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Sintesi stereoselettiva di eterocicli come intermedi di composti biologicamente attivi

"Stereoselective synthesis of heterocycles as intermediates of biologically active compounds"

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Stereoselective synthesis of heterocycles as intermediates of biologically active compounds

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List of abbreviations

aq	aqueous
Ac	Acetyl
BOC	t-Butyloxycarbonyl
Bz	Benzoyl
Bn	Benzyl
c	concentration
Cbz	Carbobenzyloxy
CSA	Camphorsulfonic acid
Δ	reflux
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
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
LiHMDSA	Lithium Hexamethyldisilazide
M	M molar mol/L
MCPBA	meta-Chloroperbenzoic Acid
Me	Methyl
min	minutes
mg	milligram
mL	millilitre
mp	melting point

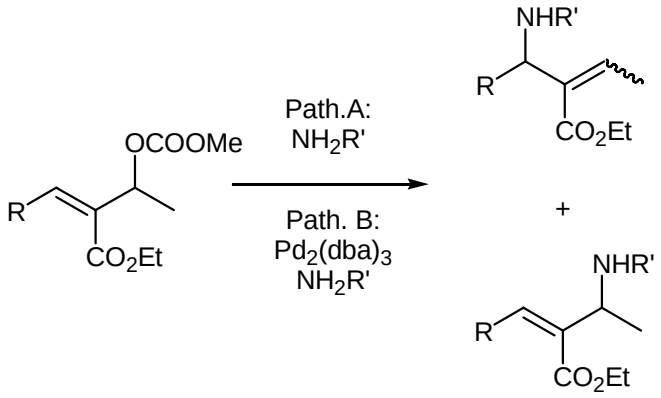
Nu	nucleophile
Ph	Phenyl
PMB	para-Methoxybenzyl
ppm	parts per million
PPTS	Pyridinium p-toulensulfonate
Py	Pyridine
rt	room temperature
TBAF	tetra-n-butylammonium fluoride
TEA	Triethylamine
Tf	Triflate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TMS	Trimethylsilyl
TS	tosyl group
Z	Zusammen (together, cis)
bs	broad singlet (NMR)
δ	Chemical shift (NMR)
13C-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
HETCOR	Heteronuclear Correlation (NMR)
HMQC	Heteronuclear Multiple Quantum Coherence (NMR)
HPLC	High Performance Liquid Chromatography

IR	Infrared
J	Coupling constant (NMR)
LC	Liquid Chromatography
m	multiplet (NMR)
MS	Mass Spectrum
NMR	Nuclear Magnetic Resonance
NOESY	Nuclear Overhauser Effect (Spectroscopy)
ORTEP	Oak Ridge Thermal Ellipse Program
q	quartet (NMR)
Rf	Retention Factor (chromatography)
s	singlet (NMR)
t	triplet (NMR)
TLC	Thin Layer Chromatography
tr	retention time (HPLC)

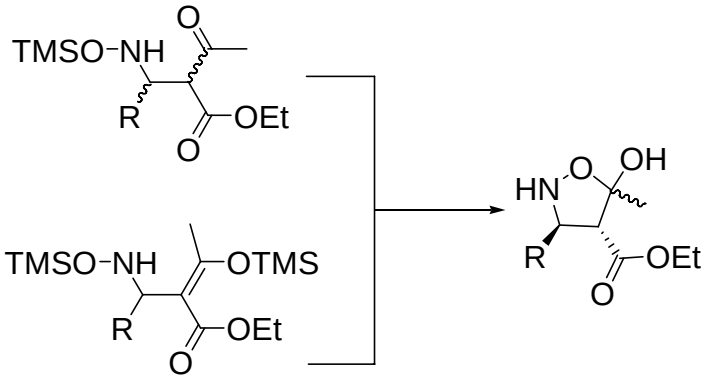
Thesis outline

The aim of this thesis was to investigate the synthesis of enantiomerically enriched heterocycles and dehydro-β-amino acid derivatives which can be used as scaffolds or intermediates of biologically active compounds, in particular as novel $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin ligands. The starting materials of all the compounds here synthesized are alkylideneacetoacetates. Alkylidene derivates are very usefull compounds, they are usually used as unsaturated electrophiles and they have the advantage of introducing different kind of functionality that may be further elaborated.

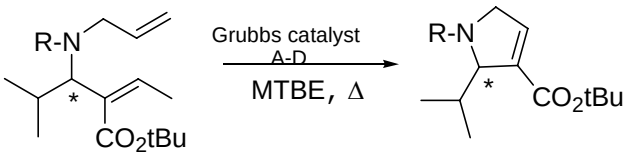
In chapter 1, regio- and stereoselective allylic amination of pure carbonates is presented. The reaction proceeds via uncatalyzed or palladium-catalyzed conditions and affords enantiopure dehydro-β-amino esters that are useful precursor of biologically active compounds.



Chapter 2 illustrates the synthesis of substituted isoxazolidines and isoxazolines via Michael addition followed by intramolecular hemiketalisation. The investigation on the effect of the Lewis acid catalysis on the regioselectivity of the addition it also reported. Isoxazolidines and isoxazolines are interesting heterocyclic compounds that may be regarded as unusual constrained β-amino acids or as furanose mimetics.



The synthesis of unusual cyclic amino acids precursors, that may be envisaged as proline analogues, as scaffolds for the design of bioactive peptidomimetics is presented in chapter 3. The synthesis of 2-substituted-3,4-dehydropyrrole derivatives starting from allylic carbonates via a two step allylic amination/ring closing metathesis (RCM) protocol is carried out. The reaction was optimized by testing different Grubbs' catalysts and carbamate nitrogen protecting groups. Moreover, in view of a future application of these dehydro-β-amino acids as central core of peptidomimetics , the malonate chain was also used to protect nitrogen prior to RCM.



R =BOC
R =Cbz
R =malonyl

Finally, chapter 4 presents the synthesis of two novel different classes of integrin antagonists, one derived from dehydro-β-amino acid prepared as described in chapter 1 and the other one has isoxazolidines synthesized in chapter 2 as rigid constrained core. Since that these compounds are promising RGD mimetics for $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, they have been submitted to biological assay and. Furthermore to interpret on a molecular basis their different affinities for the $\alpha_v\beta_3$ receptor, docking studies were performed using Glide program.

I

Regio - and stereoselective allylic carbonate amination. Synthesis of Dehydro-β-amino esters and Dehydro-β-amino acids as valuable precursors of biologically active compounds

1.1 Introduction

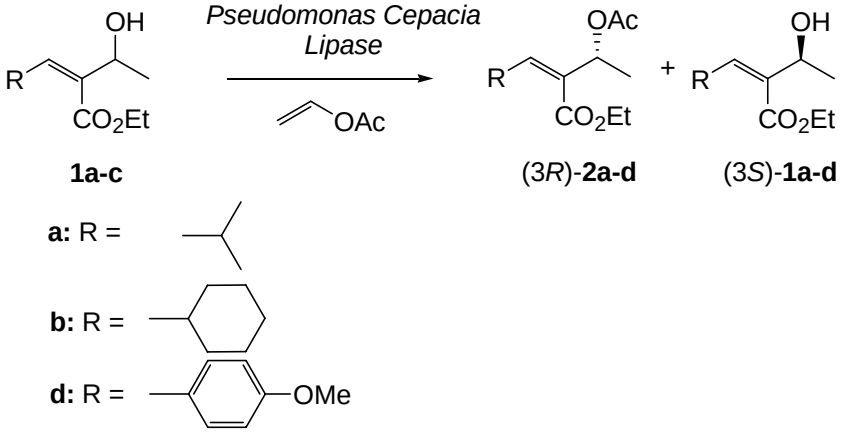
Allylic compounds are privileged substrates in organic synthesis allowing the substitution reaction to be performed with a variety of nucleophiles.[1] The C-N bond formation[2] is one of the most important linkages in organic chemistry and allows the preparation of unusual amino acids. In this contest the substitution of allylic acetates or carbonates is a powerful method for the preparation of allylic amines and represents one of the most attractive procedures for their asymmetric synthesis. While primary allylic carbonates have been widely studied, the substrates bearing substituents in Cα, Cβ and Cγ have received less attention. One of the most interesting aspects of this chemistry is the control of the regio- and the stereoselectivity on the allylic moiety. The reaction may be carried out in the absence of catalyst, depending on the nature of the nucleophile the substitution can occur *via* S_N2 or through an S_N2' process.[1b-d] Under Pd-catalyzed conditions for symmetrically substituted compounds, the step of formation of the metal complex is irrelevant for the regioselectivity of the overall reaction. On the contrary, for substrates bearing different substituents at the two allylic termini, interest increases when the regioselectivity can be controlled.[3]

In this work of thesis, the allylic amination of enantiomerically pure carbonates with proper amines, afforded dehydro-β-amino esters that are interesting precursors of unsaturated or saturated β-amino acids and also β-lactams.[4] They could be inserted as a rigid core in small constrained non-peptidic molecules mimicking the RGD (Arg-Gly-Asp) motif, present in a wide number of extracellular matrix (ECM) proteins.[5] These ligands bind to $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins, a large family of heterodimeric transmembrane glycoproteins, involved in the pathogenesis of several diseases, such as atheroschlerosis, osteoporosis, cancer and a variety of inflammatory disorders.[6] The recognition sequence binds to the receptor mainly through electrostatic interactions with regions in the protein

having opposite charges: Arg interacts with two Asp situated in the α unit of the protein and Asp with a metal cation.[7] Dehydro-β-amino acids can be used as a rigid core that may be easily linked to appendages corresponding to arginine and aspartic acid side chains.

1.2 Synthesis of optically active allylic acetates and carbonates

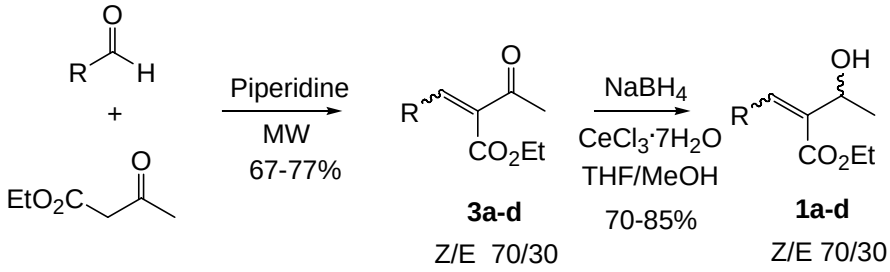
Optically active ethyl 3-hydroxy-2(1'substituted-methylidene)-butyrates, as well as their acetates, were obtained from enzymatic resolution of a series of racemic ethyl 3-Hydroxy-2(1'substituted-methylidene)-butyrates using *Pseudomonas Cepacia* lipase (EC 3.1.1.3) as a catalyst[8], with a good yield and an excellent enantiomeric excess. (Scheme 1. 1)



Scheme 1. 1

The racemic alcohols were prepared in a two steps extremely simple way by treating 10 equiv. of proper aldehyde and 1 equiv. of ethyl acetoacetate in the presence of 0.15 equiv. of piperidine under microwave assisted conditions for 7.5 min (Scheme 1. 2). Microwave assisted organic synthesis[9] is in fact a rapidly expanding area of research that offers the opportunity to strongly reduce reaction times from hours to minutes. After the usual work up, 30/ 70 mixtures of E and Z ketones were obtained in good yields.

The mixture of ketones was submitted to reduction in the presence of 1 equiv. of $CeCl_3$ and 1.1 equiv. of $NaBH_4$ in THF/MeOH 9:1 (Scheme 1. 2).[10] The corresponding Z/E alcohols **1a-d** were obtained in excellent yields and were separated by flash chromatography.



Scheme 1. 2

In order to select the most useful enzyme for the kinetic resolution of these compounds, a series of screening experiments was carried out with various lipases. Therefore, the lipases from *Candida cylindracea*, *Mucor miehei, Aspergillus niger* and *Rhizopus nivens* were tested via irreversible acylation by treating the substrates with 4 equiv. of vinylacetate and 0,5 mass equiv. of enzime in diethylether at r.t. for several days.[11]

Finally, the use of Pseudomonas cepacia proved to be very successful for the resolution of Z-1a-d alcohols, affording the acetylated compounds in good yields and high ee, although in lengthy reaction times. However, any effort to resolve the allylic alcohols E-1a-d under these conditions failed. Lipase from Pseudomonas cepacia (EC 3.1.1.3) is a well known catalyst in organic synthesis, used both for kinetic resolution of racemic mixture of secondary alcohols, as well as in hydrolysis and transesterification.12 The racemic alcohols 1a-d were submitted to catalyzed enzymatic acetylation employing vinyl acetate in diethyl ether at 30 °C (Table 1).

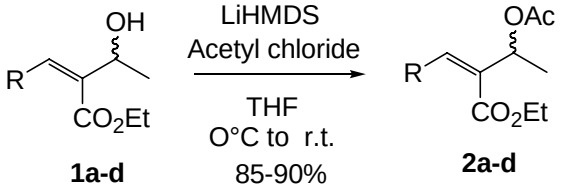
Table 1

Entry	Substrate	Products	Time (days)	Conversion (%)[b]	Isolated yield (%)[c]	$[\alpha]_D$	ee[d]	E[e]
1	**1a**	(3*S*)-**1a**	5	50	45	-11.0	>99	>1000
		(3*R*)-**2a**			34	+36.5	>99	
2	**1b**	(3*S*)-**1b**	5	47	45	-8.7	88	37
		(3*R*)-**2b**			42	+27.2	>99	
3	**1d**	(3*S*)-**1d**	5	50	36	-4.0	>99	>1000
		(3*R*)-**2d**			40	+44.3	>99	

[a] The substrate at 0.2 M concentration in diethyl ether was stirred with 0.5 mass equiv. of enzyme, 5 equiv. of vinyl acetate for five days at room temperature. [b] As monitored by GC-MS an d by ^{1}H NMR integrals [c] Determined on isolated and purified products. [d] Determined by HPLC with chiral column (see experimental) [e] Values determined using Sih's method, from the extent of conversion and enantiomeric excess of the recovered substrate as described and checked via values determined from the enantiomeric excess of the product.

Monitoring of the reaction progress by GC-MS allowed for determination of the conversion. Samples of racemic acetates **2a-d** were prepared as reference compounds, via acetylation of racemic alcohols **1a-d**.

Although several different basic conditions were tested for the reaction with acetyl chloride (TEA, NaH, K_2CO_3), good results could be obtained only when the reaction was performed in the presence of 1.5 equiv. of LiHMDS (Scheme 1. 3).



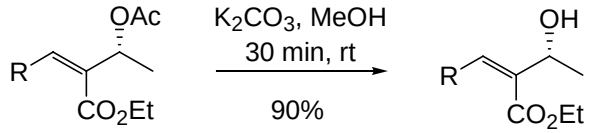
1a-d **2a-d**

Scheme 1. 3

After the required conversion in the lipase catalyzed reaction was achieved, the enzyme was filtered off and the mixture was submitted to the usual work up. The ^{1}H NMR of crude material showed a 1:1 ratio of alcohol/acetate, based on the peak area of the hydrogen adjacent to the secondary alcohol and its corresponding acetate (a quartet at 4.50-4.60 ppm for alcohols **1a-d** and a quartet at 5.60-5.70 ppm for acetate **2a-d**). The resulting acetates and the remaining alcohols were separated by flash chromatography.

The racemic alcohols were analytically resolved into their enantiomers by chiral HPLC and were compared with the alcohols that derived from enzymatic resolution.

Since the analytical resolution of acetates could not be easily achieved, the acetates (+)-**2a-d** were hydrolyzed to the corresponding alcohols. To this purpose, samples of the optically active acetates **2a-d** were treated with K_2CO_3 in methanol (Scheme 1. 4). The enatiomeric excesses of (+)-**1a-d** were determined via HPLC by comparison with the corresponding racemates.



(+)-2a-d **(+)-1a-d**
ee>99%

Scheme 1. 4

The stereoselectivity of Lipase prepared from *Pseudomonas cepacia* have been widely studied via models based on a classification of the relative size of the substituens (small and large) at the stereocenter of the secondary alcohol.[9] The Kazlauskas' rule to predict

which enantiomer of the secondary alcohol reacts faster, has received reliable application. On the basis of the data reported in the literature, we have attributed the *S* configuration to the allylic alcohols and the *R* to their corresponding acetates. In fact in our case the methyl group is unequivocally identified as the smaller substituent in comparison to the large one (Figure 1. 1).

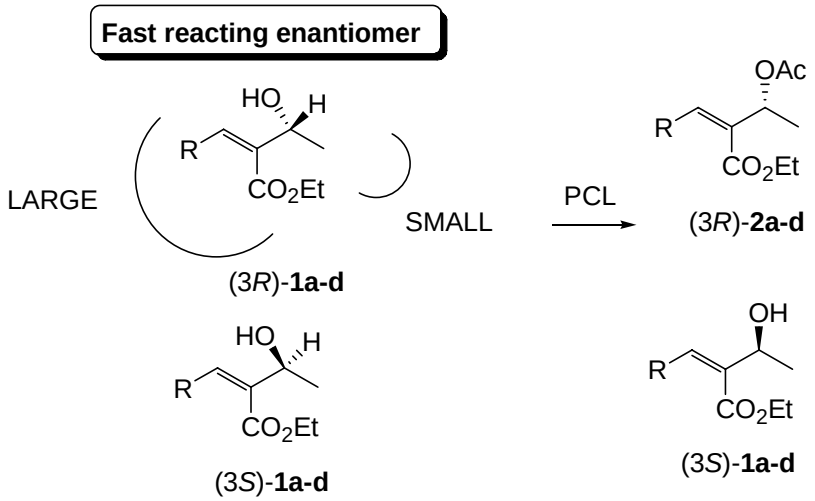
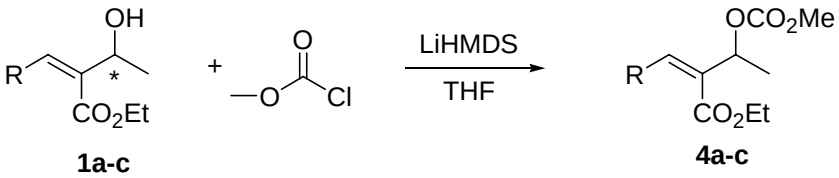


Figure 1. 1

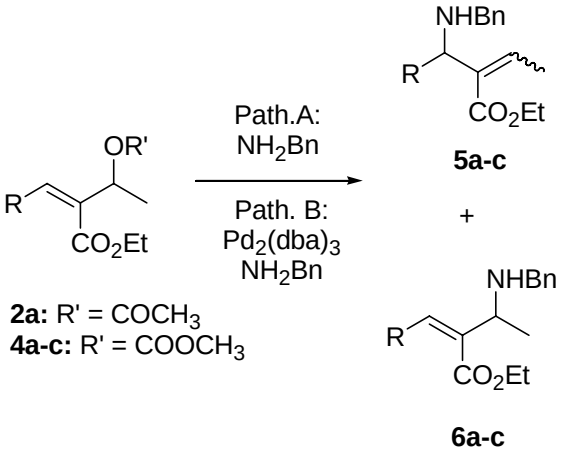
The carbonates **4a-c** have been obtained from the corresponding (*S*) and (*R*)-alcohols by treatment with LiHMDS and methyl chloroformate in dry THF. (Scheme 1. 5)



Scheme 1. 5

1.3 Solvent-dependent regio- and stereoselective amination

The reaction carried out in the absence of catalyst or under palladium-catalyzed conditions led to dehydro-β-amino esters **5** and **6**[13] interesting precursors of unsaturated β-amino acids[14] and α-alkylidene-β-lactams.[15] (Scheme 1. 6)

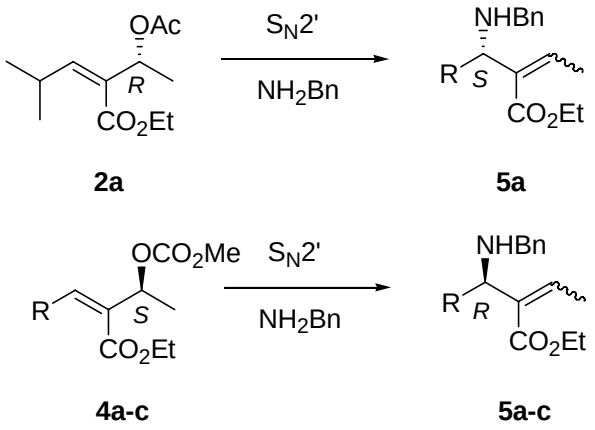


Scheme 1. 6

The optically active amino acetate **2a** and carbonate **4a-c** were synthetized as described in the previous paragraph. The amination reactions were carried out with benzylamine as nucleophile in different solvents, following pathway A or pathway B[16] (Scheme 1. 6). The regioselective nucleophilic displacement may afford respectively the dehydro-β-amino esters **5a-c** and **6a-c**.

1.3.1 Uncatalyzed amination

In the initial experiments, (±)-**2a** and benzylamine were refluxed in CH_2Cl_2, affording (±)-**5a** in 65% yield although in a very long reaction time (Table 2, entry 1). On changing the solvent to THF, faster quantitative conversion to (±)-5**a** was observed (entry 2).



Scheme 1. 7

Table 2

Entry	Substrate	Solvent[a]	Time (h)	**5**[b,c]**/6**
1	(±)-**2a**	CH_2Cl_2	96	>99/1
2	(±)-**2a**	THF	42	>99/1
3	(R)-**2a**	THF	42	>99/1
4	(R)-**2a**	CH_3CN	50	90/10
5	(±)-**4a**	THF	24	>99/1
6	(±)-**4a**	CH_3CN	40	>99/1
7	(S)-**4a**	THF	24	>99/1
8	(S)-**4a**	CH_3CN	40	>99/1
9	(±)-**4b**	THF	24	>99/1
10	(±)-**4b**	CH_3CN	40	95/5
11	(S)-**4b**	THF	24	>99/1
12	(±)-**4c**	THF	24	>99/1
13	(±)-**4c**	CH_3CN	40	88/12
14	(S)-**4c**	THF	24	>99/1

[a] The reactions were carried out in refluxing solvent. [b] The regioisomeric ratio was determined by ^{1}H NMR. Configuration of the double bond in 3 4/1 Z/E. [c] Yield of compounds purified by flash chromatography on silica gel >90% (65% only for entry 1) [d] A 12% of racemization of the benzylic position was observed by HPLC on chiral column.

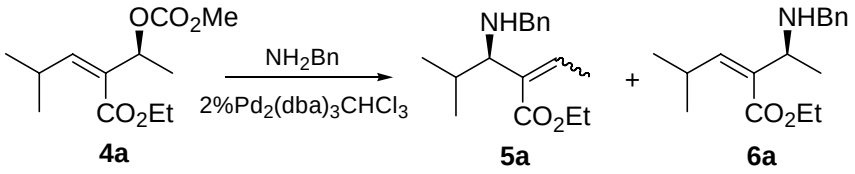
Following the same protocol on enantiomerically enriched (*R*)-**2a**, optically active **5a** was obtained as exclusive regioisomer in THF, while a 90/10 regioisomeric mixture of **5a**/**6a** was observed carrying out the reaction in acetonitrile (entries 3-4). Complete conversion of the starting material to **5a** in reduced reaction time was observed in both solvents starting from the carbonate **4a** (entries 5-6).

On repeating the reaction on (*S*)-**4a**, the chirality of the starting carbonate was completely transferred to the dehydroamino ester **5a** (99% ee determined by chiral HPLC on the pure isolated *Z*-**5a**, entries 7,8). Similar results have been obtained for compounds **4b** and **4c** (entries 9-13) although (*S*)-**4c** afforded enriched **5c** with a partial racemization of the benzylic position (entry 14).

The regiochemistry of the products accounts for a nucleophilic displacement occuring via S_N2' mechanism. Since a *syn* attack is to be predicted for neutral nucleophiles,[17] starting from (*R*)-acetate **2a**, the (*S*) configuration was attributed to the dehydro-β-amino ester **5a**, while from (*S*)-carbonates **4a-c** the (*R*) configuration was assigned to products **5a-c** (Scheme 1. 7).

1.3.2 Pd-Catalyzed amination

Treating racemic or (*S*)-4a allylic carbonate[18] with benzylamine in the presence of $Pd_2(dba)_3CHCl_3$ catalyst, either in THF or CH_3CN, the reaction show a strong solvent-dependent regiocontrol.(Table 3)



4a **5a** **6a**

Scheme 1. 8

Table 3

Entry	Substrate	Solvent[a]	**5a/6a**[b,c] (%)
1	(±)-**4a**	THF	93/7
2	(±)-**4a**	CH_3CN	20/80
3	(S)-**4a**	THF	90/10[d]
4	(S)-**4a**	CH_3CN	20/80[d]
5	(S)-**4a**	CH_3CN+ PPh_3	>99/1[d]

[a] The reactions were carried out in refluxing solvent (solution 0.1 M of 2). [b] The regioisomeric ratio was determined by ^1H NMR. Configuration of the double bond in 3a 4/1 Z/E. [c] Yield of compounds purified by flash chromatography on silica gel >90% [d] 90% ee from HPLC on chiral column, starting from (*S*)-2a having 90% ee.

The reaction carried out in THF at reflux gave compound **5a** in 93/7 regioisomeric ratio (entry 1). When (*S*)-**4a** was used as starting material, the chirality was completely retained affording 5**a** with the same enantiomeric excess of the starting carbonate (entry 3). Complete inversion of the regiochemistry occurred in CH_3CN at reflux, **6a /5a** being obtained in 80/20 regioisomeric ratio (entries 2 and 4). Similarly to what observed in THF, the reaction performed in CH_3CN on (*S*)-**4a** gave **6a** in 90% ee (entry 4). In order to enhance the regioselectivity of the reaction, the amination was performed in the presence of PPh_3.[19] A significative increase of regioselectivity was observed for the reaction carried out in CH_3CN, being **6a** formed as the exclusive regioisomer (entry 5).

Since both the oxidative addition leading to palladium complex and the nucleophilic attack normally occur stereoselectively with inversion of configuration at the reacting allylic carbon atom, the overall process proceeds with retention of configuration.[20] Therefore, starting from (*S*)-**4a**, the (*R*) configuration was assigned to **5a**, also confirmed by the observed optical rotation, and the (*S*) configuration was assigned to **6a**. Thus, starting from (*S*)-**4a** carbonate, selected conditions gave access to the two different optically active dehydroamino esters (*R*)-**5a** or (*S*)-**6a** through a solvent depending regioselective control.

On the basis of these results, **4b** and **4c** were reacted in CH_3CN (Table 4, entries 1 and 2) giving **6b** and **6c** in satisfactory regioselectivity (>85/15), that was strongly improved when PPh_3 was added to the reaction mixture, in agreement with the above reported results on the isopropyl derivative (entries 3 and 4).

Table 4

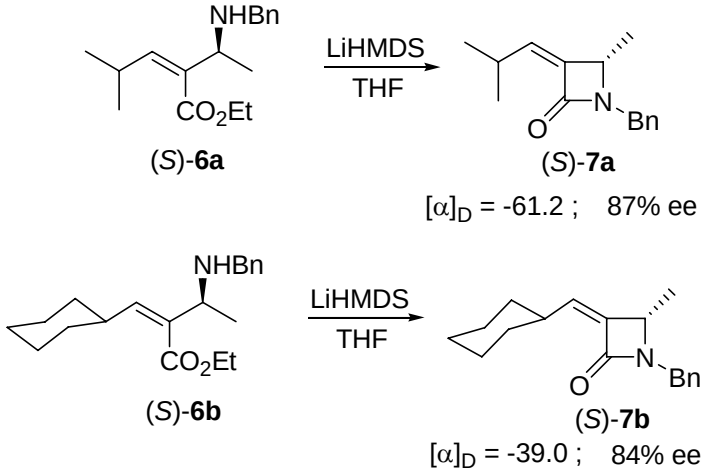
Entry	Substrate	Solvent[a]	**5/6**[bc] (%)
1	(S)-**4b**	CH_3CN	15/85
2	(S)-**4c**	CH_3CN	11/89
3	(±)-**4b**	CH_3CN+ PPh_3	5/95
4	(±)-**4c**	CH_3CN+ PPh_3	5/95

[a] The reactions were carried out in refluxing solvent (solution 0.1 M of **2**). [b] The regioisomeric ratio was determined by ^1H NMR. Configuration of the double bond in **3** 4/1 Z/E. [c] Yield of compounds purified by flash chromatography on silica gel >90% [d] 90% ee from HPLC on chiral column, starting from (*S*)-**2** having 90% ee.

1.3.3 Conversion dehydro-β-amino esters to α-alkylidene-β-lactams

The dehydro-β-amino esters were then converted to α-alkylidene-β-lactams, in order to obtain compounds of interest in our research group [21].

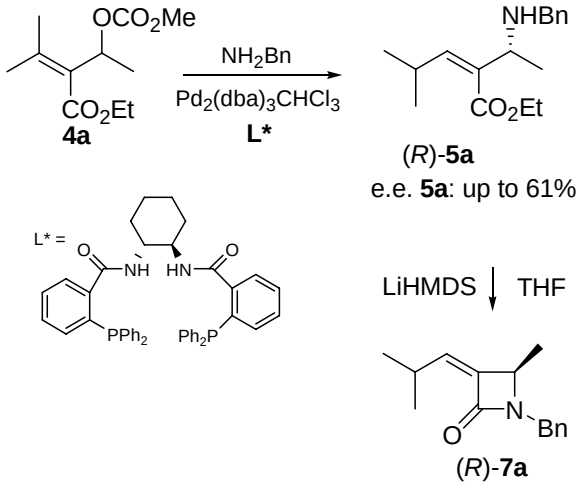
To this purpose, (*S*)-**6a-b** were easily quantitatively transformed into the corresponding Z-β-lactam (*S*)-**7a-b** by treatment with LiHMDS in anhydrous THF at –20 °C (Scheme 1. 9). The Z configuration of the double bond was attributed on the basis of vinyl proton ^{1}H NMR chemical shift (5.33-5.36 ppm)[22] and confirmed by NOE experiments.



Scheme 1. 9

1.3.4 Enantioselective allylic amination of racemic carbonates

Finally, a preliminary study on the enantioselective variant of this methodology by reaction of (±)-**4a** with benzylamine was performed using (*R*,*R*)-DACH-Phenyl Trost chiral ligand in CH_2Cl_2 and THF (Scheme 1. 10). In the presence of 5% amount of Pd and 8% amount of enantiopure chiral ligand, a strong effect on the regioselectivity could be observed. Compound **6a** was obtained as exclusive product in CH_2Cl_2, while it was isolated in 80/20 regioisomeric ratio when the reaction was performed in THF. In both cases only a moderate 40% enantiomeric excess could be observed. Otherwise, decreasing the amount of Pd/ligand (2%/6%) in THF, **6a** was isolated in 30/70 regioisomeric ratio and 61%ee, being the major isomer **5a** obtained in racemic form through uncatalyzed S_N2' reaction.



Scheme 1. 10

The (*R*) absolute configuration of the predominant enantiomer of **6a** was determined by comparison of the HPLC retention times on chiral column and optical rotation values [(*R*)-**6a** $[\alpha]_D$ = +4, 61% ee] with (*S*)-**6a** ($[\alpha]_D$ = -7, 87% ee). The attribution was further confirmed by the comparison of HPLC of the corresponding β-lactams (*S*)-**7a** and (*R*)-**7a**.

1.4 Regio- and stereoselective palladium-catalyzed allylic carbonate amination

The reaction was carried out in CH_3CN. Initially, a series of experiments was performed using racemic carbonates **8-11** with bifunctionalized amines in the presence of 2.5 mol % $Pd_2(dba)_3$-$CHCl_3$[23]. We decided to use terbutyl-ester since its hydrolysis is simpler than ethyl-ester one. The usual work-up and purification by column chromatography provided the products. (Table 5)

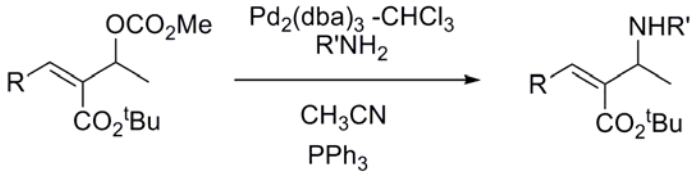
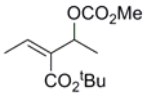
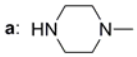
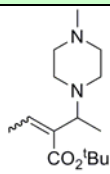
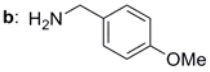
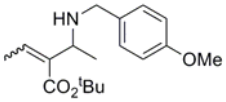
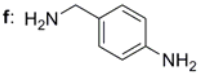
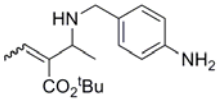
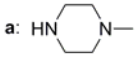
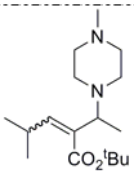
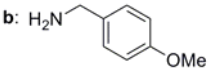
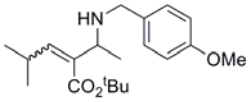
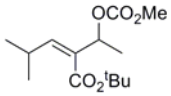
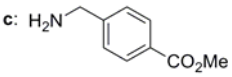
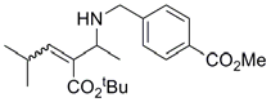
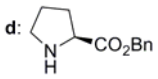
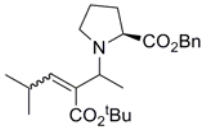
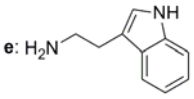
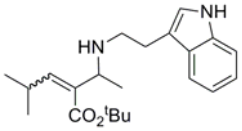
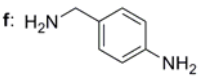
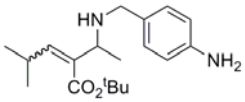
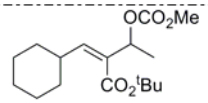
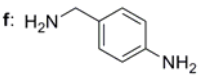
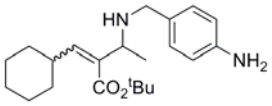
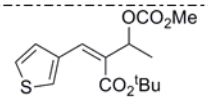
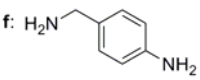
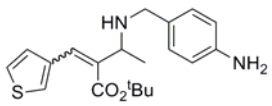


Table 5

Entry	Reagent	Amine	Product		Yield [a](%)	*Z/E*
1	 **8**	**a**: 		**12a**	40 35[b]	50/50
2		**b**: 		**12b**	35[b]	20/80
3		**f**: 		**12f**	60	30/70
4		**a**: 		**13a**	47	95/5
5		**b**: 		**13b**	45	95/5
6		**c**: 		**13c**	40	95/5
7	**9**	**d**: 		**13d**	60	95/5
8		**e**: 		**13e**	60	92/8
9		**f**: 		**13f**	65	95/5
10	 **10**	**f**: 		**14f**	70	90/10
11	 **11**	**f**: 		**15f**	87	20/80

[a] All experiments were performed using racemic carbonates **8-11** with bifunctionalized amines in the presence of 2.5% $Pd_2(dba)_3$-$CHCl_3$ and refluxing in CH_3CN for 12 h. Yields were calculated after purification of the products by flash chromatography on silica gel. [b]The reaction was conducted with 5 mol % PPh_3 as additive.

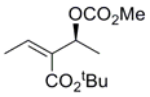
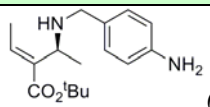
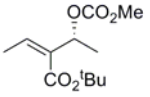
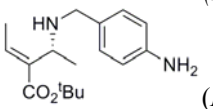
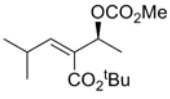
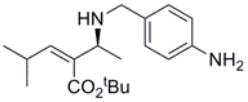
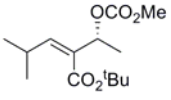
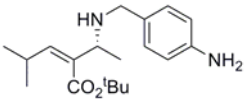
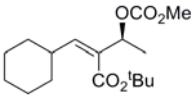
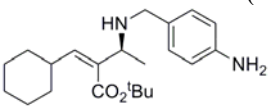
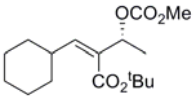
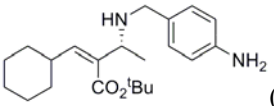
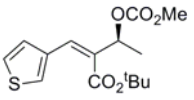
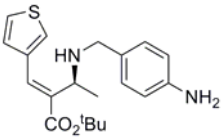
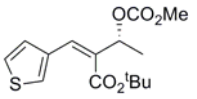
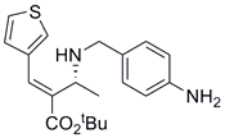
Carbonate **8** reacted with *N*-methylpiperazine in the presence, or in the absence, of PPh_3[24] giving the expected regioisomer **12a** in low yield and in both cases with complete isomerization of the double bond (*E*:*Z* ratio 1:1, entry 1).[25] Similar results have been obtained in the reaction of **8** with *p*-methoxybenzylaniline and **12b** was isolated in moderate yield as a mixture 80:20 of *E*:*Z* isomers (entry 2). The configuration of the double bond was attributed on the basis of the vinyl proton ^{1}H NMR chemical shift and confirmed by NOE experiments.[26] On reaction of **8** with 4-aminomethylaniline as a nucleophile, **12f** was obtained in higher yield, in a 70:30 *E*:*Z* ratio of geometrical isomers (entry 3).

The carbonate **9** also provided the corresponding products in good yield, but in this case with preferential formation of the *Z* isomer (*Z/E* ratio from 92/8 to 95/5). No trace of the regioisomer deriving from the S_N2^I mechanism was detected in the ^{1}H NMR of the crude, while traces of about 10-20% of the unreacted starting material were observed (entries from 4, 5, 6, 8 and 9). The benzyl L-proline (entry 7) underwent substitution giving almost exclusively the corresponding dehydro-β-amino esters with the *Z* geometry of the double bond (*Z/E* ratio 95:5) but as a 1:1 mixture of diastereoisomers, with respect to the newly formed C-N bond. Finally the reaction of carbonates **10** and **11** with 4-aminomethylaniline acting as the nucleophile gave the corresponding compounds **14f** and **15f**, isolated in good yield by flash chromatography on silica gel. The 4-aminomethylaniline exclusively reacted at the benzylamine function to give complete regioselectivity (Table 1 Entries 10-11). A good *Z/E* ratio could be observed for **14f** (90/10), while for **15f** the preferential formation of the *E* isomer was obtained (*Z/E* ratio 20:80), as already observed for **12b** and **12f**.

1.4.1 Reactions of enantiomerically pure carbonates

The substitution reactions were performed with 4-aminomethyaniline since that the corresponded products are interesting intermediates for the synthesis of potential RGD mimetics. The results obtained on treating optically active carbonates **8-11** with 4-aminomethylaniline are reported in Table 6.

Table 6

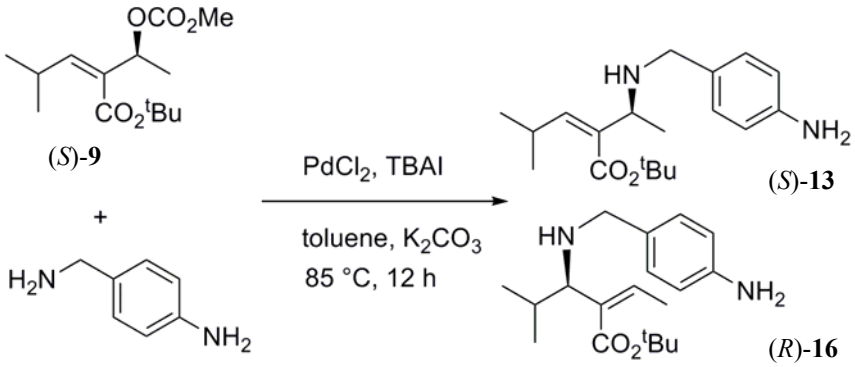
Entry	Substrate	Product[a]	ee[b](%)	Yield (%)
1	 (*S*)-**8**	 *(S)*-**12f**	>99	72
2	 (*R*)-**8**	 (*R*)-**12f**	>99	58
3	 (*S*)-**9**	 (*S*)-**13f**	>99	61
4	 (*R*)-**9**	 (*R*)-**13f**	>99	59
5	 (*S*)-**10**	 (*S*)-**14f**	>99	68
6	 (*R*)-**10**	 (*R*)-**14f**	>99	60
7	 (*S*)-**11**	 (*S*)-**15f**	>99	85
8	 (*R*)-**11**	 (*R*)-**15f**	>99	78

[a]Reaction carried out in the presence of 2.5 mol % $Pd_2(dba)_3$-$CHCl_3$ in refluxing CH_3CN. [b] Determined by HPLC on chiral column on isolated pure compounds (Chiralcel AD column for **12f** and **14f**, Chiralcel IA column for **13f**, Chiralcel OJ column for **15f**).

The enantiomeric excess of isolated products were determined by HPLC on chiral column, after separation of the *Z* isomer from the E isomer. The configuration of the newly created stereocentre was unequivocally attributed on the basis of previously reported research and on the basis of mechanistic considerations. In fact, the mechanism for the palladium-catalyzed allylic amination is generally accepted to proceed via a palladium-allyl complex that in the second step is attacked directly by the amine nucleophile, in analogy with the reaction mechanism for soft carbon nucleophiles. Since both the oxidative addition leading to palladium complex and the nucleophilic attack occur with inversion of configuration at the reacting allylic carbon atom, the overall process proceeds with retention of configuration.[27]

1.4.2 Pd-nanoparticles catalyzed reaction

In order to verify the usefulness of our procedure and to optimize our methodologies on the basis of modern techniques, we tested palladium nanoparticles as catalysts. In a recent paper Ranu and coworkers[28] explored the efficient allylic amination of allyl alcohol derivatives catalyzed by palladium nanoparticles in the presence of a base. This research group described a new protocol that gave good yields of allylated amines. On the basis of the considerable interest in the metal nanoparticles,[29] we applied the Ranu's protocol to our substrate. The reaction was first performed on the racemic **9**, then on the (***S***)-**9** carbonate. After the period of time required for completion, the reaction was worked-up as usual.



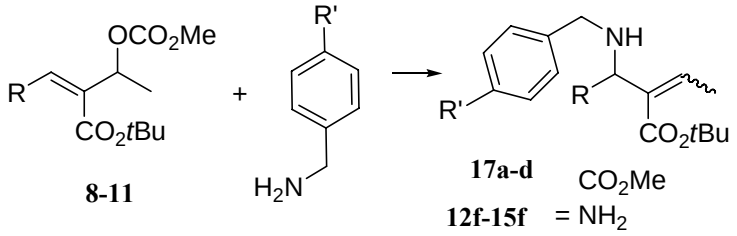
Scheme 1. 11

The regioisomers (*S*)-**13f** and (R)-**16** were obtained in 60:40 ratio and were separated by flash chromatography. The ^{1}H NMR spectrum of (S)-**13f** showed the exclusive presence of the *Z* isomer and the chiral HPLC analysis showed only the (S) enantiomer. On the contrary, (R)-**16** was isolated as a 1:1 mixture of *Z*:*E* isomers. This protocol lacked in regioselectivity, affording both **13f** and **16**, but a high stereoselectivity could be observed in the double bond and the stereocentre formation of **13f**.

1.5 Synthesis of dehydro-β-amino acids under S_N2' conditions

In initial experiments to displace the carbonate from racemic starting material, we used methyl 4-aminobenzoate in CH_3CN at reflux. The substitution reaction did not occur due to the low reactivity of the selected aromatic amine (Table 7, entry 1). On changing the nucleophile with methyl 4-aminomethyl-benzoate in refluxing CH_3CN (Scheme 1. 12), the corresponding dehydro-β-amino esters **17a-d** were obtained in good yield and

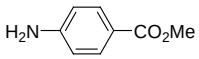
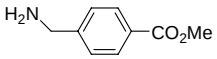
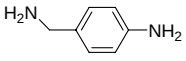
complete regioselectivity[30] (entries 2-5). The reaction was followed by TLC and stopped at disappearance of the starting material and the products were isolated and purified by flash chromatography on silica gel.



Scheme 1. 12

The regiochemistry of the products was easily attributed on the basis of their ^{1}H NMR spectra and accounts for a nucleophilic displacement occurring via S_N2' mechanism. The good reactivity of the benzylamine function, prompted us to use 4-aminomethylaniline as nucleophile in refluxing CH_3CN. Under these conditions, compounds **12f-15f** were isolated in 62-65% yield by flash chromatography on silica gel (entries 6-9).

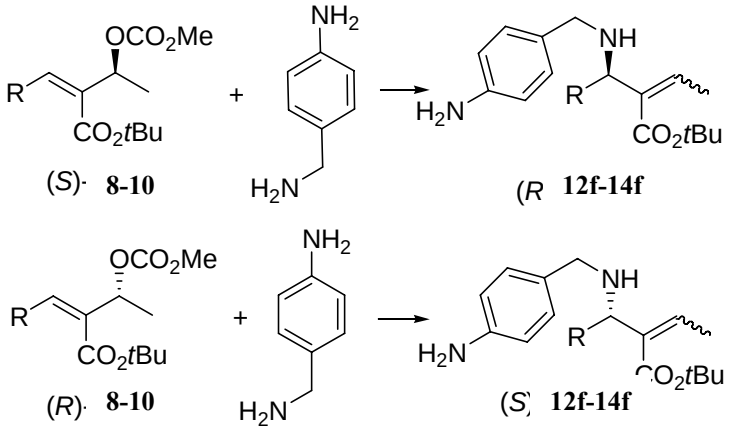
Table 7

Entry	Substrate	Nucleophile	Product	Time[a] (d)	Yield[b] (%)
1	**8**		/	4	/
2	**8**		**17a**	4	45
3	**9**	"	**17b**	4	70
4	**10**	"	**17c**	4	65
5	**11**	"	**17d**	4	35
6	**8**		**12f**	3	62
7	**9**	"	**13f**	3	65
8	**10**	"	**14f**	3	62
9	**11**	"	**15f**	3	63

[a]Reaction carried out in refluxing CH_3CN. [b] Configuration Z/E of the double bond 3/1. Yield of product purified by flash chromatography on silica gel.

Although long reaction time was required to convert the starting material into the products, the regioisomers **12f-15f** were exclusively obtained, confirming the low reactivity of the aromatic amine compared to the aliphatic one. Following the same

protocol on chiral non racemic (R)- or (S)- **8-10**, compounds (R)- or (S)- **12f-14f** were obtained with complete regio- and stereoselectivity (Scheme 1. 13, Table 8).



Scheme 1. 13

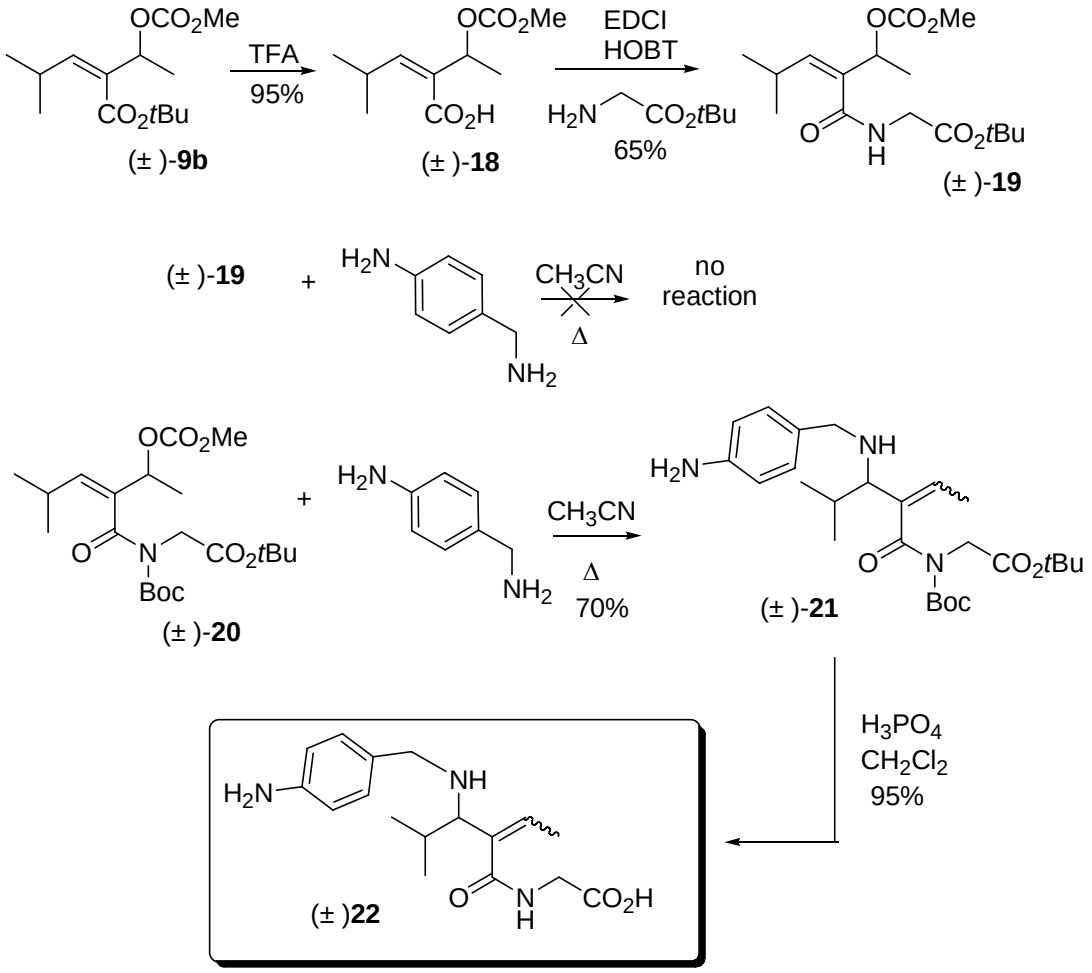
Table 8

Entry[a]	Substrate	Product	Yield[b](%)	ee[c](%)
1	(*S*)-**8**	*(R)*-**12**	65	>99
2	(*R*)-**8**	(*S*)-**12**	63	>99
3	(*S*)-**9**	(*R*)-**13**	60	>99
4	(*R*)-**9**	(*S*)-**13**	65	>99
5	(*S*)-**10**	(*R*)-**14**	63	>99
6	(*R*)-**10**	(*S*)-**14**	60	>99

[a]Reaction carried out in refluxing CH_3CN. [b] Configuration Z/E of the double bond 3/1. Yield of product purified by flash chromatography on silica gel. [c] Determined by HPLC on chiral column

The enantiomeric excesses of isolated products (Table 8) were determined by HPLC on chiral column and confirmed complete transfer of chirality of the starting carbonates to the dehydroamino esters. The stereochemistry of the products was unequivocally attributed on the basis of previously reported research.

Finally, we tried to perform the substitution reaction directly on the amide **19**, in order to obtain a peptidic sequence mimicking the RGD integrin ligand. The amide was prepared in two steps as reported in Scheme 1. 14. By treatment of **9** with trifluoroacetic acid, the corresponding acid **18** was obtained in 95% yield. The presence of the methyl carbonate and *t*-butyl ester allowed the orthogonal deprotection of one of the two moieties, by selecting basic or acid conditions.



Scheme 1. 14

Compound **18** was coupled with glycine tert-butyl ester to afford amide **19**. The S_N2' displacement of carbonate function with 4-aminomethylaniline in refluxing CH_3CN failed and the unreacted starting material was recovered almost quantitatively. To enhance the reactivity of the amide, by reducing the nitrogen back-donation, an electron withdrawing *t*-butyl carbamate group was introduced on the amide nitrogen, giving **20** in quantitative yield. This substrate showed to be much more reactive and could be converted into the corresponding amino derivative **21** by nucleophilic substitution with 4-aminomethylaniline under the above reported conditions. Finally, the simultaneous acidolysis of the *t*-butyl ester and of the Boc protection with H_3PO_4 in dichloromethane,[31] followed by treatment with Dowex 50WX2-200 ion exchange resin, eluting with NH_4OH 0.5 M, and removal of the aqueous solvent under vacuum, afforded **22** in almost quantitative yield. The ability of this new ligand to inhibit integrin

mediated cell adhesion gave promising results, having IC_{50} in the micromolar range. On this basis, a library of derivatives will be prepared and SAR studies will be performed and reported in due course.

1.6 Experimental Section

All chemicals were purchased from commercial suppliers and used without further purification. Flash chromatography was performed on silica gel (230-400 mesh). NMR Spectra were recorded with 200 or 300 MHz spectrometers. Chemical shifts were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at δ = 7.27 (^{1}H NMR) or δ = 77.0 (^{13}C NMR). GC-MS analysis were performed on HP-5 (crosslinked 5% Ph Me silicone , 30m X 0.32 mm X 0.25 μm thikness) using an injection program (initial temperature 50°C for 2 ', then 10°C/min up to 280 °C) in scan mode acquisition. Microwave assisted reactions were performed with a Milestone Mycrosynth multimode apparatus, keeping irradiation power fixed and monitoring internal reaction temperature with a Built-in ATC-FO advanced fiber optic automatic temperature control. The reactions were performed in an open vessel, equipped with a refrigerator connected to fume hood. HPLC analyses were performed an HP1100 instrument with UV-vis detector and equipped with Chiralcel OD column (25 X 0.46 cm), Chiralcel OF column (25 X 0.46 cm), Chiralcel-OJ Daicel column (Cellulose tris-4-methylbenzoatephase coated on 10μm silica gel, 100%) and Chiralcel-AD Daicel column (Amylose tris-3,5-dimethylphenylcarbamate phase coated on 10μm silica gel) eluted with n-hexane/2-propanol. Optical rotations were measured in a Perkin Elmer 343 polarimeter using a 1 dm cuvette and are referenced to the Na-D line value.

Synthesis of ketones **3a-d.** Ethyl acetoacetate (10 mmol, 1.3 g, 1.26 mL) was diluted in the proper aldehyde (10 equiv., 100 mmol) and piperidine (0.15 equiv., 1.5 mmol, 0.15 mL) was added in one portion. The mixture was submitted to microwave irradiation (Power 500W) for 7.5 minutes and then was diluted with ethyl acetate (20 mL) and washed twice with 0.1 M HCl (20 mL). The two phases were separated, the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds Z-**3a-c** and E-**3a-c** were separated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluant).

Z-3a: Yellow oil; isolated yield 61%; 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 (200 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).

E-3a: Yellow oil; isolated yield 26%; 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 (200 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).

Z-3b: Yellow oil; isolated yield 55%; 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 (200 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 16.60 min, *m/z*: 224(2), 178(100), 163(60), 149(20), 135(90), 121(29), 107(33), 95(27), 79(30), 67(21), 55(25).

E-3b: Yellow oil; isolated yield 23%; 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 (200 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 16.40 min, *m/z*: 224(5), 182(100), 163(9), 135(27), 107(42), 79(27), 67(20), 55(12).

Z-3d: Yellow oil; isolated yield 50%; 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 (200 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).

E-3d: Yellow oil; isolated yield 21%; 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 (200 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).

Synthesis of racemic alcohols **1a-d.** To a stirred solution of compound Z-**3a-d** and E-**3a-d** (5 mmol) in THF/MeOH 9:1 (10 mL) at room temperature, $CeCl_3{\cdot}7H_2O$ (1 equiv., 5 mmol, 1,86 g) and $NaBH_4$ (1.1 equiv., 5.5 mmol, 0.2 g) were 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-**1a-d** or E-**1a-d** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluant).

Synthesis of racemic acetates **2a-d.** To a solution of compound Z-**1** (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 acetates

Z-**2a-d** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluant).

Lipase catalyzed resolution of alcohols **1a-d.** To a solution of racemic alcohol **1** (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-**1a-d** and acetates (3*R*)-Z-**2a-d** were separated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 then 80/20 as eluant).

Hydrolysis of enantiomerically pure acetates 2a-d to enantiomerically pure alcohols **1a-d.** Acetate (3*R*)-Z-**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-**1a-d** were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluant).

Z-1a: 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 (200 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).
(3*S*)-Z-**1a**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 14.5 min., $[\alpha]^{25}_D$ = -11.0 (*c* 1.0 $CHCl_3$)
(3*R*)-Z-**1a**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 16.4 min., $[\alpha]^{25}_D$ = +12.4 (*c* 1.0 $CHCl_3$)

E-1a: Yellow oil; IR (film) ν/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 (200 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-1b: 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 (bs, 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 (200 MHz, $CDCl_3$) δ 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).
(3*S*)-Z-**1b**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 17.7 min., $[\alpha]^{25}_{D}$ = -8.7 (*c* 1.0 $CHCl_3$)
(3*R*)-Z-**1b**: HPLC on Chiralcel OF (97.5/2.5 *n*-hexane/2-propanol, flow 1.0 mL/min): rt 19.3 min., $[\alpha]^{25}_{D}$ = +9.4 (*c* 1.0 $CHCl_3$)

E-1b: 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 (200 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).

Z-1d: Yellow oil; IR (film) ν/cm^{-1} 3448, 2977, 2934, 1718, 1607, 1511, 1458, 1300, 1254, 1193, 1031. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.15 (t, 3H, *J* = 7.0 Hz), 1.41 (d, 3H, *J* = 6.6 Hz), 3.78 (s, 3H), 4.17 (q, 2H, *J* =7.0 Hz), 4.58 (q, 1H, *J* =6.6 Hz), 6.84 (d, 2H, *J*

= 8.8 Hz), 6.88 (s, 1H), 7.26 (d, 2H, *J* = 8.8 Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 13.9, 22.4, 55.2, 60.8, 70.2, 113.6(2), 127.8, 129.9 (2), 132.2, 135.5, 159.6, 167.6. GC-MS rt 19.60 min, *m/z*: 250(21), 232(34), 203(25), 189(75), 159(100), 144(66), 128(21), 115(57), 89(20), 77(18).

(3*S*)-Z-**1d:** HPLC on Chiralcel OD (98/2 *n*-hexane/2-propanol, flow 1.5 mL/min): rt 24.8 min., $[\alpha]^{25}_D$ = -4.0 (*c* 1.0 $CHCl_3$)

(3*R*)-Z-**1d**: HPLC on Chiralcel OD (98/2 *n*-hexane/2-propanol, flow 1.5 mL/min): rt 27.1 min., $[\alpha]^{25}_D$ = +4.8 (*c* 1.0 $CHCl_3$)

E-1d: Yellow oil; IR (film) ν/cm^{-1} 3442, 2976, 2929, 1717, 1607, 1512, 1253, 1178. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.21 (t, 3H, *J* = 7.2 Hz), 1.48 (d, 3H, *J* = 7.2 Hz), 1.90 (bs, 1H), 3.81 (s, 3H), 4.09 (q, 1H, *J* =7.2 Hz), 4.23 (q, 1H, *J* =7.2 Hz), 6.91 (s, 1H), 6.93 (d, 2H, *J* = 8.4 Hz), 7.33 (d, 2H, *J* = 8.4 Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 13.8, 22.3, 55.2, 65.0, 70.2, 113.7(2), 127.6, 128.6, 130.0, 133.0, 135.4, 159.1, 168.0. GC-MS rt 19.70 min, *m/z*: 250(11), 232(32), 203(25), 189(42), 159(100), 144(68), 128(22), 121(25), 115(60), 89(17), 77(12).

(3*R*)-Z-2a: 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 (300 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). $[\alpha]^{25}_D$ = +36.5 (*c* 1.0 $CHCl_3$)

(3*R*)-Z-2b: 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 (200 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). $[\alpha]^{25}_D$ = +27.2 (*c* 1.0 $CHCl_3$)

(3*R*)-*Z*-2d: Yellow oil; IR (film) ν/cm^{-1} 2981, 2936, 1735, 1718, 1618, 1607, 1511, 1458, 1370, 1254, 1178, 1055, 1029. ^{1}H NMR (200 MHz, $CDCl_3$) δ 1.17 (t, 3H, *J* = 7.4 Hz), 1.48 (d, 3H, *J* = 6.6 Hz), 2.05 (s, 3H), 3.79 (s, 3H), 4.17 (q, 2H, *J* =7.4 Hz), 5.68 (q, 1H, *J* =6.6 Hz), 6.77 (s, 1H), 6.82 (d, 2H, *J* = 9.2 Hz), 7.23 (d, 2H, *J* = 9.2 Hz); ^{13}C NMR (200 MHz, $CDCl_3$) δ 14.2, 20.2, 21.5, 55.5, 61.1, 71.7, 113.9(2), 127.6, 129.8(2), 130.4, 133.6, 160.0, 168.2, 170.2 GC-MS rt 20.60 min, *m/z*: 292(3), 249(6), 232(21), 203(49), 187(19), 159(100), 144(62), 128(22), 115(59), 89(14), 77(12). $[\alpha]^{25}_D$ = +44.3 (*c* 1.0 $CHCl_3$)

1c: ^{1}H NMR ($CDCl_3$) δ 1.15 (3H, t, *J*= 7.4 Hz, $OCH_2C\mathit{H_3}$), 1.54 (3H, d, *J*= 6.6 Hz, $\mathit{CH_3}CHO$), 2.07 (3H, s, $\mathit{CH_3}CO$), 4.17(2H, q, *J*= 7.4 Hz, $O\mathit{CH_2}CH_3$), 5.74 (1H, q, *J*= 6.6 Hz, $CH_3\mathit{CH}O$), 6.88 (1H, s, *CH*=C), 7.20-7.40 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C 13.7 (CH_3), 19.6 (CH_3), 21.0 (CH_3), 60.8 (CH_2), 71.0 (CH), 128.0 (CH), 128.2 (2CH), 133.3 (CH), 134.2 (C) 134.9(C), 167.6(C), 169.8(C); IR (film) ν/cm^{-1} 2983, 2936, 2360, 1740, 1447, 1371, 1239, 1151, 1055, 753; $[\alpha]_D$ = +54.7 (*c* 1 in $CDCl_3$); LC-ESI-MS rt 9.251 min, m/z 262 (M), 285 (M+Na).

General procedure for the preparation of the Carbonates. To a solution of the *Z*-allylic alcohol (1 mmol) in dry THF (10 mL), under an inert atmosphere at -78°C, LiHMDS (1.5 equiv, 1.5 mL 1 M solution in THF) was added dropwise. The solution was stirred for 30 min and then methyl chloroformate (2 equiv, 2 mmol) was added in one portion. After 40 min, the mixture was quenched with water (2mL) 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. Carbonates were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

3a: ^{1}H NMR ($CDCl_3$) δ 1.01 (6H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.30 (3H, t, *J*= 7.5 Hz, OCH_2CH_3), 1.43 (3H, d, *J*= 6.6 Hz, *CH*$_3$CHO), 3.18 (1H, m, CH_3CHCH_3), 3.77 (3H, s, *CH*$_3$OCO), 4.25 (2H, q, *J*= 7.5 Hz, OCH_2CH_3), 5.51 (1H, q, *J*= 6.6 Hz, CH_3CHO), 5.96 (1H, d, *J*= 9.9 Hz, *CH*=C); ^{13}C NMR ($CDCl_3$) δ_C 14.0 (CH_3), 20.0 (CH_3), 22.2 ($2CH_3$), 28.0 (CH), 54.5 (CH_3), 60.3 (CH_2), 73.5 (CH), 129.7 (C), 148.7 (CH), 154.7 (C), 165.9(C); IR (film) ν/cm^{-1} 2958, 2873, 1753, 1723, 1440, 1271, 1043; $[\alpha]_D$ = -21.2 (*c* 1 in $CDCl_3$); LC-ESI-MS rt 9.917 min., m/z 267 (M+Na), 511 (2M+Na).

3b: ^{1}H NMR ($CDCl_3$) δ 0.99-1.40 (4H, m, cyclohexyl), 1.30 (3H, t, *J*= 7.2 Hz, OCH_2CH_3), 1.41 (3H, d, *J*= 6.6 Hz, *CH*$_3$CHO), 1.59-1.80 (6H, m, cyclohexyl), 2.87 (1H, m, C*H*CH=C), 3.76 (3H, s, *CH*$_3$OCO), 4.22 (2H, q, *J*= 7.2 Hz, OCH_2CH_3), 5.51 (1H, q, *J*= 6.6 Hz, CH_3CHO), 6.00 (1H, d, *J*= 9.6 Hz, *CH*=C); ^{13}C NMR ($CDCl_3$) δ_C 14.1 (CH_3), 20.2 (CH_3), 25.5 ($2CH_2$), 25.8 ($2CH_2$), 32.4 (CH_2), 37.9 (CH), 54.5 (CH_3), 60.4 (CH_2), 73.8 (CH), 130.2 (C), 147.7 (CH), 166.1(C), 198.0 (C); IR (film) ν/cm^{-1} 2982, 2927, 2852, 1751, 1721, 1444, 1383, 1341, 1268, 1211, 1046; $[\alpha]_D$ = -25.8 (*c* 1 in $CDCl_3$); LC-ESI-MS rt 11.730 min., m/z 285 (M+1), 307 (M+Na) .

3c: ^{1}H NMR ($CDCl_3$) δ 1.13 (3H, t, *J*= 6.9 Hz, OCH_2CH_3), 1.57 (3H, d, *J*= 6.6 Hz, *CH*$_3$CHO), 3.79 (3H, s, *CH*$_3$OCO), 4.17 (2H, q, *J*= 6.9 Hz, OCH_2CH_3), 5.58 (1H, q, *J*= 6.6 Hz, CH_3CHO), 6.92 (1H, s, *CH*=C), 7,29 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C 13.6 (CH_3), 19.9 (CH_3), 54.5 (CH_3), 60.9 (CH_2), 74.8 (CH), 128.0 (CH), 128.3 (CH), 128.7 (CH), 133.6 (C), 134.0 (C), 154.8 (CH), 167.3(C) (C); IR (film) ν/cm^{-1} 3382, 2961, 1757, 1715, 1603, 1445, 1259, 1093, 1024, 797; $[\alpha]_D$ = -30.0 (*c* 1 in $CDCl_3$); LC-ESI-MS rt 9.58 min., m/z 279 (M+1), 301 (M+Na), 579 (2M+Na) .

8: Yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.45 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHO), 1.51 (s, 9H, OC(*CH*$_3$)$_3$), 1.99 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 3.76 (s, 3H, C*H*$_3$OCO), 5.48 (q, *J*=6.6 Hz, 1H, CH_3CHO), 6.23 (q, *J*=7.2 Hz, 1H, CH_3CHC); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 15.3 (CH_3), 20.0 (CH_3), 28.3 ($3CH_3$), 54.6 (CH_3), 74.1 (CH), 81.3 (C), 134.4 (C), 135.8 (CH), 155.0 (C), 165.5 (C). (*S*)-**8a** $[\alpha]_D$ = -24.6 (*c* 1 in $CHCl_3$); (*R*)-**8a** $[\alpha]_D$ =

+25.0 (*c* 1 in $CHCl_3$); LC-ESI-MS rt 9.77 min, m/z 244 (M), 267 (M+Na); Anal. cald. for $C_{12}H_{20}O_5$(244.13): C 59.00, H 8.25; found C 59.09, H 8.28.

9: Pale yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.01 (d, *J*=6.6 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.42 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHO), 1.50 (s, 9H, OC(*CH*$_3$)$_3$), 3.02-3.17 (m, 1H, CH_3C*H*CH_3), 3.77 (s, 3H, C*H*$_3$OCO), 5.46 (q, *J*=6.6 Hz, 1H, CH_3C*H*O), 5.84 (d, *J*=9.6 Hz, 1HCHC*H*C);^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 19.8 (CH_3), 22.5 ($2CH_3$), 26.2 (CH), 28.1 ($3CH_3$), 54.5 (CH_3), 74.0 (CH), 81.2 (C), 131.3 (C), 146.7 (CH), 155.0 (C), 165.6 (C). (*S*)-**9b** $[\alpha]_D$ = -30.0 (*c* 1 in $CHCl_3$); (*R*)-**9b** $[\alpha]_D$ = +28.9 (*c* 1 in $CHCl_3$); LC-ESI-MS rt 11.82 min, m/z 272 (M), 295 (M+Na); Anal. cald. for $C_{14}H_{24}O_5$(272.16): C 61.74, H 8.88; found C 61.76, H 8.86.

10: Pale yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.86-1.30 (m, 6H, *cyclohexyl*), 1.39 (d, *J*=6.2 Hz, 3H, C*H*$_3$CHO), 1.47 (s, 9H, OC(*CH*$_3$)$_3$), 1.66-1.79 (m, 4H, *cyclohexyl*), 2.78 (m, C*H* cyclohexyl), 3.74 (s, 3H, C*H*$_3$OCO), 5.43 (q, *J*=6.2 Hz, 1H, CH_3C*H*O), 5.83 (d, *J*=9.4 Hz, 1H, CHC*H*C); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 19.9 (CH_3), 25.5 ($2CH_2$), 25.8 (CH_2), 28.1 ($3CH_3$), 32.4 ($2CH_2$), 37.7 (CH), 54.5 (CH_3), 74.0 (CH), 81.0 (C), 131.5 (C), 145.4 (CH), 154.8 (C), 165.5 (C); (*S*)-**10** $[\alpha]_D$ = -25.2 (*c* 1 in $CHCl_3$); (*R*)-**10** $[\alpha]_D$ = +26.7 (*c* 1 in $CHCl_3$); LC-ESI-MS rt 10.81 min, m/z 312 (M), 335(M+Na); Anal. cald. for $C_{17}H_{28}O_5$(312.19): C 65.36, H 9.03; found C 65.51, H 9.05.

11: Yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.49 (s, 9H, OC(*CH*$_3$)$_3$), 1.53 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHO), 3.78 (s, 3H, C*H*$_3$OCO), 5.51 (q, *J*=6.6 Hz, 1H, CH_3C*H*O), 6.72 (s, 1H, CC*H*C), 7.14-7.31 (m, 3H, *thiophenyl*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 20.1 (CH_3), 28.0 ($3CH_3$), 54.8 (CH_3), 75.3 (CH), 82.1 (C), 125.3 (CH), 126.5 (CH), 126.7 (CH), 128.2 (C), 133.2 (CH), 136.1 (C), 155.0 (C), 166.6 (C); LC-ESI-MS rt 10.59 min, m/z 312 (M), 335(M+Na). Anal. cald. for $C_{15}H_{20}O_5S$(312.1): C 57.67, H 6.45, S 10.26; found C 57.85, H 6.44, S 10.29.

General procedure for the preparation of the dehydro-β-amino esters **5a-c** *(Pathway A).* To a solution of the corresponding carbonates (0.2 mmol) in dry THF or CH_3CN (2 mL), under nitrogen atmosphere, benzylamine (2 equiv) was added. The solution was

refluxed for 24-96 h . The solvent was removed under reduced pressure. Compounds 3a-c were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

General procedure for the preparation of the dehydro-β-amino esters **5a-c** *(Pathway B).* To a solution of the corresponding carbonates (0.2 mmol) in dry THF (2 mL), under nitrogen atmosphere, $Pd_2(dba)_3CHCl_3$ (2%, 0.004 mmol) was added; the solution was stirred at room temperature for 30 min. Then benzylamine (2 equiv) was added. The solution was refluxed for 24 h. The mixture was filtered through a celite pad and concentrated under reduced pressure. Compounds 3a-c were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

General procedure for the preparation of the dehydro-β-amino esters **5/6a-c** *(with chiral Ligand).* To a solution of dry THF (or CH_2Cl_2) under nitrogen atmosphere, $Pd_2(dba)_3CHCl_3$ (5 or 2%) and (*R,R*)-DACH-Phenyl Trost Ligand (8 or 6%) are added and stirred at room temperature until the solution turned from dark purple to orange. Then the corresponding carbonates (0.2 mmol) was added; the solution was stirred at room temperature for 30 min. Then benzylamine (2 equiv) was added. The solution was refluxed for 24 h. The mixture was pured in pentane/ether, 1:1 (10 ml), filtered through a celite pad and concentrated under reduced pressure. Compounds 3/4a-c were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

5a: ^{1}H NMR ($CDCl_3$) major isomer δ 0.77 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.16 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.32 (3H, t, *J*= 6.9 Hz, OCH$_2$C*H*$_3$), 1.70 (3H, d, *J*= 7.2 Hz, C*H*$_3$CH=C), 2.0 (1H, m, CH$_3$C*H*CH$_3$), 3.12 (1H, d, *J*= 9.6 Hz, C*H*N), 3.54 (1H, d, *J*= 13.5 Hz, C*H*$_2$Ph), 3.58 (1H, d, *J*= 13.5 Hz, C*H*$_2$Ph), 4.24 (2H, q, *J*= 6.9 Hz, OC*H*$_2$CH$_3$), 7.07 (1H, q, *J*= 6.9Hz, CH$_3$*CH*=C), 7.24-7.44 (5H, m, Ph); minor isomer δ 0.86 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.02 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.30 (3H, t, *J*= 6.9 Hz, OCH$_2$C*H*$_3$), 2.0 (1H, m, CH$_3$C*H*CH$_3$), 2.01 (3H, d, *J*= 7.2 Hz, *CH*$_3$CHO), 2.87 (1H, d, *J*= 8.4 Hz, C*H*N), 3.57 (1H, d, *J*= 13.2 Hz, C*H*$_2$Ph), 3.82 (1H, d, *J*= 13.2 Hz, C*H*$_2$Ph), 4.27 (2H, q, *J*= 6.9 Hz, OC*H*$_2$CH$_3$), 5.94 (1H, q, *J*= 6.9Hz, CH$_3$*CH*=C), 7.25-7.39 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C major isomer 14.2 (CH_3), 20.2 (CH_3), 21.0

(CH_3), 31.8 (CH), 51.1 (CH_2), 54.6 (CH_3), 60.1 (CH_2), 61.4 (CH), 126.6 (CH), 127.5 (CH), 128.1 (CH), 133.0 (C), 139.8 (C), 141.0 (CH), 167.3 (C); IR (film) ν/cm^{-1} 3360, 2958, 1946, 1711, 1653, 1445, 1267, 1093; $[\alpha]_D$ = +14.6 (*c* 1 in $CDCl_3$); HPLC analysis, 100% *n*-hexane, 0.8 ml/min, OJ column, rt 11.837 min; LC-ESI-MS rt 4.787 min., m/z 276 (M+1) .

5b: ^{1}H NMR ($CDCl_3$) major isomer δ 1.28 (3H, t, *J*= 7.2 Hz, OCH_2CH_3), 1.02-1.51 (6H, m, cyclohexyl), 1.62 (3H, d, *J*= 7.2 Hz CH_3CH=C), 1.55-1.77 (4H, m, cyclohexyl), 2.4 (1H, bs, CHCN), 3.16 (1H, d, *J*= 9.8 Hz, CHN), 3.47 (1H, d, *J*= 13.5 Hz, CH_2Ph), 3.80 (1H, d, *J*= 13.5 Hz, CH_2Ph), 4.17 (2H, q, *J*= 7.2 Hz, OCH_2CH_3), 7.01 (1H, q, *J*= 7.2 Hz, CH_3CH=C), 7.15-7.35 (5H, m, Ph); minor isomer δ 1.94 (3H, d, *J*= 7.09 Hz, CH_3CHO), 2.08 (1H, bs, CHCN), 2.85 (1H, d, *J*= 8.8 Hz, CHN), 3.50 (1H, d, *J*= 13.28 Hz, CH_2Ph), 5.85 (1H, q, *J*= 7.1 Hz, CH_3CH=C); ^{13}C NMR ($CDCl_3$) δ_C major isomer 14.1 (CH_3), 14.3 (CH_3), 26.2 (CH_2), 26.3 (CH_2), 26.7 (CH_2),31.6 (CH_2), 41.7 (CH), 51.0 (CH_2), 60.1 (CH_2), 126.5 (CH), 128.1 (CH), 128.1 (CH), 132.8 (C), 139.8 (C), 141.0 (CH), 167.3 (C); IR (film) ν/cm^{-1} 3417, 2930, 2857, 1703, 1638, 1445, 1263, 1097; $[\alpha]_D$ = +10.3 (*c* 1 in $CDCl_3$); HPLC analysis, 100% *n*-hexane, 0.8 ml/min, OJ column, rt 7.042 min; LC-ESI-MS rt 16.261 min., m/z 316 (M+1).

5c: ^{1}H NMR ($CDCl_3$) major isomer δ 1.21 (3H, t, *J*= 7.1 Hz, OCH_2CH_3), 1.91 (3H, d, *J*= 7.3 Hz, CH_3CH=C), 3.82 (1H, d, *J*= 13.3 Hz, CH_2Ph), 3.96 (1H, d, *J*= 13.3 Hz, CH_2Ph), 4.15 (2H, q, *J*= 7.1 Hz, OCH_2CH_3), 4.91 (1H, s, CHN), 7.2 (1H, q, *J*= 7.3 Hz, CH_3CH=C), 7.2-7.5 (10H, m, Ph);); minor isomer δ 1.23 (3H, t, *J*= 7.5 Hz, OCH_2CH_3), 2.08 (3H, d, *J*= 7.0 Hz, CH_3CH=C), 3.77-3.88 (2H, m, CH_2Ph), 4.65 (1H, s, CHN), 6.35 (1H, q, *J*= 7.23 Hz, CH_3CH=C); ^{13}C NMR ($CDCl_3$) δ_C major isomer 14.0 (CH_3), 51.0 (CH_2), 56.9 (CH_3), 60.3 (CH_2), 63.9 (CH), 126.5 (CH), 126.8 (CH), 127.0 (CH), 128.0 (CH), 128.2 (CH), 128.4 (CH), 133.6 (C), 139.9 (C), 140.3 (CH), 166.7 (C); minor isomer 14.2 (CH_3), 51.5 (CH_2), 54.9 (CH_3), 60.1 (CH_2), 63.9 (CH), 126.4 (CH), 126.9 (CH), 127.0 (CH), 128.1 (CH), 128.3 (CH), 128.4 (CH), 133.5 (C), 137.5 (C), 142.2 (CH), 166.9 (C); IR (film) ν/cm^{-1} 3359, 3027, 2930, 2857, 1738, 1707, 1595, 1491, 1456, 1371, 1256, 746, 700; $[\alpha]_D$ = -6.2 (*c* 1 in $CDCl_3$); HPLC analysis, 98:2 *n*-

hexane/2-propanol, 0.8 ml/min, OJ column, rt 13.463 min (major), 21.310 min (minor); LC-ESI-MS rt 9.45 min., m/z 310 (M+1).

General procedure for the preparation of the dehydro-β-amino esters **6a-c**. To a solution of the corresponding carbonates (0.2 mmol) in dry CH_3CN (2 mL), under nitrogen atmosphere, $Pd_2(dba)_3CHCl_3$ (2%, 0.004 mmol) was added; the solution was stirred at room temperature for 30 min. Then benzylamine (2 equiv) was added. The solution was refluxed for 24 h. The mixture was filtered through a celite pad and concentrated under reduced pressure. Compounds 4a-c were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluant).

6a: ^{1}H NMR ($CDCl_3$) δ 1.06 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.08 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHC*H*$_3$), 1.31 (3H, d, *J*= 6.9 Hz, *CH*$_3$CHN), 1.35 (3H t, *J*= 6.9 Hz, OCH$_2$C*H*$_3$), 1.90 (1H, s, NH), 3.01 (1H, m, CH$_3$C*H*CH$_3$), 3.43 (1H, q, *J*= 6.9 Hz, C*H*N), 3.64 (1H, d, *J*= 12.9 Hz, C*H*$_2$Ph), 3.81 (1H, d, *J*= 12.9 Hz, C*H*$_2$Ph), 4.27 (2H, q, *J*= 6.9 Hz, O*CH*$_2$CH$_3$), 5.73 (1H, q, *J*= 9.9Hz, CH*CH*=C), 7.20-7.40 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C 14.3 (CH_3), 21.3 (CH_3), 22.7 (CH_3), 23.0 (CH_3), 28.6 (CH), 51.0 (CH_2), 56.8 (CH), 60.1 (CH_2), 126.8 (CH), 128.2 (CH), 128.3 (CH), 132.2 (C), 140.4 (C), 146.2 (CH), 168.3 (C); IR (film) ν/cm^{-1} 3343, 2973, 2923, 2861, 1707, 1460, 1379, 1225, 1175, 1163, 1093, 1016; $[\alpha]_D$ = -7.0 (*c* 1 in $CDCl_3$); HPLC analysis, 100% *n*-hexane, 0.8 ml/min, OJ column, rt 11.840 min; LC-ESI-MS rt 1.507 min., m/z 276 (M+1) .

6b: ^{1}H NMR ($CDCl_3$) δ 1.29 (3H, d, *J*= 6.6 Hz, C*H*$_3$CHN), 1.35 (3H, t, *J*= 6.9 Hz, OCH$_2$C*H*$_3$), 1.05-1.41 (6H, m, cyclohexyl), 1.69-1.80 (4H, m, cyclohexyl), 2.0 (1H, bs, N*H*), 2.70 (1H, m, C*H*CH=C), 3.43 (1H, q, *J*= 6.6 Hz, CH$_3$C*H*N), 3.63 (1H, d, *J*= 12.6 Hz, C*H*$_2$Ph), 3.80 (1H, d, *J*= 12.6 Hz, C*H*$_2$Ph), 4.28 (2H, q, *J*= 6.9 Hz, O*CH*$_2$CH$_3$), 5.75 (1H, d, *J*= 9.9Hz, CH*CH*=C), 7.27-7.36 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C 14.2 (CH_3), 21.4 (CH_3), 25.6 (CH_2), 32.7 ($2CH_2$), 33.1 ($2CH_2$), 38.3 (CH), 51.0 (CH_2), 60.2 (CH_2), 126.8 (CH), 128.3 (CH), 128.8 (CH), 132.4 (C), 140.4 (C), 145.2 (CH), 168.2 (C); IR (film) ν/cm^{-1} 3346, 3027, 2924, 2851, 1709, 1600, 1450, 1366, 1118; $[\alpha]_D$ = -12.5 (*c* 1 in $CDCl_3$); HPLC analysis, 100% *n*-hexane, 0.8 ml/min, OJ column, rt 12.532 min; LC-ESI-MS rt 12.330 min., m/z 316 (M+1).

6c: ^{1}H NMR ($CDCl_3$) δ 1.40 (3H, t, *J*= 6.9 Hz, OCH_2CH_3), 1.50 (3H, d, *J*= 6.6 Hz, CH_3CHN), 2.30 (1H, bs, N*H*), 3.45 (1H, d, *J*= 13.2 Hz, CH_2Ph), 3.69 (1H, d, *J*= 13.2 Hz, CH_2Ph), 4.0 (1H, q, *J*= 6.6 Hz, CH_3CHN), 4.32 (2H, q, *J*= 6.9 Hz, OCH_2CH_3), 7.16-7.38 (10H, m, Ph) 7.83 (1H, s, C*CH*=C); ^{13}C NMR ($CDCl_3$) δ_C 14.2 (CH_3), 20.8 (CH_3), 50.4 (CH), 51.3 (CH_2), 60.5 (CH_2), 126.6 (CH), 128.0 (CH), 128.1 (CH), 128.2 (CH), 128.3 (CH), 128.9 (CH), 135.1 (C), 135.3 (C), 140.2 (C), 140.4 (CH), 168.2 (C); IR (film) ν/cm^{-1} 3348, 3027, 2981, 2927, 1689, 1499, 1456, 1244, 1117, 1020, 700; $[\alpha]_D$ = -8.0 (*c* 1 in $CDCl_3$); HPLC analysis, 98:2 *n*-hexane/2-propanol, 0.8 ml/min, OJ column, rt 14.641 min (major); LC-ESI-MS rt 11.520 min., m/z 310 (M+1).

General procedure for the preparation of the β-lactams **7a-b.** To a solution of the corresponding dehydro-β-amino esters 4a-b (0.2 mmol) in dry THF (4 mL), under an inert atmosphere at -20°C, LiHMDS (1.5 equiv, 1 M solution in THF) was added drop-wise. The solution was stirred for 50 min at the same temperature. After 40min, the mixture was quenched with water 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. Compounds 5a-b were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10 as eluant).

7a: ^{1}H NMR ($CDCl_3$) δ 1.03 (6H, d, *J*= 6.6 Hz, CH_3CHCH_3), 1.16 (3H, d, *J*= 6.6 Hz, CH_3CHN), 3.21 (1H, m, CH_3CHCH_3), 3.86 (1H, q, *J*= 6.0 Hz, C*H*N), 4.18 (1H, d, *J*= 15.0 Hz, CH_2Ph), 4.66 (1H, d, *J*= 15.0 Hz, CH_2Ph), 5.33 (1H, d, *J*= 11.1 Hz, CH*CH*=C), 7.25-7.35 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C 17.3 (CH_3), 22.9 ($2CH_3$), 28.0 (CH), 44.0 (CH_2), 54.7 (CH), 127.5 (CH), 128.2 (CH), 135.3 (CH), 136.2 (C), 140.0 (CH), 163.7 (C); IR (film) ν/cm^{-1} 3424, 2961, 2927, 1734, 1645, 1452, 1391, 1264, 1105, 1028, 792, 700; $[\alpha]_D$ = -61.2 (*c* 1 in $CDCl_3$); HPLC analysis, 98:2 *n*-hexane/2-propanol, 0.8 ml/min, AD column, rt 15.070 min; LC-ESI-MS rt 9.715 min., m/z 230 (M+1), 252 (M+Na).

7b: ^{1}H NMR ($CDCl_3$) δ 1.15 (3H, d, *J*= 6.0 Hz, CH_3CHN), 1.07-1.42 (6H, m, cyclohexyl), 1.60-1.71 (4H, m, cyclohexyl), 3.86 (1H, q, *J*= 6.0 Hz, C*H*N), 4.18 (1H,

d, *J*= 15.0 Hz, C*H*$_2$Ph), 4.66 (1H, d, *J*= 15.0 Hz, C*H*$_2$Ph), 5.36 (1H, d, *J*= 9.6 Hz, CH*CH*=C), 7.25-7.33 (5H, m, Ph); ^{13}C NMR ($CDCl_3$) δ_C 17.3 (CH_3), 25.4 ($2CH_2$), 25.8 (CH_2), 33.0 ($2CH_2$), 37.3 (CH), 44.0 (CH_2), 127.5 (CH), 128.2 (CH), 128.7 (CH), 134.2 (CH), 136.3 (C), 140.3 (CH), 163.7 (C); IR (film) ν/cm^{-1} 3390, 2923, 2846, 1738, 1452, 1379, 1264, 1098, 1028; $[\alpha]_D$ = -39.0 (*c* 1 in $CDCl_3$); HPLC analysis, 98:2 *n*-hexane/2-propanol, 0.8 ml/min, AD column, rt 16.494 min; LC-ESI-MS rt 11.773 min., m/z 270 (M+1), 292 (M+Na).

General procedure for the preparation of the dehydro-β-amino esters. To a solution of the carbonate **8-11** (0.2 mmol) in dry CH_3CN (2 mL), under nitrogen atmosphere, $Pd_2(dba)_3CHCl_3$ (2.5%, 0.005 mmol) was added in one portion. After stirring the solution at room temperature for 30 min, the amine (1.2 equiv) was added. The solution was refluxed for 12 h and then the mixture was filtered through a celite pad and concentrated under reduced pressure. The dehydro-β-amino ester was isolated by flash chromatography on silica gel.

12a: Orange oil (40%), (1:1 *E/Z* mixture); R_f (30% ethylacetate/70% cyclohexane) 0.10; ^{1}H NMR (200 MHz, $CDCl_3$) (*E*) δ 1.09 (d, *J* = 6.6 Hz, 3H, C$\underline{H}_3$CHN), 1.46 (s, 9H, OC(C$\underline{H}_3$)$_3$), 1.84 (d, *J* = 7.4Hz, 3H, C$\underline{H}_3$CHC), 2.44 (s, 3H, C$\underline{H}_3$N), 2.50–2.80 (m, 8H, *piperazine*), 3.42 (q, *J* = 7.4 Hz, 1H, $CH_3C\underline{H}N$), 6.63 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}C$); (*Z*) δ 1.26 (d, *J* = 6.6 Hz, 3H, C$\underline{H}_3$CHN), 1.42 (s, 9H, OC(C$\underline{H}_3$)$_3$), 1.78 (d, *J* = 7.4Hz, 3H, C$\underline{H}_3$CHC), 2.44 (s, 3H, C$\underline{H}_3$N), 2.50–2.80 (m, 8H, *piperazine*), 3.42 (q, *J* = 7.4 Hz, 1H, $CH_3C\underline{H}N$), 5.70 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}C$).^{13}C NMR (75 MHz, $CDCl_3$) δ 15.6, 17.7, 28.3 (3C), 47.6, 55.8, 57.0 (2C), 57.4 (2C), 90.0, 132.3, 135.9, 164.3; IR (neat, cm^{-1}) 696, 721, 802, 1014, 1119, 1152, 1261, 1367, 1392, 1454, 1622, 1712, 2795, 2852, 2930, 2964, 3364; Anal. calcd. for $C_{15}H_{28}N_2O_2$ (268.4): C 67.13, H 10.52, N 10.44; found C 67.07, H 10.52, N 10.46.

12b: Yellow oil (35%), (80:20 *E/Z* mixture); R_f (30% ethylacetate/70% cyclohexane) 0.10; ^{1}H NMR (200 MHz, $CDCl_3$) (*E*) δ 1.34 (d, *J* = 6.6 Hz, 3H, C$\underline{H}_3$CHN), 1.52 (s, 9H, OC(C$\underline{H}_3$)$_3$), 1.74 (d, *J* = 7.4 Hz, 3H, C$\underline{H}_3$CHC), 2.58 (bs, 1H, N$\underline{H}$), 3.53 (d, *J* = 12.4 Hz, 1H, HNC$\underline{H}_2$), 3.69 (d, *J* = 12.4 Hz, 1H, HNC$\underline{H}_2$), 3.73 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}N$),

3.81 (s, 3H, OC$\underline{H}_3$), 6.80–6.86 (m, 1H, $CH_3C\underline{H}C$), 6.85 (d, *J* = 8.4 Hz, 2H, *phenyl*), 7.24 (d, *J* = 8.4Hz, 2H, *phenyl*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 13.8, 20.6, 28.3 (3C), 50.0, 50.7, 55.3, 80.7, 113.8 (2C), 129.5 (2C), 132.0, 135.9, 137.9, 151.1, 166.7; IR (neat, cm^{-1}) 699, 766, 832, 1031, 1097, 1160, 1258, 1339, 1449, 1511, 1577, 1604, 1651, 1698, 2850, 2926, 2961, 3366; Anal. calcd. for $C_{18}H_{27}NO_3$ (305.4): C 70.79, H 8.91, N 4.59; found C 70.52, H 8.94, N 10.47.

12f: Yellow oil (60%), (70:30 *E/Z* mixture); R_f (50% ethylacetate/50% cyclohexane on alumina plates) 0.23; ^{1}H NMR ($CDCl_3$, 200 MHz) (*E*) δ 1.31 (d, *J* = 7 Hz, 3H, $C\underline{H}_3CHN$), 1.50 (s, 9H, $OC(C\underline{H}_3)_3$), 1.72 (d, *J* = 7.4 Hz, 3H, $C\underline{H}_3CHC$), 3.43 (d, *J* = 12.4 Hz, 1H, $HNC\underline{H}_2$), 3.60 (d, *J* = 12.4 Hz, 1H, $HNC\underline{H}_2$), 3.72 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}N$), 6.63 (d, *J* = 8 Hz, 2H, *phenyl*), 6.81 (q, *J* = 7.4 Hz, 1H, $CH_3C\underline{H}C$), 7.08 (d, *J* = 8 Hz, 2H, *phenyl*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 13.8, 20.7, 28.4 (3C), 49.9, 51.0, 80.5, 115.2 (2C), 129.4 (2C), 130.6, 136.3, 137.7, 145.2, 166.7; IR (neat, cm^{-1})1141, 1278, 1367, 1392, 1453, 1517, 1632, 1695, 2929, 2975, 3216, 3368, 3445. LC-ESI-MS rt 8.39 min, m/z 290 (M), 313 (M+Na). Chiral HPLC analysis 99:1 to 96:4 *n*-hexane/2-propanol in 30 min, 1.0 mL/min, AD column, rt 23.52 min for [*E*-(*R*)-**5f**] and 26.12 min [*E*-(*S*)-**5f**]; *E*-(*S*)-**5f** $[\alpha]_D$ = -14.0 (*c* 1 in $CHCl_3$); *E*-(*R*)-**5f** $[\alpha]_D$ = +14.0 (*c* 1 in $CHCl_3$) Anal. cald. for $C_{17}H_{26}N_2O_2$ (290.2): C 70.31, H 9.02, N 9.65; found C 70.08, H 9.00, N 9.65.

13a: Orange oil (47%), (95:5 *Z/E* mixture); R_f (40% ethylacetate/60% cyclohexane) 0.12; ^{1}H NMR (200 MHz, $CDCl_3$) (*Z*): δ 0.96 (d, *J* = 6.6 Hz, 3H, $C\underline{H}_3CHCH_3$), 0.99 (d, *J* = 6.6 Hz, 3H, $CH_3CHC\underline{H}_3$), 1.09 (d, *J* = 7 Hz, 3H, $C\underline{H}_3CHN$), 1.49 (s, 9H, $OC(C\underline{H}_3)_3$), 2.24 (s, 3H, $C\underline{H}_3N$), 2.25–2.40 (m, 8H, *piperazine*), 2.65–2.83 (m, 1H, $CH_3C\underline{H}CH_3$), 3.40 (q, *J* = 7Hz, 1H, $CH_3C\underline{H}N$), 5.33 (d, *J* = 9.6 Hz, 1H, $CHC\underline{H}C$); ^{13}C NMR (75 MHz, $CDCl_3$) δ 12.8, 22.9 (2C), 28.4 (3C), 28.6, 46.1, 55.7 (4C), 60.9, 80,3, 135.6, 140.4, 167.2 ; IR (neat, cm^{-1}) 1014, 1148, 1239, 1322, 1366, 1455, 1716, 2793, 2869, 2935, 2969. LC-MS-ESI rt 1.73, 297 (M+1) Anal. cald. for $C_{17}H_{32}N_2O_2$ (296.4): C 68.88, H 10.88, N 9.45; found C 68.93, H 10.89, N 9.43.

13b: Yellow oil (45%), (95:5 *Z/E* mixture); R_f (30% ethylacetate/70% cyclohexane) 0.33; ^{1}H NMR (200 MHz, $CDCl_3$) (*Z*): δ 1.03 (d, *J* = 6.6 Hz, 3H, $C\underline{H_3}CHCH_3$), 1.07 (d, *J* = 6.6 Hz, 3H, $CH_3CHC\underline{H_3}$), 1.25 (d, *J* = 6.6 Hz, 3H, $C\underline{H_3}CHN$), 1.53 (s, 9H, $OC(C\underline{H_3})_3$), 1.82 (bs, 1H, N<u>H</u>), 2.84–3.06 (m, 1H, $CH_3C\underline{H}CH_3$), 3.32 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}N$), 3.53 (d, *J* = 12.6 Hz, 1H, $HNC\underline{H_2}$), 3.73 (d, *J* = 12.6 Hz, 1H, $HNC\underline{H_2}$), 3.80 (s, 3H, $O\underline{CH_3}$), 5.55 (d, *J* = 10.0 Hz, 1H, CHC<u>H</u>C) 6.86 (d, *J* = 8.8 Hz, 2H, *phenyl*), 7.25 (d, *J* = 8.8 Hz, 2H, *phenyl*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 22.8, 23.2, 28.4 (3C), 28.7, 50.5, 55.3, 57.0, 80.9, 113.8 (2C), 129.5 (2C), 132.9, 133.7, 144.4, 158.6, 167.9; IR (neat, cm^{-1}) 829, 848, 1037, 1152, 1246, 1300, 1320, 1367, 1392, 1465, 1512, 1611, 1707, 2834, 2868, 2932, 2966. LC-MS-ESI rt 4.13, 334 (M+1) Anal. cald. for $C_{20}H_{31}NO_3$ (333.4): C 72.04, H 9.37, N 4.20; found C 71.95, H 9.40, N 4.18.

13c: Yellow oil (40%), (95:5 *Z/E* mixture); R_f (30% ethylacetate/70% cyclohexane) 0.35; ^{1}H NMR (200 MHz, $CDCl_3$) (*Z*): δ 0.98 (d, *J* = 6.2 Hz, 3H, $CH_3CHC\underline{H_3}$), 1.04 (d, *J* = 6.2 Hz, 3H, $C\underline{H_3}CHCH_3$), 1.24 (d, *J* = 6.6 Hz, 3H, $C\underline{H_3}CHN$), 1.51(s, 9H, $OC(C\underline{H_3})_3$), 1.77 (bs, 1H, N<u>H</u>), 2.86–3.02 (m, 1H, $CH_3C\underline{H}CH_3$), 3.27 (q, *J* = 6.6Hz, 1H, $CH_3C\underline{H}N$), 3.65 (d, *J* = 13.4 Hz, 1H, $HNC\underline{H_2}$), 3.84 (d, *J* = 13.4 Hz, 1H, $HNC\underline{H_2}$), 3.92 (s, 3H, $O\underline{CH_3}$), 5.51 (d, *J* = 9.8 Hz, 1H, CHC<u>H</u>C), 7.40 (d, *J* = 8.4 Hz, 2H, *phenyl*), 7.99 (d, *J* = 8.4 Hz, 2H, *phenyl*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.2, 22.7, 23.2, 28.4, 28.6, 50.7, 52.1, 57.4, 81.1, 128.1, 128.2 (2C), 128.8, 129.7 (2C), 144.8, 146.0, 163.5, 164.2; IR (neat, cm^{-1}) 699, 759, 1019, 1151, 1278, 1367, 1435, 1615, 1656, 1683, 1722, 2868, 2930, 2965. LC-MS-ESI rt 5.33, 362 (M+1) Anal. cald. for $C_{21}H_{31}NO_4$ (361.4): C 69.78, H 8.64, N 3.87; found C 69.52, H 8.63, N 3.87.

13d: Yellow oil (60%); (95:5 *Z/E* mixture; 1:1 diastereomeric mix); R_f (30% ethylacetate/70% cyclohexane) 0.40; *Z*-Isomer A: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.92-1.00 (m, 6H, $C\underline{H_3}CHC\underline{H_3}$), 1.25 (d, *J* = 7Hz, 3H, $C\underline{H_3}CHN$), 1.48 (s, 9H, $OC(C\underline{H_3})_3$), 1.76–1.92 (m, 4H, $NCH_2C\underline{H_2}C\underline{H_2}$), 2.63–2.83 (m, 2H, $NC\underline{H_2}$), 3.00–3.10 (m, 1H, $CH_3C\underline{H}CH_3$), 3.54–3.68 (m, 2H, $CH_3C\underline{H}N$, NC<u>H</u>CO), 5.12 (s, 2H, $C\underline{H_2}Ph$), 5.48 (d, *J* = 9.8 Hz, 1H, CHC<u>H</u>C), 7.28–7.41 (m, 5H, *phenyl*); *Z*-Isomer B: ^{1}H NMR (200 MHz, $CDCl_3$) δ 0.94 (m, 6H, $C\underline{H_3}CHC\underline{H_3}$), 1.18 (d, *J* = 7 Hz, 3H, $C\underline{H_3}CHN$), 1.50 (s, 9H, $OC(C\underline{H_3})_3$), 1.76–1.92 (m, 4H, $NCH_2C\underline{H_2}C\underline{H_2}$), 2.63–2.83 (m, 2H, $NC\underline{H_2}$), 3.00–3.10

(m, 1H, $CH_3C\underline{H}CH_3$), 3.54–3.68 (m, 2H, $CH_3C\underline{H}N$, $NC\underline{H}CO$), 5.12 (s, 2H, $C\underline{H_2}Ph$), 5.55 (d, *J* = 9.8 Hz, 1H, $CHC\underline{H}C$), 7.28–7.41 (m, 5H, *phenyl*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 17.8, 22.8, 23.4, 27.0, 28.3, 28.6, 30.1, 49.2, 59.1, 62.3, 66.0, 80.7, 128.1 (2C), 128.2(2C), 128.6(2C), 136.2, 143.3, 168.4, 175.1; IR (neat, cm^{-1}) 697, 750, 1149, 1241, 1270, 1367, 1392, 1455, 1732, 2869, 2971; LC-MS-ESI rt 5.57, 402 (M+1). LC-MS-ESI rt 5.61, 402 (M+1) Anal. cald. for $C_{24}H_{35}NO_4$ (401.5): C 71.79, H 8.79, N 3.49; found C 72.03, H 8.78, N 3.48.

13e: Brown oil (60%) (95:5 *Z/E* mixture); R_f (30% ethylacetate/70% cyclohexane) 0.12; ^1H NMR (200 MHz, $CDCl_3$) (*Z*) δ 0.97 (d, *J* = 6.6 Hz, 3H, $C\underline{H_3}CHCH_3$), 0.98 (d, *J* = 6.6 Hz, 3H, $CH_3CHC\underline{H_3}$), 1.23 (d, *J* = 6.6 Hz, 3H, $C\underline{H_3}CHN$), 1.41 (s, 9H, $OC(C\underline{H_3})_3$), 1.98 (bs, 1H, $N\underline{H}$), 2.79–3.00 (m, 5H, $NC\underline{H_2}C\underline{H_2}$, $CH_3C\underline{H}CH_3$), 3.37 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}N$), 5.52 (d, *J* = 9.4 Hz, 1H, $CHC\underline{H}C$), 7.01 (s, 1H, $NC\underline{H}$ *aromatic*), 7.01–7.21 (m, 2H, *phenyl*), 7.34 (d, *J* = 7.4 Hz, 1H, *phenyl*), 7.61 (d, *J* = 7.8 Hz, 1H, *phenyl*), 8.29 (bs, 1H, $N\underline{H}$ *aromatic*); ^{13}C NMR (75 MHz, $CDCl_3$) δ 21.4, 22.8, 23.1, 26.1, 28.2 (3C), 28.5, 47.3, 57.5, 81.0, 111.2, 114.2, 119.0, 119.2, 121.9, 128.5, 128.8, 132.1, 136.5, 144.8, 167.7; IR (neat, cm^{-1}) 739, 1120, 1152, 1244, 1367, 1455, 1703, 2868, 2927, 2971, 3057, 3408; LC-MS-ESI rt 5.57, 357 (M+1) Anal. cald. for $C_{22}H_{32}N_2O_2$ (356.5): C 74.12, H 9.05, N 7.86; found C 73,84, H 9,02, N 7,86.

13f: Yellow oil (65%), (95:5 *Z/E* mixture); R_f (40% ethylacetate/60% cyclohexane) 0.14; ^1H NMR ($CDCl_3$, 200 MHz) (*Z*): δ 1.02 (d, *J* = 5.4 Hz, 3H, $C\underline{H_3}CHCH_3$), 1.05 (d, *J* = 5.4 Hz, 3H, $CH_3CHC\underline{H_3}$), 1.23 (d, *J* = 7.8 Hz, 3H, $C\underline{H_3}CHN$), 1.51 (s, 9H, $OC(C\underline{H_3})_3$), 2.86–3.02 (m, 1H, $CH_3C\underline{H}CH_3$), 3.31 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}N$), 3.46 (d, *J* = 12.6 Hz, 1H, $HNC\underline{H_2}$), 3.66 (d, *J* = 12.6 Hz, 1H, $HNC\underline{H_2}$), 5.55 (d, *J* = 9.6 Hz, 1H, $CHC\underline{H}C$), 6.63 (d, *J* = 7.8 Hz, 2H, *phenyl*), 7.10 (d, *J* = 7.8 Hz, 2H, *phenyl*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.5, 22.8, 23.3, 28.4 (3C), 28.6, 50.6, 56.9, 80.9, 115.2 (2C), 129.4 (2C), 130.7, 133.7, 144.5, 145.3, 167.9; IR (neat, cm^{-1}) 1152, 1241, 1277, 1367, 1392, 1454, 1518, 1622, 1700, 2868, 2929, 2969, 3218, 3372. LC-MS-ESI: rt 2.9 min 319 (M+1); [*Z*-(*R*)- **13f**]: $[\alpha]_D$ = -4.8 (*c* 1 in $CHCl_3$); [*Z*-(*S*)- **13f**]: $[\alpha]_D$ = +6.6 (*c* 1 in $CHCl_3$), Chiral HPLC analysis 98:2 to 90:10 *n*-hexane/2-propanol in 25 min, 0.8mL/min, IA column, rt 15.10 min for [*Z*-(*R*)- **13f**] and 15.61 min for [*Z*-(*S*)- **13f**].

Anal. cald. for $C_{19}H_{30}N_2O_2$ (318.4): C 71.66, H 9.50, N 8.80; found C 71.69, H 9,46, N 8,82.

14f: Yellow oil (70%), (90:10 *Z/E* mixture); R_f (30% ethylacetate/70% cyclohexane) 0.13; ^{1}H NMR ($CDCl_3$, 200 MHz) (*Z*): δ 1.00–1.10, (m, 6H, *cyclohexyl*), 1.25 (d, *J* = Hz, 3H, C$\underline{H}_3$CHN), 1.52 (s, 9H, OC(C$\underline{H}_3$)$_3$), 1.60–1.80 (m, 4H, *cyclohexyl*), 2.52–2.68 (m, 1H, C$\underline{H}$ cyclohexyl), 3.31 (q, *J* = 6.6 Hz, 1H, $CH_3C\underline{H}N$),3.45 (d, *J* = 13 Hz, 1H, HNC$\underline{H}_2$), 3.65 (d, *J* = 13 Hz, 1H, HNC$\underline{H}_2$), 5.57, (d, *J* = 9.6 Hz, 1H, CHC$\underline{H}$C), 6.63 (d, *J* = 7.8 Hz, 2H, *phenyl*), 7.09 (d, *J* = 7.8 Hz, 2H, *phenyl*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 21.5, 25.8, 28.4 (3C), 32.9 (2C), 33.3 (2C), 38.3, 50.6, 56.9, 80.8, 115.2 (2C), 129.4 (2C), 130.7, 134.1, 142.9, 145.3, 167.9; IR (neat, cm^{-1}) 826, 489, 1111, 1152, 1221, 1252, 1267, 1367, 1392, 1448, 1518, 1622, 1698, 2850, 2925, 2974, 3218, 3372. [*Z*-(*R*)- **14f**]: $[\alpha]_D$ = -6.1 (*c* 1 in $CHCl_3$); [*Z*-(*S*)- **14f**]: $[\alpha]_D$ = +7.8 (*c* 1 in $CHCl_3$), LC-MS-ESI rt 5.56, 359 (M+1) Chiral HPLC analysis 95:5 to 90:10 *n*-hexane/2-propanol in 25 min, 1.0mL/min, AD column, rt 11.10 min for [*Z*-(*R*)- **14f**] and 14.08 min for [*Z*-(*S*)- **14f**]. Anal. cald. for $C_{22}H_{34}N_2O_2$ (358.5): C 73.70, H 9.56, N 7.81; found C 73.77 H 9.53, N 7.82.

15f: Orange oil (87%), (80:20 *E/Z* mixture); R_f (40% ethylacetate/60% cyclohexane) 0.12; ^{1}H NMR ($CDCl_3$, 200 MHz) (*E*): δ 1.32 (d, *J* = 7 Hz, 3H, C$\underline{H}_3$CHN), 1.57 (s, 9H, OC(C$\underline{H}_3$)$_3$), 3.42 (d, *J* = 12 Hz, 1H, HNC$\underline{H}_2$), 3.60 (d, *J* = 12 Hz, 1H, HNC$\underline{H}_2$), 4.10 (q, *J* = 7 Hz, 1H, $CH_3C\underline{H}N$), 6.57 (d, *J* = 8.4 Hz, 2H, *phenyl*), 6.66 (d, *J* = 7.6 Hz, 1H, *thiophenyl*), 6.99 (d, *J* = 8.4 Hz, 2H, *phenyl*), 7.08–7.19 (m, 1H, *thiophenyl*), 7.28–7.34 (m, 1H, *thiophenyl*), 7.61 (s, 1H, CC$\underline{H}$C); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 20.6, 28.3 (3C), 50.6, 50.9, 81.0, 115.1 (2C), 125.7, 126.4, 128.7, 129.3 (2C), 130.3, 133.4, 135.9, 136.4, 145.2, 166.9 ; IR (neat, cm^{-1}) 729, 793, 841, 1163, 1249, 1306, 1392, 1455, 1519, 1568, 1593, 1699, 2851, 2925, 2974, 3371. [*E*-(*R*)- **15f**]: $[\alpha]_D$ = +5.0 (*c* 1 in $CHCl_3$); [*E*-(*S*)- **15f**]: $[\alpha]_D$ = -4.2 (*c* 1 in $CHCl_3$), LC-MS-ESI rt 2.77, 359 (M+1) Chiral HPLC analysis 95:5 to 90:10 *n*-hexane/2-propanol in 20 min, 0.8mL/min, OJ column, rt 35.80 min for [*E*-(*S*)- **15f**] and 38.58 min for [*E*-(*R*)- **15f**]. Anal. cald. for $C_{20}H_{26}N_2O_2S$ (358.5): C 67.01, H 7.31, N 7.81; found C 66.83 H 7.28, N 7.81.

Representative procedure for Pd-nanoparticles catalyzed reaction. A mixture of amine (1 mmol), allyl carbonate (3 mmol), $PdCl_2$ (0.045 mmol), tetrabutylammonium iodide (1 mmol), and K_2CO_3 (2 mmol) in toluene (3 mL) was stirred at 85°C for 10 h. The reaction was monitored by TLC and quenched with water upon disappearance of the starting carbonate. The reaction mixture was extracted with Et_2O (3 × 10 mL). The extract was washed with water and brine then dried (Na_2SO_4). Evaporation of solvent afforded the crude products, which were purified by column chromatography on silica [hexane/ethyl acetate (70:30)-(50:50)] to provide products. The remaining black Pd nanoparticles, after extraction with ether, were further washed with ether and dried for reuse.

General procedure for the preparation of the dehydro-β-amino esters **17a-d**. To a stirred solution of the carbonate **8-11** (0.2 mmol) in dry CH_3CN (2 mL), under nitrogen atmosphere, methyl 4-aminomethylbenzoate hydrochloride (1.5 equiv, 0.3 mmol, 61 mg) and triethylamine (1.5 equiv, 0.3 mmol, 42 μL) were added. The solution was stirred at reflux for 4 days 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. Compounds **17a-d** were isolated by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluent).

17a: Yield 45%, 30 mg, isolated as a 3:1 mixture of Z/E isomers; ^{1}H NMR ($CDCl_3$, 200 MHz) major isomer δ 1.29 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHN), 1.46 (s, 9H, OC(*CH*$_3$)$_3$), 1.64 (d, *J*=7.4 Hz, 3H, C*H*$_3$CHC), 2.45 (bs, 1H, N*H*), 3.54-3.79 (m, 3H, C*H*$_2$Ph, NC*H*CH$_3$), 3.86 (s, 3H, C*H*$_3$OCO), 6.77 (q, *J*=7.4 Hz, 1H, CC*H*CH$_3$), 7.36 (d, *J*=8.4 Hz, 2H, Ph), 7.94 (d, *J*=8.6 Hz, 2H, Ph); minor isomer δ 1.23 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHN), 1.48 (s, 9H, OC(*CH*$_3$)$_3$), 1.88 (d, *J*=7.4 Hz, 3H, C*H*$_3$CHC), 3.31 (q, *J*=6.6 Hz, 1H, NC*H*CH$_3$), 5.85 (q, *J*=7.4 Hz, 1H, CC*H*CH$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C major isomer 13.7 (CH_3), 20.6 (CH_2), 28.3 ($3CH_3$), 50.1 (CH_3), 51.1 (CH), 52.0 (CH_3), 80.6 (C), 128.0 (2CH), 128.7 (C), 129.7 (2CH), 136.0 (C), 137.7 (CH), 146.2 (C), 166.5 (C), 167.1 (C); LC-ESI-MS rt 11.04 min, m/z 333 (M), 356 (M+Na). Anal. cald. for $C_{19}H_{27}NO_4$(333.19): C 68.44, H 8.16, N 4.20; found C 68.33, H 8.17, N 4.20.

17b: Yield 70%, 51 mg, isolated as a 3:1 mixture of Z/E isomers**;** ^{1}H NMR ($CDCl_3$, 200 MHz) major isomer δ 0.74 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHCH$_3$), 1.12 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHCH$_3$), 1.50 (s, 9H, OC(*CH*$_3$)$_3$), 1.60 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHC), 1.79-2.08 (m, 1H, CH$_3$C*H*CH$_3$), 3.02 (d, *J*=9.8 Hz, 1H, CHC*H*N), 3.56 (d, *J*=13.8 Hz, 1H, NHC*H*$_2$Ph), 3.86 (d, *J*=13.8 Hz, 1H, NHC*H*$_2$Ph), 3.91 (s, 3H, C*H*$_3$OCO), 6.92 (q, *J*=7.0 Hz, 1H, CC*H*CH$_3$), 7.43 (d, *J*=8.0 Hz, 2H, Ph), 7.98 (d, *J*=8.0 Hz, 2H, Ph); minor isomer δ 0.83 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHCH$_3$), 1.00 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHCH$_3$), 1.93 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHC), 2.68 (d, *J*=8.4 Hz, 1H, CHC*H*N), 5.72 (q, *J*=7.0 Hz, 1H, CC*H*CH$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C major isomer 13.7 (CH_3), 20.0 ($2CH_3$), 27.9 ($3CH_3$), 31.7 (CH), 50.1 (CH_2), 51.6 (CH_3), 61.3 (CH), 80.0 (C), 127.7 (2CH), 128.3 (C), 129.2 (2CH), 133.9 (C), 138.5 (CH), 146.5 (C), 166.5 (C), 166.7 (C); LC-ESI-MS rt 13.87 min, m/z 361 (M), 362 (M+1); Anal. cald. for $C_{21}H_{31}NO_4$(361.23): C 69.78, H 8.64, N 3.87; found C 69.75, H 8.62, N 3.88.

17c: Yield 65%, 52 mg, isolated as a 3:1 mixture of Z/E isomers**;** ^{1}H NMR ($CDCl_3$, 200 MHz) major isomer δ 1.13-1.36 (m, 4H, *cyclohexyl*), 1.50 (s, 9H, OC(*CH*$_3$)$_3$), 1.47-1.81 (m, 6H, *cyclohexyl*), 1.59 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 2.40 (m, 1H, C*H* cyclohexyl), 3.13 (d, *J*=9.4 Hz, 1H, CHC*H*N), 3.57 (d, *J*=13.8 Hz, 1H, NHC*H*$_2$Ph), 3,81 (d, *J*=13.8 Hz, 1H, NHC*H*$_2$Ph), 3.91 (s, 3H, C*H*$_3$OCO), 6.92 (m, *J*=7.2 Hz, 1H, CC*H*CH$_3$), 7.43 (d, *J*=8.2 Hz, 2H, *Ph*), 7.98 (d, *J*=8.2 Hz, 2H, *Ph*); minor isomer δ 1.92 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHC), 2.72 (d, *J*=8.8 Hz, 1H, CHC*H*N), 5.68 (q, *J*=7.0 Hz, 1H, CC*H*CH$_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C major isomer 13.9 (CH_3), 26.2 (CH_2), 26.3 (CH_2), 26.6 (CH_2), 28.2 ($3CH_3$), 30.6 (CH_2), 31.6 (CH_2), 41.4 (CH), 50.6 (CH_2), 51.8 (CH_3), 60.0 (CH), 80.3 (C), 127.9 (2CH) , 128.4 (2CH), 129.4 (CH), 133.6 (C), 138.8 (C), 146.7 (C), 166.8 (C), 167.1 (C); LC-ESI-MS rt 5.31 min, m/z 401 (M), 424 (M+Na); Anal. cald. for $C_{24}H_{35}NO_4$(401.26): C 71.79, H 8.79, N 3.49; found C 71.83, H 8.78, N 3.48.

17d: Yield 35%, 28 mg, isolated as a 3:1 mixture of Z/E isomers**;** ^{1}H NMR ($CDCl_3$, 200 MHz) major isomer δ 1.34 (s, 9H, OC(*CH*$_3$)$_3$), 1.75 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 2.56 (bs, 1H, N*H*), 3.77 (d, *J*=13.8 Hz, 1H, NHC*H*$_2$Ph), 3.90 (s, 3H, C*H*$_3$OCO), 3.94 (d,

J=13.8 Hz, 1H, NHC*H*$_2$Ph), 4.75 (s, 1H, C*H*NH), 6.95-7.05 (m, 2H, CC*H*CH$_3$, C*H thiophenyl*), 7.19-7.27 (m, 2H, *thiophenyl*), 7.48 (d, *J*=8.4 Hz, 2H, *Ph*), 8.00 (d, *J*=8.4 Hz, 2H, *Ph*); minor isomer δ 1.99 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHC), 6.04 (q, *J*=7.0 Hz, 1H, CC*H*CH$_3$); ^{13}C NMR (CDCl$_3$, 75 MHz) δ_C major isomer 13.9 (CH$_3$), 28.1 (3CH$_3$), 50.6(CH$_3$), 52.1 (CH$_2$), 54.4 (CH), 80.8 (C), 120.3 (CH), 125.2 (CH), 126.8 (CH), 128.0 (2CH), 128.9 (C), 129.7 (2CH), 134.4 (C), 139.0 (CH), 144.2 (C), 146.1 (C), 166.4 (C), 167.1 (C). LC-ESI-MS rt 12.67 min, m/z 401 (M), 424 (M+Na); Anal. cald. for $C_{22}H_{27}NO_4S$(401.17): C 65.81, H 6.78, N 3.49, S 7.99; found C 65.77, H 6.80, N 3.50, S 7.98.

Procedure for the preparation of the acid **18**. To a stirred solution of the carbonate **9** (0.2 mmol) in CH_2Cl_2 (2 mL) at 0°C, trifluoroacetic acid (15 equiv, 3 mmol, 223 μL) was added. After 2h, the mixture was washed twice with acidic water (5 mL), the organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Compound **18** was isolated in 95% yield (41 mg).

18: ^{1}H NMR (CDCl$_3$, 200 MHz) δ 0.97 (d, *J*=5.4 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.40 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHO), 3.25-3.42 (m, 1H, CH$_3$C*H*CH$_3$), 3.70 (s, 3H, C*H*$_3$OCO), 5.46 (q, *J*=6.6 Hz, 1H, CH$_3$C*H*O), 6.11 (d, *J*=10.0 Hz, 1H, CHC*H*C), 10.41 (bs, 1H, OCO*H*); ^{13}C NMR (CDCl$_3$, 200 MHz) δ_C 20.1 (CH$_3$), 21.9 (2CH$_3$), 31.8 (CH), 54.4 (CH$_3$), 73.3 (CH), 128.9 (C), 142.5 (CH), 154.7 (C), 171.4 (C); LC-ESI-MS rt 6.65 min, m/z 216 (M), 239 (M+Na); Anal. cald. for $C_{10}H_{16}O_5$(216.1): C 55.55, H 7.46; found C 55.59, H 7.49.

Procedure for the preparation of the amide **19**. To a stirred solution of the acid **18** (0.2 mmol) in dry CH_2Cl_2 (2 mL), under nitrogen atmosphere, EDCI (1.2 equiv, 0,24 mmol, 46 mg) and triethylamine (2.4 equiv, 0.48 mmol, 67 μL) 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 diluited with CH_2Cl_2 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. Compounds **19** was

isolated in 65% yield (43 mg) after flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20 as eluent).

19: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.01 (d, *J*=6.6 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.45 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHO), 1.49 (s, 9H, OC(*CH*$_3$)$_3$), 2.70-2.88 (m, 1H, CH_3CHCH_3), 3.79 (s, 3H, C*H*$_3$OCO), 4.00 (d, *J*=4.8 Hz , 2H, NHC*H*$_2$), 5.27 (q, *J*=6.6 Hz, 1H, CH_3CHO), 5.67 (d, *J*=10.2 Hz, 1H, CHC*H*C), 6.57 (bt, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 19.9 (CH_3), 22.7 ($2CH_3$), 26.8 (CH), 28.0 ($3CH_3$), 41.9 (CH_2), 54.8 (CH_3), 76.0 (CH), 82.2 (C), 131.8 (C), 137.3 (CH), 155.1 (C), 167.3 (C), 168.8 (C); LC-ESI-MS rt 10.82 min, m/z 329 (M), 352 (M+Na); Anal. cald. for $C_{16}H_{27}NO_6$(329.18): C 58.34, H 8.26, N 4.25; found C 58.31, H 8.24, N 4.25.

Procedure for the preparation of the N-BOC-protected amide **20**. To a stirred solution of the amide **19** (0.2 mmol) in dry THF (1 mL), DMAP (0.2 equiv, 0.04 mmol, 5mg), triethylamine (1.2 equiv, 0.24 mmol, 34 μL) and $(BOC)_2O$ (1.3 equiv, 0.26 mmol, 60 μL) 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. Compounds **20** was isolated in 90% yield (77 mg) by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluent).

20: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.99 (d, *J*=6.6 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.42 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHO), 1.48 (s, 18H, OC(*CH*$_3$)$_3$), 2.41-2.49 (m, 1H, CH_3CHCH_3), 3.75 (s, 3H, C*H*$_3$OCO), 4.28 (d, *J*=16.8 Hz , 1H, NC*H*$_2$), 4.45 (d, *J*=16.8 Hz , 1H, NC*H*$_2$), 5.42-5.53 (m, 2H, CH_3CHO, CHC*H*C); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C19.2 (CH_3), 22.6 ($2CH_3$), 27.2 (CH), 27.8 ($3CH_3$), 28.0 ($3CH_3$), 45.9 (CH_2), 54.5 (CH_3), 74.7 (CH), 81.8 (C), 83.8 (C), 126.7 (C), 137.6 (CH), 151.4 (C), 155.1 (C), 167.6 (C), 169.8 (C); LC-ESI-MS rt 12.26 min, m/z 429 (M), 452 (M+Na); Anal. cald. for $C_{21}H_{35}NO_8$(429.24): C 58.72, H 8.21, N 3.26; found C 58.61, H 8.19, N 3.27.

Procedure for the preparation of the amino derivative **21**. To a stirred solution of the amide **20** (0.2 mmol) in dry CH_3CN (2 mL), under nitrogen atmosphere, 4-aminomethylaniline (1.5 equiv, 0.3 mmol, 34 μL) was added. The solution was refluxed for 16h and then the solvent was removed under reduced pressure. Compound **21** was isolated in 70% yield (66 mg) by Flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5 as eluent).

21: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.83 (d, *J*=6.6 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.42 (d, *J*=7.4 Hz, 3H, C*H*$_3$CHC), 1.47 (s, 18H, OC(*CH*$_3$)$_3$), 2.60-2.81 (m, 1H, CH_3CHCH_3), 3.59 (bs, 2H, N*H*$_2$), 3.83-3.98 (m, 3H, NC*H*$_2$, C*H*NH), 4.34-4.61 (m, 2H, C*H*$_2$Ph), 6.61 (d, *J*=8.0 Hz, 2H, *Ph*), 6.83 (q, *J*=7.4 Hz, 1H, CC*H*CH$_3$), 7.01 (d, *J*=8.0 Hz, 2H, *Ph*); ^{13}C NMR ($CDCl_3$, 200 MHz) δ_C 13.5 (CH_3), 20.2 ($2CH_3$), 28.1 ($3CH_3$), 28.5 ($3CH_3$), 31.8 (CH), 42.2 (CH_2), 47.3 (CH), 50.1 (CH_2), 79.8 (C), 82.1 (C), 115.0 (2CH), 127.9 (2CH), 130.7 (C), 131.3 (C), 139.6 (CH), 144.9 (C), 156.0 (C), 169.1 (C), 169.9 (C); LC-ESI-MS rt 9.15 min, m/z 475 (M), 498 (M+Na); Anal. cald. for $C_{26}H_{41}N_3O_5$(475.3): C 65.66, H 8.69, N 8.83; found C 65.55, H 8.71, N 8.80.

Procedure for the preparation of the amino acid **22**. To a stirred solution of compound **21** (0.2 mmol), in CH_2Cl_2 (0.5mL), H_3PO_4 85% (5 equiv, 1 mmol, 69 μL) was added. After 3h the solvent was removed under reduced pressure and the crude compound was treated with Dowex 50WX2-200 ion exchange resin, eluting with NH_4OH 0.5 M. Compound **22** was isolated after emoval of the aqueous solvent in 95% yield (61 mg).

22: ^{1}H NMR (CD_3OD, 200 MHz) δ 0,93 (d, *J*=6.6 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.82 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 2.18-2.28 (m, 1H, CH_3CHCH_3), 3.83-4.38 (m, 5H, C*H*NH, NHC*H*$_2$, C*H*$_2$Ph), 6.74 (d, *J*=8.4 Hz, 2H, *Ph*), 6.90 (q, *J*=7.2 Hz, 1H, CC*H*CH$_3$), 7.21 (d, *J*=8.4 Hz, 2H, *Ph*); minor isomer δ 2.06 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 6.06 (q, *J*=7.2 Hz, 1H, CC*H*CH$_3$); ^{13}C NMR (CD_3OD, 200 MHz) δ_C major isomer 14.3 (CH_3), 19.9 ($2CH_3$), 31.9 (CH), 46.9 (CH_2), 50.9 (CH_2), 62.4 (CH), 116.3 (2CH), 121.1 (C), 128.8 (CH), 132.0 (2CH), 140.7 (C), 148.5 (C), 161.6 (C), 178.2 (C); LC-ESI-MS rt 1.06 min, m/z 319 (M), 342 (M+Na); Anal. cald. for $C_{17}H_{25}N_3O_3$(319.19): C 63.93, H 7.89, N 13.16; found C 63.99, H 7.90, N 13.11.

II

1,4- addition of nitrogen nucleophiles to unsaturated carbonyl compounds. Synthesis of substituted isoxazolidines and isoxazolines

2.1 Introduction

The conjugate addition of nitrogen containing nucleophiles to unsaturated carbonyl compounds represents one of the most employed and versatile method for C-N bond construction in organic chemistry.[32] The reaction may be performed under catalytic conditions and the use of chiral Lewis acids[33] or, more recently, of organocatalysts[34] has been described in literature.

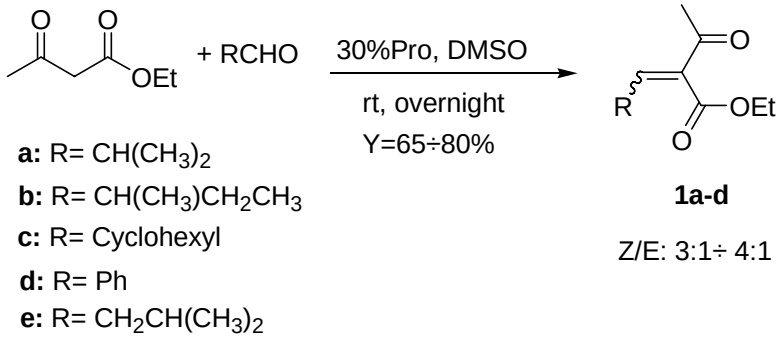
Alkylidene malonates have been intensively employed as Michael acceptors.[35] In particular, a chiral Lewis acid-catalysed Michael addition of hydroxylamino derivatives to alkylidene malonates has been reported by our group.[36] Herein, we describe the highly stereo-controlled synthesis of ethyl 5-hydroxyisoxazolidine-4-carboxylate through a Lewis acid-induced Michael addition of hydroxylamino derivatives to alkylidene acetoacetates, followed by intra-molecular hemiketal formation. The use of acetoacetates in this field is rather unusual and has the advantage of introducing a reactive keto-functionality that may be further elaborated.

Furthermore, we report a new approach to this class of compounds, using *N*-benzyl-(*tert*-butyldimethylsilyloxy)-carbamate[37] as nucleophile. This procedure directly furnished *N*-Cbz-protected-5-hydroxyisoxazolidines that can be easily transformed into the corresponding dehydrated isoxazolines, which are useful precursors of aromatic oxazoles.

Substituted isoxazolidines, isoxazolines and isoxazoles are important substrates for mechanistic studies of biologically interesting processes. For example, isoxazolidines are interesting heterocyclic compounds that may be regarded as unusual constrained β-amino acids[38] or as furanose mimetics,[39] and have been also exploited as analogues of natural products[40]. Isoxazolines have been incorporated as conformational constraint element in $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin antagonists,[41] as well as in several transcriptional activators.[42]

2.2 Preparation of alkylidene acetoacetates via Knoevenagel reaction

Alkylidene acetoacetates have been prepared through a Knøvenagel reaction between ethyl acetoacetate and various aldehydes in the presence of a catalytic amount of proline (Scheme 2. 1).[43] Proline was chosen among other bases since it was demonstrated to afford the best results in terms of yields and Z/E product selectivity.

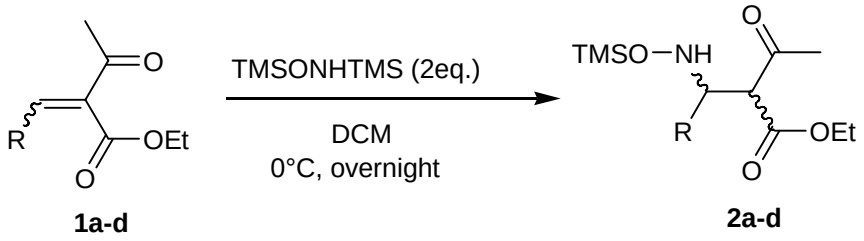


Scheme 2. 1

The stereochemical assignment for compounds **1a-d** was made 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 CH_2 group.

2.3 Michael addition of hydroxylamino derivative to alkylidene acetoacetates

The conjugate addition of bis-(*N,O*)-trimethylsilyl hydroxylamine (TMSONHTMS) to alkylidene acetoacetates was first examined in the absence of catalysts (Scheme 2. 2). Reaction conditions were optimized in order to induce the regioselective formation of 1,4-addition products versus oximes, deriving from the 1,2-addition process.



Scheme 2. 2

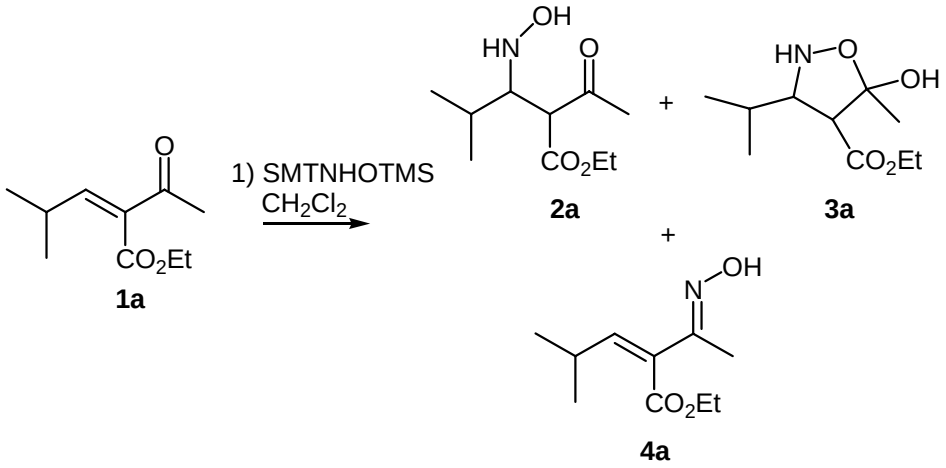
The reactions were completed in 16 hours, and the *syn* and *anti* 1,4-adducts **2a-d** were obtained in quantitative yields in 60/40 ratio, respectively, as quoted by the ^{1}H NMR of the crude reaction.

The loss of the trimethylsilyl group on nitrogen occurred during reaction work-up. In order to characterize the intermediates a very fast chromatography on silica gel allowed **2a-d** to be obtained as inseparable mixtures of *syn/anti* isomers. The stereochemical assignment was made by comparing the ^{1}H-NMR coupling constants of compounds **2a-d** with data available in literature[44] (J_{syn}=3.6Hz, J_{anti}=7.2Hz).

Notably, the reaction carried out on the pure Z or E isomer of **1a-d** gave always **2a-d** in the same *syn*/*anti* ratio.

2.3.1 Effect of the catalyst on the coniugate addition

We studied the influence exerted by the presence of different catalysts on the regioselectivity of the reaction[45]. In fact substrate **1** may be subjected either to 1,4- or 1,2-nucleophilic attack depending on the reaction conditions, thus affording the 1,4-addition product **2**, the 5-hydroxyisoxazolidine-4-carboxylate **3** or the oxime **4** respectively (Scheme 2. 3).



Scheme 2. 3

We initiated our studies by examining the addition of TMSNHOTMS to *Z*-**1a** and the ability of the Lewis acid to direct the regioselectivity (Table 9).

Table 9

	Time(h)	Catalyst[a]	Conversion (%)[b]	Yield (%)[c]		
				2	**3**	**4**
1	16	-	85	85	-	-
2	0.5	$Yb(OTf)_3$	100		100	
3	2	$BF_3{\cdot}Et_2O$	100	-	36	64
4	2	$Cu(OTf)_2$	65	-	10	55
5	3	$Cu(OTf)_2$	95	-	10	85
6	4	$Sc(OTf)_3$	100	-	10	90
7	5	$Mg(OTf)_2$	-	-	-	-

a) A 5% amount of catalyst was used in all reactions. b) The rest being unreacted starting material. c) Calculated on the basis of ^{1}H NMR signals integration

Therefore, **1a** was treated in CH_2Cl_2 in absence of the catalyst with 2 equiv. of TMSNHOTMS. Under these conditions, the adduct **2a** was obtained in 60:40 *syn*/*anti* ratio and in 85% yields (entry 1).

After that, we screened a variety of Lewis acids as catalysts and to our surprise, the expected 1,4 addition product was not observed. We found that the reaction was accelerated significantly in the presence of $Yb(OTf)_3$; in fact, addition of 5% of catalyst allowed the compound 3,4-*trans*-**3a** to be obtained in 78/ 22 anomeric ratio, in one step and in a shorter reaction time (30 min, entry 2). By using $BF_3{\cdot}EtO$ as catalyst, a mixture of **3a**, and oxime **4a** in 36/64 ratio was obtained, as detected from the ^{1}H NMR spectrum of the crude mixture (entry 3). When the reaction was carried out in the presence of $Cu(OTf)_2$, or $Sc(OTf)_3$, complete regioselectivity was observed and the *N*-protected oxime **4a** was obtained in high yield, being scandium catalyst the most selective (entries 5-6).

The unpredictability of the amount of oxime **4a** in repeated proofs, prompted us to investigate the factors that control the regioselectivity of the nucleophilic addition. Therefore a deeper inspection of the reaction conditions showed that the yields of **3a** or **4a** are strongly influenced by the presence of traces of water. In fact, when the reaction was carried out in presence of the catalyst and molecular sieves, **3a** was exclusively obtained. The experimental data show that the oxime formation is favored by the presence of water in the solution or present in the metal coordination sphere.The rate-limiting step of the oxime formation at neutral pH is the dehydration of the carbinolamine addition compound.[46] At high reactant concentration with most of the

carbonyl compound in the form of addition derivative, the rate of the oxime formation is dependent on the dehydration, that in our case seems to be favored by the presence of traces of water.

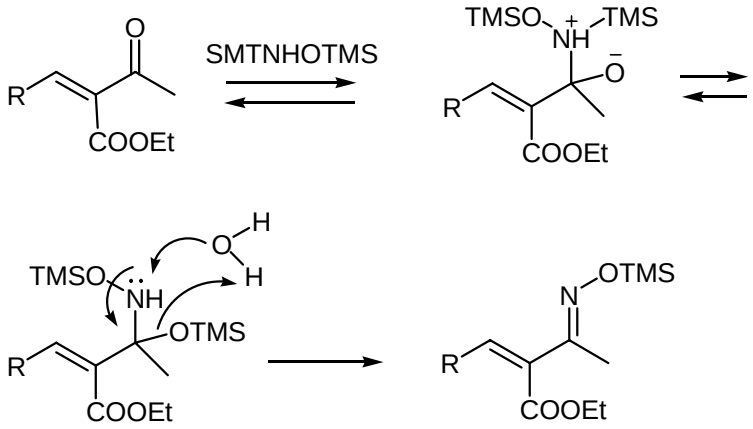
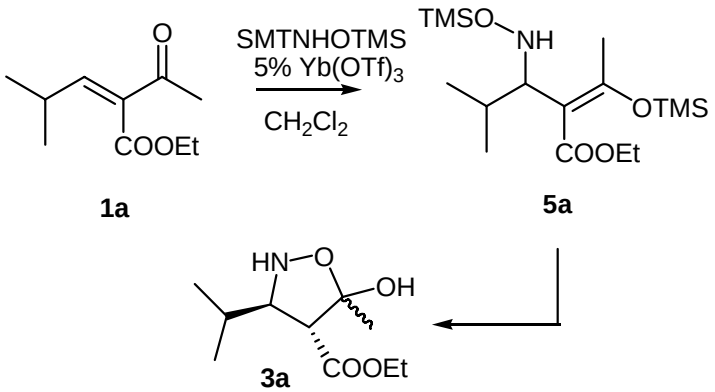


Figure 2. 1

In Figure 2. 1 a plausible mechanism is outlined, accounting for the observed results. The H_2O acting as general acid allows the TMSOH elimination to occur, shifting the equilibrium towards the oxime, that is more stable and less prone to hydrolysis in respect to the corresponding imine.

To demonstrate the generality of this method and to apply this protocol to other substrates, we investigated in detail the reaction results under optimized conditions[47].

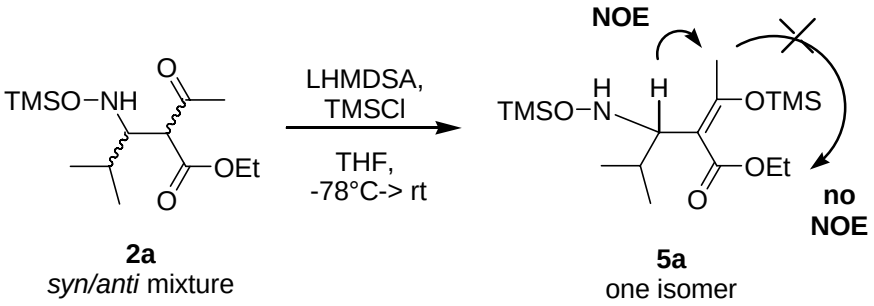


Scheme 2. 4

In the presence of 5% of ytterbium catalyst, compound **1a** was stirred in dry CH_2Cl_2 with the nucleophilic reagent at 0°C. After completion of the reaction, as indicated by TLC, the solvent was evaporated and the ^1H NMR spectrum of the crude mixture

showed the presence of the silyl enolether **5a**, that slowly converted at room temperature into 5- hydroxyisoxazolidine-carboxylate **3a** (Scheme 2. 4).

The structure of compounds **5a** has been proposed on the basis of is ^{1}H-NMR and ^{13}C-NMR spectra. A further evidence of the nature of **5a** was obtained by treating the 1,4-adduct **2a** with LHMDSA and TMSCl (Scheme 2. 5).

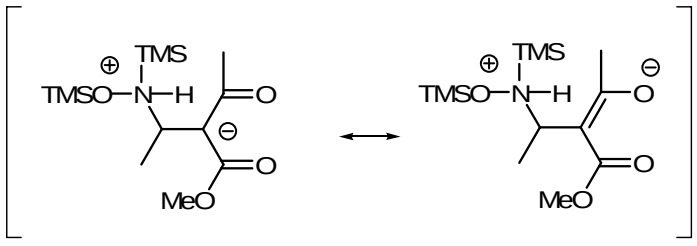


2a
syn/anti mixture

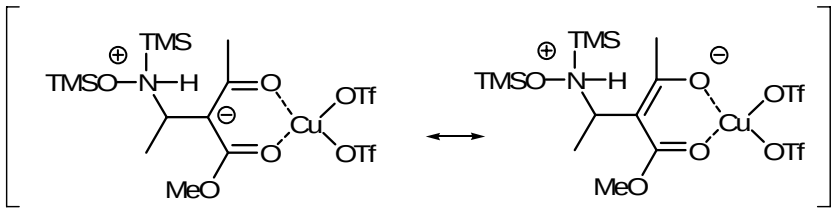
5a
one isomer

Scheme 2. 5

An explanation for the different outcomes of the uncatalysed (adducts **2a-d** in Scheme 2. 2) and catalysed (silyl enolethers **5a-d** in Scheme 2. 5) conjugate addition may be issued from the results of DFT theoretical computations on the plausible intermediates of the 1,4 addition. The conjugate addition of TMSONHTMS to the methyl ester of isopropylidene acetoacetate gave results in complete agreement with those obtained for ethyl esters.This allowed to semplify the model-system as reported in Scheme 2. 6.



IntF keto form IntF enol form



IntC keto form IntC enol form

Scheme 2. 6

It is reasonable to believe that, after the C-N bond formation, the resulting species has a zwitterionic nature, with a positive charge on the nitrogen atom and a negative charge delocalised on the enolate moiety.[48] The optimum structures of this intermediate, either as a free species (**IntF**) or forming a complex with the Lewis acid (**IntC**), have been computed (Scheme 2. 6).A schematic representation of the two possible resonance structures (ketonic and enolic) for these two intermediate species is given in Scheme 2. 6. A comparison between the C-C and the C-O enolate bond lengths in these molecules shows that in the case of the free intermediate **IntF** the keto form is dominant, while for the intermediate complex **IntC** the enol form predominates (Figure 2. 2).

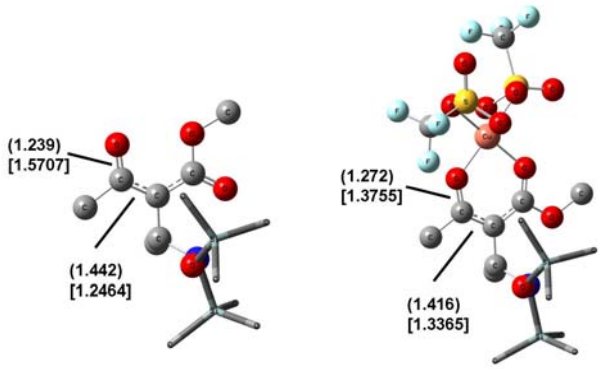
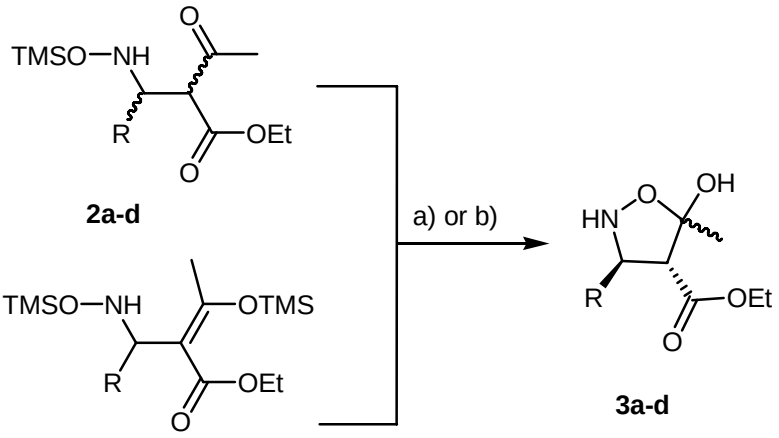


Figure 2. 2

This finding suggests that the intermediate **IntF** is likely to end up with a proton transfer to the carbon in α position, thus giving products **2a-d**. On the other hand it is reasonable to believe that intermediate **IntC**, because of its structural features, may transfer a TMS group from the nitrogen to the enol oxygen, affording products **5a-d**.

2.3.2 Intramolecular hemiketalisation

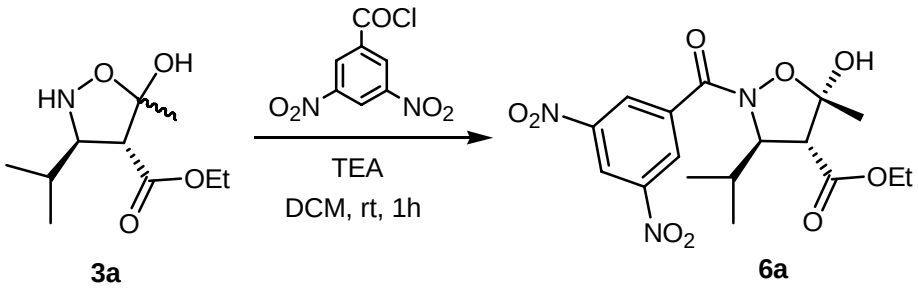
Adducts **2a-d** and silyl enolethers **5a-d** at room temperature spontaneously converted to ethyl 5-hydroxyisoxazolidine-4-carboxylates **3a-d**. In both cases the intramolecular hemiketalisation required, on the average, 24 hours to be completed. Remarkably, both classes of compounds **2a-d** and **5a-d** gave, with quantitative conversion, mixtures ranging from 80/20 to 75/25 ratio of the two stereoisomers of (3,4)-*trans* hydroxyisoxazolidines **3a-d**, differing for the stereochemistry at the C5 anomeric center (Scheme 2. 7).



a) 24h, rt; b) SiO_2, DCM, 2h;

Scheme 2. 7

The intra-molecular hemiketalisation can be accelerated by addition of silica gel to the crude mixture or using other acidity source that allows ready desilylation of the reagent (for instance, treatment with acidic water solution or acidic resins). The ethyl 5-hydroxyisoxazolidine-4-carboxylates **3a-d** were fully characterised by NMR spectrometry (DEPT, HETCOR, COSY and NOE) and thus their structures have been confirmed.(Scheme 2. 8)

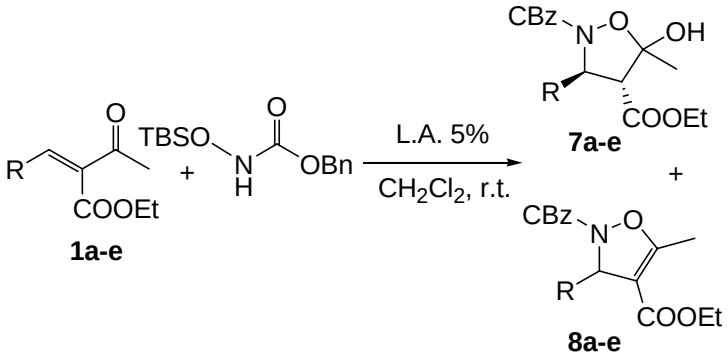


Scheme 2. 8

Once again, the derivatisation gave an unexpected result: starting from a 70/30 diastereomeric mixture of **3a**, only one (3,4)-trans isomer ($J_{3\text{-}4}$= 7.2Hz) of **6a** was obtained, probably via the isomerisation of the hemiacetalic position.

2.4 Michael addition of N-benzyl-(tert-butyldimethylsilyloxy)-carbamate to alkylidene acetoacetates

Alkylidene acetoacetates **1a-e** were treated with benzyl-(*tert*-butyldimethylsilyloxy)-carbamate in the presence of various Lewis acids in CH_2Cl_2 solvent, to afford isoxazolidine hemiketals **7** and the corresponding dehydrated isoxazoline **8** in ratios that depend on the reaction conditions[49] (Table 10). It should be noted that the TBS protecting group was removed during the usual work up procedure.



Scheme 2. 9

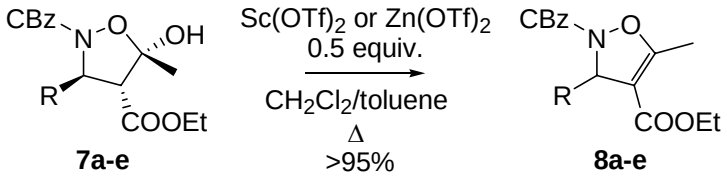
Table 10

Entry[a]	**1** S.M.	L.A. (5%)	T (°C)	t (h)	Conversion[b] **7**+**8** (%)	*trans*-**7**/**8**
1	**1a**	$Zn(OTf)_2$	0	40	/	/
2	**1a**	$Zn(OTf)_2$	40	16	23	>99:1
3	**1a**	$Cu(OTf)_2$	0	40	/	60:40
4	**1a**	$Cu(OTf)_2$	r.t.	20	>95	58:42
5	**1a**	$Cu(OTf)_2$	40	5	>95	50:50
6	**1a**	$Yb(OTf)_3$	0	40	/	/
7	**1a**	$Yb(OTf)_3$	r.t.	20	44	>99:1
8	**1a**	$Yb(OTf)_3$	40	5	>95	63:37
9	**1a**	$Sc(OTf)_3$	10	5	70	80:20
10	**1a**	$Sc(OTf)_3$	r.t	5	>95	60:40
11	**1a**	$Sc(OTf)_3$	40	5	>95	30:70
12	**1e**	$Sc(OTf)_3$	r.t.	5	94	62:38
13	**1b**	$Sc(OTf)_3$	r.t.	5	95	60:40
14	**1c**	$Sc(OTf)_3$	r.t.	5	93	60:40
15	**1d**	$Sc(OTf)_3$	r.t.	5	95	58:42

a) The reaction was performed in the presence of 1 equivalent of nucleophile in CH_2Cl_2. A 5% amount of catalyst was used in all reactions. b) The rest being unreacted starting material. The conversion was established on the basis of ^1H NMR peak integration.

From Table 10 it can be appreciated that the reaction with *Z*-**1a** $Zn(OTf)_2$ as catalyst, at 0°C or at room temperature (entries 1-2) proceeded with unsatisfactory yields. Similarly, when the reaction was run in the presence of $Cu(OTf)_2$ at 0 °C, the starting material was recovered even after long reaction times (entry 3). On the other hand, the reaction proceeded very slowly at room temperature, so it was gently warmed to 40 °C, affording the complete conversion of the starting material to a mixture of *trans*-**7a** ($J_{3,4}$ = 6.6 Hz) and **8a** (entries 4-5). The cyclization to **7a,** led to the formation of a single epimer. Compounds **7a** and **8a** were easily isolated by flash chromatography on silica gel, eluting with cyclohexane/ethyl acetate 80/20 as eluent. Similar results were observed using $Yb(OTf)_3$ as catalyst (entries 6-8). Scandium triflate showed an excellent efficiency for the activation of the desired reaction. Indeed, at ambient temperature and in the presence of $Sc(OTf)_3$, complete conversion of the starting material was observed in 5 h. A single epimer of the *trans* isoxazolidine **7a** was obtained, accompanied by the corresponding product of dehydration **8a** in 60/40 ratio (entry 10). Warming the reaction to 40°C, afforded an increased amount of **8a** (entry 11). On the other hand, in a unique experiment carried out at room temperature, a 46% conversion of *Z*-**1a** into *trans*-**7a** could be observed after 1.5 h, while only traces of dehydrated **8a** were detected in the crude ^{1}H NMR. After 3 hours of reaction, the conversion reached 90%, and *trans*-**7a** was present together with the corresponding dehydrated product **8a,** in 60:40 ratio.

The experimental conditions selected for **1a** were then explored with substrates **1b-e**. Thus, treatment of **1b-e** with carbamate at room temperature overnight in the presence of scandium catalyst, afforded essentially quantitative conversion of the starting materials to 60/40 mixtures of **7b-e** and **8b-e** (entries 12-15).

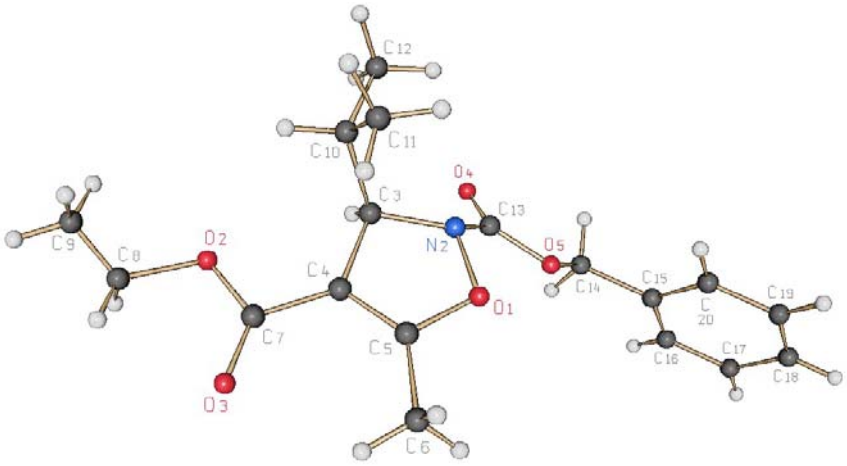


7a-e **8a-e**

Scheme 2. 10

Complete conversion of **7a-e** to **8a-e** was achieved upon addition of an additional half equivalent of $Sc(OTf)_2$ or $Zn(OTf)_2$ in toluene to the reaction mixture, while simultaneously heating to reflux for 3 h. (Scheme 2. 10).

The structures of compounds **7a** and **8a** were established by NMR techniques (DEPT, HETCOR and COSY). The formation of a single epimer of isoxazolidine **7a** was supported by the presence of a single set of signals in the ^1H NMR spectrum. The relative stereochemistry of **7a** was established by means of NOE experiments, that exhibited a strong enhancement (8%) of the isopropylic side chain upon irradiation of the C(4) hydrogen. By contrast, a weaker NOE effect (2%) was observed on the *trans* hydrogen at C(3). Furthermore, a medium NOE effect on the anomeric methyl substituent indicated a *cis* relationship between this group and the hydrogen at C(4). X-Ray analysis of **8a** secured the configuration assigned to this heterocycle (Figure 2. 3).[50]

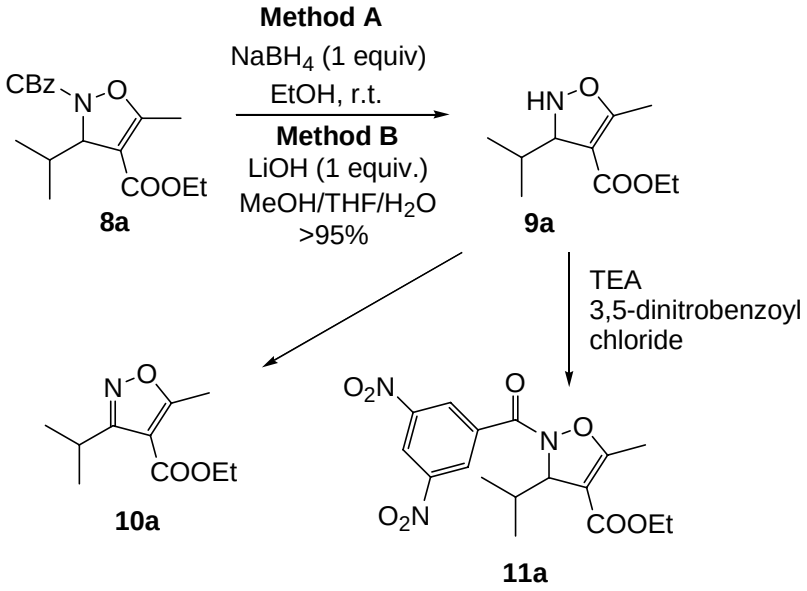


8a

Figure 2. 3

2.4.1 Reactivity of isoxazoline-4-carboxylates. Synthesis of oxazoles

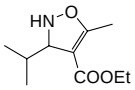
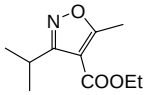
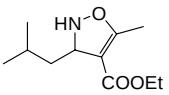
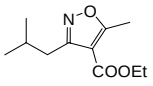
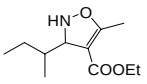
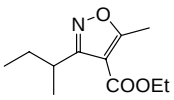
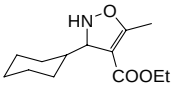
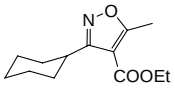
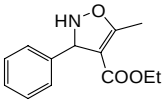
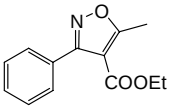
In order to remove the CBz protecting group, **8a** was stirred at room temperature with an equimolar amount of $NaBH_4$ in ethanol under argon (method A) or LiOH in $MeOH/H_2O/THF$ (method B), until the reaction was found to be complete by TLC.(Scheme 2. 11)



Scheme 2. 11

Following these protocols, isoxazoline **9a** was isolated in almost quantitative yield. Isoxazoline **9a** was characterized by spectroscopic analysis as well. This compound was unstable and spontaneously converted to the corresponding aromatic oxazole **10a**, even under inert atmosphere.[51] Therefore, as soon as formed **9a** was treated with the 3,5-dinitrobenzoyl chloride to afford derivative **11a** in quantitative yield.

Table 11

Entry	S.M	Product **9**	Yield(%)	Product **10**	Yield(%)
1	**8a**		>95		>95
2	**8b**		>95		>95
3	**8c**		>95		>95
4	**8d**		>95		>95
5	**8e**		>95		>95

Similar deprotection was carried out on **8b-e,** affording **9b-e** in quantitative yield. Rapid oxidation of isoxazolines **9b-e** took place upon exposure to air, affording **10b-e** in quantitative yield (Table 11). Compound **9c** resulted particularly unstable, being detected only in the ^{1}H NMR spectra of the crude product.

2.5 Experimental Section

All chemicals were purchased from commercial suppliers and used without further purification. Anhydrous solvents were purchased in sure seal bottles over molecular sieves and used without further drying. Flash chromatography was performed on silica gel (230-400 mesh). NMR Spectra were recorded with 200, 300 or 600 MHz spectrometers. Chemical shifts were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at δ = 7.27 (^{1}H NMR) or δ = 77.0 (^{13}C NMR).

For the preparation of alkylidene acetoacetates and the characterization of **1a** and **1c**, see paragraph 1.6.

(*Z*)-1b: Yield 66%, colorless clear oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.75 (t, *J* = 6.9 Hz, 3H, *CH*$_3$CH$_2$CH), 0.97 (d, *J* = 6.6 Hz, 3H, CH*CH*$_3$), 1.22 (t, *J* = 7.2 Hz, 3H, *CH*$_3$CH$_2$O), 1.34 (m, 2H, CH$_3$*CH*$_2$CH), 2.21 (s, 3H, CO*CH*$_3$), 2.35 (m, 1H, CH$_3$CH$_2$*CH*), 4.20 (q, *J* = 7.2 Hz, 2H, CH$_3$*CH*$_2$O), 6.50 (d, *J* = 10.8 Hz, 1H, CH=). ^{13}C NMR ($CDCl_3$, 300 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). **(*E*)-1b**: ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.74 (t, *J* = 6.9 Hz, 3H, *CH*$_3$CH$_2$CH), 0.93 (d, *J* = 6.9 Hz, 3H, CH*CH*$_3$), 1.20 (t, *J* = 7.2 Hz, 3H, *CH*$_3$CH$_2$O), 1.34 (m, 2H, CH$_3$*CH*$_2$CH), 2.25 (s, 3H, CO*CH*$_3$), 2.35 (m, 1H, CH$_3$CH$_2$*CH*), 4.16 (q, *J* = 7.2 Hz, 2H, CH$_3$*CH*$_2$O), 6.56 (d, *J* = 10.8 Hz, 1H, CH=). ^{13}C NMR ($CDCl_3$, 300 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.

(*Z*)-1d: Yield 72%, yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.28 (t, *J* = 7.2 Hz, 3H, *CH*$_3$CH$_2$O), 2.43 (s, 3H, CO*CH*$_3$), 4.34 (q, *J* = 7.2 Hz, 2H, CH$_3$*CH*$_2$O), 7.28-7.48 (m, 5H, Ph), 7.58 (s, 1H, CH=). ^{13}C NMR ($CDCl_3$, 300 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). **(*E*)-1d**: ^{1}H NMR ($CDCl_3$, 200 MHz) δ 1.34 (t, *J* = 7.2 Hz, 3H, *CH*$_3$CH$_2$O), 2.36 (s, 3H, CO*CH*$_3$), 4.30 (q, *J* = 7.2 Hz, 2H, CH$_3$*CH*$_2$O), 7.34-7.39 (m, 5H, Ph), 7.68 (s, 1H, CH=). ^{13}C NMR ($CDCl_3$, 300 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.

General procedure for the 1,4 addition of bis(N,O)-trimethylsilylhydroxylamine to alkylidene acetoacetate **1.** To a stirred solution of **1a-d** (0.5 mmol) in dry CH_2Cl_2 (2.5 mL) at 0°C under nitrogen atmosphere, bis(N,O)-trimethylsilylhydroxylamine (2 equiv, 1 mmol) was added in one portion. The reaction was followed by TLC and quenched after 16 h with water. The residue was then diluted with CH_2Cl_2 (10 mL) and washed twice with water (2 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **2a-d** were purified by flash chromatography on silica gel (eluant cyclohex-ane/EtOAc, 9/1).

(*syn*)-2a: Yield 77%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.08 (s, 9H, OSi(C*H*$_3$)$_3$), 0.95 (d, *J*=6.9 Hz, 3H, CHC*H*$_3$), 1.03 (d, *J*=6.9 Hz, 3H, CHC*H*$_3$), 1.28 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.68 (m, 1H, CH$_3$C*H*CH$_3$), 2.25 (s, 3H, OCC*H*$_3$), 3.26 (m, 1H, NC*H*), 3.45 (d, *J*=3.6 Hz, 1H, CHCO), 4.17 (m, 2H, O*CH*$_2$CH$_3$), 6.22 (d, *J*= 11.4, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -1.1 (3CH$_3$), 14.0 (CH$_3$), 20.5 (2CH$_3$), 29.4 (CH), 30.1(CH$_3$), 59.7 (CH), 60.9 (CH$_2$), 67.5 (CH), 170.1 (C), 202.9 (C).
(*anti*)-2a : ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.11 (s, 9H, OSi(C*H*$_3$)$_3$), 0.93 (m, 6H, CHC*H*$_3$), 1.26 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.94 (m, 1H, CH$_3$C*H*CH$_3$), 2.29 (s, 3H, OCC*H*$_3$), 3.37 (m, 1H, NC*H*), 3.71 (d, *J*=7.2 Hz, 1H, CHCO), 4.17 (m, 2H, O*CH*$_2$CH$_3$), 5.55 (d, *J*= 9.6, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.6 (3CH$_3$), 14.0 (CH$_3$), 18.5 (CH$_3$), 21.6 (CH$_3$), 28.6 (CH), 30.1(CH$_3$), 60.3 (CH), 61.3 (CH$_2$), 69.8 (CH), 168.7 (C), 200.6 (C).

(*syn*)-2b: Yield 70%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.09 (s, 9H, OSi(C*H*$_3$)$_3$), 0.66-1.06 (m, 8H, CHCH$_2$C*H*$_3$ + CH*CH*$_2$CH$_3$ + CHC*H*$_3$), 1.26 (t, *J*= 6.9 Hz, 3H, OCH$_2$C*H*$_3$), 1.46 (m, 1H, CH$_3$C*H*CH$_2$), 2.23 (s, 3H, OCC*H*$_3$), 3.36 (m, 1H, NC*H*), 3.42 (d, *J*= 9.6 Hz, 1H, C*H*CO), 4.18 (m, 2H, O*CH*$_2$CH$_3$), 6.0 (bs, 1H, N*H*); δ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -1.1 (3CH$_3$), 11.2 (CH$_3$), 14.0 (CH$_3$), 16.3 (CH$_3$), 26.4 (CH$_2$), 28.6(CH$_3$), 35.6(CH), 60.0 (CH$_2$), 60.9 (CH), 67.5 (CH), 170.0(C), 202.8 (C).
(*anti*)-2b ^{1}H NMR ($CDCl_3$, 300 MHz) 0.06 (s, 9H, OSi(C*H*$_3$)$_3$), 0.66-1.06 (m, 8H,

$CHCH_2C\mathit{H_3}$ + $CH\mathit{CH_2}CH_3$ + $CHC\mathit{H_3}$), 1.18 (m, 1H, $CH_3C\mathit{H}CH_2$), 1.25 (t, *J*= 6.9 Hz, 3H, $OCH_2C\mathit{H_3}$), 2.27 (s, 3H, $OCC\mathit{H_3}$), 3.69 (d, *J*=8.1 Hz, CHCO), 3.83 (m, 1H, NC*H*), 4.18 (m, 2H, $O\mathit{CH_2}CH_3$), 6.2 (bs, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.6 ($3CH_3$), 11.8 (CH_3), 13.9 (CH_3), 15.6 (CH_3), 26.7 (CH_2), 29.7(CH_3), 36.0(CH), 59.3 (CH_2), 61.2 (CH), 68.4(CH), 168.9(C), 200.3 (C).

(*syn*)-2c: Yield 75%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.07 (s, 9H, $OSi(C\mathit{H_3})_3$), 0.90-1.22 (m, 5H, cyclohexyl), 1.27 (t, *J*= 7.2 Hz, 3H, $OCH_2C\mathit{H_3}$), 1.44-1.80 (m, 6H, cyclohexyl), 2.25 (s, 3H, $OCC\mathit{H_3}$), 3.36 (m, 1H, NC*H*), 3.44 (d, *J*=3.3 Hz, 1H, CHCO), 4.12 (m, 2H, $O\mathit{CH_2}CH_3$), 6.20 (bs, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -1.1 ($3CH_3$), 14.0 (CH_3), 26.2 ($2CH_2$), 28.6 (CH), 29.2 (CH_2), 30.4 (CH_2), 30.9 (CH_2), 38.8 (CH_3), 59.2 (CH), 60.9 (CH_2), 69.0 (CH), 170.2 (C), 203.1 (C).

(*anti*)-2c : ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.13 (s, 9H, $OSi(C\mathit{H_3})_3$), 0.90-1.22 (m, 5H, cyclohexyl), 1.25 (t, *J*= 7.2 Hz, 3H, $OCH_2C\mathit{H_3}$), 1.44-1.80 (m, 6H, cyclohexyl), 2.28 (s, 3H, $OCC\mathit{H_3}$), 3.36 (m, 1H, NC*H*), 3.72 (d, *J*=3.3 Hz, 1H, CHCO), 4.20 (m, 2H, $O\mathit{CH_2}CH_3$), 6.20 (bs, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -1.2 ($3CH_3$), 14.1 (CH_3), 26.0(CH_2), 26.5(CH_2), 29.3 (CH), 30.3 (CH_2), 30.6 (CH_2), 31.0 (CH_2), 39.2 (CH_3), 59.7 (CH), 61.2 (CH_2), 67.5 (CH), 169.4 (C), 204.0 (C).

(*syn*)-2d: Yield 66%, pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.03 (s, 9H, $OSi(C\mathit{H_3})_3$), 0.94 (t, *J*= 7.2 Hz, 3H, $OCH_2C\mathit{H_3}$), 2.36 (s, 3H, $OCC\mathit{H_3}$), 3.90 (q, *J*=7.2Hz, 2H, $O\mathit{CH_2}CH_3$), 4.15 (d, *J*=7.8 Hz, 1H, CHCO), 4.68 (d, *J*=7.8 Hz, 1H, NC*H*), 5.59 (bs, 1H, N*H*), 7.20-7.50 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.8 ($3CH_3$), 13.6(CH_3), 29.6(CH_3), 61.3 (CH_2), 63.0 (CH), 65.8 (CH), 128.0(CH), 128.4(CH), 128.8(CH), 137.6(C), 167.2(C), 201.7 (C).

(*anti*)-2d : ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.01 (s, 9H, $OSi(C\mathit{H_3})_3$), 1.26 (t, *J*= 7.2 Hz, 3H, $OCH_2C\mathit{H_3}$), 2.06 (s, 3H, $OCC\mathit{H_3}$), 4.05 (d, *J*=8.7 Hz, 1H, CHCO), 4.20 (q, *J*=7.2Hz, 2H, $O\mathit{CH_2}CH_3$), 4.65 (d, *J*=8.7 Hz, 1H, NC*H*), 5.91 (bs, 1H, N*H*), 7.20-7.50 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -1.2 ($3CH_3$), 13.9(CH_3), 29.8(CH_3), 61.5 (CH_2), 62.4 (CH), 65.8 (CH), 127.8(CH), 128.2(CH), 129.5(CH), 137.6(C), 168.6 (C), 201.3 (C).

General procedure for the catalyzed 1,4 addition of bis-(N,O)-trimethylsilylhydroxylamine to alkylidene acetoacetate **1.** To a stirred solution of **1a-d** (0.5 mmol) in dry CH_2Cl_2 (2.5 mL) under nitrogen atmosphere, a catalytic amount of Lewis Acid (0.025 mmol) was added. The reaction mixture was cooled to 0°C and bis-(N,O)-rimethylsilylhydroxylamine (2 equiv, 1 mmol) was added in one portion. The mixture was stirred for 1 hour at the same temperature and then filtered through a celite pad. The solvent was removed under reduced pressure to afford compounds **5a-d**.

5a: Yield >95%, colorless clear oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.17 (s, 9H, OSi(C*H*$_3$)$_3$), 0.26 (s, 9H, OSi(C*H*$_3$)$_3$), 0.81 (d, *J*=6.9 Hz, 3H, CHC*H*$_3$), 1.07 (d, *J*=6.6 Hz, 3H, CHC*H*$_3$), 1.28 (t, *J*= 6.9 Hz, 3H, OCH$_2$C*H*$_3$), 1.54-1.64 (m, 1H, CH$_3$C*H*CH$_3$), 1.92 (s, 3H, OCC*H*$_3$), 3.12 (t, *J*= 10.8, 1H, NC*H*), 4.09-4.20 (m, 2H, O*CH*$_2$CH$_3$), 5.88 (d, *J*= 11.4, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.9 (3CH_3), 0.4 (3CH_3), 14.3 (CH_3), 20.5 (CH_3), 21.3 (CH_3), 28.9 (CH), 59.7 (CH_2), 69.7 (CH), 112.8 (C), 157.3 (C), 168.4 (C).

5b : Yield >95%, colorless clear oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.10 (s, 9H, OSi(C*H*$_3$)$_3$), 0.20 (s, 9H, OSi(C*H*$_3$)$_3$), 0.79 (d, *J*=6.9 Hz, 3H, CHCH$_2$C*H*$_3$), 0.82-0.90 (m, 2H, CH*CH*$_2$CH$_3$), 1.05 (d, *J*=6.6 Hz, 3H, CHC*H*$_3$), 1.23-1.30 (m, 3H, OCH$_2$C*H*$_3$), 1.35-1.49 (m, 1H, CH$_3$C*H*CH$_2$), 1.92 (s, 3H, OCC*H*$_3$), 3.22 (bt, *J*= 9.3, 1H, NC*H*), 4.07-4.27 (m, 2H, O*CH*$_2$CH$_3$), 5.88 (bs, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.8 (3CH_3), 0.5 (3CH_3), 10.5 (CH_3), 14.4 (CH_3), 16.9 (CH_3), 20.5 (CH), 26.6 (CH_2), 34.8 (CH_3), 59.7 (CH_2), 68.2 (CH), 112.8 (C), 156.9 (C), 168.5 (C).

5c : Yield >95%, colorless clear oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.14 (s, 9H, OSi(C*H*$_3$)$_3$), 0.24 (s, 9H, OSi(C*H*$_3$)$_3$), 0.86-1.40 (m, 6H, cyclohexyl), 1.32 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.65-1.80 (m, 4H, cyclohexyl), 1.96 (s, 3H, OCC*H*$_3$), 2.27 (bd, *J*= 13.2 Hz, 1H, cyclohexyl), 3.29 (d, *J*= 10.5, 1H, NC*H*), 4.12-4.23 (m, 2H, O*CH*$_2$CH$_3$), 5.92 (bs, 1H, N*H*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.8 (3CH_3), 0.5 (3CH_3), 14.4 (CH_3), 20.6 (CH), 25.9 (CH_2), 26.2 (CH_2), 26.6 (CH_2), 30.7 (CH_2), 31.8 (CH_2), 38.3 (CH_3), 59.7 (CH_2), 68.3 (CH), 112.5 (C), 157.3 (C), 168.5 (C).

5d : Yield >95%, colorless clear oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.21 (s, 9H, OSi(C*H*$_3$)$_3$), 0.30 (s, 9H, OSi(C*H*$_3$)$_3$, 1.08 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 2.05 (s, 3H, OCC*H*$_3$), 4.05 (q, *J*= 6.9 Hz, 2H, O*CH*$_2$CH$_3$), 4.89 (d, *J*= 11.4 Hz, 1H, HNC*H*) 6.27 (d, *J*= 11.4 Hz, 1H, N*H*), 7.29-7.35(m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C -0.9 (3CH$_3$), 0.5 (3CH$_3$), 14.0 (CH$_3$), 20.9 (CH$_3$), 59.7 (CH$_2$), 60.9 (CH), 113.13 (C), 126.8 (2CH), 128.0 (2CH), 128.6 (CH), 139.9 (C), 153.4 (C), 167.5 (C).

General procedure for the cyclization of **2a-d** *or* **5a-d** *to 5-hydroxyisoxazolidine-4-carboxylates* **3a-d:** To a stirred solution of **2a-d** or **5a-d** (0.5 mmol) in CH_2Cl_2 (5 mL), wet silica gel or Amberlist H15 (100 mg) was added and the reaction mixture was stirred for two hours at room temperature. After filtration on a Gouch filter, the silica gel was washed with methanol, (10 mL) and the mother liquors were evaporated under reduced pressure to afford compounds **3a-d**.

3a-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, CHC*H*$_3$), 0.97 (d, 3H, J = 6.6 Hz, CHC*H*$_3$), 1.67 (s, 3H, HOCC*H*$_3$), 1.69 (m, 1H, CH$_3$C*H*CH$_3$), 2.96 (d, *J* = 5.4 Hz, 1H, C*H*CO), 3.56 (dd, *J* = 5.4, 7.5 Hz, 1H, HNC*H*), 3.76 (s, 3H, O*CH*$_3$). ^{13}C NMR ($CDCl_3$, 300 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, CHC*H*$_3$), 1.00 (d, *J* = 6.6 Hz, 3H, CHC*H*$_3$), 1.50 (s, 3H, HOCC*H*$_3$), 1.70 (m, 1H, CH$_3$C*H*CH$_3$), 2.95 (d, *J* = 7.6 Hz 1H, C*H*CO), 3.58 (m, 1H, HNC*H*), 3.74 (s, 3H, O*CH*$_3$). ^{13}C NMR ($CDCl_3$, 300 MHz): δ 19.6(CH$_3$), 20.3(CH$_3$), 22.2(CH), 30.1(CH$_3$), 52.2(CH), 62.0(CH$_3$), 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.

3a-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, CHC*H*$_3$), 0.93 (t, 3H, *J* = 7.2 Hz, OCH$_2$C*H*$_3$), 0.97 (d, 3H, J = 6.6 Hz, CHC*H*$_3$), 1.51 (s, 3H, HOCC*H*$_3$), 1.64 (m, 1H, CH$_3$C*H*CH$_3$), 2.83 (d, *J* = 5.4 Hz, 1H, C*H*CO), 3.58 (dd, *J* = 5.4, 7.2 Hz, 1H, HNC*H*), 3.93 (m, 1H, O*CH*$_2$CH$_3$), 3.98 (m, 1H, O*CH*$_2$CH$_3$). ^{13}C NMR ($CDCl_3$, 300 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, CHC*H*$_3$), 0.88 (d, *J* = 6.6 Hz, 3H, CHC*H*$_3$), 0.91 (t, *J* = 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.45 (s, 3H, HOCC*H*$_3$), 1.50 (m, 1H, CH$_3$C*H*CH$_3$), 3.03 (d, *J* = 7.8 Hz 1H, C*H*CO), 3.88 (dd, *J* = 7.8, 7.2 Hz, 1H, HNC*H*), 3.92 (m, 2H, O*CH*$_2$CH$_3$). ^{13}C NMR ($CDCl_3$, 300 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.

3b: Yield 90%, pale yellow oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz): δ 0.80-1.06(m, 8H, CHCH$_2$C*H*$_3$ + CH*CH*$_2$CH$_3$ + CHC*H*$_3$) 1.30 (t, 3H, *J* = 7.2 Hz, OCH$_2$C*H*$_3$), 1.58 (m, 1H, CH$_3$C*H* CH$_2$CH$_3$), 1.67 (s, 3H, HOCC*H*$_3$),), 2.96 (d, *J* = 6.0 Hz, 1H, C*H*CO), 3.67 (t, *J* = 6.0 Hz, 1H, HNC*H*), 4.23 (m, 2H, O*CH*$_2$CH$_3$). ^{13}C NMR ($CDCl_3$, 300 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, CHCH$_2$C*H*$_3$ + CH*CH*$_2$CH$_3$ + CHC*H*$_3$) 1.29 (t, 3H, *J* = 7.2 Hz, OCH$_2$C*H*$_3$), 1.53 (s, 3H, HOCC*H*$_3$), 1.58 (m, 1H, CH$_3$C*H*CH$_2$CH$_3$), 2.95 (m, 1H, C*H*CO), 3.68 (m, 1H, HNC*H*), 4.25 (m, 2H, O*CH*$_2$CH$_3$).^{13}C NMR ($CDCl_3$, 300 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.

3c: Yield 95%, yellow oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz) 0.92-1.40 (m, 6H, cyclohexyl), 1.29 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.52-1.78 (m, 4H, cyclohexyl), 1.67 (s, 3H, OCC*H*$_3$), 2.00 (m, 1H, cyclohexyl), 3.00 (d, *J*= 5.7 Hz, 1H, C*H*CO), 3.56 (dd, *J*= 5.7, 8.1 Hz, 1H, HNC*H*), 4.24 (m, 2H, O*CH*$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 300 MHz) δ$_C$ 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, cyclohexyl), 1.33 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.52-1.78 (m, 4H, cyclohexyl), 1.64 (s, 3H, OCC*H*$_3$), 1.85 (m, 1H, cyclohexyl), 2.96 (d, *J*= 7.5 Hz, 1H, C*H*CO), 3.56 (t, *J*= 7.5 Hz, 1H, HNC*H*), 4.30(m, 2H, O*CH*$_2$CH$_3$); ^{13}C NMR ($CDCl_3$, 300 MHz) δ$_C$ 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.

3d: Yield 80%, yellow oil; Major anomer ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.31 (t, *J*= 7.2 Hz, 3H, OCH_2CH_3), 1.74 (s, 3H, $OCCH_3$), 3.24 (d, *J*= 5.4 Hz, 1H, CHCO), 4.27 (q, *J*= 7.2 Hz, 2H, OCH_2CH_3), 5.09 (d, *J*= 5.4 Hz, 1H, HNCH),7.28-7.57 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C 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, OCH_2CH_3), 1.64 (s, 3H, $OCCH_3$), 3.41 (d, *J*= 7.2 Hz, 1H, CHCO), 4.15 (q, *J*= 7.2 Hz, 2H, OCH_2CH_3), 4.94 (d, *J*= 7.2 Hz, 1H, HNCH), 7.28-7.60 (m, 5H, Ph); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C 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.

4a: ^{1}H NMR ($CDCl_3$, 300 MHz): δ 1.20 (m, 9H), 2.05 (s, 3H), 2.65 (m, 1H), 4.24 (m, 2H), 5.87 (d, 1H, *J* =10.2). ^{13}C NMR ($CDCl_3$, 300 MHz): 10.6, 14.1, 22.4, 29.4, 61.0, 131.3, 143.9154.4, 167.5.

General procedure for the conversion of compounds **3a-c** *to* **6a-c.** To a stirred solution of **3a-c** (0.5 mmol) in CH_2Cl_2 (2.5 mL) at 0°C, Et_3N (1.1 equiv, 0.55 mmol) and 3,5-Dinitrobenzoyl chloride (1.1 equiv, 0.55 mmol), were added. The solution was stirred for 1 hour and then the mixture was quenched with water. The residue was diluted with CH_2Cl_2 (5 mL) and the biphasic solution was extracted twice with water (2 x 5 mL). The organic layer was dried over Na_2SO_4, and solvent was removed under reduced pressure. Compounds **6a-c** were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 85/15 as eluant).

6a: Yield 80%, white solid; mp 148-152 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.07 (d, *J*=6.6 Hz, 6H, CH_3CHCH_3), 1.34 (t, *J*= 7.2 Hz, 3H, OCH_2CH_3), 1.65 (s, 3H, $OCCH_3$), 2.05-2.16 (m, 1H, CH_3CHCH_3)3.16 (d, *J*= 6.9, 1H, $HCCO_2$), 4.29 (q, *J*= 7.2 Hz, 2H, OCH_2CH_3), 4.94 (t, *J*= 6.9, 1H, NCH), 8.97 (s, 2H, *Ph*), 9.14 (s, 1H, *Ph*); ^{13}C NMR

($CDCl_3$, 300 MHz) δ_C 14.0 (CH_3), 18.2 (CH_3), 18.7 (CH_3), 23.0 (CH_3), 32.4 (CH), 57.7 (CH), 62.2 (CH_2), 65.9 (CH) 106.5 (C), 120.5 (CH), 129.6 (2CH), 137.6 (C), 148.0 (2C), 151.0 (C), 169.9 (C). Anal. Calcd. For $C_{17}H_{21}NO_4$: C, 49.64; H, 5.15; N, 10.21. Found: C, 49.55; H, 5.13; N, 10.26.

6b: Yield 70%, pale yellow solid; mp 120-122 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.95-1.05 (m, 6H, C*H*$_3$CHCH$_2$C*H*$_3$), 1.31 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.37-1.43 (m, 2H, CH$_3$CHC*H*$_2$CH$_3$), 1.51-1.60 (m, 1H, CH$_3$C*H*CH$_2$CH$_3$), 1.63 (s, 3H, OCC*H*$_3$), 3.15 (d, *J*= 6.9, 1H, *H*CCO$_2$), 4.26 (q, *J*= 7.2 Hz, 2H, OC*H*$_2$CH$_3$), 5.03 (q, *J*= 6.3, 1H, NC*H*), 5.84 (bs, O*H*), 8.93 (s, 2H, *Ph*), 9.19 (s, 1H, *Ph*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C 12.9 (CH_3), 14.0 (CH_3), 15.2 (CH_3), 22.7 (CH_3), 25.8 (CH_2), 39.0 (CH), 58.3 (CH), 62.1 (CH_2), 64.6 (CH) 106.5 (C), 120.4 (CH), 129.6 (2CH), 137.4 (C), 147.9 (2C), 158.6 (C), 169.4 (C). Anal. Calcd. For $C_{18}H_{23}NO_4$: C, 50.82; H, 5.45; N, 9.88. Found: C, 50.85; H, 5.42; N, 9.90.

6c: Yield 68%, white solid; mp 145-148 °C; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.02-1.31 (m, 6H, cyclohexyl), 1.29 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.59 (s, 3H, OCC*H*$_3$), 1.62-1.85 (m, 5H, cyclohexyl), 2.14 (d, *J*= 6.9, 1H, *H*CCO$_2$), 4.24 (q, *J*= 6.9 Hz, 2H, OC*H*$_2$CH$_3$), 4.88 (t, , *J*= 6.3, 1H, NC*H,*), 8.92 (s, 2H, *Ph*), 9.09 (s, 1H, *Ph*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C 14.1 (CH_3), 23.0 (CH_3), 25.8 (CH_2), 26.1 (CH_2), 26.9 (CH_2), 28.8 (CH_2), 29.3 (CH_2), 42.2 (CH), 58.0 (CH), 62.3 (CH_2), 65.2 (CH) 106.5 (C), 120.5 (CH), 129.6 (2CH), 137.6 (C), 148.0 (3C), 169.9 (C). Anal. Calcd. For $C_{20}H_{25}NO_4$: C, 53.21; H, 5.58; N, 9.31. Found: C, 53.09; H, 5.59; N, 9.28.

X-ray Crystallography: The diffraction experiments for **5a** were carried out at room temperature on a Bruker AXS Apex II CCD diffractometer using graphite-monochromated Mo-*K*α radiation (*λ*= 0.71073 Å). Intensity data were measured over full diffraction spheres using 0.3°-wide *ω* scans.. The SMART[52]software package was used for collecting frames of data, indexing reflections and determination of lattice parameters. The collected frames were then processed for integration with SAINT[27] and an empirical absorption correction was applied with SADABS[53]The structures were solved by direct methods (SIR 97)[54] and subsequent Fourier syntheses, and refined by

full-matrix least-squares calculations on F^2 (SHELXTL)[55] with anisotropic thermal parameters for the non-hydrogen atoms. The methyl and aromatic hydrogen atoms were placed in calculated positions and refined with idealized geometry, whereas the other H-atoms were located in the Fourier map and refined isotropically. CCDC-694159 for **5a** contains the supplementary crystallographic data for this paper. These data can be obtained free of charge from The Cambridge Crystallographic Data Centre via www.ccdc.cam.ac.uk/data_request/cif.

General procedure for the 1,4 addition of benzyl-(tert-butyldimethylsilyloxy)carbamate to **1a-e.** To a stirred solution of **1a-e** (0.5 mmol) and Lewis acid (0.05 equiv, 0.025 mmol), in dry CH_2Cl_2 (5 mL) at the selected temperature under nitrogen atmosphere, the carbamate (1 equiv, 0.5 mmol, 140 mg) was added in one portion. The reaction was followed by TLC and quenched with water. The residue was then diluted with CH_2Cl_2 (10 mL) and washed twice with water (2 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compounds **7a-e** and **8a-e** were purified by flash chromatography on silica gel (eluant cyclohexane/EtOAc, 85/15).

7a *:* Colorless oil; ^{1}H NMR (300 MHz, CDCl3): δH 0.86 (d, 3H, *J*=6.6 Hz), 0.89 (d, 3H, *J*=6.9 Hz), 1.22 (t, 3H, *J*=7.2 Hz), 1.70 (s, 3H), 1.81 (m, 1H), 2.90 (d, 1H, *J*=6.6 Hz), 4.23 (q, 2H, *J*=7.2 Hz), 4.60 (t, 1H, *J*=6.6 Hz), 5.10 (d, 1H, J=12.6 Hz), 5.18 (d, 1H, J=12.6 Hz), 7.27-7.34 (m, 5H).^{13}C NMR ($CDCl_3$, 75 MHz): δC 14.0(CH_3), 18.1(CH_3), 18.7(CH_3), 22.9(CH_3), 32.7(CH), 59.3(CH), 61.3(CH_2), 67.0(CH), 68.0(CH_2), 106.3(C), 127.9(CH), 128.0(CH), 128.4(CH), 135.8(C), 159.9(C), 168.2(C). LC-ESI-MS: rt 9.6 min m/z 352 (M+1), 725 (2M+Na). Anal. Calcd. For $C_{18}H_{25}NO_6$: C, 61.52; H, 7.17; N, 3.99. Found: C, 61.58; H, 7.14; N, 4.01.

7b : Pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, *J*= 6.6 Hz, 6H, C*H*$_3$CHC*H*$_3$), 1.07-1.44 (m, 2H, CHC*H*$_2$), 1.23 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.64 (m, 1H, CH$_3$C*H*CH$_3$), 1.75 (s, 3H, OCC*H*$_3$), 2.82 (d, *J*= 6.2, 1H, OCC*H*), 4.17-4.28 (m, 2H, O*CH*$_2$CH$_3$), 4.87 (dd, *J*= 6.2, 7.8, 1H, NC*H*), 5.15-5.24 (m, 2H, O*CH*$_2$Ph), 7.30-7.39 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 14.0 (CH_3), 21.9 (CH_3), 22.8 (CH_3), 23.0 (CH_3), 25.2 (CH), 26.9 (CH_2), 60.6 (CH), 61.4 (CH_2), 62.5 (CH), 68.0 (CH_2), 106.1 (C),

127.8 (CH), 128.2 (CH), 128.5 (CH), 135.6 (C), 159.8 (C),168.1 (C). LC-ESI-MS: rt 10.3 min m/z 366 (M+1). Anal. Calcd. For $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.41; H, 7.47; N, 3.84.

7c *:* Pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.86-0.91 (m, 6H, C*H*$_3$CHCH$_2$C*H*$_3$), 1.02-1.15 (m, 2H, CH$_3$CHC*H*$_2$CH$_3$), 1.28 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.46-1.54 (m, 1H, CH$_3$C*H*CH$_2$CH$_3$), 1.74 (s, 3H, OCC*H*$_3$), 2.97 (d, *J*= 6.3, 1H, OCC*H*), 3.97 (bs, 1H, O*H*), 4.18-4.24 (m, 2H, O*CH*$_2$CH$_3$), 4.74 (q, *J*= 7.2, 1H, NC*H*), 5.15-5.24 (m, 2H, O*CH*$_2$Ph), 7.32-7.35 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.4 (CH_3), 14.1 (CH_3), 14.8 (CH_3), 23.0 (CH_3), 25.7 (CH_2), 39.2 (CH), 58.7 (CH), 61.4 (CH_2), 66.2 (CH), 68.0 (CH_2), 106.5 (C), 127.9 (2CH), 128.4 (CH), 135.8 (C), 159.9 (C),168.3 (C). LC-ESI-MS: rt 10.0 min m/z 366 (M+1). Anal. Calcd. For $C_{19}H_{27}NO_6$: C, 62.45; H, 7.45; N, 3.83. Found: C, 62.48; H, 7.42; N, 3.86.

7d : Pale yellow oil; ^{1}H NMR ($CDCl_3$, 200 MHz) δ 0.98-1.32 (m, 5H, cyclohexyl), 1.29 (t, *J*= 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.44-1.82 (m, 6H, cyclohexyl), 1.74 (s, 3H, OCC*H*$_3$), 2.99 (d, *J*= 6.6 Hz, 1H, CH$_3$CC*H*), 3.81 (bs, O*H*), 4.16-4.27 (m, 2H, O*CH*$_2$CH$_3$), 4.62 (t, *J*= 6.8 Hz, 1H, NC*H*), 5.15 (d,, *J*= 18.2, Hz, 1H, O*CH*$_2$Ph), 5.25 (d,, *J*= 18.2, Hz, 1H, O*CH*$_2$Ph), 7.32-7.35 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 50 MHz) δ_C 14.1 (CH_3), 23.1 (CH_3), 25.8 (CH_2), 26.2 (CH_2), 28.8 (CH_2), 29.3 (CH_2), 29.7 (CH_2), 42.5 (CH), 59.5 (CH), 61.4 (CH_2), 66.5 (CH), 68.0 (CH_2), 106.3 (C), 127.9 (CH), 128.1 (CH), 128.4 (CH), 135.9 (C), 160.0 (C), 168.3 (C). LC-ESI-MS: rt 11.2 min m/z 414 (M+Na), 805 (2M+Na). Anal. Calcd. For $C_{21}H_{29}NO_6$: C, 64.43; H, 7.47; N, 3.58. Found: C, 64.40; H, 7.51; N, 3.56.

7e : Yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.32 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.88 (s, 3H, OCC*H*$_3$), 3.27 (d, *J*= 7.8 Hz, 1H, CH$_3$CC*H*), 4.23-4.31 (m, 2H, O*CH*$_2$CH$_3$), 4.42 (bs, O*H*), 5.22 (s, 2H, O*CH*$_2$Ph), 5.89 (d, *J*= 7.8 Hz, 1H, NC*H*), 7.28-7.46 (m, 10H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 14.0 (CH_3), 22.6 (CH_3), 61.5 (CH_2), 64.7 (CH), 65.2 (CH), 68.0 (CH_2), 105.9 (C),126.1 (CH), 127.6 (CH), 127.7 (CH), 128.1 (CH), 128.4 (CH), 128.7 (CH), 135.7 (C), 140.6 (C), 158.9 (C), 167.3 (C). LC-ESI-MS: rt 9.8 min

m/z 408 (M+Na), 793 (2M+Na). Anal. Calcd. For $C_{21}H_{23}NO_6$: C, 65.44; H, 6.02; N, 3.63. Found: C, 65.43; H, 5.99; N, 3.66.

General procedure for the conversion of **7a-e** *into* **8a-e**. To the crude reaction containing **7** and **8**, toluene (5 mL) and $Sc(OTf)_3$ or $Zn(OTf)_2$ (0.5 equiv.) were added and the solution was refluxed for 3h. The reaction was followed by TLC and quenched, after disappearance of compound 2, with water. The residue was then diluted with CH_2Cl_2 (10 mL) and washed twice with water (2 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound **8a-e** was purified by flash chromatography on silica gel (eluant cyclohexane/EtOAc, 85/15).

8a : White solid; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.71 (d, *J*= 6.6 Hz 3H, C*H*$_3$CHCH$_3$), 0.88 (d, *J*= 6.6 Hz 3H, C*H*$_3$CHCH$_3$), 1.17 (t, *J*=7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.97-2.17 (m, 1H, CH$_3$C*H*CH$_3$), 2.18 (s, 3H, OCC*H*$_3$), 4.04-4.13 (m, 2H, O*CH*$_2$CH$_3$), 4.98 (bd, *J*= 2.4 Hz 1H, NC*H*), 5.12 (s, 2H, OC*H*$_2$Ph), 7.26-7.30 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.5 (CH_3), 14.2 (CH_3), 15.3 (CH_3), 19.5 (CH_3), 31.3 (CH), 60.0 (CH_2), 68.5 (CH_2), 70.3 (CH), 103.0 (C), 128.1 (CH), 128.3 (CH), 128.5 (CH), 135.2 (C), 158.5 (C),163.4 (C), 163.7 (C). LC-ESI-MS: rt 11.7 min m/z 334 (M+1), 689 (2M+Na). Anal. Calcd. For $C_{18}H_{23}NO_5$: C, 64.85; H, 6.95; N, 4.20. Found: C, 64.84; H, 6.94; N, 4.23.

8b: Sticky yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.91 (d, *J*= 6.6 Hz 3H, C*H*$_3$CHCH$_3$), 0.95 (d, *J*= 6.6 Hz 3H, CH$_3$CHC*H*$_3$), 1.29 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.56 (m, 1H, CHC*H*$_2$), 1.77- (m, 2H, CHC*H*$_2$+ CH$_3$C*H*CH$_3$), 2.28 (s, 3H, OCC*H*$_3$), 4.19 (m, 2H, O*CH*$_2$CH$_3$), 4.87 (dd, *J*= 6.2, 7.8, 1H, NC*H*), 5.15-5.24 (m, 2H, O*CH*$_2$Ph), 7.30-7.37 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.7 (CH_3), 14.2 (CH_3), 21.3 (CH_3), 23.6 (CH_3), 24.6 (CH), 43.8 (CH_2), 60.0 (CH_2), 64.1 (CH), 68.5(CH_2), 105.1 (C), 128.2 (CH), 128.3 (CH), 128.5 (CH), 135.3 (C), 158.1 (C), 163.3(C), 163.5 (C). LC-ESI-MS: rt 12.3 min m/z 348 (M+1). Anal. Calcd. For $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.70; H, 7.22; N, 4.01.

8c : Pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.80 (d, *J*= 6.9 Hz 3H, C*H*$_3$CHCH$_2$CH$_3$), 0.88 (t, *J*= 7.2 Hz, 3H, CHCH$_2$C*H*$_3$), 1.29 (t, *J*= 7.2 Hz, 3H,

OCH_2CH_3), 1.42-1.50 (m, 2H, $CH_3CHCH_2CH_3$), 1.75-1.81 (m, 1H, $CH_3CHCH_2CH_3$), 2.30 (s, 3H, $OCCH_3$), 4.16-4.22 (m, 2H, OCH_2CH_3), 5.12 (bd, *J*= 2.7 Hz 1H, NC*H*), 5.24 (false t, *J*= 12.3 Hz, 2H, OCH_2Ph), 7.34-7.40 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.8 (CH_3), 12.7 (CH_3), 14.2 (CH_3), 16.0 (CH_3), 22.6 (CH_2), 38.5 (CH), 60.0 (CH_2), 68.5 (CH_2), 69.7 (CH), 103.1 (C), 128.3 (CH), 128.5 (CH), 129.3 (CH), 135.2 (C), 158.3 (C),163.7 (C), 164.0 (C). LC-ESI-MS: rt 12.18 min m/z 348 (M+1). Anal. Calcd. For $C_{19}H_{25}NO_5$: C, 65.69; H, 7.25; N, 4.03. Found: C, 65.71; H, 7.28; N, 4.07.

8d : Colorless clear oil; ^1H NMR ($CDCl_3$, 600 MHz) δ 1.01-1.27 (m, 5H, cyclohexyl), 1.28 (t, *J*= 7.2 Hz, 3H, OCH_2CH_3), 1.55-1.78 (m, 6H, cyclohexyl), 2.28 (s, 3H, $OCCH_3$), 4.16-4.24 (m, 2H, OCH_2CH_3), 5.05 (bd, *J*= 1.2 Hz, 1H, NC*H*), 5.23 (s, 2 Hz, 2H, OCH_2Ph), 7.35-7.38 (m, 5H, *Ph*); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.4 (CH_3), 14.1 (CH_3), 25.4 (CH_2), 25.9 (CH_2), 26.1 (CH_2), 26.2 (CH_2), 30.1 (CH_2), 41.0 (CH), 60.0 (CH_2), 68.4 (CH_2), 69.9 (CH), 102.5 (C), 128.1 (CH), 128.3 (CH), 128.4 (CH), 135.2 (C), 158.4 (C), 163.8 (C), 163.9 (C). LC-ESI-MS: rt 13.6 min m/z 396 (M+Na). Anal. Calcd. For $C_{21}H_{27}NO_5$: C, 67.54; H, 7.29; N, 3.75. Found: C, 67.56; H, 7.32; N, 3.75.

8e : Pale yellow oil; ^1H NMR ($CDCl_3$, 300 MHz) δ 1.05 (t, *J*= 7.0 Hz, 3H, OCH_2CH_3), 2.32 (s, 3H, $OCCH_3$), 3.93-4.05 (m, 2H, OCH_2CH_3), 5.10 (d, 1H, *J*= 12.6 Hz, OCH_2Ph), 5.18 (d, 1H, *J*= 12.6 Hz, OCH_2Ph), 6.00 (bs, 1H, NC*H*), 7.19-7.30 (m, 10H, *Ph*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C 11.6(CH_3), 14.0 (CH_3), 60.0 (CH_2), 68.0 (CH), 68.5 (CH), 105.0(C), 127.2 (CH), 128.2 (CH), 128.3 (CH), 128.4 (CH), 128.5 (2CH), 135.1 (C), 139.9 (C), 155.9 (C), 162.5 (C), 163.0 (C). LC-ESI-MS: rt 11.2 min m/z 390 (M+Na), 757 (2M+Na). Anal. Calcd. For $C_{21}H_{21}NO_5$: C, 68.65; H, 5.76; N, 3.81. Found: C, 68.62; H, 5.77; N, 3.84.

General procedure for the conversion of **8a-e** *into* **9a-e.** *Method A.* To a stirred solution of **8a-e** (0.5 mmol) in ethanol (5 mL) at 0°C under nitrogen atmosphere, $NaBH_4$ (1 equiv, 0.5 mmol, 18 mg) was added in one portion. The reaction was stirred at room temperature and followed by TLC. After quenching with water, the residue was diluted with EtOAc (10 mL) and washed twice with water (2 x 10 mL). The organic layer was

dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound **9a-e** was purified by flash chromatography on silica gel (eluant cyclohexane/EtOAc, 75/25).
Method B. To a stirred solution of **8a-e** (0.5 mmol) in THF7methanol/water (4/1/1, 6 mL) at room temperature, LiOH (3 equiv, 1.5 mmol, 36 mg) was added in one portion. The reaction was followed by TLC and quenched with water, the residue was diluted with EtOAc (10 mL) and washed twice with water (2 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound **9a-e** was purified by flash chromatography on silica gel (eluant cyclohexane/EtOAc, 75/25).

9a : Pale yellow oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.87 (d, *J*= 7.0 Hz 3H, C*H*$_3$CHCH$_3$), 0.97 (d, *J*= 7.0 Hz 3H, C*H*$_3$CHCH$_3$), 1.30 (t, *J*=7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.99 (m, 1H, CH$_3$C*H*CH$_3$), 2.24 (s, 3H, OCC*H*$_3$), 4.20 (m, 2H, OC*H*$_2$CH$_3$), 4.31 (bs, 1H, NC*H*),6.60-6.80 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.8 (CH_3), 14.3 (CH_3), 15.4 (CH_3), 19.7 (CH_3), 30.9 (CH), 59.6 (CH_2), 68.6 (CH), 103.9 (C), 164.8 (C), 167.2 (C). LC-ESI-MS: rt 9.3 min m/z 200 (M+1), 222 (M+Na). Anal. Calcd. For $C_{10}H_{17}NO_3$: C, 60.28; H, 8.60; N, 7.03. Found: C, 60.30; H, 8.62; N, 7.06.

9b: Yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (d, *J*= 6.6 Hz, 3H, C*H*$_3$CHCH$_3$), 0.95 (d, *J*= 6.6 Hz, 3H, CH$_3$CHC*H*$_3$), 1.19-1.33 (m, 2H, CHC*H*$_2$+ CH$_3$C*H*CH$_3$), 1.31 (t, *J*= 7.0 Hz, 3H, OCH$_2$C*H*$_3$), 1.73 (m, 1H, CHC*H*$_2$), 2.22 (s, 3H, OCC*H*$_3$), 4.20 (m, 2H, OC*H*$_2$CH$_3$), 4.37 (bs, 1H, NC*H*),6.40-6.70 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.9 (CH_3), 14.2 (CH_3), 21.5 (CH_3), 23.6 (CH_3), 25.2 (CH), 43.6 (CH_2), 60.0 (CH_2), 62.2 (CH), 106.2 (C), 164.5(C), 167.0 (C). LC-ESI-MS: rt 6.8 min m/z 214 (M+1). Anal. Calcd. For $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 61.99; H, 9.00; N, 6.52.

9c: Colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.81 (d, *J*= 6.9 Hz, 3H, C*H*$_3$CHCH$_2$CH$_3$), 0.92 (t, *J*= 7.2 Hz, 3H, CHCH$_2$C*H*$_3$), 1.20-1.31 (m, 2H, CH$_3$CHC*H*$_2$CH$_3$), 1.24 (t, *J*= 7.2 Hz, 3H, OCH$_2$C*H*$_3$), 1.74 (m, 1H, CH$_3$C*H*CH$_2$CH$_3$), 2.21 (s, 3H, OCC*H*$_3$), 4.24 (q, *J*= 7.2 Hz, 2H, OC*H*$_2$CH$_3$), 4.42 (bs, 1H, NC*H*), 6.70-6.80 (bs, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.9 (CH_3), 12.4 (CH_3), 14.3 (CH_3), 16.0 (CH_3), 22.7 (CH_2), 38.3 (CH), 59.3 (CH_2), 68.5 (CH_2), 103.7 (C), 164.7 (C), 167.5 (C).

LC-ESI-MS: rt 9.7 min m/z 214 (M+1). Anal. Calcd. For $C_{11}H_{19}NO_3$: C, 61.95; H, 8.98; N, 6.57. Found: C, 61.98; H, 8.95; N, 6.58.

9d : Colorless oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.10-1.39 (m, 5H, cyclohexyl), 1.30 (t, *J*= 7.0 Hz, 3H, $OCH_2C\mathit{H_3}$), 1.45-1.80 (m, 6H, cyclohexyl), 2.22 (s, 3H, $OCC\mathit{H_3}$), 4.21 (q, *J*= 7.0 Hz, 2H, $O\mathit{CH_2}CH_3$), 5.05 (bs, 1H, NC*H*), 6.60-6.80 (bs, 1H, NH); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.6 (CH_3), 14.2 (CH_3), 26.1 ($2CH_2$), 26.3 ($2CH_2$), 26.5 (CH_2), 41.0 (CH), 59.6 (CH_2), 68.2 (CH_2), 102.6 (C), 164.7 (C), 167.1 (C). LC-ESI-MS: rt 9.8 min m/z 240 (M+1). Anal. Calcd. For $C_{13}H_{21}NO_3$: C, 65.25; H, 8.84; N, 5.85. Found: C, 65.23; H, 8.87; N, 5.82.

9e : Pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.12 (t, *J*= 7.0 Hz, 3H, $OCH_2C\mathit{H_3}$), 2.34 (s, 3H, $OCC\mathit{H_3}$), 4.07 (m, 2H, $O\mathit{CH_2}CH_3$), 5.46 (bs, 1H, NC*H*), 7.20-7.45 (m, 10H, *Ph*); ^{13}C NMR ($CDCl_3$, 300 MHz) δ_C LC-ESI-MS: rt 7.6 min m/z 234 (M+1).

10a : Yellow oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.85 (d, *J*= 7.0 Hz 3H, $C\mathit{H_3}CHCH_3$), 0.96 (d, *J*= 7.0 Hz 3H, $C\mathit{H_3}CHCH_3$), 1.39 (t, *J*=7.2 Hz, 3H, $OCH_2C\mathit{H_3}$), 2.64 (s, 3H, $OCC\mathit{H_3}$), 3.44 (m, 1H, $CH_3C\mathit{H}CH_3$), 4.30 (m, 2H, $O\mathit{CH_2}CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 14.2 (CH_3), 17.6 (CH_3), 18.2 (CH_3), 20.9 (CH_3), 26.8 (CH), 60.5 (CH_2), 107.7 (C), 162.0(C), 168.1 (C), 173.3 (C). LC-ESI-MS: rt 3.2 min m/z 198 (M+1), 417 (2M+Na). Anal. Calcd. For $C_{10}H_{15}NO_3$: C, 60.90; H, 7.67; N, 7.10. Found: C, 60.92; H, 7.69; N, 7.06.

10b: Pale yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.96 (d, *J*= 6.6 Hz, 6H, $C\mathit{H_3}CHCH_3$), 1.18-1.40 (m, 2H, $CHC\mathit{H_2}$+ $CH_3C\mathit{H}CH_3$), 1.37 (t, *J*= 7.0 Hz, 3H, $OCH_2C\mathit{H_3}$), 2.05 (m, 1H, $CHC\mathit{H_2}$), 2.65 (s, 3H, $OCC\mathit{H_3}$), 4.30 (m, 2H, $O\mathit{CH_2}CH_3$); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 13.4 (CH_3), 14.2 (CH_3), 22.9 ($2CH_3$), 27.3 (CH), 34.5 (CH_2), 60.5 (CH_2), 108.4 (C), 162.4(C), 162.5 (C), 175.2(C). LC-ESI-MS: rt 10.6 min m/z 212 (M+1), 445 (2M+Na). Anal. Calcd. For $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.58; H, 8.09; N, 6.67.

10c: Clear colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 0.93 (t, 3H, *J*= 7.5 Hz, $CH_3CHCH_2CH_3$), 1.30 (d, *J*= 7.2 Hz, 3H, $CH_3CHCH_2CH_3$), 1.37 (t, *J*= 7.2 Hz, 3H, OCH_2CH_3), 1.57 (m, 1H, $CH_3CHCH_2CH_3$), 1.83 (m, 1H, $CH_3CHCH_2CH_3$), 2.65 (s, 3H, $OCCH_3$), 3.27 (m, 1H, $CH_3CHCH_2CH_3$), 4.32 (q, *J*= 7.2 Hz, 2H, OCH_2CH_3). ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.7 (CH_3), 13.5 (CH_3), 14.2 (CH_3), 18.2 (CH), 28.1 (CH_2), 33.0 (CH_3), 60.5 (CH_2), 108.0 (C), 162.4 (C),167.3(C), 175.1 (C). LC-ESI-MS: rt 10.1 min m/z 212 (M+1). Anal. Calcd. For $C_{11}H_{17}NO_3$: C, 62.54; H, 8.11; N, 6.63. Found: C, 62.51; H, 8.11; N, 6.58.

10d : Pale yellow oil; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 1.22-2.05 (m, 10H, cyclohexyl), 1.38 (t, *J*= 7.0 Hz, 3H, OCH_2CH_3), 2.64 (s, 3H, $OCCH_3$), 3.12 (tt, *J*= 3.2, 11.4 Hz, CHC), 4.32 (q, *J*= 7.0 Hz, 2H, OCH_2CH_3); ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 13.3 (CH_3), 14.1 (CH_3), 25.9 (CH_2), 26.3 ($2CH_2$), 31.2 ($2CH_2$), 36.1 (CH), 60.4 (CH_2), 107.6 (C), 162.3 (C), 167.3 (C), 175.0 (C). LC-ESI-MS: rt 11.3 min m/z 238 (M+1). Anal. Calcd. For $C_{13}H_{19}NO_3$: C, 65.80; H, 8.07; N, 5.90. Found: C, 65.83; H, 8.04; N, 5.96.

10e: Yellow oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 1.23 (t, *J*= 7.2 Hz, 3H, OCH_2CH_3), 2.75 (s, 3H, $OCCH_3$), 4.25 (q, *J*= 7.2 Hz, 2H, OCH_2CH_3), 7.46 (m, 3H, *Ph*), 7.63 (m, 3H, *Ph*). ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 13.6 (CH_3), 13.9 (CH_3), 60.7 (CH_2), 108.5 (C), 127.9 (CH), 128.2 (CH), 128.6 (CH), 129.6 (CH), 162.0 (C), 162.6 (C), 175.8 (C). LC-ESI-MS: rt 9.2 min m/z 232 (M+1). Anal. Calcd. For $C_{13}H_{13}NO_3$: C, 67.52; H, 5.67; N, 6.06. Found: C, 67.55; H, 6.11; N, 6.07.

Preparation of **11a** *starting from* **9a.** 3,5-dinitrobenzoyl chloride (1.2 equiv., 0.6 mmol, 138 mg) was added in one portion to a stirred solution of **9a** (0.5 mmol, 110 mg) and TEA (1.2 equiv, 84 μl) in CH_2Cl_2 (5 mL) at 0°C. The reaction was sttired at r.t. for 2h and then quenched with water. The residue was then diluted with CH_2Cl_2 (10 mL) and washed twice with water (2 x 10 mL). The organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound **11a** was purified by flash chromatography on silica gel (eluant cyclohexane/EtOAc, 98/2).

11a : Yellow solid; ^{1}H NMR ($CDCl_3$, 600 MHz) δ 0.96 (d, *J*= 7.0 Hz 3H, C*H*$_3$CHCH$_3$), 1.08 (d, *J*= 7.0 Hz 3H, C*H*$_3$CHCH$_3$), 1.34 (t, *J*=7.2 Hz, 3H, OCH$_2$C*H*$_3$), 2.24 (s, 3H, OCC*H*$_3$), 2.31 (m, 1H, CH$_3$C*H*CH$_3$), 4.24 (m, 2H, O*CH*$_2$CH$_3$), 5.64 (bs, 1H, CHN), 8.89 (m, 1H, Ph), 9.10-9.17 (m, 2H, Ph) ; ^{13}C NMR ($CDCl_3$, 75 MHz) δ_C 11.5 (CH_3), 14.2 (CH_3), 15.9 (CH_3), 19.8 (CH_3), 31.7 (CH), 56.9 (CH), 60.5 (CH_2), 104.5 (C), 121.3 (CH), 128.8 (2CH), 136.0 (C), 148.2 (2C), 162.6(C), 168.9 (C), 182.9 (C). LC-ESI-MS: rt 11.1 min m/z 394 (M+1). Anal. Calcd. For $C_{17}H_{19}N_3O_8$: C, 51.91; H, 4.87; N, 10.68. Found: C, 51.90; H, 4.89; N, 10.71.

III

Synthesis of enantiopure 2-substituted-3,4-dehydropyrrole derivatives via ring closing metathesis

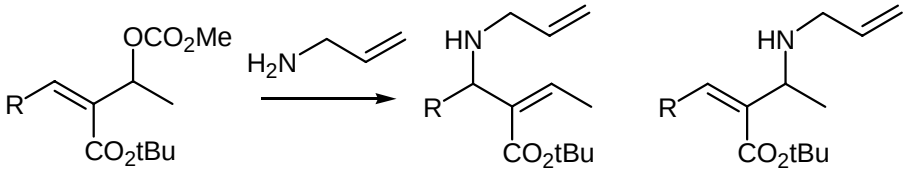
3.1 Introduction

The construction of cyclic structures is, in organic chemistry, one of the most explored field since heterocyclic rings represent the most common scaffolds for the synthesis of biologically active molecules. In this contest, ring-closing metathesis (RCM) is finding an exponential number of applications for the synthesis of various heterocycles in the fields of natural products, medicinal chemistry or material science[56]. In general, olefin metathesis always attracted chemist's attention because of its wide capability in organic synthesis. Double bonds are one of the most useful and versatile chemical functions because they are the base for various synthetic transformations. Indeed, lots of molecules having a commercial interest contain double bonds. Capability to obtain double bonds from other double bonds, allows the dramatic reduction of some synthetic pathways, especially when the scope is opening (Ring Opening Metathesis, ROM) or closing (Ring Closing Metathesis, RCM) wide-dimension rings. Ring Closing Metathesis has been particularly useful for the construction of nitrogen-containing compounds[57]. Not surprisingly, most applications have been reported for the synthesis of functionalized pyrrolidines and piperidines which constitute major classes of biologically active molecules[58]. These heterocycles are found in many alkaloid natural products, glycomimetics and drug candidates[59]. For these reasons in the past few years, RCM has become the most commonly employed metathesis in organic synthesis and has had an especially large impact on the pharmaceutical industry because the reaction allows for an efficient and direct formation of heterocycles from acyclic dienes[60]. Recently the availability of more reactive catalysts such as Grubbs-type II catalyst has opened the path to more demanding processes such as substituent bearing olefins, ruthenium catalyst have received considerable attention because of their tolerance to moisture, oxygen, and a large number of organic functional group[61].

Following the synthetic pathway already reported in chapter 1, we first synthesized dehydro-β-amino esters starting from allylic carbonates and than we envisaged *N*-allyl-dehydro-β-amino esters as suitable intermediates for the preparation of enantiopure substituted α-alkyl-dehydro-β-prolines via ring closing methatesis (RCM). These enantiopure structures may be applied as rigid scaffolds for the preparation of bioactive peptidomimetics. Synthesis of pyrrole derivatives starting from aza-Baylis-Hillman products has been already reported by Lamaty's group on a variety of racemic aromatic-substituted substrates[62], but to our knowledge no examples of enantiopure derivatives bearing aliphatic chains have been reported.

3.2 Synthesis of dehydro-β-amino esters via Ir-catalyzed allylic amination

In the past we have explored S_N2' reaction to synthesized different classes of dehydro-β-amino esters[63]. The reaction could be performed without catalyst or in presence of Pd-complexes and changing the type of solvent it is possible to have different regioselectivity. On the basis of our previously work[64], we prepared racemic and enantiopure carbonates **1a-b** that were submitted to allylic amination using allylamine as nucleophile in absence of catalyst or under Pd-catalyzed conditions (Scheme 3. 1).



1a-b **2a-b** **3a-b**

a :R = -$CH(CH_3)_2$
b: R = cyclohexil

Scheme 3. 1

Moreover, the different kind of nucleophile involved, led us to explore other catalysts in order to optimize the reaction. In particular, we found that the combination of an iridium precursor and a phosphite or phosphoramidite ligand has been described as excellent catalyst for allylic substitution with a number of different nucleophiles[65]. Iridium complexes have been also successfully applied in the stereoselective formation of C-N bonds via allylic amination[66], mostly on monosubstituted alkenyl carbonates[67]. Up to

our knowledge, iridium catalysis has been never applied to allylic carbonates having alkyl substituents on both terminals and a carboxylic group in the central position, as in compound **1**. The regioselectivity and the stereoselectivity of the substitution strongly depends on the solvent of choice and on the phosphorous ligand, that influence the Ir-allyl intermediate stability and rate of equilibration. Ethanol has been reported[68] to favor the formation of the product deriving from S_N2' mechanism and, on these bases, this solvent was used to verify the efficacy of $[Ir(COD)Cl]_2$-$P(OPh)_3$ as catalyst for allylic amination.

Table 12

Entry	Carbonate	Solvent	Allylamine (equiv)	Catalyst	Time (h)	Yield[a](%)	**2**:**3**[b]
1	*rac*-**1a**	CH_3CN	9	/	72	86	>99.1
2	(*S*)-**1a**[c]	CH_3CN	9	/	72	85	>99.1
3	(*R*)-**1a**	CH_3CN	9	/	72	85	>99.1
4	(*S*)-**1b**	CH_3CN	9	/	72	65	>99.1
5	(*R*)-**1b**	CH_3CN	9	/	72	68	>99.1
6	*rac*-**1a**	THF	15	$Pd_2(dba)_3CHCl_3$	72	35	>99.1
7	*rac*-**1a**	EtOH	3	$[Ir(COD)Cl]_2$ (2%) $P(OPh)_3$ (8%)	10	>99	>99.1
8	*rac*-**1a**	EtOH	3	/	16	52	>99.1
9	*rac*-**1a**	EtOH	3	$[Ir(COD)Cl]_2$ (2%)	16	60	>99.1

[a]Yields were calculated after purification of the products by flash chromatography on silica gel. The unreacted carbonate **1** was completely recovered after work-up. [b]Compound **2** was always obtained as 4/1mixture of Z/E isomers. [c] Enantiomeric excess of substrates and products was determined by HPLC on chiral column.

The reaction was first performed without any catalyst in refluxing acetonitrile (Table 12, entries 1-5). Under these conditions good yields were achieved only after three days, using a large excess of the amine (9 equivalents). The regioisomer **2,** deriving from S_N2' mechanism, was exclusively obtained, as 4/1 *Z*/E isomers mixture, and no traces of the product **3** could be detected. As already observed for allylic substitution with different

amines, the enantiomeric excess of the starting carbonate was completely retained and enantiopure **2a-b** was obtained starting from the corresponding chiral carbonates (entries 2-5). On the basis of our previous experience, the reaction was then performed in the presence of 3% amount of $Pd_2(dba)_3CHCl_3$ in THF. The reaction on *rac*-**1a** afforded *rac*-**2a** in modest yield, even in the presence of 15 equivalents of allylamine (entry 6). Iridium-complexes induced substitution was then performed on carbonate **1a** (entry 7). Under these conditions, the regioselective conversion of the starting carbonate into amine **2a** was observed in 10h. To verify whether the shorter reaction time and the enhanced yield had to be ascribed only to the change in solvent or also to the formation of the iridium-phosphite complex, the reaction was repeated in refluxing EtOH without any catalyst (entry 8) and in the presence of $[Ir(COD)Cl]_2$, without additional phosphine ligand (entry 9). In both cases, a lower yield was observed even after several hours, thus confirming the catalytic activity of the complex. The reaction was repeated on enantiopure (*S*)-**1a**. 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. erosion[69]. Therefore, the reaction was repeated in the presence of different catalysts and the enantiospecificity (e.s = $ee_{product}$ / $ee_{substrate}$) was determined (Table 13). The selected phosphoramidite ligands are reported in Figure 3. 1.

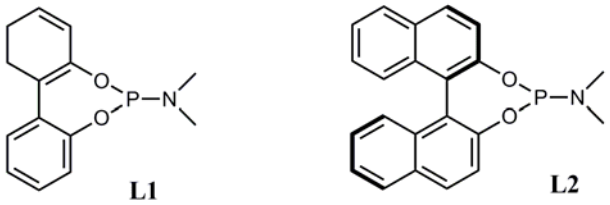


Figure 3. 1

As expected, all of the reactions were completely regioselective, affording exclusively amine **2a**. 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%.

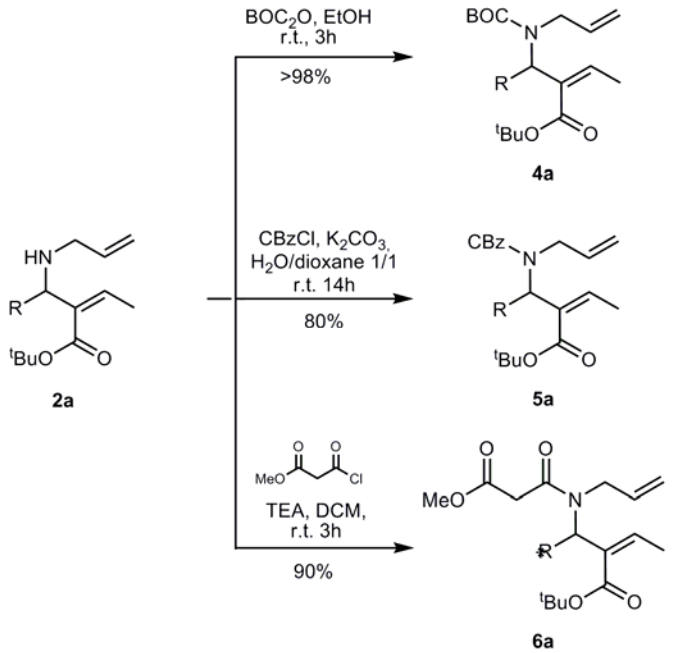
Table 13

Entry[a]	Carbonate	Ligand	e.s.
1	(*S*)-**1a**	/	72
2	(*S*)-**1a**	$P(OPh)_3$	74
3	(*S*)-**1a**	**L1**	91
4	(*S*)-**1a**	**L2**	74

[a]Compound **2** was always obtained as 4/1mixture of E/Z isomers. [b] Enantiomeric excess of substrates and products was determined by HPLC on chiral column.

3.2.1 Protection of the aminic Nitrogen

Since one of the main problem with the RCM of amines is the possibility that the aminic nitrogen poisons the ruthenium catalyst by its coordination with the nitrogen electron pair to the metal-alkylidene complex, we have protected **2a** as carbamate or amide. We decided to use three different type of N-protection in order to investigate the reactivity of these compounds and the large use that the RCM product could have. With the aim of synthesizing dihydro-pyrrole derivatives bearing more easily cleavable protecting groups, we decided to use Cbz- and BOC- as protecting group. Moreover we also synthesized the *N*-malonamide derivative **6a**, with the purpose to use it as a useful intermediate for the preparation of bioactive integrin ligands. Optimized reaction conditions for each protecting group are showed below. (Scheme 3. 2).

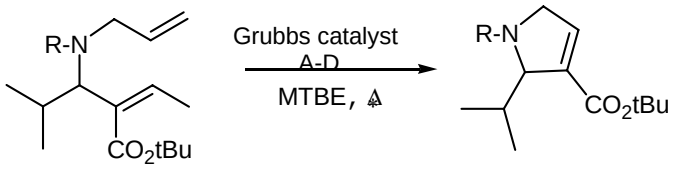


Scheme 3. 2

The *N*-BOC protected derivative **4a** was obtained in quantitative yield by treatment of **2a** with BOC_2O in ethanol at r.t. for 3h. Introduction of benzyloxycarbonyl group was accomplished by addition of the corresponding chloride to a solution of **2a** and potassium carbonate in water/dioxane (1/1 ratio) at r.t. overnight, to afford **5a** in 80% yield. Finally, *N*-malonamide derivative **6a** was obtained in 90% yield, by treatment of **2a** with methylmalonyl chloride and triethylamine in dichlorometane at r.t. for 3h.

3.3 Ring closing methatesis

Before proceeding with ring closing methatesis reactions, we have screened different types of catalysts in order to determine which is the best for our transformations. A strong effect on catalyst efficacy is linked to substrate's steric hinderance and to the electronic nature of the olefins. When steric bulk and 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[70]. On these bases, we tested, for the transformation of **4a**, **5a** and **6a**, four different catalysts (A-D). The reaction was performed in the presence of 3% of the catalyst in refluxing methyl tert-butyl ether (MTBE), since that in a recent Kuhn's work [(71)] is shown that MTBE is the ideal solvent for the synthesis of 2,5-dihydropyrroles via metathesis reaction. Selected results are reported in Table 14.

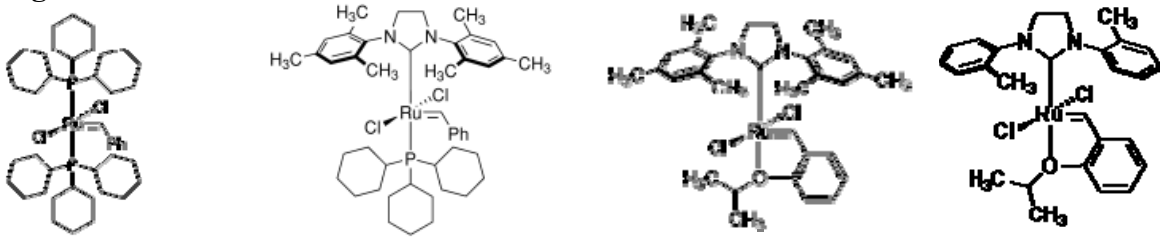


4a: R =BOC
5a: R =Cbz
6a: R =malonyl

7a: R =BOC
8a: R =Cbz
9a: R =malonyl

Scheme 3. 3

Figure 3. 2



A **B** **C** **D**

Grubbs catalyst 1° generation

Grubbs catalyst 2° generation

Grubbs-Hoveyda 2° generation

Table 14

Entry[a]	Starting material	Catalyst	t (h)	Yield[b] (%)
1	**4a**	**A**	16	/
2	**4a**[c]	**B**	2	>98
3	**5a**	**A**	16	/
4	**5a**	**B**	1.5	>98
5	**5a**	**C**	1	>98
6	**5a**	**D**	1	>98
7	**6a**[c]	**B**	16	54
8	**6a**	**C**	12	>98
9	**6a**	**D**	12	>98

[a] All experiments were performed on E/Z mixtures of starting allylamino derivatives, in the presence of 3% of the catalyst in refluxing methyl tert-butyl ether (MTBE). [b]Yields were calculated after purification of the products by flash chromatography on silica gel.[c] The reaction was performed on racemic and enantiopure substrate.

First generation Grubbs' catalyst **A** did not catalyze the ring closing metathesis on both carbamate compounds **4a** and **5a** (entries 1 and 3) allowing to recover the unreacted starting material without any trace of the heterocyclic compound, even after long reaction times. On the basis of these results, catalyst **A** was not tested on the less reactive amide **6a**. In contrast, when the reaction was performed in the presence of 3% amount of the more reactive second generation Grubbs' catalyst **B**, both carbamates **4a** and **5a** were converted into the corresponding dihydropyrroles **7a** and **8a** respectively, in quantitative yields (entries 2 and 4). The presence of Z/E mixtures of isomers did not represent a limitation, since both compounds showed the same reactivity. The reaction was monitored by TLC and complete disappearance of the starting material could be observed after only 2h for **4a** and 1.5h for **5a**. In order to complete the screening of catalysts, we performed the RCM on compound **5a** using Hoveyda-Grubbs second generation catalyst **C** and its analogue **D**, bearing 2-methylphenyl substituents on the NHC ligand in place of the 2,4,6-trimethylphenyl groups. Under these conditions, the reaction occurred slightly faster, since in both cases compound **8a** could be obtained in quantitative yield after 1h (entries 5-6). Comparison of the results obtained on the carbamate derivatives **4a** and **5a** with different catalysts, showed that no great

advantages could be observed in the use of expensive complexes **C** and **D**, since the cheaper catalyst **B** induced the complete conversion into the desired products quite rapidly. The RCM was then performed on malonamide **6a**, using the more efficient catalysts **B**,**C** and **D**. By using Grubbs's II catalyst **B,** the heterocyclic product could be obtained after 16h only in 54% yield. Enhanced reactivity could be observed in the presence of catalysts **C** and **D**, that quantitatively converted **6a** into the corresponding dihydropyrrole **9a** in 12h. Enantiopure **7a** and **9a** were obtained with the same yield through RCM of the corresponding optically active starting materials.

3.3.1 Conformational products' study

It is well known that the amide bond preceeding the proline nitrogen may adopt either the *cis* or *trans* conformation[72]. While for all the other proteinogenic amino acids the *trans* conformation is by far energetically more favorable than the *cis*, for proline the difference is much smaller. For this reason the less stable *cis* conformer is always present in peptide samples, in a percentage depending on the adjacent residues and on the polarity of the solvent[73]. Two distinct sets of resonances are commonly detected in the NMR spectrum of these compounds, as a consequence of the slow rate of interconversion[74]. This effect is enhanced for β–proline oligomers, whose *cis* and *trans* conformers are well populated at room temperature[75]. Similar considerations have been reported also for dehydroproline[76].

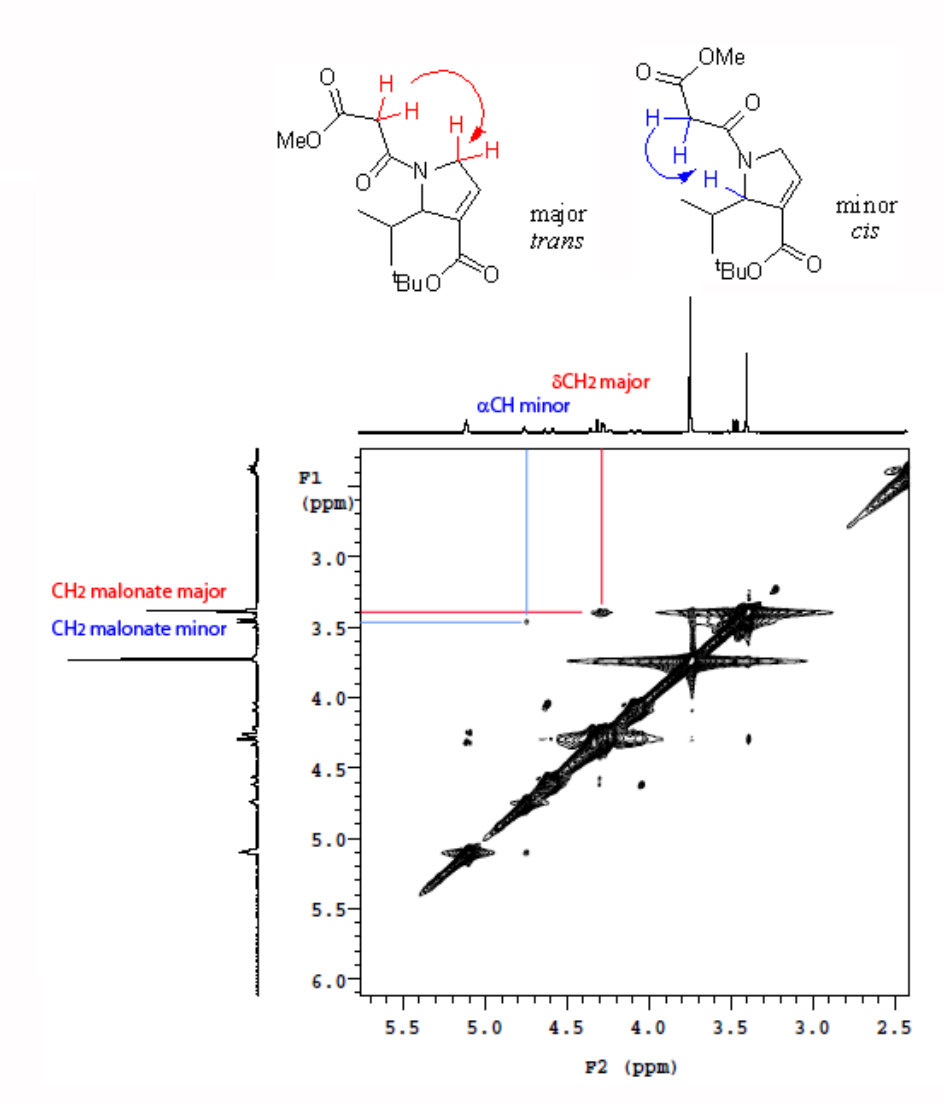


Figure 3. 3

As expected, the products of the RCM were all isolated as mixtures of two conformers. The ^{1}H NMR spectrum in $CDCl_3$ of *N*-BOC protected **7a** and *N*-CBz derivative **8a** showed the presence of the two forms as 1:1 mixtures. For compound **9a**, where dehydro-β-proline ring is linked to malonamide, the ratio between the conformers was 70:30. In order to define the conformation of amide bond, the two distinct sets of resonances were attributed by means of COSY analysis. Then, spatial arrangement of the malonic chain for the two conformers was explored through ROESY experiments (Figure 3. 3). The presence of the cross peak between malonic chain CH_2 and the δ-CH_2 protons for the major conformer allowed to attribute the *trans* conformation to the amide bond. On the contrary, the cross peak between malonic chain CH_2 and the α-CH proton confirmed the *cis* conformation of the minor isomer.

3.4 Experimental section

General: All chemicals were purchased from commercial suppliers and used without further purification. Anhydrous solvents were purchased in sure seal bottles over molecular sieves and used without further drying. Flash chromatography was performed on silica gel (230-400 mesh). NMR Spectra were recorded with Varian Gemini 200, Mercury Plus 400 or Unity Inova 600 MHz spectrometers. Chemical shifts were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at δ = 7.27 (^{1}H NMR) or δ = 77.0 (^{13}C NMR), CD_3OD set at δ = 3.31 (^{1}H NMR) or δ = 49.0 (^{13}C NMR), D_2O set at δ = 4.79 (^{1}H NMR). Coupling constants are given in Hz. The enantiomeric excesses of products were determined by HPLC analyses performed on an HP1100 instrument with UV-VIS detector and equipped with Chiralpak IC column (25 X 0.46 cm), eluted with n-hexane/2-propanol (tipical program: from 96/4 to 90/10 in 30 min, flux 0.6 mL/min). Optical rotations were measured in a Perkin Elmer 343 polarimeter using a 1 dm cuvette and are referenced to the Na-D line value. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. LC-MS analyses was performed on a HP1100 liquid chromatograph coupled with an electrospray ionization-mass spectrometer (LC-ESI-MS), using H_2O/CH_3CN as solvent at 25 °C (positive scan 100-500 m/z, fragmentor 70V).

General procedure for allylic amination without catalyst: To a stirred solution of **1** (1 mmol) in CH_3CN (5 mL) under nitrogen atmosphere, allylamine (9 mmol, 9 equiv) was added in one portion and the mixture was wormed to reflux. After 3 days, the solvent and the excess of allylamine were removed under reduced pressure and the residue was diluted with ethyl acetate (10 mL). The organic solution was extracted three times with 0.1M HCl (10 mL), then it was dried over Na_2SO_4, and solvent was removed under reduced pressure to recover unreacted **1**. 1M NaOH was added dropwise to the acid acqueous layer until basic pH was achieved and then the solution was extracted three times with CH_2Cl_2 (10 mL). The organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to afford allylamino-derivative **2** as a yellow oil (65-86%, see Table 1), that was used in the following step without further purification.

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 **1** (1 mmol) and allylamine (3 mmol, 3 equiv.) were added in one portion at r.t.. 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 0.1M HCl (10 mL) , then was dried over Na_2SO_4 and solvent was removed under reduced pressure to recover unreacted starting carbonate. 1M NaOH was added dropwise to the acid acqueous layer until basic pH was achieved and then the solution was extracted three times with CH_2Cl_2 (10 mL). The organic layers were dried over Na_2SO_4 and solvent was removed under reduced pressure to afford allylamino-derivative **2** in quantitative yield as a yellow oil that was used in the following step without further purification.

Tert-butyl 3-(allylamino)-2-ethylidene-4-methylpentanoate **2a**: Yellow oil, (80:20 *Z/E* mixture); ^{1}H NMR (200 MHz, $CDCl_3$) δ *Z* isomer: 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), 5.84-5.93 (m, 1H), 6.91 (q, *J*=7.2 Hz, 1H); *E* isomer: 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); 5.84-5.93 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ *Z* isomer 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. *E* isomer 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 14.97 min, 254 (M+1). (*S*)-**2a** $[\alpha]_D$ = +19.5 (*c* 1 in $CHCl_3$); (*R*)-**2a** $[\alpha]_D$ = -20.6 (*c* 1 in $CHCl_3$).

Tert-butyl 2-((allylamino)(cyclohexyl)methyl)but-2-enoate **2b:** Yellow oil, (80:20 *Z/E* mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ *Z* isomer: 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), 5.84-5.92 (m, 1H), 6.89 (q, *J*=7.2

Hz, 1H); *E* isomer: 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); 5.84-5.92 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ *Z* isomer 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. *E* isomer 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 16.21 min, 294 (M+1). (*S*)-**2a** $[\alpha]_D$ = +14.3 (*c* 1 in $CHCl_3$); (*R*)-**2a** $[\alpha]_D$ = -16.6 (*c* 1 in $CHCl_3$).

*Synthesis of N-BOC derivative **4a**:* Boc_2O (1mmol, 1 eq.) was added to a solution of **2a** 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 (5mL), the organic layer was dried over Na_2SO_4 and solvent was removed under reduced pressure. Compound **4a** was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70:30 as eluant).

Tert-butyl 3-(N-(tert-butoxycarbonyl)allylamino)-2-ethylidene-4-methylpentanoate **4a**: Yellow oil, (80:20 *Z/E* mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ *Z* isomer: 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), 5.68-5.79 (m, 1H), 6.83 (q, *J*=7.2 Hz, 1H); *E* isomer: 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), 5.68-5.79 (m, 1H); ^{13}C NMR (75 MHz, $CDCl_3$) δ *Z* isomer 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. *E* isomer 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 16.6 min (*Z* isomer), 14.9 min (*E* isomer), 354 (M+1), 729 (2M+23).

*Synthesis of N-CBz derivative **5a**:* Benzyloxycarbonyl chloride (1.5 mmol, 1.5 equiv.) and K_2CO_3 (2 mmol, 2 equiv) were added to a solution of **2a** (1 mmol) in dioxane/water (1:1 solution, 5 mL). The reaction mixture was stirred at r.t. 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 **5a** was purified by flash chromatography on silica gel (eluant cyclohexane /EtOAc 97:3).

Tert-butyl 3-(N-(benzyloxycarbonyl)allylamino)-2-ethylidene-4-methylpentanoate **5a:** Yellow oil, (80:20 *Z/E* mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ *Z* isomer: 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), 5.63-5.72 (m, 1H), 6.85 (q, *J*=7.2 Hz, 1H) 7.20-7.40 (m, 5H); *E* isomer: 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), 5.63-5.72 (m, 1H), 6.01 (q, *J*=7.2 Hz, 1H), 7.20-7.40 (m, 5H); ^{13}C NMR (75 MHz, $CDCl_3$) δ *Z* isomer 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. *E* isomer 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 14.7 min (*Z* isomer), 13.5 min (*E* isomer), 388 (M+1), 410 (M+23).

*Synthesis of N-malonamido derivative **6a**:* To a stirred solution of **2a** (1 mmol) in dry CH_2Cl_2(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 3h , the reaction mixture diluted with CH_2Cl_2(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 **6a**. The desired product was obtained in yields up to 95% as an yellow oil.

Tert-butyl 3-(N-allyl-3-methoxy-3-oxopropanamido)-2-ethylidene-4-methylpentanoate **6a**: Yellow oil, (80:20 *Z/E* mixture); ^{1}H NMR (400 MHz, $CDCl_3$) δ *Z* isomer: 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, 5.2 Hz, 1H), 4.44 (dd, *J*=18.2, 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), 5.65-5.74 (m, 1H), 6.91 (q, J=7.2 Hz, 1H); *E* isomer: 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), 5.65-5.74 (m, 1H), 6.18 (q, J=7.2 Hz, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) δ *Z* isomer; 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. *E* isomer: 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 10.7 min (*Z* isomer), 10.1 min (*E* isomer), 376 (M+23), 729 (2M+23). Chiral HPLC analysis (for conditions see general section), rt 32.12 min for (*S*) and 36.33 min for (*R)*; (*S*)-**6a** $[\alpha]_D$ = -30.8 (*c* 1 in $CHCl_3$); (*R*)-**6a** $[\alpha]_D$ = +26.7 (*c* 1 in $CHCl_3$).

General procedure for the ring closing metathesis (RCM) with ruthenium catalyst : Catalyst A-D (0.03 mmol) was added to a solution of **4a**, **5a** or **6a** (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).

Tert-butyl N-(tert-butoxycarbonyl)2-isopropyl-2,5-dihydro-1H-pyrrole-3-carboxylate **7a:** 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 ($CDCl_3$, 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).

Tert-butyl N-(benzyloxycarbonyl)2-isopropyl-2,5-dihydro-1H-pyrrole-3-carboxylate **8a:** 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).

Tert-butyl N-(methylmalonyl)2-isopropyl-2,5-dihydro-1H-pyrrole-3-carboxylate **9a** 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*)-**9a** $[\alpha]_D$ = +34.9 (*c* 1 in $CHCl_3$); (*R*)-**9a** $[\alpha]_D$ = -28.7 (*c* 1 in $CHCl_3$).

IV

Linear and cyclic dehydro-β-amino acid containing integrin ligands. Synthesis and bioactivity

4.1 Introduction

Integrins are a large family of heterodimeric transmembrane glycoproteins involved in the attachment of a cell to the extracellular matrix (ECM) and in signal transduction from the ECM to the cell.[77] These adhesion mechanisms are of fundamental importance in a diverse range of biological processes, including cell differentiation, apoptosis, embryonic cell migration, maintenance of tissue integrity, and blood coagulation.[78] 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, making integrins an attractive target for the development of therapeutic agents.[79] The identification of key recognition motifs within integrin ligands is the starting point for the development of antagonists. To date, these motifs have been identified for only a few subtypes. $\alpha_v\beta_3$ integrin (Figure 4. 1) has been deeply investigated as it is involved-tumor proliferation and metastasis through the formation of new blood vessels. $\alpha_v\beta_3$ integrin binds to a wide number of ECM components like fibronectin, fibrinogen, vitronectin, and osteopontin through recognition of the Arg-Gly-Asp (RGD) tripeptide sequence.[80]

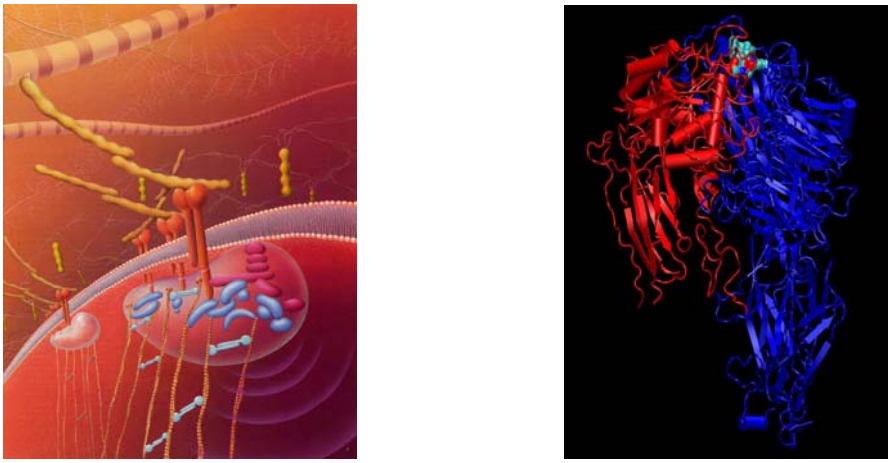


Figure 4. 1

This sequence is also essential for the binding of $\alpha_5\beta_1$ integrin to fibronectin, which has been unambiguously recognized as proangiogenic receptor.[81] $\alpha_5\beta_1$ integrin may regulate the function of integrins $\alpha_v\beta_3$ on endothelial cells during their migration in vitro or angiogenesis in vivo. Activation of $\alpha_5\beta_1$ potentiates $\alpha_v\beta_3$-mediated migration on vitronectin, whereas $\alpha_5\beta_1$ integrin antagonists inhibit $\alpha_v\beta_3$-mediated cell spreading. Therefore, antagonists of both integrins, block the same pathway of angiogenesis.[82] In this chapter, the design, synthesis, and blockade of fibronectin-mediated cell adhesion of novel $\alpha_v\beta_3/\alpha_5\beta_1$ integrin dual antagonists, whose activity could be synergistically effective in preventing angiogenesis, will be reported. The X-ray analysis[83] of the complex between $\alpha_v\beta_3$ integrin and c(RGDfV) ligand (Figure 4. 2) shows that the ligand interacts mainly through electrostatic interactions. Arg and Asp form a charged clamp that binds regions with opposite charges in the protein: Asp interacts with a metal cation in the β subunit and Arg with two Asp in the α subunit.

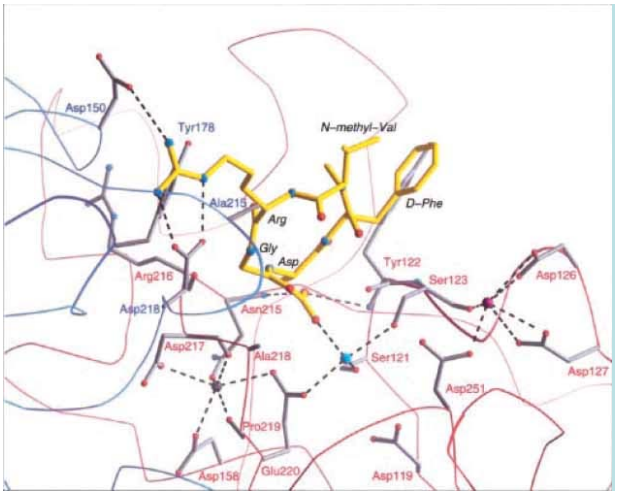


Figure 4. 2

Several efficient classes of ligands, containing the RGD sequence, have been reported in the literature.[84] These structures share as common features conformational restraints able to give a proper orientation to the peripheral substituents. Linear and cyclic peptides containing the RGD sequence, showing high affinity toward $\alpha_v\beta_3$ integrins, have significant therapeutic potential but serious limitations especially for oral dosing. The need for antagonists with higher bioavailability and lower molecular weight has prompted several research groups to develop small constrained non-peptidic molecules mimicking the RGD motif, which would be more promising for drug development.[85]

Most of the structures proposed so far consist of a polyfunctionalized rigid core, linked to appendages corresponding to arginine and aspartic acid side chains.[86] Recently we identified a series of $\alpha_v\beta_3$ integrin ligands based on 5,6-dihydropyridin-2-one scaffold that showed promising interaction with the receptor.[87] The basicity and the length of the arginine-mimicking group was found to play a central role. Moreover, the presence of a carboxylic function, mimicking the aspartic acid residue in the original binding motif, is a fundamental feature to create anionic interaction with the metal cation in the receptor active site.[88] Many heterocyclic scaffolds have been employed to maintain the acidic and the basic ends of the molecule at the appropriate distance and with the suitable conformation for binding interaction.

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, incorporation of linear or constrained amino acid into an RGD sequence may give access to simple mimetics with increased bioactivity and stability.

In this chapter, I will describe a novel class of integrin antagonists derived from dehydro-β-amino acids, as esemplified in Figure 4. 3, 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.

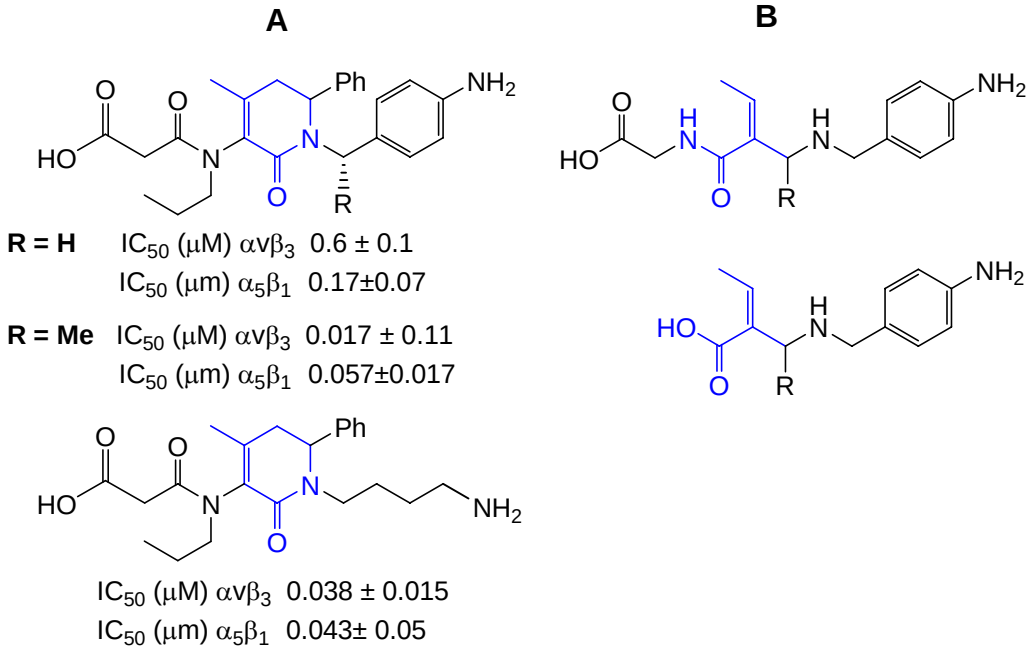


Figure 4. 3

A crucial requirement related to the bioactivity is the spatial disposition of each residue. Indeed, conformational differences may be responsible for a large variation in affinity and selectivity. We hypotesized that the restricted conformation introduced by the heterocycle in **A**, that induces a good spatial disposition of the appendages, could be substituted by the dehydro-β-amino acid, giving favorable alignment of both the basic and carboxylate moieties.

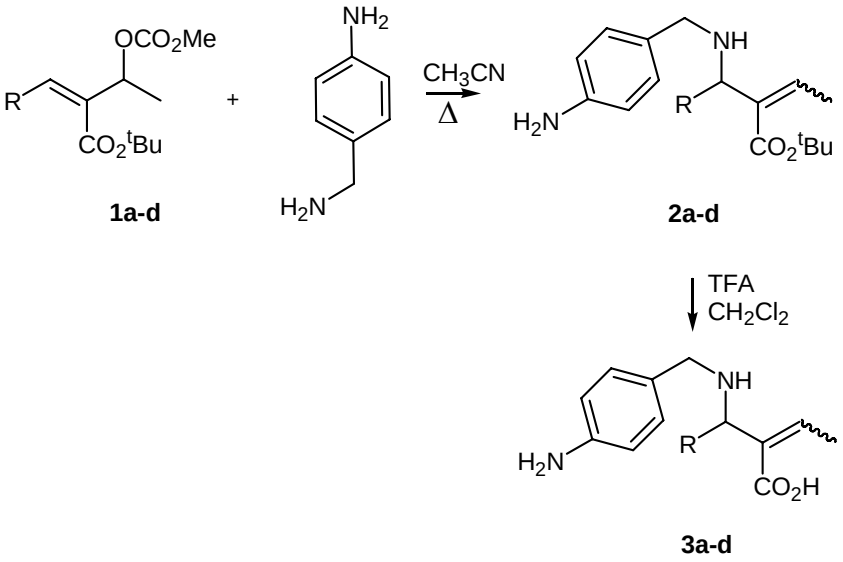
Compounds with different variations at the substituent R or in the length of the side chains, maintaining the same position as in **B**, were synthesized and analyzed as integrin antagonists.

We surmised that the global conformational constraint arising from the restriction by the dehydro-β-amino acid could result in a favorable spatial orientation of the integrin recognized functions.

Moreover I will also describe the synthesis of substituted isoxazolidines since they constitute versatile synthetic intermediates as conformational constraint. Indeed, various isoxazolidines are extensively used as 1,3-amino alcohol equivalents, as masked amino acids or as amino-sugar mimetics. Furthermore, substituted isoxazolidines, isoxazolines and isoxazoles are important substrates for mechanistic studies of biologically interesting processes. For example, isoxazolines have been incorporated as conformational constraint element in $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin antagonists, as well as in several transcriptional activators. In a similar way, constrained analogues containing the isoxazoline or isoxazole ring have been reported, and several AMPA (S-2-amino-3-(3-hydroxy-5-methyl-4-isoxazolyl)-propionic acid) receptor agonists have been synthetized and tested. On the other hand, the synthesis of unnatural nucleoside analogs containing the isoxazolidinic and isoxazolinic ring is currently of significant interest since it has been reported that the presence of an isoxazoline ring increases the antibacterial activity of carbapenem derivatives.

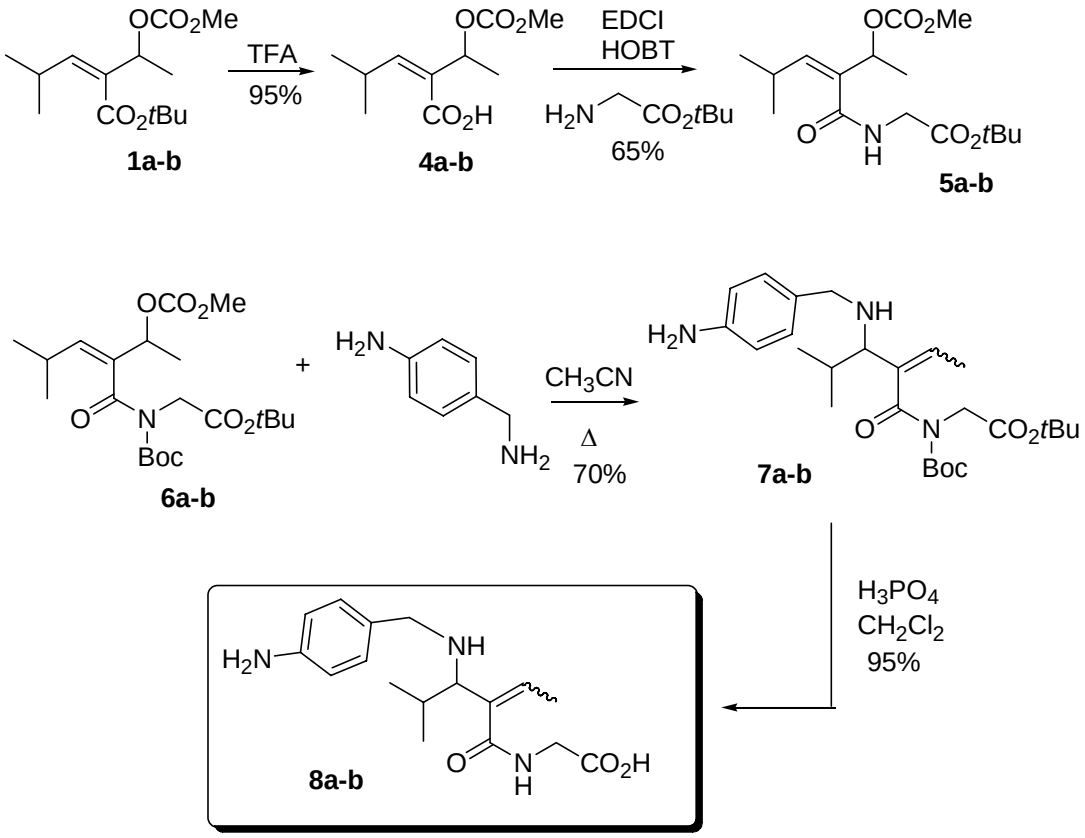
4.2 Synthesis of linear substituted dehydro-β-amino acids as RGD mimetics

Compounds **3a-d** have been prepared in order to test the regioselectivity of *p*-aminobenzylamine on carbonates **1a-d** and to verify the complete stereoselectivity of the reaction.(Scheme 4. 1) The synthesis of these compounds was based on our previously reported results and has been described in chapter 1, where each compound has been accurately analyzed. Although the compounds **3a-d** have a shorter length than that generally required for RGD mimetic ligands, they have been submitted to biological evaluation.



Scheme 4. 1

In a similar way, the RGD mimetics **8a-b** have been obtained through the S_N2' substitution on the appropriate racemic or optically active Boc-amides **6a-d**. (Scheme 4. 1).



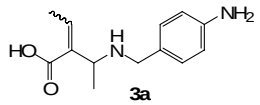
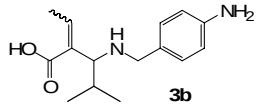
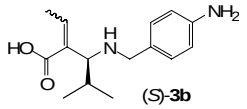
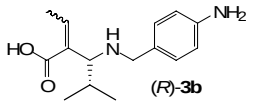
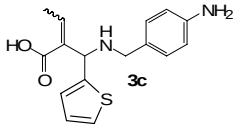
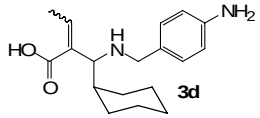
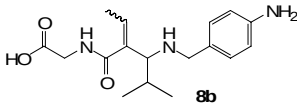
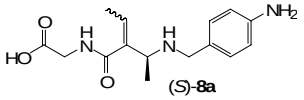
Scheme 4. 2

For details on synthetic pathway, see paragraph 1.5 and scheme 1.14

4.2.1 Biological evaluation of linear substituted dehydro-β-amino acids

In Table 15 the ability to perturb initial cell attachment mediated by $\alpha_v\beta_3$ integrin and $\alpha_5\beta_1$ integrin using cell adhesion assays of some selected compounds, is reported. The integrin ligand fibronectin (10μg/ml) was immobilized on tissue culture plates. The ability of human melanoma cell line SK-MEL 24, expressing $\alpha_v\beta_3$ integrin, and human erythroleukemic cell line K562, expressing $\alpha_5\beta_1$ integrin, to adhere to fibronectin in the presence or absence of the assayed compounds was examined. The anti-adhesion activity of the well-known integrin antagonist Ac-Asp-Arg-Leu-Asp-Ser-OH (H3534) was measured as a positive control.

Table 15

Entry	Compound	IC_{50} (μM)[a]	
		$\alpha_v\beta_3$	$\alpha_5\beta_1$
1	 **3a**	>1,000	0.019 ± 0.003
2	 **3b**	1.49	0.14
3	 (*S*)-**3b**	19.2	31.3
4	 (*R*)-**3b**	0.307	21.2
5	 **3c**	3.35	4.66
6	 **3d**	0.831	2.1
7	 **8b**	0.121	9.77
8	 (*S*)-**8a**	1.99	0.224

[a]Values are means ± standard error of three experiments.

The shorter molecules reported in entries 1-6 showed unexpected activity and selectivity depending from the substituent in position 3. For methyl derivative **3a** no adhesion could be observed on $\alpha_v\beta_3$ integrin receptor. On the other hand, the introduction of the more lipophilic *i*pr group (**3b**) gave interesting results since the racemic compound showed a good dual activity (entry 2).

The orientation provided by the configuration in position 3 seems to be crucial for the affinity on $\alpha_v\beta_3$ integrin receptors, since (*R*)-**3b** displayed increased inhibitory activity in respect to the corresponding S enantiomer.

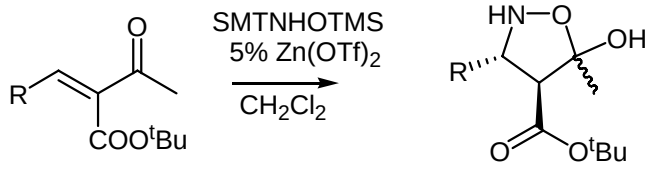
This effect is enhanced for $\alpha_v\beta_3$ receptor, being the *R* isomer about more potent than the *S* one. Comparable activity towards $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrin was observed for thiophenyl

derivative **3c** (entry 5), while the presence of a cyclohexyl group as in **3d** seems to favour a preferential affinity towards $\alpha_v\beta_3$ receptor (entry 6). This compound, indeed, resulted about more potent on $\alpha_v\beta_3$ compared to $\alpha_5\beta_1$ integrin. In entries 7 and 8, the results obtained for elongated ligands are reported. Compound **8b** showed comparable affinity to the corresponding shorter analogue **3b**. On the contrary, compound **8a** displayed good activity and a strong preference towards $\alpha_5\beta_1$ receptor, behaving as a selective not dual inhibitor.

These results seem to suggest that the activity for smaller ligands **3a-d** is not strictly connected to the substituent hinderance, while a certain effect is induced by its spatial orientation. On the other hand, a major influence of the substitution pattern can be observed for elongated compounds **8a-b**, where the introduction of the smaller substituent increases affinity towards $\alpha_5\beta_1$ integrin.

4.3 Synthesis of isoxazolidine - derivatives as rigid constrained cores of RGD mimetics

In chapter 2 I described the highly stereocontrolled synthesis of terbutyl 5-hydroxyisoxazolidine-4-carboxylate through a Lewis acid induced Michael addition of hydroxylamine derivatives to alkylideneacetoacetates, followed by intramolecular hemiketal formation. (Scheme 4. 3)



a: R= CH_3
b: R= $CH(CH_3)_2$
c: R= $CH(CH_3)CH_2CH_3$
d: R=Cyclohexyl

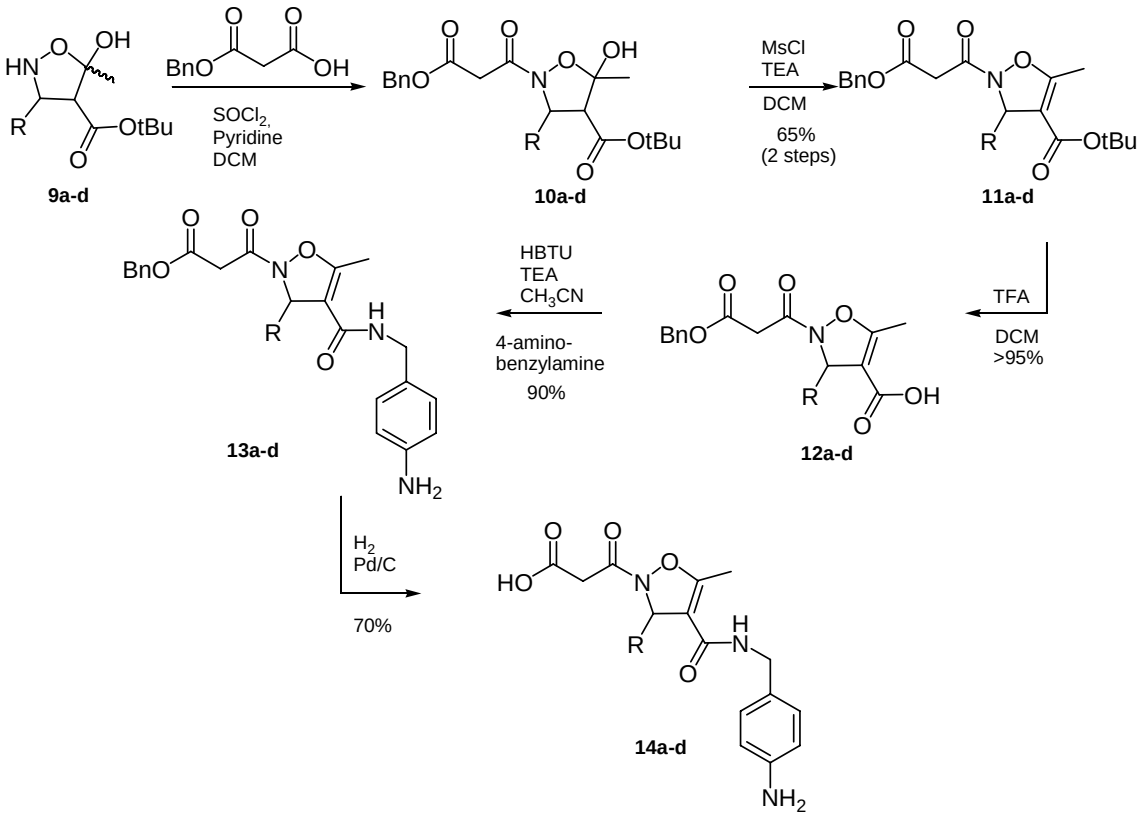
9a-d

Scheme 4. 3

The use of acetoacetates in this field is rather unusual and has the advantage of introducing a reactive keto functionality that may be further elaborated. Lewis acids have attracted much attention in organic synthesis because of their strong influence on the rate, the regio-, and the stereochemistry of numerous reactions. In this context,

carbamates have only recently been the subject of investigation as nucleophilic reagents for conjugate addition.

Herein I report a modified procedure to obtain 5-hydroxyisoxazolidine-4-carboxylates as rigid constrained cores of RGD mimetics and a simple route to obtain the ligands. We surmised that grafting of these scaffold into RGD motif could result in a favorable spatial orientation of the integrin recognition tripeptide.



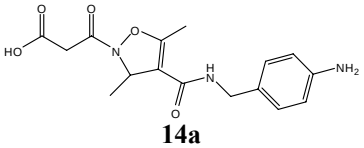
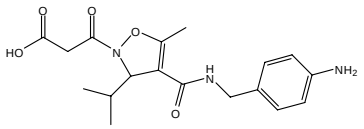
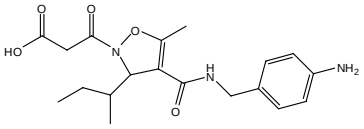
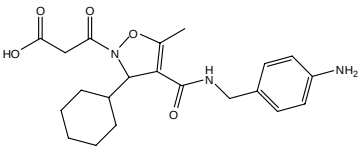
Scheme 4. 4

Terbutyl-5-hydroxyisoxazolidine-4-carboxylate **9a-d**, were synthesized as already described in chapter 2 (Scheme 4. 3), than the nitrogen was protected with the benzyl malonic derivative that will correspond to the acidic part of the final RGD mimetic (Scheme 4. 4). Adding mesyl chloride and TEA in excess, compounds **10a-d** were converted to the corresponding dehydrated isoxazoline **11a-d**. The relative acids **12a-d** were obtained by the hydrolysis of the terbutylester with trifluoroacetic acid. Compounds **12a-d** were coupled with 4-amino-benzylamine to afford amides, finally by simple hidrogenation of **13a-d** final products **14a-d** were obtained.

4.3.1 Biological evaluation of isoxazolidine- derivatives

Isoxazolidine- derivatives **14 a-d**, were evaluated for their ability to inhibit $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrin mediated cell adhesion. In Table 16 the ability to perturb initial cell attachment mediated by $\alpha_v\beta_3$ integrin and $\alpha_5\beta_1$ integrin using cell adhesion assays of some selected compounds, is reported. The integrin ligand fibronectin (10μg/ml) was immobilized on tissue culture plates. The ability of human melanoma cell line SK-MEL 24, expressing $\alpha_v\beta_3$ integrin, and human erythroleukemic cell line K562, expressing $\alpha_5\beta_1$ integrin, to adhere to fibronectin in the presence or absence of the assayed compounds was examined. The anti-adhesion activity of the well-known integrin antagonist Ac-Asp-Arg-Leu-Asp-Ser-OH (H3534) was measured as a positive control.

Table 16

Entry	Compound	$\alpha_v\beta_3$ IC_{50}, μM[a]	$\alpha_5\beta_1$ IC_{50}, μM[a]
1	 **14a**	$3.16*10^{-8}$	$1.19*10^{-8}$
2	 **14b**	$8.79*10^{-9}$	$1.05*10^{-9}$
3	 **14c**	$3.60*10^{-7}$	$1.32*10^{-6}$
4	 **14d**	$2.02*10^{-8}$	$1.03*10^{-6}$

[a]Values are means ± standard error of three experiments.

The results reported in Table 16 show that the isoxazolidine ring represents an effective scaffold to induce the proper orientation to the Asp and Arg mimicking chain, since the smaller member of the library, **14a,** is a potent ligand for both $\alpha_v\beta_3$- and $\alpha_5\beta_1$-integrin and can be regarded as a dual inhibitor. This activity could be effective in preventing

angiogenesis by blocking different pathways of the blood vessel formation in tumors. Introduction of the isopropyl group in place of the smaller methyl substituent in position 3, as in compound **14b**, resulted in a small influence on the affinity to both receptors, being the effect on $\alpha_v\beta_3$-integrin stronger in respect to $\alpha_5\beta_1$-integrin. The increased steric hinderance of the substituent in the isoxazoline ring strongly affected the activity of compound **14c**, that showed a reduction of the bioactivity from nanomolar to micromolar. Finally, the trend was confirmed by the evaluation of the bulky cyclohexyl derivative **14d**, that showed no affinity for $\alpha_v\beta_3$-integrin and only a modest preference for $\alpha_5\beta_1$-integrin.

4.4 Molecular Docking

Before submit our compounds to the biological evaluation with the isolated receptor, we decided to study the real interaction that our compounds could have inside the receptor with a molecular docking study. Two compounds for each class of product were submitted to the docking study (**3b** and **8b**, **14a** and **14d**).

Docking studies were performed using Glide (version 4.5)[89] by starting from the representative macrocycle conformations sampled during the unrestrained MD simulations. The protein binding site was derived from the X-ray crystal structure of the extracellular segment of integrin $\alpha_V\beta_3$ in complex with the cyclic pentapeptide ligand cilengitide [Protein Data Bank (PDB) entry 1L5G].[90] In this X-ray structure, the potent $\alpha_V\beta_3$ antagonist cilengitide, bound to the headgroup of the integrin, features an extended conformation of the RGD sequence with a distance of ~9 A° between the Cβ atoms of Asp and Arg. The crystal complex interaction pattern involves the formation of an electrostatic clamp between the guanidinium group of the ligand and the negatively charged side chains of Asp218 and Asp150 in the α unit and between the carboxylic group of cilengitide and the metal cation in the metal ion-dependent adhesion site (MIDAS) region of the β unit. Moreover, further stabilization occurs through hydrogen bonds between the NH group of the ligand Asp residue and the carbonyl oxygen atom of Arg216 in the β subunit as well as between the ligand carboxylate oxygen not coordinated to MIDAS and the backbone amides of Asn215 and Tyr122 in the β unit.

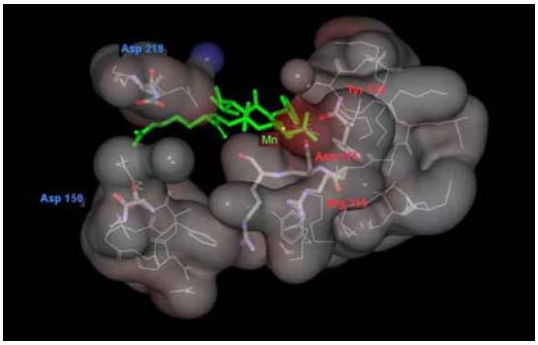


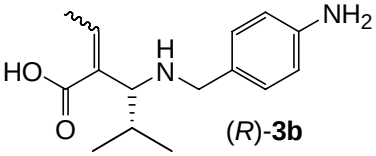
Figure 4. 4

The experimentally observed binding mode of cilengitide (Figure 4. 4) with $\alpha_V\beta_3$ integrin was taken as a reference model for the interpretation of the docking results in terms of the ligandbound conformation and ligand-protein interactions.

4.4.1 Docking results

For each ligand only the picture with the best pose and the range of the score function for the 20 poses saved (Gscore), is reported. For each 'best pose' is reported the distance from the COO- group and the nitrogen atom of NH_2 group. In fact these two groups should mimic the Asp/Arg chains of the RGD sequence. In all the pictures, in green colour, is reported the bound geometry of the cilengitide ligand, take from the X-Ray with $\alpha_v\beta_3$ integrin. Finally, the receptor is reported only with the parts that interact with the cilengitide (α chain is orange, β chain is blue and Ca-MIDAS is a green sphere) and Gscore values of our ligands can be compared with cilengitide Gscore, where in the best pose is -9,07.

Compound (*R*)-**3b** gave a Gscore of (-5,02; -3, 89)



(*R*)-**3b**

In the Best pose (Figure 4.5) the NH_2 group interacts with the C=O of the Ala215 (α chain) and there is the electrostatic interaction Ca-MIDAS/COO-.

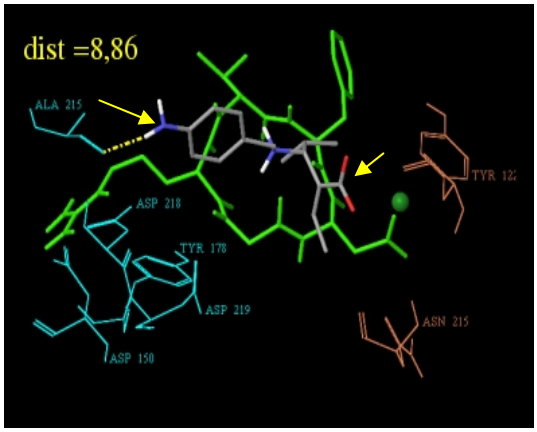


Figure 4. 5

In the next pose (Figure 4. 6) we can see a different orientation of the ligand inside the receptor, the aromatic ring seems interact with Tyr178 ring (T-shaped o stacking) of α subunit (15 to 20 poses). NH_2 group can interact with Asp218 of α subunit.

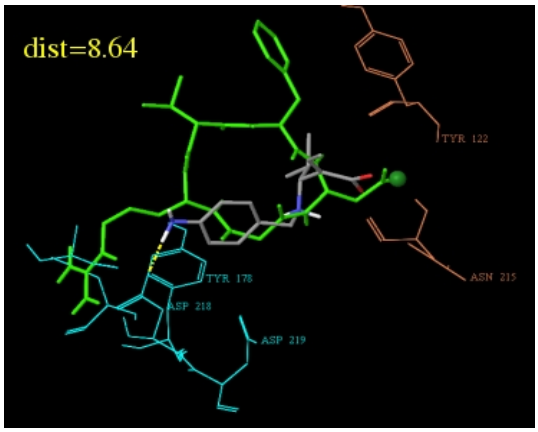
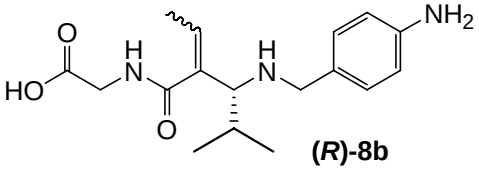


Figure 4. 6

Compound (*R*)-**8b** gave a Gscore of (-5,46; -3, 68)



(*R*)-8b

In the Best pose (Figure 4. 7) compound (*R*)-**8b** has the same interaction of the cilengitide with the COO-/ β subunit. NH_2 group interacts with Asp218 and the aromatic ring has a stacking interaction with Tyr178 of the α subunit

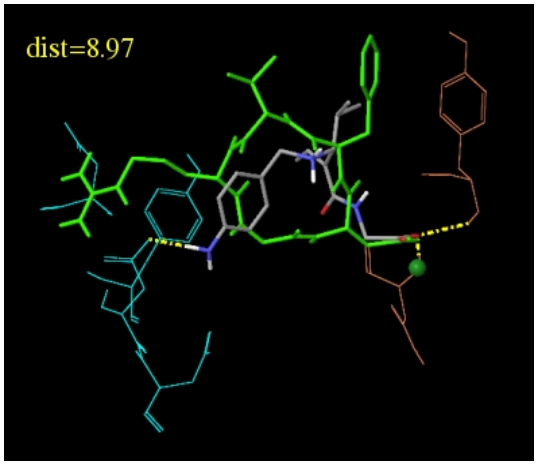
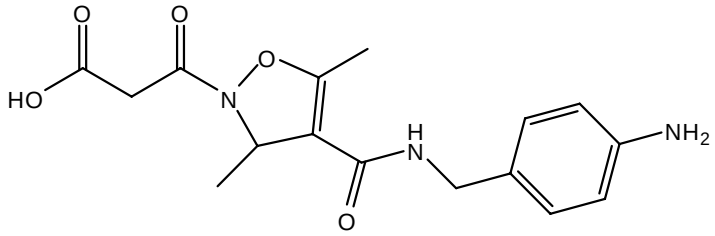


Figure 4. 7

In the other poses, there aren't hydrogen bonds with Tyr122/Asn215 of the β subunit, but there is the electrostatic interaction Ca-MIDAS/COO- (for 14 poses).
In conclusion both the ligands seem fit properly in the receptor site maintaining the key electrostatic interaction (Ca2+-MIDAS for β subunit and Asp218 for α subunit) with both the subunits but with a different distance of the pharmacophoric groups with consequent loss of important hydrogen bonds.

Since that isoxazolidine-derivatives were synthesized in racemic way, molecular docking were calculated on both the isomer (*R*)- and (*S*)- .
Moreover for all the compounds the aromatic group seems have an effect on the position inside the binding site interacting with Tyr 78 of α subunit.

Compound (*R*)-**14a** gave a Gscore of (-6.21; -4.08)



14a

In the Best pose the ligand has interactions with crystalline complex only with $–COO^-$ group (10 to 20 poses are like the best pose but not always two hydrogen bonds with Tyr122 e Asn215 are kept). The first pose in wich $–NH_2$ group fits like cilengitide is pose number 5 where we can note also the interaction of Tyr178 of α subunit (6 to 20

poses are like the 5 pose but not always two hydrogen bonds with Tyr122 e Asn215 are kept).

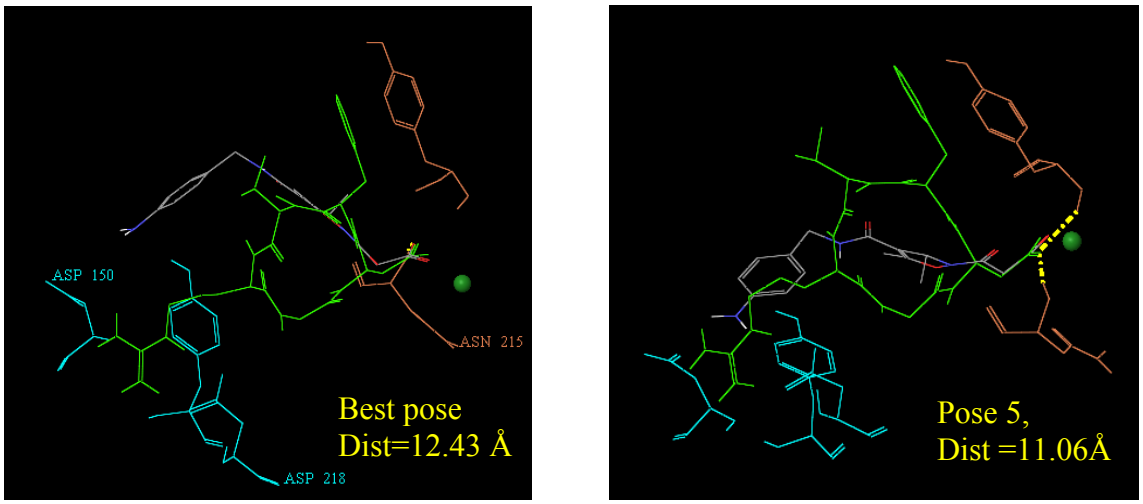


Figure 4. 8

Compound (*S*)-**14a** gave a Gscore of (-5.32;-3.40)

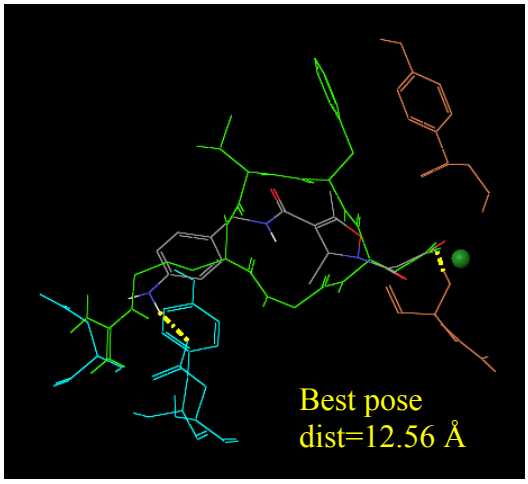
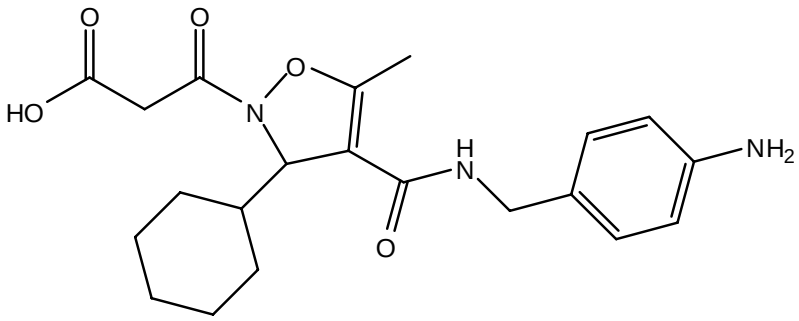


Figure 4. 9

In the Best pose the ligand has the same interaction of the cilengitide with the COO-/ β subunit. The $-NH_2$ basic group interacts with Asp218. These interactions are maintained until pose 4. 8 to 20 poses are like the best pose. In the other poses or the ligand moves to β subunit or $-NH_2$ group interacts with Asp150 of α subunit.

In conclusion compound **14a** finds poses for the interaction with $\alpha_V\beta_3$ integrin that conserve the crystallographic interactions observed in in the crystal structure of the cilengitide- $\alpha_V\beta_3$ complex.

Compound (*R*)-**14a** gave a Gscore of (-5.96;-4.20)



14d

In the Best Pose (Figure 4. 10) the ligand binds β subunit turning the cyclohexyl ring to Phe-EMD. In the next poses the ligand binds β subunit but with different orientation of cyclohexyl ring.

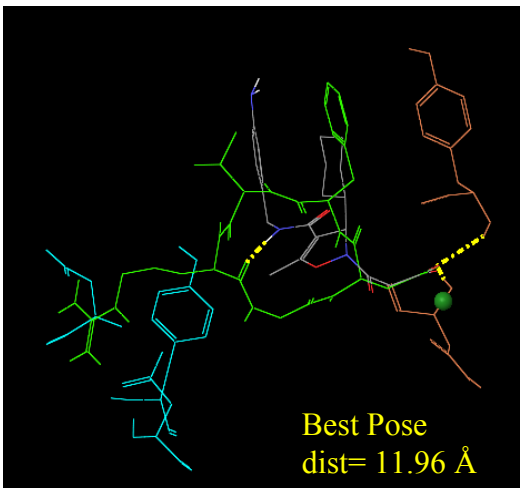


Figure 4. 10

Compound (*S*)-**14a** gave a Gscore of (-6.37; -4.20)

The ligand binds β subunit only in the first three poses turning the cyclohexyl ring to the binding site. In the pose 4 it preserves the crystallographic interactions observed in in the crystal structure of the cilengitide- $\alpha_V\beta_3$ complex for 12/20 poses.

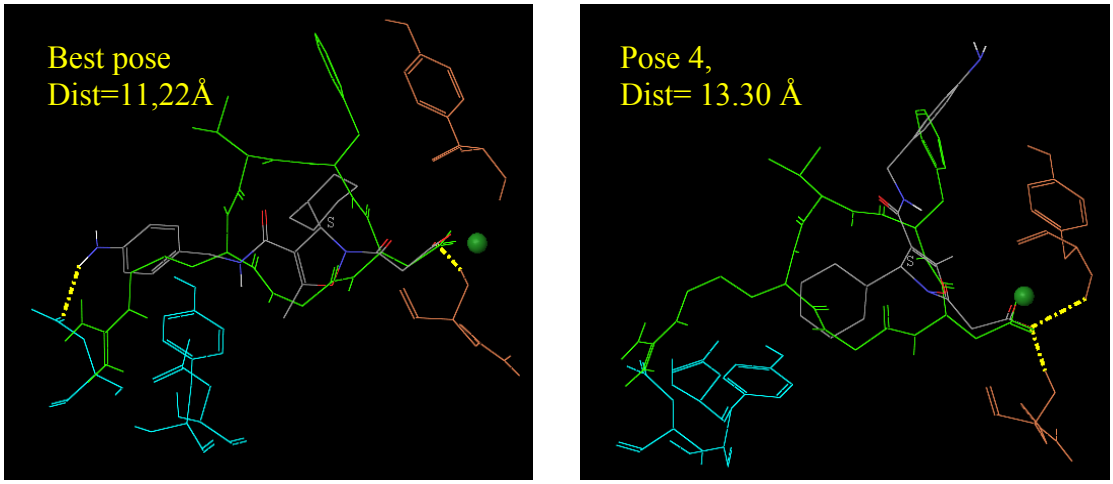


Figure 4. 11

In conclusion both the stereoisomer *R* and the stereoisomer *S* of compound **14d**, in the first poses interact with only the β subunit.

4.5 Experimental section

General: All chemicals were purchased from commercial suppliers and used without further purification. Anhydrous solvents were purchased in sure seal bottles over molecular sieves and 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 were reported as δ values (ppm) relative to the solvent peak of $CDCl_3$ set at δ = 7.27 (^{1}H NMR) or δ = 77.0 (^{13}C NMR), CD_3OD set at δ = 3.31 (^{1}H NMR) or δ = 49.0 (^{13}C NMR), D_2O set at δ = 4.79 (^{1}H NMR). Coupling constants are given in Hz. Optical rotations were measured in a Perkin Elmer 343 polarimeter using a 1 dm cuvette and are referenced to the Na-D line value. Melting points were determined on a Stuart Scientific SMP3 apparatus and are uncorrected. LC-MS analyses was performed on a HP1100 liquid chromatograph coupled with an electrospray ionization-mass spectrometer (LC-ESI-MS), using H_2O/CH_3CN as solvent at 25 °C (positive scan 100-500 m/z, fragmentor 70V).

For the synthesis and the characterization of compounds **1a-d**, **2a-d**, **4a-b**, **5a-b**, **6a-b**, **7a-b** and **8a-b**, see the experimental section of chapter 1. For the synthesis and the characterization of compounds **9a-d,** see the experimental section of chapter 2.

General procedure for the synthesis of dehydro-β-amino acids **3a-d**: TFA (7 mmol, 7 equiv.) was added to a solution of **2a-d** (1 mmol) in CH_2Cl_2(5mL) 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 **3a-d** were isolated in quantitative yield after removal of the aqueous solvent.

3a : ^{1}H NMR (CD_3OD, 200 MHz) major isomer δ 1.53 (d, *J*=6.8 Hz, 3H, C*H*$_3$CHN), 1.88 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 3.91 (d, *J*=12.8 Hz, 1H, NHC*H*$_2$Ph), 4.01 (d, *J*=12.8 Hz, 1H, NHC*H*$_2$Ph), 4.31 (q, *J*=6.8 Hz, 1H, CH_3C*H*N), 6.80 (d, *J*=8.4 Hz, 2H, Ph), 7.02

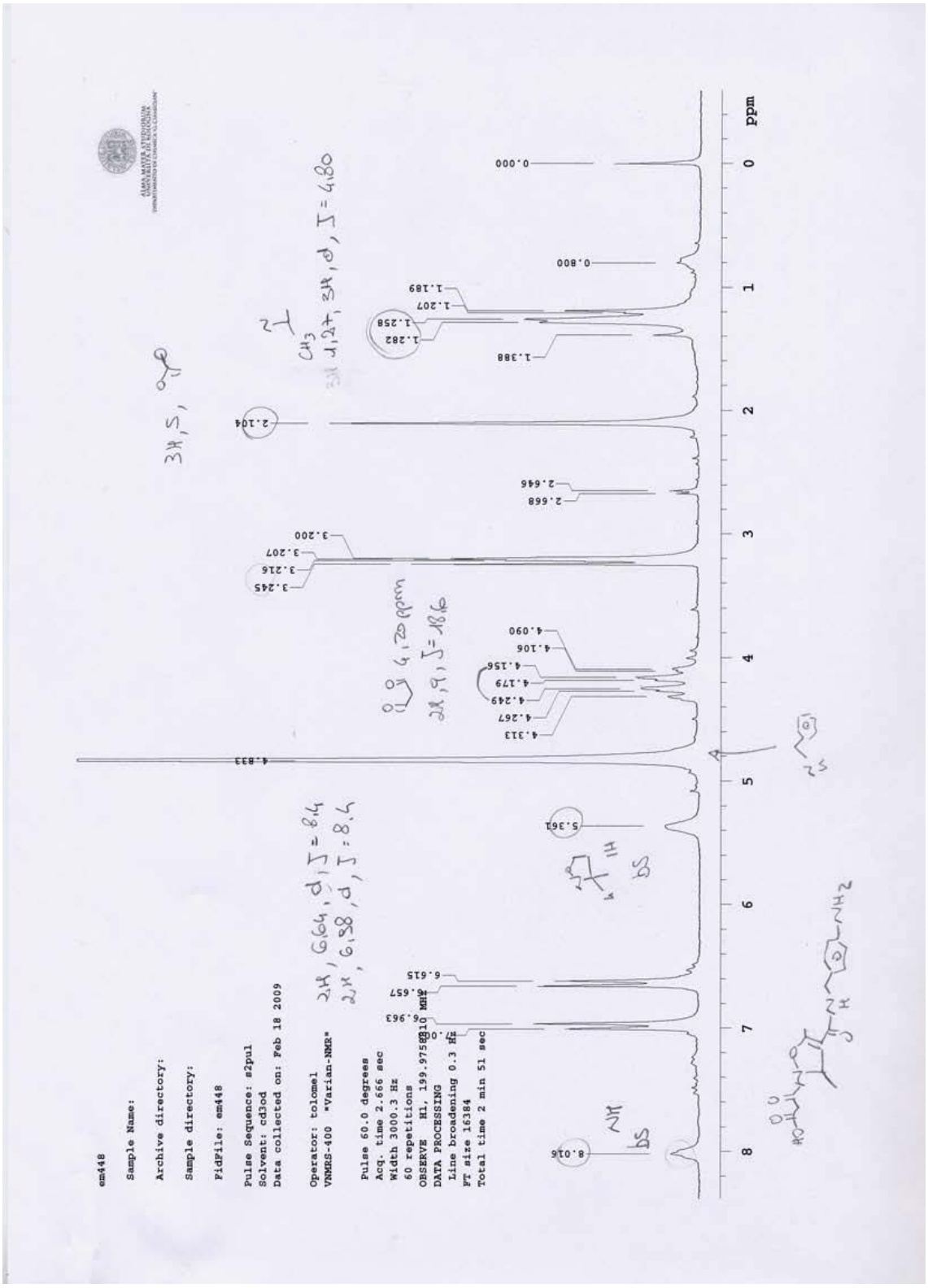
(q, *J*=7.2 Hz, 1H, CC*H*CH_3), 7.23 (d, *J*=8.4 Hz, 2H, Ph);minor isomer δ 2.12 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHC), 3.81 (q, *J*=6.6 Hz, 1H, CH_3C*H*N), 6.05 (q, *J*=7.0 Hz, 1H, CC*H*CH_3).

3b: ^{1}H NMR (CD_3OD, 200 MHz) major isomer δ 0.80 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHCH_3), 0.91 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHCH_3), 1.66 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 2.01 -2.16 (m, 1H, CH_3C*H*CH_3), 3.69-3.95 (m, 3H, CHC*H*N e NHC*H*$_2$Ph), 6.61 (d, *J*=8.4 Hz, 2H, *Ph*), 6.94 (q, *J*=7.2 Hz, 1H, CC*H*CH_3), 7.04 (d, *J*=8.4 Hz, 2H, *Ph*); minor isomer δ 1.94 (d, *J*=7.0 Hz, 3H, C*H*$_3$CHC), 5.77 (q, *J*=7.0 Hz, 1H, CC*H*CH_3).

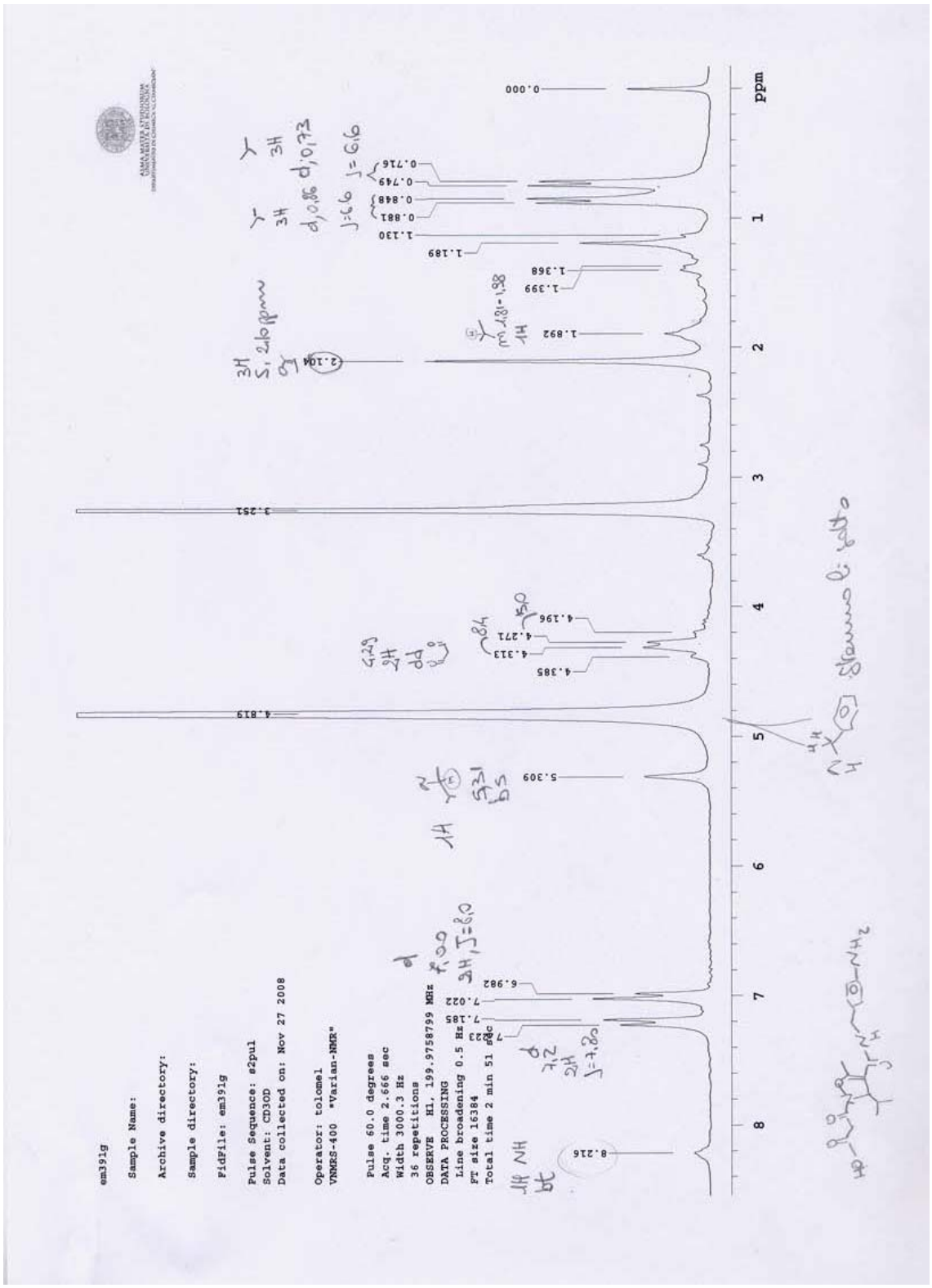
3c: ^{1}H NMR (CD_3OD, 200 MHz) major isomer δ 1.19-1.36 (m, 6H, *cyclohexyl*), 1.64-2.01 (m, 5H, *cyclohexyl*), 1.77 (d, *J*=7.2 Hz, 3H, C*H*$_3$CHC), 3.89-4.11 (m, 3H, CHC*H*N, NHC*H*$_2$Ph), 6.83 (d, *J*=7.0 Hz, 2H, *Ph*), 7.22 (d, *J*=7.0 Hz, 2H, *Ph*), 7.34 (q, *J*=7.4 Hz, 1H, CC*H*CH_3); minor isomer δ 2.13 (d, *J*=6.8 Hz, 3H, C*H*$_3$CHC), 6.33 (q, *J*=6.8 Hz, 1H, CC*H*CH_3).

3d: ^{1}H NMR (DMSO, 200 MHz) major isomer δ 1.74 (d, *J*=7.4 Hz, 3H, C*H*$_3$CHC), 3.61 (d, *J*=13.0 Hz, 1H, NHC*H*$_2$Ph), 3.78 (d, *J*=13.0 Hz, 1H, NHC*H*$_2$Ph), 5.02 (s, 1H, C*H*NH), 6.54 (d, *J*=8.4 Hz, 2H, *Ph*), 6.78 (q, *J*=7.4 Hz, 1H, CC*H*CH_3), 7.01 (d, *J*=8.4 Hz, 2H, *Ph*), 7.10 (d, 1H, *thiophenyl*), 7.44 (bs, 1H, *thiophenyl*), 7.50-7.54 (m, 1H, *thiophenyl*); minor isomer δ 1.96 (d, *J*=6.6 Hz, 3H, C*H*$_3$CHC), 4.60 (s, 1H, C*H*NH), 5.89 (q, *J*=6.6 Hz, 1H, CC*H*CH_3).

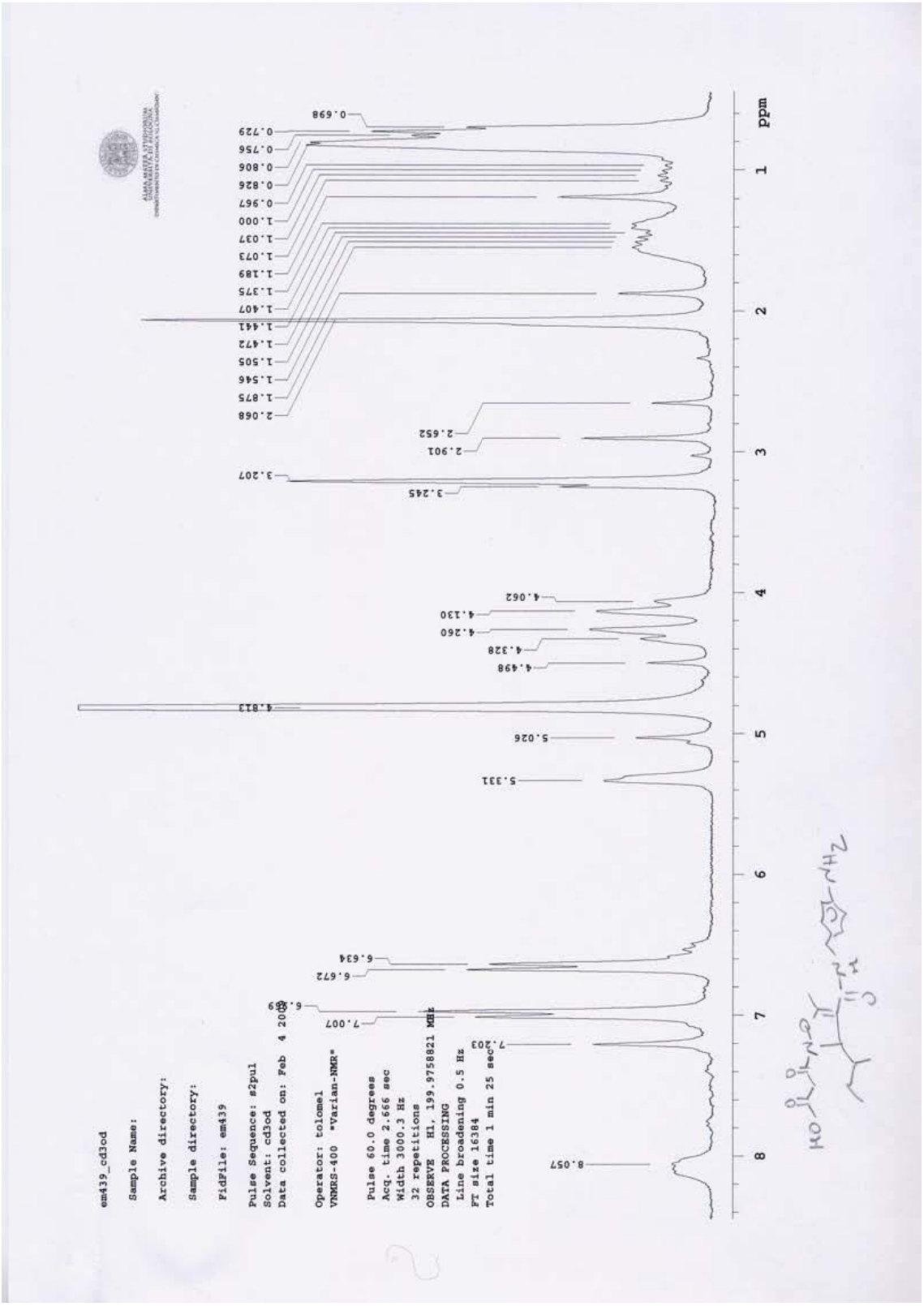
Compound **14a**



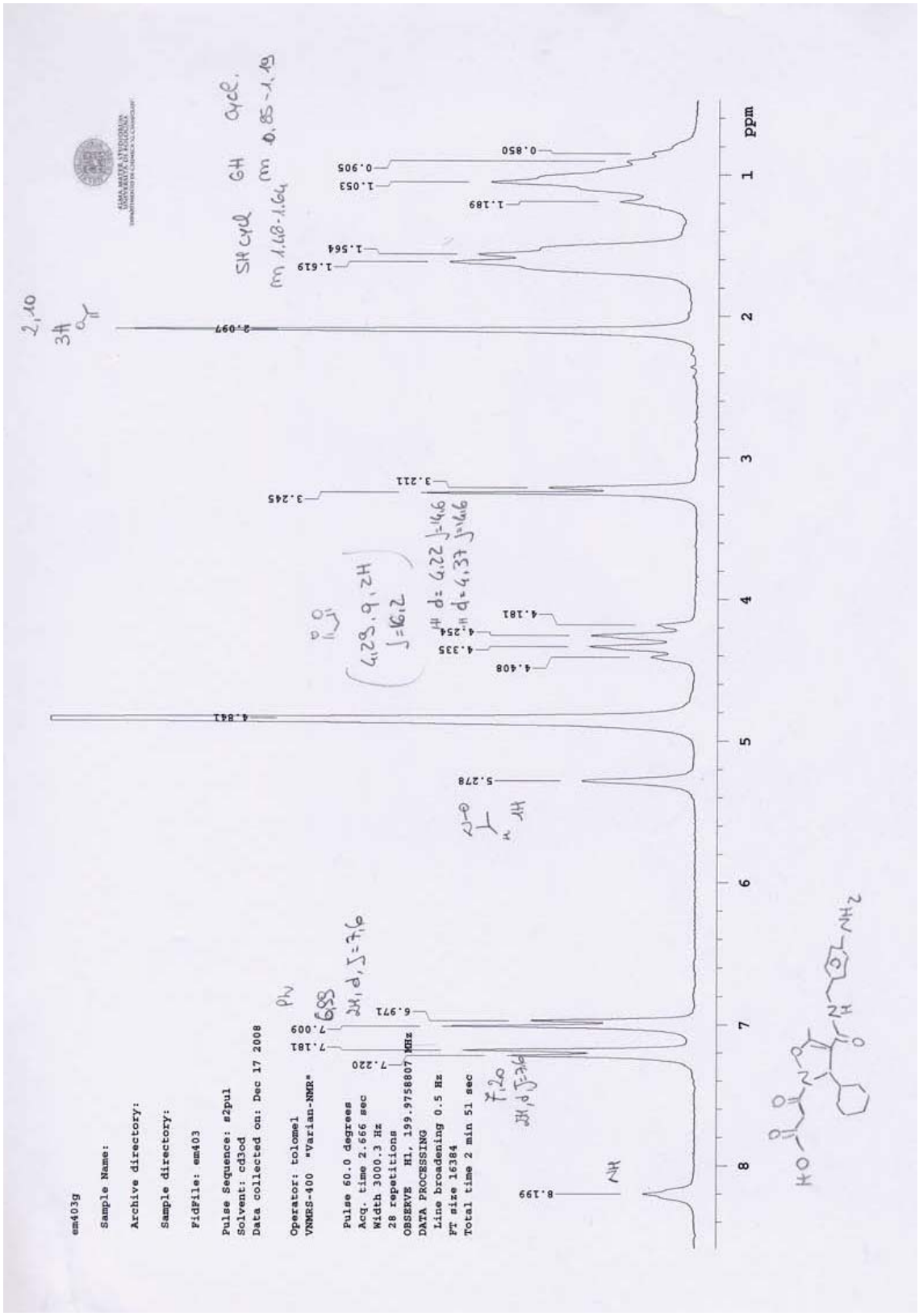
Compound **14b**



Compound **14c**



Compound **14d**



Materials for bioassays

Trypsin/EDTA, non-essential amino acids, minimum essential medium (MEM), RPMI-1640 with L-glutamine, antibiotic, and antimycotic solution, and glycine were purchased from Invitrogen (Carlsbad, CA, USA). Fetal bovine serum (FBS) and phosphate buffered saline (PBS) were from Cambrex (Walkersville, MD, USA). Citrate buffer solution, EDTA, DMSO, Triton-X-100, 4-nitrophenyl N-acetyl-b-D-glucosaminide, phorbol 12-myristate 13-acetate (PMA), pyruvic acid, fibronectin from human plasma were obtained from Sigma–Aldrich SRL (Milan, Italy). SK-MEL-24 (human malignant melanoma) and K-562 (human erythroleukemia) cell lines were obtained from American Tissue Culture Collection (ATCC, Rockville, MD, USA).

Cell culture

SK-MEL-24 cells were routinely grown in MEM medium supplemented with 10% FBS, non-essential amino acids and sodium pyruvate. K-562 cells were maintained as a stationary suspension culture in RPMI-1640 + Lglutamine with 10% FBS. Cells were kept at 37 _C in a 5% CO2 humidified atmosphere. Fourty-hour before the experiment K-562 cells were treated with 25 ng/mL of PMA to induce differentiation with increased expression of cell surface antigens.

Adhesion assays

Plates (96-well) (Corning, New York, NY, USA) were coated by passive adsorption with fibronectin (10 lg/ mL) overnight at 4 _C. Cells were counted and exposed to different concentrations of the drug for 30 min at room temperature to allow the ligand–receptor equilibrium. Stock solutions (10_2 M) of the assayed compounds were prepared in 33% DMSO and 66% PBS (v/v); further dilutions were done in PBS alone. The highest rate of DMSO in the assays was 1% of the stock solution. Control cells were exposed to the same concentration of DMSO. At the end of the incubation time, the cells were plated (50,000 cells per well) and incubated at room temperature for 1 h. Then, all the wells were washed with PBS to remove the non-adherent cells, and 50 lL of the substrate of the exosaminidase (4-nitrophenyl N-acetyl-b-D-glucosaminide dissolved at 7.5 mM in 0.09 M citrate buffer solution, pH 5, and mixed with an equal volume of 0.5% Triton X-100 in water) was added. This product is a chromogenic

substrate for b-N-acetylglucosaminidase that is transformed in 4-nitrophenol whose absorbance is measured at 405 nm. As previously described,20 there is a linear correlation between absorbance and enzymatic activity. It is, therefore, possible to identify the number of adherent cells in treated wells, interpolating the absorbance values of the unknowns in a calibration curve. The reaction was blocked by adding 100 lL of a stopping solution (50 mM glycine, 5 mM EDTA, pH 10.4) and the plate was read in a Victor2 Multilabel Counter (Perkin-Elmer, Waltham, Massachusetts, USA). Experiments were carried out in quadruplicate. Data analysis and IC50 values were calculated using Graph-Pad Prism 3.0 (GraphPad Software Incorporated, San Diego, CA, USA).

Molecular Docking. All calculations were run using the Schrodinger suite of programs (http://www.schrodinger.com) through the Maestro graphical interface. (i) Protein Setup. The recently determined crystal structure of the extracellular domain of the integrin RVβ3 receptor in complex with cilengitide and in the presence of the proadhesive ion Mn2þ (PDB entry 1L5G) was used for docking studies. Docking was performed only on the globular head of the integrin because the headgroup of integrin has been identified in the X-ray structure as the ligand-binding region. The protein structure was set up for docking as follows; the protein was truncated to residues 41-342 for chain R and residues 114-347 for chain β. Due to a lack of parameters, the Mn2þ ions in the experimental protein structure were modeled via replacement with Ca2þ ions. The resulting structure was prepared using the Protein Preparation Wizard of the graphical user interface Maestro and the OPLSAA force field. (ii) Docking. The automated docking calculations were performed using Glide72 (Grid-based Ligand Docking with Energetics) within the framework of Impact version 4.5 in a Article Journal of Medicinal Chemistry, 2010, Vol. 53, No. 1 117 standard precision mode (SP). The grid generation step started from the extracellular fragment of the X-ray structure of the $\alpha_V\beta_3$ complex with cilengitide, prepared as described in Protein Setup. The center of the grid-enclosing box was defined by the center of the bound ligand. The enclosing box dimensions, which are automatically deduced from the ligand size, fit the entire active site. For the docking step, the size of the bounding box for placing the ligand center was set to 12 A ° . No further modifications were applied to the default

settings. The Glide-Score function was used to select 20 poses for each ligand. Glide was initially tested for its ability to reproduce the crystallized binding geometry of cilengitide. The program was successful in reproducing the experimentally found binding mode of this compound, as it corresponds to the best-scored pose.

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