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List of abbreviations

Ac	Acetyl
AcIm	Acetylimidazole
AcOH	Acetic acid
AIBN	2,2'-Azobisisobutyronitrile
Ala	Alanine
Asp	Aspartic acid
Bn	Benzyl
Boc	*tert*-Butoxycarbonyl
CAN	Cerium Ammonium Nitrate
DCC	Dicyclohexylcarbodiimide
DDQ	Dichlorodicyanoquinone
DEAD	Diethylazodicarboxylate
DIC	Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMAP	4-Dimethylaminopyridine
DMF	N, N'-Dimethylformamide
DMPM	3,4-Dimethoxyphenylmethyl
DMPU	N,N'-Dimethylpropyleneureum
DMSO	Dimethylsulfoxide
EDC	N-Ethyl-N'-(3-aminopropyl)-carbodiimide hydrochloride
ES-MS	Electrospray-Mass spectrometry
Fmoc	9-Fluorenylmethoxycarbonyl
Gly	Glycine
HBTU	2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate
HF/Pyr.	Hydrogen fluoride/pyridine
His	Histidine
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
Hz	Hertz
IR	Infrared
Leu	Leucine
m.p.	Melting point
M.W.	Molecular Weight
MeOH	Methanol
MHz	MegaHertz
NMM	N-methylmorpholine
NMR	Nuclear Magnetic Resonance
Phe	Phenylalanine
Pip.	Piperidine
PPTS	Pyridinium *p*-toluenesulfonate
PTSA	*p*-Toluene sulfonic acid
ROESY	Rotational Overhauser Effect Spectroscopy
r.t.	Room temperature
Ser	Serine
TASF	Tris-(dimethylamino)sulfur trimethylsilyldifluoride
TBAF	Tetrabutylammonium fluoride
TBDPS	*tert*-Butyldiphenylsilyl
TFA	Trifluoroacetic acid

THF	Tetrahydrofuran
THP	Tetrahydropyranyl
TNBS	2,4,6-Trinitrobenzenesulfonic acid
UV/VIS	Ultraviolet/Visible
Val	Valine

Table of contents

1 Introduction

1.1 Preface

In the world of biochemistry, enzymes are molecules of primary significance. They are among the most remarkable biomolecules known because of their extraordinary specificity and catalytic power. They can catalyse biological reactions that would normally occur slowly or not at all under physiologically acceptable conditions.

One of the great intellectual challenges presented to science today is a proper understanding of how enzymes work. What an enzyme does, is bind, and thus stabilise, selectively the transition state for a particular reaction. However, our current level of understanding fails the more severe, practical test, that of designing and developing artificial enzyme systems with catalytic efficiencies which rival those of natural enzymes.[1]

The most remarkable property of proteases is the ease with which they are capable of cleaving amide bonds while a typical chemical procedure for hydrolysing aliphatic amides requires 8 N hydrochloric acid at reflux for 10 hours. The development of a catalyst for such a reaction remains one of the major challenges in organic chemistry.[2]

Until now the discovery of man-made compounds with useful catalytic properties has been tremendously slow. A strategy for the development of such a new catalyst usually starts off with the design of a specific molecule that is subsequently synthesised, purified, characterised and tested on its activity. The activity is optimised by modification of the initial molecule. In this way, the development of a new catalyst requires years of research. To render this time-and cost intensive process more efficient, combinatorial methods have been introduced to the field of catalysis since a few years.[3]

In a short period of time, combinatorial chemistry allows for the synthesis of large libraries of molecules increasing the chance of finding one or several members possessing catalytic activity. In this work, we describe a combinatorial approach towards the development of a new man-made catalyst for the hydrolysis of amide bonds, based on the well-known serine-protease α-chymotrypsin. In this chapter, the different aspects of combinatorial chemistry will be discussed. Subsequently, an introduction concerning the serine-protease, α-chymotrypsin will be presented.

---

[1] Kirby, A.J. *Angew. Chem. Int. Ed. Engl.* **1994**, *33*, 551.

[2] (a) Kirby, A.J. *Angew. Chem. Int. Ed. Engl*. **1996**, *35*, 707; (b) Motherwell, W.B.; Bingham, M.J.; Six, Y. *Tetrahedron* **2001**, *57*, 4663.

[3] Bein, T. *Angew. Chem. Int. Ed. Engl*. **1999**, *38*, 323.

1.2 Combinatorial Chemistry

For over a hundred years, the main task of the synthetic chemist has been the straightforward synthesis of one synthetic product. In contrast to classical chemistry, combinatorial chemistry allows for the rapid synthesis of large numbers of compounds. Since the early 1990's, combinatorial chemistry has attracted the attention of companies as a means of reducing the time and cost associated with the drug discovery process. The possibility to synthesise hundreds to thousands times more compounds than conventional organic synthesis increases substantially the chance of finding a new target molecule. The wide applications of combinatorial chemistry in the drug discovery process have been well documented.[4] The same way of thinking can also be applied in the search for new models mimicking the efficiency of enzymes. Synthesising a large amount of compounds increases substantially the chance of finding a catalyst compared to the design of one specific molecule.

The characteristic of combinatorial chemistry is that many different compounds are synthesised simultaneously under identical reaction conditions in a systematic manner to form a 'combinatorial library'. The library is then screened for the catalytic property in question and the active compound is identified. The total number of all synthesised compounds within a combinatorial library depends on two main factors: the number of building blocks involved in each step (y) and the number of reaction steps (z). When the number of building blocks are identical in each step, the amount of possible synthesised compounds (N) is given by the equation: $N = y^z$.

1.2.1 Solid Phase Peptide Chemistry

Almost four decades ago, Merrifield introduced the concept of solid phase synthesis in the area of peptide chemistry.[5] The revolutionary principle behind is that if the peptide is bound to an insoluble polymer, unreacted reagents at the end of each synthetic step can be removed by a simple washing procedure, substantially decreasing the time required for synthesis and purification (figure 1.1). At the end of the synthesis, the peptide is cleaved from the solid support and purified.

[4] (a) Gallop, M.A.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gordon, E.M. *J. Med. Chem.* **1994**, *37*, 1233; (b) Gordon, E.M.; Barrett, R.W.; Dower, W.J.; Fodor, S.P.A.; Gallop, M.A. *J. Med. Chem.* **1994**, *37*, 1385; (c) Terret, N.K.; Gardner, M.; Gordon, D.W.; Kobylecki, R.J.; Steele, J. *Tetrahedron* **1995**, *51*, 8135.

[5] Merrifield, R.B. *J. Am. Chem. Soc.* **1963**, *85*, 2149.

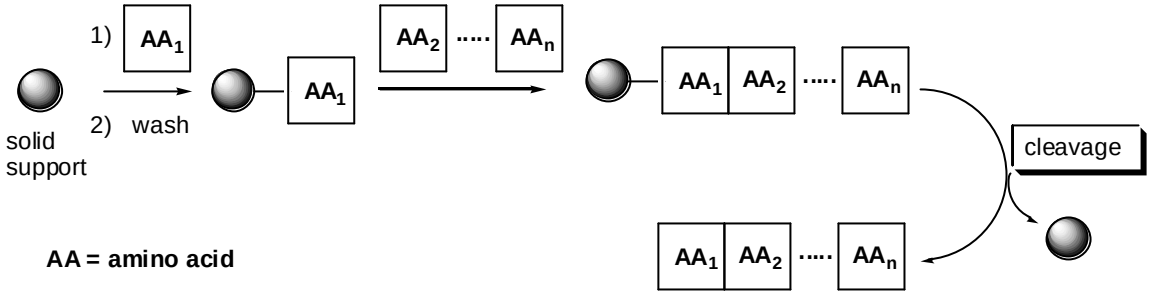


Figure 1.1: Solid phase peptide chemistry

This is only valid, however, if each individual synthetic step occurs with quantitative yields. In peptide chemistry, high yields can be achieved by using excess of reagents to push the reaction to completion. However, in order to attain such a high performance in yield, all aspects of the chemistry, from the solid support itself to the protecting groups and coupling reagents used, must be rigorously optimised.

1.2.1.1 Choice of solid support

For a solid support to be useful for SPPS (solid phase peptide synthesis), it must consist of physically robust particles that remain chemically inert to all reagents and solvent used during the synthesis. It needs functional groups allowing for the attachment of the first amino acid to the solid phase. The polystyrene-based resins cross linked with 1% or 2% divinylbenzene are the most frequently polymer supports used in SPPS. Two famous examples are the Merrifield[5] resin and the Wang[6] resin. One important feature is the swelling of the polymer in the reaction solvent allowing penetration of the reagents through the particles, ensuring accessibility of the reactive sites to the reagents.[7]

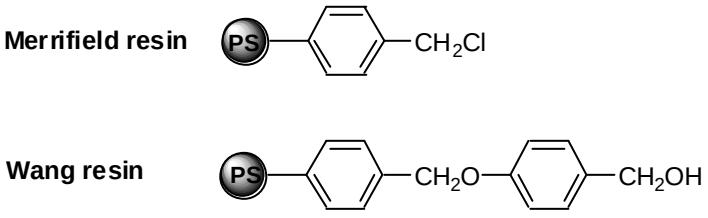


Figure 1.2

[6] Wang, S.S. *J. Am. Chem. Soc.* **1973**, *95*, 1328.

1.2.1.2 Protection schemes

In the synthesis of a dipeptide, merely mixing two free amino acids is not sufficient. This would lead to the formation of a mixture of di-, tri, and polypeptides in all possible combinations. Therefore, the choice of a suitable protection scheme is of great importance.

Although many protecting groups exist, the research and development efforts in SPPS have led to two major protection schemes that are used today. These are the so-called Boc/Bzl (*tert*-butoxycarbonyl/benzyl) approach developed by Merrifield[8] and the Fmoc/*tert*-Bu (9-fluorenylmethoxycarbonyl/*tert*-butyl) approach introduced later by Carpino[9] (figure 1.3).

In the Boc/Bzl strategy, the Boc group is used as N^{α}-protection in combination with benzyl-based side-chain protecting groups. The peptides are usually synthesised on Merrifield resin and are then cleaved from the polymer using hydrogen fluoride.

In the Fmoc/*tert*-Bu strategy, using a *tert*-butyl-based side-chain protection is obviously incompatible with the Boc group but fully compatible with the base labile protecting Fmoc group. In that case, Wang resin is used due to its higher acid lability compared to Merrifield resin. Peptides are cleaved from the solid support with trifluoroacetic acid (TFA). The superiority of the Fmoc/*tert*-Bu strategy over Boc/Bzl has already been proven. The synthesis of the same sixteen amino acids containing peptide with both methods led to a better yield and purity when the Fmoc strategy was applied.[10] In this work, the Fmoc/*tert*-Bu approach will be used.

[7] Santini, R.; Griffith, M.C.; Qi, M. *Tetrahedron Lett.* **1998**, *39*, 8951.

[8] Merrifield, R.B. *Science* **1986**, *232*, 341.

[9] (a) Carpino, L.A.; Han, G.Y. *J. Am. Chem. Soc.* **1970**, *92*, 5748; (b) Carpino, L.A.; Han, G.Y. *J. Org. Chem.* **1972**, *37*, 3404.

[10] Novabiochem catalog, **2000**, I: Introduction and background, I1-I16

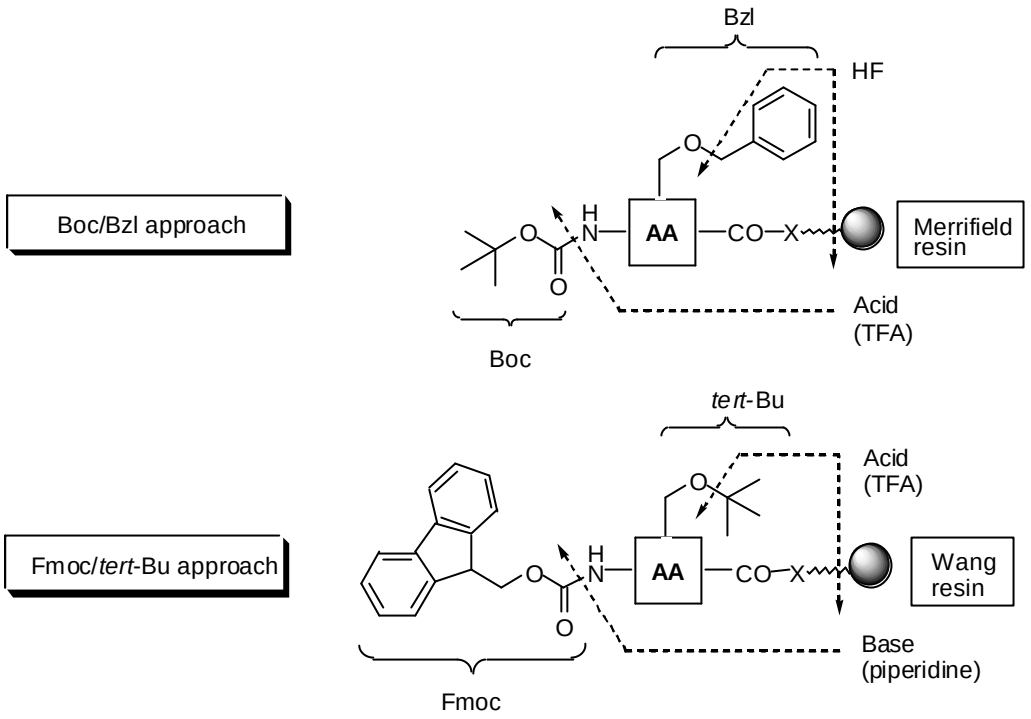


Figure 1.3: protecting group strategies

1.2.1.3 Coupling methods in SPPS

Carbodiimides continue to be the most widely used coupling reagents in SPPS (figure 1.4). Dicyclohexylcarbodiimide[11] (DCC) was the first one introduced into peptide chemistry almost 50 years ago and is actually replaced by diisopropylcarbodiimide[12] (DIC) which forms a more soluble urea by-product. The introduction of additives like 1-hydroxybenzotriazole (HOBt)[13] or 1-hydroxy-7-azabenzotriazole (HOAt)[14] into the reaction speeds up coupling processes and decreases dramatically the problem of racemisation and dehydration. Active research into the development of new carbodiimides for peptide couplings continues.[15]

[11] Sheehan, J.C.; Hess, G.P. *J. Am. Chem. Soc.* **1955**, *77*, 1067.
[12] Sarantaskis, D.; Teichman, J.; Lien, E.L.; Fenichel, R. *Biochem. Biophys. Res. Commun.* **1976**, *73*, 336.
[13] König, W.; Geiger, R. *Chem. Ber.* **1970**, *103*, 2034.
[14] Carpino, L.A. *J. Am. Chem. Soc.* **1993**, *115*, 4397.
[15] Gibson, F.S.; Park, M.S.; Rapoport, H. *J. Org. Chem.* **1994**, *59*, 7503.

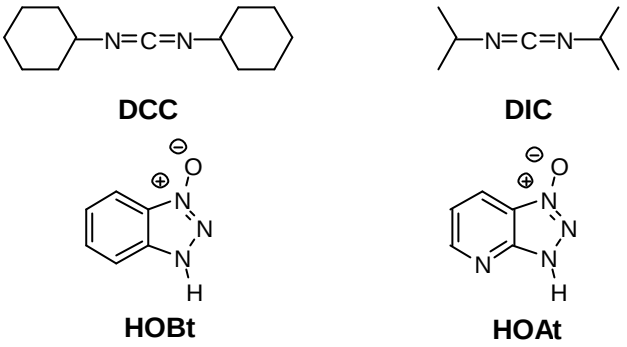


Figure 1.4

Next to carbodiimides, many new coupling reagents have been developed. Acylphosphonium salts (figure 1.5) became widely used in SPPS since their appearance in 1969.[16] However, the originally introduced 1H-benzotriazol-1-yloxy-tris(dimethylamino) phosphonium hexafluorophosphate (BOP) reagent developed by Castro[17] has been replaced by 1H-benzotriazol-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBOP), which forms a less toxic reaction-product.

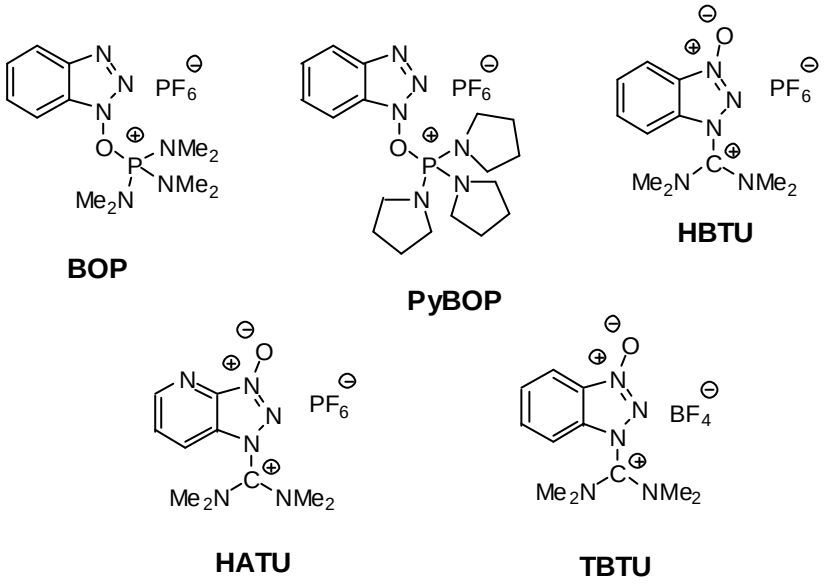


Figure 1.5

[16] Gawne, G.; Kenner, G.; Sheppard, R.C. *J. Am. Chem. Soc.* **1969**, *91*, 5669.
[17] Castro, B.; Dormoy, J.R.; Evin, G.; Selve, C. *Tetrahedron Lett.* **1975**, 1219.

Furthermore, besides these reagents uronium salts have gained importance as coupling reagents.[18] More recently, a new generation of phosphonium and uronium coupling reagents based on HOAt has been developed such as the aza analogue of 2-(1H-9-azabenzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HBTU) known as 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafluorophosphate (HATU). These coupling reagents appeared to be useful for the coupling of sterically hindered amino acids.[19,20]

1.2.2 Synthetic strategies towards combinatorial libraries

1.2.2.1 Parallel synthesis

The simplest approach in combinatorial chemistry is parallel synthesis. Molecules are simultaneously synthesised in a parallel way in distinct reaction vessels (figure 1.6). Parallel synthesis offers the advantage that each compound in each separate vessel is known at any time. This method is comparable with solution phase synthesis.

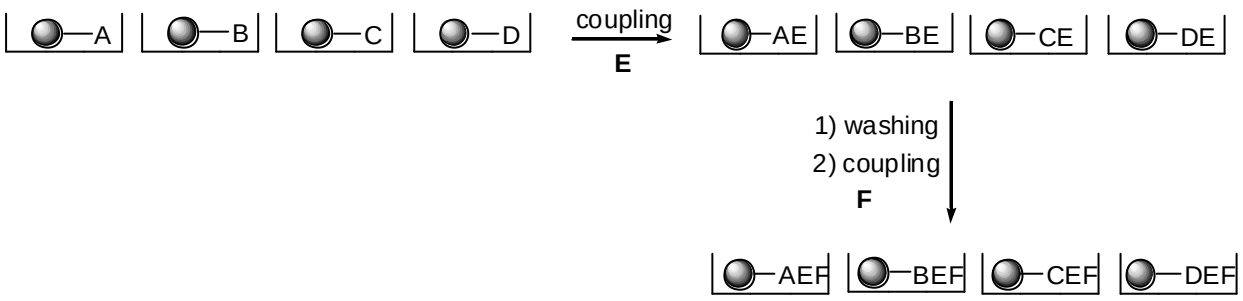


Figure 1.6: parallel synthesis on solid support

However, the easy purification in the case of solid phase chemistry explains why parallel synthesis on solid support is widely used. Several methods involving parallel synthesis on solid phase have already been described; the so-called 'Tea-Bag' method developed in 1984 by Houghten[21], the multipin-technique on polypropylene 'pins' in a 96-well plate introduced by

[18] (a) Dourtouglou, V.; Ziegler, J.-C.; Gross, B. *Tetrahedron Lett*. **1978**, 1279; (b) Dourtouglou, V.; Gross, B.; Lambropoulou, V.; Ziodrou, C. *Synthesis* **1984**, 572.
[19] Albericio, F.; Bofill, J.M.; El-Faham, A.; Kates, S.A. *J. Org. Chem.* **1998**, *63*, 9678.
[20] (a) Coste, J.; Dufour, M.N.; Pantaloni, A.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 669; (b) Frérot, E.; Coste, J.; Pantaloni, A.; Dufour, M.-N.; Jouin, P. *Tetrahedron* **1991**, *47*, 259.
[21] Houghten, R.A. *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5131.

Geysen[22] in 1984 and the spatially addressable parallel synthesis on silica wafer pioneered by Fodor in 1991.[23]

1.2.2.2 'Split and mix' synthesis

The synthesis of large libraries as a mixture of compounds using the 'split and mix' protocol was reported for the first time by Furka[24a] in 1988. But it is also referred to as 'divide, couple and recombine' by Houghten[24b] or 'the split synthesis' by Lam.[24c] A schematic illustration of the split and combine method is given in figure 1.7.

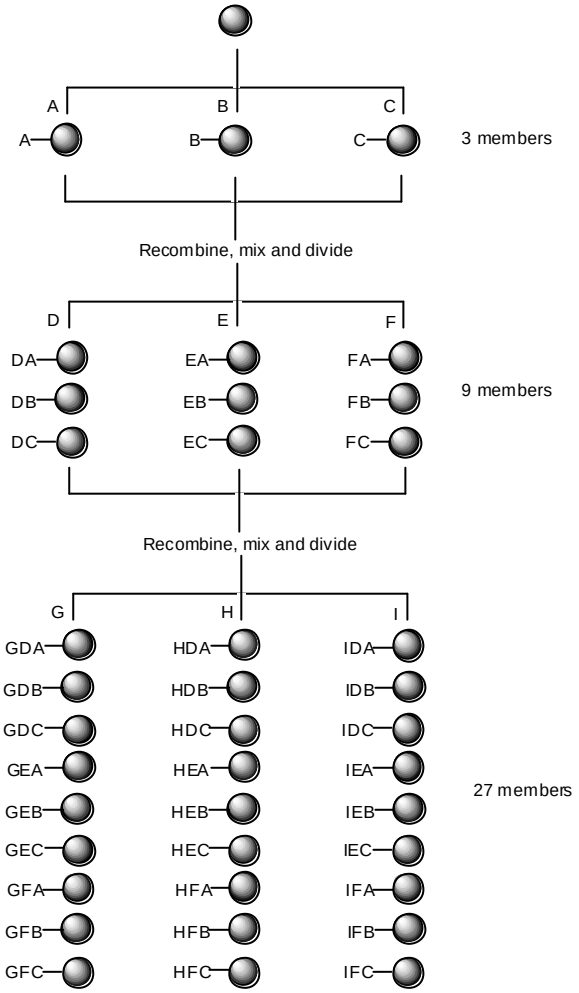


Figure 1.7 : The 'split and mix' method

[22] Geysen, H.M.; Meloen, R.H.; Barteling, S.J. *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3998.

[23] Fodor, S.P.A.; Rava, R.P.; Huang, X.C.; Pease, A.C.; Holmes, C.P.; Adams, C.L. *Nature (London)* **1993**, *364*, 555.

[24] (a) Furka, A.; Sebestyen, F.; Asgedom, M.; Dibo, G. *Int. J. Peptide Protein Res*. **1991**, *37*, 487; (b) Houghten, R.A.; Pinilla, C.; Blondelle, S.E.; Appel, J.R.; Dooley, C.T.; Cuervo, J.H. *Nature (London)* **1991**, *354*, 84; (c) Lam, K.S.; Salmon, S.E.; Hersh, E.M.; Hruby, V.J.; Kazmierski, W.M.; Knapp, R.J. *Nature (London)* **1991**, *354*, 82.

The procedure is simple and involves the division of the resin in a number (y) of equal batches. A single different monomer is then coupled in each vessel and after the coupling, all batches are mixed together thoroughly. The mixed resin is divided again into y batches and another series of coupling reactions are carried out. Repeating this protocol for z cycles can in principle produce a collection of y^z compounds. The coupling of a few building blocks can lead to the synthesis of large libraries in a short time. Furthermore, on each bead of the library, one compound is synthesised. The 'split and mix' method is also referred to as the 'one bead /one compound' concept.

1.3 Serine protease: α-chymotrypsin

1.3.1 Introduction

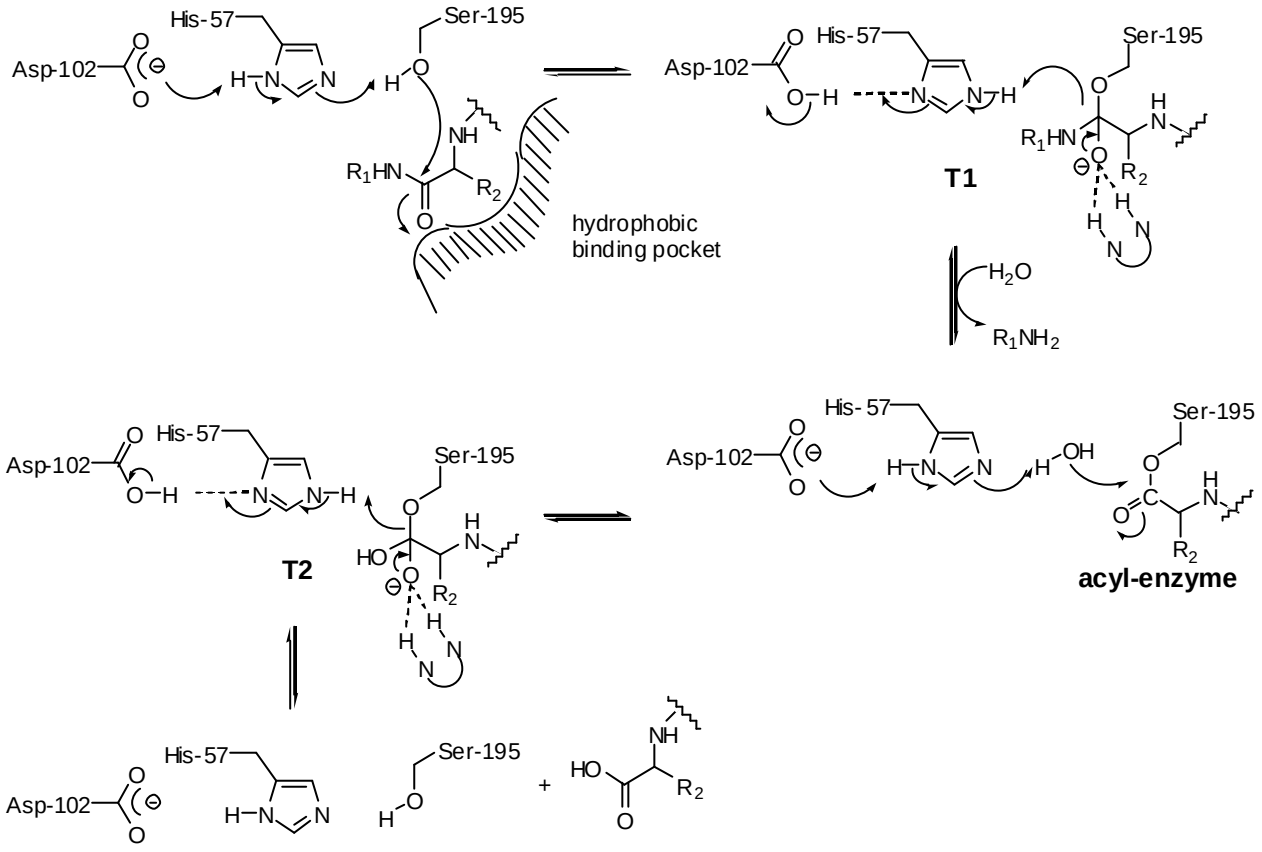
The term serine protease derives from a class of enzymes containing a serine residue in the active site. The most studied example of this group is α-chymotrypsin. The mechanism of α-chymotrypsin is probably understood in more details than any other enzyme. Its function is to catalyse the hydrolysis of peptide bonds of protein foods in the mammalian gut. It is secreted in the pancreas under an inactive form called chymotrypsinogen, a single protein chain of 245 amino acids, which is activated by trypsin in the small intestine by cleavage at two specific regions. The three-dimensional structure was elucidated by Blow in 1967.[25]

In addition to the serine-195 residue, the active site contains two other amino acids implied in the hydrolysis of amide bonds, histidine-57 and aspartate-102. They form the so-called 'catalytic triad'. α-Chymotrypsin is known to have specificity for amino acids with an aromatic side-chain like phenylalanine, tyrosine and tryptophan due to the presence of a hydrophobic pocket in the binding site. Proteins are cleaved on the carboxyl side of these aromatic amino acids.

For the hydrolytic activity of α-chymotrypsin, a 'charge relay' mechanism, which is presented in scheme 1.1, was first proposed in 1969 by Blow.[26] Two stages can be distinguished in the mechanism: the acylation step during which the acyl-enzyme is formed and the deacylation step during which the acyl-enzyme is hydrolysed.

[25] (a) Mattews, B.W.; Sigler, P.B.; Henderson, R.; Blow, D.M. *Nature (London)* **1967**, *214*, 652; (b) Blow, D.M. *Acc. Chem. Res*. **1976**, *9*, 145.
[26] Blow, D.M.; Birktoft, J.J.; Hartley, B.S. *Nature (London)* **1969**, *221*, 337.

The hydroxyl function of the amino acid serine-195 is not nucleophilic enough to attack the carbonyl function of the scissile bond but as a result of general base catalysis by the imidazole ring of the amino acid histidine-57, the nucleophilicity of the serine hydroxyl function is enhanced making a nucleophilic attack possible. The role of the carboxylate residue of aspartate-102 consists in polarising the imidazole moiety. This allows for a concerted proton transfer mechanism.



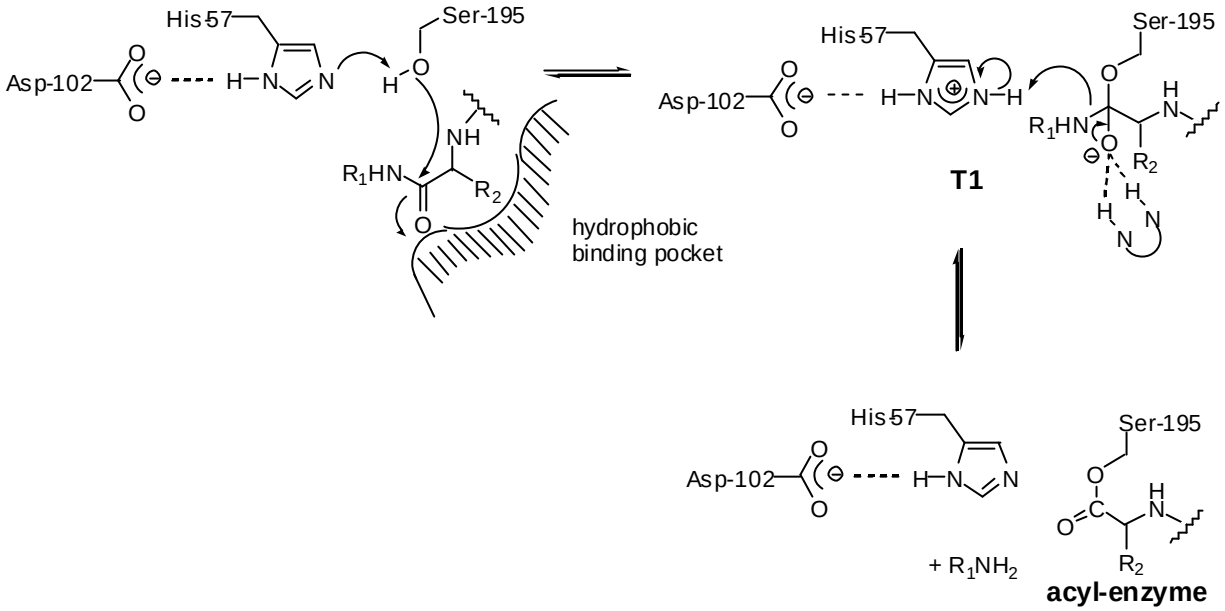
Scheme 1.1: 'charge relay' mechanism

In a first step, a tetrahedral intermediate T1 is formed, stabilised by hydrogen bond formation with the amide hydrogen of serine-195 and glycine-193 present on the backbone of the enzyme. The tetrahedral intermediate then decomposes via general acid catalysis to form the acyl-enzyme with liberation of the amine.

During the deacylation step, via essentially the same mechanism, the acyl-enzyme is hydrolysed by water. First, a molecule of water is deprotonated by the imidazole ring of the histidine-57 with the help of aspartate-102 and attacks the acyl-enzyme. A second tetrahedral intermediate

T2 is formed, which subsequently decomposes with release of the carboxylic acid and regeneration of the catalytic system.

The role of aspartate-102[27] in the mechanism of α-chymotrypsin is still under discussion.[28] An alternative mechanism has been proposed by Zimmerman and involves a single proton transfer from serine-195 to histidine-57, while the carboxylate of aspartate-102 serves to stabilize the charge of the imidazolium ion. This is the so- called 'charge stabilisation' mechanism.



Scheme 1.2: 'charge stabilisation' mechanism

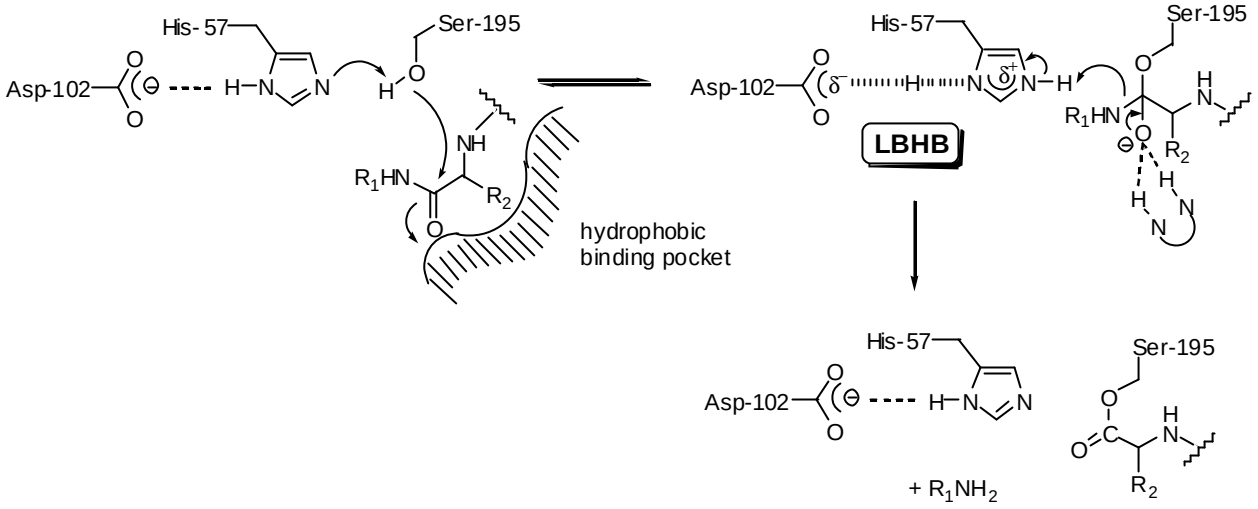
In 1994, a mechanism has been proposed in which a low-barrier hydrogen bound (LBHB) would be formed between histidine-57 and aspartate-102 (scheme 1.3).[29] However, this mechanism is still under discussion.[30]

[27] (a) Sprang, S.; Standing, T.; Fletterick, R.J.; Stroud, R.M.; Finer-Moore, J.; Xuong, N.-H.; Hamlin, R.; Rutter, W.J.; Craik, C.S. *Science* **1987**, *23*, 905; (b) Craik, C.S.; Roczniak, S.; Largman, C.; Rutter, W.J. *Science* **1987**, *23*, 909.

[28] Zimmerman, S.C.; Korthalsn, J.S.; Cramer, K.D.*Tetrahedron* **1991**, *47*, 2649.

[29] (a) Frey, P.A.; Whitt, S.A.; Tobin, J.B. *Science* **1994**, *264*, 1927; (b) Cleland, W.W.; Kreevoy, M.M. *Science* **1994**, *267*, 1887.

[30] Warshel, A.; Cleland, W.W.; Kreevoy, M.M.; Frey, P.A.; Whitt, S.A.; Tobin, J.B. *Science* **1995**, *269*, 103.



Scheme 1.3: 'low barrier hydrogen bound' mechanism

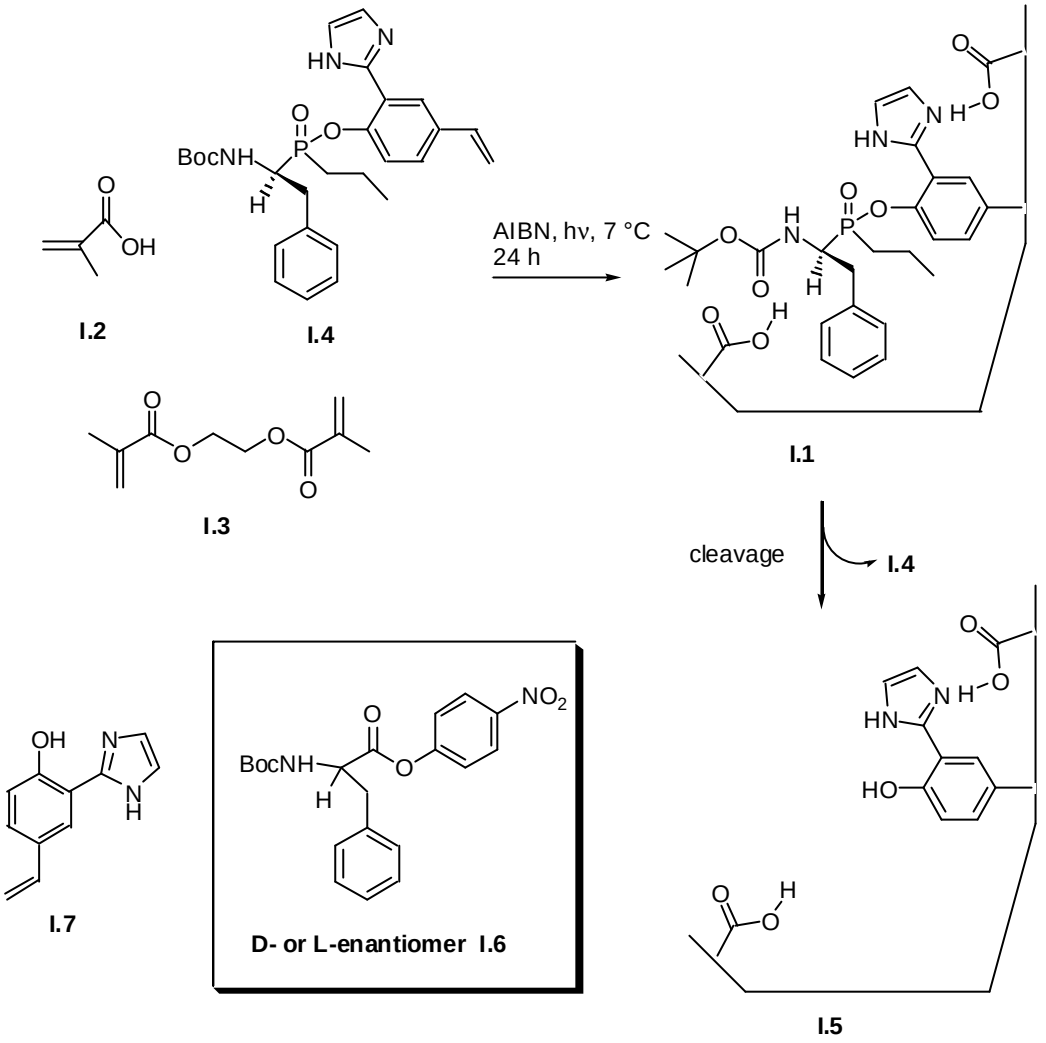
1.3.2 Artificial enzymes

The efficiency of the serine proteases has inspired scientists to the design of artificial enzymes. Past efforts to synthesise chymotrypsin models can be classified in three main classes: (1) Molecules designed with suitable functionalities which are expected to be involved in the catalysis of the chosen reaction,[31] (2) imprinted polymers and (3) pepzymes.

1.3.2.1 Mimics involving imprinted polymers

The first successful application of molecular imprinting to the design of a stereoselective catalyst with esterase activity was reported almost one decade ago.[32] Molecular imprinting is a method for obtaining specific microcavities in cross-linked polymers. The cross-link polymerisation is forced to take place around a transition state analogue of a chosen reaction. The transition state analogue is subsequently removed leaving behind an imprinted and defined shape bearing functional groups in specific positions, which are accessible for interactions. An example of molecular imprinting is given in scheme 1.4. Shea developed the network polymer **I.1** by photo-initiated free radical polymerisation of the monomers **I.2** and **I.3** in the presence of the D-enantiomer of the alkaline ester hydrolysis transition state analogue **I.4**. The D-phosphonic ester **I.4** is removed in the next stage to provide the polymer **I.5** containing a stereoselective binding site and the catalytic elements responsible for hydrolytic activity.

[31] For details concerning this approach see: Annemieke Madder, PhD-thesis '*Stapsgewijze ontwikkeling van niet-enzymatische hydrolasen*' **1997**.



Scheme 1.4

The rate of hydrolysis of both enantiomers D- and L-*tert*-butoxycarbonyl-phenylalanine *p*-nitophenyl ester **I.6** in presence of polymer **I.5** was measured and compared to reference compound **I.7.** A 10-fold acceleration was observed and as expected, polymer **I.5** proved to hydrolyse the complementary substrate D-*tert*-butoxycarbonyl-phenylalanine *p*-nitrophenyl ester faster than the L-enantiomer.

Other applications of imprinted polymers in the field of α-chymotrypsin mimics or more generally in the area of esterase mimics have been recently published.[33]

[32] Sellergren, B.; Shea, K.J. *Tetrahedron, Asymmetry* **1994**, *5*, 1403.

[33] (a) Wulff, G.; Gross, T.; Schönfeld, R. *Angew. Chem. Int. Ed. Engl*. **1997**, *36*, 1962; (b) Lele, B.S.; Kulkarni, M.G.; Mashelkar, R.A. *Reactive and functional polymers* **1999**, *39*, 37; (c) Sellergren, B.; Karmalkar, R.N.; Shea, K.J. *J. Org. Chem.* **2000**, *65*, 4009; (d) Wulff, G. *Chemical Reviews* **2002**, *102*, 1.

1.3.2.2 Mimics of α-chymotrypsin involving peptides

In 1990, Hahn and Klis designed a 73-residue peptide with enzyme-like activity.[34] With the help of computer modelling, a bundle of four short parallel amphipathic helical peptides containing the catalytic triad (serine, histidine and aspartate) in a spatial arrangement similar to that of the enzyme, was designed (figure 1.8). An 'oxyanion hole' and a binding site were also included. This assembly caused an acceleration with a factor of 10^5 compared to spontaneous hydrolysis in the cleavage of ester substrates (acetyltyrosine ethyl ester, benzyloxycarbonyltyrosine *p*-nitrophenyl ester and benzoyltyrosine ethylester). The activity of the peptide was inhibited by α-chymotrypsin inhibitors like diisopropyl fluorophosphate.

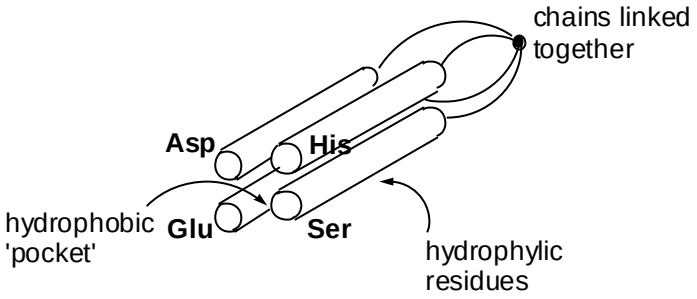


Figure 1.8

However, in two later reports, the earlier remarkable results in the hydrolysis of less labile esters and amides by the synthetic peptide were proven to be incorrect.[34b, c]

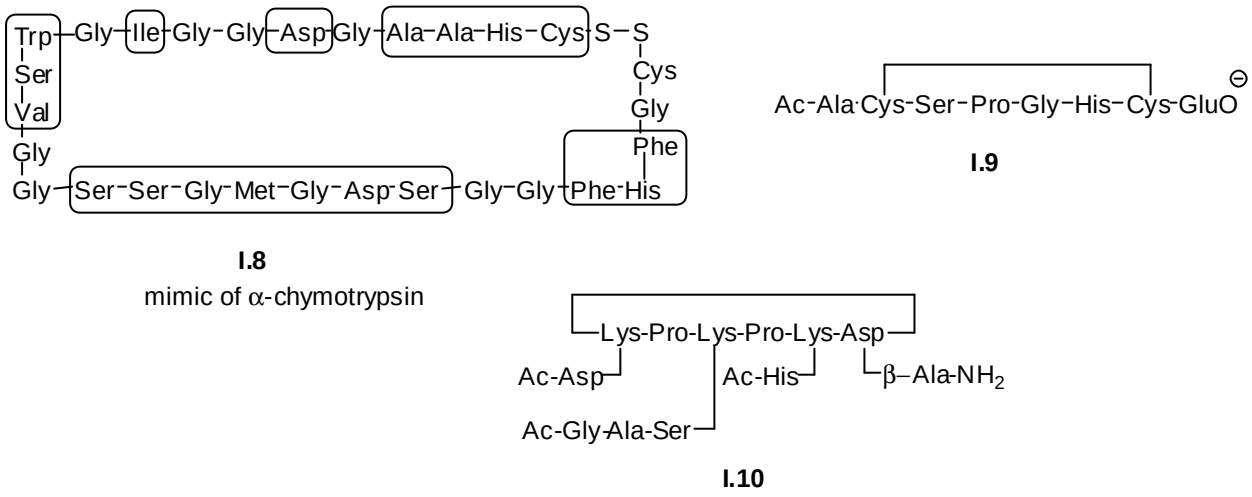
Efforts were further invested in the design and synthesis of cyclic peptides as mimics of α-chymotrypsin (figure 1.9). Atassi and Manshouri prepared cyclic peptides to mimic the active site of trypsin and α-chymotrypsin respectively.[35a] Peptide **I.8** was generated to mimic the conformation and disposition of the residues present in the active site. The components of the catalytic triad were scaffolded onto cyclic 23 and 29 residue-peptides, separated by glycine spacers. Construct **I.8** was claimed to cause the hydrolysis of several α-chymotrypsin substrates with enzyme-like efficiency and selectivity. The activity was further inhibited by

[34] (a) Hahn, K.W.; Klis, W.A.; Stewart, J.M. *Science* **1990**, *248*, 1544; (b) Corey, M.J.; Hallakova, E.; Pugh, K.; Stewart, J.M. *Appl. Biochem. Biotechnol*. **1994**, 47, 199; (c) Corey, M.J.; Corey, E. *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 11428.

[35] (a) Atassi, M.Z.; Manshouri, T. *Proc. Natl. Acad. Sci. USA* **1993**, *90*, 8282; (b) Matthews, B.W.; Craik, C.S.; Neurath, H. *Proc. Natl. Acad. Sci*. *USA* **1994**, *91*, 4103; (c) Corey, D.R.; Phillips, M.A. *Proc. Natl. Acad.*

diisopropyl fluorophosphate (DIFP) and 1-*p*-tosylamino-2-phenylethyl chloromethyl ketone (TPCK), two α-chymotrypsin inhibitors. However, independent laboratories could not confirm the unprecedented results of Atassi and Manshouri and have been unable to detect any catalytic activity.[35b-d]

Two recent examples of cyclic α-chymotrypsin mimics include the computationally designed cyclic eight-residue peptide **I.9** and the cyclic branched hexapeptide **I.10**, containing the amino acid residues of the catalytic triad. Compound **I.9** was found to hydrolyse *p*-nitrophenylacetate nine times faster compared to free histidine.[36] Hexapeptide **I.10** hydrolyzed *p*-nitrophenyl ester substrates and to a small extent a *p*-nitroanilide substrate.[37] Despite this modest activity, **I.10** represents the first example of an artificial enzyme capable of hydrolysing such an anilide substrate.



I.8
mimic of α-chymotrypsin

I.9

I.10

Figure 1.9

The work of Fukushima involves a ß-sheet polypeptide, poly(Asp-Leu-His-Leu-Ser-Leu) as an artificial hydrolytic enzyme-model for the hydrolysis of both enantiomers of N-benzyloxycarbonyl phenylalanine *p*-nitrophenyl ester.[38] The enantioselectivity and the hydrolytic rate obtained with the ß-sheet polypeptide appeared to be superior to the activity of the hexamer (Asp-Leu-His-Leu-Ser-Leu) used as reference. The influence of different parameters

Sci. USA **1994**, *91*, 4106; (d) Wells, J.A.; Fairbrother, W.J.; Otlewski, J.; Laskowski, M.; Burnier, J. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 4110.

[36] Walse, B.; Ullner, M.; Lindbladh, C.; Bülow, L.; Drakenberg, T.; Teleman, O. *Journal of Computer-Aided Molecular Design* **1996**, *10*, 11.

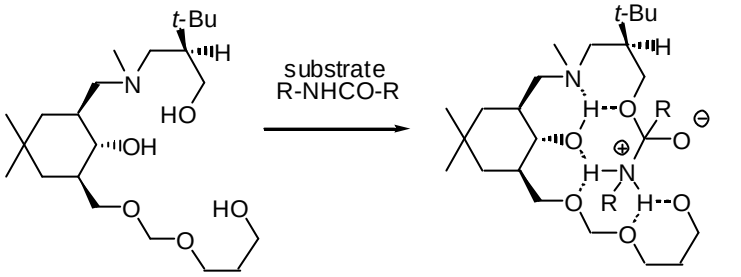
[37] Stavrakoudis, A.; Demetropoulos I.N.; Sakarellos, C.; Sakarellos-Daaitsiotis, M.; Tsikaris, V. *Lett. Pept. Sci*. **1997**, *4*, 481.

on the enantioselectivity and on the hydrolysis rate (pH, temperature and ethanol content) was studied.

Recently, a dipeptide model serine-histidine was reported to have multiple cleavage activities towards DNA, protein (Bovin Serum Albumin) and *p*-nitrophenylacetate over a wide range of pH and temperature.[39] The inverse dipeptide sequence His-Ser was shown to be inactive.

1.4 Scope and goal of this PhD work

The development of enzyme models was introduced in our laboratory about ten years ago. In the search to mimic the serine protease α-chymotrypsin, a first tentative was the design of a specific molecule **I.11** (figure 1.10).[40]



I.11 tetrahedral intermediate

Figure 1.10

A few years ago, combinatorial chemistry was introduced into this program as a method to speed up the search for new serine protease mimics. The traditional way of carefully designing a targeted molecule was replaced by the synthesis of large libraries increasing substantially the chance of finding a catalyst with potential hydrolytic activity. Moreover, the application of combinatorial chemistry for the development of hydrolase models of α-chymotrypsin has not been described in the literature so far. In an effort to explore this new field, a long-term program was set-up in our laboratory and started with the synthesis of a library **I.12** (figure 1.11) of 729 members on a rigid dipodal scaffold **I.13** using the 'split and mix' method.[41] The library was designed to contain the amino acids serine and histidine at different positions in the two independent strands to mimic the active site of the serine protease.

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[38] Fukushima, Y. *Bull. Chem. Soc. Jpn.* **1996**, *69*, 2269.

[39] Li, Y.; Zhao, Y.; Hatfield, S.; Wan, R.; Zhu, Q.; Li, X.; McMills, M.; Ma, Y.; Li, J.; Brown, K.L.; He, C.; Liu, F.; Chen, X. *Bioorg. Med. Chem.* **2000**,*8*, 2675.

[40] Madder, A.; De Clercq, P. J. *J. Org. Chem.* **1997**, *63*, 2548.

[41] De Muynck, H.; Madder, A.; Farcy, N.; De Clercq, P.; Pérez-Payán, M.N.; Öhberg, L.M.; Davis, A.P. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 145.

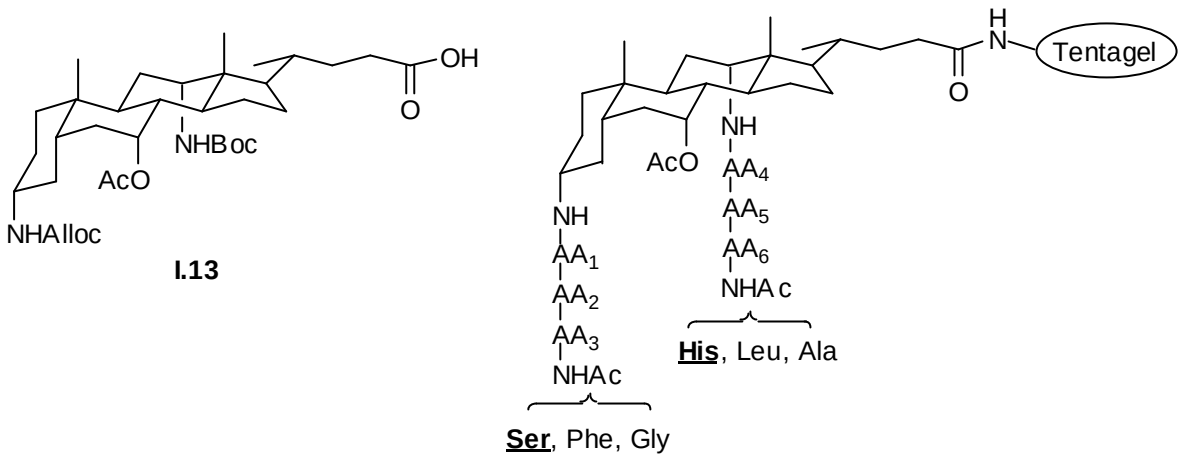


Figure 1.11

However, in the former library, the third amino acid of the catalytic triad, aspartate was omitted. As the role of aspartate-102 appears to be also important in the mechanism of α-chymotrypsin[27,28] the necessity to develop a tripodal library in which three instead of two parallel peptide strands are incorporated (figure 1.12) was fowarded as our next goal.

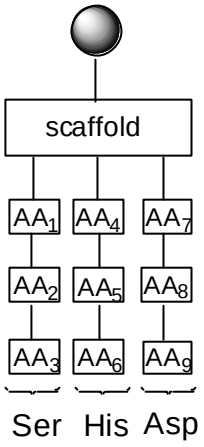


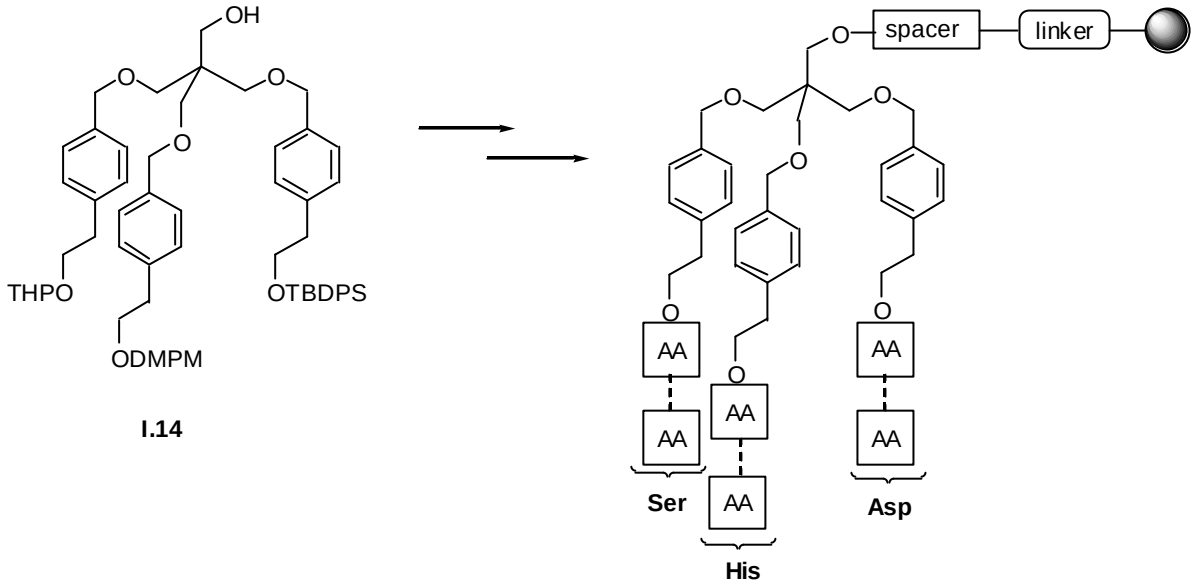
Figure 1.12

The design and the synthesis of a tripodal scaffold such as **I.14** as well as the synthesis of a library **I.15** is the subject of this thesis.

Since the scaffold **I.14** will be used on solid support, a cleavavble linker has to be introduced as represented in compound **I.15** (scheme 1.5). However, the choice of a suitable cleavable linker remains a challenge since it has to be stable to the chemistry which is performed during the synthesis and has to allow cleavage at the end of the synthesis without causing any damage to the compound. The critical selective deprotection of the three protected alcohol functions present in the scaffold **I.14** should be optimised on solid support to allow for the independent generation of three peptide strands.

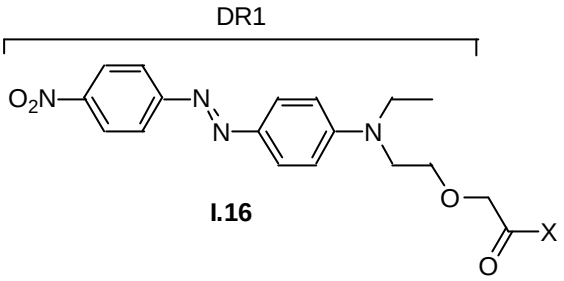
Before testing the introduction of the catalytic triad in the scaffold, a judicious choice of suitable side-chain protecting groups for the amino acids serine, histidine and aspartate should be examined. It has to be noted that the orthogonality of the side-chain protecting groups has to be compatible with the protecting groups present in the new tripodal scaffold **I.14** and with the cleavable linker.

Afterwards, the tripodal library **I.15** will be synthesised on solid support using the 'split and mix' protocol. As outlined in scheme 1.5, many members of the library will contain the three amino acids serine, histidine and aspartate at different positions of the peptide chains.



Scheme 1.5

In the next step, the tripodal library **I.15** will be tested for hydrolytic activity. For the screening of the library towards hydrolytic activity, a new type of test substrates **I.16** will be developed (figure 1.13). These screening substrates consist in the red-coloured dye Disperse Red 1 and contain an ester or amide bond. Upon exposure of the library to the dye **I.16**, the catalysts will react with the substrate and the colour will be transferred to the bead. This technique enables an easy visual detection of the polymer-bound active catalyst during the screening of the library.



I.16

a X = −OPhNO$_2$(p) = **NF31**
b X = −NHPhNO$_2$(p)
c X = −NHCH$_2$CH$_2$OPhNO$_2$(p)

Figure 1.13

2 Development of a test substrate for hydrolytic activity

2.1 Introduction

This work aims at the use of combinatorial techniques in the development of a new artificial enzyme, based on the mechanism of action of α-chymotrypsin, a well known member of the family of serine-proteases. Therefore, via the 'split and mix' protocol, a tripodal library (figure 2.1) will be constructed using one of the amino acids serine, histidine and aspartate as building block in each of the three branches. This leads to a mixture of several hundreds of compounds. Subsequently, this library will be screened for hydrolytic activity towards a chosen substrate. In this step, the use of a suitable substrate allowing for an easy detection of the solid-phase bound catalyst will be critical. Ideally, the test substrate is chosen in such a way that the catalytic activity is indicated by the appearance of a coloured bead.

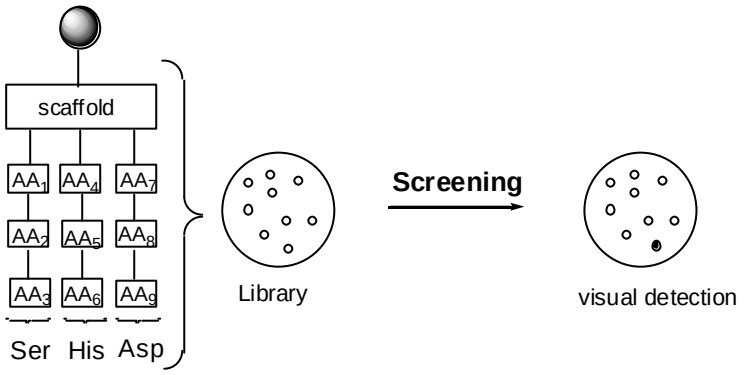
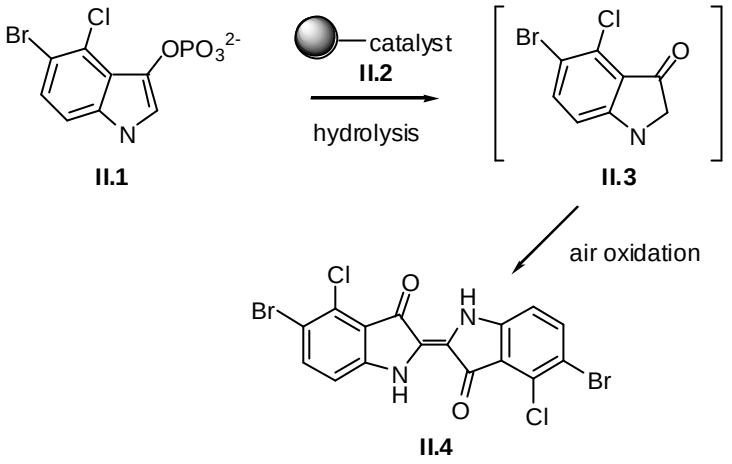


Figure 2.1

Several examples concerning the development of visual detection methods for the discovery of catalysts have already been described in the literature. A first example is the IR-thermographic technique. This method is based on the measurement of modest temperature changes due to either exothermic or endothermic processes. The most catalytically active compounds are identified by the infrared camera as 'hot' or 'cold' spots respectively.[42] Until now, this method has been applied for the screening of parallel libraries but it has not yet been implemented for the screening of libraries as compound mixtures.

[42] (a) Taylor, S.J.; Morken, J.P. *Science* **1998**, *280*, 267; (b) Reetz, M.T.; Becker, M.H.; Küling, K.M.; Holzwarth, A. *Angew. Chem. Int. Ed. Engl*. **1998**, *37*, 2647; (c) Reetz, M.T.; Becker, M.H.; Liebl, M.; Fürstner, A. *Angew. Chem. Int. Ed. Engl*. **2000**, *39*, 1236.

The use of dyes is another technique for visual detection. In two recent publications, Berkessel used dyes to detect polymer bound phosphodiesterase activity.[43] This method is presented in scheme 2.1. When the 3-hydroxylindolyl derivative **II.1** is hydrolysed by a solid-phase bound catalyst **II.2**, the resulting derivative **II.3** spontaneously oxidizes in the presence of air to afford the turquoise and insoluble dye **II.4**, which precipitates on the bead. In this way, the most active catalysts are easily detected.



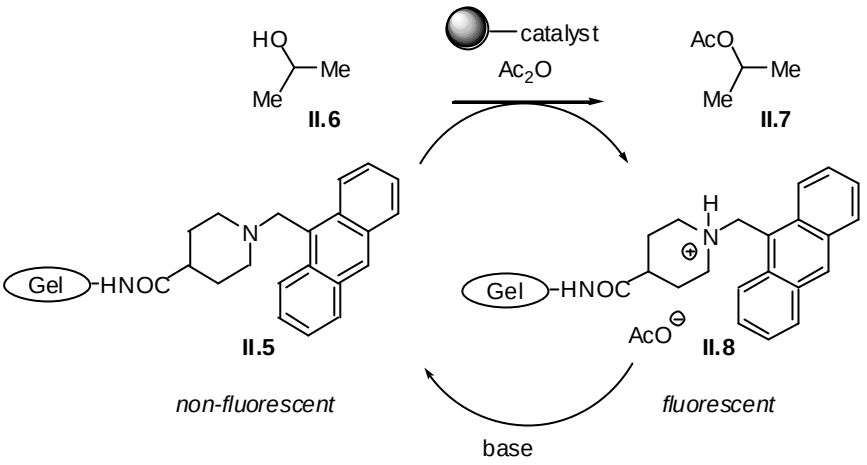
Scheme 2.1

Solid-phase bound catalyst detection can also occur by fluorescence. Miller has developed a polymeric gel containing the molecular sensor **II.5** that fluoresces in presence of a catalyst.[44] The aminomethylanthracene **II.5** is a suitable sensor to detect the acetic acid in the acylation reaction of alcohol **II.6** in presence of the solid-phase bound catalyst (scheme 2.2). The advantage of using a gel is the slow diffusion of product out of the bead affording a fluorescent zone only around the active catalyst. Interestingly, the sensor-functionalised gel is reversibly fluorescent. The presence of acetic acid induces the gel **II.5** to fluoresce due to the formation of **II.8**; washing with base results again in the gel **II.5** that is nonfluorescent. In the same area, Davis has recently published the use of a gel for the screening of combinatorial libraries in aqueous media.[45]

[43] (a) Berkessel, A.; Hérault, D.A. *Angew. Chem. Int. Ed. Engl*. **1999**, *38*, 102; (b) Berkessel, A.; Riedl, R. *J. Comb. Chem.* **2000**, *2*, 215; (c) Danek, S.C.; Queffelec, J.; Morken, J.P. *J. Chem. Soc., Chem. Commun.* **2002**, 528.

[44] Harris, R.F.; Nation, A.J.; Copeland, G.T.; Miller, S.J. *J. Am. Chem. Soc.* **2000**, *122*, 11270.

[45] Müller, M.; Mathers, T.W.; Davis, A.P. *Angew. Chem. Int. Ed. Engl*. **2001**, *40*, 3813.



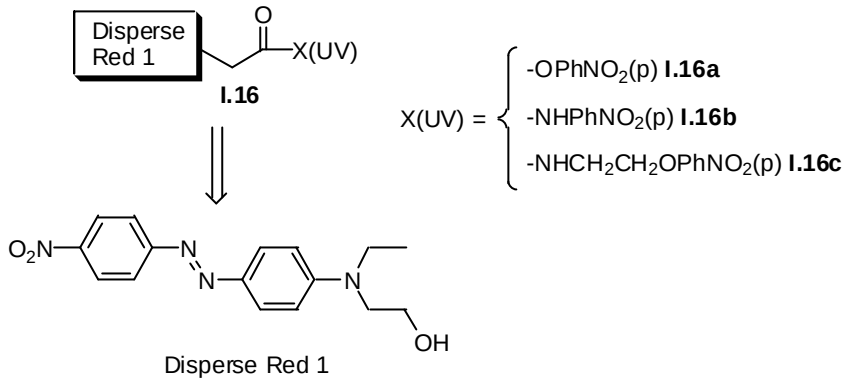
Scheme 2.2

For the screening of our combinatorial library, we chose to develop a new test-substrate answering to the following conditions:

1) the substrate should contain an ester or amide function;
2) the test substrate should allow for visual detection of the solid-phase bound catalyst;
3) the possibility of quantitative measurement for hydrolytic activity has to be taken into consideration;
4) the hydrolysis should occur according to a mechanism related to the enzymatic process observed with serine-proteases, meaning that the acylation step leads to the formation of an acyl-enzyme that is subsequently hydrolysed in the presence of water.

The new test substrates we designed have the general structure **I.16** as presented in scheme 2.3. All derivatives are composed of the same chromogenic unit covalently bound to an ester or amide function with different sensitivity towards hydrolysis: the activated *p*-nitrophenyl ester **I.16a**, the *p*-nitroanilide **I.16b** and the unactivated amide **I.16c**. The chromogenic unit is a derivative of the commercially available dye Disperse Red 1. The choice of Disperse Red 1 is based on the fact that the azo unit is reasonably chemically stable (at least under non-oxidizing conditions) and possesses a λ_{max} larger than 450 nm (λ_{max} = 503 nm).[46]

[46] Green, F.A. *The Sigma-Aldrich Handbook of Stains, Dyes and Indicators* Aldrich Chemical Company, **1990**, 290.



Scheme 2.3

In contrast to the literature examples, our new substrates were designed to allow for a qualitative (visual) and quantitative (UV/VIS spectroscopy) measurement of both the acylation and deacylation step of the enzymatic process. As shown in figure 2.2, when the solid-phase bound catalyst present in the library is exposed to the substrate in an organic solvent in the absence of water, the dye will be transferred to the catalyst resulting in a coloured bead. Moreover, the release of the UV detectable group (X) into the solution will allow for a quantitative measurement of hydrolysis using UV/VIS spectroscopy. Subsequently, when water is added, the acyl-enzyme will be hydrolysed leading to the release of the acid-dye (λ = 480 nm) into the solution and the catalyst will be regenerated.

However, the fact that the screening takes place in an organic solvent is of great importance. If the screening occurred in water, similar to an enzymatic process, the solid-phase bound catalyst would hydrolyse the substrate and the catalyst would be regenerated. It is clear that the detection of the catalyst is impossible.

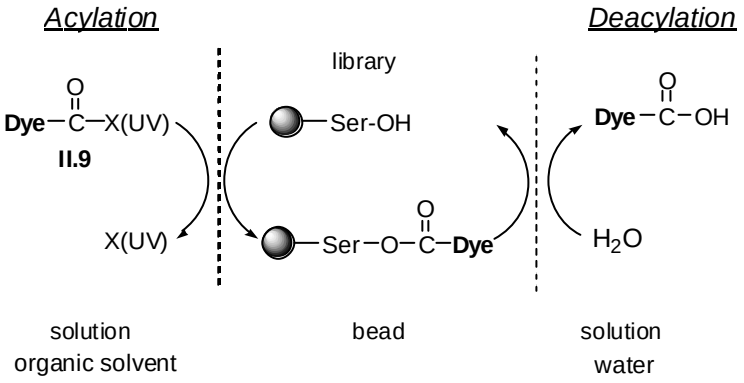


Figure 2.2

The principle of quantitative measurement of the degree of hydrolysis by UV/VIS spectroscopy is explained in more details in table 2.1. In the case of *p*-nitrophenyl ester **I.16a**, the hydrolysis of the *p*-nitrophenyl ester can be monitored at 405 nm (pH > 7) by measuring the amount of *p*-nitrophenolate present in the solution. Concerning the hydrolysis of the *p*-nitroanilide derivative **I.16b**, the release of *p*-nitroaniline is detectable and measurable at 400 nm. Finally, compound **I.16c** is a particularly interesting substrate. The hydrolysis of the amide bound results in the formation of the amine **II.10** detectable at 315 nm leading to an overlap in absorption with the amide **I.16c** at 293 nm. However, upon treatment of **II.10** with a hot aqueous solution of 6% potassium hydroxide, a 'Smiles' rearrangement will take place leading to **II.11**, which is easily detectable at 400 nm.[47]

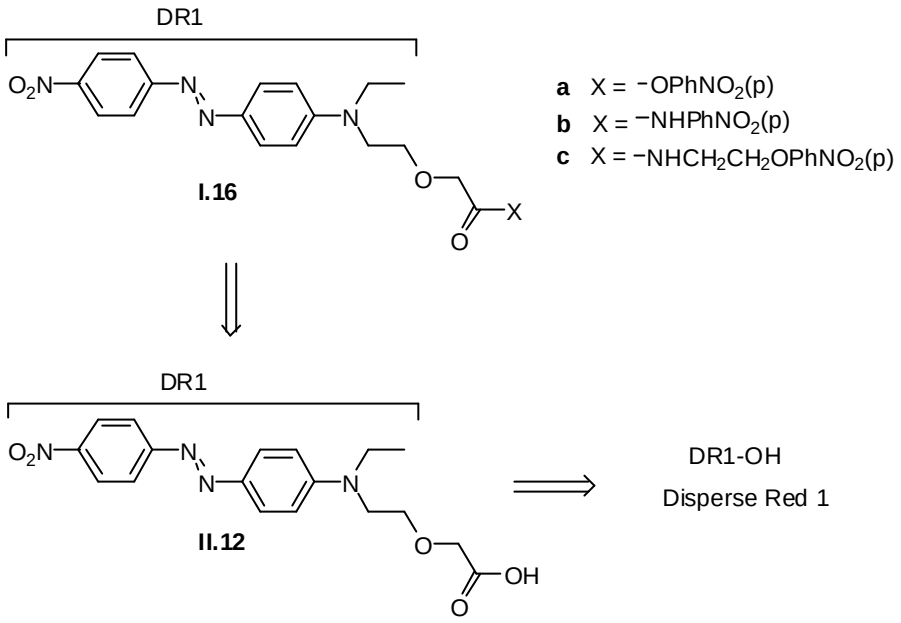
Table 2.1: Quantitative monitoring via UV/VIS spectroscopy

Substrate	λ_{max} (nm)	After hydrolysis	
		Leaving Group (X)	λ_{max} (X) (nm)
I.16a	276 nm, 480 nm	*p*-nitrophenolate	405 nm
I.16b	304 nm, 470 nm	*p*-nitroaniline	400 nm
I.16c	293 nm, 475 nm	*p*-nitrophenoxyethanamine H_2N OPhNO_2*(p)* **II.10** $OH^{\ominus}$ 'Smiles' rearrangement HO NHPhNO_2*(p)* **II.11**	315 nm 400 nm

[47] (a) Knipe, A.C. *Tetrahedron Lett*. **1975**, 3563. (b) Caldwell, W.T.; Schweiker, G.C. *J. Am. Chem. Soc.* **1952**, *74*, 5187.

2.2 Synthesis of I.16

The synthesis of compounds **I.16** was the subject of the first part of this work. *p*-Nitrophenyl ester **I.16a**, *p*-nitroanilide **I.16b** and the amide **I.16c** were prepared starting from the commercially available Disperse Red 1 as outlined in retrosynthetic scheme 2.4.



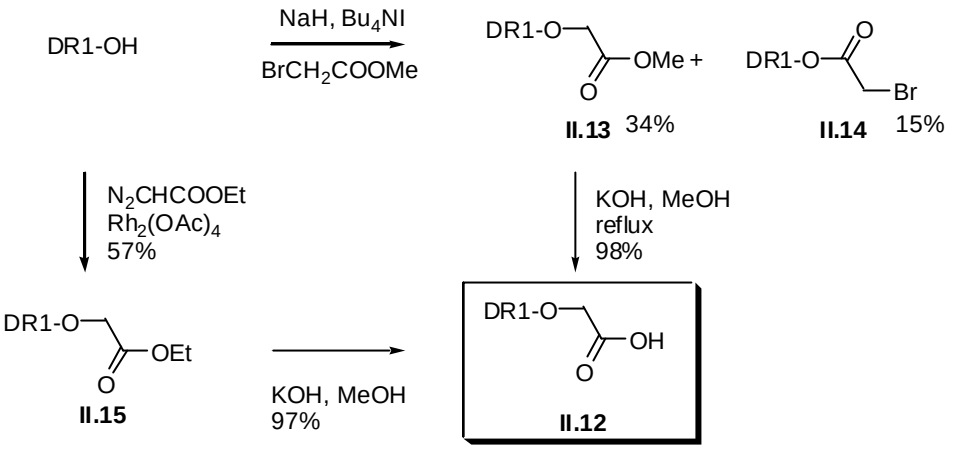
Scheme 2.4

In a first attempt to synthesise carboxylic acid **II.12**, Disperse Red 1 was deprotonated with an excess of base followed by the addition of chloro or bromoacetic acid in a classical Williamson ether synthesis. However, only starting material or a complex mixture was obtained (table 2.2).

Table 2.2: Alkylation of Disperse Red 1 with chloro or bromoacetic acid

	XCH_2COOH (eq.)	base (eq.)	solvent	temp., time	result
1	Cl (5.2 eq.)	NaH (6.2 eq.)	THF	reflux, 12 h	no reaction
2	Br (5.2 eq.)	NaH (6.2 eq.)	THF	reflux, 15 h	no reaction
3	Br (2 eq.)	KOH (4 eq.)	DMSO	23 °C, 15 h	no reaction
4	Br (2 eq.)	KOH (4 eq.)	DMSO	55 °C, 15 h	complex mixture

Alternatively, carboxylic acid **II.12** can be prepared in a two step-sequence via alkylation of Disperse Red 1 with bromomethyl acetate followed by the hydrolysis of ester **II.13** (scheme 2.5).



Scheme 2.5

Different conditions were tested and gave poor results (table 2.3). The use of silver oxide[48] as reagent gave no reaction or a complex mixture even when a catalytic amount of tetrabutylammonium iodide was added. When Disperse Red 1 was deprotonated with sodium hydride in tetrahydrofuran under reflux conditions, followed by addition of bromomethyl acetate, a complex mixture was obtained. Changing the solvent or the temperature did not lead to any improvement. Finally, the addition of a catalytic amount of tetrabutylammonium iodide led to the formation of methyl ester **II.13** in 34% yield, next to 15% of the transesterification by-product **II.14**.[49]

Finally, treatment of Disperse Red 1 with ethyl diazoacetate (scheme 2.5) in presence of a rhodium catalyst afforded ethyl ester **II.15** in a satisfactory yield of 57%.[50] Subsequently, esters **II.13** and **II.15** were transformed into the carboxylic acid **II.12** with potassium hydroxide in methanol in almost quantitative yield.

[48] Bouzide, A.; Sauvé, G. *Tetrahedron Lett*. **1997**, *38*, 5945.

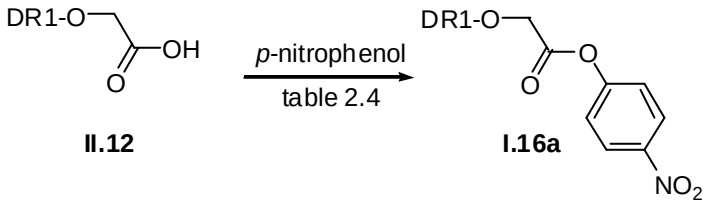
[49] (a) Cznernecki, S.; Georgoulis, C.; Provelenghiou, C. *Tetrahedron Lett*. **1976**, 3535; (b) Kanay, K.; Sakamoto, I.; Ogawa, S.; Suami, T. *Bull. Chem. Soc. Jpn*. **1987**, *60*, 1529.

[50] Paulissen, R.; Reimlinger, H.; Hayez, E.; Hubert, A.J.; Teyssié, Ph. *Tetrahedron Lett*. **1973**, *24*, 2233.

Table 2.3: Alkylation of Disperse Red 1 with bromomethyl acetate

	Base (eq.) Reagent (eq.)	Solvent	Temp. time	Results (% **II.13)**
1	Ag_2O (1.5 eq.)	CH_2Cl_2	r.t., 16 h then reflux	no reaction complex mixture
2	Ag_2O (1.5 eq.) Bu_4NI (0.01 eq.)	CH_2Cl_2	r.t.	trace of **II.13**
3	NaH (2 eq.)	THF	Reflux, 15 h	complex mixture
4	NaH (1 eq.)	DMF	r.t., 36 h	complex mixture
5	NaH (1.6 eq.) Bu_4NI (0.01 eq.)	DMF	r.t., 15 h	complex mixture
6	NaH (1.6 eq.) Bu_4NI (0.01 eq.)	THF	r.t., 15 h	**II.13** (34%) **II.14** (15%)

During the esterification of **II.12** with *p*-nitrophenol, some difficulties were encountered (table 2.4). One of the standard conditions to esterify a carboxylic acid is the use of dicyclohexylcarbodiimide in presence of a catalytic amount of 4-dimethylaminopyridine.[51] When this method was applied to the synthesis of *p*-nitrophenyl ester **I.16a**, a low yield of 35% was obtained. Next to the ester **I.16a,** 32% of a side-product was present and was identified as the O-acylurea **II.16**.



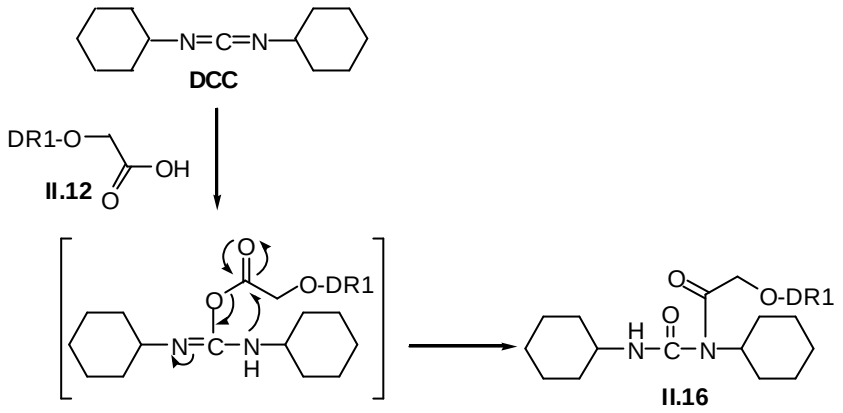
II.12 **I.16a**

Table 2.4: Conditions for the synthesis of *p*-nitrophenyl ester **I.16a**

	Reagent (1.1 eq.)	Cat. (0.01 eq.)	Solvent	Temp., time	Result (% **I.16a)**
1	DCC	DMAP	CH_2Cl_2	r.t., 18 h	35% **I.16a** 32% **II.16**
2	DCC	4-pyrrolidinopyridine	CH_2Cl_2	r.t., 24 h	42%
3	$POCl_3$		Pyridine	-15 °C, 4 h	93%

[51] Neises, B.; Andries, T.; Steglich, W. *J. Chem. Soc., Chem. Commun.* **1982**, 1132.

The mechanism of the formation of **II.16** is presented in scheme 2.6 and involves the O → N acyl transfer via a four-membered transition state. This side-reaction has been reported to be solvent independent[52] and can be avoided by decreasing the reaction temperature to 0 °C.

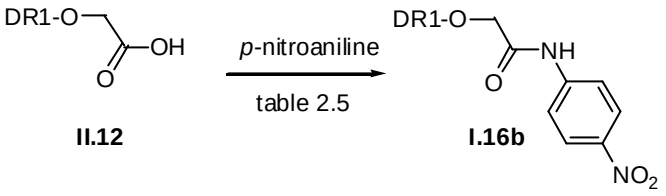


Scheme 2.6

The use 4-pyrrolidinopyridine as catalyst gave no significant improvement in yield.[53] In our case, using phosphorus oxychloride[54] turned out to be the best method for the activation of the carboxylic acid **II.12**. The *p*-nitrophenyl ester **I.16a** -also called **NF31** (see appendix)[55]- was successfully obtained in 93% yield.

Concerning the synthesis of *p*-nitroanilide **I.16b**, three different methods were tested (table 2.5). After activation of the carboxylic acid with isobutylchloroformate,[56] the anilide **I.16b** could be prepared with a poor yield of 18%. The use of carbodiimides as coupling reagent resulted in a significant improvement and afforded the anilide in 41% yield. However, the use of phosphorus oxychloride as activating reagent was also the most successful method and provided pure **I.16b** as the sole product in 57% yield after column chromatography.

[52] Hirschmann, R.; Nutt, R.F.; Veber, D.F.; Vitali, R.A.; Varga, S.L.; Jacob, T.A.; Holly, F.W.; Denkewalter, R.G. *J. Am. Chem. Soc.* **1969**, *91*, 507.
[53] Hassner, A.; Alexanian, V. *Tetrahedron Lett.* **1978,** *46*, 4475.
[54] Rijkers, D.T.S.; Hemker, H.C.; Nefkens, G.H.L.; Tesser, G.I. *Recl. Trav. Chim. Pays-Bas* **1991**, *110*, 347.
[55] Since 2001, **NF31** is commercially available from 'BACHEM', catalog 2002, p 1282, 50 mg cost 150 Euro
[56] Nedev, H.; Naharisoa, H.; Haertlé, T. *Tetrahedron Lett.* **1993**, *34*, 4201.

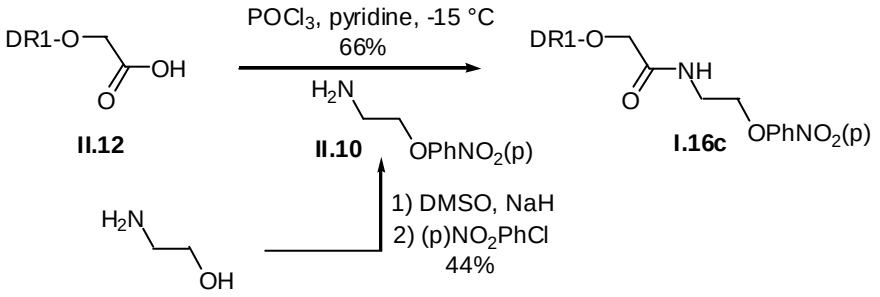


II.12 **I.16b**

Table 2.5: Synthesis of *p*-nitroanilide **I.16b**

	Reagent (1.1 eq.)	Cat. (0.01 eq.)	Solvent	Temp., time	Result (% **I.16b)**
1	*iso*BuOCOCl	-	CH_2Cl_2 / DMF 9 / 1	r.t., 24 h	18%
2	DCC	4-pyrrolidinopyridine	CH_2Cl_2	r.t., 24 h	41%
3	$POCl_3$	-	pyridine	-15 °C, 3 h	57%

Finally, phosphorus oxychloride was also the reagent of choice to synthesise the amide **I.16c** (scheme 2.7). *p*-Nitrophenoxyethanamine **II.10**, prepared from 2-aminoethanol and *p*-nitrochlorobenzene as described in the literature[57] reacted with the carboxylic acid **II.12** in presence of phosphorus oxychloride to afford the amide **I.16c** in 66% yield.



II.12 **II.10** **I.16c**

Scheme 2.7

[57] Knipe, A.C.; Sridhar, N. *Synthesis* **1976**, 606.

2.3 Applications: a novel colorimetric assay for visual detection of solid-phase bound amines

2.3.1 Introduction

Even if the enthusiasm at that time was limited, the introduction of solid phase chemistry by Merrifield in 1963 in the field of peptide synthesis was a great invention. Today, the monitoring of reactions on solid support still remains somewhat tricky. As a matter of fact, the use of traditional direct analytical methods (NMR, ES-MS, TLC) is very limited and until now, only a few techniques are available for monitoring reactions on solid support. In particular, concerning solid-phase peptide synthesis, colorimetric methods are widely used to evaluate the effectiveness of a coupling step. Since no intermediate purification is performed, it is important that every coupling step occurs in an almost quantitative yield.

A brief overview of the more common colorimetric methods is presented in table 2.6.

Picric acid[58] and bromophenol blue[59] are two colorimetric tests allowing the detection of polymer-supported amines by acid/base titration. Another method relies on a two step-process and involves attachment of a reporter followed by a selective detachment and quantitation. The dimethoxytrityl (DMT) cation test[60] and the 'nitrophenylisothiocyanate-O-trityl' (NPIT) test[61] are illustrative examples. The most popular examples of colorimetric tests with visual detection are the ninhydrin test[62], the increasingly used 2,4,6-trinitrobenzenesulfonic acid (TNBS) test[63] and the recently described '4-N,N'-dimethylaminoazobenzene-4'-isothiocyanate' (DABITC) test.[64]

[58] Gisin, B.F. *Anal. Chim. Acta* **1972**, *58*, 248.
[59] Krchnak, V.; Vagner, J.; Safar, P.; Lebl, M. *Collect. Czech. Chem. Commun.* **1988**, *53*, 2542.
[60] Reddy, M.P.; Voelker, P.J. *Int. J. Peptide Protein Res.* **1988**, *31*, 345.
[61] Chu, S.S.; Riech, S.H. *Bioorg. Med. Chem. Lett.* **1995**, 1053.
[62] Kaiser, E.; Colescott, R.L.; Bossinger, C.D.; Cook, P.I. *Anal. Biochem.* **1981**, *117*, 147.
[63] Hancock, W.S.; Battersby, J.E. *Anal. Biochem.* **1976**, *71*, 260.
[64] Shah, A.; Rahman, S.S.; De Biasi, V.; Camilleri, P. *Anal. Commun.* **1997**, *34*, 325.

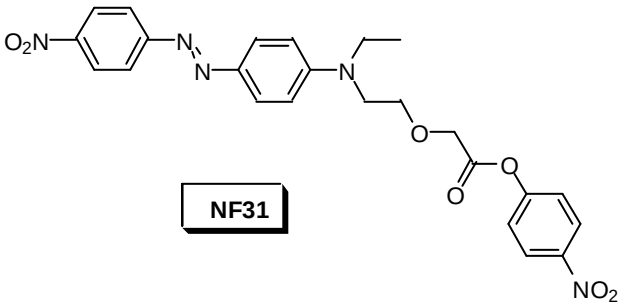
Table 2.6: Overview of monitoring methods for solid phase peptide synthesis

Test	Features
Ninhydrin[62]	Qualitative detection and quantitative determination by UV
Picric acid[58]	Quantitative determination by acid/base titration followed by UV measurement
TNBS[63]	Visual detection of primary amines (orange)
Bromophenol blue[59]	Visual detection of free amines (blue) by acid/base titration
DMT[60]	Quantitative determination by a tritylation-detritylation process followed by UV measurement
Chloranil	Visual detection of free amines (blue)
NPIT[61]	Qualitative and quantitative determination by a tritylation-detritylation process followed by UV measurement
DABITC[64]	Visual detection of primary and secondary amines (orange)

However, these latter tests are not devoid of some drawbacks. In particular, the ninhydrin test cannot be used for coupling to proline; the TNBS test gives erroneous results in the case of sterically hindered amines. So, there is still a great demand for novel sensitive colorimetric tests to monitor coupling efficiency in a simple and reliable way.

2.3.2 A new colorimetric test[65]....

Our newly developed substrate **I.16a** (**NF31**) appeared to be also very useful in the detection of solid-phase bound amines.



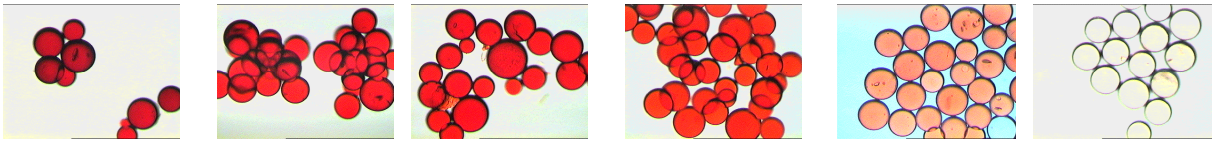
NF31

[65] Madder, A.; Farcy, N.; Hosten, N.G.C.; De Muynck, H.; De Clercq, P.J.; Barry, J.; Davis, A.P. *Eur. J. Org. Chem.* **1999**, 2787.

This new test is based on the reaction of unreacted solid-phase bound amines with **NF31**, resulting in a transfer of colour to the bead. After thorough washing, beads containing free amino functions appear as red spheres whereas completely coupled beads remain colourless. Longer reaction times or lower temperatures do not influence the outcome of the test. So far, solid supports that have been tested include TentaGel-NH_2, Novagel and Wang resins.

To be useful as a new colorimetric test, the sensitivity of the assay needed to be evaluated. Therefore, a series of resin samples of known free amine content was prepared by treatment of Wang-Gly-NH_2 with a mixture of Fmoc-Ala and Boc-Ala in different proportions. After selective removal of the Fmoc protecting group, beads were obtained with a free amine content of 50, 20, 10, 5, 2 % (see experimental part). The different samples were treated with both the **NF31** test and the TNBS test for visual comparison. From figure 2.3, it can be seen that the **NF31** test, due to the appearance of a distinct red colour in presence of free amino groups is more sensitive than the TNBS test. Since resins often become yellow when exposed to chemicals, the detection of 5% free amine using the TNBS test remains difficult.

NF31 test



50% 20% 10% 5% 2% 0%

TNBS test

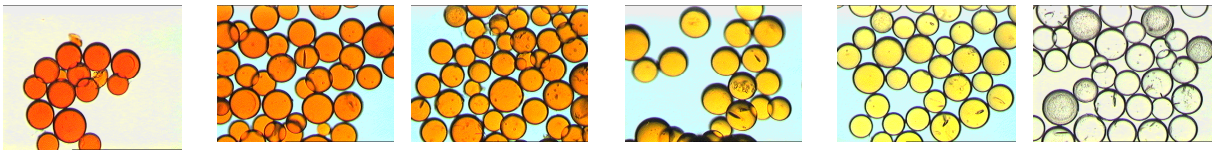
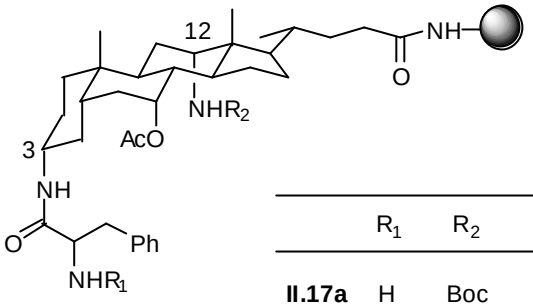


Figure 2.3

An important feature of the **NF31** test is its high reactivity towards sterically hindered amines. The following experiment illustrates this feature (figure 2.4). Derivative **II.17a** was obtained by coupling of TentaGel-NH_2 with steroid-based scaffold **I.13**.[66]



	R_1	R_2	TNBS	NF31
II.17a	H	Boc	orange	red
II.17b	Ac	Boc	colourless	colourless
II.17c	Ac	H	colourless	red

Figure 2.4

The free amino function on position 3 in **II.17a** (R_1 = H) reacts with the TNBS reagent yielding orange beads. However, the TNBS test on amine **II.17c**, obtained by capping of the free amine in **II.17a** and deprotection of the Boc-protected amine **II.17b** on position 12, did not lead to orange beads probably due to sterical hindrance. In contrast, treatment of the same resin with **NF31** resulted in intense red coloured beads proving the presence of free amines. Finally, another feature of the **NF31** test is based on the possibility to follow coupling reactions to proline. Whereas ninhydrin and TNBS tests give negative results with proline, treatment of TentaGel-Pro-NH with **NF31** leads to red beads.

[66] Hilde De Muynck, PhD-thesis *'Ontwikkeling van modelverbindingen voor Serine Proteasen via combinatorische tecknieken'*, **2000**.

2.4 Conclusion

In the first part of this work, we synthesised successfully three Disperse Red 1 derivatives **I.16**, each of them presenting a different sensitivity towards hydrolysis. The most reactive *p*-nitrophenyl ester **I.16a** is actually commercially available and is applied in two areas: 1) it is used as a new test-substrate[41] for the visual detection of active catalysts during the screening of combinatorial libraries; 2) it is used as a new colorimetric test[65] for the detection of solid-phase bound amines. Furthermore, the **NF31** test is particularly useful in the case of sterically hindered amines and for coupling reactions to proline, cases where other colorimetric tests fail.

3 The tripodal scaffold

3.1 A new tripodal scaffold

"Smaller, disordered peptides may contain all the functional groups of the enzyme active site, but even if the peptide is capable of assuming a catalytic conformation, it is conceivable, given the enormous magnitude of shape space accessible to a peptide of even thirty amino acid residues, that the age of the planet would elapse before the desired conformations were to be assumed. In short, all the major features of enzymatic catalysis are likely to be deeply flawed or entirely missing in peptides without fixed conformation".[35e] This is the reason why a multipodal scaffold is an essential element to mimic enzymes. The role of the scaffold is to mimic the three-dimensional structure of the enzyme by creating a fixed conformation in order to bring the peptide strands in proximity. One of the most important features of a scaffold is the presence of several orthogonal functionalities, allowing for the generation of independent strands in order to create an appropriate environment for catalysis similar to the active site of the enzyme.

In the literature, only a few scaffolds that allow for the independent generation of peptide strands have been described. The cholic acid derivative **III.1** developed by Still (figure 3.1) is the most famous example of a polyfunctional template which enables the generation of two parallel and different peptide strands.[67] Kemp's acid **III.2**[69b] and the triazacyclophane **III.3**[69i] are two other illustrative examples of conformationally constrained scaffolds. Both possess orthogonal protecting groups allowing for the independent introduction of parallel-oriented chains. A main feature of most scaffolds described in literature so far, is their rigidity and the limited free rotation of the peptide strands.[68]

[67] (a) Boyce, R.; Li, G.; Nestler, P.; Suenaga, T.; Still, W.C. *J. Am. Chem. Soc.* **1994**, *116*, 7955; (b) Cheng, Y.; Suenaga, T.; Still, W.C. *J. Am. Chem. Soc.* **1996**, *118*, 1813.

[68] (a) Pátek, M.; Drake, B.; Lebl, M. *Tetrahedron Lett.* **1994**, *35*, 9169; (b) Kocis, P.; Issakova, O.; Sepetov, N.; Lebl, M. *Tetrahedron Lett.* **1995**, *36*, 6623; (c) Wess, G.; Bock, K.; Kleine, H.; Kurz, M.; Guba, W.; Hemmerle, H.; Lopez-Calla, E.; Baringhaus, K.-H.; Glombik, H.; Enhsen, A.; Kramer, W. *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 2222; (d) Wunberg, T.; Kallus, C.; Opatz, T.; Henke, S.; Schmidt, W.; Kunz, H. *Angew. Chem. Int. Ed. Engl.* **1998**, *37*, 2503; (e) Broderick, S.; Davis, A.P.; Williams, R.P. *Tetrahedron Lett.* **1998**, *39*, 6083; (f) Barry, J. F.; Davis, A. P.; Pérez-Payan, M. N. *Tetrahedron Lett.* **1999**, *40*, 2849; (g) Kallus, C.; Opatz, T.; Wunberg, T.; Schmidt, W.; Henke, S.; Kunz, H. *Tetrahedron Lett.* **1999**, *40*, 7783; (h) Pattarawarapan, M.; Burgess, K. *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 4299; (i) Opatz,T.; Liskamp, R.M.J. *Org. Lett.* **2001**, *3*, 3499.

Until now, the availability of orthogonally protected flexible scaffolds is quite limited. The polymethylene derivative **III.4** (figure 3.1) developed by Nestler is an example.[69] However, due to the very high flexibility of this methylene moiety, it is unlikely that the peptide chains will adopt a parallel orientation as observed for the rigid cholic derivative **III.1**. In a comparative study, the latter flexible chain turned out to be less efficient than rigid analogue **III.1**.

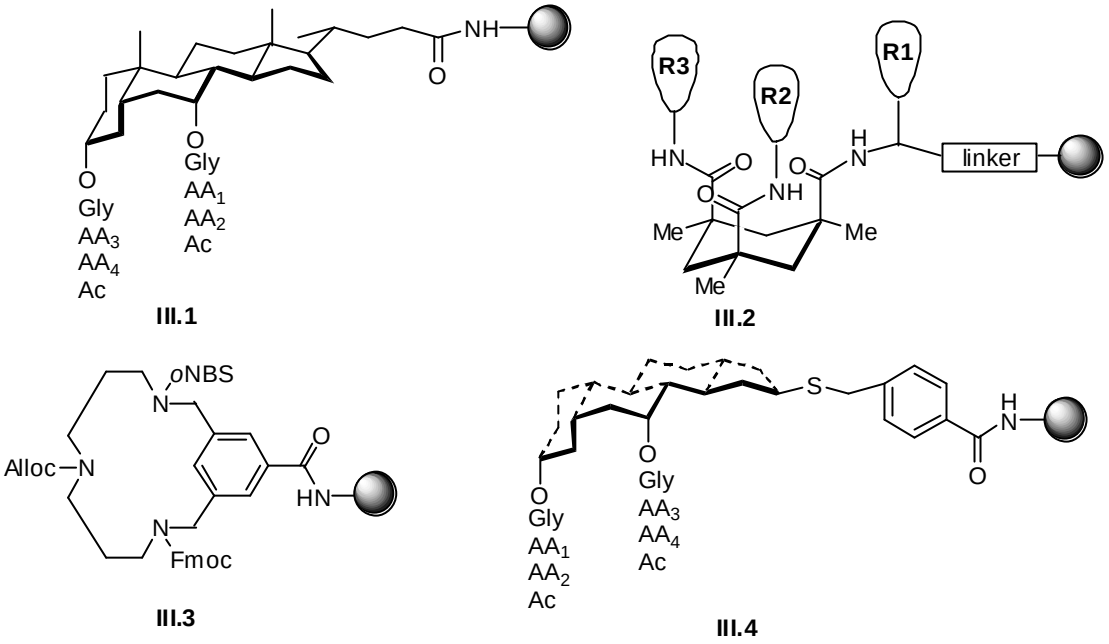
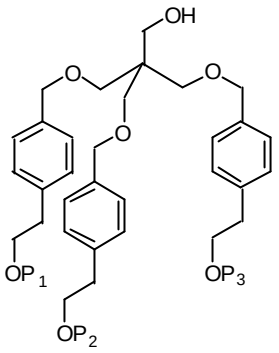


Figure 3.1

In our strategy towards the development of artificial hydrolases, a new scaffold had to be designed. In addition to our former work on a rigid dipodal scaffold, the development of a flexible tripodal scaffold was obvious. Indeed, flexible scaffolds compared to the rigid ones will have a better chance of being able to catalyse a reaction as they will be able to attain a greater diversity in conformation. On the other hand, the flexibility has to be limited in order to generate parallel peptide strands. The structure of the new flexible scaffold we designed is presented in figure 3.2.

[69] (a) Nestler, H.P. *Mol. Div.* **1996**, *2*, 35; (b) Seneci, P.; Sizemore, C.; Islam, K.; Kocis, P. *Tetrahedron Lett.* **1996**, *37*, 6319; (c) Heinonen, P.; Rosenberg, J.; Lönnberg, H. *Eur. J. Org. Chem.* **2000**, *21*, 3647; (d) Virta, P.; Rosenberg, J.; Karskela, T.; Heinonen, P.; Lönnberg, H. *Eur. J. Org. Chem.* **2001**, *18*, 3467.

The tripodal template **III.5** possesses a chemical structure that should allow for the generation of three parallel-oriented peptide strands. It was predicted and confirmed via modelling studies that the three benzylic chains adopt a parallel orientation.[66] On the other hand, due to its flexibility, the peptide strands should have enough freedom to adopt the best conformation for catalysis.



III.5

Figure 3.2

Furthermore, the protecting groups (figure 3.3) were chosen in such a way that they enable a selective deprotection: the tetrahydropyranyl (THP) ether is an acid labile protecting group and is cleaved under mild hydrolytic conditions, the 3,4-dimethoxyphenylmethyl (DMPM) ether on the other hand is removed under oxidative conditions and finally, the *tert*-butyldiphenylsilyl (TBDPS) ether is deprotected with fluoride anion. [70]

Next to the three orthogonally protected hydroxyl functions, the free hydroxyl function in scaffold **III.5** will allow the coupling to a solid support.

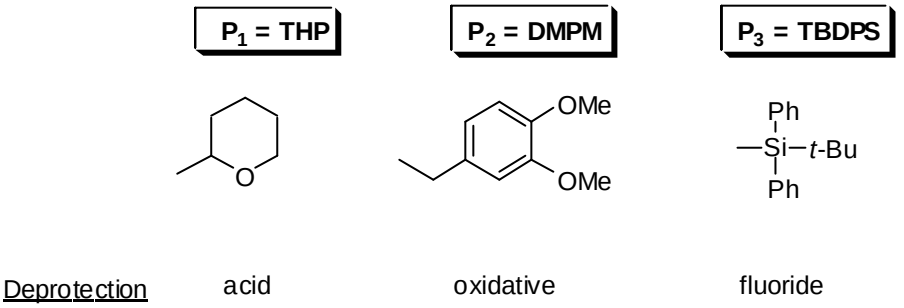
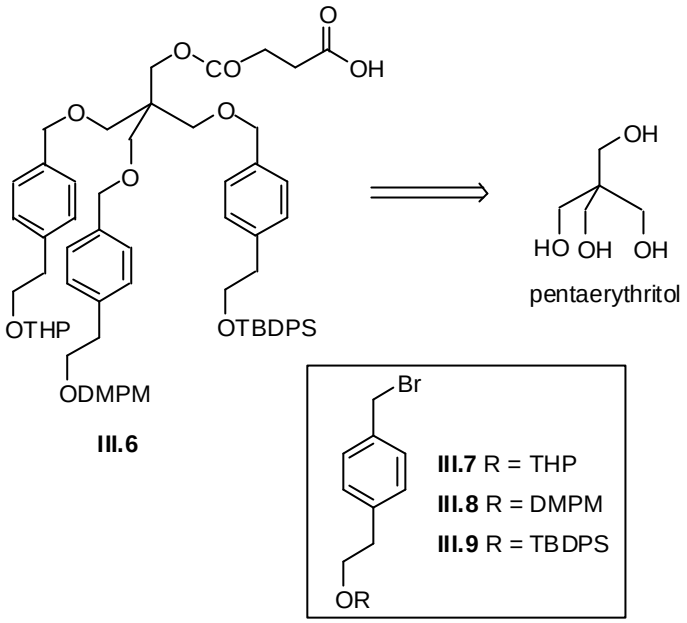


Figure 3.3

[70] Schelbaas, M.; Waldmann, H. *Angew. Chem. Int. Ed. Engl*. **1996**, *35*, 2056.

3.2 Synthesis of the tripodal scaffold **III.6**

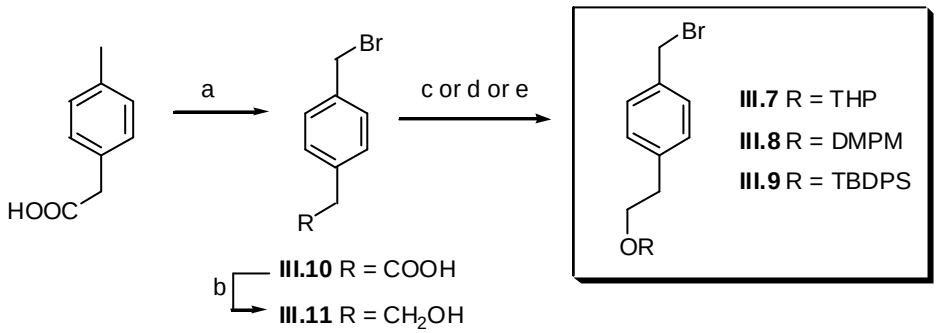
The flexible tripodal scaffold **III.6** was successfully synthesised, in its racemic form, starting from the commercially available pentaerythritol via consecutive Williamson ether syntheses with the building blocks **III.7, III.8** and **III.9** as outlined in the retrosynthetic scheme 3.1.



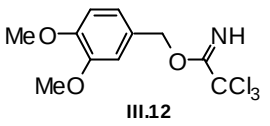
Scheme 3.1

The benzylic bromides **III.7, III.8** and **III.9** were prepared as described in scheme 3.2. The commercially available 4-methylphenylacetic acid was brominated in the presence of a catalytic amount of AIBN to afford compound **III.10**.[71] Subsequently, carboxylic acid **III.10** was reduced with borane-methyl sulfide complex to form alcohol **III.11**.[72] The introduction of the three protecting groups was performed according to known procedures.[73] Nevertheless, some problems were encountered in the introduction of the DMPM group to prepare building block **III.8**. Acceptable results were only obtained when alcohol **III.11** was reacted with an excess of acetimidate **III.12** in the presence of a catalytic amount of PPTS to afford **III.8** in 69% yield.

[71] Mitchell, A.R.; Kent, S.B.H. Engelhard, M.; Merrifield, R.B. *J. Org. Chem.* **1978**, *43*, 2845.
[72] Praly, J.P.; Descotes, G. *Tetrahedron Lett.* **1987**, *28*, 1405.
[73] (a) **THP**: Miyashita, N.; Yoshikoshi, A.; Grieco, P.A. *J. Org. Chem.* **1977**, *42*, 3772; (b) **DMPM**: Nakajima, N.; Saito, M.; Ubukata, M. *Tetrahedron Lett.* **1988**, *39*, 5565; (c) **TBDPS**: Guindon, Y.; St Denis, Y.; Daigneau, S.; Morton, H.E. *Tetrahedron Lett.* **1986**, *27*, 1237.



a) Br_2, AIBN, CCl_4, 62%;
b) $BH_3.SMe_2$, THF, 86%;
c) **THP**: PPTS, dihydropyran, CH_2Cl_2, 88%
d) **DMPM**: acetimidate **III.12**, PPTS, CH_2Cl_2, 69%
e) **TBDPS**: TBDPSCl, DIPEA, DMF, 71%

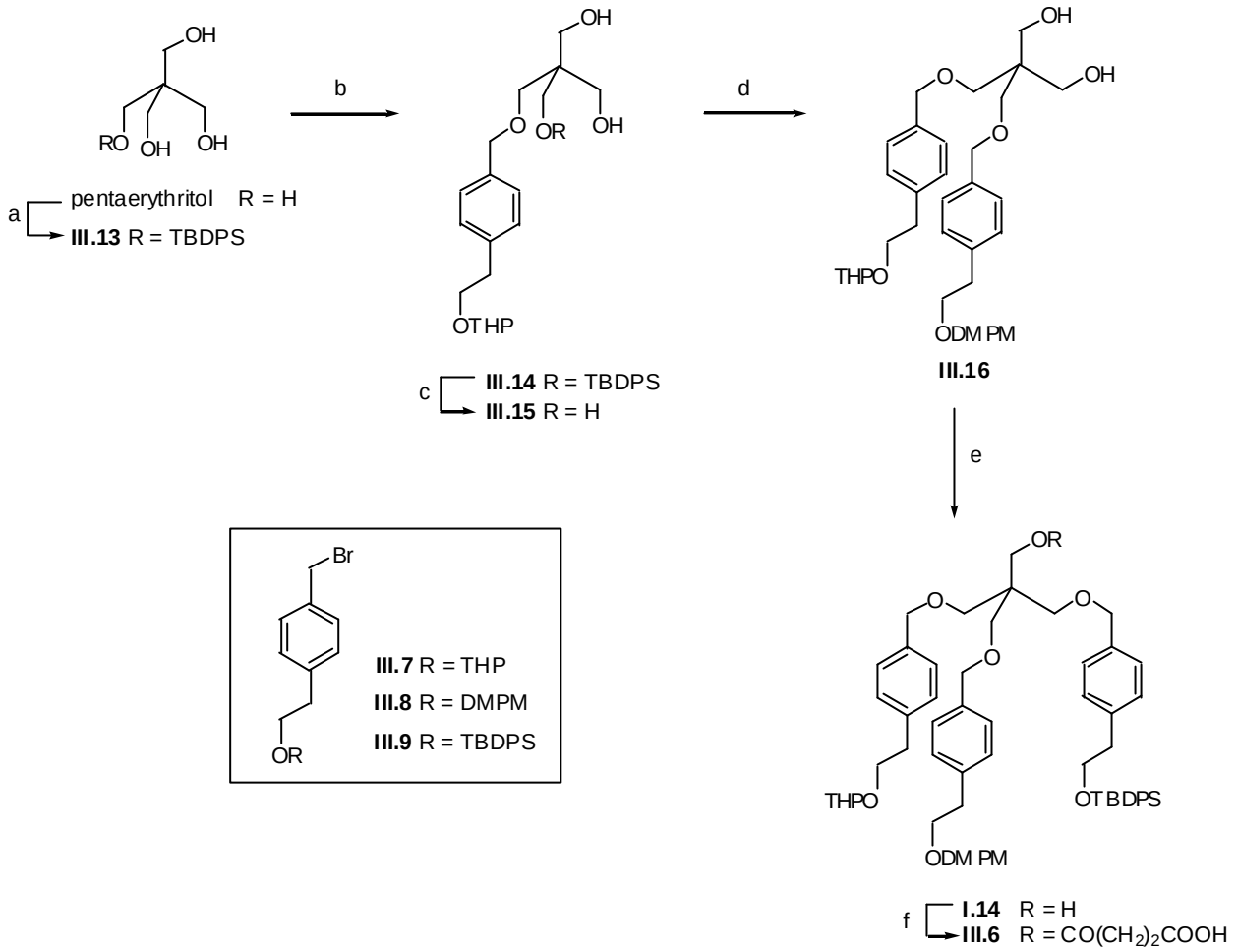


III.12

Scheme 3.2

The synthesis of the scaffold **III.6** started from the commercially available pentaerythritol. The first benzylic chain was introduced in a three-step sequence involving temporary protection of one of the hydroxymethyl groups as a *tert*-butyldiphenylsilylether to afford triol **III.13**.[74] Subsequently, a classical Williamson ether synthesis with benzyl bromide **III.7** led to the formation of **III.14**. Finally, alcohol **III.15** was obtained by deprotection of the silyl ether in **III.14** with TBAF. The two other benzylic chains were introduced in a similar way by ether formation with benzyl bromides **III.8** and **III.9** to form derivatives **III.16** and **I.14** in 32% and 49% yield respectively. The remaining alcohol function was finally treated with succinic anhydride to form target molecule **III.6** in 90% yield. The succinic moiety serves as a spacer for attachment of **III.6** to the solid phase. Incorporation of a spacer is expected to facilitate the connection to the solid support by increasing the distance between the polymer and the attachment point of the scaffold **III.6**.

[74] Hanessian, S.; Parbhanjan, H.; Qiu, D.; Nambiar, S. *Can. J. Chem.* **1996**, *74*, 1731.



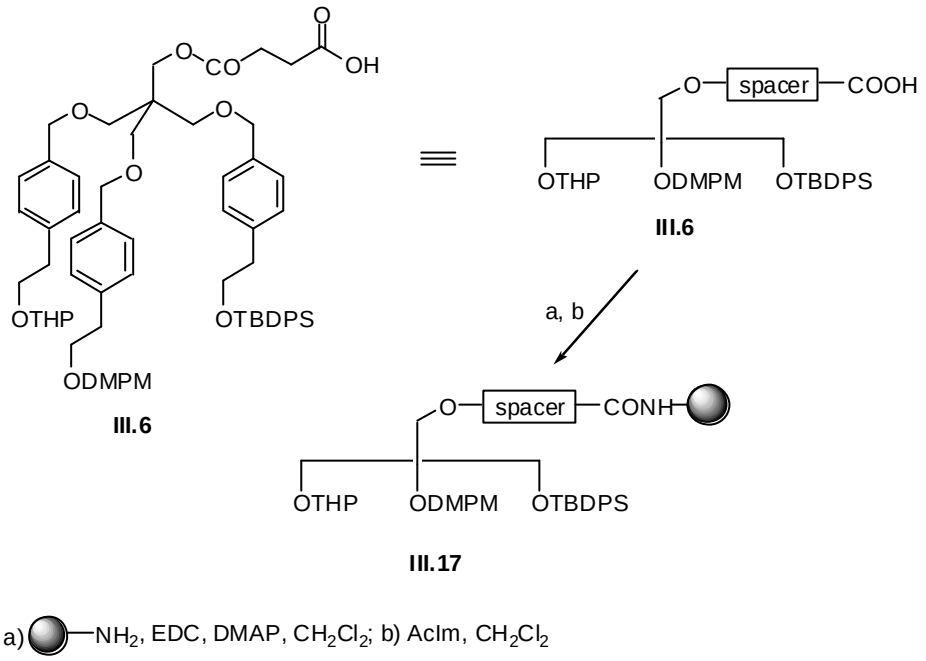
a) TBDPSCl, HIm, DMF, 68%; b) **III.7**, NaH, THF, 43%; c) TBAF, THF, 83%;
d) **III.8**, NaH, THF, 32%; e) **III.9**, NaH, THF, 49%; f) Succinic anhydride, DMAP, CH_2Cl_2, 90%

Scheme 3.3

With the scaffold in hand, we were eager to start exploring the solid phase chemistry part of this work. After attachment of scaffold **III.6** on the solid phase, we were particularly interested in the selective deprotection of the protecting groups THP, DMPM and TBDPS.

3.3 Study of the selective deprotection of the THP, DMPM and TBDPS protecting groups

Carboxylic acid **III.6** was first coupled to the solid phase TentaGel-NH_2 using EDC as coupling reagent in the presence of a catalytic amount of DMAP (scheme 3.4). The resin was then treated with a large excess of acetylimidazole to cap any remaining free amino functions to form polymer-supported scaffold **III.17**. The structure of the tripodal scaffold **III.6** will be symbolised as presented in scheme 3.4.



Scheme 3.4

TentaGel resin[75], which presents excellent swelling properties in polar and non-polar solvents, was chosen as solid support. Its solution-like behaviour allows for ^{13}C gel phase NMR monitoring in order to determine the success or failure of a chemical transformation.[76] It has to be mentioned that this technique does not require any special instrumentation.[77]

In figure 3.4a, the ^{13}C gel phase NMR spectrum of the polymer-supported **III.17** in CD_2Cl_2 is presented. Some characteristic peaks were very helpful to control the success of the following reactions. The peak at 100 ppm represents the acetal carbon of the THP protecting group. The

[75] TentaGel resin consists of grafted polyethylene glycol chains on a cross-linked polystyrene polymer.
[76] Bayer, E. *Angew. Chem. Int. Ed. Engl*. **1991**, *30*, 113.
[77] Look, G.C.; Holmes, C.P.; Chinn, J.P.; Gallop, M.A. *J. Org. Chem.* **1994**, *59*, 7588.

peaks at 112 ppm and 121 ppm are characteristic peaks for the DMPM ether and the peaks at 135 ppm (Ph) and 28 ppm (*tert*-Bu) are associated with the TBDPS ether.

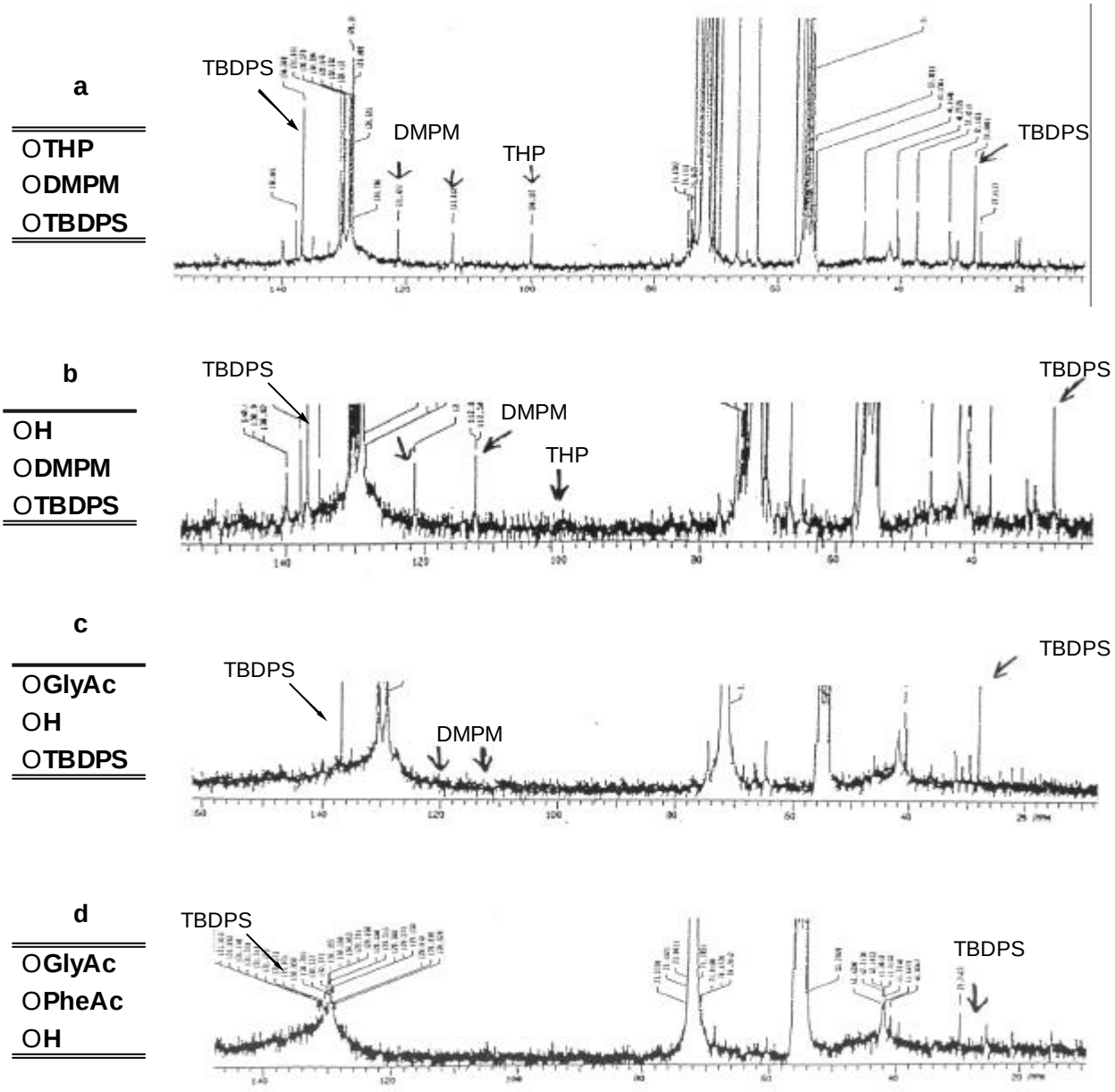
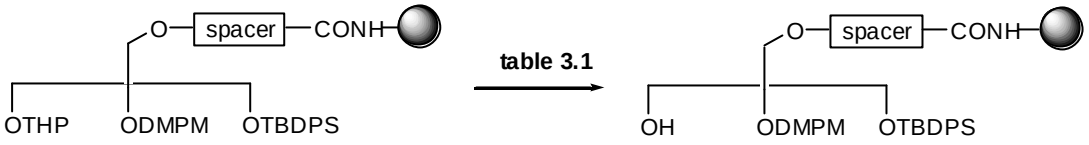


Figure 3.4: ^{13}C gel phase NMR in CD_2Cl_2

For the selective deprotection of the THP-protecting group in **III.17**, several reaction conditions were tested as presented in table 3.1. The use of excess PPTS[78a] in a mixture of methanol/1,4-dioxane at room temperature failed to give any deprotection of the THP group. On the other hand, a stronger acid such as PTSA[78b] led to the deprotection of both THP and DMPM

[78] (a) Miyashita, N.; Yoshikoshi, A.; Grieco, P.A. *J. Org. Chem.* **1977**, *42*, 3772; (b) Gala, D.; Steinman, M.; Jaret, R.S. *J. Org. Chem.* **1986**, *51*, 4488; (c) Bernady, K.F.; Floyd, M.B.; Poletto, J.F.; Weiss, M.J. *J. Org. Chem.* **1979**, *44*, 1438.

ethers. Using a solution of acetic acid in dichloromethane and water at 55 °C led to a complete deprotection of the THP group to form alcohol **III.18**.[78c]



III.17 **III.18**

Table 3.1: Selective deprotection of the tetrahydropyranyl protecting group

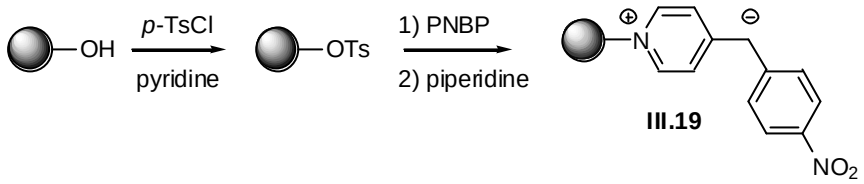
Reagent	Eq. or concentration	Solvent (ratio %)	Time, Temp.	Results
PPTS	4			
PPTS	6	MeOH / 1,4-dioxane (50/50)	Overnight, r.t.	-
PPTS	15			
PTSA	4	MeOH	Overnight, r.t.	Deprotection of THP and DMPM
AcOH	40%	CH_2Cl_2 / H_2O (35/25)	Overnight, r.t.	-
AcOH	80%	CH_2Cl_2 / H_2O (15/5)	Overnight, r.t.	-
AcOH	95%	H_2O (5)	Overnight, r.t.	-
AcOH	80%	CH_2Cl_2 / H_2O (15/5)	6 times 2h 4 h, 55-60 °C	Deprotection THP

To estimate the efficiency of a reaction on solid-phase one usually employs a colorimetric test. For the specific detection of free alcohol functions on solid support, several methods have been described in the literature and were evaluated to follow the formation of polymer-supported alcohol **III.18**.[79]

1) The method described by Riguera involves the transformation of the alcohol group into a tosylate followed by its displacement by *p*-nitrobenzylpyridine (PNBP) to form a pyridinium salt (scheme 3.5).[79a] After treatment with a base, a strongly coloured salt **III.19** is obtained. This test has been reported to be successful in monitoring the transformation of a THP ether into the alcohol.

[79] (a) Kuisle, O.; Lolo, M.; Quiñoá, E.; Riguera, R. *Tetrahedron* **1999**, *55*, 14807; (b) Attardi, M.E.; Falchi, A.; Taddei, M. *Tetrahedron Lett*. **2000**, *41*, 7395; (c) Burkett, B.A.; Brown, R.C.D.; Meloni, M.M. *Tetrahedron Lett*. **2001**, *42*, 5773.

Riguera test



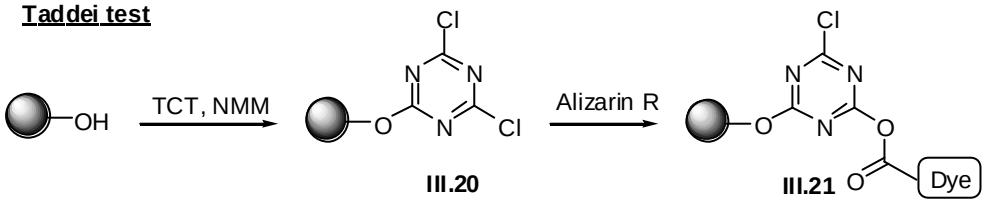
III.19

Scheme 3.5

2) The test of Taddei[79b] relies on the reaction of 2,4,6-trichloro-[1,3,5]-triazine (TCT) with a free alcohol function resulting in 2-alkoxy-4,6-dichloro-[1,3,5]-triazine **III.20**. Treatment of **III.20** with the red coloured dye Alizarin yellow R (5-(4-nitrophenyl-azo)salicylic acid, sodium salt) (scheme 3.6) results in the formation of the red coloured compound **III.21**.

3) The most recent test is one developed by Brown.[79c] The reaction of diphenyldichlorosilane with polymer-supported alcohols leads to the formation of the polymer-supported diphenylsilylchloride ether **III.22** as intermediate. Subsequent treatment of **III.22** with methyl red (2-(4-(dimethylamino)phenyl-azo)benzoic acid, sodium salt) affords tethered dye **III.23** and results in a strong orange colouration of the beads.

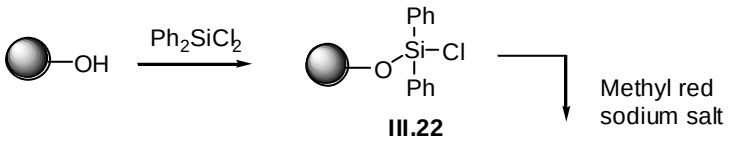
Taddei test



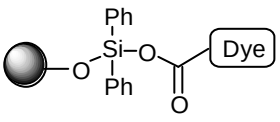
III.20 **III.21**

Red coloured beads

Brown test



III.22



III.23

Scheme 3.6

The colorimetric tests of Riguera and Taddei were used to monitor the formation of polymer-supported alcohol **III.18** but the results were disappointing. In our hands, only negative or ambiguous results were obtained.

On the other hand, monitoring the deprotection using ^{13}C gel phase NMR was examined and appeared to be the most reliable method. After deprotection of the THP group, the ^{13}C NMR spectrum (figure 3.4b) shows that the peak at 100 ppm, characteristic for the THP group disappeared whereas the characteristic peaks corresponding to the DMPM and TBDPS ethers are still present.

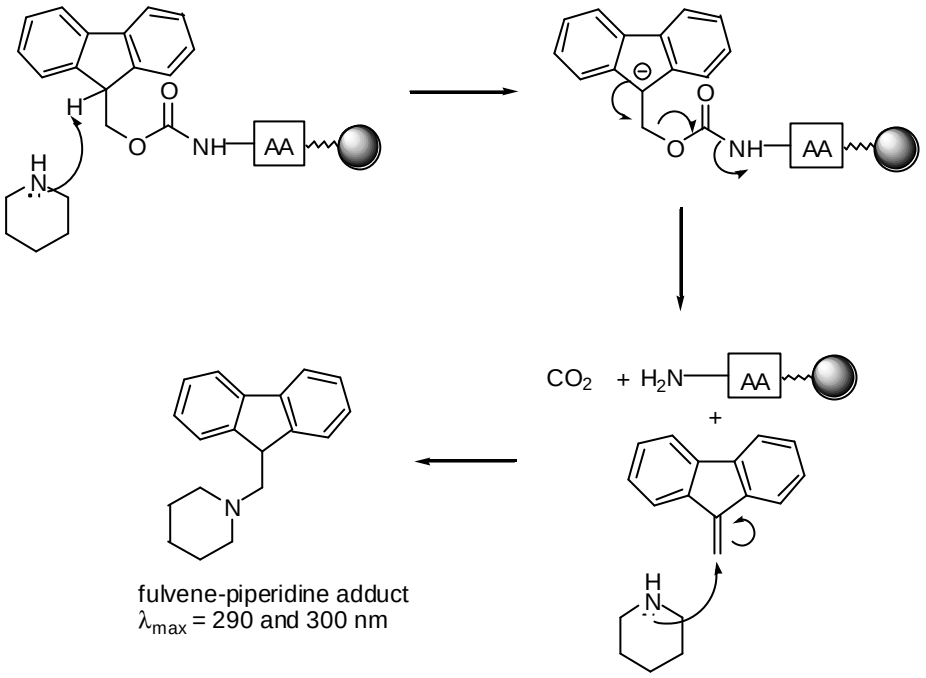
In addition, FTIR spectroscopy was also useful and showed clearly the appearance of an alcohol function.[80]

After the successful selective removal of the THP protecting group, the coupling of FmocGlyOH on the free hydroxyl function of the polymer-supported compound **III.18** was tested.

When Fmoc-protected amino acids are coupled on a polymer-supported compound, the yield of the coupling step can be determined by measuring the amount of fulvene-piperidine adduct present in the solution via UV/VIS spectroscopy.[81] The adduct is formed via an E_{1CB} reaction after treatment of the Fmoc-containing solid support with piperidine (scheme 3.7) and has characteristic absorptions at 290 nm and 300 nm. The yield of the reaction (equation (1)) is given by the ratio of the measured loading (calculated from the Lambert-Beer equation (2)) to the theoretical loading (corresponding to 100% reaction yield).

[80] One of the most common methods is the use of FTIR spectra of potassium bromide pellet: (a) Crowley, J.I.; Rapoport, H. *J. Org. Chem.* **1980**, *45*, 3215; (b) Hauske, J.R.; Dorff, P. *Tetrahedron Lett.* **1995**, *36*, 1589.

[81] (a) Meienhofer, J.; Waki, M.; Heimer, E.P.; Lambros, T.J.; Makofske, R.C.; Chang, C-D *Int. J. Prot. Res.* **1979**, *13*, 35; (b) Chang, C-D.; Waki, M.; Ahmad, M.; Meienhofer, J.; Lundell, E.O.; Haug, J.D. *Int. J. Prot. Res.* **1979**, *15*, 59.



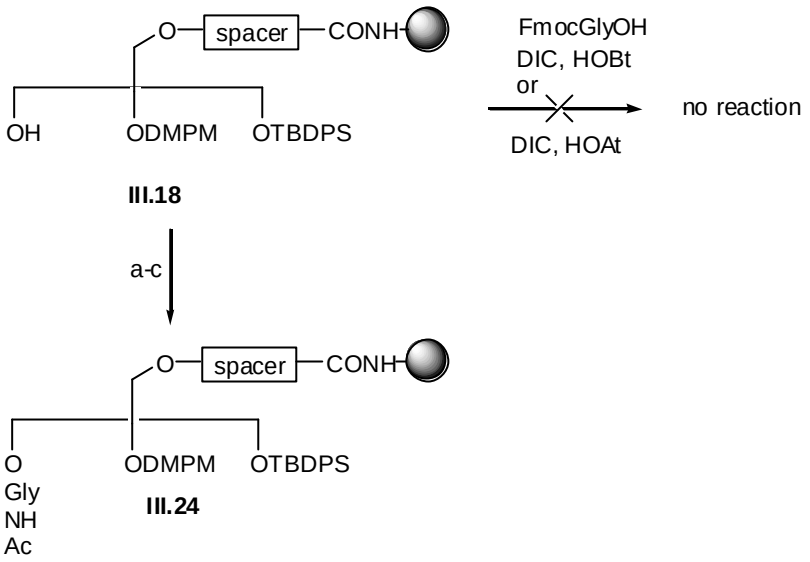
$$\text{Yield (\%)} = \frac{\text{experimental loading}}{\text{theoretical loading}} \times 100$$ equation (1)

$$\text{Experimental Loading (mmol/g)} = \frac{A \times V \text{ (ml pip./ DMF)}}{\varepsilon \text{ (ml / mmol)} \times m \text{ (g)}}$$ equation (2)

A : absorbance value of the solution
ε : absorption of 1 mmol of Fmoc-chromophore at λ nm
V : volume of solution 20% pip./DMF
m: weight of resin

Scheme 3.7

When DIC was used as the coupling reagent in the presence of HOBt or in the presence of the more reactive additive HOAt, poor results were obtained even after repetitive couplings. As mentioned in the work of Still, [67] the coupling of amino acids on a free hydroxyl function requires a more reactive reagent such as an acid chloride. Thus, the coupling reaction of the acid chloride FmocGlyCl on polymer-supported **III.18** (scheme 3.8) resulted in a quantitative conversion as determined by UV/VIS spectroscopy. After deprotection of the Fmoc-protecting group with a solution of 20% piperidine in DMF, the free amino function was capped with an excess of acetylimidazole to form **III.24**.

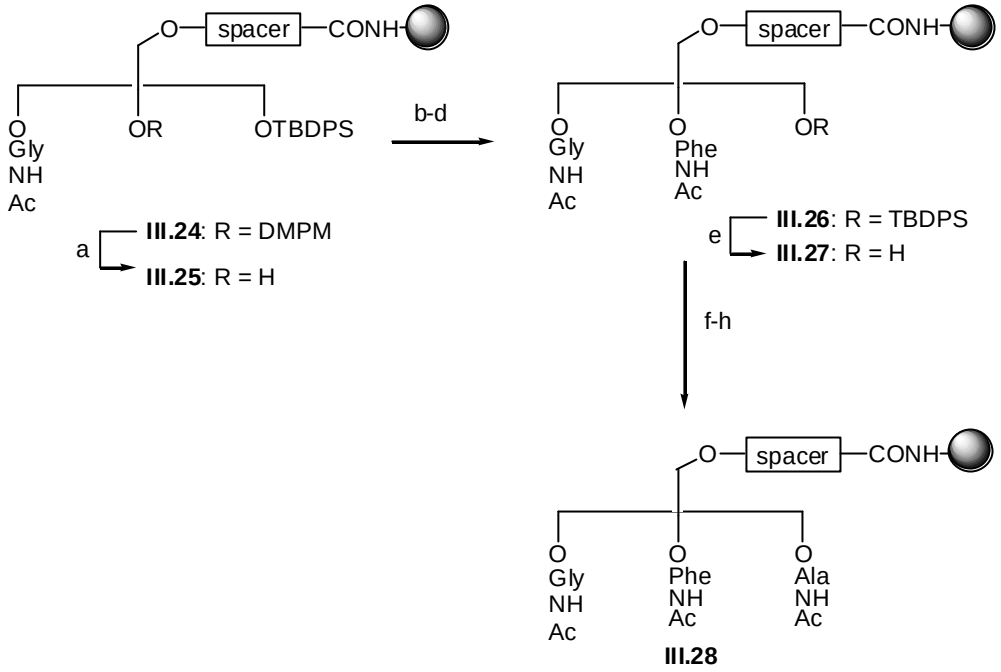


a) FmocGlyCl, DIPEA, CH_2Cl_2; b) 20% pip./DMF; c) AcIm, CH_2Cl_2

Scheme 3.8

Subsequently, the deprotection of the 3,4-dimethoxybenzyl ether (DMPM) was examined. After treatment of resin **III.24** with an excess of dichlorodicyanoquinone (DDQ),[82] analysis of the ^{13}C NMR spectrum of resin **III.25** (figure 3.4c) shows that the characteristic peaks of the DMPM protecting group at 112 ppm and 120 ppm had disappeared whereas the silyl ether was still intact. The deprotected resin **III.25** was coupled with the acid chloride FmocPheCl (scheme 3.9). Removal of the Fmoc-protecting group and capping of the free amino function with acetylimidazole afforded resin **III.26**.

[82] Oikawa, Y.; Tanaka, T.; Horita, K.; Yoshioka, T.; Yonemitsu, O. *Tetrahedron Lett.* **1984**, *25*, 5393.



a) DDQ, CH_2Cl_2 / H_2O, r.t., overnight; b) FmocPheCl, DIPEA, CH_2Cl_2, 88%; c) 20% pip. / DMF; d) AcIm, CH_2Cl_2; e) TBAF, THF, molecular sieves (400 pm); f) FmocAlaCl, DIPEA, CH_2Cl_2, 75%; g) 20% pip. / DMF; h) AcIm, CH_2Cl_2

Scheme 3.9

Finally, the silyl ether was deprotected by reaction of **III.26** with a solution of TBAF in THF (scheme 3.9) to form resin **III.27**. As indicated by the ^{13}C NMR spectrum (figure 3.4d), the characteristic peaks of the TBDPS at 28 ppm (*tert*-Bu) and 135 ppm (Ph) disappeared. The acid chloride FmocAlaCl was successfully coupled to deprotected resin **III.27**, the Fmoc-protecting group was removed and the free amino functions were capped with acetylimidazole to afford **III.28**. At this stage, the cleavage from the solid phase was the only way to prove the structure of compound **III.28** followed by analysis using traditional analytical methods. However, due to the absence of any cleavable linker, the compound could not be released from the solid phase. That is the reason why we turned our attention to the use of a cleavable linker.

3.4 Conclusion

Tripodal scaffold **III.6** was synthesised in a five-step sequence in 4.1% overall yield and was successfully coupled to the polymer-support TentaGel-NH_2 resin. Furthermore, the deprotection of the three protecting groups (THP, DMPM and TBDPS) was successfully performed in a selective and efficient way. ^{13}C gel phase NMR was the most suitable and reliable method to monitor these deprotections. The coupling of amino acids on the free hydroxyl function was examined and required the use of the reactive acid chlorides in order to obtain high coupling yields as determined by UV/VIS spectroscopy.

4 The incorporation of a cleavable linker

4.1 Introduction

Our initial experiments on solid phase revealed some difficulties in the monitoring of the deprotection and coupling steps on scaffold **III.6**. Therefore, as outlined in figure 4.1, the introduction of a linker is a solution to this problem. A linker is defined as the 'link' between the solid support and the scaffold and allows for the cleavage of the compounds attached on the solid support. In addition, the linker is a key fragment in the planning of a synthetic scheme: it needs to be stable towards the chemistry performed and it has to be possible to cleave the product from the solid phase with high efficiency at the end of the synthesis. Moreover, the synthesis of the linker, if not commercially available, needs to be straightforward. Over the past 15 years, about 2000 linkers have been developed.[83] The choice of a suitable cleavable linker for a specific synthesis is of major importance.

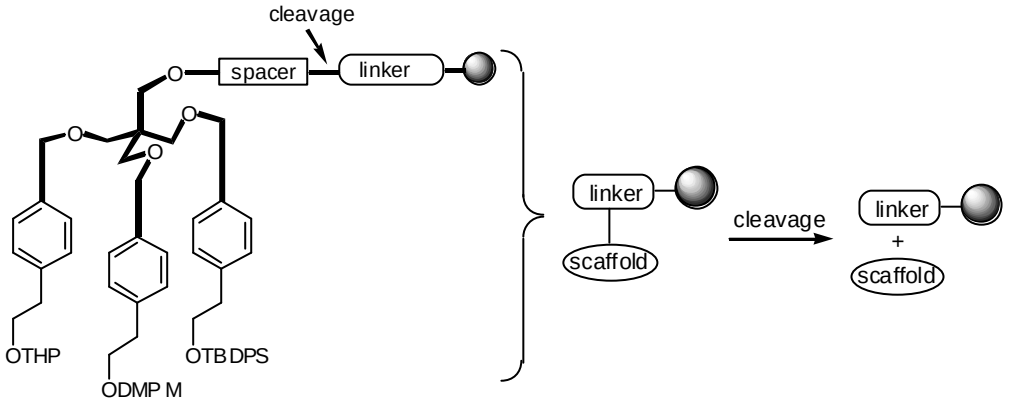


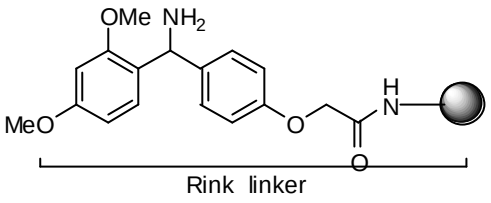
Figure 4.1

In this chapter, the efficiency of several types of linkers described in the literature is examined.

[83] Reviews about linkers: (a) James, I.W. *Tetrahedron* **1999**, *55*, 4855; (b) Gordon, K.; Balasubramanian, S. *J. Chem. Technol. Biotechnol*. **1999**, *74*, 835; (c) Guillier, F.; Orain, D.; Bradley, M. *Chemical Reviews* **2000**, *100*, 2091.

4.2 The Rink Linker: an acid labile linker

In the 60's, Merrified resin was the main solid support used for peptide synthesis and the cleavage of the peptide from the Merrifield resin required hydrogen fluoride. In 1973, Wang described an acid labile linker that requires 95% trifluoroacetic acid as cleavage conditions. Therefore, the development of highly acid labile linker **IV.1** by Rink (figure 4.2) in 1987 was announced as a first important step in the field.[84] The acid sensibility of the Rink linker is explained by the presence of the methoxy groups in ortho and para positions that stabilise the carbocation formed during the cleavage. A solution of 25% trifluoroacetic acid in dichloromethane is sufficient to release the compounds from the polymer.

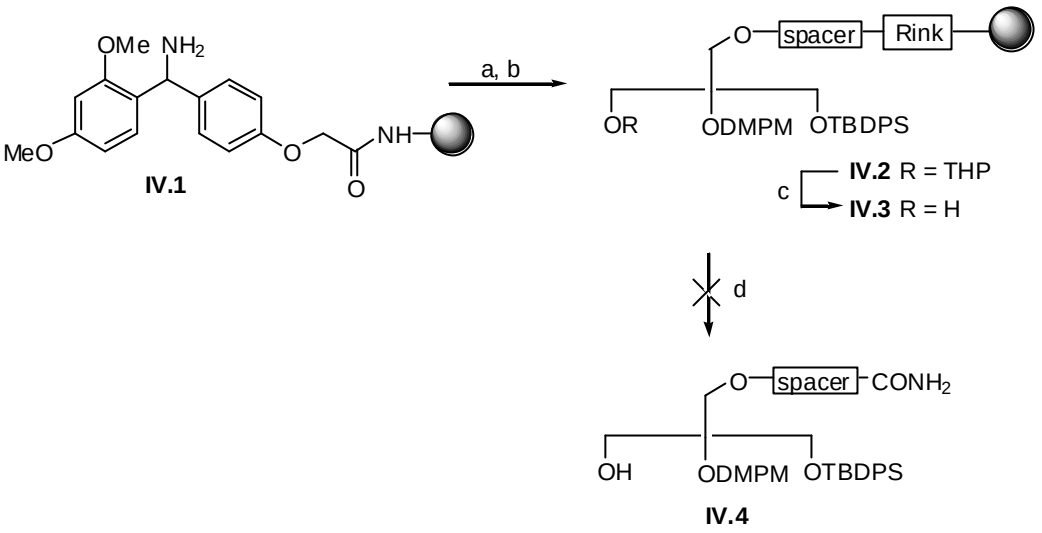


IV.1

Figure 4.2

The possibility of using the Rink linker in our synthesis was examined. Carboxylic acid **III.6** was coupled on commercially available polymer-supported Rink linker **IV.1**, using carbodiimide DIC as coupling reagent in the presence of HOAt (scheme 4.1). Any free amino functions left on the Rink linker were capped with a solution of acetic anhydride. Then, the THP protecting group in compound **IV.2** was cleaved with an aqueous solution of 80% acetic acid at 60 °C to afford alcohol **IV.3**. Subsequently, the resin was exposed to 25% trifluoroacetic acid as described in the literature to release the scaffold from the solid support. However, instead of obtaining the desired alcohol **IV.4**, a complex mixture was obtained. This observation led us to the hypothesis that our scaffold might be unstable under strong acid conditions due to the presence of the benzyl ethers functions.

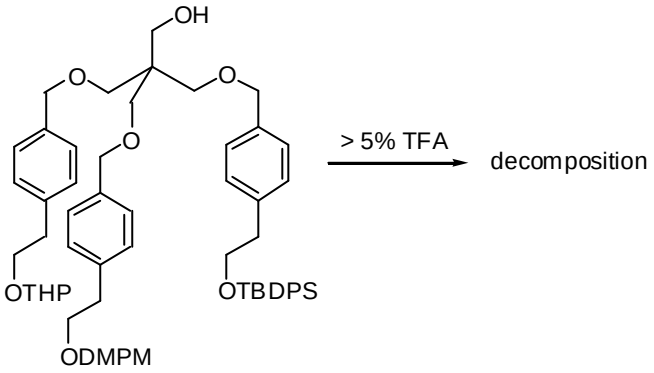
[84] (a) Rink, H. *Tetrahedron Lett.* **1987**, *28*, 3787; (b) Bernatowicz, M.S.; Daniels, S.B.; Köster; H. *Tetrahedron Lett*. **1989**, *30*, 4645.



a) DIC, HOAt, **III.6**, DMF; b) Ac_2O, DIPEA, DMAP; c) AcOH / H_2O / CH_2Cl_2: 80 / 5 / 15, 60 °C; d) TFA / TIPS / CH_2Cl_2: 25 / 2.5 / 72.5

Scheme 4.1

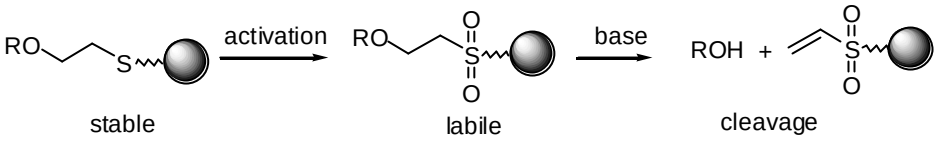
To confirm this hypothesis, the non-solid phase-bound scaffold **I.14** was treated with a series of solutions containing 50, 20, 10 and 5% trifluoroacetic acid (scheme 4.2). In each case, according to thin layer chromatographic analysis, complex mixtures were obtained. From these observations, we decided to avoid linkers involving cleavage with strong acids and we turned our attention to the so-called safety-catch linkers and photolabile linkers.



Scheme 4.2

4.3 The Kenner linker: a safety-catch linker

Over the last 10 years, a great deal of attention has been paid to the development of the so-called safety-catch linkers. Safety-catch linkers rely on a two-step cleavage process. The robust linker may be transformed after the synthesis into a more labile version, which can be cleaved under mild conditions. An example is outlined in scheme 4.3. The first step involves activation of the linker by oxidation of the sulfide to the sulfone and the second one involves the actual cleavage using a base.



Scheme 4.3

The first safety-catch linker was described by Kenner in 1971 and involves a polymer-supported sulfonamide **IV.5** (figure 4.3) that shows high stability to both acidic and basic conditions.[85] After the coupling step, (scheme 4.4), activation of **IV.6** occurs by treatment of the resin with diazomethane or iodoacetonitrile to form the labile resin **IV.7.** Nucleophilic displacement with an amine or alcohol provides the amide or ester **IV.8** respectively.

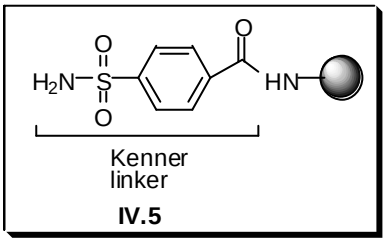
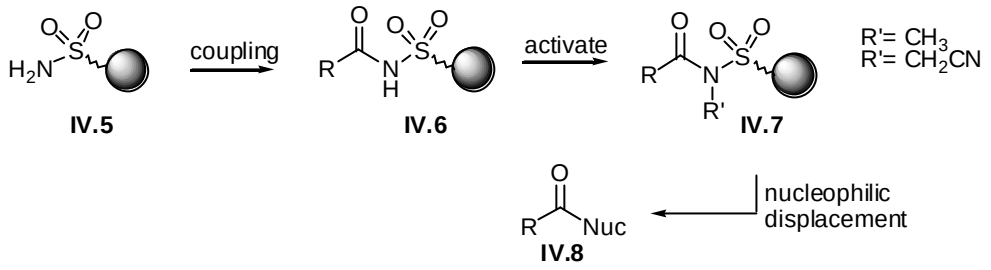


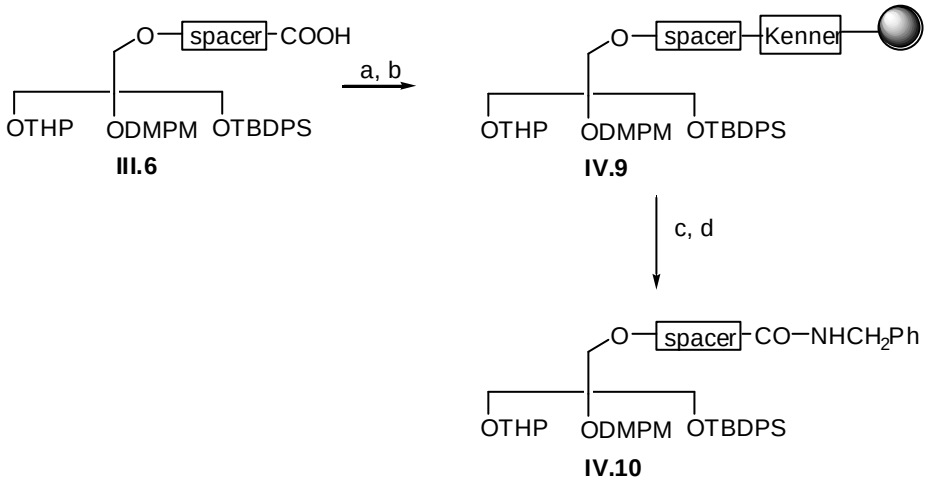
Figure 4.3

[85] (a) Kenner, G.W.; McDermott, J.R.; Shepard, R.C. *J. Chem.Soc., Chem. Commun.* **1971**, 636; (b) Backes, B.J; Ellman, J.A. *J. Am. Chem. Soc.* **1994**, *116*, 11171; (c) Backes, B.J.; Virgilio, A.A.; Ellman, J.A. *J. Am. Chem. Soc.* **1996**, *118*, 3055; (d) Backes, B.J.; Ellman, J.A. *J. Org. Chem.* **1999**, *64*, 2322.



Scheme 4.4

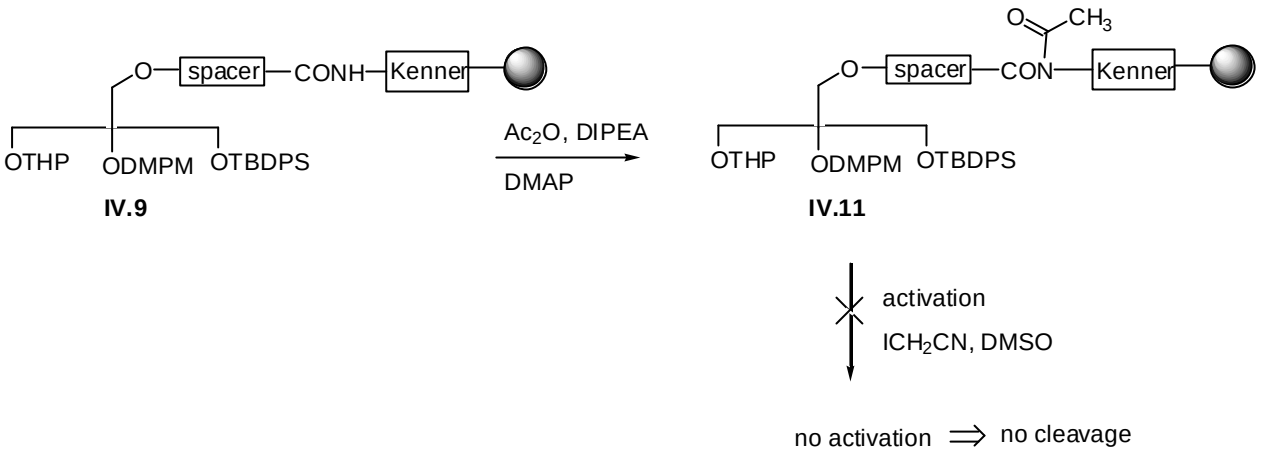
Due to its particular stability towards acids and bases, Kenner's linker **IV.5** could be a suitable linker. The coupling of the commercially available Kenner's linker **IV.5** with the carboxylic acid **III.6** was performed using EDC as coupling reagent in the presence of a catalytic amount of DMAP (scheme 4.5). The efficiency of the coupling was monitored using two colorimetric tests. The **NF31** test was successful to control the success of the coupling reaction whereas the commonly used 2,4,6-trinitrobenzenesulfonic acid (TNBS) test failed, probably due to the low nucleophilicity of the sulfonamide **IV.5**. In order to test the cleavage conditions, polymer-bound scaffold **IV.9** was activated with iodoacetonitrile in presence of diisopropylethylamine in DMSO followed by treatment with a large excess of benzylamine. Disappointingly, only some traces of the amide **IV.10** were released from the solid support.



a) EDC, DMAP, **IV.5**; b) Ac_2O, DIPEA, DMAP; c) ICH_2CN, DIPEA, DMSO;
d) Benzylamine, THF

Scheme 4.5

The low cleavage yield is probably explained by an acylation reaction of the acylsulfonamide **IV.9** that occurred during the capping of the free remaining amino functions with an excess of acetic anhydride (step b) (scheme 4.5). In this way, compound **IV.11** is blocked from activation and cleavage (scheme 4.6).



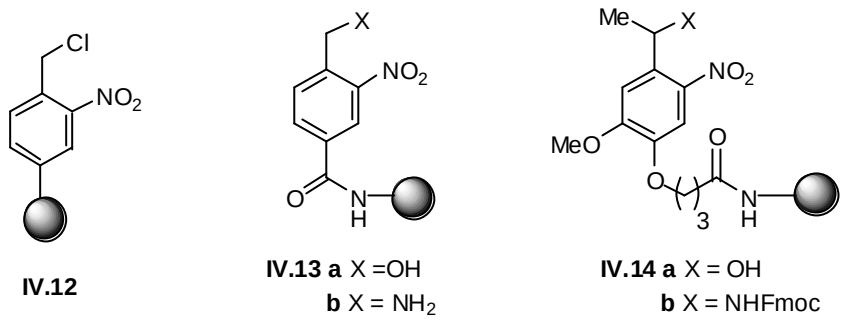
Scheme 4.6

As a result of these disappointing experiments, we turned our attention to the photolabile linkers.

4.4 Photolabile linkers

Even if photolabile linkers are not as commonly used as either acid labile or base labile linkers, their success in solid phase organic synthesis has increased.[86] Photolabile linkers offer a mild and orthogonal cleavage method that takes place under neutral conditions upon UV irradiation. However, the main drawback of photolabile linkers is the difficulty to achieve good yields for cleavage in combination with a short irradiation time. Photolytic reactions are significantly longer for polymer bound substrates than for compounds in solution because of shadowing effects of the resin and swelling properties of the support. In the 1970's, Rich was the first in developing the *o*-nitrobenzyl linkers **IV.12** and **IV.13** on polystyrene as light-sensitive linkers.[87] Unfortunately, they still required long irradiation time (18-24 h) and only moderate yields were obtained.

[86] Pillai, V.N.R. *Synthesis*, **1980**, 1.



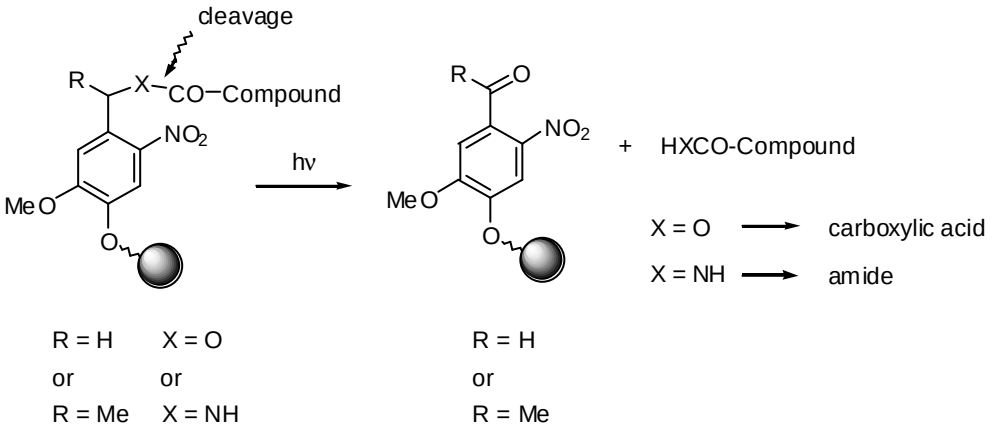
IV.12

IV.13 a X =OH
b X = NH_2

IV.14 a X = OH
b X = NHFmoc

Figure 4.4

Since then, several modifications have been brought to the original structure **IV.12** in order to increase the cleavage yield and reduce the cleavage time.[88] The most efficient photolabile linker **IV.14** reported so far is based on a veratryl derivative.[89] The incorporation of additional alkoxy groups onto the benzene ring enhances the cleavage rate due to a dramatic increase in absorbance in the deprotection wavelength region centered near 365 nm. On the other hand, it has been found that the presence of an α-methyl group, which leads to the formation, after cleavage, of a solid-supported ketone rather than a solid-supported aldehyde gives superior yields (scheme 4.7). In the case of linker **IV.13**, after cleavage, the product can be reattached to the solid support through the reactive aldehyde group.



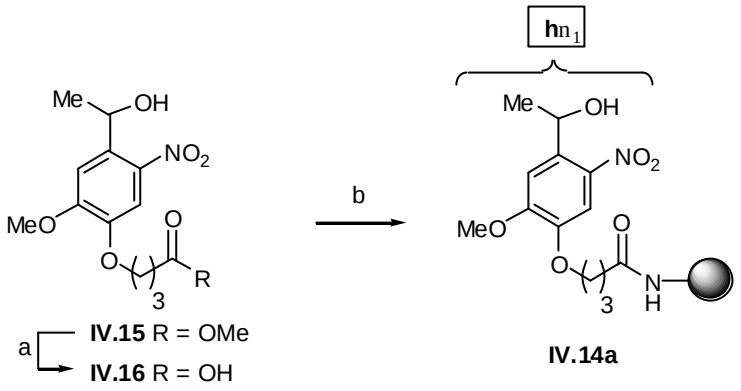
Scheme 4.7

[87] (a) Rich, D.H.; Gurwara, S.K. *J. Chem. Soc., Chem. Commun.* **1973**, 610; (b) Rich, D.H.; Gurwara, S.K. *J. Am. Chem. Soc.* **1975**, *97*, 1575.

[88] (a) Rodebaugh, R.; Fraser-Reid, B.; Geysen, H.M. *Tetrahedron Lett.* **1997**, *38*, 7653; (b) Åkerblom, A.B. *Molecular Diversity* **1999**, *4*, 53.

[89] (a) Holmes, C.P.; Jones, D.G. *J. Org. Chem.* **1995**, *60*, 2318; (b) Teague, S.J. *Tetrahedron Lett.* **1996**, *32*, 5751. (c) Holmes, C.P. *J. Org. Chem.* **1997**, *62*, 2370.

Because of their reported high efficiency, linkers **IV.14a** and **IV.14b** were tested in our project. In first instance, since we received a sample of linker **V.15** from Dr. Bart Ruttens (Laboratory of Prof. Dr. Johan Van Der Eycken), the polymer-supported alcohol derivative **IV.14a** was examined. After hydrolysis of ester **IV.15**, acid **IV.16** (scheme 4.8) was coupled on the solid support NovaGel using carbodiimide DIC as coupling reagent in the presence of HOBt to afford **IV.14a**. NovaGel resin is an interesting solid support because of its higher loading capacity compared to TentaGel resin and presents the same swelling properties.[90] The structure of **IV.14a** will be later symbolized as shown in scheme 4.8.



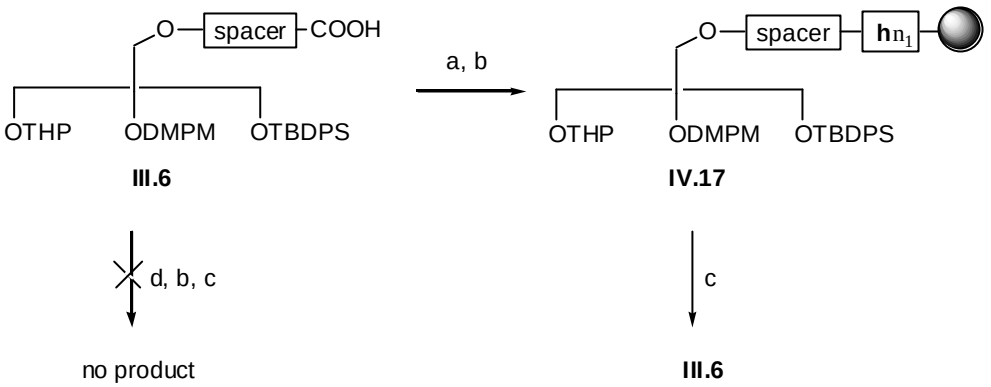
a) 1N NaOH, MeOH, 100%; b) DIC, HOBt, NovaGel-NH_2, DMF

Scheme 4.8

Subsequently, the coupling of the scaffold **III.6** on the polymer supported alcohol **IV.14a** was attempted. Following the method of Sieber, the carboxylic acid **III.6** was activated with 2,6-dichlorobenzoylchloride (scheme 4.9) in the presence of an excess of pyridine followed by the addition of the solution to the solid-supported alcohol **IV.14a**. The coupling reaction was monitored using the colorimetric DMTCl test.[60] However, the qualitative DMTCl test currently used to detect the presence of free alcohol function remained strongly positive even after a second coupling. The free alcohol functions were acylated with a solution of acetic anhydride followed by irradiation of the resin at 365 nm. Even after 3 h of irradiation, no compound **III.6** was detected according to thin layer chromatography and ^{1}H NMR. The absence of coupling is probably at the origin of this failure.

[90] Catalog Novabiochem, 2000, S1-S4

Another coupling procedure based on the use of DIC as coupling reagent in presence of a catalytic amount of DMAP was tested. After a second coupling, the free hydroxyl functions were capped with a solution of acetic anhydride and the resin **IV.17** was irradiated for 3 h affording only traces of carboxylic acid **III.6**.



a) **IV.14a**, DIC, DMAP, CH_2Cl_2; b) Ac_2O, HOBt, DMAP; c) hν (365 nm), 1,4-dioxane, 3 h;
d) **IV.14a**, 2,6-dichlorobenzoyl chloride, pyridine, DMF

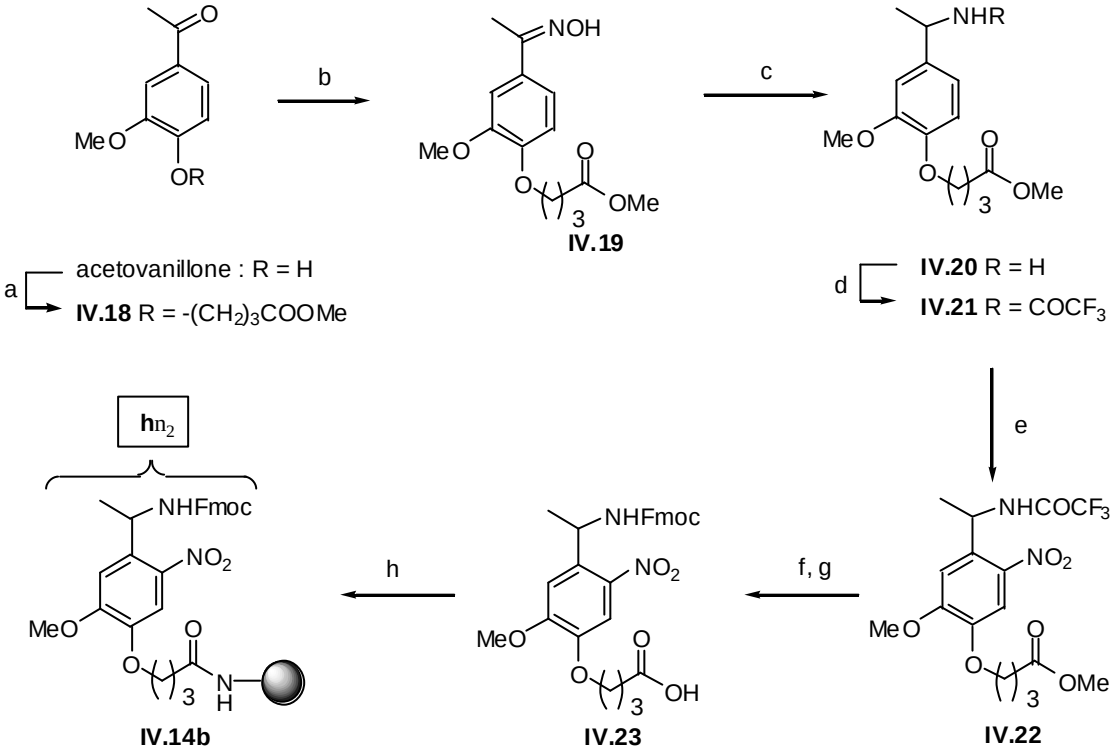
Scheme 4.9

However, the acid **III.6** was obtained in a pure form according to ^{1}H NMR, indicating that no decomposition or side-reactions took place during the irradiation, which was quite promising. On the other hand, the low yield may be explained by a slow photolytic rate or may be the result of a low coupling yield between acid **III.6** with alcohol **IV.14a** which is the most probable explanation.

To clarify these hypotheses, we turned our attention to the *o*-nitrobenzyl support **IV.14b**. In contrast to linker **IV.14a**, linker **IV.14b** possesses a protected amino function. The coupling of **III.6** on the resulting amino function was expected to occur faster and to be more efficiently monitored using the common colorimetric TNBS and **NF31** tests.

Photolabile linker **IV.14b** was synthesised, according to literature procedures in a very efficient way from the commercially available acetovanillone in a seven-step sequence (scheme 4.10).[89b] Acetovanillone reacted with methyl 4-bromobutyrate in presence of potassium carbonate to form intermediate **IV.18**. Without any purification, this keto-ester was immediately treated with hydroxylamine hydrochloride to afford oxime **IV.19** in 97% yield. The reduction of oxime **IV.19** into the amine **IV.20** was carried out under hydrogen atmosphere in methanol in the presence of 10% palladium on charcoal and hydrochloric acid. After protection of the primary amine, the nitrogroup was introduced on the benzene ring by treatment of the

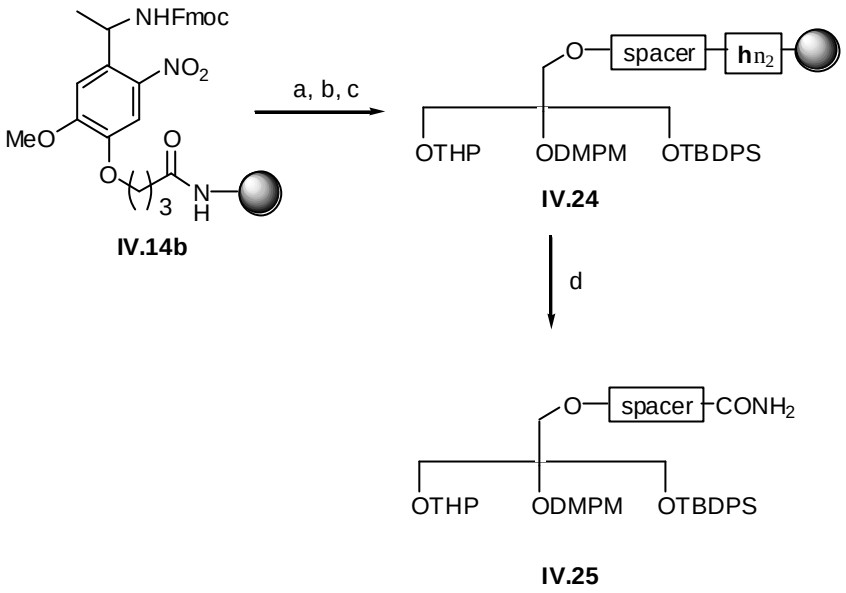
trifluoroacetamide **IV.21** with a solution of 65% of nitric acid to form intermediate **IV.22**. Afterwards, the trifluoroacetamide function and the methylester function in compound **IV.22** were hydrolysed with sodium hydroxide allowing for the introduction of the Fmoc-group leading to linker **IV.23**. Finally, the linker was coupled to the solid phase TentaGel-NH_2 using DIC as coupling reagent in the presence of HOBt to form **IV.14b**.



a) $Br(CH_2)_3COOMe$, K_2CO_3, DMF, 98%; b) $H_2NOH.HCl$, pyridine / H_2O: 2 / 1, 97%; c) H_2, Pd/C, HCl, MeOH, 71%; d) $CF_3CO)_2O$, pyridine., 0 °C, 76%; e) 65% HNO_3, 0 °C, 66%; f) 1 N NaOH, MeOH, reflux, 5 h; g) FmocCl, 1,4-dioxane / H_2O, 68% over two steps; h) DIC, HOBt, TentaGel-NH_2, DMF

Scheme 4.10

With the polymer-supported photolabile linker **IV.14b** in hand, the efficiency of this linker in our synthesis was examined. After deprotection of the Fmoc-protecting group (scheme 4.11), the scaffold **III.6** was coupled to the resulting amine, affording compound **IV.24**. The deprotection and coupling steps were monitored using the TNBS and **NF31** tests. Both tests indicated a successful coupling. Moreover, the **NF31** test appeared to be more sensitive to control the completeness of the coupling reaction involving the scaffold **III.6** with the sterically hindered amine.



IV.14b **IV.24** **IV.25**

a) 20% pip. / DMF; b) EDC, DMAP, **III.6**, CH_2Cl_2; c) AcIm, CH_2Cl_2; d) hν (365 nm), 1,4-dioxane / 1% DMSO

Scheme 4.11

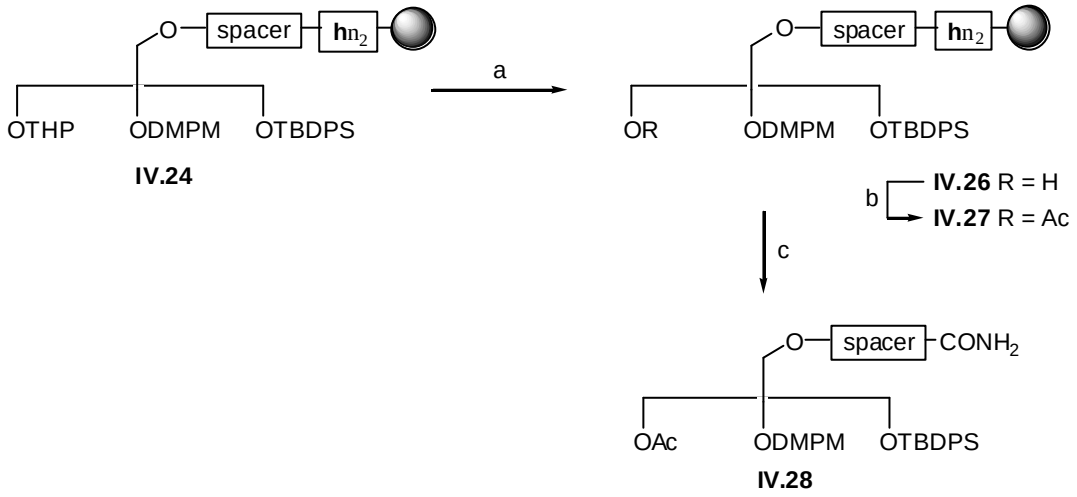
The success of the coupling could also be deduced from the ^{13}C gel phase NMR spectrum of the polymer-supported scaffold **IV.24** (figure 4.6a)**.** As mentioned previously, several characteristic peaks in the NMR spectrum were very useful to indicate the success of the coupling reaction. The peak at 100 ppm represents the carbon acetal of the THP protecting group. The peaks at 112 ppm and 121 ppm prove the presence of the DMPM ether and the peaks at 28 (*tert*-Bu) ppm and 135 ppm (Ph) are characteristic for the TBDPS ether.

At this point, the photolytic release of the scaffold was investigated. When the resin **IV.24** was exposed to UV-light (365 nm) in 1,4-dioxane, poor results were obtained. Although the literature[89b] claims that the addition of 2-aminoethanol or hydrazine to the solution could improve the yield, only impure products were obtained. The best result for the photolytic cleavage was obtained when a suspension of resin **IV.24** in 1,4-dioxane in the presence of 1% of DMSO was irradiated for 3 h affording compound **IV.25** in 86% yield.

4.5 Study of the selective deprotection of the THP, DMPM and TBDPS protecting groups

The reaction conditions described in the previous chapter were applied to deprotect the THP, DMPM and TBDPS protecting groups selectively. After each deprotection, each compound was cleaved from the polymer-support and its structure was controlled using ^{1}H NMR spectroscopy and ES-MS.

In a first stage, the deprotection of the THP protecting group was successfully performed using two methods (scheme 4.12). The first one involves treatment of the resin **IV.24** with a solution of 80% of acetic acid at 60 °C to form alcohol **IV.26** in a selective way. Another method to deprotect selectively the THP protecting group consists in using an excess of PPTS in ethanol at 55 °C for 15 h. Qualitative monitoring of this deprotection step using ^{13}C gel phase NMR (figure 4.6b) indicated a complete and selective deprotection. The peak at 100 ppm characteristic for the THP group had disappeared whereas the peaks at 110 ppm and 120 ppm characteristic for the DMPM ether and the peaks at 28 ppm and 135 ppm characteristic for the TBDPS protecting group, remained intact.



a) THP deprotection: 1) AcOH / CH_2Cl_2 / H_2O: 80 / 15 / 5, 60 °C, 28 h
2) PPTS, EtOH, 55 °C, overnight
b) Ac_2O, DIPEA, DMAP, CH_2Cl_2; c) hν (365 nm), 1,4-dioxane / 1% DMSO

Scheme 4.12

Subsequently, after capping the free hydroxyl function (scheme 4.12), resin **IV.27** was irradiated with UV light for 6 h leading to the release of compound **IV.28** in 55% yield after column chromatography. The yield involving a six-step sequence, was calculated based on the original loading of the commercial resin. The latter was determined using two quantitative methods: Fmoc UV/VIS spectroscopy and the picric acid test (see experimental part for more details).

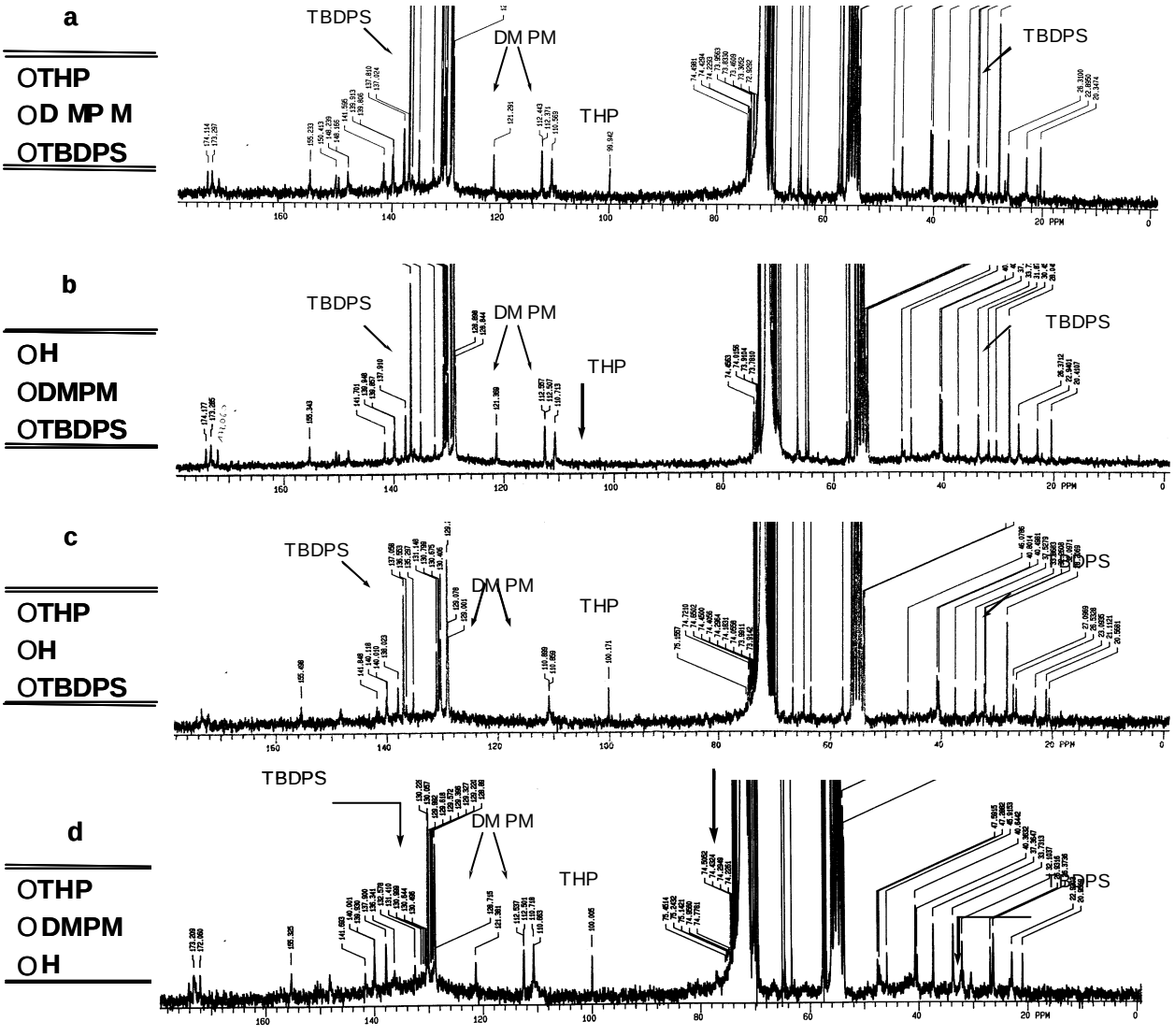
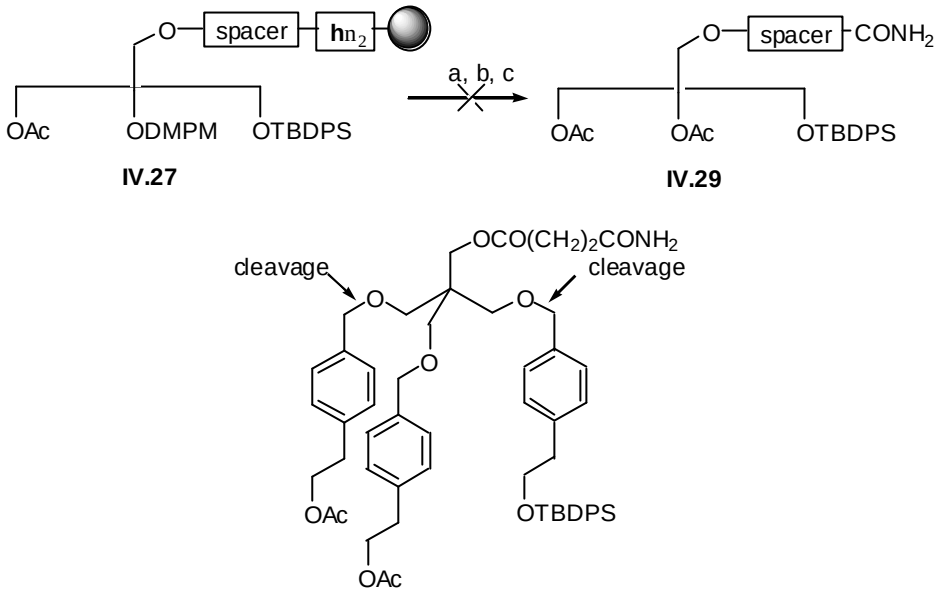


Figure 4.6

Subsequently, the selective deprotection of the DMPM ether was examined. Resin **IV.27** was treated with dichlorodicyanoquinone at room temperature for 15 h, as described in the previous chapter, followed by capping of the free hydroxyl function with acetic anhydride. ^{13}C NMR analysis indicated a successful selective deprotection of the DMPM ether function. After cleavage of the resin by irradiation with UV light, analysis via electron spray mass spectrometry showed that next to the deprotected ether **IV.29**, cleavage of the benzyl ethers present in the scaffold had occurred as shown in scheme 4.13. This side-reaction could not be detected via ^{13}C NMR spectroscopy indicating the usefulness of incorporating a cleavable linker.



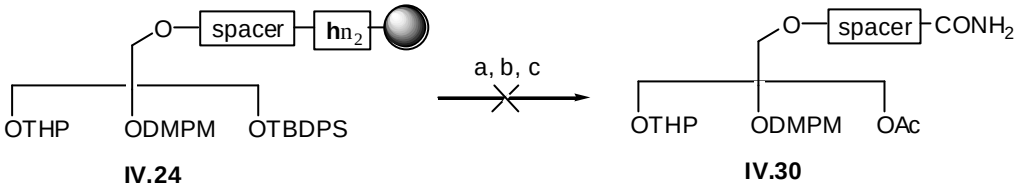
IV.27 **IV.29**

IV.29

a) DMPM deprotection: DDQ, CH_2Cl_2 / H_2O: 19 / 1, r.t., overnight;
b) Ac_2O, DIPEA, DMAP; c) hν (365 nm), 1,4-dioxane / 1% DMSO

Scheme 4.12

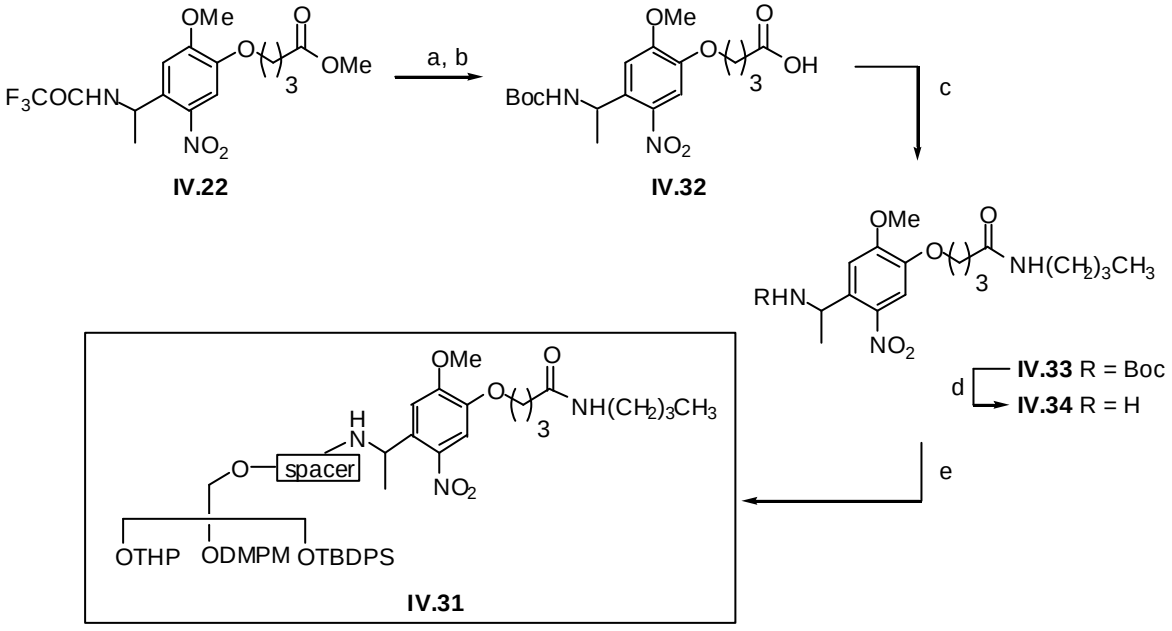
Finally, also the deprotection of the TBDPS ether was tested on compound **IV.24** using a solution of TBAF in tetrahydrofuran (scheme 4.14). After capping of the free hydroxyl function as the acetate and UV irradiation, no product **IV.30** was obtained from the photolytic deavage. As described in the literature, the basicity of the fluoride ions is responsible for the destruction of the *o*-nitrobenzyl unit of the photolabile linker.[69a]



a) TBAF, THF, molecular sieves (400 pm), overnight; b) Ac_2O, DIPEA, DMAP;
c) hν (365 nm), 1,4-dioxane / 1% DMSO

Scheme 4.13

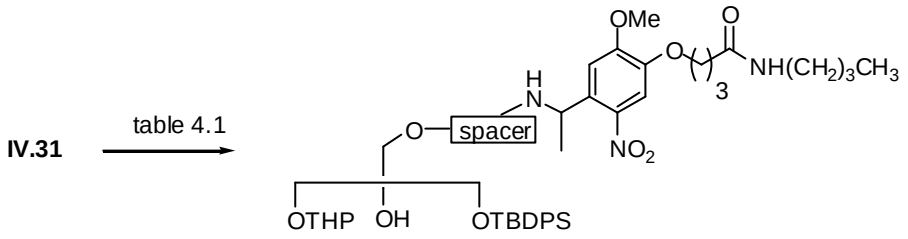
Because of the difficulties encountered in controlling the orthogonal deprotection conditions on solid support, we decided to first optimise the deprotection of the DMPM and TBDPS ethers in solution. Therefore, we synthesised model compound **IV.31** (scheme 4.14). Starting from compound **IV.22**, after hydrolysis of the trifluoroacetamide and of the methyl ester, the resulting amino function was protected as a Boc carbamate affording acid **IV.32** in 70% overall yield. Coupling of **IV.32** with *n*-butylamine led to the formation of amide **IV.33** in 46% yield. After deprotection of the Boc protecting group with a solution of TFA, the scaffold was successfully coupled to amine **IV.34** using a classical coupling procedure with EDC and DMAP to form model compound **IV.31** in 78% yield.



a) 1 N NaOH, MeOH; b) $BocCO)_2O$, 1,4-dioxane, 70% over two steps; c) *n*-butylamine, HOBt, HBTU, DMF, 46%;
d) 20% TFA, CH_2Cl_2, 98%; e) **III.6**, EDC, DMAP, CH_2Cl_2, 78%

Scheme 4.14

Compound **IV.31** was used to study and optimise the reaction conditions for the deprotection of the DMPM and TBDPS protecting group. In the case of the DMPM ether, the different conditions we tested are summarised in table 4.1. When compound **IV.31** was treated with CAN , no deprotection was observed. The use of the lewis acid $SnCl_4$ at –78 °C for 2 h in presence of thiophenol gave no reaction. Increasing the temperature to –50 °C for 15 h led to the deprotection of the THP ether. When a small excess of DDQ was added to a solution of compound **IV.31** in a mixture CH_2Cl_2 / H_2O at 5 °C for 1 h, some cleavage of the benzyl ethers present in the tripodal scaffold was still observed. Decreasing the temperature to 0 °C and shortening the reaction time to 30 min led to a selective and complete deprotection of the DMPM ether. Alcohol **IV.35** was obtained in 60% yield.

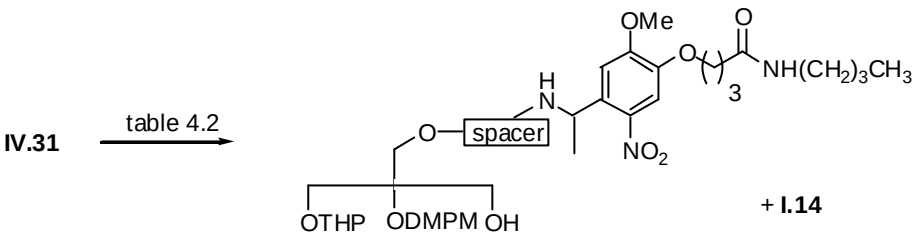


IV.35

Table 4.1: Selective deprotection of the DMPM ether in model compound **IV.31**

	Conditions	Results
1	CAN (2 eq.)	-
2	$SnCl_4$ (1.5 eq.), PhSH, -78 °C, (2 h), then –50 °C, 15 h	Deprotection of THP and DMPM ethers
3	DDQ (1.5 eq.), 5 °C, 1 h	Cleavage of the benzyl ethers (scheme 4.12)
4	DDQ (1.5 eq.), 0 °C, 30 min.	**IV.35** (60%): selective deprotection DMPM

Similarly, the deprotection of the TBDPS ether function in compound **IV.31** was investigated in solution. When compound **IV.31** was treated with an excess of TBAF, hydrolysis of the ester function present between the scaffold and the spacer was observed leading to the formation of alcohol **I.14** as a side-product, next to the desired product **IV.36**. Decreasing the reaction time gave a small improvement. A possible explanation for this hydrolysis is the presence of hydroxide ions in the solution of tetrabutylammonium fluoride, which is highly hygroscopic. This side reaction could be avoided by the addition of acetic acid which led successfully to alcohol **IV.36** in 90% yield.



IV.36

Table 4.2: Selective deprotection of the TBDPS ether in model compound **IV.31**

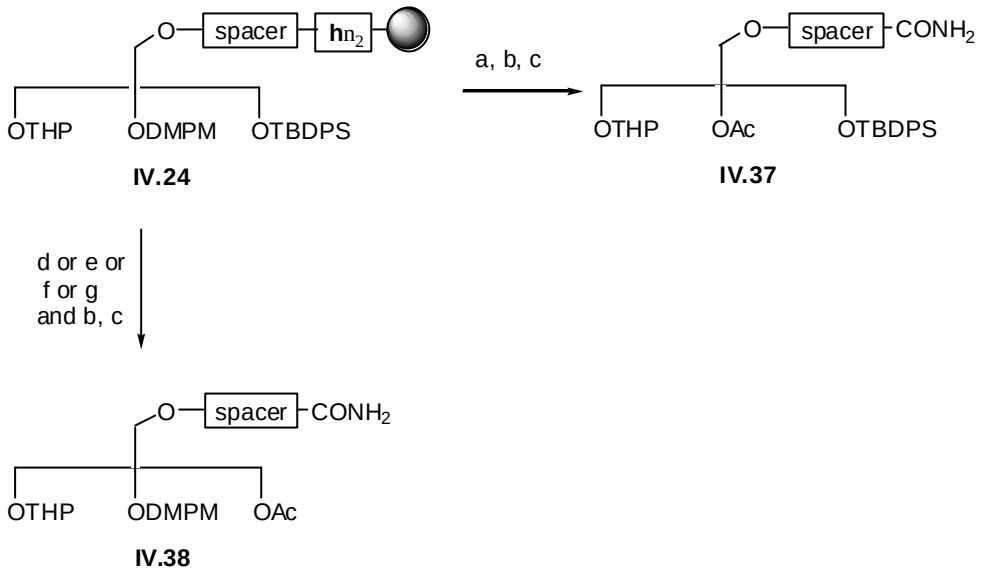
	Conditions	Results
1	TBAF (3 eq.), THF, overnight	**IV.36** + **I.14**: 1 / 1
2	TBAF (3 eq.), THF, 1 h 30	**IV.36** + **I.14**: 7 / 3
3	TBAF (3 eq.), AcOH (3 eq.), THF, overnight	**IV.36** (90%)

The newly optimised reaction conditions for the deprotection of the DMPM and TBDPS ethers were next tested on our solid-supported scaffold **IV.24**. The polymer-supported compound **IV.24** was treated with a small excess of dichlorodicyanoquinone (DDQ) for 30 min at 0 °C. Unfortunately, ^{13}C gel phase NMR analysis still indicated incomplete deprotection. However, in our case, increasing the reaction time to 1 h was sufficient to form the alcohol in a complete and selective way, as shown by ^{13}C NMR gel phase NMR (figure 4.6c): the peaks at 110 ppm and 121 ppm disappeared whereas the THP and TBDPS protecting groups are still present. After capping of the free alcohol function with a solution of acetic anhydride, the resin was exposed to UV light for 9 h to afford the acetate **IV.37** as the sole product in 62% yield after column chromatography.

Similarly, deprotection of the TBDPS ether on solid support was examined by reaction of **IV.24** with a solution of TBAF in THF in the presence of one equivalent of acetic acid overnight. Since the deprotection was incomplete according to ^{13}C gel phase NMR, the reaction was repeated overnight leading successfully to alcohol **IV.38**. ^{13}C gel phase NMR (figure 4.6d) confirmed the selective deprotection of the TBDPS ether. The peaks at 28 ppm (*tert*-Bu) and at 135 ppm (Ph) disappeared whereas the DMPM and THP protecting groups remained intact. Capping of the hydroxyl function as the acetate and irradiation of the resin for 8 h afforded acetate **IV.38** in 56% yield. Other reagents such as NH_4F,[91a] HF.pyr.,[91b-d] and TASF (tris-(dimethylamino)sulfur

[91] (a) Zhang, W.; Robins, M.J. *Tetrahedron Lett.* **1992**, *33*, 1177; (b) Newton, R.F.; Reynolds, D.P. *Tetrahedron Lett.* **1979**, *41*, 3981; (c) Nicolaou, K.C.; Seitz, S.P.; Pavia, M.R. *J. Am. Chem. Soc.* **1981**, *103*,

trimethylsilyldifluoride)[91e] were also successful in the selective deprotection of the TBDPS group in compound **IV.24**.



a) DDQ, CH_2Cl_2 / H_2O: 19 / 1, 0 °C, 1 h; b) Ac_2O, DIPEA, DMAP; c) hν (365 nm), 1,4-dioxane / 1% DMSO; d) TBAF, AcOH, THF, molecular sieves (400 pm), overnight (2x); e) NH_4F, MeOH, overnight (2x); f) HF.pyr., THF, overnight; g) TASF, DMF, 3 h

Scheme 4.15

4.6 Conclusion

In this chapter, linkers of different types were tested. The use of the Rink linker and the Kenner linker gave poor results. On the other hand, photolabile linkers gave promising results. After coupling of the scaffold to the solid-supported linker **IV.14b**, the conditions for the photolytic cleavage were optimised. This linker is stable and the polymer-supported scaffold **IV.24** was nicely cleaved from the solid phase. Subsequently, the selective deprotection of the three protecting groups (THP, DMPM and TBDPS) was optimised.[92] Furthermore, in this chapter the necessity of using a cleavable linker was shown. It has been proven that qualitative methods such as ^{13}C gel phase NMR are useful to monitor solid-phase reactions but are not always

1222; (d) Nicolaou, K.C.; Randall, J.L.; Furst, G.T. *J. Am. Chem. Soc.* **1985**, *107*, 5556; (e) Scheidt, K.A.; Chen, H.; Follows, B.C.; Chemler, S.R.; Coffey, S.; Roush, W.R. *J. Org. Chem.* **1998**, *63*, 6436.

[92] Farcy N.; De Muynck, H.; Madder, A.; Hosten, N.; De Clercq, P.J. *Org. Lett.* **2001**, 4299.

sufficient enough in the detection of side reactions. At this stage, the final goal, the synthesis of a library, could be envisaged.

5 Efforts towards the synthesis of a tripodal library

5.1 Introduction

After a careful choice of the cleavable linker and the optimisation of the selective deprotections on solid-phase bound scaffold **IV.24**, we describe here our efforts towards the synthesis of a tripodal library.

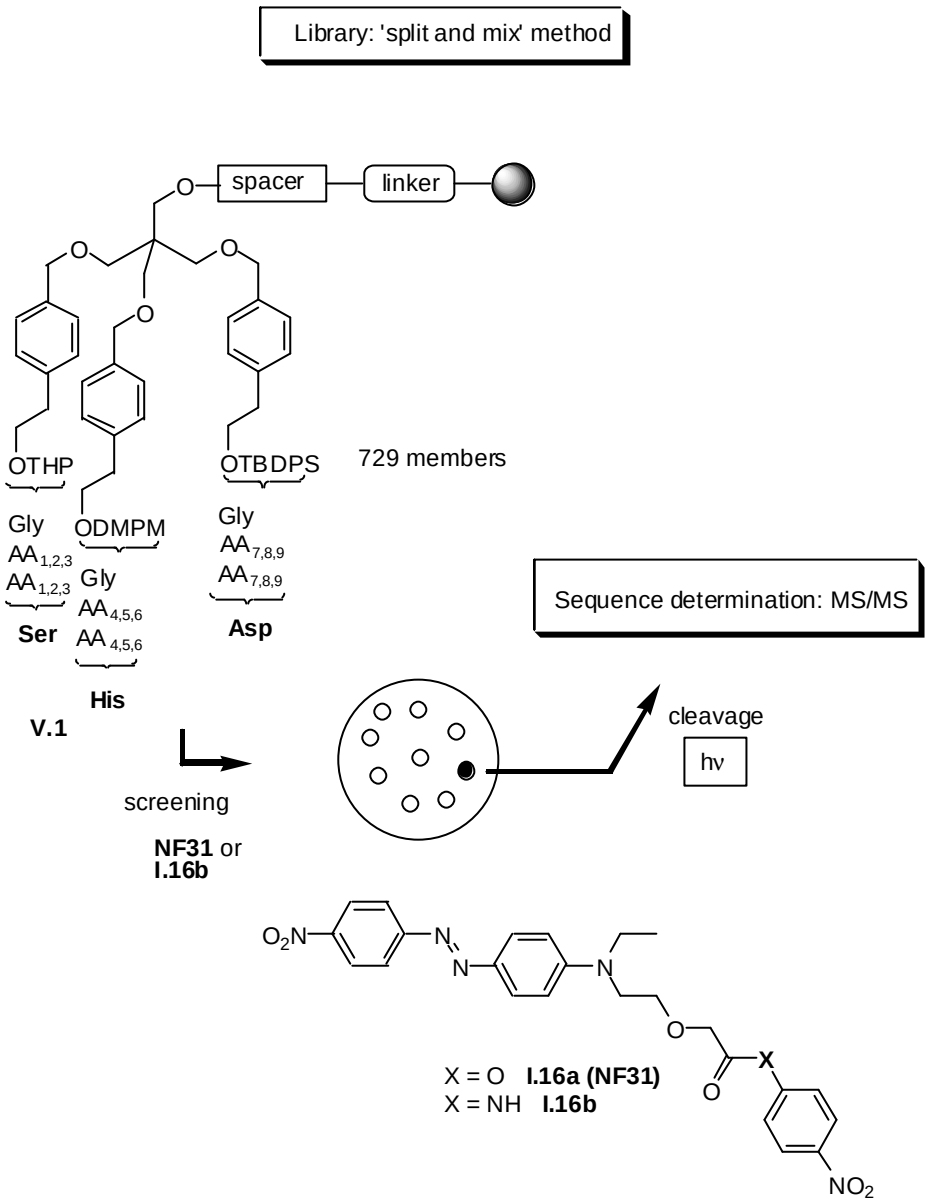
Our strategy for the synthesis of the library **V.1** is outlined in scheme 5.1. Using solid-phase bound scaffold **IV.24** as a template, the selective deprotection of the three protecting groups (THP, DMPM and TBDPS) enables an independent and consecutive construction of three peptide chains. Each strand is generated using the 'split and mix' protocol with a pool of three amino acids. For each strand, one residue of the catalytic triad, serine, histidine or aspartate is included in the pool. Except for these three essential amino acids, all others are randomly chosen among the non-functionalised side-chain amino acids.

Since Fmoc/*tert*-Bu strategy (see part 1) will be used for the synthesis of the library, Fmoc amino acids containing an acid labile protecting group on the side-chain will be selected for the catalytic triad. This explains why the THP group in **IV.24**, which is cleaved under acidic conditions, has to be removed first. Subsequently, construction of the first peptide strand using the 'split and mix' protocol with three amino acids including serine, leads to the formation of the first 9 members. Next, 81 (3^2 x 3^2) members are obtained after deprotection of for instance the DMPM ether, and generation of the second peptide strand using three other amino acids including histidine. Finally, the TBDPS ether will be removed and the last chain will be constructed including aspartate among the three amino acids used as building blocks. At this stage, the library will contain 729 (3^2 x 3^2 x 3^2) members.

Upon removal of the side-chain protecting groups the library can be tested towards hydrolytic activity. The *p*-nitrophenyl ester **I.16a** (**NF31**) and the more challenging *p*-nitroanilide **I.16b** are potential test substrates for the screening procedure. All members of the tripodal library are expected to show a different reactivity towards the substrates resulting in the appearance of more or less coloured red beads. As such, it should be possible to isolate the most active member of the library, to cleave off the product and to submit it to MS-MS tandem mass spectrometry to determine the sequence of the active member.[93] However, the determination of

[93] For single bead MS-MS analysis see: (a) Vanhoenacker, G.; Liu, L.; Lynen, F.; Madder, A.; De Clercq, P.; Sandra, P. J. Separation Science accepted; (b) Madder, A.; Li, L.; De Muynck, H.; Farcy, N.; Van Haver, D.; Fant, F.; Vanhoenacker, G.; Sandra, P.; Davis, A.P.; De Clercq, P.J. *J. Comb. Chem.* submitted.

the sequence using this method implies that all amino acids used for the construction of each peptide strand have a different molecular weight.

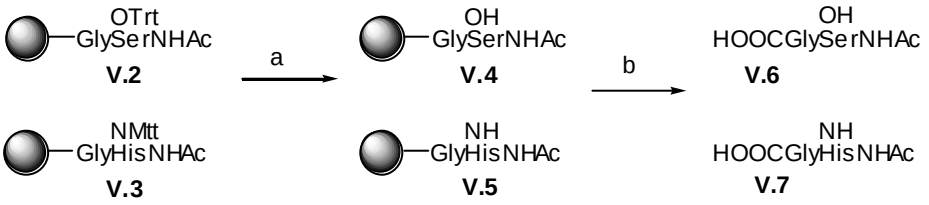


Scheme 5.1

5.2 Choice of the suitable side-chain protecting groups

A last aspect that needed to be evaluated was the choice of the side-chain protecting groups of the amino acids serine, histidine and aspartate. The principle of orthogonality between all the different protecting groups present in the scaffold and in the amino acids directed our choices.

Acid labile protecting groups were chosen to protect the serine and histidine side-chains. Since our tripodal scaffold is unstable under strongly acidic conditions (TFA), high acid labile side-chain protecting groups are preferred. That is the reason why we selected trityl side-chain protected serine and methyltrityl side-chain protected histidine.[94] Although these protecting groups, according to the literature, are usually removed with a solution of 5% TFA, we believed that weaker acids could be as efficient as TFA. To control the complete deprotection on solid support of the trityl and methyltrityl groups, polymer-supported compounds **V.2** and **V.3** were synthesised. Thus, treatment of **V.2** with a solution of 80% chloroacetic acid in a mixture CH_2Cl_2/H_2O (3/1) led to successful deprotection affording compounds **V.4** and **V.5**. After cleavage of the dipeptide from Wang resin, the complete deprotection was confirmed from ^1H NMR or/and ES-MS on the basis of the absence of aromatic peaks derived from trityl and methyltrityl groups.



a) $ClCH_2COOH$ / CH_2Cl_2 / H_2O: 80 / 15 / 5, 21 h; b) TFA / CH_2Cl_2 / H_2O: 95 / 2.5 / 2.5

Scheme 5.2

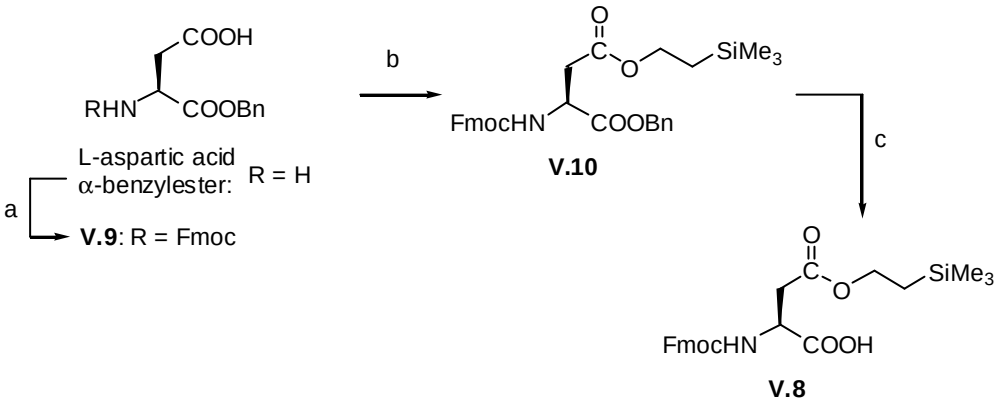
Concerning the side-chain protecting groups of aspartic acid, all commercially available N^{α} Fmoc protected aspartic acids contain a benzyl, allyl, adamantyl or *tert*-Bu ester protected side-chain. These protecting groups are removed under hydrogenolysis, palladium treatment or in presence of strong acid, respectively. All these deprotection conditions are expected to cause some damages to the benzyl ethers present in the scaffold. That is the reason why we turned our attention to an alternative protecting group, the trimethylsilylethyl ester (TMSE).[95] The

[94] Barlos, K.; Chatzi, O.; Gatos, D.; Stavropoulos, G.; Tsegenidis, T. *Tetrahedron Lett*. **1991**, *32*, 475.
[95] (a) Sieber, P. *Helv. Chim. Acta* **1977**, *60*, 2711; (b) Marlowe, C.K. *Bioorg. Med. Chem. Lett*. **1993**, *3*, 437.

deprotection of the TMSE functionality is usually carried out with a solution of 1 M TBAF in DMF for 20 min or with TASF reagent ((tris-(dimethylamino)sulfur trimethylsilyldifluoride).[91e] The TMSE group was expected to be stable during the synthesis of the library and to be removed smoothly at the end.

The trimethylsilylethyl ester **V.8** was prepared in a three-step sequence from the commercially available aspartic acid α-benzylester. Reaction with FmocOSu led to the introduction of the Fmoc-group to give **V.9**. Esterification with 2-(trimethylsilyl)ethanol using DCC as coupling reagent in the presence of DMAP afforded ester **V.10** in 70% yield. Finally, the benzyl group was removed by hydrogenolysis to afford compound **V.8** in 98% yield.

Once the side-chain protection scheme of serine, histidine and aspartate was optimised, the synthesis of our first library was attempted.



a) FmocOSu, $NaHCO_3$, 1,4-dioxane / H_2O, 100%; b) trimethylsilylethanol, DCC, DMAP, CH_2Cl_2, 70%; c) H_2, 10% Pd/C, EtOAc, 98%

Scheme 5.3

5.3 Synthesis of a dipodal library using the 'IRORI' technique

5.3.1 Introduction of the 'IRORI-technique'

The parallel and the 'split and mix' synthesis are two methods to achieve high productivity in combinatorial library synthesis. Parallel synthesis delivers discrete compounds in milligram quantities but suffers from the disadvantage of producing only a limited number of products per unit time and per reaction. The 'split and mix' technique addresses this problem of parallel synthesis but yields a mixture of compounds that needs to be deconvoluted.[96] A hybrid technique, the IRORI method as developed by Nicolaou[97] in 1995, addresses the disadvantages of both parallel and 'split-and mix' synthesis. In this technique, resin beads and a radio-frequency (RF) tag are enclosed in a porous microreactor for solid-phase reactions. The RF (radio-frequency) tag is a semiconductor device capable of receiving, storing and emitting radiofrequency signals. To each tag is assigned one compound of the library at the beginning of the synthesis meaning that for a library of n members, n microreactors containing n RF tags need to be used.

By a process called 'directed sorting', microreactors rather than individual solid phase resin beads are split and pooled. The principle of 'directed sorting' is illustrated in figure 5.1 for a library of 9 compounds. In the first step called 'sorting step', each microreactor containing the RF tag and the resin is placed on the reading station, each tag is read and all microreactors are split into three groups. Each group corresponds to a unique radiofrequency tag (i.e. **a**, **b** or **c**) corresponding to the building block to be added to that group (i.e. **A**, **B** or **C**). The reactions are carried out separately with each group. Afterwards, the microreactors are mixed together. The pool is then sorted and split again in three groups according to the second set of radiofrequency signals (**a**, **b** or **c**) corresponding to the next set of building blocks to be introduced (**A**, **B** or **C**). The chemical reactions are performed in each reaction vessel. This process is repeated until the synthesis is complete. At this stage, the identity of the compounds in each microreactor can be easily deduced by reading the RF tag.

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[96] For more informations about the recursive deconvolution see: Erb, E.; Janda, K.D.; Brenner, S. *Proc. Natl. Acad. Sci. USA* **1994**, *91*, 11422.

[97] Nicolaou, K.C.; Xiao, X.-Y.; Parandoosh, Z.; Senyei, A.; Nova, M.P. *Angew. Chem. Int. Ed. Engl.* **1995**, *34*, 2289.

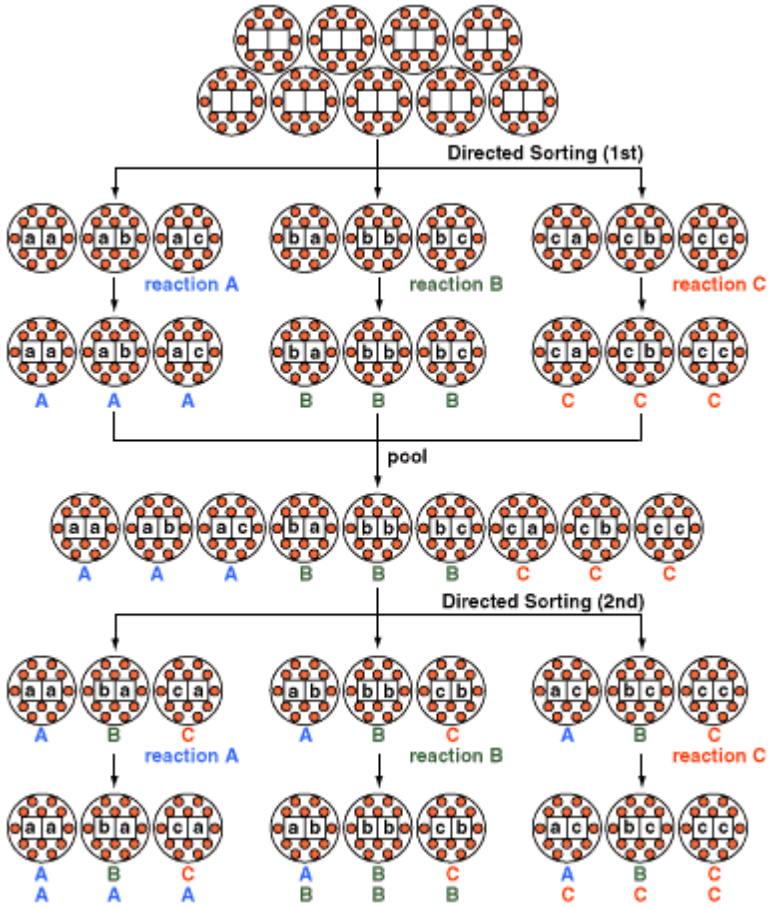


Figure 5.1: Directed-sorting

For a successful synthesis of combinatorial libraries using this technique, the bead size of the polymer support is critical. The size of the microreactor pores is 75 μm. It is therefore recommended to use beads with a diameter of at least 130 μm to avoid any loss of resin through the walls of the microreactor.

This new technique is a powerful synthetic tool for the generation of large discrete compound libraries (table 5.1). As an example, the 'IRORI' technique requires only 40 reactions to synthesise 10 000 discrete compounds in a four-step sequence with 10 building blocks for each step. In comparison, it requires at least 11 110 and up to 40 000 reactions in parallel synthesis. However, taking into consideration that for each compound of the library is assigned one radio-frequency tag, the most important limiting aspect of the 'IRORI' technique in the synthesis of large libraries is the cost of the RF tags.

Table 5.1: Synthesis of 10 000 compounds

Method	Compound form	Quantity	Number of reactions
Parallel	Discrete	Milligrams	11 110-40 000
Split-and Mix	Mixture	Nanograms	40
IRORI	Discrete	Milligrams	40

5.3.2 Synthesis of a dipodal library using the 'IRORI' technique

In collaboration with Prof. Dr. A. Berkessel (Cologne, Germany), we planned the synthesis of a small dipodal library **V.11** containing 81 members using the IRORI technique. As presented in figure 5.2, only the two most important amino acids of the catalytic triad, serine and histidine were incorporated, each of them in a separate peptide strand. As such, FmocSer(OTrt)OH, FmocPheOH and FmocValOH were used as building blocks for the synthesis of the first peptide strand and FmocHis(NMtt)OH, FmocAlaOH and FmocLeuOH for the construction of the second one.

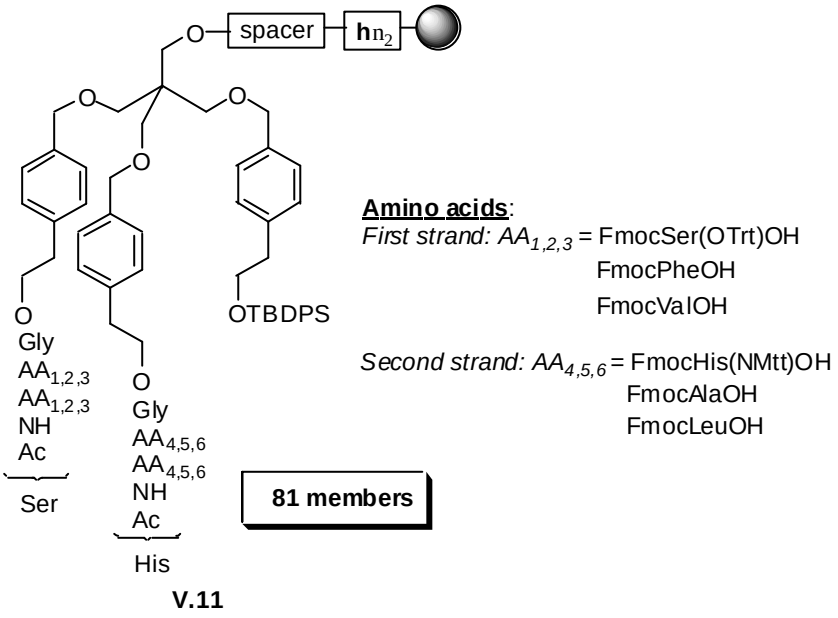
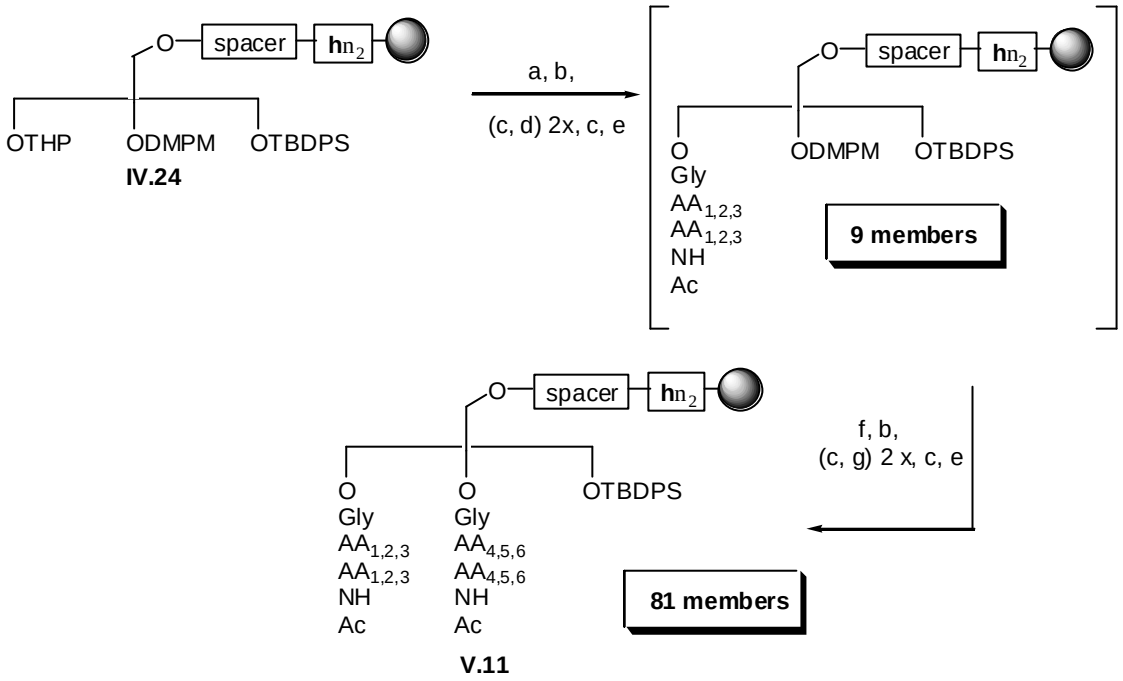


Figure 5.2

The synthesis of the library is summarised in scheme 5.3. After deprotection of the THP protecting group, the acid chloride FmocGlyCl was coupled. The resin was distributed among 81 microreactors, each of them labelled with an RF tag coding for the designated compound. The 81 microreactors were divided between three reaction vessels (27 microreactors per vessel) using

the 'directed-sorting' principle (vide supra). In each vessel, serine, phenylalanine or valine was coupled using PyBOP as coupling reagent in the presence of 3% N-methylmorpholine in dimethylformamide.[98] It has to be mentioned that the use of voluminous microreactors results in much more diluted solutions and longer reaction times (twice as long as compared to coupling reactions on loose resin). After deprotection of the Fmoc protecting group, the microreactors were sorted again in three reaction vessels for the next coupling step. In each vessel, serine, phenylalanine or valine was coupled. Finally, the Fmoc group was removed followed by capping of the free amino function with an excess of acetylimidazole. At this stage, 9 members were obtained.



a) AcOH / CH_2Cl_2 / H_2O: 80 / 15 / 5, 60 °C, 16 h; b) FmocGlyCl, DIPEA, CH_2Cl_2; c) 20% pip. / DMF; d) $AA_{1,2,3}$, PyBop, 3% NMM, DMF; e) AcIm, CH_2Cl_2; f) DDQ, CH_2Cl_2 / H_2O: 19 / 1, 2 h, 0 °C; g) $AA_{4,5,6}$, PyBop, 3% NMM, DMF

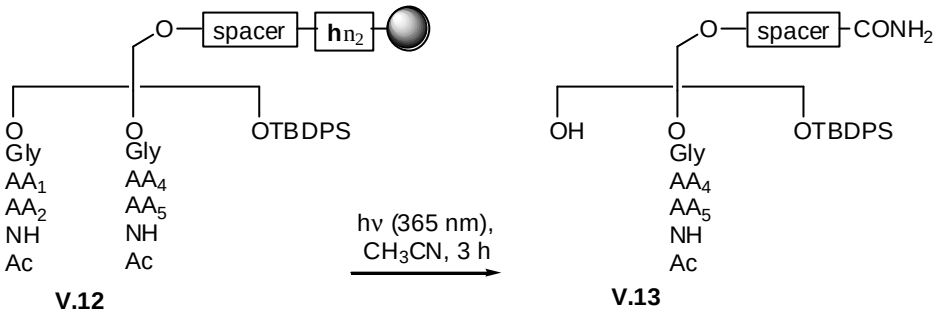
Scheme 5.4

Subsequently, all 81 microreactors were collected in a common flask and the DMPM ether was removed (scheme 5.4) using dichlorodicyanoquinone at 0 °C for 2 h. Similar to the first peptide chain, the construction of the second using the 'IRORI directed split and mix' protocol was performed with the second set of amino acids, histidine, alanine or leucine. The efficiency of all

[98] Coste, J.; Le-Nguyen, D.; Castro, B. *Tetrahedron Lett.* **1990**, *31*, 205.

coupling reactions was successfully monitored in a qualitative way using the colorimetric TNBS and **NF31** tests.

To allow the screening of the library, in the next step, the side-chain protecting groups trityl and methyltrityl were removed using a solution of 80% chlororacetic acid in a mixture of dichloromethane/water (3/1). At this stage, a representative sample (9 members) **V.12a-i** was selected from the library, submitted to photolytic cleavage and analysed (scheme 5.4). Unfortunately, the evaluation of the members by ES-MS revealed a lower molecular weight than expected corresponding to cleavage of the first introduced peptide strand affording compounds **V13a-i**. The origin of the hydrolysis of the ester bond during the synthesis of the library remains unexplained.



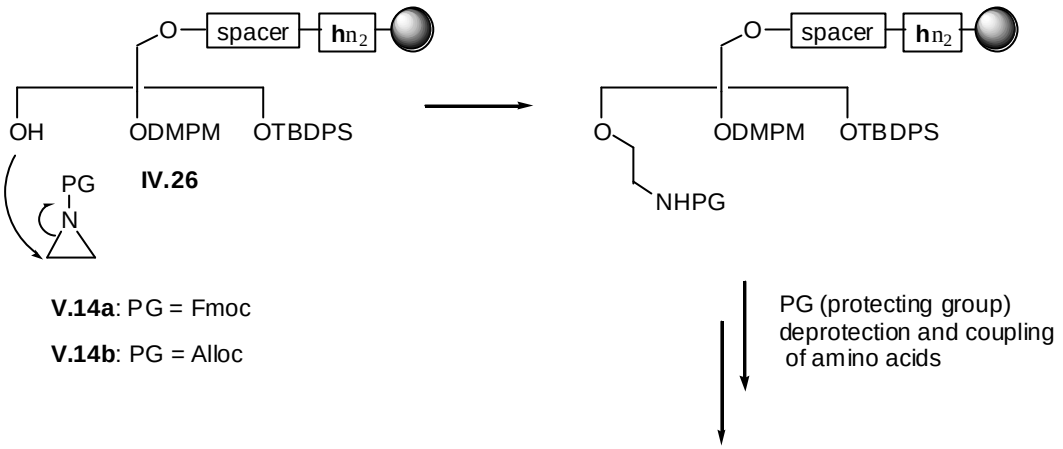
	V.12								
AA_n	**a**	**b**	**c**	**d**	**e**	**f**	**g**	**h**	**i**
1	Val	Val	Phe	Phe	Val	Val	Phe	Val	Phe
2	Phe	Phe	Val	Val	Phe	Val	Val	Phe	Phe
4	Leu	Ala	Ala	Leu	Leu	Ala	Ala	Ala	Ala
5	Ala	Leu	Leu	Leu	Leu	Ala	Ala	Ala	Ala
M_{calcd} (g/mol)	1504	1504	1504	1545	1545	1413	1462	1462	1509
M_{obsd} (g/mol)	1158	1158	1158	1200	1200	1116	1116	1116	1116
V.13									

Scheme 5.5

As a result of the apparent sensitivity of the ester bonds, it was expected that the transformation of the alcohol functions of the scaffold into amines could be the solution of this problem. This alternative pathway is explained in the next section.

5.4 Transformation of **IV.24** into an amine-functionalised tripodal scaffold

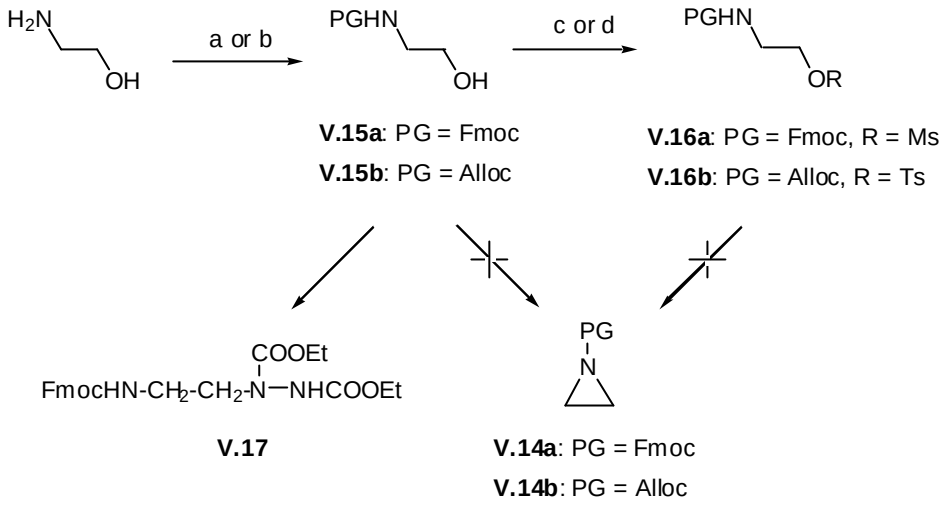
For the transformation of the alcohol functions of scaffold **IV.24** into amino functions, two strategies were taken into consideration. The first strategy involved the reaction of the polymer-supported alcohol functions of **IV.24** with a protected (PG) symmetrical aziridine derivative as outlined in scheme 5.6.



Scheme 5.6

We have chosen aziridines **V.14** protected as Fmoc or as Alloc since the deprotection of these protecting groups on solid phase are known to occur efficiently. Our attempts to synthesise aziridines **V.14** from commercially available 2-aminoethanol are presented in scheme 5.7. The free amino function was first protected with a Fmoc or Alloc group to form protected amino alcohol **V.15a** and **V.15b** in 98% and 76% yield respectively. Subsequently, the alcohol functions were transformed into good leaving groups to form the mesylate **V.16a** and the tosylate **V.16b** in high yield. Unfortunately, treatment of alcohol **V.16a** with potassium *tert*-butoxide or with lithium hexamethyldisilazane (LiHMDS) at –78 °C in tetrahydrofuran gave no ring closure but only Fmoc deprotection.[99] Moreover, when the latter conditions were applied to the tosylate **V.16b**, no aziridine formation was observed either. In an alternative attempt, treatment of **V.15a** with diethylazodicarboxylate (DEAD) in presence of triphenylphosphine under Mitsonubu reaction conditions only resulted in the isolation of the diethylazodicarboxylate derivative **V.17**.

[99] Greene, T.W.; Wuts, P.G. '*Protective groups in organic synthesis*', third edition, **2001**.: The Fmoc-protecting group is claimed to be stable to potassium *tert*-butoxide at low temperature.



a) FmocOSu, $NaHCO_3$, 1,4-dioxane / H_2O, overnight, r.t., 98%; b) AllocCl, DIPEA, CH_2Cl_2, 2 h, r.t., 76%; c) CH_3SO_2Cl, NEt_3, CH_2Cl_2, 1 h, r.t., 92%; d) TsCl, NEt_3, CH_2Cl_2, overnight., r.t., 92%

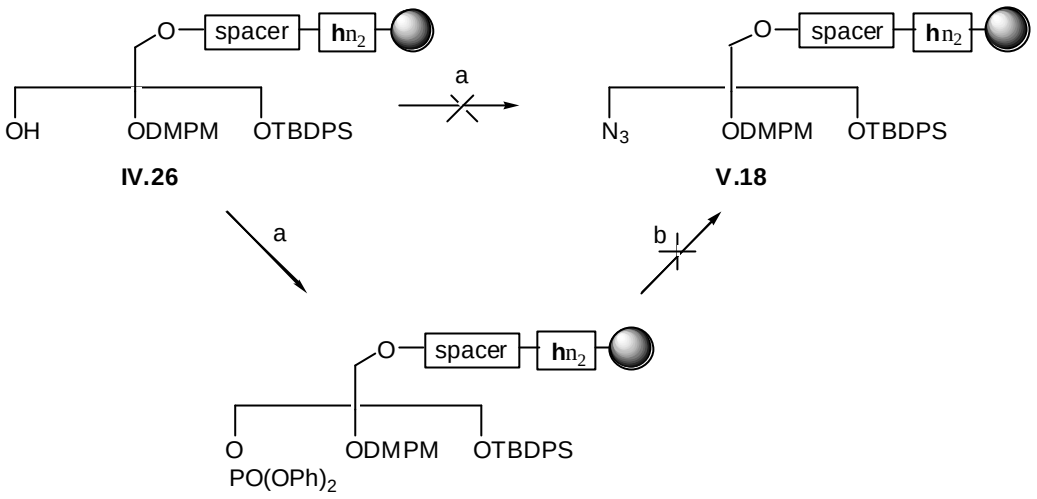
Scheme 5.7

The formation of **V.17**, resulting from a nucleophilic substitution of the mesylate by diethylazodicarboxylate has been described in the literature.[100] In this article, the authors claim that unsubstituted aziridine derivatives can only be synthesised in a very low yield (10%), whereas good yields of aziridine can be obtained if there is at least one substituent attached to either one of the two carbon atoms of the aziridine ring. We therefore concluded that this strategy was not suitable for the synthesis of an amino-functionalised scaffold.

In the second strategy, we planned the transformation of the alcohol functions of scaffold **IV.24** into azides, and subsequent reduction into the corresponding amines. As such, after removal of the THP protecting group of compound **IV.24** with PPTS at 55 °C for 15 h, the resulting alcohol **IV.26** was reacted with diphenylphosphorazidate ($(PhO)_2PON_3$) in the presence of DBU (scheme 5.8) to transform in one step the alcohol function into the corresponding azide **V.18**.[101] When the reaction was carried out at room temperature, instead of azide **V.18** only intermediate **V.19** was formed. Increasing the temperature did not give any improvement. However, when intermediate **V.19** was heated in the presence of a large excess of sodium azide in DMPU for 20 h, only partial conversion to azide **V.18** was observed.

[100] Pfister, J.R. *Synthesis* **1984**, 969.

[101] Thompson, A.S.; Humphrey, G.R.; DeMarco; A.M.; Mathre, D.J.; Grabowski, E.J.J. *J. Org. Chem.* **1993**, *58*, 5886.

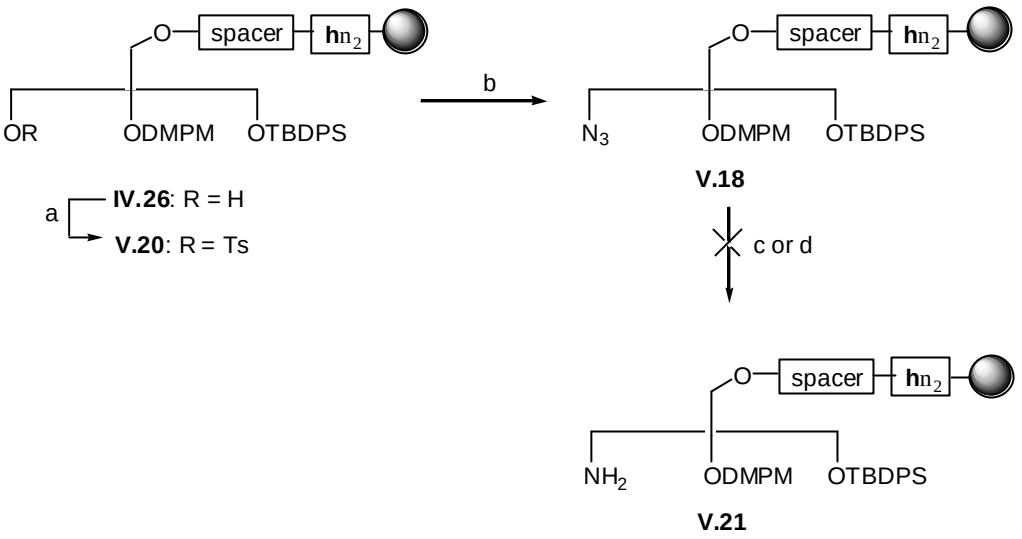


a) DPPA, DBU, toluene, overnight, r.t.; b) NaN_3, DMPU, overnight, 40 °C

Scheme 5.8

Another method involves the transformation of alcohol **IV.26** into tosylate **V.20** (scheme 5.9) followed by treatment with sodium azide in DMPU to form azide **V.18**. Concerning the reduction of azide **V.18** to amine **V.21**, due to the presence of benzyl ethers in the tripodal scaffold and an ester function between the scaffold and the spacer, neither hydrogenolysis nor reduction with lithium aluminium hydride could be used for this step. The alternative Staudinger method, involving the use of triphenylphosphine or trimethylphosphine only afforded complex reaction mixtures.[102]

[102] (a) Staudinger, H.; Meyer, J *Helv. Chim. Acta* **1919**, *2*, 635; (b) Vaultier, M.; Knouzi, N.; Carrié, R. *Tetrahedron Lett*. **1983**, *24*, 763.

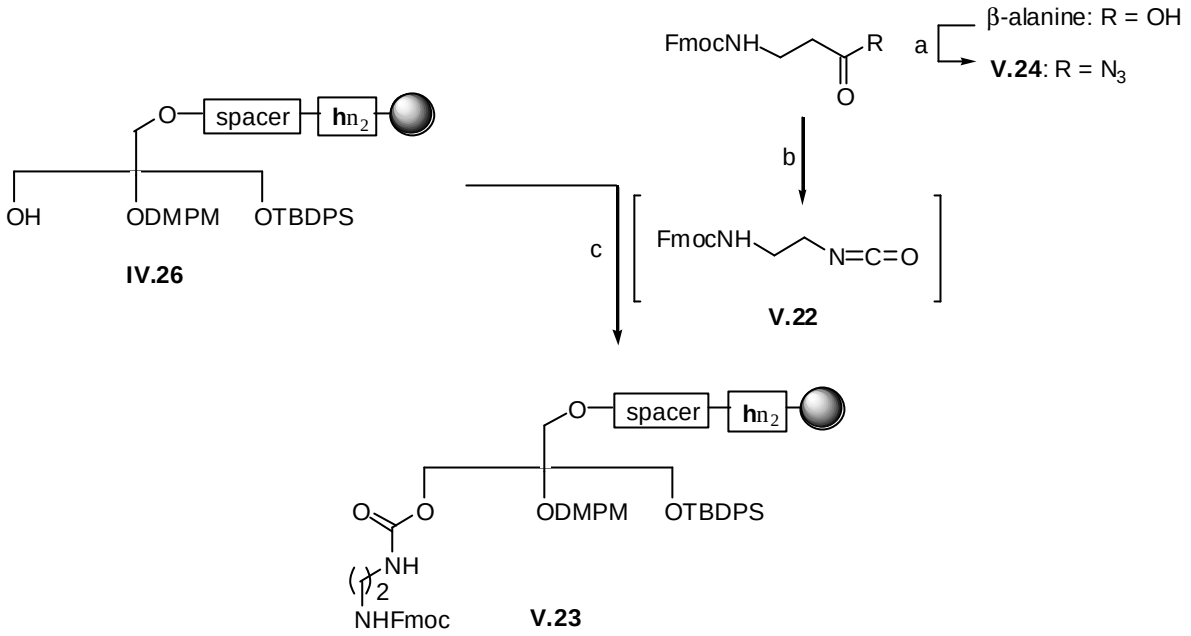


a) TsCl, NEt_3, DMAP, CH_2Cl_2, overnight, r.t.; b) NaN_3, DMPU, overnight, 40 °C; c) PPh_3, H_2O, THF, overnight, r.t.; d) PMe_3, H_2O, THF, overnight, r.t.

Scheme 5.9

In view of these disappointing results, our last tentative consisted in the reaction of alcohol **IV.26** with isocyanate **V.22** which led to the formation of the carbamate **V.23** (scheme 5.10) in 88% yield as determined by UV/VIS spectroscopy. Isocyanate **V.22** was obtained from acylazide **V.24** via a Curtius rearrangement in toluene at 65 °C. However, to our surprise, after photolytic cleavage of a sample of **V.23**, ES-MS analysis revealed this carbamate bond to be unstable. Only traces of carbamate **V.23** were observed next to a major proportion of starting material **IV.26**. The unstability of the carbamate bond can be issue from the photolytic cleavage or from the conditions used during the ES-MS analysis. Since we were unable to confirm the real origin of the failures of these strategies, no further efforts were undertaken for the synthesis of an amine-based scaffold.

At this stage, the use of the alcohol based scaffold was reconsidered. To retrace the origin of the above observed loss of one peptide strand (see synthesis of the dipodal library), a member of the library was resynthesised. After each coupling step, a resin sample was submitted to photolytic cleavage and carefully analysed by mass spectrometry.



a) EtOCOCl, NMM, NaN_3, THF / H_2O, 30 min., -20 °C, 93%; b) toluene, 1 h, 65 °C;
c) toluene, 14 h, 65 °C

Scheme 5.10

5.5 Synthesis of one member of the tripodal library

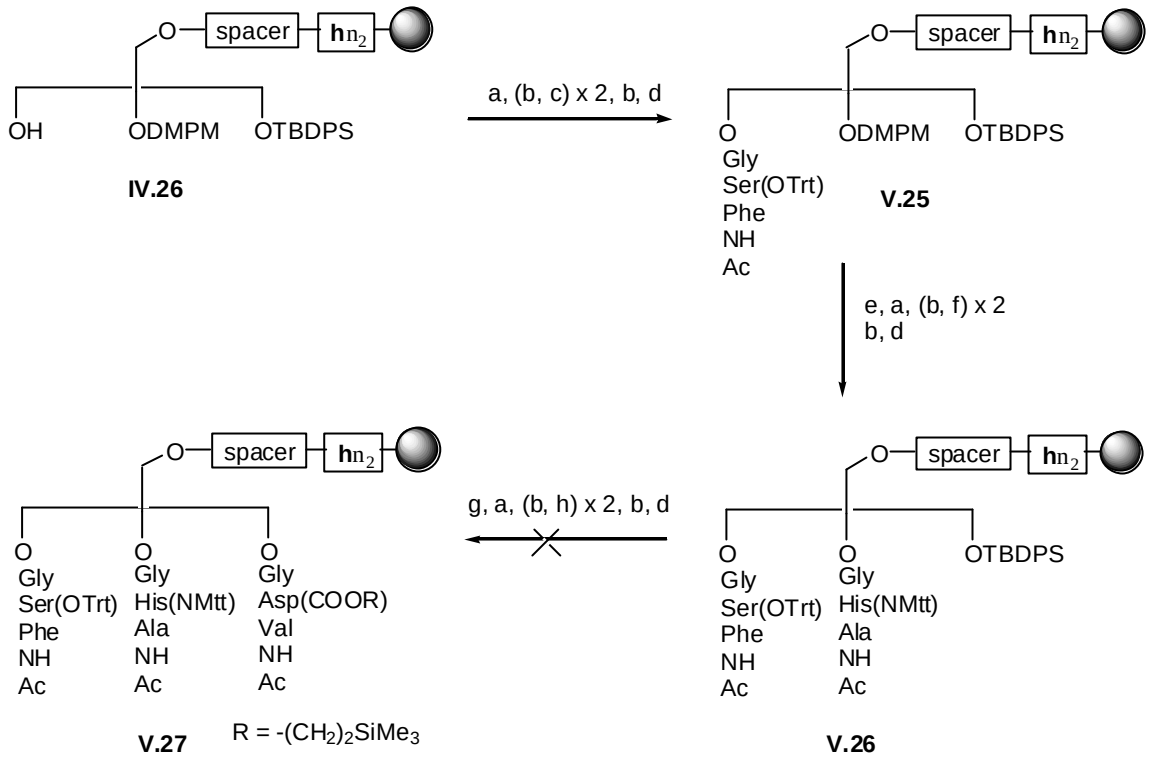
As a final stage before the actual synthesis of our tripodal library, we decided to complete the synthesis of one representative member. This synthetic exercise would serve as a final test to check all previously optimised process parameters, being:

1) the stability of the scaffold during the library synthesis (stability of the benzyl ethers);
2) the orthogonal deprotection of the scaffold protecting groups (THP, DMPM and TBDPS);
3) the usefulness of the photolabile linker for the analysis of intermediates and final products by ES-MS;
4) the monitoring of the completeness of the coupling steps using the colorimetric TNBS and **NF31** tests;
5) the stability of the glycine ester function towards hydrolytic cleavage during the synthesis of the peptide strands (hydrolysis of this ester has been previously observed during the dipodal library synthesis);
6) the stability of the serine, histidine and aspartic acid side-chain protecting groups during the synthesis of the library and the completeness of their final deprotection;

7) Evaluation of MS and NMR techniques in the determination of the peptide sequence of the final product.

The synthesis of the member of the library is outlined in scheme 5.11. After deprotection of the THP protecting group using PPTS in ethanol at 55 °C, the first peptide strand was constructed by reaction of the free alcohol function with FmocGlyCl in 95% yield followed by consecutive coupling of FmocSer(OTrt)OH and FmocPheOH using the traditional DIC/HOBt methodology. Finally, after removal of the last Fmoc protecting group, the free amino functions were capped with an excess of acetylimidazole to form resin **V.25**. The structure of **V.25**, after photolytic cleavage, was confirmed by ES-MS, ^{1}H NMR, COSY NMR, TOCSY NMR and ROESY NMR. Subsequently, the DMPM ether was removed using dichlorodicyanoquinone at 0 °C for 1 h and the second peptide strand including histidine was generated following the same strategy using the amino acids FmocHis(NMtt)OH and FmocAlaOH affording **V.26**. The photolytic cleavage of this dipodal member indicated the presence of both peptide strands meaning that no hydrolysis of the glycine ester bonds had occurred.[103] Finally, after deprotection of the TBDPS ether with a solution of TBAF in the presence of acetic acid, the third and last peptide chain containing FmocAsp(COO$(CH_2)_2SiMe_3$)OH and FmocValOH was synthesised. Unfortunately, despite the success of the dipodal derivative **V.26**, the steps leading to the construction of the third peptide strands appeared not to be compatible with the already present functionalities. Careful ^{1}H NMR analysis of the photolytically cleaved **V.27** revealed the deprotection of the side-chain protecting trityl and methyltrityl groups.

[103] It has to be noted that the ^{1}H NMR of the cleaved compound from resin **V.26** was not pure enough to attribute all peaks but we could clearly observe that the glycine ester bonds were intact due to the presence of trityl and methyltrityl peaks in the spectrum.
In addition, the limited mass-ion detection of our ES-MS equipment is 2000 g/mol and does not allow the detection of the high molecular weight of the product issue from the cleavage of resin **V.26** (2013 g/mol).



a) FmocGlyCl, DIPEA, CH_2Cl_2, 95%; b) 20% pip. / DMF; c) FmocSer(OTrt)OH or FmocPheOH, DIC, HOBt, DMF/CH_2Cl_2; d) AcIm, CH_2Cl_2; e) DDQ, CH_2Cl_2 / H_2O: 19 / 1, 1 h, 0 °C; f) FmocHis(NMtt) or FmocAlaOH, DIC, HOBt, DMF / CH_2Cl_2; g) TBAF, AcOH, THF, overnight (2x), r.t.; h) FmocAsp($COO(CH_2)_2SiMe_3$)OH or FmocValOH, DIC, HOBt, DMF / CH_2Cl_2

Scheme 5.11

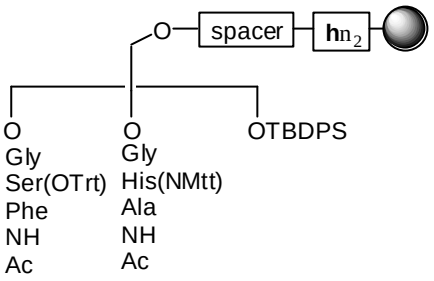
As a possible explanation, the deprotection of the trityl and methyltrityl groups might have occurred during the coupling of FmocGlyCl due to the presence of some remaining thionyl chloride from the preparation of the acid chloride of glycine. Another hypothesis for the removal of the trityl and methyltrityl group might be the long exposure time of resin **V.26** to acetic acid used as a co-solvent during the TBDPS deprotection step. To solve these problems, we decided to investigate another coupling method to couple glycine on the hydroxyl function and to avoid the use of acetic acid during the deprotection of the TBDPS ether.

Concerning the coupling of glycine on the hydroxyl function in **IV.24**, another efficient method was found to be the traditional methodology DIC/HOBt in the presence of diisopropylethylamine and a catalytic amount of DMAP.[104] Using this new method for the coupling of glycine, we were able to synthesise again successfully our dipodal member **V.26**. After removal of the trityl and

[104] The amount of DMAP needs to be carefully controlled to avoid deprotection fo the Fmoc protecting group and dicoupling.

methyltrityl group in a sample of resin **V.26**, the resulting product was cleaved by photolysis and analysed by ES-MS.

At this stage, for the deprotection of the TBDPS ether several new reaction conditions were screened (table 5.2). The use of ammonium fluoride led to hydrolysis of an ester bond in resin **V.26**. This side-reaction can be explained by the release of ammonia in the solution, which is nucleophilic enough to hydrolyse the ester bond. When potassium fluoride or cesium fluoride were used, only some traces of TBDPS deprotection were observed. On the other hand, the widely used hydrogen fluoride (70%). pyridine was efficient in the cleavage of the TBDPS ether but apparently too acidic since the trityl and methyltrityl groups were also deprotected. Finally, the less known TASF reagent (tris(dimethylamino)sulfur (trimethylsilyl)difluoride) was successful and led to a clean deprotection of the TBDPS ether after 3 h at room temperature.



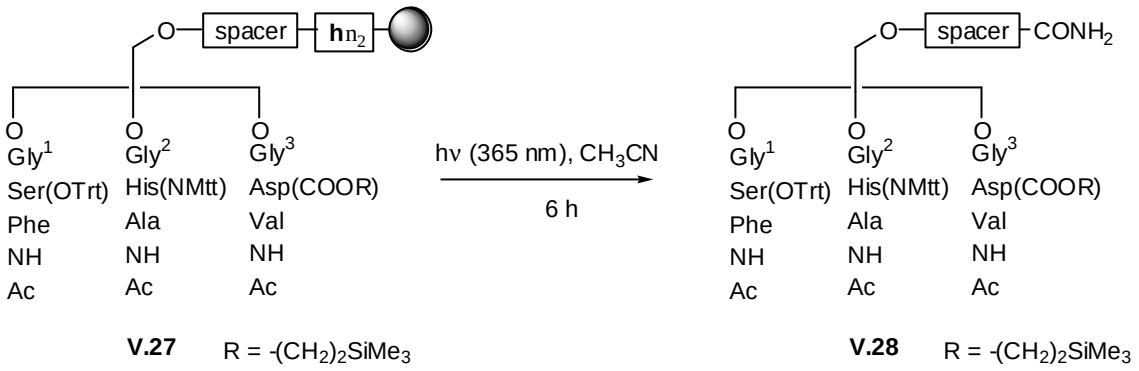
V.26

Table 5.2: Deprotection of the TBDPS ether in compound **V.26**

	Conditions	Results
1	NH_4F, MeOH	Cleavage of the ester
2	KF (10 eq.), 18-crown-6, THF, r.t.	No reaction
3	CsF (10 eq.), THF, r.t.	Trace of deprotection
4	HF.pyr. (10 eq.), THF, 4 h	Trityl and Methyltrityl deprotection
5	TASF (2 eq.) in DMF, 3 h, r.t.	Selective deprotection of the TBDPS ether

Once the TBDPS ether removed, the third and last peptide was constructed following the same strategy with the amino acids FmocAsp(COO$(CH_2)_2SiMe_3$)OH and FmocValOH.

To prove the success of the synthesis, a sample of the polymer-supported tripodal member **V.27** was cleaved (scheme 5.12) and the resulting compound **V.28** was analysed by ^{1}H NMR. The structure of the tripodal member was confirmed with the help of ^{1}H NMR, COSY NMR, TOCSY NMR, ROESY NMR.



Scheme 5.12

The ^{1}H-^{1}H COSY NMR shows the scalar coupling interactions between geminal and/or vicinal protons in one specific amino acid such as NH $\rightarrow$ Hα and Hα $\rightarrow$ Hβ. These are called the direct connections. On the other hand, the ^{1}H-^{1}H TOCSY spectrum gives correlations between any ^{1}H resonances of a spin system provided they can be linked by an uninterrupted chain of scalar couplings. The transfer of magnetisation occurs from NH $\rightarrow$ Hα $\rightarrow$ Hβ $\rightarrow$ Hγ. These are called the indirect connections.

In the ROESY spectrum, the NOE effect is used to show correlations between protons which are close together in space (distance < 500 pm). The NOE contacts between the amide, α- and β-protons can be used for the determination of the amino acid sequence. According to statistic analysis, the simultaneous observation of a NOE contact connecting the Hα and Hβ of one residue with the NH of another one (N terminal $\rightarrow$ C terminal) indicates that these residues are sequential with more than 95% probability. As illustration, the expected NOE interactions for the sequence -Val-Asp-Gly^{3}- are presented in figure 5.3.

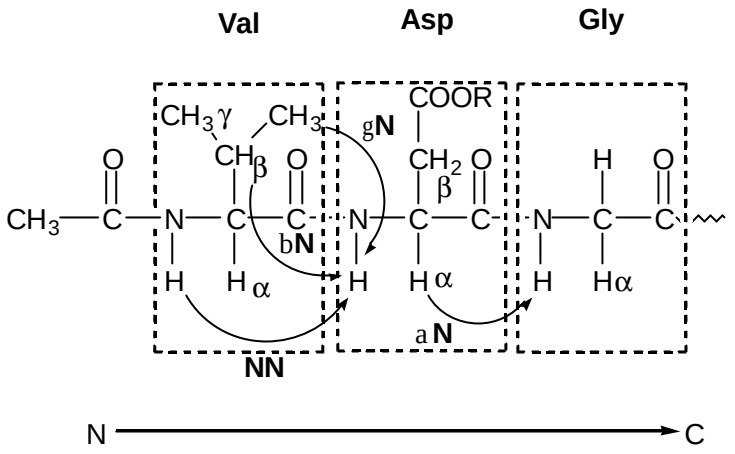
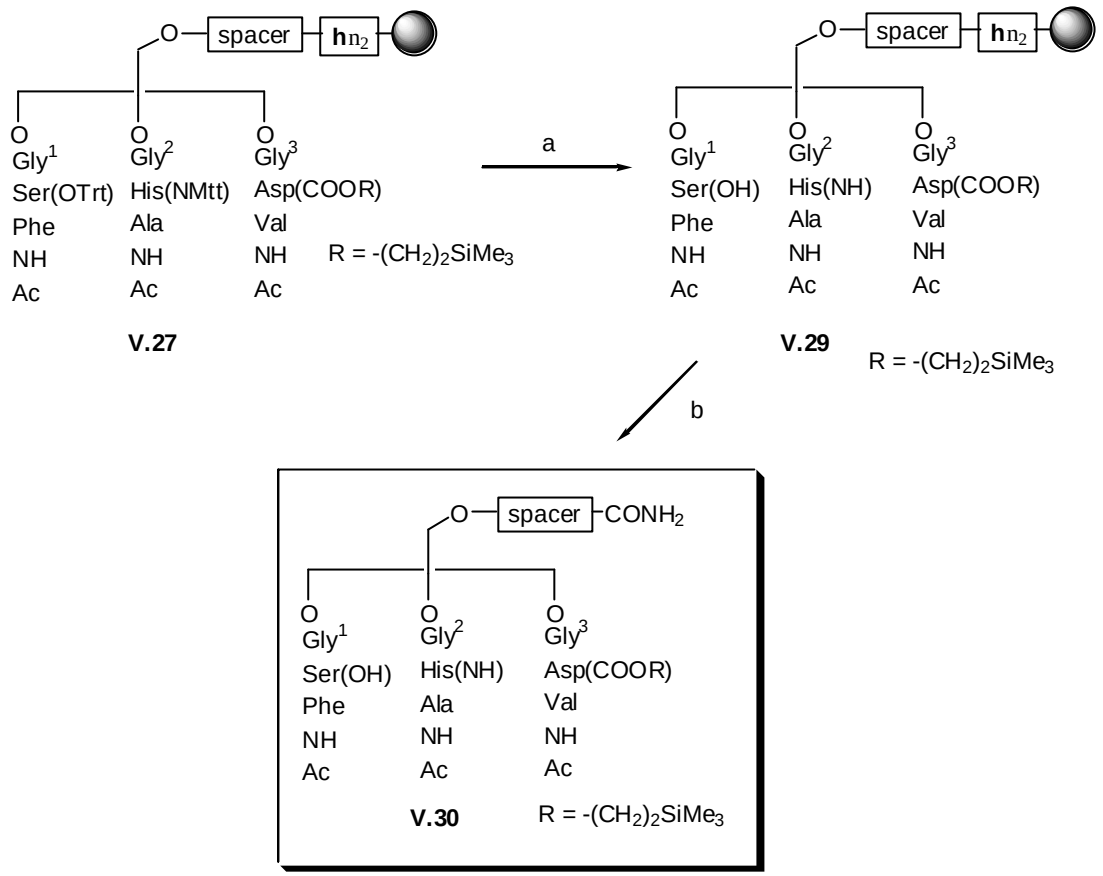


Figure 5.3: NOE's contacts in the ROESY spectrum for the sequence -Val-Asp-Gly^3-

The structure of compound **V.28** was confirmed as follows (figure 5.4): the Hγ of the valine residue is identified in the TOCSY spectrum at 0.75 ppm. The assigment of the Hα- and Hβ- are deduced from the TOCSY spectrum as indicated. On the other hand, the ROESY spectrum shows a cross-peak between the Hα of valine with the amide proton of aspartic acid at 8.14 ppm. Two other sequential interactions are present also between the Hβ and Hγ of valine with the amide proton of aspartic acid. Concerning the valine residue, the last cross peak corresponds to an interaction between the methyl from the N-terminal acetyl group at 1.8 ppm to the amide proton of valine at 7.86 ppm. The presence of a glycine residue next to aspartic acid was confirmed by the cross-peak interaction between the Hα of aspartic acid and the amide proton of glycine at 7.90 ppm. The sequence Me-Val-Asp-Gly^3- was proven to be present in compound **V.28**. The same strategy was followed to confirm the two other sequences to be consistent with -Phe-Ser-Gly^1- and -Ala-His-Gly^2. The position of all intra residue scalar coupling correlations and sequential NOE interactions are labelled on the TOCSY and ROESY spectra respectively (figure 5.4).



a) $ClCH_2COOH$ / CH_2Cl_2 / H_2O: 80 / 15 / 5, 6 h, overnight, r.t.; b) hν (365 nm), CH_3CN, 3 h

Scheme 5.13

Subsequently, the side-chain protecting groups of serine, histidine were removed (scheme 5.13) using a solution of chloroacetic acid to form resin **V.29**. This resin was cleaved by photolysis to form successfully compound **V.30**, which was analysed by ES-MS (figure 5.5). The peaks at 868, 1691 and 1713 g/mol correspond to the doubly charged adduct, the proton adduct and the sodium adduct respectively. An incomplete deprotection of the TBDPS ether explains the presence of the peak at 1538 g/mol corresponding to some remaining compound **V.26.** In the future, a longer reaction time for the TBDPS deprotection could solve this problem.

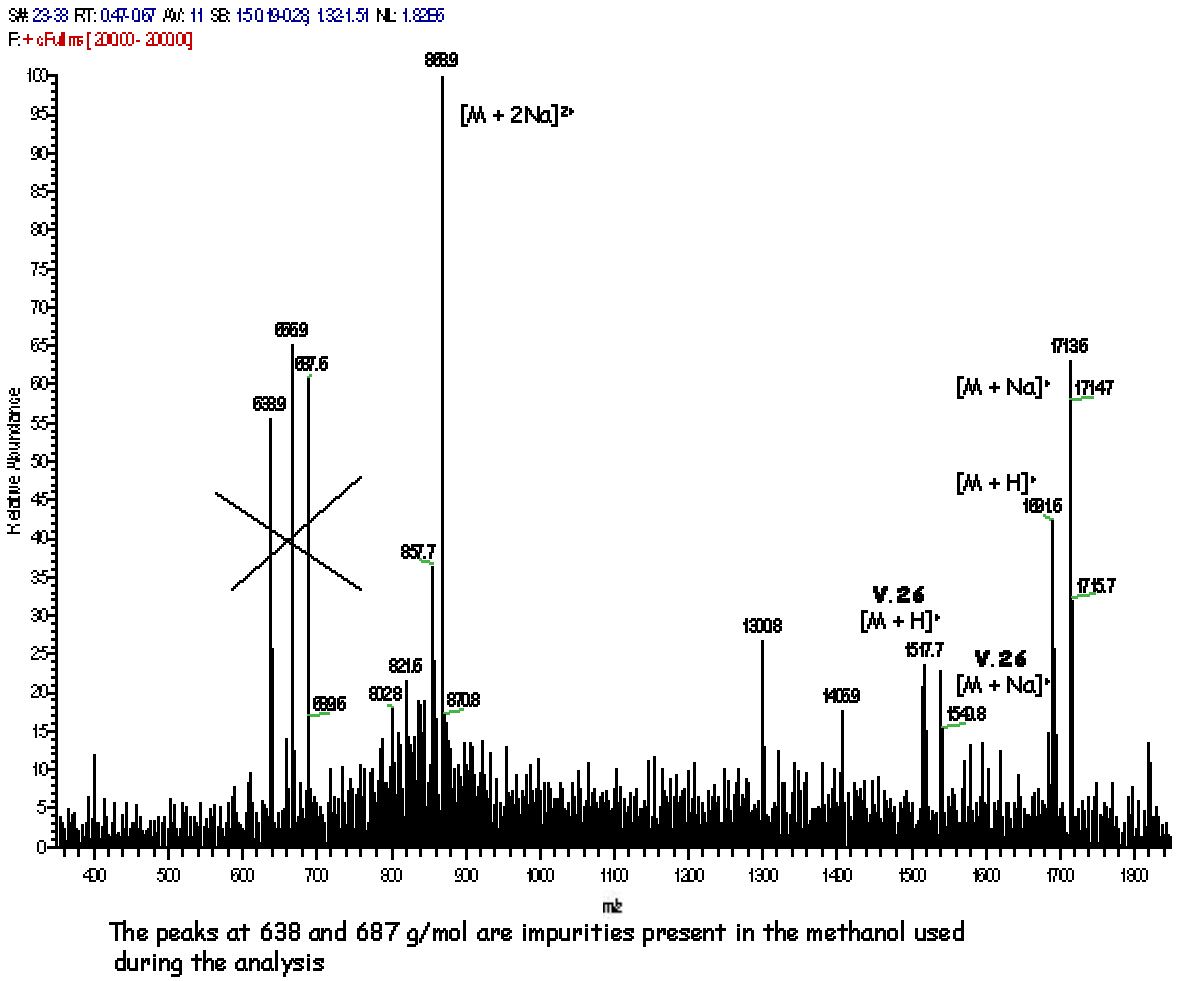


Figure 5.5

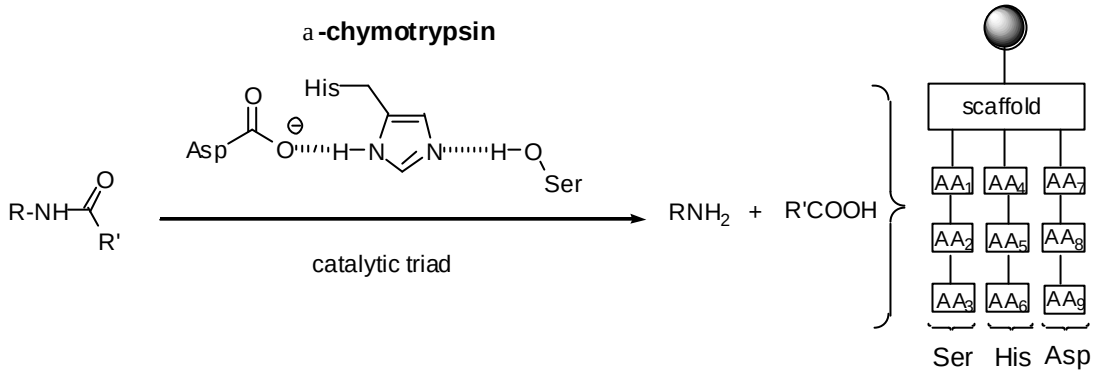
Finally, resin **V.29** was treated with fluoride anions to remove the side-chain protecting group of aspartic acid. However, the use of TBAF is known to affect the *o*-nitrobenzyl moiety blocking any possible further photolytic cleavage.[69a] When TBAF was used in the presence of acetic acid, only starting product was observed. Finally, the previously successful TASF reagent failed to deprotect the TMSE group.

5.6 Conclusion

After 'trial and error', we succeeded in the synthesis of a tripodal member of the library. All the difficulties of the orthognal protection scheme were overcome. All reactions on solid support were optimised. Our scaffold is a suitable template for the future construction of combinatorial tripodal libraries.

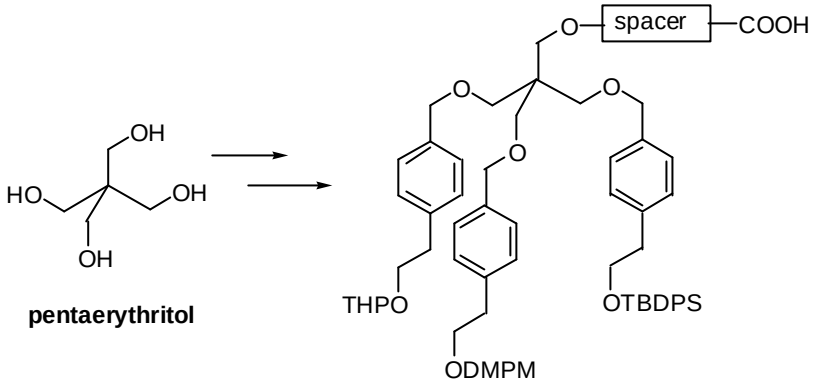
6 General conclusion and perspectives

In this work we presented our efforts towards the development of a new catalyst for the hydrolysis of amide bonds, based on the well-known serine-protease α-chymotrypsin, using combinatorial techniques (scheme 6.1). Even if the efficiency of α-chymotrypsin has inspired scientists in the design of artificial enzymes, no efficient model has been reported so far using combinatorial techniques. This PhD work involved the development of a tripodal library designed as such that each residue of the catalytic triad serine, histidine and aspartate were incorporated in one of the three peptide strands. The role of the scaffold is to induce the proximity between the three different peptide chains. By the synthesis of hundreds of compounds, the use of combinatorial chemistry gives a greater chance of finding one or several catalysts possessing hydrolytic activity.



Scheme 6.1

To attain this goal, tripodal scaffold **III.6**, was synthesised from the commercially available pentaerythritol (scheme 6.2) in 4.1% overall yield. This scaffold possesses three hydroxyl functions orthogonally protected and a carboxylic acid function allowing for the coupling to the polymer support. The polymer support used was TentaGel-NH_2.



pentaerythritol **III.6**

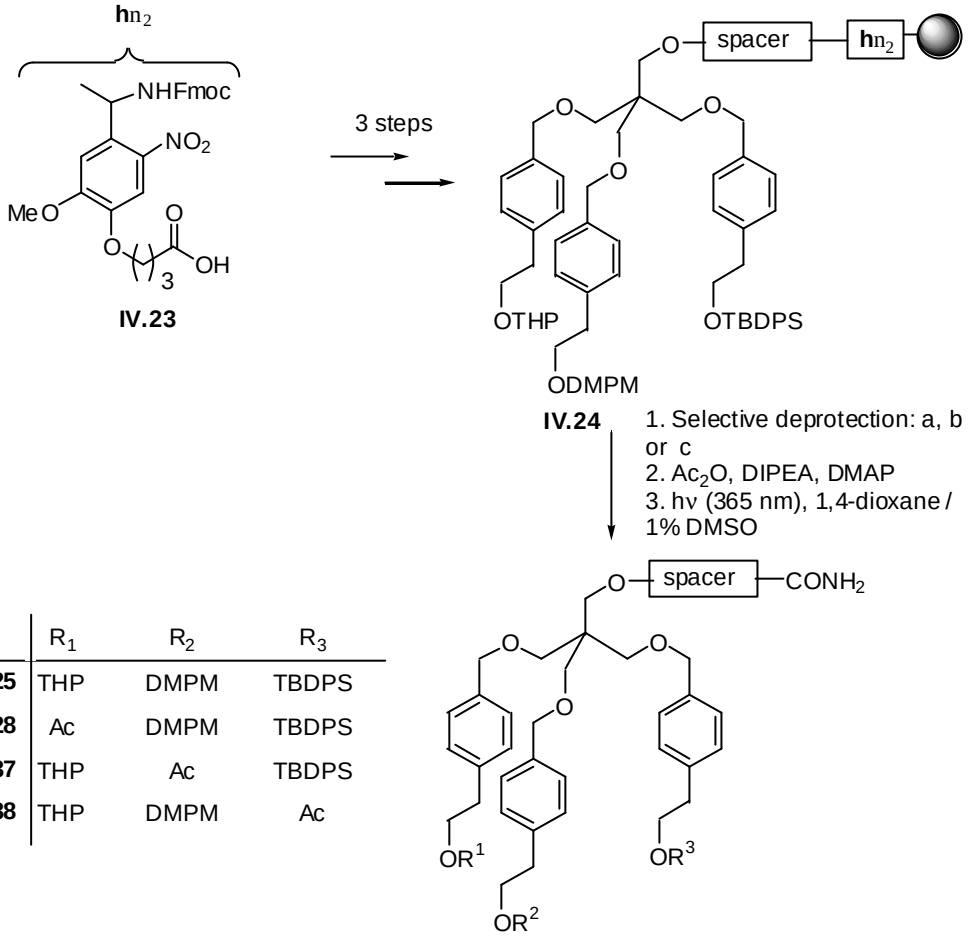
Scheme 6.2

To facilitate the solid-phase chemistry part of this work, a cleavable linker was included in the synthesis enabling the release of the compounds attached on the solid support and allowing for the analysis of the products using classical analytical methods such as ^{1}H NMR and ES-MS. The use of the *o*-nitrobenzyl photolabile linker derivative **IV.23** developed by Holmes was the most efficient. After coupling of acid linker **IV.23** to TentaGel and deprotection of the Fmoc-group, the scaffold was successfully coupled to the polymer support. The optimisation of the photolytic cleavage was performed on solid-phase bound scaffold **IV.24**. The best results were obtained when resin **IV.24** in suspension in 1,4-dioxane in presence of 1% dimethylsulfoxide was irradiated for 9 h affording compound **IV.25** in 86% yield.

The next aspect, which was evaluated, concerned the selective deprotection of the three protecting groups, THP, DMPM and TBDPS. This allowed later for the independent generation of three peptide strands. The deprotection of the THP group was performed using a solution of 80% acetic acid at 60 °C or with the help of PPTS at 55 °C. In the case of the removal of the DMPM ether in **IV.24**, complete and selective deprotection was achieved using dichlorodicyanoquinone (DDQ) at 0 °C for 1 h. Longer reaction time or higher temperature led to cleavage of the benzyl ethers present in scaffold **III.6**. Concerning the deprotection of the TBDPS ether, the procedure using tetrabutylammonium fluoride failed. However, the use of TBAF in presence of acetic acid led to a successful and selective deprotection. Although the on-resin deprotection could be followed by ^{13}C gel phase NMR, we wished to control the efficiency and the reproducibility of the above procedure in a quantitative way. Therefore, in each case, after deprotection, the free hydroxyl function was capped as the acetate, and the resulting

intermediate was subjected to photocleavage. The released acetates **IV.28**, **IV.37** and **IV.38** were obtained in 55%, 62%, and 56% respectively.

At this stage, we succeeded in the choice of a suitable cleavable linker for our synthesis, in the optimisation of the photolysis conditions and in the selective deprotection of the three protecting groups.



	R_1	R_2	R_3
IV.25	THP	DMPM	TBDPS
IV.28	Ac	DMPM	TBDPS
IV.37	THP	Ac	TBDPS
IV.38	THP	DMPM	Ac

a) AcOH / CH_2Cl_2 / H_2O: 80 / 15 / 5, 60 °C, 16 h; b) DDQ, CH_2Cl_2 / H_2O: 19 / 1, 1 h, 0 °C; c) TBAF, AcOH, molecular sieves (400 pm), THF **or** NH_4F, MeOH **or** HF/pyr., THF **or** TASF, DMF.

Scheme 6.3

Once the selective deprotection of the three protecting groups was achieved, the ultimate goal, the synthesis of a library could be envisaged. Since the Fmoc/*tert*-Bu stragegy was used, N^{α} Fmoc protected amino acids containing acid labile protected side-chains were used. The commercially available FmocSer(OTrt)OH and FmocHis(NMtt)OH were chosen. The high acid sensitive trityl and methyltrityl groups were completely removed by a solution of 80%

chloroacetic acid. In the case of aspartic acid, the trimethylsilylethyl ester (TMSE) was selected. The TMSE group can be deprotected, according to the literature using tetrabutylammonium fluoride or with the TASF reagent. Compound **V.8** could be prepared in a three-step sequence from the commercially available aspartic acid α-benzylester in 70% overall yield.

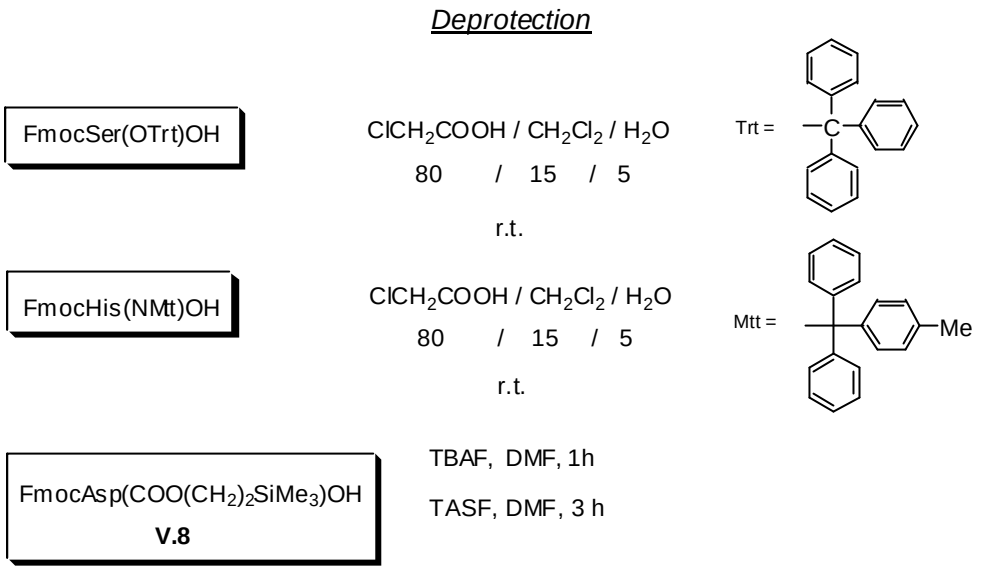
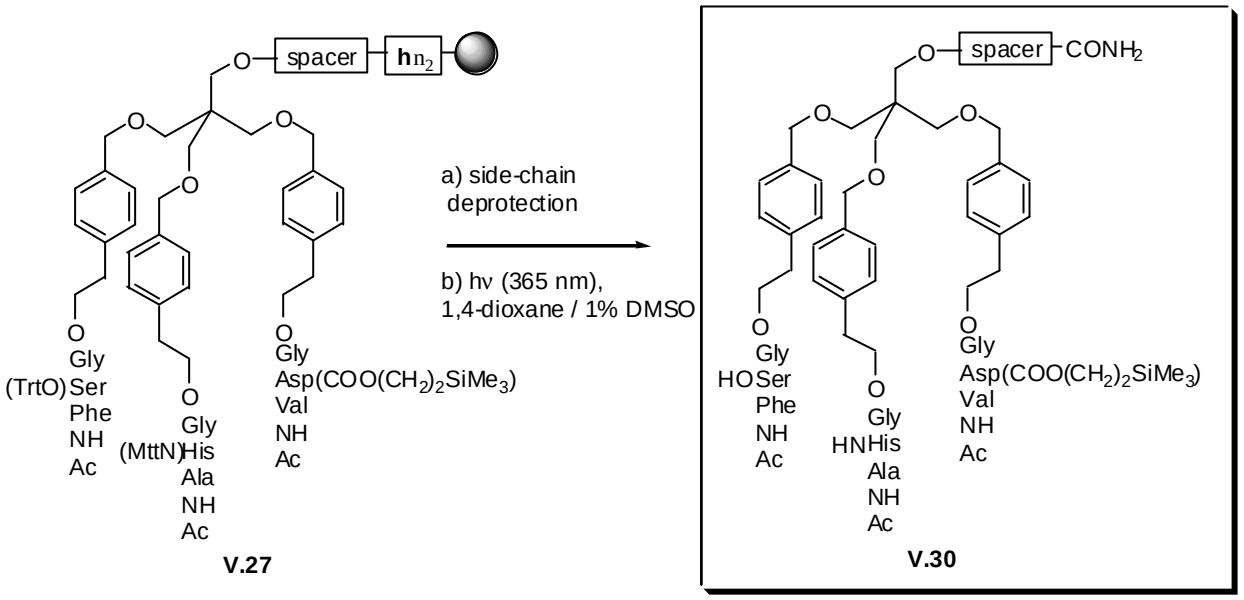


Figure 6.1

As a final stage before the actual synthesis of our tripodal library, we decided to complete the synthesis of one representative member. After removal of the THP group, glycine was coupled using traditional DIC/HOBt methodology in the presence of diisopropylethylamine and a catalytic amount of DMAP. The efficiency of the coupling reaction on the hydroxyl function was monitored quantitatively using Fmoc UV/VIS spectroscopy. The first peptide chain was constructed using FmocSer(OTrt)OH and FmocAlaOH following the DIC/HOBt protocol. After removal of the Fmoc protecting group, the free amino functions were capped with an excess of acetylimidazole. Subsequently, the DMPM ether was removed using dichlorodicyanoquinone at 0 °C for 1 h and the second peptide strand was generated following the same strategy using the amino acid building blocks FmocHis(NMtt)OH and FmocAlaOH. The TBDPS ether was deprotected with the TASF (tris-(dimethylamino)sulfur trimethylsilyldifluoride) reagent, the third peptide chain containing FmocAsp(COO(CH$_2$)$_2$SiMe$_3$)OH and FmocValOH was generated to form tripodal member **V.27**. The structure of resin **V.27** was confirmed after photolytic cleavage with the help of NMR experiments including TOCSY NMR, ROESY NMR and COSY

NMR. The completeness of all the coupling reactions was monitored using the colorimetric TNBS and **NF31** tests.

The side-chain protecting groups of serine and histidine were removed (scheme 6.4) using a solution of chloroacetic acid. After photolytic cleavage, the structure of tripodal member **V.30** was confirmed by ES-MS analysis and NMR analysis.



Scheme 6.4

In the future, the synthesis of a tripodal library using the 'split and mix' protocol will lead to a mixture of several hundreds of compounds. Subsequently, the library will be screened for hydrolytic activity towards a chosen substrate. For this step, several test substrates built up from the commercially available Disperse Red 1 were synthesised. Each of them contains an ester or amide bond representing a different reactivity towards hydrolysis: the most reactive *p*-nitrophenyl ester **I.16a**, the *p*-nitroanilide **I.16b** and the amide **I.16c**. Upon exposure of the library to the dye **I.16**, the catalyst will react with the substrate and the colour will be transferred to the bead. This technique enables an easy visual detection of the polymer-bound active catalyst upon screening of the library.

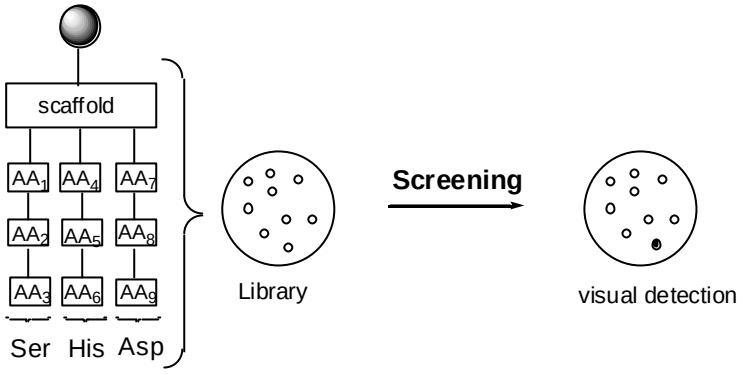
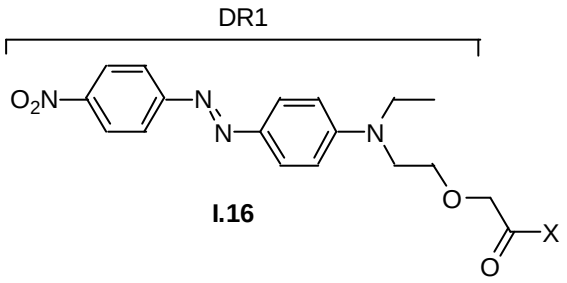


Figure 6.2

The most reactive *p*-nitrophenyl ester **I.16a** is actually commercially available and has already found a few applications: 1) it is used as a new test-substrate for the visual detection of active catalysts among the mixture of hundreds of compounds during the screening of combinatorial libraries; 2) it is widely used as a new colorimetric test for the detection of solid-phase bound amines. The **NF31** test is particularly useful in the case of sterically hindered amines and for coupling reactions to proline derivatives, cases where other colorimetric tests fail.



I.16

a X = −OPhNO$_2$(p) = **NF31**
b X = −NHPhNO$_2$(p)
c X = −NHCH$_2$CH$_2$OPhNO$_2$(p)

Figure 6.3

Following a 'trial and error' strategy, we optimised all reaction conditions to synthesise one member of our tripodal library. However, the presence of the ester bonds is the major drawback of this scaffold. The lesson, which can be drawn from the observed unstability of these glycine ester functions is that our future investigations have to be oriented to the synthesis of a more stable amino-functionalised scaffold. In this thesis, we proved that our tripodal scaffold **III.6** is a suitable template for the future construction of combinatorial tripodal libraries. The way is now clear for my successors to synthesise thousands of tripodal members.

7 Experimental part

7.1 Products, methods, chromatography, and spectroscopy

All experiments involving air moisture sensitive materials were carried out under inert atmosphere (nitrogen or argon). Tetrahydrofuran (THF) and diethyl ether were distilled from sodium benzophenone ketyl prior to use. Pyridine, triethylamine, diisopropylethylamine were distilled over calcium hydride before use. Methanol was dried on magnesium. 1,4-Dioxane was distilled from sodium borohydride. Dimethylsulfoxide and dimethylformamide were purchased from Biosolve (extra-dry). All reagents and solvents were used as received without further purification unless otherwise noted.

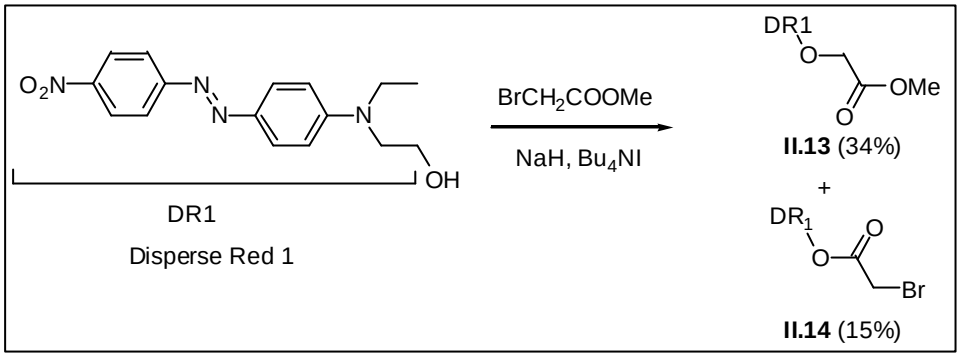
The reactions were followed by thin layer chromatography on glass plates precoated with silica gel (60F254, 0.25 mm). All compounds were purified by column chromatography on silica gel (Merck silica gel 60F254). Infrared spectra were obtained on a Perkin-Elmer 1600 series FTIR spectrophotometer using KBr plates and are reported in cm^{-1}. Ultraviolet spectra were obtained on a Varian Cary 3E UV/VIS spectrophotometer. Mass spectra were recorded on a AEI MS-50, a Finnigan 400 or a Hewlett-Packard 5988 A mass spectrometer. The electrospray spectra were recorded using a LCQ ion trap mass spectrometer equipped with a ESI source (ThermoFinnigan, San Jose, Ca, USA). The ^{1}H NMR spectra were recorded at 500 MHz on a Bruker AM-500 or at 200 MHz on a Varian Gemini-200. The chemical shifts are given in ppm and the coupling constant in Hertz (Hz). The ^{13}C NMR spectra were recorded on a Varian Gemini-200 at 50 MHz. The abbreviations used are: s (singlet), d (doublet), t (triplet), q (quadruplet). Elementary analyses were performed in the S.I.A.R.-institute of the Université Pierre et Marie Curie in Paris (France).

The reaction on solid phase were performed with a shaker (Selecta). The Perkin-Elmer Lambda 3UV/VIS spectrophotometer was used to determine the yield of the coupling reactions of amino acids on polymer-support unless otherwise noted. The pictures were taken with a Leitz microscope camera, Laborlux 12 Pol S, equipped with a JVC colour video camera. All solid phases and amino acids were purchased from Rapp polymer or Novabiochem.

Photolyses were carried out at a distance of 1 cm using a 450 W ACE GLASS incorporated 7225-34 UV lamp set at 365 nm. For small quantities of resin (2 mg), photolysis was carried out at a distance of 1 cm using a 4 W Bioblock Scientific compact UV lamp set at 365 nm (relative intensity at 15 cm of 340 $\mu W/cm^2$).

7.2 Experimental data of part 2

Synthesis of Methyl-5-{N-ethyl-N-[4-(4-nitrophenyl)azophenyl]}amino-3-oxapentanoate **II.13**



A solution of Disperse Red 1 (5.0 g, 15.90 mmol) in dry tetrahydrofuran (70 ml) was added at 0 °C to a suspension of sodium hydride (60% dispersion in oil) (1.0 g, 25.50 mmol) in dry tetrahydrofuran (20 ml). After 1 h at room temperature, a solution of bromomethyl acetate (1.78 ml, 18.10 mmol) in dry tetrahydrofuran (20 ml) and tetrabutylammonium iodide (59.0 mg, 0.16 mmol) were added. The mixture was stirred at room temperature overnight. After evaporation of the tetrahydrofuran, the residue was dissolved in dichloromethane. The organic phase was then extracted with water and the combined organic phases were dried over anhydrous magnesium sulfate. After filtration and evaporation under reduced pressure, the crude mixture was chromatographed on silica gel (isooctane / ethyl acetate: 8 / 2 to 7 / 3) to provide 2.1 g methyl ester **II.13** (34%) with 1.0 g of the transesterification product **II.14** (15%) and 2.1 g of starting material (42%).

Spectra of methyl ester **II.13**:

Bruto formula: $C_{19}H_{22}N_4O_5$

M.W.: 386.4 g/mol

m.p.: 101 °C (recrystallization from isopropanol)

R_f (toluene / ethyl acetate: 1 / 1) = 0.68

IR (film) 2916 (m), 1749 (s), 1601 (w), 1510 (s), 1212 (m), 1144 (s), 868 (m), 833 (m), 723 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.32 (2H, m), 7.91 (2H, m), 7.89 (2H, m), 6.81 (2H, m), 4.13 (2H, s), 3.77 (2H, t, J = 6.0 Hz), 3.75 (3H, s), 3.70 (2H, t, J = 6.0 Hz), 3.57 (2H, q, J = 7.0 Hz), 1.16 (3H, t, J = 7.0 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 170.6 (C), 156.8 (C), 151.3 (C), 147.2 (C), 143.5 (C), 126.2 (CH), 124.6 (CH), 122.6 (CH), 111.3 (CH), 69.0 (CH_2), 68.5 (CH_2), 51.9 (CH_3), 50.0 (CH_2), 45.9 (CH_2), 12.1 (CH_3) ppm.

ES-MS: m/z 387 $[M + H]^+$

Anal. Calcd for $C_{19}H_{22}N_4O_5$: C 59.06, H 5.74, N 114.50. Found: C 59.16, H 5.77, N 14.35.

Spectra of **II.14** :

Bruto formula: $C_{18}H_{19}N_4O_4Br$

M.W.: 435.3 g/mol

m.p.: 72 °C

R_f (toluene / ethyl acetate: 1 / 1) = 0.80

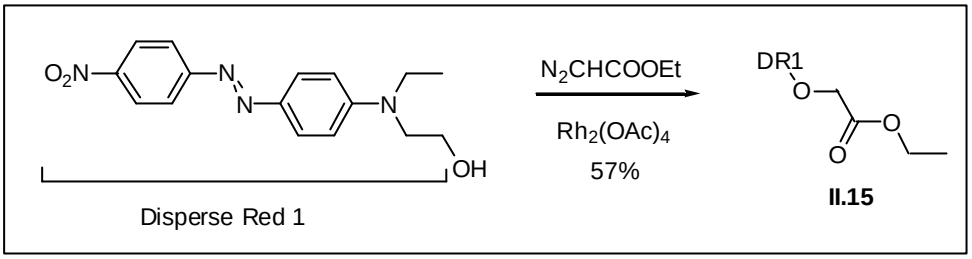
IR (KBr) 2923 (m), 1737 (m), 1602 (s), 1513 (s), 1389 (m), 1343 (m), 1277 (m), 1142 (m), 1097 (m), 1000 (w), 851 (m), 825 (m), 754 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (2H, m), 7.92 (4H, m), 6.81 (2H, m), 4.41 (2H, t, J = 6.2 Hz), 3.83 (2H, s), 3.73 (2H, t, J = 6.2 Hz), 3.55 (2H, q, J = 7.1 Hz), 1.27 (3H, t, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 167.2 (C), 156.7 (C), 151.0 (C), 147.5 (C), 143.9 (C), 126.3 (CH), 124.7 (CH), 122.7 (CH), 111.5 (CH), 63.0 (CH_2), 48.5 (CH_2), 45.8 (CH_2), 25.4 (CH_2), 12.1 (CH_3) ppm.

ES-MS: m/z 435 $[M + H]^+$, 436 $[M + H]^+$ (Br isotope)

Synthesis of Ethyl-5-{N-ethyl-N-[4-(4-nitrophenyl)azophenyl]}amino-3-oxapentanoate **II.15**



Disperse Red 1 **II.15**

To a solution of Disperse Red 1 (6.28 g, 20 mmol) and rhodium (II) acetate (150 mg, 0.34 mmol) in a mixture of dry dichloromethane (100 ml) and dry toluene (100 ml) was added at 40 °C a solution of ethyl diazoacetate (8.4 ml, 80 mmol) in toluene (40 ml) over a one-hour period. The reaction mixture was then stirred overnight at room temperature. Half of the solvent was removed under reduced pressure and the residue was purified by chromatography over a 10 cm diameter G2 fritted disc filtering column filled with silica gel, using a gradient of 10% to 25% ethyl acetate in toluene. The fractions containing the desired compound were concentrated to 20 ml and to the warm solution was slowly added 200 ml of pentane. After refrigeration overnight, the precipitate was filtered and washed with 10% diethyl ether in pentane, yielding 4.56 g (57%) of **II.15** as a bright red powder.

Bruto formula: $C_{20}H_{24}N_4O_5$

M.W.: 400.4 g/mol

m.p.: 80 °C

R_f (toluene / ethyl acetate: 1 / 1) = 0.75

IR (film) 2974 (w), 2902 (w), 1759 (s), 1603 (s), 1518 (s), 1392 (m), 1340 (m), 1209 (m), 1138 (s), 1106 (w), 1032 (w), 964 (w), 858 (m), 826 (m) cm^{-1}.

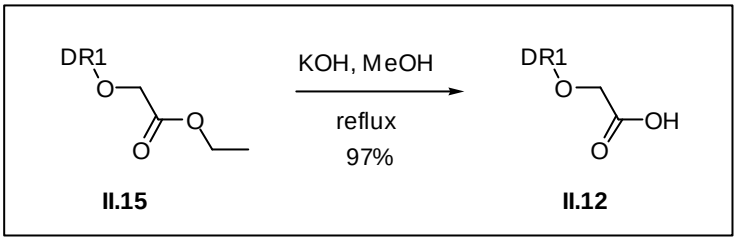
^1H NMR (500 MHz, $CDCl_3$) δ 8.32 (2H, m), 7.92 (2H, m), 7.89 (2H, m), 6.78 (2H, m), 4.22 (2H, q, J = 7.1 Hz), 4.11 (2H, s), 3.78 (2H, t, J = 6.0 Hz), 3.69 (2H, t, J = 6.0 Hz), 3.57 (2H, q, J = 7.1 Hz), 1.28 (3H, t, J = 7.1 Hz), 1.25 (3H, t, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 170.1 (C), 156.8 (C), 151.4 (C), 147.3 (C), 143.6 (C), 126.3 (CH), 124.7 (CH), 122.6 (CH), 111.3 (CH), 69.0 (CH_2), 68.7 (CH_2), 60.9 (CH_2), 50.1 (CH_2), 46.0 (CH_2), 14.2 (CH_3), 12.2 (CH_3) ppm.

ES-MS: m/z 401 (30) $[M + H]^+$, 423 $[M + Na]^+$

Anal. Calcd for $C_{20}H_{24}N_4O_5$: C 59.99, H 6.04, N 13.99. Found: C 60.12, H 6.08, N 13.82.

Synthesis of 5-{N-Ethyl-N-[4-(4-nitrophenyl)azophenyl}amino-3-oxapentanoic acid **II.12**



II.15 **II.12**

To a solution of **II.15** (5 g, 12.5 mmol) in methanol (300 ml) and toluene (70 ml) was added potassium hydroxide (4.1 g, 72.5 mmol) and the resulting mixture was refluxed under nitrogen for 1.5 h. The mixture was concentrated to 50 ml under reduced pressure, acidified with a solution (25 ml) of 10% hydrochloric acid chloride and further diluted with 120 ml of water. The aqueous phase was extracted four times with dichloromethane; the combined organic extracts were washed with water and dried over anhydrous magnesium sulfate. After filtration and concentration to 25 ml, 120 ml of diethyl ether were slowly added and the mixture was refrigerated overnight after which 4.5 g of the carboxylic acid **II.12** precipitated as a dark-red powder (97%)

Bruto formula: $C_{18}H_{20}N_4O_5$

M.W.: 372.4 g/mol

m.p.: 161 °C

R_f (dichloromethane / methanol: 9 / 1) = 0.48

UV (CH_2Cl_2): λ_{max1} = 297 nm, λ_{max2} = 478 nm

IR (KBr) 2925 (s), 2854 (m), 1721 (m), 1597 (s), 1513 (s), 1384 (m), 1337 (m), 1239 (w), 1134 (s), 1104 (m), 855 (w), 824 (m) cm^{-1}.

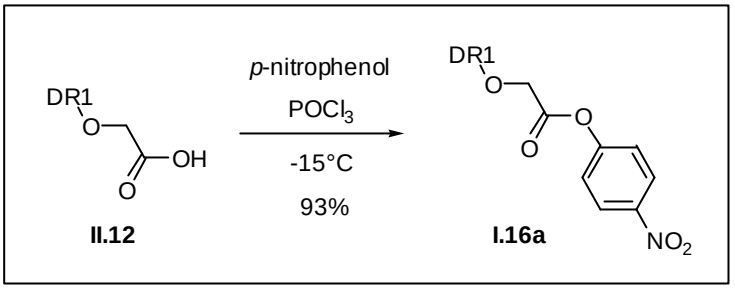
^{1}H NMR (500 MHz, DMSO-d_6) δ 8.36 (2H, m), 7.93 (2H, m), 7.83 (2H, m), 6.89 (2H, m), 4.08 (2H, s), 3.68 (2H, m), 3.65 (2H, m), 3.55 (2H, q, J = 7.0 Hz), 1.16 (3H, t, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, DMSO-d_6) + DEPT δ 171.8 (C), 156.3 (C), 151.8 (C), 146.8 (C), 142.7 (C), 126.2 (CH), 125.1 (CH), 122.6 (CH), 111.6 (CH), 68.2 (CH_2), 67.9 (CH_2), 49.5 (CH_2), 46.3 (CH_2), 12.0 (CH_3) ppm.

ES-MS: m/z 373 $[M + H]^+$

Anal. Calcd for $C_{18}H_{20}N_4O_5$**:** C 58.06, H 5.41, N 15.05. Found: C 57.65, H 5.58, N 15.22.

Synthesis of 4-nitrophenyl-5-{N-ethyl-N-[4-(4-nitrophenyl)azo]phenyl}amino-3-oxapentanoate **I.16a**



To a solution of carboxylic acid **II.12** (2.2 g, 5.9 mmol) and *p*-nitrophenol (0.84 g, 6.0 mmol) in pyridine (100 ml) and dichloromethane (120 ml) at -15 °C was added a solution of phosphorous oxychloride ($POCl_3$) (1.0 ml, 10.8 mmol) in dichloromethane (10 ml) over a one-hour period under vigorous stirring. After 4 h at –15 °C, the reaction mixture was poured into a mixture of dichloromethane and ice cold water (200 ml). The aqueous phase was extracted with dichloromethane and the combined organic layers were washed with an aqueous saturated solution of sodium bicarbonate and with water. After drying over anhydrous magnesium sulfate, the solvent was removed in vacuo and the residue was dissolved in warm acetone (50 ml). After diluting with diethyl ether (20 ml), pentane was added (150 ml) very slowly. Refrigeration overnight, filtration and washing with pentane afforded 2.7 g (93%) of **I.16a** (**NF31**) as an intense red powder.

Bruto formula: $C_{24}H_{23}N_5O_7$

M.W.: 493.5 g/mol

m.p.: 125 °C

R_f (toluene / ethyl acetate: 1 / 1) = 0.70

UV (CH_2Cl_2): λ_{max1} = 276 nm, λ_{max2} = 480 nm

IR (film) 2964 (w), 1778 (m), 1604 (s), 1588 (s), 1522 (s), 1490 (m), 1411 (w), 1389 (m), 1337 (s), 1260 (m), 1206 (m), 1120 (s), 954 (w), 916 (w), 854 (m), 818 (m), 804 (m), 755 (w), 819 (w) cm^{-1}.

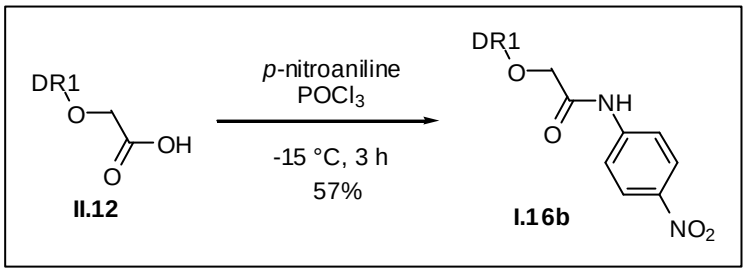
^1H NMR (500 MHz, $CDCl_3$) δ 8.33 (2H, d, J = 8.9 Hz), 8.28 (2H, d, J = 9.1 Hz), 7.92 (2H, d, J = 8.9 Hz), 7.89 (2H, d, J = 9.2 Hz, m), 7.29 (2H, d, J = 9.1 Hz), 6.79 (2H, d, J = 9.2 Hz), 4.41 (2H, s), 3.88 (2H, t, J = 5.8 Hz) 3.74 (2H, t, J = 5.8 Hz), 3.59 (2H, q, J = 7.1 Hz), 1.27 (3H, t, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 167.9 (C), 156.7 (C), 154.6 (C), 151.2 (C), 147.3 (C), 145.5 (C), 143.6 (C), 126.2 (CH), 125.3 (CH), 124,7 (CH), 122.6 (CH), 122.2 (CH), 111.3 (CH), 69.4 (CH_2), 68.4 (CH_2), 50.1 (CH_2), 46.1 (CH_2), 12.2 (CH_3) ppm.

ES-MS: m/z 494 $[M + H]^+$, 516 $[M + Na]^+$

Anal. Calcd for $C_{24}H_{23}N_5O_7$: C 58.42, H 4.70, N 14.19. Found: C 58.26, H 4.90, N 14.01.

Synthesis of 4-nitrophenyl-5-{N-ethyl-N-[4-(4-nitrophenyl)azo]phenyl}amino-3-oxapentamide **I.16b**



To a solution of **II.12** (0.54 g, 1.45 mmol) and *p*-nitroaniline (0.2 g, 1.45 mmol) in pyridine (55 ml) was added dropwise at -15 °C phosphorous oxychloride ($POCl_3$) (0.15 ml, 1.60 mmol) under vigorous stirring. The mixture was stirred at –15 °C for 3 h and was poured into a mixture of dichloromethane and ice cold water (50 ml). The aqueous phase was extracted with dichloromethane and the combined organic layers were washed with an aqueous saturated solution of sodium bicarbonate and with water. After drying over anhydrous magnesium sulfate, the solvent was removed in vacuo and the residue was purified by column chromatography (dichloromethane / isooctane: 8 / 2 to 1 / 0) affording 0.41 g of *p*-nitroanilide derivative **I.16b** in 57% yield.

Bruto formula: $C_{24}H_{24}N_6O_6$

m.p.: 176 °C

M.W.: 492.5 g/mol

R_f (toluene / ethyl acetate: 1 / 1) = 0.26

UV (CH_2Cl_2): λ_{max1} = 304 nm, λ_{max2} = 470 nm

IR (KBr) 2915 (w), 1711 (w), 1600 (m), 1584 (m), 1529 (m), 1507 (s), 1411 (w), 1379 (m), 1337 (s), 1245 (m), 1136 (m), 1103 (s), 1081 (m), 1015 (w), 856 (w), 821 (w), 754 (w), 693 (w) cm^{-1}.

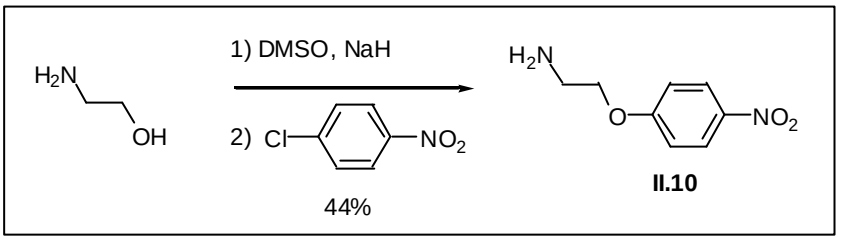
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.36 (2H, d, J = 9.0 Hz), 8.30 (1H, s), 8.05 (2H, d, J = 9.1 Hz), 7.94 (4H, dd, J = 9.2 Hz), 7.35 (2H, d, J = 9.1 Hz), 6.95 (2H, d, J = 9.2 Hz), 4.14 (2H, s), 3.88 (2H, m) 3.78 (2H, m), 3.59 (2H, q, J = 7.1 Hz), 1.30 (3H, t, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, DMSO-d_6) + DEPT δ 169.1 (C), 156.2 (C), 151.7 (C), 146.8 (C), 144.6 (C), 142.7 (C), 142.4 (C), 126.2 (CH), 125.0 (CH), 124 9 (CH), 122.5 (CH), 119.3 (CH), 111.7 (CH), 70.3 (CH_2), 68.6 (CH_2), 49.3 (CH_2), 45.2 (CH_2), 12.0 (CH_3) ppm.

ES-MS: m/z 493 $[M + H]^+$, 515 $[M + Na]^+$

Anal. Calcd for $C_{24}H_{24}N_6O_6$: C 58.53, H 4.91, N 17.05. Found: C 58.09, H 4.98, N 16.50.

Synthesis of 4-nitrophenoxyethanamine **II.10**



A suspension of sodium hydride (60% dispersion in oil) (0.83 g, 20.0 mmol) in dimethylsulfoxide (10 ml) was heated at 40 °C for 30 min. After cooling to room temperature, 2-aminoethanol (1.20 ml, 20.0 mmol) and *p*-chloronitrobenzene (3.03 g, 20.0 mmol) were added. After 20 min stirring, the mixture was diluted in 150 ml dichloromethane. The organic phase was washed twice with a 4 N aqueous solution of hydrochloric acid. The aqueous phase was washed with dichloromethane. After adding 150 ml of ethyl acetate to the aqueous phase, the solution was cooled to 6-8 °C and basified to pH = 11 under vigorous stirring with an aqueous solution of 8 N of sodium hydroxide. After washing with water, the organic phase was dried over anhydrous magnesium sulfate,, filtered and evaporated under reduced pressure to afford 1.6 g of amine **II.10** (44%).

Bruto formula: $C_8H_{10}N_2O_3$

M.W.: 182.2 g/mol

m.p.: 30 °C

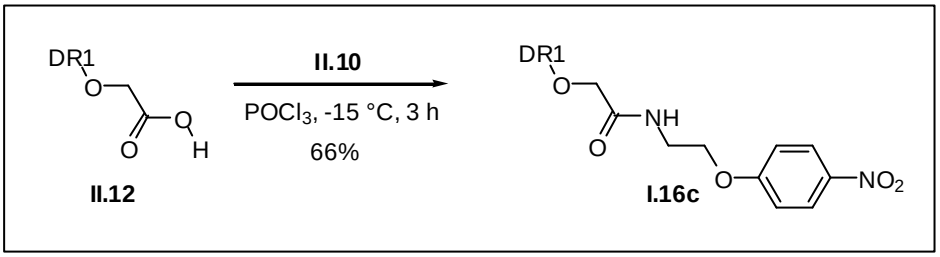
R_f (toluene / ethyl acetate: 1 / 1) = 0.26

IR (film) 3364 (w), 3113 (w), 2940 (w), 2873 (w), 1607 (s), 1593 (s), 1509 (s), 1459 (w), 1343 (s), 1299 (m), 1264 (s), 1175 (m), 1112 (s), 1010 (w), 846 (m), 753 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 8.21 (2H, d, J = 9.2 Hz), 6.97 (2H, d, J = 9.2 Hz), 4.09 (2H, t, J = 5.0 Hz), 3.15 (2H, t, J = 5.0 Hz), 1.41 (2H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 163.8 (C), 141.2 (C), 126.7 (CH), 114.3 (CH), 70.9 (CH_2), 41.0 (CH_2) ppm.

Synthesis of 2-(2-{ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethoxy)-N-[2-(4-nitro-phenoxy)-ethyl-acetamide **I.16c**



To a solution of **II.12** (0.7 g, 1.9 mmol) and 4-nitrophenoxyethanamine (0.34 g, 1.9 mmol) in pyridine (70 ml) at -15 °C was added phosphorous oxychloride ($POCl_3$) (0.35 ml, 3.8 mmol) under vigorous stirring. The mixture was stirred at –15 °C for 3 h. The reaction mixture was then poured into a mixture of dichloromethane and ice cold water (100 ml). The aqueous phase was extracted with dichloromethane and the combined organic layers were washed with an aqueous saturated solution of sodium bicarbonate and with water. After drying over anhydrous magnesium sulfate, the solvent was removed in vacuo and the residue was purified by column chromatography (dichloromethane / methanol: 99 / 1) to provide 0.67 g of the amide **I16c** as a dark red powder (66%).

Bruto formula: $C_{26}H_{28}N_6O_7$

M.W.: 536.5 g/mol

m.p.: 135 °C

R_f (toluene / ethyl acetate: 1 / 1) = 0.14

UV (CH_2Cl_2): λ_{max1} = 293 nm, λ_{max2} = 475 nm

IR (KBr) 2923 (w), 1685 (s), 1606 (s), 1507 (s), 1466 (w), 1392 (m), 1337 (s), 1251 (s), 1146 (m), 1110 (s), 1060 (m), 981 (w), 868 (w), 822 (m), 752 (w), 691 (w) cm^{-1}.

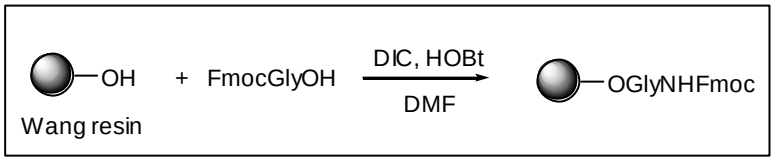
^{1}H NMR (500 MHz, $CDCl_3$) δ 8.33 (2H, d, J = 8.9 Hz), 8.15 (2H, d, J = 9.2 Hz), 7.91 (2H, d, J = 8.9 Hz), 7.88 (2H, d, J = 8.9 Hz), 6.86 (2H, d, J = 9.2 Hz), 6.81 (2H, d, J = 8.9 Hz), 6.76 (1H, t, J = 5.5), 4.01 (2H, s), 4.00 (2H, t, J = 5.4 Hz), 3.77 (2H, t, J = 5.4 Hz) 3.74 (2H, t, J = 5.4 Hz), 3.63 (2H, dt, J = 5.5 Hz), 3.54 (2H, q, J = 7.1 Hz), 1.25 (3H, t, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 169.5 (C), 163.8 (C), 156.5 (C), 151.0 (C), 147.5 (C), 143.7 (C), 141.7 (C), 126.1 (CH), 125.7 (CH), 124,7 (CH), 122.6 (CH), 114.3 (CH), 111.5 (CH), 70.5 (CH_2), 69.5 (CH_2), 67.1 (CH_2), 49.8 (CH_2), 45.6 (CH_2), 38.0 (CH_2), 12.1 (CH_3) ppm.

ES-MS: m/z 537 $[M + H]^+$

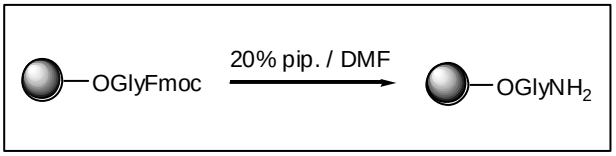
Anal. Calcd for $C_{26}H_{28}N_6O_7$: C 58.20, H 5.26, N 15.67. Found: C 58.30, H 5.57, N 15.75.

A new colorimetric test for solid-phase bound amines



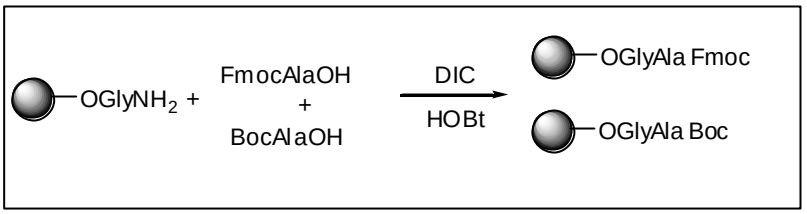
Diisopropylcarbodiimide (0.8 ml, 4.95 mmol), hydroxybenzotriazole (0.67 g, 4.95 mmol) and 4-dimethylaminopyridine (61 mg, 0.495 mmol) were added to a solution of amino acid FmocGlyOH (1.47 g, 4.95 mmol) in a mixture of dichloromethane/dimethylformamide (2/1). The carboxylic acid was activated for 30 min. The solution was then added to the Wang resin (1.5 g, 1.65 mmol, 1.1 mmol/g) and the mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 15 ml), methanol (3 x 15 ml) and dichloromethane (3 x 15 ml). The possible free remaining amino functions were capped with a solution of acetic anhydride (0.93 ml, 9.9 mmol), diisopropylethylamine (1.70 ml, 9.9 mmol) and 4-dimethylaminopyridine (1.2 g, 9.9 mmol) in dichloromethane (15 ml). After filtration, the resin was washed with dimethylformamide (3 x 15 ml), methanol (3 x 15 ml) and dichloromethane (3 x 15 ml).

Deprotection of the Fmoc-protecting group



The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml).

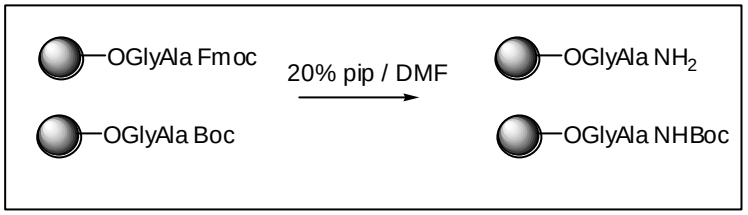
Synthesis of a series of resin samples of known free amine content:



The general coupling procedure is the following:

To a cooled (0 °C) solution of amino acids **Fmoc**AlaOH (2.5 eq.) and **Boc**AlaOH (2.5 eq.) in 0.7 ml of dimethylformamide/dichloromethane (2/5) were added diisopropycarbodiimide (5 eq.) and hydroxybenzotriazole (5 eq.). The mixture was stirred 10 min at 0 °C and 10 min at room temperature. The solution was added to the $WangGlyNH_2$ resin (20 mg, 0.017 mmol, 0.841 mmol/g, 1 eq.) and the suspension was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml).

Ratio **Fmoc**/**Boc**	**Fmoc**AlaOH (mg, mmol)	**Boc**AlaOH (mg, mmol)	DIC	HOBt
50 / 50	13.2 mg, 0.0425 mmol	8.0 mg, 0.0425 mmol		
20 / 80	5.3 mg, 0.0170 mmol	12.9 mg, 0.0680 mmol		
10 / 90	2.6 mg, 0.0085 mmol	14.5 mg, 0.0765 mmol	13.3 µl, 0.085 mmol	11.5 mg, 0.085 mmol
5 / 95	1.3 mg, 0.0043 mmol	15.3 mg, 0.0808 mmol		
2 / 98	0.5 mg, 0.0017 mmol,	15.8 mg, 0.8330 mmol		



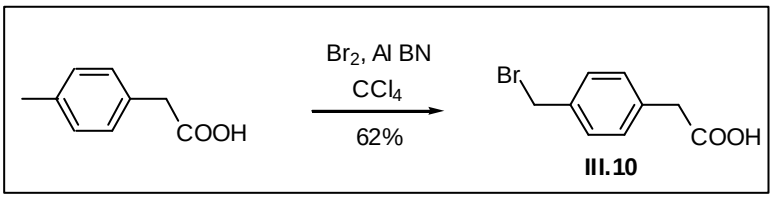
The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). The resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). Both colorimetric TNBS and **NF31** tests were performed and were compared.

TNBS test: [63] 3 drops of a solution of 10% diisopropylethylamine in dimethylformamide and 3 drops of a solution of 2, 4, 6-trinitrobenzenesulfonic acid (TNBS) in dimethylformamide were added to few beads. The appearance of an orange colour shows the presence of remaining uncoupled amino functions whereas white beads prove the completeness of the coupling.

NF31 test: [65] Few beads were suspended in 100 µl of a 0.002 M solution of **NF31** in acetonitrile. After heating for 10 minutes at 70 °C, the beads were washed with dimethylformamide, methanol and dichloromethane. Beads containing free amino functions appear as red beads while completely coupled beads remain colourless.

7.3 Experimental data of part 3

Bromination of 4-methylphenylacetic acid: Synthesis of acid **III.10**



III.10

To a solution of 4-methylphenylacetic acid (25 g, 0.17 mol) in carbon tetrachloride (300 ml) were added bromine (8.7 ml, 0.17 mol) and 2,2'-azobisisobutyronitrile (catalytic amount). The solution was irradiated with a sodium lamp (400 W) and was heated to 40 °C. After 5 min, white crystals precipitated. The mixture was cooled to 0 °C and the crystals were filtered and washed with carbontetrachloride. The residue was recrystallized from ethyl acetate to provide 24 g of carboxylic acid **III.10** (62%).

Bruto formula: $C_9H_9O_2Br$

M.W.: 229.1 g/mol

m.p.: 180 °C

R_f (toluene / ethyl acetate: 8 / 2) = 0.20

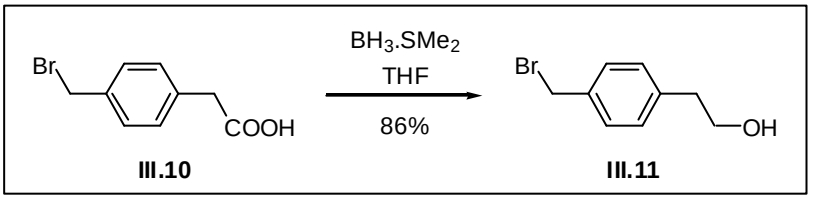
IR (film) 3027 (s), 2972 (s), 1699 (s), 1516 (w), 1408 (s), 1343 (m), 1244 (m), 1184 (m), 1093 (w), 894 (m), 838 (m), 780 (s), 675 (s), 602 (s) cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD) δ 7.36 (2H, m), 7.26 (2H, m), 4.50 (2H, s), 3.60 (2H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$ + few drops DMSO-d_6) + DEPT δ 173.0 (C), 135.9 (C), 134.6 (C), 129.4 (CH), 128.8 (CH), 40.6 (CH_2), 33.0 (CH_2) ppm.

MS (m/z): 230 ($M^{+?}Br^{81}$), 228 ($M^{+?}Br^{79}$), 150 (13), 150 (19), 149 (100), 131 (5), 104 (42), 77 (26), 63 (9), 45 (20).

Reduction of the carboxylic acid **III.10** into alcohol **III.11**



III.10 **III.11**

To a cooled (0 °C) solution of carboxylic acid **III.10** (20.0 g, 0.087 mol) in dry tetrahydrofuran (120 ml) was added dropwise borane-dimethylsulfide complex (12.3 ml, 0.13 mol). The solution was stirred at 0 °C for 2 h and at room temperature for 2 h. Water was added slowly and tetrahydrofuran was evaporated under reduced pressure. The aqueous phase was extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated. The residue was chromatographed on silica gel (ethyl acetate / isooctane: 4 / 6) to afford 16 g of alcohol **III.11** (86%).

Bruto formula: $C_9H_{11}OBr$

M.W.: 215.1 g/mol

m.p.: 76°C

R_f (isooctane / ethyl acetate: 6 / 4) = 0.28

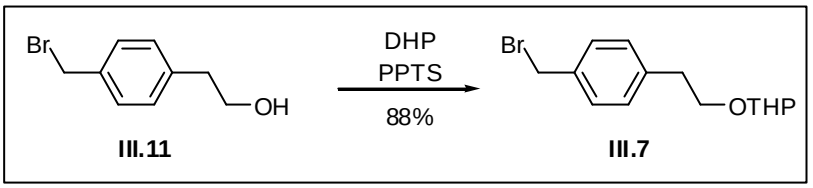
IR (film) 3395 (m), 3333 (m), 2950 (m), 1513 (w), 1477 (w), 1418 (w), 1228 (m), 1201 (m), 1100 (m), 1044 (s), 836 (m), 766 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.35 (2H, m), 7.22 (2H, m), 4.49 (2H, s), 3.86 (2H, t, J = 6.5 Hz) 2.87 (2H, t, J = 6.5 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 138.9 (C), 135.9 (C), 129.4 (CH), 129.2 (CH), 63.4 (CH_2), 38.8 (CH_2), 33.4 (CH_2) ppm.

MS (m/z): 216 ($M^{+?}Br^{81}$), 214 ($M^{+?}Br^{79}$), 135 (100), 105 (63), 104 (54), 91 (10), 77 (22), 63 (10), 51 (13).

Synthesis of building block **III.7**



III.11 **III.7**

Dihydropyran (5.10 ml, 0.056 mol) and pyridinium *p*-toluenesulfonate (1.25 g, 0.005 mol) were added to a solution of alcohol **III.11** (10 g, 0.047 mol) in dry dichloromethane (40 ml). The solution was stirred overnight at room temperature. The mixture was extracted with a saturated aqueous solution of sodium bicarbonate and with brine. The organic phase was dried over anhydrous magnesium sulfate, was filtered and evaporated under reduced pressure. The residue was purified by column chromatography (isooctane / ethyl acetate: 9 / 1) providing 12.4 g of protected alcohol **III.7** (88%).

Bruto formula: $C_{14}H_{19}O_2Br$

M.W.: 299.1 g/mol

R_f (toluene / ethyl acetate: 8 / 2) = 0.69

IR (film) 2941 (s), 2868 (m), 1514 (w), 1439 (w), 1352 (w), 1229 (w), 1200 (m), 1120 (s), 1077 (m), 1031 (s), 972 (m), 907 (w), 869 (w), 814 (w), 606 (m) cm^{-1}.

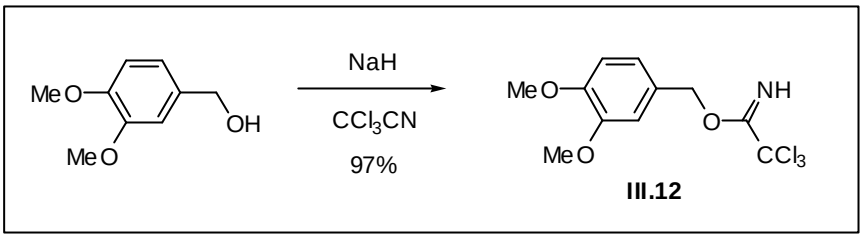
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (2H, ABd, J_{AB} = 8.0 Hz), 7.22 (2H, ABd, J_{AB} = 8.0 Hz), 4.59 (1H, m), 4.48 (2H, s), 3.94 (1H, dt, J = 9.7, 7.2 Hz), 3.73 (1H, m), 3.62 (1H, dt, J = 9.7, 7.2 Hz), 3.45 (1H, m), 2.91 (2H, t, J = 7.1 Hz), 1.80 (1H, m), 1.69 (1H, m), 1.54 (4H, m) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 139.5 (C), 135.5 (C), 129.3 (CH), 128.9 (CH), 98.6 (CH), 67.9 (CH_2), 62.1 (CH_2), 35.9 (CH_2), 33.6 (CH_2), 30.5 (CH_2), 25.3 (CH_2), 19.4 (CH_2) ppm.

MS (m/z): 299 ($M^{+?}Br^{81}$), 297 ($M^{+?}Br^{79}$), 117 (80), 85 (100), 67 (71), 41 (30).

Synthesis of building block **III.8**

Synthesis of acetimidate **III.12**



III.12

A solution of 3,4-dimethoxybenzyl alcohol (25 g, 0.149 mol) was slowly added to a suspension of sodium hydride (60% dispersion in oil) (1.19 g, 0.030 mol). The solution was then cooled to 0 °C and trichloroacetonitrile (17.6 ml, 0.175 mol) was added dropwise. The mixture was stirred 1 h at 0 °C and 2 h at room temperature. Pentane (50 ml) containing 1 ml of methanol was added followed by activated carbon. The mixture was stirred one hour before being filtered over celite. The celite was then washed with pentane. The organic phase was evaporated under reduced pressure affording 45 g of trichloroacetimidate **III.12**, which was used without further purification (97%).

Bruto formula: $C_{11}H_{12}NO_3Cl_3$

M.W.: 312.6 g/mol

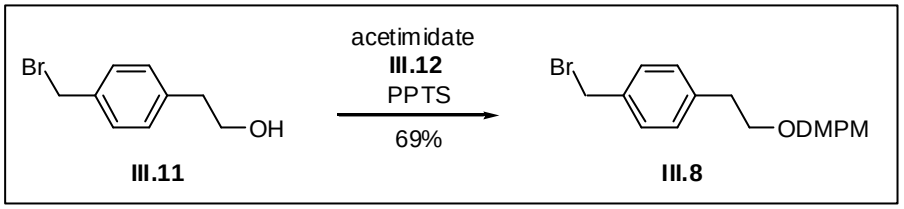
R_f (toluene / ethyl acetate: 8 / 2) = 0.59

IR (film) 3330 (w), 2935 (w), 1662 (s), 1593 (w), 1518 (s), 1463 (m), 1420 (w), 1267 (s), 1240 (m), 1160 (m), 1079 (m), 1028 (m), 828 (w), 796 (s), 648 (m) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 8.37 (1H, s), 6.98 (2H, m), 6.86 (1H, d, J = 8.1 Hz), 5.28 (2H, s), 3.88 (6H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 162.4 (C), 149.0 (C), 148.9 (C), 127.8 (C), 120.6 (CH), 111.1 (CH), 110.8 (CH), 91.4 (C), 70.7 (CH_2), 55.8 (CH_3) ppm.

Synthesis of building block **III.8**



To a solution of alcohol **III.11** (6.5 g, 0.030 mol) and pyridinium *p*-toluenesulfonate (3.8 g, 0.015 mol) in dichloromethane (48 ml) was added trichloroacetimidate **III.12** (18.8 g, 0.06 mol). The solution was stirred overnight at room temperature. After addition of an aqueous saturated solution of sodium bicarbonate, the mixture was extracted with dichloromethane, dried over magnesium sulfate and filtered. The residue was purified by column chromatography (20% ethyl acetate in toluene / pentane: 1 / 1) to afford 7.6 g of benzylbromide **III.8** (69%).

Bruto formula: $C_{18}H_{21}O_3Br$

M.W.: 365.3 g/mol

R_f (toluene / ethyl acetate: 8 / 2) = 0.61

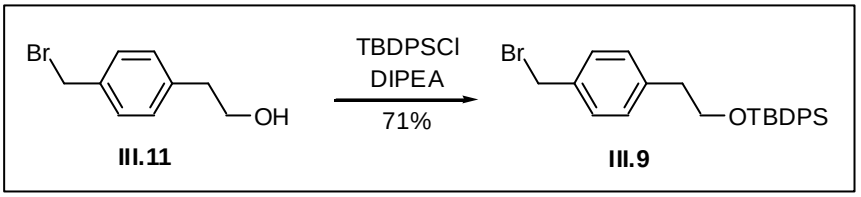
IR (film) 2935 (m), 2857 (m), 1593 (m), 1516 (s), 1464 (m), 1419 (m), 1264 (s), 1237 (s), 1157 (s), 1094 (m), 1028 (s), 809 (m), 607 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.31 (2H, ABd, J_{AB} = 8.0 Hz), 7.20 (2H, ABd, J_{AB} = 8.0 Hz), 6.82 (3H, m), 4.48 (2H, s), 4.45 (2H, s), 3.87 (3H, s), 3.84 (3H, s), 3.66 (2H, t, J = 6.9 Hz), 2.91 (2H, J = 6.9 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 148.9 (C), 148.4 (C), 139.5 (C), 135.6 (C), 130.7 (C), 129.3 (CH), 128.9 (CH), 120.0 (CH), 110.7 (CH), 72.8 (CH_2), 70.5 (CH_2), 55.8 (CH_3), 55.7 (CH_3), 35.9 (CH_2), 33.5 (CH_2) ppm.

MS (m/z): 366 ($M^{+?}Br^{81}$), 364 ($M^{+?}Br^{79}$), 151 (100), 104 (18), 77 (12), 65 (8).

Synthesis of building block **III.9**



III.11 **III.9**

tert-Butyldiphenylsilyl chloride (25.6 g, 0.093 mol) and diisopropylethylamine (28.4 ml, 0.163 mol) were added to a solution of alcohol **III.11** (10 g, 0.047 mol) in dimethylformamide (50 ml). The mixture was stirred at room temperature for 4 h. The mixture was added to ice and was extracted with diethylether. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The crude oil was purified by column chromatography (diethylether / isooctane: 1 / 9) to give 15 g of building block **III.9** (71%).

Bruto formula: $C_{25}H_{29}SiOBr$

M.W.: 453.5 g/mol

R_f (diethyl ether / isooctane: 1 / 9) = 0.58

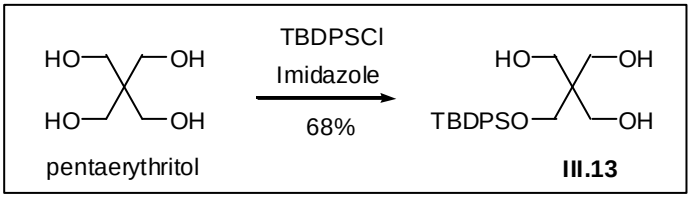
IR (film) 3047 (w), 2930 (m), 2857 (m), 1742 (w), 1427 (m), 1266 (w), 1111 (s), 823 (m), 702 (s), 505 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.60 (4H, dd, J = 6.6, 1.4 Hz), 7.42 (2H, m), 7.37 (4H, m), 7.30 (2H, ABd, J = 8.0 Hz), 7.16 (2H, ABd, J_{AB} = 8.0 Hz), 4.59 (2H, s), 3.85 (2H, t, J_{AB} = 6.8 Hz), 2.87 (2H, t, J = 6.8 Hz), 1.04 (9H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 139.6 (C), 135.5 (CH), 135.3 (C), 133.7 (C), 129.6 (CH), 128.5 (CH), 127.6 (CH), 64.9 (CH_2), 46.9 (CH_2), 38.9 (CH_2), 26.8 (CH_2), 19.1 (C) ppm.

MS (m/z): 351 (57), 247 (20), 217 (100), 181 (42), 117 (64), 91 (54), 57 (44).

Mono-TBDPS protection of pentaerythritol: synthesis of triol **III.13**



pentaerythritol **III.13**

To a solution of pentaerythritol (25 g, 0.184 mol) and imidazole (13.8 g, 0.202 mol) in dimethylformamide (1.2 l) was added dropwise *tert*-butyldiphenylsilyl chloride (26.8 g, 0.098 mol). The mixture was stirred for 60 h at room temperature. After addition of water, the solution was extracted with ethyl acetate. The organic phase was washed with water, dried over anhydrous magnesium sulfate, filtered and evaporated. The remaining dimethylformamide was removed with the Kugelrhor under reduced pressure. The oil was purified by HPLC (ethyl acetate / isooctane: 1 / 1) to provide 25 g of mono-protected triol **III.13** (68%).

Bruto formula: $C_{21}H_{30}SiO_4$

M.W.: 374.2 g/mol

m.p.: 50 °C

R_f (ethyl acetate / isooctane: 1 / 1) = 0.25

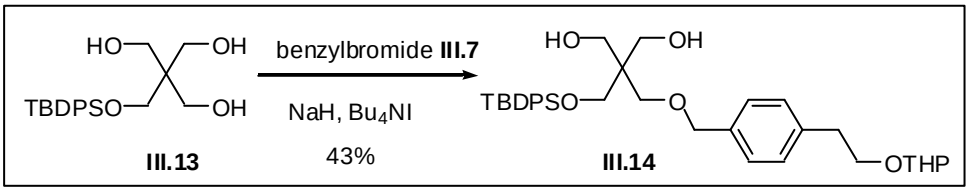
IR (film) 3386 (s), 2930 (m), 2857 (m), 1472 (m), 1428 (s), 1390 (w), 1361 (w), 1113 (s), 1036 (m), 824 (m), 740 (m), 702 (s), 504 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.64 (4H, dd, J = 8.0, 1.3 Hz), 7.41 (6H, m), 3.71 (6H, m), 3.63 (2H, m), 2.75 (3H, s), 1.07 (9H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ135.6 (CH), 132.5 (C), 130.0 (CH), 127.9 (CH), 65.3 (CH_2), 64.7 (CH_2), 64.3 (CH_2), 45.6 (C) 26.9 (CH_3), 19.1 (CH_2) ppm.

MS (m/z): 233 (10), 199 (100), 181 (19), 139 (76), 105 (25), 91 (91), 57 (51).

Alkylation of **III.13** with benzylbromide **III.7**: Synthesis of diol **III.14**



III.13 **III.14**

A solution of triol **III.13** (20 g, 0.053 mol) in dry tetrahydrofuran (32 ml) was added to a suspension of sodium hydride (60% dispersion in oil) (1.64 g, 0.041 mol) in tetrahydrofuran (80 ml). The mixture was refluxed for 30 min. and cooled at 10 °C. A solution of benzyl bromide **III.7** (12.3 g, 0.041 mol) in tetrahydrofuran (20 ml) was added followed by tetrabutylammonium iodide (738 mg, 0.002 mol). The mixture was refluxed overnight. A solution of saturated aqueous solution of ammonium chloride was added and tetrahydrofuran was evaporated. The aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over anhydrous

magnesium sulfate, filtered and evaporated under reduced pressure. The crude oil was purified by column chromatography (ethyl acetate / toluene: 1 / 9 to 5 / 5) to obtain 10.5 g of diol **III.14** (43%).

Bruto formula: $C_{35}H_{48}SiO_6$

M.W.: 592.9 g/mol

R_f (ethyl acetate / isooctane: 1 / 1) = 0.62

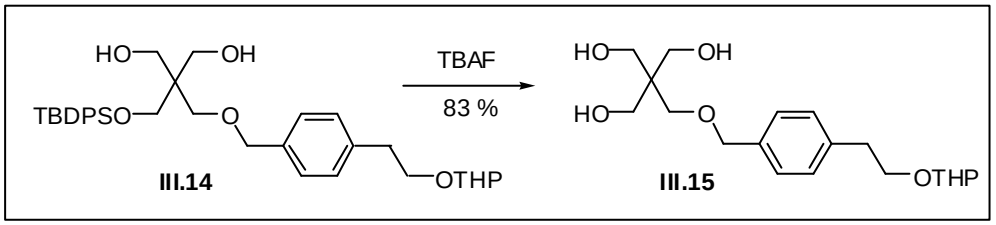
IR (film) 3449 (m), 2938 (s), 2857 (s), 1472 (w), 1428 (m), 1362 (w), 1113 (s), 1079 (m), 1031 (s), 820 (m), 703 (m), 505 (m) cm^{-1}.

^1H NMR (500 MHz, CD_3OD) δ 7.66 (4H, dd, J = 8.0, 1.3 Hz), 7.40 (2H, m), 7.33 (4H, m), 7.21 (2H, ABd, J_{AB} = 8.2 Hz), 7.20 (2H, ABd, J_{AB} = 8.2 Hz), 4.58 (1H, m), 4.46 (2H, s), 3.90 (1H, dt, J = 9.7, 7.0 Hz), 3.71 (1H, m), 3.70 (2H, s), 3.67 (4H, s), 3.60 (1H, dt, J = 9.7, 7.0 Hz), 3.55 (2H, s), 3.43 (1H, m), 2.87 (2H, t, J = 6.9 Hz); 1.80 (1H, m), 1.65 (1H, m), 1.51 (4H, m), 1.01 (9H, s) ppm.

^{13}C NMR (50 MHz, CD_3OD) δ 19.5, 19.2, 25.4, 26.8, 30.6, 36.0, 45.6, 62.2, 64.9, 68.2, 71.6, 73.6, 98.7, 127.6, 127.8, 127.9, 129.0, 129.9, 132.8, 135.6, 138.7, 145.9 ppm.

ES-MS: m/z 615 $[M + Na]^+$, 631 $[M + K]^+$

Deprotection of theTBDPS ether: Synthesis of **III.15**



To a solution of diol **III.14** (20 g, 0.034 mol) in dry tetrahydrofuran (130 ml) was added dropwise a solution 1 M of tetrabutylammonium fluoride in tetrahydrofuran (51 ml, 0.051 mol). The mixture was stirred overnight. After evaporation of the tetrahydrofuran under reduced pressure, the crude oil was purified by column chromatography (ethyl acetate / toluene: 8 / 2 to pure ethyl acetate). 10 g of desired triol **III.15** was obtained (83%).

Bruto formula: $C_{19}H_{30}O_6$

M.W.: 354.2 g/mol

R_f (dichloromethane / methanol: 9 / 1) = 0.30

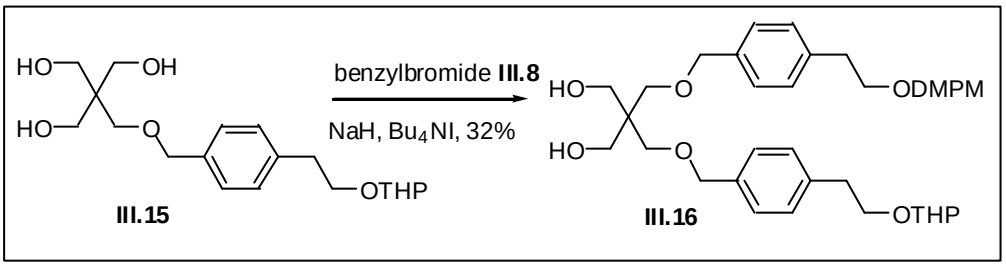
IR (film) 3407 (s), 2941 (m), 2868 (m), 1515 (w), 1365 (w), 1199 (m), 1119 (w), 1031 (s), 812 (w) cm^{-1}.

^1H NMR (500 MHz, CD_3OD) δ 7.25 (2H, ABd, J_{AB} = 8.1 Hz), 7.22 (2H, ABd, J_{AB} = 8.1 Hz), 4.58 (1H, m), 4.47 (2H, s), 3.89 (1H, dt, J = 9.7, 7.0 Hz), 3.69 (1H, m), 3.61 (6H, s), 3.60 (1H, m), 3.48 (2H, s), 3.42 (1H, m), 2.86 (2H, t, J = 6.9 Hz), 1.79 (1H, m), 1.65 (1H, m), 1.52 (4H, m) ppm.

^{13}C NMR (50 MHz, CD_3OD) + DEPT δ 139.9 (C), 137.8 (C), 130.0 (CH), 128.7 (CH), 100.0 (CH), 74.4 (CH_2), 70.9 (CH_2), 69.5 (CH_2), 63.2 (CH_2), 63.1 (CH_2), 46.8 (CH_2), 37.0 (CH_2), 31.7 (CH_2), 26.6 (CH_2), 20.4 (CH_2) ppm.

ES-MS: m/z 377 $[M + Na]^+$, 393 $[M + K]^+$

Alkylation with building block **III.8**: Synthesis of diol **III.16**



A solution of triol **III.15** (6 g, 0.017 mol) in dry tetrahydrofuran (12 ml) was added to a suspension of sodium hydride (60% dispersion in oil) (0.68 g, 0.017 mol) in tetrahydrofuran (42 ml). The mixture was refluxed for 30 min. and cooled at 10 °C. A solution of benzyl bromide **III.8** (6.21 g, 0.017 mol) in tetrahydrofuran (30 ml) was added followed by tetrabutylammonium iodide (332 mg, 0.009 mol). The mixture was refluxed overnight. A solution of saturated aqueous solution of ammonium chloride was added and tetrahydrofuran was evaporated. The aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The crude oil was purified by column chromatography (ethyl acetate / toluene: 1 / 9 to 5 / 5) to obtain 3.5 g of diol **III.16** (32%).

Bruto formula: $C_{37}H_{50}O_9$

M.W.: 638.8 g/mol

R_f (ethyl acetate / isooctane: 1 / 1) = 0.28

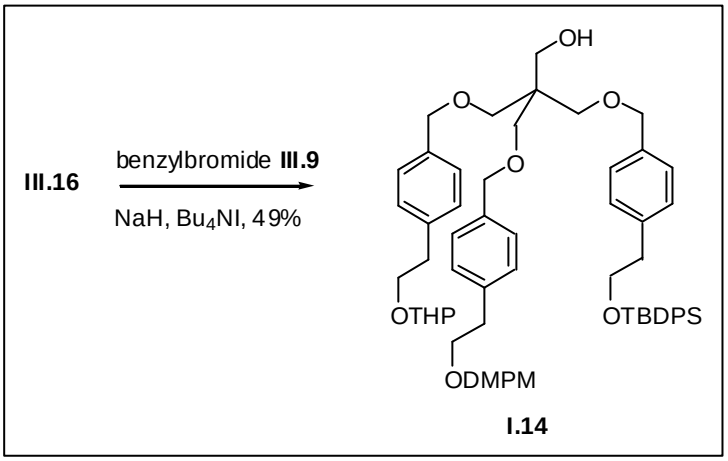
IR (film) 3458 (m), 2938 (s), 2863 (s), 1593 (w), 1516 (s), 1464 (w), 1419 (m), 1363 (m), 1363 (m), 1264 (s), 1238 (m), 1137 (m), 1083 (s), 1030 (s), 812 (m) cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD) δ 7.18 (8H, m), 6.83 (3H, m), 4.55 (1H, m), 4.43 (4H, s), 4.42 (2H, s), 3.87 (1H, dt, J = 9.6, 7.0 Hz), 3.78 (3H, s), 3.73 (3H, s), 3.67 (1H, m), 3.65 (2H, t, J = 6.8 Hz), 3.62 (4H, s), 3.57 (1H, dt, J = 9.6, 6.8 Hz), 3.48 (2H, s), 3.47 (2H, s), 3.39 (1H, m), 2.86 (2H, t, J = 6.9 Hz), 2.83 (2H, t, J = 6.9 Hz), 1.77 (1H, m), 1.62 (1H, m), 1.48 (4H, m) ppm.

APT NMR (125 MHz, CD_3OD) δ 149.1 (C), 148.7 (C), 138.5 (C), 138.4 (C), 136.5 (C), 136.4 (C), 131.2 (C), 128.7 (CH), 128.6 (CH), 127.4 (CH), 127.3 (CH), 120.2 (CH), 111.3 (CH), 111.2 (CH), 98.7 (CH), 72.9 (CH_2), 72.3 (CH_2), 70.6 (CH_2), 69.4 (CH_2), 69.3 (CH_2), 68.1 (CH_2), 61.9 (CH_2), 61.8 (CH_2), 55.1 (CH_3), 55.0 (CH_3), 45.5 (CH_2), 35.7 (CH_2), 35.6 (CH_2), 30.4 (CH_2), 25.2 (CH_2), 19.1 (CH_2) ppm.

ES-MS: m/z 661 $[M + Na]^+$, 677 $[M + K]^+$

Synthesis of tripodal scaffold **I.14**



A solution of diol **III.16** (1 g, 1.57 mmol) in dry tetrahydrofuran (1.7 ml) was added to a suspension of sodium hydride (60% dispersion in oil) (0.63 g, 1.60 mmol) in tetrahydrofuran (6 ml). The mixture was refluxed for 30 min. and cooled to 10 °C. A solution of benzyl bromide **III.9** (0.711 g, 1.57 mmol) in tetrahydrofuran (2 ml) was added followed by tetrabutylammonium iodide (33 mg, 0.089 mmol). The mixture was refluxed overnight. A solution of saturated aqueous solution of ammonium chloride was added and tetrahydrofuran was evaporated. The aqueous phase was extracted with ethyl acetate. The combined organic layers were dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The crude oil was purified by column chromatography (ethyl acetate / isooctane: 4 / 6) to obtain 0.8 g of tripodal scaffold **I.14** (49%).

Bruto formula: $C_{62}H_{78}O_{10}Si$

M.W.: 1011.39 g/mol

R_f (ethyl acetate / hexane: 4 / 6) = 0.30

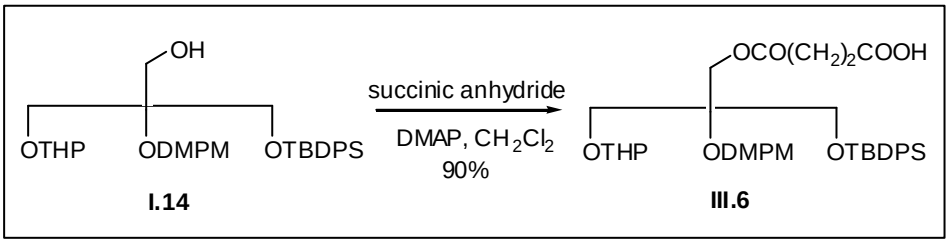
IR (film) 3518 (s), 2934 (s), 1515 (s), 1463 (m), 1428 (m), 1361 (m), 1265 (s), 1090 (s), 1030 (s) 738 (m), 704 (m) cm^{-1}.

^{1}H NMR (500 MHz, CD_2Cl_2) δ 7.60 (4H, dd, J = 7.9, 1.3 Hz), 7.41 (2H, m), 7.37 (4 H, m), 7.22 (10 H, m), 7.15 (2H, d, J = 8.0 Hz), 6.83 (3H, m), 4.58 (1H, m), 4.46 (2H, s), 4.44 (4H, s), 4.43 (2H, s), 3.91 (1H, dt, J = 9.7, 7.0 Hz), 3.86 (2H, t, J = 6.8 Hz), 3.82 (3H, s), 3.80 (3H, s), 3.76 (1H, m), 3.71 (2H, d, J = 6.3 Hz), 3.67 (2H, t, J = 6.9 Hz), 3.60 (1H, dt, J = 9.7, 7.0 Hz), 3.55 (2H, s), 3.54 (4H, s), 3.48 (1H, m), 2.90 (2H, t, J = 6.9 Hz), 2.88 (2H, t, J = 6.9 Hz), 2.87 (2H, t, J = 6.9 Hz), 1.80 (1H, m), 1.67 (1H, m), 1.52 (4H, m), 1.03 (9H, s) ppm.

APT NMR (125 MHz, CD_2Cl_2) δ 148.7 (C), 146.4 (C), 138.8 (C), 138.7 (C), 138.6 (C), 136.4 (C), 136.3 (C), 135.6 (CH), 133.8 (C), 131.3 (C), 129.6 (CH), 129.2 (CH), 129.0 (CH), 127.6 (CH), 127.5 (CH), 120.0 (CH), 111.3 (CH), 111.1 (CH), 98.7 (CH), 73.4 (CH_2), 73.3 (CH_2), 72.7 (CH_2), 70.9 (CH_2), 68.0 (CH_2), 65.2 (CH_2), 62.1 (CH_2), 55.8 (CH_3), 55.7 (CH_3), 45.1 (CH_2), 38.9 (CH_2), 36.0 (CH_2), 30.8 (CH_2), 26.6 (CH_3), 25.6 (CH_2), 19.6 (CH_2), 19.1 (CH_2) ppm.

ES-MS: m/z 1033 $[M + Na]^+$, 1050 $[M + K]^+$

Synthesis of carboxylic acid **III.6**



To a solution of the tripodal scaffold **I.14** (0.5 g, 0.495 mmol) in dichloromethane (35 ml) were added succinic anhydride (0.513 g, 4.95 mmol) and 4-dimethylaminopyridine (724 mg, 5.93 mmol). The solution was stirred at room temperature for 4 h. The mixture was extracted with an aqueous saturated solution of ammonium chloride. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated. The oil was purified by column chromatography (isooctane / ethyl acetate: 6 / 4) affording 0.5 g of acid **III.6** (90%).

Bruto formula: $C_{66}H_{82}O_{13}Si$

M.W.: 1111.46 g/mol

Rf (isooctane / ethyl acetate: 4 / 6) = 0.34

IR (film) 3400 (s), 2922 (s), 2853 (s), 1738 (w), 1712 (w), 1656 (w), 1630 (w), 1512 (w), 1456 (w), 1425 (w), 1359 (w), 1256 (w), 1153 (w), 1092 (w), 1025 (w) cm^{-1}.

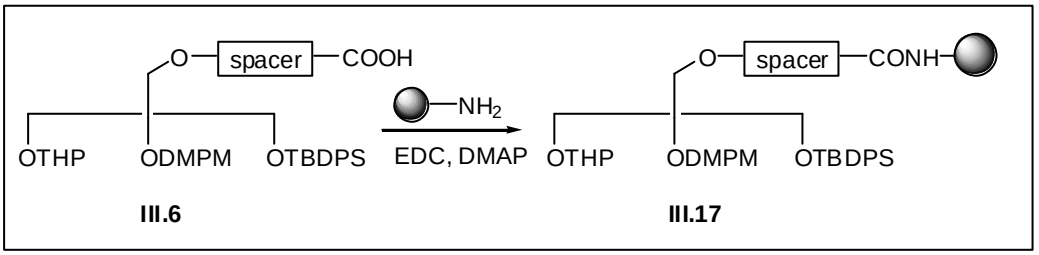
^1H NMR (500 MHz, $CDCl_3$) δ 7.60 (4H, dd, J = 7.8, 1.2 Hz), 7.41-7.33 (6H, m), 7.18 (10H, m), 7.09 (2H, d, J = 7.9 Hz), 6.82 (3H, m), 4.61 (1H, t, J = 3.3 Hz), 4.47 (2H, s), 4.42 (3H, s), 4.41 (3H, s), 4.22 (2H, s), 3.92 (1H, m), 3.87 (3H, s), 3.84 (3H, s), 3.82 (2H, t, J = 7 Hz), 3.79 (1H, m), 3.66 (2H, t, J = 7.0 Hz), 3.66 (1H, m), 3.60 (1H, m), 3.49 (4H, s), 3.47 (2H, s), 2.90 (2H, t, J = 6.8 Hz), 2.89 (2H, t, J = 7.1 Hz), 2.83 (2H, t, J = 7.0 Hz), 2.48 (2H, t, J = 5.3 Hz), 2.45 (2H, t, J = 5.3 Hz), 1.80 (1H, m), 1.70 (1H, m), 1.55 (4H, m), 1.02 (9H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 176.6, 171.7, 148.8, 148.4, 138.2, 138.0, 136.3, 136.3, 135.4, 133.6, 130.8, 129.5, 129.0, 128.8, 128.7, 127.5, 127.4, 127.3, 120.1, 110.9, 110.8, 98.7, 73.1, 72.7, 70.8, 68.9, 68.2, 65.0, 64.0, 62.1, 55.8, 55.6, 44.5, 38.9, 35.9, 30.6, 29.0, 28.6, 28.2, 26.7, 26.3, 19.4, 19.0 ppm.

ES-MS: m/z 1133 $[M + Na]^+$

Anal. Calcd for $C_{66}H_{82}O_{13}Si$: C 71.36, H 7.44. Found: C 70.72, H 7.63.

Synthesis of solid phase bound scaffold **III.17**



1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (18 mg, 0.095 mmol) and 4-dimethylaminopyridine (0.5 mg, 0.004 mmol) were added to a solution of acid **III.6** (27 mg, 0.024 mmol) in dry dichloromethane (1.5 ml) and the mixture was stirred for 30 min at room temperature. The solution was added to the resin (100 mg, 0.019 mmol, 0.19 mmol/g) and the suspension was shaken overnight at room temperature. The resin was then washed with

dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml). The coupling was performed twice.

The completeness of the coupling was controlled using the colorimetric TNBS test which resulted in white beads.

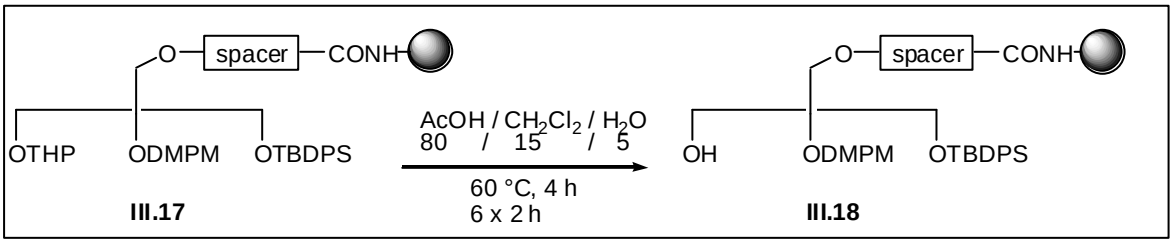
Capping of the remaining free amino functions

Acetylimidazole (21 mg, 0.19 mmol) was added to a suspension of resin **III.17** (100 mg, 0.016 mmol, 0.16 mmol/g) in dry dichloromethane (1 ml). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml). A sample of the resin was submitted to the colorimetric TNBS test and colourless beads were observed.

IR (KBr) 3018 (m), 2944 (s), 2880 (s), 1732 (w), 1669 (w), 1600 (w), 1558 (w), 1489 (m), 1452 (s), 1357 (w), 1288 (m), 1246 (m), 1146 (s), 1098 (s), 1024 (s), 951 (m), 903 (m), 840 (m), 808 (m), 761 (m), 697 (s) cm^{-1}.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 173.8, 172.4, 150.6, 139,8, 138,0, 136.9, 135.2, 132.5, 131.0, 130.6, 130.3, 129.4, 129.1, 129.0, 128.9, 121.4, 112.6, 100.1, 74.6, 74.1, 71.0 (polyethylene glycol peak), 69.5, 66.7, 63.5, 57.1, 46.0, 41.8, 40.7, 37.4, 32.2, 30.5, 28.0, 27.0, 21.0, 20.3 ppm.

Deprotection of the THP protective group in resin **III.17**



Resin **III.17** (100 mg, 0.016 mmol, 0.16 mmol/g) was treated with a solution of AcOH / H_2O / CH_2Cl_2 in the ratio 80 / 5 / 15 at 60 °C for 4 h. This procedure was repeated 6 times 2 h to obtain a complete deprotection. The resin was washed with dichloromethane (3 x 2 ml), methanol (3 x 2 ml), 5% of diisopropylethylamine in dichloromethane (3 x 2 ml), dimethylformamide (3 x 2 ml), methanol (3 x 2 ml), dichloromethane (3 x 2 ml) and was dried for 4 h under vacuum.

IR (KBr) 3585 (m), 3025 (w), 2841 (m), 1732 (m), 1668 (m), 1600 (w), 1516 (m), 1454 (s), 1346 (m), 1298 (m), 1236 (m), 1146 (s), 1110 (s), 1027 (m), 947 (m), 866 (w), 758 (m), 698 (s), 668 (m) cm^{-1}.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 173.8, 172.3, 150.6, 140.0, 138.0, 136.9, 135.3, 131.3, 131.0, 130.6, 130.0, 129.9, 129.8, 129.0, 121.4, 112.6, 77.1, 74.7, 74.2, 71.0 (polyethylene glycol peak), 66.8, 64.7, 46.1, 42.2, 42.0, 40.9, 40.5, 37.6, 32.0, 30.8, 28.3, 21.0, 20.6 ppm.

Synthesis of **III.24**

Coupling of FmocGlyCl on resin **III.18**

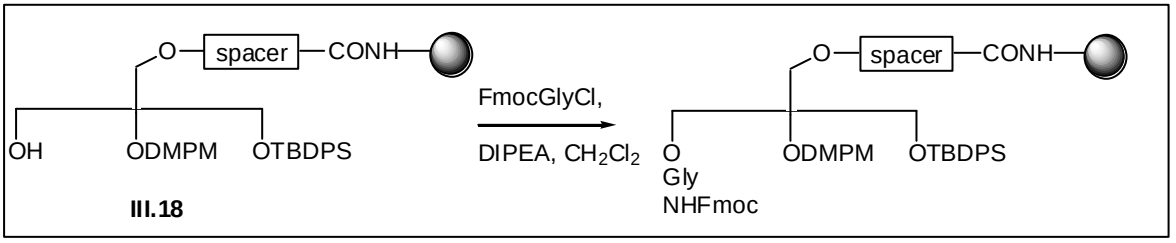
Preparation of the corresponding acid chloride

A solution of FmocGlyOH (57.0 mg, 0.19 mmol) and thionylchloride (140 µl, 1.9 mmol) in dry dichloromethane (2 ml) was heated under reflux for 2 h. The solvent was evaporated under reduced pressure. Dichloromethane (0.5 ml) was added followed by pentane (5 ml). The acid chloride precipitated, was filtered and dried under vacuum.

IR (film) 3317 (w), 1799 (s), 1713 (s), 1537 (m), 1519 (m), 1449 (m), 1347 (m), 1276 (m), 1249 (m), 1167 (w), 1044 (w), 954 (w), 759 (m), 740 (s) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (2H, d, J = 7.5 Hz), 7.59 (2H, d, J = 7.5 Hz), 7.42 (2H, dd, J = 7.5 Hz), 7.33 (2H, dd, J = 7.5 Hz), 5.41 (1H, m), 4.46 (2H, d, J = 6.8 Hz), 4.36 (2H, d, J = 5.6 Hz), 4.23 (1H, t, J = 6.8 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.1 (C), 155.9 (C), 143.5 (C), 141.3 (C), 127.8 (CH), 127.1 (CH), 124.9 (CH), 120.0 (CH), 67.5 (CH_2), 52.2 (CH_2), 46.9 (CH) ppm.



Diisopropylethylamine (0.033 ml, 0.19 mmol) was added to a solution of freshly prepared acid chloride (0.19 mmol) in dry dichloromethane (2 ml). The solution was added to resin **III.18** (100 mg, 0.016 mmol, 0.16 mmol/g) The mixture was shaken for 4 h. The coupling was repeated once

again overnight. After filtration, the resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml), and dichloromethane (3 x 2 ml).

$$\text{Loading (mmol/g)} = \frac{A \times V\,(mL)}{\varepsilon\,(mmol^{-1}L) \times m\,(mg)}$$

The yield of coupling was determined using Fmoc UV/VIS spectroscopy at 305 nm.

A = UV-absorbance

V = ml pip./DMF = 2 ml

m = weight (mg)

ε = 0.7 $mmol^{-1}L$ corresponds to the UV-absorption of 1 mmol of Fmoc-chromophore at 305 nm.

Sample: m = 5.5 mg

Absorbance: A = 0.32

Measured loading: Loading = (A x 2) / (0.7 x m) = 0.166 mmol/g

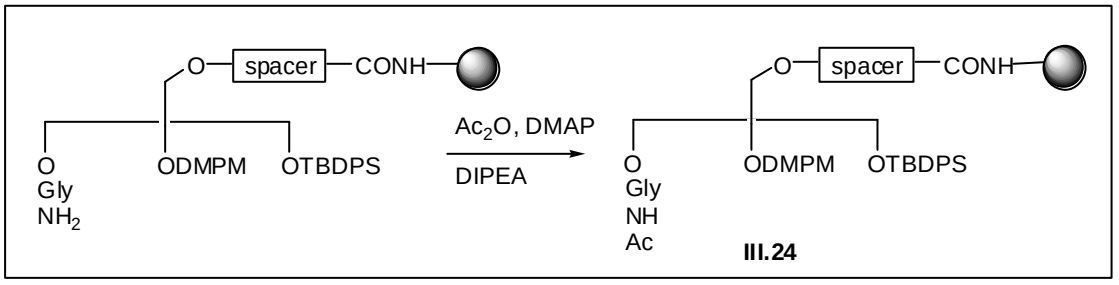
Calculated loading: 0.158 mmol/g

Yield = 105%

Deprotection of the Fmoc protective group

The resin was treated with a solution of 20% piperidine in dimethylformamide for 20 min (2x). The resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml). A sample of the resin was submitted to the colorimetric TNBS test and intense coloured beads were observed.

Capping of the free amino function



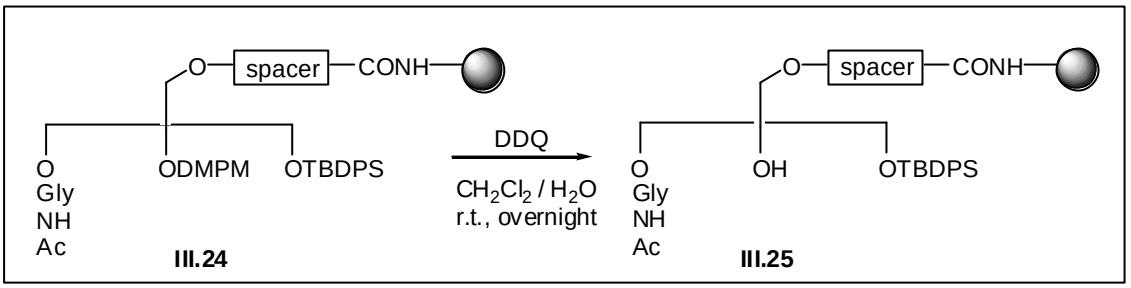
III.24

A solution of acetic anhydride (11 µl, 0.114 mmol), diisopropylethylamine (20 µl, 0.114 mmol) and 4-dimethylaminopyridine (14 mg, 0.114 mmol) in dry dichloromethane (2 ml) was added to the resin (100 mg, 0.016 mmol, 0.16 mmol/g). The mixture was shaken for 20 min at room

temperature. After filtration, the resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml). This procedure was repeated once again. A sample of the resin was submitted to the colorimetric TNBS test and colourless beads were observed.

Synthesis of **III.26**

Deprotection of DMPM protecting group: Synthesis of **III.25**

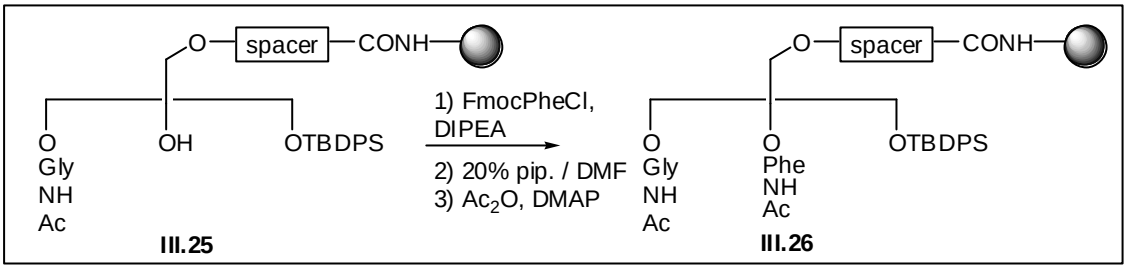


To a suspension of resin **III.24** (100 mg, 0.016 mmol, 0.16 mmol/g) in a mixture of dichloromethane / water (1.4 ml / 0.1 ml) was added dichlorodicyanoquinone (6.5 mg, 0.029 mmol). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with an aqueous saturated solution of sodium bicarbonate (3 x 5 ml), phenol / methanol: 1 / 1 (3 x 5 ml), toluene (3 x 5 ml), acetonitrile (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml).

IR (KBr) 3562 (s), 3058 (m), 3026 (m), 2876 (s), 2843 (s), 1729 (w), 1651 (s), 1480 (m), 1444 (m), 1352 (s), 1302 (m), 1145 (s), 1110 (s), 1025 (m), 945 (m), 697 (s) cm^{-1}.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 136.9, 131.2, 131.0, 130.8, 130.7, 130.6, 130.4, 130.1, 130.0, 129.8, 129.7, 129.6, 129.5, 129.1, 75.0, 71.9 (polyethyleneglycol peak), 68.6, 66.7, 64.5, 46.2, 42.1, 40.8, 31.9, 30.8, 29.6, 28.1 ppm.

Coupling of FmocPheOH on resin **III.25**



Diisopropylethylamine (0.033 ml, 0.19 mmol) was added to a solution of freshly prepared FmocPheCl (77.0 mg, 0.19 mmol) in dry dichloromethane (2 ml). The solution was added to resin (100 mg, 0.016 mmol, 0.16 mmol/g). The mixture was shaken for 4 h. The coupling was repeated once again overnight. After filtration, the resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml).

The yield of coupling was determined using Fmoc UV/VIS spectroscopy at 305 nm.

Sample: m = 5.2 mg

Absorbance: A = 0.239

Measured loading: Loading = (A x 2) / (0.7 x m) = 0.131 mmol/g

Calculated loading: 0.15 mmol/g

Yield = 88%

Deprotection of the Fmoc protective group

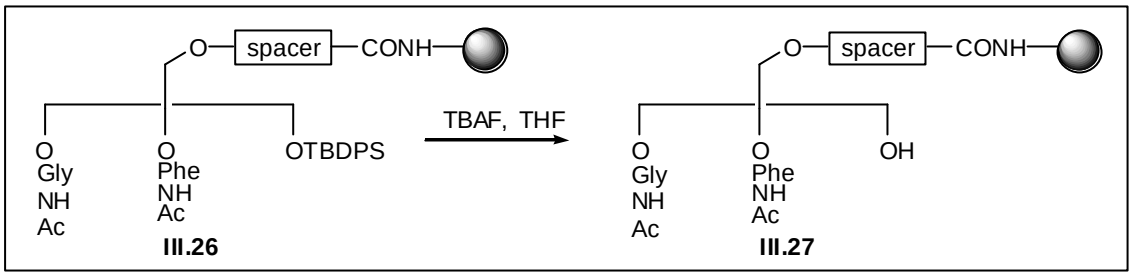
The resin was treated with a solution of 20% piperidine in dimethylformamide for twice 20 min. The resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml). A sample of the resin was submitted to the colorimetric TNBS test and intense coloured beads were observed.

Capping of the free amino function

The free amino function was capped with acetic anhydride as described previously for resin **III.24**.

Synthesis of **III.28**

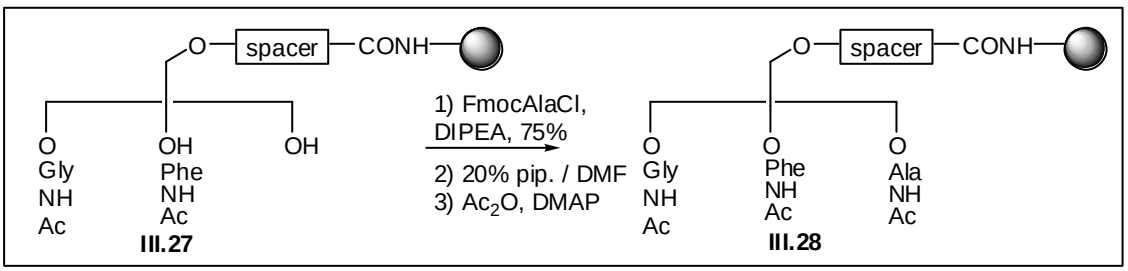
Deprotection of TBDPS ether: Synthesis of **III.27**



Tetrabutylammonium fluoride (57 µl, 0.057 mmol) and molecular sieves (400 pm) were added to a suspension of resin **III.26** (100 mg, 0.016 mmol, 0.16 mmol/g) in dry tetrahydrofuran (2 ml). The mixture was shaken overnight at room temperature. The reaction was repeated once more overnight. After filtration, the resin was washed with ethanol (3 x 5 ml), dichloromethane (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml) and was dried for 4 h under vacuum.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 131.9, 131.8, 130.7, 130.4, 130.2, 129.8, 129.6, 129.4, 129.3, 129.0, 128.8, 72.1 (polyethylene glycol peak), 43.0, 42.4, 42.2, 42.0, 41.8, 41.7, 40.8, 30.2, 26.0, 21.8, 15.5, 15.4 ppm.

Coupling of FmocAlaOH on resin **III.27**



Diisopropylethylamine (0.033 ml, 0.19 mmol) was added to a solution of freshly prepared FmocAlaCl (0.19 mmol) in dry dichloromethane (2 ml). The solution was added to resin (100 mg, 0.0162 mmol, 0.162 mmol/g). The mixture was shaken for 4 h. The coupling was repeated once

again overnight. After filtration, the resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml), and dichloromethane (3 x 2 ml).

The yield of coupling was determined using Fmoc UV/VIS spectroscopy at 305 nm.

Sample: m = 5.3 mg

Absorbance: A = 0.221

Measured loading: Loading = (A x 2) / (0.7 x m) = 0.12 mmol/g

Calculated loading: 0.162 mmol/g

Yield = 75%

Deprotection of the Fmoc protective group

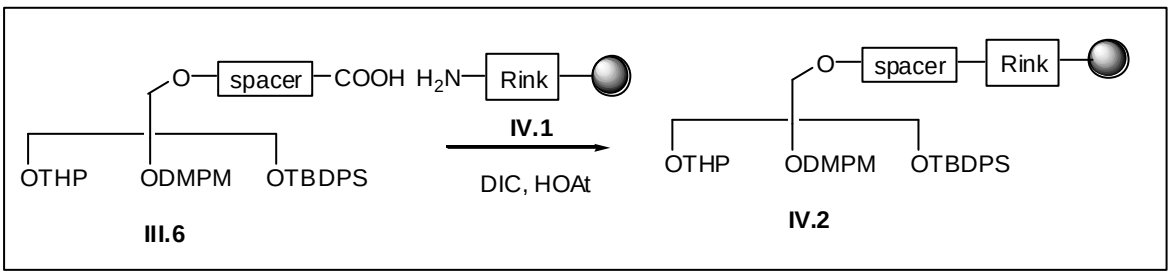
The resin was treated with a solution of 20% piperidine in dimethylformamide for 20 min (2x). The resin was washed with dimethylformamide (3 x 2 ml), methanol (3 x 2 ml) and dichloromethane (3 x 2 ml). A sample of the resin was submitted to the colorimetric TNBS test and intense coloured beads were observed.

Capping of the free amino function

The free amino function was capped with acetic anhydride as described previously for resin **III.24**.

7.4 Experimental data of part 4

Coupling of acid **III.6** to Rink resin **IV.1**: synthesis of resin **IV.2**

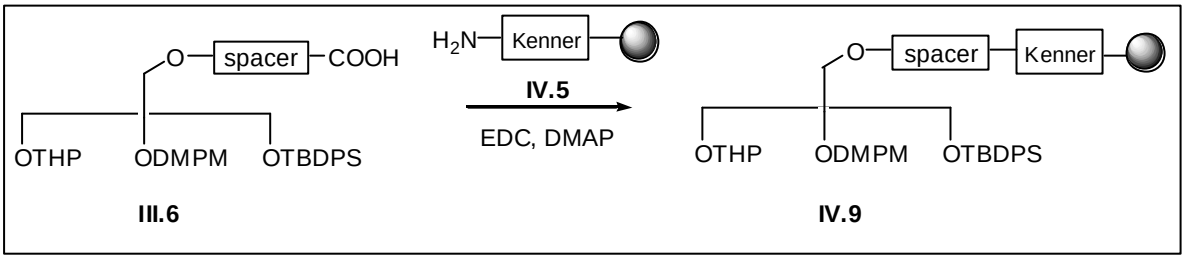


Diisopropylcarbodiimide (25 µl, 0.162 mmol) and 1-hydroxy-7-azabenzotriazole (22 mg, 0.162 mmol) were added at 0 °C to solution of acid scaffold **III.6** (0.18 g, 0.162 mmol) in dry dichloromethane (4 ml). The mixture was stirred at 0 °C for 20 min and at room temperature for

15 min. The solution was added to resin **IV.1** (0.5 g, 0.105 mmol, 0.21 mmol/g) and the mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 7 ml), methanol (3 x 7 ml) and dichloromethane (3 x 7 ml). The same procedure was repeated. The TNBS and **NF31** tests were performed resulting both in colourless beads proving a complete coupling.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 170.9, 168.7, 161.4, 158.8, 157.2, 139.4, 137.4, 136.3, 134.6, 132.0, 130.4, 130.1, 129.6, 129.2, 128.8, 128.4, 120.8, 116.8, 115.2, 112.0, 105.2, 99.9, 99.4, 73.9, 73.4, 71.3 (polyethylene glycol peak) 69.8, 68.8, 68.3, 64.5, 64.0, 63.0, 62.8, 56.6, 56.5, 56.2, 56.1, 45.4, 39.7, 39.6, 36.8, 31.7, 31.5, 30.2, 27.5, 26.4, 20.4, 19.8 ppm.

Coupling of acid **III.6** to Kenner resin **IV.5**: synthesis of resin **IV.9**



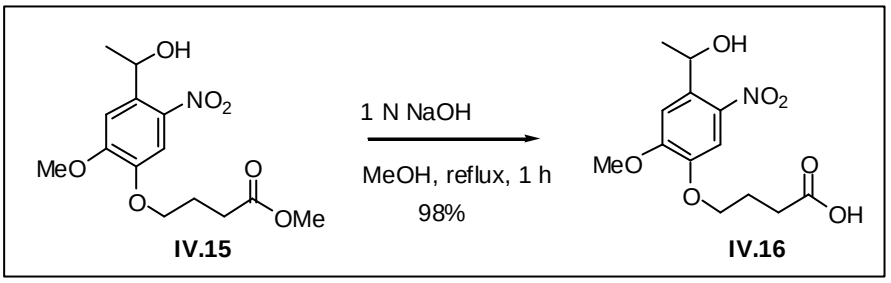
1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (60 mg, 0.312 mmol) and 4-dimethylaminopyridine (1.5 mg, 0.012 mmol) were added to a solution of acid **III.6** (90 mg, 0.078 mmol) in dry dichloromethane (2.5 ml) and the mixture was stirred for 30 min at room temperature. The solution was added to resin **IV.5** (300 mg, 0.078 mmol, 0.26 mmol/g) and the suspension was shaken overnight at room temperature. The resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). The coupling was performed twice.

The completeness of the coupling was controlled with the colorimetric **NF31** test and white beads were observed. It has to be mentioned that the TNBS test cannot be used in the case of sulfonamides.

The free amino functions were capped with an excess acetic anhydride in presence of 4-dimethylaminopyridine and diisopropylethylamine as described previously.

Synthesis of linker **IV.14a**

Synthesis of carboxylic acid **IV.16**



An aqueous solution of 1 N sodium hydroxide (5.3 ml) was added to a solution of ester **IV.15** (0.5 g, 1.6 mmol) in methanol (13 ml). The mixture was heated under reflux for one hour. The methanol was evaporated under reduced pressure. The aqueous phase was acidified at 0 °C with an aqueous solution of 6 N hydrochloric acid and was extracted with ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate, filtered and evaporated in vacuo. 0.47 g of acid **IV.16** was obtained (98%) and was used without further purification.

Bruto formula: $C_{13}H_{17}NO_7$

M.W.: 299.3 g/mol

Rf (dichloromethane / methanol: 9 / 1) = 0.32

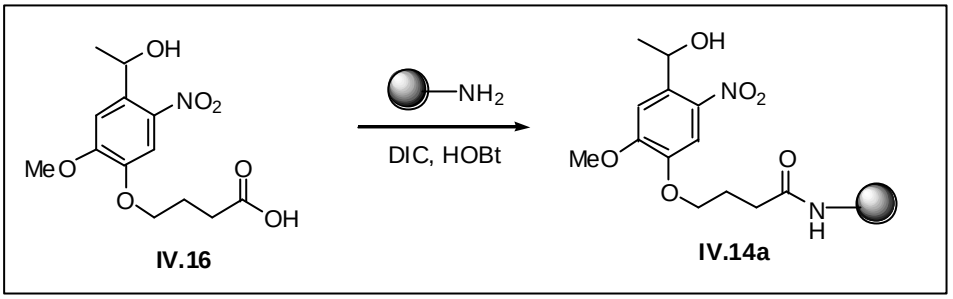
IR (KBr) 3202 (m), 2949 (m), 1698 (m), 1578 (m), 1527 (m), 1446 (w), 1402 (w), 1323 (m), 1269 (s), 1217 (m), 1100 (m), 1056 (m), 1018 (m), 898 (w), 874 (w), 819 (w), 760 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$ + few drops DMSO-d_6) δ 7.80 (1H, s), 7.45 (1H, s), 5.34 (1H, q, J = 6.1 Hz), 4.03 (2H, t, J = 6.0 Hz), 3.89 (3H, s), 2.37 (2H, t, J = 7.0 Hz), 2.02 (2H, m), 1.36 (3H, d, J = 6.1 Hz) ppm.

^{13}C NMR (50 MHz, DMSO-d_6) δ 175.4, 153.6, 146.5, 139.0, 138.3, 109.3, 108.4, 68.4, 64.2, 56.3, 31.4, 25.4, 24.8 ppm.

ES-MS: m/z 322 $[M + Na]^+$

Coupling of acid **IV.16** with Novagel



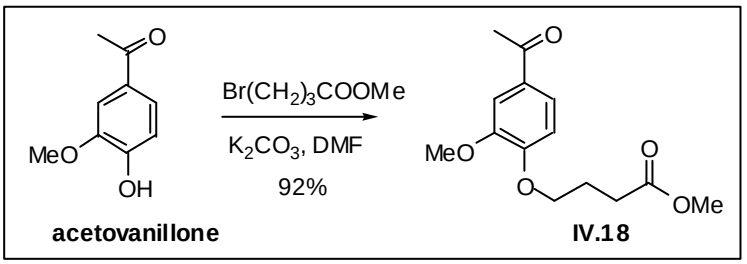
IV.16 **IV.14a**

Diisopropylcarbodiimide (48 µl, 0.304 mmol) and 1-hydroxybenzotriazole (3 mg, 0.023 mmol) were added to a solution of acid **IV.16** (91 mg, 0.304 mmol) in a mixture of dichloromethane (1 ml) / dimethylformamide (1 ml). The solution was stirred for 30 min and was added to Novagel resin (0.2 g, 0.15 mmol, 0.76 mmol/g). The mixture was shaken overnight at room temperature. After filtration, the resin was washed dimethylformamide (3 x 4 ml), methanol (3 x 4 ml) and dichloromethane (3 x 4 ml). The same procedure was repeated once.

The completeness of the coupling reaction was controlled using the colorimetric TNBS and **NF31** tests and colourless beads were observed.

Synthesis of linker **IV.14b**

Synthesis of methylester **IV.18**



acetovanillone **IV.18**

Methylbromobutyrate (30.2 g, 0.167 mol) and potassium carbonate (31.2 g, 0.226 mol) were added to a solution of acetovanillone (25 g, 0.150 mol) in dry dimethylformamide (120 ml). The mixture was stirred at room temperature overnight. Water was added to the mixture until all excess potassium carbonate was dissolved. The aqueous phase was extracted with ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate, filtered and

evaporated under reduced pressure to provide 39 g of colourless oil (98%), which easily crystallizes at 4°C.

Bruto formula: $C_{14}H_{18}O_5$

M.W.: 266.3 g/mol

m.p.: 49 °C

R_f (ethyl acetate) = 0.66

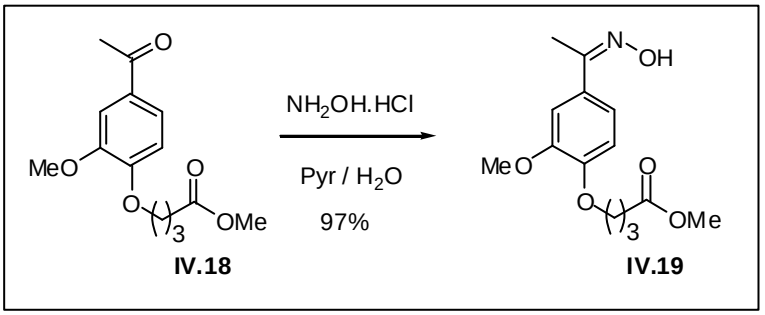
IR (film) 2925 (m), 2848 (m), 1731 (s), 1673 (m), 1587 (m), 1512 (m), 1417 (m); 1272 (m), 1219 (m), 1176 (m), 1150 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.52 (2H, m), 6.87 (1H, d, J = 8.3 Hz), 4.12 (2H, t, J = 6.3 Hz), 3.87 (3H, s), 3.67 (3H, s), 2.54 (3H, s), 2.54 (2H, t, J = 7.1 Hz), 2.17 (2H, m) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 196.3 (C), 172.9 (C), 152.2 (C), 148.9 (C), 130.1 (C), 122.8 (C), 110.9 (CH), 110.1 (CH), 67.3 (CH_2), 55.5 (CH_3), 51.2 (CH_3), 29.9 (CH_2), 25.7 (CH_3), 23.9 (CH_2) ppm.

ES-MS: m/z 267 $[M + H]^+$

Synthesis of methyl 4-(4-(1-hydroxyiminoethyl)-2-methoxyphenoxy)butanoate **IV.19**



A solution of keto-ester **IV.18** (39 g, 0.147 mol) and the chloride salt of hydroxylamine (11.3 g, 0.162 mol) in a mixture of pyridine (86 ml) / water (43 ml) was stirred overnight. A saturated aqueous solution of sodium chloride (80 ml) was added and the mixture was extracted with ethyl acetate. The organic phases were combined, dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to afford 40 g of a light yellow solid (97%).

Bruto formula: $C_{14}H_{19}NO_5$

M.W.: 281.3 g/mol

m.p.: 74 °C

R_f (ethyl acetate) = 0.66

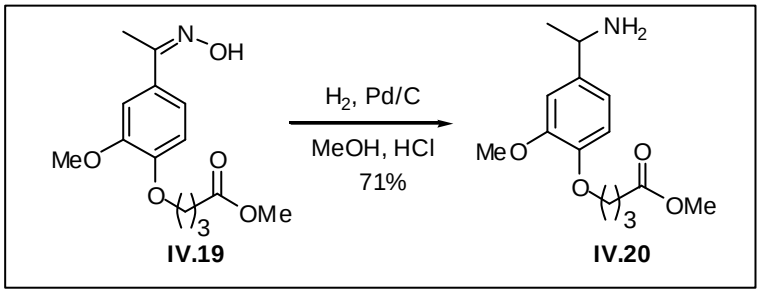
IR (film) 2924 (m), 2873 (m), 1731 (s), 1602 (w), 1578 (w), 1514 (s), 1414 (m), 1302 (m), 1244 (m), 1220 (s), 1179 (m), 1149 (m), 1085 (w), 997 (m), 944 (m), 868 (m), 733 (w), 515 (w) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.23 (1H, d, J = 2.0 Hz), 7.10 (1H, dd, J = 8.4 Hz, 2.0 Hz), 6.85 (1H, d, J = 8.4 Hz), 4.07 (2H, t, J = 6.3 Hz), 3.87 (3H, s), 3.67 (3H, s), 2.54 (2H, t, J = 7.3 Hz), 2.25 (3H, s), 2.15 (2H, m) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 173.6 (C), 155.6 (C), 149.2 (C), 149.4 (C), 129.4 (C), 119.1 (CH), 112.3 (CH), 109.0 (CH), 67.7 (CH_2), 55.8 (CH_3), 51.6 (CH_3), 30.4 (CH_2), 24.3 (CH_3), 12.0 (CH_3) ppm.

ES-MS: m/z 304 $[M + Na]^+$

Synthesis of amine **IV.20**



To a solution of oxime **IV.19** (3 g, 11.0 mmol) in methanol (30 ml) were added 10% palladium on activated carbon (1.13 g, 1.1 mmol) and 1 ml of concentrated hydrochloric acid. The flask was degassed and placed under hydrogen. The mixture was stirred overnight at room temperature. The mixture was filtered over celite. The organic phase was evaporated under reduced pressure. The residue was taken up in 20 ml water and acidified to pH 1 with an aqueous solution 6 N hydrochloric acid. The aqueous phase was extracted with ethyl acetate, basified with sodium bicarbonate until pH 9 and extracted again with ethyl acetate. The combined organic phases were dried over anhydrous magnesium sulfate, filtered and evaporated in vacuo to afford 2 g of amine **IV.20** (71%).

Bruto formula: $C_{14}H_{21}NO_4$

M.W.: 267.3 g/mol

R_f (CH_2Cl_2 / MeOH: 9 / 1) = 0.35

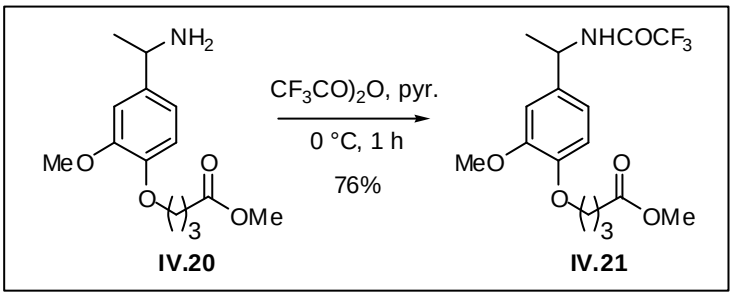
IR (film) 3588 (w), 3360 (m), 2957 (m), 1732 (s), 1592 (m), 1517 (s), 1447 (m), 1369 (w), 1321 (w), 1261 (s), 1229 (s), 1173 (s), 1140 (s), 1035 (s), 946 (w), 863 (w), 811 (w), 647 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.88 (1H, s), 6.80 (2H, s), 4.00 (3H, m), 3.83 (3H, s), 3.64 (3H, s), 2.51 (2H, t, J = 7.3 Hz), 2.09 (2H, m), 1.72 (2H, s), 1.33 (3H, d, J = 6.6 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.4, 149.3, 146.8, 140.6, 117.4, 113.2, 109.4, 67.8, 55.7, 51.3, 50.7, 30.2, 25.5, 24.3 ppm.

ES-MS: m/z 290 $[M + Na]^+$

Synthesis of methyl 4-(2-methoxy-4-(1-trifluoroacetamidoethyl)phenoxy) butanoate **IV.21**



To a cooled solution of amine **IV.20** (0.7 g, 2.62 mmol) in dry pyridine (5.5 ml) was added dropwise trifluoroacetic anhydride (0.55 ml, 3.89 mmol). The mixture was allowed to react for 1 h at 0 °C and then poured in a saturated solution of sodium chloride (30 ml), extracted with ethyl acetate, dried over anhydrous magnesium sulfate and evaporated under reduced pressure. Recrystallization from dichloromethane / pentane afforded 0.72 g of the protected amine **IV.21** (76%).

Bruto formula: $C_{16}H_{20}NO_5F_3$

M.W.: 363.2 g/mol

m.p.: 85 °C

R_f (ethyl acetate) = 0.69

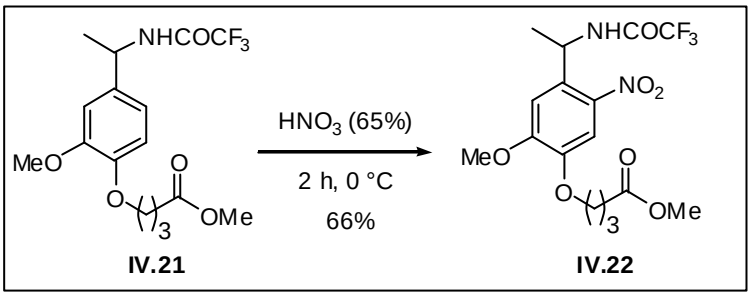
IR (KBr) 3307 (w), 2956 (m), 1714 (s), 1594 (w), 1519 (s), 1454 (m), 1422 (m), 1258 (s), 1208 (s), 1170 (s), 1034 (m), 934 (w), 809 (w), 736 (w), 702 (w), 652 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 6.82 (3H, m), 6.56 (1H, m), 5.07 (1H, dq, J = 7.0 Hz), 4.03 (2H, t J = 6.3 Hz), 3.84 (3H, s), 3.66 (3H, s), 2.52 (2H, t, J = 7.3 Hz), 2.12 (2H, m), 1.55 (3H, d, J = 6.9 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.6, 149.7 148.1, 133.7, 118.2, 113.3, 110.4, 67.9, 55.9, 51.6, 49.5, 30.4, 24.4, 20.7 ppm.

ES-MS: m/z 362 $[M - H]^-$

Synthesis of methyl 4-(2-methoxy-5-mitro-4-(1-trifluoroacetamidoethyl)-phenoxy) butanoate **IV.22**



IV.21 **IV.22**

To a cooled (0 °C) solution of protected amine **IV.21** (1.5 g, 4.13 mmol) was added slowly a solution of 65% of nitric acid (11.5 ml). The mixture was allowed to react for 2 h and was then quenched with water. The volume was adjusted to 107 ml. The mixture was kept at 4 °C overnight and was filtered to afford 1.1 g of a yellow powder (66%).

Bruto formula: $C_{16}H_{19}N_2O_7F_3$

M.W.: 408.1 g/mol

m.p.: 141 °C

R_f (ethyl acetate) = 0.68

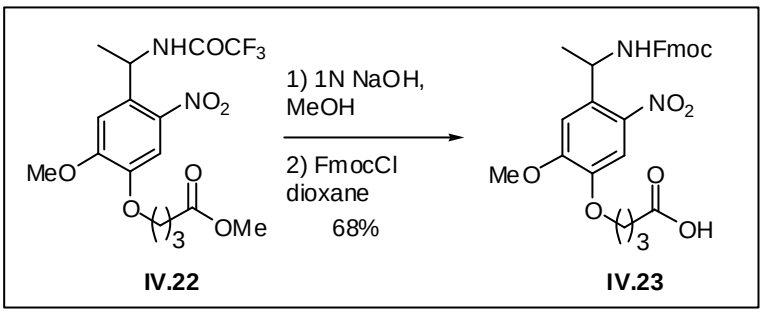
IR (KBr) 3316 (w), 2949 (w), 1720 (s), 1701 (s), 1578 (w), 1549 (m), 1519 (s), 1461 (w), 1331 (m), 1273 (s), 1214 (s), 1179 (s), 1061 (w), 938 (w), 885 (w), 703 (w), 656 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.68 (1H, s), 7.43 (1H, d, J = 7.3 Hz), 6.86 (1H, s), 5.52 (1H, dq, J = 7.2 Hz), 4.11 (2H, t, J = 6.2 Hz), 3.92 (3H, s), 3.68 (3H, s), 2.53 (2H, t, J = 7.2 Hz), 2.16 (2H, m), 1.60 (3H, d, J = 7.0 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 173.4, 154.0, 147.6, 140.5, 130.9, 111,0, 110.2, 68.2, 56.4, 51.7, 48.4, 30.3, 24.2, 20.1 ppm.

ES-MS: m/z 407 $[M - H]^-$

Synthesis of 4-(4-(1-(9-fluorenylmethoxycarbonylamino)ethyl)-2-methoxy-5-nitrophenoxy) butanoic acid **IV.23**



IV.22 **IV.23**

A solution of 1 N sodium hydroxide (7.3 ml, 7.3 mmol) and trifluoroacetamide **IV.22** (0.9 g, 2.2 mmol) in methanol (18.5 ml) was heated under reflux for 5 h. The methanol was evaporated in vacuo. 1,4-Dioxane (11.2 ml) and water (7.3 ml) were added and the pH of the solution was adjusted to pH = 9 with a solution 6 N hydrochloric acid. A solution of 9-fluorenylmethyl chloroformate (0.72 g, 2.8 mmol) in 1,4-dioxane (7.3 ml) was added. The pH was adjusted to pH = 8 over 30 min with an aqueous solution of 1 N sodium hydroxide. After two days, the reaction was quenched with 7.3 ml of a solution of 1 N hydrochloric acid and the volume was adjusted to 80 ml with water. The precipitate was filtered and dissolved in warm ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate, filtered while hot and evaporated under reduced pressure. The light yellow solid was triturated in warm ether and filtered. The residue was recrystallized from methanol to afford 0.78 g of linker **IV.23** (68%).

Bruto formula: $C_{28}H_{28}N_2O_8$

M.W.: 520 g/mol

m.p.: 200 °C

R_f (ethyl acetate) = 0.63

IR (KBr) 3352 (m); 2934 (w), 2871 (w), 1686 (s), 1654 (s), 1578 (w), 1523 (s), 1446 (w), 1368 (m), 1339 (m), 1268 (s), 1217 (m), 1172 (m), 1072 (m), 1017 (m), 880 (w), 856 (w), 742 (w), 698 (w), 575 (w) cm^{-1}.

^1H NMR (500 MHz, DMSO-d_6) δ 8.10 (1H, d, J = 8.2 Hz), 6.99 (2H, d, J = 7.6 Hz), 7.65 (2H, d, J = 7.5 Hz), 7.49 (1H, s), 7.41 (2H, td, J = 7.4, 2.2 Hz), 7.29 (2H, m), 7.25 (1H, s), 5.19 (1H, dq, J = 7.1 Hz), 4.29 (2H, t, J = 7.1 Hz), 4.18 (1H, t, J = 6.4 Hz), 4.06 (2H, t, J = 6.4 Hz), 3.89 (3H, s), 2.40 (2H, J = 7.3 Hz), 1.96 (2H, m), 1.42 (3H, d, J = 7.1 Hz) ppm.

^{13}C NMR (50 MHz, DMSO-d_6) + DEPT δ 174.1 (C), 155.3 (C), 153.5 (C), 146.4 (C), 143.9 (C), 143.7 (C), 140.7 (C), 139.9 (C), 135.5 (C), 127.6 (CH), 126.9 (CH), 125.1 (CH), 120.2 (CH), 109.4 (CH), 108.2 (CH) 67.9 (CH_2), 65.3 (CH_2) 56.2 (CH_3), 46.8 (CH), 46.0 (CH), 30.0 (CH_2), 24.1 (CH_2), 21.9 (CH_3) ppm.

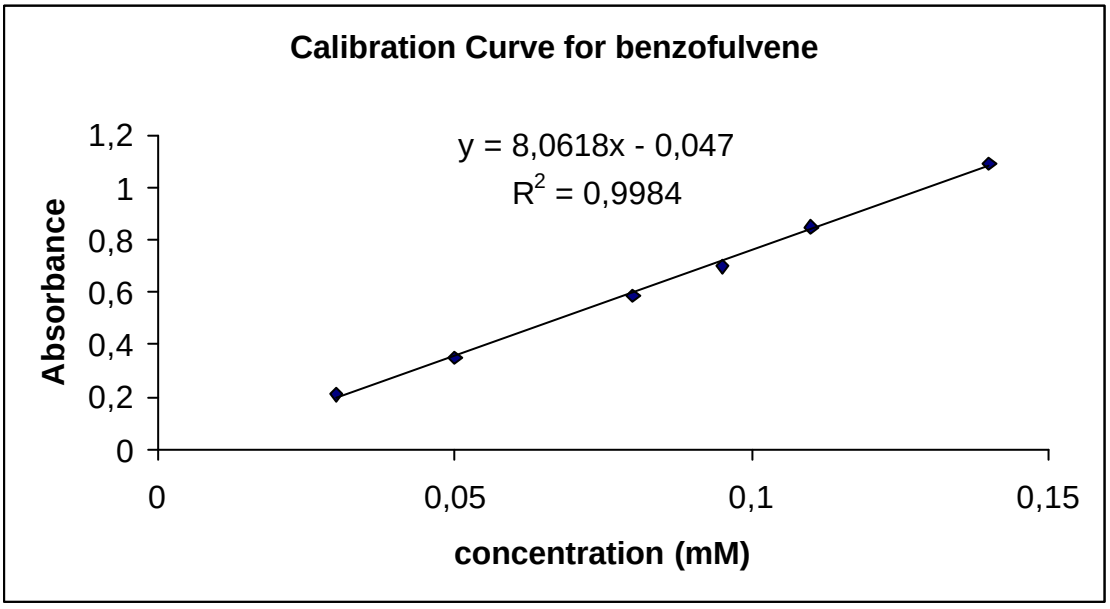
ES-MS: m/z 538 $[M + NH_4]^+$

Control of the loading of the commercial resin TentaGel

a) Using Fmoc monitoring:

Preparation of calibration curve:

The amino acid FmocValOH (50.3 mg) was dissolved in 100 ml of a solution of 20% piperidine in dimethylformamide (c = 1.482 mM). The mother solution was diluted (c = 0.14, 0.11, 0.095, 0.08, 0.05, 0.03 mM) and the absorbance of each dilution solution was measured at 300 nm. The calibration curve was then established.



Synthesis of TentaGel-ValFmoc

Diisopropylcarbodiimide (13.1 µl, 0.084 mmol) and 1-hydroxybenzotriazole (11.4 mg, 0.084 mmol) were added to a solution of amino acid FmocValOH (32.5 mg, 0.084 mmol) in dimethylformamide (2 ml). The carboxylic acid was activated for 30 min. The solution was then added to the resin TentaGel-NH_2 (100 mg, 0.28 mmol/g) and the mixture was shaken overnight at room

temperature. After filtration, the resin was washed with dimethylformamide (3 x 3 ml), methanol (3 x 3 ml) and dichloromethane (3 x 3 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Determination of the loading in a quantitative way

A sample of Tentagel-ValFmoc (37.3 mg) was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). The filtrates were collected and diluted to 100 ml with dimethylformamide.

The absorbance of the filtrate was measured at 300 nm: A = 0.626.

By using the calibration curve, the concentration of the piperidine-benzofulvene adduct can be deduced and the loading can be calculated:

Loading (mmol / g) = c (mM) x V (L) / m (g).

Absorbance: A = 0.626

Volume: V = 0.1 l

Weight of resin: m = 37.3 mg

Concentration: c = 0.0834 mM

Loading: loading = 0.22 mmol/g

b) Using picric acid

The procedure to determine the loading of the commercial resin TentaGel using picric acid is the following:

A sample of TentaGel (60.4 mg, 0.28 mmol/g) was washed with dichloromethane (3 x 5 ml), a solution of 15% triethylamine in dichloromethane (3 x 5 ml), methanol (3 x 5 ml) and with dichloromethane (5 x 5 ml). The resin was then treated with a saturated solution of picric acid (2,4,6-trinitrophenol) in dichloromethane for 30 s. The resin was then washed intensively with dichloromethane and methanol remove the excess of picric acid until an absorbance < 0.05 of the filtrate was obtained at 340 nm.

The resin was washed twice with a solution of 15% triethylamine in dichloromethane. The combined filtrates were diluted to 50 ml dichloromethane. 1 ml of the resulting bright yellow solution was diluted to 10 ml with absolute ethanol. The absorbance was measured at 358 nm and the substitution was calculated:

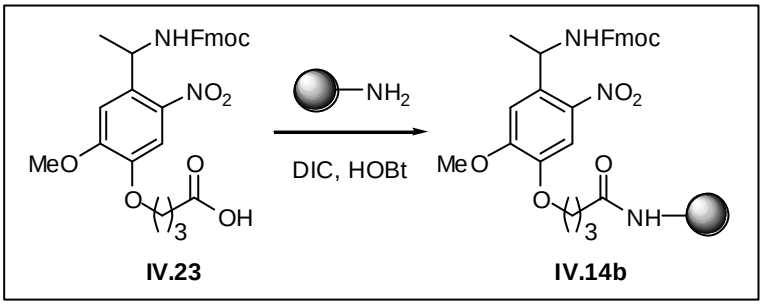
Loading (mmol/g) = 34.48 x A_{358} / m (g).

Weight of resin: m = 60.4 mg

Absorbance: A = 0.357

Substitution: loading = 0.204 mmol/g

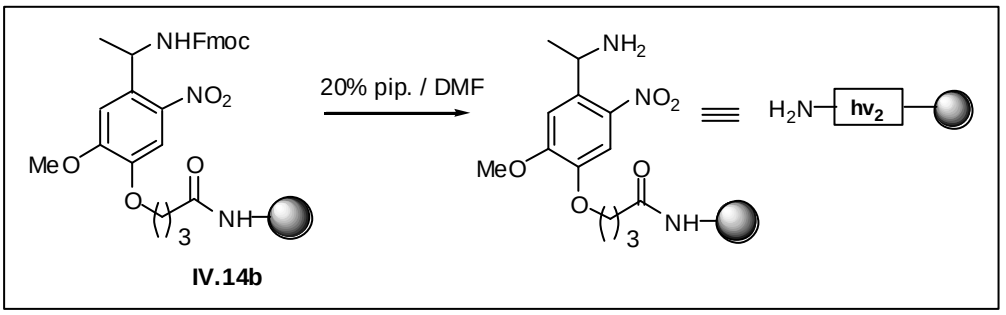
Coupling of the photolabile linker **IV.23** on TentaGel-NH_2:



IV.23 **IV.14b**

Diisopropylcarbodiimide (27 µl, 0.174 mmol) and 1-hydroxybenzotriazole (24 mg, 0.174 mmol) were added to a solution of **IV.23** (90.5 mg, 0.174 mmol) in dimethylformamide (3 ml). The mixture was stirred for 30 min at room temperature. After addition of the solution to the resin (300 mg, 0.063 mmol, 0.21 mmol/g), the suspension was shaken overnight at room temperature. The resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). To monitor the completeness of the coupling reaction, a sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

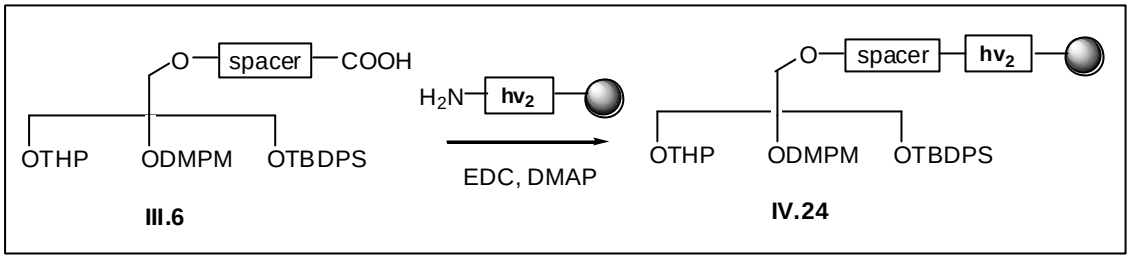
Deprotection of the Fmoc protective group



IV.14b

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). The resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). Both colorimetric TNBS and **NF31** tests were done and resulted in coloured beads.

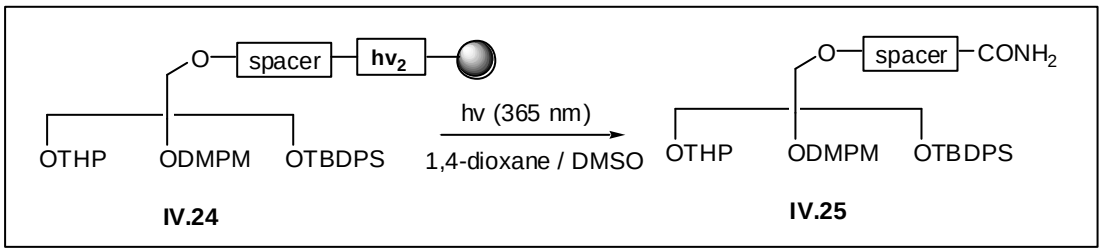
Coupling of scaffold **III.6** to polymer-supported linker **IV.14b**



1-[3-(Dimethylamino)propyl]-3-ethylcarbodiimide hydrochloride (67 mg, 0.348 mmol) and 4-dimethylaminopyridine (2 mg, 0.013 mmol) were added to a solution of scaffold **III.6** (140 mg, 0.126 mmol) in dichloromethane (3 ml). After activation of the acid for 30 min at room temperature, the solution was added to the resin (300 mg, 0.060 mmol, 0.20 mmol/g). The suspension was shaken overnight at room temperature. The resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). TNBS test and **NF31** test to control the efficiency of the coupling were preformed and showed colourless beads.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 174.1, 173.3, 172.2, 155.2, 150.4, 148.2, 148.1, 141.6, 139.9, 137.8, 137.0, 136.8, 135.0, 132.5, 131.1, 131.0, 130.8, 130.5, 130.2; 129.4, 129.3, 129.0, 128.9, 121.3, 112.5, 112.4, 110.6, 99.9, 74.5, 73.9, 71.5 (polyethylene glycol peak), 66.5, 65.0, 64.6, 63.3, 47.5, 45.8, 40.6, 40.3, 37.3, 33.6, 32.1, 31.8, 31.7, 30.4, 28.0, 26.3, 22.9, 20.3 ppm.

Cleavage of construct **IV.24**: Synthesis of **IV.25**



50 mg of resin **IV.24** (0.008 mmol, 0.163 mmol/g) in 1.5 ml of 1,4-dioxane and 15 µl of dimethylsulfoxide was exposed to UV light (365 nm) for 2 h and 6 times 3 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane

and removal of the dimethylsulfoxide with the Kugelrhor, 7.8 mg of compound **IV.25** was obtained without further purification (86%).

Bruto formula: $C_{66}H_{83}O_{12}NSi$

M.W.: 1110.5 g/mol

R_f (ethyl acetate) = 0.55

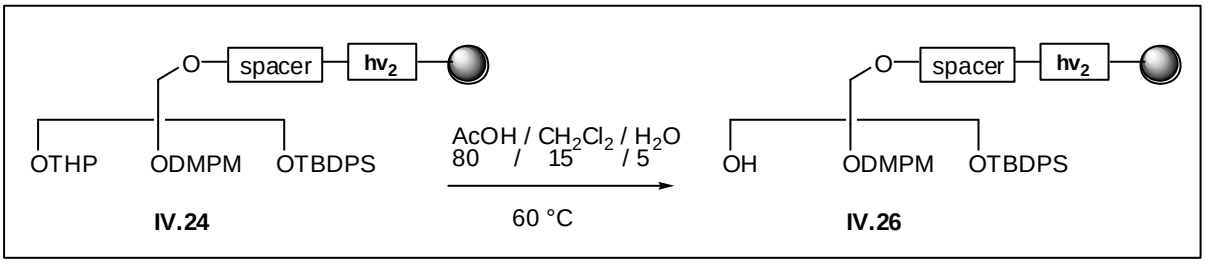
IR (film) 3424 (s), 2919 (s), 2860 (s), 1725 (s), 1672 (m), 1513 (w), 1455 (w), 1425 (w), 1260 (s), 1172 (m), 1102 (s), 1025 (s), 803 (m) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.58 (4H, dd, J = 8.0, 1.5 Hz), 7.37 (6H, m), 7.18 (10H, m), 7.09 (2H, d, J = 8.0 Hz), 6.82 (3H, m), 4.59 (1H, t, J = 3.1 Hz), 4.45 (2H, s), 4.42 (2H, s), 4.41 (4H, s), 4.21 (2H, s), 3.93 (1H, dt, J = 9.6, 7.2 Hz), 3.87 (3H, s), 3.84 (3H, s), 3.81 (2H, t, J = 7.1 Hz), 3.78 (1H, m), 3.65 (2H, t, J = 7.1 Hz), 3.60 (1H, dt, J = 9.6, 7.2 Hz), 3.49 (4H, s), 3.48 (2H, s), 3.43 (1H, m), 2.90 (2H, t, J = 6.8 Hz), 2.89 (2H, t, J = 7.0 Hz), 2.83 (2H, t, J = 7.0 Hz), 2.52 (2H, t, J = 6.9 Hz), 2.33 (2H, t, J = 6.9 Hz), 1.80 (1H, m), 1.70 (1H, m), 1.55 (4H, m), 1.02 (9H, s) ppm.

ES-MS: m/z 1212 $[M + TEA]^+$

Synthesis of acetate **IV.28**

Selective deprotection of the THP protective group:



First method: Deprotection of the THP protecting group with acetic acid

Resin **IV.24** (100 mg, 0.016 mmol, 0.163 mmol/g) was treated with 2 ml of a solution of AcOH / CH_2Cl_2 / H_2O (80 / 15 / 5) for 4 h at 60 °C. After filtration, the same reaction was repeated 6 times 2 h. The resin was washed with dichloromethane (3 x 5 ml), methanol (3 x 5 ml), 5% of diisopropylethylamine in dichloromethane, dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml) and was dried for 4 h under vacuum.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 174.2, 173.3, 172.1, 155.3, 141.7, 140.0, 139.9, 137.9, 136.9, 135.1, 132.6, 131.0, 130.8, 130.5, 130.3, 130.2, 129.7, 129.5, 129.0, 128.9, 112.6, 112.5, 110.7, 75.4, 71.5 (polyethylene glycol peak), 66.6, 65.1, 64.6, 47.6, 45.9, 40.6, 40.3, 37.4, 33.7, 31.9, 30.5, 28.0, 26.4, 22.9, 20.4 ppm.

Second method: Deprotection of the THP protecting group with PPTS

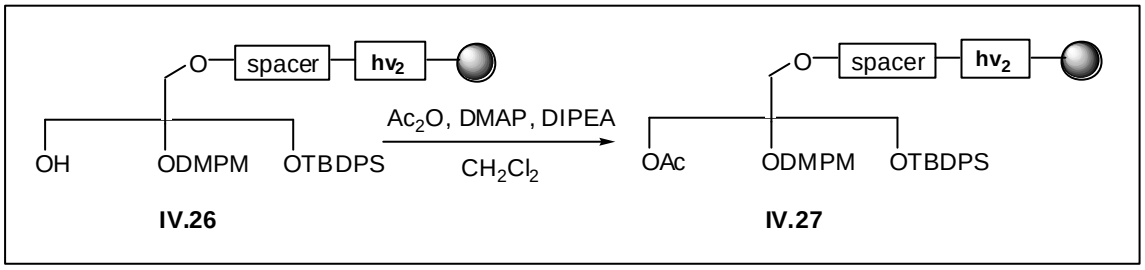
Pyridinium *p*-toluenesulfonate (0.066 g, 0.265 mmol) was added to a suspension of resin **IV.24** (0.315 g, 0.051 mmol, 0.163 mmol/g) in dry ethanol (3.2 ml). The mixture was heated at 55 °C overnight. After filtration, the resin was washed with ethanol (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml).

A sample of resin **IV.26** (2 mg, 0.33 μmol, 0.165 mmol/g) was suspended in 100 μl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analysed by ES-MS.

M.W.: 1025 g/mol

ES-MS: m/z 1026 $[M + H]^+$, 1048 $[M + Na]^+$, 1064 $[M + K]^+$

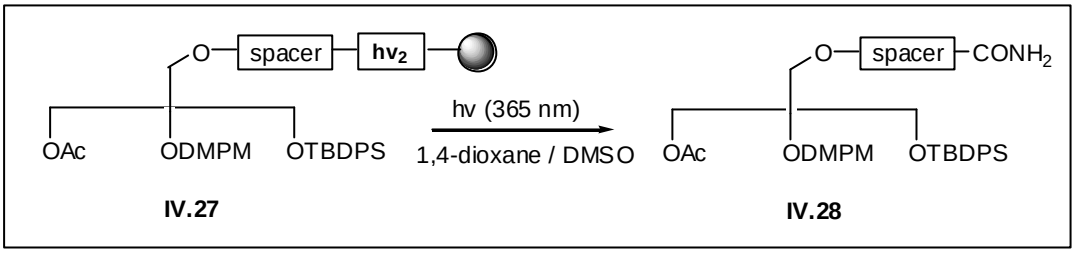
Capping of the hydroxyl function as the acetate: synthesis of **IV.27**



IV.26 **IV.27**

A solution of acetic anhydride (16 μl, 0.174 mmol), diisopropylethylamine (31 μl, 0.174 mmol) and 4-dimethylaminopyridine (21.2 mg, 0.174 mmol) in dichloromethane (2 ml) was added to the THP deprotected resin **IV.26** (0.1 g, 0.017 mmol, 0.165 mmol/g). The suspension was shaken for 20 min. The procedure was repeated once again. The resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml) and was dried for 4 h under vacuum.

Cleavage of **IV.27** from the resin: Synthesis of acetate **IV.28**



IV.27 **IV.28**

A sample of acetate **IV.27** (50 mg, 0.0082 mmol, 0.164 mmol/g) in suspension in 1 ml of 1,4-dioxane with 10 µl of dimethylsulfoxide was exposed to UV light (365 nm) for 4 times 3 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethylsulfoxide with the Kugelrhor, the sample was purified by column chromatography (ethyl acetate / isooctane: 8 / 2) to provide 4.8 mg of acetate **IV.28** (55%).

Bruto formula : $C_{63}H_{77}O_{12}NSi$

M.W.: 1067 g/mol

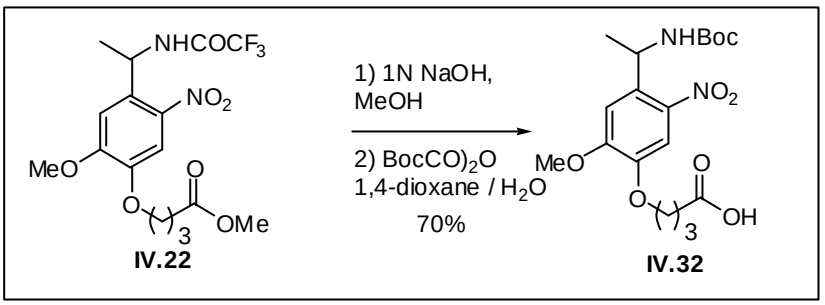
R_f (ethyl acetate) = 0.55

IR (film) 2925 (m), 2848 (m), 2860 (s), 1737 (s), 1678 (m), 1619 (w), 1507 (w), 1461 (w), 1437 (w), 1413 (w), 1360 (w), 1255 (m), 1231 (w), 1096 (m), 1025 (m), 667 (s) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.79 (4H, dd, J = 7.9, 1.5 Hz), 7.32-7.41 (6H, m), 7.17 (10H, m), 7.09 (2H, d, J = 8.0 Hz), 6.82 (3H, m), 5.47 (1H, m), 5.08 (1H, m), 4.45 (2H, s), 4.42 (2H, s), 4.41 (4H, s), 4.25 (2H, t, J = 7.1 Hz), 4.21 (2H, s), 3.87 (3H, s), 3.83 (3H, s), 3.81 (2H, t, J = 7.1 Hz), 3.64 (2H, t, J = 7.1 Hz), 3.49 (6H, s), 2.90 (4H, t, J = 7.0 Hz), 2.83 (2H, t, J = 7.0 Hz), 2.53 (2H, t, J = 6.8 Hz), 2.35 (2H, t, J = 6.8 Hz), 2.03 (3H, s), 1.02 (9H, s) ppm.

ES-MS m/z 1085 $[M + NH_4]^+$

Synthesis of 4-(4-(1-(*tert*-butylcarbonyloxyamino)ethyl)-2-methoxy-5-nitrophenoxy) butanoic acid **IV.32**



An aqueous solution of 1 N sodium hydroxide (16 ml, 16.0 mmol) and trifluoroacetamide **IV.22** (2.0 g, 4.9 mmol) in methanol (40 ml) was heated under reflux for 2 h. The methanol was evaporated in vacuo. 1,4-Dioxane (24 ml) and water (16 ml) were added and the pH of the solution was adjusted to pH = 9 with a 6 N aqueous solution hydrochloric acid. A solution of di-*tert*-butyl dicarbonate (1.7 ml, 7.36 mmol) in 1,4-dioxane (6 ml) was added and the mixture was stirred at room temperature for 2 h. After cooling to 0 °C, the pH was adjusted to 2-3 over 30 min with a solution of 10% of sodium hydrogen sulfate. The mixture was extracted with ethyl acetate. The combined organic phase were dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by recrystallization from methanol to afford 1.35 g of acid **IV.32** (70%).

Bruto formula: $C_{18}H_{26}N_2O_8$

M.W.: 398.4 g/mol

m.p.: 187 °C

R_f (ethyl acetate / AcOH: 98 / 2) = 0.73

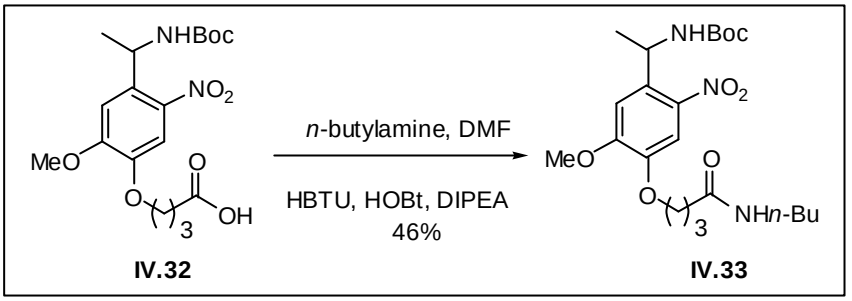
IR (KBr) 3373 (m), 3338 (m), 2999 (w), 1736 (s), 1678 (s), 1584 (m), 1517 (s), 1445 (m), 1371 (m), 1337 (s), 1288 (m), 1269 (s), 1219 (s), 1169 (s), 1072 (m), 1016 (w), 880 (w), 820 (w), 758 (w), 648 (w) cm^{-1}.

^{1}H NMR (500 MHz, DMSO-d_6) δ 12.18 (1H, s), 7.65 (1H, d, J = 8.1 Hz), 7.49 (1H, s), 7.23 (1H, s), 5.14 (1H, dq, J = 14.4, 6.6 Hz), 4.05 (2H, t, J = 6.4 Hz), 3.88 (3H, s), 2.38 (2H, t, J = 7.1 Hz), 1.94 (2H, m), 1.36 (3H, d, J = 6.6 Hz), 1.33 (9H, s) ppm.

^{13}C NMR (50 MHz, DMSO-d_6) + DEPT δ 174.0 (C), 154.8 (C), 153.5 (C), 146.3 (C), 139.9 (C), 135.9 (C), 109.4 (CH), 108.3 (CH), 78.2 (C), 67.9 (CH_2), 56.3 (CH_3), 45.6 (CH), 30.0 (CH_2), 28.2 (CH_3), 24.1 (CH_2), 22.2 (CH_3) ppm.

ES-MS: m/z 421 $[M + Na]^+$, 416 $[M + NH_4]^+$

Synthesis of amide **IV.33**



IV.32 **IV.33**

To a solution of carboxylic acid **IV.32** (0.5 g, 1.26 mmol) in dimethylformamide (12 ml) were added 2-(1H-benzotriazole-1-yl)-1,1,3,3-tetramethyluronium hexafuorophosphate (HBTU) (0.96 g, 2.52 mmol), hydroxybenzotriazole (340 mg, 2.52 mmol) and diisopropylethylamine (0.44 ml, 2.52 mmol). After stirring for 1 min, the amine (0.13 ml, 1.26 mmol) was added. The reaction was allowed to react for 3 h at room temperature. After addition of brine, the mixture was extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated in vacuo. The remaining dimethylformamide was removed with kogelruhr apparatus. The residue was purified by column chromatography (ethyl acetate / isooctane: 4 / 6). The light yellow solid obtained was recrystallized in ethyl acetate to provide 0.26 g of amide **IV.33** (46%).

Bruto formula: $C_{22}H_{35}N_3O_7$

M.W.: 453.2 g/mol

m.p.: 136 °C

R_f (ethyl acetate) = 0.55

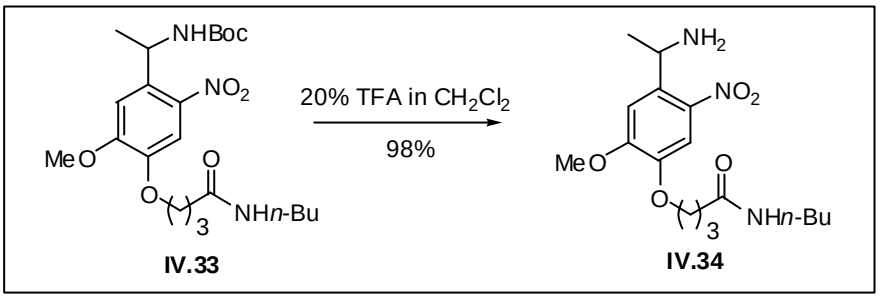
IR (film) 3364 (w), 2956 (w), 1677 (s), 1637 (m), 1518 (s), 1449 (w), 1368 (w), 1336(m), 1270 (s), 1218 (s), 1170 (m), 1073 (w), 1015 (w), 879 (w), 813 (w), 759 (w), 621 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.57 (1H, m), 6.96 (1H, s), 6.68 (1H, m), 5.33 (1H, m), 5.20 (1H, m), 4.09 (2H, t, J = 5.4 Hz), 3.93 (3H, s), 3.24 (2H, dt, J = 6.9, 5.9 Hz), 2.38 (2H, t, J = 7.2 Hz), 2.18 (2H, m), 1.48 (3H, d, J = 6.9 Hz), 1.45-1.2 (13H, m), 0.89 (3H, t, J = 7.3 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 171.9 (C), 154.8 (C), 153.7 (C), 146.7 (C), 140.3 (C), 109.8 (CH), 109.1 (CH), 79.8 (CH), 68.5 (CH_2), 56.3 (CH_3), 47.9 (CH_3), 39.2 (CH_2), 32.8 (CH_2), 31.7 (CH_2), 28.2 (CH_3), 24.9 (C), 22.0 (CH_3), 20.0 (CH_2), 13.7 (CH_3) ppm.

ES-MS: m/z 471 $[M + NH_4]^+$, 929 $[2M + Na]^+$

Synthesis of amine **IV.34**



IV.33 **IV.34**

Compound **IV.33** (0.15 g, 0.33 mmol) was treated with of a solution of 20% trifluoroacetic acid in dichloromethane (5 ml). The mixture was stirred for 20 min. The organic phase was washed with water. The aqueous phase was basified until pH = 9-10 with a saturated aqueous solution of sodium bicarbonate, the aqueous phase was washed with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure affording 114 mg of crude amine **IV.34**, which was used without further purification (98%).

Bruto formula: $C_{17}H_{27}N_3O_5$

M.W.: 353.2 g/mol

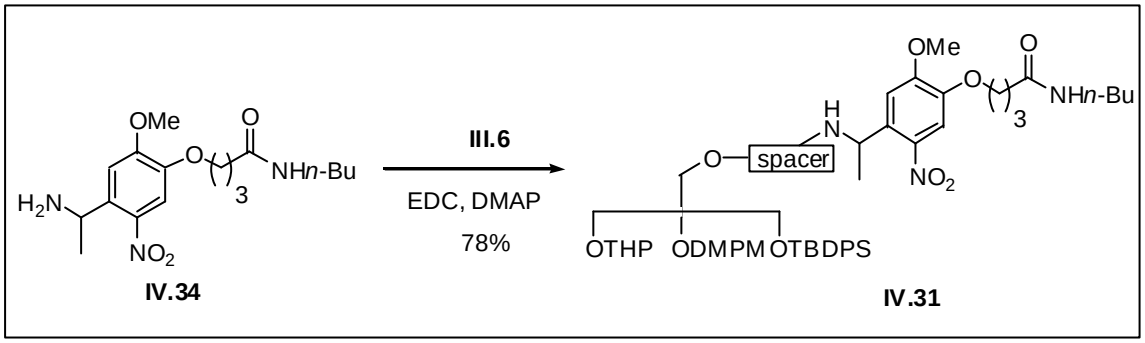
R_f (dichloromethane / methanol: 9 / 1) = 0.57

IR (film) 2959 (w), 2921 (w), 1648 (m), 1518 (s), 1455 (w), 1332 (m), 1267 (s), 1209 (m), 1049(w), 873 (w), 597 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.46 (1H, s), 7.31 (1H, s), 5.71 (1H, m), 4.79 (1H, m), 4.08 (2H, t, J = 6.0 Hz), 3.96 (3H, s), 3.23 (2H, dt, J = 6.9, 6.3 Hz), 2.38 (2H, t, J = 7.2 Hz), 2.17 (2H, m), 1.91 (2H, s), 1.44 (2H, m), 1.42 (3H, d, J = 6.5 Hz), 1.31 (2H, m), 0.89 (3H, t, J = 7.3 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 171.9 (C), 153.7 (C), 146.6 (C), 140.8 (C), 137.3 (C), 109.3 (CH), 109.0 (CH), 68.5 (CH_2), 56.3 (CH_3), 47.9 (CH_3), 39.3 (CH_2), 32.9 (CH_2), 31.7 (CH_2), 24.9 (C), 24.6 (CH_3), 20.0 (CH_2), 13.7 (CH_3) ppm.

Coupling of acid scaffold **III.6** on amine **IV.34**



To a cooled solution (0 °C) of acid scaffold **III.6** (108 mg, 0.097 mmol) and amine **IV.34** (35 mg, 0.099 mmol) in dry dichloromethane (1 ml) were added 1-[3-(Dimethylamino)propyl] -3-ethylcarbodiimide hydrochloride (19 mg, 0.099 mmol) and 4-dimethylaminopyridine (12 mg, 0.099 mmol). The solution was stirred at 0 °C for 1 h and allowed to react at room temperature overnight. The mixture was extracted with a saturated aqueous solution of ammonium chloride. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated in vacuo. The residue was purified by column chromatography (ethyl acetate / isooctane: 6 / 4) to provide 110 mg of amide **IV.31** (78%).

Bruto formula: $C_{83}H_{107}N_3O_{17}Si$

M.W.: 1445.7 g/mol

R_f (ethyl acetate / isooctane: 8 / 2) = 0.37

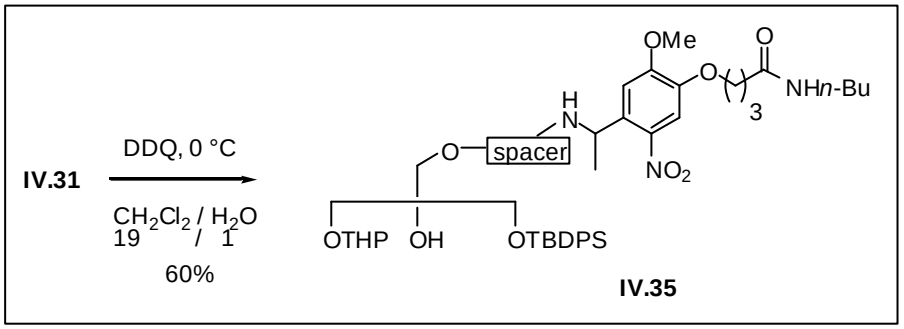
IR (film) 2932 (s), 2856 (m), 1736 (m), 1647 (m), 1517 (s), 1547 (m), 1428 (m), 1363 (m), 1268 (s), 1215 (w), 1090 (s), 1029 (m), 822 (w), 704 (m), 490 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (4H, dd, J = 7.0, 1.3 Hz), 7.53 (1H, s), 7.37 (6H, m), 7.16 (10 H, m), 7.08 (2H, d, J = 8.0 Hz), 6.91 (1H, s), 6.83 (3H, m), 6.41 (1H, d, J = 7.0 Hz), 5.64 (1H, m), 5.51 (1H, m), 4.59 (1H, m), 4.45 (2H, s), 4.42 (2H, s), 4.39 (4H, s), 4.22 (1H, ABd, J_{AB} = 10.8 Hz), 4.12 (1H, ABd, J_{AB} = 10.8 Hz), 4.05 (2H, m), 3.93 (1H, m), 3.92 (3H, s), 3.87 (3H, s), 3.84 (3H, s), 3.80 (2H, t J = 7.0 Hz), 3.77 (1H, m), 3.65 (2H, t = J = 7.1 Hz), 3.60 (1H, m), 3.47 (7H, m); 3.23 (2H, dt, J = 7.0, 6.0 Hz), 2.90 (2H, t, J = 7.0 Hz), 2.88 (2H, t, J = 7.2 Hz), 2.83 (2H, t, J = 7.0 Hz), 2.51 (2H, m), 2.36 (4H, m), 2.16 (2H, m), 1.80 (1H, m), 1.69 (1H, m), 1.56 (4H, m), 1.50 (3H, d, J = 6.9 Hz), 1.44 (2H, m), 1.33 (2H, m), 1.02 (9H, s), 0.90 (3H, t, J = 7.3 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 172.8 (C), 171.9 (C), 170.7 (C), 153.7 (C), 148.9 (C), 148.5 (C), 146.8 (C), 140.3 (C), 138.3 (C), 138.2 (C), 136.4 (C), 136.3 (C), 135.5 (CH), 134.3 (C), 133.7 (C), 130.9 (C), 129.5 (CH), 129.1 (CH), 128.6 (CH), 127.6 (CH), 127.4 (CH), 127.3 (CH), 120.1 (CH), 111.0 (CH), 110.9 (CH), 110.0 (CH), 109.9 (CH), 98.7 (CH), 73.1 (CH_2), 72.8 (CH_2), 70.9 (CH_2), 69.0 (CH_2), 68.4 (CH_2), 68.2 (CH_2), 65.1 (CH_2), 64,1 (CH_2), 62.2 (CH_2), 56.3 (CH_3), 55.9 (CH_3), 55.8 (CH_3), 47.2 (CH), 44.6 (C), 39.3 (CH_2), 38.9 (CH_2), 36.0 (CH_2), 32.8 (CH_2), 31.7 (CH_2), 30.9 (CH_2), 30.7 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 26.8 (CH_3), 25.4 (CH_2), 24.9 (CH_2), 21.4 (CH_3), 20.0 (CH_2), 19.5 (CH_2), 19.1 (CH_2), 13.7 (CH_3) ppm.

ES-MS: m/z 1462 $[M + NH_4]^+$

Deprotection of the DMPM protecting group in compound **IV.31**: synthesis of **IV.35**



To a cooled (0 °C) solution of compound **IV.31** (44.8 mg, 0.031 mmol) in 0.65 ml of a mixture of dichloromethane / water (19 / 1) was added dichlorodicyanoquinone (14 mg, 0.062 mmol). The solution was stirred at 0 °C for 30 min and the mixture was filtered over celite (wash with dichloromethane). The organic phase was washed with a saturated aqueous solution of sodium bicarbonate, dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (ethyl acetate / isooctane: 8 / 2) to give 24 mg of **IV.35** as sole product (60%).

Bruto formula: $C_{74}H_{97}N_3O_{15}Si$

M.W.: 1296.7 g/mol

R_f (ethyl acetate / isooctane: 8 / 2) = 0.25

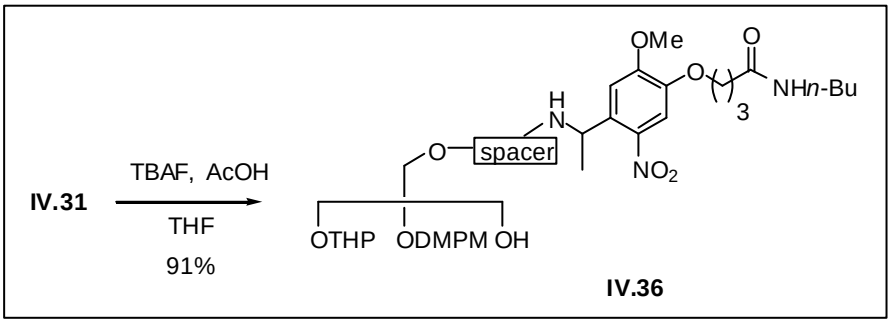
IR (film) 3313 (w), 2930 (s), 2862 (s), 1735 (m), 1647 (s), 1519 (s), 1426 (w), 1364 (w), 1271 (s), 1215 (m), 1174 (m), 1091 (s), 1026 (s), 812 (m), 704 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.59 (4H, dd, J = 7.0, 1.2 Hz), 7.53 (1H, s), 7.37 (6H, m), 7.13 (12 H, m), 6.92 (1H, s), 6.42 (1H, d, J = 7.0 Hz), 5.63 (1H, m), 5.52 (1H, dq, J = 7.0, 6.9 Hz), 4.59 (1H, m), 4.41 (6H, m), 4.19 (1H, ABd, J_{AB} = 10.8 Hz), 4.11 (1H, ABd, J_{AB} = 10.8 Hz), 4.05 (2H, m), 3.93 (1H, m), 3.91 (3H, s), 3.82 (4H, t J = 7.0 Hz), 3.76 (1H, m), 3.59 (1H, m), 3.46 (7H, m), 3.23 (2H, dt, J = 7.0, 6.0 Hz), 2.89 (2H, t, J = 7.0 Hz), 2.83 (2H, t, J = 6.3 Hz), 2.82 (2H, t, J = 6.3 Hz), 2.53 (1H, m), 2.43 (1H, m), 2.35 (4H, m), 2.15 (2H, m), 1.80 (1H, m), 1.69 (1H, m), 1.60 (1H, s), 1.56 (4H, m), 1.51 (3H, d, J = 6.9 Hz), 1.44 (2H, m), 1.31 (2H, m), 1.02 (9H, s), 0.90 (3H, t, J = 7.3 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 172.9 (C), 172.1 (C), 170.9 (C), 153.7 (C), 146.7 (C), 140.2 (C), 138.3 (C), 138.2 (C), 136.5 (C), 136.3 (C), 135.5 (CH) 133.7 (C), 129.5 (CH), 129.1 (CH), 128.6 (CH), 127.6 (CH), 127.4 (CH), 127.3 (CH), 109.8 (CH), 109.7 (CH), 98.7 (CH), 73.2 (CH_2), 72.9 (CH_2), 68.9 (CH_2), 68.4 (CH_2), 68.2 (CH_2), 65.1 (CH_2), 63.4 (CH_2), 62.2 (CH_2), 56.3 (CH_3), 47.2 (CH), 44.6 (C), 39.3 (CH_2), 38.9 (CH_2), 36.0 (CH_2), 32.8 (CH_2), 31.7 (CH_2), 30.8 (CH_2), 30.7 (CH_2), 29.7 (CH_2), 29.2 (CH_2), 26.8 (CH_3), 25.4 (CH_2), 24.9 (CH_2), 21.4 (CH_3), 20.0 (CH_2), 19.5 (CH_2), 19.1 (CH_2), 13.7 (CH_3) ppm.

ES-MS: m/z 1318 $[M + Na]^+$

Deprotection of the TBDPS protecting group in compound **IV.31**: synthesis of **IV.36**



To a solution of **IV.31** (25 mg, 0.017 mmol) in dry tetrahydrofuran (0.2 ml) were added acetic acid (1 drop) and tetrabutylammonium fluoride (1 M in tetrahydrofuran) (52 µl, 0.052 mmol). The mixture was stirred overnight at room temperature. After extraction with water, the combined organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (ethyl acetate / isooctane: 8 / 2) to afford 18.5 mg of alcohol **IV.36** (90%).

Bruto formula: $C_{67}H_{89}N_3O_{17}$

M.W.: 1208.4 g/mol

R_f (ethyl acetate / isooctane: 8 / 2) = 0.15

IR (film) 3325 (w), 2926 (s), 2866 (s), 1733 (m), 1650 (s), 1517 (s), 1452 (w), 1364 (w), 1268 (s), 1215 (w), 1158 (m), 1090 (s), 1029 (s), 802 (m), 604 (m) cm^{-1}.

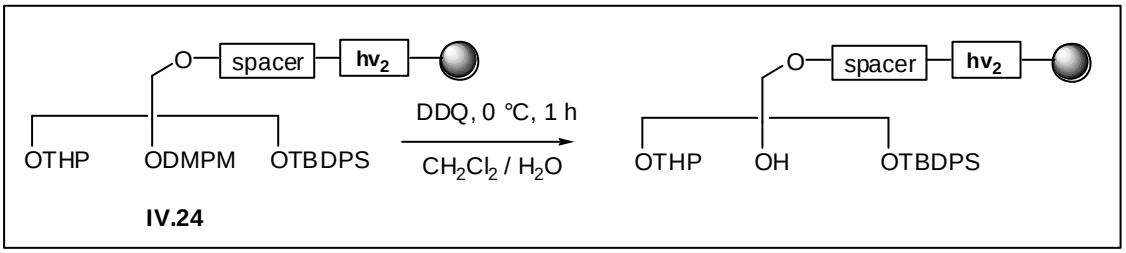
^{1}H NMR (500 MHz, $CDCl_3$) δ 7.53 (1H, s), 7.17 (12 H, m), 6.92 (1H, s), 6.83 (3H, m), 6.43 (1H, d, J = 7.0 Hz), 5.63 (1H, m), 5.52 (1H, dq, J = 7.0, 6.9 Hz), 4.59 (1H, m), 4.46 (2H, m), 4.43 (2H, s), 4.40 (2H, s), 4.38 (4H, m), 4.19 (1H, ABd, J_{AB} = 10.8 Hz), 4.11 (1H, ABd, J_{AB} = 10.8 Hz), 4.04 (2H, m), 3.93 (1H, m), 3.91 (3H, s), 3.87 (3H, s), 3.84 (3H, s), 3.83 (2H, t J = 7.0 Hz), 3.77 (1H, m), 3.66 (1H, dt, J = 7.0, 2.5 Hz), 3.60 (1H, m), 3.46 (6H, m); 3.23 (2H, dt, J = 7.0, 6.0 Hz), 2.90 (4H, m), 2.83 (2H, m), 2.53 (1H, m), 2.44 (1H, m), 2.35 (4H, m), 2.15 (2H, m), 1.80 (1H, m), 1.69 (1H, m), 1.60 (1H, s), 1.57 (4H, m), 1.51 (3H, d, J = 6.9 Hz), 1.46 (2H, m), 1.32 (2H, m), 0.90 (3H, t, J = 7.3 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) δ 172.9, 172.0, 170.8, 155.3, 148.8, 148.4, 146.7, 140.2, 138.2, 137.9, 136.5, 136.3, 134.3, 130.8, 128.9, 127.6, 127.4, 120.1, 110.8, 110.7, 109.8, 98.7, 72.9, 72.8, 70.9, 68.7, 68.3, 63.4, 62.3, 56.3, 55.8, 55.7, 44.4, 39.3, 38.7, 35.9, 32.8, 21.6, 30.8, 29.7, 25.4, 24.9, 20.0, 19.5, 13.7 ppm.

ES-MS: m/z 1230 $[M + Na]^+$

Synthesis of acetate **IV.37**

Selective deprotection of the DMPM protective group:

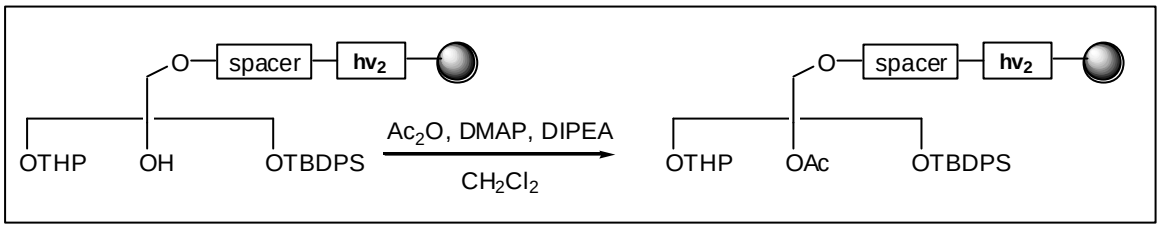


To a cooled suspension (0 °C) of resin **IV.24** (100 mg, 0.0163 mmol, 0.163 mmol/g) in a mixture of dichloromethane / water (1.4 ml / 0.1 ml) was added dichlorodicyanoquinone (7.2 mg, 0.0315 mmol). The mixture was shaken for 1 h at 0 °C. After filtration, the resin was washed with an aqueous saturated solution of sodium bicarbonate (3 x 5 ml), phenol / methanol: 1 / 1 (3 x 5 ml),

toluene (3 x 5 ml), acetonitrile (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml). The resin was then dried for 4 h at the oil pump.

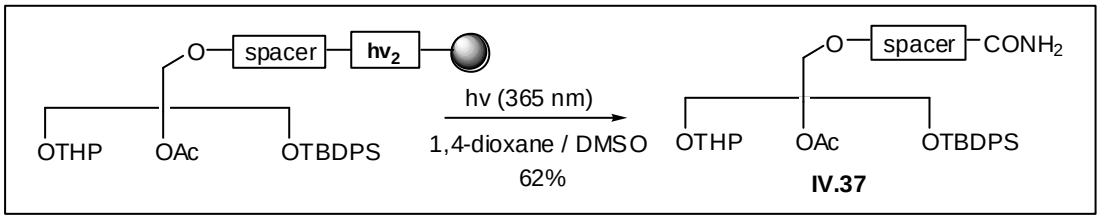
^{13}C NMR (50 MHz, CD_2Cl_2) δ 174.2, 173.3, 172.2, 155.5, 148.5, 141.9, 140.1, 138.0, 137.1, 136.6, 135.3, 131.2, 130.8, 130.7, 130.4, 129.2, 129.1, 110.9, 100.2, 75.2, 74.7, 73.2 (polyethylene glycol peak), 66.8, 64.8, 63.5, 57.8, 46.1, 40.8, 40.5, 37.5, 33.9, 32.3, 32.1, 28.2, 27.1, 26.2, 23.1, 21.1, 20.6 ppm.

Capping of the hydroxyl function as the acetate:



A solution of acetic anhydride (16 µl, 0.174 mmol), diisopropylethylamine (31 µl, 0.174 mmol) and 4-dimethylaminopyridine (21.3 mg, 0.174 mmol) in dichloromethane (2 ml) was added to the DMPM deprotected resin. The suspension was shaken for (2 x 20 min). The solid phase was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml) and dried for 4 h under vacuum.

Cleavage from the resin: Synthesis of acetate **IV.37**



A sample of the DMPM deprotected resin (48 mg, 0.008 mmol, 0.166 mmol/g) in suspension in 1 ml of 1,4-dioxane and 10 µl of dimethylsulfoxide was exposed to UV light (365 nm) for 3 times 1 h and 3 times 2 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethylsulfoxide with the Kugelrhor, the

sample was purified by column chromatography (ethyl acetate / isooctane: 8 / 2) to provide 5.1 mg of acetate **IV.37** (62%).

Bruto formula : $C_{59}H_{75}O_{11}NSi$

M.W.: 1002.2 g/mol

R_f (ethyl acetate) = 0.55

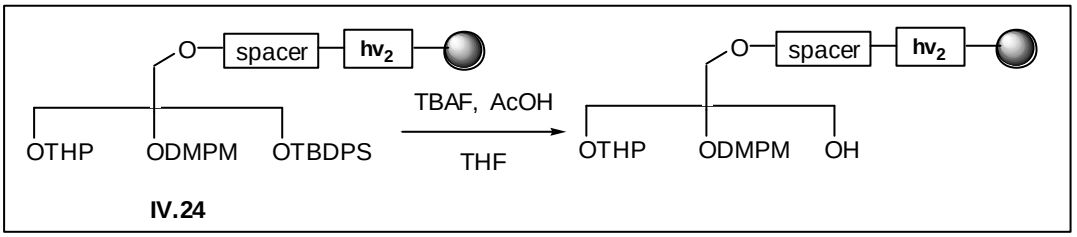
IR (film) 2939 (m), 2857 (m), 1737 (s), 1676 (m), 1514 (w), 1427 (w), 1362 (w), 1239 (w), 1089 (s), 1031 (m), 703 (m) cm^{-1}.

^1H NMR (500 MHz, CD_3OD) δ 7.52 (dd, 4H, J = 8.0, 1.4 Hz), 7.35 (6H, m), 7.17 (10H, m), 7.10 (2H, d, J = 8.0 Hz), 4.55 (1H, m), 4.42 (2H, s), 4.39 (4H, s), 4.21 (2H, t, J = 6.9 Hz), 4.14 (2H, s), 3.86 (1H, dt, J = 9.6, 7.0 Hz), 3.81 (2H, t, J = 6.5 Hz), 3.66 (1H, m), 3.57 (1H, dt, J = 9.6, 7.0 Hz), 3.46 (2H, s), 3.45 (2H, s), 3.44 (2H, s), 3.39 (1H, m), 2.87 (2H, t, J = 7.0 Hz), 2.83 (2H, t, J = 7.0 Hz), 2.81 (2H, t, J = 6.6 Hz), 2.47 (2H, m), 2.41 (2H, m), 1.97 (3H, s), 1.76 (1H, m), 1.63 (1H, m), 1.54-1.47 (4H, m), 0.96 (9H, s) ppm.

ES-MS m/z 1025 $[M + Na]^+$

Synthesis of acetate **IV.38**

Selective deprotection of the TBDPS protective group:



First method: TBAF in presence of acetic acid

Acetic acid (5 µl, 0.087 mmol), tetrabutylammonium fluoride (87 µl, 0.087 mmol) and molecular sieves (400 pm) were added to a suspension of resin **IV.24** (100 mg, 0.0163 mmol, 0.163 mmol/g) in dry tetrahydrofuran (2 ml). The mixture was shaken overnight at room temperature. The reaction was repeated overnight. After filtration, the resin was washed with ethanol (3 x 5 ml), dichloromethane (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml) and was dried for 4 h under vacuum.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 174.5, 173.2, 172.1, 155.3, 148.3, 141.7, 140.0, 139.9, 137.9, 136.3, 132.6, 130.2, 128.9, 121.4, 112.5, 110.7, 100.0, 73.2 (polyethylene glycol peak), 65.1, 63.4, 57.7, 57.0, 47.6, 47.3, 45.9, 40.4, 37.4, 33.7, 32.1, 26.4, 22.9, 20.9 ppm.

Second method: Ammonium fluoride

To a suspension of resin **IV.24** (0.100 g, 0.0163 mmol, 0.163 mmol/g) in dry methanol (1 ml) was added ammonium fluoride (14.5 mg, 0.392 mmol). The mixture was shaken overnight. The reaction was repeated again overnight and two times 4 h to get complete deprotection. After filtration, the resin was washed with methanol (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml). A sample of the resin (2 mg, 0.34 μmol, 0.170 mmol/g) was suspended in 100 μl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analysed by ES-MS.

M.W.: 871 g/mol

ES-MS: m/z 894 $[M + Na]^+$

Third method: 70% hydrogen fluoride/pyridine

To a suspension of resin **IV.24** (12.0 mg, 1.93 μmol, 0.163 mmol/g) in dry tetrahydrofuran (100 μl) was added a solution of 70% hydrogen fluoride/pyridine (5 μl, 33.6 μmol). The suspension was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml). A sample of the resin (2 mg, 0.34 μmol, 0.170 mmol/g) was suspended in 100 μl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analysed by ES-MS.

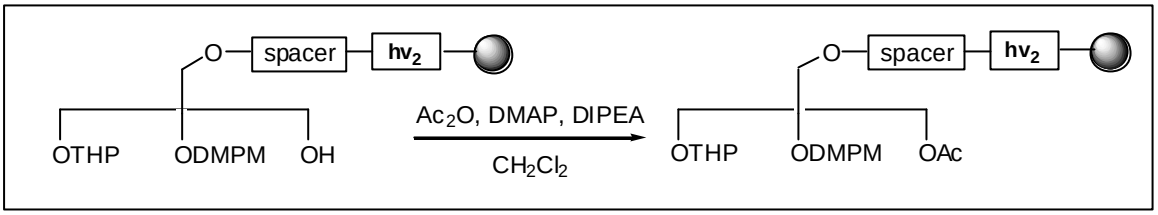
ES-MS: m/z 894 $[M + Na]^+$

Fourth method: TASF (tris-(dimethylamino)sulfur trimethylsilyldifluoride)

To a suspension of resin (10.0 mg, 1.31 μmol, 0.131 mmol/g) in dry tetrahydrofuran (100 μl) was added 0.0975 M TASF in dimethylformamide (86.1 μl, 8.4 μmol). The suspension was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml). A sample of the resin (2 mg, 0.4 μmol, 0.170 mmol/g) was suspended in 100 μl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

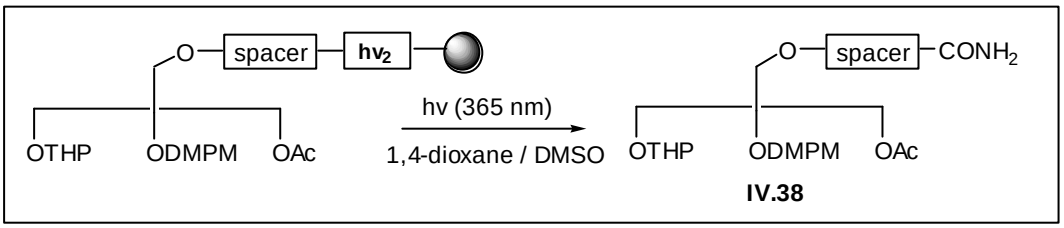
ES-MS: m/z 894 $[M + Na]^+$

Capping of the hydroxyl function as the acetate:



A solution of acetic anhydride (16 µl, 0.174 mmol), diisopropylethylamine (31 µl, 0.174 mmol) and 4-dimethylaminopyridine (21.3 mg, 0.174 mmol) in dichloromethane (2 ml) was added to the TBDPS deprotected resin (0.100 g, 0.0170 mmol, 0.170 mmol/g). The suspension was shaken for 20 min (2 times). The solid phase was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml) and dried for 4 h under vacuum.

Cleavage from the resin: synthesis of acetate **IV.38**



A sample of TBDPS deprotected resin (75 mg, 0.013 mmol, 0.170 mmol/g) in suspension in 1.5 ml of 1,4-dioxane with 15 µl of dimethylsulfoxide was exposed to UV light (365 nm) for 3 times 2 h and 2 times 1 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethylsulfoxide with the Kugelrhor, the sample was purified by column chromatography (ethyl acetate / isooctane: 8 / 2) to provide 6.5 mg of acetate **IV.38** (56%).

Bruto formula : $C_{52}H_{67}O_{13}N$

M.W.: 914.0 g/mol

R_f (ethyl acetate) = 0.55

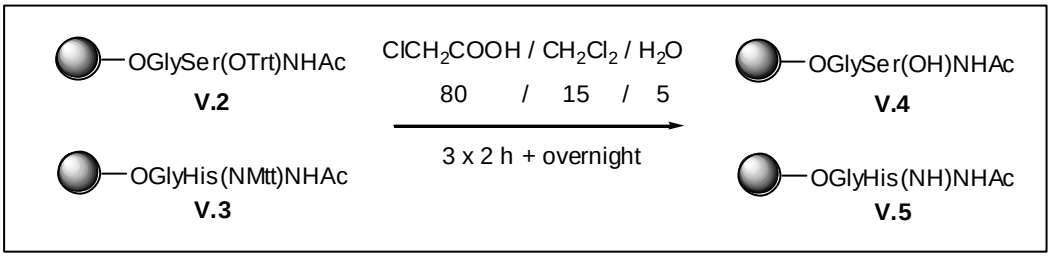
IR (film) 2924 (s), 2855 (s), 1737 (s), 1682 (m), 1515 (m), 1463 (w), 1419 (w), 1363 (w), 1264 (s), 1239 (m), 1158 (w), 1092 (s), 1030 (s), 737 (s) cm^{-1}.

^{1}H NMR (500 MHz, CD_3OD) δ 7.17 (12H, m), 6.83 (3H, m), 4.55 (1H, m), 4.42 (2H, s), 4.40 (6H, s), 4.21 (2H, t, J = 7.1 Hz), 4.13 (2H, s), 3.86 (1H, dt, J = 9.6, 7.0 Hz), 3.79 (3H, s), 3.72 (3H, s), 3.66 (2H, t, J = 6.8 Hz), 3.66 (1H, m), 3.57 (1H, dt, J = 9.6, 7.0 Hz), 3.45 (6H, m), 3.39 (1H, m), 2.88 (2H, t, J = 7.0 Hz), 2.86 (2H, t, J = 4.6 Hz), 2.83 (2H, t, J = 6.8 Hz), 2.49 (2H, m), 2.38 (2H, m), 1.97 (3H, s), 1.75 (1H, m), 1.64 (1H, m), 1.52-1.48 (4H, m) ppm.

ES-MS: m/z 931 $[M + NH_4]^+$

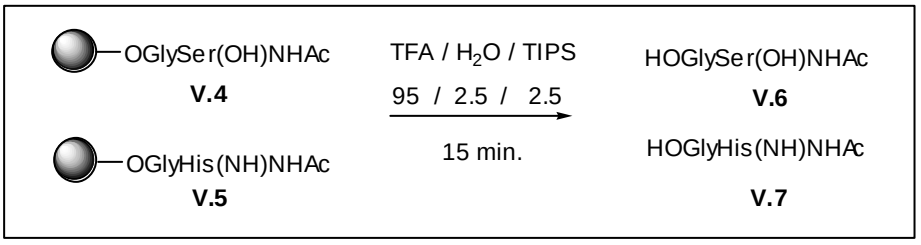
7.5 Experimental part of part 5

Deprotection of the side-chain of the amino acids Ser(OTrt) and His(NMtt)



The resin **V.2** and **V.3** (100 mg, 0.11 mmol, 1.1 mmol/g) were treated with 2 ml of a solution of chloroacetic acid / dichloromethane / water (80 / 15 / 5) overnight. After filtration, the procedure was repeated three times 2 h to obtain the complete deprotection of the side-chain of serine and histidine. The deprotection was monitored by thin layer chromatography in chloroform / acetic acid (98 / 2). The presence of trityl and methyltrityl can be easily detected on thin layer chromatography due to their characteristic yellow colour.

Cleavage of the dipeptide **V.4** and **V.5** from the resin



Resins **V.4** and **V.5** (100 mg, 0.11 mmol, 1.1 mmol/g) were treated with 2 ml of a solution of trifluoroacetic acid / water / triisopropylsilane (95 / 2.5 / 2.5) for 15 min. The resin was washed

with dichloromethane. The solution was evaporated under reduced pressure and dried under vacuum for 2 h. Analysis by ^{1}H NMR (500 MHz) spectroscopy concluded to a complete deprotection of the side-chain of the amino acids serine and histidine.

Spectral data of **V.6**

R_f (methanol / dichloromethane: 1 / 9) = 0.12

^{1}H NMR (500 MHz, CD_3OD) δ 4.49 (1H, t, J = 5.2 Hz), 3.95 (2H, s), 3.81 (2H, d, 5.2 Hz), 2.05 (3H, s) ppm.

Remark: No ES-MS has been recorded due to difficult protonation of **V.6**. The complete deprotection was only proved by ^{1}H NMR (500 MHz).

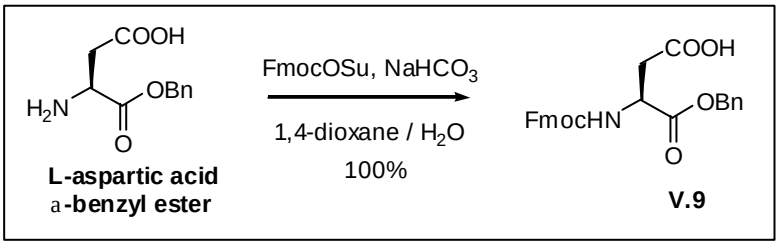
Spectral data of **V.7**

R_f (methanol / dichloromethane: 1 / 9) = 0.10

^{1}H NMR (500 MHz, CD_3OD) δ 8.80 (1H, s), 7.39 (1H, s), 4.79 (1H, t, J = 6.6 Hz), 4.00 (1H, ABd, J_{AB} = 17.7 Hz), 3.90 (1H, ABd, J_{AB} = 17.8 Hz), 3.29 (1H, m), 3.12 (1H, m), 1.99 (3H, s) ppm.

ES-MS: m/z 277 $[M + Na]^+$

Synthesis of N-(9-fluorenylmethoxycarbonyl)-L-aspartic benzyl ester **V.9**



A mixture of the commercially available L-aspartic acid α-benzyl ester (0.2 g, 0.89 mmol), N-(9-fluorenylmethoxycarbonyloxy)succinimide (0.31 g, 0.91 mmol) and sodium bicarbonate (0.226 g, 2.69 mmol) in a mixture of 1,4-dioxane (6.5 ml) / water (6.5 ml) was stirred overnight at room temperature. The mixture was cooled to 0 °C, acidified to pH = 3 with a solution of 1 N hydrochloric acid and was diluted to 25 ml with water. The mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated

under reduced pressure. The residue was purified by recrystallization from a mixture pentane/dichloromethane (9/1) to provide 0.4 g of Fmoc-protected amino acid **V.9** (100%).

Bruto formula: $C_{26}H_{23}NO_6$

M.W.: 445.4 g/mol

m.p.: 87 °C

R_f (dichloromethane / methanol / acetic acid: 9 / 0.8 / 0.2) = 0.64

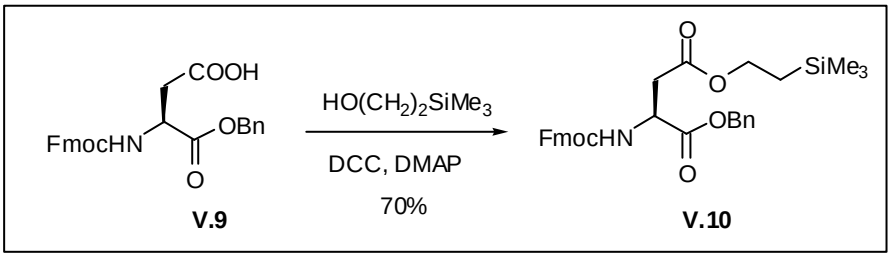
IR (film) 3415 (s), 2953 (m), 1715 (s), 1652 (m), 1511 (m), 1409 (m), 1339 (m), 1205 (m), 1041 (w), 751.1 (m), 689 (w) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, J = 7.6 Hz), 7.58 (2H, d, J = 7.1 Hz), 7.40 (2H, dd, J = 7.4 Hz), 7.32 (5H, m), 7.31 (2H, m), 5.81 (1H, d, J = 8.3 Hz), 5.20 (1H, ABd, J_{AB} = 12.6 Hz), 5.19 (1H, ABd, J_{AB} = 12.6 Hz), 4.71 (1H, dt, J = 8.3, 4.4 Hz), 4.42 (1H, ABdd, J_{AB} = 10.1, 7.2 Hz), 4.36 (1H, ABdd, J_{AB} = 10.1, 7.2 Hz), 4.22 (1H, t, J = 7.1 Hz), 3.13 (1H, ABdd, J_{AB} = 17.6, 4.6 Hz), 2.95 (1H, ABdd, J_{AB} = 17.6, 4.6 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 175.9 (C), 170.4 (C), 156.1 (C), 143.7 (C), 143.5 (C), 141.2 (C), 134.9 (C), 128.5 (CH), 128.1 (CH), 127.7 (CH), 127.0 (CH), 125.0 (CH), 119.9 (CH), 67.7 (CH_2), 67.35 (CH_2), 50.2 (CH), 46.9 (CH), 36.3 (CH_2) ppm.

ES-MS: m/z 468 $[M + Na]^+$

Synthesis of diester **V.10**



To a cooled (0 °C) solution of carboxylic acid **V.9** (2 g, 4.50 mmol), 2-trimethylsilylethanol (0.645 ml, 4.50 mmol) and 4-dimethylaminopyridine (275 mg, 2.25 mmol) was added 1,3-dicyclohexylcarbodiimide (0.93 g, 4.50 mmol). The mixture was stirred at 0 °C for 2 h and the solution was fitrated over celite. The organic phase was washed with an aqueous solution of 1 N hydrochloric acid and with water and was dried over anhydrous magnesium sulfate. After

filtration and evaporation under reduced pressure, the residue was purified by column chromatography (ethyl acetate / isooctane: 1 / 9) to provide 1.7 g of diester **V.10** (70%).

Bruto formula: $C_{31}H_{35}NO_6Si$

M.W.: 545.7 g/mol

m.p.: 82 °C

R_f (ethyl acetate / isooctane: 1 / 1) = 0.61

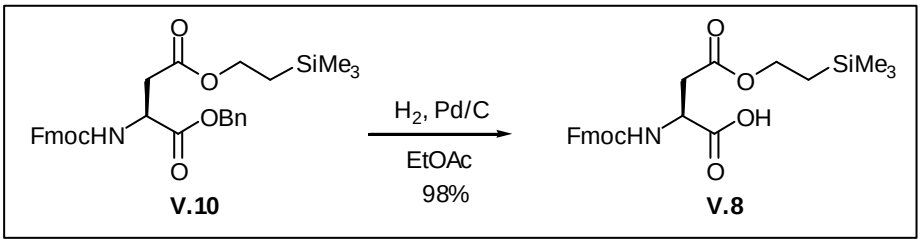
IR (film) 2954 (w), 1728 (s), 1517 (m), 1450 (m), 1414 (w), 1354 (w), 1250 (m), 1177 (w), 1057 (m), 936 (w), 859 (s), 838 (s), 759 (s), 740 (s) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J = 7.5 Hz), 7.60 (2H, d, J = 7.5 Hz), 7.40 (2H, dd, J = 7.5 Hz), 7.32 (5H, m), 7.31 (2H, m), 5.87 (1H, d, J = 8.6 Hz), 5.28 (1H, ABd, J_{AB} = 12.2 Hz), 5.16 (1H, ABd, J_{AB} = 12.2 Hz), 4.70 (1H, dt, J = 8.6, 4.4 Hz), 4.43 (1H, ABdd, J_{AB} = 10.5, 7.3 Hz), 4.36 (1H, ABdd, J_{AB} = 10.5, 7.3 Hz), 4.22 (1H, dd, J = 7.2 Hz), 4.15 (2H, m), 3.06 (1H, ABdd, J_{AB} = 17.2, 4.4 Hz), 2.86 (1H, ABdd, J_{AB} = 17.2, 4.4 Hz), 0.95 (2H, m), 0.04 (9H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 170.9 (C), 170.6 (C), 155.9 (C), 143.6 (C), 143.5 (C), 141.2 (C), 135.1 (C), 128.5 (CH), 128.1 (CH), 127.6 (CH), 127.0 (CH), 125.0 (CH), 119.9 (CH), 67.7 (CH_2), 67.2 (CH_2), 63.3 (CH_2), 50.4 (CH), 46.9 (CH), 36.6 (CH_2), 17.1 (CH_2), -1.6 (CH_3) ppm.

ES-MS: m/z 563 $[M + Na]^+$

Synthesis of N-(9-fluorenylmethoxycarbonyl)-trimethylsilylester L-aspartic **V.8**



To ester **V.10** (2.05 g, 3.760 mmol) in distilled ethyl acetate (30 ml) was added 10% palladium on activated carbon (0.4 g, 0.376 mmol). The mixture was stirred overnight at room temperature and was filtered over celite. The organic phase was evaporated under reduced pressure. The residue was purified by recrystallization from dichloromethane / pentane (1 / 9) affording 1.65 g of protected amino acid **V.8** (98%).

Bruto formula: $C_{24}H_{29}NO_6Si$

M.W.: 455.6 g/mol

m.p.: 82 °C

R_f (dichloromethane / methanol / acetic acid: 9 / 0.8 / 0.2) = 0.64

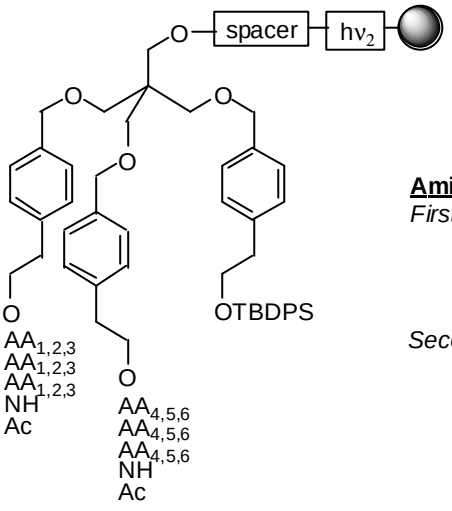
IR (film) 3352 (m), 2951 (m), 1729 (s), 1501 (w), 1447 (m), 1381 (w), 1337 (w), 1243 (m), 1210 (w), 1040 (m), 853 (m), 737 (m) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, J = 7.5 Hz), 7.60 (2H, dd, J = 7.3, 3.3 Hz), 7.40 (2H, dd, J = 7.4 Hz), 7.31 (2H, dd, J = 7.4 Hz), 5.87 (1H, d, J = 8.5 Hz), 4.69 (1H, ddd, J = 4.5 Hz), 4.45 (1H, ABdd, J_{AB} = 10.5, 7.3 Hz), 4.37 (1H, ABdd, J_{AB} = 10.5, 7.3 Hz), 4.22 (3H, m), 3.08 (1H, ABdd, J_{AB} = 17.5, 4.4 Hz), 2.87 (1H, ABdd, J_{AB} = 17.5, 4.4 Hz), 1.01 (2H, m), 0.04 (9H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 175.9 (C), 171.3 (C), 156.1, (C), 143.7 (C), 143.5 (C), 141.2 (C), 127.7 (CH), 127.0 (CH), 125.1 (CH), 119.9 (CH), 67.4 (CH_2), 63.7 (CH_2), 50.2 (CH), 47.0 (CH), 36.5 (CH_2), 17.2 (CH_2), -1.6 (CH_3) ppm.

ES-MS: m/z 478 $[M + Na]^+$

Synthesis of a 81-members dipodal library with the IRORI radio-frequency tags encoding system



V.11

Amino acids:
First strand: $AA_{1,2,3}$ = FmocSer(OTrt)OH
FmocPheOH
FmocValOH

Second strand: $AA_{4,5,6}$ = FmocHis(NMtt)OH
FmocAlaOH
FmocLeuOH

? Definition of building blocks: The building blocks (all different amino acids) were defined in the software 'ACCUTAG SYNTHESIS MANAGER'.

? Plan step: The number of steps was introduced (5 in our case) with explanation of each coupling step and a possible comment.

Step 1: coupling Val, Phe, Ser(OTrt)

Step 2: coupling Val, Phe, Ser(OTrt)

Step 3: coupling Glycine

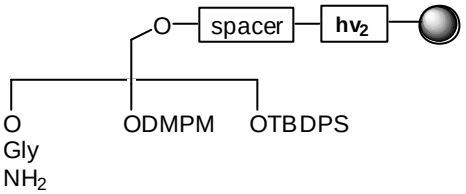
Step 4: coupling His(NMtt), Leu, Ala

Step 5: coupling His(NMtt), Leu, Ala

? Perform synthesis:

Solid-phase bound scaffold **IV.26** (0.45 g, 0.086 mmol, 0.19 mmol/g) was prepared according to the previously described procedures and was divided into 81-microkans (number of microkans = number of compounds in the library). In each microkan was introduced 5.5 mg of resin and a radio frequency tag, which encodes for one compound of the library. In the sorting step, each kan was placed on the reading station, the tag was read and the compound was sorted into the proper reactor location.

At this stage all microkans were divided in three vessels in which three different amino acids (step 1) were coupled: Val (vessel 1), Phe (vessel 2) and Ser (vessel 3). Each vessel after sorting contained 27 microkans.



1-Synthesis of the first strand

General procedure for the coupling reactions

To each vessel containing 27 microkans (148.5 mg, 0.030 mmol, 0.20 mmol/g) in dimethylformamide (25 ml) were added 3% N-methylmorpholine (0.75 ml), 1H-benzotriazol-1-yloxy-tris(pyrrolidino)phosphoniumhexafluorophosphate (PyBOP) (58 mg, 0.111 mmol) and FmocValOH (38 mg, 0.111 mmol) or FmocPheOH (43.2 mg, 0.111 mmol) or FmocSer(OTrt)OH (64 mg, 0.111 mmol). The suspension was stirred for 4-5 h. After filtration, the resin was washed for

15 min with dimethylformamide (4 x 20 ml). To control the completeness of the coupling, one microkan of each vessel was opened and few beads were submitted to **NF31** and TNBS tests. White beads were observed proving a complete coupling.

General procedure for Fmoc deprotection

The resin in each vessel was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). After filtration, the resin was washed for 15 min with dimethylformamide (4 x 20 ml). One microkan of each vessel was opened and few beads were submitted to **NF31** and TNBS tests and red and orange beads were observed respectively.

Sorting

Each kan was placed on the reading station and was sorted for the next coupling. Each radio-frequency tag was read and each kan was placed in the desired vessel.

Coupling of Val, Phe, Ser

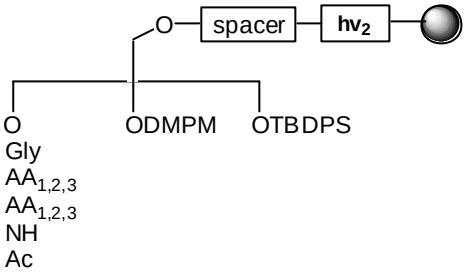
The coupling was performed as described previously.

Deprotection

The Fmoc protecting group was removed as previously. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. Red and orange beads were observed respectively.

Capping of the free terminal amino function

To each vessel containing 27 microkans (148.5 mg, 0.030 mmol, 0.20 mmol/g) in dichloromethane (30 ml) was added acetylimidazole (328 mg, 2.98 mmol). The mixture was stirred overnight. After filtration, the resin was washed with dichloromethane (1 x 25 ml) and dimethylformamide (2 x 30 ml) for 15 min. The first strand was synthesised (figure 7.1). One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. White beads were observed.



2-Synthesis of the second strand

The three following steps are identical for all members of the library and were performed in one vessel.

Deprotection of DMPM-protecting group

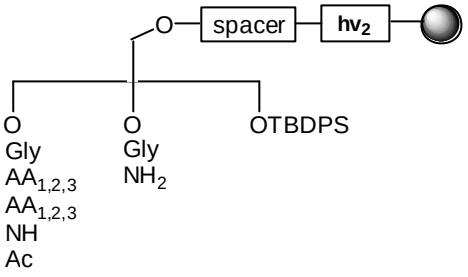
To a vessel containing 81 microkans (445.5 mg, 0.089 mmol, 0.19 mmol/g) in a 19/1 ratio of dichloromethane (95 ml) / water (5 ml) was added at 0 °C dichlorodicyanoquinone (318 mg, 1.400 mmol). The mixture was stirred for 2 h at 0 °C. After filtration, the resin was washed for 15 min with a solution of phenol/methanol (1/1), a saturated aqueous solution of sodium bicarbonate, acetonitrile, toluene, dimethylformamide, methanol and dichloromethane.

Coupling of FmocGlyCl

To a vessel containing 81 microkans (445.5 mg, 0.085 mmol, 0.19 mmol/g) in dichloromethane (90 ml) were added FmocGlyCl (1.5 g, 4.73 mmol) and diisopropylethylamine (824 µl, 4.73 mmol). The mixture was stirred at room temperature overnight. The procedure was repeated three times 6 h. After filtration, the resin was washed for 15 min with dichloromethane (1 x 25 ml) and dimethylformamide (2 x 30 ml).

Deprotection

The Fmoc protecting group was removed as previously. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. Red and orange beads were observed respectively.



Sorting

At this stage, each kan was placed on the reading station and was sorted for the next coupling. Each radio-frequency tag was read and each kan was placed in the desired vessel.

Coupling of His, Leu, Ala

To a vessel containing 27 microkans (148.5 mg, 0.028 mmol, 0.19 mmol/g) in dimethylformamide (25 ml) were coupled FmocHis(NMtt)OH (70.4 mg, 0.111 mmol), FmocLeuOH (39.2 mg, 0.111 mmol) and FmocAlaOH (34.6 mg, 0.111 mmol) in vessel 1, vessel 2 and vessel 3 respectively. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. White beads were observed.

Deprotection

The Fmoc protecting group was removed. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. Red and orange beads were observed respectively.

Sorting

Each kan was placed on the reading station and was sorted for the next coupling. Each radio-frequency tag was read and each kan was located in the desired vessel.

Coupling of His, Leu, Ala

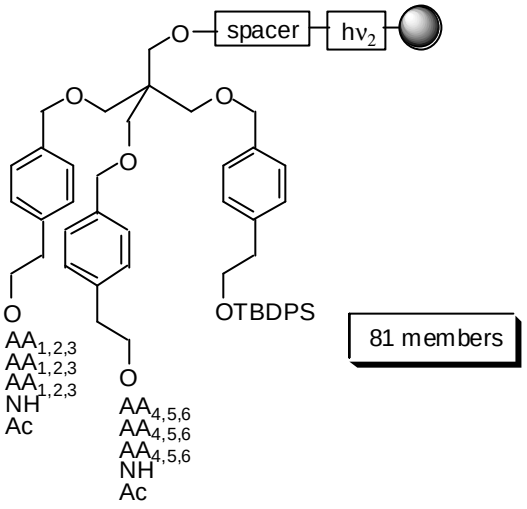
The next amino acid was coupled as previously. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. White beads were observed proving a complete coupling.

Deprotection

The Fmoc protecting group was removed. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. Red and orange beads were observed respectively.

Capping of the free terminal amino function

The free amino function was capped as described previously with acetylimidazole to obtain resin **V.1**. One microkan of each vessel was opened and a few beads were submitted to **NF31** and TNBS tests. White beads were observed.

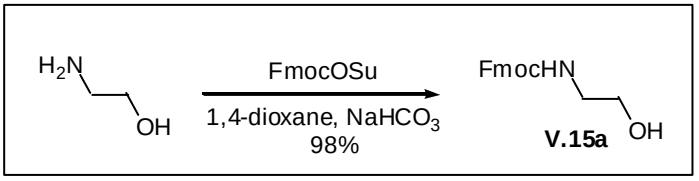


V.11

Deprotection of trityl and methyltrityl protecting groups on serine and histidine

A vessel containing 81 microkans (445.5 mg, 71.3 mmol, 0.16 mmol/g) was treated with 100 ml of a solution of chloroacetic acid (132 g, 1.4 mol) in a mixture of water (25 ml) / dichloromethane (75 ml) overnight at room temperature. The procedure was repeated for 4 h. After filtration, the resin was washed with dimethylformamide (2 x 100 ml) and with dichloromethane (2 x 100 ml).

Protection of the amino function as Fmoc in **V.15a**



V.15a

To a solution of 2-aminoethanol (2 g, 32.8 mmol) in 520 ml of a mixture of 1,4-dioxane / water (1 / 1) were added N-(9-fluorenylmethoxycarbonyloxy) succinimide (11.1 g, 33.0 mmol) and sodium bicarbonate (8.25 g, 98.1 mmol). The mixture was stirred overnight at room temperature. After cooling to 0 °C, the solution was acidified to pH = 3 with an aqueous solution of 1 N hydrochloric acid. After dilution to 100 ml with water, the aqueous phase was extracted with dichloromethane. The combined organic phases were dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The residue was recrystallized in a mixture dichloromethane/pentane (1/10), filtered and dried under vacuum providing 9.1 g of **V.15a** as a white powder (98%).

Bruto formula: $C_{17}H_{17}NO_3$

M.W.: 283.3 g/mol

m.p.: 138 °C

R_f (ethyl acetate / isooctane: 1 / 1) = 0.15

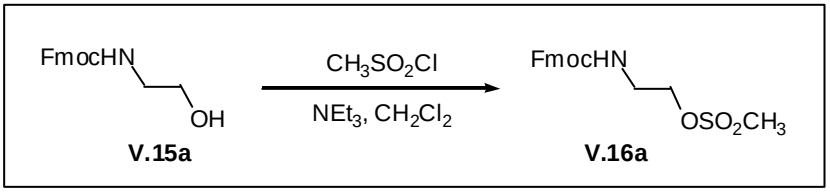
IR (film) 3476 (w), 3352 (m), 2940 (w), 1676 (s), 1542 (s), 1450 (m), 1272 (m), 1147 (w), 1069 (m), 761 (m), 740 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J = 7.5 Hz), 7.60 (2H, d, J = 7.5 Hz), 7.40 (2H, dd, J = 7.5 Hz), 7.32 (2H, dd, J = 7.5 Hz), 5.25 (1H, s), 4.43 (2H, d, J = 6.5 Hz), 4.21 (1H, t, J = 6.5 Hz), 3.70 (2H, m), 3.34 (2H, m), 2.39 (1H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$ + few drops DMSO-d_6) + DEPT δ 156.7 (C), 143.8 (C), 141.0 (C), 127.4 (CH), 126.8 (CH), 124.8 (CH), 119.7 (CH), 66.1 (CH_2), 61.4 (CH_2), 47.0 (CH_2), 43.5 (CH) ppm.

ES-MS: m/z 306 $[M + Na]^+$, 588 $[2M + Na]^+$

Formation of mesylate **V.16a**



Mesylchloride (0.82 ml, 10.6 mmol) and triethylamine (2.95 ml, 21.2 mmol) were added to a cooled (0 °C) solution of alcohol **V.15a** (2 g, 7.07 mmol) in dichloromethane (60 ml). The mixture was allowed to warm to room temperature and to react for one hour. After dilution in ethyl acetate, the mixture was extracted with brine. The organic phase was dried over anhydrous magnesium

sulfate, filtered and evaporated under reduced pressure. The residue was purified by column chromatography (ethyl acetate / isooctane: 1 / 1) to give 2.3 g of mesylate **V.16a** (90%).

Bruto formula: $C_{18}H_{19}NO_5S$

M.W.: 361.4 g/mol

m.p.: 104 °C

R_f (ethyl acetate / isooctane: 1 / 1) = 0.22

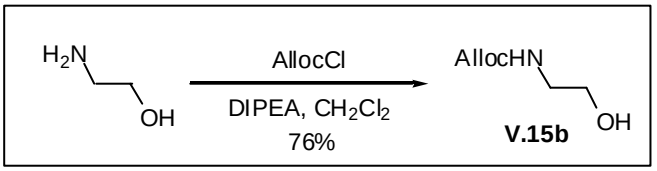
IR (film) 3476 (w), 3352 (m), 2940 (w), 1676 (s), 1542 (s), 1450 (m), 1272 (m), 1147 (w), 1069 (m), 761 (m), 740 (m) cm^{-1}.

^{1}H NMR (500 MHz, $CDCl_3$) δ 7.77 (2H, d, J = 7.5 Hz), 7.60 (2H, d, J = 7.5 Hz), 7.40 (2H, dd, J = 7.5 Hz), 7.32 (2H, dd, J = 7.5 Hz), 5.25 (1H, s), 4.43 (2H, d, J = 6.5 Hz), 4.30 (2H, t, J = 4.8 Hz), 4.21 (1H, t, J = 6.5 Hz), 3.70 (2H, m), 3.34 (2H, m), 2.39 (1H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 156.3 (C), 143.7 (C), 141.3 (C), 127.7 (CH), 127.1 (CH), 124.9 (CH), 119.9 (CH), 68.6 (CH_2), 66.9 (CH_2), 47.1 (CH), 40.4 (CH_2), 37.4 (CH_3) ppm.

ES-MS: m/z 384 $[M + Na]^+$

Synthesis of Alloc-protected amine **V.15b**



To a solution of 2-aminoethanol (2 g, 33.0 mmol) in dry dichloromethane (65 ml), were added 11.4 ml of a saturated aqueous solution of sodium bicarbonate and diisopropylethylamine (11.6 ml, 66.4 mmol). After addition of allylchloroformate (3.50 ml, 33.2 mmol), the solution was stirred overnight at room temperature. After addition of 100 ml of a saturated aqueous solution of sodium bicarbonate, the mixture was extracted with dichloromethane. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure. The crude compound was purified by column chromatography (ethyl acetate / isooctane: 1 / 1) to afford 3.6 g of the desired alcohol **V.15b** (76%).

Bruto formula: $C_6H_{11}O_3N$

M.W.: 145.1 g/mol

R_f (ethyl acetate / isooctane: 1 / 1) = 0.14

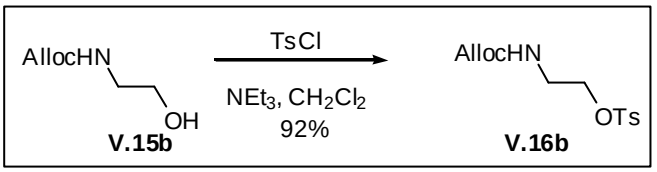
IR (film) 3349 (s), 2942 (w), 2882 (w), 1702 (s), 1542 (s), 1459 (w), 1263 (s); 1149 (m), 1069 (m), 995 (m), 995 (m), 935 (m), 778 (w) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 5.90 (1H, m), 5.39 (1H, s), 5.29 (1H, m), 5.20 (1H, m), 4.55 (2H, d , J = 5.0 Hz), 3.69 (2H, m), 3.32 (2H, m), 2.89 (1H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 157.0 (C), 132.7 (CH), 117.7 (CH_2), 65.7 (CH_2), 61.8 (CH_2), 43.3 (CH_2) ppm.

ES-MS: m/z 146 $[M + H]^+$, 168 $[M + Na]^+$

Synthesis of tosylate **V.16b**



To a cooled (0 °C) solution of alcohol **V.15b** (500 mg, 3.45 mmol) in dichloromethane were added triethylamine (1.06 ml, 7.60 mmol) and tosylchloride (725 mg, 3.80 mmol). The mixture was stirred for 2 h and was then diluted with ethyl acetate. The organic phase was washed with brine. The combined organic phases were dried over anhydrous magnesium sulfate, filtered and evaporated under vacuum. The crude oil was purified by column chromatography (ethyl acetate / isooctane: 4 / 5) to provide 944 mg of tosylate **V.16b** (92%).

Bruto formula: $C_{13}H_{17}NO_5S$

M.W.: 299.2 g/mol

R_f (ethyl acetate / isooctane: 1 / 1) = 0.38

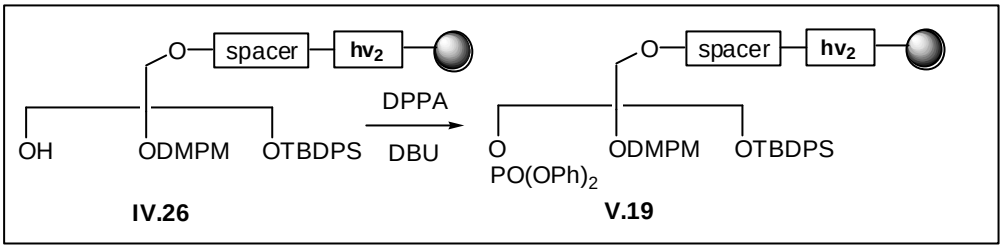
IR (film) 2955 (w), 1714 (s), 1650 (w), 1598 (w), 1531 (m), 1455 (w), 1357 (s), 1258 (m); 1177 (s), 1096 (m), 1012 (m), 917 (m), 775 (m), 664 (m), 580 (w), 554 (m) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.78 (2H, d, J = 8.2 Hz), 7.35 (2H, d, J = 8.2 Hz), 5.87 (1H, m), 5.28 (1H, d, J = 17.2 Hz), 5.21 (1H, d, J = 10.4 Hz), 5.08 (1H, s), 4.52 (2H, d , J = 5.5 Hz), 4.09 (2H, t, J = 5.5 Hz), 3.45 (2H, dt, J = 5.3 Hz), 2.45 (3H, s) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 156.0 (C), 145.0 (C), 132.5 (CH), 129.9 (CH), 127.9 (CH), 117.7 (CH_2), 69.1 (CH_2), 65.7 (CH_2), 40.10 (CH_2), 21.6 (CH_3) ppm.

ES-MS: m/z 300 $[M + H]^+$, 322 $[M + Na]^+$

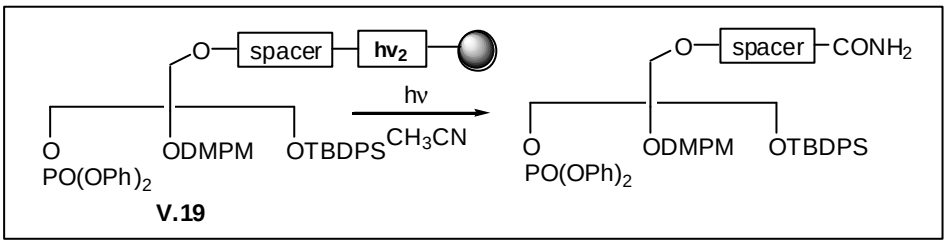
Synthesis of intermediate **V.19**



1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) (4.0 µl, 0.0272 mmol) and diphenylphosphorazidate (60 µl, 0.272 mmol) were added to a cooled (0 °C) suspension of resin **IV.26** (48.7 mg, 0.008 mmol, 0.16 mmol/g) in toluene (0.5 ml). After stirring for 2 h at 0 °C, the mixture was stirred overnight at room temperature. After filtration, the resin was washed with toluene (3 x 3 ml), dimethylformamide (3 x 3 ml), methanol (3 x 3 ml), and dichloromethane (3 x 3 ml) and was dried under vacuum for 4 h.

IR (KBr) 3413 (m), 2878 (s), 1736 (w), 1638 (m), 1618 (s), 1509 (w), 1451 (m), 1355 (m), 1250 (m), 1102 (s), 950 (s), 844 (m), 699 (s), 622 (w) cm^{-1}.

Photolytic cleavage of resin **V.19**

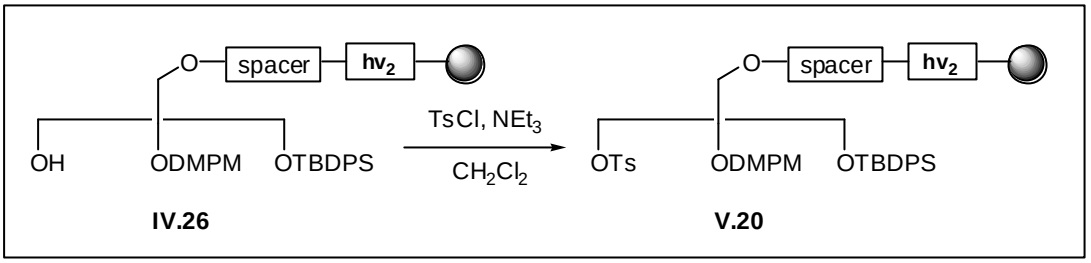


Resin **V.19** (1 mg, 0.14 10^{-3} mmol, 0.14 mmol/g) suspended in acetonitrile (100 µl) was submitted to photolytic cleavage (365 nm) for 4 h. The solution was analysed by ES-MS.

M.W.: 1257 g/mol

ES-MS: m/z 1280 $[M + Na]^+$

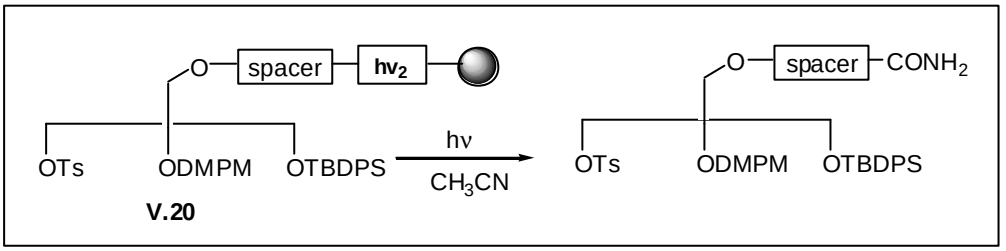
Synthesis of tosylate **V.20**



Tosylchloride (16.2 mg, 0.084 mmol) and triethylamine (12 µl, 0.084 mmol) were added to a cooled (0 °C) suspension of resin **IV.26** (50 mg, 0.011 mmol, 0.21 mmol/g) in dichloromethane (1 ml). The mixture was stirred overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml) and dried under vacuum for 4 h.

IR (KBr) 3025 (m), 3861 (s), 1737 (w), 1671 (m), 1509 (m), 1453 (s), 1347 (s), 1280 (s), 1086 (s), 949 (m), 842 (m), 699 (s) cm^{-1}.

Photolytic cleavage of resin **V.20**

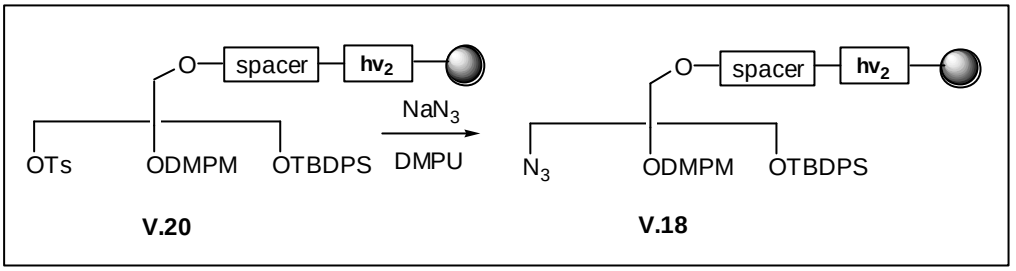


Resin **V.20** (1 mg, 0.16 10^{-3} mmol, 0.16 mmol/g) in suspension in acetonitrile (100 µl) was submitted to photolytic cleavage (365 nm) for 4 h. The solution was analysed by ES-MS.

M.W.: 1179 g/mol

ES-MS: m/z 1202 $[M + Na]^+$

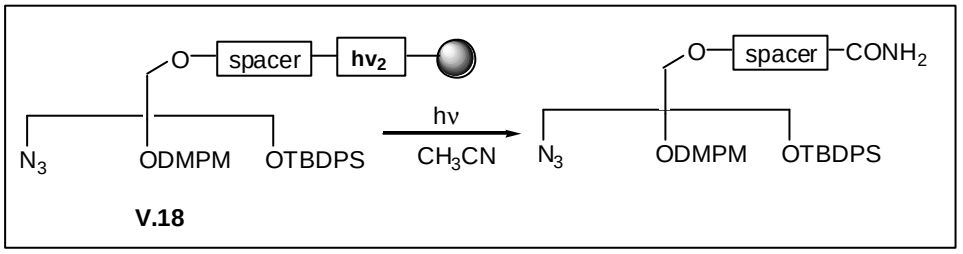
Synthesis of azide **V.18**



Sodium azide (15.5 mg, 0.24 mmol) triturated into a powder was added to a suspension of tosylate **V.20** (37.0 mg, 0.006 mmol, 0.16 mmol/g) in 1,3-dimethyl-3,4,5,6-tetrahydro-2(1H)-pyrimidone (DMPU) (0.5 ml). The suspension was heated at 40 °C overnight. The resin **V.18** was filtered, washed with DMPU (3 x 3 ml), dimethylformamide (3 x 3 ml), methanol (3 x 3 ml) and dichloromethane (3 x 3 ml) and was dried under vacuum for 4 h.

IR (KBr) 2866 (m), 2096 (w), 2023 (w), 1731 (w), 1637 (s), 1613 (s), 1514 (m), 1449 (w), 1355 (w), 1273 (w), 1102 (m), 944 (w), 621 (w) cm^{-1}.

Photolytic cleavage of azide **V.18**

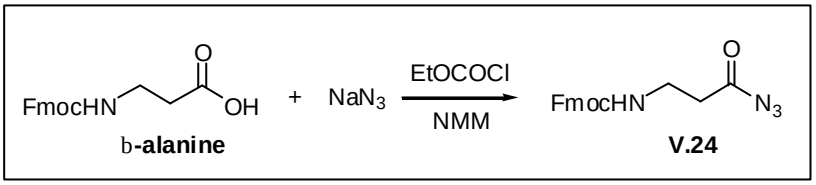


Resin **V.18** (1 mg, 0.16 10^{-3} mmol, 0.16 mmol/g) in suspension in acetonitrile (100 µl) was submitted to photolytic cleavage (365 nm) for 4 h. The solution was analysed by ES-MS.

M.W.: 1050 g/mol

ES-MS: m/z 1073 $[M + Na]^+$

Synthesis of acylazide **V.24**



To a cooled solution (-20 °C) of Fmoc-protected β-alanine (200 mg, 0.640 mmol) in dry tetrahydrofuran (1 ml) were added ethylchloroformate (68 µl, 0.704 mmol) and N-methylmorpholine (78 µl, 0.704 mmol). The mixture was stirred at -20 °C for 20 min before being allowed to warm to –5 °C. After 10 min at –5 °C, a solution of sodium azide (104 mg, 1.60 mmol) in 160 µl water was added dropwise. After 10 min, a saturated aqueous solution of sodium chloride was added and the mixture was extracted with ethyl acetate. The organic phase was dried over anhydrous magnesium sulfate, filtered and evaporated under reduced pressure to afford 208 mg of acylazide **V.24**, which was used without further purification (97%).

Bruto formula: $C_{18}H_{16}N_4O_3$

M.W.: 336.35 g/mol

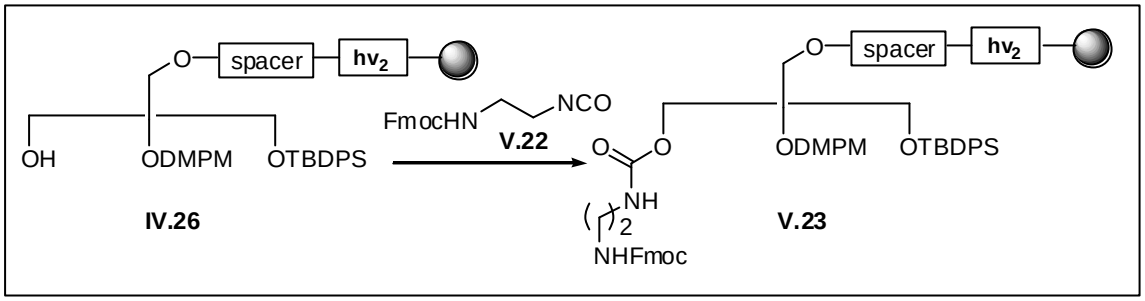
R_f (ethyl acetate / isooctane: 3 / 7) = 0.29

IR (film) 2949 (w), 2271 (s), 2139 (m), 1708 (s), 1522 (m), 1449 (m), 1255 (m), 1156 (m), 741 (m) cm^{-1}.

^1H NMR (500 MHz, $CDCl_3$) δ 7.76 (2H, d, J = 7.5 Hz), 7.71 (2H, d, J = 7.5 Hz), 7.40 (2H, ABdd, J = 7.5 Hz), 7.32 (2H, ABdd, J = 7.5 Hz), 6.20 (1H, s), 4.50 (2H, d J = 7.2 Hz), 4.31 (1H, t, J = 7.2 Hz), 3.88 (2H, dd, J = 8.4, 7.7 Hz), 3.48 (2H, dd, J = 8.4, 7.7 Hz) ppm.

^{13}C NMR (50 MHz, $CDCl_3$) + DEPT δ 156.1 (C), 151.9 (C), 143.5 (C), 141.2 (C), 127.7 (CH), 127.1 (CH), 125.2 (CH), 119.9 (CH), 68.2 (CH_2), 46.7 (CH), 43.2 (CH_2), 36.7 (CH_2) ppm.

Synthesis of carbamate **V.23**



A solution of acylazide **V.24** (50 mg, 0.149 mmol) in dry toluene (1 ml) in presence of pyridine (12 µl, 0.149 mmol) was heated to 70 °C for 1 h to form the intermediate **V.22**. The resin (30 mg, 0.005 mmol, 0.16 mmol/g) was added to the solution and the mixture was heated at 70 °C overnight. After filtration, the resin **V.23** was washed with toluene (3 x 3 ml), dimethylformamide (3 x 3 ml), methanol (3 x 3 ml) and dichloromethane (3 x 3 ml) and dried under vacuum for 4 h.

The efficiency of the reaction was monitored by UV/VIS spectroscopy at 305 nm:

Sample: m = 5 mg

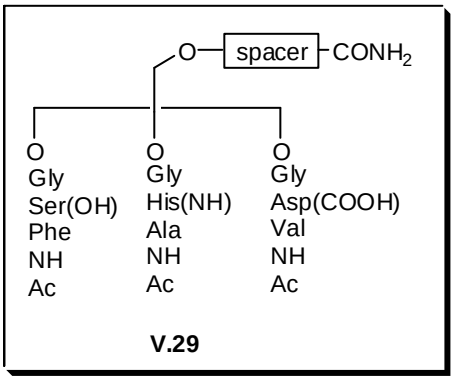
Absorbance: A = 0.239

Theoretical loading: Loading = 0.16 mmol/g

Measured loading: Loading = 0.14 mmol/g

Yield = 88%

Synthesis of one member of the library



1-Synthesis of the first strand

Coupling of FmocGlyOH on resin **IV.26**

a) Using acid chloride

A solution of FmocGlyCl (0.265 g, 0.840 mmol), diisopropylethylamine (49.0 µl, 0.28 mmol) in dicholoromethane (3 ml) was added to the resin **IV.26** (0.300 g, 0.05 mmol, 0.162 mmol/g). The suspension was skaken overnight. The coupling was repeated once again for 6 h. After filtration, the resin was washed with dimethylformamide (3 x 5 ml) and with methanol (3 x 5 ml) and dichloromethane (3 x 5 ml).

The yield of the coupling was determined by UV/VIS monitoring at 300 nm:

Sample: m = 6.0 mg

Absorbance: A = 0.96

According to the calibration curve, the experimental loading can be measured:

A = 0.96; c (mol/l) = 0.124; V (ml) = 8; loading (mmol/g) = c (mol/l) x V (l) / m (g) = 0.165 mmol/g

Calculated loading: 0.161 mmol/g

Yield = 103 %

A sample of the resin (2 mg, 0.400 µmol, 0.200 mmol/g) was suspended in 100 µl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

M.W.: 1304 g/mol

ES-MS: m/z 1327 $[M + Na]^+$

b) Using DIC, HOBt, DIPEA and DMAP

To a suspension of resin **IV.26** (0.30 g, 0.050 mmol, 0.162 mmol/g) in dry dimethylformamide (3 ml) were added FmocGlyOH (125 mg, 0.42 mmol), 1-hydroxybenzotriazole (56.7 mg, 0.42 mmol), diisopropylcarbodiimide (67 µl, 0.42 mmol), diisopropylethylamine (71 µl, 0.42 mmol) and 4-dimethylaminopyridine (0.021 mmol, 2.6 mg). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml).

The yield of the coupling was determined by UV/VIS monitoring at 300 nm.

Sample: m = 5.7 mg

Absorbance: A = 0.895

According to the calibration curve, the experimental loading can be measured:

A = 0.96; c (mol/l) = 0.116; V (ml) = 8 ; loading (mmol/g) = c (mol/l) x V (l) / m (g) = 0.162 mmol/g

Calculated loading: 0.161 mmol/g

Yield = 101 %

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Coupling of FmocSer(OTrt)OH

The carboxylic acid FmocSer(OTrt)OH (0.144 g, 0.252 mmmol) was first activated separatly with diisopropylcarbodiimide (40.0 µl, 0.252 mmol) and 1-hydroxybenzotriazole (34.0 mg, 0.252 mmol). This solution was stirred for 30 min. at room temperature and was then added to the resin (0.300 g, 0.047 mmol, 0.16 mmol/g). The mixture was skaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

A sample of the resin (2 mg, 0.300 µmol, 0.150 mmol/g) was suspended in 100 µl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

M.W.: 1633 g/mol

ES-MS: m/z 1656 $[M + Na]^+$, 1673 $[M + K]^+$

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Coupling of FmocPheOH

The carboxylic acid FmocPheOH (81.4 mg, 0.210 mmol) was first activated separately with diisopropylcarbodiimide (33.0 µl, 0.210 mmol) and 1-hydroxybenzotriazole (29.0 mg, 0.210 mmol). This solution was stirred for 30 min. at room temperature and was then added to the resin (0.30 g, 0.045 mmol, 0.150 mmol/g). The mixture was skaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

A sample of 2 mg of the resin (2 mg, 0.300 µmol, 0.147 mmol/g) was suspended in 100 µl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

M.W.: 1781 g/mol

ES-MS: m/z 1804 $[M + Na]^+$, 1820 $[M + K]^+$

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Capping of the free amino functions

Acetylimidazole (77 mg, 0.700 mmol) was added to a suspension of resin (0.250 g, 0.037 mmol, 0.147 mmol/g) in dry dichloromethane (3 ml). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed**.**

IR (KBr) 3572 (w), 3278 (w), 2860 (s), 1734 (m), 1669 (s), 1511 (s), 1453 (s), 1370 (s), 1264 (m), 1073 (s), 947 (m), 841 (w), 698 (s) cm^{-1}.

^{13}C NMR (50 MHz, CD_2Cl_2) δ 174.2, 173.3, 172.3, 171.4, 155.5, 150.0, 147,9, 145.1, 141.9, 140.2, 138.1, 137.1, 135.4, 131.2, 130.8, 130.4, 130.2, 129.9, 129.3, 129.0, 121.6, 112.8, 112.7, 111.0, 110.9, 88.3, 74.7, 74.2, 73.7, 72.1 (polyethyleneglycol peak), 71.2, 71.1, 70.2, 67.1, 66.8, 65.0, 57.9, 57.4, 47.7, 46.2, 40.9, 40.5, 37.6, 36.2, 34.0, 32.0, 28.3, 26.6, 24.4, 23.1, 20.6 ppm.

A sample of resin **V.25** (34.2 mg, 0.005 mmol, 0.147 mmol/g) in suspension in 1 ml of 1,4-dioxane containing 1% of dimethylsulfoxide was irradiated with UV light for 4 times 2 h. The solution was analyzed by ES-MS. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane and removal of the dimethylsulfoxide with the Kugelrhor, the sample was purified by column chromatography (ethyl acetate) to provide 3.7 mg of the cleaved product (45%).

Bruto formula: $C_{96}H_{108}N_4O_{16}Si$

M.W.: 1600 g/mol

R_f (ethyl acetate) = 0.53

^{1}H NMR (500 MHz, CD_3OD) δ 7.51 (4H, dd, J = 8.0, 1.4 Hz), 7.24 (38H, m), 6.81 (3H, m), 4.66 (1H, dd, J = 7.5 Hz), 4.61 (1H, dd, J = 7.5 Hz), 4.42 (2H, s), 4.40 (2H, s), 4.37 (2H, s), 4.36 (2H, s), 4.25 (2H, t, J = 6.4 Hz), 4.13 (2H, s), 3.95 (1H, ABd, J_{AB} = 17.6 Hz), 3.86 (1H, ABd, J_{AB} = 17.5 Hz), 3.79 (2H, t, J = 6.4 Hz), 3.76 (3H, s), 3.69 (3H, s), 3.63 (2H, t, J = 6.4 Hz), 3.44 (6H, m), 3.38 (1H, m), 3.34 (1H, m), 3.12 (2H, m), 2.82 (6H, m), 2.46 (2H, t, J = 6.4 Hz), 2.39 (2H, t, J = 6.4 Hz), 1.83 (3H, s), 0.95 (9H, s) ppm.

ES-MS: m/z 1623 $[M + Na]^+$, 1639 $[M + K]^+$

2-Synthesis of the second strand

Deprotection of the DMPM protecting group

To a cooled (0 °C) suspension of resin **V.25** (0.15 g, 0.023 mmol, 0.15 mmol/g) in a mixture of dichloromethane / water (1.4 ml / 0.1 ml) was added dichlorodicyanoquinone (14.3 mg, 0.063 mmol). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with an aqueous saturated solution of sodium bicarbonate (3 x 5 ml), phenol / methanol: 1 / 1 (3 x 5 ml), toluene (3 x 5 ml), acetonitrile (3 x 5 ml), dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml).

A sample of 2 mg of the resin (2 mg, 0.300 µmol, 0.15 mmol/g) was suspended in 100 µl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

M.W.: 1450 g/mol

ES-MS: m/z 1473 $[M + Na]^+$

Coupling of FmocGlyOH

To a suspension of resin (0.15 g, 0.023 mmol, 0.150 mmol/g) in dry dimethylformamide (3 ml) were added FmocGlyOH (63 mg, 0.21 mmol), 1-hydroxybenzotriazole (28.4 mg, 0.21 mmol), diisopropylcarbodiimide (33 µl, 0.21 mmol), diisopropylethylamine (36 µl, 0.21 mmol) and 4-dimethylaminopyridine (2.6 mg, 0.021 mmol). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml).

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Coupling of FmocHis(NMtt)OH

The carboxylic acid FmocHis(NMtt)OH (78 mg, 0.126 mmmol) was first activated separatly with diisopropylcarbodiimide (20 µl, 0.126 mmol) and hydroxybenzotriazole (17 mg, 0.126 mmol). This solution was stirred for 30 min. at room temperature and was then added to the resin (0.15 g, 0.021 mmol, 0.14 mmol/g). The mixture was skaken overnight at room temperature. After filtration, the resin x was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Coupling of FmocAlaOH

The carboxylic acid FmocAlaOH (39 mg, 0.126 mmmol) was first activated separatly with diisopropylcarbodiimide (20 µl, 0.126 mmol) and hydroxybenzotriazole (17 mg, 0.126 mmol). This solution was stirred for 30 min. at room temperature and was then added to the resin (0.15 g, 0.021 mmol, 0.14 mmol/g). The mixture was skaken overnight at room temperature. After filtration, the resin x was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Capping of the free amino functions

Acetylimidazole (46 mg, 0.42 mmol) was added to a suspension of the resin (0.15 g, 0.021 mmol, 0.14 mmol/g) in dry dichloromethane (3 ml). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

Deprotection of the side-chain protecting groups trityl and methyltrityl group

A sample of the resin (10 mg, 1.4 µmol, 0.14 mmol/g) was treated with a solution of 80% chloroacetic acid in a mixture dichloromethane/H_2O (3/1) overnight at room temperature. The procedure was repeated 3 times two hours.

A sample of 2 mg of the resin (2 mg, 0.280 µmol, 0.140 mmol/g) was suspended in 100 µl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

M.W.: 1515 g/mol

ES-MS: m/z 1538 $[M + Na]^+$, 1300 $[M - TBDPS + H + Na]^+$

3-Synthesis of the third strand

Deprotection of the TBDPS protecting group

To a suspension of resin **V.26** (150 mg, 0.021 mmol, 0.142 mmol/g) in 0.36 ml dimethylformamide was added TASF (0.0975 M) (tris-(dimethylamino)sulfur (trimethylsilyl)difluoride) in dimethylformamide (0.82 ml, 0.084 mmol). The suspension was shaken for 3 h at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml), dichloromethane (3 x 5 ml).

Coupling of FmocGlyOH

To a suspension of the resin (0.15 g, 0.023 mmol/g, 0.150 mmol/g) in dry dimethylformamide (3 ml) were added FmocGlyOH (63 mg, 0.21 mmol), 1-hydroxybenzotriazole (28.4 mg, 0.21 mmol), diisopropylcarbodiimide (33 µl, 0.21 mmol), diisopropylethylamine (36 µl, 0.21 mmol) and 4-dimethylaminopyridine (2.6 mg, 0.021 mmol). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml).

Deprotection of the Fmoc protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Coupling of FmocAsp(COOTMSE)OH

The carboxylic acid FmocAsp(COOTMSE)OH (57 mg, 0.126 mmmol) was first activated separatly with diisopropylcarbodiimide (20 µl, 0.126 mmol) and 1-hydroxybenzotriazole (17 mg, 0.126 mmol). This solution was stirred for 30 min. at room temperature and was then added to the resin (0.15 g, 0.021 mmol, 0.14 mmol/g). The mixture was skaken overnight at room temperature. After filtration, the resin x was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Coupling of FmocValOH

The carboxylic acid FmocValOH (43 mg, 0.126 mmmol) was first activated separatly with diisopropylcarbodiimide (20 µl, 0.126 mmol) and 1-hydroxybenzotriazole (17 mg, 0.126 mmol). This solution was stirred for 30 min. at room temperature and was then added to the resin (0.15 g, 0.021 mmol, 0.14 mmol/g). The mixture was skaken overnight at room temperature. After filtration, the resin x was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

Deprotection of the Fmoc-protecting group

The resin was treated with a solution of 20% piperidine in dimethylformamide (2 x 20 min). Then the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and intense coloured beads were observed.

Capping of the free amino functions

Acetylimidazole (46 mg, 0.42 mmol) was added to a suspension of the resin (0.15 g, 0.022 mmol, 0.14 mmol/g) in dry dichloromethane (3 ml). The mixture was shaken overnight at room temperature. After filtration, the resin was washed with dimethylformamide (3 x 5 ml), methanol (3 x 5 ml) and dichloromethane (3 x 5 ml). A sample of the resin was submitted to both colorimetric tests (TNBS and **NF31**) and colourless beads were observed.

Cleavage of tripodal member **V.27**

A sample of resin **V.27** (36.7 mg, 0.005 mmol, 0.14 mmol/g) in suspension in 1 ml of 1,4-dioxane containing 1% of dimethylsulfoxide was irradiated with UV light for 3 times 2 h. After each cleavage, the resin was filtered and washed with 1,4-dioxane. After evaporation of 1,4-dioxane

and removal of the dimethylsulfoxide with the Kugelrhor, the sample was purified by column chromatography (ethyl acetate) to provide 5.6 mg of compound **V.28** (51%).

M.W.: 2188 g/mol

R_f (methanol / dichloromethane: 1 / 9) = 0.52

Deprotection of the side-chain protecting groups trityl and methyltrityl group

A sample of the resin (10 mg, 1.4 μmol, 0.14 mmol/g) was treated with a solution of 80% chloroacetic acid in a mixture dichloromethane / H_2O (3 / 1) overnight at room temperature. The procedure was repeated 3 times two hours.

A sample of 2 mg of the resin (2 mg, 0.280 μmol, 0.140 mmol/g) was suspended in 100 μl acetonitrile and was irradiated with UV light (365 nm) for 3 h. The solution was analyzed by ES-MS.

M.W.: 1690 g/mol

ES-MS: m/z 868 $[M + 2Na]^{2+}$, 1691 $[M + H]^{+}$, 1713 $[M + Na]^{+}$

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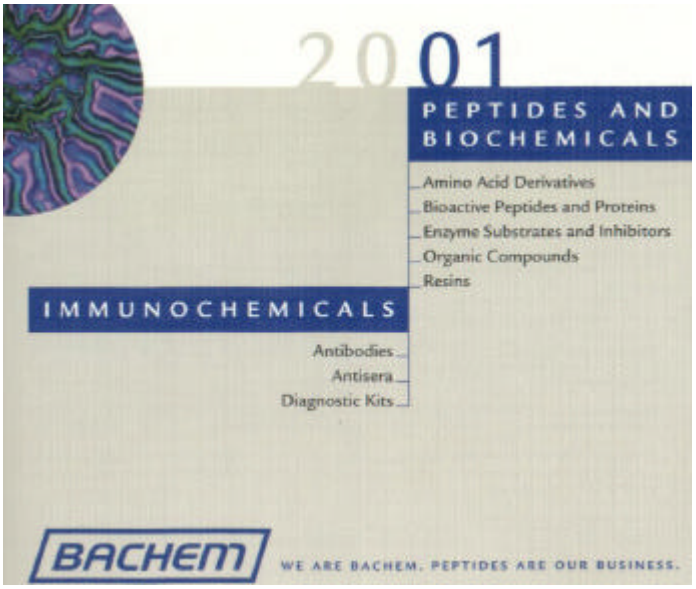
[103] It has to be noted that the ^{1}H NMR of the cleaved compound from resin **V.26** was not pure enough to attribute all peaks but we could clearly observe that the glycine ester bonds were intact due to the presence of trityl and methyltrityl peaks in the spectrum.

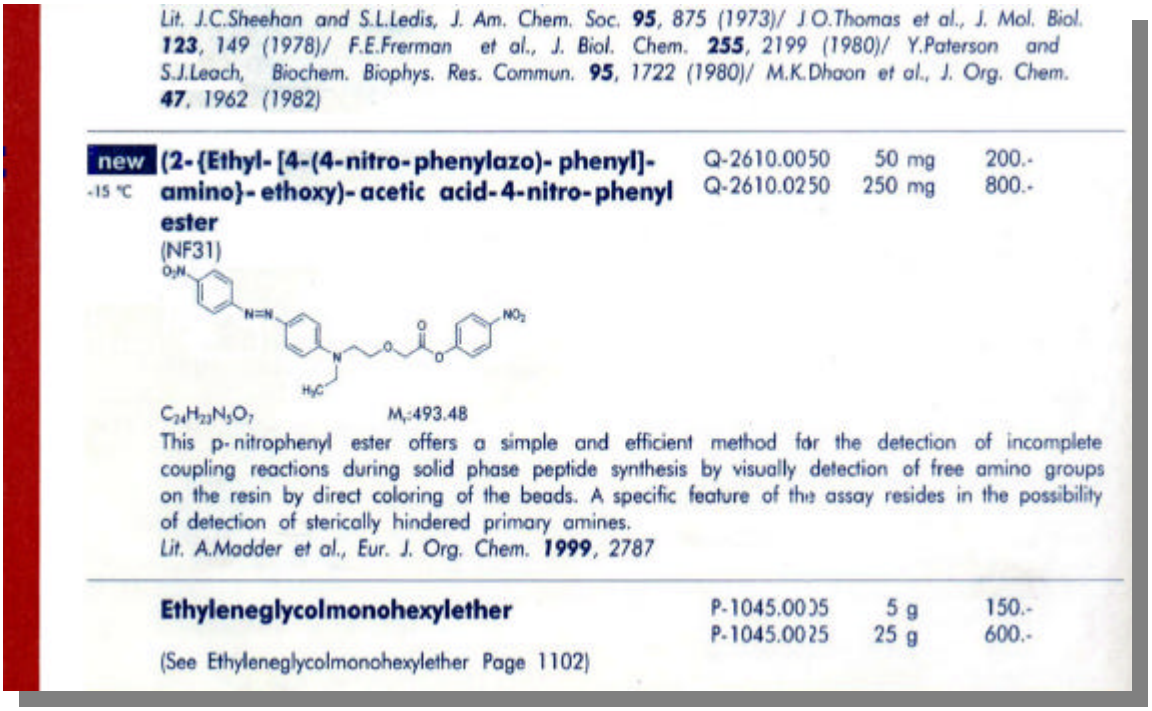
In addition, the limited mass-ion detection of our ES-MS equipment of 2000 g/mol did not allow the detection of the high molecular weight of the cleaved resin **V.25** (2013 g/mol).

[104] The amount of DMAP needs to be carefully controlled to avoid deprotection of the Fmoc group and to avoid possible bicoupling.

9 Appendix

Since 2001, **NF31** is commercially available from 'BACHEM', catalog 2002, p 1282, 50 mg cost 200 Swiss Franc (150 Euro)





Lit. J.C.Sheehan and S.L.Ledis, J. Am. Chem. Soc. ***95****, 875 (1973)/ J.O.Thomas et al., J. Mol. Biol.* ***123****, 149 (1978)/ F.E.Frerman et al., J. Biol. Chem.* ***255****, 2199 (1980)/ Y.Paterson and S.J.Leach, Biochem. Biophys. Res. Commun.* ***95****, 1722 (1980)/ M.K.Dhaon et al., J. Org. Chem.* ***47****, 1962 (1982)*

new -15 °C **(2-{Ethyl-[4-(4-nitro-phenylazo)-phenyl]-amino}-ethoxy)-acetic acid-4-nitro-phenyl ester** (NF31)

Q-2610.0050 50 mg 200.-
Q-2610.0250 250 mg 800.-

$C_{24}H_{23}N_5O_7$ M_r:493.48

This p-nitrophenyl ester offers a simple and efficient method for the detection of incomplete coupling reactions during solid phase peptide synthesis by visually detection of free amino groups on the resin by direct coloring of the beads. A specific feature of the assay resides in the possibility of detection of sterically hindered primary amines.
Lit. A.Madder et al., Eur. J. Org. Chem. ***1999****, 2787*

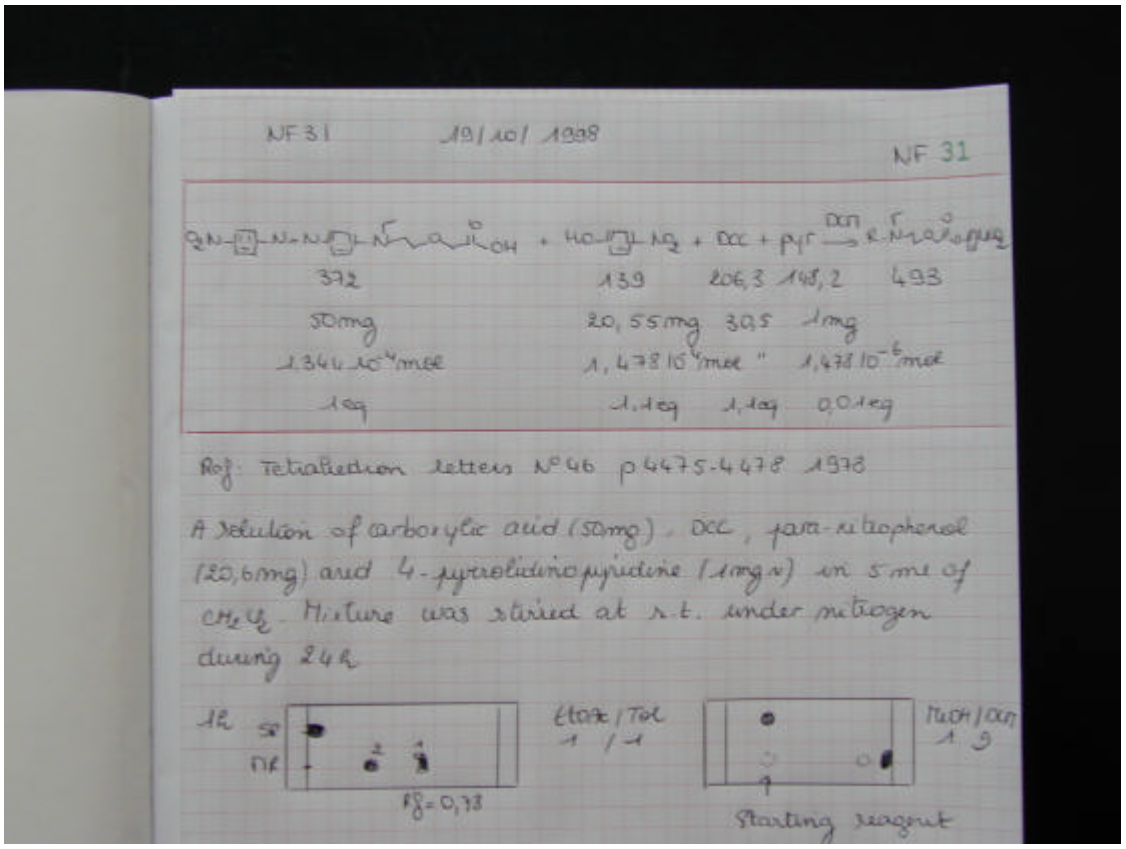
Ethyleneglycolmonohexylether

P-1045.0005 5 g 150.-
P-1045.0025 25 g 600.-

(See Ethyleneglycolmonohexylether Page 1102)

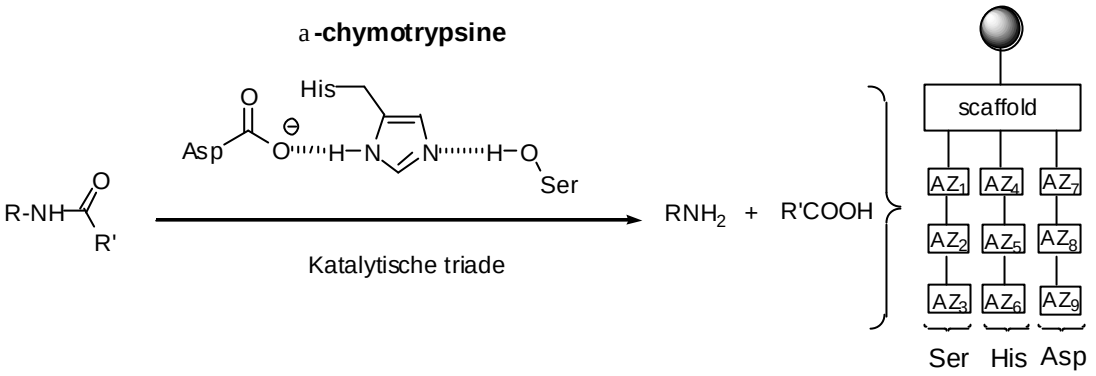
NF31

The name **NF31** comes from Nadia Farcy who prepared this compound for the first time. The number corresponds to the page of the labbook.



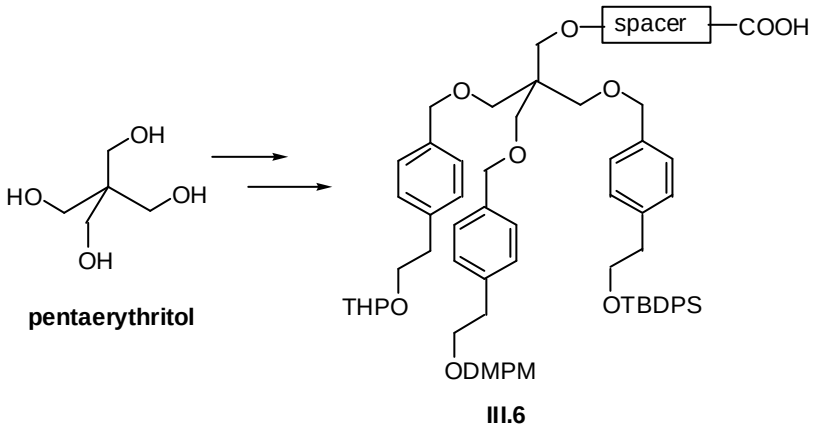
10 Samenvatting

In dit doctoraatswerk werd gewerkt aan de ontwikkeling van een nieuw niet-enzymatisch model voor het serineprotease α-chymotrypsine, gebaseerd op de katalytische triade 'serine-histidine-aspartaat'. Met behulp van een tripodale scaffold kan een combinatorische peptidebibliotheek gegenereerd worden waarin de drie aminozuren serine, histidine and aspartaat worden geïntegreerd elk in een peptideketen. De rol van de scaffold bestaat erin de nodige proximiteit te induceren tussen de verschillende katalytisch belangrijke aminozuur residues.



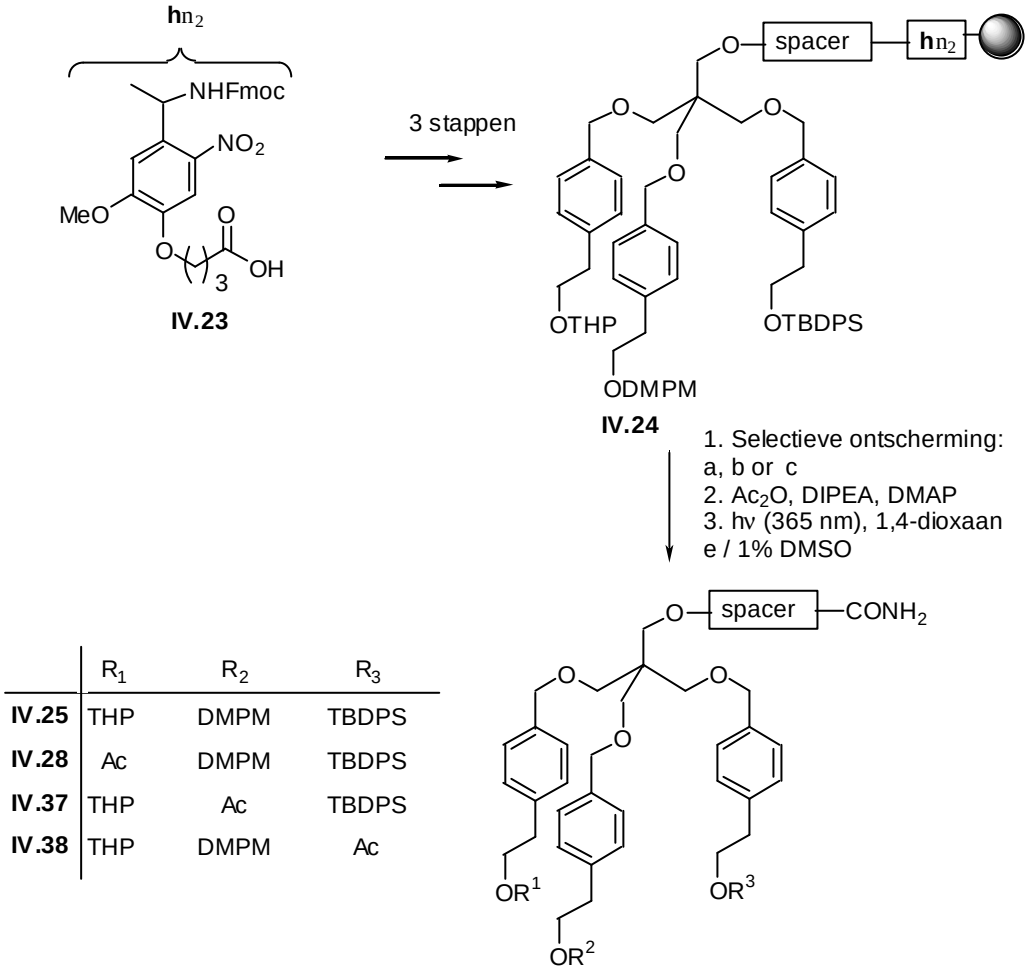
Schema 10.1

De door ons ontworpen tripodale scaffold werd bereid uitgaande van het commercieel beschikbaar en goedkoop pentaerythritol. Scaffold **III.6** bezit drie beschermde alcohol functies als aanhechtingspunten voor de peptideketens en een carbonzuur voor immobilisatie op de vaste drager.



Schema 10.2

In een eerste stadium werd de selectieve ontscherming van de drie beschermende groepen THP, DMPM en TBDPS bestudeerd. Een fotolabiele linker werd geïncorporeerd tussen de vaste fase en de pentaerytritol eenheid. Dit liet analyse en identificatie toe van de vrijgestelde producten na fotochemische afsplitsing. Het veratryl derivaat **IV.23** ontwikkeld door Holmes wordt in de literatuur beschreven als de meest efficiente fotolabiele linker. Eerst werd dit carbonzuur gekoppeld aan de vaste drager waarna de Fmoc groep ontschermd werd. Vervolgens werd de tripodale scaffold met succes aan de fotolabiele linker gehecht. Na optimalisatie van de condities bleek dat UV-bestraling (365 nm) van een suspensie van product **IV.24** in 1,4-dioxaan in aanwezigheid van 1% DMSO gedurende 9 u de beste resultaten gaf. Amide **IV.25** werd aldus bekomen met een rendement van 86%.



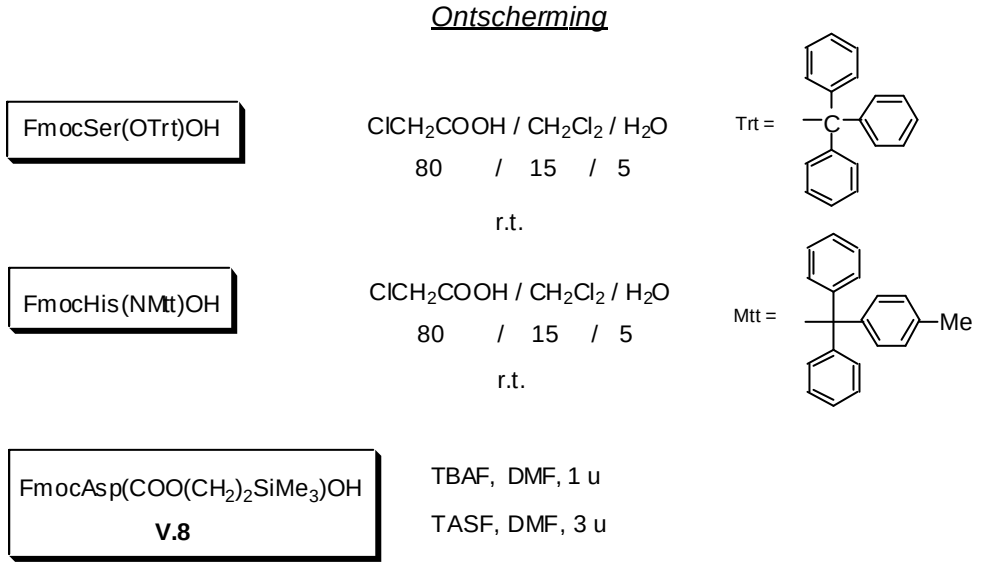
	R_1	R_2	R_3
IV.25	THP	DMPM	TBDPS
IV.28	Ac	DMPM	TBDPS
IV.37	THP	Ac	TBDPS
IV.38	THP	DMPM	Ac

a) AcOH / CH_2Cl_2 / H_2O: 80 / 15 / 5, 60 °C, 16 h; b) DDQ, CH_2Cl_2 / H_2O: 19 / 1, 1 h, 0 °C; c) TBAF, AcOH, moleculaire zeven (400 pm), THF **of** NH_4F, MeOH **of** HF/pyr., THF **of** TASF, DMF.

Schema 10.3

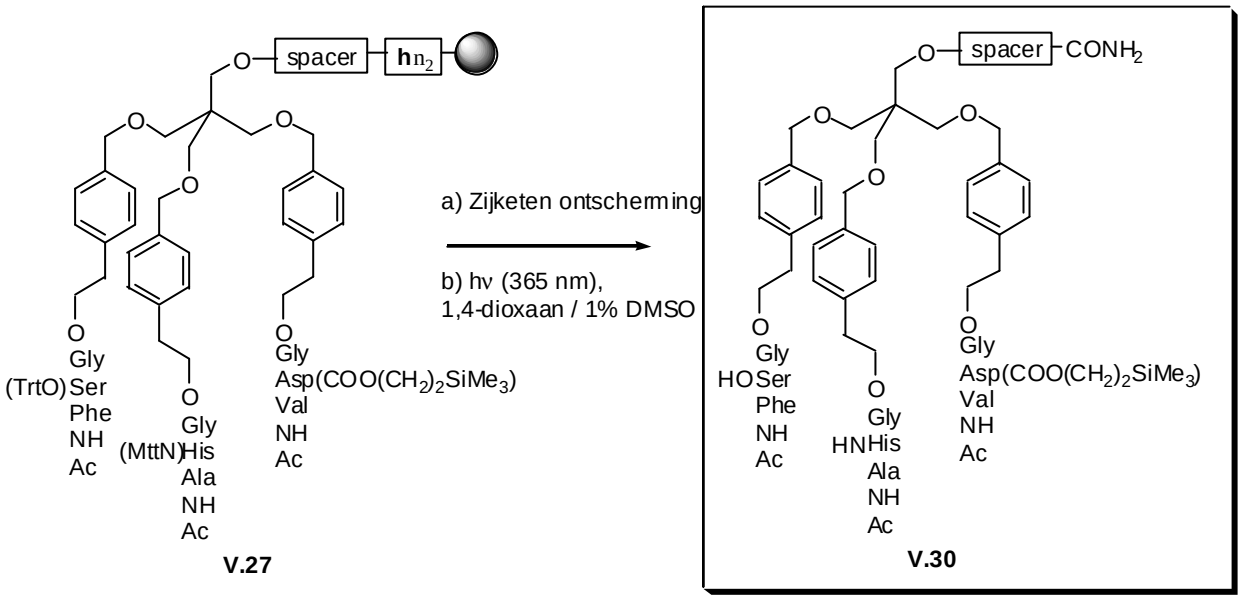
De selectieve ontscherming van de verschillende OH-beschermende groepen kon nu op een efficiënte wijze gevolgd en geoptimaliseerd worden. De afsplitsing van de THP groep werd uitgevoerd met een oplossing van azijnzuur bij 60 °C of met behulp van PPTS bij 55 °C overnacht. De DMPM ether werd op selectieve wijze onstchermd met DDQ bij 0 °C gedurende 1 u. Langere reactietijd of hogere temperatuur leidde tot nevenreacties met de benzylethers aanwezig op de scaffold. Tot slot werd TBAF met azijnzuur gebruikt voor de ontscherming van de TBDPS ether. Elke ontscherming werd gevolgd met behulp van ^{13}C NMR. De reproduceerbaarheid en efficiëntie van de beschreven procedures werd gecontroleerd na acylering van de alcoholfunctie en afsplitsing van de bekomen producten met UV-licht. De vrijgestelde acetaten **IV.28**, **IV.37** en **IV.38** werden geïsoleerd met een rendement van 55%, 62% and 56% respectievelijk.

Voor de synthese van een tripodale bibliotheek dienden vervolgens geschikte beschermende groepen voor serine, histidine and aspartaat gekozen te woorden (schema 10.4). Het commercieel beschikbare FmocSer(OTrt)OH en FmocHis(NMtt)OH werden geselecteerd. De zuurgevoelige trityl en methytrityl groepen werden afgesplitst met behulp van chloorazijnzuur. In het geval van aspartinezuur werd gekozen voor de TMSE (trimethylsilylethyl) groep. Dit beschermde aspartinezuur werd bereid uitgaande van het commercieel beschikbare α-benzyl ester aspartinezuur. Volgens de literatuur kan de TMSE groep verwijderd worden met TBAF in DMF of TASF(tris-(dimethylamin)zwavel trimethylsilyldifluoride).



Schema 10.4

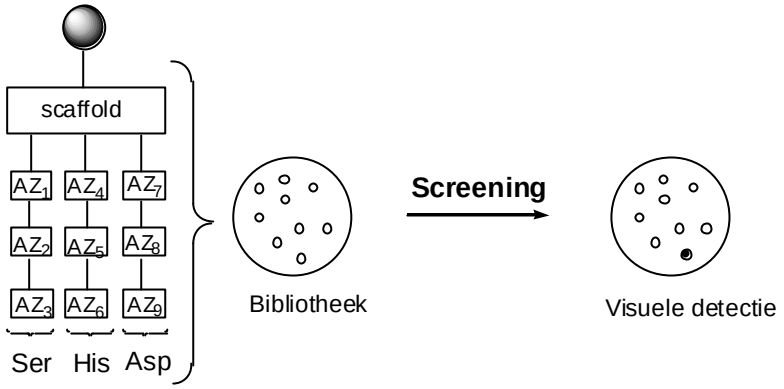
In een laatste stadium werd een representatief lid van de tripodale bibliotheek gesynthetiseerd (zie **V.27**, schema 10.5). De afsplitsing van de THP groep werd gevolgd door de constructie van de eerste peptide keten. Glycine werd gekoppeld, gebruik makende van de DIC/HOBt methode in aanwezigheid van DMAP and DIPEA. De efficiëntie van deze koppelingsreactie werd gevolgd met behulp van UV-spectroscopie. Vervolgens werden serine en fenylalanine aangehecht. Ontscherming van de Fmoc groep en acylering van de vrije amine functies gaf het monopodale lid. Daarna werd de DMPM ether verwijderd met DDQ en de tweede peptideketen werd gegenereerd via een analoge procedure. Tot slot werd de TBDPS onstchermd met het TASF reagens en de laatste peptideketen werd opgebouwd met aspartinezuur en valine als aminozuren. De efficiëntie van alle koppelingsreacties werd nagegaan door de colorimetrische TNBS en **NF31** testen. Een staal van het tripodale lid **V.27** werd op dit stadium afgesplitst van de vaste drager en de structuur van het product werd bewezen door NMR analyse. Vervolgens werden de zijketen beschermende groepen op serine en histidine afgesplitst met chloorazijnzuur. Na fotolytische afsplitsing werd het tripodal lid **V.30** geanalyseerd met behulp van massaspectroscopie.



Schema 10.5

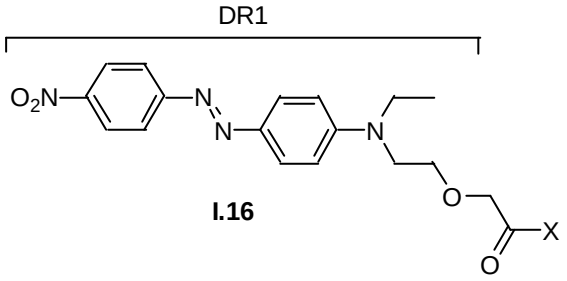
In de toekomst zal een tripodale bibliotheek opgebouwd worden op vaste fase gebruik makende van de split en mixmethode. Voor de screening van de bibliotheek werden een aantal testsubstraten **I.16** ontwikkeld. Elk van die substraten vertoont een verschillende reactiviteit in een hydrolysereactie. Naast de zeer reactieve *p*-nitrofenylester **I.16a** werden het *p*-nitroanilide **I.16b** en het amide **I.16c** gesynthetiseerd. Bij behandeling van de bibliotheek met een van de kleurstoffen **I.16** zal een potentiële katalysator met het substraat reageren waardoor een

transfer van de kleur op de vaste drager zal geobserveerd worden. Deze techniek laat een visuele detectie van eventuele katalysatoren toe.



Schema 10.6

De reactieve *p*-nitrofenylester **I.16a** werd gecommercialiseerd door Bachem (zie appendix). **NF31** wordt momenteel gebruikt als testsubstraat voor de screening van de bibliotheek ten opzichte van hydrolytische activiteit en verder als nieuwe colorimetrische test voor de detectie van vrije aminefuncties op vaste drager. Deze nieuwe test is onder andere bijzonder nuttig voor het volgen van koppelingen op prolinederivaten daar dit niet mogelijk is met de tot nu toe gekende testprocedures.



I.16

a X = −OPhNO$_2$(p) = **NF31**
b X = −NHPhNO$_2$(p)
c X = −NHCH$_2$CH$_2$OPhNO$_2$(p)

Schema 10.7

11 Curriculum Vitae

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OPLEIDING

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1995-1997 Licence et Maîtrise de Chimie Organique (mention bien), Université de Rouen, France

Stage (4/1997-8/1997): '*Kinetic study of 'in situ' polymerisation of tetrahydrofuran using different triflate esters as initiators*' (Prof. Dr. E.J. Goethals), Universiteit Gent, België

1997-1998 Diplôme d'études approfondies de chimie organique (DEA) (mention bien), Université de Rouen, France: '*Synthèse de deux polyènes : le 1-bromo-6-iodohexa-1,3,5-triène et le 1,8-dibromooctatétraène*' (Laboratoire des Fonctions Azotées et Oxygénées Complexes: Prof. Dr. G. Plé)

1998-2002 **Doctoraat Organische Scheikunde**: '*Efforts towards the synthesis of a tripodal library for the discovery of serine protease mimics*' (Prof. Dr. P. De Clercq), Universiteit Gent, België

2001: Doctoraatsopleiding

PUBLICATIES

1) 'Influence of the alkyl group of triflate esters on their initiation ability for the cationic ring-polymerization of tetrahydrofuran' M.F. Dubreuil, N.G. Farcy, E.J. Goethals *Macromol. Rapid Commun.* **1999**, *20*, 383-386.
2) 'A novel sensitive colorimetric assay for visual detection of solid phase-bound amines' A. Madder, N. Farcy, N.G.C. Hosten, H. De Muynck, P.J. De Clercq, J. Barry, A.P. Davis Eur. *J. Org. Chem.* **1999**, 2787-2791.
3) 'Application of combinatorial procedures in the search for serine-protease-like activity with focus on the acyl transfer step' H. De Muynck, A. Madder, N. Farcy, P.J. De Clercq, M.N. Pérez-Payán, L.M. Öhberg, A.P. Davis *Angew. Chem. Int. Ed. Engl.* **2000**, *39*, 145-148.

4) 'A pentaerythritol-based molecular scaffold for solid phase combinatorial chemistry' N. Farcy, H. De Muynck, A. Madder, N. Hosten, P.J. De Clercq *Org. Lett*. **2001**, 4299-4301.

CONGRESSEN EN BUITELANDSERVARINGEN

Poster presentaties

1) 10 / 1999: International Combinatorial Chemistry Symposium (Tübingen, Duitsland): *'A novel sensitive colorimetric assay for visual detection of solid phase bound amines*'
2) 7 / 2000: International: Belgium Organic Synthesis Symposium-8: '*Preliminary experiments towards the development of a tripodal library for the discovery of serine-protease mimics using combinatorial chemistry*'
3) 9 / 2001: International: Combinatorial chemistry Symposium (Southampton, Groot-Brittannië): '*Development of a tripodal library for the discovery of serine-protease mimics using combinatorial chemistry*'

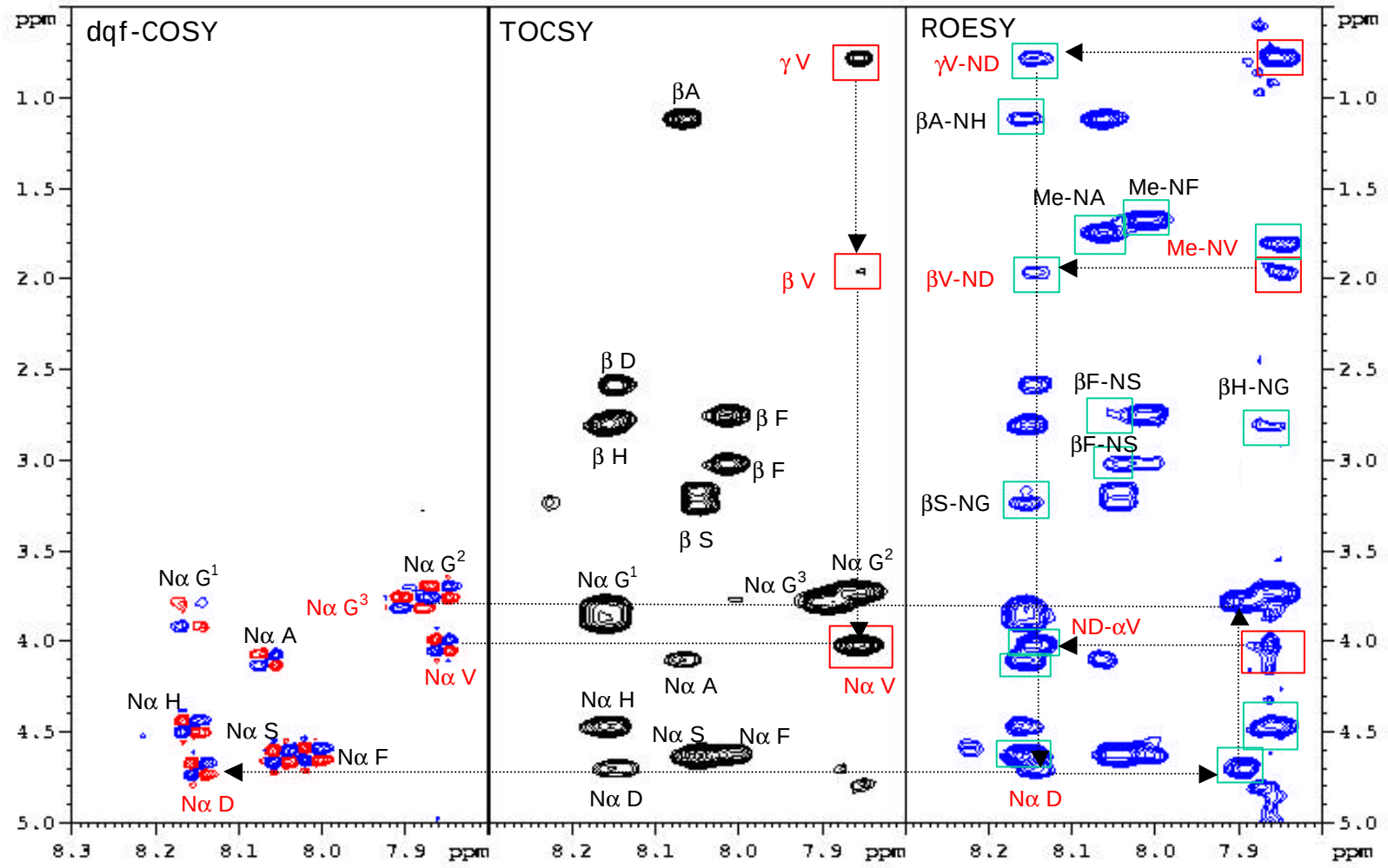
Voordrachten

1) 6 / 1999: internationaal: Voordracht in het kader van het Europese netwerk 'Training and Mobility Research' (Barcelona, Spanje)
2) 4 / 2000: nationaal: 5de Vlaamse Jongerencongres van de chemie georganiseerd door Koninklijke Vlaamse Chemische Vereniging (VUB Brussel)
3) 3 / 2001: internationaal: Voordracht in het kader van het Europese netwerk 'Training and Mobility Research' (Keulen, Duitsland)
4) 11 / 2001: internationaal: Voordracht in het kader van het Europese netwerk 'Training and Mobility Research' (Bristol, Groot-Brittannië)
5) 04/2002: internationaal: Voordracht in het kader van het Europese netwerk 'Training and Mobility Research' (Gröningen, Nederlands)

Stages

5 / 2001: synthese van een 81-leden bibliotheek op vaste fase met het gebruik van een nieuwe techniek: 'radio frequency tag system' IRORI in het labo van Prof. Dr. A. Berkessel (Keulen, Duitsland)

3 / 2002: uittesten van een nieuwe screeningsmethode ontwikkeld in het labo van Prof. Dr. A.P. Davis (Bristol, Groot-Brittannië)



G = glycine; S = serine; F= phenylalanine; H = histidine; A = alanine; D = aspartic acid; V = valine
N = NH; α = αH; β = βH

Figure 5.4