UNIVERSITY of SOUTHAMPTON

High Loading Beads for Single Bead Screening in Combinatorial Chemistry

by

Andrea Basso

Doctor of Philosophy

Department of Chemistry
Faculty of Science

October 2000

to my family

to my friends

to my land

E'nt'a barca du vin ghe navighiemu 'nsc'i scheuggi
emigranti du rìe cu'i cioi 'nt'i euggi
finché u matin crescià da puéilu rechéugge
frè di ganeuffeni e dè figge
bacan d'a corda marsa d'aegua e de sä
che a ne liga e a ne porta 'nte 'na creuza de mä

Fabrizio de André (1940-1999)

UNIVERSITY of SOUTHAMPTON

ABSTRACT

FACULTY of SCIENCE

DEPARTMENT of CHEMISTRY

Doctor of Philosophy

High Loading Beads for Single Bead Screening in Combinatorial Chemistry

by Andrea Basso

Combinatorial chemistry and solid-phase synthesis have revolutionised the process of drug discovery in the last decade. Ever since the concept of split and mix synthesis was introduced in 1988, and in particular the concept of *one bead one compound*, this approach has been associated with the possibility of generating thousands or millions of compounds in only a relatively small number of synthetic steps. With the miniaturisation of screening assays the amount of compound released by a single resin bead has become sufficient for biological tests. However, if multiple screenings, IC_{50} determination or direct structure characterisation are required, commercially available resins are not suitable. Therefore the demand for high-loading resins has drastically increased over the last years.

The research reported here is focussed on bead-loading enhancement *via* a dendrimerisation process. Two different types of high loading dendrimer resins have been synthesised.

Polyamidoamine (PAMAM) dendrimer resin has been used for the solid phase synthesis of various classes of compounds, involving many different chemical conditions. Robustness, swelling properties and suitability for single bead screening have also been investigated.

Polyether dendrimer resin has been synthesised for the first time and again chemical inertness and mechanical properties have been explored.

Both dendrimer resins can be conveniently and efficiently synthesised starting from commercially available resins, they are inert under severe chemical conditions and the final loading is sufficient for multiple single bead screenings.

Acknowledgements

First of all I would like to thank Prof. Mark Bradley, for his enthusiasm and many helpful discussions: I have always found his door open when I needed him.
Dr. Brian Evans and Dr. Neil Pegg have shown a lot of interest in my project and many times have given me good hints and help.
During these three years many people have arrived into the Bradley group and each of them has given me something special: I would like to thank them all.
I would like to thank also Mrs. Joan Street for the NMRs and Miss Julie Herniman and Dr. John Langley for the MSs.

Table of Contents

List of Abbreviations

Ac	Acetyl
Ac_2O	Acetic anhydride
AcOEt	Ethyl acetate
AcOH	Acetic acid
Ala	Alanine
APCI	Atmospheric pressure chemical ionisation
Arg	Arginine
Atm	Atmosphere
Boc	*tert*-Butoxycarbonyl
Boc-ON	2-(*tert*-butoxycarbonyloxymino)-2-phenylacetonitrile
Bu	Butyl
Cbz	Benzyloxycarbonyl
d	Doublet
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
DEAD	Diethylazodicarboxylate
DIAD	Diisopropylazodicarboxylate
DIC	Diisopropylcarbodiimide
DIPEA	Diisopropylethylamine
DMAP	Dimethylaminopyridine
DMF	N,N-Dimethylformamide
DMSO	Dimethylsulphoxide
EEDQ	2-Ethoxy-1-ethoxycarbonyl-1,2-dihydroquinoline
ES	Electrospray
Et	Ethyl
Et_2O	Diethylether
Fmoc	Fluoren-9-ylmethoxycarbonyl
Glu	Glutamic acid
Gly	Glycine
h	Hours
HATU	O-(7-Azabenzotriazol-1-yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
HOAt	1-Hydroxy-7-azabenzotriazole
HOBt	1-Hydroxybenzotriazole
HPLC	High performance liquid chromatography
IR	Infra red
J	Coupling constant (Hz)
Leu	Leucine
Lys	Lysine
m	Multiplet
Me	Methyl
MeOH	Methanol
min	Minutes
MS	Mass spectrometry
NBS	N-Bromosuccinimide
NMM	N-Methylmorpholine
NMP	1-Methyl-2-pyrrolidinone

NMR	Nuclear magnetic resonance
PEG	Poly(ethylene glycol)
PEGA	Poly(ethylene glycol) dimethylacrylamide
Ph	Phenyl
Phe	Phenylalanine
Phth	Phthalimide
ppm	Parts per million
Pro	Proline
p-TSA	*para*-Toluenesulphonic acid
PyBoP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
PyBrOP	Bromo-*tris*-pyrrolidinophosphoniumhexafluorophosphate
pyr	Pyridine
q	Quartet
quint	Quintet
R_f	Retention factor
rt	Room temperature
s	Singlet
sext	Sextuplet
SPOS	Solid phase organic synthesis
t	Triplet
TBTU	O-(Benzotriazol-1-yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TMAD	N,N,N',N'-Tetramethylazodicarboxamide
Tyr	Tyrosine
UV	Ultra violet
WSC	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
δ	Chemical shift (ppm)

Chapter One

Introduction

Part One: Combinatorial Chemistry and Biological Screenings

1.1.1 Search for Active Compounds: the Role of Organic Synthesis

One main goal of organic chemistry for the last hundred years has been the most efficient and selective synthesis possible of a single target molecule of defined structure. Since the first synthesis of urea by Wöhler in 1828,[1] chemists have found ever more efficient and selective methods enabling the synthesis of almost any compound such as vitamin B_{12},[2] Calicheamycin[3] or Taxol.[4] Once a selected target has been synthesised, purified and characterised, it is ready to be tested for specific activities. The activity is then optimised, partly by systematic and partly by intuitive variation of the initial structure. A new active substance is obtained by trial and error and many thousands of molecular variations must be synthesised before a marketable product is found.

Rapid progress in molecular biology and gene technology has made it possible to understand diseases and medical symptoms at the molecular level and has opened the door to rational drug design. Of great importance is the use of biological targets to design efficient test systems, since this enables thousands of compounds to be screened in a relatively short time and with relatively small amounts of material. This method of "High Throughput Screening" requires a large number of structurally diverse test substances, and this is where traditional organic synthesis reaches its limits. The preparation of test substances becomes the bottleneck in the search for active compounds, the speed of synthesis, or more precisely, the number of substances synthesised per time unit, thus becomes a new dimension in organic synthesis (high-throughput synthesis), in addition to yield and selectivity.

1.1.2 Combinatorial Chemistry: How and Why?

It has been estimated[5] that the number of relatively small (MW < 750) organic molecules that could be made applying the rules of valence to carbon and its neighbours in the periodic table is 10^{200}. One of these molecules will hopefully possess the optimal molecular recognition properties for binding to a given biomolecule. Moreover a different single member of this set will display the right combination of

potency, stability, water solubility, bioavailability, toxicity, ease of synthesis and patentability to be the optimal drug candidate for a biological target.

Combinatorial chemistry represents the product of matrix chemistry just as a total synthesis represents the product of linear chemistry. In total synthesis the goal is to make a single target compound whose exact structure is for some reason significant and therefore inviolate. By contrast, the goal of combinatorial chemistry is to achieve the synthesis of many related structures, none of which is critical, but which, on the whole, fills an area of so-called diversity space.

Combinatorial synthesis takes its lead from nature, which succeeds in obtaining from only a few synthetic units (for example, the twenty amino acids or the four nucleotides) a huge number of products with diverse functions (peptides, RNA) by combinatorial principles. Combinatorial chemistry is based on a principle that is both simple yet revolutionary; instead of functionalising a single starting material with two reagents X and Y and obtaining a single product, n different variants of X and m variants of Y are used. A X_n x Y_m reaction matrix can be written, in which n x m elements exist, each corresponding to a different product. A greater number of reagents and variants are used and the matrix grows exponentially. The set of all the compounds produced in a combinatorial synthesis is known as a library. In a multi-step combinatorial synthesis, the size of such a library corresponds to the total number of all the substances synthesised and is determined by the number of building blocks used per reaction (linear relation) and the number of reaction steps (exponential relation).

The first compound libraries generated by combinatorial synthesis were reported by peptide chemists in the 1980s: pioneers such as Geysen[6] and Houghten[7] developed various methods for the multiple parallel synthesis of peptides based on the original technique of solid-phase peptide synthesis developed by Merrifield.[8]

In the last ten years the focus of research in the field of combinatorial synthesis has been directed away from peptides (often having a number of disadvantages as drugs due to poor bioavailability and ready degradation) towards libraries of small organic molecules.[9]

1.1.3 High Throughput Synthesis and High Throughput Screening

Combinatorial libraries are typically created by one of two methods: split synthesis and parallel synthesis. The first is used to produce small quantities of a relatively large number of compounds (up to a hundred thousand), whereas parallel synthesis yields larger quantities of a relatively small number of compounds (usually not more than a few hundred). Split synthesis can also be used to produce mixtures rather than discrete compounds.

How big must a library be and therefore which method should be chosen for its synthesis?

A "hit" is defined as a compound of a library (or of a natural product extract) that has activity in a primary assay according to pre-established criteria (e.g.: inhibition of a ligand binding to a receptor by 50% at 5 μg/mL). The term "hit rate" is defined as the number of samples meeting pre-established activity criteria as a function of the total number of samples tested. It could be erroneously thought that the bigger the library the easier to find a hit compound. This is not necessarily true: what matters in a library is not the size but the diversity content (finding a needle in a haystack does not become easier if we increase the size of the haystack! Although one may argue that you increase also the number of needles!).

Each member of a library can be theoretically placed in a multidimentional chemical space defined by chemical descriptors such as solubility, molecular weight, partition coefficient, pKa, drug bioavailability (which can be measured or calculated for each molecule). The aim of a combinatorial library is to fill so-called diversity space, therefore a set of a thousand compounds concentrated in a small area of this space might be less useful than a set of a hundred compounds spread out on a larger area. Thus the chance of finding an active compound in a library increases with the diversity and dissimilarity of the compounds in the library. This concept can been exemplified by the following figure (Figure 1.1). One can imagine that the ideal active compound for a given biological target is represented by the top of the mountain. The members of a combinatorial library (represented by the coloured spots) will cover part of the mountain, and more chances to get closer to the top are given by the first library which is more diversified and covers a bigger area of the mountain.

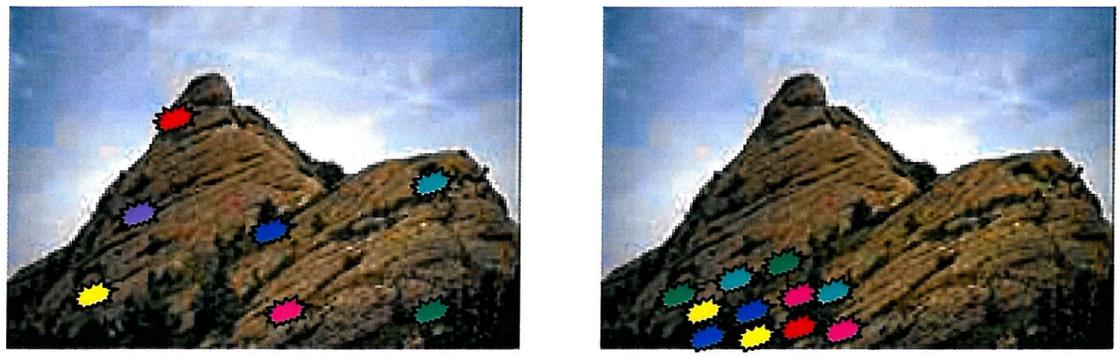


Figure 1.1: Representation of a diversity space filled by two libraries with different diversity content.

The diversity of a chemical library is not absolute but depends on the biological target. For example the compounds thiorphan and *retro*-thiorphan[10] (Figure 1.2) not only have similar appearance at first glance but they also bind to the zinc proteases thermolysin and neutral endopeptidase (NEP) with almost identical affinity. One would accordingly class them as similar. However, at another site of action, the angiotensin-converting enzyme (ACE), which is also a zinc protease, thiorphan binds by about a factor of 100 more strongly than *retro*-thiorphan. The two molecules are therefore not so similar in relative terms to this enzyme.

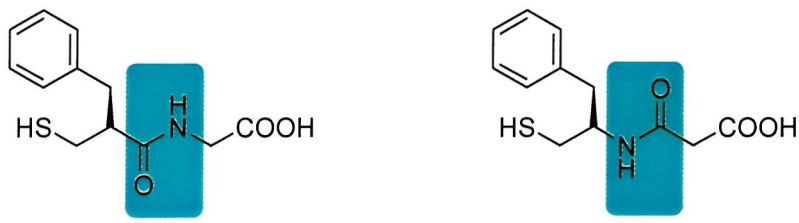


Figure 1.2: Structural similarities of thiorphan and *retro*-thiorphan.

Having said that in a chemical library diversity is often more important than size, it remains to find how diversity can be achieved. Because of the considerable amount of data required, combinatorial chemistry is unthinkable without the support of electronic data processing. Chemical databases for selection of reagents (such as the *Available Chemicals Directory, ACD*), reaction databases (such as *REACCS*, *Crossfire*,

Synopsys), virtual compound libraries databases (such as *Project Library*, *Legion* or *Unity*), programs that allow the intelligent selection of the compounds to be synthesised to achieve a maximum diversity with the minimum number of compounds (*Selector*, *Jarpat*, *C^2-Diversity*, *Chem-X*) are currently available. These, together with automated synthetic equipment developed from peptide synthesisers, are becoming more and more sophisticated. The whole of this effort can be summarised with the words *High Throughput Synthesis*. In parallel with this is *High Throughput Screening* (HTS) which is the capacity to biologically test the huge numbers of compounds synthesised. It has been hypothesised that most major pharmaceutical companies will be screening 30-60 targets per year against 500,000-1,000,000 compounds. This requires the adoption of new technologies. To screen much larger numbers of compounds whilst avoiding proportional increases in reagents and consumable costs, assay volumes must be lowered while assay plate densities have begun to be increased from 96 to 384 or 1536 wells-per-plate. This evolution is leading to what is called *ultra High Throughput Screening* (uHTS).

1.1.4 Screening Strategies for Chemical Libraries: Biochemical and Cell-based Assays

Combinatorial screening strategies are nearly as diverse as synthetic strategies, and one of the major innovations of combinatorial chemistry is that screening and synthesis can be linked together.

The simplest strategy currently considered "combinatorial" is the parallel chemical synthesis of many individual compounds, which are subsequently tested as single chemical entities. The innovation is completely on the synthesis end, and synthesis and screening are not strategically linked beyond the ability to conduct structure-activity studies more rapidly than in the past. The major advantage is that this strategy is compatible with all existing assays. The major disadvantage is that the search rate is relatively limited.

In order to exploit the potential of combinatorial methods, compounds can be screened as mixtures. Screening mixtures can be considered to be related in some way with natural product screening, where new compounds are discovered from a complex extract using analytical fractionation, retesting, purification and structural elucidation. Combinatorial methods have a number of conceptual advantages over natural product

screening: in natural product screenings the chemical composition of the active natural product is beyond the control of the chemist and requires structural elucidation while the composition of combinatorial libraries is under the complete control of the medicinal chemist. In many cases compounds isolated and identified from a natural source are too unstable or too difficult to synthesise, while unstable and unsynthesisable compounds are excluded from a combinatorial library from the beginning. While natural product extracts are not predisposed to revealing their structures, combinatorial libraries can be synthesised in a fashion that facilitate determining the structure of an active compound in a mixture using various deconvolution strategies without analytical separation, purification and structural characterisation. On the other hand one may argue that in combinatorial libraries problems related to multiple active compounds, false positive and false negative results are worse than in a natural extract because the molecules are structurally similar to each other. It is always difficult to make a comparison like this because natural extracts also often contain many types of secondary metabolites that are related. The screening strategy adopted for a given biological target depends on many different factors depending on the kind of information required, the medicinal chemistry resources available, the type and size of the library screened and the cost. Screening assays are usually divided into biochemical assays and cell-based assays; a third category, functional assays, includes all those assays performed on whole animals or tissues.

Biochemical assays are based upon defined molecular entities and usually involve the binding of a ligand to a receptor or the inhibition of an enzyme-catalysed reaction. They might involve highly-purified components, complex mixtures from cell extracts or partially-purified biochemical preparations. Most often they are designed to measure a single, well-defined and well-known molecular reaction or the interaction of two biological molecules. The amount of information that may be obtained from a biochemical assay can be limited. For example, one can readily determine if a compound inhibits a particular enzyme when present under defined and artificial conditions in the well of a microtiter plate. But the compound that is active in such a biochemical assay may not be active when applied to whole cells, due to its inability to cross the cell membrane, its susceptibility to metabolism by the cellular enzymes or its

inability to compete for binding to the molecular target in the presence of an intracellular component that was not present in the original screening assay.

In cell-based assays issues of cell-permeability, metabolism and binding in the presence of intracellular constituents are addressed during the initial screening process. Although cell-based assays have the potential to identify the same compounds as found with a specific biochemical assay, they may also uncover compounds that act at other sites, within a signal transduction pathway. Cell-based assays are therefore optimal in situations where the individual molecular steps of a pathway are not fully understood. A disadvantage of cell based assays is that once an interesting compound is identified, it is often necessary to do considerable additional research to understand the molecular site of action.

1.1.5 An Overview on Different Types of Screening Assays

Biochemical assays:

Binding assays are widely used in combinatorial chemistry: extracellular and intracellular receptors have always been very productive drug targets and can be easily converted to assays in a HTS mode. Crude cell membrane preparations are frequently used as a source of receptors, and this is usually more convenient than expensive highly purified receptors. The same membrane preparation can contain different receptors and therefore can be stored in bulk quantities and be used in different assays. The interaction of a molecule with a target receptor can be visualised and quantified using many different techniques usually based on fluorescence phenomena or scintillation.

Enzyme assays are used to discover bioactive compounds that interfere with a specific enzyme-mediated biological reaction. To inhibit the enzyme a compound may block its active site (competitive inhibition) or may interact with some other region of the enzyme that results in the perturbation of the active site (noncompetitive inhibition). The enzyme reaction may be a single-time point assay or a continuous, kinetic assay. In the former, the reaction is allowed to run for a specific period of time, then stopped and either the remaining substrate or the newly generated product is measured. In the kinetic assay, the rate of the reaction is constantly monitored over a set period of time. Until recently, the single-time point assay was used almost exclusively for HTS, but

today's advanced microplate readers can follow the rate of enzymatic reactions simultaneously in each well of a microtiter plate.

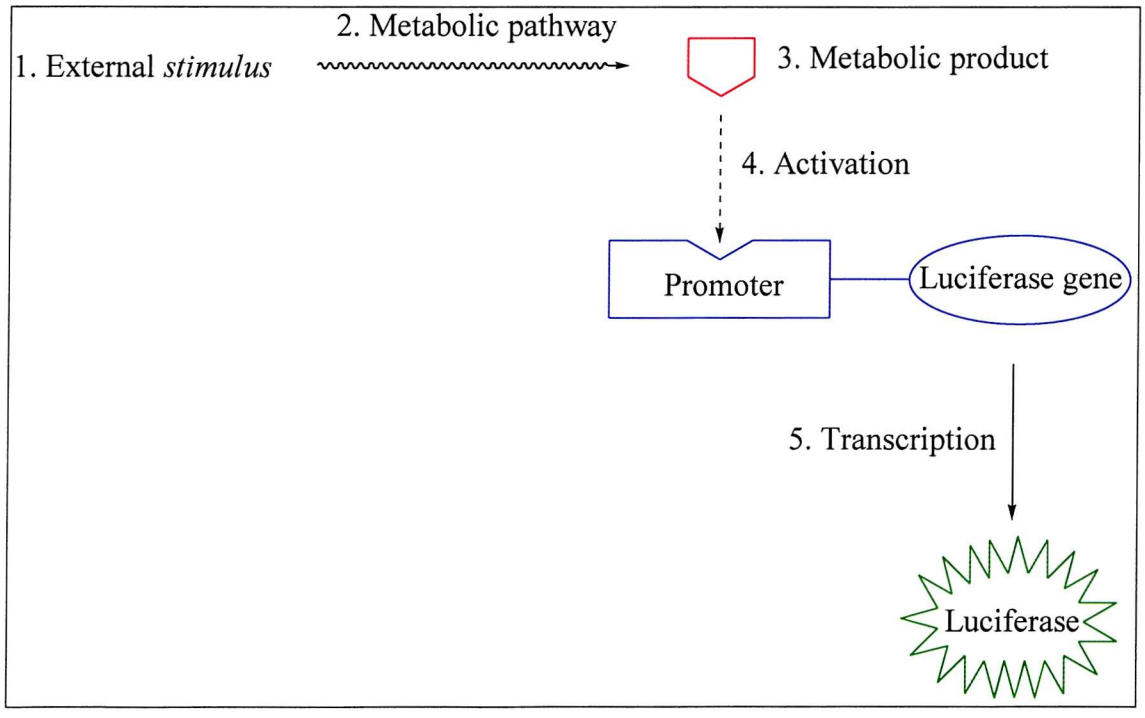
Cell-based assays:

Different cell-based assays have been successfully used in HTS. The most common ones are reporter gene assays and cell proliferation assays.

Each gene is associated with a promoter that, upon activation by an external factor, promotes the transcription of the gene and in last instance the synthesis of the enzyme the gene encodes. In *reporter gene assays* (Scheme 1.1) the presence or absence of a specific gene product is used to monitor changes in a specific metabolic pathway. The gene product is typically an easily quantifiable protein that is not usually present in the cell. The most common reporter is firefly luciferase,[11] because of its sensitivity and ease of detection. Luciferase catalyzes a reaction of luciferin with ATP resulting in the emission of photons that are readily quantified:

$$\text{ATP + luciferin} \xrightarrow{\text{luciferase}} \text{adenyl-luciferin + } PP_i$$

$$\text{adenyl-luciferin-}O_2 \longrightarrow \text{adenyloxyluciferin + } O_2 + h\upsilon$$



Scheme 1.1: Reporter gene assay.

In a typical experiment[12] a fragment of a plasmid containing the promoter for the metabolic pathway under study is transcriptionally fused to the genes coding for luciferase (the reporter genes).

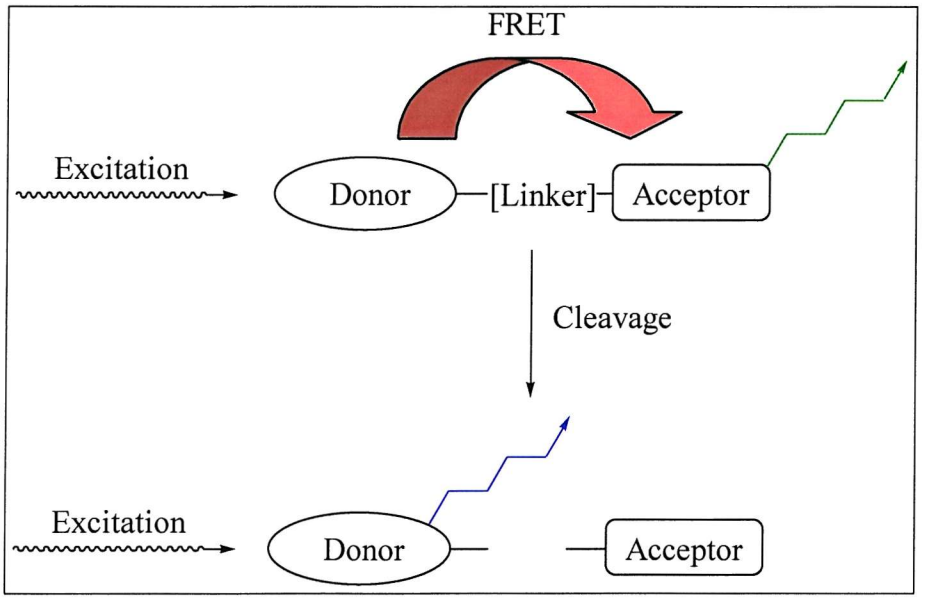
Cells can be induced to proliferate in response to the appropriate pharmacological stimuli. In *cell proliferation assays* changes in the rate of cellular proliferation are monitored and used as an index of pharmacological responses.

1.1.6 Visualisation and Quantification of Biological Responses

Visualisation and quantification of a biological response in a screening assay is usually based on fluorescence or scintillation techniques.

1.1.6.1 Fluorescence techniques

Fluorescence techniques represent one of the most important methods in high throughput screening. Moreover, due to the high sensitivity of fluorescence measurements, they seem to be the method of the future for miniaturised ultra-high-throughput screenings (i.e. 3456-well plate format). Fluorescence measurements are made in several ways and some of the macroscopic techniques can be applied in HTS: *Fluorescence Resonance Energy Transfer* (FRET)[13] can occur between two fluorophores in close proximity with suitably overlapping spectra.

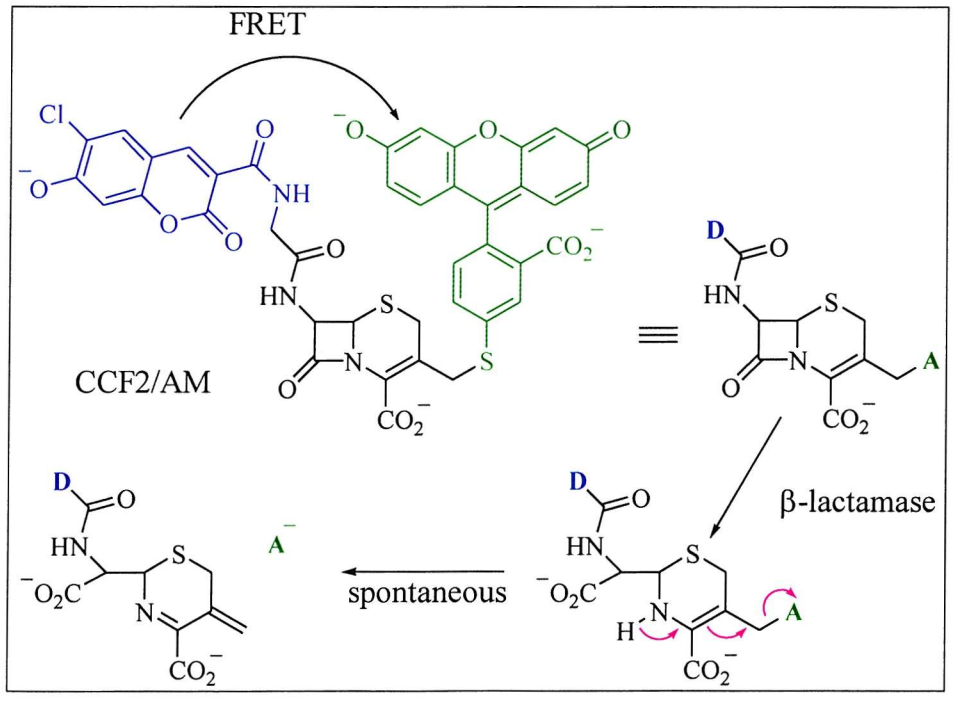


Scheme 1.2: Diagrammatic representation of FRET. When FRET occurs, the acceptor emits green light. When FRET is broken, the donor emits blue light.

Excitation of a 'donor' fluorophore can result in the transfer of energy to an 'acceptor' that has a longer wavelength of emission. An event causing an increase in distance between these two fluorophores can be measured by monitoring disruption of the FRET through re-establishment of the fluorescence emission of the donor (Scheme 1.2).

Many biological responses result in a change in distance between molecules and therefore this approach can be used in biological screening. One example of FRET-based assay is given by a chymotrypsin cleavage study of a library of FRET-peptides: these peptides contain terminal donor and acceptor. Active peptides will be cleaved by chymotrypsin causing a disruption of the FRET that can be monitored. This assay was performed on a 3456-well plate.

Cell-based assays have also been used with this technique in uHTS (3456 format). Although various concerns over the feasibility of such assays in highly miniaturised formats, a gene reporter assay had been successfully performed in which the β-lactam fluorophore CCF2/AM[14] was used to monitor the expression of β-lactamase in target cells: only cells expressing this reporter gene emitted blue light, due to disruption of FRET in the β-lactamase substrate CCF2/AM (Scheme 1.3).



Scheme 1.3: Structure of CCF2/AM and its mechanism of action.

The fundamental principle of *fluorescence polarisation* (FP)[15] is that fluorophores emit light when excited by plane-polarised light and the polarisation of the emitted light depends on how fast the fluorophore rotates during the lifetime of its excited state. FP depends therefore on the rotational characteristics of the fluorophore in solution: the smaller the molecule, the faster it rotates, and the smaller its FP will be. Binding of a fluorescence-labelled ligand to its receptor will result in a reduction of rotation and an increase of FP.

In *Fluorescence Correlation Spectroscopy* (FCS) single molecules are measured as they diffuse through the measurement volume: the speed of diffusion is dependent on their mass, therefore free ligands diffuse more rapidly than ligand-receptor complexes.

Although these methods are very efficient and sensitive, there are a few problems associated with the detection of fluorescence signals: the signal can be subject to quenching by compounds, plastics or media, fluorescence emissions can be scattered by particulates or the signal can be masked by autofluorescence (from proteins or compounds) or by high background fluorescence (from unbound labelled probes). Europium and other rare earth Lanthanides are known for the slow decay of their fluorescent signal. Fortunately interfering background fluorescence has a very short decay time, and the long-lived Eu^{+3} fluorescence can be measured after the short-lived background has decayed (usually after 50 msec).

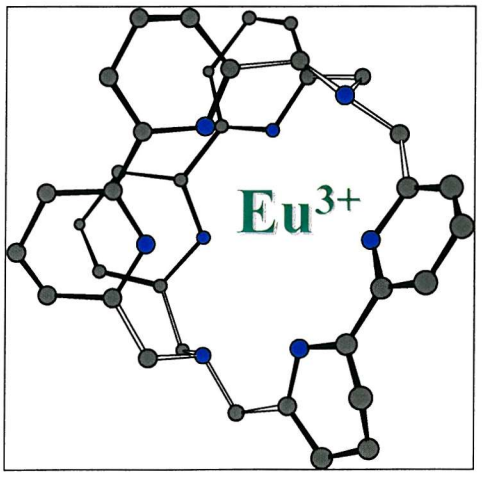
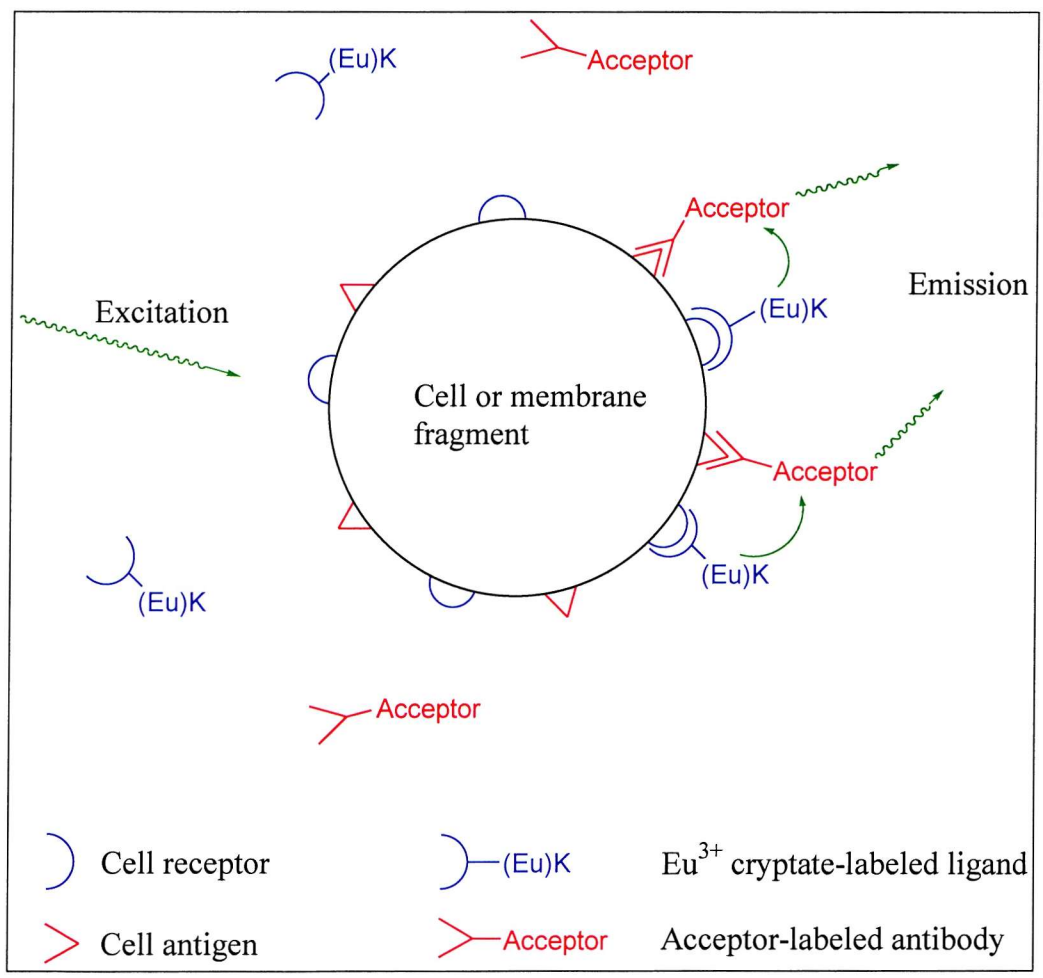


Figure 1.3: Europium cryptate ((Eu)K).

However, Eu^{3+} is not a good fluorescent label by itself, but can be complexed with macropolycyclic compounds[16] (Figure 1.3) able to collect the excitation energy and transfer it to Eu^{3+} but unable to quench the fluorescence emitted by Eu^{3+}.

Time-resolved fluorescence intensity (TRF) techniques are based on the energy transfer between a lanthanide ion chelate or cryptate to a fluorescence acceptor molecule resulting in a prolonged emission of light at a characteristic wavelength. The energy transfer is distance dependent and it is unlikely to happen when the molecule is free because it is rarely in close enough proximity over the required time scale. When two molecules, labelled respectively with the cryptate (e.g.: (Eu)K) and the acceptor, interact the fluorescence acceptor molecule will be in close enough proximity of the fluorescence emitter and therefore a signal can be detected.



Scheme 1.4: TRF technique for antibody-antigen interaction.

The molecular interactions that can be studied with TRF are those in which the association or dissociation of macromolecules labeled with (Eu)K and the acceptor are of interest, such as receptor-ligand, protein-protein, DNA-DNA, DNA-RNA, protein-DNA, antibody-antigen. Enzyme mediated reactions that cleave (proteases, nucleases) or synthesise (kinases, polymerases) compounds are also candidates for TRF. In Scheme 1.4 an example of how TRF can be used to monitor an antibody-antigen interaction is given.

1.1.6.2 Scintillation techniques

The principle of the scintillation proximity assays[17] is the emission of light when a scintillant material (usually scintillant coated or filled beads) is excited by the radiation emitted by a radioligand in its close proximity. The β particles emitted by ^{3}H and the Auger electrons released from ^{125}I by isotopic decay have very short pathlengths in water. If a molecule labeled with ^{3}H or ^{125}I is bound to the bead surface, it is in close enough proximity for the emitted radiation to activate the scintillant contained within the bead and produce a light signal. The amount of light produced is proportional to the amount of labelled molecules bound to the beads and can be measured with a scintillation counter. The energy of β particles or Auger electrons released from molecules not attached to the bead surface is absorbed by the aqueous solvent before it reaches the bead and no light is produced. The consequence of this is that ligand-receptor interaction can be quantified without the need for separation of bound from free ligand, and the whole experiment can be performed in a single-step format. The scintillant proximity approach has been used successfully in screening enzymes, receptors, protein-protein and protein-DNA interactions. Scintillant coated beads are prepared by incubating a DMSO solution of 2,5-diphenyloxazole (a well-known scintillant molecule that is a major component of most commercially available scintillation cocktails) with resin beads. Addition of water to the system results in the DMSO being washed away from the beads whilst the scintillant molecules, being insoluble in aqueous media, are precipitated within the pores of the beads. Unfortunately this manufacturing process ensures that SPA beads are completely incompatible with the use of organic solvents, where the scintillant molecules are soluble. A more elegant approach has been reported by A. J. Sutherland[18] who co-polymerised 2,5-diphenyl-4-vinyloxazole with 4-vinylbenzyl chloride, 4-ethylstyrene and divinylbenzene, using standard suspension polymerisation techniques. This

chemically-functionalised scintillant-containing resin retains the ability to scintillate even after prolonged exposure to organic solvents. A drawback of these polystyrene beads is that the assay has to be carried out in organic solvents, the resin being uncompatible with water.

A similar principle is employed with the Flashplate,[19] where the interior of each well of a microtiter plate is coated with a thin layer of polystyrene-based scintillant. The wells may then be coated with the target receptor and radiolabelled ligand binding detected through activation of the scintillant.

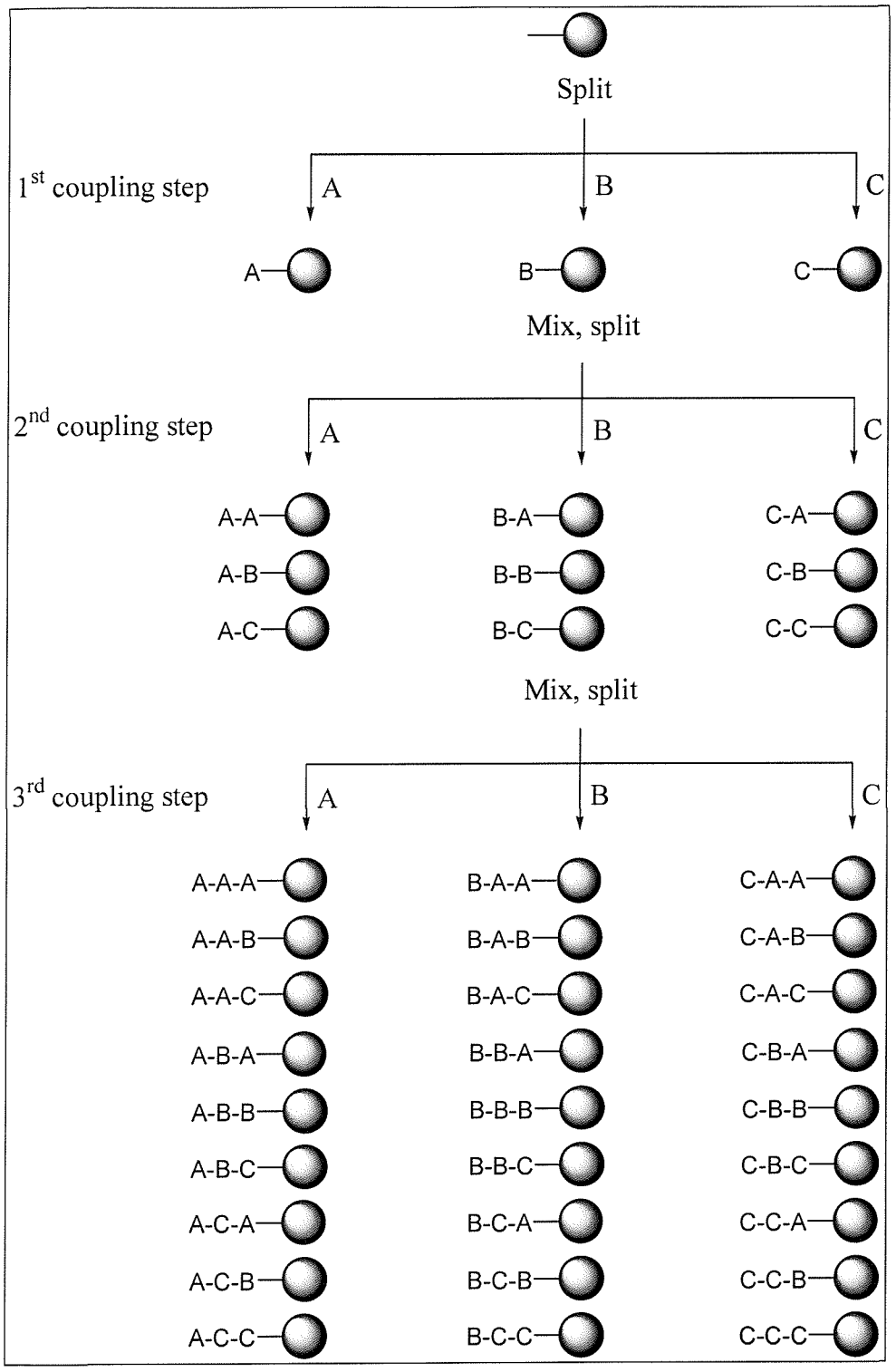
1.1.7 The One Bead-One Compound Concept

The split and mix method in combinatorial chemistry was introduced for the first time by Furka in 1988.[20] It was Lam[21] in 1991 who first recognised the one-bead-one-compound concept, based on the fact that combinatorial bead libraries contain single beads displaying only one type of compound, although in millions of copies. Different compounds are therefore spatially separable and can be tested concurrently but independently (scheme 1.5).

Moreover both on-bead binding or enzyme assays and solution-phase assays can be used by this approach. In the solid phase assays the ligands are still covalently attached to the solid support and the assays involve either direct binding of molecular target to the bead-bound ligand or detection of functional properties of the bead-bound ligand. In the solution phase assays a linker orthogonal to the synthesis is required to cleave the compounds off the beads.

1.1.8 Single Bead Screening: On-Bead and Solution-Phase Assays

A reliable high-throughput assay is essential to successfully screen a split and mix library. As mentioned before both solid-phase and solution-phase assays have been developed for the one-bead-one-compound combinatorial library method. In general it is preferred to screen libraries of small drug like molecules in solution, where the whole of the molecule is exposed to the assay target. For large peptidic libraries the on-bead format has often being preferred. Most forms of bead based screening require the distribution or 'picking' of single resin beads; this can be done by hand, however a number of different automated systems are also available.



Scheme 1.5: The *split and mix* method.

1.1.8.1 On-bead screening

The first parameter that has to be taken in account is the choice of the solid support: since most biological assays are carried out in aqueous media the solid support must

be compatible with water. TentaGel (polyethyleneglycol grafted polystyrene),[22] PepSyn gel (polydimethylacrylamide)[23] and PEGA resin are therefore the solid supports of choice. Both binding and enzyme assays have been applied to on-bead screening.

In the *binding assays* the interaction of a target with a bead-bound ligand is usually detected visually. If the molecular targets are intrinsically coloured or fluorescent a bead library can be screened directly, otherwise a reporter group such as an enzyme or a fluorescent probe is attached to the required target. In both cases the active beads will become coloured and can be picked for analysis and characterisation. There are a few examples in which the targets have been labelled with a radionuclide like ^{3}H, ^{125}I or ^{14}C:[24] the active beads are detected with an autoradiographic method. This method does not give immediate response, in fact the beads first must be immobilised and then exposed to the radiographic film. The film is then developed and the positive beads are isolated under a microscope. However, tagging with a radioisotope rather than with a large enzyme may be sterically preferable, particularly for small molecule targets.

Autoradiography has also been used in on-bead *enzyme assays*. Lam[25] reported its use to identify resin-bound peptides substrates of a protein kinase. The one-bead-one-compound library was incubated with the enzyme in the presence of the radiolabeled specie [γ-^{32}P]ATP. The beads were then immobilised and exposed to a X-ray film.

1.1.8.2 Solution-phase screening

In principle all biochemical and cell-based assays previously described can be adapted to solution-phase single-bead screenings. To maximise the power of the split and mix method it may be necessary to use a tagging system or a multiple-release technique (see below). The ligands, attached to the solid support *via* a cleavable linker, are released into solution phase where the biological assays take place. The bead that "holds" the active compound can subsequently be identified and isolated for structure determination.

If the number of beads to be screened is not big, each bead can be place in a well of a 96-well plate and the compounds screened as single entities. If the number is high (more that 5000) a two-stage release assay can be carried out, therefore double orthogonally cleavable linkers are incorporated prior to the preparation of the library. 100-500 beads are placed in a single well, part of the compounds released into solution

and tested. Beads from the positive wells are then redistributed one per well and the portions of compound still attached onto the resin beads released and tested again.

1.1.9 Structure Determination of Identified Hits in Single Bead Screenings

When the mix and split concept was first introduced, combinatorial methods were mainly applied to the synthesis of peptidic and oligonucleotidic libraries. Structural elucidation of screening "hits" was obtained simply by direct sequencing *via* Edman degradation or polymerase chain reaction (PCR). The increasing interest for the combinatorial synthesis of small organic molecules led to the need for new methods for compound identification, since direct sequencing was no longer feasible and identification problematic on the available quantities (usually of the order of pmoles) of individual library members. Different methods have been developed in the last years, and they can be divided into encoding and multiple-release strategies.

Encoding strategies were introduced for the first time by Brenner and Lerner,[26] who contemplated the use of oligonucleotides for coding peptide libraries. The basic idea behind encoding techniques is to keep record of all the synthetic steps of a library synthesis directly on the bead, using specific codes for each step. These codes are readily analysable molecules called *tags*.

The method suggested by Still[27] introduces the tags directly onto the polystyrene matrix of the resin beads: these tags are halophenol derivatives, which are chemically inert and easily analysed at subpicomolar levels by electron capture gas chromatography.

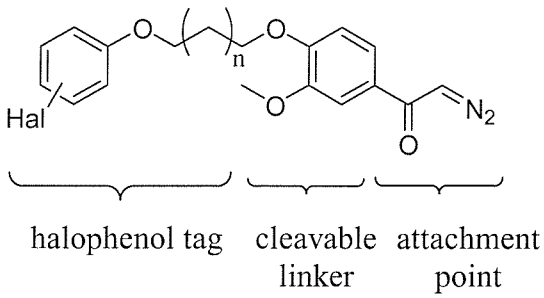


Figure 1.4: The Tag system elaborated by Still.

Figure 1.4 shows the general structure of a tag, where three distinct parts are recognisable: the halophenol ether, which contains the unique code, the catechol diether moiety, which can be cleaved oxidatively to effect tag release for analysis, the diazoketone functionality, which can be converted to a reactive acylcarbene for direct attachment to the polymer matrix.

A different tagging system has been elaborated by Gallop[28] and uses chemically robust secondary amines (Figure 1.5).

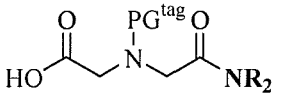
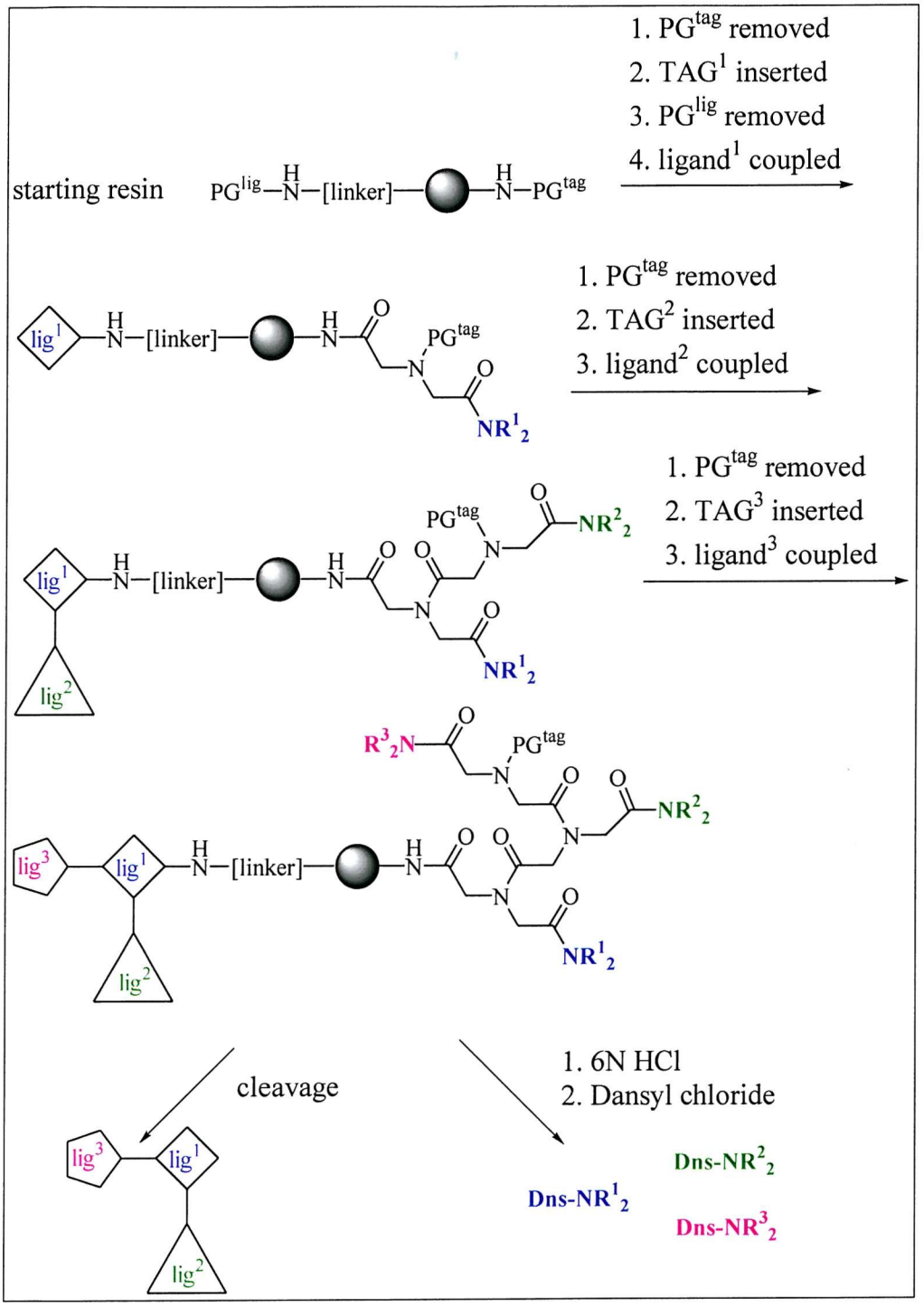


Figure 1.5: Tagging monomer unit.

These amines are incorporated into an N-((dialkylcarbamoyl)methyl)glycine oligomer through simple chemistry compatible with a wide range of polymer-supported transformations. In the decoding process, acidic hydrolysis of the tagging polymer regenerates the secondary amines, which after dansylation are resolved and detected at subpicomolar levels by fluorescence HPLC analysis. In this case the tags are introduced onto an amine-based resin that is differentially functionalised with sites for ligand synthesis and sites for tag addition. These sites are defined by orthogonal protecting groups that permit the chemistry to be addressed unambiguously at either the compound (90%) or tagging entities (10%) (Scheme 1.6).

In *multiple release* techniques compounds are released from the solid support for biological screening only partially. A fraction of the compound is still on the bead to allow identification of the structure. A combination of linkers with different cleavage sensitivities is therefore required. The first example of multiple release strategy was published by Lebl in 1993[29] and allowed five independent releases of a peptide, although with different C-terminal functionalities. In 1996 Bradley[30] published an alternative approach using a combination of three different linkers in ratio 1:1:1 (Figure 1.6). After solid phase peptide synthesis, the first portion of the peptide was released with 1% TFA in DCM. The second release was accomplished with 95% TFA in DCM, while the last portion of peptide was sequenced *via* Edman degradation.



Scheme 1.6: The encoding method developed by Gallop.

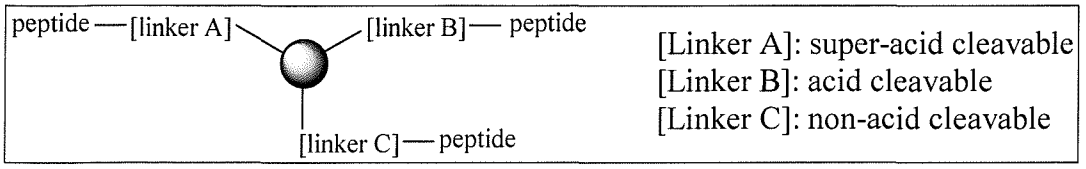


Figure 1.6: The multiple release strategy developed by Bradley.

1.1.10 Is Resin-bead Loading an Issue?

In HTS the assay volumes are nowadays in the order of microliters (2-200 in general), therefore the amount of substrate required is in the order of picomoles, for activity in the μM range. The amount of compound released by a single resin bead is usually sufficient for biological assays, however, if multiple screenings or IC_{50} determinations are required, commercially available resins are not suitable. The demand for high-loading resins has drastically increased in the last years, and different solutions have been found to overcome this deficiency.

The use of microreactors such as MicroKans[31] and NanoKans allows the synthesis of multi-milligram quantities of compounds. This method does not use single beads but groups (*bundles*) of beads kept together in a microreactor. This is labelled with a radiofrequency tag or with a barcode, therefore the concept of split synthesis can be still applied and the final compounds identified. Libraries consisting of hundreds or thousands of compounds have been synthesised with MicroKans, while NanoKans allow the synthesis of tens of thousand discrete compounds.

Another possible solution is the use of a bigger solid support: resin beads with larger size have been introduced, but usually they have shown increased fragility and lower kinetics.

The research reported in this thesis is focussed on bead-loading enhancement *via* a dendrimerisation process. In the second part of Chapter One an introduction on dendrimeric molecules will be given, while in the following chapters the synthesis, properties and applications of the so-called *dendrogel* will be given.

Part Two: Dendrimeric Molecules

1.2.1 Historical Introduction

From a mathematical/phenomenological perspective, examination of the total number of covalent bonds formed and the mode of bond formation in a reaction sequence provides a criterion by which to classify synthetic covalent chemistry into three recognisable areas:[32]

- **Traditional organic chemistry** (since 1828): incremental number of covalent bonds formed using classical reagents; relatively small molecules formed; precise control of size, shape, mass, functional groups, topology and flexibility.
- **Traditional polymer chemistry** (since 1930): large (multiple) numbers of bonds formed using reactive monomers; large molecules formed; statistical distribution of products as a function of size and mass; some control of topology and flexibility; virtually no control of shape.
- **Dendritic macromolecular chemistry** (since 1978): exponential numbers of covalent bonds formed using branch cell reagents; large molecules formed; precise control of size, shape, mass, functional groups, topology and flexibility.

The term dendrimer[33] is given in deference to the branched structure adopted by these molecules and the name comes from the prefix dendri- meaning *treelike* (from Greek *dendron*, tree) and the word polymer.

It is possible to distinguish three periods of time in which macromolecules with branched architecture have been deliberately synthesised: the first period is from the late 1860's until the early 1940's, when branched structures where considered responsible for insoluble and intractable materials formed in polymerisation reactions. In 1869 Zincke[34] and in 1885 Friedel and Crafts[35] reported the synthesis of an insoluble hydrocarbon-based material upon treatment of benzyl chloride with copper or aluminium chloride. It was speculated that its structure was polymeric, non-linear or branched.

From the early 1940's until 1978 branched structures were considered primarly from a theoretical point of view,[36] since attempts to synthesise them *via* polymerisation of functionally differentiated monomers resulted impractical and fruitless.[37]

The first who reported a dendrimer synthesis was Fritz Vögtle in 1978,[38] introducing the concept of *cascade reaction*: Michael type addition of an amine to acrylonitrile permitted the attachment of the initial two arms, or branches, subsequent reduction of

the nitriles gave the desired diamine, which was subjected to the same synthetic sequence to afford 4-cascade:benzylamine[2-N,N]:(1-azabutylidene):propylamine. At this stage the concept of control over macroassembly construction was better developed, thanks also to advances in physical isolation and purification, as well as the introduction of diverse spectroscopic procedures.

After the initial disclosure of the concept of cascade synthesis[38] ("*...reaction sequences that could be carried out repeatedly, whereby a functional group is made in such a way as to appear twice in the subsequent molecule.*") different authors explored the use of repetitive chemistry for the preparation of dendritic materials. Denkewalter, Kolc, and Lukasavage[39] patented a method for the synthesis of poly-lysine-based dendrimers. Newcome[40] reported the synthesis of polyol dendrimers relying on triester amidation with 'tris'. Tomalia[33] reported the synthesis of polyamidoamine (PAMAM) dendrimers (one of the most investigated families). Fréchet and Hawker[41] published in 1990 the synthesis of poly(aryl ether) dendrimeric architectures. Miller and Neenan[42] in the same year reported the preparation of the first aromatic-based all-hydrocarbon dendrimers. Synthesis of silyl-based dendrimers was reported by Van der Made and van Leeuwen in 1992.[43] They were obtained *via* a repetitive hydrosilylation and alkenylation sequence.

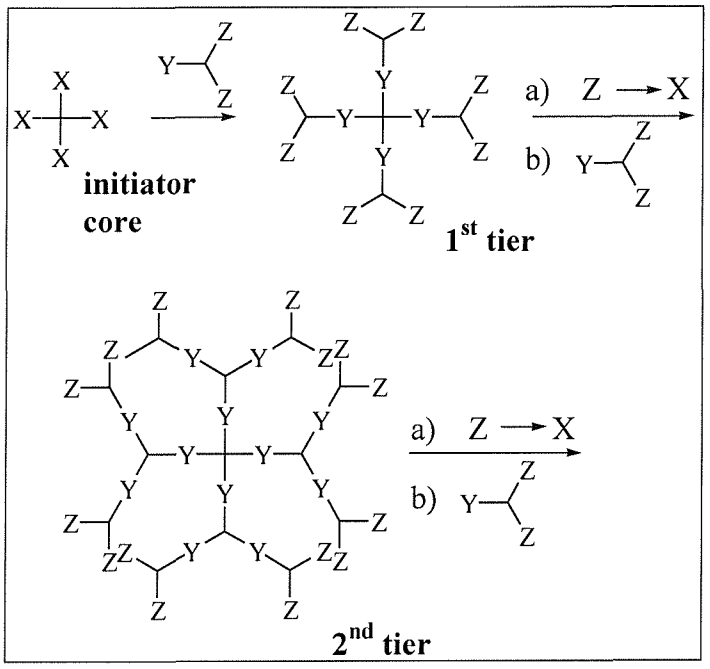
Wong[44] has reported the solid phase preparation of branched glycopeptides. A key step in the synthesis included the connection of β-1,4-galactosyl monomeric units *via* treatment with β-1,4-galactosyltransferase. Synthesis of phosphine dendrimers was firstly reported by DuBois.[45] Chapman[46] recently reported the construction of "*polycules*" which were generated from substituited 1,3,5,7-tetraphenyladamantanes.

1.2.2 Synthetic Approaches

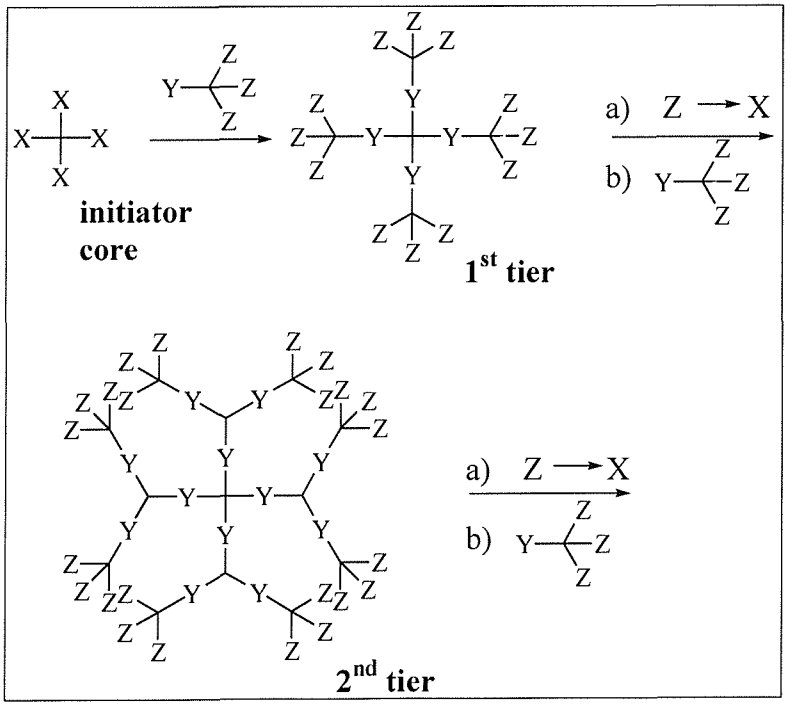
There are two main methods for assembling dendrimeric molecules: divergent synthesis and convergent synthesis.

The first method, *divergent synthesis*, involves three distinct facets: a starting point, or initiator core, that possesses multiple reactive sites, a reaction sequence so that a reactant is added to each of the reactive sites, and a branching reaction to increase the multiplicity of the molecule and generate a new branch point (Scheme 1.7 and 1.8). A key feature of the divergent method is the exponentially increasing number of

reactions that are required for the attachment of each subsequent tier (layer of generation).



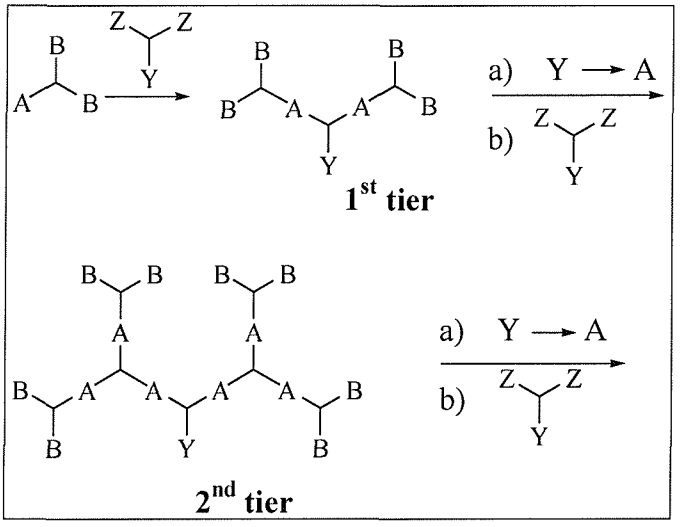
Scheme 1.7: Divergent synthesis, 1 ➡ 2 branching manner.



Scheme 1.8: Divergent synthesis, 1 ➡ 3 branching manner.

Branching is dependent on building block valency: divalent building blocks will react with two molecules of monomer, branching therefore proceedes in a 1 ➡ 2 manner. For trivalent building blocks it will proceed instead in a 1 ➡ 3 manner.

The second method, *convergent synthesis*, involves the synthesis of large dendrimeric fragments that are subsequently added to the initiator core (Scheme 1.9) and it was initially described by Fréchet in 1990.[41] A notable advantage of this procedure is the requirement of a minimum number of transformations for tier construction.

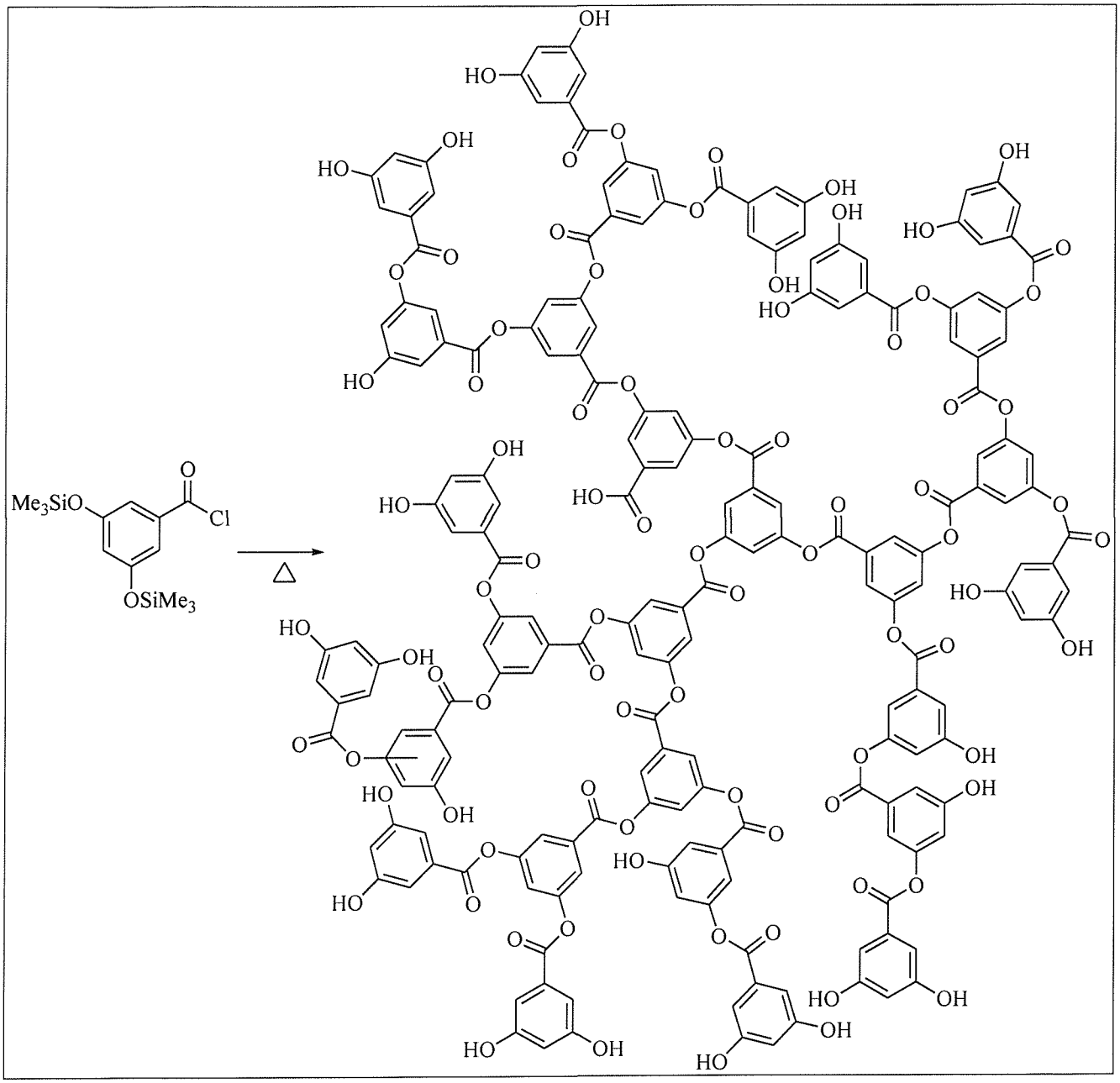


Scheme 1.9: Convergent synthesis.

The branching inherent in the synthesis leads to a geometric growth in the dendrimer for each generation and the shape of the dendrimer has been proposed to change from an open, starfish-shaped molecule to a ball-shaped structure as a consequence of steric congestion. The steric congestion at this point, the so-named *starburst*[33] limiting generation, presents synthetic problems as intramolecular reactions become favored and defects are formed.

Having described the stepwise procedures leading to the well-characterised, perfect structures of dendritic molecules, it is important also to report the synthesis of the related hyperbranched polymers. These are not monodisperse because obtained by direct, one-step polycondensation of A_xB monomers ($x \geq 2$); this synthetic procedure affords products possessing a high degree of branching, usually in the range 55-70% and is dependent of their molecular weights.

Fréchet,[47] for example, reported the high yield, one-step, reproducible preparation of hyperbranched aromatic polyesters with controllable molecular weights *via* the self-condensation of 3,5-*bis*(trimethylsislyloxy)benzoyl chloride at 200°C with a catalytic amount of DMF; the average molecular mass was 184,000 amu (Scheme 1.10).



Scheme 1.10: One-step synthesis of hyperbranched polymers.

Interesting comparisons have been made between dendritic and hyperbranched structures:[48] the thermal properties were independent of architecture and their solubilities were comparable, but greater than their linear counterparts.

1.2.3 Applications

As shown in Figure 1.7,[49] the interest towards dendrimeric molecules has highly increased in the past decade, mainly due to better knowledge of their physical/chemical properties and their structural characteristics.

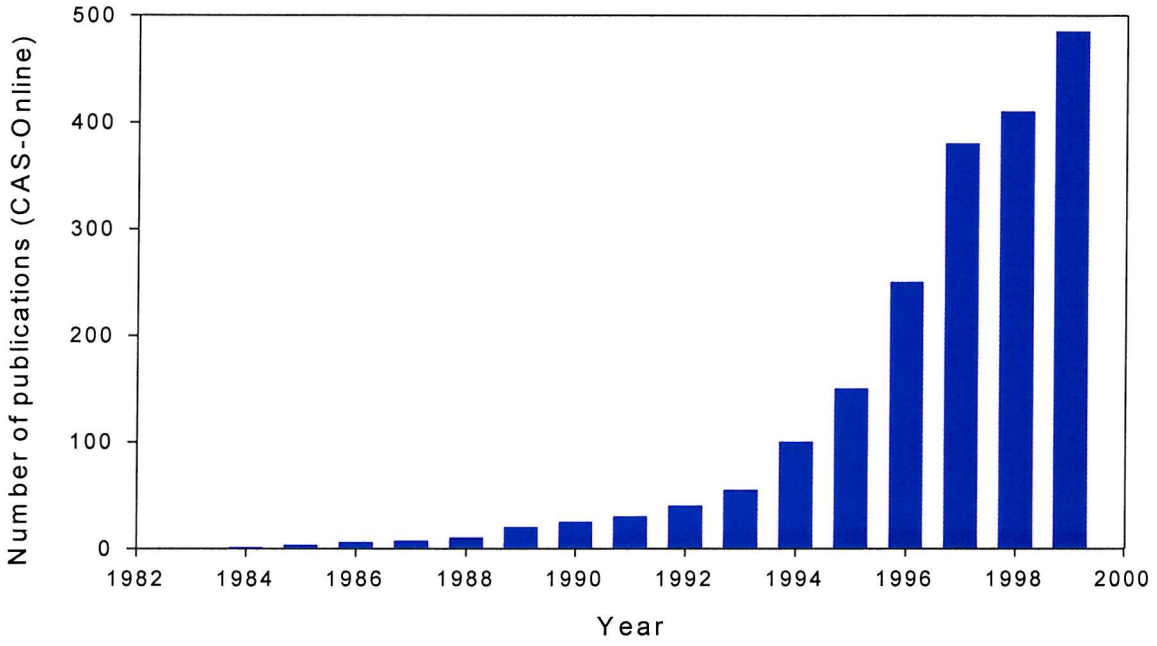


Figure 1.7: Number of Publications on Dendrimers in the last 20 years.

This has opened the way to their use for many different applications:

1. Chromatograpy (size exclusion chromatography,[50] electrokinetic capillary chromatography,[51] ion exchange processes,[52] gas separation membranes[53])
2. Pharmaceutical complexes/conjugates (sucrose mimetics,[54] MAP/antigens,[55] nucleic acid conjugates,[56] cell transfectants,[57] contrast agents,[58] drug delivery devices[59])
3. Electronic devices (semiconductors,[60] photochemical molecular devices[61])
4. Antioxidants[62]

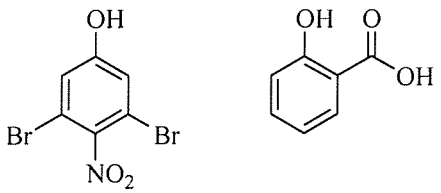
5. Inks and toners[63]
6. Liquid crystals[64]
7. Solubilisation agents[65]
8. Catalysts[66]

It is not an aim of this introduction to go through all these applications, therefore only a brief description of the different uses of polyamidoamine (PAMAM) dendrimers will be given.

The possibility of encapsulating small molecules that can then be released by removing a surface capping group (*dendritic box*) has been investigated for the first time by Jansen and Meijer[67] who enclosed Rose Bengal in a suitably derivatised PAMAM dendrimer and modulating the pH they could selectively extract the dye from a mixture with fluorescein.

Later on Crooks[68] showed that dodecanoic acid molecules can self-organise around a dendritic molecule interacting with the terminal amino groups. These non-covalent micelles are able to extract certain dyes from an organic mixture.

PAMAM dendrimers modified with *tris*(hydroxymethyl)aminomethane can solubilise organic molecules (like those shown below) in aqueous media.[69]



In the medical field PAMAM dendrimers have different interesting applications due to their low toxicity and the high number of amino-groups, that can be protonated at physiological pH and consequentially interact with polyanions like nucleic acids. Appreciable toxicity has been detected only for the seventh generation and has been attributed to the highly polycationic nature (in fact it has been found that PAMAM dendrimers with carboxylic functions as terminal groups are not toxic).[70]

PAMAM dendrimers have been used to transfer genetic material nonspecifically into mammalian cells *in vitro*.[71]

Appropriately functionalised dendrimers have also been used as devices for improving magnetic resonance imaging[72] and for boron neutron capture therapy,[73] by virtue of increasing functional density.

PAMAM dendrimers could be used also for Multiple Antigen Peptide Systems (MAPS).[55] MAPS have been used very effectively in eliciting immunogenic responses against malaria antigens in rodents,[74] as diagnostic tools[75] and for the treatment of AIDS-promoted maladies.[76] They consist of a central carrier core made up of sequential levels of lysine and an outer region of peptide antigens. The disadvantage of the present MAP systems is the nonequivalence of terminal amine sites, while it has been found that the peptides are not always able to induce antibodies capable of cross-reacting with the cognate protein, probably due to unusual conformations adopted in the MAP systems. Use of PAMAM dendrimers, homogeneous and immunologically inactive, might alleviate some of these problems.

Chapter Two

Synthesis of High Loading Tentagel Resin

2.1 Solid Phase Synthesis of Dendrimers

Solution-phase synthesis of dendrimers is often challenging, requiring long reaction times and nontrivial purification. Solid-state methodology, as outlined by Merrifield,[8] enables reactions to be driven to completion by using high concentations of reagents. Purification becomes a matter of extensive washing of the resin.

In 1988 Tam[55] reported the synthesis of resin-bound dendrimers made of Lysine. These are now commercially available for multiple-antigenic peptide (MAP) synthesis. The first solid-phase synthesis of polyamide dendrimers was attempted by Fréchet[77] in 1991 starting from 3,5-diaminobenzoic acid, but accessibility of chain ends within the solid support was found critical and reactions could not be forced to completion.

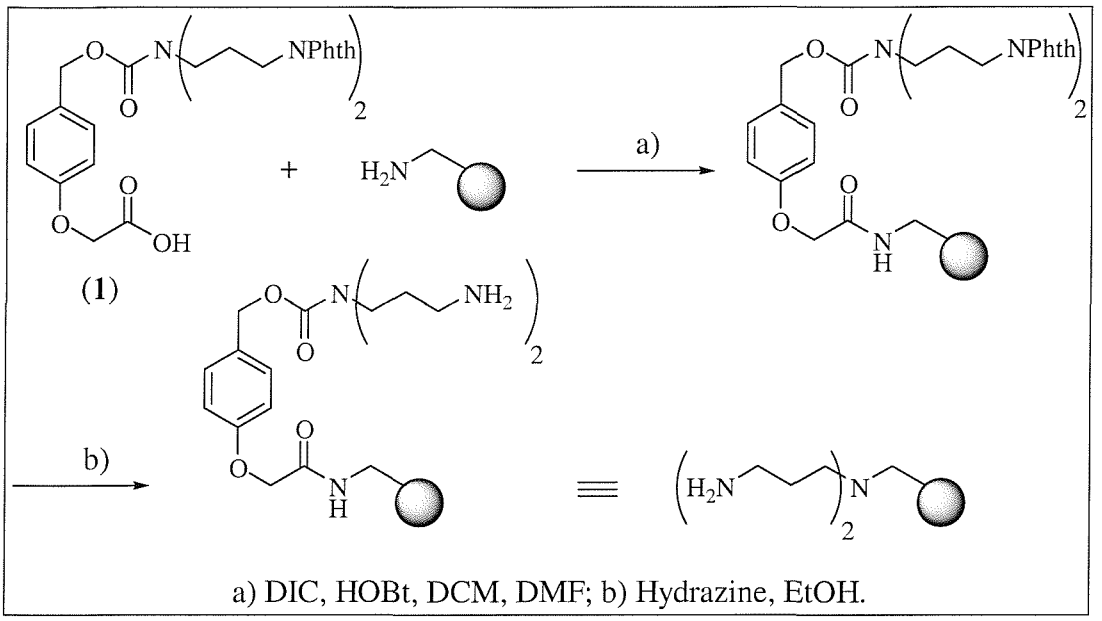
In 1993 Roy[78] described the solid-state preparation of the dendritic sialoside inhibitors of influenza A virus haemagluttinin. He employed *bis*-(N^{α},N^{ε}-Fmoc)-L-lysine benzotriazole as the building block for tier construction. The final tier (he went up to the 4th generation) was functionalised with chloroacetylglycylglycine, followed by reaction with peracetylated 2-thiosialic acid. The desired inhibitor was obtained in nearly quantitative yield.

The following year Wong[44] reported the solid-phase preparation of branched glycopeptides, partially obtained *via* enzymatic synthesis.

2.2 Solid Phase Synthesis of Polyamidoamine (PAMAM) Dendrimers

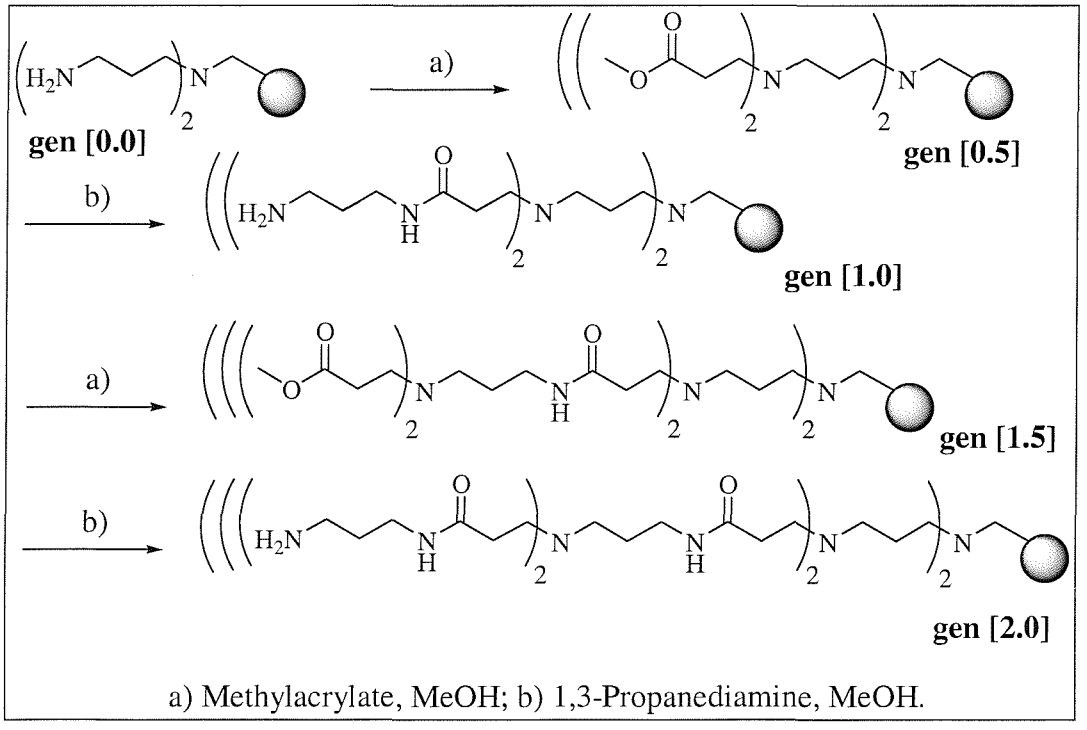
The first solid-phase synthesis of PAMAM dendrimers was reported by the group of Bradley[79] in 1997 and was initially carried out on standard-size TentaGel resin. Full characterisation of four different generations was given. The synthesis was found to be an efficient method for enhancing resin loading of at least one order of magnitude.

The first part of this project consisted in repeating the solid phase synthesis of PAMAM dendrimers on larger TentaGel beads (160 μm diameter). This was carried out using the polyamine linker (**1**) previously described (Scheme 2.1).[80]



Scheme 2.1: Synthesis of resin-bound initiator-core

The dendrimer was cleaved off the resin at different stages and analytical data compared with those previously reported, showing perfect accordance to published data (Scheme 2.2).



Scheme 2.2: Solid-phase synthesis of generation 2.0 PAMAM dendrimers.

To prove the versatility of this solid-phase synthesis two different dendrimer-conjugates were synthesised with Leu-enkephalin-Lys and Chlorambucil.[81]

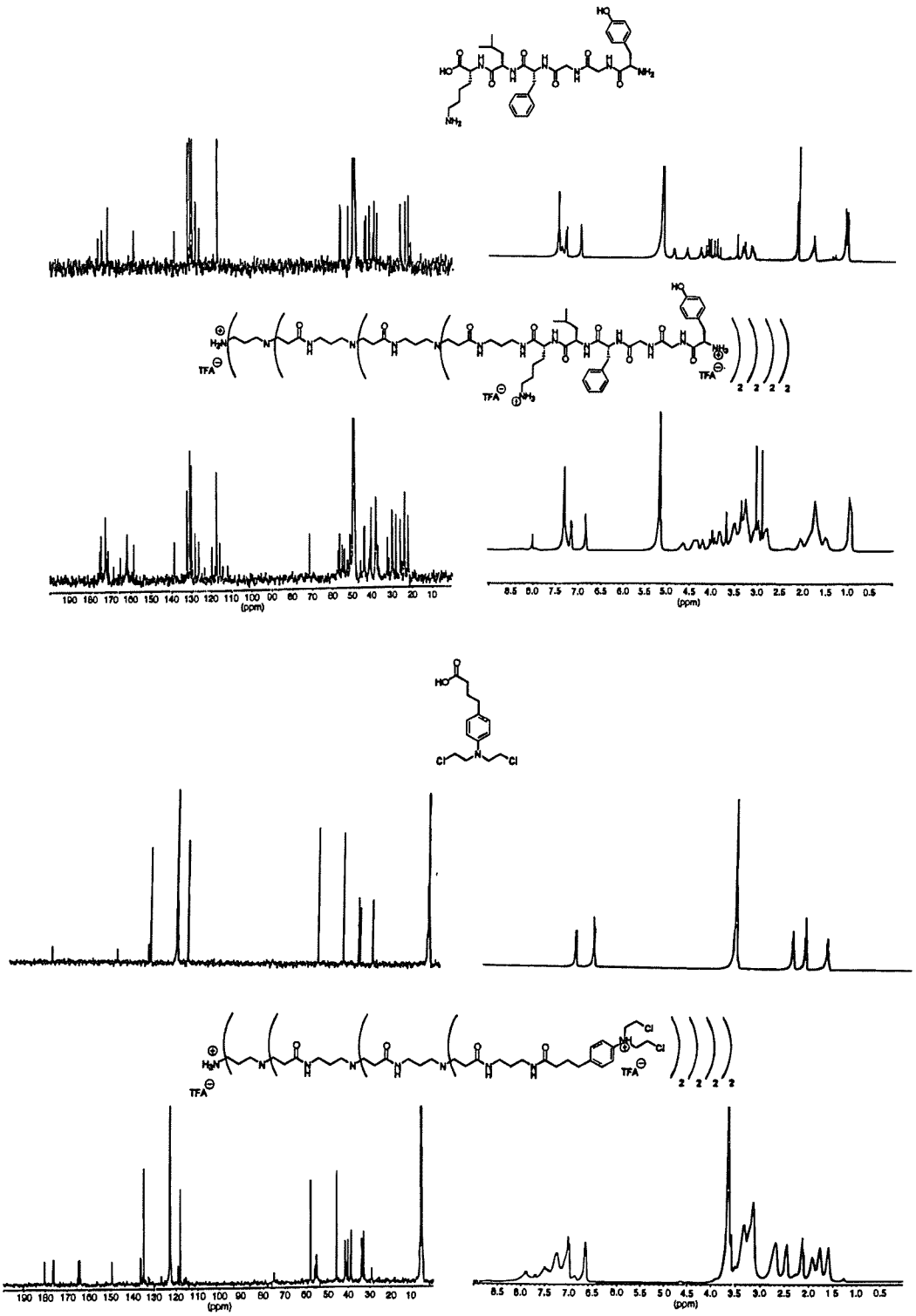


Figure 2.1: ^{1}H NMR and ^{13}C NMR of Leu-enkephaline-Lys, Chlorambucil and the correspondent dendrimer conjugates.

The ^{1}H and ^{13}C NMR of these materials were in agreement with those of authentic samples of Leu-enkephalin-Lys and Chlorambucil (Figure 2.1), taking into consideration the dendrimer resonances, showing high homogeneity of the dendrimer conjugates.

2.3 Linkers for Permanently Attached PAMAM Dendrimers

Following these preliminary studies, PAMAM dendrimers were synthesised permanently attached to the resin. The three polyamine linkers (**2**), (**3**) and (**4**) (Figure 2.2) were designed and synthesised following two different synthetic patterns.

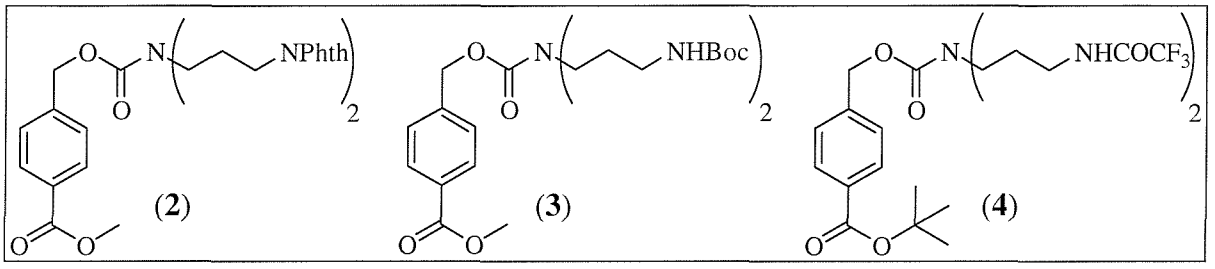
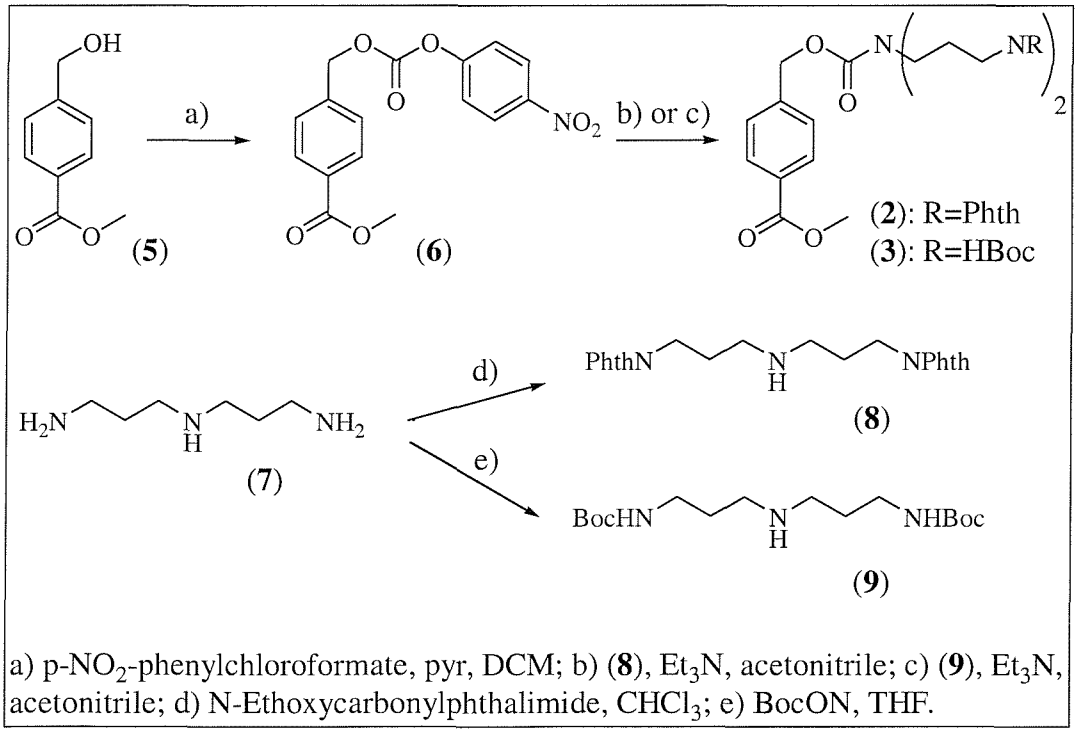


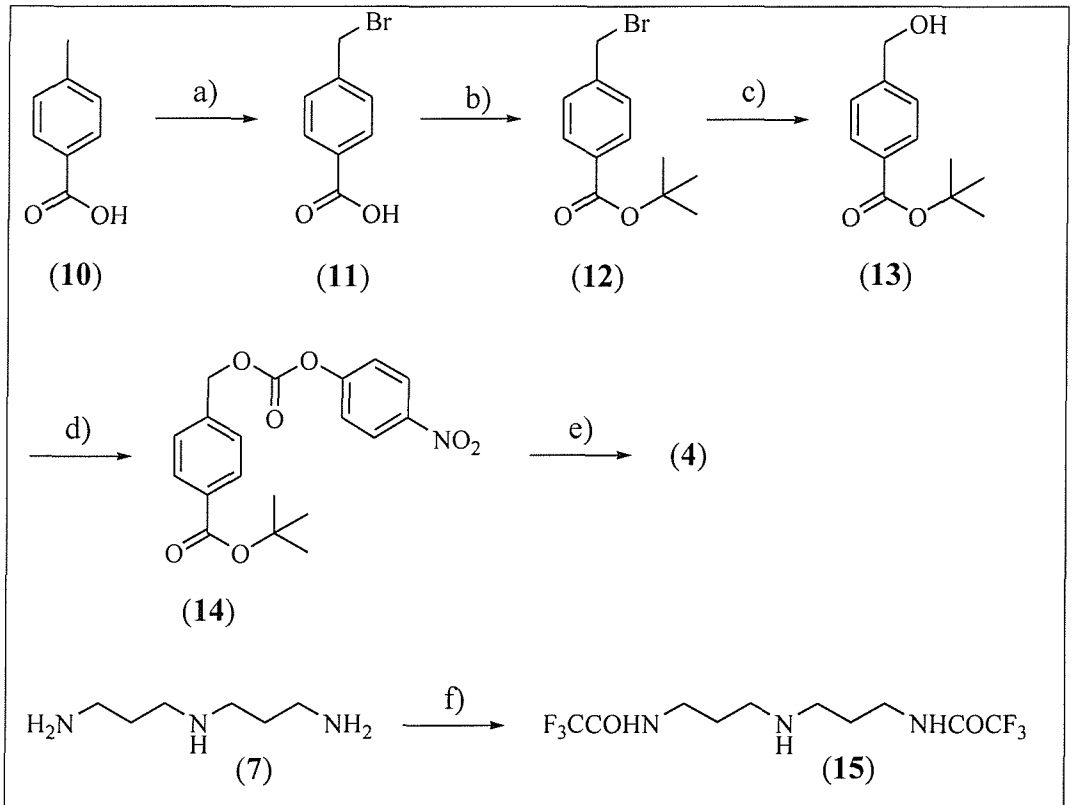
Figure 2.2: Three orthogonally protected polyamine linkers



Scheme 2.3: Synthesis of polyamine linkers (**2**) and (**3**).

For (**2**) and (**3**) the active carbonate (**6**) was obtained in one step from commercially available methyl 4-(hydroxymethyl) benzoate (**5**) and reacted respectively with the phthaloyl- and Boc-protected polyamine (**8**) and (**9**). These were synthesised in one step from *nor*-spermidine (**7**) with ethoxycarbonyl phthalimide and Boc-ON respectively (Scheme 2.3).

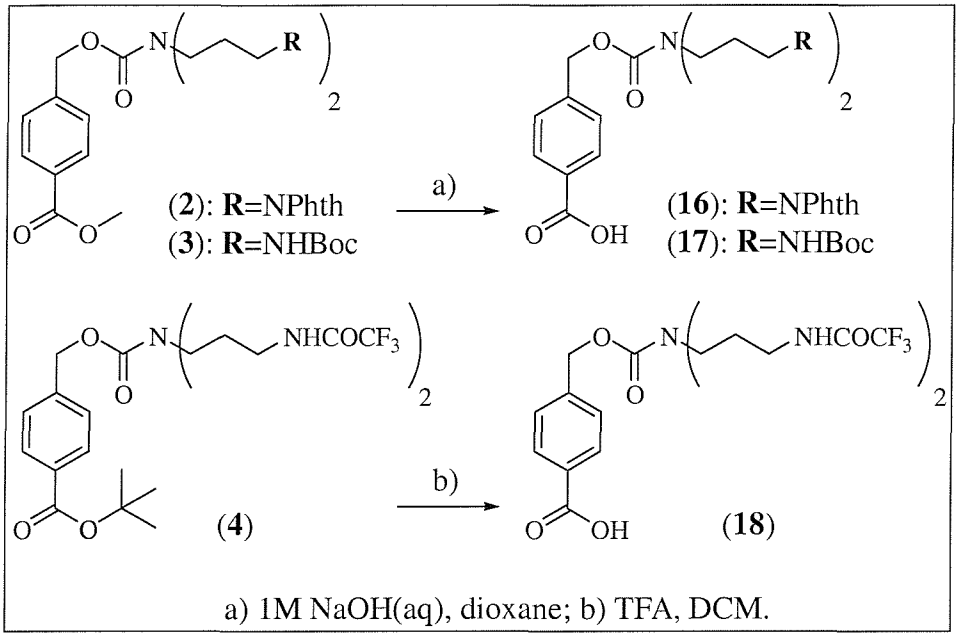
Linker (**4**) was obtained from toluic acid (**10**) *via* radical bromination at the methyl position,[82] esterification with isobutylene, conversion of the bromo-derivative (**12**) into the correspondent hydroxymethyl benzoate (**13**) and activation with *p*-nitrophenyl chloroformate. Trifluoroacetyl-protected *nor*-spermidine (**15**) was obtained upon reaction of the polyamine with ethyl trifluoroacetate and condensed with the active-carbonate (**14**) (Scheme 2.4).



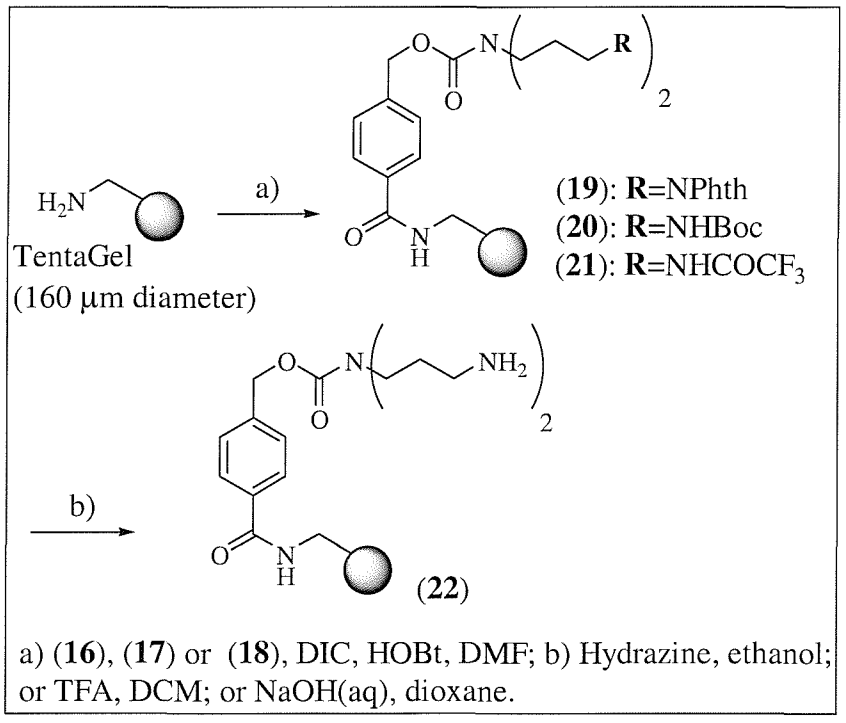
a) NBS, benzoylperoxide, chlorobenzene; b) H_2SO_4, isobutylene, DCM, dioxane; c) $AgNO_3$, dioxane, water; d) p-NO_2-phenyl chloroformate, pyr, DCM; e) (**15**), Et_3N, acetonitrile; f) Ethyl trifluoroacetate, THF.

Scheme 2.4: Synthesis of polyamine linker (**4**).

Hydrolysis of the benzoic esters was achieved with NaOH for (**2**) and (**3**) and with TFA for (**4**) (Scheme 2.5).

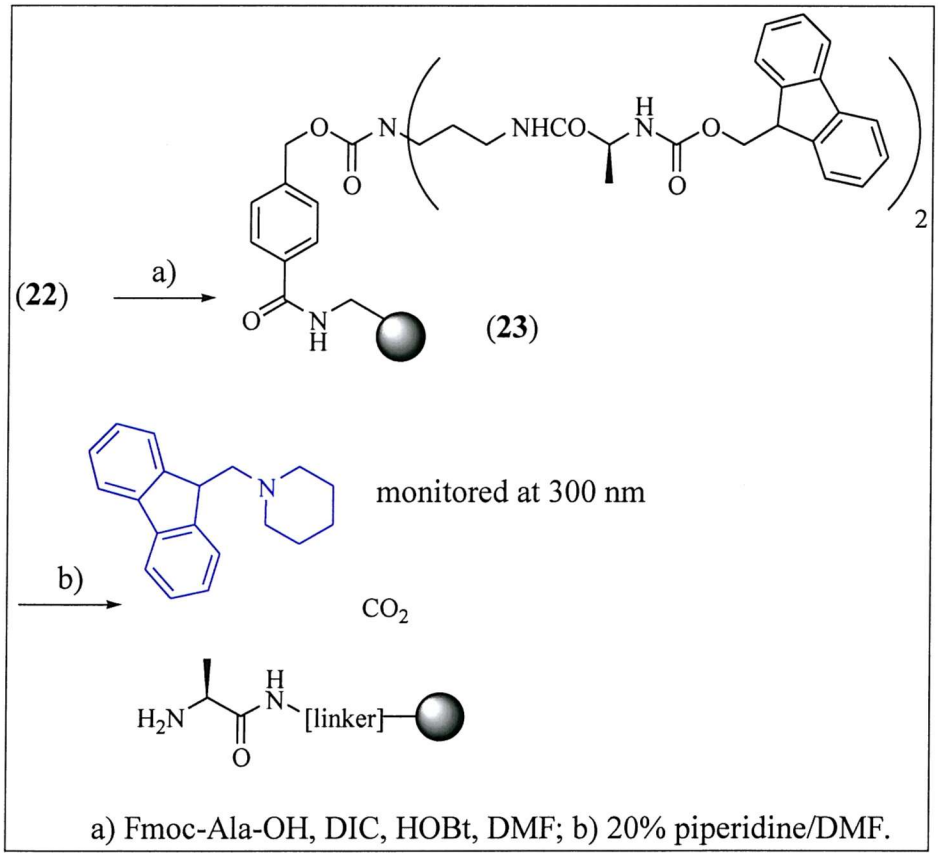


Scheme 2.5: Ester hydrolysis of the polyamine scaffolds.



Scheme 2.6: Synthesis of permanently attached initiator core

The resulting carboxylates (**16**), (**17**) and (**18**) were coupled onto aminomethyl TentaGel (0.85 nmol/bead, 0.46 mmol/g) under standard conditions and the protecting groups were removed with hydrazine/EtOH, TFA/DCM and 1M NaOH(aq)/dioxane for (**19**), (**20**) and (**21**) respectively (Scheme 2.6). Before carrying out the synthesis of the PAMAM dendrimer, the loading of the resulting resin (**22**) was checked in order to find which scaffold was giving the best results. Fmoc-Alanine was coupled onto the free amino-termini with DIC/HOBt to give (**23**) and the release of the fulvene adduct was monitored by UV after treatment with 20% piperidine in DMF (Scheme 2.7).

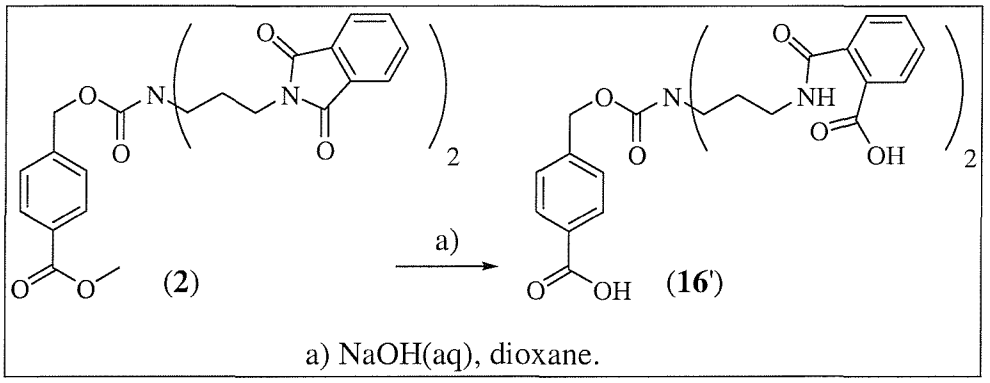


Scheme 2.7: Fmoc test for the determination of resin loading.

The values obtained were 1.2 nmol/beads, 1.2 nmol/beads and 1.7 nmol/beads for (**2**), (**3**) and (**4**) respectively.

Clearly, linker (**4**) gave the best results in terms of loading (100% of the expected value), while linkers (**2**) and (**3**) gave only 70% of the expected value.

For linker (**2**) there are two possible explanations for the lower loading: the first is that during the hydrolysis of the methyl ester the phthalimide group is hydrolysed (lability of the phthalimide moiety in basic conditions has been reported) (Scheme 2.8).[83] It is believed that upon activation with DIC/HOBt the phthalimide ring is closed again, but to some extent it could exist still as an open-chain active carboxylate able to acylate the aminomethyl resin.



Scheme 2.8: Hydrolysis of phthalimide protected polyamine linker.

In addition, the hydrazine cleavage of the polyamine protecting groups is carried out under quite strong conditions, EtOH at reflux overnight, and this could facilitate side-reactions on the solid-phase (e.g.: cyclic urea formation).

Relatively to linker (**3**), it was observed that upon treatment of Boc-protected polyamine resin (**20**) with TFA the concentration of the acid was crucial for the deprotection step. In fact, a low loading (about 0.9 nmol/bead) was obtained using DCM/TFA 7:3, even with prolonged cleavage times (5 hours), while using DCM/TFA 5:95, the loading dropped to 0.2 nmol/bead (even after extensive neutralisation of the resin with DIPEA). Better results were obtained with DCM/TFA 1:1 for 2 hours (1.2 nmol/bead), but the loading was still lower than expected. Interestingly, only PEG was observed coming off the resin during the cleavage. No trace of linker or polyamine was observed in the NMR spectrum of the filtrate.

2.4 Synthesis of PAMAM *DendroGel*

Compound (**21**) was therefore used for the synthesis of permanently attached PAMAM dendrimers. After deprotection of the trifluoroacetamide groups to give (**22**),

Generation 2.0 dendrimer resin (in batches of 2-3 grams) was obtained using the standard procedure: exhaustive Michael addition of methyl acrylate in methanol onto the resin-bound primary amines followed by displacement of the methyl ester with 1,3-propanediamine in methanol. The reactions were performed at 50°C using a peptide vessel with a water chamber. This two-step procedure was repeated twice and the final loading obtained was 5.6 nmol/bead, 82% of the theoretical maximum (Figure 2.3).

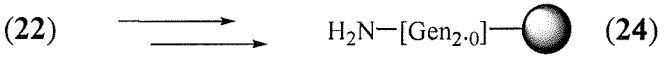


Figure 2.3: Resin-bound permanently attached generation 2.0 PAMAM dendrimers.

The swelling properties of this resin were analysed and compared with those of the starting TentaGel resin; the results are reported in Table 2.1.

Bead diameter (μm)

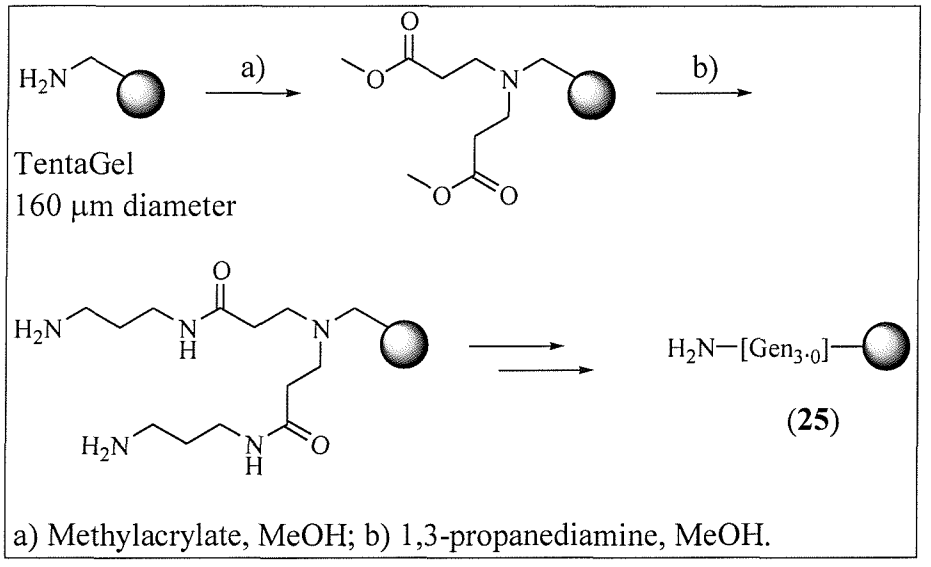
	Dry resin	DCM	DMF	MeOH	H_2O
DendroGel	170	220	210	200	220
TentaGel	160	210	200	190	200

Table 2.1: Swelling properties of TentaGel and Dendrimer resin, the diameter size is the average of 10 different beads.

It is interesting to note that dendrimer beads are slightly bigger in size than TentaGel but maintain the same swelling properties as normal TentaGel resin, with an exception for water, where dendrimer beads swell more.

The polyamine linker used for the solid-phase synthesis of PAMAM dendrimers increases the loading of a single bead by a factor of two as a result of its branched nature. Therefore, the second generation of PAMAM dendrimers attached to this core already leads to a loading that is, in theory, 8 times larger than that of the naked resin.

Another advantage is that this linker, acting as a spacer, allows the dendrimer to grow further from the resin matrix, although with TentaGel this should not be a major issue. The synthesis of this linker-molecule, however, is not completely trivial, requiring radical reaction (the bromination of toluic acid) and reaction at high-pressure (the formation of the *t*-butyl ester). Looking for an alternative method to synthesise bulk-quantities of this high-loading resin, it was decided to investigate the synthesis of the dendrimer directly attached to the amino-termini of the naked beads. The resin was therefore reacted with methyl acrylate in methanol followed by 1,3-propanediamine in methanol. This procedure was repeated three times (Scheme 2.9) and 30 g of resin were obtained starting from 20 g of commercially available TentaGel resin.



Scheme 2.9: Synthesis of permanently attached generation 3.0 PAMAM dendrimers.

The final loading of 4.0 nmol/bead (59% of the expected value) was determined with the standard procedure (coupling with Fmoc-Ala followed by treatment with piperidine/DMF). It clearly appears that the use of a polyamine linker/spacer leads to an improved loading, although other reasons can be found to interpret this result. The reactions were performed at room temperature instead of 50°C, due to the dimensions of the flask used, and also the agitation was not optimal. In the future, a more appropriate apparatus for the preparation of bulk quantities of resin will probably give results comparable with those obtained on a small scale.

2.5 Suitability of *Dendrogel* for SPOS

The loading was, however, considered satisfactory for both resins (**24**) and (**25**), therefore it was decided to investigate the mechanical and chemical properties of these beads using different chemical conditions for the synthesis of various types of molecules such as amidines, arylethers, diketopiperazines, tetrazoles, benzodiaze-pinones, amides and biaryls (Figure 2.4).

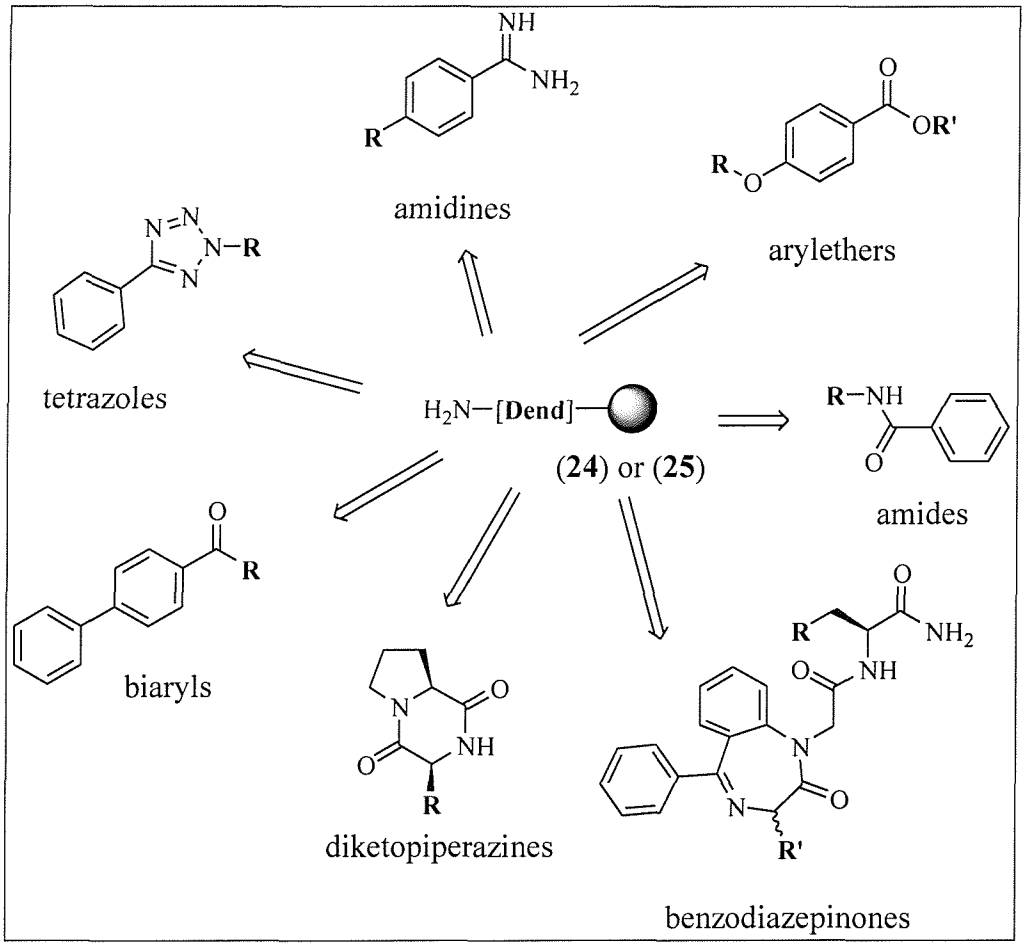


Figure 2.4: Different chemical methodologies applied to dendrimer resin.

It was believed that the PAMAM dendrimer resins would be inert towards most of the chemistries, since they are made of amide bonds and tertiary amine groups. Only synthetic procedures involving strong bases (like butyl lithium) or strong reducing agents (like lithium aluminium hydride) were therefore discarded.

In the following chapters there will be a description of the libraries and single compound syntheses on this high-loading resins.

Chapter Three

Solid Phase Synthesis of Amidine-based Antagonists of Glycoprotein IIb/IIIa

3.1 Amidines of Biological Interest, a Brief Review

Studies on the biological activity of amidines were conducted as early as 1931,[84] with compounds such as *p*-ethoxycarbonyl benzamidine and *benzenylveratryl amidine* being found to have anaesthetic properties. At the time, this was believed to be due to the amidine group that was considered to be structurally similar to the carboxyl group but readily soluble in water (many local anaesthetics contained a carboalkoxy group at the time).

Later Allen and co-workers[85] found that certain triphenylethylene-substituted amidines exhibited anti-inflammatory and anti-fungal activities. More recently the amidine moiety has been found as part of a number of pharmacologically active molecules such as fibrinogen receptor antagonists,[86] thrombin inhibitors,[87] factor Xa inhibitors[88] and S-Adenosylmethionine Decarboxylase inhibitors.[89]

3.1.1 Fibrinogen Receptor Antagonist Activity

Human blood platelets play an important role not only in normal hemostasis but also in arterial thrombosis, particularly under conditions of high shear stress typical of narrowed atherosclerotic arteries.

The deposition of platelets on thrombogenic surfaces such as ruptured atherosclerotic plaques followed by the formation of occlusive platelet-rich aggregates are crucial events leading to disorders such as unstable angina, myocardial infarction, transient ischemic attacks and stroke[90].

Recent studies on the biochemical mechanisms of platelet activation[91] indicate that the interaction of the tissue-bound von Willebrand factor (vWF) with glycoprotein Ib-IX on the platelet surface is responsible for the adhesion of platelets to exposed subendothelium. This is thought to trigger the final and most critical step in aggregation, the cross-linking of the dimeric plasma protein fibrinogen between membrane glycoprotein IIb/IIIa ($\alpha_{II}\beta_3$) receptor complexes exposed on adjacent activated platelets. In this way platelets are cross-linked and thrombus growth is initiated. Due to the release and formation of a variety of platelet activators[86] (e.g.

ADP, thrombin, collagen, serotonin, thromboxane A_2, epinephrine), additional platelets are activated and recruited to the growing thrombus. Antagonism of the fibrinogen-GP IIb/IIIa interaction represents a therapeutic target with potential utility in the treatment and prevention of diseases like unstable angina, myocardial infarction, transient ischemic attacks and stroke.[90]

Like other members of the integrin family, GP IIb/IIIa embodies a recognition site for the peptide sequence Arg-Gly-Asp (RGD). This sequence occurs four times in fibrinogen: twice in each of the two Aα chains, contribuiting four of the total six putative recognition sites within fibrinogen. The other two sites occur in the C-terminal 12 aminoacid segments HHLGGAKQAGDV of the two γ chains and are believed to be a cryptic RGD-type sequence.[91] Most of the molecules that inhibit this interaction are small linear or cyclic peptides[92] containing the RGD sequence or non-peptide molecules[93] that mimic this sequence.

According to recent studies[94] these compounds must have a carboxylic acid and an ammonium function within a distance of 11-15 Å. Moreover is has been shown that the basic function can be either a guanidine, which is the natural function of the RGD sequence, or an amine such as piperidine or a benzamidine moiety. It has been found that replacing the guanidine moiety with a benzamidine function increases the inhibitory potency a 1000-fold, due to a stronger interaction between the amidinium ion and a carboxylate function present in the glycoprotein (Figure 3.1).

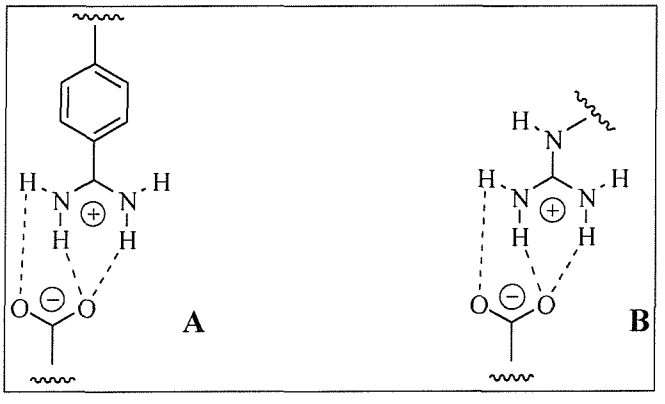


Figure 3.1: Interactions between a carboxylate and an amidinium group (**A**) and between a carboxylate and a guanidinium group (**B**).

From theoretical studies the complex **A** is 4 Kcal/mol more stable than **B**, due to better charge distribution.

Some molecules with these features are currently in preclinical or clinical trials, examples include Lamifiban (Ro44-9883) (**26**),[86] TAK-029 (**27**)[91] and Ro43-5054 (**28**)[95] (Figure 3.2).

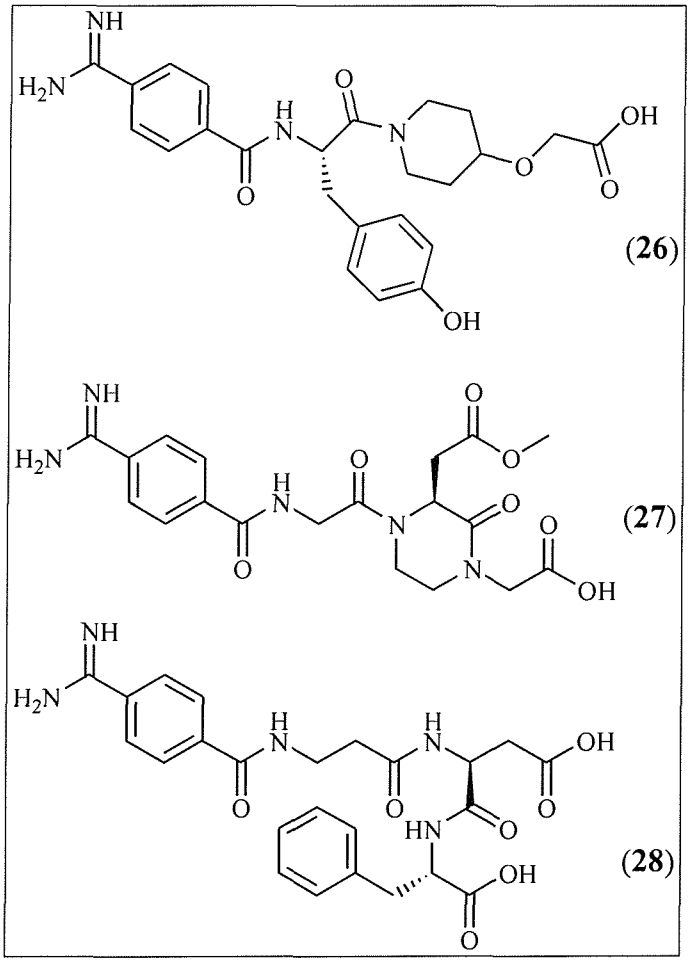


Figure 3.2: Examples of Glycoprotein IIIa-IIb antagonists.

3.1.2 Thrombin and Factor Xa Inhibitory Activity

Normal hemostasis is required for proper maintenance of the vasculature in response to routine injury and blood coagulation is one of the vital components; many proteins are involved in this process. The final step is the conversion of Factor X into its enzymatically active form Factor Xa, responsible for the conversion of prothrombin into thrombin, the final enzymatic product of blood coagulation. This not only

converts fibrinogen to fibrin for clot formation, but also strongly induces platelet aggregation.[88] The inhibition of one of these enzymes in this pathway is therapeutically important in the development of anticoagulant drugs, used to treat thrombotic diseases.[96]

Inhibition of thrombin has been the target of extensive research into the development of new anticoagulant agents,[97] but this therapeutic route has a number of potential drawbacks (thrombin inhibitors have shown a tendency to prolong bleeding time at their effective doses in experimental thrombotic models). For this reason the development of small molecules-Factor Xa inhibitors is becoming an attractive alternative to current anticoagulant therapy. It has been calculated that a molecule of Factor Xa can generate 138 molecules of thrombin;[98] thus inhibition of Factor Xa may be more efficient than inactivation of thrombin in interrupting the blood coagulation system. There are relatively few reports of small molecule inhibitors of Factor Xa and they are limited primarily to a group of amidines,[99] bis-amidines[100] and similar compounds.

A few examples are listed in Figure 3.3.

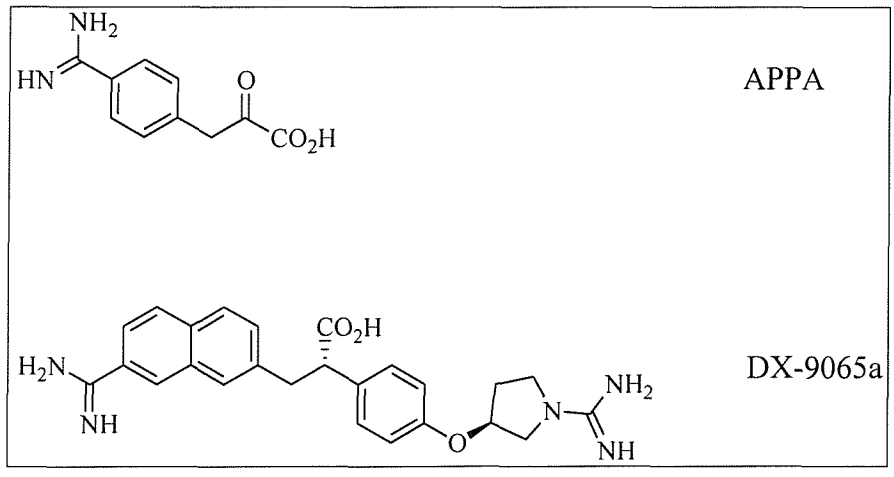


Figure 3.3: Examples of Factor Xa inhibitors.

The activity of these compounds has been proposed to be due to hydrogen-bond interactions between the amidine function and the side chain of Asp189 and the carbonyl oxygen of Gly218 in the Factor Xa active site.

3.1.3 S-Adenosylmethionine Decarboxylase Inhibitory Activity

The diamine putrescine (PUT) and the polyamines spermidine (SPD) and spermine (SPM) play an important role in cell growth. The biosynthetic pathway to SPD and SPM is controlled by S-adenosylmethionine decarboxylase (SAMDC), a rate limiting enzyme of polyamine biosynthesis. From decarboxylated S-adenosylmethionine, the aminopropyl moiety is transferred to one or both amino groups of PUT, affording SPD or SPM. The activity of SAMDC is controlled by natural polyamines and is dependent on the metabolic status of the appropriate tissue. The enzyme activity is elevated in response to proliferative stimuli, and is generally increased in rapidly growing and neoplastic cells. Inhibition of intracellular SAMDC leads to arrest of cell proliferation, in consequence of the depletion of SPD and SPM pools, or to aberrant methylation by accumulated S-adenosylmethionine.[89] Interest in SAMDC as a therapeutic target developed after the discovery that the antileukemic drug methylglyoxal bis(guanylhydrazone) (MGBG) inhibits SAMDC[101]. MGBG is the first SAMDC inhibitor to show clinical efficacy, however, owing to its severe toxicity it has not become established as an antitumor agent.

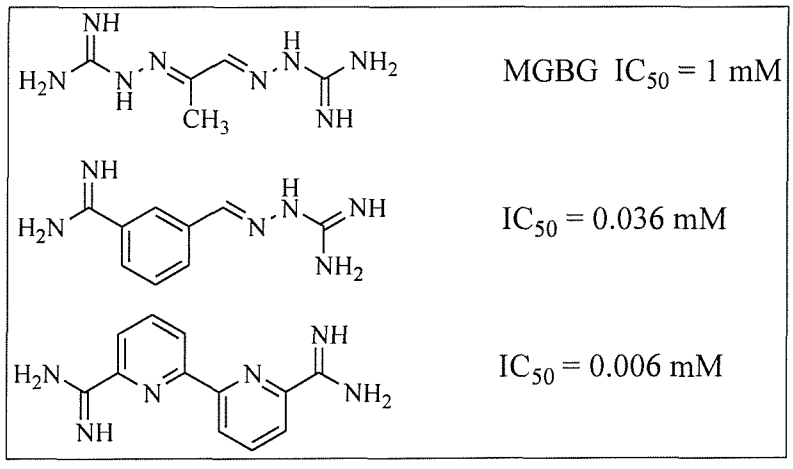


Figure 3.4: Examples of SAMDC inhibitors.

An interesting feature of the structure-activity relationship emerged from crystal structure analyses of MGBG and the inactive trifluoromethyl analogue: it was concluded that only compounds with an extended all-*trans* conformation and delocalized π electrons could inhibit SAMDC.[102] Proceeding from this hypothesis,

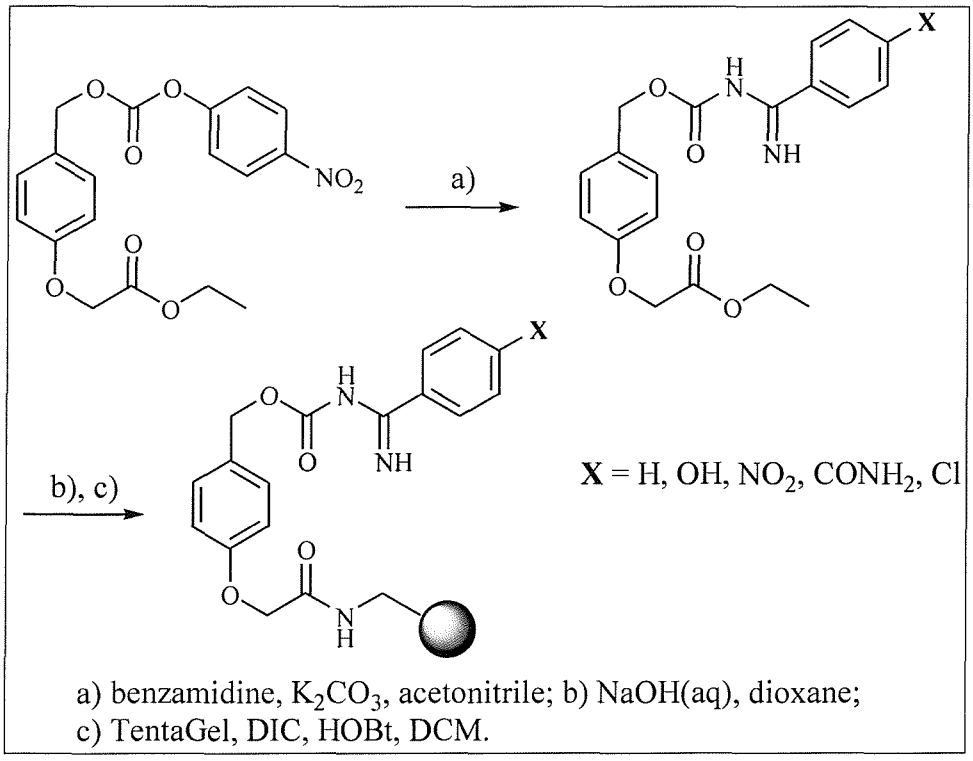
several analogues of MGBG with partially fixed all-*trans* conformations have been synthesised integrating parts of the chain into aromatic rings: some of the new molecules showed an higher inhibitory activity than MGBG (Figure 3.4).

3.2 Solid Phase Strategies for the Synthesis of Amidine-based Compounds

It is worth noting that many of the amidine-based antagonists of the fibrinogen-GP IIb/IIIa interaction present the same scaffold (see figure 3.2), consisting of a benzamidine group linked to an amino acid *via* an amide bond. The molecule is further elaborated with a piperidine moiety, in the case of Ro44-9883 or TAK-029, or with a more complex structure, for example like Ro43-5054.

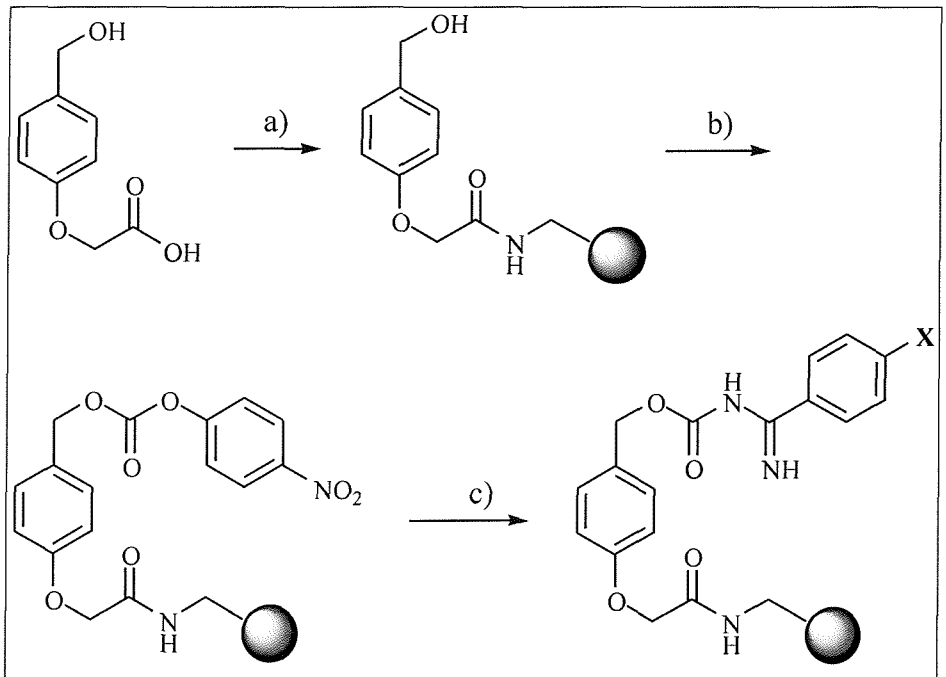
It was envisaged that compounds mimicking these structures could be easily generated by changing the amino acid and piperidine building blocks and, by using solid phase synthesis, a library of amidines could be obtained with relatively few synthetic steps.

In 1997 Bradley[103] published the solid-phase attachment of amidine-based molecules *via* a modified Wang linker.



Scheme 3.1: Synthesis of resin bound amidines *via* the amidine-linker complex route.

Two different strategies were adopted: the synthesis of the linker-amidine scaffold in solution and its attachment onto polystyrene resin (Scheme 3.1) or the activation of the resin bound linker as *p*-nitrophenyl carbonate, followed by displacement of *p*-nitrophenol by the amidine molecule (Scheme 3.2).



a) TentaGel, DIC, HOBt, DCM; b) *p*-Nitrophenyl chloroformate, NMM, DCM; c) benzamidine, K_2CO_3, DMF.

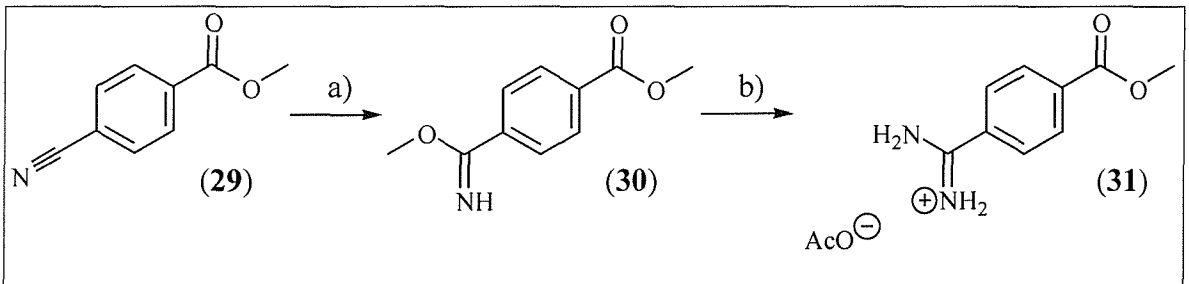
Scheme 3.2: Synthesis of resin-bound amidines *via* the resin-bound active-carbonate route

No examples of benzamidine derivatives with a carboxylic function that could be further elaborated were given, the main reason being that compounds of this type are not commercially available from common chemical suppliers.

3.3 Synthesis of the Amidine Moiety

Many procedures are known to generate the amidine moiety[104] and the most common ones start from a nitrile functionality. The classical method (Pinner reaction)[105] involves the reaction of the nitrile with saturated methanolic hydrogen chloride to generate the correspondent imidate, which is subsequently transformed into the

amidine with ammonia or ammonium carbonate. Other methods have been developed during the past decade: ethanolic ammonia and copper(I) chloride,[106] alkyl-chloroaluminum amides in toluene at 80°C,[107] amines and lanthanide(III) triflates[108] or by hydrogenation of amidoximes (obtained treating nitriles with hydroxylamine).[109] Starting from the commercially available methyl 4-cyanobenzoate (**29**), which could be easily hydrolysed in basic media, the acid labile Wang linker, already used in the previous studies, was used to attach the amidine to the dendrimerised resin. A Pinner reaction was performed, generating HCl *in situ* by reaction of acetyl chloride with methanol, and the imidate (**30**), obtained in high purity, was readily transformed into the amidinium acetate (**31**) with ammonium acetate in good yield (65% in two steps) (Scheme 3.3).

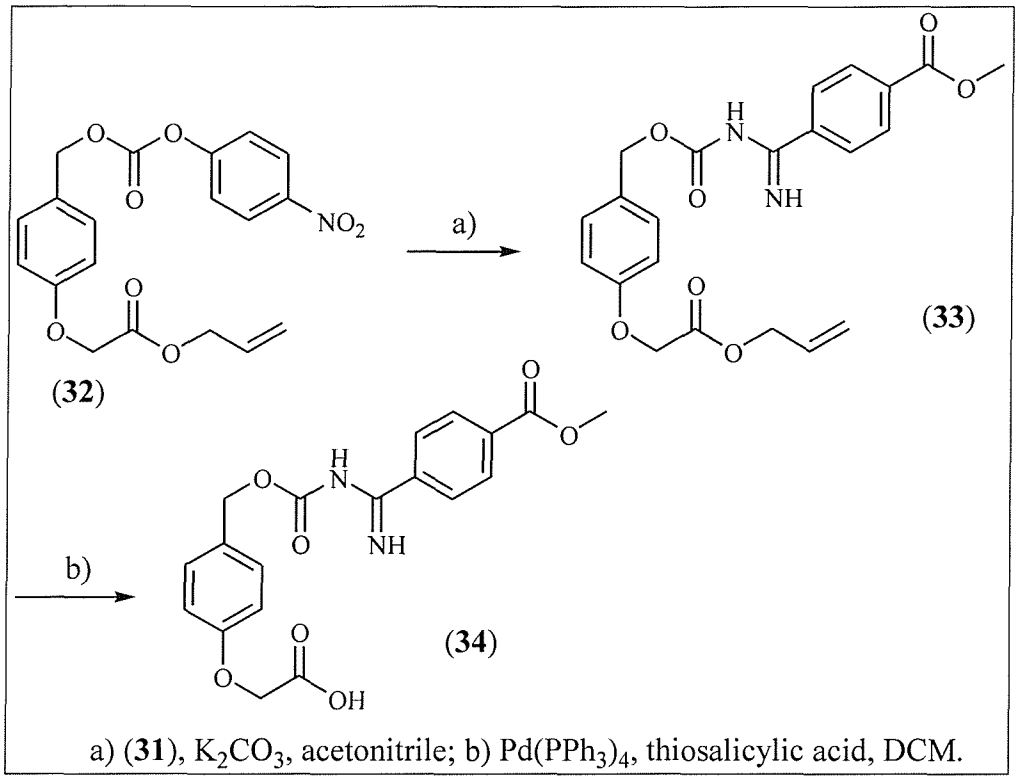


a) AcCl, MeOH; b)Ammonium acetate, MeOH.

Scheme 3.3: Synthesis of 4-(methoxycarbonyl)benzamidinium acetate.

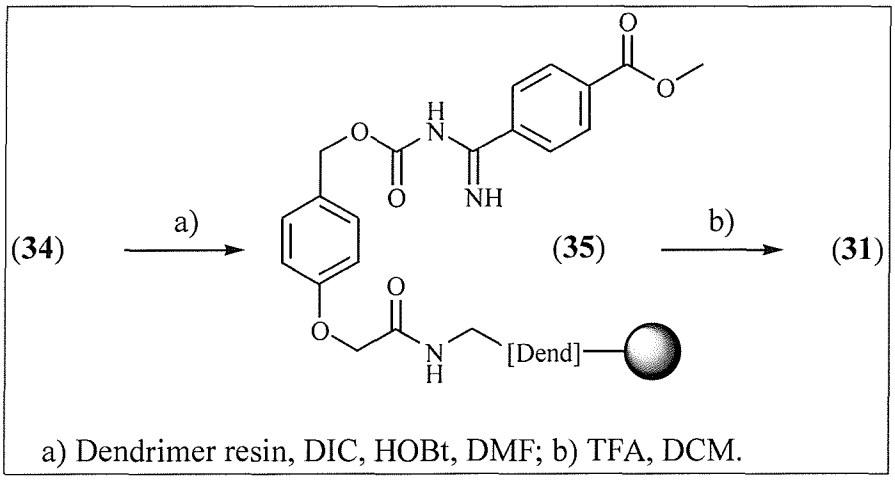
3.4 Synthesis of Resin-bound Amidine Scaffold

Compound (**31**) was then reacted with allyl 4-((4'-nitrophenoxy) carbonyloxymethyl) phenoxy acetate (**32**).[103] The linker/amidine complex (**33**) contained two *pseudo*-orthogonally[110] protected carboxylic funcions from which the allyl ester could in fact be hydrolysed selectively with $Pd(PPh_3)_4$ and thiosalicylic acid in DCM (Scheme 3.4). Purification of the resulting acid (**34**) *via* flash chromatography was not practicable due to its instability, but trituration in ethyl acetate gave the title compound in good yield (68%) and high purity. This was coupled in a straightforward manner with dendrimer resin using standard peptide coupling conditions.



Scheme 3.4: Synthesis of amidine-linker complex.

A small sample of resin was treated with TFA/DCM and the compound released from the resin analysed by HPLC, ES-MS and NMR: this, as expected, was found to be 4-methoxycarbonyl benzamidine (**31**) in very good purity and yield (Scheme 3.5).

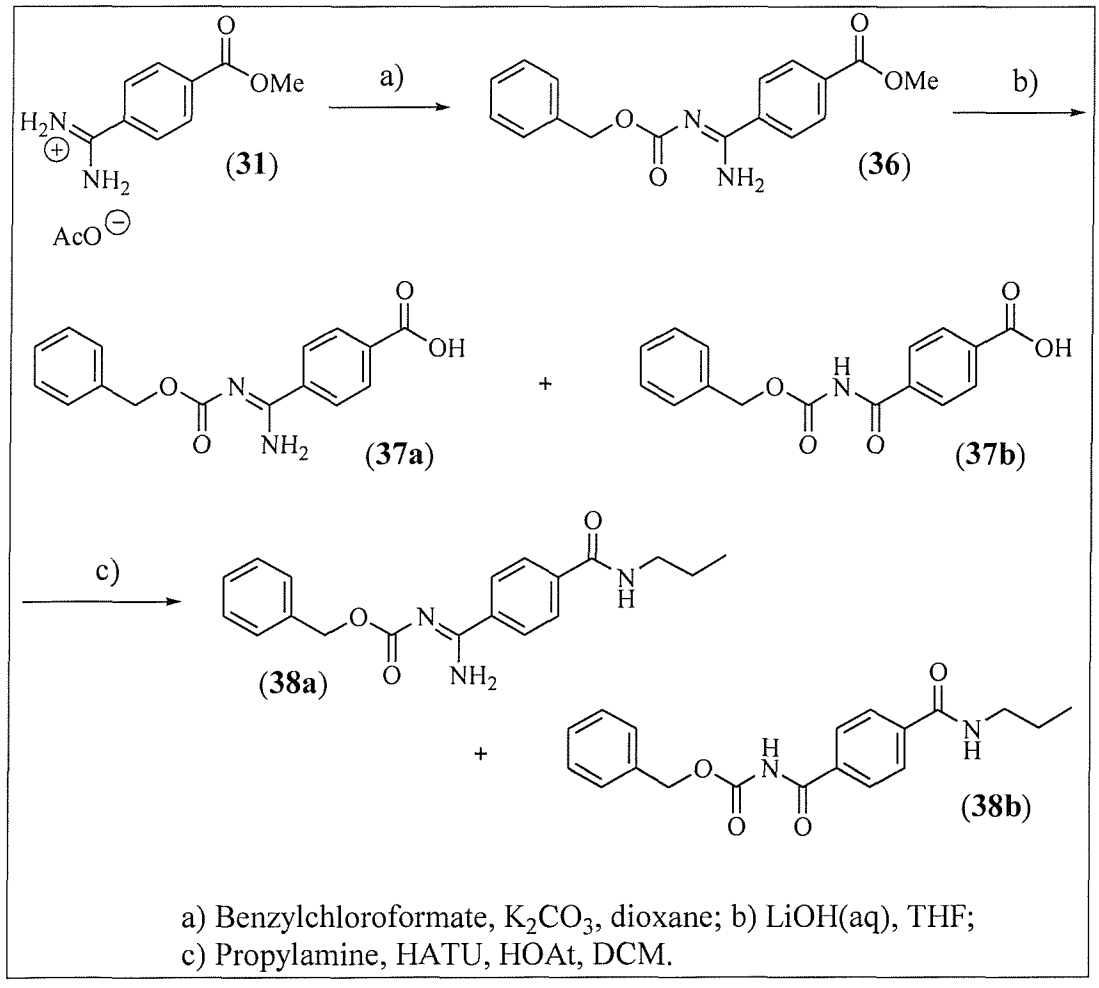


Scheme 3.5: Attachment of amidine-complex onto dendrimer resin and amidine cleavage.

3.5 Studies Towards the Hydrolysis of the Amidine Resin-bound Methyl Ester

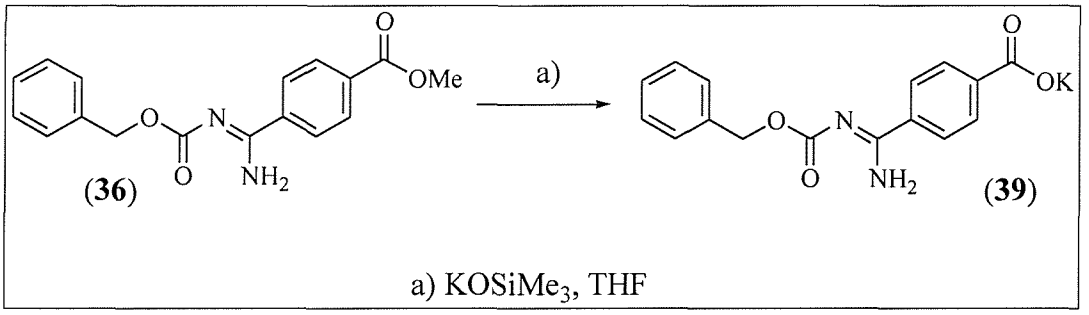
Hydrolysis of methyl benzoate (**35**) was firstly attempted with NaOH(aq)/dioxane, but the compound formed had a molecular mass of one unit higher than the expected acid. It is known that amidines are prone to react with nucleophiles, therefore we thought that the hydroxyl ions not only hydrolysed the methyl ester but also the amidine group, giving the corresponding benzamide derivative.

In order to confirm this theory and to find an alternative route, compound (**36**) was synthesised in solution and hydrolysed under the same conditions used for the solid phase synthesis. Two compounds (**37a**) and (**37b**) were detected, but they could not be separated, therefore they were coupled with propylamine using HATU/HOAt and the resulting amides (**38a**) and (**38b**) were separated and purified by flash chromatography (Scheme 3.6).



Scheme 3.6: Solution-model of amidine hydrolysis.

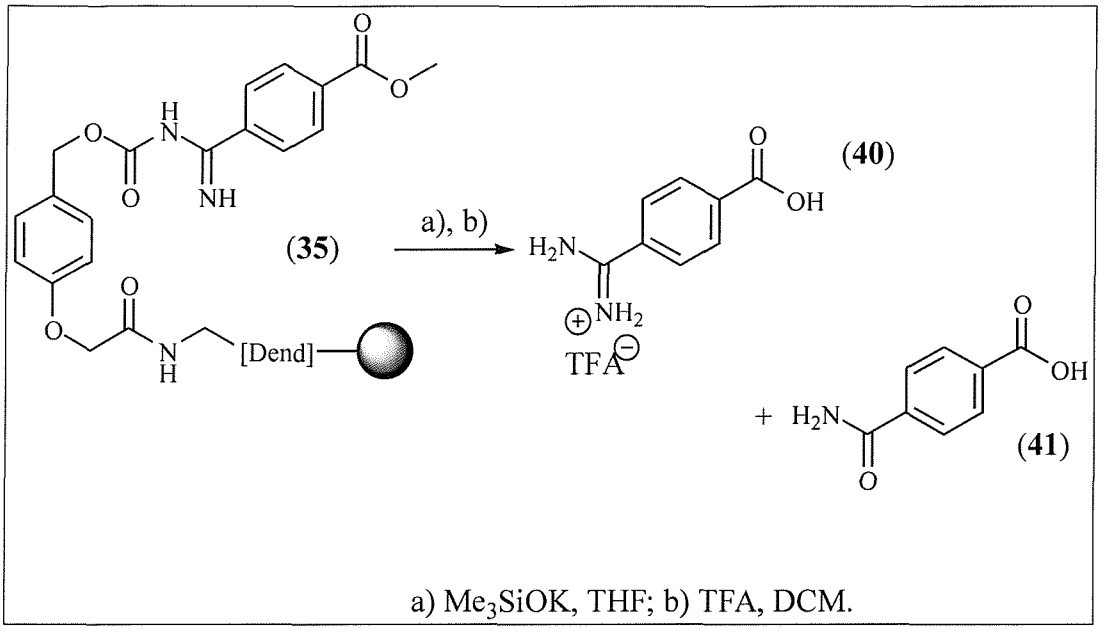
As expected the two compounds isolated were the desired benzyloxycarbonyl-protected amidine (**38a**) and the correspondent benzyloxycarbonyl-protected amide (**38b**) in ratio 7:3. It was observed that, upon treatment of compound (**36**) with potassium trimethylsilanolate[111] in THF, only the desired amidine (**39**) was obtained as the potassium salt (Scheme 3.7).



Scheme 3.7: Hydrolysis with potassium trimethylsilanolate.

Transferring this reaction in solid phase it was found to be very slow and hard to force to completion. Upon refluxing the resin overnight with 10 equivalents of $KOSiMe_3$ the starting material was completely consumed but, with the desired compound (**40**), the corresponding amide (**41**) was also detected by HPLC after TFA treatment (Scheme 3.8). The relative amounts of the two compounds were found to be variable and not reproducible. It was not possible to understand if the amidine hydrolysis was caused by traces of water present in the media or by preliminary attack of the trimethylsylanolate anion, subsequently desilylated during the TFA cleavage. However, after careful drying of the resin, the solvent and the nucleophile, the hydrolysis of the methyl ester did not proceed at all.

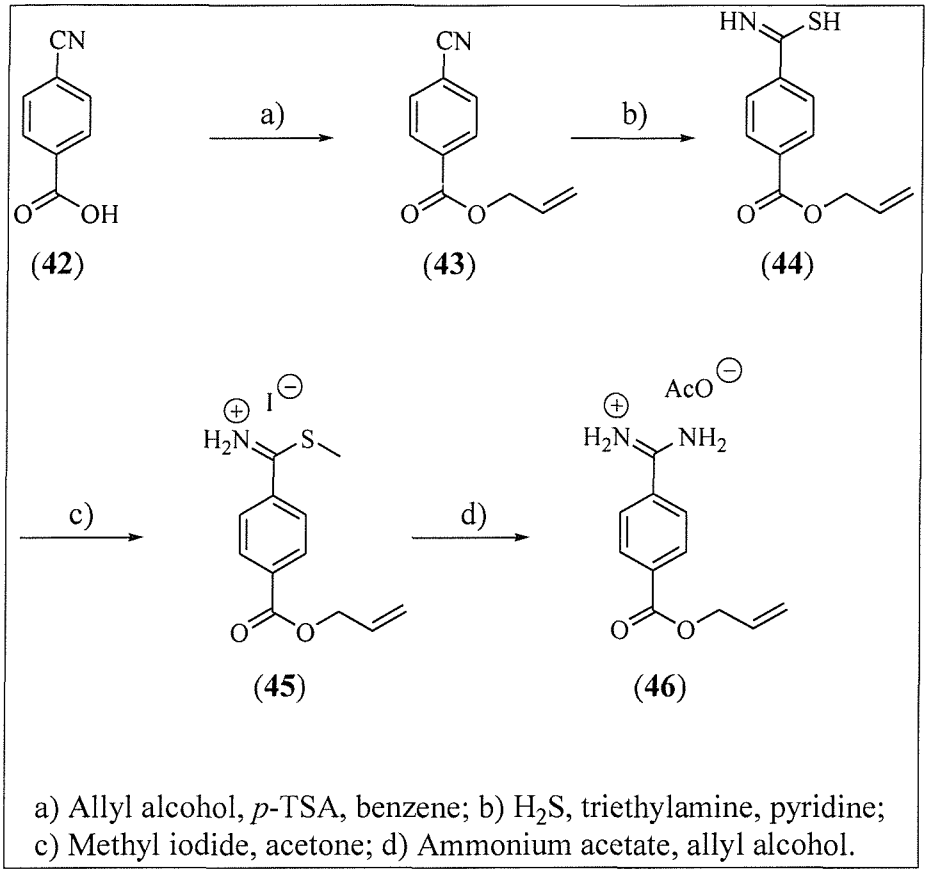
Alternative routes involving nucleophiles such as sodium iodide or trimethylsilyl iodide were investigated but without success.



Scheme 3.8: Solid-phase hydrolysis with potassium trimethylsilanolate.

3.6 Synthesis of Allyl-protected 4-Carboxybenzamidine

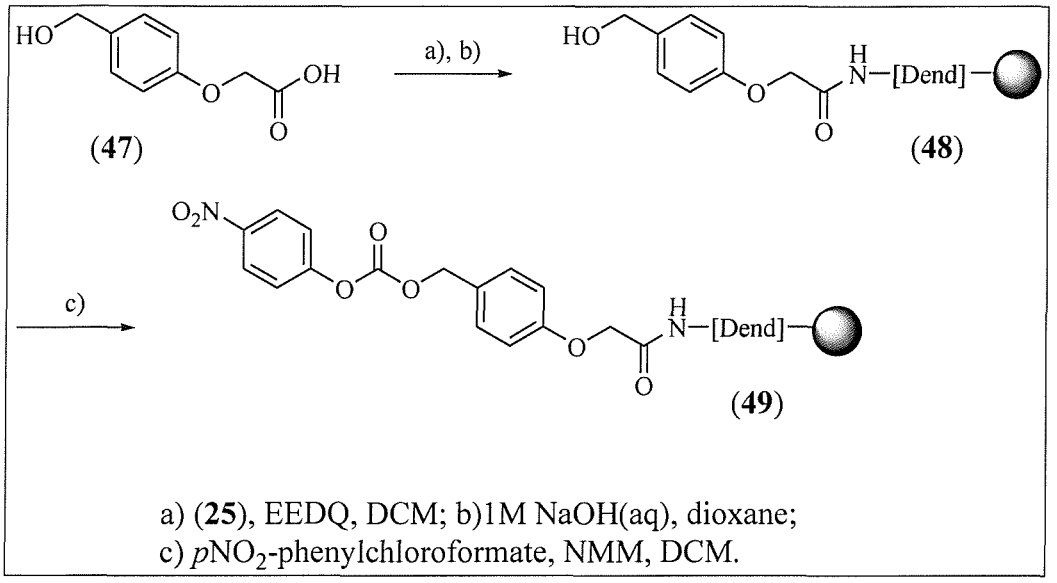
In order to avoid the side reactions related to the hydrolysis of the methyl ester, alternative protecting groups for the arylcarboxylic carbonyl function, that could be removed in milder or non-nucleophilic conditions, were investigated. Between *tert*-butyl, benzyl and allyl esters the latter appeared more promising, *tert*-butyl ester deprotection being not compatible with the acid-labile Wang linker, and the removal of a benzyl group requiring a non-trivial hydrogenolysis step on the solid phase. It was envisaged that the synthesis of allyl protected carboxybenzamidine (**46**) could not proceed *via* a classical Pinner reaction, since the double bond could react with methanol under very strongly acidic conditions. The thioimidate route[112] was therefore followed: the starting material of choice was the commercially available 4-cyanobenzoic acid (**42**). This compound was converted into the corresponding allyl ester (**43**) using a standard Fisher esterification with allyl alcohol, benzene, catalytic *p*-TSA and a Dean-Stark condenser. The cyano group was subsequently reacted with hydrogen sulfide in basic conditions to give the correspondent thioimidate. This was transformed into the amidine moiety in two steps and 58% overall yield, as shown in Scheme 3.9.



Scheme 3.9: Synthesis of 4-(allyloxycarbonyl)benzamidinium acetate.

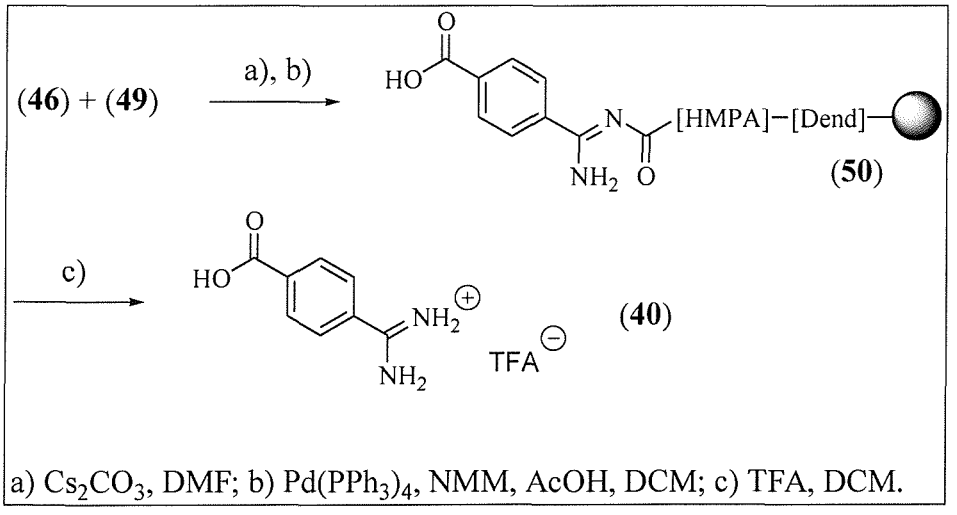
3.7 Synthesis of Resin-bound Amidine Scaffold *via* Active-carbonate Linker

The synthesis of the amidine/linker complex was now not practicable, since the allyl or methyl phenoxy acetate group could not be selectively deprotected in presence of the allyl benzoate. The 4-(hydroxymethyl)phenoxyacetic acid (HMPA) linker (**47**) was therefore attached directly to the dendrimer resin. Although EEDQ was used as the coupling agent, which is known[113] to limit ester bond formation, the presence of linker dimers was detected by HPLC (possibly due to the amine content of the dendrimer, able to catalyse ester formation). Treatment of the resin with 1M NaOH(aq)/dioxane 1:1 for two hours afforded the desired HMPA dendrimer resin. The resulting alcohol (**48**) was reacted with *p*-nitrophenyl chloroformate, leading to the active-carbonate resin (**49**) (Scheme 3.10).



Scheme 3.10: Synthesis of active-carbonate dendrimer resin.

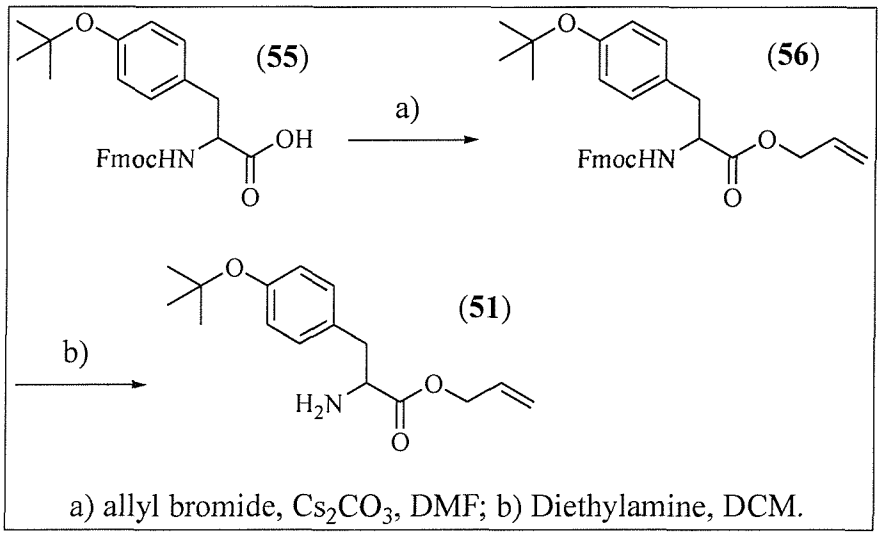
p-Nitrophenol was then quantitatively displaced by the amidine derivative (**46**) in the presence of Cs_2CO_3 in DMF, affording the desired resin-bound amidine. The cleavage of the allyl ester was performed under very mild, neutral conditions,[114] with N-methyl morpholine/acetic acid and $Pd(PPh_3)_4$ in DCM, giving the desired resin (**50**) (Scheme 3.11).



Scheme 3.11: Attachment of 4-(allyloxycarbonyl)benzamidine onto dendrimer resin and allyl-ester deprotection.

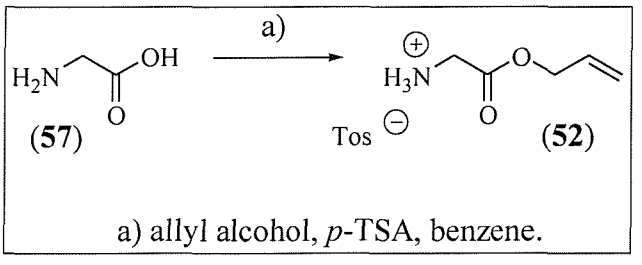
3.8 Solid Phase Synthesis of Ro44-9883 and TAK-029

The resin-bound scaffold was now ready for further elaboration. In order to validate this chemistry, it was decided to synthesise the two known compounds Ro44-9883 (**26**) and TAK-029 (**27**), to compare the solid-phase synthesis with the solution-phase synthesis reported in the literature.[86,91] The building blocks (**51**), (**52**), (**53**) and (**54**) were prepared in solution according to the following procedures. (**51**) was obtained from commercially available Fmoc-Tyr(*t*Bu)OH (**55**) in two steps: esterification with allyl bromide in DMF and deprotection of Fmoc with diethylamine in DCM (Scheme 3.12).



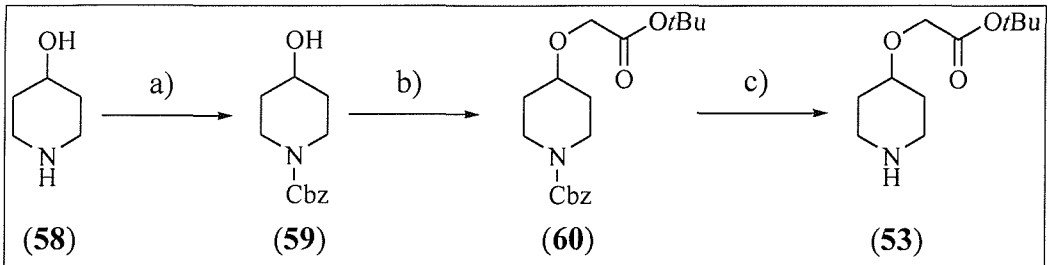
Scheme 3.12: Synthesis of Tyrosine(*t*Bu) allyl ester.

(**52**) was obtained as a tosyl salt from glycine (**57**) in one step (Scheme 3.13).



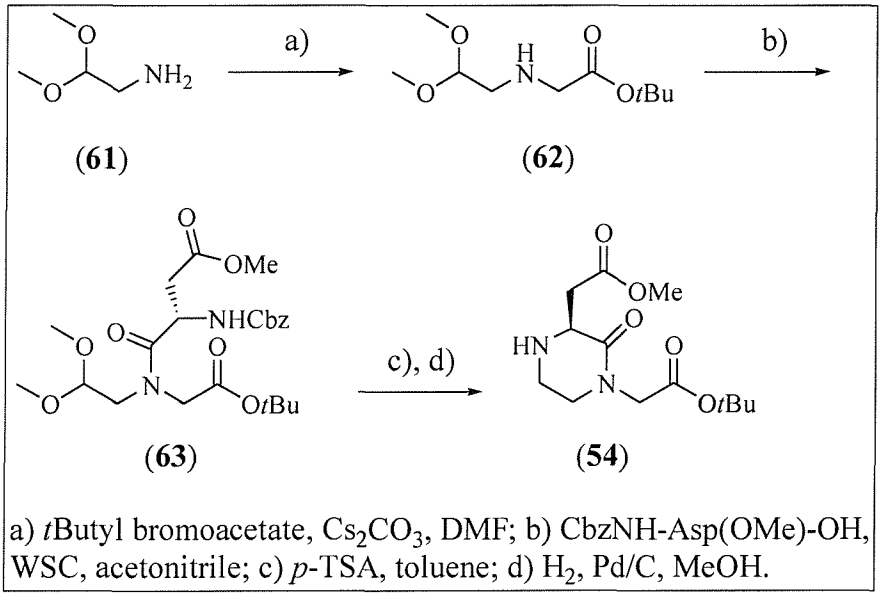
Scheme 3.13: Synthesis of Glycine allyl ester.

The literature procedures according to Weller[86] (Scheme 3.14) and Sugihara[91] (Scheme 3.15) were followed to prepare (**53**) and (**54**).



a) Cbz-Cl, triethylamine, DCM; b) *t*Butyl bromoacetate, tetrabutylammonium hydrogensulphate, NaOH(aq), toluene; c) H_2, Pd/C, MeOH.

Scheme 3.14: Synthesis of 4-(piperidyl)oxyacetate *tert*-butyl ester

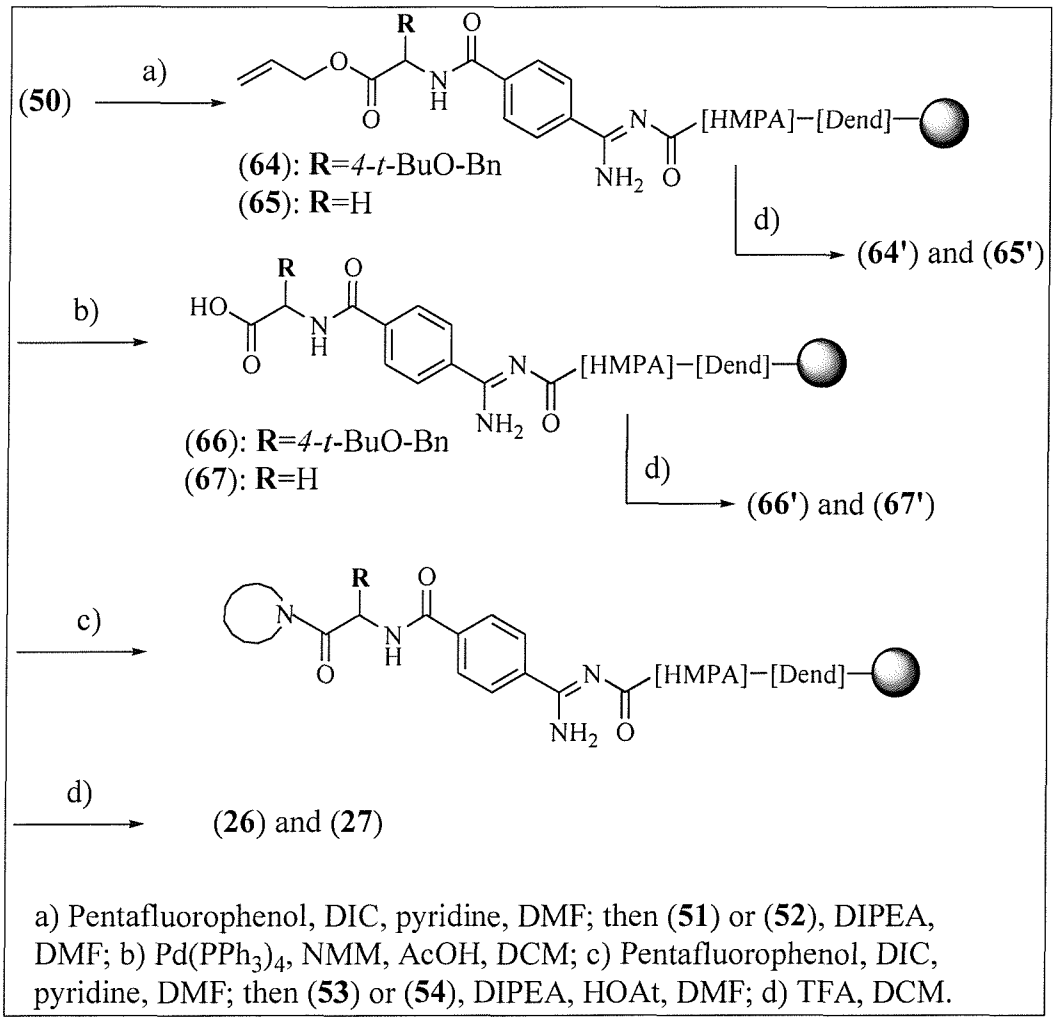


a) *t*Butyl bromoacetate, Cs_2CO_3, DMF; b) CbzNH-Asp(OMe)-OH, WSC, acetonitrile; c) *p*-TSA, toluene; d) H_2, Pd/C, MeOH.

Scheme 3.15: Synthesis of 3-((Methoxycarbonyl)methyl)-2-oxopiperazine-1-acetate *tert*-butyl ester.

The amide bond formation between the resin-bound benzoic acid (**50**) and the two amino-acids (**51**) and (**52**) was found to be difficult to drive to completion. It is known that peptide synthesis in the N-to-C direction is associated with difficulties not encountered in the standard C-to-N synthesis,[115] partially related to the fact that the activation step is performed on the resin; moreover an aromatic carboxylic acid was present, usually less reactive than an aliphatic one. A wide range of coupling

reagents/mixtures were tested (DIC/HOBt, HATU/HOAt,[116] TBTU/HOBt,[31] PyBOP,[117] PyBroP,[118] cyanuric fluoride,[119] diphenylphosphoryl azide[120]) and the best results were obtained with pentafluorophenol/pyridine/DIC: after a single overnight coupling the reactions always reached completion (Scheme 3.16).



Scheme 3.16: Solid-phase synthesis of Ro44-9883 and TAK-029.

Again the allyl esters (**64**) and (**65**) were deprotected and the resulting acids (**66**) and (**67**) reacted with the two different piperidine derivatives (**53**) and (**54**) using the same conditions as before, but with the aid of HOAt, known to be efficient in coupling reactions with secondary amines. Treatment of the resin with TFA/DCM 95:5 resulted in cleavage and deprotection of the *tert*-butyl groups, giving the final compounds (**26**) and (**27**) in 23% and 16% overall yield respectively, after purification. The overall

isolated yields of the two compounds Ro44-9883 and TAK-029 were comparable with those obtained in solution, however the synthesis did not require isolation and purification of the intermediates. In addition the amidine moiety, usually the low yielding step, was elaborated starting from a commercial inexpensive starting material, without loss of precious intermediates. Finally the synthesis was straightforward and in principle allows the generation of diversity starting from the same resin-bound amidine and different building blocks.

3.9 Parallel Synthesis of an Array of Amidine-based Compounds

Five different allyl-protected amino-acids and three piperidine derivatives were chosen to synthesise a 15-member library of amidine-based compounds mimicking the structures of Lamifiban and TAK-029. The different structures are shown in Figure 3.5.

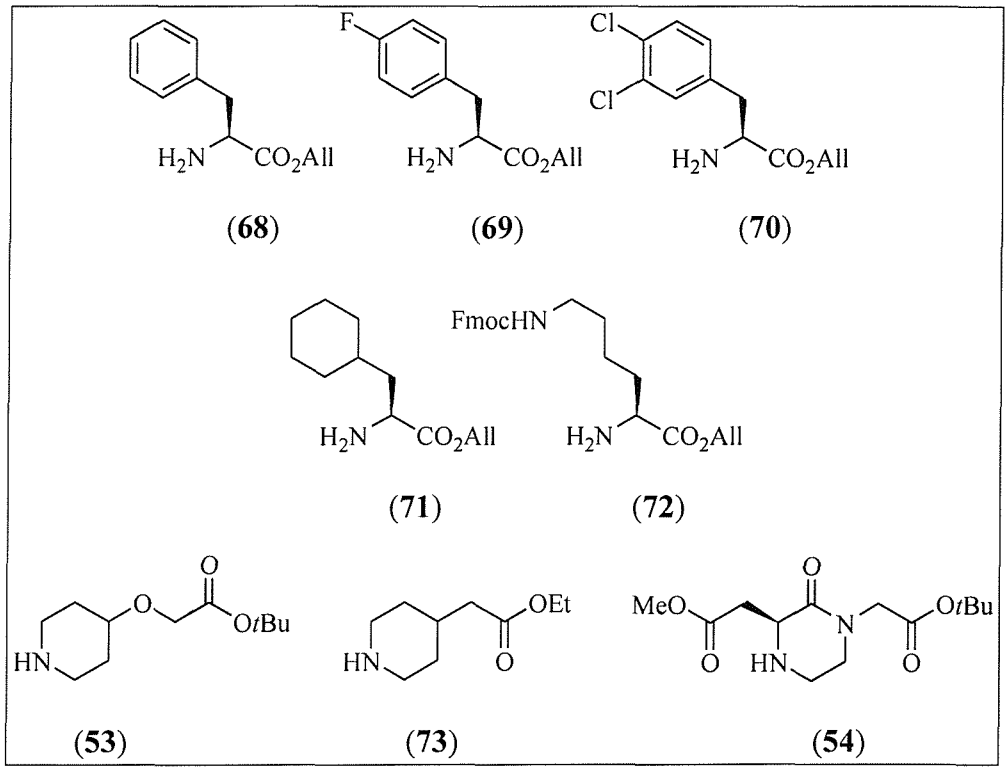
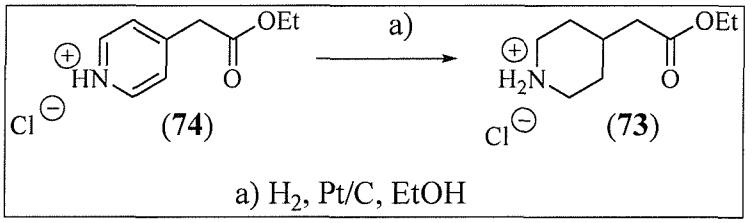


Figure 3.5: Different building-blocks for the synthesis of a library of amidines.

The allyl esters of 4-Fluorophenylalanine (**69**), 3,4-dichlorophenylalanine (**70**) and cyclohexyl alanine (**71**) were obtained in two steps from the corresponding Fmoc-protected aminoacids following the same procedure used for the tyrosine derivative

(**51**). Phenylalanine allyl ester (**68**) and ε-Fmoc-lysine allyl ester (**72**) were obtained from the corresponding aminoacids *via* esterification in benzene/allyl alcohol in the presence of 1.1 eq of *p*-toluenesulphonic acid, as for the case of the glycine derivative (**52**). The piperidine derivative (**73**) was obtained by reduction of the corresponding pyridine derivative (**74**) (Scheme 3.17).[121]



Scheme 3.17: Synthesis of 4-(piperidyl)acetate ethyl ester hydrochloride

The solid phase reactions were performed as described before and the results, in terms of HPLC purities and ES-MS analysis, are reported in Table 3.1.

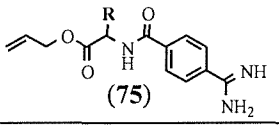
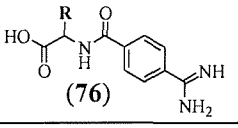
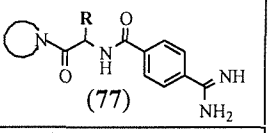
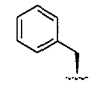
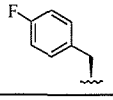
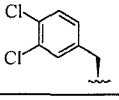
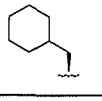
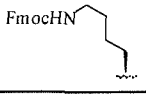
R	 (75)	 (76)	 (77)
	71%	66%	(**53**): 56% (453.2) (**73**): 52% (465.4) (**54**): 31% (524.3)
	75%	76%	(**53**): 51% (471.2) (**73**): 47% (483.4) (**54**): 26% (542.2)
	72%	63%	(**53**): 45% (521.2) (**73**): 42% (533.3) (**54**): 24% (592.6)
	80%	64%	(**53**): 49% (459.4) (**73**): 43% (471.4) (**54**): 22% (N.D.)
	75%	69%	(**53**): 34% (434.3) (**73**): 39% (446.3) (**54**): 40% (505.5)

Table 3.1: HPLC purities and ES-MS for the 15 members of the library.

The HPLC traces indicated that the purity of the crude compounds being greater than 40% in most of the cases (the purity was found to be lower for the compounds of the (**54**) series, due to the more sterically hindered piperidine derivative). The isolated yield following semi-preparative HPLC purification was approximately 30%. It is noteworthy that, without taking in account the dendrimerisation process, the final amidines were obtained after eight synthetic steps without purification of the intermediates and therefore the purities of the final crude compounds were very encouraging. Figure 3.6 shows the HPLC traces of the intermediates for the synthesis of compound (77(**69**,**53**)).

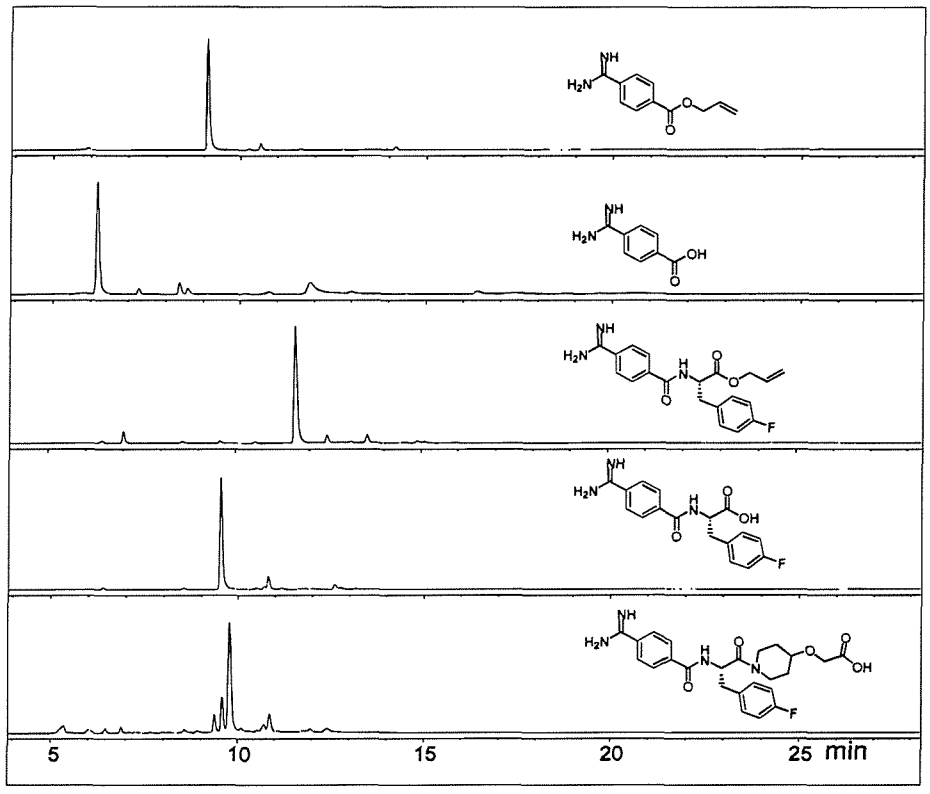


Figure 3.6: HPLC traces of the intermediates to the synthesis of one member of the library.

Combinatorial chemistry often leads to mixtures of compounds rather than single entities. The possibility of determining the components of a mixture by simple analytical techniques such as mass-spectrometry is very important. Nine members of the library were therefore analysed as a mixture using an IonSpec Ultima FT Mass Spectrometer and the resulting trace is reported in Figure 3.7. The structure of all nine compounds could be successfully determined from the analysis of that trace.

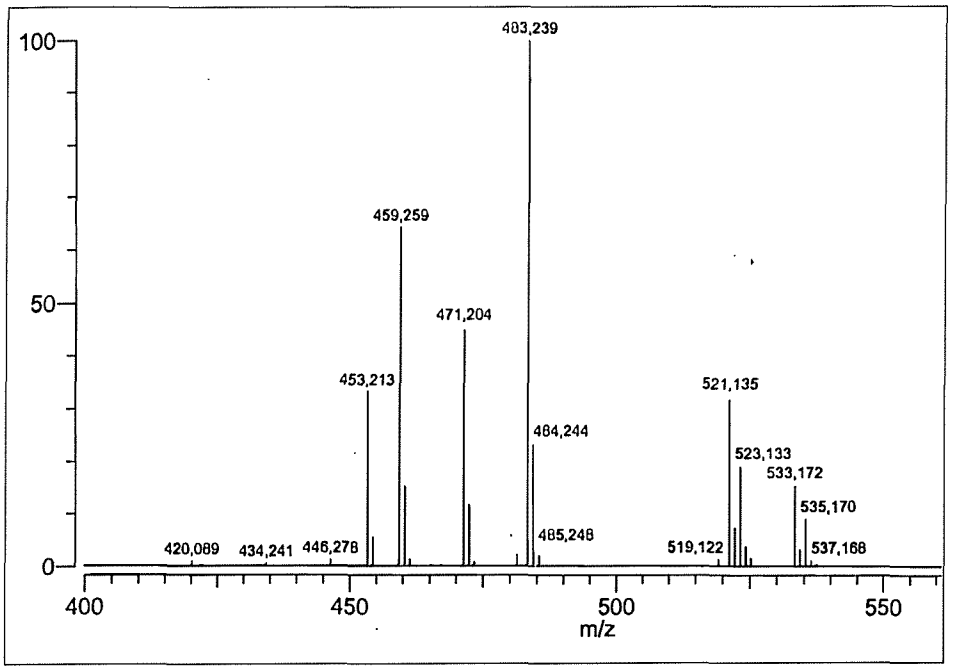


Figure 3.7: ES-MS trace of compounds (**77**(**68**),(**53**)) (453.2), (**77**(**69**),(**53**)) (471.2), (**77**(**69**),(**73**)) (483.2), (**77**(**70**),(**53**)) (521.1), (**77**(**70**),(**73**)) (533.2), (**77**(**71**),(**53**)) (459.3), (**77**(**71**),(**73**)) (471.2), (**77**(**72**),(**53**)) (434.2) and (**77**(**72**),(**73**)) (446.3).

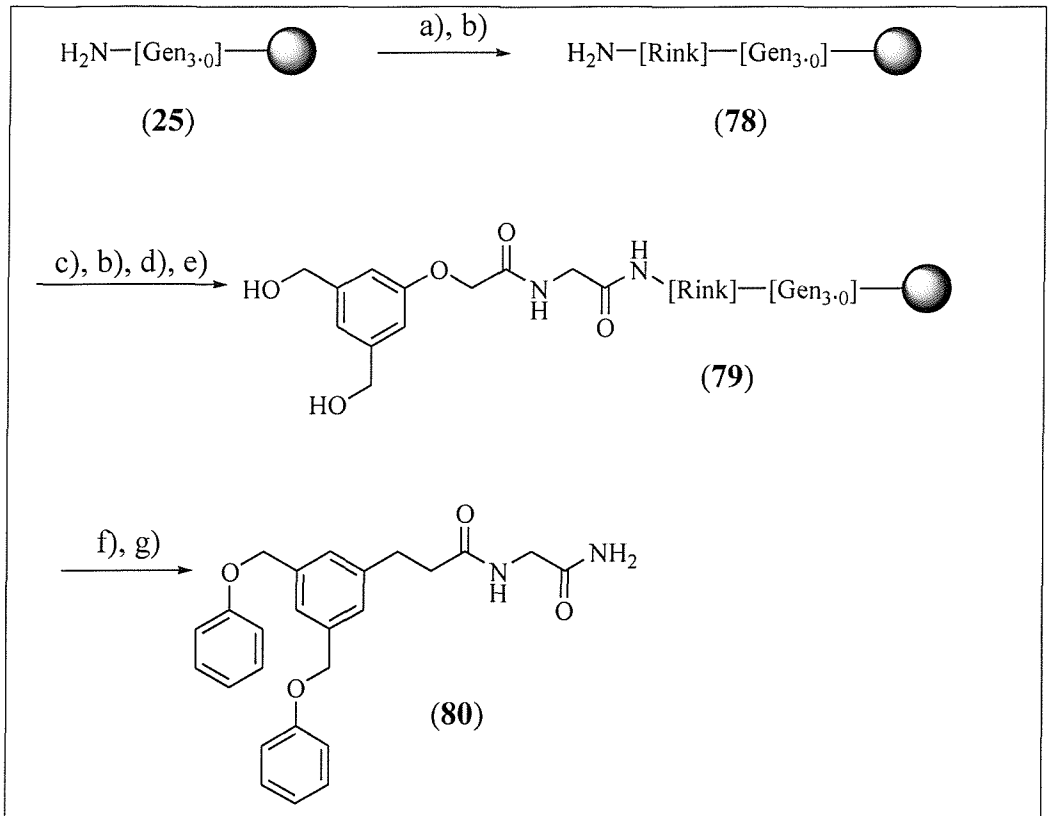
Chapter Four

Synthesis of Arylethers via Mitsunobu[122] Condensations

4.1 Preliminary Studies

The amidine chemistry was considered a severe test for dendrimer resin. The successful synthesis of amidine-based compounds proved that these new high-loading beads could be routinely utilised for amide-bond formation reactions (in both N to C and C to N direction), nucleophilic displacement of resin-bound carbonates, hydrolysis of esters and acidic cleavage. Moreover the resin-bound intermediates were stable upon storage, meaning that side-reactions between the amidine compounds and the polyamidoamine chains did not occur.

In order to extend the variety of chemical conditions compatible with this resin, the synthesis of arylethers *via* Mitsunobu condensations were chosen as the target. This choice was justified by previous experiences, in which the reaction worked satisfactorily on polystyrene but not with TentaGel resin, where often only starting material was recovered. Preliminary studies on Mitsunobu condensations were conducted following the route shown in Scheme 4.1 and were aimed at the solid-phase synthesis of arylether dendrimers (full details will be given in Chapter 6). After attachment of the Rink linker[123] onto dendrimer resin (**25**), the double Mitsunobu condensation of the resin bound 3,5-*bis*(hydroxymethyl)phenoxy derivative (**79**) with two molecules of phenol proceeded quantitatively and the final compound (**80**), after cleavage off the resin, was isolated in very good HPLC purity and yield. The reaction was routinely performed at room temperature but the portionwise addition of azodicarboxylate to the mixture of phenol, triphenylphosphine and resin-bound alcohol was found to be crucial. Moreover the resin was washed three times with freshly distilled dry THF before the reaction. After a first cycle a small amount of compound was cleaved off the resin. The HPLC analysis showed that the reaction was 80% complete, with 20% of the *mono*adduct, but no trace of the starting material. After a second cycle the reaction was complete.



a) Fmoc-Rink-OH, DIC, HOBt, DMF; b) Piperidine, DMF; c) Fmoc-Gly-OH, DIC, HOBt, DMF; d) 3,5-*bis* (acetoxymethyl)phenoxyacetic acid, DIC, HOBt, DMF; e) 1M NaOH(aq), dioxane; f) Phenol, PPh_3, DEAD, THF; g) TFA, water.

Scheme 4.1: Solid-phase Mitsunobu condensation.

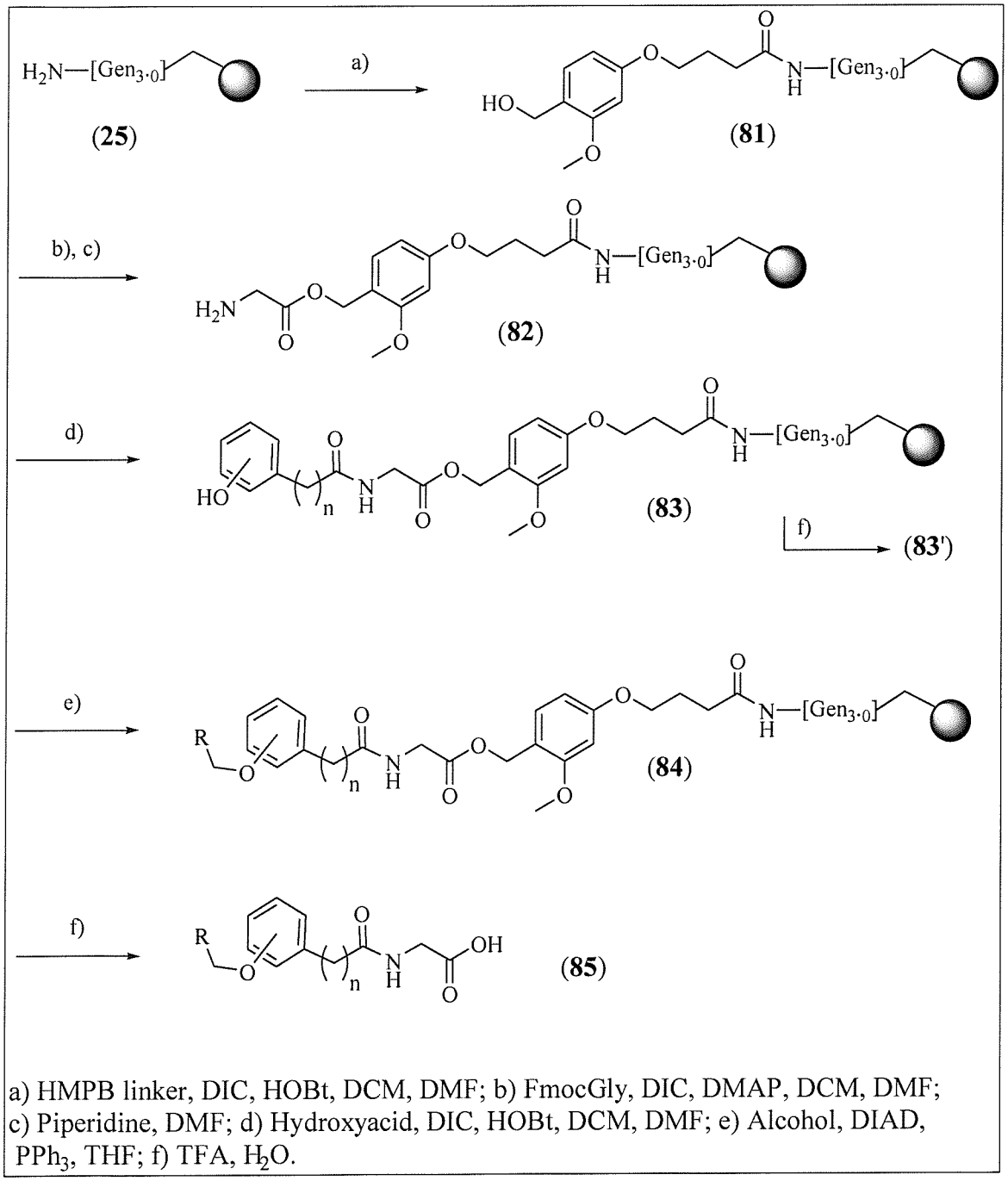
4.2 Parallel Synthesis of a 20-member Library

Following these promising results, it was decided to further investigate this reaction and to perform a parallel synthesis of compounds with possible biological activity towards cyclo-oxygenase 2.[124] A different synthetic path was chosen for the synthesis of a 20-member library of arylethers, the main difference being that this time the phenolic derivative was bound to the resin, while the primary alcohol was in solution (Scheme 4.2).

The HMPB linker was first attached to the resin, then a Fmoc-protected amino acid[125] and, after removal of the protecting group, a hydroxyphenyl carboxylic acid. These steps were all straightforward, although the amount of reagents and the reaction time were crucial to avoid phenoxy ester formation in the last reaction (detected by LC-MS and ^{1}H NMR after TFA cleavage).

Different conditions were investigated for the Mitsunobu condensation, finding that DIAD/PPh_3 worked best, while TMAD/PBu_3[126] or other combinations gave poorer results.

We also investigated if an amine could enhance the speed rate,[127] but this was not observed, probably because of the intrinsic basicity of the dendrimer itself.



Scheme 4.2: Synthesis of a 20-member library of aryl ethers.

Once the best conditions had been found, a small library was synthesised: it was decided to generate a binary library, varying the phenolic acid and the alcohol, although in principle a ternary library could have been generated. The aminoacid was kept constant for two reasons: being far from the phenolic -OH, it should have no influence on the Mitsunobu condensation. Moreover the size of the library was small enough to be carried out in a *semi*-automated fashion on an Argonaut's QUEST (20 different vials). Four different phenolic acids (with different electronic properties and position of the –OH group) were chosen together with five different alcohols (with different reactivity and steric hindrance). The results obtained are summarised in Table 4.1.

All the reactions worked very well in terms of purity (determined by HPLC with diode array UV detection) and yield (determined by ^{1}H NMR *via* d_5-DMSO calibration).[128] Only in the case of 4-(dimethylamino) phenethyl alcohol was the yield below 70%, this being attributed to the fact that the cleavage step was sluggish if a free amine was present in the cleaved compound.

All members of the library were characterised by LC-MS, ^{1}H NMR and ^{13}C NMR. Single bead MS was also carried out, but not all the compounds ionised under the experimental conditions (most of them have only an amide bond as a protonation site and the carboxylic acid does not give the corresponding carboxylate after TFA cleavage).

The amount of compound released from a single bead was determined *via* a HPLC calibration curve of compound (**85**(**1b**)). The cleavage of 10 beads afforded enough material for HPLC analysis: this was determined to be 35 nmol, therefore a single bead released 3.5 nmol, in accordance with the initial loading of dendrimer resin (**25**) of 4 nmol/bead.

During the synthesis of the library the robustness of dendrimer resin was also proven. The agitation in most of the automated systems is generated by bubbling of an inert gas, in the QUEST by magnetic bars moving up and down inside the vials. Although this method is rather harsh, the beads did not break into pieces, therefore being robust enough for automation.

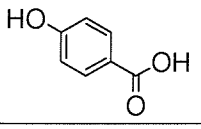
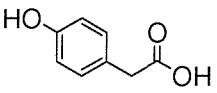
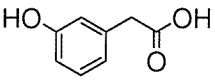
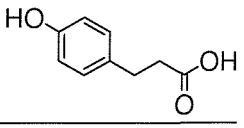
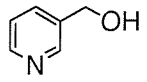
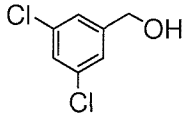
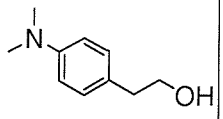
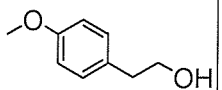
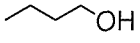
		1	2	3	4
					
a		287.2[a] 86%[b] **91%**[c]	301.2 82% **97%**	301.3 84% **85%**	315.3 79% **95%**
b		354.1 84% **100%**	368.2 82% **95%**	368.2 84% **97%**	(380.2)[d] 76% **98%**
c		343.2 68% **100%**	357.3 67% **95%**	357.3 62% **67%**	371.3 66% **88%**
d		330.1 78% **100%**	344.1 83% **91%**	344.1 78% **83%**	358.2 82% **94%**
e		252.1 85% **97%**	266.2 88% **92%**	(266.2)[d] 75% **84%**	280.2 78% **91%**

Table 4.1: [a]Single-bead MS, [b]HPLC purity and [c]yield of the 20 members of the library. [d]The expected MS peak was obtained only with higher concentration of compound.

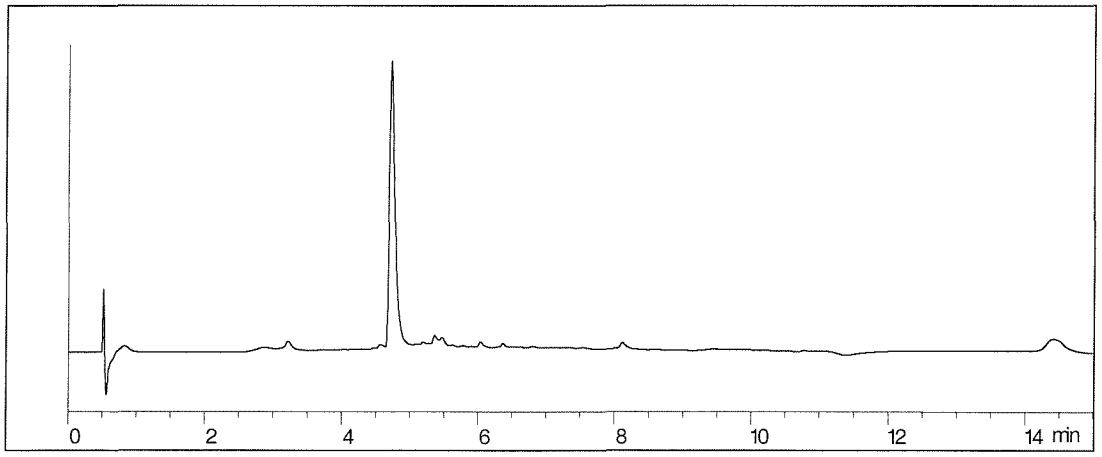


Figure 4.1: HPLC trace of compound (**85**(**1a**)).

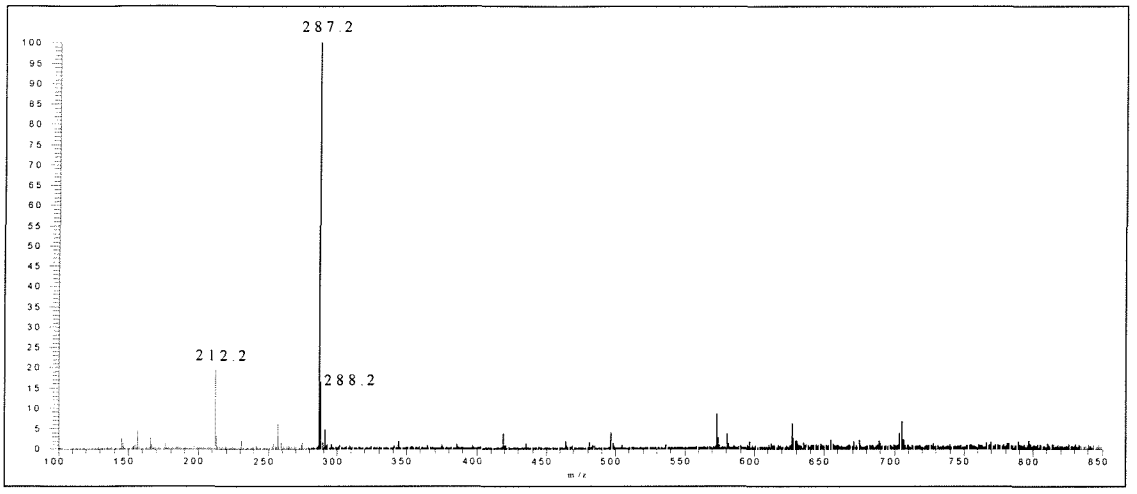


Figure 4.2: Single-bead MS of compound (**85(1a)**).

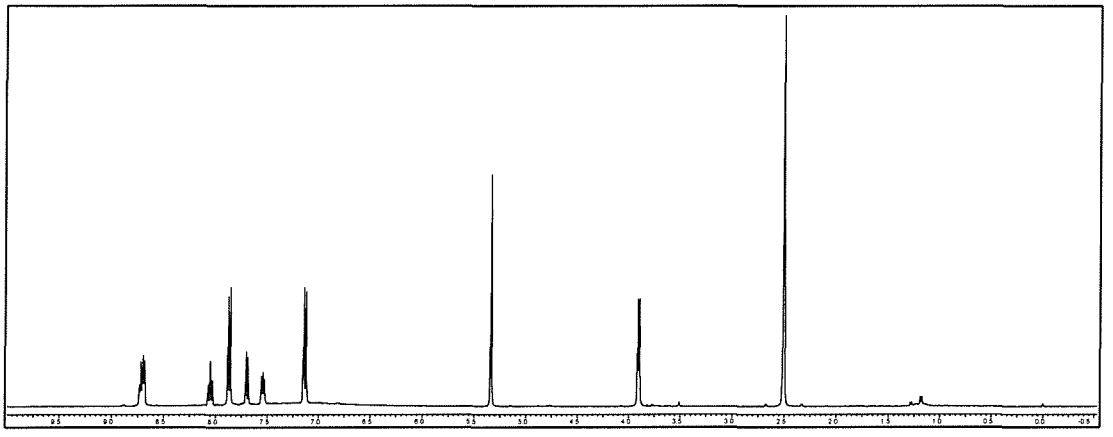


Figure 4.3: crude ^{1}H NMR of compound (**85(1a)**).

4.3 Comparison of TentaGel and Dendrimer Resin Reactivity

The Mitsunobu condensation was performed in parallel with normal TentaGel resin and dendrimer resin. The scaffold on the resin was kept constant, with Glycine and 4-hydroxyphenylacetic acid as building blocks. Four different alcohols were used: benzyl alcohol, pyridyl-2-carbinol, 3,5-dichlorobenzylalcohol and N-Boc-ethanolamine.

The results are listed in Table 4.2 and they show that, in terms of conversion, dendrimer resin worked much better. The HPLC purity was usually high in both cases.

It was thought that the basicity of the dendrimer, as mentioned before, aiding the deprotonation of the resin bound phenol, could facilitate the reaction. To prove this theory the same reactions was performed on normal TentaGel resin with N-methyl

morpholine (10 equivalents, 30 equivalents or as a solvent) or with commercially available Gen[1.0] PAMAM dendrimer (thinking that it could act as an activator), but similar or worse results were obtained.

It was also thought that water trapped in the resin could have been a problem, but both dendrimerised and normal TentaGel resin were carefully dried before the condensation. Moreover, due to the higher extent of hydrogen-bonding, dendrimer resin should have a higher tendency to trap water.

Another hypothesis was that during the manufacturing of the resin, some reactive –OH sites were left free. These could interfere with Mitsunobu-like reactions, subtracting the activated alcohol from the solution. The dendrimerisation process itself could "clean" the resin of those reactive sites.

	Conversion (%)	
Alcohol	Normal TentaGel	Dendrimer resin
Benzyl alcohol	91	91
3,5-Dichlorobenzyl alcohol	45	93
N-Boc ethanolamine	0	67
Pyridyl-2-carbinol (1^{st} coupling)	0	100
Pyridyl-2-carbinol (2^{nd} coupling)	57	100

Table 4.2: Different performances of TentaGel and dendrimer resin under Mitsunobu conditions.

The last issue to be investigated was the suitability of the beads for single-bead screening. Before submitting the single beads for biological testing, we thought that screening the compounds already released from the resin would have given an idea of their activity. Unfortunately none of the compounds showed any activity towards cyclo-oxygenase 2: single-bead screening of the same compounds was therefore not considered to be useful.

Chapter Five

Other Chemistries Applied to PAMAM Dendrimer Resin

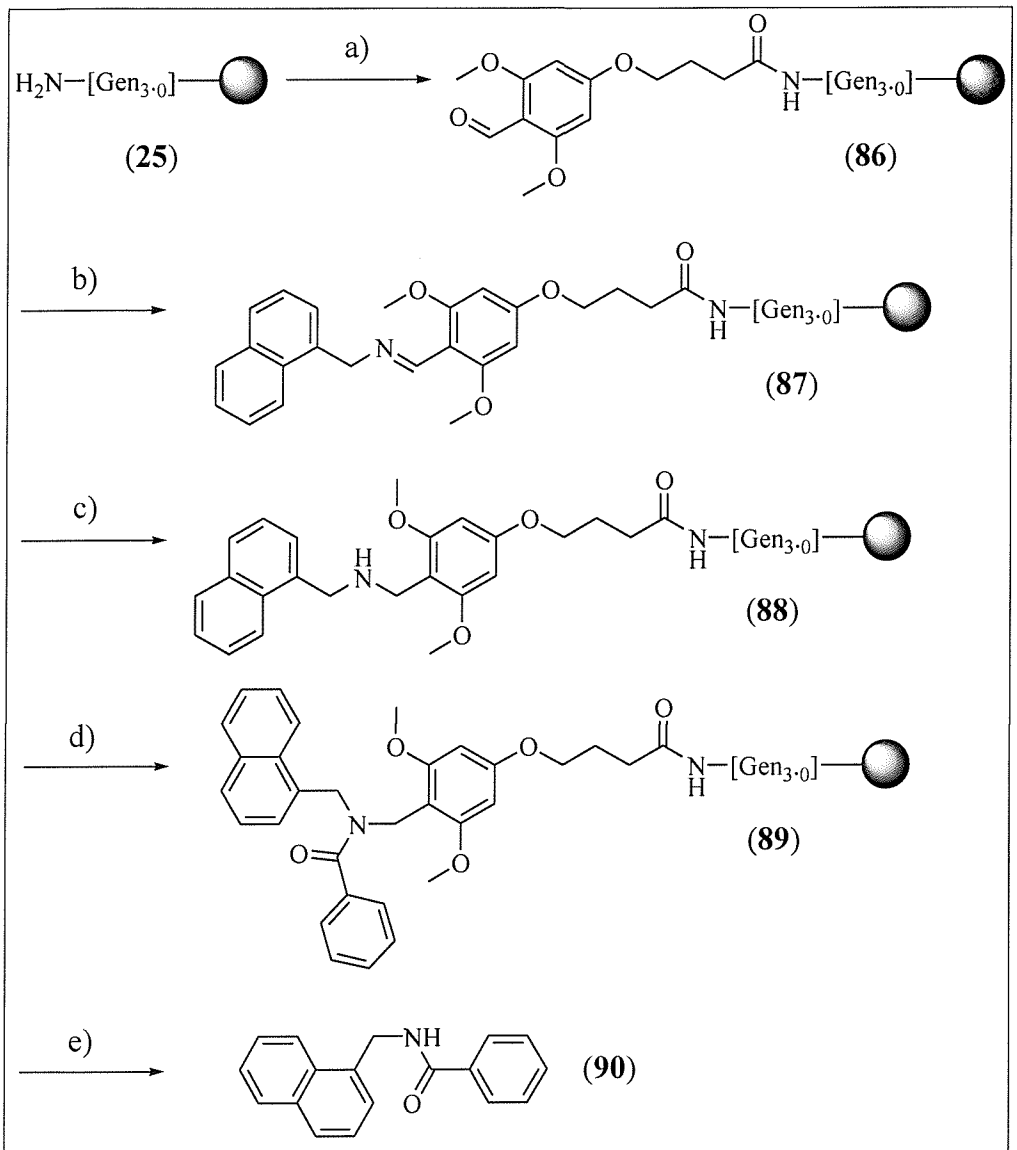
Once the syntheses of amidine-based compounds and arylethers were successfully achieved, other chemistries were investigated. Reactions commonly used in solid-phase organic chemistry that could be critical with the dendrimer backbone were chosen as targets. In this chapter six different syntheses will be illustrated and reasons will be given for the choice of the synthetic patterns followed.

5.1 Synthesis of a Secondary Amide *via* Reductive Amination

Reductive amination is a very common reaction used to synthesise secondary amines starting from an aldehyde and a primary amine. The main advantage compared to alkylation strategies is that *poly*-alkylation is usually completely suppressed. It can be performed in two steps, with isolation of the imine intermediate, or in one pot. This reaction is ideal for solid-phase chemistry because by using excesses of aldehyde (or primary amine) and reducing agent it can be easily driven to completion. The reducing agent used is usually quite mild: sodium borohydride or its derivatives (e.g.: cyanoborohydrides and acetoxyborohydrides). It was decided to investigate if the dendrimeric backbone of the resin, due to the high extent of amide bonds and tertiary amino groups, could in some way interfere with the reductive amination step, although it is known that amide bonds are usually not reduced under these conditions.

The synthetic pattern chosen is outlined in Scheme 5.1. The 4-(4'-formyl-3',5'-dimethoxy)phenoxybutiric acid linker (BAL, backbone amide linker)[129] was attached onto dendrimer resin using standard peptide coupling conditions. Reaction with (naphthylmethyl)amine in the presence of acetic acid using NMP as solvent resulted in imine formation. This was then reduced with tetrabutylammonium borohydride/acetic acid in NMP. The following steps were an acylation with benzoic acid, in order to activate to allow cleavage off the resin, and treatment with TFA/water 9:1. The resin was filtered and the solution was concentrated under a flux of nitrogen. The crude material was weighed and analysed by ES-MS, HPLC and NMR. The crude compound was essentially pure, although the yield was not very high (63%). It was decided to perform the sequence of reactions again repeating the reductive amination step twice, but no improvement of the yield was observed. Similar yields were also

obtained with normal TentaGel resin.[130] Dendrimer resin could therefore be satisfactorily used in reductive amination reactions.



a) BAL linker, DIC, HOBt, DMF; b) (1-Naphthylmethyl)amine, AcOH, NMP; c) Bu_4NBH_4, AcOH, NMP; d) Benzoic acid, PyBOP, DIPEA, DMF; e) TFA, H_2O.

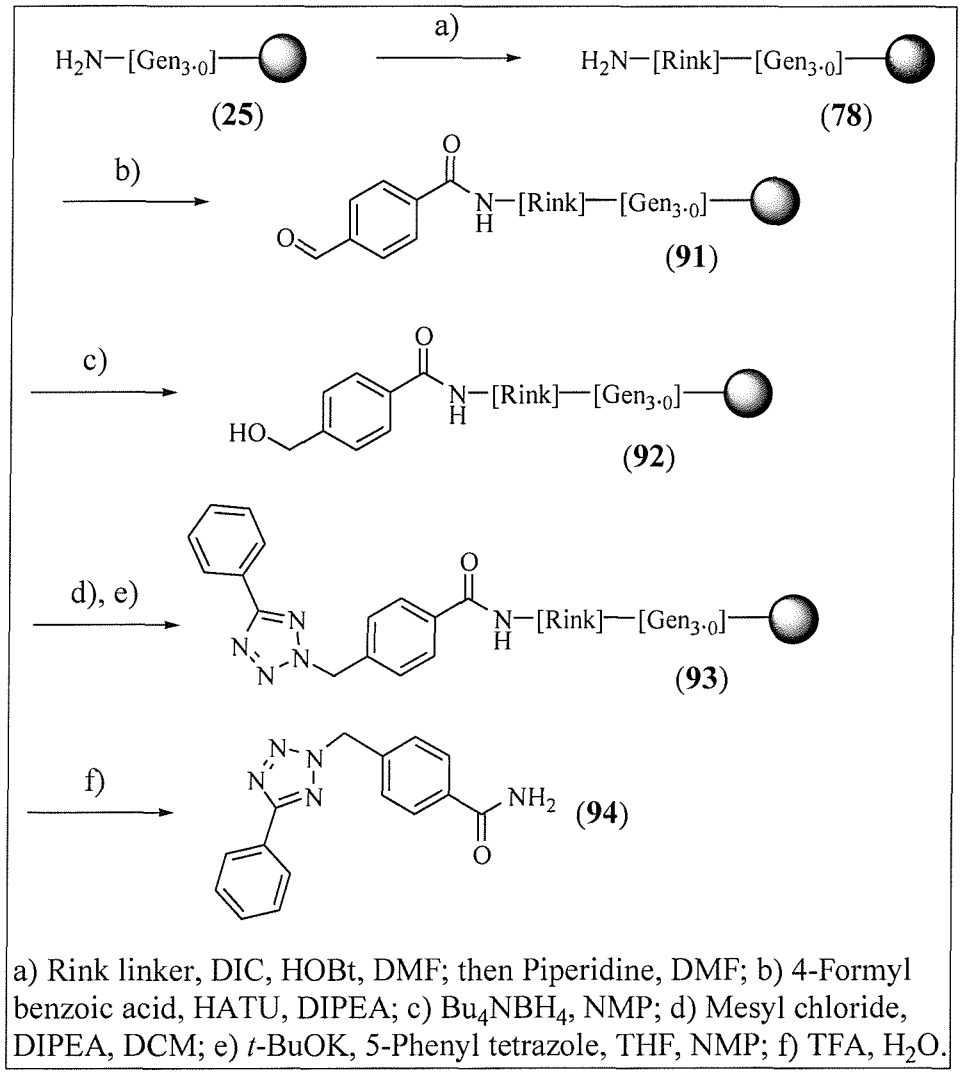
Scheme 5.1: Solid-phase synthesis of a secondary amide.

In analogy with the studies carried out during the synthesis of aryl ethers, a calibration curve was carried out to determine the amount of compound released from the dendrimer beads. Due to the presence of a strong chromophore such as a naphthalene

ring, it was possible to obtain an HPLC trace of the compound released by a single bead, with a sufficient signal/noise ratio. It was observed that a single bead released 2.0 nmol of compound, half of the theoretical value of 4.0 nmol/bead, therefore in accordance with the overall yield of the synthesis.

5.2 Synthesis of a Tetrazole Derivative *via* Displacement of a Resin-bound Mesylate

During the synthesis of amidine-based compounds we proved that reactive species like activated esters bound to dendrimer resin were stable. Due to the high content of nitrogen atoms of this resin, one could expect it to be likely to give rise to nucleophilic-attack side products to some extent.



a) Rink linker, DIC, HOBt, DMF; then Piperidine, DMF; b) 4-Formyl benzoic acid, HATU, DIPEA; c) Bu_4NBH_4, NMP; d) Mesyl chloride, DIPEA, DCM; e) *t*-BuOK, 5-Phenyl tetrazole, THF, NMP; f) TFA, H_2O.

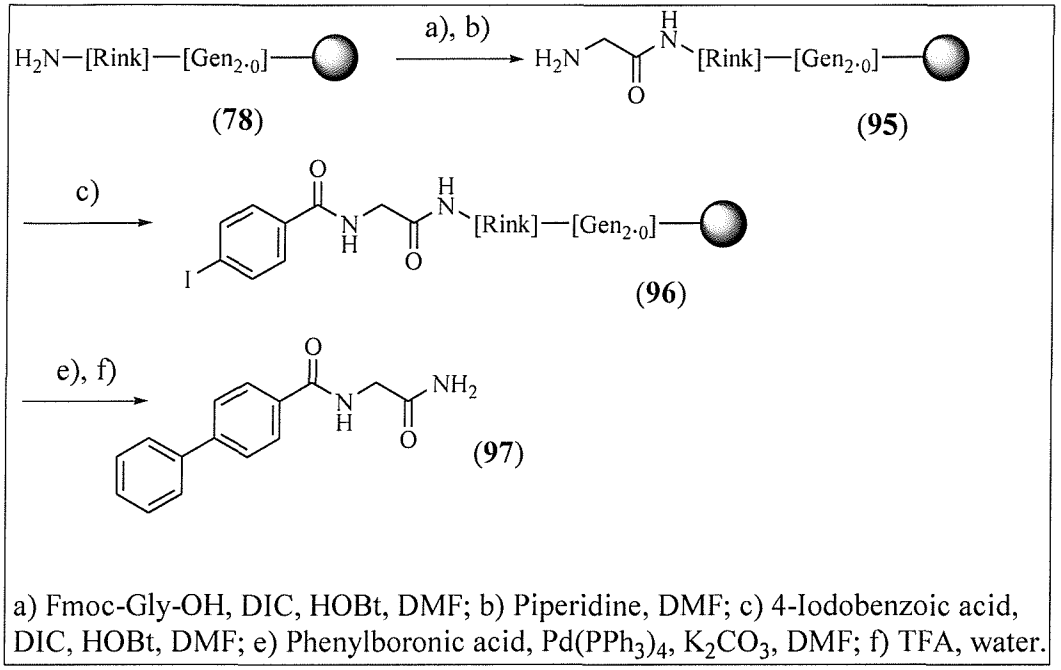
Scheme 5.2: Solid-phase synthesis of a tetrazole derivative.

In order to further investigate this possibility, it was decided to synthesise a highly reactive resin-bound benzylic mesylate and to displace it with a tetrazole anion. The synthetic path is outlined in Scheme 5.2. PAMAM dendrimer resin was reacted with Fmoc-protected Rink linker using DIC/HOBt in DMF. Fmoc deprotection with piperidine/DMF was followed by coupling with 4-formylbenzoic acid and subsequent reduction of the aldehyde to the correspondent alcohol. The alcohol was then mesylated with trifluoromethanesulphonyl chloride in DCM, using DIPEA to quench the HCl formed. The tetrazole anion was generated by reaction of phenyltetrazole with potassium *tert*-butoxide and then added to the resin. The mesylate displacement was initially kept for 7 hours, but it was found that better conversions were obtained after overnight reaction. Again the final results were satisfactory, the HPLC purity was 80% and the yield 50%, the main impurity being an isomer of the desired product, observed also with TentaGel resin.

5.3 Synthesis of a Biaryl *via* Suzuki[131] Coupling

The Suzuki reaction between an iodoaryl or iodoalkenyl derivative and a boronic acid is a common reaction used in solid phase chemistry. The main advantage compared to the solution phase version is that a larger excess of palladium catalyst can be used without the usual problems associated with purification. The reaction was therefore driven to completion easily. The experimental conditions usually involve a temperature of 80-100°C and the presence of a base like potassium carbonate. The stability of dendrimer resin to relatively high temperatures had not been investigated and it was thought that this reaction would have been an interesting test. It is known that Michael addition is a reversible reaction, therefore leakage of dendrimer branches into the solution at high temperatures in the presence of a base such as potassium carbonate could take place. The synthesis started from the Rink-dendrimer resin previously described, and continued with the couplings of Fmoc-glycine and 4-iodobenzoic acid in that order. The Suzuki condensation was performed at 100°C for 24 hours with 0.1 equivalent of $Pd(PPh_3)_4$ and 2 equivalents of potassium carbonate (relative to the amount of 4-iodobenzoic acid) in DMF (Scheme 5.3). Although at the end of the reaction the resin was completely black, the desired compound was isolated with a 90% HPLC purity and 80% overall yield after TFA cleavage. The glycine

appendage was introduced to help the ionisation of the final compound for MS analysis.



Scheme 5.3: Solid-phase synthesis of a biaryl derivative.

It was therefore concluded that PAMAM dendrimer resin was also stable at high temperatures.

5.4 Synthesis of Benzodiazepinone Derivatives for Single-bead Screening

The quantification of the amount of compound released from a single bead during the Mitsunobu and the reductive amination experiments proved that the loading of these beads is enough for single-bead screening. However, testing the beads in a real biological assay is another thing. Since it was not possible to test the library of ethers, it was decided to synthesise on the dendrimer beads a few compounds whose activity was already known: single-bead screening of these compounds should confirm this activity. It was chosen to synthesise the compounds shown in Figure 5.1. They have already been synthesised in Glaxo Wellcome[132] and have an antagonist activity towards human oxytocin ranging from 0.01 to 1 μM. The aim of the experiment was to check if it was possible to determine the IC_{50} of all three molecules with single-bead screening.

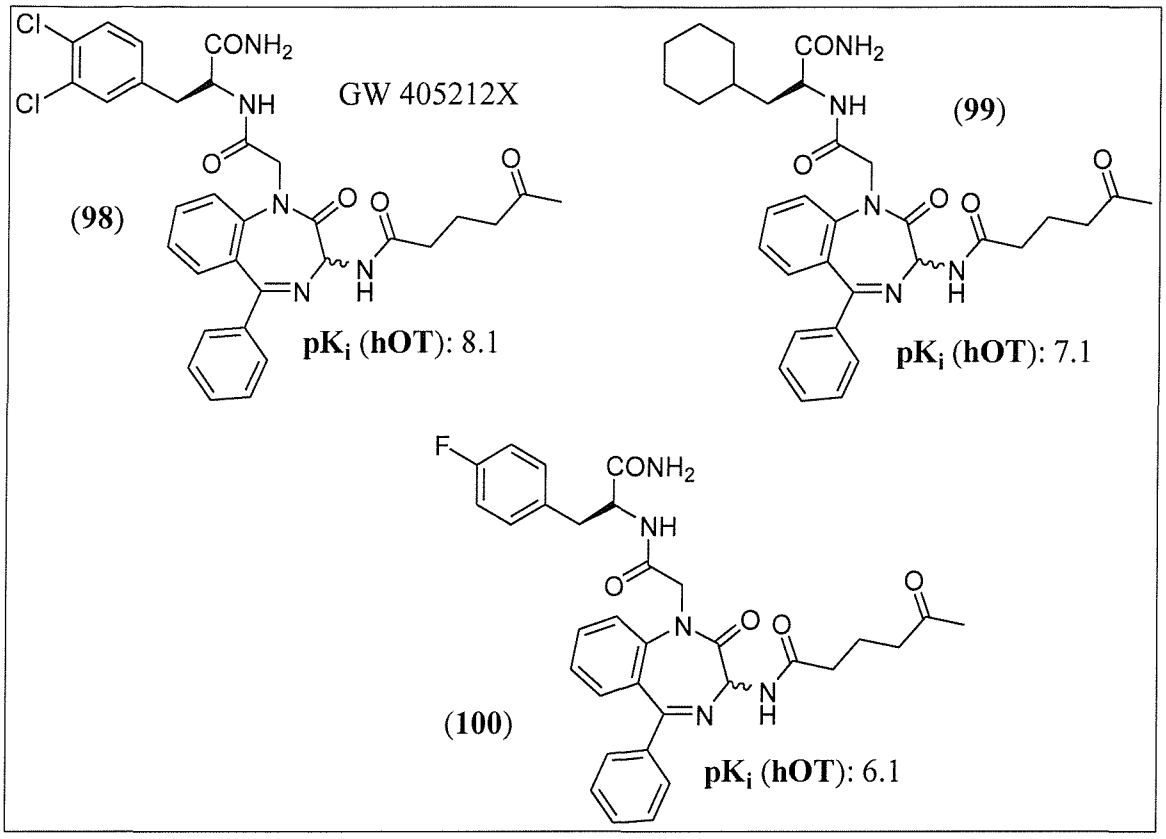
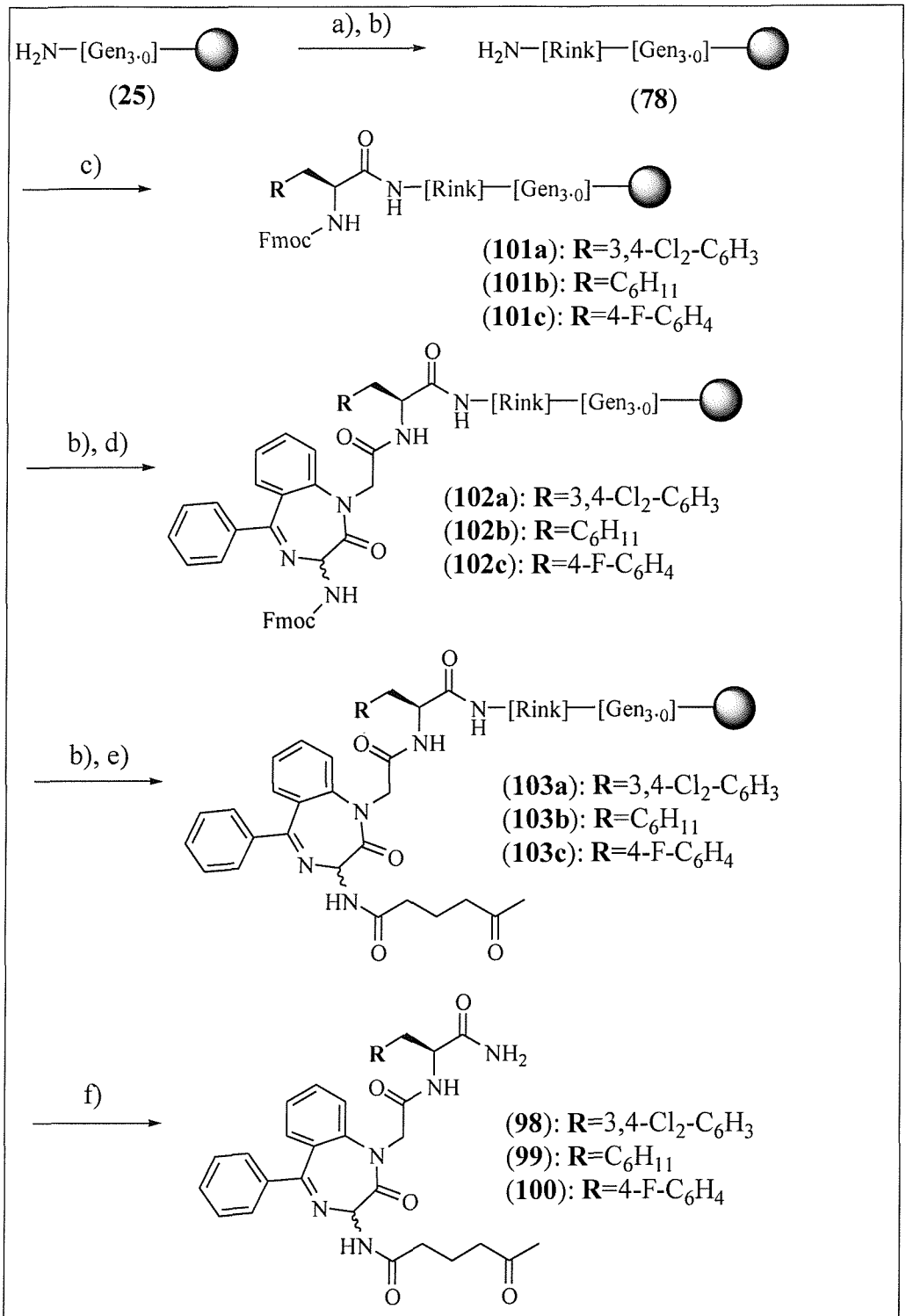


Figure 5.1: Human Oxytocin antagonists with relative inhibition potency.

The synthesis of the molecules was straightforward, following the procedure already set up at GlaxoWellcome and shown in Scheme 5.4. Rink dendrimer resin (**78**) was coupled with three different Fmoc-protected aminoacids: 3,4-dichlorophenylalanine, cyclohexylalanine and 4-fluorophenylalanine. After Fmoc deprotection 1-carboxymethyl-3-(fluorenylmethoxycarbonyl)amino-5-phenyl-1,4-benzodiazepin-2-one was coupled using HATU as coupling agent, followed again by Fmoc deprotection. HATU was used also for the final coupling of 4-acetylbutyric acid. The final compounds were obtained in very high HPLC purity after TFA cleavage and LC/MS analysis gave the expected masses.

At the moment the biological tests have not been carried out.



a) Rink linker, DIC, HOBt, DMF; b) Piperidine, DMF; c) Fmoc-aminoacid, HATU, DIPEA, DMF; d) 1-Carboxymethyl-3-(fluorenylmethoxycarbonyl) amino-5-phenyl-1,4-benzodiazepin-2-one, HATU, DIPEA, DMF; e) 5-Oxohexanoic acid, HATU, DIPEA, DMF; f) TFA, H_2O.

Scheme 5.4: Solid-phase synthesis of human oxytocin antagonists.

5.5 Investigation of Resin Cross-linking Reactions

So far, it has been demonstrated that dendrimer resin can undergo most of the chemical conditions normally used in solid phase organic synthesis, however there is still one issue to be investigated: cross-linking reactions between the branches of the dendrimer. This becomes an important matter if we consider an intramolecular reaction: due to the higher density of functional groups it is possible that molecules on different branches of the dendrimer interfere giving cross-linked products.

It was decided to investigate two different types of reaction: one in which normal resins do not give any cross-linking products (diketopiperazine synthesis) and one in which cross-linking products are usually observed to some extent (for example the reaction of chloromethylated resin with a diamine), to compare the extent of this with that obtained with dendrimer resin.

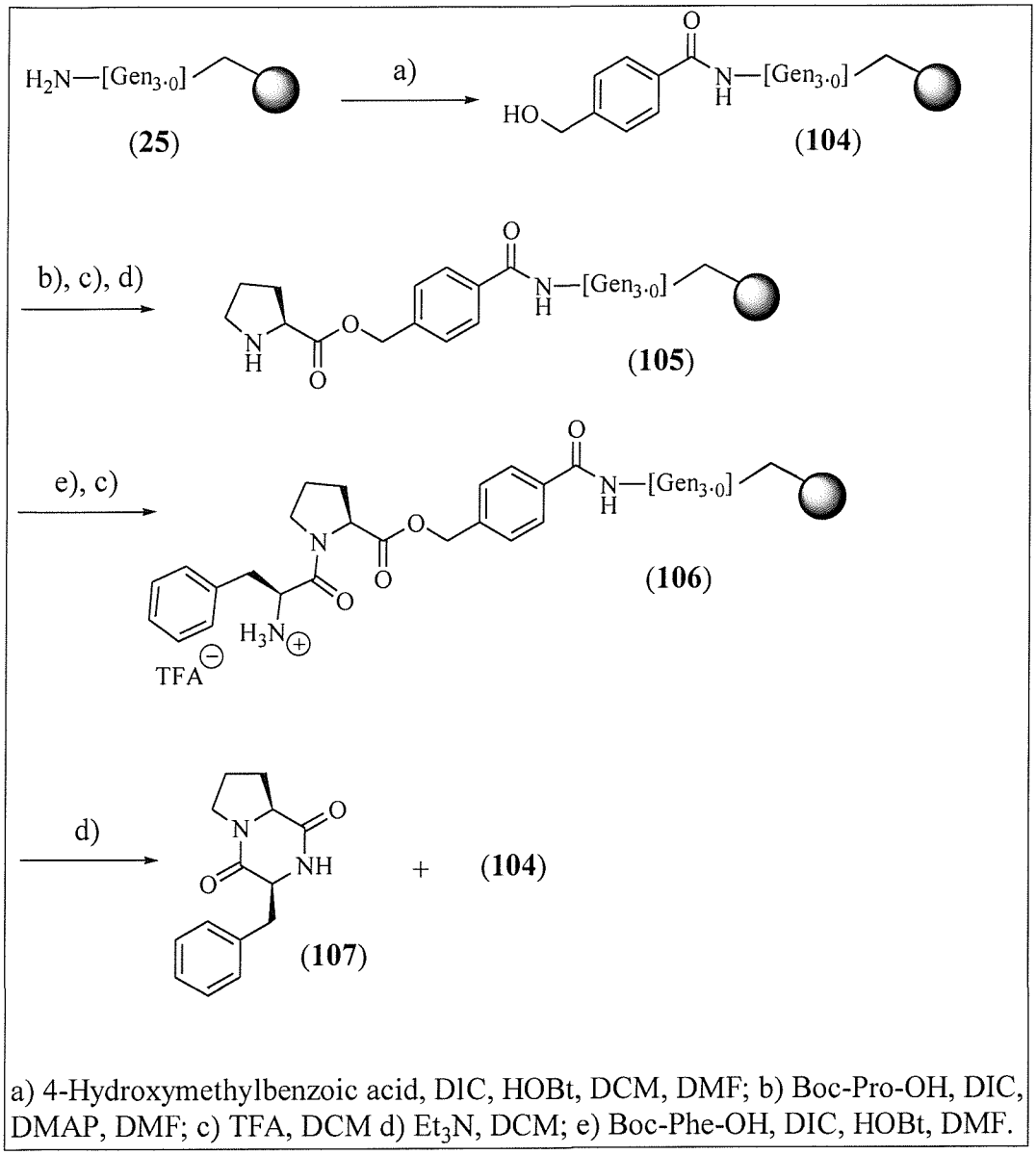
5.5.1. Diketopiperazine Synthesis

It is known that in solid phase peptide synthesis the deprotection and coupling onto the second aminoacid is crucial because the free $-NH_2$ can displace the ester bond *via* which the peptide is bound to the resin, forming a six member ring which is released into solution. This problem is generally negligible, unless one of the two aminoacids is a Proline residue that confers a β-turn onto the structure, enhancing the probability of diketopiperazine formation. During the synthesis of diketopiperazines cross-linked reactions give rise to side products such as tetrapeptides, hexapeptides or more complex structures.

The experiment started with the reaction of dendrimer resin with 4-hydroxymethyl benzoic acid as the point of attachment of the dipeptide. Together with Proline the other aminoacid chosen was phenylalanine, because of the chromophore that would aid HPLC analysis. In a first attempt Phe was first attached to the resin, and then Pro, but the release of diketopiperazine was very slow (the resin had to be suspended in neat triethylamine at 70°C overnight) due to the steric hindrance around the nitrogen of proline. However, we could manage to isolate the expected diketopiperazine as single peak by HPLC and 53% yield after *semi*-preparative HPLC purification.

In a second experiment the position of the two aminoacids was inverted (Scheme 5.5) to facilitate the ring-closure and in fact this time it took place at room temperature in DCM/triethylamine 1:1. Again the product obtained was very clean (98% purity, 60% yield after semi-preparative HPLC purification) and treatment of the resulting resin

(**104**) with 1 M NaOH(aq)/dioxane 1:1 did not release any product deriving from cross-linking reactions.

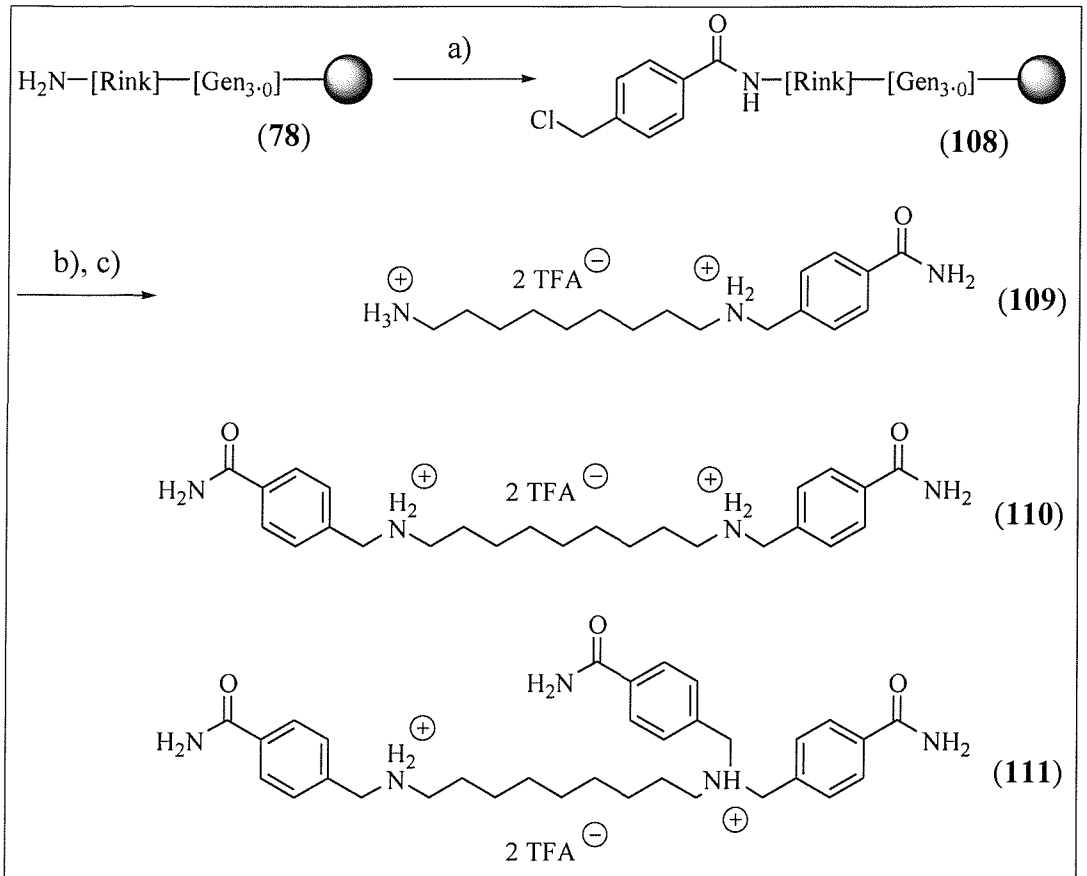


a) 4-Hydroxymethylbenzoic acid, DIC, HOBt, DCM, DMF; b) Boc-Pro-OH, DIC, DMAP, DMF; c) TFA, DCM d) Et_3N, DCM; e) Boc-Phe-OH, DIC, HOBt, DMF.

Scheme 5.5: Solid-phase synthesis of a diketopiperazine.

5.5.2 Reaction of Chloromethylated Resin with a Long-chain Diamine

After attachment of the Rink linker to TentaGel and dendrimer resin, 4-chloromethylbenzoic acid was attached. Resin (**108**) was then suspended in DMF and 1,9-diaminononane (6 equivalents compared to the amount of chlorine) was added (Scheme 5.6).



a) 4-Chloromethylbenzoic acid, DIC, HOBt, DMF; b) 1,9-Diaminononane, DMF; c) TFA, H_2O.

Scheme 5.6: Reaction of chloromethylated dendrimer resin with 1,9-diaminononane.

The resins were left shaking for two days, filtered, washed and treated with TFA/H_2O. The crude material released was analysed by HPLC, showing to be a mixture of (**109**) and (**110**), the latter being a product of a cross-linking reaction. The ratio between the two products was found to be 6:4 in both cases. In order to force cross-linking reactions to occur, a second experiment was conducted with only 4 or 2 equivalents of 1,9-diaminononane and the reaction heated at 50°C for 2 days. After cleavage off the resin, the HPLC trace of the crude material showed this time three peaks, the first two being (**109**) and (**110**). Surprisingly the third peak was found to be compound (**111**), product of a double cross-linking reaction. The relative amount of (**109**), (**110**) and (**111**) this time varied between TentaGel and dendrimer resin, the latter showing a higher extent of cross-linking reactions. The results are summarised in Table 5.1.

HPLC Area%

	(109)	**(110)**	**(111)**
6 eq. 1,9-diaminononane, room temperature			
TentaGel	63%	37%	-
Dendrimer	62%	38%	-
4 eq. 1,9-diaminononane, 50°C			
TentaGel	29%	55%	16%
Dendrimer	14%	50%	36%
2 eq. 1,9-diaminononane, 50°C			
TentaGel	21%	56%	23%
Dendrimer	8%	39%	52%

Table 5.1: Comparison of TentaGel and dendrimer resin behaviour in a cross-linking experiment.

In conclusion it was proven that in normal conditions the distance between the different branches of dendrimer resin is not a critical issue, and there is no appreciable difference compared to TentaGel resin. However, if critical conditions are chosen, the difference between TentaGel and dendrimer resin becomes more remarkable.

Chapter Six

Solid Phase Synthesis of Polyether Dendrimers

6.1 Polyether Dendrimers: Synthetic Strategies

As described in the previous chapters of this thesis, resin-bound polyaminoamide dendrimers were substantially inert towards many chemical conditions like coupling reactions, nucleophilic dispacements or borohydride reductions. However, synthetic patterns requiring the use of strong reducing agents or bases had to be avoided. Moreover, since Michael addition is a reversible reaction, long-term stability of PAMAM dendrimer resin had to be proven.

The solid phase synthesis of a more inert polyether dendrimer was therefore investigated. A Fréchet-type aryl-ether dendrimer (Figure 6.1) was chosen as the target.

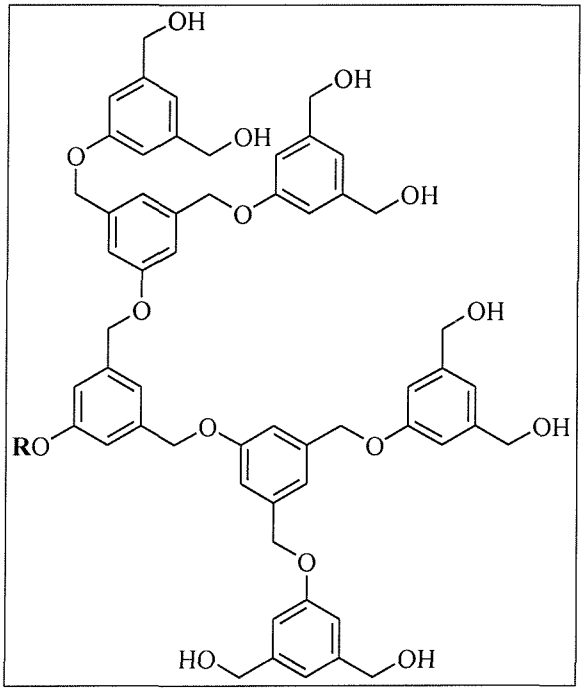
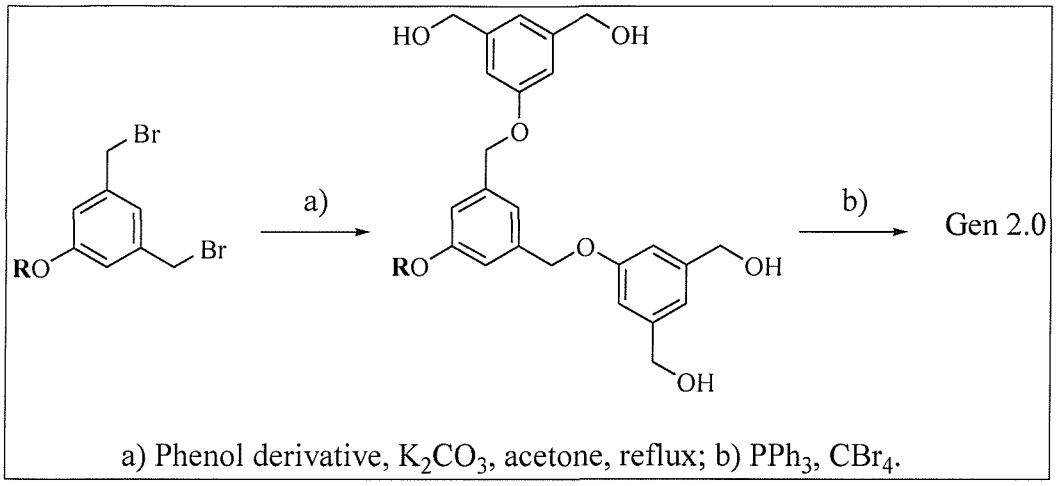


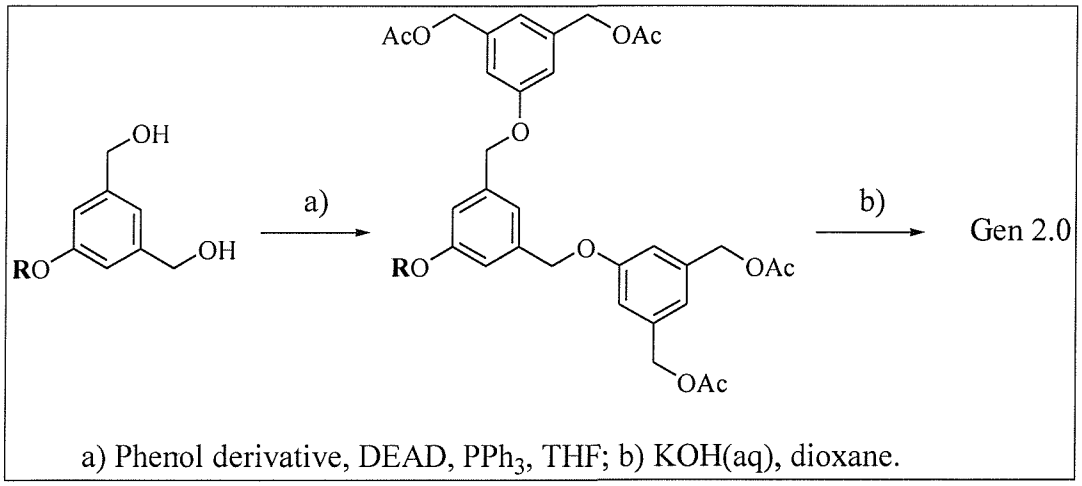
Figure 6.1: Fréchet-type polyether dendrimer.

In this kind of dendritic molecules usually the ether bond is formed *via* benzylic bromination and phenolic *O*-alkylation (Scheme 6.1),[41] and this route is preferred to the Mitsunobu condensation between a benzyl alcohol and a phenol (Scheme 6.2).[133]



a) Phenol derivative, K_2CO_3, acetone, reflux; b) PPh_3, CBr_4.

Scheme 6.1: Synthetic methodologies for aryl-ether dendrimers: O-alkylation of benzyl bromides.



a) Phenol derivative, DEAD, PPh_3, THF; b) KOH(aq), dioxane.

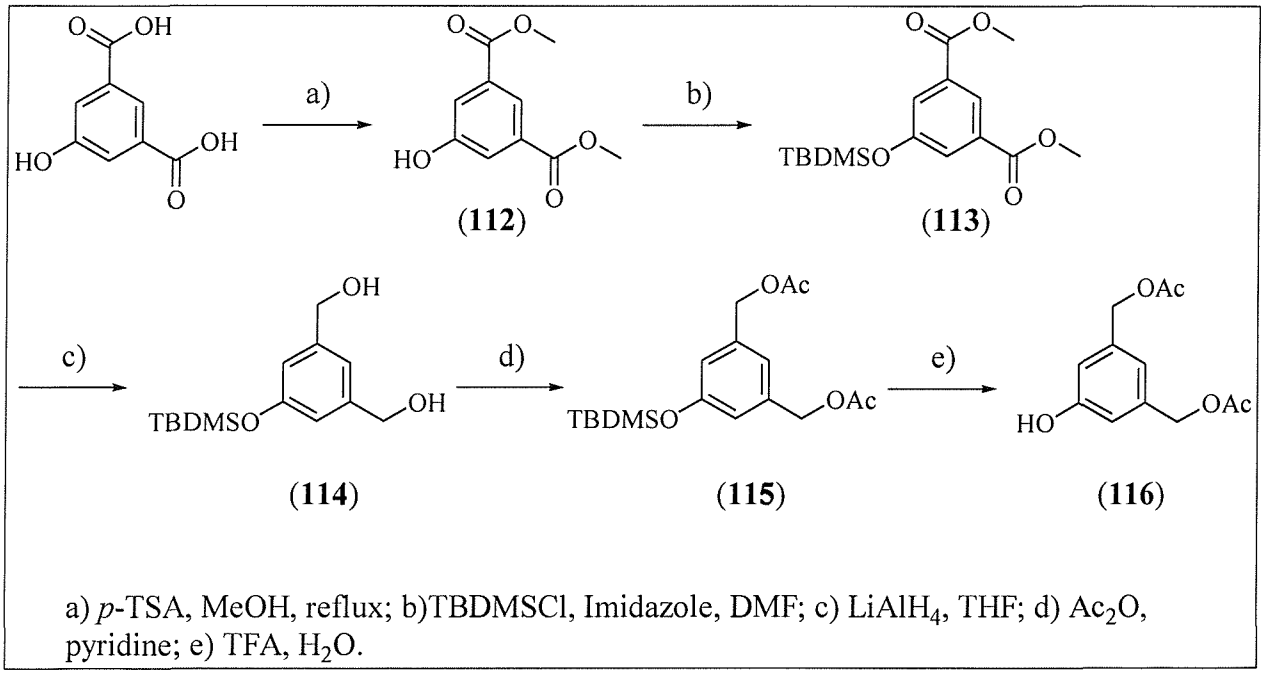
Scheme 6.2: Synthetic methodologies for aryl-ether dendrimers: Mitsunobu condensation of benzyl alcohols.

However the second strategy was believed to have some advantages when solid-phase chemistry was involved. First of all the resin-bound benzyl alcohol was less reactive than the correspondent bromide and therefore more stable. Secondly the activation of the alcohol and its displacement by the phenolic derivative could take place in a single step and side reactions would therefore be reduced. This procedure could be repeated

if the reaction did not reach completion and appearance/disappearance of C=O stretching peaks (belonging to the benzyl acetates) could be monitored by IR.

6.2 Synthesis of the Building Blocks

The building block chosen for the dendrimer construction was the previously described[134] 3,5-*bis*(acetoxymethyl)phenol (**116**). However, it was found that an alternative route to its synthesis was preferable in terms of yields, purity and time of reactions. The commercially available 5-hydroxy*iso*phthalic acid was esterified in methanol at reflux in the presence of *p*-toluenesulphonic acid. $LiAlH_4$ reduction of the ester bonds was found to proceed better if the phenolic function was protected with *tert*-butyldimethylsilyl chloride. Reduction with the free phenolic –OH was found to be sluggish and had to be heated at reflux in order to solubilise the lithium phenolate formed.

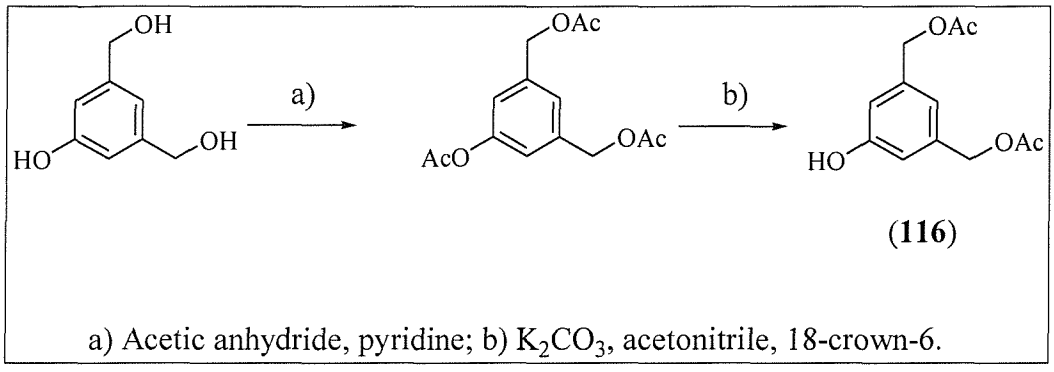


a) *p*-TSA, MeOH, reflux; b)TBDMSCl, Imidazole, DMF; c) $LiAlH_4$, THF; d) Ac_2O, pyridine; e) TFA, H_2O.

Scheme 6.3: Synthesis of 3,5-*bis*(acetoxymethyl)phenol.

On the other hand, when the phenolic –OH was protected, the reaction could be performed at room temperature and proceeded smoothly. (**114**) was then reacted with acetic anhydride in pyridine and the silyl protecting group was removed. Again the alternative route was found to be faster, since silyl deprotection with TFA proceeded in a few hours (Scheme 6.3), while in the case of O-acetoxy-3,5-(acetoxymethyl)

phenol the selective hydrolysis of the phenolic acetate with potassium carbonate in acetonitrile took more than 2 weeks to proceed to completion (Scheme 6.4).



a) Acetic anhydride, pyridine; b) K_2CO_3, acetonitrile, 18-crown-6.

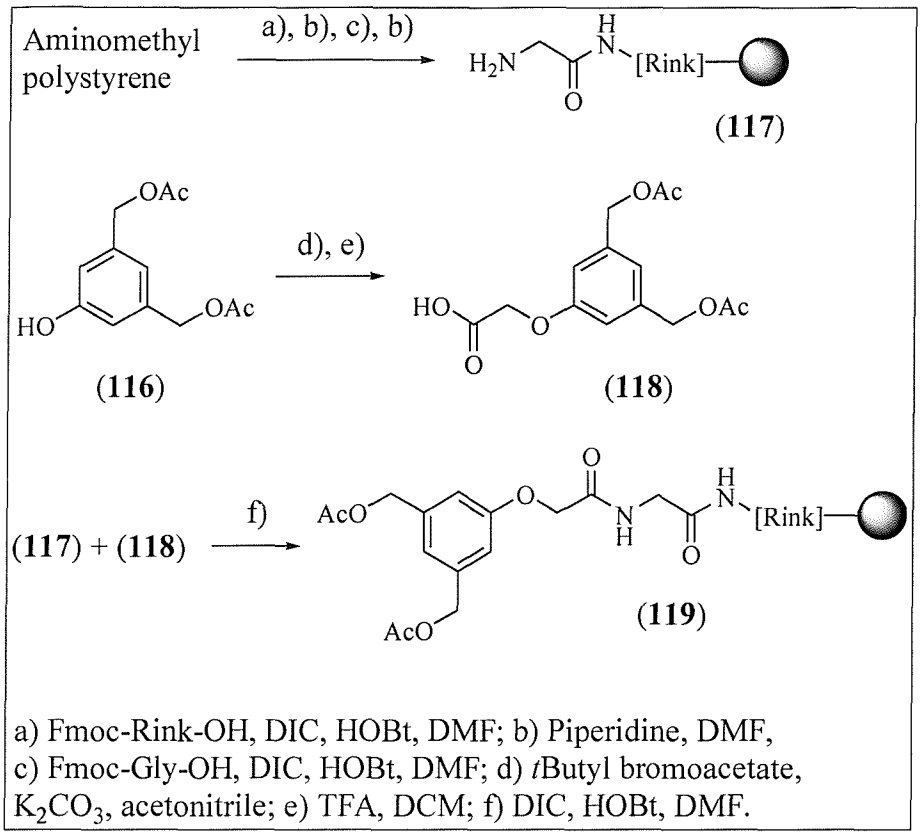
Scheme 6.4: Alternative route for the synthesis of (**116**).

6.3 Solid Phase Synthesis *via* a Cleavable Linker

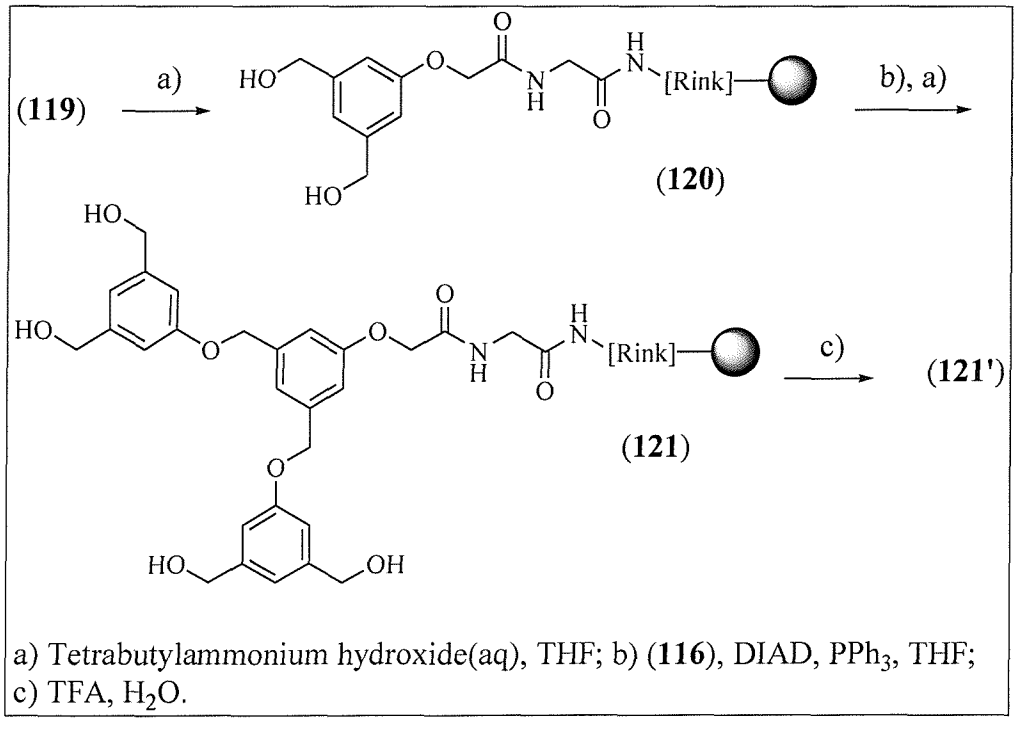
The synthesis of the dendrimer was initially carried out using a TFA-cleavable linker (Rink) to characterise the intermediates and optimise the conditions for the Mitsunobu condensation and the ester hydrolysis. An analytical construct was inserted between the linker and the dendrimer in order to facilitate MS analysis. Rink polystyrene resin was reacted with Fmoc-Gly-OH and the terminal amino group was then deprotected with 20% piperidine/DMF. Compound (**118**), obtained in two steps from (**116**), was coupled onto the resin with standard conditions DIC/HOBt (Scheme 6.5).

Hydrolysis of (**119**) was attempted with different conditions (LiOH(aq)/THF, NaOH(aq)/Dioxane, $KOSiMe_3$/THF) and best results in terms of conversion, purity and reproducibility were obtained with 40% tetrabutylammonium hydroxide(aq)/THF. The main problem was found to be the low compatibility of the polystyrene matrix with water solutions.

Initiator core (**120**) was reacted with (**116**) in the presence of triphenylphosphine and diisopropyl azodicarboxylate (DIAD) in dry THF. DIAD was preferred to the correspondent ethyl ester (DEAD) for its reduced tendency to give alkylation side-products. The azodicarboxylate was added to the suspension of resin (**120**), phenol (**116**) and triphenylphosphine in THF over a time of 2-3 h (Scheme 6.6).



Scheme 6.5: Attachment of the *initiator core* onto Rink resin.



Scheme 6.6: Synthesis of Generation 2.0 polyether dendrimer.

It was found that the slower the addition the higher the HPLC purity of the product after TFA cleavage. Generation 2.0 (**121'**) and 3.0 (**122'**) polyether dendrimer were obtained (Scheme 6.7) in high purity (Figure 6.2) and fully characterised after semi-preparative HPLC.

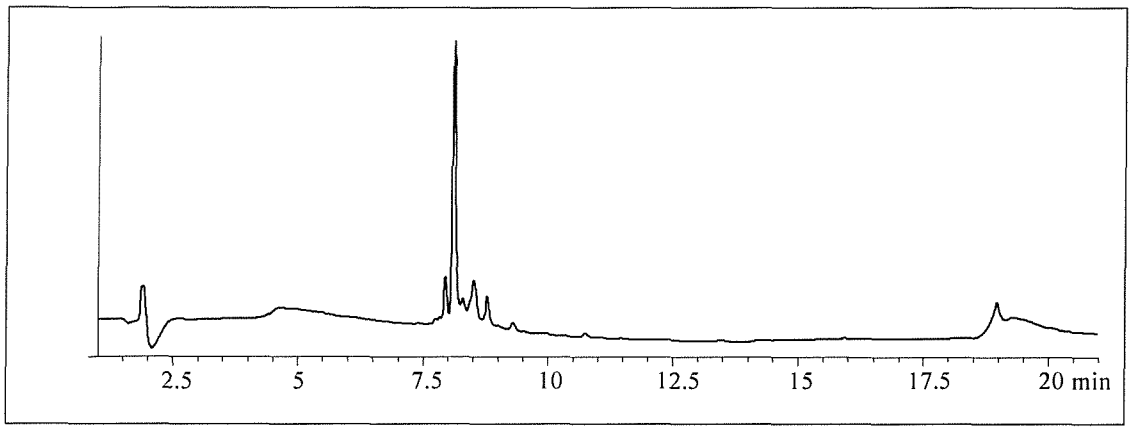
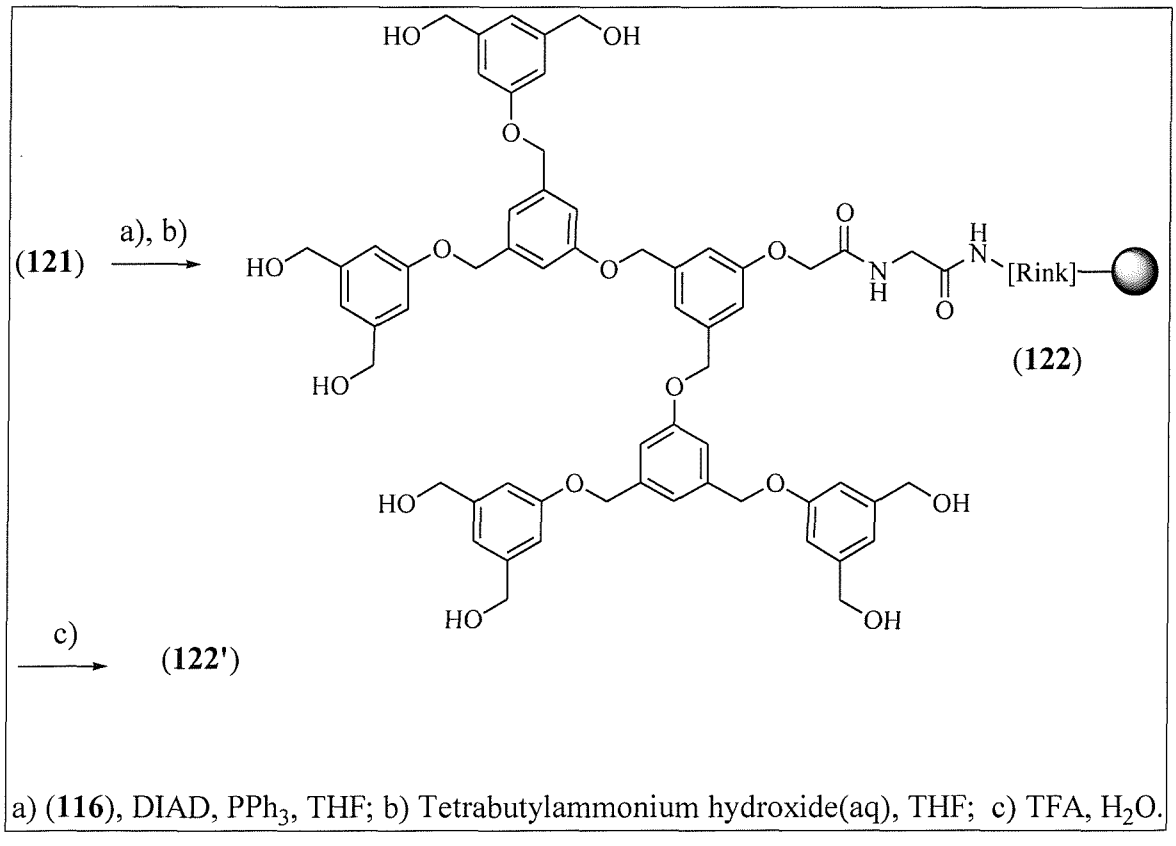


Figure 6.2: HPLC trace at 220 nm of Generation 3.0 Dendrimer (**122'**).

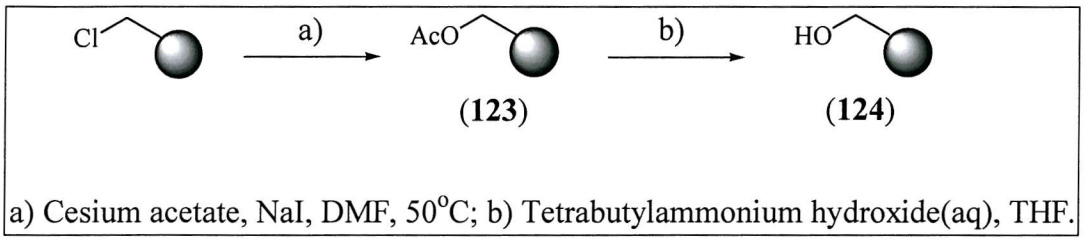


a) (**116**), DIAD, PPh_3, THF; b) Tetrabutylammonium hydroxide(aq), THF; c) TFA, H_2O.

Scheme 6.7: Synthesis of Generation 3.0 polyether dendrimer.

6.4 Synthesis of Permanently Attached Dendrimers

Permanently attached polyether dendrimers were synthesised starting from hydroxymethyl polystyrene. In order to have relatively monodispersed beads, commercially available Merrifield resin (0.93 mmol/g, diameter range 75-150 μm) was sieved and only the fraction 90-106 μm was used for the following transformations. Conversion of chloromethyl groups into the corresponding hydroxymethyl functionalities proceeded in two steps: displacement of the chloride with cesium acetate in DMF at 50°C and hydrolysis of the resulting ester with tetrabutylammonium hydroxide(aq) in THF (Scheme 6.8).



Scheme 6.8: Synthesis of hydroxymethyl polystyrene.

Completion of the hydrolysis step was monitored by IR, looking at the disappearance of the C=O stretching peak at 1735 cm^{-1} (Figure 6.3).

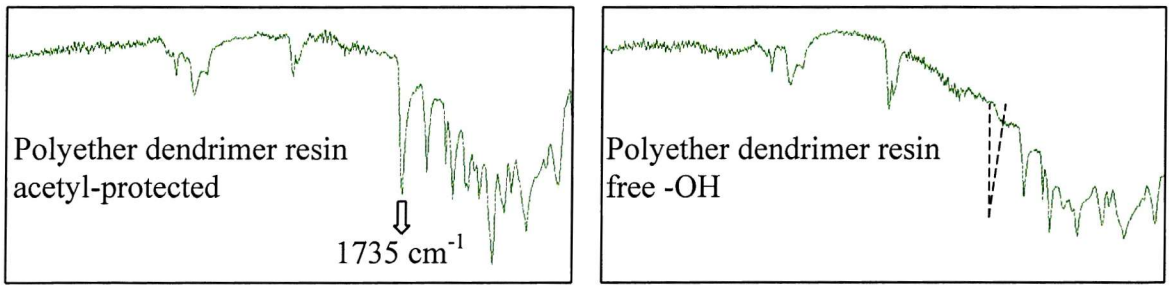
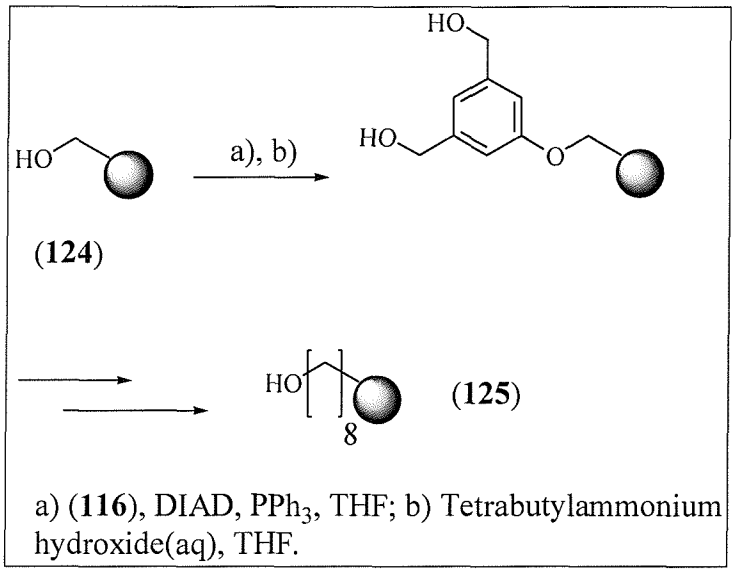


Figure 6.3: On bead IR spectra of acetyl protected and deprotected polyether dendrimers.

The final loading of the resin, by Fmoc determination, was found to be 0.82 mmol/g (86% of the theoretical value) and 0.44 nmol/bead. Synthesis of Generation 3.0

dendrimer resin proceeded as described before (Scheme 6.9) and led to a resin with a theoretical loading per bead 8 times higher than the initial one.



a) (**116**), DIAD, PPh_3, THF; b) Tetrabutylammonium hydroxide(aq), THF.

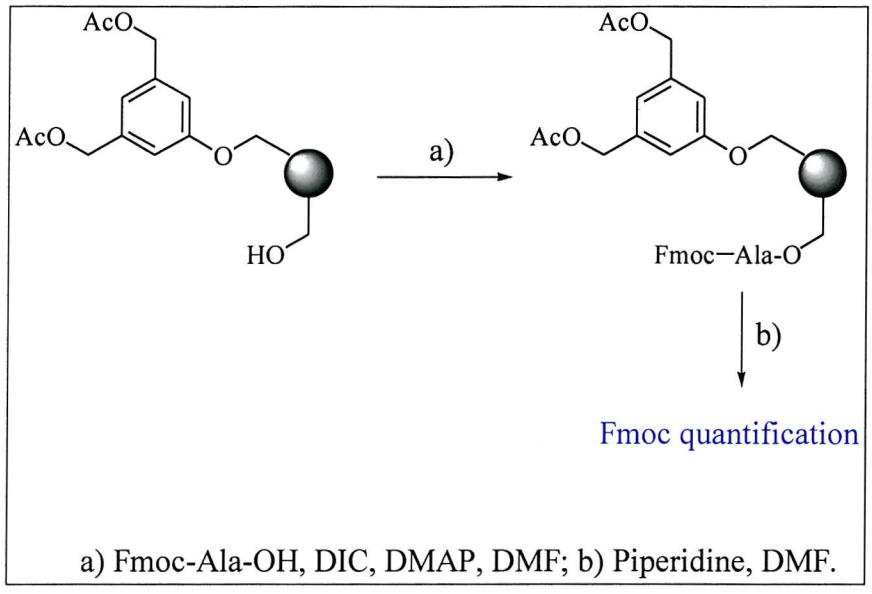
Scheme 6.9: Synthesis of permanently attached Generation 3.0 polyether dendrimer resin.

The results obtained for the different generations are reported in Table 6.1. Building block (**116**), used in a five-fold excess, was recovered after every Mitsunobu condensation *via* purification by flash chromatography.

	Experimental loading (nmol/bead)	Theoretical loading (nmol/bead)	%
Generation 1.0	0.9	0.9	100
Generation 2.0	1.8	1.8	100
Generation 3.0	3.0	3.5	86

Table 6.1: Efficiency of polyether dendrimer resin synthesis.

Completion of the Mitsunobu condensation was checked coupling Fmoc-Ala-OH onto the free –OH sites of the resin, Fmoc quantification gave for all generations negligible values, as a proof that ether-bond formation proceeded quantitatively (Scheme 6.10).



Scheme 6.10: Monitoring of completion of Mitsunobu condensation.

Completion of the hydrolysis step was checked instead by IR, monitoring the disappearance of the C=O stretching peak at 1735 cm^{-1}. The final loading of 3.0 nmol/bead was very good, taking in consideration that the synthesis involved 7 distinct Mitsnunobu condensations and 14 distinct ester hydrolysis. The final efficiency of 86% meant that each single reaction proceeded with a conversion of 99.3%.

6.4 Swelling Properties and Reaction Kinetics

The swelling properties of this resin were analysed and compared with those of hydroxymethyl polystyrene. The results are summarised in Table 6.2.

The diameter of dry resin was increased by about 25% of the original size after the dendrimerisation process. Acetyl-protected dendrimer resin was slightly bigger than the parent hydroxymethyl dendrimer resin (**125**), and this was justified by the high extent of cross-linking hydrogen bonding in the latter. Acetyl-protected dendrimer resin has the same swelling trend of hydroxymethyl polystyrene (**124**), while resin (**125**) presents some interesting features with apolar solvents like DCM and THF. In these solvents the resin swells much less than the parent acetyl-protected resin and also less than resin (**120**), probably due to the high density of hydroxyl groups present in the resin.

Bead Diameter (μm)

	Dry resin	**DCM**	**DMF**	**MeOH**	**H_2O**	**THF**
polystyrene resin	90	165	155	95	95	175
dendrimer resin –OH	110	145	185	105	110	150
dendrimer resin –OAc	115	195	190	110	115	190

Table 6.2: Swelling properties of hydroxymethyl polystyrene resin and polyether dendrimer resin.

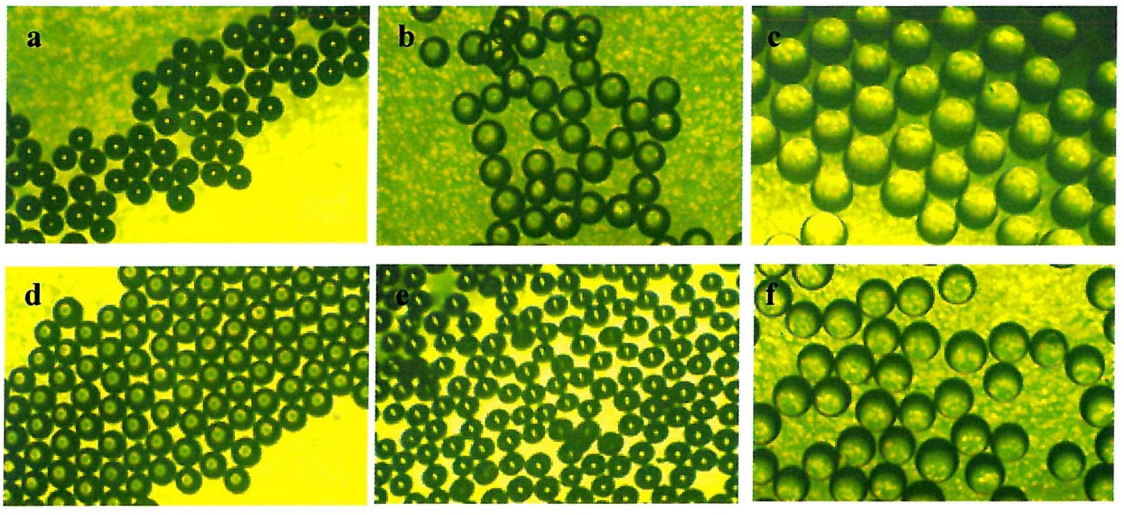


Figure 6.4: Beads (**125**) swelling: a) dry resin; b) DCM; c) DMF; d) MeOH; e) H_2O; f) THF.

Hydroxymethyl polystyrene (**124**) and polyether dendrimer resin (**125**) were also compared in terms of reaction rates. Fmoc-Ala-OH was coupled onto the two resins and Fmoc removed. Quantitative ninhydrin tests were carried out and the amount of chromophore released was monitored by UV at 570 nm after fixed intervals of time (Figure 6.5).

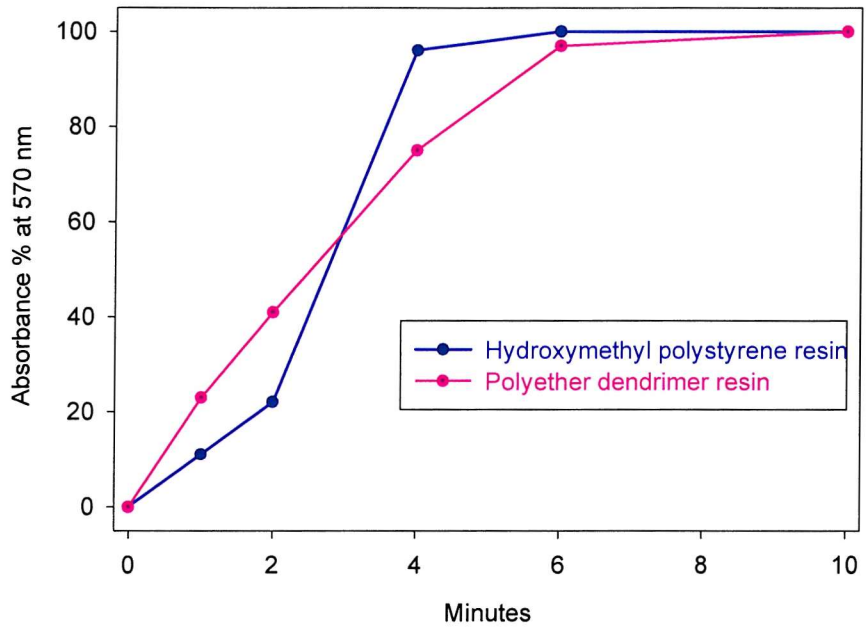
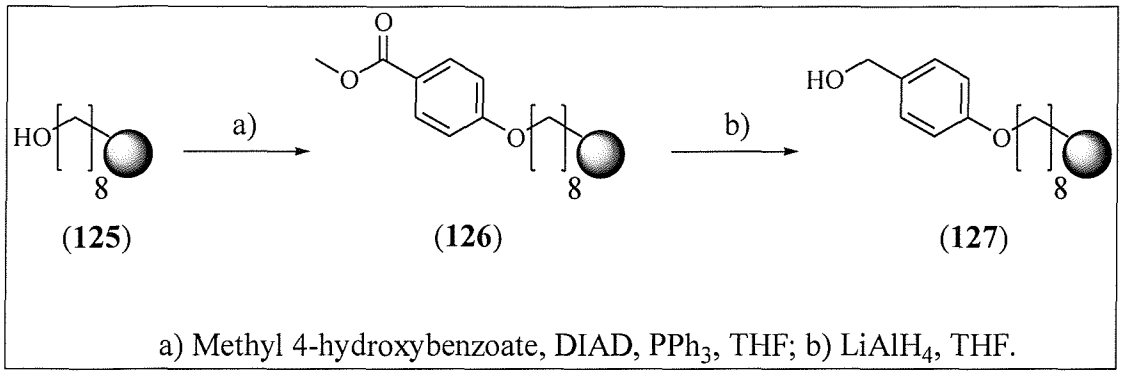


Figure 6.5: Absorbance at 570 nm *vs* time for hydroxymethyl polystyrene (**124**) and polyether dendrimer resin (**125**).

It is noteworthy that for both resins the reaction was complete after 10 min and that, taking in account experimental errors, the difference in reactivity can be considered negligible.

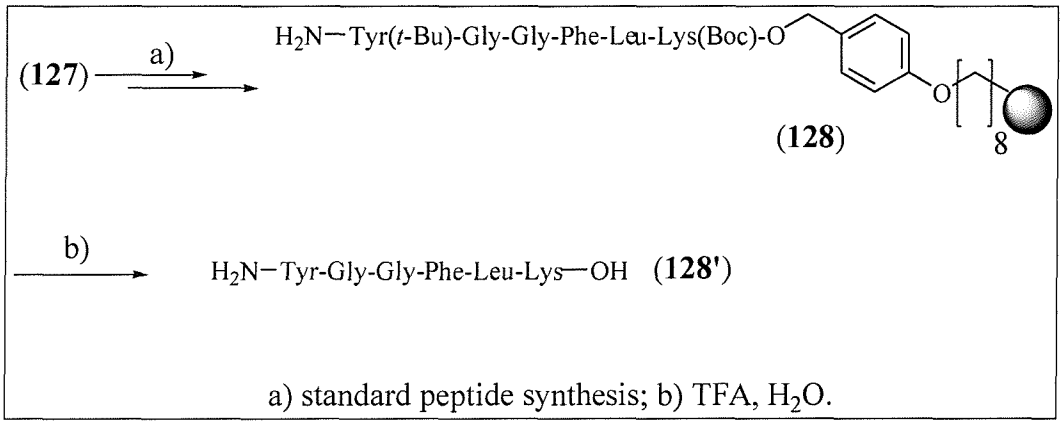
6.5 Synthesis of Leu-Enkephaline-Lys *via* the Wang Linker

To prove the versatility of this new resin, methyl 4-hydroxybenzoate was coupled *via* a Mitsunobu condensation and the resin-bound methyl ester (**126**) was then reduced with $LiAlH_4$ (Scheme 6.10). Again, completion of the reaction was monitored by IR through disappearance of the C=O stretching peak at 1713 cm^{-1}. This two-step procedure was found to be an efficient alternative method to introduce the Wang linker onto a polystyrene support. Polyether dendrimer resin was perfectly stable to the $LiAlH_4$ reduction and the final loading of dendrimer-Wang resin was found to be 2.3 nmol/bead, with 75% efficiency of this two-step procedure.



Scheme 6.10: Synthesis of Wang polyether dendrimer resin.

Hexapeptide Leu-Enkephaline-Lys was synthesised starting from resin (**127**) and cleaved off the resin with TFA/H_2O 95:5 (Scheme 6.11).



Scheme 6.11: Synthesis of Leu-Enkephaline-Lys on Wang polyether dendrimer resin.

After diethylether trituration the peptide was found to be 53% pure by HPLC and essentially pure by ^{1}H and ^{13}C NMR after semi-preparative HPLC purification. The isolated yield, relative to the loading of resin (**125**), was 66%.

Conclusions

The solid phase synthesis of different types of dendrimers has been proven to be a valid alternative to the parental solution phase synthesis. Using excess of reagents to drive the reactions to completion, dendrimers of very high purity can be easily obtained. In this thesis the use of dendrimers as resin bead-loading enhancers has been fully investigated. PAMAM dendrimer resin has been proven to be robust, inert towards many chemical conditions and suitable for multiple single-bead screenings. In alternative to this resin, polyether dendrimer resin has been shown to be the resin of choice when strong reducing agents are involved. Again, this resin has been proven to be easy to synthesise, to have high loading capacity and to maintain the same reactivity of parental polystyrene.

It is not far from reality to hypothesise that in the future dendrimer resins will be routinely used in solid phase combinatorial chemistry and single-bead screenings. However, it would be reductive to apply the solid phase synthesis of dendrimers to the sole aim of increasing the resin loading. Due to the intrinsic simplicity of the method, it could be of great support in any field where dendrimers are used. For example the preparation of PAMAM dendrimer conjugates, mentioned at the beginning of Chapter Two, allows the preparation of biologically active compounds capable of multivalent interactions. The use of unsymmetrical polyamine starting units can offer a convenient method of producing tagged or unsymmetrical dendrimeric structures. In addition, according to the convergent method for the synthesis of dendrimers outlined by Fréchet, polyether dendrimeric *wedges*[41] could be conveniently synthesised on solid phase and subsequently assembled in solution to give structures of high complexity and purity.

Chapter Seven

Experimental

General Information

^{1}H NMR and ^{13}C NMR spectra were recorded on a Bruker DPX400 (400 and 100 MHz, respectively) or with a Bruker AC300 (300 and 75 MHz, respectively) at 298 K unless otherwise stated. All chemical shifts are quoted in ppm on the δ scale using the residual protonated solvent as the internal standard. Coupling constants (*J* values) were measured in Hz. Spectra interpretation was aided by DEPT and bidimensional experiments as well as by ChemDraw software for chemical shift estimation.

Mass spectra were obtained on a VG Platform single quadrupole mass spectrometer in electrospray ionisation (ES+ or ES-) mode or atmospheric pressure chemical ionisation (APCI+) mode. High resolution accurate mass measurements were carried out on a VG Analytical 70-250-SE normal geometry double focusing mass spectrometer, fitted with an Ion-Tech saddle-field gun (for FAB), using mixtures of polyethylene glycols and/or polyethylene glycomethyl ethers (FAB) or perfluorokerosene (EI) as mass calibrants.

Analytical RP-HPLC was performed on a HP1100 system equipped with a Phenomenex Prodigy C_{18} reverse phase column (150 x 4.6 mm i.d.) with a flow rate of 1 mL/min.
Details of solvents and gradients are given below.
Method 1
monitoring at the wavelength of 220 or 254 nm and eluting with (A) 0.1% TFA in H_2O and (B) 0.042% TFA in acetonitrile, gradient 0% (B) to 100% (B) in 10 minutes.
Method 2
monitoring at the wavelength of 220 or 254 nm and eluting with (A) 0.1% TFA in H_2O and (B) 0.042% TFA in acetonitrile, gradient 0% (B) to 100% (B) in 20 minutes.

The LC/MS traces were recorded on a HP1050 chromatographer connected to a Micromass Series II mass spectrometer. The spectrometer operates in positive (ES+) and negative (ES-) modes, switching between the two modes automatically during sample analysis. The HPLC system is equipped with a 3 μm ABZ+PLUS column (3.3 cm x 4.6 mm ID) and runs a gradient from 0.1% Formic Acid in H_2O (+ 10 mM Ammonium Acetate) to 0.05% Formic Acid in Acetonitrile/H_2O 95:5 in 5 minutes. The flow rate is 3 mL/min and the injection volume 5 μL.

Semi-preparative RP-HPLC were performed on a HP1100 system equipped with a Phenomenex Prodigy C_{18} reverse phase column (250 x 10.0 mm i.d., flow rate 2.5 mL/min), monitoring at the wavelength of 220 nm or 254 nm and eluiting with (A) 0.1% TFA in H_2O and (B) 0.042% TFA in acetonitrile, gradient 0% (B) to 100% (B) over 40 minutes.

IR spectra were obtained on a BioRad FTS 135 spectrometer with a Goldengate ATR accessor with neat compounds.

UV-VIS spectra were recorded using a 8452A Diode Array Spectrophotometer. Melting points were determined using a Gallenkamp melting point apparatus.

General Resin Procedures

Qualitative Ninhydrin test[135]

The resin (1-5 mg) was poured into a test tube. Reagent A (6 drops) and reagent B (2 drops) were added and the tube heated at 100°C for 10 min. As a control the reagents were added in the same amounts as above to a test tube without any resin. The tubes were then cooled and 60% EtOH(aq) was added and the solutions mixed thoroughly. The reagents A and B are prepared as follow:

Reagent A

Solution-1: Phenol (40 g) was dissolved in absolute EtOH (10 mL) with warming and then stirred over Amberlite mixed-bed resin MB-3 (4 g) for 45 min. The mixture was then filtered.

Solution-2: Potassium cyanide (65 mg) was dissolved in water (100 mL). A 2 mL aliquot of this solution was diluited with pyridine (100 mL) (freshly distilled from ninhydrin) and stirred over Amberlite mixed-bed resin MB-3 (4 g). The solution was filtered and mixed with solution-1 to give reagent A.

Reagent B

Ninhydrin (2.5 g) was dissolved in absolute ethanol (50 mL).

Quantitative Fmoc test[136]

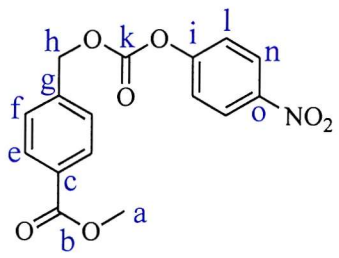
Three aliquots of dry resin (3-6 mg each or about 50 beads each) was weighed into volumetric flasks (50 mL or 1 mL) and treated with a solution of 20% piperidine in DMF (20 mL or 1 mL) for 20 min. The volume was made up to 50 mL (or 1 mL) with more 20% pieridine in DMF and the solutions mixed thoroughly. The absorbance at 302 nm was measured against a blank of 20% pieridine in DMF. The resin substitution was deduced from the following equation and calculated from the average value obtained from the three samples of resin:

$$\text{mmol/g (}\mu\text{mol/bead)} = [(A_{302} \times V) / (\varepsilon_{302} \times W)] \times 10^3$$

where A_{302} is the absorbance of the piperidyl-fulvene adduct, V is the total volume (mL), W is the weight of the resin (mg) or the exact number of beads and ε_{302} is the extinction coefficient of the adduct at 302 nm ($7800 M^{-1}cm^{-1}$).

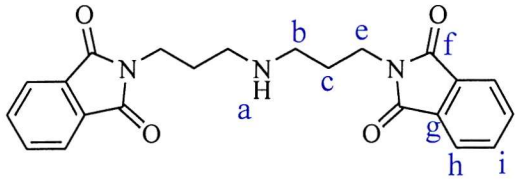
Experimental for Chapter Two

Methyl 4-{[(4'-nitrophenyl)oxycarbonyl]oxymethyl}benzoate (6)



Methyl 4-(hydroxymethyl)benzoate (30.1 mmol, 5.0 g) was dissolved in DCM (50 mL) at 0°C; pyridine (31.6 mmol, 2.6 mL) was added and the reaction stirred for a further 10 min. 4-Nitrophenylchloroformate (31.6 mmol, 6.4 g) was dissolved in DCM (50 mL) and added dropwise over 1 h. The solution became yellow. The solution was stirred at 0°C for 2 h and then at room temperature overnight. The solution was concentrated *in vacuo* and the pyridinium salt filtered. The crude material, a white needle-like solid, (9.0 g, 90%) was directly used for the following reactions. Experimental data are in accordance with the literature.[137]
TLC (3:1 PE/AcOEt) R_f: 0.5
δ_H (300MHz, $CDCl_3$): 8.27 (2H, d, **n**, *J* 10); 8.08 (2H, d, **e**, *J* 9); 7.50 (2H, d, **f**, *J* 9); 7.38 (2H, d, **l**, *J* 10); 5.36 (2H, s, **h**); 3.94 (3H, s, **a**).
δ_C (75 MHz, $CDCl_3$): 166.7 (**b**); 155.5 (**i**); 152.5 (**k**); 145.6 (**o**); 139.2 (**g**); 130.8 (**c**); 130.2 (**e**); 128.2 (**f**); 125.5 (**n**); 121.9 (**l**); 70.2 (**h**); 52.5 (**a**).
IR(ν): 1753, 1732, 1709, 1516, 1215.
Melting point: 94-97°C.

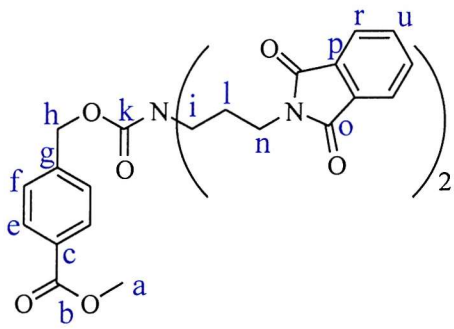
N^1,N^9-*bis*(Phthaloyl)-N-(3'-aminopropyl)-1,3-propanediamine (8)



N-(3'-aminopropyl)-1,3-propanediamine (**7**) (31.7 mmol, 4.4 mL) was dissolved in chloroform (75 mL). To this solution was rapidly added a solution of N-ethoxycarbonyl phthalimide (63.4 mmol, 13.9 g) in chloroform (75 mL). The mixture was stirred at room temperature for 2 h, then the solvent was removed *in vacuo* affording a white solid which was purified by recrystallisation in MeOH to give the pure product as a white solid (8.9 g, 72%). Experimental data are in accordance with the literature.[138]
TLC (9:1 DCM/MeOH) R_f: 0.8
δ_H (300 MHz, $CDCl_3$): 7.83 (4H, m, **h**); 7.71 (4H, m, **i**); 3.76 (4H, t, **e**, *J* 7); 2.64 (4H, t, **b**, *J* 7); 1.85 (4H, quint., **c**, *J* 7).
δ_C (75 MHz, $CDCl_3$): 168.6 (**f**); 134.0 (**i**); 132.2 (**h**); 123.4 (**g**); 47.0 (**b**); 36.0 (**e**); 29.0 (**c**).
m/z (ES+): 392.1 (100%, $[M+H]^+$).

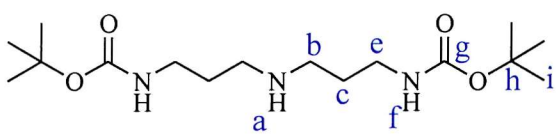
IR(ν): 1706, 1395, 1051.
Melting point: 132-136°C.

Methyl 4-{[N,N-*bis*(3'-phthalimidopropyl)amino]carbonyloxymethyl} benzoate (2)



The active ester (**6**) (8.0 mmol, 2.7g) was dissolved in acetonitrile (20 mL), triethylamine (12.0 mmol, 1.7 mL) was added and the resulting yellow solution was allowed to stir for 5 min. N^1,N^9-*bis*(phthaloyl)-N-(3'-aminopropyl)-1,3-propanediamine (**8**) (8.4 mmol, 3.3 g) was dissolved in acetonitrile (90 mL, heating) and added dropwise over 60 min to the previous solution. The mixture was heated to 50°C and left stirring for 5 h. The solution was then concentrated at reduced pressure and poured into water (75 mL), acidified to pH 6 with $KHSO_4$ and extracted with AcOEt. The organic layers were washed with brine, dried over $MgSO_4$, filtered and the solvents evaporated *in vacuo*. The crude material was purified by flash chromatography on silica gel (gradient from PE/AcOEt 1:1 to 100% AcOEt, then 100% DCM) affording the pure product as a colourless oil (3.5 g, 75%).
TLC (1:1 PE/AcOEt) R_f: 0.33
δ_H (400MHz, $CDCl_3$): 7.94 (2H, d, **e**, *J* 8); 7.85-7.75 (4H, m, **r**); 7.69 (4H, dd, **u**, *J* 6, 3); 7.32 (2H, d, **f**, *J* 8); 5.10 (2H, s, **h**); 3.80 (3H, s, **a**); 3.75-3.62 (4H, broad s, **n**); 3.43-3.30 (4H, broad s, **i**); 1.96 (4H, quint., **l**, *J* 7).
δ_C (100 MHz, $CDCl_3$): 168.4 (**o**); 166.9 (**b**); 155.8 (**k**); 141.8 (**g**); 134.1 (**u**); 132.1 (**c**); 130.0 (**e**); 129.7 (**p**); 127.4 (**f**); 123.4 (**r**); 66.6 (**h**); 52.2 (**a**); 45.6, 45.1 (**i**); 35.8 (**n**); 28.0, 27.5 (**l**).
m/z (ES+): 584.3 (100%, $[M+H]^+$), 601.4 (80%, $[M+NH_4]^+$).
IR(ν): 1771, 1701, 1390, 1275.
HR-MS (FAB+): 584.2036 (calc. 584.2033, -0.5 ppm).

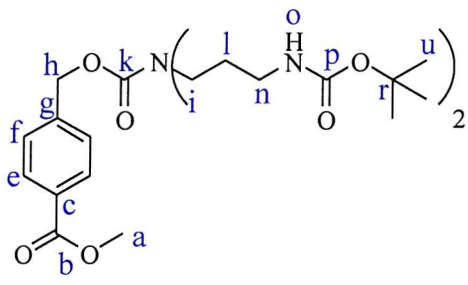
N^1,N^9-*bis*(*tert*-Butoxycarbonyl)-N-(3'-aminopropyl)-1,3-propanediamine (9)



N-(3'-Aminopropyl)-1,3-propanediamine (**7**) (7.15 mmol, 1.0 mL) was dissolved in THF (10 mL) and cooled to 0°C; BOC-ON (2-(*tert*-buthoxycarbonyloxyimino)-2-phenylacetonitrile) (15.0 mmol, 3.7 g) was dissolved in THF (20 mL) and added dropwise to the solution. The reaction was left stirring, under an atmosphere of N_2, overnight. The solvent was evaporated *in vacuo* and the resulting yellow oil dissolved

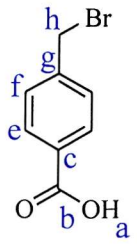
in AcOEt (50 mL) and washed with 5% NaOH(aq) (3x100mL). The organic phase was then washed with brine, dried over $MgSO_4$ and the solvent evaporated *in vacuo*, affording a white solid in quantitative yield (2.4 g). Experimental data are in accordance with the literature.[137]
TLC (95:5 AcOEt/Et_3N) R_f: 0.1
δ_H (300 MHz, $CDCl_3$): 5.25 (1.7H, broad s, **f**); 3.18 (4H, m, **e**); 2.62 (4H, t, **b**, *J* 7); 1.75 (1H, broad s, **a**); 1.62 (4H, quint., **c**, *J* 7); 1.41 (18H, s, **i**).
δ_C (75 MHz, $CDCl_3$): 156.3 (**g**); 79.0 (**h**); 47.5 (**b**); 39.0 (**e**); 29.9 (**c**); 28.6 (**i**).
m/z (ES+): 332.1 (100%, $[M+H]^+$), 354 (55%, $[M+Na]^+$), 685 (20%, $[2M+Na]^+$).
IR (cm^{-1}): 3384, 3338, 2977, 2935, 2833, 1683.
Melting point: 65-67°C.

Methyl 4-({N,N-*bis*[3'-(*tert*-butoxycarbonylamino)propyl]aminocarbonyl}oxymethyl) benzoate (3)



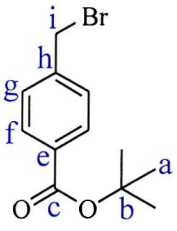
Methyl 4-[(4'-nitrophenyl)oxycarbonyloxymethyl]benzoate (**6**) (8.6 mmol, 2.8 g) was dissolved in acetonitrile (20 mL). Triethylamine (10.7 mmol, 1.5 mL) was added and the solution was stirred for 5 min. N^1,N^9-*bis*(*tert*-Butoxycarbonyl)-N-(3'-aminopropyl)-1,3-propanediamine (**7**) (7.1 mmol, 2.4 g) was dissolved in acetonitrile (30 mL) and added dropwise, over a period of 1 h, to the previous solution. The mixture was heated to 60°C and left stirring overnight. The solution was then concentrated under reduced pressure, poured into NH_4Cl_{sat} (50 mL) and extracted with AcOEt (2x50 mL). The combined organic layers were dried over $MgSO_4$, filtered and the solvents removed *in vacuo*. The crude material was purified by flash chromatography on silica gel (gradient from PE/AcOEt 1:1 to 100% AcOEt) affording the pure product as colourless oil (3.0 g, 79%). Experimental data are in accordance with the literature.[137]
TLC (6:4 PE/AcOEt) R_f: 0.70
δ_H (400 MHz, $CDCl_3$): 8.03 (2H, d, **e**, *J* 8); 7.40 (2H, d, **f**, *J* 8); 5.17 (2H, s, **h**); 4.60 (0.75H, broad s, **o**); 3.91 (3H, s, **a**); 3.37-3.25 (4H, broad s, **i**); 3.10 (4H, t, **n**, *J* 7); 1.76-1.63 (4H, broad s, **l**); 1.42 (18H, s, **u**).
δ_C (100 MHz, $CDCl_3$): 166.9 (**b**); 156.4 (**p**); 156.1 (**k**); 141.8 (**g**); 130.1 (**e**); 130.0 (**c**); 127.6 (**f**); 79.2 (**r**); 66.7 (**h**); 52.3 (**a**); 44.5 (**i**); 38.0, 37.4 (**n**); 29.2, 28.5 (**l**); 28.5 (**u**).
m/z (ES+): 524.4 (80%, $[M+H]^+$), 546.4 (100%, $[M+Na]^+$), 1069.7 (5%, $[2M+Na]^+$).
IR (cm^{-1}): 1687, 1513, 1278, 1168.
HR-MS (FAB+): 524.2990 (calc. 524.2972, -3.4 ppm).

4-(Bromomethyl)benzoic acid (11)



p-Toluic acid (0.10 mol, 13.6 g) and benzoyl peroxide (5.0 mmol, 1.2 g) were suspended in chlorobenzene (130 mL) in a round-bottom flask equipped with a reflux-condenser. The solution was heated at reflux till complete dissolution of toluic acid and then N-bromosuccinimide (0.11 mol, 19.6 g) was added portionwise. The resulting orange solution was heated at reflux for another 2 h, then cooled to room temperature to allow the precipitation of a brown solid. The solid was removed by filtration and washed twice with hot water (200 mL at 60-80°C). The solid was then recrystallised from hot MeOH giving a needle-like white solid (7.8 g, 36%). Experimental data are in accordance with the literature.[82]
TLC (AcOEt/PE 6:4) R_f: 0.45
δ_H (300 MHz, CD_3COCD_3): 8.03 (2H, d, **e**, *J* 9); 7.60 (2H, d, **f**, *J* 9); 4.71 (2H, s, **h**).
δ_C (75 MHz, CD_3COCD_3): 166.9 (**b**); 144.0 (**g**); 131.0 (**c**); 130.7 (**e**); 130.0 (**f**); 32.9 (**h**).
IR(ν): 1677, 1421, 1286.
Melting point: 228-232°C (lit.: 224-229°C).

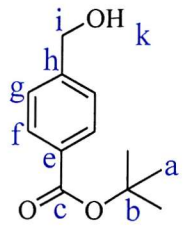
***tert*-Butyl 4-(bromomethyl)benzoate (12)**



4-(Bromomethyl)benzoic acid (**11**) (37.3 mmol, 8.0 g) was suspended in dioxane (10 mL) and DCM (20 mL) in a high pressure bottle and 0.5 mL of concentrated H_2SO_4 was added. The bottle was cooled to -40°C in a dry-ice bath and isobutylene (30 mL) was condensed inside. The suspension was left stirring at room temperature for three days. The bottle was cooled at –20°C before opening and, after addition of 1M LiOH(aq) (10 mL), it was left to warm to RT to allow slow evaporation of the isobutylene. The remaining solvents were removed *in vacuo* and the solid diluted with water (150 mL) and extracted with DCM (3x75 mL). The solvent was then evaporated and the product was purified by flash chromatography PE/AcOEt 95:5) to give a colourless oil (9.1 g, 90%). Experimental data are in accordance with the literature.[139]
TLC (AcOEt/PE 2:8) R_f: 0.75
δ_H (300 MHz, $CDCl_3$): 7.97 (2H, d, **f**, *J* 8); 7.44 (2H, d, **g**, *J* 8); 4.50 (2H, s, **i**); 1.60 (9H, s, **a**).
δ_C (75 MHz, $CDCl_3$): 165.3 (**c**); 142.2 (**h**); 132.1 (**i**); 130.1(**f**); 129.0 (**g**); 81.4 (**b**); 32.5 (**i**); 28.3 (**a**).

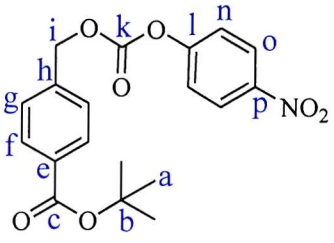
IR(ν): 2977, 1710, 1291, 1162.

***tert*-Butyl 4-(hydroxymethyl)benzoate (13)**



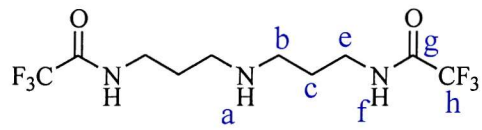
tert-Butyl 4-(bromomethyl) benzoate (**12**) (3.2 mmol, 860 mg) was dissolved in dioxane (5 mL) and water (1 mL) and treated with $AgNO_3$ (3.8 mmol, 650 mg). The solution was left stirring overnight and the solid was filtered. The solution was concentrated *in vacuo*, diluted with water (50 mL) and extracted with AcOEt (2x50 mL). The crude material was purified by flash chromatography (gradient from PE/AcOEt 95:5 to 7:3) giving the product as a colourless oil (430 mg, 65%).
TLC (AcOEt/PE 2:8) R_f: 0.33
δ_H (300 MHz, $CDCl_3$): 7.95 (2H, d, **f**, *J* 8); 7.33 (2H, d, **g**, *J* 8); 4.69 (2H, s, **i**); 2.58 (1H, s, **k**); 1.58 (9H, s, **a**).
δ_C (100 MHz, $CDCl_3$): 166.0 (**c**); 145.8 (**h**); 131.2 (**e**); 129.8 (**f**), 126.5 (**g**); 81.3 (**b**); 64.7 (**i**); 28.4 (**a**).
IR (ν): 3700-3100 (broad), 2973, 1710, 1291, 1163.

***tert*-Butyl 4-{[(4'-nitrophenyl)oxycarbonyl]oxymethyl}benzoate (14)**



tert-Butyl 4-(hydroxymethyl)benzoate (**13**) (6.0 mmol, 1.25 g) was dissolved in DCM (15 mL) at 0°C. Pyridine (6.6 mmol, 0.53 mL) was added to the solution and the reaction stirred for a further 5 min. 4-Nitrophenylchloroformate (6.6 mmol, 1.33 g) was dissolved in DCM (10 mL) and added dropwise. The solution was left stirring at room temperature overnight. The solvent was removed *in vacuo* before the crude was taken up in diethyl ether and the resulting solid filtered. The crude material, a colourless oil, (2.29 g, 100%) was used without further purification.
TLC (8:2 PE/AcOEt) R_f: 0. 50
δ_H (300 MHz, $CDCl_3$): 8.28 (2H, d, **o**, *J* 9); 8.03 (2H, d, **f**, *J* 8); 7.48 (2H, d, **g**, *J* 8); 7.38 (2H, d, **n**, *J* 9); 5.34 (2H, s, **i**); 1.61 (9H, s, **a**).
δ_C (75 MHz, $CDCl_3$): 165.3 (**c**); 155.6 (**k**); 152.5 (**l**); 145.6 (**p**); 138.6 (**h**); 132.7 (**e**); 130.0 (**f**); 128.1 (**g**); 125.7 (**o**); 121.9 (**n**); 81.5 (**b**); 70.3 (**i**); 28.3 (**a**).
IR(ν): 2978, 1764, 1710, 1527, 1212.

N^1,N^9-*bis*(Trifluoroacetyl)-N-(3'-aminopropyl)-1,3-propanediamine (15)



N-(3'-Aminopropyl)-1,3-propanediamine (**7**) (14.3 mmol, 2.0 mL) was dissolved in THF (10 mL) and cooled to 0°C. Ethyl trifluoroacetate (28.6 mmol, 3.4 mL) was added and the solution left stirring for 45 min. The solvent was then evaporated *in vacuo* and the yellow oil obtained (4.6 g, 100%) was used without further purification. Experimental data are in accordance with the literature.[140]

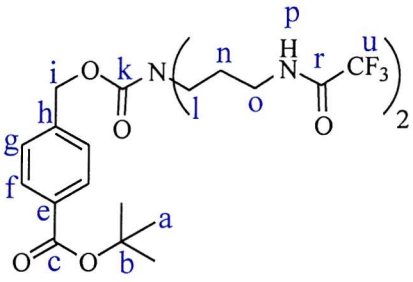
δ_H (400 MHz, $CDCl_3$): 8.52 (1.6H, broad s, **f**); 3.42 (4H, t, **e**, *J* 6); 2.71 (4H, t, **b**, *J* 6); 1.72 (4H, quint., **c**, *J* 6).

δ_C (100 MHz, $CDCl_3$): 157.5 (**g**, q, *J* 37); 116.1 (**h**, q, *J* 285); 48.0 (**b**); 39.3 (**e**); 27.9 (**c**).

m/z (ES+): 324.1 (100%, $[M+H]^+$).

IR (ν): 3303, 2938, 1705, 1552, 1182, 1148.

***tert*-Butyl 4-{N,N-*bis*[3'-(trifluoroacetylamino)propyl]aminocarbonyloxymethyl} benzoate (4)**



tert-Butyl 4-{[(4'-nitrophenyl)oxycarbonyl]oxymethyl}benzoate (**14**) (6.0 mmol, 2.24 g) was dissolved in acetonitrile (15 mL), triethylamine (9.0 mmol, 1.25 mL) was added and the resulting yellow solution was allowed to stir for 5 min. N^1,N^9-*bis*(Trifluoroacetyl)-N-(3'-aminopropyl)-1,3-propanediamine (**15**) (6.6 mmol, 2.13 g) was dissolved in acetonitrile (25 mL) and added dropwise. The mixture was heated to 60°C and left stirring overnight. The solution was then concentrated at reduced pressure, poured into NH_4Cl_{sat} (80 mL) and extracted with AcOEt (2x70 mL). The organic layers were dried over $MgSO_4$, filtered and the solvents were removed *in vacuo*. The crude material was purified by flash chromatography on silica gel (gradient from PE/AcOEt 3:1 to 1:1) affording pure product as a colourless oil (2.9g, 87%).

TLC (1:1 PE/AcOEt) R_f: 0.40

δ_H (400 MHz, $CDCl_3$): 7.94 (2H, d, **f**, *J* 8); 7.80 (1H, broad s, **p**); 7.34 (2H, d, **g**, *J* 8); 5.15 (2H, s, **i**); 3.31 (8H, broad s, **l** and **o**) 1.77 (4H, broad m, **n**); 1.56 (9H, s, **a**).

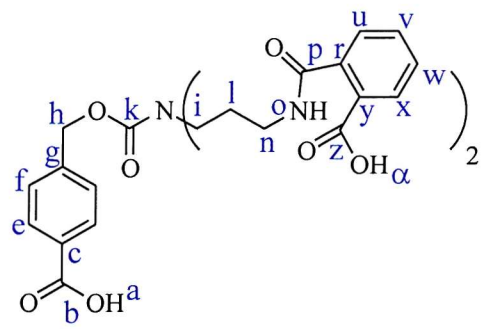
δ_C (100 MHz, $CDCl_3$): 165.6 (**c**); 157.6 (**r**, quart, *J* 37); 157.1 (**k**); 140.7 (**h**); 132.0 (**e**); 129.9 (**f**); 127.6 (**g**); 116.0 (**u**, quart, *J* 285); 81.5 (**b**); 67.2 (**i**); 44.5 (**l**); 37.5, 37.3 (**o**); 28.2 (**a**) 28.0, 27.1 (**n**).

m/z (ES+): 580.4 (100%, $[M+Na]^+$); 1137.6 (10%, $[2M+H]^+$).

IR (ν): 3313, 2977, 1705, 1162.

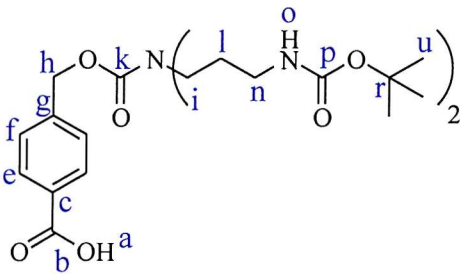
HR-MS (FAB+): 558.2018 (calc. 558.2039, 3.7 ppm).

4-[(N,N-*bis*{3'-[(2''-Carboxybenzoyl)amino]propyl}amino)carbonyloxymethyl] benzoic acid (16')



Methyl 4-{[N,N-*bis*(3'-phthalimidopropyl)amino]carbonyloxymethyl} benzoate (**2**) (0.92 mmol, 0.54 g) was dissolved in dioxane (10 mL). A solution of NaOH (2M in water, 3.7 mmol, 1.8 mL) was added dropwise and the yellow solution was stirred for 30 min and then diluted with water (15 mL). A solution of $KHSO_4$ (2M in water) was added dropwise until a white precipitate was formed (pH 5). Extraction with AcOEt afforded the triacid (**16'**) in quantitative yield.
δ_H (400MHz, CD_3OD): 7.86 (2H, d, **e**, *J* 8); 7.79 (2H, d, **x**, *J* 8); 7.45-7.35 (6H, m, **u**, **v** and **w**); 7.34 (2H, d, **f**, *J* 8); 7.23 (1.2 H, broad s, **o**); 5.10 (2H, s, **h**); 3.35 (4H, broad s, **i**); 3.26 (4H, t, **n**, *J* 7); 1.80 (4H, quint, **l**, *J* 7).
δ_C (100 MHz, CD_3OD): 173.3 (**z**); 170.0 (**b**); 169.9 (**p**); 158.3 (**k**); 143.7 (**g**); 140.2 (**y**); 133.4 (**v**); 132.6, 132.5 (**r**); 132.0 (**c**); 131.7 (**x**); 131.5 (**e**); 131.0 (**w**) 129.2 (**u**); 128.9 (**f**); 68.0 (**h**); 47.1, 46.8 (**i**); 38.9 (**n**); 29.9, 29.0 (**l**).
HPLC (method 1, 254 nm): 7.6 min (100%).
m/z (ES+): 606.5 (100%, $[M+H]^+$); (ES-): 718.3 (100%, $[M+TFA-H]^-$).

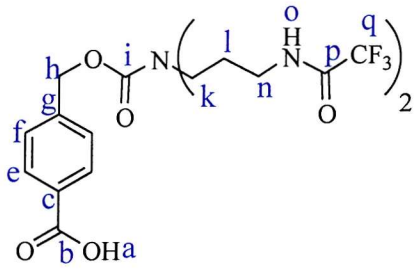
4-({N,N-*bis*[3'-(*tert*-Butoxycarbonylamino)propyl]aminocarbonyl}oxymethyl) benzoic acid (17)



Methyl 4-({N,N-*bis*[3'-(*tert*-butoxycarbonylamino)propyl]aminocarbonyl}oxymethyl) benzoate (3) (0.92 mmol, 0.48 g) was dissolved in dioxane (10 mL). A solution of NaOH (2M in water, 3.7 mmol, 1.8 mL) was added dropwise and the reaction mixture was stirred at room temperature overnight, then at 50°C for 3 h. The solution was diluted with water (35 mL) and $KHSO_4$ (2M in water) was added dropwise until a white precipitate was formed (pH 5). Extraction with Et_2O and evaporation of the solvents afforded the pure product in quantitative yield (0.47 g). Experimental data are in accordance with the literature.[137]
TLC (1:1 PE/AcOEt) R_f: 0.19

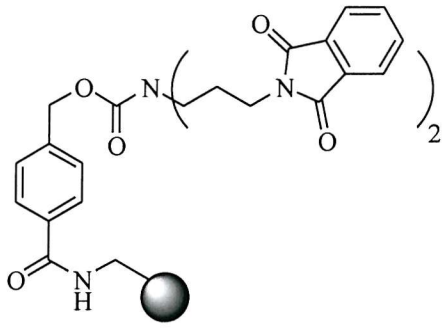
δ_H (300 MHz, $CDCl_3$): 8.09 (2H, d, **e**, *J* 8); 7.43 (2H, d, **f**, *J* 8); 5.20 (2H, s, **h**); 4.65 (0.33H, broad s, **o**); 3.33 (4H, broad s, **i**); 3.11 (4H, broad s, **n**); 1.72 (4H, broad s, **l**); 1.44 (18H, s, **u**).
δ_C (75 MHz, $CDCl_3$): 170.5 (**b**); 156.5 (**k**); 142.4 (**g**); 130.6 (**e**); 129.4 (**c**); 127.5 (**f**); 79.3 (**r**); 66.7 (**h**); 44.6 (**i**); 38.1, 37.4 (**n**); 29.3, 28.6 (**l**); 28.6 (**u**).
HPLC (method 1, 254 nm): 10.0 min (78%).
m/z (ES+): 510.5 (100%, $[M+H]^+$); (ES-): 622.4 (100%, $[M+TFA-H]^-$).

4-({N,N-*bis*[3'-(trifluoroacetylamino)propyl]aminocarbonyl}oxymethyl) benzoic acid (18)



tert-Butyl 4-({N,N-*bis*[3'-(trifluoroacetylamino)propyl]aminocarbonyl}oxymethyl) benzoate (**4**) (1.05 mmol, 585 mg) was dissolved in DCM (5 mL) and treated with TFA (5 mL) at room temperature. The reaction was left stirring for 3 h, then the solvents were removed *in vacuo* using toluene to facilitate evaporation of the TFA. The product was obtained as a pure white solid in quantitative yield.
δ_H (300 MHz, $CDCl_3$): 8.06 (1H, broad s, **o**); 7.94 (2H, d, **e**, *J* 8); 7.34 (2H, d, **f**, *J* 8); 5.15 (2H, s, **h**); 3.31 (8H, broad s, **k** and **n**) 1.77 (4H, broad m, **l**).
δ_C (75 MHz, $CDCl_3$): 165.6 (**b**); 157.6 (**p**, quart, *J* 37); 157.1 (**i**); 140.7 (**g**); 132.0 (**c**); 129.9 (**e**); 127.6 (**f**); 116.0 (**q**, quart, *J* 285); 67.2 (**h**); 44.4, 44.1 (**k**); 37.5, 36.3 (**n**); 28.0, 27.1 (**l**).
HPLC (method 1, 254 nm): 9.5 min (81%).
m/z (ES+): 502.1 (25%, $[M+H]^+$); 524.1 (100%, $[M+Na]^+$).

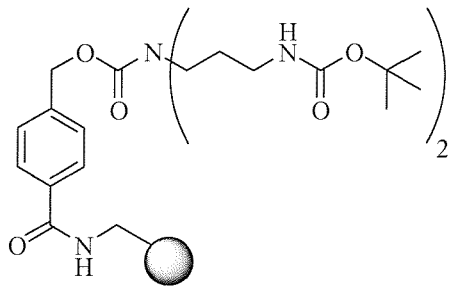
Resin-bound phthalimido-protected initiator core (19)



The triacid (**16'**) (0.92 mmol, 0.56 g) was dissolved in DCM (5 mL) and DMF (1 mL). DIC (3.7 mmol, 0.58 mL) and HOBT (3.7 mmol, 500 mg) were added and the solution stirred for 30 min. The activated acid was added to TentaGel resin (0.46 mmol/g 1.0 g) and left shaking overnight. The resin was then washed with DCM (3x10 mL), DMF

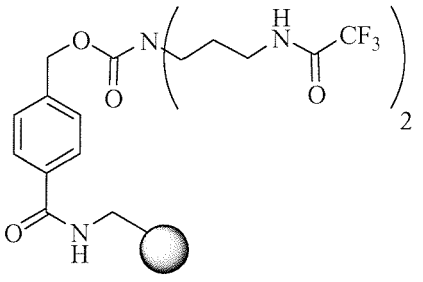
(3x10 mL) and MeOH (3x10 mL). Completion of the coupling was checked by a qualitative ninhydrin test.

Resin-bound *tert*-butoxycarbonyl-protected initiator core (20)



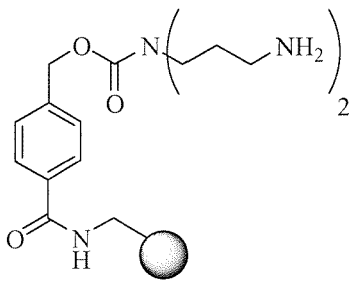
The acid (**17**) (0.92 mmol, 0.47 g) was dissolved in DCM (5 mL) and treated with DIC (0.92 mmol, 0.14 mL) and HOBT (0.92 mmol, 0.12 g). The solution was stirred for 5 min before the activated acid was added to TentaGel resin (0.46 mmol/g 1.0 g) and left shaking overnight. The resin was washed with DCM (3x10 mL), DMF (3x10 mL) and MeOH (3x10 mL). Completion of the coupling was checked by a qualitative ninhydrin test.

Resin-bound trifluoroacetyl-protected initiator core (21)



The acid (**18**) (0.92 mmol, 0.46 g) was dissolved in DCM (5 mL) and DMF (5mL). DIC (0.92 mmol, 0.14 mL) and HOBT (0.92 mmol, 0.12 g) were added and the solution stirred for 5 min. The activated acid was added to TentaGel resin (0.46 mmol/g, 1.0 g) which was then left shaking overnight. The resin was then washed with DCM (3x10 mL), DMF (3x10 mL) and MeOH (3x10 mL). Completion of the coupling was checked by a qualitative ninhydrin test.

Resin-bound initiator core (22)

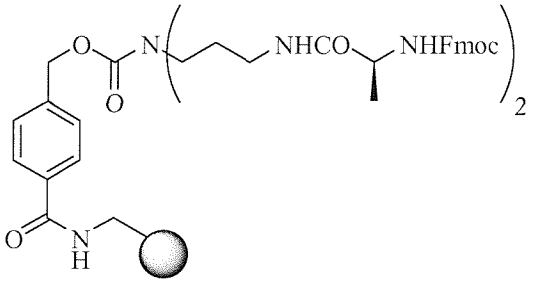


Method A:
The phthalimido-protected initiator core (**19**) (0.92 mmol of phthalimido groups) was suspended in EtOH (50 mL) and treated with hydrazine monohydrate (18.4 mmol, 0.90 mL). The reaction was heated at reflux overnight, then washed with hot water (2x20 mL), hot DMF (2x20 mL), DCM (3x10 mL), DMF (3x10 mL) and MeOH (3x10 mL). A qualitative ninhydrin test was strongly positive.

Method B:
Boc-protected initiator core (**20**) (0.92 mmol of *tert*-buthoxycarbonyl groups) was suspended in DCM (20 mL) and treated with TFA (20 mL). The resin was left shaking for 2 h, the solvents drained and the resin washed with DCM (3x20 mL), 20% DIPEA in DMF (3x20 mL), DMF (3x20 mL), MeOH (3x20 mL) and DCM (3x20 mL). A qualitative ninhydrin test was strongly positive.

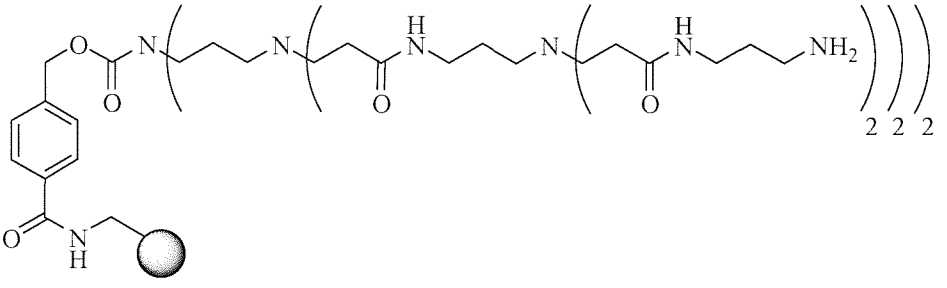
Method C:
Trifluoroacetyl-protected initiator core (**21**) (0.92 mmol of trifluoroacetyl groups) was suspended in dioxane (20 mL) and treated with 2M NaOH(aq) (20 mL). The resin was left shaking for 2.5 h, then the solvents were drained and the resin washed with water (3x20 mL), MeOH (3x20 mL), DMF (3x20 mL) and DCM (3x20 mL). A qualitative ninhydrin test was strongly positive.

Resin-bound Fmoc-Alanine-initiator core conjugate (23)



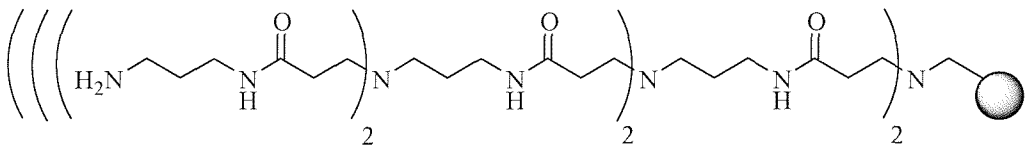
Fmoc-Ala-OH (0.040 mmol, 13 mg) and HOBt (0.040 mmol, 5 mg) were dissolved in DMF (1 mL). DIC (0.040 mmol, 6 μL) was added and the solution stirred for 5 min. The solution was then added to the resin (**22**) (0.81 mmol -NH_2/g, 25 mg) and the mixture left shaking for 3 h at room temperature. The reaction was monitored by ninhydrin test and on completion the resin was washed with DMF (3x2 mL), MeOH (3x2 mL), DCM (3x2 mL) and dried under vacuum overnight.

Resin-bound Generation [2.0] PAMAM Dendrimer (24)



Resin-bound initiator core (**22**) (0.81 mmol $-NH_2$/g, 1.0 g) was suspended in MeOH (6 mL) and treated with methyl acrylate (0.20 mol, 18 mL). The resin was left shaking for 24 h at 50°C, then the solvents were drained and the resin washed with MeOH (5x20 mL), DMF (3x20 mL) and DCM (3x20 mL). A negative qualitative ninhydrin test confirmed that the reaction was complete.
The resin was suspended in MeOH (17 mL) and treated with 1,3-propanediamine (0.20 mol, 17 mL). The resin was left shaking for 24 h at 50°C, then the solvents were drained and the resin washed with MeOH (5x20 mL), DMF (3x20 mL) and DCM (3x20 mL). A qualitative ninhydrin test was strongly positive.
This two-step procedure was repeated again with methyl acrylate/MeOH 3:1 (48 mL) and 1,3-propanediamine/MeOH 1:1 (68 mL) respectively.

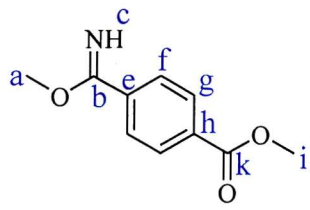
Resin-bound Generation [3.0] PAMAM Dendrimer (25)



TentaGel resin (0.46 mmol/g, 20 g) was suspended in MeOH (40 mL) and treated with methyl acrylate (1.1 mol, 100 mL). The resin was left shaking for 24 h, then filtered and washed with MeOH (5x50 mL), DMF (3x50 mL) and DCM (3x50 mL). The resin was suspended in MeOH (100 mL) and treated with 1,3-propanediamine (1.2 mol, 100 mL). The resin was shaken for 48 h, filtered and washed with MeOH (5x50 mL), DMF (3x50 mL) and DCM (3x50 mL). This two-step procedure was repeated three times, doubling the time of reaction and the amount of solvent and reagents at each step. Approximately 30 g of resin were obtained.

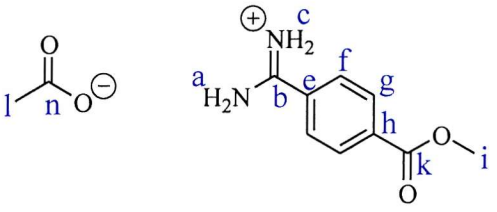
Experimental for Chapter Three

Methyl 4-(methoxycarbonyl)benzimidate (30)



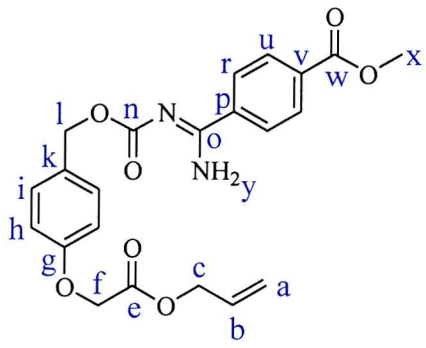
Methyl 4-cyanobenzoate (**29**) (6.2 mmol, 1.0 g) was dissolved in dry methyl acetate (0.15 mol, 12.5 mL) and freshly distilled methanol (0.19 mol, 7.5 mL). The solution was cooled to 0°C and acetyl chloride (0.15 mol, 10.7 mL) was added dropwise and the flask was then sealed. The solution was allowed to warm to room temperature and left stirring overnight. The solvents were then removed *in vacuo*, the white solid was dissolved in AcOEt and 2M ethanolic ammonia (5 mL) was added. The solution was filtered to remove the ammonium chloride and the solid was washed with AcOEt (20 mL). The solution was concentrated to a small volume (10 mL) and used without further purification.
TLC (1:1 PE/AcOEt) R_f: 0.32
δ_H (300 MHz, $CDCl_3$): 8.04 (2H, d, **g**, *J* 8); 7.78 (2H, d, **f**, *J* 8); 3.93 (3H, s, **a** or **i**); 3.91 (3H, s, **i** or **a**).
δ_C (75 MHz, $CDCl_3$): 167.3 (**k**); 166.4 (**b**); 136.6 (**e**); 132.3 (**h**); 129.8 (**g**), 127.0 (**f**); 53.6, 52.5 (**a** and **i**).
IR (ν): 2956, 1723, 1637.

4-(Methoxycarbonyl)benzamidinium acetate (31)



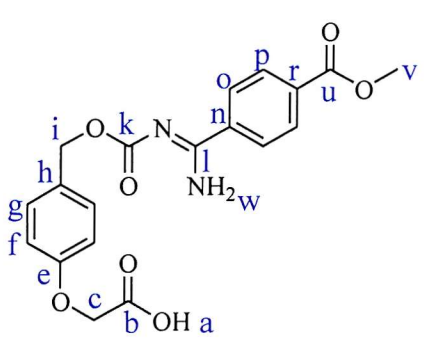
The solution of (**30**) was diluted with methanol (10 mL) and treated with ammonium acetate (18.6 mmol, 1.4 g). The solution was left stirring overnight and the white solid formed was filtered, washed with AcOEt (20 mL) and crystallised from hot methanol, yielding the product (950 mg, 65%) as a white needle-like solid. Experimental data are in accordance with the literature.[141,142]
δ_H (300 MHz, D_2O): 8.05 (2H, d, **g**, *J* 9); 7.75 (2H, d, **f**, *J* 9); 3.85 (3H, s, **i**); 1.78 (3H, s, **l**).
δ_C (75 MHz, D_2O): 184.0 (**n**); 170.5 (**b**); 168.7 (**k**); 136.9 (**e**); 134.8 (**h**); 132.6 (**f**); 130.6 (**g**); 55.6 (**i**), 25.8 (**l**).
m/z (ES+): 179.0 (100%, $[M+H\text{-}AcOH]^+$).
IR (ν): 3500-2800 (broad), 2956, 2845, 1723, 1610.
Melting point: 138°-142°C (referred to the amidine derivative).

N-({4'-[(Allyloxycarbonyl)methyloxy]phenyl}methyloxycarbonyl)-4-methoxycarbonyl benzamidine (33)



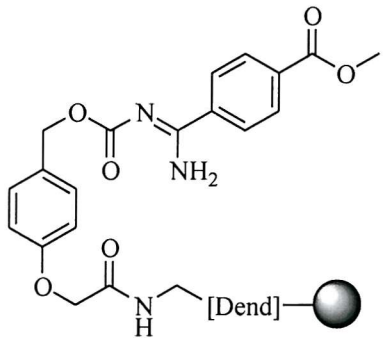
4-(Methoxycarbonyl)benzamidinium acetate (**31**) (4.2 mmol, 1.0 g) was suspended in dioxane (15 mL) and treated with potassium carbonate (12.6 mmol, 1.7 g). After 10 min allyl 4-{[(4'-nitrophenyl)oxycarbonyl]oxymethyl}phenoxyacetate (**32**) (4.6 mmol, 1.8 g) dissolved in dioxane (25 mL) was added dropwise at room temperature. The solution was left stirring overnight at room temperature. The solution was concentrated to a small volume *in vacuo*, then diluted with a saturated solution of NH_4Cl(aq) (40 mL) and extracted with AcOEt (3x30 mL) and dried over $MgSO_4$. The solvents were removed *in vacuo* and the crude material was purified by flash chromatography (gradient from PE/AcOEt 75:25 to 3:7) yielding the pure product (1.27 g, 71%) as a yellow oil.
TLC (1:1 PE/AcOEt) R_f: 0.25
δ_H (300 MHz, $CDCl_3$): 9.55 (0.4H, broad s, **y**); 8.02 (2H, d, **u**, *J* 9); 7.88 (2H, d, **r**, *J* 9); 7.37 (2H, d, **i**, *J* 9); 6.87 (2H, d, **h**, *J* 9); 5.91 (1H, ddt, **b**, *J* 17, 10, 6); 5.32 (1H, dd, $\mathbf{a_1}$, *J* 17, 1); 5.26 (1H, dd, $\mathbf{a_2}$, *J* 10, 1); 5.13 (2H, s, **f**); 4.68 (2H, d, **c**, *J* 6); 4.64 (2H, s, **l**); 3.92 (3H, s, **x**).
δ_C (75 MHz, $CDCl_3$): 168.7 (**o**); 167.4 (**w**); 166.3 (**e**); 163.6 (**n**); 157.8 (**g**); 138.7 (**p**); 133.4 (**k**); 131.5 (**b**); 130.4 (**u**); 130.0 (**i**); 127.5 (**r**); 114.7 (**h**); 129.8 (**v**); 119.4 (**a**); 67.1 (**l**); 66.1 (**c**); 65.5 (**f**); 52.6 (**x**).
m/z (ES+): 427.3 (60%, $[M+H]^+$); 449.3 (30%, $[M+Na]^+$); 465.3 (85%, $[M+K]^+$); 853.7 (100%, $[2M+H]^+$); 875.5 (25%, $[2M+Na]^+$); 891.3 (35%, $[2M+K]^+$).
HR-MS (EI+): 426.1419 (calc. 426.1427, -1.9 ppm).

N-({4'-[(carboxymethyl)oxy]phenyl}methyloxycarbonyl)-4-methoxycarbonyl benzamidine (34)



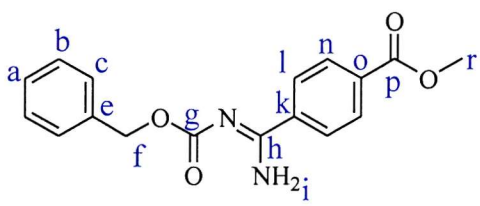
The amidine-linker complex (**33**) (1.17 mmol, 500 mg) was dissolved in 5 mL of dry THF and 5 mL of freshly distilled DCM. Nitrogen was bubbled through the solution for 45 min, then thiosalicilic acid (1.40 mmol, 220 mg) and $Pd(PPh_3)_4$ (0.06 mmol, 70 mg) were added and the solution was left stirring under N_2 for 3 h. The solvents were then removed under reduced pressure and the crude material was triturated in AcOEt. The solid was filtrated and dried *in vacuo*. The product (310 mg, 68%) could not be stored for aprolonged period of time.
TLC (1:9 MeOH/AcOEt) Rf: 0.45
δ_H (300 MHz, DMSO): 9.28 (1H, broad s, **a**); 8.16 (2H, d, **p**, *J* 9); 8.12 (2H, d, **o**, *J* 9); 7.41 (2H, d, **g**, *J* 9); 7.00 (2H, d, **f**, *J* 9); 5.12 (2H, s, **c**); 4.77 (2H, s, **i**); 3.97 (3H, s, **v**).
δ_C (75 MHz, DMSO): 170.3 (**b**); 165.7 (**l**); 165.6 (**u**); 163.8 (**k**); 157.6 (**e**); 138.6 (**n**); 132.4 (**h**); 129.9 (**p**); 129.5 (**r**); 129.2 (**g**); 128.2 (**o**); 114.3 (**f**); 65.9 (**i**); 64.5 (**c**); 52.5 (**v**).
m/z (ES+): 387.2 (100%, $[M+H]^+$), 665.3 (60%, $[M+H+OPPh_3]^+$), 773.5 (40%, $[2M+H]^+$); (ES-): 499.2 (100%, $[M-H+TFA]^-$).
HR-MS (EI+): 387.1167 (calc. 387.1192, -6.5 ppm).

Resin-bound amidine/linker complex (35)



Dendrimer resin (**24**) (1.4 mmol/g, 100 mg) were swollen in DCM (5 mL) for 10 min and the solvent was then drained. Acid (**34**) (0.51 mmol, 200 mg) was dissolved in DMF (5 mL) and treated with HOBT (0.50 mmol, 70 mg) and DIC (0.50 mmol, 80 μL). After 15 min this solution was added to the resin and left shaking overnight. The resin was then washed with MeOH (3x20 mL), DMF (3x20 mL) and DCM (3x20 mL). A qualitative ninhydrin test was negative. HPLC and ES-MS analysis of the product cleaved off the resin (with TFA/DCM 95:5) gave only the expected peak of the amidine (**31**) (HPLC (method 2, 254 nm): 7.0 min (100%); m/z (ES+): 179.1, $[M+H]^+$).

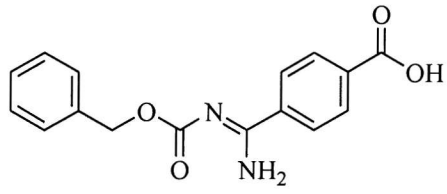
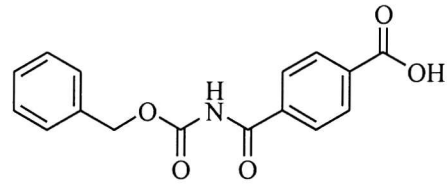
N-(Benzyloxycarbonyl)-4-(methoxycarbonyl)benzamidine (36)



4-Methoxycarbonyl benzamidinium acetate (**31**) (1.1 mmol, 250 mg) was dissolved in dioxane (2 mL) and treated with potassium carbonate (3.3 mmol, 440 mg). The

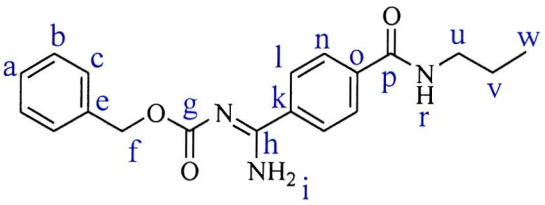
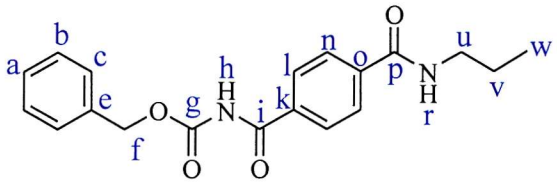
solution was cooled to 0°C in an ice-bath and benzylchloroformate (1.4 mmol, 200 μL) dissolved in dioxane (3 mL) was added dropwise. The solution was left stirring at room temperature for 4 h, then diluted with brine (30 mL) and extracted with AcOEt (3x20 mL). The crude material was purified by flash chromatography on silica gel (gradient from PE/AcOEt 3:1 to 4:6) affording the pure product (190 mg, 59%) as a colourless oil.
TLC (1:1 PE/AcOEt) Rf: 0.45
δ_H (300 MHz, $CDCl_3$): 9.59 (0.6H, broad s, **i**); 8.05 (2H, d, **n**, *J* 8); 7.89 (2H, d, **l**, *J* 8); 7.45 (2H, dd, **c**, *J* 8, 2); 6.40-6.30 (3H, m, **a** and **b**); 5.22 (2H, s, **f**); 3.93 (3H, s, **r**).
δ_C (75 MHz, $CDCl_3$): 167.2 (**h**); 166.2 (**p**); 164.4 (**g**); 138.6 (**k**); 136.5 (**e**); 133.3 (**o**); 129.8 (**n**), 128.5 (**l**); 128.2 (**b**); 128.1 (**a**); 127.3 (**c**); 67.4 (**f**); 52.3 (**r**).
m/z (ES+): 313.3 (100%, [M+H]+); 625.9 (25%, [2M+H]+); 938.3 (10%, [3M+H]+).

N-(Benzyloxycarbonyl)-4-carboxybenzamidine (**37a**) and
N-(Benzyloxycarbonyl)-4-carboxybenzamide (**37b**)

N-Benzyloxycarbonyl-4-methoxycarbonylbenzamidine (**36**) (0.80 mmol, 250 mg) was dissolved in THF (3 mL) and water (2 mL) was added together with $LiOH{\cdot}H_2O$ (1.60 mmol, 70 mg). After 1 h the solution was diluted with NH_4Cl_{sat} (20 mL) and the product extracted with AcOEt (3x15 mL). The NMR spectrum of the crude material showed the presence of two different products with very similar resonance peaks. Molecular masses of these two products, determined by ES-MS (both ES+ and ES-), were found to be 298 and 299 respectively. Separation of these two products by flash chromatography could not be achieved at this stage, due to their similar polarity.

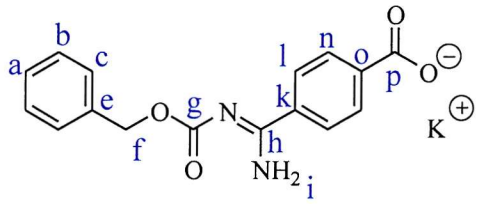
N-(Benzyloxycarbonyl)-4-[(3'-propylamino)carbonyl]benzamidine (**38a**) and
N-(Benzyloxycarbonyl)-4-[(3'-propylamino)carbonyl]benzamide (**38b**)

The mixture of benzoic acids (**37a**) and (**37b**) (0.17 mmol, 50 mg) was suspended in DCM (3 mL) and treated with HOAT (0.20 mmol, 30 mg) and HATU (0.20 mmol, 75 mg). The solution was left stirring for 5 min, then propylamine (0.85 mmol, 70 μL) was added. After 2 h the reaction was quenched with brine (20 mL) and extraction with AcOEt (3x15 mL) afforded 110 mg of crude material, purified by flash chromatography on silica gel (gradient from AcOEt/PE 1:1 to 9:1). As expected two different products were isolated: the more polar one (42 mg, 70%) turned out to be (**38a**) while the less polar one (16 mg, 30%) turned out to be (**38b**).

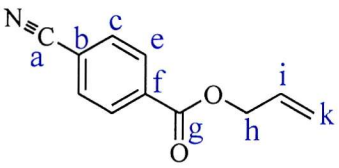
(**38a**): TLC (AcOEt) R_f: 0.65
δ_H (300 MHz, DMSO): 9.3 (1.1H, broad s, **i**); 8.62 (1H, t, **r**, *J* 5); 8.05 (2H, d, **n**, *J* 8); 7.93 (2H, d, **l**, *J* 8); 7.45-7.30 (5H, m, **a**, **b** and **c**); 5.12 (2H, s, **f**); 3.24 (2H, dt, **u**, *J* 7, 5); 1.54 (2H, sext, **v**, *J* 7); 0.89 (3H, t, **w**, *J* 7).
δ_C (75 MHz, DMSO): 166.0 (**h**); 165.5 (**p**); 163.5 (**g**); 137.7 (**k**); 137.1 (**e**); 136.4 (**o**); 128.4, 128.1, 127.8, 127.2 (**b**, **c**, **l** and **n**); 127.9 (**a**); 66.2 (**f**); 41.1 (**u**); 22.4 (**v**); 11.5 (**w**).
m/z (ES+): 340.3 (100%, $[M+H]^+$).
Amide: TLC (AcOEt) R_f: 0.70
δ_H (300 MHz, DMSO): 11.2 (1H, s, **h**); 8.63 (1H, t, **r**, *J* 5); 7.95 (2H, d, **n**, *J* 9); 7.91 (2H, d, **l**, *J* 9); 7.48-7.34 (5H, m, **a**, **b** and **c**); 5.22 (2H, s, **f**); 3.24 (2H, dt, **u**, *J* 7, 5); 1.53 (2H, sext., **v**, *J* 7); 0.89 (3H, t, **w**, *J* 7).
δ_C (75 MHz, DMSO): 165.5 (**p**), 165.4 (**i**); 151.5 (**g**); 138.2 (**k**); 135.8 (**e**); 135.3 (**o**); 128.6, 128.4, 128.3, 127.2 (**b**, **c**, **l** and **n**); 128.5 (**a**); 66.6 (**f**); 41.1 (**u**); 22.4 (**v**); 11.5 (**w**).
m/z (ES+): 341.4 (100%, $[M+H]^+$); 382.5 (50%, $[M+CH_3CN+H]^+$).

Potassium 4-[N-(benzyloxycarbonyl)amidino]benzoate (39)



N-(Benzyloxycarbonyl)-4-(methoxycarbonyl)benzamidine (**36**) (0.16 mmol, 50 mg) was dissolved in dry THF (2 mL) and treated with Me_3SiOK (0.32 mmol, 50 mg). The solution was heated at 50°C for 2 h. After cooling the white precipitate was filtered, washed with THF (10 mL) and dried under vacuum, affording quantitatively (54 mg) the desired product as its potassium salt.
δ_H (300 MHz, D_2O): 7.74 (2H, d, **n**, *J* 8); 7.57 (2H, d, **l**, *J* 8); 7.21 (5H, broad s, **a**, **b** and **c**); 4.69 (2H, s, **f**).
δ_C (75 MHz, D_2O): 177.1 (**p**); 171.9 (**h**); 166.4 (**g**); 142.4 (**o**); 138.9 (**k**); 138.6 (**e**); 131.5, 131.3, 130.1, 130.0 (**b**, **c**, **l** and **n**); 130.8 (**a**); 69.6 (**f**).
m/z (ES-): 297.4 (100%, $[M-K]^-$), 411.5 (20%, $[M+TFA-K]^-$), 595.9 (30%, $[2M-K]^-$); (ES+) 299.4 (100%, $[M-K+2H]^+$).

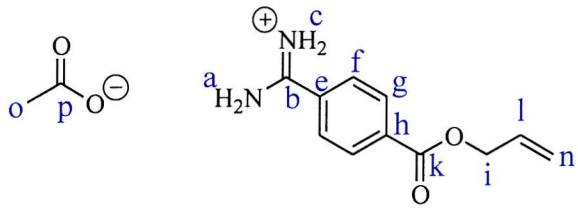
Allyl 4-cyanobenzoate (43)



4-Cyanobenzoic acid (**42**) (13.6 mmol, 2.0 g) was dissolved in allyl alcohol (30 mL) and benzene (30 mL) and *p*-toluensulfonic acid (15.0 mmol, 2.8 g) were added. The solution was heated at reflux overnight with a Dean-Stark, the solvents were evaporated at reduced pressure and the crude was dissolved in AcOEt (50 mL) and

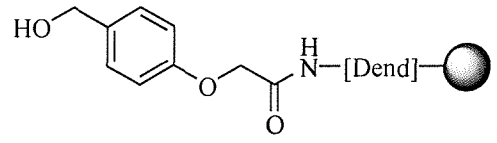
washed with 2M K_2CO_3(aq) (3x50 mL). The organic layer was dried over magnesium sulfate, filtered and evaporated to dryness affording allyl 4-cyanobenzoate (**43**) (2.5 g, 100%) as a brown oil. Experimental data are in accordance with the literature.[143]
TLC (2:1 PE/AcOEt) R_f: 0.89
δ_H (300 MHz, $CDCl_3$): 8.15 (2H, d, **e**, *J* 7); 7.74 (2H, d, **c**, *J* 7); 6.02 (1H, ddt, **i**, *J* 17, 10, 6); 5.41 (1H, dd, $\mathbf{k}_{tr}$, *J* 17, 1); 5.31 (1H, dd, $\mathbf{k}_{cis}$, *J* 10, 1); 4.84 (2H, d, **h**, *J* 6).
δ_C (75 MHz, $CDCl_3$): 164.7 (**g**); 134.1 (**f**); 132.4 (**c**); 131.7 (**i**); 130.3 (**e**); 119.2 (**k**); 118.1 (**b**); 116.6 (**a**); 66.5 (**h**).
IR (ν): 3099, 2227, 1721, 1651, 1273.
HPLC (method 2, 254 nm): 16.1 min. (92%)

4-(Allyloxycarbonyl)benzamidinium acetate (46)



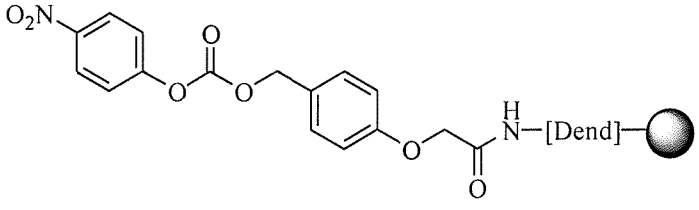
Allyl 4-cyanobenzoate (**43**) (13.6 mmol, 2.5 g) was dissolved in pyridine (50 mL) and triethylamine (10 mL) and H_2S was bubbled through the solution for 30 min. The resulting green solution was left stirring for 24 h at room temperature, then nitrogen was bubbled through the solution until it returned brown-orange. The solvents were evaporated and a yellow solid (**44**) was obtained quantitatively (HPLC (method 2, 254 nm): 14.6 min. (94%); TLC (2:1 PE/AcOEt) R_f: 0.47). This solid was dissolved in acetone (50 mL) and methyl iodide (0.14 mol, 8.4 mL) was added. The yellow solution was left stirring overnight and turned red. The solvents were evaporated yielding a red amorphous solid (**45**) (HPLC (method 2, 254 nm): 10.5 min. (68%); m/z (ES+): 236.1 ($[M+H]^+$, 100%)). This solid was dissolved in allyl alcohol (50 mL) and treated with ammonium acetate (20.4 mmol, 1.6 g). The solution was left stirring at 70°C for 24 h, then nitrogen was bubbled through it to remove methyl sulfide and the solution was concentrate to a small volume. Diethyl ether was added and the resulting brownish precipitate was filtered and triturated in AcOEt, affording 2.1 g (58%) of the desired product (**46**) as a greyish solid.
δ_H (300 MHz, CD_3OD): 8.32 (2H, d, **g**, *J* 8); 8.01 (2H, d, **f**, *J* 8); 6.17 (1H, ddt, **l**, *J* 18, 10, 5); 5.52 (1H, dd, $\mathbf{n}_{tr}$, J=18, 1); 5.40 (1H, dd, $\mathbf{n}_{cis}$, *J* 10, 1); 4.96 (2H, d, **i**, *J* 5); 1.99 (3H, s, **o**).
δ_C (75 MHz, CD_3OD): 180.0 (**p**); 167.8 (**k**); 166.3 (**b**); 136.0 (**e**); 134.6 (**h**); 133.8 (**l**); 131.4 (**f**); 129.8 (**g**); 119.4 (**n**); 67.4 (**i**); 24.9 (**o**).
HPLC (method 2, 254 nm): 9.3 min (95%).
m/z (AP+): 205.0 ($[M-AcOH+H]^+$, 100%); 246.1 ($[M-AcOH+Acetonitrile+H]^+$, 20%).
IR (ν): 3500-2500 (very broad), 1723, 1661, 1611, 1275.
HR-MS (EI+): 204.0890 (calc. 204.0899, -4.3 ppm).

Dendrimer HMPA resin (48)



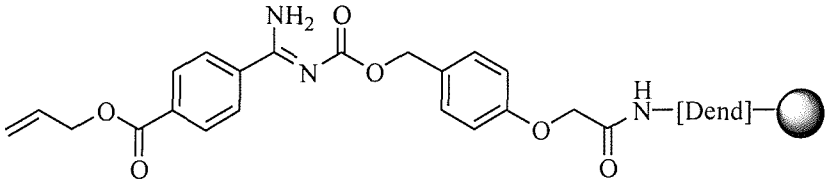
Gen[2.0] PAMAM dendrimer resin (**25**) (1.0 mmol/g, 1.0 g) was swollen in DCM (10 mL) for 5 min, then the solvent was drained and 4-(hydroxymethyl)phenoxyacetic acid (**47**) (2.8 mmol, 510 mg) and EEDQ (2.8 mmol, 690 mg) dissolved in DCM (10 mL) and DMF (2 mL) were added. The resin was shaken for 24 h, then washed with DMF (2x20 mL), MeOH (2x20 mL) and DCM (2x20 mL). A qualitative ninhydrin test was negative, confirming that the reaction reached completion. A small sample of resin (2 mg) was treated with TFA/DCM 95:5 for two hours, the solution was concentrated *in vacuo* and analysed by HPLC (method 2, 254 nm). A peak at 8.3 minutes corresponding to compound (**47**) appeared in the HPLC trace, due to formation of linker-oligomers onto the resin. The resin was treated with 1M NaOH(aq) (10 mL) and THF (10 mL) for two hours and washed with water (2x20 mL), MeOH (2x20 mL), DMF (2x20 mL) and DCM (2x20 mL). A small sample of resin (2 mg) was treated with TFA/DCM 95:5 for two hours, the solution was concentrated *in vacuo* and analysed by HPLC. Nothing appeared on the HPLC trace, meaning that any linker-oligomers were completely hydrolysed.

Active-carbonate dendrimer resin (49)



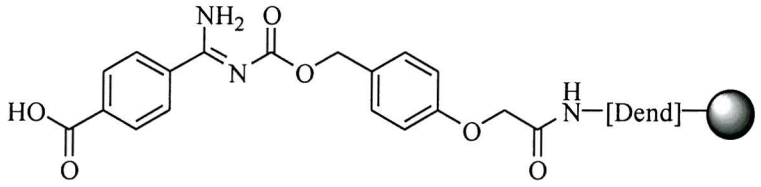
Resin (**48**) was suspended in DCM (10 mL) and N-methylmorpholine (2.0 mmol, 0.23 mL) was added. N_2 was bubbled through the resin and *p*-nitrophenyl chloroformate (2.0 mmol, 400 mg), dissolved in DCM (5 mL), was slowly added at 0°C. The resin was shaken for 30 min at room temperature, then washed with dry DCM (3x20 mL). A ^{1}H-NMR calibration analysis was carried out to determine the amount of *p*-nitrophenol released off the resin. The analysis was carried out with two different methods: with TFA/DCM 95:5 (to release *p*-nitrophenol attached to the HMPA linker) and with diethylamine/DCM 2:8 (to release all *p*-nitrophenol attached to the resin). The two values obtained were in perfect accordance and found to be 0.45 mmol per gram of resin (60% of the theoretical value).

Dendrimer resin-bound allyl-protected 4-carboxybenzamidine (50)



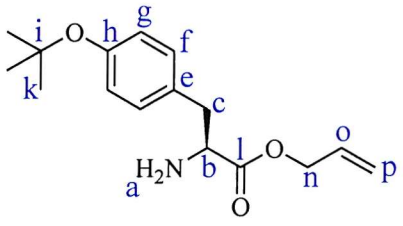
4-(Allyloxycarbonyl)benzamidinium acetate (4.4 mmol, 1.16 g) and cesium carbonate (4.4 mmol, 1.52 g) were dissolved in DMF (30 mL) and N_2 was bubbled for 30 min to eliminate possible traces of ammonia. The resin (1.1 mmol, 900 mg) was then added and left shaking for 3 days. The resin was then filtered and washed with water (3x30 mL), DMF (3x30 mL), 2M citric acid in water (3x30 mL), DMF (3x30 mL) and DCM (3x30 mL). A small sample of resin was treated with TFA and analysed by HPLC (method 2, 254 nm), giving the expected peak at 9.2 min. (93%).

Dendrimer resin-bound 4-carboxybenzamidine (50)



$Pd(PPh_3)_4$ (0.11 mmol, 120 mg) was dissolved in a degassed solution of DCM/acetic acid/N-methylmorpholine 10:2:1 (10 mL) and added to resin (**50**) (0.21 mmol of allyl groups, 300 mg) that had been previously kept under N_2 for 30 min. The resin was left shaking for 5 h, filtered and washed with 1% N,N-diisoprophylethylamine in DMF (2x25mL) and 1% sodium diethyldithiocarbamate in DMF (2x25 mL) to remove the catalyst, then washed with DMF (3x10 mL) and DCM (3x10 mL).
A small sample of resin was treated with TFA and analysed by HPLC (method 2, 254 nm), giving the expected peak of (**40**) at 6.1 min. (85%).

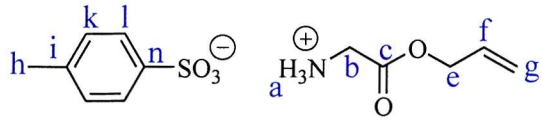
L-Tyrosine(O-*tert*-butyl) allyl ester (51)



Fmoc-L-Tyrosine(O-*tert*-Bu)-OH (**55**) (2.2 mmol, 1.0 g) was dissolved in DMF (10 mL) and cesium carbonate (1.6 mmol, 0.70 g) was added. The solution was stirred for 1 h at room temperature, then allyl bromide (10.9 mmol, 0.95 mL) was added and the reaction stirred overnight. The solution was diluted with brine (50 mL) and extracted with AcOEt (50 mL); the organic phase was then washed with brine (50 mL), dried over magnesium sulfate and the solvent removed *in vacuo*. The crude material (**56**) was dissolved in DCM (50 mL) and diethylamine (10 mL) was added. The reaction was stirred for 4 h, then the solvents were evaporated *in vacuo* and the resulting yellow oil purified by flash chromatography (ETP/AcOEt 1:1 to elute the fulvene derivative and pure AcOEt to elute the desired product). The final product was isolated as a yellow oil (560 mg, 100%). Experimental data are in accordance with the literature.[144]
δ_H (300 MHz, $CDCl_3$): 7.09 (2H, d, **f**, *J* 8); 6.92 (2H, d, **g**, *J* 8); 5.87 (1H, ddt, **o**, *J* 18, 10, 6); 5.29 (1H, dd, $\mathbf{p}_{tr}$, *J* 18, 1); 5.23 (1H, dd, $\mathbf{p}_{cis}$, *J* 10, 1); 4.59 (2H, d, **n**, *J* 6); 3.74

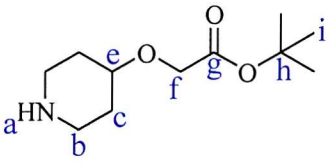
(1H, dd, **b**, *J* 8, 5); 3.05 (1H, dd, $\mathbf{c_1}$, *J* 13, 5); 2.86 (1H, dd, $\mathbf{c_2}$, *J* 13, 8); 1.73 (2H, broad s, **a**); 1.33 (9H, s, **k**).
δ_C (75 MHz, $CDCl_3$): 174.8 (**l**); 154.4 (**h**); 131.9 (**o**); 129.7 (**f**); 124.2 (**g**); 118.6 (**p**); 78.3 (**i**); 65.5 (**n**); 55.9 (**b**); 40.5 (**c**); 28.8 (**k**); **e** is overlapped.
m/z (ES+): 278.1 (100%, $[M+H]^+$); 319.0 (20%, $[M+H+acetonitrile]^+$).

Glycine allyl ester tosyl salt (52)



Glycine (**57**) (13.3 mmol, 1.0 g) was dissolved in allyl alcohol (10 mL) and benzene (15 mL). *p*-Toluensulphonic acid (14.0 mmol, 2.7 g) was added and the reaction heated at reflux overnight. The solvents were removed *in vacuo* and the tosyl salt (**52**) was used without further purification. Experimental data are in accordance with the literature.[145]
δ_H (400 MHz, CD_3OD): 7.72 (2H, d, **k**, *J* 8); 7.25 (2H, d, **l**, *J* 8); 5.97 (1H, ddt, **f**, *J* 17, 11, 6); 5.41 (1H, dd, $\mathbf{g}_{tr}$, *J* 17, 2); 5.28 (1H, dd, $\mathbf{g}_{cis}$, *J* 11, 2); 4.73 (2H, d, **e**, *J* 6); 3.88 (2H, s, **b**); 2.39 (3H, s, **h**).
δ_C (100 MHz, CD_3OD): 168.2 (**c**); 143.3 (**i**); 141.7 (**n**); 132.6 (**f**); 129.8 (**l**); 126.9 (**k**); 119.4 (**g**); 67.6 (**e**); 41.0 (**b**); 21.2 (**h**).
m/z (ES+): 115.8 (10%, $[M\text{-}TosOH+H]^+$); 156.9 (100%, $[M\text{-}TosOH+H+acetonitrile]^+$).
Melting point: 55-57°C.

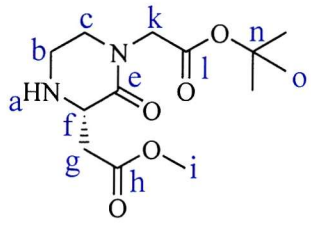
***tert*-Butyl (piperid-4-yloxy)acetate (53)**



To a solution of 4-hydroxypiperidine (**58**) (10.0 mmol, 1.0 g) in DCM (10 mL) were added successively triethylamine (11.0 mmol, 1.5 mL) and benzyl chloroformate (11.0 mmol, 1.6 mL) at 0°C. The reaction mixture was stirred at room temperature overnight, the resulting suspension was then filtered, the solvents evaporated, the residue taken up in AcOEt (50 mL) and extracted with water (2x25 mL) and 2M HCl(aq) (30 mL). The organic layer was dried over magnesium sulphate and concentrated *in vacuo*. The resulting yellow oil (**59**) (8.5 mmol, 2.0 g) was dissolved in toluene (10 mL) and *t*-butyl bromoacetate (12.7 mmol, 1.9 mL) was added, together with tetra-*n*-butylammonium hydrogensulphate (0.42 mmol, 150 mg) in 1 mL of water. A solution of sodium hydroxide (0.21 mol, 8.5 g) in water (9 mL) was added dropwise, followed by vigorous stirring for 16 h. The organic layer was then separated, dried, filtered and evaporated *in vacuo*. The crude material was purified by flash chromatography (PE/AcOEt 7:3) and the resulting white solid (**60**) (3.0 g, 8.5 mmol) was dissolved in ethanol (50 mL), treated with 10% Pd/C (Degussa type) and hydrogenated under atmospheric pressure at room temperature. After 6 h the solution

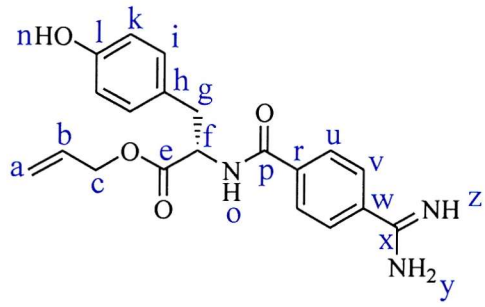
was filtered and the solvent evaporated, yielding 8.5 g (85%) of the title compound (**53**) as a pale yellow oil. Experimental data are in accordance with the literature.[86]
δ_H (400 MHz, $CDCl_3$): 4.00 (2H, s, **f**); 3.49 (1H, tt, **e**, *J* 9, 4); 3.11 (2H, dt, $\mathbf{b_1}$, *J* 13, 5); 2.63 (2H, ddd, $\mathbf{b_2}$, *J* 13, 10, 3); 1.98-1.89 (2H, m, $\mathbf{c_1}$); 1.56-1.50 (2H, m, $\mathbf{c_2}$); 1.51 (9H, s, **i**).
δ_C (100 MHz, $CDCl_3$): 170.2 (**g**); 81.6 (**h**); 76.5 (**e**); 65.8 (**f**); 44.5 (**b**); 32.8 (**c**); 28.2 (**i**).
m/z (ES+): 216.3 (100%, $[M+H]^+$).

***tert*-Butyl 3-[(Methoxycarbonyl)methyl]-2-oxopiperazine-1-acetate (54)**



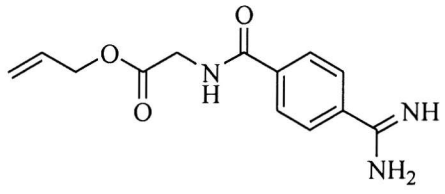
tert-Butyl bromoacetate (7.0 mmol, 1.0 mL) was dissolved in DMF (10 mL) and added dropwise to a mixture of 2,2-dimethoxyethylamine (**61**) (14.0 mmol, 1.6 mL) and cesium carbonate (7.0 mmol, 2.2 g) in DMF (10 mL) over 1 h at room temperature. The solution was stirred overnight, then diluted with water (50 mL) and extracted with AcOEt (3x50 mL). The organic phase was washed with brine (2x50 mL), dried over magnesium sulphate and concentrated *in vacuo*. The crude material was purified by flash chromatography (PE/AcOEt 1:1, 1% triethylamine) and obtained as a colourless oil (**62**) (1.2 g, 78%). This compound (5.5 mmol, 1.2 g) was dissolved in acetonitrile (25 mL) together with WSC (6.6 mmol, 1.3 g) and N-Cbz-Asp(OMe)-OH (6.1 mmol, 1.8 g) at room temperature. The reaction was stirred overnight, the solvent was then evaporated and the resulting oil dissolved in EtOAc (50 mL) and washed successively with 5% $KHSO_4$, $KHCO_{3sat}$ and brine. The organic phase was dried over magnesium sulphate and after evaporation of the solvent the resulting crude material was purified by flash chromatography (gradient from PE/EtOAc 4:1 to 3:1), yielding 2.5 g (84%) of pure product (**63**). A mixture of the amide (**63**) (4.6 mmol, 2.5 g), *p*-toluensulphonic acid (0.46 mmol, 90 mg) in toluene (50 mL) was stirred at 70°C for 2 h. After cooling the solution was washed with $NaHCO_{3sat}$ (50 mL), dried over magnesium sulphate and concentrated in vacuo. The residual oil was dissolved in methanol (50 mL), treated with 10% Pd/C (1.0 g), and hydrogenated under atmospheric pressure at room temperature overnight. The solution was filtered, concentrated in vacuo and purified by flash chromatography (PE/EtOAc 1:1), yielding 1.2 g (91%) of product (**54**). Experimental data are in accordance with the literature.[91]
δ_H (300 MHz, $CDCl_3$): 4.09 (1H, d, $\mathbf{k_1}$, *J* 17); 3.83 (1H, d, $\mathbf{k_2}$, *J* 17); 3.78 (1H, dd, **f**, *J* 9, 3); 3.66 (3H, s, **i**); 3.57-3.46 (1H, m, $\mathbf{c_1}$); 3.26-3.19 (1H, m, $\mathbf{c_2}$); 3.14-3.08 (2H, m, **b**); 3.01 (1H, dd, $\mathbf{g_1}$, *J* 17, 3); 2.71 (1H, dd, $\mathbf{g_2}$, *J* 17, 9); 2.44 (1H, broad s, **a**); 1.42 (9H, s, **o**).
δ_C (75 MHz, $CDCl_3$): 172.8 (**h**); 169.5 (**l**); 168.0 (**e**); 82.1 (**n**); 56.2 (**f**); 52.0 (**i**); 49.4 (**k**); 49.3 (**c**); 42.3 (**b**); 36.8 (**g**); 28.2 (**o**).
m/z (ES+): 287.2 (100%, $[M+H]^+$); 309.2 (40%, $[M+Na]^+$).

N-(4'-Amidinobenzoyl)tyrosine allyl ester (64')



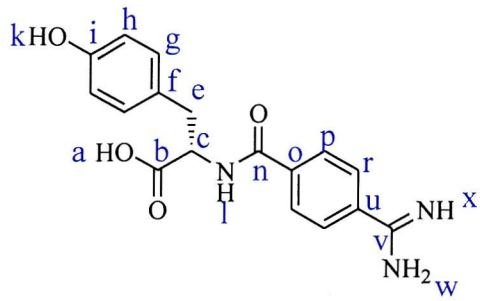
Resin (**50**) (0.035 mmol, 50 mg) was washed with freshly distilled dry THF (3x5 mL). Pentafluorophenol (0.35 mmol, 65 mg) was dissolved in dry DMF (0.5 mL) together with pyridine (0.35 mmol, 30 μL) and DIC (0.35 mmol, 60 μL). The resulting solution was added to the resin and this was then shaken for 30 min. The resin was then filtered and washed with DMF (1 mL). Compound (**51**) (0.10 mmol, 28 mg) and N,N-diisopropylethylamine (0.30 mmol, 45 μL) were dissolved in DMF (0.5 mL) and added to the resin. This was shaken overnight, filtered and washed with DMF (5x10 mL) and DCM (5x10 mL). Resin (**64**) was then treated with TFA/DCM 95:5 for three hours and the filtrate, after removal of the solvents, was purified by semi-preparative HPLC, yielding 6 mg (50%) of (**64'**) as a white solid.
δ_H (400 MHz, CD_3OD): 7.84 (2H, d, **u**, *J* 8); 7.76 (2H, d, **v**, *J* 8); 6.98 (2H, d, **i**, *J* 8); 6.60 (2H, d, **k**, *J* 8); 5.82 (1H, ddt, **b**, *J* 17, 10, 6); 5.21 (1H, ddt, $\mathbf{a}_{tr}$, *J* 17, 3, 1); 5.12 (1H, ddt, $\mathbf{a}_{cis}$, *J* 10, 3, 1); 4.74 (1H, dd, **f**, *J* 10, 6); 4.54 (2H, dt, **c**, *J* 6, 3); 3.25 (1H, dd, $\mathbf{g}_1$, *J* 14, 6); 3.06 (1H, dd, $\mathbf{g}_2$, *J* 14, 10).
δ_C (100 MHz, CD_3OD): 173.1 (**e**), 168.9 (**p**), 168.4 (**x**), 157.9 (**l**), 140.7 (**w**), 133.6 (**b**); 132.9 (**r**), 131.6, 129.7, 129.6 (**i**, **u** and **v**); 129.3 (**h**); 119.1 (**k**); 116.7 (**a**), 67.3 (**c**), 56.7 (**f**); 37.7 (**g**).
HPLC (method 2, 254 nm): 10.2 min.
m/z (ES+): 368.2 ($[M+H]^+$).
HR-MS (FAB): 368.1623 (calc. 368.1610, 3.4 ppm).

N-(4'-Amidinobenzoyl)glycine allyl ester (65')



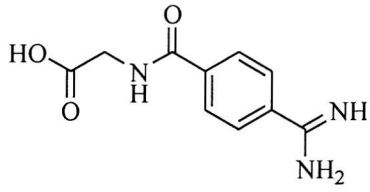
The same procedure applied for (**64'**) was followed.
HPLC (method 2, 254 nm): 8.0 min. (66%)
m/z (ES+): 262.1 ($[M+H]^+$).

N-(4'-Amidinobenzoyl)tyrosine (66')



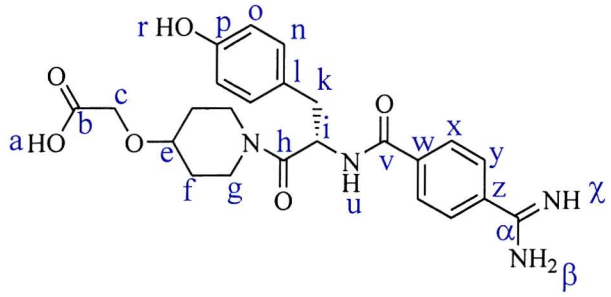
The same procedure used for (**50**) was followed for the allyl deprotection. Resin (**66**) was then treated with TFA/DCM 95:5 for three hours and the filtrate, after removal of the solvents, was purified by semi-preparative HPLC, yielding 5 mg (42%) of (**66'**) as a white solid.
δ_H (400 MHz, CD_3OD): 7.96 (2H, d, **p**, *J* 8); 7.86 (2H, d, **r**, *J* 8); 7.06 (2H, d, **g**, *J* 8); 6.63 (2H, d, **h**, *J* 8); 5.29 (1H, dd, **c**, *J* 8, 5); 3.95 (1H, dd, $\mathbf{e_1}$, *J* 14, 5); 3.71 (1H, dd, $\mathbf{e_2}$, *J* 14, 8).
δ_C (100 MHz, CD_3OD): 175.1 (**b**), 165.5 (**n**), 164.5 (**v**), 154.9 (**i**), 139.6 (**u**); 130.0 (**o**), 129.6, 127.3, 127.0 (**g**, **p** and **r**); 114.0 (**h**); 128.4 (**f**), 56.0 (**c**); 36.3 (**e**).
HPLC (method 2, 254 nm): 8.1 min.
m/z (ES+): 328.2 ($[M+H]^+$).
HR-MS (FAB): 328.1298 (calc. 328.1297, 0.2 ppm).

N-(4'-Amidinobenzoyl)glycine (67')



The same procedure applied for (**66'**) was followed.
HPLC (method 2, 254 nm): 5.9 min. (70%)
m/z (ES+): ($[M+H]^+$).

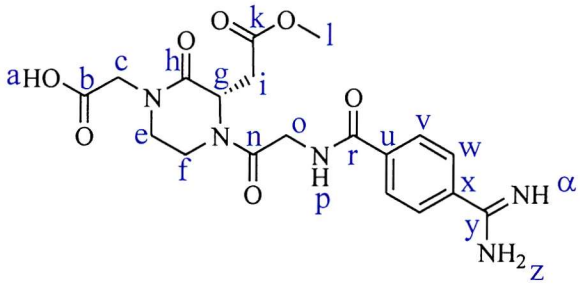
({[N-(4'-Amidinobenzoyl)Tyrosyl]-4-piperidinyl}oxy)acetic acid (Ro44-9883) (26)



Resin (**66**) (0.070 mmol, 125 mg) was washed with freshly distilled dry THF (3x5 mL). Pentafluorophenol (0.70 mmol, 130 mg) was dissolved in dry DMF (0.5 mL)

together with pyridine (0.70 mmol, 60 μL) and DIC (0.70 mmol, 120 μL). The resulting solution was added to the resin and this was then left shaking for 30 min. The resin was then filtered and washed with DMF (1 mL). Compound (**53**) (0.21 mmol, 45 mg), HATU (0.21 mmol, 29 mg) and N,N-diisopropylethylamine (0.63 mmol, 90 μL) were dissolved in DMF (0.5 mL) and added to the resin. This was shaken overnight, then filtered and washed with DMF (5x10 mL) and DCM (5x10 mL). The resin was then treated with TFA/DCM 95:5 for three hours and the filtrate, after removal of the solvents, was purified by semi-preparative HPLC, yielding 8 mg (23%) of (**26**) as a white solid. Experimental data are in accordance with the literature.[86]
Due to the presence of two different conformers the ^{1}H-NMR was recorded at 353K.
δ_H (400 MHz, DMSO d_6, 353 K): 8.73 (1H, d, **u**, *J* 8); 8.11 (2H, d, **x**, *J* 8); 7.99 (2H, d, **y**, *J* 8); 7.17 (2H, d, **n**, *J* 8); 6.75 (2H, d, **o**, *J* 8); 5.20 (1H, dt, **i**, *J* 8, 7); 4.10 (2H, s, **c**); 3.85-3.75 (2H, m, $\mathbf{g_1}$); 3.70-3.60 (2H, m, $\mathbf{g_2}$); 3.09 (1H, dd, $\mathbf{k_1}$, *J* 14, 7); 3.01 (1H, dd, $\mathbf{k_2}$, *J* 14, 8); 2.67 (1H, broad s, **e**); 1.90-1.70 (2H, m, $\mathbf{f_1}$); 1.50-1.30 (2H, m, $\mathbf{f_2}$).
δ_C (100 MHz, DMSO d_6): 172.8 (**b**); 169.6-169.4 (conformers, **h**); 165.8 (**v**); 164.9 (α); 156.3 (**p**); 138.6 (**z**); 131.3 (**w**); 130.7, 128.6, 128.3 (**l**); 128.2, 115.4, 74.7-74.2 (conformers, **e**); 65.5 (**c**); 51.5 (**i**); 43.0-42.7 (conformers, **g**); 36.9 (**k**); 31.6-31.3 (conformers, **f**).
HPLC (method 2, 254 nm): 8.4 min. (29%)
m/z (ES+): 469.3 (100%, $[M+H]^+$).
IR (ν): 3500-2800 (broad), 1660 (broad), 1434, 1182, 1129.
HR-MS (FAB): 469.2094 (calc. 469.2087, 1.5 ppm).

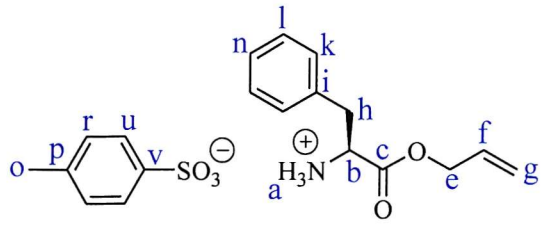
4-[(4'-Amidinobenzoyl)Glycyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid (TAK-029) (27)



The same procedure used for (**26**) was followed, 5 mg of (**27**) were isolated after purification starting from 120 mg of resin (**67**) (overall yield 16%). Experimental data are in accordance with the literature.[91]
δ_H (400 MHz, CD_3OD): 8.27 (2H, d, **v**, *J* 9); 8.08 (2H, d, **w**, *J* 9); 5.32 (1H, t, **g**, *J* 6); 4.59 (1H, d, $\mathbf{c_1}$, *J* 17); 4.49 (1H, d, $\mathbf{c_2}$, *J* 17); 4.46 (1H, d, $\mathbf{o_1}$, *J* 18); 4.30 (1H, d, $\mathbf{o_2}$, *J* 18); 4.35-4.22 (1H, m, $\mathbf{f_1}$); 4.10-3.92 (2H, m, $\mathbf{f_2}$ and $\mathbf{e_1}$); 3.85 (3H, s, **l**); 3.64 (1H, broad d, $\mathbf{e_2}$, *J* 12); 3.13 (2H, broad d, **i**, *J* 6).
δ_C (100 MHz, CD_3OD): 173.1 (**b**); 170.2 (**k**); 169.7 (**h**); 169.5 (**n**); 169.1 (**r**); 168.5 (**y**); 140.1 (**x**); 132.9 (**u**); 2x129.7 (**v** and **w**); 56.7 (**g**); 54.8 (**l**); 52.8 (**c**); 50.2 (**o**); 42.9 (**f**); 41.4 (**e**); 37.3 (**i**).
HPLC (method 2, 254 nm): 6.7 min. (29%)
m/z (ES+): 434.2 (100%, $[M+H]^+$).
IR (ν): 3500-3100 (broad), 2948, 2829, 1739, 1646, 1449.
m/z (ES+): 434.2 (100%, $[M+H]^+$).

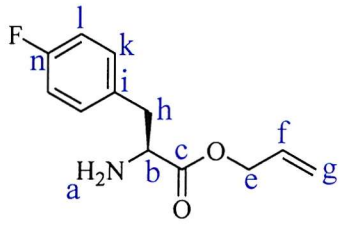
HR-MS (FAB): 434.1681 (calc. 434.1676, -1.2 ppm).

Phenylalanine allyl ester tosyl salt (68)



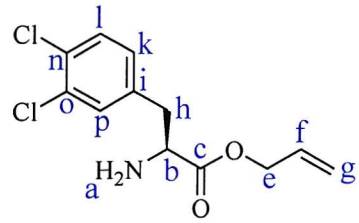
The same procedure used for (**52**) was followed. 4.1 g of product were obtained starting from 2.0 g of Phenylalanine (yield 86%). Experimental data are in accordance with the literature.[145]
δ_H (300 MHz, DMSO d_6): 8.50 (3H, s, **a**); 7.54 (2H, d, **u**, *J* 8); 7.42-7.32 (3H, m, **l** & **n**); 7.27 (2H, d, **k**, *J* 8); 7.17 (2H, d, **r**, *J* 8); 5.83 (1H, ddt, **f**, *J* 17, 11, 6); 5.29 (1H, dd, $\mathbf{g}_{tr}$, *J* 17, 1); 5.25 (1H, dd, $\mathbf{g}_{cis}$, *J* 10, 1); 4.63 (2H, d, **e**, *J* 6); 4.39 (1H, t, **b**, *J* 7); 3.19 (1H, dd, $\mathbf{h}_1$, *J* 14, 7); 3.09 (1H, dd, $\mathbf{h}_2$, *J* 14, 7); 2.33 (3H, s, **o**).
δ_C (75 MHz, DMSO): 169.1 (**c**); 145.8 (**p**); 138.2 (**v**); 134.9 (**i**); 131.8 (**f**); 129.8, 129.0, 128.5, 125.9 (**l**, **k**, **r** & **u**); 127.7 (**n**); 119.1 (**g**); 66.3 (**e**); 53.6 (**b**); 36.4 (**h**); 21.2 (**o**).
m/z (ES+): 206.1 (50%, $[M\text{-TosOH+H}]^+$); 247.2 (100%, $[M\text{-TosOH+H+acetonitrile}]^+$).
Melting point: 144-147°C.

4-(Fluoro)phenylalanine allyl ester (69)



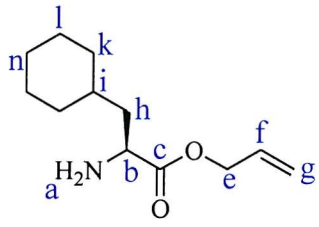
The same procedure used for (**51**) was followed. The final product was isolated as yellow oil (0.44 g, 85%).
δ_H (300 MHz, $CDCl_3$): 7.16 (2H, dd, **k**, *J* 9, 5); 6.98 (2H, t, **l**, *J* 9); 5.88 (1H, ddt, **f**, *J* 17, 10, 6); 5.29 (1H, dd, $\mathbf{g}_{tr}$, *J* 17, 1); 5.24 (1H, dd, $\mathbf{g}_{cis}$, *J* 10, 1); 4.59 (2H, d, **e**, *J* 6); 3.72 (1H, dd, **b**, *J* 7, 6); 3.05 (1H, dd, $\mathbf{h}_1$, *J* 14, 6); 2.87 (1H, dd, $\mathbf{h}_2$, *J* 14, 7); 1.66 (2H, broad s, **a**).
δ_C (75 MHz, $CDCl_3$): 174.9 (**c**); 161.8 (**n**, d, *J* 170); 133.2 (**i**); 132.1 (**f**); 125.6 (d, **k**, *J* 8); 119.2 (**g**); 110.2 (d, **l**, *J* 21); 66.0 (**e**); 56.2 (**b**); 40.5 (**h**).
m/z (ES+): 224.2 (30%, $[M+H]^+$); 265.2 (100%, $[M+H+\text{acetonitrile}]^+$).

3,4-(Dichloro)phenylalanine allyl ester (70)



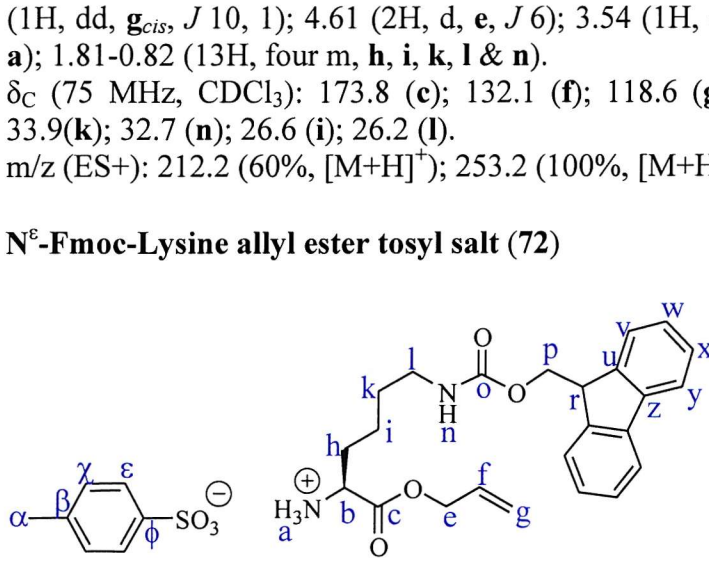
The same procedure used for (**51**) was followed. The final product was isolated as yellow oil (0.55 g, 88%).
δ_H (300 MHz, $CDCl_3$): 7.37 (1H, d, **l**, *J* 8); 7.32 (1H, d, **p**, *J* 2); 7.06 (1H, dd, **k**, *J* 8, 2); 5.88 (1H, ddt, **f**, *J* 17, 10, 6); 5.32 (1H, dd, $\mathbf{g}_{tr}$, *J* 17, 1); 5.27 (1H, dd, $\mathbf{g}_{cis}$, *J* 10, 1); 4.61 (2H, d, **e**, *J* 6); 3.73 (1H, dd, **b**, *J* 8, 6); 3.04 (1H, dd, $\mathbf{h}_1$, *J* 14, 6); 2.85 (1H, dd, $\mathbf{h}_2$, *J* 14, 8); 1.67 (2H, broad s, **a**).
δ_C (75 MHz, $CDCl_3$): 174.4 (**c**); 137.7 (**i**); 132.6 (**o**); 131.7 (**f**); 131.4 (**l**); 131.1 (**n**); 130.6 (**p**); 128.9 (**k**); 119.3 (**g**); 66.0 (**e**); 55.7 (**b**); 40.1 (**h**).
m/z (ES+): 274.2 (20%, $[M+H]^+$); 315.2 (100%, $[M+H+acetonitrile]^+$).

Cyclohexylalanine allyl ester (71)



The same procedure used for (**51**) was followed. The final product was isolated as yellow oil (0.40 g, 82%).
δ_H (300 MHz, $CDCl_3$): 5.92 (1H, ddt, **f**, *J* 17, 10, 6); 5.32 (1H, dd, $\mathbf{g}_{tr}$, *J* 17, 1); 5.25 (1H, dd, $\mathbf{g}_{cis}$, *J* 10, 1); 4.61 (2H, d, **e**, *J* 6); 3.54 (1H, dd, **b**, *J* 9, 5); 1.68 (2H, broad s, **a**); 1.81-0.82 (13H, four m, **h**, **i**, **k**, **l** & **n**).
δ_C (75 MHz, $CDCl_3$): 173.8 (**c**); 132.1 (**f**); 118.6 (**g**); 65.6 (**e**); 52.3 (**b**); 42.7 (**h**); 33.9(**k**); 32.7 (**n**); 26.6 (**i**); 26.2 (**l**).
m/z (ES+): 212.2 (60%, $[M+H]^+$); 253.2 (100%, $[M+H+acetonitrile]^+$).

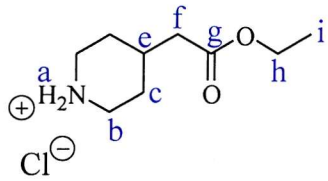
N^{ε}-Fmoc-Lysine allyl ester tosyl salt (72)



The same procedure used for (**52**) was followed. The final product was isolated as white solid (2.5 g, 98%).

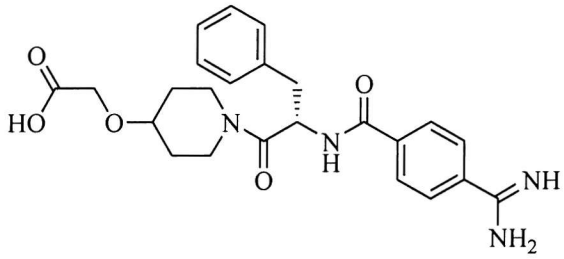
δ_H (300 MHz, DMSO d_6): 8.47 (2.1H, broad s, **a**); 8.32 (1H, broad s, **n**); 7.89 (2H, d, **y**, *J* 7); 7.68 (2H, d, **v**, *J* 7); 7.50 (2H, d, ε, *J* 8); 7.42 (2H, t, **x**, *J* 7); 7.33 (2H, t, **w**, *J* 7); 7.12 (2H, d, χ, *J* 8); 5.92 (1H, ddt, **f**, *J* 17, 11, 6); 5.37 (1H, dd, $\mathbf{g}_{tr}$, *J* 17, 1); 5.26 (1H, dd, $\mathbf{g}_{cis}$, *J* 11, 1); 4.68 (2H, d, **e**, *J* 6); 4.30 (2H, d, **p**, *J* 7); 4.21 (1H, t, **r**, *J* 7); 4.05 (1H, m, **b**); 2.97 (2H, m, **h**); 2.29 (3H, s, α); 1.86-1.65 (2H, m, **l**); 1.40 (4H, broad s, **i** & **k**).
δ_C (75 MHz, DMSO d_6): 171.2 (**c**); 156.2 (**o**); 145.6 (**u**) 144.0 (β); 140.8 (**z**); 137.8 (ϕ); 131.8 (**f**); 128.2, 127.7, 127.1, 125.6, 125.2, 120.2 (**v**, **w**, **x**, **y**, χ & ε); 118.9 (**g**); 66.0 (**p**); 65.3 (**e**); 52.0 (**r**); 46.9 (**b**); 40.4 (**l**), 29.8 (**h**), 28.9 (**k**), 21.7 (**i**); 20.9 (α).
m/z (ES+): 409.0 (100%, $[M\text{-TosOH+H}]^+$).

Ethyl 4-piperidylacetate hydrochloride (**73**)



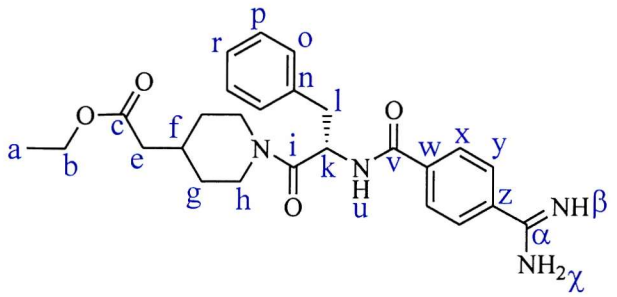
Ethyl 4-pyridylacetate (6.1 mmol, 1.0 g) was dissolved in 4M HCl in dioxane (3 mL, 12 mmol) and the solvent evaporated under reduced pressure. The resulting hydrochloride (**74**) was dissolved in EtOH (20 mL), treated with 10% Pd/C (100 mg) and hydrogenated at 50°C under 25 bars of H_2 pressure for three days. The solution was then filtered and the solvent removed *in vacuo*, yielding quantitatively the product as a yellow solid. Experimental data are in accordance with the literature.[146]
δ_H (400 MHz, CD_3OD): 9.28 (2H, broad s, **a**); 4.19 (2H, quart, **h**, *J* 7); 3.31 (2H, d, $\mathbf{b}_1$, *J* 12); 2.95 (2H, t, $\mathbf{b}_2$, *J* 12); 2.37 (2H, d, **f**, *J* 7); 2.09 (1H, broad s, **e**); 1.92 (2H, d, $\mathbf{c}_1$, *J* 13); 1.61 (2H, m, $\mathbf{c}_2$); 1.31 (3H, t, **i**, *J* 7).
δ_C (100 MHz, CD_3OD): 171.6 (**g**); 60.0 (**h**); 42.8 (**b**); 39.9 (**f**); 30.3 (**e**); 27.9 (**c**); 14.3 (**i**).
m/z (ES+): 172.1 (100%, $[M\text{-HCl+H}]^+$).

({[N-(4'-Amidinobenzoyl)Phenylalanyl]-4-piperidinyl}oxy)acetic acid (**77**(**68**,**53**))



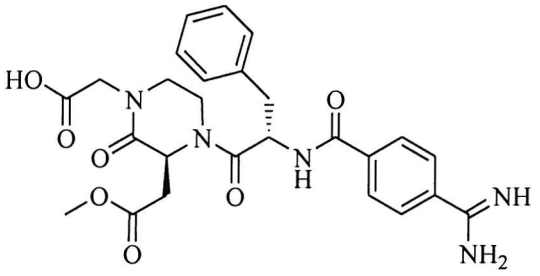
HPLC (method 2, 254 nm): 9.4 min. (56%)
m/z (ES+): 453.2, $[M+H]^+$

Ethyl {[N-(4'-amidinobenzoyl)Phenylalanyl]-4-piperidinyl}acetate (77(68,73))



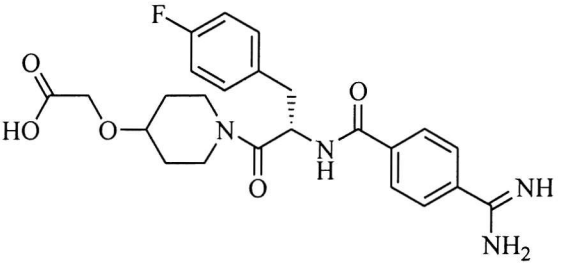
δ_H (400 MHz, CD_3OD): 8.17 (2H, d, **x**, *J* 8); 8.05 (2H, d, **y**, *J* 8); 7.46 (5H, broad s, **o**, **p** and **r**); 5.52 (1H, t, **k**, *J* 8); 4.30 (2H, q, **b**, *J* 7); 3.40-3.22 (2H, m, $\mathbf{l_1}$ and $\mathbf{l_2}$); 3.05-2.10 (8H, 3 m, **g** and **h**); 1.87 (2H, d, **e**, *J* 13); 1.85-1.70 (1H, m, **f**); 1.42 (3H, t, **a**, *J* 7).
HPLC (method 2, 254 nm): 11.7 min. (52%)
m/z (ES+): 465.4, $[M+H]^+$

4-[N-(4'-Amidinobenzoyl)Phenylalanyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid (77(68,54))



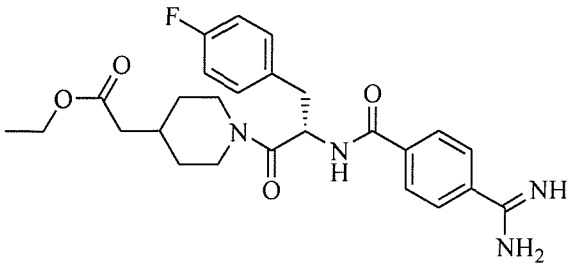
HPLC (method 2, 254 nm): 9.0 min. (31%)
m/z (ES+): 524.3, $[M+H]^+$

({[N-(4'-Amidinobenzoyl)-4-fluorophenylalanyl]-4-piperidinyl}oxy) acetic acid (77(69,53))



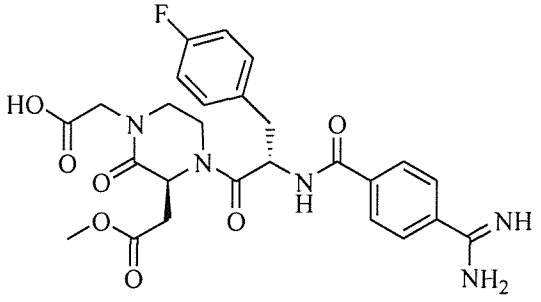
HPLC (method 2, 254 nm): 9.8 min. (51%)
m/z (ES+): 471.2, $[M+H]^+$

Ethyl {[N-(4'-amidinobenzoyl)-4-fluorophenylalanyl]-4-piperidimyl} acetate (77(69,73))



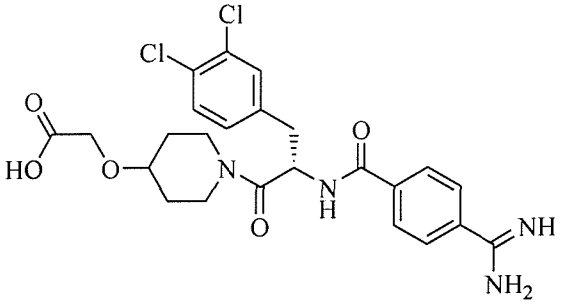
HPLC (method 2, 254 nm): 12.0 min. (47%)
m/z (ES+): 483.4, $[M+H]^+$

4-[N-(4'-Amidinobenzoyl)-4-fluorophenylalanyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid (77(69,54))



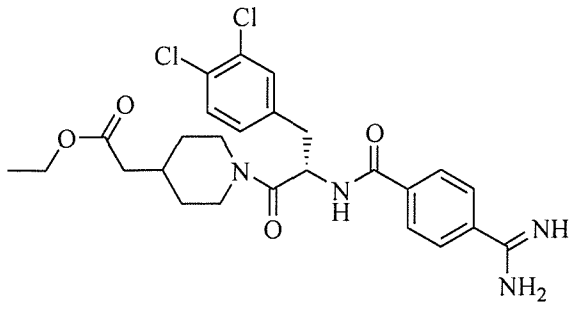
HPLC (method 2, 254 nm): 9.4 min. (26%)
m/z (ES+): 542.2, $[M+H]^+$

({[N-(4'-Amidinobenzoyl)-3,4-(dichloro)phenylalanyl]-4-piperidinyl}oxy) acetic acid (77(70,53))



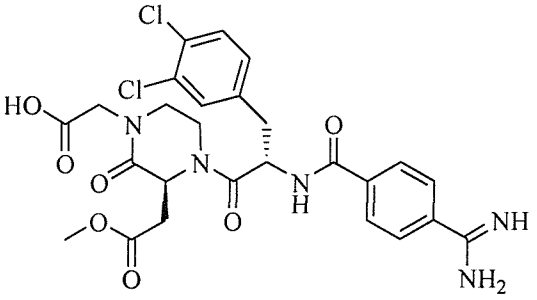
HPLC (method 2, 254 nm): 11.0 min. (45%)
m/z (ES+): 521.2, $[M+H]^+$

Ethyl {[N-(4'-amidinobenzoyl)-3,4-(dichloro)phenylalanyl]-4-piperidinyl} acetate (77(70,73))



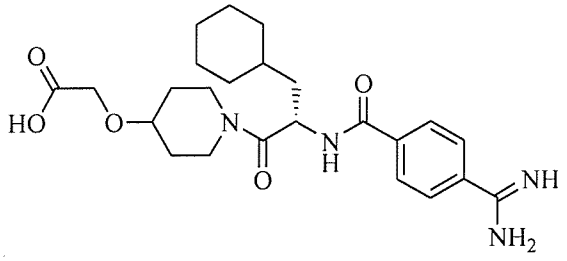
HPLC (method 2, 254 nm): 13.0 min. (42%)
m/z (ES+): 533.3, $[M+H]^+$

4-[N-(4'-Amidinobenzoyl)-3,4-(dichloro)phenylalanyl]-3-[(methoxycarbonyl) methyl] -2-oxopiperazine-1-acetic acid (77(70,54))



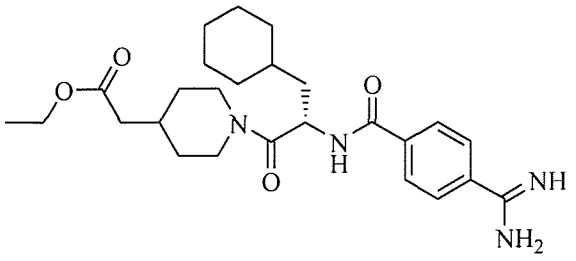
HPLC (method 2, 254 nm): 11.0 min. (24%)
m/z (ES+): 592.6, $[M+H]^+$

{[(N-(4'-Amidinobenzoyl)cyclohexylalanyl)-4-piperidinyl]oxy} acetic acid (77(71,53))



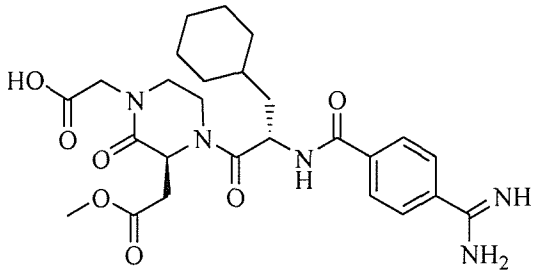
HPLC (method 2, 254 nm): 10.6 min. (49%)
m/z (ES+): 459.4, $[M+H]^+$

Ethyl {[N-(4'-amidinobenzoyl)cyclohexylalanyl]-4-piperidinyl} acetate (77(71,73))



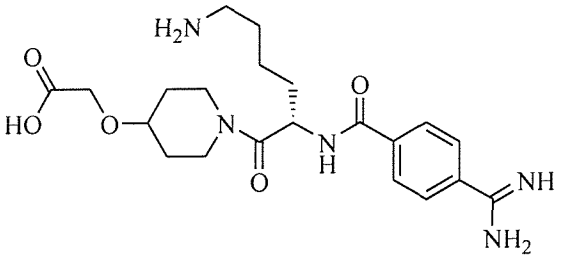
HPLC (method 2, 254 nm): 12.7 min. (43%)
m/z (ES+): 471.4, $[M+H]^+$

4-[N-(4'-Amidinobenzoyl)cyclohexylalanyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid (77(71,54))



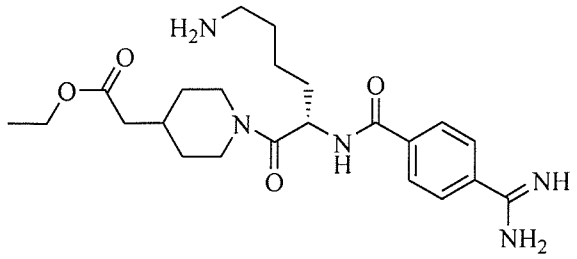
HPLC (method 2, 254 nm): 10.7 min. (22%)
m/z (ES+): N.D.

({[N^{α}-(4'-Amidinobenzoyl)lysyl]-4-piperidinyl}oxy)acetic acid (77(72,53))



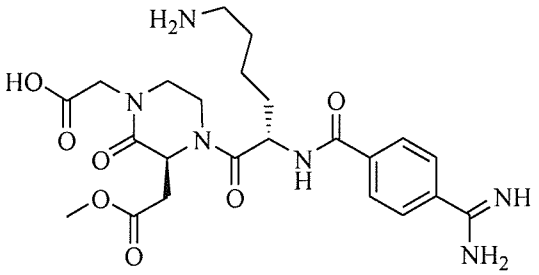
HPLC (method 2, 254 nm): 6.6 min. (34%)
m/z (ES+): 434.3, $[M+H]^+$

Ethyl {[N^{α}-(4'-amidinobenzoyl)lysyl]-4-piperidinyl}acetate (77(72,73))



HPLC (method 2, 254 nm): 13.9 min. (39%)
m/z (ES+): 446.3, $[M+H]^+$

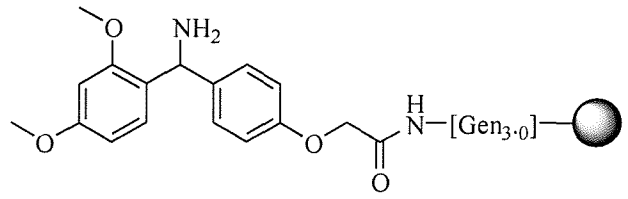
4-[N^{α}-(4'-Amidinobenzoyl)lysyl]-3-[(methoxycarbonyl)methyl]-2-oxopiperazine-1-acetic acid (77(72,54))



HPLC (method 2, 254 nm): 5.3 min. (40%)
m/z (ES+): 505.5, $[M+H]^+$

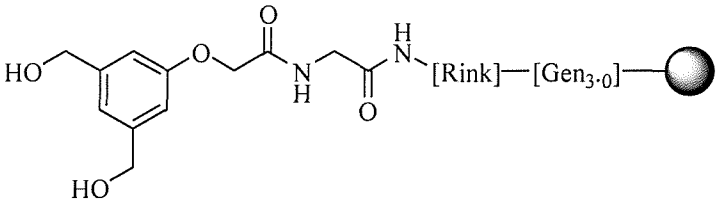
Experimental for Chapter Four

Rink dendrimer resin (78)



Dendrimer resin (**25**) (1.0 mmol/g, 1.0 g) was swollen in DCM. Fmoc-protected Rink linker (*p*-{(R,S)-α-[1-(9H-fluoren-9-yl)methoxyformamido]-2,4-dimethoxybenzyl} phenoxyacetic acid) (2.0 mmol, 1.1 g) was dissolved in DMF (10 mL) and HOBt (2.0 mmol, 300 mg) and DIC (2.0 mmol, 0.32 mL) were added. After 5 min the resulting solution was added to the resin and left shaking for 5 h. The resin was then washed with MeOH (3x10 mL), DMF (3x10 mL) and DCM (3x10 mL). A qualitative ninhydrin test resulted negative. The Fmoc protecting group was then removed treating the resin with 20% piperidine in DMF for 1 h. The Fmoc release was determined by UV for a small sample of resin, giving a loading of 0.51 mmol/g (78% of the expected value).

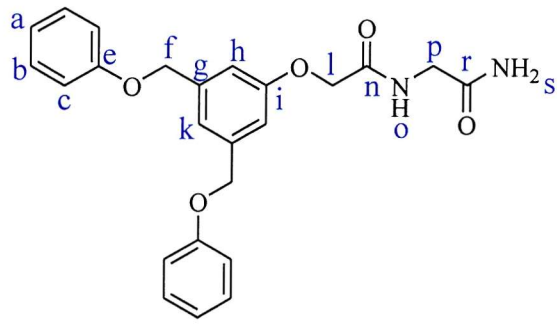
N-[3',5'-*bis*(hydroxymethyl)phenoxyacetyl]glycyl Rink dendrimer resin (79)



Rink dendrimer resin (**78**) (1.0 mmol $-NH_2$) was swollen in DCM. Fmoc-Glycine (2.0 mmol, 600 mg) was dissolved in DMF (10 mL) and HOBt (2.0 mmol, 300 mg) and DIC (2.0 mmol, 0.32 mL) were added. After 5 min the resulting solution was added to the resin and left shaking for 2 h. The coupling was repeated twice. The resin was then washed with MeOH (3x10 mL), DMF (3x10 mL) and DCM (5x10 mL). A qualitative ninhydrin test was negative. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF. A portion of the resulting resin (0.74 mmol/g, 250 mg) was swollen in DCM, 3,5-*bis* (acetoxymethyl)phenoxy acetic acid (**114**) (0.37 mmol, 100 mg) was dissolved in DMF (2 mL) and HOBt (0.37 mmol, 50 mg) and DIC (0.37 mmol, 55 μL) were added. After 5 min the resulting solution was added to the resin and left shaking for 4 h. The resin was then washed with MeOH (3x10 mL), DMF (3x10 mL) and DCM (3x10 mL). The coupling was complete by ninhydrin test. A small sample of resin was treated with TFA/H_2O 95:5 and the resulting solution analysed by HPLC and ES-MS.
HPLC (method 2, 220 nm): 10.6 min (80%).
m/z (ES+): 353.2 (100%, $[M+H]^+$); 370.2 (25%, $[M+NH_4]^+$).
The acetyl protecting groups were then removed treating the resin with 1M NaOH(aq) (2 mmol, 2mL) in dioxane (2 mL) for 2 h. A small sample of resin (**79**) was treated

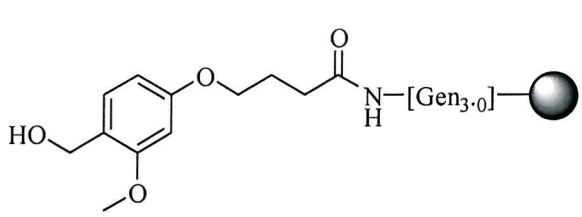
with TFA/H_2O 95:5 for 2 h, the resin was filtered and the solution evaporated and analysed by HPLC and ES-MS.
HPLC (method 2, 220 nm): 6.6 min (78%).
m/z (ES+): 269.3 (60%, $[M+H]^+$); 291.2 (100%, $[M+Na]^+$).

N-[3',5'-*bis*(Phenoxymethyl)phenoxyacetyl]glycyl amide (80)



N-[3',5'-*bis*-(Hydroxymethyl)phenoxyacetyl]glycyl Rink dendrimer resin (**79**) (1.3 mmol –OH/g, 50 mg) was washed three times with dry THF, then suspended in THF (2 mL) and phenol (0.65 mmol, 60 mg) and triphenylphosphine (0.32 mmol, 85 mg) were added. The resin was left shaking for 5 min, then DEAD (0.32 mmol, 50 μL) was added in five portions (10 μL each) at 5 min intervals. The resin was left shaking for 2 h, then filtered and the reaction was repeated a second time. The resin was then treated with TFA/H_2O 95:5 and filtered. The solution was concentrated *in vacuo*, analysed by HPLC and ES-MS and purified by flash chromatography (gradient from AcOEt/MeOH 100:0 to 1:1) yielding 12 mg of pure product as a colourless oil (90% from the loading of dendrimer resin).
δ_H (300 MHz, CD_3OD): 7.24 (4H, t, **b**, *J* 8); 7.15 (1H, s, **k**); 7.04 (2H, s, **h**); 6.95 (4H, d, **c**, *J* 8); 6.91 (2H, t, **a**, *J* 8); 5.05 (4H, s, **f**); 4.57 (2H, s, **l**); 3.92 (2H, s, **p**).
δ_C (75 MHz, CD_3OD): 171.3 (**r**); 171.2 (**n**); 160.0 (**e**); 159.4 (**i**); 141.0 (**g**); 130.4 (**b**); 121.9 (**a**); 120.5 (**k**); 115.9 (**c**); 114.0 (**h**); 70.3 (**f**); 68.1 (**l**); 42.6 (**p**).
HPLC (method 2, 220 nm): 15.8 min. (77%)
m/z (ES+): 421.4 (100%, $[M+H]^+$); 438.4 (50%, $[M+NH_4]^+$); 443.3 (30%, $[M+Na]^+$).
HR-MS (EI): 420.1687 (calc. 420.1685, 0.4 ppm).

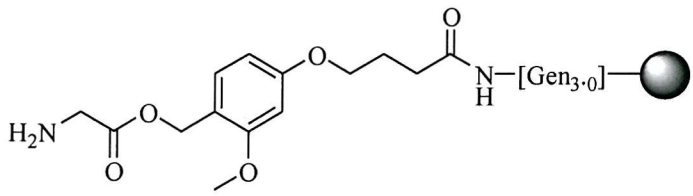
HMBA dendrimer resin (81)



Dendrimer resin (**25**) (1.0 mmol/g, 3.0 g) was swollen in DCM, the solvent drained and a solution of 4-(4'-hydroxymethyl-3'-methoxy)phenoxybutyric acid (HMBA) (9 mmol, 2.16 g) and HOBt (15 mmol, 2.03 g) in DMF/DCM 1:1 (20 mL) was added. DIC (9 mmol, 1.4 mL) was added and the resin left shaking for 2.5 h. The resin was then washed with DMF (3x20 mL) and DCM (3x20 mL) and treated with 1M NaOH(aq)/dioxane 1:1 (20 mL) for 1.5 h. This treatment was necessary to eliminate

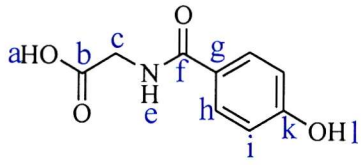
oligomers of the linker formed during the reaction. The resin was then washed with H_2O (3x20 mL), DMF (3x20 mL), MeOH (3x20 mL), DMF (3x20 mL) and DCM (3x20 mL).

Glycyl HMBA dendrimer resin (82)



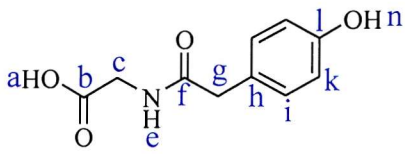
Resin (**81**) (0.82 mmol/g, 3.0 g) was swollen in DCM, the solvent drained and a solution of Fmoc-Glycine (12.3 mmol, 3.6 g), DIC (12.9 mmol, 2.0 mL) and DMAP (0.6 mmol, 75 mg) in DCM/DMF 1:1 (20 mL) was added. The reaction was kept for 3 h at room temperature, then the resin was filtered and washed with DMF (3x20 mL) and DCM (3x20 mL). The loading of the resin was checked by Fmoc determination: the value obtained (0.67 mmol/g) was in accordance with the theoretical loading meaning that the coupling was complete. The resin was treated twice with 20% piperidine in DMF (20 mL) for 20 min, then washed with DMF (3x20 mL) and DCM (3x20 mL).

N-(4'-hydroxybenzoyl)glycine (83'(1))



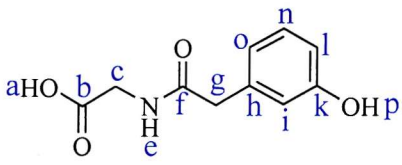
Resin (**82**) (0.78 mmol/g, 0.50 g) was coupled with 4-hydroxybenzoic acid (1.2 mmol, 161 mg) using standard conditions: DIC (1.2 mmol, 0.18 mL) and HOBt (1.9 mmol, 260 mg) in DCM/DMF 2:1 (4.5 mL). The resin was shaken for 2 h and filtered. After washing with DMF (3x5 mL), DCM (3x5 mL) and dry THF (5x5 mL), a small sample of resin (**83**(**1**)) was treated with TFA/H_2O 9:1 for 2 h. The resin was then filtered and the solution evaporated with a flow of N_2. The resulting solid (**83'**(**1**)) was analysed by LC-MS and ^{1}H NMR.
HPLC (5 min gradient, diode array): 0.95 min. (100%)
m/z (ES-): 194.1 (100%, $[M-H]^-$); 389.1 (30%, $[2M-H]^-$).
δ_H (400 MHz, DMSO d_6): 12.55 (1H, broad s, **a**); 10.02 (1H, s, **l**); 8.57 (1H, t, **e**, *J* 6); 7.73 (2H, d, **h**, *J* 9); 6.81 (2H, d, **i**, *J* 9); 3.88 (2H, d, **c**, *J* 6).

N-[(4'-hydroxyphenyl)acetyl]glycine (83'(2))



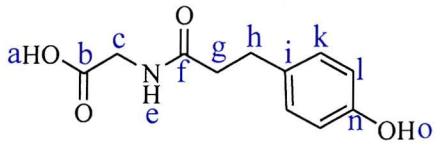
Resin (**82**) (0.78 mmol/g, 0.50 g) was coupled with 4-hydroxyphenylacetic acid (1.2 mmol, 178 mg) using standard conditions: DIC (1.2 mmol, 0.18 mL) and HOBt (1.9 mmol, 260 mg) in DCM/DMF 2:1 (4.5 mL). The resin was left shaking for 1.5 hours and filtered. After washing with DMF (3x5 mL), DCM (3x5 mL) and dry THF (5x5 mL), a small sample of resin (**83**(**2**)) was treated with TFA/H_2O 9:1 for 2 h. The resin was then filtered and the solution evaporated with a flow of N_2. The resulting solid (**83'**(**2**)) was analysed by LC-MS and ^{1}H NMR.
HPLC (5 min gradient, diode array): 0.90 min. (84%)
m/z (ES+): 210.1 (100%, $[M+H]^+$).
m/z (ES-): 208.1 (100%, $[M-H]^-$); 417.2 (20%, $[2M-H]^-$).
δ_H (400 MHz, DMSO d_6): 12.62 (1H, broad s, **a**); 9.20 (1H, broad s, **n**); 8.22 (1H, t, **e**, *J* 6); 7.05 (2H, d, **i**, *J* 8); 6.67 (2H, d, **k**, *J* 8); 3.73 (2H, d, **i**, *J* 6) 3.33 (2H, s, **g**).

N-[(3'-hydroxyphenyl)acetyl]glycine (83'(3))



Resin (**82**) (0.78 mmol/g, 0.50 g) was coupled with 3-hydroxyphenylacetic acid (1.2 mmol, 178 mg) using standard conditions: DIC (1.2 mmol, 0.18 mL) and HOBt (1.9 mmol, 260 mg) in DCM/DMF 2:1 (4.5 mL). The resin was left shaking for 2 h and filtered. After washing with DMF (3x5 mL), DCM (3x5 mL) and dry THF (5x5 mL), a small sample of resin (**83**(**3**)) was treated with TFA/H_2O 9:1 for 2 h. The resin was then filtered and the solution evaporated with a flow of N_2. The resulting solid (**83'**(**3**)) was analysed by LC-MS and ^{1}H NMR.
HPLC, (5 min gradient, diode array): 0.99 min. (89%)
m/z (ES+): 210.1 (100%, $[M+H]^+$).
m/z (ES-): 208.1 (100%, $[M-H]^-$); 417.2 (20%, $[2M-H]^-$).
δ_H (400 MHz, DMSO d_6): 8.30 (1H, t, **e**, *J* 6); 7.17 (1H, t, **n**, *J* 8); 6.68 (1H, s, **i**); 6.67 (1H, d, **o**, *J* 8); 6.60 (1H, d, **l**, *J* 8); 3.74 (2H, d, **c**, *J* 6) 3.37 (2H, s, **g**).

N-[3-(4'-hydroxyphenyl)propionyl]glycine (83'(4))



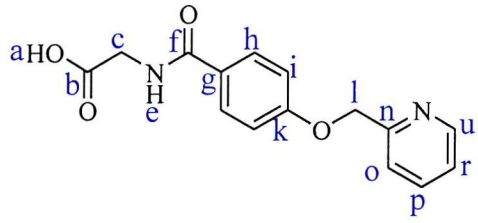
Resin (**82**) (0.78 mmol/g, 0.50 g) was coupled with 4-hydroxyphenylpropionic acid (1.2 mmol, 194 mg) using standard conditions: DIC (1.2 mmol, 0.18 mL) and HOBt (1.9 mmol, 260 mg) in DCM/DMF 2:1 (4.5 mL). The resin was shaken for 1.5 h and filtered. After washing with DMF (3x5 mL), DCM (3x5 mL) and dry THF (5x5 mL), a small sample of resin (**83**(**4**)) was treated with TFA/H_2O 9:1 for 2 h. The resin was then filtered and the solution evaporated with a flow of N_2. The resulting solid (**83'**(**4**)) was analysed by LC-MS and ^{1}H NMR.
HPLC (5 min gradient, diode array): 1.11 min. (88%)
m/z (ES+): 224.2 (100%, $[M+H]^+$).

m/z (ES-): 222.2 (100%, $[M-H]^-$); 445.2 (20%, $[2M-H]^-$).
δ_H (400 MHz, DMSO d_6): 9.10 (1H, broad s, **o**); 8.16 (1H, t, **e**, *J* 6); 6.99 (2H, d, **k**, *J* 8); 6.66 (2H, d, **l**, *J* 8); 3.73 (2H, d, **c**, *J* 6) 2.69 (2H, t, **g**, *J* 8); 2.35 (2H, t, **h**, *J* 8).

Library of arylethers (85) – general procedure

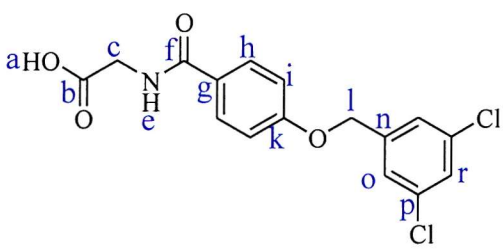
The four different resins (**83**(**1**)), (**83**(**2**)), (**83**(**3**)) and (**83**(**4**)) were divided into 5 parts each (100 mg of resin for each portion) and placed into twenty different QUEST vials. The resin was washed with dry THF (2x10 mL) and kept under vacuum overnight. The vials were then placed in the QUEST, the resin (0.7 mmol/g, 20x100 mg) was washed with dry THF (2x5 mL) and suspended in a THF solution (2 mL) of triphenylphosphine (0.7 mmol, 184 mg) and alcohol [pyridyl-2-carbinol (0.7 mmol, 68 μL), 3,5-dichlorobenzylalcohol (0.7 mmol, 124 mg), 4-(dimethylamino)phenetyl alcohol (0.7 mmol, 116 mg), 4-methoxyphenetyl alcohol (0.7 mmol, 107 mg), butyl alcohol (0.7 mmol, 64 μL)]. The solution was heated at 37°C and DIAD (0.7 mmol, 138 μL) was added in 7 portions over 30 min. The resin was left agitating for 4 h, washed with dry THF and the cycle repeated for another 3 h. The resin was finally washed with DMF (3x5 mL), MeOH (3x5 mL) and DCM (5x5 mL). Each sample of resin (50 mg) was cleaved with TFA/H_2O 9:1 for 3 h to give the compounds reported below.

N-{4'-[(2''-piridyl)methoxy]benzoyl}glycine (85(1a))



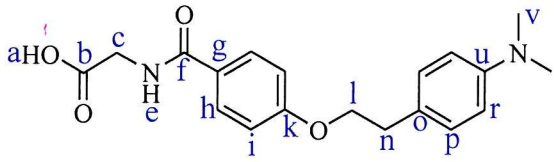
δ_H (400 MHz, DMSO-d_6): 8.75-8.60 (2H, m, **e** & **u**); 8.05 (1H, t, **p**, *J* 8); 7.87 (2H, d, **h**, *J* 9); 7.69 (1H, d, **o**, *J* 8); 7.54 (1H, dd, **r**, *J* 8, 6); 7.13 (2H, d, **i**, *J* 9); 5.34 (2H, s, **l**); 3.90 (2H, d, **c**, *J* 6).
δ_C (100 MHz, DMSO-d_6): 171.8 (**b**); 166.2 (**f**); 160.6 (**k**); 155.2 (**n**); 147.8 (**u**); 139.8 (**p**); 129.5 (**h**); 127.1 (**g**); 124.3 (**r**); 123.2 (**o**); 114.8 (**i**); 69.5 (**l**); 41.5 (**c**).
yield: 86%
HPLC (method 1, 220 nm): 4.7 min (91%).
LC-MS: 2.31 min. (287.2 $[M+H]^+$; 285.1 $[M-H]^-$).

N-{4'-[(3'',5''-dichlorophenyl)methoxy]benzoyl}glycine (85(1b))



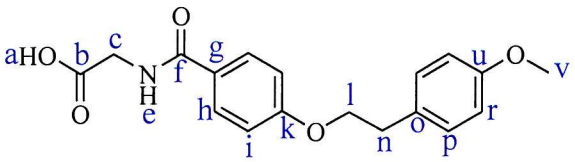
δ_H (400 MHz, DMSO-d_6): 8.71 (1H, t, **e**, *J* 6); 7.86 (2H, d, **h**, *J* 9); 7.59 (1H, s, **r**); 7.54 (1H, s, **o**); 7.11 (2H, d, **i**, *J* 9); 5.21 (2H, s, **l**); 3.90 (2H, d, **c**, *J* 6).
δ_C (100 MHz, DMSO-d_6): 172.0 (**b**); 166.4 (**f**); 160.7 (**k**); 141.6 (**n**); 134.6 (**p**); 129.6 (**h**); 128.0 (**r**); 127.1 (**g**); 114.9 (**i**); 68.2 (**l**); 41.6 (**c**).
yield: 84%.
HPLC (method 1, 220 nm): 8.3 min. (100%).
LC-MS: 3.80 min. (354.1 $[M+H]^+$; 352.1 $[M-H]^-$).

N-(4'-{2''-[4'''-(dimethylamino)phenyl]ethoxy}benzoyl)glycine (85(1c))



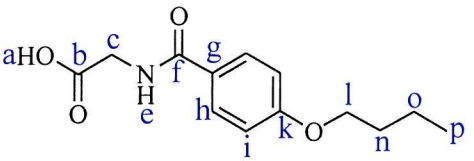
δ_H (400 MHz, DMSO-d_6): 8.69 (1H, t, **e**, *J* 6); 7.83 (2H, d, **h**, *J* 9); 7.34 (2H, d, **p**, *J* 9); 7.17 (2H, broad s, **r**); 7.01 (2H, d, **i**, *J* 9); 4.22 (2H, t, **l**, *J* 7); 3.90 (2H, d, **c**, *J* 6); 3.02 (8H, broad s, **n** & **v**).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 166.1 (**f**); 161.1 (**k**); 130.2 (**p**); 129.3 (**h**); 126.3 (**g**); 116.9 (**r**); 114.2 (**i**); 68.5 (**l**); 43.4 (**v**); 41.3 (**c**); 34.2 (**n**); **o** and **u** are missing (too weak).
yield: 68%.
HPLC (method 1, 220 nm): 5.1 min. (100%).
LC-MS: 2.58 min. (343.3 $[M+H]^+$; 341.3 $[M-H]^-$).

N-{4'-[2''-(4'''-methoxyphenyl)ethoxy]benzoyl}glycine (85(1d))



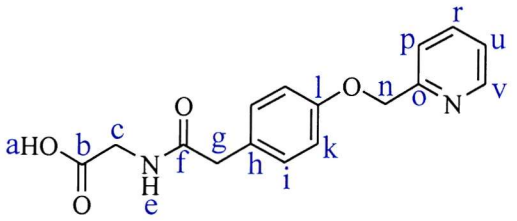
δ_H (400 MHz, DMSO-d_6): 8.68 (1H, t, **e**, *J* 6); 7.83 (2H, d, **h**, *J* 9); 7.24 (2H, d, **p**, *J* 9); 7.02 (2H, d, **i**, *J* 9); 6.87 (2H, d, **r**, *J* 9); 4.20 (2H, t, **l**, *J* 7); 3.89 (2H, d, **c**, *J* 6); 3.72 (3H, s, **c**); 2.98 (2H, t, **n**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 166.1 (**f**); 161.1 (**k**); 158.1 (**u**); 130.2 (**p**); 129.3 (**h**); 126.2 (**g**); 114.2 (**i**); 114.0 (**r**); 68.8 (**l**); 55.2 (**v**); 41.3 (**c**); 34.1 (**n**); **o** is missing (too weak).
yield: 78%.
HPLC (method 1, 220 nm): 6.9 min. (100%).
LC-MS: 3.21 min. (330.2 $[M+H]^+$; 328.2 $[M-H]^-$).

N-(4'-butoxybenzoyl)glycine (85(1e))



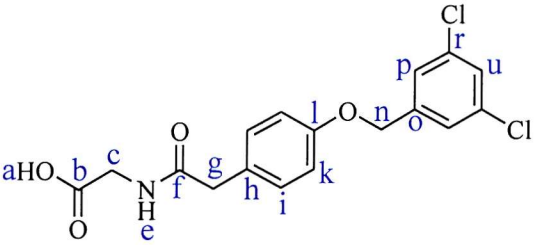
δ_H (400 MHz, DMSO-d_6): 8.67 (1H, t, **e**, *J* 6); 7.83 (2H, d, **h**, *J* 9); 7.00 (2H, d, **i**, *J* 9); 4.03 (2H, t, **l**, *J* 7); 3.89 (2H, d, **c**, *J* 6); 1.71 (2H, quint, **n**, *J* 7); 1.44 (2H, sest, **o**, *J* 7); 0.94 (3H, t, **p**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 166.2 (**f**); 161.3 (**k**); 129.3 (**h**); 126.1 (**g**); 114.2 (**i**); 67.6 (**l**); 41.3 (**c**); 30.8 (**n**); 18.9 (**o**); 13.9 (**p**).
yield: 85%.
HPLC (method 1, 220 nm): 6.8 min. (97%).
LC-MS: 3.14 min. (252.2 $[M+H]^+$; 250.2 $[M-H]^-$).

N-{[4'-(2''-piridylmethoxy)phenyl]acetyl}glycine (85(2a))



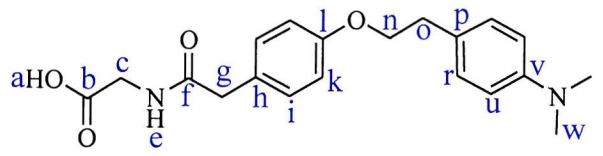
δ_H (400 MHz, DMSO-d_6): 8.69 (1H, d, **v**, *J* 5); 8.32 (1H, t, **e**, *J* 6); 8.08 (1H, t, **r**, *J* 8); 7.70 (1H, d, **p**, *J* 8); 7.56 (1H, dd, **u**, *J* 8, 5); 7.20 (2H, d, **i**, *J* 9); 6.97 (2H, d, **k**, *J* 9); 5.26 (2H, s, **n**); 3.74 (2H, d, **c**, *J* 6); 3.41 (2H, s, **g**).
δ_C (100 MHz, DMSO-d_6): 171.5 (**b**); 171.0 (**f**); 156.7 (**l**); 155.4 (**o**); 147.2 (**v**); 140.2 (**r**); 130.4 (**i**); 129.1 (**h**); 124.2 (**u**); 123.1 (**p**); 114.7 (**k**); 69.0 (**n**); 41.2 (**c**); 40.9 (**g**).
yield: 82%.
HPLC (method 1, 220 nm): 4.0 min. (97%).
LC-MS: 2.25 min. (301.2 $[M+H]^+$; 299.2 $[M-H]^-$).

N-{[4'-(3'',5''-dichlorophenyl)methoxyphenyl]acetyl}glycine (85(2b))



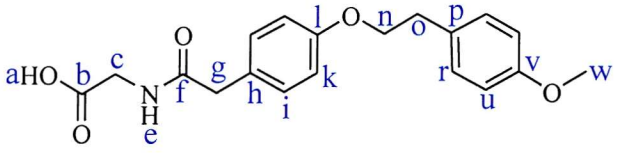
δ_H (400 MHz, DMSO-d_6): 8.31 (1H, t, **e**, *J* 6); 7.57 (1H, s, **u**); 7.50 (2H, s, **p**); 7.20 (2H, d, **i**, *J* 9); 6.94 (2H, d, **k**, *J* 9); 5.11 (2H, s, **n**); 3.74 (2H, d, **c**, *J* 6); 3.40 (2H, s, **g**).
δ_C (100 MHz, DMSO-d_6): 171.5 (**b**); 171.0 (**f**); 157.0 (**l**); 142.3 (**o**); 134.7 (**r**); 130.7 (**i**); 129.3 (**h**); 127.9 (**u**); 126.6 (**p**); 115.1 (**k**); 68.0 (**n**); 41.6 (**c**); 41.3 (**g**).
yield: 82%.
HPLC (method 1, 220 nm): 8.0 min. (95%).
LC-MS: 3.64 min. (368.2 $[M+H]^+$; 366.1 $[M-H]^-$).

N-[(4'-{2''-[4'''-(dimethylamino)phenyl]ethoxy}phenyl)acetyl]glycine (85(2c))



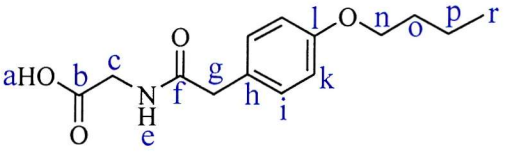
δ_H (400 MHz, DMSO-d_6): 8.29 (1H, t, **e**, *J* 6); 7.33 (2H, d, **r**, *J* 8); 7.16 (4H, d, **u** & **i**, *J* 8); 6.85 (2H, d, **k**, *J* 8); 4.12 (2H, t, **n**, *J* 7); 3.73 (2H, d, **c**, *J* 6); 3.39 (2H, s, **g**); 3.02 (6H, s, **w**); 2.98 (2H, t, **o**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 171.2 (**f**); 157.4 (**l**); 130.4, 130.3 (**i** & **r**); 128.5 (**h**); 117.1 (**u**); 114.5 (**k**); 68.4 (**n**); 43.5 (**w**); 41.4 (**c**); 41.0 (**g**); 34.5 (**o**); **p** and **v** are missing (too weak).
yield: 67%.
HPLC (method 1, 220 nm): 5.0 min. (95%).
LC-MS: 2.50 min. (357.3 $[M+H]^+$; 355.2 $[M-H]^-$).

N-({4'-[2''-(4'''-methoxyphenyl)ethoxy]phenyl}acetyl)glycine (85(2d))



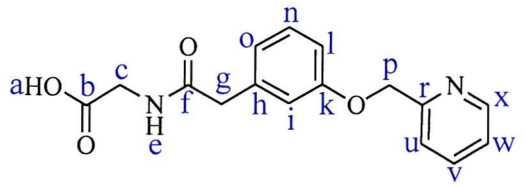
δ_H (400 MHz, DMSO-d_6): 8.28 (1H, t, **e**, *J* 6); 7.23 (2H, d, **r**, *J* 9); 7.16 (2H, d, **i**, *J* 9); 6.86 (2H, d, **k**, *J* 9); 6.84 (2H, d, **u**, *J* 9); 4.10 (2H, t, **n**, *J* 7); 3.73 (2H, d, **c**, *J* 6); 3.72 (3H, s, **w**); 3.38 (2H, s, **g**); 2.94 (2H, t, **o**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 171.2 (**f**); 158.1 (**v**); 157.4 (**l**); 130.5 (**p**); 130.4 (**k**); 130.2 (**r**); 128.4 (**h**); 114.5 (**i**); 114.1 (**u**); 68.7 (**n**); 55.3 (**w**); 41.4 (**c**); 41.0 (**g**); 34.4 (**o**).
yield: 83%.
HPLC (method 1, 220 nm): 6.8 min. (91%).
LC-MS: 3.15 min. (344.3 $[M+H]^+$; 342.3 $[M-H]^-$).

N-[(4'-butoxyphenyl)acetyl]glycine (85(2e))



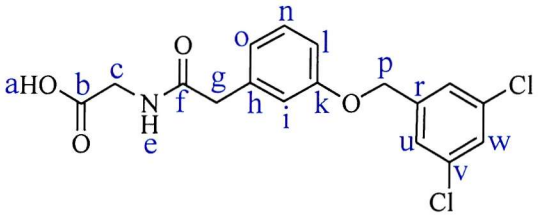
δ_H (400 MHz, DMSO-d_6): 8.28 (1H, t, **e**, *J* 6); 7.16 (2H, d, **i**, *J* 9); 6.84 (2H, d, **k**, *J* 9); 3.92 (2H, t, **n**, *J* 7); 3.73 (2H, d, **c**, *J* 6); 3.38 (2H, s, **g**); 1.67 (2H, quint, **o**, *J* 7); 1.42 (2H, sest, **p**, *J* 7); 0.92 (3H, t, **r**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 171.2 (**f**); 157.7 (**l**); 130.4(**i**); 128.3 (**h**); 114.5 (**k**); 67.4 (**n**); 41.4 (**c**); 41.0 (**g**); 31.1 (**o**); 19.1 (**p**); 14.0 (**r**).
yield: 88%.
HPLC (method 1, 220 nm): 6.6 min. (92%).
LC-MS: 3.05 min. (266.2 $[M+H]^+$; 264.2 $[M-H]^-$).

N-{[3'-(2''-piridylmethoxy)phenyl]acetyl}glycine (85(3a))



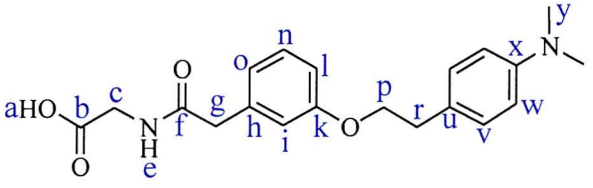
δ_H (400 MHz, DMSO-d_6): 8.70 (1H, d, **x**, *J* 5); 8.38 (1H, t, **e**, *J* 6); 8.11 (1H, t, **v**, *J* 8); 7.74 (1H, d, **u**, *J* 8); 7.59 (1H, dd, **w**, *J* 8, 5); 7.23 (1H, t, **n**, *J* 8); 7.01 (1H, s, **i**); 6.90 (2H, 2 d overlapped, **l** and **o**, *J* 8); 5.27 (2H, s, **p**); 3.76 (2H, d, **c**, *J* 6); 3.46 (2H, s, **g**).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 170.6 (**f**); 158.1 (**k**); 155.3 (**r**); 147.2 (**x**); 140.6 (**v**); 138.2 (**h**); 129.7 (**n**); 124.5 (**w**); 123.4 (**u**); 122.5 (**o**); 116.1 (**i**); 112.9 (**l**); 68.9 (**p**); 42.3 (**c**); 41.1 (**g**).
yield: 84%.
HPLC (method 1, 220 nm): 4.4 min. (85%).
LC-MS: 2.26 min. (301.2 $[M+H]^+$; 299.1 $[M-H]^-$).

N-{[3'-(3'',5''-dichlorophenyl)methoxyphenyl]acetyl}glycine (85(3b))



δ_H (400 MHz, DMSO-d_6): 8.38 (1H, t, **e**, *J* 6); 7.58 (1H, s, **w**); 7.51 (2H, s, **u**); 7.22 (1H, t, **n**, *J* 8); 6.97 (1H, s, **i**); 6.88 (2H, d, **l** and **o**, *J* 8); 5.11 (2H, s, **p**); 3.76 (2H, d, **c**, *J* 6); 3.46 (2H, s, **g**).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 170.6 (**f**); 158.1 (**k**); 141.9 (**r**); 138.2 (**h**); 134.5 (**v**); 129.6 (**n**); 127.7 (**w**); 126.4 (**u**); 122.3 (**o**); 116.0 (**i**); 112.9 (**l**); 67.8 (**p**); 42.3 (**c**); 41.1 (**g**).
yield: 84%.
HPLC (method 1, 220 nm): 7.9 min. (97%).
LC-MS: 3.61 min. (368.2 $[M+H]^+$; 366.1 $[M-H]^-$).

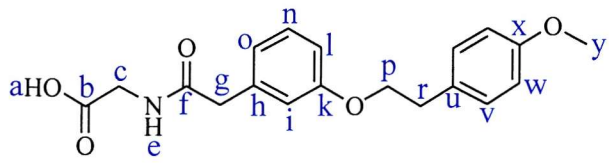
N-[(3'-{2''-[4'''-(dimethylamino)phenyl]ethoxy}phenyl)acetyl]glycine (85(3c))



δ_H (400 MHz, DMSO-d_6): 8.35 (1H, t, **e**, *J* 6); 7.36 (2H, d, **v**, *J* 8); 7.21 (2H, broad s, **w**); 7.18 (1H, t, **n**, *J* 8); 6.86 (1H, s, **i**); 6.82 (1H, d, **o**, *J* 8); 6.77 (1H, d, **l**, *J* 8); 4.13 (2H, t, **p**, *J* 7); 3.74 (2H, d, **c**, *J* 6); 3.43 (2H, s, **g**); 3.04 (6H, s, **y**); 3.00 (2H, t, **r**, *J* 7).

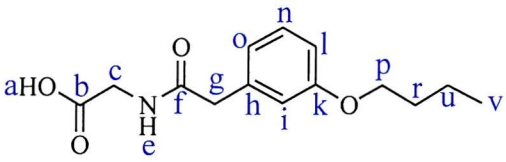
δ_C (100 MHz, DMSO-d_6): 171.6 (**b**); 170.7 (**f**); 158.6 (**k**); 138.0 (**h**); 130.4 (**v**); 129.5 (**n**); 121.7 (**o**); 117.4 (**w**); 115.6 (**i**); 112.7 (**l**); 68.3 (**p**); 43.8 (**y**); 42.3 (**c**); 41.1 (**g**); 34.5 (**r**); **u** & **x** are missing (too weak).
yield: 62%.
HPLC (method 1, 220 nm): 5.2 min. (67%).
LC-MS: 2.51 min. (357.3 $[M+H]^+$; 355.3 $[M-H]^-$).

N-({3'-[2''-(4'''-methoxyphenyl)ethoxy]phenyl}acetyl)glycine (85(3d))



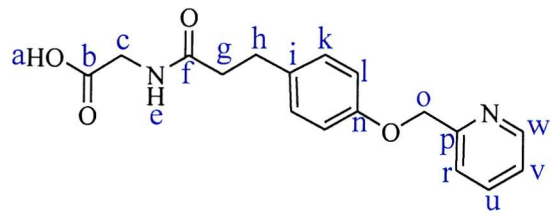
δ_H (400 MHz, DMSO-d_6): 8.34 (1H, t, **e**, *J* 6); 7.23 (2H, d, **v**, *J* 9); 7.17 (1H, t, **n**, *J* 8); 6.87 (3H, d and s overlapped, **i** and **w**, J_d 9); 6.82 (1H, d, **o**, *J* 8); 6.78 (1H, d, **l**, *J* 8); 4.10 (2H, t, **p**, *J* 7); 3.74 (2H, d, **c**, *J* 6); 3.72 (3H, s, **y**); 3.43 (2H, s, **g**); 2.95 (2H, t, **r**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.7 (**b**); 170.7 (**f**); 158.7 (**x**); 158.2 (**k**); 138.0 (**h**); 130.5 (**u**); 130.3 (**v**); 129.5 (**n**); 121.7 (**o**); 115.6 (**i**); 114.1 (**w**); 112.7 (**l**); 68.6 (**p**); 55.3 (**y**); 42.3 (**c**); 41.1 (**g**); 34.4 (**r**); **x** is missing (too weak).
yield: 78%.
HPLC (method 1, 220 nm): 6.7 min. (83%).
LC-MS: 3.14 min. (344.3 $[M+H]^+$; 342.3 $[M-H]^-$).

N-[(3'-butoxyphenyl)acetyl]glycine (85(3e))



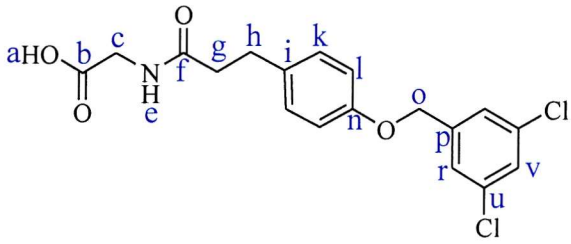
δ_H (400 MHz, DMSO-d_6): 8.34 (1H, t, **e**, *J* 6); 7.17 (1H, t, **n**, *J* 8); 6.86 (1H, s, **i**); 6.81 (1H, d, **o**, *J* 8); 6.77 (1H, d, **l**, *J* 8); 3.93 (2H, t, **p**, *J* 7); 3.75 (2H, d, **c**, *J* 6); 3.43 (2H, s, **g**); 1.68 (2H, quint, **r**, *J* 7); 1.43 (2H, sest, **u**, *J* 7); 0.93 (3H, t, **v**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 171.5 (**b**); 170.6 (**f**); 158.8 (**k**); 137.8 (**h**); 129.3 (**n**); 121.4 (**o**); 115.4 (**i**); 112.5 (**l**); 67.1 (**p**); 42.2 (**c**); 40.9 (**g**); 31.0 (**r**); 19.0 (**u**); 13.9 (**v**).
yield: 75%.
HPLC (method 1, 220 nm): 6.5 min. (84%).
LC-MS: 3.01 min. (266.2 $[M+H]^+$; 264.2 $[M-H]^-$).

N-{3-[4'-(2''-piridylmethoxy)phenyl]propionyl}glycine (85(4a))



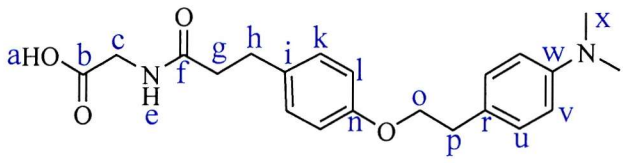
δ_H (400 MHz, DMSO-d_6): 8.68 (1H, d, **w**, *J* 5); 8.18 (1H, t, **e**, *J* 6); 8.06 (1H, t, **u**, *J* 8); 7.69 (1H, d, **r**, *J* 8); 7.54 (1H, dd, **v**, *J* 8, 5); 7.15 (2H, d, **k**, *J* 9); 6.94 (2H, d, **l**, *J* 9); 5.24 (2H, s, **o**); 3.73 (2H, d, **c**, *J* 6); 2.75 (2H, t, **h**, *J* 8); 2.39 (2H, d, **g**, *J* 8).
δ_C (100 MHz, DMSO-d_6): 172.1 (**b**); 171.7 (**f**); 156.4 (**n**); 155.7 (**p**); 147.5 (**w**); 140.1 (**u**); 134.3 (**i**); 129.6 (**k**); 124.3 (**v**); 123.1 (**r**); 115.0 (**l**); 69.2 (**o**); 40.9 (**c**); 37.3 (**g**); 30.4 (**h**).
yield: 79%.
HPLC (method 1, 220 nm): 4.8 min. (95%).
LC-MS: 2.40 min. (315.2 $[M+H]^+$; 313.2 $[M-H]^-$).

N-{3-[4'-(3'',5''-dichlorophenyl)methoxyphenyl]propionyl}glycine (85(4b))



δ_H (400 MHz, DMSO-d_6): 8.18 (1H, t, **e**, *J* 6); 7.57 (1H, s, **v**); 7.50 (2H, s, **r**); 7.14 (2H, d, **k**, *J* 9); 6.91 (2H, d, **l**, *J* 9); 5.10 (2H, s, **o**); 3.73 (2H, d, **c**, *J* 6); 2.75 (2H, t, **h**, *J* 7); 2.39 (2H, t, **g**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 172.3 (**b**); 171.9 (**f**); 156.6 (**n**); 142.2 (**p**); 134.6 (**u**); 134.4 (**i**); 129.8 (**k**); 127.8 (**v**); 126.5 (**r**); 115.1 (**l**); 68.0 (**o**); 41.0 (**c**); 37.4 (**g**); 30.6 (**h**).
yield: 76%.
HPLC (method 1, 220 nm): 8.3 min. (98%).
LC-MS: 3.77 min. (382.2 $[M+H]^+$; 380.2 $[M-H]^-$).

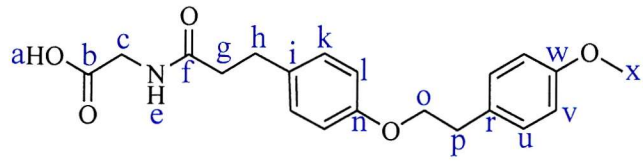
N-[3-(4'-{2''-[4'''-(dimethylamino)phenyl]ethoxy)}phenyl)propionyl]glycine (85(4c))



δ_H (400 MHz, DMSO-d_6): 8.17 (1H, t, **e**, *J* 6); 7.32 (2H, d, **u**, *J* 8); 7.15 (2H, broad s, **v**); 7.10 (2H, d, **k**, *J* 9); 6.82 (2H, d, **l**, *J* 9); 4.10 (2H, t, **o**, *J* 7); 3.73 (2H, d, **c**, *J* 6); 3.01 (6H, s, **x**); 2.97 (2H, t, **p**, *J* 7); 2.73 (2H, t, **h**, *J* 8); 2.38 (2H, t, **g**, *J* 8).

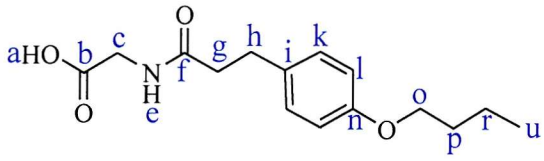
δ_C (100 MHz, DMSO-d_6): 172.0 (**b**); 171.6 (**e**); 156.8 (**n**); 133.4 (**i**); 130.2 (**u**); 129.4 (**k**); 116.6 (**v**); 114.5 (**l**); 68.3 (**o**); 43.2 (**x**); 40.7 (**c**); 37.2 (**g**); 34.4 (**p**); 30.3 (**h**); **r** and **w** are missing (too weak).
yield: 66%.
HPLC (method 1, 220 nm): 5.5 min. (88%).
LC-MS: 2.69 min. (371.3 $[M+H]^+$; 369.3 $[M-H]^-$).

N-(3-{4'-[2''-(4'''-methoxyphenyl)ethoxy]phenyl}propionyl)glycine (85(4d))



δ_H (400 MHz, DMSO-d_6): 8.17 (1H, t, **e**, *J* 6); 7.22 (2H, d, **u**, *J* 9); 7.10 (2H, d, **k**, *J* 9); 6.86 (2H, d, **v**, *J* 9); 6.81 (2H, d, **l**, *J* 9); 4.08 (2H, t, **o**, *J* 7); 3.73 (2H, d, **c**, *J* 6); 3.72 (3H, s, **x**); 2.94 (2H, t, **p**, *J* 7); 2.73 (2H, t, **h**, *J* 8); 2.38 (2H, t, **g**, *J* 8).
δ_C (100 MHz, DMSO-d_6): 172.1 (**b**); 171.7 (**f**); 158.1 (**w**); 156.9 (**n**); 133.5 (**i**); 130.5 (**r**); 130.2 (**u**); 129.4 (**k**); 114.5 (**l**); 114.0 (**v**); 68.6 (**o**); 55.3 (**w**); 40.8 (**c**); 37.3 (**g**); 34.4 (**p**); 30.4 (**h**).
yield: 82%.
HPLC (method 1, 220 nm): 7.0 min. (94%).
LC-MS: 3.29 min. (358.3 $[M+H]^+$; 356.3 $[M-H]^-$).

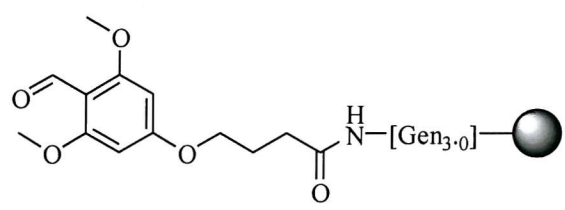
N-[3-(4'-butoxyphenyl)propionyl]glycine (85(4e))



δ_H (400 MHz, DMSO-d_6): 8.18 (1H, t, **e**, *J* 6); 7.10 (2H, d, **k**, *J* 9); 6.81 (2H, d, **l**, *J* 9); 3.91 (2H, t, **o**, *J* 7); 3.73 (2H, d, **c**, *J* 6); 2.73 (2H, t, **h**, *J* 8); 2.38 (2H, t, **g**, *J* 8); 1.67 (2H, quint, **p**, J 7); 1.42 (2H, sest, **r**, *J* 7); 0.92 (3H, t, **u**, *J* 7).
δ_C (100 MHz, DMSO-d_6): 172.2 (**b**); 171.8 (**f**); 157.3 (**n**); 133.4 (**i**); 129.5 (**k**); 114.6 (**l**); 67.3 (**o**); 40.9 (**c**); 37.4 (**g**); 31.1 (**p**); 30.4 (**h**); 19.1 (**r**); 14.0 (**u**).
yield: 78%.
HPLC (method 1, 220 nm): 7.0 min. (91%).
LC-MS: 3.22 min. (280.2 $[M+H]^+$; 278.2 $[M-H]^-$).

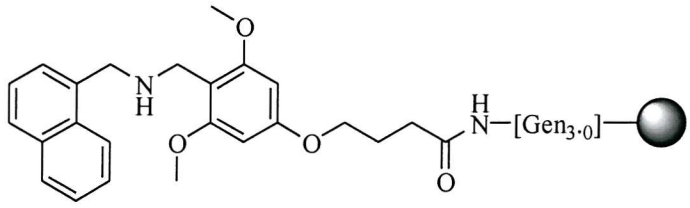
Experimental for Chapter Five

BAL dendrimer resin (86)



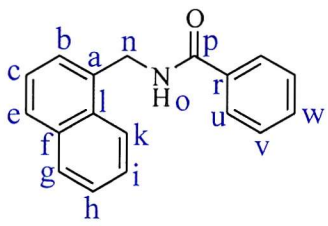
Dendrimer resin (**25**) (1.0 mmol/g, 500 mg) was swollen in DCM, then the liquid was drained. A solution of 4-(4'-formyl-3',5'-dimethoxy)phenoxybutiric acid (BAL linker) (1.5 mmol, 402 mg) and HOBt (1.5 mmol, 203 mg) in DMF (4 mL) was added together with DIC (1.5 mmol, 0.23 mL) and the resin left shaking for 4 h. The solvents were removed by filtration and the resin washed with DMF (3x10 mL) and DCM (3x5 mL). A qualitative ninhydrin test was negative, while a qualitative 2,4-dinitrophenylhydrazine test (sensitive to carbonyl groups) was strongly active.

(1-Naphthylmethyl)amine dendrimer resin (88)



Resin (**86**) (0.8 mmol/g, 100 mg) was dried under vacuum, then placed in a vial together with 1-naphthalenemethylamine (0.4 mmol, 59 μL), acetic acid (0.4 mmol, 23 μL) and N-methylpyrrolidone (0.8 mL). After 1.5 h a solution of tetrabutylammonium borohydride (0.4 mmol, 103 mg) and acetic acid (0.4 mmol, 23 μL) in DMF (0.8 mL) (left standing for 5 minutes) was added and the resin left shaking for 6.5 h. The resin was then filtered and washed with DMF (3x5 mL), 10% ethanolamine in DMF (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL) and DCM (3x5 mL). A qualitative 2,4-dinitrophenylhydrazine test was negative.

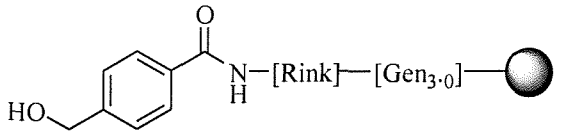
N-(1'-Naphthylmethyl)benzamide (90)



Resin (**88**) (0.71 mmol/g, 100 mg) was swollen in DCM. A solution of benzoic acid (0.24 mmol, 29 mg), PyBOP (0.24 mmol, 125 mg) and DIPEA (0.48 mmol, 84 μL) in DMF (0.8 mL) was then added and the resin left shaking for 5 h. The resin was then filtered and washed with DMF (3x5 mL), MeOH (3x5 mL) and DCM (3x5 mL). Resin

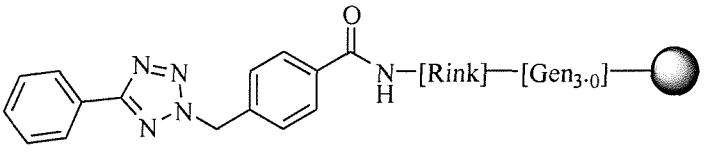
(**89**) was treated with TFA/H_2O 9:1, the solution was filtered and evaporated under a flux of N_2. The crude material (**90**) (12 mg, 63%) resulted pure by HPLC and NMR analysis.
δ_H (400 MHz, $CDCl_3$): 8.10 (1H, d, **k**, *J* 8); 7.90 (1H, d, **g**, *J* 8); 7.85 (1H, d, **e**, *J* 8); 7.76 (2H, d, **u**, *J* 8); 7.61-7.51 (3H, m, **h**, **i** and **w**); 7.49-7.44 (4H, m, **b**, **c** and **v**); 6.34 (1H, broad s, **o**); 5.11 (2H, d, **n**, *J* 5).
δ_C (100 MHz, $CDCl_3$): 167.6 (**p**); 134.7, 134.4, 133.8, (**f**, **l** and **r**); 129.2 (**e**); 129.0 (**g**); 127.4 (**w**); 127.4 (**u**); 127.2 (**v**); 132.0, 129.2, 126.5, 125.8 (**h**, **i**, **b** and **c**); 123.9 (**k**); 40.9 (**n**). **a** is missing (overlapped).
HPLC (method 1, 220 nm): 7.3 min. (100%).
LC-MS: 3.40 min. (262.2 $[M+H]^+$).
HR-MS (El+): 261.1162 (calc. 261.1154, -3.1 ppm).

4-(Hydroxymethyl)benzoyl Rink dendrimer resin (92)



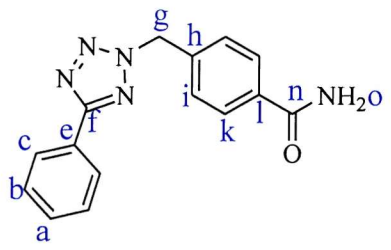
Rink-dendrimer resin (**78**) (0.75 mmol/g, 200 mg) was swollen in DCM, the solvent then drained and 4-formylbenzoic acid (0.45 mmol, 68 mg), HATU (0.45 mmol, 171 mg) and DIPEA (0.90 mmol, 157 μL) were added dissolved in DMF (2 mL). The resin was left shaking for 4 h, then washed with DMF (3x5 mL), DCM (5x5 mL) and dry THF (3x5 mL). Tetrabutylammonium borohydride (1.50 mmol, 386 mg) dissolved in DCM (2 mL) was added to resin (**91**), that was left shaking at room temperature for 4 h, then washed with MeOH (3x5 mL), DMF (3x5 mL), MeOH (3x5 mL), DCM (3x5 mL) and dry THF (3x5 mL).

4-{[5'-(Phenyl)tetrazol-2'-yl]methyl}benzoyl Rink dendrimer resin (93)



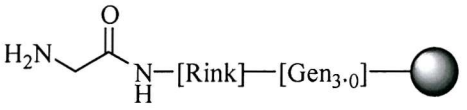
Resin (**92**) (0.67 mmol/g, 100 mg) was suspended in DCM (1 mL) and DIPEA (0.74 mmol, 129 μL) was added. After 5 min, methanesulphonyl chloride (0.67 mmol, 52 μL) in DCM (1 mL) was added and the resin left standing for 45 min. The solution was then drained and the resin washed with dry N-methylpyrrolidinone (3x2 mL). In the meanwhile 5-phenyltetrazole (0.67 mmol, 98 mg) was dissolved in N-methylpyrrolidinone (2 mL) and potassioum *tert*-butoxide (1M in THF, 0.34 mmol, 0.34 mL) was added. The resulting solution was added to the resin and this was left shaking overnight. The solvent was removed by filtration and the resin washed with MeOH (3x5 mL), DMF (3x5 mL), MeOH (3x5 mL) and DCM (3x5 mL).

4-[(5'-phenyltetrazolyl)methyl]benzamide (94)



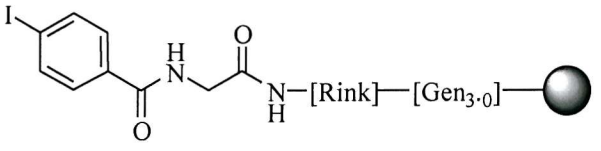
Resin (**93**) (0.62 mmol/g, 50 mg) was treated with TFA/H_2O 9:1 for 3 h, the resin was filtered and the solution evaporated under a flux of N_2. The crude material (**94**) (10 mg) was analysed by HPLC and NMR. The yield, determined by d_5-DMSO quantification, resulted to be 50%. The crude material was then purified by semi-preparative HPLC, yielding 4 mg (46%) of pure product.
δ_H (400 MHz, DMSO-d_6): 8.05 (2H, m, **c**); 7.98 (1H, broad s, $\mathbf{o_1}$); 7.88 (2H, d, **k**, *J* 8); 7.55 (3H, m, **a** and **b**); 7.46 (2H, d, **i**, *J* 8); 7.38 (1H, broad s, $\mathbf{o_2}$); 6.08 (2H, s, **g**).
δ_C (100 MHz, DMSO-d_6): 167.4 (**n**); 164.4 (**f**); 137.0 (**h**); 134.5 (**e**); 130.6 (**a**); 129.3, 128.1, 128.0, 126.3 (**b**, **c**, **i** and **k**); 126.7 (**l**); 55.7 (**g**).
HPLC (method 1, 220 nm): 6.1 min. (80%).
LC-MS: 2.92 min. (279.7 $[M+H]^+$).
HR-MS (EI+): 279.1121 (calc. 279.1120, -0.4 ppm).

Glycyl Rink dendrimer resin (95)



Rink dendrimer resin (**78**) (0.75 mmol/g, 1.35 g) was swollen in DCM; Fmoc-Glycine (2.0 mmol, 600 mg) was dissolved in DMF (10 mL) and HOBt (2.0 mmol, 300 mg) and DIC (2.0 mmol, 0.32 mL) were added. After 5 min, the resulting solution was added to the resin and left shaking for 2 h. The coupling was repeated twice. The resin was then washed with MeOH (3x10 mL), DMF (3x10 mL) and DCM (3x10 mL). A qualitative ninhydrin test was negative. The Fmoc protecting group was removed by treatment with 20% piperidine in DMF (2x20 mL) for 30 min, the resin was then washed with DMF (5x10 mL) and DCM (3x10 mL).

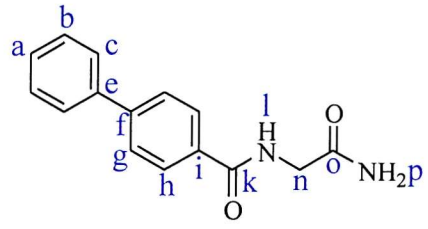
N-(4-Iodobenzoyl)glycyl Rink dendrimer resin (96)



Resin (**96**) (0.74 mmol/g, 100 mg) was swollen in DCM and after 5 min the solvent was drained. 4-Iodobenzoic acid (0.15 mmol, 37 mg) was dissolved in DMF (1 mL) and HOBt (0.15 mmol, 23 mg) and DIC (0.15 mmol, 23 μL) were added. After 5 min the resulting solution was added to the resin and left shaking for 2 h. The coupling was

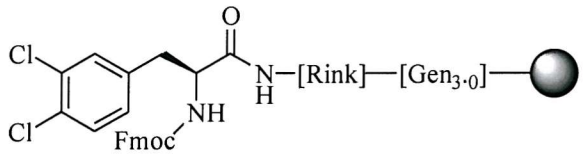
repeated twice. The resin was then filtered and washed with DMF (3x5 mL), MeOH (3x5 mL) and DCM (3x5 mL). A small sample was cleaved with TFA/H_2O 95:5 and analysed by HPLC and ES-MS.
HPLC (method 2, 220 nm): 10.6 min (72%).
m/z (ES+): 305.0 ($[M+H]^+$).

N-(4'-phenylbenzoyl)glycinamide (97)



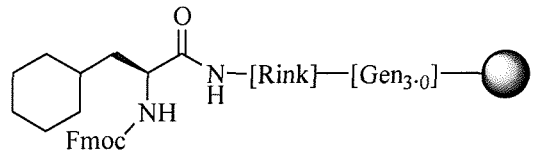
Resin (**96**) (0.62 mmol/g, 200 mg) was suspended in DMF (2 mL) and potassium carbonate (0.3 mmol, 41 mg), $Pd(PPh_3)_4$ (0.015 mmol, 17 mg) and phenyl boronic acid (0.3 mmol, 36 mg) were added. The resulting suspension was heated at 100°C for 24 h. The resin was then filtered and washed with 1% N,N-diisopropylethylamine in DMF (2x10 mL), 1% sodium diethyldithiocarbamate in DMF (2x10 mL), DMF (3x10 mL) and DCM (3x10 mL). The resin was then treated with TFA/H_2O 95:5, filtered and the solution evaporated *in vacuo*. The crude material was analysed by HPLC and ES-MS and purified by flash chromatography (gradient from AcOEt/MeOH/triethilamine 5:0.5:0.2 to 5:0.9:0.2) yielding 25 mg of pure product as a white solid (yield: 79%).
δ_H (400 MHz, DMSO-d_6): 8.84 (1H, t, **l**, *J* 6); 8.08 (2H, d, **h**, *J* 8); 7.88 (2H, d, **g**, *J* 8); 7.83 (2H, d, **c**, *J* 7); 7.59 (2H, t, **b**, *J* 7); (1H, t, **a**, *J* 7); 3.94 (2H, d, **n**, *J* 6).
δ_C (100 MHz, DMSO-d_6): 171.5 (**o**), 166.5 (**k**); 143.3 (**f**); 139.6 (**e**); 133.4 (**i**); 129.5, 128.5, 127.3, 126.9 (**a**, **b**, **c**, **g** and **h**); 42.9 (**n**).
HPLC (method 2, 254 nm): 12.0 min (88%).
m/z (ES+): 255.2 (100%, $[M+H]^+$); 509.3 (30%, $[2M+H]^+$).
HR-MS (FAB): 255.1135 (calc. 255.1134, 0.6 ppm).

N-Fmoc-(3',4'-dichloro)phenylalanyl Rink dendrimer resin (101a)



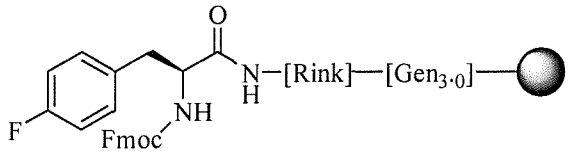
Rink dendrimer resin (**78**) (0.75 mmol/g, 200 mg) was swollen in DCM and after 5 min the solvent was drained. A solution of HATU (0.45 mmol, 170 mg), DIPEA (0.90 mmol, 0.16 mL) and *N*-Fmoc-(3,4-dichloro)Phenylalanine (0.45 mmol, 205 mg) in DMF (2 mL) was added. The resin was left shaking for 3 h. The resin was filtered, washed with DMF (3x10 mL) and DCM (3x10 mL). A qualitative ninhydrin test was negative.

N-Fmoc-(α-cyclohexyl)alanyl Rink dendrimer resin (101b)



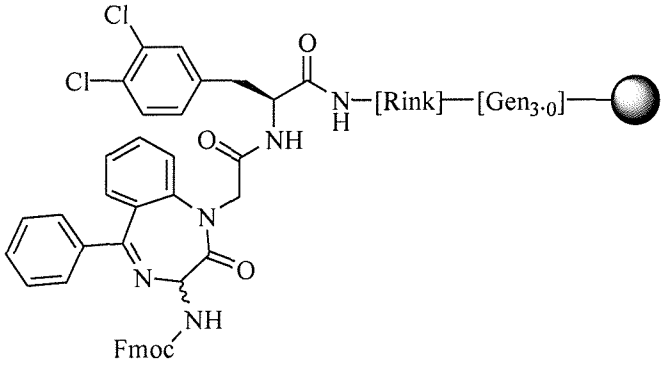
The same procedure used for (**101a**) was followed, using β-cyclohexylalanine (0.45 mmol, 177 mg).

N-Fmoc-4'-fluorophenylalanyl Rink dendrimer resin (101c)



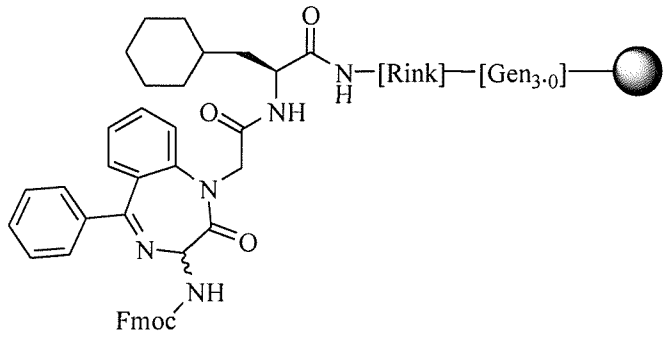
The same procedure used for (**101a**) was followed, using 4-fluorophenylalanine (0.45 mmol, 182 mg).

N-(3'-Fmoc-amino-2'-oxo-5'-phenyl-1',4'-benzodiazepin-1'-yl-acetyl)-3'',4''-(dichloro)phenylalanyl Rink dendrimer resin (102a)



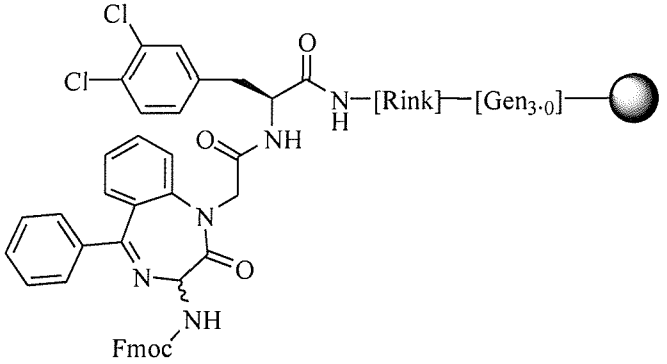
The Fmoc protection of (**101a**) was removed with 20% piperidine in DMF (2x10 mL) for 45 min. Fmoc quantification on a small sample of resin gave a loading of 3.1 nmol/bead, in accordance with the starting loading of dendrimer resin (**25**). The resin was then washed with DMF (5x10 mL) and DCM (3x10 mL). A solution of HATU (0.3 mmol, 114 mg), DIPEA (0.6 mmol, 0.10 mL) and 1-carboxymethyl-3-(fluorenylmethoxycarbonyl) amino-5-phenyl-1,4-benzodiazepin-2-one (0.3 mmol, 159 mg) in DMF (2 mL) was added to the resin. After 3.5 h, the resin was filtered and washed with DMF (3x10 mL), MeOH (3x10 mL) and DCM (3x10 mL). A qualitative ninhydrin test was negative.

N-(3'-Fmoc-amino-2'-oxo-5'-phenyl-1',4'-benzodiazepin-1'-yl-acetyl)-α-cyclohexylalanyl Rink dendrimer resin (102b)



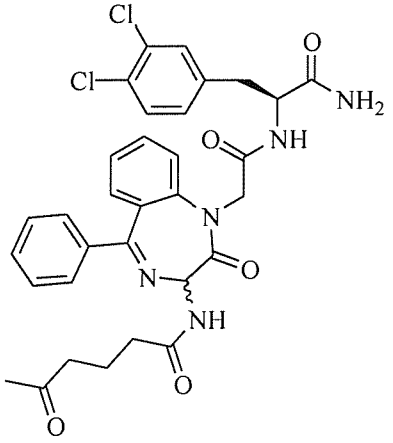
The same procedure used for (**102a**) was followed.

N-(3'-Fmoc-amino-2'-oxo-5'-phenyl-1',4'-benzodiazepin-1'-yl-acetyl)-4''-fluorophenylalanyl Rink dendrimer resin (102c)



The same procedure used for (**102a**) was followed.

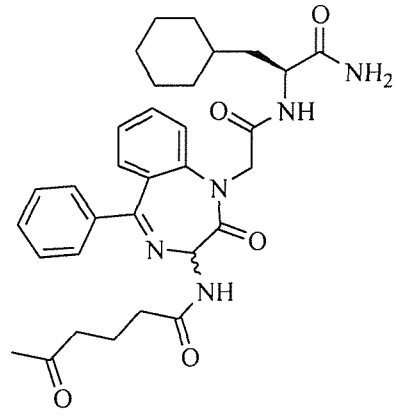
N-[3'-(5''-oxohexanoyl)amino-2'-oxo-5'-phenyl-1',4'-benzodiazepin-1'-yl-acetyl]-3''',4'''-(dichloro)phenylalanyl amide (98)



Resin (**102a**) was treated with 20% piperidine in DMF (2x10 mL) for 45 min. The resin was then washed with DMF (5x10 mL) and DCM (3x10 mL) and coupled with

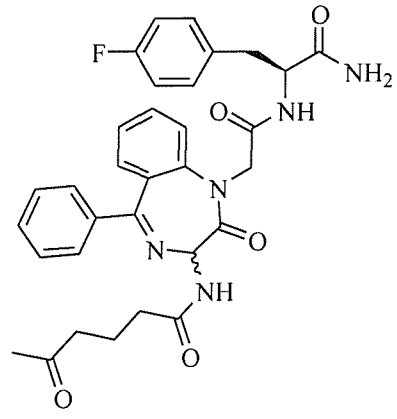
5-oxohexanoic acid (0.45 mmol, 54 μL) using HATU (0.45 mmol, 171 mg) and DIPEA (0.90 mmol, 0.16 mL) in DMF (2 mL) for 4 h. The solution was filtered and the resin was washed with DMF (3x10 mL), MeOH (3x10 mL) and DCM (3x10 mL). Resin (**103a**) was then treated with TFA/H_2O 9:1 for 2 h and the solution analysed by HPLC and MS.
HPLC (5 min gradient, diode array): 2.44 min. (87%)
LC-MS: 3.23 min. (636.3 $[M+H]^+$; 634.3 $[M-H]^-$).

N-[3'-(5''-oxohexanoyl)amino-2'-oxo-5'-phenyl-1',4'-benzodiazepin-1'-yl-acetyl]-α-cyclohexylalanyl amide (99)



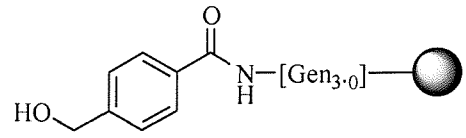
The same procedure used for (**98**) was followed.
HPLC (5 min gradient, diode array): 2.29 min. (97%)
LC-MS: 3.13 min. (574.3 $[M+H]^+$; 572.3 $[M-H]^-$).

N-[3'-(5''-oxohexanoyl)amino-2'-oxo-5'-phenyl-1',4'-benzodiazepin-1'-yl-acetyl]-4'''-fluorophenylalanyl amide (100)



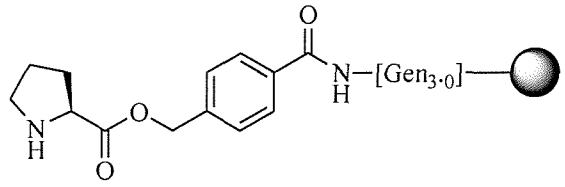
The same procedure used for (**98**) was followed.
HPLC (5 min gradient, diode array): 2.19 min. (94%)
LC-MS: 3.01 min. (586.3 $[M+H]^+$; 584.3 $[M-H]^-$).

4-(Hydroxymethyl)benzoyl dendrimer resin (104)



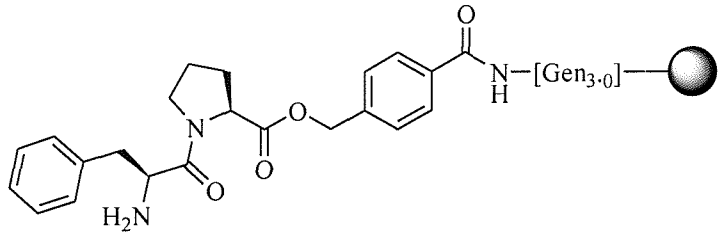
Dendrimer resin (**25**) (1.0 mmol/g, 220mg) was swollen in DCM and the solvent drained. A solution of 4-hydroxymethylbenzoic acid (0.66 mmol, 100 mg) and HOBt (1.10 mmol, 170 mg) in DMF (3 mL) was added together with DIC (0.66 mmol, 100 μL). The resin was left shaking for 2.5 h, then washed with DMF (3x5 mL) and DCM (3x5 mL) and treated with 1M NaOH(aq)/Dioxane 1:1 (4 mL) (to hydrolyse possible oligomers). The resin was then washed with H_2O (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL) and DCM (3x5 mL).

4-(Prolinylmethyl)benzoyl dendrimer resin (105)



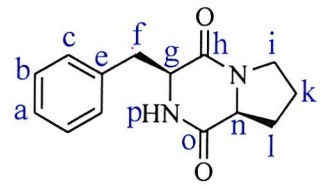
Resin (**104**) was coupled with N-Boc-Proline (0.66 mmol, 140 mg) using DIC (0.66 mmol, 100 μL) and DMAP (2 mg, cat.) in DMF (3 mL) for 3 h. The resin was then washed with DMF (3x5 mL) and DCM (3x5 mL). The Boc protecting group was removed with TFA/DCM 1:1 (4 mL) for 2 h, the solvents were drained and the resin washed with 20% DIPEA in DMF (3x3 mL) and DMF (5x5 mL).

4-[N-(Phenylalanyl)prolinylmethyl]benzoyl dendrimer resin (106)



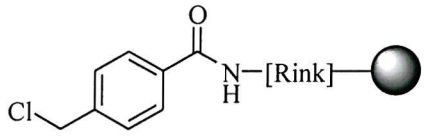
Resin (**105**) was coupled with N-Boc-Phenylalanine (0.66 mmol, 175 mg) using HATU (0.66 mmol, 251 mg) and HOAt (0.22 mmol, 30 mg) in DMF (2 mL) for 4 h. A qualitative ninhydrin test was negative. A small sample of resin was treated with 1 M NaOH(aq)/Dioxane 1:1 (4 mL): the HPLC trace (method 1, 220 nm) showed a single peak with the correct mass of the dipeptide Boc-Phe-Pro-OH: 9.40 min (98%), 363.4 $[M+H]^+$.
The Boc protecting group was removed with TFA/DCM 1:1 (4 mL) for 2 h and the solvents drained. The resin was washed with DCM (3x5 mL).

3-Benzyl-1,4-diazabicyclo[4.3.0]nonane-2,5-dione (107)



Resin (**106**) was left shaking in DCM/Et_3N 1:1 overnight. The resin was then filtered, washed with MeOH (2x1 mL) and DCM (2x1 mL) and the solvents concentrated *in vacuo*. The crude was analysed and purified by semi-preparative HPLC, giving 11 mg (61%) of pure product. Experimental data are in accordance with the literature.[147]
δ_H (400 MHz, $CDCl_3$): 7.36 (2H, t, **b**, *J* 7); 7.32 (1H, t, **a**, *J* 7); 7.23 (2H, d, **c**, *J* 7); 5.68 (1H, s, **p**); 4.28 (1H, dd, **g**, *J* 10, 3); 4.09 (1H, t, **n**, *J* 7); 3.70-3.52 (3H, m, **i** + $\mathbf{f_1}$); 2.79 (1H, dd, $\mathbf{f_2}$, J 10, 14); 2.40-1.84 (4H, 2 m, **l**, **k**).
δ_C (100 MHz, $CDCl_3$): 165.2 (**h**); 165.1 (**o**); 135.8 (**e**); 129.4 (**b**); 129.3 (**c**); 127.7 (**a**); 59.3 (**n**); 56.4 (**g**); 45.6 (**i**); 36.9 (**f**); 28.5 (**l**); 22.7 (**k**).
HPLC (method 1, 220 nm): 7.3 min (94%).
m/z (ES+): 245.2 $[M+H]^+$.
HR-MS (EI+): 244.1213 (calc. 244.1212, -0.6 ppm).

4-Chloromethylbenzoyl Rink resin (108)

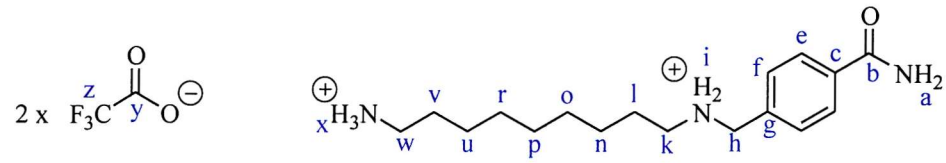


Rink TentaGel resin (0.40 mmol/g, 300 mg) and Rink dendrimer resin (**78**) (0.76 mmol/g, 300 mg) were placed in two different peptide vessels, swollen in DCM and the solvent drained. 4-Chloromethylbenzoic acid (0.36 mmol, 61 mg and 0.68 mmol, 117 mg respectively) was dissolved in DMF (2 mL and 4 mL) together with HOBt (0.36 mmol, 55 mg and 0.68 mmol, 105 mg) and DIC (0.36 mmol, 56 μL and 0.68 mmol, 107 μL). After 5 min solution was added to the resin, that was left shaking for 4 h. The solvents were removed by filtration and the resin washed with DMF (3x10 mL) and DCM (3x5mL). A qualitative ninhydrin test was negative.

Reaction with 1,9-diaminononane

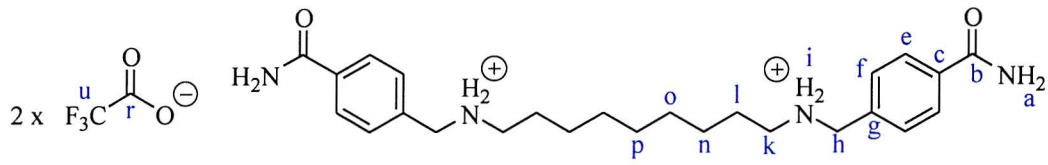
Tentagel and dendrimer resins (**108**) were divided in 3 portions each (100 mg), poured in six different vials and suspended in DMF (the amount varied in order to keep the concentration constant). Different amounts of 1,9-diaminononane were added and the resulting suspensions shaken at room temperature or stirred at 50°C for 2 days. The resin was then filtered and washed with DMF (5x5 mL), MeOH (5x5 mL), water (5x5 mL), DMF (5x5 mL) and DCM (5x5 mL). The resin was treated with TFA/H_2O 95:5 and the solution analysed by HPLC. Three different compounds were isolated in different amounts and the results are reported in Table 5.1.

N^1-(4'-Amidobenzyl)-1,9-diaminononane *bis* TFA salt (109)



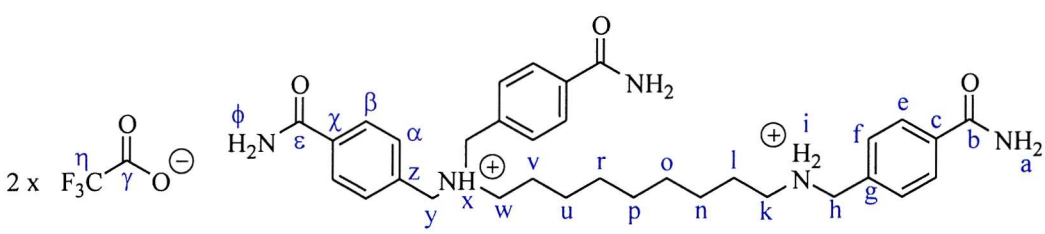
δ_H (400 MHz, DMSO-d_6): 9.05 (2H, broad s, **i**); 8.02 (1H, s, $\mathbf{a_1}$); 7.92 (2H, d, **e**, *J* 9); 7.79 (3H, broad s, **x**); 7.55 (2H, d, **f**, *J* 9); 7.42 (1H, s, $\mathbf{a_2}$); 4.19 (2H, t, **h**, *J* 6); 2.90 (2H, broad s, **k**); 2.76 (2H, m, **w**); 1.60 (2H, m, **l**); 1.50 (2H, m, **v**); 1.26 (10H, broad s, **n**, **o**, **p**, **r** and **u**).
δ_C (100 MHz, DMSO-d_6): 167.0 (**b**); 158.0 (**y**, quart, *J* 33); 134.8, 134.4 (**c** and **g**); 129.4, 127.5 (**e** and **f**); 116.3 (**z**, quart, *J* 295); 49.2 (**h**); 48.3 (**w**); 46.3 (**k**); 28.3, 28.1, 26.7, 25.6, 25.5, 25.0 (**l**, **n**, **o**, **p**, **r**, **u** and **v**).
HPLC (method 1, 220 nm): 5.1 min.
m/z (ES+): 187.7 (100%, $[M+2H+2Acetonitrile-2TFA]^{2+}$); 292.3 (15%, $[M+H-2TFA]^+$).
HR-MS (FAB+): 292.2387 (calc. 292.2389, 0.5 ppm).

N^1,N^9-*bis*(4'-Amidobenzyl)-1,9-diaminononane *bis* TFA salt (110)



δ_H (400 MHz, DMSO-d_6): 8.96 (4H, broad s, **i**); 8.01 (1H, s, $\mathbf{a_1}$); 7.92 (4H, d, **e**, *J* 9); 7.56 (4H, d, **f**, *J* 9); 7.44 (1H, s, $\mathbf{a_2}$); 4.18 (4H, m, **h**); 2.89 (4H, broad s, **k**); 1.60 (4H, m, **l**); 1.25 (10H, broad s, **n**, **o** and **p**).
δ_C (100 MHz, DMSO-d_6): 167.2 (**b**); 158.0 (**r**, quart, *J* 33); 135.0, 134.7 (**c** and **g**); 129.6, 127.7 (**e** and **f**); 116.3 (116.3 (**u**, quart, *J* 295); 49.5 (**h**); 46.6 (**k**); 28.5 (**p**); 28.4, 25.9, 25.3 (**l**, **n**, **o**).
HPLC (method 1, 220 nm): 5.4 min.
m/z (ES+): 233.8 (85%, $[M+2H+Acetonitrile-2TFA]^{2+}$); 254.4 (50%, $[M+2H+2Acetonitrile-2TFA]^{2+}$); 425.4 (100%, $[M+H-2TFA]^+$).
HR-MS (FAB+): 425.2912 (calc. 425.2916, 1.2 ppm).

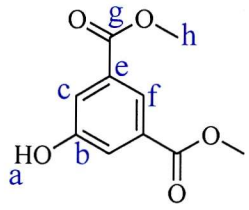
N^1,N^1,N^9-*tris*(4'-Amidobenzyl)-1,9-diaminononane *bis* TFA salt (111)



δ_H (400 MHz, DMSO-d_6): 10.2 (1H, broad s, **x**); 8.90 (2H, broad s, **i**); 8.05-8.01 (3H, m, $\mathbf{a_1}$ and ϕ_1); 7.93 (6H, m, **e** and β); 7.60 (4H, broad s, α); (2H, d, **f**, *J* 9); 7.42-7.47 (3H, m, $\mathbf{a_2}$ and ϕ_2); 4.40 (4H, broad s, **y**); 4.19 (2H, t, **h**, *J* 6); 2.89 (4H, broad s, **k** and **w**); 1.67-1.57 (4H, m, **l** and **v**); 1.25-1.16 (10H, m, **n**, **o** and **p**, **r** and **u**).
δ_C (100 MHz, DMSO-d_6): 167.7 (**b** and ε); 157.8 (γ, quart, *J* 33); 143.5 (**g** and **z**); 132.9 (**c**); 132.7 (χ); 128.1, 127.7, 127.4, 127.3 (**e**, **f**, α and β); 116.3 (η, quart, *J* 295); 52.8 (**h**); 52.3 (**y**); 48.4 (**w** and **k**); 28.9, 28.8, 28.7, 26.7, 26.3, 26.2 (**l**, **n**, **o**, **p**, **r**, **u** and **v**).
HPLC (method 1, 220 nm): 5.6 min.
m/z (ES+): 279.9 (100%, $[M+2H-2TFA]^{2+}$); 300.5 (100%, $[M+2H+Acetonitrile-2TFA]^{2+}$); 320.3 (20%, $[M+2H+2Acetonitrile-2TFA]^{2+}$); 558.5 (25%, $[M+H-2TFA]^{+}$).
HR-MS (FAB+): 558.3429 (calc. 558.3444, 2.6 ppm).

Experimental for Chapter Six

Dimethyl 5-hydroxyisophthalate (112)



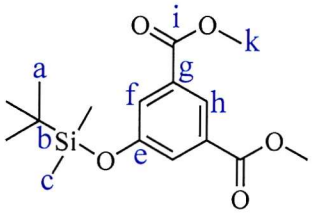
5-Hydroxyisophthalic acid (0.20 mol, 36 g) was dissolved in MeOH (300 mL) and *p*-toluensulphonic acid (1.0 mmol, 1.9 g) was added. The solution was heated at reflux overnight, the solvent was then evaporated under reduced pressure. The crude was dissolved in AcOEt (200 mL) and washed with 2M $KHCO_3$(aq) (2x200 mL). The organic phase was dried over magnesium sulphate, filtered and dried *in vacuo*. Pure product was isolated quantitatively as a white solid (41 g, 100%). Experimental data are in accordance with the literature.[134]

δ_H (300 MHz, DMSO-d_6): 10.30 (1H, broad s, **a**); 7.92 (1H, s, **f**); 7.48 (2H, s, **c**); 3.85 (6H, s, **h**).

δ_C (75 MHz, DMSO-d_6): 165.5 (**g**); 158.0 (**b**); 131.4 (**e**); 120.3 (**f**); 120.2 (**c**); 52.5 (**h**).

melting point: 161-164°C.

Dimethyl 5-(*tert*-butyldimethylsilyl)oxyisophthalate (113)

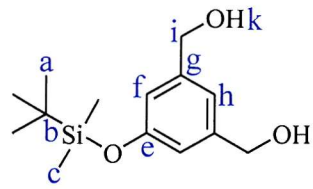


Alcohol (**112**) (51 mmol, 10.7 g) was dissolved in DMF (50 mL) and treated with imidazole (75 mmol, 5.1 g) and *tert*-butyldimethylsilylchloride (50 mmol, 7.5 g). The resulting mixture was poured into Et_2O (200 mL) and water (100 mL) and the aqueous phase was extracted with Et_2O (100 mL). The combined organic layers were washed successively with water (100 mL), 10% AcOH(aq) (5x100 mL), water (2x100 mL), 10% NaOH(aq) (5x100 mL), water (100 mL) and brine (100 mL). After drying over magnesium sulphate, the solvent was evaporated to yield a colourless oil (15.8 g, 98%) that was used without further purification. Experimental data are in accordance with the literature.[133]

δ_H (300 MHz, $CDCl_3$): 8.26 (1H, s, **h**); 7.65 (2H, s, **f**); 3.91 (6H, s, **k**); 0.97 (9H, s, **a**); 0.21 (6H, s, **c**).

δ_C (75 MHz, $CDCl_3$): 166.2 (**i**); 156.0 (**e**); 131.9 (**g**); 125.5 (**f**); 123.8 (**h**); 52.5 (**k**); 25.7 (**a**); 18.3 (**b**); -4.4 (**c**).

O-(*tert*-Butyldimethyl)silyl-3,5-*bis*(hydroxymethyl)phenol (114)



To a stirred suspension of $LiAlH_4$ (68 mmol, 2.6 g) in dry THF (70 mL) was added dropwise a solution of (**113**) (49 mmol, 15.8 g) in dry THF (80 mL) at 0°C. The suspension was allowed to reach r.t. and stirred overnight. The reaction was quenched with $NH_4Cl_{sat}(aq)$ (20 mL). After 2 h, the solution was concentrated under reduced pressure, dissolved in water (100 mL) and extracted with AcOEt (2x100 mL). The combined organic layers were dried over magnesium sulphate, filtered and evaporated *in vacuo*. The resulting white solid (10.6 g, 81%) was used without further purification. Experimental data are in accordance with the literature.[133]

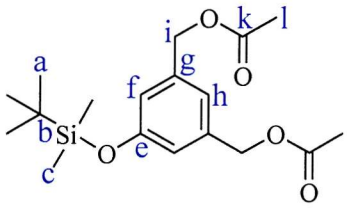
δ_H (300 MHz, $CDCl_3$): 6.88 (1H, s, **h**); 6.69 (2H, s, **f**); 4.51 (4H, s, **i**); 3.46 (2H, broad s, **k**); 0.97 (9H, s, **a**); 0.18 (6H, s, **c**).

δ_C (75 MHz, $CDCl_3$): 155.9 (**e**); 142.8 (**g**); 118.4 (**h**); 117.8 (**f**); 64.9 (**i**); 25.8 (**a**); 18.3 (**b**); -4.3 (**c**).

IR (ν): 3234 (broad), 2923, 2854, 1591, 1443, 1147.

Melting point: 86-90°C.

O-(*tert*-Butyldimethyl)silyl-3,5-*bis*(acetoxymethyl)phenol (115)

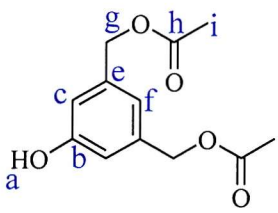


Di-alcohol (**114**) (18.6 mmol, 5.0 g) was dissolved in pyridine (30 mL) and treated with an excess of acetic anhydride (20 mL). The reaction was stirred overnight, then the solvents were evaporated with the aid of toluene (3x50 mL) and the resulting colourless oil (6.5 g, 100%) was used without further purification.

δ_H (300 MHz, $CDCl_3$): 6.85 (1H, s, **h**); 6.73 (2H, s, **f**); 5.03 (4H, s, **i**); 2.10 (6H, s, **l**); 0.97 (9H, s, **a**); 0.18 (6H, s, **c**).

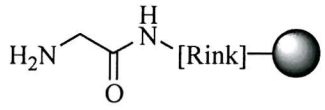
δ_C (75 MHz, $CDCl_3$): 170.9 (**k**); 156.1 (**e**); 137.9 (**g**); 120.8 (**h**); 119.6 (**f**); 66.0 (**i**); 25.8 (**a**); 21.1 (**l**); 18.3 (**b**); -4.3 (**c**).

3,5-*bis*(Acetoxymethyl)phenol (116)



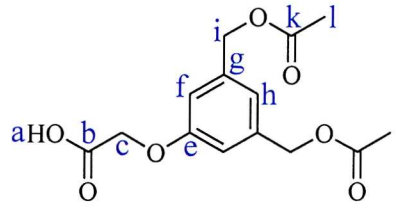
Silyl ether (**115**) (5.7 mmol, 2.0 g) was dissolved in a mixture TFA/water 95:5 (30 mL) and stirred for 3 h at rt. The solvents were then evaporated under reduced pressure with the aid of toluene (2x50 mL). The crude material was purified by flash chromatography (PE/Et_2O 1:1), yielding the correct compound (1.3 g, 96%) of product as a yellow solid. Experimental data are in accordance with the literature.[134]
TLC (PE/Et_2O 1:1): R_f 0.35.
δ_H (300 MHz, $CDCl_3$): 6.85 (1H, s, **f**); 6.77 (2H, s, **c**); 5.03 (4H, s, **g**); 2.10 (6H, s, **i**).
δ_C (75 MHz, $CDCl_3$): 171.5 (**h**); 156.6 (**b**); 138.0 (**e**); 120.0 (**f**); 115.2 (**c**); 66.1 (**g**); 21.2 (**i**).
IR (ν): 3239 (broad), 2977, 1705, 1236.
Melting point: 49-52°C.

N-Glycyl Rink-polystyrene resin (117)



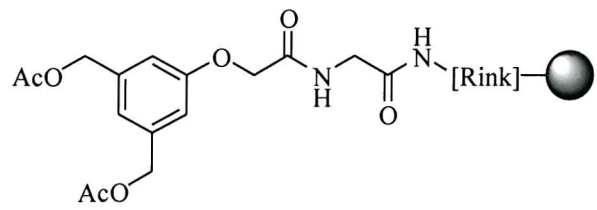
The same procedure used for (**95**) was followed, using 1.5 g of aminomethyl polystyrene (loading 0.9 mmol/g). The final loading was determined by Fmoc quantification and found to be 0.7 mmol/g.

3,5-*bis*(Acetoxymethyl)phenoxyacetic acid (118)



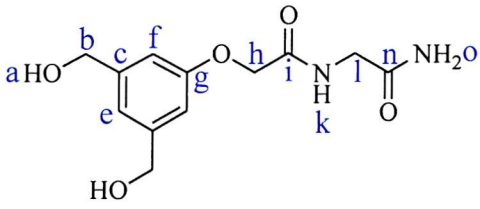
3,5-*bis*(Acetoxymethyl)phenol (**116**) (1.26 mmol, 300 mg) was dissolved in acetonitrile (30 mL), potassium carbonate (2.5 mmol, 350 mg), potassium iodide (0.13 mmol, 20 mg) and *tert*-butyl bromoacetate (1.5 mmol, 225 μL) were added and the solution left stirring for 36 h at rt. The reaction mixture was filtered and washed with acetonitrile (20 mL). The filtrate, after evaporation of the solvent, was purified by flash chromatography (gradient from PE/acOEt 4:1 to PE/AcOEt 7:3). The pure compound was obtained as a colourless oil (390 mg, 88%).
This compound (1.00 mmol, 350 mg) was dissolved in DCM (15 mL) and treated with TFA (10 mL). The solution was stirred for 1 h, then the solvents were evaporated, yielding quantitatively (325 mg) the product as a white solid.
TLC (2:1 PE/AcOEt) R_f: 0.3
δ_H (300 MHz, DMSO-d_6): 7.05 (1H, s, **h**); 6.97 (2H, s, **f**); 5.13 (4H, s, **i**); 4.79 (2H, s, **c**); 2.17 (6H, s, **l**).
δ_C (75 MHz, DMSO-d_6): 170.3, 170.1 (**b** and **k**); 158.0 (**e**); 138.0 (**g**); 120.0 (**h**); 113.6 (**f**); 65.1 (**i**); 64.5 (**c**); 20.7 (**l**).
IR (ν): 3231 (broad), 1762, 1723, 1605, 1243.

N-[3,5-*bis*(Acetoxymethyl)phenoxyacetyl]glycyl Rink resin (119)



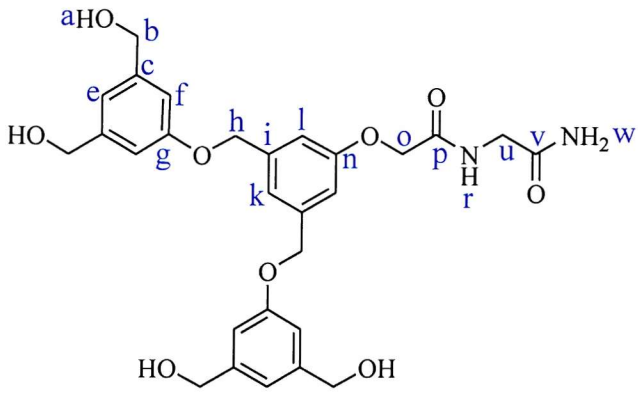
Glycyl Rink resin (0.70 mmol/g, 250 mg) was swollen in DCM. 3,5-*Bis* (acetoxymethyl)phenoxy acetic acid (**118**) (0.37 mmol, 100 mg) was dissolved in DMF (2 mL) and HOBt (0.37 mmol, 50 mg) and DIC (0.37 mmol, 55 μL) were added. After 5 min the resulting solution was added to the resin and left shaking for 4 h at rt. The solvents were then drained and the resin washed with DMF (3x5 mL), MeOH (3x5 mL) and DCM (3x5 mL). Completion of the reaction was controlled by qualitative ninhydrin test. A small sample was cleaved with TFA/H_2O 95:5 and analysed by HPLC and ES-MS.
HPLC (method 1, 220 nm): 7.6 min (88%).
m/z (ES+): 353.2 (100%, $[M+H]^+$); 370.2 (25%, $[M+NH_4]^+$).

N-[3,5-*bis*(Hydroxymethyl)phenoxyacetyl)glycyl amide (120')



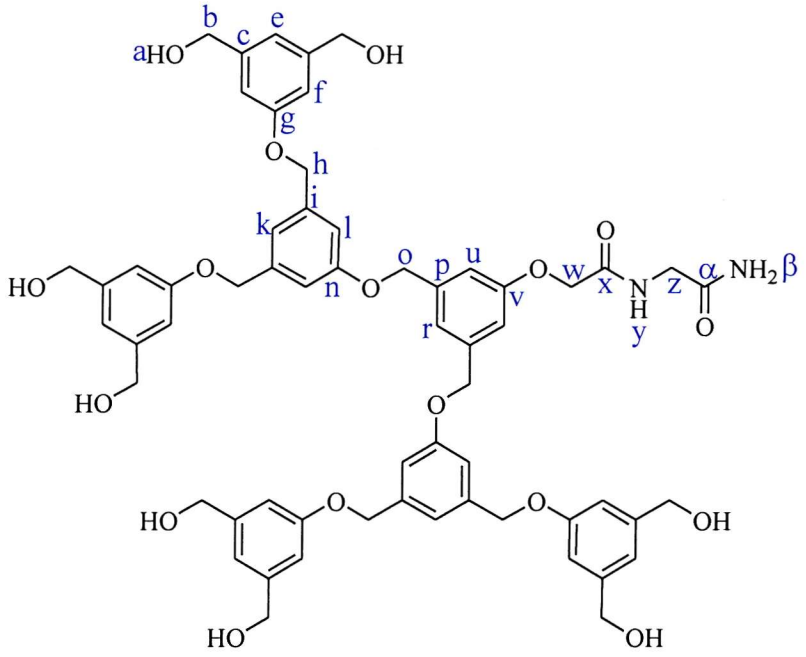
Resin (**119**) (1.2 mmol -OAc/g, 100 mg) was suspended in THF (4 mL) and treated with 40% tetrabutylammonium hydroxide(aq) (1 mL) for 2 h. The resin was filtered, washed with water (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). Resin (**116**) was then treated with TFA/H_2O 95:5 (10 mL) for 3 h. The resin was then filtered, and the solution concentrated *in vacuo* and analysed by HPLC and ES-MS. Crude material was then purified by semi-preparative HPLC yielding (**116'**) as a white solid (12 mg, 74%).
δ_H (400 MHz, DMSO): 6.88 (1H, s, **e**); 6.82 (2H, s, **f**); 4.48 (2H, s, **h**); 4.46 (4H, s, **b**); 3.77 (2H, s, **l**).
δ_C (100 MHz, DMSO): 171.8 (**n**); 169.3 (**i**); 157.9 (**g**); 143.9 (**c**); 117.9 (**e**); 111.4 (**f**); 66.9 (**h**); 63.2 (**b**); 41.4 (**l**).
HPLC (method 1, 220 nm): 5.6 min (100%).
m/z (ES+): 269.3 (60%, $[M+H]^+$); 291.3 (100%, $[M+Na]^+$).

N-{3,5-*bis*[3',5'-*bis*(Hydroxymethyl)phenoxymethyl]phenoxyacetyl}glycyl amide (121')



Resin (**120**) (1.2 mmol –OH/g, 250 mg) was washed with dry THF (3x5 mL). A solution of (**116**) (1.5 mmol, 360 mg) and triphenylphosphine (1.5 mmol, 390 mg) in THF (4 mL) was added and the resulting suspension was treated with DIAD (1.5 mmol, 300 μL). The addition of DIAD was carried out over 2 h, the reaction was then shaken overnight. The resin was filtered and the filtrate was concentrated and purified by flash chromatography (gradient from PE/Et_2O 7:3 to PE/Et_2O 3:7) to yield pure (**116**) (200 mg, 69% of recovery yield). The resin was washed with THF (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). A small sample was cleaved with TFA/H_2O 95:5 and analysed by HPLC and ES-MS.
HPLC (method 1, 220 nm): 9.8 min (89%).
m/z (ES+): 709.3 (80%, $[M+H]^+$); 731.3 (100%, $[M+Na]^+$).
The resin was then suspended in THF (3 mL) and treated with 40% tetrabutylammonium hydroxide(aq) (2 mL) overnight. The resin was then filtered, washed with water (3x5 mL) and treated with 40% tetrabutylammonium hydroxide (aq)/THF 2:3 (5 mL) for additional 4 h. The resin was filtered and washed with water (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). Resin (**121**) (0.51 mmol/g, 100 mg) was then treated with TFA/H_2O 95:5 (10 mL) for 3 h. The resin was filtered, the solution evaporated *in vacuo* and analysed by HPLC and ES-MS. Crude material was then purified by semi-preparative HPLC yielding (**121'**) as a white solid (18 mg, 67%).
δ_H (400 MHz, CD_3OD): 7.19 (1H, s, **k**); 7.07 (2H, s, **e**); 6.92 (2H, s, **l**); 6.90 (4H, s, **f**); 5.10 (4H, s, **h**); 4.59 (2H, s, **o**); 4.55 (8H, s, **b**); 3.89 (2H, s, **u**).
δ_C (100 MHz, CD_3OD): 173.2 (**v**); 170.7 (**p**); 159.8 (**g**); 159.0 (**n**); 144.2 (**c**); 140.5 (**i**); 120.1 (**k**); 117.8 (**e**); 113.7 (**l**); 112.5 (**f**); 69.9 (**h**); 67.8 (**o**); 64.4 (**b**); 42.2 (**u**).
HPLC (method 1, 220 nm): 7.0 min (77%).
m/z (ES+): 541.5 (80%, $[M+H]^+$); 563.4 (100%, $[M+Na]^+$).

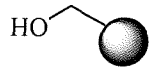
N-(3,5-*bis*{3',5'-*bis*[3'',5''-*bis*(Hydroxymethyl)phenoxymethyl]phenoxymethyl} phenoxyacetyl)glycyl amide (122')



Resin (**121**) (2.0 mmol –OH/g, 250 mg) was washed with dry THF (3x5 mL). A solution of (**116**) (2.5 mmol, 600 mg) and triphenylphosphine (2.5 mmol, 650 mg) in THF (4 mL) was added and the resulting suspension was treated with DIAD (2.5 mmol, 500 μL). The addition of DIAD was carried out over a period of 3 h, the reaction mixture was then shaken for 24 h at rt. The resin was filtered and the filtrate was evaporated to dryness and purified by flash chromatography as described before. The resin was washed with THF (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). A small sample was cleaved with TFA/H_2O 95:5 and analysed by HPLC and ES-MS.
HPLC (method 1, 220 nm): 11.8 min (46%).
m/z (ES+): 1421.4 (100%, $[M+H]^+$); 1443.3 (50%, $[M+Na]^+$).
The resin was then suspended in THF (4 mL) and treated with 40% tetrabutylammonium hydroxide(aq) (4 mL) overnight. The resin was then filtered, washed with water (3x5 mL) and treated again with 40% tetrabutylammonium hydroxide (aq)/THF 1:1 (8 mL) for additional 5 h. The resin was filtered and washed with water (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). Resin (**122**) (0.39 mmol/g, 100 mg) was then treated with TFA/H_2O 95:5 (10 mL) for 3 h. The resin was filtered, the solution evaporated *in vacuo* and analysed by HPLC and ES-MS. Crude material was then purified by semi-preparative HPLC yielding (**118'**) as a white solid (10 mg, 23%).
δ_H (300 MHz, DMSO-d_6): 7.30 (1H, s, **r**); 7.24 (2H, s, **k**); 7.19 (6H, broad s, **l** & **u**); 7.06 (2H, broad s, β); 6.97 (4H, s, **e**); 6.95 (8H, s, **f**); 5.22 (4H, s, **o**); 5.17 (8H, s, **h**); 4.68 (2H, s, **w**); 4.55 (16H, s, **b**); 3.85 (2H, broad s, **z**).
δ_C (100 MHz, DMSO-d_6): 171.1 (α); 168.3 (**x**); 159.0 (**n**); 158.8 (**g**); 158.4 (**v**); 144.3 (**c**); 139.5 (**i**); 139.3 (**p**); 120.0 (**r**); 119.3 (**k**); 117.4 (**e**); 113.8 (**u**); 113.5 (**l**); 111.4 (**f**); 69.5 (**o**); 69.3 (**h**); 67.4 (**w**); 63.2 (**b**); 41.8 (**z**).
HPLC (method 1, 220 nm): 8.1 min (52%).

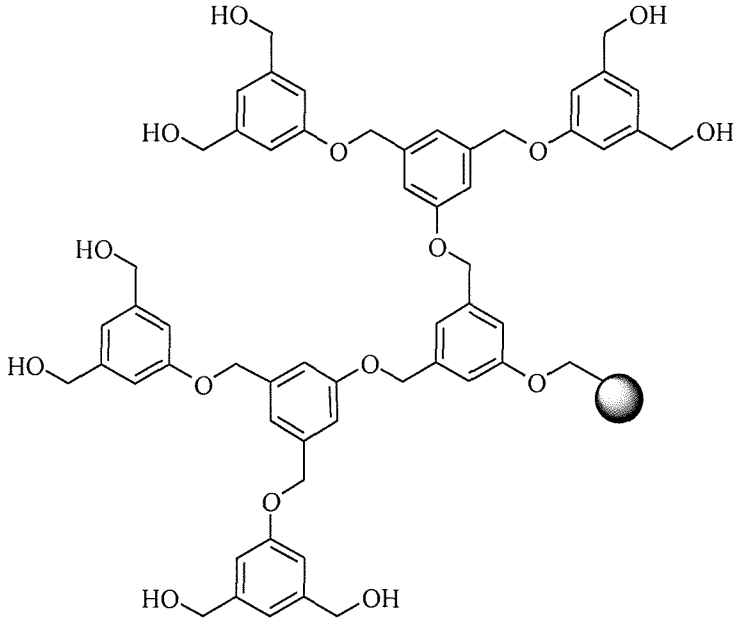
m/z (TOF LD+): 1107.5 (100%, $[M+Na]^+$); 1123.4 (30%, $[M+K]^+$).

Hydroxymethyl polystyrene (124)



Commercial Merrifield resin was sieved using a Reutsch Sieving Unit, with Endcotte Stainless Steel Sieves. The fraction of resin with bead diameter 90-106 μm was collected (0.93 mmol/g, 3.0 g) and suspended in DMF. Dry cesium acetate (13.5 mmol, 2.6 g) and sodium iodide (0.27 mmol, 40 mg) were added and the resin shaken for 48 h at 50°C. The resin was filtered and washed with water (5x30 mL), MeOH (5x30 mL), DMF (5x30 mL), DCM (5x30 mL) and Et_2O (2x 20 mL). A small sample of resin was analysed by IR and showed the characteristic C=O stretching peak at 1735 cm^{-1}. Resin (**123**) was suspended in THF (40 mL) and treated with 40% tetrabutylammonium hydroxide(aq) (15 mL) overnight. The resin was then filtered and washed with water (3x30 mL), MeOH (3x30 mL), DMF (3x30 mL), DCM (3x30 mL) and Et_2O (2x30 mL). IR analysis showed no peak at 1735 cm^{-1} to confirm complete hydrolysis.
A small sample of resin (0.95 mmol/g, 100 mg) was coupled with Fmoc-Ala-OH (0.47 mmol, 148 mg) using DIC (0.47 mmol, 74 μL), DMAP (0.047 mmol, 6 mg) and N-methylmorpholine (0.24 mmol, 26 μL). After 4 h the resin was filtered and washed. Fmoc quantification gave a loading of 0.82 mmol/g and 0.44 nmol/bead, the number of beads per gram was therefore about $2x10^6$.

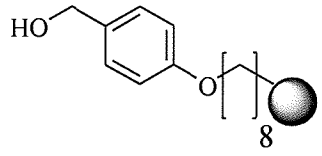
Generation 3.0 polyether dendrimer resin (125)



Resin (**124**) (0.82 mmol/g, 1.0 g) was washed with dry THF (2x10 mL). Phenol (**116**) (4.1 mmol, 980 mg) and triphenylphosphine (4.1 mmol, 1.08 g) were dissolved in dry THF (10 mL) and added to the resin. DIAD (4.1 mmol, 0.81 mL) was added portionwise over a period of 3 h. The resin was shaken overnight, then filtered and

washed with THF (3x10 mL), DMF (3x10 mL), DCM (3x10 mL) and Et_2O (2x10 mL). A small sample of resin (10 mg) was treated with Fmoc-Ala-OH (0.026 mmol, 8 mg), DIC (0.026 mmol, 4 μL) and a few crystals of DMAP dissolved in DMF (0.3 mL) for 4 h. The resin was then washed and treated with 20% piperidine/DMF. The solution was analysed by UV, showing neglectable absorbance at 300nm. A qualitative ninhydrin test on the deprotected resin was negative.
Acetyl protected resin was then treated with 40% tetrabutylammonium hydroxide(aq)/THF 2:3 (25 mL) overnight. The resin was filtered and washed with water (3x20 mL), MeOH (3x20 mL), DMF (3x20 mL), DCM (3x20 mL) and Et_2O (2x20 mL). A small sample of resin (20 mg) was treated with Fmoc-Ala-OH (0.15 mmol, 46 mg), DIC (0.15 mmol, 23 μL), DMAP (0.015 mmol, 2 mg) and N-methylmorpholine (0.075 mmol, 8 μL) dissolved in DMF (0.5 mL) for 4 h. The resin was then washed, treated with 20% piperidine/DMF and the loading determined by UV.
The whole procedure was repeated three times (doubling the amount of reagents every time) to give the final resin (**125**).

Wang polyether dendrimer resin (127)



Polyether dendrimer resin (**125**) (2.8 mmol/g, 50 mg) was washed with dry THF (2x5 mL). Methyl 4-hydroxybenzoate (1.4 mmol, 210 mg) and triphenylphosphine (1.4 mmol, 365 mg) were dissolved in dry THF (2 mL) and added to the resin. The resulting suspension was treated with DIAD (1.4 mmol, 0.28 mL), added over a time of 3 h. The resin was shaken overnight, then washed with THF (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). The IR spectrum showed a peak at 1713 cm^{-1}, corresponding to the C=O stretching of the ester group. The resin was suspended in dry THF (1 mL) and treated with $LiAlH_4$ (0.70 mmol, 27 mg) overnight. The excess of reducing agent was destroyed with 1M $NaHSO_4$(aq) (3x1 mL) and the resin was washed with water (3x5 mL), MeOH (3x5 mL), DMF (3x5 mL), DCM (3x5 mL) and Et_2O (2x5 mL). Completion of the reduction was checked by IR analysis.

Leu-enkephaline-Lys (128')

Resin (**123**) (0.14 mmol –OH) was swollen in DCM for 5 min. Fmoc-Lys(Boc)-OH (0.42 mmol, 197 mg) was dissolved in DMF (1 mL) and treated with DIC (0.42 mmol, 66 μL), DMAP (0.042 mmol, 9 mg) and N-methylmorpholine (0.21 mmol, 23 μL). The resulting solution was added to the resin and shaken for 4 h at rt. The resin was then washed with DMF (3x5 mL) and DCM (3x5 mL). Fmoc deprotection was achieved upon treatment of the resin with 20% piperidine/DMF (5 mL) for 45 min, the resin was then washed with DMF (5x5 mL) and DCM (5x5 mL).
Fmoc-Leu-OH (0.70 mmol, 247 mg) was dissolved in DMF (1 mL) and treated with DIC (0.70 mmol, 0.11 mL) and HOBt (0.70 mmol, 0.11 g). After 5 min the resulting solution was added to the resin, and this was shaken for 3 h. After the usual washing

procedure and the Fmoc deprotection, the resin was coupled with Fmoc-Phe-OH, Then with Fmoc-Gly-OH twice and finally with Fmoc-Tyr(*tert*-Bu)-OH.
After the final Fmoc deprotection, resin (**128**) was treated with TFA/H_20 95:5 (10 mL) for 3 h and filtered. The filtrated was concentrated under reduced pressure and poured into ice-cold Et_2O (40 mL). The resulting white solid was washed with Et_2O (2x40 mL) and dried *in vacuo*.
HPLC (method 1, 220 nm): 6.6 min (53%).
m/z (ES+): 684.5 (100%, $[M+H]^+$).

References

[1] F. Wohler, *Ann. Phys. Chem.* **1828**, *12*, 253.
[2] R. B. Woodward, *Pure Appl. Chem.* **1973**, *33*, 145.
[3] K. C. Nicolaou, *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 1377-1385.
[4] J. J. Masters, J. T. Link, L. B. Snyder, W. B. Young, S. J. Danishefsky, *Angew. Chem., Int. Ed. Engl.* **1995**, *34*, 1723-1726.
[5] A. W. Czarnik, *Combinatorial Chemistry* **1995**, 13-18.
[6] H. M. Geysen, R. H. Meloen, S. J. Barteling, *Proc. Natl. Acad. Sci. USA* **1984**, *81*, 3998-4002.
[7] R. A. Houghten, *Proc. Natl. Acad. Sci. USA* **1985**, *82*, 5131-5135.
[8] R. B. Merrifield, *J. Am. Chem. Soc.* **1963**, *85*, 2149-2154.
[9] E. M. Gordon, R. W. Barrett, W. J. Dower, S. P. A. Fodor, M. A. Gallop, *J. Med. Chem.* **1994**, *37*, 1385-1401.
[10] B. P. Roques, F. Noble, V. Daugé, M.-C. Fournié-Zaluski, A. Beaumont, *Pharm. Rev.* **1993**, *45*, 87-146.
[11] G. S. A. B. Stewart, P. Williams, *J. Gen. Microbiology* **1992**, *138*, 1289-1300.
[12] R. S. Burlage, G. S. Sayler, F. Larimer, *J. of Bacteriology* **1990**, *172*, 4749-4757.
[13] L. Mere, T. Bennett, P. Coassin, P. England, B. Hamman, T. Rink, S. Zimmerman, P. Negulescu, *Drug Discovery Today* **1999**, *4*, 363-369.
[14] G. Zlokarnik, P. A. Negulescu, T. E. Knapp, L. Mere, N. Burres, L. Feng, M. Whitney, K. Roemer, R. Y. Tsien, *Science* **1998**, *279*, 84-88.
[15] M. V. Rogers, *Drug Discovery Today* **1997**, *2*, 156-160.
[16] B. Alpha, J.-M. Lehn, G. Mathis, *Angew. Chem., Int. Ed. Engl.* **1987**, *26*, 266-267.
[17] M. Picardo, K. T. Hughes, *High Throughput Screening: the Discovery of Bioactive Substances (Devlin J. P. ed.)* **1997**, 307-316.
[18] B. Clapham, A. J. Sutherland, *Tetrahedron Lett.* **2000**, *41*, 2253-2260.
[19] B. A. Brown, M. Cain, J. Broadbent, S. Tompkins, G. Henrich, R. Joseph, S. Casto, H. Harney, R. Greene, R. Delmondo, S. Ng, *High Throughput Screening: the Discovery of Bioactive Substances (Devlin J. P. ed.)* **1997**, 317-328.
[20] A. Furka, F. Sebastyén, M. Asgedom, G. Dibó, *Proceedings of the 14th International Congress of Biochemistry, Utrecht (The Netherlands)* **1988**, *5*, 47.
[21] K. S. Lam, S. E. Salmon, E. M. Hersh, V. J. Hruby, W. M. Kazmierski, R. J. Knapp, *Nature* **1991**, *354*, 82-84.
[22] E. Bayer, W. Rapp, *US patent 4,908,405* **1990**.
[23] E. Atherton, D. L. J. Clive, R. C. Sheppard, *J. Am. Chem. Soc.* **1975**, *97*, 6584-6585.
[24] H. P. Nestler, H. Wennemers, R. Sherlock, D. L. Y. Dong, *Bioorg. Med. Chem. Lett.* **1996**, *6*, 1327-1330.
[25] K. S. Lam, J. Wu, *Methods (San Diego)* **1994**, *6*, 482.
[26] S. Brenner, R. A. Lerner, *Proc. Natl. Ac. Sci. USA* **1992**, *89*, 5381-5383.
[27] H. P. Nestler, P. A. Bartlett, W. C. Still, *J. Org. Chem.* **1994**, *59*, 4723-4724.
[28] Z.-J Ni, D. Maclean, C. P. Holmes, M. M. Murphy, B. Ruhland, J. W. Jacobs, E. M. Gordon, M. A. Gallop, *J. Med. Chem.* **1996**, *39*, 1601-1608.
[29] M. Lebl, M. Pátek, P. Kociš, V. Krchnák, V. J. Hruby, S. E. Salmon, K. S. Lam, *Int. J. Peptide Protein Res.* **1993**, *41*, 201-203.
[30] M. Cardno, M. Bradley, *Tetrahedron Lett.* **1996**, *37*, 135-138.
[31] X.-Y. Xiao, R. Li, H. Zhuang, B. Ewing, K. Karunaratne, J. Lillig, R. Brown, K. C. Nicolaou, *Biotechnology and Bioengineering* **2000**, *71*, 44-50.

[32] M. K. L. Tomalia, D. M. Hedstrand, D. A. Tomalia, A. B. Padias, H. K. Hall, *Tetrahedron* **1997**, *53*, 15495-15513.
[33] D. A. Tomalia, H. Baker, J. R. Dewald, M. Hall, G. Kallos, S. Martin, J. Roeck, J. Ryder, P. Smith, *Polym. J.* **1985**, *17*, 117-132.
[34] T. Zincke, *Chem. Ber.* **1869**, *2*, 737-740.
[35] C. Friedel, J. M. Crafts, *Bull. Soc. Chem.* **1885**, *43*, 53.
[36] P. J. Flory, *J. Am. Chem. Soc.* **1941**, *63*, 3083-3100.
[37] P. J. Flory, *J. Am. Chem. Soc.* **1952**, *74*, 2718-2723.
[38] E. Buhleier, W. Wehner, F. Vögtle, *Synthesis* **1978**, 155-158.
[39] R. G. Denkewalter, J. F. Kolc, W. J. Lukasavage, *U. S. Pat. 4, 410, 688* **1983**.
[40] G. R. Newkome, Z.-Q. Yao, G. R. Baker, V. K. Gupta, *J. Org. Chem.* **1985**, *50*, 2003-2004.
[41] C. J. Hawker, J.-M. J. Fréchet, *J. Chem. Soc., Chem. Comm.* **1990**, 1010-1013.
[42] T. M. Miller, T. X. Neenan, *Chem. Mat.* **1990**, *2*, 346-349.
[43] A. W. van der Made, P. W. N. M. van Leeuwen, *J. Chem. Soc., Chem. Comm.* **1992**, 1400-1401.
[44] M. Schuster, P. Wang, J. C. Paulson, C.-H. Wong, *J. Am. Chem. Soc.* **1994**, *116*, 1135-1136.
[45] A. Miedaner, C. J. Curtis, R. M. Barkley, D. L. DuBois, *Inorg. Chem.* **1994**, *33*, 5482-5490.
[46] O. L. Chapman, J. Magner, R. Ortiz, *Polym. Preprints*, **1995** *36*, 739-740.
[47] C. J. Hawker, R. Lee, J.-M. J. Fréchet, *J. Am. Chem. Soc.* **1991**, *113*, 4583-4588.
[48] K. L. Wooley, J. M. J. Fréchet, C. J. Hawker, *Polymer* **1994**, *35*, 4489-4495.
[49] S. Cicchi, *La Chimica e l'Industria*, **2000**, *82*, 433-440.
[50] P. L. Dubin, S. L. Edwards, J. I. Kaplan, M. S. Mehta, D. A. Tomalia, J. Xia, *Anal. Chem.* **1992**, *64*, 2344-2347.
[51] M. Castagnola, L. Cassiano, A. Lupi, I. Messana, M. Patamia, R. Rabino, D. V. Rossetti, B. Giardina, *J. Chrom.* **1995**, *692*, 463-469.
[52] G. R. Killat, D. A. Tomalia, *U. S. Pat. 4, 871,779* **1989**.
[53] L. F. Borisova, B. I. Nakhmanovich, A. V. Pakhomov, N. V. Platonova, G. I. Fajdel, V. K. Shatalov, *USSR Pat. 1, 808, 362* **1993**.
[54] G. E. DuBois, B. Zhi, G. M. Roy, S. Y. Stevens, M. Yalpani, *J. Chem. Soc., Chem. Comm.* **1992**, 1604-1605.
[55] J. P. Tam, *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 5409-5413.
[56] R. H. E. Hudson, M. J. Damha, *J. Am. Chem. Soc.* **1993**, *115*, 2119-2124.
[57] J. Haensler, F. C. Szoka, Jr., *Bioconj. Chem.* **1993**, *4*, 372-379.
[58] E. C. Wiener, M. W. Brechbiel, H. Brothers, R. L. Magin, O. A. Gansow, D. A. Tomalia, P. C. Lauterbur, *Magn. Reson. Med.* **1994**, *31*, 1-8.
[59] M. Ghosh, M. J. Miller, *J. Org. Chem.* **1994**, *59*, 1020-1026.
[60] H. K. Hall, Jr., D. W. Polis, *Polym. Bull.* **1987**, *17*, 409-416.
[61] P. Belser, A. von Zelewsky, M. Frank, C. Seel, F. Vögtle, L. DeCola, F. Barigelletti, V. Balzani, *J. Am. Chem. Soc.* **1993**, *115*, 4076-4086.
[62] C. A. Migdal, *U. S. Pat. 4, 938,885* **1990**.
[63] F. M. Winnik, M. P. Breton, *U. S. Pat. 5, 266,106* **1993**.
[64] V. Percec, P. Chu, M. Kawasumi, *Macromolecules* **1994**, *27*, 4441-4453.
[65] J. F. G. A. Jansen, E. M. M. de Brabander-van den Berg, E. W. Meijer, *Science* **1994**, *266*, 1226-1229.
[66] D. A. Tomalia, P. R. Dvornic, *Nature* **1994**, *372*, 617-618.

[67] J. F. G. A. Jansen, E. W. Meijer, *J. Am. Chem Soc.* **1995**, *117*, 4417-4418.
[68] V. Chechik, M. Zhao, R. M. Crooks, *J. Am. Chem. Soc.* **1999**, *121*, 4910-4911.
[69] L. J. Twyman, A. E. Beezer, R. Esfand, M. J. Hardy, J. C. Mitchell *Tetrahedron Lett.* **1999**, *40*, 1743-1746.
[70] R. Duncan, N. Malik, *Proc. Int. Symp. Control. Relat. Bioact. Mater.* **1996**, *30*, 105-106.
[71] J. F. Kukowska-Latallo, A. U. Bielinska, J. Johnson, R. Spindler, D. A. Tomalia, J. R. Baker, Jr., *Proc. Natl. Acad. Sci. USA* **1996**, *93*, 4897-4902.
[72] E. Toth, D. Pubanz, S. Vauthey, L. Helm, A. E. Merbach, *Chem. Eur. J.* **1996**, *2*, 1607-1615.
[73] G. R. Newkome, C. N. Morrefield, J. M. Keith, G. R. Baker, G. H. Escamilla, *Angew. Chem., Int. Ed. Engl.* **1994**, *33*, 666-668.
[74] A. Pessi, E. Bianchi, F. Bonelli, L. Chiappinelli, *J. Chem. Soc., Chem. Comm.* **1990**, 8-9.
[75] J. P. Defoort, B. Nardelli, W. Huang, D. D. Ho, J. P. Tam, *Proc. Natl. Acad. Sci. USA* **1992**, *89*, 3879-3883.
[76] N. Aoki, M. Mori, K. Kato, Y. Sakamoto, K. Noda, M. Tajima, H. Shimada, *Neurosc. Lett.* **1996**, *205*, 111-114.
[77] K. E. Uhrich, S. Boegeman, J. M. J. Fréchet, S. R. Turner, *Polym. Bull.* **1991**, *25*, 551-558.
[78] R. Roy, D. Zanini, S. J. Meunier, A. Romanowska, *J. Chem. Soc., Chem. Comm.* **1993**, 1869-1872.
[79] V. Swali, N. J. Wells, G. J. Langley, M. Bradley, *J. Org. Chem.* **1997**, *62*, 4902-4903.
[80] I. R. Marsh, H. Smith, M. Bradley, *J. Chem. Soc., Chem. Comm.* **1996**, 941-942.
[81] N. J. Wells, A. Basso, M. Bradley, *Biopolymers (Peptide Science)* **1998**, *47*, 381-396.
[82] D. H. Rich, S. K. Gurwara, *J. Am. Chem. Soc.* **1975**, *97*, 1575-1579.
[83] K. E. Holt, G. E. Hutton, C. N. Morfitt, G. Ruecroft, S. J. C. Taylor, P.D. Tiffin, N. Tremayne, M. Woods, *Tetrahedron Letters* **1997**, *38*, 8253-8256.
[84] A. P. Tawse Easson, F. L. Pyman, *J. Chem. Soc., Trans II* **1932**, 2991-3001.
[85] R. E. Allen, E. L. Schumann, W. C. Day, M. G. Van Campen, Jr., *J. Am. Chem. Soc.* **1958**, *80*, 591-598.
[86] L. Alig, A. Edenhofer, P. Hadvári, M. Hürzeler, D. Knopp, M. Müller, B. Steiner, A. Trzeciak, T. Weller, *J. Med. Chem.* **1992**, *35*, 4393-4407.
[87] H. Vieweg, G. Wagner, *Pharmazie* **1982**, *37*, 178-182.
[88] T. Nagahara, Y. Yokohama, K. Inamura, S. Katakura, S. Komoriya, H. Yamaguchi, T. Hara, M. Iwamoto, *J. Med. Chem.* **1994**, *37*, 1200-1207.
[89] J. Stanek, G. Caravatti, H. Capraro, P. Furet, H. Mett, P. Schneider, U. Regenass, *J. Med. Chem.* **1993**, *36*, 46-54.
[90] T. Weller, L. Alig, M. Beresini, B. Blackburn, S. Bunting, P. Hadvári, M. Hürzeler Müller, D. Knopp, B. Levet-Trafit, M. T. Lipari, N. B. Modi, M. Müller, C. J. Refino, M. Schmitt, P. Schönholzer, S. Weiss, B. Steiner, *J. Med. Chem.* **1996**, *39*, 3139-3147.
[91] H. Sugihara, H. Fukushi, T. Miyawaki, Y. Imai, Z. Terashita, M. Kawamura, Y. Fujisawa, S. Kita, *J. Med. Chem.* **1998**, *41*, 489-502.
[92] J. Samanen, F. Ali, T. Romoff, R. Calvo, E. Sorenson, J. Vasko, B. Storer, D. Berry, D. Bennett, M. Strohsacker, D. Powers, J. Stadel, A. Nichols, *J. Med. Chem.* **1991**, *34*, 3114-3125.

[93] G. D. Hartman, M. S. Egbertson, W. Halczenko, W. L. Laswell, M. E. Duggan, R. L. Smith, A. M. Naylor, P. D. Manno, R. J. Lynch, G. Zhang, C. T.-C. Chang, R. J. Gould, *J. Med. Chem.* **1992**, *35*, 4640-4642.
[94] J. A, Zablocki, M. Miyiano, S. N. Rao, S. Panzer-Knodle, N. Nicholson, L. Feigen, *J. Med. Chem.* **1992**, *35*, 4914-4917.
[95] For a review on these compounds: S. A. Mousa, D. A. Cheresh, *Drug Discovery Today* **1997**, *5*, 187-199.
[96] M. Verstraete, P. Zoldhelyi, *Drugs* **1995**, *49*, 856-884.
[97] J. J. Edmunds, S. T. Rapundalo, *Annu. Rep. Med. Chem.* **1996**, *31*, 51-60.
[98] S. Elodi, K. Varadi, *Thromb. Res.* **1979**, *15*, 617-629.
[99] S. I. Klein, M. Czekaj, C. J. Gardner, K. R. Guertin, D. L. Cheney, A. P. Spada, S. A. Bolton, K. Brown, D. Colussi, C. L. Heran, S. R. Morgan, R. J. Leadley, C. T. Dunwiddie, M. H. Perrone, V. Chu, *J. Med. Chem.* **1998**, *41*, 437-450.
[100] R. R. Tidwell, W. P. Webster, S. R. Shaver, *Thrombosis Res.* **1980**, *19*, 339-349.
[101] H. G. Williams-Ashman, A. Schenone, *Biochem. Biophys. Res. Comm.* **1972**, *46*, 288-295.
[102] W. C. Hamilton, S. J. La Placa, *Acta Crystallogr.* **1968,** *B24*, 1147-1156.
[103] P. Roussel, and M. Bradley, *Tetrahedron Lett.* **1997**, *38*, 4861-4864.
[104] for a review: I. Fleming; *Comprehensive Organic Synthesis*, vol. 6, pages 542-550
[105] R. Allen, E. Schumann, W. Day, M. Campen, Jr., *J. Am. Chem. Soc.* **1958**, *80*, 591-598.
[106] G. Rousselet, P. Capdevielle, M. Maumy, *Tetrahedron Lett.* **1993**, *34*, 6395-6398.
[107] R. S. Garigipati, *Tetrahedron Lett.* **1990**, *31*, 1969-1972.
[108] J. H. Forsberg, V. T. Spaziano, T. M. Balasubramanian, G. K. Liu, S. A. Kinsley, C. A. Duckworth, J. J. Poteruca, P. S. Brown, J. L. Miller, *J. Org. Chem.* **1987**, *52*, 1017-1021.
[109] B. D. Judkins, D. G. Allen, T. A. Cook, B. Evans, T. E. Sardharwala, *Synthetic Communications* **1996**, *26*, 4351-4367.
[110] The protecting groups are defined *pseudo*-orthogonal because only the allyl group can be selectively removed.
[111] W. J. Hoekstra, M. N. Greco, S. C. Yabut, B. L. Hulshizer, B. E. Maryanoff, *Tetrahedron Lett.* **1997**, *38*, 2629-2632.
[112] G. Wagner, B. Voigt, H. Vieweg, *Pharmazie* **1984**, *39*, 226-230.
[113] B. Belleau, G. Malek, *J. Am Chem. Soc.* **1968**, *90*, 1651-1652.
[114] S. A. Kates, S. B. Daniels, N. A. Solé, G. Barany, F. Albericio, *Proc. 13th American Peptide Symposium*, R. S. Hodges & J. A. Smith (Eds.), ESCOM, Leiden **1994**, 113-115.
[115] B. Henkel, L. Zhang, E. Bayer, *Liebigs Ann./Recueil* **1997**, 2161-2168.
[116] L. A. Carpino, *J. Am. Chem. Soc.* **1993**, *115*, 4397-4398.
[117] B. Castro, J. R. Dormoy, G. Evin, C. Selve, *Tetrahedron Lett.* **1975**, *16*, 1219-1222.
[118] J. Coste, M. Dufour, A. Pantaloni, B Castro, *Tetrahedron Lett.* **1990**, *31*, 669-672.
[119] J. Bertho, A. Loffet, C. Pinel, F. Reuther, G. Sennyey, *Tetrahedron Lett.* **1991**, *32*, 1303-1306.
[120] T. Shioiri, K. Ninomiya, S. Yamada, *J. Am. Chem. Soc.* **1972**, *94*, 6203-6205.
[121] A. K. Dutta, L. L. Coffey, M. E. A. Reith, *J. Med. Chem.* **1997**, *40*, 35-43.
[122] O. Mitsunobu, *Synthesis* **1981**, 1-28.
[123] H. Rink, *Tetrahedron Lett.*, **1987**, *28*, 3787-3790.
[124] A. S. Kalgutkar, *Opinion in Therapeutic Patents* **1999**, *9*, 831.

[125] G. Barany, R. B. Merrifield, *The Peptides*, eds. E. Gross and J. Meienhofer, Academic Press, New York, **1980**, *2*, 65.
[126] T. A. Rano, K. T. Chapman, *Tetrahedron Lett.* **1995**, *36*, 3789-3792.
[127] L. S. Richter, T. R. Gadek, *Tetrahedron Lett.* **1994**, *35*, 4705-4706.
[128] This is an approximate method of quantification. The amount of residual protonated DMSO present in the NMR solvent (constant in each bottle) is quantified with a known quantity of *p*-nitrophenol via the respective ^{1}H NMR signal integrations. This is then used as an internal standard in the calculation of the quantity of compound released by a known amount of resin.
[129] K. J. Jensen, J. Alsina, M. F. Songster, J Vagner, F. Albericio, G. Barany, *J. Am. Chem. Soc.* **1998**, *120*, 5441-5452.
[130] Brian Evans, *unpublished results.*
[131] N. Miyaura, T. Yanagi, A. Suzuki, *Syn. Commun.* **1981**, *11*, 513-519.
[132] P. G. Wyatt, G. Hickin, N. D. Miller, M. J. Allen, *unpublished results.*
[133] S. Höger, *Synthesis* **1997**, 20-22.
[134] P. R. Ashton, D. W. Anderson, C. L. Brown, A. N. Shipway, J. F. Stoddart, M. S. Tolley, *Chem. Eur. J.* **1998**, *4*, 781-795.
[135] E. Kaiser, R. L. Colescott, C. D. Bossinger, P. I. Cook, *Anal. Biochem.* **1970**, *34*, 595-598.
[136] J. Eicher, M. Bienert, A. Stierandove, M. Lebl, *Peptide Res.* **1991**, *4*, 296-301.
[137] I. R. Marsh, M. Bradley, *Tetrahedron* **1997**, *53*, 17317-17334.
[138] F. Bambino, R. T. C. Brownlee, F. C. K. Chiu, *Tetrahedron Lett.* **1994**, *35*, 4619-4622.
[139] A. Rosowsky, R. A. Forsch, R. G. Moran, *J. Med. Chem.* **1989**, *32*, 709-715.
[140] S. Carrington, A. H. Fairlamb, I. S. Blagbrough, *Chem. Comm.* **1998**, 2335-2336.
[141] G. Wagner, H. Vieweg, H. Kühmstedt, *Pharmazie* **1973**, *28*, 288-292.
[142] J. Terpinski, D. Z. Denney, R. Beveridge, D. P. Cox, R. A. Moss, *Magnetic Res. in Chem.* **1987**, *25*, 923-927.
[143] T. J. Deming, B. M. Novak, *J. Am. Chem. Soc.* **1993**, *115*, 9101-9111.
[144] C. M. Taylor, C. A. Weir, *J. Org. Chem.* **2000**, *65*, 1414-1421.
[145] H. Waldmann, H. Kunz, *Liebigs Ann. Chem.* **1983**, 1712-1725.
[146] D. Brundish, A. Bull, V. Donovan, J. D. Fullerton, S. M. German, J. F. Hayler, D. Janus, P. D. Kane, M. McDonnell, G. P. Smith, R. Wakeford, C. V. Walker, G. Howarth, W. Hoyle, M. C. Allen, J. Ambler, K. Butler, M. D. Talbot, *J. Med. Chem.* **1999**, *42*, 4584-4603.
[147] J. Šavrda, L. Chertanova, M. Wakselman, *Tetrahedron* **1994**, *50*, 5309-5322.