.9 Hz, 3H, CH_3 menthyl), 0.82-0.98 (m, 7H, H menthyl), 0.99-1.15 (m, 2H, H menthyl), 1.45 (br t, *J* = 11.5 Hz, 1H, H menthyl), 1.49-1.59 (m, 1H, H menthyl), 1.62-1.76 (m, 2H, H menthyl), 1.87-1.99 (m, 1H, H menthyl), 2.02-2.12 (m, 1H, H menthyl), 3.24 (dd, *J* = 12.3, 5.9 Hz, 1H, H-4), 3.69 (dd, *J* = 12.3, 4.7 Hz, 1H, H-4), 4.80 (td, *J* = 10.9, 4.2 Hz, 1H, H-1'), 5.54 (s, 1H, H-2), 6.42 (t, *J* = 5.3 Hz, 1H, H-5), 7.52 (t, *J* = 7.8 Hz, 2H, H-*m* Ar), 7.62 (t, *J* = 7.4 Hz, 1H, H-*p* Ar), 7.89 (d, *J* = 7.5 Hz, 3H, H-5", H-*o* Ar), 8.69 (br s, 1H, NH), 8.79 (d, *J* = 7.5 Hz, 1H, H-6").

^{13}C NMR (100.6 MHz, $CDCl_3$): δ16.1, 20.7, 21.9, 23.2, 26.2, 31.5, 34.0 (C menthyl), 37.1 (C-4), 40.8, 47.1 (C menthyl), 77.0 (C-1'), 79.7 (C-2), 90.7 (C-5"), 96.6 (C-5), 127.5 (C-*o* Ar), 129.1 (C-*m* Ar), 133.0 (C-*p* Ar), 133.2 (C-*i* Ar), 143.6 (C-6"), 154.9 (C-4"), 162.6 (C-2"), 166.4 (C=O benzoyl), 169.3 (*C*=OO-menthyl).

Elemental analysis calcd (%) for [$C_{25}H_{31}N_3O_5S$]: C 61.83; H 6.43; N 8.65; S 6.60. Found: C 61.97; H 6.41; N 8.62; S 6.57.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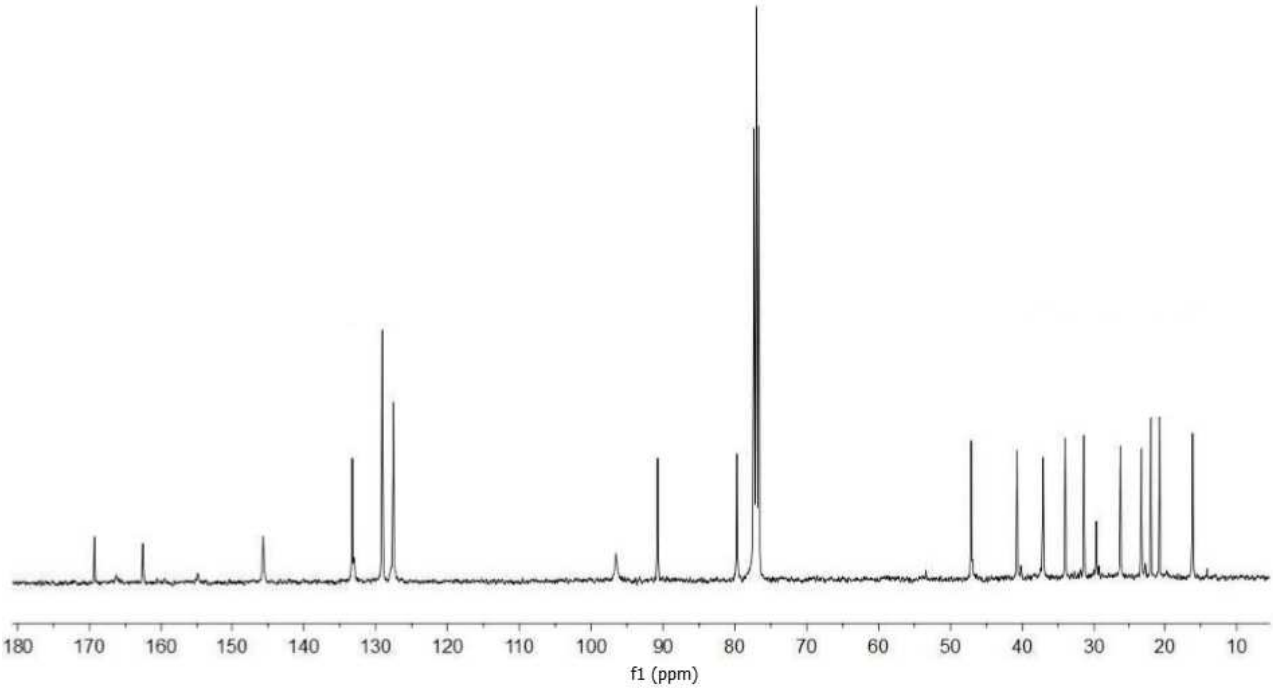
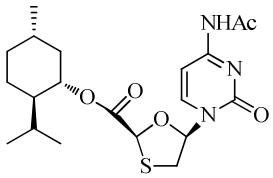
f1 (ppm)


180 170 160 150 140 130 120 110 100 90 80 70 60 50 40 30 20 10
f1 (ppm)

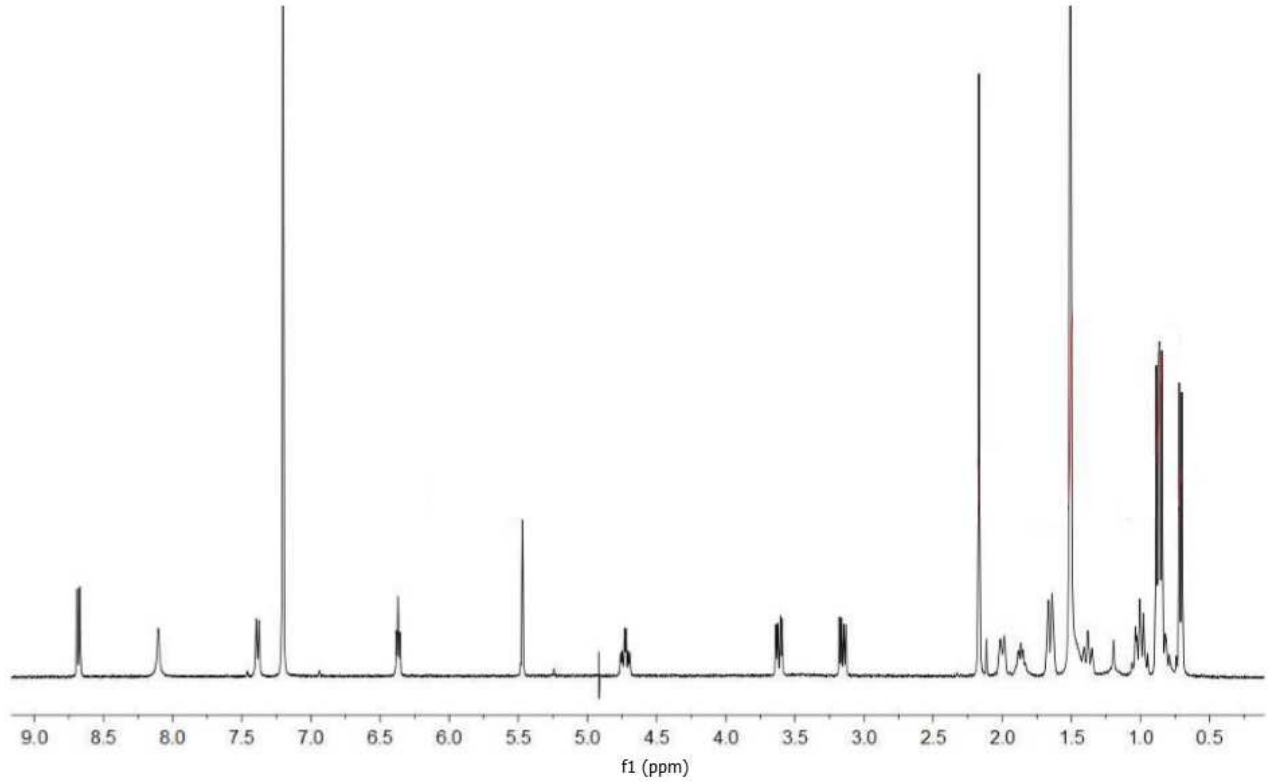


(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*R*)-5-(4-acetamido-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (30).

^{1}H NMR (500 MHz, $CDCl_3$): δ 0.74 (d, *J* = 6.9 Hz, 3H, CH_3 menthyl), 0.80-0.95 (m, 7H, H menthyl), 0.96-1.11 (m, 2H, H menthyl), 1.41 (br t, *J* = 11.7 Hz, 1H, H menthyl), 1.46-1.56 (m, 1H, H menthyl), 1.68 (br d, *J* = 11.4 Hz, 2H, H menthyl), 1.83-1.94 (m, 1H, H menthyl), 2.03 (br d, *J* = 11.6 Hz, 1H, H menthyl), 2.19 (s, 3H, C=OC*H*$_3$), 3.18 (dd, *J* = 12.3, 5.8 Hz, 1H, H-4), 3.65 (dd, *J* = 12.3, 4.6 Hz, 1H, H-4), 4.75 (td, *J* = 11.1, 4.4 Hz, 1H, H-1'), 5.55 (s, 1H, H-2), 6.40 (br t, *J* = 5.4 Hz, 1H, H-5), 7.41 (d, *J* = 7.3 Hz, 1H, H-5"), 8.13 (br s, 1H, NH), 8.71 (d, *J* = 7.3 Hz, 1H, H-6").

^{13}C NMR (100.6 MHz, $CDCl_3$) : δ 16.0, 20.6, 21.8, 23.1 (C menthyl), 24.9 (C=O*C*H_3), 26.0, 31.3 (C menthyl), 33.9 (C menthyl), 37.0 (C-4), 40.6, 46.9 (C menthyl), 76.8 (C-1'), 79.6 (C-2), 90.6 (C-5"), 96.6 (C-5), 145.4 (C-6"), 154.9 (C-4"), 162.9 (C-2"), 169.2 (*C*=OCH$_3$), 169.9 (*C*=OO-menthyl).

Elemental analysis calcd (%) for [$C_{20}H_{29}N_3O_5S$]: C 56.72; H 6.90; N 9.92; S 7.57. Found: C 56.88; H 6.87; N 9.88; S 7.54.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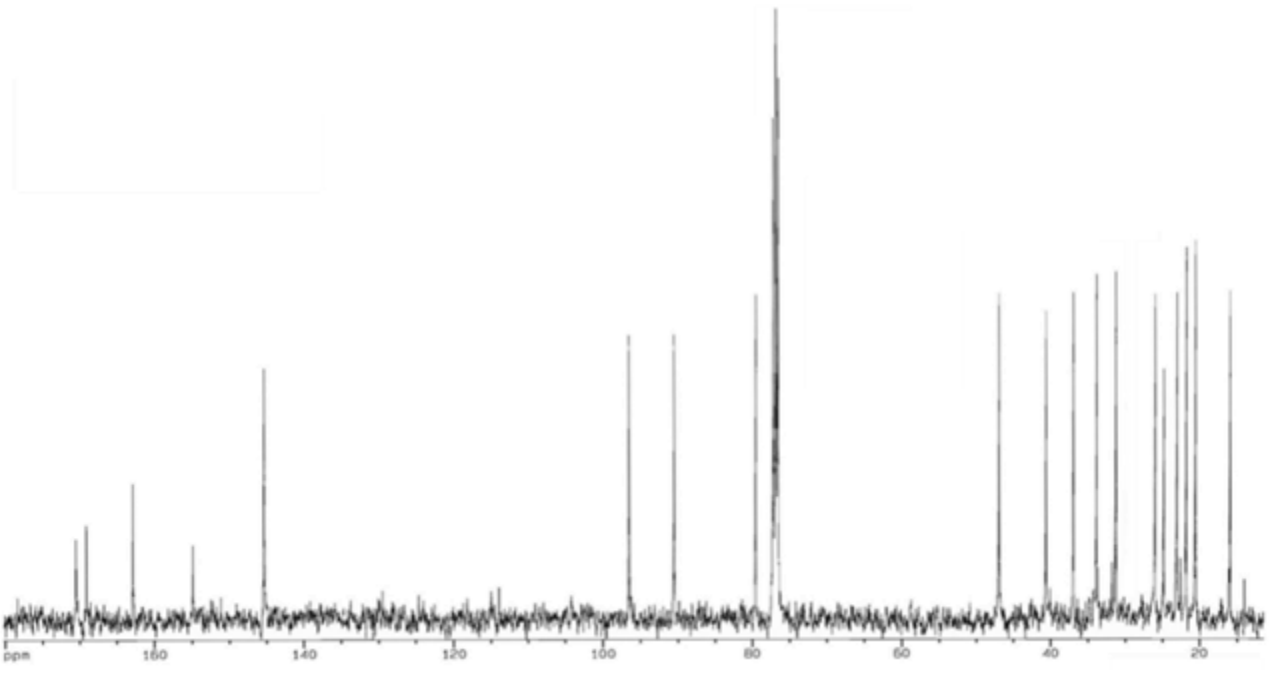
f1 (ppm)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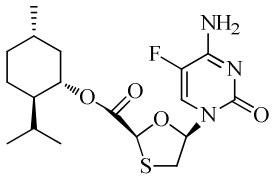
20

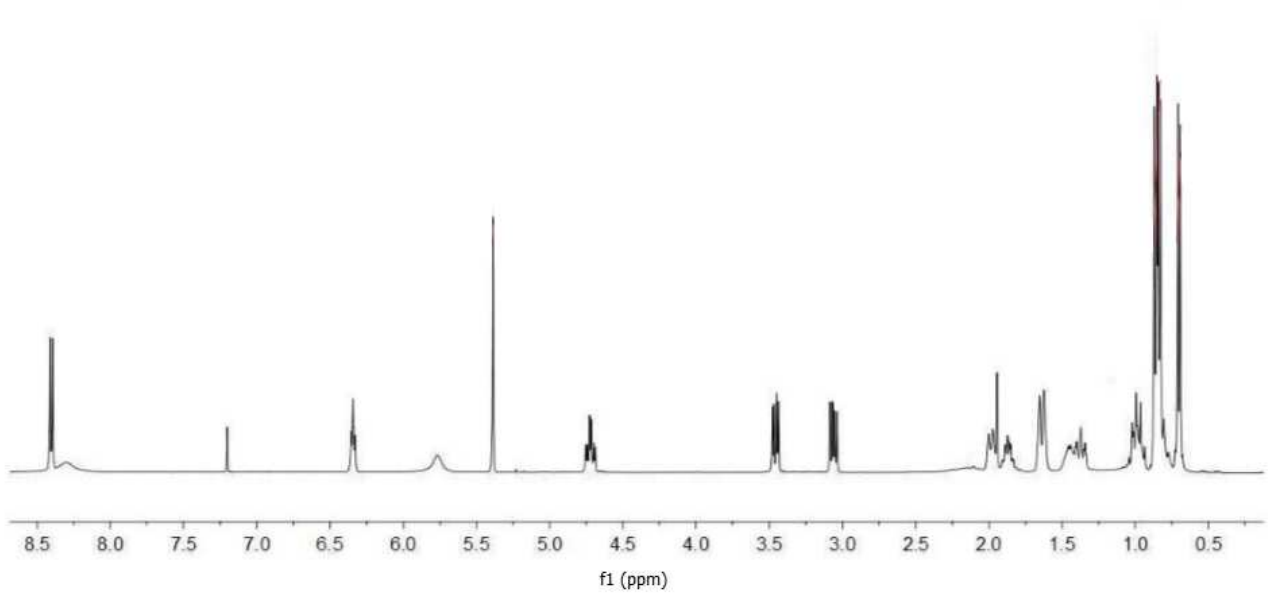


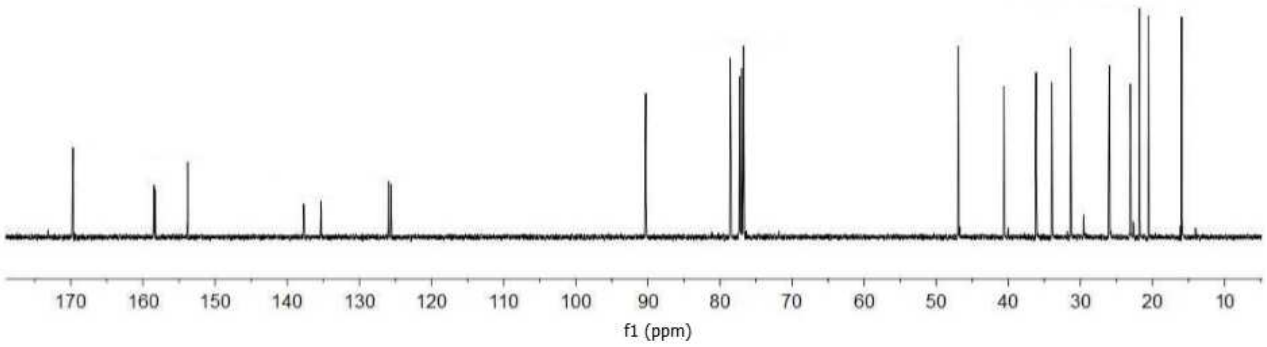
(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*R*)-5-(4-amido-5-fluoro-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (32).

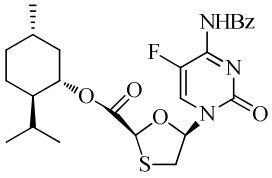
^{1}H NMR (400 MHz, $CDCl_3$): δ 0.76 (d, *J* = 6.9 Hz, 3H, CH_3 menthyl), 0.80-0.95 (m, 7H, H menthyl), 0.96-1.12 (m, 2H, H menthyl), 1.43 (bt, *J* = 11.4 Hz, 1H, H menthyl), 1.47-1.58 (m, 1H, H menthyl), 1.69 (br d, *J* = 11.4 Hz, 2H, H menthyl), 1.87-1.98 (m, 1H, H menthyl), 2.04-2.08 (m, 1H, H menthyl), 3.11 (dd, *J* = 12.1, 6.6 Hz, 1H, H-4), 3.51 (dd, *J* = 12.1, 4.7 Hz, 1H, H-4), 4.78 (td, *J* = 11.0, 4.4 Hz, 1H, H-1'), 5.44 (s, 1H, H-2), 5.82 (br s, 1H), 6.40 (ddd, *J* = 6.4, 4.8, 1.6 Hz, 1H, H-5), 8.36 (br s, 1H, NH_2), 8.46 (d, *J* = 6.6 Hz, 1H, H-6").

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 16.0, 20.6, 21.8, 23.1, 26.0, 31.4, 34.0 (C menthyl), 36.2 (C-4), 40.6, 47.0 (C menthyl), 76.7 (C-1'), 78.6 (C-2), 90.3 (C-5), 125.7 (d, *J* = 33.2 Hz, C-6"), 136.5 (d, *J* = 242.0 Hz, C-5"), 153.8 (C-2"), 158.3 (d, *J* = 14.2 Hz, C-4"), 169.7 (*C*=OO-menthyl).

Elemental analysis calcd (%) for [$C_{18}H_{26}FN_3O_4S$]: C 54.12; H 6.56; N 10.52; S 8.03. Found: C 53.97; H 6.58; N 10.55; S 8.06.







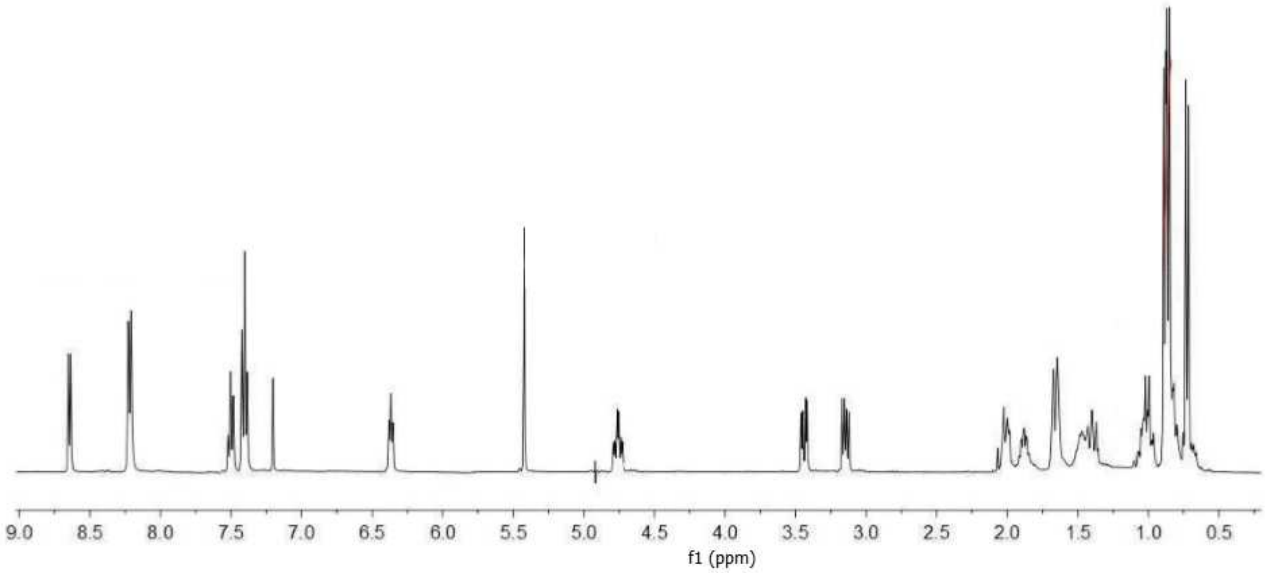
(1'*S*,2'*R*,5'*S*)-2-Isopropyl-5-methylcyclohexyl (2*S*,5*R*)-5-(4-benzamido-5-fluoro-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (33).

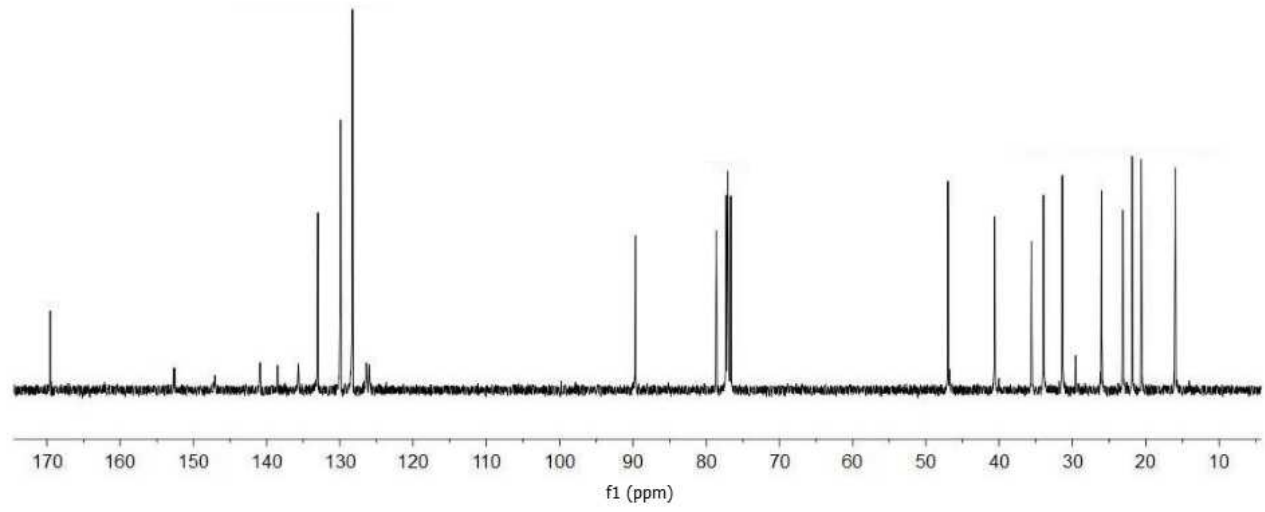
^{1}H NMR (600 MHz, $CDCl_3$): δ 0.78 (d, *J* = 7.0 Hz, 3H, CH_3 menthyl), 0.83-0.97 (m, 7H, H menthyl), 0.99-1.15 (m, 2H, H menthyl), 1.46 (br t, *J* = 12.2 Hz, 1H, H menthyl), 1.48-1.60 (m, 1H, H menthyl), 1.72 (br d, *J* = 11.3 Hz, 2H, H menthyl), 1.87-1.98 (m, 1H, H menthyl), 2.02-2.11 (m, 1H, H menthyl), 3.20 (dd, *J* = 12.2, 6.9 Hz, 1H, H-4), 3.50 (dd, *J* = 12.2, 4.7 Hz, 1H, H-4), 4.82 (dt, *J* = 11.0, 4.3 Hz, 1H, H-1'), 5.48 (s, 1H, H-2), 6.43 (br t, *J* = 5.6 Hz, 1H, H-5), 7.46 (t, *J* = 7.6 Hz, 2H, H-*m* Ar), 7.56 (t, *J* = 7.6 Hz, 1H, H-*p* Ar), 8.27 (d, *J* = 7.6 Hz, 3H, H-5", H-*o* Ar), 8.70 (d, *J* = 6.3 Hz, 1H, H-6").

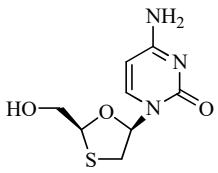
^{13}C NMR (100.6 MHz, $CDCl_3$): δ16.0, 20.6, 21.8, 23.1, 26.0, 31.4, 33.9 (C menthyl), 35.6 (C-4), 40.6, 47.0 (C menthyl), 77.0 (C-1'), 78.6 (C-2), 89.7 (C-5), 126.2 (d, *J* = 41.3 Hz, C-6"), 128.3

(C-*o* Ar), 129.9 (C-*m* Ar), 133.0 (C-*p* Ar), 135.6 (C-*i* Ar), 139.7 (d, *J* = 241.7 Hz, C-5”), 147.1 (C-2”), 152.6 (d, *J* = 19.4 Hz, C-4”), 162.1 (C=O benzoyl), 169.5 (*C*=OO-menthyl).

Elemental analysis calcd (%) for [$C_{25}H_{30}FN_3O_5S$]: C 59.63; H 6.00; N 8.34; S 6.37. Found: C 59.73; H 6.02; N 8.32; S 6.35.







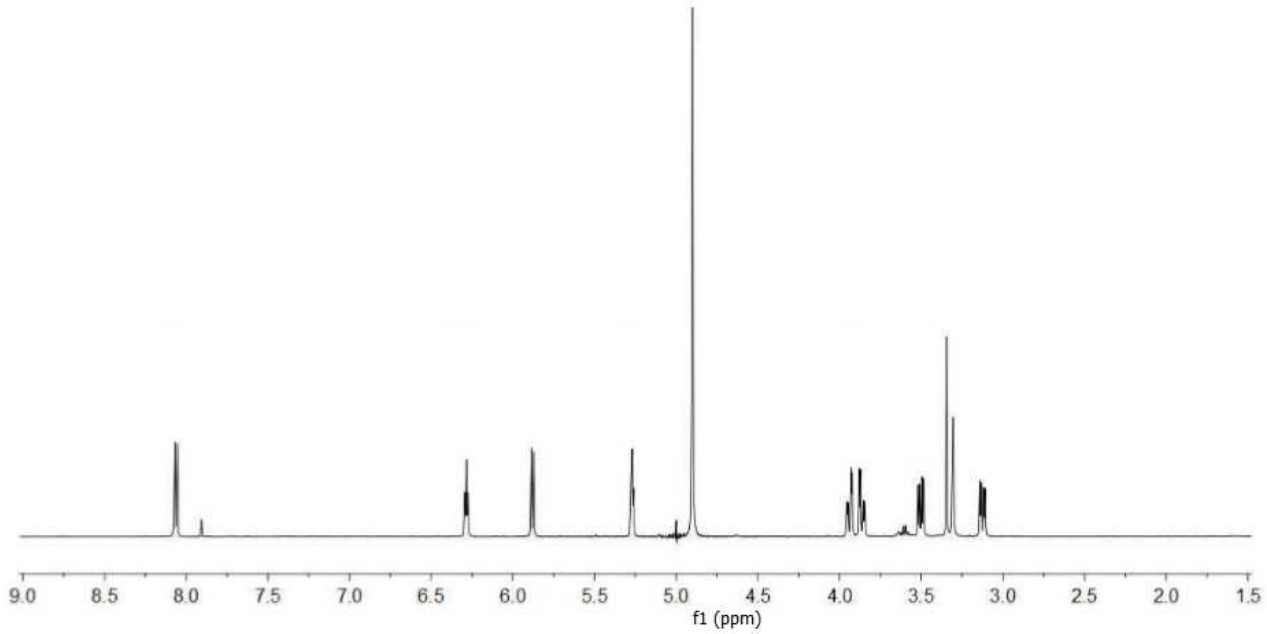
(2*S*,5*R*)-2-(Hydroxymethyl)-5-(4-amido-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (4, (+)-BCH-189).

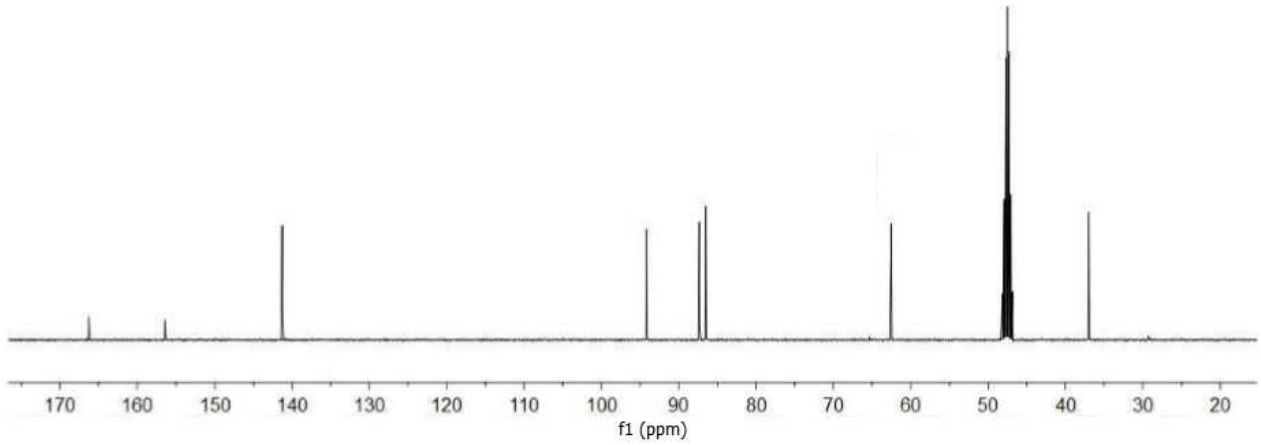
Protected nucleoside **28** (24 mg, 0.0630 mmol) was dissolved in anhydrous THF (1.5 mL) under nitrogen atmosphere, and $LiAlH_4$, suspended in THF (800 µl), was added at 0 °C. After an hour, the reaction was quenched with MeOH, was filtered through a celite pad and concentrated under reduced pressure. Chromatography of the crude residue over silica gel (95:5 DCM-MeOH) gave the pure nucleoside **4** (12 mg, 83%).

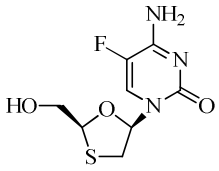
^{1}H NMR (500 MHz, MeOD): δ 3.12 (dd, *J* = 12.0, 4.1 Hz, 1H, H-4), 3.50 (dd, *J* = 12.0, 5.4 Hz, 1H, H-4), 3.86 (dd, *J* = 12.5, 4.0 Hz, 1H, CH_2OH), 3.94 (dd, *J* = 12.5, 2.8 Hz, 1H, CH_2OH), 5.27 (br s, 1H, H-2), 5.88 (d, *J* = 7.5 Hz, 1H, H-5'), 6.28 (br t, *J* = 4.6 Hz, 1H, H-5), 8.05 (d, *J* = 7.5 Hz, 1H, H-6').

^{13}C NMR (100.6 MHz, MeOD): δ 37.2 (C-4), 62.5 (CH_2OH), 86.3 (C-2), 86.6 (C-5), 94.2 (C-5'), 141.6 (C-6'), 156.6 (C-4'), 166.3 (C-2').

Elemental analysis calcd (%) for [$C_8H_{11}N_3O_3S$]: C 41.91; H 4.84; N 18.33; S 13.99. Found: C 42.03; H 4.82; N 18.27; S 14.02.







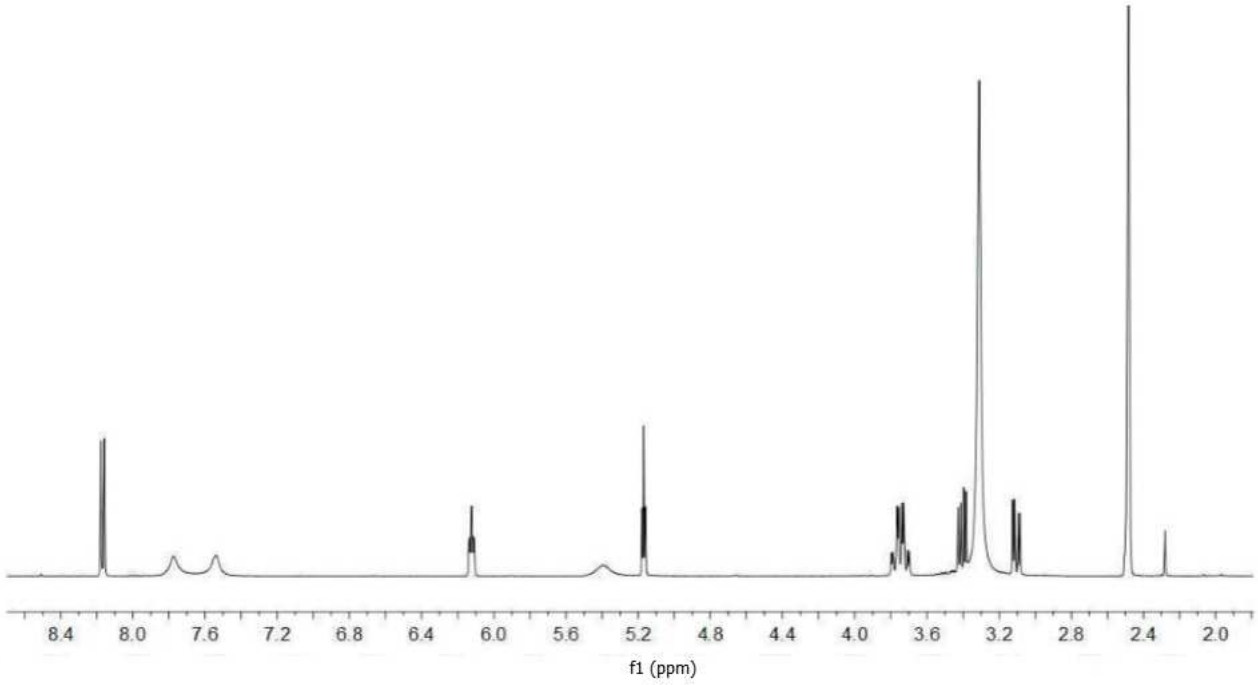
(2*S*,5*R*)-2-(Hydroxymethyl)-5-(4-amido-5-fluoro-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (34, D-FTC).

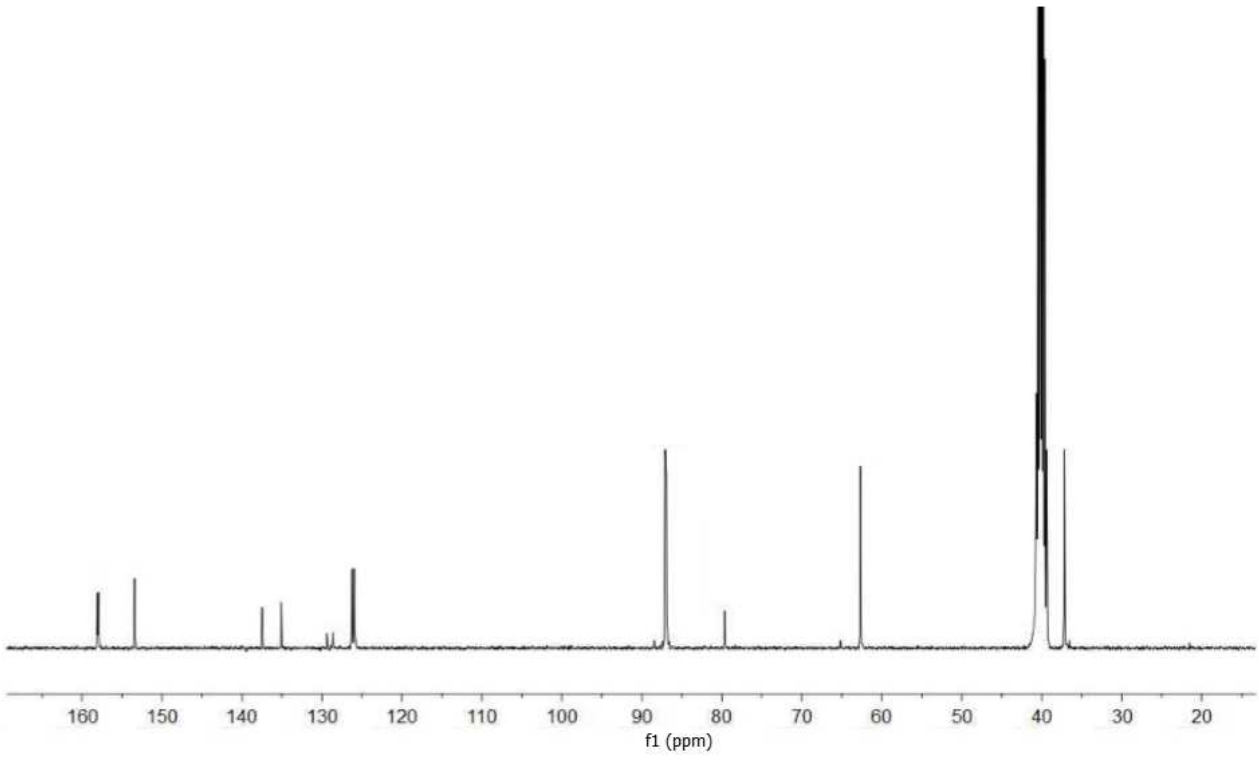
A solution of K_2HPO_4 (111 mg, 0.639 mmol) in H_2O (500 µL) was added to a suspension of protected nucleoside **32** (65 mg, 0.162 mmol) in ethanol (1 mL). Hence a solution of $NaBH_4$ (16 mg, 0.426 mmol) in H_2O (600 µL) containing 25% w/w NaOH was added to the reaction mixture. After 1 h, the reaction was quenched with diluted HCl, adjusting the pH to 4-4.5 and then to pH 7 using a saturated solution of $NaHCO_3$. The mixture was filtered through a celite pad and concentrated under reduced pressure. Chromatography of the crude residue over silica gel (9:1 to 85:15 DCM-MeOH) gave the pure nucleoside **34** (40 mg, 99%).

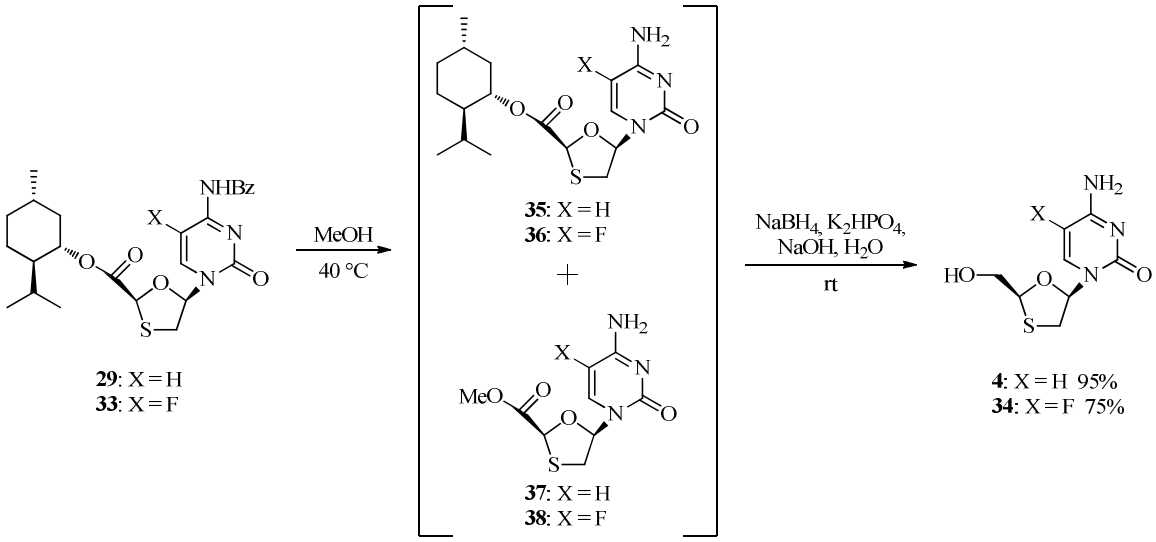
^{1}H NMR (400 MHz, DMSO): δ 3.10 (dd, *J* = 11.9, 4.4 Hz, 1H, H-4), 3.40 (dd, *J* = 11.9, 5.6 Hz, 1H, H-4), 3.72 (dd, *J* = 12.3, 4.1 Hz, 1H, CH_2OH), 3.77 (dd, *J* = 12.3, 3.8 Hz, 1H, CH_2OH), 5.17 (t, *J* = 3.9 Hz, 1H, H-2), 6.12 (ddd, *J* = 5.6, 4.8, 1.8 Hz, 1H, H-5), 7.54 (br s, 1H), 7.78 (br s, 1H), 8.17 (d, *J* = 7.2 Hz, 1H, H-6').

^{13}C NMR (100.6 MHz, DMSO): δ36.6 (C-4), 62.2 (CH_2OH), 86.4 (C-2), 86.5 (C-5), 125.6, (d, *J* = 32.6 Hz, C-6'), 136.2 (d, *J* = 240.6 Hz, C-5'), 152.9 (C-2'), 157.5 (d, *J* = 13.4 Hz, C-4').

Elemental analysis calcd (%) for [$C_8H_{10}FN_3O_3S$]: C 38.86; H 4.08; N 17.00; S 12.97. Found: C 38.74; H 4.10; N 17.05; S 13.01

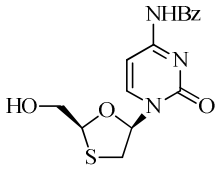






Reduction of benzoyl nucleosides: general procedure.

A suspension of protected nucleoside **29** or **33** (1 mmol) in anhydrous methanol (20 mL) was heated to 40 °C and left under stirring at the same temperature for 48h (**29**) or 72 h (**33**). The reaction mixture (containing menthyl nucleoside **35** or **36** as the main products) was then cooled to room temperature and a solution of K_2HPO_4 (3 eq) in H_2O (2 mL) was added. Hence a solution of $NaBH_4$ (2 eq) in H_2O (2 mL) containing 25% w/w NaOH was added to the reaction mixture. After 1 h, the reaction was quenched with diluted HCl, adjusting the pH to 4-4.5 and then to pH 7 using a saturated solution of $NaHCO_3$. The mixture was filtered through a celite pad and concentrated under reduced pressure. Chromatography of the crude residue over silica gel gave the pure nucleoside (**4**: 95% o.y.; **34**: 75% o.y.).



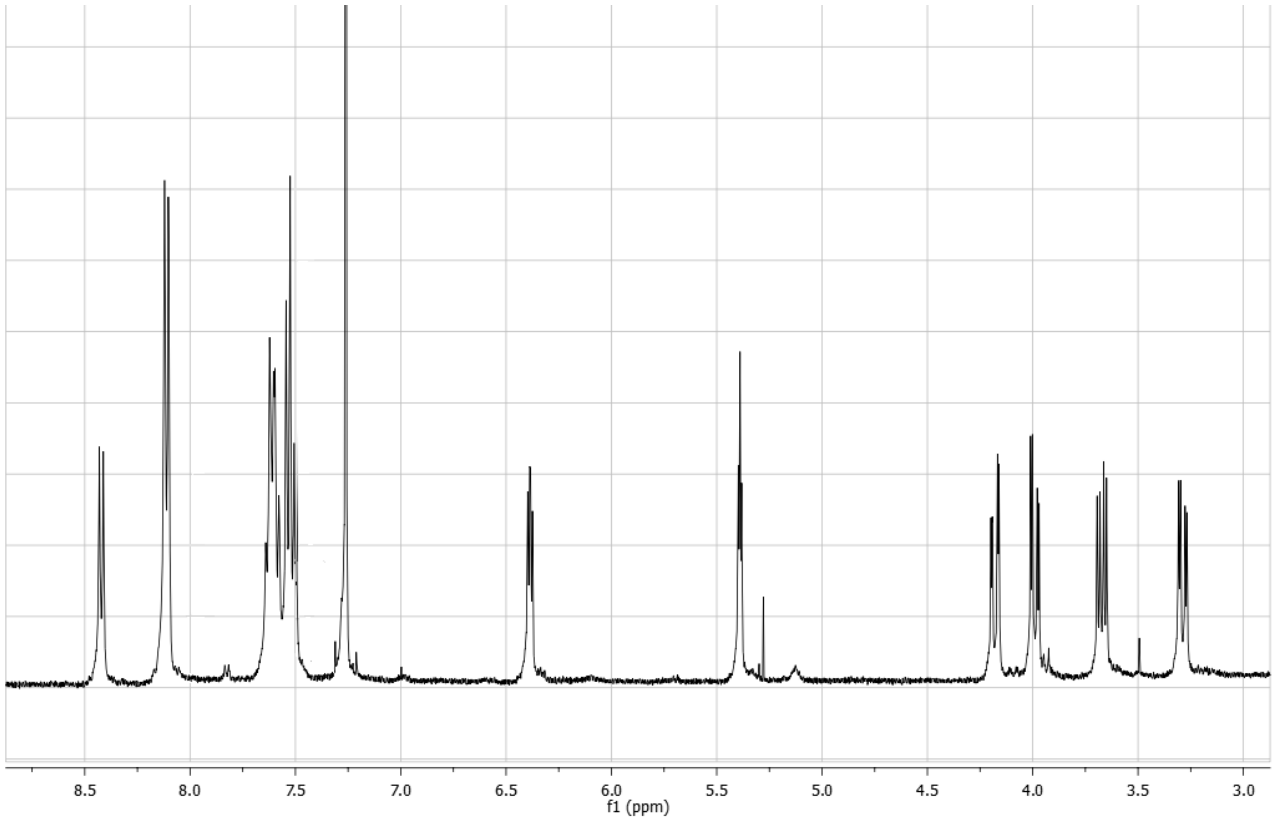
2(*S*)-(Hydroxymethyl)-5(*R*)-(4-benzamide-2-oxo-1,2-dihydropyrimidine)-1'-yl-1,3-oxathiolane (8).

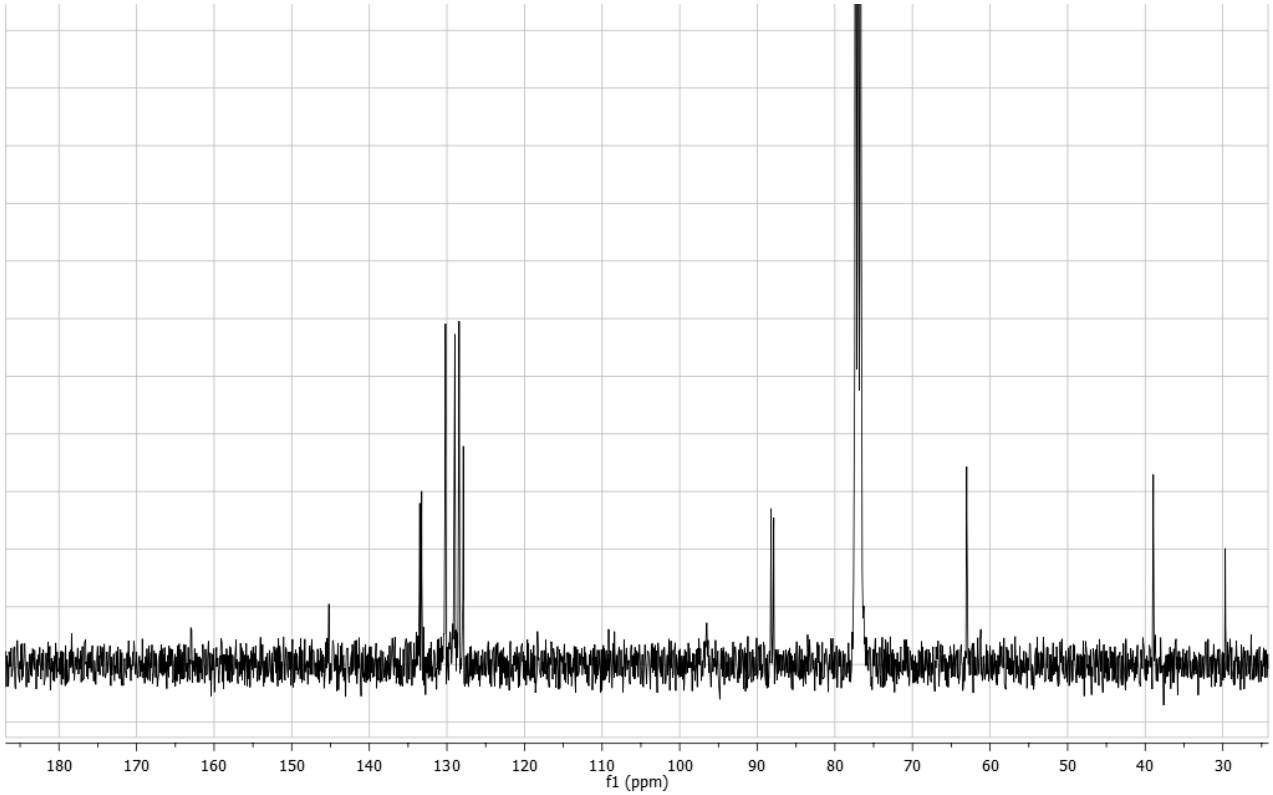
To a solution of (+)-BCH-189 (**4**, 75 mg, 0.328 mmol) in pyridine (7 ml) was added under nitrogen atmosphere TMSCl (360 µl, 2.84 mmol). After 30 minutes, benzoyl chloride (82 µl, 0.710 mmol)

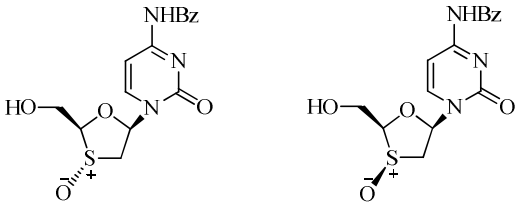
was added at 0 °C and the solution was stirred at room temperature overnight. The reaction was quenched at 0 °C with 800 µl of H_2O and 800 µl of NH_3 (aq). The mixture, after stirring for one hour, was dried under reduced pressure. Flash chromatography of the crude (97:3 DCM–MeOH to 8:2 DCM MeOH) afforded protected product **8** (108 mg, 99%).

^{1}H NMR (400 MHz, $CDCl_3$): δ3.29 (dd, *J* = 12.4, 3.6 Hz, 1H, H-4), 3.67 (dd, *J* = 12.4, 5.3 Hz, 1H, H-4), 3.99 (dd, *J* = 12.7, 3.5 Hz, 1H, CH_2OH), 4.18 (dd, *J* = 12.6, 2.6 Hz, 1H, CH_2OH), 5.39 (t, *J* = 3.2 Hz, 1H, H-2), 6.39 (dd, *J* = 5.2, 3.6 Hz, 1H, H-5), 7.52 (t, *J* = 7.9 Hz, 2H, H-*m* Ar), 7.58-7.64 (m, 2H, H-5', H-*p* Ar), 8.11 d, *J* = 7.2 Hz, 2H, H-*o* Ar), 8.42 (d, *J* = 7.5 Hz, 1H, H-6').

^{13}C NMR (100.6 MHz, $CDCl_3$): δ29.7 (C-4), 39.0 (CH_2OH), 87.9 (C-2), 88.2 (C-5), 127.9 (C-5'), 128.4 (C-*m* or *o* Ar), 129.0 (C-*m* or *o* Ar), 130.2 (C-*p* Ar), 133.2 (C-6'), 133.5 (C-*i* Ar), 145.2 (C-4'), 163.0 (C-2'), 178.4 (C=O benzoyl).







2(*S*)-(Hydroxymethyl)-5(*R*)-(4-benzamide-2-oxo-1,2-dihydropyrimidine)-1'-yl-3-oxido-1,3-oxathiolane (11 and 12).

Nucleoside **8** (169 mg, 0.484 mmol) was dissolved in anhydrous DCM (6 ml) under nitrogen atmosphere, and the mixture was cooled to -78 °C. A suspension of *m*-CPBA (108 mg, 0.629 mmol) in DCM (1 ml) was added to the starting product, and the reaction was stirred for 30 minutes. A solution of $NaHCO_3$ was then added, and the mixture was concentrated under reduced pressure. Flash chromatography (95:5 DCM-MeOH to 9:1 DCM-MeOH), preparative TLC, HPLC of the residue could not separate the sulfoxides **11** and **12**.

5. REFERENCES

1. Nair, V.; Chi, G. *Rev. Med. Virol.* **2007**, *17*, 277.

2. Arts, E. J.; Wainberg, M.A. *Antimicrob. Agents Chemother.* **1996**, *40*, 527.

3. Tomasselli, A.G.; Heinrikson, R.L. *Biochim. Biophys. Acta.* **2000**, *1477*, 189.

4. De Clercq, E.; Field, H.J. *Antivir. Chem. Chemother.* **2008**, *19*, 75.

5. Hao, Z.; Cooney, D.A.; Hartman, N.R.; Perno, C.F.; Fridland, A.; Devico, A.L.; Sarngadharan, M.G.; Broder, S.; Johns, D.G. *Mol. Pharmacol*. **1988**, *34*, 431.

6. "Fact sheet". *UNAIDS.org*. Retrieved November 29, **2015**.

7. Sarafianos, S.G.; Marchand, B.; Das, K.; Himmel, D.M.; Parniak, M.A.; Hughes, S.H.; Arnold, E. *J. Mol. Biol*. **2009**, *385*, 693.

8. Kohl, N.E.; Emini, E.A.; Schleif, W.A.; Davis, L.J.; Heimbach, J.C.; Dixon, R.A.F.; Scolnick, E.M.; Sigal, I.S. *Proc. Natl. Acad. Sci. USA.* **1988**, *85*, 4686.

9. Muesling, M.A.; Smith, D.H.; Cabradilla, C.D.; Benton, C.V.; Lasky, L.A.; Capon, D.J. *Nature.* **1985**, *313*, 450.

10. Coffin, J.M. *Science*. **1995**, *267*, 483.

11. (a) Kitchen, C.M.; Kitchen, S.G.; Dubin, J.A.; Gottlieb, M.S. *Clin. Infect. Dis*. **2001**, *33*, 466. (b) Valenti, W.M. *AIDS Reader* **2001**, *11*, 260. (c) King, J.T., Jr.; Justice, A.C.; Roberts, M.S.; Chang, C.C.; Fusco, J.S. *Med. Decis. Making.* **2003**, *23*, 9.

12. (a) Vacca, J.P.; Condra, J.H. *Drug Discov. Today.* **1997**, *2*, 261. (b) Wlodawer, A.; Vondrasek, J. *Annu. Rev. Biophys. Biomol. Struct.* **1998**, *27*, 249. (c) Gago, F. *IDrugs.* **1999**, *2*, 309. (d) Holtzer, C.D.; Roland, M. *Ann. Pharmacother.* **1999**, *33*, 198.

13. Steigbigel, R.T. *et al. N. Engl. J. Med*. **2008**, *359*, 339.

14. Carr, A. *Nat. Rev. Drug Discovery.* **2003**, *2*, 624.

15. Martinez-Picado, J.; De Pasquale, M.P.; Kartsonis, N.; Hanna, G.J.; Wong, J.; Finzi, D.; Rosenberg, E.; Gunthard, H.F.; Sutton, L.; Savara, A.; Petropoulos, C.J.; Hellmann, N.; Walker, B.D.; Richman, D.D.; Siliciano, R.; D'Aquila, R.T. *Proc. Natl. Acad. Sci. U.S.A.* **2000**, *97*, 10948.

16. Little, S.J.; Holte, S.; Routy, J.P.; Daar, E.S.; Markowitz, M.; Collier, A.C.; Koup, R.A.; Mellors, J.W.; Connick, E.; Conway, B.; Kilby, M.; Wang, L.; Whitcomb, J.M.; Hellmann, N.S.; Richman, D.D. *N. Engl. J. Med*. **2002**, *347*, 385.

17. De Clercq, E. *Nat. Rev. Drug Discovery*. **2002**, *1*, 13.

18. Meadows, D.C.; Gervay-Hague, J. *ChemBioChem.* **2006**, *1*, 16.

19. Mitsuya, H.; Weinhold, K.J.; Furman, P.A., St; Clair, M.H.; Lehrman, S.N.; Gallo, R.C.; Bolognesi, D.; Barry, D.W.; Broder, S. *Proc. Natl. Acad. Sci. U.S.A.* **1985**, *82*, 7096.

20. Balzarini, J.; Kang, G.J.; Dalal, M.; Herdewijn, P.; DeClercq, E.; Broder, S.; Johns, D.G. *Mol. Pharmacol.* **1987**, *32*, 162.

21. Mitsuya, H.; Broder, S. **1989**. U.S. Patent 4,861,759.

22. Daluge, S.M.; Good, S.S.; Faletto, M.B.; Miller, W.H., St; Clair, M.H.; Boone, L.R.; Tisdale, M.; Parry, N.R.; Reardon, J.E.; Dornsife, R.E.; Averett, D.R.; Krenitsky, T.A. *Antimicrob. Agents Chemother*. **1997**, *41*, 1082.

23. Gosselin, G.; Schinazi, R.F.; Sommadossi, J.P.; Mathé, C.; Bergogne, M.C.; Aubertin, A.M.; Kirn, A.; Imbach, J.L. *Antimicrob. Agents Chemother*. **1994**, *38*, 1292.

24. Schinazi, R.F.; Chu, C.K.; Peck, A.; McMillan, A.; Mathis, R.; Cannon, D.; Jeong, L.S.; Beach, J.W.; Choi, W.B.; Yeola, S.; Liotta, S.C. *Antimicrob. Agents Chemother*. **1992**, *36*, 672.

25. Moore, R.D.; Chaisson, R.E. *Ann. Intern. Med*. **1996**, *124*, 633.

26. Hammer, S.M.; Squires, K.E.; Hughes, M.D.; Grimes, J.M.; Demeter, L.M.; Currier, J.S.; Eron, J.J.; Feinberg, J.E.; Balfour, H.H.; Deyton, L.R.; Chodakewitz, J.A.; Fischl, M.A. *N. Engl. J. Med.* **1997**, *337*, 725.

27. Gulick, R.M.; Mellors, J.W.; Havlir, D.; Eron, J.J.; Gonzalez, C.; McMahon, D.; Richman, D.D.; Valentine, F.T.; Jonas, L.; Meibohm, A.; Emini, E.A.; Chodakewitz, J.A.; Deutsch, P.; Holder, D.; Schleif, W.A.; Condra, J.H. *N. Engl. J. Med.* **1997**, *337*, 734.

28. Moore, R.D.; Chaisson, R.E. *AIDS.***1999**, *13*,1933.

29. (a) Balzarini, J. *Pharm. World Soc.* **1994**, *16*, 113. (b) Hao, Z.; Cooney, D.A.; Farquhar, D.; Perno, C.F.; Zhang, K.; Masood, R.; Wilson, Y.; Hartman, N.R.; Balzarini, J.; Johns, D.G. *Mol. Pharmacol*. **1990**, *37*, 157.

30. Eriksson, S.; Munch-Petersen, B.; Johansson, K.; Eklund, H. *Cell. Mol. Life Sci*. **2002**, *59*, 1327.

31. Perno, C.F.; Yarchoan, R.; Cooney, D.A.; Hartman, N.R.; Gartner, S.; Popovic, M. *et al*. *J. Exp. Med*. **1988**, *168*, 1111.

32. Furman, P.A.; Fyfe, J.A.; St Clair, M.H.; Weinhold, K.; Rideout, J.L.; Freeman, G.A. *et al. Proc. Natl Acad. Sci. USA.* **1986**, *83*, 8333.

33. De Muys, J.M.; Gourdeau, H.; Nguyen-Ba, N.; Taylor, D.L.; Ahmed, P.S.; Mansour, T.; Locas, C.; Richard, N.; Wainberg, M.A.; Rando, R.F. *Antimicrob. Agents Chemother.* **1999**, *43*, 1835.

34. Schinazi, R.F.; McMillan, A.; Cannon, D.; Mathis, R.; Lloyd, R.M.; Peck, A.; Sommadossi, J.P.; St Clair, M.; Wilson, J.; Furman, P.A.; Painter, G.; Choi, W.B.; Liotta, D.C. *Antimicrob. Agents Chemother.* **1992**, *36*, 2423.

35. Kim, H.O.; Schinazi, R.F.; Shanmuganathan, K.; Jeong, L.S.; Beach, J.W.; Nampalli, S.; Cannon, D.L.; Chu, C.K. *J. Med. Chem*. **1993**, *36*, 519.

36. Lin, T.S.; Luo, M.Z.; Liu, M.C.; Pai, S.B.; Dutschman, G.E.; Cheng, Y.C. *Biochem. Pharmacol*. **1994**, *47*, 171.

37. Flexner, C. *Nat. Rev. Drug Discov*. **2007**, *6*, 959.

38. (a) Bethell, R.C.; Lie, Y.S.; Parkin, N.T. *Antiviral Chem. Chemother*. **2005**, *16*, 295. (b) Gu, Z.; Allard, B.; de Muys, J.M.; Lippens, J.; Rando, R.F.; Nguyen-Ba, N.; Ren, C.; McKenna, P.; Taylor, D.L.; Bethell, R.C. *Antimicrob. Agents Chemother*. **2006**, *50*, 625.

39. Cahn, P.; Cassetti, I.; Wood, R.; Phanuphak, P.; Shiveley, L.; Bethell, R.C.; Sawyer, J. *AIDS.* **2006**, *20*, 1261.

40. (a) Hurwitz, S.J.; Otto, M.J.; Schinazi, R.F. *Antiviral Chem. Chemother*. **2005**, *16*, 117. (b) Herzmann, C.; Arastèh, K.; Murphy, R.L.; Schulbin, H.; Kreckel, P.; Drauz, D.; Schinazi, R. F.; Beard, A.; Cartee, L.; Otto, M.J. *Antimicrob. Agents Chemother*. **2005**, *49*, 2828.

41. (a) Bazmi, H.Z.; Hammond, J.L.; Cavalcanti, S.C.; Chu, C.K.; Schinazi, R.F.; Mellors, J.W. *Antimicrob. Agents Chemother*. **2000**, *44*, 1783. (b) Gu, Z.; Wainberg, M.A.; Nguyen-Ba, N.; L'Heureux, L.; De Muys, J.M.; Bowlin, T.L.; Rando, R.F. *Antimicrob. Agents Chemother*. **1999**, *43*, 2376. (c) Chong, Y.; Borroto-Esoda, K.; Furman, P.A.; Schinazi, R.F.; Chu, C.K. *Antiviral Chem. Chemother*. **2002**, *13*, 115. (d) Lin, T.S.; Chen, M.S.; McLaren, C.; Gao, Y.S.; Ghazzouli, I.; Prusoff, W.H. *J. Med. Chem.* **1987**, *30*, 440. (e) Dutschman, G.E.; Bridges, E.G.; Liu, S.H.; Gullen, E.; Guo, X.; Kukhanova, M.; Cheng, Y.C. *Antimicrob. Agents Chemother*. **1998**, *42*, 1799. (f) Hammond, J.L.; Parikh, U.M.; Koontz, D.L.; Schlueter-Wirtz, S.; Chu, C.K.; Bazmi, H.Z.; Schinazi, R F.; Mellors, J.W. *Antimicrob. Agents Chemother.* **2005**, *49*, 3930.

42. Maury, G. *Antiviral Chem. Chemother*. **2000**, *11*, 165.

43. Smejkal, J.; Sorm, F. *Collect. Czech. Chem. Commun.* **1964**, *29*, 2809.

44. (a) Belleau, B.; Dixit, D.; Nguyen-Bu, N.; Kraus, J.-L. International Conference on AIDS. **1989**. Montreal, Canada, 4–9 June, paper no T.C.O.1. (b) Belleau, B.; Dixit, D.; Nguyen-Bu, N. **1989**. Eur. Patent Appl., 337713.

45. Coates, J.A.V.; Cammack, N.; Jenkinson, H.J. *et al*. *Antimicrob. Agents Chemother*. **1992**, *36*, 202.

46. Wengel, J.; Lau, J.; Pederson, E.B.; Nielson, C.M. *J. Org. Chem.* **1991**, *56*, 3591.

47. Severini, A.; Liu, X.-Y.; Wilson, J.S.; Tyrrell, D.L.J. *Antimicrob. Agents Chemother*. **1995**, *39*, 1430.

48. Shewach, D.S.; Liotta, D.C.; Schinazi, R.F. *Biochem. Pharmacol.* **1993**, *45*, 1540.

49. Chang, C.-N.; Doong, S.-L.; Zhou, J.H. *et al. J. Biol. Chem.* **1992**, *267*, 13938.

50. Cammack, N.C.; Rouse, P.L.; Marr, C.L.P.; *et al. Biochem. Pharmacol.* **1992**, *43*, 2059.

51. Cui, L.; Schinazi, R.F.; Gosselin, G.; Imbach, J.-L.; Chu, C.K.; Rando, R.F.; Revankar, G.R.; Sommadossi, J.-P. *Biochem. Pharmacol.* **1996**, *52*, 1577.

52. Feng, J.Y.; Johnson, A.A.; Johnson, K.A.; Schinazi, R.F.; Anderson, K.S.; Furman, P.A. In: *Program and abstracts of the 2nd International Workshop on Adverse Drug Reactions and Lipodystrophy in HIV* (Toronto, Canada). **2000**.

53. Schinazi, R.F.; Boudinot, F.D.; Ibrahim, S.S.; Manning, C.; McClure, H.M.; Liotta, D.C. *Antimicrob. Agents Chemother*. **1992**, *36*, 2432.

54. (a) Wilson, J.E.; Martin, J.L.; Borroto-Esoda, K.; Hopkins, S.; Painter, G; Liotta, D.C.; Furman, P.A. *Antimicrob. Agents Chemother.* **1993**, *37*, 1720. (b) Wilson, J.E.; Aulabaugh, A.; Caligan, B.; McPherson, S.; Wakefield, J.K.; Jablonski, S.; Morrow, C.D.; Reardon, J.E.; Furman, P.A. *J. Biol. Chem.* **1996**, *271*, 13656.

55. Furman, P.A.; Davis, M.; Liotta, D.C. *et al. Antimicrob. Agents Chemother.* **1992**, *36*, 2686.

56. Wang, P.; Hong, J.H.; Cooperwood, J.S.; Chu C.K. *Antiviral Res.* **1998**, *40*, 19.

57. Kohlstaedt, L.A.; Wang, J.; Friedman, J.M.; Rice, P.A.; Steitz, T.A. *Science*. **1992**, *256*, 1783.

58. (a) Tisdale, M.; Kemp, S.D.; Parry, N.R.; Larder, B.A. *Proc. Natl. Acad. Sci.* **1993**, *90*, 5653. (b) Schinazi, R.F.; Lloyd, R.M.; Nguyen, M.-H. *et al. Antimicrob. Agents Chemother.* **1993**, *37*, 875.

59. Choi, W.B.; Wilson, L.J.; Yeola, S.; Liotta, D.C. *J. Am. Chem. Soc.* **1991**, *113*, 9377.

60. Jeong, L.S.; Schinazi, R.F.; Beach, J.W. *et al. J. Med. Chem.* **1993**, *36*, 2627.

61. Jeong, L.S.; Schinazi, R.F.; Beach, J.W. *et al. J. Med. Chem.* **1993**, *36*, 181.

62. Beach, J.W.; Jeong, L.S.; Alves, A.J. *et al. J. Org. Chem.* **1992**, *57*, 2217.

63. Jin, H.M.; Siddiqui, A.; Evans, C.A.; Tse, H.L.A.; Mansour, T.S.; Goodyear, M.D.; Ravenscroft, P.; Beels, C.D. *J. Org. Chem.* **1995**, *60*, 2621.

64. (a) Milton, J.; Brand, S.; Jones, M.F.; Rayner, C.M. *Tetrahedron Lett.* **1995**, *36*, 6961. (b) Cousins, R.P.C.; Mahmoudian, M.; Youds, P.M. *Tetrahedron: Asymm.* **1995**, *6*, 393. (c) Dionne, G. **1996**. US Patent 5,538,975. (d) Caputo, R.; Guaragna, A.; Palumbo, G.; Pedatella, S. *Eur. J. Org. Chem.* **1999**, 1455.

65. Hill, M.L.; Whitehead, A.J.; Hornby, R.; Hallett, P.; Goodyear, M.D.; Dwyer, P.O. **2001**. US Patent 6,329,522 B1.

66. Murth, K.S.K.; Horne, S.E.; Reddy, G.V.; Senanayake, C.B.W.; Radatus, B.K. **2002**. US Patent 6,380,388 B1.

67. Xie, Y.; Hritzko, B.; Chin, C.Y.; Wang, N.-H.L. *Ind. Eng. Chem. Res.* **2003**, *42*, 4055.

68. Robins, M.J.; MacCoss, M.; Naik, S.R.; Ramani, G. *J. Am. Chem. Soc.* **1976**, *98*, 7381. (b) Heidelberger, C.; Duschinsky, R. **1957**. US Patent 2,802,005. (c) Duschinsky, R.; Heidelberger, C. **1960**. US Patent 2,945,038.

69. Roy, B.N.; Singh, G.P.; Srivastava, D.; Aher, U.P.; Patil, S.U. **2013**. PCT Int. Appl. WO2013021290A1 IB2012/050117.

70. (a) Fernández, I.; Khiar, N. *Chem. Rev.* **2003**, *103*, 3651. (b) Wojaczyńska, E.; Wojaczyński, J. *Chem. Rev*. **2010**, *110*, 4303.

71. (a) Pitchen, P.; Dunach, T.; Deshmukh, N.N.; Kagan, H.B. *J. Am. Chem. Soc*. **1984**, *106*, 8188. (b) Brunel, J.M.; Diter, P.; Duetsch, M.; Kagan, H B. *J. Org. Chem*. **1995**, *60*, 8086.

72. (a) Di Furia, F.; Modena, G.; Seraglia, R. *Synthesis.* **1984**, 325. (b) Massa, A.; Mazza, V.; Scettri, A. *Tetrahedron: Asymmetry.* **2005**, *16*, 2271.

73. Goodyear, M.D.; Hill, M.L.; West, J.P.; Whitehead, A.J. *Tetrahedron Lett.* **2005**, *46*, 8535.

74. Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 7863.

75. Pastore, A.; Valerio, S.; Adinolfi, M.; Iadonisi, A. *Chem. Eur. J*. **2011**, *17*, 5881.

76. Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett*. **2002**, 269.

77. Yadav, J.S.; Reddy, S.B.V.; Premalatha, K.; Swamy, T. *Tetrahedron Lett.* **2005**, *46*, 2687.

78. Giordano, M.; Iadonisi, A. *Eur. J. Org. Chem*. **2013**, 125.

79. Laval S.; Dayoub, W.; Pehlivan, L.; Metay, E.; Favre-Reguillon, A.; Delbrayelle, D.; Mignani, G.; Lemaire, M. *Tetrahedron.* **2014**, *70*, 975.

80. Yan Chan, L.; Seh Kai Lim, J.; Kim, S. *Synlett.* **2011**, *19*, 2862.

81. Patel, J.P.; Li, A.; Dong, H.; Korlipara V.L.; Mulvihill, M.J. *Tetrahedron Lett.* **2009**, *50*, 5975.

82. Plitta, B.; Adamska, E.; Giel-Pietraszuk, M.; Fedoruk-Wyszomirska, A.; Naskret-Barciszewska, M.; Markiewicz, W.T.; Barciszewski, J. *Eur. J. Med. Chem*. **2012**, *55*, 243.

83. Nowak, I.; Conda-Sheridan, M.; Robins, M.J. *J. Org. Chem.* **2005**, *70*, 7455.

Section 2

1. INTRODUCTION

Iminosugars are carbohydrate derivatives having the endocyclic oxygen replaced by a nitrogen atom. This substitution makes iminosugars compounds of difficult preparation and profoundly changes their biological properties. In particular, iminosugars are the most interesting carbohydrate mimics, especially for their ability to act as glycosidase inhibitions, arising from their resemblance to sugars. Glycosidases are found in a number of important biological processes, such as intestinal digestion, post-translational processing of glycoproteins and the lysosomal catabolism of glycoconjugates; for this reason sugar mimics might have enormous therapeutic potential in many diseases such as diabetes, viral infection and lysosomal storage disorders.

The inherited metabolic disorders of glycosphingolipid (GSL) metabolism are a rare group of diseases that cause different dysfunctions, often neurodegenerative. Generally, problems in catabolic enzyme activity lead to lysosomal storage of GSL substrates and, in many diseases, other glycoconjugates. Today there are not cures for lysosomal storage diseases, and treatment is mostly symptomatic, although bone marrow transplantation and enzyme replacement therapy have been tried with some success. An approach to treating these diseases could be the substrate reduction therapy (SRT), in this regard there is the discovery and development of *N*-alkylated iminosugars, more lipophilic and, consequently, better absorbed, as effective drugs.

1.1. IMINOSUGARS

For many years iminosugars were also denominated “azasugars” to distinguish them from general amino sugars, such as glucosamine derivatives or other carbohydrate analogues, but commonly the term *aza* is used when a nitrogen replaces a carbon, not an oxygen. They can also be defined polyhydroxylated alkaloids, but their biogenetic pathway is completely different: the starting product of the route is always a carbohydrate[1].

Iminosugars are classified in five classes, according to their structure: polyhydroxylated piperidines, pyrrolidines, indolizidines, pyrrolizidines and nortropanes (**Figure 1.1**). They can also occur in a glycosylated form.

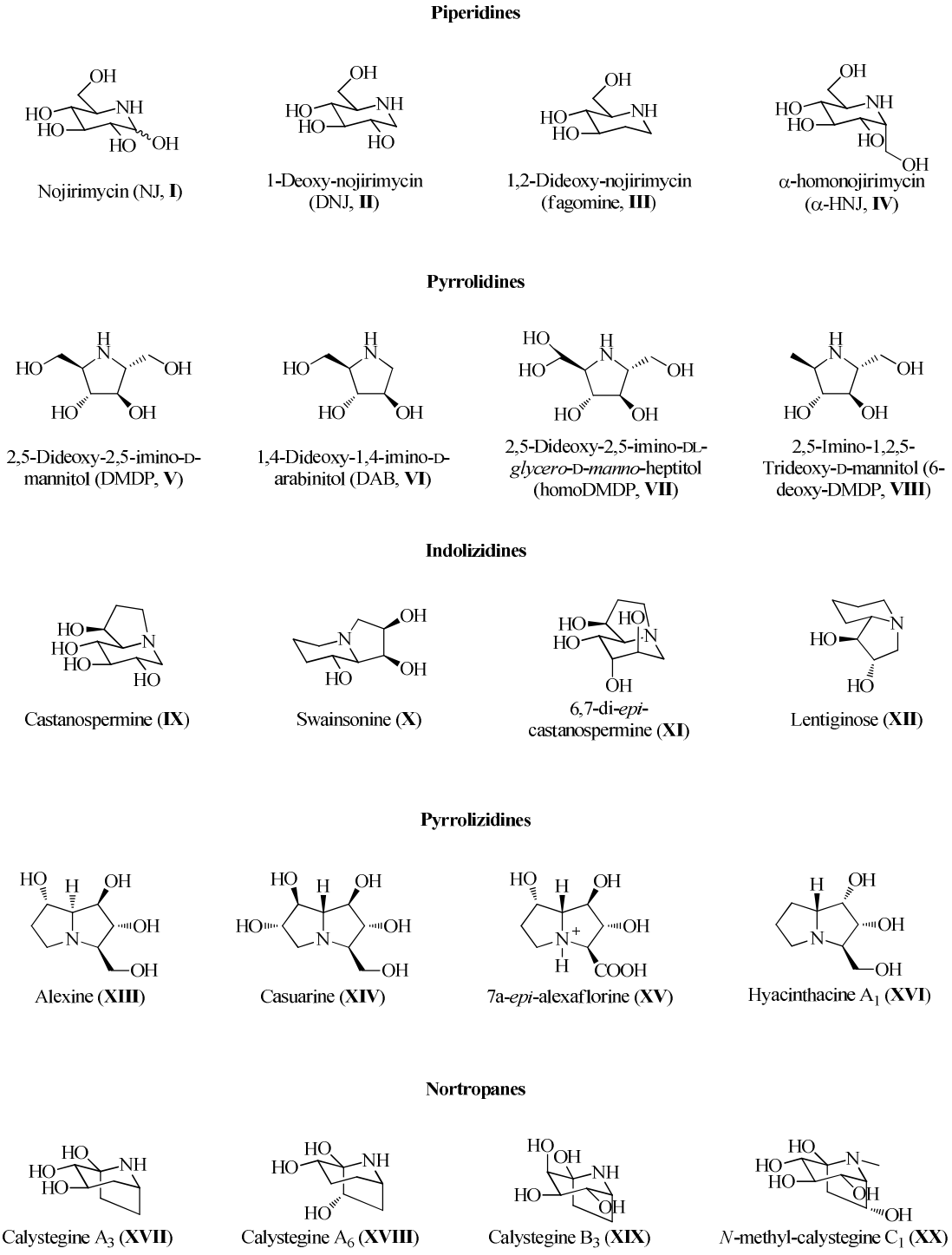


Figure 1.1: The five classes of iminosugars.

The study of the iminosugars led to a noteworthy progress in glycobiology, especially after the investigation of their inhibition mechanisms on glycosyltransferases[2], glycogen phosphorylases[3], nucleoside-processing enzymes[4] and metalloproteinases[5]. New therapies for numerous diseases have been developed thanks to the ability of iminosugars to influence the folding of abnormal glycosidases, avoiding their degradation, and their resemblance, in the protonated form, to the transition state of the glycosidic bond hydrolysis. Because of these properties, iminosugars are among the most powerful inhibitors of glycosydases[6] (**Figure 1.2**). Although the lack of the sp^2 character in the nitrogen-C-1 bond affects the mimicking of transition state, iminosugars bind the active sites of glycosidases 10^3 times better than carbohydrates, probably because nitrogen participates to interaction.

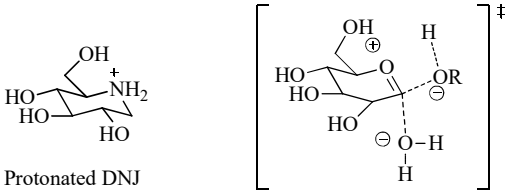


Figure 1.2: Resemblance between protonated DNJ and carbohydrate hydrolysis transition state.

Nojirimycin (NJ, **I**), an analog of glucose, is the first discovered iminosugar, isolated from Streptomyces roseochromogenes R-468 in 1966[7], and is characterized by the presence of a nitrogen atom in place of the endocyclic oxygen. NJ is a strong inhibitor of α- and β-glucosidases[8] with IC_{50} = 9 and 19 μM respectively. However, NJ was never approved as antibiotic because of the instability of the hydroxyl group at C-1. Its reduction with $NaBH_4$ lead to 1-deoxynojirimycin (DNJ, **II**)[7], isolated in 1966 from the roots of mulberry trees as fagomine (**III**), but also produced by many strains in the genera Bacillus and Streptomyces[9]. DNJ displayed α-glucosidase inhibitory activity *in vitro* but not *in vivo*[10], and for this reason a large number of its derivatives (as Emiglitate or Miglitol) was synthetized trying to increase the *in vivo* activity. From mulberry trees was also isolated DAB (**VI**), a potent inhibitor of α-glucosidases and α-mannosidase, while α-homonojirimycin (α-HNJ, **IV**), isolated from the neotropical liana *Omphalea diandra*, the first natural substituted DNJ at C-1, is a specific strong inhibitor of only α-glucosidases.

Swainsonine (**X**) can inhibit lysosomal and Golgi α-mannosidases, and castanospermine (**IX**), casuarine (**XIV**) and DMDP (**V**) show potent inhibitory activity against α-glucosidases, but, curiously, the L-enantiomer of DMDP is more powerful and specific than the D-enantiomer[11].

Many D-iminosugars are competitive inhibitors of D-glycohydrolases, but their L-enantiomers are noncompetitive inhibitors of the enzymes[11,12].

Recently, L-iminosugars were synthetized and tested in a large number of diseases; although the active site of enzymes generally accepts only the D-enantiomer, L-enantiomers can inhibit glycosidases with different mechanisms of action and a comparable potency[13].

1.1.1. APPLICATIONS OF *N*-ALKYLATED IMINOSUGARS

As seen above, the strong inhibitory activity of DNJ *in vitro* attracted the attention of many chemists who focused on production of its *N*-alkylated derivatives, in an attempt to increase their activity *in vivo*. *N*-alkylated iminosugars present an amphiphilic character that increases the inhibitory potency because the carbon chain takes part in the recognition of the active site of glucosylceramidase[14] and the protonated nitrogen forms an ion pair with a carboxylate present in the active site. Deoxynojirimycin derivatives (**Figure 1.3**) are endowed with the highest activity among *N*-alkylated iminosugars and the elongation of the alkyl chain brings about an improvement in the activity.

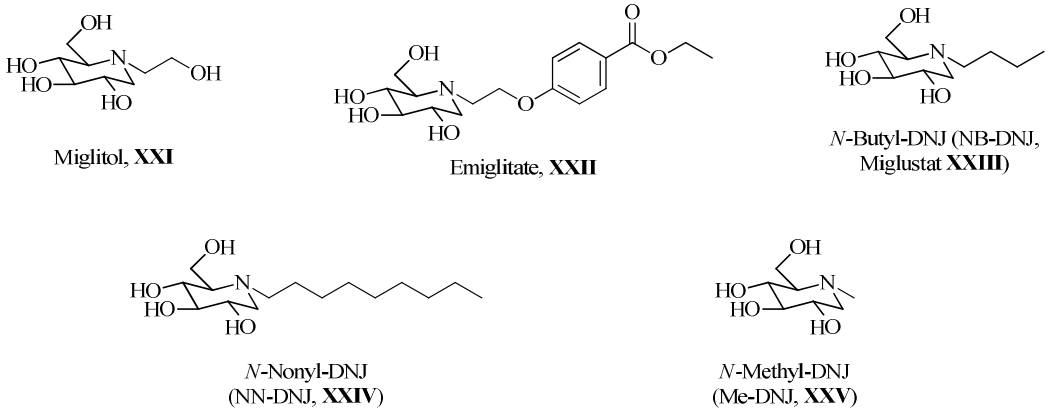


Figure 1.3: *N*-substituted DNJ derivatives.

Iminosugars can prevent the glycosylation of *N*-linked glycoproteins or alter the structure of carbohydrates, affecting cell functions. DNJ, HNJ strongly inhibit α-glucosidases II, their alkylation shifts their effect on α-glucosidases I, but their activity is lower than castanospermine,

probably because **IX** has a fixed axial position of the C-1 group, that correspond to the C-6 group of DNJ and HNJ[15]. The C-6 OH group of **II** and C-1 OH group of **IV** are in equatorial position, and the NH···O-6 in DNJ or NH···O-1 in HNJ intramolecular interaction stabilizes the C-6—O-6 or C-1—O-1 conformation. The *N*-alkylation increases the efficiency of DNJ or HNJ derivatives favoring the C-6 OH and C-1 OH axial conformation, breaking the intramolecular interactions[16].

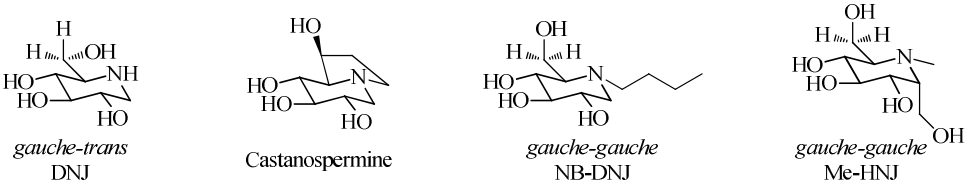


Figure 1.4: Preferred conformation of **II**, **IX**, **XXIII** and *N*-substituted HNJ (**IV**).

In 1996 FDA approved *N*-hydroxyethyl-deoxynojirimycin (Miglitol, **XXI**, **Figure 1.3**) as a second generation α-glucosidase inhibitor for its potent activity associated with a few intestinal side effects[17], due to the complete absorption of the drug. In fact, Miglitol is able to reduce the postprandial elevation of blood glucose by 50% and is metabolized (absorbed from the intestinal tract), in contrast to Acarbose (another α-glucosidase inhibitor approved in 1990 as anti-diabetes drug). It was introduced in the market in 1999 for the treatment of type 2 diabetes mellitus, and is prescribed also for heart diseases due to diabetic complications. Emiglitate (**XXII**), as DAB (**VI**) or isofagomine, showed similar activities with a long lasting effect *in vivo*, caused by a quasi-irreversible binding to α-glucosidases[18]. The higher reactivity of Emiglitate over Miglitol is due to its greater lipophilicity of the former, which can reach lysosomes more easily than Miglitate[19].

Some iminosugars as DNJ (**II**), *N*-butyl-DNJ (NB-DNJ, **XXIII**) or castanospermine (**IX**) can feature antiviral activity for their capacity to interfere in glycosylation processes, and viral envelope glycoproteins are essential for virion assembly. These iminosugars can inhibit HIV replication, a property probably related to the potent inhibitor activity of α-glucosidases I. Unfortunately, despite their good anti-HIV activity *in vitro*, the *in vivo* activity is not comparable to zidovudine[20] (EC_{50} = 560, 56 and 29 μM for **II**, **XXIII** and **IX** against 0.1 μM for AZT), and high concentration of the drugs are needed, but side effects can occur.

Conversely, NB-DNJ, and in particular *N*-nonyl-DNJ (NN-DNJ, **XXIV**) are good *in vitro* inhibitors of *N*-linked glycosylations involved in the transport of HBV virus out of the cell[21] and their administration results in a high percentage of virus particles retained in the host cell. They

also feature interesting activity against bovine viral diarrhea virus (BVDV), a culture surrogate of hepatitis C virus (HCV).

Owing to the important role of membrane-anchored oligosaccharides in tumor growth, substrates as iminosugars, capable of altering the glycosidase and glycosyltransferase structures, could be employed in tumor treatments. Castanospermine and *N*-methyl-DNJ (Me-DNJ, **XXV**) inhibit platelet aggregation of metastatic cells and cellular transformation by altering oncogene glycosylation, and reduce the adhesion of cancer cells to the vascular endothelium[22].

1.2. LYSOSOMAL STORAGE DISORDERS

1.2.1. GLYCOSPHINGOLIPIDS METABOLISM

Glycosphingolipids (GSLs) are composed of the hydrophobic amino alcohol sphingosine or ceramide, situated ubiquitously in the lipid bilayer of the cellular membrane, linked to a hydrophilic carbohydrate, with about 300 known structural variants[23]. Their function is to shield eukaryotic cells from chemical and mechanical damage through a protective carbohydrate external layer. If GSLs metabolism is altered, a series of diseases can develop[24]; these are rare in incidence (1 in 18000 live births worldwide) but severe and challenging to be treated. The glycolipid lysosomal storage disease are the most frequent cause of pediatric neurodegenerative disease.

All glycosphingolipids share a common biosynthetic pathway, that starts from the condensation between palmitoyl-CoA and serine. Then, a long string of metabolic steps in the cytosol leads to the generation of ceramide, which can be converted to sphingomyelin, galactosylceramide or glucosylceramide. The two glycosyltransferases (galactosyl and glucosyl) that promote these last two transformations are localized in the endoplasmic reticulum[25] and in the cytosol/Golgi membrane, respectively[26]. The glucosylceramide moves across the Golgi membrane where a galactose residue is added, under the agency of a glucosylceramide-specific galactosyltransferase[27]; the resulting lactosylceramide is the precursor of several different groups of glycosphingolipids (**Figure 1.5**).

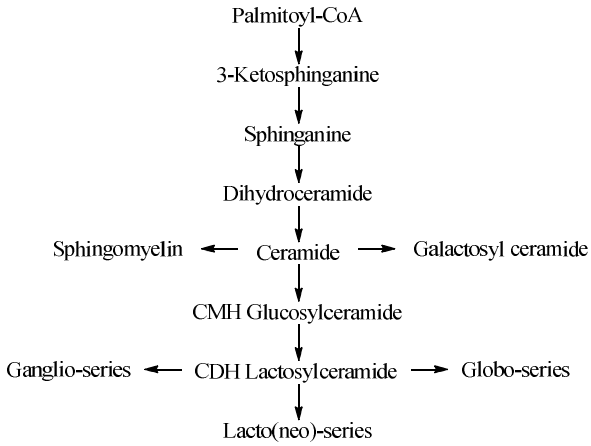


Figure 1.5: Glycosphingolipid Synthesis.

While the biosynthetic pathway proceeds in the cytosol, endoplasmic reticulum, and Golgi apparatus, the catabolism takes place in the lysosome (**Figure 1.6**). Specialized proteins, called sphingolipid activator proteins (SAPs), catalyze the hydrolysis of GSLs from the non-reducing terminus[28]; their inherited lack or malfunction results in the accumulation of the catabolism intermediates and, consequently, in the lysosomal storage disorders of glycolipids. The most common of these disorders is known as the Gaucher disease.

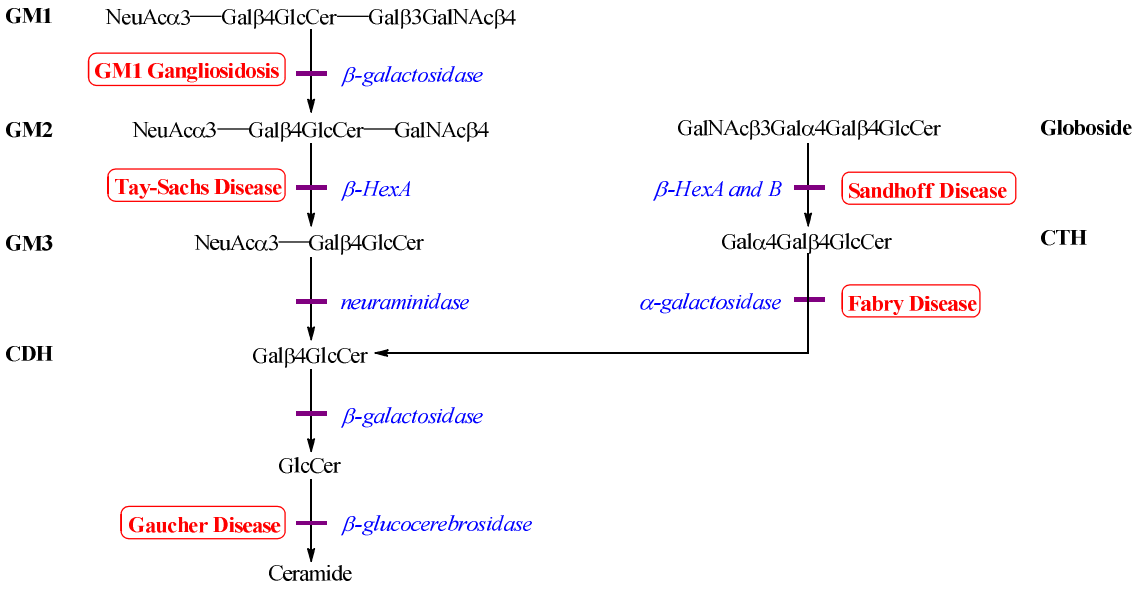


Figure 1.6: Glycosphingolipid Catabolism. For every defect in a lysosomal glycosidase (in blue), there is an inherited pathology (in red).

1.2.2. GAUCHER DISEASE: CERAMIDE *β*-GLUCOSIDASE DEFICIENCY

More than 75 gene mutations on the chromosome 1 lead to an impaired activity of ceramide *β*-glucosidase, the enzyme which catalyzes the hydrolysis of glucosylceramide to glucose and ceramide, (EC 3.2.1.45). Depending on the combination of the alleles (null, severe or mild), the clinical phenotypes can be predicted (Type I, Type II/III)[29]. This disorder is called Gaucher disease, after Philippe Gaucher, the French doctor who first described its symptoms[30] (anemia, fatigue, bruising, low blood platelet, skeletal disorders, enlargement of liver and spleen and, as described before, neurological pathologies), but its biochemical bases were elucidated just a century later.

The lack or reduced activity of ceramide *β*-glucosidase cause an accumulation of glucocerebroside in macrophages of liver, lungs, spleen and bone marrow. There are three types of Gaucher disease[31]: 1) the non-neuropathic, the most common form, with enlargements of liver and spleen, anemia and skeletal weaknesses; 2) the acute infantile neuropathic, that is revealed after 6 months from the birth with a progressive brain damage, the enlargement of liver and spleen and spasticity; 3) the chronic neuropathic, characterized by progressive neurologic damages (arising also in adulthood), anemia, enlargement of liver and spleen and respiratory problems.

There is not a cure for this disease, but some therapies have been developed to overcome the deficit of the enzyme capacity, in particular: 1) the direct administration of enzyme, 2) the bone marrow replacement, 3) the gene delivery and 4) the substrate deprivation (inhibiting the synthesis of glycosphingolipids).

The enzyme replacement therapy can be successful with some forms of the disorder. In particular, the size of the liver and the spleen can be decreased with an enzyme replacement treatment, but this is really expensive and life-long lasting. The research for new drugs is hampered by the rarity of the disease and high costs.

1.2.3. EMPLOYMENT OF *N*-SUBSTITUTED IMINOSUGARS IN GAUCHER DISEASE THERAPY

The commercially available drugs for the Gaucher disease therapy are recombinant glucocerebrosidase, and are Ceredase, Imiglucerase, Velaglucerase, Taliglucerase alfa and

Eliglustat, approved from 1991 to 2014 by FDA. NB-DNJ (**XXIII**), previously developed as anti-HIV agent, was approved after multiple clinical studies for the substrate reduction therapy against type I Gaucher disease thanks to its low toxicity, proving less expensive than enzyme replacement treatment. **XXIII** prevents accumulation of glucosylceramide (GlcCer) inhibiting its glucosyltransferase-mediated biosynthesis. Tests on Tay-Sachs mice showed that the treatment with NB-DNJ could prevent the accumulation of GM2 ganglioside in the brain[32], while the same treatment on healthy mice did not cause diseases, the only side effect being the disappearance of GSL in many organs[33].

Unfortunately, the lack of information about the enzyme structure and its binding sites makes the research of new GCS inhibitors more complicated. For this reason, a large number of DNJ derivatives, mimicking the ceramide substrate in the GCS active site, have been synthetized to be tested for their therapeutic potential on different diseases. The increase of the chain length led to stronger inhibitions toward lysosomal β-glucosidase (GCase), perhaps for of a better mimicking of ceramide. Substituents at C-1 and C-6 led to a loss of transferase inhibition, while the presence of the hydroxyl at C-4 is fundamental for the bioactivity. These results highlight the importance of three chiral centers for the mimicking and the potency[34] (**Figure 1.7**).

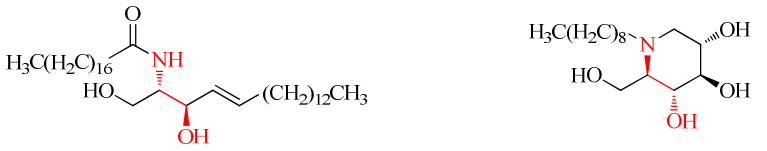
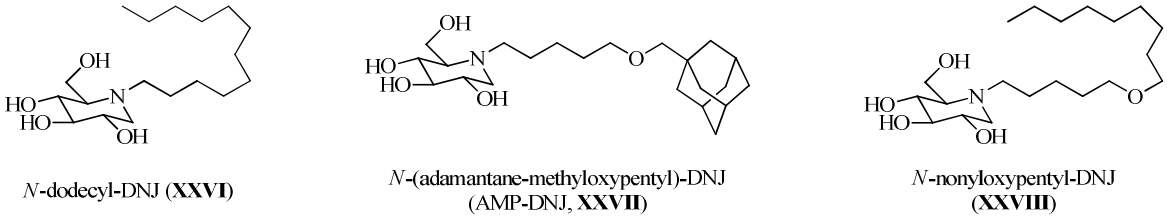


Figure 1.7: Mimicking of active moieties of ceramide.

N-nonyl-DNJ (**XXIV**) is a good inhibitor for GCase with an IC_{50} = 1 μM; *N*-decyl and *N*-dodecyl-DNJ (**XXVI**, **Figure 1.7**) are predominantly inhibitory. In 1993, a second generation of iminosugar inhibitors has been reported; these can inhibit glucosylceramide[35], and include *N*-(5-adamantane-1-yl-methoxypentyl)-DNJ (AMP-DNJ, **XXVII**), a very powerful inhibitor of non-lysosomal and membrane glucosylceramidases (IC_{50} = 1.7 and 48 nM respectively). In the inhibition of glucosylceramide synthase, **XXVI** is 100 times more potent than **XXIII** and, considering the important role of GSLs in many other diseases, it may find several different therapeutic applications. In fact, the administration of **XXVI** to mice with hapten-induced ulcerative colitis resulted in beneficial anti-inflammatory responses[36]. A recent study on a large number of *N*-substituted DNJ and L-*ido*-DNJ[37] indicated that the highest GCS, GBA1 and GBA2

inhibitory activity was showed by *N*-nonyloxypentyl-DNJ (**XXVIII**) and its L-epimer, suggesting their employment in lysosomal storage disorders, in particular Gaucher disease.



N-dodecyl-DNJ (**XXVI**)

N-(adamantane-methyloxypentyl)-DNJ (AMP-DNJ, **XXVII**)

N-nonyloxypentyl-DNJ (**XXVIII**)

Figure 1.7: Active *N*-substituted DNJ derivatives.

1.3. CONCLUSIONS AND FUTURE PERSPECTIVES

Iminosugars are potent inhibitors of α- and β-glucosidases, and have been approved by FDA as antidiabetic and antiviral agents. More recently, a new use of iminosugars, in particular if *N*-substituted, has been proposed: considering the fundamental role of glycosidases and glycosyltransferases in carbohydrate metabolism, in particular in glycosphingolipid catabolism, these inhibitors can be used as therapeutic agents in lysosomal storage disorders, in particular in type I Gaucher disease.

The *N*-alkylated iminosugars potentially can be tested in further diseases involving carbohydrates: their substrate mimicking attitude can be refined with the employment of L-iminosugars, that in several cases showed a higher activity than the corresponding D-enantiomers.

2. OBJECTIVES AND SYNTHETIC STRATEGY

This section of the thesis was addressed to the synthesis of both the enantiomers of the *N*-(5-adamantane-1-yl-methoxypentyl)-DNJ and the *N*-nonyloxypentyl-DNJ with the development of a convenient synthetic route. These DNJ derivatives will be tested for their potential biological activity in other lysosomal storage diseases.

The stepwise strategy can be summarized in a few critical points:

- The construction of a common precursor constituted by a protected pentanediol (**Figure 2.1**).

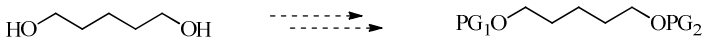


Figure 2.1: Construction of protected pentanediol.

- The coupling of the common precursor with adamantane-methanol and nonanol, and subsequent oxidation to aldehyde of the terminal hydroxyl group in the alkyl chain (**Figure 2.2**).

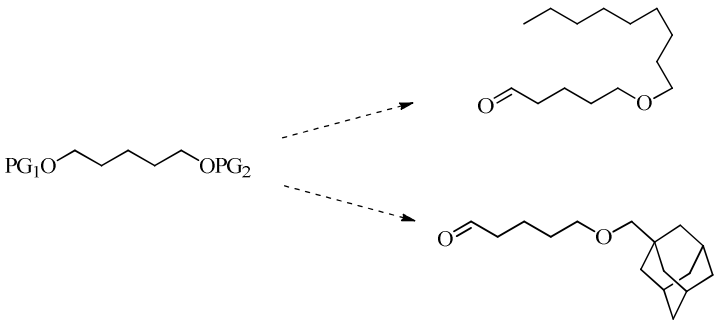


Figure 2.2: Coupling of protected pentanediol and oxidation.

- The final coupling between the two obtained aldehydes with the previously synthetized D- and L-DNJ (**Figure 2.3**).

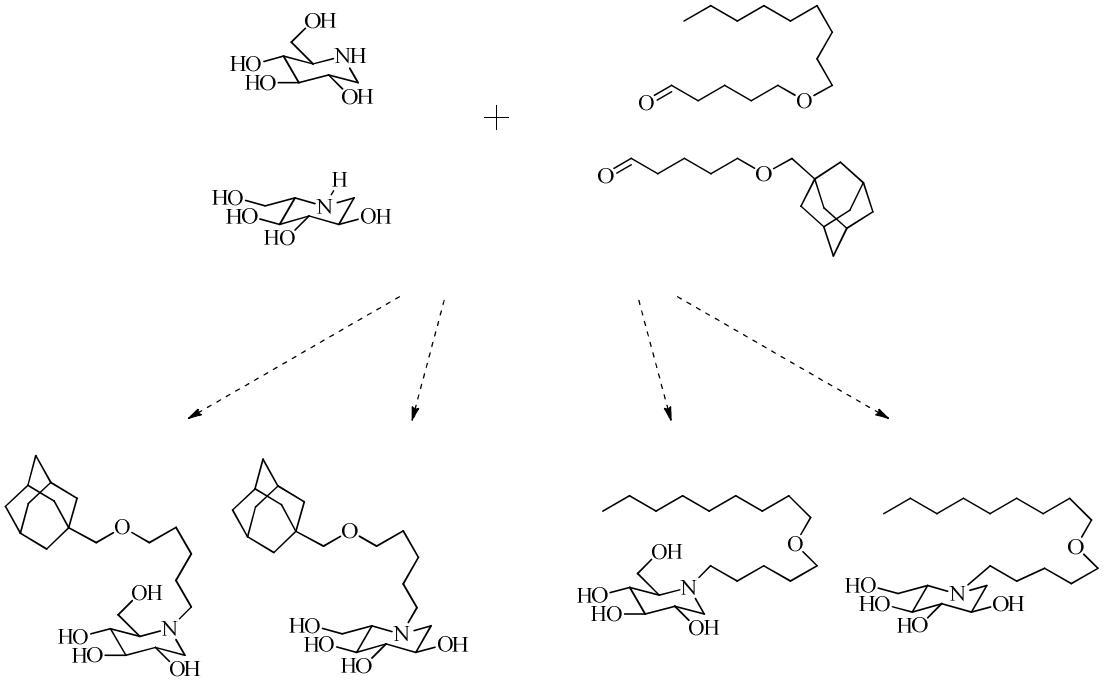
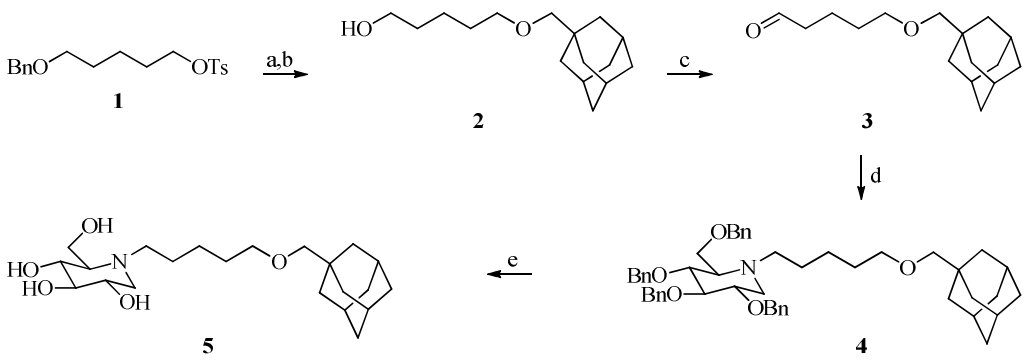


Figure 2.3: Final coupling to obtain D- and L- AMP-DNJ and *N*-nonyloxypentyl-DNJ.

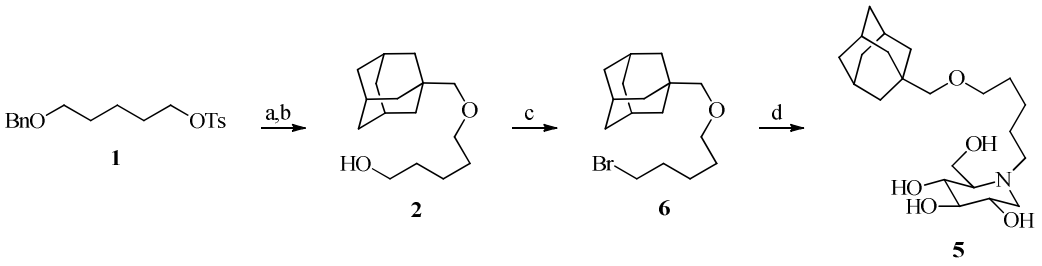
3. RESULTS AND DISCUSSION

The syntheses of *N*-(5-adamantane-1-yl-methoxypentyl)-DNJ[38] and *N*-nonyloxypentyl-DNJ[36] have been already accomplished and described in literature. The synthetic route to AMP-DNJ is reported in **Scheme 3.1**:



Scheme 3.1: (a) Adamantane-methanol, NaH; (b) Pd/C, H_2; (c) DMSO, $(COCl)_2$, TEA; (d) tetra-Bn-DNJ, Pd/C, H_2, AcOH; (e) Pd/C, H_2, HCl.

The pathway to *N*-nonyloxypentyl-DNJ is described in **Scheme 3.2**:



Scheme 3.2: (a) Adamantane-methanol, NaH; (b) Pd/C, H_2; (c) PPh_3, CBr_4; (d) DNJ, K_2CO_3.

To avoid the demanding reaction conditions required for the multiple hydrogenolytic de-*O*-benzylations (**Scheme 3.1**) and the coupling between the alkyl bromide and the DNJ (80 °C for 16 h, **Scheme 3.2**), the synthetic route developed in this thesis contemplates the use of *p*-

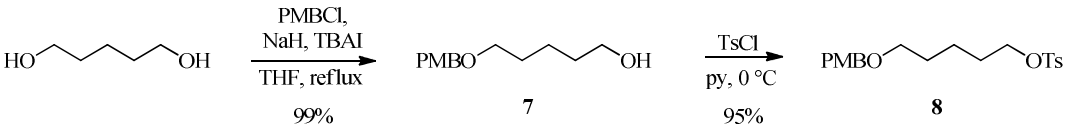
methoxybenzyl as a transient protecting group for pentanediol, in combination with a tosyl leaving group.

3.1. CONSTRUCTION OF THE PROTECTED COMMON PRECURSOR

The former explored strategy to obtain **2** involved a two-step sequence already described in literature[39]; in the first step, the adamantane-methanol reacted with 3,4-dihydro-2*H*-pyran in presence of *p*-toluenesulfonic acid to give a pyran adduct undergoing, in the second step, a reductive opening upon exposure to BH_3 in THF. Unfortunately the second step met with failure, probably because in the literature is reported the reaction with the borane tetrahydrofuran complex stabilized with *N*-isopropyl-*N*-methyl-*tert*-butylamine, a reactive no more commercially available. Indeed, addition of DIPEA to the reaction mixture did not give results.

The latter explored route involved the strategy described in **Scheme 3.1**, with the combined use of the *p*-methoxybenzyl and the tosyl group in the derivatization of starting pentanediol.

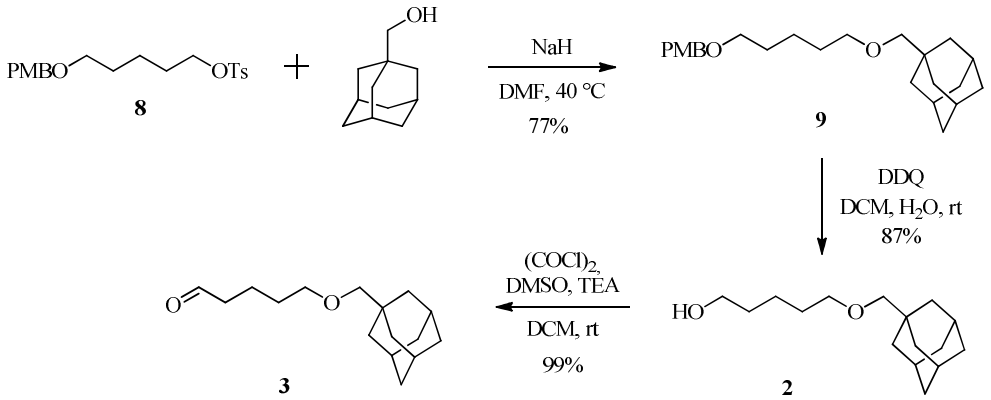
This latter was sequentially protected in excellent yield with a *p*-methoxybenzyl (to give **7**) and a tosyl group (to give **8**) (**Scheme 3.3**). Use of a large stoichiometric excess of pentanediol over PMBCl ensured the protection of only one hydroxyl group.



Scheme 3.3: Synthesis of common hydrophobic intermediate **8**.

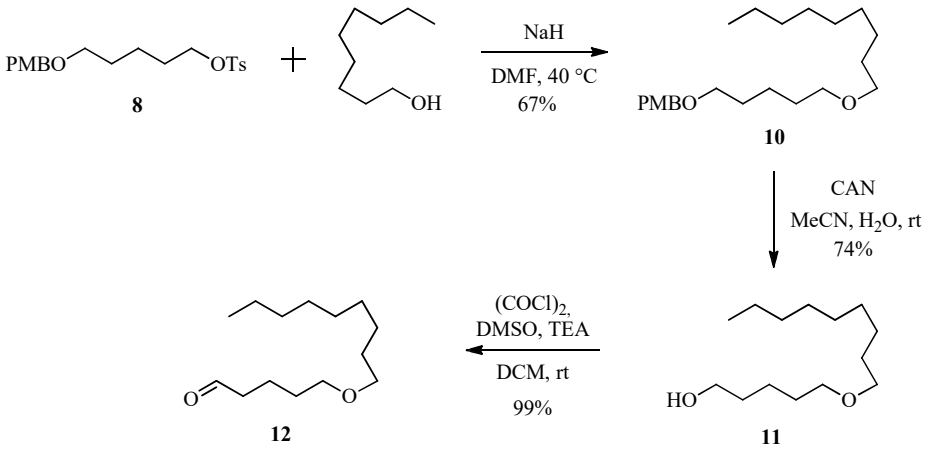
3.2. SYNTHESES OF ADAMANTANE- AND NONYL-ALDEHYDES

Coupling of **8** and adamantane-methanol, activated by NaH, was carried out under highly anhydrous conditions to obtain ether **9**. The radical deprotection with DDQ afforded alcohol **2**, which was oxidized almost quantitatively to aldehyde **3** under Swern conditions (**Scheme 3.4**).



Scheme 3.4: Synthesis of adamantane aldehyde **3**.

Compound **8** was also coupled with nonanol to obtain the precursors of *N*-nonyloxypentyl-DNJ. As with **9**, ether **10** was exposed to DDQ in order to remove the PMB protecting group, but the radical reaction led to the cleavage of the internal ether moiety, giving nonanol and other byproducts. TFA was also unsuccessful for the purpose, whereas ceric ammonium nitrate (CAN) eventually afforded the desired alcohol **11**. This latter intermediate **11** was then oxidized to aldehyde **12**, again under Swern conditions (**Scheme 3.5**).

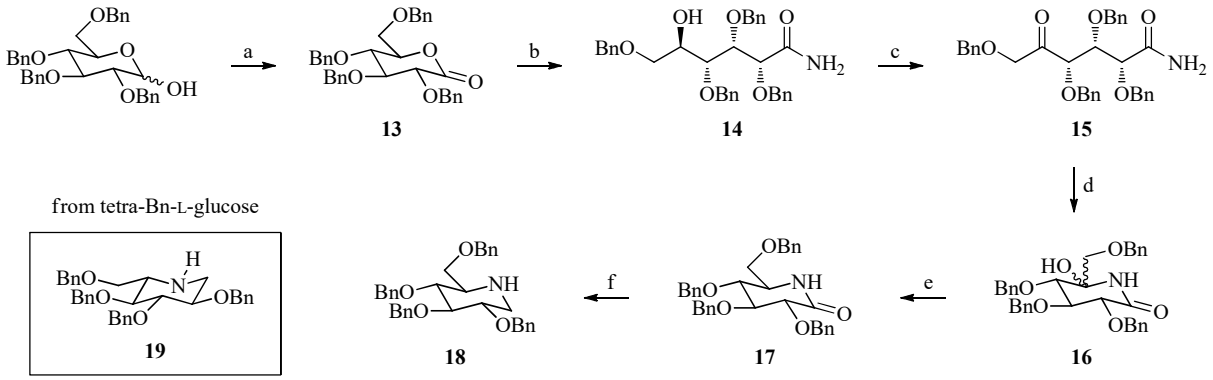


Scheme 3.5: Synthesis of nonyl aldehyde **12**.

3.3. COUPLING OF ALDEHYDES WITH D- AND L-DNJ

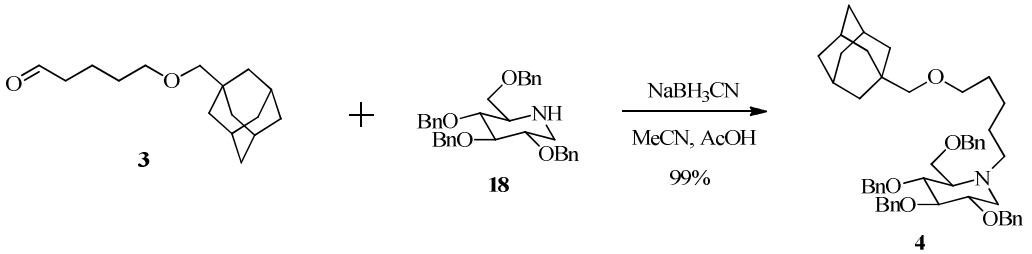
Benzylated DNJ and L-DNJ (**18**, **19**) were synthesized starting from commercially available 2,3,4,5-tetra-*O*-benzyl-D-glucopyranose and 2,3,4,5-tetra-*O*-benzyl-L-glucopyranose, according

to Overkleeft *et al*.[40] by the research group were this section of the thesis was carried out (**Scheme 3.6**).



Scheme 3.6: (a) DMSO, Ac_2O; (b) NH_3/MeOH; (c) DMSO, Ac_2O; (d) NH_3/MeOH; (e) $NaBH_3CN$, HCO_2H; (f) $LiAlH_4$.

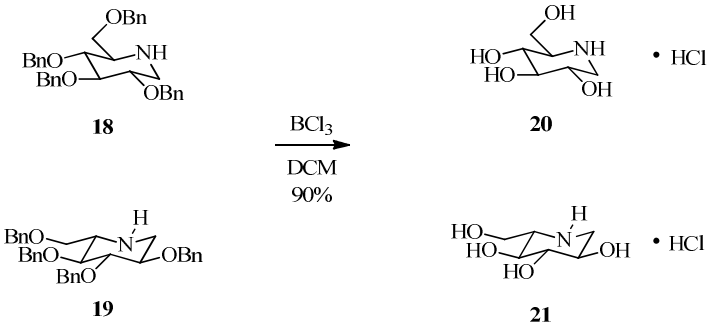
The coupling carried out with benzylated DNJ (**18**) and the adamantane-aldehyde **3** in presence of $NaBH_3CN$ gave the protected *N*-(5-adamantane-1-yl-methoxypentyl)-DNJ with an excellent yield (**Scheme 3.7**).



Scheme 3.7: Construction of protected adamantane-DNJ (**4**).

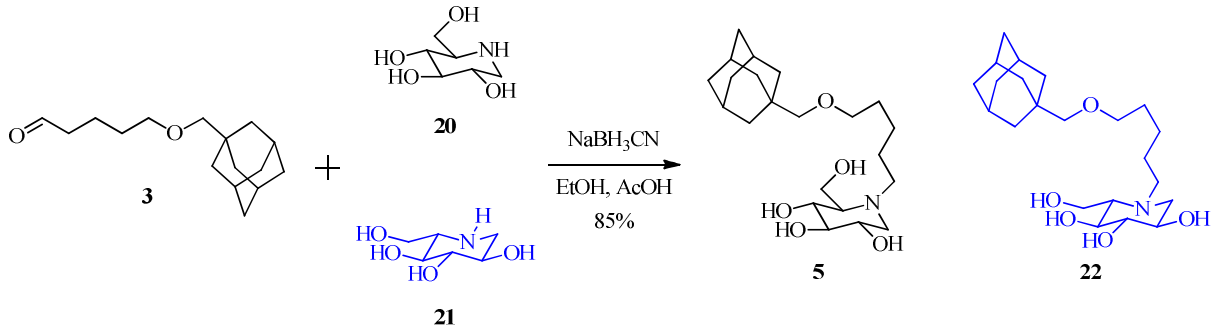
Unfortunately the deprotection of **4** carried out with BCl_3 gave only byproducts. A change of strategy was then necessary, and it was decided to conduct the coupling with D- and L-DNJ after their deprotection.

Indeed, benzylated DNJ and L-DNJ (**18**, **19**) were successfully *O*-deprotected with an excess of BCl_3 (**Scheme 3.8**).



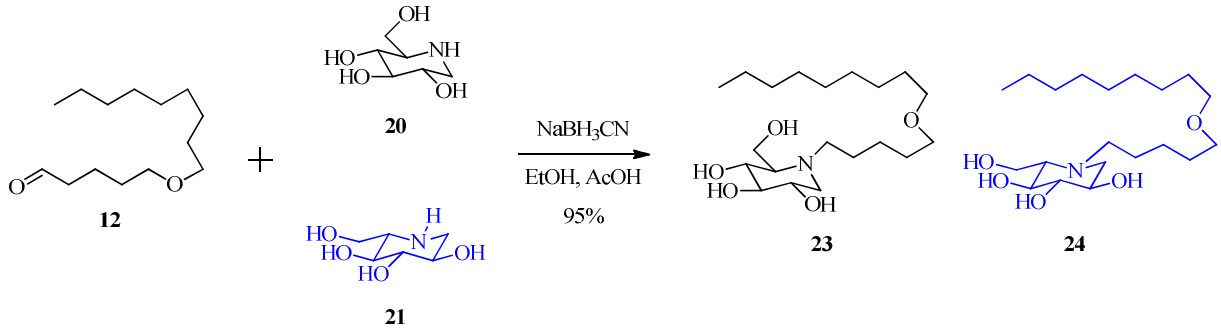
Scheme 3.8: Syntheses of DNJ (**21**) and L-DNJ (**24**).

The coupling between aldehyde **3** and either the DNJ enantiomers was carried out under mildly acidic conditions in presence of $NaBH_3CN$, to provide products **5** and **22** (**Scheme 3.9**).



Scheme 3.9: Synthesis of the final products **5** and **22**.

Final reductive amination between aldehyde **12** and either D- and L-DNJ (yielding **23** and **24**) was carried out as with the adamantane derivative (**Scheme 3.10**).



Scheme 3.10. Synthesis of final products **23** and **24**.

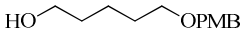
3.4. CONCLUSIONS AND FUTURE PERSPECTIVES

An efficient synthetic route to *N*-(5-adamantane-1-yl-methoxypentyl)-DNJ and *N*-nonyloxypentyl-DNJ and their enantiomers was accomplished, involving the construction of a common intermediate for the hydrophobic moiety. This pathway avoided the demanding reaction conditions required in previous described routes[36,38], for example in the multiple hydrogenolytic de-O-benzylation of the iminosugar moiety or the long-lasting coupling between DNJ and a suitable alkyl bromide. The obtained products will be tested for their biological activity.

4. EXPERIMENTAL SECTION

All air sensitive manipulations were carried out under dry nitrogen atmosphere. Solvents and all standard reagents were purchased from Sigma Aldrich, Alfa Aesar of VWR and were used without further purification.

Analytical thin-layer chromatography was performed on SiO_2 (Merck silica gel 60 F_{254}), and the spots were located with ultraviolet radiation, iodine vapor and chromic mixture. Chromatography refers to flash chromatography and was carried out on SiO_2 (Merck Kieselgel 60, 230-400 mesh). Drying of organic extracts during workup of reactions was performed over anhydrous Na_2SO_4. Evaporation of solvent was accomplished with a rotary evaporator. NMR spectra were recorded at 400 MHz (^1H) and 100.6 MHz (^{13}C) (Bruker DRX, Bruker Avance) and chemical shifts are reported in δvalues downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, integrated intensity, multiplicity, coupling constant (*J*) in Hertz (Hz), and assignment (when possible).

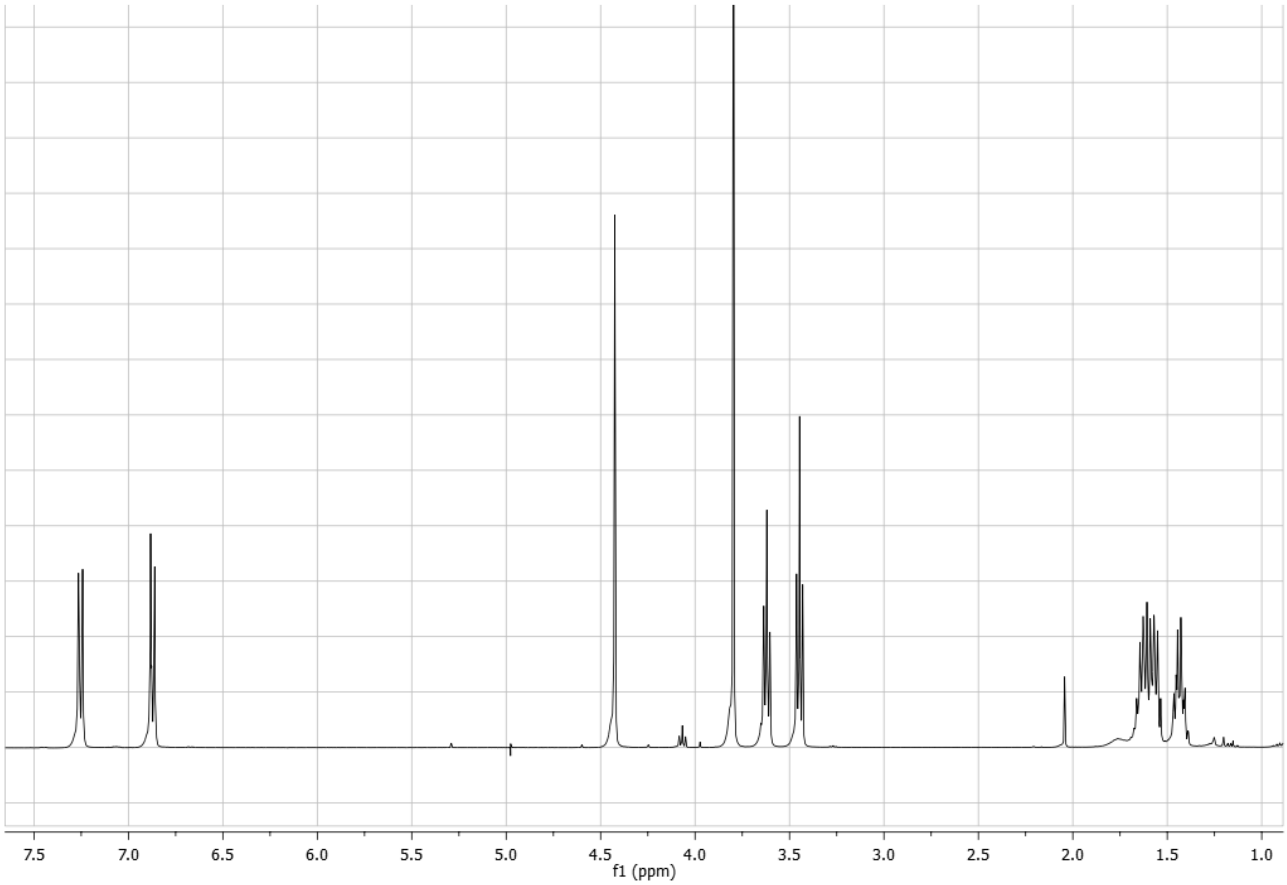


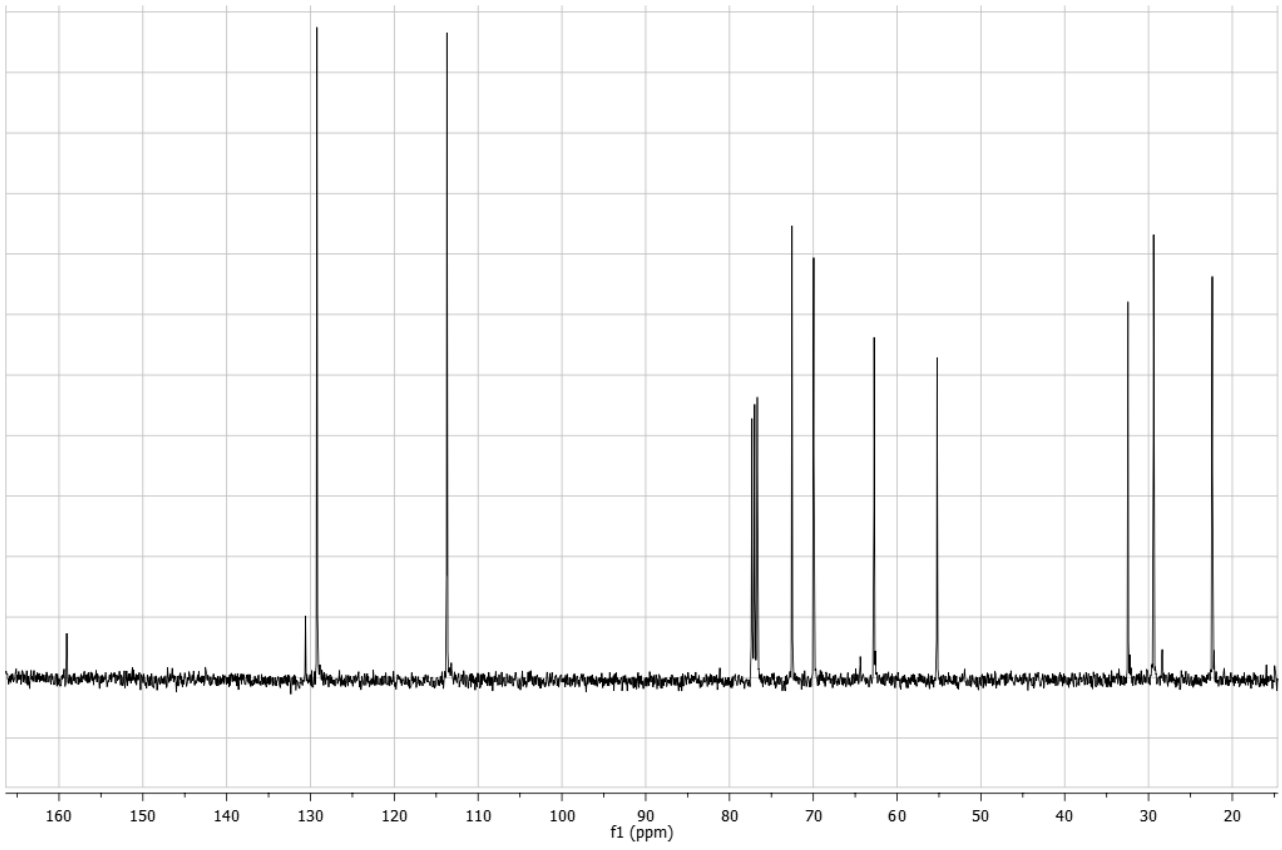
5-((4-Methoxybenzyl)oxy)pentan-1-ol (7).

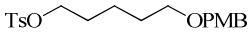
To a stirred suspension of NaH (376 mg, 9.40 mmol, 60% dispersion in mineral oil) in THF (13.5 ml) under argon at 0 °C was added via cannula a solution of 1,5-pentanediol (3.0 ml, 28.6 mmol) in THF (4.5 ml). The reaction was heated to reflux for 3 h, then allowed to cool to rt. TBAI (79 mg, 0.214 mmol) and PMBCl (591 µl, 4.27 mmol) were added, and the mixture was again heated to reflux for 12 h. Water was then added, and the crude was extracted with EtOAc. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Flash chromatography (7:3 hexane-EtOAc) of the residue afforded **7** (949 mg, 99%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41-1.47 (m, 2H, H-3), 1.54-1.67 (m, 4H, H-2, H-4), 3.45 (t, 2H, *J* = 6.5 Hz, H-5), 3.62 (t, *J* = 6.4 Hz, 2H, H-1), 3.80 (s, 3H, OCH_3), 4.42 (s, 2H, C*H*$_2$-Ar), 6.87 (d, *J* = 8.7 Hz, 2H, H-*m* Ar), 7.25 (d, *J* = 8.7 Hz, 2H, H-*o* Ar).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 22.4 (C-3), 29.6 (C-4), 29.4 (C-2), 55.2 (O*C*H$_3$), 62.7 (C-1), 70.0 (C-5), 72.5 (*C*H$_2$-Ar), 113.7 (C-*m* Ar), 129.2 (C-*o* Ar), 130.6 (C-*i* Ar), 159.1 (C-*p* Ar).


7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
f1 (ppm)


160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
f1 (ppm)

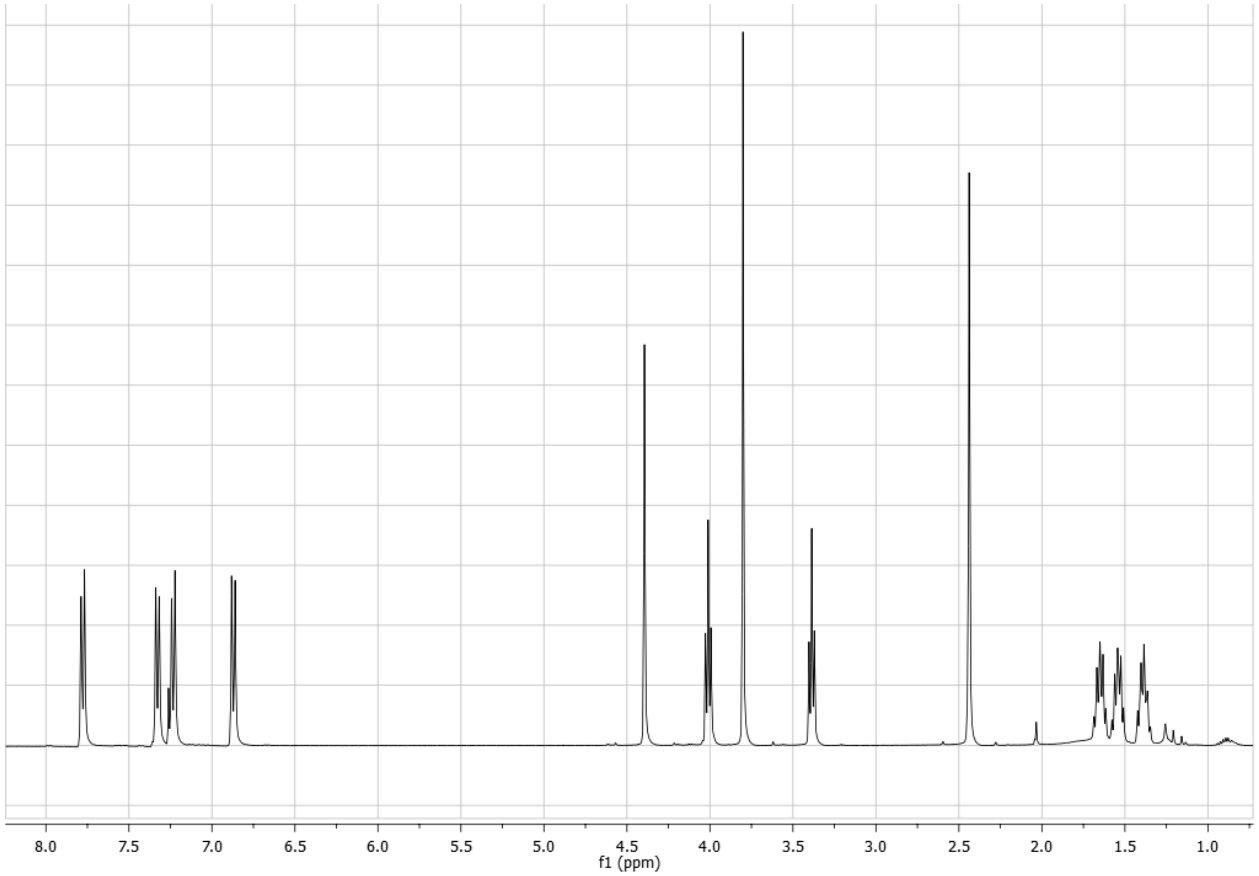


5-((4-Methoxybenzyl)oxy)pentan-1-yl 4-methylbenzenesulfonate (8).

To a cooled (0 °C) solution of **7** (958 mg, 4.27 mmol) in pyridine (30 ml) was added *p*-toluenesulfonyl chloride (1.61 g, 8.45 mmol), and the reaction mixture was stirred at the same temperature overnight. Ethyl acetate and water were added, and the crude was extracted. Combined organic extracts washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (8:2 hexane-EtOAc) of the residue afforded compound **8** (1.535 g, 95%).

^{1}H NMR (400 MHz, $CDCl_3$): δ1.35-1.42 (m, 2H, H-3), 1.51-1.58 (m, 2H, H-4), 1.62-1.69 (m, 2H, H-2), 2.44 (s, 3H, CH_3 Ts), 3.39 (t, *J* = 6.3 Hz, 2H, H-5), 3.80 (s, 3H, OCH_3), 4.01 (t, *J* = 6.5 Hz, 2H, H-1), 4.40 (s, 2H, C*H*$_2$-Ar), 6.87 (d, *J* = 8.5 Hz, 2H, H-*m* PMB), 7.23 (d, *J* = 8.3 Hz, 2H, H-*o* PMB), 7.33 (d, *J* = 8.2 Hz, 2H, H-*m* Ts), 7.78 (d, *J* = 8.1 Hz, 2H, H-*o* Ts).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ21.5 (CH_3 Ts), 22.1 (C-3), 28.5 (C-2), 28.9 (C-4), 55.2 (O*C*H$_3$), 69.5 (C-1), 70.4 (C-5), 72.4 (*C*H$_2$-Ar), 113.7 (C-*m* PMB), 127.8 (C-*o* Ts), 129.1 (C-*o* PMB), 129.8 (C-*m* Ts), 130.4 (C-*i* PMB), 133.0 (C-*p* Ts), 144.6 (C-*i* Ts), 159.0 (C-*p* PMB).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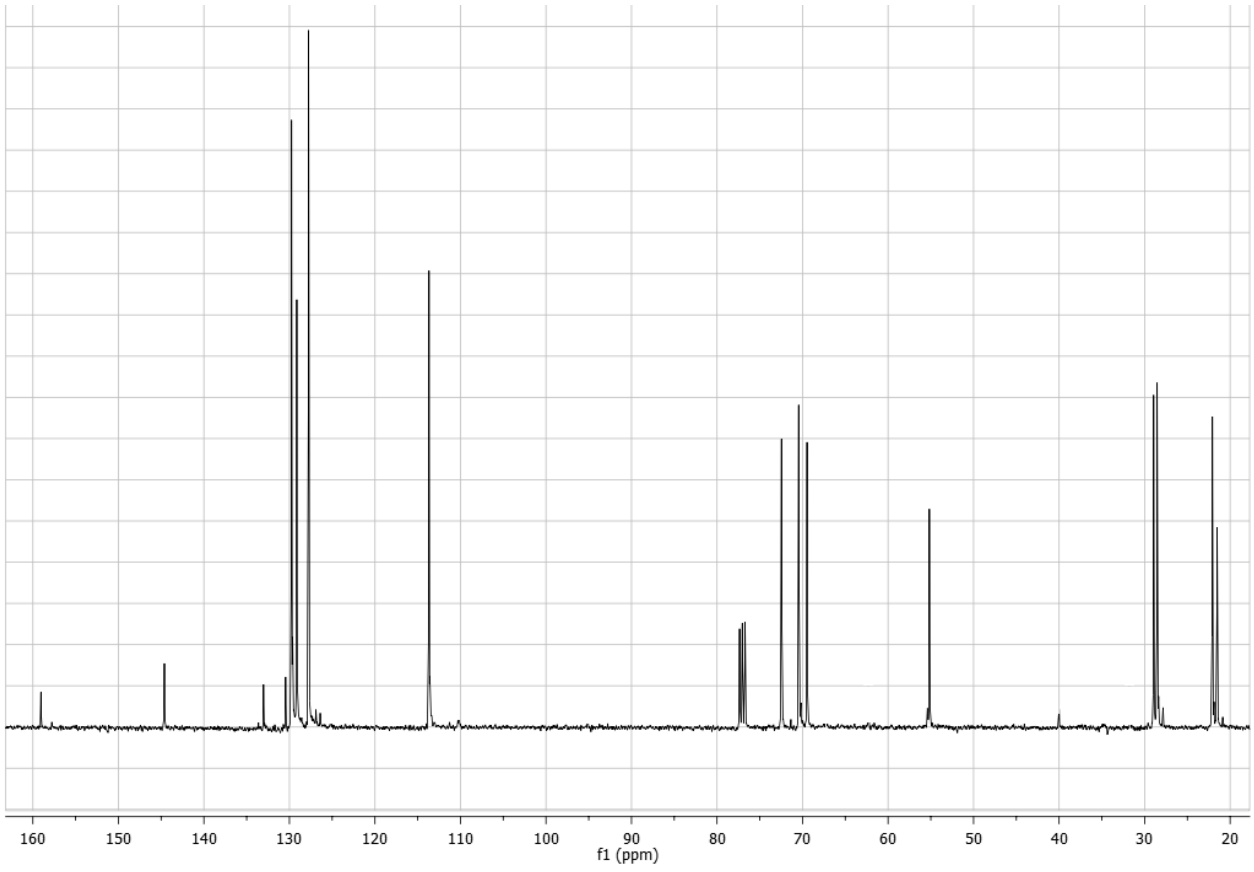
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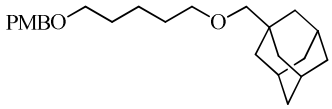
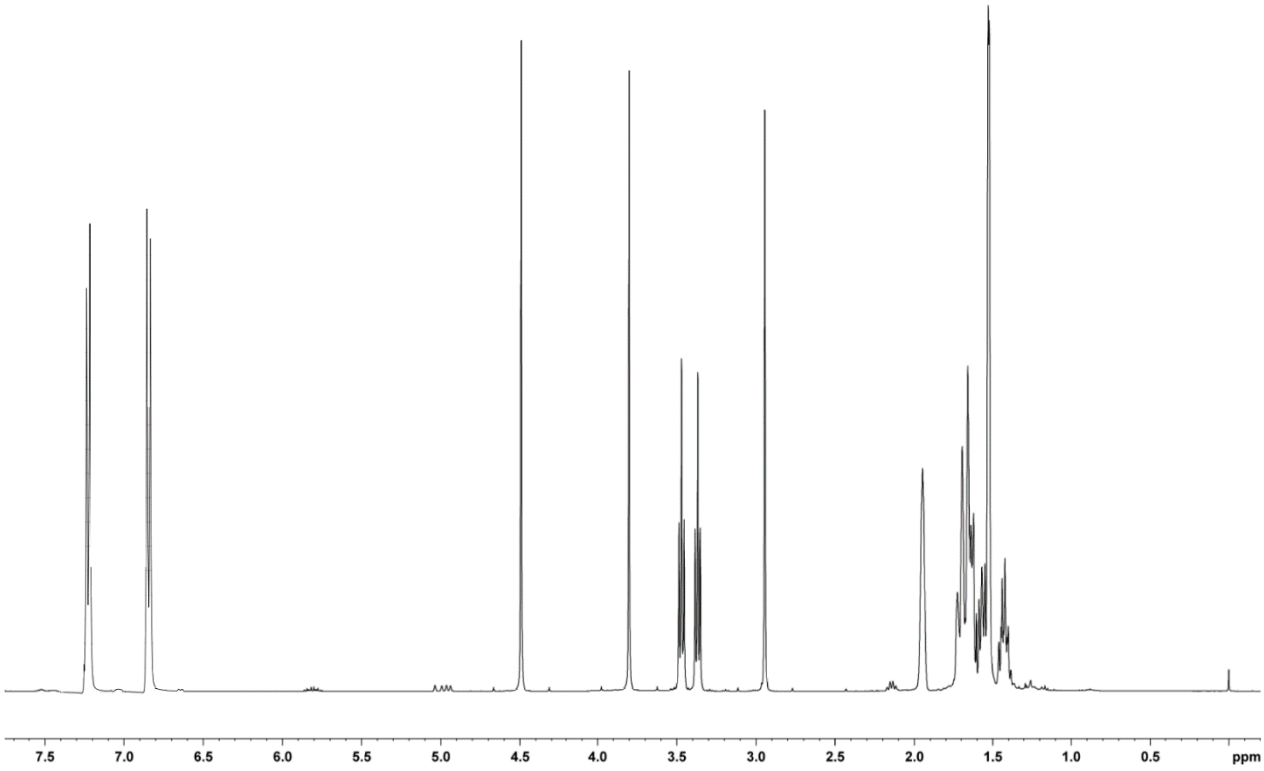


5-(Adamantane-1-yl-methoxy)-1-((4-methoxybenzyl)oxy)pentane (9).

To a dry solution of adamantanemethanol (438 mg, 2.63 mmol) in DMF (4.5 ml) was added at 0 °C NaH (210 mg, 5.26 mmol, 60% dispersion in mineral oil), and the mixture was heated to 40 °C for one hour. Next, a dry solution of tosylate **8** (829 mg, 2.19 mmol) in DMF (1.5 ml) was added to the reaction and the mixture was stirred at the same temperature overnight. The reaction was then quenched at 0 °C with MeOH and extracted with EtOAc. The combined organic layers were dried (Na_2SO_4), filtered, concentrated under reduced pressure and the resulting residue was purified by flash chromatography (97:3 hexane-EtOAc) to furnish **9** (626 mg, 77%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.39-1.47 (m, 2H, H-3), 1.52 (br d, *J* = 2.4 Hz, 6H, H adamantane), 1.55-1.60 (m, 2H, H adamantane), 1.62-1.72 (m, 8H, H-2, H-4, H adamantane), 1.95 (br s, 3H, H adamantane), 2.94 (s, 2H, C*H*$_2$-adamantane), 3.37 (t, *J* = 6.6 Hz, 2H, H-5), 3.47 (t, *J* = 5.8 Hz, 2H, H-1), 3.80 (s, 3H, OCH_3), 4.49 (s, 2H, C*H*$_2$-Ar), 6.87 (d, *J* = 8.7 Hz, 2H, H-*m* Ar), 7.25 (d, *J* = 8.7 Hz, 2H, H-*o* Ar).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 22.8 (C-3), 28.3 (C adamantane), 29.4 (C-4), 29.6 (C-2), 34.0 (C adamantane), 37.2 (C adamantane), 39.7 (C adamantane), 55.3 (O*C*H_3), 70.1 (C-1), 71.9 (C-5), 72.5 (*C*H_2-adamantane), 81.9 (*C*H_2-Ar), 113.7 (C-*m* Ar), 129.2 (C-*o* Ar), 130.7 (C-*i* Ar), 139.0 (C-*p* Ar).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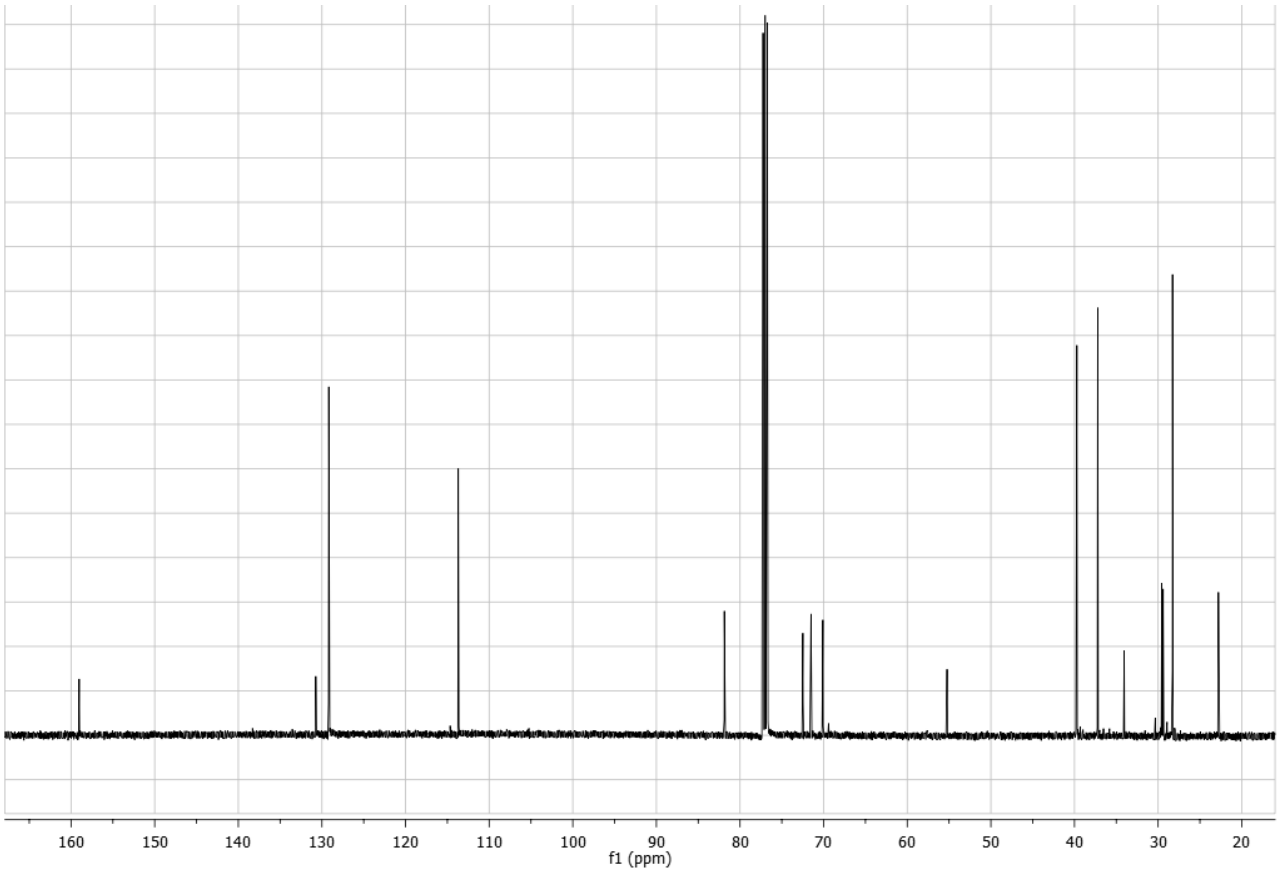
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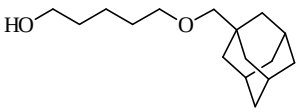
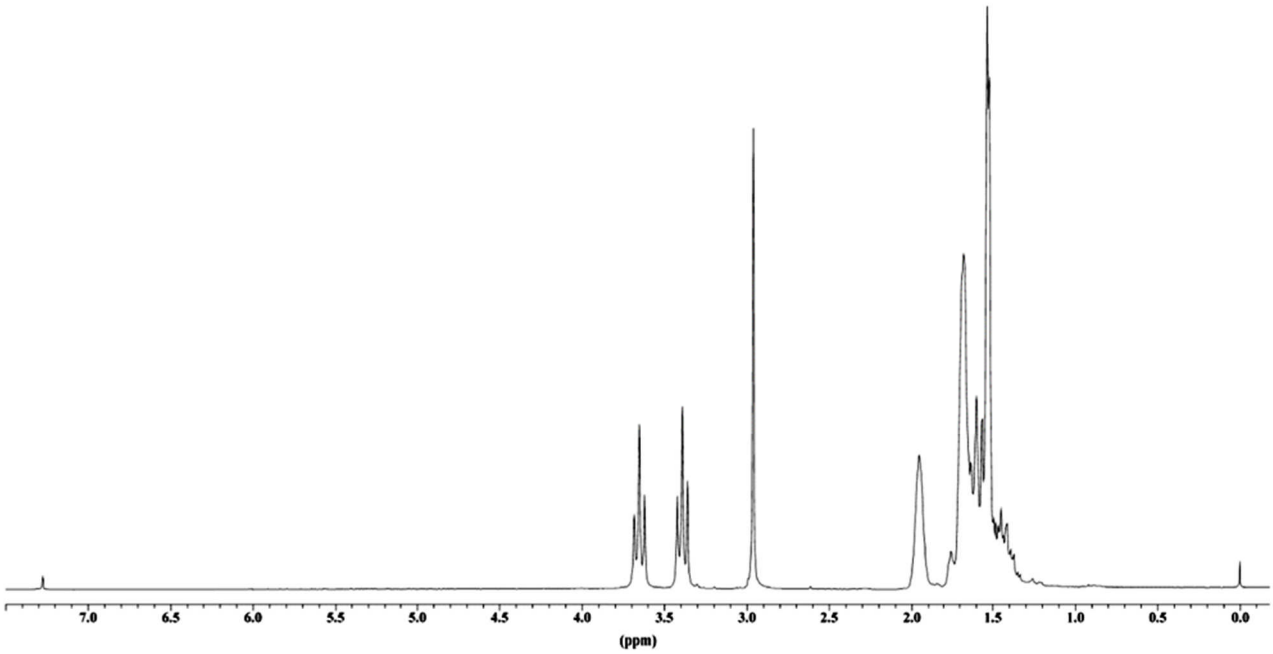


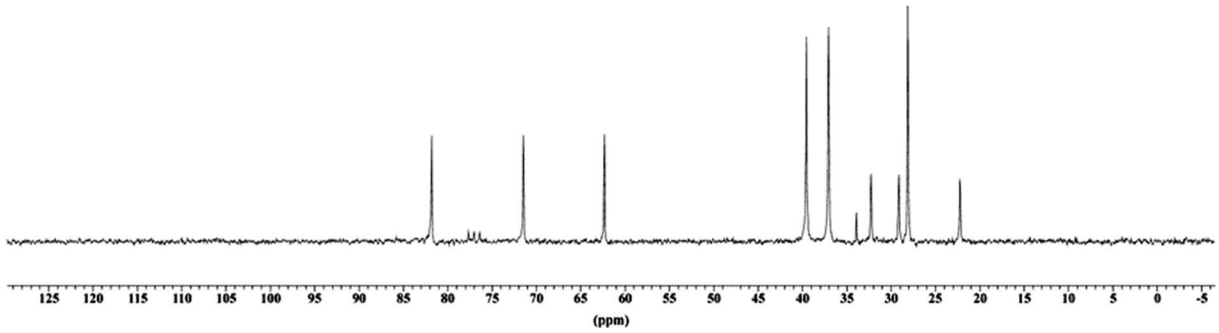
5-(Adamantane-1-yl-methoxy)pentan-1-ol (2).

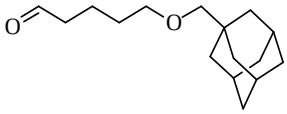
Compound **9** (616 mg, 1.66 mmol) was dissolved in CH_2Cl_2 (36 mL), and water (4 ml) and DDQ (752 mg, 3.31 mmol) were added. The resulting mixture was stirred at rt for 5 hours. The two phases were separated, and the organic layer was washed with water, dried and concentrated under reduced pressure. The crude **5** was purified by flash chromatography (hexane-EtOAc 8:2) to give pure **2** (363 mg, 87%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.41-1.70 (m, 18H, H-2, H-3, H-4, H adamantane), 1.95 (br s, 3H, H adamantane), 2.96 (s, 2H, C*H*$_2$-adamantane), 3.39 (t, *J* = 6.6 Hz, 2H, H-5), 3.65 (t, *J* = 5.8 Hz, 2H, H-1).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 22.2 (C-3), 28.0 (C adamantane), 29.1 (C-4), 32.2 (C adamantane), 33.9 (C-2), 37.0 (C adamantane), 39.5 (C adamantane), 62.3 (C-1), 71.4 (C-5), 81.8 (*C*H$_2$-adamantane).





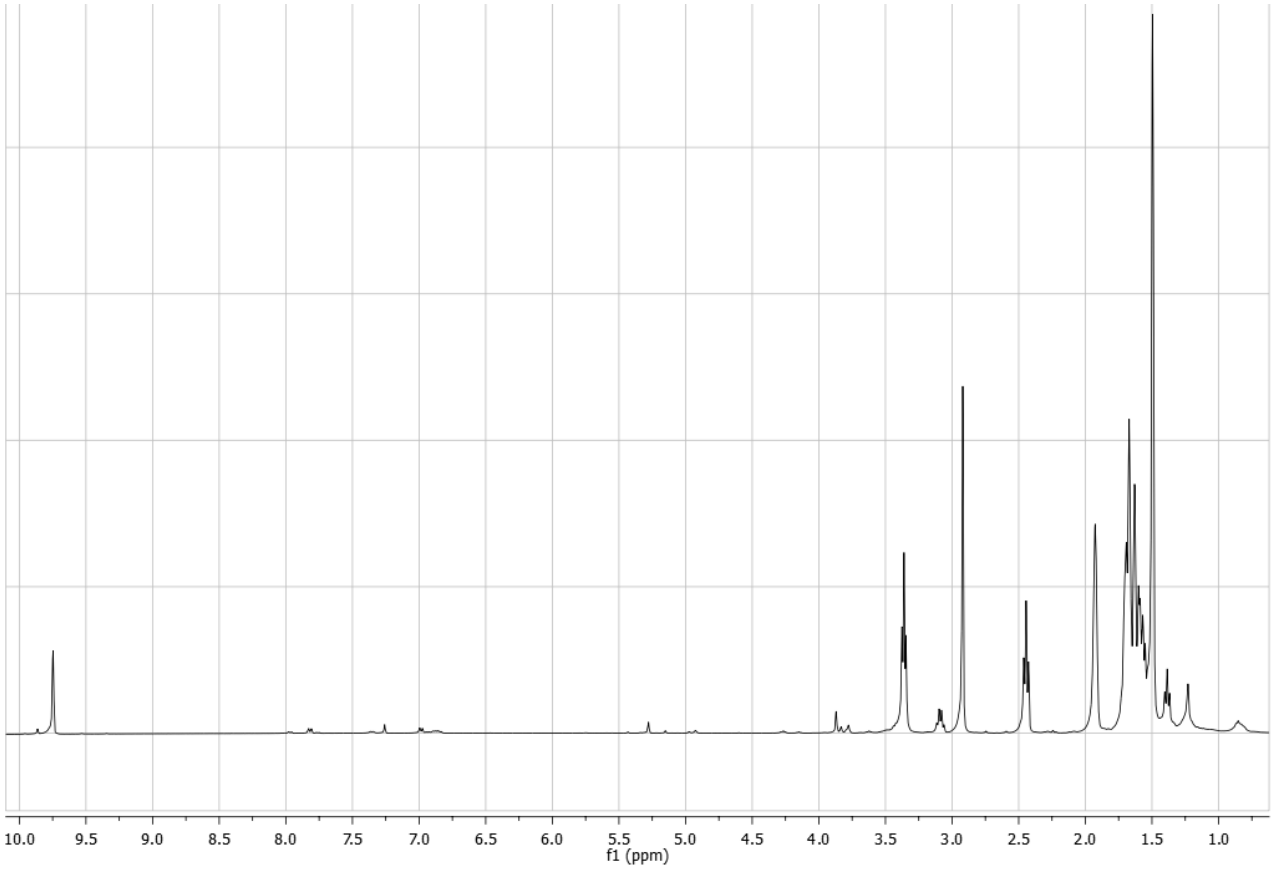


5-(Adamantane-1-yl-methoxy)pentan-1-al (3).

A solution of oxalylchloride (32 µl, 0.375 mmol) in DCM (5 µl) was cooled to –78 °C. After dropwise addition of DMSO (53 µl, 0.750 mmol) in DCM (500 µl), the mixture was stirred for 30 min. A dry solution of **2** (86 mg, 0.341 mmol) in DCM (500 µl) was added dropwise to the reaction at –78 °C. After two hours, TEA (237 µl, 1.70 mmol) was added, and the mixture was allowed to warm to rt over two hours. The reaction was quenched with few drops of aqueous HCl 0.01 M and extracted. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure, affording pure aldehyde **3** (86 mg, 99% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ 1.50 (br s, 6H, H adamantane), 1.55-1.69 (m, 10H, H-3, H-4, H adamantane), 1.93 (br s, 2H, H adamantane), 2.44 (app t, J = 7.1 Hz, 2H, H-2), 2.92 (s, 2H, CH_2-adamantane), 3.36 (t, J = 5.9 Hz, 2H, H-5), 9.75 (s, 1H, H-1).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 18.9 (C-3), 28.2 (C adamantane), 28.9 (C-4), 34.0 (C adamantane), 37.1 (C adamantane), 39.6 (C adamantane), 43.6 (C-2), 70.8 (C-5), 81.9 (*C*H_2-adamantane), 202.8 (C-1).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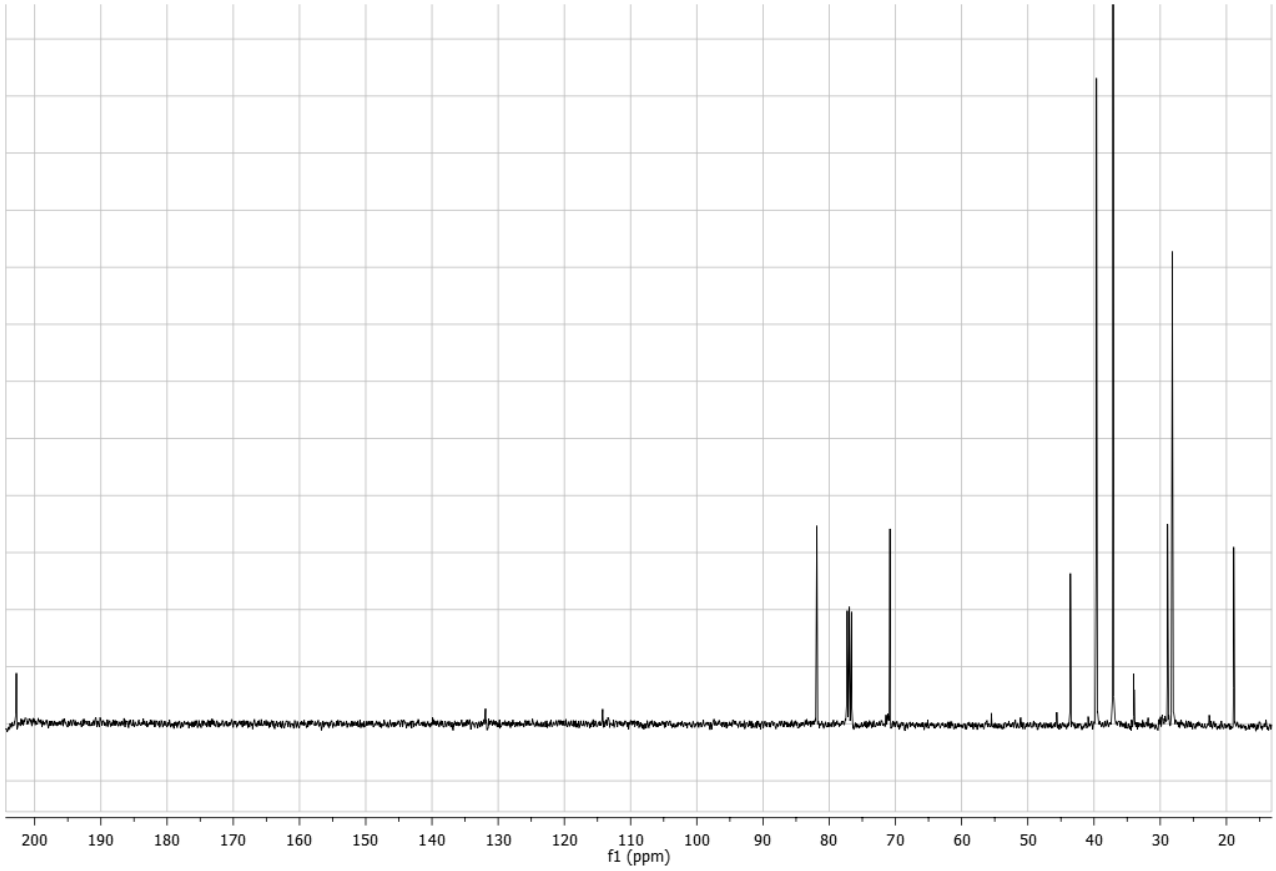
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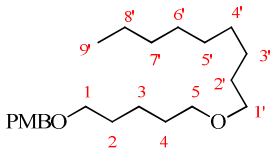
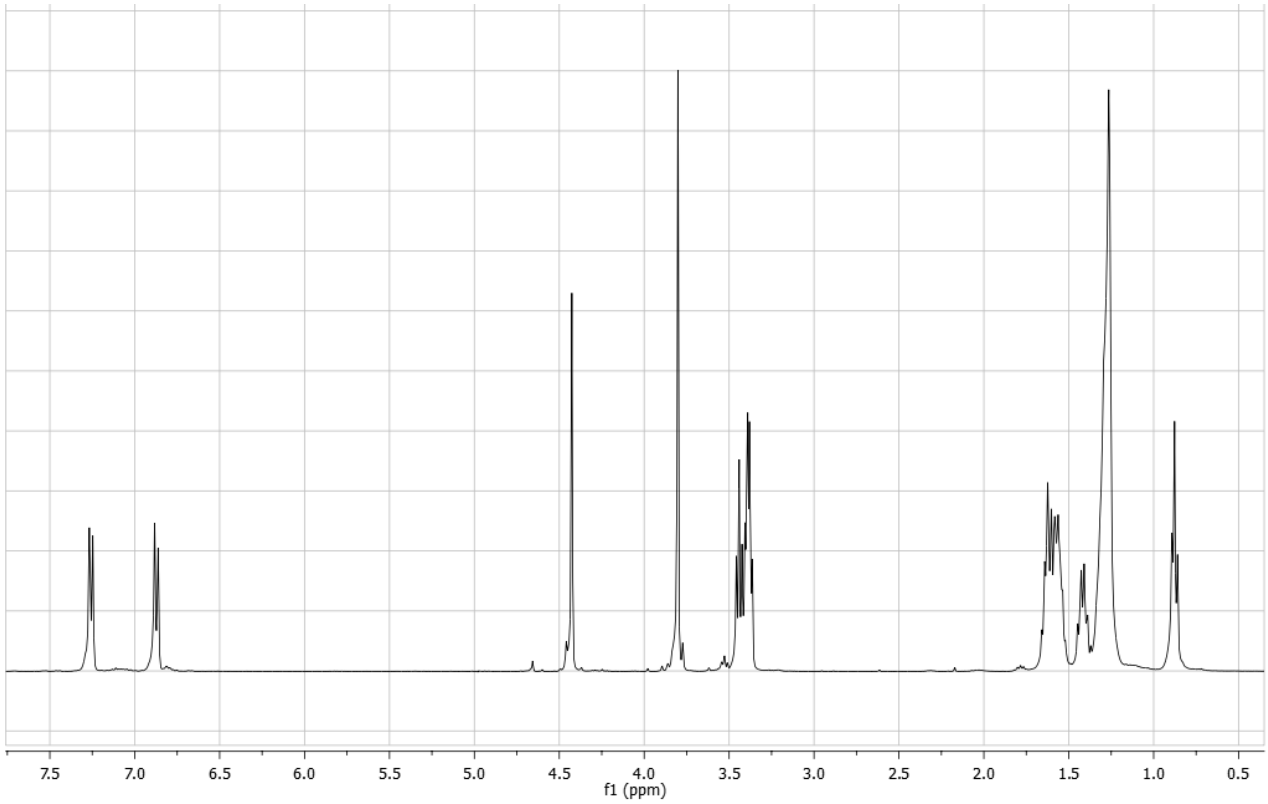


5-(Nonyloxy)-1-((4-methoxybenzyl)oxy)pentane (10).

To a dry solution of nonanol (1.15 ml, 6.59 mmol) in DMF (30 ml) was added at 0 °C NaH (527 mg, 13.9 mmol, 60% dispersion in mineral oil), and the mixture was heated to 50 °C for 3 hour (until the end of the gas evolution). Next, a dry solution of tosylate **8** (1.66 g, 4.39 mmol) in DMF (10 ml) was added to the reaction at 0 °C and the mixture was stirred at the same temperature overnight. The reaction was then quenched with MeOH and concentrated under reduced pressure. The resulting residue was purified by flash chromatography (97:3 hexane-EtOAc) to furnish **10** (1.032 g, 67%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (t, *J* = 6.2 Hz, 3H, H-9'), 1.26 (br s, 10H, H-4', H-5', H-6', H-7', H-8'), 1.39-1.45 (m, 2H, H-3'), 1.54-1.66 (m, 8H, H-2, H-3, H-4, H-2'), 3.36-3.41 (m, 4H, H-5, H-1'), 3.44 (t, *J* = 6.6 Hz, 2H, H-1), 3.80 (s, 3H, OCH_3), 4.43 (s, 2H, C*H*$_2$-Ar), 6.87 (d, *J* = 8.4 Hz, 2H, H-*m* Ar), 7.25 (d, *J* = 8.3 Hz, 2H, H-*o* Ar).

^{13}C NMR (100,6 MHz, $CDCl_3$): δ 14.0 (C-9'), 22.6 (C-3), 22.7 (C-8'), 28.1, 29.2, 29.5, 29.6, 29.7 (C-2, C-4, C-2', C-3', C-4', C-5', C-6'), 31.8 (C-7'), 55.2 (OCH_3), 70.0 (C-1), 70.8 (C-1'), 70.9 (C-5), 72.4 (*C*H_2-Ar), 113.6 (C-*m* Ar), 129.1 (C-*o* Ar), 130.7 (C-*i* Ar), 159.0 (C-*p* Ar).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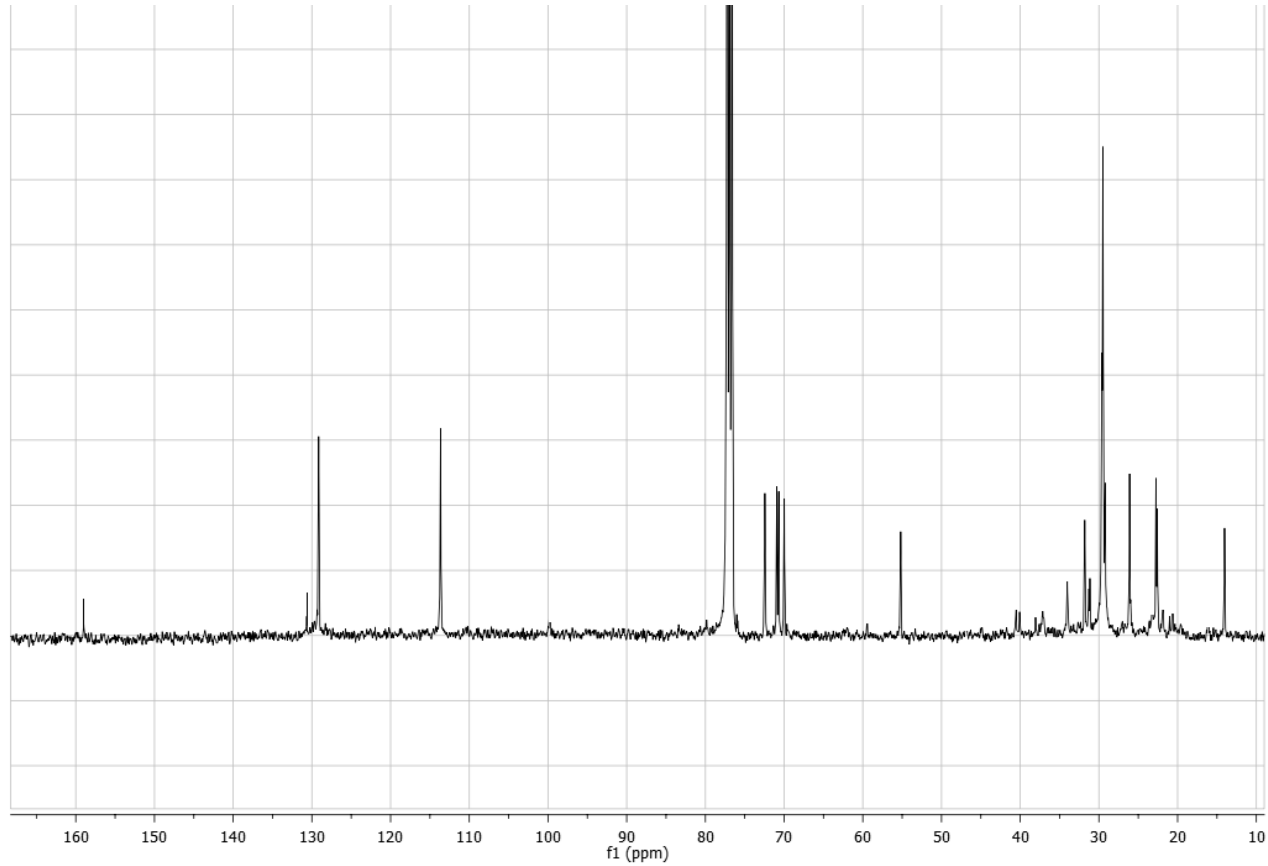
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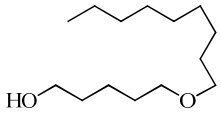
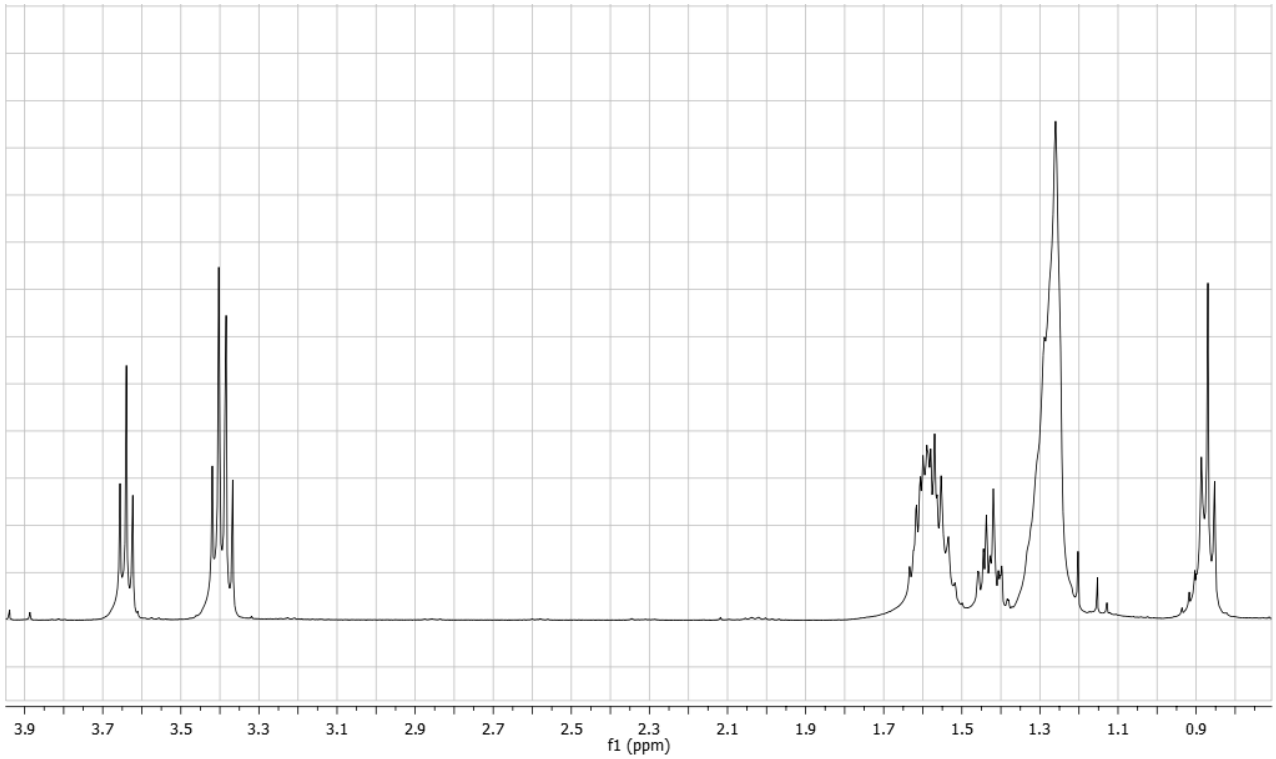


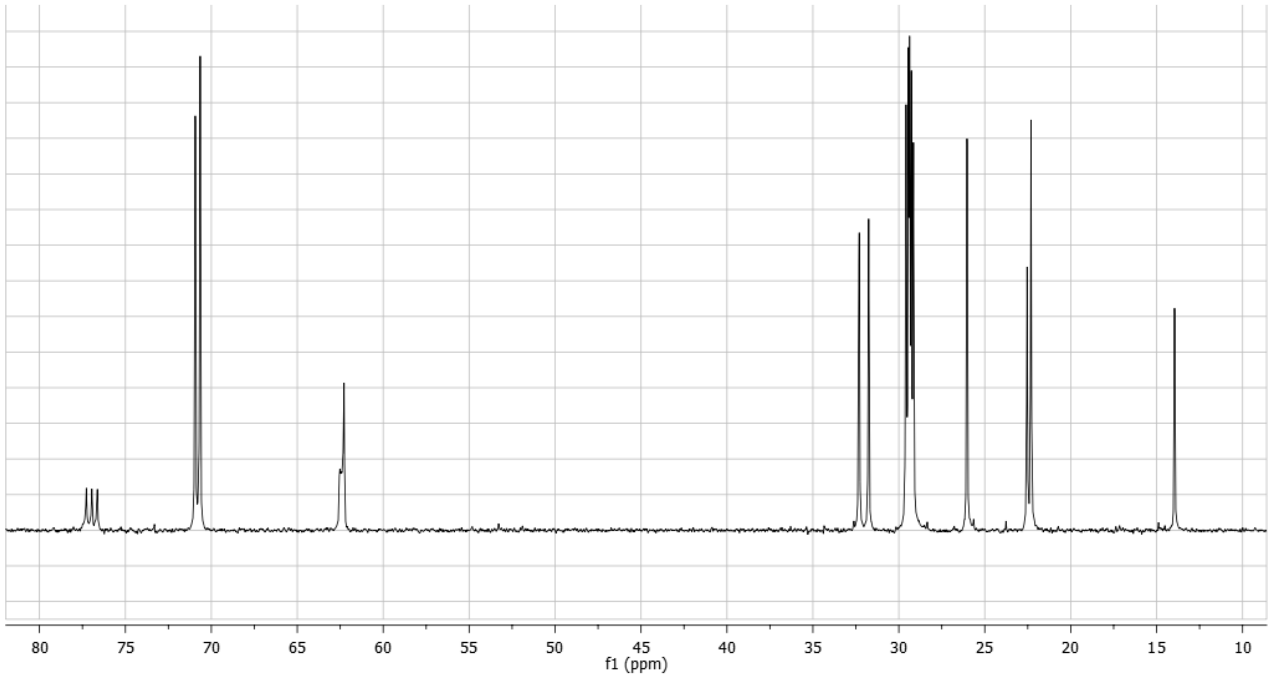
5-(Nonyloxy)pentan-1-ol (11).

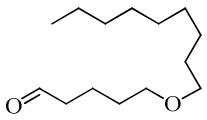
Compound **10** (49 mg, 0.140 mmol) was suspended in a mixture of acetonitrile and water in ratio 9:1 (1.5 ml), and CAN (383 mg, 0.699 mmol) was added. After 30 minutes the reaction mixture was extracted with EtOAc and aqueous $NaHCO_3$. The combined organic extracts were dried, filtered and concentrated under pressure. Flash chromatography (9:1 to 8:2 hexane-EtOAc) of the residue gave the compound **11** (24 mg, 74%).

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.87 (t, *J* = 6.7 Hz, 3H, H-9'), 1.26 (br s, 10H, H-4', H-5', H-6', H-7', H-8'), 1.40-1.45 (m, 2H, H-3'), 1.53-1.62 (m, 8H, H-2, H-3, H-4, H-2'), 3.39 (app q, *J* = 14.2, 6.6 Hz, 4H, H-5, H-1'), 3.64 (t, *J* = 6.5 Hz, 2H, H-1).

^{13}C NMR (100,6 MHz, $CDCl_3$): δ 14.0 (C-9'), 22.3 (C-3), 22.5 (C-8'), 28.0, 29.2-29.6 (C-2, C-4, C-3', C-4', C-5', C-6'), 31.8 (C-2'), 32.3 (C-7'), 62.3 (C-1), 70.6 (C-1'), 70.9 (C-5).





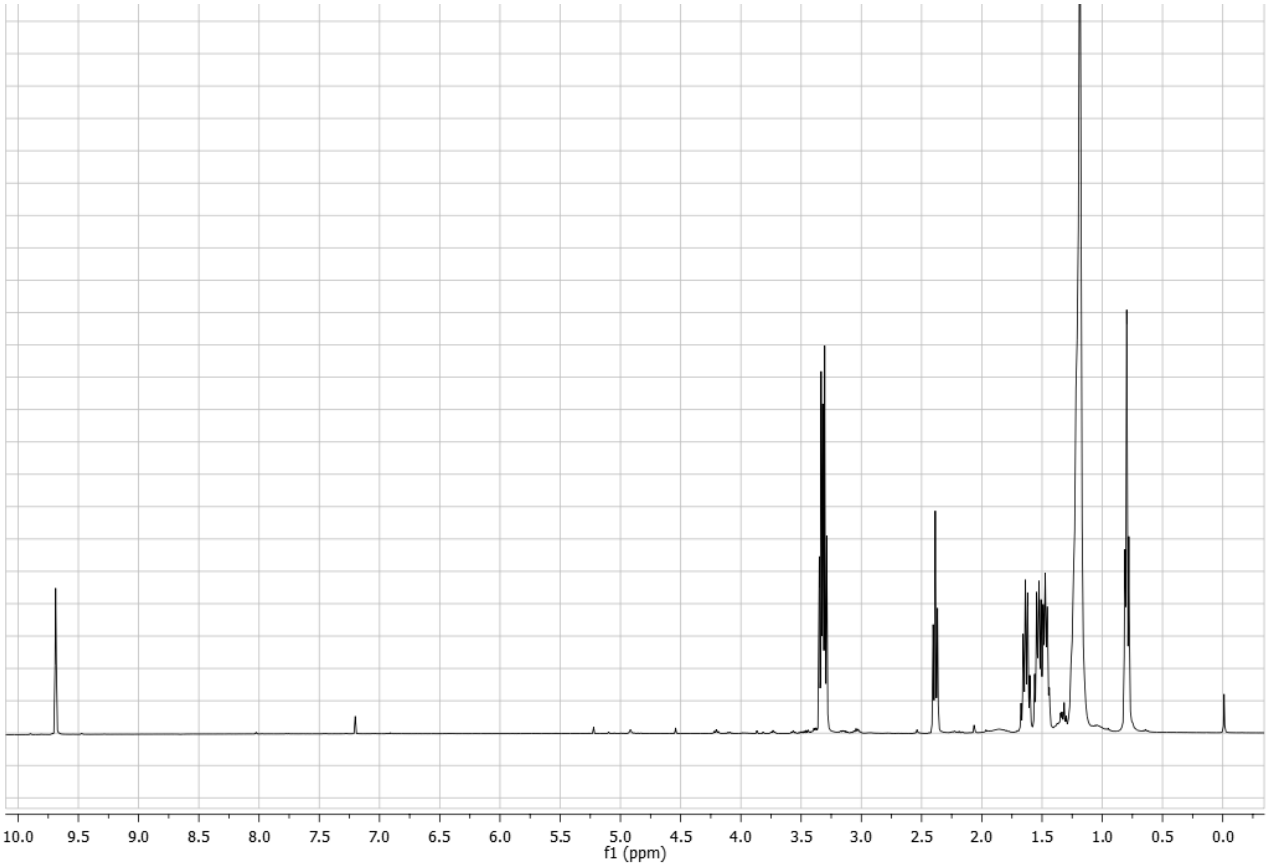


5-(Nonyloxy)pentan-1-al (12).

A solution of oxalylchloride (34 µl, 0.406 mmol) in DCM (10 µl) was cooled to –78 °C. After dropwise addition of DMSO (58 µl, 0.812 mmol) in DCM (500 µl), the mixture was stirred for 30 min. A dry solution of **11** (85 mg, 0.369 mmol) in DCM (500 µl) was added dropwise to the reaction at –78 °C. After two hours, TEA (257 µl, 1.84 mmol) was added, and the mixture was allowed to warm to rt over 90 minutes. The reaction was quenched with few drops of aqueous HCl 0.01 M and extracted. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure, affording pure aldehyde **12** (70 mg, 99% yield).

^{1}H NMR (400 MHz, $CDCl_3$): δ0.80 (t, *J* = 6.2 Hz, 3H, H-9’), 1.19 (br s, 12H, H-3’, H-4’, H-5’, H-6’, H-7’, H-8’), 1.47 (dt, *J* = 13.5, 6.7 Hz, 2H, H-2’), 1.52 (dt, *J* = 14.3, 6.2 Hz, 2H, H-4), 1.64 (dt, *J* = 14.4, 6.9 Hz, 2H, H-3), 2.39 (t, *J* = 7.1 Hz, 2H, H-2), 3.30 (t, *J* = 6.7 Hz, 2H, H-5), 3.33 (t, *J* = 6.3 Hz, 2H, H-1’), 9.69 (s, 1H, H-1).

^{13}C NMR (100,6 MHz, $CDCl_3$): δ14.0 (C-9’), 18.9 (C-3), 22.6 (C-8’), 26.1, 29.0-29.6 (C-4, C-3’, C-4’, C-5’, C-6’, C-7’), 31.8 (C-2’), 43.5 (C-2), 70.1 (C-5), 71.0 (C-1’), 202.5 (C-1).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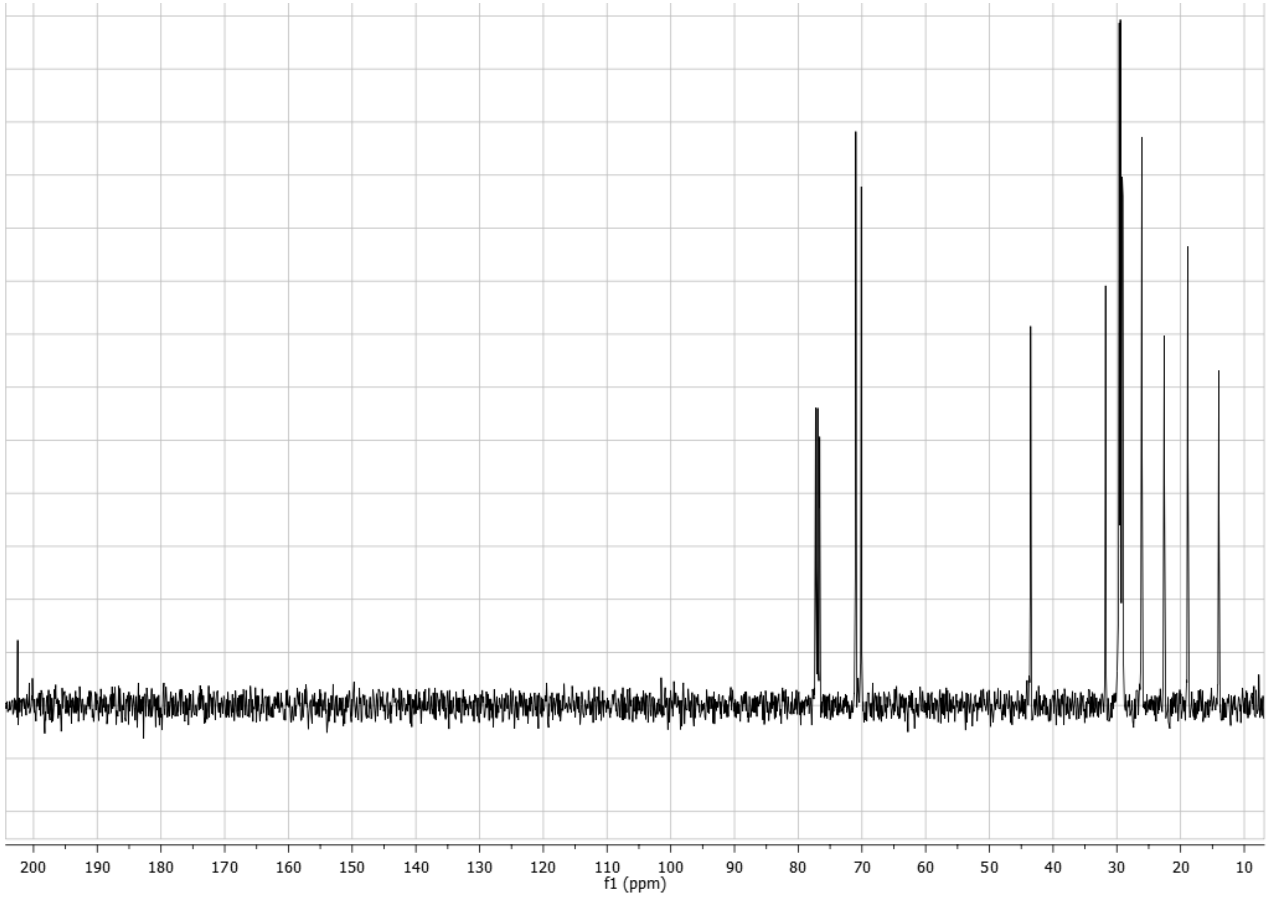
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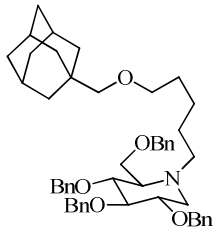


(2*R*,3*R*,4*R*,5*S*)-1-(*N*-(5-(adamantan-1-ylmethoxy)pentyl))-2-((benzyloxy)methyl)piperidine-3,4,5-tris(benzyloxy)) (4).

A reaction flask was charged with aldehyde **3** (36 mg, 0.143 mmol), 2,3,4,5-tetra-*O*-benzyl-D-nojirimycin (**18**) (30 mg, 0.0574 mmol), MeCN (966 µL) and glacial AcOH (34 µl), and finally was added $NaBH_3CN$ (7 mg, 0.115 mmol) under inert atmosphere. The reaction was stirred overnight, then was quenched with an aqueous solution of $NaHCO_3$ and extracted. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (95:5 to 8:2 hexane-EtOAc) of the resulting residue afforded compound **4** (56 mg, 99%).

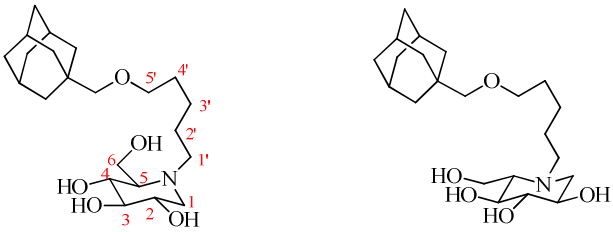
For NMR spectra and data see: *J. Org. Chem.* **2007**, *72*, 1088.

(2*R*,3*R*,4*R*,5*S*)-2-(Hydroxymethyl)piperidine-3,4,5-triol), 1-deoxynojirimycin, DNJ (20).

(2*S*,3*S*,4*S*,5*R*)-2-(Hydroxymethyl)piperidine-3,4,5-triol), L-1-deoxynojirimycin, L-DNJ (21).

To a solution of 2,3,4,5-tetra-*O*-benzyl-D-nojirimycin (**18**) or 2,3,4,5-tetra-*O*-benzyl-L-nojirimycin (**19**) (246 mg, 0.470 mmol) in DCM (10 ml) was added at 0 °C BCl_3 (4.70 ml, 4.70 mmol, solution 1 M in DCM) and the mixture was allowed to warm to rt overnight. The reaction was quenched with MeOH at 0 °C and concentrated under reduced pressure. The residue was washed with Et_2O, EtOAc and MeOH, and the resulting precipitate was dried, affording **20** or **21** (69 mg, 90%).

For NMR spectra and data see: *Org. Biomol. Chem.* **2011**, *9*, 1372; *Tetrahedron.* **2007**, *63*, 6827.



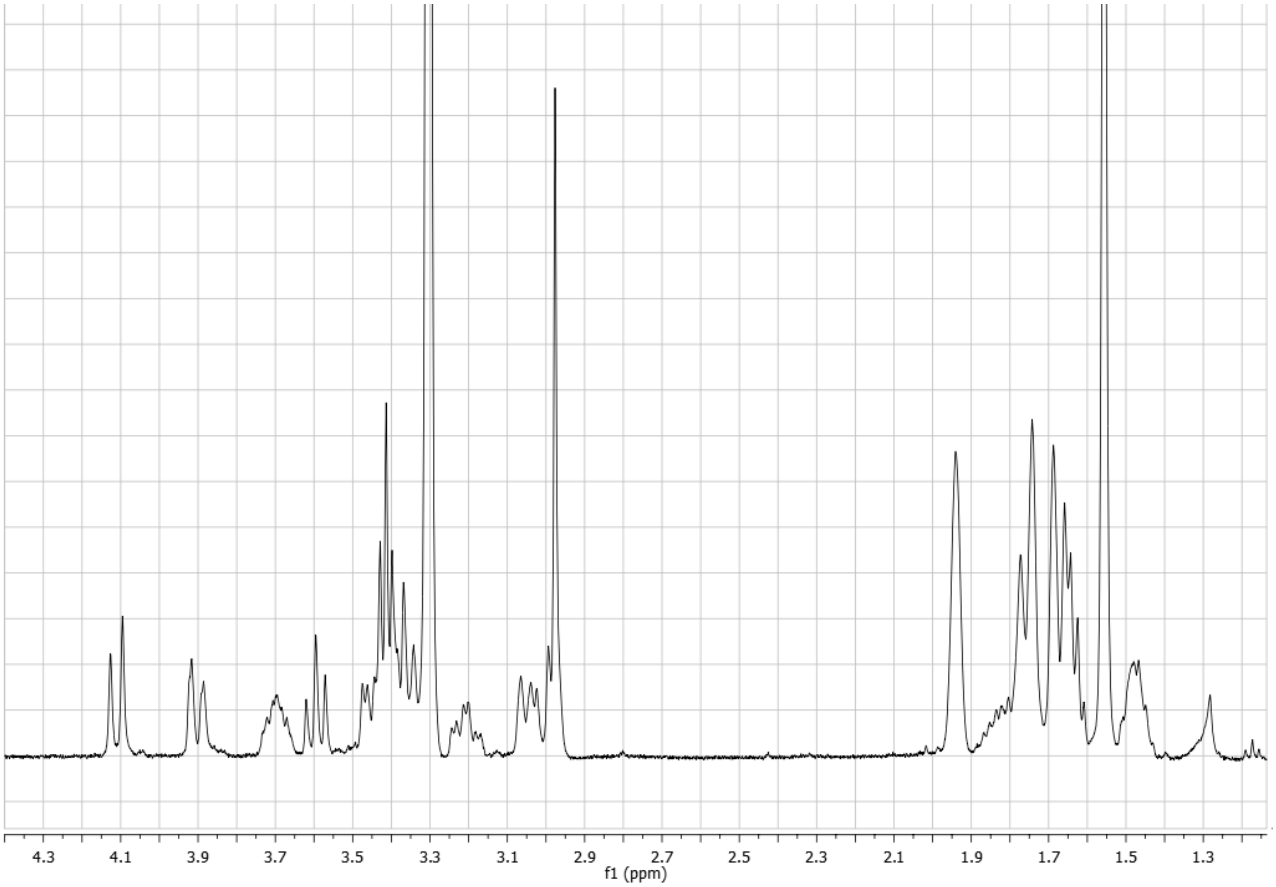
***N*-(5-(adamantan-1-ylmethoxy)pentyl)-1-deoxynojirimycin, AMP-DNM (5).**

***N*-(5-(adamantan-1-ylmethoxy)pentyl)-1-L-deoxynojirimycin, L-AMP-DNM (22).**

A reaction flask was charged with aldehyde **3** (29 mg, 0.116 mmol), DNJ (**20**) or L-DNJ (**21**) (13 mg, 0.0773 mmol), EtOH (1 mL) and glacial AcOH (10 µl), and finally was added $NaBH_3CN$ (7 mg, 0.116 mmol) under inert atmosphere. The reaction was stirred for 2 days, then was concentrated under reduced pressure. The residue was suspended in HCl 5% (500 µl), neutralized to pH 7.5 with solid Na_2CO_3 and extracted with DCM. Combined organic extracts washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (7.6:2.2:0.2 DCM-MeOH-NH_3 aq) of the resulting residue afforded compounds **5** and **22** (23 mg, 85%).

^{1}H NMR (400 MHz, MeOD): δ 1.43-1.51 (m, 2H, H-3'), 1.56 (br s, 6H, H adamantane), 1.61-1.69 (m, 4H, H-2', H-4'), 1.74-1.87 (m, 6H, H adamantane), 1.94 (br s, 3H, H adamantane), 2.98 (br s, 2H, C*H*$_2$-adamantane), 2.99-3.07 (m, 2H, H-1'), 3.20 (dt, *J* = 12.1, 5.2 Hz, 1H, H-5), 3.30-3.48 (m, 5H, H-1, H-2, H-3, H-4), 3.60 (t, *J* = 9.8 Hz, 1H, H-5'), 3.67-3.72 (m, 1H, H-5'), 3.90 (app d, *J* = 12.2 Hz, 1H, H-6), 4.11 (d, *J* = 12.2 Hz, 1H, H-6).

^{13}C NMR (100,6 MHz, MeOD): δ 22.6 (C-2'), 23.2 (C-3'), 28.2 (C adamantane), 28.7 (C adamantane), 36.8 (C adamantane), 39.3 (C adamantane), 52.6 (C-4'), 53.6 (C-1'), 54.2 (C-1), 65.9 (C-6), 66.6 (C-5), 67.7 (C-2), 70.7 (C-4), 71.1 (C-5'), 76.9 (C-3), 81.6 (*C*H$_2$-adamantane).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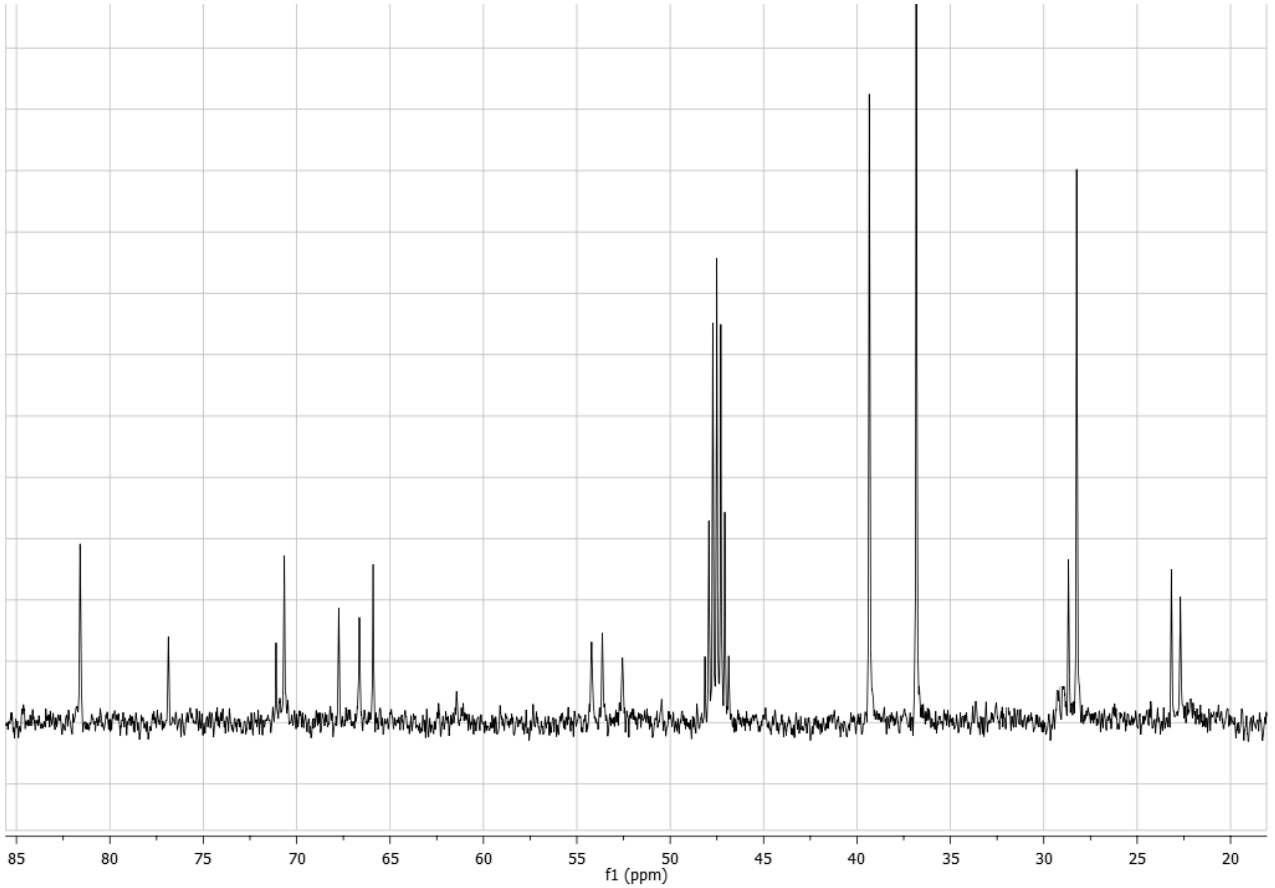
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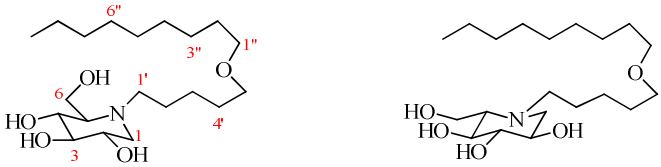


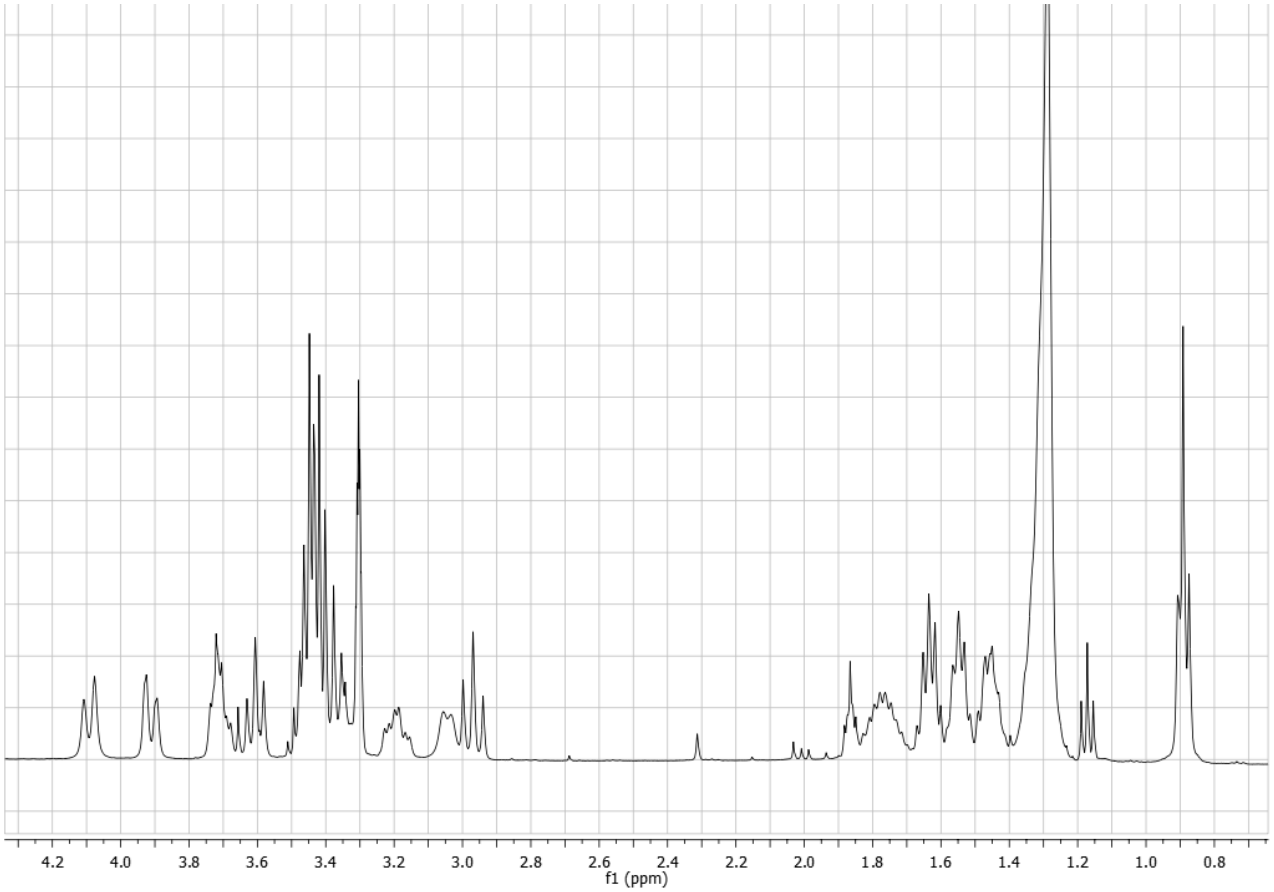
***N*-(Nonyloxypentyl)-1-deoxynojirimycin (23).**

***N*-(Nonyloxypentyl)-1-L-deoxynojirimycin (24).**

A reaction flask was charged with aldehyde **3** (45 mg, 0.197 mmol), DNJ (**20**) or L-DNJ (**21**) (21 mg, 0.132 mmol), EtOH (1.6 mL) and glacial AcOH (10 µl), and finally was added $NaBH_3CN$ (12 mg, 0.197 mmol) under inert atmosphere. The reaction was stirred for 2 days, then was concentrated under reduced pressure. The residue was suspended in HCl 5% (800 µl), neutralized to pH 7.5 with solid Na_2CO_3 and extracted with DCM. Combined organic extracts washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (DCM to 7:3 DCM-MeOH) of the resulting residue afforded compounds **23** and **24** (31 mg, 95%).

^{1}H NMR (400 MHz, MeOD): δ 0.89 (t, *J* = 7.1 Hz, 3H, H-9”), 1.29 (br s, 12H, H-3”, H-4”, H-5”, H-6”, H-7”, H-8”), 1.43-1.49 (m, 2H, H-3’), 1.52-1.58 (m, 2H, H-2”), 1.60-1.67 (m, 2H, H-4’), 1.70-1.83 (m, 2H, H-2’), 2.97 (t, *J* = 11.9 Hz, 1H, H-1), 3.04 (br. d, *J* = 9.4 Hz, 1H, H-5), 3.19 (dt, *J* = 12.1, 5.3 Hz, 1H, H-1’), 3.30-3.31 (m, 2H, H-1’, H-3), 3.34-3.49 (m, 5H, H-1, H-5’, H-1”), 3.61 (t, *J* = 9.8 Hz, 1H, H-4), 3.68-3.74 (m, 1H, H-2), 3.91 (br d, *J* = 12.6 Hz, 1H, H-6), 4.09 (br d, *J* = 12.4 Hz, 1H, H-6).

^{13}C NMR (100.6 MHz, MeOD): δ 14.6 (C-9”), 23.0 (C-8”), 23.9 (C-3’), 24.7 (C-2’), 25.0 (C-3”), 27.4 (C-6”), 30.4, 30.5, 30.7, 30.9, 33.2 (C-4’, C-2”, C-4”, C-5”, C-7”), 54.1 (C-1’), 56.5 (C-1), 57.6 (C-6), 67.5 (C-5), 69.5 (C-2), 70.7 (C-4), 71.8 (C-5’), 72.2 (C-1”), 79.6 (C-3).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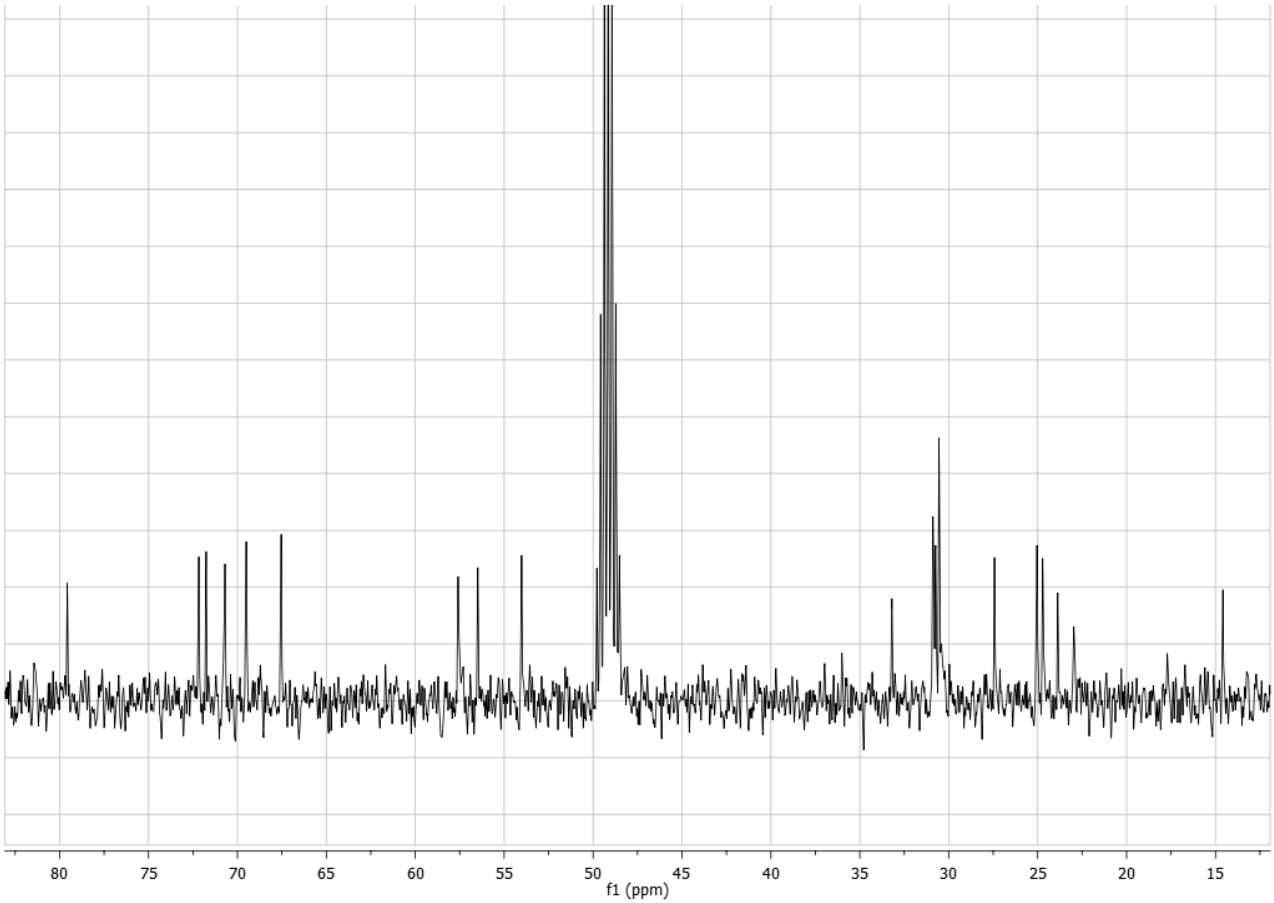
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5. REFERENCES

1. Hardicka, D.J.; Hutchinson, D.W. *Tetrahedron.* **1993**, *49*, 6707.

2. (a) Wong, C.-H.; Dumas, D.P.; Ichikawa, Y.; Koseki, K.; Danishefsky, S.J.; Weston, B.W.; Lowe, J.B. *J. Am. Chem. Soc.* **1992**, *114*, 7321. (b) Compain, P.; Martin, O.R. *Curr. Top. Med. Chem.* **2003**, *3*, 541.

3. (a) Bols, M.; Hazelle, R.; Thomsen, I.B. *Chem. Eur. J.* **1997**, *3*, 940. (b) Heightman, T.D.; Vasella, A.; Tsitsanou, K.E.; Zographos, S.E.; Skamnaki, V.T.; Oikonomakos, N.G. *Helv. Chim. Acta.* **1998**, *81*, 853. (c) Somsak, L.; Nagy, V.; Hadady, Z.; Docsa, T.; Gergely, P. *Curr. Pharm. Des.* **2003**, *9*, 1177.

4. (a) Horenstein, B.A.; Zabinski, R.F.; Schramm, V.L. *Tetrahedron Lett.* **1993**, *34*, 7213. (b) Schramm, V.L.; Tyler, P.C. *Curr. Top. Med. Chem.* **2003**, *3*, 525.

5. Moriyama, H.; Tsukida, T.; Inoue, Y.; Yokota, K.; Yoshino, K.; Kondo, H.; Miura, N.; Nishimura, S.-I.. *J. Med. Chem.* **2004**, *47*, 1930.

6. Cipolla, L.; Lay, L.; Nicotra, F.; Pangrazio, C.; Panza, L. *Tetrahedron*. **1995**, *51*, 4679.

7. Inoue, S.; Tsuruoka, T.; Ito, T.; Niida, T. *Tetrahedron.* **1967**, *23*, 2125.

8. Niwa, T.; Inoue, S.; Tsuruoka, T.; Koaze, Y.; Niida, T. *Agric. Biol. Chem.* **1970**, *34*, 966.

9. (a) Yagi, M.; Kouno, T.; Aoyagi, Y.; Murai, H. *Nippon Nogei Kagaku Kaishi*. **1976**, *50*, 571. (b) Schmidt, D.D.; Frommer, W.; Muller, L.; Truscheit, E. *Naturwissenshaften*. **1979**, *66*, 584. (c) Murao, S.; Miyata, S. *Agric. Biol. Chem.* **1980**, *44*, 219. (d) Ezure, Y.; Murao, S.; Miyazaki, K.; Kawamata, M. *Agric. Biol. Chem.* **1985**, *49*, 1119.

10. Junge, B.; Matzke, M.; Stltefuss, J. *Handbook of experimental pharmacology*. Springer-Verlag, New York. **1996**, *119*, 411.

11. Yu, C.-Y.; Asano, N.; Ikeda, K.; Wang, M.-X.; Butters, T.D.; Wormald, M.R.; Dwek, R.A.; Winters, A.L.; Nash, R.J.; Fleet, G.W.J. *Chem. Commun.* **2004**, 1936.

12. Asano, N.; Ikeda, K.; Yu, L.; Kato, A.; Takebayashi, K.; Adachi, I.; Kato, I.; Ouchi, H.; Takahata, H.; Fleet, G.W.J. *Tetrahedron: Asymmetry.* **2005**, *16*, 223.

13. Kato, A.; Kato, N.; Kano, E.; Adachi, I.; Ikeda, K.; Yu, L.; Okamoto, T.; Banba, Y.; Ouchi, H.; Takahata, H.; Asano, N. *J. Med. Chem.* **2005**, *48*, 2036.

14. Legler, G.; Liedtke, H. *Biol. Chem. Hoppe-Seyler.* **1985**, *366*, 1113.

15. (a) Saunier, B.; Kilker Jr., R.D.; Tkacz, J.S.; Quarini, A.; Herscovics, A. *J. Biol. Chem.* **1982**, *257*, 14155. (b) Zeng, Y.; Pan, Y.T.; Asano, N.; Nash, R.J.; Elbein, A.D. *Glycobiology.* **1997**, *7*, 297. (c) Szumilo, T.; Kaushal, G.P.; Elbein, A.D. *Arch. Biochem. Biophys.* **1986**, *247*, 261. (d) Schweden, J.; Borgmann, C.; Legler, G.; Bause, E. *Arch. Biochem. Biophys.* **1986**, *248*, 335.

16. (a) Hempel, A.; Camerman, N.; Mastropaolo, D.; Camerman, A. *J. Med. Chem.* **1993**, *36*, 4082. (b) Tan, A.; van den Broek, L.; van Boekel, S.; Ploegh, H.; Bolscher, J. *J. Biol. Chem.* **1991**, *266*, 14504.

17. (a) Joubert, P.H.; Foukaridis, G.N.; Bopape, M.L. *Eur. J. Clin. Pharmacol.* **1987**, *31*, 723. (b) Joubert, P.H.; Venter, H.L.; Foukaridis, G.N. *Br. J. Clin. Pharmacol.* **1990**, *30*, 391.

18. Robinson, K.M.; Begovic, M.E.; Rhinehart, B.L.; Heineke, E.W.; Ducep, J.B.; Kastner, P.R.; Marshall, F.N.; Danzin, C. *Diabetes.* **1991**, *40*, 825.

19. Bomhard, E.M. *Handbook of Experimental Pharmacology*; Kuhlmann, J.; Puls, W., Eds; Springer: Berlin, Heidelberg, New York. **1996**, *119*, 557.

20. Taylor, D.L.; Sunkara, P.S.; Liu, P.S.; Kang, M.S.; Bowlin, T.L.; Tyms, A.S. *AIDS.* **1991**, *5*, 693.

21. (a) Block, T.M.; Lu, X.; Platt, F.M.; Foster, G.R.; Gerlich, W.H.; Blumberg, B.S.; Dwek, R.A. *Proc. Natl Acad. Sci. USA.* **1994**, *91*, 2235. (b) Block, T.M.; Lu, X.; Mehta, A.S.; Blumberg, B.S.; Tennant, B.; Ebling, M.; Korba, B.; Lansky, D.M.; Jacob, G.S.; Dwek, R.A. *Nat. Med.* **1998**, *4*, 610. (c) Mehta, A.; Zitzmann, N.; Rudd, P.M.; Block, T.M.; Dwek, R.A. *FEBS Lett.* **1998**, *430*, 17.

22. (a) Humphries, M.J.; Matsumoto, K.; White, S.L.; Olden, K. *Cancer Res*. **1986**, *46*, 5215. (b) Spearman, M.A.; Ballon, J.M.; Gerrard, J.M.; Greenberg, A.H.; Wright, J.A. *Cancer Lett.* **1991**, *60*, 185. (c) Hadwiger, A.; Niemann, H.; Kabish, A.; Bauer, H.; Tamura, T. *EMBO J.* **1986**, *5*, 689.

23. Chester, M.A. *Glycoconjugate J.* **1999**, *16*, 1.

24. Kolter, T.; Sandhoff, K. *Angew. Chem., Int. Ed*. **1999**, *38*, 1532.

25. Sprong, H.; Kruithof, B.; Leijendekker, R.; Slot, J.W.; van Meer, G.; van der Sluijs, P. *J. Biol. Chem*. **1998**, *273*, 25880.

26. Marks, D.L.; Wu, K.J.; Paul, P.; Kamisaka, Y.; Watanabe, R.; Pagano, R.E. *J. Biol. Chem.* **1999**, *274*, 451.

27. Nomura, T.; Takizawa, M.; Aoki, J.; Arai, H.; Inoue, K.; Wakisaka, E.; Yoshizuka, N.; Imokawa, G.; Dohmae, N.; Takio, K.; Hattori, M.; Matsuo, N. *J. Biol. Chem.* **1998**, *273*, 13570.

28. Furst, W.; Sandhoff, K. *Biochim. Biophys. Acta.* **1992**, *1126*, 1.

29. Beutler, E.; Gelbart, T. *Blood Cells Molecules Dis*. **1997,** *23*, 2.

30. Gaucher P.C.E. *De l'epithelioma primitif de la rate, hypertrophie idiopathique de la rate sans leucemie [academic thesis]*. **1882**, Paris, France.

31. Nagral, A. *J. Clin. Exp. Hepatol.* **2014**, *4*, 37.

32. Platt, F.M.; Neises, G.R.; Reinkensmeier, G.; Townsend, M.J.; Perry, V.H.; Proia, R.L.; Winchester, B.; Dwek, R.A.; Butters, T.D. *Science*. **1997**, *276*, 428.

33. Platt, F.M.; Reinkensmeier, G.; Dwek, R.A.; Butters, T.D. *J. Biol. Chem.* **1997**, *272*, 19365.

34. Platt, F.M.; Neises, G.R.; Karlsson, G.B.; Dwek, R.A.; Butters, T.D. *J. Biol. Chem.* **1994**, *269*, 27108.

35. (a) Vanweely, S.; Brandsma, M.; Strijland, A.; Tager, J M.; Aerts, J. *Biochim. Biophys. Acta.* **1993**, *1181*, 55. (b) Overkleeft, H.S.; Renkema, G.H.; Neele, J.; Vianello, P.; Hung, I.O.; Strijland, A.; van der Burg, A.M.; Koomen, G J.; Pandit, U.K.; Aerts, J.M. *J. Biol. Chem*. **1998**, *273*, 26522.

36. Ghisaidoobe, A.; Bikker, P.; de Bruijn, A.C.J.; Godschalk, F.D.; Rogaar, E.; Guijt, M.C.; Hagens, P.; Halma, J.M.; van't Hart, S.M.; Luitjens, S.B.; van Rixel, V.H.S.; Wijzenbroek, M.; Zweegers, T.; Donker-Koopman, W.E.; Strijland, A.; Boot, R.; van der Marel, G.; Overkleeft, H.S.; Aerts, J.M.F.G.; van den Berg, R.J.B.H.N. *Med. Chem. Lett.* **2011**, *2*, 119.

37. Butters, T.D.; van den Broek, L A.G.M.; Fleet, G.W.J.; Krulle, T.M.; Wormald, M.R.; Dwek, R.A.; Platt, F.M. *Tetrahedron: Asymmetry*. **2000**, *11*, 113.

38. Wennekes, T.; van den Berg, R.J.B.H.N.; Donker, W.; van der Marel, G.A.; Strijland, A.; Aerts, J.M.F.G.; Overkleeft, H.S. *J. Org. Chem.* **2007**, *72*, 1088.

39. Cooper, C.G.F.; Lee, E.R.; Silva, R.A.; Bourque, A.J.; Clark, S.; Katti, S.; Nivorozhkin, V. *Org. Process Res. Dev.* **2012**, *16*, 1090.

40. Wennekes, T.; Lang, B.; Leeman, M.; van der Marel, G.A.; Smits, E.; Weber, M.; van Wiltenburg, J.; Wolberg, M.; Aerts, J.M.F.G.; Overkleeft, H.S. *Org. Process Res. Dev*. **2008**, *12*, 414.

Section 3

1. INTRODUCTION

Alkaloids are a group of natural products that contain mostly basic nitrogen atoms. They are found in a large variety of organisms from bacteria to animals, but also in fungi, plants, and sponges. Owing to their basic nature, alkaloids can be purified from crude extracts of source organisms by acid-base extraction. Despite several efforts, a true exhaustive classification of these compounds is lacking because of their great structural diversity.

Some alkaloids are commonly known for their psychotropic and stimulant activities (cocaine, caffeine, nicotine), but they also have a wide range of pharmacological activities, including antimalarian (quinine), antiasthma (ephedrine)[1], analgesic (morphine)[2] or anticancer (homoharringtonines)[1]. Most of the pharmacological activities of alkaloids are still unknown, as well as their biological role for living organism that produce them. In last years the attention is focused on the possible exploitation of alkaloids in still incurable diseases, like cancer.

Cancer collects different diseases involving abnormal cell growth with the potential to invade or spread from one tissue to another the body[3]. There are over 100 different known cancers that affect humans[4]. In 2012 about 14.1 million new cases of cancer occurred globally (not including skin cancer other than melanoma); it caused about 8.2 million deaths or 14.6% of all human deaths[1]. Because cancer is a class of diseases[5], there will not ever be a single cure, and new different treatments are now being tested in clinical trials to compare the proposed treatment to the best existing one[6]. There are intense efforts in scientific research to understand the disease processes and discover possible therapies. Madangamines[7] constitute a relatively new family of marine alkaloids isolated from Haplosclerida sponges. Some members of this class of natural products show significant anticancer activity which can be of therapeutic interest in a future.

1.1. MARINE SPONGES OF HAPLOSCLERIDA AS A SOURCE OF BIOACTIVE ALKALOIDS

Sponges (phylum Porifera) are considered the most primitive multicellular form of life. They first appeared on Earth almost 600 million years ago and had just a little structural evolution since

that time[8]. Sponges, as well as other sessile marine invertebrates, are filter feeders that have developed efficient defense mechanisms against foreign aggressors including viruses, bacteria, or eukaryotic organisms[8]. Marine sponges are among the richest sources of pharmacologically-active chemicals from marine organisms and more than half of all known alkaloids of marine origin have been isolated from sponges. Often the bioactive secondary metabolites isolated from sponges are the outcome of functional enzyme clusters, which originated from the sponges and their associated microorganisms. More than five-thousand different products are known from sponges and their associated microorganisms, and their number increases of more than 200 new products each year. These large numbers are expected to come from the need of the sponges to acquire novel leads against bacterial, viral, fungal and parasitic diseases, as these infectious microorganisms evolve and develop resistance to existing compounds.

Chemical studies carried out over the last decades proved that sponges are important sources of novel steroids, terpenoids, peptides, macrolides, and alkaloids[9] featuring potential clinical interest. Indeed, several marine natural products have successfully advanced to the late stages of clinical trials and moreover a growing number of candidates is constantly considered as promising leads for extended preclinical assessment.

In this context, marine sponges in the order Haplosclerida are the source of more than one hundred biogenetically related alkaloids, in particular of 3-alkylpyridine and 3-alkylpiperidine alkaloids. The first 3-alkylpiperidine sponge alkaloid, the halitoxin, was discovered in 1978 by Schmitz *et al.*[10], and since then other chemical studies performed on Haplosclerida sponges allowed the isolation of a large variety of alkaloids.

As a group, the 3-alkylpiperidines are characterized by the diversity and complexity of their chemical structures and by the range of biological activities that they exhibit[11]. There are eleven macrocyclic skeletons known within the 3-alkylpiperidine family: these include the haliclamine/cyclostellettamine, ingenamine, madangamine, ircinal, manzamine, halicyclamine, saraine 1-3, saraine A to C, petrosin, xestospongin/araguspongine and aragupetrosine skeletons (**Figure 1.1**). Three of these skeletal types, belonging to the ingenamine[12], madangamine[7] and halicyclamine[13] alkaloids, were first isolated and all reported in 1994. For this reason, this is considered a relatively young line of research, and the potential of these alkaloids has not been still completely explored.

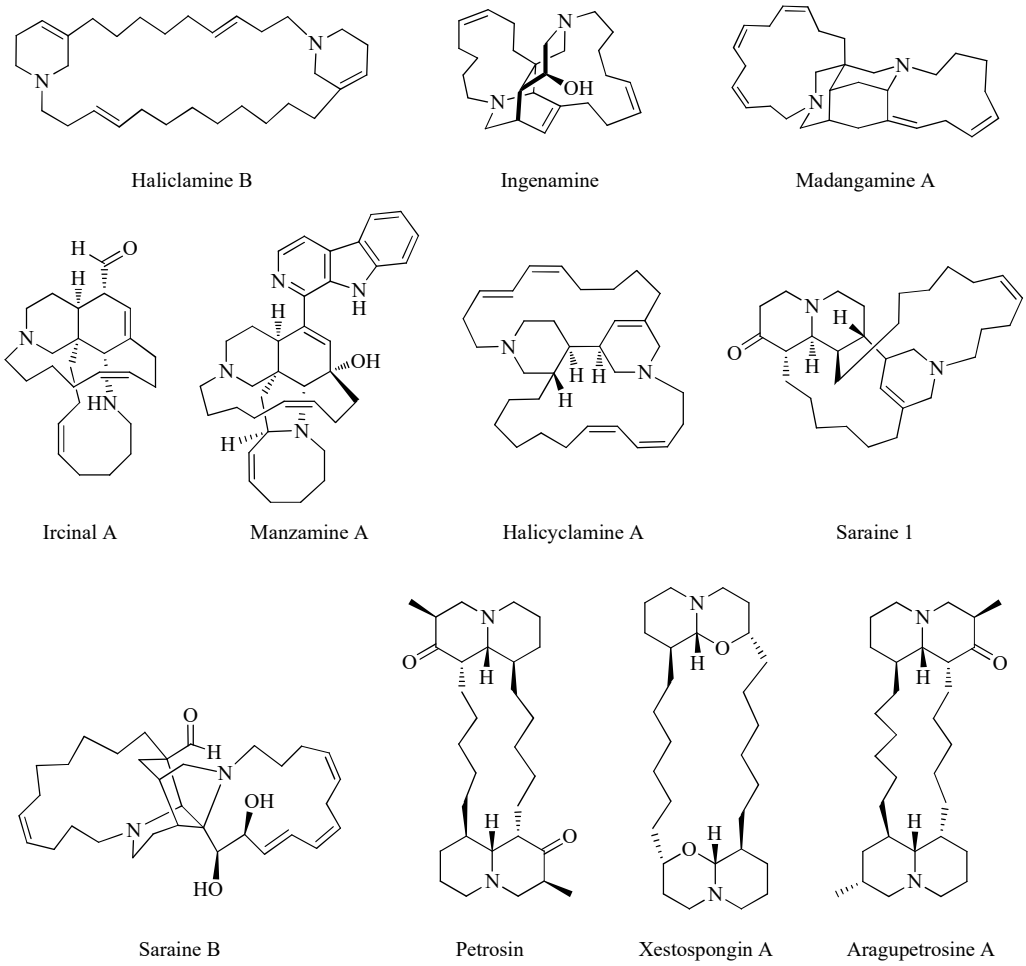


Figure 1.1: 3-alkylpiperidine alkaloids.

The 3-alkylpiperidine alkaloids stand, from the beginning, as a challenge to the extant methodology for chemical structure elucidation. In the case of most of the macrocyclic 3-alkylpiperdine alkaloids, the difficulties associated with analyzing the NMR data associated to the long chain alkyl bridges spanning the nitrogen containing polycyclic cores have complicated the structure elucidation leading occasionally to incorrect structural hypothesis. It is interesting to note that the saraines, a class of alkaloids isolated from *Reniera sarai*, a sponge extensively found in the Bay of Naples, have a chemical structure so complicated that was elucidated after 9 years of investigations by Cimino and co-workers[14]. The availability of 2D experiments conducted on high field NMR spectrometers and X-rays diffraction analysis finally proved to be a structural tool powerful enough to meet the challenge of unambiguously determining the connectivity in the long chain alkyl bridges[14].

Many types of biological activity were found in 3-alkylpiperidine alkaloids suggesting their potential use as source of drugs. In particular, the antileukaemic activities of the manzamines[15],

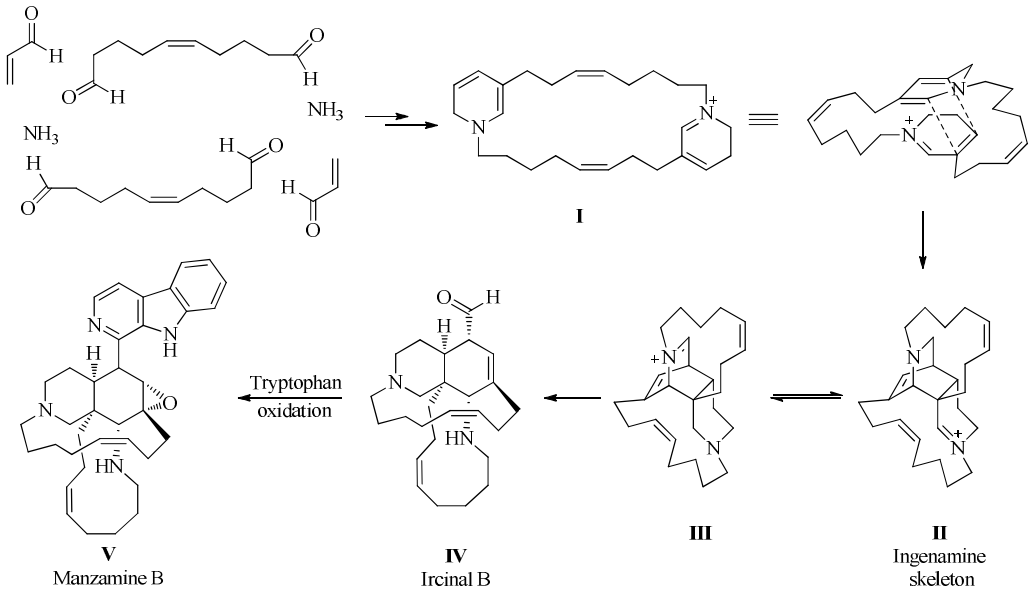
the antibacterial and antitumoral properties of the saraines[16] and madangamines[17], have attracted much attention.

The combination of potent biological activity and structural complexity found in the 3-alkylpiperidine alkaloids has spurred the interest synthetic chemists. To date synthetic efforts were undertaken towards the synthesis of each component of the family, in particular the most active manzamines[18], saraines[19] and madangamines[20].

1.2. BIOGENETIC THEORIES

The first tentative biogenetic hypothesis for the 3-alkylpiperidine family was suggested by Cimino *et al.*[14]. The origin of these alkaloids can be envisaged in the reduced bis-3-alkylpyridine macrocycles suggesting a biogenetic relationship between the oligomeric halitoxins and the 3-alkylpiperidine alkaloids.

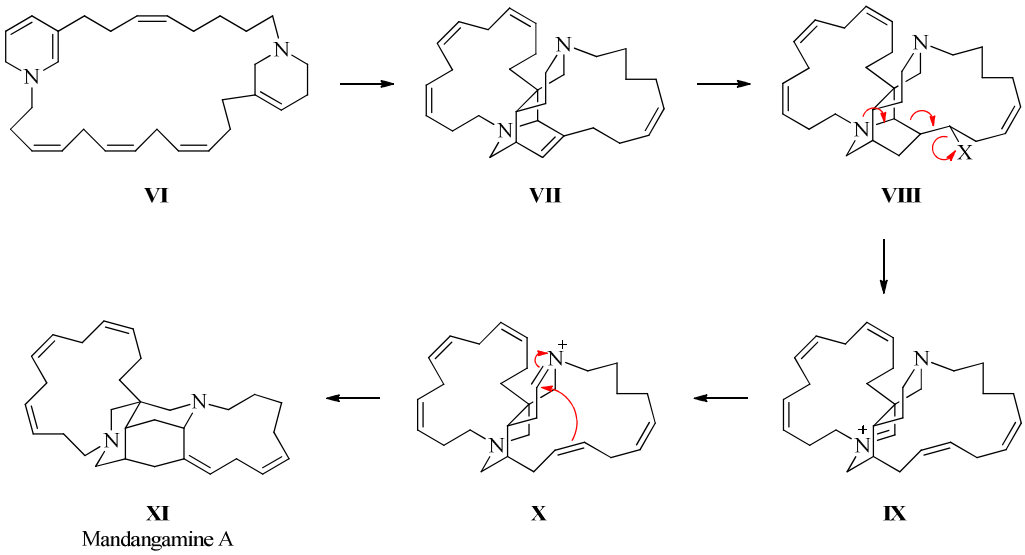
Indeed, it was from the isolation of manzamine A in 1988 that researchers began to realize that the biogenesis of all these types of polycyclic alkaloids could have a common path. An elegant hypothesis by Whitehead and Baldwin[21] proposed that the manzamines arise from a bis-3-alkyldihydropyridine precursor (**Scheme 1.1**). Curiously, this hypothesis anticipated the occurrence of two new classes of alkaloids corresponding to the pentacyclic and tetracyclic intermediates in their biogenetic scheme, the ircinal B (**IV**)[22], the tetracyclic product, and ingenamines (**II**)[12], the pentacyclic intermediate.



Scheme 1.1: Biogenetical proposal of Whitehead and Baldwin.

According to the proposal, the bis-dihydropyridinium cycles arise from ammonia, a C_{10} di-aldehyde and a C_3 acrolein equivalent. These units are assembled into a 3-alkylpiperidine monomer that can either polymerize leading the oligomeric halitoxins or dimerize into a bis(3-alkylpiperidine) macrocycle (**I**). The dimer (featuring tautomerism) evolved into intermediate **II** via an *endo* intramolecular [4+2] Diels-Alder cycloaddition. Final intermediate **IV**, corresponding to ircinal B, was originated via an hydrolytic ring-opening and a condensation with tryptophan. A further oxidation step led to the manzamine B (**V**) and similar paths lead to related polycyclic skeletons found in many of these complex alkaloids.

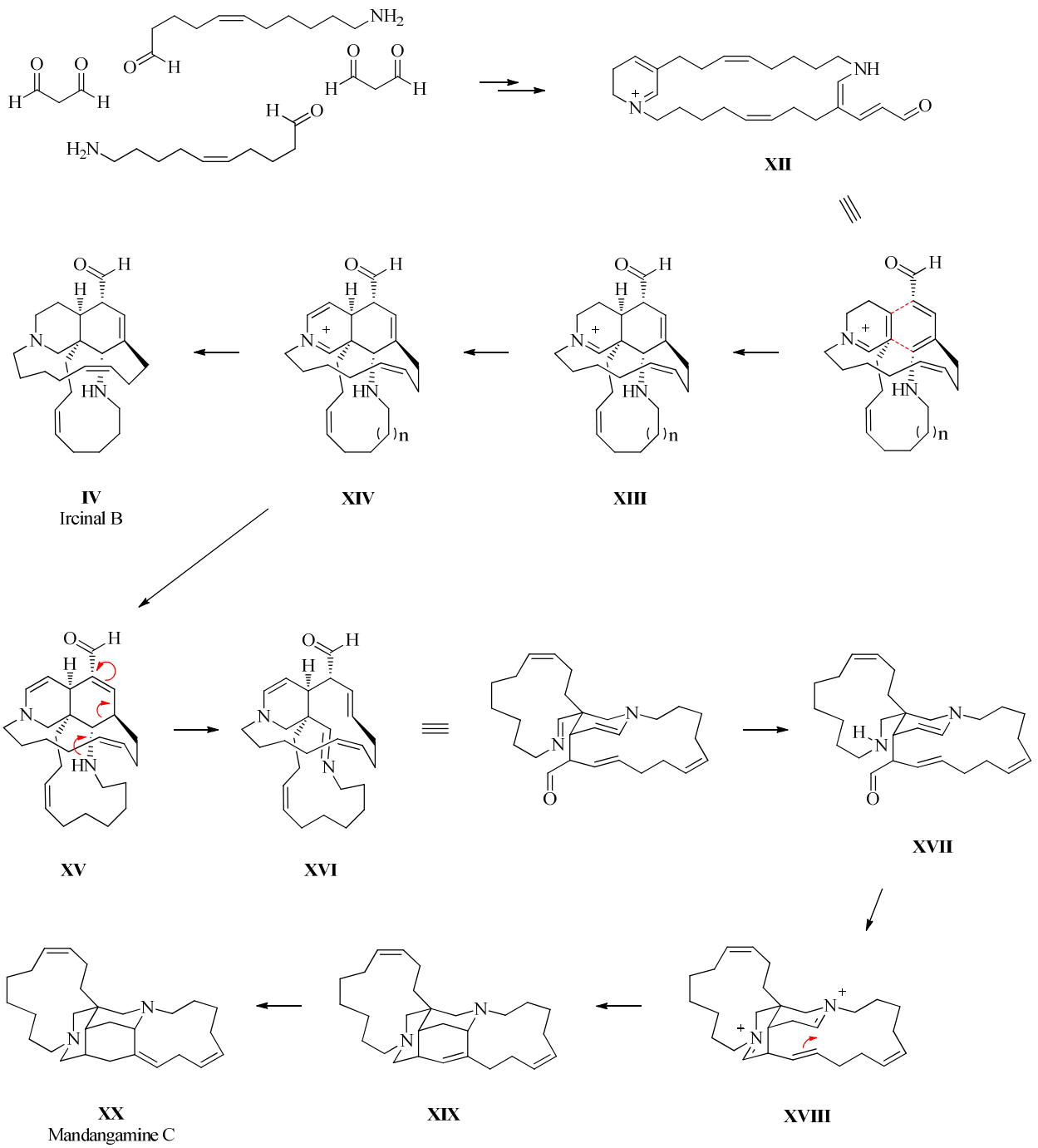
In 1994, Kong *et al.*[7,24] conjectured a different biosynthetic pathway to 3-alkylpiperidine and in particular to madangamines, outlined in **Scheme 1.2**.



Scheme 1.2: Kong's biogenesis for madangamines.

In the Kong model, in analogy with the Whitehead and Baldwin proposal, the biogenesis starts from ammonia, a C_{10} di-aldehyde, and a C_3 acrolein equivalent and proceeds through a partially reduced bis-3-alkylpyridine (**VI**), which undergoes a [4+2] cycloaddition to generate the intermediate **VII**, related to the ingenamine class of alkaloids. This ingenamine-type intermediate can then undergo a fragmentation to give the tetracyclic intermediate **IX**, that gives the regioisomeric iminium ion **X** with a redox exchange between the two nitrogen atoms. Subsequent "aza-Prins-type" reaction generate the madangamine skeleton **XI**. It may be that enzyme(s) catalysing this rearrangement has a specific requirement for a particular chain length and functionality in the C-3 to N-11 bridge in the putative ingenamine precursor. Thus, most of the resulting madangamines have identical N-1 to C-3 bridges but show variations in the N-7 to C-9 bridge.

An alternatively theory, suggested by Marazano *et al.*[25], is illustrated in **Scheme 1.3**.



Scheme 1.3: Marazano's proposal for madangamines' biosynthesis.

In this proposal, Marazano suggests that manzamine alkaloids can alternatively be viewed as derived from two unsaturated long-chain aminoaldehydes and two malono-dialdehyde units. Natural 3-alkylpyridinium salt derivatives (**XII**) are obtained with a cyclization in an acidic medium; the resulting macrocycle with an intramolecular Diels-Alder reaction gives the tetracyclic intermediate **XIII**. An oxidation leads to the advanced intermediate of the syntheses of manzamines and ircinals (**XIV**): in fact, reduction of this tetracyclic compound followed by

oxidation can give access to ircinal derivatives such as ircinal B (*n* = 1, **IV**), precursor of manzamine.

As an alternate pathway, intermediate **XIV** (*n* = 3) was reduced to give, after double-bond migration, amino aldehyde **XV**. Ring opening could then occur, leading to the imine derivative **XVI** whose reduction would afford secondary amine **XVII**. Cyclization to the corresponding double-iminium salt derivative **XVIII**, obtained by condensation, could then produce pentacyclic intermediate **XIX**, which with a final double-bond migration affords madangamine C.

1.3. MADANGAMINE ALKALOIDS

Madangamines, as seen above, are a group of complex optically active pentacyclic diamine alkaloids isolated from the marine sponge *Xestospongia ingens* of the order Haplosclerida, collected by hand using scuba on reefs off Madang, Papua New Guinea. They are apparently related to macrocycles like saraines, manzamines, ircinals, petrosins, through a common biogenetic bis-3-alkylpiridine precursor, as emerges from their structures and proposed biogenetic theories.

Andersen *et al.*[7] isolated in 1994 the first madangamine alkaloid, the A (**XI**, **Figure 1.2**), that showed *in vitro* cytotoxicity against murine leukemia P388 (ED_{50} 0.93 µg/mL) and human lung A549 (ED_{50} 14 µg/mL), brain U373 (ED_{50} 5.1 µg/mL), and breast MCF-7 (ED_{50} 5.7 µg/mL) cancer cell lines. The isolation was conducted with multiple extractions of specimens of *X. ingens* with MeOH, and the combined MeOH extracts were reduced *in vacuo* to an aqueous suspension. This suspension was diluted with distilled H_2O and then partitioned sequentially against hexane and EtOAc. Repeated fractionation of the hexane-soluble materials using silica gel flash chromatography and normal-phase HPLC led to the isolation of madangamine A[7].

Madangamines from B to E (**XX-XXIII**) were isolated via the same way by Andersen *et al.* in 1998[24]. A detailed analysis of the NMR data and mass spectrometry identified the constitution and relative configurations of the whole structure of madangamines[7,24]. The basic madangamine skeleton consists of a tricyclic core (ABC rings) and two linear bridges (DE rings). The central tricyclic core, unprecedented among natural products, is constituted by two piperidine rings both in chair conformation cis-fused, attached to a carbocyclic ring with a slightly flattened chair

conformation. The E ring (N-1 to C-3 bridge) consists of two *Z* skipped double bonds, one connected to the carbocyclic ring, and is identical for A-E madangamines, while D ring (N-7 to C-9 bridge) shows variations both in size (13 to 15-membered) and in the position and degree of unsaturation (**Figure 1.2**).

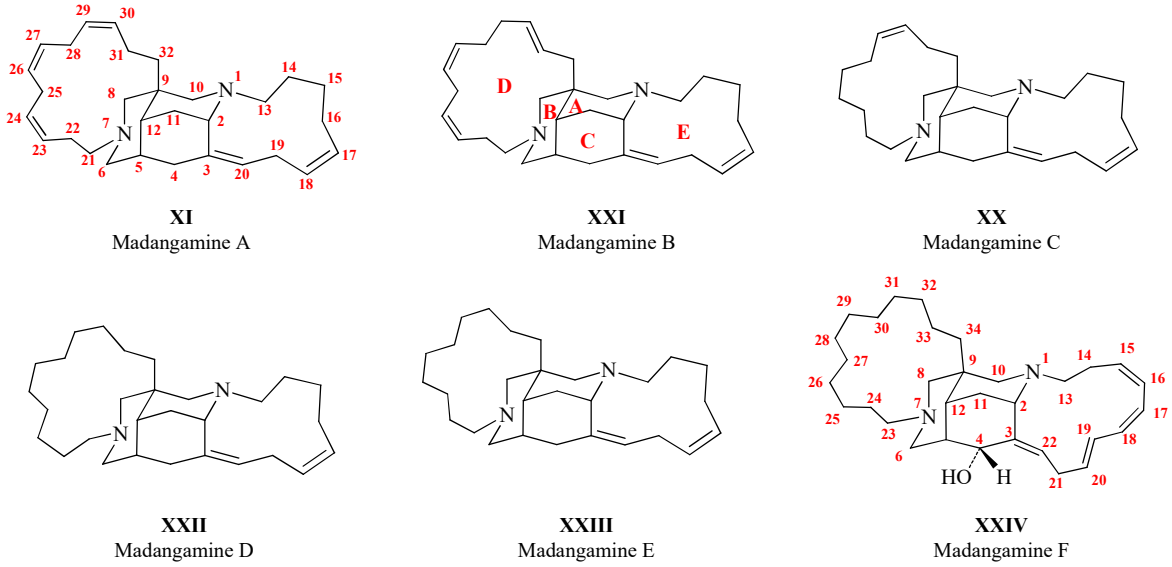


Figure 1.2: Madangamine alkaloids.

All the madangamines are extremely non polar compounds, in fact they dissolve in the hexane layer, and this is probably related to their core structure. One of the two nitrogen atoms, the N-7, cannot readily invert because the cavity of the central core is too small to easily accommodate the C-21 alkyl group. Therefore, the lone pair on the N-7 nitrogen atom is locked in the center of the tricyclic core where it is relatively inaccessible for protonation and hydrogen bonding. The anticipated result would be a decreased basicity for the N-7 amine and consequently a reduced polarity for the madangamines.

No data regarding the biological activity of B-E madangamines have been reported until now, but these alkaloids with their peculiar structure could be an interesting target for both the synthetic organic chemists and the pharmaceutical research.

Another madangamine, the F (**XXIV**), was isolated in 2007 by Berlink *et al.*[26] from *Pachychalina alcaloidifera*, a different Haplosclerid sponge, collected in Ilha do Pai, Niterói, Rio de Janeiro, Brazil[27]. The crude extract presented a very complex mixture of alkaloids difficult to separate. Madangamine F was isolated from the MeOH crude extract by column chromatography on silica gel using small amounts (10 g or less) of stationary phase, with a gradient of MeOH in

CH_2Cl_2 or a gradient of 1:1 MeOH/MeCN in CH_2Cl_2. **XXIV** shows a remarkable polarity respect to the other members of the madangamine group[26].

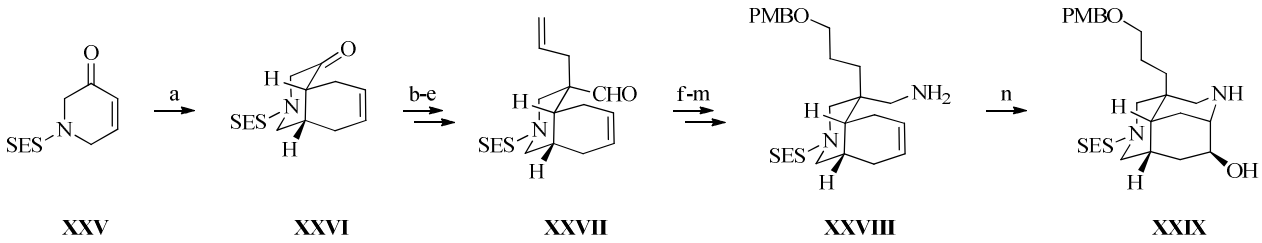
The comparison of NMR spectra of **XXIV** with those of other madangamines confirmed the structural identity of the madangamine central core. Nevertheless, madangamine F is the first member of the madangamine group of alkaloids featuring a C10 (instead of a C8) bridge bearing four insaturations between N-1 and C-3 and a hydroxyl group at C-4 (**Figure 1.2**). The occurrence of a hydroxyl group is noteworthy, because confirms that the madangamine skeleton biogenetically derives from ingenamines: its position corresponds to the C-4 position in the putative ingenamine precursor, which is commonly unsaturated at $\Delta^{3,4}$, and, therefore, is susceptible to an enzyme-mediated addition of H_2O.

According to Kumar, also madangamine F shows cytotoxic activity against human central nervous system SF295 (ED_{50}19.8 µg/mL), breast MDA-MB435 (ED_{50} 16.2 µg/mL), colon HCT8 (ED_{50}>25 µg/mL), and leukemia HL60 (ED_{50}16.7 µg/mL) cancer cell lines[26].

1.4. PREVIOUS SYNTHETIC APPROACHES

Nowadays, several total syntheses are reported for almost every member of the 3-alkylpiperide group, especially for the most representative alkaloids with potent pharmaceutical activity[18,28]. Nonetheless, in the case of the madangamine family, only one total synthesis has been reported just for madangamine D and was carried out by the research group were this thesis was partially developed[20]. However, several syntheses addressing various madangamine substructures have been previously reported by other groups[25,29-37].

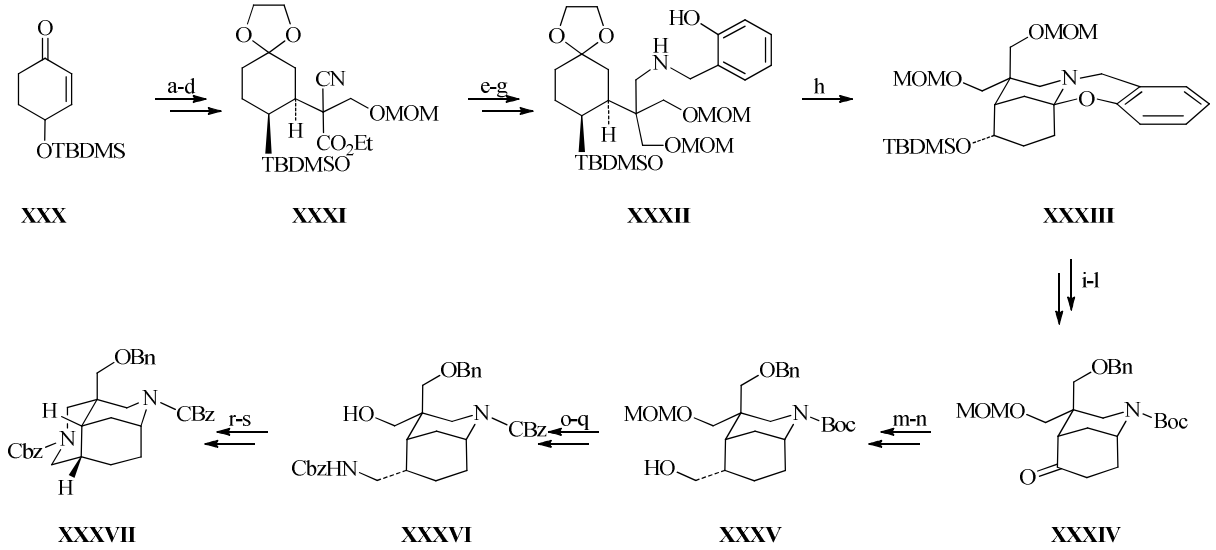
The first approach to the tricyclic core of madangamine was reported in 1997 by Weinreb[29], starting from enone key intermediate **XXV** (**Scheme 1.4**), obtained with Hiemsra's metodology[30].



Scheme 1.4: (a) 1,3-butadiene; (b) TosMIC, *t*-BuOK; (c) DIBAL-H; (d) diallylamine, PPh_3, $Pd(OCOCF_3)_2$; (e) HCl; (f) $NH_2OH \cdot HCl$; (g) $LiAlH_4$; (h) *p*-$BrC_6H_4SO_2Cl$, TEA, DMAP;(i) CsF/DMAP, TEA, TsCl; (j) $NH_2OCH_2Ph \cdot HCl$ (k) disiamylborane/H_2O_2, NaOH; (l) NaH, TBAI, PMBCl; (m) $LiAlH_4$; (n) $Hg(OCOCF_3)_2/(CF_3)_2CHOH$, $NaBH_4$.

Though a [4+2] cycloaddition of enone **XXV** with butadiene at high pressure (12 kbar), the desired cis keto azadecalin derivative **XXVI** was obtained. The ketone functionality was then converted to nitrile and subsequently to aldehyde, at the same time an allyl chain was introduced selectively giving the appropriate stereochemistry to C-9 (**XXVII**). Further transformation led to primary amine **XXVIII**, and a two-step reaction with mercuric trifluoroacetate and sodium borohydride yielded the tricycle **XXIX**. The amino alcohol had appropriate functionalities for the subsequent construction of both macrocyclic rings, but the large number of steps required to get XXIX makes this strategy not suitable.

Kibayashi[31] in 2004 proposed a synthesis for the diazatricyclic core involving an intramolecular *N*,*O*-acetalization of a keto-aminophenol (**Scheme 1.5**).

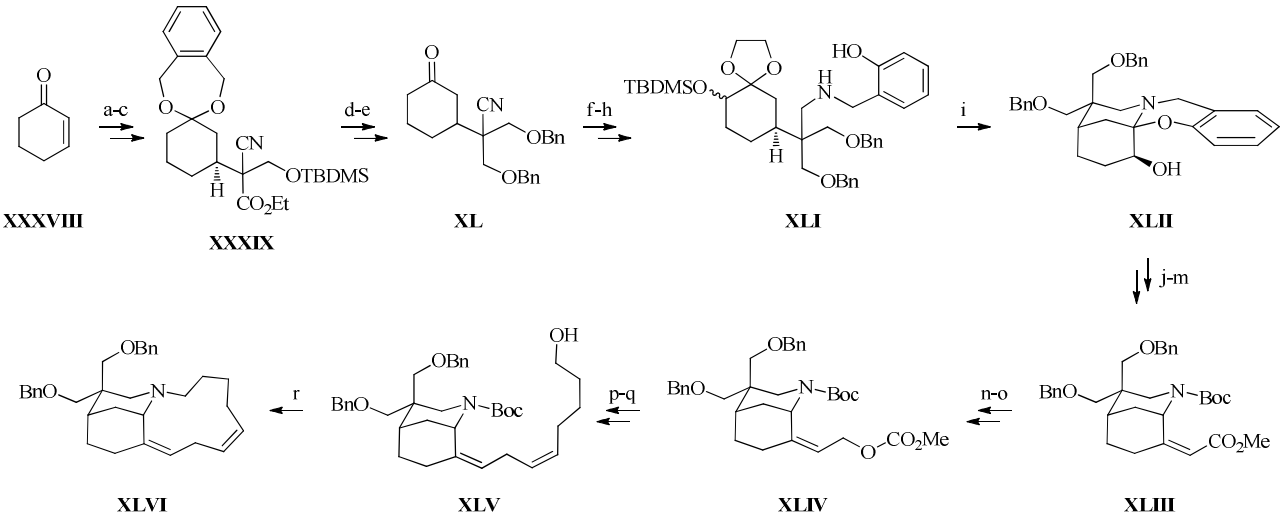


Scheme 1.5: (a) $CH_2(CN)CO_2Et$, *t*-BuOK; (b) $(CH_2OH)_2$, *p*-TsOH; (c) 35% HCHO, $KHCO_3$; (d) MOMCl, *i*-Pr_2EtN; (e) (i) $LiBH_4$; (ii) MOMCl, *i*-Pr_2EtN; (f) DIBAL-H i; (g) salicylaldehyde/$NaBH_4$; (h) PPTS; (i) AlH_3; (j) H_2, Pd/C; (k) (i) Boc_2O, NaH; (ii) BnBr, NaH;(iii) TBAF;(l) PCC;(m) Tebbe reagent; (n) (i) 9-BBN;(ii) NaOH, H_2O_2;(o) PhthNH, DEAD, Ph_3P; (p) (i) HCl;(ii) $H_2NNH_2 \cdot xH_2O$; (q) CbzCl;(r) MsCl, TEA; (s) *t*-BuOK.

Cyclohexanone **XXX** was subjected to Michael addition and appropriate protections to give nitrile ester **XXXI** that, via several reductions and a final reductive amination with salicylaldehyde, was converted to aminophenol **XXXII**. Upon treatment of **XXXII** with pyridinium *p*-toluenesulfonate in acetone–H_2O under reflux, the transiently formed keto-aminophenol undergoes intramolecular condensation to give the tetracyclic *N,O*-acetal **XXXIII** consisting of the 2-azabicyclo[3.3.1]nonane nucleus. Subsequent processes led to ketone **XXXIV** and then to primary alcohol **XXXV** as a single diastereoisomer, through a hydroboration with 9-BBN from the less congested convex β-face. The Mitsunobu reaction, followed by deprotection and protection steps, converted the intermediate **XXXV** to the primary alcohol **XXXVI**, which via a mesylation and treatment to *t*-BuOK led to the tricyclic compound **XXXVII**.

This strategy, despite the efficiency, led to a tricyclic compound that lacked a functionalization on C-3, making the construction of E ring impossible.

Two years later Kibayashi published another paper[32] with a different approach to the synthesis of madangamine, synthetizing the ACE ring system (**Scheme 1.6**).



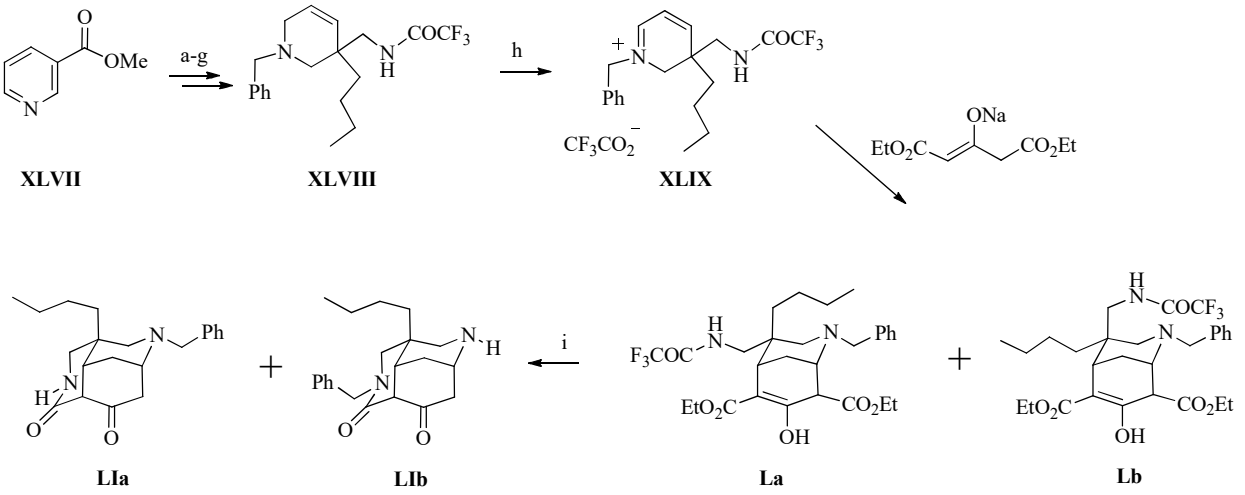
Scheme 1.6: (a) $CH_2(CN)CO_2Et$; (b) *o*-$C_6H_4(CH_2OH)_2$, *p*-TsOH; (c) (i) HCHO; (ii) TBDMSCl, imidazole; (d) (i) $LiBH_4$; (ii) TBAF; (e) (i) BnBr, NaH; (ii) PPTS; (f) (i) TBDMSCl; (ii) OsO_4, NMO; (g) (i) $(CH_2OH)_2$, TMSCl; (ii) TBDMSOTf, 2,6-lutidine; (h) DIBAL-H/*o*-$(OH)C_6H_4CHO$, $NaBH_4$; (i) HCl; (j) $LiAlH_4$, $AlCl_3$; (k) H_2, $Pd(OH)_2$: (l) (i) $(Boc)_2O$; (ii) DMP; (m) $(CF_3CH_2O)_2P(=O)CH_2CO_2Me$, KHMDS, 18-crown-6; (n) DIBAL-H; (o) $MeCO_2Cl$; (p) (*Z*)-$Bu_3SnCH=CH(CH_2)_4OTBDPS$, $Pd(dba)_2$; (q) TBAF; (r) (i) DMP; (ii) TFA; (iii) $NaBH(OAc)_3$.

The cyclohexanone **XXXVIII** was subjected to Michael addition, protections and hydroxymethylation to give nitrile ester **XXXIX**, that was converted to **XL** via cleavage of the

cyclic acetal. With a strategy similar to the previous one, Kibayashi obtained the reductive amination product **XLI** and then the tetracyclic *N,O*-acetal **XLII** as a single diastereoisomer. After reductive cleavage of the acetal, hydrogenation, protection and oxidation of **XLII**, a Still's *Z*-selective Wittig-Horner olefination was accomplished, giving the (*Z*)-exo-olefin **XLIII** as major product with undesired (*E*)-isomer in ratio 11:1. The mixture was reduced and methoxycarbonylated, leading to **XLIV**, and to the skipped diene **XLV** as a single stereoisomer after a palladium-catalyzed coupling with the (*Z*)-vinylstannane, (1,1-dimethylethyl)(dimethyl){[(5*Z*)-6-(tributylstannanyl)hex-5-enyl]oxy}silane. Finally, the 11-membered ring was fabricated by a sequential reaction involving oxidation of **XLV** with Dess–Martin periodinane followed by deprotection and intramolecular reductive amination giving rise to the expected tricyclic product **XLVI**.

This strategy also led to a tricyclic compound lacking a functionalization on C-5, and thus unusable to give the pentacyclic madangamine.

A different proposal came from Marazano[25] in 2005, who basing upon his biogenetic theory, linked madangamines to ircinals. The condensation of the sodium salt of diethylacetonedicarboxylate with a dihydropyridinium salt derivative is the key reaction of the scheme (**Scheme 1.7**).

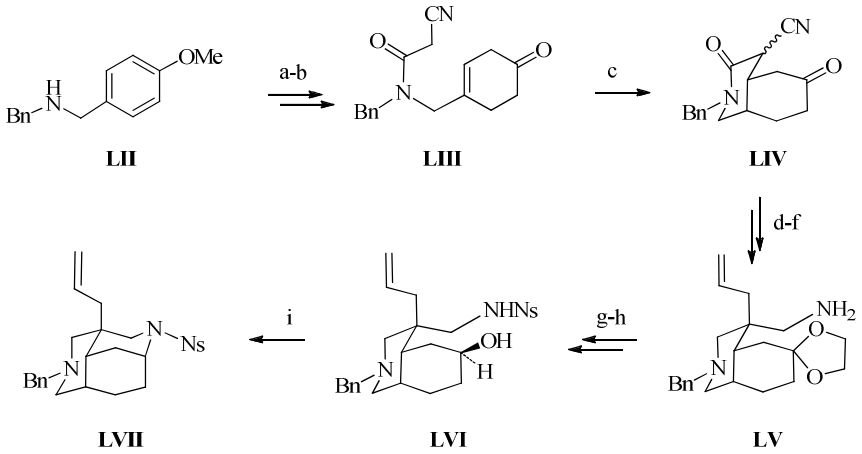


Scheme 1.7: (a) $BnBr/NaBH_4$; (b) LDA/*n*-BuBr; (c) DIBAH; (d) $BnNH_2$; (e) *t*-BuOK; (f) oxalic acid; (g) py, $(CF_3CO)_2O$; (h) *m*-CPBA/$(CF_3CO)_2O$; (i) acetone dicarboxylate diethyl ester sodium salt/K_2CO_3.

Nicotinic acid methyl ester (**XLVII**) after seven step gave the tetrahydropyridine **XLVIII**, that afforded derivative **XLIX** after a treatment with *m*-CPBA and trifluoroacetic anhydride. Dihydropyridinium salt **XLIX** was treated with the sodium salt of acetone dicarboxylate diethyl ester at ambient temperature, and provided adducts **La** and **Lb** in an inseparable mixture. Treatment of the crude mixture with an alkaline solution provided two tricyclic products **LIa** and **Lib** via rearrangement.

This biogenetically inspired strategy demonstrated the possibility of obtaining the tricyclic system with a stereoselective approach with a little number of steps, but unfortunately also in this case the C-9 lacks of functionalization required to complete the synthesis of madangamines.

In 2008 Bonjoch[33] proposed a synthesis of the tricyclic core of madangamines in a 10-step sequence starting from a 4-(aminomethyl)anisole derivative (**Scheme 1.8**).

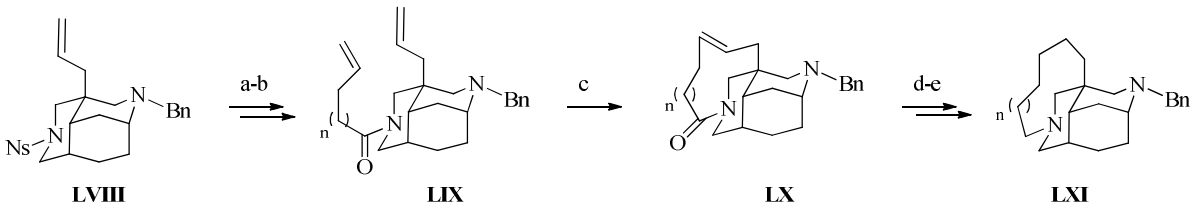


Scheme 1.8: (a) Li, NH_3; (b) (i) $NCCH_2CO_2H$, PyBOP, TEA; (ii) HCl; (c) NaOEt; (d) AllBr, LDA; (e) $(CH_2OH)_2$, *p*-TsOH; (f) AlH_3; (g) (i) NsCl, TEA; (ii) HCl; (h) L-selectride; (i) DEAD; PPh_3; TEA.

The synthesis began with a Birch reduction of the 4-methoxybenzylamine[34] (**LII**), followed by a reductive amination of the resulting dihydroanisole then coupled with cyanoacetic acid. After an acid treatment intermediate **LIII** was obtained. Reaction of **LIII** with NaOEt induced the isomerization of the double bond and then an intramolecular Michael process to diastereoselectively give the cis-perhydroisoquinoline **LIV**. An allylation, followed by a protection and a reduction led to diamino derivative **LV**. After nosylation and cleavage of the

acetal group, the resulting ketone was reduced with L-selectride giving the axial alcohol **LVI**, that finally gave the target tricycle **LVII** with a S_N2 substitution.

In 2015 Bonjoch[35] accomplished the synthesis of the tetracyclic ABCD ring system of madangamines D-F, starting from a tricyclic derivative synthetized with a different strategy respect to the previous one (**Scheme 1.9**).



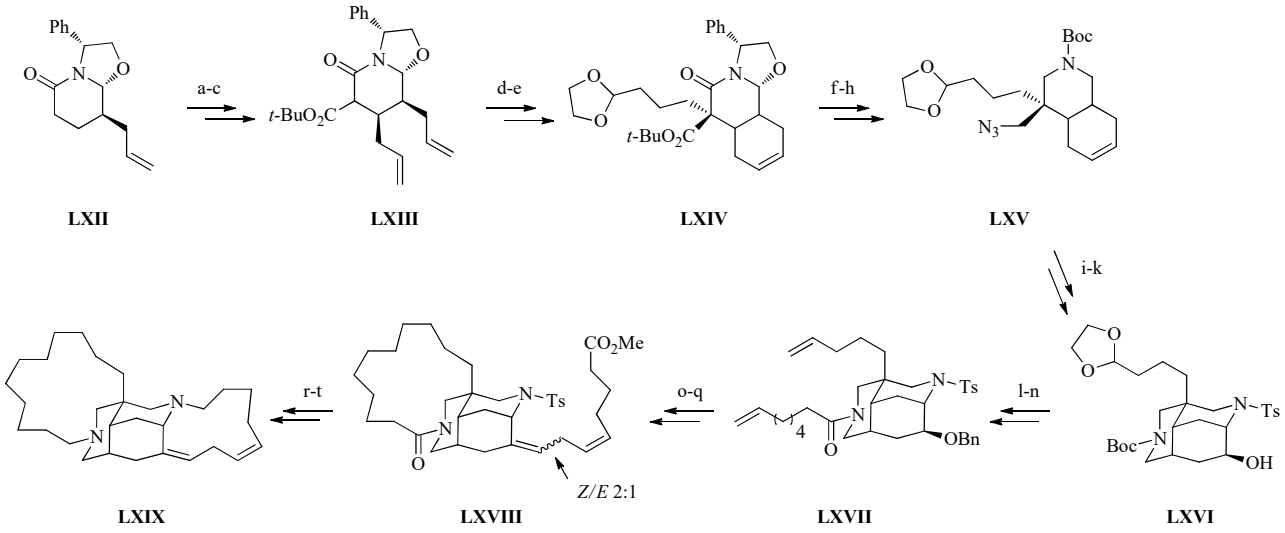
LVIII **LIX** **LX** **LXI**

Scheme 1.9: n = 5,6 or 7. (a) PhSH, K_2CO_3; (b) carboxylic acid chloride, TEA; (c) Grubbs II; (d) H_2, Pd/C; (e) $LiAlH_4$.

Removal of nosyl group followed by amidation between secondary amine and a carboxylic acid with a terminal double bond (constituted by 9, 10 or 11 members, to obtain D ring of madangamine E, D or F respectively) afforded intermediate **LIX**. The RCM was undertaken using Grubbs second-generation catalyst, giving **LX**. Finally, reductions and *N*-debenzylation led to tetracyclic system **LXI**.

Akin to other reported strategies, the favorable number of steps cannot compensate the lacking of functionalization at C-3 that makes the final annulation of E ring impossible.

Finally, in 2014, the research group of Mercedes Amat and Joan Bosch accomplished the first total synthesis of a member of madangamine family[20], with a strategy tuned during a work of many years (**Scheme 1.10**). The construction of madangamine D allowed to obtain the absolute configuration of madangamines which, until that moment, was only been inferred by correlation with that of their presumed biosynthetic precursors, the ingenamines. Furthermore, the obtaining of pure madangamine D allowed to run a cytotoxicity test, because it was always isolated in a mixture with other madangamines. Madangamine D showed significant in vitro cytotoxic activity against human colon HT29 (GI_{50} 4.4 µg mL^{-1}) and pancreas PSN1 (GI_{50} 7.4 µg mL^{-1}) cancer cell lines.

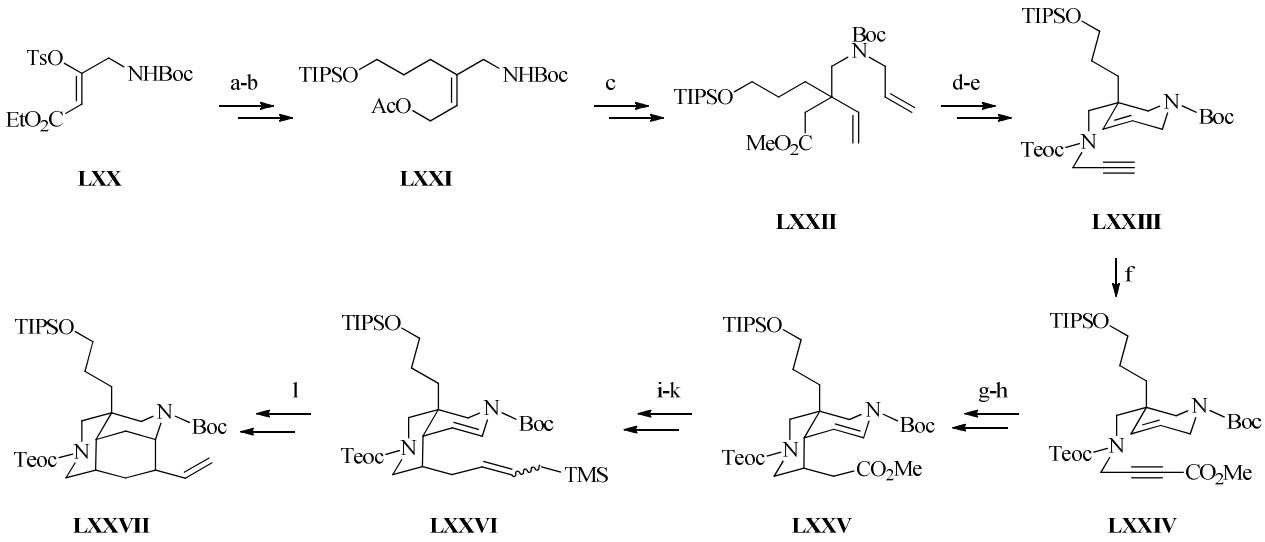


Scheme 1.10: (a) LiHMDS, $(Boc)_2O/C_6H_5SeCl$; (b) H_2O_2; (c) AllMgBr, TMSCl; (d) Grubbs II; (e) NaH, $(CH_2O)_2CH(CH_2)_3Br$; (f) Na, $NH_3/LiAlH_4/(Boc)_2O$; (g) TEA, MsCl; (h) NaN_3; (i) *m*-CPBA; (j) Me_3P; (k) TsCl, TEA; (l) NaH, BnBr; (m) TFA/$ClCO(CH_2)_5CH=CH_2$, TEA; (n) HCl/*t*-BuOK, Br^- $Ph_3P^+CH_3$; (o) Grubbs I; (p) H_2, Pd/C/DMP; (q) NaHMDS, Br^- (*Z*)-$Ph_3P^+CH_2CH_2CH=CH(CH_2)_3CO_2Me$;(r) Na, naphthalene; (s) LiOH/EDCI, HOBt; (t) $LiAlH_4$.

In the Amat-Bosch scheme, the enantiopure lactam[36] LXII was converted to LXIII with a conjugate addition of an allyl chain and then the intermediate LXIV was obtained by a RCM and a stereoselective alkylation. At this point, the removal of the chiral auxiliary followed by protection of the resulting nitrogen with Boc and the conversion of hydroxyl to azide afforded product LXV. Oxidation of bicyclic LXV led to the formation of an epoxide, and the azide moiety was reduced with a Staudinger reaction to a primary amine. The spontaneous cyclization and protection of the resulting secondary amine moiety afforded the diazatricyclic core of madangamines (**LXVI**). The next step was the construction of D ring, achieved with a RCM between the terminal double bonds of two alkyl chains (**LXVII**). One of the chains was obtained with an acylation of nitrogen with 7-octenoyl chloride, and the other via a Wittig methylenation of unprotected acetal moiety. Reduction of the resulting double bond led to saturated ring. The last E ring was assembled with a Wittig reaction of the ketone moiety anchored to the tetracyclic system, followed by the amidation between the carboxyl and the cyclic secondary amine.

The construction of E ring in this strategy shows some weaknesses: the Wittig reaction between the alkyl chain and the ketone moiety of the tetracyclic system has just a selectivity *Z/E* 2:1.

The most recent approach to the tricyclic core of madangamines was reported by Chida[37] in 2015, who started from the synthesis of the *cis* fused diazadecalin structure (**Scheme 1.11**).



LXX **LXXI** **LXXII** **LXXIII** **LXXIV** **LXXV** **LXXVI** **LXXVII**

Scheme 1.11: (a) AllOTIPS, 9-BBN/$PdCl_2$, Cs_2CO_3; (b) (i) DIBAL-H, $BF_3 \cdot Et_2O$; (ii) Ac_2O, py, DMAP; (c) (i) AllBr, NaH; (ii) $MeC(OMe)_3$, *t*-$BuCO_2H$; (d) (i) $HCONH_2$, NaOMe; (ii) 2-TMS-ethanol, $PhI(OAc)_2$; (e) (i) Grubbs I; (ii)$HC{\equiv}CCH_2Br$, NaH; (f) $PdCl_2$, CO, NaOAc; (g) $Pd_2dba_3 \cdot CHCl_3$, HCO_2H; (h) $NaBH_4$; (i) MeNHOMe·HCl, *i*-PrMgCl; (j) DIBAL-H; (k) (i) Ph_3PCH_3Br, *n*-BuLi; (ii) AllTMS, Grubbs II; (l) $BF_3 \cdot Et_2O$.

Enol tosylate **LXX** gave, via a Suzuki-Miyaura coupling, a reduction of the ester and an acetylation, the allylic acetate **LXXI**. The *N*-allylation of **LXXI**, followed by acetate methanolysis, and a subsequent Johnson-type Claisen rearrangement with $MeC(OMe)_3$ provided intermediate **LXXII**. The resulting methyl ester **LXXII** was subjected to a sequence of amidation and Hofmann rearrangement. A subsequent RCM, followed by *N*-propargylation, afforded enyne **LXXIII**. This alkyne was then carbonylated to give **LXXIV** that led to the AB-ring system in presence of $Pd_2dba_3 \cdot CHCl_3$. Methyl ester **LXXV** was converted to **LXXVI** via a Wittig reaction and a cross-metathesis reaction. A stepwise cyclization finally provided the diazatricyclic core.

This synthetic sequence led to a product with functionalization both on C-3 and C-9, but the transformation of the single bond on C-3 to a *Z* double bond remains a really hard task, especially without changing the procedure for the closure of C ring.

1.5. CONCLUSIONS AND FUTURE PERSPECTIVES

Beyond the intrinsic interest connected with their nature and chemistry, madangamines may also result a possible tool in cancer therapy. Cancer is a complex mixture of diseases without a definitive cure and cause millions deaths per years. A solution could be found in scientific research

and in testing new isolated natural substances with interesting pharmaceutical properties. In this context, madangamines, a class of alkaloids recently isolated from a marine sponge, are showing interesting cytotoxic activities *in vitro*, and new tests *in vivo* could confirm the possible potential application of these compounds as drugs. Unfortunately, the current methods of isolation of these alkaloids cannot permit to have enough material for an extensively use of these substances. For this reason, organic chemists are searching new strategies to obtain madangamines. The synthetic routes reported in literature address mainly the diazatricyclic core of madangamines, contemplate a large number of steps and not always allow the construction of last two external macrocycles. Moreover, although some approaches are reported to get the two external macrocycles, a comprehensive convenient madangamines synthesis integrating the different cyclic and macrocyclic units is still lacking.

2. OBJECTIVES AND SYNTHETIC STRATEGY

The approach of the Mercedes Amat group to madangamine alkaloids can be divided in two synthetic parts which include: 1) the construction of the fully-functionalized bridged diazatricyclic central core, common to all madangamines from A to E, using a general methodology for synthesis of enantiopure *aza*-heterocycles from phenylglycinol-derived oxazolopiperidone lactams[38] as chiral scaffolds, and 2) the subsequent annulations of the macrocyclic rings D and E. In this context, during my stage carried out in her research group, my studies focused on the development of a new and efficient methodology for the assembly of the macrocyclic E ring and the construction of the fully functionalized tricyclic core of these alkaloids as key steps for the enantioselective synthesis of the ABCE system of madangamines A-E.

The stepwise strategy can be outlined in few critical points:

- The assembly of the common chiral diazatricyclic nucleus of madangamines, taking advantage of the chiral induction given by (*R*)-phenylglycinol which can be considered a source of chiral ammonia (**Figure 2.1**).

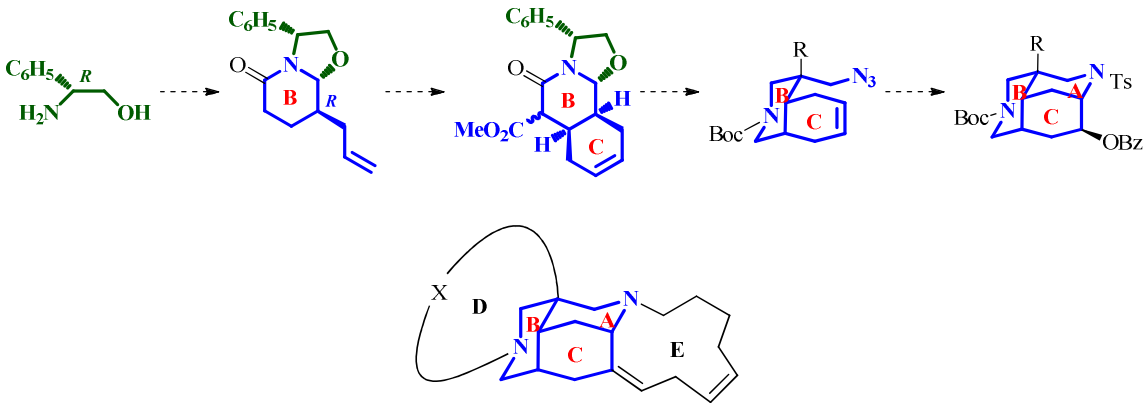


Figure 2.1: Assembly of ABC system.

- The synthesis of a model system of these alkaloids constituted by A and C rings (**Figure 2.2**).

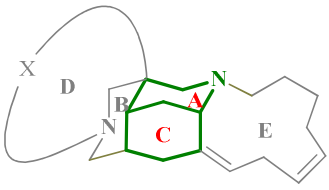


Figure 2.2: Construction of the model system.

- The construction of the unsaturated eastern E ring of madangamines with a stereo-controlled formation of the (Z)-double bond between C-3 and C-20 and its eventual incorporation to the ABC tricyclic system (**Figure 2.3**).

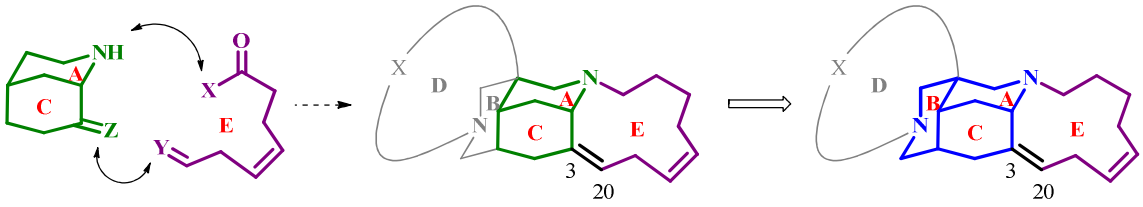


Figure 2.3: Construction E ring.

Starting from the total synthesis of madangamine A, the most biologically active member of this group of alkaloids, these efforts will pave the way to a comprehensive access route to the other members of the madagamine family.

3. RESULTS AND DISCUSSION

3.1. CONSTRUCTION OF THE DIAZATRICYCLIC CORE

Piperidine rings are commonly present in many bioactive natural products, in particular in alkaloids, and their obtaining by a simple synthesis could be a powerful tool in the construction of a large plethora of potential drugs owning this kind of structure. In recent years, the Amat-Bosch research group focused its attention on the synthesis of oxazolopiperidone lactams starting from phenylglycinol[38], as source of enantiopure substituted piperidines.

These lactams were synthetized for the first time by Meyers in 1984 via a cyclocondensation between a 1,5 dicarbonylic ester and phenylglycinol paving the way to the preparation of iminosugars and piperidine and tetrahydroisoquinoline alkaloids[39]. These "first-generation oxazolopiperidone lactams" can undergo a stereoselective introduction of various substituents at different positions (except position 5) of the piperidine ring, controlled by their conformational rigidity. The Amat-Bosch research group prepared many enantiopure piperidine derivatives following this synthetic strategy (**Figure 3.1**).

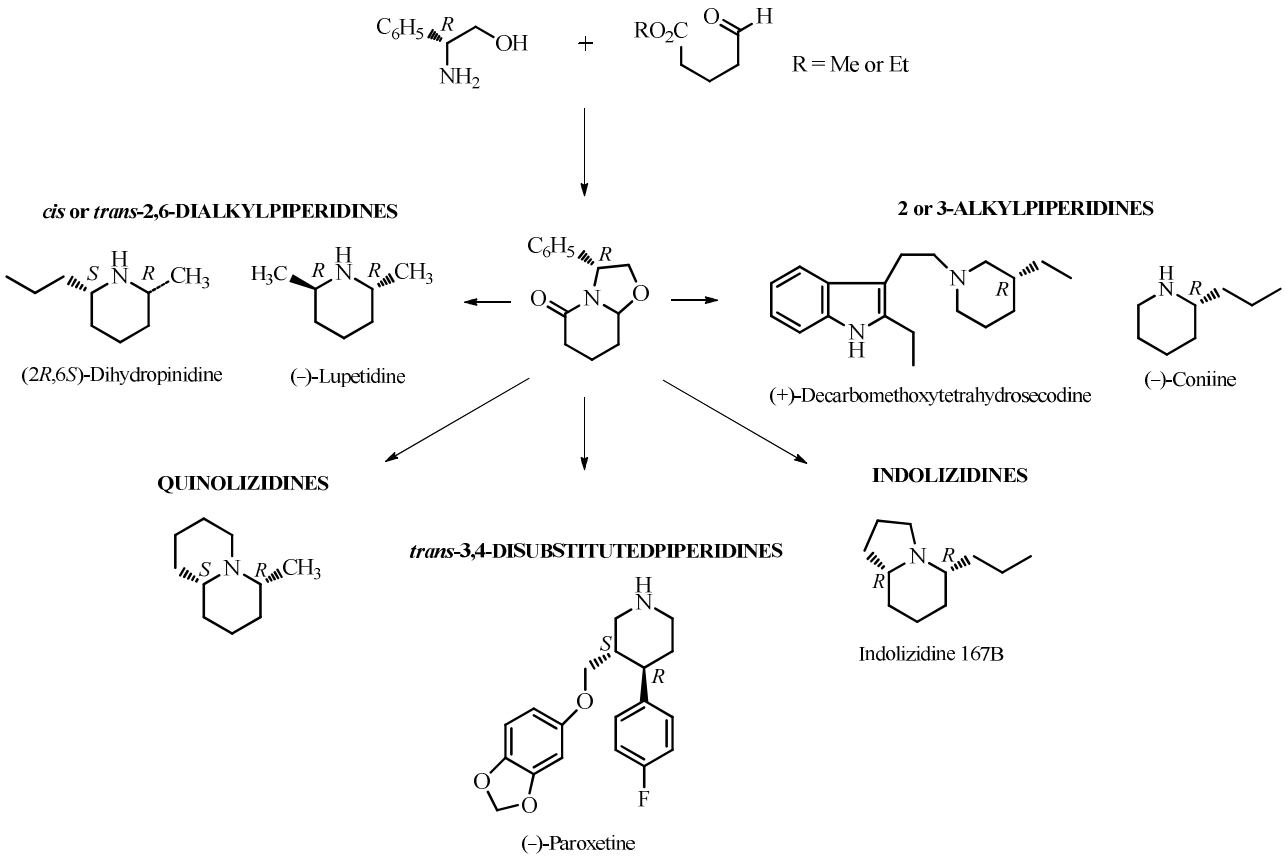


Figure 3.1: Few examples of first generation oxazolopiperidone lactams prepared by the research group starting from enantiomer *R* of phenylglycinol.

Later on, a more straightforward procedure for the synthesis of enantiopure polysubstituted piperidines was developed by the research group, leading to the construction of "second-generation oxazolopiperidone lactams". Phenylglycinol reacts with a dicarbonylic ester already bearing appropriate substituents that will give a α,β and/or γ enantiopure polysubstituted piperidines. Amat-Bosch research group synthetized a huge library of enantiopure alkaloids featuring higher complexity with respect to the previous synthetized ones (**Figure 3.2**).

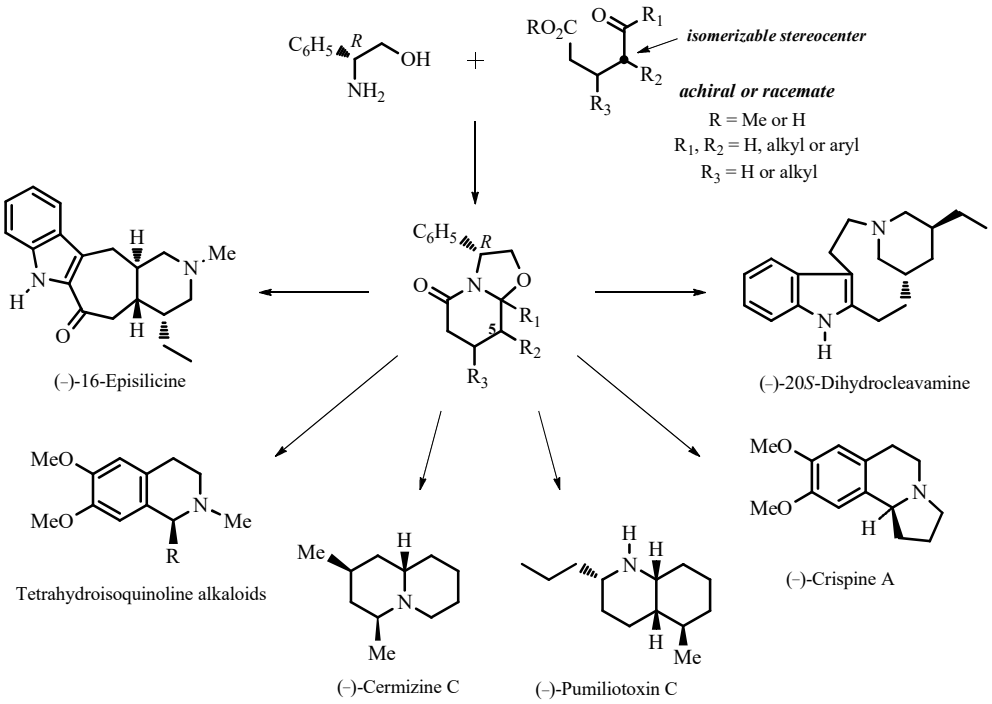
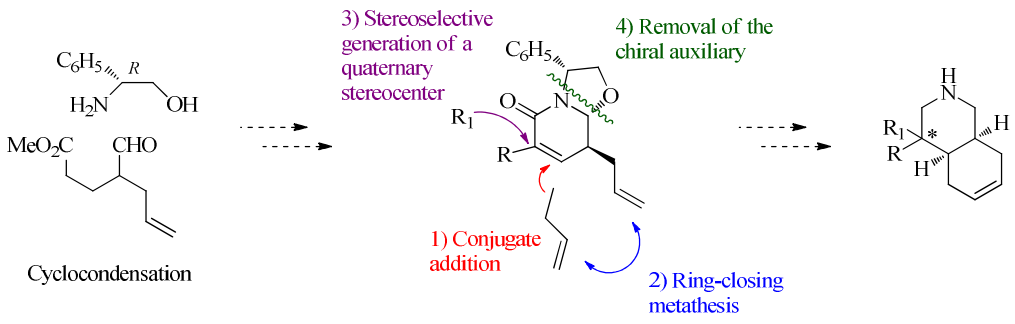


Figure 3.2: Few examples of second generation oxazolopiperidone lactams prepared by the group starting from enantiomer *R* of phenylglycinol.

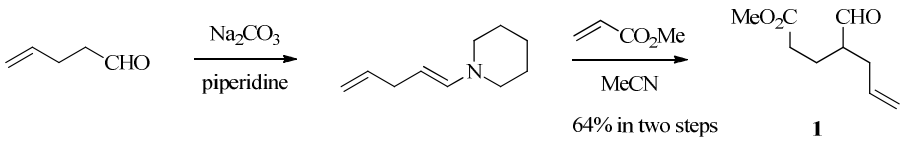
In light of these results, it was decided to take advantage form the experience of the group using this strategy to construct the BC ring system of madangamines, via a synthetic pathway allowing a multi-gram scale production of the bicyclic system[20]. The (*R*)-phenylglycinol and a dicarbonylic racemic ester bearing an allylic chain after a cyclocondensation gave access to functionalized piperidinic rings. The subsequent stereoselective conjugate addition stereoselectively introduces an allyl substituent required for the construction of the ciclohexene ring, built by a ring-closing metathesis. The final removal of the phenylethanol moiety of the chiral inductor produce a *cis* bicyclic system (**Scheme 3.1**).



Scheme 3.1: Synthesis of BC ring system.

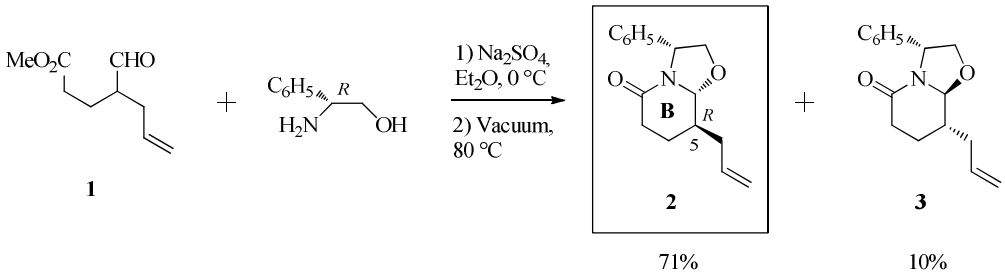
The first challenge of this thesis has been the construction of the chiral oxazolopiperidone lactam from a racemic oxoester, exploiting a dynamic kinetic resolution.

In the first approach 4-pentenal reacted with piperidine and then with methyl acrylate, producing the racemic 4-formyl-6-heptenoate (**1**) in an overall 64% yield (**Scheme 3.2**).



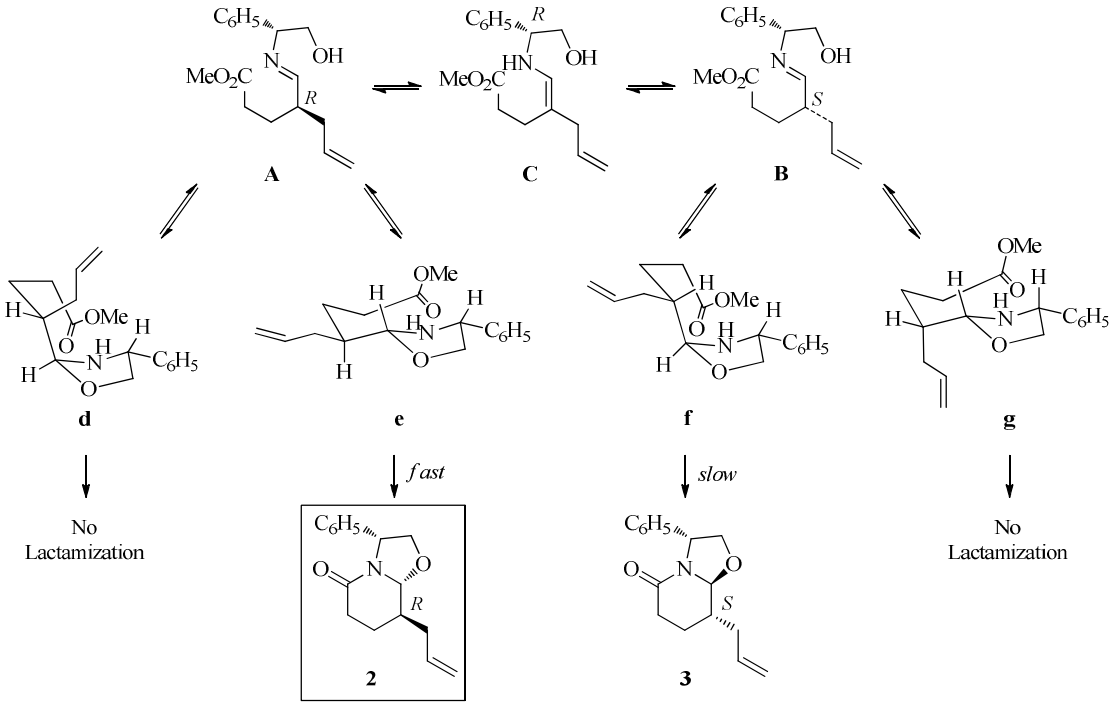
Scheme 3.2: Synthesis of the dicarbonylic racemic ester.

The cyclocondensation of this product with (*R*)-phenylglycinol in presence of Na_2SO_4 afforded the couple of isomeric lactams **2** and **3** in ratio 8.8:1.2 with an yield of 81%, leading to the B ring of madangamines and the first stereocenter *R* in position 5 (**Scheme 3.3**).



Scheme 3.3: Assembly of B ring.

These results indicated that a dynamic kinetic resolution had occurred during the cyclodehydration reaction (**Scheme 3.4**): the interaction of (*R*)-phenylglycinol and **1** generate two imines (**A** and **B**) in equilibrium via enamine **C**. These two imines can generate four diastereoisomeric oxazolidines (**d**-**g**), but only the two isomers in with the allylic chain is in equatorial position in the chair-like transition step (**e** and **f**) lead to an irreversible lactamization. The formation of oxazolidine **2** is faster than its diastereoisomer **3** because the methyl ester approaches to the nitrogen atom from the less hindered face of the oxazolidine ring (**Scheme 3.4**).

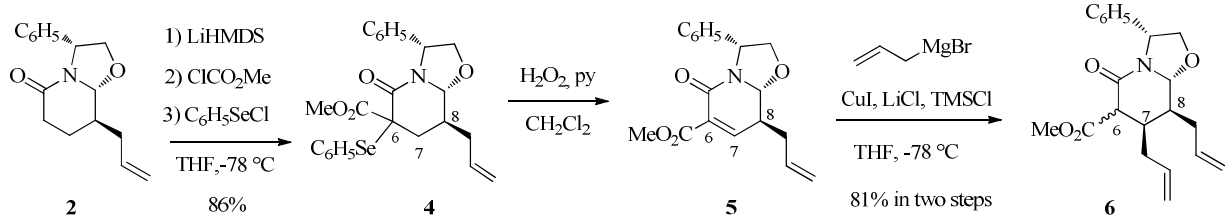


Scheme 3.4: Dynamic kinetic resolution for B ring fabrication.

The next addressed step was the construction of carbocyclic C ring.

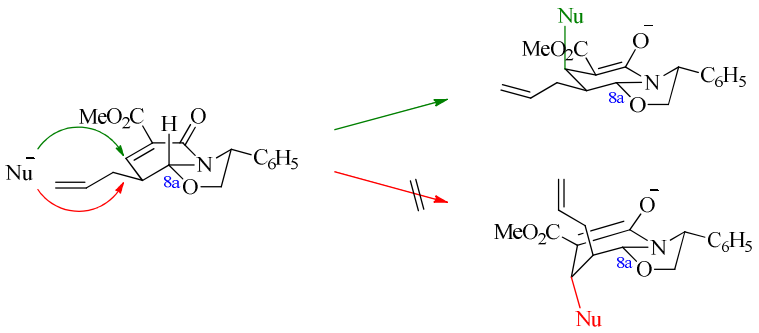
To build the skeleton of the ring it has been employed a highly stereoselective conjugated addition of organocuprates to this unsaturated lactam **5** with another procedure developed by Amat-Bosch research group[38].

Since α,β-unsaturated lactams are poor Michael acceptors, the introduction of an electron withdrawing group[40] as methoxy carbonyl at the C-6 position was required in order to improve the reactivity of these compounds towards the addition of organocuprates[41], and of a phenylselenium group that could lead, via an oxidation step, to unsaturated lactam **5**. The conjugate addition of the allylcuprate (formed *in situ*) afforded the mixture of isomerizable epimers at C-6 (**6**) with an *exo*-facial diastereoselectivity (**Scheme 3.5**). This process allowed to synthesize an important intermediate, direct precursor of the BC ring system, and to install the second contiguous stereocenter at the C-7 position of madangamine.



Scheme 3.5: Construction of intermediate **6**.

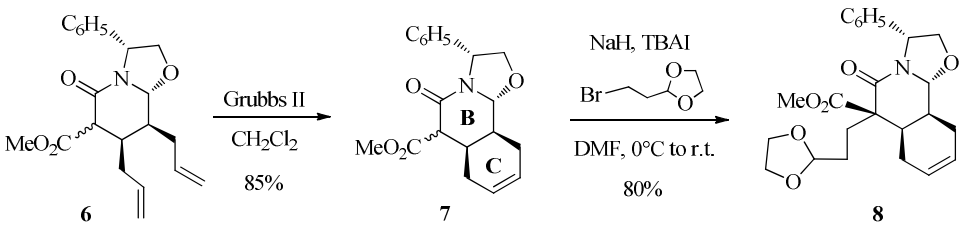
The stereoselectivity of the process is due to the rigid structure of the molecule caused by the amide bond. The conformation of the six-membered ring is determined by the configuration of C-8a (**Scheme 3.6**).



Scheme 3.6: Stereoselective control in conjugate addition.

The attack of the nucleophile is axial to the electrophilic carbon leading a chair-like transition state (the lower energy intermediate at low temperatures), in a process that involves a stereoelectronic control. The attack on the endo face would lead to a boat-like transition state and is kinetically disfavored.

The synthesis of the tricyclic lactams **7**, including the generation of C ring of madangamines, occurred with a ring closing metathesis catalyzed by a 2nd generation Grubbs catalyst. The alcoxycarbonyl group present in starting lactam **6** not only increased the acidity of the C=O α position, to permit the conjugate addition and the subsequent alkylation, but also acted as a precursor of the aminomethyl chain required for the synthesis of the A ring of the tricyclic core. The alkylation of **7** occurred from the more accessible face, the less hindered *exo*-face, giving compound **8** as single product, producing the chiral center in position 6, that coincide with sterocenter in position 9 of madangamine (**Scheme 3.7**).

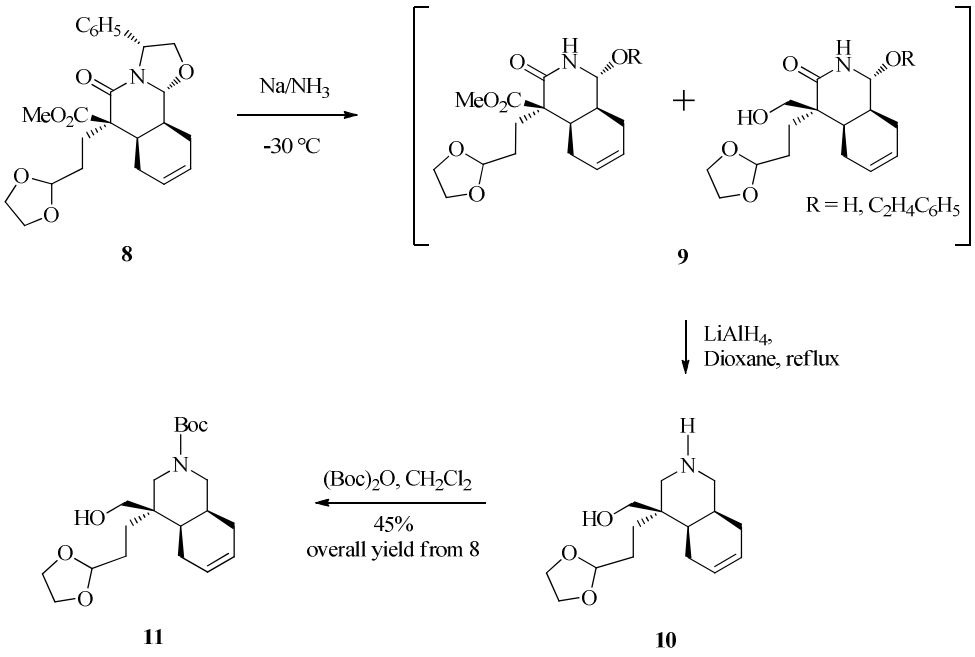


Scheme 3.7: RCM and alkylation.

The next step is the removal of the chiral inductor. Previous studies on similar molecules demonstrated that the treatment of the lactam with sodium metal in liquid ammonia followed by a

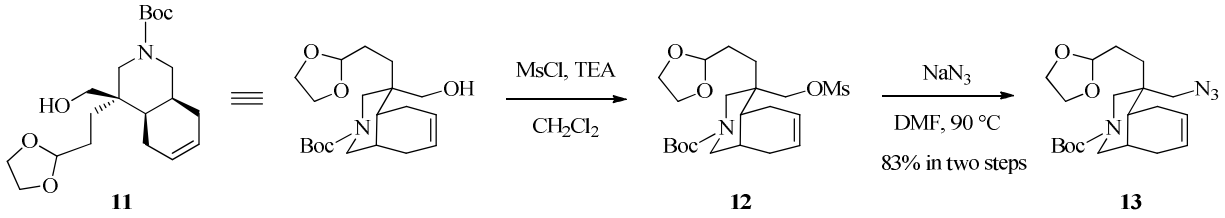
strong reduction with $LiAlH_4$ gave the best results. In fact, reaction with $Pb(OAc)_4$, CAN, HCl, Lewis acids, alkali metals encapsulated in silica gel (Na-K-SG)[42], *trans*-acylating reagents (1-chloroethyl chloroformate)[43], did not give the desired product. Reaction with $LiAlH_4$ followed by mesylation and elimination gave only byproducts.

Thus, the reaction with sodium in liquid ammonia gave a mixture of unstable alkoxy(hydroxy) lactams **9**, that were immediately treated with an excess of $LiAlH_4$ to give, as only product, the aminoalcohol **10**, which was finally converted in to the *N*-Boc derivative **11** with an overall yield of 45% (**Scheme 3.8**).



Scheme 3.8: Removal of the chiral inductor.

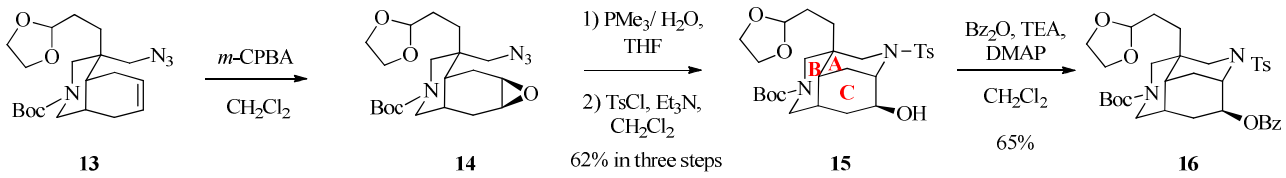
To build the A ring it has been necessary the conversion of the hydroxylmethyl group in position 4 to an azidomethyl chain, that could constitute afterwards the secondary amine moiety of the A piperidine ring. This interconversion has been accomplished via mesyl derivative, leading to **13** via a long reaction due to the steric hindrance of the bicyclic system (**Scheme 3.9**).



Scheme 3.9: Conversion of alcoholic moiety to azide.

The annulation of A ring was previously accomplished by Amat-Bosch research group with Weinreb methodology[29], which involves an aminomercuriation reaction followed by oxidation, but the complicate experimental protocol led the group to a change of strategy with a more wieldy one.

Azide **13** was oxidized stereoselectively to the single isomer epoxide **14** with *m*-CPBA. The azide moiety of **14** was converted to amine with a Staudinger reduction in presence of PMe_3, and spontaneously attacked the epoxide ring, leading to the ABC tricyclic system. The secondary amine of this derivative was immediately protected with a tosyl, affording **15**, and the alcoholic moiety at C-3 was converted to benzoyl ester, giving finally the desired protected ABC diazatricyclic core of madangamines **16** (**Scheme 3.10**).



Scheme 3.10: Construction of the ABC system.

3.2. ASSEMBLY OF E RING

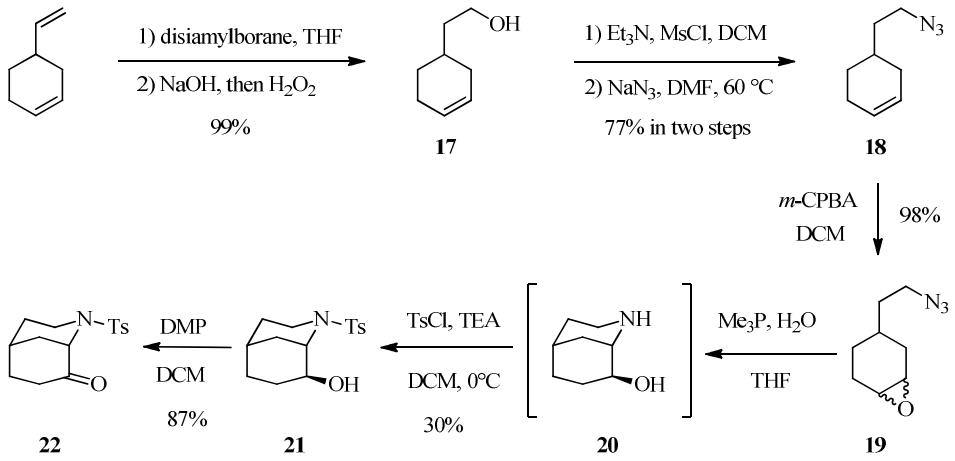
Having achieved the first purpose, new procedures to synthetize stereoselectively the E macrocycle have been explored. The construction of this ring is the most complicated step in the synthesis of madangamine, because it includes the challenging skipped (*Z*,*Z*)-diene. To avoid the waste of the ABC diazatricyclic core, a model compound was employed to evaluate the synthetic strategies only involving the AC ring moiety (**Figure 3.3**).

Figure 3.3: Model system.

3.2.1. PREPARATION OF THE MODEL SYSTEM

In order to explore as much as possible different strategies, the synthesis of a model system, less expensive and more rapid than the diazatricyclic core of madangamine, was required to get a morphan derivative including the AC ring system of the nucleus of the alkaloids.

To this aim, the exocyclic double bond of commercially available 4-vinylcyclohexene was selectively hydroborated, and after an oxidative work-up the alcohol **17** was obtained, that was converted to azide **18** with a similar but easier procedure used for the previously described **13**. The annulation of the piperidine ring was achieved after the oxidation of the endocyclic double bond to the corresponding diastereoisomeric epoxides **19** and the Staudinger reduction of azide moiety intermediates followed by a spontaneous cyclization. The nitrogen of the obtained unstable intermediate **20** was selectively protected with a tosyl group with a satisfying overall yield, considering the impossibility of the epoxide *syn*-oriented to the alkyl chain to cyclize. Mild oxidation of this alcohol **21** using Dess-Martin periodinane provided the target AC bicyclic compound **22** in 87% yield (**Scheme 3.16**).

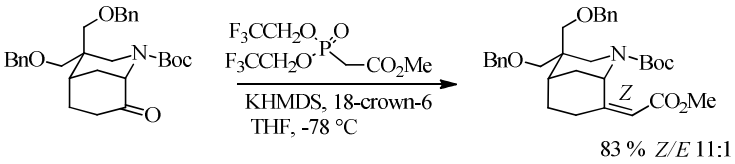


Scheme 3.16: Construction of the model system.

3.2.2. PREVIOUS SYNTHETIC APPROACHES

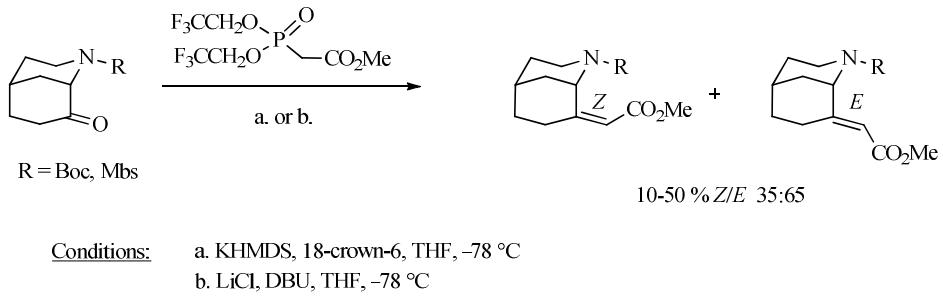
In early studies, the Amat and Bosch research group had investigated different approaches to the construction of the E ring. The first strategy contemplated the closure of the ring via a stepwise

strategy, because the hardest challenge was the build-up of the exocyclic *Z* double bond. It was initially considered the use of the Still-Gennari modification (HWE-SGM)[44] of the Horner-Wadsworth-Emmons reaction (HWE), a method that contemplate the use of methyl [bis(trifluoroethyl)phosphono]acetate in presence of KHMDS/18-crown-6 in THF. The mechanism of this reaction is not completely understood, but there is a general consensus that the stereoselectivity is thermodynamically controlled. This reaction had been successfully reported by Kibayashi on a similar morphan skeleton[32] (**Scheme 3.11**).



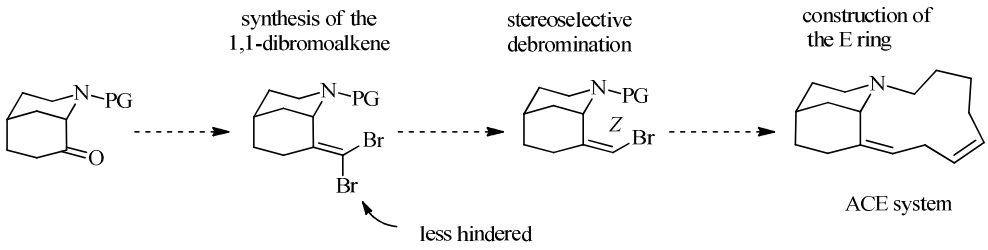
Scheme 3.11: Kibayashi proposal.

Disappointingly, although excellent stereoselectivity was reported by Kibayashi, any attempt to induce the same *Z* stereoselectivity in the model compounds failed, as a mixture of isomeric compounds was obtained in very low yields and the desired *Z*-olefin was the minor product of the reaction with protected morphan systems. Probably steric factors play an important role in this step. The employment of a modified protocol (**b**), reported by Masaume and Roush[45], gave just an insufficient slight improvement (**Scheme 3.12**).



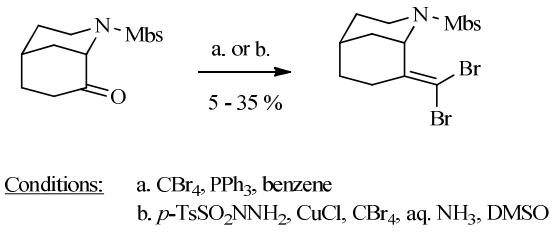
Scheme 3.12: Still-Gennari reaction on AC ring system.

In a further approach, the conversion of the ketone into a dibromoalkene moiety was tried, in the hypothesis that the most accessible Br atom might be prone to a chemoselective debromination into *E*- and *Z*-isomers of terminal vinyl bromides for the steric hindrance of the molecule (**Scheme 3.13**).



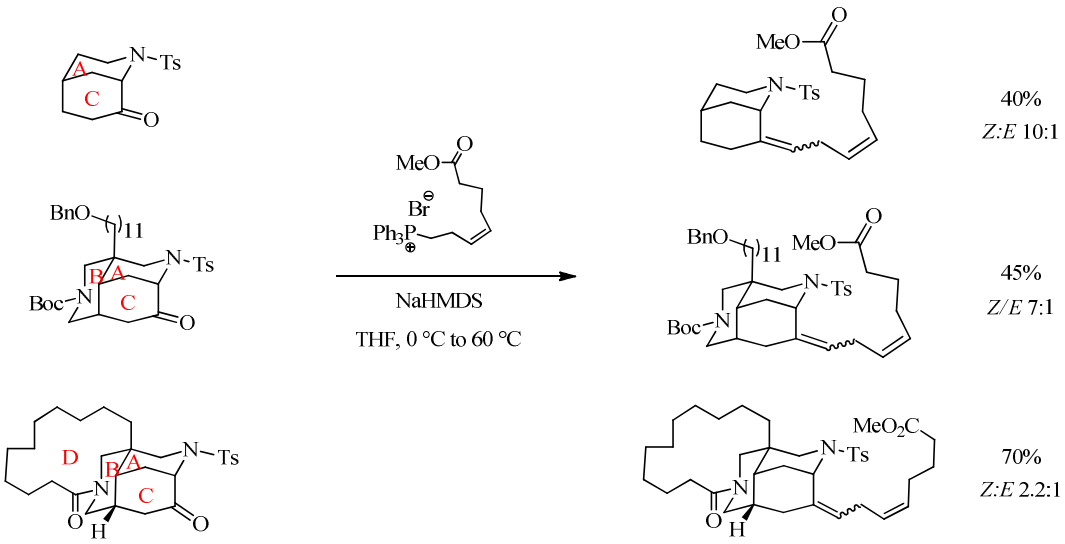
Scheme 3.13: Strategy for chemoselective debromination.

Both the approaches, the Wittig-type reaction with CBr_4[46] and the catalytic methodology involving the conversion of a tosylhydrazone derivative[47], gave very low yields (**Scheme 3.14**).



Scheme 3.14: Formation of the dibromoalkene derivative.

Following these attempts, a more direct route was explored, that involves the installation of a carbon chain with a *Z*-selective Wittig reaction and the annulation of the E ring through an intramolecular macrolactamization. This strategy was tested on the same AC model system, and a more complicated system bearing the ABC ring core, and in both cases the desired *Z*-exo-olefin was obtained as major product. Unfortunately, when the Wittig reaction was tested on the tetracyclic system, a strong erosion of stereoselectivity was observed[20] (**Scheme 3.15**).



Scheme 3.15: Wittig reaction.

In light of these results, a completely different strategy was clearly needed.

3.2.3. NEW SYNTHETIC APPROACHES

Three different strategies are in study to face this hard challenge, based on a former amidation between a long linear alkyl chain and the nitrogen of the morphan and a subsequent reaction of annulation. The new synthetic proposal are outlined below:

- The RCM metathesis via Grubbs catalysts strategy: a construction of a linear alkyl chain of nine carbons bearing in positions 5 and 8 two double bonds (the former *Z*), followed by amidation with the nitrogen of the morphan and a final ring-closing metathesis promoted by Grubbs catalysts between the two double bonds.
- The McMurry strategy: The synthesis of a similar alkyl chain of eight carbons bearing in position 5 a *Z* double bond and in position 8 a protected (or masked) aldehyde moiety, which could give, after the amidation, a McMurry reaction with the carbonyl of the morphan.
- The olefin metathesis with Schrock catalyst: The amidation of the former alkyl chain with the two double bonds and the model system, followed by an olefin metathesis promoted by Schrock catalyst (**Figure 3.4**).

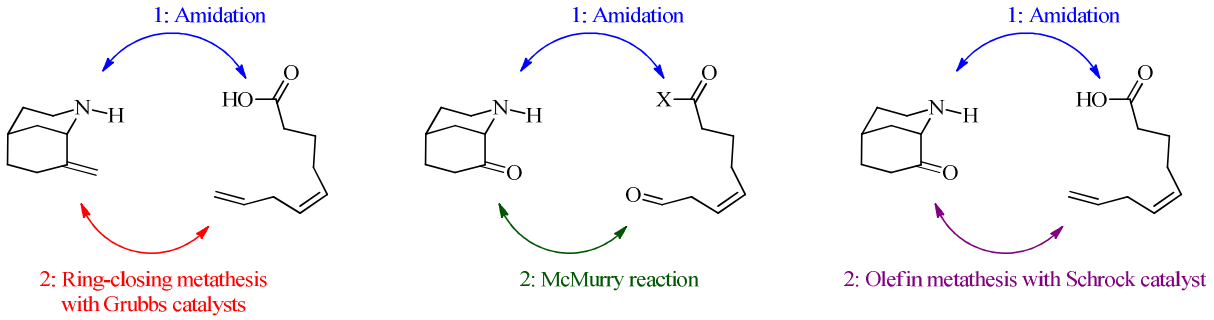


Figure 3.4: General synthetic plan.

3.2.4. THE RING-CLOSING METATHESIS VIA GRUBBS CATALYSTS STRATEGY

Olefin metathesis involves a rearrangement of unsaturated carbon-carbon bonds in presence of metal carbene catalysts[48]. In most of cases this reaction is used to form C-C bonds with the production, as the sole byproduct, of the volatile and easy to remove ethylene thus increasing the interest of both academic and industrial scientists. Generally we find the olefin metathesis in three related groups of reaction, the ring-opening metathesis polymerization, the ring-closing metathesis and acyclic cross metathesis.

The ring-closing metathesis (RCM) is employed for the synthesis of medium and large sized rings starting from acyclic diene precursors, and is preferred to other reaction for its adaptability to different substrates matched to the tolerance towards many functional groups. Furthermore, a large number of catalysts can be employed in this reaction, though the molybdenum and ruthenium complexes are the most utilized (**Figure 3.5**).

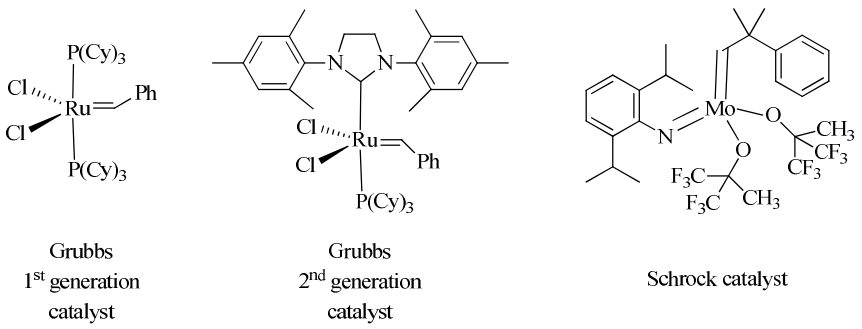


Figure 3.5: Examples of olefin metathesis catalysts.

These catalysts (in particular the Ru-carbene systems), are active also in presence of air, moisture or low impurities, and can be stored in air atmosphere, and for this reason are the most used catalysts.

A series of [2+2] cycloadditions and cycloreversion compose the general mechanism of olefin metathesis[49]. An equilibrium is established between all the intermediates, and for this reason is necessary to move this equilibrium in favour of the products, to avoid to get a mixture of olefins. One of the products of the general mechanism is the volatile ethene, so the process is entropically shifted on the desired cycloalkane (**Figure 3.6**).

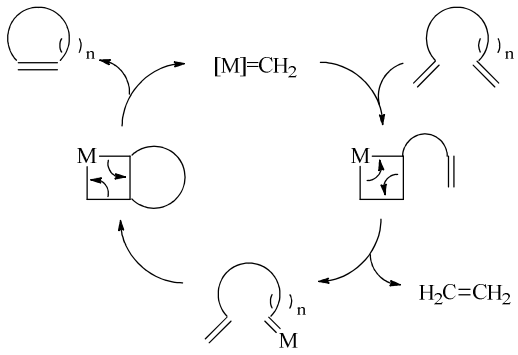


Figure 3.6: Olefin metathesis catalytic cycle.

As depicted in the above figure, a [2+2] cycloaddition between the catalyst and the diene leads to a metallocyclobutane intermediate, which evolves to a metal-carbene compound via a [2+2] cycloreversion with a loss of ethylene. This intermediate gives another intramolecular cycloaddition affording an instable bicyclic derivate, which opens giving the desired cyclic product and the starting catalyst.

If the reaction is not sufficient diluted, the acyclic diene metathesis polymerization can compete with the ring-closing metathesis, in particular in case of macrocyclization. The competing reaction is more important with substrates featuring an increased ring size and conformational renstriction, but the slow addition of the substrate and high temperatures can favour the ring closure[50].

The first explored strategy for the cyclization of E ring was the one concerning the RCM. According to this approach, the final annulation of the E ring would involve a terminal olefinic chain (previously enchained to the system with an amidation) and a double bond anchored to the morphan **(Figure 3.7).**

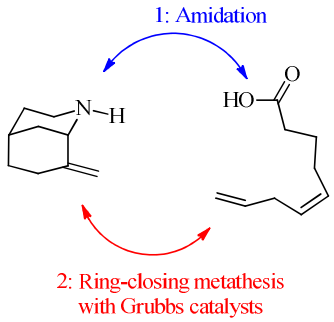
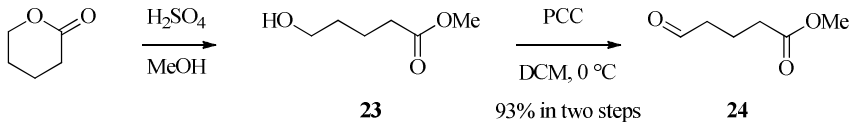


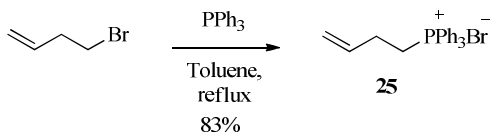
Figure 3.7: RCM strategy.

The first step was the construction of the alkyl chain that will constitute the skeleton of E ring. To build a long linear alkyl chain with a *Z* double bond, it was necessary a Wittig reaction between smaller carbon fragments. Moreover, the chain needs an ester at an extremity so to allow an amidation reaction with the secondary amine of the model system. The linear C-5 fragment with a terminal ester (methyl 5-oxopentanoate (**24**)) was easily prepared by methanolysis of commercial *δ*-valerolactone and subsequent oxidation of the instable intermediate **23** with PCC (**Scheme 3.17**).



Scheme 3.17: Synthesis of ester aldehyde **24**.

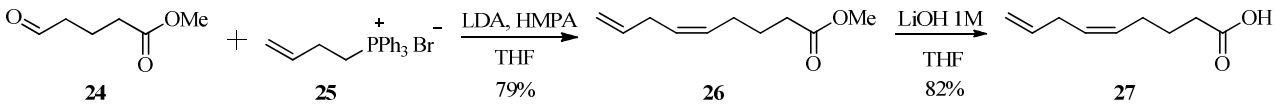
To carry out a Wittig reaction it was necessary the generation of a phosphonium salt. Salt **25** was easily obtained by reacting 4-bromo-1-butene with triphenylphosphine in refluxing toluene (**Scheme 3.18**).



Scheme 3.18: Preparation of the phosphonium salt **25**.

Different bases were investigated to promote the Wittig reaction between **24** and **25**. Use of NaHMDS produced compound **26** only in traces, whereas *n*-BuLi allowed a slight increase in yield (40%). Finally, the use of LDA, in presence of HMPA, afforded ester **26** in a good 79% yield.

Basic hydrolysis of alkyl chain **26** with an aqueous solution of LiOH produced acid **27** in a fairly good yield (82%) (**Scheme 3.19**).



24 **25** **26** **27**

Scheme 3.19: Construction of acid **27**.

The ring-closing metathesis involves double bonds, so it was necessary to transform the ketone moiety of the bicyclic model system **22** (**Scheme 3.16**) into an alkene.

One of the most explored reactions in organic synthesis is the alkylidenation of aldehydes and ketones. The most applied technique is the Wittig reaction, that presents various limitations associated to the steric environment and to moisture sensibility of the intermediates. In 1978 Tebbe[51] synthetized a library of titanium-based reagents that could transfer a methylene via a tetracyclic intermediate with a compound bearing a carbonyl with a mechanism similar to one of the olefin metathesis (**Figure 3.8**).

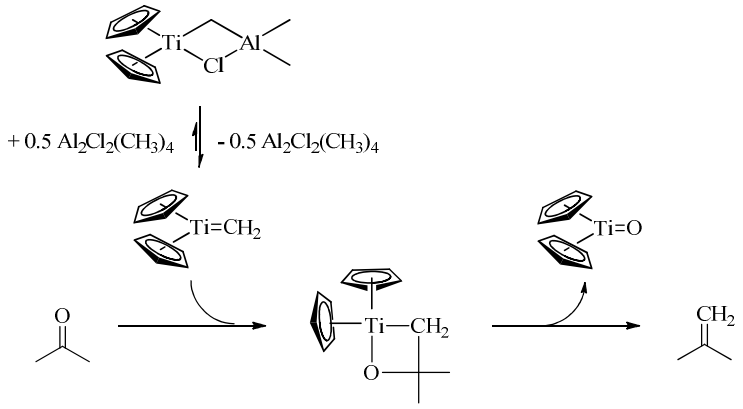


Figure 3.8: Tebbe reagent mechanism.

In presence of a Lewis base, a carbene is generated, that give a cycloaddition with a carbonyl, affording the unstable tetracyclic intermediate. The cycloreversion [2+2], particularly favoured by the oxophilicity of the Ti(IV), leads to the alkene and a titanium oxide species. The advantages of Tebbe reagent over the Wittig reaction are the requirement of less basic condition of reactions and the lower occurrence of β-elimination. Furthermore, the Tebbe reagent can add a methylene also to esters, lactones and amides and does not racemize chiral α carbons.

Petasis in 1990[52] proposed an alternative to the classical Tebbe reagent. The Petasis catalyst, a dimethyltitanocene, is less expensive, needs shorter preparation time, is less air or moisture sensitive and does not produce residual aluminium reagents. The mechanism of reaction is really similar to the Tebbe's one (**Figure 3.9**).

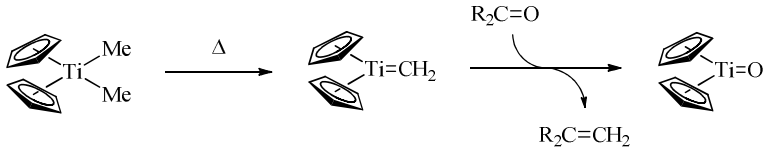
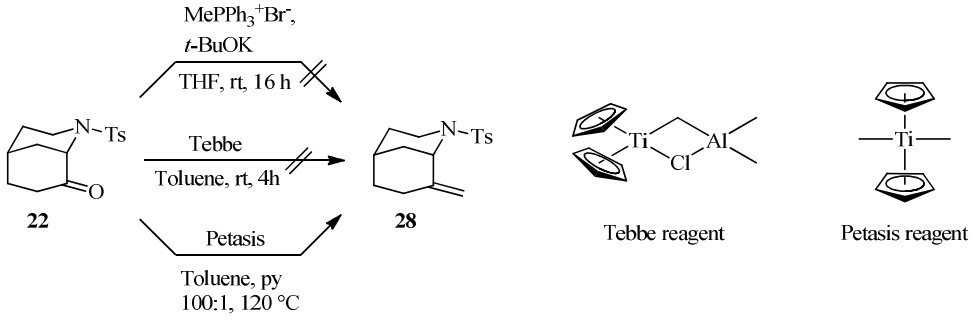


Figure 3.9: Petasis reagent mechanism.

The dimethyltitanocene is easily converted to the active carbenolic complex via a thermal α-elimination, that gives the same tetracyclic intermediate previously described with a carbonylic compound, and the same cycloreversion.

To convert the ketone moiety of the model system **22** (**Scheme 3.16**) to an olefin, a first route was explored, using a Wittig reaction, but only by-products were recovered. The second strategy involved the Tebbe reagent, but the unreacted starting product was always recovered. Petasis reagent was then examined under the conditions reported by Bennasar in 2006 for methylenation of *N*-acylamides[53]. This method provided the desired product **28** (**Scheme 3.20**), but an equilibrium between the starting material and the final product was established, limiting the production of the alkene; nevertheless, unreacted starting product can be recovered. Increase of either the catalyst loading or the reaction time only led to the formation of by-products (**Table 3.1**).



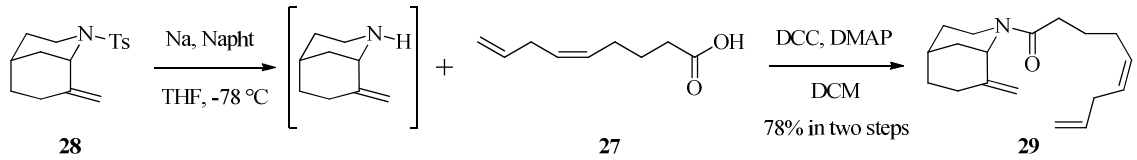
Scheme 3.20: Olefination of ketone **22**.

Equiv. Petasis	Reaction time	Yield (%)	Conversion	By-products
1.5	3h	39	65	Yes
2	5h	46	56	No
2	6h	47	69	No
2.5	5h + night	Traces	Traces	Yes
2.5 + 2.5[a]	5h + 5h[b]	38	60	No
5	4h + 4h[b]	29	34	Yes

a: Added after one day of reaction; *b*: reaction in two days, with night at room temperature

Table 3.1: Explored conditions for the olefination.

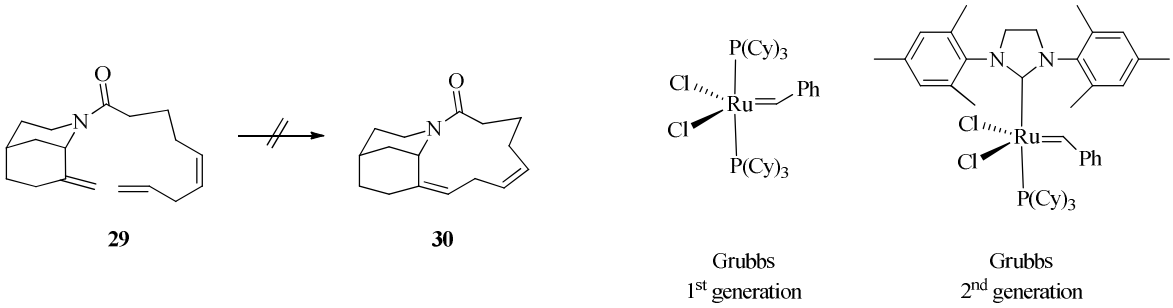
The synthetic route involves at this point the removal of the tosyl protecting group from alkene **28** upon exposure to sodium naphtalenide. The subsequent amidation with alkyl chain **27**, promoted by DCC and DMAP, led to olefinic compound **29** in a good yield (**Scheme 3.21**).



28 **27** **29**

Scheme 3.21: Synthesis of amide **29**.

A wide set of ring-closing metathesis conditions were screened on amide **29**, with both first and second generation Grubbs catalysts (**Scheme 3.22**); unfortunately, all these attempts met with failure (**Table 3.2**), giving byproducts or dimers, probably because of the low accessibility of the exocyclic double bond attached to the ring.



Scheme 3.22: Failed ring-closing metathesis.

Catalyst (%)	Solvent	Temperature / Reaction time	Dilution	Dimers and by-products
2nd gen. (10 + 10)[a]	Toluene	25 °C/ 4 hours 50 °C / 1 night 70 °C / 1 day 90 °C/ 1 day	0.1 mmol per 5 mL (0.02 M)	Yes
2nd gen. (10)	Toluene	120 °C / 2 days	0.1 mmol per 10 mL (0.01 M)	Yes
1st gen. (10)	CH_2Cl_2	40 °C / 24 hours	0.1 mmol per 200 mL (0.5 mM)	Yes
1st gen. (10 + 10 + 5)[a]	Toluene	50 °C / 1 day 70 °C/ 1 night 90 °C/ 1 day 120 °C / 5 days	0.1 mmol per 1 L (0.1 mM)	Yes

a: portionwise addition in different days

Table 3.2: Explored conditions for the ring-closing metathesis.

In the light of all these results, the strategy was slightly changed, planning to perform the closure of the E ring with a McMurry reaction.

3.2.5. THE MCMURRY STRATEGY

The second explored proposal was the one concerning the final annulation of the E ring via a McMurry reaction involving two carbonyls, one at the end of an alkyl chain (previously attached onto the bicyclic system with an amidation) and the second anchored to the morphan (**Figure 3.10**).

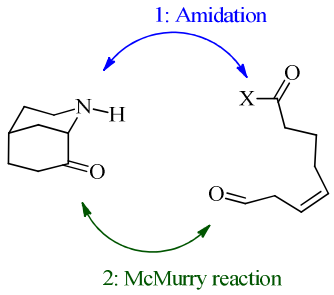


Figure 3.10: McMurry strategy.

The McMurry reaction was firstly described in 1974[54] and since then has a relevant role in organic chemistry. Both ketone and aldehydes in presence of low-valent titanium reagents can give a reductive dimerization by single electron transfer to afford alkenes. The original reagent system was composed of $TiCl_3$ and $LiAlH_4$, but the low reproducibility of the reaction lead McMurry to look for a new system, composed of $TiCl_3$ and Li, Zn-Cu, Mg or alkali (or the most used $TiCl_4$/Zn). The mechanism involves two step, the formation of the carbon-carbon bond via reductive dimerization and the deoxygenation of the pinacol intermediate (**Figure 3.11**).

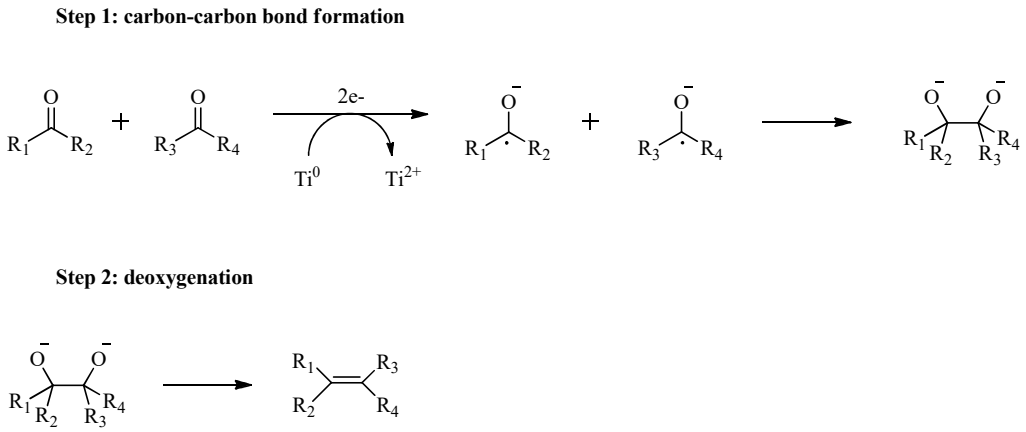


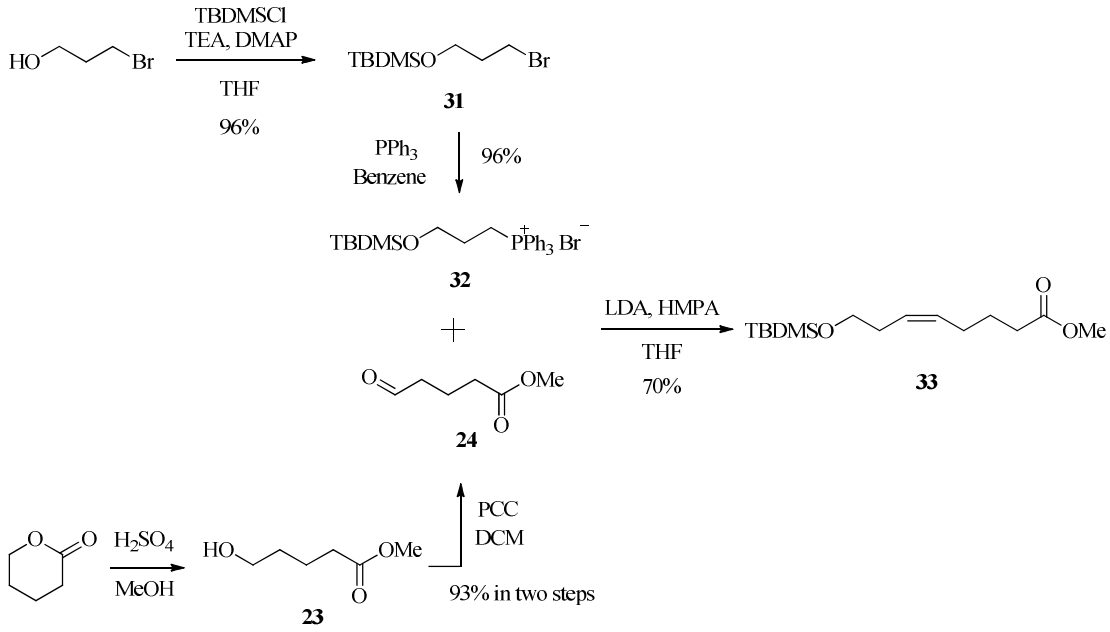
Figure 3.11: McMurry reaction mechanism.

The stereochemical outcome of the reaction is not controlled, because a mixture of threo and erythro diols is obtained in the first step, and in the second step the cleavage of the two C-O bonds is not concerted, leading then to a mixture of *Z* and *E* alkenes. The intramolecular reactions have more predictable stereoselectively and always high yields. This reaction is compatible with a large

number of functional groups and is proving increasingly valuable in the synthesis of natural products requiring the formation of six to fifteen-membered rings[55].

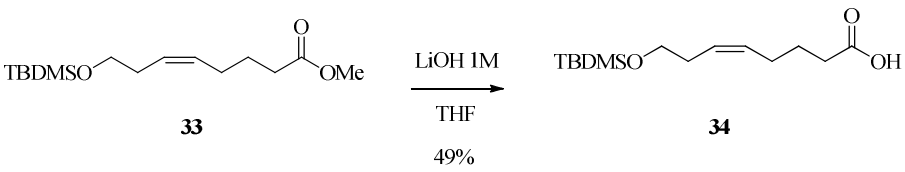
In the case of the eleven-membered E ring of madangamines, the formation of the double bond between C-3 and C-20 with stereochemistry *Z* could be favoured by the presence of the other *Z* double bond between C-17 and C-18. The reagent systems applicable that will be applied are $TiCl_4$/Zn, the most utilized; the $TiCl_3(DME)_2$/Zn-Cu, more soft and specific for intramolecular couplings, and the $TiCl_3$/Li, preferable in presence of amides. The difference between these systems is not due to the reactive species, that is presumed to be the Ti(0), but their preparation, that probably leads to distinct particle sizes, surface area, physical nature of the surface (edges, corners, holes), and in particular solvent. Indeed, the solvent can stabilize the Ti(0) during its formation; the most used solvent is THF, that can also help the electron transfer by solubilizing the intermediates and is not reduced in the reaction conditions.

The following scheme shows the synthetic route leading to the requisite alkyl chain (**Scheme 3.23**). To get the new target compound, a different phosphonium salt of three carbons with a protected alcohol (**31**) was synthesized starting from 3-bromopropan-1-ol. Different bases were investigated to promote the Wittig coupling between the phosphonium salt and the previously employed aldehyde **24**. The use of NaHMDS gave the desired product but only in low amounts (13%), whereas *n*-BuLi allowed a slight increase in yield (43%). Finally, the use of LDA, in presence of HMPA, afforded the ester **33** in a satisfying of 70%.



Scheme 3.23: Synthesis of alkyl chain **33**.

Basic hydrolysis of ester **33** with an aqueous solution of LiOH in a two-phase system with THF produced acid **34** with a slow reaction and a modest yield (**Scheme 3.24**); for this reason a systematic study of the reaction was performed (**Table 3.3**).



Scheme 3.24: Basic hydrolysis of ester **33**.

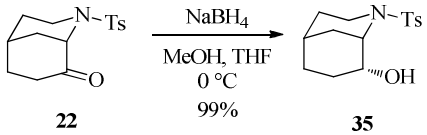
Entry	Base	Eq.	Solvents	M of base	Time	Product	Desilylated acid
1	LiOH	20	THF/H_2O	0.3	3 days	Yes (49%)	No
2	LiOH	20+20	THF/H_2O	0.3	24 h	Yes (37%)	Yes (Traces)
3	LiOH	40	THF/H_2O	0.3	24 h	Yes (7%)	Yes (Traces)
4	LiOH	40+40	THF/H_2O	0.6→1.2	36 h	No	Yes
5	LiOH	60	THF/H_2O	0.6	6 h	No	Yes
6	LiOH	20	THF/MeOH/H_2O	0.2	1h	Yes (Traces)	Yes (Major)
7	LiOH	5	THF/MeOH/H_2O	0.2	1 h	Yes (Traces)	Yes (Major)
8	LiOH	1	THF/MeOH/H_2O	0.1	1 h	Yes (Traces)	Yes (Major)
9	KOH	20	THF/MeOH	0.3	3h	No	Yes
10	KOH, NH_2OH	20, 10	THF/MeOH	0.6	2h	Yes (Traces)	Yes (Major)
11	LiOH, H_2O_2	2, 4	THF/H_2O	1	2h	No	Yes

Table 3.3: Studies of basic hydrolysis.

Starting from the observation that the standard hydrolysis with LiOH (entry 1) in a biphasic water/THF mixture required a long reaction time and didn't provide high yields, the first attempt was addressed to an increase of the amount of base (entries 3-5). Unfortunately, the yield of the desired product decreased in favor of a desilylated acid. The addition of methanol[56], allowed to get a monophasic system, with a dramatic decrease of the reaction time and an increase of the desilylated byproduct, even with 1 equivalent of LiOH (entries 6-8). Other bases were

investigated[57] (entries 9-11), but the best results were obtained with LiOH. Consequently, it was decided to utilize the classic biphasic reaction, with the idea of revisiting this step in case of a successful McMurry strategy.

Coming back to the overall synthesis of the model system (**Scheme 3.16**), the impossibility of employing compound **22** in this strategy to avoid its breaking with sodium naphtalenide solution (required for the removal of the tosyl group) forced the use of a protected alcoholic function instead of the ketone moiety. For this reason, the protected model compound **21** previously synthesized was exploited, and ketone moiety of **22** was reduced to alcohol (**Scheme 3.25**) in different reaction conditions.



22 **35**

Scheme 3.25: Reduction of ketone **22**.

Borohydride reduction proceeded smoothly (10 minutes) with a variety of solvents (MeOH/DCM, MeOH/THF, EtOH) and only in the presence of DCM the reaction were slower. Other reducing agents were used ($NaBH_3CN$, $LiAlH_4$), always obtaining good results and quantitative yields, and the combination of PMHS (polymethylhydrosiloxane) and TBAF[58] that produced a mixture of the two alcohols in ratio 1:1.

Curiously, the reduction led to the other isomer of the alcohol **21** obtained from previously described epoxide opening (**Scheme 3.16**). In fact, in that synthetic approach the cyclization occurs with the attack of the primary amine to the epoxide on the opposite side of the ring, producing a cycle with the hydroxyl group and the hydrogen in a cis relationship; consequently, in the resulting bicyclic product the hydroxyl group is axially oriented. In the reduction the hydride attacks the most accessible face, with an axial orientation, giving an equatorial hydroxyl (**Figure 3.12**).

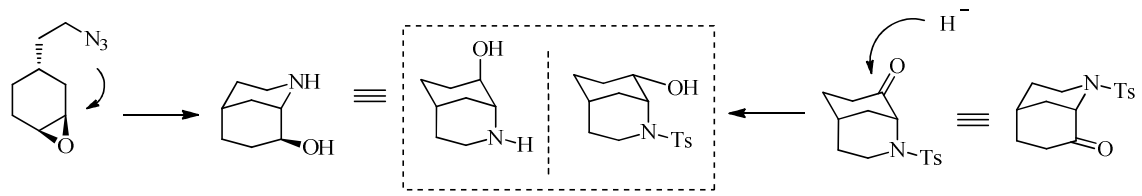
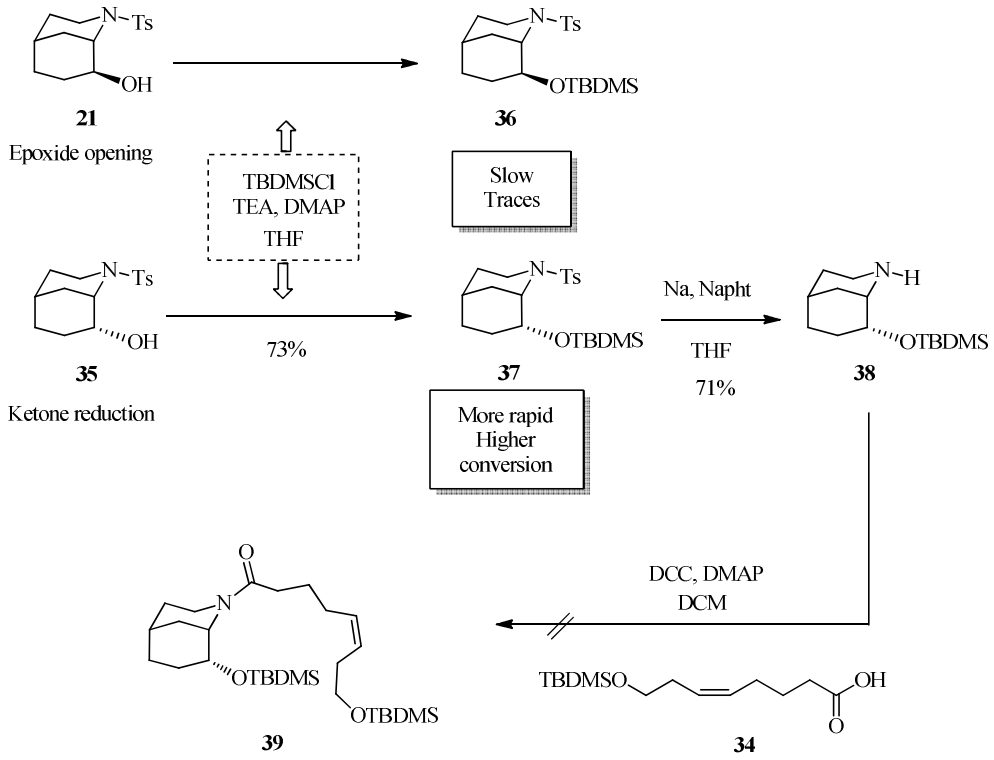


Figure 3.12: Isomers of alcohol **21**.

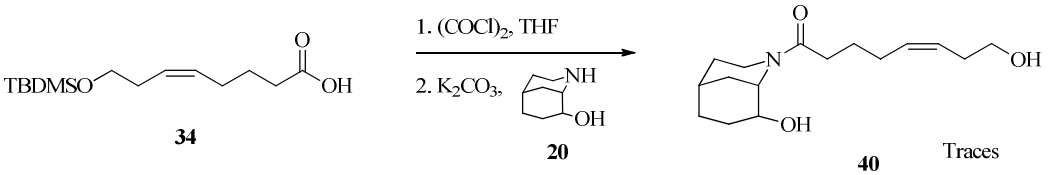
To pursue the McMurry strategy, both morphan alcohols **21** and **35** were protected with the TBDMS group, namely the same protecting group planned to be introduced in the alkyl chain precursor **34**. Surprisingly, while the protection of the morphan **21** (derived from the epoxide opening) was slow and afforded the product in traces, the protection of the morphan **35** (obtained via hydride reduction) was faster and gave much better yield. Considering that the alcoholic moiety should be oxidized to ketone, the synthesis proceed from protected compound **35**. *N*-detosylation of **35** was successfully accomplished by sodium naphtalenide reduction producing **38** with an excellent yield without any loss of the protecting group. Carbodiimide-mediated amidation of **38** with **34** gave the desired product **39** in traces with a large number of byproducts (**Scheme 3.26**).



Scheme 3.26: Failed synthesis of protected dialcohol **39**.

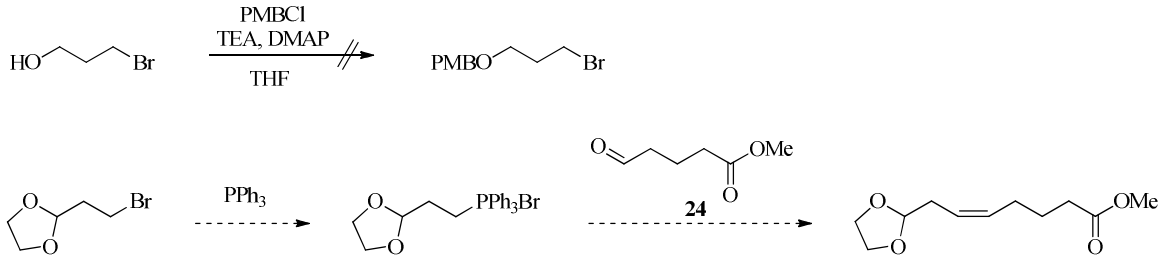
At this stage, a more direct route to the desired keto-aldehyde was undertaken. Thus, acylation of the *aza*-bicycle **20**, resulting from the intramolecular epoxide opening, was attempted trying to take advantage of the higher reactivity of the amino function compared with that of the secondary hydroxyl group. The route contemplates the conversion of the acid alkyl chain **34** to acyl chloride, and in a second step the amidation with the unprotected morphan **20**, using a mild base as K_2CO_3 to suppress the esterification reaction of the alcoholic function, and thus avoid the redundant steps of *N*-protection and deprotection (**Scheme 3.27**). Unfortunately, during the generation of the acyl

chloride, the strong acidic medium desilylated the alkyl chain, leading to a mixture of products (the desired one was present in traces).



Scheme 3.27: Amidation on unprotected morphan **20**.

Considering the unsatisfactory results, a new alkyl chain is going to be synthetized with a different protecting group. After unsuccessful attempts of protecting the alcoholic moiety of 3-bromopropan-1-ol with a *p*-methoxybenzyl group, a new strategy will be explored, starting from 2-(2-bromoethyl)-1,3-dioxolane (**Scheme 3.28**).



Scheme 3.28: Synthesis of new alkyl chains.

3.2.6. THE OLEFIN METATHESIS WITH SCHROCK CATALYST

A final approach was investigated, involving a different olefin metathesis between a carbonyl (the ketone moiety of the morphan system) and a double bond (on an alkyl chain previously attached to the model system with an amidation), promoted by Schrock catalyst[59] (**Figure 3.13**).

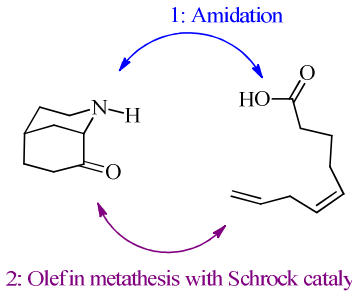


Figure 3.13: Cyclization promoted by Schrock catalyst.

The first annulations between an alkene and a carbonyl moiety were carried out by Clive, oxidizing with the ozone the double bond to carbonyl and then inducing coupling between the carbonyls in presence of low-valent titanium reagents[60]. This titanium catalyst is prepared under milder conditions than the ones described by McMurry[55] (**Figure 3.14**).

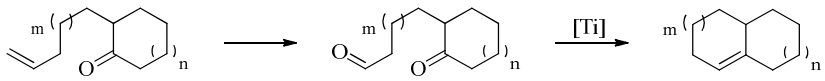


Figure 3.14: Clive's annulation mechanism.

The introduction of the olefin metathesis in this process afforded the innovative direct one-step reaction that does not need the oxidation of the double bond to carbonyl. The mechanism of this new olefin metathesis is really close to the classical one (**Figure 3.15**).

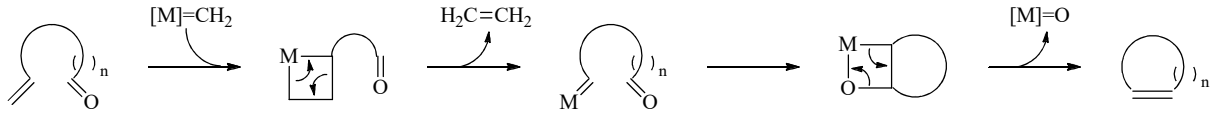


Figure 3.15: Carbonyl olefination mechanism.

A substantial difference is the absence of a catalytic cycle: the catalyst during the process is oxidized and is not more usable. Another weakness of the carbonyl olefination is the need for a higher temperature and longer reaction time respect to the classical olefin metathesis.

The promoters employed in carbonyl olefination are alkylidene complexes of molybdenum, already successfully used in the synthesis of tetracyclic ABCE system of manzamine A[61], like Schrock catalyst (**Figure 3.16**),.

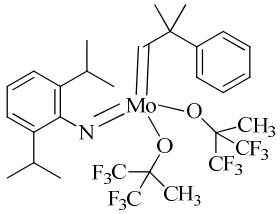
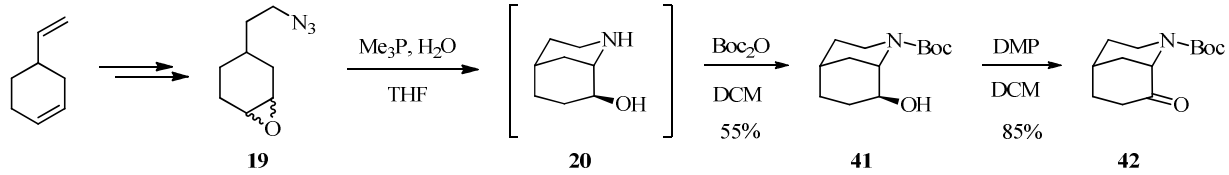


Figure 3.16: Schrock catalyst.

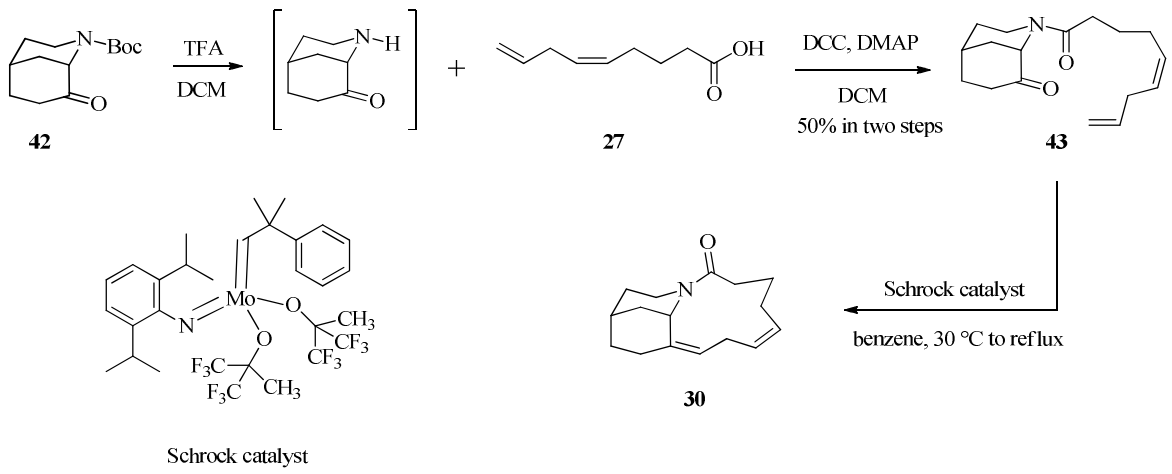
To get the desired ketone moiety on the morphan system without a protecting group on the nitrogen, the mixture of epoxides **19** was reduced to amine in presence of Me_3P and, after the spontaneous cyclization, unstable intermediate **20** was obtained. Compound **20** was then protected with a *tert*-butyloxycarbonyl group, easier to remove than tosyl group. The alcoholic moiety of **41** was then converted to ketone with DMP, affording product **42** (**Scheme 3.29**).



19 **20** **41** **42**

Scheme 3.29: Synthesis of the Boc-protected model system.

Removal of *tert*-butyloxycarbonyl protecting group was easily achieved in presence of TFA and amidation with the previously synthetized alkyl chain **27** (in ring-closing metathesis strategy, **Scheme 3.19**) gave intermediate **43**. Schrock catalyst proved capable to promote the intramolecular olefin metathesis in a low yield and on a small scale reaction, leading to the desired ACE tricyclic system (**Scheme 3.30**). The reaction conditions will be further optimized.

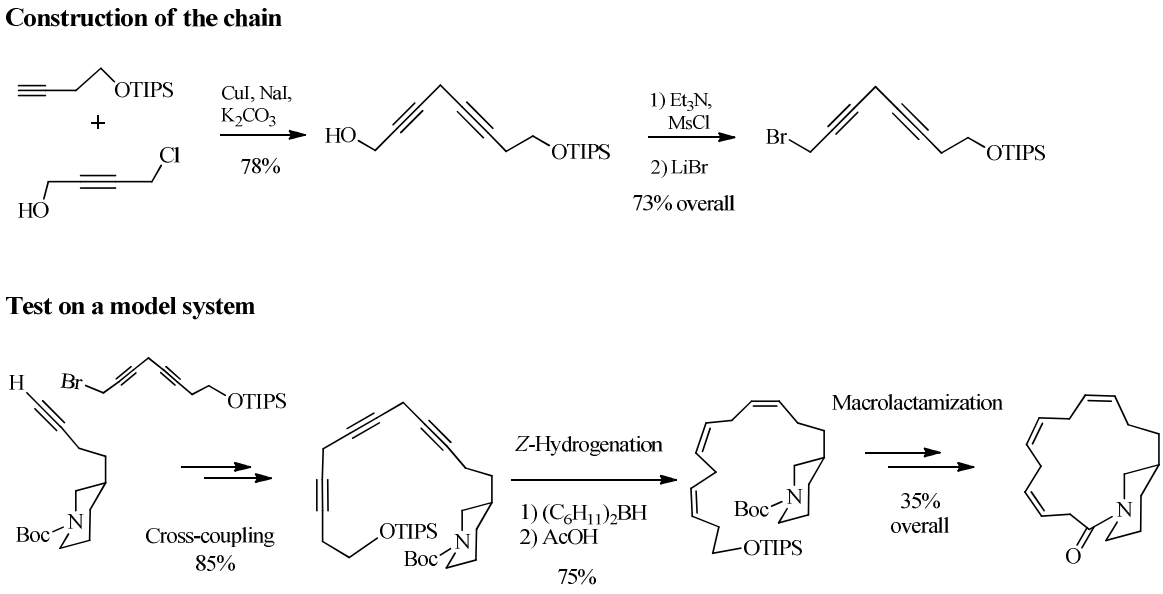


Scheme 3.30: Synthesis of the ACE system.

3.3. CONCLUSIONS AND FUTURE PERSPECTIVES

For their structural complexity, madangamine construction is still a challenge for synthetic chemists. After the total synthesis of madangamine D, the assembly of other members of this family are now under investigation by Amat-Bosch research group. An improved synthetic pathway for its diazatricyclic core has been developed respect to described one[20], that could be easily adapted to other members of madangamine family. The fully functionalized core common to all madangamines has been assembled, having now in hand considerable amounts of this three bridged diazatricyclic ABC ring systems in enantioselective fashion. A model system of the AC ring moiety has been designed and synthetized, that has been employed for the assessment of as many synthetic approaches as possible for the construction of E ring, avoiding the excessive consumption of the precious ABC tricyclic system. Several ring-closing strategies were investigated for the E ring build-up and different protecting groups were tested. The use of Grubbs catalysts in the metathesis approach and the use of TBDMS protecting group for the alkyl chain to react with the morphan system proved unfruitful. The search thus moved toward the synthesis of another carboxylic acid derivative with a dioxolane as protecting group for the carbonyl moiety at the terminal position of the alkyl chain, that in future could react with the morphan system and essay the final cyclization with a McMurry reaction. Moreover, the Schrock catalyst has been explored to promote ring-closing metathesis between the ketone moiety of the morphan and the alkene moiety of the alkyl chain, that finally gave the desired ACE system.

Optimization of the reaction with Schrock catalyst is in progress in the Amat-Bosch research to achieve a scalable procedure. In case of success, the reaction will be applied on the fully functionalized diazatricyclic system. In parallel, the McMurry strategy, that involves less expensive reactives, is under scrutiny and will be selected in case of promising results. Also, the final annulation of D ring has already be planned, evaluated on a model system (made by B ring) and reported in literature by Amat-Bosch research group[60]. The approach is based on a chain bearing two skipped triple bonds to get a cross-coupling with the alkyne moiety anchored to the model system. The reduction of the alkyne led to the selective formation of three *Z* double bonds. The final macrolactamization produced the precursor of D ring (**Scheme 3.31**).



Scheme 3.31: Construction of D ring on a model system.

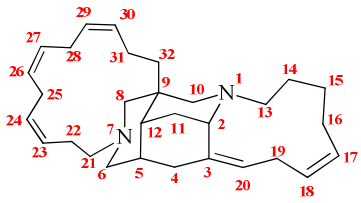
4. EXPERIMENTAL SECTION

All air sensitive manipulations were carried out under dry argon or nitrogen atmosphere. Solvents and all standard reagents were purchased from Sigma Aldrich, Alfa Aesar of Acros and were used without further purification.

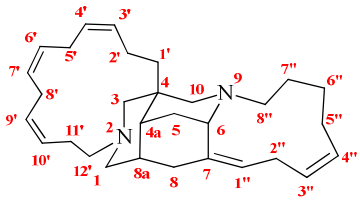
Analytical thin-layer chromatography was performed on SiO_2 (Merck silica gel 60 F_{254}), and the spots were located with 1% aqueous $KMnO_4$ or hexachloroplatinate. Chromatography refers to flash chromatography and was carried out on SiO_2 (SDS silica gel 60 ACC, 35-75 mm, 230-240 mesh ASTM). Drying of organic extracts during workup of reactions was performed over anhydrous $MgSO_4$ or Na_2SO_4. Evaporation of solvent was accomplished with a rotary evaporator. NMR spectra were recorded at 300 or 400 MHz (^1H) and 75.4 or 100.6 MHz (^{13}C), and chemical shifts are reported in δ values downfield from TMS or relative to residual chloroform (7.26 ppm, 77.0 ppm) as an internal standard. Data are reported in the following manner: chemical shift, integrated intensity, multiplicity, coupling constant (*J*) in Hertz (Hz), and assignment (when possible). Assignment and stereochemical determinations are given only when they are derived from definitive two-dimensional NMR experiments (HSQC-COSY). Optical rotations were measured on Perkin-Elmer 241 polarimeter. $[\alpha]_D$ values are given in 10^{-1} deg cm^2 g^{-1}.IR spectra were performed in a spectrophotometer Nicolet Avantar 320 FT-IR and only noteworthy IR absorptions (cm^{-1}) are listed. High-resolution mass spectra (HMRS; LC/MSD TOF Agilent Technologies) were performed by Centres Científics i Tecnològics de la Universitat de Barcelona. Mass spectra (MS) data are reported as m/z (%).

The IUPAC nomenclature was used in the NMR assignation of all compounds whereas for the sake of clarity, madangamine biogenetic numbering has been employed throughout the dissertation.

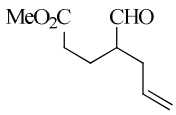
Madangamine A



Biogenetic numbering



IUPAC numbering

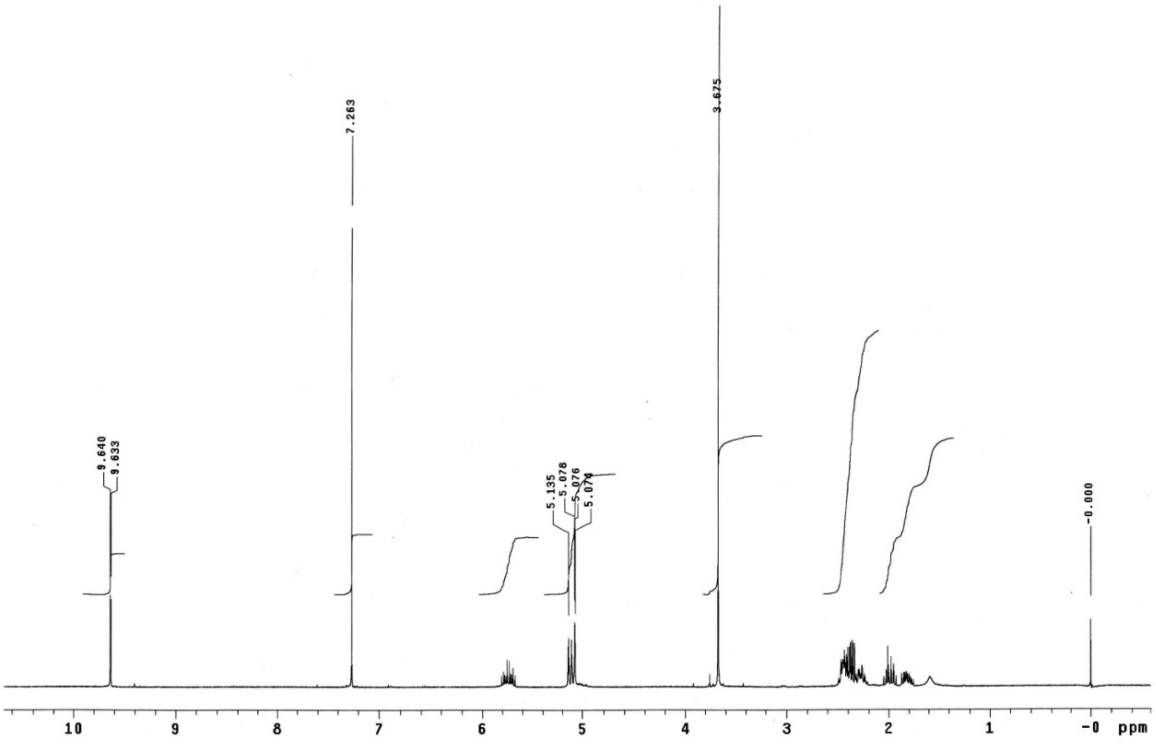


Methyl-4-formyl-6-heptenoate (1).

4-Pentenal (4.64 mL, 0.0470 mol) was added dropwise during 30 minutes to a stirred, ice-cold suspension of anhydrous potassium carbonate (2.21 g, 0.0160 mol) in piperidine (12 mL). Stirring was continued at room temperature for 2 days and the mixture filtered through sintered glass. The residue was washed thoroughly with ether which was subsequently removed under reduced pressure affording the piperidine enamine of 4-pentenal, as a pale yellow oil. To a solution of methyl acrylate (7.62 mL, 0.0846 mol) in 75 mL of dry acetonitrile, cooled below 5 °C, was added the above enamine in 19 mL of dry acetonitrile during 30 min. The mixture was stirred at room temperature for 5 hours and then refluxed for 72 hours. Glacial acetic acid (10.5 mL) in water (75 mL) was added and the mixture refluxed for further 8 hours. Ether (250 mL) was added to the residue and the organic layer was washed with 3 N HCl (100 mL), saturated $NaHCO_3$ (100 mL), brine (100 mL) and finally dried with $MgSO_4$ and filtered. Removal of the organic solvents left methyl-4-formyl-6-heptenoate (**1**, 5.10 g, 64%) as a brown oil that was used in the next reaction without further purification.

^{1}H NMR (300 MHz, $CDCl_3$): δ1.80 (m, 1H, H-3), 1.98 (m, 1H, H-3), 2.21-2.51 (m, 5H, 2H-2, H-4, 2H-5), 3.67 (s, 3H, CH_3), 5.09 (m, 2H, H-7), 5.76 (m, 1H, H-6), 9.63 (d, J = 2.1 Hz, 1H, CHO).

^{13}C NMR (75.4 MHz, $CDCl_3$): δ23.2 (C-3), 31.2 (C-5), 32.9 (C-2), 50.3 (CH_3), 51.7 (C-4), 117.6 (C-7), 134.3 (C-6), 173.4 (C-1), 203.7 (CHO).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5.074
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-0.000
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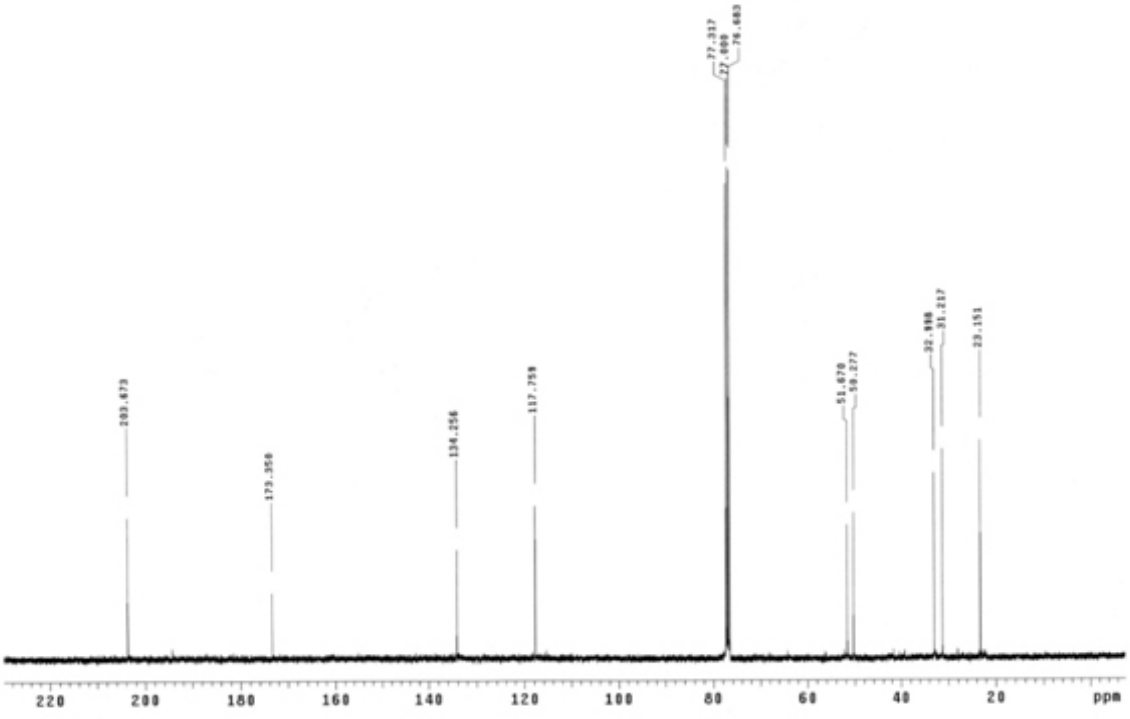
8
7
6
5
4
3
2
1
-0
ppm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76.663
193.673
173.358
134.256
117.759
51.670
50.277
32.998
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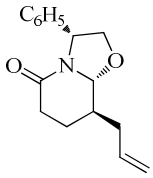
200
180
160
140
120
100
80
60
40
20
ppm



(3*R*,8*R*,8a*R*)-8-Allyl-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (2).

A mixture of racemic methyl 4-formyl-6-heptenoate**1** (4.60 g, 29.0 mmol), (*R*)-phenylglycinol (3.98 g, 29.0 mmol), and anhydrous Na_2SO_4 (17.0 g, 120 mmol) in Et_2O (115 mL) was stirred at 0 °C for 2 hours. The resulting suspension was filtered through celite and concentrated under reduced pressure. The residue was heated at 80 °C for 18 hours under vacuum (10-15 mmHg). Flash chromatography (8:2 hexane-EtOAc to EtOAc) of the residue afforded oxazolopiperidone **2** (5.30 g, 71%) and its (8*S*,8a*S*)-diastereoisomer **3** (753 mg, 10%).

Compound **2** (major isomer, lower R_f):

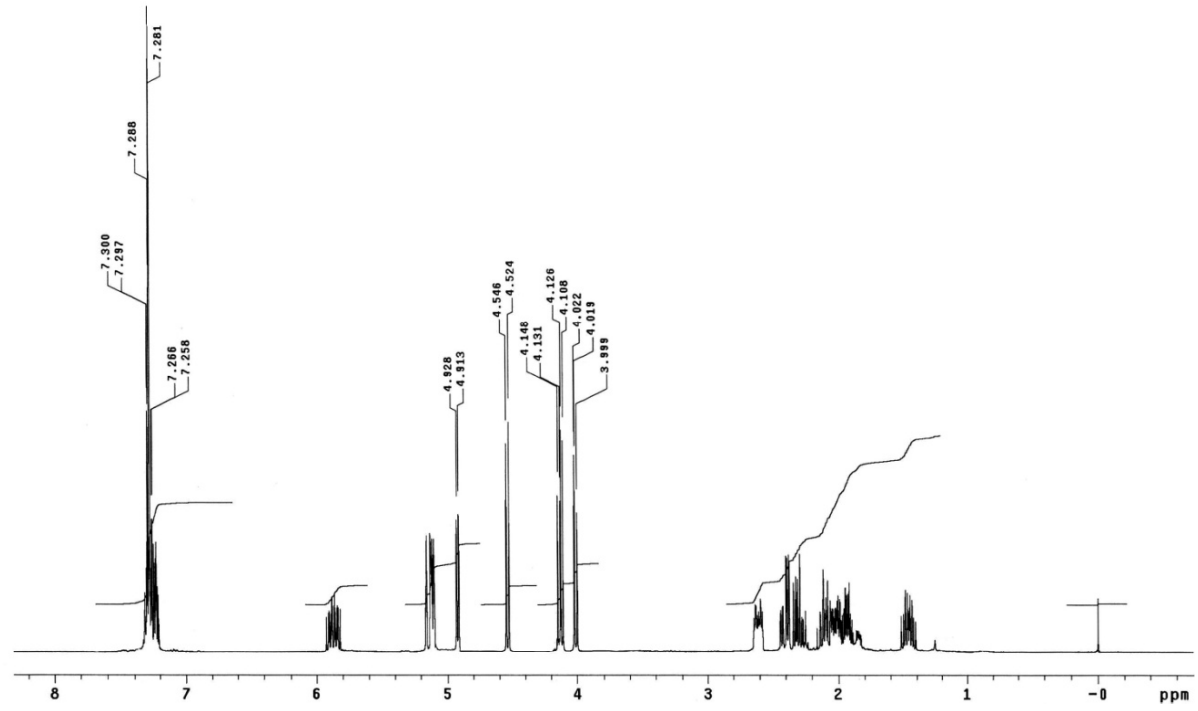
$[\alpha]_D^{22} = -32.8$ (*c* 1.0, EtOH).

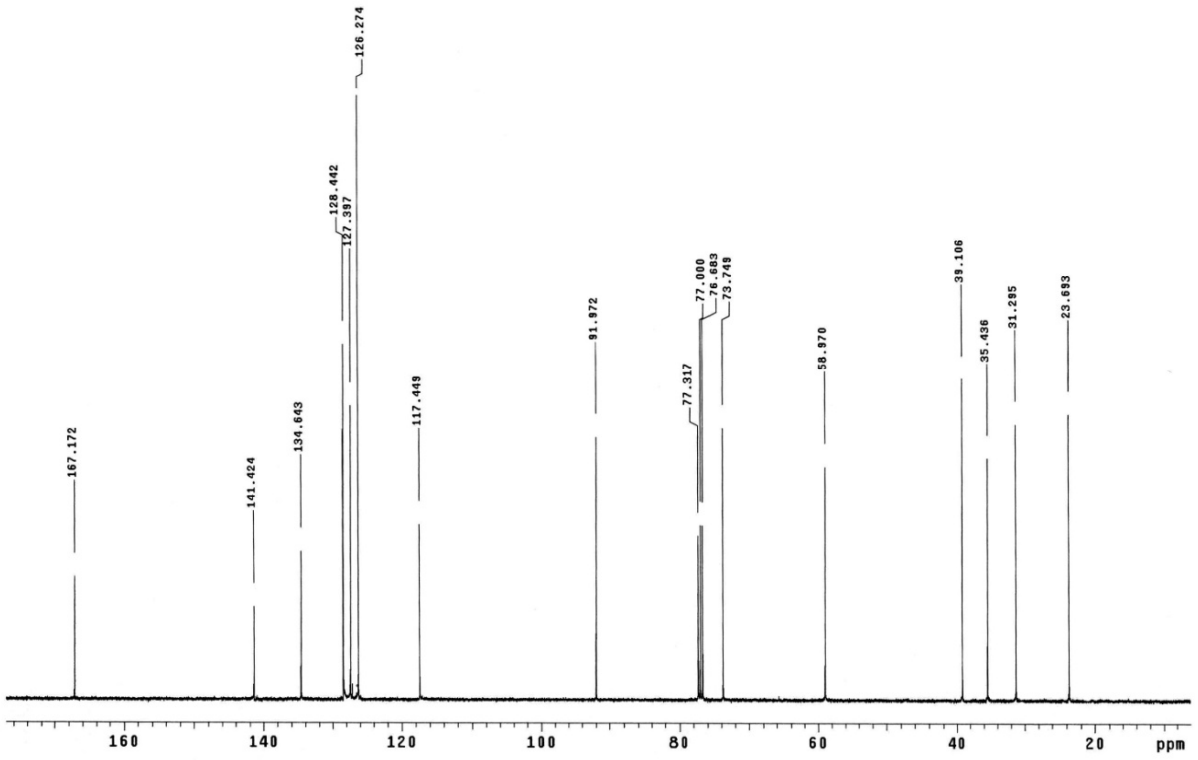
IR (film): 1655 (NCO) cm^{-1}.

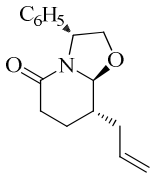
^{1}H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ 1.45 (dddd, *J* = 13.8, 13.8, 12.0, 7.2 Hz, 1H, H-7), 2.02 (m, 3H, H-7, H-8, CH_2 allyl), 2.30 (ddd, *J* = 18.0, 12.0, 6.6 Hz, 1H, H-6), 2.42 (ddd, *J* = 18.0, 7.2, 1.8 Hz, 1H, H-6), 2.62 (m, 1H, CH_2 allyl), 4.01 (dd, *J* = 9.0, 1.2 Hz, 1H, H-2), 4.11 (dd, *J* = 9.0, 6.9 Hz, 1H, H-2), 4.53 (d, *J* = 8.7 Hz, 1H, H-8a), 4.92 (d, *J* = 6.6 Hz, 1H, H-3), 5.12 (m, 2H, CH_2=), 5.86 (dddd, *J* = 16.5, 10.2, 7.8, 6.0 Hz, 1H, CH=), 7.20-7.30 (m, 5H, C_6H_5).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 23.6 (C-7), 31.2 (C-6), 35.4 (CH_2 allyl), 39.1 (C-8), 58.9 (C-3), 73.7 (C-2), 91.9 (C-8a), 117.4 (CH_2=), 126.2, 128.4 (C-*o*, *m* C_6H_5), 127.4 (C-*p* C_6H_5), 134.6 (CH=), 141.4 (C-*i* C_6H_5), 167.1 (NCO).

Elemental analysis calcd (%) for [$C_{16}H_{19}NO_2 \cdot ¼\ H_2O$]: C 73.40, H 7.51, N 5.35.Found: C 73.71, H 7.25, N 5.41.







Compound **3** (minor isomer, higher R_f):

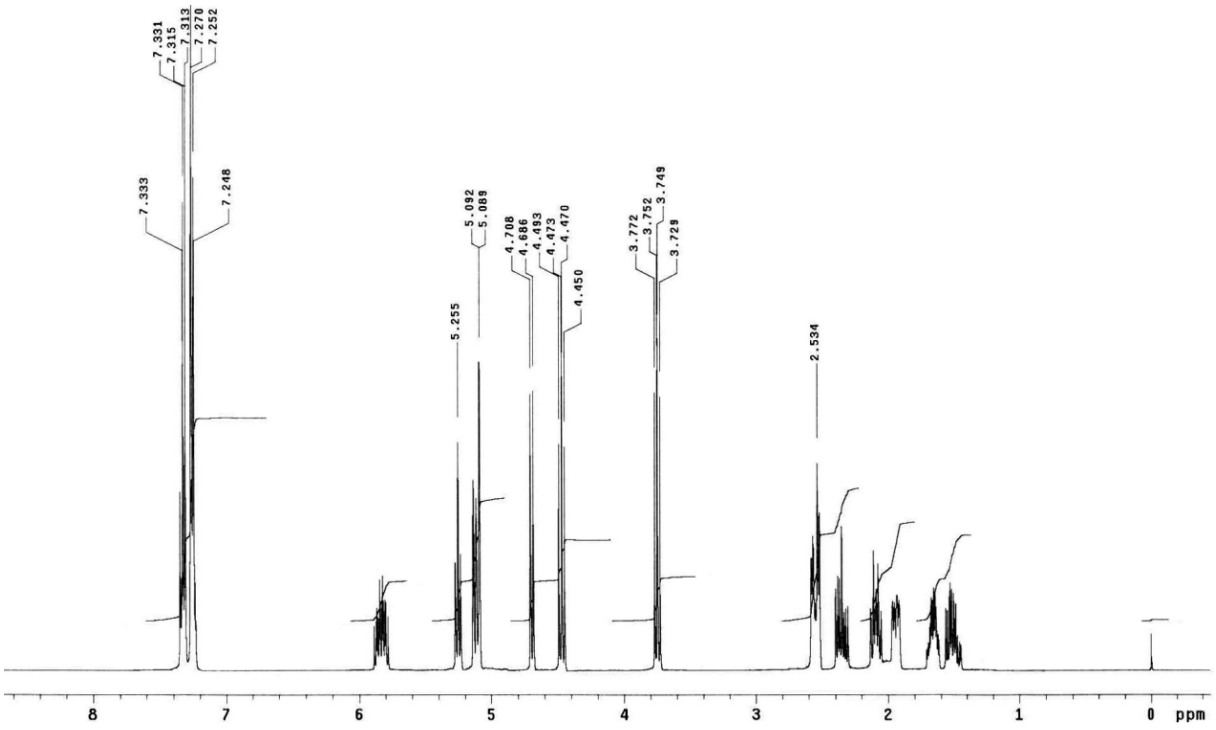
$[\alpha]_D^{22} = -59.9$ (*c* 1.0, EtOH).

IR (film): 1658 (NCO) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ 1.53 (m, 1H, H-7), 1.66 (m, 1H, H-8), 1.96 (m, 1H, H-7), 2.07 (dt, *J* = 16.5, 8.4, 8.4 Hz, 1H, CH_2 allyl), 2.35 (ddd, *J* = 18.6, 12.0, 6.6 Hz, 1H, H-6), 2.56 (m, 2H, H-6, CH_2 allyl), 3.75 (dd, *J* = 9.0, 7.8 Hz, 1H, H-2), 4.47 (dd, *J* = 9.0, 8.1 Hz, 1H, H-2), 4.69 (d, *J* = 8.4 Hz, 1H, H-8a), 5.13 (m, 2H, CH_2=), 5.25 (t, *J* = 7.8 Hz, 1H, H-3), 5.83 (dddd, *J* = 16.5, 10.2, 8.1, 6.0 Hz, 1H, CH=), 7.25-7.34 (m, 5H, C_6H_5).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 22.7 (C-7), 31.3 (C-6), 35.8 (CH_2 allyl), 39.5 (C-8), 58.3 (C-3), 72.3 (C-2), 92.0 (C-8a), 117.4 (CH_2=), 126.0, 128.7 (C-*o*, *m* C_6H_5), 127.5 (C-*p* C_6H_5), 134.6 (CH=), 139.4 (C-*i* C_6H_5), 168.7 (NCO).

Elemental analysis calcd (%) for [$C_{16}H_{19}NO_2 \cdot ¼\ H_2O$]: C, 73.40; H, 7.51; N, 5.35. Found: C, 73.27; H, 7.25; N, 5.51.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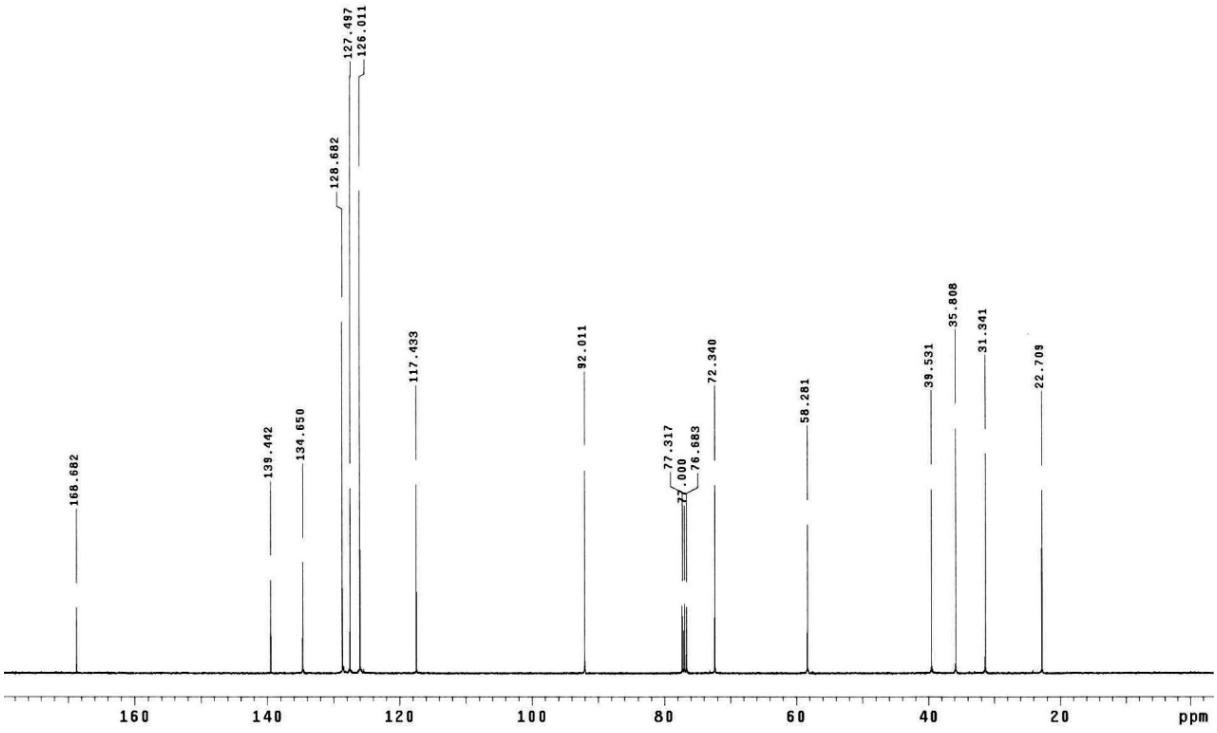
4.470
4.450
5.255
3.772
3.752
3.749
3.729
2.534
8
7
6
5
4
3
2
1
0
ppm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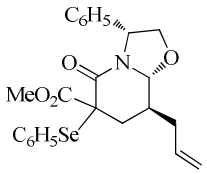


(3*R*,8*S*,8a*R*)-8-Allyl-6-(methoxycarbonyl)-5-oxo-3-phenyl-6-(phenylselenyl)-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo-[3,2-*a*]pyridine (4).

Lithium bis(trimethylsilyl)amide (1M in THF, 17.1 mL, 17.09 mmol) was slowly added at –78 °C to a solution of lactam **2** (2.0 g, 7.77 mmol) in anhydrous THF (120 mL), and the resulting mixture was stirred for 90 minutes. Then, methyl chloroformate (0.600 mL, 7.77 mmol) and, after 90 minutes of continuous stirring at –78 °C, phenylselenyl chloride (2.09 g, 10.9 mmol) were added to the solution. The resulting mixture was stirred for a further 1 hour and poured into saturated aqueous NH_4Cl. The aqueous layer was extracted with EtOAc, and the combined organic extracts were dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (9:1 hexane-EtOAc to 1:1 hexane-EtOAc) of the resulting oil afforded **4** as a mixture of C-6 epimers (3.14 g, 86% overall yield).

Epimer **4a** (higher R_f):

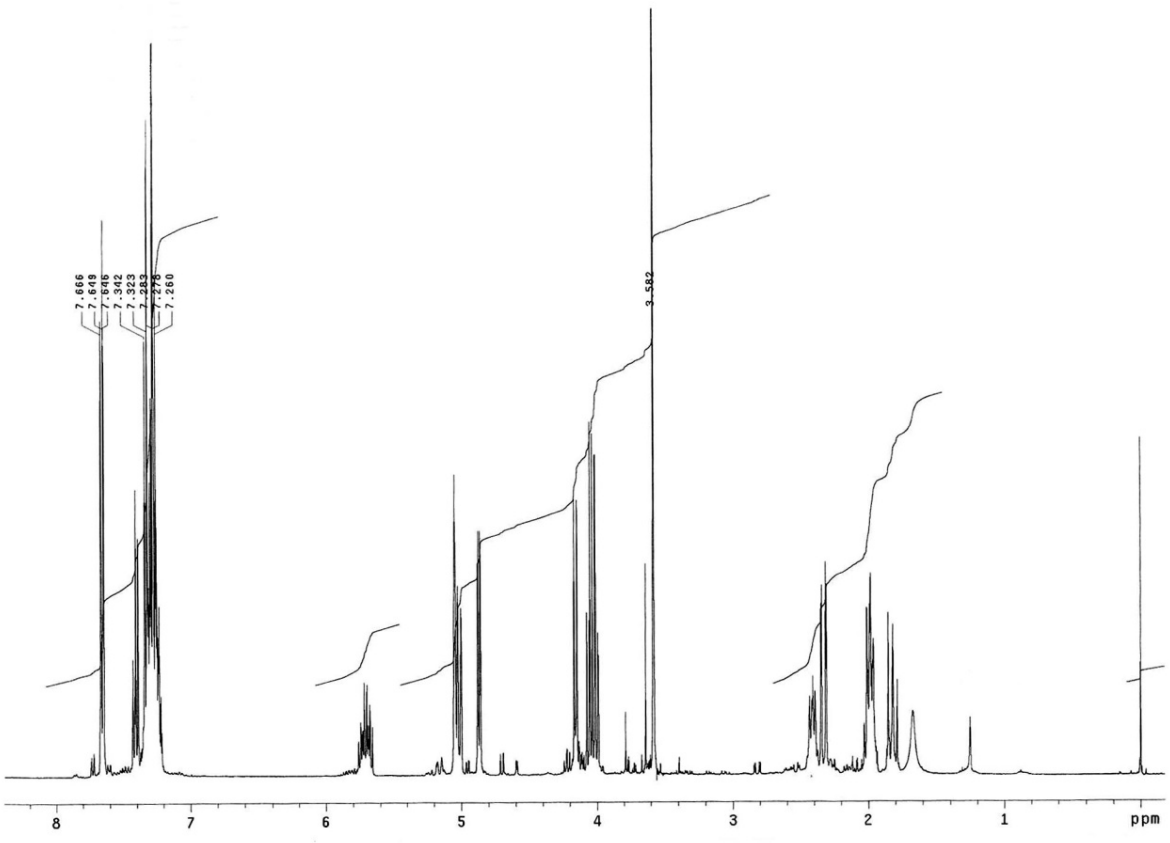
$[\alpha]_D^{22}$= -99.3 (*c* 0.7, $CHCl_3$).

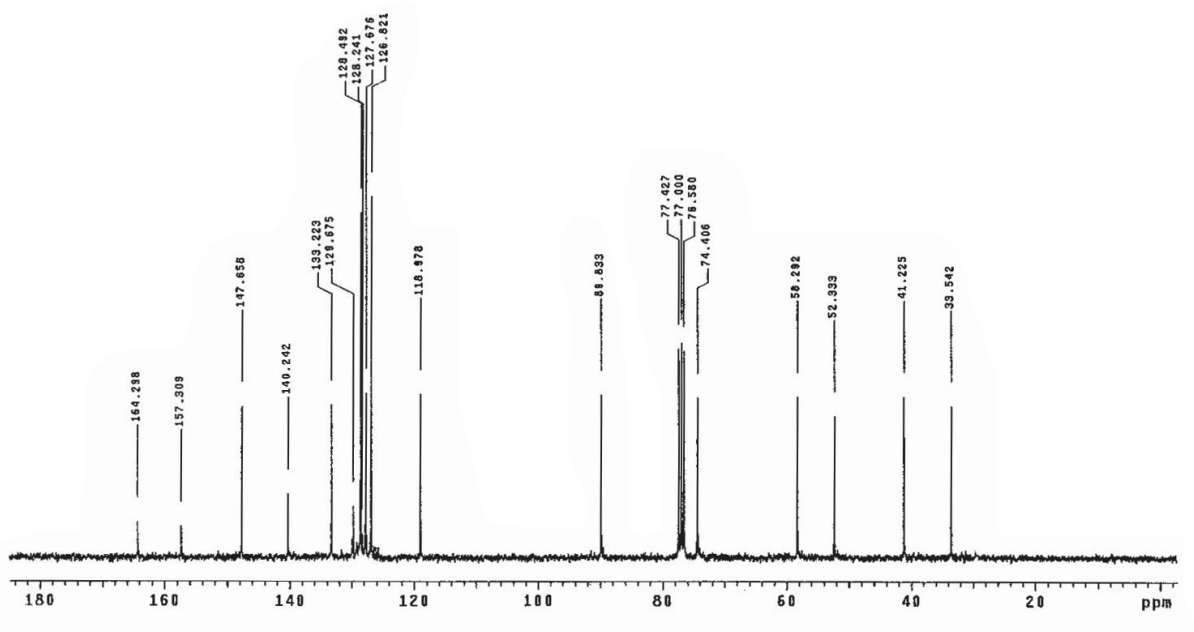
IR (film): 1667 (NCO), 1725 (COO) cm^{-1}.

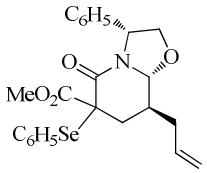
^{1}H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ1.82 (dd, *J*=14.0, 12.4 Hz, 1H, CH_2 allyl), 1.98(m, 2H, H-7, H-8), 2.33 (dd, *J* = 14.0, 2.8 Hz, 1H, CH_2 allyl), 2.41 (m, 1H, H-7), 3.58 (s, 3H, CH_3), 4.00 (dd, *J*= 9.2, 2.0 Hz, 1H, H-2), 4.05 (dd, *J*= 9.2, 6.8 Hz, 1H, H-2), 4.15 (d, *J*= 8.8 Hz, 1H, H-8a), 4.87 (dd, *J*= 6.8, 2.0 Hz, 1H, H-3), 5.03 (m, 2H, CH_2=), 5.17 (dddd, *J*= 16.8, 10.4 7.6, 6.4 Hz, 1H, CH=), 7.26-7.66 (m, 10H, ArH).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ34.8 (C-7), 36.6 (CH_2), 37.7 (C-8), 53.0 (CH_3), 54.0 (C-6), 59.4 (C-3), 74.0 (C-2), 91.8 (C-8a), 117.7 (CH_2=), 126.4-138.2 (C-*o*, *m*, *p*), 133.7 (CH=), 140.5 (C-*i*), 163.3 (NCO), 170.8 (COO).

HRMS (ESI) calcd for $[C_{24}H_{25}NO_4Se + H]^+$: 471.0948, found: 471.0955.







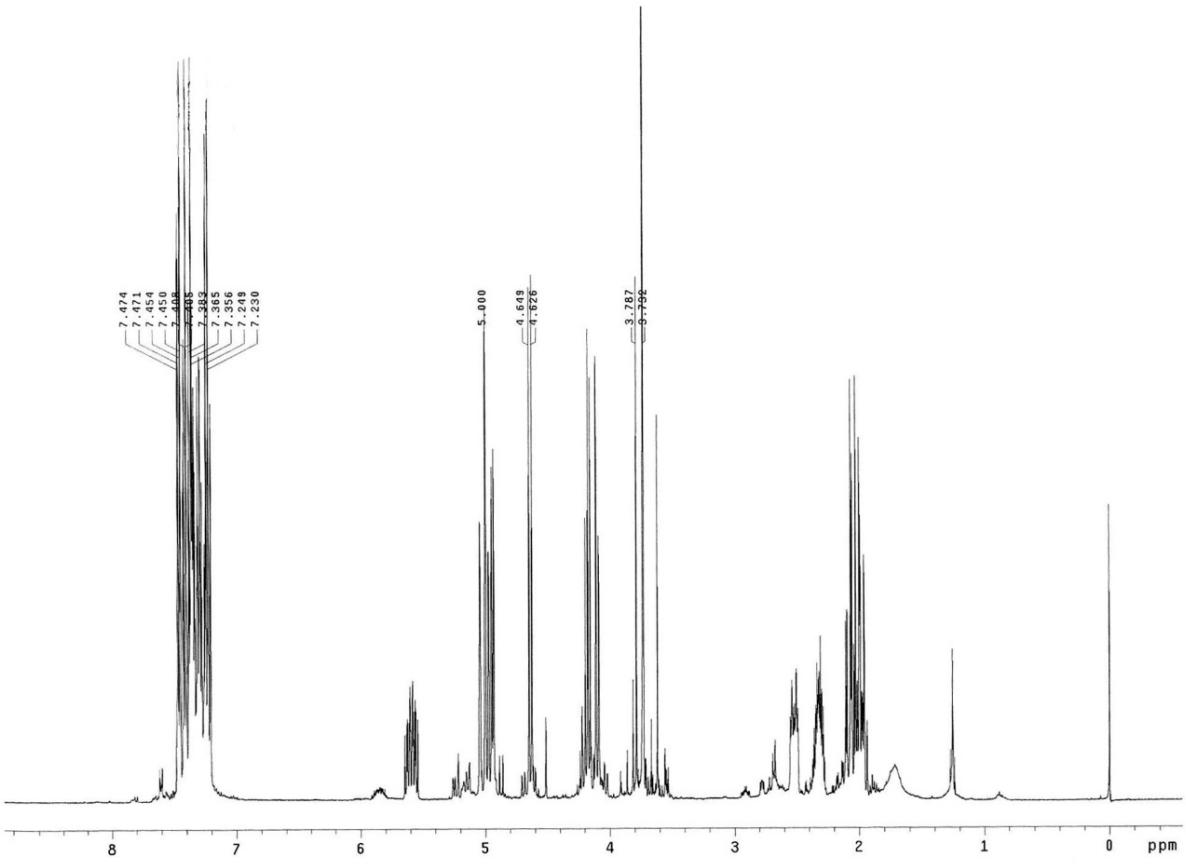
Epimer**4b** (lower R_f):

$[\alpha]_D^{22}$ = +18.5 (*c* 0.5, $CHCl_3$).

^{1}H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ1.98 (m, 1H, CH_2 allyl), 2.00 (dd, *J* = 15.2, 11.6 Hz, 1H, H-7), 2.08 (dd, *J*= 15.2, 4.0 Hz, 1H, H-7), 2.32 (m, 1H, H-8), 2.52 (m, 1H, CH_2 allyl), 3.73 (s, 3H, CH_3), 4.10 (dd, *J* = 9.2, 1.2 Hz, 1H, H-2), 4.17 (dd, *J* = 9.2, 6.8 Hz, 1H, H-2), 4.63 (d, *J* = 9.2 Hz, 1H, H-8a), 4.97 (m, 3H, H-3, CH_2=), 5.59 (dddd, *J* = 16.0, 10.0, 8.0, 6.0 Hz, 1H, CH=), 7.23-7.47 (m, 10H, ArH).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ33.6 (C-7), 35.0 (CH_2), 36.5 (C-8), 53.3 (CH_3), 55.6 (C-6), 59.6 (C-3), 73.8 (C-2), 91.6 (C-8), 117.8 (CH_2=), 126.8-138.3 (C-*o*, *m*, *p*), 134.0 (CH=), 140.5 (C-*i*), 162.8 (NCO), 171.0 (COO).

HRMS (ESI) calcd for $[C_{24}H_{25}NO_4Se + H]^+$: 471.0948, found: 471.0946.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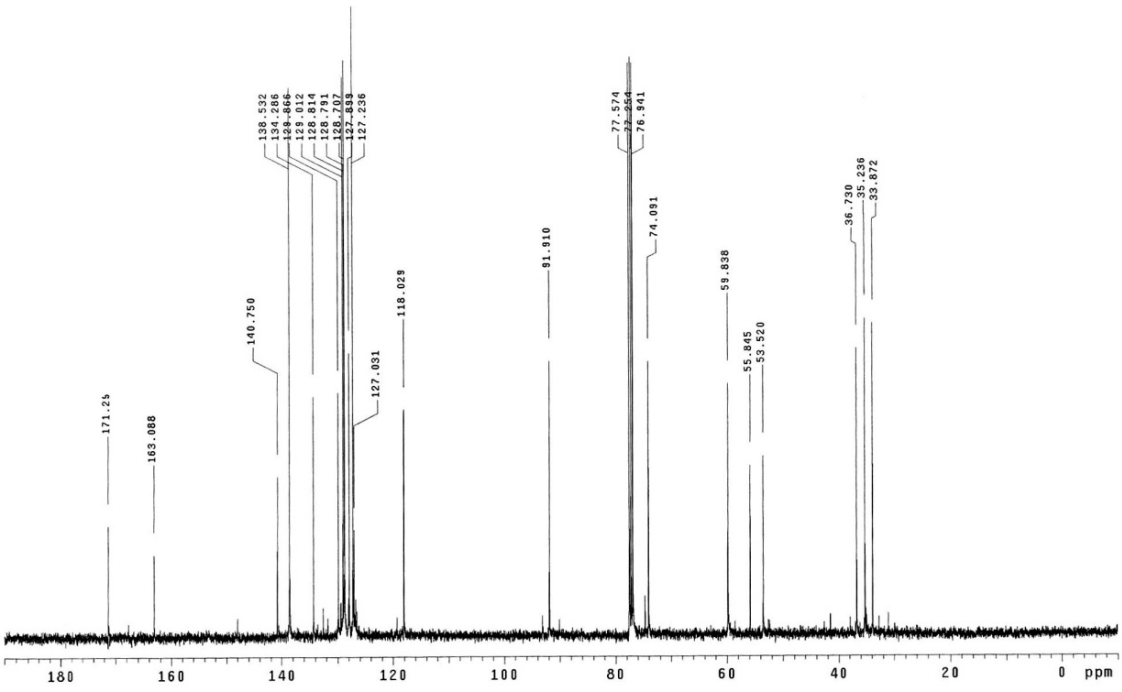
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59.838
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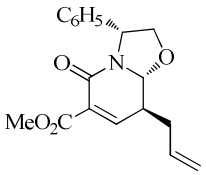


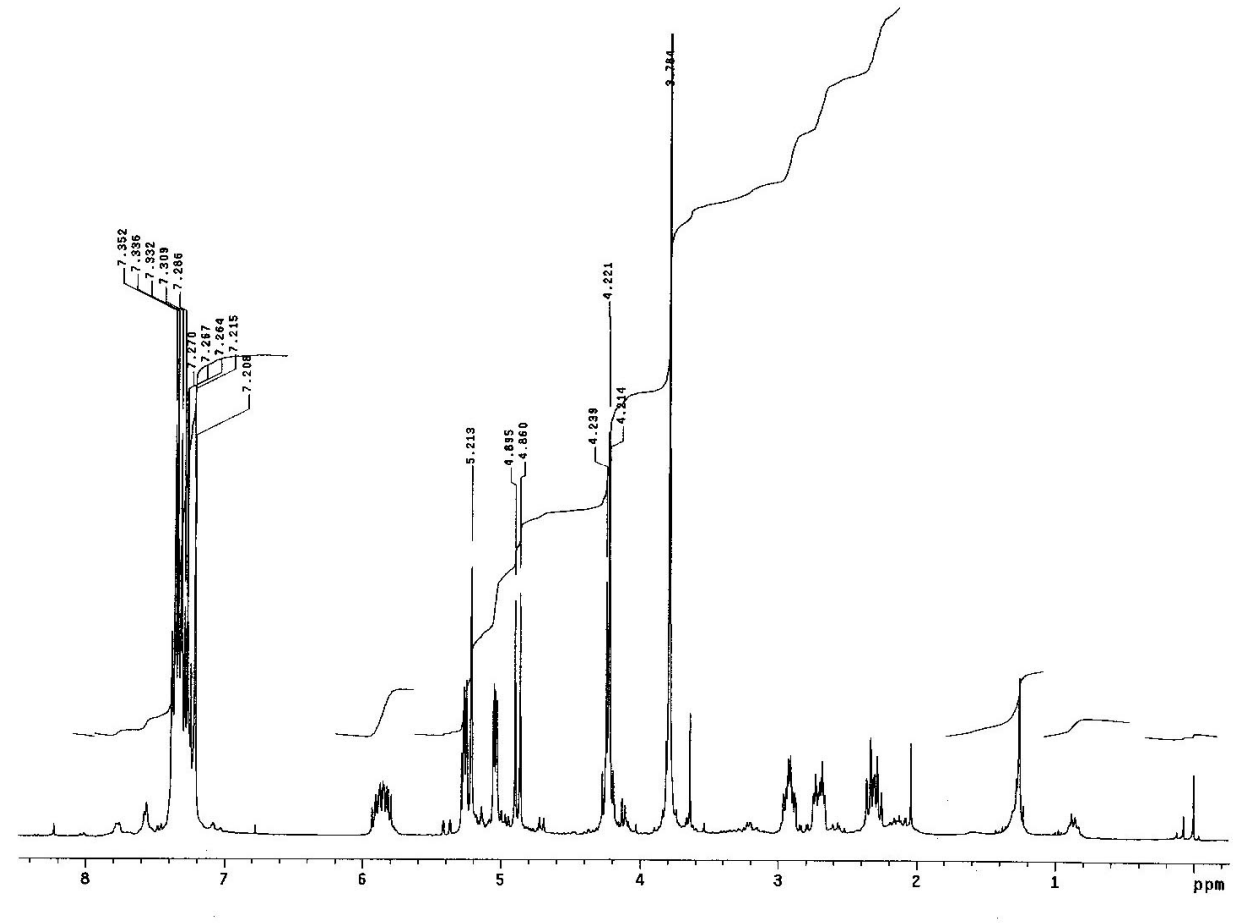
(3*R*,8*S*,8a*R*)-8-Allyl-6-(methoxycarbonyl)-5-oxo-3-phenyl-2,3,8,8a-tetrahydro-5*H*-oxazolo[3,2-*a*]pyridine (5).

30% aqueous H_2O_2 (0.729 mL) and pyridine (0.358 mL, 4.42 mmol) were added to a solution of selenides **4** (1.60 g, 3.40 mmol) in CH_2Cl_2 (231 mL), and the resulting mixture was stirred at rt for 2 hours. The two phases were separated, andthe organic layer was washed with water, dried and concentrated to give crude **5** (1.65 g) as an oil, wich was used in the next reaction without further purification.

IR (film): 1673 (NCO), 1741 (COO) cm^{-1}.

^{1}H NMR (300 MHz, $CDCl_3$): δ2.31 (dt, *J* = 14.4, 8.7 Hz, 1H, CH_2 allyl), 2.70 (dm, *J* = 14.4, 1H, CH_2 allyl), 2.91 (m, 1H, H-8), 3.78 (s, 3H, CH_3), 4.20 (dd, *J*= 9.0, 2.4 Hz, 1H, H-2), 4.24 (dd, *J*= 9.0, 6.0 Hz, 1H, H-2), 4.87 (d, *J*= 10.5 Hz, 1H, H-8a), 5.03 (dd, *J*= 6.0, 2.4 Hz, 1H, H-3), 5.27 (m, 2H, CH_2=), 5.86 (dddd, *J*= 15.3, 10.88.7, 5.7 Hz, 1H, CH=), 7.20-7.36 (m, 5H, C_6H_5).

^{13}C NMR (75.4 MHz, $CDCl_3$): δ33.5 (CH_2), 41.2 (C-8), 52.3 (CH_3), 58.3 (C-3), 74.4 (C-2), 89.8 (C-8a), 119.0 (CH_2=), 126.8-128.5 (C-*o*, *m*, C_6H_5), 127.7 (C-*p*, C_6H_5), 129.7 (C-6), 133.3 (CH=), 140.2 (C-*i*C_6H_5), 147.6 (C-7), 157.3 (COO), 164.3 (NCO).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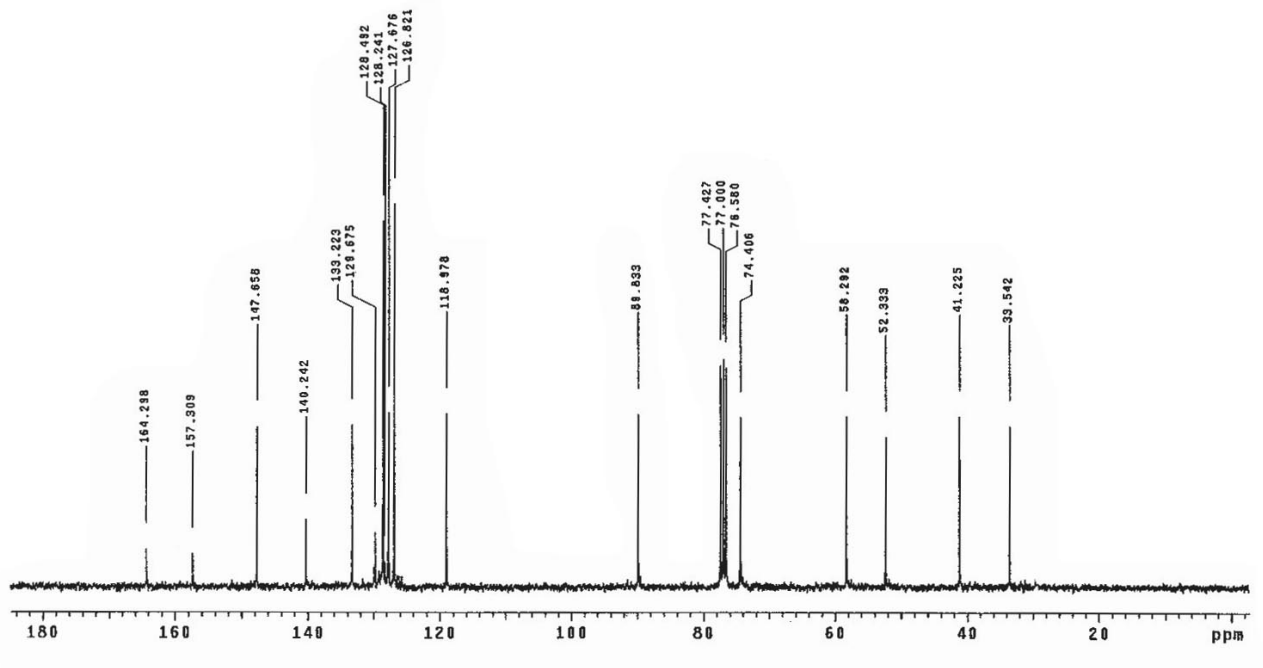
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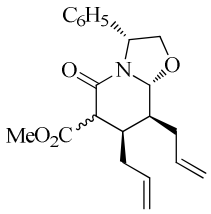


(3*R*,7*R*,8*S*,8a*R*)-7,8-Diallyl-6-(methoxycarbonyl)-5-oxo-3-phenyl-2,3,6,7,8,8a-hexahydro-5*H*-oxazolo[3,2-*a*]pyridine (6).

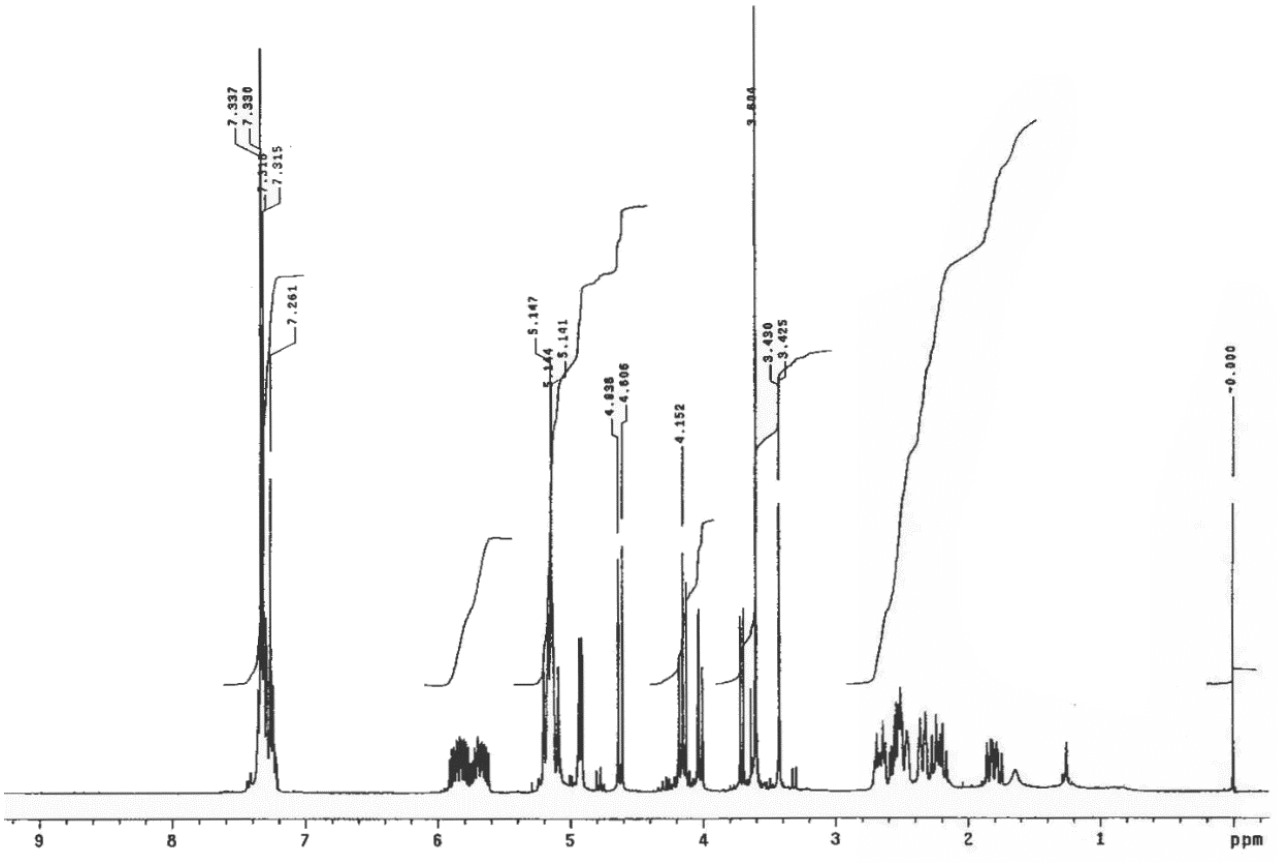
LiCl (1.20 g, 28.4 mmol) was dried at 80 °C for 1 hour under vacuum (10–15 mmHg) in a three-necked, 250 mL round-bottomed flask. Then, CuI (5.40 g, 28.4 mmol) and THF (150 mL) were added under an inert atmosphere, and the mixture was stirred at room temperature for 5 minutes. The suspension was cooled to –78 °C, and allylmagnesium bromide (28.4 mL of a 1 M solution in Et_2O, 28.4 mmol), TMSCl (3.60 mL, 28.4 mmol), and unsaturated lactam **5** (7.11 mmol) in THF (5 mL) were successively added. The resulting mixture was stirred at –78 °C for 18 hours. The reaction was quenched with saturated aqueous NH_4Cl, and the resulting mixture was filtered through celite. The aqueous layer was extracted with EtOAc, and the combined organic extracts were dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography (9:1 to 7:3 hexane-EtOAc) of the resulting oil gave **6** (2.05 g, 81% yield) as a mixture of C-6 epimers (ratio 2:1).

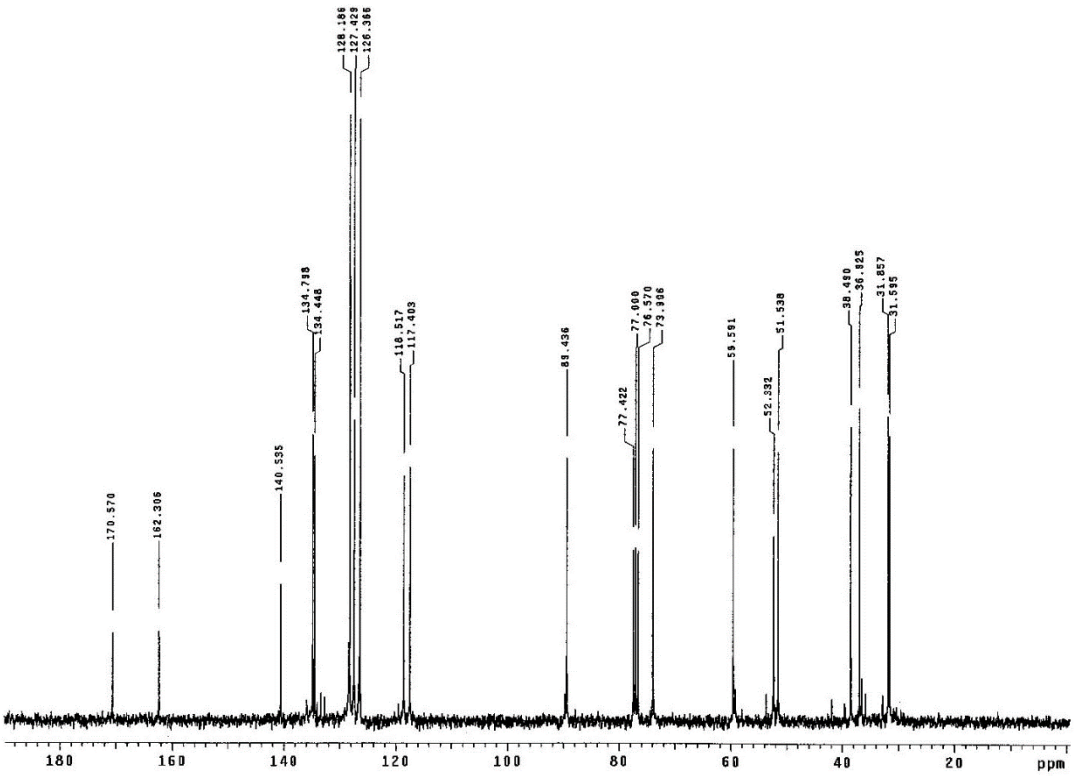
IR (film): 1665 (NCO), 1736 (COO) cm^{-1}.

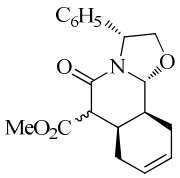
^{1}H NMR (300 MHz, $CDCl_3$, COSY, gHSQC): δ1.80 (ddd, *J* = 14.1, 12.0, 9.0 Hz, 1H, CH_2 allyl), 2.16 (dt, *J* = 14.1, 9.3, 9.3 Hz, 1H, CH_2 allyl), 2.34 (dm, *J* = 12.0 Hz, 1H, H-7), 2.44-2.70 (m, 3H, H-8, CH_2 allyl), 3.43 (d, *J* = 1.5 Hz, 1H, H-6), 3.60 (s, 3H, CH_3O), 4.02 (dd, *J* = 9.3, 1.8 Hz, 1H, H-2), 4.15 (dd, *J* = 9.3, 7.2 Hz, 1H, H-2), 4.62 (d, *J* = 9.6 Hz, 1H, H-8a), 4.91 (dd, *J* = 7.2, 1.8 Hz, 1H, H-3), 5.14 (m, 4H, CH_2=), 5.68 (dddd, *J* = 15.0, 10.2, 9.0, 4.8 Hz, 1H, CH=), 5.84 (dddd, *J* = 15.3, 9.9, 8.7, 5.1 Hz, 1H, CH=), 7.26-7.33 (m, 5H, C_6H_5).

^{13}C NMR (75.4 MHz, $CDCl_3$): δ31.6, 31.8 (CH_2), 36.9 (C-7), 38.5 (C-8), 51.5 (C-6), 52.3 (CH_3O), 59.6 (C-3), 73.9 (C-2), 89.4 (C-8a), 117.4, 118.5 (CH_2=), 126.4, 128.2 (C-*o*, *m*), 127.4 (C-*p*), 134.4, 134.8 (CH=), 140.5 (C-*i*), 162.3 (NCO), 170.6 (COO).

HRMS (ESI) calcd for $[C_{21}H_{25}NO_4 + H]^+$: 356.1783, found: 356.1779.







(3*R*,6a*R*,10a*S*,10b*R*)-6-(Methoxycarbonyl)-5-oxo-3-phenyl-2,3,6,6a,7,10,10a,10b-octahydro-5*H*-oxazolo[2,3-*a*]isoquinoline (7).

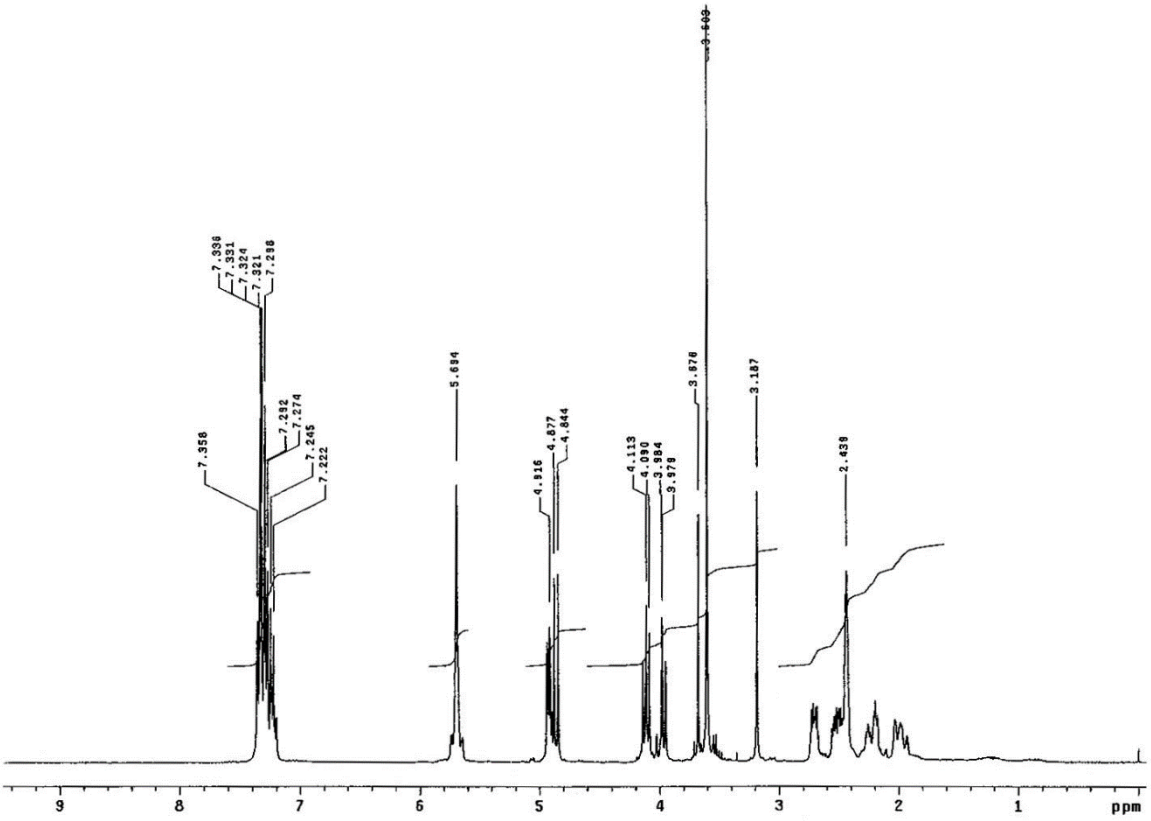
Second generation Grubbs catalyst (642 mg, 0.756 mmol) was added to a solution of lactam **6** (3.58 g, 10.1 mmol) in CH_2Cl_2 (1.44 L). The mixture was stirred for 18 hours at room temperature, and the resulting suspension was concentrated. Flash chromatography (4:1 to 3:2 hexane-EtOAc) of the residue gave tricyclic lactam **7** as a mixture of C-6 epimers (2.80 g, 85% yield).

IR (film): 1667 (NCO), 1738 (COO) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ 2.00 (m, 1H, H-7), 2.20 (m, 1H, H-7), 2.43 (m, 2H, H-10), 2.50 (m, 1H, H-6a), 2.70 (m, 1H, H-10a), 3.18 (s, 1H, H-6), 3.60 (s, 3H, CH_3O), 3.96 (dd, *J* = 9.0, 1.2 Hz, 1H, H-2), 4.12 (dd, *J* = 9.0, 6.9 Hz, 1H, H-2), 4.85 (d, *J* = 9.9 Hz, 1H, H-10b), 4.92 (dd, *J* = 6.9, 1.2 Hz, 1H, H-3), 5.69 (m, 2H, H-8, H-9), 7.22-7.35 (m, 5H, C_6H_5).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.1 (C-10a), 28.0 (C-7), 32.6 (C-10), 33.5 (C-6a), 52.2 (CH_3O), 53.9 (C-6b), 59.4 (C-3), 73.6 (C-2), 87.1 (C-10b), 124.4, 124.8 (C-8, C-9), 126.8, 128.0 (C-*o*, *m*), 127.2 (C-*p*), 140.6 (C-*i*), 162.0 (NCO), 170.2 (COO).

Elemental analysis calcd (%) for [$C_{19}H_{21}O_4N \cdot ¼ H_2O$]: C, 68.76; H, 6.53; N, 4.22. Found: C, 68.82; H, 6.90; N, 4.20.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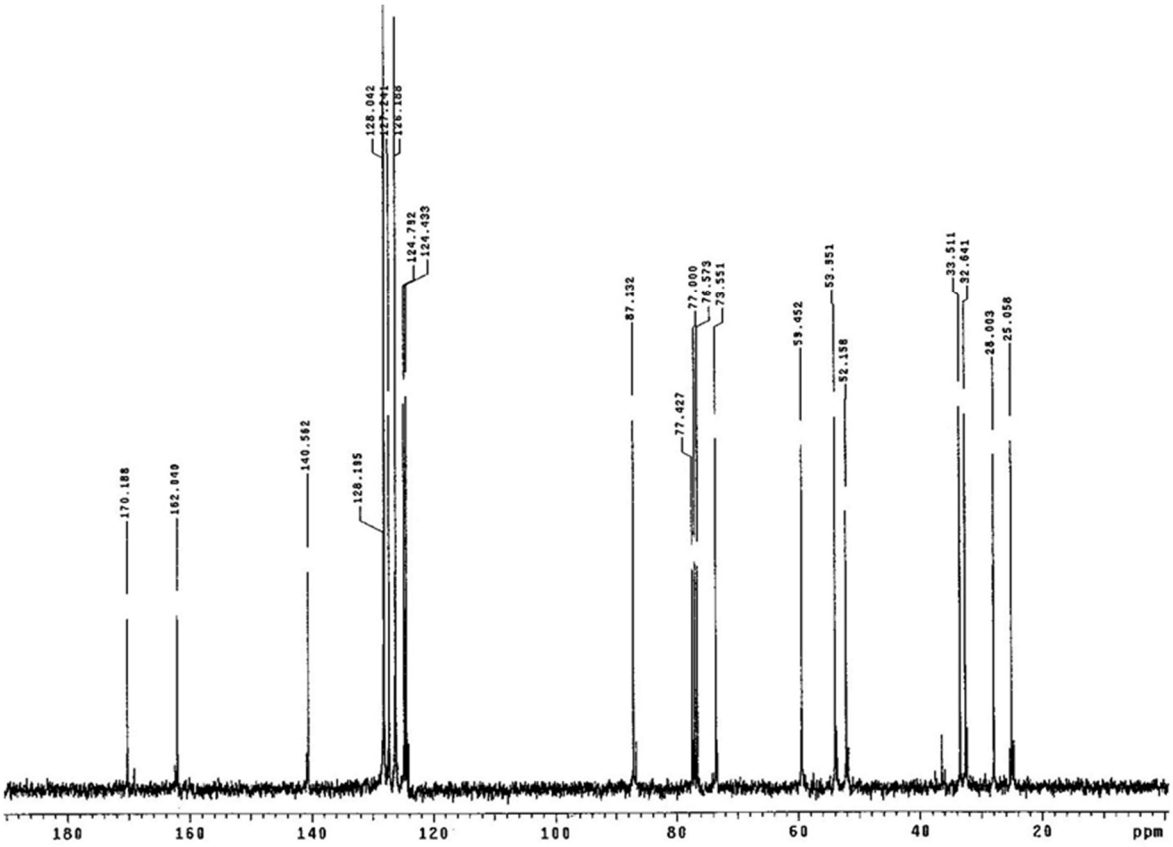
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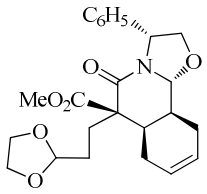


(3*R*,6*R*,6a*R*,10a*S*,10b*R*)-6-[3-(1,3-Dioxolan-2-yl)ethyl]-6-(methoxycarbonyl)-5-oxo-3-phenyl-2,3,6,6a,7,10,10a,10b-octahydro-5*H*-oxazolo[3,2-*a*]isoquinoline (8).

A solution of isoquinoline **7** (880 mg, 2.70 mmol) in dry DMF (2 mL) was added to a cooled (0º C) suspension of NaH (161 mg of a 60% dispersion in mineral oil, 4.02 mmol) in dry DMF under an inert atmosphere, and the resulting mixture was stirred at 0º C for 1 hour. Then TBAI (198 mg, 0.536 mmol) and 2-(2-bromoethyl)-1,3-dioxolane (1.59 ml, 13.5 mmol) were added to the solution and the mixture was stirred overnight at room temperature. The reaction was quenched with aqueous NH_4Cl, extracted firstly with diethyl ether and then with CH_2Cl_2. Combined organic extracts washed with brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Flash chromatography (7:3 to 1:1 hexane-EtOAc) of the resulting residue afforded compound **8** (905 mg, 78%).

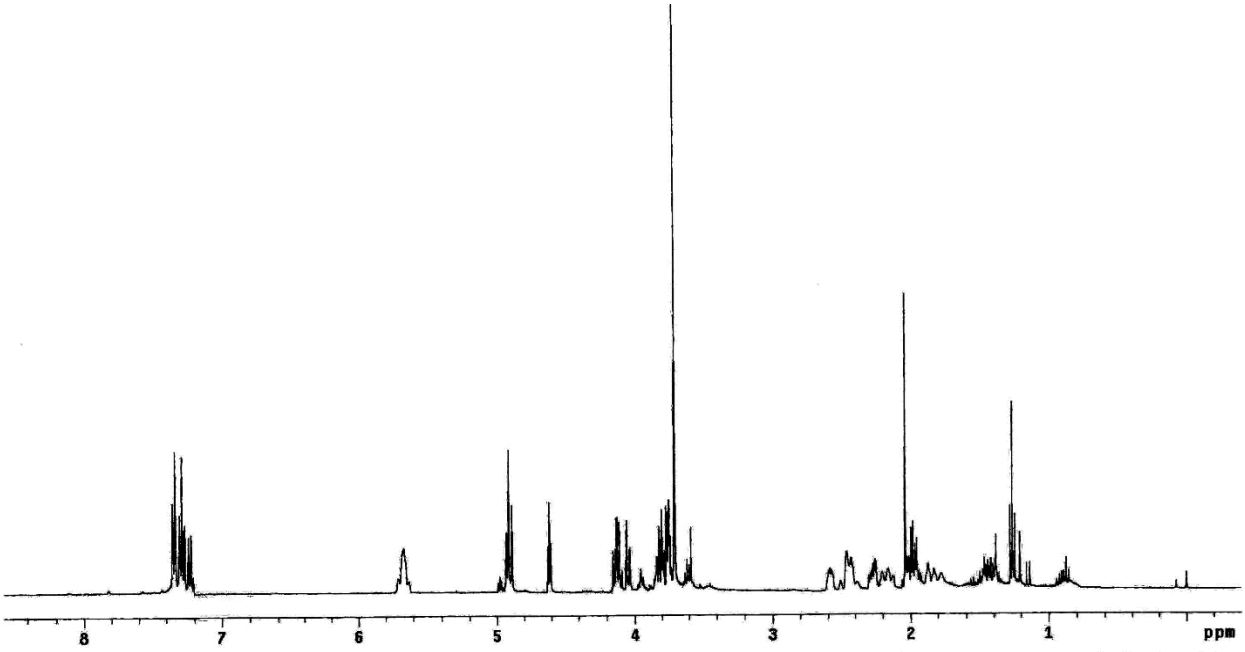
$[\alpha]^{22}_D$ = –35.2 (*c* 0.7, $CHCl_3$).

IR (film): 1655 (NCO), 1738 (COO) cm^{-1}.

^{1}H NMR (400 MHz,$CDCl_3$, COSY, gHSQC): δ 1.44 (m, 2H, H-2'), 1.85 (dm, *J* = 17.2 Hz, 1H, H-7), 1.99 (m, 2H, H-1'), 2.16 (tm, *J* = 17.2 Hz, 1H, H-7), 2.27 (ddd, *J* = 11.6, 5.6 , 3.6 Hz, 1H, H-6), 2.46 (m, 2H, H-10), 2.58 (dd, *J* = 9.6, 4.8 Hz, 1H, H-10a), 3.71 (s, 3H, CH_3O), 3.75, 3.80 (2m, 4H, CH_2O), 4.05 (dd, *J* = 9.2, 1.6 Hz, 1H, H-2), 4.13 (dd, *J* = 9.2, 6.8 Hz, 1H, H-2), 4.61 (t, *J* = 4.8 Hz, 1H, H-3'), 4.90 (d, *J* = 9.6 Hz, 1H, H-10b), 4.92 (dd, *J* = 6.8, 1.6 Hz, 1H, H-3'), 5.67 (m, 2H, H-8, H-9), 7.10-7.20 (m, 5H, C_6H_5).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 25.6-25.7 (C-7, C-10), 29.4 (C-2'), 31.4 (C-1'), 33.4 (C-10a), 40.0 (C-6a), 51.9 (CH_3O), 58.4 (C-6b), 59.7 (C-3), 64.6 (CH_2O), 73.5 (C-2), 87.0 (C-10b), 104.1 (C-3'), 124.3-124.6 (C-8 C-9), 126.6 -128.3 (C-*o*, *m* C_6H_5), 127.4 (C-*p*), 141.5 (C-*i*), 165.1 (NCO), 171.8 (COO).

HRMS (ESI) calcd for $[C_{24}H_{30}NO_6 + H]^+$: 428.2068, found: 428.2062.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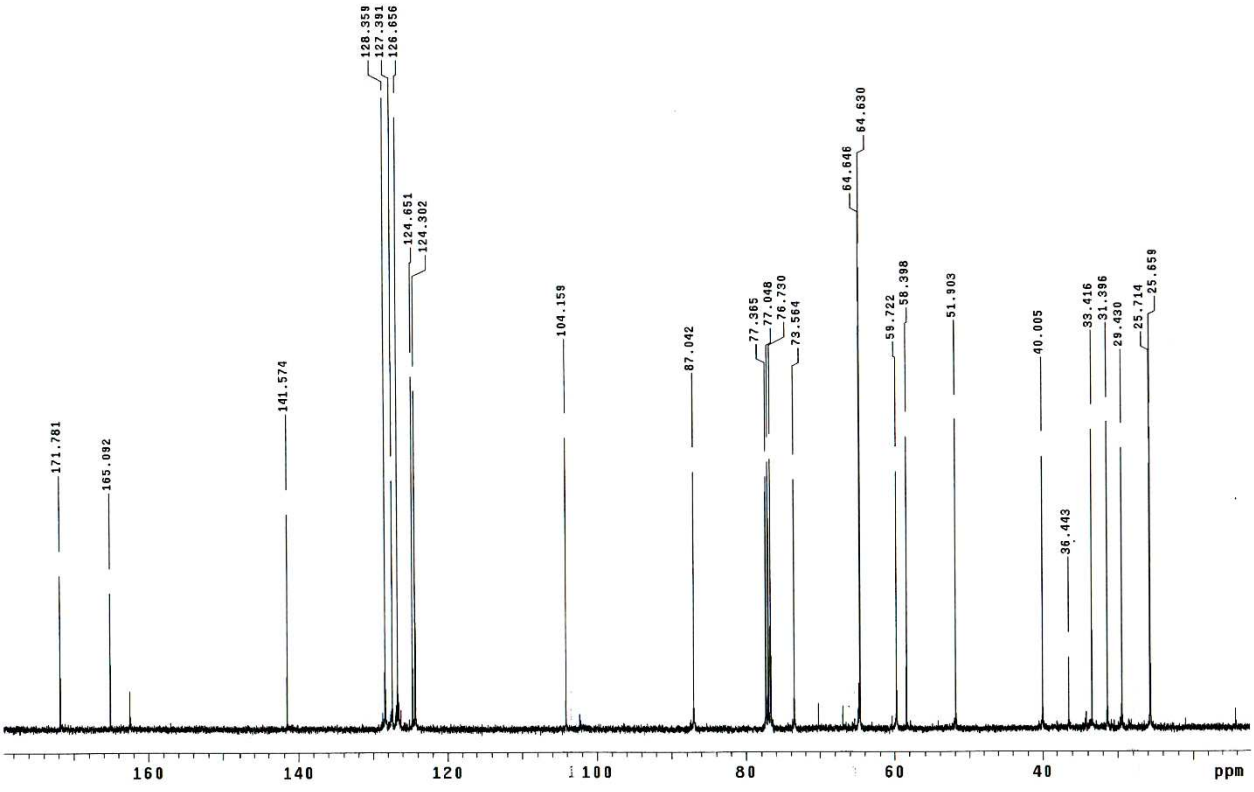
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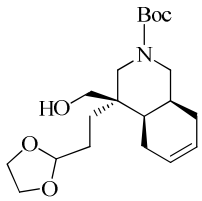


(4*R*,4a*R*,8a*S*)-2-(*tert*-Butoxycarbonyl)-4-[3-(1,3-dioxolan-2-yl)ethyl]-4-(hydroxymethyl)-1,2,3,4,4a,5,8,8a-octahydroisoquinoline (11).

First step: Liquid ammonia (15 mL) was condensed at -78 ºC in a three-necked, 100 mL round-bottomed flask equipped with a coldfinger condenser charged with dry ice-acetone, and then a solution of lactam **8** (200 mg, 0.454 mmol) in THF (10 mL) was added. The temperature was raised to -30 ºC and sodium metal was added in small portions until the blue color persisted for 2 minutes. The reaction was quenched by the addition of solid NH_4Cl until the blue color disappeared, and the mixture was stirred at room temperature for 4 hours. The residue was digested at room temperature with CH_2Cl_2, and the resulting suspension was filtered through celite. The solution was concentrated under reduced pressure.

Second step: A solution of the above residue (mixture of compounds **9**) in anhydrous dioxane (14 mL) was added dropwise to lithium aluminium hydride (235 mg, 6.19 mmol) in a 3 necked flask at 0 ºC, under argon atmosphere and the resulting mixture was stirred overnight at reflux. The reaction was quenched with distilled water and NaOH 10%, dried with $MgSO_4$, filtered and concentrated to give the amino alcohol **10**, which was used in the next step without further purification.

Third step: Di-*tert*-butyl-dicarbonate (0.103 mL, 0.449 mmol) was added dropwise to a solution of the above amino alcohol **10** in anhydrous CH_2Cl_2 (7 mL) at room temperature under inert atmosphere and the resulting mixture was stirred for 20 hours. The solution was poured into saturated aqueous NH_4Cl and extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried, filtered, and concentrated. Flash chromatography (9:1 to 1:1 hexane-EtOAc) of the residue gave the compound **11** (71 mg, 45% overall yield from **8**).

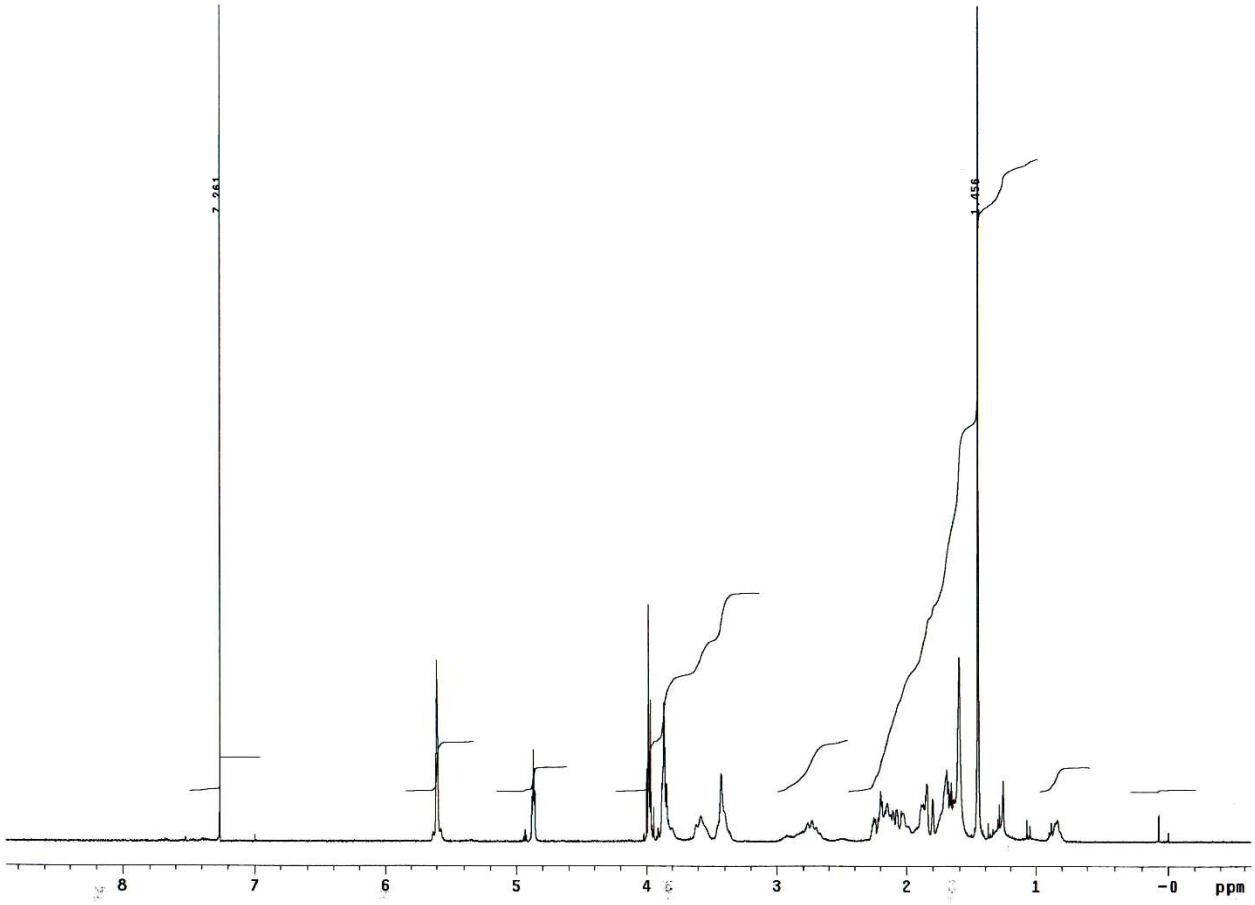
$[\alpha]^{22}_D$ = -8.34 (*c* 0.44, CH_2Cl_2).

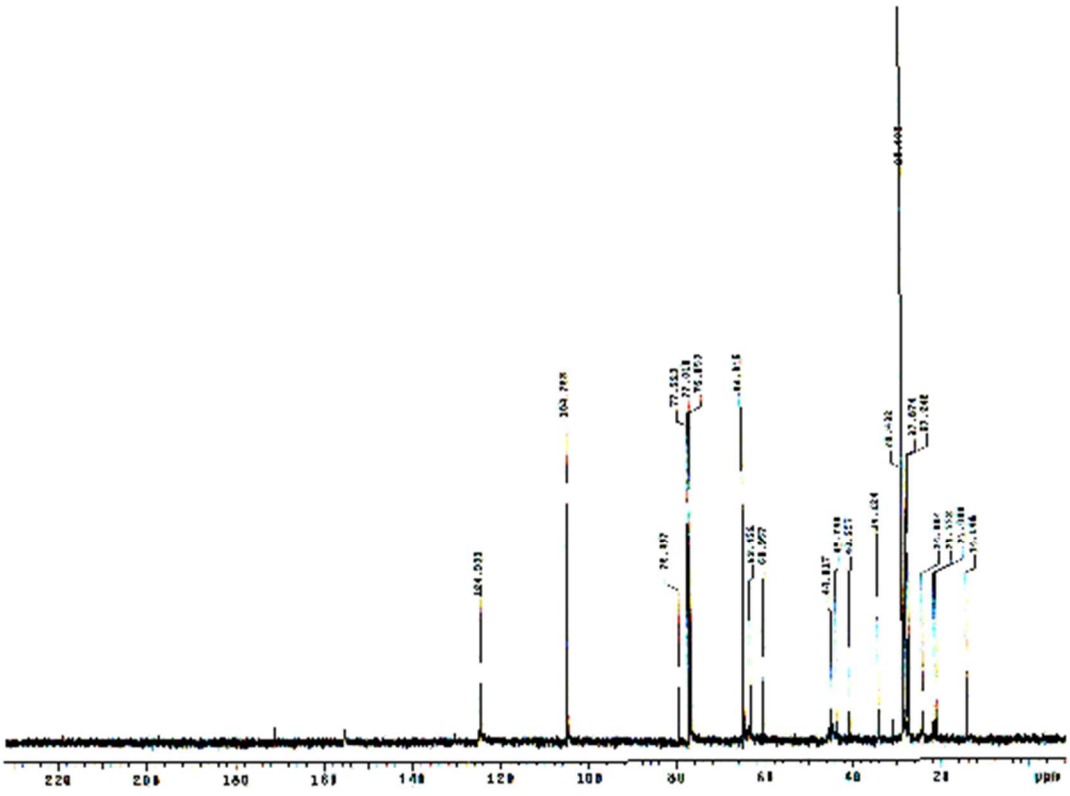
IR (film): 1683 (C=O), 3480 (OH) cm^{-1}.

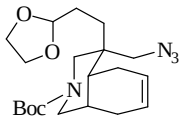
^{1}H NMR (400 MHz, $CDCl_3$,COSY, gHSQC):δ 1.45 (s, 9H, [(CH$_3$)$_3$C]), 1.58-1.76 (m, 4H, H-1', H-2'), 1.78-1.96(m, 2H, H-8, H-4a), 1.98-2.30 (m, 4H, H-5, H-8, H-8a), 2.67-2.77 (m, 2H, H-1, H-3), 3.42(s, 2H, CH_2OH), 3.59-3.62 (m, 2H, H-1,H-3), 3.85-3.98 (2m, 4H, CH_2O), 4.87(t, J = 4.8 Hz, 1H, H-3'), 5.60 (m, 2H, H-6, H-7).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.6(C-5), 24.0 (C-1'), 27.2 (C-2'), 27.9 (C-8a), 28.4 (C-8,[(CH$_3$)$_3$C]), 34.2(C-4), 45.4(C-1,C-3),64.9 (CH_2O), 68.2 (CH_2OH), 79.5 ([(CH_3)$_3$$C$]), 104.7 (C-3'), 124.8 (C-6, C-7), 155.0 (NCOO).

HRMS (ESI) calcd for $[C_{20}H_{33}NO_5 + Na]^+$: 390.2251, found: 390.2263.







(4*R*,4a*R*,8a*S*)-4-(Azidomethyl)-2-(*tert*-butoxycarbonyl)-4-[3-(1,3-dioxolan-2-yl)ethyl]-1,2,3,4,4a,5,8,8a-octahydroisoquinoline (13).

First Step: Anhydrous TEA (2.18 ml, 15.6 mmol) and methanesulfonyl chloride (1.21 ml, 15.6 mmol) were added at 0 ºC under an inert atmosphere to a stirred solution of alcohol **11** (1.98 g, 5.19 mmol) in anhydrous CH_2Cl_2 (86 ml), and the resulting mixture was stirred at room temperature for 4 hours. The reaction was quenched with a saturated aqueous NH_4Cl solution and extracted with CH_2Cl_2. The combined organic extracts were dried, filtered and concentrated to give a yellow oil which was used in the next step without purification.

Second Step: NaN_3 (2.03 g, 31.2 mmol) was added to a solution of the above mesylate **12** in anhydrous DMF (15 ml) and the mixture was heated to 90 ºC. After 48 hours, more NaN_3 (2.03 g, 31.2 mmol) was added and the resulting mixture was stirred at 90 ºC for an additional 24 hours. Then the reaction was quenched with distilled water, extracted with CH_2Cl_2, The organic extracts

were dried, filtered and concentrated under pressure to give an oil. Flash chromatography (hexane to 9:1 hexane-EtOAc) of the residue gave the azido compound **13** (1.69 g, 83%).

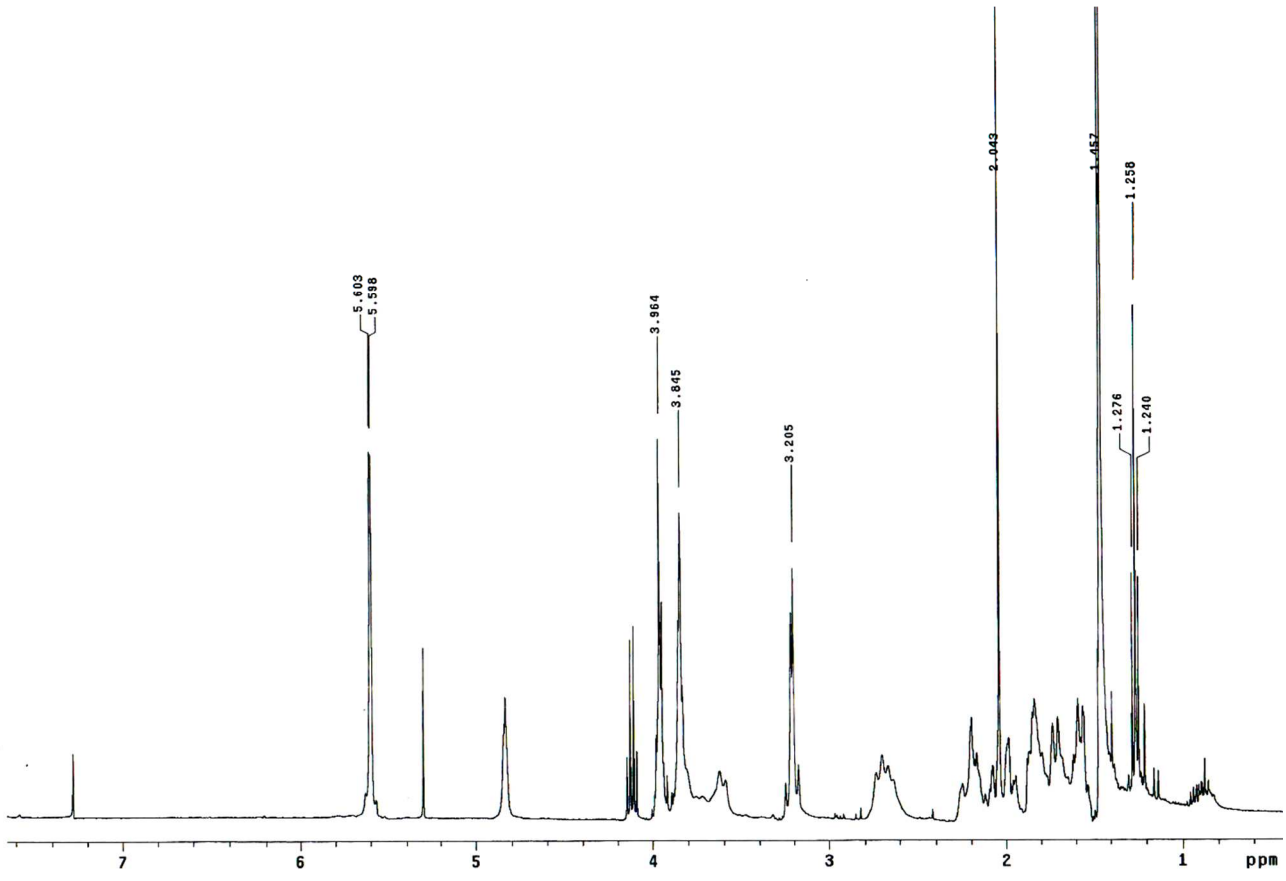
$[\alpha]^{22}_D$ = -35.2 (*c* 0.595, CH_2Cl_2).

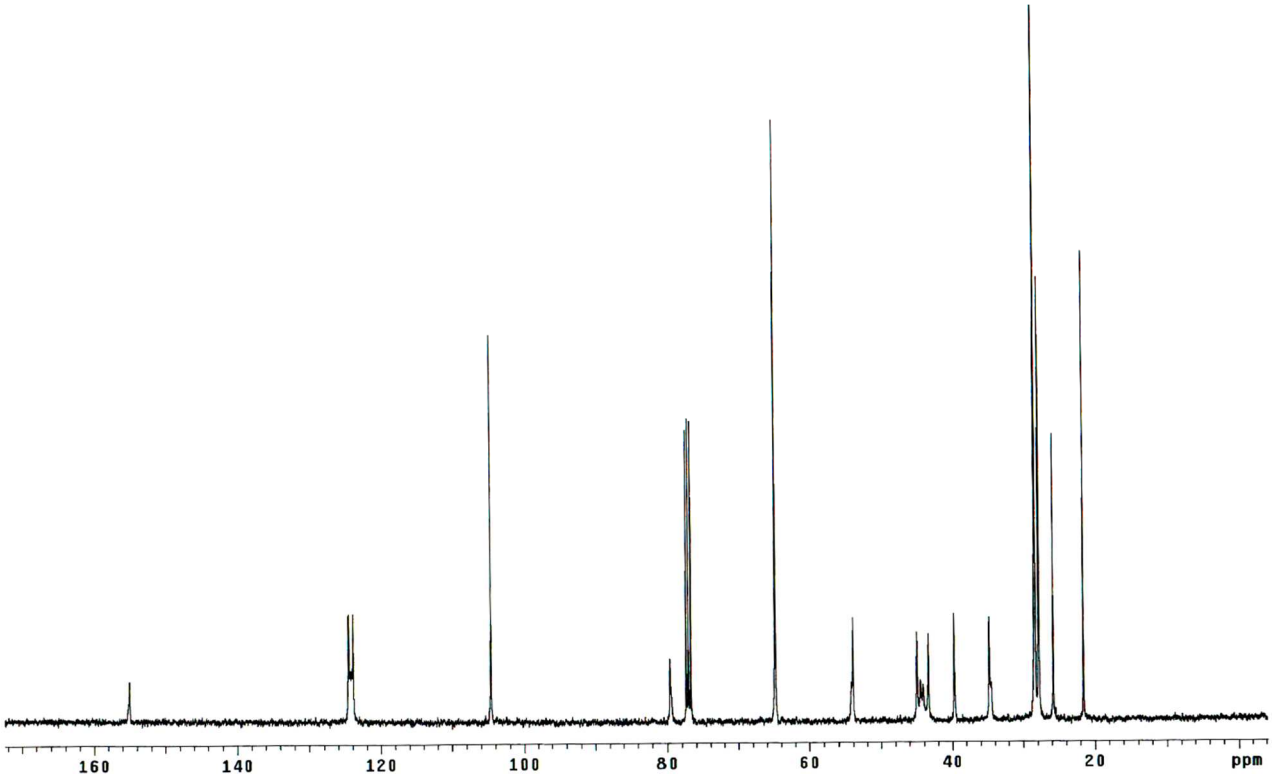
IR (film): 1691 (C=O), 2100 (N_3) cm^{-1}.

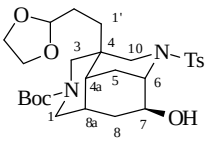
^{1}H NMR (400 MHz, $CDCl_3$,COSY, gHSQC):δ1.35 (s, 9H, [$(CH_3)_3C$]), 1.44-2.08 (m, H, 10H, H-1', H-2', H-4, H-4a, H-5, H-8a), 1.88-2.08 (m, 4H, H-5, H-8, H-8b), 2.49-2.63 (m, 2H, H-1, H-3), 3.10 (s, 2H, CH_2N_3), 3.59-3.62 (m, 2H, H-1,H-3), 3.72- 3.85 (2m, 4H, CH_2O), 4.72 (t, *J* = 4.8 Hz, 1H, H-3'), 5.48 (m, 2H, H-6, H-7).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ21.6 (C-2'), 25.8 (C-5), 27.8 (C-8a), 27.9 ([(CH_3)C], C-8), 28.4 (C-1'), 34.8 (C-4a), 39.7 (C-4), 43.4-45.0 (C-1, C-3), 54.0 (CH_2N_3), 64.9 (CH_2OH), 79.7 [$(CH_3)_3C$], 104.6 (C-3'), 123.9, 124.6 (C-6, C-7), 155.0 (NCOO).

HRMS (ESI) calcd for $[C_{20}H_{32}N_4O_4 + Na]^+$: 415.2316, found: 415.2334.







(4*R*,4a*R*,6*S*,7*S*,8a*S*)-2-(*tert*-Butoxycarbonyl)-4-[3-(1,3-dioxolan-2-yl)ethyl]-7-hydroxy-6,4-(iminomethano)-9-(*p*-toluenesulfonyl)perhydroisoquinoline (15).

First step: *m*-Chloroperoxybenzoic acid (1.48 g, 77% of purity, 6.61 mmol) was added to a solution of azide **13** (1.22 g, 3.00 mmol) at 0 °C in CH_2Cl_2, (43 mL) and the mixture was allowed to warm slowly to room temperature. After 5 hours, the reaction was quenched with saturated aqueous $NaHCO_3$, and the mixture was extracted with CH_2Cl_2. The organic extracts were dried, filtered and concentrated under reduced pressure to give azido epoxide **14**, which was used in the next step without purification.

Second step: Me_3P (4.8 mL of a 1 M solution in THF, 4.80 mmol) was added to a solution of the above azido epoxide **14** in THF (60 mL), and the mixture was stirred at room temperature for 2 hours. Water (6 mL) was added, and the resulting mixture was stirred overnight at room temperature and concentrated under reduced pressure to afford a diazatricyclic alcohol.

Third step: TEA (0.410 mL, 3.00 mmol) was added dropwise to a stirring solution of the above aminoalcohol in anhydrous CH_2Cl_2 (50 mL) at 0 ºC. A solution of *p*-toluenesulfonyl chloride (572 mg, 3.00 mmol) in anhydrous CH_2Cl_2 (5 mL) was transferred via cannula, and the stirring was continued at 0 ºC for 2.5 hours. The reaction was then quenched with saturated aqueous NH_4Cl, and the aqueous layer was extracted with CH_2Cl_2. The combined organic extracts were dried, filtered and concentrated under reduced pressure to afford the protected tricyclic compound **15** (998 mg, 62% in three steps) after flash chromatography (9:1 to 1:1 hexane-EtOAc).

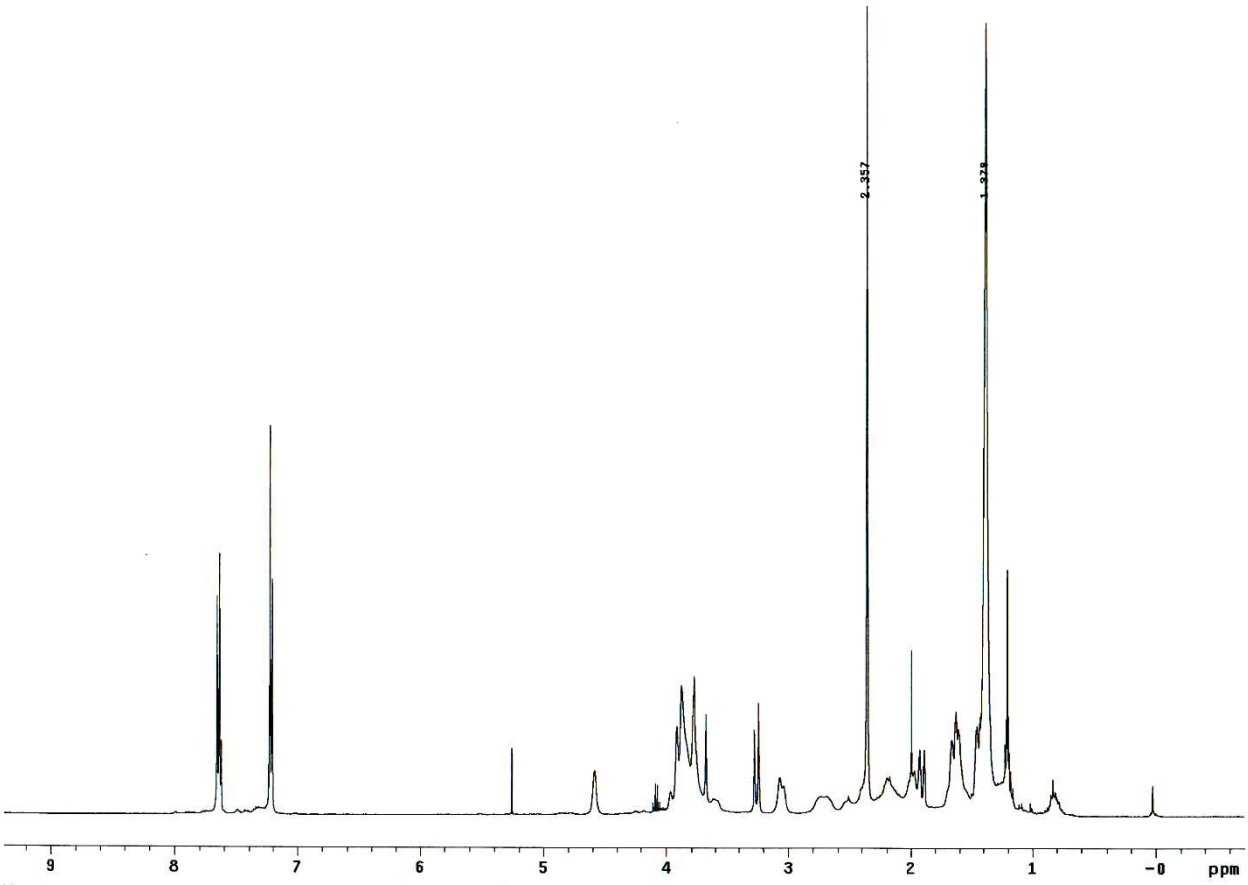
$[\alpha]_D^{22}$ = +24.01 (*c* 1.5, $CHCl_3$).

IR (film): 1689 (C=O), 3490 (OH) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ 1.35, 1.41 (m, 13H, [$(CH_3)_3$C], H-1',H-2'), 1.45 (m, 1H, H-4a), 1.59-1.64 (m, 3H, 2H-8, H-5a), 1.92 (d, *J* = 16.0 Hz, 1H, H-5b), 1.97 (m, 1H, H-8a), 2.33 (s, 3H, CH_3Ts), 2.60-2.78 (m, 2H, H-1a, H-3b), 3.03 (d, *J* = 12.5 Hz, 1H, H-10a), 3.24 (d, *J* = 12.9 Hz, 1H, H-10b), 3.76 (m, 2H, CH_2O), 3.83 (masked, 1H, H-7), 3.86 (m, 2H, CH_2O), 3.90 (masked, 2H, H-1a, H-3b), 3.96 (s, 1H, H-6), 4.60 (s, 1H, H-3'), 7.22 (d, *J* = 8.1 Hz, 2H, H-*m* Ts), 7.64 (d, *J* = 8.2 Hz, 2H, H-*o* Ts).

^{13}C NMR (100.6 MHz, $CDCl_3$) : δ 21.1 (CH_3Ts), 21.9 (C-5), 27.3 (C-1'), 28.2 [(*C*H_3)C],29.4 (C-2'), 30.7 (8a), 32.3 (C-8), 32.4 (C-4), 35.2 (C-4a), 46.8 (C-10), 50.8 (C-6), 48.4-49.6 (C-1, C-3), 64.8 (2CH_2O),67.3 (C-7), 80,0 [(CH_3)*C*], (104.4 (C-3'), 127.2 (C-*o* Ts), 129.9 (C-*m* Ts), 137.6 (C-*p* Ts), 143.3 (C-*i* Ts), 155.3 (NCOO).

HRMS (ESI) calcd for $[C_{27}H_{40}N_2O_7S + H]^+$: 537.2629, found: 537.2613.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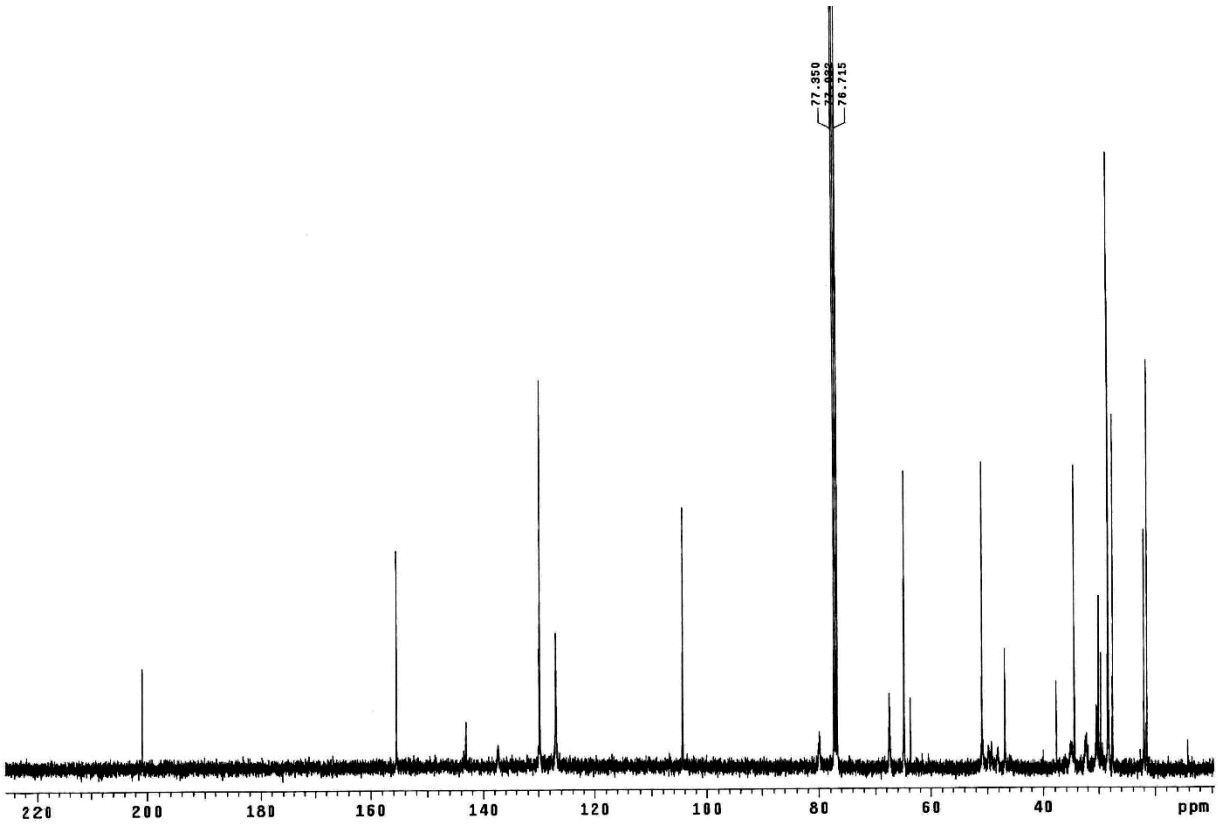
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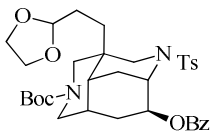


(4*R*,4a*R* ,6*S*,7*S*,8a*S*)-7-(*p*-Benzoyloxy)-2-(*tert*-butoxycarbonyl)-4-[3-(1,3-dioxolan-2-yl)ethyl]-6,4-(iminomethano)-9-(*p*-toluensulfonyl)perhydroisoquinoline (16).

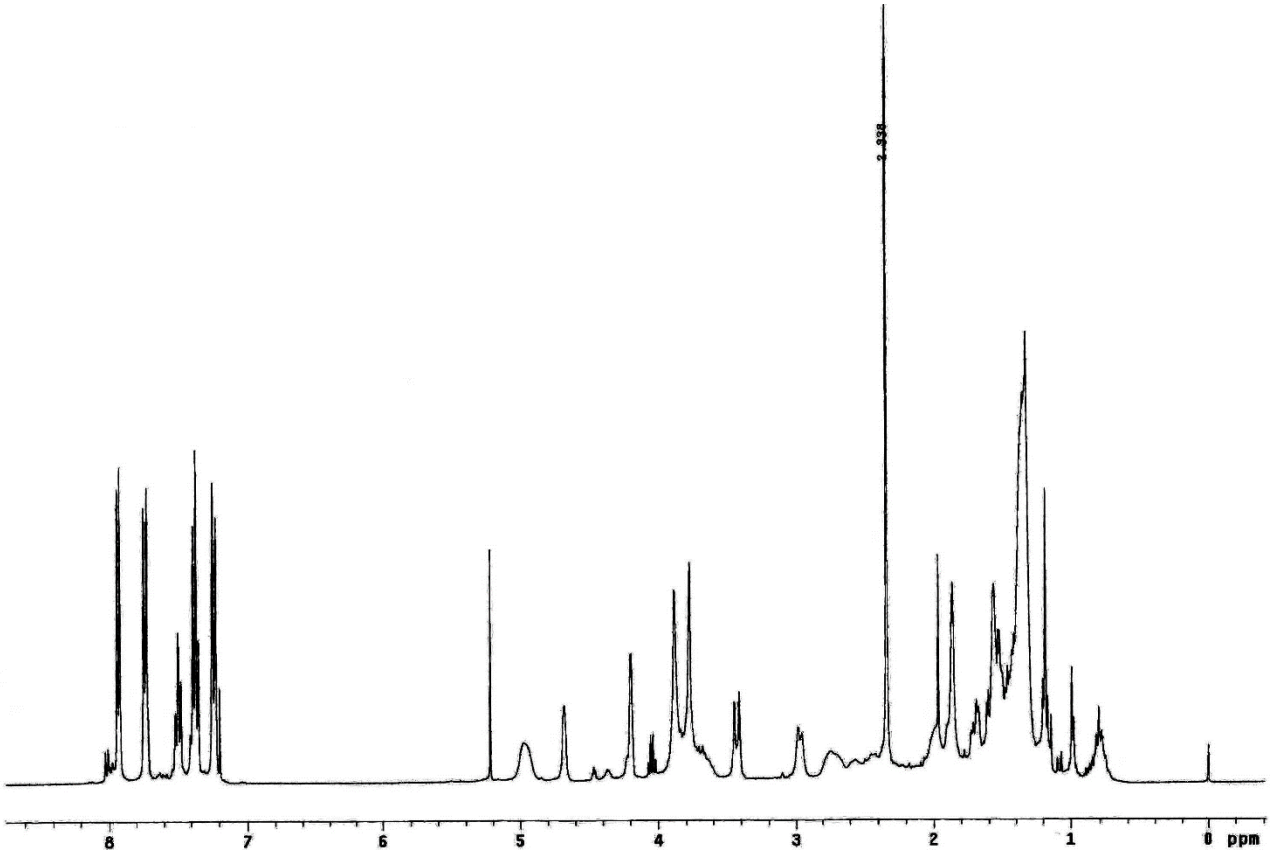
Triethylamine (40 µL, 0.296 mmol) and DMAP (2.4 mg, 0.0198 mmol) were added to a stirred solution of tricyclic compound **15** (56 mg, 0.0990 mmol) in CH_2Cl_2 (2.5 mL). After 30 minutes benzoic anhydride (34 mg, 0.150 mmol) was added and the resulting mixture was stirred at room temperature overnight. The reaction was quenched by the addition of a solution of NH_4Cl and the aqueous layer was extracted with CH_2Cl_2. The combine organic extracts were dried over $MgSO_4$, filtered, and concentrated under reduced pressure. Flash chromatography (9:1 to 1:1 hexane-EtOAc) of the residue afforded compound benzoic compound **16** (40 mg, 65%) as a white foam.

IR (film): 1715 (C=O) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, COSY, gHSQC): δ 1.22 (m, 2H, H-2'), 1.29-1.45 (m, 11H, H-1', [$(CH_3)_3C$]), 1.49-1.62 (m, 2H, H-4a, H-8), 1.66-1.74 (m, 1H, H-8), 1.86 (s, 2H, H-5), 1.97 (s, 1H, H-8a), 2.34 (s, 3H, CH_3Ts), 2.50-2.81 (m, 2H, H-1, H-3), 3.03 (d, *J* = 12.5 Hz, 1H, H-10), 3.47 (d, *J* = 12.9 Hz, 1H, H-10), 3.73-3.89 (masked, 2H, H-1, H-3), 3.77, 3.87 (2m, 4H, $2CH_2O$), 4.24 (s, 1H, H-6), 4.72 (s, 1H, H-3'), 5.03 (s, 1H, H-7), 7.23 (d, *J* = 8.1 Hz, 2H, H-*m* Ts), 7.38 (t, *J* = 7.8 Hz, 2H, H-*o* C_6H_5), 7.50 (t, *J* = 7.4 Hz, 1H, H-*p* C_6H_5), 7.74 (d, *J* = 8.2 Hz, 2H, H-*o* Ts), 7.93 (dd, *J* = 8.2, 1.5 Hz, 2H, H-*m* C_6H_5).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.8 (CH_3Ts), 23.7 (C-5),27.3 (C-1'), 28.1 [(CH_3)C], 28.2 (C-2'), 29.6 (C-8), 30.9 (C-8a), 31.1 (C-4), 34.7 (C-4a), 47.2 (C-10), 48.3 (C-6), 49.3-49.8 (C-1, C-3), 64.7 ($2CH_2O$),69.2 (C-7), 80.0 [$(CH_3)C$], 104.2 (C-4'), 127.2 (C-*o* Ts), 128.4 (C-*m* C_6H_5), 129.5 (C-*o* C_6H_5), 129.7 (C-*m* Ts), 133.1 (C-*p* C_6H_5), 137.1 (C-*p* Ts), 143.4 (C-*i* Ts), 155.5 (NCOO).

HRMS (ESI) calcd for $[C_{34}H_{44}N_2O_8S + H]^+$: 641.2891, found: 641.2877.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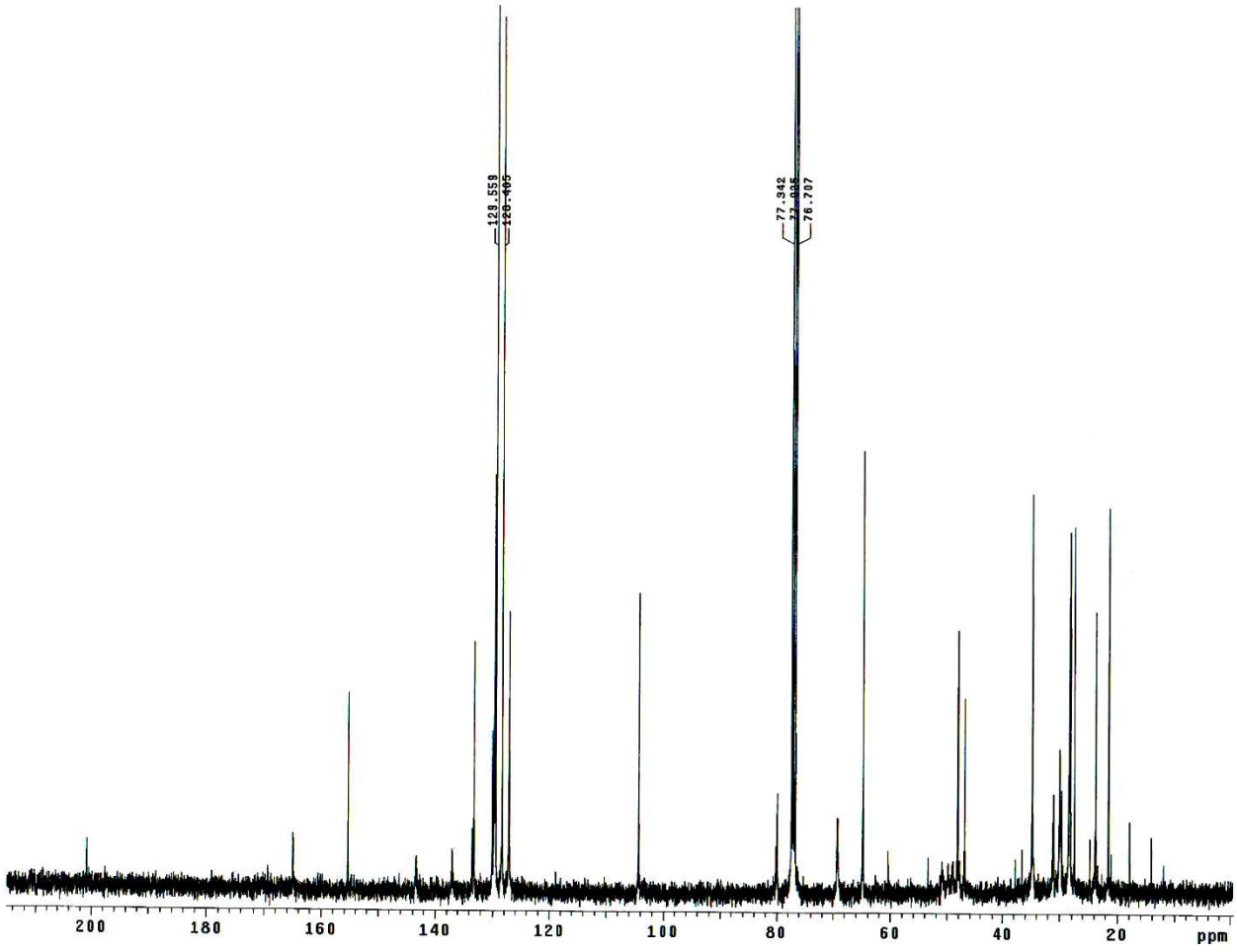
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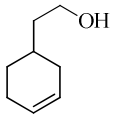


2-(3-Cyclohexenyl)ethanol (17).

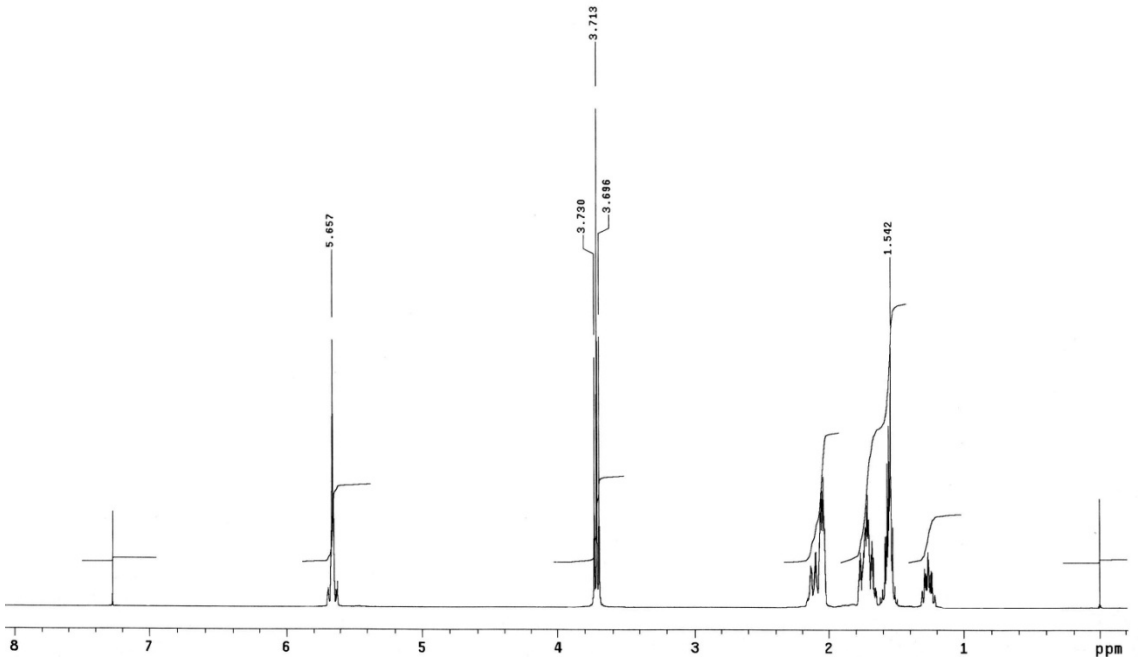
$BF_3 \cdot Et_2O$ (3.8 mL, 30.5 mmol) was added dropwise over 30 minutes under inert atmosphere to a cooled (0 °C) solution of 2-methyl-2-butene (30.5 mL, 61.0 mmol of a 2 M solution in THF) and $NaBH_4$ (855mg, 22.2 mmol) in anhydrous THF (14 mL), and the resulting mixture was stirred at 0 °C for 1 h. 4-Vinylcyclohexene (3.6mL, 27.7 mmol) was then added dropwise over a 5 minutes and the solution was stirred at room temperature for 2 h. The borane intermediate was oxidized by slowly addition at 0 °C of NaOH 3 N (25 mL) and H_2O_2 30% (25 mL), and the resulting solution was stirred overnight at 70 °C. The mixture was extracted with Et_2O and the combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure affording alcohol **17** (3.4 g, 99%).

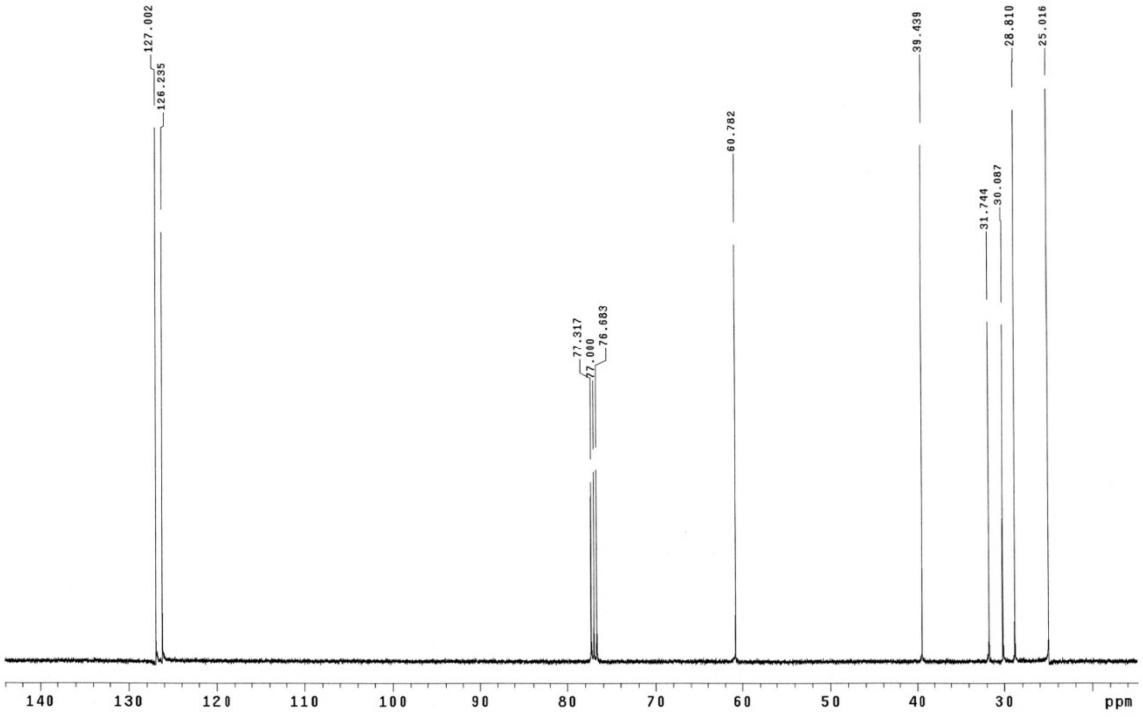
IR (film):3400 (OH) cm^{-1}.

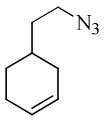
^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ1.26 (m, 1H, H-5), 1.54 (m, 2H, H-1'), 1.69 (dm, *J* = 14.8 Hz, 1H, H-3), 1.72 (m, 2H, H-4, H-5), 2.05 (m, 2H, H-6), 2.11 (dm, *J* = 14.8 Hz, 1H, H-3), 3.71 (t, *J* = 6.8 Hz, 2H, H-2'), 5.65 (m, 2H, H-1, H-2).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ25.0 (C-6), 28.8 (C-5), 30.1 (C-4), 31.7 (C-3), 39.4 (C-1'), 60.8 (C-2'), 126.0, 127.0 (C-1,C-2).

HRMS (ESI) calcd for $[C_8H_{14}O + H]^+$: 127.1117, found: 127.1114.







4-(2-Azidoethyl)-1-cyclohexene (18).

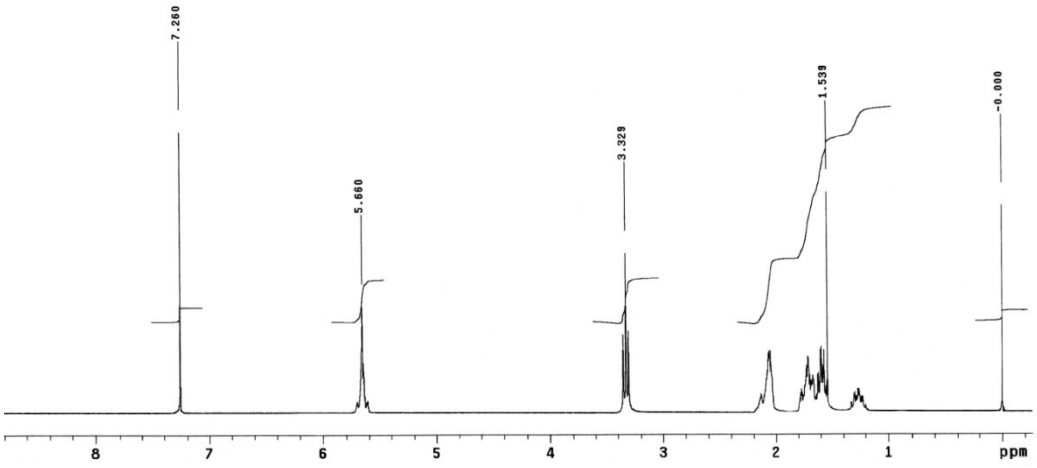
First step: Triethylamine (6.52 mL, 46.8 mmol) and mesyl chloride (4.83 mL, 62.4 mmol) were added dropwise to a stirring solution of alcohol **17** (3.4 g, 27.4 mmol) in anhydrous CH_2Cl_2 (400

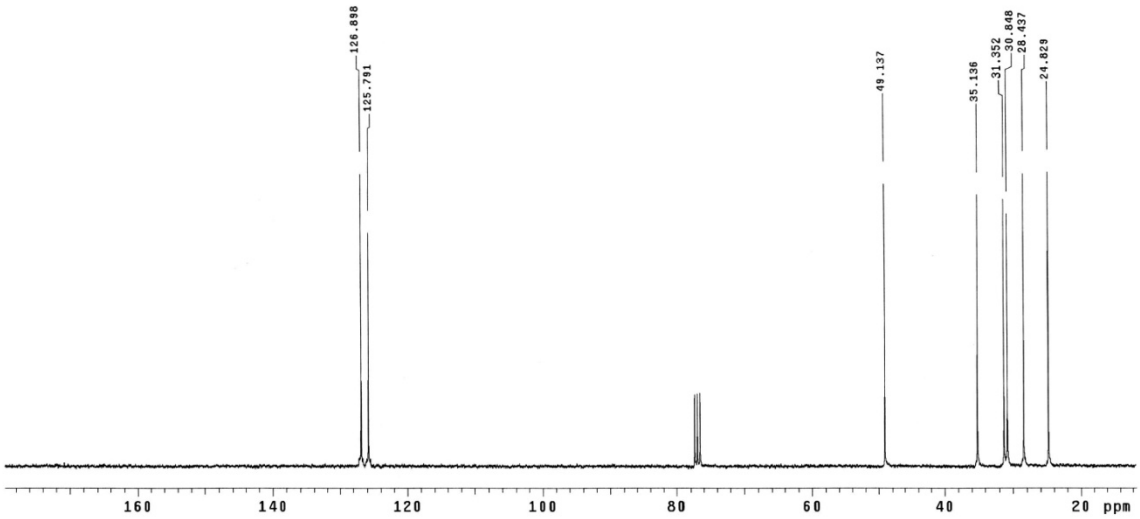
mL) cooled to 0 °C under inert atmosphere. After the end of addition, the mixture was allowed to heat slowly to room temperature and was stirred for additional 2 h. Saturated aqueous NH_4Cl was added and the mixture was extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered and the solvent was concentrated under reduced pressure. Flash chromatography (hexane to 8:2 hexane – ethyl acetate) affords the corresponding mesyl derivative (6.42 g, 99%).

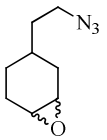
Second step: NaN_3 (10.9 g, 167.7 mmol) was added to a solution of the above compound (9.809 g, 41.9 mmol) in anhydrous DMF (250 mL) and the mixture was heated at 60 °C for 3 h. The resulting mixture was quenched with distilled water. The aqueous layer was extracted with CH_2Cl_2, and the combined organic extracts were washed 6 times with water, dried with Na_2SO_4 and concentrated under reduced pressure to give an oil. Flash chromatography (hexane) afforded azide derivative **18** (5.068 g, 80%) as a colourless oil.

^{1}H NMR (300 MHz, $CDCl_3$): δ 1.29 (m, 1H, H-5), 1.53-1.80 (m, 5H, H-5, H-4, H-3, 2H-1'), 2.05 (m, 3H, 2H-6, H-3), 3.33 (t, J = 6.9 Hz, 2H, H-2'), 5.66 (m, 2H, H-1, H-2).

^{13}C NMR (75.4 MHz, $CDCl_3$): δ 24.8 (C-6), 28.4 (C-5), 30.8 (C-4), 31.3 (C-3), 35.1 (C-1'), 49.1 (C-2'), 125.8, 126.9 (C-1, C-2).







3-(2-Azidoethyl)-7-oxabicyclo[4.1.0]heptane (19).

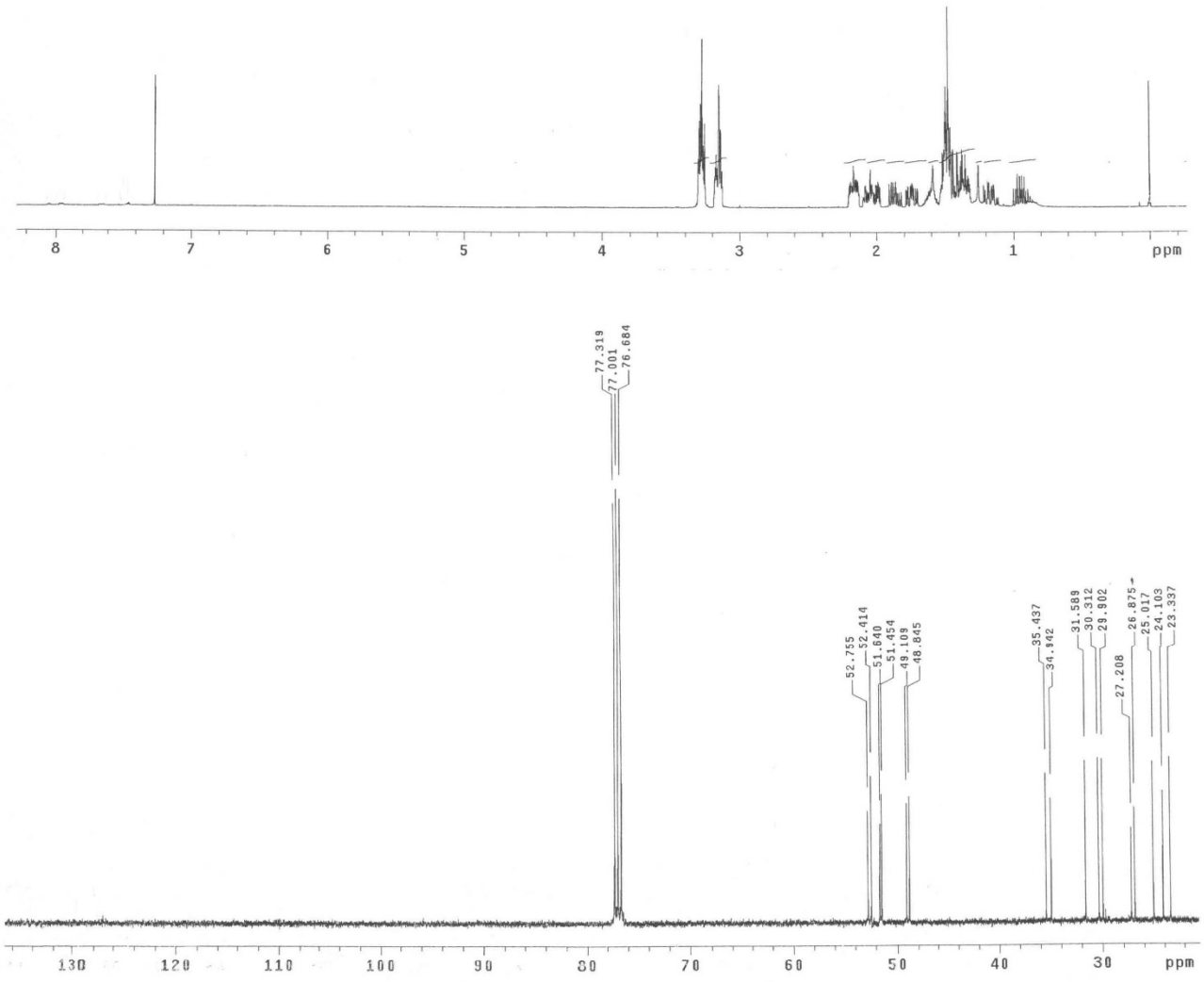
m-Chloroperoxybenzoic acid (2.2 g, technical grade 77%, 9.934 mmol) was added to a cold (0 °C) solution of azide **18** (1.0 g, 6.622 mmol) in CH_2Cl_2 (50 mL), and the mixture was allowed to warm slowly to room temperature. After 4 h under stirring at room temperature, the mixture was washed with saturated aqueous $NaHCO_3$, then saturated aqueous $Na_2S_2O_3$, dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure to give a mixture of epoxydes (1.1 g, 99%) which was used in the next step without further purification.

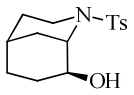
IR (film): 1262 (C-O-C), 2097 (N=N=N) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ0.88-1.00 (m, 2H, 2H-5), 1.11-1.21 (m, 1H, H-5), 1.31-1.53 (m, 8H, 2H-3, 1H-4, 1H-5, 4H-1'), 1.56-1.65 (m, 1H, H-4), 1.70-1.78 (m, 1H, H-6), 1.82-1.90 (m, 1H, H-6), 1.97-2.10 (m, 2H, 1H-3, 1H-6), 2.13-2.21 (m, 2H, 1H-3, 2H-6), 3.12-3.18 (m, 4H, 2H-1, 2H-2), 3.26-3.30 (m, 4H, H-2').

^{13}C NMR (100.6 MHz, $CDCl_3$): δ23.3 (C-6), 24.1 (C-5), 25.0 (C-6), 26.9 (C-5), 27.2 (C-4), 29.9 (C-4), 30.3 (C-3), 31.6 (C-3), 34.9 (C-1'), 35.4 (C-1'), 48.8 (C-2'), 49.1 (C-2'), 51.4 (C-1), 51.6 (C-1), 52.4 (C-2), 52.8 (C-2).

HRMS (ESI) calcd for $[C_8H_{13}N_3O + H]^+$: 168.1131, found: 168.1136.





8-Hydroxy-2-(toluenesulfonyl)-2-azabicyclo[3.3.1]nonane (21).

First step: Me_3P (2.40 mL of a 1 M solution in THF, 2.40 mmol) was added to a solution of the above epoxydes **19** (150 mg, 0.904 mmol) in THF/H_2O (22 mL, 9:1), and the mixture was stirred at room temperature for one night. The resulting solution was concentrated under reduced pressure to afford crude 2-ABN framework (**20**) as pale yellow oil.

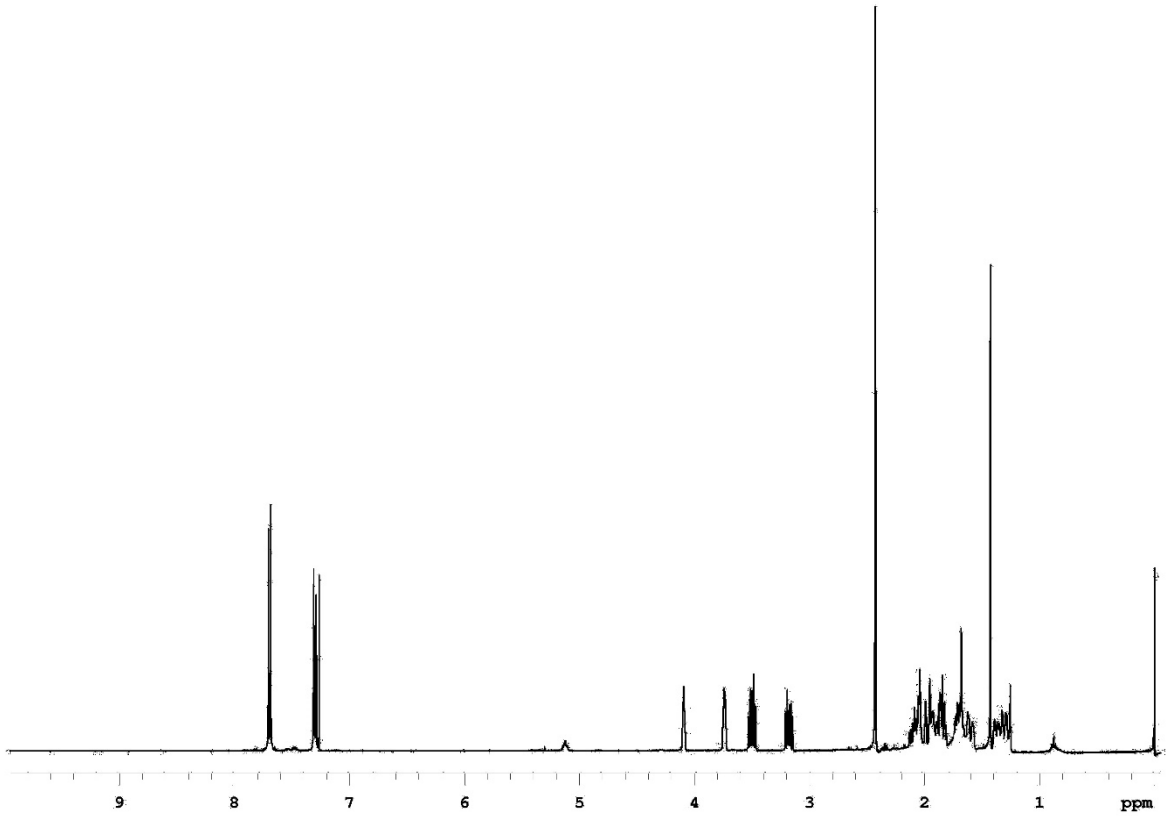
Second step: A solution of toluenesulfonyl chloride (297mg, 1.56 mmol) in anhydrous CH_2Cl_2 (8 mL) and triethylamine (185µL, 1.32 mmol) were added dropwise to a solution of the above amino alcohol (**20**) in anhydrous CH_2Cl_2 (4.5 mL) at 0 °C under inert atmosphere. The mixture was stirred for 4 h at 0 °C, poured into saturated NH_4Cl, and the resulting solution was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, and concentrated under reduced pressure. Flash chromatography (hexane to 1:1 hexane-EtOAc) gave alcohol **21**(80mg, 30% overall yield from epoxides **19**).

IR (film): 3600 (OH) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$): δ 1.26-2.05 (m, 9H, 2H-4, 2H-9, H-5, 2H-6, 2H-7), 2.43 (s, 3H, CH_3), 3.18 (qp, *J*= 6.3 Hz, 1H, H-3), 3.50 (qp, *J*= 6.3 Hz, 1H, H-3), 3.75 (m, 1H, H-1), 4.10 (br. s, 1H, H-8), 7.30 (d,*J*= 8.1 Hz, 2H, H-*o* Ts), 7.70 (d,*J*= 8.1 Hz, 2H, H-*m* Ts).

^{13}C NMR (75.4 MHz, $CDCl_3$): δ 21.4 (CH_3 Ts), 23.3 (C-5), 24.1 (C-7), 25.8 (C-6, C-4), 27.6 (C-9), 40.9 (C-3), 53.3 (C-1), 68.7 (C-8), 127.1 (C-*o* Ts), 129.6 (C-*m* Ts), 136.8 (C-*i* Ts), 143.2 (C-*p* Ts).

HRMS (ESI) calcd for $[C_{15}H_{22}NO_3S + H]^+$: 296.1215, found: 296.1208.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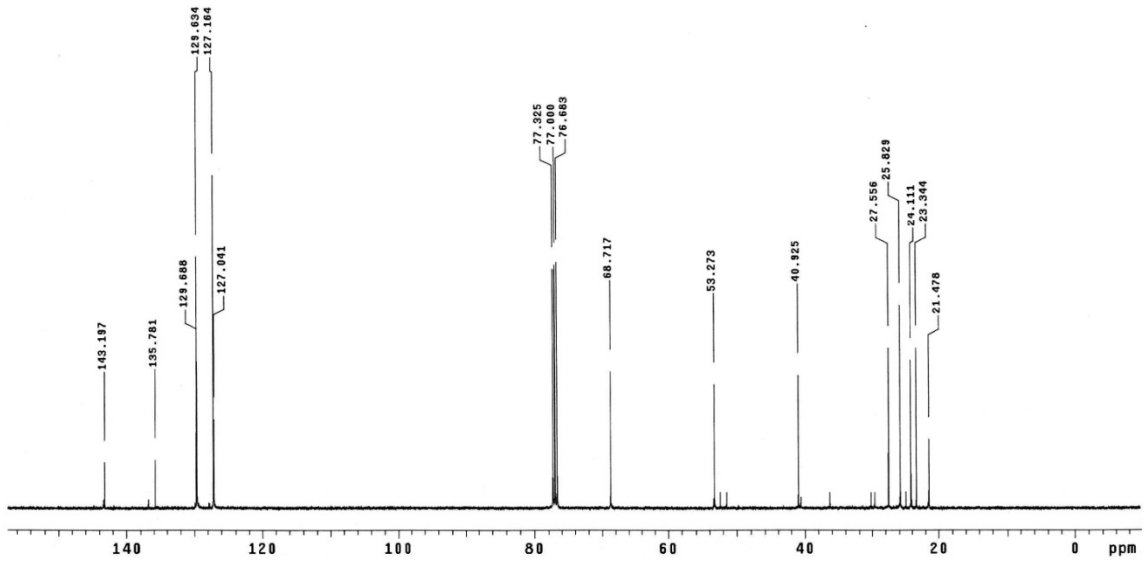
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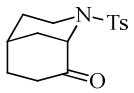


8-Oxo-2-(toluenesulfonyl)-2-azabicyclo[3.3.1]nonane (22).

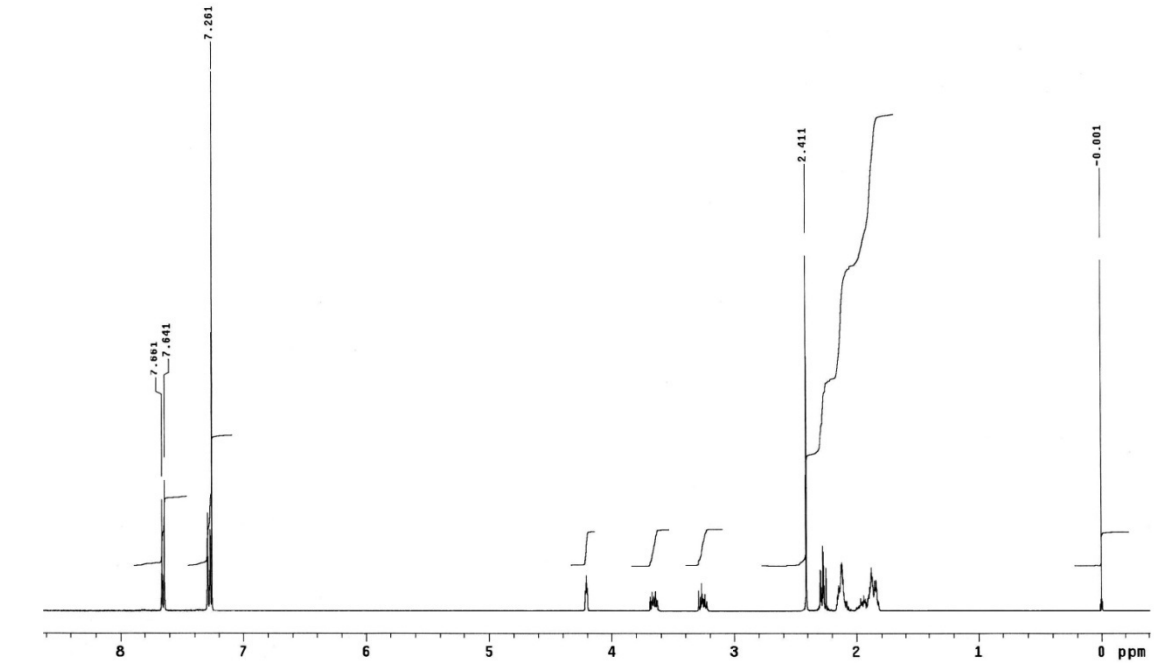
Dess-Martin Periodinane (2.957 g, 6.971 mmol) was added at room temperature to a stirring solution of bicyclic alcohol **21** (1.371 g, 4.647 mmol) in CH_2Cl_2 (100 mL) under inert atmosphere. After one night of stirring at room temperature, a saturated (1:1) solution of $NaHCO_3$-$Na_2S_2O_3$was slowly added. The resulting mixture was stirred vigorously for 45 min and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was purified by flash chromatography (hexane–hexane/EtOAc 7:3), affording ketone **22** (1.183 g, 87%).

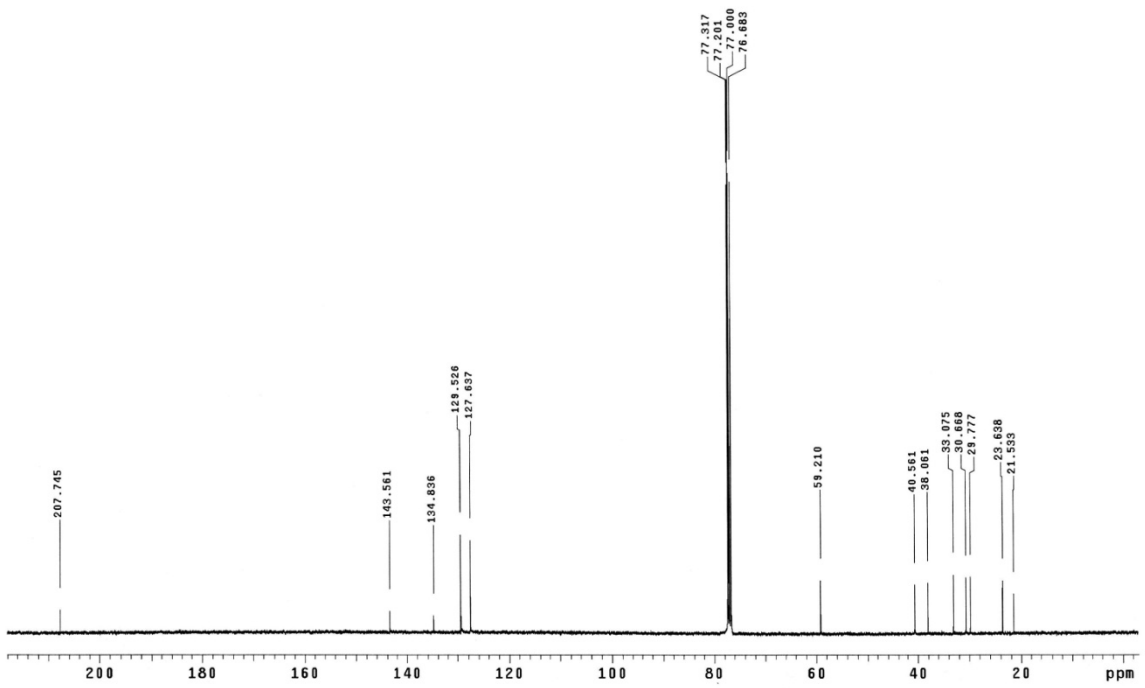
IR (film): 1716 (C=O) cm^{-1}.

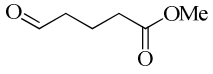
^{1}H NMR (400 MHz, $CDCl_3$): δ1.84-1.89 (m, 3H, H-4, H-9, H-6), 1.95 (m, 1H, H-6), 2.12 (m, 3H, H-5, H-4, H-9), 2.28 (m, 2H, 2H-7), 2.41 (s, 3H, CH_3 Ts), 3.27 (m, 1H, H-3), 3.65 (dt, *J* = 12.4, 6.0 Hz, 1H, H-3), 4.21 (t, *J* = 2.8 Hz, 1H, H-1), 7.28 (dd, *J* = 8.0, 0.8 Hz, 2H, H-*o* Ts), 7.65 (d,*J*= 8.0 Hz, 2H, H-*m* Ts).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ21.5 (CH_3 Ts), 23.6 (C-5), 29.8 (C-4), 30.6 (C-6), 33.1 (C-9), 38.1 (C-7), 40.6 (C-3), 59.2 (C-1), 127.6 (C-*o* Ts), 129.5 (C-*m* Ts), 134.8 (C-*i* Ts), 143.5 (C-*p* Ts), 207.7 (C-8).

HRMS (ESI) calcd for $[C_{15}H_{19}NO_3S + H]^+$: 294.1158, found: 294.1150.







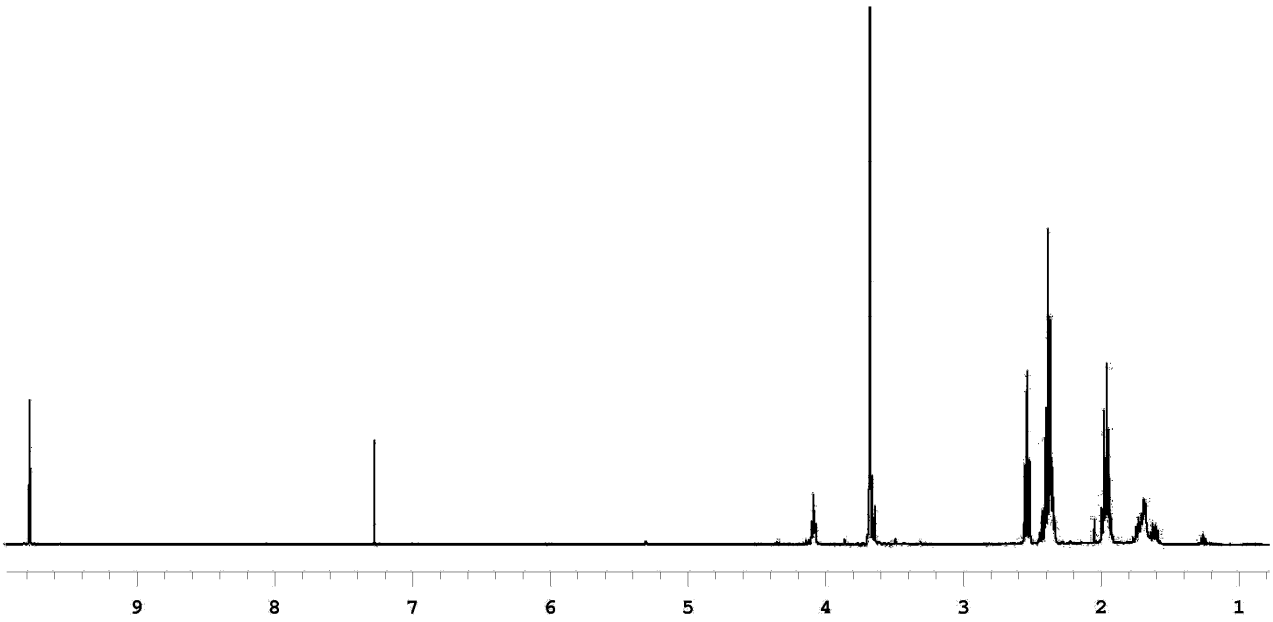
Methyl 5-oxopentanoate (24).

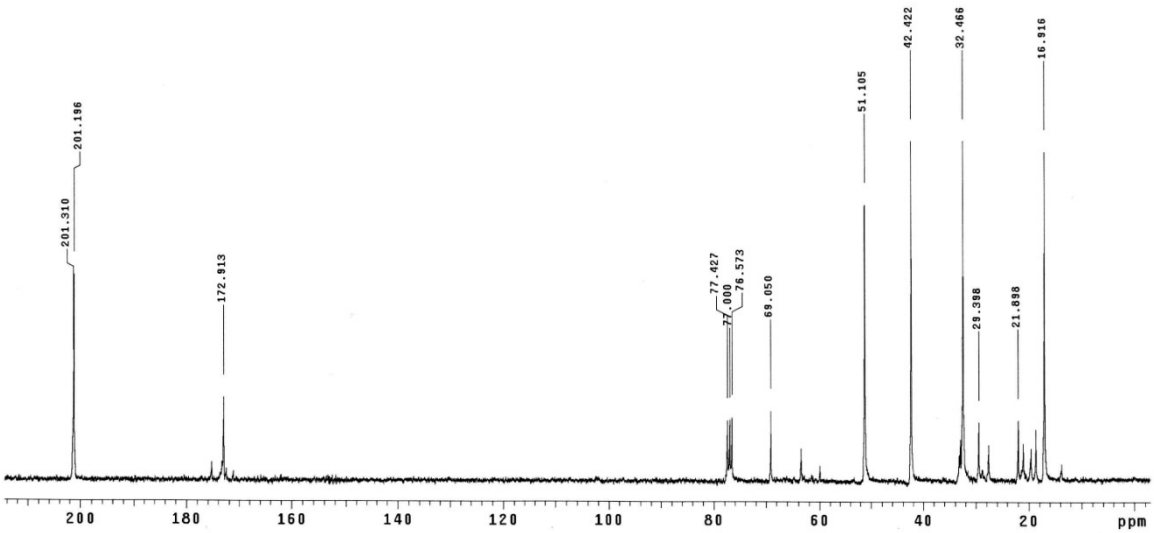
Sulfuric acid (200 µL) was slowly added to a solution of *δ*-valerolactone (9.27 mL, 99.9 mmol) in MeOH (50 mL). After one night, the reaction was quenched with $CaCO_3$ and filtered on celite

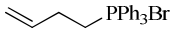
(washings with diethyl ether). The filtered was dried at 0 °C under reduced pressure and dissolved in 500 mL of CH_2Cl_2. PCC (32.3 g, 149.8 mmol) and celite (32 g) were added to this solution at 0 °C and the reaction was stirred overnight. The reaction mixture was filtered on silica (washings with hexane and hexane-EtOAc 9:1) and dried, giving methyl 5-oxopentanoate **24** (12.076 g,93% in two steps).

^{1}H NMR (300 MHz, $CDCl_3$): δ1.96 (t, *J*=7.5 Hz, 2H, 2H-3), 2.39 (t, *J*=7.5 Hz, 2H, 2H-2), 2.56 (td, *J*= 7.5, 1.5 Hz, 2H, 2H-4), 3.69 (s, 2H, OCH_3), 9.78 (t, *J*=1,5 Hz, 1H, CHO).

^{13}C NMR (74.6 MHz, $CDCl_3$): δ16.8 (C-3), 32.4 (C-2), 42.3 (C-4), 51.0 (OCH_3), 172.8 (CO_2Me), 201.1 (CHO).

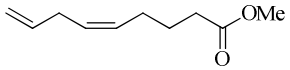






3-Butenyltriphenylphosphonium bromide (25).

A mixture of 4-bromo-1-butene (3.2 mL, 31.5 mmol) and triphenylphosphine (8.27 g, 31.5 mmol) in toluene (21 mL) was heated at reflux for 24 h. The reaction mixture was then cooled to rt and filtered over a Buchner funnel. A white solid was collected, which was washed with toluene and dried under high vacuum to afford desired product (8.5 g, 68%).
Experimental procedure from: *Org. Biomol. Chem.* **2014**,*12*, 330.
For NMR and mass data see: *Org. Lett.* **2007**, *9*, 445.



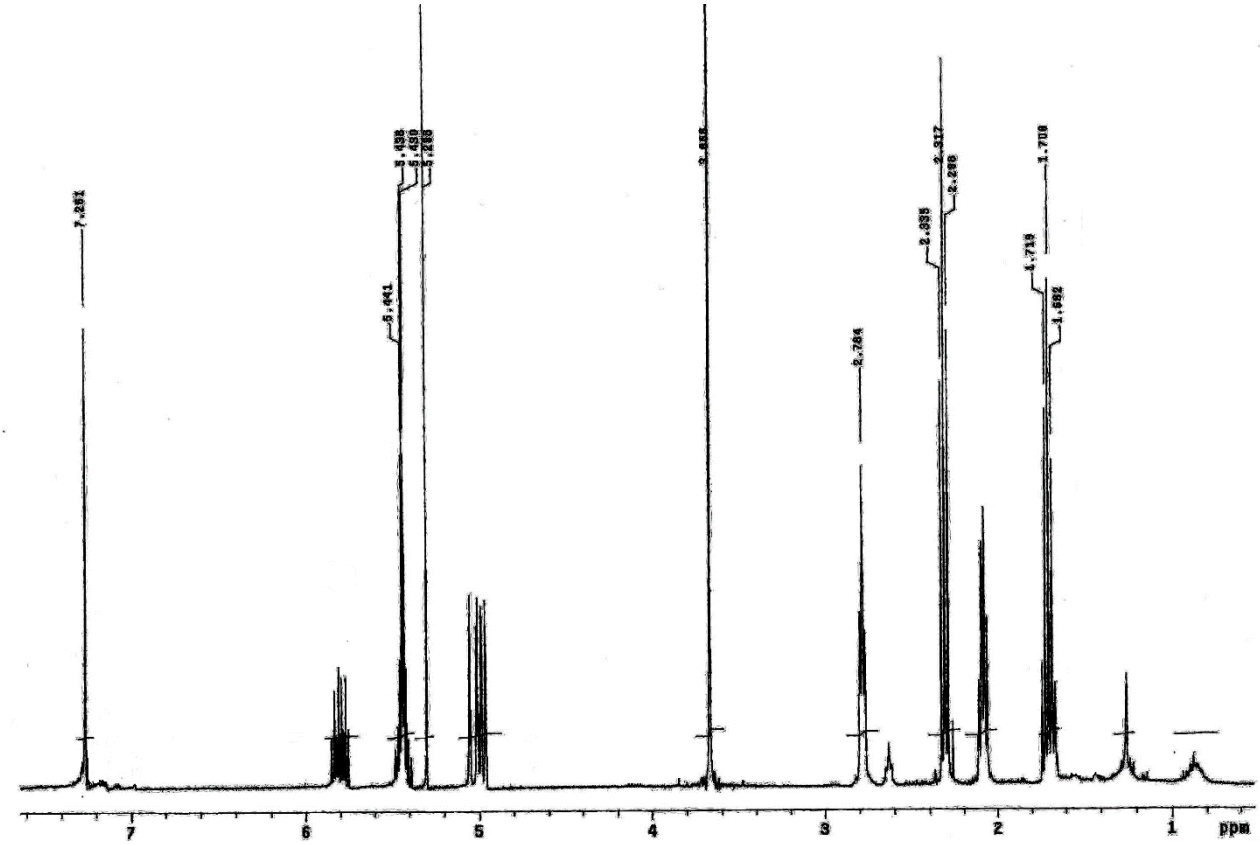
(*Z*)-Methyl 5,8-nonadienoate (26).

LDA (138 µL, 0.277 mmol) was slowly added at –40 °C to a mixture of the phosphonium salt **25** (120 mg, 0.302 mmol), previous dried at 40 °C under vacuum with a trap of P_2O_5 over a weekend, in HMPA (190 µL, 0.110 mmol) and THF (1 mL). After stirring at –40 °C for one hour, a solution of aldehyde **24** in THF (250µL) was added to the mixture cooled at –78 °C and the reaction was stirred overnight, allowing the temperature raise to rt. The reaction was then quenched with NH_4Cl and extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (hexane to hexane – Et_2O 95:5) gave the desired product **26** (33 mg, 79%).

IR (film): 1741 (O-C=O) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ1.70 (dt, *J* = 14.4,8.0 Hz, 2H, H-3), 2.09 (appdd, *J* = 13.2,6.4 Hz, 2H, H-4), 2.32 (t, *J* = 7.2 Hz, 2H, H-2), 2.78 (appt, *J* = 6.0 Hz,2H, H-7), 3.67 (s, 3H, OCH_3), 4.97 (dq, *J* = 10.4, 3.2,1.6 Hz, 1H, H-9), 5.03 (dq, *J* = 15.2, 4.0,2.0 Hz, 1H, H-9), 5.39-5.48 (m, 2H, H-5, H-6), 5.80 (ddt, *J* = 16.4, 10.0,6.4 Hz, 1H, H-8).

^{13}C NMR (400 MHz, $CDCl_3$): δ4.7 (C-3), 26.4 (C-4), 31.4 (C-7), 33.4 (C-2), 51.4 (OCH_3), 114.7 (C-9), 127.8 (C-5), 129.6 (C-6), 136.8 (C-8), 174.1 (C-1).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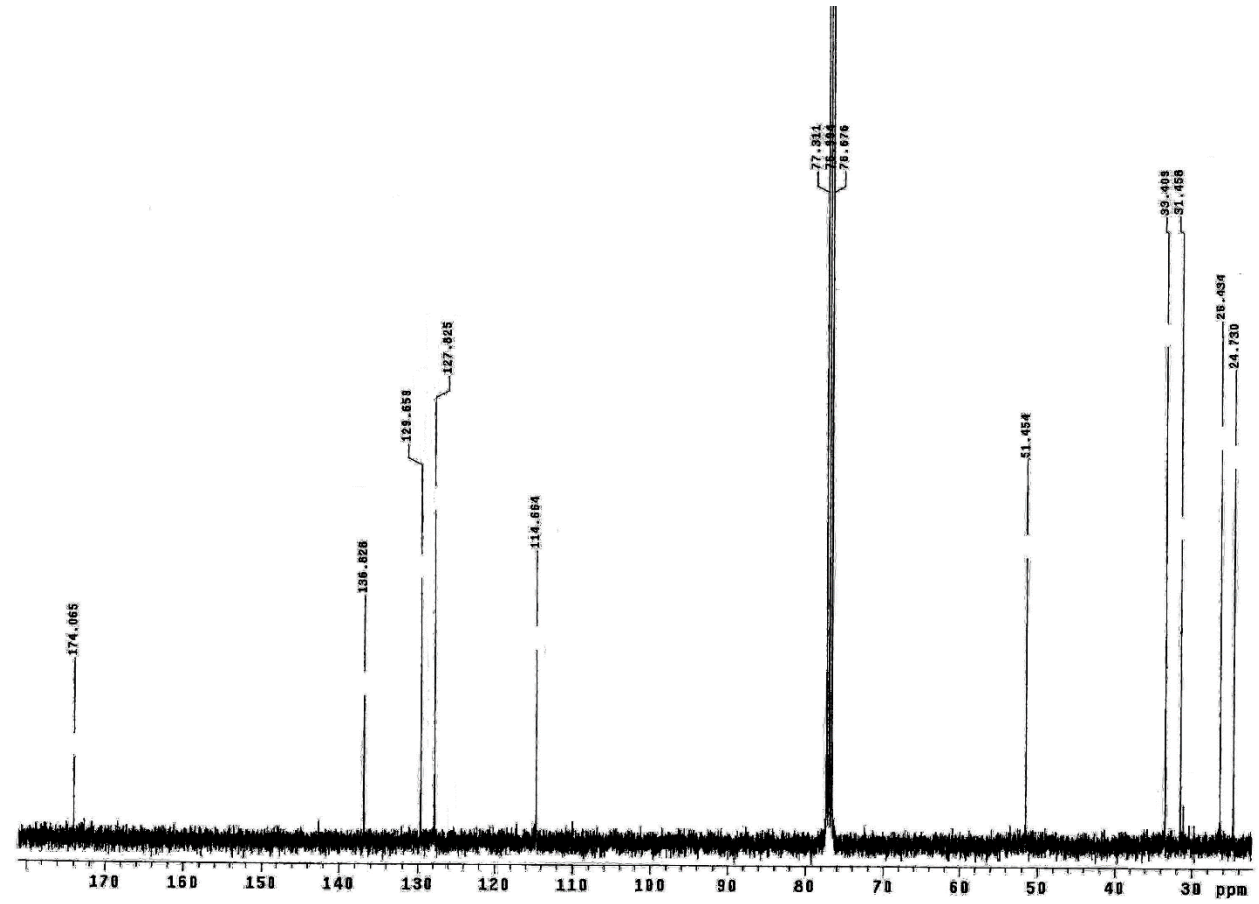
ppm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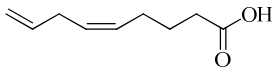


(*Z*)-5,8-Nonadienoic acid (27).

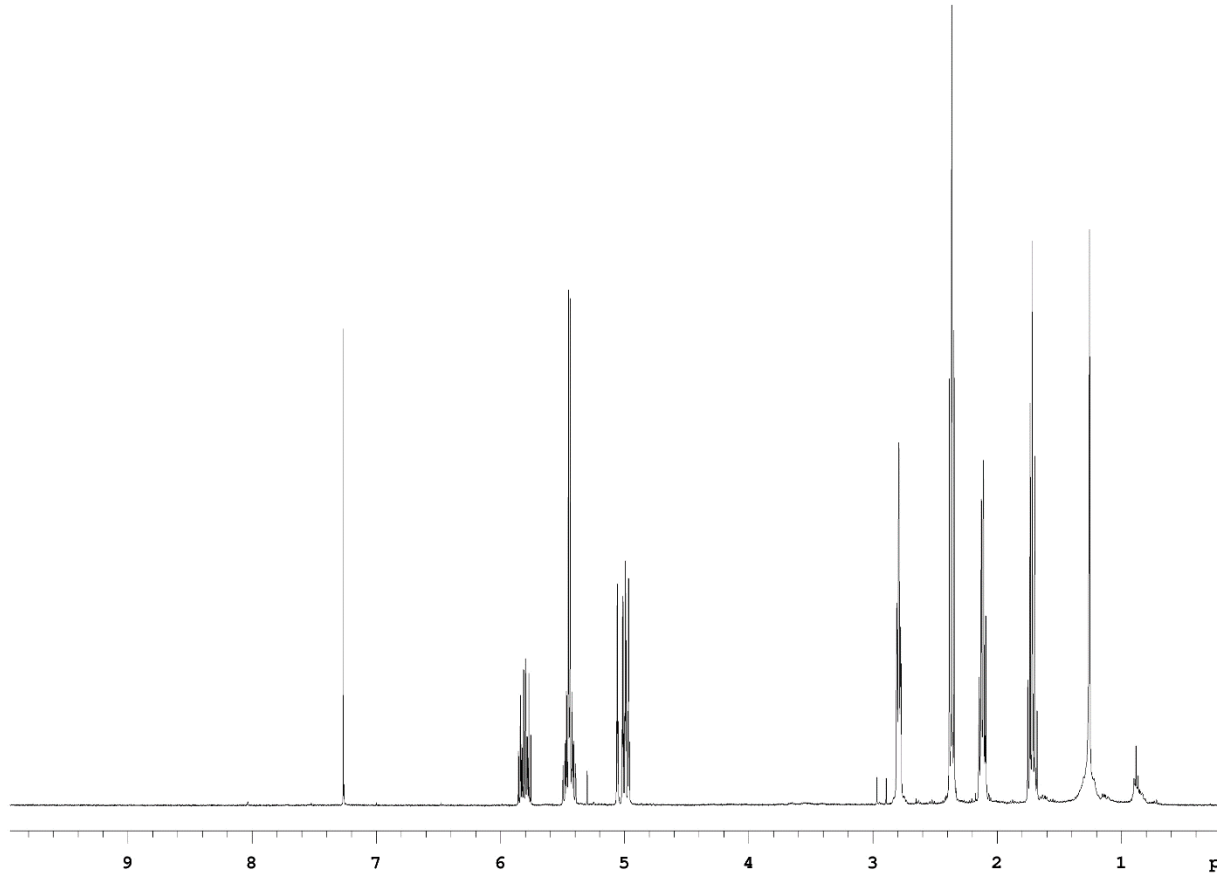
An aqueous solution of LiOH (1 M, 31 mL, 30.95 mmol) was added to a solution of the ester **26** (130 mg, 0.774 mmol) in THF (25 mL), and the resulting mixture was stirred at room temperature for 4 h. The reaction was quenched by addition of saturated aqueous NaCl, and the solution was washed with Et_2O. The aqueous layer was then acidified until pH 5 and extracted with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure to afford the corresponding acid **27**, which was used in the next step without further purification (98 mg, 82%).

IR (film): 1709 (O-C=O) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ 1.71 (dt, *J* = 14.8, 7.2 Hz, 2H, H-3), 2.12 (appdd, *J* = 14.0, 7.2 Hz, 2H, H-4), 2.36 (t, *J* = 7.2 Hz, 2H, H-2), 2.79 (appt, *J* = 6.0 Hz, 2H, H-7), 4.98 (dq, *J* = 10.0, 2.8, 1.2 Hz, 1H, H-9), 5.03 (dq, *J* = 17.2, 3.2, 1.6 Hz, 1H, H-9), 5.39-5.49 (m, 2H, H-5, H-6), 5.80 (ddt, *J* = 17.2, 10.8, 6.0 Hz, 1H, H-8).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 24.4 (C-3), 26.3 (C-4), 31.5 (C-7), 33.2 (C-2), 114.7 (C-9), 128.0 (C-5), 129.5 (C-6), 136.8 (C-8), 179.3 (C-1).

HRMS (ESI) calcd for $[C_9H_{12}O_2 + H]^+$: 153.0921, found: 153.0916.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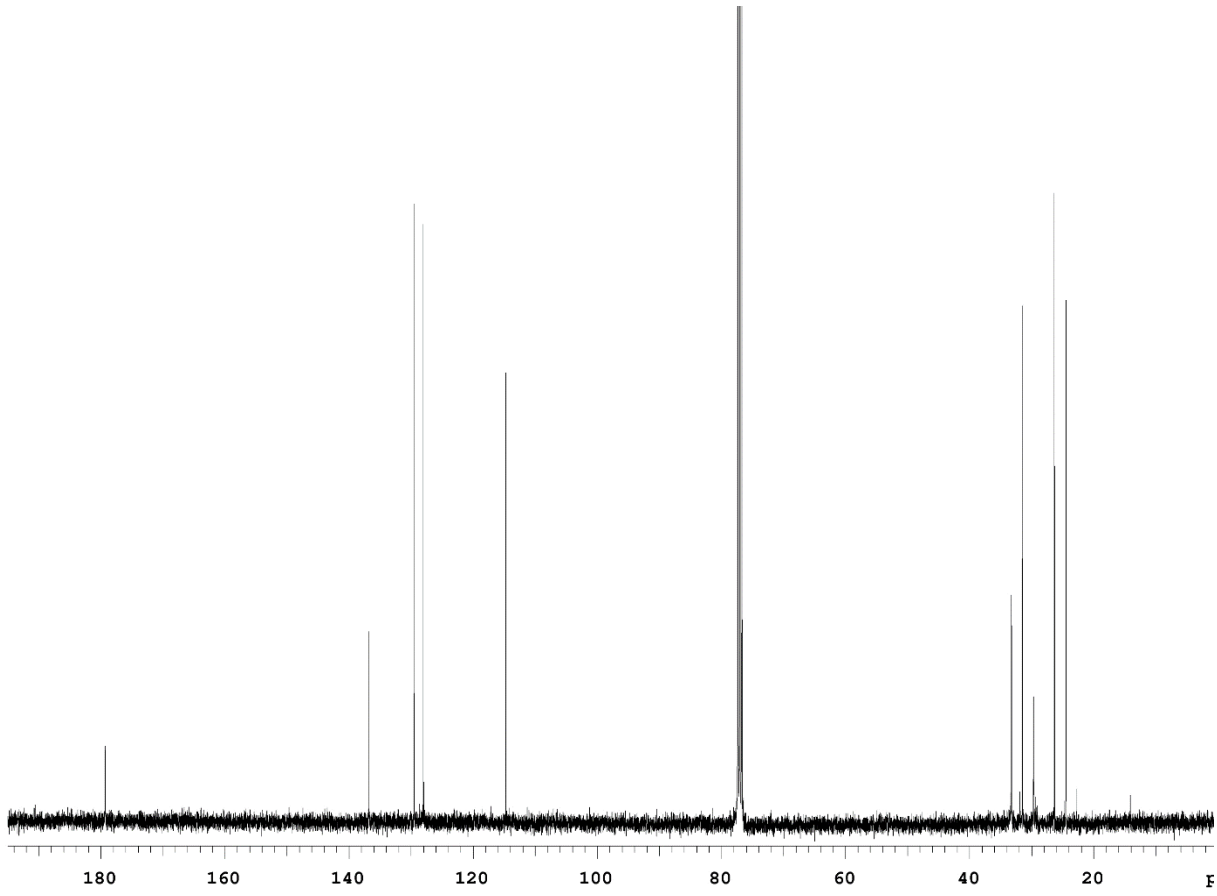
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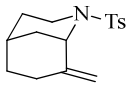


8-Methylene-2-(toluenesulfonyl)-2-azabicyclo[3.3.1]nonane (28).

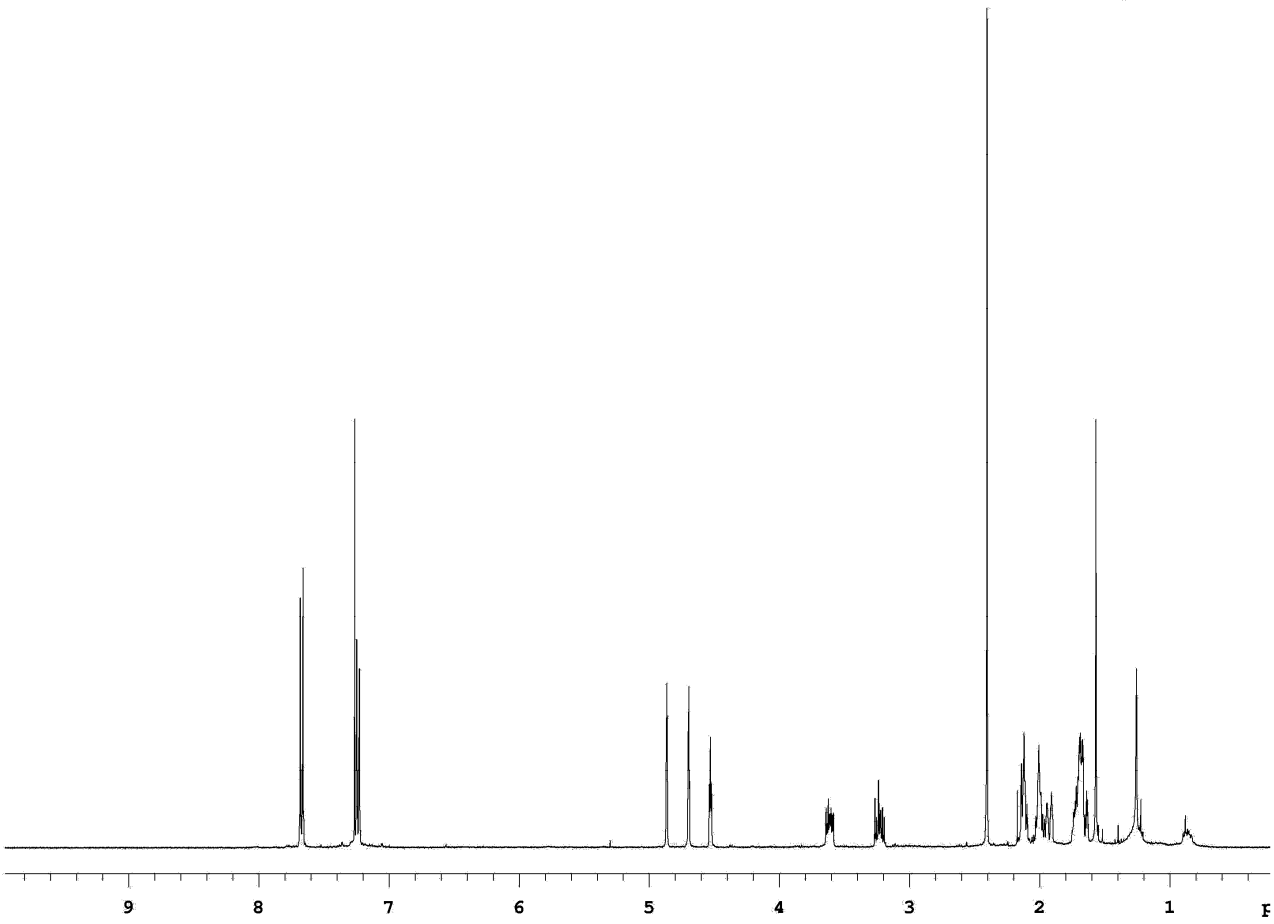
MeLi (457 µL of a solution 1.6 M in Et_2O, 0.376 mmol) was slowly added at 0 ºC in the dark under inert atmosphere to a suspension of Cp_2TiCl_2 (87 mg, 0.348 mmol) in anhydrous Et_2O (1.5 mL) cooled at 0 °C. After stirring at this temperature for 1h, the reaction mixture was quenched with H_2O and extracted in the dark with Et_2O. The combined organic extracts were dried and concentrated under reduced pressure in absence of light to give Cp_2TiMe_2 as an orange solid. A solution of above solid (0.348 mmol) in anhydrous toluene/pyridine (100:1, 2 mL) was added under argon atmosphere to a solution of bicyclic ketone **22** (51 mg, 0.174 mmol) in anhydrous toluene (500 µL) at room temperature, and the resulting mixture was stirred at reflux temperature in the dark for 6h. The solvent was then removed, and the resulting residue was treated with 8:2 Et_2O/CH_2Cl_2. The precipitate was filtered and the liquid filtrate concentrated. Purification by flash chromatography (hexane to 9:1 hexane–EtOAc) afforded alkene **28** (24 mg), recovering 16 mg of the starting ketone **22** (47%, 69% considering the unreacted ketone).

IR (film): 1160 (N-SO_2-C) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ 1.63-1.75 (m, 4H, H-4, 2H-6, H-9), 1.90-1.95 (m, 1H, H-9), 1.98-2.03 (m, 2H, H-4, H-5), 2.09-2.14 (m, 2H, 2H-7), 2.40 (s, 3H, CH_3 Ts), 3.19-3.27 (m, 1H, H-3), 3.58-3.64 (m, 1H, H-3), 4.53 (t, *J* = 3.3 Hz, 1H, H-1), 4.70 (dt, *J* = 1.9,1.3 Hz, 1H, =CH_2), 4.86 (t, *J* = 1.8 Hz, 1H, =CH_2), 7.24 (dd, *J* = 8.0,0.6 Hz, 2H, H-*m* Ts), 7.67 (dt, *J* = 8.6,2.0 Hz, 2H, H-*o* Ts).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.5 (CH_3 Ts), 24.5 (C-5), 29.6 (C-4), 30.3 (C-7), 30.9 (C-6), 34.9 (C-9), 41.9 (C-3), 55.1 (C-1), 111.7 (=CH_2), 127.7 (C-*m* Ts), 129.1 (C-*o* Ts), 136.1 (C-*p* Ts), 142.8 (C-*i* Ts), 146.1 (C-8).

HRMS (ESI) calcd for $[C_{16}H_{21}NO_2S + H]^+$: 292.1366, found: 292.1367.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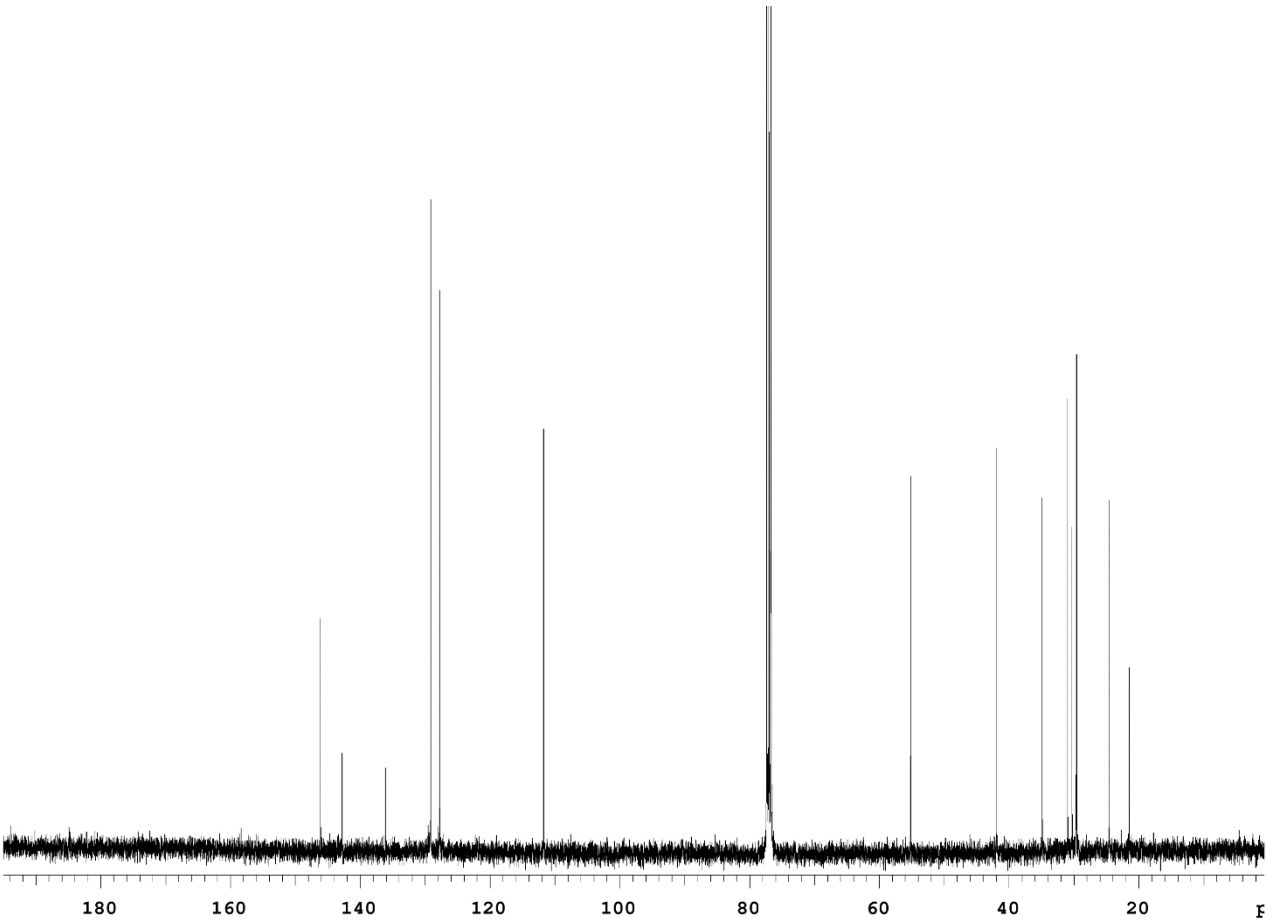
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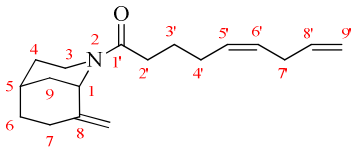


(*Z*)-8-Methylene-2-nona-5,8-dien-1-oxo-2-azabicyclo[3.3.1]nonane (29).

First step: Sodium metal (384 mg, 16.70 mmol) was added at room temperature to a solution of naphthalene (1068 mg, 8.33 mmol) in THF (24 mL). After stirring for 2 h, part of the mixture (10.04 mL) was added at -78 °C to a solution of the tosyl derivative **28** (225 mg, 0.773 mmol) in THF (20 mL). After 10 min at -78 °C, a few drops of saturated aqueous NH_4Cl were carefully added, and the resulting solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. This crude was used without further purification in the next step.

Second step: To a stirred solution of acid **27** (62 mg, 0.4 mmol) and above amine (0.4 mmol) in CH_2Cl_2 (2 mL) was added dropwise a solution of DCC (99 mg, 0.480 mmol) and DMAP (5 mg, 0.04 mmol) in CH_2Cl_2 (1.5 mL) at -20 °C under argon. After the end of addition, the mixture was allowed to heat slowly to room temperature and was stirred for one night. The dicyclohexylurea was then filtered off and the filtrate was extracted with HCl 0.5 M and then with Na_2CO_3. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. The product was purified by flash chromatography (hexane-EtOAc 98:2), affording amide **29** (85 mg, 78% in two steps).

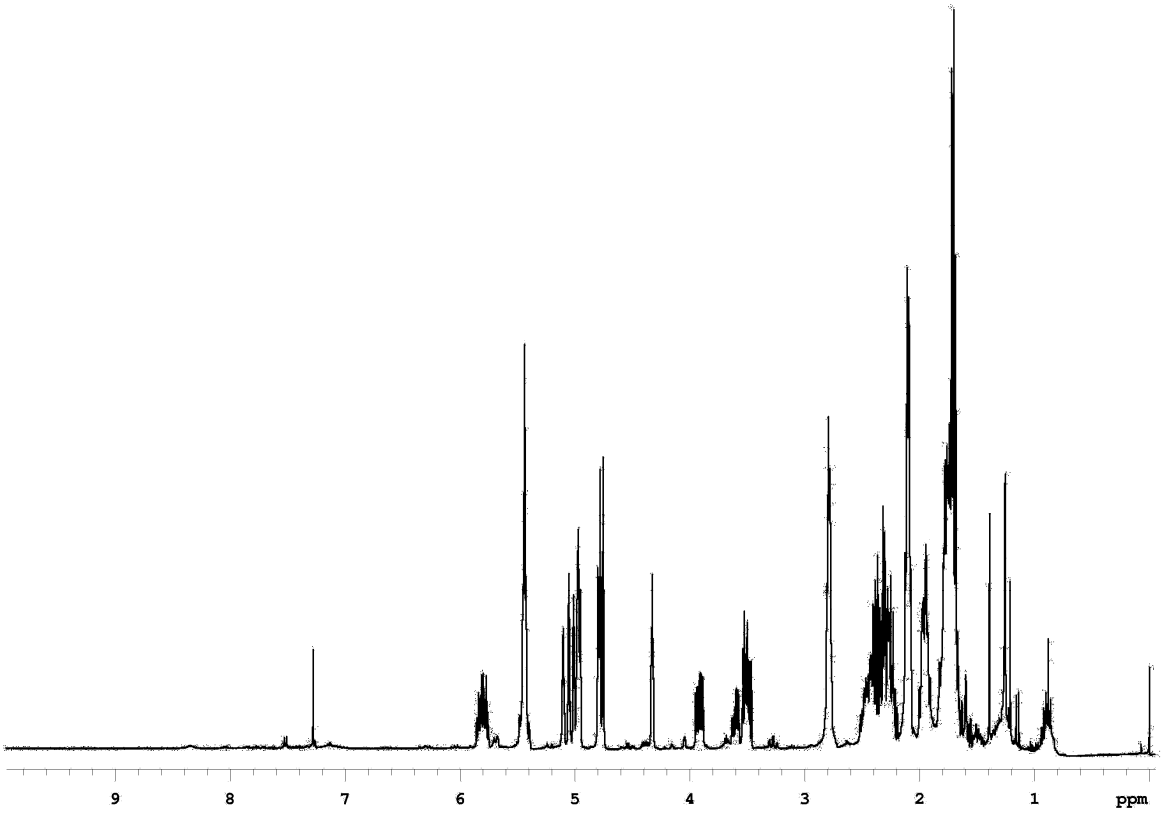
IR (film): 1410 (CH_2-C=C), 1644 (N-C=O) cm^{-1}.

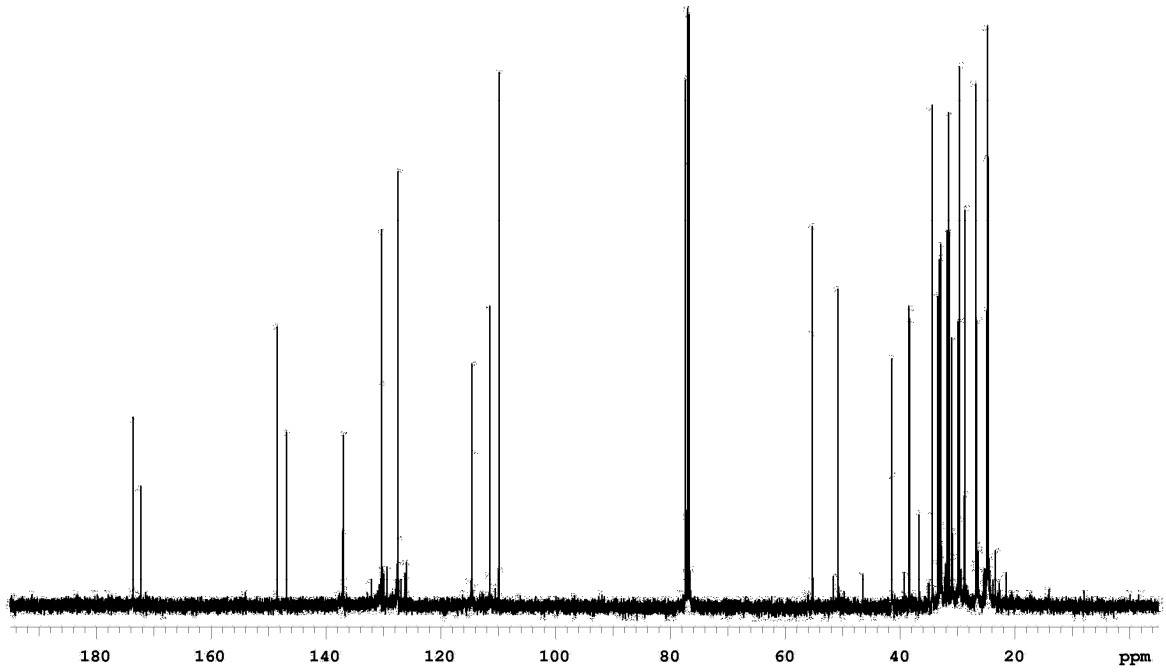
^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC, rotamers): δ 1.68-1.79 (m, 12H, 2H-4, 2H-6, 2H-7, 2H-9, 4H-3'),1.91-1.99 (m, 4H, 2H-4, 2H-9), 2.08-2.13 (m, 6H, 2H-5, 4H-4'), 2.23-2.52 (m, 6H, 2H-7, 4H-2'), 2.76-2.81 (m, 4H, 4H-7'), 3.46-3.54 (m, 2H, 2H-3), 3.60 (ddd, *J* = 12.8, 7.6, 3.6 Hz, 1H, 1H-3), 3.92 (ddd, *J* = 14.4, 7.6, 4.4 Hz, 1H, 1H-3), 4.33 (t, *J* = 2.8, 1H, 1H-1), 4.75-4.80 (m, 3H, =CH_2), 4.95-5.11 (m, 4H, 1H-1, 2H-9', =CH_2), 5.39-5.48 (m, 4H, 2H-5', 2H-6'), 5.75-5.85 (m, 2H, 2H-8').

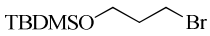
^{13}C NMR (100.6 MHz, $CDCl_3$, rotamers): δ 24.6, 24.7 (C-5), 24.9 (C-3'), 26.7, 26.8 (C-4'), 28.7 (C-9), 29.7 (C-7), 29.9 (C-9), 31.0 (C-3'), 31.4, 31.5 (C-7'), 31.8 (C-6), 32.9 (C-7), 33.2, 33.4 (C-

2'), 34.4 (C-7), 38.4, 41.4 (C-3), 50.8, 55.3 (C-1), 109.8, 111.4 (=CH_2), 114.5, 114.6 (C-9'), 127.3, 127.4 (C-5'), 130.2, 130.3 (C-6'), 136.9, 137.0 (C-8'), 146.8, 148.4 (C-8), 172.2, 173.5 (C-1').

HRMS (ESI) calcd for $[C_{18}H_{27}NO + H]^+$: 274.2165, found: 274.2166.





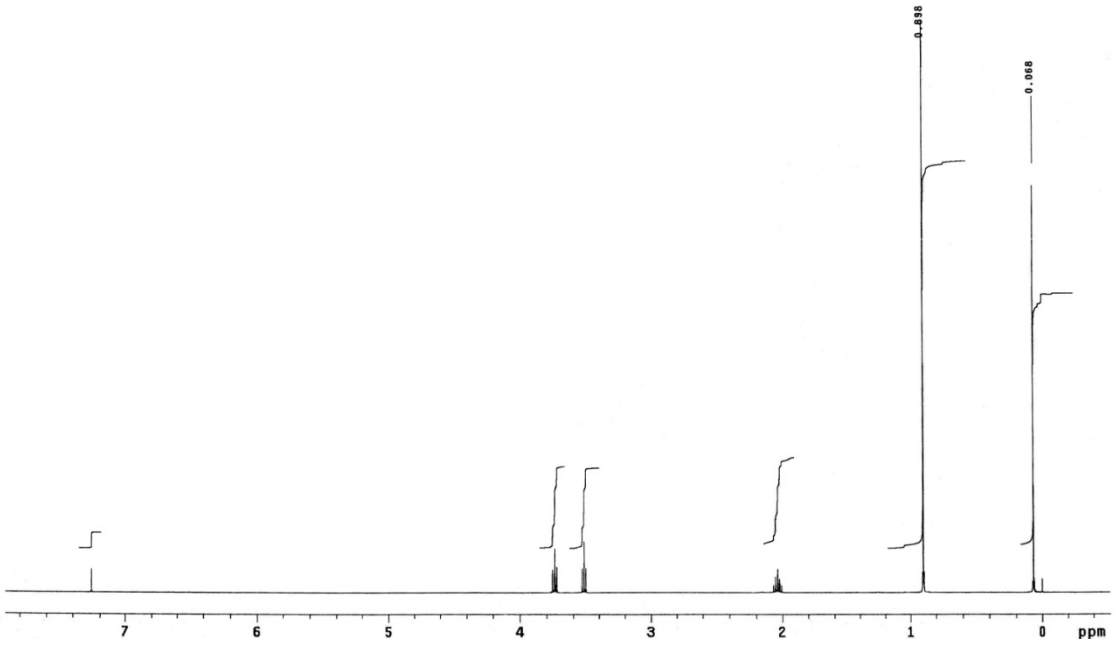


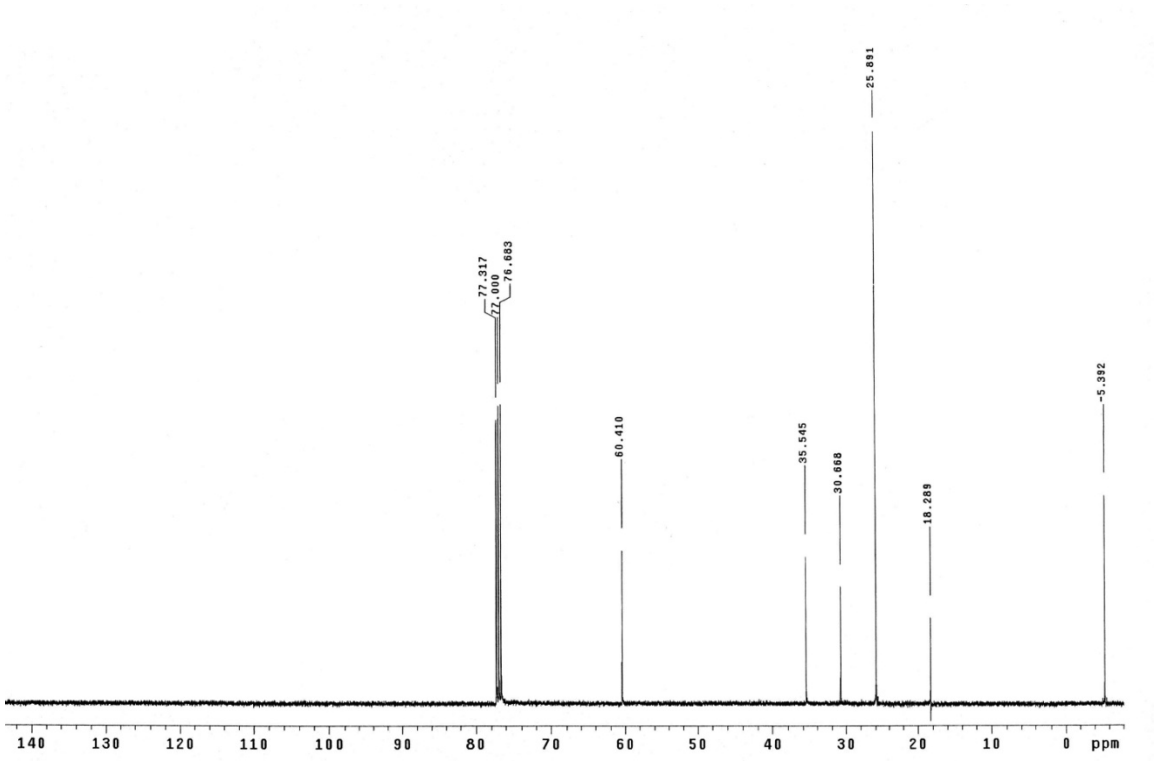
3-Bromo-1-(*tert*-butyldimethylsilyloxy)propane (31).

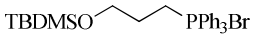
Triethylamine (3.6 mL, 26.0 mmol), dimethylaminopiridine (122 mg, 1.0 mmol) and *tert*-butyldimethylsilane chloride (3.6 g, 24.0 mmol) were added to a solution of 3-bromopropanol (1.8 mL, 20 mmol) in anhydrous THF (30 mL) under inert atmosphere at room temperature. After 18 h stirring at room temperature, saturated NH_4Cl was added and the resulting mixture was extracted with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4, and concentrated under reduced pressure. Flash chromatography (hexane) gave protected alcohol **31** (4.8 g, 96%) as colourless oil.

^{1}H NMR (400 MHz, $CDCl_3$): δ 0.07 (s, 6H, CH_3Si), 0.90 [s, 9H, $(CH_3)_3C$], 2.03 (m, 2H, H-2), 3.51 (t, J = 6.6 Hz, 2H, H-1), 3.73 (t, J = 5.4 Hz, 2H, H-3).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ -5.4 ($2CH_3Si$), 18.3 [$(CH_3)_3CSi$], 25.9 [$(CH_3)_3CSi$], 30.7 (CH_2Br), 35.5 (CH_2), 60.4 (CH_2O).







3-(*tert*-Butyldimethylsilyloxy)propyltriphenyl-phosphonium bromide (32).

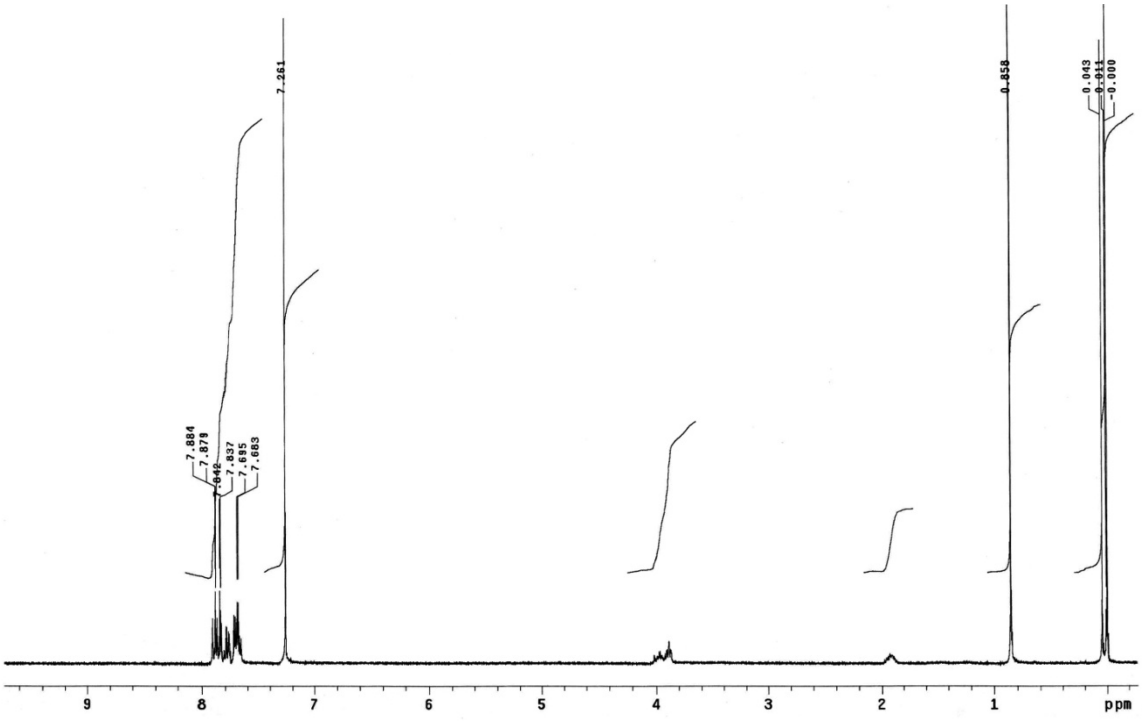
Triphenylphosphine (5.0 g, 19.1 mmol) was added to a solution of protected alcohol **31** (4.0 g, 15.9 mmol) in anhydrous benzene (3 mL) under argon atmosphere. The mixture was heated at 85 °C for 18 h and then was cooled to room temperature. Ether was added to the resulting solution and a white solid was formed. The residue was filtered and washed several times with ether. Flash chromatography of the resulting solid (CH_2Cl_2 to 9:1 CH_2Cl_2-MeOH) afforded phosphonium salt **32** (7.9 g, 96%).

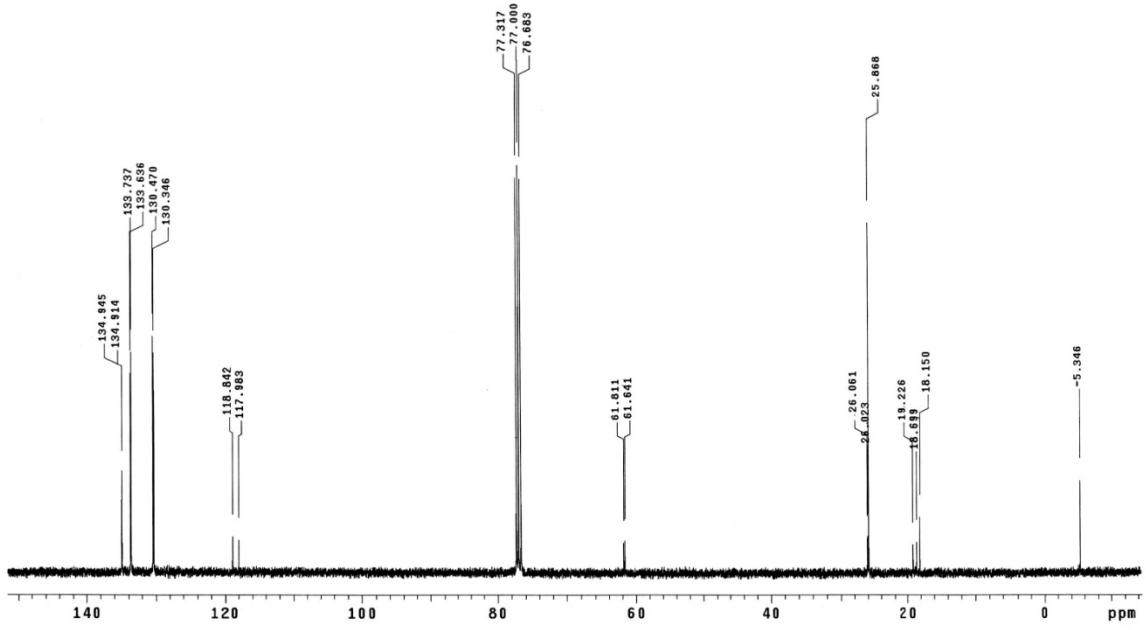
^{1}H NMR (300 MHz, $CDCl_3$): δ 0.04 (s, 6H, CH_3Si), 0.85 [s, 9H, $(CH_3)_3C$], 1.95 (m, 2H, H-2), 3.98 (m, 4H, H-1, H-3), 7.69-7.90 (m, 15H, C_6H_5).

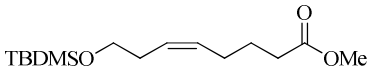
^{13}C NMR (75.4 MHz, $CDCl_3$): δ -5.3 ($2CH_3Si$), 18.2 [$(CH_3)_3CSi$], 18.7, 19.2 (CH_2P), 25.9 [$(CH_3)_3CSi$], 26.0, 26.1 (CH_2), 61.6, 61.8 (CH_2O), 118.0, 118.8 (C-*i*C_6H_5), 130.3-133.7 (C-*o*, *m* C_6H_5), 134.9 (C-*p* C_6H_5).

m.p. 216 °C.

HRMS (ESI) calcd for $[C_{21}H_{22}OP - TBDMSi + H]^+$: 321.1403, found: 321.1398.







(*Z*)-Methyl 8-(*tert*-butyldimethylsilyloxy)-5-octenoate (33).

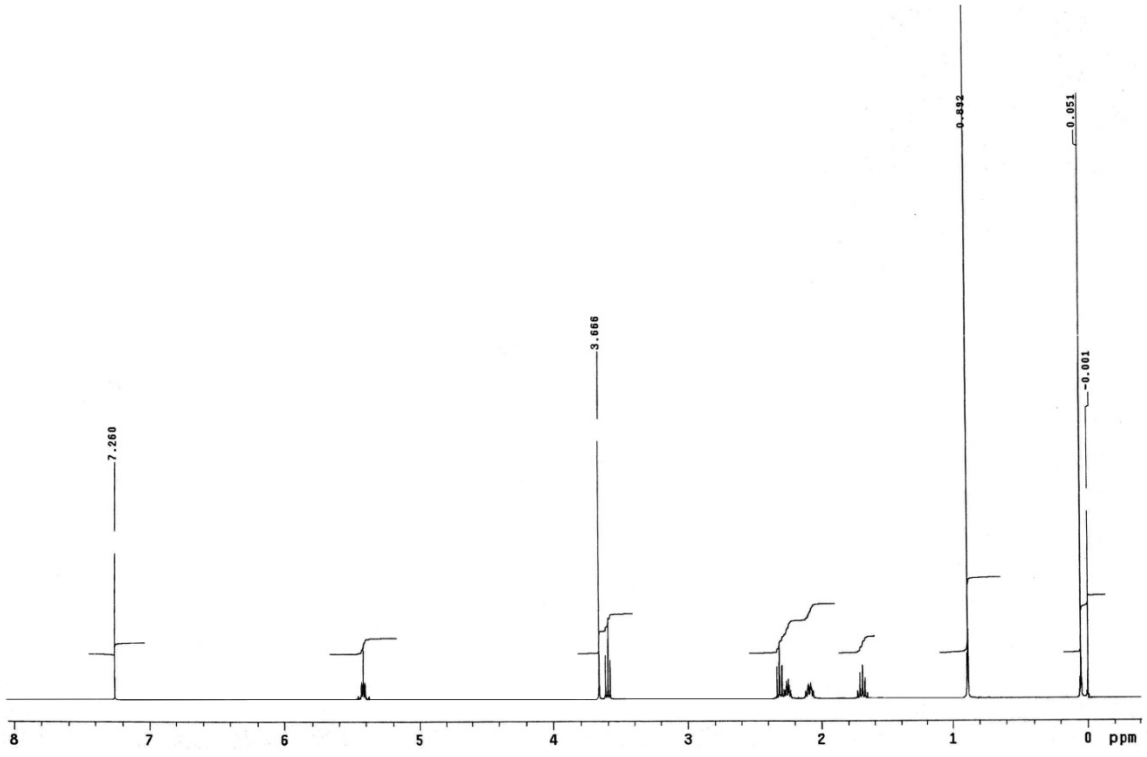
LDA (890 µL, 1.424 mmol) was slowly added at -40 °C to a mixture of the phosphonium salt **32** (1 g, 1.940 mmol), previous dried at 40 °C under vacuum with a trap of P_2O_5 over a weekend, in HMPA (1.225 mL, 7.055 mmol) and THF (6 mL). After stirring at -40 °C for one hour, a solution of aldehyde **24** in THF (2.50 mL) was added to the mixture cooled at -78 °C and the reaction was stirred overnight, allowing the temperature raise to room temperature. The reaction was then quenched with NH_4Cl and extracted with diethyl ether. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (hexane to hexane–Et_2O 98:2) gave the desired product **33** (390 mg, 70%).

IR (film): 1742 (COO) cm^{-1}.

^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ0.05 (s, 6H, CH_3Si), 0.89 [s, 9H, $(CH_3)_3CSi$], 1.69 (m, 2H, 2H-3), 2.08 (m, 2H, 2H-4), 2.25 (m, 2H, 2H-7), 2.31 (t, *J* = 8.0 Hz, 2H, 2H-2), 3.60 (t, *J* = 6.4 Hz, 2H, CH_2OSi), 3.67 (s, 3H, CH_3O), 5.40 (m, 2H, H-5, H-6).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ-5.3 (CH_3Si), 18.3 [$(CH_3)_3CSi$], 24.8 (C-3), 25.9 [$(CH_3)_3CSi$], 26.7 (C-4), 31.1 (C-7), 33.5 (C-2), 51.5 (CH_3O), 62.9 (C-8), 126.9, 130.3 (C-5, C-6), 174.1 (NCO).

HRMS (ESI) calcd for $[C_{15}H_{30}O_3Si + H]^+$: 287.2000, found: 287.2042.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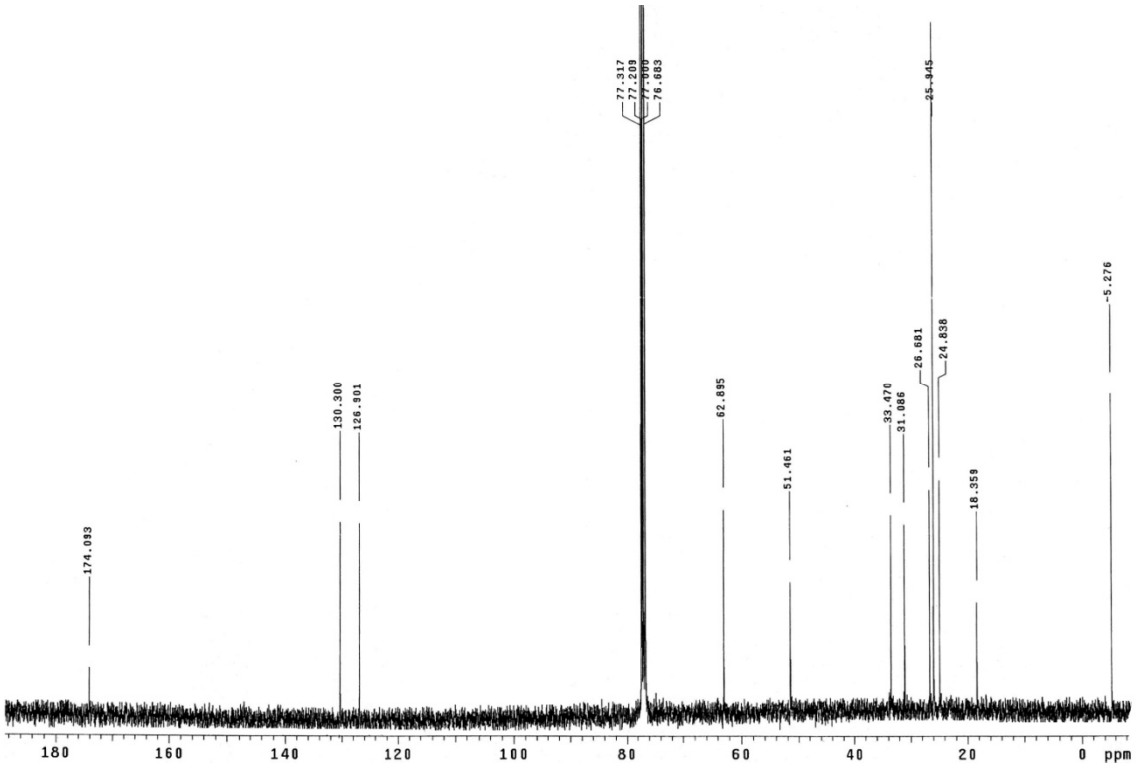
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174.093
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126.901
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26.681
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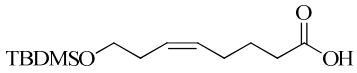
0
ppm



(*Z*)-8-(*tert*-Butyldimethylsilyloxy)-5-octenoic acid (34).

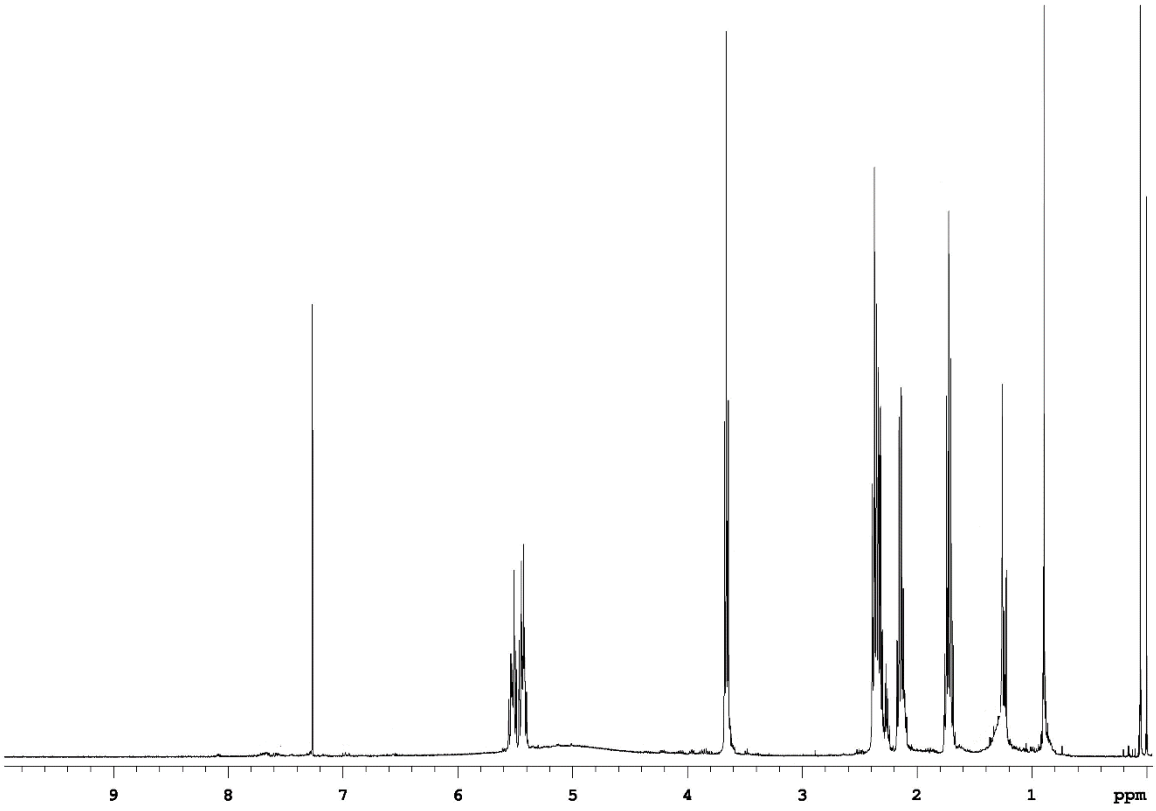
An aqueous solution of LiOH (1 M, 28.5 mL, 28.5 mmol) was added to a solution of the ester **33** (407 mg, 1.423 mmol) in THF (41 mL), and the resulting mixture was stirred at room temperature overnight. The reaction was quenched by addition of saturated aqueous NaCl, and the solution was extracted first with Et_2O, then the combined aqueous extracts were acidified until pH 5 and extracted again with Et_2O. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (EtOAc) afforded the corresponding acid **34**, without further purification (190 mg, 49%).

IR (film): 1708 (COO) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ0.05 (s, 6H, CH_3Si), 0.89 [s, 9H, $(CH_3)_3CSi$], 1.69-1.76 (m, 2H, 2H-3), 2.12-2.17 (m, 2H, 2H-4), 2.30-2.39 (m, 4H, 2H-7, 2H-2), 3.66 (t, J = 6.4 Hz, 2H, 2H-8), 5.40-5.46 (m, 1H, H-6), 5.49-5.56 (m, 1H, H-5).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ-5.3 (CH_3Si), 18.4 [$(CH_3)_3CSi$], 24.5 (C-3), 25.9 [$(CH_3)_3CSi$], 26.5 (C-4), 30.7 (C-7), 33.2 (C-2), 62.2 (C-8), 126.6 (C-6), 131.6 (C-5), 178.9 (C-1).

HRMS (ESI) calcd for $[C_{14}H_{27}O_3Si - H]^+$: 271.1735, found: 271.1738.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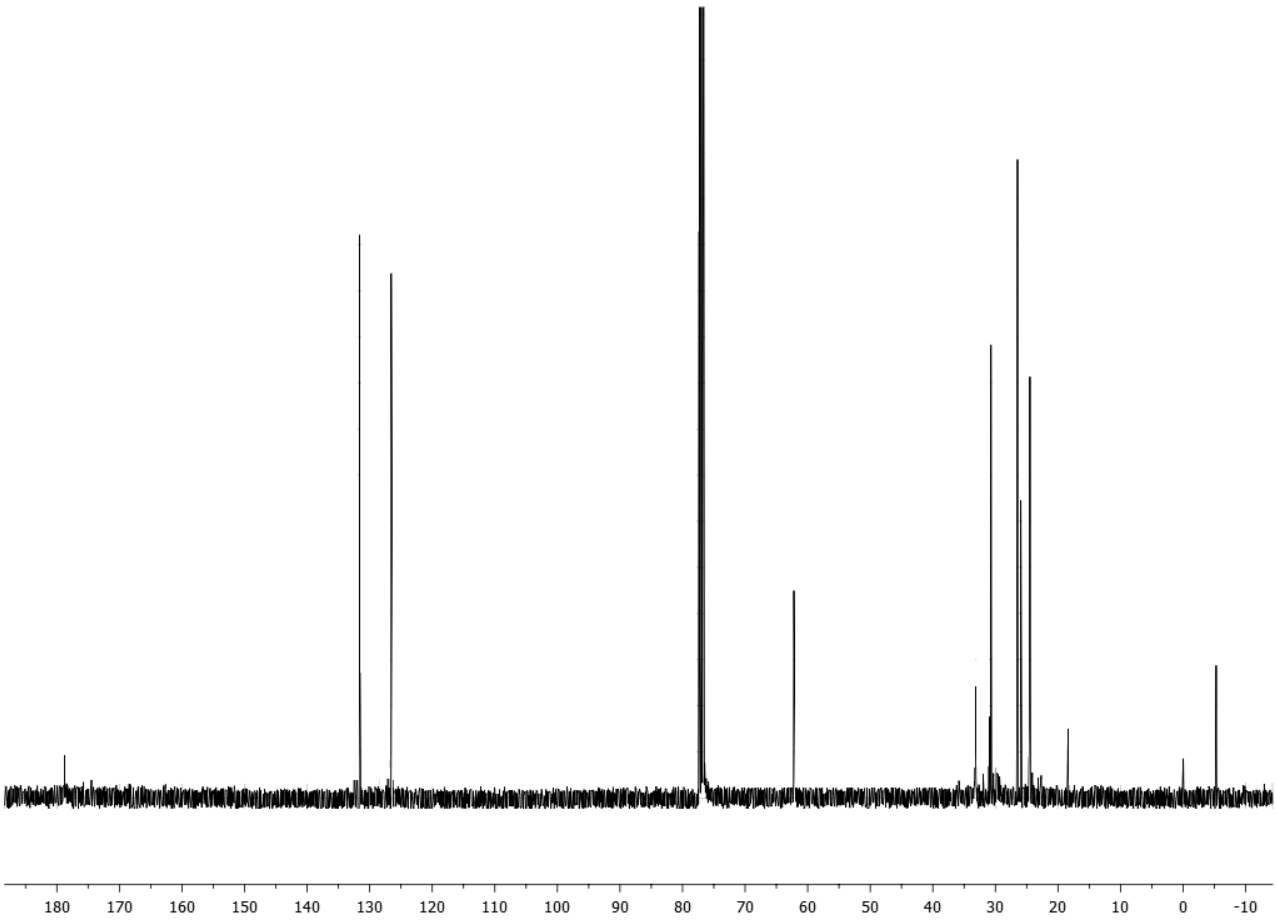
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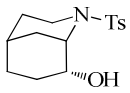


8-Hydroxy-2-(toluenesulfonyl)-2-azabicyclo[3.3.1]nonane (35).

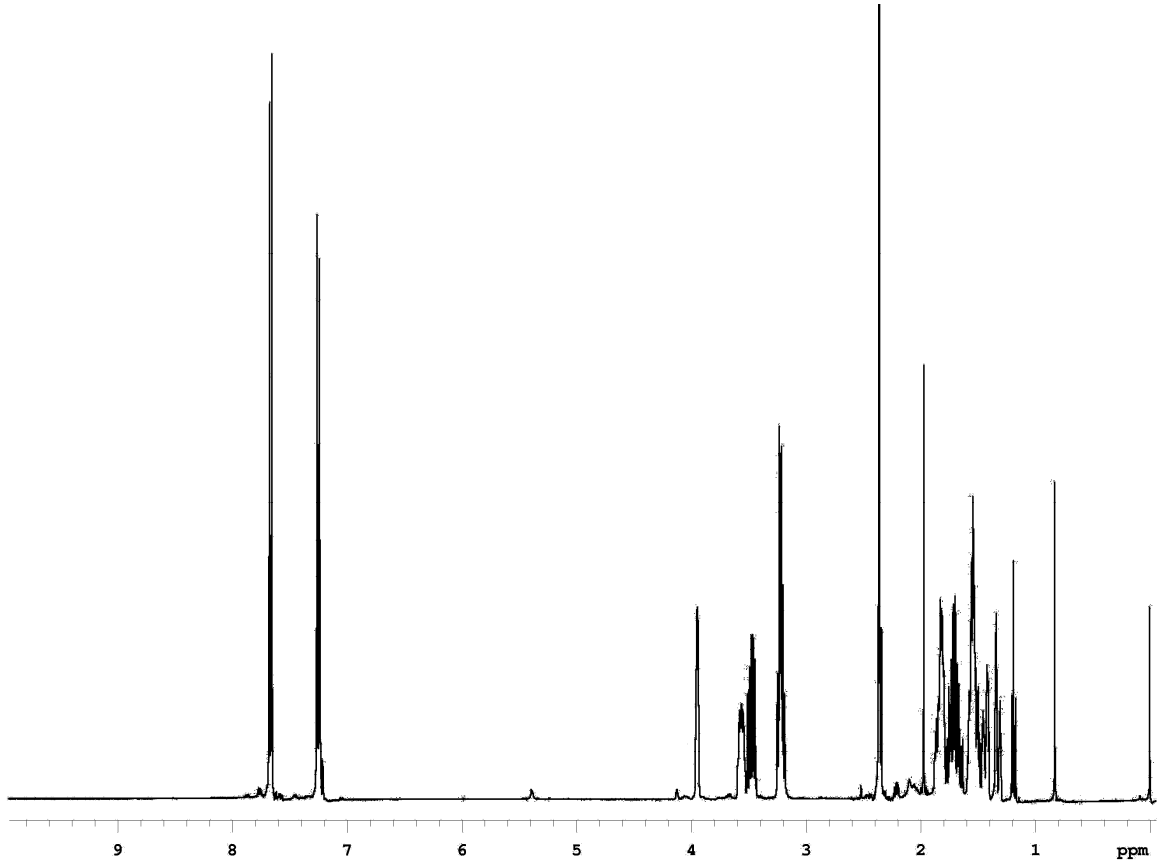
$NaBH_4$ (183 mg, 4.833 mmol) was added to a solution of ketone **22** (944 mg, 3.222 mmol) in THF (9 mL) and MeOH (9 mL) at 0 °C, and the resulting mixture was for 10 minutes. The reaction was quenched by addition of saturated aqueous $NaHCO_3$, and the solution was extracted with Et_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Alcohol **35** was obtained without further purification (866 mg, 91%).

IR (film): 3600 (OH) cm^{-1}.

^1H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ 1.30-1.35 (m, 1H, H-9), 1.41-1.46 (m, 1H, H-9), 149-1.58 (m, 3H, H-4, 2H-6), 1.63-1.77 (m, 2H, H-4, H-7), 1.79-1.86 (m, 2H, H-5, H-7), 2.36 (s, 3H, CH_3), 3.18-3.25 (m, 1H, H-3), 3.44-3.51 (m, 1H, H-3), 3.54-3.58 (m, 1H, H-8), 3.95 (br. s, 1H, H-1), 7.25 (d, *J* = 8.0 Hz, 2H, H-*o* Ts), 7.70 (d, *J* = 8.8 Hz, 2H, H-*m* Ts).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 21.3 (CH_3 Ts), 22.7 (C-5), 27.6 (C-4), 28.3 (C-7), 29.3 (C-6), 29.4 (C-9), 41.6 (C-3), 53.7 (C-1), 71.0 (C-8), 127.1 (C-*o* Ts), 129.6 (C-*m* Ts), 135.7 (C-*i* Ts), 143.4 (C-*p* Ts).

HRMS (ESI) calcd for $[C_{15}H_{22}NO_3Si + H]^+$: 296.1315, found: 296.131.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3
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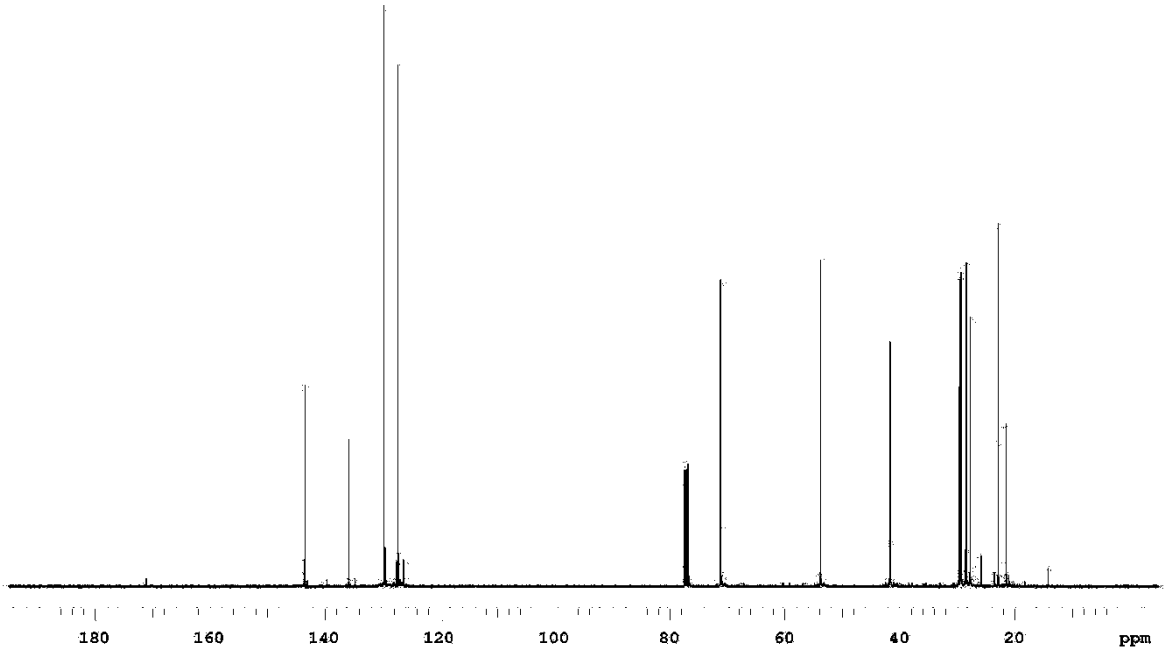
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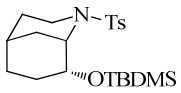
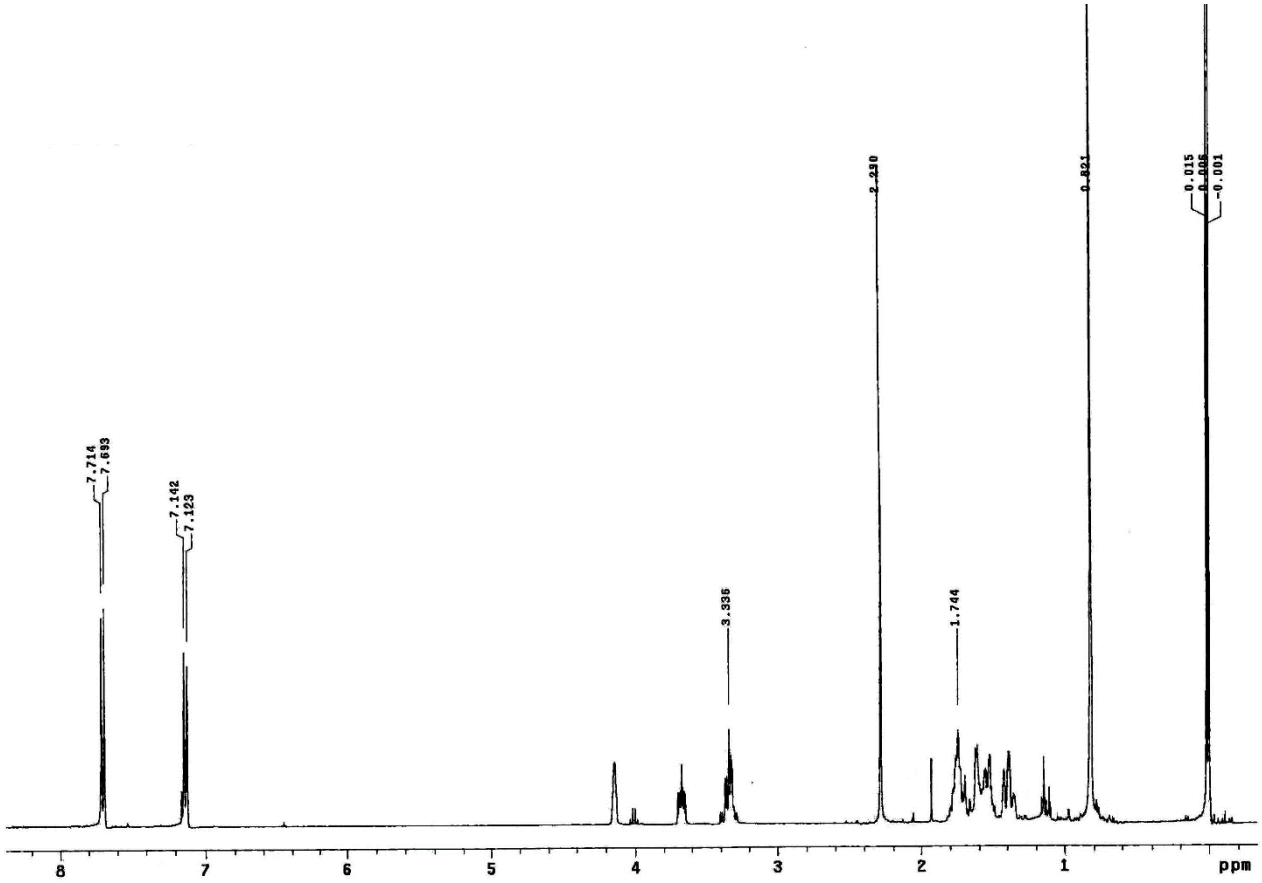


8-(*tert*-Butyldimethylsilyloxy)-2-(toluenesulfonyl)-2-azabicyclo[3.3.1]nonane (37).

TEA (123 µL, 0.881 mmol) and DMAP (4 mg, 0.0339 mmol) were added under inert atmosphere to a solution of alcohol **35** (200 mg, 0.678 mmol) and TBDMSCl (123 mg, 0.814 mmol) in THF (6 mL), and the reaction was stirred at reflux overnight. The reaction mixture was quenched by addition of saturated aqueous NH_4Cl, and the solution was extracted with Et_2O, dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Purification by flash chromatography (hexane to 7:3 hexane-EtOAc) afforded compound **37** (92 mg), recovering 108 mg of the starting alcohol **35** (33%, 72% considering the unreacted alcohol).

^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ0.06 (s, 6H, CH_3Si), 0.82 [s, 9H, $(CH_3)_3CSi$], 1.36-1.43 (m, 4H, H-6, H-9), 1.51-1.63 (m, 3H, H-4, H-7), 1.69-1.74 (m, 2H, H-5, H-7), 2.29 (s, 3H, CH_3), 3.32-3.37 (m, 2H, H-3), 3.67 (ddd, *J* = 11.2, 7.2,4.4 Hz, 1H, H-8), 4.14 (br. s, 1H, H-1), 7.13 (d, *J* = 7.6 Hz, 2H, H-*o* Ts), 7.70 (d, *J* = 8.4 Hz, 2H, H-*m* Ts).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ0.2 (CH_3Si), 23.4 (CH_3 Ts), 26.3 (C-5), 29.4 [$(CH_3)_3CSi$], 30.9 (C-4), 33.9 (C-7), 36.4 (C-6), 36.9 (C-9), 47.2 (C-3), 57.6 (C-1), 76.8 (C-8), 132.3 (C-*o* Ts), 134.2 (C-*m* Ts), 143.7 (C-*i* Ts), 147.3 (C-*p* Ts).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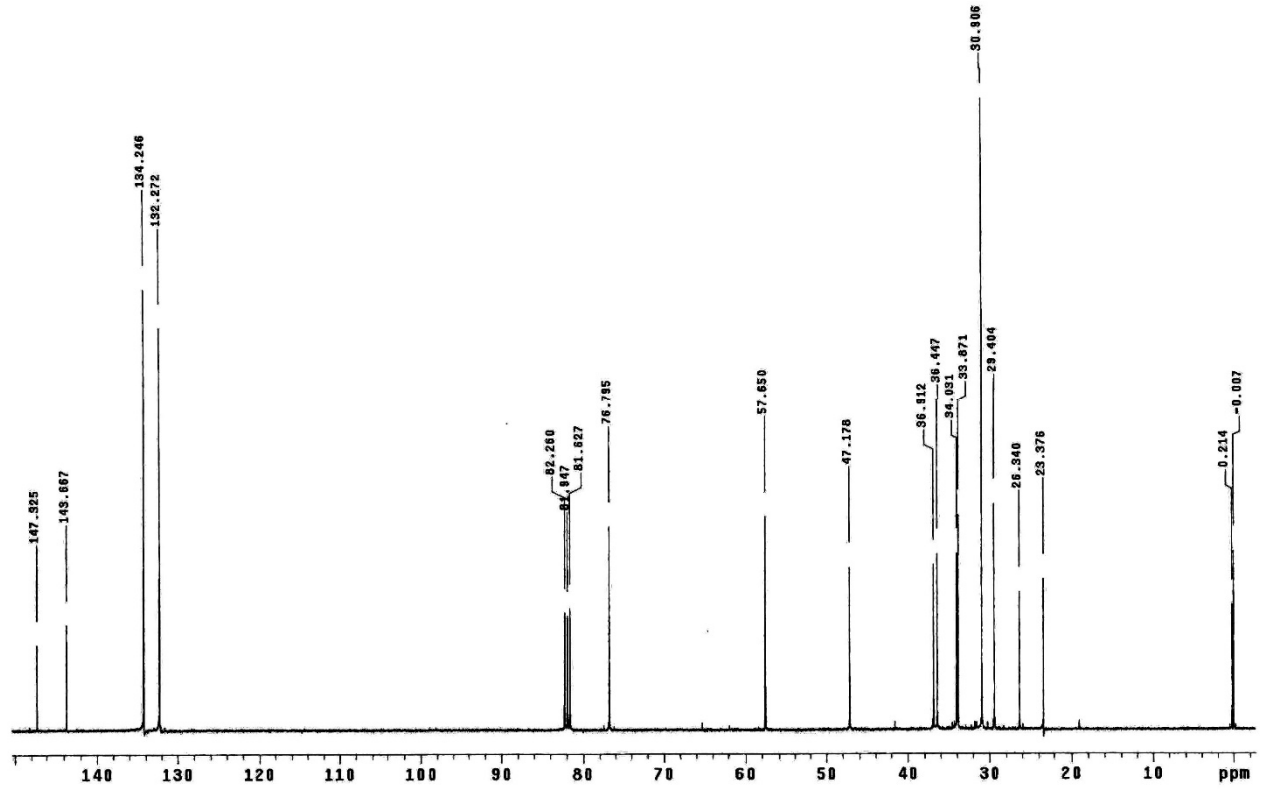
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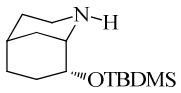


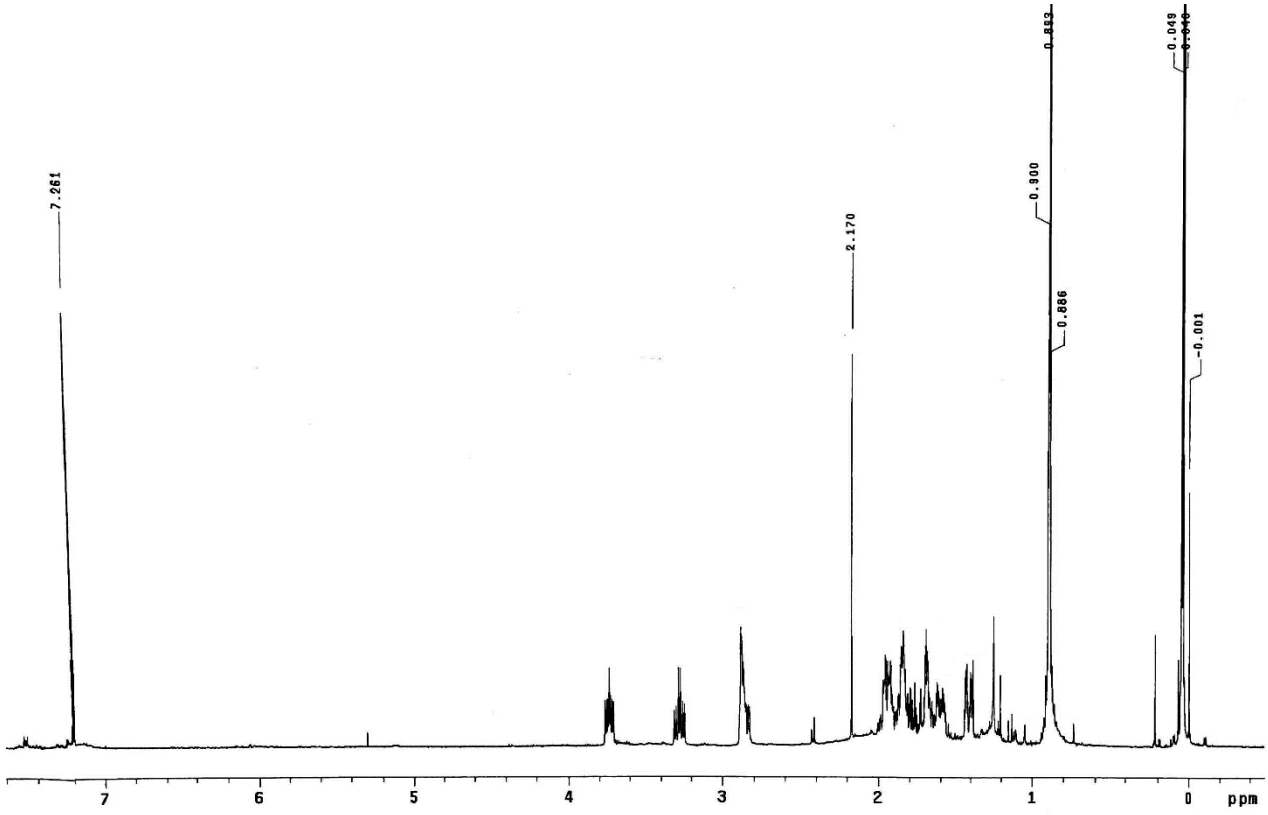
8-(*tert*-Butyldimethylsilyloxy)-2-azabicyclo[3.3.1]nonane (38).

Sodium metal (96 mg, 4.176 mmol) was added at room temperature to a solution of naphthalene (267 mg, 2.083 mmol) in THF (6 mL). After stirring for 2 h, part of the mixture (800 µL) was added at -78 °C to a solution of the tosyl derivative **37** (25 mg, 0.0611 mmol) in THF (1.6 mL). After 10 min at -78 °C, a few drops of saturated aqueous NH_4Cl were carefully added, and the resulting solution was dried over anhydrous Na_2SO_4, filtered and concentrated under reduced pressure. Flash chromatography on basic alumina (EtOAc to 8:2 EtOAc-methanol) afforded compound **38** (11 mg, 71%).

^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ 0.04 (s, 3H, CH_3Si), 0.05 (s, 3H, CH_3Si), 0.89 [s, 9H, $(CH_3)_3CSi$], 1.39-1.44 (m, 2H, 2H-9), 1.58-1.63 (m, 2H, 2H-6), 1.68-1.71 (m, 1H, H-4a), 1.83-1.87 (m, 2H, H-5, H-7a), 1.91-1.97 (m, 2H, H-4b, H-7b), 2.83-2.88 (m, 2H, H-1, H-3a), 3.28 (dt, J = 12.4, 5.6 Hz, 1H, H-3b), 3.74 (ddd, J = 10.8, 7.6, 4.0 Hz, 1H, H-8).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ -4.7 (CH_3Si), -4.5 (CH_3Si), 18.1 [$(CH_3)_3CSi$], 25.0 (C-5), 25.9 [$(CH_3)_3CSi$], 29.6 (C-6), 30.5 (C-4), 32.0 (C-7), 32.1 (C-9), 41.3 (C-3), 52.1 (C-1), 73.6 (C-8).

HRMS (ESI) calcd for $[C_{14}H_{30}NOSi + H]^+$: 256.2091, found: 256.2084.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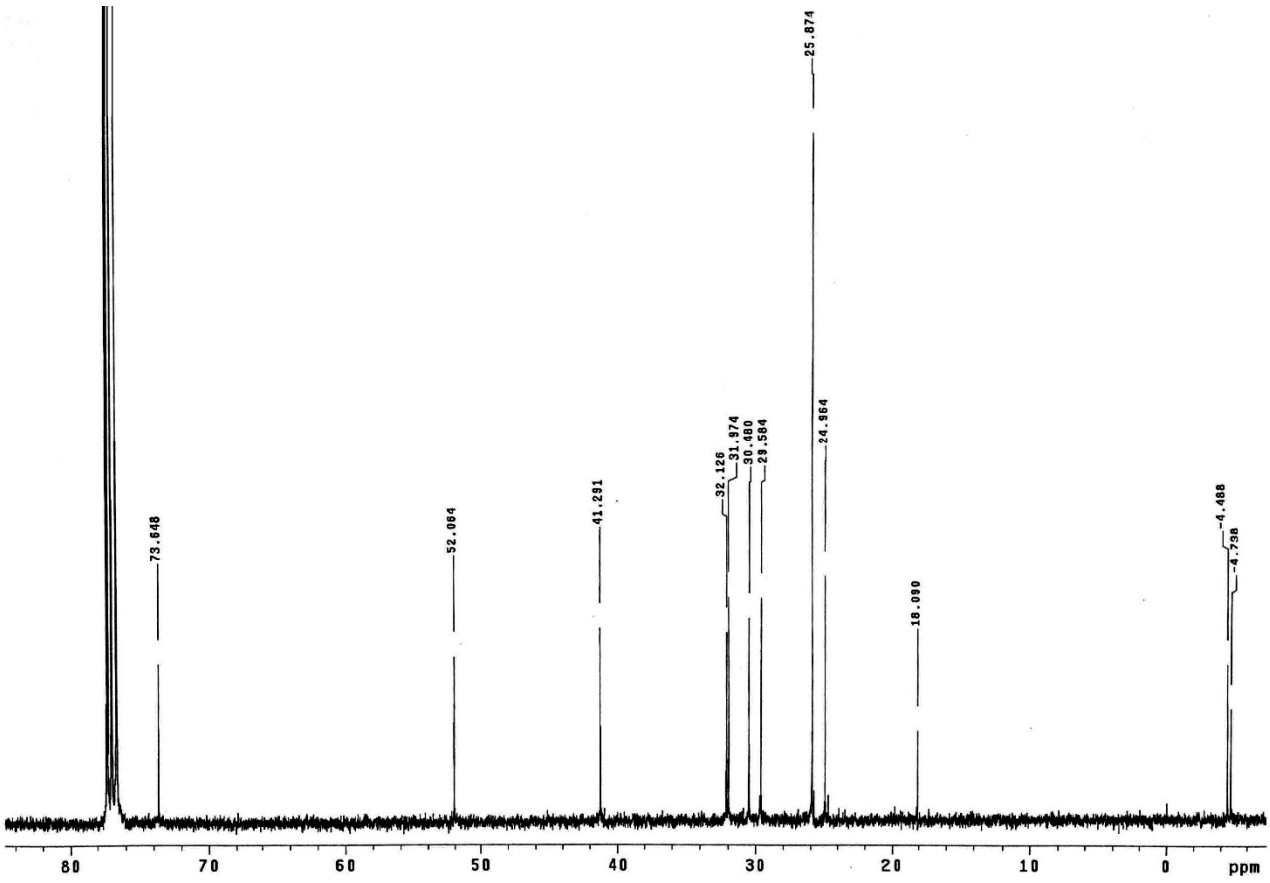
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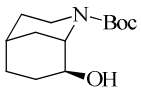


2-(*tert*-Butoxycarbonyl)-8-hydroxy-2-azabicyclo[3.3.1]nonane (41).

First step: Me_3P (12.5 mL of a 1 M solution in THF, 12.5 mmol) was added to a solution of the epoxydes **19** (1.67 g, 10.0 mmol) in THF/H_2O (170 mL, 6:1) and the mixture was stirred overnight at 70 °C. The resulting solution was concentrated under reduced pressure to afford crude (2-ABN) framework as pale yellow oil.

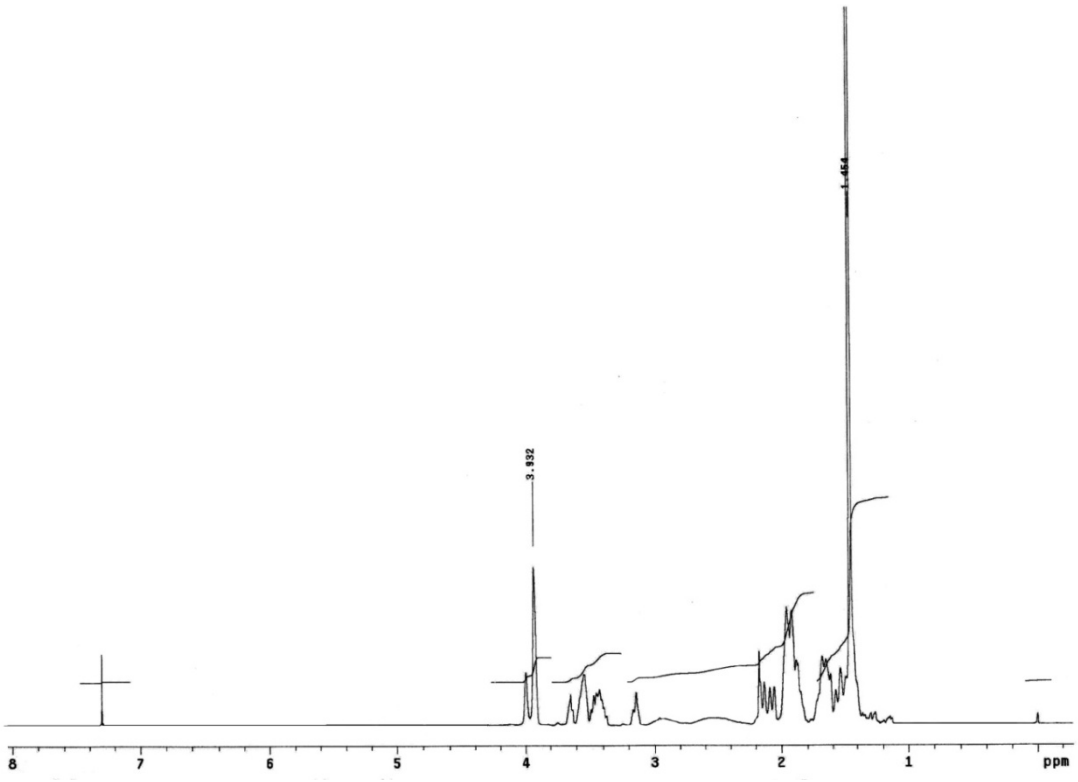
Second step: A solution of di-*tert*-butyl dicarbonate (2.1 g, 10.0 mmol) in anhydrous CH_2Cl_2 (3 mL) was added dropwise to a solution of the above amino alcohol (10.0 mmol) in anhydrous CH_2Cl_2 (222 mL) at room temperature under inert atmosphere. The mixture was stirred for 2 h, poured into water, and the resulting solution was extracted with CH_2Cl_2. The combined organic extracts were washed with brine, dried over anhydrous $MgSO_4$, filtered and concentrated under reduced pressure. Flash chromatography (9:1 to 6:4 hexane-EtOAc) gave alcohol **41** (1.28 g, 55% overall yield from **19**) as colourless oil.

IR (film): 3453 (OH), 1689, 1666 (C=O) cm^{-1}.

^1H NMR (300 MHz, $CDCl_3$, rotamers): δ 1.45 [s, 9H, $C(CH_3)_3$], 1.45 (masked, 2H, H-6), 1.57 (m, 1H, H-7), 1.65 (m, 2H, H-4, H-9), 1.91 (m, 3H, H-4, H-9, H-5), 2.06, 2.14 (d, J = 13.2 Hz, 1H, H-7), 2.50 (br. s, 1H, OH), 3.42 (m, 1H, H-3), 3.53 (m, 1H, H-3), 3.93 (s, 1H, H-1), 3.99 (s, 1H, H-8).

^{13}C NMR (75.4 MHz, $CDCl_3$, rotamers): δ 24.0, 24.2 (C-5), 25.0, 25.1 (C-7), 25.6, 25.8 (C-6), 26.6, 26.8 (C-4), 27.9, 28.2 (C-9), 28.4 [$C(CH_3)_3$], 39.1, 40.0 (C-3), 50.9, 51.3 (C-1), 66.6, 67.6 (C-8), 79.3, 79.4 [$C(CH_3)_3$], 156.0, 156.1 (CO).

HRMS (ESI) calcd for $[C_{13}H_{23}NO_3 + H]^+$: 264.1570, found: 264.1577.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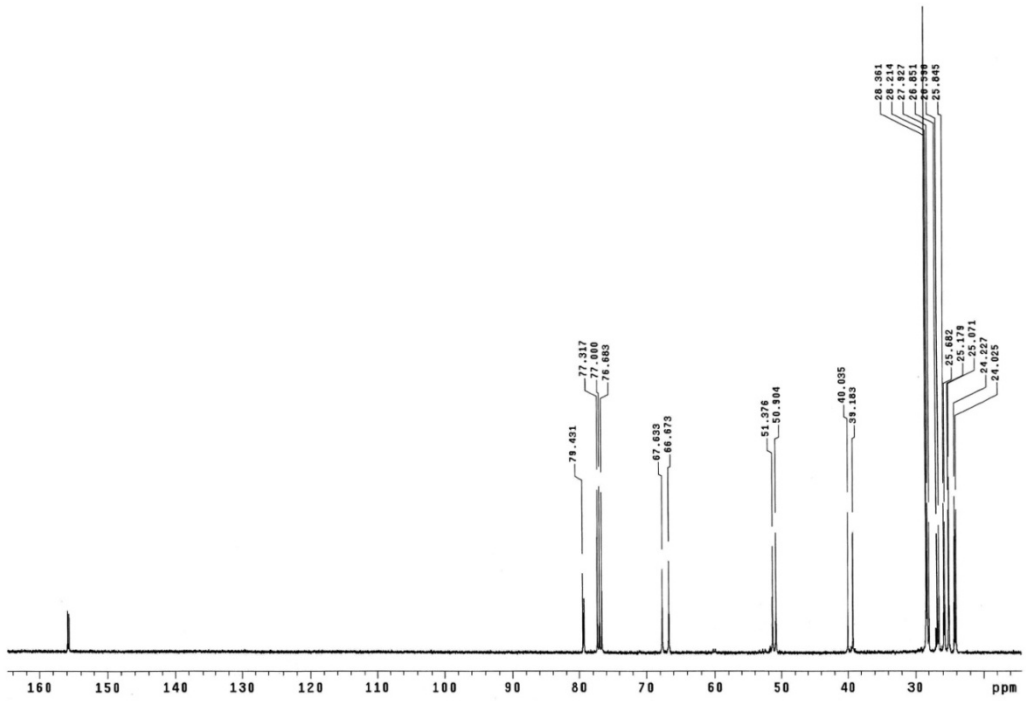
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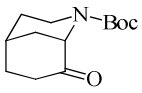
ppm



2-(*tert*-Butoxycarbonyl)-8-oxo-2-azabicyclo[3.3.1]nonane (42).

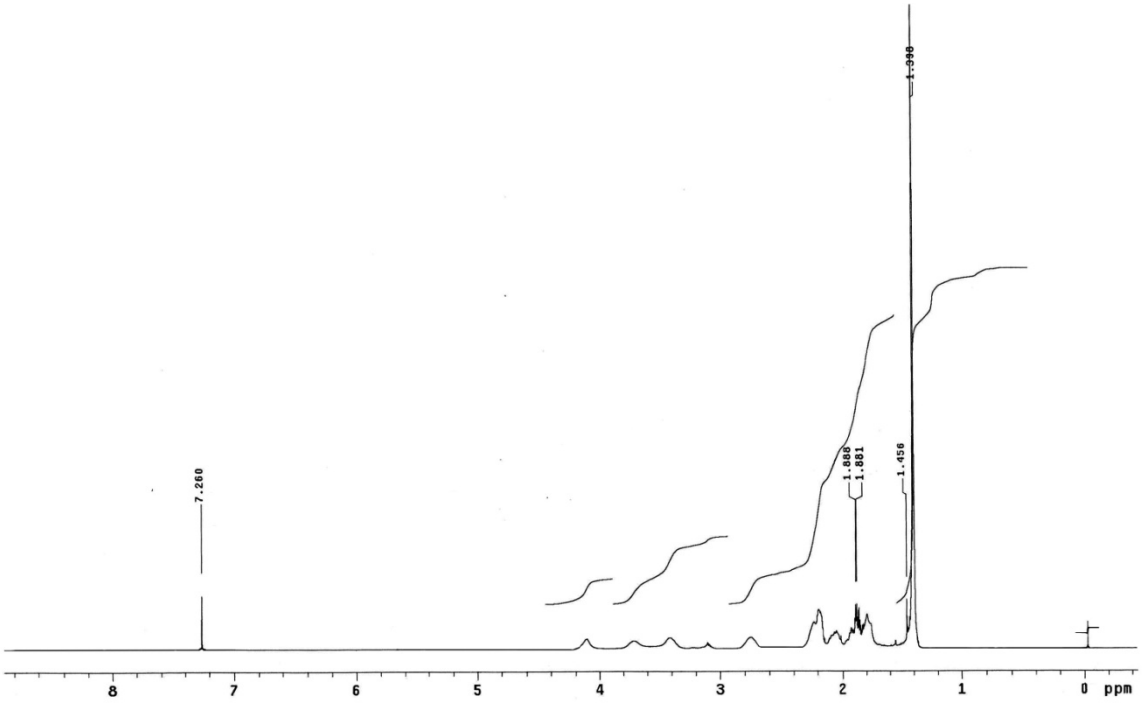
Dess-Martin Periodinane (5.7 g, 13 mmol) was added at room temperature to a solution of bicyclic alcohol **41** (1.28 g, 5.3 mmol) in CH_2Cl_2 (196 mL). After 18 h of stirring at room temperature, a saturated (1:1) solution of $NaHCO_3$-$Na_2S_2O_3$ was slowly added. The resulting mixture was stirred vigorously for 1 h and extracted with CH_2Cl_2. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and concentrated under reduced pressure. Flash chromatography (hexane to 1:1 EtOAc-hexane) afforded ketone **42** (1.07 g, 85%) as a colorless oil.

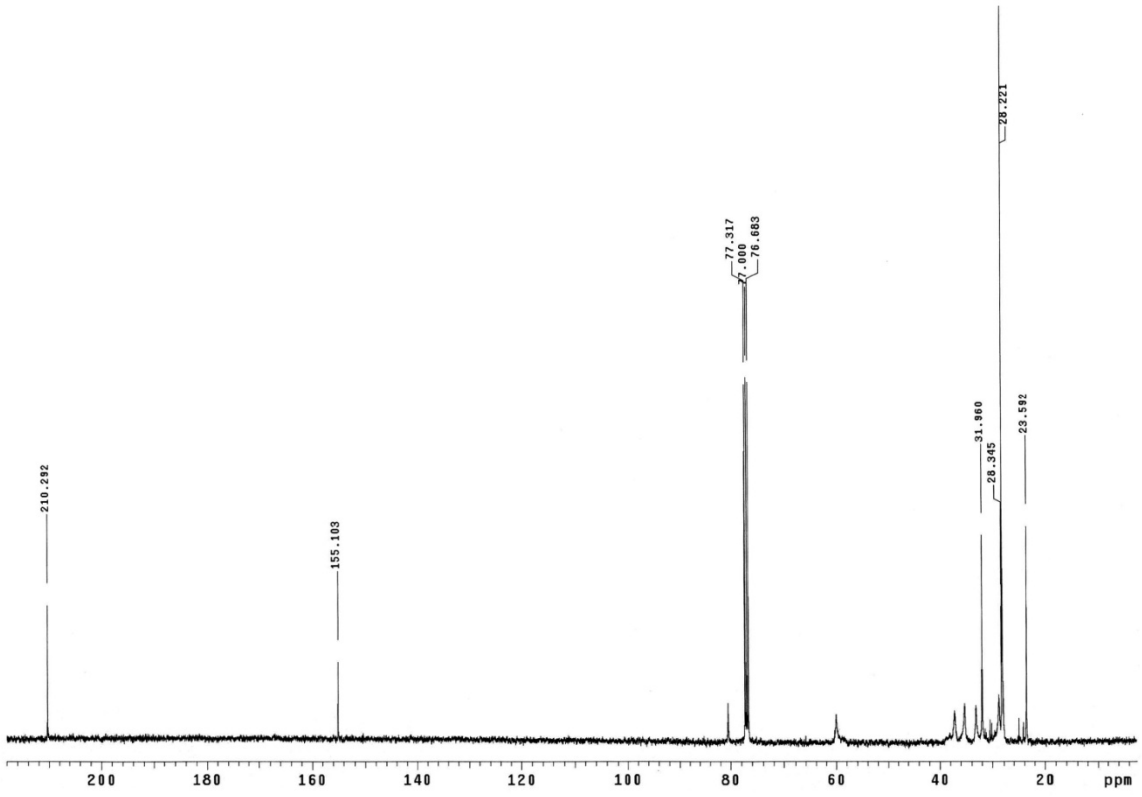
IR (film): 1696, 1705 (C=O) cm^{-1}.

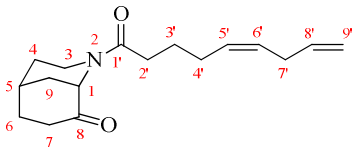
^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC): δ 1.40 [s, 9H, $(CH_3)_3C$], 1.79, 1.81 (2m, 2H, H-6, H-4), 1.91 (m, 2H, H-9), 2.08 (m, 1H, H-6), 2.10-2.20 (m, 3H, H-5, H-4, H-7), 2.76 (m , 1H, H-7), 3.43 (m, 1H, H-3), 3.73 (m, 1H, H-3), 4.20 (m, 1H, H-1).

^{13}C NMR (100.6 MHz, $CDCl_3$): δ 22.6 (C-5), 28.2 [$(CH_3)_3C$], 28.3 (C-4), 31.9 (C-6), 35.3 (C-9), 35.3 (C-7), 37.2 (C-3), 60.0 (C-1), 80.6 [$(CH_3)_3C$], 155.1 (COO), 210.3 (CO).

HRMS (ESI) calcd for $[C_{13}H_{21}NO_3 + Na]^+$: 262.1419, found: 262.1416.







(*Z*)-8-Oxo-2-nona-5,8-dien-1-oxo-2-azabicyclo[3.3.1]nonane (43).

First step: TFA (1.1 ml) was added at room temperature to a solution of ketone **42** (69 mg, 0.289 mmol) in CH_2Cl_2 (3.2 mL). After stirring for 30 min, the mixture was concentrated under reduced pressure. This crude was used without further purification in the next step.

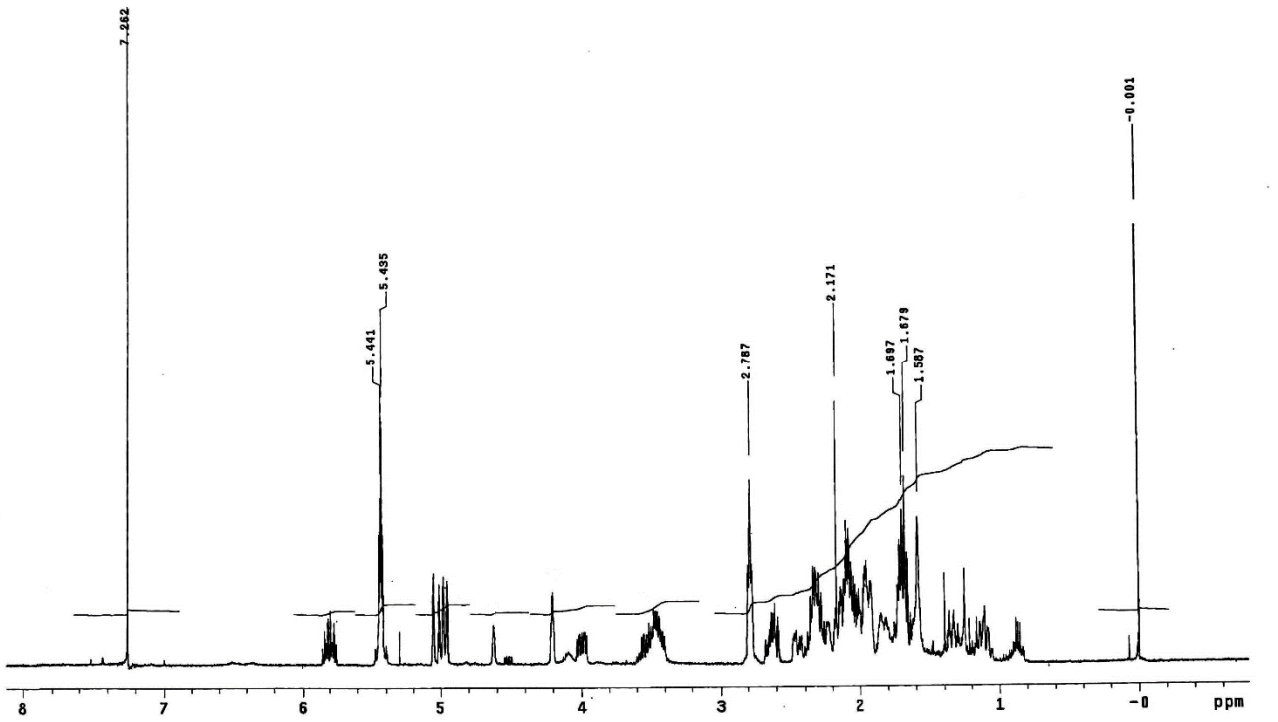
Second step: To a stirred solution of acid **27** (45 mg, 0.292 mmol) and above amine (0.289 mmol) in CH_2Cl_2 (1.6 mL) was added dropwise a solution of DCC (71 mg, 0.345 mmol) and DMAP (4 mg, 0.03 mmol) in CH_2Cl_2 (1.1 mL) at -20 °C under argon. After the end of addition, the mixture was allowed to heat slowly to room temperature and was stirred for one night. The dicyclohexylurea was then filtered off and the filtrate was extracted with HCl 0.5 M and then with Na_2CO_3. The combined organic extracts were dried over anhydrous Na_2SO_4, filtered, and

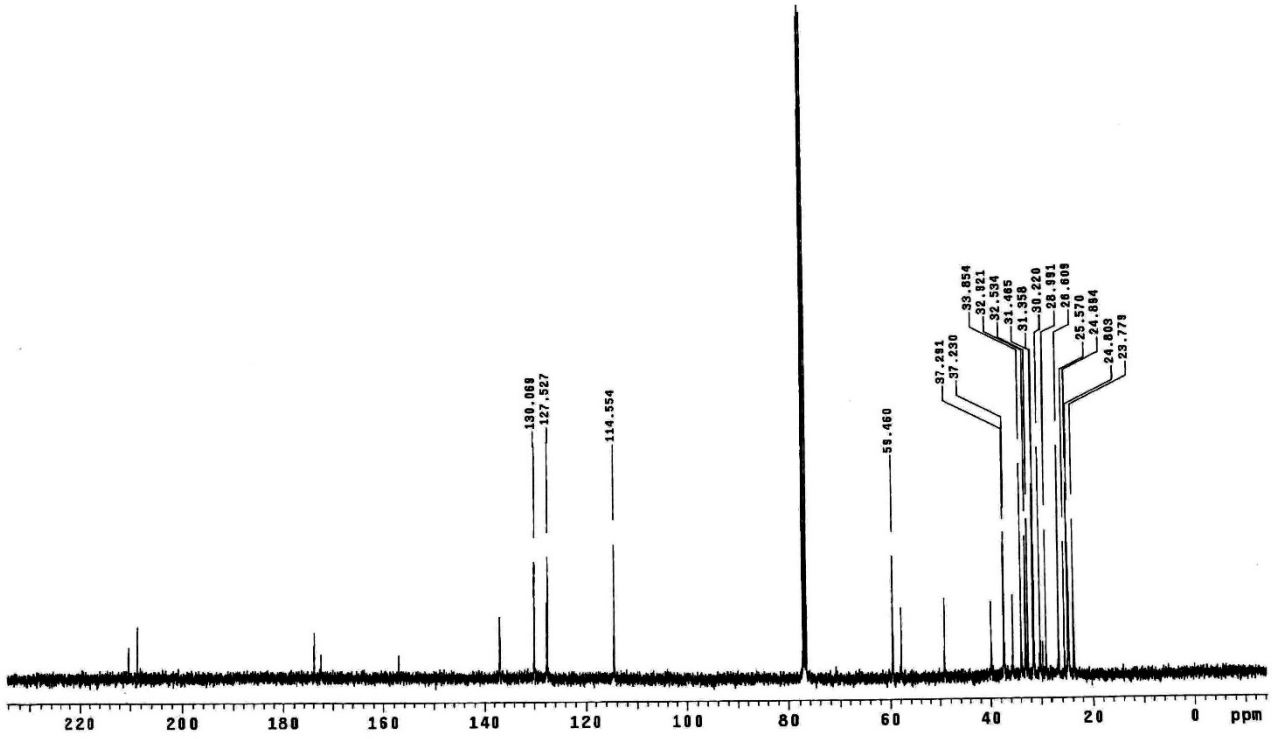
concentrated under reduced pressure. The product was purified by flash chromatography (hexane-EtOAc 8:2), affording amide **43** (40 mg, 50% in two steps).

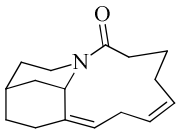
^{1}H NMR (400 MHz, $CDCl_3$, COSY, HSQC, rotamers): δ1.66-1.73 (m, 2H, H-3'),1.83 (dm, 2H, H-4), 1.90-1.98 (m, 4H, H-4, H-6, H-9), 2.00-2.12 (m, 3H, 2H-4', H-6), 2.13-2.19 (dm, 1H, H-9), 2.22-2.24 (m, 1H, H-5), 2.28-2.36 (m, 2H, H-2'), 2.42-2.48 (m, 1H, H-7), 2.59-2.68 (m, 1H, H-7), 2.79 (t, *J* = 5.6 Hz, 2H, H-7'), 3.41-3.55 (m, 3H, H-3), 3.99 (ddd, *J* = 12.0, 7.2, 4.8 Hz, 1H, H-3), 4.21 (br s, 1H, H-1), 4.62 (br s, 1H, H-1), 4.96 (dm, *J* = 10.0 Hz, 1H, $=CH_2$), 5.03 (dm, *J* = 16.8 Hz, 1H, $=CH_2$), 5.42-5.45 (m, 2H, H-5', H-6'), 5.81 (ddt, *J* = 16.8, 10.0, 6.8, 6.8 Hz, 1H, H-8').

^{13}C NMR (100.6 MHz, $CDCl_3$, rotamers): δ23.7, 23.8 (C-5), 24.9 (C-3'), 26.6 (C-4'), 29.0 (C-4), 30.2 (C-6), 31.4, 31.5 (C-7'), 32.5 (C-9), 32.9, 33.0 (C-2'), 33.8 (C-4), 35.6, 37.2 (C-7), 37.3, 39.8 (C-3), 57.8, 59.5 (C-1), 114.5 ($=CH_2$), 127.5, 127.6 (C-6'), 130.0, 130.1 (C-5'), 136.9-137.0 (C-8'), 172.5-173.8 (C-1'), 208.7-210.3 (C-8).

HRMS (ESI) calcd for $[C_{17}H_{25}NO_2 + H]^+$: 276.1958, found: 276.1955.



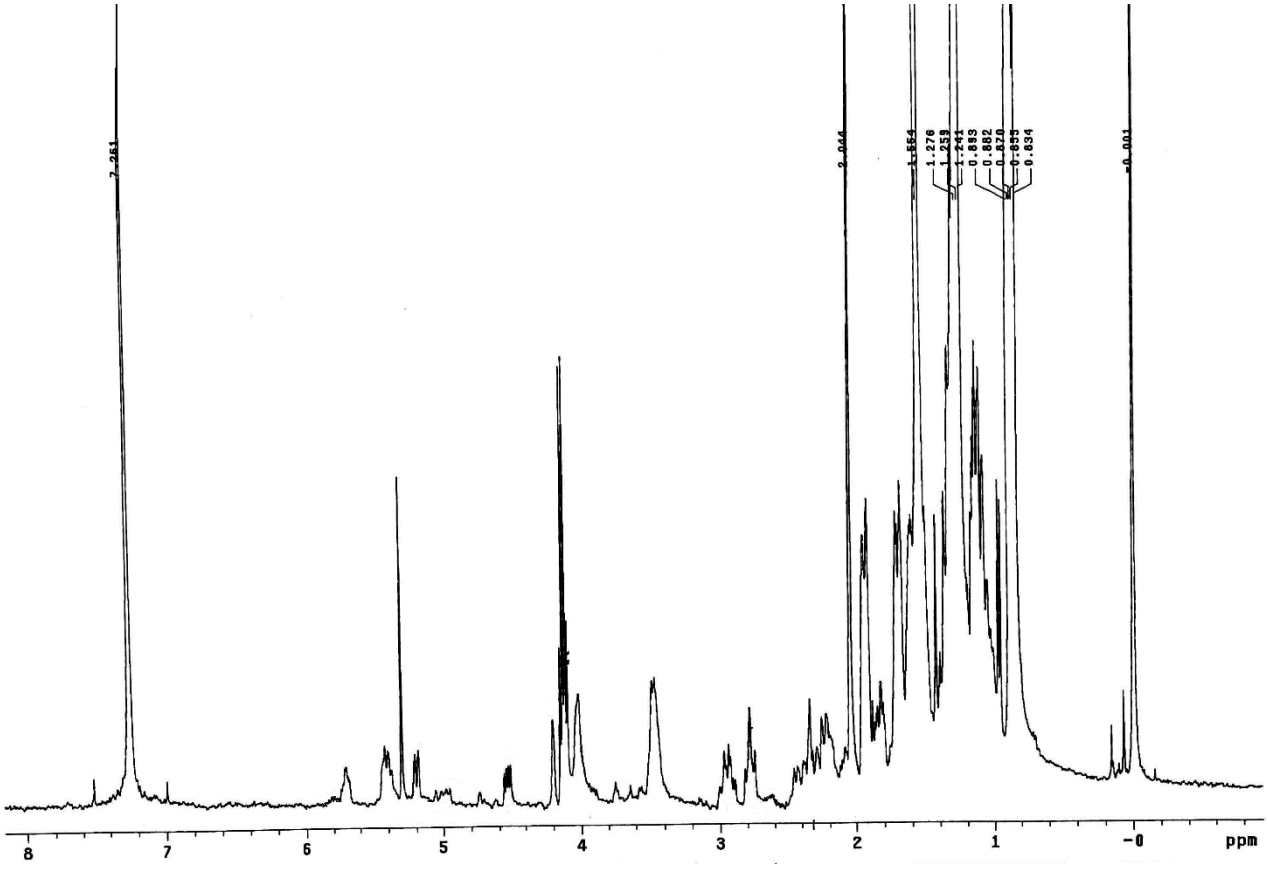


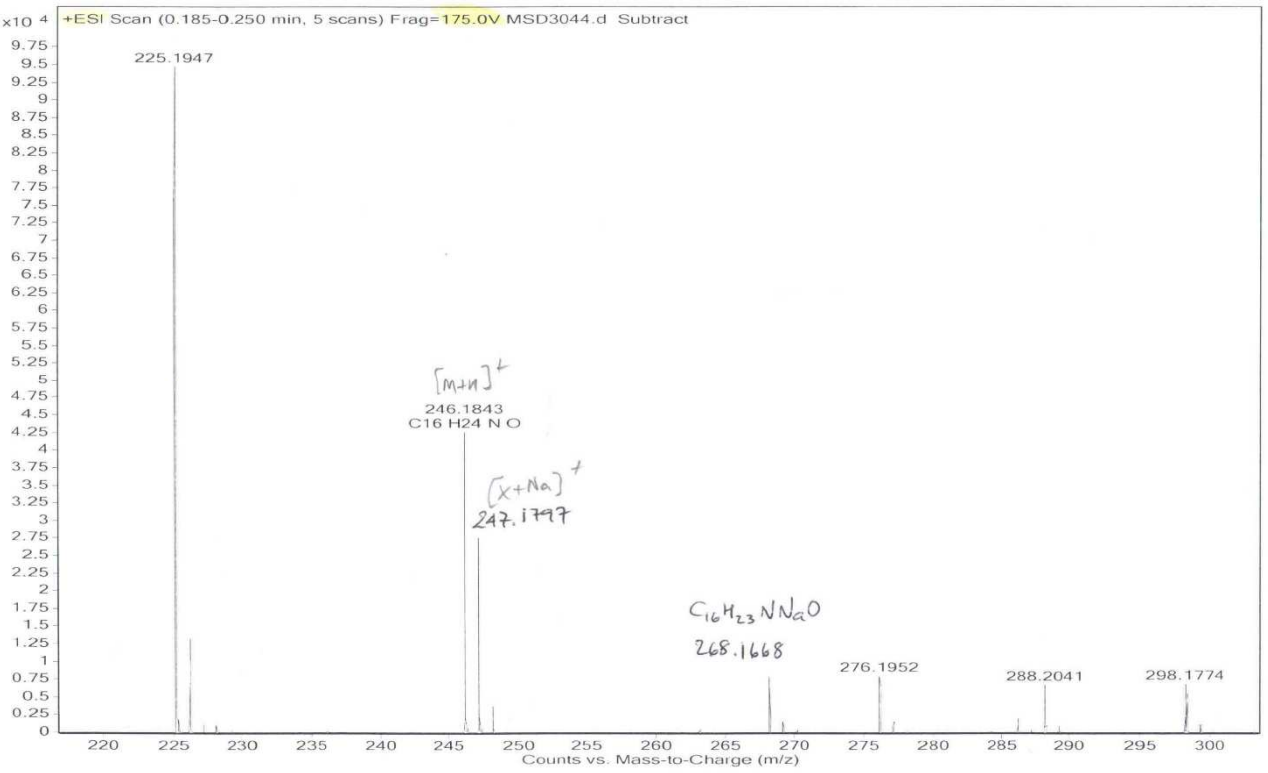


BCE system (30).

Schrock catalyst (36 mg, 0.0470 mmol) was added under inert atmosphere to a solution of **43** (15 mg, 0.0470 mmol) in benzene (5.2 ml), and the mixture was heated to 30 °C for one night, and to reflux for additional 24 hours. The reaction was then concentrated under reduced pressure. Flash chromatography (hexane to 2:8 EtOAc-hexane) afforded product **30** in traces.

HRMS (ESI) calcd for $[C_{16}H_{24}NO + H]^+$: 246.1852, found: 246.1845.


7.261
2.044
1.554
1.276
1.253
1.241
0.883
0.882
0.820
0.835
0.834
0.001
8
7
6
5
4
3
2
1
0
ppm


x10 4
+ESI Scan (0.185-0.250 min, 5 scans) Frag=175.0V MSD3044.d Subtract
225.1947
[M+H]+
246.1843
C16 H24 N O
[X+Na]+
247.1797
C16H23NNaO
268.1668
276.1952
288.2041
298.1774
Counts vs. Mass-to-Charge (m/z)

5. REFERENCES

1. Kittakoop, P., Mahidol, C., Ruchirawat, S. *Curr. Top. Med. Chem.* **2014**, *14*, 239.

2. Sinatra, R.S.; Jahr, J.S.; Watkins-Pitchford, J.M. **2010**. *The Essence of Analgesia and Analgesics*. Cambridge University Press. p. 82.

3. Winther, H.; Jorgensen, J.T. *Pharm. Med.* **2010**, *24*, 363.

4. "Cancer Fact sheet N°297". *World Health Organization*. Retrieved June 10, **2014**.

5. "Defining Cancer". *National Cancer Institute*. Retrieved June 10, **2014**.

6. (a) "What Is Cancer?". *National Cancer Institute*. Retrieved August 17, **2009**. (b) "Cancer Fact Sheet". *Agency for Toxic Substances & Disease Registry*. Retrieved August 17, **2009**.

7. Kong, F.; Andersen, R J.; Allen, T.M. *J. Am. Chem. Soc.* **1994**, *116*, 6007.

8. Custódio, M.R.; Lôbo-Hajdu, G.; Hajdu, E.; Muricy, G., Eds.; Museu Nacional: Rio De Janeiro, Brazil. **2007**, 173.

9. (a) D'Auria, M.; Minale, L.; Riccio, R. *Chem. Rev.* **1993**, *93*, 1839. (b) Minale, L. In *Marine Natural Products, Chemical and Biological Perspectives*; Scheuer, P. J., Ed.; Academic Press: New York, **1978**, Vol. 1, Chapter 4. (c) Fusetani, N.; Matsunaga, S. *Chem. Rev.* **1993**, *93*, 1793. (d) Fusetani, N.; Shinoda, K.; Matsunaga, S. *J. Am. Chem. Soc.* **1993**, *115*, 3977. (e) Molinski, T.F. *Chem. Rev.* **1993**, *93*, 1825.

10. Schmitz, F.J.; Hollenbeak, K.H.; Campbell, D.C. *J. Org. Chem.* **1978**, *43*, 3916.

11. Andersen, R.J.; Van Soest, R.W.M.; Kong, F. In *Alkaloids: Chemical and Biological Perspectives*; Pelletier, S. W., Ed.; Pergamon Press: New York, **1996**; Vol. 10, 301.

12. Kong, F.; Andersen, R.J.; Allen, T.M. *Tetrahedron Lett*. **1994**, *35*, 1643.

13. Jaspars, M.; Pasupathy, V.; Crews, P. *J. Org. Chem.* **1994**, *59*, 3253.

14. (a) Cimino, G.; De Stefano, S.; Scognamiglio, G.; Sodano, G.; Trivellone, E. *Bull. Soc. Chim. Belg.* **1986**, *95*, 783. (b) Cimino, G.; Mattia, C. A.; Mazzarella, L.; Puliti, R.; Scognamiglio, G.; Spinella, A.; Trivellone, E. *Tetrahedron.* **1989**, *45*, 3863. (c) Cimino, G.; Fontana, A.; Scognamiglio, G.; Trivellone, E. *Mag. Res. Chem.* **1989**, *29*, 327. (d) Cimino, G.; Scognamiglio, G.; Spinella, A.; Trivellone, E. *J. Nat. Prod*. **1990**, *53*, 1519.

15. Sakai, R.; Higa, T.; Jefford, C. W.; Bernardinelli, G. *J. Am. Chem. Soc*. **1986**, *108*, 6404.

16. Guo, Y.; Madaio, A.; Trivellone, E.; Scognamiglio, G.; Cimino, G. *Tetrahedron.* **1996**, *52*, 8341.

17. Yoshimura, Y.; Inoue, J.; Yamazaki, N.; Aoyagi, S.; Kibayashi, C. *Tetrahedron Lett.* **2006**, *47*, 3489.

18. Winkler, J.D.; Axten, J.M. *J. Am. Chem. Soc.* **1998**, *120*, 6425.

19. Denhart, D.J.; Griffith, D.A.; Heathcock, C.H. *J. Org. Chem.* **1998**, *63*, 9616.

20. Ballette, R.; Pérez, M.; Proto, S.; Amat, M.; Bosch, J. *Angew. Chem. Int. Ed*. **2014**, *53*, 6202.

21. Baldwin, J.E.; Whitehead, R.C. *Tetrahedron Lett.* **1992**, *33*, 2059.

22. Kondo, K.; Shigcmori, H.; Kikuchi, Y.; Ishibashi. M.; Sasaki, T.; Kobayashi, J. *J. Org. Chem.* **1992**, *57*, 2480.

23. Baldwin, J.E.; Bischoff, L.; Claridge, T.D.W.; Heupel, F.A.; Spring, D.R.; Whitehead R.C. *Tetrahedron*. **1997**, *53*, 2271.

24. Kong, F.; Graziani, E.I.; Andersen, R.J. *J. Nat. Prod.* **1998**, *61*, 267.

25. (a) Jakubowicz, K.; Abdeljelil, K.B.; Herdemann, M.; Martin, M.T.; Gateau-Olesker, A.; Mourabit, A.A.; Marazano, C.; Das B.C. *J. Org. Chem.* **1999**, *64*, 7381. (b) Hourcade, S.; Ferdenzi, A.; Retailleau, P.; Mons, S.; Marazano, C. *Eur. J. Org. Chem.* **2005**, 1302. (c) Tong, H.M.; Martin, M.T.; Chiaroni, A.; Bénéchie, M.; Marazano, C. *Org. Lett.* **2005**, *7*, 2437. (d) Ge, C.S.; Hourcade, S.; Ferdenzi, A.; Chiaroni, A.; Mons, S.; Delpech, B.; Marazano, C. *Eur. J. Org. Chem.* **2006**, 4106.

26. Oliveira, J.H.H.L.; Nascimento, A.M.; Kossuga, M.H.; Cavalcanti, B.C.; Pessoa, C.O.; Moraes, M.O.; Macedo, M.L.; Ferreira, A.G.; Hajdu, E.; Pinheiro, U.S.; Berlinck, R.G.S. *J. Nat. Prod*. **2007**, *70*, 538.

27. (a) Pinheiro, U.S.; Berlinck, R.G.S.; Hajdu, E. *Contrib. Zool*. **2005**, *74*, 271. (b) Oliveira, J. H.H.L.; Grube, A.; Köck, M.; Berlinck, R.G.S.; Macedo, M.L.; Ferreira, A.G.; Hajdu, E.J. *Nat. Prod*. **2004**, *67*, 1685. (c) Oliveira, J.H.H.L.; Seleghim, M.H.R.; Timm, C.; Grube, A.; Köck, M.; Nascimento, G.G.F.; Martins, A.C.T.; Silva, E.G.O.; Souza, A.O.; Galetti, F.C.S.; Minarini, P.R.R.; Silva, C.L.L.; Hajdu, E.; Berlinck, R.G.S. *Mar. Drugs.* **2006**, *4*, 1. (d) Oliveira, M.F.; Oliveira, J.H.H.L.; Galetti, F.C.S.; Souza, A.O.; Silva, C.L.; Hajdu, E.; Peixinho, S.; Berlinck, R.G.S. *Planta Med*. **2006**, *72*, 437.

28. (a) Morimoto, Y.; Yokoe, C. *Tetrahedron*. **1997**, *38*, 8981; (b) Baldwin, J.E.; James, D.A.; Lee, V. *Tetrahedron Lett.* **2000**, *41*, 733; (c) Martin, S.F.; Humphrey, J.M.; Ali, A.; Hillier, M.C. *J. Am. Chem. Soc.* **1999**, *121*, 866. (d) Jacubec, P.; Hawkins, A.; Felzmann, W.; Dixon, D.J. *J. Am. Chem. Soc.* **2012**, *134*, 17482. (e) Torisawa, Y.; Hashimoto, A.; Nakagawa, M.; Hino, T. *Tetrahedron Lett.* **1989**, *30*, 6549. (f) Nakagawa, M. *J. Heterocyclic Chem.* **2000**, *37*, 567. (g) Becker, M.H.; Chua, P.; Downham, R.; Douglas, C.J.; Garg, N.K.; Hiebert, S.; Jaroch, S.; Matsuoka, R.T.; Middleton, J.A.; Ng, F.W.; Overman, L.E. *J. Am. Chem. Soc.* **2007**, *129*, 11987. (h) Garg, N.K.; Hiebert S.H.; Overman, L.E. *Angew. Chem. Int. Ed*. **2006**, *45*, 2912. (i) Scott, R.W.; Epperson, J.; Heathcock, C.H. *J. Org. Chem*. **1998**, *63*, 5001. (j) Hoye, T.R.; North, J.T.; Yao, L.J. *J. Am. Chem. Soc.* **1994**, *116*, 2617.

29. Matzanke, N.; Gregg, R.J.; Weinreb R.J.; Parvez, M. *J. Org. Chem*. **1997**, *62*, 1920.

30. Hopman, J.C.P.; van den Berg, E.; Ollero, L.O.; Hiemstra, H.; Speckamp, W.N. *Tetrahedron Lett.* **1995**, *36*, 4315.

31. Yamazaki, N.; Kusanagi, T.; Kibayashi, C. *Tetrahedron Lett.* **2004**, *45*, 6509.

32. Yoshimura, Y.; Inoue, J.; Yamazaki, N.; Aoyagi, S.; Kibayashi, C. *Tetrahedron Lett.* **2006**, *47*, 3489.

33. Quirante, J.; Paloma, L.; Diaba, F.; Vila, X.; Bonjoch, J. *J. Org. Chem.* **2008**, *73*, 768.

34. Quirante, J.; Vila, X.; Paloma, L.; Guiu, J.M.; Bonjoch, J. *Tetrahedron*. **2007**, *63*, 1372.

35. Diaba, F.; Pujol-Grau, C.; Martínez-Laporta, A.; Fernández, I.; Bonjoch, J. *Org. Lett.* **2015**, *17*, 568.

36. (a) Amat, M.; Pérez, M.; Minaglia, A.T.; Peretto, B.; Bosch, J. *Tetrahedron*. **2005**, *63*, 5839. (b) Amat, M.; Pérez, M.; Proto, S.; Bosch, J. *Chem. Eur. J.* **2010**, *16*, 9438. (c) Amat, M.; Pérez, M.; Bosch, J. *Chem. Eur. J.* **2011**, *17*, 7724. (d) Amat, M.; Ballette, R.; Proto, S.; Pérez, M.; Bosch, J. *Chem. Commun*. **2013**, *49*, 3149.

37. Yanagita, Y.; Suto, T.; Matsuo, N.; Kurosu, Y.; Sato, T.; Chida, N. *Org. Lett.* **2015**, *17*, 1946.

38. (a) Escolano, C.; Amat, M.; Bosch, J. *Chem. Eur. J.* **2006**, *12*, 8198. (b) Amat, M.; Bassas, O.; Llor, N.; Cantó, M.; Pérez, M.; Molins, E.; Bosch, J. *Chem. Eur. J.* **2006**, *12*, 7872. (c) Amat, M.; Lozano, O.; Escolano, C.; Molins, E.; Bosch, J. *J. Org. Chem*. **2007**, *72*, 4431. (d) Amat, M.; Griera, R.; Fabregat, R.; Bosch, J. *Angew. Chem.* **2008**, *120*, 3396; *Angew. Chem. Int. Ed.* **2008**, *47*, 3348. (e) Amat, M.; Gómez-Esqué, A.; Escolano, C.; Santos, M.M.M.; Molins, E.; Bosch, J. *J. Org. Chem.* **2009**, *74*, 1205. (f) Amat, M.; Llor, N.; Checa, B.; Molins, E.; Bosch, J. *J. Org. Chem.* **2010**, *75*, 178. (g) Amat, M.; Elias, V.; Llor, N.; Subrizi, F.; Molins, E.; Bosch, J. *Eur. J. Org. Chem*. **2010**, *41*, 4017. (h) Amat, M.; Pérez, M.; Bosch, J. *Synlett*. **2011**, *2*, 143. (i) Pérez, M.; Arioli, F.; Rigacci, G.; Santos, M.M.M.; Gómez-Esqué, A.; Escolano, C.; Florindo, P.; Ramos, C.; Bosch, J.; Amat, M. *Eur. J. Org. Chem.* **2011**, 3858. (j) Amat, M.; Pérez, M.; Bosch, J. *Chem. Eur. J.* **2011**, *17*, 7724. (k) Amat, M., LLor, N.; Griera, R.; Pérez, M.; Bosch, J. *Nat. Prod. Commun.* **2011**, *4*, 515.

39. (a) Meyers, A.I.; Harre, M.; Garland, R. *J. Am. Chem. Soc*. **1984**, *106*, 1146. (b) Romo, D.; Meyers, A.I. *Tetrahedron*. **1991**, *47*, 9503. (c) Meyers, A.I.; Brengel, G.P. *Chem. Comm*. **1997**, 1. (d) Groaning, M.D.; Meyers, A.I. *Tetrahedron.* **2000**, *56*, 9843.

40. (a) Nagashima, H.; Washiyama, M.; Itoh, K. *Tetrahedron Lett.* **1985**, *26*, 657. (b) Hagen, T. J. *Synlett.* **1990**, 63.

41. (a) Overman, L.E.; Robichaud, A.J. *J. Am. Chem. Soc.* **1989**, *111*, 300. (b) Herdeis, C.; Kaschinski, C.; Karla, R. *Tetrahedron: Asymmetry* **1996**, *7*, 867. (c) Muller, M.; Schoenfelder, A.; Didier, B.; Mann, A.; Wermuth, C.-G. *Chem. Commun.* **1999**, 683. (d) Carreira, E.M.; Lerchner, A. *J. Am. Chem. Soc.* **2002**, *124*, 14826. (e) Hanessian, S.; van Otterlo, W.A.L.; Nilsson, I.; Bauer, U. *Tetrahedron Lett.* **2002**, *43*, 1995. (f) Cossy, J.;

Mirguet, O.; Gomez Pardo, D.; Desmurs, J.-R. *New J. Chem.* **2003**, *27*, 475. (g) Pineschi, M.; Del Moro, F.; Gini, F.; Minnaard, A.J.; Feringa, B.L. *Chem. Commun.* **2004**, 1244. (h) Deiters, A.; Pettersson, M.; Martin, S.F. *J. Org. Chem.* **2006**, *71*, 6547.

42. Nandi, P.; Dye, J.L.; Jackson, J.E. *Tetrahedron Lett.* **2009**, *50*, 3864.

43. (a) Ennis, M.D.; Hoffman, R.L.; Ghazal, N.B.; Old, D.W.; Mooney, P.A. *J. Org. Chem.* **1996**, *61*, 5813. (b) Olofson, R.A.; Abbott, D.E. *J. Org. Chem.***1984**, *49*, 2795. (c) Olofson, R.A.; Martz, J.T.; Senet, J.P.; Piteau, M.; Malfroof, T. *J. Org. Chem.* **1984**, *49*, 2081.

44. Still, W.C.; Gennari, C. *Tetrahedron Lett.* **1983**, *24*, 4405.

45. Blanchette, M.A.; Choy, W.; Davis, J.T.; Essenfeld, A.P.; Masamune, S.; Roush, W.R.; Sakai T. *Tetrahedron Lett.* **1984**, *25*, 2183.

46. (a) Posner, G.H.; Loomis, G.L.; Sawaya, H.S. *Tetrahedron Lett.* **1975**, *16*, 1373. (b) Rule, M.; Mondo, J.A.; Berson, J.A. *J. Am. Chem. Soc.* **1982**, *104*, 2209.

47. Korotchenko, V.N.; Shastin, A.V.; Nenajdenko, V.G.; Balenkova, E. *Org. Biomol. Chem.* **2003**, *1*, 1906.

48. (a) Grubbs, R.H.; Pine, S.H. *Comprehensive Organic Synthesis*, Trost, B.M.; Fleming, I.; Paquette, L.A. Eds.; Pergamon: New York, **1991**, Vol. 5, Chapter 9.3. (b) Schrock, R.R. *The Strem Chemiker*, Vol. XIV, Strem Chemicals, Newburgport, **1992**, *1*, 1. (c) Ivin, K.J.; Mol, J.C. *Olefin Metathesis and Metathesis Polymerization*, Academic Press, San Diego, **1997**.

49. Fürstner, A. *Angew. Chem.* **2000**, *112*, 3140; *Angew. Chem. Int. Ed.* **2000**, *39*, 3012.

50. Grubbs, R.H.; Chang, S. *Tetrahedron.***1998**, *54*, 4413.

51. Tebbe, F.N.; Parshall, G.W.; Reddy, G.S. *J. Am. Chem. Soc.* **1978**, *100*, 3611.

52. Petasis, N.A.; Bzowej, E.I. *J. Am. Chem. Soc.* **1990**, *112*, 6392.

53. Bennasar, M.L.; Roca, T.; Monerris, M.; García-Díaz, D. *J. Org. Chem.* **2006**, *71*, 7028.

54. McMurry, J.E.; Fleming, M.P. *J. Am. Chem. Soc.* **1974**, *96*, 4708.

55. McMurry, J.E. *Chem. Rev.* **1989**, *89*, 1513.

56. Sabino, A.A.; Pilli, R.A. *Tetrahedron Lett.* **2002**, *43*, 2819.

57. (a) Bowen, E.G.; Wardrop, D.J. *Org. Lett.* **2010**, *12*, 5330. (b) Zhu, L.; Lauchli, R.; Loo, M.; Shea, K.J. *Org. Lett.* **2007**, *9*, 2269. (c) Fürstner, A.; Aïssa, C.; Riveiros, R.; Ragot, J. *Angew. Chem. Int. Ed.* **2002**, *41*, 4763.

58. Nadkarni, D.; Hallissey, J.; Mojica, C. *J. Org. Chem.* **2003**, *68*, 594.

59. Fu, G.C.; Grubbs, R.H. *J. Am. Chem. Soc.* **1993**, *115*, 3800.

60. Clive, D.L.J.; Zhang, C.; Murthy, K.S.K.; Hayward, W.D.; Daigneault, S. *J. Org. Chem.* **1991**, *56*, 6447.

61. Martin, S.F.; Liao, Y.; Wong, Y.; Rein, T. *Tetrahedron Lett.* **1994**, *35*, 691.

62. Proto, S.; Amat, M.; Pérez, M.; Ballette, R.; Romagnoli, F.; Mancinelli, A.; Bosch, J. *Org. Lett.* **2012**, *14*, 3916.

LIST OF ABBREVIATIONS

$(CF_3)_2CHOH$	1,1,1,3,3,3-hexafluoro-2-propanol
$(C_6H_{11})_2BH$	dicyclohexylborane
$(CF_3CH_2O)_2P(=O)CH_2CO_2Me$	methyl bis(2,2,2-trifluoroethyl)phosphonoacetates
$(CF_3CO)_2O$	trifluoroacetic anhydride
$(CH_2O)_2CH(CH_2)_3Br$	2-(3-bromopropyl)-1,3-dioxolane
$(CH_2OH)_2$	1,2-etanediol
$(COCl)_2$	oxalyl chloride
(Z)-$Bu_3SnCH=CH(CH_2)_4OTBDPS$	(1,1-dimethylethyl)(dimethyl){[(5Z)-6-(tributylstannanyl)hex-5-enyl]oxy}silane
3TC	lamivudine
9-BBN	9-borabicyclo(3.3.1)nonane
ABC	abacavir
Ac	acetyl
Ac_2O	acetic anhydride
AcOH	acetic acid
AIDS	human acquired immunodeficiency syndrome
$AlCl_3$	aluminium chloride
AlH_3	aluminium hydride
AllBr	allyl bromide
AllMgBr	allyl magnesium bromide
AllOTIPS	(allyloxy)triisopropylsilane
AllTMS	allyltrimethylsilane
AMP-DNJ	*N*-(5-adamantane-1-yl-methoxypentyl)-1-deoxy-nojirimycin
Ar	aryl
ART	anti-retroviral therapy
AZT	zidovudine
AZTTP	zidovudine triphosphate
B	nucleobase
BCH-189	dideoxy thiacytidine
BCl_3	boron trichloride
$BF_3 \cdot Et_2O$	boron trifluoride diethyl etherate
BH_3	borane
Bn	benzyl
BnBr	benzyl bromide
$BnNH_2$	benzylamine
Boc	t-butyloxycarbonyl
Boc_2O	di-t-butyl dicarbonate

BSA	*N*,*O*-bis(trimethylsilyl)acetamide
Bu	buthyl
BVDV	bovine viral diarrhea virus
Bz	benzoyl
Bz_2O	benzoic anhydride
BzCl	benzoyl chloride
C_6H_5	phenyl
C_6H_5SeCl	phenylselenenyl chloride
$CaCO_3$	calcium carbonate
CAN	ceric ammonium nitrate
CBr_4	carbon tetrabromide
CBz	benzyloxycarbonyl
Cbz	carboxybenzyl
CbzCl	benzyl chloroformate
CbzCl	benzyl chloroformate
CCR5	C-C chemokine receptor type 5
CD4	cluster of differentiation 4
$CDCl_3$	deuterated chloroform
$CH_2(CN)CO_2Et$	ethyl cyanoacrylate
$CH_2(CN)CO_2Et$	ethyl cyanoacrylate
CH_2Cl_2	dichloromethane
$CHCl_3$	chloroform
$ClCO_2Me$	methyl chloroformate
CO	carbon monoxide
CO_2Me	methoxy carbonyl
CoA	coenzyme A
COSY	correlation spectroscopy
Cp_2TiCl_2	bis(cyclopentadienyl)titanium(IV) dichloride
Cp_2TiMe_2	Petasis reagent
CRIs	co-receptor inhibitors
Cs_2CO_3	caesium carbonate
CsF	caesium fluoride
CuCl	copper(I) chloride
CuI	copper(I) iodide
CuOOH	cumene hydroperoxide
CXCR4	C-X-C chemokine receptor type 4
Cy	cyclohexyl
d4T	stavudine
DAB	1,4-dideoxy-1,4-imino-D-arabinitol
DBU	1,8-diazabicyclo[5.4.0]undec-7-ene

DCC	N,N'-dicyclohexylcarbodiimide
dCK	deoxycytidine kinase
DCM	dichloromethane
ddc	zalcitabine
ddI	didanosine
DDQ	2,3-Dichloro-5,6-dicyano-1,4-benzoquinone
DEAD	diethyl azodicarboxylate
DET	diethyl tartrate
DIBAH	diisobutylaluminium hydride
DIBAL-H	diisobutylaluminium hydride
DIPEA	N,N-diisopropylethylamine
DMAP	4-dimethylaminopyridine
DMDP	2,5-dideoxy-2,5-imino-D-mannitol
DME	dimethoxyethane
DMF	dimethylformamide
DMP	Dess-Martin periodinane
DMSO	dimethyl sulfoxide
DNA	deoxyribonucleic acid
DNJ	1-deoxy-nojirimycin
dNTP	deoxynucleotide triphosphates
dr	diastereoisomeric ratio
EC_{50}	half maximal effective concentration
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
ee	enantiomeric excess
env	envelope
ERG	electron releasing group
ESI	electrospray ionization mass spectrometry
Et	ethyl
Et_2O	diethyl ether
Et_3N	triethylamine
Et_3SiH	triethylsilane
Et_3SiI	triethylsilyl iodide
EtOAc	ethyl acetate
EtOH	ethanol
E-TP	emtricitabine triphosphate
EWG	electron withdrawing group
FDA	Food and Drug Administration
FIs	fusion inhibitors
FTC	emtricitabine
FT-IR	Fourier transform infrared spectroscopy

gag	group-specific antigen
GBA	glucocerebrosidase
GCase	lysosomal β-glucosidase
GCS	glucosyl ceramide synthase
GlcCer	glucosylceramide
GM	monosialodihexosylganglioside
gp	glycoprotein
GSL	glycosphingolipid
H_2	hydrogen
H_2NNH_2	hydrazine
H_2O_2	hydrogen peroxide solution (30 wt. % in H_2O)
H_2SO_4	sulfuric acid
HAART	highly active anti-retroviral therapy
HBr	hydrobromic acid
HBV	hepatitis B virus
$HC{\equiv}CCH_2Br$	3-bromoprop-1-yne
HCHO	formaldehyde
HCl	hydrochloric acid
HCO_2H	formic acid
$HCONH_2$	formamide
HCV	hepatitis C virus
$Hg(OCOCF_3)_2$	mercury(II) trifluoroacetate
HI	hydroiodic acid
HIV	human immunodeficiency virus
HMPA	hexamethylphosphoramide
HNJ	homonojirimycin
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
HRMS	high-resolution mass spectrometry
HSAB	hard and soft acids and bases
HSQC	heteronuclear single quantum coherence
HWE	Horner-Wadsworth-Emmons
HWE-SGM	Horner-Wadsworth-Emmons Still-Gennari modification
I_2	iodine
IC_{50}	half maximal inhibitory concentration
IN	integrase
INIs	integrase inhibitors
i-Pr_2EtN	N,N-diisopropylethylamine
i-PrMgCl	isopropylmagnesium chloride
IUPAC	international union of pure and applied chemistry

K_2CO_3	potassium carbonate
K_2HPO_4	dipotassium phosphate
KEX	potassium ethyl xanthate
$KHCO_3$	potassium bicarbonate
KHMDS	potassium bis(trimethylsilyl)amide
$KMnO_4$	potassium permanganate
KOH	potassium hydroxide
LDA	lithium diisopropylamide
$LiAlH_4$	lithium aluminium hydride
$LiBH_4$	lithium borohydride
LiBr	lithium bromide
LiCl	lithium chloride
LiHMDS	lithium bis(trimethylsilyl)amide
LiOH	litium hydroxide
m.p.	melting point
Mbs	*p*-methoxybenzenesulfonyl
m-CPBA	*m*-chloroperoxybenzoic acid
Me	methyl
Me	methyl
Me_2S	dimethyl sulfide
Me_3P	trimethylphosphine
$MeC(OMe)_3$	1,1,1-trimethoxyethane
MeCN	acetonitrile
$MeCO_2Cl$	methyl chloroformate
Me-DNJ	*N*-methyl-1-deoxy-nojirimycin
MeLi	methyllitium
MeNHOMe·HCl	N,O-dimethylhydroxylamine hydrochloride
MeOD	deuterated methanol
MeOH	methanol
$MePPh_3Br$	methyltriphenylphosphonium bromide
$MeSO_3H$	methanesulfonic acid
$MgSO_4$	magnesium sulfate
MOM	methoxymethyl
MOMCl	chloromethyl methyl ether
Ms	mesyl
MS	mass spectra
MsCl	methanesulfonyl chloride
N_3	azide
$Na_2S_2O_3$	sodium thiosulfate
Na_2SO_4	sodium sulfate

$NaBH(OAc)_3$	sodium triacetoxyborohydride
$NaBH_3CN$	sodium cyanoborohydride
$NaBH_4$	sodium borohydride
NaCl	sodium chloride
NaH	sodium hydride
$NaHCO_3$	sodium bicarbonate
NaHMDS	sodium bis(trimethylsilyl)amide
NaI	sodium iodide
$NaIO_4$	sodium periodate
NaN_3	sodium azide
NaOAc	sodium acetate
NaOEt	sodium ethoxide
NaOH	sodium hydroxide
NaOMe	sodium methoxide
Napht	naphtalene
NAs	nucleoside analogues
NB-DNJ	*N*-butyl-1-deoxy-nojirimycin
n-BuBr	1-bromobutane
n-BuLi	*n*-butyllithium
$NCCH_2CO_2H$	cyanoacetic acid
NH_2OCH_2Ph	O-benzylhydroxylamine
$NH_2OH \cdot HCl$	hydroxylamine hydrochloride
NH_3	ammonia
NH_4Cl	ammonium chloride
NH_4OH	ammonium hydroxide
NJ	nojirimycin
NMO	N-methylmorpholine oxide
NMR	nuclear magnetic resonance
NN-DNJ	*N*-nonyl-1-deoxy-nojirimycin
NNRTIs	non-nucleoside reverse transcriptase inhibitors
NRTIs	nucleoside reverse transcriptase inhibitors
Ns	nosyl
NsCl	nosyl chloride
NtRTIs	nucleotide reverse transcriptase inhibitors
Nu^-	nucleophile
o-$(OH)C_6H_4CHO$	salicylaldehyde
o.y.	overall yield
o-$C_6H_4(CH_2OH)_2$	1,2-benzenedimethanol
OH	hydroxyl
OsO_4	osmium tetroxide

P_2O_5	phosphorus pentoxide
$Pb(OAc)_4$	lead(IV) acetate
p-$BrC_6H_4SO_2Cl$	4-bromobenzenesulfonyl chloride
PCC	pyridinium chlorochromate
$Pd(dba)_2$	bis(dibenzylideneacetone)palladium(0)
$Pd(OCOCF_3)_2$	palladium(II) trifluoroacetate
$Pd(OH)_2$	palladium hydroxide
Pd/C	palladium on carbon
$Pd_2dba_3 \cdot CHCl_3$	tris(dibenzylideneacetone)dipalladium(0)-chloroform adduct
PDC	pyridinium dichromate
$PdCl_2$	palladium(II) chloride
Ph	phenyl
Ph_3P	triphenylphosphine
Ph_3PCH_3Br	methyltriphenylphosphonium bromide
$PhI(OAc)_2$	(diacetoxyiodo)benzene
PhSH	thiophenol
PhthNH	phthalimide
PIs	protease inhibitors
PMB	4-methoxybenzyl
PMB cells	primary human lymphocytes
PMBCl	4-methoxybenzyl chloride
PMe_3	trimethylphosphine
PMHS	polymethylsiloxane
pol	polymerase
pol γ	DNA polimerase γ
PPh_3	triphenyl phosphine
PPTS	pyridinium *p*-toluenesulfonate
PR	protease
p-TsOH	4-toluenesulfonic acid
py	pyridine
PyBOP	(benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
RCM	ring-closing metathesis
rev	regulator of expression of virion proteins
R_f	retardation factor
RNA	ribonucleic acid
Rnase	ribonuclease
RT	reverse transcriptase
rt	room temperature
SAP	sphingolipid activator protein
SES	trimethylsilylethylsulfonyl

SiO_2	silicon dioxide
S_N2	bimolecular nucleophilic substitution
$SnCl_4$	tin(IV) tetrachloride
$SOCl_2$	thionyl chloride
SRT	substrate reduction therapy
tat	trans-activator of transcription
TBAF	tetra-*n*-butylammonium fluoride
TBAI	tetrabutylammonium iodide
TBDMS	*tert*-butyldimethylsilyl
TBDMSCl	*t*-butyldimethylsilyl chloride
TBDMSOTf	*t*-butyldimethylsilyl trifluoromethanesulfonate
TBDPS	*tert*-butyldiphenylsilyl
TBDPSCl	*tert*-butyldiphenylsilyl chloride
t-$BuCO_2H$	pivalic acid
t-BuOK	potassium *t*-butoxide
t-BuOMe	methyl *tert*-butyl ether
TEA	triethylamine
Teoc	2-(trimethylsilyl)ethoxycarbonyloxy
TFA	trifluoroacetic acid
THF	tetrahydrofuran
$Ti(Oi\text{-}Pr)_4$	titanium(IV) isopropoxide
$TiCl_3$	titanium(III) chloride
$TiCl_4$	titanium(IV) chloride
TIPS	triisopropylsilyl
TLC	thin-layer chromatography
TMS	trimethylsilyl
TMSCl	chlorotrimethylsilane
TMSI	trimethylsilyl iodide
TMSOTf	trimethylsilyl trifluoromethanesulfonate
TosMIC	*p*-toluenesulfonylmethyl isocyanide
TsCl	4-toluenesulfonyl chloride
ZDV	zidovudine
$ZrCl_4$	zirconium(IV) chloride

SUPPORT LETTER

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To Whom It May Concern

It is with great pleasure that I provide this letter about the work developed by Federica Caso during her stay in the laboratory of Organic Chemistry of the Faculty of Pharmacy of the University of Barcelona.

Federica Caso joined my group to do a stay on January 12, 2015 and she was working interruptedly till July 10th, 2015. She came back on September 1st, 2015, and she was working interruptedly till February 26, 2016. During her stay in Barcelona she was involved in a project devoted to the enantioselective total synthesis of marine alkaloids of the madangamine group. In particular, she was working on the search of alternative procedures to improve the stereocontrolled synthesis of the 11-membered E ring of this class of alkaloids.

Federica was a very motivated and responsible student, with a strong interest in her research work and excellent work habits. She has good intellectual ability, resourcefulness, initiative and a good knowledge of the field. She is skilled at synthetic manipulation, purification, separation techniques, operating infrared and nuclear magnetic resonance equipment, and interpretation of mass spectrometry and spectroscopic data. She has experience in literature searches and good general computer skills. In addition, she is able to plan her laboratory work, interpret results and meet deadlines.

On a personal level, Federica is a kind and helpful person, with an open character. She has a good capacity for teamwork and always got along very well with the members of the research group and the professors of the Department.

Working with Federica Caso has left me with the most favorable impression of her commitment to chemical research and of her personal qualities.

Sincerely,

Mercedes Amat
Professor of Organic Chemistry
University of Barcelona

Barcelona, March 19, 2016

COMMUNICATIONS AND PUBLICATION

PUBLICATION

Maria Federica Caso, Daniele D'Alonzo, Stefano D'Errico, Giovanni Palumbo, Annalisa Guaragna. "Highly Stereoselective Synthesis of Lamivudine (3TC) and Emtricitabine (FTC) by a novel *N*-glycosylation Procedure".

Org. Lett. **2015**, *17*, 2626.

COMMUNICATIONS

"Glycosciences: a complex discipline with a central role in modern biology, biotechnology and medicine".

Summer Trainin School- COST BM1003. Napoli (Italy). June 3-5, 2014.

Ischia Advanced School of Organic Chemistry - IASOC 2014. Ischia (Italy). Sept. 21-25, 2014.

Maria Federica Caso, Daniele D'Alonzo, Giovanni Palumbo, Annalisa Guaragna. "The Iodine/Silane system: a novel exploitation in nucleoside synthesis".

XXXVI Convegno della Divisione di Chimica Organica CDCO 2015. Bologna (Italy). Sept. 13-17, 2015.

Oral Communication

Celeste Are, Roberto Ballette, Maria Pérez, Maria Federica Caso, Joan Bosch, Mercedes Amat. "Enantioselective synthetic approach to the marine alkaloids of the madangamine group".

XII Simposio de Investigadores Jóvenes de la Real Sociedad Española de Química. Barcelona (Spain). Nov. 3-6, 2015.

Poster Communication

Celeste Are, Roberto Ballette, Maria Pérez, Maria Federica Caso, Joan Bosch, Mercedes Amat. "A unified strategy for the enantioselective synthesis of madangamines A, C and E".

IX Trobada de Joves Investigadors dels Països Catalans. Perpignan (France). Feb 3-5, 2016.

Oral Communication

Organic LETTERS

Letter

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Highly Stereoselective Synthesis of Lamivudine (3TC) and Emtricitabine (FTC) by a Novel *N*-Glycosidation Procedure

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Supporting Information

ABSTRACT: The combined use of silanes (Et_3SiH or PMHS) and I_2 as novel *N*-glycosidation reagents for the synthesis of bioactive oxathiolane nucleosides 3TC and FTC is reported. Both systems (working as anhydrous HI sources) were devised to act as substrate activators and *N*-glycosidation promoters. Excellent results in terms of chemical efficiency and stereoselectivity of the reactions were obtained; surprisingly, the nature of the protective group at the N4 position of (fluoro)cytosine additionally influenced the stereochemical reaction outcome.

In the absence of effective vaccines able to control viral infections, clinical use of chemically modified nucleosides currently represents the core of any chemotherapeutic treatment aiming at a substantial and prolonged suppression of viral replication.[1,2] Because of the structural relationship with their natural counterparts, synthetic nucleosides can deeply interfere with various viral life cycles, mainly at a transcriptional level, by blocking the information flow enclosed in the viral genomes.[1a] Among sugar-modified nucleosides, those having a 1,3-oxathiolane moiety as deoxyribose bioisostere have received considerable attention over the last two decades, owing to their remarkable antiviral properties, especially as reverse transcriptase inhibitors (RTIs).[3−5] As result of the powerful biological activities and favorable toxicological profiles,[3] the two oxathiolane nucleosides Lamivudine (3TC, **1**) and Emtricitabine (FTC, **2**) have been licensed for the treatment of human immunodeficiency viruses (HIV-1 and HIV-2) and hepatitis B virus (HBV) infections, whether administered individually or in combination with other inhibitors[5,6] (Figure 1). In addition, a number of other promising oxathiolane nucleosides including Apricitabine (ATC, **3**) and Racivir (RCV, **4**) (Figure 1) are currently undergoing clinical evaluation as antiretroviral agents.[7,8]

Over the years, the increasing clinical request for oxathiolane nucleosides has justified the development of a wide variety of synthetic approaches allowing their preparation in high purity and on large scale.[4,9,10] Despite their structural simplicity, access to these compounds has represented a major synthetic challenge, because of (a) the need for a chemistry enabling stereochemical control of two potentially epimerizable stereocenters and (b) the need for stereoselective *N*-glycosidation methodologies required in the absence of directing groups adjacent to the glycosidation site.

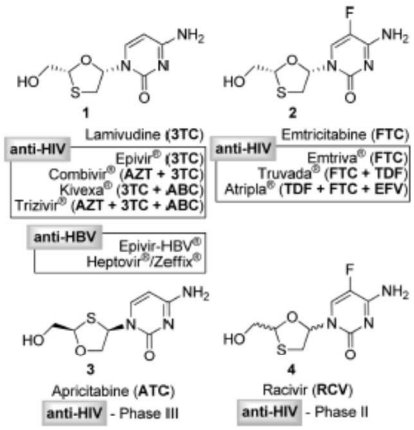

Figure 1. Oxathiolane nucleosides currently approved or undergoing clinical evaluation as antiviral agents.

Some among the most efficient approaches aimed at the stereoselective oxathiolane nucleoside synthesis have been focused on preparation of nucleoside precursors **8** and **9** (Scheme 1).[11−15] One of the methods of choice for the industrial production of 3TC and FTC follows the procedure suggested by Whitehead et al.,[13,15] which involves the reaction between L-menthyl ester-containing (5*S*)-5-chlorooxathiolane **7a** (prepared from 5-hydroxyoxathiolane **5a** with 2*R*,5*R* configuration at the oxathiolane ring by reaction with $SOCl_2$) and presilylated cytosine or 5-fluorocytosine, leading to nucleoside precursors **8a**

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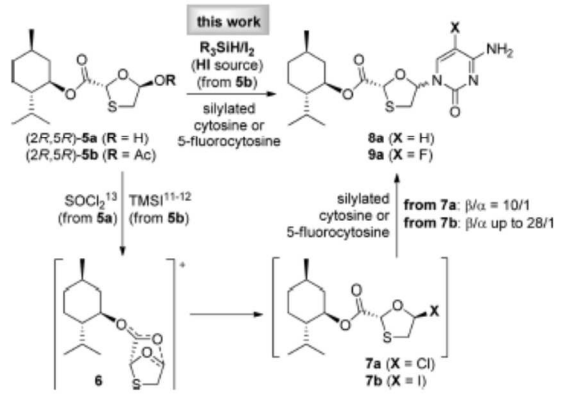
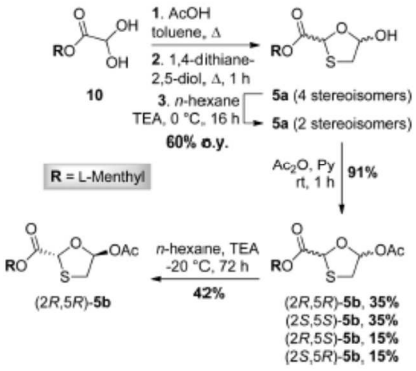
Scheme 1. Synthetic Approaches to Oxathiolane Nucleoside Precursors 8 and 9



Scheme 2. Synthesis of (2*R*,5*R*)-5b from Glyoxylate 10



and **9a** in satisfying yields (**8a**: 66%;[13] **9a**: 81%[15a]) and anomeric selectivity (**8a**: α:β = 10:1;[13] **9a**: not given) (Scheme 1). This approach represented a convenient alternative to the previous methodology by Tse et al.,[11,12] who used the more unstable and expensive (although more efficient) TMSI as *N*-glycosidation reagent (**8a**: 75%, β:α = 23:1;[12] **9a**: 91%, β:α = 28:1[11]) starting from acetate (2*R*,5*R*)-**5b** (Scheme 1). As widely reported, in both cases the high β-selectivity is due to formation of oxonium ion **6**, stabilized through anchimeric assistance by the menthyl ester function.[13]

In the framework of our interest into new *N*-glycosidation methods,[16] the search for more efficient, stereoselective, and cost-effective variants of the above approaches caused us to explore the synthetic potential of the *N*-glycosidation reagent based on the combined use of silanes [especially triethylsilane (Et_3SiH) and polymethylhydrosiloxane (PMHS)] and iodine (I_2). Use of these systems in organic synthesis already has numerous precedents in the literature, especially in carbohydrate chemistry.[17] The Et_3SiH/I_2 system is an established reagent devised for a wide variety of transformations, including iodination,[18] regioselective Bn group removal,[19] *O*-glycosylation[20a] (even in stereoselective fashion),[20b] and rearrangement reactions.[21] The PMHS/I_2 system has demonstrated an analogous synthetic potential;[22] the very low cost of PMHS[23] makes the latter an even more convenient option for large-scale applications.

As widely reported, both Et_3SiH/I_2 and PMHS/I_2 systems act as sources of anhydrous HI, which is believed to be the actual reagent in most of the above trasformations.[17] Herein, the latter was conceived to be used both as acetate **5b** activator (enabling its conversion into 5-α-iodooxathiolane **7b**) and as glycosidation promoter (Scheme 1).

Access to enantiopure 5-acetoxyoxathiolane (2*R*,5*R*)-**5b** was explored using the synthetic strategy reported by Whitehead et al.[13] (Scheme 2). The coupling reaction between commercially available L-menthyl glyoxylate monohydrate (**10**) and 1,4-dithiane-2,5-diol, followed by treatment of the crude mixture with a TEA-containing *n*-hexane solution provided hemiacetal **5a** (60% yield). Literature procedure reports an early formation of four stereoisomers **5a** [with the following isomeric distribution: *trans*-(2*R*,5*R*)-**5a**, 35%; *trans*-(2*S*,5*S*)-**5a**, 15%; *cis*-(2*R*,5*S*)-**5a**, 35%; *cis*-(2*S*,5*R*)-**5a**, 15%], while the subsequent addition of *n*-hexane/TEA is claimed to enable both the rapid interconversion among the stereoisomers and the selective precipitation of the sole (2*R*,5*R*)-**5a**.[13] However, in our hands, the exact repetition of this methodology (as well as that of a more recent variation)[14] on laboratory scale (10 mmol) always gave a roughly equimolar mixture of two hemiacetal stereoisomers **5a** (60% overall yield) containing the (2*R*,5*R*) isomer. In addition, hydroxyl group acetylation of the last ones (Ac_2O/Py) led again to a base-mediated isomerization, providing a mixture of four acetates **5b** (91%). Differently from the aforementioned Whitehead's distribution[13] of hemiacetals **5a**, we identified[24] as the two major acetate stereoisomers **5b** those having the C2 and C5 stereocenters in a *trans* relationship [*trans*-(2*R*,5*R*)-**5b**, 35%; *trans*-(2*S*,5*S*)-**5b**, 35%; *cis*-(2*R*,5*S*)-**5b**, 15%; *cis*-(2*S*,5*R*)-**5b**, 15%] (Scheme 2). Accordingly, an accurate separation of the two main isomers was mandatory to ensure a high optical purity to the target nucleosides: indeed, while the *trans* acetate with the (2*R*,5*R*) configuration represented a suitable building block en route to (−)-oxathiolane nucleosides, conversely the use of the stereoisomer with (2*S*,5*S*) configuration allows the synthesis of the corresponding (+)-enantiomers. Gratifyingly, selective recrystallization of the sole (2*R*,5*R*)-**5b** could be achieved by treatment of the mixture with *n*-hexane containing a catalytic amount of TEA (42% yield). Isomerization at C2 (but not at C5) position, enabling conversion of the minor *cis*-(2*S*,5*R*)-**5b** into the corresponding *trans*-(2*R*,5*R*)-**5b**, was also observed at this stage.

Silane/I_2-mediated *N*-glycosidation was then tested by reaction of (2*R*,5*R*)-**5b** with cytosine (**11a**), 5-fluorocytosine (**12a**), and their N^4-benzoyl and N^4-acetyl derivatives **11b**,**c** and **12b** (Table 1). The reaction involved the treatment of **5b** with a premixed silane/I_2 solution followed by the *one-pot* addition of presilylated nucleobase solutions. First attempts with unprotected cytosine **11a** using Et_3SiH/I_2 and PMHS/I_2 as *N*-glycosidation reagents provided, already after 1 h at rt, the corresponding nucleoside **8a** in excellent yields (Et_3SiH: 98%; PMHS: 86%) and, somewhat in line with previous methods, with high anomeric selectivities (β:α = 15:1, 88% de) (entries 1 and 2).

Under the same conditions, the reaction of **5b** with 5-fluorocytosine **12a** gave higher selectivities (β:α = 35:1, 94% de) and similar yields (Et_3SiH: 91%; PMHS: 84%) (entries 10 and 11). The reaction of **5b** with protected nucleobases was then considered: surprisingly, we found a dramatic improvement in the β/α ratio when N^4-benzoyl- and N^4-acetyl(5-fluoro)-cytosines **11b**,**c** and **12b** were used (entries 3−8, 12, and 13). As an example, treatment of **5b** with Et_3SiH/I_2 or PMHS/I_2 and **11c** gave the corresponding nucleoside precursor **8c** with a 80−

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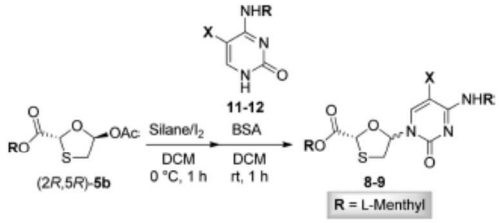
Table 1. Silane/I_2-Mediated *N*-Glycosidation Reaction



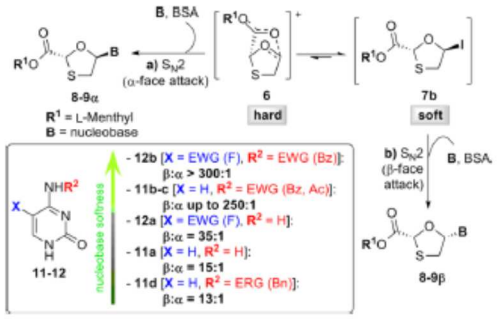
		nucleobase					
entry	silane		R	X	nucleoside	yield (%)	selectivity[a,b] (de, %)
1	Et_3SiH	**11a**	H	H	**8a**	98	88
2	PMHS	**11a**	H	H	**8a**	86	88
3	Et_3SiH[c]	**11b**	Bz	H	**8b**	72	95
4	PMHS[c]	**11b**	Bz	H	**8b**	73	95
5	Et_3SiH[d]	**11b**	Bz	H	**8b**	87	98
6	PMHS[d]	**11b**	Bz	H	**8b**	94	>99
7	Et_3SiH	**11c**	Ac	H	**8c**	95	97
8	PMHS	**11c**	Ac	H	**8c**	80	98
9	Et_3SiH[d]	**11d**	Bn	H	**8d**	62[e]	86
10	Et_3SiH	**12a**	H	F	**9a**	91	94
11	PMHS	**12a**	H	F	**9a**	84	94
12	Et_3SiH	**12b**	Bz	F	**9b**	85	98
13	PMHS	**12b**	Bz	F	**9b**	90	>99

[a]Determined by ^1H NMR analysis (400–600 MHz). [b]The structure of β- and α-nucleosides was determined by reduction of the menthyl ester group of **8a** and **9a** and subsequent NMR comparison of the corresponding reduction products with literature data.[25] [c]Silane/I_2 addition to the reaction mixture immediately after premixing of the two reagents. [d]Silane/I_2 addition to the reaction mixture carried out 15 min after premixing of the two reagents. [e]Mixture of regioisomeric N^4 and N^3 nucleosides (as suggested by NMR analysis).

95% yield and β/α ratios of 68:1–85:1 (97–98% de) (entries 7 and 8). Notably, the stereochemical outcome of the reactions was significantly influenced by the mixing times of silanes with I_2. As an example, when addition of Et_3SiH/I_2 to **5b** was performed immediately after premixing of the two reagents (entry 3), the reaction with N^4-benzoyl cytosine **11b** gave the corresponding nucleoside **8b** with an anomeric ratio of about 36:1 (95% de); in the same reaction, a much higher β-selectivity (β:α = 83:1, 97% de) was instead observed when Et_3SiH and I_2 were mixed for 15 min before addition[26] (entry 5). With PMHS/I_2, the same conditions provide even larger selectivity differences, with the β/α ratios increasing from 35:1 (95% de; entry 4) to 250:1 (>99% de; entry 6). The reaction with N^4-benzoylfluorocytosine **12b** similarly provided exceedingly high β-selectivities using either Et_3SiH/I_2 (98% de; entry 12) and PMHS/I_2 (>99% de; entry 13).

The results reported in Table 1 highlight the superiority of PMHS/I_2 regarding the stereoselectivity of the reactions, while with Et_3SiH/I_2 higher yielding conversions were observed in almost all cases. It is worth noting that, considering the selectivity differences between the two systems, the participation of further reactive species other than HI in the reaction mechanism cannot be excluded. The dramatic stereoselectivity improvement of the reactions with N^4-protected cytosines **11b,c** and **12b** compared to those with **11a** and **12a** also deserve some comments. At first glance, these results even appeared counterintuitive (the bulkier the N^4-amino group in the nucleobase, the higher the *cis*-selectivity). A good agreement between experimental data and

Scheme 3. Silane/I_2-Mediated *N*-Glycosidation and "Protective Group Effect": A Working Hypothesis



the HSAB (hard and soft acids and bases) theory was instead found. Accordingly, starting from the well-established[13] statement that an equilibrium exists between a minor, "hard" β-oxonium ion **6** (leading to α-configured S_N2 products) and a major, "soft" α-glycosyl iodide **7** (leading to β-configured products), we reasoned that the installation of an electron-withdrawing group (EWG) at the N4 position of the nucleobases **11** and **12** (e.g., in **11b,c** and **12b**) would make the corresponding N1 position a "softer" site than in **11a** and **12a**. This would explain the large preference of **11b,c** and **12b** for **7b** (as result of preponderant soft–soft interactions), whereas in the case of **11a** and **12a** a minor β-selectivity would be based on a non-negligible hard–hard interaction with **6** (Scheme 3). It should be also noted that, along the same lines, the higher selectivity in the reaction of **12a** compared with that in the reaction of **11a** can be similarly explained as a consequence of the presence of the fluorine atom at C5 position.

On the basis of these assumptions, the presence of an electron-releasing group (ERG) such as in the N^4-benzylcytosine (**11d**), able to reduce (albeit slightly) the softness of the corresponding persilylated nucleobase, was supposed to worsen the β-selectivity. As a result, treatment of **5b** with Et_3SiH/I_2 and persilylated **11d**[27] gave nucleoside precursor **8d** with an anomeric ratio of ca. 13:1 (86% de), additionally in low yield[28] (Table 1, entry 9).

With nucleoside precursors **8** and **9** in hand, attention was then turned to L-menthyl group reduction to afford target 3TC and FTC (Scheme 4). In line with previous reports,[12] treatment of **8a** (R = H) with $LiAlH_4$ provided, after 1 h at 0 °C, the corresponding free nucleoside **1** in a satisfying 83% yield. Conversely, the same procedure, starting from N^4-Bz or N^4-Ac derivatives **8b,c**, only provided the corresponding ester reduction products in low (58% from **8c**) or even negligible amounts (<10% from **8b**). Alternatively, reduction of **8b** and **9b** (R = Bz) was conceived by two-stages, one-pot procedure involving early N^4-acyl group removal by "superheated" MeOH[29] to give **8a** and **9a**, followed by addition of a reducing agent to the crude reaction mixture (Scheme 4). However, treatment of **8c** with MeOH at 100 °C as described by Robins et al.[29] provided, already after 1 h, large amounts of the epimerization product (2*S*,5*S*)-**8a** along with the expected nucleoside with (2*R*,5*S*) configuration [(2*R*,5*S*)-**8a**: (2*S*,5*S*)-**8a** = 2.2:1]; incidentally, detection in the crude of other nucleoside species containing methyl ester groups also suggested formation of transesterification products **13**. On the other hand, a temperature decrease led to a substantial minimization of the epimerization degree [T = 50 °C, (2*R*,5*S*)-**8a**: (2*S*,5*S*)-**8a** = 24:1; T = 40 °C, (2*R*,5*S*)-**8a**: (2*S*,5*S*)-**8a** =

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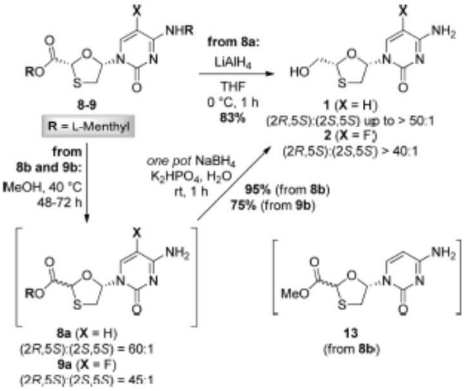
Scheme 4. 3TC and FTC Synthesis by One-Pot Reduction of Nucleoside Precursors 8 and 9



60:1]. Eventually, one-pot addition of $NaBH_4$ and K_2HPO_4 to the crude reaction mixture gave lamivudine (**1**) in an excellent 95% overall yield and with a high anomeric selectivity (β:α > 50:1). The same protocol, repeated on nucleoside **9b**, provided unprotected emtricitabine **2** with similar results (75% overall yield; β:α > 40:1) (Scheme 4).

In summary, we have provided a first look at the synthetic potential of Et_3SiH/I_2 and PMHS/I_2 as novel *N*-glycosidation reagents, as exemplified by the synthesis of the antiviral drugs oxathiolane nucleosides 3TC and FTC. Because of the low cost and high stability of the reagents, the chemical efficiency, and the exceedingly high stereoselectivity of the reactions, this approach can be reasonably considered as an effective alternative to the existing methodologies and reagents devoted to the same end. Crucial in influencing the stereochemical reaction outcome, an unprecedented role played by the N4 protective groups of (fluoro)cytosine has been also observed and rationalized on the basis of their capacity to increase nucleobase softness. Far beyond the scope of this work, this last finding opens up new opportunities in the stereoselective synthesis of β-nucleosides not relying on the presence of stereodirecting groups. A more comprehensive investigation on this topic aimed at studying scope and limitations of silane/I_2 in nucleoside synthesis is ongoing and will be published elsewhere.

■ ASSOCIATED CONTENT

Supporting Information

Experimental procedures and copies of NMR spectra. The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.5b00982.

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Notes

The authors declare no competing financial interest.

■ REFERENCES

(1) (a) De Clercq, E. *Nat. Rev. Drug Discovery* **2002**, *1*, 13−25. (b) Jordheim, L. P.; Durantel, D.; Zoulim, F.; Dumontet, C. *Nat. Rev. Drug Discovery* **2013**, *12*, 447−464.

(2) *Modified Nucleosides: in Biochemistry, Biotechnology and Medicine*; Herdewijn, P., Ed.; Wiley−VCH: Weinheim, 2008.

(3) (a) Mansour, T. S.; Storer, R. *Curr. Pharm. Design* **1997**, *3*, 227−264. (b) Rando, R. F.; Nguyen-Ba, N. *Drug Discovery Today* **2000**, *5*, 465−476. (c) Schinazi, R. F.; Hernandez-Santiago, B. I.; Hurwitz, S. J. *Antiviral Res.* **2006**, *71*, 322−334. (d) Wainberg, M. A. *Antiviral Res.* **2009**, *81*, 1−5.

(4) *Antiviral Nucleosides: Chiral Synthesis and Chemotherapy*; Chu, C. K., Ed.; Elsevier: Amsterdam, 2003.

(5) Cihlar, T.; Ray, A. S. *Antiviral Res.* **2010**, *85*, 39−58.

(6) Férir, G.; Kaptein, S.; Neyts, J.; De Clercq, E. *Rev. Med. Virol.* **2008**, *18*, 19−34.

(7) Ghosh, R. K.; Ghosh, S. M.; Chawla, S. *Expert Opin. Pharmacother.* **2011**, *12*, 31−46.

(8) Cox, S.; Southby, J. *Expert Opin. Invest. Drugs* **2009**, *18*, 199−209.

(9) (a) Wilson, L. J.; Hager, M. W.; El-Kattan, Y. A.; Liotta, D. C. *Synthesis* **1995**, 1465−1479. (b) Romeo, G.; Chiacchio, U.; Corsaro, A.; Merino, P. *Chem. Rev.* **2010**, *110*, 3337−3370.

(10) D'Alonzo, D.; Guaragna, A. In *Chemical Synthesis of Nucleoside Analogues*; Merino, P., Ed.; John Wiley & Sons: Hoboken, 2013.

(11) Mansour, T.; Jin, H.; Tse, A. H. L.; Siddiqui, M. A. PCT Int. Appl. WO 92/20669, 1992.

(12) Jin, H.; Siddiqui, A.; Evans, C. A.; Tse, A.; Mansour, T. S.; Goodyear, M. D.; Ravenscroft, P.; Beels, C. D. *J. Org. Chem.* **1995**, *60*, 2621−2623.

(13) Goodyear, M. D.; Hill, M. L.; West, J. P.; Whitehead, A. J. *Tetrahedron Lett.* **2005**, *46*, 8535−8538.

(14) Roy, B. N.; Singh, G. P.; Srivastava, D.; Aher, U. P.; Patil, S. U. PCT Int. Appl. WO2013021290 A1, 2013.

(15) (a) Richhariya, S.; Singh, K.; Prasad, M. PCT Int. Appl. WO2007077505 A2, 2007. (b) dos Santos Pinheiro, E.; Antunes, O. A. C.; Fortunak, J. M. D. *Antiviral Res.* **2008**, *79*, 143−165.

(16) (a) D'Alonzo, D.; Amato, J.; Schepers, G.; Froeyen, M.; Van Aerschot, A.; Herdewijn, P.; Guaragna, A. *Angew. Chem., Int. Ed.* **2013**, *52*, 6662−6665. (b) Paolella, C.; D'Alonzo, D.; Palumbo, G.; Guaragna, A. *Org. Biomol. Chem.* **2013**, *11*, 7825−7829.

(17) Adinolfi, M.; Iadonisi, A.; Pastore, A.; Valerio, S. *Pure Appl. Chem.* **2012**, *84*, 1−10.

(18) Adinolfi, M.; Iadonisi, A.; Ravidà, A.; Schiattarella, M. *Tetrahedron Lett.* **2003**, *44*, 7863−7866.

(19) Pastore, A.; Valerio, S.; Adinolfi, M.; Iadonisi, A. *Chem.—Eur. J.* **2011**, *17*, 5881−5889.

(20) (a) Adinolfi, M.; Barone, G.; Iadonisi, A.; Schiattarella, M. *Synlett* **2002**, 269−270. (b) Tanaka, H.; Yoshizawa, A.; Takahashi, T. *Angew. Chem., Int. Ed.* **2007**, *46*, 2505−2507.

(21) (a) Yadav, J. S.; Reddy, S. B. V.; Premalatha, K.; Swamy, T. *Tetrahedron Lett.* **2005**, *46*, 2687−2690. (b) D'Alonzo, D.; Froeyen, M.; Schepers, G.; Di Fabio, G.; Van Aerschot, A.; Herdewijn, P.; Palumbo, G.; Guaragna, A. *J. Org. Chem.* **2015**, DOI: 10.1021/acs.joc.5b00406.

(22) Giordano, M.; Iadonisi, A. *Eur. J. Org. Chem.* **2013**, 125−131.

(23) An average cost of ca. 0.2 €/g has been estimated according to the main suppliers of chemicals (Mar 3, 2015).

(24) The structures of (2*R*,5*R*)-**5b** and (2*S*,5*S*)-**5b** were established by 1D and 2D NMR studies (see the Supporting Information) and confirmed by NMR and $[\alpha]_D$ comparison with literature data (ref 11).

(25) Jeong, L. S.; Shinazi, R. F.; Beach, J. W.; Kim, H. O.; Nampalli, S.; Shanmuganathan, K.; Alves, A. J.; McMillan, A.; Chu, C. K.; Mathis, R. *J. Med. Chem.* **1993**, *36*, 181−195.

(26) In this case, complete reaction mixture discoloration was observed, suggesting quantitative reduction of starting I_2.

(27) Barciszewski, J.; Markiewicz, W. T.; Adamska, E.; Plitta, B.; Giel-Pietraszuk, M.; Wyszko, E.; Markiewicz, M.; Fedoruk-Wyszomirska, A.; Kulinski, T.; Chmielewski, M. PCT Int. Appl. WO2011115513 A2, 2011.

(28) A considerable amount (28%) of a side product, which NMR signals were consistent with the structure of the regioisomeric N^3-nucleoside, was also formed.

(29) Nowak, I.; Conda-Sheridan, M.; Robins, M. J. *J. Org. Chem.* **2004**, *70*, 7455−7458.

DOI: 10.1021/acs.orglett.5b00982
Org. Lett. 2015, 17, 2626−2629