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Synthesis and Application of Fluorinated Carbohydrates and other Bioactive Compounds

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Erfolgsrezept

Ich will das Geheimnis verraten,
dass mich zum Ziel geführt hat.
Meine Stärke liegt einzig und allein
in meiner Beharrlichkeit.

LOUIS PASTEUR

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Die vorliegende Arbeit entstand im Zeitraum von Feburar 2005 bis Oktober 2009 am Institut für Organische Chemie der Universität Wien.

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SHORT SUMMARY

This PhD Thesis describes the synthesis and application of various fluorinated carbohydrate derivatives and the synthesis of a Diclofenac metabolite to investigate a potential immunologic mechanism underlying Diclofenac-hypersensitivity.

The increasing interest in fluorinated organic compounds is due to the fact that these derivatives not only exhibit challenging biological and physico-chemical properties, but also allow the application of high end NMR techniques as a result of the highly sensitive ^{19}F nucleus.

The development of a novel reporter system to study protein interactions via ^{19}F-NMR was established. This approach uses 2-^{19}F-labeled maltose as a spy ligand to indirectly probe protein-protein interactions of proteins fused or tagged to maltose binding protein. For that purpose, a variety of stereoselective fluorinated carbohydrate derivatives was synthesized. (Chapter A)

Synthetic efforts to 6-deoxy-6-fluoro-glucose and –galactose derivatives as potential substrates for enzymatic synthesis of disaccharides are described. (Chapter B)

Diclofenac (Voltaren™) is a member of nonsteroidal anti-inflammatory drugs (NSAIDs) and has been used for its antipyretic, analgetic and anti-inflammatory activities in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute muscle pain. Although this drug has proven to be highly effective and secure, various adverse drug reactions have been reported in the past, including hepatotoxicity. There are several postulations of an involvement of IgE, but mechanistic evidence is still not available.

To prove drug-specific IgE mediated reactions against the nonsteroidal anti-inflammatory drug Diclofenac, 5-Hydroxy-Diclofenac metabolites were synthesized. (Chapter C)

ZUSAMMENFASSUNG

Die vorliegende Doktorarbeit beschäftigt sich sowohl mit der Synthese und Anwendung von fluorierten Kohlenhydraten als auch mit der Herstellung von Diclofenac Metaboliten für mechanistische Untersuchungen in der Allergieforschung.

Das Interesse an fluorierten organischen Verbindungen ist in den letzten Jahren stark gestiegen. Diese Entwicklung wird einerseits auf die veränderten biologischen und physikalisch-chemischen Eigenschaften zurückgeführt, andererseits ermöglicht der hoch empfindliche ^{19}F Kern die Anwendung von speziellen NMR Techniken.

Zur Untersuchung von Proteinbindungen mittels ^{19}F-NMR wurde eine neue Strategie entwickelt. 2-^{19}F-markierte Maltose wird als Sonde genutzt, um Protein-Protein Wechselwirkungen zum „maltose binding protein“ (MBP) mittels ^{19}F-NMR zu verfolgen. Für diese Zwecke wurde eine Vielzahl von stereoselektiv fluorierten Kohlenhydraten hergestellt. (Chapter A)

Weiters wird die Herstellung von 6-Desoxy-6-fluor-glukose und –galaktose Derivaten als potentielle Substrate zur enzymatischen Herstellung von fluormarkierten Disacchariden beschrieben. (Chapter B)

Diclofenac (Voltaren™) gehört zu der Gruppe der nichtsteroidalen Antirheumatika und wird aufgrund der antipyretischen, analgetischen und entzündungshemmenden Wirkung zur Behandlung von Rheuma, Arthritis, Spondylitis ankylopoetica sowie bei leichten bis mittleren Schmerzen und Entzündungen eingesetzt. Obwohl Diclofenac schon seit vielen Jahren ein sehr beliebter und häufig angewandter Arzneistoff ist, gibt es immer wieder Meldungen über allergische Reaktionen, einschließlich Leberversagen. Trotz zahlreicher mechanistischer Vermutungen konnte ein IgE-basierender Mechanismus nie bewiesen werden.

Um mechanistische Studien in diese Richtung voranzutreiben, wurde ein 5-Hydroxy-Diclofenac Metabolit hergestellt. (Chapter C)

LIST OF ABBREVIATIONS

Ac	acetyl
Ac_2O	acetic acid anhydride
AcOH	acetic acid
b	broad
cat	catalytic
collidine	2,4,6-trimethylpyridine
CSA	chemical shift anisotropy
d	doublet
2D	two dimensional
DAST	diaminosulfur trifluoride
DCM	methylene chloride
DMAP	4-(dimethylamino)-pyridine
DMF	dimethylformamide
DMSO	dimethylsulfoxide
EE	ethyl acetate
eq (equiv.)	equivalent
Et	ethyl
MBP	maltose binding protein
Me	methyl
MW	microwave conditions
NaOMe	sodium methoxide
NIS	*N*-iodosuccinimide
Ph	phenyl
PE	petrol ether
ppm	parts per million
Pyr	pyridine
RT	room temperature
s	singlet
SET	single electron transfer
t	triplet
TBAF	tetrabutylammonium fluoride
TBDMS	*t*-butyldimethylsilan

TFA	trifluoroacetic acid
Tf	trifluoromethanesulfonyl (= triflate)
THF	tetrahydrofuran
TLC	thin layer chromatography
TsOH	toluenesulfonic acid

CHAPTER- A

SYNTHESIS AND APPLICATION OF FLUORINATED MALTOSE DERIVATIVES

ABSTRACT

The concept presented here relies on the development of an indirect ^{19}F-detected NMR reporter system with internal control possibilities for studying protein binding events.

The benefits of fluorine (^{19}F) NMR detection for ligand-based NMR screening applications have been convincingly demonstrated in the past. The usage of the fluorine NMR alleviates most of the problems encountered with ^{1}H observation, such as signal overlap and dynamic range problems. Additionally, the ^{19}F nucleus with 100% natural abundance and a magnetogyric ratio comparable to ^{1}H is highly sensitive and due to its large chemical shift anisotropy (CSA) very responsive to molecular weight changes accompanied by binding events.

A novel reporter system for ^{19}F-NMR investigation of protein interactions is presented. This approach uses 2-F-labeled maltose as spy ligand to indirectly probe protein-protein interactions of proteins fused or tagged to maltose binding protein. The key feature is the simultaneous NMR observation of both ^{19}F-NMR signals of gluco/manno-type-2-F-maltose-isomers; one isomer (α-gluco-type) senses the protein interaction and the non-binding isomers (β-gluco- and/or α/β manno-type) are utilized as internal references.

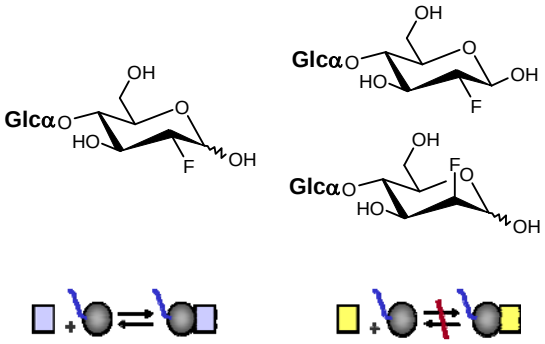


Figure 1. Reporter system for the binding studies using ^{19}F-NMR.

Moreover, this reporter system was used for relative affinity studies of fluorinated and non-fluorinated carbohydrates to the maltose binding protein.

Syntheses of various fluorinated maltose derivatives, observed DAST induced rearrangement and their application for NMR measurements will be presented.

THEORETICAL BACKGROUND

HISTORY OF FLUORINE

The discovery and first isolation of fluorine has a long history.[1] In 1530, Georgius Agricola described the use of fluorspar (CaF_2, fluorite) as a flux, which promotes the fusion of metals or minerals. In 1670, Schwanhard performed the first glass etching with acid treated fluorspar. In 1764, A. S. Marggraf carried out the first synthesis of hydrofluoric acid with fluorspar and sulfuric acid. In 1808, H. Davy postulated the existence of a new element and suggested the name *fluorine* from Latin *fluere* for *to flow*. Berzelius recommended the chemical symbol *F* in 1814. But the real breakthrough was made by Henri Moisson in 1886. He realized the first synthesis of the element fluorine by electrolysis of an HF-KF system. In 1906, Moissan was awarded the Nobel Prize in chemistry for this.

In 1930s, the first fluorinated organic compounds were used for industrial purpose: refrigerants (*Freon*), fire extinguishing chemicals (*Halon*) and aerosol propellants. In 1941, the beginning of the Manhattan project for the development of nuclear weapons required the first large-scale production of fluorine. On the one hand fluorine was used as uranium hexafluoride (UF_6) for uranium isotope separation via gas diffusion. Then again the application of very corrosive fluorine revealed the need of highly resistant materials. The development of electrochemical fluorination and fluorine containing polymers (PTFE, *teflon*) was expedited by that need. Only in the 1950s and 60s application of fluorine in other industrial sectors was applied (pharmaceutical and material science). Nowadays, the technical production of fluorine is still based on the original method of Moissan, the so-called middle-temperature procedure.

Figure 2. Moissan stamp: To honor the 100th Anniversary of the isolation of fluorine by Moissan, the French government issued a special postage stamp featuring a picture of Henri Moissan together with the reaction scheme. The stamp was quite spectacular, because the reverse reaction of what Moissan had accomplished was on top of it.[2]

APPEARANCE

Due to its high reactivity, the natural abundance of fluorine[1] is as minerals, e.g. fluorspar, fluoroapatite ($Ca_5(PO_4)_3F$), cryolith (Na_3AlF_6). Fluorine has several isotopes (^{17}F-^{21}F), but only the natural occurring stable isotope ^{19}F and the ^{18}F isotope with a half life of 109.77 minutes are relevant in chemistry. Despite of the relatively high abundance of fluorine in the lithosphere, natural fluorinated compounds are rare in nature. Of this very few natural products, most are

handled as xenobiotic to biological systems. An exception is fluoroacetic acid, the first isolated fluoro-organic compound (South African gifblaar shrub- *Dichapetalum cymosum*), which mimics acetic acid so perfectly that it can be infiltrated in the Krebs cycle, therefore this compound is highly toxic.[8]

FLUORINATED ORGANIC COMPOUNDS

Fluorine is the most electronegative of all elements, causing highly polar and very stable carbon-fluorine bonds. Its strongly localized electrons render very low polarizability and a relatively small size. The excellent match between the fluorine 2s and 2p orbitals with the corresponding orbitals of carbon are unique. Fluorine has a slightly larger size compared to the hydrogen atom (1.20 Å), but with a van der Waals radius of 1.47 Å, it occupies a smaller volume than a methyl, amino or hydroxyl group (1.52 Å). The short carbon-fluorine bond length (1.39 Å) is similar to the carbon-oxygen (1.43 Å) one. These key points are the reason why the replacement of some functional groups, including C-H, C-OH, C=O by fluorine are so attractive.

The introduction of fluorine into organic compounds is widely used in pharmaceutical and medicinal chemistry in the last 30 years. Fluorine substitution allows the simultaneous modulation of electronic, lipophilic and steric parameters. Physico-chemical properties such as metabolic stability, enzyme substrate recognition, bioavailability, basicity, hydrolytic stability can be fine tuned.[3,4]

Some of the challenging biological and physico-chemical properties[5,6]are shortly described above, using some examples of fluorine-containing pharmaceuticals.[7,8,9,10]

METABOLIC STABILITY[3]

One of the main parameters in many drug-discovery projects is the metabolic stability which determines the bioavailability of a drug. Lipophilic compounds have the tendency to be oxidized by cytochrome P450 or other enzymes in the liver. One strategy to counteract that problem is to make the molecule more polar, another one is to block the metabolic labile site with a fluorine substituent. An example for the increase of metabolic stability by fluorine is the cholesterol-absorption inhibitor *Ezetimibe*[11,12]. Two metabolic labile sites are substituted by fluorine to avoid oxidation of the phenyl ring to phenol.

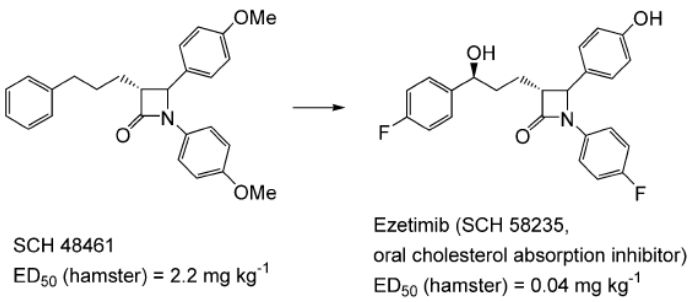


Figure 3. Optimization of metabolic properties of *Ezetimibe* by the incorporation of fluorine.[11,12]

PHYSICOCHEMICAL PROPERTIES[3]

Due to its very high electronegativity, fluorine causes extremely strong effects on the basicity and acidity of nearby functional groups. Shifts in the pK_a of several log units can be observed, depending on the fluorine position relative to the basic or acidic group, shown in Figure 4. The introduction of fluorine significantly lowers the pK_a of such compounds and the reduced basicity affects quite often the affinity to receptors, which affect oral absorption. Since strongly basic groups are often required for receptor binding, the real challenge is to find an optimum between good pharmacokinetic properties of a molecule and its binding affinities.

pK_a		**pK_a of protonated amines**	
CH_3COOH	4.76	$CH_3CH_2NH_2$	10.7
CH_2FCOOH	2.59	$CH_2FCH_2NH_2$	8.98
CHF_2COOH	1.24	$CHF_2CH_2NH_2$	7.52
CF_3COOH	0.23	$CF_3CH_2NH_2$	5.7

Figure 4. Examples of pk_a's of acetic acid and their fluorinated derivatives as well as the basicity of ethylamine and its fluorinated analogs.[13,14]

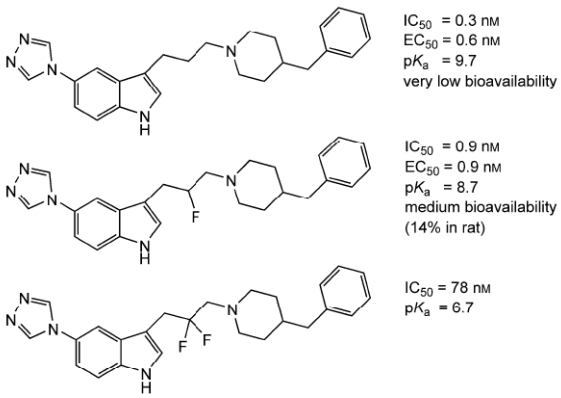


Figure 5. Effects of pK_a values on the bioavailability and receptor binding of a $5HT_{1D}$ agonist.[3,15]

Another important parameter in medicinal chemistry is the need for groups of substantial lipophilicity to gain good binding affinities to proteins.[16] But a high lipophilicity is often accompanied by a reduced solubility. Again, the right balance between these two conflicting effects is important.

MOLECULAR CONFORMATION[3]

The insertion of fluorine induces changes in the preferred molecular conformation. Again, size and electronegativity of fluorine are crucial. The volume of a trifluoromethyl group compared with a methyl group is roughly twice as high. Spectroscopic studies and high-level quantum-mechanical calculations demonstrate that the exchange of a methoxy for a trifluoromethyl group

in a compound is not simple isosteric replacement, because they adopt different conformations. The R-group in R-CF_2O-Ph will point in a different direction from that of the R-group in R-CH_2O-Ph.

An example illustrating this point is a cholesteryl ester transfer protein (CETP) including a 3-tetrafluoroethoxy substituent. Massa et al.[17] implies that the steric and electronic properties of Ph-OCF_2CF_2H are very similar to 2-phenyl-furan, which is non-planar. This is quite interesting for a medicinal chemist, because monosubstituted furan is usually considered to be an unfavorable group due to its metabolic instability and its potential to produce reactive metabolites. The OCF_2CF_2H side chain is therefore a promising route forward to transferring a biologically active furane into a more stable group.

PROTEIN-LIGAND INTERACTIONS[3]

The binding affinity in protein-ligand complexes can be significant influenced by fluorine. This effect can be direct by interaction of the fluorine with the protein, or it can be indirect by modulation of the polarity of other groups of the ligand that interacts with the protein. Prevalently, a fluorine substituent causes a slight enhancement of the binding affinity due to an increased lipophilicity of the molecule. This results in an increased non-specific affinity to the protein. The probably strongest indirect effect of fluorine on binding affinity is the change of basicity or acidity of the ligand molecule. In the before mentioned example of $5HT_{1D}$ agonist[15] (Figure 5), the difluoro compound is no longer basic enough to achieve high binding affinities for the $5HT_{1D}$ receptor.

Polar interactions can play an important role in protein-ligand interactions and can cause significant increased binding affinities. In the case of fluorine substituted thrombin inhibitors the mono-fluorinated compound binds five times stronger to thrombin than the non-fluorinated compound. Its binding mode was determined by X-ray structure analysis and demonstrates that the fluorine atom is in extremely close contact with the H-C_α-C=O moiety of Asn98 of thrombin. Such fragments offer several promising polar interactions with fluorine, therefore they can be considered as fluorophilic.

Does fluorine form hydrogen bonds? This question is part of a considerable debate.[18,19,20] The number of found cases, in which fluorine atoms engage in a nonbonding interaction which can be legitimately called hydrogen bonds, is very small. In most instances, the interactions of carbon-fluorine units are better described as weak polar interactions. An intensive search of the Cambridge Crystallographic

Structure Database (CSD), including detailed inspection of individual crystal structures and backed by ab initio calculations on model systems, confirmed that organic fluorine hardly ever accepts hydrogen bonds, unless in the absence of a better acceptor. With its low polarizability and tightly localized lone pairs, fluorine is incapable to compete with stronger hydrogen-bond acceptors such as oxygen or nitrogen.

INTRODUCTION OF FLUORINE INTO ORGANIC COMPOUNDS

In the first experiments several organic substrates were treated with highly reactive fluorine gas.[1] All these experiments, either at room temperature or cooled with liquid nitrogen, had something in common: they resulted in explosions and no major product could be isolated. Plausible first explanations were on basis of thermo-chemical considerations:

- By the formation of the highly stable carbon-fluorine (116 kcal/mol) bond the energy release is considerably greater than the energy needed for the dissociation of carbon-carbon (83 kcal/mol) or carbon-hydrogen (99 kcal/mol) bonds.
- Initiation of uncontrolled radical chain reactions by the extremely low homolytic dissociation energy of elemental fluorine (37 kcal/mol) even at low temperatures and in the absence of light.

Various approaches to handle fluorine gas and control the immense reaction enthalpy have been made in the last 80 years. The prevalent methods of the present status[21,22] will be discussed on the following pages with a main focus on used reagents.

DIRECT FLUORINATION[1]

On lab scale the application of elemental fluorine is rare, even the initiation of radical chain reactions can be controlled by appropriate choice of solvent, because highly safety precautions due to the highly toxic gas (LC_{50} 185 ppm) have to be provided. Solvent systems like $CFCl_3$/$CHCl_3$, sometimes with additional 10% ethanol, serve as effective radical scavengers. Reaction enthalpy can be handled by dilution of fluorine gas with nitrogen or helium and by the usage of low temperature.

One of the first projects of selective direct fluorination as industrial applications was the production of the cytostatic 5-fluorouracil.[23] In the most common process the uracil precursor is treated with nitrogen-diluted fluorine in hot water and the intermediate fluorohydrin is subsequently dehydrated with sulfuric acid or by heating the aqueous solution to 100°C.

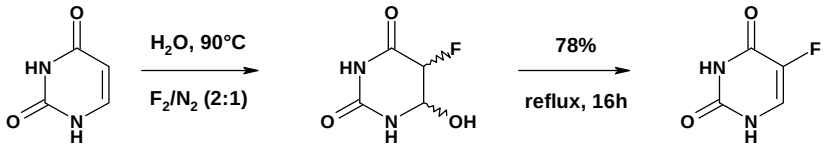


Figure 6. Direct fluorination process for industrial-scale production of 5-fluorouracil.

During the last few years, there have been great advances in the selective direct fluorination, even of sensitive organic compounds.[24] Especially, the developed methods of R.D. Chambers and coworkers are noteworthy because they are fulfilling the requirements of robust and reproducible industrial processes.

For modern fluorination procedures on lab scale a variety of specialized fluorination technologies and reagents were developed. Preferably they are divided into two main categories: electrophilic insertion of fluorine or nucleophilic attack. A short overview of possible strategies is resumed herein.[25]

NUCLEOPHILIC FLUORINATION

Fluoride can act as an extremely poor nucleophile in protic solvents (solvatisation) but really powerful in polar aprotic solvents (formation of tight ion pairs), especially with large sterically demanding lipophilic cations (delocalization of the positive charge by reduction of ion pairing). One such popular example of "naked" fluorine is tetrabutyl ammonium fluoride (TBAF). To overcome problems such as poor solubility, substitution versus elimination, lowering of toxicity and price, as well as increasing stability a variety of fluorinating agents were developed over the time, such as Olah's reagent, diethylaminosulfur trifluoride (DAST), TBAF, KF, CsF and others.

- ***HF reagents***: Hydrogen fluoride itself is a rather ineffective and aggressive fluorinating agent. With the application of HF-amine complexes not only the corrosive and reactive nature of HF can be tamed, but also the nucleophilicity can be reduced as well as an activation of substrate can be required, e.g. pyridinium poly(hydrogen fluoride), PPHF, Olah's reagent[26].

- ***Inorganic fluorides***: Especially alkali metal fluorides have been used to substitute fluorine for other halogens in a variety of compounds such as aromatic halides, alkyl halides, α-halo esters, nitriles and amides, as well as ω–halo alcohols, esters and nitriles. The main driving force in these reactions is the formation of the thermodynamically favorable carbon-fluorine bond (116 kcal/mol). These fluorinations are often carried out in high boiling solvents which aid solubility of the ionic fluorides or in anhydrous solvents. Under these conditions, the unsolvated fluoride ion, also called "naked" ion is formed. Crown ethers have also been used to solvate inorganic fluorides (KF, CsF) by complexation, thus enhancing the reactions rate. Solvation of metal cations is not just limited to crown ethers but can be extended to using donor solvents such as glymes or glycols.

- ***Sulfur fluorides***:[27] An increase of nucleophilicity could be also achieved by pairing the hard Lewis base (fluoride) with a soft Lewis acid. Various sulfur-fluoride reagents were developed starting from the SF_4. But despite its versatility, SF_4 is a highly toxic gas and must be handled under reduced pressure in an autoclave. In order to simplify the handling, less volatile analogs have been developed by exchanging one fluorine by a dialkylamino group, well known as DAST reagent. The sulfur-nitrogen bond is relatively instable, therefore the reagent can explode violently when heated over 90°C. Third generations reagents for larger scale are morpholino sulfurtrifluoride (MOST) and the methoxyethyl derivative (BAST, Deoxofluor). Deoxofluor also decomposes at elevated temperature, but it does so without any thermal run-away reactions and subsequent explosions.

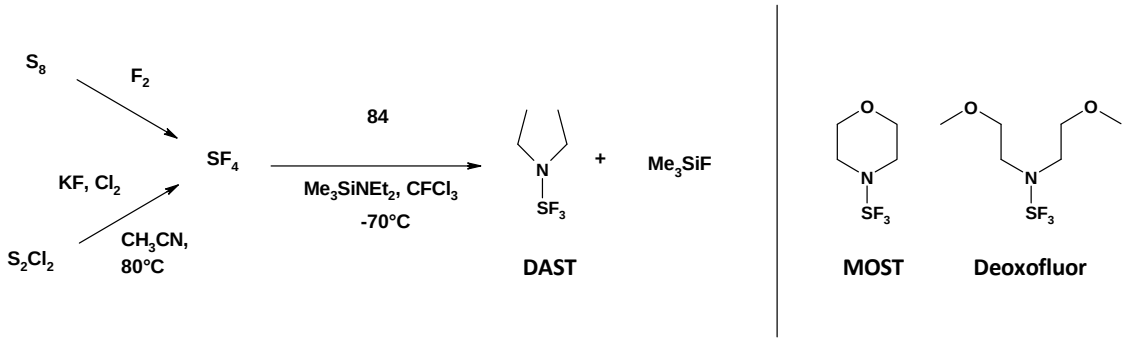


Figure 7. Synthesis of DAST and its derivatives.[28]

ELECTROPHILIC FLUORINATION[29]

For the fluorination of electron-rich centers, especially direct conversion of carbon-hydrogen to carbon-fluorine linkages, a positive fluorine source is needed. For a long time, elemental fluorine was the sole source of electrophilic fluorination and is still used in pharmaceutical chemistry. But the danger and problems of fluorine gas made the development of a set of easily and safely applicable electrophilic reagents essential.

The ability of fluorine to behave as an electrophil is not easy to accomplish since it is the most electronegative element known. By withdrawing electronic charge from fluorine through inductive effects or by the presence of a good leaving group next to fluorine or by a combination of both, this problem could be circumvented. The first reagent for electrohilic fluorination was CF_3COOF, followed by others such as perchloryl fluoride ($FClO_3$), xenon difluoride (XeF_2), nitrogen oxide fluorides and several other hypofluorides. Although these reagents are safer and better to handle than elemental fluorine, many of them are strong oxidizing agents and can cause side products. Major progress in this field came by the implementation of N-F reagents. They are prepared from relatively inexpensive starting materials by reacting the corresponding N-H compound with F_2. Umemoto et al. developed the first reagents including *N*-fluoropyridinium triflate and its derivatives, followed by *N*-fluoro-*N*-alkylsulfonimides, *N*-fluoroperfluoroalkylsulfonamides and *N*-fluorobenzenesulfonimide (NFSI).

- **Organo-fluoroxy reagents**: An early example of electrophilic fluorinations through nucleophilic attack on fluorine with displacement of a highly electronegative leaving group, is the fluoroxytrifluoromethane, developed by Barton[30]. Acetyl hypofluorite[31] is used since the 1980's for a variety of fluorination reactions, followed by many others.

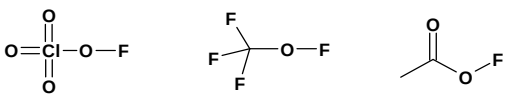


Figure 8. Examples of electrophilic organo-fluoroxy reagents.

- **N-F reagents**: Nitrogen has not only a lower electronegativity as oxygen but also the bond strength of nitrogen-fluorine is higher compared to oxygen-fluorine, decreasing the electrophilicity of such reagents and making them rather stable and convenient to handle.

  N-FLUOROPYRIDINIUM TRIFLATES: Umemoto et al.[32,33,34] extensively explored the use of these salts as fluorinating agents. They discovered that the non-nucleophilic counterions were essential for the stability and triflates was particularly benefiting. The electrophilic power increases with decreasing electron density of the N^+-F site, modifiable by varying the ring substituents. Thereby reactivity and selectivity can be fine tuned.

  SELECTFLUOR AND ITS DERIVATIVES:[36] F-TEDA-BF_4 or also called Selectfluor was developed by Banks[35] and coworkers as part of their research on N-F chemistry. It is a very stable, user-friendly and versatile fluorinating agent. The fluorinating power can be modulated by variation of the electron-withdrawing alkyl side chain as well as the counterion. Selectfluor is the most important and commercially available derivative.

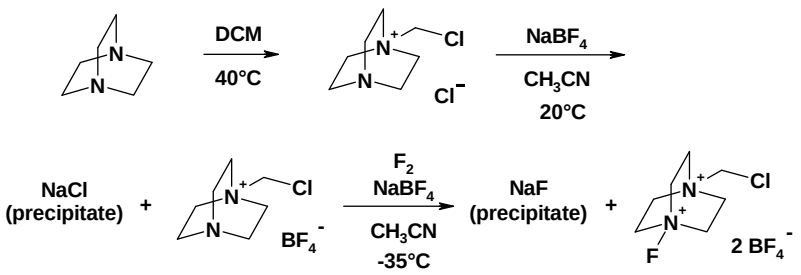


Figure 9. Synthesis of Selectfluor.[36,37]

SULFONYL DERIVATIVES $RSO_2N(F)R'$: In 1984 Barnette[38] showed that *N*-alkyl-*N*-fluorosulfonamides can be used as versatile and effective fluorinating agents. They are easily to prepare by treatment of the precursor amide with one equivalent of 1-5% fluorine in nitrogen. DesMarteau[39] reported in 1987 the synthesis and application of *N*-fluoro-trifluoromethylsulfonimide, the most powerful electrophilic fluorinating agents known. In the 1990's, Davis and Han[40] developed *N*-fluoro-*o*-benzenedisulfonimide (NFOBS) and *N*-fluorobenzenesulfonimide (NFSI) was established by Differding et al[41].

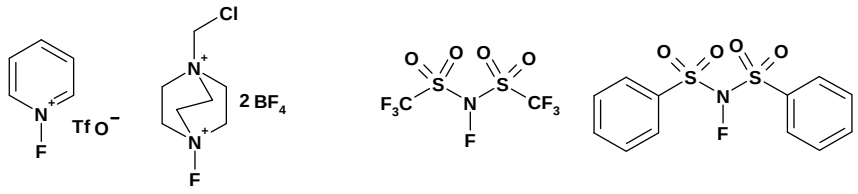


Figure 10. Examples of N-F reagents for electrophilic fluorination.

NUCLEAR MAGNETIC RESONANCE

Nuclear magnetic resonance (NMR) is the most important technique to analyze and elucidate the structure and stereochemistry in organic chemistry.

In the past decade significant advances in NMR spectroscopy could be witnessed. Based on tremendous gains in sensitivity due to high-field spectrometers and cryogenic probe technologies as well as the introduction of new correlation and higher-dimensional methods[42], unprecedented structural and functional information could be obtained on biological important systems, especially as a powerful tool for studying protein-protein interactions.[43,44]

To overcome the well known and inherent problem of molecular weight limitation of current NMR spectroscopy, which renders direct observation of the interaction partners unfeasible, an indirect observation technique for the detection of protein interactions has been recently established.[45] It utilizes the relaxation properties of a small molecular weight reporter ligand which reversibly binds to a ligand binding domain which in turn is fused to the interacting protein of interest. Subsequent protein-protein interaction leads to an additional increase of the molecular weight and can efficiently be probed by following NMR relaxation changes of the ligand (e.g. selective T_1 or T_2 which reflect the effective molecular weight). Due to this indirect detection scheme no isotope labeling of the protein interaction partners is required and consumption of protein material is reduced.

FLUORINE NMR

Analysis of fluorinated organic compounds by ^{19}F-NMR is an invaluable method to determine the structure. Depending on the chemical environment, the ^{19}F resonances of fluorinated organic and inorganic compounds cover a huge range of up to 900 ppm.

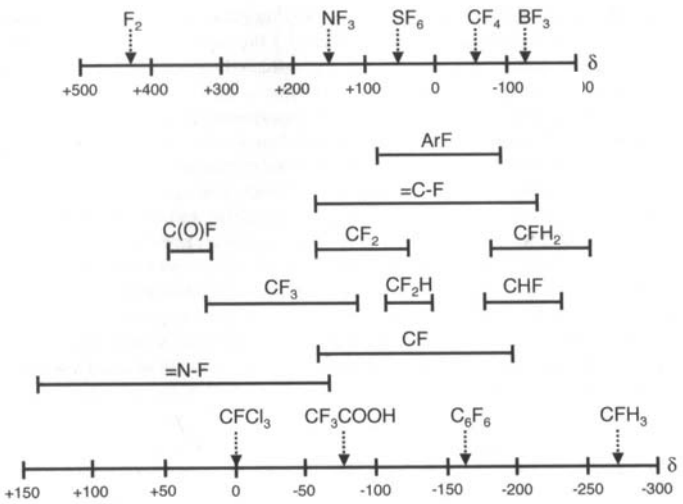


Figure 11. ^{19}F chemical shifts for different fluorinated chemicals and fragments.[1]

The natural occurring stable isotope ^{19}F has a nuclear spin of ½, a sensitivity of 0.83 relative to ^{1}H nucleus and a high gyromagnetic ratio (γ) of 40.05 MHz/T. Due to their close γ-values, ^{19}F-NMR can be measured with most ^{1}H-NMR instruments by tuning the RF coils appropriately. $CFCl_3$ is typically used as reference standard, next to CF_3COOH, C_6F_6 and others.

nucleus	spin	γ [10^7 rad T^{-1} s^{-1}]	$\gamma_{rel.}$	$S_{abs.}$	natural abundance [%]
^{1}H	½	26.75	1	1	99.99
^{19}F	**½**	**25.18**	**1**	**0.83**	**100**
^{13}C	½	6.73	0.25	0.000176	1.07
^{15}N	½	-2.71	0.1	0.00000385	0.37
^{31}P	½	10.84	0.41	0.0663	100

Figure 12. Overview of spin characteristics of the most important NMR-active nuclei.

Due to the characteristics of the ^{19}F nucleus, this technique is now finding increasing applications in biological chemistry, especially to study aspects in chemical biology. Due to the high sensitivity of the fluorine nucleus, the detection of µM concentrations is possible for analysis at physiological concentrations. Analyses are possible without the need of purification, because the resonances of fluorinated organic compounds do not overlap with those of ^{13}C and ^{1}H. The relatively large chemical shifts resulting from only minor changes in the chemical environment, lead to little or no peak overlap. As further advantage can be mentioned that dynamic range problems do not occur in aqueous solution (e.g. intense buffer and solvent peak).

This is why fluorinated organic compounds and consequently ^{19}F-NMR have very important roles in research at the chemical-biological interface. They are widely used as probes to explore enzyme mechanisms, metabolic pathways, biomolecular interactions and structural analysis of macromolecules.[46,47,48,49,50,51]

GRAPHICAL ABSTRACT

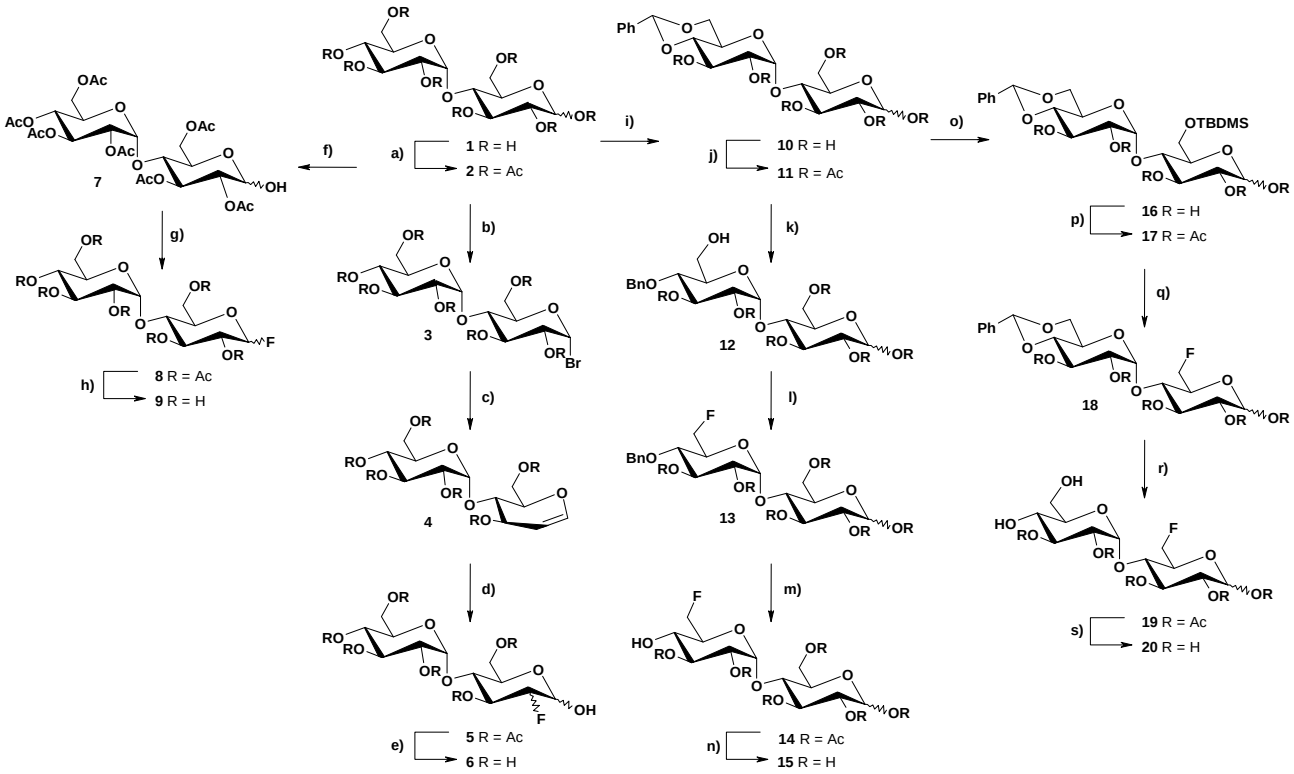


Figure 13. (a) Ac_2O, Pyr, 97%; (b) HBr, AcOH, 99%; (c) Zn, *N*-methylimidazol, ethyl acetate, 74%; (d) Selectfluor, CH_3NO_2, 40%; (e) NaOMe, MeOH, 99%; (f) NH_2NH_2*HOAc, DMF, 94%; (g) DAST, CH_2Cl_2, 89%; (h) NaOMe, MeOH, 99%; (i) α,α-dimethoxytoluene, *p*-TsOH, DMF, 79%; (j) Ac_2O, Pyr, 93%; (k) BH_3*THF, Bu_2BOTf, THF 56%; (l) microwave reaction, DAST, CH_2Cl_2, 79%; (m) Pd/C, H_2, ethylacetate, 64%; (n) NaOMe, MeOH, 75%; (o) TBDMS-Cl, imidazol, DMF, 43%; (p) Ac_2O, Pyr, quant.; (q) Deoxofluor, CH_2Cl_2, 17%; (r) conc. AcOH, 73%; (s) NaOMe, MeOH, 40%.

STRATEGY FOR THE 'GALACTO-TYPE'-6-F-MALTOSE DERIVATIVE

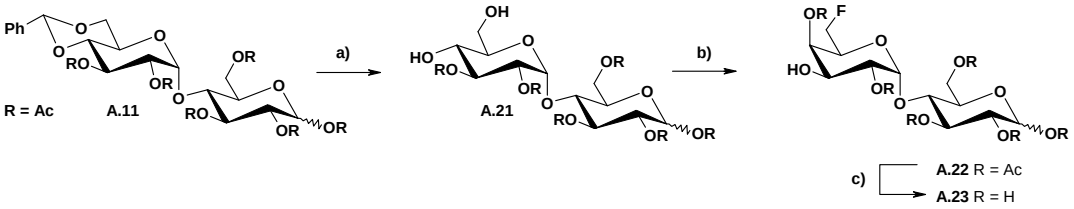


Figure 14. (a) conc. AcOH, 76%; (b) DAST, collidine, CH_2Cl_2, 30%; (c) NaOMe; MeOH, quant.

RESULTS AND DISCUSSION

The concept of this part of my PhD relies on the development of an indirect ^{19}F-detected NMR reporter system with internal control possibilities for studying protein binding events. The benefits of fluorine (^{19}F) NMR detection for ligand-based NMR screening applications as well as for ^{19}F magnetic resonance imaging (MRI) have been convincingly demonstrated in the past.[52,53,54,55,56,57,58] The usage of the fluorine NMR alleviates most of the problems encountered with ^{1}H observation, such as signal overlap and dynamic range problems. Additionally, the ^{19}F nucleus with 100% natural abundance and a magnetogyric ratio comparable to ^{1}H is highly sensitive and due to its large chemical shift anisotropy (CSA) very responsive to molecular weight changes accompanied by binding events.

Thus ^{19}F detection can be anticipated to be a general and versatile probe for indirect NMR studies of protein binding and interaction events. Biological systems often require sophisticated buffer systems for stabilization and solubility, thus leading to severe spectral overlap and dynamic range problems (e.g. intense buffer and solvent peaks). These drawbacks are particular present in the case of membrane-bound (or attached) proteins where additional peaks originating from membrane lipids raise severe technical problems. However, indirect detection techniques should always be cross-checked with reference experiments to demonstrate selectivity of binding and to exclude systematic errors (e.g. unspecific binding/aggregation and/or viscosity changes due to increased protein concentration). Ideally, the system of choice would thus be a mixture of reporter ligands consisting of one ^{19}F-labeled reporter ligand and another chemically similar (also ^{19}F-labeled) reference compound lacking the affinity to the ligand binding domain.

The rationale for choosing maltose lies in the fact that maltodextrin/maltose-binding protein (MBP) is a generally applicable protein fusion tag with beneficial solution properties and is therefore widely used in molecular biology.[59,60]

MALTOSE BINDING PROTEIN

MBP belongs to the family of periplasmic binding proteins which are involved in active transport processes of small molecules into gram negative bacteria as initial high-affinity binding components; furthermore these proteins participate as sensors for signaling through chemotaxis.[61] MBP binds maltodextrin and linear oligosaccharides of up to eight α(1-4)-linked glucose (Glc) units with micromolar affinities.[62,63] X-ray structural data (PDB ID codes 1DMB and 1ANF) demonstrated that the MBP (370 residues, Mr = 41 kDa) consists of two globular domains joined by a hinge-bending region, where the ligand-binding site is located in a cleft between the two domains. MBP exists in two different conformations: the ligand-free “open” form, exposing the binding site and, in the presence of a ligand, the “closed” form, trapping the ligand to provide contacts from both domains.[64,65] The number of protein-sugar hydrogen bonds associated with maltose and MBP is 12 excluding those with water and between glucose units. The reducing glucose unit (g_1) makes about twice as many direct hydrogen bonds with MBP as the non-reducing glucose unit (g_2) (Figure 15). But there is some evidence for the importance of van der Waals interaction and aromatic residue stacking for the oligosaccharide binding, too.[66,67,68]

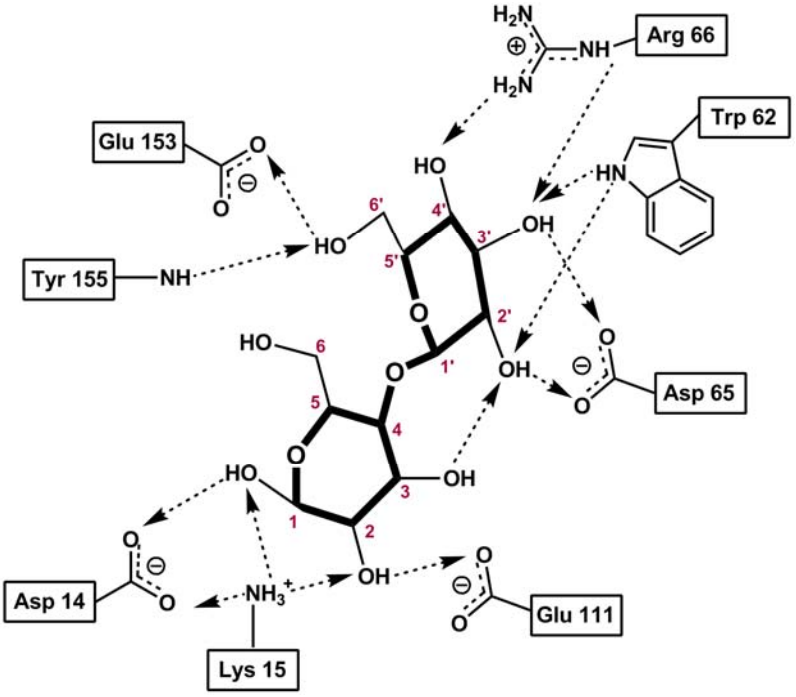


Figure 15. Schematic diagram of the hydrogen bonds between MBP and maltose; hydrogen bonds are shown as dashed lines.

Specifically, the 2-OH and the 2'-OH moieties are involved in an intricate hydrogen bonding network including the carboxyl group of Glu111 and Asp65 and the amino group of Lys15 and Trp62, respectively. We thus decided to synthesize 2-^{19}F-labeled maltose. By replacing the OH group by fluorine and modifying the stereochemistry at position 2, different binding affinities of the anomeric mixture of the two resulting diastereomers were expected (Figure 16). The gluco-type 2-F-maltose in which the fluorine atom occupies the equatorial position of g_1 of maltose should display comparable binding affinities as maltose itself, whereas the manno-type 2-F-maltose was expected to lose its affinity due to the axial orientation.

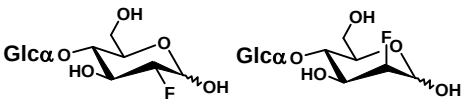


Figure 16. 2-^{19}F-maltose reporter system: non-stereoselective fluorine labeling at the 2-position of maltose leads to a 2/1 mixture of two epimeric forms [left: gluco-type; right: manno-type]. Only the gluco-type isomer of 2-deoxy-2-fluoro-maltose retains the affinity to maltose binding protein (MBP).

SYNTHESES OF THE REPORTER SYSTEM

The synthesis of the 2-F-maltose reporter system was performed following a modified protocol developed by Dax et al.[73] Starting from maltose **A.1**, disaccharide α-bromide **A.3** was obtained in excellent yield by standard acetylation procedure and subsequent treatment with hydrobromic acid in glacial acetic acid.[69] Treatment of bromide **A.3** with Zn and *N*-methyl imidazol[70] afforded the protected maltal derivative **A.4**, which was transformed to the target compounds utilizing Selectfluor® as fluorinating agent[71] in a nitromethane solution.[72,73] Final deprotection with sodium methoxide yielded the deprotected fluoro- derivatives **A.6**.

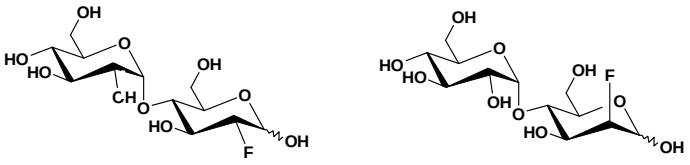


Figure 17. 2-F maltose mixture **A.6** (reporter system).

By variation of solvent and protecting group the sterioselectivity of the fluorination reaction could be influenced. The results of these anomeric mixtures of 2-fluoro maltose derivatives **A.5** with gluco- and manno- type stereochemistry are summarized in the following table. (Figure 18). With the peracetylated maltal **A.4** best results could be obtained using nitromethane. Promising gluco- to manno ratios were achieved using the steric demanding pivaloyl protecting group, but the equatorial product could never be isolated exclusively.

Protecting group	Solvent	Glc/ Man	Glc [α/ β]	Man [α/ β]
acetyl	**CH_3NO_2**	**2/ 1**	**3/ 1**	**12/ 1**
acetyl	CH_3NO_2/H_2O = 5/1	2/ 1	2/ 1	12/ 1
acetyl	acetone	1/ 2	2/ 1	13/ 1
acetyl	acetone/H_2O = 5/1	1/ 1	2/ 1	10/ 1
acetyl	[MMIM][$MeSO_4$]	1/ 1	30/ 1	10/ 1
pivaloyl	**CH_3NO_2**	**5/ 1**	**3/ 1**	**1/ 0.1**
pivaloyl	CH_3NO_2/H_2O = 5/1	4/ 1	3/ 1	50/ 1

Figure 18. Reactions conditions for the fluorination with Selectfluor; Abbreviations are: **Glc** for gluco-type maltose; **Man** for manno-type maltose.

NMR spectra of the peracetylated 2-F-maltose mixture **A.5** are shown in the following figures. (Figure 24) Assignments of the signals were achieved using 2D NMR techniques and are summarized in the HSQC spectra. (Figure 21 to Figure 23)

MECHANISM OF THE SELECTFLUOR REACTION

Two possible mechanistic pathways are proposed for fluorination with Selectfluor: single electron transfer (SET) and nucleophilic S_N2 substitution. Both strategies lead to the same 2-fluoro-oxo-carbenium ion. (Figure 19) This highly reactive intermediate reacts immediately with either the tertiary amine funcionality of Selectfluor or any other nucleophile available (e.g. water). (Figure 20)

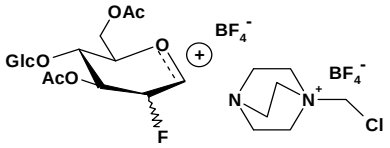


Figure 19. 2-Fluoro-oxo-carbenium intermediate.

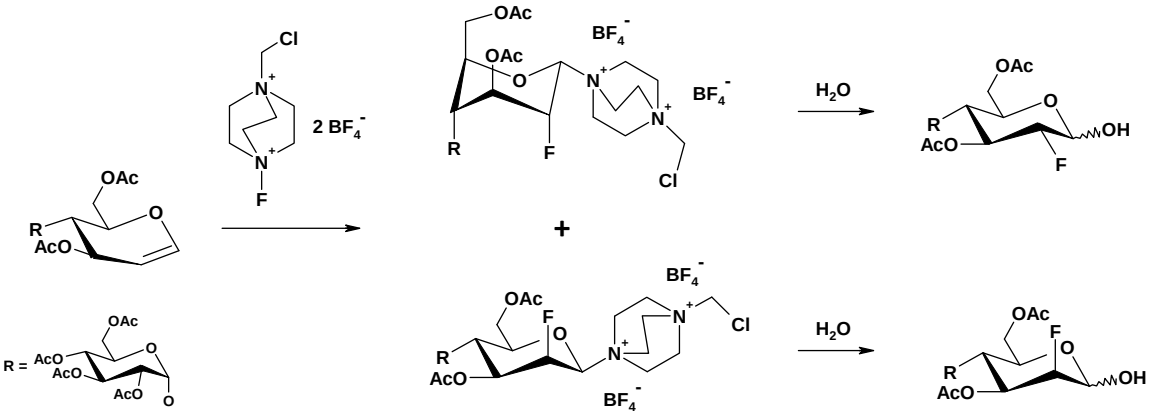


Figure 20. Proposed products of the syn-addition using Selectfluor.

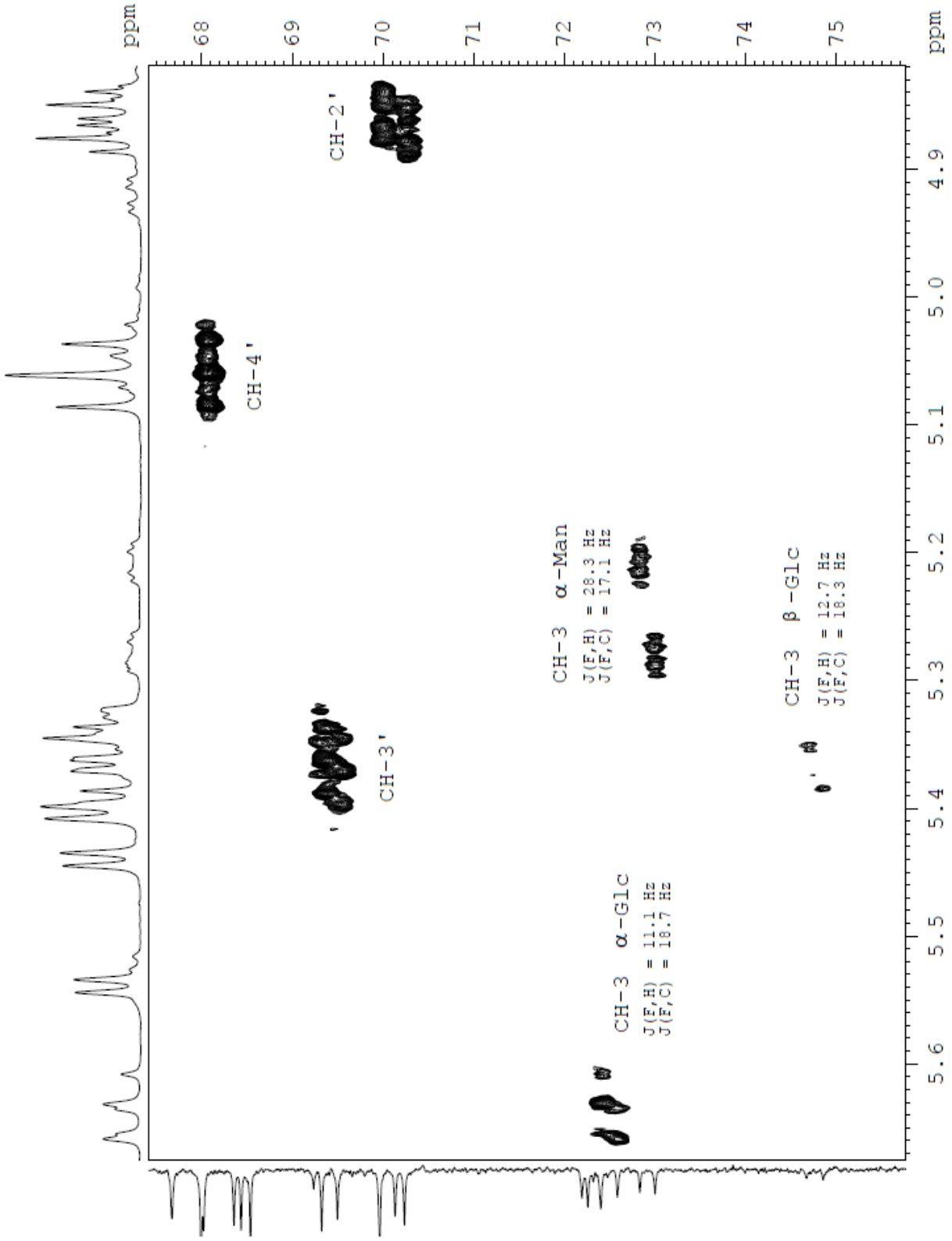


Figure 21. Assignments of the peracetylated 2-F-maltose mixture **A.5** (part 1).

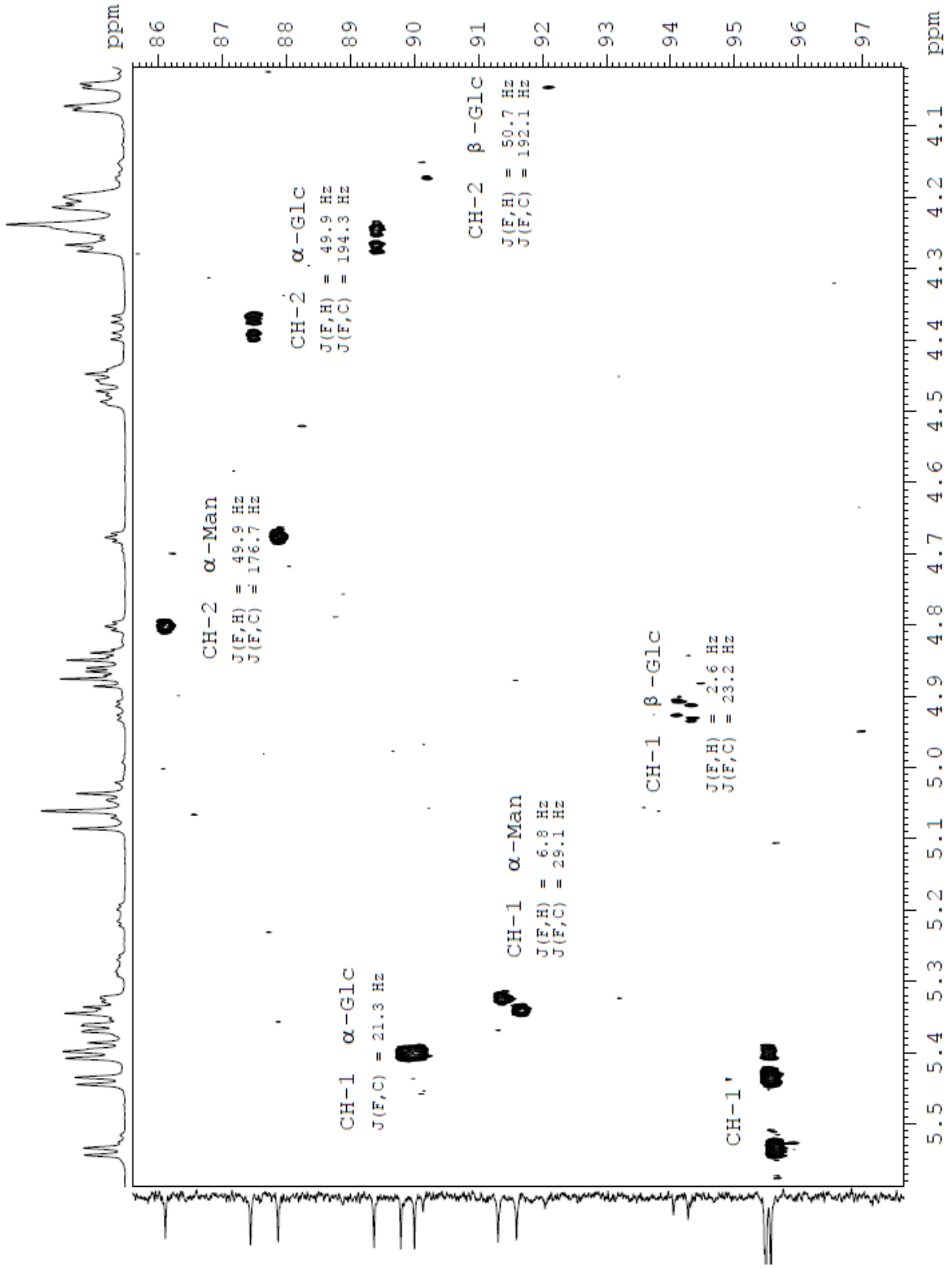


Figure 22. Assignments of the peracetylated 2-F-maltose mixture **A.5** (part 2).

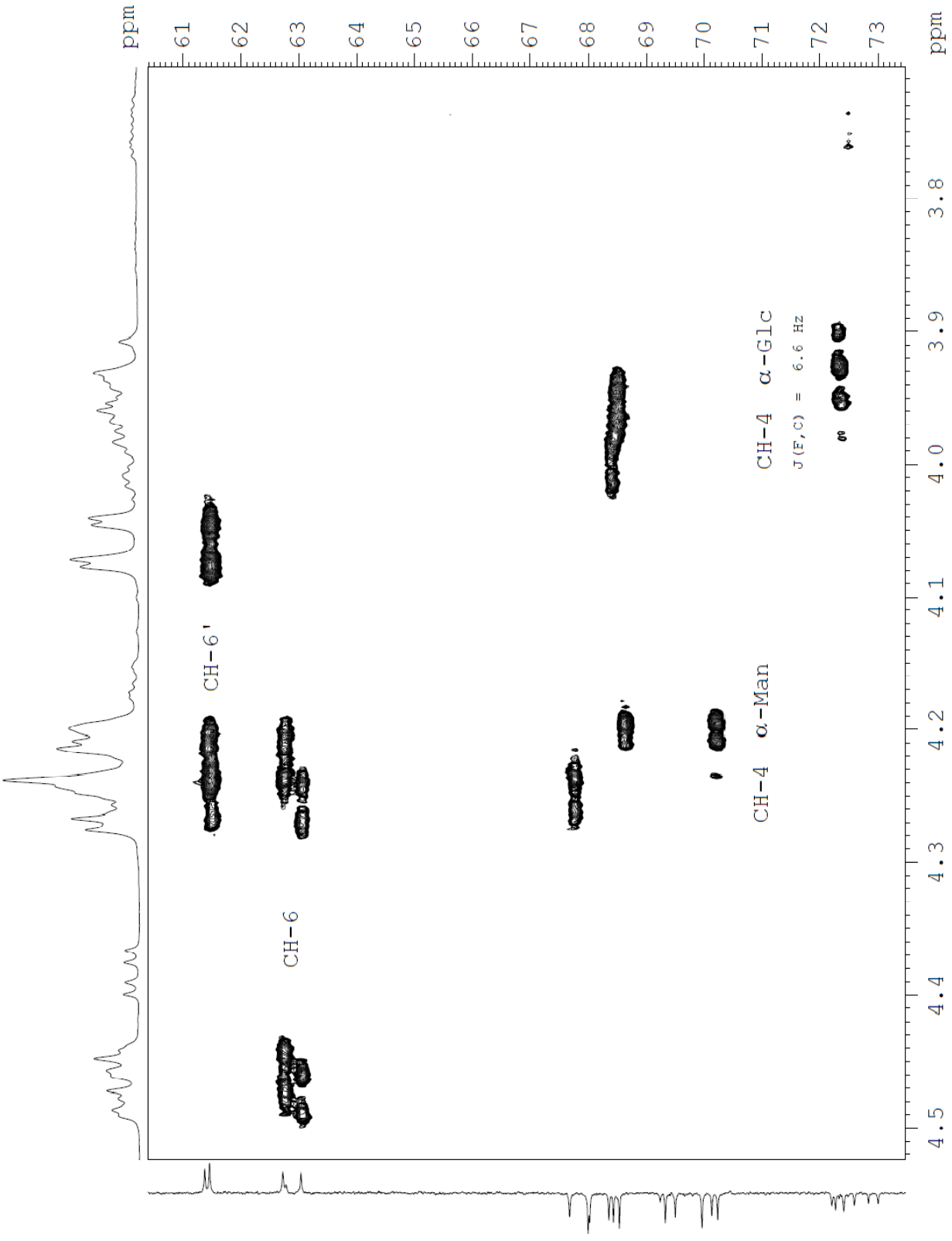


Figure 23. Assignments of the peracetylated 2-F-maltose mixture **A.5** (part 3).

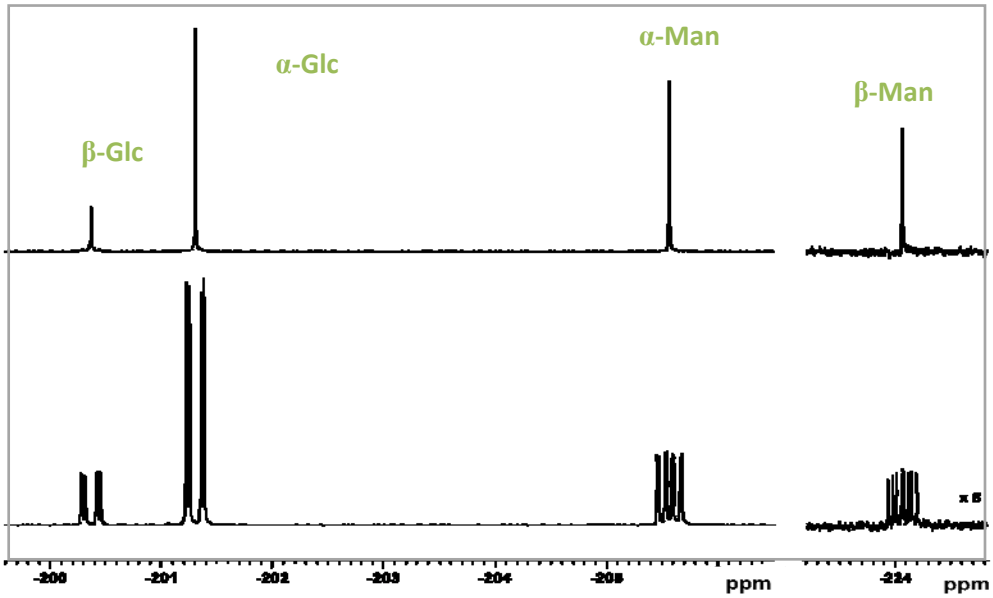
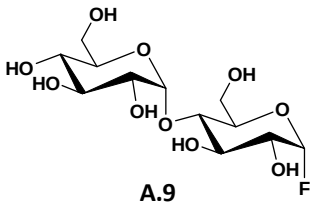


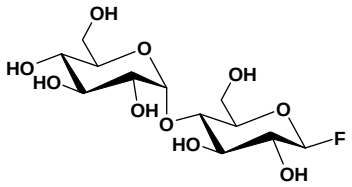
Figure 24. Typical 1D ^{19}F-NMR spectra (uncoupled and ^{1}H coupled) of the peracetylated 2-F-maltose mixture **A.5**. The ^{19}F resonances for the different compounds are as follows: gluco: α (-201.37 ppm), β (-200.43 ppm); manno: α (-205.67 ppm), β (-224.17 ppm).

SYNTHESIS OF FLUORINATED MALTOSE DERIVATIVES

Maltosyl fluoride **A.9** was obtained by deprotection of the anomeric acetyl group of compound **A.2** with hydrazine acetate[74] yielding derivative **A.7**, followed by nucleophilic fluorination with DAST[75,76] generating the anomeric mixture **A.8**. The α-anomer could be isolated by HPLC and subsequent Zemplén saponification of the remaining acetate protecting groups yielded the α-maltosyl fluoride **A.9**.

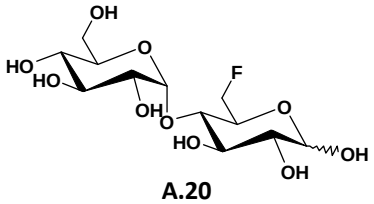


A.9



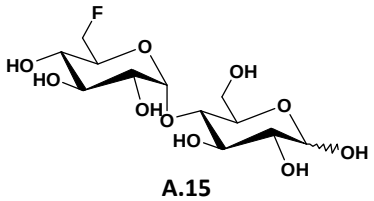
However, the β-maltosyl fluoride turned out to be rather unstable. Decomposition of the unprotected fluorinated sugar to maltose and hydrofluoric acid started immediately in D_2O-solution. Therefore only the α-maltosyl fluoride was used for the binding studies.

Starting from 4',6'-benzylidene maltose[77] **A.10**, the primary alcohol was protected as *t*-butyldimethylsilyl ether followed by standard peracetylation. Treatment of the silyl protecting group with an excess of Deoxofluor[78] yielded the 6-F-maltose derivatives **A.18**. Final deprotection with acetic acid[77,79] and sodium methoxide respectively yielded compound **A.20**.



A.20

The regioselective reductive ring opening of benzylidene acetals in the maltose derivative **A.11** was performed with a complex of BH_3/Bu_2BOTf at -70°C.[80,81] Fluorination with DAST[82,83] was performed in a sealed tube for 1 hour at 80°C under microwave conditions. The deprotection of the benzyl group was achieved with Pd/C[84], followed by a Zemplèn saponification to obtain product **A.15**.



A.15

The synthesis of the galacto-type derivative **A.23** started from peracetylated benzylidene maltose **A.11**.[77] Deprotection[77] with acetic acid followed by microwave fluorination with DAST[82] yielded a mixture of fluorinated disaccharides including the estimated 6'-fluoro-maltose, the 4',6'-difluoro maltose and the 6'-F-'galacto'-maltose derivatives. This crude product mixture could be separated by column chromatography to yield the desired product **A.22**[79] in 30% yield followed by Zemplèn deprotection to derivative **A.23**.

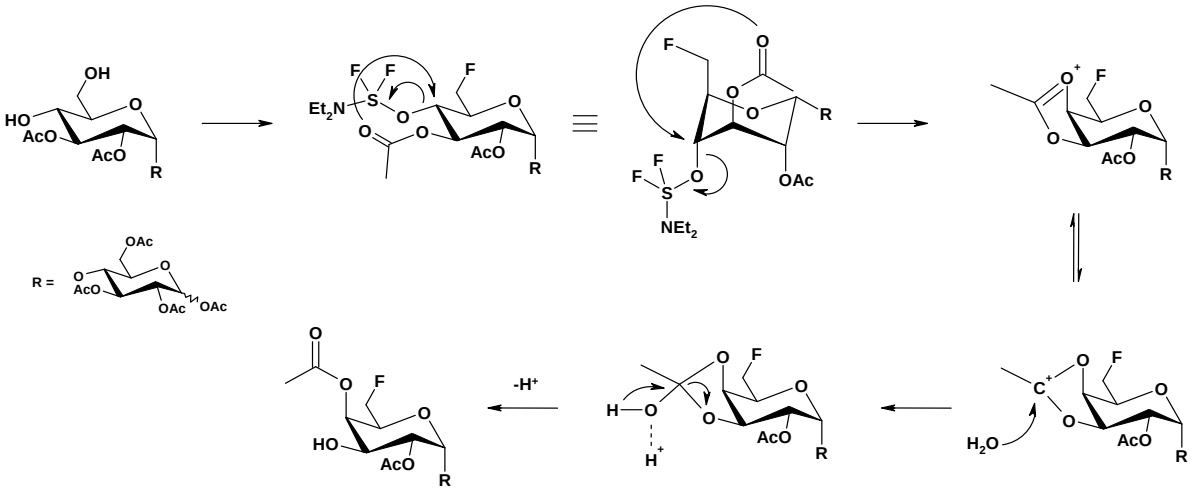


Figure 25. Proposed DAST induced rearrangement of the acetyl protecting group in position-3.

BINDING STUDIES USING THE 2-F-MALTOSE REPORTER SYSTEM

The binding properties of the two stereoisomers of 2-^{19}F-labeled maltose (gluco- and manno-type) to the maltose binding protein and a MBP-fusion protein comprising the LDL receptor fragment VR53 were analyzed.

As can be seen in Figure 26 and Figure 27, the stereoisomers of 2-F labeled maltose clearly exhibit different changes in transverse relaxation rates upon addition of MBP. The significant change in line width was only observed for the interacting α-2-F-maltose. In contrast the transverse relaxation remained nearly unchanged for the manno-type epimers and the β-gluco-type isomer. This observation corresponds to the anomeric preference described by Gehring et al.[85] The numeric specifity of MBP with a 2.7-fold higher affinity for α- versus β-maltose was demonstrated via tritium NMR spectroscopy.[85,86,87] In addition, the β-anomer can be bound in two different modes, probably corresponding to the closed and open domain conformations of MBP; but only the α-anomer complex has been observed in X-ray structures of MBP with maltose.[67]

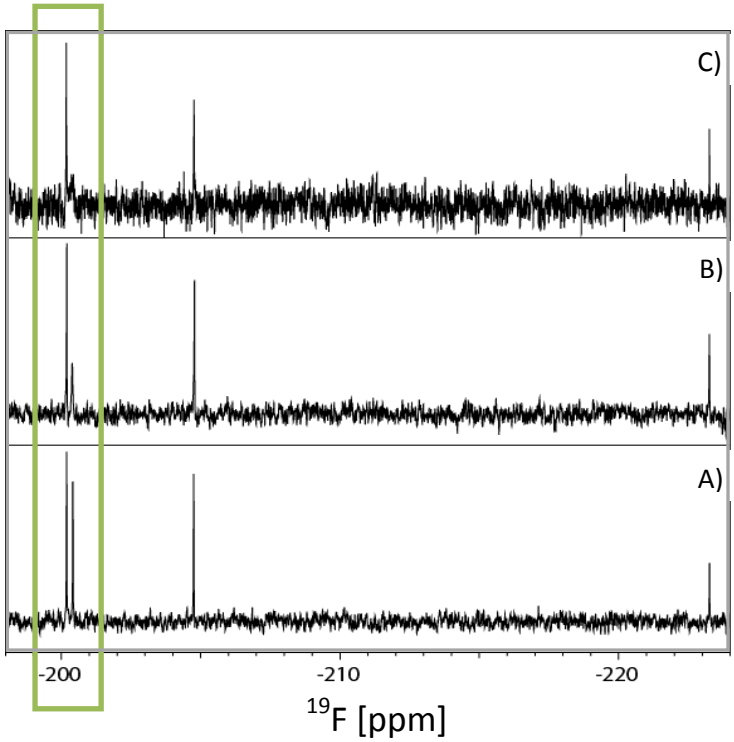


Figure 26. 1D ^{19}F NMR: experimental demonstration of differential binding of gluco- and manno-type 2-F-labeled maltose in the free form (A); bound to maltose binding protein (B), and bound to MBP-VR-53 fusion protein (C). Highlighted area shows the gluco-type region.

Furthermore this technique was used for probing the interactions between 2-F-maltose and the MBP-VR53[88,89] fusion protein, which has roughly almost twice the molecular weight of MBP alone. As expected, an increase of the transverse relaxation rate could be observed through the specific and significant binding of the α-gluco-type isomer to the MBP-VR53 fusion protein. The higher resulting molecular weight is reflected in a further (proportional) increase of line broadening

(Figure 27). In a similar way non-covalent protein-protein interactions will increase the effective molecular weight by transient binding and result in a consequently increased line-width, which can be quantified to derive affinities.

This clearly demonstrates both the binding selectivity of the α-gluco-type and the feasibility of the β-gluco-type and manno-type isomers respectively, serving as internal reference compounds to rule out unspecific binding and interactions (e.g. changes in viscosity).

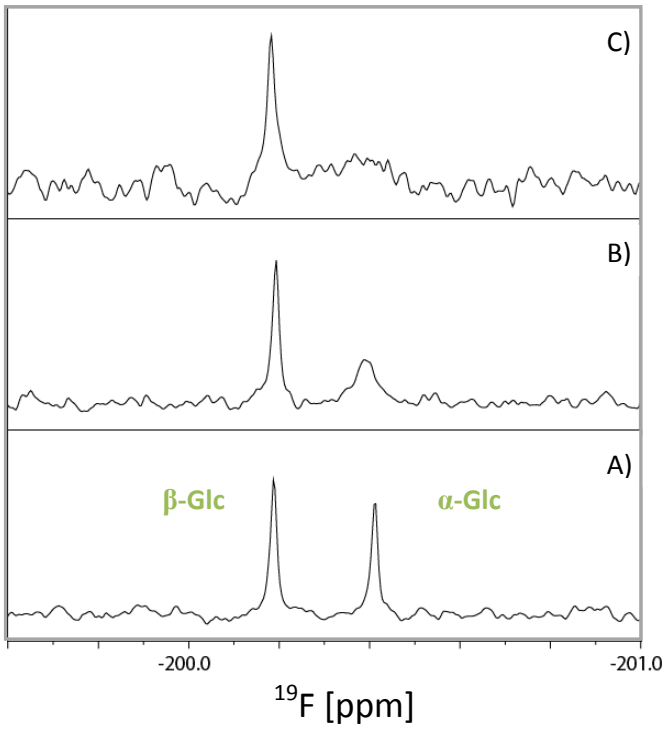


Figure 27. ^{19}F-NMR expansion of the gluco-type region of the 2-F-maltose reporter system.

RELATIVE AFFINITY STUDIES USING THE 2-F-MALTOSE REPORTER SYSTEM

This ^{19}F-labeled reporter system was additionally used to measure relative binding affinities of various fluorinated and non-fluorinated maltose derivatives to MBP in competition titration experiments. The incorporation of fluorine in different positions into maltose allows fine tuning of the carbohydrate affinities to the maltose binding protein.

The initial experiments have been performed using maltose, maltotriose, maltohexose and cellobiose, as well as the artificial α-methyl glucoside. The well-known ability of MBP to bind exclusively to linear maltooligosaccharides or maltodextrins of up to eight α(1-4)-linked glucose units could be confirmed by competition titration and ^{19}F-NMR experiments. The displacement of α-gluco-2-F-maltose could be observed already by the addition of 1/25 equivalents of maltose.

Similar results were obtained for the malto-oligosaccharides, maltotriose and maltohexose as well. In contrast, α-methyl glucoside and cellobiose showed no binding.

To specify the precise hydroxyl groups that are directly involved in hydrogen bonding to MBP, further competition experiments were performed with different fluorinated maltose derivatives. Change i.e. reduction of line width of the α-2-F-maltose signal could be observed if the competitor had a higher affinity as the α-2-F-maltose itself; caused by the release of α-2-F-maltose from the binding pocket of the maltose binding protein.

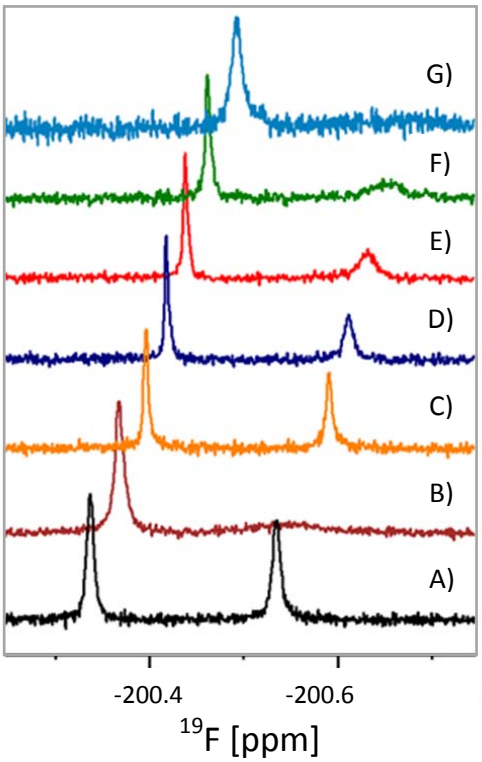


Figure 28. Competition titration using the 2-F-maltose reporter system and ^{19}F NMR: only the important sector of the gluco-type isomers is shown (staggered). (A) 2-F-maltose, (B) 2-F-maltose bound to MBP, (C-G) addition of 0.12 equiv. of the following maltose derivatives: (C) 6-F-maltose, (D) maltose, (E) α–maltosyl fluoride, (F) 6'-F-maltose, (G) 6'-F-'galacto'-maltose.

An overview of the results of the titration experiments are shown in Figure 28. Stepwise addition of equivalent amounts of single fluorinated maltose derivatives to the 2-F-maltose reporter system allows direct comparison, of the competitor's relative affinity to MBP. The 6-F-maltose is the most efficient competitor with an affinity equal to maltose followed by α-maltosyl fluoride and 6'-F-maltose. The 6'-F-'galacto'-maltose derivative does not bind to MBP at all.

Fluorinated substrate analogues perturb the hydrogen bonding network in the substrate binding pocket to a certain extent. Therefore it is not always possible to bind the ligand with an optimal hydrogen bonding geometry. These results are fully consistent to published X-ray data.[67] In the case of 2-F-maltose the 2-OH which acts at the same time as hydrogen bond acceptor for the Nε of Lys15 and as a bond donor to the carboxylate of Glu111, a fluorine in position-2 can be only a (limited) acceptor leaving some of the H-bonds 'frustrated'.

In that respect, introducing the fluorine into the 6-position results in a smaller energetic penalty (compared the 2-F-maltose), because no direct H-bonds between the ligand and MBP are involved, only indirect water mediated interactions are concerned (data not shown). Therefore the affinity is higher in that case. Similar arguments apply in the other cases.

It is however possible to 'fine tune' the affinity between the ligand binding domain and the reporter ligand by using differently fluorinated maltose derivatives in which different hydroxyl group are substituted by fluorine.

Thus the affinity of the reporter ligand can be 'customized' for a specific study of protein-protein interaction to match the affinity between the protein interaction partners. Proteins with low affinities or with a relatively small molecular weight are better detectable with high affinity ligands, whereas strongly interacting proteins or high molecular weight protein ligands are better studied with low-affinity ligands.

CONCLUSION

In summary, it was shown that 2-deoxy-2-F-maltose can be effectively used as a reporter system to study protein binding interactions by ^{19}F-NMR. The particular benefit of this novel reporter system is the simultaneous accessibility of reference molecules (non-binding manno-type and β-gluco-type 2-F-maltose isomers) which serve as internal standards, to rule out unspecific binding and interactions and thus increasing the reliability of this method.

The 2-F-maltose reporter system was used to study ligand binding affinity to MBP. 'Fine tuning' by regioselective fluorination of single hydroxyl groups of maltose were used to define the important hydroxyl groups, which are responsible for the hydrogen bonding network and therefore for bonding to the protein. The results of the competition titration are in perfect agreement to the X-ray data published[66] earlier.

Applications of the reporter system to biological material inherently giving strong background signals (e.g. membrane-bound protein receptors) should be straightforward, have the advantage that ^{19}F signals can be detected with high sensitivity and without any background and broaden the applicability of this approach.

EXPERIMENTAL PROCEDURES

GENERAL METHODS

Solvents were purified by distillation and dried by standard procedures. Thin layer chromatography (TLC) was performed on precoated silica gel plates 60 F254 (Merck), detected with UV light (254 nm), ceric ammonium molybdate as well as 5% vanillin/sulfuric acid and heated by a hotgun. For preparative column chromatography silica gel 60M (230-400 mesh, Macherey-Nagel) was used.

^{1}H and ^{13}C NMR spectra were recorded on Bruker AVANCE DPX 250, AV 400, DRX 400 WB or DRX 600 NMR spectrometers (Bruker BioSpin, Germany). Chemical shifts δ are expressed as parts per million (ppm) and were referenced to residual solvent signals at 7.26 ppm ($CDCl_3$) and 4.79 ppm (D_2O) for the proton NMR spectra as well as to the solvent signal 77.16 ppm ($CDCl_3$) or in D_2O to external dioxane at 67.2 ppm for ^{13}C NMR spectra. Coupling constants are quoted in Hz.

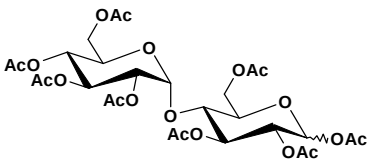
^{19}F NMR spectra were recorded on a Bruker AVANCE DRX 600 NMR spectrometer equipped with 5 mm QNP probe (^{1}H, ^{13}C, ^{19}F, ^{31}P) at a ^{19}F frequency of 564.69 MHz. Proton decoupling, when applied, was achieved by a Waltz-16 composite pulse decoupling sequence with a γB_1 of 1KHz. ^{19}F resonances were referenced relative to external CCl_3F.

Mass spectra were recorded on electron spray ionization Finnigan MAT 8230 mass spectrometer.

Microwave heating was performed with a Biotage initiator synthesizer.

GENERAL PROCEDURES

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-β-D-glucopyranoside



A.2

To a cooled solution of maltose monohydrate **A.1** (20 g, 55.5 mmol) in dry pyridine (120 mL) containing *N,N*-dimethylaminopyridine (cat.), acetic anhydride (105 mL, 1.1 mol) was added dropwise. The mixture was stirred for 15 h at room temperature and was then poured into ice/water (1000 mL) to precipitate the peracetylated maltose**.** Filtration and drying under reduced pressure afforded the product **A.2** as colourless foam (36.5 g, 97%).

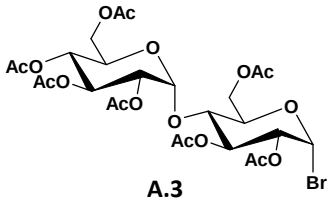
$C_{28}H_{38}O_{19}$ $M_r = 678.59$

^{1}H (400.13 MHz, $CDCl_3$): δ 5.73 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.39 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.34 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{3',4'}$ 9.7 Hz, H-3'), 5.28 (dd, 1H, $J_{2,3}$ 9.1 Hz, $J_{3,4}$ 8.7 Hz, H-3), 5.05 (dd, 1H, $J_{3',4'}$ 9.7 Hz, $J_{4',5'}$ 10.2 Hz, H-4'), 4.96 (dd, 1H, $J_{1,2}$ 8.2 Hz, $J_{2,3}$ 9.1 Hz, H-2), 4 85 (dd, 1H, $J_{1',2'}$ 4.0 Hz, $J_{2',3'}$ 10.5 Hz, H-2'), 4.44 (dd, 1H, $J_{5,6a}$ 2.5 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.22 (dd, 1H, $J_{5,6b}$ 4.4 Hz, $J_{6a,6b}$ 12.3 Hz, H-6b), 4.23 (dd, 1H, $J_{5',6'a}$ 3.7 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 4.02 (dd, 1H, $J_{3,4}$ 8.7 Hz, $J_{4,5}$ 9.6 Hz, H-4), 4.03 (dd, 1H, $J_{5',6'b}$ 2.4 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), 3.93 (ddd, 1H, $J_{4',5'}$ 10.2 Hz, $J_{5',6'a}$ 2.4 Hz, $J_{5',6'b}$ 3.7 Hz, H-5'), 3.83 (ddd, $J_{5,6a}$ 2.6 Hz, $J_{5,6b}$ 4.4 Hz, $J_{4,5}$ 9.6 Hz, H-5), 2.13 (s, 3H, CH_3), 2.09 (s, 6H, 2 CH_3), 2.04 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.99 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.70 (C=O), 170.63 (C=O), 170.56 (C=O), 170.19 (C=O), 170.01 (C=O), 169.72(C=O), 169.57(C=O), 168.93 (C=O), 95.88 (C-1'), 91.43 (C-1), 75.39 (C-3), 73.16 (C-5), 72.61 (C-4), 71.10 (C-2), 70.16 (C-2'), 69.46 (C-3'), 68.74 (C-5'), 68.13 (C-4'), 62.68 (C-6), 61.61 (C-6'), 21.00 (CH_3), 20.93 (2 CH_3), 20.80 (CH_3), 20.71 (3 CH_3), 20.67 (CH_3).

MS: Calcd for [$C_{28}H_{38}O_{19}$]: *m/z* 678:59: ESIMS found: $[M+Na]^+$ 700.9

2,3,6-Tri-*O*-acetyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-α-D-glucopyranosyl bromide



A.3

Octaacetylmaltose **A.2** (10.5 g, 15.4 mmol) was dissolved in dry methylene chloride (DCM, 50 mL) and glacial acetic acid (50 mL). The solution was cooled to 0°C and hydrobromic acid in glacial acetic acid (5.7 M, 16.3 mL, 92.6 mmol) was added dropwise. The reaction mixture was stirred for 3 h at this temperature and quenched with ice/water (300 mL) and DCM (100 mL). The water layer was extracted with DCM (3x 50 mL). The combined organic extracts were washed with water (70 mL), dried over $MgSO_4$, the solids were filtrated off and the solution was concentrated under reduced pressure. The light sensitive α-bromide **A.3** was obtained as colourless foam (10.7 g, 99%).

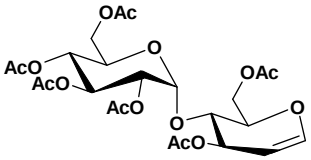
$C_{26}H_{35}BrO_{17}$ M_r = 699.45

^{1}H (250.13 MHz, $CDCl_3$): δ 6.46 (d, 1H, $J_{1,2}$ 4.0 Hz, H-1), 5.57 (dd, 1H, $J_{2,3}$ 9.8 Hz, $J_{3,4}$ 8.9 Hz, H-3), 5.37 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.33 (dd, 1H, $J_{2',3'}$ 10.5 Hz, $J_{3',4'}$ 9.4 Hz, H-3'), 5.03 (dd, 1H, $J_{3',4'}$ 9.4 Hz, $J_{4',5'}$ 10.1 Hz, H-4'), 4.82 (dd, 1H, $J_{1',2'}$ 4.0 Hz, $J_{2',3'}$ 10.5 Hz, H-2'), 4 67 (dd, 1H, $J_{1,2}$ 4.0 Hz, $J_{2,3}$ 9.9 Hz, H-2), 4.47 (dd, 1H, $J_{5,6a}$ 3.6 Hz, $J_{6a,6b}$ 13.6 Hz, H-6a), 4.25-4.17 (m, 3H, H-5, H-6b, H-6'a), 4.02 (dd, 1H, $J_{3,4}$ 8.9 Hz, $J_{4,5}$ 8.9 Hz, H-4), 4.01 (dd, 1H, $J_{5',6'b}$ 2.1 Hz, $J_{6'a,6'b}$ 12.9 Hz, H-6'b), 3.91 (ddd, 1H, $J_{4',5'}$ 10.1 Hz, $J_{5,6'a}$ 3.7 Hz, $J_{5',6'b}$ 2.1 Hz, H-5'), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.96 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.81 (C=O), 170.62 (C=O), 170.41 (C=O), 169.99 (2 C=O), 169.64 (C=O), 169.56 (C=O), 95.93 (C-1'), 86.19 (C-1), 72.69 (C-5), 72.49 (C-3), 71.75 (C-4), 71.17 (C-2),

70.16 (C-2'), 69.40 (C-3'), 68.80 (C-5'), 68.08 (C-4'), 62.00 (C-6), 61.50 (C-6'), 20.99 (CH_3), 20.90 (CH_3), 20.80 (CH_3), 20.76 (CH_3), 20.72 (3 CH_3).

3,6-Di-*O*-acetyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-D-glucal



A.4

To the vigorous stirred solution of α-maltosyl bromide **A.3** (2 g, 2.9 mmol) in ethyl acetate (10 mL), zinc powder (1.87 g, 28.6 mmol) and *N*-methyl imidazol (0.34 mL, 4.3 mmol) was added. The reaction mixture was refluxed for 4 h, cooled to room temperature and filtered over Celite to remove solid zinc. The organic layer was washed twice with saturated aqueous $NaHSO_4$ (20 mL), 10% aqueous $NaHCO_3$ (25 mL) and brine (25 mL), dried over $MgSO_4$ and concentrated under vacuum. The residue was purified by chromatography over silica gel ($CHCl_3$/Et_2O 1:2) to obtain the peracetylated maltal **A.4** as colorless foam (1.18 g, 74%).

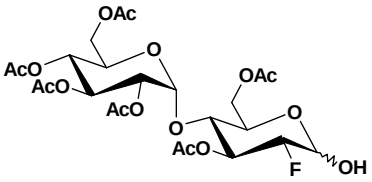
$C_{24}H_{32}O_{15}$ M_r = 560.50

^{1}H (400.13 MHz, $CDCl_3$): δ 6.43 (dd, 1H, $J_{1,2}$ 6.1 Hz, $J_{1,3}$ 1.1 Hz, H-1), 5.50 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.41 (dd, 1H, $J_{2',3'}$ 10.4 Hz, $J_{3',4'}$ 9.4 Hz, H-3'), 5.18 (m, 1H, H-3), 5.06 (dd, 1H, $J_{3',4'}$ 9.4 Hz, $J_{4',5'}$ 10.3 Hz, H-4'), 4.83 (dd, 1H, $J_{1',2'}$ 4.0 Hz, $J_{2',3'}$ 10.4 Hz, H-2'), 4.82 (dd, 1H, $J_{1,2}$ 6.1 Hz, $J_{2,3}$ 3.5 Hz, H-2), 4 81-4.78 (m, 2H, H-2', H-2), 4.41-4.32 (m, 2H, H-6a, H-6b), 4.31-4.27 (m, 1H, H-5), 4.28 (dd, 1H, $J_{5',6'a}$ 4.2 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 4.10 (dd, 1H, $J_{5',6'b}$ 2.3 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), 4.06-4.01 (m, 2H, H-4, H-5'), 2.12 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.054 (s, 3H, CH_3), 2.048 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.01 (6 s, 18H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.72 (C=O), 170.61 (C=O), 170.52 (C=O), 170.50 (C=O), 170.16 (C=O), 169.69 (C=O), 145.74 (C-1), 98.78 (C-2), 96.00 (C-1'), 74.26 (C-5), 72.70 (C-4), 70.59 (C-2'), 69.78 (C-3'), 69.66 (C-3), 68.45 (C-5'), 68.37 (C-4), 62.03 (C-6), 61.81 (C-6'), 21.24 (CH_3), 20.94 (CH_3), 20.82 (CH_3), 20.81 (CH_3), 20.75 (CH_3), 20.69 (CH_3).

MS: Calcd for [$C_{24}H_{32}O_{15}$]: *m/z* 560.50: ESIMS found: $[M+Na]^+$ 582.9

3,6-Di-*O*-acetyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-2-deoxy-2-fluoro-D-gluco- and D-mannopyranoses



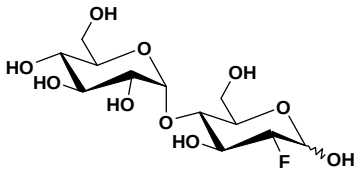
A.5

To a solution of protected maltal **A.4** (131 mg, 0.23 mmol) in nitromethane (2.5 mL), Selectfluor™ (99 mg, 0.28 mmol) was added. The reaction mixture was stirred vigorously by room temperature for 15 h and then heated to reflux for 1 h. After cooling to room temperature, the solvent was removed under reduced pressure and the residue was redissolved in DCM (20 mL). The organic layer was washed with 10% aqueous $NaHCO_3$ solution (20 mL) and water (20 mL), dried over $MgSO_4$ and the solvent was removed under vacuum. The remaining material was purified by chromatography over silica gel (PE/EE 1:1). The mixture of product diastereomers **A.5** was isolated as colourless foam (78 mg, 56%) with a gluco to manno ratio of 2:1.

$C_{24}H_{33}FO_{16}$ $M_r = 586.51$

^{19}F NMR (564.69 MHz, $CDCl_3$): glucose type, α-anomer δ -201.22 (dd, $J_{F,2}$ 49.7 Hz, $J_{F,3}$ 11.0 Hz), glucose type, β-anomer δ -200.41 (ddd, $J_{F,1}$ 2.4 Hz, $J_{F,2}$ 50.7 Hz, $J_{F,3}$ 12.9 Hz), mannose type, α-anomer δ -205.44 (dddd, $J_{F,1}$ 6.8 Hz, $J_{F,2}$ 50.0 Hz, $J_{F,3}$ 28.3 Hz, $J_{F,4}$ 2.3 Hz), mannose type, β-anomer δ -223.89 (ddd, $J_{F,1}$ 18.0 Hz, $J_{F,2}$ 50.9 Hz, $J_{F,3}$ 28.4 Hz).

4-*O*-(α-D-glucopyranosyl)-2-deoxy-2-fluoro-D-gluco- and D-mannopyranoses



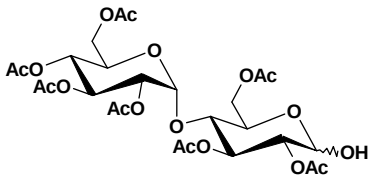
A.6

Deprotection was performed according to the Zemplén protocol: the peracetylated 2-F-maltose mixture **A.5** (78 mg, 0.13 mmol) was suspended in dry methanol (2 mL). The sodium methoxide (0.5 mL of a freshly prepared 0.1 M stock solution) was added and stirred for 6 h at room temperature. By addition of dry ice or Dowex H^+ (pH 6-7), the excess of $NaOCH_3$ was quenched. Lyophilisation of this alcoholic solution yielded a colourless foam (45 mg, quant.).

$C_{12}H_{21}FO_{10}$ $M_r = 344.29$

^{19}F NMR (564.69 MHz, D_2O): glucose type, α-anomer δ -200.70 (dd, $J_{F,2}$ 49.3 Hz, $J_{F,3}$ 13.6 Hz), glucose type, β-anomer δ -200.48 (m), mannose type, α-anomer δ -205.08 (ddd, $J_{F,1}$ 7.5 Hz, $J_{F,2}$ 49.1 Hz, $J_{F,3}$ 31.4 Hz), mannose type, β-anomer δ -223.49 (ddd, $J_{F,1}$ 20.2 Hz, $J_{F,2}$ 51.4 Hz, $J_{F,3}$ 31.2 Hz).

2,3,6-Tri-*O*-acetyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-α/β-D-glucopyranoside



A.7

A solution of octaacetylmaltose **A.2** (5 g, 7.37 mmol) in dry DMF (25 mL), was stirred in the presence of hydrazine acetate (8.11 mmol) for 1½ h. (Hydrazine acetate was freshly prepared by

combining equimolar amounts of hydrazine hydrate and acetic acid in methanol). The solvent was removed under reduced pressure to yield the anomeric mixture (α/β = 2:1) of the 1-hydroxy-maltose derivative **A.7** (4.4 g, 94%) as a colourless solid.

$C_{26}H_{36}O_{18}$ M_r = 636.55

α-Anomer:

^{1}H (600.13 MHz, $CDCl_3$): δ 5.58 (dd, 1H, $J_{2,3}$ 10.1 Hz, $J_{3,4}$ 9.0 Hz, H-3), 5.44 (d, 1H, $J_{1',2'}$ 4.1 Hz, H-1'), 5.37 (dd, 1H, $J_{3',2'}$ 10.6 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 5.36 (d, 1H, $J_{1,2}$ 3.6 Hz, H-1), 5.07 (dd, 1H, $J_{4',3'}$ 9.5 Hz, $J_{4',5'}$ 10.1 Hz, H-4'), 4.86 (dd, 1H, $J_{2,1}$ 4.1 Hz, $J_{2,3}$ 10.6 Hz, H-2'), 4.78 (dd, 1H, $J_{2,1}$ 3.6 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4.50 (dd, 1H, $J_{5,6a}$ 2.21 Hz, $J_{6a,6b}$ 11.9 Hz, H-6a), 4.27-4.21 (m, 3H, H-6'a, H-6b, H-5), 4.05 (dd, 1H, $J_{5',6'b}$ 2.3 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6b), 3.99 (dd, 1H, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 9.5 Hz, H-4), 3.97 (m, 1H, H-5'), 2.15-2.00 (7 s, 21H, CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 170.97-169.58 (C=O), 95.68 (C-1'), 90.20 (C-1), 72.74 (C-4), 72.41 (C-3), 71.64 (C-2), 70.15 (C-2'), 69.54 (C-3'), 68.59 (C-5'), 68.15 (C-4'), 67.95 (C-5), 62.89 (C-6), 61.56 (C-6'), 21.19-20.73 (CH_3).

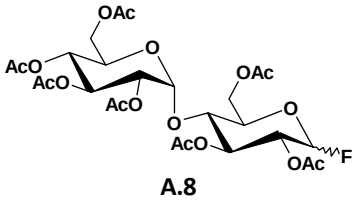
β-Anomer:

^{1}H (600.13 MHz, $CDCl_3$): δ 5.40 (d, 1H, $J_{1',2}$ 4.1 Hz, H-1'), 5.35 (dd, 1H, $J_{3',2'}$ 10.5 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 5.30 (dd, 1H, $J_{3,2}$ 9.4 Hz, $J_{3,4}$ 8.9 Hz, H-3), 5.05 (dd, 1H, $J_{4',3'}$ 9.5 Hz, $J_{4',5'}$ 10.5 Hz, H-4'), 4.86 (dd, 1H, $J_{1',2'}$ 4.2 Hz, $J_{2',3'}$ 10.5 Hz, H-2'), 4.79 (d, 1H, $J_{1,2}$ 7.9 Hz, H-1), 4 73 (dd, 1H, $J_{2,1}$ 7.9 Hz, $J_{2,3}$ 9.4 Hz, H-2), 4.48 (dd, 1H, $J_{5,6a}$ 2.6 Hz, $J_{6a,6b}$ 12.1 Hz, H-6a), 4.27-4.21 (m, 2H, H-6b, H-6'a), 4.05 (dd, 1H, $J_{5',6'b}$ 2.4 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), 4.00 (dd, 1H, $J_{3,4}$ 8.9 Hz, $J_{4,5}$ 9.6 Hz, H-4), 3.97-3.94 (m, 1H, H-5'), 3.74 (ddd, 1H, $J_{5,6a}$ 2.6, $J_{5,6b}$ 4.4, $J_{4,5}$ 9.6, H-5), 2.15-2.00 (7 s, 21H, CH_3).

^{13}C (150.91 MHz, $CDCl_3$): δ 170.97-169.58 (C=O), 95.72 (C-1'), 95.12 (C-1), 74.85 (C-3), 74.00 (C-2), 72.80 (C-4), 72.59 (C-5), 70.13 (C-2'), 69.44 (C-3'), 68.71 (C-5'), 68.13 (C-4'), 62.99 (C-6), 61.61 (C-6'), 21.19-20.73 (CH_3).

MS: Calcd for $[C_{26}H_{36}O_{18}]$: *m/z* 636.55: ESIMS found: $[M+Na]^+$ 658.9

2,3,6-Tri-*O*-acetyl-4-*O*-(2',3',4',6'-tetra-*O*-acetyl-α-D-glucopyranosyl)-α/β-D-glucopyranosyl fluorides



A.8

Fluorination of the anomeric hydroxyl group was performed with DAST under argon atmosphere. To a solution of peracetylated 1-hydroxy-maltose derivative **A.7** (1 g, 1.57 mmol) in dry DCM (25 mL), DAST (0.23 mL, 1.73 mmol) was added dropwise. The reaction mixture was stirred for 2 h at room temperature, quenched with 10% aqueous $NaHCO_3$ solution (15 mL), extracted with DCM

(3x 15 mL), dried over $MgSO_4$ and concentrated under vacuum. The crude product was purified by chromatography over silica gel ($CHCl_3$/Et_2O 1:1) to furnish the anomeric mixture of maltosyl fluoride **A.8** (0.89 g, 89%) as colourless foam (α/β = 1:3). Separation of the diastereomers could be achieved by HPLC.

$C_{26}H_{35}FO_{17}$ M_r = 638.54

α-Anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -149.21 ($J_{F,1}$ 53.1 Hz, $J_{F,2}$ 24.1 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 5.64 (dd, 1H, $J_{1,F}$ 53.1 Hz, $J_{1,2}$ 2.7 Hz, H-1), 5.53 (dd, 1H, $J_{2,3}$ 10.1 Hz, $J_{3,4}$ 9.0 Hz, H-3), 5.42 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.34 (dd, 1H, $J_{3',2'}$ 10.6 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 5.05 (dd, 1H, $J_{4',3'}$ 9.5 Hz, $J_{4',5'}$ 10.2 Hz, H-4'), 4.85 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.6 Hz, H-2'), 4.82 (ddd, 1H, $J_{2,F}$ 24.1 Hz, $J_{2,1}$ 2.7 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4 52 (dd, 1H, J 2.4 Hz, J 12.5 Hz, H-6a), 4.23 (dd, 2H, J 3.6 Hz, J 12.6 Hz, H-6a', H-6b), 4.16 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6a}$ 2.4 Hz, $J_{5,6b}$ 3.6 Hz, H-5), 4.05 (dd, 1H, $J_{3,4}$ 9.0 Hz, $J_{4,5}$ 10.0 Hz, H-4), 4.03 (dd, 1H, $J_{5'6'b}$ 2.4 Hz, $J_{6'a,6'b}$ 12.5 Hz, H-6'b), 3.92 (ddd, $J_{4',5'}$ 10.2 Hz, $J_{5',6'a}$ 3.5 Hz, $J_{5',6'b}$ 2.4 Hz, H-5'), 2.14 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.05(s, 3H, CH_3), 2.014 (s, 3H, CH_3), 2.01 (s, 3H, CH_3), 1.99 (s, 3H, CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ δ 170.75 (C=O), 170.62 (C=O), 170.46 (C=O), 170.21 (C=O), 169.97 (C=O), 169.86 (C=O), 169.55 (C=O), 103.72 (d, $J_{1,F}$ 229.5 Hz, C-1), 95.79 (C-1'), 71.84 (C-3), 71.73 (C-4), 70.71 (d, $J_{2,F}$ 24.5 Hz, C-2), 70.32 (d, $J_{5,F}$ 3.9 Hz, C-5), 70.12 (C-2'), 69.36 (C-3'), 68.71 (C-5'), 68.00 (C-4'), 62.14 (C-6), 61.45 (C-6'), 21.15-20.61 (CH_3).

β-Anomer:

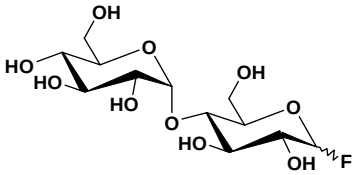
^{19}F (564.69 MHz, $CDCl_3$): δ -132.35 ($J_{F,1}$ 52.5 Hz, $J_{F,2}$ 8.7 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 5.42 (dd, 1H, $J_{1,2}$ 4.9 Hz, $J_{1,F}$ 52.5 Hz, H-1), 5.41 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.37 (dd, 1H, $J_{2',3'}$ 10.6 Hz, $J_{3',4'}$ 9.5 Hz, H-3'), 5.13 (dd, 1H, $J_{2,3}$ 6.4 Hz, $J_{3,4}$ 7.8 Hz, H-3), 5.06 (dd, 1H, $J_{3',4'}$ 9.5 Hz, $J_{4',5'}$ 10.3 Hz, H-4'), 4.95 (dd, 1H, $J_{2,F}$ 8.7 Hz, $J_{2,1}$ 4.9 Hz, $J_{2,3}$ 6.3 Hz, H-2), 4 84 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.6 Hz, H-2'), 4.54 (dd, 1H, $J_{5,6a}$ 3.4 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 4.24 (dd, 1H, $J_{5,\ 6'a}$ 4.1 Hz, $J_{6'a,6'b}$ 12.5 Hz, H-6'a), 4.22 (dd, 1H, $J_{5,6b}$ 4.6 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 4.15 (ddd, 1H, $J_{4,3}$ 7.8 Hz, $J_{4,\ 5}$ 8.7 Hz, $J_{4,F}$ 0.6 Hz, H-4), 4.07 (dd, 1H, $J_{5,\ 6'b}$ 2.4 Hz, $J_{6'a,6'b}$ 12.5 Hz, H-6'b), 4.00-3.98 (m, 2H, H-5, H-5'), 2.15 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 2.00 (s, 3H, CH_3).

^{13}C (150.91 MHz, $CDCl_3$): δ 170.68 (C=O), 170.58 (C=O), 170.56 (C=O), 170.16 (C=O), 170.11 (C=O), 169.57 (C=O), 169.49 (C=O), 105.61 ($J_{1,F}$ 219.8 Hz, C-1), 96.06 (C-1'), 74.17 ($J_{3,F}$ 5.5 Hz, C-3), 72.45 ($J_{5,F}$ 1.7 Hz, C-5), 72.14 (C-4), 71.37 ($J_{2,F}$ 31.9 Hz, C-2), 70.30 (C-2'), 69.45 (C-3'), 68.73 (C-5'), 68.15 (C-4'), 62.79 (C-6), 61.65 (C-6'), 20.98-20.68 (CH_3).

MS: Calcd for [$C_{26}H_{35}FO_{17}$]: *m/z* 638.54: ESIMS found: $[M+Na]^+$ 661.0

4-*O*-(α-D-glucopyranosyl)-α-D-glucopyranosyl fluorides



A.9

According to the procedure described for compound **A.6**, the deprotection of peracetylated α-maltosyl fluoride (100 mg, 0.16 mmol) with sodium methoxide (0.4 equiv.) afforded α-maltosyl fluoride **A.9** in quantitative yield.

$C_{12}H_{21}FO_{10}$ $M_r = 344.29$

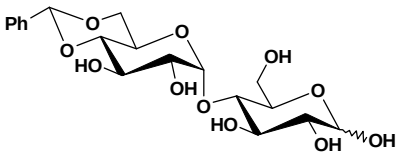
α-Anomer:

^{19}F (564.69 MHz, D_2O): δ -150.92 ($J_{F,1}$ 53.5 Hz, $J_{F,2}$ 26.4 Hz).

^{1}H (600.13 MHz, D_2O): δ 5.69 (dd, 1H, $J_{1,F}$ 53.5 Hz, $J_{1,2}$ 2.8 Hz, H-1), 5.42 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1'), 4.00 (dd, 1H, $J_{3,2}$ 9.9 Hz, $J_{3,4}$ 9.2 Hz, H-3), 3.95 (ddd, 1H, $J_{4,5}$ 10.0 Hz, $J_{5,6a}$ 2.0 Hz, $J_{5,6b}$ 4.2 Hz, H-5), 3.88 (dd, 1H, $J_{5,6a}$ 2.0 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 3.85 (dd, 1H, $J_{5',6'a}$ 2.0 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 3.83 (dd, 1H, $J_{5,6b}$ 4.2 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 3.76 (dd, 1H, $J_{5',6'b}$ 5.1 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'b), 3.75 (dd, 1H, $J_{3,4}$ 9.2 Hz, $J_{4,5}$ 10.0 Hz, H-4), 3.71 (ddd, 1H, $J_{4',5'}$ 9.9 Hz, $J_{5',6'a}$ 2.0 Hz, $J_{5'6'b}$ 5.1 Hz, H-5'), 3.68 (dd, 1H, $J_{3',2'}$ 9.9 Hz, $J_{3',4'}$ 9.9 Hz, H-3'), 3.65 (ddd, 1H, $J_{2,F}$ 26.4 Hz, $J_{2,1}$ 2.8 Hz, $J_{2,3}$ 9.9 Hz, H-2), 3.58 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 9.9 Hz, H-2'), 3.41 (dd, 1H, $J_{4',3'}$ 9.9 Hz, $J_{4',5'}$ 9.9 Hz, H-4').

^{13}C (150.90 MHz, D_2O): δ 107.47 (d, $J_{1,F}$ 223.3 Hz, C-1), 100.09 (C-1'), 75,97 (C-4), 73.23(C-3), 73.17 (C-5'), 73.07 (C-3'), 73.04 (d, $J_{5,F}$ 3.2 Hz, C-5), 72.06 (C-2'), 71.23 (d, $J_{2,F}$ 24.9 Hz, C-2), 69.63 (C-4'), 60.78 (C-6'), 60.45 (C-6).

4-*O*-(4',6'-benzylidene-α-D-glucopyranosyl)-D-glucopyranoside



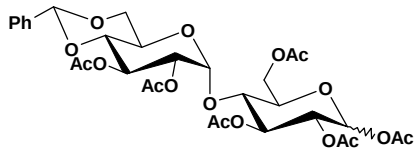
A.10

In a 100 mL round-bottomed flask maltose monohydrate **A.1** (5 g, 13.9 mmol), α,α-dimethoxytoluene (4.9 mL) and p-toluene sulfonic acid (0.25 g) were suspended in dry DMF (45 mL). The flask was attached to a rotary evaporator. The reaction mixture was stirred by rotation for 5 hours at 50 C and under 39 mbar vacuum. After neutralization with conc. NH_3 (pH~7) the solvents were removed under reduced pressure. The crude product was purified by chromatography over silica ($CHCl_3$/MeOH 3:1) to yield benzylidenemaltose **A.10** in 79% (4.7 g).

$C_{19}H_{26}O_{11}$ $M_r = 430.40$

MS: Calcd for $[C_{19}H_{26}O_{11}]$: *m/z* 430.40: ESIMS found: $[M+Na]^+$ 452.9

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2',3'-di-*O*-acetyl-4',6'-benzylidene-α-D-glucopyranosyl)-D-glucopyranoside



A.11

According the procedure described for compound **A.2**, the peracetylation of benzylidenemaltose **A.10** (4.3 g, 10 mmol) with acetic anhydride (19 mL, 200 mmol) and catalytic amount of DMAP in dry pyridine (20 mL), followed by purification by chromatography over silica gel (PE/EE 1:1) afforded compound **A.11** in 93% yield (6.4 g).

$C_{31}H_{38}O_{17}$ M_r = 682.62

α-anomer:

^{1}H (400.13 MHz, $CDCl_3$): δ 7.44-7.41 (m, 2H, H-2,6 phenyl), 7.35-7.33 (m, 3H, H-3,4,5 phenyl), 6.23 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.52 (dd, 1H, $J_{3,4}$ 8.7 Hz, $J_{3,2}$ 10.1 Hz, H-3), 5.47 (dd, 1H, $J_{2',3'}$ 10.2 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.47 (s, 1H, CH-phenyl), 5.37 (d, 1H, $J_{1',2'}$ 4.2 Hz, H-1'), 4.97 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4.90 (dd, 1H, $J_{2',1'}$ 4.2 Hz, $J_{2',3'}$ 10.2 Hz, H-2'), 4.50 (dd, 1H, $J_{5,6a}$ 2.4 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.28-4.22 (m, 2H, H-6b, H-6'a), 4.12 (ddd, 1H, $J_{4,5}$ 9.7 Hz, $J_{5,6a}$ 2.4 Hz, $J_{5,6b}$ 3.5 Hz, H-5), 4.04 (dd, 1H, $J_{3,4}$ 8.7 Hz, $J_{4,5}$ 9.7 Hz, H-4), 3.89-3.81 (m, 1H, H-5'), 3.71 (dd, 1H, $J_{6'b,5'}$ 4.3 Hz, $J_{6'b,6'a}$ 10.2 Hz, H-6'b), 3.64 (dd, 1H, $J_{4',3'}$ 9.6 Hz, $J_{4',5'}$ 9.6 Hz, H-4'), 2.22 (s, 3H, CH_3), 2.11 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.99 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.97 (C=O), 170.04 (C=O), 169.88 (C=O), 169.82 (C=O), 169.77 (C=O), 169.09 (C=O), 136.81 (C1 phenyl.), 129.26 (C-4 phenyl), 128.35 (C-3,5 phenyl), 126.34 (C-2,6 phenyl), 101.78 (CH-phenyl), 96.89 (C-1'), 89.02 (C-1), 78.89 (C-4'), 72.65 (C-4), 72.53 (C-3), 71.06 (C-2'), 70.36 (C-5), 69.91 (C-2), 68.58 (C-3'), 68.55 (C-6'), 64.00 (C-5'), 62.43 (C-6), 21.14-20.58 (CH_3).

β-anomer:

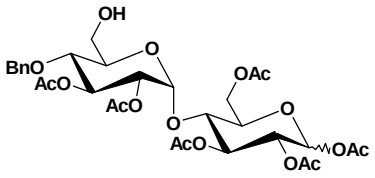
^{1}H (400.13 MHz, $CDCl_3$): δ 7.44-7.41 (m, 2H, H-2,6 phenyl), 7.35-7.33 (m, 3H, H-3,4,5 phenyl), 5.74 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.47 (s, 1H, CH-phenyl), 5.45 (dd, 1H, $J_{2',3'}$ 10.2 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.35 (d, 1H, $J_{1',2'}$ 4.2, H-1'), 5.30 (dd, 1H, $J_{3,2}$ 9.2 Hz, $J_{3,4}$ 8.8 Hz, H-3), 4.97 (dd, 1H, $J_{2,1}$ 8.2 Hz, $J_{2,3}$ 9.2 Hz, H-2), 4.88 (dd, 1H, $H_{2',1'}$ 4.2 Hz, $H_{2',3'}$ 10.2 Hz, H-2'), 4.49 (dd, 1H, $J_{5,6}$ 2.6 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.28-4.22 (m, 2H, H-6b, H-6'a), 4.04 (dd, 1H, $J_{3,4}$ 8.8 Hz, $J_{4,5}$ 9.9 Hz, H-4), 3.89-3.81 (m, 2H, H-5, H-5'), 3.73 (dd, 1H, $J_{6'b,5'}$ 4.3 Hz, $J_{6'b,6'a}$ 10.3 Hz, H-6'b), 3.62 (dd, 1H, $J_{4',3'}$ 9.6 Hz, $J_{4',5'}$ 9.6 Hz, H-4'), 2.10 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.06 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 2.01 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.95 (C=O), 170.37 (C=O), 170.36 (C=O), 170.17 (C=O), 170.05 (C=O), 168.90 (C=O), 136.81 (C-1 phenyl), 129.26 (C-4 phenyl), 128.35 (C-3,5 phenyl), 126.34 (C-2,6 phenyl), 101.78 (CH-phenyl), 96.76 (C-1'), 91.40 (C-1), 78.91 (C-4'), 75.47 (C-3), 73.19 (C-5), 72.69

(C-4), 71.17 (C-2'), 70.98 (C-2), 68.58 (C-3'), 68.55 (C-6'), 63.91 (C-5'), 62.51 (C-6), 21.14-20.58 (CH_3).

MS: Calcd for $[C_{31}H_{38}O_{17}]$: *m/z* 682.62: ESIMS found: $[M+Na]^+$ 705.7

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2',3'-di-*O*-acetyl-4'-*O*-benzyl-α-D-glucopyranosyl)-D-glucopyranoside



A.12

In an oven-dried round-bottomed reaction flask compound **A.11** (123 mg, 0.18 mmol) was dissolved in freshly distilled THF under argon atmosphere. The solution was cooled to -70°C and was then treated with borane-THF complex (0.9 mL of a 1M solution in THF). After 15 min, the reaction mixture was treated with Bu_2BOTf (0.45 mL of a 1M solution in DCM) and stirred for 4 hours. The temperature was brought to 10°C over this periode of time. The reactions was quenched with NH_3 (0.2 mL) and dropewise addition of MeOH (6.0 mL) until effervescence ceased. The product was concentrated in vacuum and purified by flash column chromatography (silicagel, PE/EE 1:1) to yield the regioselectively deprotected compound **A.12** in 56% (69 mg).

$C_{31}H_{40}O_{17}$ M_r = 684.64

α-anomer:

^{1}H (400.13 MHz, $CDCl_3$): δ 7.35-7.24 (m, 5H, H-phenyl), 6.21 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.48 (dd, 1H, $J_{3,4}$ 8.6 Hz, $J_{3,2}$ 10.1 Hz, H-3), 5.39 (dd, 1H, J 10.7 Hz, J 8.7 Hz, H-3'), 5.34 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.94 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.0 Hz, H-2), 4.74 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.7 Hz, H-2'), 4.62 (d, 1H, J 11.4 Hz, CH_2-phenyl), 4.58 (d, 1H, J 11.4 Hz, CH_2-phenyl), 4.41 (dd, 1H, $J_{6a,5}$ 2.5 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.16 (dd, 1H, $J_{6a,5}$ 3.3 Hz, $J_{6b,6a}$ 12.3 Hz, H-6b), 4.07 (ddd, 1H, $J_{4,5}$ 9.9 Hz, $J_{5,6a}$ 2.5 Hz, $J_{5,6b}$ Hz, H-5), 4.00 (dd, 1H, $J_{3,4}$ 8.6 Hz, $J_{4,5}$ 9.9 Hz, H-4), 3.77-3.71 (m, 2H, H-6'a, H-6'b), 3.70-3.61 (m, 2H, H-4', H-5'), 2.20-1.92 (CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.97 (C=O), 170.68 (C=O), 169.94 (C=O), 169.91 (C=O), 169.75 (C=O), 169.07 (C=O), 137.54 (C-1 phenyl), 128.64/128.15 (C-2,3,5,6 phenyl), 128.16 (C-4 phenyl), 96.16 (C-1'), 88.96 (C-1), 75.33 (C-4'), 74.85 (CH_2-phenyl), 72.54 (C-3), 72.43 (C-4), 72.30 (C-5'), 71.20 (C-3'), 70.83 (C-2'), 70.29 (C-5), 69.83 (C-2), 62.61 (C-6), 61.25 (C-6'), 21.11-20.51 (CH_3).

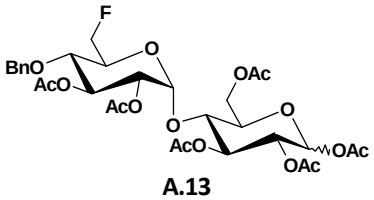
β-anomer:

^{1}H (400.13 MHz, $CDCl_3$): δ 7.35-7.24 (m, 5H, H-phenyl), 5.71 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 5.41 (dd, 1H, $J_{3,2}$ 10.7 Hz, $J_{3,4}$ 8.7 Hz, H-3'), 5.31 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.27 (dd, 1H, $J_{3,2}$ 9.3 Hz, $J_{3,4}$ 8.7 Hz, H-3), 4.94 (dd, 1H, $J_{2,1}$ 8.1 Hz, $J_{2,3}$ 9.3 Hz, H-2), 4.73 (dd, 1H, $H_{2',1'}$ 4.0 Hz, $H_{2',3'}$ 10.7 Hz, H-2'), 4.61 (d, 1H, J 11.4 Hz, CH_2-Phenyl), 4.47 (d, 1H, J 11.4 Hz, CH_2-Phenyl), 4.42 (dd, 1H, $J_{6a,5}$ 2.5 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.16 (dd, 1H, $J_{6a,5}$ 4.3 Hz, $J_{6b,6a}$ 12.4 Hz, H-6b), 3.79 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6a}$ 2.5 Hz, $J_{5,6b}$ 4.3 Hz, H-5), 3.77-3.71 (m, 2H, H-6'a, H-6'b), 3.70-3.61 (m, 2H, H-4', H-5'), 2.20-1.92 (CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.94 (C=O), 170.71 (C=O), 170.06 (C=O), 169.98 (C=O), 169.77 (C=O), 168.85 (C=O), 137.54 (C-1 phenyl), 128.63 / 128.14 (C-2,3,5,6 phenyl), 128.18 (C-4 phenyl), 96.13 (C-1'), 91.35 (C-1), 75.41 (C-3), 75.34 (C-4'), 74.81 (CH_2-phenyl), 73.13 (C-5), 72.46 (C-5'), 72.38 (C-4), 71.20 (C-3'), 71.09 (C-2), 70.79 (C-2'), 62.73 (C-6), 61.25 (C-6'), 21.11-20.51 (CH_3).

MS: Calcd for [$C_{31}H_{40}O_{17}$]: *m/z* 684.64: ESIMS found: $[M+Na]^+$ 707.2

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2',3'-di-*O*-acetyl-4'-*O*-benzyl-6'-deoxy-6'-fluoro-α-D-glucopyranosyl)-D-glucopyranoside



A.13

A solution of compound **A.12** (62 mg, 0.09 mmol), DAST (0.024 mL, 0.18 mmol) and collidine (0.024 mL, 0.18 mmol) in anhydrous DCM was heated in the microwave generator for 60 minutes at 80°C. TLC control showed no further starting material. The reaction was quenched by saturated $NaHCO_3$. The solution was extracted trice with DCM (à 10 mL). The organic layers were washed with water, dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (silicagel, PE/EE 1:1) to give 49 mg (79%) of compound **A.13**.

$C_{31}H_{39}FO_{16}$ M_r = 686.63

α-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -234.82 (td, $J_{F,6'}$ 47.6 Hz, $J_{F,5'}$ 28.8 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 7.34-7.22 (m, 5H, H-phenyl), 6.21 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.48 (dd, 1H, $J_{3,4}$ 8.5 Hz, $J_{3,2}$ 10.1 Hz, H-3), 5.42 (dd, 1H, $J_{3',2'}$ 10.6 Hz, $J_{3',4'}$ 9.3 Hz, H-3'), 5.38 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.94 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4.78 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.6 Hz, H-2'), 4.61 (d, 1H, J 11.3 Hz, CH_2-phenyl), 4.59 (ddd, 1H, $J_{F,6'a}$ 47.6 Hz, $J_{6'a,5'}$ 2.7 Hz, $J_{6'a,6'b}$ 10.6 Hz, H-6'a), 4.58 (d, 1H, J 11.3 Hz, CH_2-phenyl), 4.49 (ddd, 1H, $J_{F,6'b}$ 47.6 Hz, $J_{6'a,5'}$ 1.4 Hz, $J_{6'b,6'a}$ 10.6 Hz, H-6'b), 4.36 (dd, 1H, $J_{6a,5}$ 2.1 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.15 (dd, 1H, $J_{6b,5}$ 3.0 Hz, $J_{6b,6a}$ 12.4 Hz, H-6b), 4.06-4.01 (m, 2H, H-4, H-5), 3.81-3.69 (m, 1H, H-5'), 3.65 (dd, 1H, $J_{4',3'}$ 9.3 Hz, $J_{4',5'}$ 9.9 Hz , H-4'), 2.20 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.04 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.97 (s, 3H, CH_3), 1.93 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.85-168.85 (C=O), 137.27 (C-1 phenyl), 128.67 / 128.11 (C-2,3,5,6 phenyl) 128.25 (C-4 phenyl), 96.13 (C-1'), 88.97 (C-1), 81.38 (d, $J_{F,6'}$ 173.9 Hz, C-6'), 75.08 (CH_2-phenyl), 74.86 (d, $J_{F,4'}$ 6.1 Hz, C-4'), 72.51 (C-3), 72.15 (C-4), , 71.30 (C5'), 71.16 (C3'), 70.54 (C2'), 70.21 (C5), 69.85 (C2), 62.39 (C-6), 21.10-20.51 (CH_3).

β-anomer:

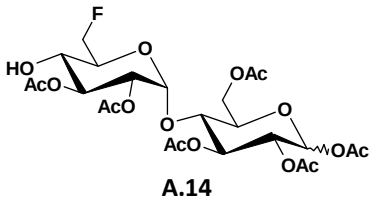
^{19}F (564.69 MHz, $CDCl_3$): δ -234.90 (td, $J_{F,6'}$ 47.7 Hz, $J_{F,5'}$ 28.9 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 7.34-7.22 (m, 5H, H-phenyl), 5.72 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 5.39 (dd, 1H, $J_{3',2'}$ 10.5 Hz, $J_{3',4'}$ 9.3, H-3'), 5.34 (d, 1H, $J_{1',2'}$ 4.0, H-1'), 5.27 (dd, 1H, $J_{3,2}$ 9.1 Hz, $J_{3,4}$ 8.7 Hz, H-3), 4.94 (dd, 1H, $J_{2,1}$ 8.1 Hz, $J_{2,3}$ 9.1 Hz, H-2), 4.77 (dd, 1H, $H_{2',1'}$ 4.0 Hz, $H_{2',3'}$ 10.5 Hz, H-2'), 4.60 (d, 1H, J 11.3 Hz, CH_2-phenyl), 4.59 (ddd, 1H, $J_{F,6'a}$ 47.7 Hz, $J_{6'a,5'}$ 2.7 Hz, $J_{6'a,6'b}$ 10.6 Hz, H-6'a), 4.49 (ddd, 1H, $J_{F,6'b}$ 47.7 Hz, $J_{6'a,5'}$ 1.4 Hz, $J_{6'b,6'a}$ 10.6 Hz, H-6'b), 4.47 (d, 1H, J 11.3 Hz, CH_2-phenyl), 4.37 (dd, 1H, $J_{6a,5}$ 2.5 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.155 (dd, 1H, $J_{6b,5}$ 4.2 Hz, $J_{6b,6a}$ 12.4 Hz, H-6b), 4.02 (dd, 1H, $J_{4,3}$ 8.7 Hz, $J_{4,5}$ 9.6 Hz, H-4), 3.79 (ddd, 1H, $J_{4,5}$ 9.6 Hz, $J_{5,6a}$ 2.5 Hz, $J_{5,6b}$ 4.2 Hz, H-5), 3.80-3.70 (m, 1H, H-5'), 3.64 (dd, 1H, $J_{4',3'}$ 9.3 Hz, $J_{4',5'}$ 9.9 Hz, H-4'), 2.075 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.98 (s, 3H, CH_3), 1.92 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.85-168.85 (C=O), 137.27 (C-1 phenyl), 128.66 / 128.09 (C-2,3,5,6 phenyl), 128.23 (C-4 phenyl), 96.12 (C-1'), 91.32 (C-1), 81.38 (d, $J_{F,6'}$ 173.9 Hz, C-6'), 75.50 (C-3), 75.04 (CH_2-phenyl), 74.85 (d, $J_{F,4'}$ 6.1 Hz, C-4'), 73.04 (C-5), 72.42- (C-4), 71.16 (C-3'), 71.10 (C-2), 70.51 (C-2'), 62.49 (C-6), 21.10-20.51 (CH_3).

MS: Calcd for [$C_{31}H_{39}FO_{16}$]: *m/z* 686.63: ESIMS found: $[M+Na]^+$ 709.2

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2',3'-di-*O*-acetyl-6'-deoxy-6'-fluoro-α-D-glucopyranosyl)-D-glucopyranoside



A.14

To a solution of compound **A.13** (100 mg, 0.15 mmol) in 10 mL ethyl acetate, 10 mg of Pd/C (10 mg) was added. The reaction mixture was flushed twice with argon and stirred over the weekend under hydrogen atmosphere at room temperature. The suspension was filtered over a celite pad and the concentrated in vacuum. Purification by column chromatography (silica gel, PE/EE 1:2) afforded compound **A.14** in 64% (56 mg) yields.

$C_{24}H_{33}FO_{16}$ M_r = 596.51

α-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -235.52 ($J_{F,6'a}$ 47.4 Hz, $J_{F,6'b}$ 47.4 Hz, $J_{F,5'}$ 25.4 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 6.21 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.50-5.47 (m, 1H, H-3), 5.40 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1'), 5.21 (dd, 1H, $J_{3',2'}$ 10.6 Hz, $J_{3',4'}$ 9.3 Hz, , H-3'), 4.94 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.2 Hz, H-2), 4.77 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 10.6 Hz, H-2'), 4.62 (ddd, 1H, $J_{F,6'a}$ 47.4 Hz, $J_{6'a,5'}$ 3.9 Hz, $J_{6'a,6'b}$ 10.3 Hz, H-6'a), 4.54 (ddd, 1H, $J_{F,6'}$ 47.4 Hz, $J_{6'b,5'}$ 1.7 Hz, $J_{6'b,6'a}$ 10.3 Hz, H-6'b), 4.40 (dd, 1H, $J_{6a,5}$ 1.8 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.17 (dd, 1H, $J_{6b,5}$ 2.9 Hz, $J_{6b,6a}$ 12.4 Hz, H-6b), 4.01-4.07 (m, 2H, H-4, H-5), 3.74 (dddd, 1H, $J_{F,5'}$ 25.4 Hz, $J_{5',4'}$ 10.1 Hz, $J_{5',6'a}$ 3.9 Hz, $J_{5',6'b}$ 1.7 Hz, H-5'), 3.63 (ddd, 1H, $J_{4',3'}$ 9.3 Hz, $J_{4',5'}$ 10.1 Hz, $J_{4',OH}$ 6.3 Hz, H-4'), 3.25 (d, 1H, $J_{4',OH}$ 6.3 Hz, 4'-OH), 2.19 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 1.99 (s, 3H, CH_3), 1.96 (s, 3H, CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 171.47 (C=O), 171.00 (C=O), 170.81 (C=O), 170.10 (C=O), 169.98 (C=O), 169.12 (C=O), 95.84 (C-1'), 88.97 (C-1), 81.66 (d, $J_{F,6'}$ 173.6 Hz, C-6'), 72.48 (C-3), 72.15 (C-3'), 72.09 ($J_{F,5'}$ 18.0 Hz, C-5'), 71.55 (C-4),70.27 (C-5), 70.07 (C-2'), 69.80 (C-2), 68.12 (d, $J_{F,4'}$ 7.4 Hz, C-4'), 62.33 (C-6), 21.12-20.51 (CH_3).

β-anomer:

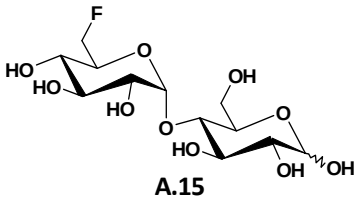
^{19}F (564.69 MHz, $CDCl_3$): δ -235.65 (td, $J_{F,6'}$ 47.3 Hz, $J_{F,5}$ 25.1 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 5.73 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.39 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.29 (dd, 1H, $J_{3,2}$ 9.3 Hz, $J_{3,4}$ 8.8 Hz, H-3), 5.18 (dd, 1H, $J_{3',2'}$ 10.5 Hz, $J_{3',4'}$ 9.4 Hz, H-3'), 4.97 (dd, 1H, $J_{2,1}$ 8.2 Hz, $J_{2,3}$ 9.3 Hz, H-2), 4.80 (dd, 1H, $H_{2',1'}$ 4.0 Hz, $H_{2',3'}$ 10.5 Hz, H-2'), 4.64 (ddd, 1H, $J_{F,6'a}$ 47.3 Hz, $J_{6'a,5}$ 3.9 Hz, $J_{6'a,6'b}$ 10.3 Hz, H-6'a), 4.57 (ddd, 1H, $J_{F,6'}$ 47.3 Hz, $J_{6'b,5}$ 1.7 Hz, $J_{6'b,6'a}$ 10.3 Hz, H-6'b), 4.46 (dd, 1H, $J_{6a,5}$ 2.3 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.18 (dd, 1H, $J_{6b,5}$ 4.0 Hz, $J_{6b,6a}$ 12.3 Hz, H-6b), 4.08 (dd, 1H, $J_{4,3}$ 8.8 Hz, $J_{4,5}$ 9.7 Hz, H-4), 3.81 (ddd, 1H, $J_{5,4}$ 9.7 Hz, $J_{5,6a}$ 2.3 Hz, $J_{5,6b}$ 4.0 Hz, H-5), 3.75 (dddd, 1H, $J_{F,5'}$ 25.1 Hz, $J_{5',4'}$ 10.1, $J_{5',6'a}$ 3.9 Hz, $J_{5',6'b}$ 1.7 Hz, H-5'), 3.65 (ddd, 1H, $J_{4',3'}$ 9.4 Hz, $J_{4',5'}$ 10.1 Hz, $J_{4',OH}$ 6.3 Hz, H-4'), 2.94 (d, 1H, $J_{OH,4'}$ 6.3 Hz, 4'-OH), 2.11 (s, 3H CH_3), 2.09 (s, 6H CH_3), 2.05 (s, 3H CH_3), 2.00 (s, 6H, CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 171.84 (C=O), 170.93 (C=O), 170.79 (C=O), 170.27 (C=O), 169.76 (C=O), 169.03 (C=O), 95.78 (C-1'), 91.39 (C-1), 81.66 (d, $J_{F,6'}$ 173.6 Hz, C-6'), 75.56 (C-3), 73.21 (C-5), 72.52 (C-3'), 72.12 (d, $J_{F,5'}$ 18.0 Hz, C-5'), 71.84 (C-4), 71.08 (C-2), 69.93 (C-2'), 68.38 (d, $J_{F,4'}$ 7.5 Hz, C-4'), 62.47 (C-6), 21.00-20.69 (CH_3).

MS: Calcd for [$C_{24}H_{33}FO_{16}$]: *m/z* 596.51: ESIMS found: $[M+Na]^+$ 619.1

4'-*O*- (6'-deoxy-6'-fluoro-α-D-glucopyranosyl)-D-glucopyranoside



A.15

According to the general procedure for compound **A.6,** deprotection of the acetyl groups of compound **A.14** (14 mg, 0.023 mmol) yielded 6 mg (75%) of the 6'-F-maltose.

$C_{12}H_{21}FO_{10}$ M_r = 344.29

α-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -235.79 (td, $J_{F,6'}$ 47.3 Hz, $J_{F,5'}$ 28.1 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 5.44 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1'), 5.22 (d, $J_{1,2}$ 3.8 Hz, H-1), 4.73 (ddd, 1H, $J_{6'a,F}$ 47.3 Hz, $J_{5',6'a}$ 3.7 Hz, $J_{6'a,6'b}$ 10.7 Hz, H-6'a), 4.68 (ddd, 1H, $J_{6'b,F}$ 47.3 Hz, $J_{5',6'b}$ 1.8 Hz, $J_{6'a,6'b}$ 10.7 Hz, H-6'b), 3.96 (dd, 1H, $J_{3,2}$ 9.9 Hz, $J_{3,4}$ 9.1 Hz, H-3), 3.93 (ddd, 1H, $J_{5,4}$ 10.0 Hz, $J_{5,6a}$ 2.3 Hz, $J_{5,6b}$ 4.6 Hz, H-5), 3.91-3.82 (m, 1H, H-5'), 3.82 (dd, 1H, $J_{6a,5}$ 2.3 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.77 (dd, 1H, $J_{6b,5}$ 4.6 Hz, $J_{6b,6a}$ 12.2 Hz, $J_{6a,6b}$ H-6b), 3.71 (dd, 1H, $J_{2',3'}$ 9.9 Hz, $J_{3',4'}$ 9.3 Hz, H-3'), 3.65 (dd, 1H, $J_{4,3}$ 9.1 Hz,

$J_{4,5}$ 10.0 Hz, H-4), 3.59 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 9.9 Hz, H-2'), 3.56 (dd, 1H, $J_{2,1}$ 3.8 Hz, $J_{2,3}$ 9.9 Hz, H-2), 3.50 (dd, 1H, $J_{3',4'}$ 9.3 Hz, $J_{4',5'}$ 10.2 Hz, H-4').

^{13}C (150.90 MHz, $CDCl_3$): δ 100.03 (C-1'), 92.24 (C-1), 82.54 (d, $J_{F,6'}$ 167.8 Hz, C-6'), 77.24 (C-4), 73.60 (C-3), 73.03 (C-3'), 71.99 (C-2'), 71.89 (C-2), 71.77 ($J_{F,5'}$ 17.4 Hz, C-5'), 70.19 (C-5), 68.63 (d, $J_{F,4'}$ 6.9 Hz, C-4'), 60.87 (C-6).

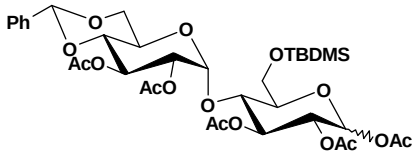
β-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -235.77 (td, $J_{F,6'}$ 47.3 Hz, $J_{F,5'}$ 28.0 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 5.44 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1'), 4.725 (ddd, 1H, $J_{6'a,F}$ 47.3 Hz, $J_{5',6'a}$ 3.7 Hz, $J_{6'a,6'b}$ 10.7 Hz, H-6'a), 4.68 (ddd, 1H, $J_{6'b,F}$ 47.3 Hz, $J_{5',6'b}$ 1.8 Hz, $J_{6'a,6'b}$ 10.7 Hz, H-6'b), 4.64 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 3.91-3.82 (m, 1H, H-5'), 3.88 (dd, 1H, $J_{6a,5}$ 2.1 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.73 (dd, 1H, $J_{6b,5}$ 5.2 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.76 (dd, 1H, $J_{3,2}$ 9.5 Hz, $J_{3,4}$ 9.1, H-3), 3.70 (dd, 1H, $J_{2',3'}$ 9.9 Hz, $J_{3',4'}$ 9.3 Hz, H-3'), 3.65 (dd, 1H, $J_{4,3}$ 9.1 Hz, $J_{4,5}$ 9.8 Hz, H-4), 3.59 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 9.9 Hz, H-2'), 3.70-3.60 (m, 1H, H-5), 3.50 (dd, 1H, $J_{3',4'}$ 9.3 Hz, $J_{4',5'}$ 10.2 Hz, H-4'), 3.26 (dd, 1H, $J_{2,1}$ 8.0 Hz, $J_{2,3}$ 9.5 Hz, H-2).

^{13}C (150.90 MHz, $CDCl_3$): δ 99.92 (C-1'), 96.12 (C-1), 82.54 (d, $J_{F,6'}$ 167.8 Hz, C-6'), 77.02 (C-4), 76.57 (C-3), 74.80 (C-5), 74.35 (C-2), 73.01 (C-3'), 71.78 ($J_{F,5'}$ 17.4 Hz, C-5'), 71.64 (C-2'), 68.60 (d, $J_{F,4'}$ 6.8 Hz, C-4'), 60.99 (C-6).

1,2,3-Tri-*O*-acetyl-4-*O*-(2',3'-di-*O*-acetyl-4',6'-benzylidene-α-D-glucopyranosyl)-6-deoxy-6-*t*-butyldimethylsilyl-D-glucopyranoside



A.17

To a cooled solution of 4',6'-benzyliden maltose **A.10** (579 mg, 1.38 mmol) and imidazol (219 mg, 1.61 mmol) in dry DMF (10 mL), *t*-butyldimethylsilyl chloride (218 mg, 1.61. mmol) was added and the reaction mixture was stirred for 24 h at room temperature. Then the solvents were removed in vacuum, the residue **A.16** was redissolved in dry pyridine (10 mL), cooled to 0°C and then acetic anhydride (2.5 mL, 26 mmol) was added. After 12 h stirring at room temperature, the mixture was poured into ice/water (100 mL). The precipitate was removed by filtration and purified by column chromatography (PE/EE 3:2) to afford the product **A.17** as colourless foam (437 mg, 43%) with an α/β = 1/0.8.

$C_{35}H_{50}O_{16}Si$ M_r = 754.85

α-anomer:

^{1}H (600.13 MHz, $CDCl_3$): δ 7.44-7.41 (m, 2H, H-phenyl), 7.36-7.34 (m, 3H, H-phenyl), 6.25 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.53 (dd, 1H, $J_{3,2}$ 10.2 Hz, $J_{3,4}$ 9.2 Hz, H-3), 5.48 (s, 1H, CH-phenyl), 5.47 (dd, 1H, $J_{3',2'}$ 10.2 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.39 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 4.881 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.2 Hz,

H-2), 4.879 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.2 Hz, H-2'), 4.32 (dd, 1H, $J_{6'a,5'}$ 4.8 Hz, $J_{6'a,6'b}$ 10.2 Hz, H-6'a), 4.15 (dd, 1H, $J_{4,3}$ 9.2 Hz, $J_{4,5}$, 9.2 Hz, H-4), 4.00-3.91 (m, 1H, 6a), 3.96-3.91 (m, 1H, H-5'), 3.87-3.85 (m, 2H, H-5, H-6b), 3.72 (dd, 1H, $J_{6'b,5'}$ 8.1 Hz, $J_{6'a,6'b}$ 10.2 Hz, H-6'b), 3.63 (dd, 1H, $J_{4',3'}$ 9.6 Hz, $J_{4',5'}$ 9.6 Hz, H-4'), 2.20-1.98 (5s, 15H, CH_3), 0.90 (s, 9H, $C(CH_3)_3$), 0.08 (s, 3H, Si- CH_3), 0.07 (s, 3H, Si- CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 170.89-169.16 (C=O), 137.03 (C-1 phenyl), 128.32/126.37 (C-2,3,5,6 phenyl), 101.76 (CH-phenyl), 96.41 (C-1'), 89.29 (C-1), 79.16 (C-4'), 73.22 (C-5), 72.50 (C-3), 70.84 (C-4), 70.17 (C-2), 68.82 (C-6'), 68.72 (C-3'), 63.76 (C-5'), 61.73 (C-6), 26.14 ($C(\underline{C}H_3)_3$), 21.23-20.63 (CH_3), 18.58 ($\underline{C}(CH_3)_3$), 4.86 (Si-CH_3).

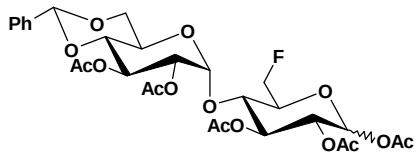
β-anomer:

^{1}H (600.13 MHz, $CDCl_3$): δ 7.44-7.41 (m, 2H, H-phenyl), 7.36-7.34 (m, 3H, H-phenyl), 5.70 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.47 (s, 1H, CH-phenyl), 5.45 (dd, 1H, $J_{3',2'}$ 10.2 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.39 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.30 (dd, 1H, $J_{3,2}$ 9.4 Hz, $J_{3,4}$ 9.0, H-3), 4.91 (dd, 1H, $J_{2,1}$ 8.2 Hz, $J_{2,3}$ 9.4 Hz, H-2), 4.86 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.2 Hz, H-2'), 4.31 (dd, 1H, $J_{6'a,5'}$ 4.8 Hz, $J_{6'a,6'b}$ 10.2 Hz, H-6'a), 4.145 (dd, 1H, $J_{4,3}$ 9.9 Hz, $J_{4,5}$ 9.5 Hz, H-4), 4.00-3.91 (m, 1H, 6a,6b), 3.96-3.91 (m, 1H, H-5'), 3.70 (dd, 1H, $J_{6'b,5'}$ 8.1 Hz, $J_{6'a,6'b}$ 10.2 Hz, H-6'b), 3.61 (dd, 1H, $J_{4',3'}$ 9.6 Hz, $J_{4',5'}$ 9.6 Hz, H-4'), 3.56 (dt, 1H, $J_{5,4}$ 9.5 Hz, $J_{5,6}$ 2.3 Hz, H-5), 2.20-1.98 (5s, 15H, CH_3), 0.89 (s, 9H, $C(CH_3)_3$), 0.06 (s, 3H, Si-CH_3), 0.05 (s, 3H, Si-CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 170.89-169.16 (C=O), 137.04 (C-1 phenyl), 128.31 (C-2,3,5,6 phenyl), 129.9 (C-4 phenyl), 101.77 (CH-phenyl), 96.13 (C-1'), 91.56 (C-1), 79.17 (C-4'), 75.63 (C-3), 75.65 (C-5), 71.42/71.36/71.31/70.27 (C-2'α/β, C-2, C-4), 68.81 (C-6'), 68.70 (C-3'), 63.66 (C-5'), 61.70 (C-6), 26.16 ($\underline{C}(CH_3)_3$), 21.23-20.63 (CH_3), 18.66 ($C(\underline{C}H_3)_3$), 4.88 (Si-CH_3).

MS: Calcd for [$C_{35}H_{50}O_{16}Si$]: *m/z* 754.85: ESIMS found: $[M+Na]^+$ 777.3

1,2,3-Tri-O-acetyl-4-O-(2',3'-di-O-acetyl-4',6'-benzylidene-α-d-glucopyranosyl)-6-deoxy-6-fluoro-d-glucopyranoside



A.18

Deprotection of the silyl group and subsequent fluorination was performed in a one pot procedure using Deoxofluor. The TBDMS protected maltose derivative **A.17** (190 mg, 0.25 mmol) was refluxed with 50% Deoxofluor solution (in toluene, 1.1 g, 2.5 mmol) and dry CH_2CL_2 (5 mL) for 48 h. The solution was cooled to room temperature, quenched with methanol (2 mL) neutralized with conc. ammonia (pH~7) and concentrated under vacuum. The residue was chromatographed on silica gel (PE/EE 3:2) to obtain product **A.18** as colorless foam (27 mg, 17%).

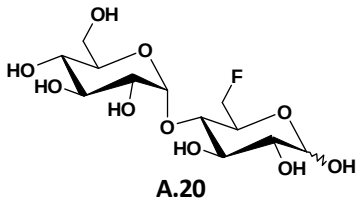
$C_{29}H_{35}FO_{15}$ M_r = 642.58

^{19}F (564.69 MHz, $CDCl_3$): δ -237.43 (ddd, $J_{F,6a}$ 47.1 Hz, $J_{F,6b}$ 48.0 Hz, $J_{F,5}$ 30.2 Hz).

^{1}H (400.13 MHz, $CDCl_3$): δ 7.45-7.43 (m, 2H, H-phenyl), 7.37-7.34 (m, 3H, H-phenyl), 6.28 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.56 (ddd, 1H, $J_{3,2}$ 10.1 Hz, $J_{3,4}$ 9.3 Hz, $J_{3,F}$ 0.8 Hz, H-3), 5.50 (s, 1H, CH-phenyl), 5.48 (dd, 1H, $J_{3',2'}$ 10.2 Hz, $J_{3',4'}$ 9.6 Hz, H-3'), 5.43 (d, 1H, $J_{1',2'}$ 4.2 Hz, H-1'), 4.97 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.1 Hz, H-2), 4.90 (dd, 1H, $J_{2',1'}$ 4.2 Hz, $J_{2',3'}$ 10.2 Hz, H-2'), 4.79 (ddd, 1H, $J_{F,6a}$ 47.1 Hz, $J_{6a,5}$ 2.2 Hz, $J_{6a,6b}$ 10.9 Hz, H-6a), 4.63 (ddd, 1H, $J_{F,6b}$ 48.0 Hz, $J_{6b,5}$ 1.0 Hz, $J_{6a,6b}$ 10.9 Hz, H-6b), 4.31 (dd, 1H, $J_{6'a,5'}$ 94.3Hz, $J_{6'a,6'b}$ 9.8 Hz, H-6'a), 4.21 (dd, 1H, $J_{4,3}$ 9.3 Hz, $J_{4,5}$ 9.9 Hz, H-4), 4.03 (dddd, 1H, $J_{F,5}$ 30.2 Hz, $J_{5,4}$ 9.9 Hz, $J_{5,6a}$ 2.2 Hz, $J_{5,6b}$ 1.0 Hz, H-5), 3.81 (ddd, $J_{5',4'}$ 9.8 Hz, $J_{5',6'a}$ 4.3 Hz, $J_{5',6'b}$ 9.6 Hz, H-5'), 3.74 (t, $J_{6'b,5'}$ 9.8 Hz, $J_{6'b,6'a}$ 9.8 Hz, H-6'b), 3.66 (t, 1H, $J_{4',3'}$ 9.6 Hz, $J_{4',5'}$ 9.6 Hz, H-4'), 2.21 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.07 (s, 3H, CH_3), 2.03 (s, 3H, CH_3), 1.99 (s,3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 170.92 (C=O), 170.18 (C=O), 169.97 (C=O), 169.88 (C=O), 169.07 (C=O), 136.87 (C-1 phenyl), 128.38/126.22 (C-2,3,5,6 phenyl), 129.24 (C-4 phenyl), 101.53 (CH-phenyl), 96.57 (C-1'), 89.21 (C-1), 81.36 (d, $J_{F.6}$ 176.8 Hz, C-6), 78.75 (C-4'), 72.52 (C-3), 71.44 (d, $J_{F,5}$ 18.3 Hz, C-5), 71.01 (C-2'), 70.05 (d, $J_{F,4}$ 7.6 Hz, C-4), 69.98 (C-2), 68.72 (C-3'), 68.56 (C-6'), 64.00 (C-5'), 21.13 (CH_3), 21.09 (CH_3), 20.92 (CH_3), 20.73 (CH_3), 20.55 (CH_3).

4-*O*-(α-D-glucopyranosyl)-6-deoxy-6-fluoro-D-glucopyranoside



A.20

Deprotection was performed by refluxing compound **A.18** (13 mg, 0.02 mmol) with conc. acetic acid (2 mL) for ½ h. The reaction mixture was poured into water (2 mL) and extracted with ethyl acetate (3x 5 mL). The combined organic layers were washed with 10% aqueous $NaHCO_3$ and brine (à 5 mL), dried over $MgSO_4$, concentrated under reduced pressure and chromatographed over silica (PE/EE 2:7) to yield product **A.19** (8 mg, 73%). Deprotection of the acetyl groups (8 mg, 0.014 mmol) with sodium methoxide (0.4 equiv.) according to the procedure reported for compound **6** afforded 6-deoxy-6-fluoro maltose **A.20** (2 mg, 40%).

$C_{12}H_{21}FO_{10}$ M_r = 344.29

α-anomer:

^{19}F (564.69 MHz, D_2O): δ -234.88 (m).

^{1}H (600.13 MHz, D_2O): δ 5.46 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1'), 5.24 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.74 (ddd, 1H, $J_{6a,F}$ 47.0 Hz, $J_{6a,5}$ 3.0 Hz, $J_{6a,6b}$ 10.7 Hz, H-6a), 4.64 (ddd, 1H, $J_{6b,F}$ 48.1 Hz, $J_{6b,5}$ 1.7 Hz, $J_{6a,6b}$ 10.7 Hz, H-6b), 4.11-4.03 (m, 1H, H-5), 3.98 (dd, 1H, $J_{3,2}$ 9.8 Hz, $J_{3,4}$ 8.9 Hz, H-3), 3.85 (dd, 1H, $J_{6'a,5'}$ 2.2 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6'a), 3.77-3.67 (m, 3H, H-6'b, H-4, H-5'), 3.69 (dd, 1H, $J_{3',2'}$ 9.9 Hz, $J_{3',4'}$ 9.3 Hz, H-3'), 3.575 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 9.9 Hz, H-2'), 3.57 (dd, 1H, $J_{2,1}$ 3.8 Hz, $J_{2,3}$ 9.8 Hz, H-2), 3.41 (dd, 1H, $J_{4',3'}$ 9.3 Hz, $J_{4',5'}$ 9.9 Hz, H-4').

^{13}C (150.90 MHz, D_2O): δ 99.77 (C-1'), 92.39 (C-1), 82.76 (d, $J_{F,6}$ 168.2 Hz, C-6), 75.47 (d, $J_{F,4}$ 6.4 Hz, C-4), 73.56 (C-3), 73.14-73.10 (C-3',5'), 72.02 (C-2'), 71.60 (C-2), 69.68 (C-4'), 69.14 (d, $J_{F,5}$ 17.3 Hz, C-5), 60.83 (C-6).

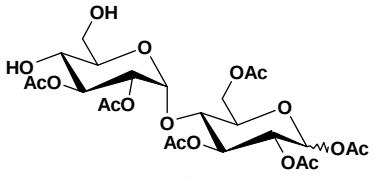
β-anomer:

^{19}F (564.69 MHz, D_2O): δ -235.41 (t, $J_{F,6}$ 47.5 Hz, $J_{F,5}$ 30.7 Hz).

^{1}H (600.13 MHz, D_2O): δ 5.457 (d, 1H, $J_{1',2'}$ 3.9 Hz, H-1'), 4.80 (ddd, 1H, $J_{6a,F}$ 47.2 Hz, $J_{6a,5}$ 2.9 Hz, $J_{6a,6b}$ 10.7 Hz, H-6a), 4.70 (ddd, 1H, $J_{6b,F}$ 47.9 Hz, $J_{6b,5}$ 1.7 Hz, $J_{6a,6b}$ 10.7 Hz, H-6b), 4.69 (d, 1H, $J_{1,2}$ 8.1 Hz, H-1), 3.84 (dd, 1H, $J_{6'a,5'}$ 2.3 Hz, $J_{6'a,6'b}$ 12.4 Hz, H-6a'), 3.79 (dd, 1H, $J_{3,2}$ 9.0 Hz, $J_{3,4}$ 8.0 Hz, H-3), 3.77-3.67 (m, 4H, H-6'b, H-4, H-5, H-5'), 3.68 (dd, 1H, $J_{3',2'}$ 10.0 Hz, $J_{3',4'}$ 9.3 Hz, H-3'), 3.576 (dd, 1H, $J_{2',1'}$ 3.9 Hz, $J_{2',3'}$ 10.0 Hz, H-2'), 3.41 (dd, 1H, $J_{4',3'}$ 9.3 Hz, $J_{4',5'}$ 9.9 Hz, H-4'), 3.28 (dd, 1H, $J_{2,1}$ 8.1 Hz, $J_{2,3}$ 9.0 Hz, H-2).

^{13}C (150.90 MHz, D_2O): δ 99.70 (C-1'), 96.27 (C-1), 82.50 (d, $J_{F,6}$ 168.6 Hz, C-6), 76.45 (C-3), 75.26 (d, $J_{F,4}$ 6.6 Hz, C-4), 74.27 (C-2), 73.43 (d, $J_{F,5}$ 17.8 Hz, C-5), 73.14-73.10 (C-3',5'), 71.94 (C-2'), 69.65 (C-4'), 60.83 (C-6).

1,2,3,6-Tetra-*O*-acetyl-4'-*O*-(2',3'-di-*O*-acetyl-α -D-glucopyranosyl)-D-glucopyranoside



A.21

Maltose derivative **A.10** was peracetylated under standard conditions. The benzylidene group was then selectively cleaved, starting from 868 mg (1.27 mmol) of derivative **A.11** and according the procedure described for compound **A.20**. The crude mixture was purified by chromatography over silica (PE/EE 1:5) to yield product **A.21** (576 mg, 76%) with a α/β ratio of 5:2.

$C_{24}H_{34}O_{17}$ M_r = 594.52

α-anomer:

^{1}H (600.13 MHz, $CDCl_3$): δ 6.24 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.51 (dd, 1H, $J_{3,2}$ 10.2 Hz, $J_{3,4}$ 8.8 Hz, H-3), 5.37 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.23 (dd, 1H, $J_{3',2'}$ 10.6 Hz, $J_{3',4'}$ 8.8 Hz, H-3'), 4.97 (dd, 1H, $J_{2,1}$ 3.7 Hz, $J_{2,3}$ 10.2 Hz, H-2), 4.78 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.6 Hz, H-2'), 4.47 (dd, 1H, $J_{6a,5}$ 2.3 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.22 (dd, 1H, $J_{6b,5}$ 3.7 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.10 (ddd, 1H, $J_{5,4}$ 10.0 Hz, $J_{5,6a}$ 2.3 Hz, $J_{5,6b}$ 3.7 Hz, H-5), 4.04 (dd, 1H, $J_{4,3}$ 8.8 Hz, $J_{4,5}$ 10.0 Hz, H-4), 3.84-3.81 (m, 2H, H-6'a, H-6'b), 3.71-3.65 (m, 2H, H-4', H-5'), 2.93 (d, 1H, $J_{4',OH}$ 4.9 Hz, 4'-OH), 2.22 (s, 3H, CH_3), 2.14 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.08 (s, 3H, CH_3), 2.02 (s, 3H, CH_3), 1.99 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 171.56 (C=O), 171.22 (C=O), 170.95 (C=O), 170.10 (C=O), 170.04 (C=O), 169.14 (C=O), 96.07 (C-1'), 89.01 (C-1), 72.76 (C-5'), 72.53 (C-3), 72.19 (C-3'), 72.00 (C-4), 70.42 (C-5), 70.28 (C-2'), 70.09 C-4'), 69.44 (C-3'), 69.83 (C-2), 62.74 (C-6), 62.45 (C-6'), 21.21-20.60 (CH_3).

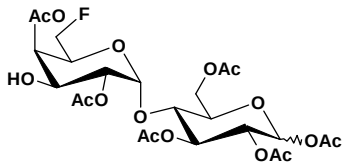
β-anomer:

^{1}H (60013 MHz, $CDCl_3$): δ 5.74 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.35 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.29 (dd, 1H, $J_{3,2}$ 9.3 Hz, $J_{3,4}$ 8.8 Hz, H-3), 5.19 (dd, 1H, $J_{3',2'}$ 10.6 Hz, $J_{3',4'}$ 9.1 Hz, H-3'), 4.98 (dd, 1H, $J_{2,1}$ 8.2 Hz, $J_{2,3}$ 9.3 Hz, H-2), 4.77 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.6 Hz, H-2'), 4.49 (dd, 1H, $J_{6a,5}$ 2.4 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.21 (dd, 1H, $J_{6b,5}$ 4.4 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.03 (dd, 1H, $J_{4,3}$ 8.8 Hz, $J_{4,5}$ 9.7 Hz, H-4), 3.84-3.78 (m, 3H, H-5, H-6'a, H-6'b), 3.71-3.65 (m, 2H, H-4', H-5'), 2.96 (d, 1H, $J_{4',OH}$ 4.9 Hz, 4'-OH), 2.13 (s, 3H, CH_3), 2.09 (s, 3H, CH_3), 2.10 (s, 3H, CH_3), 2.05 (s, 3H, CH_3), 2.012 (s, 3H, CH_3), 2.01 (s, 3H, CH_3).

^{13}C (100.61 MHz, $CDCl_3$): δ 171.63 (C=O), 171.23 (C=O), 170.91 (C=O), 170.24 (C=O), 170.04 (C=O), 168.99 (C=O), 95.96 (C-1'), 91.40 (C-1), 75.53 (C-3), 73.30 (C-5), 72.78 (C-5'), 72.23 (C-3'), 72.12 (C-4), 71.08 (C-2), 70.20 (C-2'), 70.13 (C-4'), 62.82 (C-6), 62.42 (C-6'), 21.21-20.60 (CH_3).

MS: Calcd for [$C_{24}H_{34}O_{17}$]: *m/z* 594.52: ESIMS found: $[M+Na]^+$ 617.6

1,2,3,6-Tetra-*O*-acetyl-4-*O*-(2',4'-di-*O*-acetyl-6'-deoxy-6'-fluoro-α-D-galactopyranosyl)-D-glucopyranoside



A.22

According to the general procedure for compound **A.13,** fluorination of the 6'-hydroxy group was carried out under microwave promotion, starting from 170 mg (0.29 mmol) of derivative **A.21**. The crude mixture was purified by flash column chromatography (silica gel, PE/EE 1:2) to give 50 mg (30%) of compound **A.22**.

$C_{24}H_{33}FO_{16}$ M_r = 596.51

α-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -230.88 (td, $J_{F,6'}$ 46.8 Hz, $J_{F,5'}$ 15.1 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 6.24 (d, 1H, $J_{1,2}$ 3.7 Hz, H-1), 5.51 (dd, 1H, $J_{3,2}$ 10.2 Hz, $J_{3,4}$ 8.7 Hz, H-3), 5.47 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.39 (dd, 1H, $J_{4',3'}$ 3.4 Hz, $J_{4',5'}$ 1.4 Hz, H-4'), 5.00 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.7 Hz, H-2'), 4.98 (dd, 1H, $H_{2,1}$ 3.7 Hz, $H_{2,3}$ 10.2 Hz, H-2), 4.48 (dd, 1H, $J_{6a,5}$ 2.1 Hz, $J_{6a,6b}$ 12.4 Hz, H-6a), 4.41 (ddd, 1H, $J_{F,6'a}$ 46.2 Hz, $J_{6'a,5'}$ 4.3 Hz, $J_{6'a,6'b}$ 9.9 Hz, H-6'a), 4.38 (ddd, 1H, $J_{F,6'b}$ 47.4 Hz, $J_{6'b,5'}$ 6.6 Hz, $J_{6'b,6'a}$ 69.9 Hz, H-6'b), 4.15 (dd, 1H, $J_{3',2'}$ 10.7 Hz, $J_{3',4'}$ 3.4 Hz, H-3'), 4.17-4.12 (m, 1H, H-5'), 4.10 (dd, 1H, $J_{6b,5}$ 3.5 Hz, $J_{6a,6b}$ 12.4 Hz, H-6b), 4.08 (dd, 1H, $J_{4,3}$ 8.7 Hz, $J_{4,5}$ 10.0 Hz, H-4), 4.04 (ddd, 1H, $J_{5,4}$ 9.9 Hz, $J_{5,6a}$ 2.1 Hz, $J_{5,6b}$ 3.5 Hz, H-5), 2.19-1.99 (CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 171.57 (C=O), 170.99 (2 C=O), 170.17 (C=O), 169.96 (C=O), 169.04 (C=O), 96.27 (C-1'), 89.05 (C-1), 81.78 (d, $J_{F,6'}$ 172.5 Hz, C-6'), 72.43 (C-3), 71.70 (C-4), 70.52 (C-5), 70.48 (C-2'), 70.39 (d, $J_{F,4'}$ 6.1 Hz, C-4'), 69.80 (C-2), 68.94 (d, $J_{F,5'}$ 21.6 Hz, C-5'), 65.99 (C-3'), 62.27 (C-6), 21.18-20.59 (CH_3).

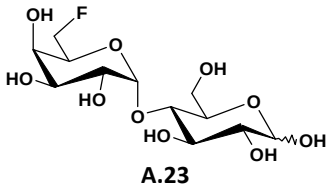
β-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -230.81 (td, $J_{F,6'}$ 46.8 Hz, $J_{F,5'}$ 15.3 Hz).

^{1}H (600.13 MHz, $CDCl_3$): δ 5.73 (d, 1H, $J_{1,2}$ 8.2 Hz, H-1), 5.45 (d, 1H, $J_{1',2'}$ 4.0 Hz, H-1'), 5.39 (dd, 1H, $J_{4',3'}$ 3.5 Hz, $J_{4',5'}$ 1.4 Hz, H-4'), 5.28 (dd, 1H, $J_{3,2}$ 9.5 Hz, $J_{3,4}$ 8.8 Hz, H-3), 4.99 (dd, 1H, $J_{2',1'}$ 4.0 Hz, $J_{2',3'}$ 10.7 Hz, H-2'), 4.98 (dd, 1H, $H_{2,1}$ 8.2 Hz, $H_{2,3}$ 9.5 Hz, H-2), 4.50 (dd, 1H, $J_{6a,5}$ 2.3 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 4.42 (ddd, 1H, $J_{F,6'a}$ 46.2 Hz, $J_{6'a,5'}$ 4.3 Hz, $J_{6'a,6'b}$ 9.9 Hz, H-6'a), 4.38 (ddd, 1H, $J_{F,6'b}$ 47.4 Hz, $J_{6'b,5'}$ 6.7 Hz, $J_{6'b,6'a}$ 9.9 Hz, H-6'b), 4.14 (dd, 1H, $J_{3',2'}$ 10.7 Hz, $J_{3',4'}$ 3.5 Hz, H-3'), 4.18-4.11 (m, 1H, H-5'), 4.09 (dd, 1H $J_{6b,5}$ 4.4 Hz, $J_{6b,6a}$ 12.3 Hz, H-6b), 4.07 (dd, 1H, $J_{4,3}$ 8.8 Hz, $J_{4,5}$ 9.7 Hz, H-4), 3.77 (ddd, 1H, $J_{5,4}$ 9.7 Hz, $J_{5,6a}$ 2.2 Hz, $J_{5,6b}$ 4.4 Hz, H-5), 2.14-2.01 (CH_3).

^{13}C (150.90 MHz, $CDCl_3$): δ 171.59 (C=O), 170.97 (C=O), 170.96 (C=O), 170.27 (C=O), 169.73 (C=O), 169.04 (C=O), 96.13 (C-1'), 91.41 (C-1), 81.83 (d, $J_{F,6'}$ 172.6 Hz, C-6'), 75.59 (C-3), 73.43 (C-5), 71.73 (C-4), 71.06 (C-2), 70.49 (C-2'), 70.40 (d, $J_{F,4'}$ 6.2 Hz, C-4'), 68.95 (d, $J_{F,5'}$ 21.5 Hz, C-5'), 66.05 (C-3'), 62.42 (C-6), 21.05-20.69 (CH_3).

4'-*O*-(6'-deoxy-6'-fluoro-α-D-galacatopyranosyl)-D-glucopyranoside



A.23

According to the general procedure for compound **A.6**, deprotection of the acetyl groups of compound **A.22** (37 mg, 0.062 mmol) afforded derivative **A.23** in 99% (21 mg).

$C_{12}H_{21}FO_{10}$ M_r = 344.29

α-anomer:

^{19}F (564.69 MHz, D_2O): δ -230.42 (ddd, $J_{F,6'a}$ 45.4 Hz, $J_{F,6'b}$ 48.1 Hz, $J_{F,5'}$ 16.4 Hz).

^{1}H (600.13 MHz, D_2O): δ 5.412 (d, 1H, $J_{1',2'}$ 3.0 Hz, H-1'), 5.22 (d, 1H, $J_{1,2}$ 3.8 Hz, H-1), 4.66 (ddd, 1H, $J_{F,6'a}$ 45.4 Hz, $J_{5',6'a}$ 3.5 Hz, $J_{6'a,6'b}$ 10.1 Hz, H-6'a), 4.60 (ddd, 1H, $J_{F,6'b}$ 48.1 Hz, $J_{5',6'b}$ 7.7 Hz, $J_{6'a,6'b}$ 10.1 Hz, H-6'b), 4.31-4.25 (m, 1H, H-5'), 4.04-4.03 (m, 1H, H-4'), 3.95 (dd, 1H, $J_{3,2}$ 10.0 Hz, $J_{3,4}$ 8.9 Hz, H-3), 3.90-3.85 (m, 3H, H-5, H-2', H-3'), 3.85 (dd, 1H, $J_{6a,5}$ 2.4 Hz, $J_{6a,6b}$ 12.2 Hz, H-6a), 3.78 (dd, 1H, $J_{6b,5}$ 5.1 Hz, $J_{6a,6b}$ 12.2 Hz, H-6b), 3.60 (dd, 1H, $J_{4,3}$ 8.9 Hz, $J_{4,5}$ 10.0 Hz, H-4), 3.57 (dd, 1H, $J_{2,1}$ 3.8 Hz, $J_{2,3}$ 10.0 Hz, H-2).

^{13}C (150.90 MHz, D_2O): δ 100.70 (C-1'), 92.22 (C-1), 83.92 (d, $J_{F,6'}$ 165.4 Hz, C-6'), 78.24 (C-4), 73.61 (C-3), 71.52 (C-2), 70.46 (d, $J_{F,5'}$ 19.7 Hz, C-5'), , 70.35 (C-5), 69.39 (d, $J_{F,3'}$ 1.7 Hz, C-3'), 69.22 (d, $J_{F,4'}$ 7.6 Hz, C-4'), 69.92 (C-2'), 61.01 (C-6).

β-anomer:

^{19}F (564.69 MHz, $CDCl_3$): δ -230.41 (ddd, $J_{F,6'a}$ 45.4 Hz, $J_{F,6'b}$ 48.1 Hz, $J_{F,5'}$ 16.4 Hz).

^{1}H (600.13 MHz, D_2O): δ 5.416 (d, 1H, $J_{1',2'}$ 3.0 Hz, H-1'), 4.66 (ddd, 1H, $J_{F,6'a}$ 45.4 Hz, $J_{5',6'a}$ 3.5 Hz, $J_{6'a,6'b}$ 10.1 Hz, H-6'a), 4.64 (d, 1H, $J_{1,2}$ 8.0 Hz, H-1), 4.60 (ddd, 1H, $J_{F,6'b}$ 48.1 Hz, $J_{5',6'b}$ 7.7 Hz, $J_{6'a,6'b}$ 10.1 Hz, H-6'b), 4.31-4.25 (m, 1H, H-5'), 4.04-4.03 (m, 1H, H-4'), 3.90 (dd, 1H, $J_{6a,5}$ 2.1 Hz, $J_{6a,6b}$ 12.3 Hz, H-6a), 3.88-3.85 (m, 2H, H-2', H-3'), 3.74 (dd, 1H, $J_{6b,5}$ 5.1 Hz, $J_{6a,6b}$ 12.3 Hz, H-6b), 3.76-3.73 (m, 1H, H-3), 3.60 (dd, 1H, $J_{4,3}$ 8.9 Hz, $J_{4,5}$ 10.0 Hz, H-4), 3.60-3.56 (m, 1H, H-5), 3.27 (dd, 1H, $J_{2,1}$ 8.0 Hz, $J_{2,3}$ 9.6 Hz, H-2).

^{13}C (150.90 MHz, D_2O): δ 100.59 (C-1'), 96.12 (C-1), 83.92 (d, $J_{F,6'}$ 165.4 Hz, C-6'), 77.99 (C-4), 76.58 (C-3), 74.94 (C-5), 74.22 (C-2), 70.48 (d, $J_{F,5'}$ 19.7 Hz, C-5'), 69.38 (d, $J_{F,3'}$ 1.7 Hz, C-3'), 69.20 (d, $J_{F,4'}$ 7.7 Hz, C-4'), 68.82 (C-2'), 61.16 (C-6).

MS: Calcd for $[C_{12}H_{21}FO_{10}]$: *m/z* 344.29: ESIMS found: $[M+Na]^+$ 367.2

CHAPTER- B

PRELIMINARY EXPERIMENTS REGARDING ENZYMATIC SYNTHESIS OF FLUORINATED DISACCHARIDES

ABSTRACT

Glycosidases have been used for the synthesis of a variety of disaccharides, oligosaccharides and their derivatives. Syntheses of disaccharides using β–galactosidase from *Aspergillus oryzae* have been successfully performed by transglycosidation strategies by various groups including Nilsson[90], Schmid[91], Thiem[92], and many others.

The regioselectivity of glycosidase-catalysed reactions can be changed by manipulation of the acceptor glycoside and by varying the aglycon structure.[93,94,95] Recently it was found that the introduction of fluorine in position-6 of the glycosyldonor enhances the selectivity of this chemical glycosylation reaction. It is hypothesized that the nature of the substituent in position-6 affects the stereochemical outcome of these chemical glycosylation reactions by influencing the stability of the oxocarbenium intermediate and therefore the key equilibrium by its electron withdrawing properties.[96]

The initial idea behind this project was to explore the influence of fluorinated glycosyl donors and acceptors on the enzymatic reaction with β–galactosidase. In case that the enzyme accepts the fluorinated substrates, the preferred β(1-6) linkage is blocked and enzymatic reactions should result in normally discriminated linkage or eventually in new combinations. Furthermore, the fluorine nucleus allows the application of ^{19}F-NMR and therefore the detection of conversion, position and possibly interactions of labeled carbohydrates.

Synthetic strategies for the glycosidation reaction include the application of the well known and very effective *p*-nitrophenyl-β-galactoside as donor substrate (native and fluorinated) and β-thiophenylglucosides and -galactosides and their fluorinated derivatives as acceptors. The reasons for using thioglycosides as acceptor molecules for the enzymatic reaction are on one hand their stability and on the other hand the possibility to subsequently activating them as donor for chemical glycosylation procedures.[97,98] Aglycons containing a large and non-polar phenyl group normally increase the yield of such reactions.

This chapter focuses on the syntheses of potential monosaccharide precursors for β-galactosidase catalyzed transglycosylation.

THEORETICAL BACKGROUND

FLUORINE IN ORGANIC CHEMISTRY

Figure 29. Of course the elements are earth, water, fire and air. But what about fluorine? Surely you can't ignore fluorine.[99,2]

For more details to fluorine, fluorinated organic compounds, fluorination methods and ^{19}F-NMR please have a look at Chapter A's theoretical background.

ENZYME-CATALYZED GLYCOSIDE SYNTHESIS

In the last two decades, impressive syntheses of complex oligosaccharides have been established by chemical methods. The limitations of these multistep approaches are still unsolved: the requirement of extensive protection-deprotection sequences. To accomplish these difficulties, enzymes catalyzed glycosidic bond formations are introduced for synthetic strategies. Approaches using *glycosyltransferases* are promising and offer regioselective transfer and high yields, but they are usually expensive, not available in large quantities and highly selective with respect to the glycosyl acceptors. Methods using the transglycosylation activity of *glycoside hydrolases (glycosidases)* are a promising alternative.

GLYCOSYLTRANSFERASES

The biosynthesis of oligosaccharides in nature is achieved by glycosyltransferases. These enzymes are divided into two groups: enzymes of the Leloir pathway and those of non-Leloir pathways.

- **Leloir transferases** utilize activated glycosyl esters of nucleoside mono- or diphosphates as glycosyl donors. Enyzmes of this pathway are responsible for the synthesis of most *N*- and *O*-linked glycoproteins and other glycoconjugates in the endoplasmic reticulum and the Golgi apparatus.

- **Non-Leloir transferases** typically operate with glycosyl phosphates as activated donors. Glucan phosphorylases are a general example of this group of enzymes catalyzing the synthesis of oligo- and polysaccharides, including sucrose and trehalose phosphorylase.

The main advantage of this method is the high regio- and stereoselectivity induced by the enzymes. Cost of the enzymes and the nucleoside donors as well as unique substrate specificity for single enzymes are unfavorable.

GLYCOSIDASES

Nature provides plenty of enzymes that can hydrolyze the glycosidic linkage. Practically all organisms have the need to degrade polysaccharides into small molecules which can pass membranes and therefore provide a source of energy. Glycosidases can be divided into two major mechanistic classes by the method how they hydrolyze glycosidic linkage: under retention or inversion of the anomeric configuration.

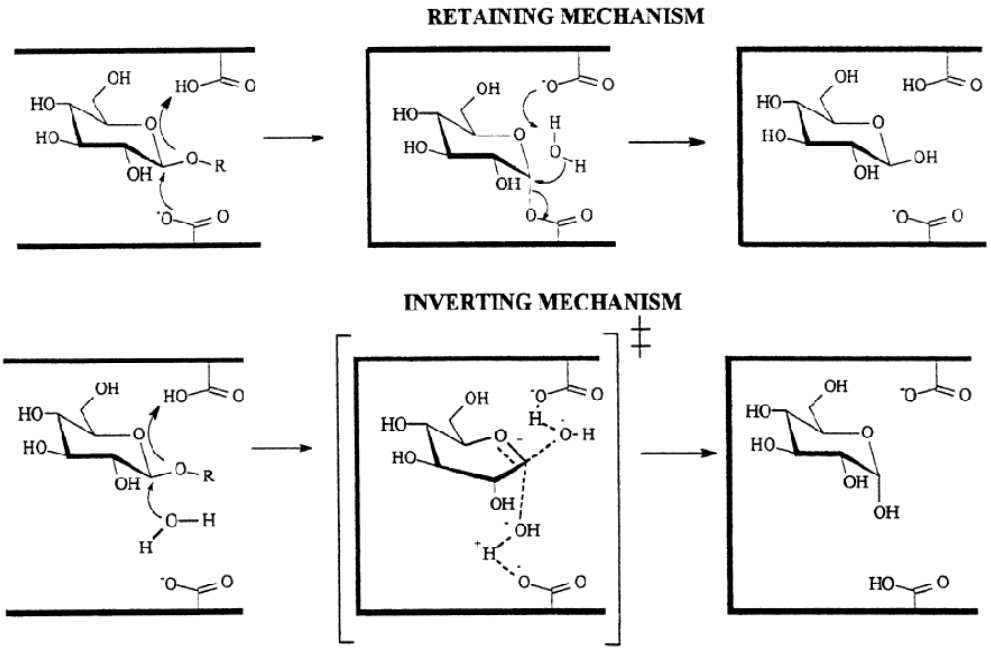
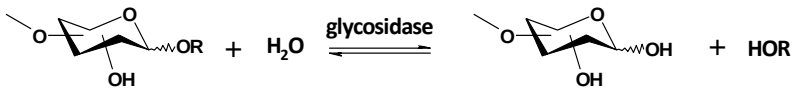


Figure 30. Mechanism of inverting and retaining glycosidases.[100]

Glycosidases are stereospecific catalysts, available in large quantities and allow for application on less complex donor substrates. They usually cleave glycosidic bonds, but can also be used for the reversed reactions of glycosidic bond formation under equilibrium (thermodynamic) or kinetic controlled conditions.[101,102,103,104,105]



In the *thermodynamic approach* control of the equilibrium in the desired direction is necessary. This can be achieved by increasing concentrations of alcohol acceptor and sugar donor as well as reduction of the amounts of water and variation of temperature. This method provides poor yields of desired products and purification is often difficult due to excess of starting material and possible side products.

The more successful *kinetically controlled approach* can be achieved by modulation of the substrate with superior leaving groups, thus changing the kinetics of the enzyme and substrate binding. The efficiently formation of the "glycosyl enzyme" intermediate from a reactive donor is crucial, followed by rapid transfer of the glycosyl residue to an acceptor alcohol rather than water. Therefore the enzyme must have a binding site for the acceptor alcohol. This method is also called *transglycosylation.*

Appropriate glycosyl donors are aryl glycosides, glycosyl fluorides and di- or oligosaccharides. These reactions need to be controlled carefully to reduce glycoside hydrolysis. Therefore these reactions must be quenched when the glycosyldonor is consumed. These enzymes recognize broad spectra of glycosylacceptors, including other monosaccharides, diols, oximes, allyl and propargyl alcohols, steroids, alkaloids and amino acids.

GLYCOSYNTHASES

A new approach was developed by Steve Withers et al. using a protein engineered glycosidase mutant that could carry out the desired glycosyl transfer reaction but which could not hydrolyze these products once formed.[106] The best method for a complete elimination of this activity in a retaining glycosidase through a single mutation is to change the catalytic nucleophile (-COOH) to an non-nucleophile residue (e.g. alanine). This kind of glycosynthase could accept glycosyl fluorides of the wrong configuration.

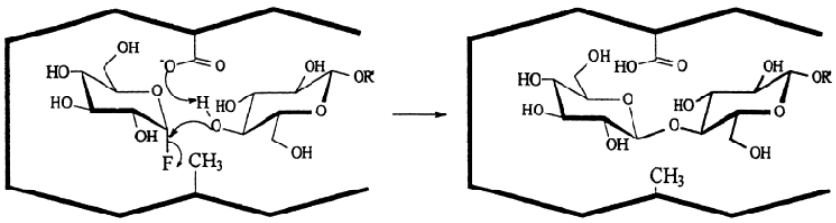


Figure 31. Mechanism of glycoside synthesis with a glycosynthase.[107]

ENZYMATIC GLYCOSYLATION USING β-GALACTOSIDASE

Complex carbohydrates and carbohydrate conjugates have structural functions or serve as information carriers in biological systems. Many cell-surface carbohydrates of glycoconjugates are involved in a variety of fundamental biochemical recognition processes, including development, growth, cell adhesion, metastasis, immune response, infection and numerous signal

transductions. The elucidation of these compounds and their function greatly depends on the availability of the corresponding oligosaccharide structure. Their isolation is very difficult, because they exist in minuscule quantities. Although only a limited number of monosaccharides are commonly found as building blocks in glycoproteins and glycolipids in mammalian systems, their multifunctional linkage allows the assembly of an immense variety of complex structures considering the branching and stereochemistry of glycosylation and modification. The enzymes involved in formation and degradation of oligosaccharides are very interesting for many scientists to mimic nature. These glycoenzymes are also of great interest for medicinal chemists as targets for inhibition due to their ability to cause diseases and metabolic disorders.

β-*GALACTOSIDASE*

β-Galactosidase is one of the best characterized glycosylhydrolase catalyzing *in vivo* the hydrolysis of lactose to glucose and galactose.[108] A second catalytic activity of this enzyme is the conversion of lactose in allolactose, which is the natural inducer for the *lac* operon. However, this enzyme is widely distributed in nature and can be found in yeast, bacteria, fungi, animal organs and plants (especially peaches, apricots, almonds and apples). β-galactosidase from different origins varies considerably in their mode of action, although the specific properties of the enzyme remains nearly the same. Bacterial β-galactosidase from *Escherichia coli* and fungal β-galactosidase from *Aspergillus oryzae* are the most prominent ones. Traditionally, the most intense research work has been done on the enzyme of *Escherichia coli.* For this representative crystal structural data are available and therefore mechanistic questions can be partially solved.

The crystal structure[109,110] of β–galactosidase of *Escherichia coli* (product of the *lacZ* operon) shows a 464 kDa tetramer of four identical 1023 amino acid chains. Each chain consists of five domains; every third is an eight-stranded α/β barrel that comprises much of the active site.

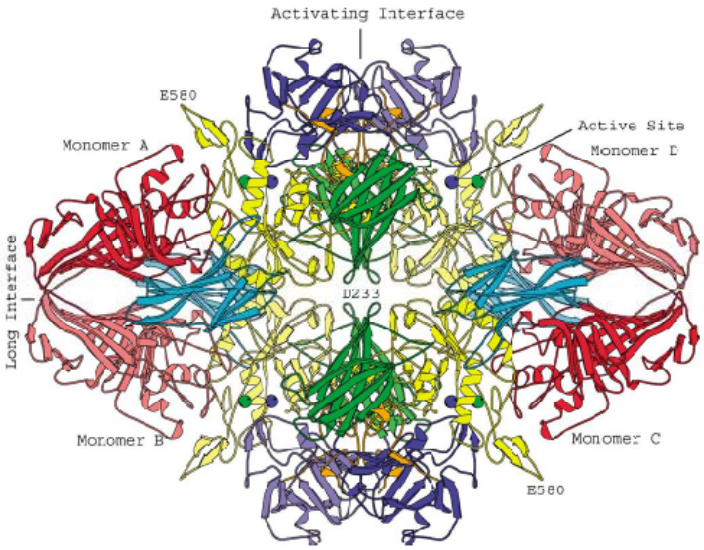


Figure 32. View of the β–galactosidase tetramer looking down one of the two-fold axes.[110]

These active sites are well separated and supposedly act independently. Individual monomers of the enzyme are probably inactive due to the fact that it takes two monomers to complete an active site. Mg^{2+} and Na^{+} are required for full activity of the enzyme; one of each was identified in the active site. Important residues for catalysis in or near the active site are Glu 461, Glu 537, Tyr 503. Catalytic activity proceeds via the formation of a covalent galactosyl intermediate with Glu537 including "shallow" and "deep" modes of substrate binding.

MECHANISM

β-galactosidase is a member of the class of retaining glycosidases, meaning at the anomeric position the *beta*-conformation is obtained. Catalysis proceeds via the double-displacement mechanism generally accepted for all glycosidases shown in Figure 33.[111,112] Formation of the covalent glycosyl-enzyme intermediate is followed by hydrolysis via oxocarbenium ion-like transition state. The active site of the enzyme contains a pair of carboxylic acids. Acid/base catalysis is provided by a single carboxyl group at the active side functioning as an acid catalyst for the first glycosylation step and as a base catalyst in the second deglycosylation step. By double inversion at the anomeric center, retention of conformation is obtained.

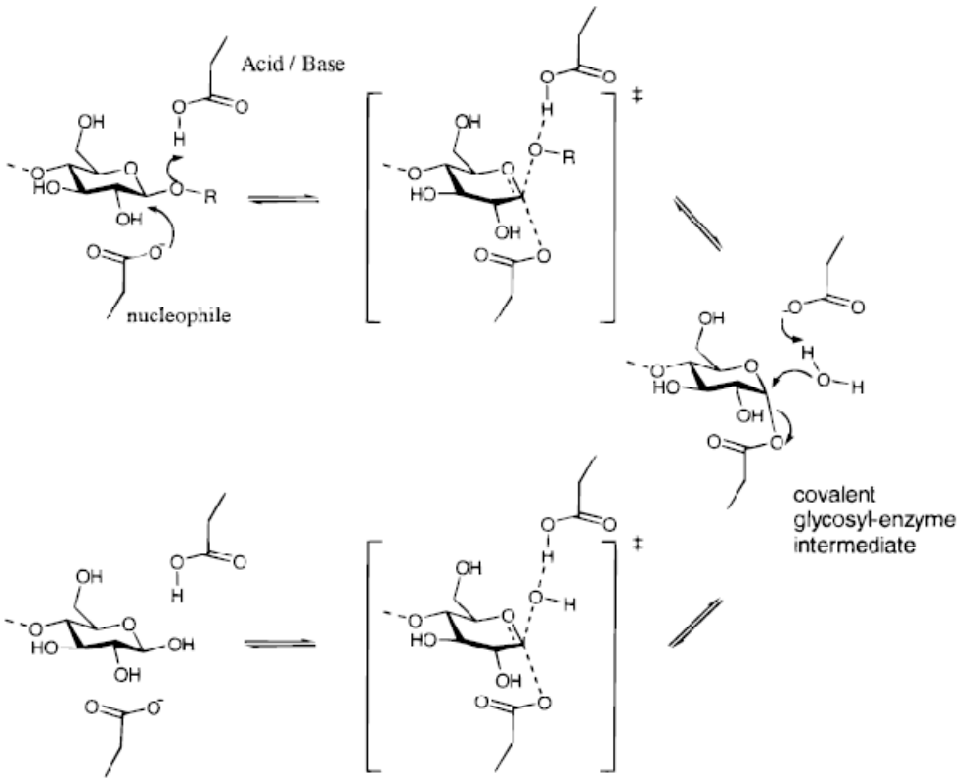


Figure 33. Mechanism of retaining glycosidase as well as for transglycosylases.[113]

GRAPHICAL ABSTRACT

STRATEGY FOR THE GALACTOSE DERIVATIVES

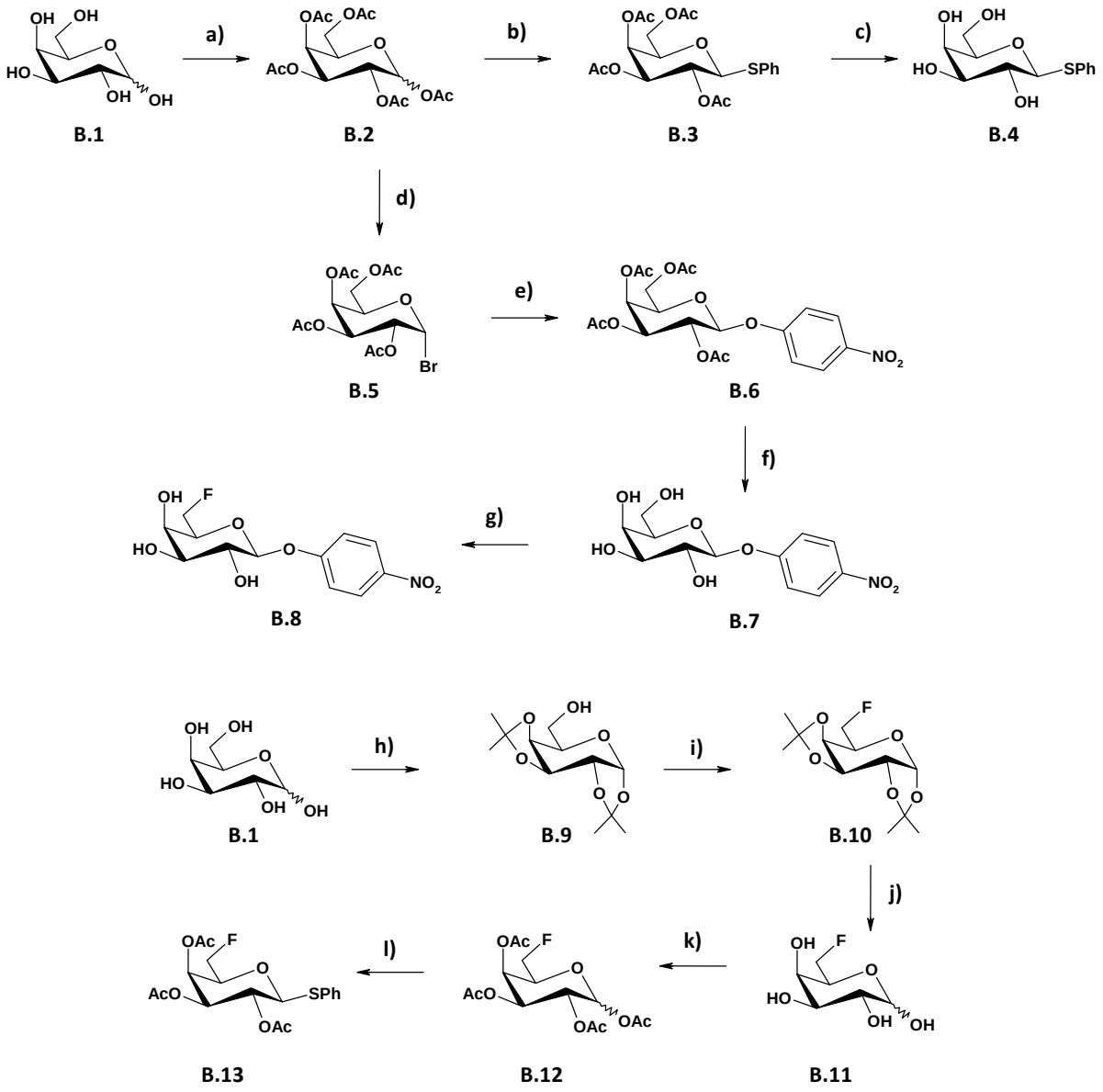


Figure 34. (a) Ac_2O, DMAP, pyridine, 98%; (b) thiophenol, BF_3*Et_2O, chloroform, 43%; (c) NaOMe, MeOH, 91%; (d) HBr, AcOH, 79%; (e) *p*-nitrophenol, K_2CO_3, acetone, 45%; (f) NaOMe, MeOH, 92%; (g) DAST, DCM, 26%; (h) $CuSO_4/H_2SO_4$, acetone, 87%; (i) DAST, collidine, DCM, microwave, 78%; (j) CF_3COOH, H_2O; (k) Ac_2O, DMAP, pyridine, 78%; (l) thiophenol, BF_3*Et_2O, chloroform, 49%.

STRATEGY FOR THE GLUCOSE DERIVATIVES

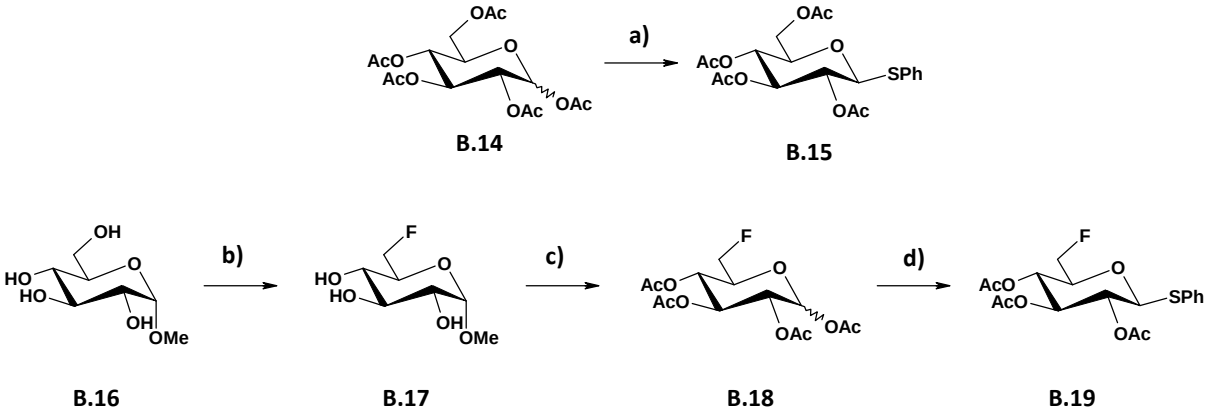


Figure 35. (a) thiophenol, BF_3*Et_2O, chloroform, 43%; (b) DAST, DCM, 85%; (c) H_2SO_4, Ac_2O, 75%; (d) thiophenol, BF_3*Et_2O, chloroform, 28%.

RESULTS AND DISCUSSION

Aim of this part of the PhD thesis was the synthesis of native and fluorinated galactose and glucose derivatives for their possible enzymatic application with β–galactosidase from *Aspergillus oryzae* (EC 3.2.1.23). [90,91,92,93,94,110,114]

The very common *p*-nitrophenyl group was chosen as aglycon for the galactosyldonor substrate. On one hand it is a good leaving group due to the electron withdrawing effects of the nitro group, and on the other hand kinetic observation of the enzymatic reactions can be achieved by the detection of released *p*-nitrophenolate using an UV-spectrometer.[115,116]

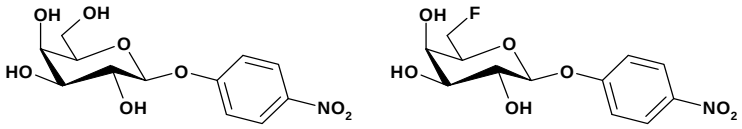
The motives for choosing the thiophenyl group as aglycon for the glycosylacceptors were their extreme stability due to the sulfur atom and the often observed increase of yield in enzymatic reactions due to the large and non-polar phenyl group.[95,117,118] As further advantage is the possible activation of such thioglycosyls as donors for further chemical glycosylation.[97,98,119]

Fluorination of the primary hydroxyl group in position-6 was chosen because the β(1-6) linkage is the preferred connection in enzymatic reactions with β–galactosidase from *Aspergillus oryzae* for these substrate molecules.[91] Furthermore, fluorine provides the possibility to investigate conversion, position and possible interactions of the enzymatic reactions, as well as the design of other linkage possibilities by fluorine NMR.

Deprotection of monosaccharide precursors **B.13**, **B.15** and **B.19** as well as the very promising enzymatic transglycosylation experiments could not be realized until date.

SYNTHESIS OF DONOR SUBSTRATES

Starting from peracetylated galactose **B.2,** bromination was performed with hydrobromic acid in glacial acetic acid under standard conditions. α–bromide **B.5** was converted into its *p*-nitrophenyl-derivative **B.6** by nucleophilic displacement under inversion of the anomeric center with potassium carbonate and *p*-nitrophenol in acetone.[120,121] Deprotection according to the Zemplén protocol afforded the *p*-nitrophenolgalactoside **B.7**.



B.7 **B.8**

Figure 36. Glycosyldonors: native and fluorinated *p*-nitrophenylgalactoside.

Nucleophilic fluorination of the deprotected galactoside **B.7** using DAST afforded the desired product **B.8** in 26% yield. Yield optimization especially of the fluorination reaction could be accomplished by lowering the reaction temperature to -78°C. Therefore the primary hydroxyl group should be favored in comparison to the secondary ones. Variation of the amounts of DAST should minimize other side products.

SYNTHESIS OF ACCEPTOR SUBSTRATES

The synthetic strategy for the thiophenyl derivatives **B.3** and **B.15** was the Lewis acid catalysed S_N1-reactions of the peracetylated glucose **B.14** and galactose **B.2** with boron trifluoride diethyl etherate and thiophenol.

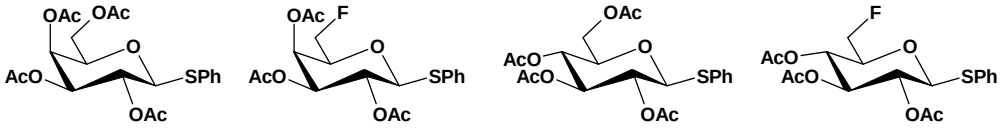


Figure 37. Glycosylacceptors of glucose and galactose.

Synthesis of the 6-deoxo-6-fluoro-galactose **B.11** was performed starting from galactose **B.1** itself.[122,123] The secondary hydroxyl groups were protected as isopropylidene ketals **B.9** using acetone, copper sulfate and sulfuric acid. The free primary hydroxyl group was fluorinated with DAST. Due to poor yields reaction conditions had to be optimized. Variation of solvents, base and temperature etc. are summarized in the following table. (Figure 38) Best results could be obtained using two equivalents of DAST and collidine in anhydrous methylene chloride under microwave conditions by a constant temperature of 80°C during a time period of 60 minutes.

Solvent (anhydrous)	DAST	Base	Temperature	Time	Yield
diglyme[124]	4 equiv.	-	60°C	4 h	27%
diglyme	5 equiv.	-	RT	15 h	30%
diglyme	5 equiv.	-	60°C	2 h	30%
DCM	4 equiv.	-	RT	3 h	19%
THF	4 equiv.	-	RT	15 h	12%
diglyme	**2 equiv.**	**2 equiv. DMAP**	**RT / reflux**	**24 h / 4 h**	**50%**
DCM	2 equiv.	2 equiv. DMAP	RT /50°C	24 h / 2 h	31%
DCM	2 equiv.	2 equiv. collidine	RT / 50°C	24 h / 4 h	53%
DCM	**2 equiv.**	**2 equiv. collidine**	**microwave / 80°C**	**60 min.**	**78%**

Figure 38. Reaction conditions for the fluorination using DAST.

Variation of protecting groups to afford compound **B.12** was established by the cleavage of the isopropylidene groups using 80% aqueous trifluoractic acid to compound **B.11**, followed by direct peracetylation under standard conditions using acetic anhydride and catalytic amounts of *N*,*N*-

dimethylamino pyridine in anhydrous pyridine. Introduction of the thiophenyl aglycon was performed under Lewis acid catalyzed conditions yielding the 6-fluoro-thiophenyl-β-galactoside **B.13** in 49% yield.

Synthesis of the 6-fluoro-β-thioglucoside **B.19** was performed starting from α-methyl glucoside **B.16**. Nucleophilic fluorination using DAST afforded the 6-fluoro derivative **B.17** in 85% yield. Variation of the protecting group was achieved using sulfuric acid and acetic anhydride to afford compound **B.18**.[125] Derivatisation of the anomeric position under standard conditions afforded the desired fluorinated β-thiophenylglucoside **B.19** in 28% yield.

MECHANISM OF THE DAST REACTION

In the first step nucleophilic displacement of fluorine on sulfur by oxygen of the hydroxyl group occurs, followed by the substitution of the leaving group by fluoride. The existence of the shown intermediate is supported by ^{19}F-NMR spectral data. As a consequence, the fluorination of optically active alcohols by using DAST generally proceeds with inversion of configuration.

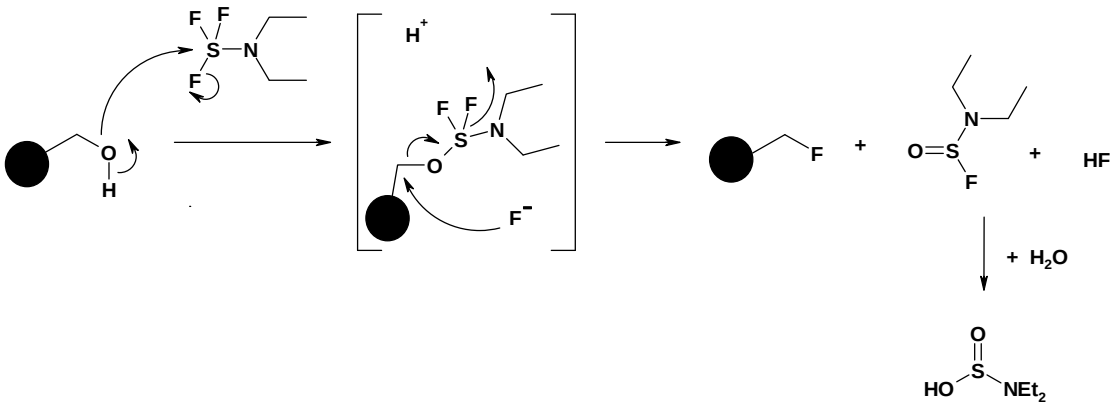


Figure 39. Mechanism of fluorination reactions using DAST or one of its analogs.

POSSIBLE ACTIVATION MECHANISM USING COLLIDINE

The fluorination is activated by the addition of a base, e.g. collidine or DMAP to the reaction. Even though multiple possible activation mechanisms are imaginable, probably not a single mechanism is the determining factor but a combination of all of them: function of an acid scavenger, deprotonation of the hydroxyl group and activation of the DAST reagent, etc.

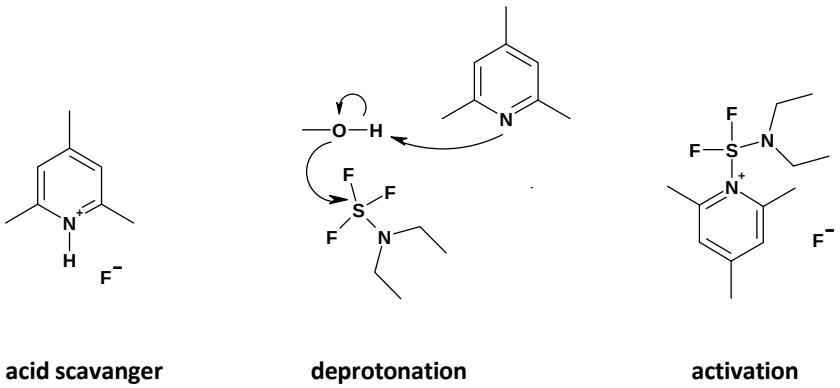


Figure 40. Possible activation mechanisms of collidine.

EXPERIMENTAL PROCEDURES

GENERAL METHODS

Solvents were purified by distillation and dried by standard procedures. Thin layer chromatography (TLC) was performed on precoated silica gel plates 60 F254 (Merck), detected with UV light (254 nm), ceric ammonium molybdate as well as 5% vanillin/sulfuric acid and heated by a hotgun. For preparative column chromatography silica gel 60M (230-400 mesh, Macherey-Nagel) was used.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX 400 NMR spectrometer. Chemical shifts are expressed as parts per million (ppm) and were referenced to 7.26 ($CDCl_3$), 4.79 (D_2O), 2.50 (d_6-DMSO) and 2.05 ($(CD_3)_2CO$) for the proton spectra as well as to 77.16 ($CDCl_3$), 39.52 (d_6-DMSO) and 29.84 ($(CD_3)_2CO$) for ^{13}C spectra. Coupling constants are quoted in Hertz (Hz).

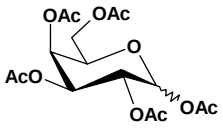
Mass spectra were recorded on spectrometers from Micro Mass (Fissions Instrument Trio200) in electron impact (EI) mode (70 eV) and on a Finnigan MAT 8230 in electron spray ionization mode.

Microwave heating was performed with a Biotage initiator synthesizer.

All chemicals used were purchased by Aldrich or Acros.

GENERAL PROCEDURES

1,2,3,4,6-Penta-*O*-acetyl-α/β-D-galactopyranoside



B.2

To a cooled solution of D-galactose **B.1** (10 g, 55.5 mmol, 1 eq) in anhydrous pyridine (65 mL) containing catalytic amounts of DMAP, acetic anhydride (46 mL, 490 mmol, 10 eq) was added dropwise. The mixture was stirred overnight at room temperature and was quenched with ice/water (200 mL), extracted trice with ethyl acetate (à 100 mL). The combined organic layers were washed five times with 1N HCl and twice with water, dried over $MgSO_4$ and the solvents were removed under reduced pressure. Coevaporation with toluene afforded compound **B.2** in 98% (21.67 g) yield.

$C_{16}H_{22}O_{11}$ $M_r = 390.34$

α-anomer:

^{1}H (400 MHz, $CDCl_3$): δ 6.37 (d, 1H, $J_{1,2}$ 1.76 Hz, H-1), 5.35-5.33 (m, 1H, H-2), 5.35-5.33 (m, 1H, H-3), 5.50-5.49 (m, 1H, H-4), 4.36-4.32 (m, 1H, H-5), 4.23-4.03 (m, 2H, H-6a, H-6b), 2.152 (3H, CH_3), 2.088 (3H, CH_3), 2.036 (3H, CH_3), 2.014 (3H, CH_3), 1.998 (3H, CH_3).

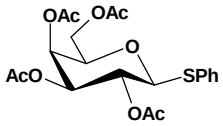
^{13}C (400 MHz, $CDCl_3$): δ 170.53 (C=O), 170.34 (C=O), 170.29 (C=O), 170.03 (C=O), 169.08 (C=O), 89.90 (C-1), 68.90 (C-5), 67.56 (C-4), 67.50 (C-2), 66.59 (C-3), 61.40 (C-6), 20.93 (CH_3), 20.78 (CH_3), 20.76 (CH_3), 20.73 (CH_3), 20.66 (CH_3).

β-anomer:

^{1}H (400 MHz, $CDCl_3$): δ 5.69 (d, 1H, $J_{1,2}$ 8.32 Hz, H-1), 5.42 (dd, 1H, $J_{4,3}$ 3.40 Hz, $J_{4,5}$ 0.96 Hz, H-4), 5.35-5.33 (m, 1H, H-3), 5.08 (dd, 1H, $J_{3,4}$ 3.40 Hz, $J_{3,2}$ 10.40 Hz, H-3), 4.07-4.05 (m, 1H, H-5), 4.23-4.03 (m, 2H, H-6), 2.16-1.98 (15H, CH_3).

^{13}C (400 MHz, $CDCl_3$): δ 170.51-169.06 (C=O), 92.30 (C-1), 71.84 (C-5), 70.98 (C-3), 67.98 (C-2), 66.94 (C-4), 61.20 (C-6), 21.12-20.65 (CH_3).

Thiophenyl-2,3,4,6-tetra-*O*-acetyl-β-D-galactopyranoside



B.3

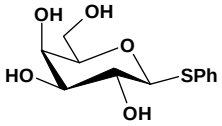
To a stirred solution of compound **B.2** (10.8 g, 27.7 mmol, 1 eq) in chloroform (100 mL) thiophenol was added (3.66 g, mmol, 1.2 eq) at 10°C, followed by dropwise addition of BF_3*Et_2O (17.4 mL, mmol, 5 eq). The reaction mixture was stirred overnight at room temperature, quenched with saturated $NaHCO_3$ solution until formation of gas subsided. The layers were separated and the organic layer was washed with water twice, dried over $MgSO_4$, and the solvents were removed under reduced pressure. The crude product mixture was purified by column chromatography (silica gel, hexane/ethyl acetate=2/1) to yield 5.2 g (43%) of compound **B.3.**

$C_{20}H_{24}O_9S$ M_r = 440.46

^{1}H (600 MHz, $CDCl_3$): δ 7.523-7.499 (m, 2H, H-arom.), 7.318-7.306 (m, 3H, H-arom.), 5.412 (bd, 1H, $J_{4,3}$ 3.36 Hz, H-4), 5.234 (dd, 1H, $J_{2,3}$ 9.92 Hz, $J_{2,1}$ 10.04 Hz, H-2), 5.048 (dd, 1H, $J_{3,4}$ 3.34 Hz, $J_{3,2}$ 9.94 Hz, H-3), 4.714 (d, 1H, $J_{1,2}$ 9.92 Hz, H-1), 4.187 (dd, 1H, $J_{6a,6b}$ 11.40 Hz, $J_{6a,5}$ 6.88 Hz, H-6a), 4.112 (dd, 1H, $J_{6b,6a}$ 12.10 Hz, $J_{6b,5}$ 6.58 Hz, H-6b), 3.935 (t, 1H, $J_{5,6a}$ 6.58 Hz, $J_{5,6b}$ 6.58 Hz), 2.115 (CH_3), 2.036 (2 CH_3), 1.970 (CH_3).

^{13}C (600 MHz, $CDCl_3$): δ 170.50 (C=O), 170.32 (C=O), 170.18 (C=O), 169.56 (C=O), 132.73 (C-arom.), 129.03 (C-arom.), 128.30 (C-arom.), 86.75 (C-1), 74.57 (C-5), 72.15 (C-3), 67.42, 67.37 (C-4, C-2), 61.77 (C-6), 20.97 (CH_3), 20.79 (CH_3), 20.76 (CH_3), 20.71 (CH_3).

Thiophenyl-β-D-galactopyranoside



B.4

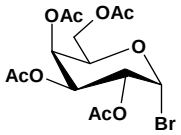
Deprotection was performed according to the Zemplén protocol: the peracetylated thiogalactoside **B.3** (5 g, 11.35 mmol) was suspended in anhydrous methanol (80 mL). The sodium methoxide (23 mL of freshly prepared 0.1M stock solution) was added and stirred for 5 hours at room temperature. The reaction mixture was quenched by addition of dry ice (pH 6-7). Lyophilisation of the aqueous solution yielded 2.8 g (91%) colorless foam **B.4**.

$C_{12}H_{16}O_5S$ $M_r = 272.32$

^1H (600 MHz, D_2O): δ 7.580-7.564 (m, 2H, H-arom.), 7.423-7.354 (m, 3H, H-arom.), 4.769 (d, 1H, $J_{1,2}$ 9.72 Hz, H-1), 3.983 (d, 1H, $J_{4,3}$ 3.30 Hz, H-4), 3.772-3.696 (m, 3H, H-5, H-6a, H-6b), 3.681 (dd, 1H, $J_{3,4}$ 3.33 Hz, $J_{3,2}$ 9.45 Hz, H-3), 3.624 (t, 1H, $J_{2,3}$ 9.53 Hz, $J_{2,1}$ 9.54 Hz, H-2).

^{13}C (600 MHz, D_2O): δ 133.08 (C_1-arom.), 131.42, 129.69, 128.20 (C-arom.), 88.41 (C-1), 79.33 (C-5), 74.32 (C-3), 69.57 (C-2), 69.03 (C-4), 61.30 (C-6).

2,3,4,6-Tetra-*O*-acetyl-α-D-galactosyl bromide



B.5

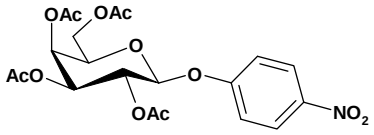
Peracylated galactose **B.2** (32.5 g, 83 mmol, 1 eq) was dissolved in anhydrous methylene chloride (75 mL) and glacial acetic acid (75 mL). The solution was cooled to 0°C and hydrobromic acid in glacial acetic acid (5.7 M solution, 89 mL, 0.5 mol, 6 eq) was added dropwise. The reaction mixture was stirred for 4 hours at room temperature and quenched with ice/water (600 mL). The aqueous solution was extracted six times with methylene chloride (à 80 mL). The combined organic extracts were washed with 4 times with saturated $NaHCO_3$ solution (150 mL) and twice with water, dried over $MgSO_4$ and the solvents were removed *in vacuo.* Compound **B.5** was obtained in 79% (26.8 g) yield and was used without any further purification.

$C_{14}H_{19}BrO_9$ $M_r = 411.20$

^1H (400 MHz, $CDCl_3$): δ 6.69 (d, 1H, $J_{1,2}$= 3.96 Hz, H-1), 5.51 (dd, 1H, $J_{3,4}$= 3.3 Hz, $J_{4,5}$= 1.23 Hz, H-4), 5.40 (dd, 1H, $J_{2,3}$= 10.62 Hz, $J_{3,4}$= 3.3 Hz, H-3), 5.04 (dd, 1H, $J_{1,2}$= 3.96 Hz, $J_{2,3}$= 10.64 Hz, H-2), 4.48 (btd, 1H, $J_{5,6}$= 6.60 Hz, H-5), 4.18 (dd, 1H, $J_{5,6}$= 6.34 Hz, $J_{6,6}$= 11.41 Hz, H-6a), 4.10 (dd, 1H, $J_{5,6}$= 6.76 Hz, $J_{6,6}$= 11.44 Hz, H-6b), 2.15-2.01 (12H, CH_3).

^{13}C (400 MHz, $CDCl_3$): δ 170.43-169.87 (C=O), 88.26 (C-1), 71.21 (C-5), 68.14 (C-3), 67.92 (C-2), 67.13 (C-4), 60.96 (C-6), 20.87-20.67 (CH_3).

_p_-Nitrophenol-2,3,4,6-tetra-_O_-acetyl-β-D-galactopyranoside



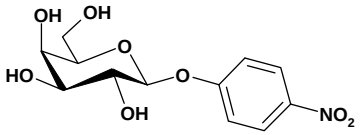
B.6

A mixture of compound **B.5** (1 g, 2.43 mmol, 1 eq), *p*-nitrophenol (0.57 g, 4.1 mmol, 1.7 eq) and anhydrous K_2CO_3 (0.5 g, 3.6 mmol, 1.5 eq) in anhydrous acetone (10 mL) was refluxed overnight. The reaction mixture was cooled to room temperature and diluted with water (10 mL) while shaking. The obtained precipitate was recrystallized with ethanol to yield 0.51 g (45%) of compound **B.6**.

$C_{20}H_{23}NO_{12}$ M_r = 469.40

^1H (250 MHz, $CDCl_3$): δ 8.214 (d, 2H, J 9.15 Hz, H-arom.), 7.085 (d, 2H, J 9.20, H-arom.), 5.522 (dd, 1H, $J_{2,3}$ 10.32 Hz, $J_{2,1}$ 7.87 Hz, H-2), 5.480 (d, 1H, $J_{4,3}$ 3.20 Hz, H-4), 5.169 (d, 1H, $J_{1,2}$ 7.98 Hz, H-1), 5.139 (dd, 1H, $J_{3,4}$ 3.20 Hz, $J_{3,2}$ 10.43 Hz, H-3), 4.237-4.095 (m, 3H, H-5, H-6a, H-6b), 2.191 (CH_3), 2.071 (2 CH_3), 2.022 (CH_3).

_p_-Nitrophenyl-β-D-galactopyranoside



B.7

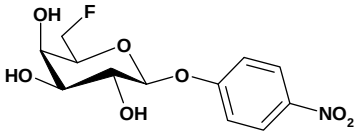
Deprotection was performed according to the Zemplén protocol: the peracetylated galactoside **B.7** (0.51 g, 1.1 mmol, 1 eq) was suspended in anhydrous methanol (22 mL). The sodium methoxide (5.5 mL of freshly prepared 0.1 M stock solution) was added and stirred for 2 hours at room temperature. The reaction mixture was quenched by addition of dry ice (pH 6-7). The solvents were removed under reduced pressure to afford 0.3 g (92%) of compound **B.7** as yellow solid.

$C_{12}H_{15}NO_8$ M_r = 301.25

^1H (400 MHz, D_2O): δ 8.300 (d, 2H, J 9.24 Hz, H-arom.), 7.291 (d, 2H, J 9.35 Hz, H-arom.), 5.246 (d, 1H, $J_{1,2}$ 7.60 Hz, H-1) 4.067 (d, 1H, $J_{4,3}$ 3.28 Hz, H-4), 3.979 (t, 1H, J 6.18, J 6.18, H-5), 3.909 (dd, 1H, $J_{2,1}$ 7.60 Hz, $J_{2,3}$ 9.96 Hz, H-2), 3.853-3.826 (m, 3H, H-5, H-6a, H-6b).

^{13}C (400 MHz, D_2O): δ 162.27 (C_O-arom.), 142.93 (C_N-arom.), 126.50 (C-arom.), 116.86 (C-arom.), 100.43 (C-1), 76.06 (C-5), 72.84 (C-3), 70.75 (C-2), 68.79 (C-4), 61.10 (C-6).

6-Deoxy-6-fluoro-*p*-nitrophenyl-β-D-galactopyranoside



B.8

To a cooled (-40°C) suspension of compound **B.7** (287 mg, 0.95 mmol, 1 eq) in anhydrous methylene chloride (20 mL) under argon atmosphere DAST (0.75 mL, 5.7 mmol, 6 eq) was slowly added. The reaction mixture was stirred for 30 minutes at -40°C and for 4 hours at room tempearture. The reaction was cooled and quenched with methanol until formation of gas was subsided. The solvents were evaporated and the crude product mixture was purified by column chromatography (silica gel, chloroform/methanol=10/1) to yield 75 mg (26%) of compound **B.8**

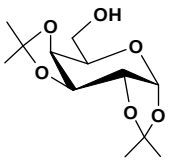
$C_{12}H_{14}FNO_7$ $M_r = 303.25$

^{19}F (600 MHz, D_2O): δ -230.802 (ddd, $J_{F,6a}$ 47.73 Hz, $J_{F,6b}$ 45.42 Hz, $J_{F,5}$ 15.18 Hz).

^{1}H (600 MHz, D_2O): δ 8.268 (d, 2H, J 9.42 Hz, H-arom.), 7.261 (d, 2H, J 9.48 Hz, H-arom.), 5.266 (d, 1H, $J_{1,2}$ 7.92 Hz, H-1), 4.713 (dd, 1H, $J_{F,6a}$ 48.36 Hz, $J_{6a,5}$ 3.60 Hz, $J_{6a,6b}$ 10.38 Hz, H-6a), 4.638 (dd, 1H, $J_{F,6b}$ 45.66 Hz, $J_{6b,5}$ 7.74 Hz, $J_{6b,6a}$ 10.01 Hz, H-6b), 4.239 (ddd, $J_{F,5}$ 15.40 Hz, $J_{5,6a}$ 7.35 Hz, $J_{5,6b}$ 3.57 Hz, H-5), 4.080 (d, $J_{4,3}$ 3.06 Hz, H-4), 3.884 (dd, 1H, $J_{2,3}$ 10.02 Hz, $J_{2,1}$ 7.74 Hz, H-2), 3.820 (dd, 1H, $J_{3,2}$ 9.99 Hz, $J_{3,4}$ 3.21 Hz, H-3).

^{13}C (600 MHz, d6-DMSO): δ 162.32 (C_O-arom.), 141.71 (C_N-arom.), 125.77 (C-arom.), 116.44 (C-arom.), 99.99 (C-1), 83.19 (d, $J_{F,6}$ 165.72 Hz, C-6), 73.61 (d, $J_{F,5}$ 20.34 Hz, C-5), 72.63 (C-3), 69.80 (C-2), 67.95 (d, $J_{F,4}$ 6.39 Hz, C-4).

1,2:3,4-Di-*O*-isopropylidene-α-D-galactopyranoside



B.9

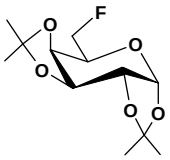
A suspension of D-galactose **B.1** (10 g, 55.5 mmol, 1 eq), anhydrous $CuSO_4$ (22 g, 137.8 mmol, 2.5 eq) and conc. H_2SO_4 (1.1 mL) in dry acetone was stirred at room temperature for two days. The reaction mixture was filtered over a pad of celite and washed several times with acetone. After the addition of saturated $NaHCO_3$ solution (65 mL), acetone was evaporated. The residue was extracted five times (à 100 mL) with methylene chloride. The combined organic layers were washed once with water, dried over $MgSO_4$ and concentrated *in vacuo*. The crude product was purified by column chromatography (silica gel, chloroform/acetone=4/1 to 2/1) to give 12.6 g (87%) of compound **B.9**.

$C_{12}H_{20}O_6$ $M_r = 260.29$

^{1}H (400 MHz, $CDCl_3$): δ 5.562 (d, 1H, $J_{1,2}$ 5.04 Hz, H-1), 4.609 (dd, 1H, $J_{3,2}$ 2.46 Hz, $J_{3,4}$ 7.90 Hz, H-3), 4.330 (dd, 1H, $J_{2,1}$ 5.04 Hz, $J_{2,3}$ 2.52 Hz, H-2), 4.268 (dd, 1H, $J_{4,3}$ 7.82 Hz, $J_{4,5}$ 1.78 Hz, H-4), 3.894-3.828 (m, 2H, H-5, H-6a), 3.767-3.705 (m, 1H, H-6b), 1.529 (s, 3H, 1,2-CH_3), 1.454 (s, 3H, 3,4-CH_3), 1.333 (s, 6H, 1,2:3,4-CH_3).

^{13}C (400 MHz, $CDCl_3$): δ 109.62 (3,4-C_q), 108.82 (1,2-C_q), 96.45 (C-1), 71.77 (C-4), 70.92 (C-3), 70.74 (C-2), 68.23 (C-5),62.50 (C-6), 26.18 (1,2-CH_3), 26.08 (3,4-CH_3), 25.08, 24.45 (1,2-CH_3, 3,4-CH_3).

6-Deoxy-6-fluoro-1,2:3,4-di-*O*-isopropylidene-α-D-galactopyranoside



B.10

A solution of compound **B.9** (1.88 g, 7.2 mmol, 1 eq), DAST (1.9 mL, 14.4 mmol, 2 eq), and collidine (1.9 mL, 14.4 mmol, 2 eq) in anhydrous methylene chloride (15 mL) was heated in the microwave generator for 60 minutes at 80°C. TLC control showed no remaining starting material. The reaction was quenched with saturated $NaHCO_3$ solution. The reaction mixture was extracted with DCM (à 30 mL). The combined organic layers were washed with water, dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by column chromatography (silicagel, hexane/ethyl acetate=6/1) to yield 1.47 g (78%) of fluorinated galactose derivative **B.10**.

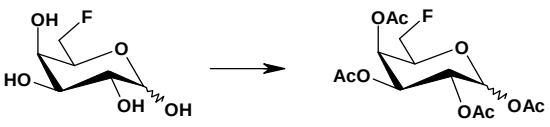
$C_{12}H_{19}FO_5$ M_r = 262.28

^{19}F (600 MHz, $CDCl_3$): δ -231.599 ($J_{F,6a}$ 47.76 Hz, $J_{F,6b}$ 46.18 Hz, $J_{F,5}$ 13.34 Hz, $J_{F,4}$ 0.90 Hz).

^{1}H (600 MHz, $CDCl_3$): δ 5.562 (d, 1H, $J_{1,2}$ 5.04 Hz, H-1), 4.632 (ddd, 1H, $J_{3,2}$ 2.54 Hz, $J_{3,4}$ 8.03 Hz, $J_{F,3}$ 0.92 Hz, H-3), 4.580 (ddd, 1H, $J_{F,6b}$ 46.02 Hz,$J_{6b,6a}$ 9.59 Hz, $J_{6b,5}$ 5.06 Hz, H-6b), 4.534 (ddd, 1H, $J_{F,6a}$ 47.94 Hz, $J_{6a,6b}$ 9.59 Hz, $J_{6a,5}$ 6.98 Hz, H-6a), 4.344 (dd, 1H, $J_{2,1}$ 5.01 Hz, $J_{2,3}$ 2.49 Hz, H-2), 4.267 (dd, 1H, $J_{4,3}$ 7.86 Hz, $J_{4,5}$ 1.92 Hz, H-4), 4.079 (ddd, 1H, $J_{F,5}$ 13.33 Hz, $J_{5,6a}$ 6.95 Hz, $J_{5,6b}$ 5.00 Hz, $J_{5,4}$ 1.94 Hz, H-5), 1.546 (s, 3H, 1,2-CH_3), 1.450 (s, 3H, 3,4-CH_3), 1.340 (s, 6H, 1,2:3,4-CH_3).

^{13}C (600 MHz, $CDCl_3$): δ 109.81 (3,4-C_q), 108.96 (1,2-C_q), 96.33 (C-1), 82.21 (d, $J_{F,6}$ 167.92 Hz, C-6), 70.73 (C-3), 70.92 (d, $J_{F,4}$ 2.72 Hz, C-4), 70.57 (C-2), 66.77 (d, $J_{F,5}$ 21.82 Hz, C-5), 26.17 (1,2-CH_3), 26.06 (3,4-CH_3), 25.06, 24.56 (1,2-CH_3, 3,4-CH_3).

6-Deoxy-6-fluoro-1,2,3,4-tetra-*O*-acetyl-α/β-D-galactopyranoside



B.11 **B.12**

Compound **B.10** (242 mg, 0.9 mmol, 1 eq) was dissolved in 80% trifluoroacetic acid (5 mL) and stirred for one hour at room temperature. Evaporation of the reaction mixture, followed by coevaporation with water (3x 5 mL) and toluene (3x 5 mL) gave compound **B.11**. This crude mixture was redissolved in anhydrous pyridine (5 mL), cooled to 0°C and acetic anhydride (1 mL, 10.6 mmol, 11.8 eq) and catalytic amount of DMAP was added. The reaction mixture was stirred overnight at room temperature, quenched with ice water. The water layer was extracted three times with methylene chloride. The combined organic layers were washed twice with 1N HCl and once with water, dried over $MgSO_4$ and the solvent were removed *in vacuo*. Purification with column chromatography over silica gel (hexane/ethyl acetate=3/2) afforded compound **B.12** in 78% yield (247 mg).

$C_{14}H_{19}FO_9$ M_r = 350.29

α-anomer:

^{19}F (600 MHz, $CDCl_3$): δ -232.291 ($J_{F,6a}$ 46.84 Hz, $J_{F,6b}$ 46.84 Hz, $J_{F,5}$ 12.17 Hz).

^{1}H (600 MHz, $CDCl_3$): δ 6.403 (bs, 1H, H-1), 5.564-5.558 (m, 1H, H-3), 5.366-5.335 (m, 2H, H-2, H-4), 4.543-4.329 (m, 3H, H-6a, H-6b, H-5), 2.167-1.997 (CH_3).

^{13}C (600 MHz, $CDCl_3$): δ 170.17-169.05 (C=O), 89.80 (C-1), 80.84 (d, $J_{F,6}$ 171.43 Hz, C-6), 69.65 (d, $J_{F,5}$ 24.22 Hz, C-5), 67.62, 67.59, 67.43, 66.92 (C-2, C-3), 21.16-20.67 (CH_3).

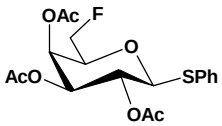
β-anomer:

^{19}F (600 MHz, $CDCl_3$): δ -232.150 ($J_{F,6a}$ 46.39 Hz, $J_{F,6b}$ 46.39 Hz, $J_{F,5}$ 11.60 Hz).

^{1}H (600 MHz, $CDCl_3$): δ 5.729 (d, 1H, $J_{1,2}$ 8.28 Hz, H-1), 5.496-5.489 (m, 1H, H-4), 5.366-5.335 (m, 2H, H-2), 5.095 (dd, 1H, J 10.44 Hz, J 3.42 Hz, H-3), 4.543-4.329 (m, 3H, H-6a, H-6b),4.087 (ddd, 1H, $J_{F,5}$ 11.70 Hz, $J_{5,6a}$ 6.14 Hz, $J_{5,6b}$ 5.60 Hz, $J_{5,4}$ 0.89 Hz, H-5), 2.167-1.997 (CH_3).

^{13}C (600 MHz, $CDCl_3$): δ 170.17-169.05 (C=O), 92.29 (C-1), 80.32 (d, $J_{F,6}$ 172.31 Hz, C-6), 72.38 (d, $J_{F,5}$ 24.14 Hz, C-5), 70.91 (C-3), 67.93 (C-2), 66.88 (C-4), 21.16-20.67 (CH_3).

Thiophenyl-2,3,4-tri-O-acetyl-6-deoxy-6-fluoro-β-D-galactopyranoside



B.13

According the procedure described for compound **B.3**, synthesis of the fluorinated thiogalactoside was carried out using compound **B.12** (243 mg, 0.7 mmol, 1 eq), thiophenol (100 µL, 0.98 mmol, 1.4 eq) and BF_3*Et_2O (0.45 mL, 3.6 mmol, 5 eq) in 4 mL of chloroform. The crude product was purified by column chromatography (silica gel, hexane/ethyl acetate=3/1) to yield 137 mg (49%) of compound **B.13.**

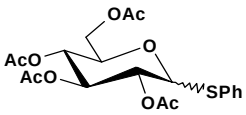
$C_{18}H_{21}FO_7S$ M_r = 400.42

^{19}F (600 MHz, $CDCl_3$): δ -231.072 ($J_{F,6a}$ 46.73 Hz, $J_{F,6b}$ 46.27 Hz, $J_{F,5}$ 12.15 Hz).

^{1}H (600 MHz, $CDCl_3$): δ 7.518-7.502 (m, 2H, H-arom.), 7.329-7.301 (m, 3H, H-arom.), 5.459 (dd, 1H, $J_{4,3}$ 3.39 Hz, $J_{4,5}$ 1.11 Hz, H-4), 5.250 (t, 1H, $J_{2,3}$ 9.96 Hz, $J_{2,1}$ 9.96 Hz, H-2), 5.062 (dd, 1H, $J_{3,4}$ 3.33 Hz, $J_{3,2}$ 9.96 Hz, H-3), 4.742 (d, 1H, $J_{1,2}$ 10.02 Hz, H-1), 4.505 (ddd, 1H, $J_{F,6a}$ 46.98 Hz, $J_{6a,6b}$ 9.71 Hz, $J_{6a,5}$ 6.59 Hz, H-6a), 4.407 (ddd, 1H, $J_{F,6b}$ 46.08 Hz, $J_{6b,6a}$ 9.71 Hz, $J_{6b,5}$ 5.18 Hz, H-6b), 3.989 (dddd, 1H, $J_{F,5}$ 12.08 Hz, $J_{5,6a}$ 6.62 Hz, $J_{5,6b}$ 5.36 Hz, $J_{5,4}$ 1.16 Hz, H-5), 2.114 (CH_3), 2.093 (CH_3), 1.973 (CH_3).

^{13}C (600 MHz, $CDCl_3$): δ 170.23 (C=O), 170.13 (C=O), 169.55 (C=O), 132.58 (C-arom.), 129.09 (C-arom.), 128.30 (C-arom.), 86.92 (C-1), 80.96 (d, $J_{F,6}$ 172.35 Hz, C-6), 75.31 (d, $J_{F,5}$ 23.04 Hz, C-5), 72.03 (C-3), 67.33, 67.31, 67.26 (C-4, C-2), 20.96 (CH_3), 20.71 (CH_3), 20.70 (CH_3).

Thiophenyl-2,3,4,6-tetra-*O*-acetyl-α/β-D-glucopyranoside



B.15

According the procedure described for compound **B.3**, synthesis of the peracetylated thioglucoside was carried out using compound **B.14** (10.8 mg, 27.67 mmol, 1 eq), thiophenol (3.4 mL, 33.2 mmol, 1.2 eq) and BF_3*Et_2O (17.4 mL, 138.4 mmol, 5 eq) in 110 mL of chloroform. The crude product was purified by column chromatography (silica gel, hexane/ethyl acetate=1/1) to yield 5.2 g (43%) of compound **B.15** (α/β = 1/12).

$C_{20}H_{24}O_9S$ M_r = 440.46

α-anomer:

^{1}H (400 MHz, $CDCl_3$): δ 7.421-7.398 (m, 2H, H-arom.), 7.289-7.235 (m, 3H, H-arom.), 5.885 (d, 1H, $J_{1,2}$ 5.76 Hz, H-1), 5.409 (dd, 1H, $J_{3,4}$ 9.76 Hz, $J_{3,2}$ 9.84 Hz, H-3), 5.073 (dd, 1H, $J_{2,3}$ 9.96 Hz, $J_{2,1}$ 5.94 Hz, H-2), 5.044 (dd, 1H, $J_{4,5}$ 9.88 Hz, $J_{4,3}$ 9.22 Hz, H-4), 4.536 (ddd, 1H, $J_{5,6a}$ 5.15 Hz, $J_{5,6b}$ 2.19 Hz, $J_{5,4}$ 10.25 Hz, H-5), 4.245 (dd, 1H, $J_{6a,6b}$ 12.32 Hz, $J_{6,5}$ 5.20 Hz, H-6a), 4.001 (dd, 1H, $J_{6b,6a}$ 12.34 Hz, $J_{6,5}$ 2.18 Hz, H-6b), 2.065 (CH_3), 2.017 (CH_3), 2.003 (CH_3), 1.981 (CH_3).

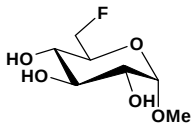
^{13}C (400 MHz, $CDCl_3$): δ 170.49 (C=O), 169.90 (C=O), 169.82 (C=O), 169.59 (C=O), 133.13 (C-arom.), 131.90 (C-arom.), 129.17 (C-arom.), 127.82 (C-arom.), 85.00 (C-1), 70.75 (C-2), 70.45 (C-3), 68.62 (C-4), 68.21 (C-5), 61.94 (C-6), 20.73 (CH_3), 20.65 (2 CH_3), 20.60 (CH_3).

β-anomer:

^{1}H (400 MHz, $CDCl_3$): δ 7.506-7.482 (m, 2H, H-arom.), 7.324-7.307 (m, 3H, H-arom.), 5.221 (t, 1H, $J_{3,4}$ 9.34 Hz, $J_{3,2}$ 9.34 Hz, H-3), 5.037 (t, 1H, $J_{4,5}$ 9.76 Hz, $J_{4,3}$ 9.76 Hz, H-4), 4.971 (dd, 1H, $J_{2,3}$ 9.36 Hz, $J_{2,1}$ 10.00 Hz, H-2), 4.705 (d, 1H, $J_{1,2}$ 10.08 Hz, H-1), 4.223 (dd, 1H, $J_{6a,6b}$ 12.28 Hz, $J_{6,5}$ 5.00 Hz, H-6a), 4.175 (dd, 1H, $J_{6b,6a}$ 12.28 Hz, $J_{6,5}$ 2.68 Hz, H-6b), 3.723 (ddd, 1H, $J_{5,6a}$ 5.02 Hz, $J_{5,6b}$ 2.68 Hz, $J_{5,4}$ 10.04 Hz, H-5), 2.083 (CH_3), 2.076 (CH_3), 2.015 (CH_3), 1.986 (CH_3).

^{13}C (400 MHz, $CDCl_3$): δ 170.70 (C=O), 170.31 (C=O), 169.52 (C=O), 169.39 (C=O), 133.28 (C-arom.), 131.79 (C-arom.), 129.07 (C-arom.), 128.56 (C-arom.), 85.89 (C-1), 75.96 (C-5), 74.12 (C-3), 70.10 (C-2), 68.37 (C-4), 62.30 (C-6), 20.88 (CH_3), 20.85 (CH_3), 20.72 (CH_3).

_O_-Methyl-6-deoxy-6-fluoro-α-D-glucopyranoside



B.17

To a cooled (-40°C) solution of α–methyl glucoside **B.16** (1.5 g, 7.7 mmol, 1 eq) in anhydrous methylene chloride (30 mL) under argon atmosphere DAST (5.8 mL, 44.3 mmol, 5.7 eq) was slowly added. The reaction mixture was brought to room temperature and stirred for 3 hours. The reaction was cooled and quenched with methanol until formation of gas was subsided. The solvents were evaporated and the crude product mixture was purified by column chromatography (silica gel, ethyl acetate/methanol=9/1) to yield 1.28 g (85%) of compound **B.17**.

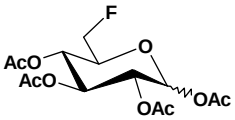
$C_7H_{13}FO_5$ M_r = 196.17

^{19}F (600 MHz, D_2O): δ -235.759 (ddd, $J_{F,6a}$ 47.42 Hz, $J_{F,6b}$ 47.41 Hz, $J_{F,5}$ 28.63 Hz).

^{1}H (600 MHz, D_2O): δ 4.851 (d, 1H, $J_{1,2}$ 3.78 Hz, H-1), 4.745 (ddd, 1H, $J_{F,6a}$ 46.96 Hz, $J_{6a,6b}$ 10.80 Hz, $J_{6a,5}$ 3.62 Hz, H-6a), 4.709 (ddd, 1H, $J_{F,6b}$ 47.89 Hz, $J_{6b,6a}$ 10.77 Hz, $J_{6b,5}$ 1.83 Hz, H-6b), 3.812 (dddd, 1H, $J_{F,5}$ 28.53, $J_{5,6a}$ 3.25 Hz, $J_{5,6b}$ 1.81 Hz, $J_{5,4}$ 10.23 Hz, H-5), 3.708 (dd, 1H, $J_{3,4}$ 9.51 Hz, $J_{3,2}$ 9.62 Hz, H-3), 3.594 (dd, 1H, $J_{2,3}$ 9.81 Hz, $J_{2,1}$ 3.81 Hz, H-2), 3.525 (dd, 1H, $J_{4,5}$ 10.20 Hz, $J_{4,3}$ 9.18 Hz, H-4), 3.443 (s, 3H, CH_3).

^{13}C (400 MHz, D_2O): δ 99.86 (C-1), 82.57 (d, $J_{F,6}$ 167.51 Hz, C-6), 73.30 (C-3), 71.50 (C-2), 70.76 (d, JF,5 17.31 Hz, C-5), 68.78 (d, JF,4 6.75 Hz, C-4), 55.59 (CH_3).

1,2,3,4-Tetra-_O_-acetyl-6-deoxy-6-fluoro-D-glucopyranoside



B.18

To a cooled (0°C) solution of compound **B.17** (1.2 g, 6.1 mmol, 1 eq) in acetic anhydride (11.6 mL, 123 mmol, 20 eq) conc. H_2SO_4 (0.4 mL) was added dropwise. The reaction mixture was stirred for one hour at 0°C and overnight at room temperature. The reaction was quenched with ice/water (200 mL). The aqueous solution was extracted five times with ethyl acetate (50 mL), the combined organic layers were washed trice with saturated $NaHCO_3$ solution and once with water (100 mL), dried over $MgSO_4$ and the solvents were removed *in vacuo*. Purification with column chromatography (silica gel, hexane/ethyl acetate=1/1) afforded 1.15 g of compound **B.18** in 75% yield (α>>β).

$C_{14}H_{19}FO_9$ M_r = 350.29

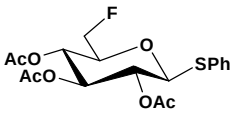
α-anomer:

^{19}F (600 MHz, $CDCl_3$): δ -233.822 (ddd, $J_{F,6a}$ 47.12 Hz, $J_{F,6b}$ 47.12 Hz, $J_{F,5}$ 23.52 Hz).

^{1}H (400 MHz, $CDCl_3$): δ 6.347 (d, 1H, $J_{1,2}$ 3.66 Hz, H-1), 5.489 (dd, 1H, $J_{3,2}$ 9.90 Hz, $J_{3,4}$ 9.84 Hz, H-3), 5.147 (dd, 1H, $J_{4,3}$ 9.48 Hz, $J_{4,5}$ 10.38 Hz, H-4), 5.080 (dd, 1H, $J_{2,3}$ 10.32 Hz, $J_{2,1}$ 3.66 Hz, H-2), 4.477 (ddd, 1H, $J_{F,6a}$ 47.26 Hz, $J_{6a,6b}$ 10.59 Hz, $J_{6a,5}$ 2.37 Hz, H-6a), 4.434 (ddd, 1H, $J_{F,6b}$ 47.04 Hz, $J_{6b,6a}$ 10.61 Hz, $J_{6b,5}$ 3.95 Hz, H-6b), 4.095 (dddd, 1H, $J_{F,5}$ 23.56 Hz, $J_{5,6a}$ 2.37 Hz, $J_{5,6b}$ 3.65 Hz, $J_{5,4}$ 10.38 Hz, H-5), 2.176 (CH_3), 2.061 (CH_3), 2.030 (CH_3), 2.012 (CH_3).

^{13}C (400 MHz, $CDCl_3$): δ 170.41 (C=O), 169.72 (C=O), 169.46 (C=O), 168.83 (C=O), 89.12 (C-1), 81.02 (d, $J_{F,6}$ 176.51 Hz, C-6), 70.69 (d, $J_{F,5}$ 19.52 Hz, C-5), 69.90 (C-3), 69.28 (C-2), 67.81 (d, $J_{F,4}$ 6.88 Hz, C-4), 20.98 (CH_3), 20.79 (CH_3), 20.70 (CH_3), 20.56 (CH_3).

Thiophenyl-2,3,4-tri-*O*-acetyl-6-deoxy-6-fluoro-β-D-glucopyranoside



B.19

According the procedure described for compound **B.3**, synthesis of the fluorinated thioglucoside was carried out using compound **B.18** (356 mg, 1.02 mmol, 1 eq), thiophenol (170 µL, 1.66 mmol, 1.6 eq) and BF_3*Et_2O (0.65 mL, 5.2 mmol, 5 eq) in 6 mL of chloroform. The crude product was purified by column chromatography (silica gel, hexane/ethyl acetate=2/1) to yield 112 mg (28%) of compound **B.19**.

$C_{18}H_{21}FO_7S$ M_r = 400.42

^{19}F (600 MHz, $CDCl_3$): δ -231.667 (ddd, $J_{F,6a}$ 46.99 Hz, $J_{F,6b}$ 46.99 Hz, $J_{F,5}$ 20.49 Hz).

^{1}H (600 MHz, $CDCl_3$): δ 7.500-7.482 (m, 2H, H-arom.), 7.333-7.310 (m, 3H, H-arom.), 5.245 (t, 1H, $J_{3,4}$ 9.36 Hz, $J_{3,2}$ 9.36 Hz, H-3), 5.009 (t, 1H, $J_{4,5}$ 9.87 Hz, $J_{4,3}$ 9.87 Hz, H-4), 4.953 (dd, 1H, $J_{2,3}$ 9.30 Hz, $H_{2,1}$ 10.08 Hz, H-2), 4.725 (d, 1H, $J_{1,2}$ 9.96 Hz, H-1), 4.488 (ddd, 1H, $J_{F,6a}$ 46.84 Hz, $J_{6a,6b}$ 10.44 Hz, $J_{6a,5}$ 2.79 Hz, H-6a), 4.462 (ddd, 1H, $J_{F,6b}$ 46.18 Hz, $J_{6b,6a}$ 10.40 Hz, $J_{6b,5}$ 4.88 Hz, H-6b), 3.747 (dddd, 1H, $J_{F,5}$ 20.49 Hz, $J_{5,6a}$ 2.75 Hz, $J_{5,6b}$ 4.79 Hz, $J_{5,4}$ 10.21 Hz, H-5), 2.081 (CH_3), 2.030 (CH_3), 1.987 (CH_3).

^{13}C (600 MHz, $CDCl_3$): δ 170.32 (C=O), 169.51 (C=O), 169.33 (C=O), 133.26 (C-arom.), 131.59 (C-arom.), 129.16 (C-arom.), 128.62 (C-arom.), 85.94 (C-1), 81.48 (d, $J_{F,6}$ 176.12 Hz, C-6), 76.63 (d, $J_{F,5}$ 19.76 Hz, C-5), 73.98 (C-3), 69.98(C-2), 67.99 (d, JF,4 6.66 Hz, C-4), 20.87 (CH_3), 20.72 (CH_3), 20.69 (CH_3).

CHAPTER- C

SYNTHESIS AND APPLICATION OF 5-HYDROXY-DICLOFENAC METABOLITE

for mechanistic investigation
of systemic hypersensitivity caused by
the non steroidal anti-inflammatory drug Diclofenac

in cooperation with

Martin Himly, PhD
Division of Allergy and Immunology
Department of Molecular Biology
University of Salzburg

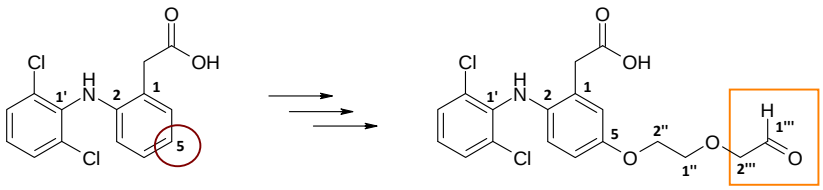
ABSTRACT

Diclofenac (Voltaren™) **C.1** is a member of nonsteroidal anti-inflammatory drugs (NSAIDs) and has been used for its antipyretic, analgetic and anti-inflammatory activities in the treatment of rheumatoid arthritis, osteoarthritis, ankylosing spondylitis, and acute muscle pain. Although this drug has been proven to be highly effective and secure, various adverse drug reactions have been reported in the past, including hepatotoxicity. A lot of research has been done in the last two decades to investigate immune interactions of Diclofenac. There are several postulations of an involvement of IgE, but mechanistic evidence is still not available.

The aim of this project was to investigate a potential immunologic mechanism against the nonsteroidal anti-inflammatory drug Diclofenac.

In this approach Diclofenac was covalently bound to human serum albumin (HSA) and the direct interaction of the resulting conjugates with effector cells will be investigated.

The following chapter focuses on the synthesis of Diclofenac metabolite **C.9**, which was later used in immunization experiments. For this approach it was important to retain the carboxylic acid. This could be obtained by amplifying position 5 in the molecule with a phenolic group. The hydroxyl function was prolonged using a polyethylene glycol spacer, with subsequent oxidation of position 1''' to afford a terminal carbonyl moiety.



C.1 **C.9**

Figure 41. Chemical structure of Diclofenac **C.1** and Diclofenac derivative **C.9**.

To selectively bind the Diclofenac derivative to the HSA protein, reductive amination was chosen as a coupling method. The aldehyde group of the Diclofenac derivative was conjugated to the lysines of the carrier protein with sodium cyanoborohydride while the carboxylic acid function remained untouched.

SHORT SUMMARY OF IMMUNOLOGIC TESTS

In line with the concept of bioactivation and haptenation, Diclofenac and five phase-I Diclofenac metabolites were covalently coupled to primary amines of human serum albumin (HSA) via their carboxylic acid function. Furthermore, the 5-hydroxy-diclofenac metabolite **C.9** was successfully conjugated using reductive amination with sodium cyanoborohydride. The HSA conjugates were characterized for Diclofenac-load and aggregation state, and their IgE-receptor cross-linking capacity was controlled in a mouse model. Using these conjugates, presence of drug-specific IgE was investigated in sera of 59 patients with mild to severe hypersensitivity reactions to either Diclofenac only or multiple reactivity to various NSAIDs. The array of *in vitro* assays included ELISA, mediator release assay, and basophil activation test (BAT).

Using these methods no IgE specific for Diclofenac or the five phase-I metabolites was detected neither in serum nor bound to basophils of hypersensitivity patients. Further negative results obtained with HSA conjugates of alternatively linked 5-hydroxy-diclofenac **C.9** eliminated an assumed presence of carboxylic acid-specific IgE.

IgE-mediated mechanism for Diclofenac hypersensitivity can be excluded considering native and metabolized Diclofenac coupled via the carboxylic group or the alternative linkage in position 5.

In conclusion, effector mechanism involving bioactivation and haptenation for Diclofenac hypersensitivity seems to be unlikely.

All immunological data were produced in the Department of Molecular Biology, Division of Allergy and Immunology at the University of Salzburg under the supervision of Dr. Martin Himly together with Dr. Andrea Harrer and Helene Damhofer.

THEORETICAL BACKGROUND

DEFINITION OF ALLERGY

The term Allergy was first defined by the Viennese pediatrician Clemens von Pirquet in 1906[126]: "An altered capacity of the body to react to a foreign substance after prior experience with the same material."

Presently: "An altered immune reaction to a spectrum of environment antigens mediated by IgE antibodies causing activation of mast cells, basophils, eosinophils.

HYPERSENSITIVITY - DISORDERS CAUSED BY IMMUNE RESPONSES

The major task of the immune system in human beings is the defense against infections, but sometimes diseases and tissue injuries can be caused by immune responses themselves. An immune response to an antigen results in intolerance to that antigen, a so called hypersensitivity. Such abnormal immune responses can be caused by antigens of two different origins: uncontrolled responses to foreign antigens or immune responses directed against autologous antigens (autoimmunity).

There is a clinically heterogeneous group of disorders, based on the type of immune response and the nature and location of the antigen. Hypersensitivity reactions are commonly classified on their principal immunologic mechanism, which causes the tissue injury and disease. (Figure 42)

Type of hypersensitivity	**Pathologic immune mechanisms**	**Mechanisms of tissue injury and disease**
Immediate hypersensitivity (type I)	IgE antibody leading to mast cell/basophil activation	Mast cells and their mediators (vasoactive amines, lipid mediators, cytokines)
Antibody-mediated (type II)	IgG, IgM antibodies against extracellular matrix antigens or cell surface	Opsonization and phagocytosis of cells Complement- and Fc receptor- mediated recruitment and activation of leukocytes (neutrophils, macrophages) Abnormalities in cellular functions, e.g., hormone receptor signaling
Immune complex-mediated (type III)	Immune complexes of circulating antigens and IgM or IgE antibodies	Complement- and Fc receptor- mediated recruitment and activation of leukocytes
T cell-mediated (type IV)	1. $CD4^+$ T cells (delayed- type hypersensitivity) 2. $CD8^+$ CTLs (T cell- mediated cytolysis)	1. Macrophage activation, cytokine- mediated inflammation 2. Direct target cell killing, cytokine-mediated inflammation

Figure 42. Types of hypersensitivity reactions according to Gel and Coombs.[127,128]

Immediate hypersensitivity, type I hypersensitivity is caused by the release of mediators from mast-cells. These reactions are activated by the production and cross linking of preexisting IgE antibodies bound to mast cells in diverse tissues.

If the disease is caused by another antibody class, it is classified as type II or type III hypersensitivity. Antibodies directed against cell or tissue antigens can harm these cells or tissues or even change their cellular functions, and are called antibody-mediated (type II). Immune complex-mediated (type III) hypersensitivity is causing such diseases, where antibodies form complexes with soluble antigens, which are deposited in blood vessels in various tissues. Reactions of T lymphocytes mostly against self antigens in tissues symbolize type IV or T-cell mediated hypersensitivity.

Immediate hypersensitivity is the most prevalent type of hypersensitivity; it affects 20-25% of the population. In clinical medicine, these reactions are commonly called *allergy* or *atopy*.

NATURE OF ALLERGENS

DEFINITION OF ALLERGENS: "Antigens that elicit immediate hypersensitivity (allergic) reactions are environmental proteins or chemicals bound to proteins to which the atopic individual is chronically exposed." (Figure 43)

Until now, it is not known why some antigens induce strong allergic reactions whereas some of them do not. But there are two important characteristics of all allergens: Individuals have to be exposed to them repeatedly and they do not stimulate the innate immune response, unlike microbes. Although some features may be representative of many common allergens, there are no general structural attribute of proteins to predict their atopic potential. These features comprise low molecular weight, glycosylation and high solubility in body fluids. Because immediate hypersensitivity reactions are reliant on T cells, T-cell-independent antigens, e.g. polysaccharides cannot generate such allergic reactions unless they are bound to proteins.

1.	**Inhalants**	Pollen Fungi (moulds) Animal products
2.	**Ingestants**	Foods Drugs
3.	**Contactants**	Plants Chemicals Latex
4.	**Injectans**	Insect venom Drugs

Figure 43. Classification of allergens according to route of exposure.

The natural history of contact is an important determinant of the amount of specific IgE antibodies produced. (Figure 44) For the development of an atopic reaction to a specific antigen repeated exposure to this antigen is necessary, because sensitization of mast cells with IgE must happen before a hypersensitivity reaction to an allergen can arise.

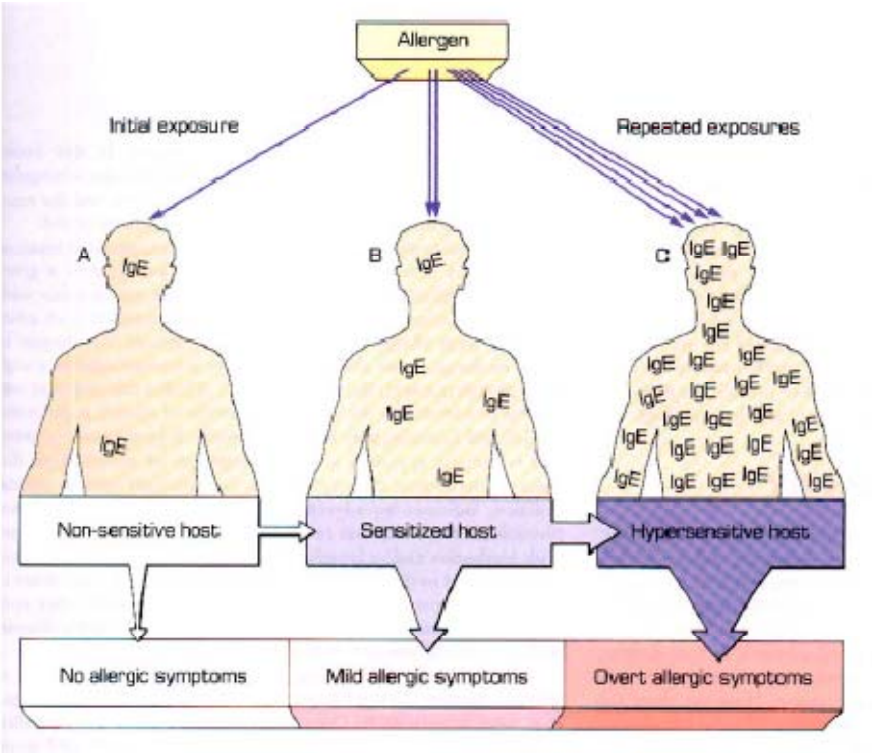


Figure 44. Schematic starting with first exposure with an allergen.

IMMUNOTHERAPY

The only curative approach for allergic diseases is immunotherapy. In *desensitization*, the most prominent method with promising results, increasing quantities of allergen are repeatedly administered subcutaneously. As a consequence specific IgE levels decrease.

IMMEDIATE HYPERSENSITIVITY – MOST PREVALENT TYPE OF HYPERSENSITIVITY DISEASE

Immediate hypersensitivity is initiated by the introduction of an allergen (protein antigens or chemicals bound to proteins), which stimulates the development of T_H2 cells. (Figure 45) Interleukin (IL)-4 and IL-13, T_H2 cell released cytokines stimulate B lymphocytes specific for the foreign antigens. Immunoglobulin E (IgE) binds to Fc receptors (FcεRI) on mast cell (or basophils, eosinophils), and subsequent exposure to an allergen activates the mast cells by cross-linking of bound IgE to secrete preformed and newly produced mediators that are responsible for the pathologic reactions.

The cross-linking of IgE by an allergen initiates multiple signaling pathways. (Figure 46) These signals lead to different responses of the mast cells (basophils): degranulation of vasoactive amines and proteases, the synthesis and secretion of arachidonic acid metabolites (prostaglandines, leukotrienes), as well as various cytokines.

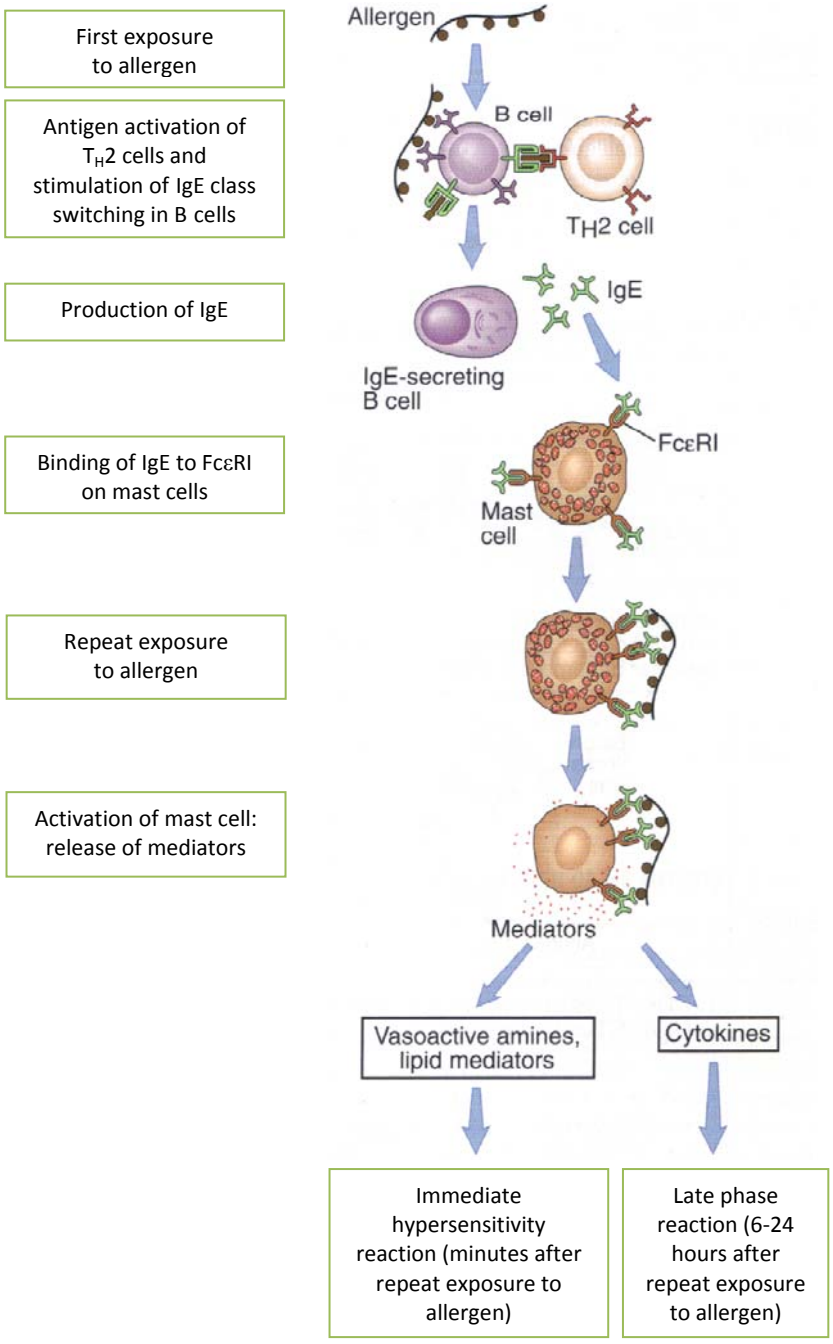


Figure 45. Typical sequence of events in immediate hypersensitivity.[129]

Some mast cell mediators are responsible for acute vascular and smooth muscle reactions and inflammation which may occur within minutes of reintroduction of antigen into a previously sensitized individual. Other mast cell mediators, such as cytokines cause so called late phase reactions (inflammation) by additionally recruiting neutrophils and eosinophils over several hours.

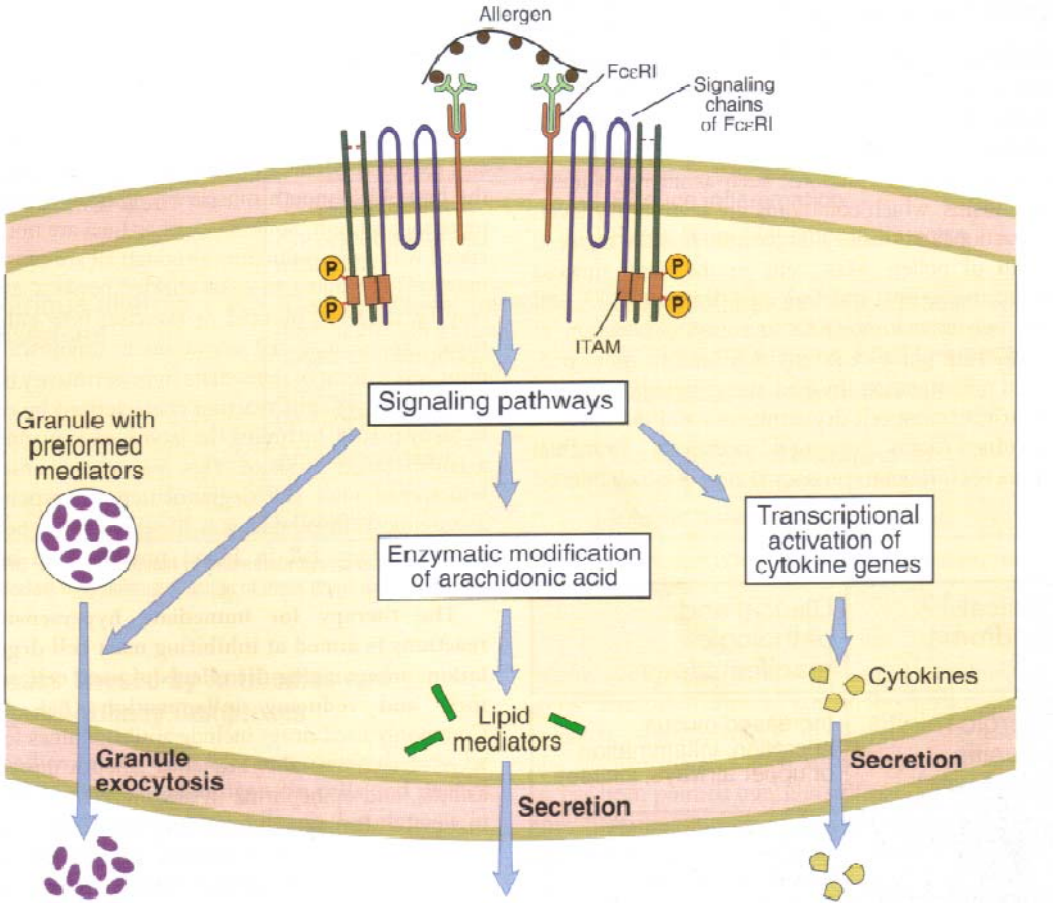


Figure 46. Biochemical events in mast cell activation.[127]

There are several clinical and pathologic features caused by releasing various mast cell mediators in different tissues. (Figure 47) Some of them are mild reactions, such as allergic rhinitis and sinusitis (hay fever), but there are more severe forms of immediate hypersensitivity too, such as anaphylaxis caused by fall in blood pressure (shock) and airway obstruction. (Figure 48)

The aim of drug therapy is the inhibition of mediator production, blockage of the release of granule contents, neutralization or counteracting the effects of the already liberated harmful mediators on target organs and reducing inflammation.

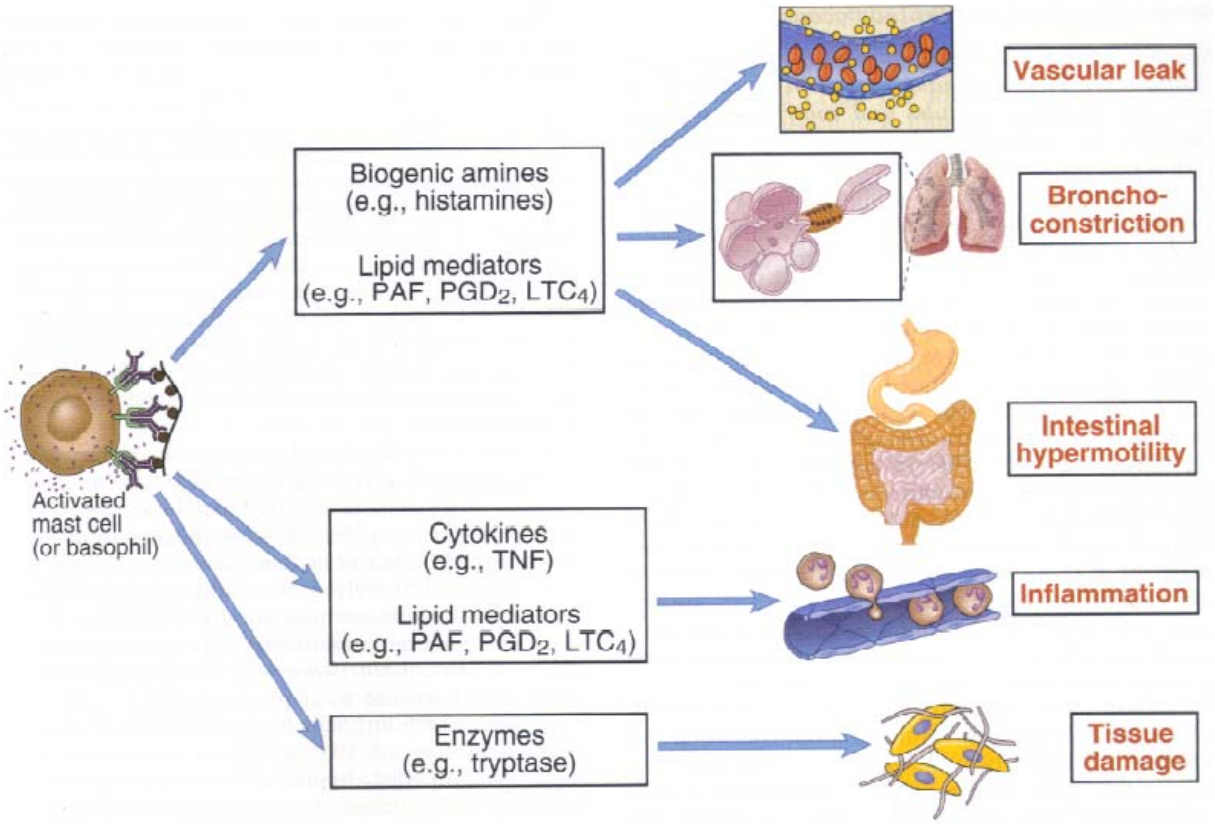


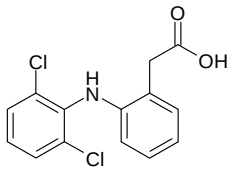
Figure 47. Biological effects of mediators of immediate hypersensitivity.[129]

Clinical syndrome	Clinical and pathologic manifestations	Therapy
Allergic rhinitis, sinusitis, (hay fever)	Increased mucus secretion; inflammation of upper airways, sinuses	"Desensitization" (repeated administration of low doses of allergens) Anti-IgE antibody (in clinical trials) Antihistamines Cromolyn
Food allergies	Increased peristalsis due to contraction of intestinal muscles	
Bronchial asthma	Bronchial hyper- responsiveness caused by smooth muscle contraction; inflammation and tissue injury caused by late phase reaction	Corticosteroids Phosphodiesterase inhibitors
Anaphylaxis (may be cause by drugs, bee sting, food)	Fall in blood pressure (shock) caused by vascular dilation; airway obstruction due to laryngeal edema	Epinephrine

Figure 48. Clinical manifestations of immediate hypersensitivity reactions.[127]

METABOLISM AND POTENTIAL IMMUNE REACTIONS OF DICLOFENAC

Diclofenac (2-[2-(2',6'-**dichlo**rophenyl)amino**phen**yl] **ac**etic acid)[130] **C.1** is a member of the nonsteroidal anti-inflammatory drug (NSAID) family. Diclofenac, the active compound of Voltaren™, is used as standard treatment of osteoarthritis, rheumatoid arthritis, ankylosing spondylitis, and acute muscle pain.[131] The compound has both analgesic and antipyretic activity that is achieved by competing with arachidonic acid for binding to cylcooxygenase (COX) and resulting in decreased formation of prostaglandins.[132] Among other duties, prostaglandins act as messenger molecules in inflammation process.[133]



C.1

Figure 49. Chemical structure of Diclofenac.

The COX enzyme exists in two isomeric forms with different functions: COX-1 protects the gastric mucosa, regulates the blood flow in kidneys and supports platelet aggregation; the role of COX-2 is associated with pain and swelling resulting from inflammation.[134] Diclofenac binds to both isoforms, unselectively.

For the metabolism of Diclofenac in humans, phase I reactions such as phenyl hydroxylation and phase II reactions including acyl glucuronidation and glutathione conjugation are described.[135] As the major route of clearance of Diclofenac in human bodies, enzymes catalysed phase II reactions including uridine 5'-diphosphoglucuronyl transferase and glutathione S-transferase are essential. Further oxidation (phase I) catalyzed by cytochrome P450 leads to a various hydroxylated derivatives of Diclofenac.[136,137,138,139] The major metabolites, found *in vitro* and *in vivo* in rat,[140] monkey and human hepatocytes[141,142,143,144], bile and urine[145,146] are outlined in Figure 50 and Figure 51.

It has been hypothesized that benzoquinone imines of Diclofenac are produced as glutathione metabolites of the phase II bioactivation process, which can covalently bind to cysteine residues of proteins (Figure 52). The accumulation of such protein conjugates linked to Diclofenac in the liver could result in immune-mediated hepatotoxicity.

Another bioactivation mechanism has been described where the transacylation or glycation potential of reactive acyl glucuronide intermediates to produce conjugates with proteins are discussed (Figure 53). An involvement in drug hypersensitivity[147] of covalently bound adducts of various NSAIDs (naproxen, zomepirac, fenoprofen, tolmetin, benoxaprofen, etc,) and human serum albumin (HSA) was hypothesized, after their detection by tandem mass spectrometry.[148,149,150,151,152] Protein adducts with Diclofenac located in livers of treated mice and rats could be verified by immunoblotting with a polyclonal antibody.[153,154] These modified

proteins included plasma membrane proteins (110, 140, 220 KDa) and one microsomal protein (50 KDa).

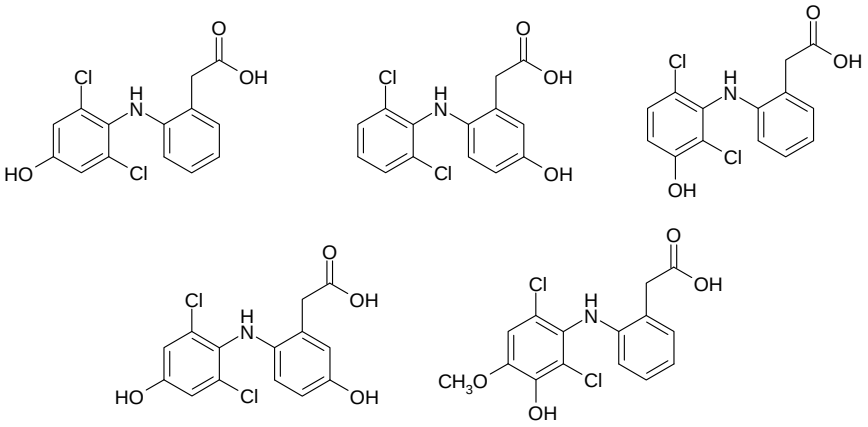


Figure 50. Main phase I metabolites of Diclofenac.

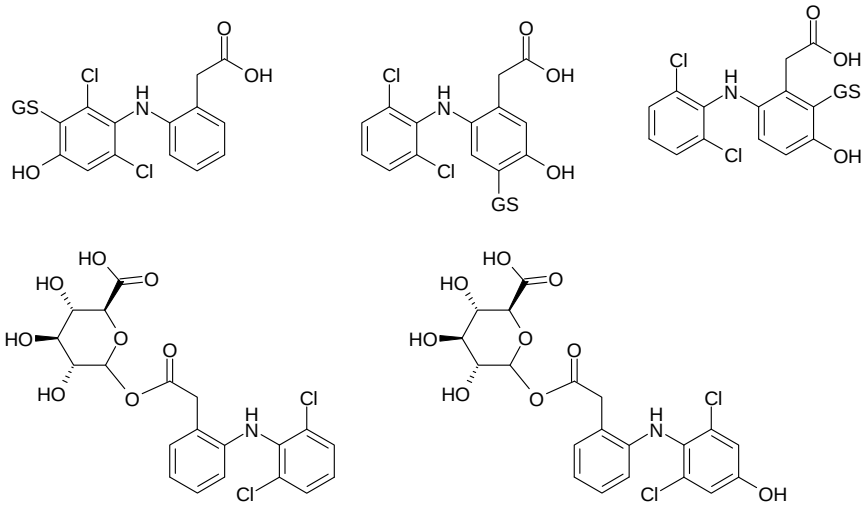


Figure 51. Main phase II metabolites of Diclofenac with glutathione abbreviated as GS.

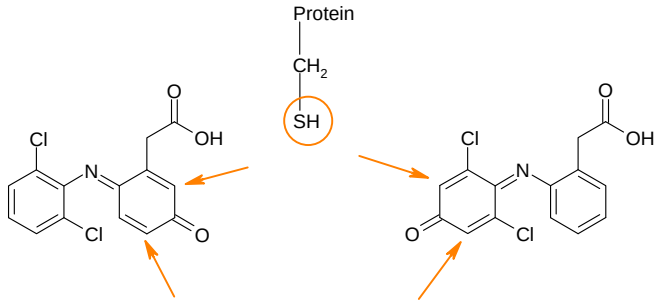


Figure 52. Benzoquinone imine intermediates of Diclofenac. Reactive sites for protein conjugation are shown in orange.

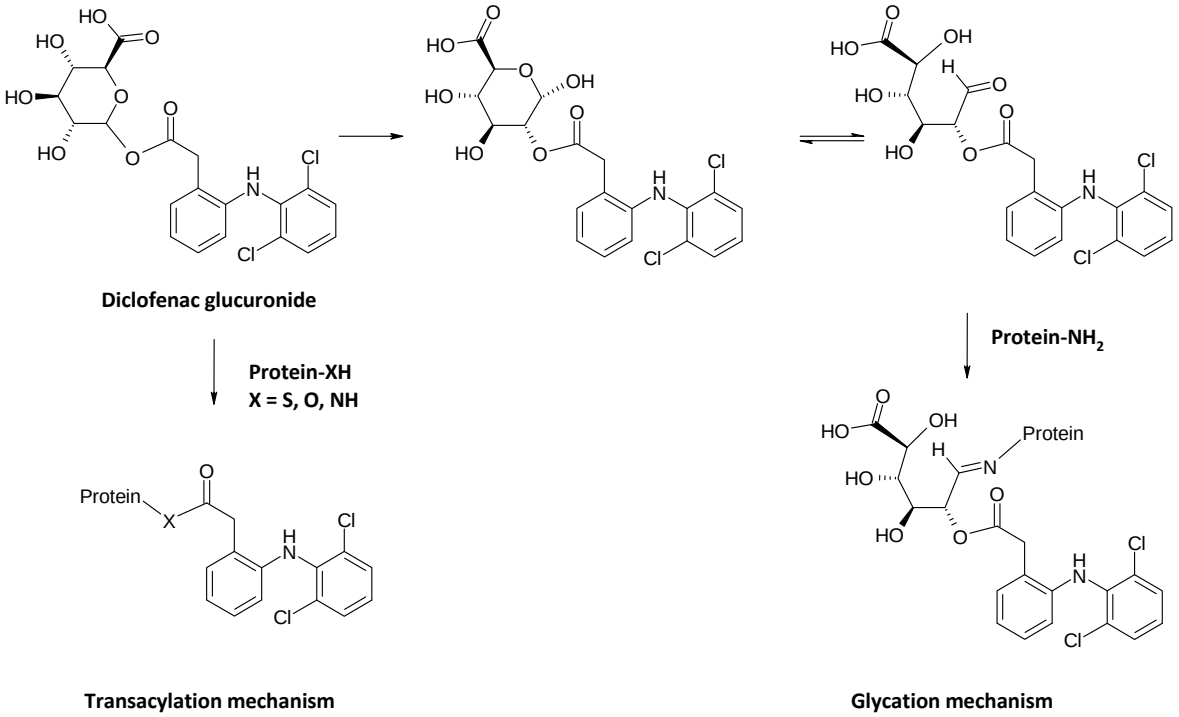


Figure 53. Proposed mechanisms for chemical protein modifications by reactive acyl glucuronide intermediates.

The proposed mechanisms for Diclofenac and their metabolites (Figure 53) show the possible alternative pathways compared to physiological mercapturic acid or acyl glucoronide routes, resulting in adverse reactions including severe hepatotoxicity or immunological reactions. Cases of acute immune hemolytic anemia against Diclofenac glucuronide and 4'-Hydroxy-Diclofenac have been reported.[155,156]

Previous postulations assume an involvement of IgE in intense systemic reactions like anaphylaxis upon oral or topical administration of Diclofenac. However, mechanistic evidence is still not available.[157,158,159]

More than 7 million people worldwide are considered to be treated with Diclofenac every year. Within 992 cases of drug-induced anaphylaxis, 30 cases were associated with this drug. Even lethal drug reactions to Diclofenac[160,161] as well as positive results of skin tests have been reported, suggesting an IgE-mediated mechanism.[162,163,164] The lack of convincing data on the existence of Diclofenac-specific IgE antibodies do not allow classification as type I hypersensitivity.

The aim of this project was the investigation of a potential immunologic mechanism underlying Diclofenac hypersensitivity. For this purpose a variety of *in vitro* immunological assays, including ELISA, mediator release assays and basophil activation test (BAT)[165] were performed, including different Diclofenac derivatives.[166] The synthesis of the used 5-hydroxy-diclofenac derivative is described in this chapter.

GRAPHICAL ABSTRACT

FIRST DIMETHYL AMIDE APPROACH

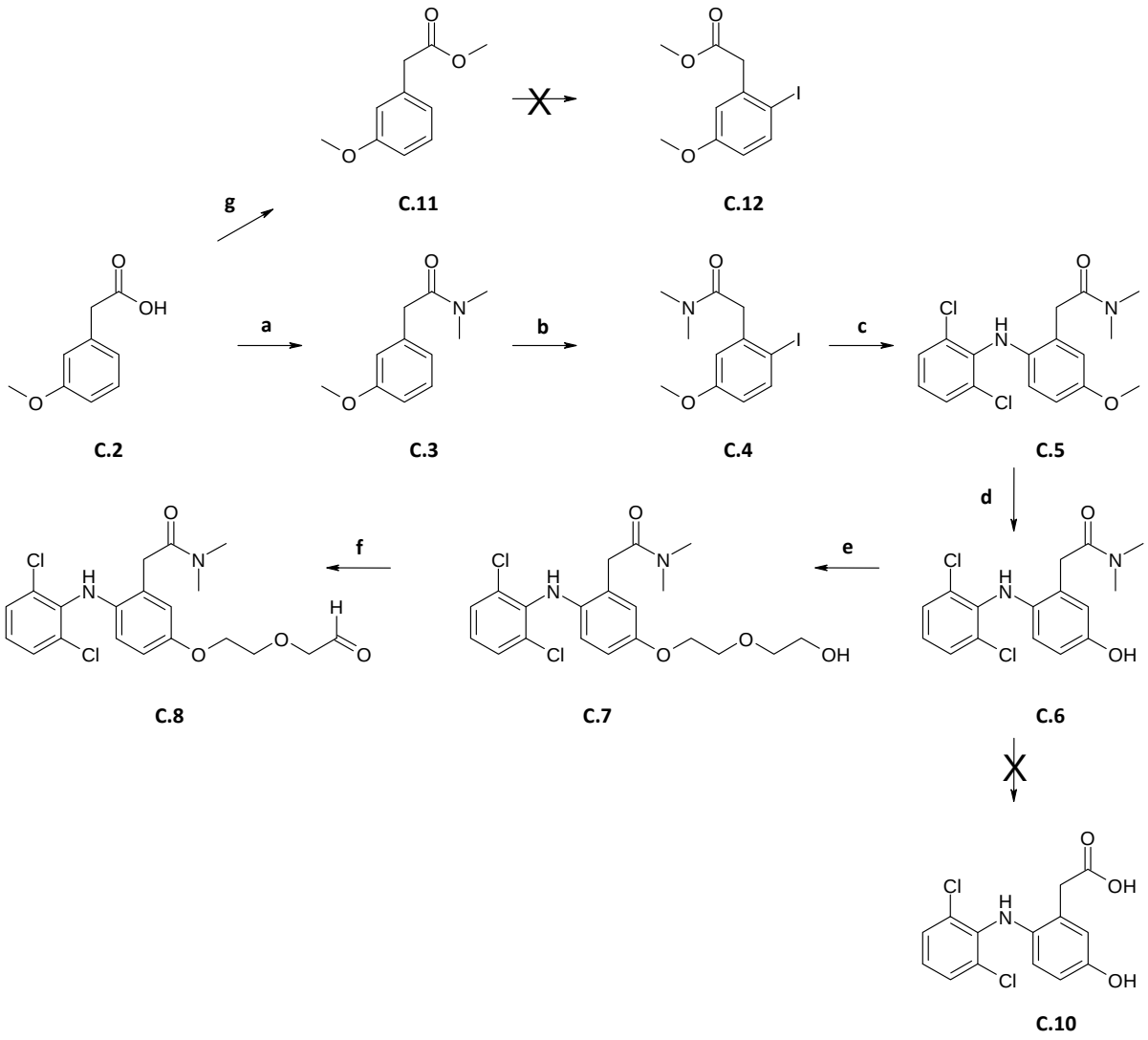


Figure 54. (a) oxalyl chloride, aqueous dimethylamine, DMF, DCM, 98%; (b) *N*-iodosuccinimide, CH_3CN, 71%; (c) 2,6-dichloroaniline, Cu-powder, CuI, K_2CO_3, toluene, 56%; (d) BBr_3, DCM/DCE, 97%; (e) diethylenglycol monochloride, K_2CO_3, DMF, 54%; (f) oxalyl chloride, DMSO, DCM, 78%; (g) Amberlite H^+, MeOH, quant.

FINAL SYNTHETIC STRATEGY

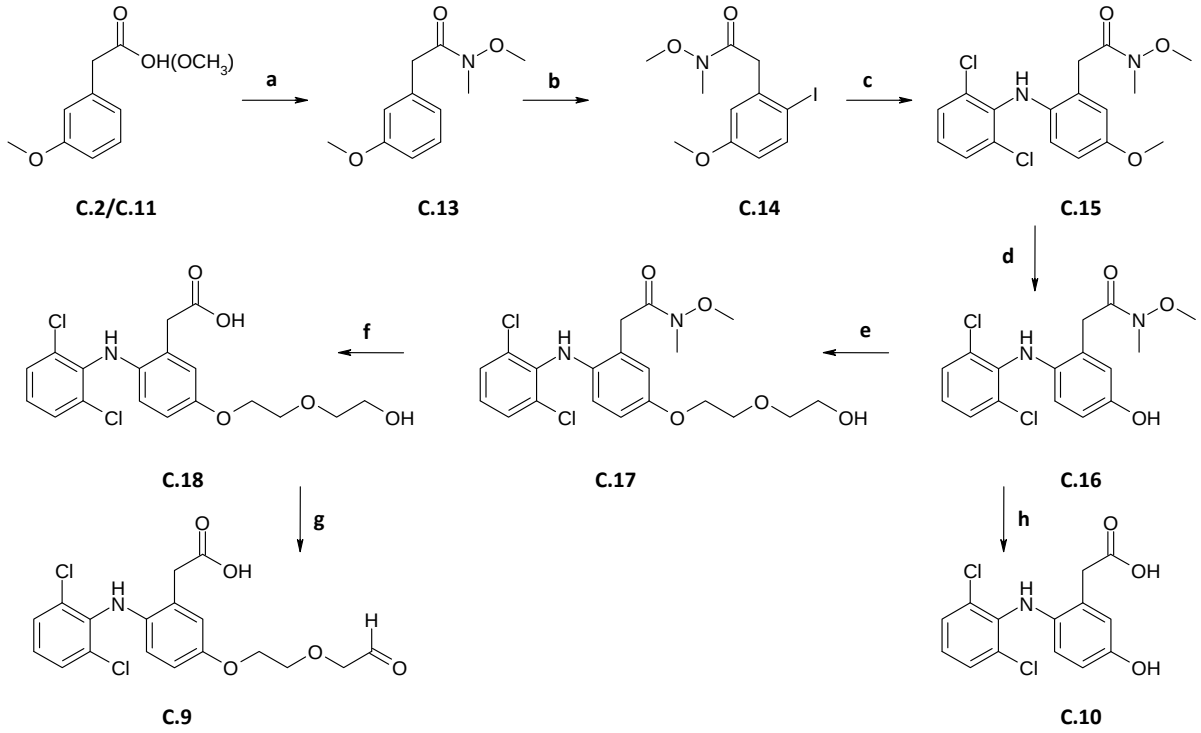


Figure 55. (a) ester: DIBAL-H, *N,O*-dimethylhydroxylamin hydrochloride, THF, 55% or acid: DCC, *N,O*-dimethylhydroxylamin hydrochloride, triethylamine, chloroform, 34%; (b) *N*-iodosuccinimide, CH_3CN, 84%; (c) 2,6-dichloroaniline, Cu-Bronze, CuI, K_2CO_3, toluene, 85%; (d) BBr_3, DCM/DCE, 97%; (e) diethylenglycol monochloride, K_2CO_3, DMF, 58%; (f) KOH, MeOH/H_2O, 46%; (g) IBX, TFA, DMSO, 11%; (h) KOH, MeOH/H_2O, 36%.

RESULTS AND DISCUSSION

Target of this synthesis was the derivatization of Diclofenac **C.1** in position 5 with a spacer handle for the conjugation to human serum albumin (HSA), leaving the carboxylic acid function intact. 5-Hydroxydiclofenac **C.10** is an oxidation product of the metabolism of Diclofenac in humans catalyzed by cytochrome P450. To match nature as exactly as possible, the synthetic strategy was precised by amplifying position 5 with a phenolic group, followed by prolongation using a polyethylene glycol spacer and subsequent oxidation of the spacer ending.

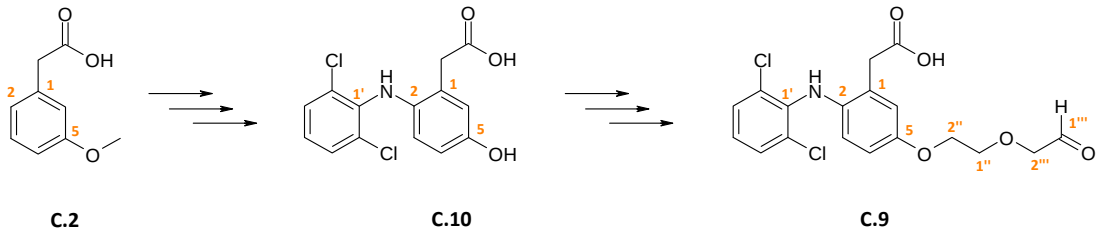


Figure 56. Schematic strategy for amplifying position 5.

The introduction of the hydroxyl group in position 5 of Diclofenac **C.1** was performed following a modified protocol developed by Kenny et al.[167]. This approach is starting from the 5-hydroxy derivative of phenylacetic acid, followed by subsequent buildup of the Diclofenac structure.

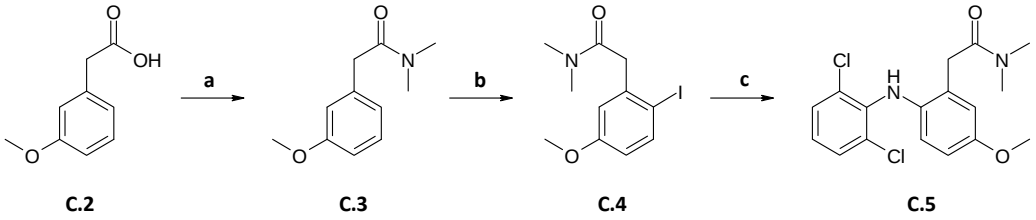


Figure 57. (a) oxalyl chloride, aqueous dimethylamine, DMF, DCM, 98%; (b) *N*-iodosuccinimide, CH_3CN, 71%; (c) 2,6-dichloroaniline, Cu-powder, CuI, K_2CO_3, toluene, 56%.

Starting from 3-methoxyphenylacetic acid **C.2** the carboxyl group was protected as amide **C.3** from the corresponding acid chloride with oxalyl chloride[168] and aqueous dimethylamide using the strategy of Schotten-Baumann. To introduce the amino bridge connecting the two aromatic rings, iodine was introduced regioselectively with *N*-iodosuccinimide (NIS) at the *ortho*-position. As side product of the *ortho/para* activation a 2,6-diiodocompound could be isolated in 3% yield.

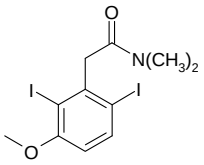


Figure 58. 2,3-Diiodocompound as side product.

Diaryl-coupling of the 6-iodo compound **C.4** to 2,6-dichloroaniline was accomplished under Ullmann conditions. Yields between 30-56% of compound **C.5** could be achieved using activated copper powder[172] under anhydrous conditions. Therewith the structure of Diclofenac was generated in three steps and an overall yield of 39%.

Cleavage of the methylether with BBr_3 afforded the protected 5-hydroxy diclofenac **C.6** in almost quantitative yields.

Hydrolysis of the amide at this stage proved to be very troublesome.[169,170,171] Excessive degradation or traces of product could be found, but even very careful, controlled conditions and rigorous exclusion of oxygen (Figure 59) could not led to satisfactory yields. As probable malefactor the free phenolic group is hypothesized.

Solvent	Reagent	Temperature	Time	Result
EtOH	1M NaOH (8 eq)	reflux	36 hours	traces
EtOH	1M NaOH (2-8 eq)	Schlenk/ reflux	36 hours	traces
EtOH	1M KOH (2-8 eq)[172]	reflux	36 hours	traces
H_2O	KO-*t*-BuOH (6.6 eq)	RT	15 hours	decomposition
MeOH	*p*-TosOH (3.5 eq)	105°C/ MW	1 hour	cyclization (indolone)
THF/H_2O	H_2O_2 (4 eq)[173,174]	RT	15 hours	no reaction

Figure 59. Reaction conditions for the hydrolysis of the dimethylamide.

Variation of the protecting group using methylester[153] **C.11** instead of dimethylamide was considered for a short time period, because already the introduction of iodine under standard conditions could not be achieved regioselectively. (Figure 60)

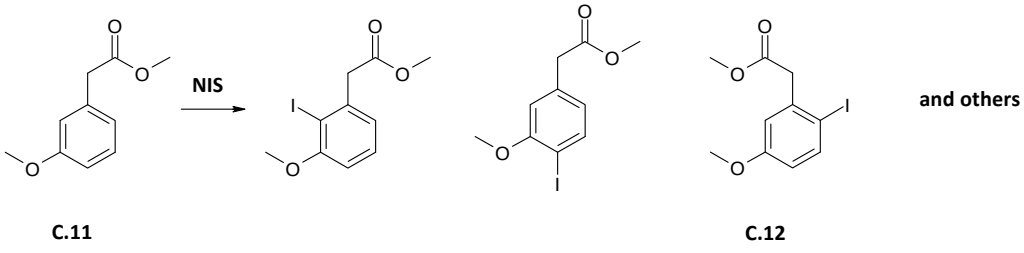


Figure 60. Three identified iodo-compounds of the product mixture.

Modifications using the Weinreb amide as protecting group for the carboxylic acid were successful. The syntheses followed the same synthetic strategy, starting from 3-methoxyphenylacetic acid **C.2**. The carboxyl group was protected as Weinreb[175] amide **C.13** via two different methods. The standard procedures with DCC[176,177] showed only 34% yield in contrast to the method with DIBAL-H[178], but the lower yields were acceptable due to the

possibility of reisolation of starting material **C.2**. Afterwards iodine was introduced regioselectively at the *ortho*-position in 84% yield. Diaryl-coupling of the 6-iodo compound **C.14** to 2,6-dichloroaniline was accomplished under modified Ullmann conditions. Yields around 80% could be achieved, if freshly activated copper-bronze was used instead of copper-powder.[179] Treatment of diarylamine **C.15** with BBr_3 afforded the protected 5-hydroxy-diclofenac **C.16** in almost quantitative yield. Even the cleavage of the Weinreb amide proved to be a little sophisticated and could be achieved under harsh microwave conditions with 2N KOH to yield the desired 5-hydroxydiclofenac **C.10** in 36%. Finally with the attempt to introduce the linker before deprotection, the yield could be improved to 46% for compound **C.18**.

For the selective bonding of 5-hydroxydiclofenac to HSA using position 5, a spacer molecule had to be attached. For the success of the conjugation spacer properties as length, magnitude, structure, hydrophobic character, charge and others, have to be considered. The length of a spacer molecule used for protein bonding should not be too short, so that the sterical hindrance could be reduced to a minimum. Five to six atom long spacer molecules are most frequently used in literature. To achieve better water solubility for protein coupling, a polyethylene glycol was used as a linker[180] instead of a linear alkyl chain.[181,182,183] This reaction was carried out under microwave conditions to yield 54% and 58% of prolonged alcohol **C.7** and **C.17**.[184,185]

To leave the carboxylic acid group untouched, reductive amination[186,187,188] was chosen as a coupling method instead the very common EDC conjugation.

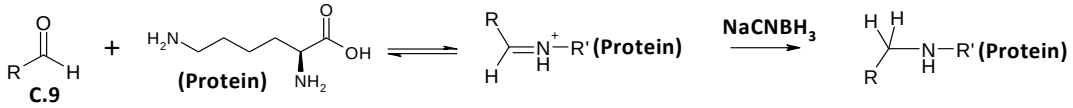


Figure 61. Reaction scheme for the coupling with sodium cyanoborohydride to the lysines of the HSA protein.

For an effective coupling of the Diclofenac derivative to the lysines of the carrier protein with sodium cyanoborohydride[189] , the linker must contain a terminal aldehyde function. The final synthetic steps included deprotection[190] of Weinreb amide **C.17** under basic conditions and oxidation using IBX[191,192,193] to the desired aldehyde **C.9**. (Figure 62) Primary Swern oxidation[194,195,196] was only successful with a protected carboxylic acid function; aldehyde **C.8** was used for first experiments and optimization of the reductive amination procedure.

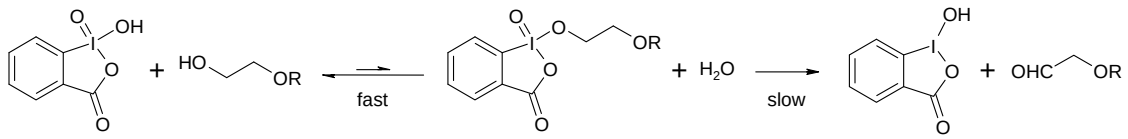


Figure 62. Proposed mechanism of the IBX oxidation.

In conclusion, synthesis of the desired 5-hydroxydiclofenac derivative **C.9** was successful using the Weinreb amide as protecting group.

EXPERIMENTAL PROCEDURES

GENERAL METHODS

Solvents were purified by destillation and dried by standard procedures. Thin layer chromatography (TLC) was performed on precoated silica gel plates 60 F254 (Merck), detected with UV light (254 nm), ceric ammonium molybdate as well as 5% vanillin/sulfuric acid and heated by a hotgun. For preparative column chromatography silica gel 60M (230-400 mesh, Macherey-Nagel) was used.

Cu-bronze was activated by a treatment with a 2% solution of iodine in acetone for 5-10 minutes. The product was filtered over a Büchner funnel, removed and washed by stirring into a slurry with a 1/1 solution of conc. hydrochlorid/acetone and filtered again. This product was dried in a vacuum desiccators and should be immediately used.

^{1}H and ^{13}C NMR spectra were recorded on a Bruker AVANCE DRX 400 NMR spectrometer. Chemical shifts are expressed as parts per million (ppm) and were referenced to 7.26 ($CDCl_3$), 4.79 (D_2O), 2.50 (d_6-DMSO) and 2.05 ($(CD_3)_2CO$) for the proton spectra as well as to 77.16 ($CDCl_3$), 39.52 (d_6-DMSO) and 29.84 ($(CD_3)_2CO$) for ^{13}C spectra. Coupling constants are quoted in Hertz (Hz).

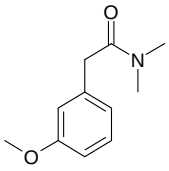
Mass spectra were recorded on spectrometers from Micro Mass (Fissions Instrument Trio200) in electron impact (EI) mode (70 eV) and on a Finnigan MAT 8230 in electron spray ionization mode.

Microwave heating was performed with a Biotage initiator synthesizer.

All chemicals including the starting compound **C.2** were purchased by Aldrich.

GENERAL PROCEDURES

2-(3-methoxyphenyl)-*N,N*-dimethyl acetamide



C.3

To a solution of *p*-methoxyphenylacetic acid **C.2** (11 g, 66 mmol, 1 eq) in dry methylene chloride (100 mL) was added dropwise oxalyl chloride (6.2 mL, 1.2 eq) and catalytic amounts of dry DMF (5 drops). The solution was stirred until formation of gas subsided and the solvents were removed. The crude product was redissolved in dry methylene chloride (50 mL) and added dropwise to a cooled vigorously stirred two-phase system of 40%w/w aqueous dimethylamine (45 mL) and 50 mL methylene chloride at 0°C for one hour. The organic layer was separated, washed with 1N

HCl, saturated Na_2CO_3 solution and water (à 25 mL) and dried over $MgSO_4$, to afford quantitative yields of product **C.3**, which was used without any further purification.

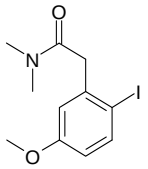
$C_{11}H_{15}NO_2$ M_r = 193.25

^1H (400 MHz, $CDCl_3$): δ 7.215 (bt, 1H, $J_{5,4/5,6}$ 7.76 Hz, H-5), 6.833-6.764 (m, 3H, H-2, H-4, H-6), 3.783 (s, 3H, OCH_3), 3.684 (s, 2H, CH_2), 2.981 (s, 3H, NCH_3), 2.954 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 171.00 (C=O), 159.96 (C-3), 136.70 (C-1), 129.71 (C-5), 121.21 (C-2), 114.42 (C-6), 112.41 (C-4), 55.32 (CH_3O), 41.25 (CH_2), 37.86 (NCH_3), 35.74 (NCH_3).

MS: Calcd for [$C_{11}H_{15}NO_2$]: *m/z* 193.25: found: [M+H] 194.2

2-(2-iodo-5-methoxyphenyl)-*N,N*-dimethyl acetamide



C.4

A suspension of amide **C.3** (5 g, 25.9 mmol, 1 eq) and *N*-iodosuccinimide (13.4 g, 59.5 mmol, 2.3 eq) was refluxed for 20 hours, concentrated under reduced pressure and redissolved in diethyl ether. The organic layer was washed twice with 10% $Na_2S_2O_3$ solution (25 mL) and once with brine, dried over $MgSO_4$ and the solvents were removed *in vacuo*. The crude product was purified by flash column chromatography (silicagel, hexane/ethyl acetate=1/1) to give 5.84 g (71%) of iodo-compound **C.4**.

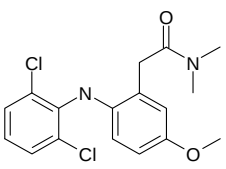
$C_{11}H_{14}INO_2$ M_r = 319.14

^1H (400 MHz, $CDCl_3$): δ 7.680 (d, 1H, $J_{3,4}$ 8.73 Hz, H-3), 6.855 (d, 1H, $J_{6,4}$ 2.97 Hz, H-6), 6.555 (dd, 1H, $J_{4,3}$ 8.71 Hz, $J_{4,6}$ 2.98 Hz, H-4), 3.763 (s, 3H, OCH_3), 3.758 (s, 2H, CH_2), 3.027 (s, 3H, NCH_3), 2.999 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 170.24 (C=O), 160.26 (C-5), 139.82 (C-3), 115.95 (C-6), 114.97 (C-4), 89.67 (C-2), 55.50 (CH_3O), 45.99 (CH_2), 37.85 (NCH_3), 37.84 (NCH_3).

MS: Calcd for [$C_{11}H_{14}INO_2$]: *m/z* 319.14: found: [M-I] 192

2-(2-(2',6'-dichlorophenylamino)-5-methoxyphenyl)-*N,N*-dimethyl acetamide



C.5

A mixture of the iodo-compound **C.4** (0.79 g, 2.45 mmol, 1 eq), 2,6-Dichloranilin (0.76 g, 4.67 mmol, 1.9 eq), anhydrous K_2CO_3 (263 mg, 1.84 mmol, 0.75 eq), CuI (26 mg, 0.14 mmol) and Cu powder (86 mg, 1.4 mmol, 0.6 eq) in anhydrous toluene was refluxed for 2½ days under argon atmosphere. The mixture was cooled, filtered over a Celite pad, washed with toluene and concentrated *in vacuo*. The crude product was dissolved in ethyl acetate, washed with water and brine (à 10 mL), dried over $MgSO_4$ and the solvents were removed under reduced pressure. The product was purified by flash column chromatography (silica gel, hexane/ethyl acetate=3/2) to yield 490 mg (56%) of compound **C.5**.

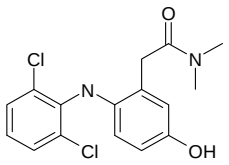
$C_{17}H_{18}Cl_2N_2O_2$ M_r = 353.25

^{1}H (400 MHz, $CDCl_3$): δ 7.293 (d, 2H, $J_{3'/5',4'}$ 8.04 Hz, H-3', H-5'), 7.145 (bs, 1H, NH), 6.889 (dd,1H, $J_{4',3'/5'}$ 8.04 Hz, H-4'), 6.743 (d, 1H, $J_{6,4}$ 2.80 Hz, H-6), 6.660 (dd, $J_{4,6}$ 2.82 Hz, $J_{4,3}$ 8.62 Hz, H-4), 6.525 (d, 1H, $J_{3,4}$ 8.72 Hz, H-3), 3.818 (s, 2H, CH_2), 3.756 (s, 3H, CH_3O), 3.184 (s, 3H, NCH_3), 3.000 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 171.42 (C=O), 154.93 (C-5), 139.10 (C-1'), 137.03 (C-1), 128.95 (C-3', C-5'), 128.67 (C-2', C-6'), 127.99 (C-2), 122.94 (C-4'), 120.21 (C-3), 116.61 (C-6), 112.23 (C-4), 55.67 (OCH_3), 38.12 (NCH_3), 37.63 (CH_2), 36.06 (NCH_3).

MS: Calcd for [$C_{17}H_{18}Cl_2N_2O_2$]: *m/z* 353.25: found: [M-H] 352

2-(2-(2',6'-dichlorophenylamino)-5-hydroxyphenyl)-*N,N*-dimethyl acetamide



C.6

To a solution of methoxyamide **C.5** (1.3 g, 3.57 mmol, 1 eq) in 1,2-dichloroethane (50 mL) a 1M solution of BBr_3 (13 mL, 13 mmol, 3.6 eq) in methylene chloride was added dropwise. The reaction was stirred for 1½ hours at room temperature and was quenched by the addition of half saturated $NaHCO_3$ solution. The mixture was extracted trice with methylene chloride, the combined organic layers were washed with water and dried over $MgSO_4$. Evaporation of the solvents afforded compound **C.6** in 97% (1.16 g) without any further purification.

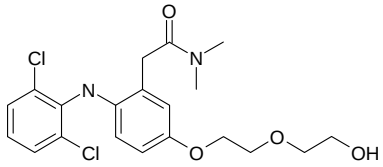
$C_{16}H_{16}Cl_2N_2O_2$ M_r = 339.22

^{1}H (400 MHz, d6-DMSO): δ 8.942 (s, 1H, OH), 7.428 (d, 2H, $J_{3'/5',4'}$ 8.07 Hz, H-3', H-5'), 7.275 (bs, 1H, NH), 7.024 (t, 1H, $J_{4',3'/5'}$ 8.04 Hz, H-4'), 6.645 (d, 1H, $J_{6,4}$ 2.74 Hz, H-6), 6.481 (dd, $J_{4,6}$ 2.81 Hz, $J_{4,3}$ 8.59 Hz, H-4), 6.235 (d, 1H, $J_{3,4}$ 8.56 Hz, H-3), 3.719 (s, 2H, CH_2), 3.118 (s, 3H, NCH_3), 2.870 (s, 3H, NCH_3).

^{13}C (400 MHz, d6-DMSO): δ 170.88 (C=O), 152.20 (C-5), 138.57 (C-1'), 134.85 (C-1), 129.11 (C-3', C-5'), 128.13 (C-2', C-6'), 127.57 (C-2), 123.35 (C-4'), 119.14 (C-3), 117.11 (C-6), 113.48 (C-4), 37.44 (NCH_3), 36.39 (CH_2), 35.17 (NCH_3).

MS: Calcd for [$C_{16}H_{16}Cl_2N_2O_{22}$]: *m/z* 339.22: found: [M+Na] 361.1/ 363.1

2-(2-(2',6'-dichlorophenylamino)-5-(2''-(2'''-hydroxyethoxy)ethoxy)phenyl)-*N,N*-dimethyl acetamide



C.7

A suspension of amide **C.6** (266 g, 0.78 mmol, 1 eq) and anhydrous K_2CO_3 (120 mg, 0.87 mmol, 1.1 eq) in dry DMF (10 mL) was heated under microwave conditions for two minutes at 100°C and after addition of diethylenglycol monochloride (92µL, 0.87 mmol, 1.1 eq) via a syringe for 60 minutes at 100°. TLC control still showed starting material **C.6**, therefore further diethylenglycol monochloride (100 µL, 0.94 mmol, 1.2 eq) was added and the mixture was heated again under microwave conditions for 60 minutes at 100°C, followed by standard heating overnight at 100°C. The reactions was quenched by 1N HCl until a pH value of 1 was reached and the solution was extracted six times with ethyl acetate (à 15 mL). The combined organic layers were washed with water, dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (silicagel, hexane/ethyl acetate=1/3) to afford 180 mg (54%) of compound **C.7**.

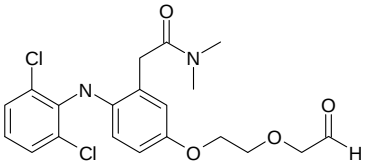
$C_{20}H_{24}Cl_2N_2O_4$ M_r = 427.33

^{1}H (400 MHz, $CDCl_3$): δ 7.290 (d, 2H, $J_{3'/5',4'}$ 8.04 Hz, H-3', H-5'), 7.133 (bs, 1H, NH), 6.891 (t,1H, $J_{4',3'/5'}$ 8.00 Hz, H-4'), 6.788 (d, 1H, $J_{6,4}$ 2.80 Hz, H-6), 6.669 (dd, $J_{4,6}$ 2.94 Hz, $J_{4,3}$ 8.70 Hz, H-4), 6.507 (d, 1H, $J_{3,4}$ 8.68 Hz, H-3), 4.095-4.072 (m, 2H, H-1''), 3.840-3.816 (m, 2H, H-2''), 3.805 (s, 2H, CH_2),3.760-3.738 (m, 2H, H-2'''), 3.666-3.643 (m, 2H, H-1'''), 3.174 (s, 3H, NCH_3), 2.994 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 171.42 (C=O), 153.95 (C-5), 138.99 (C-1'), 137.35 (C-1), 128.95 (C-3', C-5'), 128.70 (C-2', C-6'), 127.97 (C-2), 123.037 (C-4'), 120.14 (C-3), 117.53 (C-6), 113.12 (C-4), 72.69 (C-1'''), 69.95 (C-2''), 67.99 (C-1''), 61.93 (C-2'''), 38.15 (NCH_3), 37.53 (CH_2), 36.07 (NCH_3).

MS: Calcd for [$C_{20}H_{24}Cl_2N_2O_4$]: *m/z* 427.33: found: [M-H] 425.2/427.2

2-(2-(2',6'-dichlorophenylamino)-5-(2''-(2'''-oxoethoxy)ethoxy)phenyl)-*N,N*-dimethyl acetamide



C.8

To a cooled solution (-70°C) of oxalyl chloride (0.84 mL of 1M solution, 0.84 mmol, 2 eq) was added a DMSO solution (1.26 mg of 1M solution, 1.26 mmol, 3 eq) slowly thus keeping the temperature constant. After stirring for 30 minutes, a solution of compound **C.7** (180 mg, 0.42 mmol, 1 eq) in dry methlyene chloride (3 mL) was added dropwise and stirring was continued for further 15 minutes at -70°C. Slow addition of trimethylamin (2.1 mL of 1M solution, 2.1 mmol, 5 eq) and stirring of the reaction mixture for one hour (-70°C to -10°C) showed on the TLC control full consumption. The reaction was quenched with water and the layers were separated. The organic layer was washed twice with 1N HCl and was dried over $MgSO_4$. Evaporation of the solvents and purification by column chromatography (silica gel, pure ethyl acetate) afforded aldehyde **C.8** 78% (139 mg) yield.

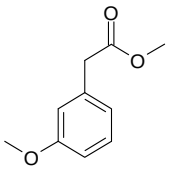
$C_{20}H_{22}NCl_2N_2O_2$ M_r = 425.32

^{1}H (400 MHz, $CDCl_3$): δ 9.684 (bs, 1H, H-1'''), 7.254 (d, 2H, $J_{3'/5',4'}$ 8.00 Hz, H-3', H-5'), 6.858 (t,1H, $J_{4',3'/5'}$ 8.06 Hz, H-4'), 6.747 (d, 1H, $J_{6,4}$ 2.84 Hz, H-6), 6.631 (dd, $J_{4,6}$ 2.86 Hz, $J_{4,3}$ 8.70 Hz, H-4), 6.472 (d, 1H, $J_{3,4}$ 8.73 Hz, H-3), 4.169 (s, 2H, 2'''), 4.090-4.067 (m, 2H, H-1''), 3.860-3.838 (m, 2H, H-2''), 3.775 (s, 2H, CH_2), 3.137 (s, 3H, NCH_3), 2.951 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 200.586 (C-1'''), 171.21 (C=O), 153.57 (C-5), 138.75 (C-1'), 137.29 (C-1), 128.79 (C-3', C-5'), 128.52 (C-2', C-6'), 127.81 (C-2), 122.94 (C-4'), 119.86 (C-3), 117.29 (C-6), 112.87 (C-4), 76.88 (C-2'''),70.44 (C-2''), 67.89 (C-1''), 37.97 (NCH_3), 37.34 (CH_2), 35.87 (NCH_3).

MS: Calcd for [$C_{20}H_{22}NCl_2N_2O_2$]: *m/z* 425.32: found: [M-H] 423.2/425.2

Methyl-2-(3-methoxyphenyl) acetate



C.11

p-Methoxyphenylacetic acid **C.2** (10 g, 60 mmol, 1 eq) was stirred with Amberlite H^+ in anhydrous methanol overnight at 70°C. Amberlite was filtered and the solvents were removed *in vacuo*. The crude product was redissolved in ethyl acetate, washed trice with 1N HCl and was dried over $MgSO_4$. Solvents were removed under reduced pressure to afford the methyl ester **C.11** in quantitative yield (10.8 g).

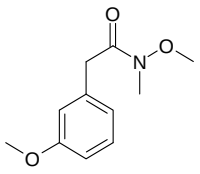
$C_{10}H_{12}O_3$ M_r = 180.21

^{1}H (400 MHz, $CDCl_3$): δ 7.238 (t, 1H, J 7.68, H-5), 6.875-6.803 (m, 3H, H-4, H-2, H-6), 3.803 (s, 3H, CH_3O), 3.695 (s, 3H, $COOCH_3$), 3.604 (s, 2H, CH_2).

^{13}C (400 MHz, $CDCl_3$): δ 172.03 (C=O), 159.87 (C-3), 135.53 (C-1), 129.69 (C-5), 121.73 (C-6), 115.06 (C-2), 112.79 (C-4), 55.33 (CH_3O), 52.18 ($COOCH_3$), 41.37 (CH_2).

MS: Calcd for [$C_{10}H_{12}O_3$]: *m/z* 180.21: found: [M^+] 180.0

_N_-Methoxy-2-(3-methoxyphenyl)-_N_-methyl acetamide



C.13

Method a)

To a suspension of *N,O*-Dimethylhydroxylamin hydrochloride (3.9 g, 40 mmol, 6 eq) in dry THF (8 mL) was slowly added a solution of DIBAL-H in toluene (25.8 mL, 38.7 mmol, 5.8 eq) at 0°C. The reaction mixture was brought to room temperature and stirred for one hour before ester **C.11** (1.2 g, 6.7 mmol, 1 eq) in dry THF (7 mL) was added dropwise. Afterwards stirring was continued over night. The reaction was cooled to 0°, quenched with water (15 mL) and 1M $KHSO_4$ (40 mL) solution and extracted five times with methylene chloride (à 15 mL). The combined organic layers were washed with water, dried over $MgSO_4$. Solvents were removed under reduced pressure and the crude product was purified by column chromatography (silica gel, hexane/ethyl acetate=2/1) to yield 0.77 g (55%) of Weinreb amide **C.13**.

Method b)

Phenylacetic acid **C.2** (5 g, 30 mmol, 1 eq) and *N,O*-Dimethylhydroxylamin hydrochloride (2.94 g, 30 mmol, 1.1 eq) were dissolved in chloroform (250 mL) and stirred under argon atmosphere at room temperature. To the reaction mixture was first added dropwise a solution of DCC (6.8 g, 33 mmol, 1.1 eq) in chloroform (100 mL) followed by triethylamine (4.7 mL, 33 mmol, 1.1 eq). The reaction mixture was stirred overnight at 50°C. After filtration of the precipitate the solution was washed with 1N HCl (30 mL) twice, followed by 1N NaOH (3x à 30 mL) and 1N HCl (3x à 30 mL),dried over $MgSO_4$ and concentrated *in vacuo*. Purification by column chromatography (silicagel, hexane/ethyl acetate=1/1) yielded 2.14 g (34%) of Weinreb amide **C.13**.

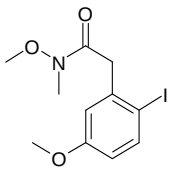
$C_{11}H_{15}NO_3$ M_r = 209.25

^{1}H (400 MHz, $CDCl_3$): δ 7.222 (t, 1H, $J_{5,4/5,6}$ 7.84 Hz, H-5), 6.883-6.857 (m, 2H, H-2, H-6), 3.788 (dd, 1H, $J_{4,5}$ 8.13 Hz, J 0.06 Hz, H-4),3.795 (s, 3H, OCH_3), 3.745 (s, 2H, CH_2), 3.609 (s, 3H, $NOCH_3$), 3.193 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 172.35 (C=O), 159.83 (C-3), 136.54 (C-1), 129.55 (C-5), 121.80 (C-2), 114.98 (C-6), 112.53 (C-4), 61.42 ($NOCH_3$), 55.32 (OCH_3), 39.57 (CH_2), 32.38 (NCH_3).

MS: Calcd for [$C_{11}H_{15}NO_3$]: *m/z* 209.25: found: [M] 209

2-(2-Iodo-5-methoxyphenyl)-*N*-methoxy-*N*-methyl acetamide



C.14

Weinreb amide **C.13** (2 g, 9.6 mmol, 1 eq) was dissolved in 25 mL acetonitrile and *N*-iodosuccinimide (3.23 g, 14.3 mmol, 1.5 eq) was added. The suspension was refluxed for 18 hours, concentrated under reduced pressure and taken up in diethyl ether. The organic layer was washed four times with 10% $Na_2S_2O_3$ solution (25 mL) and once with brine, dried over $MgSO_4$ and the solvents were removed *in vacuo*. The crude product was purified by flash column chromatography (silicagel, hexane/ethyl acetate=1/1) to give 2.7 g (84%) of iodo-compound **C.14**.

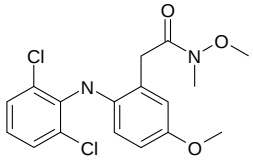
$C_{11}H_{14}INO_3$ M_r = 335.14

^{1}H (400 MHz, $CDCl_3$): δ 7.687 (d, 1H, $J_{3,4}$ 8.72 Hz, H-3), 6.869 (d, 1H, $J_{6,4}$ 3.04 Hz, H-6), 6.559 (dd, 1H, $J_{4,3}$ 8.74 Hz, $J_{4,6}$ 3.02 Hz, H-6) 3.884 (s, 2H, CH_2), 3.766 (s, 3H, OCH_3), 3.723 (s, 3H, $NOCH_3$), 3.226 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 171.47 (C=O), 160.10 (C-5), 139.86 (C-3), 139.49 (C-1), 116.54 (C-6), 115.01 (C-4), 89.96 (C-2), 61.59 ($NOCH_3$), 55.47 (OCH_3), 44.62 (CH_2), 32.58 (NCH_3).

MS: Calcd for [$C_{11}H_{14}INO_3$]: *m/z* 335.14: found: [M-I] 208.35

2-(2-(2',6'-dichlorophenylamino)-5-methoxyphenyl)-*N*-methoxy-*N*-methyl acetamide



C.15

A mixture of the iodo-compound **C.14** (2.7 g, 8 mmol, 1 eq), 2,6-Dichloroaniline (2.5 g, 15.3 mmol, 1,9 eq), anhydrous K_2CO_3 (830 mg, 6 mmol, 0.75 eq), CuI (92 mg, 0.5 mmol, 0.06 eq), freshly activated[179] Cu-bronze powder (305 mg, 5 mmol, 0.6 eq) and 4Å molecular sieve in 30 mL anhydrous toluene was refluxed for 4 days under argon atmosphere. The mixture was cooled, filtered over a pad of Celite, washed with toluene and concentrated. The crude product was dissolved in ethyl acetate, washed with water and brine once (à 25 mL), dried over $MgSO_4$ and the solvents were removed under reduced pressure. The product could be purified by decoating with hexane/ethyl acetate=2/1 and yielded 2.55 g (85%) of compound **C.15**.

$C_{17}H_{18}Cl_2N_2O_3$ M_r = 369.25

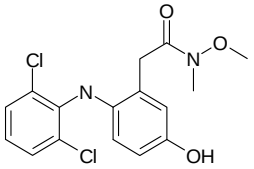
^{1}H (400 MHz, $CDCl_3$): δ 7.296 (d, 2H, $J_{3'/5',4'}$ 8.04 Hz, H-3', H-5'), 7.106 (bs, 1H, NH), 6.893 (t,1H, $J_{4',3'/5'}$ 8.02 Hz, H-4'), 6.802 (d, 1H, $J_{6,4}$ 2.92 Hz, H-6), 6.664 (dd, $J_{4,6}$ 2.94 Hz, $J_{4,3}$ 8.66 Hz, H-4), 6.527

(d, 1H, $J_{3,4}$ 8.72 Hz, H-3), 3.940 (s, 2H, CH_2), 3.787 (s, 3H, $NOCH_3$), 3.761 (s, 3H, OCH_3), 3.241 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 172.68 (C=O), 155.00 (C-5), 139.10 (C-1'), 136.97 (C-1), 128.96 (C-3', C-5'), 128.61 (C-2', C-6'), 127.59 (C-2), 122.93 (C-4'), 120.29 (C-3), 116.87 (C-6), 112.54 (C-4), 61.68 ($NOCH_3$), 55.66 (OCH_3), 35.95 (CH_2), 31.06 (NCH_3).

MS: Calcd for [$C_{17}H_{18}Cl_2N_2O_3$]: *m/z* 369.25: found: [M-Amin] 307.35/309.38

2-(2-(2',6'-dichlorophenylamino)-5-hydroxyphenyl)-*N*-methoxy-*N*-methyl acetamide



C.16

To a solution of amide **C.15** (1.6 g, 4.3 mmol, 1 eq) in 1,2-dichloroethane (30 mL) was added drop wise a 1M solution of BBr_3 (15.6 mL, 15.6 mmol, 3.6 eq) in methylene chloride. The reaction was stirred for 30 minutes at room temperature and was quenched with half saturated aqueous $NaHCO_3$ solution (300 mL). The reaction mixture was extracted with ethyl acetate (5x à 80 mL), the combined organic layers were washed with water twice, dried over $MgSO_4$ and the solvents were removed *in vacuo* to yield 1.56 g (99%) of pure compound **C.16**.

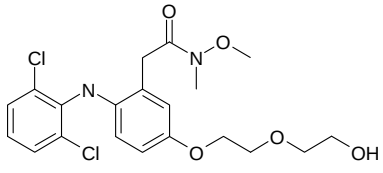
$C_{16}H_{16}Cl_2N_2O_3$ M_r = 355.22

^1H (400 MHz, d-6 DMSO): δ 8.996 (s, 1H, OH), 7.443 (d, 2H, $J_{3'/5',4'}$ 8.04 Hz, H-3', H-5'), 7.080 (bs, 1H, NH), 7.040 (t,1H, $J_{4',3'/5'}$ 8.08 Hz, H-4'), 6.662 (d, 1H, $J_{6,4}$ 2.76 Hz, H-6), 6.503 (dd, $J_{4,6}$ 2.76 Hz, $J_{4,3}$ 8.48 Hz, H-4), 6.265 (d, 1H, $J_{3,4}$ 8.67 Hz, H-3), 3.797 (s, 2H, CH_2), 3.761 (s, 3H, $NOCH_3$), 3.163 (s, 3H, NCH_3).

^{13}C (400 MHz, d-6 DMSO): δ 172.68 (C=O), 152.35 (C-5), 138.53 (C-1'), 134.71 (C-1), 129.12 (C-3', C-5'), 127.62 (C-2', C-6'), 127.48 (C-2), 123.43 (C-4'), 119.45 (C-3), 117.46 (C-6), 113.75 (C-4), 61.95 ($NOCH_3$), 35.46 (CH_2), 32.40 (NCH_3).

MS: Calcd for [$C_{16}H_{16}Cl_2N_2O_3$]: *m/z* 355.22: found: [M] 353.2/355.2

2-(2-(2',6'-dichlorophenylamino)-5-(2''-(2'''-hydroxyethoxy)ethoxy)phenyl)-*N*-methoxy-*N*-methyl acetamide



C.17

A suspension of Weinreb amide **C.16** (365 mg, 1.03 mmol, 1 eq) and anhydrous K_2CO_3 (156 mg, 1.13 mmol, 1.1 eq) in anhydrous DMF was heated in the microwave for 2 minutes at 100°C. The diethylenglycol monochloride (120 µL, 1.13 mmol. 1.1 eq) was added via a syringe to the reaction mixture and was heated for 60 minutes at 100°C. TLC control still showed starting material **C.16**, therefore further diethylenglycol monochloride (60 µL, 0.57 mmol, 0.5 eq) was added and the reaction was heated again in the microwave for 60 minutes at 100°C, followed by normal heating for 18 hours at 100°C. The reaction was quenched by 1N HCl until a pH value of 1 was reached. The solution was extracted four times with ethyl acetate (à 20 mL). The organic layers were washed with water, dried over $MgSO_4$ and the solvents were removed under reduced pressure. The crude product was purified by flash column chromatography (silicagel, hexane/ethyl acetate=1/1 to pure ethyl acetate) to give 266 mg (58%) of compound **C.17**.

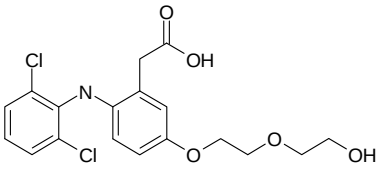
$C_{20}H_{24}Cl_2N_2O_5$ M_r = 443.33

^{1}H (400 MHz, $CDCl_3$): δ 7.286 (d, 2H, $J_{3'/5',4'}$ 8.00 Hz, H-3', H-5'), 7.109 (bs, 1H, NH), 6.887 (t, 1H, $J_{4',3'/5'}$ 8.00 Hz, H-4'), 6.834 (d, 1H, $J_{6,4}$ 2.84 Hz, H-6), 6.667 (dd, 1H, $J_{4,3}$ 8.72 Hz, $J_{4,6}$ 2.88 Hz, H-4), 6.502 (d, 1H, $J_{3,4}$ 8.72 Hz, H-3), 4.090-4.067 (m, 2H, H-1''), 3.919 (bs, 2H, CH_2), 3.834 (m, 2H, H-2''), 3.774 (s, 3H, $NOCH_3$), 3.752-3.730 (m, 2H, H-2'''), 3.659-3.636 (m, 2H, H-1'''), 3.227 (s, 3H, NCH_3).

^{13}C (400 MHz, $CDCl_3$): δ 172.26 (C=O), 153.98 (C-5), 138.96 (C-1'), 137.25 (C-1), 128.93 (C-3', C-5'), 128.60 (C-2', C-6'), 127.52 (C-2), 122.99 (C-4'), 120.16 (C-3), 117.72 (C-6), 113.37 (C-4), 72.66 (C-1'''), 69.91 (C-2''), 67.92 (C-1''), 61.90 (C-2'''), 61.67 ($NOCH_3$), 35.82 (CH_2), 32.43 (NCH_3).

MS: Calcd for [$C_{20}H_{24}Cl_2N_2O_5$]: *m/z* 443.33: found: [M+Na] 465.2/467.2

2-(2-(2',6'-dichlorophenylamino)-5-(2''-(2'''-hydroxyethoxy)ethoxy)phenyl)acetic acid



C.18

A solution of 2N KOH (166 µL, 0.33 mmol, 2.3 eq) was added to a suspension of Weinreb amide **C.17** (64 mg, 0.14 mmol, 1eq) in a one to one mixture of methanol and water (1+1 mL). The reaction mixture was stirred at room temperature until TLC control showed no remaining starting material (1 ½ hours). The reaction was acidified with 1N HCl (pH=1), extracted with ethyl acetate for five times (à 15 mL). The organic layer was washed with brine once, dried over $MgSO_4$ and the solvents were removed under vacuum. Crude product could be purified by column chromatography (silicagel, ethyl acetate/methanol=5/1) to afford 25 mg (43%) of acid **C.18**.

$C_{18}H_{19}Cl_2NO_5$ M_r = 400.26

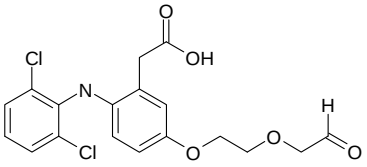
^{1}H (400 MHz, $(CD_3)_2CO$)): δ 7.373 (d, 2H, $J_{3'/5',4'}$ 7.96 Hz, H-3', H-5'), 7.200 (bs, 1H, NH), 7.017 (bt, 1H, $J_{4',3'/5'}$ 7.98 Hz, H-4'), 6.911 (bs, 1H, H-6), 6.674 (bd, 1H, $J_{4,3}$ 8.40 Hz, H-4), 6.424 (d, 1H, $J_{3,4}$ 8.60

Hz, H-3), 4.062-4.027 (m, 2H, H-1''), 3.755 (bs, 4H, CH_2,H-2''), 3.651-3.627 (m, 2H, H-2'''), 3.576-3.552 (m, 2H, H-1''').

^{13}C (400 MHz, $(CD_3)_2CO$)): δ 176.42 (COOH), 155.24 (C-5), 139.92 (C-1'), 137.36 (C-1), 129.85 (C-3', C-5'), 129.15 (C-2', C-6'), 129.09 (C-2), 124.17 (C-4'), 120.43 (C-3), 118.02 (C-6), 114.03 (C-4), 73.60 (C-1'''), 70.42 (C-2''), 68.66 (C-1''), 61.98 (C-2'''), 40.76 (CH_2).

MS: Calcd for [$C_{18}H_{19}Cl_2NO_5$]: *m/z* 400.26: found: [M] 398.0/400.0

2-(2-(2',6'-dichlorophenylamino)-5-(2''-(2'''-oxoethoxy)ethoxy)phenyl)acetic acid



C.9

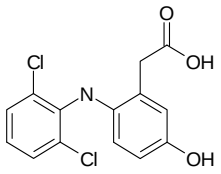
Alcohol **C.18** (22 mg, 55 µmol, 1 eq) was dissolved in 1 mL DMSO and trifluoroacetic acid (6.3 µL, 82 µmol, 1.5 eq) was added. To this reaction mixture a solution of IBX (23 mg, 82 µmol, 1.5 eq) in 1 mL DMSO was added and stirred at room temperature for 6 hours. The reaction was cooled, quenched with water and filtered. The filtrate was extracted three times with ethyl acetate and the combined organic layers were washed with brine once. Drying over $MgSO_4$, evaporation of the solvents and purification by column chromatography (silica gel, hexane/ethyl acetate=1/2) afforded aldehyde **C.9** in 11% (2.5 mg) yield.

$C_{18}H_{17}Cl_2NO_5$ M_r = 398.25

^{1}H (400 MHz, $(CD_3)_2CO$)): δ 9.686 (t, 1H, $J_{1''',2'''}$ 0.76 Hz, CHO), 7.426 (d, 2H, $J_{3'/5',4'}$ 8.08 Hz, H-3', H-5'), 7.071 (t, 1H, $J_{4',3'/5'}$ 8.06 Hz, H-4'), 6.957 (d, 1H, $J_{6,4}$ 2.92 Hz, H-6), 6.852 (bs, 1H, NH), 6.755 (dd, 1H, $J_{4,3}$ 8.70 Hz, $J_{4,6}$ 2.94 Hz, H-4), 6.487 (d, 1H, $J_{3,4}$ 8.68 Hz, H-3), 4.227 (d, 2H, $J_{2''',1'''}$ 0.80 Hz, H-2'''), 4.167-4.143 (m, 2H, H-2''), 3.924-3.900 (m, 2H, H-1''), 3.817 (s, 2H, CH_2COOH).

^{13}C (400 MHz, $(CD_3)_2CO$)): δ 201.57 (CHO), 173.81 (COOH), 155.53 (C-5), 139.94 (C-1'), 137.48 (C-1), 130.10 (C-3', C-5'), 129.23 (C-2', C-6'), 128.74 (C-2), 124.64 (C-4'), 121.07 (C-3), 118.18 (C-6), 114.47 (C-4), 77.51 (C-2'''), 71.12 (C-1''), 68.83 (C-2''), 38.84 (CH_2COOH).

2-(2-(2',6'-dichlorophenylamino)-5-hydroxyphenyl) acetic acid



C.10

To a suspension of Weinreb amide **C.16** (130 mg, 0.36 mmol, 1 eq) in methanol/water (5 mL/5 mL) was slowly added 2N KOH (0.7 mL, 1.5 mmol, 2 eq) at room temperature. The blue

colored reaction mixture was refluxed overnight, brought to room temperature, quenched with 1N HCl until a pH value of 1 was reached and extracted five times with ethyl acetate (à 10 mL). The combined orgnic layers were washed once with water and brine, dried over $MgSO_4$ and the solvents were removed *in vacuo*. Purification of the crude mixture with column chromatography (silicagel, gradient of hexane/ethyl acetate=1/1 to pure ethyl acetate) yielded compound **C.19** in 36% (41 mg).

$C_{14}H_{11}Cl_2NO_3$ M_r = 312.15

^1H (400 MHz, $CDCl_3$): δ 7.291 (d, 2H, $J_{3'/5',\ 4'}$ 8.01 Hz, H-3', H-5'), 6.907 (t, 1H, $J_{4',\ 3'/5'}$ 8.00 Hz, H-4'), 6.779 (d, 1H, $J_{6,4}$ 2.68 Hz, H-6), 6.632 (dd, 1H, $J_{4,3}$ 8.67 Hz, $J_{4,6}$ 2.74 Hz, H-4), 6.500 (d, 1H, $J_{3,4}$ 8.61 Hz, H-3), 6.400 (bs, 1H, NH), 3.802 (s, 2H, CH_2COOH).

MS: Calcd for [$C_{14}H_{11}Cl_2NO_3$]: *m/z* 180.21: found: [M-H] 310.1/312.1

NMR SPECTRA APPENDIX

of selected compounds

LIST OF SELECTED NMR SPECTRA

CHAPTER – A

A.8 ^{13}C, ^{19}F, HSQC

A.9 ^{13}C, ^{19}F, HSQC

A.13 ^{13}C, ^{19}F, HSQC

A.15 ^{13}C, ^{19}F, HSQC

A.18 ^{13}C, ^{19}F, HSQC

A.20 ^{13}C, ^{19}F, HSQC

A.22 ^{13}C, ^{19}F, HSQC

A.23 ^{13}C, ^{19}F, HSQC

COMPETITION TITRATION

2-F-maltose reporter system and 6-F-maltose (**A.20**)

2-F-maltose reporter system and 6'-F-maltose (**A.15**)

2-F-maltose reporter system and α-maltosylfluoride (**A.9**)

2-F-maltose reporter system and maltose

2-F-maltose reporter system and 6'-F-'galacto'-maltose (**A.23**)

CHAPTER – B

B.3 ^{1}H, ^{13}C, HSQC

B.7 ^{1}H, ^{13}C, HSQC

B.8 ^{1}H, ^{13}C, ^{19}F, HSQC

B.10 ^{13}C, ^{19}F, HSQC

B.13 ^{13}C, ^{19}F, HSQC

B.15 ^{13}C, HSQC

B.18 ^{13}C, ^{19}F, HSQC

B.19 ^{13}C, ^{19}F, HSQC

CHAPTER – C

C.8 ^{1}H, ^{13}C

C.11 ^{1}H, ^{13}C

C.13 ^{1}H, ^{13}C

C.14 ^{1}H, ^{13}C

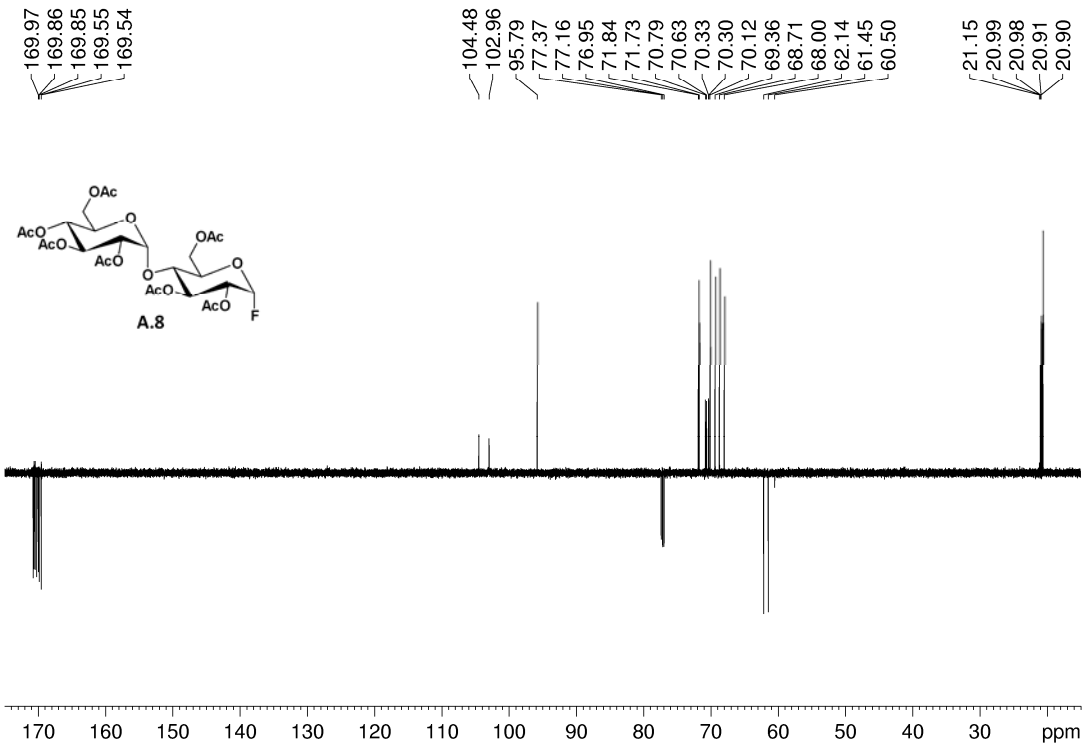
C.15 ^{1}H, ^{13}C

C.16 ^{1}H, ^{13}C

C.17 ^{1}H, ^{13}C

C.18 ^{1}H, ^{13}C

C.9 ^{1}H, ^{13}C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70.79
70.63
70.33
70.30
70.12
69.36
68.71
68.00
62.14
61.45
60.50
21.15
20.99
20.98
20.91
20.90
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AcO
AcO
AcO
O
OAc
O
AcO
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F
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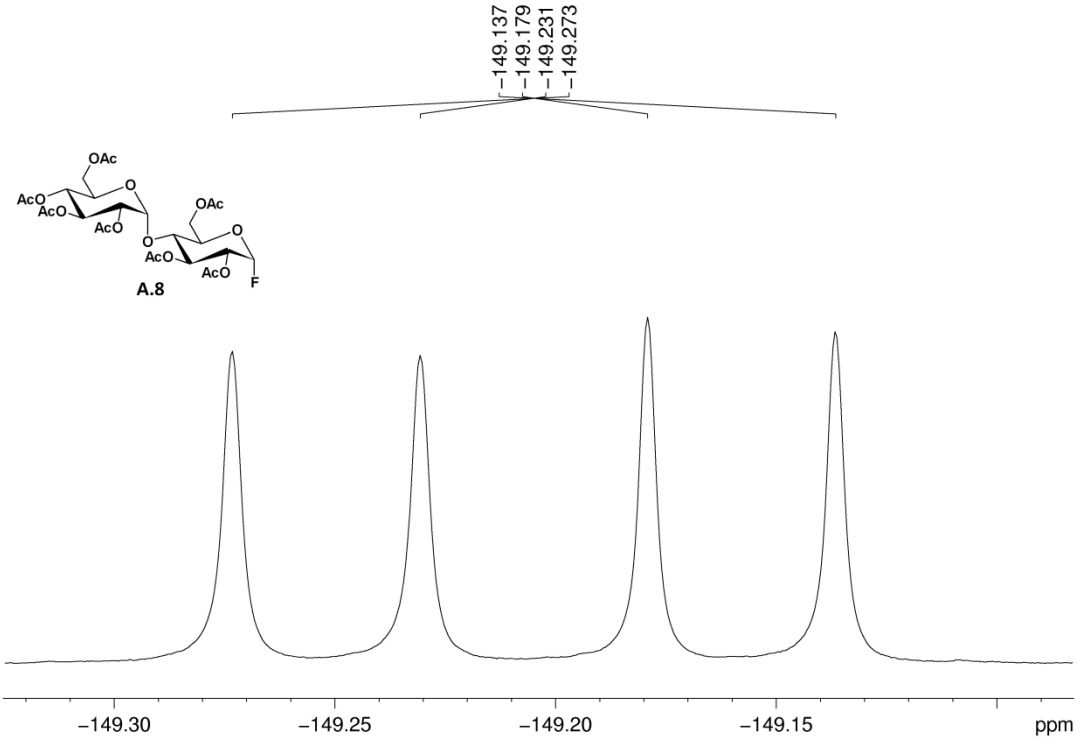
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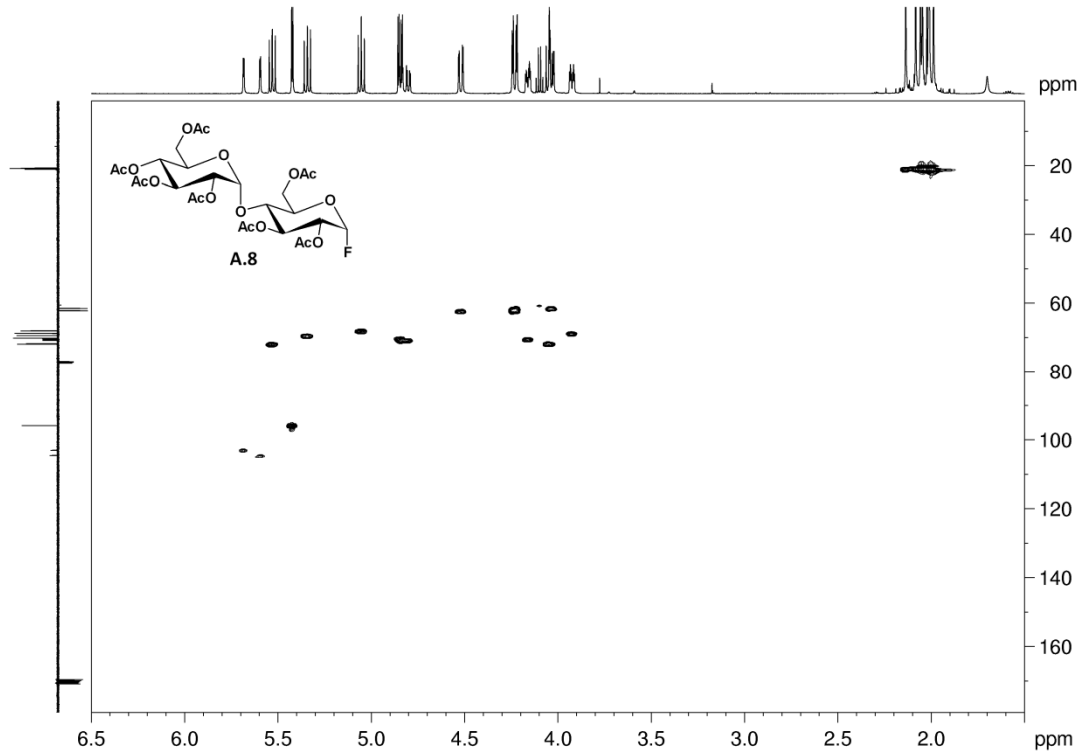
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2.0
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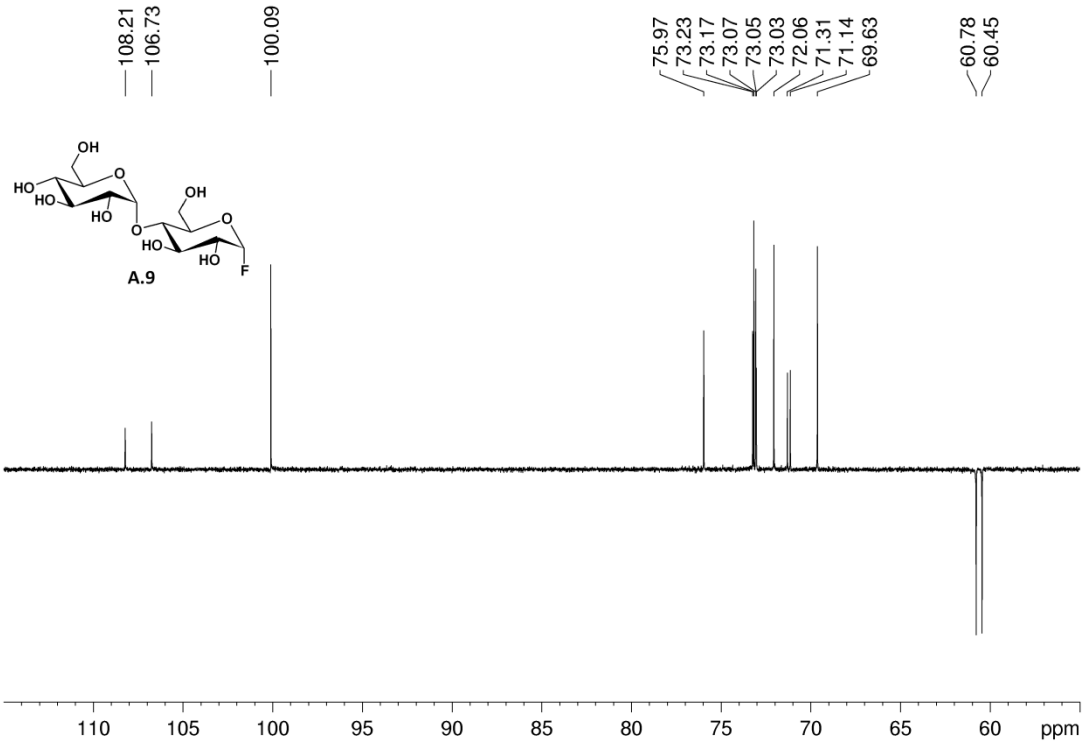
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72.06
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60.78
60.45
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HO
HO
HO
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HO
HO
F
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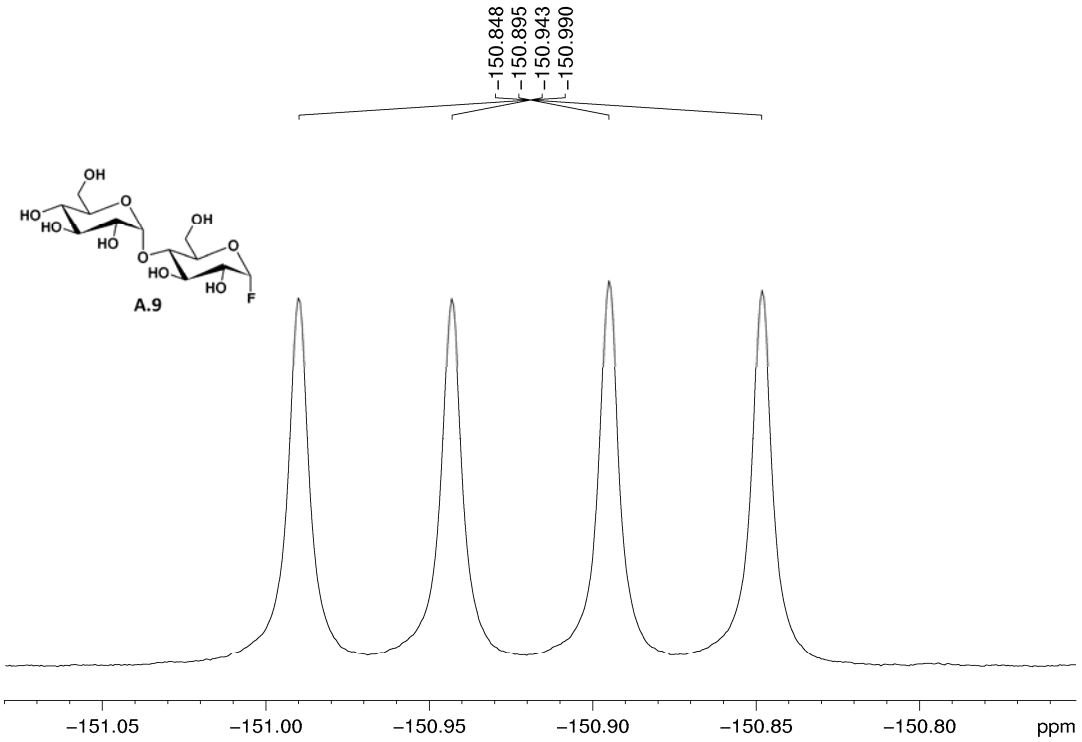
60
ppm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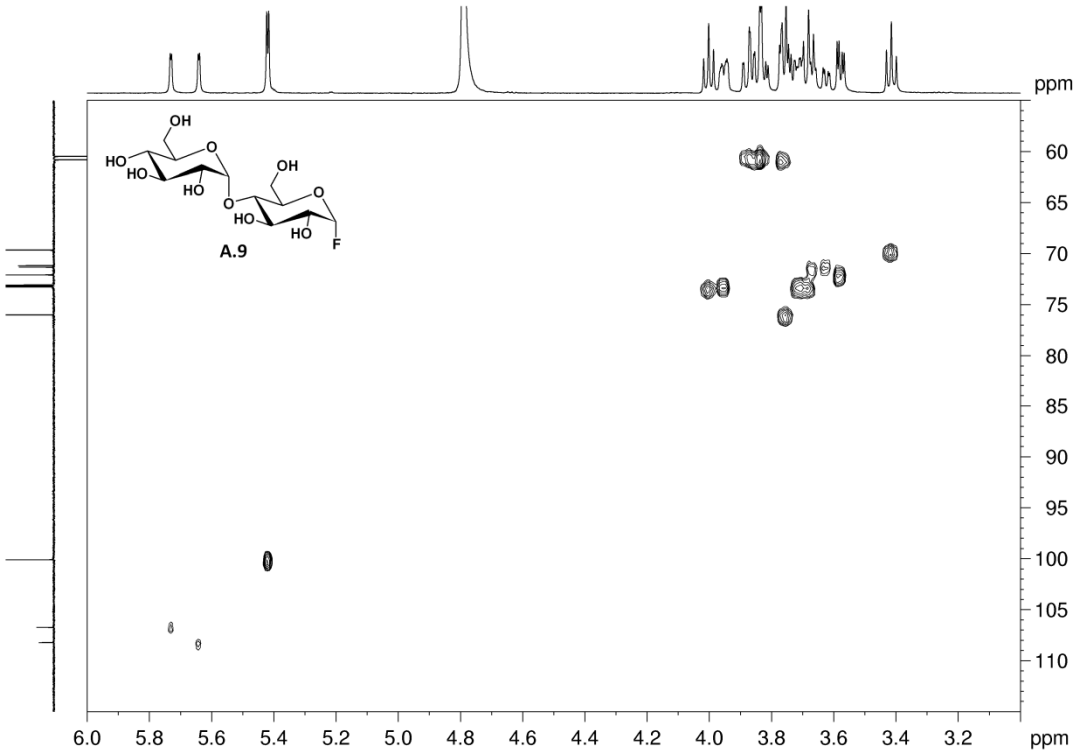
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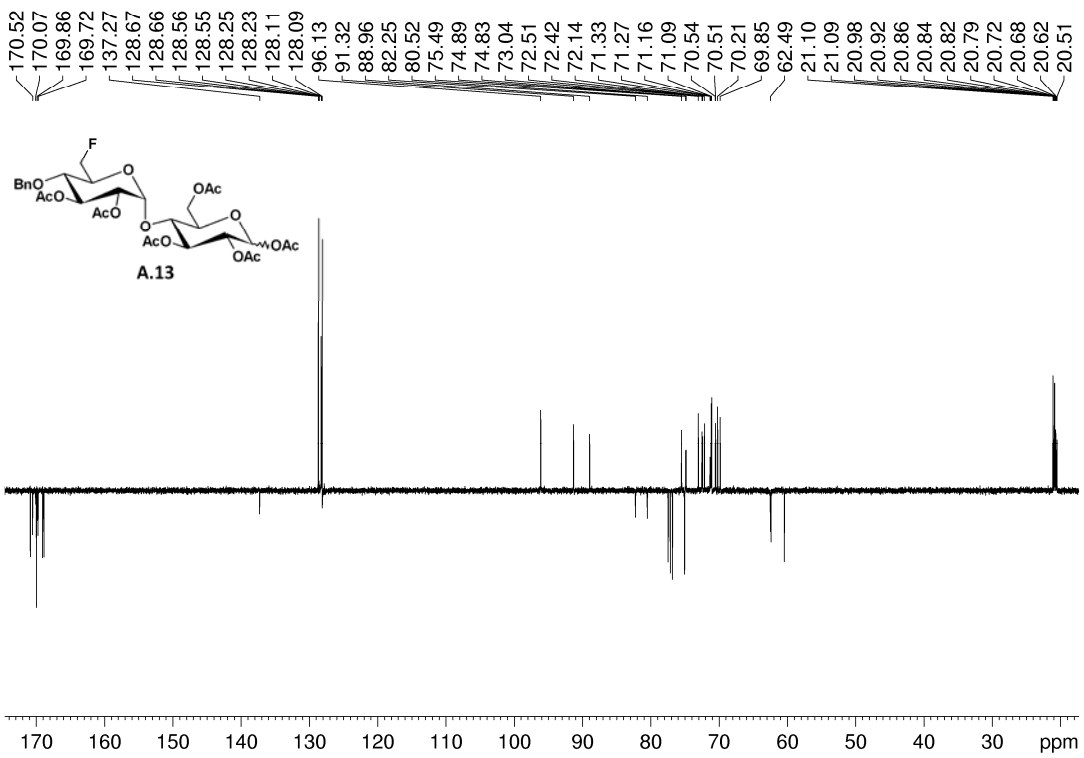
3.2
ppm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91.32
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75.49
74.89
74.83
73.04
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72.42
72.14
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71.16
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69.85
62.49
21.10
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20.92
20.86
20.84
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20.79
20.72
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20.62
20.51
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AcO
O
OAc
O
AcO
OAc
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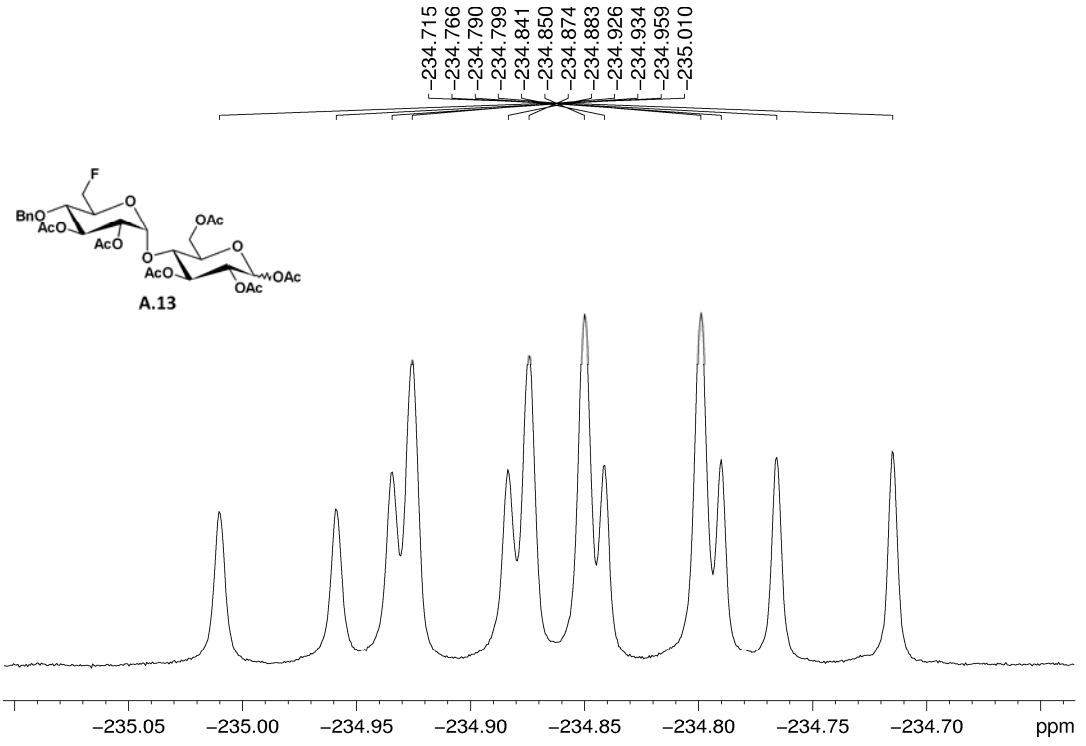
ppm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AcO
OAc
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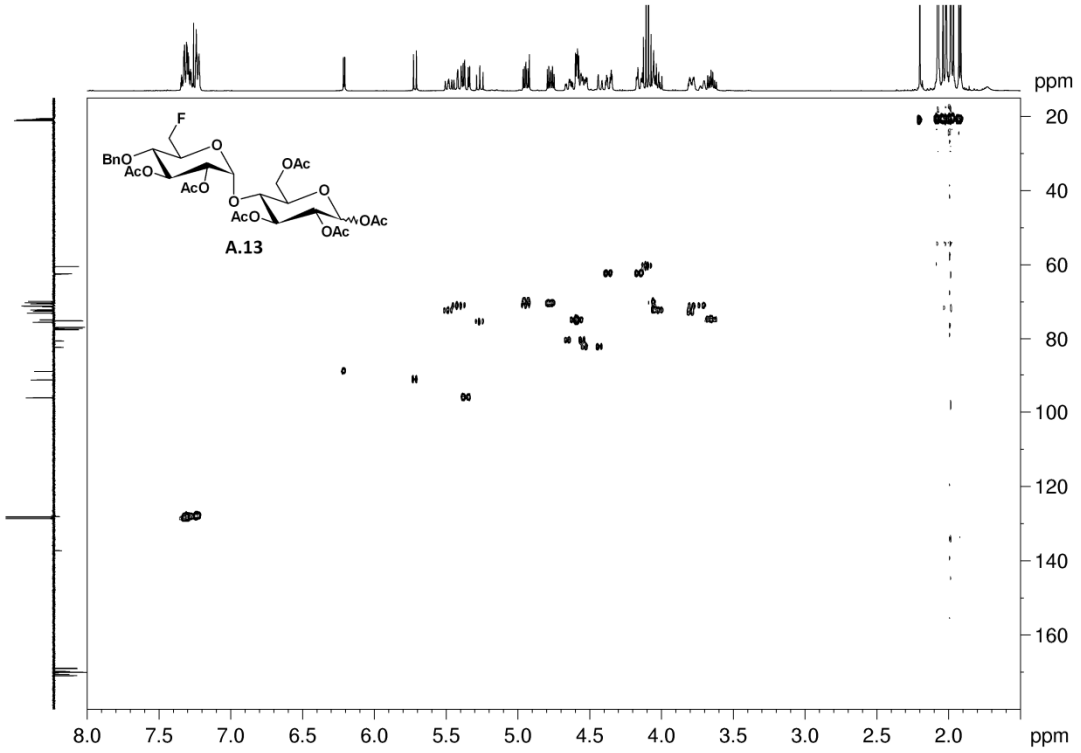
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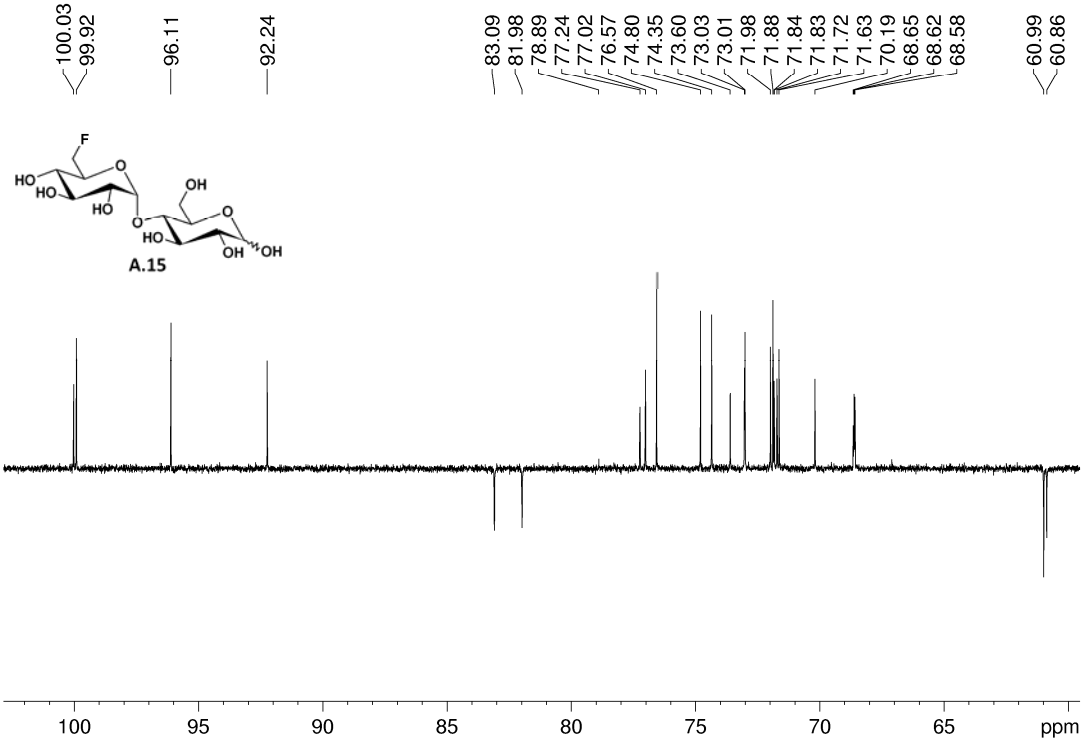
A.13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71.83
71.72
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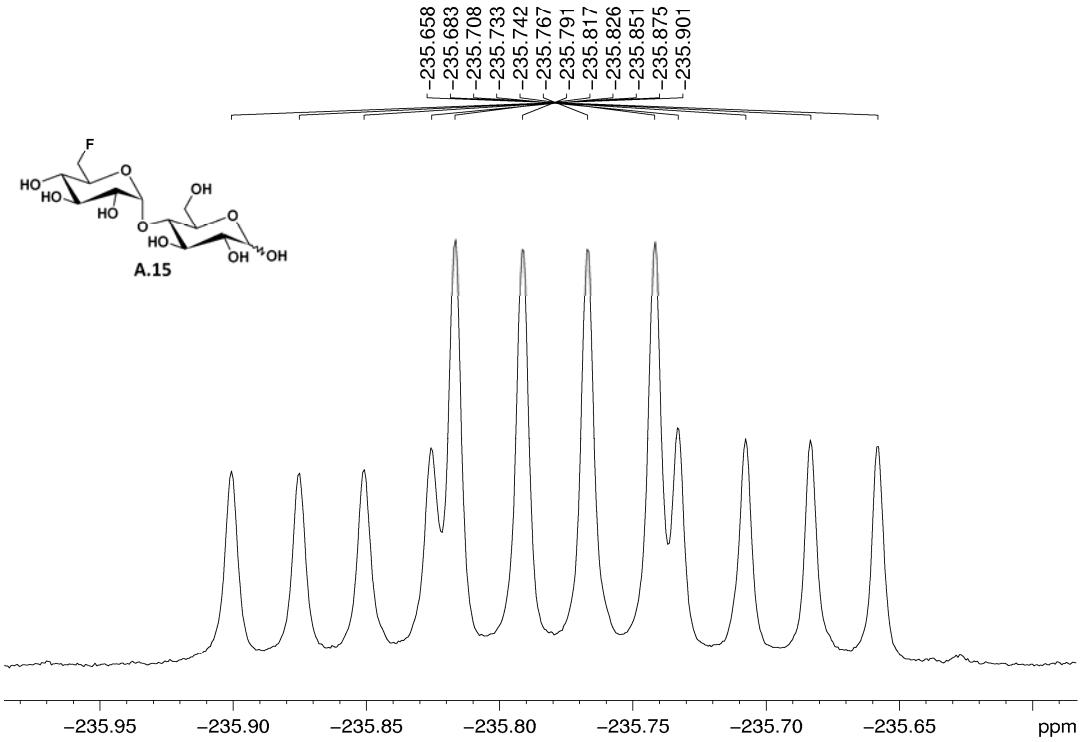
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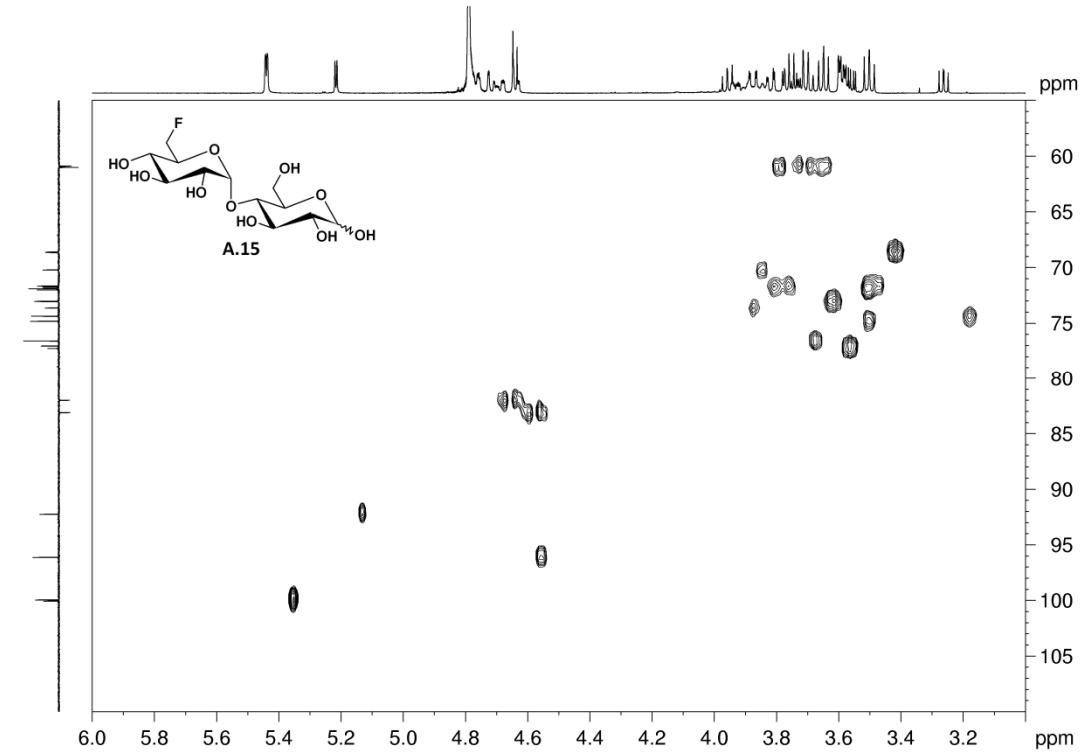
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4.4
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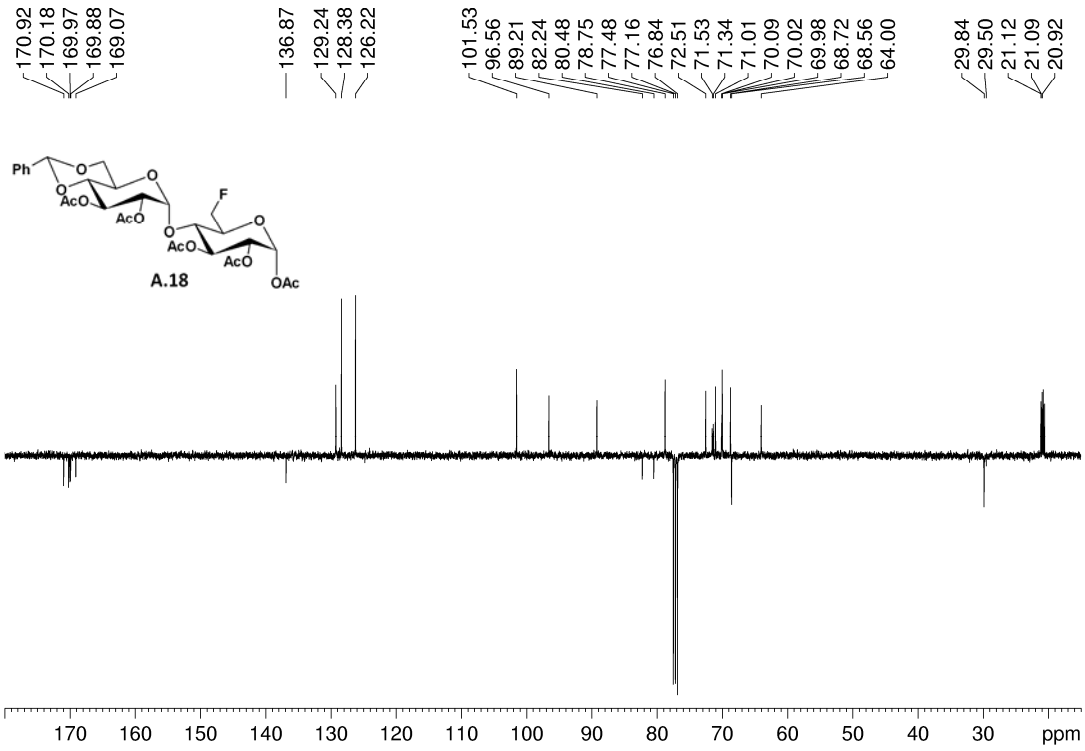
3.4
3.2
ppm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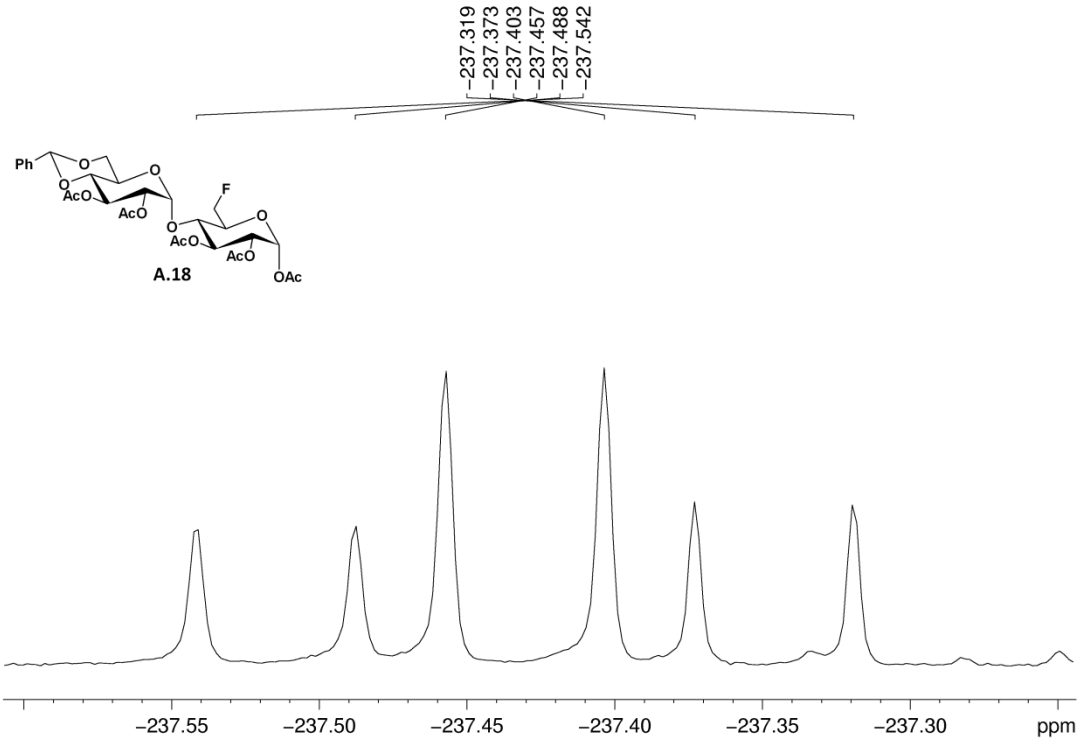
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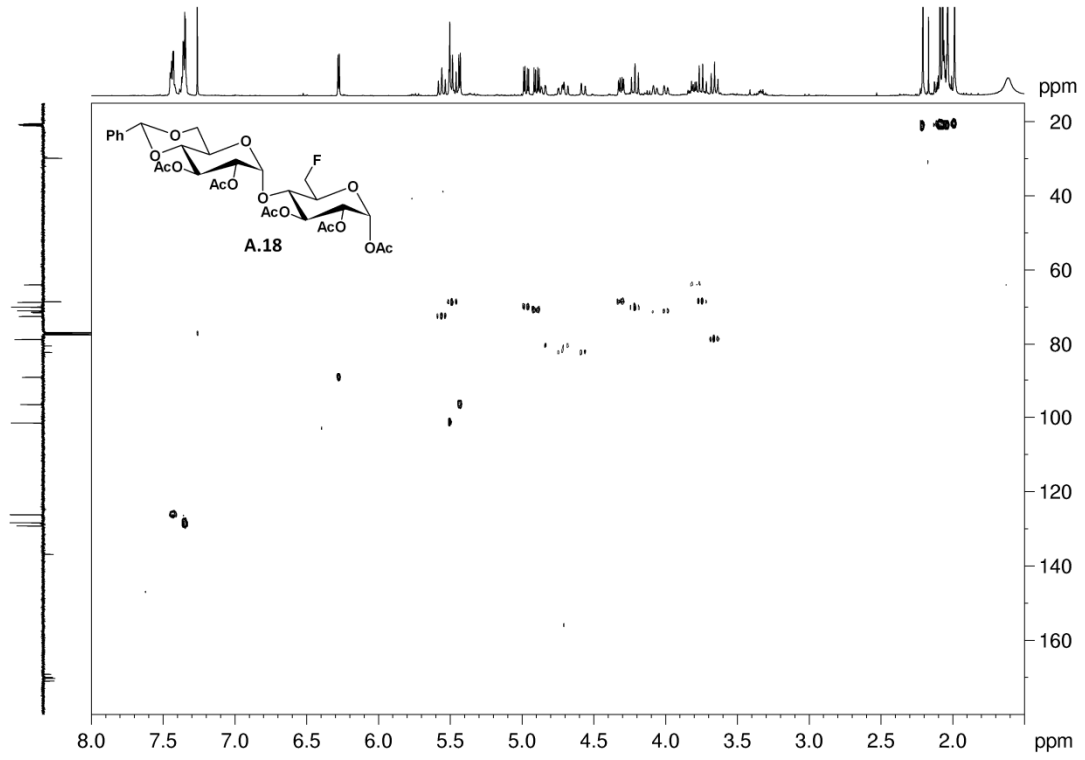
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AcO
AcO
AcO
F
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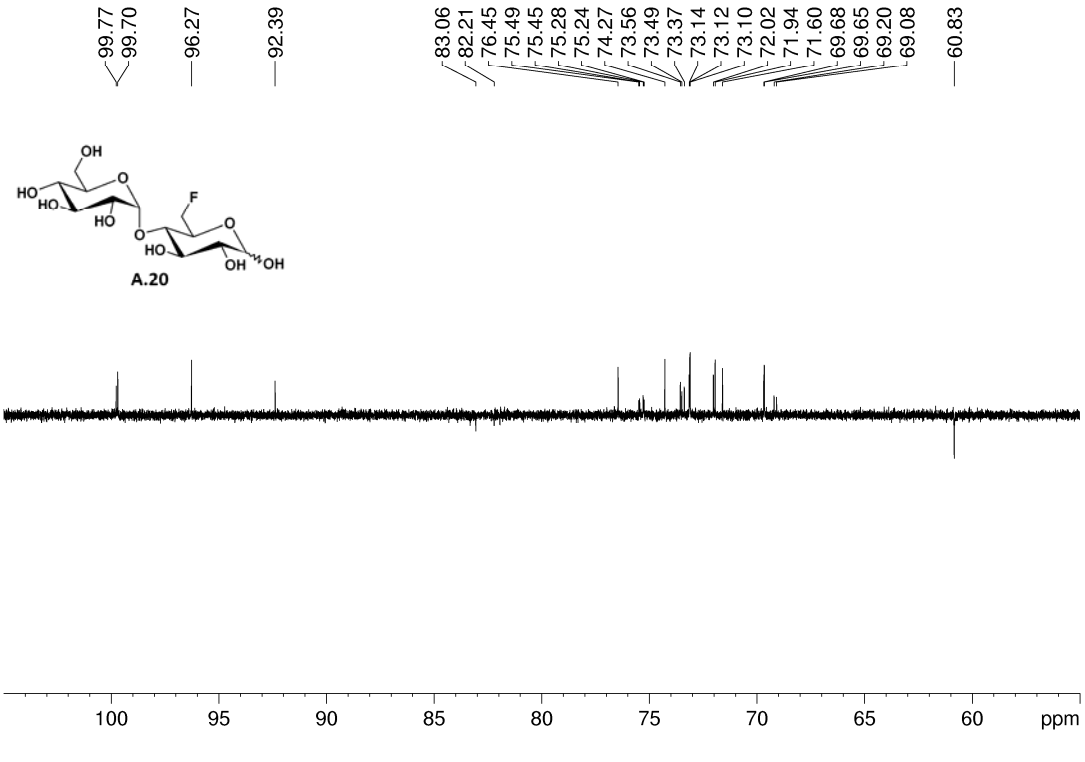
A.18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73.49
73.37
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69.08
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HO
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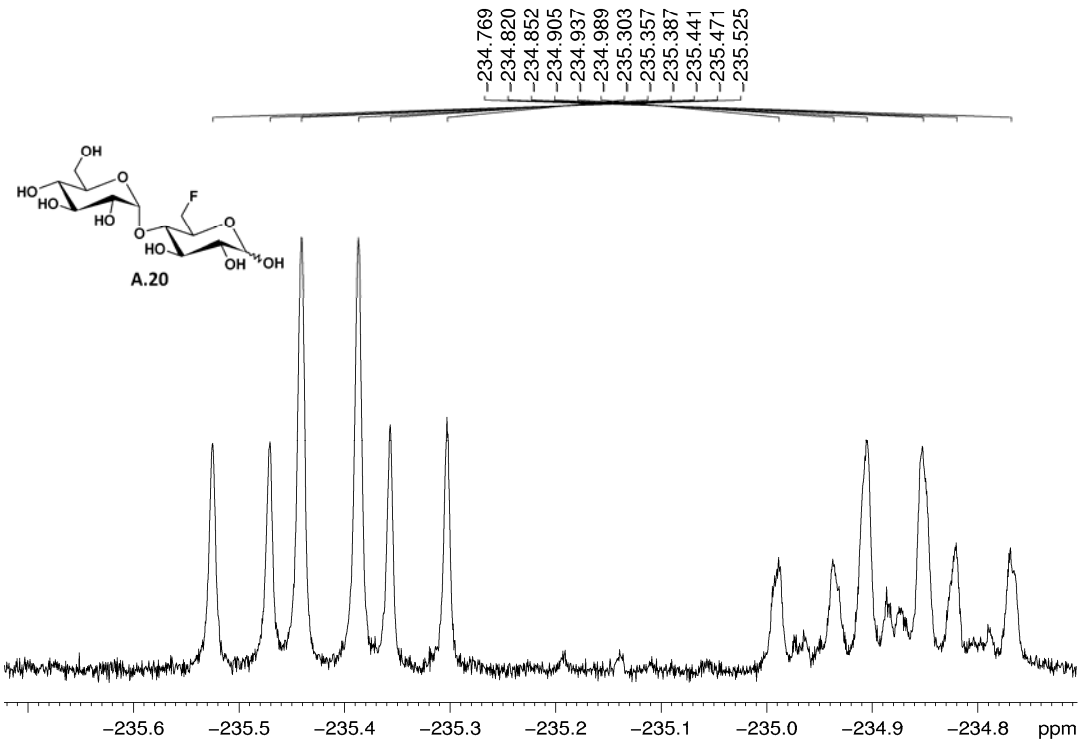
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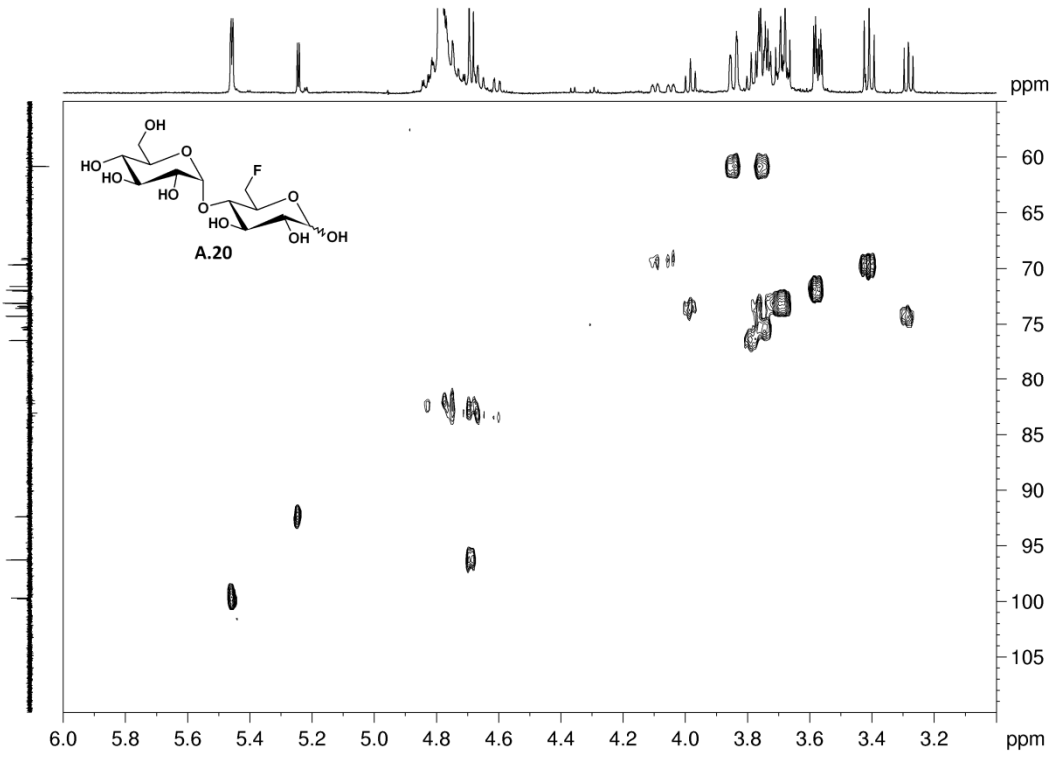
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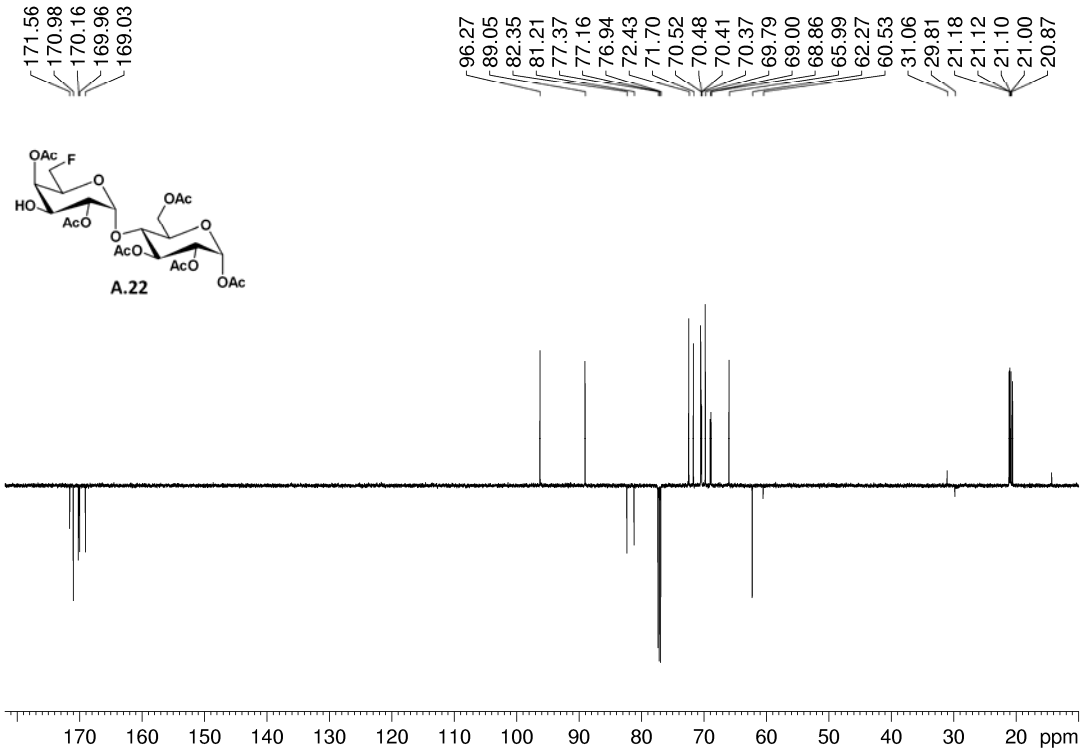
ppm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62.27
60.53
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21.12
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A.22
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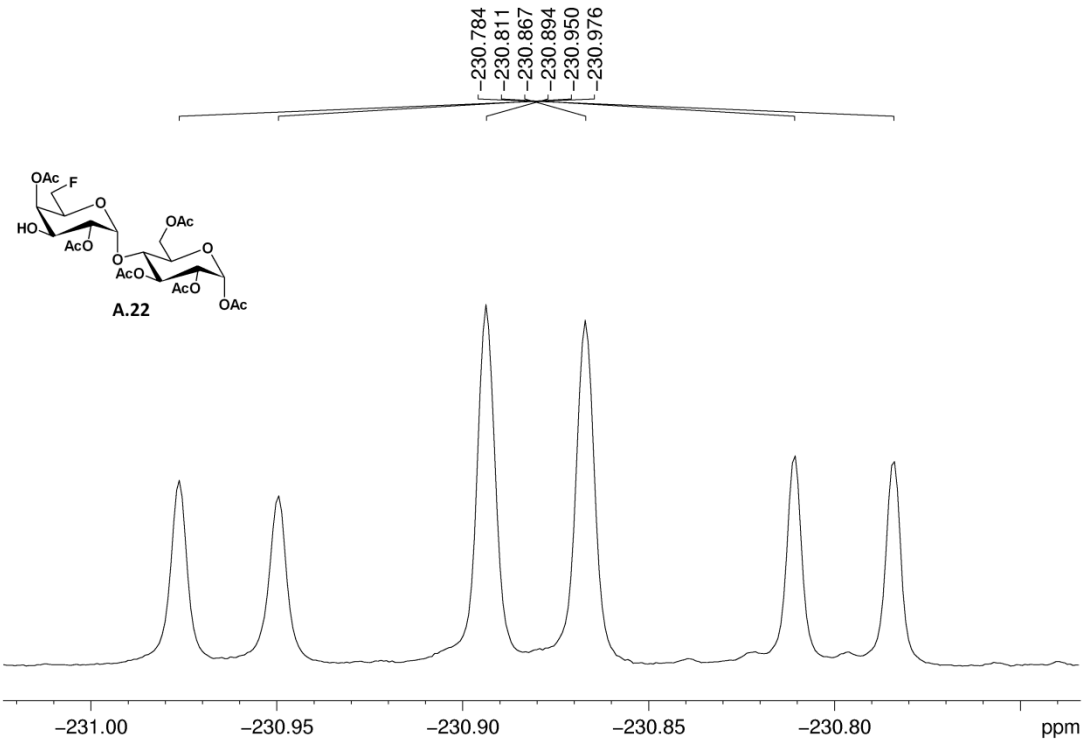
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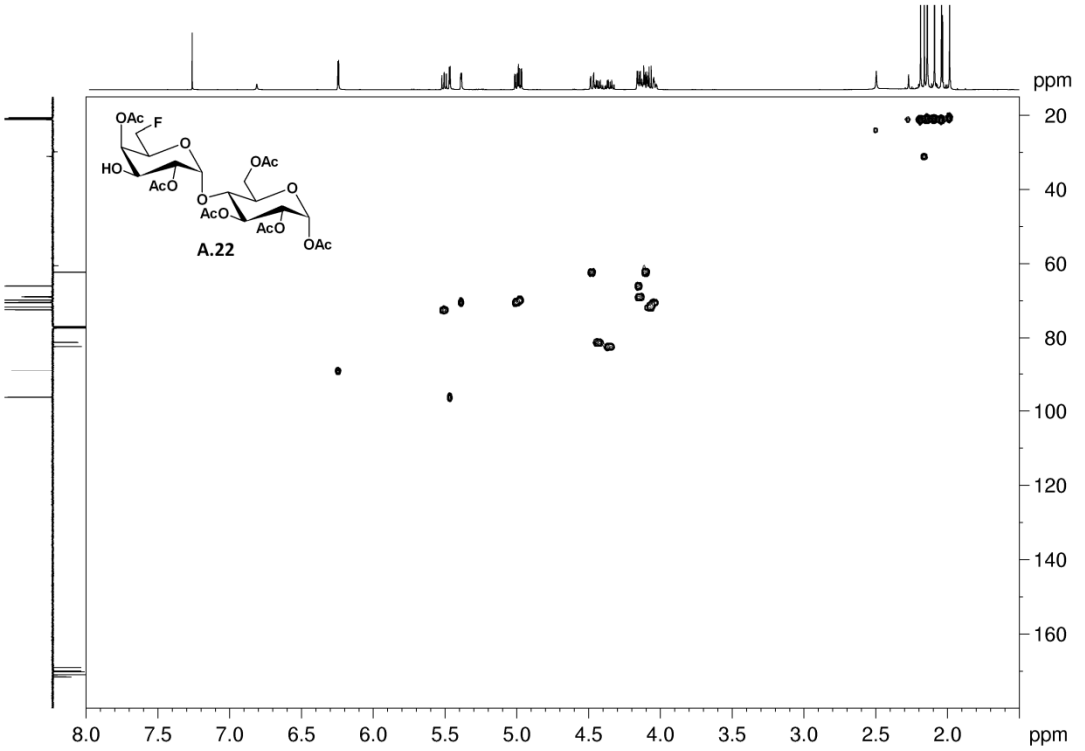
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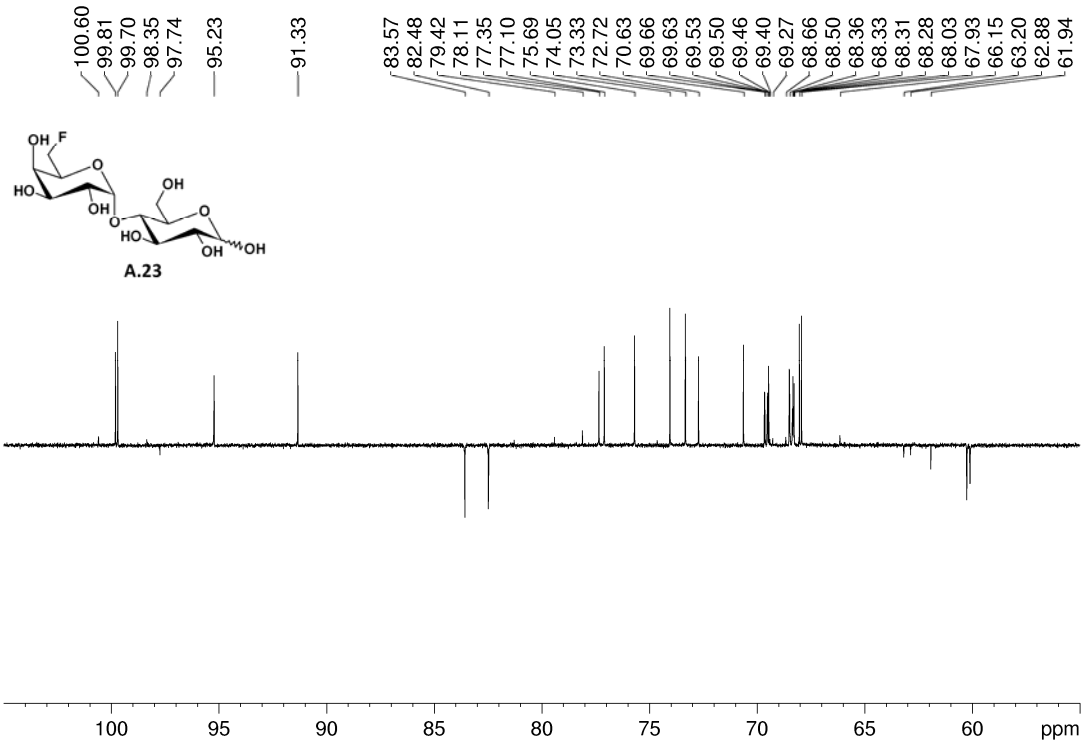
ppm
A.22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77.10
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69.63
69.53
69.50
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68.66
68.50
68.36
68.33
68.31
68.28
68.03
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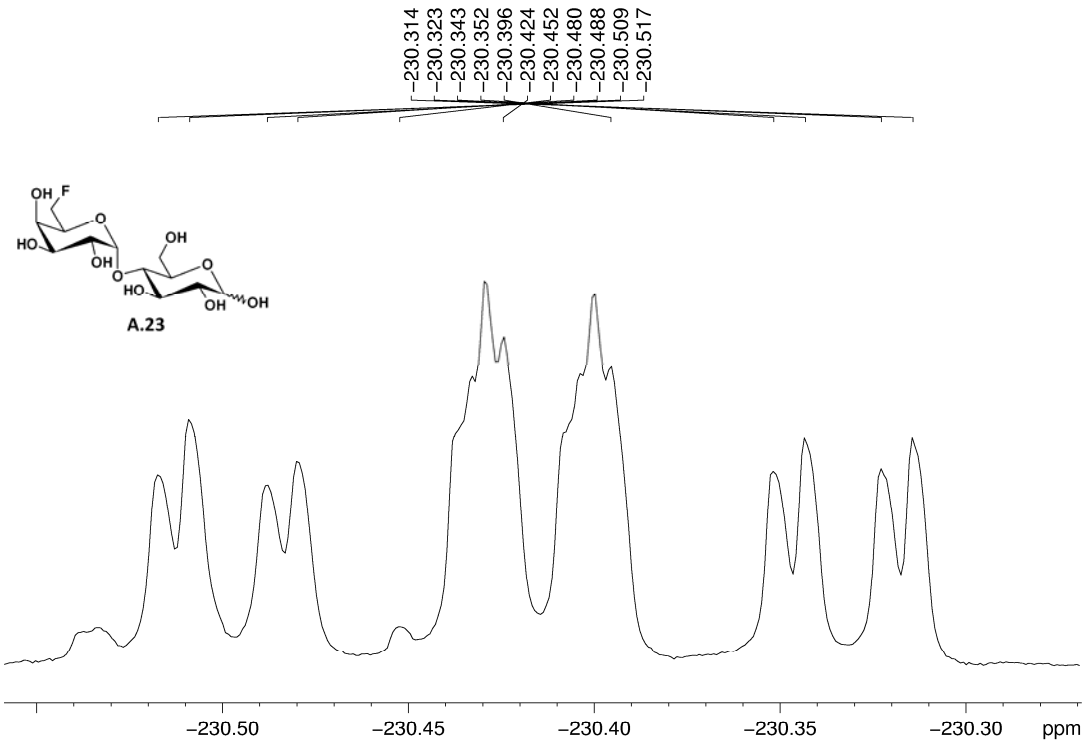
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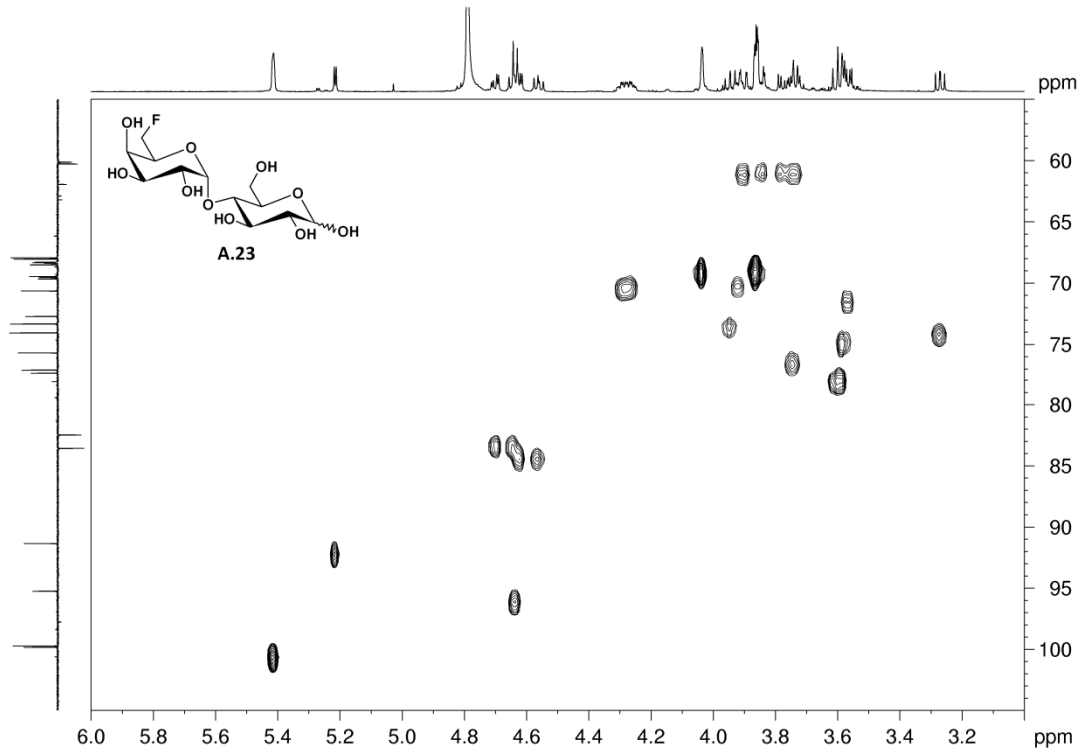
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5.4
5.2
5.0
4.8
4.6
4.4
4.2
4.0
3.8
3.6
3.4
3.2
ppm

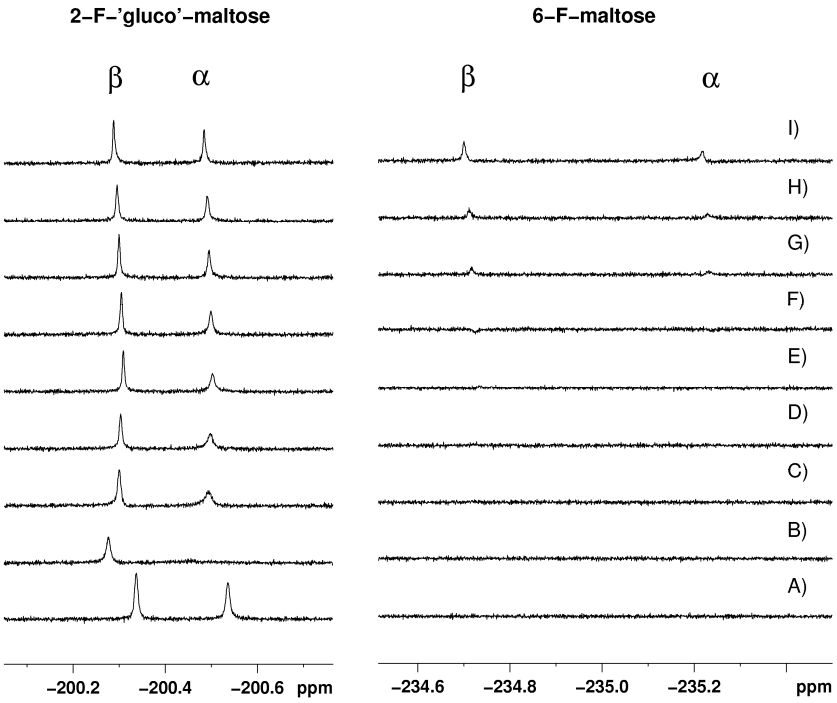


Figure 63. Competition titration using the 2-F-maltose reporter system and ^{19}F NMR: only the important sector of the gluco-type isomers is shown. (A) 2-F-maltose, (B) 2-F-maltose bound to MBP, (C-I) addition of 0.05, 0.06, 0.07, 0.12, 0.15, 0.2 and 0.35 equiv. of 6-F maltose **A.20**.

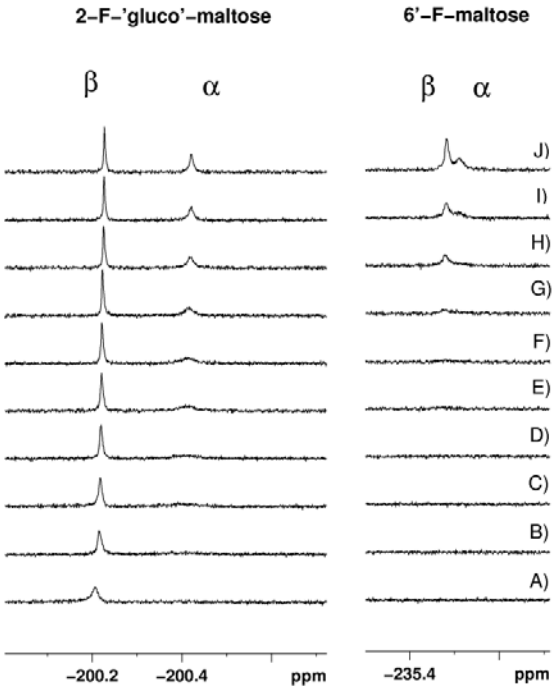


Figure 64. Competition titration using the 2-F-maltose reporter system and ^{19}F NMR: only the important sector of the gluco-type isomers is shown. (A) 2-F-maltose bound to MBP, (B-J) addition 0.04, 0.06, 0.08, 0.12, 0.16, 0.24, 0.44, 0.6, 1 equiv. of 6'-F-maltose **A.15**.

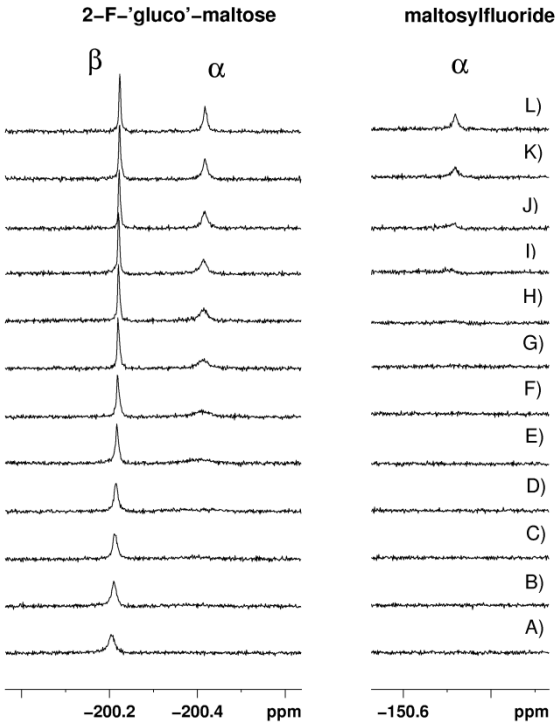


Figure 65. Competition titration using the 2-F-maltose reporter system and ^{19}F NMR: only the important sector of the gluco-type isomers is shown. (A) 2-F-maltose bound to MBP, (B-L) addition of 0.015, 0.02, 0.03, 0.04, 0.06, 0.08, 0.12, 0.16, 0.2, 0.28, 0.36 equiv. of α-maltosylfluoride **A.9**.

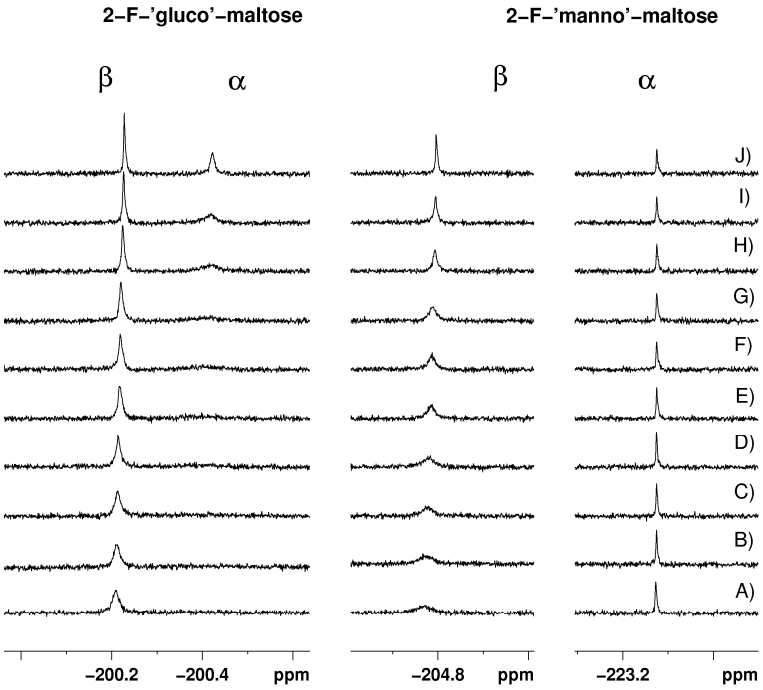


Figure 66. Competition titration using the 2-F-maltose reporter system and ^{19}F NMR: only the important sector of the gluco-type isomers is shown. (A) 2-F-maltose bound to MBP, (B-J) addition of 0.003, 0.007, 0.012, 0.019, 0.023, 0.025, 0.04, 0.05, 0.12 equiv. of maltose.

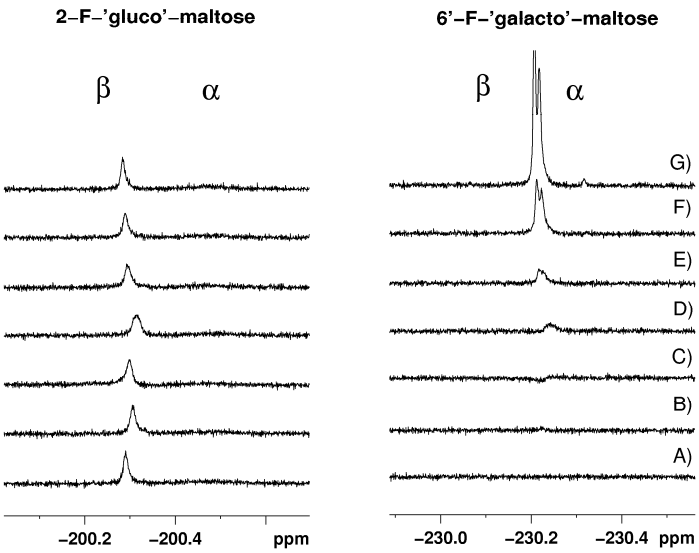
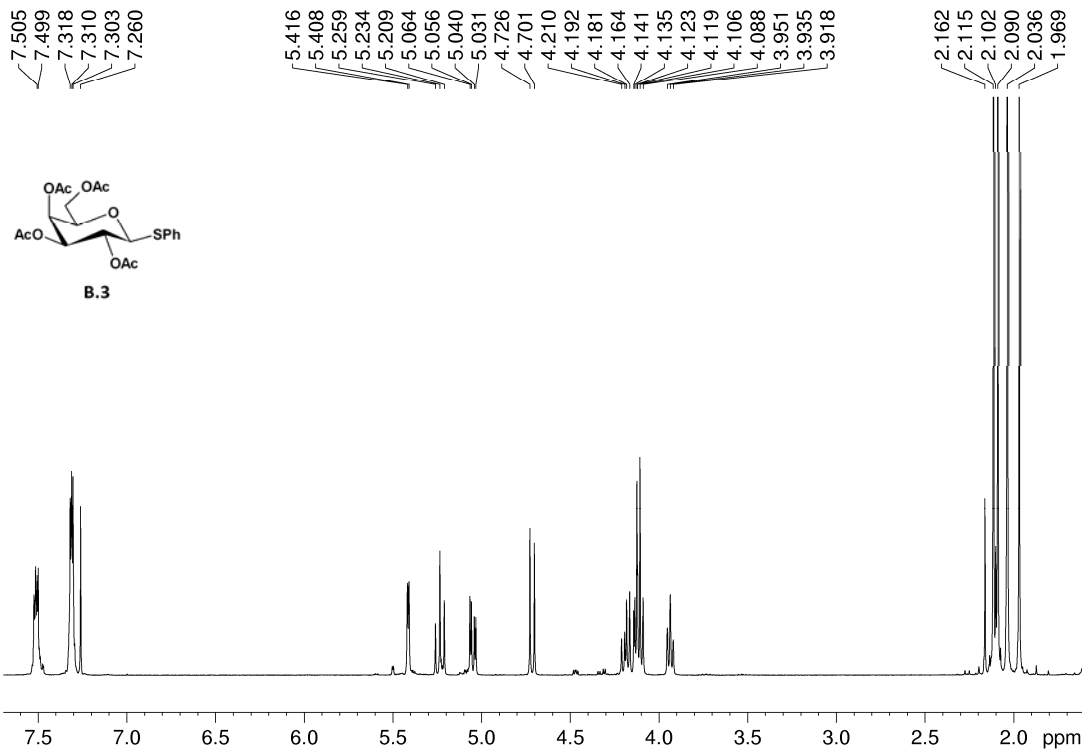
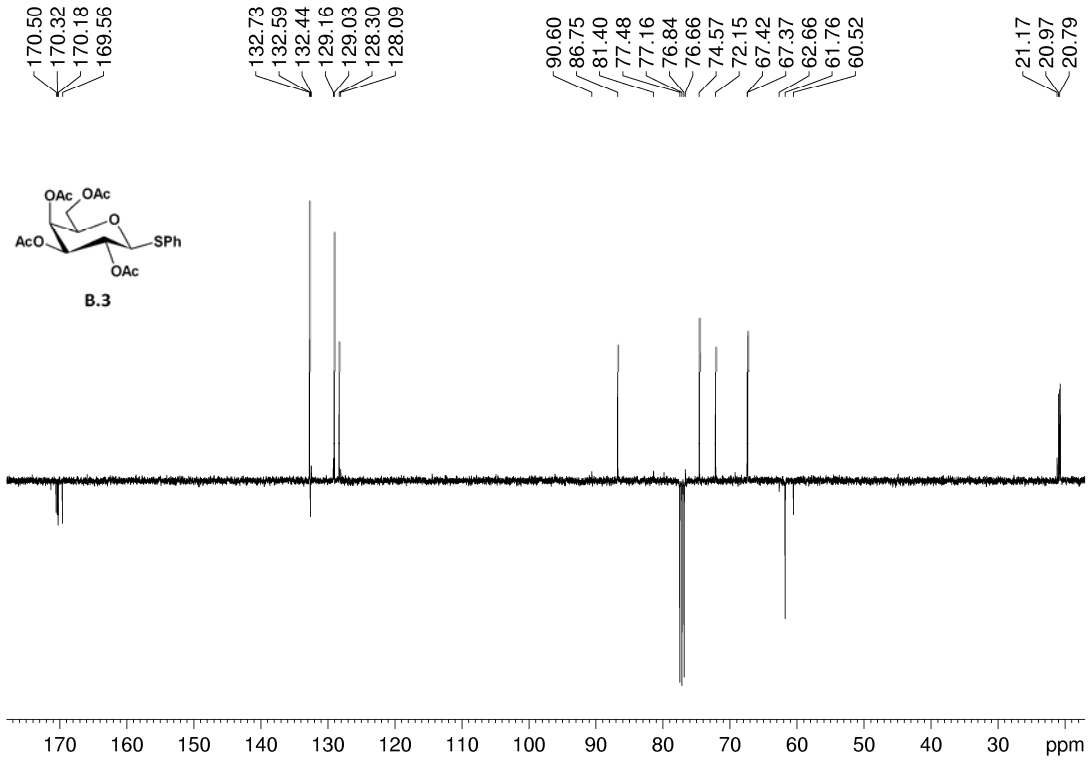
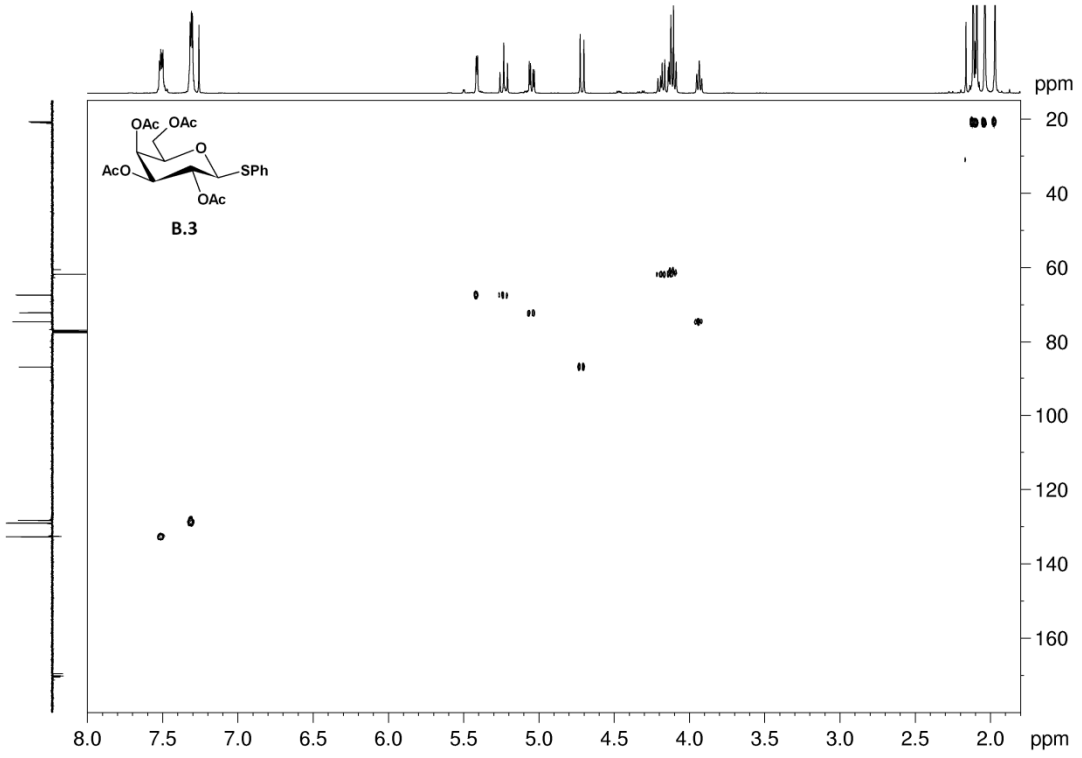
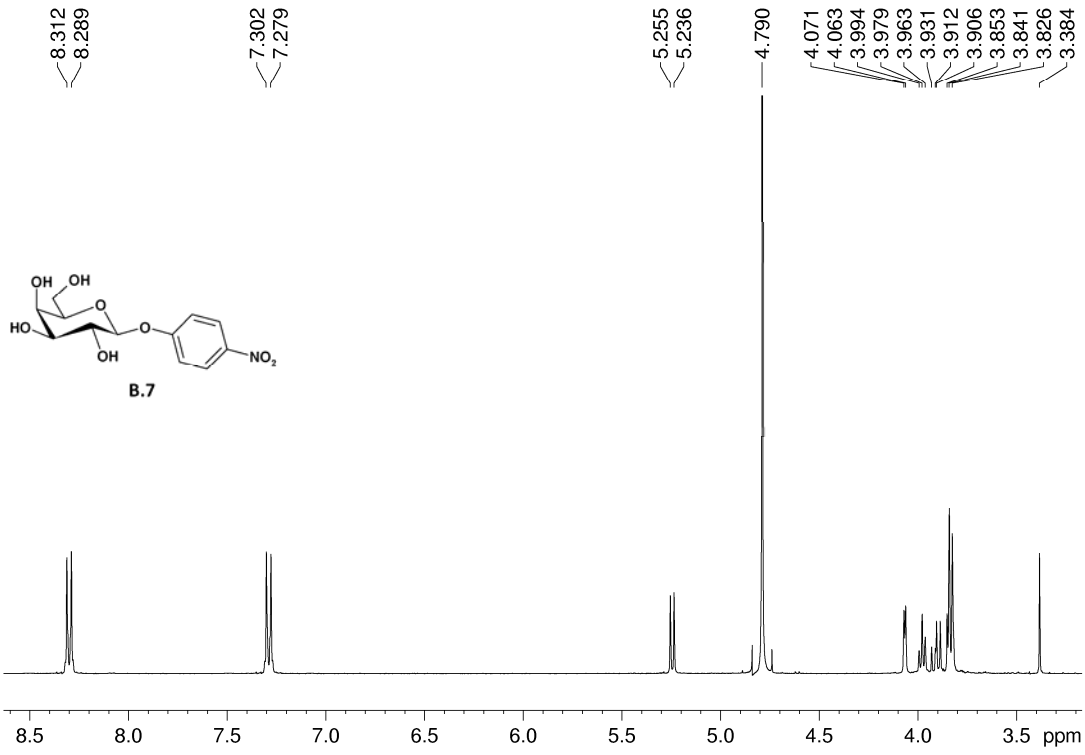


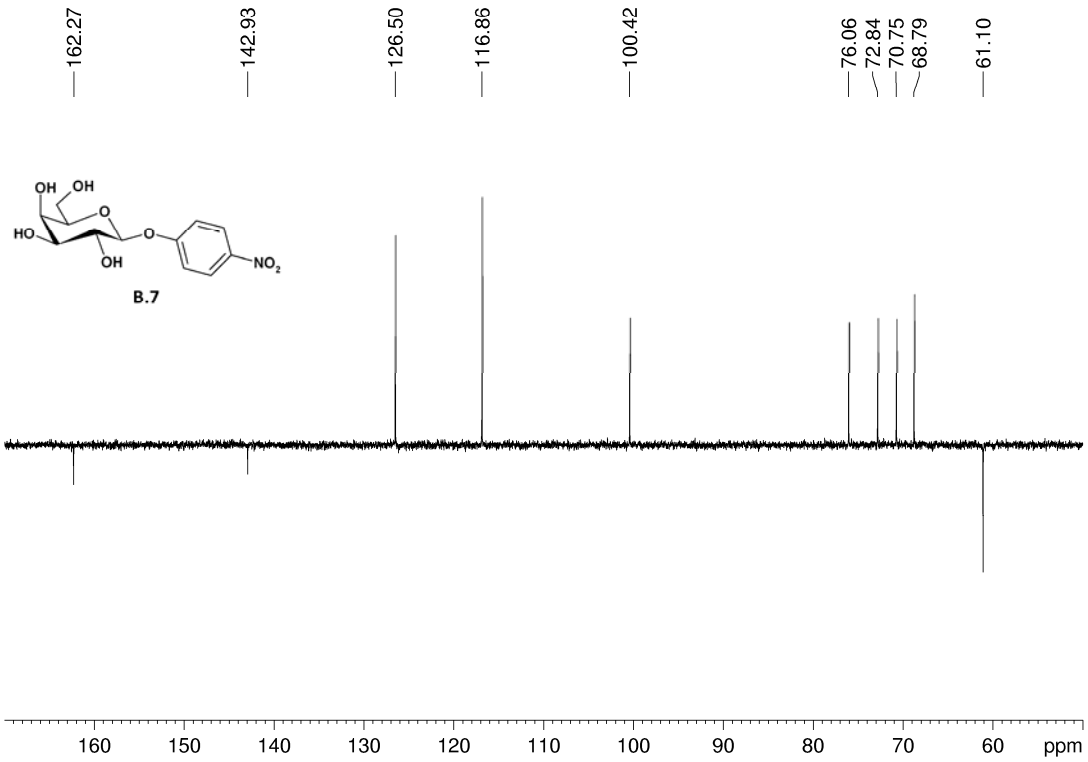
Figure 67. Competition titration using the 2-F-maltose reporter system and ^{19}F NMR: only the important sector of the gluco-type isomers is shown. (A) 2-F-maltose bound to MBP, (B-G) addition of 0.01, 0.04, 0.12, 0.2, 0.7, 1.7 equiv. of 6'-F-'galacto'-maltose **A.23**.

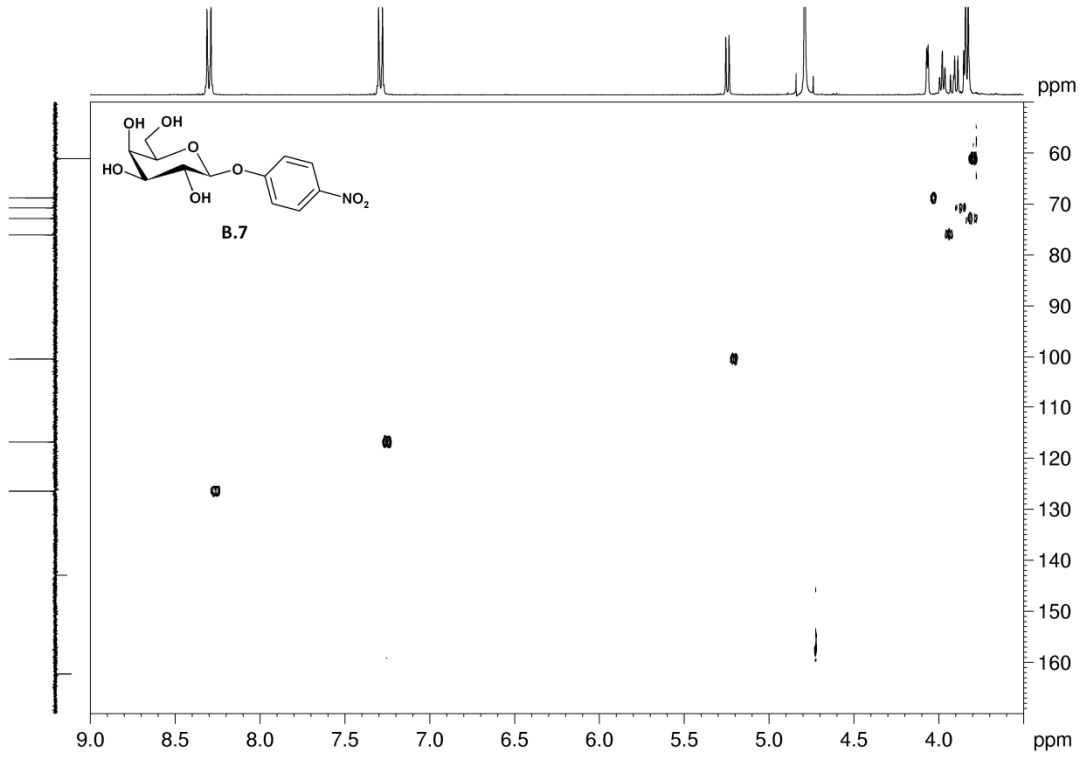

7.505
7.499
7.318
7.310
7.303
7.260
5.416
5.408
5.259
5.234
5.209
5.064
5.056
5.040
5.031
4.726
4.701
4.210
4.192
4.181
4.164
4.141
4.135
4.123
4.119
4.106
4.088
3.951
3.935
3.918
2.162
2.115
2.102
2.090
2.036
1.969
OAc
OAc
O
AcO
SPh
OAc
B.3
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm

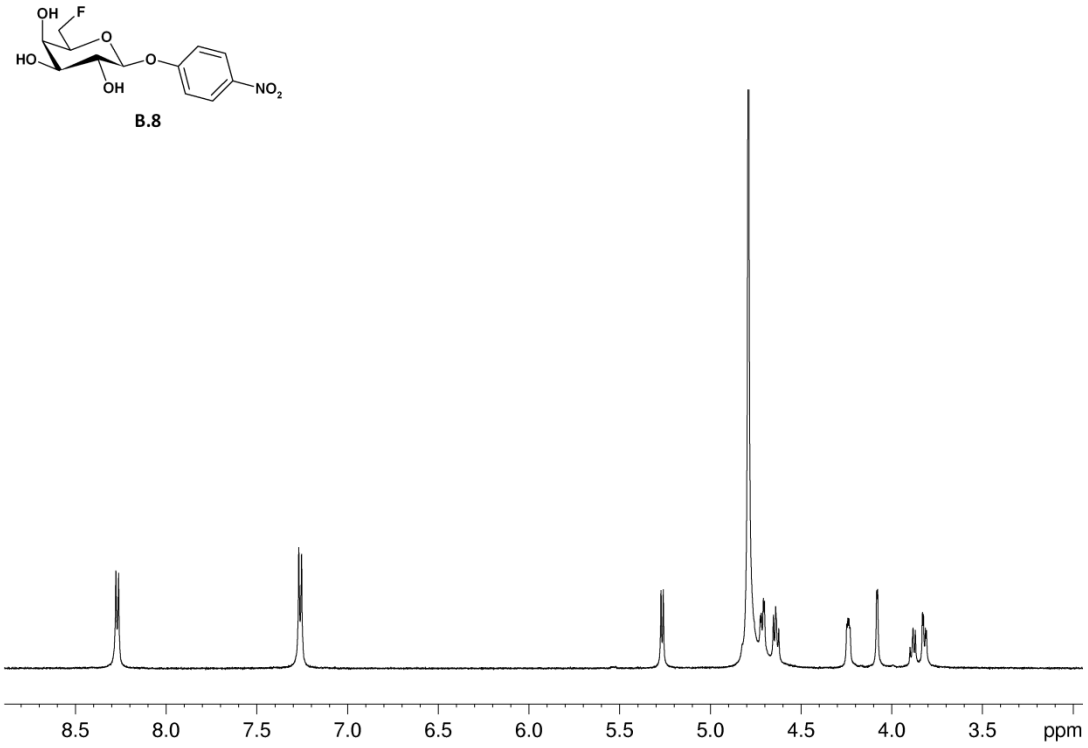

170.50
170.32
170.18
169.56
132.73
132.59
132.44
129.16
129.03
128.30
128.09
90.60
86.75
81.40
77.48
77.16
76.84
76.66
74.57
72.15
67.42
67.37
62.66
61.76
60.52
21.17
20.97
20.79
OAc
OAc
O
AcO
SPh
OAc
B.3
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

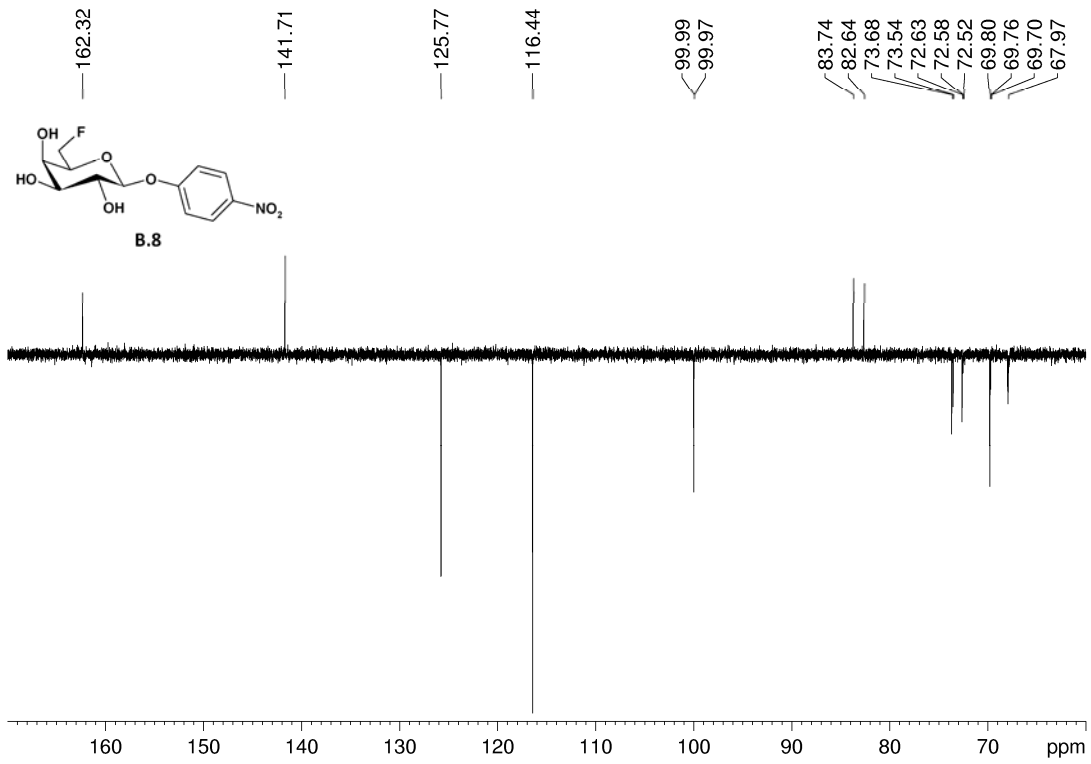

OAc
OAc
O
AcO
SPh
OAc
B.3
ppm
20
40
60
80
100
120
140
160
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm

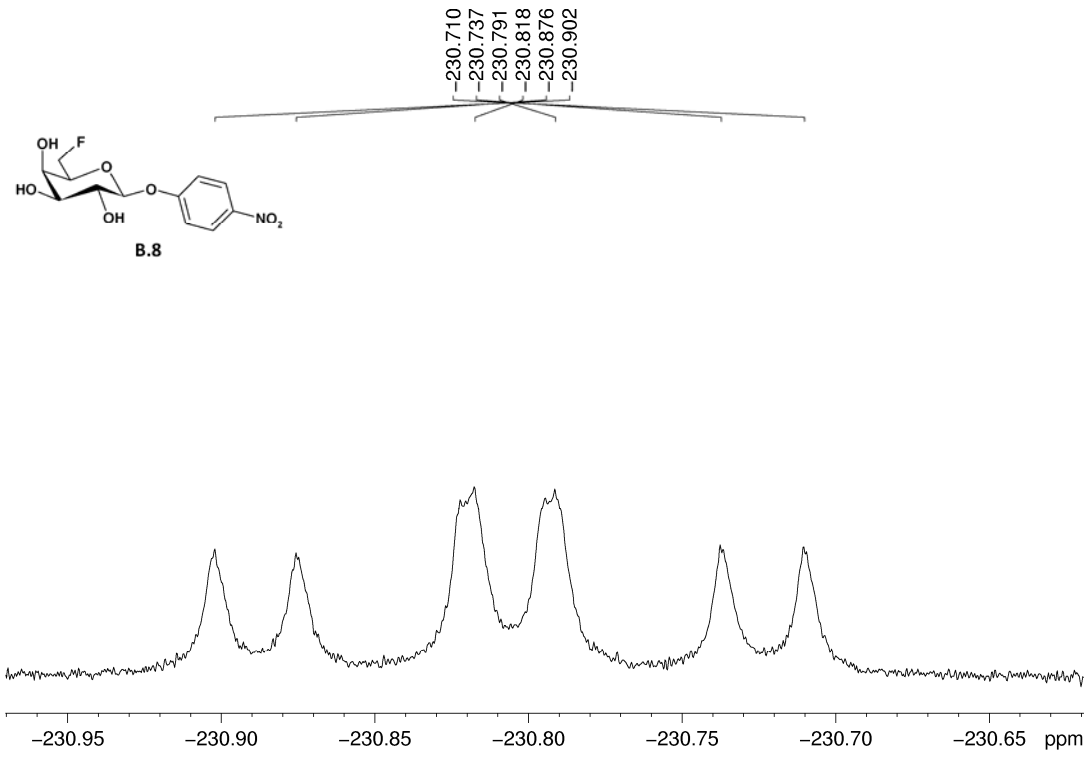

8.312
8.289
7.302
7.279
5.255
5.236
4.790
4.071
4.063
3.994
3.979
3.963
3.931
3.912
3.906
3.853
3.841
3.826
3.384
OH
OH
O
HO
O
OH
NO2
B.7
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm


162.27
142.93
126.50
116.86
100.42
76.06
72.84
70.75
68.79
61.10
B.7
160
150
140
130
120
110
100
90
80
70
60
ppm


ppm
B.7
60
70
80
90
100
110
120
130
140
150
160
9.0
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
ppm


OH
F
HO
O
OH
O
NO2
B.8
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm


162.32
141.71
125.77
116.44
99.99
99.97
83.74
82.64
73.68
73.54
72.63
72.58
72.52
69.80
69.76
69.70
67.97
OH
F
HO
O
OH
O
NO2
B.8
160
150
140
130
120
110
100
90
80
70
ppm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-230.737
-230.791
-230.818
-230.876
-230.902
OH
F
O
HO
O
OH
NO2
B.8
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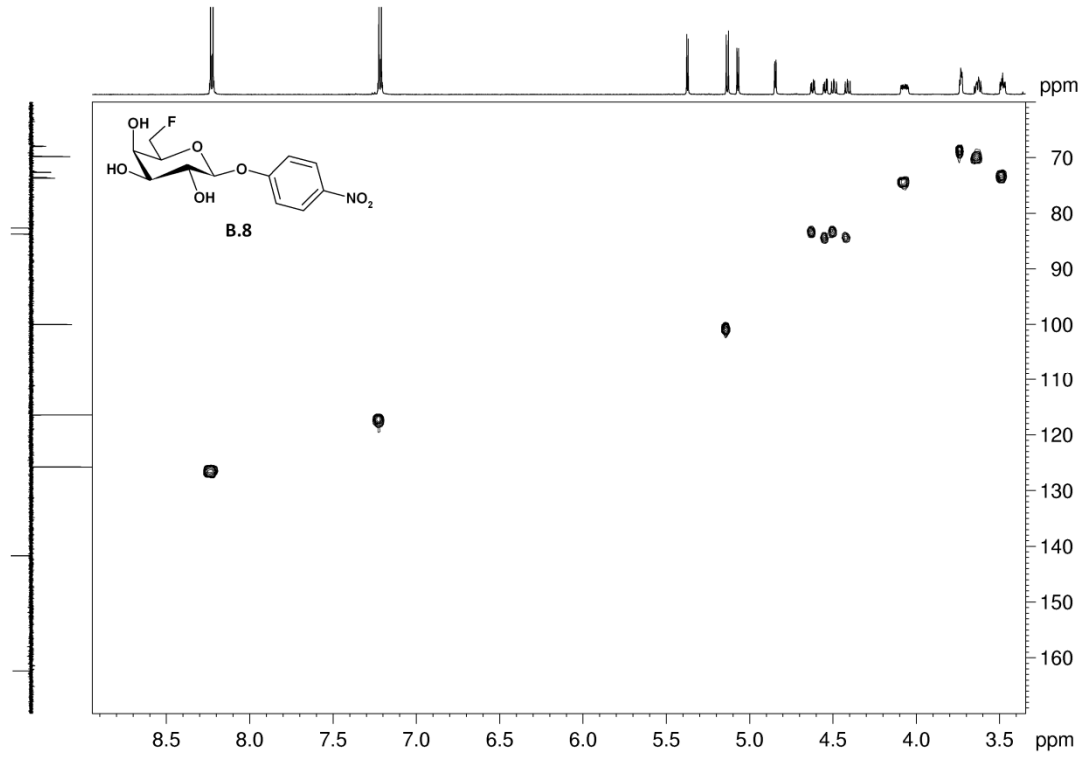
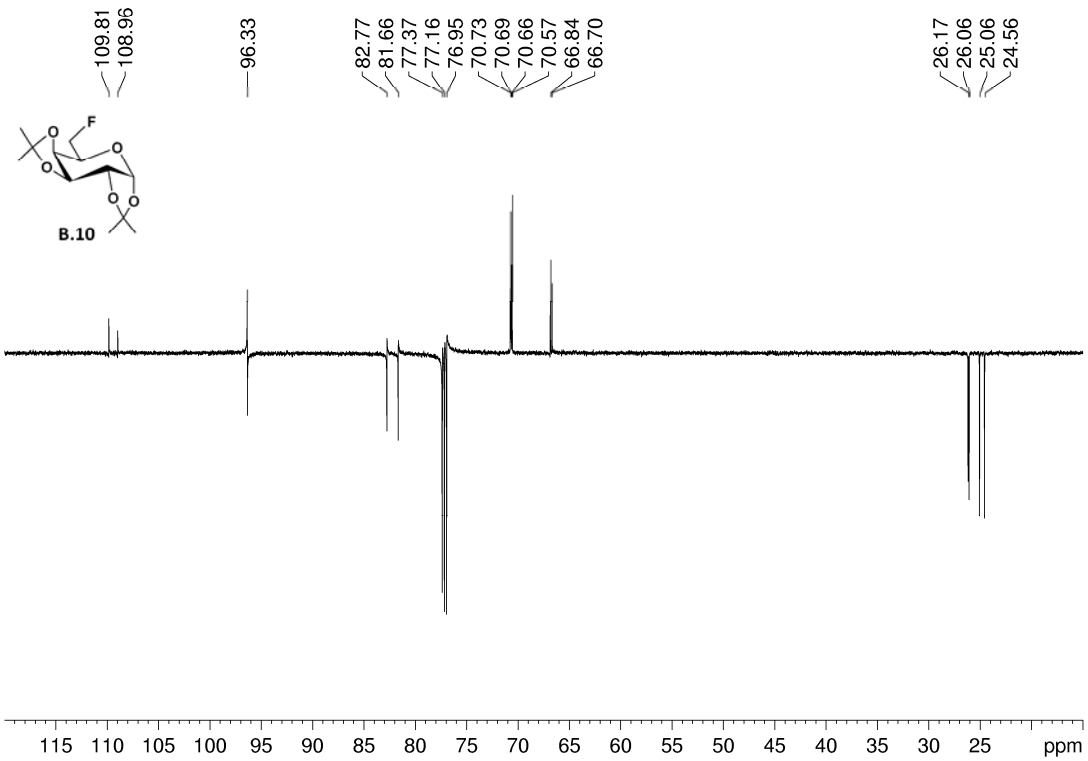
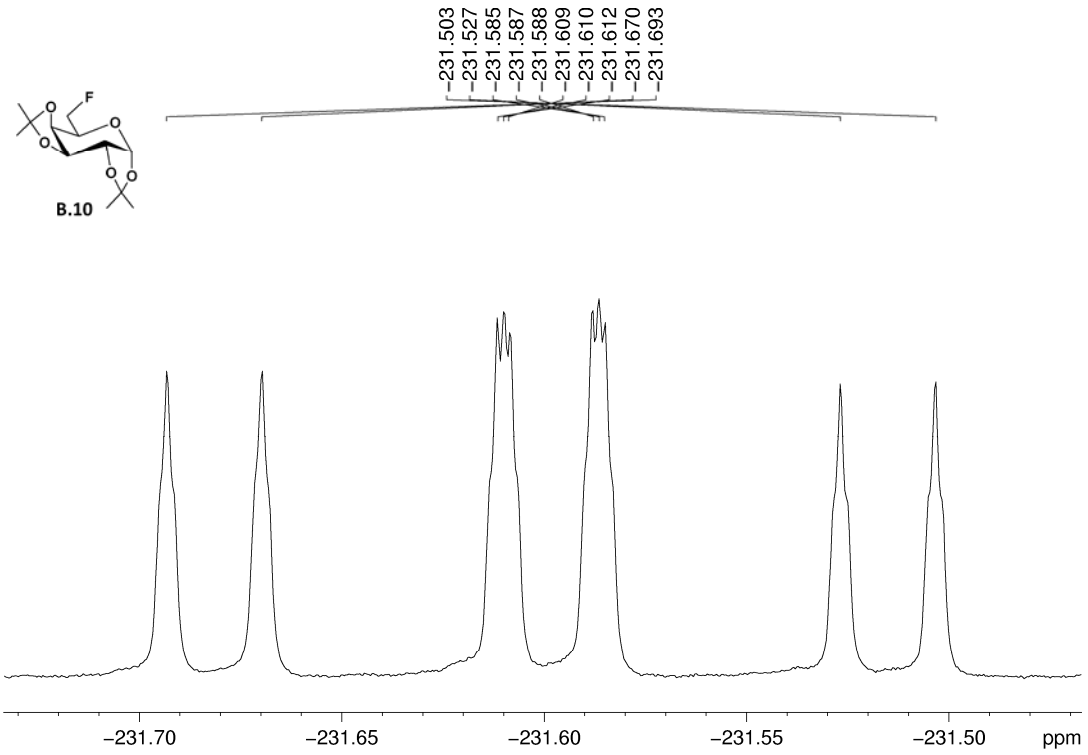
−230.90
−230.85
−230.80
−230.75
−230.70
−230.65
ppm


ppm
OH
F
O
HO
O
OH
NO2
B.8
70
80
90
100
110
120
130
140
150
160
8.5
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
ppm


109.81
108.96
96.33
82.77
81.66
77.37
77.16
76.95
70.73
70.69
70.66
70.57
66.84
66.70
26.17
26.06
25.06
24.56
B.10
115 110 105 100 95 90 85 80 75 70 65 60 55 50 45 40 35 30 25 ppm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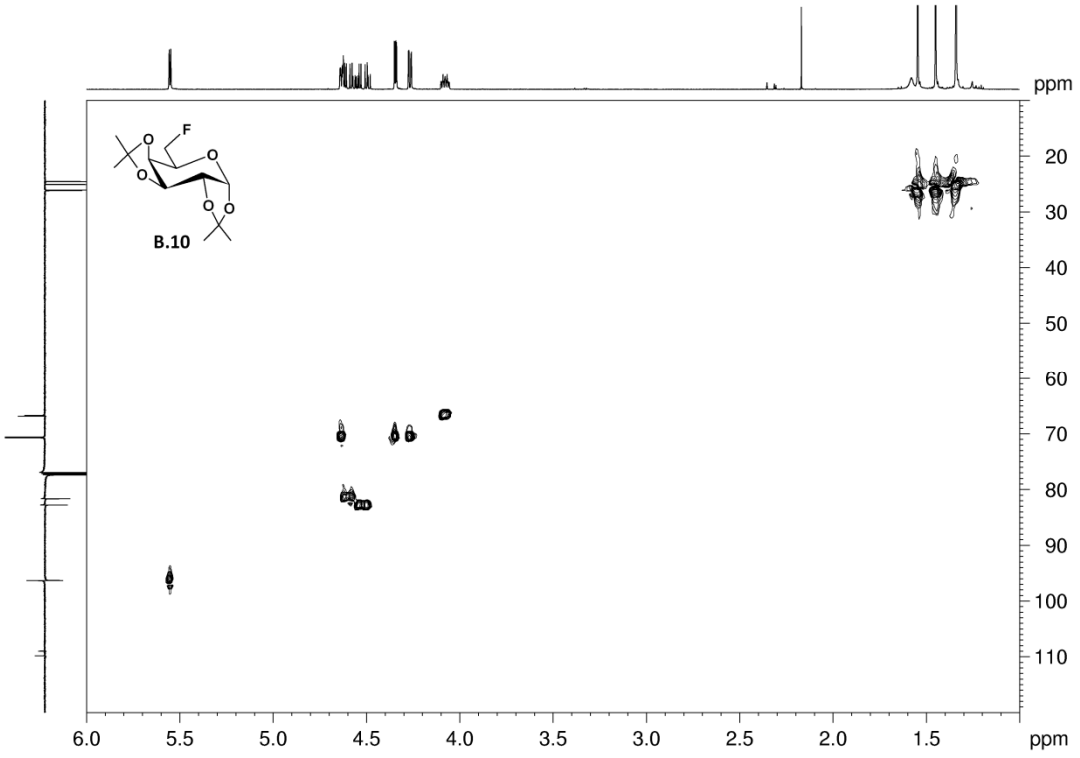
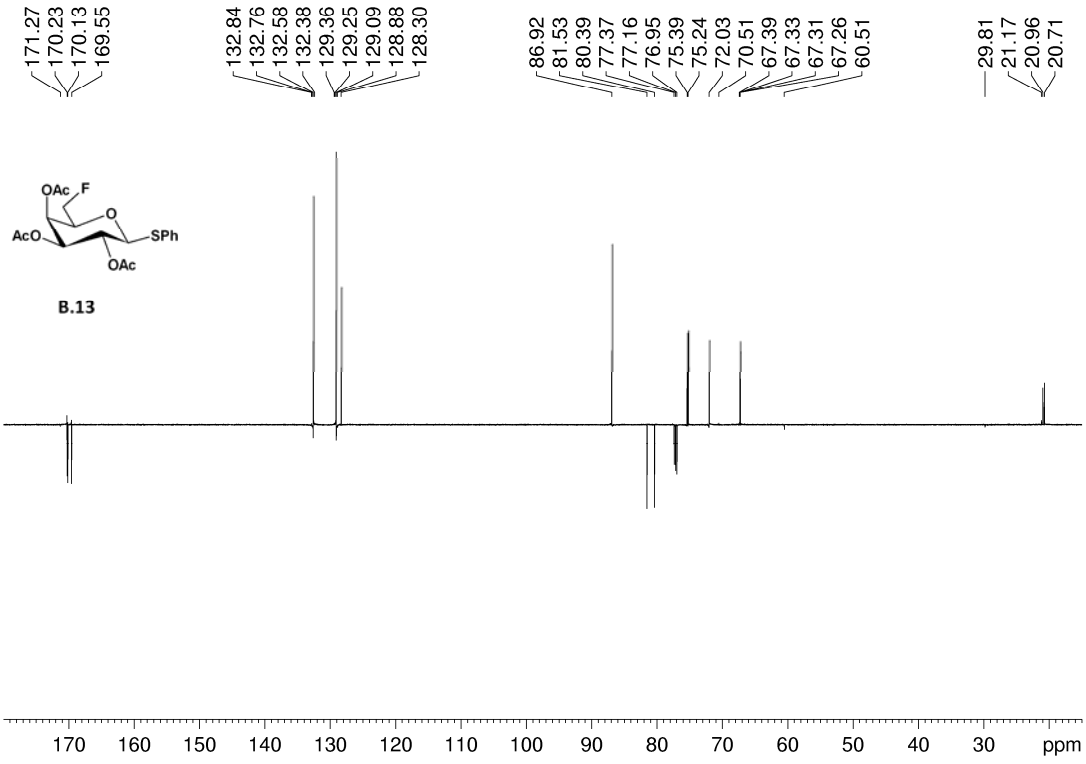
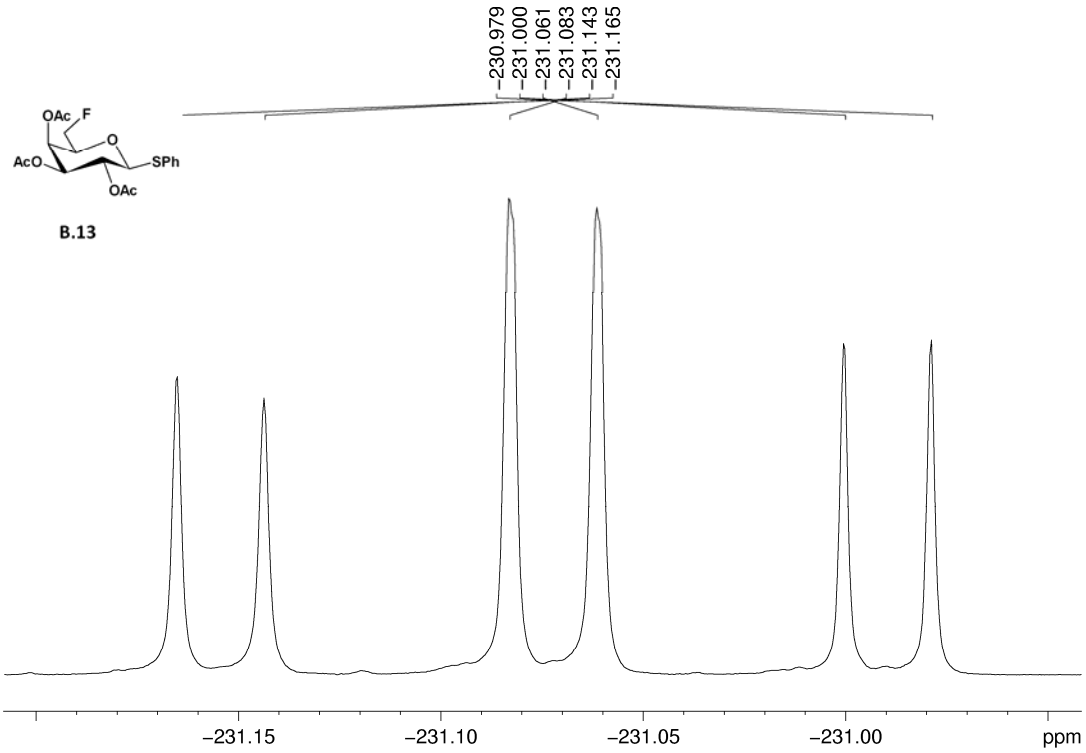
231.527
231.585
231.587
231.588
231.609
231.610
231.612
231.670
231.693
B.10
−231.70 −231.65 −231.60 −231.55 −231.50 ppm


B.10
ppm
20
30
40
50
60
70
80
90
100
110
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
ppm


171.27
170.23
170.13
169.55
132.84
132.76
132.58
132.38
129.36
129.25
129.09
128.88
128.30
86.92
81.53
80.39
77.37
77.16
76.95
75.39
75.24
72.03
70.51
67.39
67.33
67.31
67.26
60.51
29.81
21.17
20.96
20.71
B.13
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm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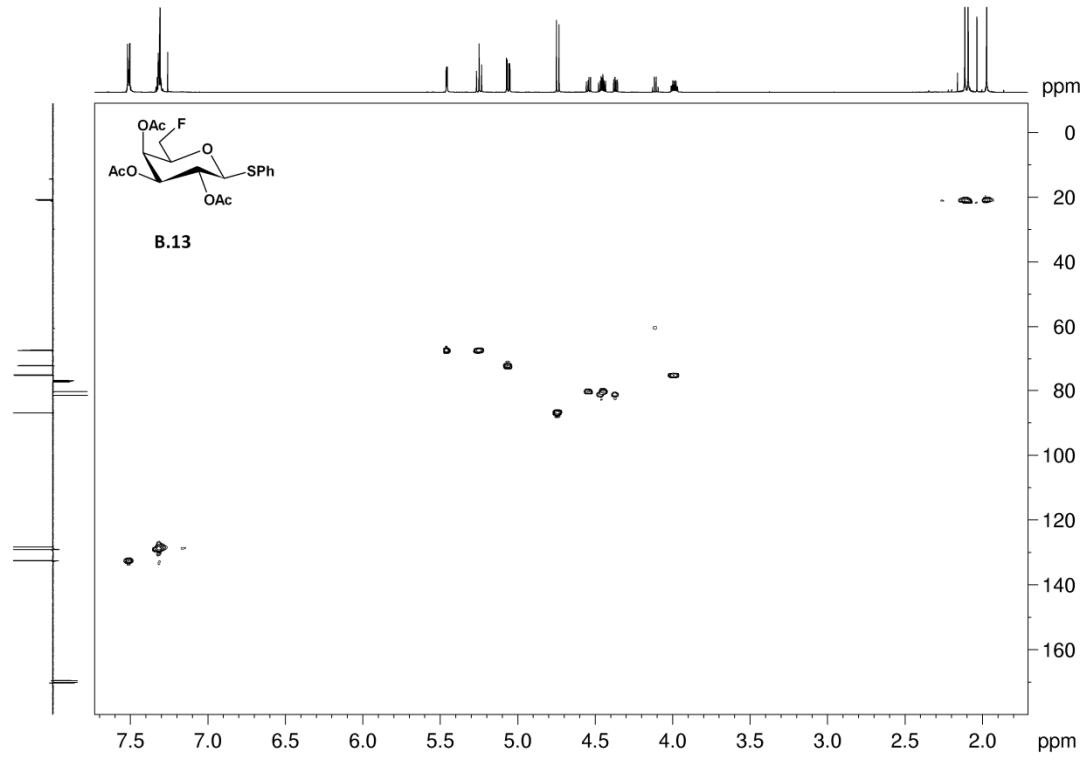
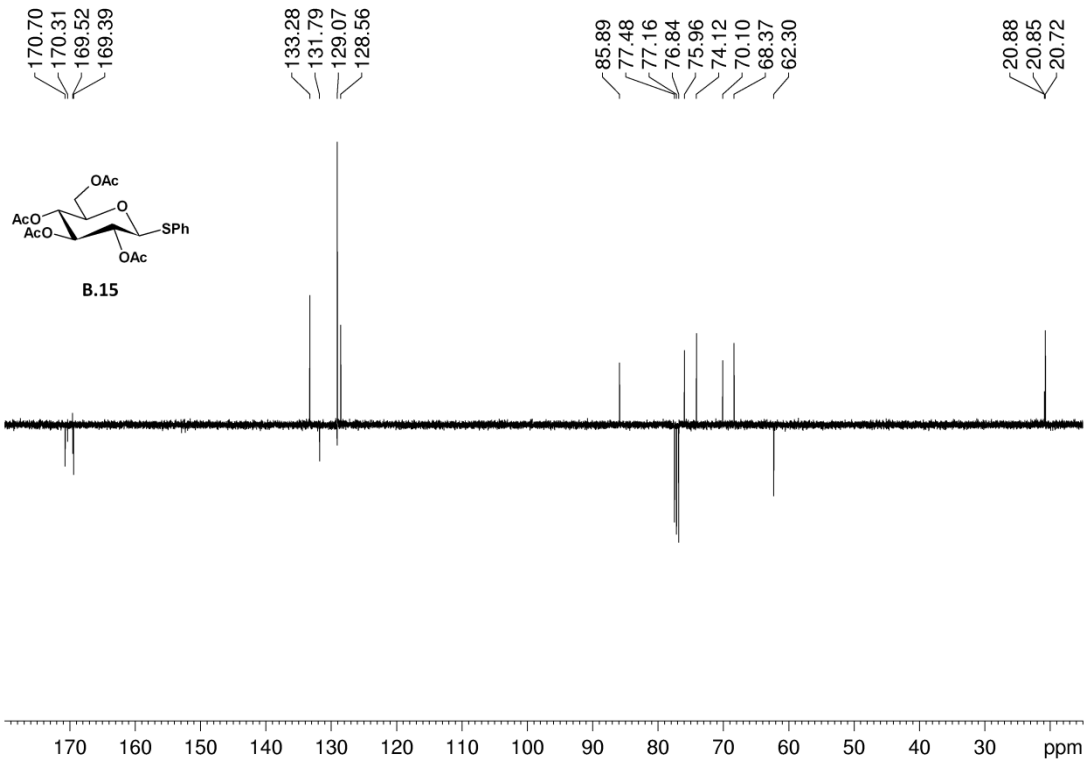
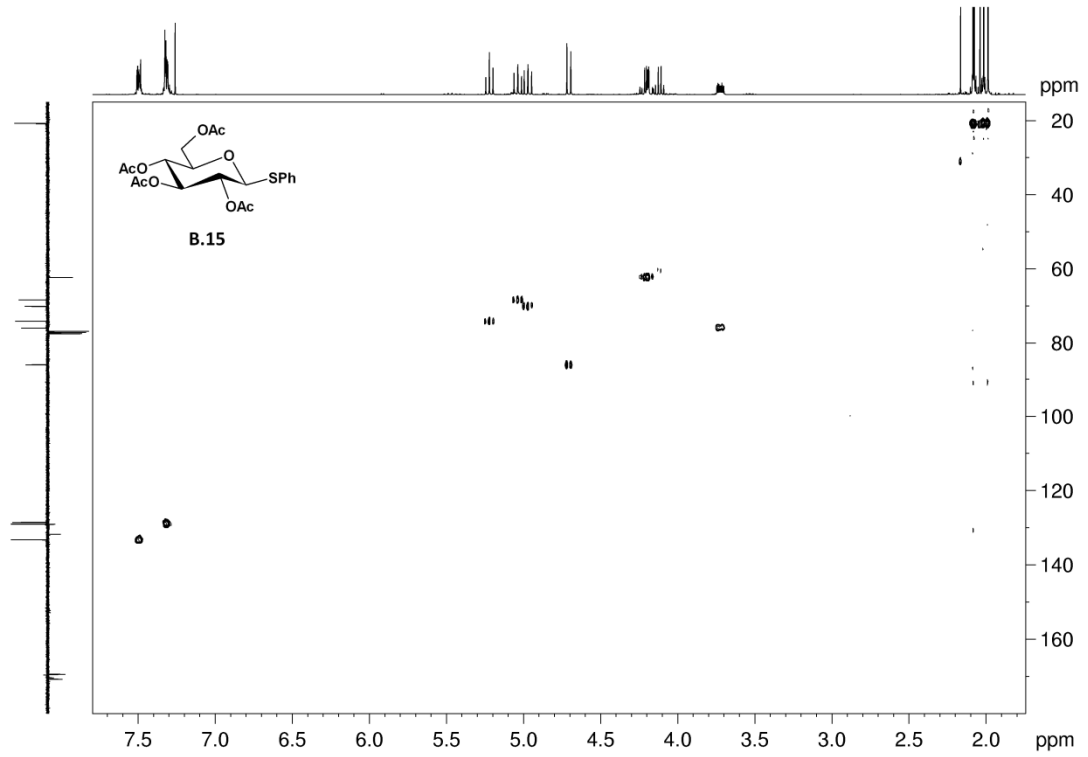
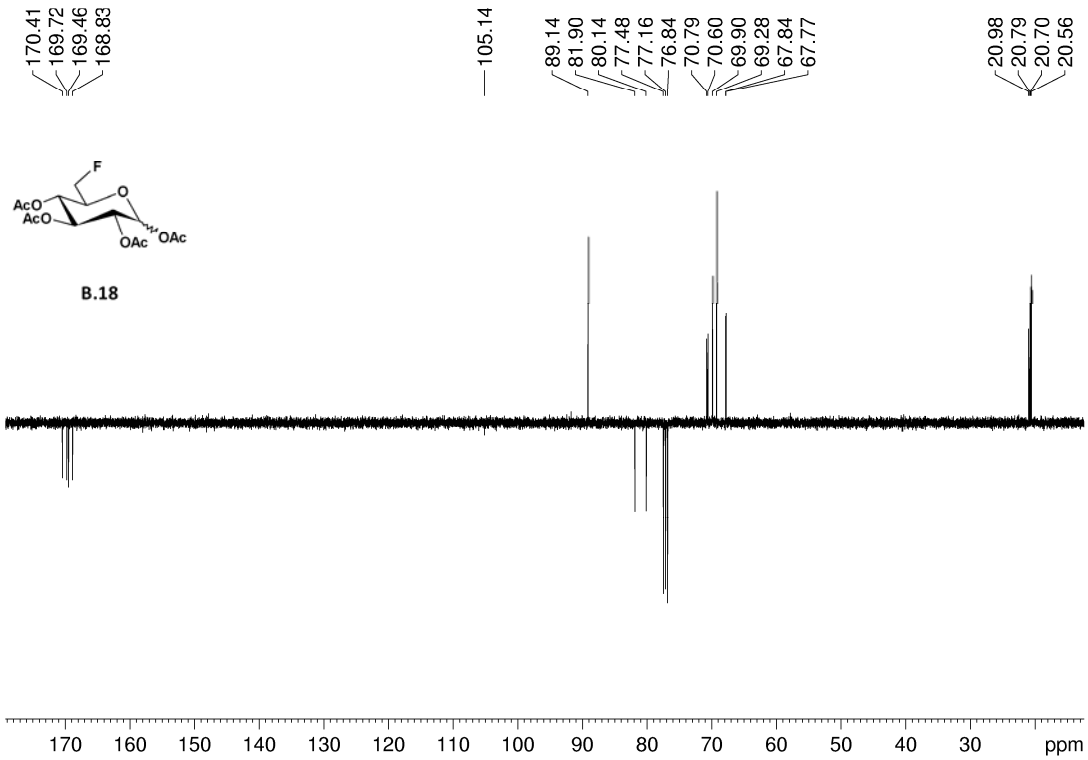
-231.083
-231.143
-231.165
OAc
F
O
AcO
SPh
OAc
B.13
−231.15
−231.10
−231.05
−231.00
ppm


ppm
OAc
F
O
AcO
SPh
OAc
B.13
0
20
40
60
80
100
120
140
160
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm


170.70
170.31
169.52
169.39
133.28
131.79
129.07
128.56
85.89
77.48
77.16
76.84
75.96
74.12
70.10
68.37
62.30
20.88
20.85
20.72
OAc
AcO
AcO
O
SPh
OAc
B.15
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm


ppm
20
40
60
80
100
120
140
160
OAc
AcO
AcO
O
SPh
OAc
B.15
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm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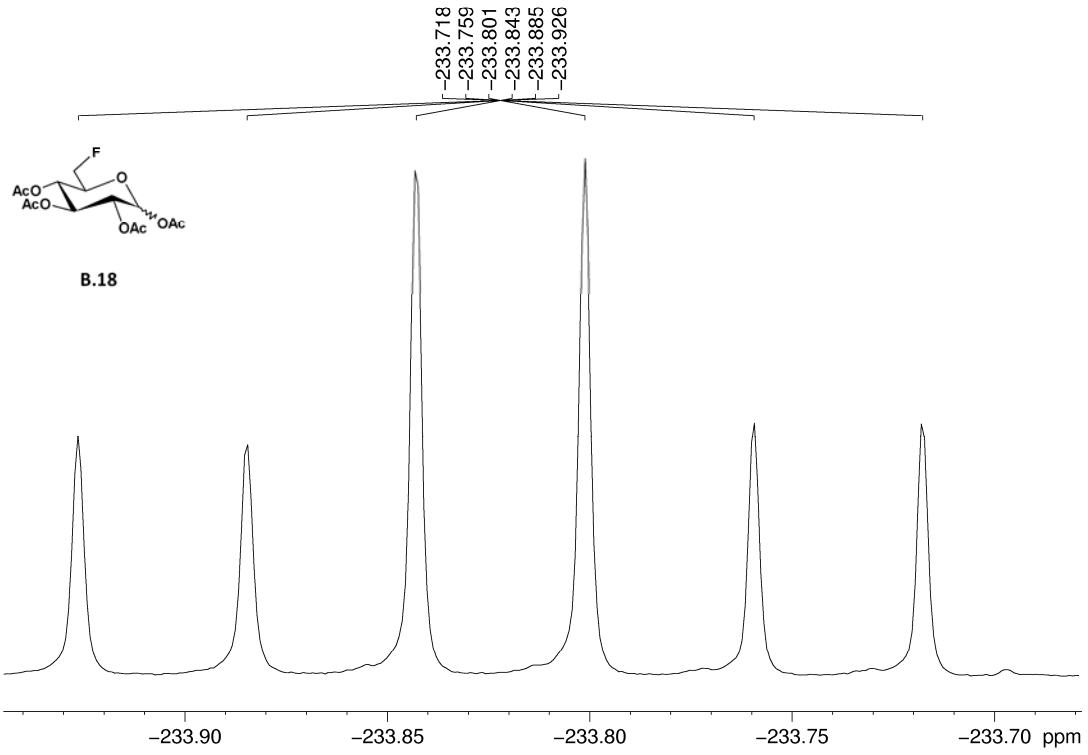
169.72
169.46
168.83
105.14
89.14
81.90
80.14
77.48
77.16
76.84
70.79
70.60
69.90
69.28
67.84
67.77
20.98
20.79
20.70
20.56
F
O
AcO
AcO
OAc
OAc
B.18
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm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-233.759
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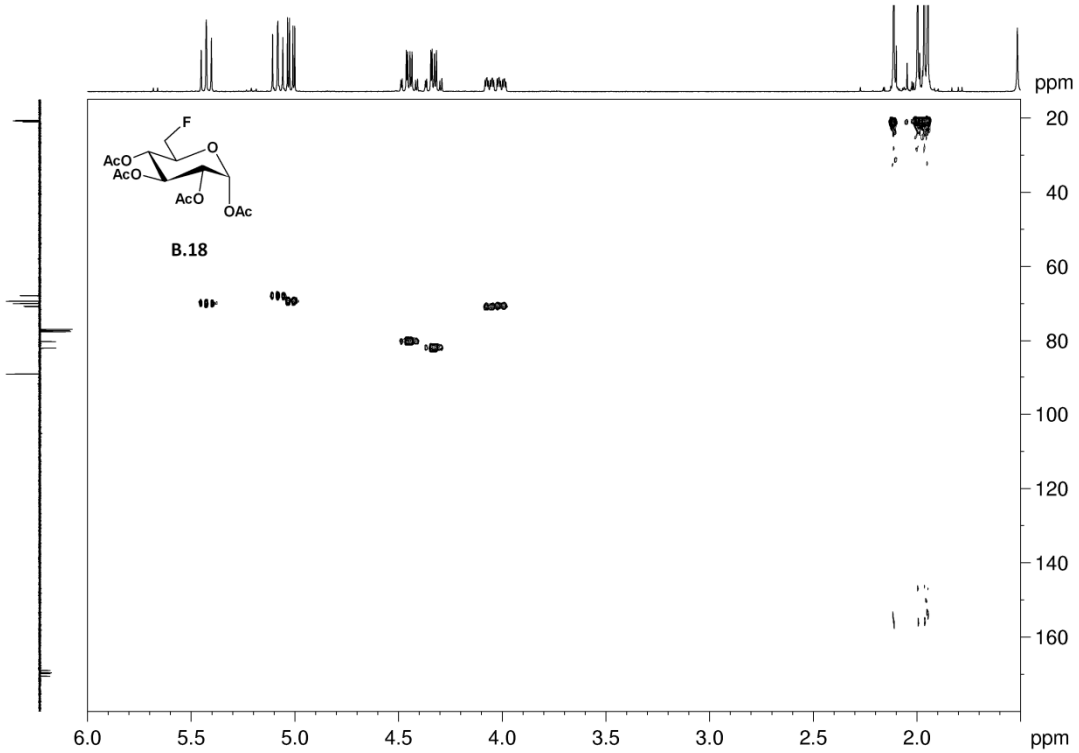
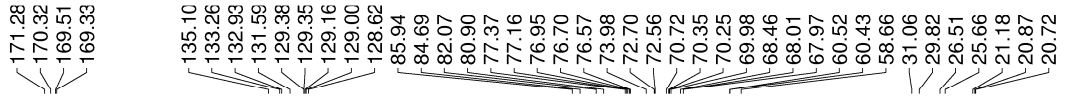
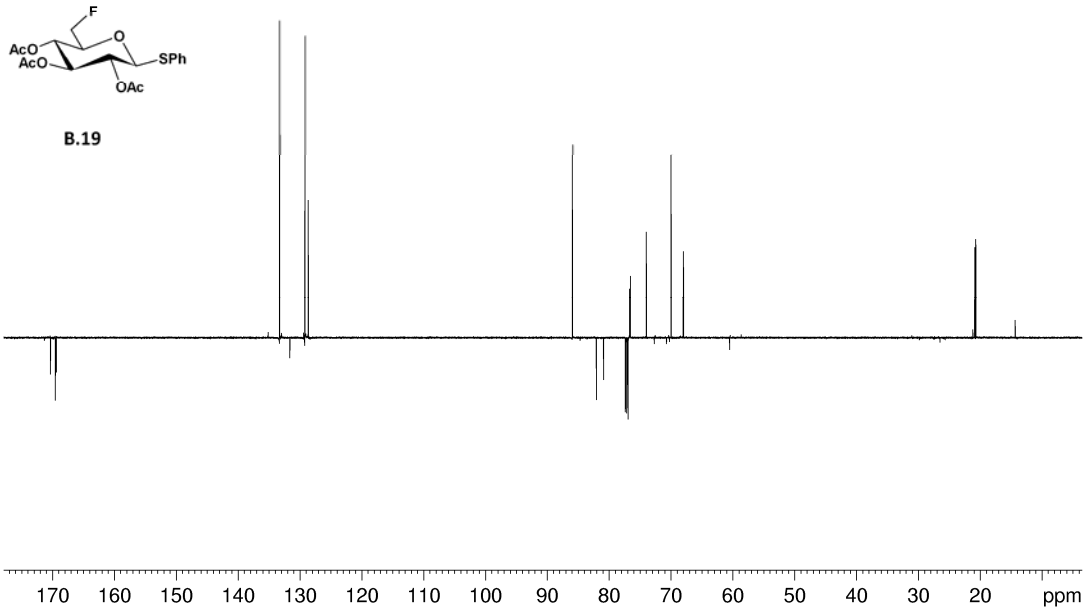
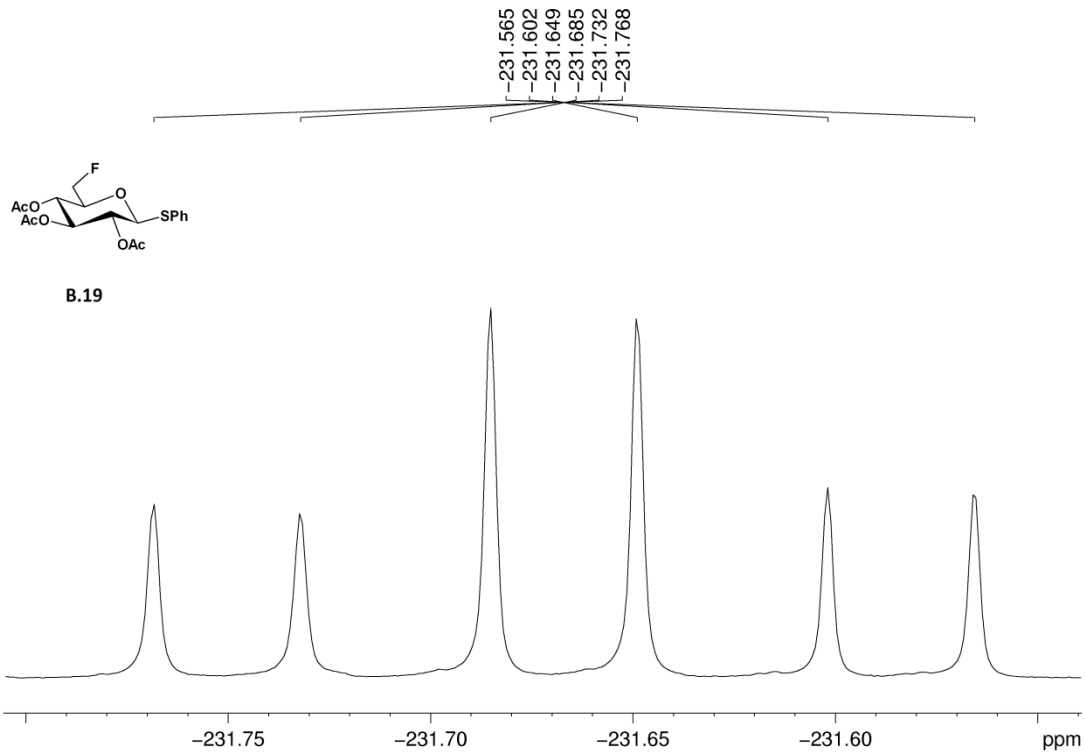
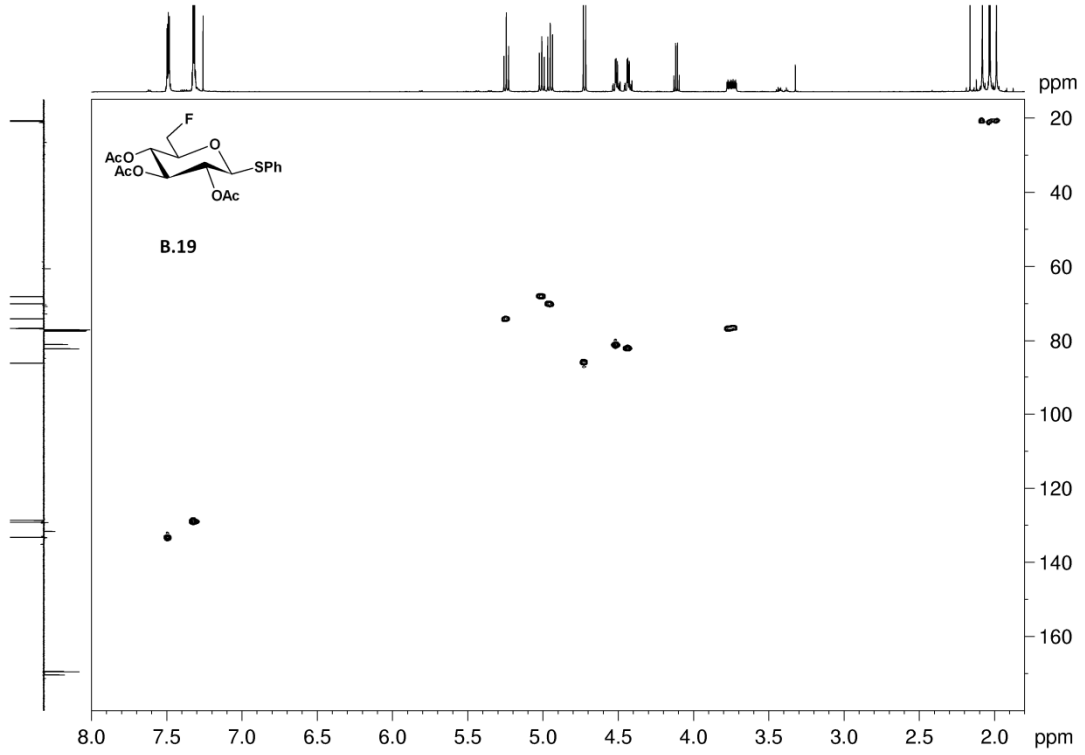
-233.885
-233.926
F
O
AcO
AcO
OAc
OAc
B.18
−233.90
−233.85
−233.80
−233.75
−233.70
ppm

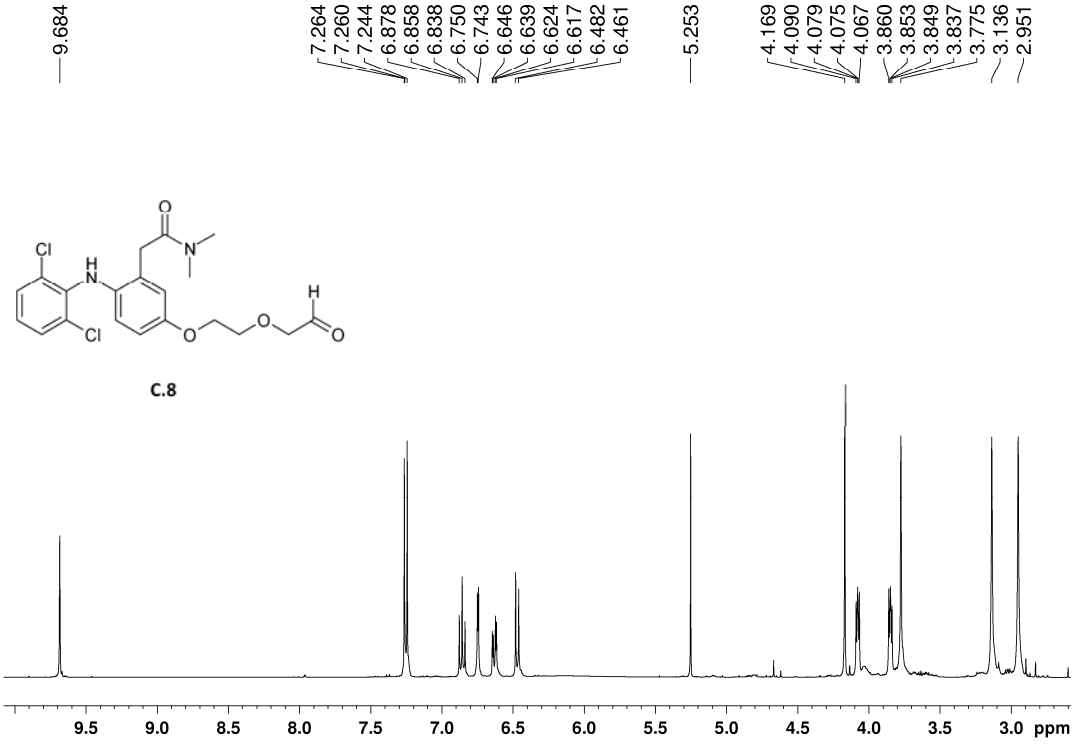

F
O
AcO
AcO
AcO
OAc
B.18
ppm
20
40
60
80
100
120
140
160
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm


171.28
170.32
169.51
169.33
135.10
133.26
132.93
131.59
129.38
129.35
129.16
129.00
128.62
85.94
84.69
82.07
80.90
77.37
77.16
76.95
76.70
76.57
73.98
72.70
72.56
70.72
70.35
70.25
69.98
68.46
68.01
67.97
60.52
60.43
58.66
31.06
29.82
26.51
25.66
21.18
20.87
20.72


F
O
AcO
AcO
SPh
OAc
B.19
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
ppm


−231.565
−231.602
−231.649
−231.685
−231.732
−231.768
F
O
AcO
AcO
SPh
OAc
B.19
−231.75
−231.70
−231.65
−231.60
ppm


ppm
F
O
AcO
AcO
SPh
OAc
B.19
20
40
60
80
100
120
140
160
8.0
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
ppm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7.244
6.878
6.858
6.838
6.750
6.743
6.646
6.639
6.624
6.617
6.482
6.461
5.253
4.169
4.090
4.079
4.075
4.067
3.860
3.853
3.849
3.837
3.775
3.136
2.951
C.8
9.5
9.0
8.5
8.0
7.5
7.0
6.5
6.0
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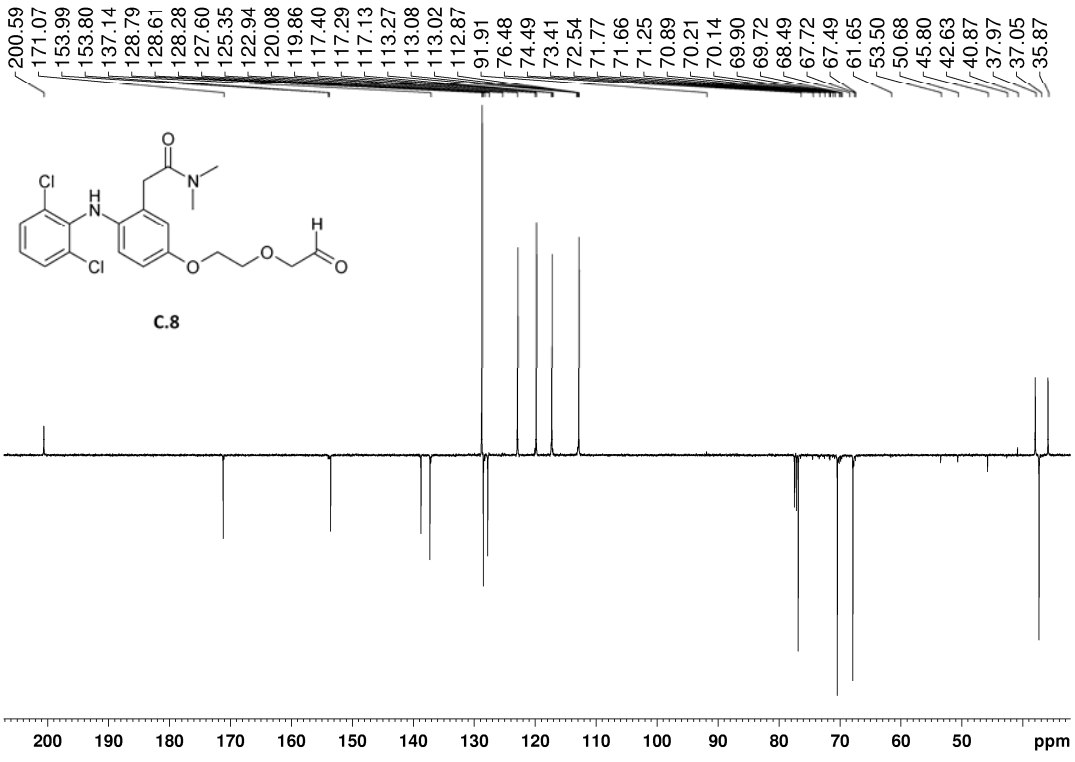
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117.40
117.29
117.13
113.27
113.08
113.02
112.87
91.91
76.48
74.49
73.41
72.54
71.77
71.66
71.25
70.89
70.21
70.14
69.90
69.72
68.49
67.72
67.49
61.65
53.50
50.68
45.80
42.63
40.87
37.97
37.05
35.87
C.8
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180
170
160
150
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130
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110
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70
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50
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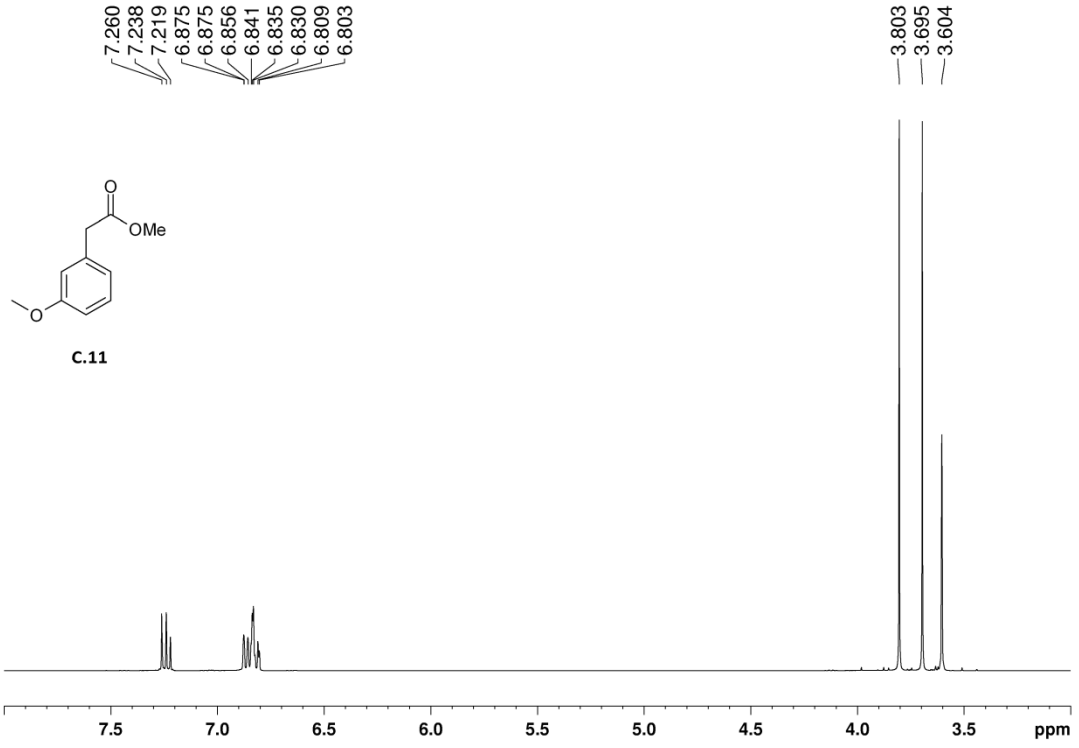
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3.695
3.604
O
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O
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5.5
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4.5
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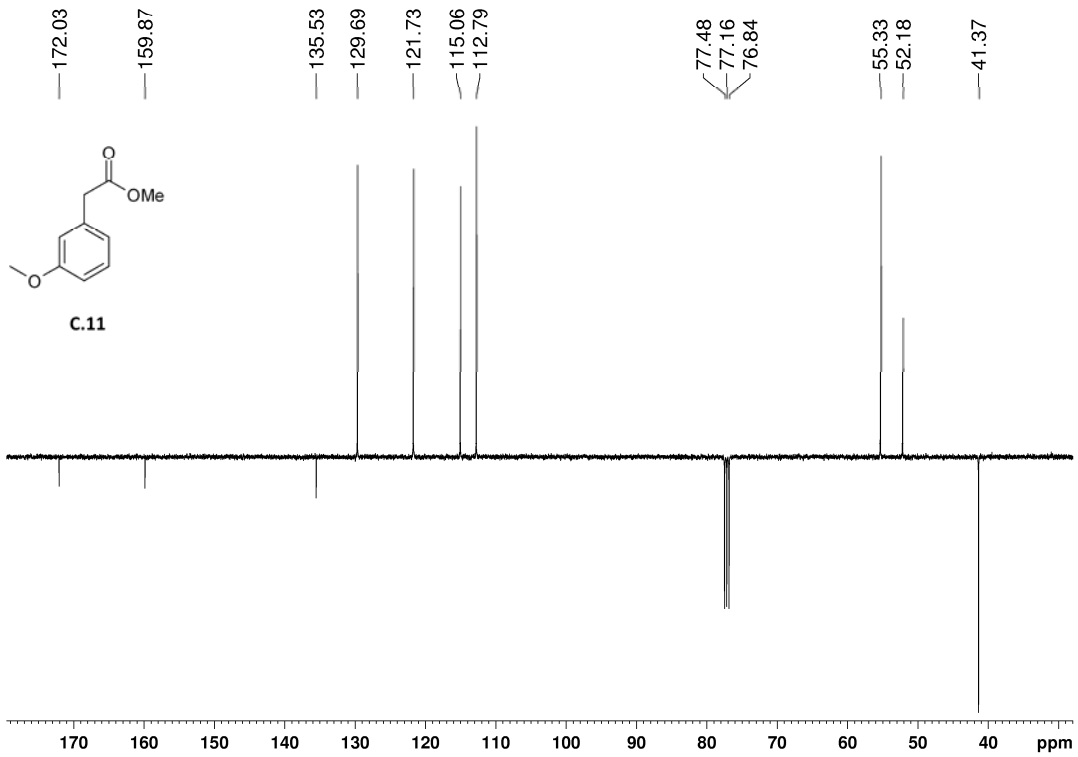
3.5
ppm

C.11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115.06
112.79
77.48
77.16
76.84
55.33
52.18
41.37
O
OMe
O
170
160
150
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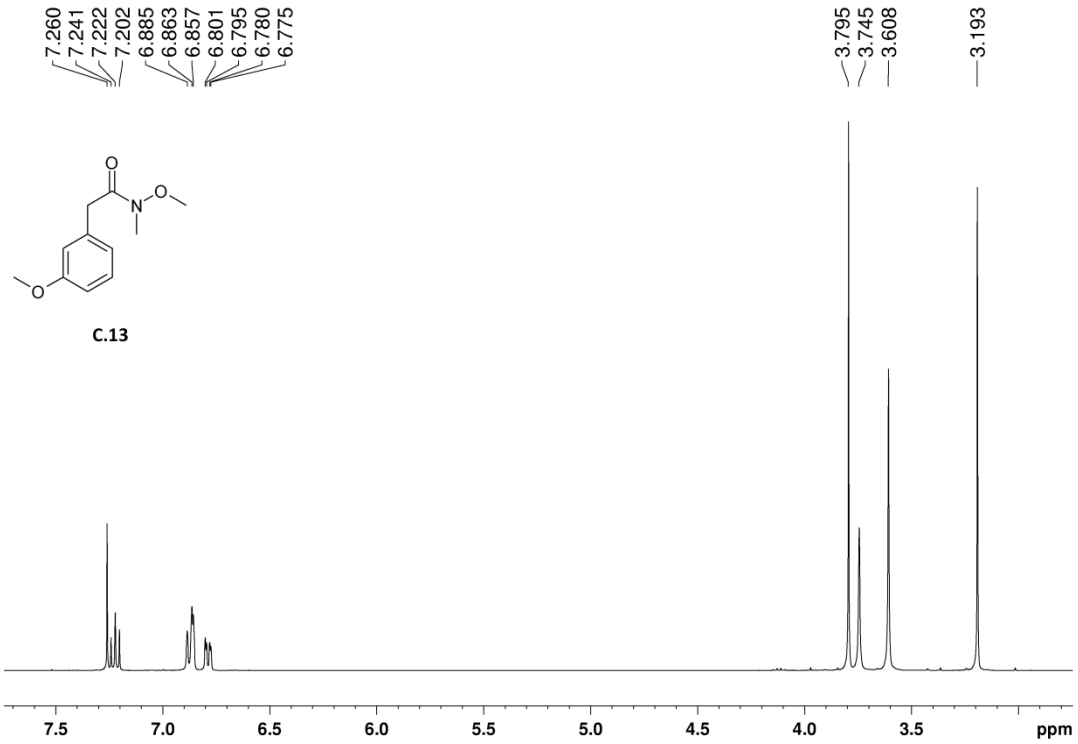
130
120
110
100
90
80
70
60
50
40
ppm

C.11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6.863
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6.801
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3.745
3.608
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5.0
4.5
4.0
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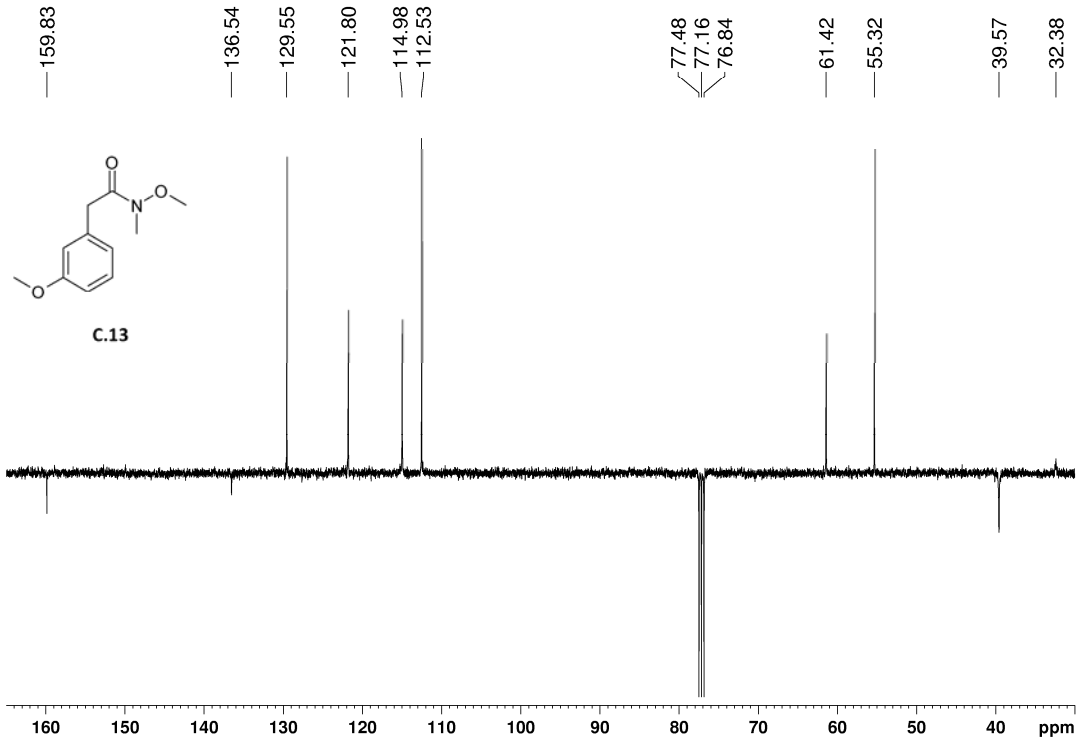
ppm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129.55
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112.53
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77.16
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61.42
55.32
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C.13
160
150
140
130
120
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100
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60
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40
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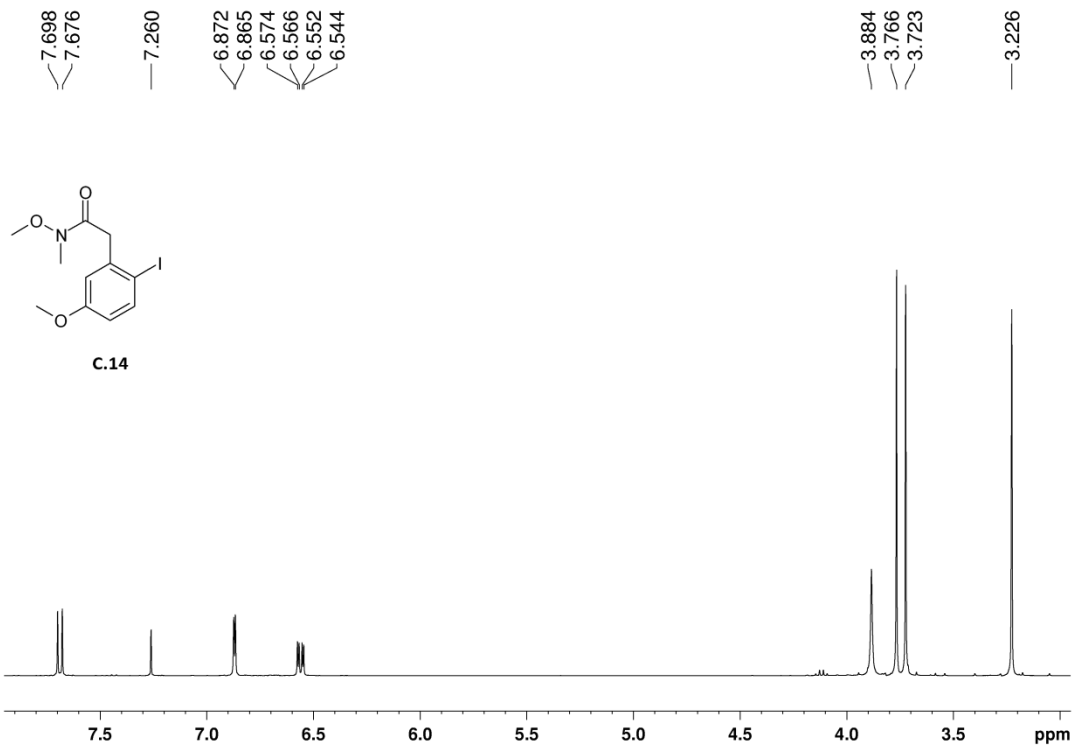
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6.552
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3.884
3.766
3.723
3.226
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7.5
7.0
6.5
6.0
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5.0
4.5
4.0
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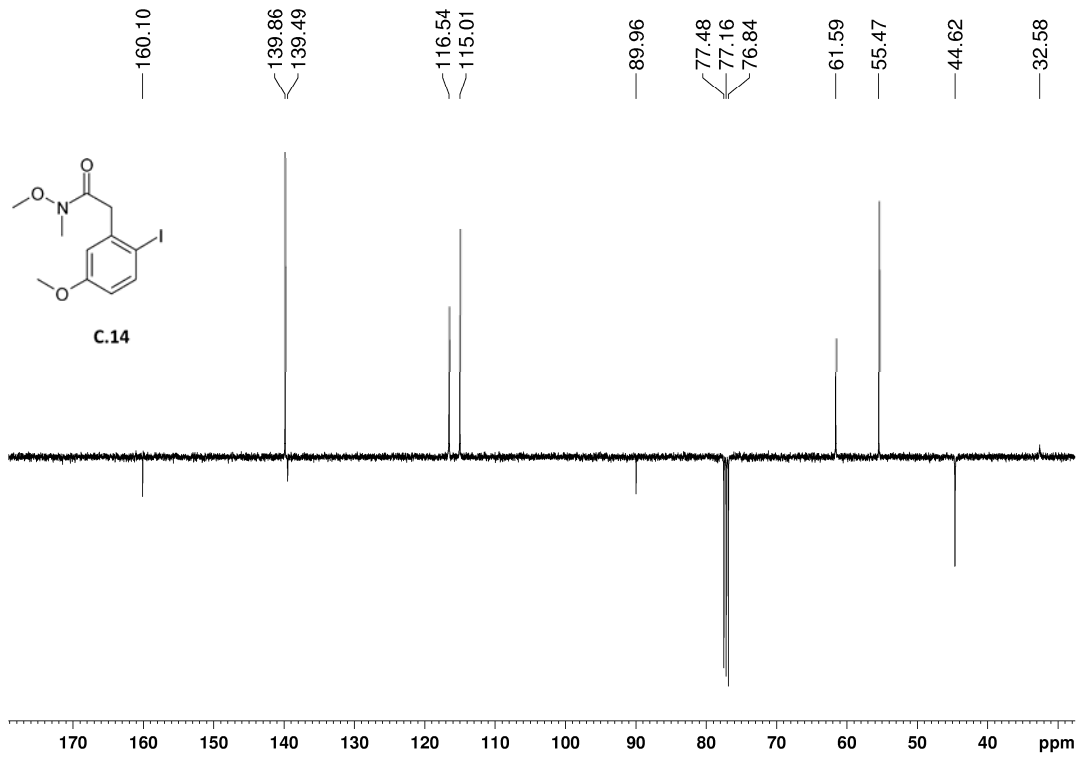
ppm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89.96
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77.16
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61.59
55.47
44.62
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C.14
170
160
150
140
130
120
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100
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80
70
60
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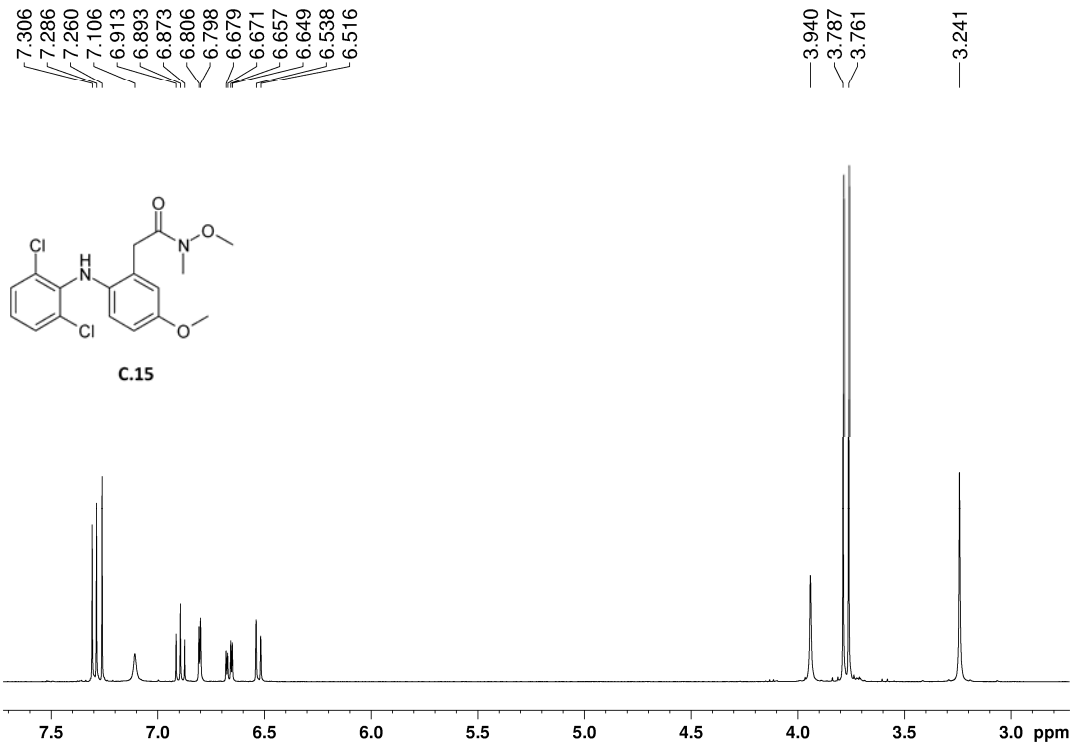
40
ppm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7.286
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6.679
6.671
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6.649
6.538
6.516
3.940
3.787
3.761
3.241
Cl
H
N
O
Cl
O
C.15
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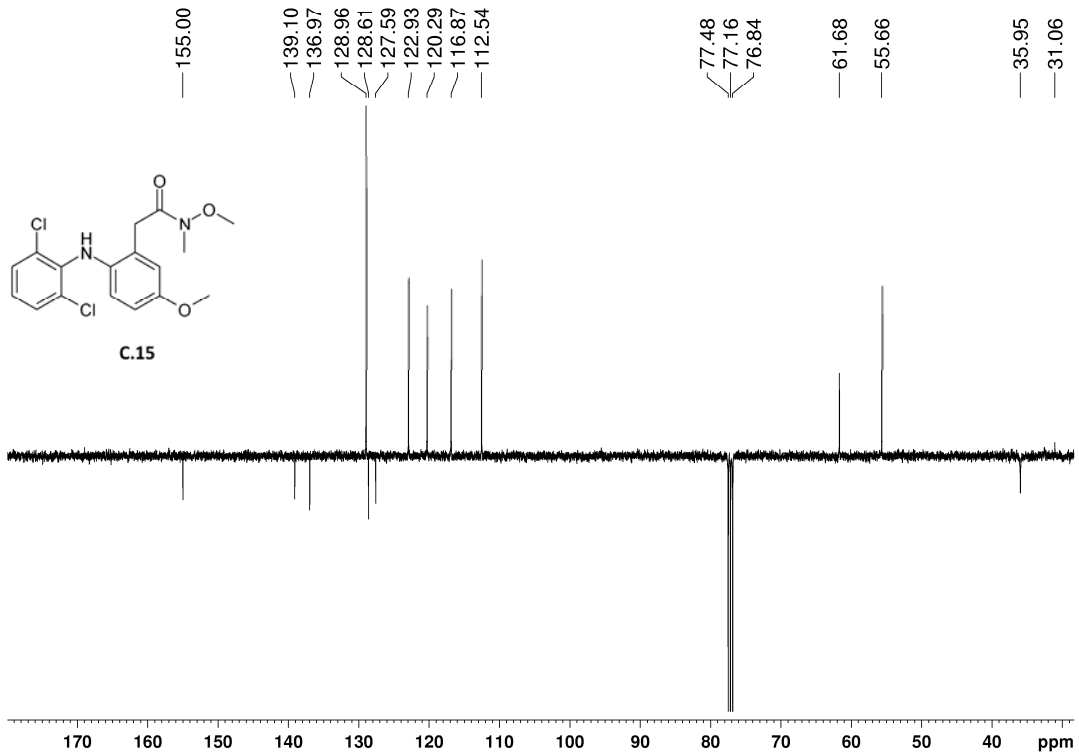
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0 ppm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136.97
128.96
128.61
127.59
122.93
120.29
116.87
112.54
77.48
77.16
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61.68
55.66
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Cl
H
N
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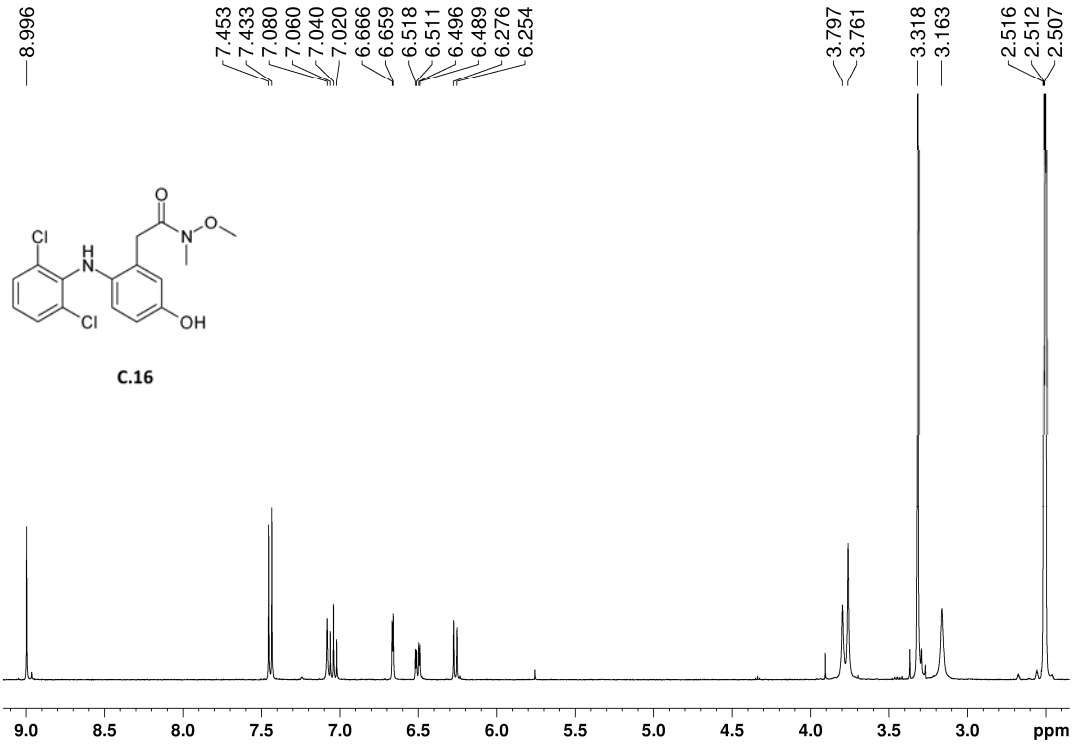
Cl
O
C.15
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm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7.453
7.433
7.080
7.060
7.040
7.020
6.666
6.659
6.518
6.511
6.496
6.489
6.276
6.254
3.797
3.761
3.318
3.163
2.516
2.512
2.507
Cl
H
N
O
N
O
Cl
OH
9.0
8.5
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6.0
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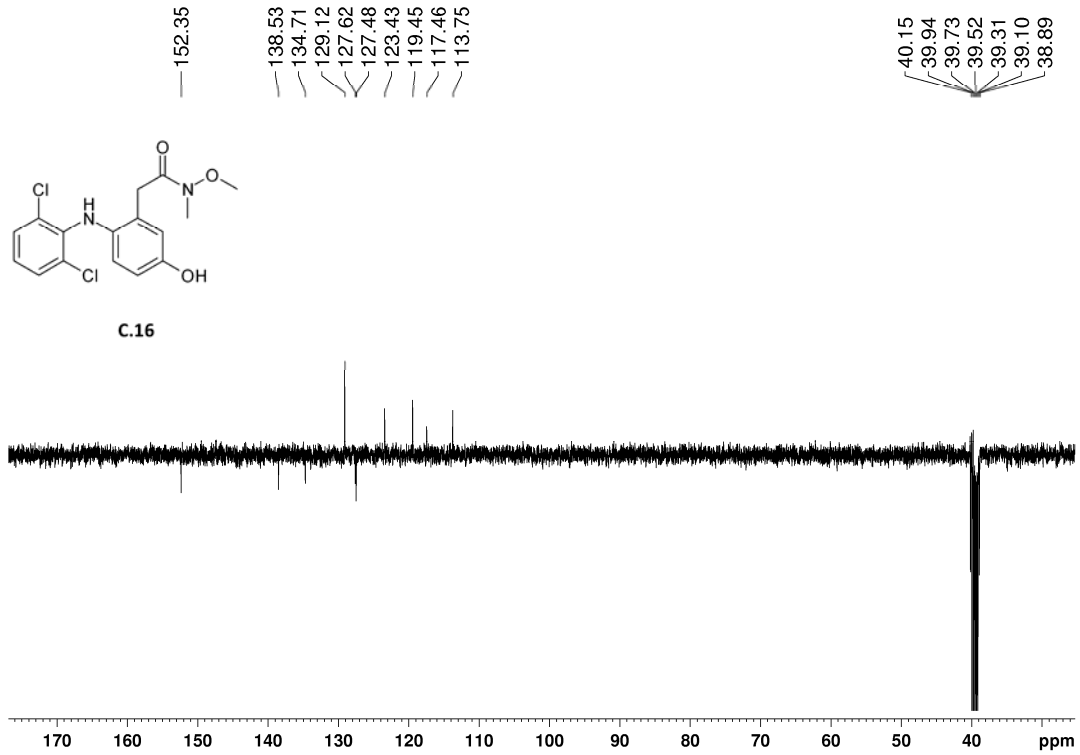
3.0
ppm

C.16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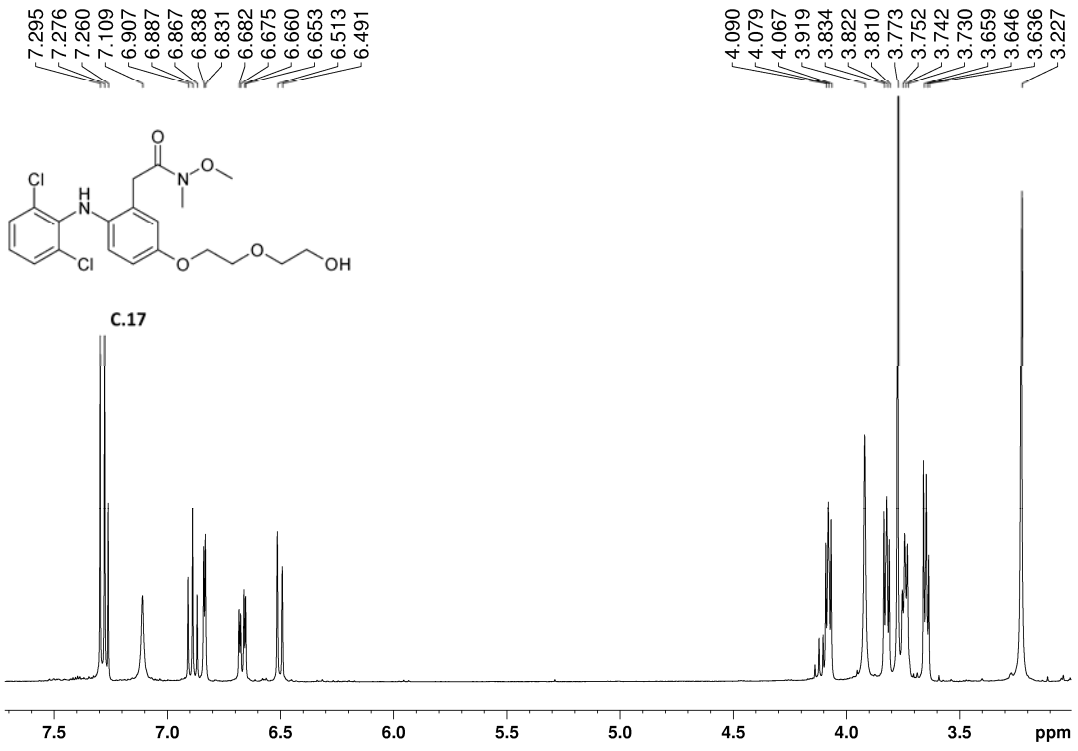
138.53
134.71
129.12
127.62
127.48
123.43
119.45
117.46
113.75
40.15
39.94
39.73
39.52
39.31
39.10
38.89
Cl
H
N
O
N
O
Cl
OH
170
160
150
140
130
120
110
100
90
80
70
60
50
40
ppm

C.16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7.276
7.260
7.109
6.907
6.887
6.867
6.838
6.831
6.682
6.675
6.660
6.653
6.513
6.491
4.090
4.079
4.067
3.919
3.834
3.822
3.810
3.773
3.752
3.742
3.730
3.659
3.646
3.636
3.227
Cl
H
N
O
N
O
Cl
O
O
OH
C.17
7.5
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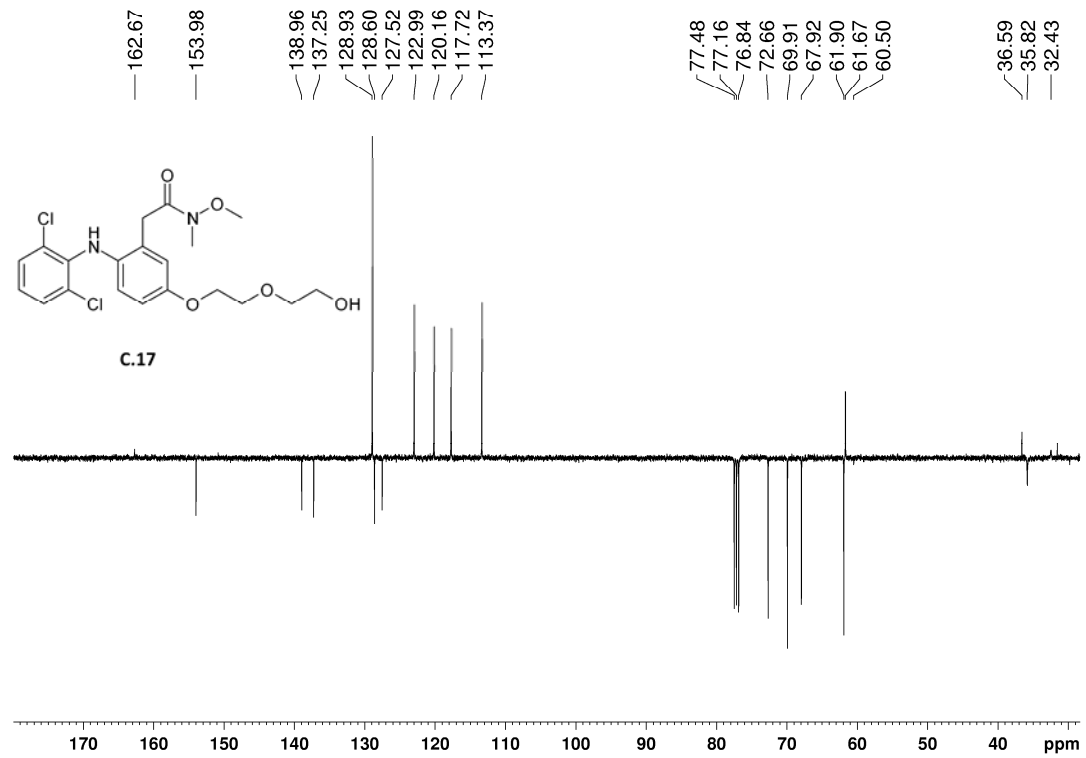
5.0
4.5
4.0
3.5
ppm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137.25
128.93
128.60
127.52
122.99
120.16
117.72
113.37
77.48
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72.66
69.91
67.92
61.90
61.67
60.50
36.59
35.82
32.43
Cl
H
N
O
N
O
Cl
O
O
OH
C.17
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130
120
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100
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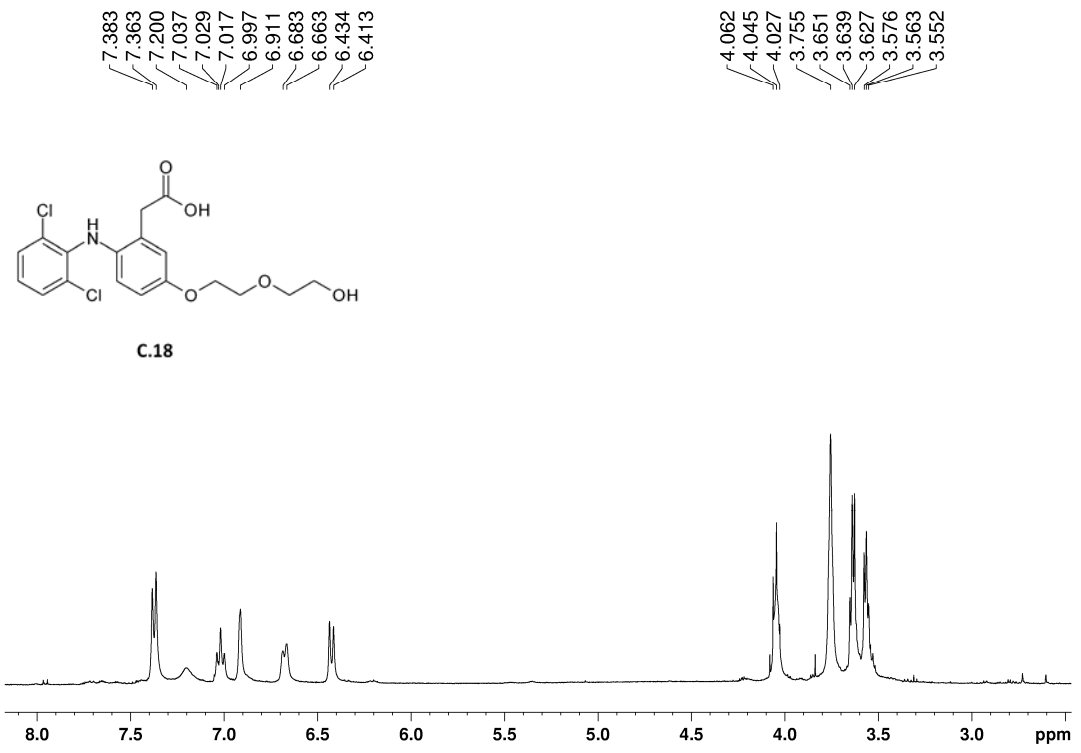
70
60
50
40
ppm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7.363
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6.911
6.683
6.663
6.434
6.413
4.062
4.045
4.027
3.755
3.651
3.639
3.627
3.576
3.563
3.552
Cl
H
N
Cl
O
OH
O
O
OH
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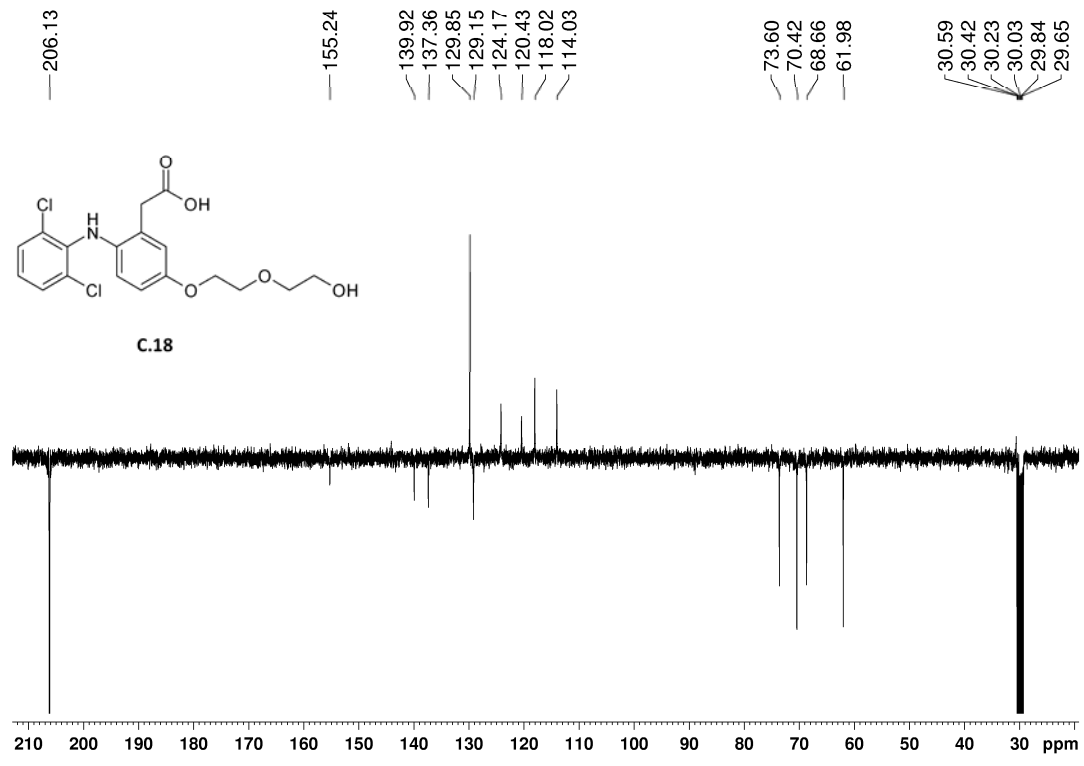
6.0
5.5
5.0
4.5
4.0
3.5
3.0
ppm

C.18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129.85
129.15
124.17
120.43
118.02
114.03
73.60
70.42
68.66
61.98
30.59
30.42
30.23
30.03
29.84
29.65
Cl
H
N
Cl
O
OH
O
O
OH
210
200
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180
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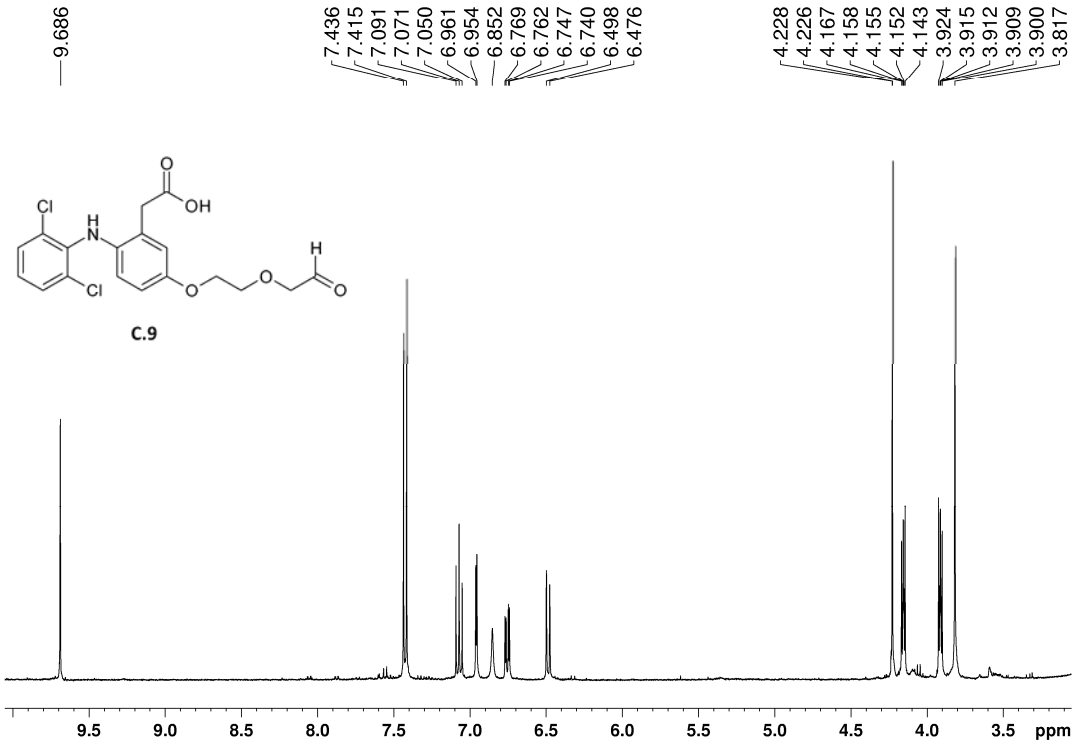
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

C.18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6.954
6.852
6.769
6.762
6.747
6.740
6.498
6.476
4.228
4.226
4.167
4.158
4.155
4.152
4.143
3.924
3.915
3.912
3.909
3.900
3.817
Cl
H
N
Cl
O
OH
O
O
O
H
O
C.9
9.5
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7.5
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6.5
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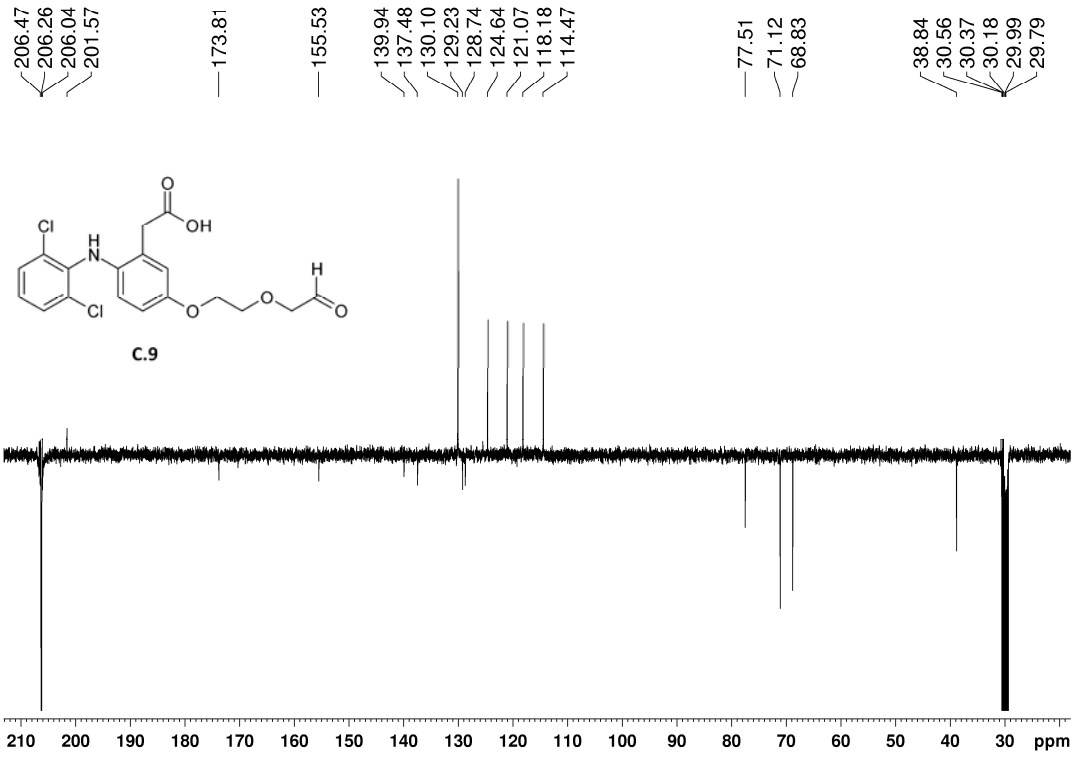
ppm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201.57
173.81
155.53
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129.23
128.74
124.64
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118.18
114.47
77.51
71.12
68.83
38.84
30.56
30.37
30.18
29.99
29.79
Cl
H
N
Cl
O
OH
O
O
O
H
O
C.9
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
ppm

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'Diclofenac hypersensitivity is immune to hapten concept of drug allergy'
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Bioconjugate Chemistry, in preparation