MODIFIED AMINO ACIDS: SYNTHESIS AND APPLICATIONS

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A mio fratello e ai
miei genitori.

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2. Poster Presentations

- Sintesi stereoselettiva di 2,3-diamminoacidi, Stefania Capone, Romualdo Caputo, Giovanni Palumbo, Silvana Pedatella. *XXVIII Corso Estivo di Sintesi in Chimica Organica "A. Corbella"*, Gargnano (Italy), June 16th-20th, 2003.
- Synthesis of dipeptide taste ligands containing $\beta^{2,3}$-disubstituted amino acids: mimics of aspartame, Stefania Capone, Romualdo Caputo, Annalisa Guaragna, Giovanni Palumbo, Silvana Pedatella. *XXIX Corso Estivo di Sintesi in Chimica Organica "A. Corbella"*, Gargnano (Italy), June 14th-18th, 2004.
- β^2- and $\beta^{2,2}$- long chain substituted amino acids towards amphiphilic peptides. Stefania Capone, Romualdo Caputo, Annalisa Guaragna, Giovanni Palumbo Silvana Pedatella. *Ischia Advanced School of Organic Chemistry 2004,* Ischia (Italy), September 18th-23th, 2004 (selected for oral presentation).
- Una facile sintesi di *sin*- e *anti*-$\beta^{2,3}$-ammino acidi enantiopuri, Adele Bolognese, Stefania Capone, Romualdo Caputo, Antonio Lavecchia, Luigi Longobardo, Ettore Novellino, Antonello Santini, Angela Tuzi. *Giornate Scientifiche Interpolo - Polo delle Scienze e delle Tecnologie per la Vita - Polo delle Scienze e delle Tecnologie*, Napoli (Italy), May 26th-27th, 2005.
- Synthetic efforts towards oligomers of amino acids bearing two hexadecyl or two arginine side chains for cell-wall interaction and cell penetration. Albert. K. Beck; Stefania Capone, Romualdo Caputo, Dieter Seebach. *FEBS Summer School – Chemistry Meets Biology*, Spetses Island (Greece), July 17th-29th, 2005.

3. Oral Presentations

- β^2- and $\beta^{2,2}$- long chain substituted amino acids towards amphiphilic peptides. *Ischia Advanced School of Organic Chemistry 2004,* Ischia (Italy), September 18th-23th, 2004.

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Abbreviations

AcOH	Acetic Acid
ADDA	3-amino-10-phenyl-2,6,8-trimethyl-9-metoxy-4,6-decadienoic acid
ADI	Acceptable Daily Intake
Aib	Amino isobutyric acid
cAMP	cyclic Adenosyl Monophosphate
AP-M	Aminopeptidases-M
Boc	*tert*-butoxycarbonyl-
bw	body weight
CAN	Cerium Ammonium Nitrate
Cbz	Benzyloxycarbonyl-
CD	Circular Dicroism
DAB	Diaminobutanoic acid
DAP	Diaminopropanoic acid
DBAD	Di-*tert*-butylazadicarboxylate
DIPEA	Diisopropylethylamine
DGI	Direct G-protein Interaction
DMAP	*N*,*N*-dimethylaminopyridine
DMF	Dimethylformamide
DMSO	Dimethylsulfoxyde
DNA	Deoxyribonucleic Acid
EtOAc	Ethylacetate
FDA	Food and Drug Adminisrtation
Fmoc	Fluorenylmethyloxycarbonyl-
GPCRs	G-Protein-Coupled Receptors
GR	Glycine Receptor
GRAS	Generally Regarded As Safe
GTP	Guanosine Triphosphate
ICAT	Isotope Coded Affinity Tag
IP3	Inositol Trisphosphate
IR	Infra Red Spectroscopy
KHMDS	Potassium hexamethyldisilylamide
LDA	Lithium diisopropylamide
LiHMDS	Lithium hexamethyldisilylamide
MeOH	Methanol
Mp	Melting Point
MS	Mass Spectrometry
NKA	Neurokinin A
NMM	*N*-methylmorpholine
NMR	Nuclear Magnetic Resonance
NRPSs	Non-Ribosomal Peptide Synthases
PDPI	Polystyryl Diphenylphosphine-Iodine complex
PKU	Phenylketonuria
RNA	Ribonucleic Acid
mRNA	messanger RNA
tRNA	transfer RNA
SAR	Structure-Activity Relationship
SIDA	Stable Isotope Dilution Assay
SR	Sugar Receptor
TBAF	Tetrabutylammoniumfluoride
TEA	Triethylamine
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography
TRCs	Taste Receptor Cells
Z	Benzyloxycarbonyl-

Panoramica Generale delle Tematiche Trattate nel Lavoro di Tesi

Introduzione

La ricerca di farmaci nuovi e stabili spinge sempre più i chimici di sintesi alla ricerca di molecole in grado di mimare la struttura e l'attività delle molecole naturali biologicamente attive, migliorandone le caratteristiche di stabilità nel mezzo biologico. Nell'ambito, quindi, delle scienze biologiche, chimiche e farmacologiche, si fa sempre più forte l'interesse verso la peptidomimetica. I peptidomimetici, infatti, sono molecole ispirate a peptidi naturali, ma in grado di offrire numerosi vantaggi, se paragonati ai farmaci peptidici classici; oltre a presentare un'accresciuta efficienza e selettività, i peptidomimetici possono avere minori effetti collaterali, presentare una migliorata somministrazione orale e una prolungata attività farmacologica, non essendo soggetti alla degradazione enzimatica.

Un approccio peptidomimetico che è emerso negli ultimi anni con particolare veemenza, consiste nell'uso di amminoacidi non-proteinogenici nella costruzione di foldameri, specialmente peptidici. L'uso di tali amminoacidi è giustificato dal fatto che i peptidi che li contengono formano strutture secondarie stabili, presentano nuove attività biologiche e possono essere usati per il design di strutture molecolari e catalizzatori.

Inoltre amminoacidi non-proteinogenici o non-ribosomiali possono avere pronunciati effetti sulla conformazione degli scheletri peptidici.

Per esempio, amminoacidi α,α-disostituiti, come l'Aib e la α-etilalanina, conferiscono rigidità allo scheletro peptidico attraverso la formazione di α-eliche e β-turn. Queste strutture organizzate sono responsabili dell'attività biologica dei 'peptaboil', un gruppo di peptidi antibiotici isolati da funghi e caratterizzati da una larga percentuale di residui Aib. Nei doppi strati lipidici delle membrane biologiche, i 'peptaboil' danno origine a canali ionici voltaggio-dipendente che sono simili a quelli ritrovati nelle sinapsi neuronali e, ad alte concentrazioni, possono causare la lisi cellulare. I residui α,α-disostituiti sono inoltre trovati in peptidi di natura differente, come nella clamidocina, un tetrapeptide ciclico inibitore della proliferazione cellulare, nella quale essi hanno simili effetti conformazionali.

I β-amminoacidi sono ritrovati in animali, microrganismi, organismi marini, sia in forma libera che come derivati, e posseggono un'ampia varietà di proprietà farmacologiche. Essi vengono rinvenuti in peptidi naturali ad attività antibiotica e antimicotica, e vengono inoltre utilizzati per costruire β-peptidi per uso terapeutico. Piccole catene di β-peptidi, ad esempio, hanno mostrato attività inibitrice verso una proteina di membrana coinvolta nei processi di assorbimento di colesterolo e lipidi, mentre altre posseggono attività antimicrobica e antiemolitica. β-Peptidi con un'alta concentrazione di cariche positive attraversano le membrane biologiche di batteri e mammiferi e possono essere considerati un nuovo gruppo di 'cell penetrating peptides'. Inoltre essi posseggono un'elevata stabilità contro le peptidasi e le proteasi, sia *in vitro* che *in vivo*. Infine, essi possono adottare strutture secondarie stabili come α-eliche, β-turn e sheet.

L'importanza farmacologica dei peptidi non-ribosomali giustifica la considerevole attenzione rivolta alla loro preparazione; quest'ultima richiede efficienti sintesi degli amminoacidi costituenti. Sebbene non sia sempre tenuto nella dovuta attenzione, il coupling di questi residui inusuali è spesso difficoltoso, a causa della ridotta reattività dei residui amminoacidici nella formazione dei legami peptidici.

Linee Generali

Tenendo presente tutto quanto sopra, come parte del nostro studio di amminoacidi non-naturali, abbiamo deciso di mettere a punto nuovi e semplici metodi di sintesi stereoselettiva di amminoacidi non-proteinogenici; inoltre abbiamo preso in considerazione lo studio delle loro possibili applicazioni nel campo delle tecnologie alimentari e farmaceutiche.
In particolare, gli obiettivi raggiunti sono:

- Sintesi stereoselettiva di $\beta^{2,3}$-amminoacidi (2,3-disostituiti) attraverso l'introduzione sulla posizione C-2 di β^3-amminoacidi di:

1. Gruppi polari (OH, NH_2, CH_2OH)
2. Gruppi non polari (CH_3, CH_2Ph)
3. Deuterio, con completa diteurazione della posizione C-2.

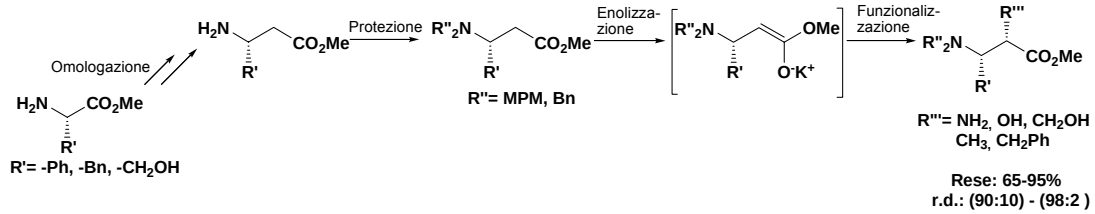
- Sintesi di nuovi mimici dell' aspartame:
  1. Sintesi in soluzione di diversi dipeptidi contenenti β-amminoacidi 2,3-disostituiti
  2. Studi conformazionali, attraverso dicroismo circolare (CD) e dinamica molecolare, di questi dipeptidi
- Sintesi di amminoacidi 'lipidici':
  1. Introduzione di lunghe catene idrofobiche sulla posizione C-2 di β-alanina
  2. Sintesi di peptidi contenenti questo tipo di β-amminoacidi 2,2-disostituiti
- Sintesi di α-amminoacidi α,α-disostituiti:
  1. Sintesi di peptidi contenenti α-amminoacidi α,α-disostituiti: nuovi 'cell penetrating peptides (CPPs)'

Resultati e discussione

Nella prima fase dei nostri studi, abbiamo messo a punto un'introduzione stereocontrollata sulla posizione C-2 di β^3-amminoacidi di gruppi polari (OH, NH_2, CH_2OH) e non polari (CH_3, CH_2Ph).

Recentemente, abbiamo riportato la preparazione di 2-idrossi-β^3-amminoacidi enantiomericamente puri attraverso l'ossidrilazione di enolati chirali di β^3-amminoacidi.

Benzil *N,N*-diprotetti β^3-ammino metil esteri, preparati dai loro corrispondenti α-amminoacidi, sono stati trattati con potassio esametildisilazanuro (KHMDS), e il risultante enolato è stato fatto reagire con un elettrofilo per ottenere i derivati 2-sostitutiti.



Schema 1

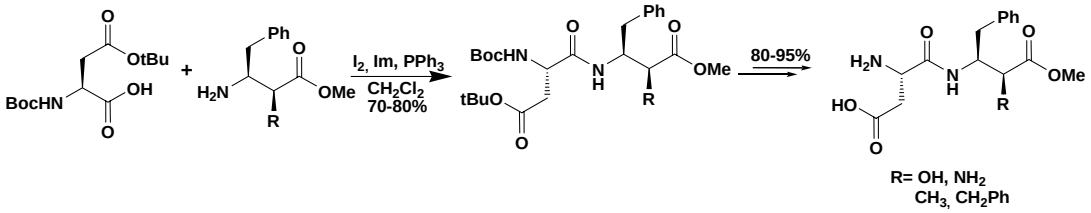
L'induzione asimmetrica è esercitata dal centro stereogenico dell'amminoacido di partenza, il contributo del quale è amplificato dalla presenza di gruppi protettori ingombranti sulla funzione amminica. Le rese e i rapporti diastereoisomerici sono molto elevati in tutti i casi da noi presi in considerazione.

Nella seconda parte del lavoro di tesi, i $\beta^{2,3}$-amminoacidi sono stati usati per la sintesi di una serie di dipeptidi del gusto. La scoperta del dolcificante aspartame [H-(L)-Asp-(L)-Phe-OMe] ha infatti ispirato la sintesi di un largo numero di analoghi peptidici con l'obiettivo di trovare nuovi composti, non solo con un migliorato profilo dolcificante, ma specialmente più stabili alla degradazione proteolitica.

Infatti è noto che l'aspartame è idrolizzato dalle esterasi dell'intestino ad acido aspartico, metanolo e fenilalanina. Quindi, come fonte di fenilalanina, l'aspartame risulta pericoloso per persone affette da fenilchetonuria.

In accordo con i modelli proposti per le interazioni dolcificante-recettore, la disponibilità di analoghi conformazionalmente rigidi è molto importante per stabilire le caratteristiche molecolari richieste per esplicare un gusto dolce o amaro. L'incorporazione di amminoacidi conformazionalmente costretti costituisce uno strumento essenziale per esplorare questo aspetto. Molti analoghi conformazionalmente costretti dell'aspartame sono stati sintetizzati e studiati: in ognuno di questi, il residuo di L-Phe è stato sostituito da diversi residui amminoacidici, inclusi β-amminoacidi. In questo modo, inoltre, si è eliminata la produzione di fenilalanina, generata dall'idrolisi dell'aspartame. E' riportato che β-amminoacidi 2,3-disostituiti sono conformazionalmente più rigidi dei semplici β^2 e/o β^3-amminoacidi. Come

estensione del nostro studio sulla sintesi di $\beta^{2,3}$-amminoacidi, abbiamo quindi preso in considerazione il loro uso nella sintesi di nuovi peptidi analoghi dell'aspartame, in cui il $\beta^{2,3}$-amminoacido è utilizzato per la sostituzione del residuo di L-Phe.
Si sono quindi preparati diversi peptidi, come mostrato nello schema 2.



Schema 2

La ricerca volta alla delucidazione dei dettagli delle relazioni struttura-attività dell'aspartame e dei suoi analoghi ha condotto all'ampia accettazione della teoria di Goodman per la predizione del gusto dolce. Per poter comparare le strutture dei nostri peptidi con le ben note strutture di altri peptidi ad attività dolcificante, abbiamo combinato alla sintesi, studi di dinamica molecolare e di dicroismo circolare.

I profili CD dei nostri peptidi sono sovrapponibili a quelli dell'aspartame. Le uniche differenze possono essere interpretate alla luce di un diverso numero di conformeri a bassa energia, i cui contributi CD si annullano reciprocamente. I nostri peptidi risultano quindi più flessibili rispetto all'aspartame, sebbeno le loro caratteristiche strutturali siano più vicine a quelle dell'aspartame di altri peptidi noti in letteratura.

Dai dati di dinamica molecolare risulta che i peptidi presentano diverse conformazioni che corrispondono a minimi di energia; tra queste in ogni caso c'è anche la conformazione a "L-shape", necessaria per l'interazione della molecole con il recettore del gusto.

Una terza parte del progetto di dottorato è stata, infine, focalizzata allo studio della sintesi e della reattività di α- e β-amminoacidi α,α-disostituiti.

La sintesi di peptidi contenenti residui α,α-disostitutiti ingombranti, come l'Aib, è infatti complicata da numerosi problemi. Ciclizzazioni a dichetopiperazine, attribuite all'effetto dei gruppi alchilici geminali, possono avvenire facilmente con certi peptidi contenenti Aib e residui analoghi, in seguito alla deprotezione del sito *N*-terminale.

La congestione sterica dei residui α,α-disostituiti impedisce inoltre la formazione dei legami peptidici e, come atteso, i coupling sono particolarmente difficili quando entrambi i gruppi amminico e carbossilico sono ingombrati.

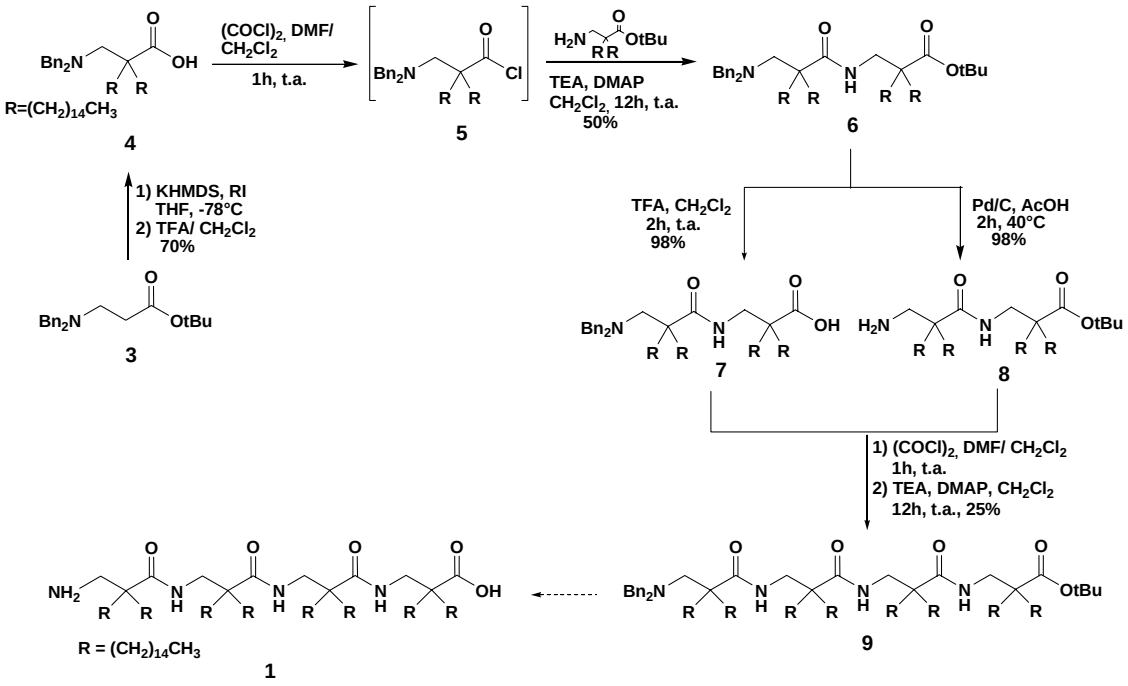
D'altra parte i β-amminoacidi α,α-disostituiti dovrebbero poter essere accoppiati mediante i metodi di sintesi convenzionali (EDC/HOBt), senza incorrere in particolari complicazioni, come quelle che invece si sperimentano con α-amminoacidi α,α-disostituiti stericamente ingombrati.

Quindi abbiamo deciso di introdurre, sulla posizione C-2 di β-amminoacidi, lunghe catene idrofobiche, in quanto è riportato che peptidi 'coniugati' con α-amminoacidi lipidici mostrano una migliorata permeabilità verso le membrane cellulari e un'accresciuta stabilità biologica.

Per la preparazione di β-amminoacidi α,α-disostituiti sono noti molti metodi; noi abbiamo scelto quello sviluppato nel nostro laboratorio: alchilazione dell'estere metilico dell'acido 3-amminopropanoico *N*-benzil protetto **3** *via* doppia deprotonazione.

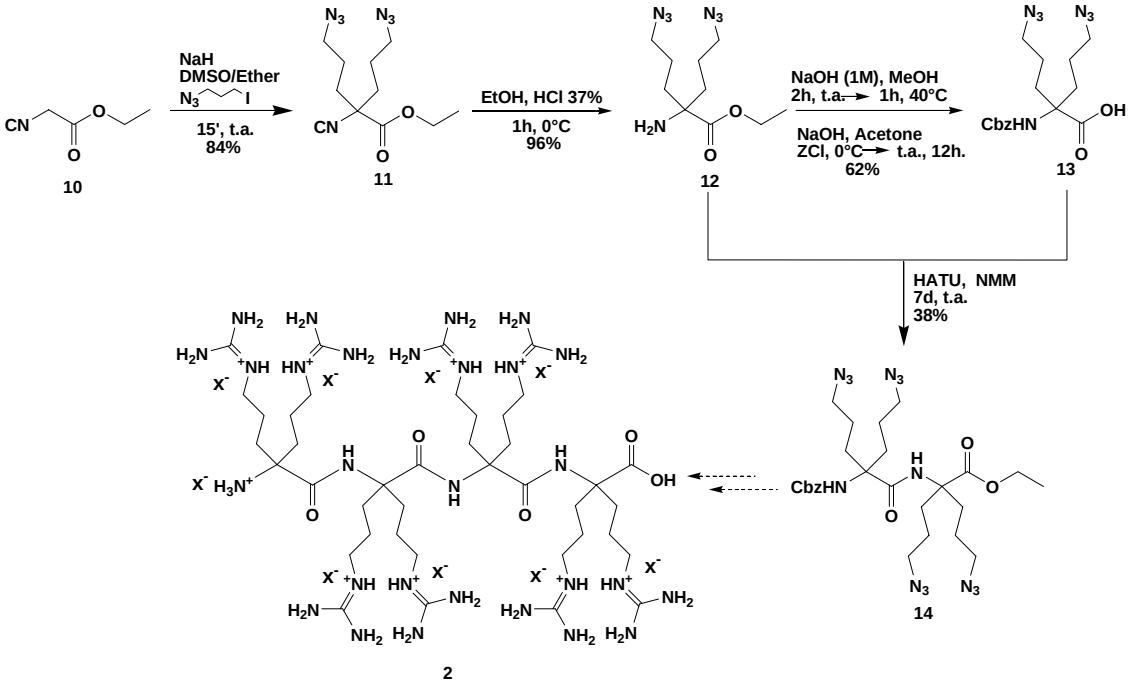
Per la preparazione del β-tetrapeptide α,α-disostituito **1** abbiamo quindi usato come building block la β-alanina 2,2-disostitutita protetta **4**, ottenuta per doppia alchilazione dell'estere *tert*-butilico dell'acido 3-(dibenzylamino)propanoico **3**, e successiva idrolisi del gruppo estereo.

Come in parte atteso, il coupling condotto in maniera convenzionale non ha funzionato sul nostro $\beta^{2,2}$-amminoacido; l'attivazione della funzione carbossilica è stata realizzata con ossalil cloruro per generare un acil cloruro più reattivo, come è riportato nello Shema 3.



Schema 3

Infine, come parte di un progetto sviluppato in collaborazione con il Prof. Seebach, presso lo Swiss Federal Institute of Technology (ETH) in Zurigo, abbiamo preparato un derivato α,α-dialchilato della glicina, recante catene laterali che verranno poi convertite in analoghe catene laterali dell'arginina (Schema 4). Tali amminoacidi costituiscono i building block per la sintesi di peptidi ad alta presenza di cariche positive; questo tipo di peptidi è in grado di legare e attraversare le membrane biologiche, costituendo così una potenziale nuova classe di 'cell penetrating peptides (CPPs)'. Per tale motivo, il primo passo è stato lo sviluppo di una procedura per la preparazione dell'isocianato **11**: la doppia alchilazione dell'etil isocianato **10** ha fornito il prodotto di dialchilazione con due gruppi ω-azido-propilici, in eccellenti rese (Schema 4). Le opportune modificazioni dei gruppi funzionali hanno poi condotto all'ammino etil estere **12** e all'acido *N*-Cbz protetto **13**, che sono stati poi accoppiati con HATU/NMM (Schema 4). Come da attendersi per quanto accennato in precedenza sulla reattività di residui amminoacidici a,a-disostitutiti, questa reazione di coupling è molto lenta (7 giorni) e procede con solo il 38% di resa.


NaH
DMSO/Ether
15', t.a.
84%
EtOH, HCl 37%
1h, 0°C
96%
NaOH (1M), MeOH
2h, t.a. → 1h, 40°C
NaOH, Acetone
ZCl, 0°C → t.a., 12h.
62%
HATU, NMM
7d, t.a.
38%
10
11
12
13
14
2

Schema 4

Summary

The peptidomimetic research continues to inspire medicinal chemists for seeking either potential drugs or pharmacological tools.

A peptidomimetic approach that has emerged in recent years, with significant potential, consists of the use of non-proteinogenic amino acids in the construction of foldamers, especially peptides. The use of non-proteinogenic amino acids is accounted for by the capability of non-natural amino acid containing peptides to form unique secondary structures, to show novel biological activities, and to be used as rulers for the design of molecular devices and catalysts.

Having all this in mind, as a part of our interest in the chemistry of non-natural amino acids, we planned to devise new and simple methods for stereoselective synthesis, readily applicable also on large scale, of non-proteinogenic amino acids; moreover, we decided to study their possible exploitation in food technologies and pharmaceutical applications.

In particular, the goals we have reached so far are:

- Stereoselective synthesis of $\beta^{2,3}$-amino acids (2,3-disubstituted) by introduction at the C-2 position of β^3-amino acids of:
    1. Polar groups (OH, NH_2, CH_2OH)
    2. Non polar groups (CH_3, CH_2Ph)
    3. Deuterium, with total dideuteration of C-2 position.
- Synthesis of new mimics of aspartame:
    3. Solution-phase synthesis of different dipeptides containing $\beta^{2,3}$-disubstituted β-amino acids
    4. Conformational studies, carried out by circular dicroism (CD) and molecular dynamics, of such dipeptides
- Synthesis of 'lipidic' amino acids:
    1. Introduction of long hydrophobic chains at C-2 position of β-alanine
    2. Synthesis of peptides containing this kind of 2,2-disubstituted β-alanine
- Synthesis of α,α-disubstituted α-amino acids
    1. Synthesis of peptides containing α,α-disubstituted α-amino acids: new cell penetrating peptides (CPPs)

Chapter 1

Proteins and Peptides

Proteins constitute a class of biomacromolecules which virtually affect every feature that characterizes a living organism.

All organisms make use of many chemical reactions to supply themselves continually with energy, but these reactions, by themselves, could not occur fast enough under physiological conditions to sustain life. The rate of these reactions in organisms is increased, of many orders of magnitude, by the presence of enzymes, which are proteins. Expression of the encoded genetic information of nucleic acids, essential for life and mostly specific for the structure of a protein, depends almost entirely on proteins. Proteins store and transport a variety of particles ranging from macromolecules to electrons. They are the crucial components of muscles and other systems for converting chemical energy into mechanical energy. They also are necessary for sight, hearing, and the other senses. Many others are simply structural, providing the filamentous architecture within cells and the materials that are used in hair, nails, tendons and bones of animals.

In spite of these diverse biological functions, proteins represent a relatively homogeneous class of molecules. All are the same type of linear polymer, built on various combinations of 20 amino acids. They differ only for the sequence in which the amino acids are assembled into polymeric chains. The secret of their functional diversity lies partly in the chemical diversity of the amino acids but even more in the diversity of the three-dimensional structures that these building blocks can form, simply by being linked in different sequences. The awesome functional properties of proteins can be understood only in terms of relationship to their three-dimensional structures.

A series of incisive studies in the late 1950s and early 1960s revealed that the amino acid sequences of proteins are genetically determined. Amino acid sequences are important for several reasons. First, knowledge of the sequence of a protein is very helpful, indeed usually essential, in elucidating its mechanism of action, and proteins with novel properties can be generated by varying the sequence of known structures. Secondly, analyses of relations between amino acid sequences and three-dimensional structures of proteins are uncovering the rules that govern the folding of polypeptide chains. Amino acid sequence is the link between the genetic message in DNA and the three-dimensional structure that performs a protein's biological function. Third, alterations in amino acid sequences can produce abnormal function and disease. Fatal diseases, such as sickle-cell anaemia and cystic fibrosis, can result from a change in a single amino acid in a single protein. Fourth, the sequence reveals much about the evolutionary history of a protein. Consequently, molecular events in evolution can be traced from amino acid sequences.

Peptidomimetics

The peptidomimetic research continues to inspire medicinal chemists for seeking either potential drugs or pharmacological tools.[1] In biological, chemical, and pharmaceutical areas, in fact, they offer interesting advantages over physiologically active peptides, which as active substances are crucial for living organisms. Beside increasing efficiency and selectivity of natural peptides, peptidomimetics may decrease side effects, improving oral bioavailability and prolonging the pharmacological activity by hindering enzymatic degradation within the organism.[2]

Natural peptides and proteins are of key importance in many chronic and infectious disorders. Prion diseases, for instance, are a result of conformational changes of a protein, while the development of Alzheimer's disease is associated with deposition of β-amyloid peptides in insoluble form. Autoimmune diseases are characterized by recognition dysfunction, self-peptides being attacked by immune system as foreign molecules.

At the same time, peptides as therapeutic agents are of very limited use. Peptides are highly flexible molecules, easily degraded by proteases and usually too polar to pass membranes that separate them from their targets in the cells.

Considerable efforts have been made in designing compounds with improved bioavailability and capability to mimic peptide functions, resulting in a heterogeneous class of compounds known as peptidomimetics.

From a structural point of view peptidomimetics can be prepared by approaches ranging from the slight modification of the initial structure of a peptide to the generation of a pure non-peptide.

The very first steps in peptidomimetic design were based on simple N– or C–terminus modifications, single amino acid replacement or exchange of natural with unnatural side chains as well as of L- with D-amino acids.

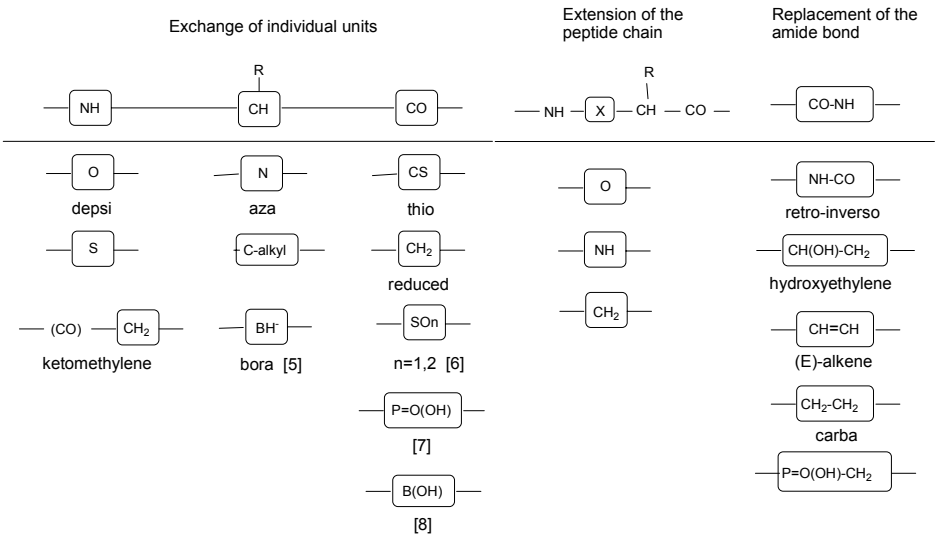


Table 1.1 The most frequent modifications of the peptide backbones (modified from Wiley-VCH Angewante Chemie, Int. Ed., ref. 2)

Modification of the peptide backbone generally involves the exchange of units in the peptide chain with sterically or electronically equivalent units, as well as the introduction of additional functions. The most general modifications[2] are synoptically shown in Table 1.1.

Cyclization reactions are very useful in the synthesis of peptidomimetic. The conformation of a peptide can be stabilized or blocked by the introduction of bridges of various lengths between different parts of the molecule. The bridging can either

occur within a single amino acid residue, or involve several amino acid residues. Bridges can be introduced at various sites and involve various backbone regions. The bridge can be a link between two side chains, between two backbone units, or between a side chain and backbone unit. In addition the peptide bond can be completely or exclusively incorporated into a ring.

There are also interesting examples of cyclic peptides and peptidomimetics having a sugar moiety. Sugar amino acids offer many possibilities in the preparation of peptidomimetics with predictable conformational characteristics.

Few examples of the tremendous amount of available peptidomimetic compounds with structural motifs ranging from peptide chains to completely non-peptidic components are shown[2] in Figure 1.1.

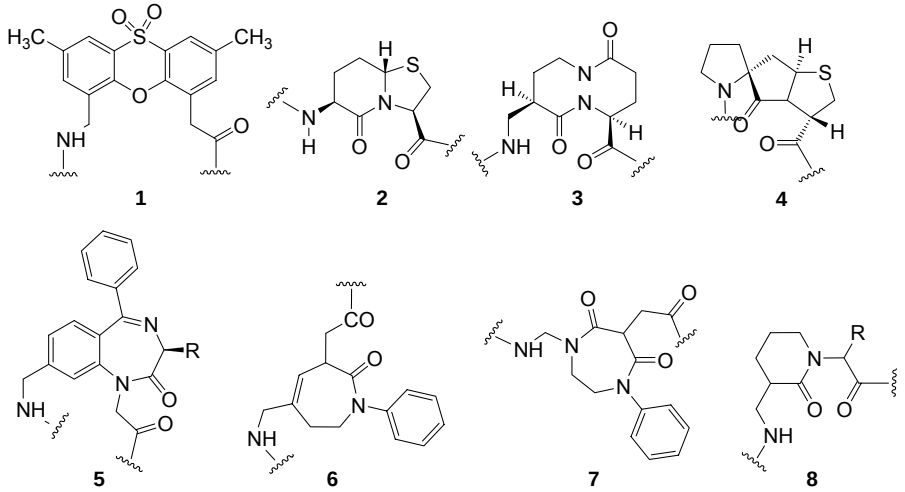


Figure 1.1 Structural motifs of some peptidomimetics.

Finally, peptides can be constructed with completely non-natural amino acids, such as β-amino acids,[3] γ-amino acids,[3] and α,α-disubstituted amino acids.[4] The use of non-proteinogenic amino acids has aroused much attention among organic as well as peptide and medicinal chemists, because non-natural amino acid containing peptides form unique secondary structures,[3] show novel biological activities,[5] and can be used as rulers for the design of molecular devices and catalysts.[6]

Peptidomimetic Drug Design

de novo Design

A goal, that has not been achieved yet, is the *de novo* design of peptidomimetics.[2] Presently, natural substrates or model structures identified by screenings (plant or animal extracts, substances isolated from microorganisms, compound libraries) are required as the starting point to develop new bioactive compounds. In contrast, *de novo* design implies development without such a starting point. Thus, the structure of a peptidomimetic drug would be developed, for example, based only on the known amino acid sequence of the natural peptide or the natural interaction partner. Then the final goal of *de novo* design would be of immense practical value in medicinal chemistry; it is enough to consider that the research in drug development is still based on empirical findings: thousands of compounds must be synthesized and tested before one of them is deemed marketable by FDA. The other compounds are discarded during stringent pharmacological, toxicological and clinical tests. Certainly with the help of *de novo* development this rejection rate could be drastically reduced.

A step in the direction of *de novo* design has been taken by various computer programs which have the task of calculating new active structures based only on the active site of the enzyme.

Rational Drug Design

Rational drug design is the oldest alternative to medicinal chemistry drug development procedures. Thanks to the recent technological progresses, it became an integral part of the drug development process. Highly refined methods of conformational analysis (e.g. NMR, X-ray structural analysis) and the new computer programs have made an important contribution to the current state of the art. Coupled with rapid through-put screening technology, this approach is becoming the predominant method used in drug discovery programs. In addition, synthetic chemistry has also made great progress; without the modern methods developed by organic chemists the construction of complex, stereochemically defined molecules with particular functional groups and hydrophobic elements attached to a non-peptidic scaffold would hardly have been possible.

A peptidomimetic approach that has emerged in recent years with significant potential, is the incorporation of non-natural amino acids into proteins. In this way it is possible to give the protein structure specific properties, that increase its stability to proteolytic degradation and its activity as drug.

Some of the techniques used to develop new peptidomimetics are briefly described below.[7]

Bioconjugation

Bioconjugation[8] is the simplest and longest standing method for the introduction of non-natural amino acids into proteins. The approach relies on the existing functionality of a protein, and through the use of appropriate chemical reagents, some chosen amino acid side chains can be modified. All side chains displaying reactive functionality can be modified, and, depending on the functionality in question and the choice of modifying reagents, highly selective, quantitative modifications can take place.

The most widely used bioconjugation strategy exploits the latent nucleophilicity of the thiol side chain of cysteine. Through the use of appropriate electrophiles thiol groups can be rapidly, selectively and quantitatively modified. Cysteine modifications fall into two major categories: alkylation and mixed disulfide formation. Within these categories further subdivisions can be made according to the choice of modifying reagent employed.

Non-Ribosomal Peptide Synthesis

In eukaryotes, the synthesis of cellular peptides and proteins is conducted by the ribosomal machinery, in which the genetic information encoded in mRNA is translated into specific peptide sequences by a protein/RNA complex that moves progressively along each mRNA strand. In prokaryotes, a number of biologically important peptides are synthesised by a different mechanism, in which the entire sequence of a peptide is dictated by the structure of the enzyme that synthesizes it. This process, non-ribosomal peptide synthesis,[9] is directed by large modular enzymes termed non-ribosomal peptide synthases (NRPS). NRPSs are responsible for the synthesis of a diverse set of peptides, including several antimicrobial agents, and peptide fragments that are subsequently incorporated into

other secondary metabolites, such as the tripeptide δ-(L-α-aminoadipoyl)-L-cysteinyl-D-valine (Figure 1.2),[10] a precursor in the synthesis of penicillins and cephalosporins.

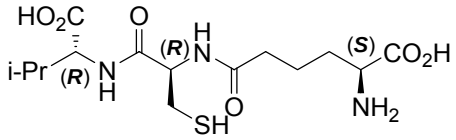


Figure 1.2 δ-(L-α-Aminoadipoyl)-L-cysteinyl-D-valine.

A unique feature of NRPSs is the diversity of the peptides that can be synthesised. Whereas ribosomal peptide synthesis produces linear peptides composed of all L-amino acids, containing only minor post-translational modifications, the non-ribosomal peptide synthesis produces an array of chemically diverse peptides that frequently contain drastic modifications, including cyclic structures, D-amino acid presence, *N*-methylation, unusual heteroaromatic groups, and series of *N*- and *C*-terminal modifications. The synthesis of each of these peptides is catalysed by a unique non-ribosomal peptide synthase. Each module of the synthase contains the requisite components for the activation and reaction of a single amino acid. Diversity is achieved by the recruitment of different components in each module according to the amino acid to be incorporated.

tRNA Engineering Method

Over the course of the last twenty-five years the research of several groups has been focused on finding a way to use the natural coded protein synthesis machinery to produce proteins that contain non-natural amino acids.

During the protein synthesis, the coded message of DNA is transcribed by RNA-polymerase in messenger RNA molecules (mRNA). Every mRNA is made up of 'codons'. Each codon codes for one of the twenty natural amino acids; it is made up of three nucleic-bases that are recognised by complementary three bases constituting an 'anti-codon' within a transfer RNA molecule (tRNA).

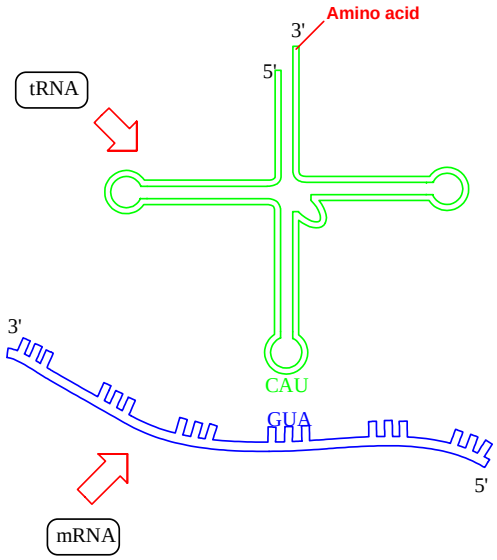


Figure 1.3 The codon reading event of coded protein synthesis.

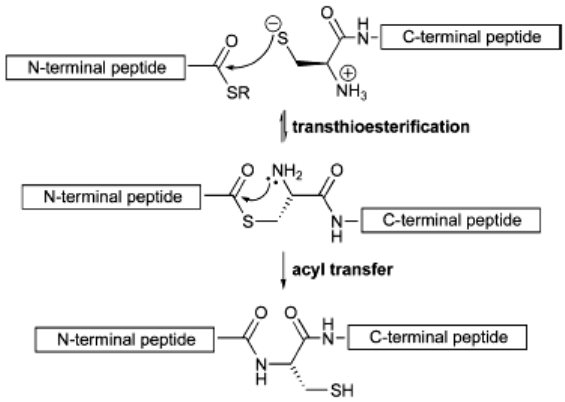
The recognition is made through Watson–Crick base-pairing. Each tRNA molecule is chemically linked to a specific amino acid that corresponds to the specific anti-codon present in that tRNA molecule as shown in Figure 1.3.

Thus, it would be possible to attach a non-natural amino acid to the tRNA to insert that non-natural amino acid in place of the originally expected one. However, in so doing a naturally occurring amino acid would be replaced by a non-natural amino acid in all instances, and the number of possible amino acids within a protein would still be limited to twenty. The idea is, thus, to attach non-natural amino acids to the anti-codon that possesses the complementary sequence to the stop-codon. The genetic code, indeed, also contains three codons that are used to send ‘‘stop’’ signals to the protein synthesis machinery and thus terminate translation when required. By using a tRNA molecule that possesses an anti-codon that is complementary to one of the stop codon signals it is possible to ‘‘suppress’’ the stop signal and in addition, insert an amino acid that is attached to the suppressor tRNA in place of the stop signal. There have been two main obstacles on this pathway: first, charging by chemical or enzymatic methods the tRNA molecules with non-natural amino acids can be challenging. Second, the existing tRNA aminoacylation system must not charge the suppressor tRNA with any other amino acid. This is critical in order to maintain the fidelity in terms of the amino acid composition of translated proteins. So the use of coded protein synthesis for the production of proteins containing non-natural amino acids opens up an immense number of possibilities.[3]

Peptide Ligation

The chemical ligation[11] is one of the most useful and important approach for the synthesis of proteins; it consists in the coupling of unprotected peptide fragments in acqueous solution trough the intervention of selective covalent bond-forming reaction. A number of selective reagents suitable for the covalent modification of proteins have been known for many years, but general methodologies for the ligation of peptide fragments through the formation of natural backbone amides have only recently become widely available. These methods have enormous potential for the preparation of macromolecules containing novel peptide or protein domains, and have proven particularly useful for the synthesis of proteins that are traditionally difficult to obtain by *in vivo* molecular biology-based approaches, such as membrane proteins.

The most widely applied ligation method is the reaction of an N-terminal cysteine of a peptide or protein with a C-terminal thioester of another peptide (Scheme 1.1).[11,12]



Scheme 1.1 Native chemical ligation: thioester-based method.

Following a reversible intramolecular transthioesterification to form a thioester bridge between the two peptides, intramolecular attack of the α-amino group of the C-terminal peptide furnishes the desired peptide bond. This approach, often termed 'native chemical ligation', requires that one of the peptides to be fused has a cysteine at the N-terminus, and produces a ligated product containing at least one cysteine. N-terminal cysteines are readily prepared by a number of conventional *in vitro* and *in vivo* methods. The major challenge with this approach is the synthesis of a peptide or protein with a C-terminal thioester.

Peptides Constructed from non-Proteinogenic Amino Acids

A peptidomimetic approach, which has emerged in recent years with significant potential, is the use of non-proteinogenic amino acids in the construction of foldamers, especially peptides.

As mentioned before, the use of non-proteinogenic amino acids has became important because non-natural amino acid containing peptides present peculiar characteristics.[3,5,6] Non-proteinogenic or non-ribosomal amino acids can have pronounced effects on the conformation of the peptide backbone.

For example, *N*-alkyl amino acids (or imino acids) exhibit reduced preferences for the *trans* conformation normally assumed by secondary amides, and this effect can lead to biologically relevant β-turn structures, similar to those often induced by proline residues.[13] *N*-Methyl peptides can form less intermolecular hydrogen bonds and this can significantly affect peptide secondary and tertiary structures, and as a result these peptides are useful for activity-structure relationship studies. *N*-Substituted peptides may also exhibit enhanced hydrophobicity and improved stability to proteolytic enzymes, which can increase bioavailability and therapeutic potential.[14] Bioactive natural peptides containing *N*-methyl residues are widespread and commonly occur in bacterial antibiotics, fungal metabolites such astentoxin and cyclosporine,[15] marine natural products such as dolastatins, jaspamides, and many others, and the cyanobacterial microcystins and nodularin, among others (see also Chapter 2 and NRPS).

α,α-Disubstituted amino acids, such as Aib and α-ethylalanine,[16] rigidify the peptide backbone through the formation of helixes and β-turns.[17] These organized structures are responsible for the interesting biological activity of the peptaibols,[18] a group of peptide antibiotics isolated from soil fungi and characterized by a high percentage of Aib residues. In bilayer membranes, the peptaibols give rise to voltage dependent ion channels that are reminiscent of those found at neuronal synapses[19] and, at high cellular concentrations, can cause cell lysis.[18c] α,α-Disubstituted residues are also found in non-peptaibol peptides, such as chlamydocin,[20] where they may have similar conformational effects.

β-Amino acids are found in animals, microorganisms, marine organisms, either in free form or as derivatives, and possess a wide range of pharmacological properties.[21] They are found in peptide natural products that exhibit antibiotic and antifungal, as well as cytotoxic activity. They are also employed to develop β-peptides for use as therapeutics. Short chain β-peptides have been shown to inhibit an intestinal membrane bound cholesterol- and lipid-transporting protein,[22] while others have been shown to possess antimicrobial and haemolytic activities.[5a,23] Cationic β-peptides cross bacterial and mammalian cell membranes and can be considered a new group of cell-penetrating peptides.[24] β-Peptides also show high stability against peptidases and proteases, both *in vitro* and *in vivo*,

compared with that of their α-peptide equivalents.[25] Finally, the early investigation of polymeric β-peptides indicated that they are able to adopt stable helical structures,[22,26] with later studies revealing that sheet structures and turns can also be adopted.[3,27]

The pharmacological importance of non-ribosomal peptides justifies the considerable effort devoted to their synthesis.

Chemical synthesis is useful for structure proof and for providing ample quantities of compounds that might not be available in suitable amounts from natural sources or through fermentation procedures. For peptide-based pharmaceuticals, synthetic manipulation of a lead compound may be required to reduce its toxicity or enhance its activity, selectivity, or bioavailability, or in some other way modify its pharmacological profile. An efficient laboratory synthesis of these peptides requires efficient syntheses of the constituent amino acids. Although it is often underestimated, the coupling of these unusual residues is sometimes difficult, due to the amino acid structures that can complicate the amide bond formation by conventional synthetic methods.

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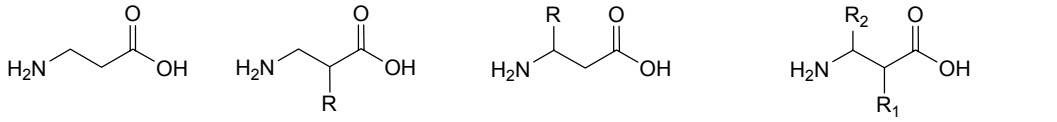
Chapter 2

β-Amino Acids and Derivatives

The use of peptidomimetics has emerged as a powerful mean for overcoming the limitations typical of peptides, thus for improving the therapeutic potential of the peptides themselves. A peptidomimetic approach that has emerged in recent years with significant potential, is the use of β -amino acids. β -Amino acids are similar to α -amino acids have two carbon atoms that separate amino group and carboxyl function, as represented by compound **1**; more in particular, the compounds with general formula **2**, that have a substituent at the C-2 position, are generally defined β^2-amino acids or α-substituted β-amino acids; compounds with general formula **3**, with a substituent at C-3 position are usually called β^3-amino acids or β-substituted β-amino acids and compound as **4** with both the C-2 and C-3 positions substituted, are usually named 2,3-disubstituted β-amino acids or α,β-disubstituted β-amino acids.[1]



β-amino acid (β-alanine) **1**

β^2-amino acid or α-substituted β-amino acid **2**

β^3-amino acid or β-substituted β-amino acid **3**

$\beta^{2,3}$-amino acid or α,β-disubstituted β-amino acid **4**

In all these compounds there is no direct interaction between carboxyl function and amino group: that obviously leads to significant differences between β- and α-amino acids, considering both physical properties and chemical reactivity.

β-Amino Acids in Nature

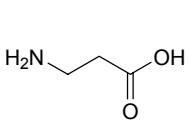
β-Amino acids, although not as abundant as their α-parents, are crucial structural subunits of numerous biologically active and natural products,[2] and have recently emerged as increasingly important class of compounds in medicinal chemistry.

In nature, β-amino acids are present either in free form or as building blocks in natural products and possess a wide range of pharmacological properties. They also exist in space, and have been detected, together with various α-amino acids, in meteorites.[3]

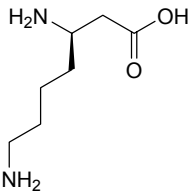
Probably the most common, and perhaps the most important, β-amino acid is H-βhGly-OH, β-alanine (**4**), an 'essential' amino acid component of pantothenic acid, coenyzme A, and carnosine in muscle tissue. Its conformational flexibility renders β-alanine a building block not only suited for helices and turns, but also for incorporation into linear peptide segments.[4]

Several β-amino acids are components of important antibiotics:[5] for example (3*S*)-β^3-lysine (**5)** is largely produced in *Streptomices* species and is present in

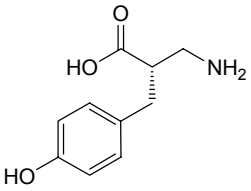
antibiotics such as Streptomycin F and Streptocrocyn.[6] β[2]-Tyrosine (**6**) and isoserine (**7**) are present in *Edeines*,[7] a huge group of peptidic antibiotic.



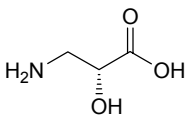
β-alanine
4



β-lysine
5



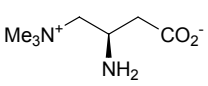
β-tyrosine
6



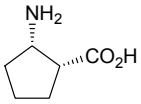
isoserine
7

Emeriamine (**8**) has hypoglycaemic and antiketogenic activities in rats after oral intake.[8] Cispentacin (**9**) is an antifungal antibiotic.[1]

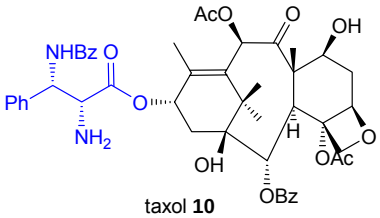
Functionalized β-amino acids are key components of a variety of bioactive molecules such as taxol (**10**) one of the most active antitumor agents which contains phenylisoserine as its side chain.[2c]



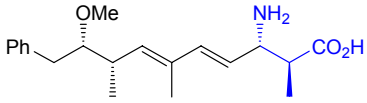
emeriamine **8**



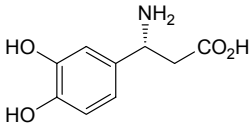
cispentacin **9**



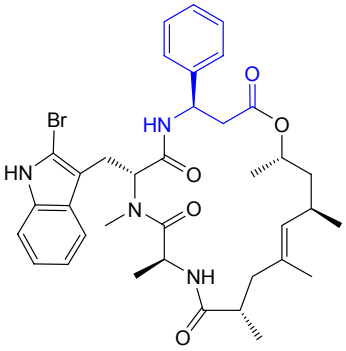
taxol **10**



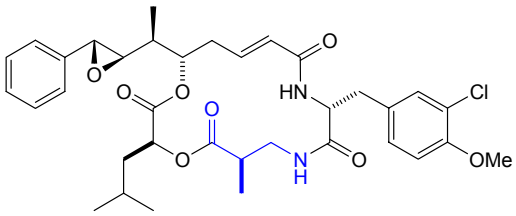
ADDA **11**



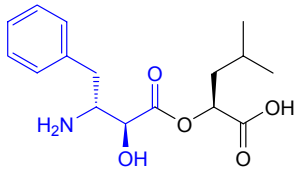
(*R*)-β-dopa **12**



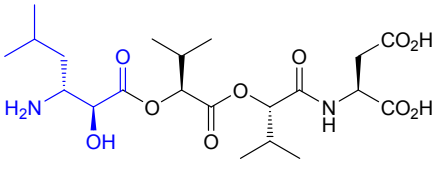
jaspakinolide **13**



cryptophycin **14**



bestatin **15**



amastatin **16**

The unsaturated β-amino acid ADDA (**11**) is present in the antibiotics cyanovinfin RR, nodularin, and microcystin LR.[9]

Furthermore, β-amino acids are also segments in peptidic natural products with various biological activities.

Steglich et al. have demonstrated that (*R*)-β-dopa, 3,4-dihydroxy-β-phenylalanine (**12**;) is present in mushroom *Cortinarius violaceus* as the Fe(III)–catechol complex, which gives to the fruit its blue-violet color.[10] Moreover several cyclic peptides, characterized by the presence of at least one β-amino acid residue are known; β-tyrosine, a β-aryl-β-amino acid, for example, is present in jasplakinolide (**13**), a sponge metabolite with potent insecticidal and antifungal properties.[11]

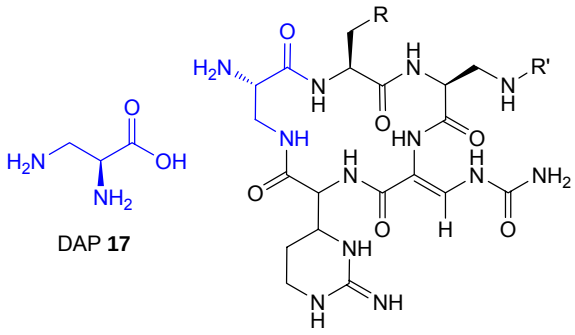
Other representative examples include cryptophycin 1 (**14**) a potent tumor-selective depsipeptide,[12] and the aminopeptidase inhibitors bestatin (**15**) and amastatin (**16**).[13]

2,3-Diamino Acids

2,3-Diamino acids are important non-proteinogenic amino acids, usually components of both natural and synthetic bioactive compounds.[14] Their origins and their functions are highly diversified: several studies have shown that a great number of 2,3-diamino acids are present in plants and microorganisms and show interesting pharmacological properties. For instance, they act as antibiotics, anticancer drugs, inhibitors of aminotransferases and inhibitors of proteases.

They are currently well recognized as key structural moieties in a variety of biologically active molecules.

(*S*)-2,3-Diaminopropanoic acid (DAP) (**17**), for example, represents an important structural element of capreomycin[15] (**18**) that, isolated from *Streptomyces capreolus*, represents a potent antitubercular drug employed as second-line therapeutic agent in combination with other antibiotics.



DAP **17**

capreomycin **18**

2,3-Diaminobutanoic acids[16] DABs (Figure 2.1) are present in several molecules with biological activity. The (2*S*,3*S*) diastereomer (**19**) is a component of several tuberculostatic heptapeptide antibiotics, including antrimycin[17] (**23**), cirratiomycin[18] (**24**), isolated from *Streptomyces xanthocidicus* and *S. cirratus* respectively. It was also found in the highly potent antibiotic hexapeptide lavendomycin[19] (**25**), isolated from culture filtrates of *Streptomyces lavendulae*; the latter has a low toxicity and a high antibiotic activity towards Gram-positive bacteria both *in vivo* and *in vitro*.

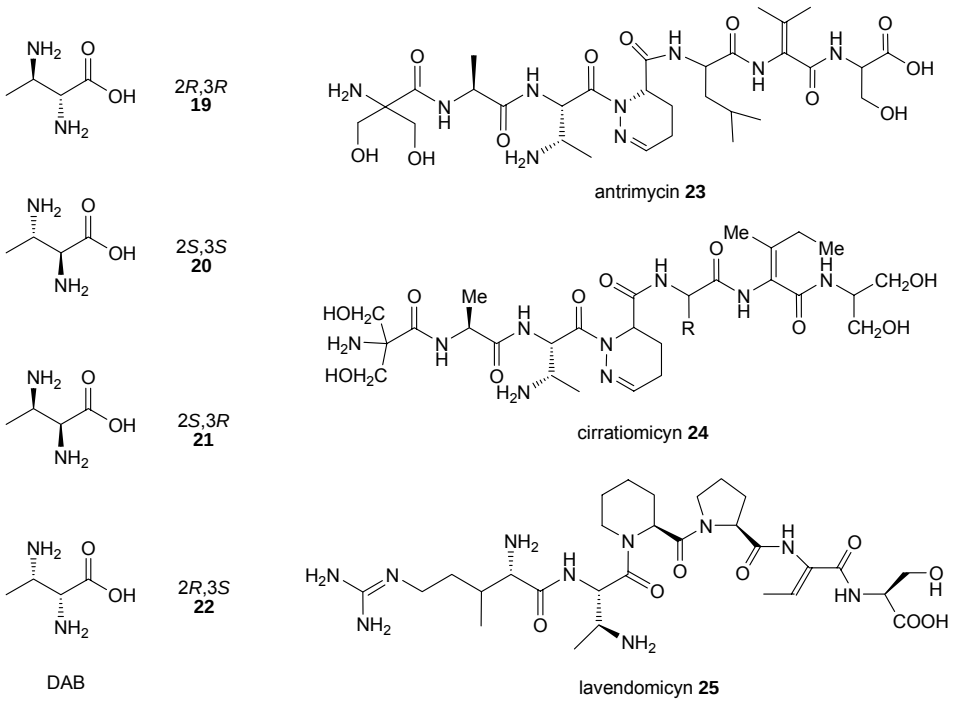
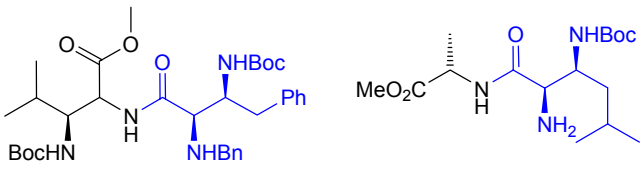


Figure 2.1 The four isomers of 2,3-Diaminobutanoic acids (DAB) and the natural molecules of which DAB is a component

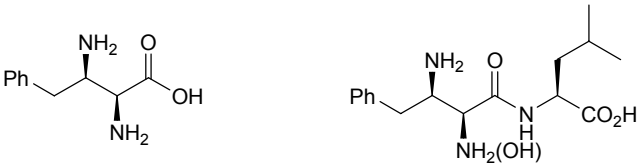
Moreover, DAB is present in plant extracts of acacia[20] and in some dipeptides with antimycotic activity,[19] such as **26** and **27**, isolated from *Micromonospora* and *Streptomyces spp*.; the antifungal activity is partially due to their ability to inhibit selectively glucosamine-6-phosphate synthetase of *Candida albicans*.



26 **27**

2,3-Diaminoacids are also used in the synthesis of mimics of biological active molecules.

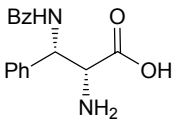
(2*S*,3*R*)-2,3-Diamino-4-phenylbutanoic acid[21] (**28**), for instance, is incorporated[22] in aminodeoxybestatin (**29**). The latter is a modified version of bestatin (**30**), a dipeptide isolated from *Streptomyces olivoreticuli*, with immune modifying, anticancer and analgesic activity. In aminodeoxybestatin, the hydroxy function is substituted by an amine function; this modification does not change the biological activity of the molecule that acts as an aminopeptidases-M (AP-M) inhibitor like bestatin itself.



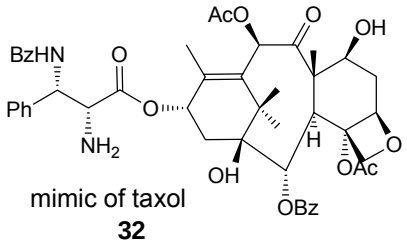
(2*S*,3*R*)-2,3-diamino-4-phenyl butanoic acid
28

aminodeoxybestatin (bestatin)
29 (**30**)

(2*R*,3*S*)-2,3-Diamino-3-phenylpropanoic acid (**31**) has been considered as an alternative side chain in the anticancer drug Taxol,[23] (**32**) in order to improve its solubility in biological media.



31



32

Day by day, the synthesis of 2,3-diamino acids is becoming more and more interesting mainly because of their use as building blocks in peptidomimetic synthesis; diamino-acid-containing peptides, indeed, have significant resistance to proteases[24] and are conformationally constrained.[25] When incorporated into peptide, with either β- or α-amino group as part of the backbone, this class of amino acids might provoke changes in polarity, additional hydrogen-bonding interactions, and basic character. Interest in 2,3-diamino acids also stems from their occurrence in cyclic peptides.

2,3-Diaminopropanoic acid, for example, is a component of MEN 10627, a highly constrained molecule corresponding to two 14-membered bicyclic peptides fused together in a bicyclic structure, made up of a peptidic bond between the amino group of DAP and the carboxylic function of aspartic acid side chain. MEN10627 is a potent, highly selective and long lasting, peptide-based neurokinin A receptor antagonist.[26] NKA receptor antagonists may represent a new class of agents, useful in inflammatory diseases of first respiratory ways, such as asthma, and in other diseases, in which smooth muscle contraction caused by Tachykinins (a family of peptides widely distributed in the central and peripheral nervous system of amphibians and mammals) play a pathogenic role, for example exaggerated intestinal motility. The biological and pharmacological features of MEN 10627 are closely related to the conformational rigidity of its bicyclic structure.[27]

Moreover, the use of 2,3-diaminoacids for the synthesis of β-lactams is noteworthy,[14] main structures of several natural and synthetic antibiotics.

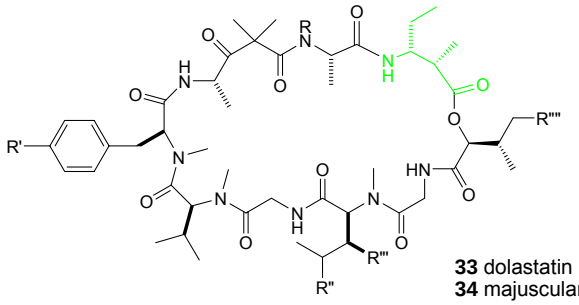
Finally, the usefulness of simple chiral 1,2-diamines as auxiliaries and controller groups in asymmetric synthesis (e.g., dihydroxylation,[28] conjugate addition,[29] olefination,[30] allylation,[31] epoxidation,[32] and aldol reactions[33]) is also well documented. Chiral diamines from 1,2-diaminocyclohexane are used as chiral ligands to co-ordinate osmium during *cis*- hydroxylation of olefins with OsO_4.[34] With the same aim are also used some derivatives of *N*,*N*'-dipentyl-2,2'-bipyrrolidine.[35] Finally chiral bicyclic phosphonamides present a remarkable diastereofacial selectivity in asymmetric olefination and alkylation of ketones.[17]

The use of chiral 1,2-diamines to resolve racemic mixtures of chiral allylic alcohols has been reported as well.[36] Chiral recognition in the coordination of olefins to transition-metal fragments is a topic of great interest[37] because it can offer simple procedures for thermodynamic[36c] or kinetic[38] resolutions of racemic mixtures or for analytical determination of enantiomeric abundances.[39]

2-Alkyl-β³-Amino Acids

This class of β-amino acids are components of important natural substances of biological and pharmacological interest. Their origin and their functions are several:

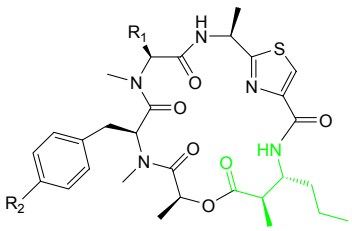
most of them are generated in microorganisms and have a variety of pharmacological properties. There are several examples where these molecules are components of antibiotics,[40] anticancer and antifungal drugs.



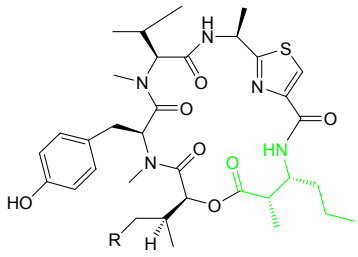
	R	R'	R''	R'''	R''''
33 dolastatin 11	H	OMe	Me	H	Me
34 majusculamide C	H	OMe	H	Me	Me

3-Amino-2-methylpentanoic acid, for instance, is an important structural element of dolastatin 11 (**33**), a depsipeptide with potent anticancer activity, originally isolated from the mollusk *Dolabella auricularia*.[41]

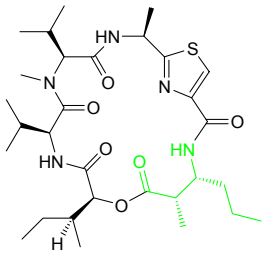
The same $\beta^{2,3}$-amino acid is a component of another peptidic structure, the majusculamide[42] C (**34**), a cyclic depsipeptide that, isolated from a toxic blue-green alga, *Lyngbya majuscule*, inhibits the growth of a number of fungal plant pathogens.[43] (2*R*,3*R*)-3-amino-2-methylhexanoic acid and its diastereomer, (2*S*,3*R*)-3-amino-2-methylhexanoic acid, are components of a series of bioactive peptides[44] called ulongamides A-F and are isolated from cyanobacteria *Lyngbya sp*. The first of these amino acids is found in compounds **35**-**37** and the other one in compounds **38-40**. All of them, except the last one, exhibit a slight cytotoxicity towards tumoral cells such as KB and LoVo.[45]



35 R_1 = i-Pr; R_2= H
36 R_1 = i-Pr; R_2= OH
37 R_1 = Bn; R_2= OH

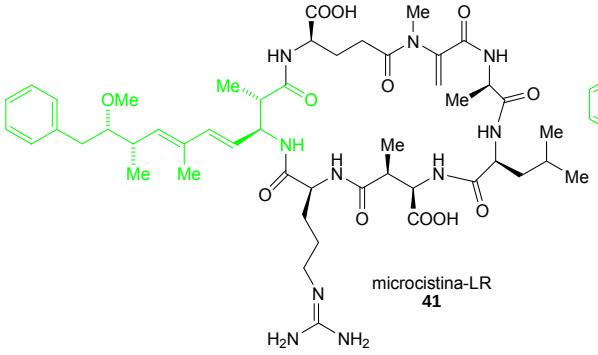


38 R = H
39 R = Me

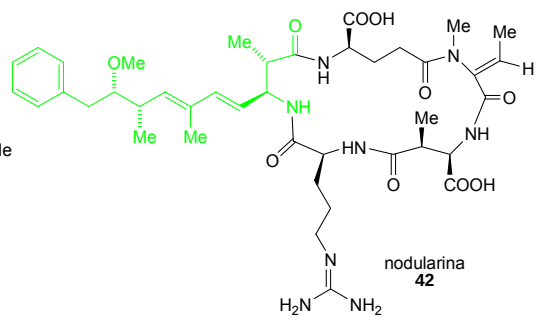


40

The $\beta^{2,3}$-amino acid ADDA (3-amino-10-phenyl-2,6,8-trimethyl-9-methoxy-4,6-decadienoic acid, **11**) was also found as an essential constituent in complex structures, such as cyclic peptides microcistyne-LR (**41**) and nodularine[46] (**42**). These structures, originated from bacteria *Microcystis aeruginosa* and *Nodularia spumigena,*[47] are toxins that act on the hepatic human system.



microcistina-LR
41



nodularina
42

Finally, the major interest in $\beta^{2,3}$-amino acids is due to some peculiar characteristics: they increase the proteolytic stability of the peptides made up of them and promote formation of stable secondary structures such as helices, β-turns and β-sheets;[48] these special properties of $\beta^{2,3}$-amino acids depend on their conformational rigidity, that is also a consequence of their configuration.[25]

Isotopically Labeled β-Amino Acids

Isotopically labeled molecules are important synthetic targets, as they reveal valuable information regarding the mechanism of enzymatic processes.[49] Especially attractive are the chiral isotopically labeled amino acids, which have been used to obtain firsthand knowledge about the stereochemistry of such processes.[50]

Due to the interesting biological properties[51] of β-amino acids, and their role in natural product biosynthesis,[52] isotopically labeled β-amino acids are logical synthetic targets. They have been used to determine the stereochemical outcome and mechanism of various enzymatic processes as well as for the synthesis of important biosynthetic intermediates.[53]

The simplest member in the β-series is 3-amino propanoic acid, or β-alanine; for its stereocontrolled labeling different approaches were developed, from biocatalytic approaches to asymmetric syntheses, involving self-regeneration of stereogenic centers, using a method developed by Seebach and co-workers.[54]

Apart from β-alanine, other similar isotopically labeled β-amino acids are also invaluable entities for revealing their role in natural product biosynthesis. They have mostly been prepared by enzyme-mediated transformations[55] or chemical methods.[56]

Moreover, isotopically labeled compounds are used in several analytical techniques: two of the most important are the ICAT method[57] (Isotope Coded Affinity Tag), for the accurate quantification and concurrent sequence identification of the individual proteins within complex mixtures., and the SIDA method (Stable Isotope Dilution Assay), for the analytical determination of trace compounds in food. Both these methods use isotopically labeled molecules as synthons for the preparation of more complex reagents (see ICAT) or as internal standard (see SIDA).[58]

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Chapter 3

Synthesis of β-Amino Acids

Introduction

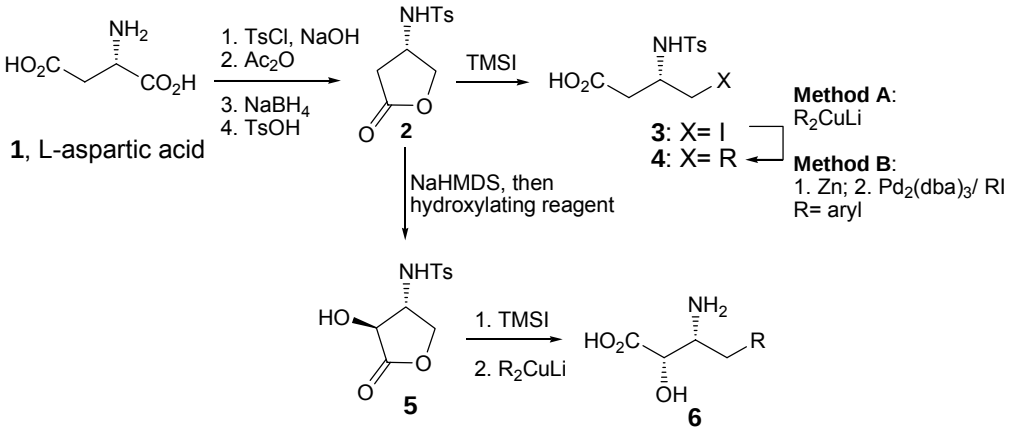
Given the significance of β-amino acids, it is not surprising that the development of their syntheses, mainly stereoselective ones, has become an important and challenging goal for organic chemists in recent years. Numerous methodologies have emerged, and most of the work prior to 1998 has been extensively reviewed.[1] There are eight main approaches available to date for stereoselective synthesis of β-amino acids including homologation of α-amino acids, enzymatic resolution, addition of enolates (or equivalents) to imines, Curtius rearrangement, conjugate addition of a nitrogen nucleophile to α,β-unsaturated esters or imides, hydrogenation, amino hydroxylation and β-lactam synthesis.[2]

Each approach has its own advantages and limitations. The development of an efficient process suitable for large-scale synthesis, which is easy to operate, practical and inexpensive, remains a significant challenge. No doubt, the growing interest in enantiopure β-amino acids will stimulate the developement of new and improved methods for their synthesis in the near future.

The Chiral Pool Approach: α-Amino Acids as Starting Materials

Stereoselective Synthesis of β-Amino Acids Starting from Aspartic Acid

Aspartic acid is a naturally occurring α-amino acid which possesses a β-amino carboxylic acid moiety. This feature makes aspartic acid an attractive precursor for the preparation of many β-amino acid derivatives. Jefford and co-workers discovered that 3-(*N*-tosylamino)-butano-4-lactone **2**, which is derived from aspartic acid **1** *via* tosylation, anhydride formation and selective $NaBH_4$ reduction, was a useful template for the stereoselective synthesis of β-amino acid derivatives (Scheme 3.1, **4**, method A).[3] The same methodology was also extended to the synthesis of α-hydroxy β-amino acid, where lactone **2** was hydroxylated followed by ring opening with TMSI and substitution with organocuprates to yield **6** with *syn* configuration (Scheme 3.1).

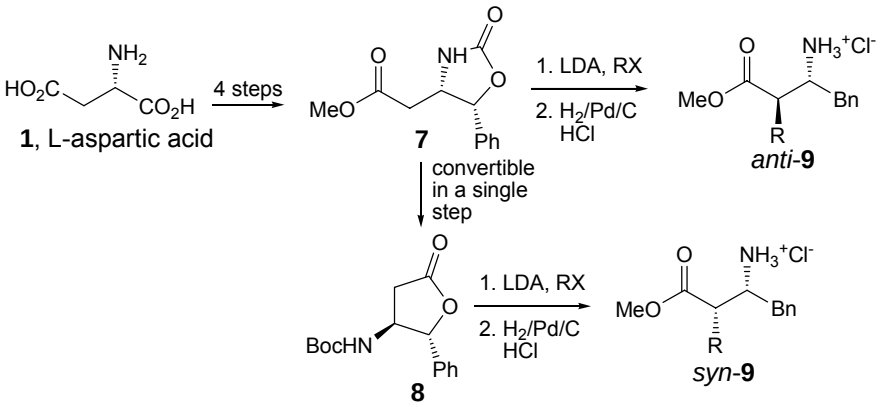


Scheme 3.1 Stereoselective synthesis of β-amino acids starting from aspartic acid.

Dexter and Jackson reported a mild and convenient method for the synthesis of functionalized zinc reagents replacing organo-cuprates.[4] The zinc reagent obtained from **3** is allowed to undergo a palladium catalyzed coupling reaction with aromatic iodides giving β–amino esters **4** in moderate to good yields (Scheme 3.1, Method B).

Seki and Matsumoto have explored the use of oxazolidin-2-one-4-acetic acid derivative **7** (Scheme 3.2), prepared from L-aspartic acid, in β-amino acid synthesis.[5]

The compound **7** is prepared from L-aspartic acid through the acylation of benzene or phenyllithium with α-amino carboxyl group of the L-aspartic acid skeleton. Alkylation of the dianion derivatives of **7** with alkyl halides and subsequent hydrogenation afforded *anti*-disubstituted β-amino acids in high stereoselectivities. Complete reversal of stereoselection is realized by the alkylation[6] of 4-phenyl-3-*tert*-butoxycarbonylamino-4-butanolide **8** which was obtained in a single step from **7**. The 2,3,4-trisubstituted amino lactone thus obtained is hydrogenated to give *syn*-disubstituted β-amino acids **9**.

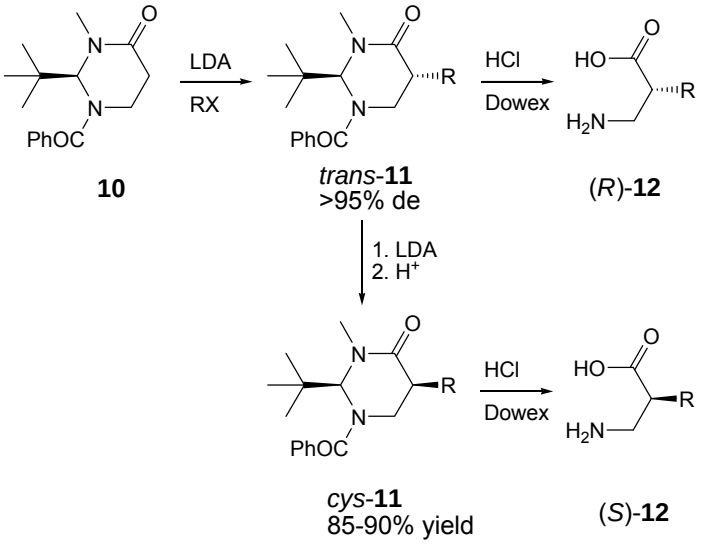


Scheme 3.2 Seki and Matsumoto approach to 2,3-disubstituted β-amino acids.

Stereoselective Synthesis of β-Amino Acids from Asparagine and Derivatives

Diastereoselective alkylation to introduce substitution at the α-carbon is by far the most efficient method for preparing 2,2-disubstituted β-amino acid derivatives. Better diastereoselectivities are often obtained using cyclic auxiliaries, which give higher differentiation of the two diastereotopic faces. The alkylation of heterocyclic compound perhydropyrimidin-4-one **10** (Scheme 3.3), has been extensively studied.[7] The high trans diastereoselectivity found in the addition of enolate **10**-Li to

electrophiles is probably due to the axial orientation of the *tert*-butyl group, as revealed by an X-ray crystallographic structure of perhydropyrimidinone **10**. In this way, the chirality center at C(2) induces the stereoselective formation of the new stereogenic center at C(5) of the heterocycle.
Various electrophiles were allowed to react with **10** with almost complete stereocontrol (95% de) providing the trans alkylated product **11** in good yields, which upon hydrolysis using HCl gave 2-substituted β-amino acids **12** with *R* configuration (Scheme 3.3).

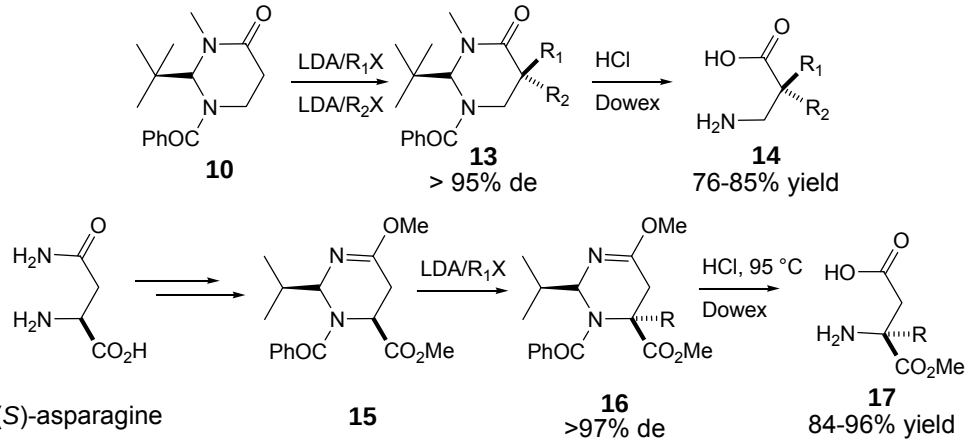


Scheme 3.3 Diastereoselective alkylation of heterocyclic compounds according to Seebach (see ref. 7).

Alternatively, the β-amino acids with *S* configuration were conveniently prepared by epimerization of the trans adducts. The key step in this transformation was the highly diastereoselective protonation of enolates generated from trans-**11**.

In an extension to their methodology, Juaristi and co-workers demonstrated the application of the same chiral 3-amino-propionic acid derivative **10** in the synthesis of 2,2-disubstituted β-amino acids **14** (Scheme 3.4).[8]

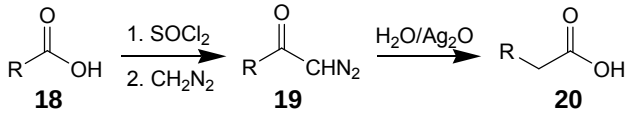
Furthermore, the same authors[9] investigated the alkylation of iminoester **15** using different electrophiles: the reactions furnished the trans products **16**, precursors to 3,3-disubstituted β-amino acids **17**, with excellent diastereoselectivities (Scheme 3.4).



Scheme 3.4 $\beta^{2,2}$- and $\beta^{3,3}$-amino acids according to Juaristy (references 8, 9).

Arndt–Eistert Homologation

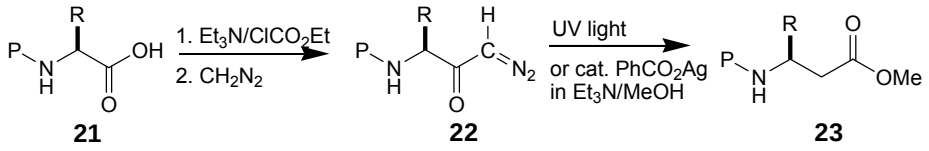
The Arndt–Eistert reaction is the oldest method for one carbon chain elongation of a carboxylic acid (Scheme 3.5).[10]



Scheme 3.5 Arndt-Eistert homologation.

Taking the advantage of ready availability, low cost, and high enantiomeric purity of α-amino acids, the direct homologation to prepare β-amino acids following the Arndt–Eistert procedure has found many applications in small molecule as well as natural product synthesis.

Seebach and co-workers have extensively utilized this approach in β-peptide synthesis. As outlined in Scheme 3.6, the *N*-protected α-amino acids **21** are converted to mixed anhydrides using $Et_3N/ClCO_2Et$. The addition of CH_2N_2 then affords diazoketones **22** with good yields and without any racemization. Wolff rearrangement of diazoketones to β-amino acid derivatives is then achieved by UV light initiation or by the use of a catalytic amount of silver benzoate in triethylamine and methanol.[11] In addition, diazoketones **22** may be trapped with the amine terminus of another carboxyl protected β-amino acid fragment to provide β-peptides directly. A modified procedure using *N*-Fmoc-protected α-amino acids and i-BuOCOCl/*N*-methylmorpholine to form the mixed anhydride was later proposed by Seebach *et al.* for solid phase synthetic applications.[12]



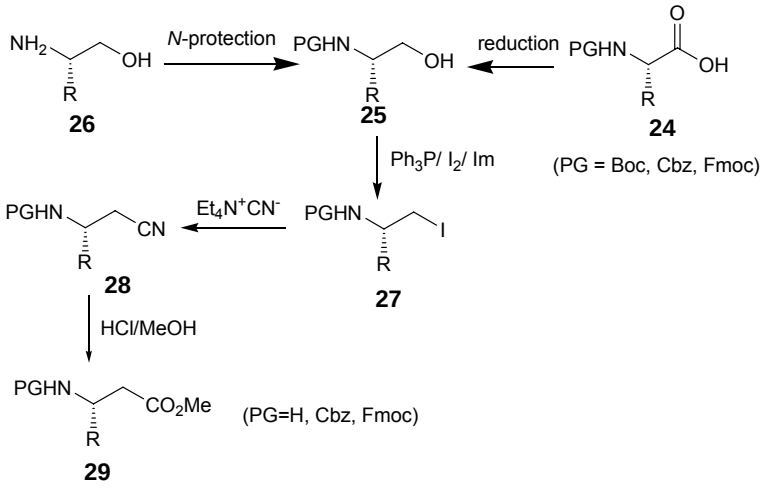
Scheme 3.6 Homologation of α-amino acids by the Ardnt-Eistert homologation procedure.

Several modifications were then proposed to convert diazoketones **22** to β-amino esters[13] and to apply this homologation procedure to the synthesis of 2-substituted β-amino acids[14] and β-lactams[15] as well as to the total synthesis of compounds such as dipeptides containing a β-arginine fragment, or TAN-1057 A,B, anti MRSA (methicillin-resistant Staphylococcus aureus) antibiotics.[16]

Results and Discussion

The Arndt–Eistert protocol works very well for the preparation of enantiomerically pure substituted β-amino acids from α-amino acids. Unfortunately, it is not suitable for large scale preparations due to the high cost of the silver catalyst and, even more, the difficult handling of the hazardous reagent CH_2N_2.

A few years ago an alternative way to homologate α-amino acid was developed in our labs [17]. It consisted of the reduction of *N*-protected α-amino acids **24**, followed by conversion of the resulting β-amino alcohols **25** to β-amino iodide **27**, and then to β-amino cyanides **28** (Scheme 3.7). The key step of this transformation was the generation of the iodides in high yields and without racemization using triphenylphosphine-iodine complex.



Scheme 3.7 Homologation of α-amino acids.

The subsequent replacement of the iodine atom in **27** by a cyano group, and hydrolysis (or alcoholysis) of the latter, complete the synthetic sequence leading to *N*-protected and/or *C*-protected β-amino acids.

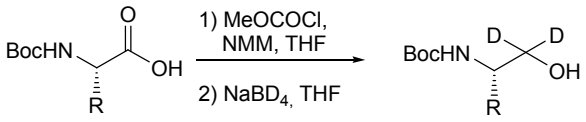
This method allows the direct conversion of *N*-protected α-amino acids into their β-homologs with complete retention of configuration, using the common alkoxycarbonyl protections of the amino function. This method is extremely useful: all the reactions are really simple, the reagents being also cheap and easy to handle.

Synthesis of Isotopically Labeled β-Amino Acids

The method described above was also modified to be adapted to the preparation of deuterium labeled molecules, the interest of which was already discussed in Chapter 2.

Thus, 2,2-^{2}H-β^{3}-amino acids were directly and easily prepared from α-amino acids.

N-protected 2,2-^{2}H β-amino alcohols were prepared from *N*-protected α-amino acid mixed anhydrides by sodium borodeuteride reduction.



30a-e **31**a-e

a) R= Me
b) R= CH_2Ph
c) R= $(CH_2)_4NHBoc$
d) R= H
e) R=CH_2OBn

Scheme 3.8 Synthesis of isotopically labeled *N*(Boc)-β-amino alcohols.

In Table 3.1 we have summarized the results obtained using as starting materials some proteinogenic α-amino acids.

It is noteworthy that during the reduction, the percentage of bis-deuteration is excellent, being in all instances higher than 98% (^{1}H- and ^{13}C-NMR, MS measurements).

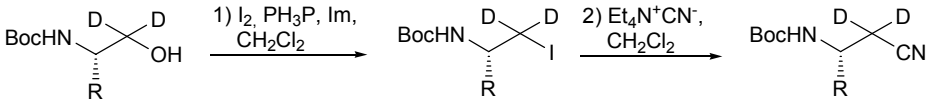
Table 3.1 2,2-^{2}H *N*-(Boc)-β-amino alcohols from *N*(Boc)-α-amino acids (Scheme 3.8)

	R[a]	P[b]	m.p. (°C)	$[\alpha]_D^{25}$ (c)[c]	Yield %[d]	^{2}H %
31a	Ala		58.2-59.1	-9.8 (1.32)	92	>99
31b	Phe		97.3-98.9	-23.1 (1.15)	90	>99
31c	Lys	Boc	oil	-10.1 (1.92)	85	>99
31d	Gly		oil		75	>99
31e	Ser	Bn	64.9-65.7	+14.8 (1.71)	75	>99

[a] Side chain of starting α-amino acids. [b] Protection at side chain. [c] $CHCl_3$ solutions (cell sample with a pathlenght of 1 dm). [d] Yield of the pure product, after recrystallization. The yields are the same as those reported for non-deuterated *N*(Boc)-amino alcohols.[18]

The *N*-protected β-amino alcohols **31a-e** are then converted to *N*-protected β-amino iodides **32a-e** as reported in Scheme 3.9.

Triphenylphosphine-iodine complex, in the presence of imidazole, accomplishes the OH→I replacements in high yields and under very mild conditions. This preserves *N*- and side chain- protections, and does not affect the configuration of the C-3 stereogenic center as well as the percentage of deuteration at the C-2 position. Moreover, it is possible to use a polymer bound triarylphosphine, like polystyryl diphenylphosphine; this permits that phosphine oxide, which is formed as the only by-product, is linked to a polymeric matrix and, thus, can be separated by simple filtration. This avoids time consuming circumstantial purification procedures to obtain the pure products. If isolation of iodide derivatives is not necessary, the use of dichloromethane soluble and much less expensive triphenylphosphine-iodine complex is anyway the best choice and the reaction is also successful.



31a-e **32a-e** **33a-e**

a) R= Me
b) R= CH_2Ph
c) R= $(CH_2)_4NHBoc$
d) R= H
e) R=CH_2OBn

Scheme 3.9 Synthesis of 2,2-^{2}H-β-amino cyanides

When we used *N*-protected β-amino iodides as such in the conversion to *N*-protected β-amino cyanides without any purification, soluble triphenylphosphine could be used (Scheme 3.9).

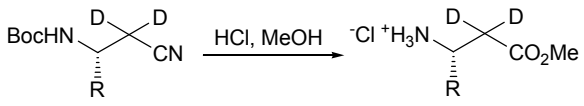
Table 3.2 2,2-^{2}H-*N*-(Boc)-β-amino cyanides from 2,2-^{2}H-*N*-(Boc)-β-amino alcohols

	R[a]	P[b]	m.p. (°C)	$[\alpha]_D^{25}$ (c)[c]	Yield %[d]	^{2}H %
33a	Ala		68.2-70.0	-120.1 (1.38)	80	>99
33b	Phe		123.8-125.0	-18.2 (1.15)	74	>99
33c	Lys	Boc	60.7-64.0	-45.2 (0.57)	70	>99
33d	Gly		42.4-45.0		88	>99
33e	Ser	Bn	oil	-9.7 (1.62)	65	>99

[a] Side chain of starting α-amino acids. [b] Protection at side chain. [c] $CHCl_3$ solutions (cell sample with a pathlenght of 1 dm). [d] Yield of the pure product, after recrystallization. The yield are the same of that already reported for not deuterated *N*(Boc)-amino alcohols.[18]

The preparation of *N*-protected β-amino cyanides has been very seldom reported. *N*-tosyl β-amino nitriles[18] were prepared from *N*-tosyl aziridines by cyanotrimethylsilane in the presence of lanthanoid tricyanide as catalyst; *N*,*N*-dibenzyl β-amino cyanides have also been obtained by deoxygenation of *N*,*N*-dibenzyl α-amino aldehyde cyanohydrins[19] or by an intriguing monosubstitution operated by LiCN in DMF onto the dimethyl diester of the *N*,*N*-diprotected amino diol coming from aspartic acid reduction.[20] To the best of our knowledge, the conversion of *N*(Boc)-(*R*)-phenylglycinol tosyl ester to the corresponding β-amino cyanide by NaCN (or KCN) in DMSO (90 °C, 1h) represents (although questionable) the only example of synthesis of *N*(alkoxycarbonylamino)-protected β-amino cyanides.[21] In our hands, *N*(Boc)-protected β-amino iodides were smoothly converted to their corresponding cyanides by 4h reflux with tetraethylammonium cyanide[22] in dichloromethane (Scheme 3.9). The work-up of the reaction is also very simple: when the starting β-amino iodide is fully consumed (TLC monitoring), silica gel is added to the reaction mixture and the solvent is evaporated under reduced pressure. The solid is then mechanically collected from the reaction flask and transferred directly onto a short silica gel column (petroleum ether-ethyl acetate) to afford the product that is finally crystallized from dichloromethane-hexane. This is the only chromatographic purification step of the whole process.

Thus, the *N*(Boc)-protected β-amino cyanides **33**a-e were hydrolyzed under acid conditions with HCl in methanol 10 N for 12 h at room temperature; under those conditions the Boc-group is cleaved, and the C-2 fully deuterated β^3-amino methyl esters **34**a-e were obtained.



33a-e **34**a-e

a) R= Me
b) R= CH_2Ph
c) R= $(CH_2)_4NHBoc$
d) R= H
e) R=CH_2OBn

Scheme 4.0 Synthesis of 2,2-^{2}H-β-amino methyl esters.

The deuterium content does not change throughout the whole process and the isotopic labelling is always higher then 99% (Table 3.3).

Table 3.3 2,2-^{2}H-*N*-(Boc)-β-amino cyanides from 2,2-^{2}H-*N*-(Boc)-β-amino alcohols

	R[a]	P[b]	m.p. (°C)	$[\alpha]_D^{25}$ (c)[c]	Yield %[d]	^{2}H %
34a	Ala		250 dec.	+0.19 (2.5 in MeOH)	87	>99
34b	Phe		255 dec.	+4.28 (1.38)	85	>99
34c	Lys		oil	+8.49 (0.56)	82	>99
34d	Gly		102.4-106.0		90	>99
34e	Ser	Bn	oil	+0.68 (0.74)	80	>99

[a] Side chain of starting α-amino acids. [b] Protection at side chain. [c] $CHCl_3$ solutions (cuvette with a pathlenght of 1 dm). [d] Yield of the pure product, after recrystallization. The yields are the same as those reported for not deuterated *N*(Boc)-amino cyanides.[18]

In conclusion the entire process is very simple and general; it gives excellent yields and the reactions are easy to handle. Moreover it is possible to scale up the process, without encountering particular difficulties.

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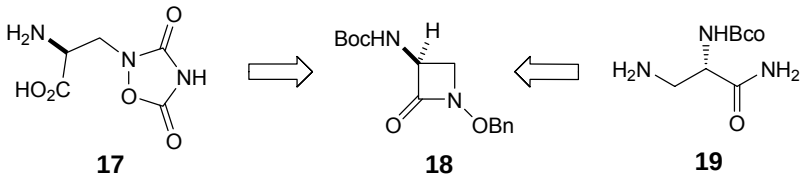
Chapter 4

Synthesis of 2,3-Diamino Carboxylic Acids

The development of simple and efficient methods to produce enantiomerically pure 2,3-diamino carboxylic acids from readily available starting materials represents a fascinating goal from both chemical and pharmaceutical point of view. Several asymmetric syntheses have been reported so far.

One of the most practical approaches to 2,3-diamino acids expolits β-lactams as starting materials. The synthesis of β-aminoalanine derivatives, such as the one reported by Baldwin,[1] involves formation of L-quisqualic acid **17** from *N*-benzyloxy β-lactam **18**, in excellent yield.

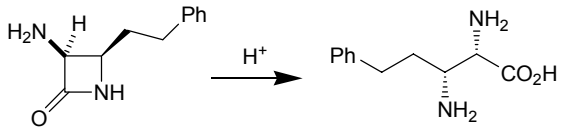


17 **18** **19**

Scheme 3.1 Retrosynthetic analysis of β-aminoalanine as reported by Baldwin[1]

In a similar way, Hecht[2] reported the synthesis of (2*S*)-3-amino-2-[(*tert*-butoxy-carbonyl)amino]propionamide **19** from (2*S*)-*N*-(benzyloxy)-2-azetidin-one **18** (Scheme 3.1).

More recently, Ojima and co-workers[3] have illustrated the use of enantiomerically enriched 3-amino-β-lactams for the construction of 3-substituted 2,3-diamino acids *via* acid catalyzed ring opening.



20 **21**

Scheme 3.2 3-Substituted 2,3-diamino acids *via* acid catalyzed ring opening

Many other procedures for the synthesis of 2,3-diamino acids from β-lactams have been reported.[4]

Several methodologies use proteinogenic α-amino acids to prepare 2,3-diamino acids; they are often quite expensive because of the use of stoichiometric amounts of chiral reagents.

For instance, 2,3-diaminopropanoic acid and 2,3-diaminobutanoic acid are synthesized from aspartic acid,[5] L-serine[6] and treonine.[7]

Rapoport and co-workers developed a general method for the stereoselective synthesis of 3-substituted 2,3-diamino acids *via* alkylation of an aspartic acid derivative. The Rapoport-type aspartate alkylation consisted of the incorporation of the C-3 substituent as a positive synthon (a halide was used as a suitable synthetic equivalent).

An alternative pathway consists in diastereoselective addition of Grignard reagents, negative synthons, to α-amino nitrones from L-serine,[6] that provides a method for the synthesis of optically active 3-substituted 2,3-diamino acids

Both diastereoisomers of 2,3-diaminobutanoic acids are easy accessible through a nucleophilic substitution reaction on derivatives of β-substituted L- or D-threonines,[7] but in that case the methodology is not versatile.

The Mitsunobu reaction on serine,[8] the Hofmann and Curtius rearrangements of asparagine derivatives,[9] and the Schmidt reaction of aspartic acid[10] were used to access chiral 2,3-diaminopropanoic acids.

A variety of other syntheses have also been reported: the conjugate addition[11] of homochiral lithium *N*-benzyl-*N*-α-methylbenzylamide to α,β-unsaturated esters and *in situ* amination with trisyl azide, the asymmetric Rh(I)-phosphine-catalyzed hydrogenation of diastereoisomeric enamides,[12] and the ring opening of *cis*-3-alkylaziridine-2-carboxylates coming from Sharpless asymmetric aminohydroxylation of α,β-unsaturated esters.[13]

Although several methods for preparing chiral diamino acids are known, our interest was to find a general procedure for stereoselective formation of C-N bonds, for the direct introduction of an amino function onto C-2 position of β^3-amino acids.

Electrophilic Amination of Nucleophilic Carbons

Stereoselective formation of C-N bonds on starting materials easy to handle and available is an important goal of organic synthesis.

Various stereoselective procedure for the introduction of an amino group onto the C-α of carbonyl compounds are reported in the literature;[14] in general, direct stereoselective addition of nitrogen-containing functional groups onto carbon atoms is the simplest approach for the formation of stereogenic centers having nitrogen atoms.

Results and Discussion

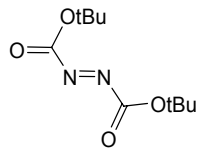
The synthetic approach we decided to follow, is based on a direct stereoselective reaction of α-amination of β-amino methyl esters using di-*tert*-butylazodicarboxylate (DBAD) as nitrogen source (Figure 3.6), as already reported in literature by Seebach *et al*.[15]

Recently, we have described the preparation of enantiomerically pure 2-hydroxy-β^3-amino acids by hydroxylation of chiral enolates of β^3-amino acids.[16] We found that the replacement of Boc- or Cbz-groups, used by Seebach[15] as protections of the amino function of methyl 3-amino-butanoate in a similar reaction, with benzyl groups gave better results, assuring good yields and excellent diastereoselectivity.

According to the same scheme, we decided to introduce different electrophile to generalize the use of this kind of protections.

Thus, we have prepared 2,3-diamino acids through electrophilic amination.

For the preparation of the starting materials we used the methodology[17] devised in our own lab and described in this thesis (Chapter 2).[18]

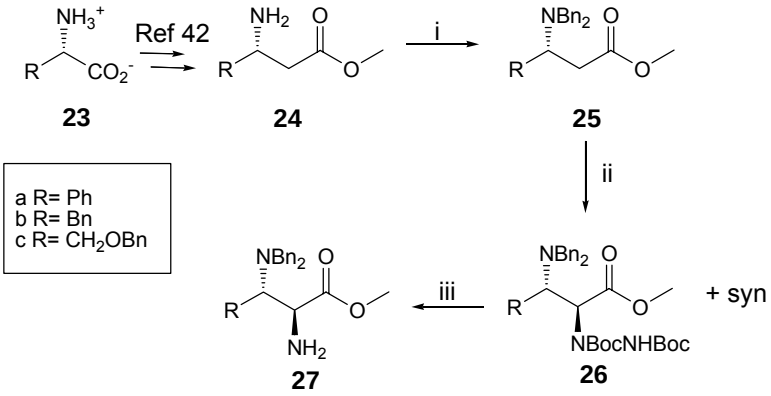


Di-tert-butylazodicarboxylate
DBAD

22

Figure 3.6 DBAD

In the literature several reagents for electrophilic amination, such as derivatives of hydroxylamines,[19] sulphonylazides,[20] oxaziridines,[21] and azodicarboxylate esters. We have reported the use of the latter because especially the di-*tert*-butyl ester is a crystalline solid, stable and commercially available; moreover it reacts with remarkable diastereofacial selectivity in reactions with different kinds of chiral enolates.[22] Finally, the *tert*-butyloxycarbonyl groups on the nitrogen atoms are easily removed under mild conditions and the reductive cleavage of N-N bonds is possible in good yields.



i. BnBr, DIPEA, toluene, reflux; ii. KHMDS, DBAD, dry THF, -78°C;
iii. a) TFA, CH_2Cl_2; b) H_2, Ni(Ra), MeOH,)))

Scheme 3.3 Conversion of α-amino acids into 2,3-diamino acids

Thus, *N*,*N*-dibenzylated β^3-amino esters (**25a–c**), in anhydrous THF at –78 °C and under anhydrous nitrogen atmosphere, were treated with potassium bis(trimethylsilyl)amide (KHMDS) to generate the corresponding enolates. The use of more common bases, such as LiHMDS and LDA, for the enolate generation was neglected since in our experience[16] such bases lead to significantly poorer results. After 1 h, solid di-*tert*-butyl azodicarboxylate (DBAD) was added to the reaction mixture that was kept at –78 °C for an additional hour. Under such conditions, the Boc-protected hydrazino derivatives of the starting **25a–c** were obtained. The double protection of the amino group is necessary to avoid formation of by-products coming from the abstraction of the N–H proton in the enolate generation step. Consequently, common protecting groups that are stable under basic conditions, such as Boc or Cbz, could not be used under our reaction conditions. Therefore, in a first attempt, the 4-methoxybenzyl group, we had already used elsewhere,[16] was chosen for its peculiar removal conditions (CAN, CH_3CN/H_2O). Unfortunately, although the group turned out to be stable under the reaction conditions, we could not use it because the deprotection of the final 3-[di(4-methoxybenzyl)amino]-2,3-diamino esters led to a plethora of products coming from oxidative cleavage of the C2–C3 bond. Eventually, we used a double benzylic protection that eliminated such deprotection problems and represented at the same time a very bulky nitrogen substituent, suitable to affect the stereochemical outcome[23] of the enolate coupling with the electrophile DBAD. As a matter of fact, the coupling afforded a mixture of anti:syn Boc-protected 2-hydrazino derivatives of **25a–c** with excellent diastereoisomer ratios.

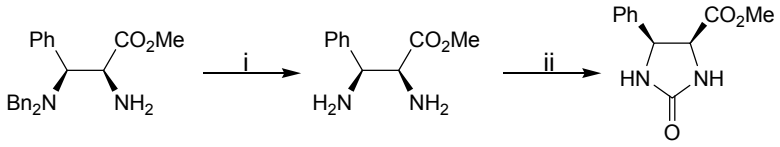
Table 3.1. Functionalization at C-2 of the fully protected β^3-amino esters **25a–c**

Protected β^3-amino ester	R	Boc protected 2-hydrazino derivatives **26a–c**		anti-2,3-Diamino esters (**27a–c**), yield (%)[a]
		Yield[b](%)	*anti/syn*	Yield (%)
25a	Ph	92	93:7	70
25b	Bn	90	97:3	78
25c	CH_2OBn	90	94:6	65

a) Overall yield after Boc removal and reductive cleavage of the hydrazine moiety in the anti diastereoisomers 8a–c.
b) Yield of both diastereoisomers.

Due to the complexity of the ^{1}H NMR spectra of the Boc containing hydrazino derivatives, they were converted into the corresponding diastereoisomeric mixtures of diamino esters (e.g., **27**) to determine the diastereoisomeric ratio. The synthetic path is depicted in Scheme 3.3 and the results obtained for selected *N*-protected β^3-amino esters, namely the methyl esters of β^3-phenylglycine **25a**, β^3-phenylalanine **25b**, and β^3-serine **25c**, are reported in Table 3.1.

The more abundant anti diastereoisomers were submitted to removal of the Boc protections (TFA in CH_2Cl_2) and cleavage of the N–N bond by hydrogenolysis with Ni(Ra) at low pressure and room temperature in an ultrasound bath. The reduction of hydrazines to amines is reported to be accomplished at high temperature, under high hydrogen pressure.[24] The use of ultrasound reduces significantly both temperature and pressure.[25] As a matter of fact, the hydrogenolysis under such conditions was complete after only 4 h and no traces of C-2 epimerization products could be detected by ^{1}H NMR spectroscopic analysis. The *anti* configuration of the more abundant diastereoisomers coming from the couplings of **25a–c** with DBAD could be attributed, in the case of **26a**, as follows: the final product **27a** was debenzylated and treated, without isolation, with 1,1'-carbonyldiimidazole to afford the imidazolidinone **29** (Scheme 3.4). The ^{1}H NMR coupling constant of 9.6 Hz supported[26] the *cis* configuration of the H-4 and H-5 protons and, thus, the *anti* configuration of the starting diamino compound.



27 **28** **29**

i. Pd/C, H_2, AcOH, 50°C, 90%; ii. 1,1'-carbonyldiimidazole, TEA, THF, 0 °C, 85%.

Scheme 3.4 Synthesis of *cis*-imidazolidinone (**29**).

In the light of this result, in agreement with our previous work on the hydroxylation at C-2 of β^3-amino esters, it seems likely that the stereochemical outcome of the functionalization at C-2 is independent of the nature of the electrophile used, being only a function of the relative stabilities of the enolate conformations.

The high diastereoselectivity, found in all the cases examined, could be explained on the basis of the same theory, already used for the interpretation of the

stereochemical outcome of the α-hydroxylation reaction, in which the *anti* diastereoisomer is also the major product.

The asymmetric induction is exerted by the stereogenic center of the starting amino acid, the contribution of which is, in fact, probably amplified by the presence of the cumbersome amine protection. As a matter of fact, PM3 calculations carried out for the enolate ion produced from the starting compound **25b** showed that its more favoured conformation was the one with the enolate group between the hydrogen atom and the protected amino group (Figure 3.7, **A**).

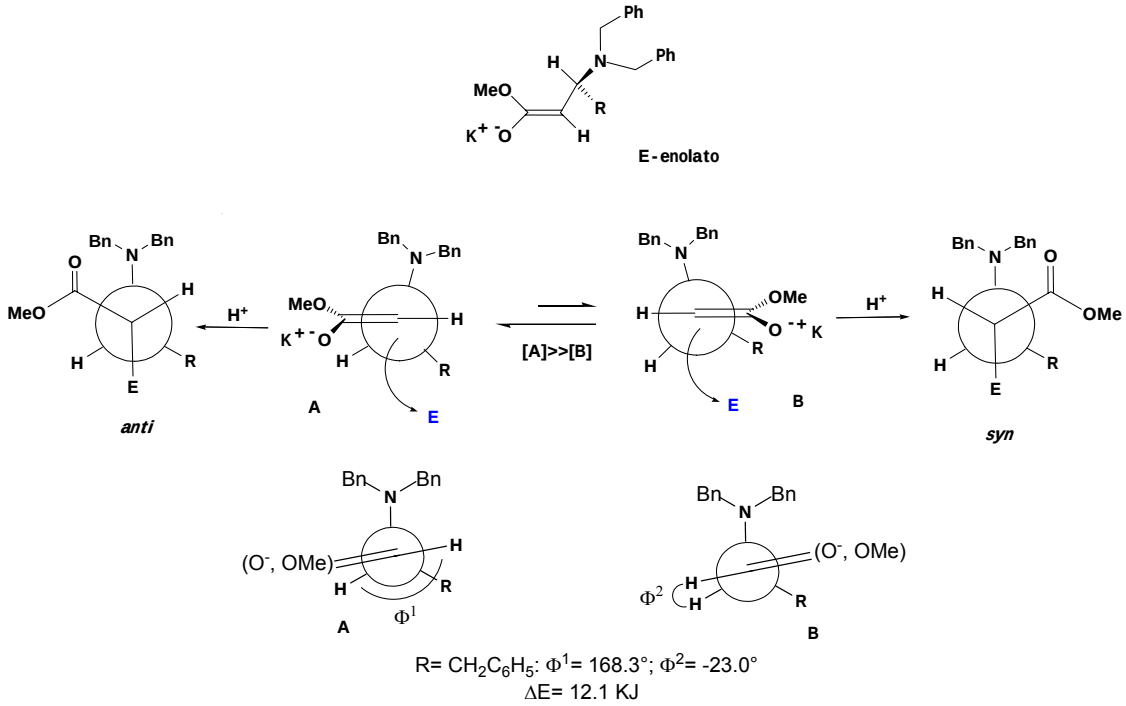


Figure 3.7 Conformational energies for the enolates

Its energy is lower than the energy of **B**. The attack of the DBAD reagent takes place from the less hindered face of the enolate moiety (opposite to the bulky substituted nitrogen group), it provides an excess of the *anti* diastereisomer. Accordingly, in the case of the enolate from **25a**, the same conformation had a much smaller energy advantage, and this may account for the lower diastereisomeric ratio achieved in its amination.

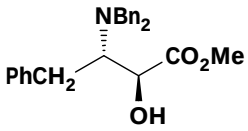
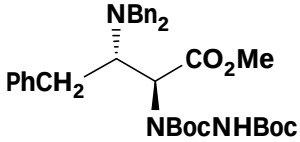
This model explains how the diastereoisomeric ratio is related to the hindrance of alkyl residue present on the asymmetric carbon; indeed, the bigger R, the more stable is the conformer **A** in respect with conformer **B,** with formation of the isomer *anti* as major product.

Models based on cyclic intermediates were discarded: previous studies, carried out on the α-hydroxylation, demonstrated that the use of lithium bis(trimethylsilyl)amide (LHMDS) instead of potassium bis(trimethylsilyl)amide (KHMDS) gives the same diastereoisomeric ratio. It is known that K^+ ion is scantly co-ordinated, as compared to the smaller Li^+ ion, and the amine function, with double cumbersome protections, such as benzyl groups, is not a good electron donor.

Moreover, the type of electrophile used in the reaction with the enolates influences little the stereochemical outcome of the reaction. In Table 3.2 are shown the diastereoisomer ratios of both α-hydroxylation and α-amination processes with

the methyl esters of *N*-protected β^3-phenylalanine **25b**: both the processes give rise to comparable diastereoisomer ratios.

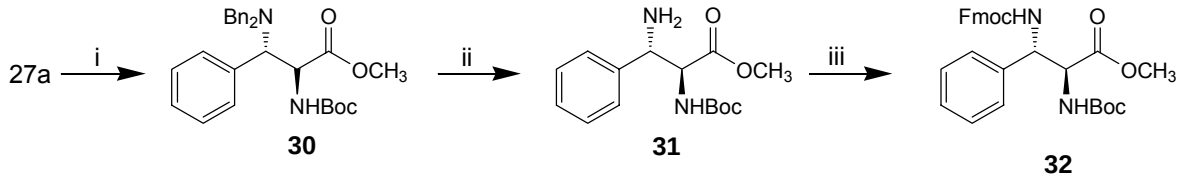
Table 3.2. Functionalization at C-2 of fully protected β^3-amino esters

α-Hydroxylation product	Yield[1] %	d.r.[2] %	α-Amination product	Yield[1] %	d.r.[2] %
	63	99:1		90	97:3

[1] Total yield of both diastereoisomers.
[2] Diastereomeric ratio determined by 400 MHz ^{1}H NMR and by GC-MS.

Conclusions

This amination procedure of β^3-amino esters offers several advantages, if compared with many other reported procedures. First of all, it does not require the use of either chiral reagents or chiral auxiliaries: in fact, the observed selection in the coupling step is merely due to the influence of the existing stereogenic center of the starting β^3-amino ester, enhanced by the presence of two bulky substituents on the nitrogen atom. Moreover, it is noteworthy that the amino groups in the final 2,3-diamino esters have a different protection status: this implies a broad flexibility of their use in peptide synthesis. For instance, the free amino group can be Boc protected and the benzyl groups then removed hydrogenolytically ready for a Fmoc protection, or vice versa. Accordingly, in connection with our current interest in the synthesis of glycosyl amino acids, we have prepared the compound **32** as shown in Scheme 3.4



i. Boc_2O, TEA, dioxane, 0 °C, 98%; ii. Pd/C, H_2, methanol, 50 °C, 90%;
iii. FmocOSu, Na_2CO_3, DMF/dioxane, 0 °C, 65%.

Scheme 3.4 Preparation of orthogonally protected 2,3-diamino acid ester **32**.

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Chapter 5

Synthesis of 2,3-Disubstituted β-Amino Acids

Introduction

Stereoselective synthesis of β-amino acids represents an important task for organic chemists, especially considering the growing interest for the application of β-amino acids in various areas. The peculiar characteristics of β-peptides that can be obtained stimulated scientists to look for new methodologies to obtain enantio-pure β-amino acids. In this respect several articles,[1] reviews[2] and even books[3] were dedicated to the synthesis of highly pure β^2-, β^3- and $\beta^{2,3}$-aminoacids.[4,5,6]

For the synthesis of the latter compounds several methods are known; some of these use, as starting materials, natural α-aminoacids,[7] and the construction of the second stereogenic center by hydroboration[1e] or via *N*-tosylated allyl amine intermediates.[8] In a second approach L-aspartic acid[9a,b,d,10] is used to prepare *syn*- and *anti*-$\beta^{2,3}$-amino acids, via an oxazolidone intermediate,[11] as already discussed in Chapter 3, and ester enolates have been used in conjunction with tert-butylsulfinyl imines to afford these compounds.[12]

Asymmetric synthesis of $\beta^{2,3}$-aminoacids can be obtained by conjugated addition of chiral lithium amides on methyl and *tert*-butyl crotonates and cinnamate esters,[13] by chiral Lewis acids mediated conjugate addition of amines on imides and oxazolidinones,[14] by 1,3-dipolar cycloadditions of oximes and allylic alcohols,[15] and by conversion of the Baylis-Hillmann adducts.[16] An extension of Davies protocol[1c,d] is reported by Gellman[17] *et al.* to prepare protected *syn*-2,3-dialkyl β-amino acids that contain lysine-like side chains.

Great part of these synthetic methodologies have the advantage of being highly diastereo- or enantio-selective; however they are often limited by low yields and high number of synthetic steps.

Recently new strategies have been developed for the synthesis of $\beta^{2,3}$-aminoacids using both homogeneous[18] and heterogeneous catalysis of specific β-amino acid precursors.[2d,6c] These approaches are, however, limited by the need of expensive chiral auxiliaries.

Finally, the alkylation of β^3-amino acids, readily obtained from α-amino acids via Arndt-Eistert homologation (Chapter 3), was investigated by Seebach as a mean to obtain 2,3-dialkyl β-amino acids.[19]

Since in our lab a stereoselective method for the synthesis of 2-hydroxy β^3-aminoacids[20] had been devised, and since a suitable procedure was optimized as part of this thesis work to prepare 2-amino β^3-amino acids (Chapter 4), we thought to exploit this methodology to the synthesis of 2-alkyl-β^3-amino acids as well.

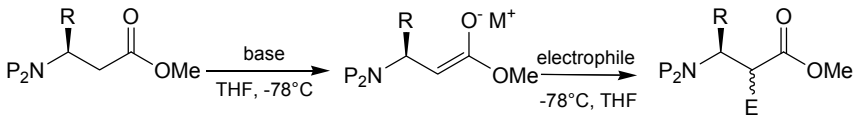
Synthetic Strategy

Stereoselective carbon-carbon bond forming reactions in α-position to carbonyl groups and their derivatives, is one of the most important tools in asymmetric synthesis.[21] Indeed diastereoselective reaction of chiral molecules, bearing nucleophylic carbons to electrophylic alkylating agents, represents an easy approach to the formation of new stereogenic centers.

β^3-Aminoesters present relatively acidic protons in α position to the ester function and, in the presence of strong bases, form enolates that are able to undergo coupling reactions with different electrophiles.[22] The synthetic strategy we developed, inspired by former studies on α-hydroxylation[20] and α-amination of β^3-aminomethylesters, exploits the reactivity of β^3-aminoesters to undergo stereoselective coupling reactions using different and economic commercially available carbon electrophiles such as methyliodide, benzylbromide and formaldehyde, in order to introduce at C-2 of β^3-aminoacids, side-chain analogues to those present in natural α-aminoacids, such as methyl (alanine), benzyl (phenylalanine) and hydroxymethyl (serine) groups.

Moreover, alkyl chains with a large number of carbon atoms could be introduced which is interesting, since it is reported that peptides[23] "conjugated" with lipidic α-amino acids show increased permeability through cell membranes and enhanced biological stability.

Under the optimized reaction conditions (Scheme 5.1), a base was added at -78 °C in anhydrous THF to the fully protected β^3-aminomethylester, to generate an enolate, that is in turn allowed to react with an electrophile, added in situ at -78 °C under nitrogen atmosphere, to yield the desired $\beta^{2,3}$-amino methyl ester.



Scheme 5.1 Conditions for α-alkylation of β^3-amino methyl esters

Yields and stereochemical outcome of these reactions were highly dependent up on the experimental conditions chosen.

A first factor to be considered is the selection of the correct enolate counterion, because of the different ability of lithium and potassium to co-ordinate the enolate anion.

Crucial for yields are the ratios of β-aminoester, base, and electrophile.

Finally the amino protecting group has to be considered. In such a case the benzyl group, as reported in the literature,[24] showed the best results in terms of yields and diastereomeric ratios. First of all, the double protection avoided side reactions with the strong base used, and then the steric clash on the diastereotopic faces induced a specific bias toward the addition to the enolate.[25] Since we chose to alkylate β^3-serine having a benzyl protection on the OH of the side chain, we considered to use the *p*-methoxybenzyl group as protection of the β-amino function in all the substrates employed, to have all orthogonal protecting groups on the β-amino acid.

The high diastereoselectivity of these reactions could be explained on the basis of the theory, already used for the interpretation of the stereochemical outcome of the α-hydroxylation and the α-amination reactions, as already illustrated in Chapter 4.

Models based on cyclic intermediates were discarded: first studies conducted on the α-hydroxylation process showed, indeed, that the use of LHMDS in the place of KHMDS causes in some case a decrease in diastereoselection; furthermore, it is

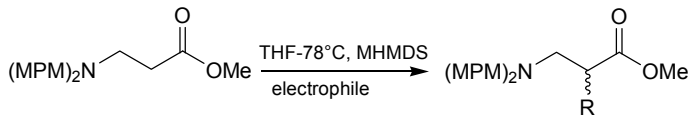
known that K^+, being bigger than Li^+, has a weaker coordination character; finally the *N*,*N*-dibenzylated amino function is not prone to act as electron donor.

Reagent ratios, nature of the base and temperature were optimized on β-alanine as a model substrate for α-alkylation and α-hydroxymethylation reactions. The best conditions devised were then applied to more complex β^3-aminoacids such as β^3-homophenylalanine, β^3-homophenylglycine and β^3-homoserine.

α-Alkylation Reactions

As mentioned above, reaction conditions were optimized on β-alanine bearing two *p*-methoxybenzyl protecting groups at the amino function. As depicted in Table 2, reaction conditions already optimized for the other hetero-functionalizations (1:2:1.8; substrate:base:electrophile) yielded a dialkylated product, together with the expected monoalkylated one that suggested us to reduce the equivalent of electrophile used to 1.1.

Table 5.1 α-Alkylation reactions of *N,N* -(MPM)-β-alanine methylester with KHMDS and LiHMDS



1

2 R = CH_3

3 R = CH_2Ph

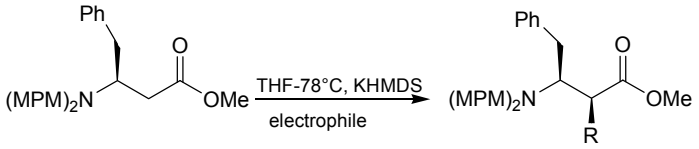
Base	Electrophile	Subs:Base:Electr. Equivalents	Time (min) and Temperature (°C)	Yield%[a]
KHMDS	CH_3I	1 : 2 : 1.8	60, -78	40[b]
KHMDS	CH_3I	1: 2 : 1.1	90, -78	61
LiHMDS	CH_3I	1 : 2 : 1.1	90, -78	45
LiHMDS	$PhCH_2Br$	1 : 2 : 2.2	150, -78	30[b]
LiHMDS	$PhCH_2Br$	1 : 2 : 1.1	150, -78	40
KHMDS	$PhCH_2Br$	1 : 2 : 1.1	150, -78	65
KHMDS	$PhCH_2Br$	1 : 2 : 2.2	150, -78	50[b]

[a]Yields of the product isolated and characterized after chromatographic purification.
[b]Dialkylation product formation was observed.

Chemical yields are good, and confirm also the outcome of some monodeuteration experiments (not reported), KHMDS affords greater yields than LHMDS, especially if one considers some recovered unreacted starting materials. This result is explained in terms of co-ordination ability of the enolate counterion: different from Li^+, K^+ is weakly co-ordinating and determines higher electron density on the $C^{\delta-}$ of the enolate, that is therefore more easily attached by electrophiles.

The optimized conditions were applied to more complex substrates. The results of α-alkylation reaction on *N,N*-(MPM)-β^3-phenylalanine methylester (**4**) are reported in Table 5.2. KHMDS was chosen as base, and 1:2:1.1 ratios between starting material, base, and electrophile were used.

Table 5.2 α-Alkylation reactions of *N,N* -(MPM)-β^3-phenylalanine methylester with KHMDS



4 **5** R = CH_3

6 R = CH_2Ph

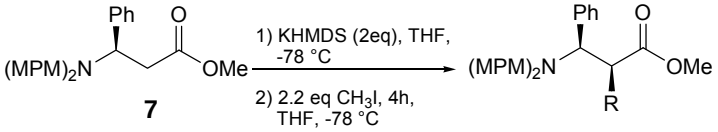
Electrophile	Subs:Base:Electr. Equivalents	Time (min) and Temperature (°C)	Yield%[a]	d.r.%[b]
CH_3I	1 : 2 : 1.1	120,-78	54	93:7
CH_3I	1: 2.8 : 1.1	180,-78	36	93:7
CH_3I	1 : 2 : 2.2	120,-78	97	93:7
$PhCH_2Br$	1 : 2 : 2.2	150,-78	90	97:3

[a]Total yield considering both diastereoisomers. [b]Diastereomeric ratios were determined by GC-MS and ^{1}H NMR analysis.

In order to improve the yields of the reactions, we tried to increase the equivalents of both base and electrophile, hoping that the steric hindrance of the residue at C-3 position of the β^3-amino acid could prevent formation of dialkylated products.

It is noteworthy, in fact, that the excess of the electrophile (2.2 equiv. versus 1.1) did not yield dialkylation, and afforded only a diastereomeric mixture of monoalkylated products.

The results obtained for *N,N*-(MPM)-β^3-phenylglycine methylester (**7**) are reported in Scheme 5.2



7

8 R = CH_3
80%, [a]d.r. 9:1

9 R = CH_2Ph
75%, [a]d.r. 92:8

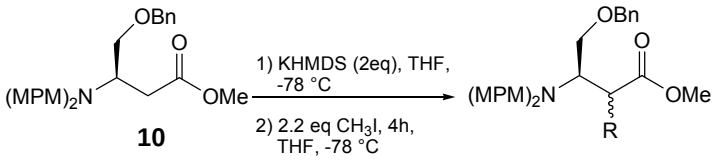
[a]Diastereomeric ratios were determined by GC-MS and ^{1}H NMR analysis.

Scheme 5.2 α-Alkylation reactions of *N,N* -(MPM)-β^3-phenylglycine methylester with KHMDS.

In this case, the best conditions for α-methylation and α-benzylation were the same as for β^3-phenylalanine.

The lower diastereoisomer ratio is due to the lower hindrance exerted by the phenyl group of β-phenylglycine in comparison with the benzyl group of phenylalanine.

Finally in Scheme 5.3 are reported the results obtained in the alkylation of *N,N*-(MPM)-β^3-serine methylester (**10**), under the conditions already experienced.



11 R = CH_3
89%, [a]d.r. 94:6

12 R = CH_2Ph
78%, [a]d.r. 96:4

[a]Diastereomeric ratios were determined by GC-MS and ^1H NMR analysis.

Scheme 5.3 α-Alkylation reactions of *N,N* -(MPM)-β^3-serine methylester with KHMDS

α-Hydroxymethylation Reaction

The introduction of polar chains at C-2 position of β^3-amino acids is of great interest for creating peptidic scaffolds with different side chains and there are only few methods to do it.[17,26]

For this reason, we considered, besides the α-alkylation reactions, the introduction of a group at C-2 position.

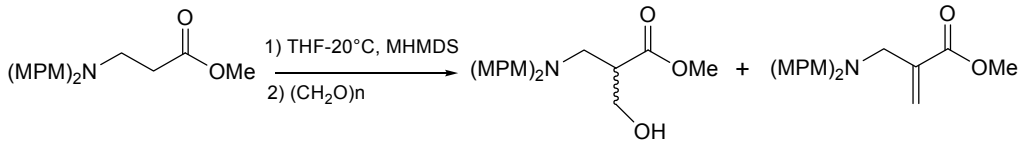
Also in this case, the best conditions were found using β-alanine as model. The electrophile was paraformaldehyde, that is cracked to formaldehyde, before the use and transfer into the reaction flask with a nitrogen flow.

The results are summarized in Table 5.3.

The difference between KHMDS and LHMDS was confirmed in this reaction as well. As shown in the Table, in fact, under the same reaction conditions, KHMDS reacted faster and gave better yields.

The temperature plays a crucial role; the enolate formation has to be carried out at -78 °C, but formaldehyde has to be added at higher temperature, to prevent its polymerization during the transfer from the cracking flask into the reaction system; at this temperature the reaction product **13** undergoes elimination to give **14**. In order to minimize this elimination process, cooling the system immediately after the addition of formaldehyde was necessary.

Table 5.3. α-Hydroxymethylation reaction of *N,N*- (MPM)-β-alanine methylester



1 **13** **14**

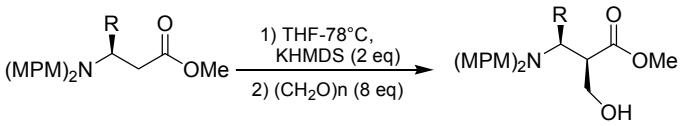
Base	Subs:Base:Electr. Equivalents	Time (min) and Temperature (°C)	Yield of 13[a]	Yield of 14[a]
LiHMDS	1 : 2 : 4	10, -20	32	30
KHMDS	1: 2 : 4	10, -20	40	50
KHMDS	1 : 2 : 8	10, -20	50	30
KHMDS	1 : 2 : 8	90, -78	85	--

[a]Yields after chromatographic purifications.

One more time, the reaction was extended to other β^3-amino acids, used in the other functionalizations as well.

As shown in Table 5.4, the diastereoisomeric ratios, as well as the yields are really good, especially considering that similar reactions operated on different starting materials work worst.[26]

Table 5.4 α-Hydroxymethylation reaction of *N,N*- (MPM)-β^3-phenylalanine, phenylglycine and serine methylesters



15-17

R	Time (min) and Temperature (°C)	Yield%[a]	d.r.%[b]
CH_2Ph	60, -78	65	99:1
Ph	60, -78	88	97:3
CH_2OBn	60, -78	78	95:5

[a]Yield of products **15-17** after chromatographic purification. [b]Estimated by GC-MS and ^{1}H NMR.

Lipo-amino Acids and Lipo-peptides

Lipo-conjugates prepared by introduction of a lipophilic moiety into a peptide sequence have been demonstrated to increase its overall hydrophobicity. This represents a powerful tool to solve the problem of insufficient bio-availability of some potentially bioactive peptides that may be related mainly to their poor membrane solubility.[23d]

In this respect, particular attention has been focused on lipidic α-amino acids. These unnatural α-amino acids contain long hydrocarbon side chains and have an amphiphilic nature that influences their physical properties.[23a]

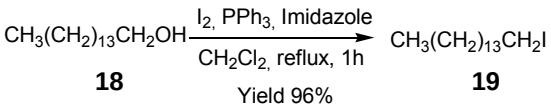
Peptides conjugated with lipo-amino acids show increased permeability through cell membranes and increased biological stability, both of which are essential properties for good drug delivery.[23b,c]

Moreover, lipo-conjugated peptides show better resistance towards proteolytic enzymes than the unmodified sequences.[27]

To the best of our knowledge, there are no examples in the literature of β-analogues of lipo-amino acids, having hydrophobic side chains on the 2-position of β-aminoacids.

For this reason, having optimized a method to introduce stereoselectively different kinds of substituents on the C-2 position of β-amino acids, we tried to use the same conditions to introduce one or two hydrophobic chains on β-amino acids.

First, we prepared a good electrophile: using a reaction devised in our lab, we accomplished the OH→I replacement exploiting the formation of PPh_3/I_2 complex as described in Chapter 3 and as shown in the Scheme 5.4:



Scheme 5.4 Synthesis of pentadecyl iodide

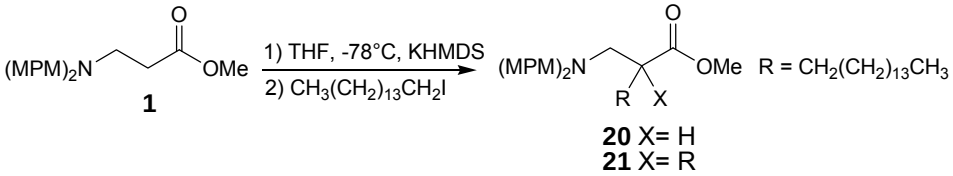
This reaction confirms that the method works really well also on simple alcohols, besides β-amino alcohols.

The compound **19** was used as electrophile in the alkylation of the enolate of β-alanine methyl ester.

It is noteworthy that the enolate is generated at -78 °C and, at this temperature, the pentadecyl iodide is not soluble in THF. For this reason, we were forced to operate at higher temperature (-20→0 °C). The difficulty to operate at these temperatures is due to the intrinsic instability of the enolate. Moreover, the iodide also becomes less stable; indeed, due to the basicity of the solution, the elimination process on the electrophile becomes more probable and competes with the substitution reaction.

Having all this in mind, we tried to use the same conditions devised for the other α-alkylations, except for higher temperatures.

Table 5.5 Alkylation of *N*,*N*-di-*p*-methoxylbenzyl β-AlaOMe



Subs:Base:Electr. Equivalents	Time and Temperature	Yield [a]%
1 : 2 : 2.2	120 min, 0 °C	50
1 : 2 : 1.1	180 min, 0 °C	13
1 : 1.1 : 1.1	90 min, 0 °C	4

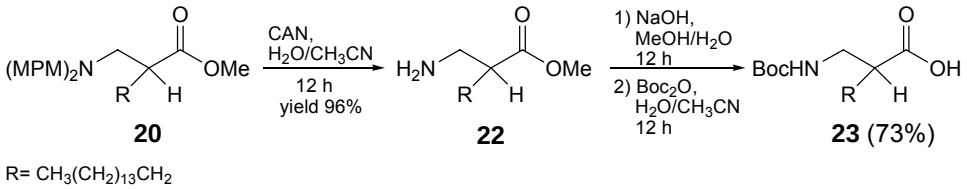
[a]Overall yield of mono- and di-alkylation products.

As shown in the table, under all the conditions already tested for the other alkylations, we had formation of di-alkylation product in addition to the mono-alkylated one, as already experienced for the methylation and benzylation of β-alanine; this could also not be prevented by reduction of the amount of both base and electrophile.

With the aim to use both the products for the preparation of peptides, it was necessary to optimize the process to discriminate mono- and di-alkylation.

However the first problem, that we considered, was the possibility to remove from the final products all the protections. It is known, in fact, that hydrolysis of hindered esters could be difficult; if we consider that our lipoamino acids are scarcely soluble in water, this deprotection is very intriguing. Thus, we tried, first, to isolate the mono- and di-alkylated products, to optimize their deprotections.

Actually, whereas the product **20** (Scheme 5.5) is easily deprotected on both amino and carboxyl functions, for the product **21**, especially the hydrolysis of the methyl ester group was hard to accomplish.

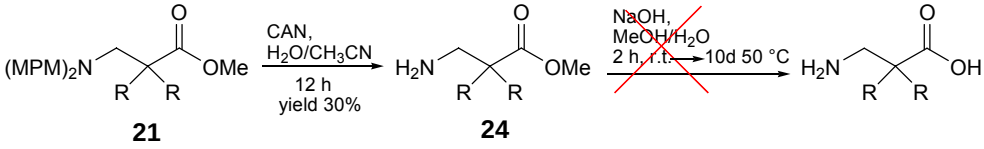


20 **22** **23** (73%)

R= $CH_3(CH_2)_{13}CH_2$

Scheme 5.5 Deprotection of mono-alkylated product.

As shown in Scheme 5.6, we first attempted to deprotect the amino group, using CAN in a mixture of H_2O/CH_3CN (1:4, v:v). The reaction run for 2 days giving the product **24** with only 30% yield. Moreover, the hydrolysis of **24** with NaOH in $MeOH/H_2O$ attempted for 12 h at room temperature and for 10 days at 50 °C without giving any result. Also hydrolysis with Me_3SiCl/NaI, that proceeds via an acyl iodide intermediate, did not afford any product.



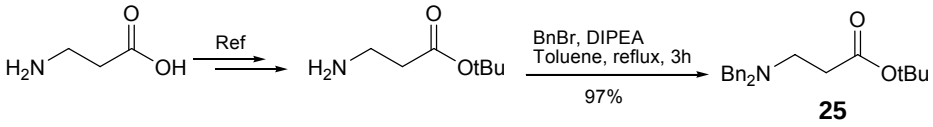
21 **24**

R= $CH_3(CH_2)_{13}CH_2$

Scheme 5.6 Deprotection of di-alkylated product.

For this reason, we replaced the methyl ester with the more easy to cleave *tert*-butyl ester. The latter can be cleaved under mild acid conditions. It was also necessary to replace the MPM protections with simple benzyl groups. MPM deprotection, in fact, is carried out with CAN in H_2O/CH_3CN, namely strong oxidative conditions. Instead, the benzyl group is cleaved by hydrogenation on Pd/C in AcOH.

According to a published procedure,[28] we prepared the *N*,*N*-dibenzyl-β-alanine-*tert*-butyl ester **25**, as shown in Scheme 5.7.



25

Scheme 5.7 Preparation of protected β-alanine

The compound **25** was then used as starting material for optimization of the reaction with pentadecyl iodide.

Mono-α-alkylation

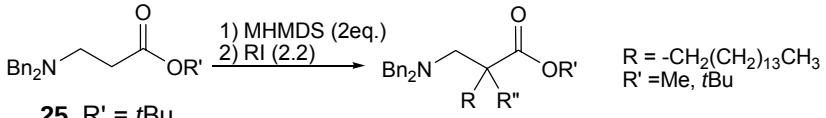
From the first experiments, it was possible to conclude that, under any conditions, with KHMDS, both mono- and di-alkylated products are always obtained.
Thus, we considered the use of LHMDS.

Li^+ ion, in fact, is smaller than K^+ ion, and it is able to form aggregates in solution. Its co-ordinating character is stronger than that of K^+ and, thus, Li^+ is able to form with the enolate a more covalent (compared with that produced by K^+) bond, decreasing in this way nucleophilicity and thus reactivity of the $C^{\delta-}$ of the enolate.

Actually, treating **25** with LHMDS (2 eq) and electrophile (2.2 eq), as reported in entry 2 of Table 5.6, we obtained only mono-alkylated product with 95% yield. Also reiteration of the reaction on **26** did not lead to the di-alkylated product.

Using, under the same conditions, KHMDS as base (entry 1, Table 5.6), we had an excellent yield, 95%, but a mixture of products, with 25% of di-alkylation.

Table 5.6 Alkylation of *N*,*N*-dibenzyl β-AlaOtBu and *N*,*N*-dibenzyl β-AlaOMe



25 R' = *t*Bu
28 R' = Me

26 R'= *t*Bu, R"= H **MONO**
27 R'= *t*Bu, R"= R **DI**
29 R'= Me, R"= H **MONO**
30 R'= Me, R"= R **DI**

Subs.	Base	Time and Temperature	Total Yield	MONO/DI Ratio
25	KHMDS	60 min, 0° C	95%	3:1
25	LiHMDS	30 min, 0° C	95%	> 99:1
28	KHMDS	60 min, 0° C	70%	2.3:1
28	LiHMDS	3 h, 0° C	60%	> 99 : 1

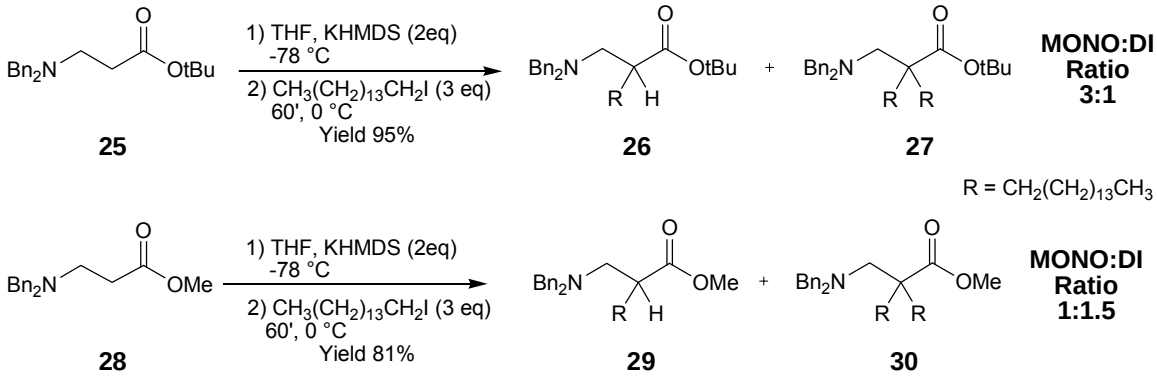
We also wanted to compare the reactivity of *N*-protected β-homoalanine having different ester groups, namely the reactivity of **25** with that of **28** (Table 5.6); thus, we prepared **28** from H-β-Ala-OMe, and then we alkylated **28**, exploiting the same conditions used to alkylate **25**: employing LHMDS as base, we had again only mono-alkylation but with lower yield; instead, the use of KHMDS gave both the products with total yield of 70%.

The different reactivity shown by **25** and **28** with LHMDS is probably due to the steric hindrance of *tert*-butyl ester; the enolate coming from **25** is less co-ordinated Li^+ and thus more reactive.

Double-alkylation in the α-position

In order to accomplish the double alkylation, obviously we used KHMDS as base, because only with this we observed traces of dialkylated product.

Using the conditions shown in Scheme 5.8, we afforded mixtures of mono- and di-alkylated products, for both **25** and **28** as starting materials.



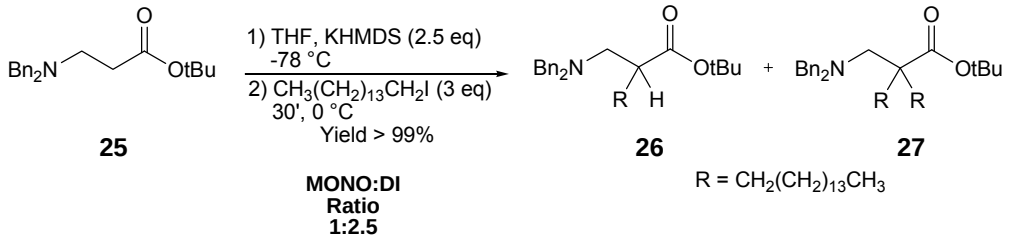
Scheme 5.8 Double alkylation of *N*,*N*-dibenzyl β-AlaOtBu and *N*,*N*-dibenzyl β-AlaOMe.

Also in this case, we could note a different reactivity between **26** and **29**. The latter reacts faster to give the di-alkylated product **30**, the major product of the double alkylation reaction of **28**. The compound **26**, instead, is alkylated with more difficulty and only 25% of it is converted to di-alkylated product **27**. This different behaviour is again due to the larger steric hindrance of *tert*-butyl ester compared with that of methyl ester; *tert*-butyl ester on one hand facilitates the entrance of the first chain onto C-2 of **25** (for the reason already discussed above), but on the other hand,

makes difficult the access of the second chain on the already alkylated C-2 position of **26**.

However, we decided to use the compound **25** as starting material for the preparation of lipo-amino acids, because of the problem met during the hydrolysis of methyl ester of highly hindered substrates such as **30**.

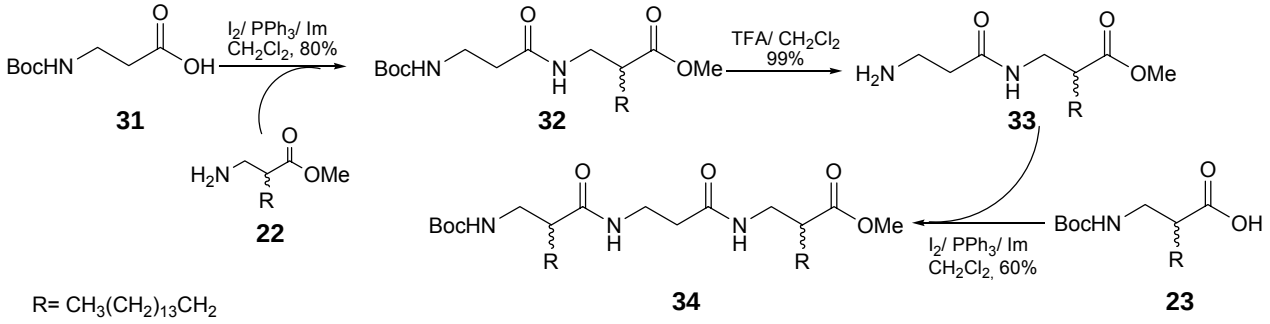
To improve the amount of di-alkylated product we increased the amount of base used, from 2 to 2.5 equivalents, and the results are shown in Scheme 5.9.



Scheme 5.9 Double alkylation of *N*,*N*-dibenzyl β-AlaOtBu.

Peptide Coupling

The first experiments were carried out on the mono-alkylated product. The latter consist of a racemic mixture, which was used as such, without performing any resolution, because this compound was considered just as a model for couplings. We tested, in fact, the reactivity of both its amino and carboxyl functions, making them to react with carboxyl and amino groups of a protected β-alanine, respectively. Thus, using the mono-alkylated amino acid as building block, we prepared a dipeptide and a tripeptide still made up of it but containing a spacer of β-alanine in the middle.

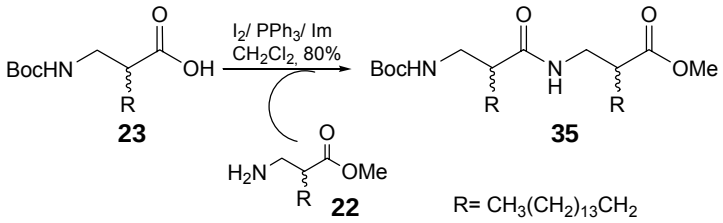


Scheme 5.10 Synthesis of a tripeptide

The activation of carboxyl function for all the couplings was performed using PPh_3/I_2 complex to form an acyl iodide intermediate, that reacts in turn with the amino function of another amino acid.

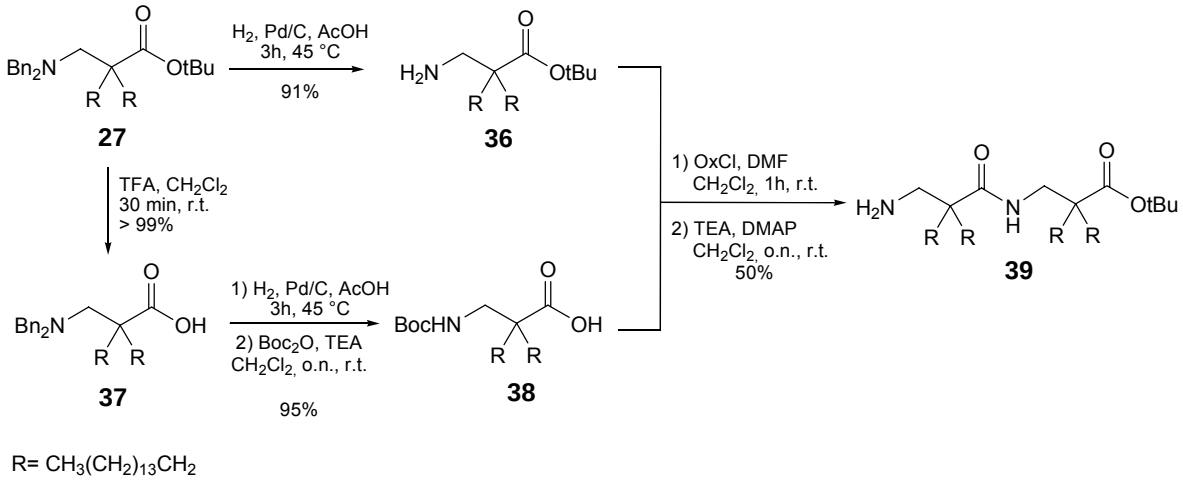
As shown in Scheme 5.10, the amino function of the mono-alkylated β-alanine **22** reacts well, without suffering from any steric hindrance; the same is true for the carboxyl function of **23** that reacts with good yield. Thus, we obtained the tripeptide with a total yield of ~50%.

The dipeptide was prepared using the same conditions, with 80% of yield (Scheme 5.11).



Scheme 5.11 Synthesis of a dipeptide

The same procedure was followed for the di-alkylated β-alanine.
At first, we prepared the starting materials for the couplings, as shown in Scheme 5.12.



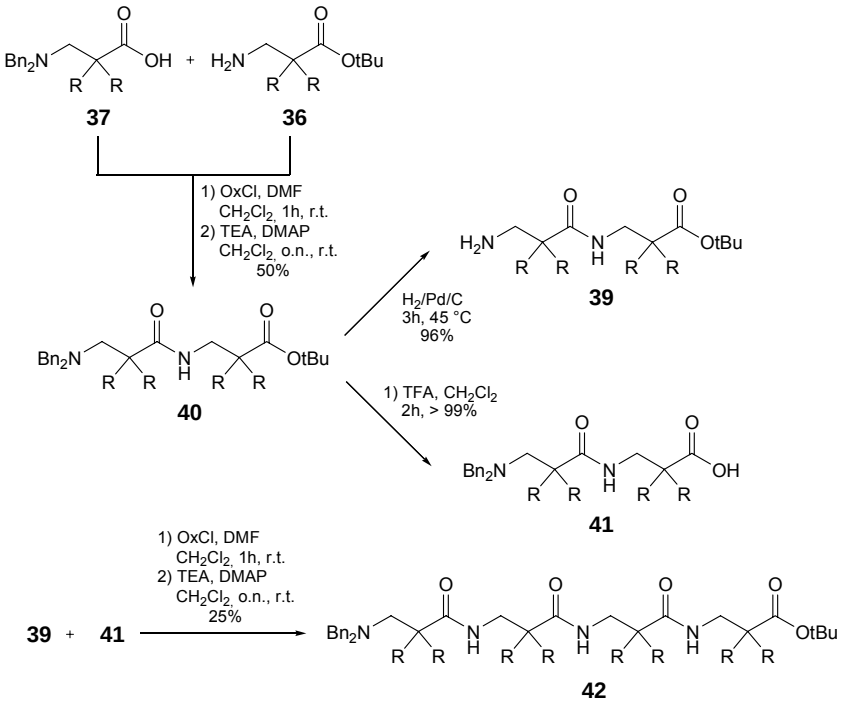
Scheme 5.12 Synthesis of a dipeptide.

As could be expected, the activation of the carboxyl function in **38** was difficult, because of the hindrance of the two long-hydrophobic chains. Some classical methods for peptide-coupling failed (PPh_3/I_2, DCC/HOBt) and also the coupling with a stronger activating agent, such as HATU, proceeded with modest yield (58%).

The use of $SOCl_2$ as activating agent was, instead, successful. The activation gave acyl chloride in quantitative yield, as tested by quenching the reaction with MeOH.

The next step was the reaction of the acyl chloride intermediate with the amino function of **36** that proceeded with 50% yield.

Under these conditions, it was, however, precluded the possibility to use the Boc group as *N*-protection of the β-amino acid. In fact, the acidity of the medium during the activation step causes the loss of the protecting group.



Scheme 5.13 Synthesis of a tetrapeptide.

So, we decided to keep the benzyl group and to use directly the compound **37** in the coupling with **36** to prepare dipeptide **40** and then tetrapeptide **42**.

Conclusion

The method described represents a convenient synthesis of protected lipophilic amino acids containing one or two alkyl side chains (C 15). The length of the chain can be modulated just choosing the right electrophile, and the method is general.

Resolution of the racemic mixture of **26** or **29** and extensive application of the pure enantiomers of these lipophilic amino acids in peptide synthesis is in progress in our laboratories.

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Chapter 6

Sweetness and Artificial Sweeteners

The sense of taste plays a critical role in the life and nutritional status of humans and animals as well. The ability to identify sweet-tasting foodstuffs is particularly important as it provides us and other vertebrates with a mean to seek out needed carbohydrates with high nutritive value. Sweetness can be a sensory cue for energy to fuel metabolic needs and physical activity. Foods that are naturally sweet, such as fruit and breast milk, contain important nutrients to support health. Sweet foods and beverages offer a pleasurable addition to a meal or a snack. Sweet-tasting compounds help mask unpleasant tastes, thereby enabling the development of more palatable foods, health care products, and medicines. The perception of bitter, on the other hand, is essential for its protective value, enabling humans to avoid potentially deadly plant alkaloids and other environmental toxins.

Sweet taste is stimulated by a wide variety of compounds including sugars, sugar alcohols, and dipeptides, that elicit pleasurable sensations with (nutritive) or without (non-nutritive) energy. Nutritive sweeteners provide a sweet taste and a source of energy; non-nutritive sweeteners are sweet without energy.

Because of an exponential growth in the number of patients suffering from diseases caused by the consumption of sugar, such as obesity[1] or diabetes[2] mellitus, there is increased interest in dietary factors that cause energy intake to exceed energy expenditure[3].

Existing evidence does not support the claim that diets high in nutritive sweeteners *by themselves* have caused an increase in obesity rates or other chronic conditions[4] (eg, hyperlipidemia, diabetes, dental caries, behavioral disorders). Nonetheless, consumers who want the taste of sweetness without added energy may select non-nutritive sweeteners to assist in the management of weight, diabetes, and other chronic diseases. Non-nutritive sweeteners also have, in fact, the potential to assist in control of blood glucose, and in prevention of dental caries.

The FDA has approved five non-nutritive sweeteners and regulates them as food additives: saccharin, aspartame, acesulfame potassium (or acesulfame K), sucralose, and most recently neotame.

Mode of Action

Compounds that stimulate the sweet sensation interact with taste receptors in the mouth and throat. Through a transduction mechanism, the sweet chemical message is changed to a nerve signal for the perception of sweet taste. Models of sweet transduction are being tested under speculation that nutritive sweeteners have different mechanisms than non-nutritive sweeteners.[5]

First, the diversity of chemical structures (including sugars, sulfamates, heterocycles, ureas, arylguanidines, peptides, proteins, oximes, and terpenes) which can evoke sweet taste is really huge. This suggests that there must be more than one receptor

type.[6] It is unlikely that a single receptor binding site could accommodate all of the known sweet-tasting compounds.

Secondly, we know that it is easy for humans to distinguish different taste qualities for different high-potency sweeteners. Most consumers can readily tell the difference between sugar and high-potency sweeteners and trained panellists can detect taste differences among different high-potency sweeteners. Third, blends of structurally different sweeteners (aspartame plus acesulfame, for example) produce synergy, a higher-than-predicted sweetness intensity based on potencies of the two individual sweeteners.[7] On the other hand, structurally similar sweeteners, such as saccharin and acesulfame, do not show synergy; they are simply additive. Many more studies of this type would be required to get an estimate of the number of different types of sweetness receptors that might be involved.

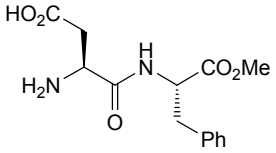
Finally there are reasons to suspect that sugars do not bind to a specific receptor site. First, sugars taste sweet only at high concentration (about 0.1 M); this is in sharp contrast to the concentrations required for hormones, neurotransmitters, etc. to trigger their receptors (typically nanomolar or picomolar concentrations). Furthermore, it is well known that D- and L-sugars have comparable sweetness potencies. These observations suggest that sugars might act indirectly on receptors or ion channels, through effects on the properties of the surrounding membrane lipids.

In summary, the available evidence indicates that high-potency sweeteners utilize a different mechanism than sugars to trigger the response of taste cells. In addition, it appears that the receptors which mediate the sweetness of aspartame are different from those which respond to saccharin and acesulfame.

Mechanism for Sweet Transduction

Taste receptor proteins have not yet been isolated or unequivocally identified. There are two major reasons for that:

1. Traditional receptor isolation methods have usually involved the use of a high-affinity ligand (half-maximal binding at nanomolar concentrations or less) which can be used to monitor the progress of purification. Sugars trigger sweetness only at concentrations of ~0.1 M; aspartame (**1)**, saccharin (**2**), and acesulfame-K (**3**) elicit sweetness at concentrations of few millimoles per liter. At these concentrations, it is nearly impossible to distinguish receptor-specific binding from non-specific binding to other components of the tissue from which the receptors might be extracted.



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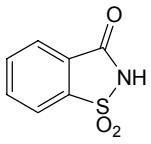

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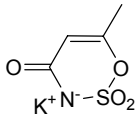


3

2. The difficulty in isolating taste-active cells. These are specialized epithelial cells which are embedded in taste buds among a large number of no taste-active cells. When these cells are separated from their nerve cells, they rapidly de-differentiate back into ordinary epithelial cells. Thus, it has not been possible to grow taste-active cells in cell cultures.

Despite these difficulties there is now good evidence indicating that sweetness is mediated in many cases by G-protein-coupled receptors[8] (GPCR), that are responsible for the detection of many neurotransmitters, hormones, odorants, and

even photons. They are proteins having seven α-helical segments embedded in the cell membrane.

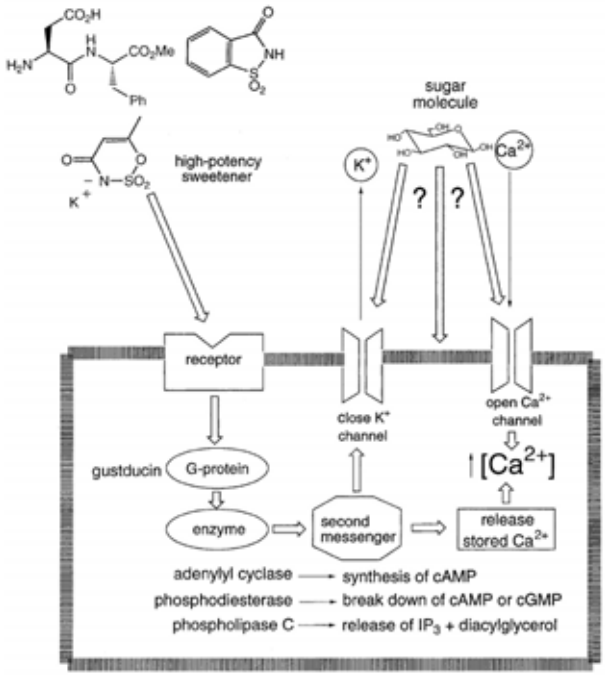


Figure 6.1 Schematic representation of a taste cell, indicating likely components of the taste transduction system. The initial stimulus may be either a high potency sweetner or a sugar; the final step is cell depolarization, which may involve loss of potassium ions and a sharp increase in the concentration of free intracellular calcium ions. [from Wiley-VCH Angewante Chemie Int. Ed. (ref. 8)].

When extracellular ligands bind to the receptor (Figure 6.1), they induce a conformational change in the protein causing the intracellular portion to interact with one or more GTP-binding proteins (the G proteins). The G proteins may, in turn, modify the activity of an enzyme, which increases or decreases the amount of a 'second messenger' molecule: adenylyl cyclase may be induced to make higher levels of cyclic AMP (cAMP), or a phosphodiesterase may degrade cyclic nucleotides, or a phospholipase may convert phosphatidylinositol lipids to diacylglycerol and inositol trisphosphate (IP3). These second messengers may alter calcium ion concentrations or modify ion channel activities leading to depolarization of the taste receptor cells (TRCs) which, in turn, sends a nerve signal to the brain. It seems[5] that sucrose and other sugars trig sweet transduction *via* adenylyl cyclase and cAMP, whereas artificial sweeteners *via* IP_3 production; all transduction pathways are proposed to converge on common elements that mediate a rise in intracellular Ca^{2+} followed by neurotransmitter release. These two pathways (diagrammed in Fig. 6.2) coexist in the same TRCs.

It is presently unclear how these receptors could selectively mediate cAMP responses to sugars and IP_3 responses to artificial sweeteners, but recently sweet-responsive receptors have been cloned and expressed[5] and so it should be possible, with next studies, to test definitively these various models of sweet transduction.

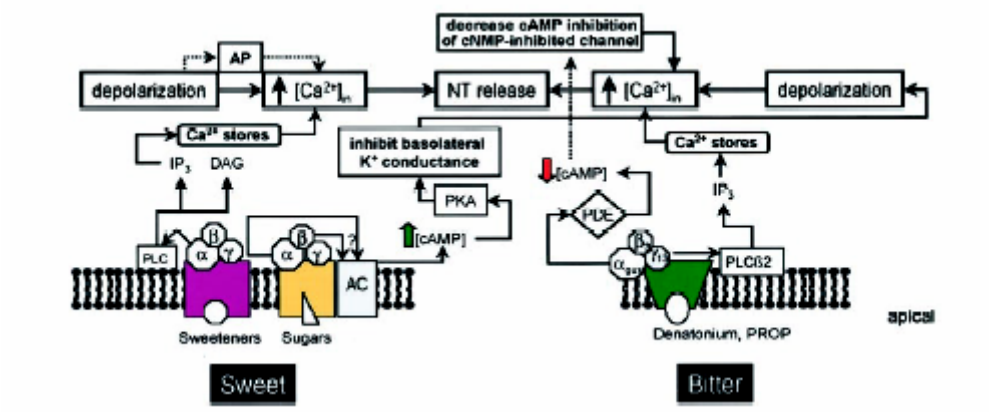


Figure 6.2 Proposed transduction mechanisms in vertebrate taste receptor cells underlying taste qualities [from The American Society for Biochemistry and Molecular Biology, The Journal of Biological Chemistry (ref. 5)].

Structure-Activity Relationship

As illustrated above, the perception of sweetness is initiated by an interaction between a ligand and a receptor on the surface of a taste cell. In 1967 Shallenberger and Acree proposed that the ligands involved in this reaction must have a bipolar functional group capable of forming a cyclic hydrogen bonded transition state.[9]

In order to explain the initial chemistry of the transduction mechanism in rodents, three proposals have been made, as already briefly mentioned above and summarized below:

1. The sugar receptor (SR) mechanism: a cellular response is brought about by a specific 7-trans-membrane receptor protein, coupled to a G-protein and a second messenger cascade. Certain polyol structures (sweet ligands) interact with the protein receptors causing a G protein to release the intracellular second messenger cAMP (Hepler & Gilman, 1992).
2. The glycine receptor (GR) mechanism: amino acid-like ligands bind to a second 7-transmembrane receptor protein interacting with another G-protein to release the intracellular second messenger IP3 (Naim, Bernhardt, Zehavi & Levinson, 1996).
3. The direct G-protein interaction (DGI) mechanism: certain ``amphiphilic'' compounds (having both polar and non-polar functions) penetrate the cell and interact with the G proteins causing second messenger release, usually IP3, in much the same way that some drugs behave pharmacologically (Naim et al., 1996).

Relating the structure of sweet tasting molecules to their sensory properties, the most commonly used tool to generate hypotheses about ligand binding in human systems is a process called structure-activity relationship (SAR).

The four most frequently mentioned SAR models, consistent with both SR and GR receptor mechanisms, are: the Shallenberger theory or the bipolar hydrogen bonding (*AH-B* theory);[9] the three-point attachment theory (*AH-B-*γ);[10] the multi-point attachment theory;[11] the α-helix receptor protein theory.[12]

All these theories[13] are consistent with the idea that sweet-tasting compounds contain a hydrogen bond donor (AH) and a hydrogen bond acceptor (B) that reacts with a complementary AH-B pair on the receptor, forming two hydrogen bonds and/or interrupting an intramolecular hydrogen bond on the receptor protein.

Shallenberger and Acree utilized this model to analize different kinds of sweetners and to establish if their molecules had the intramolecular system *AH-B.* (Figure 6.3)

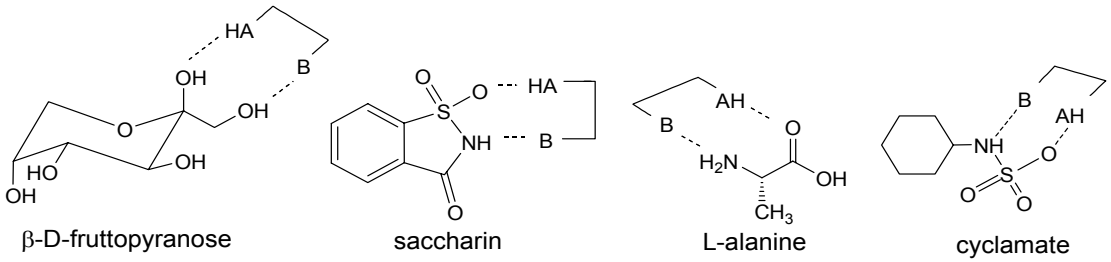


Figure 6.3 AH-B system in some sweeteners

This theory was then developed in 1972 by Kier,[11] who proposed that a potent sweetener must interact with the sweetness receptor through three interactions, two of which by hydrogen bonding according to Shallenberger and Acree and the third one by dispersion (van der Waals) or hydrophobic interaction with the so called γ site. This interaction is to increase the affinity of an amino acid with an AH-B glycophore at the receptor site, thus enhancing the sweet taste potency. Therefore, if γ is not required for binding, it may function to modulate the potency of the ligand. The degree of enhancement is governed by the distance and position of γ from AH-B system, as evident from observations on the taste of aspartame analogues.[14] Goodman[15] *et al.* in 1987 sought to deduce basic principles of taste perception from consideration of the energy-minimized structures and the tastes of a series of dipeptide isomers and derivatives. It was found that the structures of sweet-tasting dipeptides adopt the L-shape shown in Figure 6.4 while their retro-inverso analogues do not. Only the L,L-isomer of aspartame tastes sweet, since only this isomer can adopt the L-shape prerequisite for sweet taste while the other three isomers are bitter.

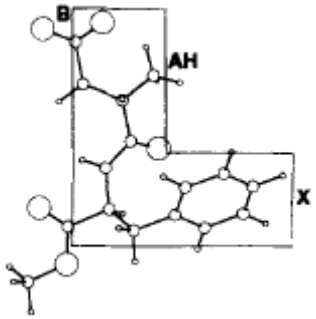


Figure 6.4 Required L-shaped molecule for sweet aspartyl compounds.[18]

In 1991, this model was developed by Tinti and Nofre[12] who suggested that, when a sweetener interacts with its receptor, it splits both ionic and H-bonding interactions in the sites, triggering profound conformational changes in the receptor and allowing it to expand. Presumably, this allosteric effect is responsible for initiating transduction. Sweetness potency can then be correlated with the number of additional sites involved at the receptor during interaction. This is consistent with the role of γ as an amplifier of potency but unnecessary for sweetness.

Another model for the sweet taste receptor has been introduced by Suami and Hough[13] in 1991 who postulate that the AH-B interaction of sweet molecules is at the *N*-terminus of a receptor protein which has a right-handed α-helical conformation. These ideas are consistent with the chiral specificity of the sweet taste response.

So all these models, although they differ in details, include an AH-B interaction and the modulation of hydrogen bonds as essential to the initiation transduction.

Commercial Synthetic non-Nutritive Sweeteners

In the last few years, an intense research effort has been expressed for the discovery of new intensive sweeteners. [8] The screening of new active compounds has been made by systematic examination of natural compounds from plants and classical methods of drug design of synthetic compounds, from known active leads. Despite these efforts, to date the two synthetic compounds, aspartame and saccharin, remain the undisputed leads of the market of intensive sweeteners.

Aspartame

Aspartame (**1**) is currently the most widely used non-nutritive sweetener worldwide. Although this compound was known in the literature, its sweetness was discovered by James Schlatter, who in 1965 while working on an antiulcer compound licked his fingers.[16] Several analogues, especially more stable esters,[17] were made and evaluated for sweetness potencies, taste profile, and toxicities. In the end, however, it was the first compound to be chosen for commercial development.

At high pH aspartame cyclizes to the corresponding diketopiperazine. In aqueous solution, the maximum stability[18] is at pH 4.3. At higher or lower pH, the half-life of aspartame diminishes quickly. The ester group of the aspartame is very susceptible to hydrolysis and hence avoidance of excessive heat is desirable since degradation with concomitant loss of sweetness can be rapid. It can be used in almost all food categories, but the major consumption is in beverages.[19]

Intestinal esterases hydrolyze aspartame to aspartic acid, methanol, and phenylalanine.[20] These components are found in much greater amounts in the normal diet in fruit, vegetables, meat, and milk. For example, a serving of non-fat milk provides about six times more phenylalanine and 13 times more aspartic acid, whereas a serving of tomato juice has about six times more methanol than an equal volume beverage sweetened 100% with aspartame.[21]

Detailed studies have been conducted to determine how ingestion of aspartame influences plasma levels of aspartic acid, phenylalanine, and methanol (or the byproduct formate). In studies with healthy adults,[22] plasma aspartate concentrations or blood levels of formate did not change with a bolus load up to four times the ADI (Acceptable Daily Intake) for aspartame (i.e., 200 mg/kg).

Plasma phenylalanine response to aspartame (as well as to other dietary sources of phenylalanine) varies in persons with phenylketonuria (PKU), a homozygous recessive inborn error of metabolism, that prevent metabolism of phenylalanine. People with this rare (frequency is approximately one in 10,000 whites) inborn error, in consequence of excess intake of this amino acid, can suffer an increasing of plasma phenylalanine levels and then its adverse effects.[23] For this reason, the FDA requires that foods containing aspartame have the prominent display of the following label: “PHENYLKETONURICS: CONTAINS PHENYLALANINE”.[24]

Aspartame breaks down to diketopiperazine in liquid systems with excessive heat exposure. Animal toxicity studies show that, even if all aspartame were converted to diketopiperazine in beverages, the amount would be well below the FDA-established ADI of 3 g/kg bw/day for this compound.[25] Some individuals report allergic reactions to aspartame, including edema of the lips, tongue, and throat, besides dermatologic reactions and respiratory problems.[26] However, two double-blinded challenge studies

report difficulty in recruiting individuals who claim an allergic response to aspartame and a failure to reproduce the allergic reaction in controlled experimental conditions.[27]

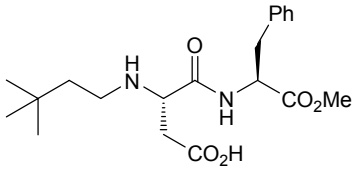
A comprehensive review of the safety of aspartame has recently been published,[28] that support the safety of aspartame as a food additive and negates claims of its association with a range of health problems including brain tumors.

Towards New Sweeteners

Although aspartame can be used in a wide variety of foods and beverages, its stability remains a problem. Extending shelf life by improving the stability of sweeteners, in general, in different food products, in particular diet beverages, would have significant economic impact.

The stability of aspartame can be improved forming inclusion complexes with β-cyclodextrin that are able to complex a wide variety of guest molecules,[29] and the formation of inclusion complexes with aspartame has been demonstrated[30]. For aspartame, studies have indicated that the β-CyD/aspartame complexation occurs via the inclusion of the phenyl ring within the β-CyD cavity, with the methyl ester exposed outward, away from the complex. On the basis of this model,[30] it is possible to explain the decrease in the rate of formation of diketopiperizine that takes place, because formation of the inclusion complex in this manner prohibits intramolecular cyclization. However, the model cannot entirely account for the concomitant decrease of α-L-aspartyl-L-phenylalanine formation because the methyl ester group is expected to be still exposed to the solution environment.

A number of companies and universities are still actively pursuing the search for, and development of, new non-nutritive synthetic sweeteners. Economic issues are driving these compounds to be extremely potent on a sweetness-to-weight basis when compared to sucrose. Since the discovery[31] of aspartame, the special interest in dipeptide sweeteners has increased and a large number of analogues related to aspartame have been synthesized.[8] These potential next-generation sweeteners contain a diverse array of functionality ranging from dipeptide derivatives[32] such as **7** and **8** to guanidines[33] and ureas.[34]



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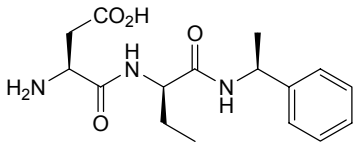

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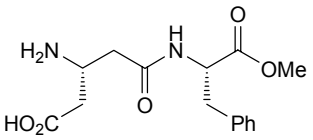
Neotame (**7**), for example, is a derivative of the dipeptide phenylalanine and aspartic acid. This compound is 8000 times sweeter than sucrose and can be prepared by a reductive alkylation of aspartame (**1**).[24] This new sweetener appears to have increased stability under a variety of conditions relative to **1**. It is partially absorbed in the small intestine, rapidly metabolized by esterases, and excreted in urine and feces. Methanol is released during the hydrolysis; the amount released is insignificant even at the 90th percentile of estimated daily intake of neotame. A small percentage (<20%) of phenylalanine from the ingested neotame may be released into plasma. This amount is not clinically significant for individuals with PKU. Thus, the label for products with neotame does not need to alert phenylketonurics that the product contains phenylalanine. Furthermore, no organs were found to concentrate neotame or its metabolites. [35-36]

Dipeptide Taste Ligands Containing homo-β-Amino Acid Residues

Recently Miyoshi[37] *et al* reported the synthesis of several aspartame dipeptide analogues, whose backbone was elongated by one methylene group, to analyse the steric effect in relation with the potency of sweetness. It has been demonstrated[32k] that the sweet-tasting activity of the dipeptide is preserved if the aspartyl moiety is restricted to L-Asp or to the shorter homologue aminomalonic acid in which the side chain methylene group is missing; elongation of the side chain as in the higher homologue NH_2-L-Glu-L-Phe-OMe led to bitter compounds.[38]

Later in 1999, Benedetti[39] *et al.* described the synthesis and conformational properties of a series of dipeptide taste ligands, differing from the commercial aspartame by the presence of a methylene group between the C-1 and the C-2 carbon atoms (as in β^3-residues) in either L-Asp or L-Phe residues (**9**-**11**).



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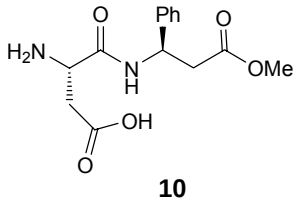

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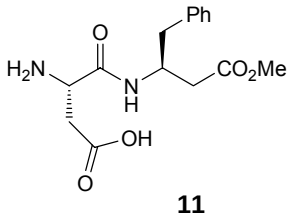

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The relationship between conformation and sweet taste showed that the lengthening of the peptide skeleton at the L-Asp site (as in **9**) results in a loss of sweetness with the production of tasteless compounds, thus confirming that (i) the *C*-terminal end is the most important function in imparting the sweet taste to the molecule and (ii) the orientation of the amide group linking Asp and Phe residues of aspartame analogues plays a decisive role in obtaining very sweet compounds. On the other hand, lengthening of the skeleton at the *C*-terminal L-Phe site (as in **10** and **11**) maintains the sweet character in both NH_2-L-Asp-β^3-L-Phe-OMe and NH_2-L-Asp-β^3-L-Phg-OMe. Anyway, all these compounds present a decreased sweetness potency with respect to aspartame; this could be ascribed to an increase of the skeleton chain flexibility due to the methylene insertion.

The conclusion was that the sweet power of aspartame analogues can only be modulated by modifying the *C*-terminal moiety of the molecule.

For a sweet-tasting ligand the 'bioactive conformation' was proposed to be an 'L-shaped' structure,[39-40] as mentioned above. The analysis of the conformational behaviour of the synthesized dipeptides showed that all of the compounds can adopt preferentially extended and 'L-shaped' conformations.

Data of structure–taste relationships accumulated so far prompted us to investigate the effect on taste attained by insertion of a substituent at C-2 position of a β^3-amino acid, that replaces the L-Phe residue in dipeptide analogues. For this purpose, using our homologation procedure[40] in the preparation of β^3-amino acids, and the already illustrated reaction for the introduction of substituents onto C-2 position of β^3-amino acids, also devised in our lab, we prepared a series of new taste ligand dipeptide analogues, that will be presented in the next section.

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Chapter 7

Synthesis of Aspartame Analogues

Following the discovery of aspartame, the interest in dipeptide sweeteners has increased, and the possibility of designing and synthesizing new analogues[1] of this molecule has stimulated numerous studies of structure-activity relationship.

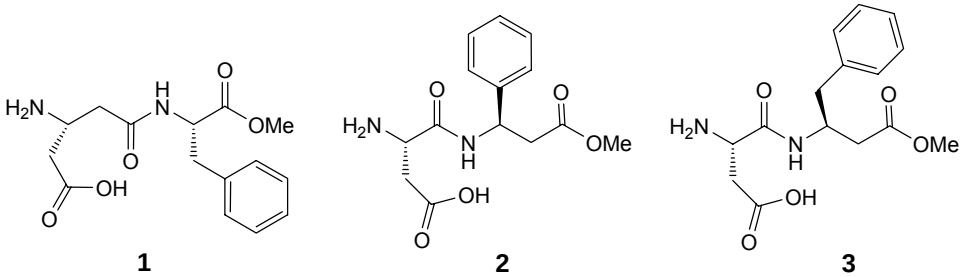
The strongest interest is in finding aspartame analogues that are more stable to enzymatic degradation: as mentioned in chapter 6, indeed, aspartame is hydrolyzed by intestinal esterases to aspartic acid, methanol, and phenylalanine. Thus, as source of phenylalanine, aspartame could be dangerous for people with phenylketonuria.

In recent years, several efforts have been made in order to elucidate the stereochemical basis of sweet taste and two models have emerged as the most appropriates: Temussi's[2] and Goodman's[3]. The first one proposes as bioactive conformation of aspartame an extended structure; the second suggests that a sweet-tasting molecule possesses an L-shape conformation: the stem of the L is formed by the zwitterionic (AH and B) ring of the aspartyl residue and the base of the L is formed from the hydrophobic group X (see Fig. 6.4, Chapter 6). The difference between the two models is then the bioactive conformation of peptide tastants like aspartame and consequently there is the difficulty to elucidate which is the bioactive conformation of these aspartame-like sweeteners.

In order to develop a three-dimensional receptor model, several conformationally restricted analogues of aspartame have been synthesised and studied. Since it has been shown that many changes in the Phe-residue[4] of the dipeptide are well tolerated, and taking into consideration that one approach involves the use of constrained amino acids,[5] the Phe residue of aspartame has been replaced by different conformationally restricted amino acids.[6]

On the other hand, the synthesis of peptides in which each amino acid residue is replaced by a β-amino acid has also been reported.[7] (**1**-**3**).

The insertion of β-amino acids as new molecular tools in bioactive products represents a way to improve both resistance to biodegradation and pharmacokinetic properties of these molecules.



1 **2** **3**

Conformational studies[7] of the dipeptides **1**-**3** showed that they are able to adopt an L-shape conformation, as required for a good interaction with the receptorial sites of the sweet taste. Moreover it is evident from these studies, as it was shown by

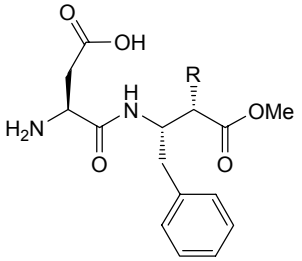
others,[8] that the elongation of the peptide skeleton at the L-Asp site (like in **1**) results in a drastic loss of sweetness with the production of tasteless compounds, showing the importance of the C-terminal site of the peptide for its interaction with the receptor.

On the other hand, lengthening of the skeleton at the C-terminal L-Phe site (like in **2** and **3**) preserves the sweet taste, causing only a slightly decrease of sweet-tasting activity compared with that of aspartame. This loss of activity is probably due to an increasing flexibility of the peptide, caused by the introduction of a methylene group that allows C2-C3 bond of β^3-Phe to be less rigid.

To the best of our knowledge, Phe residue of aspartame has never been replaced by 2,3-disubstituted β-amino acids. Since it is reported that 2,3-disubstituted β-amino acids are more conformationally constrained than simple β^2 and/or β^3-amino acids,[24] we decided to investigate the effect on taste of the systematic insertion of a substituent at C-2 position of the β^3-phenylalanine that replaces the Phe-residue of aspartame.

Results and Discussions

We planned to investigate the design and synthesis of $\beta^{2,3}$-disubstituted amino acid containing peptides as new aspartame analogues: the L-Asp residue is linked to a β^3-Phe functionalised at C-2 position with both polar and non-polar groups. In particular, we prepared the dipeptides **4**-**7**, containing respectively hydroxyl, amino, methyl and benzyl groups.



4 R= OH
5 R= NH_2
6 R= CH_3
7 R= CH_2Ph

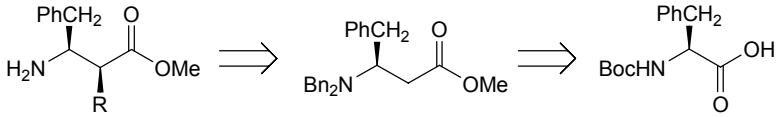
The presence of one substituent at C-2 position of the β^3-Phe should reduce, as mentioned before,[24] the flexibility of the β-amino acid residue, and should not compromise the formation of L-shape, requested by bioactive conformation.

Moreover, the presence of a polar substituent, like in **4** and **5**, could improve water solubility of the molecules, whereas the non-polar residues, like in **6** and **7**, could influence the interactions of the compounds with the hydrophobic γ site within taste-receptors.

Preparation of 2,3-Disubstituted β-Amino Acids

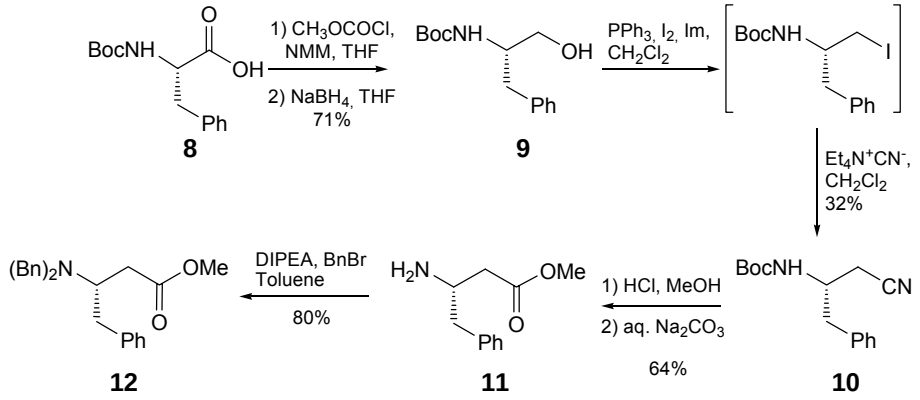
The 2,3-disubstituted β-amino acids are prepared as described in Chapters 4 and 5.

For the introduction of both polar and non-polar groups, we devised a stereoselective coupling reaction between the enolate of *N*,*N*-diprotected β^3-phenylalanine methyl ester and appropriate electrophiles. The starting β-amino acid is prepared, as described in Chapter 3, for direct homologation of α-phenylalanine, using the procedure reported in 1995 by Caputo[9] and co-workers (Scheme 7.1).



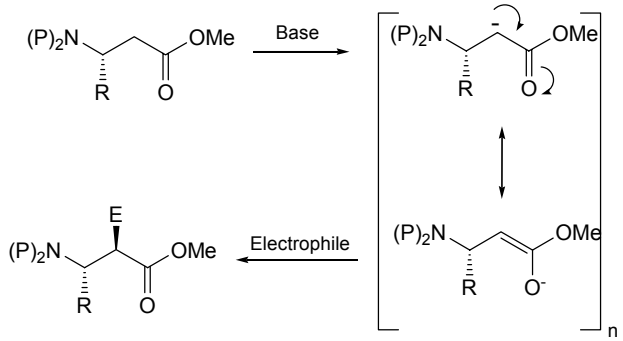
Scheme 7.1 Retrosynthetic scheme for the preparation of 2,3-disubstituted β-amino acids.

Thus, *N*(Boc)-phenylalanine **8** is converted (Scheme 7.2), into a mixed anhydride by methylchloroformate and *N*-methylmorpholine. Reduction by $NaBH_4$ of the mixed anhydride then gave phenylalaninol used to prepare the corresponding α-amino iodide by reaction with triphenylphosphine/iodine complex in the presence of imidazole. The iodide obtained is then treated with tetraethylammonium cyanide to lead to β-amino cyanide **10**. The methanolysis of the cyano group with HCl (12 N) gives, after alkaline work-up, the β-amino methyl ester in 35% overall yield. Finally, the amino function of β-phenylalanine methyl ester is protected as *N*,*N*-dibenzyl amine with benzyl bromide and diisopropylethylamine.



Scheme 7.2 Synthesis of *N*,*N*-dibenzyl β^3-phenylalanine by homologation of *N*(Boc)-α-phenylalanine.

Exploiting the acidity of the two protons at C-2 of β-amino methyl ester, it is possible to introduce onto this position miscellaneus substituents, entrapping the enolate with appropriate electrophiles (Scheme 7.3).



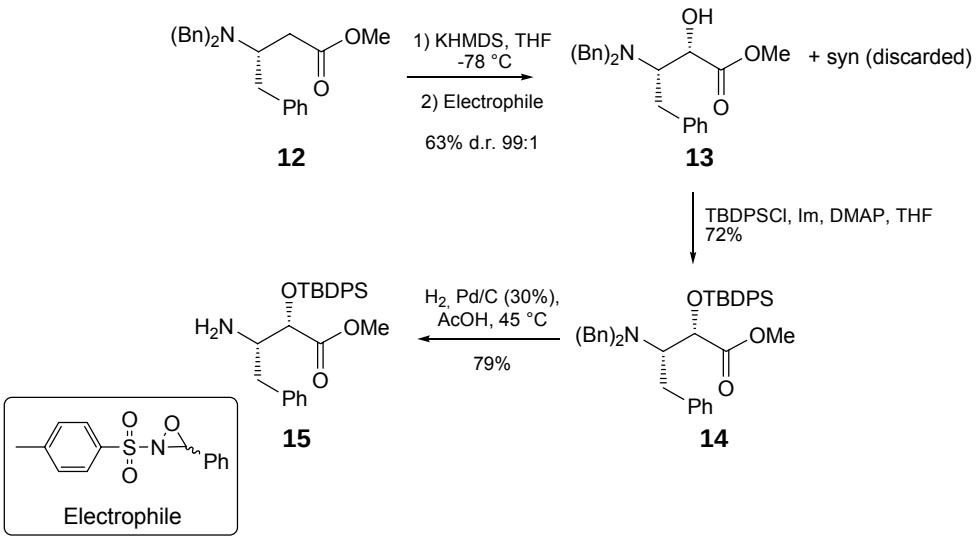
E = OH, NH_2, CH_3, CH_2Ph

Scheme 7.3 Functionalization of β-amino methyl esters.

Synthesis of *anti*-2-Hydroxy-β^3-Phenylalanine Methyl Ester

The introduction[10] of the hydroxyl group is carried out by treating *N*,*N*-dibenzyl β^3-phenylalanine methyl ester **12** with potassium bis(trimethylsilyl)-amide (KHMDS), and allowing the resulting enolates to react with racemic 2-[(4-methylphenyl)sulfonyl]-3-phenyloxaziridine to afford the α-hydroxy derivatives **13** (Scheme 7.4). α-Hydroxylation proceeded smoothly at −78 °C with high yields and good diastereomeric ratios.

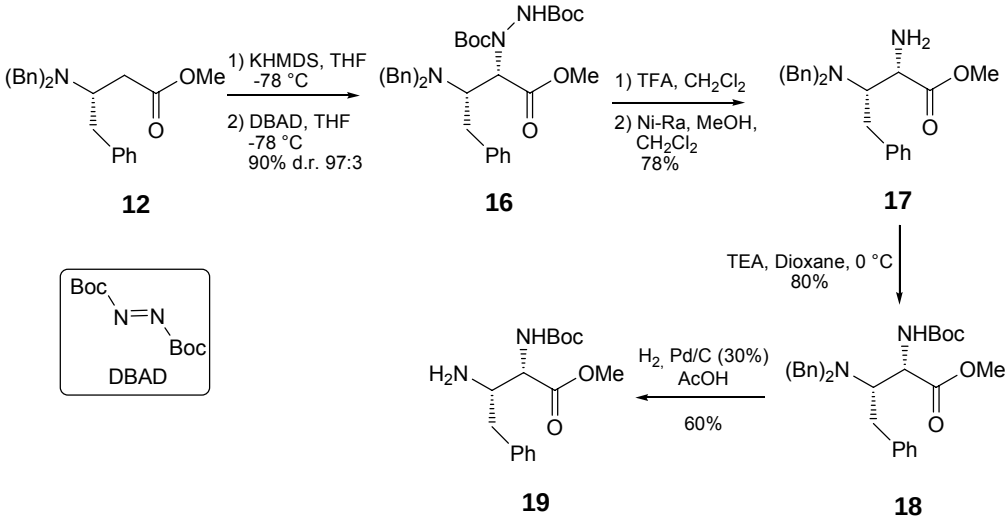
The compound **13** is then protected at the hydroxyl group with tert-butyldiphenylsilyl chloride (TBDPSCl), giving **14**, that in turn is deprotected with H_2 and Pd/C to give **15**, then used for coupling with aspartic acid.



Scheme 7.4 Asymmetric hydroxylation of homo-β-amino methyl esters.

Synthesis of *anti*-2-Amino-β^3-Phenylalanine methyl ester

As reported in Chapter 4, under the conditions we have devised, the enolate of *N*,*N*-diprotected β^3-phenylalanine is treated with KHMDS in anhydrous THF and at -78 °C; under nitrogen flow, solid DBAD is then added to obtain after 1h the compound **17** in good yield and high diastereoisomeric ratio (Scheme 7.5).

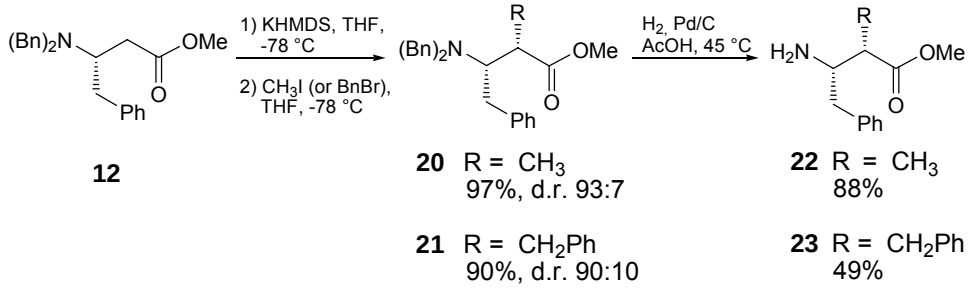


Scheme 7.5 Asymmetric amination of β^3-phenylalanine methyl ester.

With the aim to use 2,3-diamino methyl esters in place of the Phe-residue in the aspartame dipeptide, the compound **17** is first protected at the free amino group as tert-butylcarboxylate, and then deprotected at β-amino group, with H_2 and Pd/C, to give compound **19** ready for coupling with aspartic acid.

Synthesis of *anti*-2-Substituted-β^3-Phenylalanine Methyl Ester

Following the procedure already described for the preparation of both 2-hydroxy and 2-amino β^3-phenylalanine methyl esters, the enolate of **12** is treated with CH_3I or BnBr, as shown in Scheme 7.6.



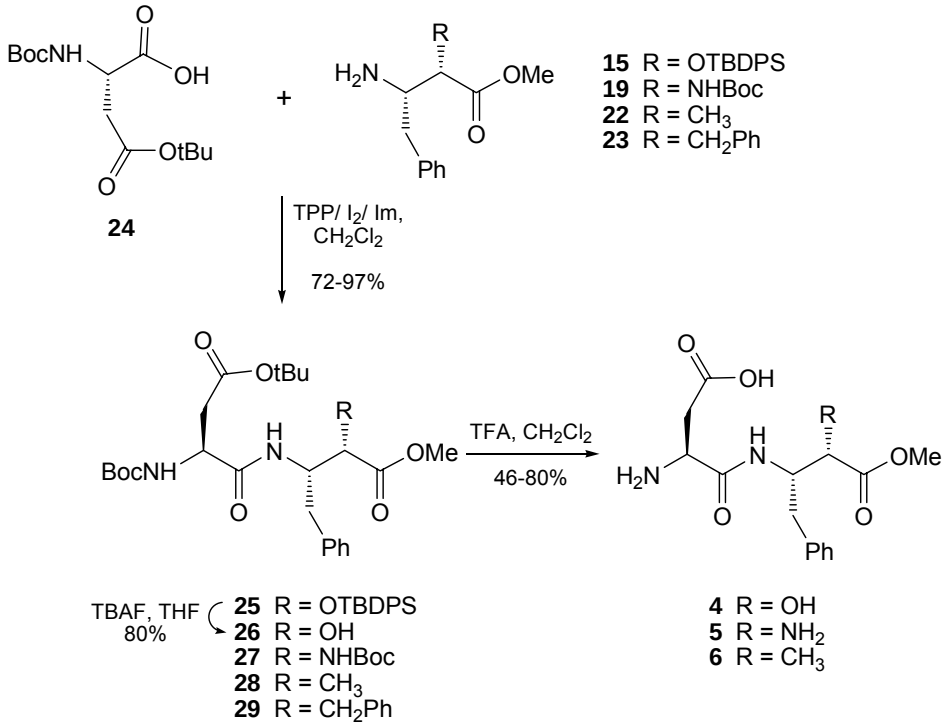
Scheme 7.6 Asymmetric alkylation of β^3-phenylalanine methyl ester.

The products **20** and **21** are obtained in good yields and high diastereoisomeric ratio, as already shown for the other functionalizations as well.

For the use in coupling reactions with aspartic acid, both products are deprotected with H_2 and Pd/C to lead to **22** and **23**, ready for the coupling step.

Peptide Synthesis

2-Substituted β^3-amino acids **15**, **19**, **22**, and **23**, prepared as described above, were then used in coupling reactions with the aspartic acid derivative **24** (Scheme 7.7), in which both β-carboxyl and amino functions are protected as Boc and tert-butyl ester. The α-carboxyl function is activated as acyl iodide, using triphenylphosphine/iodine complex in the presence of imidazole to accomplish the conversion of the acid into its acyl derivative, that is not isolated, due to its readily hydrolysable nature. Its formation is monitored by TLC and the reaction works smoothly in 4 hours; then compounds **15**, **19**, **22**, or **23** are added to the reaction mixture to give the peptides **25**, **27**-**29**, respectively, in good to excellent yields (Scheme 7.7).



Scheme 7.7 Coupling reaction between the aspartic acid derivative **24** and α-substituted β^3-phenylalanine.

Peptides **4**-**6** are all white crystalline solids with good water solubility; all of them will be tested in toxicological assays and in taste assessment using a volunteer taste panel.

Circular Dicroism Analyses

In order to investigate the structure–activity relationships (SAR) of the dipeptide taste ligands with respect to the original dipeptide aspartame, conformational analyses in solution were carried out by CD.

For CD experiments all peptides were dissolved in a 1:1 (v/v) TFE–H_2O mixture to improve the solubility of the dipeptides and to have results comparable with that reported in literature.[7]

In figure 7.2 are reported the spectra for peptides **4**-**6**, and for aspartame itself.

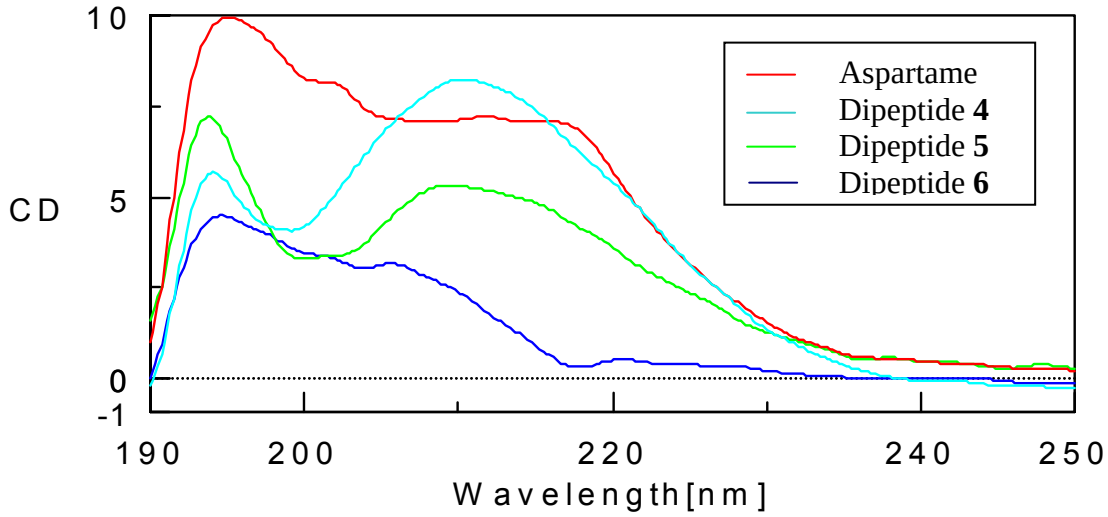


Figure 7.2 CD spectra for aspartame and its analogues.

All the spectra may be considered as linear combinations of a medium-intensity CD band with a maximum at about 195 nm and a second positive band with a maximum between 210 and 220 nm. As reported in literature,[7] the CD band between 210 and 230 nm could be interpreted as the result of superposition of two different contributions: a band distinctive of the spectra of β^3-amino acid containing peptides and a positive contribution of the aromatic phenyl side-chain.[11] The band near 195 nm is a characteristic feature of the spectra of peptides containing a phenyl substituent.[11] Since the chromophores are more or less the same, the differences in intensity among CD spectra should arise from a difference in the number of conformers which contribute to the CD. A less intense signal corresponds to a wider set of conformers, whose CD contributions tend to cancel with each other. Thus, it is possible to conclude that the new peptides are slightly more flexible than aspartame.

As reported in the literature,[7] it is not always possible, anyway, to link a CD result with sweetness: for example, the dipeptides containing β^3-aspartic acid replacing the Asp-residue in aspartame show a CD spectrum that closely resembles that of aspartame; however, this replacement causes a loss of taste.

So, our analysis can only give information related to the conformational flexibility of the different peptides as compared to aspartame.

Molecular Modelling Study

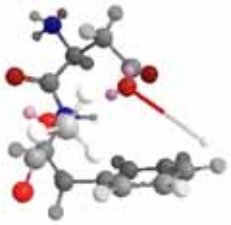
According to the model proposed by Goodman[3] *et al.*, the sweet taste is associated with an ‘L-shaped conformation’, in which the hydrophobic group projects along the +X axis (Fig. 2). A reverse ‘L-shaped’ structure, in which the hydrophobic group

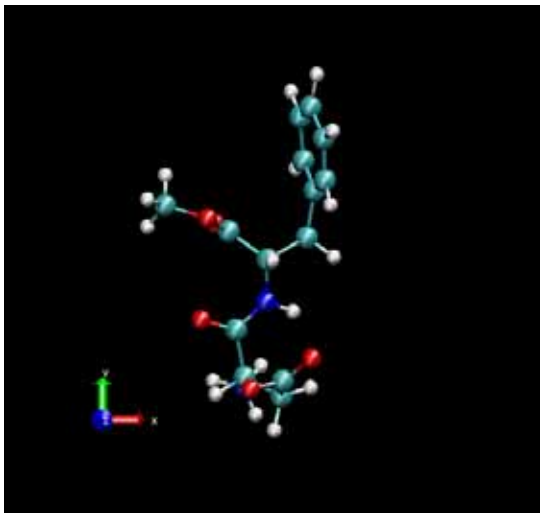
points to the - Z axis, is associated with a bitter taste. Other possible topochemical arrays would lead to tasteless compounds.

On the other hand, the model of Temussi[2] *et al.* proposes an extended conformation as the active conformation.

For aspartame as such, it is not possible to discriminate which of the two models is valid for the sweet taste responce, because aspartame presents both conformations as low energie-conformations, as shown by Molecular Dinamics.

L-Shape for Aspartame





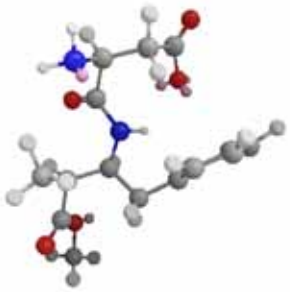
Extended structure for aspartame

From here, the interest of synthetic chemists in synthesizing restricted analogues.

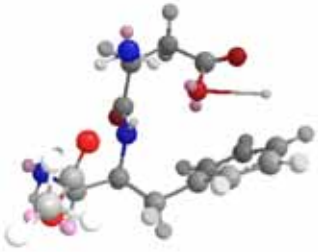
Unfortunately, our dipeptides are not more rigid then aspartame: indeed, for all our dipeptides are possible both extended and 'L-shape' low energy type conformations. Here the L-Shape conformations of all the dipeptides are reported, and those extended only for the dipeptide **6**.

All computer simulations were carried out using the Chem3D program and software Amber, using Parm99 as force field.

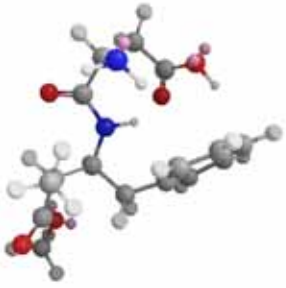
L-Shape Dipeptide 4

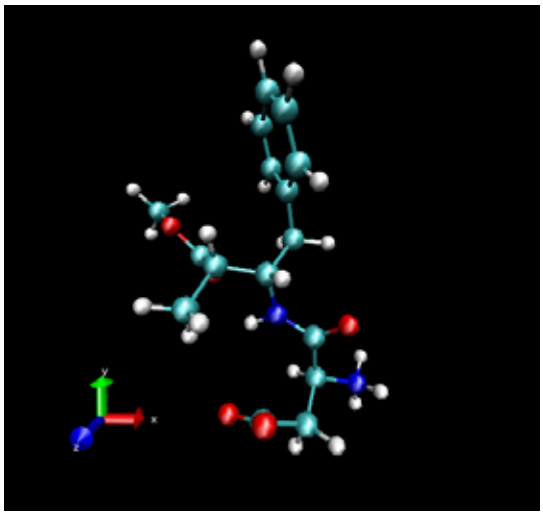


L-Shape Dipeptide 5



L-Shape Dipeptide 6





Extended Conformation for Dipeptide 6

Conclusions

Herein we have reported the synthesis of new aspartame analogues in which the Phe-residue is replaced by a $\beta^{2,3}$-disubstituted amino acid with the aim to decrease conformation flexibility and, as a consequence, to improve sweetness potency.

Moreover the introduction of polar substituents on the β-amino acid residue afforded encreased water solubility. However, analogues bearing non-polar substituents could better fit the γ-site of the taste receptor, improving the sweet-taste response.

Furthermore, the replacement of the α-Phe residue with the β-homologue allows the use of these potential sweeteners from phenylketonurics.

Finally, the more stable α-β peptide bond to typical hydrolysis conditions, could lead to the use of these compounds in a wide range of foods and beverages.

However, from the molecular modelling data, it is not possible to derive which is, between the extended and the L-Shape conformations, the active structure for a dipeptide taste-ligand.

Toxicological and sweet taste potency tests are in progress to establish if our dipeptides are sweet and can be used in food technology.

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H. J.; Kamphuis, J.; Quaedflieg, P. J. L. M.; Temussi, P. *Tetrahedron: Asymmetry* **1997**, *8*, 1305. d) Mapelli, C; Newton, M. G.; Ringold, C. E.; Stammer, C. H.; *Int. J. Pept. Protein Res.* **1987**, *30*, 498. e) Culberson, J. C.; Walters, D. E. In *Sweeteners: Discovery, Molecular Design and Chemoreception* (Eds Walters, D. E.; Orthoefer, F. T.), Washington DC, **1991**; pp 162. f) Mapelli, C.; Stammer, C. H.; Lok, S.; Mierke, D. F.; Goodman, M. *Int. J. Pept. Protein Res.* **1988**, *32*, 484.

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Chapter 8

Cell Penetrating Peptides

Introduction

The cell membrane is a component of every biological cell; it is a thin and structured bilayer of phospholipid and protein molecules that envelopes the cell. The several hundred lipid species[1] form bilayers of a few nanometer thickness with the lipidic alkyl chains pointing towards the center plane and the polar headgroups facing the aqueous phases on either side of the membrane. It separates a cell interior from its surroundings and controls what moves in and out.

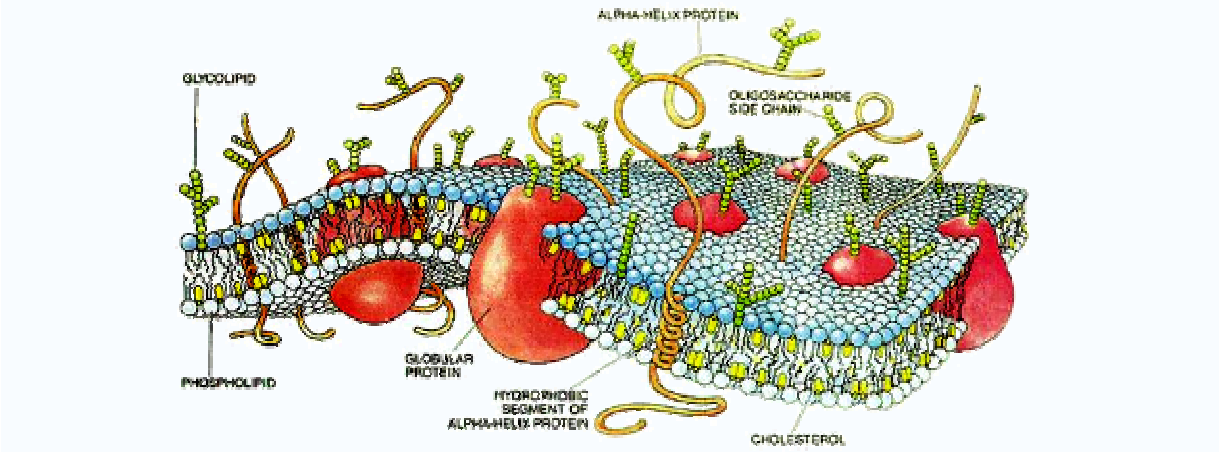


Figure 8.1 Cartoon.type drawing of a biological membrane.

As a lipid bilayer, the cell membrane is selectively permeable. This means that only some molecules can pass unhindered in or out of the cell. These molecules are either small or lipophilic. Other molecules can pass in or out of the cell, if there are specific transport molecules. Depending on the molecule, transport occurs by different mechanisms, which can be separated into those that do not consume energy in the form of ATP (passive transport) and those that do (active transport).

Membranes of eukaryotic cells and organelles, as well as the cell wall and membrane of pathogenic microorganisms, thus, constitute a serious barrier for the access of hydrophilic drugs to their target molecules inside the cells. To overcome problems of conventional and gene drug delivery, various techniques have been developed. A conventional procedure for delivering genetic material is to use viral vectors, but treating genetic disorders with this method has met with only limited success.[2] Alternative non-viral methods, such as electroporation, microinjection, and the use of liposomes, have been developed for conventional and gene drug delivery. These methods have been proved to be effective in vitro and for research purposes, but show limited potential for delivery in vivo due to toxicity, cell damage, and immunogenicity. They are also technically demanding in their application, lack of tissue and cell specificity, and can deliver material to only a limited number of cells.

In view of these considerations, transduction peptides and proteins offer a promising new tool for non-invasive delivery of hydrophilic drugs and genetic material. Over the last 10 years, it has been found that certain peptides and proteins can penetrate the cell membrane and enter the cell. A variety of cargo molecules can be attached to these peptides and proteins and translocated into the cell. Carrier peptides and proteins thus constitute a new class of potential drug delivery vectors.

Cell-Penetrating Peptides: an Overview

The commonly used terminology of the carrier peptides is “cell-penetrating peptide” (CPP).[3] Very generally, CPPs are peptides, which can be internalized by cells by mechanisms that require no energy and are receptor mediated or not. The common features of all CPPs appear to be a positive charge and amphipathicity. All known CPPs are net positively charged at physiological pH. Most CPPs have only one negative charge (at the C-terminus) or even none, when the C-terminus is amidated. The negative charge does not prevent internalization of CPP into the cells; on the contrary, a peptide is known that carries four negative charges in addition to five positive ones, and that is effectively internalized.[4] With the exception of polycationic homopolymers (polyarginines, polylysines and polyornithines),[5] all other CPPs are amphipathic. Some of them adopt amphipathic character when in an α-helical structure, while others have distinct hydrophobic and hydrophilic parts.

Mechanism of Penetration

The transport of CPPs across membranes has been studied using cultured cells, artificial lipid vesicles, tissues and in vivo. CPPs were successfully internalized in a number of different mammalian cells.

The mechanism of internalization of CPPs has not been resolved yet. In spite of some common features of these peptides, particularly their highly cationic nature, their structural diversity has fuelled the idea that the transduction mechanism is not the same for CPPs of different types. A number of investigations of CPP internalization in cell lines have been carried out under conditions that should prevent active transport of CPPs and their translocation by endocytotic pathways. Efficient translocation was observed at low temperatures (0 to +4 °C) and in the presence of many different inhibitors of endocytosis.[3a] Recent results showed, however, that the role of endocytosis in internalization of CPP is not negligible.[6] At least for some CPPs endocytosis could be an exclusive or alternative mechanism of internalization.

In some cases, an inverted micelle mechanism was suggested, in which positively charged peptides interact with negatively charged phospholipids to convert part of the membrane into an inverted micelle structure that can open on either the intracellular or the extracellular side of the membrane. [6a,7]

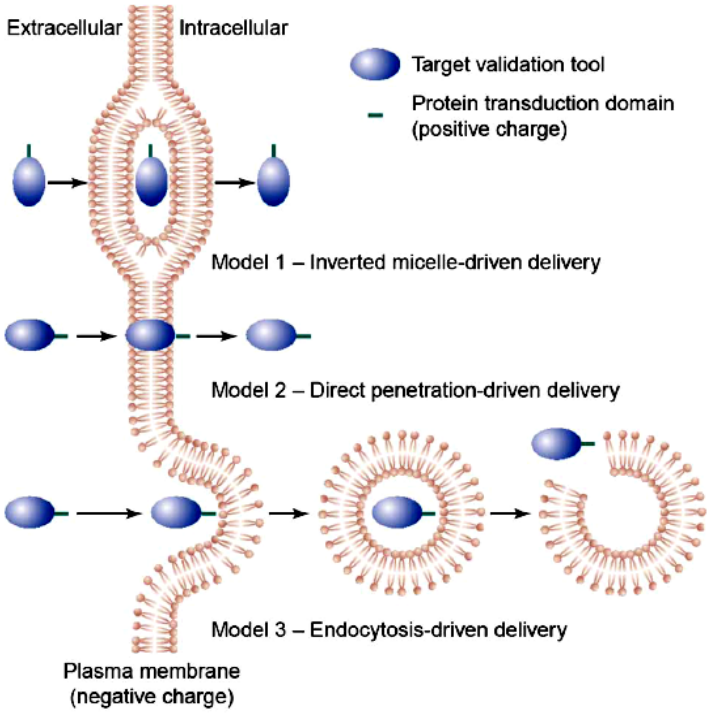


Figure 8.2 Proposed mechanisms of cellular delivery of cargos mediated by cell penetrating peptides.[8]

Cationic CPPs conjugated to macromolecules such as peptides, proteins or oligonucleotides seem to firstly bind to the plasma membrane via an initial rapid electrostatic interaction. Subsequently the complexes are internalized by cells through various hypothetical mechanisms.

On the other hand, internalization of some CPPs appears to be very much affected by the membrane composition. The internalization of CPP is a multistep process, a sequence of equilibrium states with a non-equilibrium step, namely the degradation of CPP in the cell. The first step of internalization is the interaction of CPP with the cell surface. After the internalization, there is a fraction of CPP that interacts with inner cell structures (intracellular membranes, proteins, etc), and a portion that is internalized but not bound (for instance in cytosol). Finally, the proteolytic cleavage of the CPP in the cell produces degradation products that afterwards are expelled out of the cell (the non-equilibrium step).

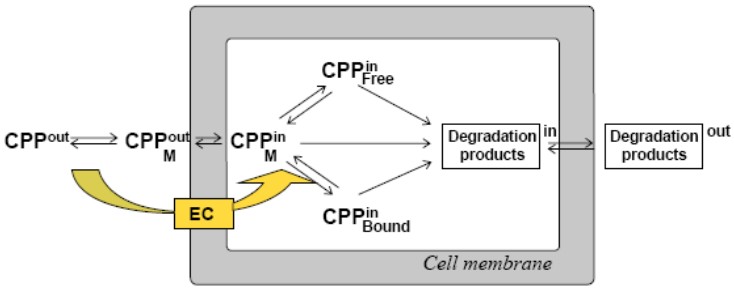


Figure 8.3. Simplified kinetic scheme for cell-penetrating peptide internalization [from Elsevier-Advanced Drug Delivery Reviews (ref. 3a)].

Cargo Delivery Using Cell Penetrating Peptides

The most interesting application of CPPs is the possibility of attaching on them biologically active cargos that have to be translocated into cells.

Indeed, since different CPPs have been demonstrated to enter different cells, tissues and organs, their use to promote translocation of various types of useful cargoes is increased.

Different types of cargoes can be attach to CPPs, ranging from small molecules to proteins and large supramolecular particles, and the translocation works with great efficiency and reasonable velocity.

Cargos can be attached in different ways.

The link between CPP and cargo is usually a covalent bond. When the cargo is a peptide or a protein, CPP and cargo are most often synthesized or expressed in tandem as fusion protein.[9] Alternatively, a suitable amino acid side-chain or bifunctional spacer molecule can be used. For example, the thiol group of cysteines is often used to attach cargos: after translocation, the reductive environment in the cell readily cleaves to the disulphide bridge between CPP and cargo, causing release of the cargo.

Attachment of cargo to CPP can be achieved also by non-covalent bonds, employing for instance the interaction of avidin to a biotin-CPP construct;[10] this method has been used very seldom.

Physical interaction between CPP and cargo is even possible. This third category has already been used for the cellular delivery of nucleotides.[11] To produce the CPP–cargo construct, the CPP can simply be mixed with the oligonucleotide cargo to form a complex through electrostatic interactions between the positively charged domain of the peptide and the negatively charged regions of the nucleotide phosphate backbone.

To use CPPs as drug delivery vehicles, CPPs should be:

– small and simple to synthesize
– able to be coupled to different cargoes without losing their translocation properties
– tissue, cell and cell-compartment selective
– stable cargo carriers but without side effects

Some of these properties have already been successfully realized. Most CPPs are relatively small and can be synthesized using traditional methods that can be automated.

The coupling of cargo is not a problem in most cases but the decrease of rate and efficiency of translocation in the case of large cargoes has not been overcome yet.

The same is true with the selectivity of CPPs. Incorporation of signalling peptide sequences into CPPs was suggested and undertaken in order to provide selective targeting of the cell nucleus in the case of gene delivery.[12] Selectivity toward cancer cells would be of great interest. For this purpose, the proteins specifically overexpressed in these cells could be used.[13] A similar approach could be exploited for antiviral drugs carried by CPPs designed to target specific surface proteins of virus infected cells.[14]

The metabolic stability of CPP is an important biopharmaceutical factor since the peptides should carry their cargoes to the targets before they are metabolically cleaved.

If on one hand, good extracellular stability would be profitable for efficient cargo delivery, on the other hand, high intracellular stability could result in accumulation of CPP inside the cells with all the potential, undesirable side effects. In general, the stability of peptides, both extracellularly and intracellularly, is poor due to the presence of peptidases and proteinases, whose activity leads to rapid hydrolysis of most peptides. Stability could be enhanced by protecting the C-terminus by amidation, by replacement of the naturally occurring L-amino-acids of the sequence

by their non-physiological D-counterparts,[15] or by using non-natural amino acids, such as β-amino acids.[16]

Nevertheless, in addition to a negative connotation, metabolization of CPP is also a prerequisite for the release of chemically ligated cargoes after internalization, and has important consequences for the physiological clearance of CPP and their acute and chronic toxicity when used for therapeutic purposes.

The development of carefully engineered CPP has to require a balance between both aspects, in order to avoid premature cleavage of the cargo and to free the cargo once internalized.

Biological Effects: Toxicity and Immunogenicity

The use of CPPs as drug delivery vehicles needs to keep to a minimum the toxic effects of CPPs. Side effects of CPPs have not been explored in detail yet; in fact if the *in vitro* toxicity of CPPs has been frequently characterized, the *in vivo* studies are limited. In general, two types of toxic effect have been observed: toxic effects on membranes of cells and organelles, and toxic effects resulting from the specific interaction of CPPs with cell components.[3a]

However, most CPPs show only moderate or very limited side effects when applied *in vivo* and *in vitro*.

Polycationic Peptides: Oligoarginine

A promising approach directed at improving or enabling the cellular uptake of drugs or drug candidates involves the use of peptide-based molecular transporters to carry these agents actively into cells. Representative of this approach is the use of short oligomers of L-arginine (**1**), that are remarkably efficient molecular transporters of drug and probe molecules into cells and tissue. They provide highly water-soluble conjugates that rapidly enter cells. In particular, drug conjugates of these arginine transporters have been shown to exhibit novel and significant penetration into human skin and to release their drug cargoes in targeted T cells.[17]

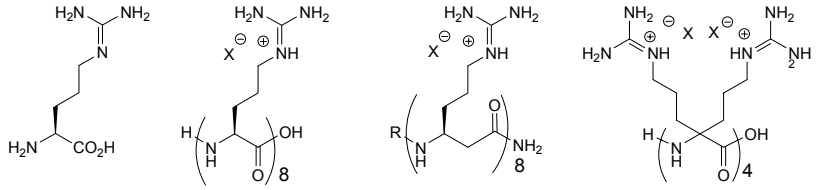
The guanidinium headgroups are principally responsible for the uptake of these oligomers into cells according to the model that provides initial cell-surface binding of the peptide carrying positively charged side chains followed by endocytosis or direct penetration-driven delivery and cytoplasmic trafficking. Indeed, the polarity of the guanidinium groups could be attenuated through association with cell surface groups bearing a complementary charge (phospholipids, fatty acid salts, and sulfates), thereby producing a less polar ion pair complex capable of diffusing into the membrane.

Several modifications can be made to the peptide structure, such as the backbone chirality, the position of attachment and the length of side chains, the backbone composition (for example, branched guanidinium reach oligosaccarides and dendrimers), without altering the uptake properties of these molecules.[18]

Several mechanisms could accommodate the structure-function relationships of such a huge range of different compounds, and some could operate concurrently. A receptor-mediated process is inconsistent with the broad range of structural modifications that promote uptake. Conventional passive diffusion across the non-polar interior of the plasma membrane is difficult to reconcile with the polarity of the arginine oligomers and the dependency of uptake on the number of charges.

Wender *et al.*[19] provided a mechanistic hypothesis for how short oligomers of arginine (such as **1**) can migrate across the plasma membrane of a cell. The water-soluble, positively charged guanidinium headgroups of the transporter form bidentate

hydrogen bonds with H-bond acceptor functionality on the cell surface. The resultant ion pair complexes partition into the lipid bilayer and migrate across at a rate proportional to the membrane potential. The complex dissociates on the inner leaf of the membrane and the transporter enters the cytosol. This mechanism is consistent with the highly permissive structure-function relationships, the apparent lack of cell-type specificity and uptake being slowed but not inhibited at 4°C. This hypothesis does not preclude competing uptake by other mechanisms including endocytosis, which is likely to dominate with large cargoes.[20]



Finally, due to the unusual properties of β-peptides built of β^2- or β^3-homologues of proteinogenic α-amino acids (for reviews, see ref 21), β-oligohomoarginine were considered suitable candidates as CPPs. Seebach[16] *et al.* have already reported the synthesis of short oligomers of β^3-arginine, showing that short fluorescently labelled oligomers of β^3-homoarginine of type **2** are also able to enter living cells efficiently, presumably by a non-endocytotic uptake mechanism.[16]

As part of a project focused on the understanding of uptake pathways of cell penetrating peptides (CPPs), we have prepared, in collaboration with Prof. Seebach, at Swiss Federal Institute of Technology in Zurich, α,α-dialkylated glycine carrying special chains, precursors of arginine side chains. The goal of this study is the preparation of α,α-disubstituted α-peptides of type **3**, that could be efficient molecular transporters of drugs and probe molecules into cells and tissues.

The synthesis of compounds such as **3** and subsequently the study of their binding and cell permeability properties could shed new light on alternative uptake pathways and on the understanding of the role of overall charge, charge distribution and chain length in the cell-penetration ability of this class of CPPs.

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Chapter 9

Attempts at Synthesizing α-Substituted Arginine Derivatives

Introduction

The increasing number of papers, dealing either with applications of C-α-disubstituted α-amino acids (α,α-disubstituted glycines) or with new synthetic methodologies for their incorporation into peptide sequences, is due to several factors. In particular, these non-natural amino acids occur in an important family of membrane-active natural antibiotics,[1] such as peptaiboils;[2] moreover, they are used for the construction of conformationally constrained, enzyme-resistant agonists and antagonists of bioactive peptides.[3] Their severely restricted conformational freedom allows the exploitation of such compounds as precise molecular rulers,[4] scaffolding blocks in the *de novo* design of protein and enzyme mimetics,[5] or suitable models for spectroscopic studies.[6]

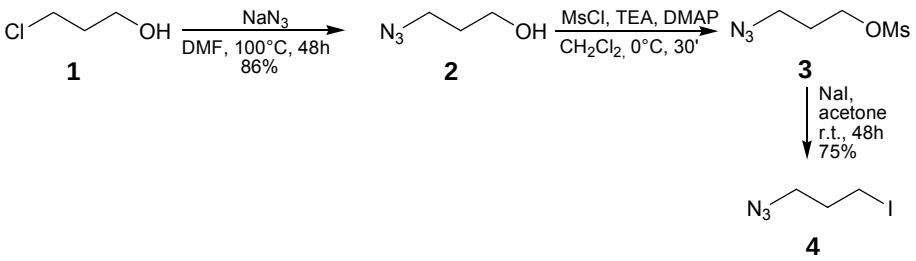
As only a few α,α-disubstituted α-amino acids are commercially available, many synthetic methods have been devised for their preparation.[7]

The main problem a peptide chemist has to tackle with α,α-disubstituted α-amino acids is their rather poor reactivity, particularly at their nucleophilic amino function, due to high steric hindrance at α-carbon of the these residues.[8] This prevents one from preparing a homo-oligomeric series in good yield and in a relatively short time with the coupling methods available to date.[4,9]

Results and Discussion

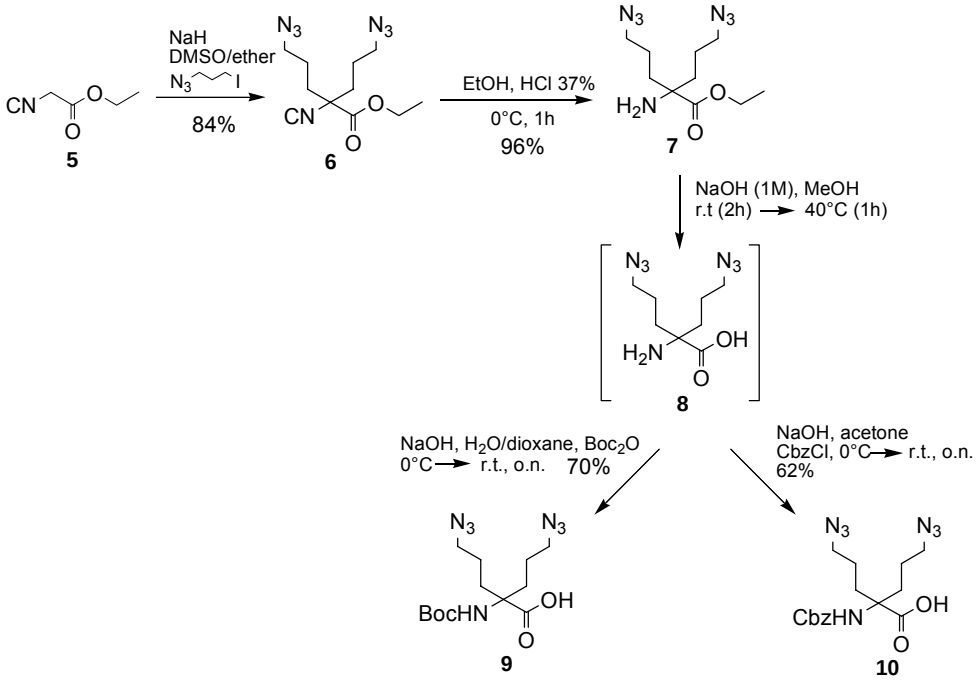
We were interested in the preparation of short oligomers of α,α-dialkylated analogues of arginine, as potential efficient cell penetrating peptides (Chapter 8).

We planned the synthesis of the dialkylated analogue of arginine adapting a procedure reported in literature.[10] In particular, we thought to introduce the guanidinium head by reaction of guanidinylating reagents with an amino group of a propyl amino group.[11] The amino group can be introduced as azido function that can be, in turn, easily reduced by hydrogenolysis or by Staundinger reaction. Thus, we prepared the azide **4** (Scheme 9.1) from 3-chloropropanol **1**, and we used it as 'protected' electrophile[12] in the dialkylation reaction of ethyl isocyanoacetate **5** (Scheme 9.2).



Scheme 9.1 Preparation of ω-iodoazide from 3-chloropropanol.

To obtain successful and reproducible results, oil-free NaH is necessary in the alkylation reaction: in this way, 2 eq. of base are enough to have the dialkylated product (**6**) in 84% yield (Scheme 9.2).



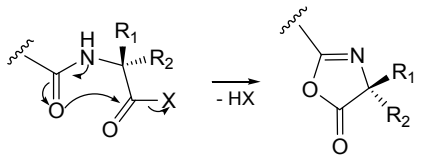
Scheme 9.2 Synthetic results.

The compound **6** was then converted into α-amino ethyl ester **7** by acid hydrolysis in 96% yield (Scheme 9.2).

Finally, the ethyl ester function of **7** was hydrolysed with aq. NaOH in MeOH and in turn the amino function protected to have the second building block for peptide coupling (**9** or **10)**.

Early attemps of coupling were carried out using Boc as *N*-protection. This urethane protection is, indeed, as easily introduced as easily removed, being especially useful in solution-phase peptide synthesis.

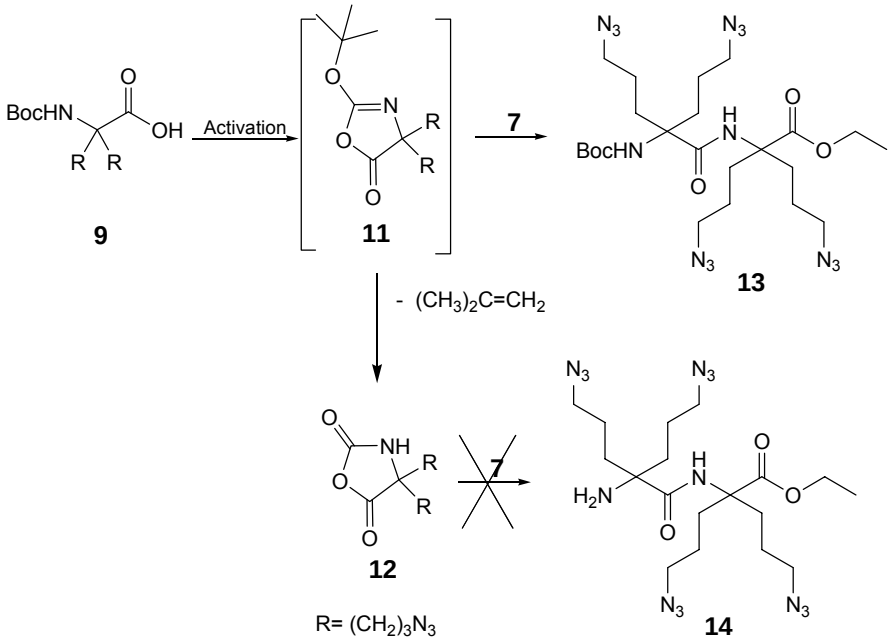
It is known that, during the peptide coupling, the activation of carboxyl function of both peptides and urethane or amide *N*-protected derivatives leads, *via* an intramolecular reaction, to the oxazol-5(4*H*)-one heterocyclic skeleton, whose the formation of which is greatly favoured by the *gem*-dialkyl effect (Scheme 9.3).[13]



Scheme 9.3 Oxazol-5(4*H*)-one formation.

Unlike oxazolones formed from α-amino acids,[14,15] those formed from α,α-disubstituted α-amino acid derivatives are not prone to racemization (or epimerization) since that would require a C-C or C-N bond cleavage. Indeed, the shelf-stable oxazolones from α,α-disubstituted α-amino acids and peptides are easily isolated and, despite their relatively poor reactivity at room temperature, can be directly exploited in peptide synthesis, since the nucleophilic attack of an amino group at the C-5 forms a peptide bond. [8]

However, when we allowed to react the carboxylic acid derivative **9** with the amine **7** under peptide coupling conditions (Scheme 9.4), the ligated product **13** was isolated in poor yield (Table 9.1). The oxazolin-2,5-dione derivative **12** (Leuch's anhydride) was observed as main product. As already reported in literature,[16] compound **11** decomposes to give **12** *via* retro-ene reaction (Scheme 9.4). Emploiment of Leuchs anhydride in peptide synthesis is also reported;[16,17] however, in our experience, compound **12** is unreactive under the coupling conditions we used, probably due to steric hindrance.



Scheme 9.4 Attempted coupling reaction with **9**.

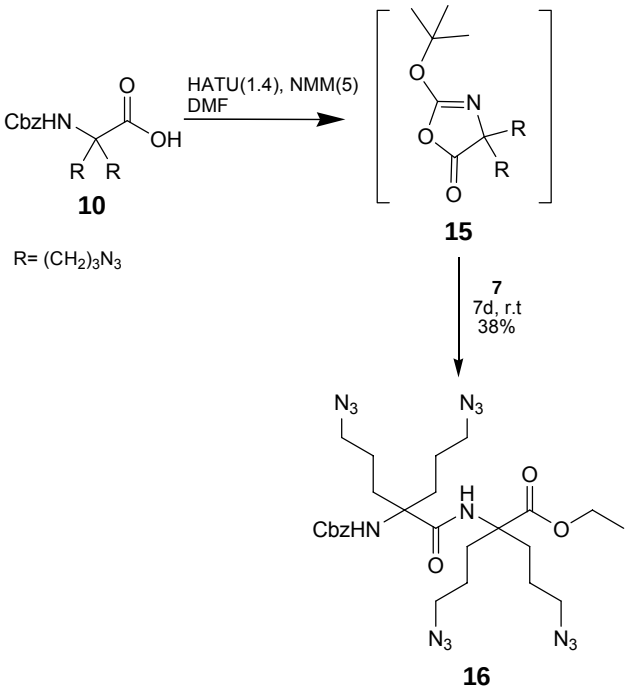
Under all the reaction conditions tested, we could not obtain the dipeptide **13** in good yield (Table 9.1). Both HATU and EDC in dichloromethane afforded mainly the anhydride by-product (entries 1-2). Moving from dichloromethane to dimethylformamide little amounts of the desired product were isolated (entries 3-4). DIPEA instead of NMM gave the worst results even after 5 days (entry 5). Taking into account that the main product isolated was the unproductive Leuch's anhydride and that many side product were observed as well, we decided to use the Z-protected amino acid **10.**

Table 9.1 Coupling reaction of **7** and **9**.

Reaction Conditions (eq)	Solvent	Yield of 12(%)	Yield of 13(%)
HATU(1.2)/NMM(3) o.n., r.t.	CH_2Cl_2	31	/
EDC(1.3)/HOAt(1)/NMM(1) 5d, .r.t.→ 5d, 55 °C→ MW	CH_2Cl_2	Quant.	/
HATU(2)/NMM(3) 4h, r.t.	DMF	70	2
HATU(1.2)/NMM(3) o.n., r.t.	DMF	24	5
HATU(2)/DIPEA(5) 5d, r.t. →2d, 50 °C→MW	DMF	8[a]	/

[a]Together with **12**, 30% of **7** was recovered, as well as other products.

Also in this case, an oxazolone intermediate **15** is expected (Scheme 9.5), but should not decompose under the coupling conditions, to the Leuch's anhydride; thus we could recover the peptide product **16** together with the starting materials (Table 9.2).



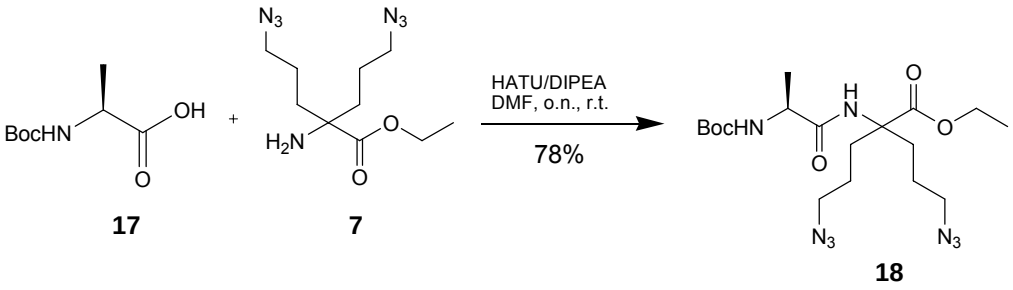
Scheme 9.5 Coupling reaction using Z-protection.

Table 9.2 Coupling reactions between **7** and **10**.

Reaction Conditions (eq)	Solvent	Yield of 16%
HATU(1.4)/NMM(5), 4d, r.t.	DMF	38
EDC(1)/HOAt(1)/NMM(1), 7d, r.t	CH_2Cl_2	15
T3P(1.4)/NMM(5), 4d, r.t.	DMF	/

This coupling reaction is also extremely slow: in a test experiment we allowed **7** to react with Boc-Ala-OH, and the desired product could be isolated in good yields (Scheme 9.6). This result rules-out the hypothesis that the poor reactivity of the used sterically hindered amino acids could be ascribed to the depleted nucleophilicity of the amino function.

Together, these results suggest that our hypothesis about the poor reactivity of oxazolone intermediates of this type could be correct, although no investigations on its behaviour with less hindered amino acids were carried out.



Scheme 9.6 Coupling of **7** with Boc-Ala-OH.

As reported by Toniolo, with the coupling methods available to date, the high steric hindrance of these residues makes it difficult to prepare homo-oligomeric compounds in good yield and in a relatively short time.[4,9]

Finally, preliminary studies of hydrolysis of the ethyl ester function in peptide **16** demostrated the necessity to replace this ester function with a more reactive ester such as *tert*-butyl or trimethylsilyl ethyl ester that can be removed under mild conditions.

Instead, the first attempts of reduction of azido group, with in situ protection of the amino function, were encouraging, but also this reaction needs some improvement.

These experiments are still in progress and will be further carried out by the author in following years.

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Experimental Part

General

NMR spectra were recorded on Varian Inova 500 MHz, Bruker DRX 400 MHz, Varian Gemini 300 MHz, Varian Gemini 200 MHz spectrometers: chemical shifts are in ppm (δ) and *J* coupling constants in Hz; , spectral splitting patterns are designated as singulet (s), doublet (d), triplet (t), quadruplet (q), multiplet or more overlapping signals (m), broad signal (b); solvent $CDCl_3$, unless otherwise specified. GC/MS analyses were performed on Hewlett–Packard 6890 GC/5973N MS. Optical rotations were determined on Jasco P-1010 polarimeter (1.0 dm cell); solvent $CHCl_3$, unless otherwise specified. Infrared spectra were recorded using JASCO FT/IR-430 Spectrometer. Ms: *IonSpec Ultima 4.7-T-FT* ion cyclotron resonance (MALDI or HR-MS in a 2,5-dihydroxybenzoic acid matrix) mass spectrometer; in m/z (% of basis peak). Mps were taken on a Gallenkamp apparatus. Elemental analyses were performed on a Perkin–Elmer Series II 2400, CHNS analyzer. TLC were carried out on silica gel Merck 60 F254 plates (0.2 mm layer) and column chromatographies on Merck Kieselgel 60 (70–230 mesh). Reactions involving air- or moisture-sensitive reagents or intermediates were performed under argon or nitrogen atmosphere in glassware which had been heat gun or flame-dried under high vacuum. Indicated reaction temperatures refer to those of the reaction bath, while room temperature (rt) is noted as 25 °C. Preparative reactions were stirred magnetically. Tetrahydrofuran (THF), diethyl ether (Et_2O), methylene chloride (CH_2Cl_2), and toluene were obtained from a anhydrous solvent system (activated alumina columns, positive pressure of argon). All other solvents were used as received in Sure/Seal bottles (Aldrich). Triethylamine (Et_3N), diisopropylethylamine (i-Pr_2NEt), pyridine and chlorotrimethylsilane (TMSCl) were distilled from CaH_2 immediately prior to use. All other reagents were purchased from Aldrich at the highest commercial quality and used without further purification.

Preparation of Dideuterated β-Amino Acids

Reduction of Carboxyl Function

1,1-^{2}H (*S*)-*tert*-butyl 1-hydroxypropan-2-ylcarbamate (31a). General procedure

To a solution of *N*-methyl-morpholine (NMM) (2.8 mL, 25.4 mmol) and **30a** (4.0 g, 21.2 mmol) in anhydrous THF (92 mL), under magnetic stirring and at 0 °C, methylchloroformiate was added (MeOCOCl) (2.0 mL, 25.4 mmol) dropwise. After 40 minutes, the solution was filtered on Celite® and the salts of *N*-methyl morpholinium were washed with anhydrous THF (3 x 70 mL). To the flow through, under magnetic stirring and at 0 °C, a soluction of $NaBD_4$ (0.9 g, 21.2 mmol) in D_2O (7.7 mL) was added dropwise. The mixture was kept for 10 minutes at room temperature. The solvent was then evaporated under reduced pressure, the residue was diluted with EtOAc (2x100 mL) and washed with 10% aq NH_4Cl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated in vacuo to afford the crude reaction product whose chromatography on silica gel ($CHCl_3$:CH_3OH-95:5) gave the compound **31a** (3.47 g, yield 92%). Mp 58.2-59.1 °C (from EtOAc/Hexane); $[\alpha]_D^{25}$-9.8 (*c* 1.32). ^{1}H NMR (300 MHz) : δ 1.15 (d, $J_{CH_3,CH}$ = 6.8, 3H, CH_3), 1.43 (s, 9H, CH_3Boc), 2.95 (bs, 1H, OH), 3.71 (dd, *J*=6.6, *J*=6.8, 1H, CH), 4.75 (bd, *J*=6.6, 1H, NH). ^{13}C NMR (75 MHz): δ 17.1, 28.2, 48.3, 66.5, 79.5, 156.2.

Under the same conditions, the other dideuterated *N*-protected β-amino alcohols were also obtained.

1,1-^{2}H (*S*)-*tert*-butyl 1-hydroxy-3-phenylpropan-2-ylcarbamate (31b)

Prepared starting from **30b** (yield 90%); mp 97.3–98.9 °C (from EtOAc/Hexane); $[\alpha]_D^{25}$-23.1 (*c* 1.15). ^{1}H NMR (300 MHz) : δ 1.43 (s, 9H, CH_3Boc), 1.92 (bs, 1H, OH), 2.82 (d, *J*=7.1, 2H, CH_2Ph), 3.78-3.92 (m, 1H, CH), 4.72 (bs, 1H, NH), 7.18-7.28 (m, 5H, H-Ar). ^{13}C NMR (75 MHz) : δ 28.2, 37.3, 49.1, 53.4, 79.6, 126.4, 128.4, 129.1, 137.6, 157.7.

6,6-^{2}H (*S*)-*tert*-butyl 6-hydroxyhexane-1,5-diyldicarbamate (31c)

Prepared starting from **30c** (yield 85%); oil; $[\alpha]_D^{25}$-10.1 (*c* 1.92, CH_3OH). ^{1}H NMR (200 MHz; C_6D_6, 75 °C) : δ 0.93-1.32 (m, 6H, 3xCH_2), 1.43 (s, 18H, 2xCH_3Boc), 2.14 (bs, 1H, OH), 2.78-2.98 (m, 2H,

C*H*$_2$NH), 3.43-3.58 (m, 1H, CH), 4.13 (bs, 1H, N*H*CH$_2$), 4.51 (bs, N*H*CH). ^{13}C NMR (125 MHz) : δ 23.0, 28.7, 30.2, 30.9, 40.0, 52.6, 65.8, 79.4, 79.7, 156.6, 156.7.

2,2-^{2}H *tert*-butyl 2-hydroxyethylcarbamate (31d)

Prepared starting from **30d** (yield 75%); oil. ^{1}H NMR (200 MHz): δ 1.43 (s, 9H, CH$_3$Boc), 3.22 (d, *J*=5.5, 2H, CH$_2$NH), 3.86 (bs, 1H, OH), 5.10 (bt, *J*=5.5, 1H, NH). ^{13}C NMR (50 MHz): δ 28.2, 29.5, 42.8, 79.6, 156.8.

3,3-^{2}H (*R*)-*tert*-butyl 1-(benzyloxy)-3-hydroxypropan-2-ylcarbamate (31e)

Prepared starting from **30e** (yield 75%); mp 64.9–65.7 °C (from EtOAc/Hexane); $[\alpha]_D^{25}$+14.8 (*c* 1.71). ^{1}H NMR (300 MHz) : δ 1.45 (s, 9H, CH$_3$Boc), 2.60 (bs, 1H, OH), 3.67 (dd, *J*=4.3, *J*=9.3, 1H, CH$_a$O), 3.71 (dd, *J*=3.8, *J*=9.3, 1H, CH$_b$O), 3.80-3.90 (m, 1H, CH), 4.38 (d, *J*=12.0, 1H, CH$_2$Ph), 4.62 (d, *J*=12.0, 1H, CH$_2$Ph), 5.22 (bs, 1H, NH), 7.27-7.42 (m, 5H, H-Ar). ^{13}C NMR (75 MHz) : δ 28.8, 51.7, 63.1, 70.6, 73.4, 79.5, 127.0, 127.2, 127.8, 136.9, 155.1.

Conversion of *N*-Protected β-Amino Alcohols into *N*-Protected β-Amino Nitriles

1,1-^{2}H (*S*)-*tert*-butyl 1-cyanopropan-2-ylcarbamate (33a)

N-protected β-amino iodide. General procedure

To a solution of iodine (2.7 g, 10.5 mmol) in anhydrous CH_2Cl_2 (72 mL) was added dropwise a solution of triphenylphosphine (2.8 g, 10.5 mmol) in CH_2Cl_2 at room temperature, in inert atmosphere and under megnetic stirring; after 15 minutes solid imidazole (1.4 g, 21.1 mmol) was added. The misture was then kept for 15 minutes at room temperature, and then the compound **31a** (1.5 g, 8.4 mmol), in anhydrous CH_2Cl_2, was finally added to the suspension and the reaction was allowed to reflux until the starting material was completely consumed (~3h, TLC, $CHCl_3$/CH_3OH=8:2). The solvent was then evaporated under reduced pressure and the residue, diluted with EtOAc (50 mL) was washed with 10% aq. $Na_2S_2O_4$ (40 mL) and then brine (3 x 50 mL) until neutral. The organic layer was finally dried (Na_2SO_4) and evaporated in vacuo to give the compound **32a** in mixture with white solid Ph_3PO. The crude was used as such in the next step of cyanide-substitution.

N-protected β-amino nitriles. General procedure

$Et_4N^+CN^-$ (1.4 g, 9.1 mmol) in anhydrous CH_2Cl_2 was added in one portion to a solution of the crude **32a** (2.2 g, 7.6 mmol) in anhydrous CH_2Cl_2 (90 mL) at room temperature, under nitrogen atmosphere and magnetic stirring. The mixture was refluxed until the starting material was completely consumed (~4 h, TLC, Hexane/EtOAc 7:3). The solution was then directly transfered onto a silica gel column and eluted with $CHCl_3$, to remove the salts coming from $Et_4N^+CN^-$. The solid residue (4.4 g), was finally purified by chromatography (Hexane:AcOEt 7:3) to give **33a** as a white solid, recristallized from EtOAc/Hexane (1.1 g, yield 80%). Mp 68.2-70.0 °C. $[\alpha]_D^{25}$-120.2 (*c* 1.38). ^{1}H NMR (400 MHz) : δ 1.33 (d, 3H, *J*=6.8, CH$_3$), 1.43 (s, 9H, CH$_3$Boc), 3.95-4.15 (m, 1H, CH), 4.69 (bd, 1H, *J*=7.05, NH). ^{13}C NMR (75 MHz) : δ 19.4, 25.0, 28.3, 43.0, 80.1, 117.4, 154.8.

Under the same conditions, the other dideuterated *N*-protected β-amino alcohols were also obtained.

1,1-^{2}H (*S*)-*tert*-butyl 1-cyano-3-phenylpropan-2-ylcarbamate (33b)

Prepared starting from **31b** (yield 74%); mp 123.8–125.0°C (from EtOAc/Hexane); $[\alpha]_D^{25}$-18.2 (*c* 1.15). ^{1}H NMR (200 MHz) : δ 1.43 (s, 9H, CH$_3$), 2.86 (dd, *J*=7.8, *J*=13.6, 1H, CH$_a$Ph), 2.99 (dd, *J*=5.8, *J*=13.6, 1H, CH$_b$Ph), 4.00-4.17 (m, 1H, CH), 4.72 (bs, 1H, NH), 7.18-7.28 (m, 5H, H-Ar). ^{13}C NMR (75 MHz) : δ 28.2, 29.9, 39.3, 48.3, 79.6, 117.3, 126.4, 128.4, 129.1, 137.6, 154.8.

6,6-^{2}H (*S*)-*tert*-butyl 6-cyanohexane-1,5-diyldicarbamate (33c)

Prepared starting from **31c** (yield 74%); mp 60.7–64.0°C; $[\alpha]_D^{25}$-45.2 (*c* = 0.57). ^{1}H NMR (200 MHz; C_6D_6, 75 °C) : δ 0.72-1.18 (m, 6H, 3xCH$_2$), 1.38 (s, 9H, CH$_3$Boc), 1.44 (s, 9H, CH$_3$Boc), 2.64-2.81 (m, 2H, CH$_2$NH), 3.29-3.42 (m, 1H, CH), 3.92 (bs, 1H, CH$_2$NH), 4.21 (bs, 1H, CHNH). ^{13}C NMR (50 MHz) : δ 23.0, 28.5, 28.6, 29.9, 30.0, 33.1, 40.0, 47.3, 79.5, 80.3, 117.5, 155.4, 156.4.

2,2-^{2}H *tert*-butyl 2-cyanoethylcarbamate (33d)

Prepared starting from **31d** (yield 88%); m.p. 42.4–45.0°C (from EtOAc/Hexane). ^{1}H NMR (200 MHz): δ 1.45 (s, 9H, CH$_3$Boc), 3.38 (d, *J*=6.3, 2H, CH$_2$NH), 4.90 (bs, 1H, NH). ^{13}C NMR (50 MHz): δ 28.1, 29.5, 36.5, 80.1, 118.0, 155.0.

3,3-^{2}H (*R*)-*tert*-butyl 1-(benzyloxy)-3-cyanopropan-2-ylcarbamate (33e)

Prepared starting from **31e** (yield 65%); oil; $[\alpha]_D^{25}$-9.7 (*c* = 1.62). ^{1}H NMR (300 MHz) : δ 1.50 (s, 9H, CH_3Boc), 3.60 (dd, *J*=4.9, *J*=9.6, 1H, CH_aO), 3.70 (dd, *J*=3.8, *J*=9.6, 1H, CH_bO), 4.05-4.18 (m, 1H, CH), 4.58 (s, 2H, CH_2Ph), 5.10 (bd, *J*=6.4, 1H, NH), 7.31-7.40 (m, 5H, H-Ar). ^{13}C NMR (75 MHz) : δ 28.1, 29.6 46.8, 69.5, 73.4, 80.1, 117.1, 127.6, 127.9, 128.4, 137.1, 154.8.

Conversion of *N*-Protected β-Amino Nitriles into β-Amino Methyl Esters.

4,4-^{2}H (*S*)-4-methoxy-4-oxobutan-2-aminium chloride (34a)

General procedure

A solution of *N*-Boc-amino nitrile **33a** (0.36 g, 1.95 mmol) in anhydrous methanol (11.5 mL), at –15 °C, was saturated with anhydrous HCl (g) and kept at room temperature for 12 h. To this solution few water drops (53 mg, 2.93 mmol) were added. After 1 h, the solvent was co-evaporated with anhydrous Et_2O under reduced pressure (3 x 25 mL), to give the product **34a** as a crystalline solid (0.3 g, yield 87%).

Mp 250 °C dec. (from MeOH/$CHCl_3$); $[\alpha]_D^{25}$+0.19 (*c* 2.5, CH_3OH). ^{1}H NMR (200 MHz; CD_3OD) : δ 1.47 (d, *J*=6.8, 3H, CH_3), 3.48 (t, *J*=6.8, 1H, CH), 3.72 (s, 3H,CH_3O), 8.35 (bs, NH_3^+). ^{13}C NMR (50 MHz; CD_3OD) : δ 18.3, 38.2, 44.7, 52.2, 170.8.

Under the same conditions, the other dideuterated *N*-protected β-amino methyl esters were also obtained.

4,4-^{2}H (*S*)-4-methoxy-4-oxo-1-phenylbutan-2-aminium chloride (34b)

Prepared starting from **33b** (yield 85%); mp 255.0°C dec (from MeOH/$CHCl_3$); $[\alpha]_D^{25}$+4.28 (*c* 1.38, CH_3OH). ^{1}H NMR (500 MHz; CD_3OD): δ 2.94 (dd, 1H, *J*=8.3, *J*=14.16, CH_aPh), 3.79 (dd, 1H, *J*=6.35, *J*=14.16, CH_bPh), 3.70 (s, 1H, CH_3O), 3.82 (t, 1H, *J*=7.32, CH), 7.05-7.38 (m, 5H, H-Ar). ^{13}C NMR (75 MHz; CD_3OD): δ 39.5, 48.3, 50.3, 52.8, 128.7, 129.4, 130.1, 136.7, 206.2.

7,7-^{2}H (*S*)-7-methoxy-7-oxoheptane-1,5-diaminium chloride (34c)

Prepared starting from **33c** (yield 82%); oil; $[\alpha]_D^{25}$+8.49 (*c* 0.56, CH_3OH). ^{1}H NMR (500 MHz; CD_3OD) : δ 1.48-1.58 (m, 2H, CH_2), 1.68-1.72 (m, 2H, C*H*$_2$CH), 2.93 (t, *J*=7.8, 2H, C*H*$_2$NH), 3.57 (t, *J*=6.8, 1H, CH), 3.75 (s, 3H, OCH_3).

3,3-^{2}H 3-methoxy-3-oxopropan-1-aminium chloride (34d)

Prepared starting from **33d** (yield 90%); mp 102.4-106.0 °C (from MeOH/$CHCl_3$). ^{1}H NMR (200 MHz; CD_3OD): δ 3.18 (bs, 2H, C*H*$_2$NH), 3.73 (s, 3H, OCH_3). ^{13}C NMR (50 MHz; CD_3OD): δ 32.1, 36.4, 52.7, 172.6.

4,4-^{2}H (R)-1-(benzyloxy)-4-methoxy-4-oxobutan-2-aminium chloride (34e)

Prepared starting from **33e** (yield 80%); oil; $[\alpha]_D^{25}$+0.68 (*c* 0.74, CH_3OH). ^{1}H NMR (300 MHz; CD_3OD): δ 3.63-3.78 (m, 1H, CH_2O), 3.63 (s, 3H, CH_3O), 4.48 (d, *J*=12.0, 1H, CH_aPh), 4.54 (d, *J*=12.0, 1H, CH_bPh), 4.78 (m, 1H, CH), 7.31-7.40 (m, 5H, H-Ar). ^{13}C NMR (75 MHz): δ 37.0, 48.0, 51.8, 72.4, 73.1, 127.2, 127.5, 127.8, 138.2, 171.1.

Synthesis of *anti*-2,3-Diamino Acids

N,*N*-Dibenzyl Protections of β^3-Amino Methyl Esters

Methyl (*R*)-3-(dibenzylamino)-3-phenylpropanoate (25a): typical procedure.

A magnetically stirred suspension of β^3-phenylglycine methyl ester **24a** (8.38 mmol; 1.50 g) and diisopropylethylamine (DIPEA) (7.3 mL; 41.90 mmol) in toluene (18.0 mL) was warmed gently until a clear solution was obtained. Then, benzyl bromide (6.0 mL; 50.28 mmol) was added in one portion and the resulting solution was refluxed for 4 h. The reaction mixture was then cooled in an ice bath, diluted with EtOAc (2 x 100 mL) and extracted with 10% aq NH_4Cl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to afford a crude reaction product whose chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave the pure cristalline compound **25a**, after recrystallization from EtOAc/Hexane (2.56 g; yield 85%). Mp 51.8–53.0 °C. $[\alpha]_D^{25}$ +71.6 (*c* 2.0). ^{1}H NMR (500 MHz): δ 2.73 (dd, *J*=7.3, *J*=14.6 , 1H, H-2a), 3.14 (dd, *J*=8.8, *J*=14.6, 1H, H-2b), 3.18 (d, *J*=13.7, 2H, NCHPh), 3.64 (s, 3H, OCH_3), 3.78 (d, *J*=13.7, 2H, NCHPh), 4.33 (dd, *J*=7.3, *J*=8.8, 1H, H-3), 7.20–7.41 (m, 15H, H-Ar). ^{13}C NMR (125 MHz): δ 36.9, 51.8, 53.9, 59.1, 127.2, 127.7,

128.3, 128.4, 128.8, 129.1, 137.6, 139.8, 172.3. IR (KBr, cm^{-1}): ν 1714. Anal. Calcd for $C_{24}H_{25}NO_2$: C 80.19, H 7.01, N 3.90. Found: C 80.30, H 7.05, N 3.92.

Under the same conditions, the following *N,N*-diprotected esters were also obtained.

Methyl (*S*)-3-(dibenzylamino)-4-phenylbutanoate (25b)

Oil (yield 83%). $[\alpha]_D^{25}$ -5.4 (*c* 1.0). ^{1}H NMR (500 MHz): δ 2.33 (dd, *J*=6.4, *J*=14.2, 1H, H-2a), 2.56 (dd, *J*=8.8, *J*=13.2, 1H, H-4a), 2.65 (dd, *J*=8.3, *J*=14.2, 1H, H-2b), 3.12 (dd, *J*= 5.7, *J*=13.2, 1H, H-4b), 3.40–3.50 (m, 1H, H-3), 3.56 (s, 3H, OCH_3), 3.62 (d, *J*=13.7, 2H, NCHPh), 3.76 (d, *J*=13.7, 2H, NCHPh), 7.20–7.70 (m, 15H, H-Ar). ^{13}C NMR (125 MHz): δ 35.9, 36.3, 51.6, 53.7, 57.8, 126.4, 127.2, 128.4, 128.6, 129.1, 129.5, 139.7, 139.8, 172.9. IR (KBr, cm^{-1}): ν 1712. Anal. Calcd for $C_{25}H_{27}NO_2$: C 80.40, H 7.29, N 3.75. Found: C 80.25, H 7.32, N 3.77.

Methyl (*R*)-4-(benzyloxy)-3-(dibenzylamino)butanoate (25c)

Oil (yield 86%). $[\alpha]_D^{25}$ +31.6 (*c* 1.8). ^{1}H NMR (500 MHz): δ 2.55 (dd, *J*=6.3, *J*=14.6, 1H, H-2a), 2.68 (dd, *J*=7.8, *J*=14.6, 1H, H-2b), 3.47–3.54 (m, 1H, H-3), 3.56–3.62 (m, 4H, H-4a and OCH_3), 3.66 (d, *J*=13.7, 2H, NCHPh), 3.71 (dd, *J*=9.8, *J*=5.4, 1H, H-4b), 3.75 (d, *J*=13.7, 2H, NCHPh), 4.49 (d, *J*=12.7, 1H, OCHPh), 4.52 (d, *J*= 12.7, 1H, OCHPh), 7.20–7.45 (m, 15H, H-Ar). ^{13}C NMR (125 MHz): δ 34.7, 51.7, 54.5, 55.2, 70.3, 73.3, 127.1, 127.8, 128.4, 128.6, 129.1, 138.6, 140.1, 173.0. IR (KBr, cm^{-1}): ν 1715. Anal. Calcd for $C_{26}H_{29}NO_3$: C 77.39, H 7.24, N 3.47. Found: C 77.25, H 7.27, N 3.48.

Reactions of Enolates of β^3-Amino Methyl Esters with DBAD.

Methyl (2*S*,3*S*)-3-(dibenzylamino)-2-[N',N"-(di-*tert*-butoxycarbonyl)-hydrazino]-3-phenylpropanoate (26a): typical procedure.

To a magnetically stirred solution of **25a** (2.56 g; 7.12 mmol) in anhydrous THF (75 mL), at -78 °C and under anhydrous argon atmosphere, 0.5 M KHMDS in toluene (28.5 mL; 14.24 mmol) was added dropwise. After 1 h solid di-*tert*-butyl azodicarboxylate (2.85 g; 12.82 mmol) was added in one portion to the reaction mixture, kept at -78 °C under stirring. Within 1 h the reaction was quenched by addition of glacial AcOH (1.1 mL) and diluted with EtOAc. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvents evaporated in vacuo. The oily residue, after chromatography on silica gel (hexane/EtOAc, 9:1), afforded the pure title compound **26a** (foam; 3.86 g; yield 92%; d.r. 93:7). $[\alpha]_D^{25}$ +65.7 (*c* 1.6).

The ^{1}H NMR data were not significant, apparently due to the occurrence of mixtures of rotamers. IR (KBr, cm^{-1}): ν 3260, 1740, 1720. Anal. Calcd for $C_{34}H_{43}N_3O_6$: C 69.25, H 7.35, N 7.13. Found: C 69.17, H 7.31, N 7.15.

Under the same conditions, the following Boc-diprotected 2-hydrazino derivatives were also obtained.

Methyl (2*S*,3*S*)-3-(dibenzylamino)-2-[N',N"-(di-*tert*-butoxycarbonyl)- hydrazino]-4-phenylbutanoate (26b)

Foam (yield 90%). $[\alpha]_D^{25}$ -2.3 (*c* 0.3). IR (KBr, cmK1): ν 3250, 1730, 1712. Anal. Calcd for $C_{35}H_{45}N_3O_6$: C 69.63, H 7.51, N 6.96. Found: C 69.60, H 7.49, N 7.01.

Methyl (2*S*,3*R*)-4-(benzyloxy)-3-(dibenzylamino)-2-[N',N"- (di-tert-butoxycarbonyl)-hydrazino]butanoate (26c)

Foam (yield 90%). $[\alpha]_D^{25}$ +45.0 (c 1.5). IR (KBr, cm^{-1}): ν 3270, 1728, 1715. Anal. Calcd for $C_{36}H_{47}N_3O_7$: C 68.22, H 7.47, N 6.63. Found: C 68.19, H 7.40, N 6.68.

Reductive cleavages of the hydrazino bonds.

Methyl (2*S*,3*S*)-2-amino-3-(dibenzylamino)-3-phenylpropanoate (27a): typical procedure.

To a magnetically stirred solution of **26a** (3.86 g; 6.55 mmol) in anhydrous CH_2Cl_2 (54 mL), TFA (54 mL) was added in one portion. After 2 h, the solvent was evaporated under reduced pressure. The crude reaction product, redissolved in MeOH (26 mL), was transferred into a flask containing W-2 Raney nickel (3.86 g, wet) and equipped with a hydrogen inflated balloon. The flask was dipped into an ultrasound bath filled with water and sonicated for 4 h at rt till the starting product was completely consumed (TLC). The reaction mixture was then filtered through Celite® washing with MeOH (100 mL). Removal of the solvent under reduced pressure gave a residue that was redissolved in EtOAc (200 mL), washed with 10% aq Na_2CO_3 (2x100 mL), dried (Na_2SO_4), and evaporated in vacuo, to

afford an oil whose chromatography on silica gel (hexane/EtOAc, 7:3) led to the pure title compound **27a** (oil; 1.71 g; yield 70%). $[\alpha]_D^{25}$ +62.4 (*c* 1.1). ^{1}H NMR (300 MHz): δ 1.97 (bs, 2H, NH_2), 3.05 (d, *J*=13.5, 2H, NCHPh), 3.82 (s, 3H, OCH_3), 3.83–3.88 (m, 3H, H-2 and NCHPh), 4.26 (d, *J*=10.3, 1H, H-3), 7.20–7.60 (m, 15H, H-Ar). ^{13}C NMR (50 MHz): δ 51.7, 54.0, 56.3, 67.3, 126.9, 128.1, 128.3, 128.8, 129.7, 133.5, 139.0, 174.1. IR (KBr, cm^{-1}): ν 3500–3200, 1714. Anal. Calcd for $C_{24}H_{26}N_2O_2$: C 76.98, H 7.00, N 7.48. Found: C 76.81, H 7.06, N 7.52.

Under the same conditions, the following 2-amino esters were also obtained.

Methyl (2*S*,3*S*)-2-amino-3-(dibenzylamino)-4-phenylbutanoate (27b)

Oil (yield 78%). $[\alpha]_D^{25}$ +7.9 (*c* 0.4, MeOH). ^{1}H NMR (500 MHz): δ 1.63 (bs, 2H, NH_2), 2.93 (dd, *J*=7.3, *J*=13.7, 1H, H-4a), 3.09 (dd, *J*=6.3, *J*=13.7, 1H, H-4b), 3.23–3.29 (m, 1H, H-3), 3.60 (s, 3H, OCH_3), 3.61–3.67 (m, 3H, H-2 and NCHPh), 3.70 (d, *J*=13.7, 2H, NCHPh), 7.10–7.40 (m, 15H, H-Ar). ^{13}C NMR (100 MHz): δ 32.7, 52.2, 55.1, 55.6, 63.8, 126.5, 127.4, 128.5, 128.7, 129.4, 129.9, 139.9, 140.5, 175.9. IR (KBr, cm^{-1}): ν 3530–3210, 1712. Anal. Calcd for $C_{25}H_{28}N_2O_2$: C 77.29, H 7.26, N 7.21. Found: C 77.19, H 7.25, N 7.23.

Methyl (2*S*,3*R*)-2-amino-4-(benzyloxy)-3-(dibenzylamino)-butanoate (27c)

Oil (yield 65%). $[\alpha]_D^{25}$ +30.9 (*c* 1.0). ^{1}H NMR (500 MHz, C_6D_6): δ 1.48 (bs, 2H, NH_2), 3.22–3.28 (m, 1H, H-3), 3.29 (s, 3H, OCH_3), 3.58 (d, *J*=6.8, 1H, H-2), 3.60 (dd, *J*=5.8, *J*=9.8, 1H, H-4a), 3.65 (d, *J*=13.7, 2H, NCHPh), 3.72 (dd, *J*=4.9, *J*=9.8, 1H, H-4b), 3.84 (d, *J*=13.7, 2H, NCHPh), 7.05–7.40 (m, 15H, H-Ar). ^{13}C NMR (100 MHz): δ 52.1, 55.4, 55.6, 61.1, 67.2, 73.7, 127.3, 127.9, 128.0, 128.5, 128.8, 129.4, 138.7, 140.2, 175.7. IR (KBr, cm^{-1}): ν 3510–3200, 1716. Anal. Calcd for $C_{26}H_{30}N_2O_3$: C 74.61, H 7.22, N 6.69. Found: C 74.59, H 7.20, N 7.01.

Methyl (4*S*,5*S*)-4-methoxycarbonyl-5-phenyl-2-imidazolidinone (29)

A magnetically stirred solution of **27a** (0.020 g; 0.053 mmol) in glacial AcOH (0.5 mL) was hydrogenolysed over 30% Pd/C catalyst (0.006 g) for 2 h at 50 8C, under a slightly positive pressure given by an inflated balloon (~3 bar). The mixture was then filtered through Celite® washing with MeOH (10 mL). Removal of the solvents under reduced pressure gave a residue that was redissolved in anhydrous THF (1.1 mL). The solution was cooled to 0 °C. Et_3N (0.063 mL, 0.053 mmol) and 1,1'-carbonyldiimidazole (0.013 g; 0.079 mmol) were then added in sequence. After 30 min at 0 °C and 2 h at rt the solvents were evaporated under reduced pressure and the remaining crude residue was dissolved in EtOAc and filtered on a short silica gel plug (~3 cm^3) with the same solvent (3x10 mL). By partial evaporation of the solvent under reduced pressure, a semicrystalline residue could be collected whose recrystallization by the same solvent afforded the pure **29** (0.010 g; yield 88%) as a white solid. Mp 202–203 °C dec. (lit.[1] 203–205 °C). ^{1}H, ^{13}C NMR and IR spectra were superimposable to those reported.

Methyl (2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-(dibenzylamino)-3-phenylpropanoate (30)

To a solution of compound **27a** (0.67 g; 1.80 mmol) in dioxane (21 mL) at 0 °C, Et_3N (0.42 mL; 2.70 mmol) and Boc_2O (0.89g; 3.60 mmol) were added in sequence. The reaction mixture, warmed up to room temperature and stirred for 1 h, was then diluted with EtOAc. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvents evaporated in vacuo to give an oil. Its chromatography on silica gel (hexane/EtOAc, 9:1) afforded the pure title compound **30** (oil; 0.78 g; yield 92%). $[\alpha]_D^{25}$ +50.7 (*c* 1.5). ^{1}H NMR (400 MHz): δ 1.55 (s, 9H, Boc), 3.04 (d, *J*=13.5, 2H, NCHPh), 3.86 (s, 3H, OCH_3), 3.97–4.02 (m, 3H, H-3, NCHPh), 4.58 (bd, *J*=8.3, 1H, NHBoc), 5.14 (bt, *J*=9.7, 1H, H-2), 7.24–7.44 (m, 15H, H-Ar). ^{13}C NMR (125 MHz): δ 28.3, 52.3, 54.1, 54.7, 65.0, 80.2, 127.3, 128.3, 128.5, 129.2, 130.1, 132.6, 139.1, 146.9, 154.9, 172.3. IR (KBr, cm^{-1}): ν 1718, 1705. Anal. Calcd for $C_{29}H_{34}N_2O_4$: C 73.39, H 7.22, N 5.90. Found: C 73.25, H 7.20, N 5.92.

Methyl (2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-amino-3-phenylpropanoate (31)

A magnetically stirred solution of **30** (0.78 g; 1.66 mmol) in glacial AcOH (7.4 mL) was hydrogenolysed over 30% Pd/C catalyst (0.23 g) for 2 h at 50 °C, under a slightly positive pressure given by an inflated balloon (~3 bar). The mixture was then filtered trough Celite® and washed with MeOH (100 mL). The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (2x100 mL). The organic layer was washed with 10% aq Na_2CO_3 (300 mL), dried (Na_2SO_4), and the solvent evaporated in vacuo to give compound **31** as a white crystalline solid, after recrystallization from

[1] Alexakis, A.; Lensen, N.; Tranchier, J.-P.; Mangeney, P.; Feneau-Dupont, J.; Declercq, J. P. *Synthesis* **1995**, *8*, 1038–1050.

hexane/acetone 9:1 (0.44 g; yield 90%). Mp 110.3–112.3 °C. $[\alpha]_D^{25}$ +29.0 (*c* 0.9). ^{1}H NMR (400 MHz, C_5D_5N): δ 1.35 (s, 9H, Boc), 3.62 (s, 3H, OCH_3), 5.34 (d, *J*=7.1, 1H, H-3), 5.77 (bt, *J*=8.0, 1H, H-2), 7.21–7.35 (m, 3H, H-Ar), 7.89 (d, *J*=7.3, 2H, H-Ar), 8.80 (bd, *J*=8.6, 1H, NHBoc). ^{13}C NMR (100 MHz, C_5D_5N): δ 28.3, 52.4, 57.1, 59.4, 79.3, 128.6, 128.9, 138.0, 140.2, 156.8, 172.0. IR (KBr, cm^{-1}): ν 3350, 3200, 1765, 1715. Anal. Calcd for $C_{15}H_{22}N_2O_4$: C 61.21, H 7.53, N 9.52. Found: C 61.32, H 7.57, N 9.58.

Methyl (2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-(9H-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoate(32)

To a stirred solution of **31** (0.44 g; 1.48 mmol) in dioxane (5.8 mL) and 10% aq Na_2CO_3 (0.31 g; 2.96 mmol) at 0 °C, Fmoc–OSu (0.41 g; 1.18 mmol) dissolved in DMF (1.5 mL) was added slowly. The reaction mixture, after 30 min at 0 °C and 2 h at rt, was extracted with CH_2Cl_2. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvents evaporated under reduced pressure to give an oil. The chromatography on silica gel ($CHCl_3$) afforded the pure compound **32**, white solid after recrystallization from hexane/acetone 9:1 (0.50 g; yield 65%). Mp 166.6–168.1 °C. $[\alpha]_D^{25}$ +38.7 (*c* 0.3). ^{1}H NMR (500 MHz, C_5D_5N): δ 1.20 (s, 9H, Boc), 3.42 (s, 3H, OCH_3), 4.14 (t, *J*=6.8, 1H, Fmoc), 4.30 (dd, *J*=6.8, *J*=10.3, 1H, CHFmoc), 4.45 (dd, *J*=6.8, *J*=10.3, 1H, CHFmoc), 5.31 (t, *J*=9.3, 1H, H-2), 5.69 (t, *J*=9.3, 1H, H-3), 7.05–7.70 (m, 14H, H-Ar and NHBoc), 9.17 (d, *J*=9.3, 1H, NHFmoc). ^{13}C NMR (125 MHz): δ 28.5, 47.5, 52.9, 57.7, 58.0, 67.3, 120.2, 125.4, 126.8, 127.3, 128.4, 128.9, 141.5, 144.1, 155.9, 156.5, 170.4. IR (KBr, cm^{-1}): ν 3395 (br), 1705. Anal. Calcd for $C_{30}H_{32}N_2O_6$: C 69.75, H 6.24, N 5.42. Found: C 69.82, H 6.27, N 5.43.

Synthesis of 2,3-Disubstituted β-Amino Acids

Full Protection of the Amino Group of β-amino acids

Methyl 3-[Bis(4-methoxybenzyl)amino]propanoate (1)

A magnetically stirred suspension of β-alanine hydrochloride methyl ester (0.39 g; 2.84 mmol) and diisopropylethylamine (DIPEA, 2.9 mL; 14.20 mmol) in toluene (10 mL) was gently warmed until a clear solution was obtained. 4-Methoxybenzyl chloride (2.3 mL; 17.04 mmol) was then added in one portion, and the resulting solution was heated under reflux until the starting homo-β-glycine, and also the intermediate N-monosubstituted derivative, were completely consumed (ca. 5 h, TLC monitoring). The reaction mixture was then cooled in an ice bath, diluted with ethyl acetate (0.2 L), and extracted with 10% aq. NH4Cl. The organic layer was washed with brine and dried (Na_2SO_4), and the solvents were evaporated under reduced pressure to afford a crude reaction product, chromatography of which on silica gel (petroleum ether/ Et_2O, 8:2) gave the pure, oily title compound **1** (0.83 g; yield 85%). ^{1}H NMR (500 MHz): δ 2.41 (t, *J*=7.08, 2H, H-2), 2.77 (t, *J*=7.08, 2H, H-3), 3,45 (s, 3H, OMe), 3.60 (s, 4H, 2xMpm-CH_2), 3.80 (s, 6H, 2xMpm-OMe), 6.85 (d, *J*=8.2, 4H, 2xMpm-H-3 and H-5), 7.45 (d, *J*=8.2, 4H, 2xMpm-H-2 and H-6). ^{13}C NMR (75 MHz): δ 32.4, 48.7, 51.7, 55.0, 57.0, 113.4, 129.7, 131.2, 158.5, 172.7. Anal. Calcd. for $C_{20}H_{25}NO_4$: C 69.95, H 7.34. Found C 70.20, H 7.30.

Under the same conditions, the following N-protected esters were also obtained.

Methyl (*S*)-3-[Bis(4-methoxybenzyl)amino]-4-phenylbutanoate (4)

Oil (yield 90%). $[\alpha]_D^{25}$ -6.2 (*c* 1.89). ^{1}H NMR (400 MHz): δ 2.31 (dd, *J*=6.1, *J*=14.1, 1H, H-2_a), 2.53 (dd, *J*=8.8, *J*=13.5, 1H, H-4_b), 2.63 (dd, *J*=8.6, *J*=14.1, 1H, H-2_b), 3.08 (dd, *J*=5.7, *J*=13.5, 1H, H-4_b), 3.46 (m, 1H, H-3), 3.52 (d, *J*=13.4, 2H, 2xMpm-CH_aH$_b$), 3.56 (s, 3H, OMe), 3.66 (d, *J*=13.4, 2H, 2xMpm-CH$_a$*H$_b$*), 3.80 (s, 6H, 2xMpm-OMe), 6.83 (d, *J*=8.7, 4H, 2xMpm-H-3 and H-5), 7.06-7.26 (m, 9H, 5xH-Ar, 2x Mpm-H-2 and H-6). ^{13}C NMR (100 MHz): δ 36.0, 51.2, 52.4, 55.1, 57.1, 113.4, 125.9, 128.2, 129.1, 129.8, 131.5, 139.4, 158.5, 172.7. Anal. Calcd. for $C_{27}H_{31}NO_4$: C 74.80, H 7.21. Found C 74.69, H 7.23.

Methyl (*R*)-3-[Bis(4-methoxybenzyl)amino]-3-phenylpropanoate (7)

Oil (yield 83%).$[\alpha]_D^{25}$ +77.5 (*c* 2.11). ^{1}H NMR (200 MHz): δ 2.71 (dd, *J*=7.2, *J*=14.5, 1H, H-2_a), 3.09 (d, *J*=13.6, 2H, 2xMpm-CH_aH$_b$), 3.07-3.18 (m, 1H, H-2_b), 3.64 (s, 3H, OMe), 3.69 (d, *J*=13.6, 2H, 2xMpm-CH$_a$*H$_b$*), 3.79 (s, 6H, 2xMpm-OMe), 4.29 (dd, *J*=7.2, *J*=8.7, 1H, H-3), 6.85 (d, *J*=8.7, 4H, 2xMpm-H-3 and H-5), 7.23-7.41 (m, 9H, 5xH-Ar, 2xMpm-H-2 and H-6). ^{13}C NMR (100 MHz): δ 36.4, 51.4, 52.6, 55.1, 58.5, 113.5, 127.3, 128.0, 128.5, 129.8, 131.6, 137.6, 158.6, 172.0. Anal. Calcd. for $C_{26}H_{29}NO_4$: C 74.44, H 6.97. Found C 74.79, H 6.93.

Methyl (*R*)-4-(Benzyloxy)-3-[bis(4-methoxybenzyl)amino]butanoate (10)

Oil; (yield 85%). ^{1}H NMR (400 MHz): δ 2.51 (dd, *J*=6.4, *J*=14.3, 1H, H-2$_a$), 2.64 (dd, *J*=7.7, *J*=14.3, 1H, H-2$_b$), 3.48 (m, 1H, H-3), 3.52-3.57 (m, 3H, 2xMpm-C*H$_a$*H$_b$, H-4$_a$), 3.58 (s, 3H, OMe), 3.63-3.68 (m, 3H, 2xMpm-CH$_a$*H$_b$*, H-4$_b$), 3.79 (s, 6H, 2xMpm-OMe), 4.48 (s, 2H, 2xH-5), 6.83 (d, *J*=8.7, 4H, 2xMpm-H-3 and H-5), 7.21 (d, *J*=8.7, 4H, 2xMpm-H-2 and H-6), 7.30-7.36 (m, 5H, H-Ar). ^{13}C NMR (75 MHz): δ 34.5, 51.4, 53.4, 54.7, 55.2, 70.2, 73.1, 113.5, 127.5, 128.3, 129.9, 132.0, 138.3, 158.6, 172.8. Anal. Calcd. for $C_{20}H_{25}NO_4$: C 72.55, H 7.18. Found C 72.31, H 7.22.

Introduction of methyl group

Methyl 3-(bis(4-methoxybenzyl)amino)-2-methylpropanoate (2)

To a magnetically stirred solution of **1** (0.45 mmol, 0.15 g) in anhydrous THF (8 mL), 0.5 M KHDMS in THF (1.80 mL, 0.93 mmol) was added dropwise, at -78 °C and under anhydrous argon,. After 1 h, CH_3I (0.49 mmol, 0.31 mL) was added to the solution in one portion, and the reaction mixture was kept at -78 °C whilst stirring. Within 90 min, the reaction was quenched by addition of 10% aq NH_4Cl (50 mL) and extracted with ethyl acetate. The organic layer was washed with brine until neutral and dried (Na_2SO_4), and the solvents were evaporated in vacuo. The residue (180 mg) was purified by chromatography on silica gel (hexane/ethyl acetate, 9:1) afforded the pure, yellow oily title compound **2** (0.10 g, yield 61%). ^{1}H NMR (500 MHz): δ 1.10 (d, *J*=6.3, 3H, CH_3), 2.35-2.43 (m, 1H, H-2), 2.70-2.80 (m, 2H, H-3$_a$ and H-3$_b$), 3.40 (d, *J*=13.3, 2H, 2xMpm-C*H$_a$*H$_b$), 3.54 (d, *J*=13.3, 2H, 2xMpm-CH$_a$*H$_b$*), 3.64 (s, 3H, OMe), 3.80 (s, 6H, 2xMpm-OMe), 6.83 (d, *J*=8.4, 4H, 2xMpm-H-3 and H-5), 7.20 (d, *J*=8.4, 4H, 2xMpm-H-2 and H-6). ^{13}C NMR (125 MHz): δ 15.5, 38.9, 51.7, 55.5, 57.3, 57.8, 113.7, 130.2, 131.6, 158.8, 176.5. Anal. Calcd. for $C_{21}H_{27}NO_4$: C 70.56, H 7.61, N 3.92. Found C 70.53, H 7.59; N 3.90.

Under the same conditions (but 2.2 eq. of CH_3I instead 1.1), the other α-methyl-β^3-amino acids were also obtained.

(2*S*,3*S*)-Methyl 3-(bis(4-methoxybenzyl)amino)-2-methyl-4-phenylbutanoate (5)

Oil; (yield 97%, d.r. 93:7). ^{1}H NMR (400 MHz): δ 1.03 (d, *J*=6.9, 3H, CH_3), 2.74 (dd, *J*=7.1, *J*=14.2, 1H, H-4$_a$), 2.83-2.88 (m, 1H, H-2), 3.10 (dd, *J*=6.1, *J*=14.2, 1H, H-4$_b$), 3.24-3.27 (m, 1H, H-3), 3.41 (d, *J*=13.5, 2H, 2xMpm-C*H$_a$*H$_b$), 3.63 (s, 3H, OMe), 3.69 (d, *J*=13.5, 2H, 2xMpm-CH$_a$*H$_b$*), 3.81 (s, 6H, 2xMpm-OMe), 6.84 (d, *J*=8.6, 4H, 2xMpm-H-3 and H-5), 7.15 (d, *J*=8.6, 4H, 2xMpm-H-2 and H-6), 7.17-7.30 (m, 5H, 5xH-Ar). ^{13}C NMR (100 MHz): δ 16.0, 33.6, 43.2, 51.8, 53.8, 55.6, 62.4, 113.8, 126.3, 128.7, 129.7, 130.5, 132.1, 141.4, 158.9, 176.2. Anal. Calcd. for $C_{28}H_{33}NO_4$: C 75.14, H 7.43, N 3.13. Found C 75.17, H 7.45, N 3.15.

(2*S*,3*R*)-Methyl 3-(bis(4-methoxybenzyl)amino)-2-methyl-3-phenylpropanoate (8)

Oil; (yield 80%, d.r. 9:1). ^{1}H NMR (500 MHz): δ 1.46 (d, *J*=6.8, 3H, CH_3), 2.92 (d, *J*=13.7, 2H, 2xMpm-C*H$_a$*H$_b$), 3.30 (s, 3H, OMe), 3.31-3.37 (m, 1H, H-2), 3.74-3.86 (m, 9H, H-3, 2xMpm- CH$_a$*H$_b$* and 2xMpm-OMe), 6.88 (d, *J*=8.8, 4H, 2xMpm-H-3 and H-5), 7.18-7.40 (m, 9H, 5xH-Ar, 2x Mpm-H-2 and H-6).^{13}C NMR (125 MHz): δ 16.5, 42.1, 51.5, 53.1, 55.5, 64.2, 114.0, 127.6, 128.0, 129.6, 130.1, 131.9, 135.8, 158.8, 175.9. Anal. Calcd. for $C_{27}H_{31}NO_4$: C 74.80, H 7.21, N 3.23. Found C 74.82, H 7.24, N 3.25.

(2*S*,3*R*)-Methyl 4-(benzyloxy)-3-(bis(4-methoxybenzyl)amino)-2-methylbutanoate (11)

Oil; (yield 89%, d.r. 94:6). ^{1}H NMR (400 MHz): δ 1.23 (d, *J*=7.0, 3H, CH_3), 2.74-2.84 (m, 1H, H-2), 3.05-3.10 (m, 1H, H-3), 3.49 (d, *J*=13.6, 2H, 2xMpm-C*H$_a$*H$_b$), 3.55 (s, 3H, OMe), 3.65 (dd, *J*=4.6, *J*=10.0, 1H, H-4$_a$), 3.73 (dd, *J*=5.5, *J*=10.0, 1H, H-4$_b$), 3.78-3.83 (m, 8H, 2xMpm-CH$_a$*H$_b$* and 2xMpm-OMe), 4.46 (d, *J*=12.0, 1H, Bzl-H$_a$), 4.53 (d, *J*=12.0, 1H, Bzl-H$_b$), 6.85 (d, *J*=8.6, 4H, 2xMpm-H-3 and H-5), 7.22 (d, *J*=8.6, 4H, 2xMpm-H-2 and H-6), 7.30-7.45 (m, 5H, H-Ar). ^{13}C NMR (100 MHz): δ 16.3, 40.9, 51.8, 54.2, 55.7, 59.5, 68.4, 73.6, 113.9, 127.8, 127.9, 128.7, 130.4, 132.5, 138.8, 158.9, 176.7. Anal. Calcd. for $C_{29}H_{35}NO_5$ C 72.93, H 7.39, N 2.93. Found C 72.95, H 7.41, N 2.96.

Introduction of benzyl group

Under the same conditions of α-methylation (but using BnBr instead CH_3I as electrophile) the α-benzyl-β-amino acids were also obtained (1.1 eq. of BnBr for benzylation of β-alanine and 2.2 eq. for the other β^3-amino acids).

Methyl 2-benzyl-3-(bis(4-methoxybenzyl)amino)propanoate (3)

Oil, (yield 65%). ^{1}H NMR (500 MHz): δ 2.49 (dd, *J*=6.1, *J*=12.4, 1H, H-4$_a$), 2.74 -2.82 (m, 3H, H-4$_b$, H-3$_a$ and H-3$_b$), 2.94-3.10 (m, 1H, H-2), 3.37 (d, *J*=13.6, 2H, 2xMpm-C*H$_a$*H$_b$), 3.57 (s, 3H, OMe), 3.58 (d, *J*=13.6, 2H, 2xMpm-CH$_a$*H$_b$*), 3.80 (s, 6H, 2xMpm-OMe), 6.84 (d, *J*=8.3, 4H, 2xMpm-H-3 and H-5), 7.08 (d, *J*=7.6, 2H, 2xPh-3H and H-5), 7.16-7.31 (m, 7H, 3xPh-H, 2xMpm-H-2 and H-6). ^{13}C NMR (125

MHz): δ 36.7, 47.2, 55.4, 55.5, 57.8, 57.9, 113.7, 128.9, 130.2, 130.4, 131.3, 139.5, 158.8, 175.2. Anal. Calcd. for $C_{27}H_{31}NO_4$: C 74.80, H 7.21, N 3.23. Found C 74.83, H 7.19, N 3.26.

(2*S*,3*S*)-Methyl 2-benzyl-3-(bis(4-methoxybenzyl)amino)-4-phenylbutanoate (6)

Oil, (yield 90%, d.r. 97:3). ^{1}H NMR (400 MHz): δ 2.62 (dd, *J*=5.0, *J* =13.7, 1H, H-5$_a$), 2.70 (dd, *J*=10.7, *J*=13.7, 1H, H-5$_b$), 2.85 (dd, *J*=7.7, *J*=14.2, 1H, H-4$_a$), 3.04-3.10 (m, 1H, H-2), 3.25 (dd, *J*=5.5, *J*=14.2, 1H, H-4$_b$), 3.34- 3.40 (m, 1H, H-3), 3.42 (d, *J*=13.3, 2H, 2xMpm-C*H$_a$*H$_b$), 3.50 (s, 3H, OMe), 3.76 (d, *J*=13.3, 2H, 2xMPM-CH$_a$*H$_b$*), 3.80 (s, 6H, 2xMpm-OMe), 6.85 (d, *J*=8.7, 4H, 2xMpm-H-3 and H-5), 6.80-7.7 (m, 14H, 10xH-Ar, 2x Mpm-H-2 and H-6). ^{13}C NMR (125 MHz): δ 33.8, 36.6, 51.3, 51.8, 53.6, 55.4, 61.5, 113.6, 126.3, 128.4, 128.7, 128.8, 129.4,129.6, 130.2, 130.4, 131.7, 139.7, 158.8, 174.3. Anal. Calcd. for $C_{34}H_{37}NO_4$: C 77.98, H 7.12, N 2.67. Found C 78.00, H 7.15, N 2.70.

(2*S*,3*R*)-Methyl 2-benzyl-3-(bis(4-methoxybenzyl)amino)-3-phenylpropanoate (9)

Oil, (yield 75%, d.r. 92:8). ^{1}H NMR (500 MHz): δ 2.69 (dd, *J*=11.6, *J* =13.6, 1H, H-4$_a$), 3.02 (d, *J*=13.6, 2H, 2xMpm-C*H$_a$*H$_b$), 3.08 (s, 3H, OMe), 3.49 (ddd, *J* =11.6, *J*=11.7, 1H, H-2), 3.79-3.92 (m, 9H, H-4$_b$, 2xMPM-C*H$_a$H$_b$* and 2xMpm-OMe), 3.90 (d, *J*=11.7, 1H, H-3), 6.90 (d, *J*= 8.7, 4H, 2xMpm-H-3 and H-5), 7.10-7.42 (m, 14H, 10xH-Ar, 2x Mpm-H-2 and H-6). ^{13}C NMR (125 MHz): δ 37.5, 51.0, 51.1, 53.3, 55.4, 64.0, 114.1, 126.5, 127.7, 128.0, 128.6, 129.0, 129.8, 130.2, 131.7, 135.0, 140.0, 158.9, 174.3. Anal. Calcd. for $C_{33}H_{35}NO_4$: C 77.77, H 6.92, N 2.75. Found C 77.80, H 6.94, N 2.79.

(2*S*,3*R*)-Methyl 2-benzyl-4-(benzyloxy)-3-(bis(4-methoxybenzyl)amino)butanoate (12)

Oil, (yield 78%, 96:4). ^{1}H NMR (400 MHz): δ 2.55 (dd, *J*=11.2, *J*=13.6, 1H, H-5$_a$), 2.98 (ddd, *J*=3.5, *J*=11, *J*=11.2, 1H, H-2), 3.18-3.25 (m, 1H, H-3), 3.39 (s, 3H, OMe), 3.51 (dd, *J*=3.5, *J*=13.6, 1H, H-5$_b$), 3.59 (d, *J*=13.5, 2H, 2xMpm-C*H$_a$*H$_b$), 3.71 (dd, *J*=4.61, *J*=10.1, 1H, H-4$_a$), 3.80 (dd, *J*=5.85, *J*=10.1, 1H, H-4$_b$), 3.86 (s, 6H, 2xMpm-OMe), 3.94 (d, *J*=13.5, 2H, 2xMPM-CH$_a$*H$_b$*), 4.50 (d, *J*=11.9, 1H, Bzl-H$_a$), 4.56 (d, *J*=11.9, 1H, Bzl-H$_b$), 6.91 (d, *J*=8.6, 4H, 2xMpm-H-3 and H-5), 7.10 (d, *J*= 6.8, 2 H, H-Ar), 7.19-7.45 (m, 12 H, 8xH-Ar, 2 x Mpm-H-2 and H-6). ^{13}C NMR (75 MHz): δ 36.7, 49.3, 51.0, 53.8, 55.1, 58.6, 67.8, 73.1, 113.6, 126.0, 127.4, 128.2, 130.0, 131.8, 138.2, 139.9, 158.5, 174.6. Anal. Calcd. for $C_{35}H_{39}NO_5$: C 75.92, H 7.10, N 2.53. Found C 75.94, H 7.06, N 2.55.

Introduction of hydroxymethyl group

Methyl 3-(bis(4-methoxybenzyl)amino)-2-(hydroxymethyl)propanoate (13)

To a magnetically stirred solution of **1** (0.48 mmol, 0.16 g) in anhydrous THF (8.3 mL), 0.5 M KHDMS in THF (1.92 mL, 0.96 mmol) was added dropwise, at -78 °C and under anhydrous argon,. After 1 h, the solution is let to reach -20 °C; HCHO (g), generated by cracking of paraformaldehyde (3.84 mmol, 0.12 mg) at 220 °C is then bubbled into the solution by nitrogen flow and the system is cooled again at -78 °C. Within 90 min, the reaction was quenched by addition of 10% aq NH_4Cl (50 mL) and extracted with ethyl acetate. The organic layer was washed with brine until neutral and dried (Na_2SO_4), and the solvents were evaporated in vacuo. The residue (265 mg) was purified by chromatography on silica gel (hexane/ethyl acetate, 7:3) afforded the pure, yellow oily title compound **13** (0.15 mg, yield 85%). ^{1}H NMR (500 MHz): δ 2.76 (dd, *J*=4.6, *J*=12.9, 1H, H-3$_a$), 2.86 (dd, *J*=10.2, *J*=12.9, 1H, H-3 $_b$), 2.94-3.10 (m, 1H, H-2), 3.28 (d, *J*=13.2, 2H, 2xMpm-C*H$_a$*H$_b$), 3.65 (s, 3H, OMe), 3.73-3.90 (m, 10H, 2xMpm-OMe, 2xMpm-CH$_a$*H$_b$*, 2xCH$_a$H$_b$OH), 6.82 (d, *J*=8.4, 4H, 2xMpm-H-3 and H-5), 7.20 (d, *J*=8.4, 4H, 2xMpm-H-2 and H-6). ^{13}C NMR (125 MHz): δ 44.3, 51.6, 53.8, 55.1, 57.8, 63.9, 113.7, 129.8, 130.4, 158.7, 172.9. Anal. Calcd. for $C_{21}H_{27}NO_5$: C 67.54, H 7.29, N 3.75. Found C 67.57, H 7.31, N 3.77.

Methyl 2-((bis(4-methoxybenzyl)amino)methyl)acrylate (14)

Oil. ^{1}H NMR (400 MHz): δ 3.27 (s, 2H, 3'-H), 3.50 (s, 4H, 2xMpm-CH$_2$), 3.72 (s, 3H, OMe), 3.79 (s, 6H, 2xMpm-OMe), 6.00 (s, 1H, H-3$_a$), 6.25 (s, 1H, H-3$_b$), 6.82 (d, *J*=8.4, 4H, 2xMpm-H-3 and H-5), 7.20 (d, *J*=8.4, 4H, 2xMpm-H-2 and H-6). ^{13}C NMR (50 MHz): δ 51.5, 53.5, 55.1, 57.1, 113.4, 125.8, 129.5, 131.2, 138.2, 158.5, 167.4. Anal. Calcd. for $C_{21}H_{25}NO_4$: C 70.96, H 7.09, N 3.94. Found C 70.99, H 7.11, N 3.97.

Under the same conditions, the other α-hydroxymethyl-β^3-amino acids were also obtained.

(2*R*,3*S*)-Methyl 3-(bis(4-methoxybenzyl)amino)-2-(hydroxymethyl)-4-phenylbutanoate (15)

Oil, (yield 65%, d.r. 99:1). ^{1}H NMR (500 MHz): δ 2.66-2.68 (m, 1H, H-2), 2.92 (dd, *J*=9.06, *J*=13.6, 1H, H-4$_a$), 3.14 (dd, *J*=4.7, *J*=13.6, 1H, H-4$_b$), 3.32-3.40 (m, 3H, H-3 and 2xMPM-C*H$_a$*H$_b$), 3.52 (dd, *J*=4.89, *J* =11.4,1H, C*H$_a$*H$_b$*OH*), 3.61 (dd, *J*=6.8, *J* =11.4, CH$_a$*H$_b$*OH), 3.70 (s, 3H, OMe), 3.80 (m, 8H, 2xMpm-CH$_a$*H$_b$* and 2xOMe-Mpm), 6.57 (d, *J*=8.7, 4H, 2xMpm-H-3 and H-5), 6.80-7.70 (m, 9H, 5xH-Ar, 2xMpm-H-2 and H-6). ^{13}C NMR (175 MHz): δ 32.7, 49.5, 51.8, 54.1, 55.4, 59.6, 62.4, 113.9, 126.4,

128.5, 129.5, 129.6, 131.6, 140.2, 159.9, 174.2. Anal. Calcd. for $C_{28}H_{33}NO_5$: C 72.55, H 7.18, N 3.02. Found C 72.58, H 7.22, N 3.07.

(2*R*,3*R*)-Methyl 3-(bis(4-methoxybenzyl)amino)-2-(hydroxymethyl)-3-phenylpropanoate (16)

Oil, (yield 88%, 97:3). ^{1}H NMR (500 MHz): 2.89 (d, *J*=13.4, 2H, 2xMpm-CH_aH$_b$), 3.39-3.47 (m, 2H, 2xCH_2OH), 3.53-3.59 (m, 1H, H-2), 3.80 (s, 6H, 2xMpm-OMe), 3.84 (s, 3H, OMe), 3.90 (d, *J*=13.4, 2H, 2xMpm-CH$_a$$H_b$), 4.10 (d, *J*=12.2 1H, H-3), 6.70 (d, *J*=8.7, 4H, 2xMpm-H-3 and H-5), 7.19-7.32 (m, 9H, 5xH-Ar, 2x Mpm-H-2 and H-6). ^{13}C NMR (125 MHz): 50.2, 51.7, 52.8, 55.9, 60.8, 61.9, 113.5, 127.7, 128.2, 129.2, 129.7, 131.1, 133.6, 158.4, 174.1. Anal. Calcd. for $C_{27}H_{31}NO_5$: C 72.14, H 6.95, N 3.12. Found C 72.17, H 6.99, N 3.15.

(2*R*,3*R*)-Methyl 4-(benzyloxy)-3-(bis(4-methoxybenzyl)amino)-2-(hydroxymethyl)butanoate (17)

Oil, (yield 78%, d.r. 95:5). ^{1}H NMR (500 MHz): 3.08-3.13 (m, 1H, H-2), 3.31-3.36 (m, 1H, H-3), 3.36 (d, *J*=13.7, 2H, 2xMPM-CH_aH$_b$), 3.65 (s, 3H, OMe), 3.66-3.71 (m, 2H, H-4$_a$, 1xCH_aH$_b$OH), 3.73- 3.78 (m, 2H, H-4$_b$, 1xCH$_a$$H_b$OH), 3.79 (s, 6H, 2xMpm-OMe), 3.85 (d, *J*=13.7, 2H, 2xMPM-CH$_a$$H_b$), 4.50 (d, *J* =12.2, 1H, Bzl-H$_a$), 4.55 (d, *J*=12.2, 1H, Bzl-H$_b$), 6.82 (d, *J*=8.3, 4H, 2xMpm-H-3 and H-5), 7.15 (d, *J*=8.3, 4H, 2xMpm-H-2 and H-6), 7.30- 7.40 (m, 5H, 5xBzl-aromatici-H). ^{13}C NMR (50 MHz): 49.0, 51.5, 53.7, 55.1, 56.4, 61.5, 66.5, 113.4, 127.5, 127.7, 128.4, 130.0, 131.4, 137.7, 158.5, 174.2. Anal. Calcd. for $C_{29}H_{35}NO_6$: C 70.57, H 7.15, N 2.84. Found C 70.59, H 7.18, N 2.87.

Synthesis of Lipo-β-Amino Acids

Benzylation of the Amino Group of β-Alanine

tert-Butyl 3-(dibenzylamino)propanoate (25)

A magnetically stirred suspension of β-alanine *tert*-butyl ester (0.39 g; 3.40 mmol) and diisopropylethylamine (DIPEA, 2.9 mL; 17.0 mmol) in toluene (10 mL) was gently warmed until a clear solution was obtained. Benzyl chloride (2.43 mL; 20.4 mmol) was then added in one portion, and the resulting solution was heated under reflux until the starting β-alanine *tert*-butyl ester, and also the intermediate N-monosubstituted derivative, were completely consumed (ca. 3 h, TLC monitoring). The reaction mixture was then cooled in an ice bath, diluted with ethyl acetate (50 mL), and extracted with 10% aq. NH4Cl. The organic layer was washed with brine and dried (Na_2SO_4), and the solvents were evaporated under reduced pressure to afford a crude reaction product, chromatography of which on silica gel (petroleum ether/ Et_2O, 8:2) gave the pure, oily title compound **25** (0.35 g; yield 97%). ^{1}H NMR (500 MHz): δ 1.41 (s, 9H, ^{t}Bu-H), 2.41 (t, *J*=7.3, 2H, H-2), 2.80 (t, *J*=7.3, 2H, H-3), 3.60 (s, 4H, 2xCH_2-Ph), 7.20-7.40 (m, 10H, H-Ar). ^{13}C NMR (125 MHz): δ 28.3, 34.1, 49.6, 58.2, 80.4, 127.1, 128.4, 129.0, 139.6, 172.2. Anal. Calcd. for $C_{21}H_{27}NO_2$: C 77.50, H 8.36, N 4.30. Found C 77.53, H 8.38, N 4.33.

Under the same conditions, the N-benzyl-β-ala-OMe was also obtained.

Methyl 3-(dibenzylamino)propanoate (28)

Oil, (yield 90%). ^{1}H NMR (500 MHz): δ 2.50 (t, *J*=7.3, 2H, H-2), 2.80 (t, *J*=7.3, 2H, H-3), 3.58 (s, 4H, 2xCH_2-Ph), 3.62 (s, 3H, OMe), 7.41-7.70 (m, 10H, H-Ar). ^{13}C NMR (50 MHz): δ 32.6, 49.1, 51.4, 58.0, 127.0, 128.1, 128.7, 139.2, 172.9. Anal. Cald. for $C_{18}H_{21}NO_2$: C 76.29, H 7.47, N 4.94. Found C 76.31, H 7.49, N 4.97.

Preparation of the electrophile

1-Iodopentadecane (19)

To solid iodine (4.2 g, 16.4 mmol) was slowly added a solution of triphenylphosphine (4.3 g, 16.4 mmol) in anhydrous CH_2Cl_2 (120 mL) at room temperature, in inert atmosphere and under magnetic stirring; after 15 minutes imidazole (2.2 g, 32.9 mmol) was added. The mixture was then left for other 15 minutes at room temperature. 1-Pentadecanol **18** (3.0 g, 13.1 mmol), in anhydrous CH_2Cl_2, was finally added to the suspension and the reaction was allowed to reflux until the starting material was completely consumed (1h, TLC, petroleum ether). The solvent was then evaporated under reduced pressure and the residue, diluted with EtOAc (150 mL) was washed with 10% aq. $Na_2S_2O_4$ (40 mL) and then brine (3 x 50 mL) until neutrality. The organic layer was finally dried (Na_2SO_4) and evaporated in vacuum to give a crude that is finally easily purified by chromatography in petroleum ether to give the iodopentadecane as colourless oil **19** (4.3 g; yield 97%). ^{1}H NMR: δ 0.88 (t, *J*=6.8, 3H,

H-15), 1.20-1.44 (m, 24 H, 12xCH_2), 1.82 (dt, *J*=7.3, 2H, H-2), 3.19 (t, *J*=7.3, 2H, H-1). ^{13}C NMR: δ 7.6, 14.3, 22.9, 28.8, 29.6-29.9, 30.7, 32.1, 33.8.

tert-Butyl 2-((dibenzylamino)methyl)heptadecanoate (26)

To a magnetically stirred solution of hexamethyldisilazane (HMDS, 0.12 mL, 0.58 mmol) and butyllithium (1.6 M in hexane, 0.36 mL, 0.582 mmol) in anhydrous THF (5 mL), was added dropwise at -78 °C a solution of **25** (0.29 mmol, 0.024 g) in anhydrous THF. After 1 h, iodopentadecane (0.64 mmol, 0.22 mg) was added to the solution in one portion, and the reaction mixture was let to reach 0 °C whilst stirring. Within 30 min, the reaction was quenched by addition of 10% aq NH_4Cl (50 mL) and extracted with ethyl acetate. The organic layer was washed with brine until neutral and dried (Na_2SO_4), and the solvents were evaporated in vacuo. The residue was purified by chromatography on silica gel (hexane/ethyl acetate, 99:1) afforded the pure, yellow oily title compound **2** (0.14 g, yield 95%). ^{1}H NMR (400 MHz): δ 0.88 (t, *J*=6.7, 3H, H-17), 1.26-1.30 (s, 26H, H-alifatici), 1.35-1.55 (m, 11H, H-3 and OtBu), 2.37 (dd, *J*=5.5, *J*=12.4, 1H, H-3'a), 2.53-2.60 (m, 1H, H-2), 2.76 (dd, *J*=9.1, *J*=12.4, 1H, H-3'b), 3.43 (d, *J*=13.6, 1H, 2xC$H_a$$H_b$-Ph), 3.65 (d, *J*=13.6, 1H, 2xC$H_a$$H_b$-Ph), 7.18-7.35 (m, 10H, H-Ar) ^{13}C NMR (50 MHz): δ 14.5, 23.1-32.3, 45.7, 56.9, 58.8, 80.3, 127.2, 128.5, 129.3, 139.7, 175.3. Anal. Calcd. for $C_{36}H_{57}NO_2$: C 80.69, H 10.72, N 2.61. Found C 80.71, H 10.74, N 2.63.
Under the same conditions, the mono-alkylated **29** was also obtained.

Methyl 2-((dibenzylamino)methyl)heptadecanoate (29)

Oil, (3h, yield 60%). ^{1}H NMR (300 MHz): δ 0.78 (m, 3H, H-17), 1.10-1.26 (m, 26H), 1.28-1.41 (m, 2H, H-3), 2.38 (dd, 1H, *J*=4.3Hz, *J*=11.5, H-3'a), 2.54-2.72 (m, 2H, H-2 and H-3'b), 3.30 (d, *J*=13.4, 2H, 2x-C$H_a$$H_b$Ph), 3.53 (s, 3H, OMe), 3.58 (d, *J*=13.4, 2H, 2xC$H_a$$H_b$Ph), 7.05-7.30 (m, 10H, H-Ar). ^{13}C NMR (75 MHz): δ 13.9, 22.5-31.7, 44.7, 51.1, 56.1, 58.2, 126.7, 127.9, 128.8, 139.1, 175.6.
Under the same condition, but using KHMDS as base and the equivalents reported in Table 5.11 (Chapter 5), the dialkylated products **27** was obtained.

tert-Butyl 2-((dibenzylamino)methyl)-2-pentadecylheptadecanoate (27)

Oil, (Total yield 99%, di-alkylation percentage 75%). ^{1}H NMR (400 MHz): δ 0.88 (t, *J*=6.8, 6H, 2x H-17), 1.18-1.34 (m, 52H), 1.39-1.60 (m, 13H, 2x H-3 and OtBu), 2.68 (s, 2H, H-3'), 3.48 (s, 4H, 2xCH_2-Ph), 7.18-7.35 (m, 10H, H-Ar). ^{13}C NMR (50 MHz): δ 14.1, 22.7-32.6, 50.2, 58.8, 60.4, 80.0, 127.0, 128.1, 129.2, 139.6, 176.8.
Under the same condition, but using KHMDS as base and the equivalents reported in Table 5.10 (Chapter 5), the dialkylated products **30** was obtained.

Methyl 2-((dibenzylamino)methyl)-2-pentadecylheptadecanoate (30)

Oil, (Total yield 81%, dialkylation percentage 60%). ^{1}H NMR (400 MHz): δ 0.87 (t, *J*=6.8, 6H, 2x H-17), 1.10-1.35 (m, 52H), 1.40-1.60 (m, 4H, 2x H-3), 2.72 (s, 2H, H-3'), 3.45 (s, 4H, 2xCH_2-Ph), 3.58 (s, 3H, OMe), 7.18-7.33 (m, 10H, H-Ar). ^{13}C NMR (50 MHz): δ 14.5, 23.1-32.7, 50.5, 51.8, 59.4, 57.7, 59.5, 127.3, 128.5, 129.6, 139.9, 178.4.

Methyl 2-((bis(4-methoxybenzyl)amino)methyl)heptadecanoate (20)

Oil. ^{1}H NMR (500 MHz): δ 0.95 (t, 3H, *J*=6.6, H-17), 1.10-1.36 (m, 26H), 1.37-1.56 (m, 2H, H-3), 2.41 (dd, 1H, *J*=4.4, *J*=11.2, H-3'a), 2.65-2.76 (m, 2H, H-2 and H-3'b), 3.31 (d, *J*=13.4, 2H, 2xMpm-C$H_a$$H_b$), 3.60 (d, *J*=13.4, 2H, 2xCH_2-Mpm), 3.65 (s, 3H, OMe), 3.80 (s, 6H, 2xOMe-MPM), 6.85 (d, *J*=8.3, 4H, 2xH-2 and H-6-MPM), 7.20 (d, *J*=8.3, 4H, 2xH-3 and H-5-Mpm). ^{13}C NMR (125 MHz): δ 14,6, 23.1, 27.7, 30.7, 32.2, 45.0, 51.4, 55.3, 56.0, 57.6, 112.9, 129.3, 130.6, 157.6, 174.7.

Methyl 2-((bis(4-methoxybenzyl)amino)methyl)-2-pentadecylheptadecanoate (21)

Oil. ^{1}H NMR (500 MHz): δ 0.90 (t, *J*=6.8, 6H, 2x H-17), 1.10-1.40 (m, 52H), 1.41-1.60 (m, 4H, 2x H-3), 2.64 (s, 2H, H-3'), 3.36 (s, 4H, 2xCH_2-Mpm), 3.60 (s, 3H, OMe), 3.80 (s, 6H, 2xOMe-Mpm), 6.82 (d, *J*=8.7, 4H, 2xH-2 and H-6-Mpm), 7.20 (d, *J*=8.7, 4H, 2xH-3 and H-5-Mpm).
^{13}C NMR (200 MHz): δ 14.1, 22.6-32.4, 49.8, 51.6, 55.2, 57.7, 58.1, 113.7, 129.5, 130.9, 159.0, 177.5.

Deprotection Reactions

N-Deprotection

Methyl 2-(aminomethyl)heptadecanoate (22)

A magnetically stirred solution of **26** (0.61 mmol, 300 mg) in glacial AcOH (3.1 mL) was hydrogenolysed over 30% Pd/C catalyst (90 mg) for 2 h at 50 °C, under a slightly positive pressure given by an inflated balloon (~3 bar). The mixture was then filtered trough Celite® and washed with MeOH (100 mL). The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (2x100 mL). The organic layer was washed with 10% aq Na_2CO_3 (300 mL), dried (Na_2SO_4),

and the solvent evaporated in vacuo to give the pure, title compound **22**, as dark oil (172 mg, yield 90%). ^{1}H NMR (400 MHz): δ 0.89 (t, *J*= 6.5, 3H, H-17), 1.23-1.37 [m, 26 H, $(CH_2)_{13}$], 1.39-1.50 (m, 1H, H-3), 1.52-1.63 (m, 1H, H-3b), 2.41-2.47 (m, 1H, H-2), 2.82 (dd, *J*=4.7, *J*=12.8, 1H, H-3'a), 2.92 (dd, *J*=8.3, *J*=12.8, 1H, H-3'b), 3.71 (s, 3H, OMe). ^{13}C NMR (100 MHz): δ 14.5, 23.1, 27.7, 29.5-30.5, 32.3, 44.4, 49.5, 51.9, 176.3.
Under the same conditions, the dialkylated product **36** was also obtained.

tert-Butyl 2-(aminomethyl)-2-pentadecylheptadecanoate (36)

Yellow solid (yield 91%). Mp 46.5-47.8 (from CH_3CN). ^{1}H NMR (400 MHz): δ 1.10-1.40 [m, 52 H, $2x(CH_2)_{13}$], 1.45 (s, 9H, OtBu), 1.46-1.62 (m, 4H, H-3, H-3"), 2.90 (s, 2H, H-3'). ^{13}C NMR: δ 14.1, 16.9, 22.6, 23.8, 28.0, 29.3, 29.4, 29.7-31.9, 33.4, 45.3, 48.1, 80.7, 177.9.

Hydrolysis of methyl ester and Boc- protection of amino function

2-((*tert*-Butoxycarbonylamino)methyl)heptadecanoic acid (23)

To a solution of compound **22** (96 mg; 0.31 mmol) in MeOH (1.8 mL), NaOH (52mg; 1.3 mmol) in H_2O (1 mL) was added. The reaction mixture was kept at room temperature and magnetic stirring for 12h. The solvent was then removed under reduced pressure; the residue was then diluted with H_2O (7 mL) and Boc_2O (135 mg; 0.62mmol) in CH_3CN (2 mL). The reaction mixture was kept for other 12 h at room temperature and under magnetic stirring. Thus the solvent was removed under reduced pressure and the residue was dissolved in EtOAc (2x50 mL). The organic layer was washed with iced 0.1 M HCl (2x25 mL), dried (Na_2SO_4), and the solvent evaporated in vacuo to give a crude whose purification by chromatography afford to the pure, compound **23** (90 mg; yield 73%). Crystalline white solid. Mp 73.2-74.3 (from CH_3CN). ^{1}H NMR (400 MHZ) (Mixture of conformers): δ 0.85 (bt, 3H, H-17), 1.23-1.78 [m, 37H, $(CH_2)_{14}$, Boc], 2.62 (bs, 1H, H-2), 3.10-3.50 (m, 2H, H-3'), 5.00 (bs, 1H, NH). ^{13}C NMR (100 MHz): δ (The minor conformer is labelled with *) 14.1, 22.7, 27.0, 28.3, 29.4-29.7, 31.9, 41.4, 42.7*, 45.6, 79.5, 81.0*, 155.9, 158.0*, 179.0*, 180.4.

Hydrolysis of *tert*-butyl ester and Boc- protection of the amino function

2-((dibenzylamino)methyl)-2-pentadecylheptadecanoic acid (37)

To a solution of compound **27** (314 mg; 0.42 mmol) in CH_2Cl_2 (2 mL) was added TFA (1 mL) and the reaction was kept at room temperature for 30 min. The solvent is then removed in vacuo and the residue diluted with EtOAc. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvent evaporated in vacuo to give a yellow solid. Its recrystallization from CH_3CN afforded the pure title compound **37** (290 mg; yield >99%). Mp 38.0-39.0. ^{1}H NMR (400 MHz): δ 0.88 (t, *J*=6.6, 6H, H-17, H-17"), 1.10-1.36 [m, 52H, $2x(CH_2)_{13}$], 1.38-1.62 (m, 4H, H-3, H-3"), 2.87 (s, 2H, H-3'), 3.79 (s, 4H, $2xCH_2Ph$), 7.28-7.41(m, 10H, H-Ar). ^{13}C NMR (75 MHz): δ 13.8, 22.4, 23.5, 29.1-29.9, 31.7, 35.5, 48.0, 56.9, 59.0, 60.1, 127.9, 128.6, 129.5, 135.5, 178.1.

2-((*tert*-Butoxycarbonylamino)methyl)-2-pentadecylheptadecanoic acid (38)

Under the same conditions used to deprotect **26** and **27**, the compound **37** (180 mg; 0.21 mmol) was also deprotected. The residue coming from the debenzylation of **37** (162 mg) was in turn protected as Boc derivative **38**. Thus, the crude was diluted with dioxane (3.0 mL); to this solution, at 0 °C, Et_3N (0.06 mL; 0.27 mmol) and Boc_2O (128 mg; 0.78 mmol) were added in sequence. The reaction mixture was warmed up to room temperature and stirred for 12 h; then the solvent was removed in vacuo and the residue diluted with EtOAc. The organic layer was washed with aq NH_4Cl and then brine until neutral, dried (Na_2SO_4), and the solvent evaporated in vacuo to give a oil. Its chromatography on silica gel (petroleum ether/Et_2O) afforded the pure title compound **38** (122 mg; yield >95%). Oil. ^{1}H NMR (200 MHz): δ 0.87 (bt, 6H, H-17, H-17"), 1.10-1.38 [m, 52H $2x(CH_2)_{13}$], 1.39-1.60 (m, 13H, H-3, H-3", OtBu), 3.26 (d, *J*=6.2, 2H, H-3'), 4.79 (t, *J*=6.2, 1H, NH). ^{13}C NMR (50 MHz): δ 14.1, 22.7, 23.9, 28.0-29.7, 30.2, 31.9, 34.2, 43.8, 50.2, 80.5, 155.9, 181.0.

Peptide Synthesis

Preparation of Tripeptide 34

Coupling product from the reaction between the compounds 22 and 31.

Methyl 2-((3-(tert-butoxycarbonylamino)propanamido)methyl)heptadecanoate (32)

To a solution of triphenylphosphine (0.15 mmol, 38.5 mg) in anhydrous CH_2Cl_2 (5 mL) at room temperature, under inert atmosphere of N_2 and under magnetic stirring, solid I_2 is added (0.15 mmol,

37.3 mg) in one portion. After 15 minutes, when the colour of the solution changes from violet to orange, solid imidazole is added (0.47 mmol, 32 mg) and the colour changes again to yellow. After 15 minutes, compound **31** (0.12 mmol, 23.0 mg), is finally added in one portion to the suspension, and the mixture goes at room temperature until the end of the starting acid (~4 h, TLC $CHCl_3$:CH_3OH 98:2). At that point, the compound **22** (0.15 mmol, 46.0 mg) is added and the reaction goes for 12h at room temperature. Then the solvent is removed under reduced pressure and the residue, diluted in EtOAc (10 mL), is washed with 10% aq. $Na_2S_20_3$ (2x10 mL). The organic layer is then washed with brine, dried (Na_2SO_4), and evaporated in vacuo to afford a crude reaction product whose chromatography on silica gel ($CHCl_3$) gave the pure **32** (47.2 mg, yield 80%) white solid. Mp 59.5-62.5 (from CH_3CN). ^{1}H NMR (500 MHz): δ 0.84 (t, *J*=6.5, 3H, H-17), 1.02-1.80 [m, 37H, $(CH_2)_{14}$, Boc], 2.27- 2.35 (m, 2H, H-6'), 2.55-2.65 (m, 1H, H-2), 3.28-3.42 (m, 3H, H-3'a, H-7'), 3.47-3.57 (m, 1H, H-3'b), 3.70 (s, 3H, OMe), 5.18 (s, 1H, NHBoc), 6.02 (s, 1H, NH). ^{13}C NMR (125 MHz): δ 14.3, 22.9, 27.3, 28.6- 29.9, 32.1, 36.5, 36.9, 40.5, 40.6, 45.2, 45.4, 52.0, 52.2, 76.5, 156.3, 171.7, 175.9.

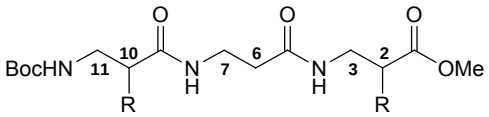
Deprotection of 32.

Methyl 2-((3-aminopropanamido)methyl)heptadecanoate (33)

The same conditions used to obtaine **37** were also used to obtaine **33** (yield >99%). Mp 94.0-96.2 (from MeOH/Et_2O). ^{1}H NMR (500 MHz, CD_3OD): δ 0.90 (t, *J*=6.6, 3H, H-17), 1.21-1.42 [m, 26H, $(CH_2)_{13}$], 1.43-1.54 (m, 1H, H-3a), 1.55-1.65 (m, 1H, H-3b), 2.50-2.70 (m, 3H, H-2, H-6'), 3.16 (t, *J*=6.3, 2H, H-7'), 3.30-3.36 (m, 1H, H-3'a), 3.39 (dd, *J*=5.4, *J*=13.7, 1H, H-3'b). ^{13}C NMR (50 MHz, CD_3OD): δ 14.3, 23.6, 28.1, 30.4-30.9, 32.6, 33.0, 37.1, 42.0, 46.7, 52.3, 172.4, 176.6.

Coupling product from the reaction between the compounds 33 and 23

Methyl 2,2-dimethyl-4,8,12-trioxo-7-pentadecyl-3-oxa-5,9,13-triazatriacontane-15-carboxylate (34)

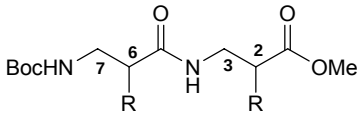


It was followed the same procedure used to prepare **32** (55.1 mg; yield 80%). Mp 86.9-89.2 (from CH_2Cl_2). ^{1}H NMR (500 MHz): δ 0.83 (t, *J*=6.9, 6H, 2xCH_3), 0.98-1.97 [m, 65H, 2x$(CH_2)_{14}$, Boc], 2.20-2.40 (m, 3H, H-2), 2.45-2.65 (m, 1H, H-10), 3.10-3.60 (m, 6H, H-3, H-7, H-11), 3.68 (s, 3H, OMe), 5.10 (bs, 1H, NH), 6.30 (bd, 1H, NH), 6.50 (bd, 1H, NH). 13C NMR (75 MHZ): d 14.1, 22.6, 27.1, 27.3, 28.4, 29.3-30.1, 31.9, 35.5, 35.9, 40.4, 42.5, 45.2, 47.2, 51.9, 79.2, 156.2, 171.7, 174.6, 175.7. Mass (ESI+): M/z 765 (M+) for $C_{45}H_{87}N_3O_6$.

Preparation of Dipeptide 35

Coupling product from the reaction between the compounds 22 and 23

Methyl 2-((2-((tert-butoxycarbonylamino)methyl)heptadecanamido)methyl)heptadecanoate (35)

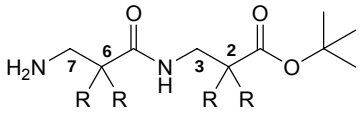


Mp 80.0-82.1 (form CH_3CN). ^{1}H NMR (400 MHz): δ 0.89 (t, *J*=6.9, 6H, 2XCH_3), 1.17-1.72 [m, 65H, 2x$(CH_2)_{14}$, Boc], 3.17 (dd, *J*=8.6, *J*=13.7, 1H, H-7a), 3.25-3.42 (m, 2H, H-7b, H-3a), 3.47-3.60 (m, 1H, H-3b), 3.71 (s, 3H, OMe), 4.81-5.10 (m, 1H, NHBoc), 5.95-6.05 (m, 1H, NH). ^{13}C NMR (100 MHz): δ 14.1, 22.7, 27.1, 27.3, 28.4, 29.3, 29.4-30.2, 31.9, 40.2, 42.7, 44.9, 47.5, 51.8, 79.2, 156.1, 174.6, 175.7.

Preparation of Dipeptide 39

Coupling product from the reaction between the compounds 36 and 38

***tert*-Butyl 2-((2-(aminomethyl)-2-pentadecylheptadecanamido)methyl)-2-pentadecylheptadecanoate (39)**

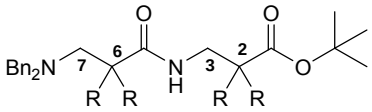


To a solution of **38** (96 mg, 0.16 mmol) in anhydrous CH_2Cl_2 (2.5 mL) at rt was added oxalyl chloride (0.07 µL, 0.8 mmol) and DMF [1 drop (0.0016 mmol)]. After stirring for 1 h, the reaction mixture was concentrated under reduced pressure and put under high vacuum for 15 min. The residual yellow oil was dissolved in CH_2Cl_2 (4 mL) and at rt was added DMAP (cat.), TEA (45 µL; 0. 83 mmol) and **36** (83 mg; 16 mmol). After stirring for 5 h, the reaction mixture was washed with brine. The organic layer was separated and the aqueous layer was extracted with CH_2Cl_2 (3x). The combined organic layers were dried (Na_2SO_4) and concentrated under reduced pressure. Purification of the crude product by FC (petroleum ether/Et_2O 100:0 → 95:5) afforded dipeptide **39** (31 mg, yield 69%) as a white solid. ^{1}H NMR (500 MHz): δ 0.84 (t, *J*=6.3, 12H, 4xCH_3), 1.10-1.73 [m, 121H, 4x(CH2)14, OtBu], 3.38 (s, 2H, H-7), 3.48 (d, *J*=6.3, 2H, H-3), 6.87 (t, *J*=6.3, 1H, NH). ^{13}C NMR (125 MHz): δ 14.0, 26.6, 22.6, 23.9, 24.3, 28.0, 29.3-30.0, 31.8, 32.6, 34.2, 42.6, 47.9, 49.9, 58.1, 80.5, 175.7, 176.2. Mass (HiResMALDI): 1058.092 (M+1) for $C_{70}H_{140}N_2O_3$.
The same product can be prepared with a yield of 96% from **40**, following the same procedure reported to have **36.**

Preparation of Tetrapeptide 42

Coupling product from the reaction between the compounds 36 and 37

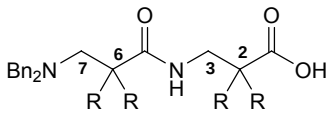
***tert*-Butyl 2-((2-((dibenzylamino)methyl)-2-pentadecylheptadecanamido)methyl)-2-pentadecylheptadecanoate (40)**



The same conditions used to prepare the dipeptide **39** were also used to obtaine the dipeptide **40** (yield >99%). Mp 36.8-37.5 (from CH_3CN). ^{1}H NMR (500 MHz): δ 0.88 (t, *J*=7.0, 12H, 4xCH_3), 1.02-1.60 [m, 121H, 4x$(CH_2)_{14}$, OtBu], 2.64 (s, 2H, H-7), 3.27 (d, *J*=5.8, 2H, H-3), 3.48 (s, 4H, H-Bn), 6.55 (t, *J*=5.8, 1H, NH), 7.18-7.34 (m, 10H, H-Ar). ^{13}C NMR (125 MHz): δ 14.0, 22.6, 23.3, 23.8, 28.0, 29.3, 29.4, 29.5, 29.6-29.7, 30.2, 30.7, 31.8, 32.5, 34.2, 42.2, 49.1, 49.7, 59.2, 59.5, 80.5, 126.7, 128.0, 129.1, 139.3, 175.7, 176.8. Mass (HiResMALDI): 1238.185 (M+1) for $C_{84}H_{152}N_2O_3$.

Hydrolysis of *tert*-butyl ester

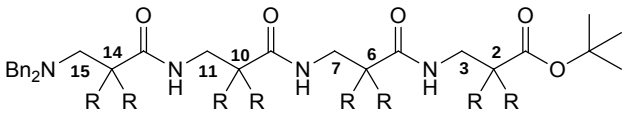
2-((2-((Dibenzylamino)methyl)-2-pentadecylheptadecanamido)methyl)-2-pentadecylheptadecanoic acid (41)



The same procedure to have **37** was used to prepare **41** (yield >99%). Mp 62.9-64.7 (from CH_2Cl_2). ^{1}H NMR (300 MHz): δ 0.84 (t, *J*=6.6, 12H, 4xCH_3), 1.00-2.00 [m, 112H, 4x$(CH_2)_{14}$], 2.40-3.80 (m, 6H, H-3, H-7, H-Bn), 7.03-7.43 (m, 10H, H-Ar). ^{13}C NMR (75 MHz): δ 14.1, 22.6, 23.9, 29.3-31.9, 42.6, 45.8, 46.9, 49.2, 59.2, 127.3, 128.5, 128.8, 135.5, 172.0, 181.0. Mass (HiResMALDI): 1182,123 (M+1) for $C_{80}H_{144}N_2O_3$.

Coupling product from the reaction between the compounds 39 and 41

***tert*-Butyl 2-benzyl-5,9,13-trioxo-4,4,8,8,12,12,16,16-octapentadecyl-1-phenyl-2,6,10,14-tetraazaheptadecan-17-oate (42)**



The same coupling procedure used to prepare **40** was also used to prepare **42** (yield 25%). Mp 62.8-64.8 (from CH_3CN). ^{1}H NMR (300 MHz): δ 0.87 (t, *J*=6.6, 24H, 8xCH_3), 1.05-1.60 [m, 224H, 8x$(CH_2)_{14}$, OtBu], 2.64 (bs, 2H, H-15), 3.23-3.30 (m, 2H, H-3), 3.31-3.40 (m, 4H, H-7, H-11), 3.41-3.48 (m, 4H, H-Bn), 6.38-6.46 (m, 2H, NH), 7.04-7.50 (m, 11H, NH, H-Ar). ^{13}C NMR (75 MHz): δ 14.1, 22.7, 23.5, 24.0, 28.0, 29.3-31.9, 32.6, 34.6, 34.8, 42.6, 43.3, 48.3, 48.5, 49.1, 49.7, 59.2, 59.5, 80.9, 126.7, 128.1, 129.3, 139.5, 176.1, 176.5, 177.1. Mass (HiResMALDI): 2222.195 (M+1) for $C_{150}H_{282}N_4O_5$.

Synthesis of Aspartame Analogues

Synthesis of *anti*-2-Hydroxy-β^3-Phenylalanine Methyl Ester

Introduction of hydroxyl group

(2*S*,3*S*)-methyl 3-(dibenzylamino)-2-hydroxy-4-phenylbutanoate (13)

To a magnetically stirred solution of **12** (0.54 mmol, 200 mg) in anhydrous THF (4 mL), 0.5 M KHDMS in THF (2.14 mL, 1.07 mmol) was added dropwise, at -78 °C and under anhydrous argon. After 1 h, solid 2-[(4-methylphenyl)sulfonyl]-3-phenyloxaziridine (0.9 mmol, 264 mg) was added to the solution in one portion, and the reaction mixture was kept at -78 °C whilst stirring. Within 30 min, the reaction was quenched by addition of 10% aq NH_4Cl (200 mL) and extracted with ethyl acetate. The organic layer was washed with brine until neutral and dried (Na_2SO_4), and the solvents were evaporated in vacuo. The residue (470 mg) was purified by chromatography on silica gel (hexane/ethyl acetate, 8:2) afforded the pure, oily title compound **13** (130 mg, yield 63%[2]; d.r. 99:1). $[\alpha]_D^{25}$ +17.8 (*c* 0.5). ^{1}H NMR (400 MHz): δ 2.87 (dd, *J*=7.3, *J*=14.0, 1H, H-4a), 3.08 (dd, *J*=7.3, *J*=14.0, 1H, H-4b), 3.49 (dt, *J*=2.6, *J*=7.3, 1H, H-3), 3.56 (s, 3H, OMe), 3.72 (d, *J* =14.0, 2H, Ha-Bn), 3.88 (d, *J*=14.0, 2H, Hb-Bn), 4.57 (d, *J*=2.6, 1H, H-2), 7.00-7.40 (m, 15H, H-Ar). ^{13}C NMR (125 MHz); δ 31.8, 52.6, 55.1, 62.7, 69.7, 126.6, 127.7, 128.4, 128.6, 129.4, 129.8, 174.7. Anal. Calcd. for $C_{25}H_{27}NO_3$: C 77.09, H 6.99, N 3.60. Found C 77.13, H 6.96, N 3.65.

Under the same conditions, the other α-substituted compounds were also obtained.

Protection of the hydroxyl group

(2*S*,3*S*)-methyl 2-(*tert*-butyldiphenylsilyloxy)-3-(dibenzylamino)-4-phenylbutanoate (14)

To a solution of **13** (0.32 mmol, 125 mg), in anhydrous THF (4 mL) at room temperature, imidazole (1.92 mmol, 130 mg) and DMAP (0.014 mmol, 2.2 mg) were added. After complete solubilization of imidazole, under inert atmosphere of N_2, *tert*-buthyldiphenilsilylcloride (TBDPSCl, 0.57 mmol, 156.5 mg), was added with formation of a white precipitate (imidazolium choride). The suspention was kept under magnetic stirring until the starting material was completely consumed (~6h, TLC). The solution, diluted with EtOAc (200 mL), was washed with 10% aq NH_4Cl (2 x 100 mL) and then with brine. The organic layer was finally dried (Na_2SO_4), and the solvent was evaporated under reduced pressure to afford a crude reaction product (664 mg), chromatography of which on silica gel (petroleum ether/ Et_2O, 95:5) gave the pure, oily title compound **14** (145 mg, yield 72%). $[\alpha]_D^{25}$ -43.32 (*c* 1.53). ^{1}H NMR (500 MHz): δ 1.10 (s, 9H, 3xCH_3), 3.00 (dd, *J*=9.8, *J*=14.6, 1H, H-4a), 3.16 (dd, *J*=3.9, *J*=14.6, 1H, H-4b), 3.22 (s, 3H, OMe), 3.41-3.45 (m, 1H, H-3), 3.48 (d, *J*=13.7, 2H, Ha-Bn), 3.65 (d, *J*=13.7, 2H, Hb-Bn), 4.56 (d, *J*=5.4, 1H, H-2), 7.02-7.75 (m, 25H, H-Ar). ^{13}C NMR (125 MHz): δ 19.8, 27.3, 33.4, 51.3, 54.6, 63.7, 73.4, 126.1, 126.9, 127.6, 127.8, 128.1, 128.4, 129.1, 129.9, 130.1, 136.2, 136.3, 139.6, 140.9, 172.8. Anal. Calcd. for $C_{42}H_{47}NO_3Si$: C 78.59, H 7.38, N 2.23. Found C 78.62, H 7.41, N 2.28.

Deprotection of the β-amino function

(2*S*,3*S*)-methyl 3-amino-2-(*tert*-butyldiphenylsilyloxy)-4-phenylbutanoate (15)

A magnetically stirred solution of **14** (0.23 mmol, 145 mg) in glacial AcOH (4.3 mL) was treated with H_2 over 30% Pd/C catalyst (37 mg) for 2 h at 50 °C, under a slightly positive pressure given by an inflated balloon (~3 bar). The mixture was then filtered through Celite® and washed with MeOH (30 mL). The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (2x25 mL). The organic layer was washed with 10% aq Na_2CO_3 (25 mL), dried (Na_2SO_4), and the solvents evaporated in vacuo to give the pure, oily title compound **15**, (102 mg, yield 99%). $[\alpha]_D^{25}$ -1.1 (*c* 0.2). ^{1}H NMR (400

[2] Total yield for both diastereoisomers.

MHz): δ 1.12 (s, 9H, CH_3-O^{t}Bu-TBDPS), 1.68 (bs, 2H, NH_2), 2.48 (dd, *J*=9.2, *J*=13.6, 1H, H-4a), 2.86 (dd, *J*=5.1, *J*=13.6, 1H, H-4b), 3.31 (m, 1H, H-3), 3.46 (s, 3H, OMe), 4.20 (d, *J*=4.3, 1H, H-2), 7.16-7.80 (m, 15H, 5xH-Ar, 10xH-Ar-TBDPS). ^{13}C NMR: δ 19.2, 26.7, 39.1, 51.2, 57.0, 76.3, 126.1, 127.3, 127.5, 128.2, 129.0, 129.6, 129.7, 132.7, 135.6, 135.7, 138.6, 171.5. Anal. Calcd. for $C_{27}H_{33}NO_3Si$: C 72.44, H 7.43, N 3.13. Found C 72.46, H 7.39, N 3.09.

Synthesis of *anti*-2-Amino β^3-Phenylalanine Methyl Ester

Introduction of amino function group

The procedures to prepare the compounds **16** and **17** have already been reported for the compounds **26b** and **27b** in Synthesis of *anti*-2,3-diamino acids.

Protection of the α-amino function

Under the same conditions used to prepare compound **30** in Synthesis of *anti*-2,3-diamino acids, compound **18** was also synthesised.

(2*S*,3*S*)-methyl 2-(*tert*-butoxycarbonylamino)-3-(dibenzylamino)-4-phenylbutanoate (18)

Chromatography on silica gel of the crude product in Etp/AcOEt = 95/5 gave the compound **18** (yield 80%) as pure, oily title compound. $[\alpha]_D^{25}$ +14.6 (*c* 0.6). ^{1}H NMR (500 MHz): δ 1.44 (s, 9H, CH_3-Boc), 2.80 (dd, *J*=7.3, *J*=13.9, 1H, H-4a), 3.07 (dd, *J*=6.8, *J*=13.9, 1H, H-4b), 3.33-3.38 (m, 1H, H-3), 3.63 (d, *J*=13.2, 2H, Ha-Bn), 3.63 (s, OMe), 3.71 (d, *J*=13.7, 2H, Hb-Bn), 4.61-4.64 (m, 1H, H-2), 5.09 (d, *J*=9.3, 2H, NH-Boc), 7.05-7.40 (m, 15H, H-Ar). ^{13}C NMR (125 MHz)**:** δ 28.6, 33.0, 52.3, 53.8, 54.1, 61.7, 80.00, 126.5, 127.3, 128.4, 128.6, 129.3, 129.6, 139.5, 155.3, 172.7. Anal. Calcd. for $C_{30}H_{36}N_2O_4$: C 73.74, H 7.43, N 5.73. Found C 73.72, H 7.40, N 5.70.

Deprotection of the β-amino function

Under the same conditions used to prepare compound **15**, compound **19** was also synthesised.

(2*S*,3*S*)-methyl 3-amino-2-(*tert*-butoxycarbonylamino)-4-phenylbutanoate (19)

Pure oil (yield 60%). $[\alpha]_D^{25}$ +21.9 (*c* 0.3). ^{1}H NMR (500 MHz)**:** δ 1.44 (s, 9H, CH_3-Boc), 2.52 (dd, *J*=9.8, *J*=14.2, 1H, H-4a), 2.91 (dd, *J*=3.9, *J*=14.2, 1H, H-4b), 3.23-3.38 (m, 1H, H-3), 3.76 (s, OMe), 4.38 (m, 1H, H-2), 5.45-5.55 (m, 1H, NH-Boc), 7.18-7.36 (m, 5H, H-Ar). ^{13}C NMR (125 MHz)**:** δ 28.6, 41.1, 52.5, 55.6, 58.4, 80.34, 126.6, 128.9, 129.5, 138.5, 155.8, 171.9. Anal. Calcd. for $C_{16}H_{24}N_2O_4$: C 62.32, H 7.84, N 9.08. Found C 62.32, H 7.84, N 9.05.

Synthesis of *anti*-2-Substituted-β^3-Phenylalanine Methyl Ester

Introduction of alkyl groups.

The same procedure of the hetero-substitution was followed, starting from compound **12**, 2 eq of KHMDS and 2.2 eq of CH_3I (or BnBr) as electrophile.

(2*S*,3*S*)-methyl 3-(dibenzylamino)-2-methyl-4-phenylbutanoate (20)

Purified by flash chromatography (silica gel; Hexane /Et_2O = 97:3); d.r. 93:7; compound **20:** (144 mg, yield 97%). Oil; $[\alpha]_D^{25}$ -6.93 (*c* 0.2) ^{1}H NMR (300 MHz) :δ 0.94 (d, *J*=6.9, 3H, CH_3), 2.68 (dd, *J*=6.9, *J*=14.3, 1H, H-4a), 2.72-2.84 (m, 1H, H-2), 3.04 (dd, *J*=6.04, *J*=14.3, 1H, H-4b), 3.21 (m, 1H, H-2), 3.42 (d, *J*=13.6, 2H, Ha-Bn), 3.54 (s, 3H, OMe), 3.70 (d, *J*=13.6, 2H, Hb-Bn), 7.00-7.30 (m, 15H, H-Ar). ^{13}C NMR (75 MHz): δ 15.3, 33.2, 42.7, 51.2, 54.1, 62.2, 125.8, 126.7, 127.8, 128.2, 129.0, 192.2, 139.4, 140.7, 175.6. Anal. Calcd. for $C_{26}H_{29}NO_2$: C 80.59, H 7.54, N 3.61. Found C 80.62, H 7.58, N 3.59.

(2*S*,3*S*)-methyl 2-benzyl-3-(dibenzylamino)-4-phenylbutanoate (21)

Purified by flash chromatography (silica gel; Hexane /Et_2O = 98:2); d.r. 9:1; compound **21**: (0.477 mmol, 220 mg, yield 90%). Oil; $[\alpha]_D^{25}$ -58.9 (*c* 0.20). ^{1}H NMR (400 MHz): δ 2.61 (dd, *J*=4.6, *J*=13.7, 1H, H-4a), 2.65-2.75 (m, 1H, H-4b) 2.87 (dd, *J*=7.4, *J*=14.3, 1H, C*Ha*HbPh), 3.04-3.09 (m,1H, H-2), 3.25 (dd, *J*=5.5, *J*=14.3, 1H, CHa*Hb*Ph), 3.36-3.41 (m, 1H, H-3), 3.49 (s, 3H, OMe), 3.52 (d, *J*=13.6, 2H, Ha-Bn), 3.86 (d, *J*=13.6, 1H, Hb-Bn), 6.90-7.40 (m, 20H, H-Ar). ^{13}C NMR (125 MHz): δ 29.7, 34.1, 42.5, 51.3, 52.9, 54.2, 126.3, 128.4, 128.5, 128.8, 129.2, 138.6, 174.5. Anal. Calcd. for $C_{32}H_{33}NO_2$: C 82.90, H 7.17, N 3.02. Found C 82.88, H 7.15, N 3.04.

Deprotection of the β-amino function

Under the same conditions used to prepare compound **15**, compound **22** and **23** were also synthesised.

(2*S*,3*S*)-methyl 3-amino-2-methyl-4-phenylbutanoate (22)

Pure oil (42 mg, yield 88%). $[\alpha]_D^{25}$ -14.7 (*c* 0.2). ^{1}H NMR (500 MHz)**:** δ 1.30 (d, *J*=7.9, 3H, CH_3), 2.48 (dd, *J*=2.4, *J*=13.2, 1H, H-4a), 2.51-2.59 (m, 1H, H-2), 2.95 (dd, *J*=4.4, *J*=13.2, 1H, H-4b), 3.16-3.22 (m, 1H, H-3), 3.73 (s, 3H, OMe), 7.20-7.38 (m, 5H, H-Ar). ^{13}C NMR (125 MHz)**:** δ 14.3, 40.7, 44.5, 51t.7, 55.3, 126.6, 128.6,129.3,138.4, 175.6. Anal. Calcd. for $C_{12}H_{17}NO_2$: C 69.54, H 8.27, N 6.76. Found C69.58, H 8.27, N 6.73.

(2*S*,3*S*)-methyl 3-amino-2-benzyl-4-phenylbutanoate (23)

Pure oil (10 mg, yield 49%). $[\alpha]_D^{25}$ -17.1 (*c* 0.7). ^{1}H NMR (500 MHz)**:** δ 2.58-2.90 (m, 2H, NH_2), 2.61 (bs, 1H, H-4a), 2.75-2.83 (m, 1H, C*Ha*HbPh), 2.90-3.15 (m, 3H, H-2, H-4b, CHa*Hb*Ph), 3.18-3.26 (m, 1H, H-3), 3.72 (s, 3H, OMe), 7.12-7.33 (m, 5H, H-Ar). ^{13}C NMR (125 MHz): δ 29.9, 34.3, 35.9, 51.7, 54.5, 126.6, 126.7, 128.6, 128.7, 129.0, 129.5, 138.8, 139.2, 174.7. Anal. Calcd. for $C_{18}H_{21}NO_2$: C 76.26, H 7.47, N 4.94. Found C 76.34, H 7.44, N 4.90.

Peptide Synthesis

Coupling product from the reaction of the compounds 24 with 15. General Procedure

(*S*)-*tert*-butyl 3-(*tert*-butoxycarbonylamino)-4-((2*S*,3*S*)-3-(*tert*-butyldiphenylsilyloxy)-4-methoxy-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoate (25)

To a solution of triphenylphosphine (0.38 mmol, 99.0 mg) in anhydrous CH_2Cl_2 (1 mL) at room temperature, under inert atmosphere of N_2 and under magnetic stirring, solid I_2 is added (0.38 mmol, 96.0 mg) in one portion. After 15 minutes, when the colour of the solution changes from violet to orange, solid imidazole is added (0.65 mmol, 44 mg) and the colour changes again to yellow. After 15 minutes, compound **24** (0.32 mmol, 93.7 mg), is finally added in one portion to the suspension, and the mixture runs at room temperature until the end of the starting acid (~1 h, TLC $CHCl_3$:CH_3OH 98:2). The compound **15** (0.22 mmol, 97 mg) is then added and the reaction was allowed to react for 12h at room temperature. Then the solvent is removed under reduced pressure and the residue, diluted with EtOAc (10 mL), is washed with 10% aq. $Na_2S_2O_3$ (2x10 mL). The organic layer is then washed with brine, dried (Na_2SO_4), and evaporated in vacuo to afford a crude reaction product whose chromatography on silica gel (petroleum ether/EtOAc, 90:10) gave the pure **25** (80 mg, yield 72%) as colourless oil. $[\alpha]_D^{25}$ -25.3 (*c* 0.6). ^{1}H NMR (400 MHz)**:** δ 1.13 (s, 9H, CH_3-OtBu-TBDPS), 1.43 (s, 9H, CH_3-Boc), 1.47 (s, 9H, CH_3-O^{t}Bu-$CO_2{}^t$Bu), 2.51 (dd, *J*=5.1, *J*=16.6, 1H, H-3'a), 2.70 (dd, *J*=5.5, *J*=16.6, 1H, H-3'b), 2.86 (d, *J*=7.8, 2H, H-4), 3.38 (s, 3H, OMe), 4.23-4.39 (m, 2H, H-2, H-2'), 4.50 (ddd, *J*=3.8, *J*=7.8, *J*=8.8, 1H, H-3), 5.40-5.46 (m, 1H, NHBoc), 6.91 (d, *J*=8.8, 1H, NHCO), 7.09-7.65 (m, 15H, H-Ar). ^{13}C NMR (125 MHz)**:** δ 19.9, 27.3, 28.4, 28.7, 36.5, 37.3, 51.1, 52.1, 54.2, 73.8, 80.6, 81.9, 126.9, 127.9, 128.2, 128.8, 129.6, 130.2, 132.8, 133.2, 136.3, 137.6, 155.8, 170.9, 171.6, 172.5. Anal. Calcd. for $C_{40}H_{54}N_2O_8Si$: C, 66.82; H, 7.57; N, 3.90. Found C 66.77, H 7.53, N 3.89.

Coupling product from the reaction of the compounds 24 with 19

(*S*)-*tert*-butyl 3-(*tert*-butoxycarbonylamino)-4-((2*S*,3*S*)-3-(*tert*-butoxycarbonylamino)-4-methoxy-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoate (27)

Chromatography on silica gel of the crude product (petroleum ether/EtOAc, 95:5) gave the pure **27** (yield 80%) as colourless oil. $[\alpha]_D^{25}$ -0.7 (*c* 5.5). ^{1}H NMR (500 MHz)**:** δ 1.38 (s, 9H, Boc), 1.44 (s, 18H, 2xCH_3-Boc), 2.50 (dd, *J*=5.9, *J*=16.6, 1H, H-3'a), 2.77-2.80 (m, 2H, H-4a, H-3'b), 2.86 (dd, *J*=6.3, *J*=13.7, 1H, H-4b), 3.59 (s, 3H, OCH_3), 4.32-4.40 (m, 1H, H-2), 4.40-4.50 (m, 1H, H-2'), 4.50-4.60 (m, 1H, H-3), 5.50-5.70 (m, 2H, 2 x NHBoc), 7.15-7.39 (m, 5H, H-Ar), 7.4 (m, 1H, NHCO). ^{13}C NMR (100 MHz)**:** δ 28.0, 37.3, 51.1, 52.4, 53.3, 55.7, 80.2, 81.6, 126.8, 128.4, 129.3, 136.7, 155.4, 155.8, 170.9, 170.9, 171.0. Anal. Calcd. for $C_{29}H_{45}N_3O_9$: C, 60.09; H, 7.82; N, 7.25. Found C 60.07, H 7.80, N 7.28.

Coupling product from the reaction of the compounds 24 with 22

(*S*)-*tert*-butyl 3-(*tert*-butoxycarbonylamino)-4-((2*S*,3*S*)-4-methoxy-3-methyl-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoate (28)

Chromatography on silica gel of the crude product (petroleum ether/EtOAc, 9:1) gave the pure **28** (yield 85%) as colourless oil. $[\alpha]_D^{25}$ -23.54 (*c* 0.3). ^{1}H NMR (400 MHz): δ 1.15 (d, *J*=7.2, 3H, CH_3), 1.43 (s, 3H, Boc), 1.45 (s, 9H, OtBu), 2.56-2.65 (m, 2H, H-2, H-3'a), 2.68 (dd, *J*=8.7, *J*=13.6, 1H, H-4a), 2.75-2.91 (m, 2H, H-3'b, H-4b), 3.72 (s, 3H, OMe), 4.20-4.28 (m, 1H, H-3), 4.40-4.48 (m, 1H, H-1'), 5.60 (bd, *J*=6.6, 1H, NHBoc), 7.18-7.31 (m, 5H, H-Ar), 7.44 (bd, *J*=9.5, 1H, NHCO). ^{13}C NMR (75 MHz): δ 15.1, 37.2, 39.7, 40.2, 46.1, 51.6, 52.8, 81.4, 126.4, 128.4, 129.1, 137.6, 170.6, 175.5. Anal. Calcd. for $C_{25}H_{38}N_2O_7$: C, 62.74; H, 8.00; N, 5.85. Found C 69.58, H 8.27, N 8.02.

Coupling product from the reaction of the compounds 24 with 23

(*S*)-*tert*-butyl 4-((2*S*,3*S*)-3-benzyl-4-methoxy-4-oxo-1-phenylbutan-2-ylamino)-3-(*tert*-butoxycarbonylamino)-4-oxobutanoate (29)

Chromatography on silica gel of the crude product (petroleum ether/EtOAc, 9:1) gave the pure **29** (yield 97%) as colourless oil. $[\alpha]_D^{25}$ -50.84 (*c* 0.5). ^{1}H NMR (400 MHz): δ 1.43 (s, 9H, Boc), 1.49 (s, 9H, OtBu), 2.61-2.98 (m, 7H, H-2, H-4, C*H*$_2$Ph, H-3'), 3.62 (s, 3H, OMe), 4.27-4.29 (m, 1H, H-3), 4.47-4.48 (m, 1H, H-2'), 5.60 (bd, 1H, NHBoc), 6.76-7.26 (m, 10H, H-Ar), 7.44 (bd, *J*=8.8, 1H, NHCO). ^{13}C NMR (100 MHz): δ 28.4,28.7, 36.6, 37.6, 40.6, 41.3, 48.6, 51.5, 52.0, 80.6, 81.9, 126.9, 128.8, 129.2, 129.6, 137.8, 138.6, 155.8, 171.0, 171.7, 174.5. Anal. Calcd. for $C_{31}H_{42}N_2O_7$: C 67.13, H 7.63, N 5.05. Found C 67.18, H 7.59, N 5.03.

Deprotection Reactions of Coupling Products

Deprotection of hydroxyl function of 25

(*S*)-*tert*-butyl 3-(*tert*-butoxycarbonylamino)-4-((2*S*,3*S*)-3-hydroxy-4-methoxy-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoate (26)

To a solution of **25** (0.152 mmol, 110 mg) in THF (2.5 mL), tetrabutylammoniumfluoride 1M (TBAF, 0.152 mmol, 0.138 mL) is added dropwise, keeping the solution at 0 °C under magnetic stirring. After 10 minutes the starting material is completely consumed (TLC: $CHCl_3/CH_3OH$ = 8:2); the solvent is then removed under reduced pressure and the residue, diluted with EtOAc (25 mL) and H_2O (25 mL), is washed with a saturated solution of $NaHCO_3$ and dried on Na_2SO_4. The crude product is finally purify on silica gel column in CH_2Cl_2→ CH_2Cl_2/MeOH (9:1), to give the product **26** (58 mg; yield 80 %) as a colourless oil. $[\alpha]_D^{25}$ -4.0 (*c* 0.4). ^{1}H NMR (400 MHz)**:** δ 1.45 (s, 9H, CH_3-Boc), 1.47 (s, 9H, CH_3-O^{t}Bu), 2.60 (dd, *J*=6.5, *J*=16.8, 1H, H-3'a), 2.78-2.84 (m, 1H, H-3'b), 2.85 (d, 2H, *J*=7.2, H-4), 3.59 (s, 3H, OMe), 4.28 (d, *J*=3.0, 1H, H-2), 4.35-4.45 (m, 1H, H-2'), 4.52-4.68 (m, 1H, H-3), 5.50-5.65 (m, 1H, NHBoc), 6.88 (d, *J*=7.9, 1H, NHCO); 7.18-7.35 (m, 5H, H-Ar). ^{13}C NMR**:** δ 28.4, 28.7, 35.9, 37.7, 51.3, 52.9, 53.8, 72.2, 80.8, 82.2, 127.1, 128.8, 129.8, 137.2, 155.9, 171.3, 171.5, 173.2. Anal. Calcd. for $C_{24}H_{36}N_2O_8$: C 59.98, H 7.55, N 5.83. Found C 59.94, H 7.50, N 5.81.

Removal of *tert*-butoxycarbonyl protections

(*S*)-3-amino-4-((2*S*,3*S*)-3-hydroxy-4-methoxy-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoic acid (4)

To a solution of compound **26** (0.121 mmol, 58.4mg) in CH_2Cl_2 (3 mL), at 0 °C under magnetic stirring, trifluoroacetic acid is added dropwise (TFA 1.5 mL). The reaction goes until all the starting material was consumed (TLC: $CHCl_3/CH_3OH$ = 8:2). The solvent is then evaporated under reduced pressure and the TFA is co-evaporated several times with CH_2Cl_2. The solid residue is diluted with acetone and recrystallized from Et_2O, giving the product **4** as a pure hygroscopic crystalline solid (31 mg, yield 80%). ^{1}H NMR (400 MHz)**:** δ 2.54 (dd, *J*=7.7, *J*=16.5, 1H, H-3'a), 2.79 (dd, *J*=4.3, *J*=16.5, 1H, H-3'b), 3.13 (dd, *J*=5.2, *J*=13.7, 1H, H-4a), 3.51 (dd, *J*=9.7, *J*=13.7, 1H, H-4b), 3.67 (s, 3H, OMe), 3.78-3.83 (m, 1H, H-3), 3.92 (dd, *J*=4.3, *J*=7.7, 1H, H-2'), 4.63 (d, *J*=3.4, 1H, H-2), 7.20-7.30 (m, 5H, H-Ar). ^{13}C NMR (100 MHz): δ 42.6 (2xC), 51.9, 52.2, 53.7, 63.3, 126.9, 128.1, 128.9, 129.7, 130.2, 136.0, 169.7, 170.1, 174.1. Anal. Calcd. for $C_{15}H_{20}N_2O_6$: C 55.55, H 6.22, N 8.64. Found C 55.59, H 6.17, N 8.68.

Under the same conditions, the following deprotected dipeptides were also obtained.

(*S*)-3-amino-4-((2*S*,3*S*)-3-amino-4-methoxy-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoic acid (5)

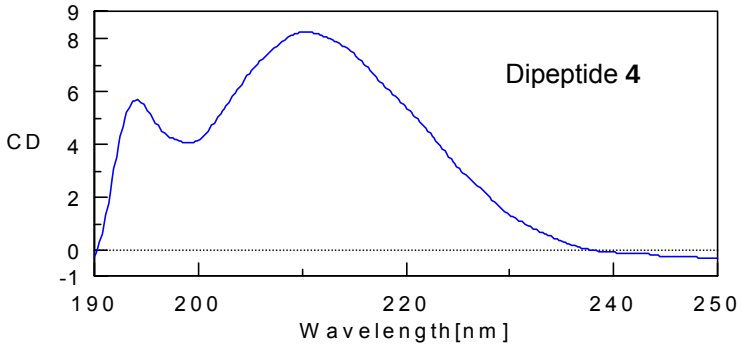
Dipeptide **5** (40 mg; yield 80%). Crystalline solid, mp 134.2-136.4 °C (from Acetone/Et_2O). $[\alpha]_D^{25}$ -13.9 (*c* 0.3, H_2O). ^{1}H NMR (400 MHz)**:** δ 2.76 (dd, *J*=5.1, *J*=17.7, 1H, H-3'a), 2.83 (dd, *J*=6.2, *J*=17.7, 1H, H-3b'), 2.98 (dd, *J*=10.1, *J*=14.1, 1H, H-4a), 3.14 (dd, *J*=5.9, *J*=14.1, 1H, H-4b), 3.86 (s, 3H, OMe), 4.06 (bt, *J*=5.5, 1H, H-2'), 4.31 (d, *J*= 2.6, 1H, H-2), 4.61-4.82 (m, 1H, H-3), 7.28-7.37 (m, 5H, H-Ar). ^{13}C NMR (100 MHz): δ 36.5, 36.6, 50.3, 52.1, 54.3, 56.6, 127.8, 129.4, 129.6, 136.7, 168.2, 170.5, 175.8. Anal. Calcd. for $C_{15}H_{21}N_3O_5$: C 55.72, H 6.55, N 13.00. Found C 55.77, H 6.59, N 13.03.

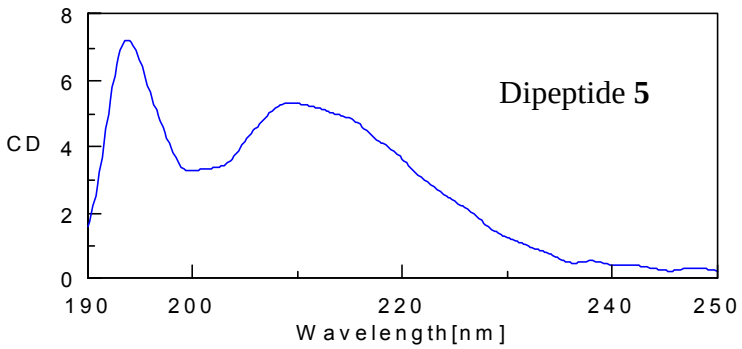
(*S*)-3-amino-4-((2*S*,3*S*)-4-methoxy-3-methyl-4-oxo-1-phenylbutan-2-ylamino)-4-oxobutanoic acid (6)

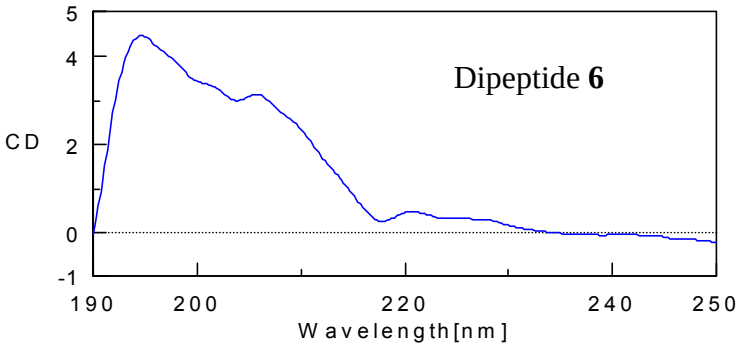
Dipeptide **6** (yield 46%). Crystalline solid, mp 91-93 °C (from Acetone/Et_2O). $[\alpha]_D^{25}$ -8.3 (*c* 0.1). ^{1}H NMR (400 MHz, D_2O)**:** δ 1.11 (d, *J*=7.0, 3H, CH_3), 2.57-2.70 (m, 4H, H-4a, H-3b, H-3'a, H-2), 2.93 (dd, *J*=4.6, *J*=14.1, 1H, H-4b), 3.57 (s, 3H, OMe), 3.92 (m, 1H, H-2'), 4.22 (m, 1H, H-3), 7.11-7.86 (m, 5H, H-Ar) ^{13}C NMR (100 MHz, D_2O): δ 13.8, 37.5, 44.0, 44.1, 50.4, 52.8, 53.8, 127.1, 129.0, 138.3, 168.7, 174.1, 177.3. Anal. Calcd. for $C_{16}H_{22}N_2O_5$: C 59.61, H 6.88, N 8.69, found C 59.65, H 6.82, N 8.65.

CD Studies

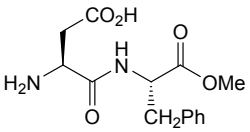
CD measurements were carried out on a Jasco J715 spectropolarimeter. A single Jasco cylindrical cuvette with a pathlenght of 1 cm was used for all measurements. The spectra were recorded using a scan speed of 5 nm min^{-1}, with a resolution of 0.2 nm, a sensitivity di 50 mdeg, a time costant of 16 sec and a spectral window from 260 to 190 nm.
Peptides **8** and **9**, and aspartame wild-type were dissolved in water-trifluoroethanol 1:1 (v:v) at concentrations of 6.2 x 10^{-4} mM.
The spectra for these peptides are shown below.

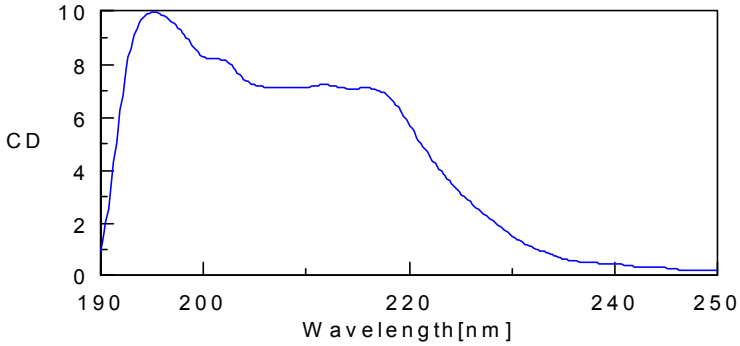






Aspartame





Molecular dynamics

All computer simulations were carried out using the Chem3D program.
Molecular Mechanics and Molecular Dynamics were achieved by means of MM2 force field, as implemented in Chem3D. Molecular Dynamics trajectories were collected in periods of 1ns, using a step interval of 1 fs, and a target temperature of 373 K.
The extended conformations of aspartame and dipeptide 6 were found using software Amber, emploing as Force Field Parm99, specific for amino acidic structurs.

Synthesis of Dialkylated Analogue of Arginine

Alkylation of ethyl isocyanoacetato

Ethyl 5-azido-2-(3-azidopropyl)-2-cyanopentanoate (6)

To a solution of ethyl isocyanoacetate (1 g; 8.8 mmol) in anhydrous DMSO (9 mL) and ether (89 mL) was added the 1-azido-3-iodopropane (3.7 g, 17.6 mmol) and NaH (0.6 g; 26.5 mmol washed from mineral oil) at room temperature. The reaction mixture was allowed to react for 15 min at r.t. then H_2O (5 mL) was added and the mixture was washed with ether. The organic layer was then washed with brine, dried (Na_2SO_4) and the solvent removed to afford 2.5 g of the crude product. Purification by flash cromatography (silica gel, hexane:ether 9:1→ 7:3) gave the compound **6** (2.1 g; 85%) as colorless oil. IR (neat): 2938.9*m*, 2100.2*s*, 1748.6*s*. ^{1}H NMR (400 MHz): δ 1.34 (t, *J*=7.1, 3H, CH_3), 1.50-1.65 (m, 2H, CH_2), 1.70-1.85 (m, 4H, CH_2), 2.00-2.12 (m, 2H, CH_2), 3.25-3.45 (m, 2H, CH_2N_3), 4.29 (q, *J*=7.1, 2H, CH_2O). ^{13}C NMR (100 MHz): δ 14.12, 23.90, 36.32, 50.61, 63.08, 67.59, 160.51, 168.12. Anal. Calcd for $C_{11}H_{17}N_7O_2$: C 47.30, H 6.13, N 35.10. Found: C 47.52, H 6.07, N 34.82

Hydrolysis of isocyanate[3]

[3] Ref Procedure: *J. Org. Chem.* **1989**, *9*, 2170-2178.

Ethyl 2-amino-5-azido-2-(3-azidopropyl) pentanoate (7)

The compound **6** (2 g; 7.2 mmol) was dissolved in 16 mL of 5% HCl in EtOH (5.5 mL of 38% acqueus HCl in 60 mL of absolute ethanol) and the reaction was stirred at 40°C for 12 h. Then the solvent was removed under vacuum, the crude was dissolved in EtOAc and washed with HCl 1M to remove other organic residue. The aqueous layer was then basified with NaOH 1M (pH≈10) and washed with EtOAc. Finally, the organic layer was washed with brine and dried to give 1.8 g of product **7** as colorless oil (yield 96%). IR (neat): 2934.5*s*, 2094*s*, 1726.1*s*. ^{1}H NMR (400 MHz): δ 1.29 (t, *J*=7.1, 3H, CH_3), 1.44-1.72 (m, 8H, 2xCH_2, 2xC*Ha*Hb and NH_2), 1.79-1.86 (m, 2H, 2xCHa*Hb*), 3.22-3.34 (m, 4H, 2xCH_2N_3), 4.20 (q, *J*=7.1, 2H, CH_2O). ^{13}C NMR (100 MHz): δ 14.3, 23.6, 37.1, 51.5, 60.4, 61.4, 96.9, 176.2. Anal. Calcd for $C_{10}H_{19}N_7O_2$: C 44.60, H 7.11, N 36.41. Found: C 44.63, H 7.13, N 36.19.

Hydrolysis of ester function and Boc-protection

5-Azido-2-(3-azidopropyl)-2-(tert-butoxycarbonylamino)pentanoic acid (9)

To a solution of compound **7** (0.4 g; 1.5 mmol) in 9.8 ml of MeOH, 1 N NaOH (3 ml) is added and the solution is stirred at 40°C untill the disappearance of the starting material (TLC). Then the solvent is removed under vacuum and the crude is dissolved in 5.3 ml of dioxane and 1.7 ml of 1 N NaOH. To this solution 2 g of Boc_2O are added in tree portions. The reaction is allowed to react over night at room temperature. Then the solvent is removed under vacuuo and the residue dissolved with EtOAc and washed with 10% $KHSO_4$ and brine. Purification by FC (Silica gel, CH_2Cl_2: MeOH 9:1) gives 0.35 g of product **9** as white solid (yield 70%). ^{1}H-NMR (400 MHz): δ 1.36-1.45 (m, 11H, Boc, CH_2), 1.54-1.63 (m, 2H, CH_2), 1.91 (ddd, *J*=4.5, *J*=12.3, 2H, CH_2), 2.30-2.50 (m, 2H, CH_2), 3.20-3.30 (m, 4H, CH_2), 5.79 (s, 1H, NHBoc). ^{13}C-NMR (100 MHz): δ 23.5, 27.7, 32.5, 51.2, 65.7, 80.1, 155.2, 175.1. ESI-MS: 365.18 [$(M+Na)^+$], 342.18 [$(M+H)^+$].

Coupling reaction between 9 and 7

To a solution of **9** (50 mg, 0.15 mmol) in DMF (0.5 mL) was added the amine **7** (40 mg, 0.15 mmol) and NMM (0.05 mL, 1.76 mmol). After 5 min, HATU (68 mg, 0.18 mmol) was added and the reaction was allowed to react for 12h at 25 °C. The solvent was then removed, the residue was dissolved in EtOAc and washed with 10% KHSO4, brine, 10% KHCO3, brine; then dried to recover 108 mg of crude. Purification by flash cromatography (Hexane:EtOAc / 8:2→7:3) afforded to coupling product (4 mg, 5%).

4,4-bis(2-Azidoethyl)oxazolidine-2,5-dione (12)

(Yield 24%). ^{1}H NMR (300 MHz): δ 1.42-2.10 [m, 8H, 2x$(CH_2)_2$], 3.28-3.47 (m, 4H, 2xCH_2), 5.78 (bs, 1H, NH). ^{13}C NMR (75 MHz): δ 23.3, 34.4, 50.7, 66.7, 152.1, 171.7. ^{13}C (75 MHz): 23.3, 34.4, 50.7, 66.7, 152.1, 171.7.

Ethyl 5-azido-2-(5-azido-2-(3-azidopropyl)-2-(*tert*-butoxycarbonylamino)pentanamido)-2-(3-azidopropyl)pentanoate (13)

^{1}H NMR (300 MHz): δ 1.29 (t, *J*=7.1, 3H, CH_3), 1.45-1.65 (m, 17H, Boc, 4xCH_2), 1.70-1.95 (m, 2H, 4xCH_2), 2.10-2.30 (m, 2H,CH_2), 2.40-2.58 (m, 2H, CH_2), 3.11-3.40 (m, 8H, 4xCH_2N_3), 4.28 (q, *J*=7.1, 2H, CH_2O), 5.40 (s, 1H, NHBoc), 7.11 (s, 1H, NH).ESI-MS: 615.2 [$(M+Na)^+$], 593.3 [$(M+H)^+$], 493.4 [$(M^+$-Boc)].

Hydrolysis of ester function and Cbz-protection

5-Azido-2-(3-azidopropyl)-2-(benzyloxycarbonylamino)pentanoic acid (10)

To a solution of compound **7** (1 g; 3.7 mmol) in 22 mL of MeOH, 1M NaOH (7 mL) was added and the solution was stirred at 40°C until the starting material was completely consumed (TLC). Then the solvent was removed under reduced pressure and the crude was dissolved in 1:1 (v:v) 1M NaOH (3.4 mL):Acetone (3.4 mL) and kept at 0 °C. To this solution CbzCl (4.2 mmol; 1.3 mL) were added in tree portions and the pH was adjusted to 10-11 with 2M NaOH. The reaction was allowed to react over night at room temperature. Then the solvent is removed under vacuo and the residue dissolved with Et_2O and washed with 2M NaOH to remove organic stuff. The aqueous layer is then acidified with iced 6N HCl and extracted with EtOAc. Finally the organic layer is washed with brine, dried on Na_2SO_4 and removed under reduced pressure to give a crude, whose purification by FC (Silica gel, CH_2Cl_2: MeOH 9:1) gave 0.86 g of product **10** as white solid (yield 62%). ^{1}H-NMR (400 MHz): δ 1.36-1.45 (m, 2H, CH_2), 1.54-1.63 (m, 2H, CH_2), 1.91 (ddd, *J*=4.5, *J*=12.3, 2H, CH_2), 2.30-2.50 (m, 2H, CH_2), 3.20-3.30 (m, 4H, CH_2), 5.09 (s, 2H, OCH_2Ph), 5.79 (s, 1H, NHCbz) 7.30-7.51 (m, 5H, H-Ar). ^{13}C-NMR (100

MHz): δ 23.7, 32.7, 50.9, 63.2, 66.7, 128.0, 128.3, 128.6, 136.5, 154.2, 175.5. ESI-MS: 398.1 [$(M+Na)^+$], 376.1 [$(M+H)^+$].

Coupling reaction between 10 and 7

ethyl 5-azido-2-(5-azido-2-(3-azidopropyl)-2-(benzyloxycarbonylamino)pentanamido)-2-(3-azidopropyl)pentanoate (16)

To a solution of **10** (165 mg, 0.44 mmol) in DMF (1 mL) was added the amine **7** (118 mg, 0.44 mmol) and NMM (0.2 mL, 1.76 mmol). After 5 min, HATU (234 mg, 0.62 mmol) was added and the reaction was allowed to react for 7 d at 25 °C. The solvent was then removed, the residue was dissolved in EtOAc and washed with 10% $KHSO_4$, brine, 10% $KHCO_3$, brine; then dried to recover 290 mg of crude. Purification by flash cromatography (Hexane:EtOAc / 8:2→7:3) afforded to coupling product (105 mg, yield 39%). ^{1}H-NMR (300 MHz): δ 1.20-1.60 (m, 11H, 4xCH_2 and CH_3), 1.70-1.95 (m, 4H, 2xCH_2), 2.25-2.53 (m, 4H, 2xCH_2), 3.12-3.39 (m, 8H, 4xCH_2), 4.28 (q, 2H, O*C*$H_2$$CH_3$), 5.07 (s, 2H, OCH_2Ph), 5.83 (s, 1H, NHCbz), 6.97 (s, 1H, NH), 7.30-7.40 (m, 5H, H-Ar). ^{13}C-NMR (100 MHz): δ 23.7, 32.7, 50.9, 63.2, 66.7, 128.0, 128.3, 128.6, 136.5, 154.2, 175.5. Mass (HiResMALDI): 649.303 for ($C_{26}H_{38}N_{14}O_5$+Na^+).

Coupling reaction between 17 and 7

ethyl 5-azido-2-(3-azidopropyl)-2-(2-(*tert*-butoxycarbonylamino)propanamido)pentanoate (18)

To a solution of **17** (35 mg, 0.18 mmol) in DMF (2 mL) was added the amine **7** (50 mg, 0.18 mmol) and DIPEA (0.15 mL, 0.9 mmol). After 5 min, HATU (138 mg, 0.36 mmol) was added and the reaction was allowed to react for 12h at 25 °C. The solvent was then removed, the residue was dissolved in EtOAc and washed with 10% $KHSO_4$, brine, 10% $KHCO_3$, brine; then dried to recover 84 mg of crude. Purification by flash cromatography (CH_2Cl_2:EtOAc / 8:2) afforded to coupling product (62 mg, yield 78%). ^{1}H-NMR (300 MHz): δ 1.15-1.35 (m, 8H, CH_2, 2xCH_3), 1.40-1.68 (m, 11H, Boc, CH_2), 1.78-1.90 (m, 2H, CH_2), 2.42-2.61 (m, 2H, CH_2), 3.08-3.35 (m, 4H, 2xCH_2N_3), 4.01-4.15 (m, 1H, CH), 4.23 (q, *J*=7.1, 2H, CH_2O), 4.95 (m, 1H, NHBoc), 7.09 (s, 1H, NH).

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Efficient synthesis of orthogonally protected *anti*-2,3-diamino acids

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Abstract—An asymmetric synthesis of *anti*-2,3-diamino acids is reported. The enolates of *N*,*N*-dibenzylated β^3-amino esters were treated with di-*tert*-butyl azodicarboxylate (DBAD) to afford their N',N''-di-Boc-2-hydrazino derivatives with excellent *anti* diastereoisomeric ratio. Final Boc removal and reductive cleavage of the hydrazino bond led to the expected 2,3-diamino esters having only one free amino group. In comparison with other asymmetric C-2 amination procedures, this method does not need the use of expensive chiral reagents and/or chiral auxiliaries, while leads to products which can be orthogonally protected.
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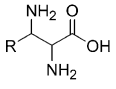
1. Introduction

2,3-Diamino acids are important non-protein amino acids, usually components of both natural and synthetic bioactive compounds.[1] In fact, they are currently well recognized as key structural moieties in a variety of biologically active molecules: (*S*)-2,3-diamino propanoic acid (DAP, **1**),[2] 2,3-diamino butanoic acids (DAB, **2** and **3**)[3] and (2*S*,3*R*)-2,3-diamino-4-phenylbutanoic acid (**4**)[4] are present in some antifungal dipeptides[5] and in peptide antibiotics, like aspartocin,[3] glumamycin,[6] lavendomycin[7] and aminodeoxybestatin.[8] Furthermore, (2*R*,3*S*)-2,3-diamino-3-phenylpropanoic acid (**5**) has been considered as an alternative side chain in the anticancer drug Taxol.[9]



1 R=H (2*S*)
2 R=CH_3 (2*S*,3*S*)
3 R=CH_3 (2*R*,3*S*)
4 R=CH_2Ph (2*S*,3*R*)
5 R=Ph (2*R*,3*S*)

The elementary, polyfunctional 2,3-diamino acid unit has been frequently used to probe several aspects of peptide and protein structures. In addition, the usefulness of simple chiral 1,2-diamines as auxiliaries and controller groups in asymmetric synthesis (e.g., dihydroxylation,[10] conjugate addition,[11] olefination,[12] allylation,[13] epoxidation,[14] and aldol reaction[15]) is also well documented. Their use to resolve racemic mixtures of chiral allylic alcohols has been reported as well.[16]

The development of simple and efficient methods to produce enantiomerically pure 2,3-diamino acids from readily available starting materials represent a fascinating goal and several asymmetric syntheses have been reported so far. The Mitsunobu reaction on serine,[17] the Hofmann and Curtius rearrangements of asparagine derivatives,[18] and the Schmidt reaction on aspartic acid[19] were used to access chiral 2,3-diaminopropanoic acid. A variety of other syntheses have been also reported: the conjugate addition[20] of homochiral lithium *N*-benzyl-*N*-α-methylbenzylamide to α,β-unsaturated esters and in situ amination with trisyl azide, the asymmetric Rh(I)-phosphine-catalyzed hydrogenation of diastereoisomeric enamides,[21] and the ring opening of *cis*-3-alkylaziridine-2-carboxylates coming from Sharpless asymmetric aminohydroxylation of α,β-unsaturated esters.[22]

In this paper we report a new inexpensive, general and highly stereoselective synthesis of *anti*-2,3-diamino acids via amination of β^3-amino esters.[23]

2. Results and discussion

N,*N*-Dibenzylated β^3-amino esters (**7a–c**), in dry THF at −78 °C and under dry nitrogen stream, were treated with potassium bis(trimethylsilyl)amide (KHMDS) to get the corresponding enolates. The use of more common bases, such as LiHMDS and LDA, for the enolate generation was

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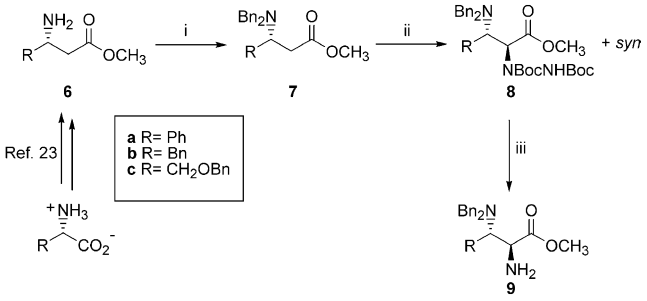
neglected since in our experience[24] such bases lead to significantly poorer results. After 1 h, solid di-*tert*-butyl azodicarboxylate (DBAD) was added to the reaction mixture that was kept at −78 °C for an additional hour. Under such conditions, the Boc-diprotected hydrazino derivatives of the starting **7a–c** were obtained.

The double protection of the amino group is necessary to avoid formation of by-products coming from the abstraction of the N–H proton in the enolate production step. Consequently, common protecting groups that are stable under basic conditions, such as either Boc or Cbz, could not be used under our reaction conditions. Therefore, in a first attempt, the 4-methoxybenzyl group, we had already used elsewhere,[24] was chosen for its peculiar removal conditions (CAN, CH_3CN/H_2O). Unfortunately, although the group turned out to be stable under the reaction conditions, we could not use it because the deprotection of the final 3-[di(4-methoxybenzyl)amino]-2,3-diamino esters led to a plethora of products coming from oxidative cleavage of the C2–C3 bond. Eventually, we used a double benzylic protection that eliminated such deprotection problems and represented at the same time a very bulky nitrogen substituent, suitable to affect the stereochemical outcome[25] of the enolate coupling with the electrophile DBAD. As a matter of fact, the coupling afforded a mixture of *anti*:*syn* Boc protected 2-hydrazino derivatives of **7a–c** with excellent diastereoisomeric ratio. Due to the complexity of the ^{1}H NMR spectra of the Boc containing hydrazino derivatives, they were converted into the corresponding diastereoisomeric mixtures of diamino esters (e.g., **9**) to determine accurately the diastereoisomeric ratio.

The synthetic path is depicted in Scheme 1 and the results obtained for selected β^3-amino esters, namely the methyl esters of β^3-phenylglycine (**6a**), β^3-phenylalanine (**6b**), and β^3-serine (**6c**), are reported in Table 1.



i. BnBr, DIPEA, toluene, reflux; ii. KHMDS, DBAD, dry THF, -78 °C;
iii. a) TFA, CH_2Cl_2; b) H_2, Ni(Ra), MeOH, ultrasound

Scheme 1. Conversion of α-amino acids into monoprotected 2,3-diamino esters.

The more abundant *anti* diastereoisomers were submitted to removal of the Boc protections (TFA in CH_2Cl_2) and cleavage of the N–N bond by hydrogenolysis with Ni(Ra) at low pressure and room temperature in an ultrasound bath.

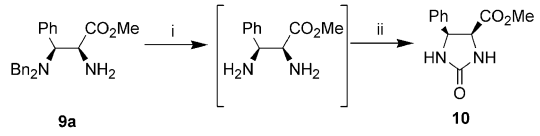
The reduction of hydrazines to amines is reported to be accomplished at high temperature, under high hydrogen pressure.[26] The use of ultrasound reduces significantly both temperature and pressure.[27] As a matter of fact, the hydrogenolysis under such conditions was complete after only 4 h and no traces of C-2 epimerization products could be detected by ^{1}H NMR spectroscopic analysis.

Table 1. Functionalization at C-2 of the fully protected β^3-amino esters **7a–c**

Protected β^3-amino ester	R	Boc protected 2-hydrazino derivatives of **7a–c**		*anti*-2,3-Diamino esters (**9a–c**), yield (%)[a]
		Yield (%)[b]	*anti*/*syn*	
7a	Ph	92	93:7	70
7b	Bn	90	97:3	78
7c	CH_2OBn	90	94:6	65

[a] Overall yield after Boc removal and reductive cleavage of the hydrazine moiety in the *anti* diastereoisomers **8a–c**.
[b] Yield of both diastereoisomers.

The *anti* configuration of the more abundant diastereoisomers coming from the couplings of **7a–c** with DBAD could be attributed, in the case of **8a**, as follows: the final product **9a** was debenzylated and treated, without isolation, with 1,1′-carbonyldiimidazole to afford the imidazolidinone **10** (Scheme 2).



i. Pd/C, H_2, AcOH, 50 °C, 90%; ii. 1,1'-carbonyldiimidazole, TEA, THF, 0 °C, 85%

Scheme 2. Synthesis of *cis*-imidazolidinone (**10**).

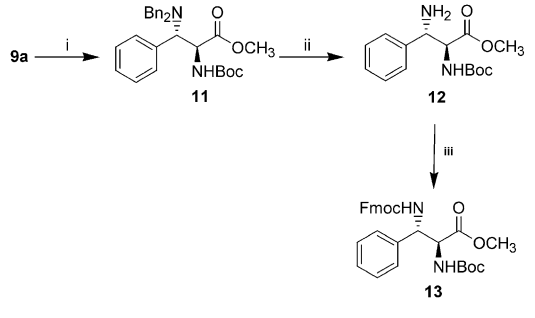
The ^{1}H NMR spectroscopy coupling constant of 9.6 Hz supported[28] the *cis*-configuration of the H-4 and H-5 protons and, thus, the *anti*-configuration of the starting diamino compound.

In the light of this result and in agreement with our previous work on the hydroxylation at C-2 of β^3-amino esters, it seems likely that the stereochemical outcome of the functionalization at C-2 is independent of the nature of the electrophile used, being only a function of the relative stabilities of the enolate conformations.[24]

3. Conclusion

This amination procedure of β^3-amino esters offers several advantages, if compared with many other reported procedures. First of all, it does not require the use of either chiral reagents or chiral auxiliaries: in fact, the observed selection in the coupling step is merely due to the influence of the existing chiral center of the starting β^3-amino ester, enhanced by the presence of two bulky substituents on the nitrogen atom. Moreover, it is noteworthy that the amino groups in the final 2,3-diamino esters have a different protection status: this implies a broad flexibility of their use in peptide synthesis. For instance, the free amino group can be Boc protected and the benzyl groups then removed hydrogenolitically to host an Fmoc protecting group, or vice

versa should either Boc- or Fmoc-strategy be used. Accordingly, in connection with our current interest in the synthesis of glycosyl amino acids, we have prepared the compound **13** as shown in Scheme 3.



Scheme 3. Preparation of the orthogonally protected 2,3-diamino acid **13**.

4. Experimental

4.1. General

NMR spectra were recorded on Varian Inova 500 MHz, Varian Gemini 200 MHz, Varian Gemini 300 MHz, Bruker DRX 400 MHz spectrometers: chemical shifts are in ppm (δ) and J coupling constants in Hz; solvent $CDCl_3$, unless otherwise specified. GC/MS analyses were performed on Hewlett–Packard 6890 GC/5973N MS. Optical rotations were determined on Jasco P-1010 polarimeter (1.0 dm cell); solvent $CHCl_3$, unless otherwise specified. Infrared spectra were recorded using JASCO FT/IR-430 Spectrometer. Mps were taken on a Gallenkamp apparatus. Elemental analyses were performed on a Perkin–Elmer Series II 2400, CHNS analyzer. TLC were carried out on silica gel Merck 60 F_{254} plates (0.2 mm layer) and column chromatographies on Merck Kieselgel 60 (70–230 mesh). Dry solvents were distilled immediately before use.

4.1.1. *N,N*-Dibenzyl protections of 6a–c. *Methyl (R)-3-(dibenzylamino)-3-phenylpropanoate* (**7a**): *typical procedure*. A magnetically stirred suspension of β^3-phenylglycine methyl ester **6a** (1.50 g; 8.38 mmol) and diisopropylethylamine (DIPEA, 7.3 mL; 41.90 mmol) in toluene (18.0 mL) was warmed gently until a clear solution was obtained. Then, benzyl bromide (6.0 mL; 50.28 mmol) was added in one portion and the resulting solution was refluxed for 4 h. The reaction mixture was then cooled in an ice bath, diluted with EtOAc (2×100 mL) and extracted with 10% aq NH_4Cl. The organic layer was washed with brine, dried (Na_2SO_4), and evaporated under reduced pressure to afford a crude reaction product whose chromatography on silica gel (petroleum ether/EtOAc, 95:5) gave the pure crystalline compound **7a**, after recrystallization from hexane (2.56 g; 7.12 mmol; 85%). Mp 51.8–53.0 °C. $[\alpha]_D^{20}$ +71.6 (c 2.0). ^{1}H NMR (500 MHz): δ 2.73 (dd, J=7.3, 14.6 Hz, 1H, H-2a), 3.14 (dd, J=8.8, 14.6 Hz, 1H, H-2b), 3.18 (d, J=13.7 Hz, 2H, NC*H*Ph), 3.64 (s, 3H, OCH_3), 3.78 (d, J=13.7 Hz, 2H, NC*H*Ph), 4.33 (dd, J=7.3, 8.8 Hz, 1H, H-3), 7.20–7.41 (m, 15H, *H*-Ar). ^{13}C NMR (125 MHz): δ 36.9, 51.8, 53.9, 59.1, 127.2, 127.7, 128.3, 128.4, 128.8, 129.1, 137.6, 139.8, 172.3. IR (KBr, cm^{-1}): ν 1714. Anal. Calcd for $C_{24}H_{25}NO_2$: C 80.19, H 7.01, N 3.90. Found: C 80.30, H 7.05, N 3.92.

Under the same conditions, the following *N,N*-diprotected esters were also obtained.

Methyl (S)-3-(dibenzylamino)-4-phenylbutanoate (**7b**). Oil (83%). $[\alpha]_D^{20}$ −5.4 (c 1.0). ^{1}H NMR (500 MHz): δ 2.33 (dd, J=6.4, 14.2 Hz, 1H, H-2a), 2.56 (dd, J=8.8, 13.2 Hz, 1H, H-4a), 2.65 (dd, J=8.3, 14.2 Hz, 1H, H-2b), 3.12 (dd, J=5.7, 13.2 Hz, 1H, H-4b), 3.40–3.50 (m, 1H, H-3), 3.56 (s, 3H, OCH_3), 3.62 (d, J=13.7 Hz, 2H, NC*H*Ph), 3.76 (d, J=13.7 Hz, 2H, NC*H*Ph), 7.20–7.70 (m, 15H, *H*-Ar). ^{13}C NMR (125 MHz): δ 35.9, 36.3, 51.6, 53.7, 57.8, 126.4, 127.2, 128.4, 128.6, 129.1, 129.5, 139.7, 139.8, 172.9. IR (KBr, cm^{-1}): ν 1712. Anal. Calcd for $C_{25}H_{27}NO_2$: C 80.40, H 7.29, N 3.75. Found: C 80.25, H 7.32, N 3.77.

Methyl (R)-4-(benzyloxy)-3-(dibenzylamino)butanoate (**7c**). Oil (86%). $[\alpha]_D^{20}$ +31.6 (c 1.8). ^{1}H NMR (500 MHz): δ 2.55 (dd, J=6.3, 14.6 Hz, 1H, H-2a), 2.68 (dd, J=7.8, 14.6 Hz, 1H, H-2b), 3.47–3.54 (m, 1H, H-3), 3.56–3.62 (m, 4H, H-4a and OCH_3), 3.66 (d, J=13.7 Hz, 2H, NC*H*Ph), 3.71 (dd, J=9.8, 5.4 Hz, 1H, H-4b), 3.75 (d, J=13.7 Hz, 2H, NC*H*Ph), 4.49 (d, J=12.7 Hz, 1H, OC*H*Ph), 4.52 (d, J=12.7 Hz, 1H, OC*H*Ph), 7.20–7.45 (m, 15H, *H*-Ar). ^{13}C NMR (125 MHz): δ 34.7, 51.7, 54.5, 55.2, 70.3, 73.3, 127.1, 127.8, 128.4, 128.6, 129.1, 138.6, 140.1, 173.0. IR (KBr, cm^{-1}): ν 1715. Anal. Calcd for $C_{26}H_{29}NO_3$: C 77.39, H 7.24, N 3.47. Found: C 77.25, H 7.27, N 3.48.

4.1.2. Reactions of 7a–c with DBAD. *Methyl (2S,3S)-3-(dibenzylamino)-2-[N′,N″-(di-tert-butoxycarbonyl)-hydrazino]-3-phenylpropanoate* (**8a**): *typical procedure*. To a magnetically stirred solution of **7a** (2.56 g; 7.12 mmol) in dry THF (75 mL), at −78 °C and under dry argon atmosphere, 0.5 M KHMDS in toluene (28.5 mL; 14.24 mmol) was added dropwise. After 1 h solid di-*tert*-butyl azodicarboxylate (2.85 g; 12.82 mmol) was added in one portion to the reaction mixture kept at −78 °C under stirring. Within 1 h the reaction was quenched by addition of glacial AcOH (1.1 mL) and diluted with EtOAc. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvents evaporated in vacuo. The oily residue, after chromatography on silica gel (hexane/EtOAc, 9:1), afforded the pure title compound **8a** (foam; 3.86 g; 6.55 mmol; 92%). $[\alpha]_D^{20}$ +65.7 (c 1.6). The ^{1}H NMR data were not significant, apparently due to the occurrence of mixtures of rotamers. IR (KBr, cm^{-1}): ν 3260, 1740, 1720. Anal. Calcd for $C_{34}H_{43}N_3O_6$: C 69.25, H 7.35, N 7.13. Found: C 69.17, H 7.31, N 7.15.

Under the same conditions, the following Boc diprotected 2-hydrazino derivatives were also obtained.

Methyl (2S,3S)-3-(dibenzylamino)-2-[N′,N″-(di-tert-butoxycarbonyl)-hydrazino]-4-phenylbutanoate (**8b**). Foam (90%). $[\alpha]_D^{20}$ −2.3 (c 0.3). IR (KBr, cm^{-1}): ν 3250, 1730, 1712. Anal. Calcd for $C_{35}H_{45}N_3O_6$: C 69.63, H 7.51, N 6.96. Found: C 69.60, H 7.49, N 7.01.

Methyl (2S,3R)-4-(benzyloxy)-3-(dibenzylamino)-2-[N′,N″-(di-tert-butoxycarbonyl)-hydrazino]butanoate (**8c**). Foam

(90%). $[\alpha]_D^{20}$ +45.0 (*c* 1.5). IR (KBr, cm^{-1}): ν 3270, 1728, 1715. Anal. Calcd for $C_{36}H_{47}N_3O_7$: C 68.22, H 7.47, N 6.63. Found: C 68.19, H 7.40, N 6.68.

4.1.3. Reductive cleavages of the hydrazino bond in 8a–c. *Methyl (2S,3S)-2-amino-3-(dibenzylamino)-3-phenylpropanoate* (**9a**): *typical procedure*. To a magnetically stirred solution of **8a** (3.86 g; 6.55 mmol) in dry CH_2Cl_2 (54 mL), TFA (54 mL) was added in one portion. After 2 h, the solvent was evaporated under reduced pressure. The crude reaction product, redissolved in MeOH (26 mL), was transferred into a flask containing W-2 Raney nickel (3.86 g, wet) and equipped with a hydrogen inflated balloon. The flask was dipped into an ultrasound bath filled with water and sonicated for 4 h at rt till the starting product was completely consumed (TLC). The reaction mixture was then filtered through Celite® washing with MeOH (100 mL). Removal of the solvents under reduced pressure gave a residue that was redissolved in EtOAc (200 mL), washed with 10% aq Na_2CO_3 (2×100 mL), dried (Na_2SO_4), and evaporated in vacuo, to afford an oil whose chromatography on silica gel (hexane/EtOAc, 7:3) led to the pure title compound **9a** (oil; 1.71 g; 4.58 mmol; 70%). $[\alpha]_D^{20}$ +62.4 (*c* 1.1). ^{1}H NMR (300 MHz): δ 1.97 (bs, 2H, NH_2), 3.05 (d, J=13.5 Hz, 2H, NC*H*Ph), 3.82 (s, 3H, OCH_3), 3.83–3.88 (m, 3H, H-2 and NC*H*Ph), 4.26 (d, J=10.3 Hz, 1H, H-3), 7.20–7.60 (m, 15H, *H*-Ar). ^{13}C NMR (50 MHz): δ 51.7, 54.0, 56.3, 67.3, 126.9, 128.1, 128.3, 128.8, 129.7, 133.5, 139.0, 174.1. IR (KBr, cm^{-1}): ν 3500–3200, 1714. Anal. Calcd for $C_{24}H_{26}N_2O_2$: C 76.98, H 7.00, N 7.48. Found: C 76.81, H 7.06, N 7.52.

Under the same conditions, the following 2-amino esters were also obtained.

Methyl (2S,3S)-2-amino-3-(dibenzylamino)-4-phenylbutanoate (**9b**). Oil (78%). $[\alpha]_D^{20}$ +7.9 (*c* 0.4, MeOH). ^{1}H NMR (500 MHz): δ 1.63 (bs, 2H, NH_2), 2.93 (dd, J=7.3, 13.7 Hz, 1H, H-4a), 3.09 (dd, J=6.3, 13.7 Hz, 1H, H-4b), 3.23–3.29 (m, 1H, H-3), 3.60 (s, 3H, OCH_3), 3.61–3.67 (m, 3H, H-2 and NC*H*Ph), 3.70 (d, J=13.7 Hz, 2H, NC*H*Ph), 7.10–7.40 (m, 15H, H-Ar). ^{13}C NMR (100 MHz): δ 32.7, 52.2, 55.1, 55.6, 63.8, 126.5, 127.4, 128.5, 128.7, 129.4, 129.9, 139.9, 140.5, 175.9. IR (KBr, cm^{-1}): ν 3530–3210, 1712. Anal. Calcd for $C_{25}H_{28}N_2O_2$: C 77.29, H 7.26, N 7.21. Found: C 77.19, H 7.25, N 7.23.

Methyl (2S,3R)-2-amino-4-(benzyloxy)-3-(dibenzylamino)-butanoate (**9c**). Oil (65%). $[\alpha]_D^{20}$ +30.9 (*c* 1.0). ^{1}H NMR (500 MHz, C_6D_6): δ 1.48 (bs, 2H, NH_2), 3.22–3.28 (m, 1H, H-3), 3.29 (s, 3H, OCH_3), 3.58 (d, J=6.8 Hz, 1H, H-2), 3.60 (dd, J=5.8, 9.8 Hz, 1H, H-4a), 3.65 (d, 2H, J=13.7 Hz, NC*H*Ph), 3.72 (dd, 1H, J=4.9, 9.8 Hz, H-4b), 3.84 (d, 2H, J=13.7 Hz, NC*H*Ph), 7.05–7.40 (m, 15H, *H*-Ar). ^{13}C NMR (100 MHz): δ 52.1, 55.4, 55.6, 61.1, 67.2, 73.7, 127.3, 127.9, 128.0, 128.5, 128.8, 129.4, 138.7, 140.2, 175.7. IR (KBr, cm^{-1}): ν 3510–3200, 1716. Anal. Calcd for $C_{26}H_{30}N_2O_3$: C 74.61, H 7.22, N 6.69. Found: C 74.59, H 7.20, N 7.01.

4.1.4. Methyl (4*S*,5*S*)-4-methoxycarbonyl-5-phenyl-2-imidazolidinone (10). A magnetically stirred solution of **9a** (0.020 g; 0.053 mmol) in glacial AcOH (0.5 mL) was hydrogenolysed over 30% Pd/C catalyst (0.006 g) for 2 h at 50 °C, under a slightly positive pressure given by an inflated balloon (~3 bar). The mixture was then filtered through Celite® washing with MeOH (10 mL). Removal of the solvents under reduced pressure gave a residue that was redissolved in dry THF (1.1 mL). The solution was cooled to 0 °C. Et_3N (0.063 mL, 0.053 mmol) and 1,1′-carbonyldiimidazole (0.013 g; 0.079 mmol) were then added in sequence. After 30 min at 0 °C and 2 h at rt the solvents were evaporated under reduced pressure and the remaining crude residue was dissolved in EtOAc and filtered on a short silica gel plug (~3 cm^3) with the same solvent (3×10 mL). By partial evaporation of the solvent under reduced pressure, a semicrystalline residue could be collected whose recrystallization by the same solvent afforded the pure **10** (0.010 g; 0.045 mmol; 88%) as a white solid. Mp 202–203 °C dec. (lit.[27] 203–205 °C). ^{1}H, ^{13}C NMR and IR spectra were superimposable to those reported.

4.1.5. Methyl (2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-(dibenzylamino)-3-phenylpropanoate (11). To a solution of compound **9a** (0.67 g; 1.80 mmol) in dioxane (21 mL) at 0 °C, Et_3N (0.42 mL; 2.70 mmol) and Boc_2O (0.89 g; 3.60 mmol) were added in sequence. The reaction mixture, warmed up to room temperature and stirred for 1 h, was then diluted with EtOAc. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvents evaporated in vacuo to give an oil. Its chromatography on silica gel (hexane/EtOAc, 9:1) afforded the pure title compound **11** (oil; 0.78 g; 1.66 mmol; 92%). $[\alpha]_D^{20}$ +50.7 (*c* 1.5). ^{1}H NMR (400 MHz): δ 1.55 (s, 9H, Boc), 3.04 (d, J=13.5 Hz, 2H, NC*H*Ph), 3.86 (s, 3H, OCH_3), 3.97–4.02 (m, 3H, H-3, NC*H*Ph), 4.58 (bd, J=8.3 Hz, 1H, N*H*Boc), 5.14 (bt, J=9.7 Hz, 1H, H-2), 7.24–7.44 (m, 15H, *H*-Ar). ^{13}C NMR (125 MHz): δ 28.3, 52.3, 54.1, 54.7, 65.0, 80.2, 127.3, 128.3, 128.5, 129.2, 130.1, 132.6, 139.1, 146.9, 154.9, 172.3. IR (KBr, cm^{-1}): ν 1718, 1705. Anal. Calcd for $C_{29}H_{34}N_2O_4$: C 73.39, H 7.22, N 5.90. Found: C 73.25, H 7.20, N 5.92.

4.1.6. Methyl (2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-amino-3-phenylpropanoate (12). A magnetically stirred solution of **11** (0.78 g; 1.66 mmol) in glacial AcOH (7.4 mL) was hydrogenolysed over 30% Pd/C catalyst (0.23 g) for 2 h at 50 °C, under a slightly positive pressure given by an inflated balloon (~3 bar). The mixture was then filtered trough Celite® and washed with MeOH (100 mL). The solvent was removed under reduced pressure and the residue was dissolved in EtOAc (2×100 mL). The organic layer was washed with 10% aq Na_2CO_3 (300 mL), dried (Na_2SO_4), and the solvents evaporated in vacuo to give compound **12** as a white crystalline solid, after recrystallization from hexane/acetone 9:1 (0.44 g; 1.49 mmol; 90%). Mp 110.3–112.3 °C. $[\alpha]_D^{20}$ +29.0 (*c* 0.9). ^{1}H NMR (400 MHz, C_5D_5N): δ 1.35 (s, 9H, Boc), 3.62 (s, 3H, OCH_3), 5.34 (d, J=7.1 Hz, 1H, H-3), 5.77 (bt, J=8.0 Hz, 1H, H-2), 7.21–7.35 (m, 3H, *H*-Ar), 7.89 (d, J=7.3 Hz, 2H, Ar-H), 8.80 (bd, J=8.6 Hz, 1H, N*H*Boc). ^{13}C NMR (100 MHz, C_5D_5N): δ 28.3, 52.4, 57.1, 59.4, 79.3, 128.6, 128.9, 138.0, 140.2, 156.8, 172.0. IR (KBr, cm^{-1}): ν 3350, 3200, 1765, 1715. Anal. Calcd for $C_{15}H_{22}N_2O_4$: C 61.21, H 7.53, N 9.52. Found: C 61.32, H 7.57, N 9.58.

4.1.7. Methyl (2*S*,3*S*)-2-(*tert*-butoxycarbonylamino)-3-(9*H*-fluoren-9-ylmethoxycarbonylamino)-3-phenylpropanoate (13). To a stirred solution of **12** (0.44 g; 1.48 mmol) in dioxane (5.8 mL) and 10% aq Na_2CO_3 (0.31 g; 2.96 mmol) at 0 °C, Fmoc–OSu (0.41 g; 1.18 mmol) dissolved in DMF (1.5 mL) was added slowly. The reaction mixture, after 30 min at 0 °C and 2 h at rt, was extracted with CH_2Cl_2. The organic layer was washed with brine until neutral, dried (Na_2SO_4), and the solvents evaporated under reduced pressure to give an oil. The chromatography on silica gel ($CHCl_3$) afforded the pure compound **13**, white solid after recrystallization from hexane/acetone 9:1 (0.50 g; 0.96 mmol; 65%). Mp 166.6–168.1 °C. $[\alpha]_D^{20}$ +38.7 (*c* 0.3). ^{1}H NMR (500 MHz, C_5D_5N): δ 1.20 (s, 9H, Boc), 3.42 (s, 3H, OC*H*$_3$), 4.14 (t, *J*=6.8 Hz, 1H, Fmoc), 4.30 (dd, *J*=6.8, 10.3 Hz, 1H, C*H*Fmoc), 4.45 (dd, *J*=6.8, 10.3 Hz, 1H, C*H*Fmoc), 5.31 (t, *J*=9.3 Hz, 1H, H-2), 5.69 (t, *J*=9.3 Hz, 1H, H-3), 7.05–7.70 (m, 14H, *H*-Ar and N*H*Boc), 9.17 (d, *J*=9.3 Hz, 1H, N*H*Fmoc). ^{13}C NMR (125 MHz): δ 28.5, 47.5, 52.9, 57.7, 58.0, 67.3, 120.2, 125.4, 126.8, 127.3, 128.4, 128.9, 141.5, 144.1, 155.9, 156.5, 170.4. IR (KBr, cm^{-1}): ν 3395 (br), 1705. Anal. Calcd for $C_{30}H_{32}N_2O_6$: C 69.75, H 6.24, N 5.42. Found: C 69.82, H 6.27, N 5.43.

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