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Alkylidene malonates and acetoacetates as intermediates for the preparation of bioactive molecules

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Contents

List of abbreviations

Aq	aqueous
Ac	Acetyl
BOC	t-Butyloxycarbonyl
Bz	Benzoyl
Bn	Benzyl
c	concentration
Cbz	Carbobenzyloxy
Δ	reflux
DCM	Dichloromethane
de	diastereomeric excess
DMF	Dimethylformamide
DMSO	Dimethyl Sulfoxide
dr	diastereomeric ratio
E	Entgegen (opposite, trans)
eq	equivalent
Et	Ethyl
g	gram
h	hours
iPr	isopropyl
LiHMDA	Lithium Hexamethyldisilamide
M	molar mol/L
Me	Methyl
min	minutes
mg	milligram
mL	milliliter
mp	melting point
Nu	nucleophile
Ph	Phenyl

ppm	parts per million
Py	Pyridine
rt	room temperature
TBAF	tetra-n-butylammonium fluoride
TEA	Triethylamine
Tf	Triflate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl
Z	Zusammen (together, cis)
bs	broad singlet (NMR)
δ	Chemical shift (NMR)
^{13}C-NMR	Carbon-13 Nuclear Magnetic Resonance
COSY	Correlation Spectroscopy (NMR)
d	doublet (NMR)
dd	doublet of a doublet (NMR)
DEPT	Distorsionless Enhancement by Polarisation Transfer (NMR)
EI	Electronic Impact (MS)
ESI	Electron Spray Ionisation
FID	Free Induction Decay (NMR)
FT	Fourier Tranform
Hz	Hertz
HBTU	*N*,*N*,*N*′,*N*′-Tetramethyl-*O*-(1*H*-benzotriazol-1-yl)uronium hexafluorophosphate
HETCOR	Heteronuclear Correlation (NMR)
HMQC	Heteronuclear Multiple Quantum Coherence (NMR)
HPLC	High Performance Liquid Chromatography

General experimental procedure

All chemicals were purchased from commercial suppliers and were used without further purification. Anhydrous solvents were purchased in sure-seal bottles over molecular sieves and were used without further drying. Flash chromatography was performed on silica gel (230–400 mesh). Dowex 50WX2-200(H) ion-exchange resin was used for purification of free amino acids. NMR Spectra were recorded with Varian Gemini 200, Mercury Plus 400, or Unity Inova 600 MHz spectrometers. Chemical shifts (δ) are reported in ppm relative to the solvent peak of $CDCl_3$ set at δ= 7.27 ppm (^{1}H NMR) or δ=77.0 ppm (^{13}C NMR), CD_3OD set at δ= 3.31 ppm (^{1}H NMR) or δ=49.0 ppm (^{13}C NMR), or D_2O set at δ= 4.79 ppm (^{1}H NMR). Coupling constants (J) are given in Hz. LC–MS analyses were performed on an HP1100 liquid chromatograph coupled with an electrospray ionization mass spectrometer (LC-ESI-MS), using H_2O/CH_3CN as solvent at 258°C (positive scan 100– 500 m/z, fragmentor 70 V).

Thesis outline

In this thesis we investigated the versatility and the potential applications of different kinds of alkylidene malonates, acetoacetates, malonamides and acetoacetoamides. Our research group devoted great attention to this kind of compounds since alkylidenes can be considered important intermediates in the synthesis of several scaffolds, to be inserted into molecules of potential biological and pharmaceutical interest. The increasing use of alkylidenes is due to their ability to react as unsaturated electrophiles and to the possibility to exploit them as intermediates for the introduction of different kind of functionalities, as will be discussed later on.

The preparation of alkylidene malonates, acetoacetates, malonamides and acetoacetoamides is presented in ***chapter 1.***

This section deals with different preparation methods of alkylidenes that we developed during the last few years and to the technologies involved for each synthetic protocol.

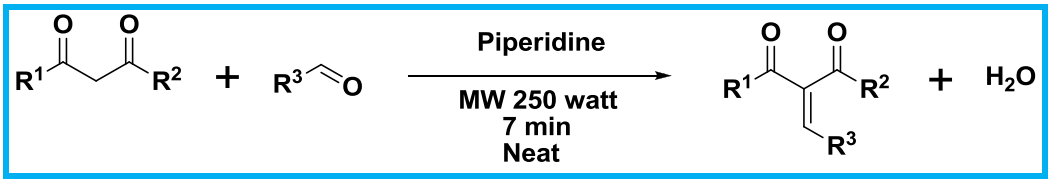


Fig.1. Knoevenagel reaction carried under microwave irradiation.

The reactivity that allowed to use the alkylidenes as intermediates in the synthesis of scaffolds for biologically active compounds is shown in ***chapter 2***. In particular, we will discuss the most important reactions used to obtain the desired molecules, and we will focus on the most interesting aspects of these latter ones.

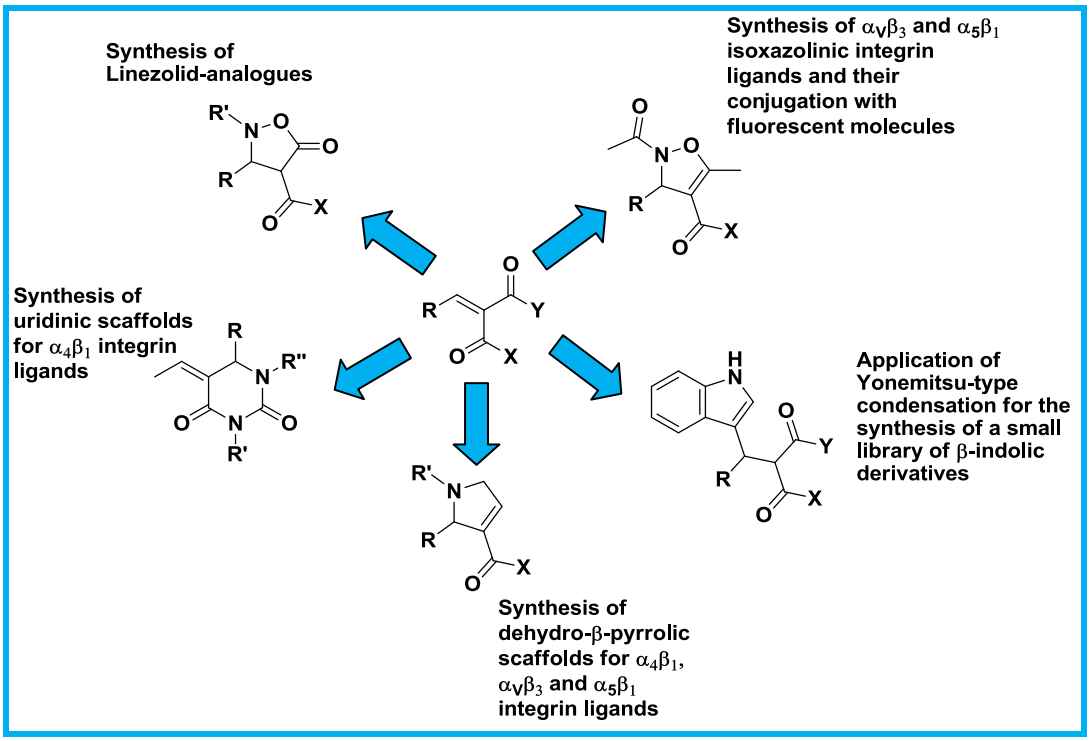


Fig.2 Developed projects starting from alkylidenes.

Finally, ***chapter 3*** will illustrate the potential applications and the related syntheses of potential bioactive compounds. The synthesized molecules find application in several fields and for this reason we considered each class of compounds in its related branch of interest.

Chapter 1

Preparation of Alkylidenes Malonates, Acetoacetates, Malonamides, Acetoacetoamides

1.1 Introduction

The α,β-unsaturated compounds have been considered since many decades the starting materials for the preparation of polyfunctionalized products through simple and economic transformations. The alkylidenes' ability to be easily converted into a wide range of compounds with potential biological interest, position them – somehow –in a high position in the list of privileged structures. Indeed, starting from these kind of compounds, it is possible to obtain several scaffolds, which are part of some more complicated molecules and may be used in the biological or pharmaceutical field.

Due to their high reactivity, alkylidenes take part to several reactions. However, we focused our attention on two of them: the conjugate Michael addition and the allylic substitution on the alcohol deriving from acetoacetic ketone reduction.

To synthesize various alkylidenes (acetoacetates, malonates, acetoacetoamides and malonamides) we used the Knoevenagel reaction, a widely studied process that we have slightly modified by using a recently discovered and ever-increasing technology: the MAOS (Microwave-Assisted Organic Synthesis).

In the Knoevenagel condensation, an aldehyde or a ketone react with an activated methylene compound to give a substituted olefin by using an amine base as catalyst. By deprotonation of the activated methylene the stabilized enolate is generated. The amine catalyst also reacts with the aldehyde or ketone to form an iminium ion, which then is attacked by the enolate. The obtained intermediate compound after base induced β-elimination, yields the final olefin product.

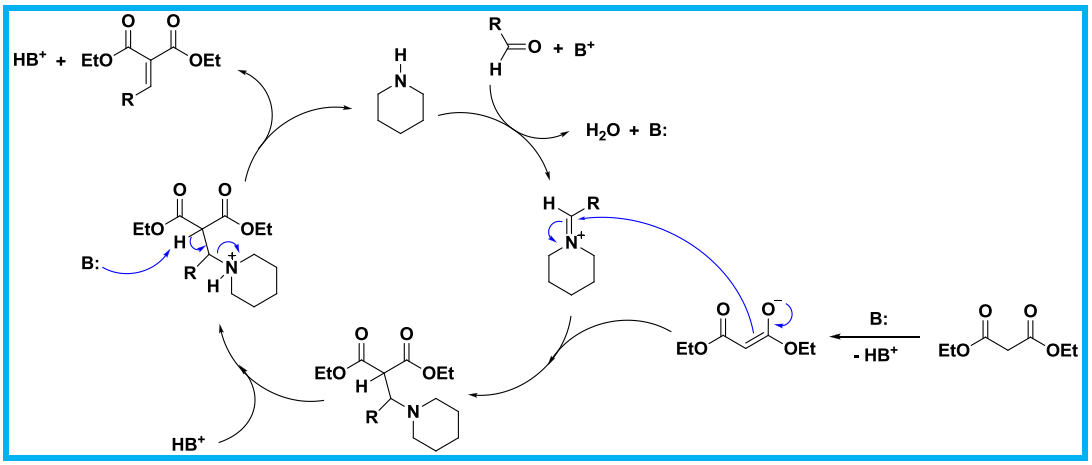


Fig.3. Mechanism of Knoevenagel reaction.

1.1.1 Microwaves in organic synthesis

The ever-increasing demand of techniques to obtain a large number of compounds in relatively short time, allowed microwaves to gain importance among the technologies used in organic synthesis. In fact, with MAOS (Microwave-Assisted Organic Synthesis), it is possible to significantly reduce the reaction time because this kind of technique, raising the temperature very quickly, allows to fasten the reaction and may give access to different reaction routes, leading to unexpected yields, conversions and selectivities.

In the initial experiments, organic chemists used MWs simply to obtain a rapid and constant heating, but very soon they realized that this technology was able to give additional benefits. Later on, in fact, the scientific community suggested that a chemical reaction could also be triggered by acting directly on bonds with an electromagnetic radiation exposure, and not merely by an increase of temperature as in the conventional heating. This microwave reaction is called “non-thermal effect”, and it is widely discussed in literature.[1,2]

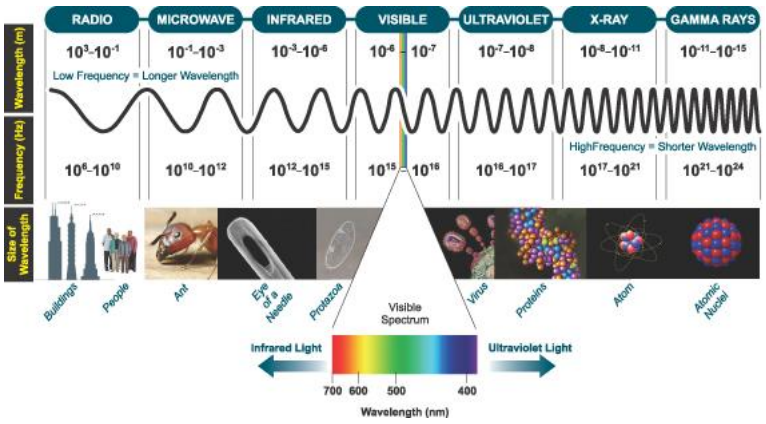


Fig.4 Electromagnetic spectrum.

Microwaves are electromagnetic radiations that range from 300 to 300 000 MHz on the electromagnetic spectrum. For this reason, they do not possess enough energy to break, rotate or to strongly vibrate chemical bonds, but they allow molecules to orientate their permanent dipolar moment parallel toward the applied electric field. In a simplified description, we can state that molecules with dipolar moment will try to realign themselves with the electric field they are continuously submitted to, that is oscillating. This generated motion turns into an increase of single molecules kinetic energy, which is transferred to the system as heat. Accordingly, we can state that microwaves induce heating that generates from "inside" the reaction mixture rather than from "outside", as in a conventional heating.

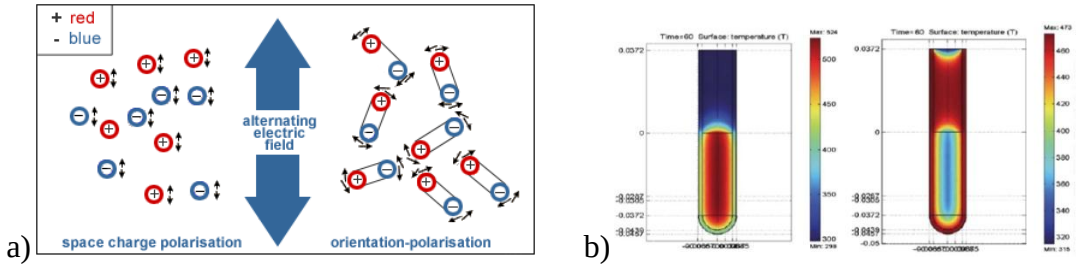
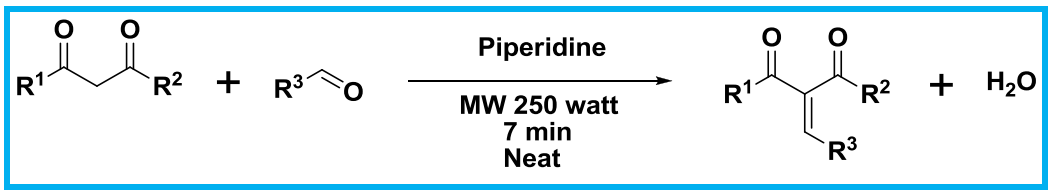


Fig.5 a) Orientation of molecules with a dipolar moment subjected to an alternating electric field and b) difference between dipolar and conventional heating.

1.2 Preparation of Alkylidene Malonates and Acetoacetates

As we said before, alkylidenes represent excellent substrates able to take part in reactions we are very interested in. They may be involved in different kind of reactions and, in particular, two of them are significant for us: the Michael addition of nucleophile and the allylic nucleophilic substitution (S_N2').

First of all, we have developed a methodology based on the Knoevenagel reaction to obtain the desired alkylidenic compounds. This reaction has been widely studied under a variety of conditions, by changing solvent and introducing a base or a Lewis acid as catalyst. In addition, we combined the Knoevenagel reaction with a new and efficient technology as MAOS.



Scheme 1. Synthetic route used to obtain alkylidene malonates and acetoacetates.

Alkylidene malonates and acetoacetates are prepared by using an excess of the proper aldehyde in respect to methylene active compound (malonate or acetoacetate), in the presence of 15% of piperidine under microwave irradiation for 7 minutes (Scheme 1). Mixtures of E and Z isomers (1/4) were obtained in good yields after work up performed by extraction with EtOAc and water.

The mixture of isomers was easily separated through flash chromatography and the single isomers were stored in a freezer to prevent isomerization.

Following this method, alkylidene malonates and acetoacetates showed in Fig. 6 were synthesized and used for several purposes, which will be taken into consideration later in this thesis. The stereochemical assignment for compounds **1a-l** was realized on the basis of DPFGSE-NOE (Double Pulse Field Gradient Spin Echo NOE) experiments. We found that the major isomer (Z) exhibits a strong

NOE effect between the vinyl hydrogen and the ketone CH_3 group, while the minor isomer (E) shows a strong NOE effect between the vinyl hydrogen and the ester chain.

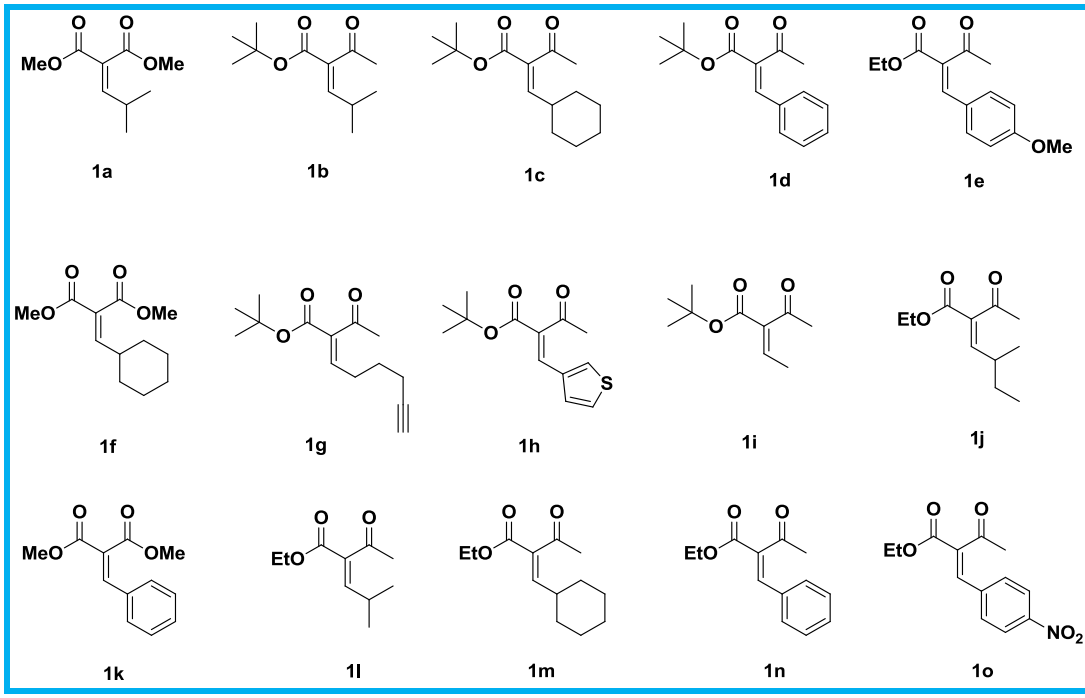
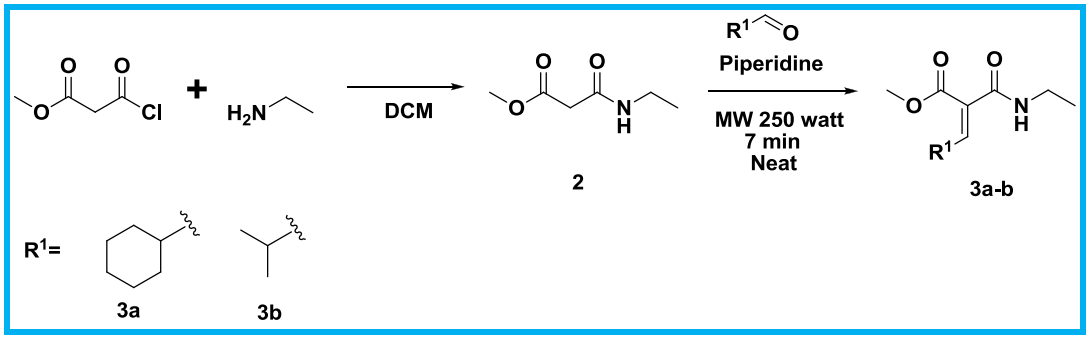


Fig. 6 Alkylidene malonates and acetoacetates synthesized through Knoevenagel reaction under microwave irradiation.

1.3 Preparation of Alkylidene Malonamides and Acetoacetoamides.

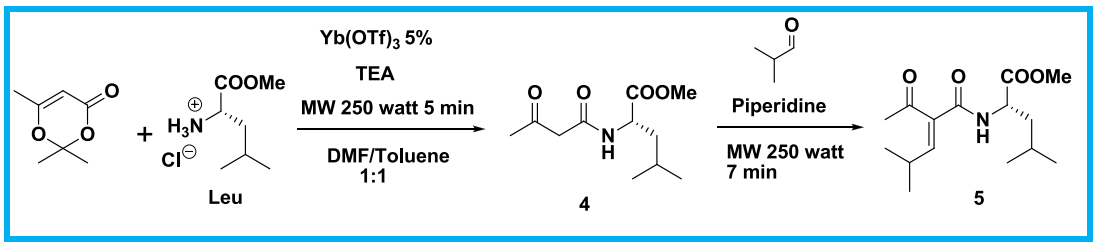
The alkylidene amides that result from the condensation among aldehyde, respective malonamides and acetoacetoamides are undoubtedly another important class of molecules that can be used as a potential starting material for synthetizing heterocyclic scaffolds. Regarding a synthesis of alkylidene malonamides, we started from methyl malonyl chloride, which, reacting with an amine, ethylamine in particular, afforded the corresponding amide through nucleophilic acylic substitution. The following Knoevenagel reaction with a proper aldehyde under

microwave assisted condition and in the presence of a catalytic amount of piperidine (15% mol), provided the corresponding alkylidene malonamides in satisfactory yields (Scheme 2).



Scheme 2. Synthetic route to synthesize alkylidene malonamides.

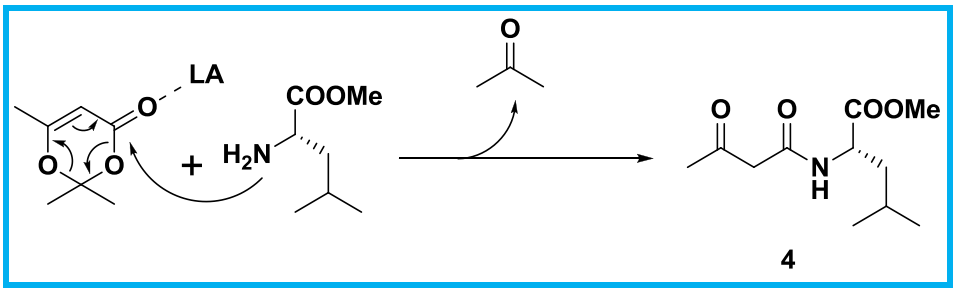
On the other hand, to obtain alkylidene acetoacetoamides we followed a different approach. Starting from 2,2,6-trimethyl-4H-1,3-dioxin-4-one, a masked acetoacetate, and Leucine as amine, by means of Ytterbium triflate as catalyst and TEA, we obtained acetoacetoamides with good yield. The reaction was performed under microwave irradiation for 5 minutes. The presence of triethylamine is fundamental to relieve the Leucine from salt. The obtained acetoacetoamide was then once again irradiated for other 7 minutes after adding isobuthyraldehyde and piperidine in the same vessel in order to obtain the desired unsaturated compound in good yield (Scheme 3).



Scheme 3. Synthetic route to synthesize alkylidene acetoacetoamides.

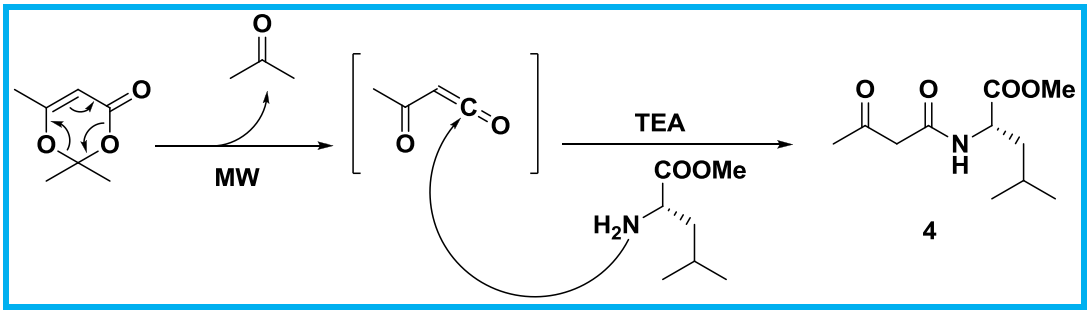
The raction time for the Knoevenagel condensation is very short, and that is also true for the same reaction carried out on the previously treated malonic esters and acetoacetate derivatives. This consideration let us state that the reported method is quite reproducible on different substrates.

Concerning the one-pot-two-steps protocol for the preparation of alkylidene acetoacetamides, there are some dubts on the effective mechanism followed in particular in the first step, in the opening of the heterocycle. The mechanism hypothesized by us, envisage a direct attack of amine on carbonilic carbon activated by Lewis acid and subsequently rearrangement with loss of acetone (Scheme 4).



Scheme 4.

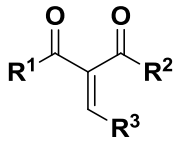
In literature a different mechanism is reported were the first loss of acetone obtained by heating, provides a very reactive ketene that undergoes a nucleophilic attack by amine to get the final amide (Scheme 5).



Scheme 5

To better understand the real mechanism we tried to trap the ketene through a 2+2 cycloaddition, using for instance benzylketimine, but we never found any trace of products deriving from reactions involving ketene. This attempt allows to suggest that the correct mechanism could be the one proposed by us.

1.4 Experimental section



General procedure for the preparation of α,β-unsatured compounds 1a-o: Aldheyde (1,3eq), piperidine (0,15eq) and methylene active compound (1eq) were irradiated with microwaves for 7 minutes at 250 watt in neat conditions. The reaction mixture was then diluted with ethyl acetate and washed 3 times with water. The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds Z and E were separated by flash chromatography on silica gel (c-Hex/EtOAc 95:5). The desired product was obtained in range yield of 60-90% with relative Z/E ratio depending from substrate.

*For **1i** and **1g** the same procedure was performed at room temperature because the aldehyde is very volatile.

1a: ^{1}H-NMR (400 MHz, $CDCl_3$) δ 6.85 (d, J= 10.8 Hz, 1H), 3.80 (s, 3H), 3.76 (s, 3H), 2.60-2.73 (m, 1H), 1.08 (d, J= 6.6 Hz, 6H).

1b:^{1} H-NMR (400 MHz, $CDCl_3$) *Z isomer*: δ 1.09 (d, 6H, J = 6.45 Hz), 1.55 (s, 9H), 2.30 (s, 3H), 2.65 (m, 1H), 6.53 (d, 1H, J = 10.4 Hz); LC-MS rt 9.5 min, m/z : 235; yield 80%.

E isomer: δ 1.45 (d, 6H, J = 6.6 Hz), 1.51 (s, 9H), 2.34 (s, 3H), 2.60 (m, 1H), 6.58 (d, 1H, J = 10.6 Hz); LC-MS rt 10.2 min, m/z 235; yield 20%.

1c: : *Z isomer*: ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21-1.43 (m, 5H), 1.45 (s, 9H), 1.60-1.77 (m, 5H), 2.31 (s, 3H), 2.37 (m, 1H), 6.63 (d, 1H, *J* = 9.8 Hz); GC-MS rt 9.3 min, *m/z*: 252yellow oil; isolated yield 55%

E isomer: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.14-1.26 (m, 5H) 1.45 (s, 9H), 1.66-1.77 (m, 5H), 2.38 (s, 3H), 2.42 (m, 1H), 6.72 (d, 1H, *J* = 10.6 Hz). GC-MS rt 10.0 min, *m/z*: 252, yellow oil; isolated yield 23%.

1d: *Z isomer*:; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.45 (s, 9H, O*tBu*)2.43 (s, 3H, COCH_3), 7.28-7.48 (m, 5H, Ph), 7.58 (s, 1H, CH=). Yellow oil. Yield 72%.

E isomer: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.45 (m, 9H, O*tBu*), 2.36 (s, 3H, COCH_3), 7.34-7.39 (m, 5H, Ph), 7.68 (s, 1H, CH=). Yield 23%;

1e: *Z isomer*: IR (film) ν/cm^{-1}2980, 2936, 2841, 1726, 1655, 1601, 1513, 1452, 1260, 1222, 1176, 1028. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.33 (t, 3H, *J* = 7.2 Hz), 2.44 (s, 3H), 3.88 (s, 3H), 4.40 (q, 2H, *J* =7.2 Hz), 6.93 (d, 2H, *J* = 7.2 Hz), 7.46 (d, 2H, *J* = 7.2 Hz), 7.50 (s, 1H); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.0, 26.2, 55.2, 61.4, 114.2(2), 125.0, 131.6(2), 132.1, 140.9, 161.6, 168.1, 194.6. GC-MS rt 20.30 min, *m/z*: 248(100), 233(80), 217(21), 203(30), 189(8), 174(24), 161(55), 137(18), 117(9), 89(12). Yellow oil; isolated yield 50%.

E isomer: IR (film) ν/cm^{-1}2979, 2935, 2841, 2739, 1700, 1601, 1577, 1512, 1315, 1258, 1174, 1026. ^{1}H NMR (300 MHz, $CDCl_3$) δ1.31 (t, 3H, *J* = 7.2 Hz), 2.39 (s, 3H), 3.89 (s, 3H), 4.27 (q, 2H, *J* =7.2 Hz), 7.00 (d, 2H, *J* = 7.2 Hz), 7.61 (s, 1H), 7.83 (d, 2H, *J* = 7.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ14.1, 26.5, 55.4, 61.6, 114.4(2), 125.5, 131.7(2), 132.3, 140.3, 161.5, 168.3, 194.6; GC-MS rt 20.20 min, *m/z*: 248(100), 233(88), 217(26), 203(35), 189(12), 174(29), 161(76), 137(31), 117(17), 103(10), 89(31). Yellow oil; isolated yield 21%.

1f: *Z isomer*: ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21-1.43 (m, 5H), 1.60-1.77 (m, 5H), 2.31 (s, 3H), 2.37 (m, 1H), 3.82 (s, 6H), 6.63 (d, 1H, *J* = 9.8 Hz); GC-MS rt 9.3 min, *m/z*: 252yellow oil; isolated yield 63%

E isomer: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.14-1.26 (m, 5H), 1.66-1.77 (m, 5H), 2.38 (s, 3H), 2.42 (m, 1H),3.83 (s, 6H), 6.72 (d, 1H, *J* = 10.6 Hz). GC-MS rt 10.0 min, *m/z*: 252, yellow oil; isolated yield 20%.

1g:see chapter 3 because this product was obtained at room temperature to avoid side product.

1h: *Z isomer*: ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.30 (s, 3H), 7,2-7,6 (m, 3H), 8.27 (s, 1H).

E isomer: ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.32 (s, 3H), 7,2-7,6 (m, 3H), 8.25 (s, 1H).

1i: *Z isomer*: ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.10 (d, J= 7.2 Hz, 3H), 2.30 (s, 3H), 7,10 (q, J= 10.4 Hz, 1H). Yield 20%.

E isomer: ; ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.45 (s, 9H), 2.10 (d, J= 7.2 Hz, 3H), 2.30 (s, 3H), 7,20 (q, J= 10.4 Hz, 1H). Yield 5%.

1j: *Z isomer*: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (t, *J* = 6.9 Hz, 3H, CH_3CH_2CH), 0.97 (d, *J* = 6.6 Hz, 3H, $CHCH_3$), 1.22 (t, *J* = 7.2 Hz, 3H, CH_3CH_2O), 1.34 (m, 2H, CH_3CH_2CH), 2.21 (s, 3H, $COCH_3$), 2.35 (m, 1H, CH_3CH_2CH), 4.20 (q, *J* = 7.2 Hz, 2H, CH_3CH_2O), 6.50 (d, *J* = 10.8 Hz, 1H, CH=). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.5(CH_3), 13.9(CH_3), 19.3(CH), 26.5(CH_2), 29.1(CH_3), 36.3(CH_3), 60.9(CH_2), 136.0(C), 152.8(CH), 166.4(C), 195.0(C). Colorless clear oil. Yield 66%.

E isomer: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.74 (t, *J* = 6.9 Hz, 3H, CH_3CH_2CH), 0.93 (d, *J* = 6.9 Hz, 3H, $CHCH_3$), 1.20 (t, *J* = 7.2 Hz, 3H, CH_3CH_2O), 1.34 (m, 2H, CH_3CH_2CH), 2.25 (s, 3H, $COCH_3$), 2.35 (m, 1H, CH_3CH_2CH), 4.16 (q, *J* = 7.2 Hz, 2H, CH_3CH_2O), 6.56 (d, *J* = 10.8 Hz, 1H, CH=). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.6(CH_3), 13.9(CH_3), 19.2(CH), 26.3(CH_2), 29.0(CH_3), 35.4(CH_3), 61.0(CH_2), 134.5(C), 153.0(CH), 164.1(C), 201.2(C). Anal. Calcd. For $C_{11}H_{18}O_3$: C, 66.64; H, 9.15. Found: C, 66.72; H, 9.14.

1k: : *Z isomer*:; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.77 (s, 6H), 7.28-7.48 (m, 5H, Ph), 7.58 (s, 1H, CH=). Yield 73%.

E isomer: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 3.77 (s, 6H), 7.34-7.39 (m, 5H, Ph), 7.68 (s, 1H, CH=). Yield 15%.

1l: *Z isomer*: IR (film) ν/cm^{-1} 2967, 2936, 2872, 1731, 1698, 1670, 1467, 1321, 1258, 1212, 1036. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.07 (d, 6H, *J* = 6.6 Hz), 1.31 (t, 3H, *J* = 7.4 Hz), 2.30 (s, 3H), 2.65 (m, 1H), 4.29 (q, 2H, *J* =7.4 Hz), 6.60 (d, 1H, *J* = 10.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 21.9 (2), 26.8, 29.6, 61.2, 134.9,

154.0, 166.6, 195.4; GC-MS rt 11.74 min, *m/z*: 184(2), 138(100), 123(60), 110(15), 96(57), 81(24), 67(32), 55(26). Yellow oil; isolated yield 61%.

E isomer: IR (film) ν/cm^{-1} 2965, 2933, 2872, 1705, 1638, 1467, 1367, 1239, 1200, 1051. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.02 (d, 6H, *J* = 6.6 Hz), 1.30 (t, 3H, *J* = 7.4 Hz), 2.37 (s, 3H), 2.62 (m, 1H), 4.24 (q, 2H, *J* =7.4 Hz), 6.69 (d, 1H, *J* = 10.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 13.9, 21.6, 21.8, 26.5, 29.4, 61.1, 134.8, 154.0, 166.5, 197.9; GC-MS rt 11.45 min, *m/z*: 184(2), 138(100), 123(55), 110(15), 96(64), 81(26), 67(31), 55(19). Yellow oil; isolated yield 26%.

1m: *Z isomer*: %; IR (film) ν/cm^{-1}2981, 2928, 2852, 1731, 1698, 1673, 1635, 1448, 1380, 1305, 1210, 1150, 1035. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21-1.43 (m, 5H), 1.34 (t, 3H, *J* = 7.0 Hz), 1.60-1.77 (m, 5H), 2.31 (s, 3H), 2.37 (m, 1H), 4.30 (q, 2H, *J* =7.0 Hz), 6.63 (d, 1H, *J* = 9.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 25.1(2), 25.3, 26.7, 31.7, 31.8, 39.1, 61.0, 135.2, 152.5, 166.6, 197.9. GC-MS rt 9.3 min, *m/z*: 225, yellow oil; isolated yield 55%

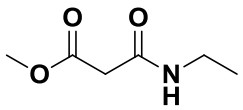
E isomer: IR (film) ν/cm^{-1} 2927, 2853, 2360, 2342, 1701, 1654, 1637, 1448, 1274, 1253. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.14-1.26 (m, 5H), 1.27 (t, 3H, *J* = 7.0 Hz), 1.66-1.77 (m, 5H), 2.38 (s, 3H), 2.42 (m, 1H), 4.26 (q, 2H, *J* =7.0 Hz), 6.72 (d, 1H, *J* = 10.6 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 25.0 (2), 25.6, 31.3, 31.9 (2), 38.3, 61.0, 133.9, 152.8, 164.7, 201.3. GC-MS rt 10.0 min, *m/z*: 225, yellow oil; isolated yield 23%;

1n: *Z isomer*:; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.28 (t, *J* = 7.2 Hz, 3H, CH_3CH_2O), 2.43 (s, 3H, $CO\mathit{CH_3}$), 4.34 (q, *J* = 7.2 Hz, 2H, $CH_3\mathit{CH_2}O$), 7.28-7.48 (m, 5H, Ph), 7.58 (s, 1H, CH=). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 13.8(CH_3), 26.5(CH_3), 61.7(CH_2), 128.8 (CH), 129.5(CH), 130.7(CH), 132.9(C), 134.6(C), 141.2(CH), 167.7(C), 194.6(C). Yellow oil. Yield 72%.

E isomer: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.34 (t, *J* = 7.2 Hz, 3H, CH_3CH_2O), 2.36 (s, 3H, $CO\mathit{CH_3}$), 4.30 (q, *J* = 7.2 Hz, 2H, $CH_3\mathit{CH_2}O$), 7.34-7.39 (m, 5H, Ph), 7.68 (s, 1H, CH=). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.1(CH_3), 31.1(CH_3), 61.5(CH_2),

128.8 (CH), 129.6(CH), 130.3(CH), 132.8(C), 134.0(C), 140.4, (CH), 164.3(C), 203.3(C). Anal. Calcd. For $C_{13}H_{14}O_3$: C, 71.54; H, 6.47. Found: C, 71.59; H, 6.48. isolated yield 23%;

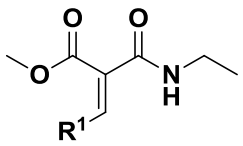
1o: *Z isomer*:; ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.20 (t, *J* = 7.2 Hz, 3H, CH_3CH_2O), 2.38 (s, 3H, $COCH_3$), 4.27 (q, *J* = 7.2 Hz, 2H, CH_3CH_2O), 7.50 (d, J= 8.4 Hz, 1H, Ph), 7.56 (d, J= 8.4 Hz, 1H,Ph), 7.62 (s, 1H, CH=), 8.16 (m, 2H). Yellow oil. Yield 80%.



2

Procedure for the preparation of 2: Under inert conditions, were added ethylamine (1.2eq), triethylamine (2eq) and THF (1M). The reaction mixture, maintained under stirring, was cooled to a temperature of 0°C, and then proceed with the gradual addition of methyl malonylchloride (1eq). The reaction was carried at room temperature maintaining the stirring for 24 h. His course was followed by TLC. The work-up was performed concentrating the THF and performing extractions with H_2O/DCM. The recovered organic phase was dried with Na_2SO_4, concentrated and dried by vacuum pump. Yellow oil. Yield 79%.

2: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.18 (t, 3H, J=8 Hz), 3.35 (m, 4H), 3.78 (s, 3H), 7.25 (bs, 1H);



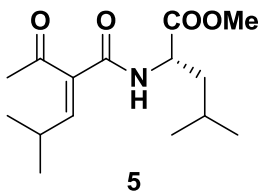
3a-b

Procedure for the preparation of 3a-b: Methyl-3-(ethylamino)-3-ossopropanoato (1eq), proper aldehyde (6eq) and piperidine (15%) were added to perform a

reaction to microwave, setting the instrument to a power of 250W for 7 min. The work-up was performed by extraction with H_2O/AcOEt. The recovered organic phase was dried with Na_2SO_4, concentrated and dried by vacuum pump. Yellow oil. The crude was subjected to purification by flash chromatography on silica gel with eluent 8:2 c-Hex/AcOEt in order to separate the two geometric isomers that are obtained. The isomer (E) appears as a yellow solid, while the isomer (Z) appears as a white solid. The total yield, calculated with respect to methyl malonylchloride not having performed in any previous step purification, amounts to 79%.

3a: ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.2 (t, 3H, J=3.7 Hz), 1.75 (m, 11H), 3.4 (m, 2H), 3.78 (s, 3H), 6.98 (d, 1H, J=5 Hz, isomero (E));

3b: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.04 (d, 6H, J=6.8 Hz), 1.15 (t, 3H, J=7.6 Hz), 3.00 (m, 1H), 3.33 (m, 2H), 3.81 (s, 3H), 6,93 (d, 1H, J=10,8 Hz, isomero (Z)), 7.24 (d, 1H, J=5.2 Hz, isomero (E)), 7.73 (bs, 1H).



5

Procedure for the preparation of 5: In a flask were added in order L-Leucine methyl ester (2,76 mmol, 1eq) or HN-Gly-OtBu (1eq), 10 ml of toluene, 10 ml of DMF, 1eq of 2,2,6-trimethyl-1,3-dixin-4-one, 1eq of TEA and 5% of $Yb(OTf)_3$. The mixture was irradiated by microwave at 250 watt for 5 min with strirring bar. After cooling of the mixture were added 1.78ml (7eq) of isobutyraldehyde and submitted another time under microwave irradiation for 30 min at 150 watt. The work-up was performed by extraction with H_2O/AcOEt. The recovered organic phase was dried with Na_2SO_4, concentrated and dried by vacuum pump. Brown oil. The crude was subjected to purification by flash chromatography on silica gel with

eluent 6:4 c-Hex/AcOEt in order to separate the two geometric isomers that are obtained.Yield 41% over two steps.Z/E ratio 9/1. E-isomer not isolated.

Z-5: ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.97 (δ, 6H, J= 6Hz), 1.11 (d, 6H, J= 6.8), 1.65 (m, 1H, J= 6.8 Hz), 1.73 (m, 1H, J= 6.8 Hz), 1.74 (m, 1H, J= 6.8), 2.37 (s, 3H), 3.16 (m, 1H, J= 3.2), 3.75 (t, 3H, J= 2.4 Hz), 4.71 (m, 1H, J= 5.2Hz), 6.71 (d, 1H, J= 10Hz), 7.10 (br d, 1H, J= 7.6Hz). ^{13}C NMR ($CDCl_3$, 100 MHz): δ 198.1, 173.1, 165.1, 158.3, 135.1, 52.2, 50.8, 41.2, 29.1, 26.9, 24.9, 22.8, 22.1, 21.8.HPLC-MS: rt 8.9 min, m/z 284 (M+H).

Chapter 2

Reactivity of alkylidene derivatives

2.1 Introduction

In this second chapter, we will examine the most important reactions performed starting from alkylidene malonates, acetoacetates and malonamides, that afforded the polyfunctionalized molecules with potential bioactivity discussed in the next chapter. In this context, we have explored three main routes, leading to different scaffolds for bioactive derivatives.

The ways that we have followed are:

- the Michael conjugate addition with *N*,*O*-Bis(trimethylsilyl)hydroxylamine, used to obtain isoxazolinic and isoxazolidinonic compounds,
- the same reaction with several indole derivatives to get a library of β-tryptophan-like molecules,
-the Luche reduction of alkylidene derivatives to allylic alcohols and the following nucleophilic allylic substitution as steps to achieve a cyclic β-dehydropyrrolic derivative.

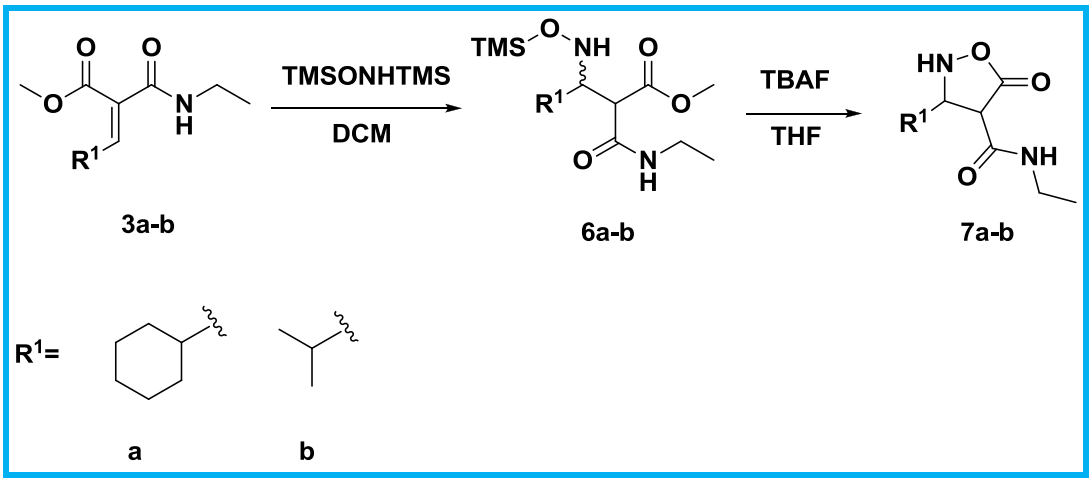
Later on, specific reactivity of alkylidene derivatives in the above mentioned reactions will be discussed individually.

2.2 Michael conjugate addition

From a biological and pharmaceutical point of view, the1,4-Michael addition is one of the most used reactions to obtain interesting compounds. In literature there are several synthetic procedures to carry out this kind of reaction. Over the last few years, in our research group, methods to synthesize isoxazolines[3] and isoxazolidinones[4] have been developed starting from alkylidene acetoacetates and malonamides respectively.

2.2.1 Michael conjugate addition on alkylidene malonamides

Alkylidene malonamides are starting materials used in the synthesis of isoxazolidinones. In this reaction, the nucleophilic attack of TMSONHTMS to electrophilic position of the unsaturated alkene allowed to obtain 1,4-adducts that, after treatment with TBAF, gave the final isoxazolidinone through intramolecular acyl substitution. It is interesting to note that during the reaction work-up of the Michael reaction, the loss of trimethylsilyl group on nitrogen spontaneously occurred.



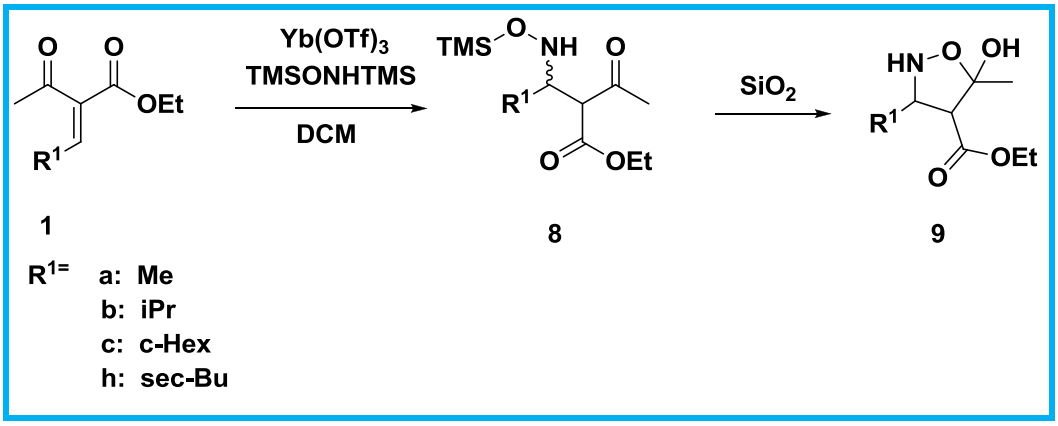
Scheme 6. Synthetis of isoxazolidinones through Michael addition.

Differently from the reaction already carried out on alkylidene acetoacetates, we observed that the conjugate addition on alkylidene malonamides proceeds also without catalyst. Preliminary tests have demonstrated that the presence of the catalyst does not afford benefit to the correct reaction course but, on the contrary, favors quick degradation of the products in the reaction vessel.

2.2.2 Michael conjugate addition on alkylidene acetoacetates

The same procedure seen for alkylidenes malonamides, was applied to alkylidene acetoacetates in order to obtain isoxazolines. The reaction was performed this time in the presence of a catalyst. As our research group recently published, the Lewis acid ($Yb(OTf)_3$) employed in the reaction allows to reduce the overnight reaction time by 1-2 hours depending on the substrate, and it directs the regioselectivity of the reaction.[5] In fact, the initial alkylidene, depending on the reaction conditions, may be subjected either to 1,4- or 1,2-nucleophilic attack, thus providing respectively the 1,4- addition product desired, the 5-hydroxyisoxazolidine-4-carboxylate, or the oxime.

For these reasons, reaction conditions were previously optimized in our research group to induce the regioselective formation of 1,4-addition products versus oximes deriving from the 1,2-addition process. On the basis of this study, we carried out the following syntheses using the catalyst in 5% molar amount to limit the oxime formation. The resulting adduct spontaneously rearranges to give the isoxazolidine **9**, a scaffold that was used to obtain the integrin ligands discussed in chapter 3.



Scheme 7. Synthetis of isoxazolines through Michael addition.

2.3 Synthesis of dehydro-pyrrole scaffold

2.3.1 Luche reduction

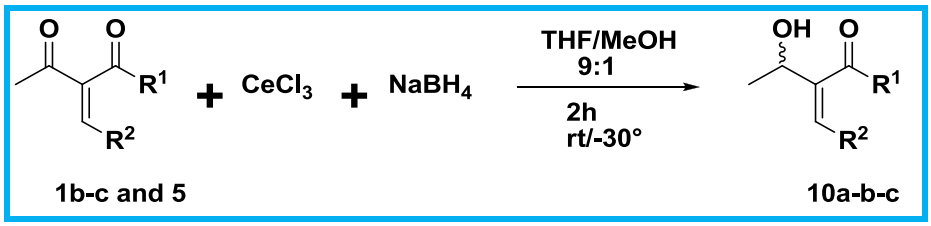
The Luche reduction is a highly regioselective 1,2-reduction of unsaturated ketones, aldehydes, or enones and it is very useful to selectively reduce the carbonyl moiety is the presence of a double bond. In fact, this reaction is able to transform α,β-unsatured compounds into allylic alcohols by using $NaBH_4/CeCl_3$ in alcoholic solvent.

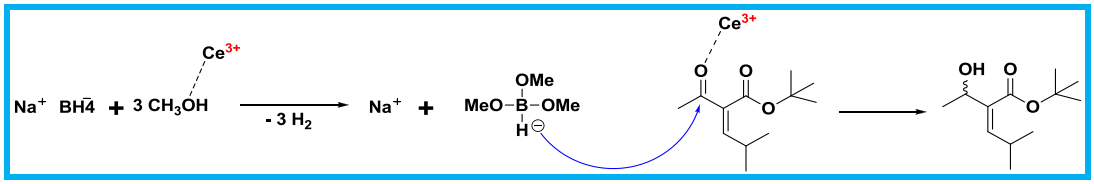
$NaBH_4$ is the main reagent in the reaction, and it is responsible for adding a hydride to the carbonyl group. It is a soft nucleophile and the selective reduction of the carbonyl group requires a harder nucleophile to get a 1,2 addition.

The role of lanthanoid chloride $CeCl_3$ is to transform the reducing agent from a hydrogen-based reducing agent to a –OR group based compound. This hardens the reducing agent, making it more likely to add a hydride specifically to the carbonyl group.

An alcohol solvent (methanol or ethanol) provides the hydrogen atom necessary to add onto the alkoxide group after that reducing agent has added hydride to the carbonyl group. The alcohol solvent is activated by the Ce^{3+} ion, making its proton very acidic, which then comes off and is added to the oxygen atom of the ketone.

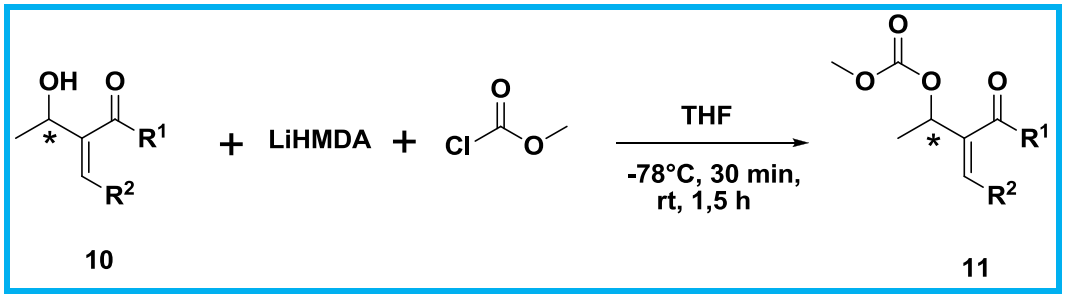
The effective reaction mechanism is still not very clear but Luche himself has suggested the following hints: in summary, Ce^{3+} catalyzes the transformation of the reducing borohydride by the alcohol solvent and also activate the latter, making it easier for the oxygen of the carbonyl group to pick up the hydrogen atom from alcohol. The novel reducing agent adds a hydride to the carbon atom of the carbonyl group, creating an alkoxide. The alkoxide then is protonated by the activated alcohol solvent, completing the reduction of the carbonyl to an alcohol. In such a way, we can justify the reduction selectivity with the HSAB theory. However, for further precautions, the reaction was carried out at low temperature to avoid side attacks on the present double bond.





Scheme 8. Luche reduction and relative mechanism.

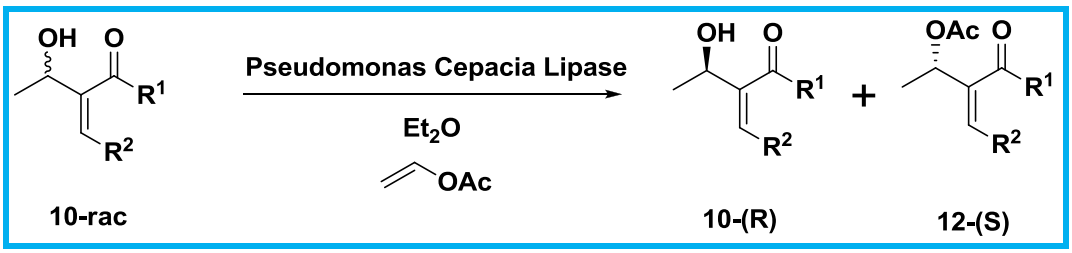
Therefore, from the alkylidene acetoacetates and acetoacetoamides we obtained the related racemic alcohols that we needed to transform to the corresponding enantiopure carbonates. To this purposes we performed an enzymatic resolution followed by treatment with methyl chloroformiate, as we will explain in the next section.



Scheme 9. Synthesis of carbonates enantiomerically pure starting from alcohol obtained by Luche reaction and solved by enzymatic resolution.

2.3.2 Kinetic enzymatic resolution

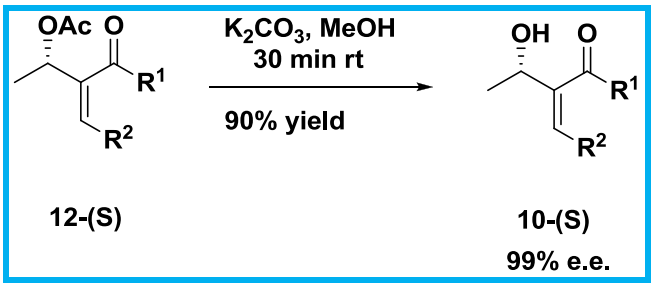
Following the procedure previously optimized in our research group (after enzyme and conditions screening),[6] the resolution of racemic mixtures of the allylic alcohols shown before have been performed using *Pseudomonas Cepacia lipase* (EC 3.1.1.3) as catalyst.[7]



Scheme 10. Kinetic enzymatic resolution of racemic alcohols deriving from Luche reduction.

The selected lipase proved to be very successful for the resolution of Z-alcohols, giving the acetylated compounds in good yields and high e.e., although in sluggish reaction times. Indeed, racemic alcohols were submitted to enzyme catalyzed acetylation employing vinyl acetate in diethyl ether at 30 °C for 30 hours. Under these conditions, both products were isolated in good yields and excellent enantiomeric excesses. The reaction can be monitored by ^{1}H-NMR and GC-MS, which allow the determination of the conversion. The racemic alcohols were analytically resolved into their enantiomers by chiral HPLC and were compared with the alcohols that derived from enzymatic resolution.

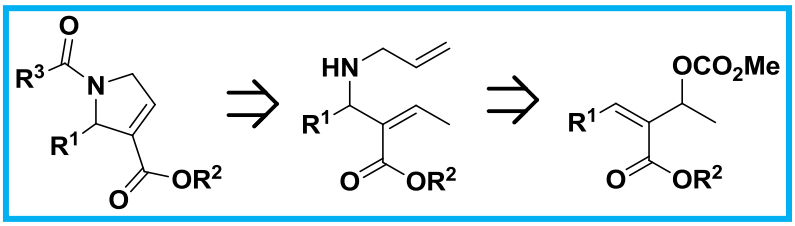
Acetates **12 (R)** were hydrolyzed to corresponding alcohols with a K_2CO_3 in methanol treatment (Scheme 11). Enatiomeric excesses of resulted alcohols were determined via HPLC by comparison with corresponding racemates. As we mentioned in the previous paragraph, enantiopure alcohols were transformed into the corresponding carbonates by treatment with methyl chloroformate (Scheme 9).



Scheme 11. Hydrolysis of acetates obtained by enzymatic resolution.

2.3.3 Nucleophilic allylic substitution (S_N2')

As illustrated in Scheme 12, the allylic carbonate can be used as intermediate for a synthesis of interesting heterocyclic scaffolds and, in particular, it is possible to utilize it to obtain a dehydropyrrole ring. The retrosynthetic pathway shows that, if the desired heterocycle can be synthesized by ring closing methatesis, the substrate for this reaction has to be prepared through a S_N2' reaction on the allylic carbonate. On the basis of our research group's experience, the racemic and enantiopure carbonates, that we have seen above, have been subjected to nucleophilic allylic substitution with allyl amine. The reaction was carried out in absence of catalyst or under Ir-catalyzed conditions.[8]

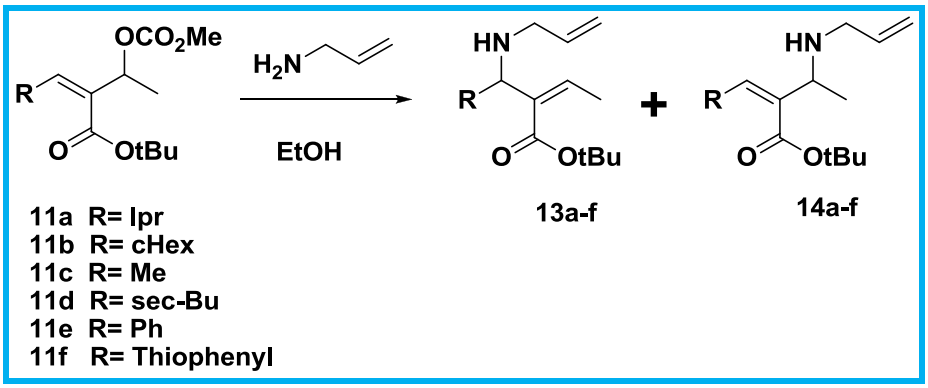


Scheme 12. Retrosynthetic route to synthesize optically active dehydropyrroles.

Recently, we observed that Iridium precursor and phosphate or phosphoramidite ligands combination have been described as excellent catalytic system with a number of different nucleophiles for allylic substitution.[9] Iridium complexes have been also successfully applied in the stereoselective formation of C-N bonds via

allylic amination,[10]– mostly on monosubstituted alkenyl carbonates,[11]– but they have been never applied to allylic carbonates such as compound **11**, which presents alkyl substituents on both terminals and a carboxylic group in the central position.

In an initial screening we observed that the choice of solvent and of the phosphorous ligand strongly influence the regioseletivity and the stereoselectivity. Among the other, we tested also ethanol as solvent because it has been reported[12] to favor the formation of the product deriving from S_N2' mechanism. Some selected results are shown in Table 2.



Scheme 13. Nucleophilic allylic substitution on racemic and enantiopure carbonates.

Reactions performed without any catalyst in refluxing acetonitrile (Table 2, entries 1-7) afforded good yields but only after three days and by using a large excess of amine (9 equivalents). Deriving from S_N2' mechanism, the regioisomer **13** was exclusively obtained, as 4/1 *Z*/E isomers mixture, and no traces of the product **14** could be detected. The enantiomeric excess of the initial carbonate was completely retained and enantiopure **13a-f** was obtained starting from the corresponding chiral carbonates (entries 2-5). When using ethanol as solvent in presence of Ir-phosphorous ligand as catalytic couple, we observed an increase of yield with regioselectivity retention. To confirm the good action of the combination between Ir and phosphourus ligand, we tried to perform the reaction only with ethanol and

then without phosphourus ligand. In both cases, the obtained results weren't comparable to those provided with Ir-phosphorous catalytic couple.

Entry	Carbonate	Solvent	Allylamine(eq)	Catalyst	Time	Yield[a] (%)	13:14
1	rac-Z-11a	MeCN	9	/	72	86	>99:1[b]
2	(S)-Z-11a[c]	MeCN	9	/	72	85	>99:1[b]
3	(R)-Z-11a[c]	MeCN	9	/	72	85	>99:1[b]
4	(S)-Z-11b[c]	MeCN	9	/	72	65	>99:1[b]
5	(R)-Z-11b[c]	MeCN	9	/	72	68	>99:1[b]
6	rac-Z-11e	MeCN	9	/	72	50	>99:1[b]
7	rac-Z-11f	MeCN	9	/	72	85	>99:1[b]
8	rac-Z-11a	THF	15	$Pd_2(dba)_3CHCl_3$	72	35	>99:1
9	rac-Z-11a	EtOH	3	$[Ir(COD)Cl]_2$(2%) $P(OPh)_3$(8%)	10	>99	>99:1
10	rac-Z-11a	EtOH	3	/	16	52	>99:1
11	rac-Z-11a	EtOH	3	$[Ir(COD)Cl]_2$(2%)	16	60	>99:1
12	rac-Z-11c	EtOH	3	$[Ir(COD)Cl]_2$(2%) $P(OPh)_3$(8%)	8	>99	>99:1[d]
13	rac-Z-11d	EtOH	3	$[Ir(COD)Cl]_2$(2%) $P(OPh)_3$(8%)	10	95	>99:1[e]
14	rac-Z-11e	EtOH	3	$[Ir(COD)Cl]_2$(2%) $P(OPh)_3$(8%)	10	>99	75:25
15	rac-Z-11f	EtOH	3	$[Ir(COD)Cl]_2$(2%) $P(OPh)_3$(8%)	10	90	60:40

Table 2.

[a] Yields were calculated after purification of the products by flash chromatography on silica gel. The unreacted carbonate **11** was completely recovered after work-up as pure Z-isomer.

[b] Compound **13** was always obtained as 4/1 mixture of Z/E isomers.

[c] Enantiomeric excess of substrates and products was determined by HPLC on chiral column (always >95% ee).

[d] In this case only the one product is formed for the symmetry of the molecule.

[e] Obtained as a 5/1 mixture of Z/E isomers, each as 1:1 diastereomeric mixture.

At this point it has been opportune to consider a possible screening of phosphorous ligands. The reaction was repeated on enantiopure (*S*)-**11a**. The "memory effect" of allylic substitution leading to conservation of the enantiomeric purity depends on

the rate of isomerization of Ir-allyl intermediates and the selection of the proper phosphorous ligand may influence the e.e. decrease.[13] Therefore, the reaction was repeated in the presence of different catalysts and the enantiospecificity (e.s. = $ee_{product}$ / $ee_{substrate}$) was determined (Table 3). The selected phosphoramidite ligands are reported in Figure 7.

As expected, all reactions are completely regioselective, affording exclusively compound **13a**. The enantiomeric excess of the product was determined by HPLC on chiral column and the enantiospecificity was calculated. The result, obtained without phosphorous ligand (entry 1), was quite similar to those obtained with triphenylphosphite or phosphoramidite **L2** (entries 2 and 4). The best result was observed for the reaction performed with ligand **L1**, that allowed to enhance the enantiospecificity up to 91%.

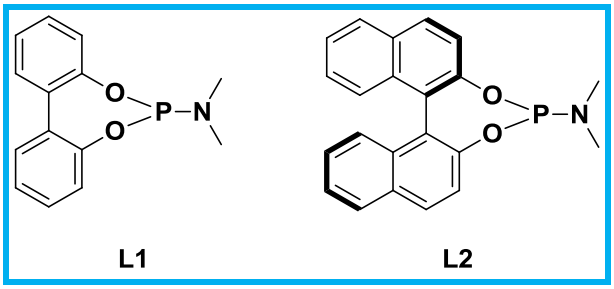


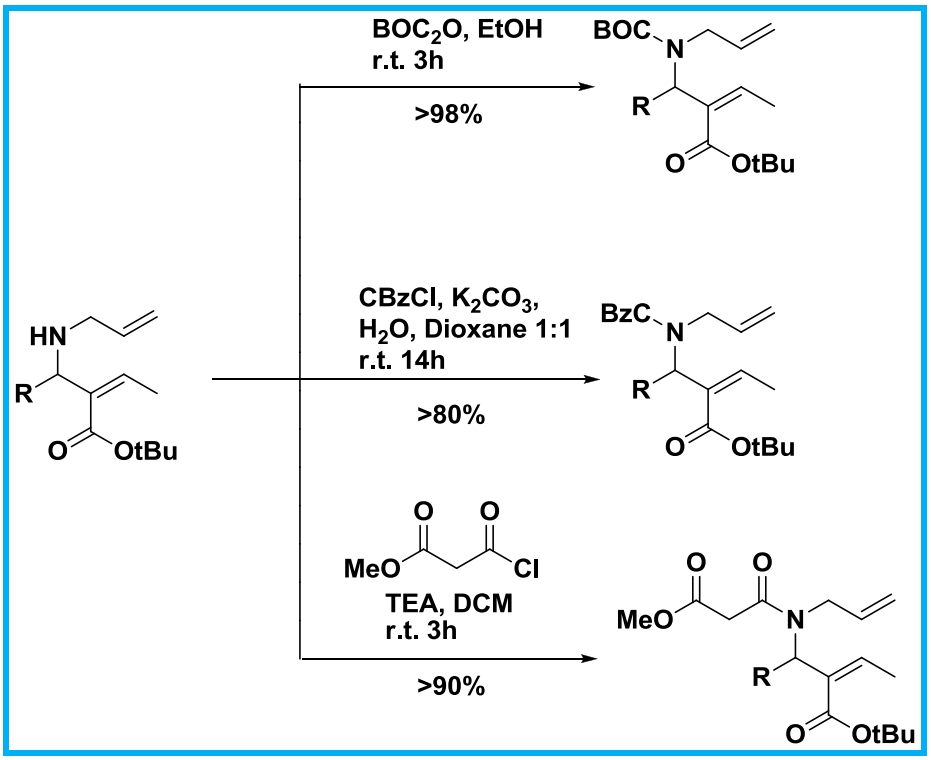
Fig. 7. Phosphorous ligands used to optimized the S_N2'.

Entry[a]	Carbonate	Lignd	(R)-Z-13a/(S)-E-13a
1	(S)-Z-11a	/	86/14
2	(S)-Z-11a	$P(OPh)_3$	87/13
3	(S)-Z-11a	L1	95/5
4	(S)-Z-11a	L2	87/13

Table 3. [a] Enantiomeric excess of substrates and products was determined by HPLC on chiral column.

2.3.4. Ring closing methatesis (RCM)

As previously suggested, ring closing methatesis (RCM) is useful method to synthesize cyclic scaffolds for potential biological or pharmaceutical applications. This reaction allows to obtain from 5- to 30-membered cyclic alkenes and the E/Z selectivity depends on the ring strain. The reaction proceeds in presence of a catalyst and most widely employed of them are Ruthenium catalysts shown in.



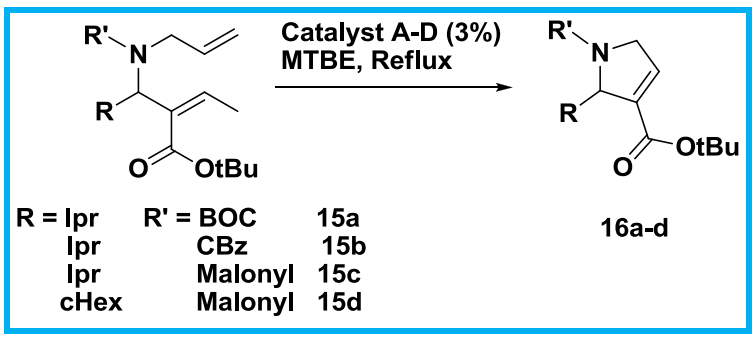
Scheme 14. Protecting group employed to perform RCM.

Exploiting the ability of this reaction to easily provide an unsatured ring, we thought to utilize the diene amine synthesized through the S_N2' reaction discussed above, to obtain 2,5-dihydropyrrole derivatives. Before performing RCM, we needed to protect the nitrogen, because it may poison the ruthenium catalyst by coordination of the nitrogen electron pair to the metal-alkylidene complex. Therefore, we decided to protect the nitrogen with three different N-protecting

groups in order to investigate the reactivity of these compounds and the large use that the RCM products could have. The employed protecting groups include an amide and two carbamates, as shown in scheme 14.

Taking the synthesis of dihydropyrrole derivatives into consideration as final target, we selected more easily cleavable protecting groups as –BOC and –CBz. Among the inserted protecting group, there is the N-malonamide derivative in order to use it as a useful intermediate for the preparation of bioactive integrin ligands.

This derivative was synthesize in good yield by treatment of starting amine with methylmalonyl chloride and TEA in DCM at rt for 3h. The N-BOC derivative was obtained after a treatment with BOC_2O in ethanol at rt for 3h in excellent yield and finally the synthesis of N-CBz derivative was performed using the corresponding chloride in a solution of starting amine and K_2CO_3 in water/dioxane (1:1 ratio) at rt overnight. The reaction has led to 80% yield after isolation.



Scheme 15. Ring closing methatesis on protected allylic amines.

We then turned our attention to RCM. To determine the best catalyst for our transformation we screened different commercially available compounds (Figure 8). A strong effect on catalyst efficacy is linked to steric hinderance of substrate and to the electronic nature of the olefins. When the steric bulk and the electron withdrawing character of the olefin substituents are combined in the same

substrate, the rate of RCM is strongly reduced and the metal-complex may decompose before reacting.[14]

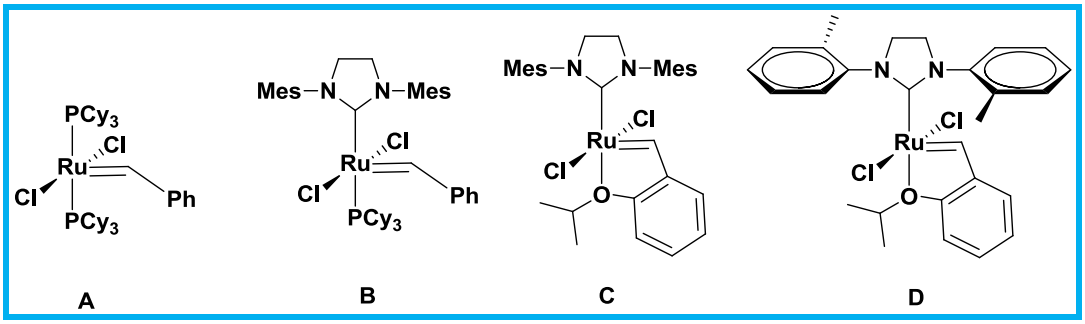


Fig. 8. Ruthenium catalysts employed.

In a recent Kuhn's work [15]it is shown to what extent methyl tertbutyl ether (MTBE) is the ideal solvent for the synthesis of 2,5-dihydropyrroles via metathesis reaction. Afterwards, we chose it for our reactions in presence of 3% of catalyst.

On both carbamates, catalyst A doesn't provide the product allowing the recovery of starting materials without any trace of the heterocyclic compound, even after long reaction times. On these grounds, catalyst A was not tested on the amide derivative. However, performing the reactions on the carbamates with second-generation Grubbs' catalyst B, cyclic products have been obtained quantitatively. The same reactivity is registered on the Z/E mixture of isomers and the complete transformation to the desired products was observed in 1,5-2h on the N-CBz and N-BOC compounds respectively. On N-CBz derivatives were also tested Hoveyda-Grubbs' second-generation catalysts C and D providing the dihydropyrrole compounds slightly faster. Comparing the results using catalyst B with the one obtained by employing the more expensive catalysts C and D, it is possible to see how there is a lack of significant improvement but for a few time. In a similar way, the reaction on malonamide was carried out with catalysts B,C and D. With the use of Hoveyda-Grubbs' second-generation catalysts C and D, quantitative yields of product have been obtained over a lapse of 12h of reaction whereas, by employing catalyst B, after 16h we recovered dihydropyrrole derivative only in 54% yield.

Entry	Starting Material	Catalyst	Time (h)	Yield
1	15a	A	16	/
2	15a	B	2	>98
3	15b	A	16	/
4	15b	B	1.5	>98
5	15b	C	1	>98
6	15b	D	1	>98
7	15c	B	16	54
8	15c	C	12	>98
9	15c	D	12	>98
10	15d	C	12	>98

Table 4. Ring closing methatesis on protected diene amines.

2.4 Multicomponent reactions

Nowadays, Multicomponent Reactions (MCRs) represent a novel and challenging frontier in synthetic organic chemistry. In this kind of processes three or more components react in the same vessel leading to the final product without the need of isolating any intermediate. Since the very beginning of their validation and application, MCRs proved to be a very useful tool for the synthesis of structurally complex molecules (in particular natural products) and in the field of drug discovery.

2.4.1 Yonemitsu-type trimolecular condensation

One kind of MCRs is the Yonemitsu Reaction by which differently substituted indoles, aldehydes and dicarbonylic compounds react to give tryptophan derivatives.[16] In the late seventies, Yonemitsu and coworkers reported a new trimolecular condensation among the Meldrum's acid, an indole and an aldehyde, which provided 3-substituted indols. During the following years and up to the present day, this reaction has been reinvestigated in order to overcome its limitations. In fact, replacing Meldrum's acid with another active methylene compound, which possess a different pK_a value, poor results could be obtained under standard conditions. As explained by the reaction sequence reported below, the Yonemitsu reaction consists in two different steps: first the formation of the

alkylidene derivative occurs through a Knoevenagel reaction and than it is followed by indole addition to the double bond by Michael conjugate addition. Each single step has been optimized by changing L.A., method, solvent, temperature and time.
In literature several examples are reported about this process but they present some negative aspects such as a stoichiometric amount of catalyst [17](using $TiCl_4$) or long reaction times and low yields. From these groundwork and our research group's past experience on microwave assisted reactions we have developed an improved protocol to obtain greater efficiency for the Yonemitsu-type trimolecular condensation.

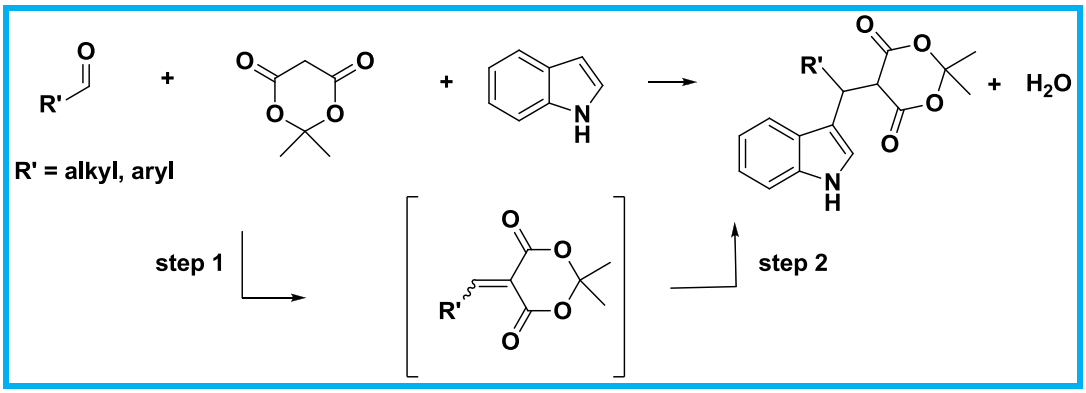
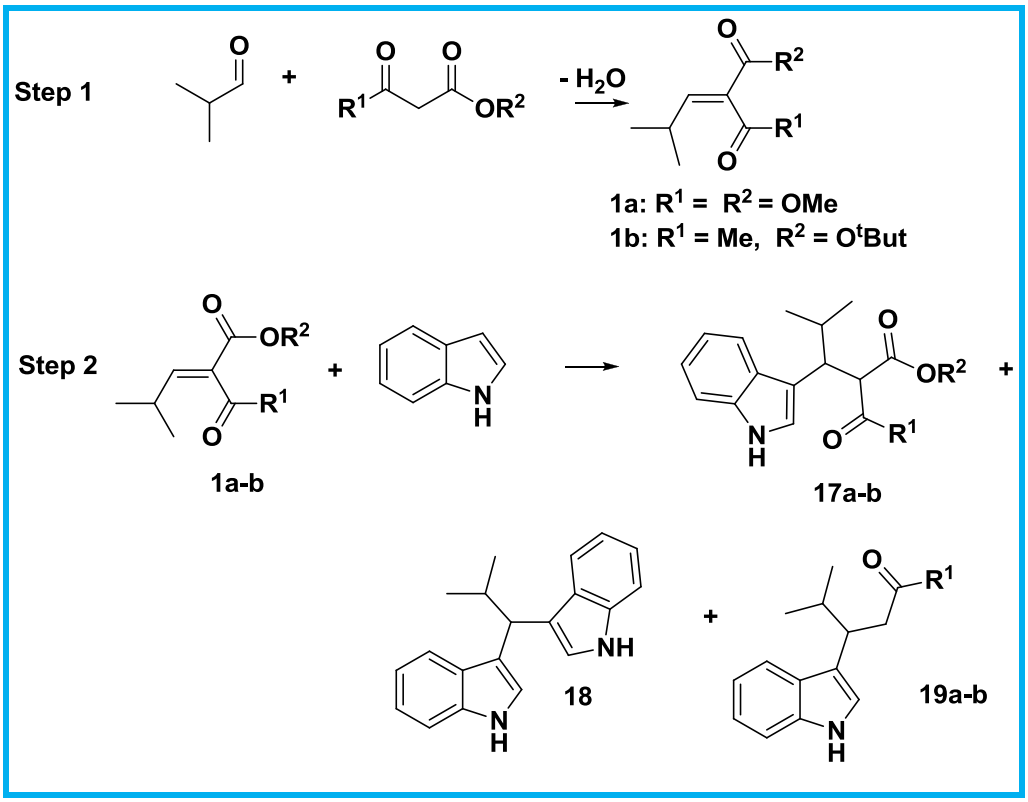


Figure 9. Knoevenagel reaction represented by steps.

The MCR was performed following a sequential protocol, by synthesizing the intermediates **1a-b** in few minutes using microwaves under solvent free conditions and adding in the same reaction vessel L.A., solvent and indole to obtain **17a-b**. For the reaction performed using acetoacetate derivative as starting material, we also tried to improve diasteroselectivity finding the best conditions.
To optimize conditions, we choose as stardard reaction the condensation of dimethyl malonate with isobutylaldehyde and indole (Scheme 16). Initially, we tried to perform a domino MCR by adding all reagents simultaneously in the microwave reactor and irradiating at 250W for 20 minutes without solvent. Under these conditions very dirty reaction crudes were always observed since a number of

condensations among reagents could occur, and for this reason we decided to optimize the reaction by searching the best conditions for each step.

The best conditions for the first step had been already discussed in a previous part of this thesis, and by following them, equimolar amounts of dimethyl malonate or acetoacetate were reacted with isobutyraldehyde, using 15% of piperidine as catalyst under microwave irradiation at 250W for 7 minutes. Alkylidene malonate **1a** or alkylidene acetacetate **1b** were obtained in 90% yield after isolation by flash chromatography.



Scheme 16. One pot-two steps Yonemitsu-type condensation.

We then focused our attention on the second step, that was initially performed on purified **1a.** Some selected results are reported in Table 1.

On the basis of previously reported methods, we choose to begin our screening by using $Yb(OTf)_3$ as catalyst in different solvents (entries 1-3). By using toluene or acetonitrile to dissolve reagents, side product **18** was mainly obtained. On the other hand, using DMF as solvent better results were obtained, achieving adduct **17a** in 41% yield. The formation of bis-indolic derivative **18** could be easily explained in the one pot reaction, where a double addition of indole to the aldehyde may be supposed, but is quite unexpected starting from alkylidene malonate. As already suggested by Gao and Wu,[18] the adduct **17** is probably converted into a reactive indolenine derivative[19] by the loss of the active methylene fragment, which reacts with another molecule of indole (Figure 10).

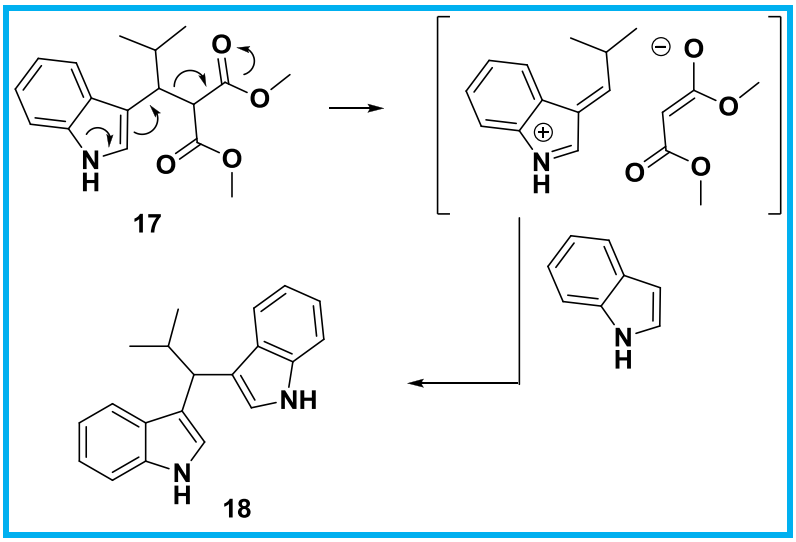


Fig. 10. Hypothetical formation mechanism of bis-indole compound starting from intermediate 2.

To select the optimal catalyst several L.A. have been screened. In the presence of $Cu(OTf)_2$, compound **17a** could be isolated in 51% yield, but evolution into compound **18** (21%) and decarboxylation to **19a** (5%) was always observed (entry 5). On the contrary, $Zn(OTf)_2$ was not an efficient catalyst affording a very low yield under the same conditions (entry 6). The reaction performed in the presence

of $Sc(OTf)_3$ afforded a satisfactory amount of product (entry 7), without presence of undesired bis-indolic derivative **18**. No advantages have been registered attempting to enhance the yield by changing microwave power (entries 8 and 9) or reaction times (entries 10-12), since an increase of side-products was always observed.

Entry	Reagent	Power (W)	Time (min)	Solvent	Lewis Acid	Yield **17**(%)[a]	Yield **18**(%)[a]	Yield **19**(%)[a]
1	**1a**	250	20	Toluene	$Yb(OTf)_3$	28	36	/
2	**1a**	250	20	DMF	$Yb(OTf)_3$	41	21	15
3	**1a**	250	20	CH_3CN	$Yb(OTf)_3$	6	29	/
4	**1a**	250	20	CH_3CN	$Sc(OTf)_3$	6	42	/
5	**1a**	250	20	DMF	$Cu(OTf)_2$	51	21	7
6	**1a**	250	20	DMF	$Zn(OTf)_2$	18	/	5
7	**1a**	250	20	DMF	$Sc(OTf)_3$	57	/	16
8	**1a**	150	20	DMF	$Sc(OTf)_3$	20	6	5
9	**1a**	300	20	DMF	$Sc(OTf)_3$	45	9	20
10	**1a**	250	20	DMF	$Sc(OTf)_3$	45	v	12
11	**1a**	250	20	DMF	$Sc(OTf)_3$	31	16	35
12	**1b**	250	20	DMF	$Yb(OTf)_3$	19	9	55
13	**1b**	250	20	DMF	$Cu(OTf)_2$	/	12	75
14	**1b**	250	20	DMF	$Zn(OTf)_2$	/	9	38
15	**1b**	250	20	DMF	$Sc(OTf)_3$	41	7	44
16[b]	**1b**	250	20	DMF	$Sc(OTf)_3$	30	5	16
17	**1b**	250	20	DMF	/	/	18	/

Table 5.Screening of Lewis acid in the second step in the Yonemitsu-type condensation.

With the same step by step approach, the reaction that use acetoacetate **1b** as starting material has been studied and it must be specified that this reaction has been less investigated than the corresponding on alkylidene malonate. This is probably due to the fact that, with acetoacetate, the newly formed adduct has two stereocenters and, unfortunately, maintainance of diastereocontrol is quite difficult

due to the strong acidity of the acetoacetic proton. Equilibration to a 60/40 diastereomeric mixture indeed often occurs by storing compounds at room temperature. For these reasons the conjugate addition of indole to alkylidene acetoacetate **1b** required milder conditions.

Skipping the first step discussed before[20] we focused our attention on the conjugate addition. Microwave irradiation at 250W for 20 minutes in the presence of Yb, Cu or Zn triflates always afforded complete conversion of the starting material mainly to bis-indole derivative **18** or decarboxylated adduct **19b** (entries 12-14). Only $Sc(OTf)_3$ allowed to isolate **17b** in 41% yield, together with an equal amount of **19b** (entry 15). Increasing the dilution from 1M to 0.1 M we registered similar results but a lower conversion of the starting material that was recovered in 50% amount (entry 16). When the reaction was performed without Lewis acid, the presence of 84% of starting material was observed in the crude mixture together with 16% of bis-indole compound **18**, but no traces of the adduct **17b** could be detected (entry 17). These results showed that isolation of satisfactory amount of **17b** is quite difficult, since evolution to **18** or **19b** always occurs before complete transformation of the starting material. On these basis, we thought that microwave activation should be avoided in the second step for alkylidene acetoacetate **1b**.

Basing on registered information from the two distinct step, we performed the one-pot two steps reaction. In this protocol, reagents are added in a sequential way without isolating the unsaturated intermediate. Some selected results are reported in Table 2. The reactions were performed by adding the reagents for the second step directly into the vessel, without performing any work-up to the first step crude. We started to studied conditions for dimethyl malonate reaction. Initially it has been verified that simple introduction of pure indole, without adding solvent or catalyst, is not sufficient to induce product formation but only the bis-indole derivative was observed in small amount (entry 1). Considering the unreacted starting materials in the first step that could be still present in the vessel, we checked for products deriving from the condensation of aldehyde, indole and piperidine,[21] but no traces

of these derivatives were ever observed. The reaction has been performed under the same conditions (250W, 10 min, neat) in the presence of $Yb(OTf)_3$, but the complete recover of the alkylidene intermediate **1a** (entry 2) suggesting us that second step requires a solvent.

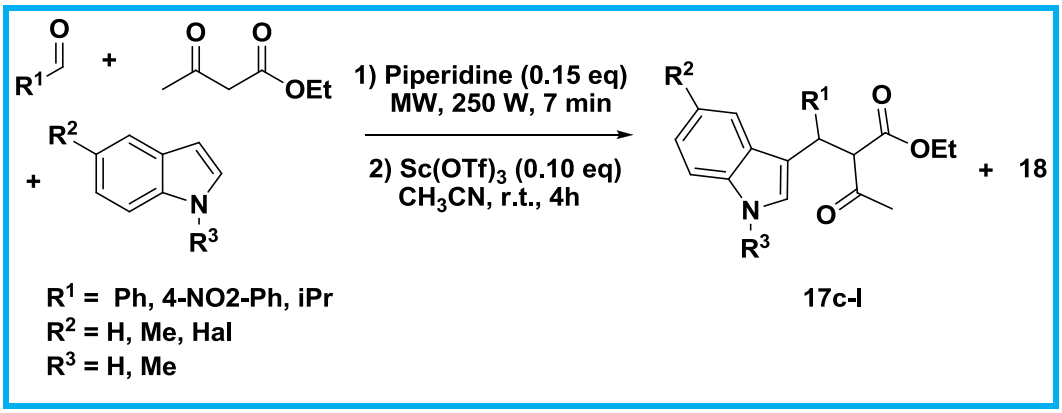
Entry	Reagent	1st step	2nd step	Solvent	Lewis Acid	Yield 17(%)[a]	Yield 18(%)[a]	Yield 1(%)[a]
1	A	250W-7min	250W-10min	/	/	/	11	42
2	A	250W-7min	250W-10min	/	$Yb(OTf)_3$	/	/	90
3	A	250W-7min	250W-20min	DMF	$Sc(OTf)_3$	33	16	16
4	A	250W-7min	250W-20min	DMF	$Yb(OTf)_3$	45	25	8
5	A	250W-7min	r.t.-4h	DMF	$Yb(OTf)_3$	/	/	90
6	A	250W-7min	r.t.-4h	CH_3CN	$Yb(OTf)_3$	30	3	9
7[b]	B	250W-7min	250W-20min	DMF	$Sc(OTf)_3$	/	5	12
8	B	250W-7min	r.t.-4h	DMF	$Yb(OTf)_3$	/	6	77
9	B	250W-7min	r.t.-4h	DMF	$Sc(OTf)_3$	/	6	85
10	B	250W-7min	r.t.-4h	CH_3CN	$Yb(OTf)_3$	67(51/49)	/	5
11	B	250W-7min	r.t.-4h	CH_3CN	$Sc(OTf)_3$	75(63/37)	7	16
12	B	250W-7min	r.t.-4h	CH_3CN	$Cu(OTf)_2$	47(50/50)	16	12
13	B	250W-7min	r.t.-4h	CH_3CN	$Zn(OTf)_2$	23(61/49)	8	43
14	**B**	250W-7min	0°C-17h	CH_3CN	$Sc(OTf)_3$	71(70/30)	7	16
15	B	250W-7min	0°C-17h	CH_3CN	$Yb(OTf)_3$	66(60/40)	8	12
16	B	250W-7min	-20°C-4h	CH_3CN	$Yb(OTf)_3$	29(75/25)	2	34

Table 6.One pot-two steps optimization in the Yonemitsu-type condensation.

The experiments carried out under MW irradiation in DMF afforded yields in the range between 33% and 45% and, as expected, a large amount of compound **18** was

also obtained both with scandium or ytterbium triflates (entries 3-4). On the basis of these results, we performed the second step at room temperature (entries 5-6) using acetonitrile as solvent, since it is resulted to be the more suitable to our purposes under these conditions, even if the yield was comparable to those obtained under microwave conditions.

At this point, the one-pot two step reaction starting from ethyl acetoacetate has been studied following the same approach. Performing both steps under microwave catalyzed conditions, the reaction didn't afford satisfactory results (entry 7), because decarboxylation of **17b** cannot be avoided, as previously observed when the second step was performed on isolated **1b**. By conducting the second step at room temperature in DMF we could observe the presence of the unsaturated intermediate **1b** as major product, thus confirming that under these conditions DMF is not the solvent of choice (entries 8-9). The reaction carried out in CH_3CN provided the desired product **2b** in satisfactory yield. By changing the Lewis acid (entries 10-13) we could verify that $Yb(OTf)_3$ or $Sc(OTf)_3$ are the best catalysts, since from the reaction with copper or zinc, bis-indole **18** and alkylidene intermediate **1b** were isolated in considerable amount. The diastereomeric ratio has been improved lowering the reaction temperature in the second step. In fact, we obtained an improvement from 51/49 at room temperature up to 75/25 at -20°C (entry 14-16). The (2*S**,3*S**) stereochemistry of the preferred stereoisomer was attributed by comparison with the literature.[22] Anyway, the diastereomeric excess of the mixture is unstable due to the acidity of the α-proton, and fast equilibration to 60/40 mixture occurs under mild basic conditions.



Scheme 17. MCR via one-pot two steps method with substituted indoles on aliphatic and aromatic aldehydes.

Applying the optimized conditions to the condensation of ethyl acetoacetate with aromatic aldehydes and substituted indoles, we developed a small library of compounds (Scheme 17). In the reactions performed with benzaldehyde (entries 1-4), we obtained modest yields till maximum 39% when 5-F-indole was employed (entries 2). The reaction of indole with 4-NO_2-benzaldehyde afforded a better result (55% yield, entry 5).

On the contrary, from the condensation with isobutyraldehyde and 5-substituted indoles, quite satisfactory yields have been observed. The reactions with this aldehyde led to a reduced amount of side products (entries 6-8). In particular, the reaction with 5-F-indole gave an excellent result, because the product **17h** was obtained in 55% yield and no traces of bis-indolic derivative could be detected in the crude by ^{1}H NMR nor it could be isolated after flash chromatography. The reaction performed with 1-Me-indole and isobutyraldehyde afforded **17l** in 61% yield (entry 8), a result similar to that obtained with unsubstituted indole, while a very unsatisfactory yield was observed when the same reaction was performed with benzaldehyde (entry 4).

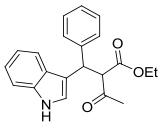
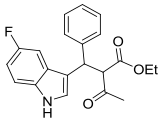
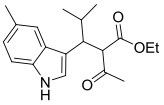
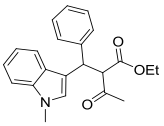
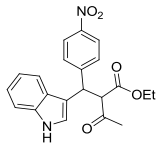
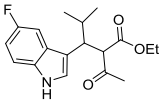
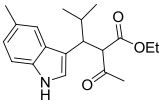
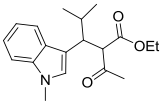
Entry	R^1	R^2	R^3	Product		Yield **17**(%)[a]	Yield **bisindole**(%)[a]
1	Ph	H	H	17c		36	27
2	Ph	F	H	17d		39	30
3	Ph	Me	H	17e		32	29
4	Ph	H	Me	17f		23	33
5	4-NO_2- Ph	H	H	17g		55	22
6	iPr	F	H	17h		55	< 5
7	iPr	Me	H	17i		50	18
8	iPr	H	Me	17l		61	18

Table 7. Small library produced by Yonemitsu-type condensation protocol.

2.5 Experimental section

Addition on malonamides:

General procedure for the 1,4 addition of N,O bis(trimethylsilyl)hydroxylamine to compound 3a-b (compounds 6a-b).

Lewis acid (0.25 mmol) was added to a stirred solution of alkylidene malonamide 1 (5mmol, 1.3 g) in dry DCM (25 mL) at -40°C. After ten minutes, N,O-bis(trimethylsilyl)hydroxylamine (2.2 equiv., 11 mmol, 2.35 mL) was added dropwise and the solution was monitored by TLC and quenched with water (20 mL) at appearance of degradation products. After washing twice with water (20 mL), the organic layer was dried with Na_2SO_4 and solvent was removed under reduced pressure. Compounds 6a-b were obtained as yellow oil with 83-76% of yield respectively.

6a: ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.1 (s, 9H), 1.18 (m, 3H), 1.7 (m, 11H), 3.4 (m, 4H), 3.78 (s, 3H);

6b: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 0.1 (s, 9H), 0.94 (t, 6H, J=6.7 Hz), 1.18 (m, 3H), 1.87 (m, 1H), 3.28 (m, 3H), 3.74 (d, 1H, J=3 Hz), 3.78 (s, 3H).

General procedure to obtain isoxazolidinones 7a-b.

Tetrabutylammonium fluoride (2 equiv., 1.5 g) was added to a solution of 6 (3 mmol, 0.92 g) in THF at room temperature. The solution was stirred overnight and then the solvent was removed under reduced pressure. The residue was diluted with diethyl ether (10 mL) and washed twice with water (10 ml). The organic layers were dried with Na_2SO_4 and solvent was removed under reduced pressure. Isoxazolidinones 7a-b were obtained in 43-26% yield respectively after purification by flash chromatography on silica gel (cyclohexane/EtOAc, 8:2).

7a: ^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.21 (t, 3H, J=4 Hz), 1.77 (m, 11H), 3.38 (m, 3H), 4.01 (m, 1H), 6.3 (bs, 1H).

7b: ^{1}H-NMR (200 MHz, $CDCl_3$): δ 0.96 (d, 6H, J=3.4 Hz), 1.19 (t, 3H, J=3.9 Hz), 1.6 (bs, 1H), 1.89 (m, 1H), 3.32 (m, 3H), 3.99 (m, 1H), 6.26 (bs, 1H).

Addition on alkylidene acetoacetates:

General procedure for the 1,4 addition of N,O bis(trimethylsilyl)hydroxylamine to compound 1(various) (compounds 9)

To a stirred solution of 1 (0.5 mmol) in dry DCM (2.5 mL) at 0 °C under nitrogen, N,O-bis(trimethylsilyl)hydroxylamine (2 equiv., 1 mmol) was added in one portion. The reaction was monitored by TLC and quenched after 16 h with H_2O. The residue was then diluted with DCM (10 mL) and washed with H_2O (2X10 mL). The organic layer was dried with Na_2SO_4, and the solvent was removed under reduced pressure. Compounds 8 were purified by flash chromatography with silica gel (cyclohexane/EtOAc, 9:1).

9a-methyl ester: Yield >95%, colorless clear oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.88 (d, *J* = 6.6 Hz, 3H), 0.97 (d, 3H, J = 6.6 Hz), 1.67 (s, 3H), 1.69 (m, 1H), 2.96 (d, *J* = 5.4 Hz, 1H), 3.56 (dd, *J* = 5.4, 7.5 Hz, 1H), 3.76 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 18.7(CH_3), 19.2(CH_3), 23.8(CH), 32.5(CH_3), 52.4(CH), 60.2(CH_3), 67.9(CH), 106.5(C), 171.0(C). Minor anomer ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.92 (d, *J* = 6.9 Hz 3H), 1.00 (d, *J* = 6.6 Hz, 3H), 1.50 (s, 3H), 1.70 (m, 1H), 2.95 (d, *J* = 7.6 Hz 1H), 3.58 (m, 1H), 3.74 (s, 3H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 19.6(CH_3), 20.3(CH_3), 22.2(CH), 30.1(CH_3), 52.2(CH), 62.0(CH3), 71.3(CH), 108.4(C), 172.0(C). Anal. Calcd. For $C_9H_{17}NO_4$: C, 53.19; H, 8.43; N, 6.89. Found: C, 53.14; H, 8.45; N, 6.92.

9a-ethyl ester: Yield 95%, yellow oil; Major anomer ^{1}H NMR (C_6D_6, 600 MHz): δ 0.82 (d, *J* = 6.6 Hz, 3H), 0.93 (t, 3H, *J* = 7.2 Hz), 0.97 (d, 3H, J = 6.6 Hz), 1.51 (s, 3H), 1.64 (m, 1H), 2.83 (d, *J* = 5.4 Hz, 1H), 3.58 (dd, *J* = 5.4, 7.2 Hz, 1H), 3.93 (m, 1H), 3.98 (m, 1H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.1(CH_3), 18.7(CH_3), 19.4(CH_3), 23.8(CH), 32.6(CH_3), 60.1(CH_2), 61.3(CH), 67.9(CH), 106.5(C),

170.7(C). Minor anomer ^{1}H NMR (C_6D_6, 600 MHz): δ 0.86 (d, *J* = 7.2 Hz 3H), 0.88 (d, *J* = 6.6 Hz, 3H), 0.91 (t, *J* = 7.2 Hz, 3H), 1.45 (s, 3H), 1.50 (m, 1H), 3.03 (d, *J* = 7.8 Hz 1H), 3.88 (dd, *J* = 7.8, 7.2 Hz, 1H), 3.92 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 14.2(CH_3), 19.6(CH_3), 20.4(CH), 22.2(CH_3), 30.1(CH_3), 61.1(CH_2), 62.0(CH), 71.9(CH), 108.4(C), 171.5(C). Anal. Calcd. For $C_{10}H_{19}NO_4$: C, 55.28; H, 8.81; N, 6.45. Found: C, 55.31; H, 8.79; N, 6.43.

9b: Yield 90%, pale yellow oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80-1.06(m, 8H) 1.30 (t, 3H, *J* = 7.2 Hz), 1.58 (m, 1H), 1.67 (s, 3H), 2.96 (d, *J* = 6.0 Hz, 1H), 3.67 (t, *J* = 6.0 Hz, 1H), 4.23 (m, 2H). ^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.2(CH_3), 14.1(CH_3), 15.2(CH_3), 23.8(CH), 26.2(CH_2), 39.0(CH_3), 59.8(CH), 61.3(CH_2), 66.6(CH), 106.1(C), 170.7(C). Minor anomer ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80-1.06(m, 8H) 1.29 (t, 3H, *J* = 7.2 Hz), 1.53 (s, 3H), 1.58 (m, 1H), 2.95 (m, 1H), 3.68 (m, 1H), 4.25 (m, 2H).^{13}C NMR ($CDCl_3$, 75 MHz): δ 11.8(CH_3), 13.6(CH_3), 18.2(CH_3), 23.1(CH), 26.6(CH_2), 40.1(CH_3), 60.3(CH), 61.9(CH_2), 70.1(CH), 105.0(C), 167.2(C). Anal. Calcd. For $C_{11}H_{21}NO_4$: C, 57.12; H, 9.15; N, 6.06. Found: C, 57.11; H, 9.19; N, 6.08.

9c: Yield 95%, yellow oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz) 0.92-1.40 (m, 6H), 1.29 (t, *J*= 7.2 Hz, 3H), 1.52-1.78 (m, 4H), 1.67 (s, 3H), 2.00 (m, 1H), 3.00 (d, *J*= 5.7 Hz, 1H), 3.56 (dd, *J*= 5.7, 8.1 Hz, 1H), 4.24 (m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1 (CH_3), 23.9(CH), 25.8 (CH_2), 26.0 (CH_2), 26.3 (CH_2), 29.4(CH_2), 29.9 (CH_2), 42.3 (CH_3), 60.1(CH), 61.4 (CH_2), 67.2 (CH), 106.3 (C), 170.8 (C). Minor anomer ^{1}H NMR ($CDCl_3$, 300 MHz) 0.92-1.40 (m, 6H), 1.33 (t, *J*= 7.2 Hz, 3H), 1.52-1.78 (m, 4H), 1.64 (s, 3H), 1.85 (m, 1H), 2.96 (d, *J*= 7.5 Hz, 1H), 3.56 (t, *J*= 7.5 Hz, 1H), 4.30(m, 2H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1 (CH_3), 22.0(CH), 25.1 (CH_2), 25.6(CH_2), 26.9 (CH_2), 30.9(CH_2), 31.0 (CH_2), 40.0 (CH_3), 61.0(CH), 62.1 (CH_2), 70.2(CH), 108.2 (C), 171.4 (C). Anal. Calcd. For $C_{13}H_{23}NO_4$: C, 60.68; H, 9.01; N, 5.44. Found: C, 60.75; H, 9.00; N, 5.41.

9h: Yield 80%, yellow oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, *J*= 7.2 Hz, 3H), 1.74 (s, 3H), 3.24 (d, *J*= 5.4 Hz, 1H), 4.27 (q, *J*= 7.2 Hz, 2H), 5.09 (d,

J= 5.4 Hz, 1H),7.28-7.57 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.4 (CH_3), 24.4 (CH_3), 61.9 (CH_2), 65.0 (CH), 70.2(CH), 107.0 (C), 126.3 (2CH), 127.3 (2CH), 128.7 (CH), 134.1 (C), 178.0 (C). Minor anomer ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.27 (t, *J*= 7.2 Hz, 3H), 1.64 (s, 3H), 3.41 (d, *J*= 7.2 Hz, 1H), 4.15 (q, *J*= 7.2 Hz, 2H), 4.94 (d, *J*= 7.2 Hz, 1H), 7.28-7.60 (m, 5H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 14.1 (CH_3), 25.1 (CH_3), 60.9 (CH_2), 64.2 (CH), 69.3(CH), 105.8 (C), 126.5 (2CH), 127.1 (2CH), 128.9 (CH), 136.1 (C), 169.4 (C). Anal. Calcd. For $C_{13}H_{17}NO_4$: C, 62.14; H, 6.82; N, 5.57. Found: C, 62.19; H, 6.80; N, 5.53.

Luche reduction:

General procedure for Luche reduction to compounds 10.

To a stirred solution of compound Z-**1** and E-**1** (5 mmol) in THF/MeOH 9:1 (10 mL) at room temperature, $CeCl_3{\cdot}7H_2O$ (1 equiv., 5 mmol, 1,86 g) was added. After 30 min the solution was carried at -30°C and $NaBH_4$ (1.1 equiv., 5.5 mmol, 0.2 g) was added in one portion. The solution was monitored by TLC and quenched after disappearence of the starting ketone by addition of water (5 mL). After removal of THF and MeOH under reduced pressure, the residue was diluted with ethyl acetate (10 ml) and washed twice with water (5 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Racemic alcohols **Z-10-rac** or **E-10-rac** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluant).

Z-10a. Yellow oil; IR (film) ν/cm^{-1} 3437, 2964, 2933, 2870, 1717, 1467, 1372, 1228, 1178, 1159. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.00 (d, 6H, J = 6.6 Hz), 1.24 (m, 6H), 3.10 (m, 1H), 4.23 (q, 2H, J = 6.6 Hz), 4.42 (q, 1H, J = 6.2 Hz) 5.87 (d, 1H, J = 10.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 22.4, 22.5, 28.2, 60.3, 69.2, 133.1, 146.9, 167.7. GC–MS rt 11.62 min, m/z: 186(2), 171(36), 144(21), 125(100), 115(20), 97(61), 79(35), 67(25), 55(24). (3S)-Z-10a: HPLC on Chiralcel OF (97.5/2.5 n-hexane/ 2-propanol, flow 1.0 mL/min): rt 14.5 min, [α]= -11,0 (c 1.0,

$CHCl_3$).(3R)-Z-10a: HPLC on Chiralcel OF (97.5/2.5 n-hexane/2-propanol, flow 1.0 mL/min): rt 16.4 min, [α]= +12,4 (c 1.0, $CHCl_3$).

E-10a. Yellow oil; IR (film) m/cm^{-1} 3452, 2963, 2871, 1693, 1641, 1466, 1368, 1261, 1176, 1190. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.03 (d, 3H, J = 6.6 Hz), 1.06 (d, 3H, J = 6.6 Hz), 1.24 (t, 3H, J = 7.2 Hz), 1.42 (d, 3H, J = 6.6 Hz), 2.77 (m, 1H), 4.22 (q, 2H, J = 7.2 Hz), 4.72 (m, 1H), 6.52 (d, 1H, J = 10.2 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 22.2(2), 23.8, 27.1, 60.4, 65.0, 131.7, 149.2, 167.5. GC–MS rt 11.80 min, m/z: 186(2), 171(64), 143(27), 125(100), 115(11), 97(46), 79(20), 67(18), 55(15).

Z-10b. Yellow oil; IR (film) ν/cm^{-1} 3438, 2926, 2851, 1714, 1448, 1373, 1303, 1263, 1208, 1178, 1153. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01–1.36 (m, 5H), 1.25 (t, 3H, J = 7.0 Hz), 1.25 (d, 3H, J = 6.6 Hz), 1.60–1.73 (m, 5H), 2.30 (br s, 1H), 2.75 (m, 1H), 4.24 (q, 2H, J = 7.0 Hz), 4.41 (q, 1H, J = 6.6 Hz), 5.88 (d, 1H, J = 10.0 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) d 14.0, 22.5, 25.4, 25.7, 25.9, 32.4, 32.5, 37.9, 60.2, 69.2, 133.4, 145.6, 167.7. GC–MS rt 16.40 min, m/z: 226(2), 208(38), 179(33), 162(100), 147(28), 133(51), 119(37), 107(20), 99(29), 91(36), 81(87), 67(38), 55(34). (3S)-Z-1b: HPLC on Chiralcel OF (97.5/2.5 n-hexane/ 2-propanol, flow 1.0 mL/min): rt 17.7 min, α= -8,7 (c 1.0, $CHCl_3$). (3R)-Z-10b: HPLC on Chiralcel OF (97.5/2.5 n-hexane/ 2-propanol, flow 1.0 mL/min): rt 19.3 min, α=+9,4 (c 1.0, $CHCl_3$).

E-10b: Yellow oil; IR (film) ν/cm^{-1} 3423, 2926, 2852, 1691, 1448, 1368, 1263. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.11–1.25 (m, 5H), 1.29 (t, 3H, J = 7.4 Hz), 1.39 (d, 3H, J = 6.6 Hz), 1.44–1.73 (m, 5H), 2.34 (m, 1H), 4.19 (q, 2H, J = 7.4 Hz), 4.70 (q, 1H, J = 6.6 Hz) 6.50 (d, 1H, J = 9.8 Hz); ^{13}C NMR (50 MHz, $CDCl_3$) δ 14.1, 23.9, 25.4 (2), 25.7, 32.1(2), 37.0, 60.6, 65.1, 132.1, 147.8, 167.7. GC–MS rt 16.81 min, m/z: 226(2), 211(100), 179(18), 165(68), 143(55), 129(38), 119(35), 105(19), 91(32), 81(70), 67(45), 55(43).

10c: Yellow oil, ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, J= 6.8 Hz, 6H), 0.95 (d, J= 6.4 Hz, 6H), 1.46 (d, J= 6.8, 3H, major isomer), 1.48 (d, J= 6.8 Hz, 3H, minor isomer), 1.50-190 (m, 3H), 2.00 (m, 1H), 3.74 (s, 3H), 3.79 (s, 3H), 4.41 (m, 1H), 5.26 (m, 1H). LC-MS-ESI rt 9.0 min, m/z 344 (M+1).

Synthesis of racemic and enantiomerically pure acetates:

General procedure for the synthesis of racemic acetates 12.

To a solution of compound Z-**10** (1 mmol) in dry THF (5 mL), under inert atmosphere at 0°C, LiHMDS (1.5 equiv., 1.5 mL 1M solution in THF) was added dropwise. The solution was stirred for 30 minutes and then acetyl chloride (1.5 equiv., 1.5 mmol, 0.1 mL) was added in one portion and ice bath was removed. After one hour, the mixture was quenched with water (5 mL) and THF removed under reduced pressure. The residue was diluted with ethyl acetate (10 ml) and washed twice with water (5 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Racemic and enantiomerically pure acetates Z-**12** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluant).

12a: (3R)-Z-12a: Yellow oil; IR (film) ν/cm^{-1} 2963, 1644, 1466, 1241, 1178, 1097. ^{1}H NMR (300 MHz, $CDCl_3$) δ 1.05 (d, 6H, J = 6.6 Hz), 1.34 (m, 6H), 2.08 (s, 3H), 3.15 (m, 1H), 4.26 (q, 2H, J = 7.2 Hz), 5.65 (q, 1H, J = 6.6 Hz) 5.91 (d, 1H, J = 9.9 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 19.8, 21.2, 22.4, 22.5, 28.2, 60.4, 69.9, 130.5, 147.9, 169.9,170.5. GC–MS rt 12.89 min, m/z: 213(2), 185(24), 168(35), 143(100), 123(57), 95(83), 79(54), 67(35), 55(28). α=+36:5 (c 1.0, $CHCl_3$).

12b: (3R)-Z-12b. Yellow oil; IR (film) ν/cm^{-1} 2926, 2852, 1690, 1448, 1368, 1303, 1263, 1222, 1155, 1064. ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.89–1.25 (m, 5H), 1.26 (t, 3H, J = 7.0 Hz), 1.33 (d, 3H, J = 6.6 Hz), 1.55–1.77 (m, 5H), 2.02 (s, 3H), 2.80 (m, 1H), 4.19 (q, 2H, J = 7.0 Hz), 5.60 (q, 1H, J = 6.6 Hz), 5.88 (d, 1H, J = 10.0 Hz); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 19.9, 21.2(2), 25.5(2), 26.0, 32.4, 32.6, 37.8,

60.2, 69.9, 130.8, 146.7, 166.4, 170.1. GC– MS rt 17.40 min, m/z: 268(2), 225(18), 208(55), 179(63), 162(100), 151(10), 143(95), 133(57), 119(35), 105(33), 91(41), 81(84), 67(38), 55(31). α=+27:2 (c 1.0, $CHCl_3$).

Enzymatic resolution:

General procedure for Enzymatic resolution

To a solution of racemic alcohol **10-rac** (5 mmol) in diethyl ether (25 mL) at 40 °C, vinylacetate (4 equiv., 4 mmol, 0.37 mL) and Lipase from *Pseudomonas Cepacia* (46 U/mg, 0.2 mass equiv.) were added. The progress of the reaction was assessed every 12 h by GC-MS. The reaction was stopped by filtration of the enzyme and elimination of solvent and byproducts under reduced pressure. Enantiomerically pure alcohols (3*S*)-Z-**10** and acetates (3*R*)-Z-1**2** were separated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 then 80/20 as eluant). For NMR spectra of alcohol see before (Luche reduction).

Hydrolysis of enantiomerically pure acetates 12 to enantiomerically pure alcohols 10.

Acetate (3*R*)-Z-1**2** (0.5 mmol) was stirred in MeOH (5 mL) in the presence of K_2CO_3 (1 equiv., 0.5 mmol, 70 mg) for 30 min. at room temperature. The reaction was quenched by addition of 0.1 M HCl (5 mL). After removal of MeOH under reduced pressure, the residue was diluted with ethyl acetate (10 mL) and washed twice with water. The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Enantiomerically pure alcohols (3*R*)-Z-**10** were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluant). For NMR spectra see before)Luche reduction).

General procedure for the synthesis of racemic and enantiopure carbonate 11.

To a solution of LiHMDS (1.5 equiv., 1.5 mL 1M solution in THF) in dry THF (5 mL), compound Z-**10** (1 mmol) under inert atmosphere at -78°C, was added dropwise and stirred for 30 min. Then methyl chloroformiate (2 equiv.) was added in one portion and the reaction mixture was stirred at -78°C for . After one hour, the mixture was quenched with water (5 mL). The mixture was diluted with ethyl acetate (10 ml) and washed twice with water (5 ml). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Racemic and enantiomerically pure acetates Z-**12** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).Yield 65-70%.

11a: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.01 (d, J= 6.6 Hz, 6H), 1.42 (d, J= 6.6 Hz, 3H), 1.50 (s, 9H), 3.02-3.17 (m, 1H), 3.77 (s, 3H), 5.46 (q, J= 6.6 Hz, 1H), 5.84 (d, J= 9.6 Hz, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ 19.8, 22.5, 26.2, 28.1, 54.5, 74.0, 81.2, 131.3, 146.7, 155.0, 165.6

11b: ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.02-1.14 (m, 5H), 1.50 (s, 9H), 1.60-1.82 (m, 6H). 3.77 (s, 3H), 5.46 (q, J= 6.6 Hz, 1H), 5.84 (d, J= 9.6 Hz, 1H).

11c: ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92-0.99 (m, 12H), 1.44 (d, J= 6.0 Hz, 3H, major isomer), 1.45 (d, J= 6.8 Hz, 3H, minor isomer), 1.59 (m, 1H), 1.83 (m, 1H), 2.01 (m, 1H), 2.49 (m, 1H), 3.70 (s, 3H, major isomer), 3.72 (s, 3H, minor isomer), 3.75 (s, 3H, minor isomer), 3.76 (s, 3H, major isomer), 3.77 (s, 3H, minor isomer), 5.04 (dd, J= 9.6 Hz, 5.2 Hz, 1H), 5.41-5.50 (m, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ 171.1, 171.0, 169.4, 167.7, 153.9 major isomer, 153.2 minor isomer, 137.1 major isomer, 134.6 minor isomer, 57.3, 54.6 minor isomer, 54.1 major isomer, 54.0 major isomer, 53.6 minor isomer, 52.4 major isomer, 52.3 minor isomer, 38.7 minor isomer, 38.6 major isomer, 38.4 major isomer, 38.2 minor isomer, 29.7, 28.6, 25.1, 23.1, 22.3 minor isomer, 22.2 major isomer, 21.8 minor isomer, 21.7

major isomer, 19.5 major isomer, 19.3 minor isomer. LC-MS-ESI rt 10.5 min, m/z 424 (M+Na).

Nucleophilic allylic substitution:

General procedure for allylic amination in the presence of Iridium catalyst

To a stirred solution of $[Ir(COD)(Cl)]_2$ (0.02 mmol) and phosphorous ligand (0.08 mmol) in EtOH (5 mL) under nitrogen atmosphere, carbonate 11 (1 mmol) and allylamine (3 mmol, 3 equiv.) were added in one portion at room temperature. The solution was stirred under refluxing conditions and monitored by TLC. After removal of the solvent and the excess of allylamine under reduced pressure, the residue was diluted with ethyl acetate (10 mL). The organic solution was extracted three times with 0.1 M HCl (10 mL), then was dried over Na_2SO_4 and solvent was removed under reduced pressure to recover unreacted starting carbonate. 1 M NaOH was added dropwise to the acid aqueous layer until basic pH was achieved and then the solution was extracted three times with DCM (10 mL). The organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to afford allylamino derivative 13 in quantitative yield as a yellow oil. Compound 13 was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70:30 as eluant).

Z-13a: Yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.68 (d, J = 6.6 Hz, 3H), 1.02 (d, J = 6.6 Hz, 3H), 1.41 (s, 9H), 1.69 (d, J = 7.4 Hz, 3H), 1.97 (m, 1H), 2.91 (dd, J = 14.0, 6.2 Hz, 1H), 3.05 (d, J = 9.2 Hz, 1H), 3.16 (dd, J = 14.0, 5.4 Hz, 1H), 5.03–5.18 (m, 2H), 5.89 (m, 1H), 6.91 (q, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 20.0, 21.0, 28.1, 32.0, 49.7, 61.4, 80.4, 115.0, 133.3, 134.3, 138.4, 166.7. LC–MS–ESI rt 14.97 min, 254 (M+1). **(S)-Z-13a** $[a]^D$ =+19.5 (c 1 in CHCl3); **(R)-Z-13a** $[a]^D$ = -20.6 (c 1 in $CHCl_3$); Anal. cald. for $C_{15}H_{27}NO_2$ (253.20): C 71.10, H 10.74, N 5.53; found C 70.84, H 10.65, N 5.57.

E-13a: Yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.77 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H), 1.40 (s, 9H), 1.84 (d, J = 7.0 Hz, 3H), 1.97 (m, 1H), 2.72 (d, J = 8.2 Hz, 1H), 2.91 (dd, J = 14.0, 6.2 Hz, 1H), 3.16 (dd, J = 14.0, 5.4 Hz, 1H), 5.03-5.18 (m, 2H), 5.68 (q, J = 7.0 Hz, 1H); 5.89 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.8, 20.0, 21.0, 28.0, 32.1, 49.8, 61.4, 80.0, 115.0, 133.3, 134.8, 137.8, 167.8. LC–MS–ESI rt 15.84 min, 254 (M+1). Anal. cald. for $C_{15}H_{27}NO_2$ (253.20): C 71.10, H 10.74, N 5.53; found C 71.22, H 10.68, N 5.50.

Z-13b: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (m, 1H), 0.92 (m, 1H), 1.11–1.25 (m, 4H), 1.42 (s, 9H), 1.49 (m, 4H), 1.76 (d, J = 6.8 Hz, 3H), 2.28 (m, 1H), 2.96 (dd, J = 17.6, 6.6 Hz, 1H), 3.20 (d, J = 9.2 Hz, 1H), 3.22 (dd, J = 17.6, 5.6 Hz, 1H), 5.04 (dd, J = 1.4, 10.0 Hz, 1H), 5.13 (dd, J = 1.4, 17.2 Hz, 1H), 5.88 (m, 1H), 6.89 (q, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.0, 26.3, 26.7, 28.2, 30.7, 31.7, 41.6, 60.1, 80.2, 115.2, 134.0, 137.9, 138.6, 166.9. LC–MS–ESI rt 16.21 min, 294 (M+1). **(S)-Z-13b** $[a]^D$ = + 14.3 (c 1 in $CHCl_3$); **(R)-Z-13b** $[a]^D$ = -16.6 (c 1 in $CHCl_3$). Anal. cald. for $C_{18}H_{31}NO_2$ (293.24): C 73.67, H 10.65, N 4.77; found C 73.91, H 10.61, N 4.78.

E-13b: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.78 (m, 1H), 0.92 (m, 1H), 1.11–1.25 (m, 4H), 1.42 (s, 9H), 1.49 (m, 4H), 1.90 (d, J = 6.8 Hz, 3H), 2.28 (m, 1H), 2.82 (d, J = 8.6 Hz, 1H), 2.96 (dd, J = 17.6, 6.6 Hz, 1H), 3.20 (d, J = 9.2 Hz, 1H), 3.22 (dd, J = 17.6, 5.6 Hz, 1H), 5.04 (dd, J = 1.4, 10.0 Hz, 1H), 5.13 (dd, J = 1.4, 17.2 Hz, 1H), 5.78(q, J = 7.2 Hz, 1H); 5.89 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 15.0, 26.4, 26.7, 28.2, 30.6, 31.7, 41.8, 68.1, 80.6, 115.2, 133.6, 137.8, 138.6, 166.9. LC–MS–ESI rt 16.95 min, 294 (M+1). Anal. cald. for $C_{18}H_{31}NO_2$ (293.24): C 73.67, H 10.65, N 4.77; found C 73.39, H 10.60, N 4.75.

Z-13c::Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.33 (d, J = 7.2 Hz, 3H), 1.50 (s, 9H), 1.79 (d, J = 7.2 Hz, 3H), 3.07 (dd, J = 14.0, 6.0 Hz, 1H), 3.18 (dd, J = 14.0, 6.0 Hz, 1H), 3.75 (q, J = 7.2 Hz, 1H), 5.06–5.17 (m, 2H), 5.92 (m, 1H), 6.81 (q, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 20.5, 28.3, 50.1, 59.8, 81.4, 117.4,

132.3, 135.8, 137.6, 166.5. LC–MS–ESI rt 10.32 min, 226 (M+1). Anal. cald. For $C_{13}H_{23}NO_2$ (225.17): C 69.29, H 10.29, N 6.22; found C 69.03, H 10.10.27, N 6.22.

E-13c: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (d, J = 6.8 Hz, 3H), 1.53 (s, 9H), 1.91 (d, J = 6.8 Hz, 3H), 3.11 (dd, J = 13.8, 6.0 Hz, 1H), 3.24 (dd, J = 13.8, 6.0 Hz, 1H), 3.36 (q, J = 6.8 Hz, 1H), 5.06-5.17 (m, 2H), 5.86-5.96 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 20.2, 28.3, 51.1, 60.0, 81.2, 116.9, 132.2, 135.6, 137.8, 167.4. LC–MS–ESI rt 11.44 min, 226 (M+1). Anal. cald. for $C_{13}H_{23}NO_2$ (225.17): C 69.29, H 10.29, N 6.22; found C 69.58, H 10.24, N 6.25.

Z-13d: Yellow oil; ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.70 d, J = 7.2 Hz, 3H), 1.05 (d, J = 7.2 Hz, 3H), 1.00–1.82 (m, 3H), 1.46 (s, 9H), 1.74 (d, J = 7.2 Hz, 3H), 2.82–3.28 (m, 3H), 4.99–5.16 (m, 2H), 5.82 (m, 1H), 6.87 (q, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 11.6, 13.9,16.2, 26.5, 28.4, 38.7, 50.4, 59.6, 80.8, 115.8, 132.6, 134.9, 137.8, 164.1. LC–MS–ESI rt 16.11 min, 268 (M+1). Anal. cald. for $C_{16}H_{29}NO_2$ (267.22): C 71.86, H 10.93, N 5.24; found C 71.64, H 10.95, N 5.25.

E-13d: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.77 (d, J = 7.0 Hz, 3H), 0.84 (d, J = 6.8 Hz, 3H), 1.00–1.82 (m, 3H), 1.48 (s, 9H), 1.90 (d, J = 7.2 Hz, 3H), 2.82–3.28 (m, 3H), 4.99–5.16 (m, 2H), 5.71–5.96 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.2, 13.3,18.2, 26.1, 28.3, 36.2, 50.0, 60.1, 81.6, 118.0, 132.1, 133.7, 138.1, 167.3. LC–MS–ESI rt 13.34 min, 268 (M?1). Anal. cald. for $C_{16}H_{29}NO_2$ (267.22): C 71.86, H 10.93, N 5.24; found C 72.00, H
10.88, N 5.21.

Z-13e: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.31 (s, 9H), 1.93 (d, J = 7.6 Hz, 3H), 3.21 (dd, J = 14.0, 6.0 Hz, 1H), 3.32 (dd, J = 14.0, 5.6 Hz, 1H), 4.83 (s, 1H), 5.11 (dd, J = 1.6, 10.4 Hz, 1H), 5.22 (dd, J = 1.6,16.8 Hz, 1H), 5.99 (m, 1H), 7.01 (q, J = 7.6 Hz, 1H), 7.18-7.43 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 28.8, 49.8, 60.6, 81.6, 116.8, 127.4, 127.9, 128.5, 132.1, 133.9, 136.0, 139.2, 166.4. LC–MS–ESI rt 20.22 min, 288 (M?1). Anal. cald. for $C_{18}H_{25}NO_2$ (287.19): C 75.22, H 8.77, N 4.87; found C 75.40, H 8.72, N 4.84.

E-13e: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.30 (s, 9H), 1.94 (d, J = 7.2 Hz, 3H), 3.12 (dd, J = 14.0, 6.4 Hz, 1H), 3.20 (dd, J = 14.0, 6.0 Hz, 1H), 4.45 (s, 1H), 5.05 (dd, J = 1.6, 10.4 Hz, 1H), 5.15 (dd, J = 1.6,17.2 Hz, 1H), 5.88 (m, 1H), 6.09 (q, J = 7.2 Hz, 1H), 7.18–7.43 (m, 5H); ^{13}C NMR(75 MHz, $CDCl_3$) δ 16.1, 28.7, 50.1, 60.2, 83.4, 117.9, 127.5, 127.8, 128.2, 132.6, 134.6, 137.2, 138.9, 167.9. LC–MS–ESI rt 21.56 min, 288 (M+1). Anal. cald. For $C_{18}H_{25}NO_2$ (287.19): C 75.22, H 8.77, N 4.87; found C 75.01, H 8.75, N 4.86.

Z-13f: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (s, 9H), 1.97 (d, J = 7.2 Hz, 3H), 3.26 (dd, J = 14.0, 5.6 Hz, 1H), 3.38 (dd, J = 14.0, 5.6 Hz, 1H), 4.89 (s, 1H), 5.16–5.31 (m, 2H), 6.03 (m, 1H), 7.09 (q, J = 7.2 Hz, 1H), 7.10 (m, 1H), 7.24–7.35 (m, 2H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.9, 28.4, 49.9, 57.6, 81.3, 115.9, 120.3, 125.4, 128.1, 132.4, 134.4, 136.5, 139.0, 168.0. LC–MS–ESI rt 19.02 min, 294 (M+1). Anal. cald. for $C_{16}H_{23}NO_2S$ (293.14): C 65.49, H 7.90, N 4.77, S 10.93; found C 65.64, H 7.91, N 4.76, S 10.98.

E-13f: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (s, 9H), 2.05(d, J = 7.2 Hz, 3H), 3.22–3.34 (m, 2H), 4.57 (s, 1H), 5.16–5.31 (m, 2H), 5.98–6.15 (m, 2H), 7.12 (m, 1H), 7.24–7.35 (m, 2H); $_{13}$C NMR (75 MHz, $CDCl_3$) δ 14.6, 28.3, 49.7, 57.0, 80.9, 117.1, 122.2, 125.6, 128.0, 132.1, 135.0, 136.9, 140.2, 166.5. LC–MS–ESI rt 16.43 min, 294 (M+1). Anal. cald. for $C_{16}H_{23}NO_2S$ (293.14): C 65.49, H 7.90, N 4.77, S 10.93; found C 65.77, H 7.88, N 4.74, S 10. 94.

E-14e: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (d, J = 6.8 Hz, 3H), 1.49 (s, 9H), 2.83 (dd, J = 14.0, 6.4 Hz, 1H), 3.03 (dd, J = 14.0, 7.6 Hz, 1H), 3.84 (q, J = 6.8 Hz, 1H), 4.83–4.90 (m, 2H), 5.71 (m, 1H), 7.18-7.31 (m, 5H), 7.61 (s, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 28.4, 51.3, 58.2, 80.9, 117.4, 127.5, 127.7, 128.0, 129.1, 134.9, 136.7, 138.0, 165.9. LC–MS–ESI rt 18.44 min, 288 (M+1). Anal. cald. for $C_{18}H_{25}NO_2$ (287.19): C 75.22, H 8.77, N 4.87; found C 75.33, H 8.77, N 4.85.

Z-14e: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (s, 9H), 1.45 (d, J = 6.8 Hz, 3H), 2.84 (dd, J = 14.0, 6.4 Hz, 1H), 3.02 (dd, J = 14.0, 7.6 Hz, 1H), 4.59 (q, J = 6.8 Hz, 1H), 4.83–4.90 (m, 2H), 5.78 (m, 1H), 6.82 (s, 1H), 7.15–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 19.2, 28.3, 51.3, 58.6, 81.2, 116.9, 127.5, 127.6, 128.1, 129.1, 134.6, 135.9, 138.6, 167.0. LC–MS–ESI rt 17.10 min, 288 (M?1). Anal. cald. for $C_{18}H_{25}NO_2$ (287.19): C 75.22, H 8.77, N 4.87; found C 75.11, H 8.81, N 4.87.

Synthesis of N-BOC derivative 15a:

Boc_2O (1 mmol, 1 eq.) was added to a solution of 13a in EtOH (5 mL) at room temperature and the reaction mixture was left stirring for 3 h. Then, the solvent was removed under reduced pressure and the residue was diluted with EtOAc (10 mL). After washing three times with water (5 mL), the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound 15a was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70:30 as eluant).

Z-15a: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.79 (d, J = 6.4 Hz, 3H), 0.90 (d, J = 6.4 Hz, 3H), 1.48 (s, 9H), 1.53 (s, 9H), 1.92 (d, J = 7.4 Hz, 3H), 2.62 (m, 1H), 3.82-4.01 (m, 2H), 4.81(d, J = 11.2 Hz, 1H), 4.94-5.04 (m, 2H), 5.74 (m, 1H), 6.83 (q, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 19.7, 27.7, 27.9, 28.1, 45.6, 58.4, 78.8, 79.9, 114.4, 133.9, 136.1, 142.4, 155.8, 166.7. LC–MS–ESI rt 16.6 min 354 (M+1), 729 (2M+23). Anal. cald. for $C_{20}H_{35}NO_4$ (353.26): C 67.95, H 9.98, N 3.96; found C 67.77, H 10.00, N 3.94.

E-15a: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.92(d, J = 6.8 Hz, 6H), 1.48 (s, 9H), 1.53 (s, 9H), 1.92 (d, J = 7.4 Hz, 3H), 2.62 (m, 1H), 3.82–4.01 (m, 2H), 4.62(d, J = 10.8 Hz, 1H), 4.94-5.04 (m, 2H), 5.48 (q, J = 7.2 Hz, 1H), 5.76 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.2, 20.1, 27.7, 27.9, 29.4, 45.3, 59.6, 78.8, 79.4, 114.4, 133.9, 135.5, 141.6, 155.4, 166.7 LC– MS–ESI rt 14.9 min 354 (M+1), 729

(2M+23). Anal. cald. for $C_{20}H_{35}NO_4$ (353.26): C 67.95, H 9.98, N 3.96; found C 68.26, H 9.95, N 3.96.

Synthesis of N-CBz derivative 15b:

Benzyloxycarbonyl chloride (1.5 mmol, 1.5 equiv.) and K_2CO_3 (2 mmol, 2 equiv) were added to a solution of 13a (1 mmol) in dioxane/ water (1:1 solution, 5 mL). The reaction mixture was stirred at room temperature overnight. The solution was diluted ethyl acetate (10 mL) and washed three times with water (10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound 15b was purified by flash chromatography on silica gel (eluant cyclohexane/EtOAc 97:3).

Z-15b: : Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.79 (d, J = 6.8 Hz, 3H), 0.92 (d, J = 6.8 Hz, 3H), 1.46 (s, 9H), 1.90 (d, J = 7.2 Hz, 3H), 2.68 (m, 1H), 3.94-4.06 (m, 2H), 4.83 (d, J = 9.8 Hz, 1H), 4.90–5.18 (m, 4H), 5.67 (m, 1H), 6.85 (q, J = 7.2 Hz, 1H) 7.20–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.5, 19.8, 20.0, 27.8, 28.0, 45.7, 59.7, 67.0, 80.3, 115.0, 127.6, 128.2, 128.5, 133.6, 135.8, 137.0, 143.0, 156.8, 166.8. LC–MS–ESI rt 14.7 min 388 (M+1), 410 (M+23). Anal. cald. for $C_{23}H_{33}NO_4$ (387.24): C 71.29, H 8.58, N 3.61; found C 71.43, H 8.56, N 3.96.

E-15b: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.91 (d, J = 6.8 Hz, 6H), 1.44 (s, 9H), 1.90 (d, J = 7.2 Hz, 3H), 2.68 (m, 1H), 3.94–4.06 (m, 2H), 4.62 (d, J = 9.2 Hz, 1H), 4.90–5.18 (m, 4H), 5.68 (m, 1H), 6.01 (q, J = 7.2 Hz, 1H), 7.20–7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ 14.1, 19.9, 20.3, 27.8, 29.7, 46.2, 59.9, 67.3, 80.4, 115.3, 127.6, 128.2, 128.5, 133.5, 135.2, 136.6, 142.0, 156.0, 166.8. LC–MS–ESI rt 13.5 min 388 (M+1), 410 (M+23). Anal. cald. for $C_{23}H_{33}NO_4$ (387.24): C 71.29, H 8.58, N 3.61; found C 71.07, H 8.62, N 3.95.

Synthesis of N-malonamido derivative 15c-d:

To a stirred solution of 13a (1 mmol) in dry DCM (5 mL), methyl malonyl chloride (1.5 mmol, 1.5 equiv.) and triethylamine (1.5 mmol, 1.5 equiv.) were added at room temperature. After stirring for 3 h, the reaction mixture diluted with DCM (10 mL) and washed twice with water (10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Purification of the crude residue by flash chromatography on silica gel (eluant cyclohexane/EtOAc 9:1) afforded compound 15c-d. The desired product was obtained in yields up to 95% as an yellow oil.

Z-15c: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ: 0.81(d, J = 6.6 Hz, 3H), 0.92 (d, J = 6.6 Hz, 3H), 1.45 (s, 9H), 1.95 (d, J = 7.4 Hz, 3H), 2.67 (m, 1H), 3.38 (d, J = 15.4 Hz, 1H), 3.53 (d, J = 15.4 Hz, 1H), 3.74 (s, 3H), 3.94 (dd, J = 18.2 Hz, 5.2 Hz, 1H), 4.44 (dd, J = 18.2 Hz, 2.4 Hz, 1H), 5.04 (d, J = 17.2 Hz, 1H), 5.13 (d, J = 10.4 Hz, 1H), 5.33 (d, J = 11.6 Hz, 1H), 5.70 (m, 1H), 6.91 (q, J = 7.2 Hz, 1H); ^{13}C NMR (75 MHz, $CDCl_3$,) δ 14.6, 19.7, 27.8, 28.0, 41.2, 46.8, 52.1, 57.0, 80.4, 115.7, 132.9, 135.0, 143.9, 166.6, 167.0, 168.4. LC–MS–ESI rt 10.7 min 376 (M+23), 729 (2M+23). Chiral HPLC analysis (Chiralpak IC, eluted with n-hexane/2-propanol, typical program: from 96/4 to 90/10 in 30 min, flux 0.6 mL/min) rt 33.24 min for **(R)-Z-15c** and 36.62 min for **(S)-Z-15c**; **(R)-Z-15c** $[a]^D$ = +26.7 (c 1 in $CHCl_3$). **(S)-Z-15c** $[a]^D$ = -30.8 (c 1 in $CHCl_3$); Anal. cald. for $C_{19}H_{31}NO_5$ (353.22): C 64.56, H 8.84, N 3.96; found C 64.89, H 8.90, N 3.95.

E-15c: Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 0.94 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 1.92 (d, J = 7.4 Hz, 3H), 2.67 (m, 1H), 3.44 (d, J = 15.4 Hz, 1H), 3.54 (d, J = 15.4 Hz, 1H), 3.76 (s, 3H), 4.02 (dd, J = 18.2, 5.0 Hz, 1H), 4.23 (dd, J = 18.2, 2.0 Hz, 1H), 5.04 (d, J = 17.2 Hz, 1H), 5.13 (d, J = 10.4 Hz, 1H), 5.15 (d, J = 10.0 Hz, 1H), 5.70 (m, 1H), 6.18 (q, J = 7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.3, 19.8, 27.8, 29.2, 41.0, 46.9, 52.1, 57.3, 80.2, 115.0, 133.1, 135.0, 142.7, 166.6, 167.1, 168.4. LC–MS–ESI rt 10.1 min 376 (M+23), 729

(2M+23). Chiral HPLC analysis (Chiralpak IC, eluted with n-hexane/2-propanol, typical program: from 96/4 to 90/10 in 30 min, flux 0.6 mL/min) rt 48.40 min for **(R)-E-15c** and 59.28 min for **(S)-E-15c**. Anal. cald. for $C_{19}H_{31}NO_5$ (353.22): C 64.56, H 8.84, N 3.96; found C 64.58, H 8.82, N 3.98.

15d: : Yellow oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.02-1.21 (m, 5H), 1.45 (s, 9H), 1.50-165 (m, 6H), 1.92 (d, J = 7.4 Hz, 3H), 3.35 (d, J= 15.2 Hz, 1H), 3.48 (d, J=15.2 Hz, 1H), 3.94 (m, 1H), 4.39 (m, 1H), 4.85-5.10 (m, 2H), 5.38 (d, J= 11.2 Hz, 1H), 5.66 (m, 1H), 6.85 (q, J= 6.8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.7, 25.8, 26.3, 28.0, 29.8, 30.2, 36.9, 41.1, 46.9, 52.1, 53.4, 55.7, 80.4, 115.8, 132.6, 135.0, 144.0, 166.7, 167.0, 168.4. LC-MS-ESI rt 10.3 min 394 (M+1).

Ring closing metathesis:

General procedure for the ring closing metathesis (RCM) with ruthenium catalyst:

Catalyst A–D (0.03 mmol) was added to a solution of 15a-d (1 mmol) in MTBE and the reaction mixture was stirred at reflux for 3 h. Then the reaction cooled to room temperature and was quenched by addition of ethyl vinyl ether (0.1 mL). The solvent was removed under reduced pressure and the crude residue was purified through by chromatography on silica gel (cyclohexane/ EtOAc 95:5).

16a: Yellow oil (1:1 trans/cis conformers mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ Trans conformer: 0.82(d, J = 6.6 Hz, 3H), 0.94 (d, J = 6.6 Hz, 3H), 1.46 (s, 18H), 2.21 (m, 1H), 3.98 (bd, J = 18.6 Hz, 1H), 4.31 (bd, J = 18.6 Hz, 1H), 4.91 (bm, 1H), 6.76 (bm, 1H); Cis conformer: 0.84 (d, J = 7.0 Hz, 3H), 0.86 (d, J = 7.0 Hz, 3H), 1.43 (s, 9H), 2.21 (m, 1H), 4.08 (bd, J = 18.0 Hz, 1H), 4.42 (bd, J = 18.0 Hz, 1H), 4.78 (bm, 1H), 6.63 (bm, 1H); ^{13}C NMR (CDC_{l3}, 100 MHz) δ Trans conformer: 17.0, 19.1, 25.0, 27.4, 31.7, 53.5, 65.3, 78.6, 80.2, 135.3, 136.0, 153.7, 161.9. Cis conformer: 16.6, 19.2, 27.2, 27.9, 32.1, 53.5, 67.2, 79.1, 82.4, 135.7,

136.1, 154.0, 161.1. LC–MS–ESI rt 12.6 min, 334 (M+23). Anal. cald. for $C_{17}H_{29}NO_4$ (311.21): C 65.57, H 9.39, N 4.50; found C 65.80, H 9.35, N 4.47.

16b: Yellow oil (1.1 trans/cis conformers mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ Trans conformer: 0.83 (d, J = 7.2 Hz, 3H), 0.96 (d, J = 7.2 Hz, 3H), 1.49 (s, 9H), 2.53 (m, 1H), 4.10 (bd, J = 18.0 Hz, 1H), 4.39 (bd, J = 18.0 Hz, 1H), 4.91 (bm, 1H), 6.64 (bm, 1H), 7.28–7.41 (m, 5H); Cis conformer: 0.88 (d, J = 6.6 Hz, 3H), 0.89 (d, J = 6.6 Hz, 3H), 1.43 (s, 9H), 2.21 (m, 1H), 4.10 (bd, J = 18.0 Hz, 1H), 4.48 (bd, J = 18.0 Hz, 1H), 4.84 (bm, 1H), 6.71 (bm, 1H), 7.28-7.41 (m, 5H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ Trans conformer: 17.6, 19.0, 28.0, 32.3, 54.6, 66.8, 67.9, 81.2, 127.9, 128.0, 128.1, 128.5, 135.7, 136.8, 154.9, 162.6. Cis conformer: 17.4, 19.2, 29.6, 32.7, 54.0, 67.1, 68.5, 81.2, 127.9, 128.0, 128.1, 128.5, 136.1, 136.6, 155.3, 162.6. LC–MS–ESI rt 12.2 min, 368 (M+23). Anal. cald. For $C_{20}H_{27}NO_4$ (345.19):C69.54,H7.88,N4.05; found C 69.33, H 7.92, N 4.06. tert-**16c:** Yellow oil (70:30 trans/cis conformers mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ Trans conformer: 0.91(d, J = 7.0 Hz, 3H), 0.92 (d, J = 7.0 Hz, 3H), 1.50 (s, 9H), 2.41 (m, 1H), 3.41 (s, 2H), 3.75 (s, 3H), 4.27 (ddd, J = 16.6, 2.0, 4.4 Hz, 1H), 4.35 (dd, J = 16.6, 2.4 Hz, 1H), 5.12 (bt, J = 3.2 Hz, 1H), 6.64 (bm, 1H); Cis conformer: 0.92 (d, J = 6.6 Hz, 3H), 0.96 (d, J = 6.6 Hz, 3H), 1.51 (s, 9H), 1.96 (m, 1H), 3.45 (d, J = 15.0 Hz, 1H), 3.51 (d, J = 15.0 Hz, 1H), 3.76 (s, 3H), 4.08 (ddd, J = 18.8, 1.6, 3.0 Hz, 1H), 4.35 (dd, J = 18.8, 2.8 Hz, 1H), 4.77 (bt, J = 3.2 Hz, 1H), 6.76 (bm, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ Trans conformer:18.3, 18.4, 28.0, 31.8, 42.2, 52.5, 54.6, 68.0, 81.6, 134.1, 136.7, 162.5, 164.3, 167.7. Cis conformer:17.5, 18.7, 28.0, 34.5, 40.8, 52.5, 53.8, 68.4, 81.5, 134.9, 137.3, 162.5, 164.8, 167.5. LC–MS–ESI rt 8.25 min, 312 (M+1), 334 (M+23), 645 (2M+23). **(S)-16c** $[a]^D$ = + 34.9 (c 1 in $CHCl_3$); **(R)-16c** $[a]^D$ = -28.7 (c 1 in $CHCl_3$). Anal. cald. for $C_{16}H_{25}NO_5$ (311.17): C 61.72, H 8.09, N 4.50; found C 61.98, H 8.11, N 4.52.

Yonemitsu:

Representative procedure for one-pot two steps trimolecular condensation (second step with microwaves)

Dimethyl malonate or ethyl acetoacetate (1 mmol), aldehyde (1 equiv, 1 mmol) and piperidine (0.15 equiv, 0.15 mmol, 15 μL) were mixed in the microwave reactor and submitted to irradiation at 250 W for 7 minutes. The mixture was allowed to reach room temperature and diluted with DMF (1 mL). Indole (1 equiv) and Lewis acid (0.1 equiv) were then added and the solution was submitted to microwave irradiation at 250W for 20 minutes. After cooling to room temperature, the reaction mixture was diluted with water and extracted with EtOAc (3 × 10 mL). The extract was washed with water and brine then dried (Na_2SO_4). Evaporation of solvent left the crude products, which were purified by column chromatography over silica (cyclohexane/ ethyl acetate 90:10).

Representative procedure for one-pot two steps trimolecular condensation (second step at r.t.)

Dimethyl malonate or ethyl acetoacetate (1 mmol), aldehyde (1 equiv, 1 mmol) and piperidine (0.15 equiv, 0.15 mmol, 15 μL) were mixed in the microwave reactor and submitted to irradiation at 250 W for 7 minutes. The mixture was allowed to reach room temperature and diluted with DMF (1 mL). Indole (1 equiv) and Lewis acid (0.1 equiv) were then added and the solution was left stirring at room temperature for 4 h. The reaction was monitored by TLC and stopped at disappearance of the starting material. The reaction mixture was diluted with water and then extracted with EtOAc (3 × 10 mL). The extract was washed with water and brine then dried (Na_2SO_4). Evaporation of solvent left the crude products, which were purified by column chromatography over silica (cyclohexane/ ethyl acetate 90:10).

17a: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.08 (s, 1H), 7.68 (dd, *J* = 7.8, 1.5, Hz, 1H), 7.38 – 7.29 (m, 1H), 7.14 (m, 2H), 7.04 (d, *J* = 2.5 Hz, 1H), 4.00 (d, *J* = 11.0

Hz, 1H), 3.83 (dd, *J* = 11.0, 4.9 Hz, 1H), 3.75 (s, 3H), 3.36 (s, 3H), 2.17 – 1.97 (m, 1H), 0.88 (d, *J* = 6.7 Hz, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) δ 169.3, 168.7, 135.6, 128.4, 122.6, 121.8, 119.6, 119.3, 113.0, 110.8, 56.3, 52.6, 52.2, 42.1, 30.6, 21.9, 17.9. LC-ESI-MS rt 10.41 min, m/z 304 (M+1), 326 (M+Na), 629 (2M+Na). Anal. calcd. for $C_{17}H_{21}NO_4$ (303.1): C 67.31, H 6.98, N 4.62; found C 67.50, H 6.99, N 4.62.

17b: ^{1}H NMR (400 MHz, Chloroform-*d*) mixture of isomers δ 8.19 (s, 0.4H, minor), 8.14 (s, 0.6H, major), 7.75 – 7.56 (m, 1H), 7.33 (dd, *J* = 8.0, 3.8, Hz, 1H), 7.21 – 7.04 (m, 2H), 6.99 (d, *J* = 2.5 Hz, 0.6H), 6.89 (d, *J* = 2.0 Hz, 0.4H), 4.02 (d, *J* = 11.5 Hz, 0.6H), 3.95 (d, *J* = 12.1 Hz, 0.4H), 3.86 (dd, *J* = 11.5, 4.1 Hz, 0.6H), 3.86 (dd, *J* = 12.1, 4.1 Hz, 0.4H), 2.32 (s, 3H), 2.10 (m, 0.6H), 2.02 – 1.95 (m, 0.4H), 1.90 (s, 3H), 1.50 (s, 9H), 0.99 – 0.75 (m, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) mixture of isomers δ 203.9, 203.2, 168.5, 167.5, 135.5, 135.4, 128.9, 128.4, 123.3, 122.9, 121.9, 121.7, 119.9, 119.6, 119.3, 119.2, 113.3, 112.4, 111.0, 110.7, 82.0, 81.3, 66.3, 66.0, 41.9, 41.4, 30.6, 30.4, 29.0, 27.9, 27.4, 27.1, 22.2, 22.1, 17.5, 17.2. LC-ESI-MS rt 10.54 min (major), 11.05 min (minor), m/z 352 (M+Na). Anal. calcd. for $C_{18}H_{23}NO_3$ (329.2): C 72.92, H 8.26, N 4.25; found C 72.43, H 8.24, N 4.24.

18: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 7.77 (s, 2H), 7.67 (d, *J* = 8.2 Hz, 2H), 7.25 (dd, *J* = 8.0, 1.0 Hz, 2H), 7.21 – 7.03 (m, 4H), 6.99 (d, *J* = 2.4 Hz, 2H), 4.27 (d, *J* = 8.4 Hz, 1H), 2.76 – 2.54 (m, 1H), 1.03 (d, *J* = 6.6 Hz, 6H). ^{13}C NMR (100 MHz, Chloroform-*d*) mixture of isomers δ 137.0, 136.1, 123.6, 121.5, 119.2, 118.7, 110.9, 110.8, 58.9, 41.0, 21.8. LC-ESI-MS rt 10.91 min, m/z 289 (M+1), 311(M+Na). Anal. calcd. for $C_{20}H_{20}N_2$ (288.2): C 83.30, H 6.99, N 9.71; found C 83.23, H 6.97, N 9.73.

19a: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.00 (s, 1H), 7.65 (dd, *J* = 8.0, 1.3 Hz, 1H), 7.35 (dd, *J* = 8.2, 1.0 Hz, 1H), 7.14-7.20 (m, 2H), 6.99 (d, *J* = 2.4 Hz, 1H), 3.53 (s, 3H), 3.42 – 3.29 (m, 1H), 2.83 (dd, *J* = 15.0, 5.7 Hz, 1H), 2.71 (dd, *J* = 15.0, 9.6 Hz, 1H), 2.20 – 1.97 (m, 1H), 0.92 (d, *J* = 6.7 Hz, 3H), 0.94 (d, *J* = 6.7

Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) 170.6, 136.1, 127.5, 121.7, 120.9, 119.2, 119.0, 117.9, 110.9, 49.6, 40.1, 32.6, 30.3, 20.5, 20.1 LC-ESI-MS rt 9.36 min, m/z 246 (M+1), 268 (M+Na). Anal. calcd. for $C_{15}H_{19}NO_2$ (245.1): C 73.44, H 7.81, N 5.71; found C 73.61, H 7.82, N 5.70.

19b: ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.01 (s, 1H), 7.66 (dd, *J* = 8.1, 1.3 Hz, 1H), 7.44 – 7.29 (d, 2H, *J* = 7.2 Hz), 7.24 – 7.03 (m, 1H), 6.95 (d, *J* = 2.4 Hz, 1H), 3.35 (dt, *J* = 8.0, 6.6 Hz, 1H), 2.95 (dd, *J* = 15.0, 8.0 Hz, 1H), 2.77 (dd, *J* = 15.0, 6.6 Hz, 1H), 2.18 – 2.02 (m, 1H), 1.99 (s, 3H), 0.94 (d, *J* = 6.7 Hz, 3H), 0.89 (d, *J* = 6.7 Hz, 3H). ^{13}C NMR (100 MHz, Chloroform-*d*) 209.4, 136.3, 127.2, 121.9, 121.7, 119.3, 119.0, 117.3, 111.2, 47.0, 39.3, 32.7, 30.1, 20.5, 20.3.LC-ESI-MS rt 8.71 min, m/z 230 (M+1), 252 (M+Na), 268 (M+K). Anal. calcd. for $C_{15}H_{19}NO$ (229.1): C 78.56, H 8.35, N 6.11; found C 78.36, H 8.34, N 6.13.

17c: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.03 (bs,NH); 7.55 (d, 1H, J= 5.0 Hz); 7.35-7.02 (m, 9H); 5.06 (d, 1H, J= 8.0 Hz); 4.39 (d, 1H, J= 8.0 Hz); 3.96 (q, 2H, J= 7.2 Hz); 2.16 (s, 3H); 1.01 (t, 3H J= 7.2 Hz)). ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 203.1, 168.1, 141.3, 136.2, 128.3, 128.0, 126.7, 126.4, 122.4, 121.4, 119.4, 111.2, 66.6, 61.4, 43.0, 30.3, 13.7 LC-ESI-MS rt 9.8 min; m/z= 358 (M+23); 693 (2M+23). Minor isomer: isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.00 (bs,NH); 7.55 (d, 1H, J=5.0 Hz); 7.35-7.02 (m, 9H); 5.09 (d, 1H, J= 8.0 Hz); 4.51 (d, 1H, J= 8.0 Hz); 3.98 (q, 2H, J= 7.2 Hz); 2.03 (s, 3H); 0.99 (t, 3H J= 7.2 Hz)). ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 202.2, 168.0, 141.3, 136.1, 128.6, 128.1, 126.7, 126.6, 122.2, 120.7, 119.6, 117.2, 111.0, 65.8, 61.6, 42.6, 30.9, 13.6. LC-ESI-MS rt 9.6 min; m/z= 358 (M+23); 693 (2M+23). Anal. calcd. for $C_{21}H_{21}NO_3$ (335.1): C 75.20, H 6.31, N 4.18; found C 75.15, H 6.29, N 4.20.

17d: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.00 (bs, NH); 7.39-7.11 (m, 7H); 6.99 (d, 1H, *J*=2.0 Hz), 6.85 (m, 1H); 6.68 (s, 1H), 4.96 (d, 1H, J= 12.0 Hz); 4.37 (d, 1H, J= 12.0 Hz); 3.95 (q, 2H, J= 7.2 Hz); 2.20 (s, 3H); 0.98 (t, 3H, J=7.2 Hz). ^{13}C NMR (100 MHz,

Chloroform-*d*) δ: 202.1, 167.9, 157.8 (d, J_{C-F} = 214 Hz), 140.9, 132.7, 128.4(d, J_{C-F} = 10 Hz), 128.0, 127.3, 126.9, 123.0, 119.3, 111.7, 110.4 (d, J_{C-F} =9 Hz), 104.6 (d, J_{C-F} = 23 Hz), 66.3, 61.5, 29.6, 28.4, 13.9. LC-ESI-MS rt 9.7 min; m/z= 376 (M+23); 729 (2M+23). Minor isomer: ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.97 (bs, NH); 7.39-7.11 (m, 7H); 6.98 (d, 1H, *J*=2.0 Hz), 6.68 (s, 1H), 6.85 (m, 1H); 5.00 (d, 1H, J= 11.6 Hz); 4.48 (d, 1H, J=11.6 Hz); 3.97 (q, 2H, J=7.2 Hz); 2.20 (s, 3H); 0.98 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 202.9, 168.0, 157.5 (d, J_{C-F} = 214 Hz), 143.3, 133.2, 128.5(d, J_{C-F} = 10 Hz), 127.9, 127.2, 126.4, 122.5, 119.3, 111.6, 110.1 (d, J_{C-F} =8 Hz), 104.8 (d, J_{C-F} = 23 Hz), 65.6, 61.5, 30.4, 28.4, 13.7. LC-ESI-MS rt 9.9 min; m/z= 376 (M+23); 729 (2M+23). isomer Anal. calcd. for $C_{21}H_{20}FNO_3$ (353.1): C 71.37, H 5.70, N 3.96; found C 71.22, H 5.71, N 3.97.

17e: Major isomer: ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.95 (bs, NH); 7.37-6.95 (m, 9H); 5.04 (d, 1H, J= 12.0 Hz); 4.36 (d, 1H, J=12.0 Hz); 3.96-4.00 (m, 2H); 2.40 (s, 3H), 2.05 (s, 3H); 0.98 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 202.3, 168.2, 141.4, 134.5, 128.7, 128.2, 127.9, 127.8, 126.6, 124.0, 121.4, 118.7, 115.7, 110.6, 66.0, 61.4, 30.3, 28.0, 21.6, 13.6. LC-ESI-MS rt 10.4 min; m/z= 372 (M+23); 721 (2M+23). Minor isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.00 (bs, NH); 7.37-6.95 (m, 9H); 5.07 (d, 1H, J= 11.8 Hz); 4.49 (d, 1H, J=11.8 Hz); 3.96-4.00 (m, 2H); 2.40 (s, 3H), 2.05 (s, 3H); 1.01 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 203.1, 168.0, 141.4, 134.5, 128.9, 128.5, 127.9, 127.8, 126.8. 124.1, 121.5, 118.9, 116.7, 110.7, 66.8, 61.2, 30.4, 28.2, 21.4, 13.8. LC-ESI-MS rt 10.2 min; m/z= 372 (M+23); 721 (2M+23). Anal. calcd. for $C_{22}H_{23}NO_3$ (349.4): C, 75.62; H, 6.63; N, 4.01; found C 75.64, H 6.60, N 4.02.

17f: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.69-7.11 (m, H); 7.00 (m, 1H), 6.94 (s, 1H); 5.07 (d, 1H, J= 11.0 Hz); 4.39 (d, 1H, J=11.0 Hz); 3.98 (m, 2H); 3.70 (s, 3H), 2.06 (s, 3H); 0.98 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 202.0, 167.9, 141.2, 136.9, 130.6, 128.0, 127.7, 126.6, 125.5, 121.7, 119.1, 118.9, 115.6, 109.0, 65.9, 61.3, 42.6, 30.1, 13.8. Minor isomer: ^{1}H

NMR (400 MHz, Chloroform-*d*) δ: 7.69-7.11 (m, 8H); 7.00 (m, 1H), 6.94 (s, 1H); 5.10 (d, 1H, J= 11.2 Hz); 4.50 (d, 1H, J=11.2 Hz); 3.98 (m, 2H); 3.70 (s, 3H), 2.18 (s, 3H); 1.00 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 202.9, 168.0, 141.6, 136.9, 129.5, 128.2, 127.9, 126.8, 126.0, 121.9, 119.3, 118.9, 114.7, 109.1, 66.6, 61.3, 43.0, 30.8, 13.7. LC-ESI-MS rt 10.7 min; m/z= 372 (M+23). LC-ESI-MS rt 11.2 min; m/z= 372 (M+23). Anal. calcd. for $C_{22}H_{23}NO_3$ (349.2): C 75.62, H 6.63, N 4.01; found C 75.49, H 6.62, N 4.03.

17g: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.47 (bs, NH); 8.05 (m, 2H), 7.45-7.00 (m, 8H); 5.20 (d, 1H, *J*=12.0 Hz), 4.45 (d, 1H, *J*=12.0 Hz); 4.03 (m, 2H); 2.18 (s, 3H); 1.07 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 201.7, 167.6, 149.3, 146.5, 136.2, 126.2, 123.7, 122.7, 121.8, 119.9, 118.5, 114.6, 111.3, 65.3, 61.8, 42.2, 30.9, 13.8. LC-ESI-MS rt 9.7 min; m/z= 398 (M+18), 403 (M+23). Minor isomer: isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.42 (bs, NH); 8.05 (m, 2H), 7.45-7.00 (m, 8H); 5.23 (d, 1H, *J*=12.0 Hz), 4.55 (d, 1H, *J*=12.0 Hz); 4.05 (m, 2H); 2.16 (s, 3H); 0.99 (t, 3H, J=7.2 Hz) ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 201.0, 167.4, 149.5, 146.6, 136.3, 129.0, 125.9, 123.6, 122.5, 121.2, 119.7, 118.7, 115.4, 111.5, 65.4, 61.7, 41.8, 30.2, 13.6. LC-ESI-MS rt 9.6 min; m/z= 398 (M+18), 403 (M+23). Anal. calcd. for $C_{21}H_{20}N_2O_5$ (380.1): C 66.31, H 5.30, N 7.36; found C 66.10, H 5.29, N 7.37.

17h: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.32 (bs, NH); 7.29 (dd, 1H, $J_{H\text{-}H}$ = 1 Hz, $J_{H\text{-}F}$ = 9.6 Hz), 7.21 (dd, 1H, $J_{H\text{-}H}$ = 9.2 Hz, $J_{H\text{-}F}$ = 4.4 Hz), 6.89 (d, 1H, *J* = 2.4 Hz), 6.87 (dd, 1H, $J_{H\text{-}H}$ = 9.2 Hz, $J_{H\text{-}F}$ = 2.4 Hz), 4.22 (m, 2H), 4.02 (d, 1H, *J*=12.0 Hz), 3.77 (m, 1H), 2.03 (m, 1H), 1.94 (s, 3H), 1.31 (t, 3H, *J*=7.2 Hz), 0.85 (d, 6H, *J* =6.6. Hz). ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 203.3, 169.1, 157.9 (d, $J_{C\text{-}F}$ = 233 Hz), 132.1, 129.0 (d, $J_{C\text{-}F}$ = 10 Hz), 125.1, 113.4, 111.6, 110.6 (d, $J_{C\text{-}F}$ =12 Hz), 104.5 (d, $J_{C\text{-}F}$ = 18 Hz), 64.7, 61.5, 42.0, 30.6, 27.2, 21.9, 17.7, 14.0. LC-ESI-MS rt 10.1 min; m/z= 342 (M+23), 661 (2M + 23). Minor isomer: isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ 8.20 (1H, bs), 7.29 (dd, 1H, $J_{H\text{-}H}$ = 1 Hz, $J_{H\text{-}F}$ = 9.6 Hz), 7.21 (dd, 1H, $J_{H\text{-}H}$ = 9.2 Hz, $J_{H\text{-}F}$ = 4.4 Hz), 7.02 (d, 1H, *J* = 2.8

Hz), 6.87 (dd, 1H, J_{H-H} = 9.2 Hz, J_{H-F} = 2.4 Hz), 4.22 (m, 2H), 4.10 (d, 1H, *J*=10.8 Hz), 3.77 (m, 1H); 2.31 (s, 3H), 1.98 (m, 1H), 0.83 (d, 6H, *J* =6.6. Hz). 0.77 (t, 3H, *J*=7.2 Hz). ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 202.8, 168.5, 158.0 (d, J_{C-F} = 233 Hz), 132.0, 128.7 (d, J_{C-F} = 10 Hz), 124.6, 113.3, 112.4, 110.1 (d, J_{C-F} =12 Hz), 103.4 (d, J_{C-F} = 18 Hz), 64.8, 61.0, 41.7, 30.9, 29.1, 22.0, 17.2, 13.4. LC-ESI-MS rt 9.8 min; m/z= 342 (M+23), 661(2M + 23). Anal. calcd. for $C_{18}H_{22}FNO_3$ (319.1): C 67.69, H 6.94, N 4.39; found C 67.71, H 6.90, N 4.38.

17i: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.97 (bs, NH); 7.45 (s, 1H); 7.23-6.86 (m, 3H); 4.25 (q, 2H, J=7.2); 4.10 (d, 1H, J= 11.0 Hz); 3.83 (dd, 1H, J= 2.8 Hz, 11.0 Hz); 2.47 (s, 3H); 2.31 (s, 3H); 2.04 (m, 1H); 1.31 (t, 3H, J= 7.2 Hz), 0.88 (d, 6H, J= 6.6 Hz); ^{13}C NMR (100 MHz, Chloroform-*d*) δ: : 203.6, 169.4, 134.6, 128.8, 128.3, 123.6, 121.9, 119.2, 112.5, 110.6, 65.0, 61.0, 41.6, 31.0, 29.2, 21.9, 17.8, 17.3, 13.5. LC-ESI-MS rt 10.5 min; m/z= 338 (M+23); Minor isomer: isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 8.02 (bs, NH); 7.45 (s, 1H); 7.23-6.86 (m, 3H); 4.02 (d, 1H, J=12.0 Hz); 3.86 (dd, 1H, J=4.0 Hz, 12.0 Hz); 3.80 (q, 2H, J= 7.6 Hz); 2.43 (s, 3H); 1.98 (m, 1H); 1.92 (s, 3H); 1.02 (d, 3H, J= 6.6 Hz); 0.88 (d, 6H, 6.6 Hz); 0.81 (t, 3H, J=7.6 Hz); ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 203.3, 168.6, 134.0, 128.7, 128.1, 123.3, 121.8, 119.0, 111.5, 110.4, 64.9, 61.4, 41.8, 30.8, 29.0, 21.5, 17.6, 17.1, 14.1. LC-ESI-MS rt 10.8 min; m/z= 338 (M+23). Anal. calcd. for $C_{19}H_{25}NO_3$ (315.2): C 72.35, H 7.99, N 4.44; found C 72.42, H 7.96, N 4.45.

17l: Major isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.65 (d, 1H, J= 8.0 Hz); 7.25-7.08 (m, 3H); 6.74 (s, 1H); 4.23 (q, 2H, J=7.2 Hz); 4.09 (d, 1H, J= 10.8 Hz); 3.81 (dd, 1H, J= 4.4 Hz, 10.8 Hz); 3.70 (s, 3H); 1.91 (s, 3H); 1.96 (m, 1H); 1.28 (t, 3H, J=7.2 Hz); 0.84 (d, 3H, J=6.6 Hz); 0.83 (d, 3H, J= 6.6 Hz); ^{13}C NMR (100 MHz, Chloroform-*d*) δ: 203.3, 1695, 128.7, 127.5, 121.5, 119.7, 119.0, 110.4, 109.1, 64.8, 61.4, 41.7, 32.8, 30.6, 29.0, 22.1, 17.7, 14.1 LC-ESI-MS rt 10.8 min; m/z= 338 (M+23). Minor isomer: isomer ^{1}H NMR (400 MHz, Chloroform-*d*) δ: 7.65 (d, 1H, J= 8.0 Hz); 7.25-7.08 (m, 3H); 6.88 (s, 1H); 4.20 (q, 2H, J=7.2 Hz);

4.01 (d, 1H, J= 12.0 Hz); 3.86 (dd, 1H, J= 4.0 Hz, 12.0 Hz); 3.71 (s, 3H); 2.28 (s, 3H); 2.02 (m, 1H); 0.84 (d, 3H, J= 6.6 Hz); 0.83 (d, 3H, J= 6.6 Hz);0.73 (t, 3H, J=7.2 Hz).^{13}C NMR (100 MHz, Chloroform-*d*) δ: 203.2, 168.5, 136.4, 129.0, 121.2, 119.3, 118.8, 111.4, 108.8, 65.0, 60.8, 42.0, 32.7, 30.4, 27.6, 22.0, 17.2, 13.4. LC-ESI-MS rt 10.5 min; m/z= 338 (M+23). Anal. calcd. for $C_{19}H_{25}NO_3$ (315.2): C 72.35, H 7.99, N 4.44; found C 72.60, H 7.98, N 4.43.

Chapter 3

Applications of synthesized molecules as scaffolds for bioactive compounds generation

In this part of the thesis we will discuss about applications of synthesized compounds during the last three years. Classes of compound will be divided into topics and discussed within their relative field of interest.

We will begin by talking about $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands, whose potential applications are as anticancer compounds and we will consider their biological evaluations. For this class of compounds, we report also the syntheses conjugated molecules with a fluorescent fragment to obtain a diagnostic tool. The second topic taken into account deals with $\alpha_4\beta_1$ integrin ligands, which differ from those mentioned above for the insertion of a further pharmacophoric moiety to fit in the different binding site. Also for this class, related biological tests and syntheses are reported. Potential Linezolid-like antibiotics have been finally developed and the corresponding syntheses and biological evaluations will be discussed in the third part of this chapter.

3.1 $\alpha_V\beta_3$ and $\alpha_5\beta_1$ Integrin Ligands

3.1.1 Introduction to integrins $\alpha_V\beta_3$ and $\alpha_5\beta_1$

Integrins are a large family of heterodimeric transmembrane glycoproteins and they are involved in several cellular activities as adhesion, differentiation, proliferation and cellular migration.[23] Alterations or aberrations in integrin-mediated cell adhesion have been connected with the pathogenesis of several diseases such as atherosclerosis, osteoporosis, cancer and a variety of inflammatory disorders and for these reasons integrins are an attractive target for the development of therapeutic agents.[24] In general, every development of an antagonist compound with a potential biological effect is based on the identification of key-recognition motifs between the target molecule and the natural ligand. Actually, the above-mentioned motifs have been identified for only few subtypes of integrins. Among different classes of these proteins, the $\alpha_V\beta_3$ has been deeply investigated as it is involved in tumor proliferation and metastasis through the formation of new blood vessels. It is known that $\alpha_V\beta_3$ integrin interacts with extracellular matrix (ECM) through a specific recognition motif: the RGD tripeptide sequence (Arg-Gly-Asp). Using this recognition triad, the protein is able to bind a wide number of extracellular matrix components like fibronectin, fibrinogen, vitronectin and osteopontin.[25]

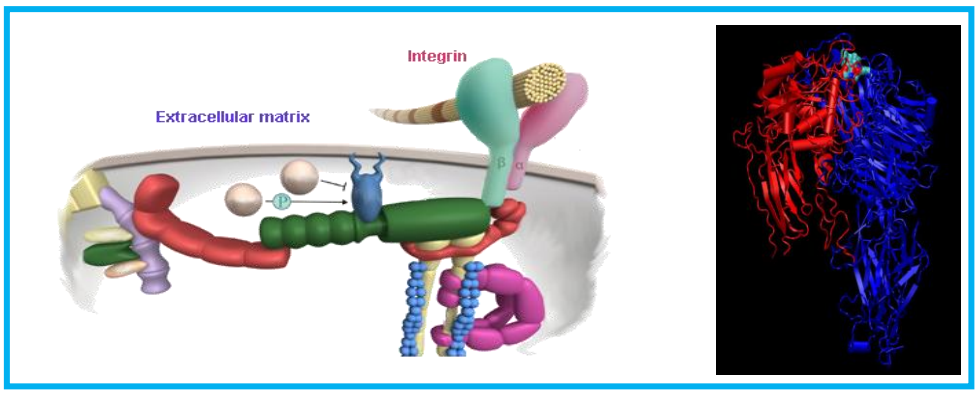


Fig. 11. Integrin $\alpha_V\beta_3$ into membrane (left) and isolated representation (right).

Another integrin family, recognized as proangiogenic receptor[26], use the same recognition sequence to bind fibronectin: the $\alpha_5\beta_1$.[27] During their migration in vitro or angiogenesis in vivo, this integrin may regulate the function of integrin $\alpha_V\beta_3$ on endothelial cells. Activation of $\alpha_5\beta_1$ potentiates $\alpha_V\beta_3$-mediated migration, whereas $\alpha_5\beta_1$ integrin antagonists inhibit $\alpha_V\beta_3$-mediated cell spreading. On this base, it is possible to realize that a single antagonist that mime a RGD sequence, could mediate the activity of both types of integrine and then block the same pathway of angiogenesis.[27]

In literature several classes of integrin ligands that contain the RGD sequence have been reported and the X-ray analysis[28] of the complex between one of them (RGDfV) and $\alpha_V\beta_3$ integrin, shows that the ligand interacts manly through electrostatic forces. To bind the protein, the ligand uses a charged clamp that is attracted by regions with an opposite charge in the binding site: Asp interact with a metal cation in the β subunit and Arg with two Asp in the α subunit.

Integrin ligands containing the RGD sequence have important problems that - only recently – the scientific community had to face and overcome. The major problem is their oral bioavailability and this makes the good activity registered by linear and cyclic antagonist less interesting. This is mainly due to the degradation of peptide ligand by peptidase enzyme that occurs under physiological conditions. Research groups that work on this field are motivated to invest their force in finding a good compound able to retain high activity versus integrins and, at the same time, to provide a good bioavailability by possessing a low molecular weight and a non-peptidic backbone that mimicks the RGD sequence. A large part of developed ligands is based on a central rigid scaffold, to which it is possible to insert appendages that are able to mimic Arg and Asp side chains.[29]

As reported in the literature, the arginine-mimiking group and the carboxylic aspartate-mimiking group are essential to bind the active site on protein.[30]

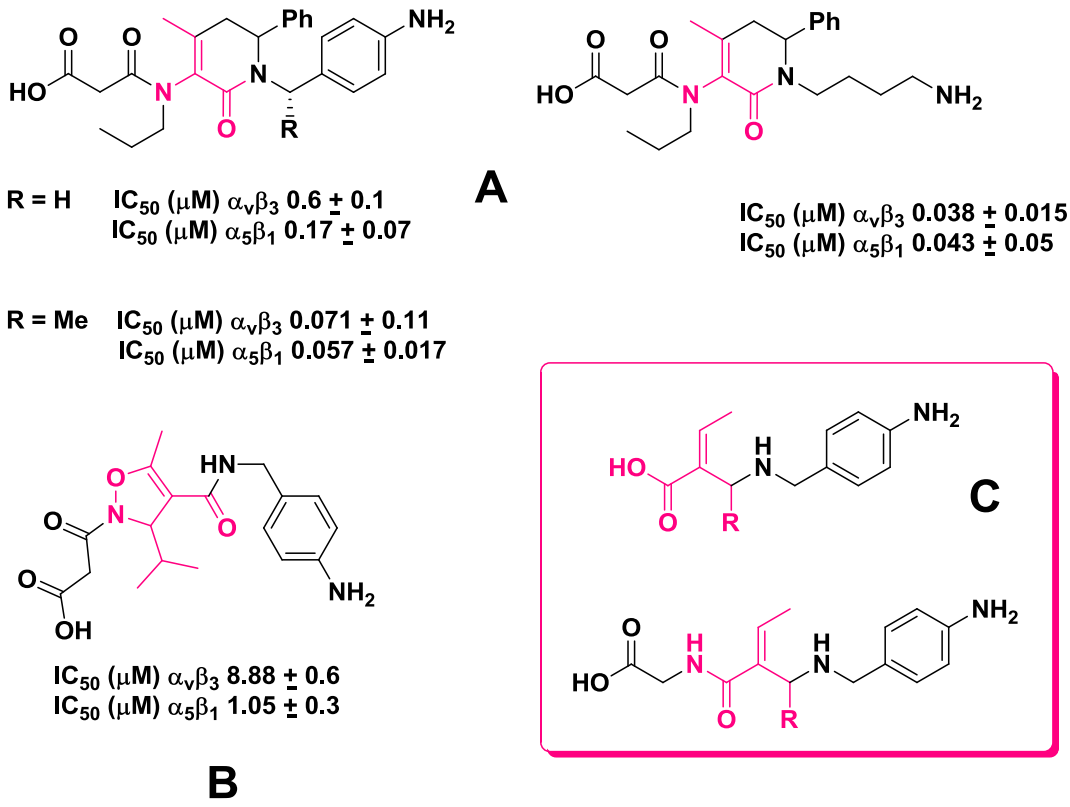


Fig.12. $\alpha_v\beta_3/\alpha_5\beta_1$ integrin ligands containing an unsaturated α- or β-amino acid fragment (A, B) and novel linear dehydro-β-amino acid structures (C).

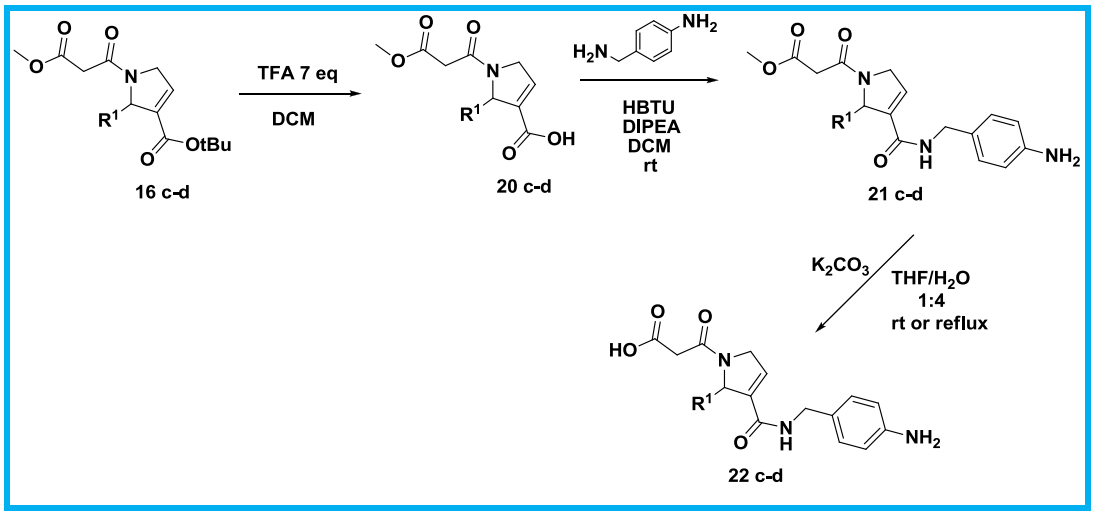
Some years ago, in our group, a series of $\alpha_V\beta_3$ integrin ligands based on 5,6-dihydropyridin-2-one were identified as scaffold and they showed promising interactions with integrin receptors.[31] The introduction of a β-amino acid into RGD-containing cyclic peptides has been already reported with the aim to stabilize distinct conformations, having a γ-turn with centrally positioned glycine. On the other hand, the incorporation of a linear or constrained amino acid into an RGD sequence may give access to simple mimetics with an increased bioactivity and stability. From that experience we tried to develop new classes of integrin ligands by changing the central heterocyclic core and by inserting β-aminoacids also in the structure of linear antagonist.

3.1.2 Synthesis of heterocyclic $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands

Novel synthetic pathways for the preparation of bioactive small molecules are based on heterocyclic scaffolds[32] and for this reason their syntheses receive ever-increasing interest. The central rigid core, represented by the heterocycle, is often the initial point from which more complex molecules with specific activity begin to be prepared. Moreover, inserting a lateral appendage on hetereocycle allows easy construction of small libraries, differing for the substitution pattern. Recently, the term "privileged structure" has been frequently reported to define recurring structural elements that are likely to facilitate bindings with biological target, inducing ordered spatial disposition of side chains[33]. In this context, the proline represents a good starting point to synthesize potential molecules with biological activity. In fact, the cyclic structure of this amino acid gives to the residue a particular conformational rigidity due to the reduced allowed rotations[34] and it is possible to use it where a turned peptide backbone is required. Several modifications have been performed on the proline scaffolds and, among these, β-proline has received great attention since the substitution of an α-amino acid with the corresponding β-analog in key position of a bioactive sequence still affords conformational restrictions. These modifications represent a winning approach to develop mimetics, overcoming the low bioavailability limitations of natural peptides.[35] Our research group showed that the peptides containing the β-analog of proline recorded a good receptor affinity together with enhanced stability towards enzymatic hydrolysis.[36] On the other hand, unsaturated α-amino acids have been also explored and 3,4-dehydroproline (Dhp), the analog of proline having a double bond between Cβ and Cγ, showed to induce changes in the structure and biological properties of collagen, proteins and peptides.[37] On this base, we tried to combine the notions mentioned above by synthesizing a dehydro-β-proline employable as scaffold for our researches.

To synthesize ***dehydro-β-pirrole*** ligands we started from intermediates **16c-d** discussed in section 2.6 that, through a deprotection of t-Butyl ester carried out

with TFA in dichloromethane in 24h, provided corresponding acids **20c-d** (Scheme 18). Crude reaction mixtures could be directly used for the subsequent reaction. The following step is the coupling with 4-aminobenzylamine. Even if in theory both the amine functions could give this reaction, the primary amine's reaction rate was remarkably faster, and only products **21c-d** were observed in good yields. This synthetic pathway allowed us to obtain the final products in yields included between 50-80%, after methyl ester hydrolysis by treatment with K_2CO_3 in THF/H_2O (1:4 ratio). Compounds **22c-d** were purified by DOWEX ion exchange resin.

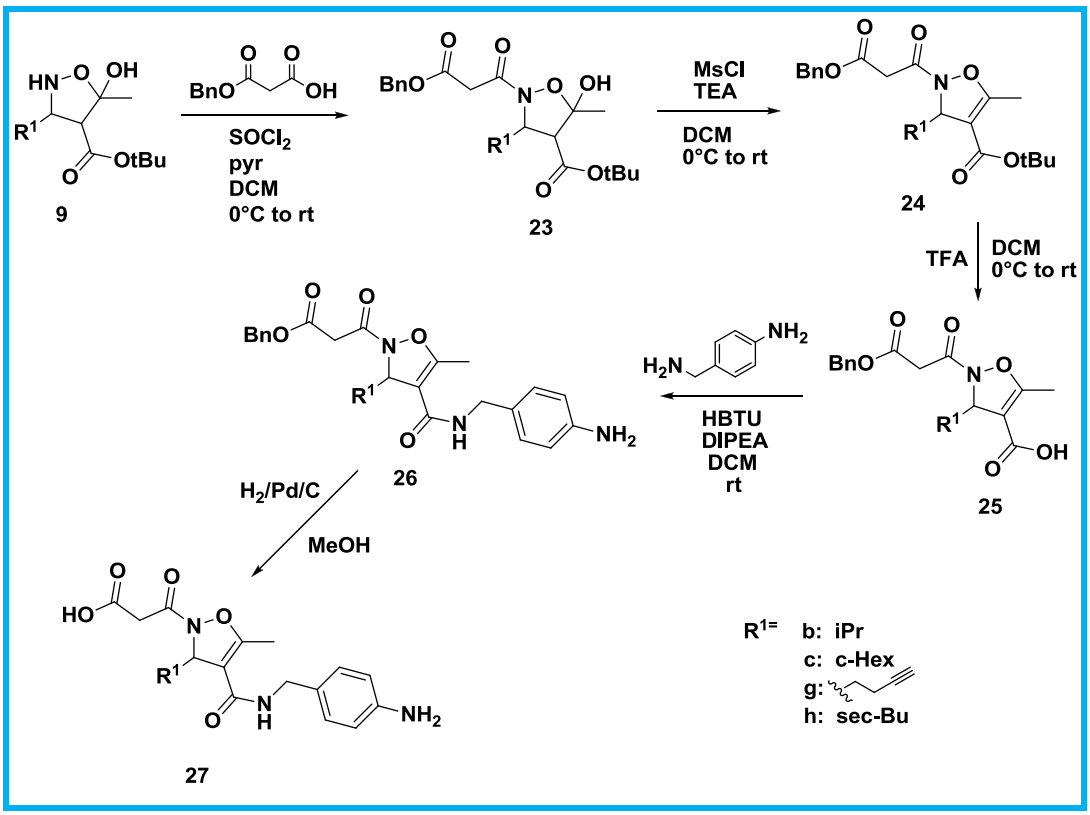


Scheme 18. Synthesis of β-dehydropyrrolic ligands.

Another class of integrin ligands developed by us, contains the ***isoxazoline*** scaffold.

Willing to test a different scaffold for integrin ligands, our choice has fallen on isoxazoline because its isoxazolidine precursor may be consider as a masked amino acid, as a 1,3 amino alcohol equivalent, or as a furanose ring mimetic in the preparation of bioactive compounds. Indeed, several transcriptional activators contain the isoxazolinic nucleus as conformational constrain element. In the

literature compounds containing isoxazoline and isoxazolidine ring are reported as antimitotic combrestatin analogues. Aware of that, we developed a small library of integrin ligands that present isoxazoline core as scaffold and the same lateral appendages inserted in a dehydro-β-pyrrolic ligands discussed before, to retain the pharmacophoric entity

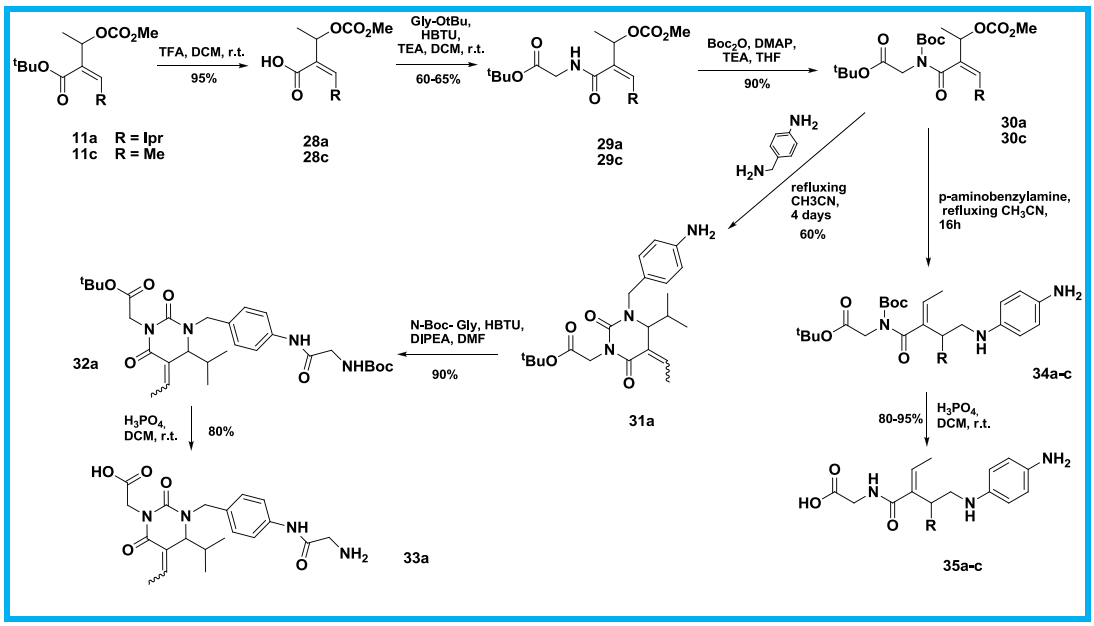


Scheme 19. Synthesis of isoxazolinic compounds.

The synthesis of these class of compounds began from intermediates **9** met in section 2.2.2. The nitrogen on the ring has been protected with benzyl malonic derivative that will correspond to the acidic part of the final RGD mimetic (Scheme 19) to get **23**. After the treatment of this compound with mesyl chloride and TEA in excess, the corresponding dehydrate isoxazoline **24** has been obtained. The relative acids **25** were obtained by the hydrolysis of the terbutylester with

trifluoroacetic acid. Compounds **25** were coupled with 4-amino-benzylamine to afford amides, finally by simple hydrogenation of **26** final products **27** were obtained.

The third class of heterocyclic compound developed is relative to ***pyrimidindione derivatives***. After the deprotection of t-Bu ester on carbonate **11a-c** with TFA in DCM, we coupled the resulted acid with Gly-OtBu using HBTU and TEA in DCM to obtain compound **29a-c** (Yield 60-65%).



Scheme 20.Synthesis of pyrimidindione and linear derivatives.

This was protected on nitrogen with Boc using Boc_2O, DMAP and TEA in DCM to provide **30a-c**. Performing the S_N2' with p-aminobenzylamine on these compounds, we have seen a different behavior, in comparison with the one observed for similar reactions on different substrates. After prolonged reaction times (4 days), spontaneous intramolecular cyclization to compound **31a** occurred (60% yield). On the contrary, starting from **30c**, compound **34c** was isolated in 70% yield, and no traces of the cyclic derivative could be detected even after

longer refluxing times. Simultaneous removal of the ester and carbamate protections by treatment of **34a** e **c** in a phosphoric acid/dichloromethane biphasic mixture, allowed to obtain **35a** e **c** in 95 e 80% yield.

The different behaviors of **30a** and **30c** in the intramolecular cyclization can be ascribed to the different conformational arrangement induced by the steric hindrance of methyl and isopropyl chains. The rotation of the C2eC3 bond in 7 that may occur to minimize steric hindrance, displays for **30a** the amine nitrogen in the proper position to act as nucleophile on the carbamate carboxylic group (Fig. 13).

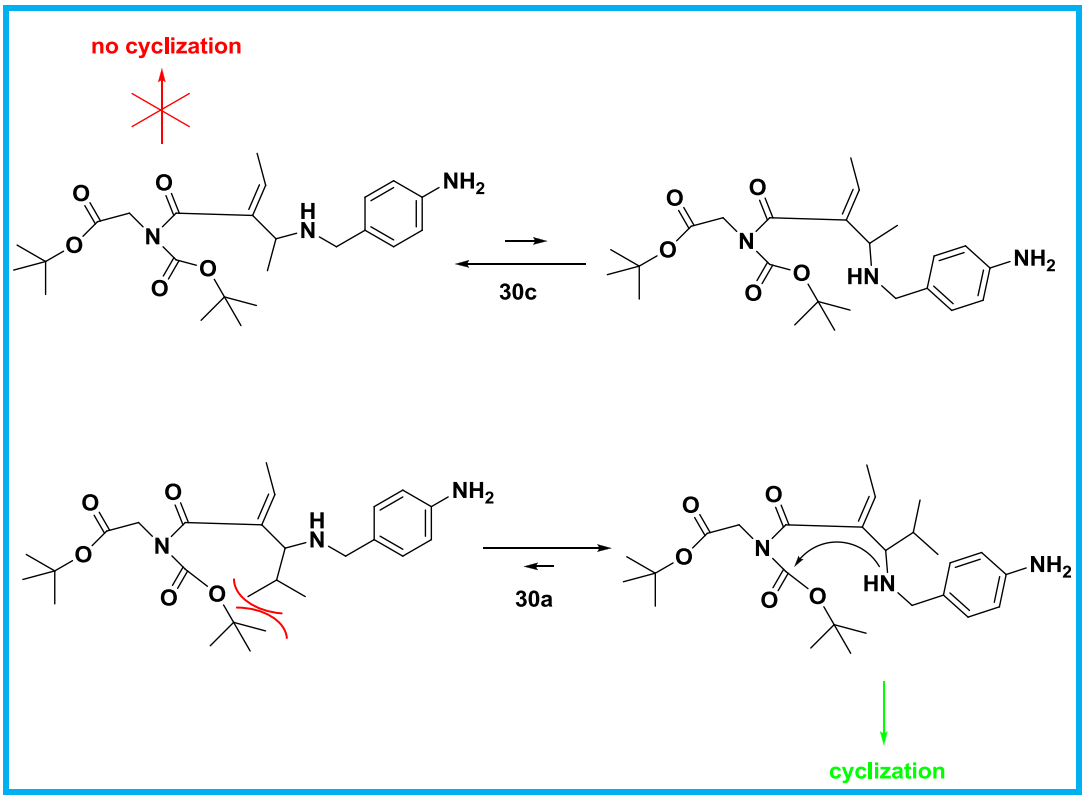


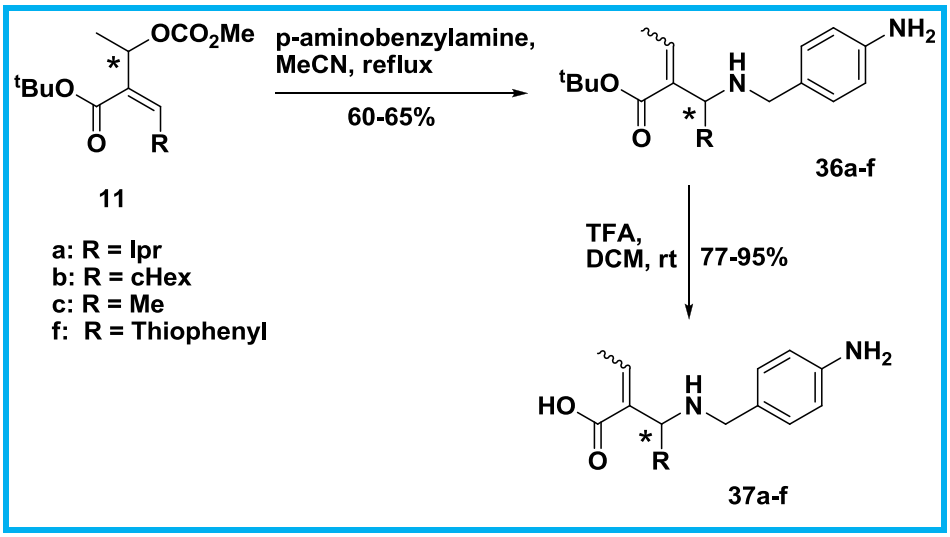
Fig. 13. Proposed conformational arrangements of major isomers Z-**30a** and Z-**30c**, explaining the alternative behaviors in intramolecular cyclization.

Since this reaction affords dihydropyrimidindiones, that have been successfully introduced as rigid scaffolds in bioactive molecules of considerable interest for the

pharmaceutical industry, this process deserves further investigation. The effect of the carbamate leaving group, amine nucleophile, C3 stereochemistry and amino acid chain will be evaluated by computational studies and reported in due course. To explore whether the cyclic structure could be suitable for the interaction with the receptor, dihydropyrimidindione **31a** was further decorated with a second glycine residue. Compound **32a** was isolated in 90% yield, following the usual coupling protocol and then deprotected to afford the free amino acid **33a** (80% yield).

3.1.3 Synthesis of linear $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands

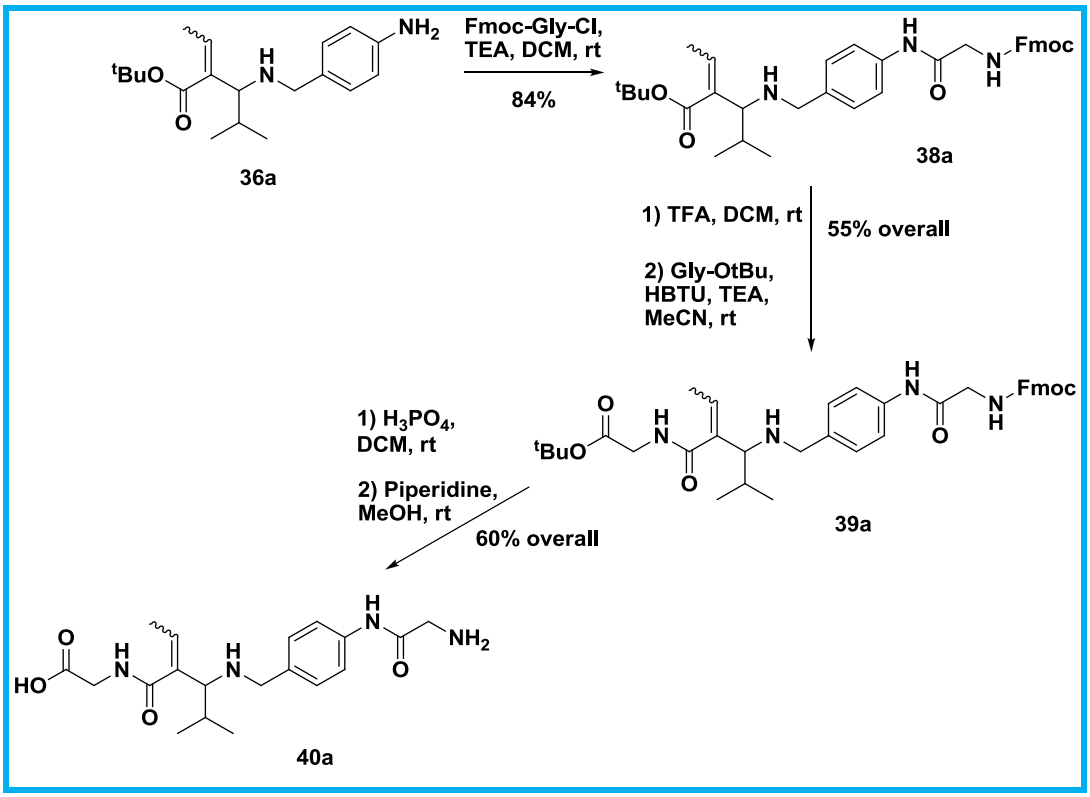
In previous papers, we have identified a series of $\alpha_v\beta_3/\alpha_5\beta_1$ integrin dual ligands, sharing as a common feature the presence of an unsaturated α- or β-amino acid fragment, inserted into a cyclic backbone (Fig. 12, compounds A and B).[38] The introduction of a β–amino acid into RGD-containing cyclic peptide has been already reported in the literature with the aim to stabilize distinct conformations, having a β-turn with centrally positioned glycine. In this part, we will describe a novel class of linear integrin antagonists derived from dehydro-β-amino acids, as esemplified in Figure 12, where the heterocycle in **A** is substituted by the dehydro-β-amino acid linked to a carboxylic acid and a basic function to mimic aspartic and guanidinic appendages. Dehydro-β-amino acid containing molecules, with different variations at the nature and length of the side chains were synthesized through S_N2' substitution of chiral carbonate-esters or amides and analyzed as integrin antagonists. At first, the small library of N-p-aminobenzyl-dehydro-β-amino acids **37a-c** and **37f** was prepared through an easy two step procedure starting form carbonates **11a-f** (Scheme 21). The regio- and stereoselective synthesis of amino esters **36a-f** was based on our previously reported results and has been described in due papers, where each compound has been accurately analyzed.[39]



Scheme 21. Synthesis of linear compounds.

Removal of the ester protection to obtain free amino acids **37a-f** was performed by treatment with an excess of trifluoroacetic acid in DCM at room temperature (77 e 95% yield). Although the compounds **37a-f** have a shorter length than that generally required for RGD mimetic ligands, their low molecular weight and the respect of the commonly accepted rules for bioavailability [40], suggested that they could represent valuable lead compounds. On the other hand, the shorter length may lead to a partial ligand/receptor interaction and to an uncommon effect on adhesion and intracellular signaling.

The spontaneous cyclization occurring during the substitution reaction on N-Boc amide **30c** described before, suggested that inversion of the steps in the synthetic protocol could allow the easier isolation of the linear compound. Thus, compound **38a** was isolated in 84% yield by treatment of **36a** with Fmoc-Gly-Cl in the presence of triethylamine. After removal of the tert-butyl ester, coupling with Gly-OtBu under the usual peptide synthesis conditions, allowed to obtain **39a** in 55% overall yield. Selective removal of Fmoc and t-butyl protection afforded **40a** in 60% yield (Scheme 22).



Scheme 22. Synthesis of modified linear compounds

3.1.4 Biological evaluations

Inhibition of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin-mediated cell adhesion (Isoxazoline derivatives)

The ability of racemic compounds shown in table 8 to inhibit the adhesion of K562 cells (human erythroleukemia expressing $\alpha_5\beta_1$ integrin) or SK-MEL-24 cells (human malignant melanoma expressing $\alpha_v\beta_3$ integrin) to immobilized fibronectin was evaluated. These models are widely used to investigate potential antagonists of $\alpha_v\beta_3$ integrin-mediated SK-MEL-24 cell adhesion and $\alpha_5\beta_1$ integrin-mediated K562cell adhesion.[41]

As reported in this table, only the final products have been tested, which possess the functional groups and spacing required for receptor affinity. In these experiments, cells were seeded onto plates coated with different substrata and allowed to adhere before quantitation of the number of adherent cells in the presence of increasing concentrations of test compounds. Under these conditions, no significant cell adhesion was observed for bovine serum albumin (BSA)-coated plates (negative control) or nonspecific substrate- coated plates (i.e., collagen I for SK-MEL-24 expressing $\alpha_v\beta_3$ integrin and poly-l-lysine for K562 expressing $\alpha_5\beta_1$ integrin; data not shown). The registered inhibition of the adhesion of SK-MEL-24 and K562 cells to fibronectin or vitronectin was compared with that of the standard compounds, Ac-Asp-Arg-Leu-Asp-Ser-OH and H-Gly-Arg-Gly-Asp-Asn-Pro-OH, known to be potent inhibitors of cell adhesion mediated by $\alpha_v\beta_3$ integrin (**entry 6-7**).[42]

Among the five new RGD mimetics tested, the compound **27b** in entry 2 exhibited the highest potency as an inhibitor of cell adhesion mediated by $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins because its IC_{50} values are in the nanomolar range.

The compound **27a** (entry 1), which has a smaller methyl substituent on the isoxazoline ring, maintained notable efficacy, showing IC_{50} values only tenfold less potent than the isopropyl substituted analogue (IC_{50} = 32 nm versus $\alpha_v\beta_3$ integrin

and 12 nm versus $\alpha_5\beta_1$ integrin). The sec-butyl-substituted derivative **27h** (entry 3) showed decreased inhibitory activity, but an increase in selectivity toward $\alpha_v\beta_3$ integrin. A strong preference of compound **27c** (entry 4) for $\alpha_v\beta_3$ integrin was observed, as it has an IC_{50} value of 20 nm as an $\alpha_v\beta_3$ integrin antagonist, whereas its IC_{50} value toward $\alpha_5\beta_1$ is 1 m. Finally, the compound in reported entry 5, which was designed to verify the effect of elongation and a terminal aliphatic amine on adhesion inhibition, exhibited poor IC_{50} values, in the millimolar to sub-millimolar range.

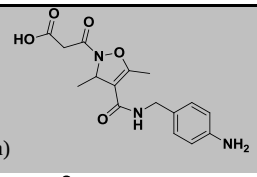
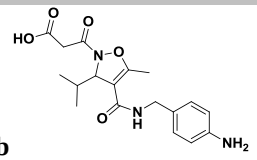
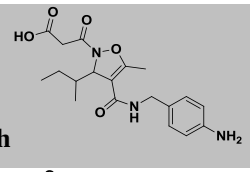
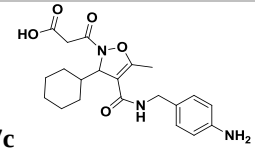
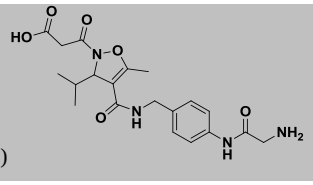
Entry	**Compound**	**IC_{50}[nM] $\alpha_v\beta_3$**	**IC_{50}[nM] $\alpha_5\beta_1$**
1	 a)	32±3	12±4
2	 **27b**	8.8±0.6	1.05±0.3
3	 **27h**	360±70	1320±80
4	 **27c**	20±6	1030±50
5	 a)	15500±900	>100000
6	Ac-Asp-Arg-Leu-Asp-Ser-OH	25±3	>100000
7	H-Gly-Arg-Gly-Asp-Asn-Pro-OH	234±32	>100000

Table 8. a) Synthesized in the past in our laboratory.

The results listed in Table 8 show that the isoxazoline ring is an effective scaffold for inducing the proper orientation of the Asp and Arg-mimicking chain, as smaller members of the library displayed excellent inhibitory activities toward adhesion mediated by both receptors, and can thus be regarded as dual inhibitors.

Interestingly, compounds **6a** and **6b** are more potent than the reference compounds Ac-Asp-Arg-Leu-Asp-Ser-OH and H-Gly-Arg-Gly-Asp-Asn-Pro-OH, as they inhibit cell adhesion mediated by $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin with IC_{50} values lower than those of the reference compounds. Moreover, as **27b** exhibited the highest potency as an inhibitor of cell adhesion to fibronectin, we tested its ability to block the adhesion of SK-MEL-24 cells to vitronectin relative to the standard peptide Ac-Asp-Arg-Leu-Asp-Ser-OH. The compound showed an IC_{50} value of 3.14 nm, while the reference peptide showed an IC_{50} value in the sub-micromolar range (IC_{50}= 0.33μm) versus vitronectin mediated cell adhesion.

Effect of an integrin antagonist on fibronectin-induced ERK phosphorylation (Isoxazoline derivatives)

Connection of the extracellular matrix components with integrins generates an intracellular signaling that involves increased phosphorylation of cytoplasmic second messengers. In this contest, the phosphorylation of ERK plays a central role in fibronectin-mediated survival signaling through integrins: in fact, cell adhesion activates ERK by binding of $\alpha_5\beta_1$ integrins at the cell surface to ECM proteins such as fibronectin.[43] Therefore, we investigated the effect of our most effective compound **27b** on fibronectin-induced phosphorylation of ERK in K562 cells, which express $\alpha_5\beta_1$ integrin.

K562 cells were serum-starved in RPMI-1640 containing 1% fetal bovine serum (FBS) for 16 h; thereafter, they were pre-incubated with our compound for 30 min in suspension and plated for 30 min on fibronectin or poly-l-lysine (used as non-specific substrate).

In agreement with other studies, the ERK pathway is activated in K562 cells exposed to phorbol-12-myristate- 13-acetate (PMA, 65 nm; Figure 2). In cells exposed to fibronectin, a significant increase in phosphorylated ERK is observed after 60 min, whereas no change is detected for shorter exposure times, as previously described. This late ERK phosphorylation induced by fibronectin has been previously reported.[43]

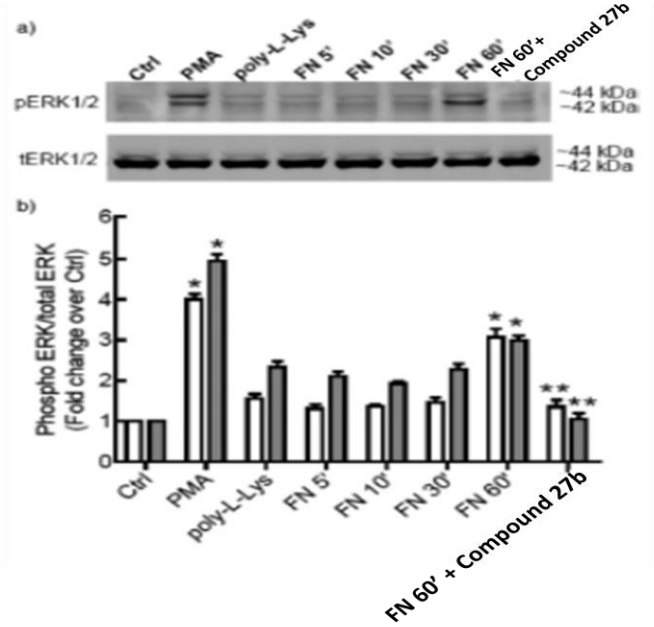


Fig. 14.

Compound **27b** prevents fibronectin-induced phosphorylation of ERK. K562 cells were serum-starved in RPMI-1640 containing 1% FBS for 16 h; cells were then pre-incubated with our compound for 30 min in suspension. The cells were kept in suspension (Ctrl) or plated on fibronectin (FN) or on poly-l-lysine (poly-l-Lys). After 30 min, cells were lysed, and lysates were analyzed by Western blot using an

antibody directed against phosphorylated ERK (pERK) or total ERK (tERK). The information reported in figure 14 may be resumed as reported below:

a) Western blot shows that control cells exposed to PMA (65 nm) or plated on FN had a much stronger signal for phosphorylated ERK than those plated on poly-l-lysine (used as nonspecific substrate). Pre-incubation with our compound before plating on fibronectin caused a significant decrease in the amount of phosphorylated ERK in K562 cells.

b) The densitometric analysis of the bands (mean ±SEM; n=6): p42 (white color), p44 (grey color) is also reported; the amount of phosphorylated ERK is normalized to the total amount of ERK. Densitometric quantification confirmed that our compound specifically decreases fibronectin-induced phosphorylation of ERK. In control cells, as expected, PMA induced a significant increase in ERK phosphorylation; *$p<0.01$ versus Ctrl, **$p<0.001$ versus FN60' (Newman−Keuls test after ANOVA).

Inhibition of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin-mediated cell adhesion (β-dehydro-pyrrole derivatives)

Relatively to the results reported in Table 9 the β-dehydro-pyrrole derivatives have been submitted to the same kind of biological assays already presented for definition of activity against $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins., Derivatives with c-Hex side chain afforded no activity (**entry 4-5**) while derivatives bearing iPr side chain possess a good activity (**entry 1-3**)

In particular, the racemic mixture (**entry 1**) showed two IC_{50} values comparable to the isoxazolinic compounds seen before, while the isolated enantiomers have a different behavior. The R-enantiomer presents a selective inhibition versus $\alpha_v\beta_3$ than $\alpha_5\beta_1$ (**entry 2**) and the S-enantiomer is not active to $\alpha_v\beta_3$ and showed a moderate inhibition value versus $\alpha_5\beta_1$. From these results we can suppose a

possible synergic action between enantiomers difficult to understand only on the data that we have to date.

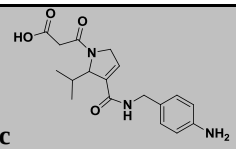
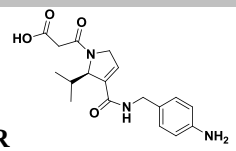
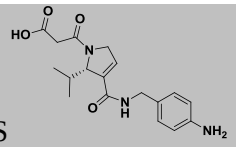
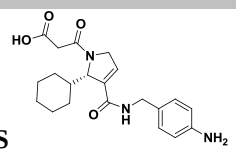
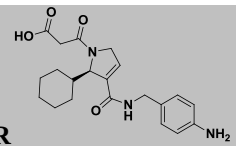
Entry	Compound	IC_{50}[μM] $\alpha_v\beta_3$	IC_{50}[μM] $\alpha_5\beta_1$
1	 **22c-rac**	0.01142	0.0036
2	 **22c-R**	0.0376	90.5
3	 **22c-**S	Not active	0.531
4	 **22d-S**	Not active	Not active
5	 **22d-R**	Not active	Not active

Table 9. $\alpha_v\beta_3$ integrin and $\alpha_5\beta_1$ integrin-mediated cell adhesion to fibronectin in the presence of dehydro-β-amino acid containing RGD mimetics (dehydro-pyrrole derivatives).

Inhibition of $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin-mediated cell adhesion (linear and uridinic derivatives)

In table 10 the activities of linear dehydro-β-amino acids and of six membered ring derivative **33a** are presented. The shorter molecules reported in **entries 1-4** showed unexpected activity and selectivity depending on the substituent in position 3.

Treatment with methyl derivative (**entry 1**) did not affect $\alpha_v\beta_3$ integrin-mediated cell adhesion, but excellent affinity toward $\alpha_5\beta_1$ integrin was observed. On the other hand, the introduction of the isopropylic group, gave better results since comparable affinity could be observed toward both integrins (**entry 2**). Increased activity toward $\alpha_5\beta_1$ integrin was observed for thiophenyl derivative (**entry 3**), while for c-Hex derivative a preferential affinity toward $\alpha_v\beta_3$ receptor could be observed (**entry 4**). Anyway, the difference in the affinity among the members of this class of compounds is not sufficient to deduce strong structure-activity relationship effects.

Moreover, these molecules are quite smaller than the usual RGD mimetics as cilengitide, and for this reason difference in the steric hindrance of the side chain may not be crucial for receptor affinity. Bringing the molecules to a more appropriate length, it has been possible to observe an increase in the anti-adhesion activity (**entries 5-6**). In particular the iPr-derivate (**entry 6**) showed an increased $\alpha_5\beta_1$ integrin-mediated inhibitory activity respect to the corresponding shorter analog (**entry 2**). On the contrary, **35c** displayed good activity and a strong preference toward $\alpha_v\beta_3$ integrin, behaving as a selective not dual inhibitor. Introduction of a second further glycine unit allowed elongation of the molecule. Moreover in this ligand the interaction with the α chain of the receptor should no longer occur through the aniline group but with the glycine amino moiety. These modification allowed to obtain the most potent dual antagonist (**entry 7**). Finally, the last tested molecule **33a** does not have significant activity to the studied integrins, because a dramatic decrease in affinity toward both integrins occurred for this compound (**entry 8**), thus suggesting that the conformational constraint introduced by the cyclic scaffold doesn't allow pharmacophore's proper orientation.

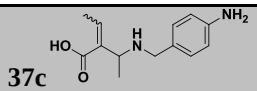
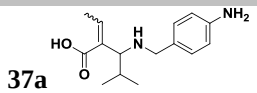
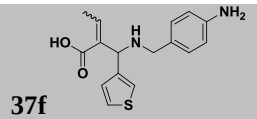
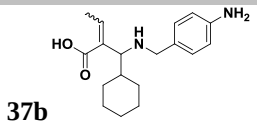
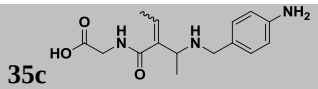
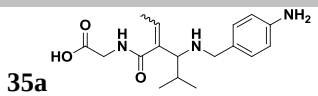
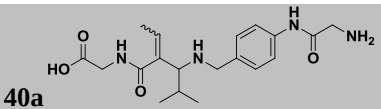
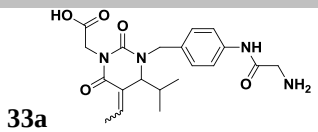
Entry	Compound	IC_{50}[nM][a] $\alpha_v\beta_3$	IC_{50}[nM][a] $\alpha_5\beta_1$
1	 **37c**	>100	0.019±0.003
2	 **37a**	1.49±0.05	9.8±0.6
3	 **37f**	3.3±0.3	4.7±0.2
4	 **37b**	0.83±0.08	2.1±0.8
5	 **35c**	0.12±0.03	9.8±0.3
6	 **35a**	2.0±0.1	0.22±0.05
7	 **40a**	0.27±0.06	0.17±0.03
8	 **33a**	>100	>100
9	Gly-Arg-Gly-Asp-Thr-Pro[b]	0.00062±0.00003	0.93±0.08
10	H-Gly-Arg-Gly-Asp-Asn-Pro-OH[b]	0.23±0.03	>100

Table 10. $\alpha_v\beta_3$ integrin and $\alpha_5\beta_1$ integrin-mediated cell adhesion to fibronectin in the presence of dehydro-β-amino acid containing RGD mimetics (linear and pyrimidinone derivatives).

[a] Values are means of three experiments.

[b] Reference compounds.

Effect of dehydro-β-amino acid containing RGD mimetics on fibronectin-induced ERK phosphorylation in DAOY or K562 cells (linear derivatives)

To investigate the effect of the most effective compounds (**entry 5 and 7** in table 10) on intracellular integrin-mediated signaling activation we employed DAOY medulloblastoma cells, which express $\alpha_v\beta_3$ integrin (Fig. 15) .[44] The tested compounds showed a similar potency as cell adhesion inhibitors in DAOY cells (data not shown).

DAOY cells were serum-starved in DMEM for 16 h; thereafter, they were detached and pre-incubated with the two our integrin antagonists for 30 min and plated for 90 min on fibronectin. As shown in Fig. 15, in cells exposed to fibronectin, a significant increase in phosphorylated ERK1/2 is observed after 90 min, while after 120 min ERK1/2 activation is completely abolished (ERK1/2 phosphorylation was not detected in DAOY cells exposed for 30 or 60 min to fibronectin; data not shown). Pre-incubation with both compounds caused a significant decrease in the amount of fibronectin-induced ERK phosphorylation in DAOY cells.

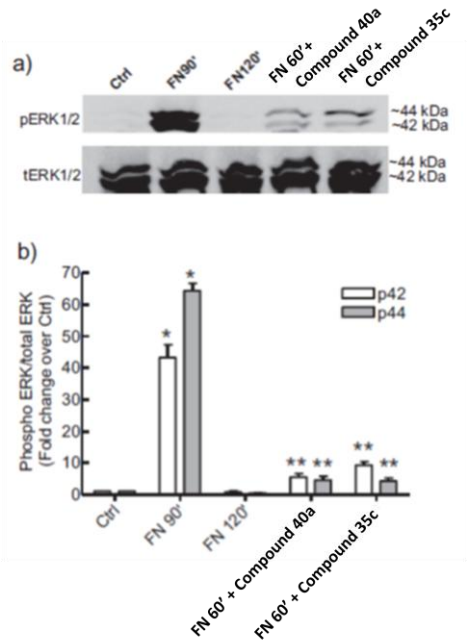


Fig. 15.

To investigate the effect of the most effective compounds **37c** and **40a** on intracellular $\alpha_5\beta_1$ integrin-mediated signaling activation we employed K562 cells. As previously described, K562 cells were serum-starved in RPMI-1640 containing 1% fetal bovine serum (FBS) for 16 h; thereafter, they were pre-incubated with compound **37c** or **40a** (10 μM) for 60 min in suspension and plated for 60 min on fibronectin. In agreement with other studies,[45] the ERK signaling is activated in K562 cells exposed to phorbol-12-myristate-13- acetate positive control (PMA, 65 nm; Fig. 15). Cells exposed to PMA or fibronectin showed a significant increase in phosphorylated ERK after 60 min whereas compound **40a** was able to revert fibronectin-induced ERK1/2 phosphorylation increment (Fig. 16). On the contrary, compound **37c** was not able to block ERK pathway activation. The behavior of compound **37c**, that is ineffective toward $\alpha_v\beta_3$ integrin-mediated cell adhesion and shows high affinity for $\alpha_5\beta_1$ integrin but is not able to block signaling, suggest that probably, it could act through a different mechanism of action that deserves more investigation in future studies. Integrins and their endogenous ligands are large and complex molecules and so there is the potential for a competitive antagonists to interact with more than one site on the integrin to block ligand binding and to prevent the downstream sequela of integrin activation.[46] It is conceivable that small molecules, like **37c** described in this study, can prevent fibronectin-mediated cell adhesion in cells expressing $\alpha_5\beta_1$ integrin, whereas they are not capable to block fibronectin-induced cell signaling. Integrin-mediated adhesion often occurs under tensile forces such as fluid flow or myosin-mediated contractions that cells exert to sample the rigidity of their surroundings. Thus, to enable mechanosensing, integrins could not be constitutively active.[47] These events are mediated by fibronectin and seem to be antagonized by the compound **40a**. On the contrary, compound **37c** may not prevent fibronectin-initiated cell signaling which requires more complex integrin conformational modifications.

In the literature, examples of small molecule ligands that may only partially interact with the binding site and for this reason show an uncommon effect when compared with classical RGD ligands, have been reported.[48] Moreover, several studies have also described compounds that could be classified as allosteric antagonist or allosteric effector: these molecules could not directly compete with the binding of integrin specific ligands.[49]

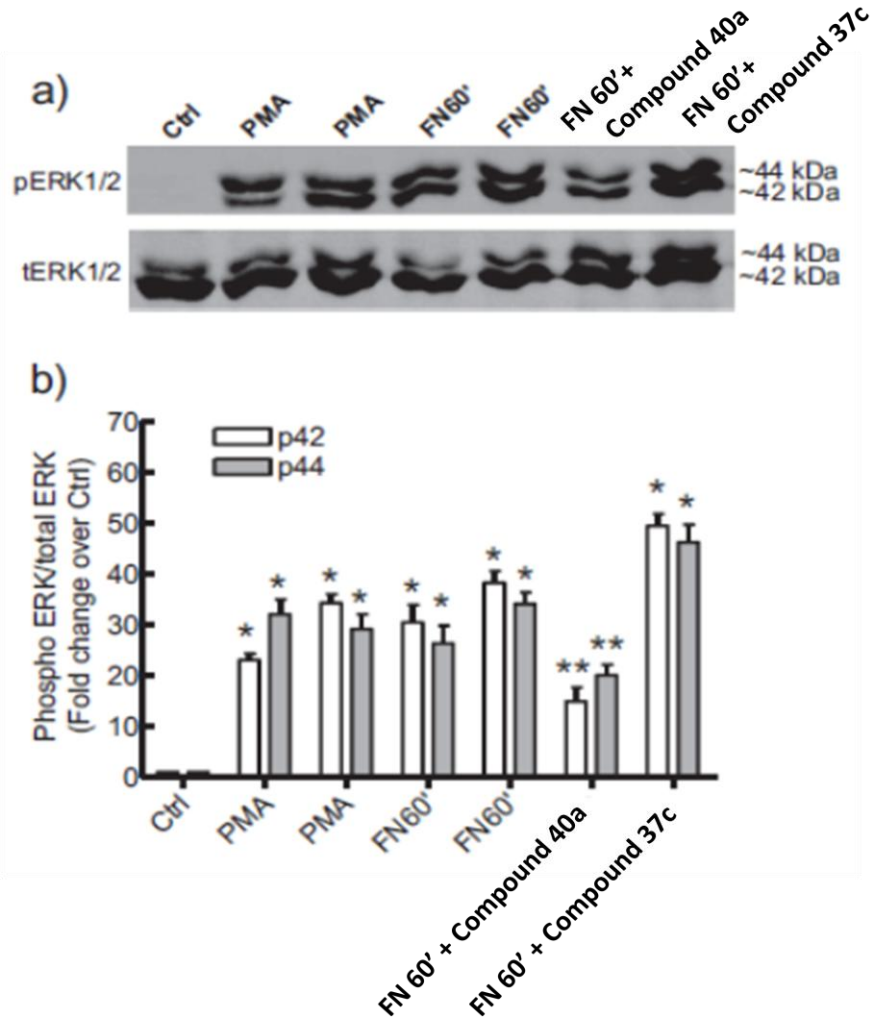


Fig. 16.

3.1.5 Synthesis of fluorescent compounds

In order to develop a new drug delivery system, we searched a molecule able to achieve this new challenge. If among the different classes of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ the choice fells easily on the most active isoxazolinic compounds, suitably modified, for the fluorescent compound to link to the latter, a class of compound developed in the Professor Luc Brunsveld's laboratory in Eindhoven (NL) was chosen. These kind of compounds present different points of interest (Figure 17).

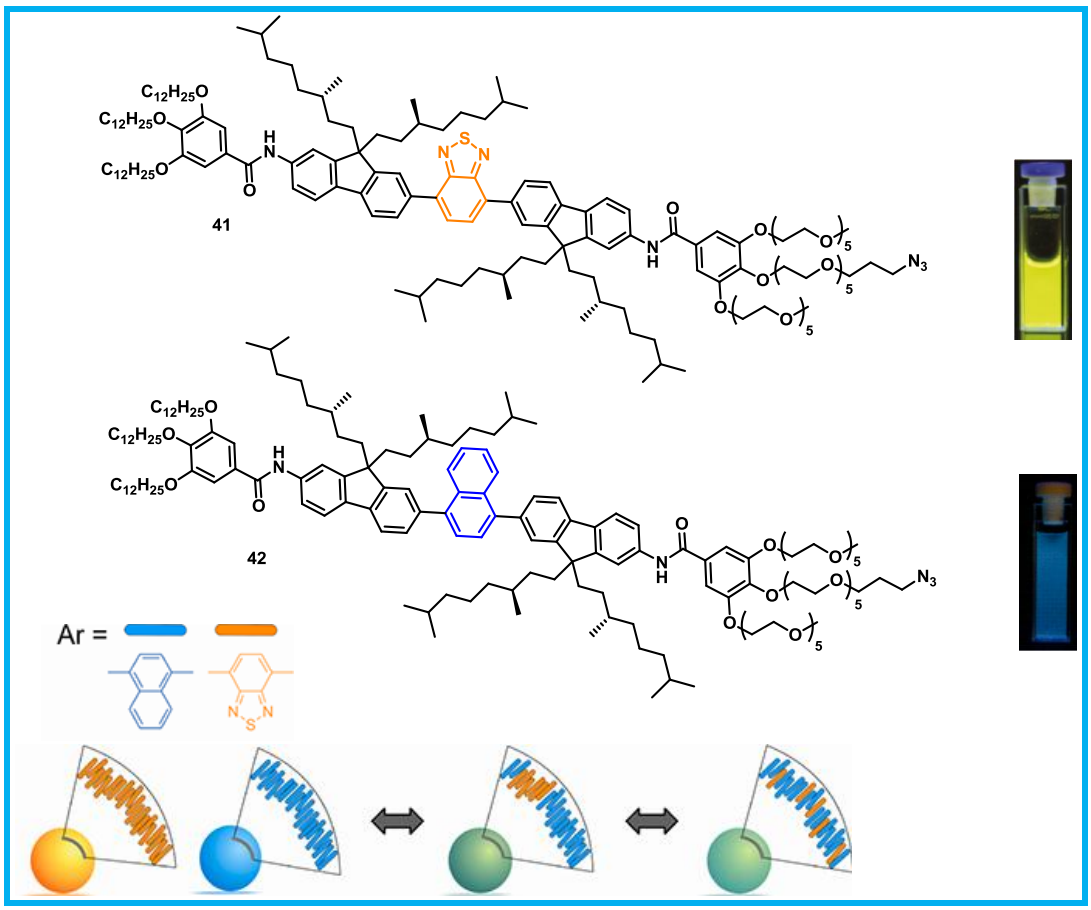
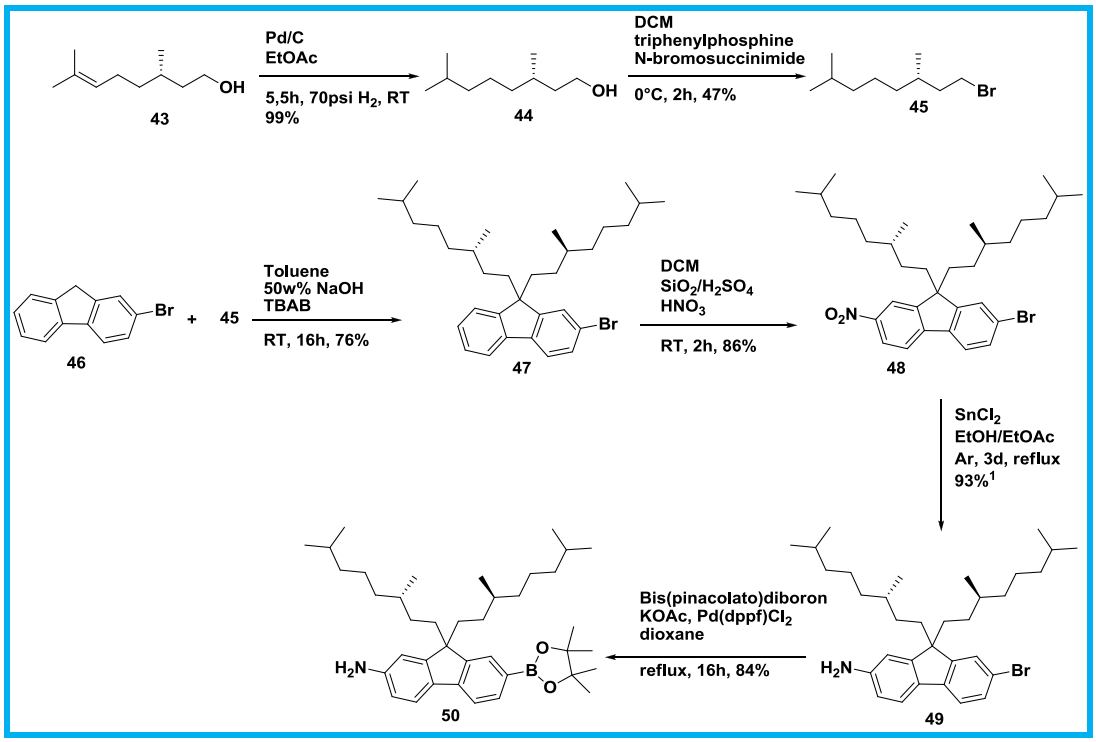


Fig. 17. Fluorescent molecules synthesized and relative nanoparticles formation.

How is possible to see in Figure 17, these molecules consist of a bicyclic aromatic central core on which are linked two fluorenyl entity which in turn are linked to two different gallic acid derivatives. In fact, the chains linked to the gallic acid

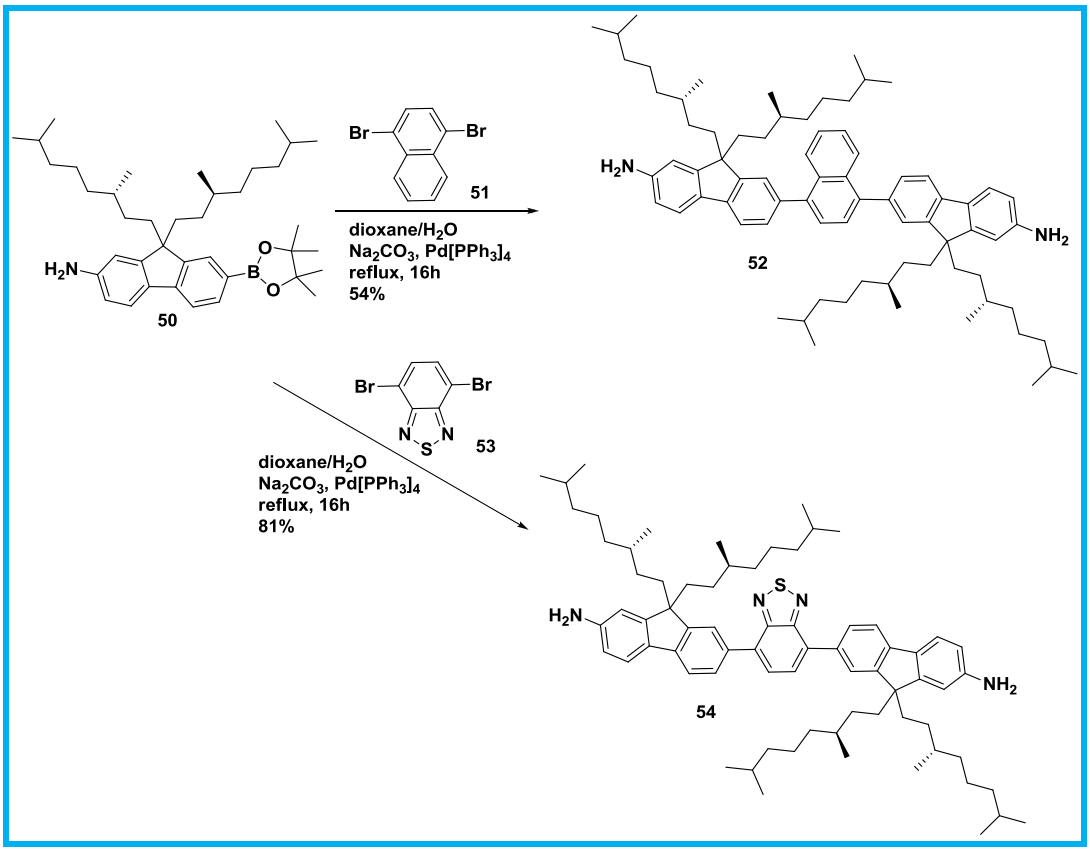
portions, have different nature: one is hydrophobic, since formed by a lipophilic satured chain, and the other is hydrophilic, because formed by a polyethylene glycol chain. The specific affinity towards different kind of solvents of these two portions allows us to obtain amphiphilic nanoparticles.[50]The presence of the fluorenyl moieties and the bicyclic aromatic central core provide a very good detection system under UV irradiation and, in addition, by changing the byciclic aromatic central core, it is possible to obtain a different color under UV lamp. It is also interesting to note that a terminal azide is present on the hydrophilic chain, which allows us the easy connection with an integrin ligand presenting an alkyne side chian, via a simple click reaction. During the PhD period spent abroad, the two fluorescent molecules described before have been synthesized.



Scheme 23.

First, we synthesized the lipophilic chain to insert on fluorenyl group. Starting from alcohol **43**, by reduction of the double bond with H_2/Pd/C **44** has been obtained in

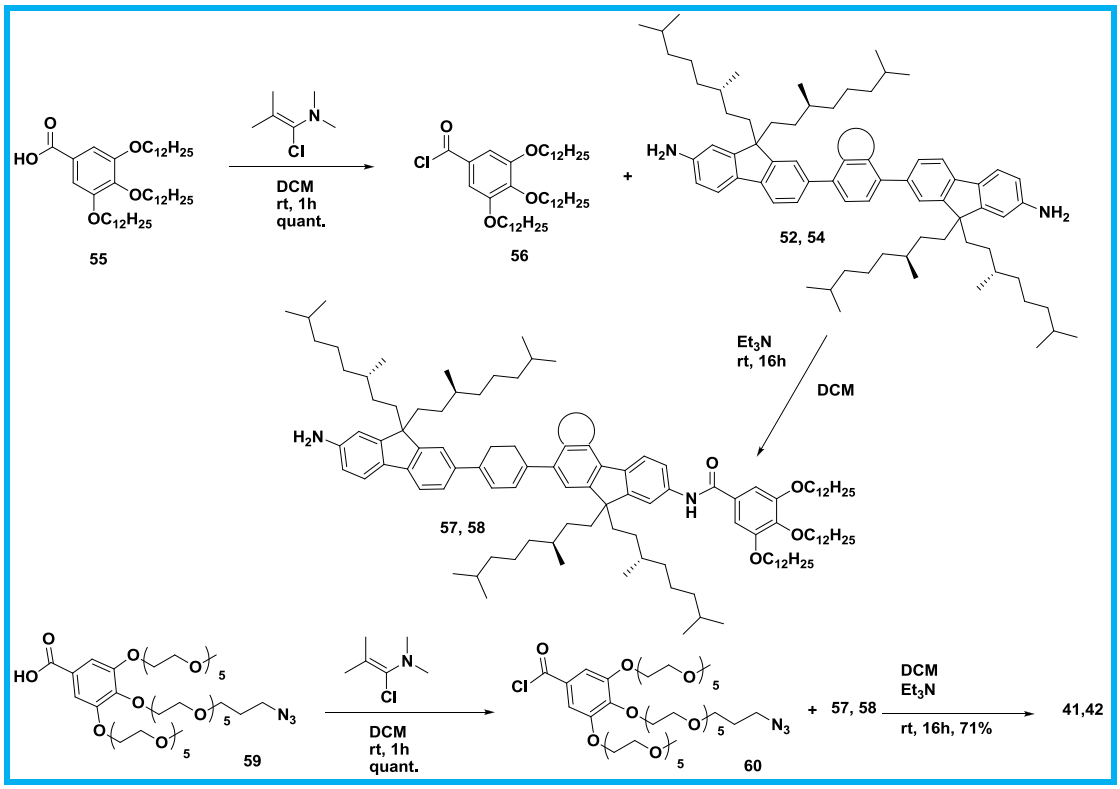
99% yield, which, after treatment by N-bromosuccinimide and tryphenylphosphine, provided bromide **45**. This bromo derivative has been inserted on fluorenyl group **46** using TBAB (Tetra-n-butylammonium bromide) and 50 w% of NaOH in toluene, giving **47** in good yield. By nitration with $SiO_2/H_2SO_4/HNO_3$ of the latter compound, it has been possible to obtain **48** that later provided the amine **49** in 93% yield, by reduction of nitro group with $SnCl_2$ in EtOH/EtOAc after three days of reflux. This amino-bromide has been used to obtain the boronic ester **50** in good yield by treatment with Bis(pinacolato)diboron, KOAc, $Pd(dppf)Cl_2$ in dioxane after 16 hours of reflux (Scheme 23).



Scheme 24.

At this point, the two different bicyclic aromatic core able to give a different color under UV irradiation have been inserted through a Suzuki coupling, using a

mixture of dioxane/H_2O as solvent, Na_2CO_3, $Pd[PPh_3]_4$ and employing the corresponding bicyclic-dibromide **51** and **53**. After 16h of reflux, 54% of **52** and 81% of **54** have been recovered respectively (Scheme 24). Treating the gallic acid derivative **55** with Ghosez's reagent, we obtained the chloride **56** that presents the hydrophobic chains. This chloride has been immediately added to **52** or **54** by syringe pump over 16 hours to obtain the mono-acylation of amine and to provide **57** and **58** selectively (Scheme 25).



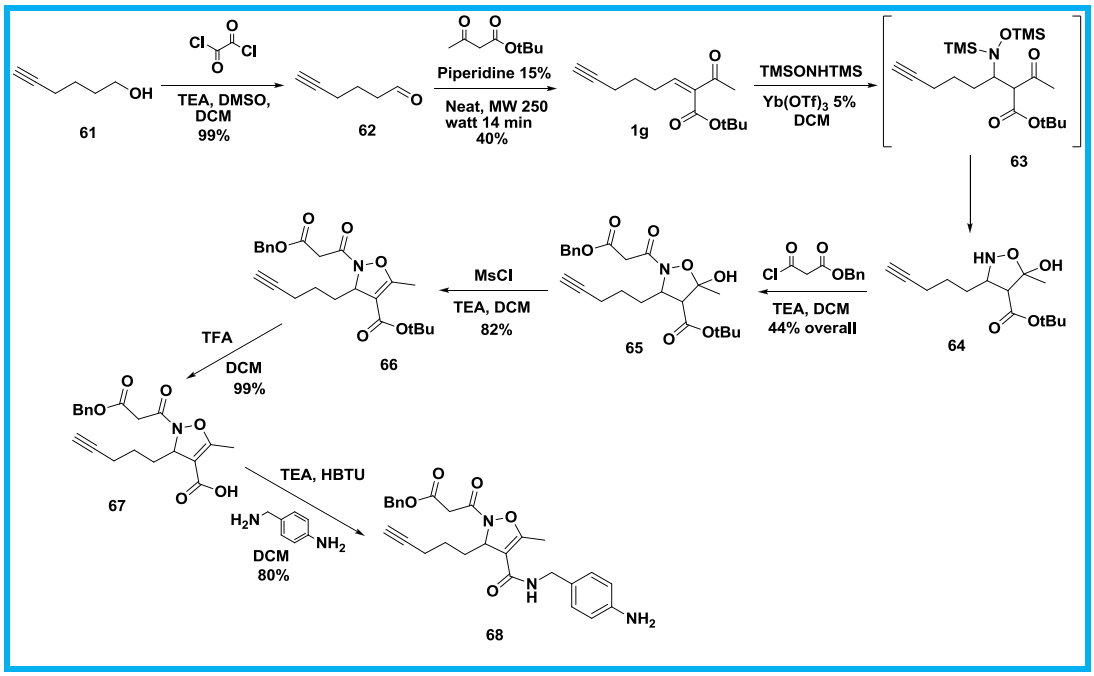
Scheme 25.

Finally, chloride **60** has been prepared following the same approach with Ghosez's reagent on the gallic acid derivative possessing the hydrophilic moiety and then added to **57** and **58** to get **42** and **41** respectively (Scheme 25).

For the experimental section see J. Am. Chem. Soc. 2011, 133, 17063–17071 and Chem. Commun., 2009, 1697–1699.

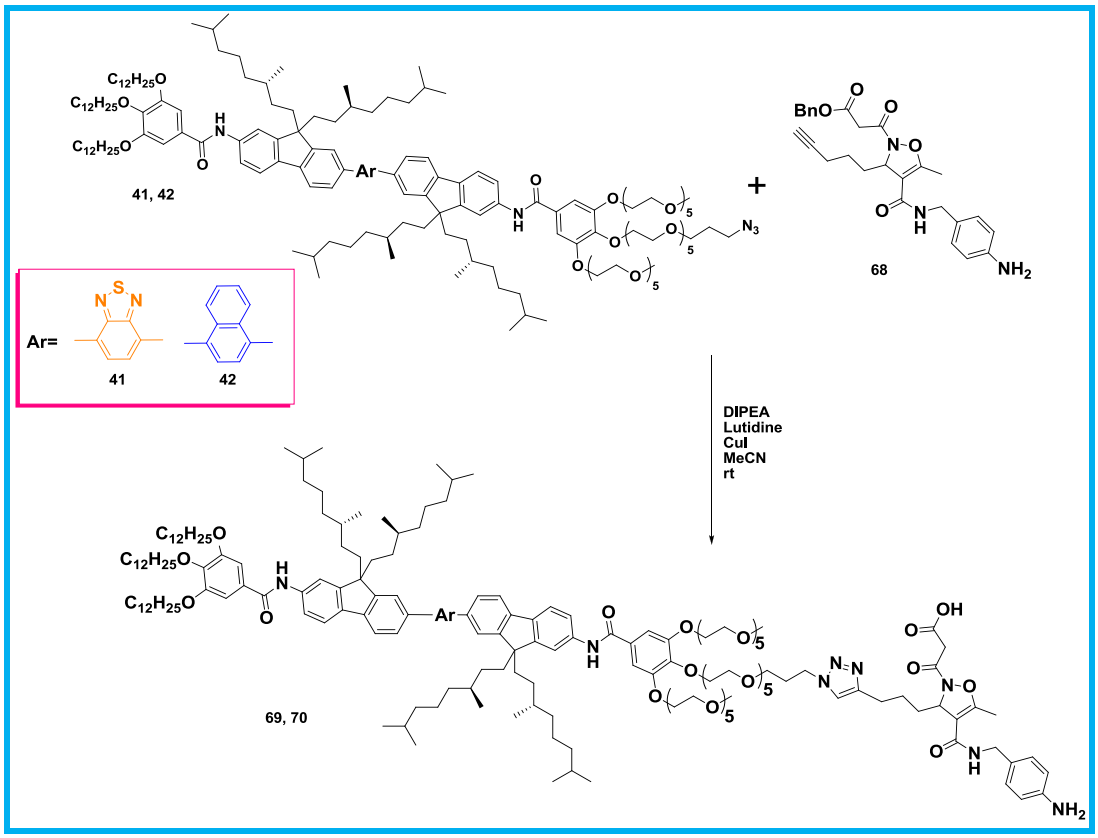
3.1.6 Conjugation of integrin ligands with fluorescent molecules

As previously said, the class of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligand chosen has been the class of isoxazolines and, in particular, the candidate molecule to link the fluorescent compound presents in its structure an alkyne able to react with an azide by click reaction. To synthesize this kind of isoxazolinic ligand, we prepared the aldehyde **62** by Swern oxidation using oxalyl chloride (99% yield) and then subjected it to Knoevenagel reaction with t-Butyl acetoacetate in presence of a catalytic amount of piperidine under MW irradiation following the procedure explained in chapter 1. From this reaction we obtained the corresponding alkylidene acetoacetate **1g** in 40% and probably the not excellent yield is due to a polymerization of the aldehyde during the reaction. The α,β-unsatured compound obtained was subjected to Michael addition with TMSONHTMS and ytterbium triflate as catalyst to obtain the intermediate **63** that spontaneously provided isoxazolidine **64** (Scheme 26).



Scheme 26. Synthesis of functionalized isoxazolinic ligand.

It has not been possible to isolate the product of this step because several intermediates resulting from hemiacetalic nature of isoxazolidine are present and for this reason we could not calculate the yield, so we performed the subsequent reaction using the crude product. After treatment with benzyl malonyl chloride and TEA, we obtained **65** in 44% yield after two steps. Using mesyl chloride and TEA in DCM isoxazoline **66** was finally obtained with good yield (82%). At this point the t-butylester hydrolysis, carried out with TFA in DCM, provided the corresponding acid **67** in 99% yield and the final coupling with p-aminobenzylamine performed with HBTU and TEA in dichloromethane allowed us to obtain the desired product **68** in 80% yield (Scheme 26).



Scheme 27. Conjugation of integrin ligand with fluorescent molecules by click reaction.

Having in our hand the two building block to link, we performed a click reaction between alkyne **68** and fluorescent molecule **41**/**42**. The reaction was carried out using CuI as catalyst, DIPEA and Lutidine in MeCN at room temperature for two hours providing the final product **69**/**70** in good yield (Scheme 27).

3.1.7 Experimental section

Dehydro pyrrole derivatives:

General procedure for the deprotection of OtBu ester 16c-d to obtain 20c-d:

TFA (7eq) was dissolved in a 0.1M solution of proper ester in DCM and the reaction mixture was stirred until complete consumpyion of the starting material (generally 12-24h). The solvent was removed in vacuo and the crude mixture was used for the following step. Quantitative yield of 20c-d..

20c: Yellow oil ,^{1}H NMR (400 MHz, CD_3OD) δ = 0.90 (d, J=7.2 Hz, 3H), 0.94 (d, J=7.2 Hz, 3H), 0.98 (d, J=7.2 Hz, minor isomer on amide bond 0.35H), 2.22 (m, minor isomer on amide bond 0.35H), 2.35 (m, major isomer on amide bond 1H), 3.73 (s, 3H), 4.10 (d, J=14, minor isomer on amide bond 0.35H), 4.35 (dd, J=20.5 Hz, 4.4 Hz, 1H), 4.52 (dd, J =20.8 Hz, 8.8Hz, 1H), 4.93 (s, minor isomer on amide bond 0.35H), 5.05 (d, J= 2.8 Hz, 1H), 6.8 (s, major isomer on amide bond, 1H), 6.90 (s, minor isomer on amide bond, 0.35H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 17.7, 18.2, 18.9, 19.2, 33.2, 35.5, 39.4, 52.9, 55.1, 56.1, 58.3, 62.0, 69.1, 69.7, 137.7, 138.8, 166.2, 167.5, 179.6.LC-MS ESI: r.t. 12.2 min m/z 256.1 [M+1], 278.1 [M+Na].α(MeOH, c=1): (S)-20c: +19.5, (R)-20c: -26-1.

20d: Yellow oil ,^{1}H NMR (400 MHz, CD_3OD) δ = 0.80-0.91 (m, 5H), 1.40-1.55 (m,6H), 3.73 (s, 3H), 4.10 (d, J=14, minor isomer on amide bond 0.35H), 4.35 (dd, J=20.5 Hz, 4.4 Hz, 1H), 4.52 (dd, J =20.8 Hz, 8.8Hz, 1H), 4.93 (s, minor isomer on amide bond 0.35H), 5.05 (d, J= 2.8 Hz, 1H), 6.8 (s, major isomer on amide bond, 1H), 6.90 (s, minor isomer on amide bond, 0.35H). α: **S-20d**= +33.9, **R-20d**= -30.3. LC-MS-ESI rt 8.7 min 296 (M+1).

General procedure for the preparation of 21c-d:

Under nitrogen atmosphere, the acid 20c-d was dissolved in dry DCM in order to create a 0.2M solution, and TEA (2eq) followed by HBTU (1eq) were added. Finally, 4-aminobenzylamine (1,5eq) was added to reaction mixture and it was stirred overnight. Then other DCM was added to reaction mixture and the organic layer was washed once with water, dried by Na_2SO_4 and the DCM was evaporated in vacuo and the crude product was purified through flash chromatography on silica gel (c-Hex/EtOAc 2:8) to obtain a dark orange dense oil in yield up of 90%.

21c: Yellow oil ,^{1}H NMR (400 MHz, CD_3OD) δ 0.87 (d, J=10 Hz, 3H), 0.9 (d, J=9.2 Hz, 3H), 2.38 (m, 1H), 3.37 (s, 2H), 3.75 (s, 3H), 4.31 (s, minor isomer on amide bond 0.35H), 4.4 (s, 2+1H), 4.46 (s, major isomer on amide bond 1H), 4.54 (s, minor isomer on amide bond 0.35H), 5.05 (d, J=12 Hz, minor isomer on amide bond 0.35H), 5.18 (s, major isomer on amide bond 1H), 6.45 (s, major isomer on amide bond 1H), 6.52 (s, minor isomer on amide bond 0.35H), 7.05 (d, J=20 Hz, 2H), 7.31 (d, J=16 Hz, 2H). ^{13}C NMR (100 MHz, $CDCl_3$) δ = 15.3, 18.8, 19.7, 20.1, 21.7, 33.3, 36.3, 44.3, 53.7, 55.9, 57.0, 62.3, 70.5, 70.8, 122.1, 131.0, 131.7, 139.6, 167.3, 196.2. LC-MS rt 12.2 min, m/z 360, acid injection. α (MeOH, c=1): (S)-21c: +27.5, (R)-21c: -26.5.

21d: Yellow oil ,^{1}H NMR (400 MHz, CD_3OD_3) δ 0.8-1.2 (m, 5H), 1.45-1.80 (m, 6H), 3.15 (d, J= 7.6 Hz, 1H), 3.19 (d, J= 7.2 Hz,1H), 4.20-4.74 (m, 4H), 4.93 (s, 1H. minor), 5.06 (s, 1H, major), 6.33 (s, 1H, major), 6.41 (s, 1H, minor), 6.67 (d, J= 6.8 Hz, 2H), 7.05 (d, J= 6.8 Hz, 2H), 7.94 (s, 2H), 8.55 (bs, 1H). ^{13}C NMR (100 MHz, CD_3OD) minor and major δ 11.1, 15.2, 16.6, 25.4, 25.7, 27.1, 28.1, 40.5, 40.8, 41.6, 41.7, 46.4, 46.6, 50.8, 53.7, 54.0, 67.3, 114.6, 127.33, 127.7, 127.8, 128.0, 137.2, 145.5, 164.5, 164.9, 167.5. LC-MS-ESI rt 4.9, 400 (M+1), 822 (2M+Na). α (MeOH, c=1): (S)-21c: +27.0, (R)-21c: -25.2.

General procedure for the preparation of 22c-d:

Compound 21c-d was dissolved in a 1:4 mixture of THF and water in order to create a 0.02M solution. Then K_2CO_3 was added and the reaction mixture was stirred under refluxing conditions for 4h. After that, the pH was turn acid and the solvent was removed in vacuo. Crude reaction mixture was dissolved in the fewer quantity as possible of an acidic water/MeOH solution and was then purified on ion exchange DOWEX resin to afford a dark green vitreous solid in typical yields of 50-80%.

22c: Yellow oil ,^{1}H NMR (400 MHz, D_2O) δ = 0.81 (d, J=6.8 Hz, 3H), 0.83 (d, J=7.6 Hz, 3H), 0.86 (d, J= 6.8 Hz, minor isomer on amide bond 0.35H), 2.20 (m, minor isomer on amide bond 0.35H), 2.38 (m major isomer on amide bond 1H), 4.14 (dd, J= 17 Hz, 2.8 Hz, minor isomer on amide bond 0.35H) 4.37 (s, 2H), 4.43 (m, 2H), 4.99 (s, minor isomer on amide bond 0.35H), 5.07 (s, major isomer on amide bond 1H), 6.48 (s, major isomer on amide bond 1H), 6.55 (s, minor isomer on amide bond 0.35H), 6.92 (d, J=8 Hz 2H. ^{13}C NMR (100 MHz, D_2O) δ = 14.9, 15.7, 18.1, 18.3, 30.5, 33.5, 42.5, 53.7, 54.9, 68.1, 68.9, 117.7, 128.7, 128.8, 130.4, 130.5, 130.9, 131.4, 135.5, 135.9, 141.9, 166.5, 166.6, 169.1, 174.0. LC-MS rt 4.4 min, m/z 346, acid injection.

22d: Vitreous yellow solid ,^{1}H NMR (400 MHz, CD_3OD) δ 0.9-1.2 (m, 5H), 1.5-1.75 (m, 6H), 3.29 (s, 2H), 4.2-4.5 (m, 4H), 4.96 (s, 1H, minor), 5.08 (s, 1H, major), 6.32 (s, 1H, major), 6.39 (s, 1H, minor), 6.65 (d, J= 8 Hz, 2H), 6.05 (d, J= 8.4 Hz, 1H) ^{13}C NMR (100 MHz, CD_3OD) minor and major δ 25.2, 25.5, 25.8, 27.2, 28.3, 40.9, 41.7, 43.8, 54.1, 67.2, 67.8, 114.6, 127.4, 127.8, 127.9, 128.2, 137.4, 145.7, 164.6, 167.0, 170.8. LC-MS-ESI rt 11.2 386 (M+1), 795 (2M+Na).

Isoxazolines derivatives:

General procedure for the synthesis of N-malonamido derivatives 23:

$SOCl_2$ (1.5 mmol, 1.5 equiv) was added dropwise to a stirred solution of benzyl malonic acid (1.5 mmol, 1.5 equiv) in dry DCM (5 mL) at 0°C. After removing the

ice bath, the solution was for 30 min at room temperature, and then pyridine (5 mmol, 5 equiv) and proper isoxazolidine 9 (1 mmol) were added. The solution was left to stir overnight, and then the reaction mixture was diluted with DCM (10 mL) and washed with H_2O (2 X 10 mL). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo. Purification of the crude residue by flash chromatography on silica gel, (eluent cyclohexane/EtOAc 9:1) afforded compounds 23 as racemic mixtures of trans isomers.

23a: Yellow oil (236 mg, 60%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.43 (d, J=6.8 Hz, 3H), 1.50 (s, 9H), 1.63 (s, 3H), 2.84 (d, J = 8.0 Hz, 1H), 3.54 (d, J =15.8 Hz, 1H), 3.65 (d, J =15.8 Hz, 1H), 4.57 (bs, 1H), 4.71 (m, 1H), 5.15 (d, J =13.2 Hz, 1H), 5.22 (d, J =13.2 Hz, 1H), 7.37 (s, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 20.4, 23.0, 27.9, 41.1, 55.8, 62.9, 67.2, 83.0, 104.8, 128.3, 128.4, 128.7, 135.3, 160.3, 167.4, 168.1. LC-MS ESI: r.t. 9.14 min m/z 394 [M+1], 416 [M+Na], 809 [2M+Na].

23b: Yellow oil (292 mg, 66%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.71 (d, J=7.0 Hz, 3H), 0.88 (d, J=7.0 Hz, 3H), 1.40 (s, 9 H), 1.54 (s, 3H), 1.88 (m, 1 H), 2.87 (d, J=6.6 Hz, 1H), 3.43 (d, J=15.6 Hz, 1 H), 3.60 (d, J=15.6 Hz, 1H), 4.51 (m, 1 H), 4.61 (bs, 1H), 5.08 (s, 2H),7.29 ppm (s, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): δ=17.7, 18.6, 23.0, 27.9, 32.2, 41.2, 58.8, 65.0, 67.1, 82.8, 105.7, 128.2, 128.5, 128.6, 135.4, 168.0, 168.7, 169.2 ppm; LC–MS (ESI): t_R=10.4 min, m/z: 444 [M+Na], 865 [2M+Na].

23c: Yellow oil (290 mg, 63%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.97-1.26 (m, 6H), 1.42-1.51 (m, 2H), 1.46 (s, 9H), 1.58-1.85 (m, 3H), 1.63 (s, 3H), 2.97 (d, J = 6.6 Hz, 1H), 3.55 (d, J =15.4 Hz, 1H), 3.68 (d, J =15.4 Hz, 1H), 4.58 (m, 1H), 5.12 (d, J =12.4 Hz, 1H), 5.20 (d, J =12.4 Hz, 1H), 7.34 (s, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 23.6, 27.7, 27.9, 28.3, 29.1, 29.5, 38.0, 42.1, 66.9, 67.1, 82.0, 105.7, 128.1, 128.3, 128.4, 136.2, 168.1, 169.2, 169.8. LC-MS ESI: r.t. 13.1 min m/z 462

[M+1], 484 [M+Na].

23g: See compound **65**.

23h: Yellow oil (265 mg, 61%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.86-0.94 (m, 6H), 1.44-1.55 (m, 12H), 1.64 (s, 3H), 2.98 (d, J = 7.0 Hz, 1H), 3.64 (s, 2H), 4.57 (bs, 1H), 4.69 (m, 1H), 5.18 (s, 2H), 7.36 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 11.1, 11.9, 13.1, 16.2, 23.1, 27.9, 31.9, 41.1, 66.7, 67.2, 81.0, 105.9, 128.3, 128.4, 128.6, 135.2, 162.5, 162.7, 166.9. LC-MS ESI: 10.9 min m/z 436 [M+Na], 893 [2M+Na].

General procedure for the synthesis of dehydration of 23 to 24:

CH_3SO_2Cl (116 mL, 1.5 mmol, 1.5 equiv) and Et_3N (348 mL, 2.5 mmol, 2.5 equiv) were added in a single portion to a stirred solution of 23 (1 mmol) in dry DCM (5 mL) at room temperature. The solution was stirred at room temperature and stopped upon disappearance of the starting material spot (by TLC), typically after 1 h. The reaction mixture was then diluted with DCM (10 mL) and washed with H_2O (2X10 mL). The organic layer was dried (Na_2SO_4) and filtered, and the solvent was removed in vacuo. Purification of the crude residue by flash chromatography on silica gel (eluent cyclohexane/ EtOAc 9:1) afforded compounds 24.

24a: Yellow oil (330 mg, 88%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.43 (d, J=6.8 Hz, 3H), 1.49 (s, 9H), 2.08 (s, 3H), 3.49 (d, J =15.8 Hz, 1H), 3.62 (d, J =15.8 Hz, 1H), 5.17 (s,2H), 5.28 (q, J =6.8 Hz, 1H), 7.20-7.35 (m, 5H). ^{13}C NMR (50 MHz, CDCl3) δ = 10.7, 19.3, 28.4, 30.6, 64.5, 66.1, 82.7, 110.3, 128.2, 128.3, 128.5, 135.1, 150.5, 163.9, 165.3, 165.9. LC-MS ESI: r.t. 11.00 min m/z 376 [M+1], 398 [M+Na], 773 [2M+Na].

24b: Yellow oil (359 mg, 89%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.71 (d, J=6.6 Hz, 3 H), 0.89 (d, J=6.6 Hz, 3H), 1.41 (s, 9H), 1.55 (m, 1H), 2.01 (s, 3 H), 3.44 (d, J=15.8 Hz, 1 H), 3.60 (d, J=15.8 Hz, 1H), 5.08 (s, 2 H), 5.12 (bs, 1 H), 7.36 ppm (m, 5H); ^{13}C NMR (50 MHz, $CDCl_3$): d=10.9, 15.6, 19.7, 29.0, 31.3, 40.9, 66.9,

68.1, 80.7, 105.3, 128.1, 128.2, 128.4, 135.0, 161.1, 162.2, 166.3, 169.4 ppm; LC–MS (ESI): t_R= 12.4 min, m/z: 404 [M+1], 829 [2M+Na].

24c: Yellow oil (376 mg,

85%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.94-1.85 (m, 11H), 1.47 (s, 9H), 2.27 (s, 3H), 3.48 (d, J =15.4 Hz, 1H), 3.66 (d, J =15.4 Hz, 1H), 5.14 (s, 2H), 5.16 (bs, 1H), 7.32 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 11.1, 25.3, 26.3, 26.9, 29.7, 30.5, 41.1, 67.2, 67.8, 82.1, 105.8, 128.3, 128.5, 131.9, 136.3, 151.0, 162.5, 165.7, 169.1. LC-MS ESI: 12.3 min m/z 444 [M+1], 466 [M+Na].

24g: See compound **66**.

24h: Yellow oil (354 mg, 85%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.76 (d, J=7.0 Hz, 3H), 0.94 (t, J=7.2 Hz, 3H), 1.38-1.54 (m, 2H), 1.48 (s, 9H), 1.81 (m, 1H), 2.09 (s, 3H), 3.51 (d, J =16.2 Hz, 1H), 3.60 (d, J =16.2 Hz, 1H), 5.16 (s, 2H), 5.31 (bs, 1H), 7.28-7.42 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 11.0, 11.1, 13.0, 23.8, 28.2, 38.4, 41.1, 66.7, 67.1, 81.0, 105.4, 128.3, 128.4, 128.6, 135.1, 150.9, 162.4, 166.6, 169.7. LC-MS ESI: r.t. 14.6 min m/z 418 [M+1], 440 [M+Na].

General procedure for tert-butyl ester hydrolysis (compounds 25):

TFA (15 equiv, 15 mmol, 1.15 mL) was added to a stirred solution of 24 (1 mmol) in DCM (2 mL) at 0°C. After 2 h, the mixture was washed with H_2O (2_5 mL), the organic layer was dried (Na_2SO_4), filtered, and the solvent was removed in vacuo. The acids 25 were used in the following step without further purification.

25a: Yellow oil (305 g, 95%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.47 (d, J=6.8 Hz, 3H), 2.09 (s, 3H), 3.48 (d, J =15.8 Hz, 1H), 3.66 (d, J =15.8 Hz, 1H), 5.09 (s, 2H), 5.18 (m, 1H), 7.29 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ =11.0, 20.6, 31.6, 42.1, 66.9, 104.8, 127.9, 128.1, 128.5, 135.0, 163.0, 167.1, 167.9, 169.1. LC-MS ESI: r.t. 8.9 min m/z 320 [M+1], 342 [M+Na], 661 [2M+Na].

25b:: Yellow oil (340 mg, 98%): ^{1}H NMR (200 MHz, $CDCl_3$): δ=0.80 (d, J=6.8 Hz, 3 H), 0.98 (d, J= 6.8 Hz, 3 H), 2.12 (s, 3 H), 2.20 (m, 1 H), 3.53 (d, J=16.0 Hz,

1H), 3.74 (d, J=16.0 Hz, 1 H), 5.16 (s, 2 H), 5.26 (bs, 1H), 7.34 ppm (m, 5H); ^{13}C NMR (50 MHz, CDCl3): d=11.4, 15.4, 19.8, 23.9, 31.2, 40.9, 67.3, 103.7, 128.3, 128.4, 128.5, 134.9, 164.8, 166.4, 168.6, 169.9 ppm; LC–MS (ESI): t_R=9.2 min, m/z: 348 [M+1], 370 [M+Na], 717 [2M+Na].

25c: Yellow oil (354 mg, 98%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 1.08-1.36 (m, 5H), 1.49-1.78 (m, 6H), 2.39 (s, 3H), 3.45 (d, J =15.8 Hz, 1H), 3.65 (d, J =15.8 Hz, 1H), 5.07 (s, 2H), 5.14 (bs, 1H), 7.19-7.26 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 11.6, 24.9, 25.5, 25.6, 25.8, 26.0, 26.1, 31.7, 67.3, 67.7, 103.1, 128.3, 128.5, 128.6, 135.0, 165.6, 166.4, 166.7, 168.5. LC-MS ESI: r.t. 10.9 min m/z 362 [M+1], 384 [M+Na], 745 [2M+Na].

25g: See compound **67**.

25h: Yellow oil (375 mg, 97%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.67-0.92 (m, 6H), 1.06-1.27 (m, 2H), 1.77 (m, 1H), 2.03 (m, 3H), 3.42 (d, J =15.6 Hz, 1H), 3.64 (d, J =15.6 Hz, 1H), 5.07 (s, 2H), 5.24 (d, J =10.2 Hz, 1H), 7.18-7.25 (m, 5H), 8.80-9.20 (bs, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 11.4, 12.6, 16.1, 23.6, 26.9, 31.4, 38.0, 67.2, 68.0, 103.7, 128.2, 128.4, 128.5, 134.9, 164.7, 166.4, 168.3, 170.0. LC-MS ESI: r.t. 11.3 min m/z 388 [M+1], 410 [M+Na], 797 [2M+Na].

General procedure for coupling of 25 with p-aminobenzylamine (compounds 26): Under nitrogen atmosphere, the acid 25 was dissolved in dry DCM in order to create a 0.2M solution, and TEA (2eq) followed by HBTU (1eq) were added. Finally, 4-aminobenzylamine (1,5eq) was added to reaction mixture and it was stirred overnight. Then other DCM was added to reaction mixture and the organic layer was washed once with water, dried by Na_2SO_4 and the DCM was evaporated in vacuo and the crude product was purified through flash chromatography on silica gel (c-Hex/EtOAc 2:8) to obtain a dark orange dense oil in yield up of 90%. Data available for Isopropyle derivatives 2b and 2g.

26b: Yellow oil (180 mg, 80%): ^{1}H NMR (200 MHz, $CDCl_3$) δ = 0.82 (d, J=6.6 Hz, 3H), 0.93 (d, J=6.6 Hz, 3H), 2.04 (m, 1H), 2.13 (s, 3H), 3.48 (d, J =16.2 Hz,

1H), 3.68 (d, J =16.2 Hz, 1H), 4.35 (s, 2H), 5.14 (s, 2H), 5.19 (bs, 1H), 5.81 (bt, 1H), 6.64 (d, J = 6.2 Hz, 2H), 7.06 (d, J = 6.2 Hz, 2H), 7.42 (m, 5H). ^{13}C NMR (50 MHz, $CDCl_3$) δ =11.6, 15.9, 19.0, 28.6, 32.0, 41.7, 66.9, 67.4, 105.9, 118.7, 128.2, 128.5, 128.6, 128.7, 129.7, 134.8, 137.6, 149.8, 163.2, 165.4, 167.9. LC-MS ESI: r.t. 8.35 min m/z 452 [M+1], 473 [M+Na], 925 [2M+Na].

2g: See compound **68**.

General procedure for hydrogenation of 26 to 27:

To a solution of 26 (0.5 mmol) in MeOH, Pd/C (10 mg) was added in one portion. The reaction mixture was stirred vigorously at room temperature under H_2 atmosphere overnight. The solution was filtered to remove catalyst, and the solvent was evaporated in vacuo. The crude compound was treated with Dowex 50WX2-200 ion-exchange resin, eluting with NH_4OH (0.5m). Compounds 27 were isolated after removal of the aqueous solvent in vacuo.

27a: Yellow oil (126 mg, 76%): ^{1}H NMR (200 MHz, CD_3OD) δ = 1.68 (d, J=7.2 Hz, 3H), 2.07 (s, 3H), 4.18 (d, J =15.8 Hz, 1H), 4.27 (d, J =15.8 Hz, 1H), 4.82 (s, 2H), 5.33 (m, 1H), 7.04 (d, J =8.4 Hz, 2H), 7.18 (d, J =8.4 Hz, 2H), 8.07 (bt, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 11.6, 21.9, 32.6, 44.9. 68.2, 115.6, 121.0, 128.7, 129.3, 135.8, 148.1, 161.9, 164.0, 169.2. LC-MS ESI: r.t. 0.98 min m/z 334 [M+1], 689 [2M+Na].

27b: Yellow oil (143 mg, 79%): ^{1}H NMR (200 MHz, CD_3OD): δ=0.73 (d, J=6.6 Hz, 3 H), 0.86 (d, J= 6.6 Hz, 3 H), 1.89 (m, 1 H), 2.10 (s, 3 H), 4.23 (d, J=15.0 Hz, 1H), 4.35 (d, J=15.0 Hz, 1 H), 4.81 (s, 2H), 5.31 (bs, 1H), 7.00 (d, J=8.0 Hz, 2 H), 7.20 (d, J=8.0 Hz, 2 H), 8.21 ppm (bt, 1H); ^{13}C NMR (50 MHz, $CDCl_3$): d=11.4, 17.0, 20.1, 27.8, 30.7, 45.5, 69.8, 117.0, 120.7, 129.5, 129.7, 136.3, 146.3, 161.6, 163.4, 167.1 ppm; LC–MS (ESI): t_R= 1.46 min, m/z: 362 [M+1], 745 [2M+Na].

27c: Yellow oil (162 mg, 81%): ^{1}H NMR (200 MHz, CD_3OD) δ = 0.82-1.25 (m, 6H), 1.44-1.70 (m, 6H), 2.10 (s, 3H), 3.24 (s, 2H), 4.21 (d, J =14.6 Hz, 1H), 4.37

(d, J =14.6 Hz, 1H), 5.28 (bs,1H), 6.99 (d, J =7.8 Hz, 2H), 7.20 (d, J =7.8 Hz, 2H), 8.20 (bt, 1H). ^{13}C NMR (50 MHz, $CDCl_3$) δ = 9.8, 25.8, 25.9, 26.4, 29.6, 30.9, 42.0, 66.6, 115.4, 118.8, 128.6, 129.8, 138.9, 146.2, 163.5, 164.1, 166.0. LC-MS ESI: r.t. 2.21 min m/z 402 [M+1], 803 [2M+1]

27g: The deprotection didn't work on this substrate.

27h: Yellow oil (160 mg, 85%): ^1H NMR (200 MHz, CD_3OD) δ = 0.77-0.89 (m, 6H), 1.03-1.57 (m, 3H), 2.13 (s, 3H), 3.27 (s, 2H), 4.17 (d, J =13.6 Hz, 1H), 4.37 (d, J =13.6 Hz, 1H), 5.39 (bs,1H), 6.72 (d, J =7.6 Hz, 2H), 7.41 (d, J =7.6 Hz, 2H), 8.12 (bt, 1H). ^{13}C NMR (50 MHz, CD_3OD) δ = 11.9, 12.3, 23.2, 26.8, 30.7, 45.5, 69.8, 117.0, 120.7, 129.5, 129.7, 136.3, 146.3, 161.6, 163.4, 167.1. LC-MS ESI: r.t. 1.54 min m/z 376 [M+1], 773 [2M+Na].

Pyrimidindione derivatives

Procedure for the preparation of the acid 28a e c:

To a stirred solution of the carbonate 11a e c (0.2 mmol) in DCM (2 mL) at 0°C, trifluoroacetic acid (15 equiv, 3 mmol, 223 mL) was added. After 2 h, the mixture was washed twice with water (5 mL), the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure.

28a: Yield 95%, pale yellow solid (75:25, Z/E mixture), ^1H NMR ($CDCl_3$, 400 MHz) Z isomer δ 1.57 (d, J = 6.8 Hz, 3H,), 2.05 (d, J = 7.2 Hz, 3H), 3.79 (s, 3H,), 5.76 (q, J = 6.8 Hz, 1H), 7.20 (q, J = 7.2 Hz, 1H,); E isomer δ 1.49 (d, J = 6.8 Hz, 3H), 1.92 (d, J = 7.2 Hz, 3H), 5.53 (q, J = 6.8 Hz, 1H), 6.67 (q, J = 7.2 Hz, 1H). IR (neat, cm^{-1}) 1105, 1172, 1288, 1463, 1671, 1750, 2855, 2915, 2961, 3317. ^{13}C NMR (CDCl3, 200 MHz) d Z isomer 14.3, 19.5, 50.9, 53.6, 117.1, 130.6, 139.6, 151.3, 174.3. LC-MS-ESI rt 1.22 min, 189 (M + 1). Anal. cald. for $C_8H_{12}O_5$ (188.18): C 51.06, H 6.43; found C 50.95, H 6.45.

28c: Yield 95% (>99:1, Z/E mixture), pale yellow solid, ^1H NMR ($CDCl_3$, 200 MHz) δ 0.97 (d, J = 5.4 Hz, 6H), 1.40 (d, J = 6.6 Hz, 3H), 3.25e3.42 (m, 1H), 3.70

(s, 3H), 5.46 (q, J = 6.6 Hz, 1H), 6.11 (d, J = 10.0 Hz, 1H,), 10.41 (bs, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 20.1, 21.9, 31.8, 54.4, 73.3, 128.9, 142.5, 154.7, 171.4; IR (neat, cm^{-1}) 1010, 1168, 1287, 1691, 1742, 2845, 2921, 2961, 3323. LC-ESI-MS rt 6.65 min, m/z 216 (M), 239 (M + Na). Anal. cald. for $C_{10}H_{16}O_5$ (216.23): C 55.55, H 7.46; found C 55.57, H 7.45.

Procedure for the preparation of the amide 29a e c:

To a stirred solution of the acid 28 (0.2 mmol) in dry DCM (2 mL), under nitrogen atmosphere, EDCI (1.2 equiv, 0.24 mmol, 46 mg) and triethylamine (2.4 equiv, 0.48 mmol, 67 mL) were added. After 30 min HOBT (1.2 equiv, 0.24 mmol, 33 mg) and glycine tbutyl ester hydrochloride (1.2 equiv, 0.24 mmol, 41 mg) were added. The solution was stirred overnight and then the mixturewas diluted with DCM and washed twice with acidic water (5 mL) and twice with basic water (5 mL). The two phases were separated, the organic layer was dried over Na2SO4, and solvent was removed under reduced pressure. Compound 29 was isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluent).

29a: Yield 60% (75:25, Z/E mixture), yellow oil, $_1H$ NMR ($CDCl_3$, 200 MHz) d 1.41 (s, 9H),1.43 (d, J = 6.6 Hz, 3H),1.83 (d, J = 7.2 Hz, 3H), 3.74 (s, 3H), 3.94-4.01 (m, 2H), 5.25 (q, J = 6.8 Hz, 1H, CHO), 5.96 (q, J = 7.2 Hz, 1H), 6.61 (bt, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 15.0, 20.0, 28.0 (3C), 42.0, 54.8, 76.1, 82.2, 131.8, 137.3, 155.1, 167.3, 168.8. IR (neat, cm^{-1}) 938, 1040, 1158, 1267, 1371, 1524, 1612, 1674, 1744, 1750, 28 661, 2926, 2971, 3336. LC-MS-ESI rt 6.8 min, m/z 301 (M + 1). Anal. cald. for $C_{14}H_{23}NO_6$ (301.34): C 55.80, H 7.69, N 4.65; found C 55.60, H 7.68, N 4.66.

29c: Yield 65% (>99:1, Z/E mixture), yellow oil, ^1H NMR ($CDCl_3$, 200 MHz) δ 1.01 (d, J = 6.6 Hz, 6H), 1.45 (d, J = 7.0 Hz, 3H), 1.49 (s, 9H), 2.70-2.88 (m, 1H), 3.79 (s, 3H), 4.00 (d, J = 4.8 Hz, 2H), 5.27 (q, J = 6.6 Hz, 1H), 5.67 (d, J = 10.2 Hz, 1H), 6.57 (bt, 1H); ^{13}C NMR ($CDCl_3$, 50 MHz) δ 19.9, 22.7 (2C), 26.8, 28.0 (3C),

41.9, 54.8, 76.0, 82.2, 131.8, 137.3, 155.1, 167.3, 168.8; IR (neat, cm_1) 942, 1046, 1157, 1267, 1367, 1442, 1519, 1605, 1667, 1736, 1750, 2861, 2924, 2960, 3348. LC-ESI-MS rt 10.82 min, m/z 329 (M), 352 (M + Na); Anal. cald. for $C_{16}H_{27}NO_6$ (329.39): C 58.34, H 8.26, N 4.25; found C 58.20, H 8.28, N 4.26.

Procedure for the preparation of the N-BOC-protected amide 30a and c:

To a stirred solution of the amide 29 a and c (0.2 mmol) in dry THF (1 mL), DMAP (0.2 equiv, 0.04 mmol, 5 mg), triethylamine (1.2 equiv, 0.24 mmol, 34 mL) and $(BOC)_2O$ (1.3 equiv, 0.26 mmol, 60 mL) were added. The solution was stirred overnight and then the solvent was removed under reduced pressure. The residue was diluted with ethyl acetate (10 mL) and washed twice with water (5 mL). The two phases were separated, the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Compound 30 a and c was isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluent).

30a: Yield 88% (70:30, Z/E mixture), yellow oil, ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.42 (s, 18H), 1.46 (d, J = 6.6 Hz, 3H), 1.72 (d, J = 7.2 Hz, 3H), 3.69 (s, 3H), 4.19-4.41 (m, 2H), 5.41 (q, J = 6.6 Hz, 1H), 5.80 (q, J = 7.2 Hz, 1H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 14.6, 19.2, 27.7 (3C), 28.0 (3C), 45.9, 54.6, 74.8, 81.8, 83.8, 126.7, 137.6, 151.4, 155.1, 167.6, 169.8; IR (neat, cm^{-1}) 790, 849, 1034, 1160, 1448, 1592, 1657, 1679, 1746, 1762, 2866, 2928, 2979. LC-MS-ESI rt 11.0 min, m/z 424 (M + Na), 440 (M + K). Anal. cald. for $C_{19}H_{31}NO_8$ (401.45): C 56.84, H 7.78, N 3.49; found C 57.00, H 7.76, N 3.48. 6b: Yield 90% (70:30, Z/E mixture), yellow oil, ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.99 (d, J = 6.6 Hz, 6H), 1.42 (d, J = 6.6 Hz, 3H), 1.48 (s, 18H), 2.41-2.49 (m, 1H), 3.75 (s, 3H), 4.28 (d, J = 16.8 Hz, 1H), 4.45 (d, J = 16.8 Hz, 1H), 5.42-5.53 (m, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) d 19.2, 22.6 (2C), 27.2, 27.8 (3C), 28.0 (3C), 45.9, 54.5, 74.7, 81.8, 83.8, 126.7, 137.6, 151.4, 155.1, 167.6, 169.8; IR (neat, cm^{-1}) 745, 848,1030, 1159, 1442,1588,1652,1677,1743, 1756, 2859, 2928, 2978. LC-ESI-MS rt 12.26 min,

m/z 429 (M), 452 (M + Na); Anal. cald. for $C_{21}H_{35}NO_8$ (429.50): C 58.72, H 8.21, N 3.26; found C 58.78, H 8.19, N 3.25.

Procedure for the preparation of the dihydropyrimidindione 31:

To a stirred solution of 30 (0.2 mmol) in dry CH_3CN (2 mL), under nitrogen atmosphere, 4-aminobenzylamine (1.5 equiv, 0.3 mmol, 34 mL) was added. The solution was refluxed for 4 days and then the solvent was removed under reduced pressure. Compound 31 was isolated by flash chromatography on silica gel (DCM/ ethyl acetate 90/10 as eluent).

31: Yield 60% (89:11, Z/E mixture), yellow sticky oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.79 (d, J = 6.8 Hz, 3H), 0.96 (d, J = 6.8 Hz, 3H), 1.42 (s, 9H), 1.48 (d, J = 7.2 Hz, 3H), 2.13 (m, 1H), 3.77 (d, J = 7.6 Hz, 1H), 3.90 (d, J = 14.8 Hz, 1H), 4.38 (d, J = 16.8 Hz, 1H), 4.45 (d, J = 16.8 Hz, 1H), 5.28 (d, J = 14.8 Hz, 1H), 6.57 (d, J = 6.8 Hz, 2H), 6.77 (q, J = 7.2 Hz, 1H), 6.94 (d, J = 6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 18.5, 19.7, 28.0 (3C), 33.0, 42.9, 51.0, 57.0, 81.6, 115.2 (2C), 126.1, 129.0 (2C), 130.7, 137.2,146.0,153.1,164.9,167.6. IR (neat, cm^{-1}) 948,1196,1345,1463, 1581,1658,1705, 2853, 2922, 2950, 3366. LC-ESI-MS rt 9.5 min, m/z 424 (M + Na), 825 (2M + Na); Anal. cald. for $C_{22}H_{31}N_3O_4$ (401.50): C 65.81, H 7.78, N 10.47; found C 65.84, H 7.81, N 10.51.

Procedure for the preparation of the 32:

To a stirred solution of the N-Boc-Gly (0.2 mmol, 35 mg) in dry DMF (2 mL), under nitrogen atmosphere, N,N-diisopropylethylamine (2 equiv, 0.4 mmol, 69 mL) and HBTU (1 equiv, 0.2 mmol, 76 mg) were added. After 30 min, compound 31 (1.2 equiv, 0.24 mmol, 96 mg) was added. The solution was stirred for 3 h and then the mixture was diluted with ethyl acetate and washed twice with acidicwater (5 mL) and twice with basicwater (5 mL). The two phases were separated, the organic layer was dried over Na_2SO_4, and solvent was removed under reduced

pressure. Compound 32 was isolated by flash chromatography on silica gel (DCM/ethyl acetate 80/20 as eluent).

32: Yield 90% (91:9, Z/E mixture), yellow oil, $_1$H NMR ($CDCl_3$, 400 MHz) δ 0.77 (d, J = 7.2 Hz, 3H), 0.94 (d, J = 7.2 Hz, 3H), 1.39 (s, 9H), 1.41 (s, 9H), 1.49 (d, J = 7.2 Hz, 3H), 2.10 (m, 1H), 3.73 (d, J = 7.6 Hz, 1H), 3.84 (d, J = 6.0 Hz, 2H), 3.98 (d, J = 15.6 Hz, 1H), 4.36 (d, J = 16.6 Hz, 1H), 4.42 (d, J = 16.6 Hz, 1H), 5.20 (bs, 1H), 5.32 (d, J = 15.6 Hz, 1H), 6.79 (q, J = 7.2 Hz, 1H), 7.09 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H), 8.10 (bs, 1H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.2,18.4,19.7, 28.0 (3C), 28.2 (3C), 33.1, 42.9, 45.4, 51.0, 57.8, 81.7, 120.1 (2C), 127.8, 128.1 (2C), 132.5, 137.0, 137.6, 152.7, 156.5, 164.8, 167.5, 167.9. IR (neat, cm^{-1}) 961, 1200, 1377, 1463, 1552, 1632, 1651, 1660, 1700, 2884, 2924, 2956, 3500. LC-ESI-MS rt 9.97 min, m/z 581 (M + Na). Anal. cald. for $C_{29}H_{42}N_4O_7$ (558.67): C 62.35, H 7.58, N 10.03; found C 62.53, H 7.58, N 10.01.

Procedure for the preparation of the amino acid 33:

To a stirred solution of compound 32 (0.2 mmol, 112 mg), in DCM (0.5 mL), H_3PO_4 (85% solution in water, 7.5 equiv, 1.5 mmol, 207 mL) was added. After 12 h the organic solvent was removed under reduced pressure and the residue was treated with Dowex 50WX2-200 ion exchange resin, eluting with NH_4OH 0.5 M. Compound 33 was isolated after removal of the aqueous solvent.

33: Yield 80% (88:12, Z/E mixture), yellow solid, m.p. 130-132 °C (dec.) ^{1}H NMR (D_2O, 400 MHz) δ 1.68 (d, J = 6.8 Hz, 3H), 1.85 (d, J = 6.8 Hz, 3H), 2.55 (d, J = 7.6 Hz, 3H), 3.03 (m, 1H), 4.79 (s, 2H), 5.01 (d, J = 6.8 Hz, 1H), 5.09 (d, J = 16.4 Hz, 1H), 5.11 (d, J = 16.4 Hz, 1H), 5.23 (d, J = 15.8 Hz, 1H), 6.02 (d, J = 15.8 Hz, 1H), 7.79 (q, J = 7.6 Hz, 1H), 8.18 (d, J = 8.4 Hz, 2H), 8.33 (d, J = 8.4 Hz, 2H); ^{13}C NMR (D_2O, 100 MHz) δ 13.9, 17.1, 18.3, 32.7, 41.3, 44.6, 51.2, 58.4, 121.4 (2C), 126.6, 128.0 (2C), 133.7, 135.7, 140.6, 154.0, 166.3, 167.2, 175.3. IR (neat, cm^{-1}) 846, 1157, 1367, 1470, 1538, 1635, 1667, 1698, 2841, 2875, 2924, 2948, 3363,

3421. LC-ESI-MS rt 1.23 min, m/z 403 (M + 1), 425 (M + Na). Anal. cald. for $C_{20}H_{26}N_4O_5$ (402.44): C 59.69, H 6.51, N 13.92; found C 59.75, H 6.48, N 13.96.

Linear derivatives:

Procedure for the preparation of the amino derivative 34:

To a stirred solution of 30 (0.2 mmol) in dry CH_3CN (2 mL), under nitrogen atmosphere, 4-aminobenzylamine (1.5 equiv, 0.3 mmol, 34 mL) was added. The solution was refluxed for 16 h and then the solvent was removed under reduced pressure. Compound 34 was isolated by flash chromatography on silica gel (cyclohexane/ ethyl acetate 95/5 as eluent).

34a: Yield 70% (81:19, Z/E mixture), yellow sticky oil, ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.39 (s, 18H), 1.48 (d, J = 7.2 Hz, 3H), 1.73 (d, J = 7.2 Hz, 3H), 3.90 (t, J = 6.2 Hz, 2H), 4.44 (s, 2H), 5.03 (q, J = 7.2 Hz, 1H), 6.15 (q, J = 7.2 Hz, 1H), 6.50 (bt, 1H), 6.59 (d, J = 8.4 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H). ^{13}C NMR ($CDCl_3$, 50 MHz) δ 13.6, 18.0, 28.1(3C), 28.4 (3C), 42.2, 47.3, 50.1, 79.8, 82.1, 115.0, 127.9, 130.7, 131.3, 139.6, 144.9, 155.9, 169.1, 169.9. IR (neat, cm^{-1}) 741, 848, 1163, 1371, 1458, 1519, 1673, 1718, 1741, 2864, 2887, 2907, 2962, 3061, 3379, 3464. LC-MS-ESI rt 9.1 min, m/z 448 (M + 1); Anal. cald. For $C_{24}H_{37}N_3O_5$ (447.57): C 64.41, H 8.33, N 9.39; found C 64.35, H 8.33, N 9.36.

34c: Yield 65% (78:22, Z/E mixture), yellow sticky oil, ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.83 (d, J = 6.6 Hz, 6H), 1.42 (d, J = 7.4 Hz, 3H), 1.47 (s, 18H), 2.60-2.81 (m, 1H), 3.59 (bs, 2H), 3.83-3.98 (m, 3H), 4.34-4.61 (m, 2H), 6.61 (d, J = 8.0 Hz, 2H), 6.83 (q, J = 7.4 Hz, 1H), 7.01 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 50 MHz) d 13.5, 20.2 (2C), 28.1 (3C), 28.5 (3C), 31.8, 42.2, 47.3, 50.1, 79.8, 82.1, 115.0, 127.9, 130.7, 131.3, 139.6, 144.9, 156.0, 169.1, 169.9; IR (neat, cm^{-1}) 737, 847, 1157, 1367, 1463, 1519, 1674, 1715, 1738, 2863, 2902, 2962, 3047, 3373, 3467. LC-ESI-MS rt 9.15 min, m/z 476 (M + 1), 498 (M + Na); Anal. cald. for $C_{26}H_{41}N_3O_5$ (475.62): C 65.66, H 8.69, N 8.83; found C 65.58, H 8.70, N 8.86.

Procedure for the preparation of the amino acid 35:

To a stirred solution of compound 34 (0.2 mmol), in DCM (0.5 mL), H_3PO_4 (85% solution in water, 5 equiv, 1 mmol, 69 mL) was added. After 3 h the organic solvent was removed under reduced pressure and the residue was treated with Dowex 50WX2-200 ion exchange resin, eluting with NH_4OH 0.5 M. Compound 35 was isolated after removal of the aqueous solvent.

35a: Yield 80% (77:23, Z/E mixture), pale yellow solid, m.p. 92-94 °C (dec.) ^{1}H NMR (CD_3OD, 200 MHz) δ 1.38 (d, J = 7.0 Hz, 3H), 1.79 (d, J = 6.8 Hz, 3H), 3.70-4.25 (m, 5H), 6.70-6.74 (m, 3H), 7.01-7.25 (m, 3H). ^{13}C NMR (CD_3OD, 50 MHz) d 14.1, 18.7, 44.7, 49.8, 53.7, 115.1 (2C), 128.0, 130.4, 132.0 (2C), 137.8, 144.6, 164.2,179.7; IR (neat, cm^{-1}) 1109,1215,1299,1467,1578,1662,1746, 2824, 2956, 3026, 3325, 3366. LC-MS-ESI rt 0.98 min, m/z 292 (M + 1). Anal. cald. for $C_{15}H_{21}N_3O_3$ (291.35): C 61.84, H 7.27, N 14.42; found C 62.02, H 7.25, N 14.43.

35c: Yield 95% (80:20, Z/E mixture), pale yellow solid, m.p. 96-98 °C (dec.) ^{1}H NMR (CD_3OD, 200 MHz) δ 0.93 (d, J = 6.6 Hz, 6H), 1.82 (d, J = 7.2 Hz, 3H), 2.18-2.28 (m, 1H), 3.83-4.38 (m, 5H), 6.74 (d, J = 8.4 Hz, 2H), 6.90 (q, J = 7.2 Hz, 1H), 7.21 (d, J = 8.4 Hz, 2H); ^{13}C NMR (CD_3OD, 50 MHz) δ 14.3, 19.9 (2C), 31.9, 46.9, 50.9, 62.4,116.3 (2C),121.1,128.8,132.0 (2C),140.7,148.5,161.6, 178.2; IR (neat, cm^{-1}) 1107, 1206, 1289, 1455, 1574, 1662, 1750, 2821, 2842, 2926, 2981, 3015, 3317, 3368. LC-ESI-MS rt 1.06 min, m/z 320 (M + 1), 342 (M + Na). Anal. cald. for $C_{17}H_{25}N_3O_3$ (319.40): C 63.93, H 7.89, N 13.16; found C 64.12, H 7.90, N 13.20.

General procedure for the synthesis of 36a-f:

To a stirred solution of carbonate **11** (0.2 mmol) in dry CH_3CN (2 mL), under a nitrogen atmosphere, was added 4-aminobenzylamine (1.5 equiv., 0.3 mmol, 34 μL). The solution was heated at reflux for 3 d and then the solvent was removed

under reduced pressure. Compounds **36a-f** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate, 85:15).

36a: Yield: 62–65%, 35–37 mg, isolated as a 3:1 mixture of *Z*/*E* isomers. ^{1}H NMR (200 MHz, $CDCl_3$) major isomer: δ = 1.47 (d, *J* = 6.8 Hz, 3 H), 1.49 (s, 9 H,) 1.73 (d, *J* = 7.4Hz, 3 H), 3.52 (d, *J* = 12.6 Hz, 1 H), 3.68 (d, *J* = 12.6 Hz, 1 H), 3.81 (q, *J* = 6.8 Hz, 1 H), 6.59 (d, *J* = 8.4 Hz, 2 H), 6.93 (q, *J* = 7.4 Hz, 1 H), 7.16 (d, *J* = 8.4 Hz, 2 H) ppm. ^{1}H NMR (200 MHz, $CDCl_3$) minor isomer: δ = 1.37 (d, *J* = 6.8 Hz, 3 H), 1.95 (d, *J* = 7.0 Hz, 3 H), 4.02 (q, *J* = 6.8 Hz, 1 H), 6.23 (q, *J* = 7.0 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) major isomer: δ = 13.7 (CH_3), 20.6 (CH_3), 28.3 (3 CH_3), 49.9 (CH), 50.9 (CH_2), 80.5 (C), 115.2 (2 CH), 129.4 (2 CH), 130.5 (C), 136.3 (C), 137.7 (CH), 145.3 (C), 166.7 (C) ppm. (*S*)-**36a** [α]D = –14.0 (*c* = 1, $CHCl_3$); (*R*)-**36a** [α]D = +14.0 (*c* = 1, $CHCl_3$). HPLC (*n*-hexane/2-propanol, 99:1 to 96:4 over 30 min; 1.0 mLmin–1; AD column; r.t): *t*R = 23.52 [(*R*)-**36a**], 26.12 min [(*S*)- **36a**]. LC–ESI-MS (r.t.): *t*R = 8.39 min, *m*/*z* = 290 [M], 313 [M + Na]. $C_{17}H_{26}N_2O_2$ (290.2): calcd. C 70.31, H 9.02, N 9.65; found C 70.08, H 9.00, N 9.65.

36b: Yield: 60–65%, 38–41 mg, isolated as a 3:1 mixture of *Z*/*E* isomers. ^{1}H NMR (200 MHz, $CDCl_3$) major isomer: δ = 0.74 (d, *J* = 6.8Hz, 3 H), 1.10 (d, *J* = 6.8Hz, 3 H), 1.49 (s, 9 H), 1.67 (d, *J* = 7.2 Hz, 3 H), 1.80– 2.05 (m, 1 H), 3.06 (d, *J* = 9.6 Hz, 1 H), 3.38 (d, *J* = 12.8 Hz, 1 H), 3.70 (d, *J* = 12.8 Hz, 1 H), 6.63 (d, *J* = 8.2 Hz, 2 H), 6.92 (q, *J* = 7.2 Hz, 1 H), 8.20 (d, *J* = 8.2 Hz, 2 H) ppm. ^{1}H NMR (200 MHz, $CDCl_3$) minor isomer: δ = 0.83 (d, *J* = 6.6Hz, 3 H), 0.97 (d, *J* = 6.6Hz, 3 H), 1.93 (d, *J* = 7.0 Hz, 3 H), 2.77 (d, *J* = 8.6 Hz, 1 H), 5.77 (q, *J* = 7.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major isomer: δ = 14.0 (CH_3), 20.1 (2 CH_3), 28.3 (3 CH_3), 32.1 (CH), 50.7 (CH_2), 61.3 (CH), 80.2 (C), 114.9 (2 CH), 129.1 (2 CH), 131.1 (C), 134.4 (C), 138.7 (CH), 144.9 (C), 167.0 (C) ppm. (*S*)-**36b** [α]D = –11.7 (*c* = 1, CHCl3); (*R*)-**36b** [α]D = +12.0 (*c* = 1, CHCl3). HPLC (*n*-hexane/ 2-propanol, 99:1 to 90:10 over 30 min; 1.0 mLmin–1; AD column; r.t.): *t*R = 10.39 [(*R*)-**36b**], 10.95 min [(*S*)-**36b**]. LC–ESI-MS (r.t.): *t*R = 5.87 min, *m*/*z* = 318 [M], 341 [M + Na].

$C_{19}H_{30}N_2O_2$ (318.23): calcd. C 71.66, H 9.50, N 8.80; found C 71.52, H 9.51, N 8.83.

36c: Yield: 60–63%, 43–46 mg, isolated as a 3:1 mixture of *Z*/*E* isomers. ^{1}H NMR (200 MHz, $CDCl_3$) major isomer: δ = 1.07–1.27 (m, 4 H), 1.41–1.81 (m, 6 H), 1.50 (s, 9 H), 1.65 (d, *J* = 7.2 Hz, 3 H), 2.36 (m, 1 H), 3.17 (d, *J* = 9.6 Hz, 1 H), 3.39 (d, *J* = 13.2 Hz, 1 H), 3.70 (d, *J* = 13.2 Hz, 1 H), 6.64 (d, *J* = 8.4 Hz, 2 H), 6.92 (q, *J* = 7.2Hz, 1 H), 7.12 (d, *J* = 8.4 Hz, 2 H) ppm. ^{1}H NMR (200 MHz, $CDCl_3$) minor isomer: δ = 1.93 (d, *J* = 7.4 Hz, 3 H), 2.10 (m, 1 H), 2.80 (d, *J* = 8.8 Hz, 1 H), 3.41 (d, *J* = 12.8 Hz, 1 H), 3.72 (d, *J* = 12.8 Hz, 1 H), 5.73 (q, *J* = 7.4 Hz, 1 H) ppm. ^{13}C NMR (75 MHz, $CDCl_3$) major isomer: δ = 13.9 (CH_3), 26.0 (CH_2), 26.2 (CH_2), 26.5 (CH_2), 28.0 (3 CH_3), 30.3 (CH_2), 31.4 (CH_2), 41.3 (CH), 50.2 (CH_2), 59.6 (CH), 80.2 (C), 114.8 (2 CH), 129.0 (2 CH),
130.3 (C), 133.5 (C), 138.9 (CH), 144.9 (C), 166.9 (C) ppm. (*S*)-**36c** [α]D = –9.0 (*c* = 1, CHCl3); (*R*)-**36c** [α]D = +13.4 (*c* = 1, CHCl3). HPLC (*n*-hexane/2-propanol, 99:1 to 90:10 over 30 min; 1.0 mLmin–1; AD column; r.t.): *t*R = 12.44 [(*S*)-**36c**], 15.14 min [(*R*)- **36c**]. LC–ESI-MS (r.t.): *t*R = 5.28 min, *m*/*z* = 358 [M], 381 [M + Na]. $C_{22}H_{34}N_2O_2$ (358.26): calcd. C 73.70, H 9.56, N 7.81; found C 73.83, H 9.54, N 7.83.

36f: Yield: 63%, 45 mg, isolated as a 3:1 mixture of *Z*/*E* isomers. ^{1}H NMR (200 MHz, $CDCl_3$) major isomer: δ = 1.35 (s, 9 H), 1.82 (d, *J* = 7.4 Hz, 3 H), 3.61 (d, *J* = 13.0 Hz, 1 H), 3.78 (d, *J* = 13.0 Hz, 1 H), 4.81 (s, 1 H), 6.66 (d, *J* = 8.4 Hz, 2 H), 6.96–7.06 (m, 2 H), 7.13–7.26 (m, 4 H) ppm. ^{1}H NMR (200 MHz, $CDCl_3$) minor isomer: δ = 2.00 (d, *J* = 7.0Hz, 3 H), 4.51 (s, 1 H), 6.09 (q, *J* = 7.0 Hz, 1 H) ppm. ^{13}C NMR (50 MHz, $CDCl_3$) major isomer: δ = 14.1 (CH_3), 28.1 (3 CH_3), 50.6 (CH_2), 54.3 (CH), 80.7 (C), 115.2 (2 CH), 120.3 (CH), 125.0 (CH), 127.0 (CH), 129.4 (2 CH), 130.4 (C), 134.8 (C), 138.9 (CH), 144.6 (C), 145.4 (C), 166.6 (C) ppm. LC–ESI-MS (r.t.): *t*R = 10.22 min, *m*/*z* = 358 [M], 381 [M + Na]. $C_{20}H_{26}N_2O_2S$ (358.17): calcd. C 67.01, H 7.31, N 7.81, S 8.94; found C 67.19, H 7.32, N 7.84, S 8.96.

General procedure for the synthesis of dehydro-β-amino acids 37a-f:

Trifluoroacetic acid (7 mmol, 7 equiv.) was added to a solution of 36a-f (1 mmol) in DCM (5 mL) and the reaction mixture was stirred at room temperature until complete consumption of the starting material. The solvent and the excess of reagent were removed under reduced pressure. The crude residue was treated with Dowex 50WX2-200 ion exchange resin, eluting with NH_4OH 0.5 M. Compounds 37a-f were isolated in 77-95% yield after removal of the aqueous solvent.

37a: Yield 77% (75:25, Z/E mixture), pale yellow solid, m.p. 96-98 °C (dec.) ^{1}H NMR (CD_3OD, 200 MHz) Z isomer δ 1.53 (d, J = 6.8 Hz, 3H), 1.88 (d, J = 7.2 Hz, 3H), 3.91 (d, J = 12.8 Hz, 1H), 4.01 (d, J = 12.8 Hz, 1H), 4.31 (q, J = 6.8 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 7.02 (q, J = 7.2 Hz, 1H), 7.23 (d, J = 8.4 Hz, 2H); E isomer δ 1.53 (d, J = 6.8 Hz, 3H), 2.12 (d, J = 7.0 Hz, 3H), 3.81 (q, J = 6.6 Hz, 1H), 3.91 (d, J = 12.8 Hz, 1H), 4.01 (d, J = 12.8 Hz, 1H), 6.05 (q, J = 7.0 Hz, 1H), 6.80 (d, J = 8.4 Hz, 2H), 7.23 (d, J= 8.4 Hz, 2H). ^{13}C NMR (CD_3OD, 50 MHz) d Z isomer 14.3, 19.5, 50.9, 53.6, 117.1, 122.1, 130.6, 132.6, 139.6, 151.3, 174.3; IR (neat, cm^{-1}) 1081, 1392, 1590, 1651, 1688, 2848, 2921, 2950, 3368, 3399. LC-MS-ESI rt 1.18 min (Z isomer), 1.53 min (E isomer), 235 (M + 1), 257 (M + 23), 491 (2M + 23). Anal. cald. for $C_{13}H_{18}N_2O_2$ (234.29): C 66.64, H 7.74, N 11.96; found C 66.40, H 7.76, N 11.99.

37b: Yield 95% (70:30, Z/E mixture), white solid, m.p. 108-110 °C (dec.) ^{1}H NMR (CD_3OD, 200 MHz) Z isomer δ 0.80 (d, J = 7.0 Hz, 3H), 0.91 (d, J = 7.0 Hz, 3H), 1.66 (d, J = 7.2 Hz, 3H), 2.01-2.16 (m, 1H), 3.69-3.95 (m, 3H), 6.61 (d, J= 8.4 Hz, 2H), 6.94 (q, J = 7.2 Hz, 1H), 7.04 (d, J = 8.4 Hz, 2H); E isomer δ 0.81 (d, J = 7.0 Hz, 3H), 0.89 (d, J = 7.0 Hz, 3H), 1.94 (d, J = 7.0 Hz, 3H), 2.01-2.16 (m, 1H), 3.69-3.95 (m, 3H), 5.77 (q, J = 7.0 Hz, 1H), 6.61 (d, J = 8.4 Hz, 2H), 7.06 (d, J = 8.4 Hz, 2H). ^{13}C NMR (CD_3OD, 50 MHz) δ Z isomer 14.3, 19.0, 32.6, 50.2, 62.0, 116.3, 121.0, 131.9, 132.0, 142.0, 150.3, 176.1; E isomer 14.9, 20.0, 31.8, 50.3, 62.0, 116.3, 121.0, 131.8, 132.0, 142.1, 151.0, 177.0. IR (neat, cm^{-1}) 846, 1372,

1610, 1684, 2852, 2957, 3366, 3421. LC-MS-ESI rt 1.13 min (Z isomer),1.59 min (E isomer), 263 (M + 1), 547 (2M + 23). Anal. cald. for $C_{15}H_{22}N_2O_2$ (262.35): C 68.67, H 8.45, N 10.68; found C 68.88, H 8.45, N 10.71.

37c: Yield 88% (70:30, Z/E mixture), yellow solid, m.p. 195-197 °C (dec.) ^{1}H NMR (DMSO, 200 MHz) Z isomer δ 1.74 (d, J = 7.4 Hz, 3H), 3.60 (d, J = 13.2 Hz, 1H), 3.72 (d, J = 13.2 Hz, 1H), 5.02 (s, 1H), 6.53 (d, J = 8.6 Hz, 2H), 6.78 (q, J = 7.4 Hz, 1H), 7.01 (d, J = 8.4 Hz, 2H), 7.10 (d, 1H), 7.47 (bs, 1H), 7.50-7.54 (m, 1H); E isomer δ 1.96 (d, J = 6.8 Hz, 3H), 3.60 (d, J = 13.2 Hz, 1H), 3.72 (d, J = 13.2 Hz, 1H), 4.60 (s, 1H), 5.89 (q, J = 6.8 Hz, 1H), 6.53 (d, J = 8.6 Hz, 2H), 7.01 (d, J = 8.4 Hz, 2H), 7.10 (d, 1H), 7.47 (bs, 1H), 7.50-7.54 (m, 1H); ^{13}C NMR (CD_3OD, 50 MHz) δ Z isomer 14.4, 51.2, 56.4, 117.1, 121.7, 126.2, 126.7, 128.7, 130.0, 132.8, 132.9, 134.3, 138.8, 140.5, 151.3, 174.6; E isomer 16.7, 50.5, 56.4, 117.1, 122.0, 126.3, 126.7, 128.7, 130.0, 132.6, 132.9, 134.3, 138.6, 140.5, 153.3, 170.7; IR (neat, cm^{-1}) 760, 823, 1027, 1160, 1368, 1442, 1678, 1741, 2824, 2924, 2978, 3364, 3437. LC-MS-ESI rt 1.22 min (E isomer), 1.63 min (Z isomer), 303 (M þ 1). Anal. cald. For $C_{16}H_{18}N_2O_2S$ (302.39): C 63.55, H 6.00, N 9.26, S 10.60; found C 63.32, H 5.99, N 9.24, S 10.57.

37f: Yield 78% (75:25, Z/E mixture), pale yellow solid, m.p. 134-136 _°C (dec.) ^{1}H NMR (CD_3OD, 200 MHz) Z isomer δ 0.80-1.36 (m, 6H), 1.64-1.88 (m, 4H), 1.77 (d, J = 7.2 Hz, 3H), 1.99-2.01 (m, 1H), 3.89-4.11 (m, 3H), 6.83 (d, J = 7.0 Hz, 2H), 7.22 (d, J = 7.0 Hz, 2H), 7.34 (q, J = 7.4 Hz, 1H); E isomer δ 0.80-1.36 (m, 6H), 1.64-1.88 (m, 4H), 1.99-2.01 (m, 1H), 2.13 (d, J = 6.8 Hz, 3H), 3.89-4.11 (m, 3H), 6.33 (q, J = 6.8 Hz, 1H), 6.83 (d, J = 7.0 Hz, 2H), 7.22 (d, J = 7.0 Hz, 2H). ^{13}C NMR (CD_3OD, 50 MHz) Z isomer δ 15.1, 27.8 (2C), 27.9 (2C), 32.0, 43.1, 51.1, 62.3, 117.1, 121.9, 131.3, 132.7, 141.7, 151.2, 175.7; E isomer δ 15.6, 27.8 (2C), 28.0 (2C), 30.7, 42.1, 51.0, 62.3, 117.1, 122.1, 131.3, 132.7, 140.8, 151.2, 175.7; IR (neat, cm^{-1}) 1095, 1178, 1370,1455, 1614, 1651, 2841, 2922, 2939, 3307, 3409. LC-MS-ESI rt 1.68 min (Z isomer), 1.80 min (E isomer), 303 (M + 1), 325 (M +

23), 341 (M + 39), 627 (2M + 23). Anal. cald. for $C_{18}H_{26}N_2O_2$ (302.41): C 71.49, H 8.67, N 9.26; found C 71.25, H 8.69, N 9.24.

Procedure for the preparation of 38a:

To a stirred solution of the 36a (0.2 mmol) in dry DCM (5 mL), triethylamine (1.2 equiv, 0.24 mmol, 34 mL) and Fmoc-Gly-Cl (1.1 equiv, 0.22 mmol, 69 mL) were added. The solution was stirred for 12 h and then washed twice with water (5 mL). The two phases were separated, the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Compound 38a was isolated by flash chromatography on silica gel (cyclohexane/ ethyl acetate 90/10 as eluent).

38a: Yield 84% (75:25, Z/E mixture), pale yellow solid, m.p. 95-97 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.74 (d, J = 6.6 Hz, 3H), 1.14 (d, J = 6.6 Hz, 3H), 1.49 (s, 9H),1.66 (d, J = 7.2 Hz, 3H), 1.93 (m, 1H), 3.08 (d, J = 9.2 Hz, 1H), 3.52 (d, J = 12.8 Hz, 1H), 3.81 (d, J = 12.8 Hz, 1H) 4.00 (bs, 2H), 4.25 (t, J = 9.6 Hz, 1H), 4.42 (d, J = 9.6 Hz, 2H), 7.14 (q, J = 7.2 Hz, 1H), 7.30-7.43 (m, 8H), 7.70 (d, J = 7.2 Hz, 2H), 7.74 (d, J = 7.2 Hz, 2H); ^{13}C NMR ($CDCl_3$,100 MHz) δ 14.1, 20.1, 21.2, 28.2 (3C), 32.0, 46.1, 50.4, 53.4, 65.0, 80.3, 114.9, 120.6, 122.2, 123.3, 124.5, 128.8, 129.2, 130.2, 130.6, 138.0, 144.0, 149.0, 156.2,167.0,168.8. IR (neat, cm^{-1}) 1159,1276,1367,1455,1651,1682, 1698, 1738, 2823, 2861, 2923, 2982, 3018, 3450. LC-ESI-MS rt 5.46 min, m/z 620 (M + Na). Anal. cald. for $C_{35}H_{41}N_3O_5$ (597.32): C 72.34; H 7.25; N 7.03; found C 72.62, H 7.28, N 7.05.

Procedure for the preparation of the acid 39a:

To a stirred solution of 38a (0.2 mmol) in DCM (2 mL) at 0 °C, trifluoroacetic acid (15 equiv, 3 mmol, 223 mL) was added. After 2 h, the mixture was washed twice with water (5 mL), the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. The obtained acid was diluted in dry DCM (2 mL), under nitrogen atmosphere, and EDCI (1.2 equiv, 0.24 mmol, 46 mg) and

triethylamine (2.4 equiv, 0.48 mmol, 67 mL) were added. After 30 min HOBT (1.2 equiv, 0.24 mmol, 33 mg) and glycine t-butyl ester hydrochloride (1.2 equiv, 0.24 mmol, 41 mg) were added. The solution was stirred overnight and then the mixture was diluted with DCM and washed twice with acidic water (5 mL) and twice with basic water (5 mL). The two phases were separated, the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Compound 39a was isolated by flashchromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluent).

39a: Yield 55% (95:5, Z/E mixture), yellow solid, m.p.119-121 °C, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 0.82 (d, J = 7.2 Hz, 3H), 1.07 (d, J = 7.2 Hz, 3H),1.49 (s, 9H),1.71 (d, J = 7.6 Hz, 3H), 2.10 (m, 1H), 3.34-3.95 (m, 7H), 4.28 (t, J = 6.4 Hz, 1H), 4.42 (d, J =6.4 Hz, 2H), 6.85 (q, J = 7.6 Hz, 1H), 7.31-7.44 (m, 6H), 7.55 (d, J =8.0 Hz, 2H), 7.71 (d, J = 7.6 Hz, 2H), 7.83 (d, J = 8.0 Hz, 2H); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 14.0, 20.1, 20.4, 28.3 (3C), 32.2, 38.8, 42.8, 48.3, 51.2, 62.4, 68.2, 82.9, 120.6, 121.0, 121.3, 122.0, 126.2, 128.7, 129.8, 130.2, 130.3, 138.9, 142.5, 145.2, 158.3, 170.5, 171.4 (2C); IR (neat, cm^{-1}) 1161, 1278, 1368, 1463, 1651, 1673, 1684, 1698, 1732, 2822, 2861, 2918, 2980, 3020, 3391. LC-ESI-MS rt 6.54 min, m/z 655 (M + 1). Anal. cald. For $C_{38}H_{46}N_4O_6$ (654.80): C 69.70; H 7.08; N 8.56; found C 69.89, H 7.05, N 8.58.

Procedure for the preparation of the amino acid 40a:

To a stirred solution of compound 39a (0.2 mmol, 112 mg), in DCM (0.5 mL), H_3PO_4 (85% solution in water, 3 equiv, 0.6 mmol, 83 mL) was added. After 16 h the organic solvent was removed under reduced pressure. The residue was diluted in methanol (2 mL), and piperidine (0.1 equiv, 0.02 mmol, 2 mL) was added. After 8 h the organic solvent was removed under reduced pressure, the residue was diluted in 1 N HCl (0.5 mL) and with treated with Dowex 50WX2-200 ion

exchange resin, eluting with NH_4OH 0.5 M. Compound 40a was isolated after removal of the aqueous solvent.

40a: Yield 60% (90:10, Z/E mixture), yellow solid, m.p. 141-143 °C (dec.) ^{1}H NMR (CD_3OD, 400 MHz) δ 0.78 (d, J = 7.2 Hz, 3H), 0.91 (d, J = 7.2 Hz, 3H), 1.64 (d, J = 7.2 Hz, 3H), 2.21 (m, 1H), 3.33-3.39 (m, 2H), 3.94-4.02 (m, 2H) 4.04 (s, 2H), 4.41 (d, J = 7.8 Hz, 1H), 6.78 (q, J = 7.2 Hz, 1H), 7.33 (d, J = 8.4 Hz, 2H), 7.40 (d, J = 8.4 Hz, 2H); ^{13}C NMR (CD_3OD, 100 MHz) δ 14.7, 19.7, 19.9, 31.9, 42.9, 43.4, 52.6, 67.1, 121.3 (2C), 128.5 (2C), 132.1, 132.4, 138.1, 154.0, 165.3, 168.6, 171.9. IR (neat, cm^{-1}) 1160, 1276, 1367, 1444, 1671, 1682, 1698, 1701, 2824, 2855, 2921, 3368, 3410. LC-ESI-MS rt 1.04 min, m/z 377(M + 1), 399 (M + Na). Anal. cald. for $C_{19}H_{28}N_4O_4$ (376.45): C 60.62, H 7.50, N 14.88; found C 60.43, H 7.51, N 14.91.

***Procedure for the synthesis of aldehyde 62*:**

Under inert conditions and at -78°C, dry DMSO (5.88ml, 3eq) was added dropwise to a solution of oxalyl chloride (2eq) in dry DCM (317 ml) and stirred for 2 h. After this time hex-5-yn-1-ol (1eq) in DCM (80 ml) was added dropwise to reaction mixture over 30 min. The mixture was stirred for further 30 minutes and then TEA dry (6eq) was added dropwise. The reaction was immediately warmed to room temperature and stirred for 1 h. After this time the solution was diluted with Et_2O (322 ml) and water (161 ml), extract with Et_2O and washed with brine. The organic layer were dried with anhydrous sodium sulfate and concentrated in vacuo.

62: Yield 99% , yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.86 (m, 2H), 1.99 (t, J= 2.8 Hz, 1H), 2.28 (m, 2H), 2.62 (m, 2H), 9.81 (s, 1H). LC-MS-ESI rt 6.4 min, 97 (M+1), 215 (2M+Na).

Synthesis of unsatured compound 1g:

In a round-bottom flask aldehyde 62 (1eq, 28.83mmol), tert-butyl acetoacetate (1eq) and piperidine (0,15 eq) were added and stirred under neat conditions for 2 days. After this time the mixture was extracted with EtOAc and water, the organic layer were dried by anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (c-Hex/EtOAc 98:2).

1g-major isomer: Yield 35% , yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.56 (s, 9H), 1.74 (m, 2H), 1.99 (s, 1H), 2.26 (m, 2H), 2.31 (s, 3H), 2.44 (m, 2H), 6.75 (t, J= 8 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 18.13, 27.00, 27.11, 27.84, 28.69, 69.15, 82.39, 83.31, 138.57, 145.32, 165.70, 195.07. LC-MS-ESI rt 9.2 min, 259 (M+Na).

1g-minor isomer: Yield 10% , yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.50 (s, 9H), 1.67 (m, 2H), 2.02 (s, 1H), 2.25 (m, 4H), 2.36 (s, 3H), 6.79 (t, J= 7.6 Hz, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.75, 25.40, 30.45, 69.00, 81.51, 82.97, 137.68, 145.62, 163.08, 200.74. LC-MS-ESI rt 9.1 min, 259 (M+Na).

Synthesis of compound 64:

Under inert conditions and at 0°C, $Yb(OTf)_3$ (5% mol) was added to a stirred solution of compound 1g (1eq, 6.08 mmol) in dry DCM (111.2 ml). After 10 minutes TMSONHTMS was added and the reaction mixture stirred for 30 minutes. After this time the reaction was quenched by water and extracted with DCM/water. The organic layer were dried with anhydrous sodium sulfate and concentrated in vacuo. The crude product was used for the next step.

Synthesis of compound 65:

Under inert conditions, $SOCl_2$ (1,5eq) was added dropwise to a solution of mono-benzyl-malonyl acid (1eq, 1,86 mmol) in dry DCM (35.7 ml) at 0°C and then stirred at room temperature for 30 min. 9,3 mmol of pyridine (5eq) and compound

64 in 17,7 ml of dry DCM were added. The reaction mixture was stirred overnight and followed by TLC. The reaction mixture was quenched with water and extracted with DCM. The organic layer were dried by anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (c-Hex/EtOAc 95:5).

65: Yield 44% , yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.49 (s, 9H), 1.57 (m, 2H), 1.75 (m, 2H), 1.95 (t, J= 2.8 Hz, 1H), 2.17 (s, 3H), 2.23 (m, 2H), 2.87 (d, J= 7.2 Hz, 1H), 3.63 (d, J= 16 Hz, 1H), 3.57 (d, J=15.6 Hz, 1H), 4.67 (m, 1H), 5.14 (d, J=12 Hz, 1H), 5.18 (d, J=12 Hz, 1H), 7.36 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 17.9, 22.7, 24.4, 27.6, 33.7, 40.9, 58.8, 66.7, 68.7, 82.2, 83.7, 105.3, 128.1, 135.2, 167.1, 167.6, 168.9. LC-MS-ESI rt 10.5 min, 446 (M+1), 913 (2M+Na).

Synthesis of compound 66:

Compound 65 (1.86 mmol, 1eq) in dry DCM (9.6 ml) was stirred with TEA (2eq) at 0°C for 10 min. Mesyl chloride (2eq) was added and the reaction mixture was stirred overnight at room temperature. After this time the reaction was diluted with DCM and washed with water. The organic layer were dried with anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (c-Hex/EtOAc 8:2).

66: Yield 82% , yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.5 (s, 9H), 1.56 (m, 2H), 1.75 (m, 1H), 1.93 (t, J= 2.8, 1H), 1.99 (m, 1H), 2.08 (s, 3H), 2.21 (m, 2H), 3.65 (d, J= 16 Hz, 1H), 3.51 (d, J= 16 Hz, 1H), 5.17 (s, 1H), 5.30 (bs, 1H), 7.35 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.1, 17.9, 23.7, 26.8, 28.0, 32.6, 40.9, 62.3, 81.0, 84.0, 105.8, 128.4, 135.1, 161.2, 162.1, 166.4, 167.8. LC-MS-ESI rt 11.5 min, 428 (M+1), 450 (M+Na).

Synthesis of compound 67:

TFA (15 eq) was added to a solution of compound 66 (0.56 mmol, 1eq) in DCM (3ml) at 0°C and the reaction was stirred at room temperature overnight. The mixture was washed with water and the organic layer were dried with anhydrous sodium sulfate and concentrated in vacuo. The crude product was used for the next step.

67: Yield 99% , yellow oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.57 (m, 2H), 1.78 (m, 1H), 1.94 (s, 1H), 2.02 (m, 1H), 2.13 (s, 3H), 2.21 (m, 2H), 3.55 (d, J= 16 Hz, 1H), 3.71 (d, J= 16 Hz, 1H), 5.17 (s, 2H), 5.36 (bs, 1H), 7.37 (m, 5H), 11.56 (bs, 1H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.5, 17.9, 23.6, 32.5, 40.7, 67.2, 68.9, 83.7, 104.1, 128.5, 135.0, 164.3, 166.2, 168.0, 168.1. LC-MS-ESI rt 10.9 min, 372 (M+1), 394 (M+Na).

Synthesis of compound 68:

Under inert conditions, TEA (1.5eq) and HBTU (1.5eq) were added to a solution of compound 67 (1 eq, 0.46 mmol) in dry DCM (5.8 ml) and stirred for 5 min. Then 4-aminobenzylamine (2eq) was added and the reaction was stirred at room temperature for 4 h. After this time the reaction was extract with DCM/water, the organic layer were dried with anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (c-Hex/EtOAc from 6:4 to 5:5).

68: Yield 80% , orange oil, ^{1}H NMR ($CDCl_3$, 400 MHz) δ 1.51 (m, 2H), 1.65 (m, 1H), 1.70 (t, J= 2.8 Hz, 1H), 1.94 (m, 1H), 2.12 (s, 3H), 2.14 (m, 1H), 2.26 (m, 1H), 3.41 (d, J= 16Hz, 1H), 3.57 (d, J= 16 Hz, 1H), 4.24 (dd, J= 14.8 Hz, J= 5.2 Hz, 1H), 4.39 (dd, J= 14.4 Hz, J= 5.6Hz, 1H), 5.11 (s, 2H), 5.32 (bd, J= 6.8 Hz, 1H), 5.99 (bs, 1H), 6.60 (d, J= 9.2 Hz, 2H), 7.03 (d, J= 8 Hz, 2H), 7.31 (m, 5H). ^{13}C NMR ($CDCl_3$, 100 MHz) δ 11.1, 17.3, 22.9, 32.6, 38.6, 43.0, 67.1, 69.1, 83.7,

106.4, 114.9, 128.5, 135.0, 145.9, 158.8, 161.9, 166.2, 168.3. LC-MS-ESI rt 8.5 min, 476 (M+1), 498 (M+Na), 973 (2M+Na).

3.2 $\alpha_4\beta_1$ Integrin Ligands

3.2.1 Introduction to integrins $\alpha_4\beta_1$

As previously said regarding $\alpha_5\beta_1$ and $\alpha_V\beta_3$ integrins, the $\alpha_4\beta_1$ integrins are heterodimeric transmembrane proteins as well. Unlike the former, this class of integrins has a fundamental role in white blood cells migration to the inflammatory site and for this reason it is involved in several pathologies – including asthma, multiple sclerosis and rheumatoid arthritis. Different studies have shown that $\alpha_4\beta_1$ integrin bind VCAM (vascular adhesion molecule) and fibronectin, and the incorrect operation of this interaction causes the pathologies mentioned earlier. To better understand the mechanism by which an hypothetical inhibitor could block the activity of the integrin, a representative model of $\alpha_4\beta_1$ integrin structure has been constructed. The design of a synthetic inhibitor is of crucial importance to mimick the right interactions involved between natural ligand and protein. The studies carried out on ligand-protein interactions have shown that both integrin subunits take part to ligand binding. For this kind of integrins, the complete interaction mechanism between the two involved parts is still unknown.

Integrin $\alpha_4\beta_1$ binds to fibronectin through recognition of three amino acids situated on the ligand surface: Leu-Asp-Val (LDV). This sequence can be considered equivalent to the RGD sequence for integrins $\alpha_V\beta_3$ and $\alpha_5\beta_1$, and, in view of the similarities with the latter, the possibility to use RGD mimetics as $\alpha_4\beta_1$ integrin inhibitor has been considered. Initial attemps tested with $\alpha_4\beta_1$ integrin inhibitor mimicking RGD sequence, have not provided desired results probably because side chains in used ligands are too flexible. During the years, it was understood that LDV sequence is fundamental to obtain the interaction with integrin, and using this sequence a potent and selective inhibitor for $\alpha_4\beta_1$ integrin has been selected: [N-(2-methylphenyl)-ureido]phenylacethyl-Leu-Asp-Val shown in Figure 18.

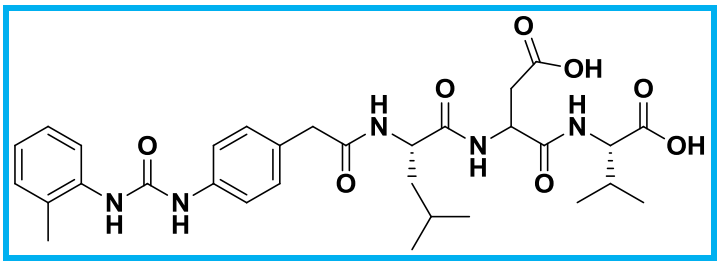


Fig. 18. [N-(2-methylphenyl)-ureido]phenylacethyl-Leu-Asp-Val

The identified interactions between protein and inhibitor show that an acidic unit linked to a spacer core is fundamental in order to generate hydrophilic interactions and an amide or an another group has to be able to establish hydrogen bond. This has been understood by X-ray analysis. The mentioned inhibitor is not resistant to enzyme hydrolysis because of the presence of a peptidic part. For this reason, it has been designed a non-peptidic structure to replace a LDV sequence, attempting to maintain the high activity on targed. Several developed antagonists contain the PUPA residue (Figure 19) to a structure ending with a carboxylate.

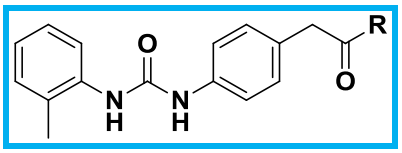


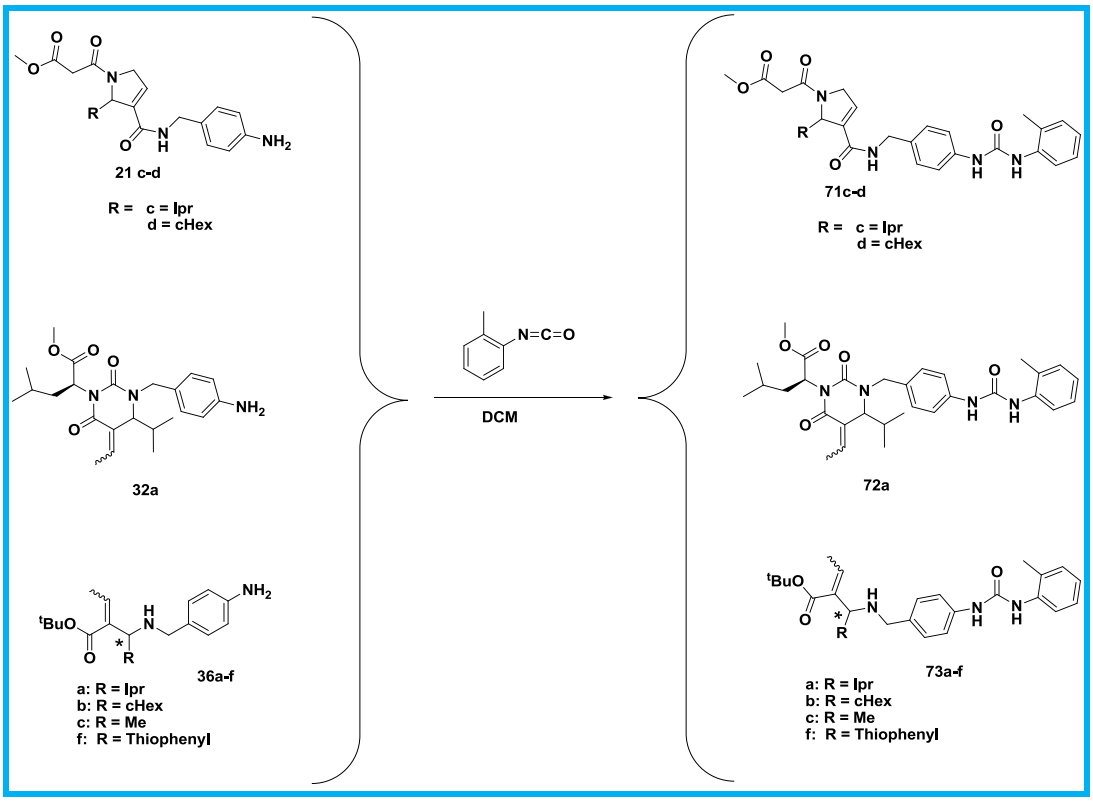
Fig. 19. 2-methyl PUPA.

Reported results suggested that the more active compounds present a PUPA residue along with a donor and an acceptor of hydrogen bond separated by hydrophobic portion. Usually, a terminal carboxylate is the acceptor and a nitrogen acts is the donor. After these considerations, we developed a series of compounds with appropriate features to be $\alpha_4\beta_1$ integrin inhibitors.

3.2.2 Synthesis of heterocyclic and linear integrin ligands

To obtain the $\alpha_4\beta_1$ integrin ligands we decided to insert the PUPA residue on the intermediate of $\alpha_V\beta_3$ and $\alpha_5\beta_1$ integrin ligands mentioned in the last section.

In particular, some heterocyclic ligands from the classes of dehydro-pyrrole and dihydropyrimidindione and some linear ligands have been studied. A simple protocol was used to introduce the fundamental PUPA pharmacophore moiety, which employs the O-tolyl-isocyanate in DCM at room temperature for 12 hours (Scheme 28).

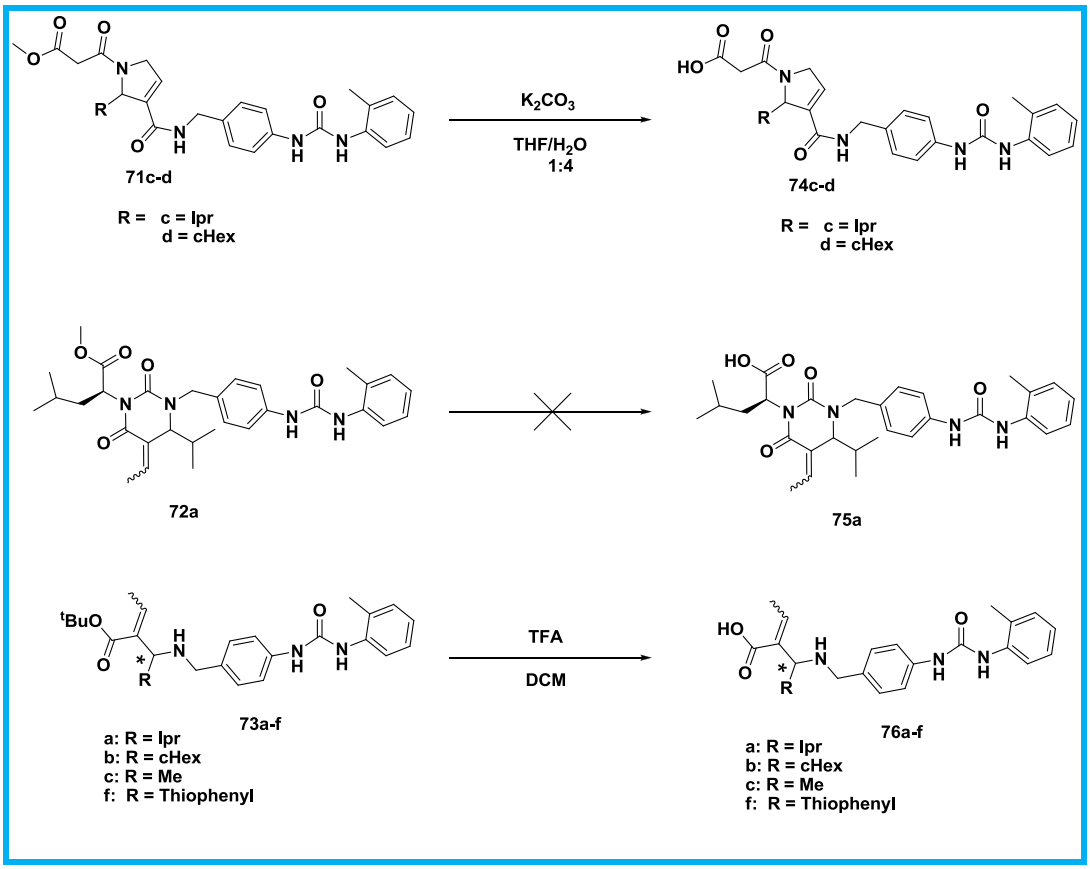


Scheme 28. Insertion of PUPA moiety on integrin ligand.

The compounds thus obtained, have been deprotected on the ester through defferent hydrolysis procedure, depending on the alkyl chain of the ester (Scheme 29). To hydrolyze the methyl ester present on the dehydropyrrole derivatives treatment

with potassium carbonate in THF/H_2O for four hours under reflux allowed us to obtain the free acids **74c-d**.

Also for the cleavage of the same ester on the dihydropyrimidinone derivative **72a**, several kind of methodologies have been tested but the product has never been observed. Finally, the acids **76a-f** have been obtained by treatment with TFA in DCM at room temperature overnight.



Scheme 29. Cleavage of esters.

3.2.3 Biological evaluations

Jurkat cell adhesion assays were done by coating 96-well plates were coated at 4 C overnight with 2 mg/mL of VCAM-1 and plotting a saturation curve for the ligand to establish the best signal-to-noise ratio. Non-specific hydrophobic binding sites were blocked by incubation with a BSA (1%)/HBSS (w/v) solution for 30 min at 37 C. The day of the assay, the cells were counted and stained with 12.5 μM CMFDA (30 min at 37°C). After three rinses with BSA/HBSS to wash away the excess dye, aliquots of 500,000 (Jurkat) cells were divided among a number of tubes corresponding to the number of treatments. For inhibition experiments, cells were mixed with the drug and pre-incubated at 37°C for 30 min to reach equilibrium before being plated.

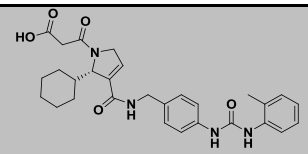
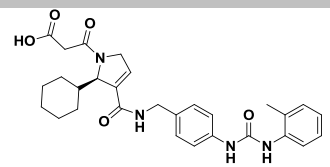
Entry	Compound	IC_{50}[nM] $\alpha_4\beta_1$
1		No active
2		No active

Table 11. At this stage, only the two dehyropyrroles reported in table X have been tested and unsatisfactory results have been observed.

After 30 min incubation at 37°C in the coated wells, the non-specifically bound cells were washed away with BSA/HBSS solution. Adherent cells were lysed by the addition of 0.5% Triton-X-100 in PBS (30 min at 4°C). Released CMFDA was quantified by fluorescence imaging at Ex485 nm/Em535 nm (Wallac ARVO 1420 multilabel counter) and adherent cells was counted by interpolation on a standard

curve. The fluorescence intensity with and without VCAM-I was taken as respectively 100% and 0%. Alternatively, the number of adherent cells was calculated by comparison with a standard curve prepared in the same plate using known concentrations of labeled cells.

3.2.4 Experimental section

General procedure for the preparation of urea-derivatives 71-73:

In a roun-bottom flask were added in order 249 mg (0.6 mmol, 1eq) of compound 21, 1 ml of DCM and 75 μl (0.6 mmol, 1eq) of O-tolyl isocyanate. The reaction was stirred at room temperature and followed by TLC. After 20h the reaction was complete and then has been performed an extraction with EtOAc/water. The organic layer were dried with anhydrous sodium sulfate and concentrated in vacuo. The crude product was purified by flash chromatography (c-Hex/EtOAc 8:2). Orange oils. Yields 80-88%.

71c major and minor isomer on amidic bond: ^{1}H NMR (acetone-d^{6}, 400 MHz) δ 0.87 (d, J= 5.6 Hz, 3H), 0.89 (d, J= 5.6 Hz, 3H), 2.03 (s, 3H), 2.36 (m, 1H), 4.43 (d, J= 6 Hz, 2H), 3.7 (s, 3H), 4.27 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 4.31 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 5.13 (s, 1H), 6.47 (s, 1H), 6.94-7.88 (m, 8H), 7.68 (bs, 2H), 8.38 (bs, 1H).

71d: ^{1}H NMR (acetone-d^{6}, 400 MHz) δ 0.89-1.32 (m, 5H), 1.40-1.55 (m, 6H), 2.03 (s, 3H), 4.43 (d, J= 6 Hz, 2H), 3.7 (s, 3H), 4.27 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 4.31 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 5.13 (s, 1H), 6.35 (s, 1H), 6.94-7.88 (m, 8H), 7.68 (bs, 2H), 8.38 (bs, 1H). α: **S-71d**=+20.47, **R-71d**= - 17.50. LC-MS-ESI rt 7.7 min, 533 (M+1), 555 (M+Na).

72a: NMR (acetone-d^{6}, 400 MHz) δ 0.86 (d, J= 4.8 Hz, 3H), 0.87 (d, J= 6.8 Hz, 3H), 0.92-0.96 (m, 6H), 1.00 (d, J= 6.4 Hz, 3H), 1.58 (d, J= 6.4 Hz, 3H), 1.94 (m, 1H), 2.05 (m, 1H), 2.20 (s, 3H), 3.64 (s, 3H, major), 3.66 (s, 3H, minor), 3.82 (d, J= 7.2 Hz, 1H), 3.84 (d, J= 7.6 Hz, 1H, minor), 3.97 (d, J= 15.6 Hz, 1H, major), 4.01 (d, J= 15.2 Hz, 1H, minor), 5.30 (m, 1H), 5.33 (d, J= 1.2 Hz, 1H, major), 5.35 (d, J= 4.8 Hz, 1H), 6.86 (q, J= 7.2 Hz, 2H), 6.89 (m, 1H), 6.95 (m, 1H), 7-7.2 (m, 4H), 7.59 (d, J= 8.0 Hz, 1H, major), 7.63 (d, J= 7.4 Hz, 1H).

73a: Yield: 62–65%, ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.47 (d, *J* = 6.8 Hz, 3 H), 1.49 (s, 9 H,) 1.73 (d, *J* = 7.4Hz, 3 H), 3.52 (d, *J* = 12.6 Hz, 1 H),3.64 (s, 3H), 3.68 (d, *J* = 12.6 Hz, 1 H), 3.81 (q, *J* = 6.8 Hz, 1 H), 6.50-7.4 (m, 9H).

Hydrolisis for methyl and tert-butyl ester already reported in chapter 2.

74c: major and minor isomer on amidic bond: ^{1}H NMR (acetone-d^6, 400 MHz) δ 0.87 (d, J= 5.6 Hz, 3H), 0.89 (d, J= 5.6 Hz, 3H), 2.03 (s, 3H), 2.36 (m, 1H), 4.43 (d, J= 6 Hz, 2H), 4.27 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 4.31 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 5.13 (s, 1H), 6.47 (s, 1H), 6.94-7.88 (m, 8H), 7.68 (bs, 2H), 8.38 (bs, 1H), 8.6 (bs, 1H).

74d: ^{1}H NMR (acetone-d^6, 400 MHz) δ 0.89-1.32 (m, 5H), 1.40-1.55 (m, 6H), 2.03 (s, 3H), 4.43 (d, J= 6 Hz, 2H), 4.27 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 4.31 (dd, J= 16 Hz, J= 4,4 Hz, 1H), 5.13 (s, 1H), 6.35 (s, 1H), 6.94-7.88 (m, 8H), 7.68 (bs, 2H), 8.38 (bs, 1H), 8.6 (bs, 1H).

3.3 Analogues of Linezolid

3.3.1 Introduction to antibiotics

In nature a great number of bacteria are know to be responsible of a huge number of diseases by combining their action. Bacteria are however divided in two big families, depending on the Gram's test response: Gram positive and Gram negative (Figure 20). Among Gram positive bacteria appear *Enterococcus*, *Streptococcus* and *Staphylococcus*. These three classes of bacteria proved to be able to develop resistance to a large part of developed antibiotics and, with them, the battle against antibiotic resistance became an important challenge for researchers working in this field.

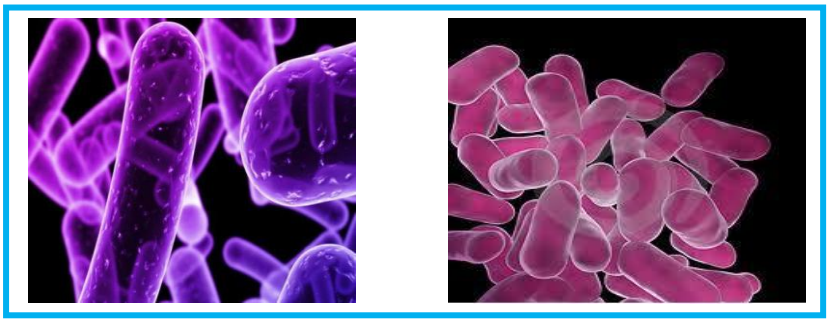


Fig. 20. Gram positive (left) and negative (right).

The first compound that was able to contrast some bacteria, Penicillin G, has been introduced to the drug market approximately 60 years ago, but, after some years later, microorganisms developed resistance to its action. During the sixties the first Penicillase-resistant penicillins arrived on the market The enzyme Penicillase is produced by the bacteria and it is able to hydrolyze penicillins on the lattamic ring and make them inactive. Examples of these kinds of antibiotics are Meticillin and Oxacillin (shown in Figure 21) and later Vancomicin, but after some years resistance has been registered for them too. Subsequently, several drugs have

provided good activities against different families of bacteria, but their exploitation has been often limited by resistance.

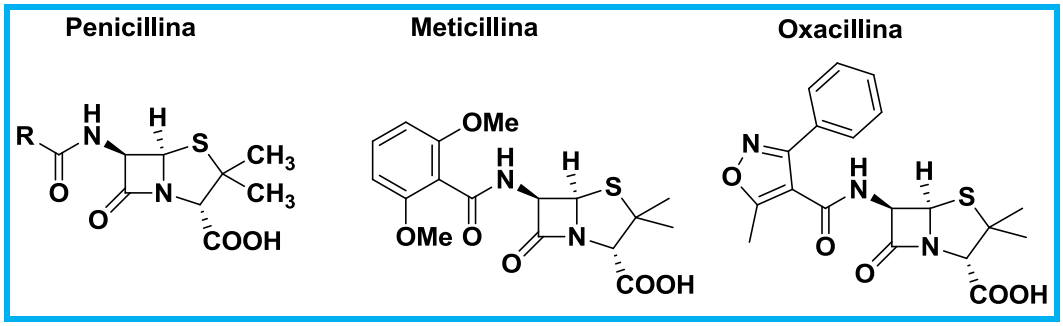


Fig. 21. Various classes of antibiotics.

In the nineties, to face this problem, a new class of compounds has been developed: the *Oxazolidinones*. The action mechanism of this class of molecules seems to be due to the inhibition of proteic synthesis[51] and it appears that these compounds do not gave cross-resistance with other antibiotics with already known mechanisms, until their admission on the market. The increasing interest in these compounds is justified by their large action on several Gram positive bacteria, including the ones that demonstrate resistance to date. An important aspect of oxazolidinones is that they are orally active and this would allow continuing the treatment autonomously without resort to hospital. From the DuPont company, in the 1987, two structure for oxazolidinones have been introduced: Dup-105 and Dup-721(Figure 22). Expecially the second one demonstrated to have a good activity against a large part of Gram-positive bacteria, but still not comparable to Vancomicin efficacy. The reactivity of these compounds is closely linked to their structure and after several changes on molecules backbone, *Eperezolid* has been synthesized, which presents a right balance among antibacterial activity, pharmacokinetic, solubility in water and other proper properties. Modifications on piperazinic ring have led to *Linezolid*, which has in its structure a morpholinic ring functioning as a electron-donor group

and it presents a of fluoride atom on the aromatic ring. Mentioned moieties are known to be fundamental in order to obtain good activity against bacteria.

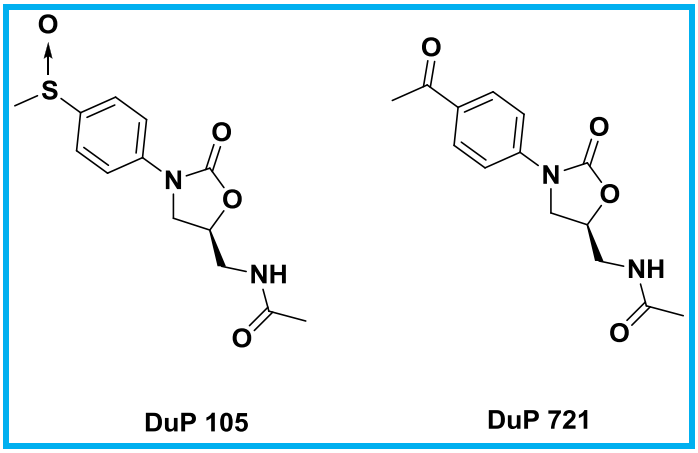


Figure 22. Isoxazolidinonic compound synthesized by DuPont company.

Linezolid inhibits the protein synthesis by blocking the aggregation between ribosomal subunits. In particular, it binds to ribosome on subunit 50S through its pharmacophores: three rings and one tail highlighted in Figure 23.

During the interaction with proteins, the morpholinic ring is orientated to the subunit interface, the oxazolidinic ring and the tail are orientated to ribosomal tunnel interacting with U2504 and A2503 residues respectively and the aromatic ring is between A2451 and U2506 residues (Figure 24).

Despite the good activity registered and the demonstrated mechanism, Linezolid presented some cases of resistance as well. In fact, mutations related to rRNA on subunit 23S interfere with Linezolid action overcoming the block given by drug.

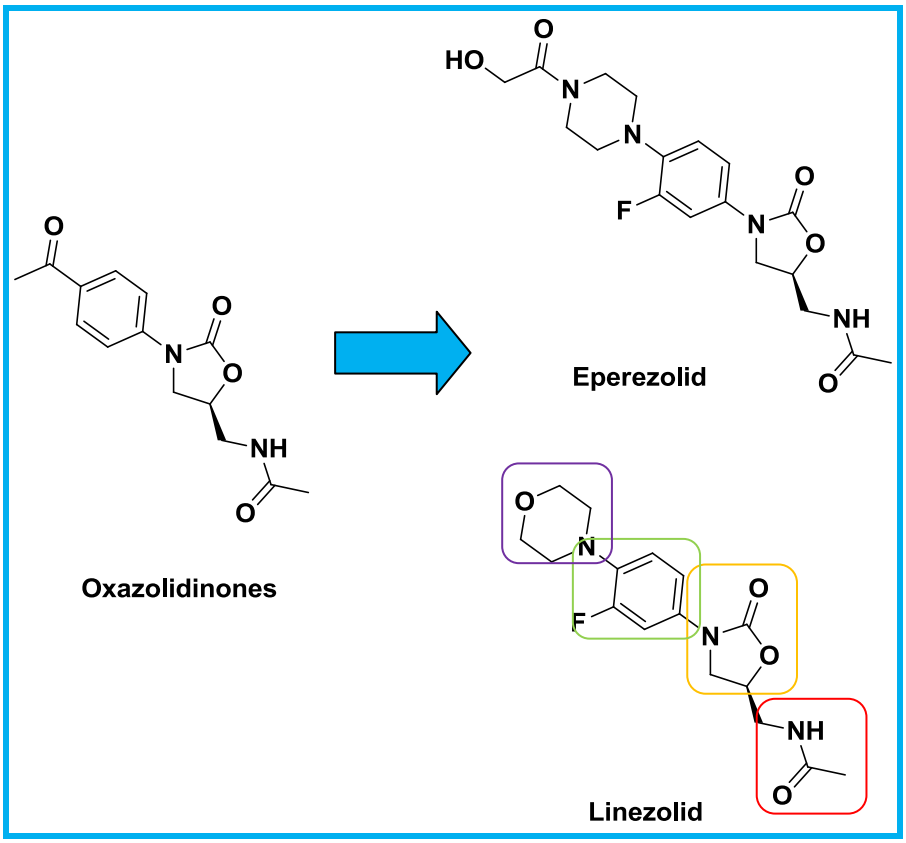


Fig. 23. Oxazolidinonic compounds

The continuous increase of resistance cases to Linezolid by several bacterial families has risen the need of new molecules to counteract this propensity. Basing on Linezolid structure we developed compounds that retain pharmacophores, with some modifications though.

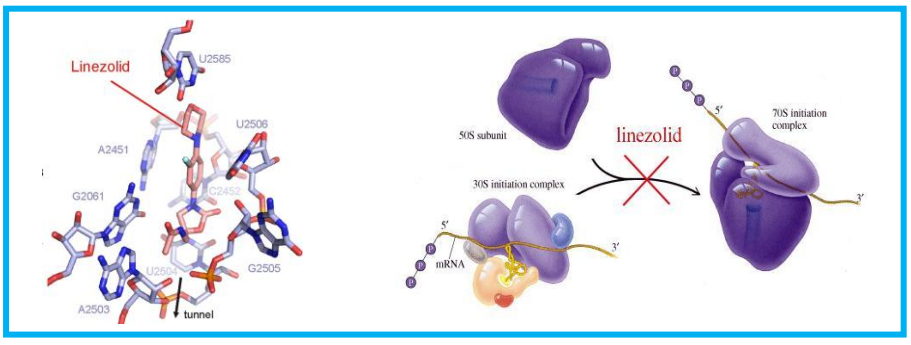
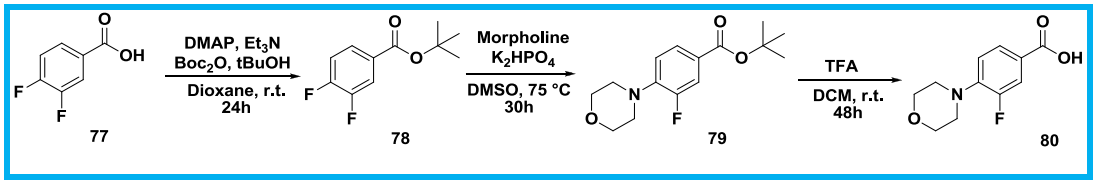


Fig. 24. Linezolid in the binding site and its action mechanism.

3.3.2 Synthesis of Linezolid analogues

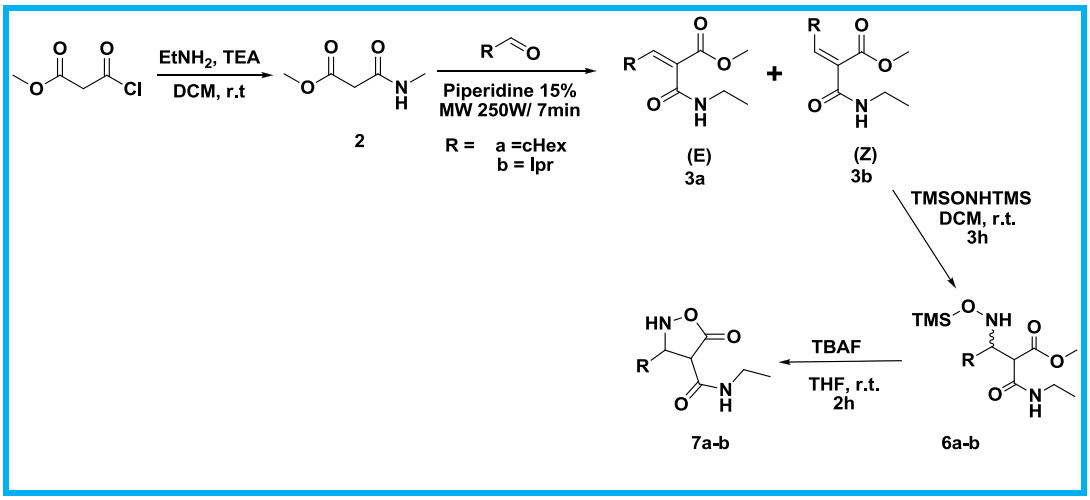
Linezolid analogues were synthesized starting from 3,4-difluorobenzoic acid **77** which has been protected as t-Butyl ester using Boc_2O, tBuOH, TEA and DMAP. The reaction was carried out in dioxane at room temperature and, after 24 hours, provided the corresponding ester **78** in 66% of yield. By treating this ester with K_2HPO_4 and morpholine, via a nucleophilic aromatic substitution in DMSO at 75°C for 30 hours, the compound **79** has been obtained in 82% of yield. The cleavage of t-butyl ester carried out in DCM with trifluoroacetic acid at room temperature for 48 hours provided the compound **80** quantitatively (Scheme 30).

In parallel, two isoxazolidinonic ring have been synthesized, differing from each other only for the alkyle side chain: we selected the structure with isopropyl chain and cyclohexyl chain as model compounds.



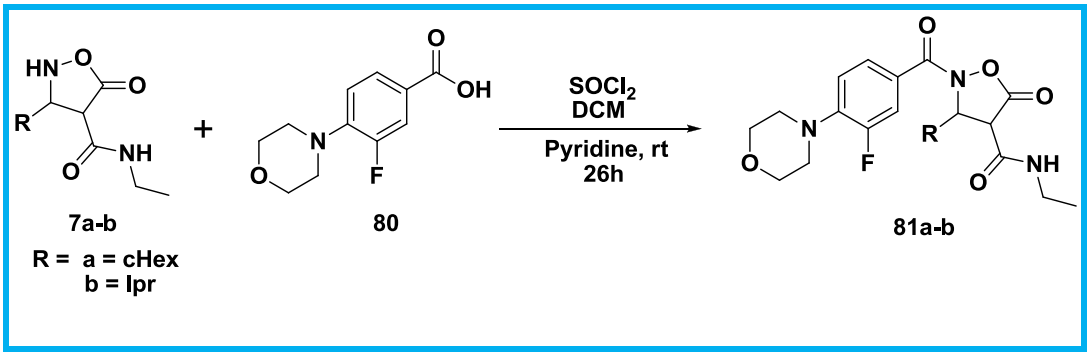
Scheme 30. Synthesis of 3-fluoro-4-morpholinobenzoic acid.

To obtain these rings, we first prepared malonamide **2** by a simple acylation of ethylamine with methylmalonyl chloride using TEA as a base in dichloromethane. After 24 hours at room temperature, the reaction provided us the malonamide in 79% of yield. By microwave assisted procedure, it has been possible to perform a very fast Knoevenagel reaction between the malonamide obtained and two different aldheyde (Isopropylaldehyde and cyclohexyl aldehyde) using piperidine as catalyst. In fact, in only 7 minutes without solvent, related alkylidenes **3a-b** have been provided in 29% and 79% of yield respectively. From this reaction we obtained a mixture of isomers with an abundance of Z one (Z/E ratio 4:1).



Scheme 31. Synthesis of isoxazolidinonic scaffold.

At this point we took advantage of the ability of alkylidene malonamides to take part in Michael addition as acceptor to introduce TMSONHTMS and obtain the adduct **6a-b**. After the addition of TMSONHTMS performed at -40°C in dichloromethane, the reaction was left stirring at room temperature for 3 hours and provided the product **6a** in 76% and **6b** in 83% of yield. By treatment of these adducts with TBAF with THF as solvent, after 2 hours at room temperature, isoxazolidinones **7a-b** have been isolated in 26% and 43% respectively (Scheme 31).



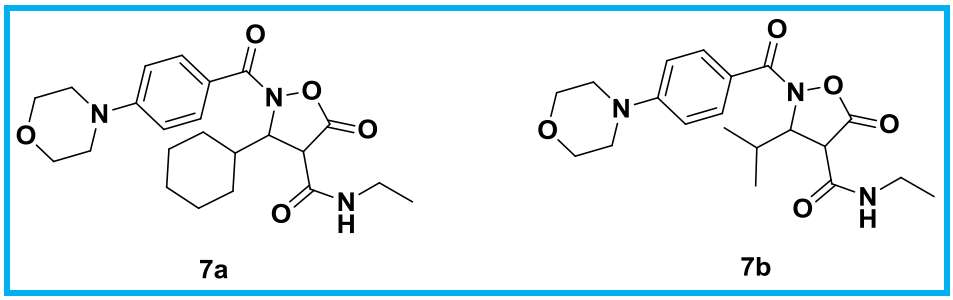
Scheme 32. Coupling between isoxazolidinonic scaffold and 3-fluoro-4-morpholinobenzoic acid.

Finally, a coupling between isoxazolidinones **7ab** and acid **80** using thionyl chloride and pyridine in dichloromethane allowed us to obtain the final compounds **81a-b** in 14% and 37% of yield respectively (Scheme 32).

3.3.3. Biological evaluations

To test the antibiotic efficiency of **7a** and **7b** a *dilution method* was used, which measures the minimum concentration of antibiotic able to inhibit the growth of the microrganism (MIC). This is an important assay because it provides informations about the conditions that lead to the death of the bacterium: once inhibited its growth it can be expelled from the body by the immune system. For the two molecules synthesized, the antibacterial activity in vitro by determination their MICs, using the method namely Broth Dilution was evaluated. The test was performed on Gram-positive bacteria such as *S. aureus, S. epidermidis, S. hominis, P. aeruginosa, P. mirabilis, E. faecalis and E. mirabilis*.

The first phase of testing involved the use of commercial strains of these bacteria.



Family	Molecule 7a	Molecule 7b	Linezolid	Ciprofloxacine	Cefuroxime
ATCC S. aureus 29213	>128	>128	2	2	4
ATCC S. aureus 25923	>128	>128	1	2	2
BAA 977 S. aureus	>128	>128	2	1	2
MU 50 S. aureus	>128	>128	2	128	4
ATCC 43300 S. aureus	>128	>128	0.5	1	>128
BAA-1026 S. aureus		>128	2		
ATCC 35218 E. coli		>128			8
ATCC 12453 P. mirabilis		>128			0.5
ATCC E. faecalis 29212	>128	>128	1	4	>128
S. hominis α 26	>128	>128	32	>128	8
S. epidermis α 86	>128	128	16	>128	16
S. epidermis α 99	>128	128	16	>128	128
S. epidermis α 560	>128	128	16	>128	>128
S. epidermis α 657	>128	128	16	>128	>128
S. epidermis α 758	>128	128	16	>128	>128
S. epidermis α 1453	>128	128	16	>128	128
S. epidermis G 220	>128	128	16	>128	>128
S. epidermis G 1027	>128	>128	16	>128	16
S. epidermis G 1054	>128	128	16	>128	128
S. epidermis CT 20 (2) cfr+	>128	128	16	>128	54

Table 12. Minimum concentration of antibiotic able to inhibit the growth of the microrganism (MIC). Compound 7a-b.

For MIC values higher than 128 mg/L, the molecule is considered devoid of antibacterial activity to the considered strain. So the molecule (a) can not be classified as an antibiotic to the strains used. Concerning the molecule (b), for nine strains of S. epidermidis it has a limit MIC of 128 mg/L so, it can be regarded as an antibiotic also if it shows a MIC considerably higher than the drugs currently used.

At this point, only for the molecule (b), the same analysis using bacterial strains isolated from patients with cystic fibrosis were performed. The results are reported in Table 13.

Family	Molecule (b)	Linezolid
S. aureus AA1	64	2
S. aureus AA2	>128	2
S. aureus AA3	64	2
S. aureus FB1	64	2
S. aureus FB2	32	2
S. aureus FB3	32	2
S. aureus DD1	>128	1
S. aureus DD2	>128	1
S. aureus DD3	>128	1
S. aureus VV1	128	2
S. aureus VV2	>128	2
S. aureus VV3	64	1

Table 13. Analysis on bacterial strains isolated from patients with cystic fibrosis.

From these further tests, we observe an improvement of the MIC value against strains of S. aureus, in particular, the best results are observed for strains FB2 and FB3. However, also in this case, the values are much higher than those of Linezolid compared to the same strain. On the basis of these data, we can consider that our molecules should not be descarted but they may be considered as lead compounds, whose activity has to be increased by modifications of the backbone.

3.3.4 Experimental section

Synthesis of compound 78:

Under nitrogen atmosphere, 3,4-difluorobenzoic acid (1eq), tert-butanol (1.1eq), TEA (2eq), DMAP (0.1eq) and dioxane (1eq) were added. The reaction mixture was stirred at 0°C until the addition of Boc_2O (1.3eq). The reaction was stirred at room temperature following it by TLC. The work-up was performed by quenching with water and extraction with EtOAc. The organic layer were collected and dried with sodium solfate anidrous, concentrated in vacuo. Yellow oil with 66% of yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.6 (s, 9H), 7.19 (m, 1H), 7.54 (m, 2H);

Synthesis of compound 79:

In a flask for reflux were added compound 78 (1 eq), DMSO (0.3 M), K_2HPO_4 (4 eq) e morpholine (2 eq). The reaction mixture was stirred at reflux for 30h at 75°C, following it by TLC. The work-up was performed by quenching with water and extraction with EtOAc. The organic layer were collected and dried with sodium solfate anidrous, concentrated in vacuo. Yellow oil with 82% of yield.

^{1}H-NMR (200 MHz, $CDCl_3$): δ 1.59 (s, 9H), 3.2 (t, 4H, J=2.4 Hz), 3.95 (t, 4H, J=2.4 Hz), 6.94 (t, J=4 Hz), 7.68 (m, 2H);

Synthesis of compound 80:

Under inert conditions 79 (1 eq) DCM (0.2 M) e K_2HPO_4 (4 eq) were added. The reaction mixture was stirred at 0°C to add TFA dropwise (25eq). The reaction was warmed at room temperature and stirred for 30h following it by TLC. The work-up was performed by acidification by HCl 1M, then an extraction with H_2O/DCM. The organic layer were collected and dried with sodium solfate anidrous, concentrated in vacuo. Yellow oil with 46% of yield.

^{1}H-NMR (400 MHz, $CDCl_3$): δ 3.31 (t, 4H, J=4.8 Hz), 3.89 (t, 4H, J=4.8 Hz), 6.93 (t, 1H, J=8 Hz), 7.76 (dd, 1H, J^1=14.4 Hz, J^2=2.4 Hz), 7.87 (dd, 1H, J^1=8.4 Hz, J^2=2.4 Hz);

General procedure for the synthesis of compounds 81a-b:

Under inert conditions, compound 80 (1,5 eq) and DCM (0.1 M) were added. The reaction mixture was carried at 0°C to add $SOCl_2$ (1.5eq) and stirred for 1h. After this time, pyridine (5eq) was added and the reaction mixture was stirred for further 20 min. Finally was added isoxazolidinone 7a-b (1eq) and the reaction was stirred for 24h following it by TLC. The work-up was performed by quenching with basic water and extraction with DCM. The organic layer were collected and dried with sodium solfate anidrous, concentrated in vacuo. Crude orange oil . After flash chromatography 8:2 c-Hex/EtOAc has been obtained the 37% and 14% of yield respectively for 81a-b.Withe olid

81a: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1.05 (d, 6H, J=6.4 Hz), 1.16 (t, 3H, J=7.6 Hz), 2.17 (m, 1H), 3.22 (t, 4H, J=4.4 Hz), 3.31 (m, 2H), 3.43 (d, 1H, J=3.2 Hz), 3.88 (t, 4H, J=4,4 Hz), 5.3 (dd, 1H, J^1=5.6 Hz, J^2=2.8 Hz), 6.16 (bs, 1H), 6.92 (t, 1H, J=8.8 Hz), 7.52 (dd, 1H, J^1=8 Hz, J^2=1.6 Hz), 7.58 (dd, 1H, J^1=8 Hz, J^2=1.6 Hz);

81b: ^{1}H-NMR (400 MHz, $CDCl_3$): δ 1,8 (m, 11H), 3.16 (m, 4H), 3.31 (m, 2H), 3.46 (d, 1H, J=2 Hz), 3.84 (m, 4H), 5.22 (m, 1H), 6.88 (t, 1H, J=8.4 Hz), 7.48 (d, 1H, J=9 Hz), 7.54 (d, 1H, J=8 Hz);

References

[1] M. Dressen,, B. H. P. Van De Kruijs, J. Meuldijk, J. Vekemans, L. A. Hulshof, *Org. Process Res. Dev.*, **2007**, 11, 865-869.

[2] A. De la Hoz, A. Diaz-Horitz, A. Moreno, *Chem. Soc. Rev.*, **2005**, 34, 164-178.

[3] F. Benfatti, G. Cardillo, S. Contaldi, L. Gentilucci, E. Mosconi, A. Tolomelli, E. Juaristi, G. Reyes-Rangel, *Tetrahedron*, **2009**, 65,2478-2483.

[4] A Tolomelli, G. Cardillo, L.Gentilucci, R. Juris, A. Viola and E. Juaristi, *Arkivoc*, **2012**, V, 196-209.

[5] F. Benfatti, G. Cardillo, L. Gentilucci, E. Mosconi, A. Tolomelli, *Synlett*, **2008**, 17, 2605-2608.

[6] Klibanov, A. M. *Acc. Chem. Res.* **1990**, 23, 114-120

[7] F. Benfatti, G. Cardillo, L. Gentilucci, E. Mosconi, A. Tolomelli, *Tetrah. Asymm.* **2007**, 18, 2227-2232.

[8] A. Tolomelli, L. Gentilucci, E, Mosconi, A. Viola, E. Paradisi, *Amino Acids*, **2011**, 41, 575-586.

[9] Bartels B, Garcia-Yebra C, Rominger F, Helmchen G, *Eur J Inorg Chem* **, 2002**, 2569-2586; Takeuchi R, Kashio M, *Angew Chem Int Ed Engl***, 1997,** 36, 263-265.

[10] Roggen M, Carreira EM , *J Am Chem Soc* , **2010,** 132, 11917-11919.

[11] Gnamm C, Franck G, Miller N, Stork T, Brödner K, Helmchen G, *Synthesis* , **2008,** 20, 3331-3350; Leitner A, Shu C, Hartwig JF, *Proc Nat Ac Sc* **, 2004**, 101, 5830-5833; R. Takeuchi Naoki U, Tanabe K, Yamashita K, Shiga N, *J Am Chem Soc* **, 2001,** 123, 9525-9534.

[12] R, Takeuchi Naoki U, Tanabe K, Yamashita K, Shiga N, *J Am Chem Soc* **, 2001,** 123,9525-9534.

[13] Bartels B, Garcia-Yebra C, Rominger F, Helmchen G, *Eur J Inorg Chem* **, 2002,** 2569-2586.

[14] Kirkland T. A, Grubbs RH, *J Org Chem* **, 1997,** *62*, 7310-7318.

[15] K. Kuhn, T. Champagne, S. Hong,, W. Wei, A. Nickel, C. Lee, S. Virgil, R. Grubbs, R. Pederson, *Org. Lett*., **2010**, 12, 5, 984-987.

[16] F. Epifanio, S. Genovese, O. Rosati, S. Tagliapietra , C. Pelucchini, M. Curini, *Tetrehedron Letters,* **2011**, 52; 568-571.

[17] S.Gerard, A. Renzetti, B. Lefevre, A. Fontana, P. De Maria; J. Sapi, *Tetrahedron;* **2010**, 66, 3065-3069.

[18] K.Gao, J. Wu, *Org. Lett*. **2008**, 10, 2251-2254.

[19] (a) J.Matsuo, Y. Tanaki, H. Ishibashi, *Tetrahedron* **2008**, 5262-5267. (b) J. D Albright, H. R. Snyder, *J. Am. Chem. Soc*. **1959**, 81, 2239-2245 (c) R. Ballini, A. Palmieri, M. Petrini, R.R. Shaikh, *Adv. Synth. Catal*. **2008**, 350, 129-134.

[20] (a) F. Benfatti,; G. Cardillo,; L. Gentilucci,; E. Mosconi,; A. Tolomelli, *Synlett* **2008**, 2605–2608.

[21] C.Acharya, S. Dey, P. Jaisankar, *Tetrahedron lett*., **2012**, 53, 5548-5551.

[22] (a) A. Renzetti, E. Dardennes, A. Fontana, P. De Maria, J. Sapi, S. Gerard, *J. Org. Chem*. **2008**, 73, 6824-6827. (b) S. Gerard, A.Renzetti, B. Lefevre, A. Fontana, P. De Maria, J. Sapi, *Tetrahedron*, **2010**, *66*, 3065-3069.

[23] (a) Hynes, R. O. *Cell* **1992**, 69, 11–25; (b) Curley, G. P.; Blum, H.; Humphries, M. J. *Cell. Mol. Life Sci.* **1999**, 56, 427–441; (c) Gottschalk, K.-E.; Kessler, H. *Angew. Chem. Int. Ed.* **2002**, 41, 3767–3773.

[24] (a) Lampugnani, M. G.; Resnati, M.; Dejana, E.; Marchisio, P. C. J. *Cell Biol*. **1991**, 112, 479–490; (b) Coutre, S.; Leung, L. *Ann. Rev. Med*. **1995**, 46, 257–265;

(c) Ojima, I.; Chakravarty, S.; Dong, Q. *Bioorg. Med. Chem*. **1995**, 3, 337–360; (d) Georges- Labousse, E.; Messaddeq, N.; Yehia, G.; Cadalbert, L.; Dierich, A.; LeMeur, M. *Nature Gen.* **1996**, 13, 370–373; (e) Rozzo, C.; Chiesa, V.; Ponzoni, M. *Cell Death Differ*., **1997**, 4, 713–724.

[25] (a) Henry, C.; Moitissier, N.; Chapleur, Y. *Mini Rev. Med. Chem*. **2002**, 2, 531–542; (b) Horton, M. A. *Int. J. Biochem. Cell Biol*. **1997**, 29, 721–725; (c) Varner, J. A.; Brooks, P. C.; Cheresh, D. A. *Cell Adhes. Commun*. **1995**, 4, 367–374

[26] (a) Zimmermann, D.; Guthohrlein, E. W.; Malsevic, M.; Sewald, K.; Wobbe, L.; Heggermann, C.; Sewald, N. *ChembioChem* **2005**, 6, 272–276; (b) Sudhakar, A.; Sugimoto, H.; Yang, C.; Lively, J.; Zeisberg, M.; Kalluri, R. *Proc. Nat. Acad. Sci*. **2003**, 100, 4766–4771; (c) Marinelli, L.; Meyer, A.; Heckmann, D.; Lavecchia, A.; Novellino, E.; Kessler, H. *J. Med. Chem*. **2005**, 48, 4204–4207; (d) Addicks, E.; Mazitschek, R.; Giannis, A. *ChembioChem* **2002**, 3, 1078–1088.

[27] Kim, S.; Harris, M.; Varner, J. A. *J. Biol. Chem*. **2000**, *275*, 33920–33928.

[28] Xiong, J.-P.; Stehle, T.; Zhang, R.; Joachimiak, A.; Frach,M.; Goodman, S. L.; Arnaout, M. A. *Science* **2002**, *296*, 151–155, PDB file deposited with code 1L5G.

[29] (a) Nicolaou, K. C.; Trujillo, J. I.; Jandeleit, B.; Chibale, K.; Rosenfeld, M.; Diefenbach, B.; Cheresh, D. A.; Goodman, S. L. *Bioorg. Med. Chem*. **1998**, *6*, 1185-1208; (b) Pitts, W. J.; Wityak, J.; Smallheer, J. M.; Tobin, A. E.; Jetter, J. W.; Buynitsky, J. W.; Harlow, P. P.; Solomon, K. A.; Corjay, M. A.; Mousa, S. A.; Wexler, R. R.; Jadhav, P. K. *J. Med. Chem.* **2000**, *43*, 27–40; (c) Trabocchi, A.; Menchi, G.; Guarna, F.; Machetti, F.; Scarpi, D.; Guarna, A. *Synlett*. **2006**, 331–353.

[30] Takagi, J.; Springer, T. A. *Immunol. Rev*. **2002**, *186*, 141–163.

[31] F. Benfatti, G. Cardillo, S. Fabbroni, P. Galzerano, L. Gentilucci, R. Juris, A. Tolomelli, M. Baiula, A. Spartà, S. Spampanato, *Bioorg. Med. Chem.* **2007** (*15*), 7380-7390

[32] Schuffenhauer A, Ertl P, Roggo S, Wetzel S, Koch MA, Waldmann H, The Scaffold tree-visualization of the scaffold universe by hierarchical scaffold classification. *J Chem Inf Model*, **2007**, 47, 47–58.

[33] Müller G., Drug Disc Today, **2003**, 8,681–691.

[34] McDonald D.Q., Still W.C., *J Org Chem*, **1996**, 61, 1385–1391.

[35] Cheng R.P., Gellman S.H., DeGrado WF, *Chem Rev*, **2001**, 101, 3219–3232

[36] Cardillo G., Gentilucci L., Tolomelli A., Calienni M., Qasem A.R., Spampinato S.,*Org Biomol Chem*, **2003**, 1, 1498–1502

[37] Kang Y.K., Park H.S., *Pept. Sci.*, **2009**, 92, 387–398

[38] (a) F. Benfatti, G. Cardillo, S. Fabbroni, L. Gentilucci, R. Perciaccante, A. Tolomelli, M. Baiula, S. Spampinato, *Tetrahedron Asymmetry*,**2006**, 17, 167-170;

(b) A. Tolomelli, L. Gentilucci, E. Mosconi, A. Viola, S.D. Dattoli, M. Baiula, S. Spampinato, L. Belvisi, M. Civera, *ChemMedChem*, **2011**, 6, 2264-2272.

[39] (a) G. Cardillo, A. Gennari, L. Gentilucci, E. Mosconi, A. Tolomelli, S. Troisi, *Eur. J. Org. Chem*, **2009**, 5991-5997;

(b) G. Cardillo, L. Gentilucci, E. Mosconi, A. Tolomelli, S. Troisi, E. Juaristi, *Tetrahedron*, **2010**, 66, 4994-4999.

[40] C.A. Lipinski, F. Lombardo, B.W. Dominy, P.J. Feeney, *Adv. Drug Del. Rev.*, **2001**, 46, 3-26.

[41] S. Caltabiano, W. T. Hum, G. J. Attwell, D. N. Gralnick, L. J. Budman, A. M. Cannistraci, F. J. Bex, *Biochem. Pharmacol*,. **1999**, 58, 1567 –1578.

[42] H. Fujii, H. Komazawa, H. Mori, M. Kojima, I. Itoh, J. Murata, I. Azuma, I. Saiki, *Biol. Pharm. Bull*., **1995**, 18, 1681– 1688.

[43] a) B. D. Cox, M. Natarajan, M. R. Gladson, L. Candece, J. Cell. Biochem. 2006, 99, 35–52; b) D. D. Schlaepfer, C. R. Hauck, D. J. Sieg, *Progr. Biophys. Mol. Biol.* **1999**, 71, 435 –478.

[44] T. Taga, A. Suzuki, I. Gonzalez-Gomez, F.H. Gilles, M. Stins, H. Shimada,L. Barsky, K.I. Weinberg, W.E. Laug, *Int. J. Cancer*, **2002,** 98 690-697.

[45] (a) B.D. Cox, M. Natarajan, M.R. Gladson, L. Candece, J. Cell. Biochem. 99 (2006) 35-52;(b) D.D. Schlaepfer, C.R. Hauck, D.J. Sieg, *Progr. Biophys. Mol. Biol.*, **1999**, 71, 435-478.

[46] D. Cox, M. Brennan, N. Moran, *Nat. Rev. Drug Discov*., **2010**, 9, 804-820.

[47] E. Puklin-Faucher, V. Vogel, *J. Biol. Chem.*, **2009**, 52,36557-36568.

[48] (a) J. Zhu, J. Zhu, A. Negri, D. Provasi, M. Filizola, B.S. Coller, T.A. Springer, Blood, **2010**, 116, 5050-5059.

[49] T. Xiao, J. Takagi, B.S. Coller, J.-H. Wang, T.A. Springer, *Nature*, **2004**, 43259-67.

[50] K. Petkau, A Kaeser, I. Fischer, L. Brunsveld and A. P. H. J. Schenning, *JACS*, **2011**, 133, 17063-17071.

[51] E. Skripin, T. S. McConnell, J. De Vito, L. Lawrence, J. A. Ippolito, E. M. Duffy, J. Sutcliffe, F. Franceschi, *Antimicrobial Agents and Chemotherapy*, **2008** 52, 350-357.