Towards Synthetic Phage Libraries

by

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List of Abbreviations

AA	Amino acid
Ac	Acetyl
(Abz)	2-aminobenzoic acid
AcOEt	Ethyl acetate
AcOH	Acetic acid
Ala	Alanine
APCI	Atmospheric pressure chemical ionisation
Arg	Arginine
Atm	Atmosphere
Bp	Base pair
BSA	Bovine serum albumin
Boc	*tert*-Butyloxycarbonyl
Boc-ON	2-(*tert*-Butoxycarbonyloxymino)-2-phenylacetonitrile
Boc_2O	Di-*tert*-butyldicarbonate
CAMS	Cell adhesion molecules
CPG	Control pore glass
CS	Connecting segment
d	Doublet
D	Aspartic acid
DNA	Deoxyribonucleic acid
Dns	Dansyl chloride
DCC	Dicyclohexylcarbodiimide
DCM	Dichloromethane
Dde-OH	2-Acetyl dimedone
Ddz	*N*-((2-(3, 5-Dimethoxyphenyl)prop-2-yl)oxy)carbonyl
DEAD	Diethylazodicarboxylate
DIC	Diisopropylcarbodiimide
DIPEA	*N*, *N*-Diisopropylethylamine
DKP	Diketopiperazine
DMAP	Dimethylaminopyridine
DMF	Dimethylformamide

DMSO	Dimethylsulphoxide
dNTP	Deoxynucleoside triphosphate
DVB	Divinyl benzene
E	Glutamic acid
EC-GC	Electron capture gas chromatography
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme linked immunosorbent assay
ELLA	Enzyme linked lectin assay
ELS	Evaporative light scattering detector
ES	Electrospray
Et_2O	Diethylether
Fmoc	Fluoren-9-ylmethoxycarbonyl
G	Glycine
Gen	Generation
Glu	Glutamic acid
Gly	Glycine
hrs	hours
HA	Hemagglutinin
HOBt	1-Hydroxybenzotriazole
HMPB	Hydroxymethylmethoxyphenoxybutyric acid
HMPA	Hydroxymethyphenoxy acetic acid
HPLC	High-performance liquid chromatography
(HSQC)	Heteronuclear single quantum correlation
I	Isoleucine
IC_{50}	Inhibition concentration (50 %)
ICAM	Immunoglobulin cell adhesion molecule
IR	Infrared
J	Coupling constant
Kd	Kilodaltons
L	Leucine
Leu	Leucine
m	Multiplet
MAS	Magic angle spinning
MALDI-TOF	Matrix assisted laser desorption ionisation time of flight

MeOH	Methanol
min	Minutes
mM	Millimolar
Mmt	Methoxy trityl
MS	Mass spectrometry
nM	Nanomolar
NMR	Nuclear magnetic resonance
OPD	*o*-phenylenediamine dihydrochloride
PAMAM	Polyamido amine
PBS	Phosphate bovine serum
PCR	Polymerase chain reaction
PEG	Poly (ethylene glycol)
PG	Protecting group
Ph	Phenyl
Phth	Phthalimide
ppm	Parts per million
Pro	Proline
PyBoP	(Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate
q	Quartet
R_f	Retention factor
ROMP	Ring opening metathesis polymerisation
RP	Reverse phase
RT	Room temperature
s	Singlet
SA	Sialic acid
SCAL	Saftey catch acid linker
Ser	Serine
SPOS	Solid-phase organic synthesis
t	Triplet
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
Thr	Threonine
TLC	Thin layer chromatography

TMS	Trimethyl silyl
TsOH	Tosic acid
UV	Ultraviolet
V	Valine
VRE	Vancomycin-resistant enterococci
VCAM	Vascular cell adhesion molecule
VLA	Very late antigen
$\in$Ahx	6-α-aminohexanoic acid

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Chapter 1: Combinatorial Libraries, Screening and the Identification of Active Compounds.

1.1 Chemical Libraries

Combinatorial chemistry which came to the fore in the 1990`s and has been the subject of many reviews[1-3] derives its power and usefulness from its ability to generate large numbers of compounds relatively quickly. There are 3 main categories of chemical libraries (a) primary or unbiased libraries (b) focused libraries and (c) biased targeted libraries.

Primary or Unbiased Libraries

These are libraries that are not designed on the basis of structural information of any specific target and are useful for targets with no known ligands.[4-5] These libraries are therefore intended to be diverse collections of molecules as sources of active compounds for a range of different targets. The compounds can be prepared on the solid-phase by split and mix synthesis (Figure 1) and hits from these libraries represent the starting point for further structure optimisation. Synthesis of these kinds of libraries requires much ground work, testing the synthetic route in solution initially and preparation of a model library eliminating poorly performing monomers in a process known as monomer rehearsal. The monomer sets in libraries of this kind are usually commercially available.

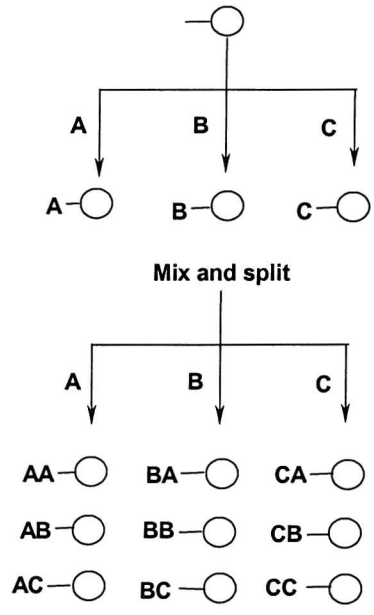


Figure 1: Split and mix synthesis.

Focused Libraries

Focused libraries are libraries designed using structural information gained from the literature or hits from a primary library screen.[6-7] These libraries are usually quite small containing between 10 to 100 compounds and are almost always prepared as discrete compounds using parallel synthesis. Combinatorialisation of a focused library is much less demanding than for a primary library due to the reduced diversity of the monomers. In a focused library it is advantageous to spend time to rationally select and synthesise monomers that are not commercially available as it could result in a novel structure thus giving an advantage over competitors in the same area.

Biased Targeted Libraries

A compromise between large unbiased and small focused libraries has become popular especially in pharmaceutical applications.[8] The libraries are inspired not by structural information but by general information regarding similar classes of targets or by the desired profile that a drug must posses for example (molecular weight, partition coefficient, water solubility and other physicochemical properties). While the number of monomer sets employed for the synthesis of biased targeted libraries is similar to that seen for primary libraries some additional criteria are applied. If the library design is driven by structural information the monomers will be chosen accordingly. The scaffold becomes important when the library individuals are filtered using physicochemical parameters. For example log p of the functionalised scaffold may limit the selection of monomers to either highly hydrophobic or hydrophilic structures. The same is true of monomers and their molecular weights.

1.2 Structure Determination of Hits from Solid-Phase Libraries

Medium to large bead-based libraries are often prepared in large quantities with up to 500 library equivalents. This allows the library to be tested in a number of different screens for a number of different targets. However, methods are required which allow the fast and reliable identification of active compounds. At present there are a number of structure determination methods available.

Bead based approaches consist of submitting bead-based libraries to assays that allow the detection of active beads and finally the determination of the structure of the

compound on the active beads. Screening of libraries for biological targets usually occurs in solution where both the library individuals and the target are dissolved in the same assay medium. On bead methods are attractive as they offer distinct advantages over screening a cleaved compound. Firstly when a complex is formed between the target and the on bead library member the target can be washed away and the same library can be tested on other targets. Secondly positive hits are easily spotted and these active beads can be physically separated from the other inactive beads and their structures determined by appropriate analytical methods.

On-bead screening and structure determination was first described by Lam *et al* using an enzyme linked colorimetric assay[9] (Figure 2) and the method was hailed as "a new type of synthetic peptide library for identifying ligand binding activity." Acceptor molecules were coupled to an enzyme, alkaline phosphatase and fluorescein and added to the peptide bead-based library. A few beads were intensely stained and visible to the naked eye seen with a low power microscope. Forceps were then used to remove the beads for analysis.

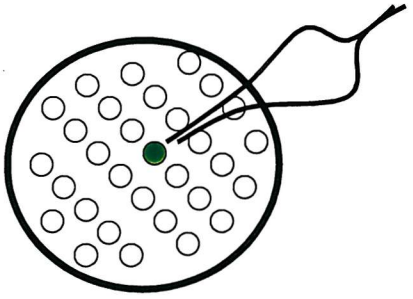


Figure 2: On-bead screening.

Lam`s group and other workers used the method extensively. Workers at Affymax[10] used fluorescently labelled antibodies, with active beads being identified by fluorescence when an antibody bound to the beads. Meldal and co-workers used fluorescence quenching to characterise the substrate specificity of cysteine proteases[11] with fluorescence being observed when the protease cleaved specific peptide sequences which had held the fluorescence donor 2-aminobenzoic acid (Abz) and fluorescent quencher 3-nitrotyrosine (Y (NO_2)) in close proximity.

To date on bead screening has been used to detect and identify biological interactions using isolated receptors, enzymes and antibodies as targets. Since screening takes place

in an aqueous environment it is necessary to use hydrophilic supports. Studies have been performed to determine the accessibility of the resin sites to macromolecular targets. The results show that hydrophilic resins such as TentaGel and Argogel are not accessible to enzymes except on their surface while access to the inner sites is possible only with resins such as PEGA.[11, 12-14]

1.3 Deconvolution Methods for Solid-Phase Libraries.

Houghten *et al* devised two iterative methods[15-17] of screening solid-phase peptide libraries in solution and these were termed "dual defined iterative methodology" and combinatorial positional scanning libraries.

In the seminal paper[15] a dual defined hexapeptide library containing 18 amino acids (excluding cysteine and tryptophan) was constructed. After the first round of synthesis the library existed as 324 (18 x 18) mixtures, each composed of 104,976 different peptide sequences, Ac-O_1O_2XXXX-NH_2. O_1 and O_2 were defined in each of the mixtures and each X was a mixture of all 18 amino acids. A library of >34 million members was obtained. These 324 pools were assayed and positive results for the first two residues (O_1 O_2) were noted. Next, 18 new libraries were synthesised with the formula DVO_3XXX, one for each amino acid at position 3 and tested to define O_3. The process was repeated until all the positions (X) were defined. Essentially this methodology is an iterated search process that consists of making the library in a number of segregated pools, finding the active pool that defines the entity for the position on the molecule and then repeating the process until the active component has been identified. A virtue of this methodology is that the multiplicity of the components decreases with each step, so that an enrichment process occurs and because molecules can be assayed in solution, it allows functional as well as binding assays.

The positional scanning[16] format is again based on soluble combinatorial libraries and is a related technique to the dual defined iterative methodology and is based on screening a set of mixtures, represented by the formulas O_1XXXXX, XO_2XXXX, XXO_3XXX, $XXXO_4$XX, $XXXXO_5$X, and $XXXXXO_6$. The screening of each library determines the most active peptide at that position. The method also alleviates the need for iterative synthesis.

1.4 Structure Determination Using a Multiple Release Strategy.

In the multiple release strategy[18] the compound is released from the support for biological screening but the release is only partial. A fraction of the compound remains on the bead to allow the identification of the active structure and this is achieved by employing a combination of orthogonal linkers.

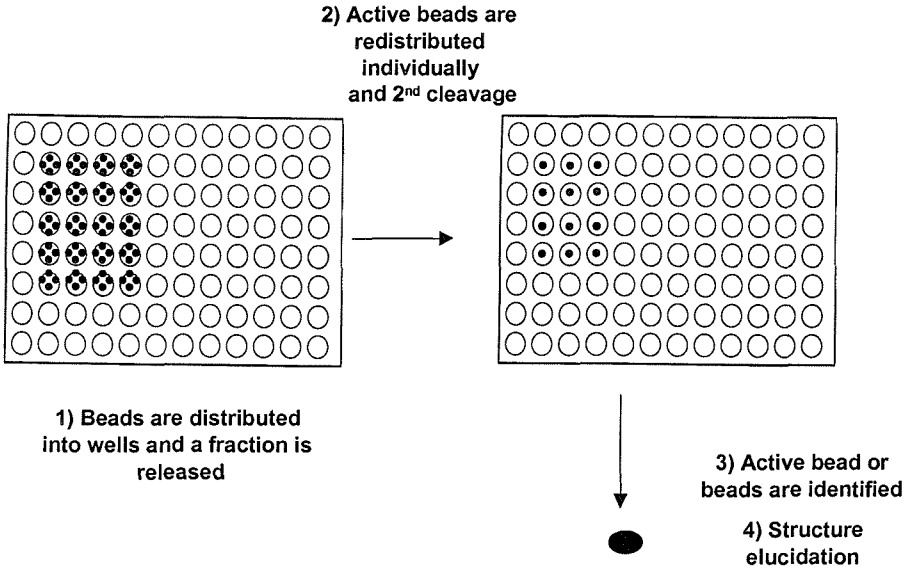


Figure 3: Multiple release strategy.

(Figure 3) shows an example of the screening process where the library is distributed into a 96-well microtiter plate format with several beads per well. The compounds are then partially released into the wells liberating only a fraction of the material fom the beads. After the screen the beads from the active wells are redistributed individually and a second release allows the screening and identification of the single compound or compounds associated with the active bead or beads. The initial linker construct (Figure 4) designed by Lam *et al* [18] provided five independent releases of compound but the drawbacks of this method was that the C-termini of the released peptides were different and this could therefore effect the activity of the peptides.

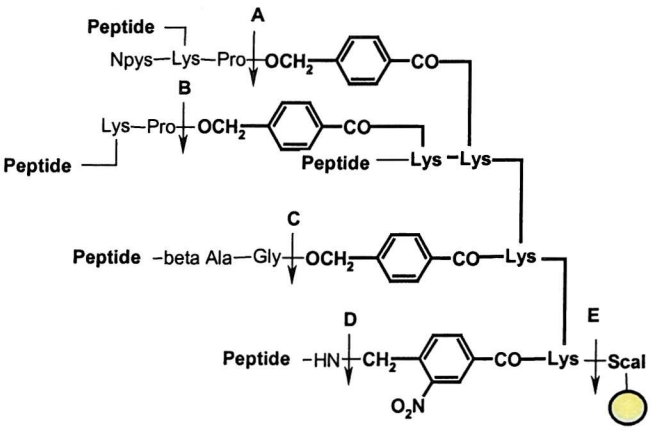


Figure 4: Release A) 0.1M Triphenylphosphine/tosic acid/dimethylformamide via diketopiperazine formation; Release B) 50% Trifluoroacetic acid via diketopiperazine formation; Release C) Sodium hydroxide; Release D) Photolysis; Release E) 1M Trimethylsilylbromide/thioanisole/trifluoroacetic acid.

The problem was overcome using a modified approach where the peptides were released with NaOH and TFA promoted diketopiperazine formation and the active compound being determined by Edman degradation of the third strand still attached to the solid-phase. Lam`s group designed another generation of multiple cleavable linkers using iminodiacetic acid as the key component.[19] After synthesis the first release occurred at pH 8.5 after activation with TFA and diketopiperazine formation.
Bradley[20] has used an alternative approach such as the use of a mixture of 3 different linkers added in a 1:1:1 ratio onto amino methyl resin (Figure 5).

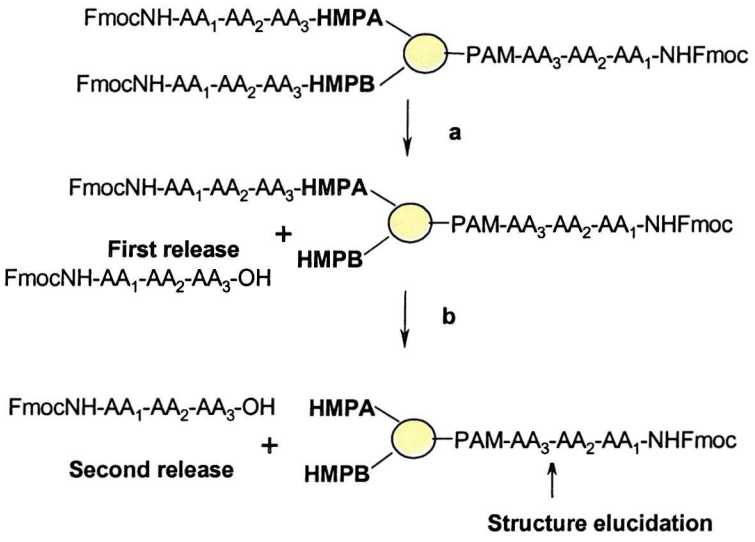


Figure 5: a) 1% Trifluoroacetic acid/dichloromethane; b) 95% Trifluoroacetic acid/dichloromethane.

Bradley[21] also provided an example of a multiple release and encoding strategy with a two-linker system used for screening of an inverted C-terminal modified peptide library.

1.5 Encoding Strategies

Structure determination of positive hits from pool libraries can also be made through the coupling of a tag or code.

1.5.1 Peptide and DNA encoding

The first encoding approaches used peptides[26] and oligonucleotides as tags.[27-29] Oligonucleotide tagging will be discussed in more detail chapter 5. With an amino acid encoding strategy (Figure 7) instead of defining a small number of the sites for the coding strand a bifunctional molecule such as lysine was used. The α-amine was protected with a base labile Fmoc group and the ε-amine (used for the coding strand synthesis) was protected with the mild acid labile Ddz group (removed with 5% TFA). Four amino acids (Phe, Ala, Leu and Gly) were used to code each building block by a sequence of three amino acids for example Phe-Ala-Leu for one building block. The coding technique gave rise to 64 coding possibilities. At the end of the synthesis phenylalanine was added to the coding strand to serve as an internal standard during Edman sequencing. Edman degradation of the coding strand provided the sequence.

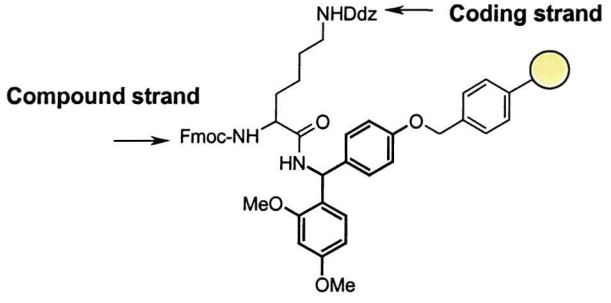


Figure 7: Amino acid coding strategy.

Lam and co-workers[19] employed a similar approach with Fmoc protection for the coding strand and Boc protection for the compound strand. The screening assay was carried out in solution with the compound being released from the bead and the coding

strand remaining on the resin. The major drawback of this technique was that it required the compound to be spatially addressable to the bead from which it came. The same group also developed another approach[13] whereby the coding area and screening area were actually physically differentiated. To do this they used an enzyme shaving method, which allowed the interior of the bead to the coding region and the surface of the bead to be the screening area. Boc chemistry was used for the internal sites and Fmoc chemistry for the external sites.

After selection of the active bead the interior sequence was determined by Edman degradation and the active sequence therefore deduced. "Encoded amino acid scanning" was a technique described by Camarero and co-workers.[30] It is a method where, defined sequences of N-Boc amino acids containing a cysteine with an Fmoc protected thiol were attached to a PAM derivatised resin. A library with two modified residues was then synthesised. When a modification occurred at one position the cysteine was deprotected and encoded with the appropriate amino acid. After selection of the active peptides the tags were removed from cysteine by treatment with Hg $(OAc)_2$ revealing the modification on the active peptide.

1.5.2 Haloaromatic Tags for Binary Encoding.

Oligonucleotide and peptide codes however have their drawbacks for use in the synthesis of small molecule libraries. This is due to the sensitivity of peptides and even more so the oligonucleotides to some common reaction conditions such as strong bases reducing agents and acidic conditions. Much more stable tags were therefore required and a major achievement was reported by Ohlmeyer and Still[31-32] with the introduction of electrophoric tags. (Figure 8).

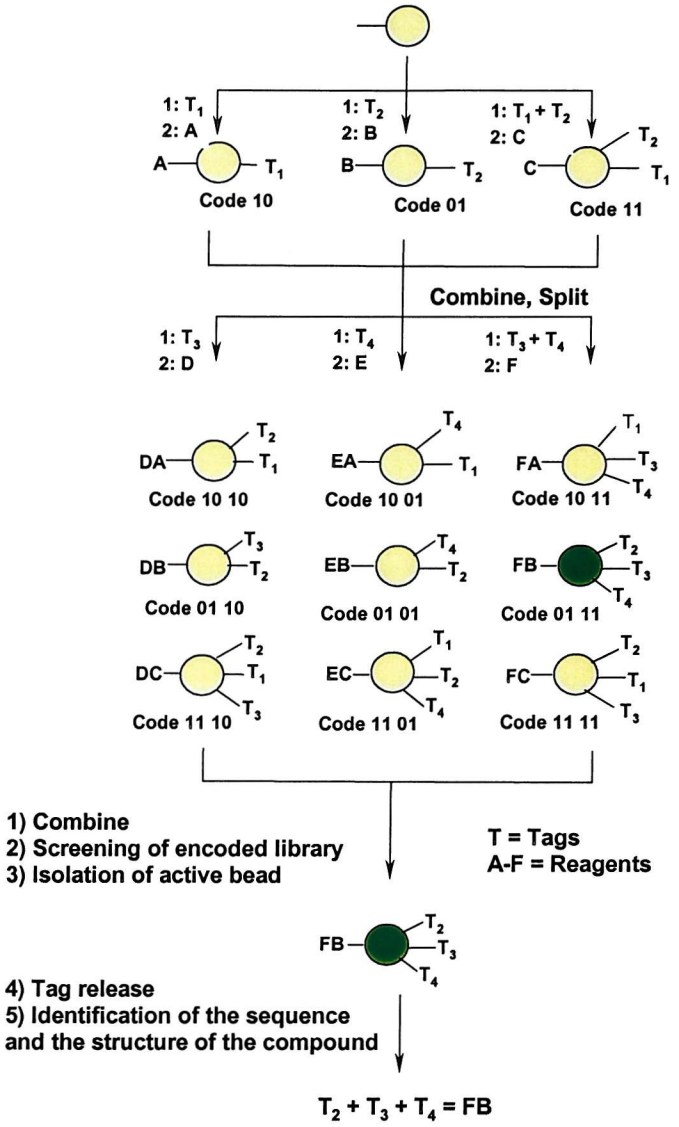


Figure 8: Haloaromatic tags for binary encoding

In this coding technique (Figure 8) chemical tags recording the sequence are attached to the resin bead concurrently with the synthesis of the compound on the bead. The encoding sequence is based on a binary approach. At the end of the synthesis each bead will have its own collection of tags. A big advantage of the approach is that only a small proportion of the resin needs to be functionalised. Still used the strategy preparing haloaromatic molecules by alkylation of commercially available halophenols with ω-bromo-1-alkanols. The tags could be detected at levels less than 0.1 pmol using electron capture gas chromatography. This meant that levels of only 1% molecular tags were required for encoding. This therefore minimized the problems in library evaluation resulting from tag-receptor interactions using support bound assays.[34]

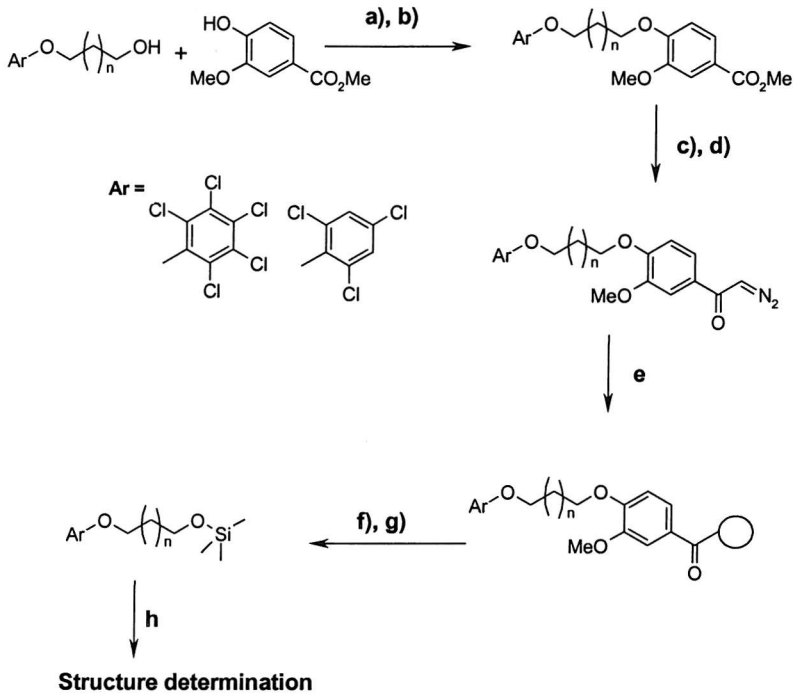


Figure 9: Haloaromatic Tags: a) Triphenylphosphine, diethyl azodicarboxylate, rt; b) Lithium hydroxide; c) Oxalyl chloride; d) Diazomethane; e) resin, rhodium trifluoroacetate; f) cerium ammonium nitrate; g) Bis-trimethylsilane acetamide; h) EC-GC characterisation.

The tags (Figure 9) were modified so that when they were added to the support as acylcarbenes generated by the treatment of diazaacetates with rhodium trifluoroacetate, with no need for orthogonal sites on the resin beads as the carbenes react in a non-specific manner with the beads and the library members, however the amount of modified library member was negligible. Tag cleavage was performed via oxidative cleavage with cerium ammonium nitrate and silylation of the resulting alcohols. The silyl derivatives were then characterised by EC-GC. This system has been widely used by Still and other groups.[34-36]

1.5.3 Secondary Amine Tags

In 1996 workers at Affymax reported an encoding strategy based on the use of chemically robust secondary amines.[37]

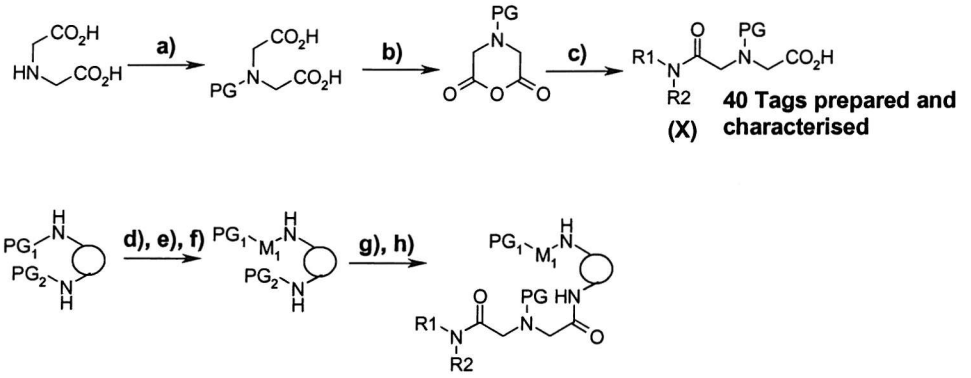


Figure 10: Secondary amine tags a) N-Protection; b) Triphosgene; c) Coupling with R_1R_2NH; d) PG_1 deprotection; e) Resin portioning (1 to n); f) Coupling with M_1 (PG_1); g) PG_2 deprotection; h) Coupling with (X).

These tags (Figure 10) and (Figure 11) were incorporated as part of a polyamide backbone. The technique employed amino functionalised resin that was then differentially functionalised with sites for ligand synthesis (90%) and sites for tag addition (10%) using orthogonal protecting groups. At each step of the split and mix synthesis the addition of a building block was recorded by coupling the appropriate mixture of tag. After screening the active beads were isolated and treated with 6N (HCL) in a capillary tube in order to release the secondary amine tags (Figure 10) which were reacted with Dansyl chloride (Dns) and analysed using an HPLC fitted with a fluorescence detector. Affymax has used and optimised the approach.[38-40]

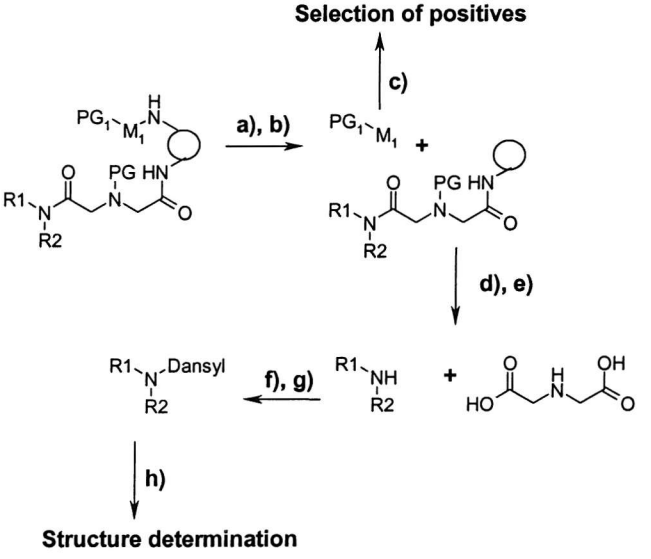


Figure 11: Secondary amine tags: decoding protocols. a) PG_1 deprotection; cleavage of library components; c) screening; d) PG_2 deprotection; e) Hydrochloric acid 6N, reflux, 15 hrs; f) Lithium carbonate; g) Dansyl chloride; h) HPLC/CEC decoding.

1.5.4 Ladder Scanning Strategy.

This is a technique first reported by Youngquist[22] that allows the determination of a peptide sequence on a single bead by mass spectrometry. At each step of the synthesis a small part of the peptide is capped to block the synthesis. After screening, the active bead is removed and the peptide cleaved furnishing a series of truncated products, which contain the final compound and the family of its capped precursors. The structure is identified due to the mass difference between the ladder fragments. The method can be compared to the sequencing of peptides by enzymatic degradation (Figure 6).

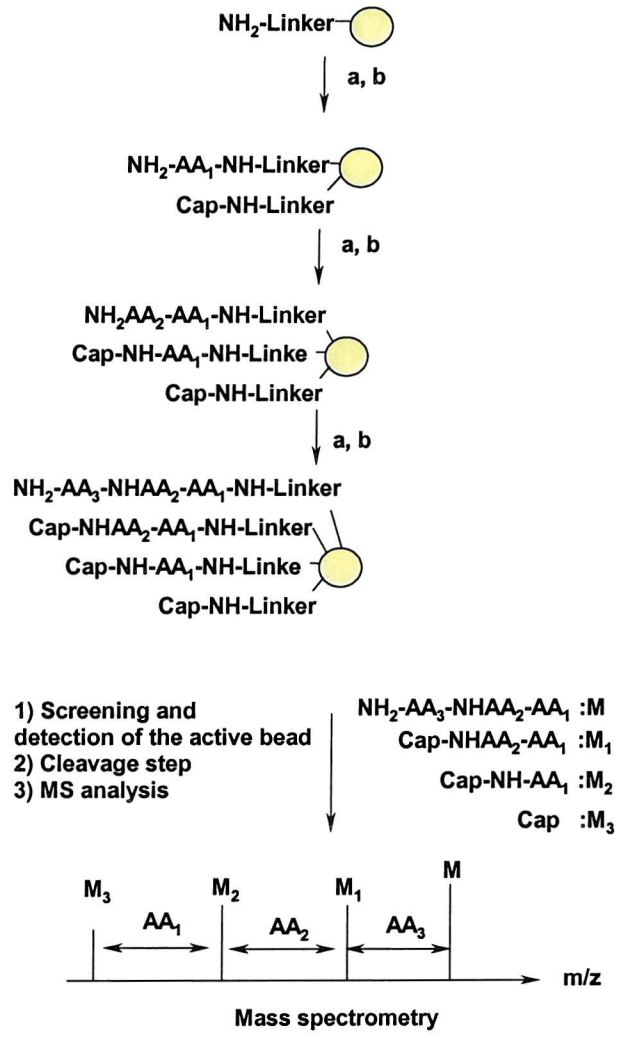


Figure 6: Ladder scanning strategy.

Burgess[23] has employed a modified approach for the synthesis of a library of tetrapeptides on TentaGel. Here the truncation was not performed by partial capping but

by insertion of a photolabile linker on 5 % of the amino sites. At the end of the synthesis photolytic cleavage produced a mixture of different fragments, which were analysed by MALDI-TOF. Bradley has also reported a method[24] of ladder identification based on the incorporation of Fmoc methionine 5-10% with each amino acid. Treatment with BrCN in TFA/H_20 for 24 hours resulted in cleavage of a mixture of different sized peptides. Bradley[25] has reviewed mass spectrometric analysis in combinatorial chemistry.

1.6 Non-chemical encoding

Using tagging methods that do not require a covalent bond between the tag and the solid support is an appealing approach as it reduces the chemical complexity and it ensures a direct link between the code and each library member. Several tagging procedures of this kind have been reported.[41-44] The most important non-chemical encoding technique uses radiofrequency tags to encode chemical libraries.[43-44] In the technique each compound is prepared in a specific reaction vessel/device where a radiofrequency tag is located and each tag is pre-encoded before synthesis with a readable signal. At any stage of a solid-phase synthesis step the code can be read on a coding station and the structure of the compound can be determined.

1.7 Solid-Supports

Hydrophobic polystyrene beads has been the subject of many studies and reviews[45-50] and are the most commonly used support in solid-phase synthesis. They are representative of a class of so-called gelatinous solid supports consisting of polystyrene with 1-2% DVB.[51] These supports swell in most organic solvents. Swelling depends on the solvent used and apolar solvents can cause the polystyrene resin to swell 3 to 8 times the dry volume. Swelling is however poor in polar solvents. This problem of polar solvent incompatibility has been overcome by the grafting of hydrophilic monofunctional or bifunctional PEG chains on to the polystyrene resin producing hybrid supports (Figure 12) which allow the use of hydrophilic solvents.[52]

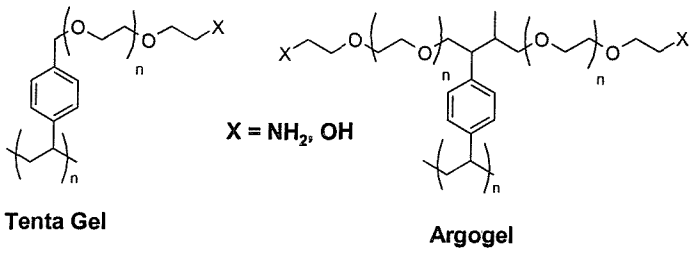


Figure 12: Commercially available hybrid PEG-PS resins.

The higher degree of flexibility of the terminal PEG chain produces a real solution like microenvironment and this allows on-bead reaction monitoring by ^{13}C NMR and MAS ^{1}H NMR. Loading of grafted resin is however low in comparison to polystyrene resins for example between 0.2 and 0.3 mmol per gram for TentaGel.

PEGA resin which is produced by radical polymerisation of acrylamide substituted PEG chains is ideal for aqueous solvents and allows inner penetration of macromolecular reagents.[53]

1.8 High Throughput Screening and Screening technologies.

High throughput screening (HTS) is the process of testing a large number of diverse chemical structures against disease targets to identify hits.[54, 55] Central to the HTS process is an *in vitro* biochemical or cell-based assay using a validated biological target which represents a disease state. Standard (HTS) assays are currently run in 96-well microtitier plates in batch formats although there has recently been a move towards miniturization.

Common therapeutic targets for (HTS) are enzymes, cell surface receptors, nuclear receptors, ion channels and signal transduction proteins. Compounds that interact with these targets are usually identified using *in vitro* biochemical assays, however cell based assays using engineered mammalian cell lines are now widely employed. The target does not need to be purified extensively in order to be compatible with the *in vitro* screening conditions. A major disadvantage however is the cost and difficulty of producing stable engineered eukaryotic cell lines.

Detection Technologies

The detection technologies employed depend on the type of biochemical pathway under investigation. For example *in vitro* receptor binding assays with Kd values in the nano

to pico molar range generally employ radiometric detection. The same is true for protein-protein interaction assays with Kd values in the micro to nano molar range. Enzymatic assays however employ colorimetric, fluorimetric and radiometric detection.

1.8.1 Radiochemical Methods

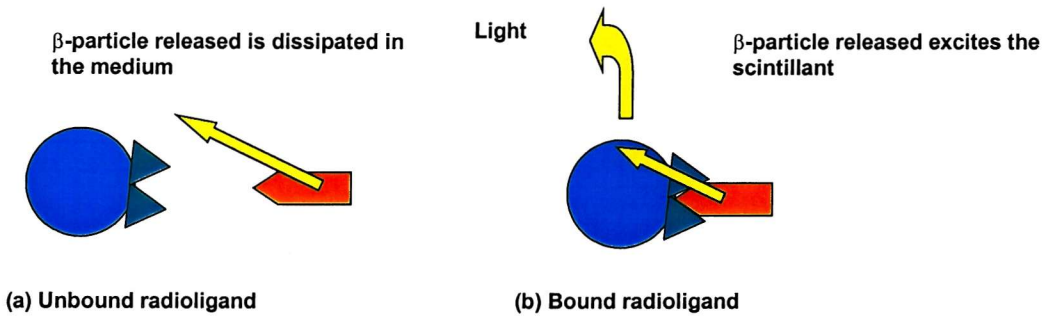


Figure 13: Principles of scintillation proximity assay (SPA) technology. (a) The path length of decay for the β-particle released by the isotope is not close enough to the SPA bead (shown in blue) and the energy is dissipated in the aqueous medium resulting in little or no detection. (b) When the radioligand (shown in red) is bound to the SPA bead (through a specific capture molecule shown in green) the β-particle released is capable of exciting the scintillant contained within the bead and detectable light is emitted.

The scintillation proximity assay (SPA) (Figure 13) has been the standard assay in many (HTS) operations, mainly because it does not require a separation step and can be easily automated. It can also be adapted to a variety of enzyme and protein-protein interaction assays. One version of the (SPA) utilises polyvinyl toluene microspheres or beads (5 μM diameter) into which a scintillant has been incorporated. The outer surface is coated with a hydrophilic polyhydroxy film that reduces the hydrophobicity of the bead and hence non-specific interactions. Ligands are usually radiolabelled with ^{3}H or ^{124}I. These elements are used due to their relatively short path lengths ^{3}H emits β-particles with a path length of 1.5 μm and ^{125}I between 1 and 17.6 μm. When a radiolabelled ligand is captured on the outer surface of the bead, radioactive decay occurs in close proximity to the bead and effectively transfers energy to the scintillant, which results in light emission. When the radiolabel is displaced or inhibited from binding to the bead it

remains free in solution and is too distant from the scintillant for efficient energy transfer. Energy dissipated into solution results in no light emission from the beads.

1.8.2 Non-Isotopic Detection Methods

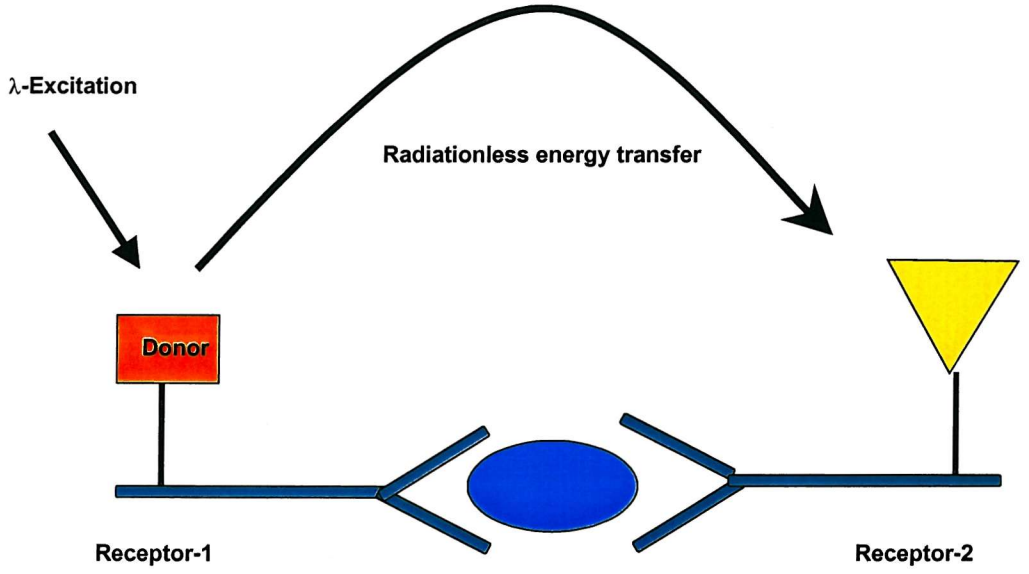


Figure 14: Principles of fluorescence energy transfer. The transfer only occurs when the donor (shown in red) and acceptor (shown in orange) are in close proximity via binding to the same ligand (shown in blue).

For fluorescence based binding assays in homogeneous format, three assay techniques are available to quantify bound from unbound species. Fluorescence resonance energy transfer (FRET) assays (Figure 14) can be designed using a donor and an acceptor. This system was one of the earliest methods developed for (HTS). A peptide substrate for a HIV protease was synthesised with EDANS (at the amino terminus) as the donor fluorophore and DABCYL (at the carboxy terminus) as the acceptor chromophore.[56] The disadvantages of this method is that in the EDANS-DABCYL pair many organic and natural products absorb the absorption and emission maxima of EDANS which is (λ_{ab} ~ 340 nm λ_{em} = 490 nm). They can also quench the EDANS fluorescence generating false positives.

Time Resolved Fluorescence

Homogenous time resolved fluorescence addresses the problems described with the EDANS-DABCYL system. The assay uses FRET between two fluorophores (a europium cryptate and a 105 Kda phycobiliprotein, allophycocyanin) as labels. The Eu-trisbipyridine cryptate (TBP-Eu^{3+} λ_{ex} = 337 nm) has two bipyridyl groups that harvest light and channel it to the caged Eu.$^{3+}$ It has a long fluorescence lifetime and non-radiatively transfers energy to allophycocyanin when the two labels are in close proximity (50 % transfer efficiency at a donor acceptor distance of 9.5 nm) The resulting fluorescence of allophycocyanin (λ_{ex} = 665 nm) retains the long lifetime of the donor TBP-Eu^{3+} allowing a time resolved measurement. The first (HTS) assay for a protease enzyme (Herpes simplex virus type-1) was described by Kolb *et al.*[57]

Fluorescence Polarisation

Fluorescence polarisation[58] is another technique that has gained popularity. The technique utilises fluorescently tagged molecules and when fluorescently labelled molecules in solution are illuminated with plane polarised light the emitted fluorescence will be in the same plane provided the molecules remain stationary. As molecules are not at rest depolarisation of fluorescence emission occurs. At a constant viscosity and temperature, polarisation is directly proportional to molecular volume. Hence changes in molecular volume or weight due to binding interactions can be detected as a change in polarisation.

1.9 Cell Adhesion

Several physiological processes such as the immune response, wound healing, embryogenesis and cell differentiation require and are strongly influenced by cell-cell and cell-extracellular matrix interactions. These interactions are also very important in various disease states, most notably cancer. Cell-cell, cell-matrix interactions are mediated through four different families of cell adhesion molecules (CAMS) selectins, cadherins, integrins and immunoglobulins.[59-60] There have recently been many advances made in understanding several CAMS most notably the $\alpha_v\beta_3$, $\alpha_v\beta_5$, $\alpha_4\beta_1$, $\alpha_{IIIb}\beta_3$, integrin[61-63] receptors and they represent tremendous therapeutic and diagnostic opportunities in the treatment of cancer, cardiovascular, inflammatory, bone and pulmonary disorders. Table 1.

Table 1

Therapeutic area	**Diseases**	**CAM**
Cardiovascular	Thromboembolic disorders	$\alpha_{IIIb}\beta_3$, selectins
	Restenosis	$\alpha_v\beta_3$, selectins
	Atherosclerosis	β_1, β_2, VCAM-1
Inflammatory	Rheumatoid arthritis, osteoarthritus	β_1, β_2, selectins, ICAM-1
	Transplantation	
Cancer	Metastasis	$\alpha_v\beta_3$, β_1, β_4, β_5,
Ocular	Diabetic retinopathy, Macular degeneration	$\alpha_v\beta_3$, $\alpha_v\beta_5$
Pulmonary	Asthma, allergy	$\alpha_4\beta_1$, VCAM-1
Bone	Osteoporosis	$\alpha_v\beta_3$, β_1, β_5
Central nervous system	Multiple sclerosis, neurological disorders	$\alpha_4\beta_1$, VCAM-1, ICAM-4
Gasterointestinal tract	Bowel diseases	β_1, β_4, β_6, β_7
Kidney	Renal failure	β_1

1.10 Integrins

The recognition of integrins as a widely expressed family of cell surface adhesion receptors began 1987.[63] There are now approximately 20 distinct vertebrae integrins which have been identified and the number is still rising. Integrins appear to be the major receptors by which cells attach to the extracellular matrix and some integrins mediate important cell-cell adhesion events (Figure 15). They therefore play important roles in development and in adult organisms. The ability to affect integrin function using antibodies or peptides offers many opportunities for therapeutic intervention in the disease states already mentioned. Due to their many roles integrins have been intensively studied by scientists in many different fields. Below the figure shows the overall shape of integrins deduced by electron microscopy.

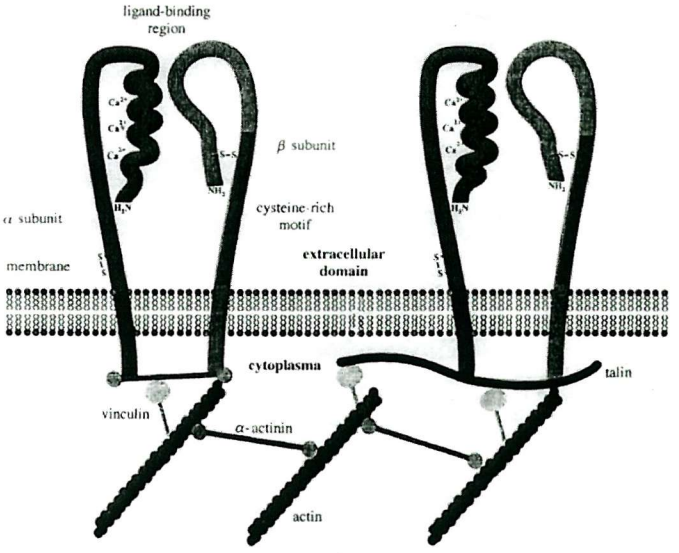


Figure 15: Shape of integrins as deduced by electron microscopy. Used with permission.[62]

All integrins are α, β heterodimers. The α subunits vary in size between 120 to 180 Kd and are covalently associated with a β- subunit 90-110 Kd in size. Most integrins are expressed on a wide variety of cells and most cells express several integrins. Table 2 shows the diversity of vertebrae integrins. Although eight β subunits and fourteen α subunits could in theory associate to give more than 100 integrin heterodimers the actual diversity appears to be much more restricted. Many α subunits can associate with

only a single β subunit however several α subunits for example (α_4, α_6, α_v) can associate with more than one β subunit and α_v is the most promiscuous in this respect. Integrins bind divalent cations and it is known that these are essential for receptor function, the nature of the cations can effect both affinity and specificity for ligands.[64-68] The position of the ligand binding site(s) within which the integrin subunits have been deduced.[69-71] Chemical crosslinking data, puts the ligand in close proximity to a segment of the β subunit. The evidence thus points to a model in which both α and β subunits contribute to a ligand binding site that lies at the interface between the two subunits.

1.11 Ligands for integrins.

The RGD cell adhesion sequence was discovered in fibronectin in 1984.[72-73] The finding that only three amino acids would form an essential recognition site for cells in a very large protein was initially viewed with scepticism, but this observation was later confirmed with fibronectin and further proteins.[74] (Figure 16) shows fibronectin and the various regions where the integrin ligands are found.

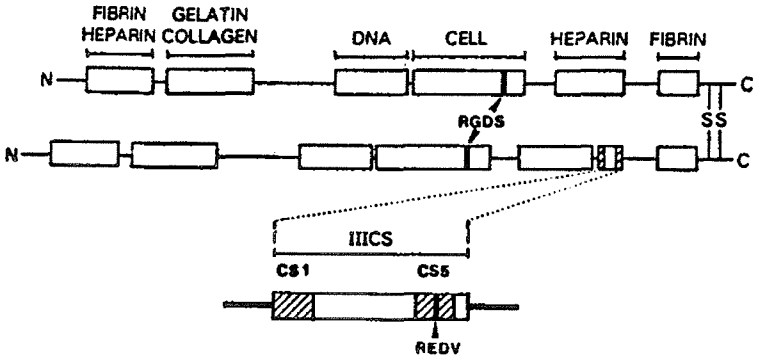


Figure 16: Representation of fibronectin. Used with permission.[77]

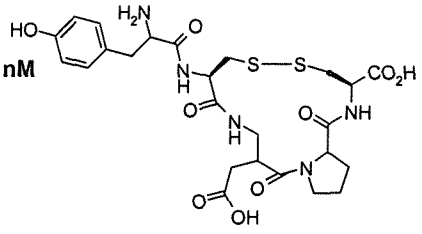
Fibronectin (Figure 16) is a cell adhesion protein widely distributed in the tissues of all vertebrates. It is present as a polymeric fibrillar network in the extracellular matrix and as soluble protomers in the body fluids.[75-77] Fibronectin contains at least two major domains that support cell adhesion. The first is the central domain recognised by a variety of cell types, (RGD is located here) and the second is located in the alternatively spliced type III connecting segment IIICS the first cell type-specific adhesion site within this region is called CS1 and it comprises 25 amino acids. From this it was found that

the EILDVPST peptide sequence supported melanoma cells spreading as well as EILDV and LDVPS. The importance of this LDV sequence is also evident if the human amino acid sequence is compared with other species. It can be seen that it is completely preserved in the III CS regions of rat, bovine and avian fibronectins. The receptor for CS1 is the integrin $\alpha_4\beta_1$.

Synthetic peptides containing these sequences are competitive inhibitors of cell adhesion to fibronectin. They can also mimic cell adhesion proteins when coated on a surface. The affinity of these peptides is however low and RGD is about 1000 times less effective in cell adhesion assays than fibronectin itself.[78] LDV and RGD ligands are specific in their activity, changes in as much as the replacement of Asp with Glu or Gly with Ala can significantly reduce activity and the Asp residue must be of the L form as the D-form is inactive. In order to increase this low affinity cyclic versions of the peptide sequences have been investigated.[79-83] **(A), (B)** (Figure 17) as well as rational modifications to the peptide backbone.[84-85]

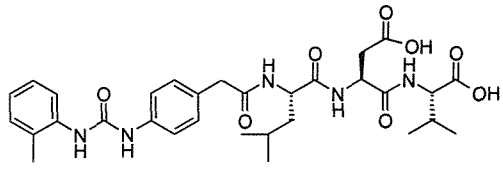
Fibronectin IC_{50} = 9 nM

(A) IC_{50} = 4 nM



(B) IC_{50} = 4 micro M **Cyclo(lLeu-Leu-Val-NH$(CH_2)_2$-S$(CH_2)_2$CO)**

(C) IC_{50} = 6 nM



(D) IC_{50} = 1-100 nM

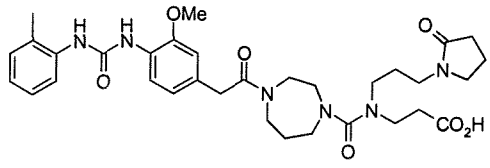


Figure 17: RGD and LDV ligands.

1.12 Multivalent compounds.

Many biological binding events operate through multivalent interactions, for example the adhesion of the influenza virus to epithelial cells,[86-87] (Figure 18) the attachment of neutrophils to cells close to a site of inflammation[88-90] and the attachment of antibodies to antigens.[91-94] The importance of these interactions have only begun to be unravelled, particularly as some multiple binding interactions between a multivalent ligand and multivalent receptor can be orders of magnitude stronger than the binding of the corresponding monovalent ligand.

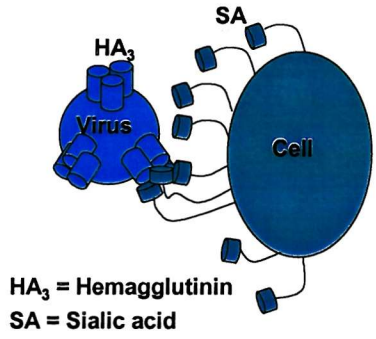


Figure 18: The influenza virus attaches to cells by the interaction of trimeric hemagglutinin (HA_3, shown as protruding cylinders on the virus) with sialic acid (SA, shown as caps on the cell).

Multivalency can be viewed as a means by which nature has amplified weak interactions, and this is the case with the exceptionally weak affinity of native saccharide ligands for their receptors.

Mechanism

The mechanism for the increases in affinity has mostly been attributed to entropically enhanced binding due to a chelate-type effect.

Enhanced affinity in polyvalent interactions

Understanding the entropy of polyvalent interactions is essential to understanding the relationship of monovalent to polyvalent binding.[100] Polyvalent systems have entropically enhanced binding. The total entropy of a polyvalent interaction ΔS^{poly} can be considered in terms of contributions from changes in translational, rotational and conformational entropies as well as a contribution accounting for changes in the entropy

of surrounding water. The most important for understanding why we get stronger interactions is conformational entropy. We can dicuss the role of entropy in polyvalent interactions using a simple bi-valent system as a model.

Case 1: $\Delta S_{conf} = 0$

For the association of two monovalent receptors with two monovalent ligands (Figure 19) the total entropic cost is 2 ΔS_{trans} + 2 ΔS_{rot}. If the ligands and the receptors are both connected by a rigid linking group, having precisely the correct spacing to match the two receptor ligand sites then the total entropic cost of assembling these two bivalent species is approximately ΔS_{trans} + ΔS_{rot} (half the entropic cost of association of two independent pairs of molecules and the same as that of one monovalent interaction. This is due to a neighbouring group effect because once a single ligand–receptor interaction occurs between these two rigid bivalent species, subsequent *intra*molecular interactions between the second ligand moiety on the rigid bivalent species and the receptor site can occur without additional translational and rotational entropic cost, and at no cost to conformational entropy. If there are no additional enthalpic costs the binding of the second ligand to the second receptor (intramolecularly) occurs with a greater change in free energy than does the first. Such binding is entropically enhanced.

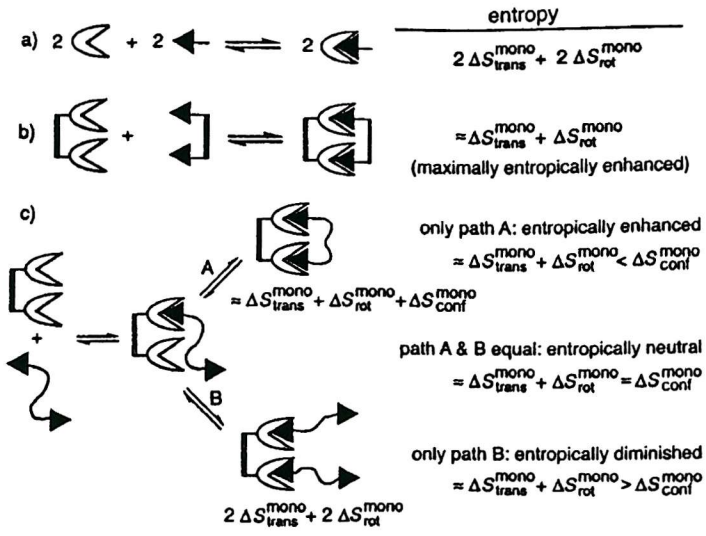


Figure 19: Relationships between translational, rotational and conformational entropies for a divalent system with a rigid and flexible linking group. Used with permission.[100]

Case 2: $\Delta S_{conf} \neq 0$

Case 1 is in general unrealistic: All linking groups are somewhat flexible and ΔS_{con} is almost always less than zero on complexation. This means the number of conformations open to a bi-valent ligand before complexation is greater than after complexation. If this conformational cost is less than the total translational and rotational cost, then the total entropic cost of bivalent association is still less than for the monovalent case and binding is still entropically enhanced. If the translational and rotational costs equal the conformational cost then the binding is entropically neutral and the total energetic cost of complexing a second ligand on the dimeric species is the same as complexing the second dimeric species.

Although conformational flexibility increases the conformational entropic cost of association, the same flexibility increases the likelihood that all ligand receptor interactions can occur without energetic strain.

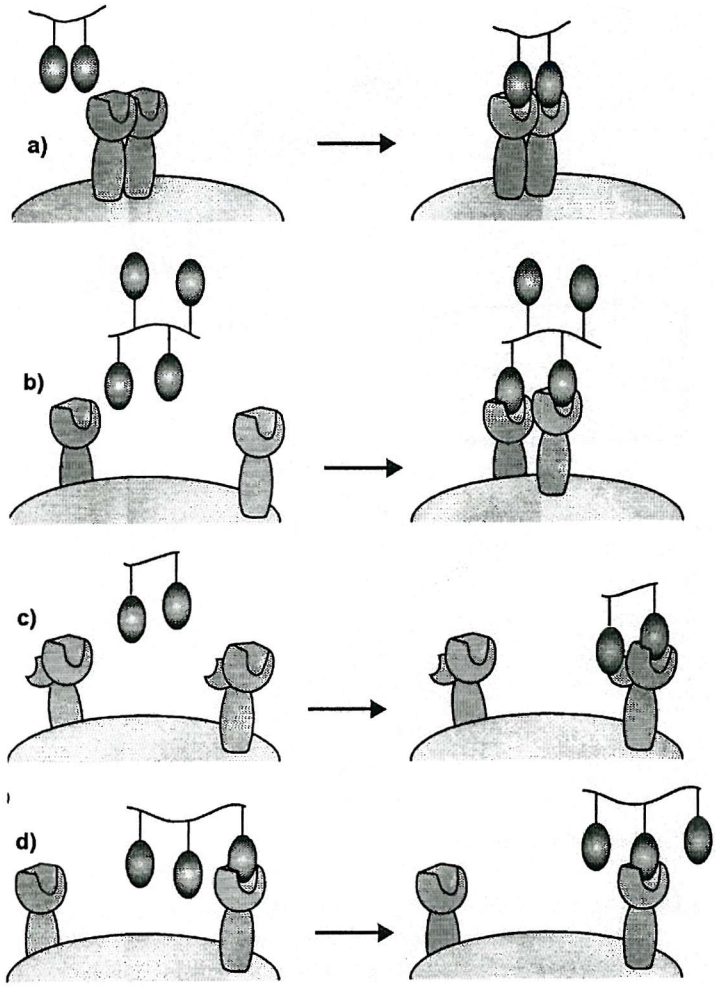


Figure 20: a) Multivalent ligands can bind oligomeric receptors by occupying multiple binding sites (chelate effect; b) Multivalent ligands can cause receptors to cluster on the cell surface ; c) Multivalent ligands can occupy primary and secondary binding sites on

a receptor; d) Multivalent ligands display higher local concentrations of binding epitopes, which can result in higher apparent affinities. Used with permission.[95]

Other mechanisms (Figure 20) that are attributed to the increased affinity of multivalent receptor-ligand interactions[95] are that multivalent compounds cause receptors to cluster on the cell surface, which leads to the activation of signalling pathways. Multivalent ligands can occupy primary and secondary binding sites on a receptor and multivalent ligands display higher local concentrations of binding epitopes. Although the mechanisms for the increased affinity of multivalent receptor-ligand interactions are not fully understood they are certainly stronger than monovalent interactions.

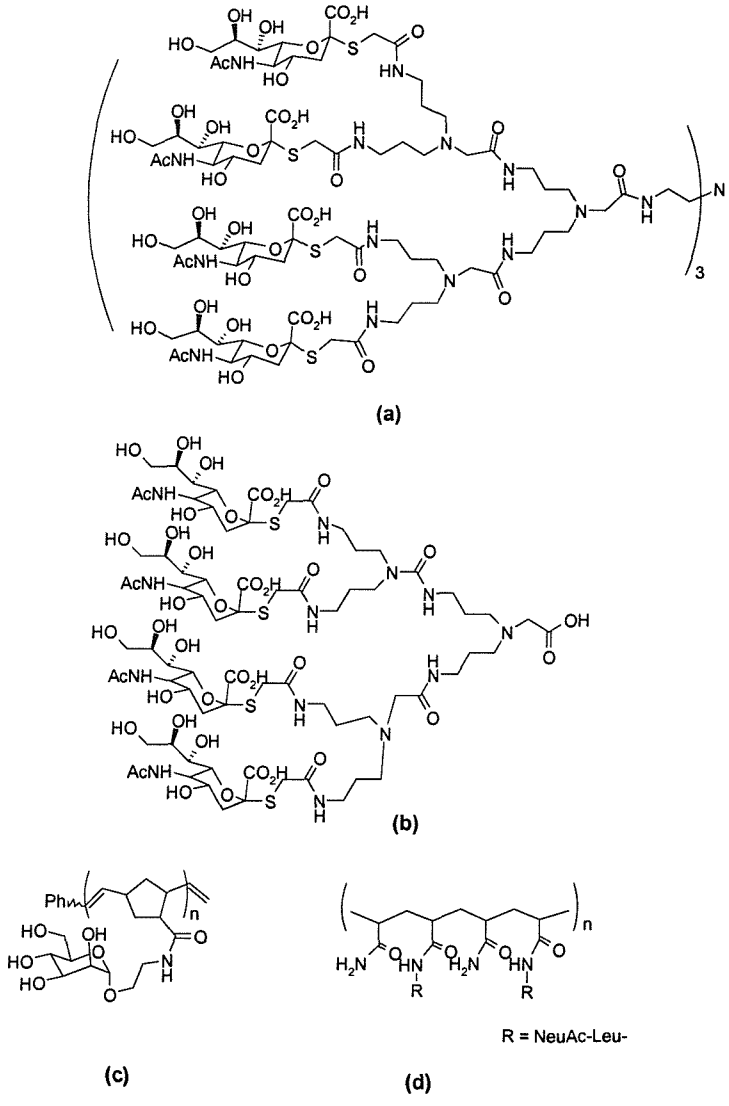


Figure 21: Multivalent inhibitors.

Random/Statistical Multivalency

Realising that multivalency can improve weak affinity many groups have developed high affinity lectin ligands through multivalent expression.[95-116]

Roy and co-workers[106-113] have developed several types of neoglycoconjugates including random coil glycoconjugates, hyperbranched polymers, glycodendrons and glycodendrimers. The dendrimers used included a tethered variety, **(a)** (Figure 21), a divergent variety **(b)** (Figure 21) that display the sugar-binding moiety in a "random" high-density fashion. The biological testing of the structurally similar tethered and divergent α-sialodendrimers in an ELLA assay revealed compounds with nM potency but with an increase in relative potency in comparison to monovalent forms of 127 for the tetra-valent divergent dendrimer **(b)** yet 182-fold for the 12-valent tethered dendrimer **(a)**.

Kiessling and co-workers[95-99] favoured the linear polymer approach and have used ROMP to produce defined length polymers with active *N*-hydroxysuccinimide esters, which allowed the attachment of α-mannose derivatives **(c)** (Figure 21). When tested for their ability to inhibit cell agglutination using a Concavalin A inhibition assay the relative potency of the best polymer to the monomer was 550-fold.

Whitesides[100-105] and co-workers generated linear polymers derivatised from poly (acrylic acid) having multiple units of *N*-acetylneuraminic acid (NeuAc-L-NH_2) on the side chain **(d)** (Figure 21). The polymers were assayed for their ability to inhibit hemagglutination of chicken erythrocytes by influenza virus A (X-31). Potent inhibitors were found with affinities 10^6 times that of the monomer.

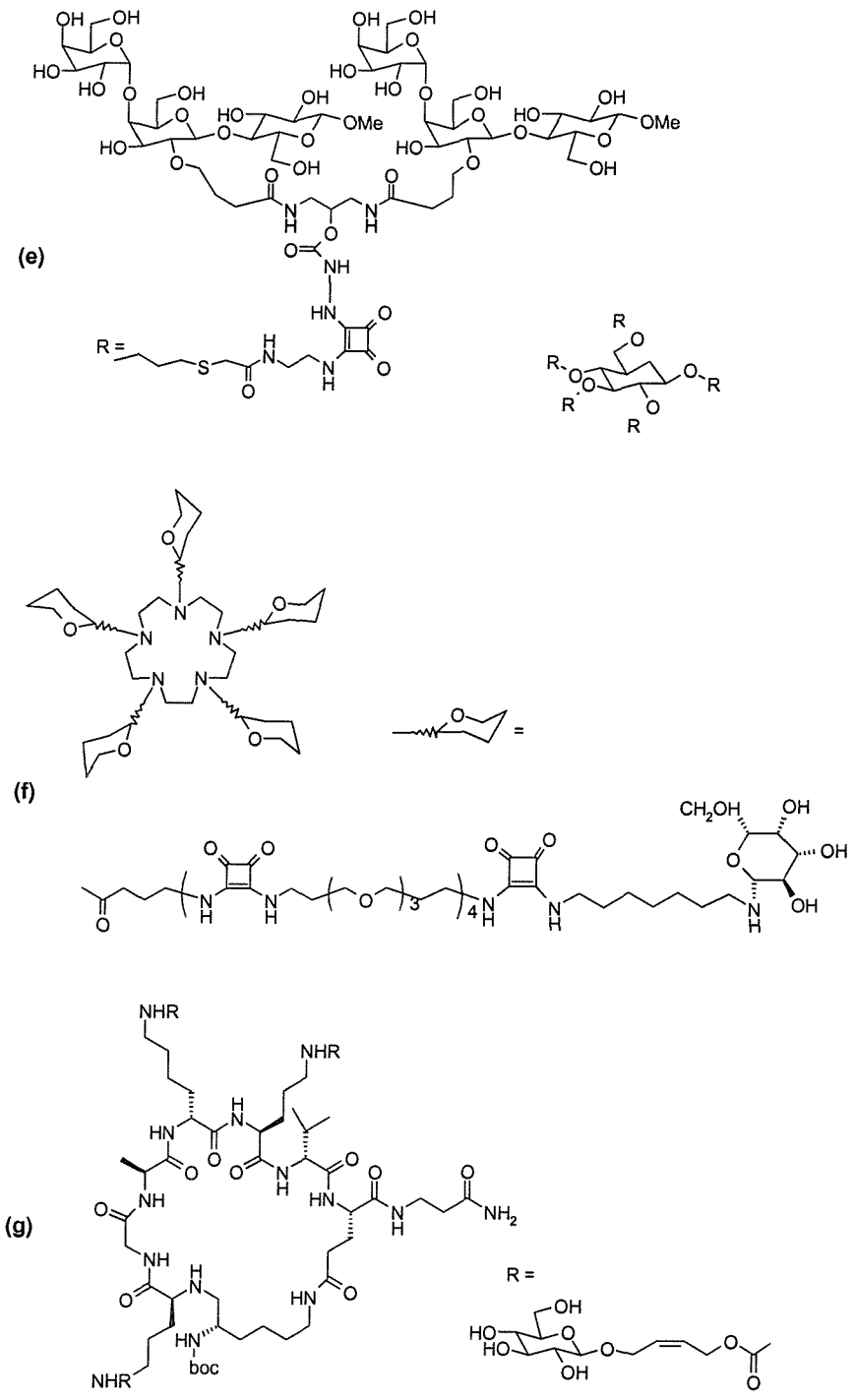


Figure 22: Multivalent ligands synthesised with more design.

Tailored multivalency.

As described above many strategies have been employed in order to enhance interactions between receptors and glyco-conjugates. The glycopolymer conjugates employed are able to cover large areas of cell surfaces providing (statistical multivalency). Another approach, (which came to the fore in the early work by Lee[116]

and co-workers was to take a more rational approach designing the ligand in order to achieve the best fit for the binding site. This approach has recently led to the synthesis of ligands with astonishing affinity, a decavalent molecule[118] **(e)** (Figure 22) with a 10 million fold and a pentavalent molecule[119] **(f)** (Figure 22) with a 104,000-fold increase in potency was observed. This is in contrast to the glycopolymers mentioned earlier which rarely achieved greater than a 1,000-fold improvement over the monovalent ligand.

Combinatorial approach.

The idea that the fit/orientation of the ligands being important has inspired a combinatorial approach[120] to the problem in order to try and "hit" the required orientation of the sugar residues, using the conformational diversity of D- and L- amino acids arranged in a cyclic structure **(g)** (Figure 22). No affinities of these compounds however have been reported.

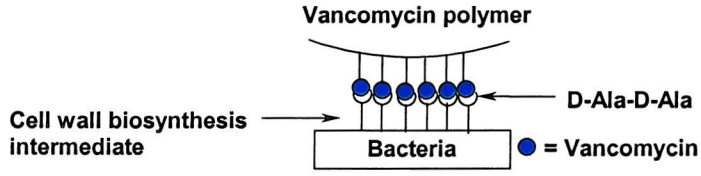


Figure 23: Multivalent vancomycin.

Non-carbohydrate multivalent compounds.

It is evident from the sheer number of papers in the area of carbohydrate multivalent compounds, that the enhancement of weak non-bonding interactions of sugar ligands can be improved by the "cluster effect" the idea however has not been widely extended to the recognition of complex natural products or peptides. An example of a multivalent polymer of vancomycin[121] (Figure 23) synthesised by ROMP has shown significant 8-6-fold enhancement of antibacterial activity against vancomycin-resistant enterococci (VRE). Synthesis of multivalent biocompatible polyamides[122] with a defined number of repeat units has been reported with a view to publishing the biological assays. Although not multivalent there is current interest in dimeric analogues of DNA mono-intercalating agents[123] as potential anti-cancer drugs. The compounds, which were

evaluated for their ability to inhibit growth activity in tumor cell lines, were found to be 2-9.5-fold better than monomeric counter parts.

Multivalent effectors

Multivalent effectors have an application in understanding signal transduction pathways.[124] This approach has been used in the mimicry of erythropoeitin (EPO), which upon binding induces activation of many intracellular signalling molecules.[124] However the most common synthetic effectors have been those that provoke an immune response and attempts to produce an anticancer vaccine particularly with carbohydrate antigens has generated a lot of synthetic effort.[125-126]

Chapter 2:
Solid-Phase Synthesis of Dendrimers for the Preparation of a Multivalent Ligand Scaffold

Chapter 2 Synthesis of Dendrimers on the Solid-Phase for the Preparation of a Multivalent Ligand Scaffold.

2. 1 Introduction to Dendrimers

Dendrimers are three-dimensional highly ordered oligomeric compounds. The term "dendrimer" comes from the greek word for tree "dendron" and was first used by Tomalia to describe his polyamidoamine (PAMAM) dendrimers.[127, 133] Although the concept of highly branched structures had been around since the early 1940`s they were really only considered from a theoretical point up until the 1970`s[128] the main rationale for this being that synthetic attempts via polymerisation of functionally differentiated monomers were fruitless.[129]

In 1978 Vögtle carried out the first successful dendrimer synthesis[130] (Figure 24) introducing the concept of a "cascade reaction"

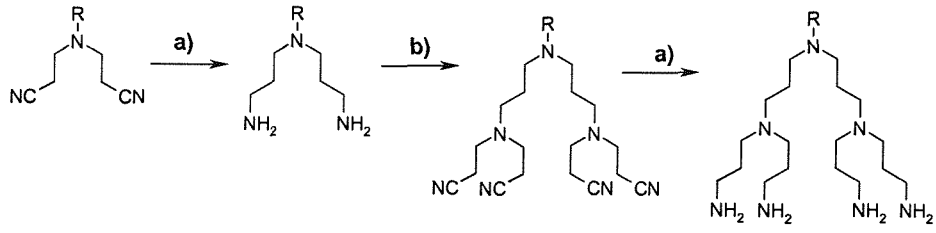


Figure 24: a) Cobalt$^{(II)}$ sodium borohydride, methanol; b) Acrylonitrile, acetic acid.

After this initial success with the "cascade" concept other workers soon explored the use of repetitive chemistry for the preparation of dendritic materials. Denkewalter[131] for example patented a method for the synthesis of poly-lysine-based dendrimers. Newkome[132] reported polyol dendrimers and Tomalia[133] the synthesis of polyamidoamine PAMAM dendrimers. In 1990 Fréchet[134] published the synthesis of poly(aryl ether) dendrimeric architectures and in the same year Miller[135] prepared the first aromatic based all hydrocarbon dendrimers. Silyl based dendrimers were reported in 1992 by van Leeuwen.[136]

There are two main strategies for the synthesis of dendrimers: Divergent and Convergent methods (Figure 25).

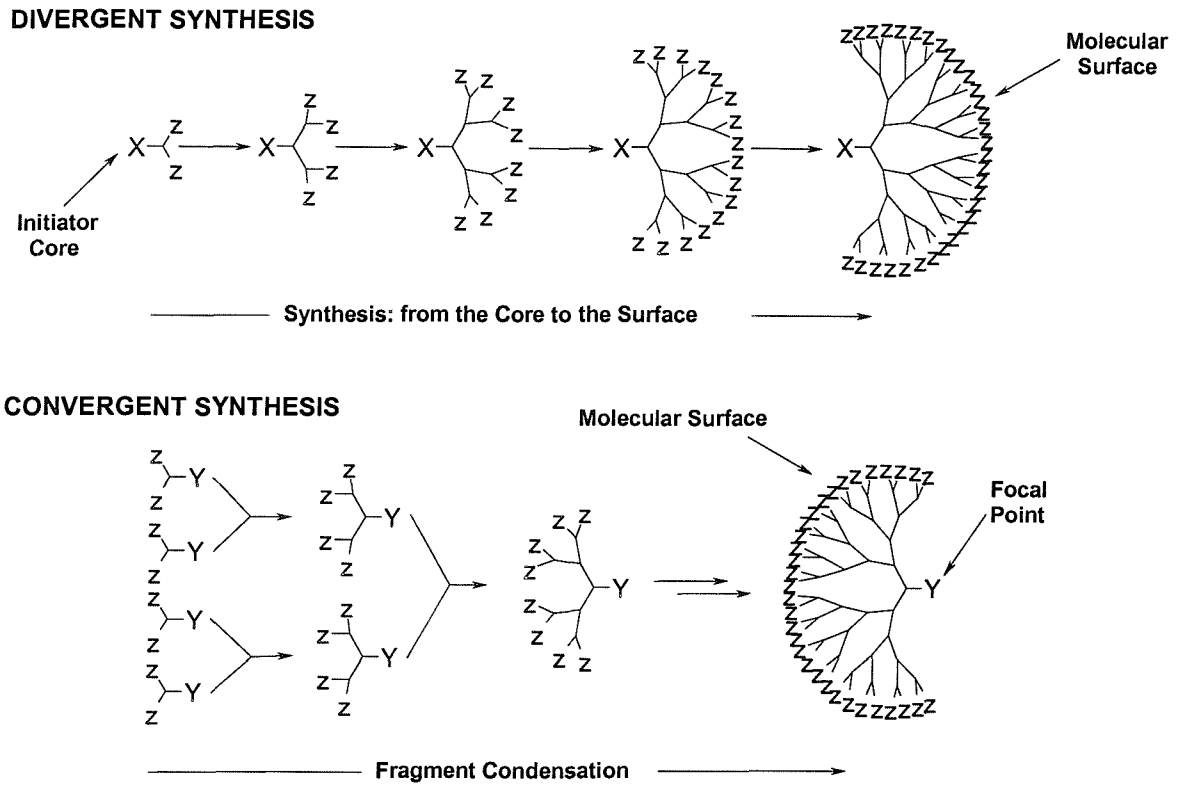


Figure 25: Dendrimer synthesis by divergent and convergent synthesis.

Divergent synthesis involves a starting point or initiator core. The initiator core can be seen to be like a seed or germ which gives rise to a huge number of branches. Steric saturation can be reached with this method after a number of generations and defects begin to appear. Convergent synthesis involves the synthesis of large dendrimeric fragments that are subsequently added to the initiator core and was initially described by Fréchet in 1990.[134]

Applications of Dendrimers

Dendrimers are very useful macromolecules and an important reason for this is that they have many advantages over classical random macromolecules, which can have a coiled conformation and buried functionality. Dendrimers in contrast with their radial orientation present their terminal functionality in a very favourable way accessible to incoming reagents. Dendrimers also have three distinct microenvironments (core, branched shell and external surface) and these unique physical, chemical and structural properties convey upon them many useful attributes. Dendrimers have been used for a wide variety of applications.

1: Chromatography: (SEC)[137] ion exchange processes[138]

2: Pharmaceutical complexes/conjugates: (MAP/antigens),[139] nucleic acid conjugates,[140] cell transfectants,[141] drug delivery devices,[142] photochemical molecular devices,[143] inks and toners,[144] liquid crystals,[145]catalysts[146] and solubilisation agents.[147]

PAMAM dendrimers have been the dendrimer class that has been the most investigated especially for medical applications. This has been due to the fact that they are soluble in aqueous solution, it has been shown that generation five or smaller dendrimers do not present any toxicity problems *in vitro* or *in vivo*. PAMAM dendrimers are also non-immunogenic.[148] They are commercially available and have also been used as devices for improving magnetic resonance imaging[149] and for boron neutron capture therapy.[150] Kim *et al*[151] have used dendrimers for solution-phase combinatorial chemistry their approach has the advantage that size exclusion chromatography may be used to separate products from reactants.

2.2 Solid Phase Synthesis of Dendrimers

Solution-phase synthesis of dendrimers is often challenging, requiring long reaction times and non-trivial purification. The solid-phase approach as outlined by Merrifield therefore is an attractive strategy for the synthesis of such molecules, as it allows reactions to be driven to completion using high concentrations of reagents and purification being a matter of resin washing. The first reported synthesis of resin-bound dendrimers was by Tam.[152] These were prepared using lysine residues and are now commercially available for multiple-antigenic peptide (MAP) synthesis.

It was Fréchet in 1991[153] who attempted the first synthesis of polyamide dendrimers with limited success. It was found that the reactions could not be forced to completion.

In 1993 Roy described the first solid-state preparation of the dendritic sialoside inhibitors of influenza A virus haemagluttinin by a fragment condensation route.[154] The following year Wong reported the solid-phase synthesis of branched glycopeptides obtained in part *via* enzymatic synthesis.[155]

2.3 Solid-Phase Synthesis of Dendrimers to Obtain High Loading Resins for Single Bead Screening in Combinatorial Chemistry.

In 1997 Bradley[156-158] reported the first solid-phase synthesis of PAMAM dendrimers up to generation [4.0] for the application of a high-loading support for combinatorial

synthesis. The group also reported an AB_3-type[159] dendrimer and polyether dendrimer[160] synthesis on the solid-phase. These high loading resins allowed cleavage of sufficient material from a single resin bead for structure analysis by 500 MHz ^1H NMR and screening. RP-HPLC analysis revealed that approximately 32 nmoles of pure peptide was released from the bead.[158] These resins were also used for the synthesis of various classes of compounds (Figure 26) such as a library of aryl ethers prepared via the Mitsonubu reaction in a semi-automated fashion.[161]

A library of amidines as potential GP IIb-IIa antagonists were also synthesised. Suzuki reactions were also carried out on the resin using 0.1 equivalents of $Pd(PPh_3)_4$ and 2 equivalents of K_2CO_3 in DMF at 100°C for 2 hrs. The final compounds were obtained in a purity of 90% and in 80% overall yield.[162-163]

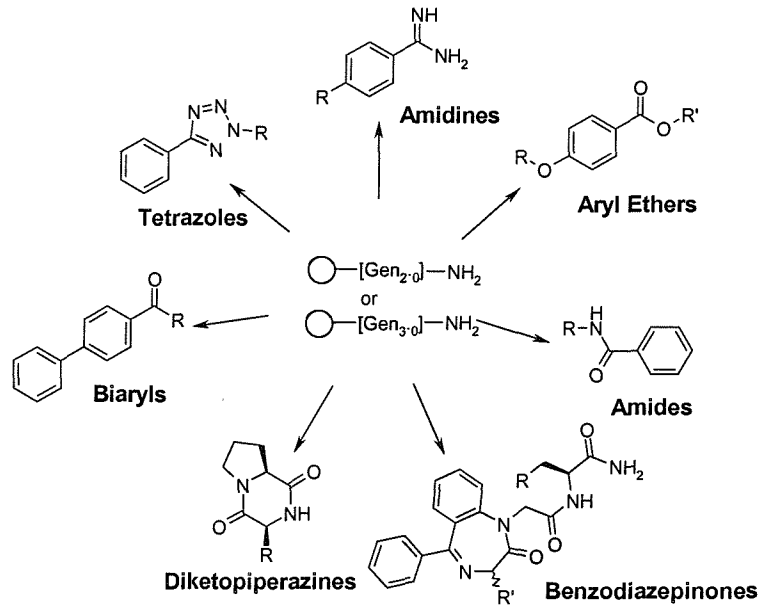


Figure 26: Different Chemistries Applied to Generation [2.0] or [3.0] PAMAM dendrimer resins.

2.4 Solid-Phase Synthesis of PAMAM Dendrimers

Solid-phase dendrimer synthesis began (Figure 28) from a resin immobilised initiator core, from the two primary amine groups of a 1,5,9-triazanonane according to a published procedure.[164-165] The initiator core is immobilised on the resin via an acid-labile construct **(8)** the synthesis of which is shown (Figure 27).

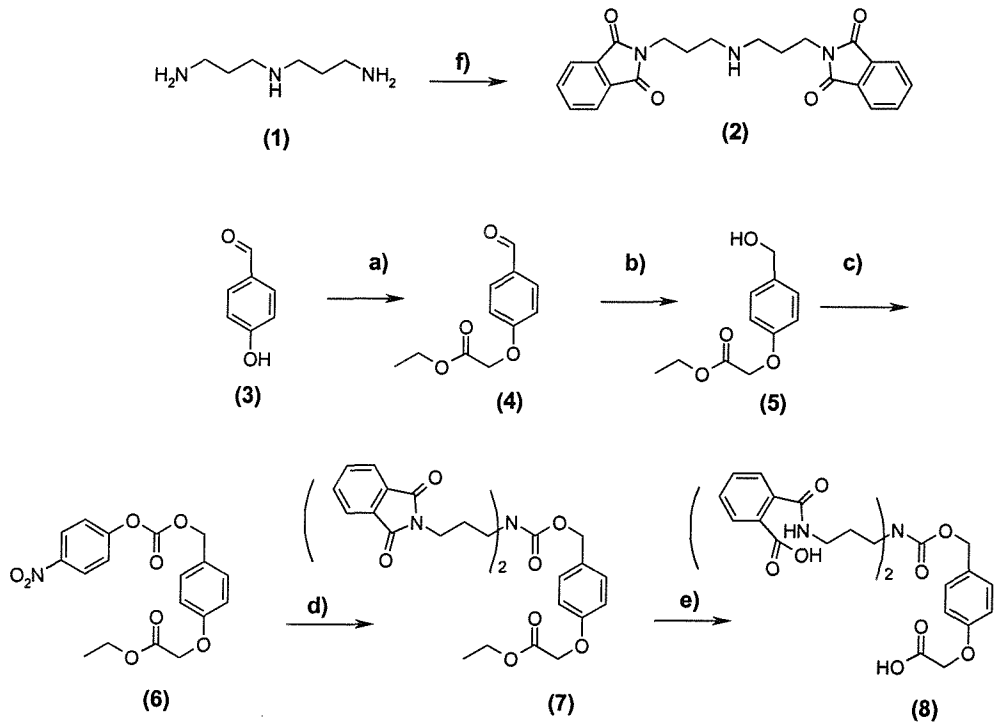


Figure 27: The synthesis of protected polyamine-acid-labile unloaded linker **(8)**: a) Potassium carbonate, potassium iodide, ethyl bromoacetate, acetonitrile, reflux; b) Ethanol, sodium borohydride; c) Pyridine, 4-nitrophenyl chloroformate, dichloromethane; d) Acetonitrile, triethylamine, **(6)**, **(2)**; e) Dioxane, sodium hydroxide, potassium hydrogen sulphate; f) *N*-ethoxycarbonylpthalimide, chloroform.

A problem encountered with the synthetic route chosen was that the phthalimide rings of compound **(7)** were opened upon saponification. It was therefore necessary to reform the phthalimide ring and this was performed by activation of the triacid **(8)** with DIC/HOBt for 40-60 minutes prior to addition to the resin. Following deprotection of the phthalimide protecting group with 5% hydrazine dendrimer synthesis began according to the published procedures.[166-167]

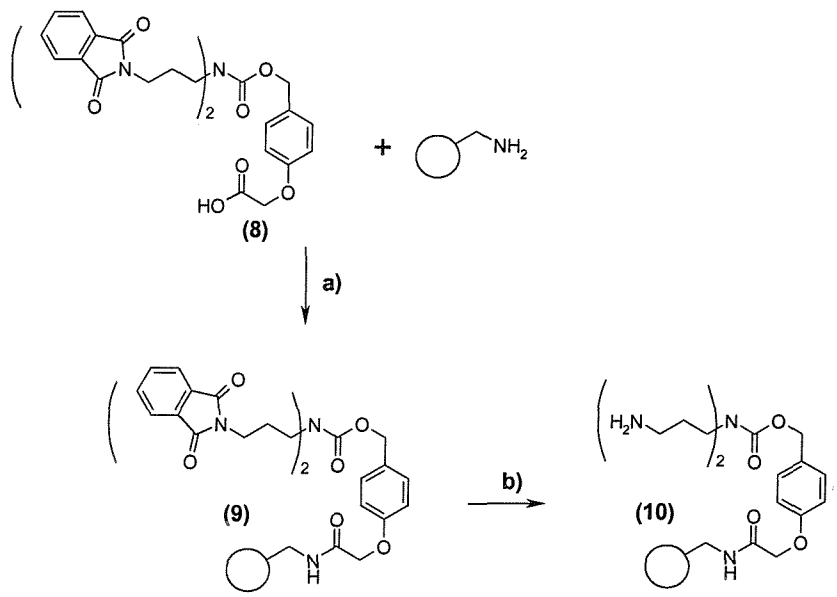


Figure 28: Synthesis of resin-bound initiator core: a) 1-Hydroxybenzotriazole, diisopropylcarbodiimide, dichloromethane; 5% hydrazine, ethanol.

Starting from the initiator core conjugate **(10)** (Figure 28) treatment with 250 equivalents of methyl acrylate in methanol twice for 48 hours at 50°C followed by removal of excess reagents by filtration and thorough washing (DCM, DMF, MeOH, Et_2O) formed the various generation [X.5] resin bound PAMAM dendrimers (Figure 29).

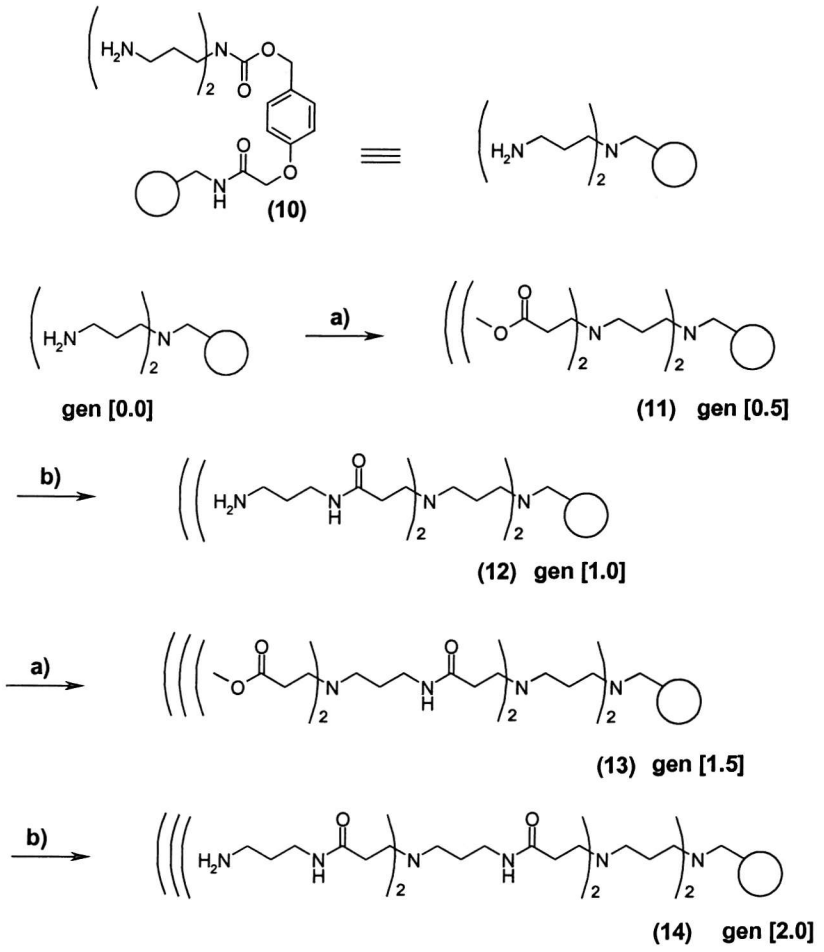


Figure 29: Solid-Phase Dendrimer synthesis: a) Methyl acrylate, methanol; b) 1, 3-diaminopropane, methanol.

To monitor the formation of half-generation dendrimers **(11)** and **(13)** electrospray mass spectrometry was used (Figure 31) as it could readily detect the presence of any defects. This was important, as defect structures arising from retro Michael reactions **(A)** and intramolecular cyclic amide formation **(B)** (Figure 30) would cause a problem with dendrimer homogeneity.[168]

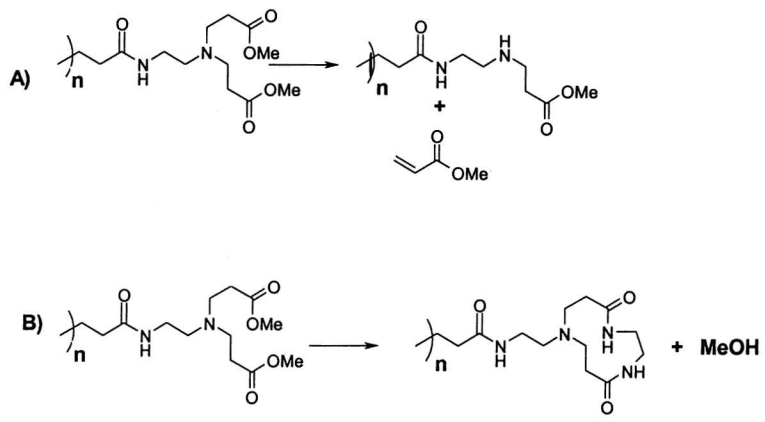


Figure 30: Defects that occur in PAMAM synthesis. A: Retro Michael; B: Cyclic amide formation.

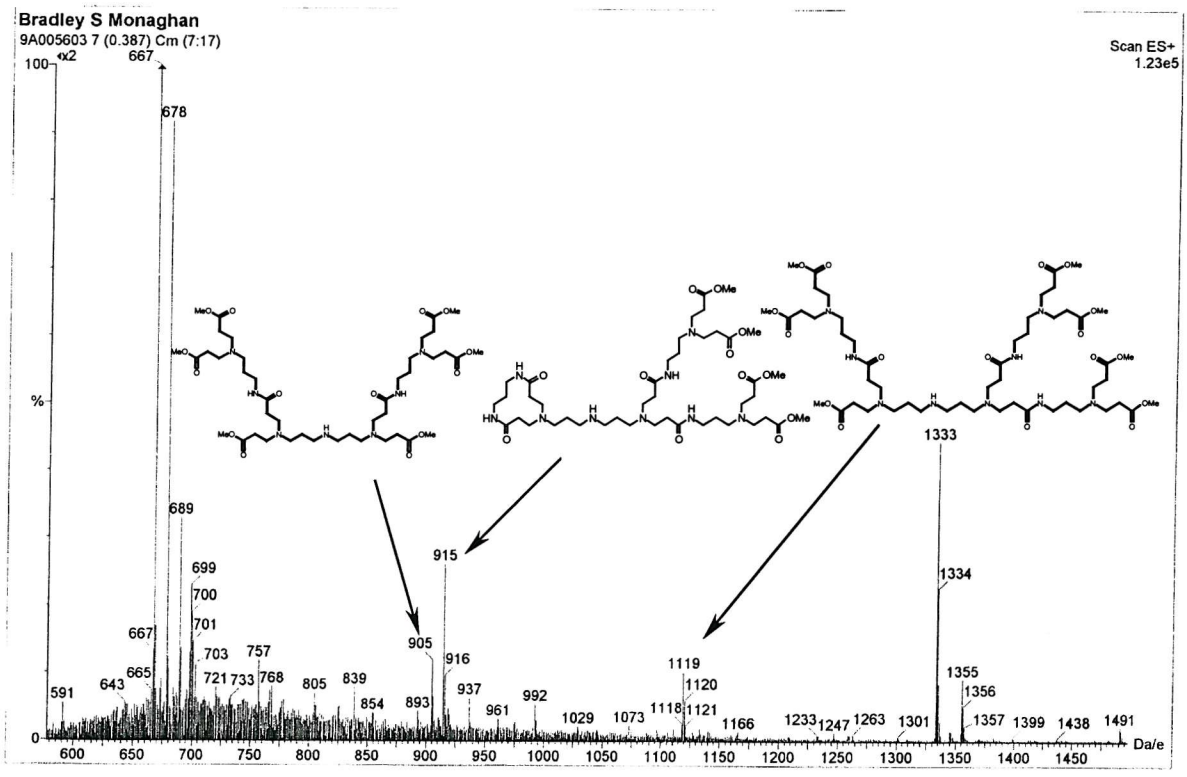


Figure 31: Mass spectra showing presence of defects.

The half generation dendrimers were then treated with 1, 3-diaminopropane for 72 hrs at room temperature and then again for a further 72 hrs to form the next full generation dendrimer. For full generation dendrimers **(12)**, **(14)**, **(18a)**, **(18b)** and **(19)** the best way to obtain an insight into dendritic purity was to attach a chromophore such as

fluorescein or 4-hydroxyphenylacetic acid to a small amount of resin then cleavage with 90 % TFA in DCM and analysis by RP-HPLC (Figure 32).

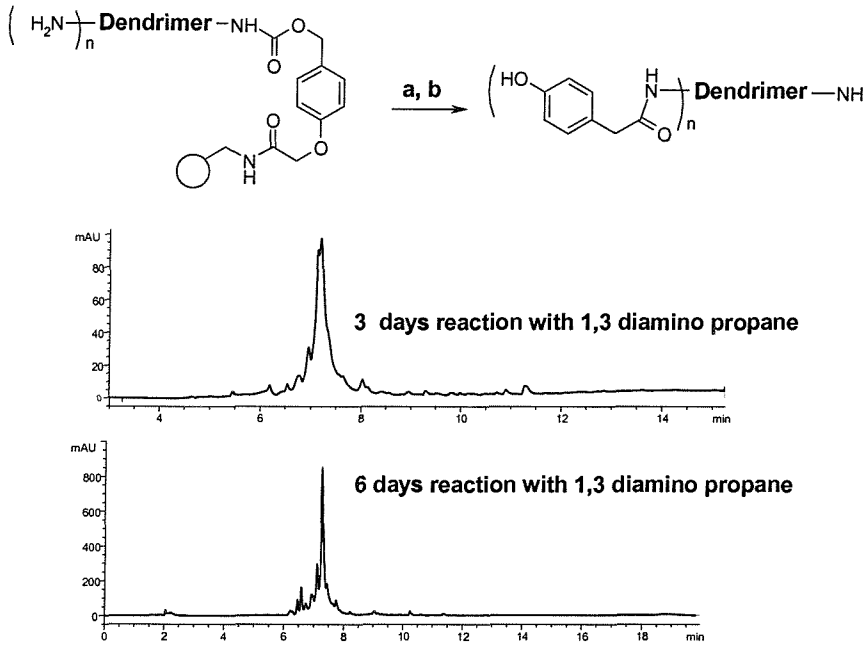


Figure 32: Dendrimer purity can be monitored by coupling a chromophore to full generation dendrimers and analysing the cleaved compound by RP-HPLC. It can be clearly seen how after 6 days the reaction has almost gone to completion. a) 4-Hydroxy-phenyl-acetic acid, diisopropylcarbodiimide, 1-hydroxybenzotriazole, dichloromethane; b) 90% trifluoroacetic acid in water.

2.5 Synthesis of Hybrid AB_3-Type Dendrimer.

The dendrimer building block, dimethyl 6-isocyanato-6-(4-carbomethoxy-2-oxabutyl)-4, 8-dioxaundecanedioate, **(16)** pioneered by Newkome *et al*,[169] (Figure 33) has been used previously by Bradley for the synthesis of high loading beads.[159] The advantages of the building block **(16)** are that it allows the facile multi-functionalisation of the resin. The precursor was available in large quantities so this was used to form the building block in one step according to a published procedure for the conversion of amines to isocyanates.[170]

(15) (16)

Figure 33: Synthesis of isocyanate building block a) Di-*tert*-butyl dicarbonate, dimethyl amino pyridine, dichloromethane.

Treatment of the PAMAM initiator core-acid labile linker conjugate **(10)** with the building block **(16)**, *N,N*-diisopropylethylamine (DIPEA) and a catalytic amount of 4-(dimethylamino)pyridine (DMAP) in dichloromethane afforded AB^3-type generation [0.5] dendrimer **(17)**. Displacement of the methyl ester by 1, 3-diaminopropane or ethylene diamine in DMSO provided the AB_3-type dendrimers up to generation [2.0] shown (Figure 34). Dendrimers produced in this way were of much higher purity with significantly less defects than the PAMAM series synthesised previously. They were therefore used to a much greater extent throughout the project. The chemistry for the dendrimer synthesis shown had previously been developed by other members of the Bradley group. However little attention had been paid to dendrimer purity as the dendrimers had not been intended for the applications described in this thesis. The achievement in this chapter is the attention paid to producing consistently high purity dendrimers.

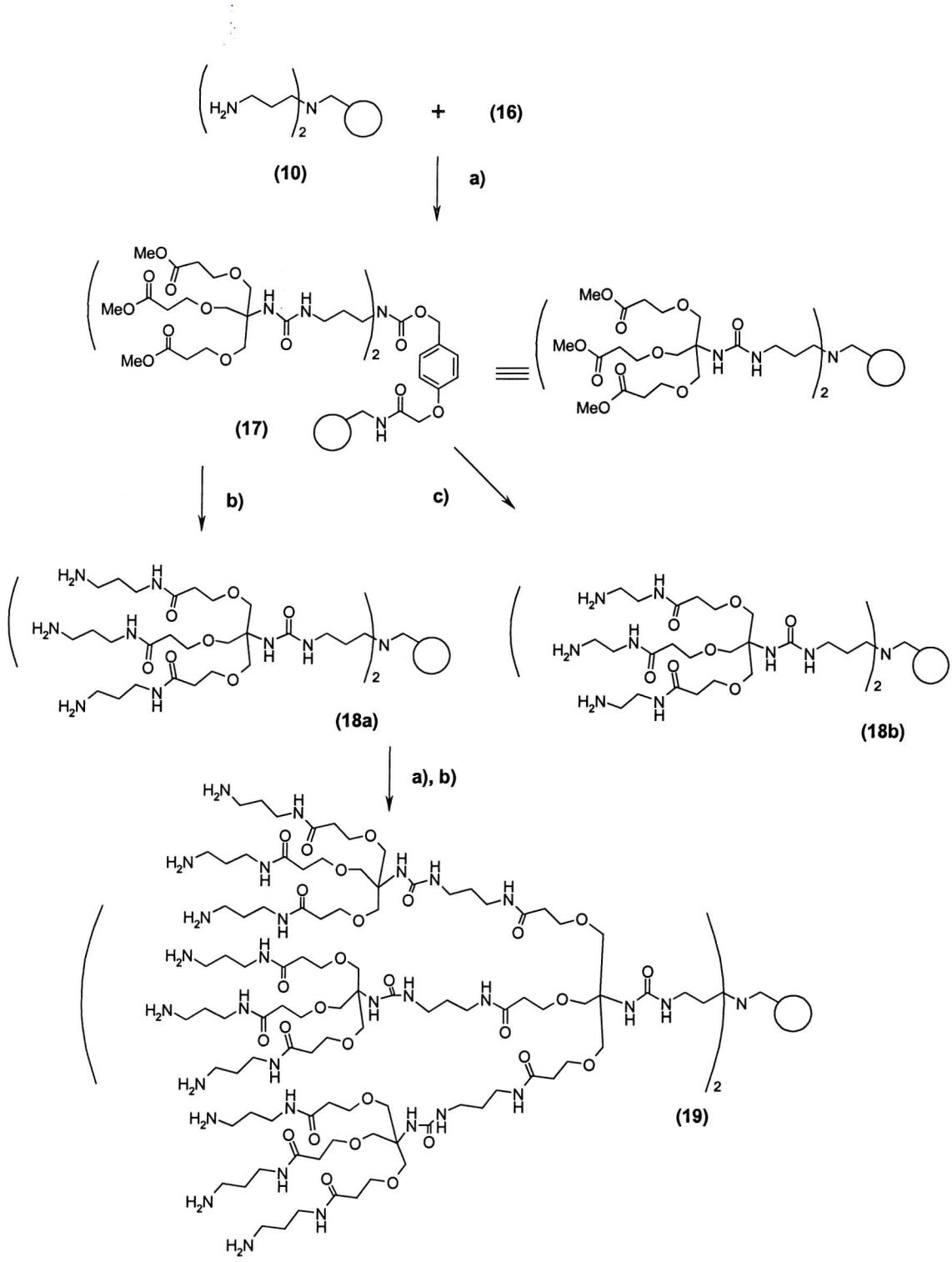


Figure 34: Synthesis of AB_3-type dendrimers **(18a), (18b), (19)**: a) **(16)**, diisopropylethylamine, dimethylaminopyridine, dichloromethane; b) 1, 3 diamino propane, dimethyl sulphoxide or methanol; c) Ethylene diamine, methanol.

2.6 Conclusions

Published procedures developed within the Bradley group were followed in order to synthesise dendrimers on the solid-phase with high purity. Initially mass spectral analysis was used as a means to monitor reactions however coupling of a chromophore to the dendrimerised resin and cleavage and RP-HPLC analysis combined with mass spectral analysis was found to be the most efficient way of monitoring the purity of the dendrimers. The solid-phase immobilised dendrimers are an efficient multivalent scaffold for the presentation of biologically active ligands. In theory any active sequence could now be attached to the dendrimers.

Chapter 3:
Synthesis of Multivalent Peptide-Dendrimer Conjugates for an Investigation of Integrin Binding

Chapter 3 Synthesis of Multivalent Peptide-Dendrimer Conjugates for an Investigation of Integrin Binding.

3.1 Introduction to LDV Ligands

As stated in the introduction (chapter 1.9) cell-cell and cell-matrix interactions are involved in a number of disease states, most notably cancer metastasis and various inflammatory conditions for example rheumatoid arthritis and asthma.[60-64] They are also involved in many physiological processes including the immune response, wound healing, embryogenesis and cell differentiation. Integrins are the major receptors by which cells attach to the extracellular matrix. The integrin VLA-4 or $\alpha_4\beta_1$ (the integrin studied here) is found on numerous cell types including tumor cells, lymphocytes and eosinophils.

Fibronectin[76-78] is a cell adhesion protein widely distributed in the tissues of all vertebrates and is present as a polymeric fibrillar network in the extracellular matrix and as soluble protomers in body fluids. Significantly, it contains at least two major domains that support cell adhesion. The counter ligand for VLA-4 includes both VCAM-1 (found on vascular endothelial cells) and a peptide motif found within fibronectin. The minimal essential peptide sequence in both ligands is LDV (this is analogous to the RGD motif, which is a ligand for a wide variety of integrin receptors in particular $\alpha_{IIb}\beta_3$ and $\alpha_v\beta_3$). The affinity of these tripeptides is however low when compared to fibronectin in cell adhesion assays[78] which has an IC_{50} value of 9 nM. This is because high avidity interactions result due to multivalent interactions. Recently various polymers have been used to improve carbohydrate protein interactions using the multivalent effect.[96] Dendrimers are mono-disperse multivalent polymers and provide excellent templates for the multivalent presentation of biologically active ligands. Dendrimer conjugates were investigated in this chapter as a possible way to increase the affinity of the weak binding ligand, (LDV), to the $\alpha_4\beta_1$ integrin using solid-phase synthesis to allow efficient dendrimer conjugation.

3.2 Synthesis of LDV-Dendrimer Conjugates

Peptide-dendrimer synthesis began from the dendrimer resins **(10)**, **(12)**, **(14)** and **(18b)** described in chapter 2 with the tripeptide Leu-Asp-Val being synthesised directly onto

the dendrimer resins using standard solid-phase peptide chemistry. Coupling was performed with 5 equivalents of HOBt, DIC and amino acid, in DMF (Figure 35).

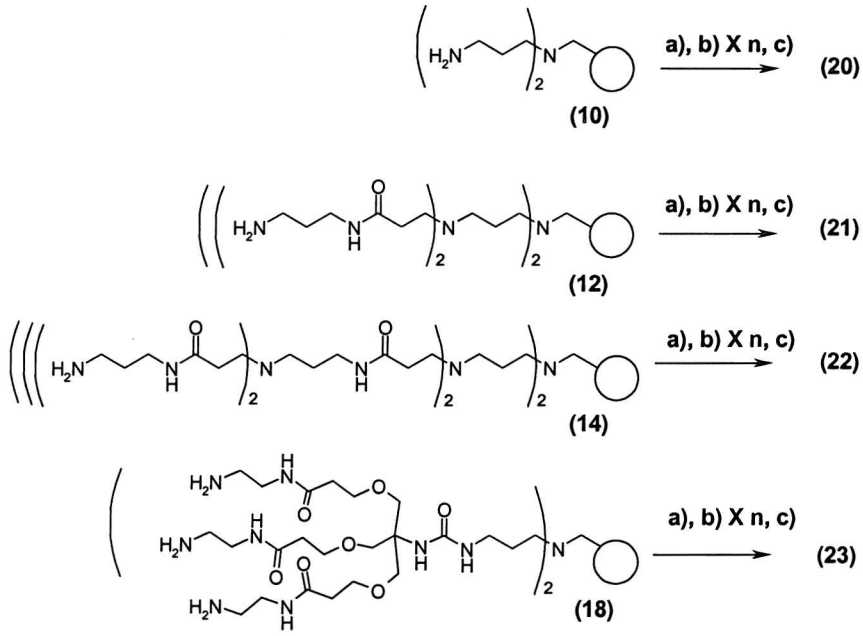


Figure 35: Synthesis of peptide-dendrimer conjugates. a) Fmoc-amino acid, diisopropylcarbodiimide, 1-hydroxybenzotriazole; b) 20% piperidine/dimethyl formamide; c) 90% trifluoroacetic acid/dichloromethane 2hrs.

Following dendrimer-peptide conjugate synthesis deprotection and cleavage with 90% TFA in DCM, the cleaved products were dissolved in acetic acid, concentrated and then precipitated with ether, centrifuged and freeze dried. The compounds **(20)-(23)** (Figure 36) were subsequently purified by semi-preparative RP-HPLC. It can be seen clearly from the RP-HPLC data shown in (Figure 37) that the LDV-dendrimer conjugates synthesised by the solid phase route were of exceptional purity, without any defect structures, something, which is difficult to obtain in large molecules of this kind. The yields given in (Table 2) represent between 8-12 synthetic steps.

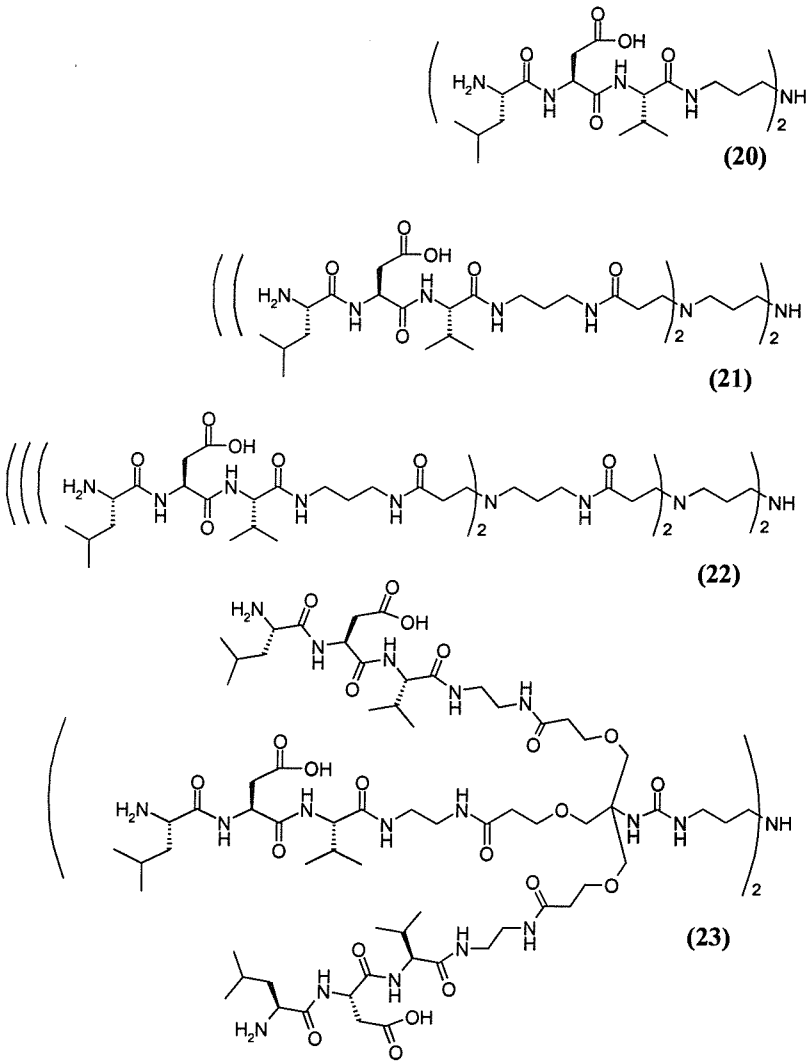


Figure 36: compounds **(20)-(23)**.

Table 2

Peptide-Dendrimer	Synthetic steps	%Yield (Isolated).
(20)	8	21
(21)	10	11
(22)	12	8
(23)	8	26

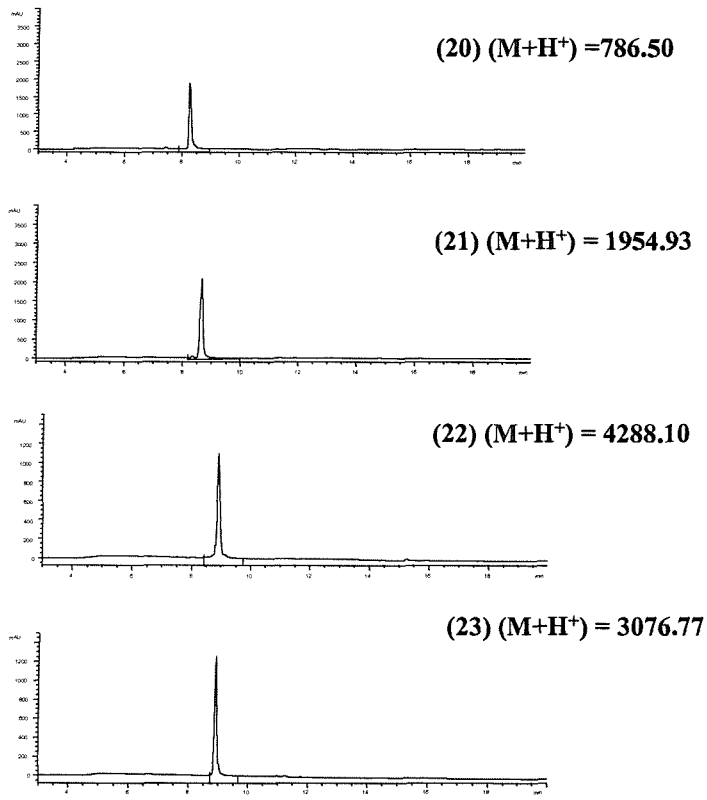


Figure 37: RP-HPLC purities after semi-preparative chromatography of compounds **(20)-(23).**

It was clear from the crude RP-HPLC traces of the peptide-conjugates that the AB_3-type hybrid dendrimer produced much purer compounds as evidenced by RP-HPLC and isolated yield.

3.3 Screening of LDV-Dendrimer Conjugates

The LDV-dendrimer conjugates were evaluated for their ability to inhibit the binding of the biotinylated peptide EILDVPST-NH_2 to the $\alpha_4\beta_1$ integrin adhesion receptor, expressed on cancer cells that had been grown on 96 well-plates. The peptide EILDVPST-NH_2 contains the cell-binding motif LDV and is part of the CS1 domain of fibronectin. The amount of bound biotinylated peptide was quantified using an avidin-labelled peroxidase, with a substrate that resulted in the formation of a coloured reaction product. The biotinylated peptide EILDVPST-NH_2 bound ~ 8 times better to the cells than the non-biotinylated compound. The screen therefore was a very stringent one where the compound was not only competing against the binding of the peptide motif EILDVPST-NH_2 but binding also enhanced by biotin.

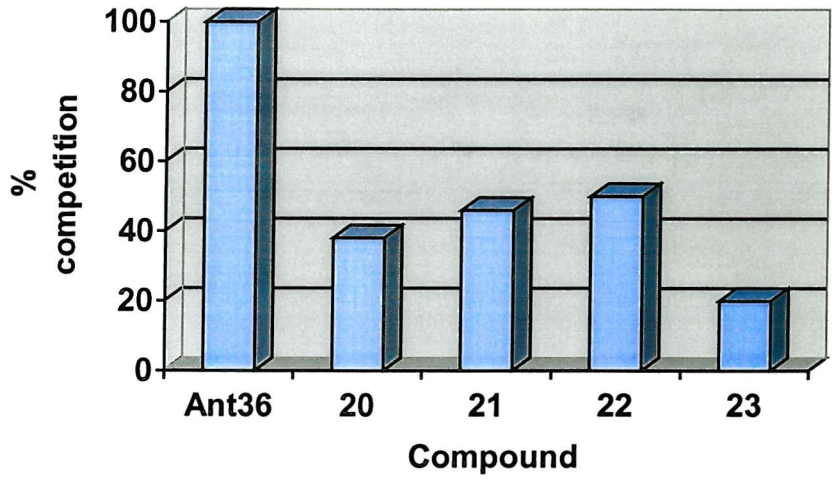


Figure 38: The % inhibition of 100 μM biotinylated EILDVPST-NH_2 by 1 mM of test compounds **(20)**, **(21)**, **(22)**, **(23)** and internal standard **Ant 36**.

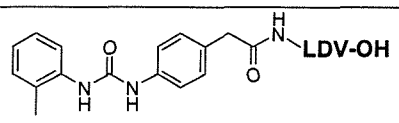
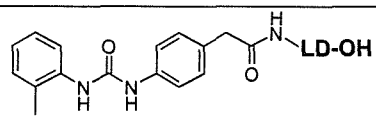
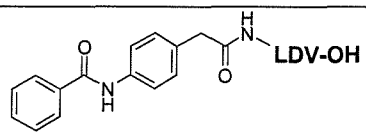
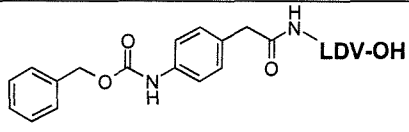
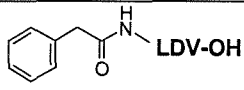
The results show clearly that the LDV containing peptide **ANT 36** (used as the positive control sequence, KYCLDVLDVLDV-NH_2) was the best inhibitor, with 100% inhibition of the biotinylated peptide at 1 mM concentration. The graph also shows that the percentage inhibition by the LDV conjugates increased slightly with higher generation dendrimers. However, these results showed that even the divalent PAMAM dendrimer **(20)** was a more effective inhibitor than **(23)**, which has six copies of the LDV peptide. This may indicate as hypothesised by Roy *et al*[111] that increased inhibitory capacity cannot solely be attributed to an increase in multivalency. The results also highlight the fact that although the LDV minimal sequence is essential for binding, surrounding amino acids or alternative spacers are required for optimal activity.

3.4 Ligand Optimisation

In the initial screening experiment it was found that the compounds were not as potent inhibitors as was hoped however, some evidence did exist that the multivalent effect could increase the affinity of the relevant ligands. It was decided therefore that in order to proceed to the next stage of the project much firmer evidence of increased avidity was needed. The next approach was to synthesise a series of divalent ligands for the integrin receptors being targeted and again screen for activity. The ligands synthesised

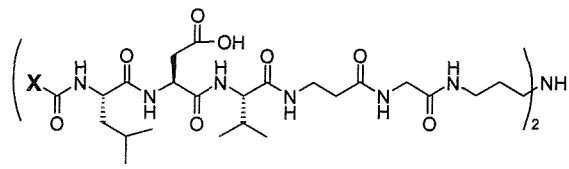
were based on studies of some reported compounds, which were screened in adhesion assays by ELISA and direct binding studies using scintillation counting. (Table 3).[171]

Table 3

Inhibitor	IC_{50} (nM)	
	Binding	**Adhesion**
	0.6	6
	18	68
	295	3,250
	30	200
	1,000	40,000

As these compounds were high affinity inhibitors (best IC_{50}, 6 nM) it was assumed that this should produce a positive lead for a comparison of multivalent binding.

The first ligand dendrimer conjugates synthesised consisted simply of attaching the LDV sequence to the dendrimer surface however, surrounding residues may be important for ligand conformation. Thus for the next series of compounds spacer residues glycine and β-alanine were also employed. The synthesis of compounds such as those shown in (Figure 39) was proposed.



X = Capping group

Figure 39: Design of di-valent ligands.

3.4.1 Synthesis of Capping Groups.

Three of the eight proposed capping groups were not available commercially. Synthesis of **(26)** and **(28)** involved the addition of the corresponding isocyanate to a suspension of *p*-amino-phenyl acetic acid followed by precipitation from ether to afford **(26)**, **(28)** in 70% and 75% yield respectively. **(31)** was obtained by the addition of the *N*-hydroxysuccinimide formate **(30)** to a solution of ethyl 4-aminophenylacetate **(29)** followed by recrystallisation to afford **(31)** in (70%) yield. Saponification afforded **(32)** in 60% yield.

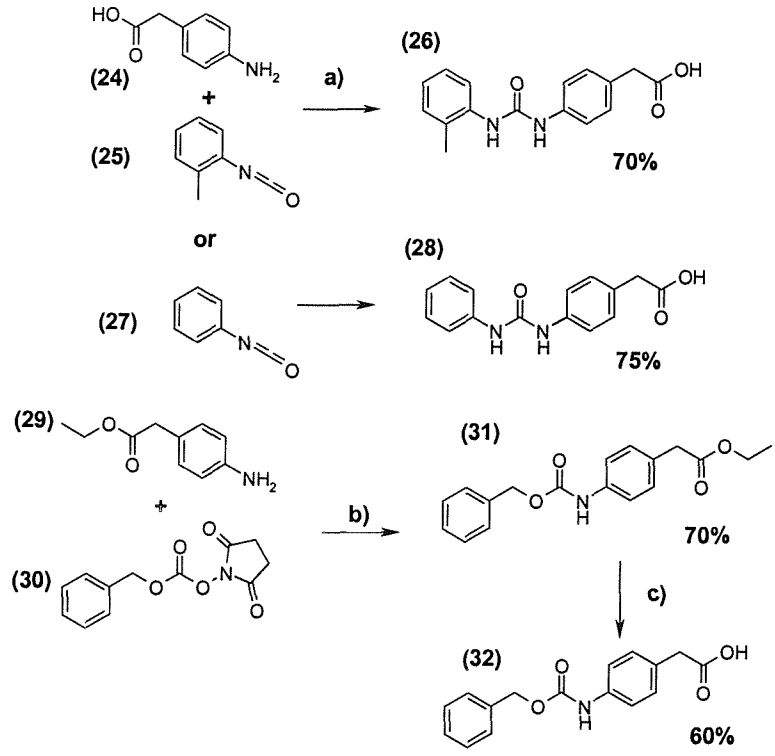


Figure 40: Synthesis of capping groups. a) *p*-Amino-phenyl-acetic acid, *o*-tolyl isocyanate, dimethylformamide 50°C; b) 4-Amino-phenyl-acetate, benzyloxycarbonyl *N*-hydroxysuccinimide ester, dichloromethane; c) 1N Sodium hydroxide, ethanol.

3.4.2 Synthesis of Capped Di-Valent Peptide-Dendrimer Conjugates.

Synthesis began from the dendrimer resin (initiator core) **(10)** and subsequent coupling using standard peptide coupling conditions of the Fmoc amino acids Leu, Asp(OtBu), Val, β-Ala and Gly. The resin **(33)** was split into eight portions and each of the carboxylic acids shown (Figure 41) were coupled overnight with DIC/HOBt. Cleavage with the optimal cleavage mixture, TFA/thioanisole/phenol/water/ethane-dithiol 82.5 %/5%/5%/5%/2.5% and precipitation in ether afforded the target compounds **34-41**, typical crude yields being ~ 40%.

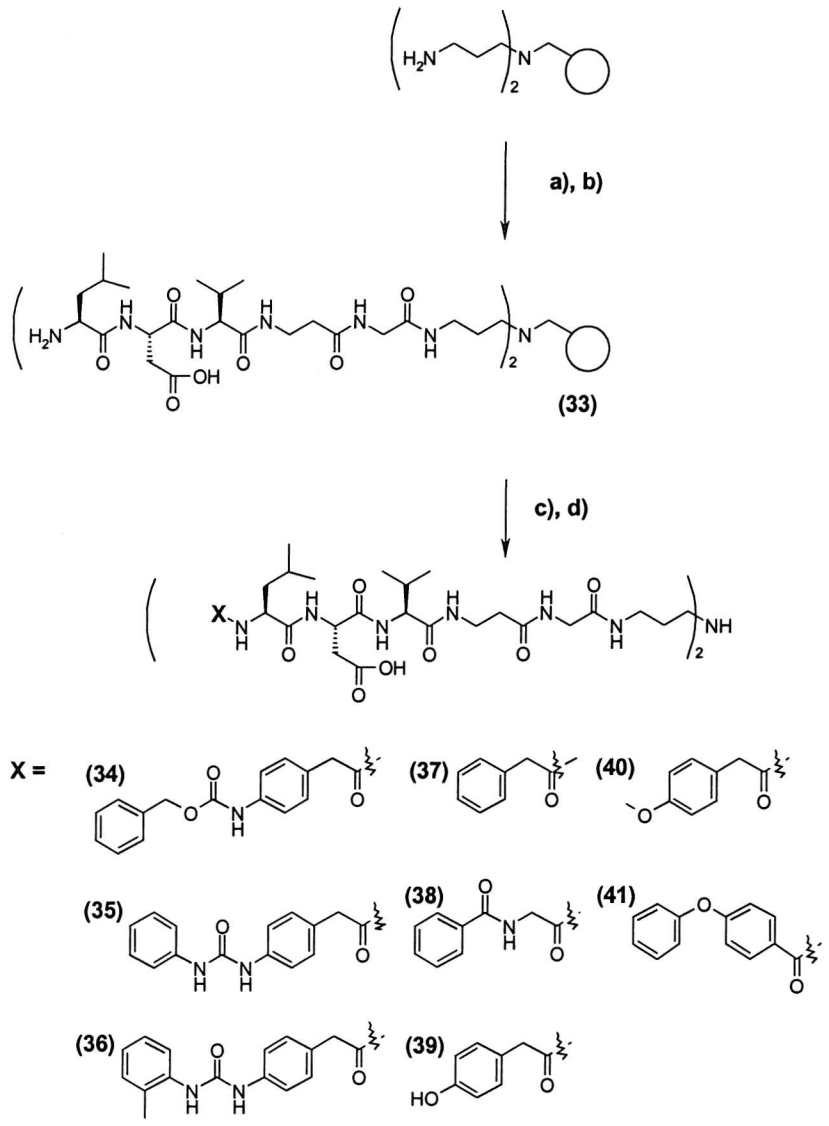


Figure 41: Synthesis of capped di-valent peptide-dendrimer conjugates: a) Fluoren-9-ylmethoxycarbonyl amino acids, diisopropylcarbodiimide, 1-hydroxybenzotriazole, dichloromethane; b) 20% Piperidine in dimethyl formamide c) Capping group, diisopropylcarbodiimide, 1-hydroxybenzotriazole, dichloromethane; d) 90% trifluoroacetic acid in dichloromethane.

Only compound **(39)** was sufficiently soluble in water to purify by semi-preparative RP-HPLC. However it was decided that since the synthesis was straightforward and highly efficient the compounds were of sufficient purity for screening without further purification.

3.5 Screening of Capped Di-Valent Peptide-Dendrimer Conjugates.

Due to the insolubility of the compounds **(34)-(42)** in water they were dissolved in PBS/TWEEN peptide diluent containing 10% DMSO. This level of DMSO is the maximum permissible that does not affect the screen. In the screen it could be seen quite clearly with the naked eye that the di-valent peptide-dendrimer conjugates were effectively competing with the biotinylated peptide EILDVPST-NH_2. The results of the screen are shown in Table 4.

Table 4

Compound	IC50 (μM)	% Inhibition 1000(μM)	% Inhibition 100(μM)	% Inhibition 50(μM)
(34)	5.55	88	28	0
(35)	14.89	92	78	13
(36)	31.64	88	64	0
(37)	17.68	88	59	3
(38)	12.51	75	63	0
(39)	25.32	81	52	0

(40)	5.62	58	70	17
(41)	4.50	90	75	40
(42)	-	68	14	0
(Ant35)	10.28	82	63	30

(42) LDV-NH_2

(Ant35) = KYCILDVILDVILDVNH_2.

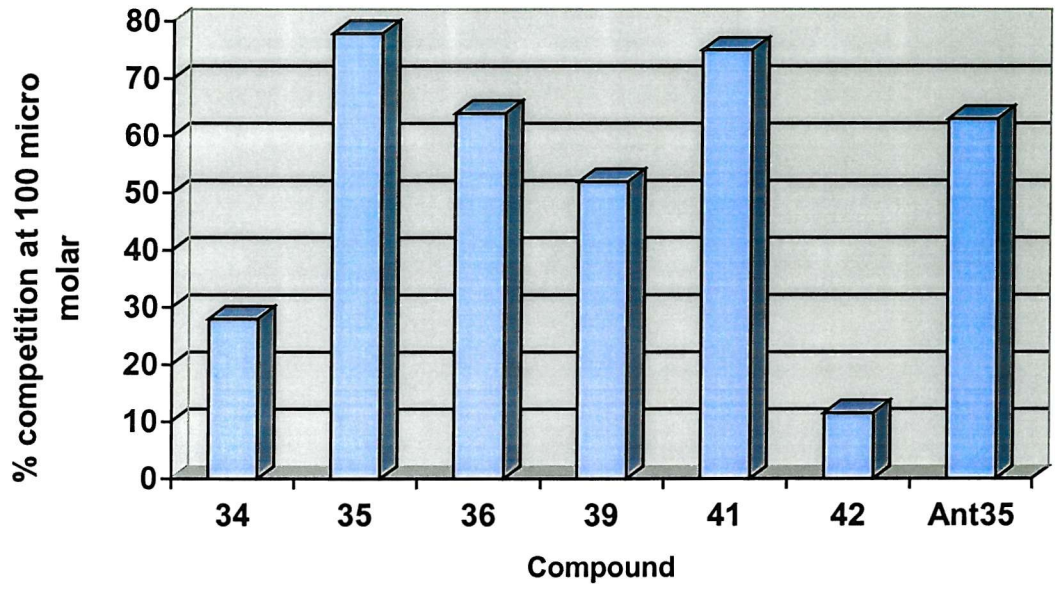


Figure 42: The % inhibition of 100 μM biotinylated EILDVPST-NH_2 by 100 μM of test compounds **(34)**, **(35)**, **(36)**, **(39)**, **(41)**, **(42)** and internal standard **Ant 35**.

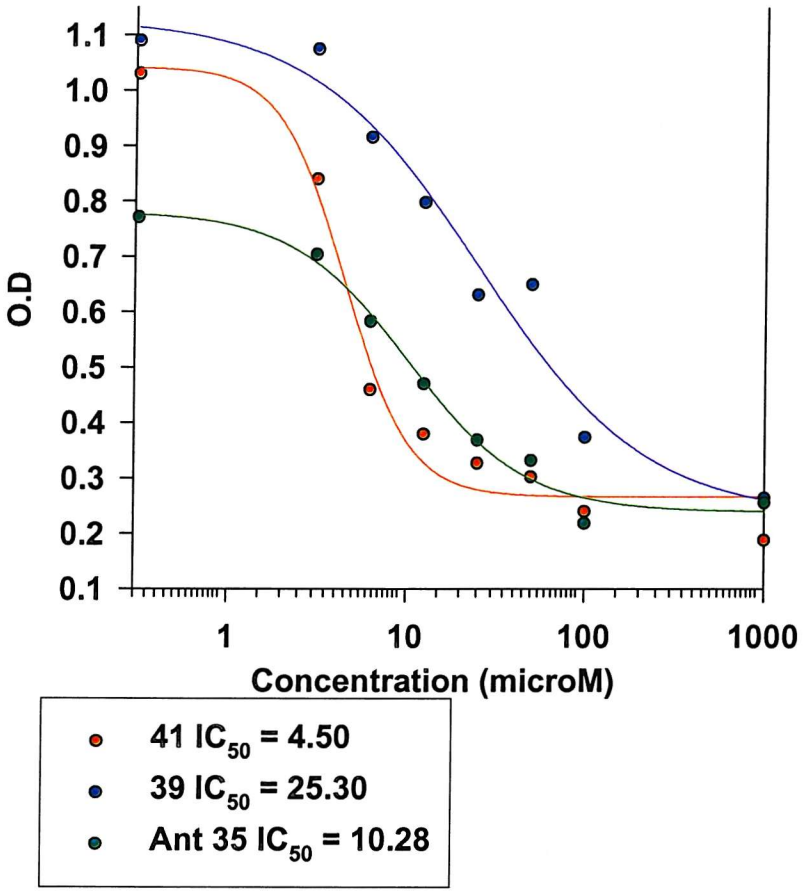


Figure 43: Standard curves for **(39)**, **(41)**, **(Ant 35)**.

From the results of the screening experiment we can conclude many positive things. Firstly the addition of a capping group significantly increases the activity of the LDV sequence as all the capped compounds had greater activity at 100 μM than LDV-NH_2. It can be seen that the rigid phenyl urea compound **(35)** was more potent than the benzyloxy-carboxamido compound **(34)** which was also found to be the case by the workers at Biogen *inc*[171] however the 2-methylphenylurea containing compound **(36)** was unexpectedly less active than **(35)** and this was probably due to the insolubility of the compound. It can also be seen from the results that compound **(41)** was the most active. (Figure 43) shows the standard curves obtained for **(41)** the most active compound and **(39)** the most water soluble compound as well as the internal standard KYCILDVPST-NH_2 (ANT 35).

3.6 Synthesis of Optimised Ligand-Dendrimer Conjugates.

As the preparatory screening experiment of the di-valent compounds showed convincingly that adding a capping group imparted activity to our LDV sequence the next step was to investigate whether presenting these active ligands on higher generation dendrimers would increase the binding through a multivalent effect.
Therefore the active ligands were synthesised directly onto dendrimer resins **(12)** and **(18a)** (Figure 44).

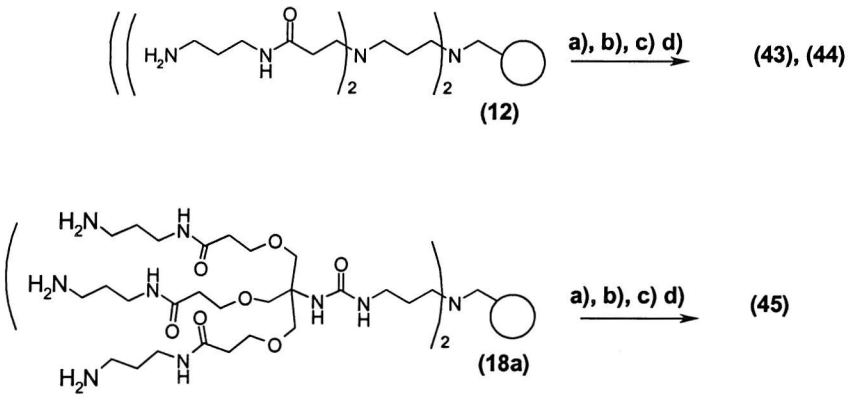


Figure 44: Synthesis of **(43)-(45)**: a) Fluoren-9-ylmethoxycarbonyl amino acids, diisopropylcarbodiimide, 1-hydroxybenzotriazole, dichloromethane; b) 20% Piperidine in dimethyl formamide c) Capping group, diisopropylcarbodiimide, 1-hydroxybenzotriazole, dichloromethane; d) 90% trifluoroacetic acid in dichloromethane.

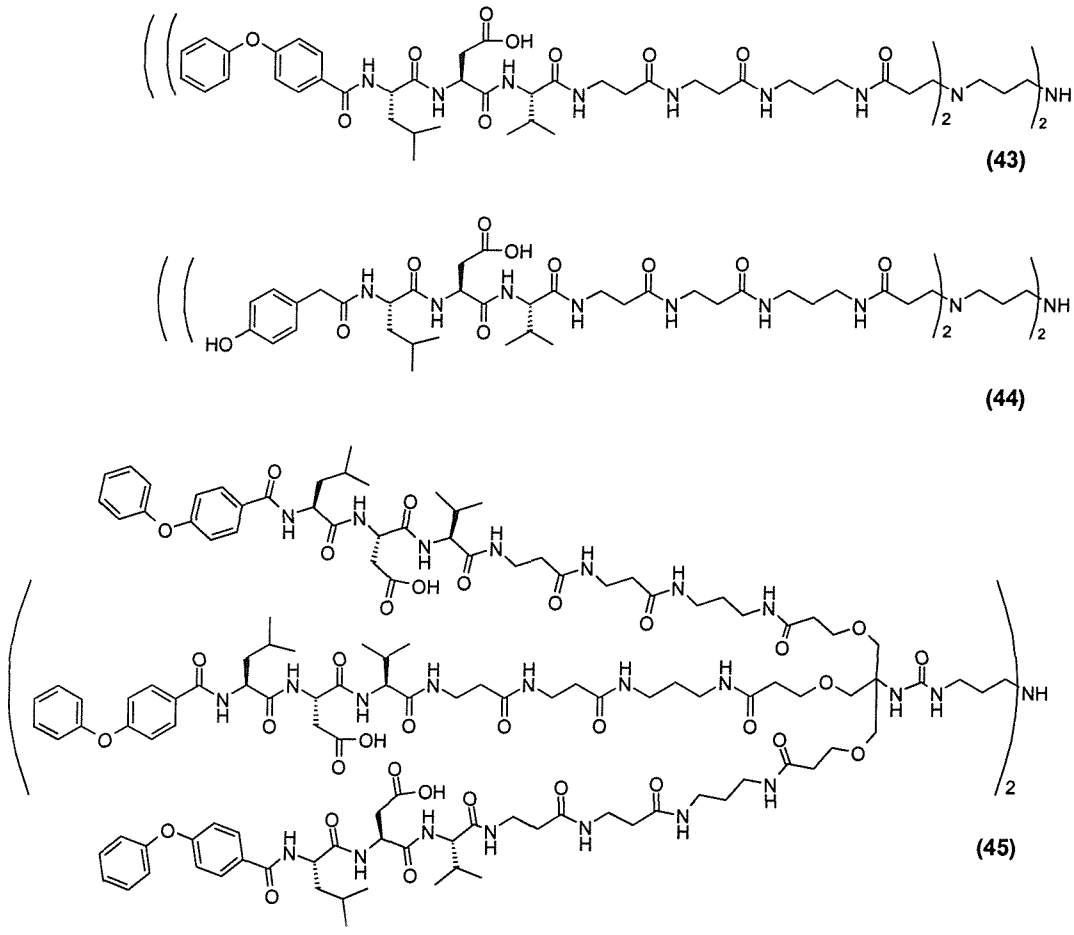


Figure 45: Optimal ligands presented on PAMAM and AB_3-type dendrimers

A so-called approach of "tailoring" oligovalency with optimal length spacers, in order to fit the exact spacing of receptors on a surface has been shown to produce a dramatic increase in avidity.[118] The "tailoring" oligovalency approach described by Kitov[118] *et al* makes use of information gathered from a crystal structure and thus is a much more rational approach compared to the "random" multivalency approach applied with previous dendrimer-sugar conjugates. It was decided, based on the fact that spacer length can effect affinity, to investigate the effect of different length spacers on the affinity of the peptide-dendrimer conjugates. One, two or three residues of ∈Ahx were introduced between the dendrimer backbone and the active ligand (Figure 46).

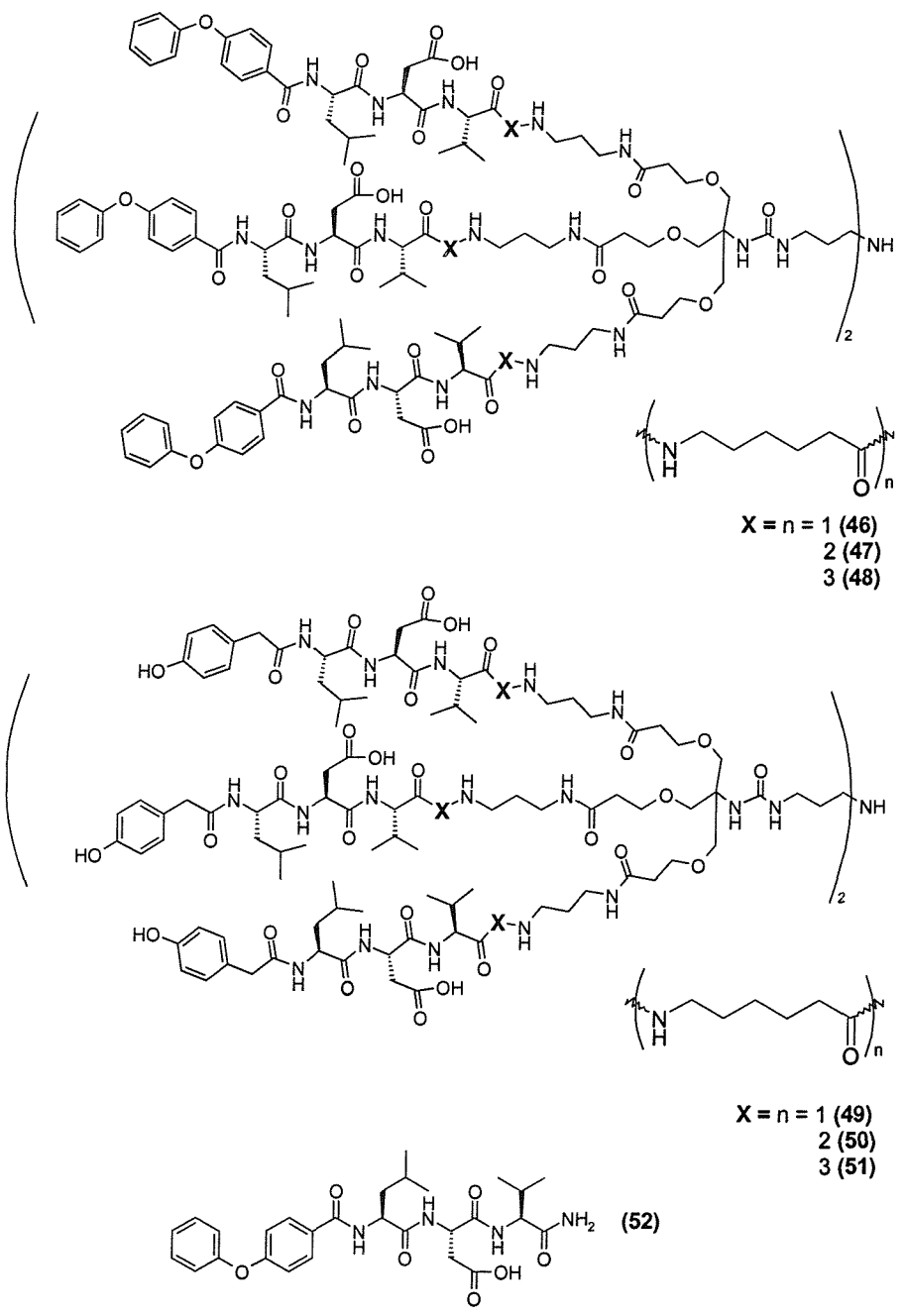


Figure 46: Dendrimer compounds **(46)-(51)** with one, two or three residues of ∈Ahx between the dendrimer backbone and the active ligand.

3.7 Screening of Optimised Ligand-Dendrimer Conjugates (43)-(51).

Compounds **(43)-(52)** were screened as before. The problem of insolubility was however a lot greater for these optimised ligand-dendrimer conjugates and IC_{50} values could not be obtained. However it could be seen that the compounds at the higher concentrations were active. Compound **(52)** is shown (Figure 46).

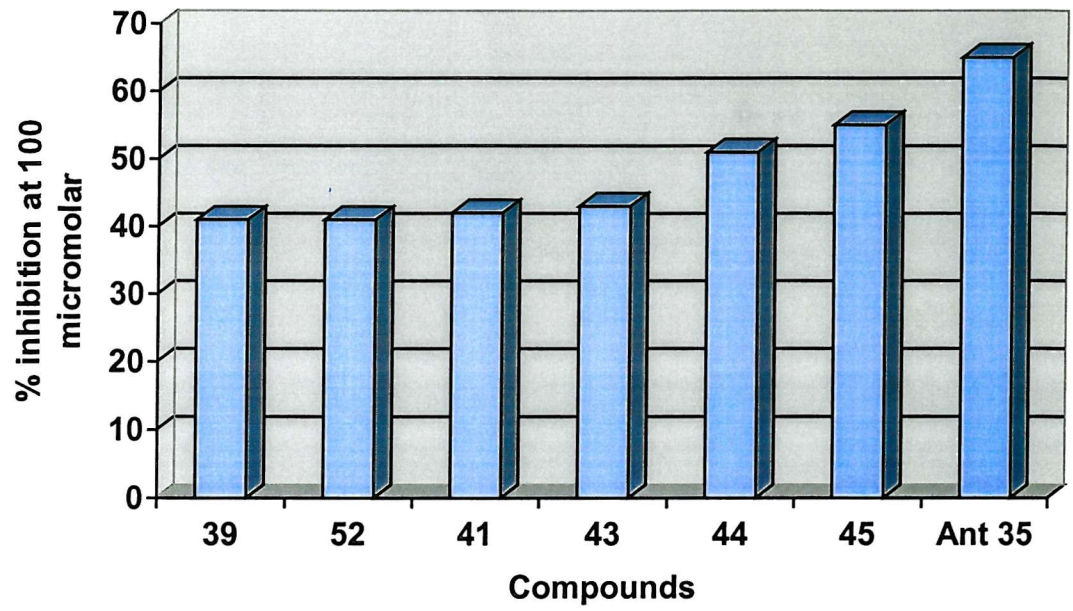


Figure 47: The % inhibition of 100 μM biotinylated EILDVPST-NH_2 by 100 μM test compounds **(39)**, **(41)**, **(43)**, **(44)**, **(45)**, **(52)** and internal standard **Ant 35**.

What the graph (Figure 47) shows is that all the compounds have activity at 100 μM. However it could be seen that there was little difference between **(52)** and **(41)** which are monovalent and divalent versions of the same compound. The graph also shows that with ligand-dendrimer conjugates **(44)** and **(45)** there is an increase in the affinity in comparison to the dimeric **(39)** and **(41)**, however it not a very significant increase in binding affinity, certainly not as great as was hoped. As the compounds were so insoluble it was impossible to obtain IC_{50} values, which would give us a real indication of the effect of dendrimeric presentation.

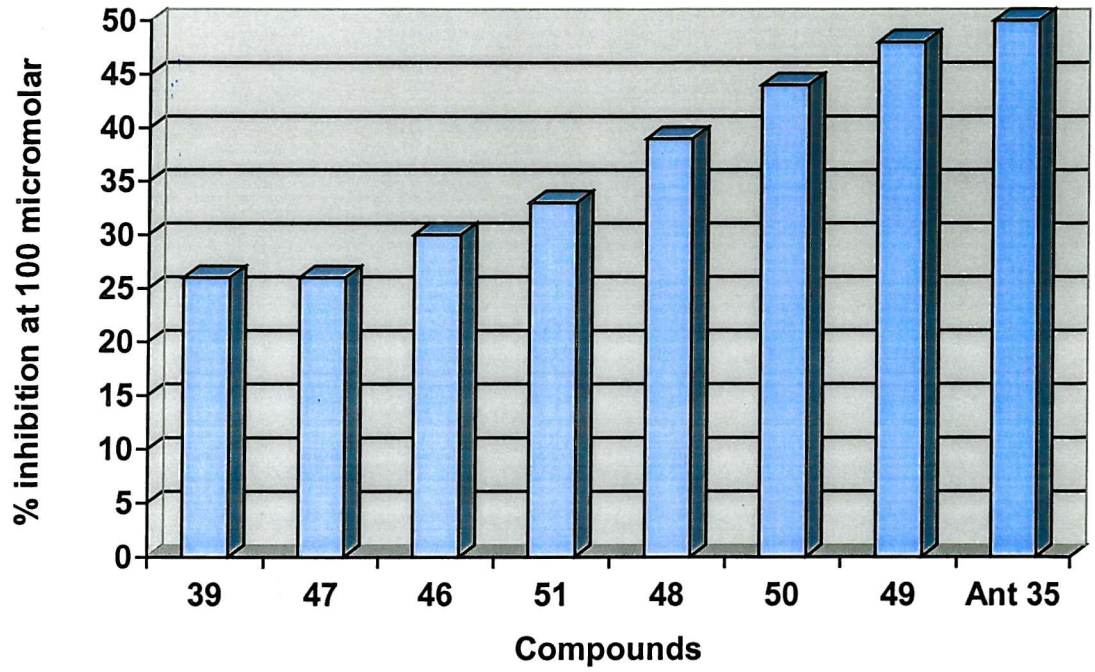


Figure 48: Screening of compounds **(39), (46)-(50), Ant 35**

In the screening experiment performed on compounds **(46)-(50)** (Figure 48) all the compounds had activity at 100 μM. All the dendrimer compounds were more potent than the divalent compound **(39)**. It would also appear that there is no significant pattern to the variation of spacer length except that the compounds with the 3 residues of ∈Ahx were the most insoluble and this may explain the fact that **(49)** was apparently more potent than **(51)**.

From both screening experiments it would appear that the AB_3-type dendrimeric scaffold is the best for the presentation of active ligands as in both cases these conjugates had the most significant increase in avidity in comparison to the divalent compounds.

3.8 Synthesis and Screening of EILDVPST-Dendrimer Conjugates.

As it was discovered in the previous screens that LDV on its own was not sufficient for good binding activity and that the capping groups although enhancing activity decreased the solubility of the compounds. It was decided to combine this knowledge to obtain meaningful IC_{50} values from which some real conclusions about the potential of multivalency to increase the binding affinity of an LDV containing ligand to its integrin

receptor could be derived. Since the biotinylated EILDVPST-NH_2 peptide used in the screen was known to be active and a quite soluble compound due to the Asp, Ser and Thr residues. It was therefore decided to couple this to our dendrimer resins **(18a)** and **(19)**. This was achieved using standard Fmoc chemistry. EILDVPST-NH_2 **(55)** and biotinylated EILDVPST-NH_2 **(56)** were also synthesised using Fmoc chemistry on polystyrene resin derivatised with a rink linker (Figure 50).

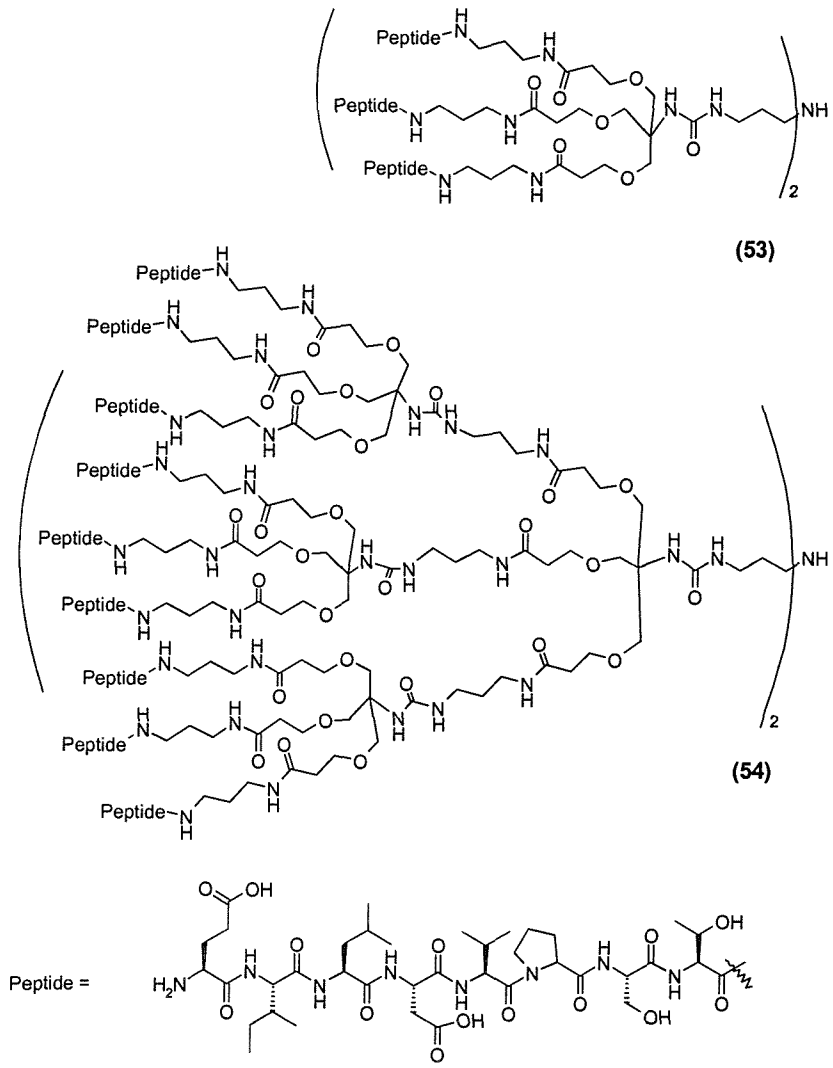


Figure 49: EILDVPST-dendrimer conjugates synthesised for screening.

The biotinylated peptide was also synthesised as the company sponsoring the work had exhausted its supply of biotinylated EILDVPST-NH_2. The exact structure of the biotinylating component of the sponsoring company's compound was unknown, as this

information was not available. As the biotinylated peptide **(56)** was different from the previous compound used (the biotinylation reagent was not the same) this had implications for the screen. It was found that the newly synthesised biotinylated peptide EILDVPST-NH_2 **(56)** when used in the screening experiment did not give as intense a colour as in previous screens for the step where the OPD was converted to an orange colour by the horseradish peroxidase. Therefore a higher concentration was needed to obtain sufficient intensity of colour from which to make comparisons. The subsequent screens therefore used biotinylated peptide EILDVPST-NH_2 **(56)** at a conc of 1 mM rather than 100 μM. This however meant that larger amounts of test compound were required, in order to allow higher concentrations of test compound. This was necessary to compete effectively with the higher concentration of competition biotinylated peptide.

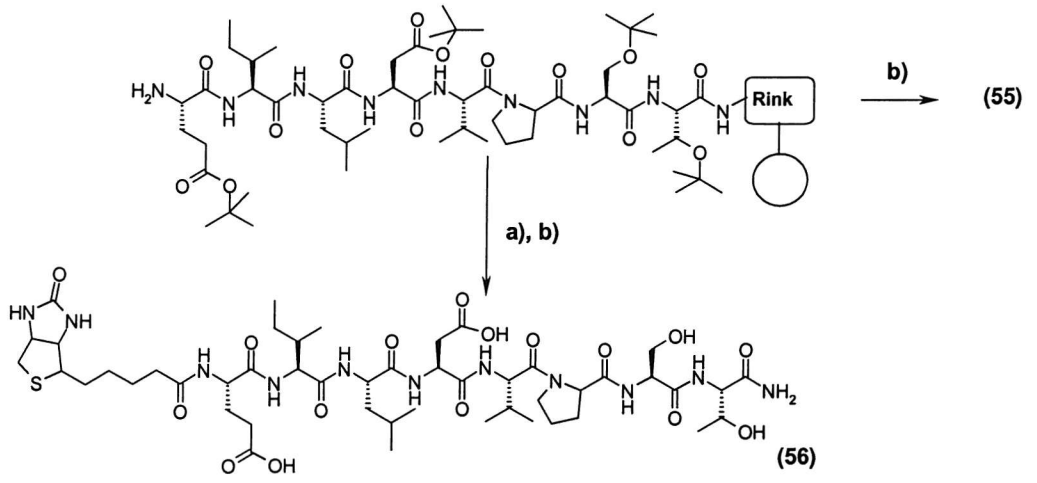


Figure 50: Synthesis of EILDVPST-NH_2 **(55)** and biotinylated-EILDVPST-NH_2 **(56)**: a) d-biotin, diisopropyl carbodiimide, hydroxybenzotriazole, Dimethylsulphoxide; b) 90% Trifluoroacetic acid/dichloromethane.

The hexavalent compound **(53)** was water soluble and had an IC_{50} value of 0.70 mM this compound was therefore 12-fold better at competing with biotinylated EILDVPST-NH_2 compound than the non-biotinylated peptide EILDVPST-NH_2 (which had an IC_{50} value of 8.52 mM).

EILDVPST-Gen [2.0]-AB_3-type dendrimer conjugate **(54)** was not soluble in the diluent and therefore was screened in the peptide diluent plus 10% DMSO as before. However

insufficient compound was available to obtain enough points for a curve and hence (Figure 51) does not show a curve for this compound.

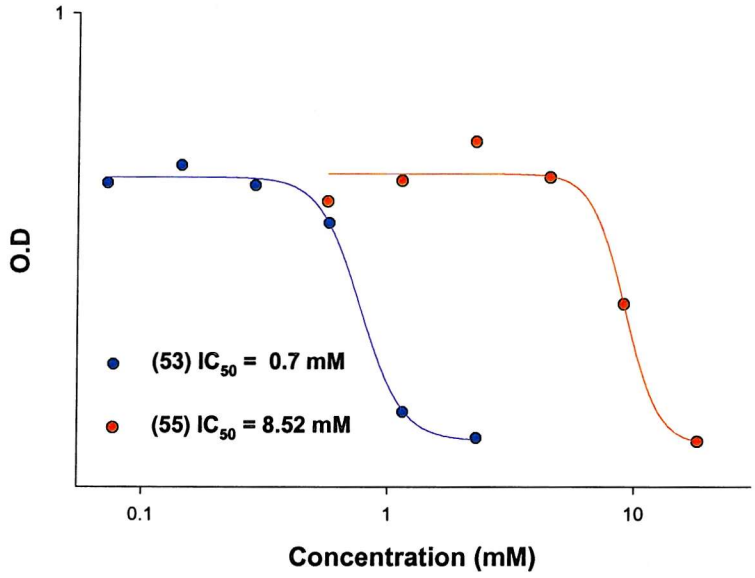


Figure 51: Standard curves for **(53)** and **(55)**.

From these results for the first time there is definite evidence that multivalent presentation of the peptide ligand EILDVPST-NH_2 can increase its affinity for its integrin receptor. It can also be said that a multivalent effect is in operation for the hexavalent form as the increase in binding affinity is 12 times that of the monovalent form, which represents more than the increase of 6-fold due to ligand number.

Although unable to obtain an IC_{50} value for compound **(54)** it would seem from the points obtained that there is little or no improvement in activity going from the hexavalent to the 18-valent compound in contrast to the improvement from monovalent to the hexavalent. This would suggest that there might be a ceiling to the increase in affinity that can be obtained with an increase in ligand presentation.

3.9 Conclusions

In this chapter dendrimer conjugates were investigated as a possible way to increase the affinity of the weak binding ligand, (LDV), to the $\alpha_4\beta_1$ integrin. It was found in an initial competitive screening experiment of a series of LDV-dendrimer conjugates that the presentation of the LDV motif itself did not produce potent competitive binding molecules for the $\alpha_4\beta_1$ integrin. It was concluded that other residues or spacer molecules would be required. The subsequent screening of a small library of di-valent

capped LDV compounds proved these conclusions, as the compounds were active. It was next investigated if the activity of these optimised ligands could be enhanced through dendrimeric presentation and this was found to be the case although limited due to solubility issues. Finally a multivalent competitive binding molecule for the $\alpha_4\beta_1$ integrin was found **(53)** which was 12-fold more potent than the monovalent EILDVPST-NH_2 **(55)**. The results although promising did not have the dramatic increases in affinity between multivalently presented ligands for the integrin receptor that other groups have found with multivalently presented sugar ligands for selectin receptors. This may be due to the insensitivity of the screen used, which was originally designed as a stringent first round screen to identify potent hits. The reason may lie in the nature of the receptor itself. Selectins, the receptor studied with multivalent carbohydrate ligands, possess multiple binding sites and are tightly packed in bunches on the surfaces of cells lending itself to multivalent interactions and this may not be the case for the integrin $\alpha_4\beta_1$

Chapter 4:
Synthetic Methods for the Construction of Multivalent Ligand Conjugates

Chapter 4 Synthetic Methods for the Construction of Multivalent Ligand Conjugates

4.1 Introduction to DNA Encoding Strategies

Oligonucleotides and peptides were the first entities employed for the chemical tagging of compound libraries. In 1993 workers at Affymax[10] published a paper wherein they had constructed and screened a bead based library of 7^7 different heptapeptides each encoded with a oligonucleotide tag present on a portion of the beads sites (Figure 52). A fluorescently labelled antibody was screened for its ability to bind to the beads and the sequence of the active peptides was deduced through PCR amplification and sequencing. A year previous to this in 1992[28] Brenner and Lerner published a theoretical paper describing how an oligonucleotide encoded library could be constructed and the following year in 1993[29] they described the design and synthesis of a set of base cleavable orthogonally protected matrices for the alternating bi-directional synthesis of encoded peptide entities (Figure 52). They also performed binding assays on five peptide sequences conjugated to an encoding oligonucleotide three of which contained a known active peptide epitope for β-endorphin. Oligonucleotide encoding is a powerful technique for encoding peptide-based libraries and the following studies were carried out to allow the synthesis of constructs, which would facilitate the use of this DNA encoding technique in the area of combinatorial chemistry.

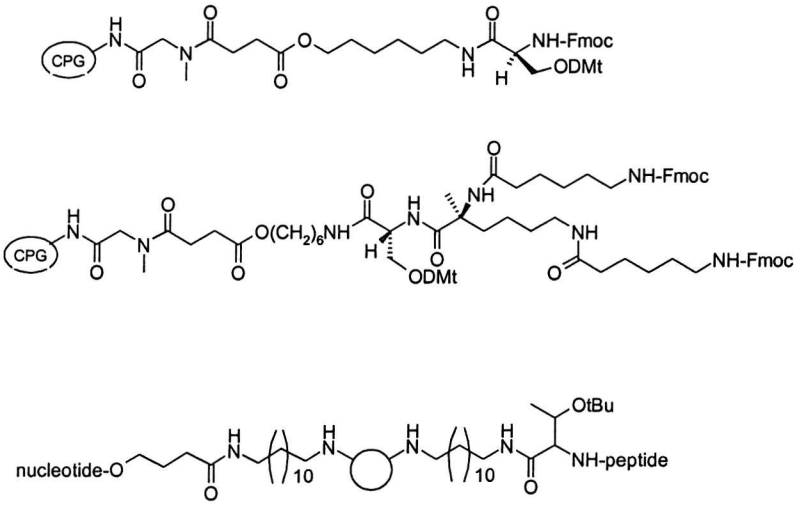


Figure 52: DNA encoding technologies previously developed.

Bradley has shown the utility of polyamines that are selectively functionalised and immobilised on a solid support by means of a Wang "oxycarbonyl" linker for the synthesis of polyamine conjugates and the library synthesis of polyamine conjugates.[164, 165, 172] It was a good starting point for the design of constructs, which would facilitate the synthesis of oligonucleotide encoded multivalent ligand libraries. The first synthesis to be described in this chapter will be the synthesis of a polyamine-linker construct, which would allow the selective functionalisation of one arm of an immobilised 1,5,9 triazanonane and then allow the subsequent functionalisation of the other arm. The construct design is shown in (Figure 53). Dendrimers prepared in this way could be used for the construction of a library, which could be encoded with a tag on the other arm. This would require orthogonal protecting groups stable to Fmoc peptide chemistry and oligonucleotide synthesis (as it was anticipated that the tag would consist of a DNA strand) (Figure 54) and finally cleaved with acid.

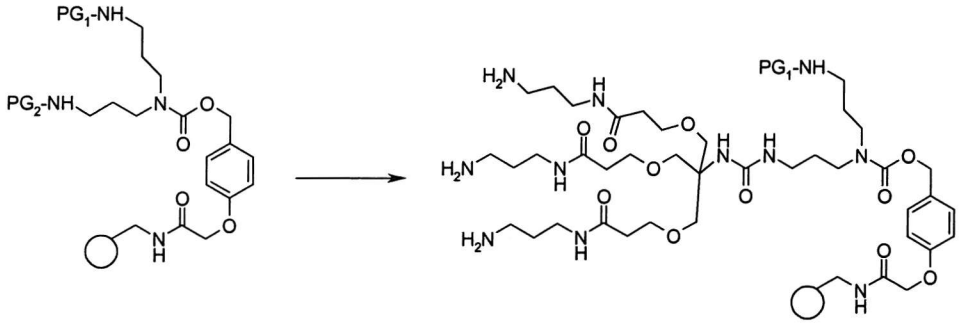


Figure 53: Proposed preparation of dendrimers on one arm of 1,5,9-triazanonane.

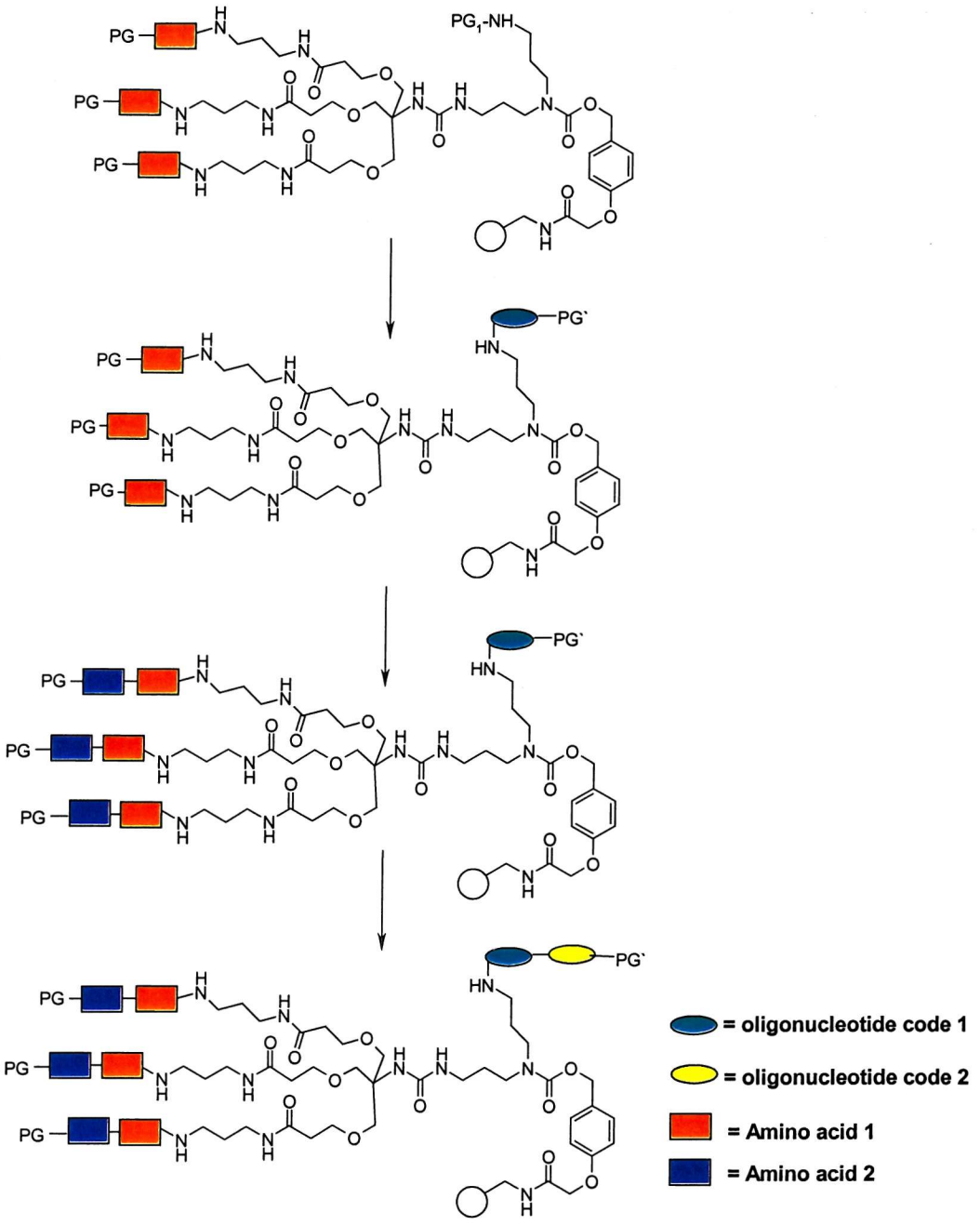


Figure 54: Proposed synthetic method to produce an encoded peptide-dendrimer conjugate library.

Once a suitable construct was synthesised peptide-dendrimer conjugates could be generated on one arm of the immobilised 1,5,9-triazanonane and then screened in order to discover potent new ligands for a chosen receptor.

4.2 Synthesis of a Tetra-Orthogonal Polyamine Linker Construct

The synthesis of the immobilised 1,5,9 triazanonane required in reality the use of four "protecting groups" which were selectively cleavable and two targets **A** and **B** were proposed. (Figure 55) shows the conditions that allow the selective removal of each "protecting group."

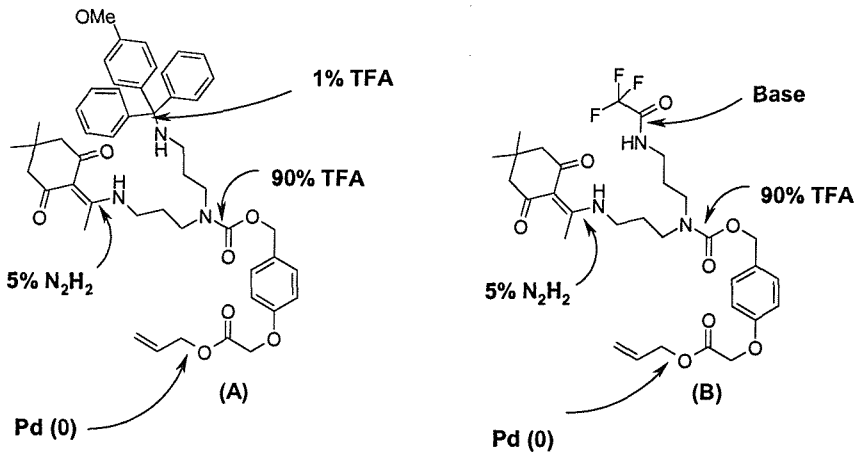


Figure 55: Conditions allowing the selective removal of each "protecting group."

4.2.1 Synthesis of N^1-(4, 4-Dimethyl-2, 6-Dioxocyclohexlidene)ethyl-N^9-Methoxy Trityl-1,5,9 Triazanonane (60).

The synthesis of protected polyamine **(60)** began with the selective mono-protection of 1,5,9-triazanonane **(1)** by the addition of (1 eq) of ethyl trifluoroacetate at low temperature according to a published procedure used for spermidine.[173] The remaining amine functionalities of this intermediate were protected by the action of an excess of Boc-anhydride to give the fully protected 1,5,9 triazanonane derivative **(57)**. Selective trifluoroacetamide deprotection of **(57)** lead to the primary amine **(58)**, which was then protected with the Dde-protecting group to give **(59)** in 96% yield. Both Boc groups were cleaved following treatment with acetyl chloride in methanol and then selective protection of the primary amine was achieved using Mmt-Cl according to a literature procedure[174] producing the desired product **(60)** in 40% yield (Figure 56).

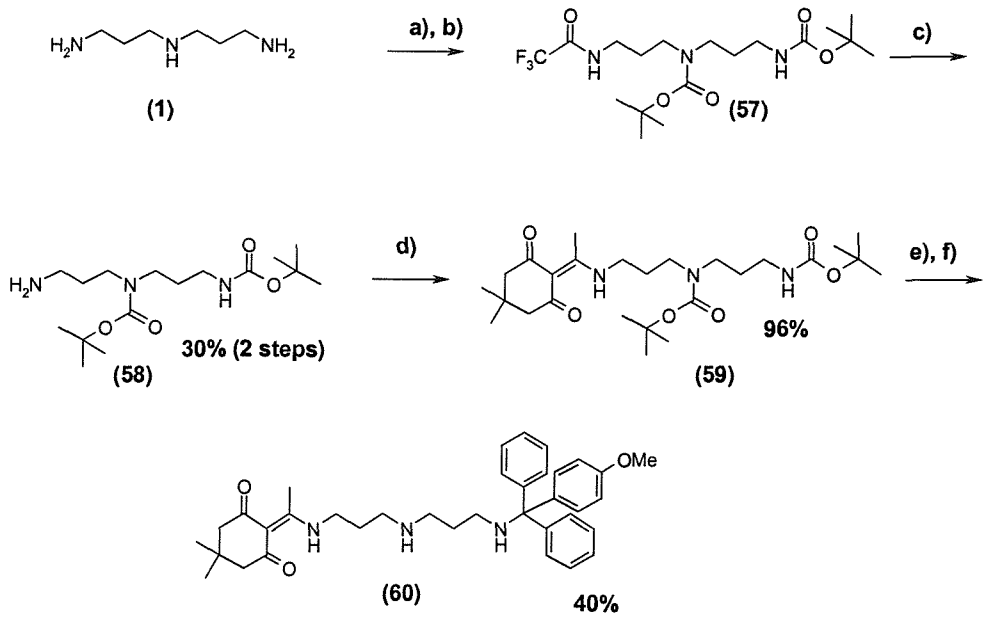


Figure 56: a) Ethyltrifluoroacetate, methanol -78°C; Di-*tert*-butyl dicarbonate, methanol; c) Potassium carbonate, methanol/water; d) 2-Acetyl dimedone, triethylamine, dichloromethane; e) Acetyl chloride, methanol; f) Methoxytritylchloride, triethylamine, dichloromethane 0°C.

4.2.2 Synthesis of N^1-(4, 4-Dimethyl-2, 6-Dioxocyclohexlidene)ethyl-N^9-Trifluoroacetyl-1,5,9 Triazanonane (63).

1,5,9 triazanonane **(1)** was selectively protected at one of the primary amines with ethyltrifluroacetate at -78°C after removal of solvent the crude compound had its remaining primary amine selectively protected with 2-acetyldimedone in the same reaction vessel to afford compound **(62)** in 51% yield (Figure 57).

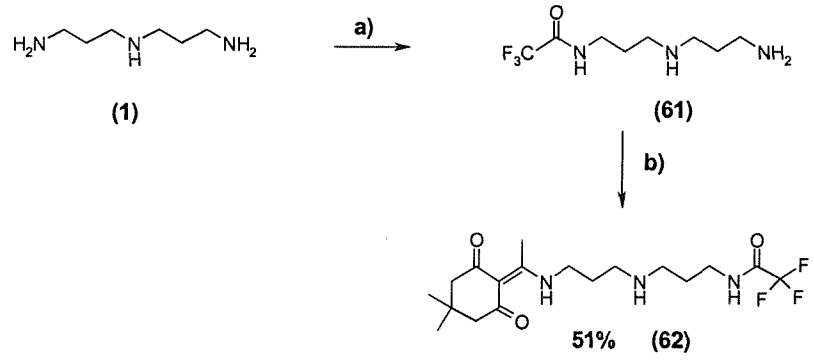


Figure 57: a) Ethyl trifluoroacetate, methanol, -78°C; b) 2-Acetyl dimedone, dichloromethane.

It can be clearly seen from the synthesis that the second synthesis of compound **(62)** was much quicker and efficient. Compound **(62)** was also much more stable to column chromatography than compound **(60)** which frequently decomposed on the column due to the acid sensitivity of the Mmt group, therefore **(62)** was prepared on a large scale for the synthesis of the linker-polyamine conjugates.

4.3 Synthesis of Polyamine Linker Construct (B)

The synthesis began with alkylation of aldehyde **(1)** followed by reduction of **(63)** to the alcohol **(64)** with cyanoborohyride in 80% yield. Subsequent reaction with nitro-phenylchloroformate afforded the reactive carbonate **(65)**. This was reacted with the protected polyamine **(62)** to afford the allylester protected linker construct **(66)** in 46% yield.

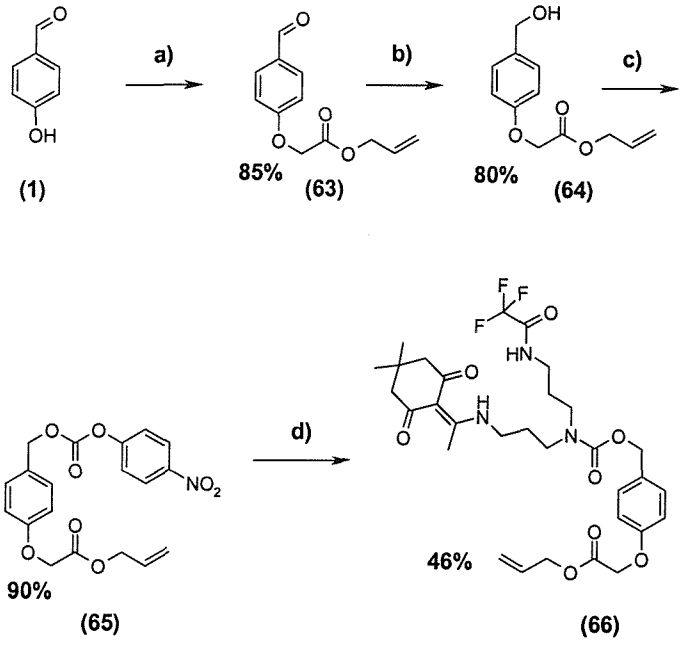


Figure 58: Synthesis of orthogonally protected polyamine- linker conjugate **(66)**: a) Potassium iodide, potassium carbonate, acetonitrile, chloroacetic acid allyl ester, reflux; b) tetrahydrofuran/water, sodium cyanoborohydride; c) Dichloromethane, Pyridine, *p*-nitro-phenylchloroformate; d) **(62)**, triethylamine, dimethylformamide.

Deprotection of an Allyl Ester Using a Plug Supported Pd Catalyst.

Deprotection of the allyl ester required the use of Pd (0) catalyst. The catalytic cycle is shown (Figure 59).

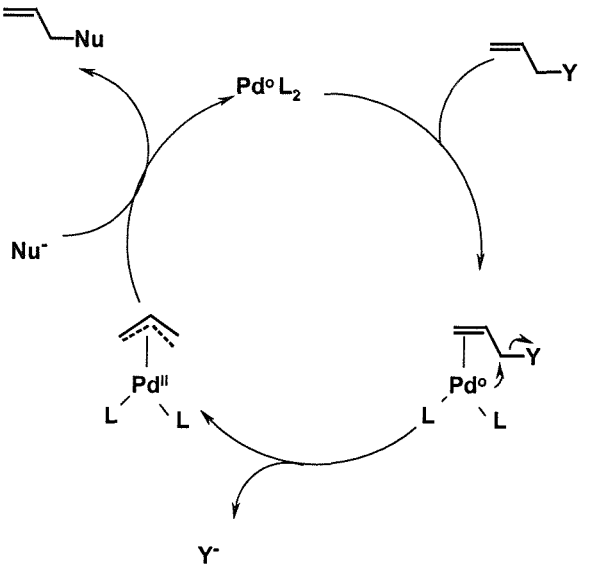


Figure 59: Catalytic cycle of allyl deprotection.

The product after reaction in solution and column chromatography was still contaminated with Pd compounds. It was decided therefore to investigate a supported catalyst to perform the step. Supported catalysts have become very popular recently with a number of reviews being published.[175] The supported catalyst used was a Pd (0) catalyst, which has been developed within the Bradley group.[176] The reaction using the supported Pd (0) catalyst was very straightforward involving refluxing **(66)** (Figure 60) overnight in THF with the plug and pyrrolidine. The product **(67)** was obtained in high purity by removal of the "Plug" then adjustment of the pH and extraction with ethyl acetate. This reaction was superior to the first method producing the desired product in good yield and significantly greater purity without the need for purification by column chromatography.

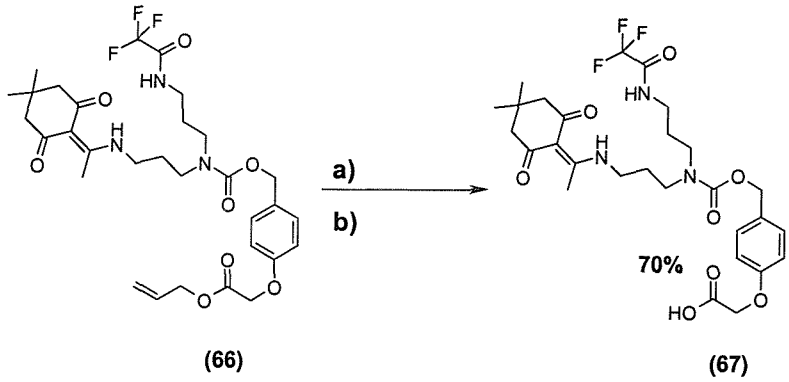


Figure 60: a) Pd (0), pyrrolidine, tetrahydofuran reflux; b) 1M potassium hydrogensulphate/water/dioxane

4.4 Synthesis of Dendrimer-Peptide Conjugates on Dendrimer Resins (71), (72), (73).

Coupling of **(67)** to TentaGel resin with HOBt, DIC, in DCM afforded the resin bound 1,5,9 triazanonane **(68)** (Figure 61). This resin bound 1,5,9 triazanonane construct with protecting groups orthogonal to each other at the N^1, N^5 and N^9 positions was suitable for the synthesis of a DNA encoded peptide-dendrimer library. However the initial aim was the synthesis of dendrimers, which could then be conjugated to a peptide sequence, which could be screened for their ability to compete with the biotinylated peptide EILDVPST-NH_2. This was to determine if this type of peptide dendrimer conjugate behaved in similar fashion to those investigated previously, see (chapter 3).

The synthesis began with deprotection of the Dde protecting group with 5% hydrazine, completion of the reaction was checked by RP-HPLC (by the disappearance of starting material). As a library was not going to be synthesised the Dde group was replaced with an acid-labile Boc group. Removal of the trifluoroacetyl group afforded resin bound dendrimer initiator core **(71)** (Figure 61).

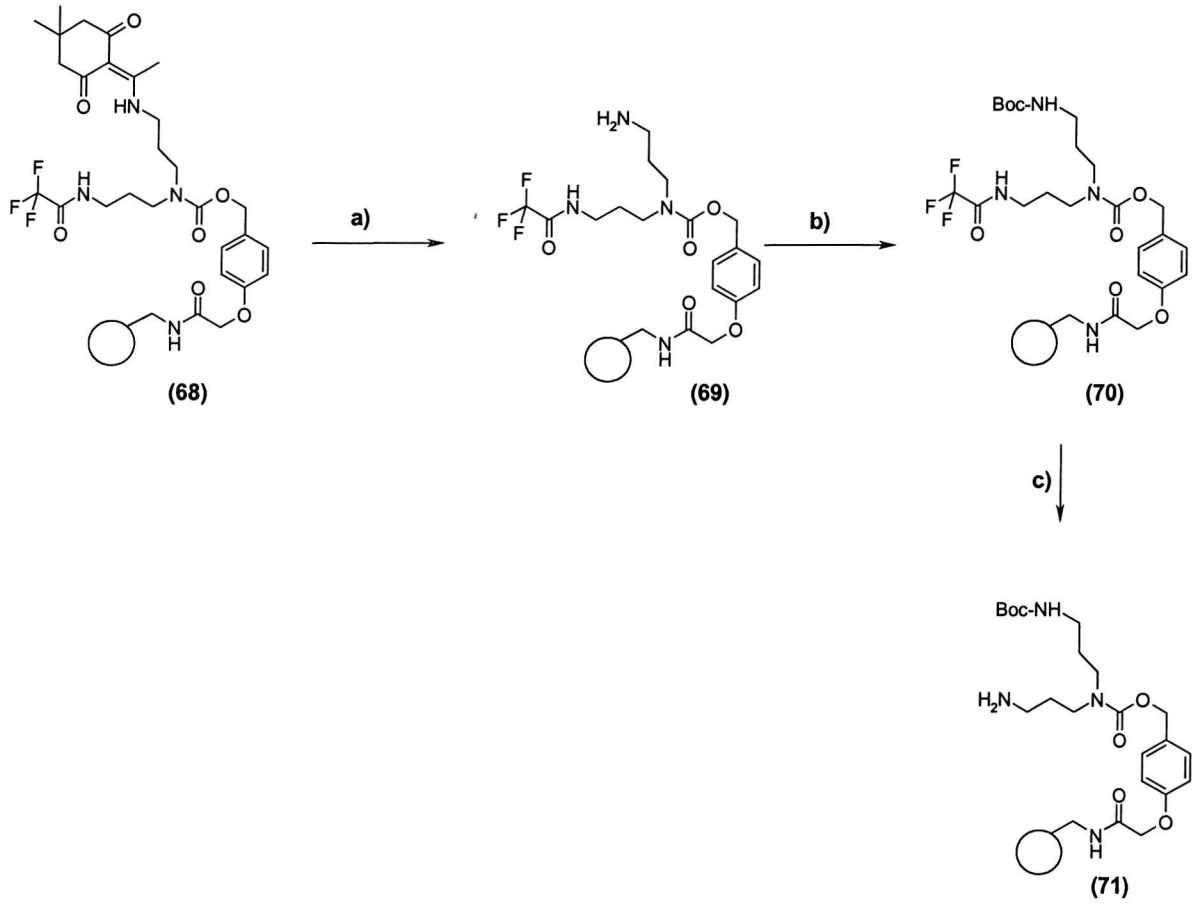


Figure 61: Synthesis of dendrimer initiator core **(71)**. a) 5% hydrazine, dimethylformamide; b) Di-*tert*-butyl dicarbonate, diisopropylethylamine, dioxane; c) 1M potassium hydroxide/tetrahydrofuran:methanol.

From this initiator core dendrimer synthesis was carried out as before with repetitive coupling of the isocyanate **(16)** and displacement of the methyl esters (Figure 62).

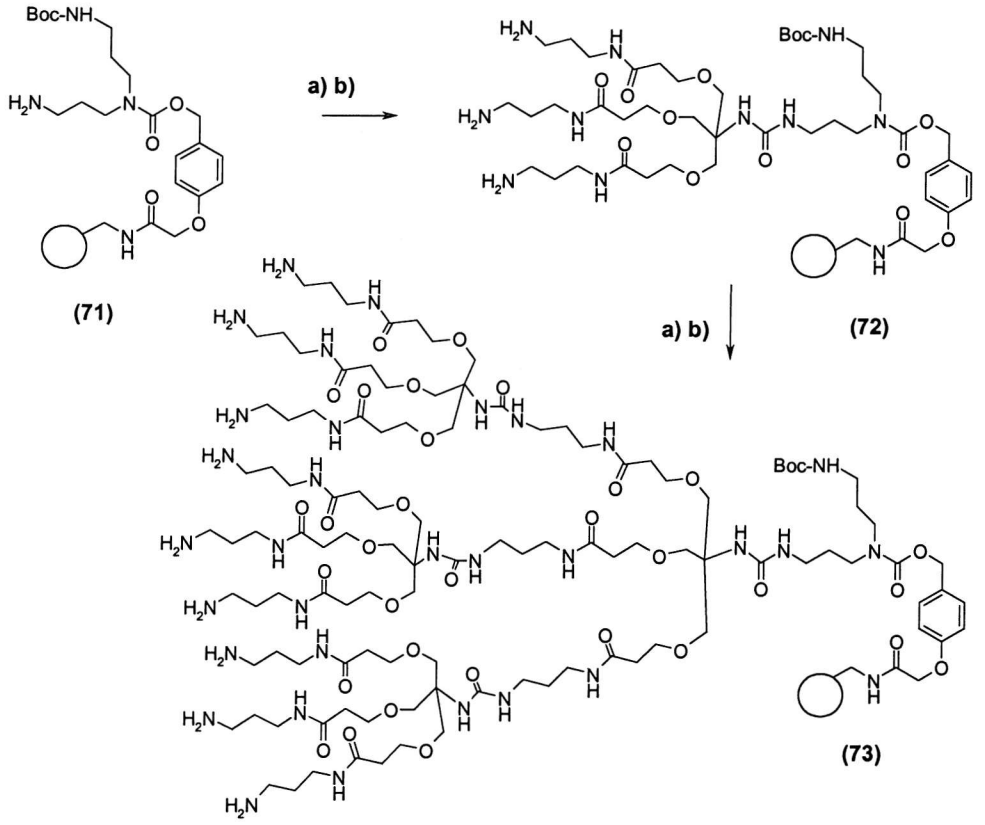


Figure 62: Dendrimer synthesis a) **(16)**, dimethylaminopyridine, diisopropylethylamine, dichloromethane b) 1,3-propanediamine, dimethylsulphoxide.

4.5 Attempts to Improve the Solubility of the Peptide–Dendrimer-Conjugates.

A major problem observed when screening the peptide-dendrimer conjugates was due to the fact that they were increasingly insoluble at the higher generations mainly due to the hydrophobic nature of the $\alpha_4\beta_1$ ligands. The optimised ligand-dendrimer conjugates in chapter 3 were so insoluble that it was impossible to obtain IC_{50} values for them however this was overcome by the incorporation of polar residues such as Ser and Thr into the full length peptide. This strategy allowed good standard curves to be obtained and hence meaningful IC_{50} values. However when constructing a library of ligands this would only allow residues, which were polar in nature to be used. It was therefore decided to investigate 4,7,10-trioxa-1, 13-tridecanediamine as the diamine to displace the methyl esters. This would then improve the water solubility of the dendrimer scaffold whilst still maintaining the homogeneous nature of the dendrimer. Poly(ethylene glycol) has been frequently used as a hydrophilic polymer for protein modification and it has been found that "pegylated" proteins antibodies, enzymes and dendrimers[177] etc are more resistant to proteolytic degradation than unmodified ones, as well as having a reduced immunogenicity. The added length of the diamine would also

add greater flexibility and space the ligands further apart. (Figure 63) shows the reaction products from the resin after 3 weeks at room temperature and (Figure 64) shows the corresponding HPLC trace.

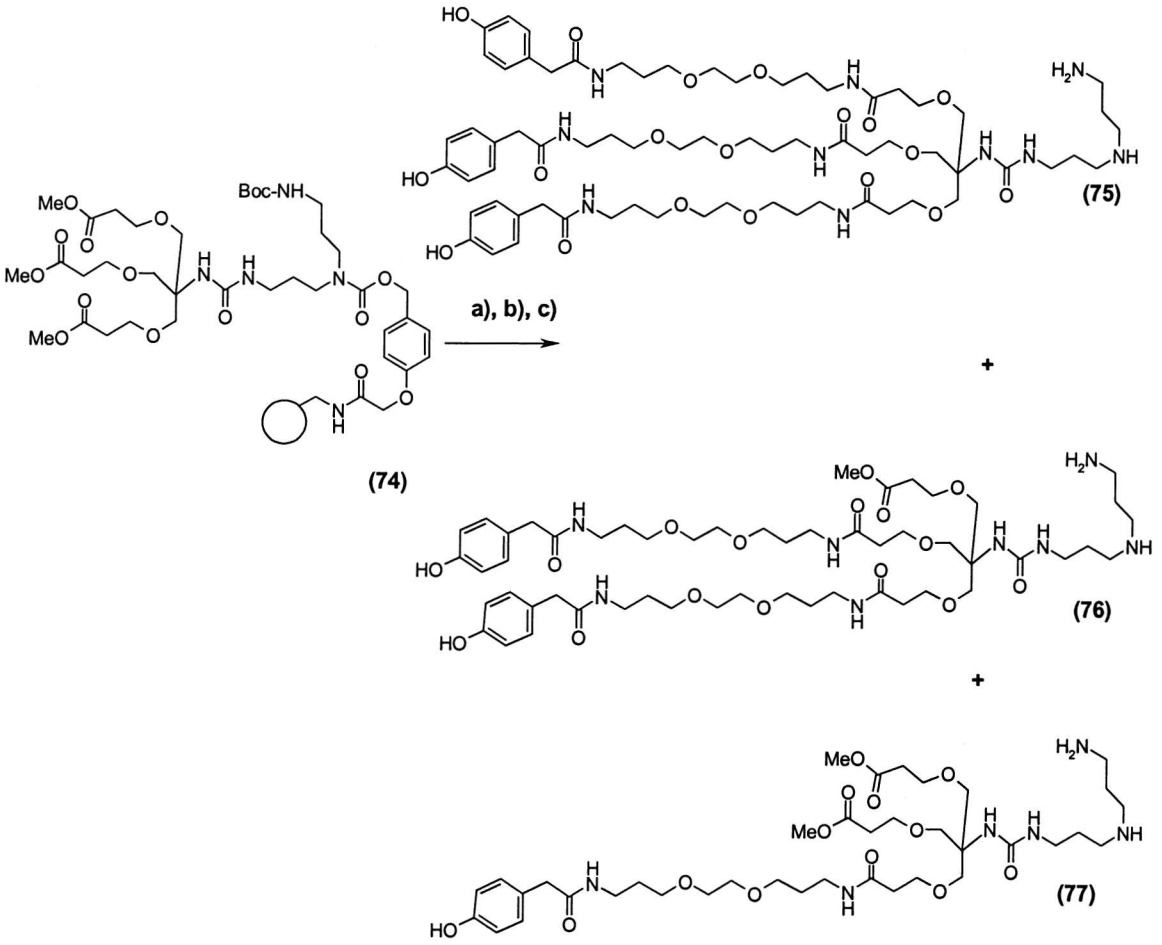


Figure 63: a) 7, 10-trioxa-1, 13-tridecanediamine, dimethylsulphoxide; b) (4-hydroxy-phenyl)-acetic acid, diisopropylcarbodiimide, hydroxybenzotriazole; dichloromethane c) 90% trifluoroacetic acid/dichloromethane

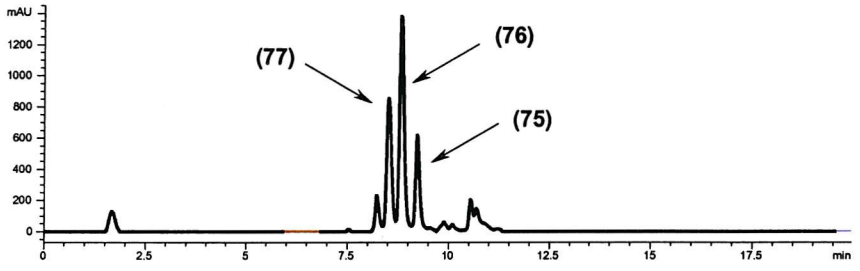


Figure 64: HPLC of **(77), (76), (75).**

4.6 Synthesis of Unsymmetrical Peptide-Dendrimer Conjugates.

As attempts to produce more soluble peptide-dendrimer conjugates using PEG spacers in the dendrimer architecture did not produce dendrimers as homogeneous as those already synthesised, it was decided to continue with resins **(71)**, **(72)**, **(73)** for the synthesis of peptide-dendrimer conjugates on one arm of the 1,5,9 triazanonane construct. The peptide sequence chosen for these experiments was a combination of the optimised capping group (4-hydroxyphenyl)acetic acid with the more water-soluble ligand. The capping group was useful as it would not only increase binding potential, but would also allow the synthesis of peptide-dendrimer conjugates with only one free primary amine. The modified peptide sequence (4-hydroxyphenyl)acetic acid-LDVPST was constructed onto the dendrimer resins **(71)**, **(72)** and **(73)** using standard Fmoc chemistry as previously described (Figure 65).

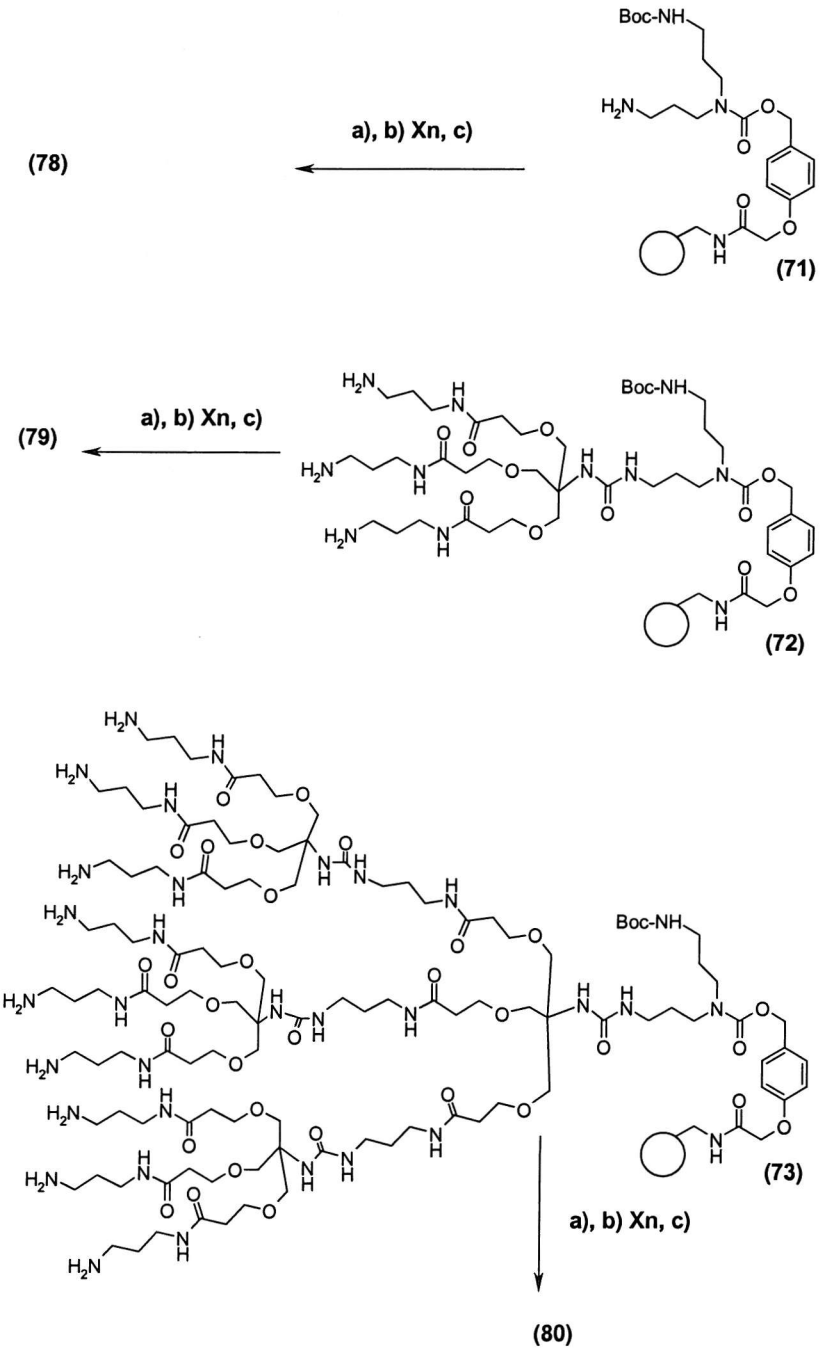


Figure 65: a) Fmoc-AA-OH, diisopropylcarbodiimide, hydroxybenzotriazole, dimethylformamide; b) 20% piperidine DMF; c) 90% Trifluoroacetic acid/dichloromethane.

After synthesis the conjugates were cleaved with 90% TFA/DCM providing the desired compounds which had excellent crude purities. HPLC traces are shown (Figure 66). Compounds **(78)** and **(79)** were water-soluble whereas **(80)** was soluble in 10 % DMSO/H_2O.

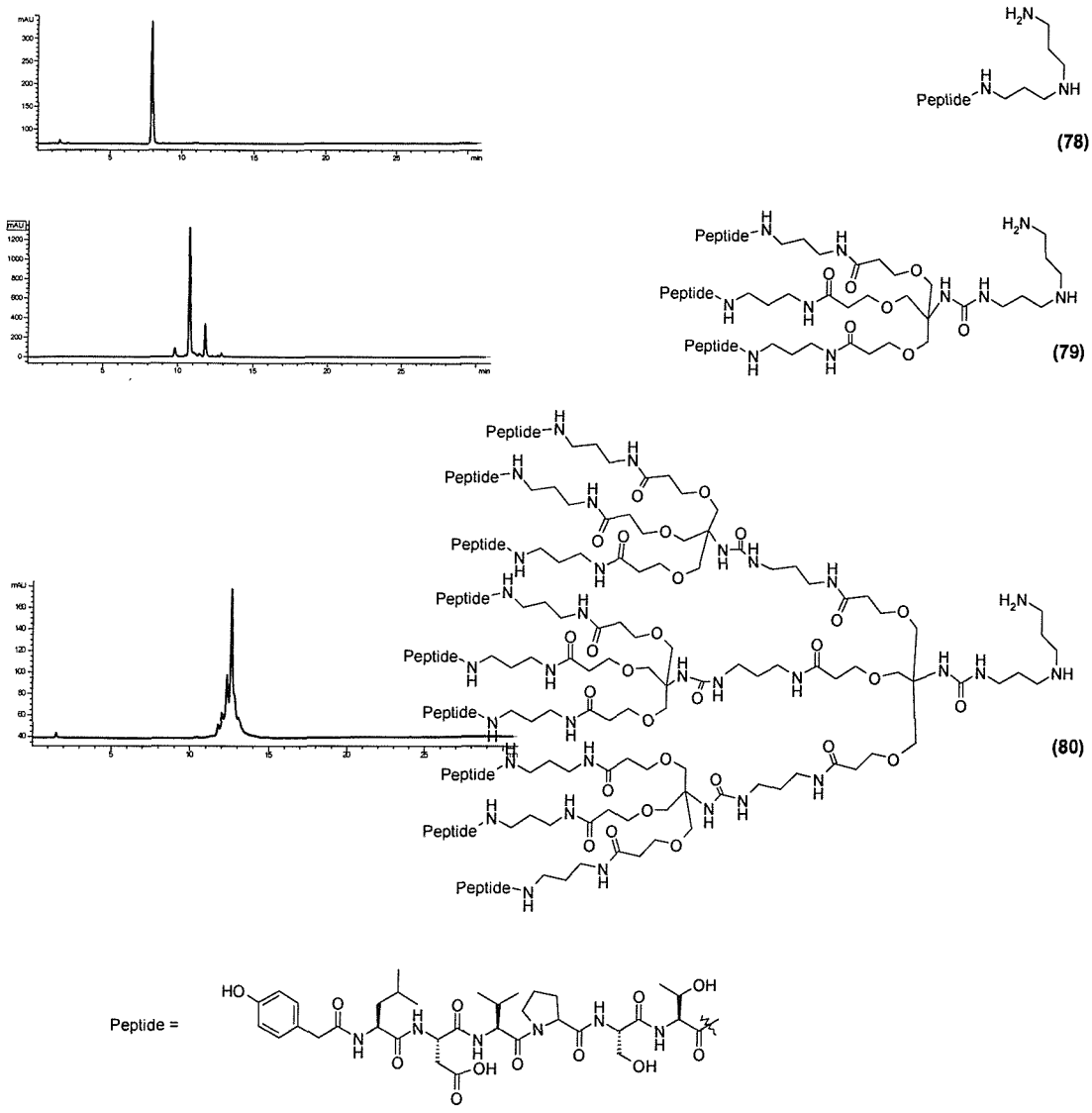


Figure 66: Crude RP-HPLC purities of compounds **(78)-(80)**.

4.7 Screening of Peptide-Dendrimer Conjugates (78)-(80).

The compounds **(55)**, **(78)**, **(79)** and **(80)** were then screened as previously described and were found to be much more soluble than the previous conjugates which enabled calculation of IC_{50} values.

The compounds were subjected to the same screen and (Figure 67) shows the curves obtained. The curve for the monovalent compound **(78)** is not shown as there was insufficient compound to obtain enough points on the curve however its projected curve was almost identical to EILDVPST-NH_2 **(55)**.

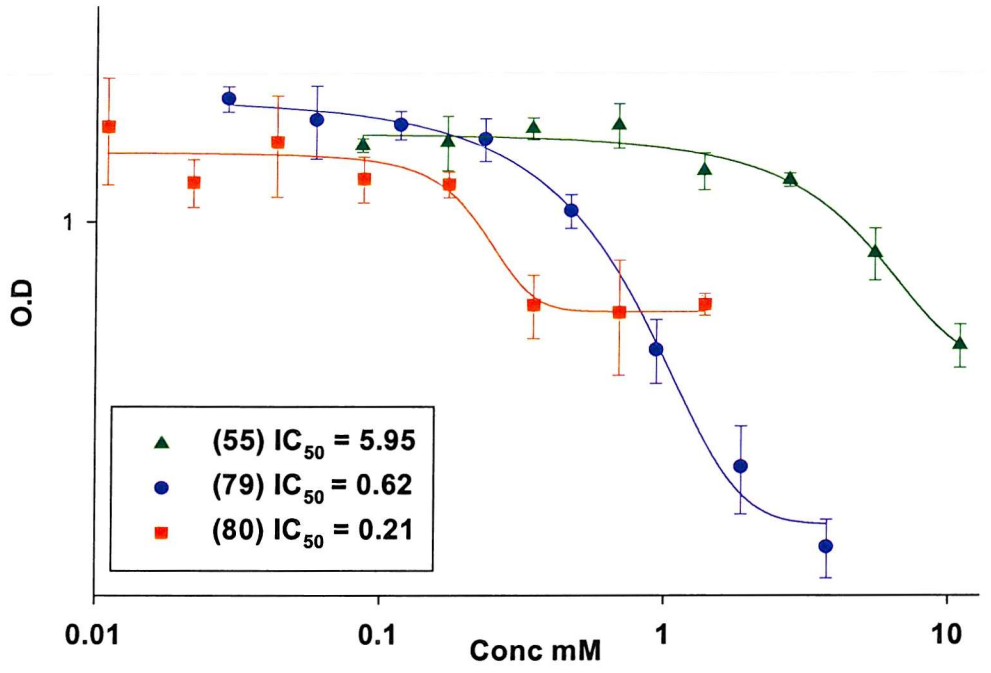


Figure 67: Standard curves for **(55)**, **(79)** and **(80)**.

It can be seen that as expected compounds **(79)** and **(80)** behaved like the compounds screened in Chapter 3 in that there was an increase in affinity going from mono to tri to nono-valent compounds. The increase in affinity for **(79)** was 9-fold whereas **(80)** was 28-fold. It can be seen therefore that the increase in affinity was greatest going from monovalent form to trivalent rather than from the trivalent to nano valent. The same effect was seen with screening of **(53)** and **(54)**, where the increase in affinity was greatest going from the monovalent form to the hexavalent than from the hexavalent to 18-valent. This may be due to the fact that there is an optimal limit to the affinity enhancement obtained with a valency of this type of molecule. The latter issue is something that Roy and co-workers have also observed.[111] The increase in affinity with the peptide-dendrimer conjugates **(79)** and **(80)** however was not as dramatic as those reported with carbohydrate ligands. A whole host of effects could contribute to this for instance the assay sensitivity, or the ligand itself perhaps the RGD ligand would behave differently as it binds a whole range of integrin receptors.

4.8 Conclusions

1,5,9 triazanonane was successfully protected with orthogonal protecting groups at N^1 and N^9 and at the resin attachment site $N.^5$ **(62)** was used for the synthesis of construct **(66)**, which allowed the preparation of peptide-dendrimers on only one arm of 1,5,9 triazanonane. Highly homogeneous peptide-dendrimer conjugates were synthesised, and screened for their ability to compete with biotinylated EILDVPST-NH_2 **(56)** these were observed to behave as the peptide–dendrimer conjugates previously synthesised and described in chapter 3. The construct synthesised is suitable for the synthesis of encoded peptide-dendrimer libraries.

Chapter 5:

Development of a Synthetic Phage

Chapter 5 Development of a Synthetic Phage.

5.1 Introduction to Phage display.

Filamentous phage are viruses that infect bacterial cells[178-179] and are flexible rods around 1 μm long and 6 nm in diameter, composed mainly (87% by mass) of a tube of helically arranged molecules of 50-residue major coat protein pVIII, encoded by a single phage gene VIII. Inside this tube lies the single stranded DNA (ssDNA; 6407-8 nucleotides in wild-type strains) (Figure 68).

Significantly phage can accommodate "foreign" DNA, namely stretches of human DNA or stretches of chemically synthesised DNA. When phage therefore infect and replicate inside a host cell the insert DNA is also replicated along with the rest of the phage DNA. In the technique of "phage display" the foreign gene is spliced into the gene for one of the viral coat proteins. This means that the foreign DNA sequence will encode the amino acid sequence of the coat protein and the peptides thus produced will be displayed on the surface of the viral coat protein. (Figure 68) below shows some of the different types of phage display systems that are used.

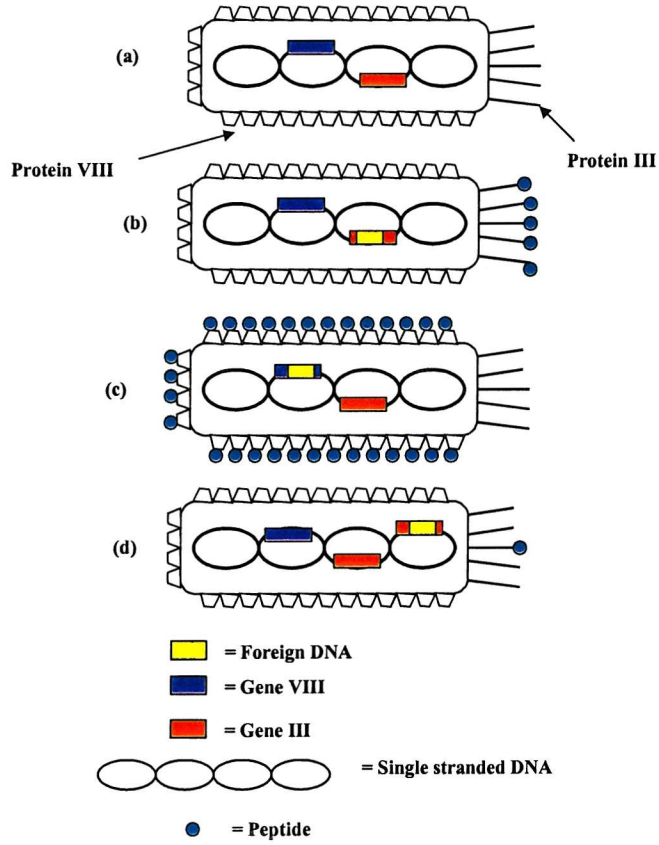


Figure 68: (a) Wild type; (b) recombinant type 3; (c) recombinant type 8 and (d) recombinant type 33 bacteriophage M13 viral particles. Viral particles are composed of one copy of single-stranded DNA and five capsid proteins. DNA can be inserted into genes III or VIII to generate chimeric proteins with the resulting peptides displayed at the *N*-terminus of either protein. The green circles on the surface of the virions represent the foreign peptides specified by the foreign coding sequences represented by the yellow box. The foreign peptide can be displayed once, five times or ~2500 times.

This technology therefore allows the production of phage display libraries, which are heterogeneous mixtures of phage clones but each carries a different peptide on its surface due to the fact that each will have a different DNA insert. Although phage are relatively large the displayed peptides are very accessible to the solvent medium and they behave much like they do free in solution when not attached to the virion surface. A feature of phage display however is that in many phage display systems for example type 3 and type 8 the peptide sequence is displayed in a multivalent fashion. In the case of type 3 the peptide is displayed 5 times and in type 8 is displayed all over the coat ~2500 times, this is significant because the binding affinity is coming not only from the ligand itself but also from a multivalent effect which is operating by the display of multiple copies of the peptide. It is therefore better in a phage display assay, in order to introduce more stringent criteria for the binding motif, to use a low density of the target receptor[180] and monovalent display of the foreign peptide[181] as in type 33 phage.

5.2 Screening phage libraries.

Phage libraries are typically screened by a process called affinity selection. The principle of affinity selection is to reduce an initial population of 10^9 phage clones each with different displayed peptides to a sub-population of phage of ~ 10^6 with increased "fitness." This sub-population can be amplified by infecting fresh bacteria so that the amplified population can be subjected to further selection to obtain a fitter subset. The ultimate goal is the survival of the fittest phage clone after a series of rounds of selection. The most common selection pressure is affinity of the phage for a target receptor. Thus in this case, phage whose displayed peptides bind the receptor are captured on the surface allowing unbound phage to be washed away. Bound phage are

recovered by a simple phage denaturation step and the recovered phage, which are still infective are amplified and the process shown in (Figure 69) is continued.

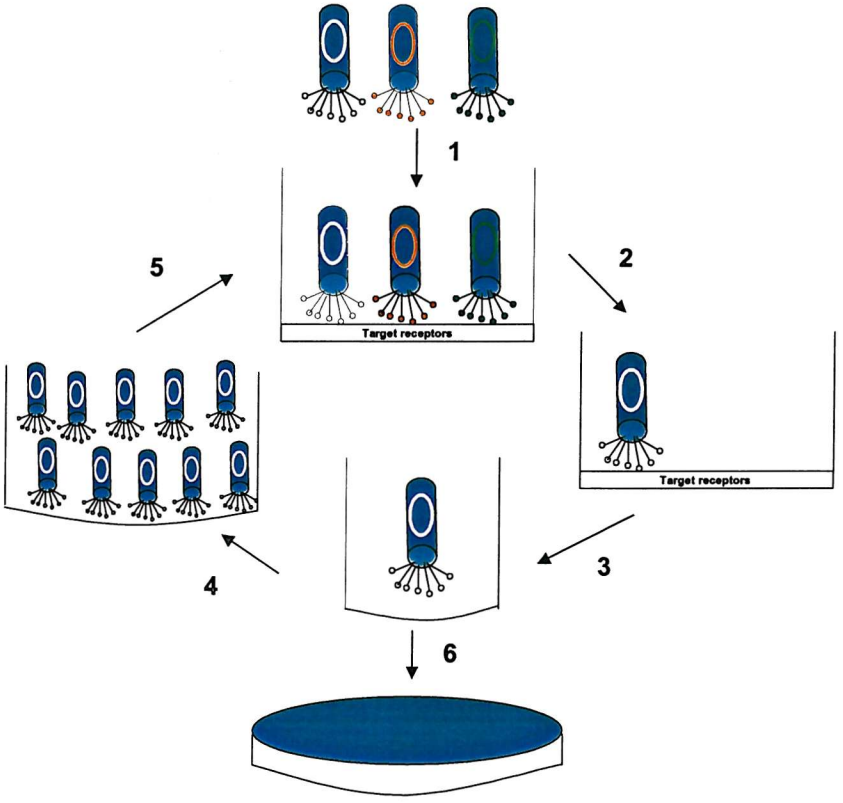


Figure 69: A library of recombinant bacteriophages displaying different peptide sequences can be fractionated by affinity selection in 6 steps. **1 :** The phage particles are added to a microtitier well coated with target protein. **2:** After incubation non-binding phage are washed away. **3:** The bound phage are released from the target by pH denaturation. **4:** The recovered phage are used to infect *E. coli* and 6 hours later each phage is amplified > 10^7 fold. **5:** An aliquot of amplified phage is added to a well containing fresh target. **6:** After repeating steps **2-5** twice more the recovered phage are plated out to yield individual plaques on bacterial lawns on petri dishes. Once the binding of clones has been confirmed their DNA is sequenced and the displayed peptide deduced.

5.3 PCR (Polymerase Chain Reaction).

PCR or the polymerase chain reaction was invented in 1985 by Kary Mullis when working for the Cetus corporation in California[182] and who later in 1993 was awarded

the noble prize for chemistry for this invention. PCR is a technique which allows the amplification of a specific DNA region that lies between two regions of known DNA sequence, to produce up to 100 billion copies of target DNA starting from only a single copy in approximately 3 hours. The original PCR protocol has been adapted for a range of specialist applications ranging from the characterisation of genes, their cloning and expression to DNA diagnostics where PCR is used for the detection of pathogens, identifying mutations responsible for inherited diseases and DNA finger printing. It has been used for the amplification of DNA tags on peptide bead libraries.[10] PCR amplification of DNA is achieved using short DNA primers which are complementary to the ends of a defined sequence of template DNA. The primers are extended on a single stranded denatured DNA (template) by a DNA polymerase (originally Klenow polymerase but replaced by the more thermally stable *Taq* polymerase) in the presence of deoxynucleoside triphosphates (dNTPs) under suitable reaction conditions. This results in the synthesis of new DNA strands complementary to the template strands. Strand synthesis is repeated by heat denaturation of the double stranded DNA, annealing the primers by cooling the mixture and primer extension by DNA polymerase at a temperature suitable for enzyme reaction. Each new strand synthesised becomes a template in further cycles of amplification. Potentially after 20 cycles there will be 2^{20} fold amplification assuming 100 % efficiency at each cycle. A number of factors however, act against the process being 100 % efficient. For example the enzyme is a limiting factor after 25-30 cycles its activity is also affected due to thermal denaturation. Secondly reannealing of target strands after their concentration increases competes with primer annealing.

A phage is essentially made up of a single-strand of DNA and a protein coat, which displays mutivalently a peptide that is encoded for by the single strand of DNA. In this chapter the aim was to synthesise a synthetic phage, comprising of a peptide sequence displayed on a monovalent, trivalent or nonavalent dendrimeric scaffold coupled to an oligonucleotide sequence (upon which PCR could be performed), encoding the peptide sequence (Figure 70). This would be used to discover if an affinity assay could be performed on the synthetic phage using cancer cells plated in the microtitier plates used for the competitive ELISA`s in chapter 3.

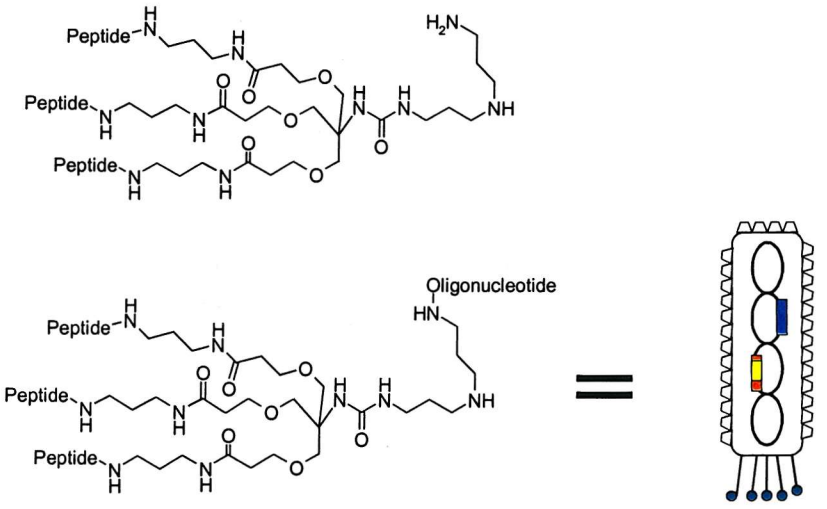


Figure 70: Design of a synthetic Phage

An oligonucleotide sequence was required that could:

1: Undergo PCR amplification therefore needing to have primer sites of at least 20 bp and a coding region for each AA.

2: Be coupled to the primary amine of the dendrimer.

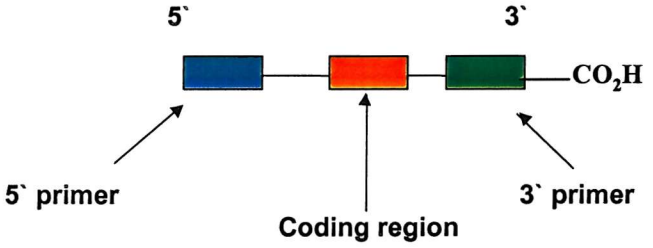


Figure 71: Design of an oligonucleotide tag.

The sequence selected was that utilised by Affymax[10] with minor alterations and was as follows.

*ATCCAATCTCTCCAC*ATCTCTATACTATCATCACC**TATCCTATCATTAC**CTCA CT*CACTTCCATTCCAC*-CO_2H

The region in bold is the coding region and regions in italics are the primer regions.

5.4 Preparation of Peptide-Dendrimer-DNA Conjugates.

The synthetic phage was synthesised by a coupling reaction between the oligonucleotide and the mono or multivalent peptide-dendrimer conjugate in solution following a published procedure[183] using 1eq of PyBop, HOBt and DIPEA (Figure 72). After coupling for 3-4 hrs at room temperature the compounds were passed through a sephedex G-50 spin column to separate the oligonucleotide-peptide dendrimer conjugates and uncoupled DNA from the coupling reagents and side products. The eluant was then freeze dried.

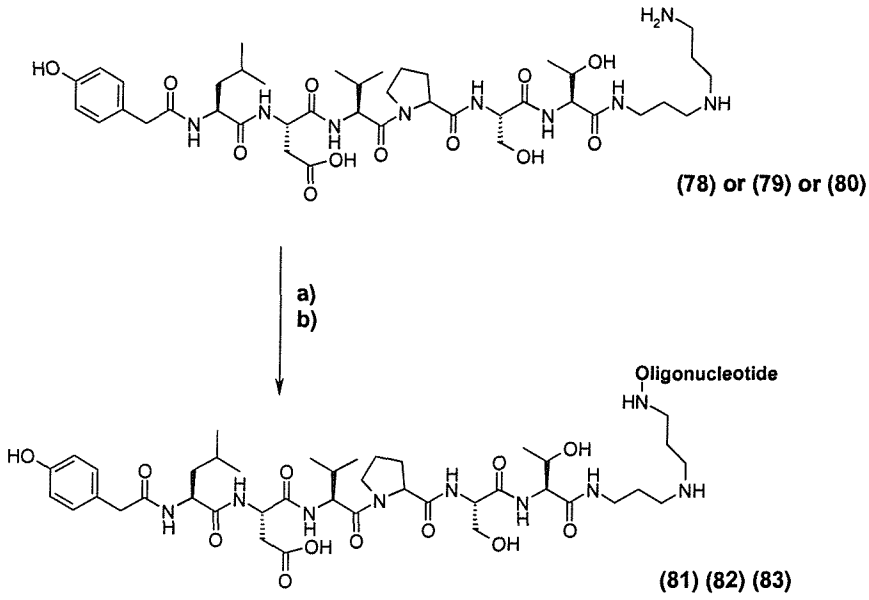


Figure 72: shows the coupling of the oligonucleotide to the monovalent peptide polyamine conjugate: a) Benzotriazol-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate, 1-Hydroxybenzotriazole, *N*, *N*-diisopropylethylamine, oligonucleotide; b) Purification by sephedex G-50 spin column.

5.5 Affinity Assay

Initial PCR experiments proved that the PCR conditions were working and that PCR could be carried out on the oligonucleotide that was coupled to the peptide dendrimer conjugates. With the affinity assay it was hoped that the peptide-dendrimer conjugates would stick to the cells and remain attached after a washing step. PCR of the scraped cells would then afford the PCR product proving that the ligands were in fact binding to the cells. If successful this methodology could be used to discover the strongest binding peptide-dendrimer compounds from a library of these compounds.

The DNA-Peptide-Dendrimer conjugates were dissolved in PBS/BSA 1% (50 μL) and added to the immobilised cells. Controls included DNA template, PBS blank and dendrimer. The compounds were incubated for 1 hour at 37°C. After washing 4 μL of water was added to each of the cells and the plate was heated at 70°C for 20 mins. After heating the cells were scraped from the cell plate in 10 μL of water and placed in an eppendorf tube. PCR was performed as before on scraped samples from the well. The PCR product was then analysed on a 2% agarose gel. (Figure 73) shows the results of the affinity assay.

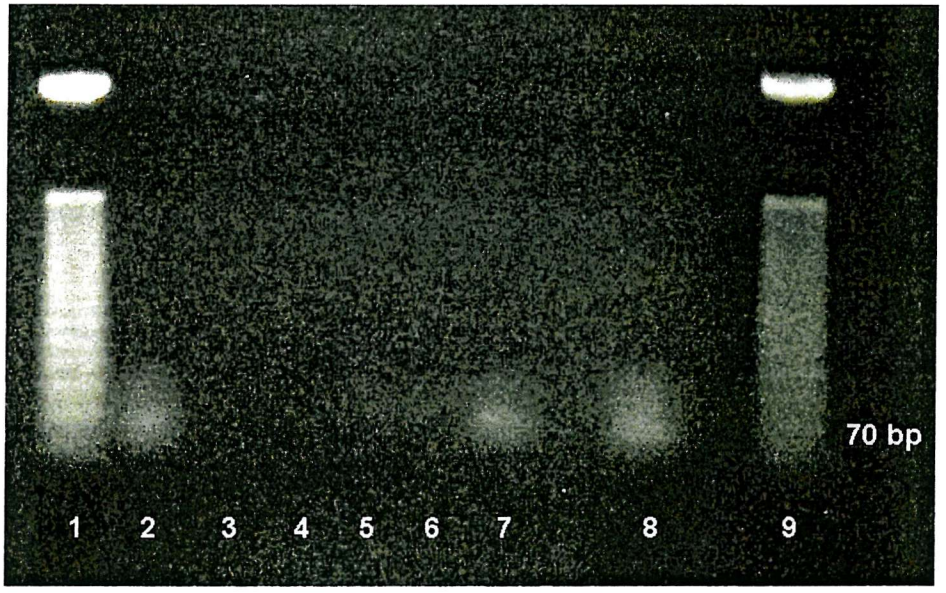


Figure 73: Ethidium bromide stained agarose gel electrophoresis of products from PCR amplifications of affinity-based assay. 1) 50 bp ladder, 2) Template control, 3) Template added to cells, 4) Negative Control PBS, 5) DNA-Dendrimer-Peptide conjugate **(83)**, 6) DNA-Dendrimer-Peptide conjugate **(82)**, 7) DNA-Dendrimer-Peptide conjugate **(81)**, 8) Template control, 9) 50 bp ladder.

The gel (Figure 73) shows that the monovalent DNA-peptide-dendrimer conjugate compound **(81)** is the only compound to have produced a PCR product. We can therefore assume that this is occurring because it is the only conjugate that has bound to the cells. This was a somewhat unexpected result as during the screening experiment described in chapter 4 it was observed that although all the peptide-dendrimer

conjugates did bind competitively to these cells the monovalent form bound less strongly than the multivalent forms. Why the peptide-dendrimer conjugates did not produce any PCR product may have been because the coupling reaction failed due to the increased steric hinderance of the peptide-dendrimer compounds. (Thus as in the control experiments only peptide-dendrimer and oligonucleotide was added to the cells and not in fact peptide-dendrimer-oligonucleotide conjugates).

As the assay itself did work it was decided to reproduce the results and also to do a further experiment to prove that the PCR product was being obtained due to binding via the peptide ligand. The conjugate **(81)** was added to a cell plate, as before but in one of the wells an excess of peptide-dendrimer conjugate was added to compete with the peptide-dendrimer-DNA conjugate. Thus when PCR was performed on the cells from this well no PCR product would be expected. Whereas in the wells without added peptide-dendrimer conjugate a PCR product would be observed. (Figure 74) shows the results of the experiment.

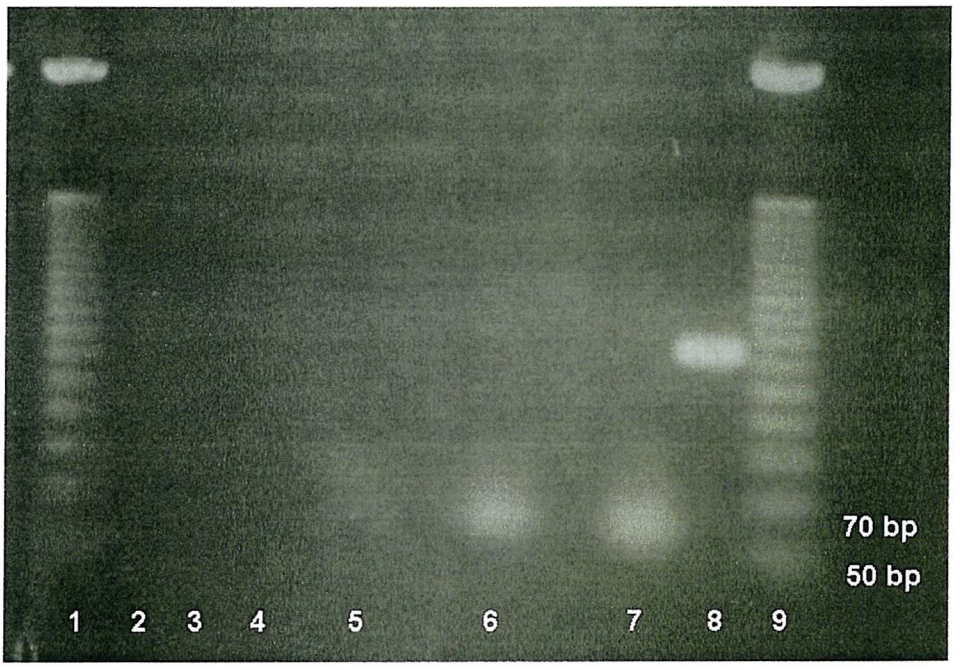


Figure 74: Agarose gel shows the result of the PCR experiment 1) 50 bp ladder, 2) Control peptide only, 3) Control Template only, 4) Blank, 5) Well with peptide-DNA conjugate **(81)** added then displaced with peptide **(78)** only, 6) Well with peptide-DNA

conjugate **(81)** only, 7) Positive control template DNA, 8) Positive control plasmid DNA, 9) 50 bp ladder.

The agarose gel shows clearly that the peptide-dendrimer conjugate successfully competed with the peptide-dendrimer-DNA conjugate as evidenced by the lack of PCR product in lane 5, whereas the PCR product was obtained in lane 6 proving the interaction was with the peptide and not the DNA.

5.6 Conclusion.

An assay that could be used for the identification of the best binding synthetic phage from a synthetic phage library has been developed. In order to achieve this a peptide-dendrimer-DNA conjugate **(81)** was synthesised. PCR was successfully performed on the cells that bound the peptide-dendrimer-DNA conjugate **(81)**. It has been shown that the PCR product was obtained due to a binding event between the peptide-dendrimer-DNA conjugates and the cells and not due to non-specific binding of the oligonucleotide.

Chapter 6.1: Chemistry Experimental

Chapter 6.2: Biological Experimental

Chapter 2 Experimental

General information.

^{1}H NMR and ^{13}C NMR were recorded on Brüker DPX400 (400 and 100 MHz respectively) or Brüker AC 300 (300 and 75 MHz respectively) spectrometers 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 reference. 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 electrospray measurements were carried out on a Brüker Apex III fourier transform mass spectrometer.

MALDI-TOF spectra were obtained from a Micro mass TOF-Spec 2E mass spectrometer using di-hydroxybenzoic acid as the matrix.

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 monitoring at 220 nm and 254 nm and using an ELS detector.

Solvent A: Water/TFA 0.1%.

Solvent B: MeCN/TFA 0.1%.

Method 1: 10% B/90% A to 90% B/10% A over ten minutes finishing with 10% A/90% B to 90% A/10% B over five minutes.

Method 2: 10% B/90% A to 90% B/10% A over 20 minutes finishing with 10% A/90% B over five minutes.

Semi-preparative RP-HPLC were performed as above with a phenomenex prodigy C_{18} 100 A, column, of size 250 x 10 mm 5 micron. Gradients used for semi-preparative RP-HPLC.

Method 3: 0% B/100% A to 50% B/50% B over 55 minutes then 50%B/50% A to 51% B/49% A over 5 mins then 51% B/49% A to 0% B/100% A in 5 mins.

IR spectra were obtained on a BioRad FTS 135 spectrometer with a Goldengate ATR 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[184]

The resin (1-5 mg) was put into a test-tube. Reagent A (6 drops) and reagent B (2 drops) were added and the tube heated at 100°C for 10 minutes. The reagents A and B were prepared as follows:

Reagent A

Solution–1: Phenol (40g) was dissolved in absolute ethanol (10 mL) by warming and then stirred over Amberlite mixed-bed resin MB-3 (4g) for 45 minutes. 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 diluted with pyridine (100 ml) (freshly distilled from ninhydrin) and stirred over Amberlite mixed bed resin MB-3 (4g). The solution was filtered and mixed with solution-1 to give reagent A.

Reagent B

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

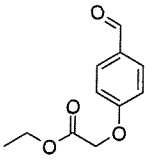
Quantitative Fmoc test[185]

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

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

Where A_{302} is the absorbance of the piperidyl-fulvene adduct, V is the total volume (mL), W is the mass of 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}).

Synthesis of (4-formylphenoxy)acetic acid ethyl ester (4)[165]



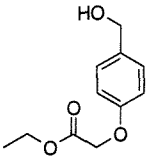
A mixture of 4-hydroxybenzaldehyde (10.00g, 81.80 mmol), potassium carbonate (22.60g, 16.36 mmol) and potassium iodide (1.49g, 8.18 mmol) in acetonitrile (200 mL) was stirred for 10 minutes. Ethyl bromoacetate (10 mL, 93.70 mmol) was added dropwise to the solution. The brown mixture was then allowed to reflux overnight during which time the solution became yellow. After cooling the salts were removed by filtration and the solvent removed *in vacuo*. The crude product was purified by column chromatography on SiO_2 eluting with petroleum ether/ethyl acetate (3:2) to afford the title compound 16.00g as a yellow/orange oil, in 94% yield. Spectral data in accordance with literature.[165]

TLC: Ether/ethyl acetate (3:2) $R_f = 0.75$.

δ_H (300 MHz, $CDCl_3$): 9.78 (1H, s, C<u>H</u>O); 7.74 (2H, d, *J* 9, ArC^{3,} ^{5}<u>H</u>,); 6.91 (2H, d, *J* 9, ArC^{2,} ^{6}<u>H</u>); 4.62 (2H, s, OC<u>H</u>$_2$CO$_2$); 4.19 (2H, q, *J* 7, OC<u>H</u>$_2$CH$_3$); 1.20 (3H, t, *J* 7, OCH$_2$C<u>H</u>$_3$,).

δ_C (75 MHz, $CDCl_3$): 190.78 (<u>C</u>HO); 169.79 (<u>C</u>O$_2$CH$_2$CH$_3$); 162.69 (Ar<u>C</u>^{1}O); 131.96 (Ar<u>C</u>^{4}CHO); 130.7 (Ar<u>C</u>^{3,} ^{5}H); 114.95 (Ar<u>C</u>^{2,} ^{6}H); 64.79 (O<u>C</u>H$_2$CO$_2$); 61.41 (CO$_2$<u>C</u>H$_2$CH$_3$); 14.16 (CO$_2$CH$_2$<u>C</u>H$_3$).

Synthesis of (4-hydroxymethylphenoxy)acetic acid ethyl ester (5)[165]



(4-Formylphenoxy)acetic acid ethyl ester (16.62g, 79.90 mmol) was dissolved in ethanol (200 mL). The solution was cooled to 0°C and sodium borohydride (1.51g, 39.95 mmol) was slowly added. The temperature was allowed to rise to room temperature. The reaction was monitored by TLC and after 1½ hours the reaction was carefully quenched with a solution of HCl (2M in ethanol) (8 mL), which was added dropwise, until evolution of hydrogen gas had stopped. The solvents were removed *in*

vacuo. Water was added (200 mL) and extracted twice with ethyl acetate (3 x 100 mL). The crude product was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (3:1), to (3:2), to afford the title compound 7.76g as a pale yellow oil in 46% yield.

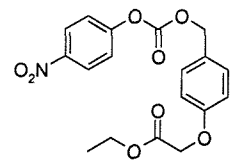
Spectral data in accordance with literature.[165]

TLC: Hexane/ethyl acetate (1:1) Rf = 0.51.

δ_H (300 MHz, $CDCl_3$): 7.27 (2H, d, *J* 8, $ArC^{3,5}\underline{H}$); 6.89 (2H, d, *J* 8, $Ar^{2,6}C\underline{H}$); 4.60 (2H, s, $C\underline{H}_2CO_2$); 4.59 (2H, s, $C\underline{H}_2OH$); 4.25 (2H, q, *J* 7, $CO_2C\underline{H}_2CH_3$); 1.26 (3H, t, *J* 7, $CH_2C\underline{H}_3$)

δ_C (75 MHz, $CDCl_3$): 169.13 ($\underline{C}O_2CH_2CH_3$); 157.45 ($Ar\underline{C}^1O$);134.46 ($Ar\underline{C}^4CH_2OH$); 128.74 ($Ar\underline{C}^{3,5}H$); 114.79 ($Ar\underline{C}^{2,6}H$); 65.50 ($O\underline{C}H_2CO_2$); 64.84 ($Ar\underline{C}H_2OH$); 61.55 ($CO_2\underline{C}H_2CH_3$); 14.29 ($CO_2CH_2\underline{C}H_3$).

Synthesis of [4-(4-nitro-phenoxycarbonyloxymethyl)phenoxy]acetic acid ethyl ester (6)[165]



(4-Hydroxymethylphenoxy)acetic acid ethyl ester (5.30g, 25.20 mmol): was dissolved in dichloromethane (100 mL) at 0°C. Pyridine (2.13 mL, 26.40 mmol) was added and the reaction stirred for two minutes, the solution remained a pale yellow colour throughout. 4-nitrophenyl chloroformate (5.33g, 26.42 mmol) was dissolved in dichloromethane (100 mL) and added dropwise over 50 minutes, (the solution remained yellow throughout). The solution was stirred at 0°C for 2 hours and then at room temperature overnight. The solution was filtered to remove the salts and the solvent removed *in vacuo*. The crude product was purified by column chromatography on SiO_2 eluting with a gradient of petroleum ether/ethyl acetate, (4:1) to (1:1), to afford the title compound 7.58g as a pale yellow oil, in 80% yield.

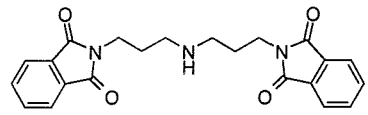
Spectral data in accordance with literature.[165]

TLC: Hexane/ethyl acetate (3:1) Rf = 0.39.

δ_H (300 MHz, $CDCl_3$): 8.27 (2H, d, *J* 8, $O_2NArC^{3,5}\underline{H}$); 7.40 (2H, d, *J* 7, $O_2NArC^{2,6}\underline{H}$); 7.37 (2H, d, *J* 7, $ArC^{3,5}\underline{H}$); 6.94 (2H, d, *J* 8, $ArC^{2,6}\underline{H}$); 5.24 (2H, s, $OC\underline{H}_2CO$); 4.65 (2H, s, $C\underline{H}_2OCO$); 4.27 (2H, q, *J* 7, $CO_2C\underline{H}_2CH_3$); 1.31 (3H, t, *J* 7, $CO_2CH_2C\underline{H}_3$).

δ_C (75 MHz, $CDCl_3$): 168.81 ($\underline{C}O_2$); 158.59 ($Ar\underline{C}^1$-O); 155.68 ($NO_2Ar\underline{C}^1$-O); 152.59 (O-$\underline{C}$O-O); 145.50 ($Ar\underline{C}^4$-NO_2); 130.87 ($Ar\underline{C}^{2,6}H$); 127.54 ($Ar\underline{C}^4CH_2O$); 125.44 ($NO_2Ar\underline{C}^{3,5}H$); 121.94 ($NO_2Ar\underline{C}^{2,6}H$); 115.00 ($Ar\underline{C}^{3,5}H$); 70.85 ($Ar\underline{C}H_2O$); 65.47 ($O\underline{C}H_2CO_2$); 61.65 ($O\underline{C}H_2CH_3$); 14.32 ($OCH_2\underline{C}H_3$).

Synthesis of *N*^{1},*N*^{9}-*bis*(Phthloyl)-1,5,9 triazanonane (2)[186]



1,5,9 Triazanonane (31.70 mmol, 4.40 mL) was dissolved in chloroform (75 mL). To this solution was rapidly added a solution of *N*-ethoxycarbonylphthlimide (63.40 mmol, 13.90g) in chloroform (75 mL). The mixture was stirred at room temperature for 2 h, the solvent was removed *in vacuo* to afford the title compound 8.90g as a white solid in 72% yield. Experimental data was in accordance with the literature.[186]

TLC: DCM/MeOH (9:1) R_f = 0.80.

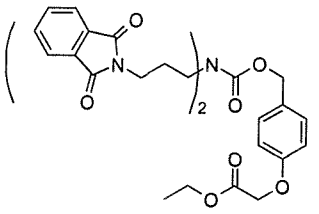
δ_H (300 MHz, $CDCl_3$): 7.86-7.80 (4H, m, $C^{1,4}\underline{H}$); 7.71 (4H, m, $ArC^{2,3}\underline{H}$); 3.76 (4H, t, *J* 7, $C\underline{H}_2NC{=}O$); 2.64 (4H, t, *J* 7, $NHC\underline{H}_2CH_2$); 1.85 (4H, quin, *J* 7, $CH_2C\underline{H}_2CH_2$) 1.60 (1H, br s, N$\underline{H}$).

δ_C (75 MHz, $CDCl_3$): 168.59 ($\underline{C}{=}O$); 134.02 ($Ar\underline{C}^{2,3}H$); 132.26 ($Ar\underline{C}^{1,4}H$); 123.33 ($Ar\underline{C}^{5,6}$); 46.99 ($NH\underline{C}H_2CH_2$); 36.03 ($\underline{C}H_2NC{=}O$); 29.00 ($CH_2\underline{C}H_2CH_2$).

m/z (ES+): 392.1 (100%, $M+H^+$).

Melting point: 132-136°C.

Synthesis of *N*^{1},*N*^{9}-*bis*(Phthloyl)-*N*^{5}-(4-Benzyloxycarbonyl-1-oxyethyl acetate)-1,5,9 triazanonane (7)[165]



[4-(4-Nitro-phenoxycarbonyloxymethyl)phenoxy]acetic acid ethyl ester (7.58g, 20.20 mmol) was dissolved in acetonitrile (30 mL), triethylamine (4.20 mL, 30.00 mmol) was added and the solution stirred for five minutes. N^1,N^9-*bis*(Phthloyl)-1,5,9 triazanonane (9.60g, 24.70 mmol) was dissolved in acetonitrile (200 ml) (heat necessary) and added dropwise. The mixture was heated to 50°C and allowed to stir overnight. The solution was concentrated *in vacuo* and poured into water (75 ml) acidified to pH 6 with $KHSO_4$ (1 molar) and extracted with ethyl acetate (3 x 50 mL). The organic layers were washed with brine and then dried over $MgSO_4$ and filtered. The crude product was purified by column chromatography on SiO_2 eluting with a gradient of petroleum ether/ethyl acetate (3:1) to ethyl acetate (0:1), to afford the title compound 10.97g as a pale yellow oil in 86% yield.

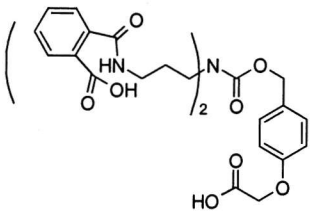
Spectral data in accordance with literature.[157]

TLC: Petroleum ether/ethyl acetate (1:1) $R_f = 0.20$

δ_H (300 MHz, $CDCl_3$): 7.81-7.78 (4H, m, ArC<u>H</u>Pth); 7.71-7.70 (4H, m, ArC<u>H</u>Pth); 7.21 (2H, d, *J* 9, $ArC^{2,\ 6}\underline{H}$); 6.83 (2H, d, *J* 9, $ArC^{3,\ 5}\underline{H}$); 5.00 (2H, s, $OC\underline{H}_2CO_2$); 4.60 (2H, s, $ArC\underline{H}_2O$); 4.27 (2H, q, *J* 7, $OC\underline{H}_2CH_3$); 3.67 (4H, br m, $C\underline{H}_2NPth$); 3.34 (4H, br m, $C\underline{H}_2NHCO_2$); 1.93 (4H, br m, $CH_2C\underline{H}_2CH_2$); 1.30 (3H, t, *J* 7, $CH_2C\underline{H}_3$).

δ_C (75 MHz, $CDCl_3$): 169.00, ($\underline{C}O_2CH_2CH_3$); 168.41 ($\underline{C}$=O Pth); 157.69 ($Ar\underline{C}^1$-O); 156.13 (NH$\underline{C}$O-O); 134.08 ($Ar\underline{C}^{4,\ 5}$H Pth); 132.20 ($Ar\underline{C}^{1,\ 2}$H Pth); 130.04 ($Ar\underline{C}^4$-CH_2); 129.72 ($Ar\underline{C}^{3,\ 5}$H); 123.39 ($Ar\underline{C}^{3,\ 6}$H Pth); 114.77 ($ArC^{2,\ 6}$H); 66.88 ($\underline{C}H_2CO_2$); 65.58 (O$\underline{C}H_2$O); 61.54 ($CO_2\underline{C}H_2CH_3$); 45.50, (CON$\underline{C}H_2$); 45.20 (PthN$\underline{C}H_2$); 35.82 ($CH_2\underline{C}H_2CH_2$); 14.32 ($OCH_2\underline{C}H_3$).

Synthesis of N^1,N^9-*bis*(2-acetyl-benzoic acid)-N^5-(4-Benzyloxycarbonyl-1-oxyacetic acid)-1,5,9 triazanonane (8)[157]



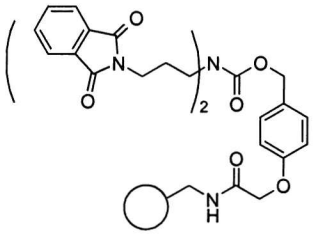
N^1,N^9-*bis*(Phthloyl)-N^5-(4-Benzyloxycarbonyl-1-oxyethyl acetate)-1,5,9 triazanonane (2g, 3.18 mmol) was dissolved in dioxane (10 mL) and NaOH (2M, 6.36 mL, 12.76 mmol) was added dropwise. The yellow solution was stirred for 30 minutes, (disappearance of starting material was followed by TLC), then diluted with water (100 mL) and carefully acidified with $KHSO_4$ (1 molar) until pH 5 when the solution became white and cloudy. Extraction with ethyl acetate (3 x 50 mL) gave the triacid as a yellow oil. The solvent was removed *in vacuo* to afford the title compound as a colourless oil in a quantitative yield.

Spectral data in accordance with literature.[157]

δ_H (300 MHz, DMSO): 8.27 (2H, br s, O<u>H</u>) 7.75 (2H, d, *J* 7, $Ar^{1,\ 4}C\underline{H}$ Pth); 7.60-7.31 (6H, m, $C^{2,\ 3}\underline{H}$ Pth, N<u>H</u>); 7.28 (2H, d, *J* 9, $ArC^{2,\ 6}\underline{H}$); 6.85 (2H, d, *J* 9, $ArC^{3,\ 5}\underline{H}$); 4.99 (2H, s, $ArC\underline{H}_2OCO$); 4.65 (2H, s, $OC\underline{H}_2$) 3.29 (4H, m, $CH_2CH_2C\underline{H}_2NH$); 3.18 (4H, m, $NC\underline{H}_2CH_2CH_2NH$); 1.73 (4H, m, $CH_2C\underline{H}_2CH_2$).

δ_C (75 MHz, DMSO): 170.26 ($ArC\underline{O}_2H$); 168.61 ($Ar\underline{C}O_2H$); 167.98 ($Ar\underline{C}ONH$); 157.46 (ArC^1); 155.48 ($N\underline{C}OO$); 138.80 (ArC^4); 131.33 ($Ar\underline{C}^2NH$); 130.62 (ArC^5); 129.59 ($Ar\underline{C}^4$); 129.27 ($Ar\underline{C}^3$); 129.14 (ArC^1); 127.66 (ArC^6); 114.38 ($ArC^{2,\ 6}$); 66.43 ($\underline{C}H_2CO_2H$); 65.92 ($\underline{C}H_2OH$); 64.48 ($\underline{C}H_2NHCO$); 54.99 ($\underline{C}H_2NH$); 36.87 ($CH_2\underline{C}H_2CH_2$).

Synthesis of resin bound N^1,N^9-*bis*(Phthloyl)-N^5-(4-Benzyloxycarbonyl-1-oxyamide)- 1,5,9 triazanonane (9)

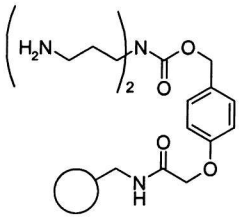


N^1,N^9-*bis*(2-Acetyl-benzoic acid)-N^5-(4-Benzyloxycarbonyl-1-oxyacetic acid)-1,5,9 triazanonane (1.39 g, 2.22 mmol) was dissolved in CH_2Cl_2 (10 mL) and DMF (2 mL), DIC (4 eq, 1.50 mL) and HOBt (4 eq, 1.20g) were added and the solution stirred for 40

minutes. The activated mixture was added to the resin (TentaGel 3.30g, 0.27 mmol/g free $-NH_2$ loading) and shaken overnight. The resin was washed with DCM, DMF, MeOH and ether (each 3 x 10 mL). Completion of the coupling was checked by a qualitative ninhydrin test.

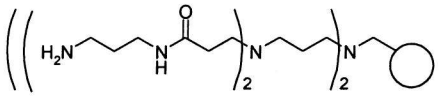
IR: (υ cm^{-1}): (1669 C=O imide).

Synthesis of resin bound N^5-(4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (10)



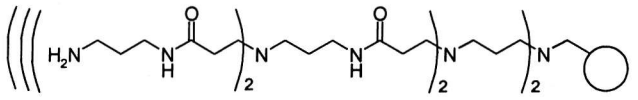
The phthalimide-protected initiator core **(9)** (0.92 mmol of phthlimido groups) was suspended in EtOH (50 mL) and treated with hydrazine monohydrate (18.40 mmol, 0.90 mL). The reaction was heated at reflux overnight, then washed with hot water (2 x 20 mL), hot DMF (2 x 20 mL), DCM (3 x 10 mL), DMF (3 x 10 mL) and MeOH (3 x 10 mL).

Synthesis of resin-bound Generation [1.0] PAMAM dendrimer (12)[157]



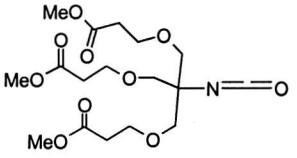
Generation [0.0] dendrimer resin (1.40g, 0.69 mmol free amine) was suspended in MeOH (0.50 eq v/v, 30 mL) and treated with methyl acrylate (250 eq, 15.60 mL). The resin was left shaking for 24 hours at 50 °C, the solvents were removed and the resin was washed extensively with MeOH, DMF and DCM and ether (each 3 x 10 mL). A negative qualitative ninhydrin test confirmed reaction was complete. The generation [0.5] dendrimer resin (0.70g, 0.49 mmol reactive sites) was suspended in MeOH (10 mL, 0.50 v/v) and treated with 1, 3-propanediamine (250 eq reactive sites, 10 mL, 0.12 mol). The resin was left shaking for 72 hours at room temperature after which time the solvents were removed and the resin washed extensively with MeOH, DMF, DCM and ether (each 3 x 10 mL). Qualitative ninhydrin test proved strongly positive. Spectral data in accordance with literature.[157]

Synthesis of resin bound Generation [2.0] dendrimer (14)[157]



Generation [1.0] dendrimer resin (1.00g, 0.88 mmol free amine) was suspended in MeOH (6 mL) and treated with methyl acrylate (250 eq, 19.80 mL). The resin was left shaking for 24 hours at 50 °C the solvents were removed and the resin was washed extensively with MeOH, DMF and DCM and ether (each 3 x 10 mL). A negative qualitative ninhydrin test confirmed reaction was complete. The generation [1.5] dendrimer resin (800 mg, 0.87 mmol reactive sites) was then suspended in MeOH (35 mL, 0.50 eq v/v) and treated with 1, 3-propanediamine (73 mL, 1000 eq of reactive sites). The resin was shaken for 72 hours at room temperature after which time the solvents were removed and the resin washed extensively with MeOH, DMF, DCM and ether (each 3 x 10 mL). Qualitative ninhydrin test proved strongly positive. Spectral data in accordance with literature.[157]

Synthesis of 6-Isocyanato-6-(4-carbomethoxy-2-oxabutyl)-4, 8-dioxaundecanedioate (16)[169]



To a stirred solution of trimethyl 6-amino-6-(4-carbomethoxy-2-oxabutyl)-4, 8-dioxaundecanedioate (3.79g, 10 mmol) and 4-dimethylaminopyridine (1.22g, 10 mmol) in DCM (40 mL) a solution of $(Boc)_2O$ (3.06g, 14 mmol) in DCM (50 ml) was added at 25°C. After stirring for 2 hours the solution was washed with aqueous HCl (1 M, 2x 40 mL) water (2x 10 mL) and then dried over Na_2SO_4. The solvent was removed *in vacuo* to afford the title compound (4.04g, 9.98 mmol) as a colourless oil in 99% yield.
The compound was sufficiently pure to be coupled onto the resin.
Spectral data in accordance with literature.[169]

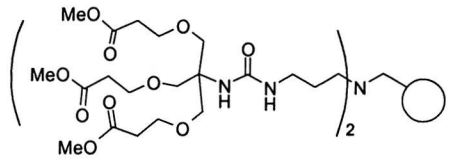
δ_H (300 MHz, $CDCl_3$): 3.75-3.71 (6H, m, $C\underline{H}_2CH_2CO_2$); 3.70 (9H, s, $OC\underline{H}_3$); 3.44 (6H, s, CH_2O); 2.59-2.55 (6H, m, $C\underline{H}_2CO_2$).

δ_C (75 MHz, $CDCl_3$): 171.35 ($\underline{C}O_2$); 126.80 (N$\underline{C}$O); 70.80 ($\underline{C}H_2O$); 66.60 (O$\underline{C}H_2CH_2$); 63.40 (N$\underline{C}CH_2$); 51.80 (OCH_3); 19.70 ($\underline{C}H_2CO_2$).

m/z (ES+): 428 ($M+Na^+$ 100%).

IR: (υ cm^{-1}): Characteristic peaks, 2953, 2874, (CH); 2245 (NCO); 1735 (COOMe).

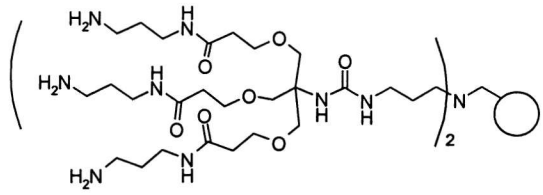
Synthesis of resin bound Ab_3 type dendrimer generation [0.5] (17)



3-[2-Isocyanato-3-(2-methoxycarbonyl-ethoxy)-2-(2-methoxycarbonyl-ethoxymethyl)-propoxy]-propionic acid methyl ester (2eq free amine sites, 0.10g) was dissolved in DCM (3 mL) with DIPEA (2 eq free amine sites, 0.43 mL) and a catalytic amount of DMAP. The reaction mixture was then added to the resin (250 mg, 0.12 mmol) and shaken overnight. After washing extensively with MeOH, DMF, DCM and ether (each 3 x 10 mL) a qualitative ninhydrin test was negative.

IR (υ cm^{-1}): 2867 (NH) (s); 1735 (C=O) (s); 1653 (OMe) (s).

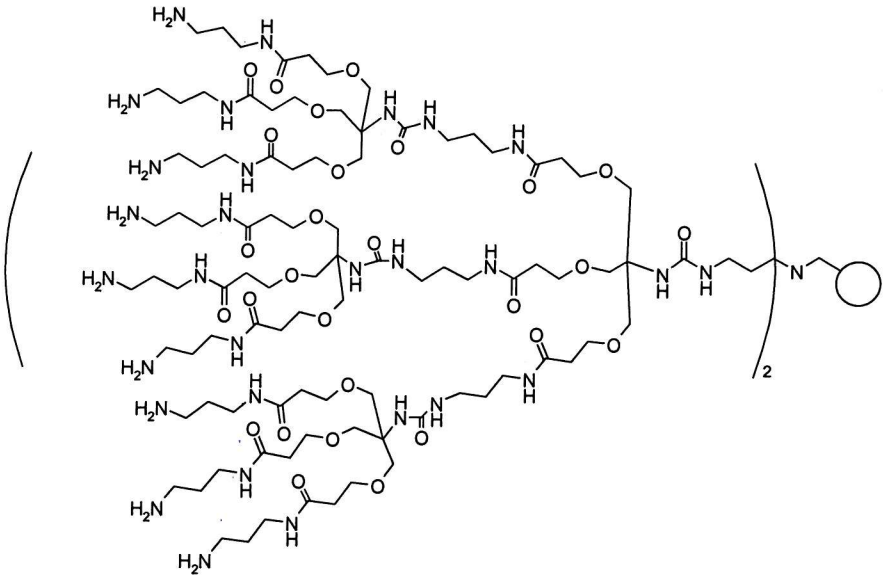
Synthesis of resin bound Ab_3 type dendrimer generation [1.0] (18a)



Generation [0.5] AB_3-type resin (200 mg, 0.30 mmol reactive sites) was suspended in DMSO for 30 mins before addition of a solution of 1, 3-diamino propane (8.30 mL, 250 eq) in DMSO (4mL, 0.50 eq v/v). The resin was then shaken for 3 days then washed with DMSO before the addition of a fresh solution of 1, 3 diamino propane (8.30 mL, 250 eq) DMSO (4 mL, 0.50 eq v/v). After a further 3 days reaction the resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL).

m/z (MALDI-TOF): 1216.97 ($M+Na^+$).

Synthesis of resin bound dendrimer generation [2.0] (19)



3-[2-Isocyanato-3-(2-methoxycarbonyl-ethoxy)-2-(2-methoxycarbonyl-ethoxymethyl)-propoxy]-propionic acid methyl ester (3eq free amine sites, 0.36g) was dissolved in DCM (3 mL) with DIPEA (3 eq free amine sites, 0.16 mL) and a catalytic amount of DMAP. The reaction mixture was then added to the generation [1.0] AB_3-type resin (250 mg, 0.30 mmol) and shaken overnight. After washing extensively with MeOH, DMF, DCM and ether (each 3 x 10 mL) a qualitative ninhydrin test was performed which proved negative. Generation [1.5] AB_3-type resin was then suspended in DMSO for 30 mins before addition of a solution of 1, 3 diamino propane (16 mL) in DMSO (8 mL, 0.50 v/v). The resin was then shaken for 3 days at room temperature. Then washed with DMSO before the addition of a fresh solution of 1, 3 diamino propane (250 eq free amine) DMSO (0.5 eq v/v). After a further 3 days reaction the resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). Reaction completion was checked by taking 10 mg of resin and coupling (4-hydroxyphenyl)-acetic acid with DIC HOBt (5 eq resin loading) for 2 hours at room temperature. Completion of the reaction was checked by qualitative ninhydrin test. After washing as before the derivatised compound was cleaved from the resin with 90% TFA/DCM, 1 drop of water as a scavenger and analysed by HPLC.

RP-HPLC: (Method 2, λ= 254) 7.58 mins.

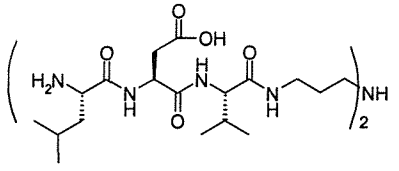
m/z: Molecular ion was not observed.

Chapter 3 Experimental

General procedure for the preparation of LDV Gen [0.0], [1.0] Peptide - Dendrimer conjugates (10), (12), (18a) and (18b).

Generation [0.0], [1.0] dendrimer resins **(10)**, **(12)**, **(18a)** and **(18b)** were swollen for 30 minutes in DCM. Fmoc amino acids (5eq), with reference to the theoretical number of free amine groups on the resin, were activated with HOBt, (5eq), DIC (5eq), in DCM. Fmoc groups were removed by shaking twice in 20% piperidine in DMF (5 mL) for 20 minutes. The activated mixture was then added to the resin and shaken overnight. Compounds were cleaved from the resin using two cycles of 90 % TFA in DCM with 1 drop of water as a scavenger for 2 hours. The solvents were removed under vacuum, dissolved in the minimum amount of acetic acid, then precipitated in ether and centrifuged. The pellets produced were washed with ether, dissolved in water and freeze dried to obtain white fluffy compounds. The crude compounds were purified by semi-preparative RP-HPLC.

Synthesis of LDV-Gen [0.0] Peptide Dendrimer conjugate (20).



δ_H (400 MHz, D_2O): 4.80 (2H, t, *J* 7, C<u>H</u>, Asp); 3.98 (2H, m, C<u>H</u>, Leu); 3.96 (2H, t, *J* 7, C<u>H</u>, Val); 3.27 (4H, m, CONHC<u>H</u>$_2$CH$_2$CH$_2$NH); 2.97 (4H, m, CONHCH$_2$CH$_2$C<u>H</u>$_2$NH); 2.90 (2H, dd, J_{AB} 18, J_{AX} = 7, CH<u>H</u>, Asp); 2.76 (2H, dd, J_{BA} 18, J_{BX} 7, CH<u>H</u>, Asp); 2.04 (2H, m, C<u>H</u>(CH$_3$)$_2$); 1.85 (4H, m, NHCH$_2$C<u>H</u>$_2$CH$_2$NH); 1.68 (4H, m, C<u>H</u>$_2$, Leu); 1.57 (2H, m, C<u>H</u>(CH$_3$)$_2$, Leu); 0.88 (24H, m, C<u>H</u>$_3$).

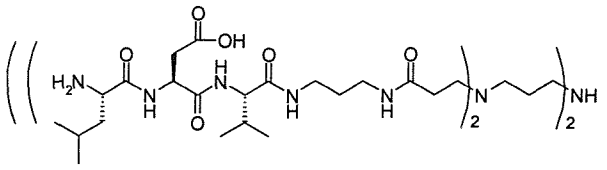
δ_C (100 MHz, D_2O): 175.04, 173.86, 172.40, 170.43 (<u>C</u>O); 60.37 (<u>C</u>H, Val); 52.14 (<u>C</u>H, Leu); 50.51 (<u>C</u>H, Asp); 45.52 (CONHCH$_2$CH$_2$<u>C</u>H$_2$NH); 40.27 (<u>C</u>H$_2$); 36.46 (<u>C</u>H$_2$); 36.34 (CONH<u>C</u>H$_2$CH$_2$CH$_2$NH); 30.14 (<u>C</u>H(CH$_3$)$_2$, Val); 25.85 (NHCH$_2$<u>C</u>H$_2$CH$_2$NH); 24.26 (<u>C</u>H(CH$_3$)$_2$, Leu); 21.88, 21.64 (<u>C</u>H$_3$); 18.79, 17.89 (<u>C</u>H$_3$).

RP-HPLC (Method 1 λ= 220): 8.28 mins.

m/z (ES+): 786.70 $(M+H)^+$ 50%, 394.00 $(M+2H)^{2+}$ 100%.

Resin (loading, 0.496 mmol/g, 500 mg) afforded 39 mg of the title compound in 21% yield.

Synthesis of LDV - Gen [1.0] Peptide Dendrimer conjugate (21).



δ_H (400 MHz, D_2O): 4.71 (4H, t, *J*, C<u>H</u>, Asp); 3.91 (4H, d, *J* 7, C<u>H</u>, Leu); 3.89 (4H, m, C<u>H</u>, Val); 3.37 (8H, m, NC<u>H</u>$_2$CH$_2$CO); 3.23 (4H, m, NHCH$_2$CH$_2$C<u>H</u>$_2$N) 3.17-3.09 (20H, m, NHC<u>H</u>$_2$CH$_2$C<u>H</u>$_2$N); 2.82 (4H, dd, J_{AB} 16, J_{AX} 7, C<u>H</u>H, Asp); 2.69 (4H, dd, J_{BA} 16, J_{BX} 7, CH<u>H</u>, Asp); 2.68 (8H, m, NCH$_2$C<u>H</u>$_2$CO); 2.12 (4H, m, NHCH$_2$C<u>H</u>$_2$CH$_2$N); 1.94 (4H, m, C<u>H</u>(CH$_3$)$_2$, Val); 1.60 (8H, m, C<u>H</u>$_2$, Leu); 1.59 (8H, m, NHCH$_2$C<u>H</u>$_2$CH$_2$N); 1.50 (4H, m, C<u>H</u>(CH$_3$)$_2$, Leu); 0.80 (48H, m, C<u>H</u>$_3$).

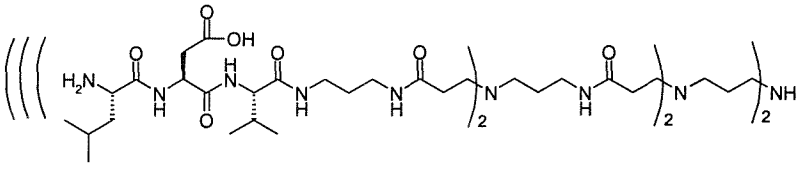
δ_C (100 MHz, D_2O): 175.04, 173.86, 172.40, 170.43 172.50 (<u>C</u>O) 60.27 (<u>C</u>H, Val); 52.13 (<u>C</u>H, Leu); 50.53 (<u>C</u>H, Asp); 50.28 (<u>C</u>H$_2$, Gen [1.0]); 44.29 (<u>C</u>H$_2$, Gen [0.0]); 40.28 (<u>C</u>H$_2$); 37.25 (<u>C</u>H$_2$); 37.11 (<u>C</u>H$_2$, Gen [0.0]); 35.89 (<u>C</u>H$_2$, Gen [1.0]); 30.33 (<u>C</u>H(CH$_3$)$_2$, Val); 28.97 (<u>C</u>H$_2$, Gen [1.0]); 28.27 (<u>C</u>H$_2$, Gen [0.0]); 24.26 (<u>C</u>H(CH$_3$)$_2$, Leu); 21.90, 21.63 (<u>C</u>H$_3$); 21.63 (<u>C</u>H$_2$, Gen [1.0]); 18.82, 17.90 (<u>C</u>H$_3$).

RP-HPLC (Method 1, λ= 220): 8.68 mins.

m/z (MALDI-TOF): 1954.93 $(M+H)^+$ 100%, 1975.94 $(M+Na)^+$.

Resin (loading, 0.88 mmol/g, 500 mg) afforded 94 mg of the title compound in 11 % Yield.

Synthesis of LDV-Gen [2.0] Peptide-Dendrimer conjugate (22).



δ_H (400 MHz, D_2O): 4.85 (8H, t, *J* 7, C<u>H</u>, Asp); 4.06 (8H, d, *J* 7, C<u>H</u>, Val); 4.01 (8H, t, *J* 7, C<u>H</u>, Leu); 3.55-3.45 (24H, br m, CH$_2$C<u>H</u>$_2$CO); 3.38-3.18 (56H, m, NHC<u>H</u>$_2$CH$_2$C<u>H</u>$_2$NH); 2.92 (8H, dd, J_{AB} 16, J_{AX} 7, C<u>H</u>H, Asp); 2.83-2.78 (32H, m, C<u>H</u>$_2$CH$_2$CO, CH<u>H</u>, Asp); 2.22 (4H, br m, NHCH$_2$C<u>H</u>$_2$CH$_2$NH, Gen [0.0]) 2.10 (8H, m,

C<u>H</u>$(CH_3)_2$, Val); 2.09 (8H, br m, NHCH_2C<u>H</u>$_2$CH_2NH, Gen [1.0]); 1.79-1.58 (40H, m, NHCH_2C<u>H</u>$_2$CH_2NH, Gen [2.0], C<u>H</u>$_2$, C<u>H</u>, Leu); 0.93 (96H, m, C<u>H</u>$_3$).

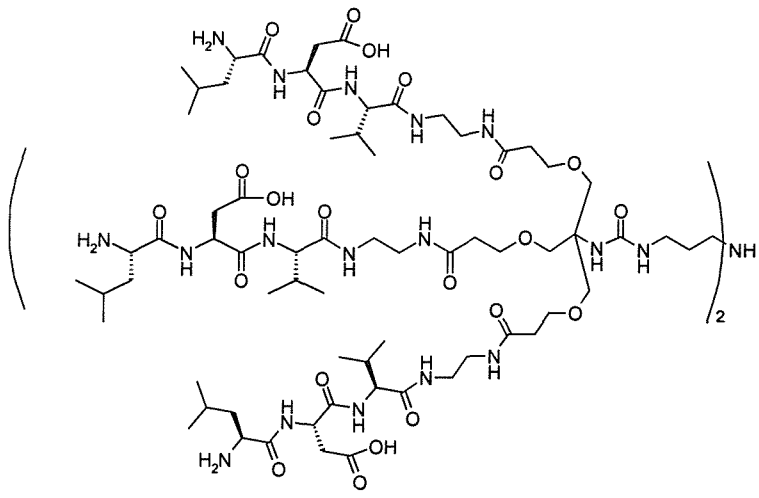
δ_C (100 MHz, D_2O): 174.66, 173.29, 172.15, 171.89, 170.41 (<u>C</u>O); 60.21, (<u>C</u>H, Val); 52.04 (<u>C</u>H, Leu); 51.49 (<u>C</u>H_2 Gen [2.0]); 50.37 (<u>C</u>H, Asp); 49.96, 49.77 (<u>C</u>H_2 Gen [1.0]); 45.0 (<u>C</u>H_2, Gen [0.0]); 40.23 (<u>C</u>H_2) 37.19, 37.04 (<u>C</u>H_2); 36.7 (<u>C</u>H_2, [Gen 2.0]); 35.99 (<u>C</u>H_2 Gen [0.0]); 30.26 (<u>C</u>H$(CH_3)_2$, Val); 29.09 (<u>C</u>H_2 Gen [1.0]); 28.20 (<u>C</u>H_2 Gen [1.0]) 27.5 (<u>C</u>H_2 Gen [2.0]); 24.21 (<u>C</u>H$(CH_3)_2$, Leu); 23.60 (<u>C</u>H_2 Gen [1.0]); 21.85, 21.57 (<u>C</u>H_3, Leu); 18.78, 17.84 (<u>C</u>H_3, Val).

m/z: (MALDI-TOF); 4288.10 ($M+H^+$).

RP-HPLC (Method 1, λ= 220): 8.91 minutes. Analytical HPLC gradient **1**

Resin (loading, 2.16 mmol/g, 390 mg) afforded 288 mg of the title compound in 8% yield.

Synthesis of LDV-Gen [1.0] AB_3-type Dendrimer Conjugate (23).



Generation [0.5] AB_3-type resin was swollen in DMSO for 30 minutes and then ethylene diamine (250 eq, 12 mL), in DMSO (0.5 eq v/v, 6 mL), was added, and the resin shaken for 72 hours. The resin was thoroughly washed and swollen again in DMSO before addition of the same quantities of reactants and shaken for a further 72 hours. The resin was washed thoroughly with DMSO, DCM, DMF, MeOH and ether (each 3 x 10 mL) and dried under vacuum. The dendrimerised resin was subjected to standard Fmoc peptide chemistry as described previously. 200 mg of resin was cleaved with 90 % TFA in DCM.

δ_H (400 MHz, D_2O): 4.77 (6H, t, *J* 7, C<u>H</u>, Asp); 3.97 (6H, d, *J* 7, C<u>H</u>, Val); 3.92 (6H, t, *J* 7, C<u>H</u>, Leu); 3.63 (12H, m, $COC\underline{H}_2CH_2CO$); 3.52 (12H, s, $C\underline{H}_2OCH_2$); 3.32–3.16 (24H, m, $NHC\underline{H}_2C\underline{H}_2NH$); 2.94 (4H, m, $(NHCH_2CH_2C\underline{H}_2NH$); 2.913-2.83 (6H, dd, J_{AB} 17, J_{AX} 7, CH<u>H</u>, Asp); 2.75 (6H, dd, J_{BA} 17 J_{XA} 7, C<u>H</u>H, Asp); 2.38 (12H, m, $COCH_2C\underline{H}_2CO$); 1.98 (6H, m, $C\underline{H}(CH_3)_2$, Val); 1.74 (4H, m, $C\underline{H}_2CH_2CH_2NH$); 1.64 (12H, m, $C\underline{H}_2$, Leu); 1.55 (6H, m, $C\underline{H}(CH_3)_2$, Leu); 0.83 (72H, m, $\underline{C}H_3$).

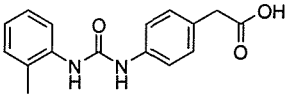
δ_C (100 MHz, D_2O): 174.59 174.17 173.49 171.99 170.43 (<u>C</u>O); 159.86 (NH<u>C</u>ONH); 69.79 ($\underline{C}H_2OCH_2CH_2$); 67.80 ($CO\underline{C}H_2CH_2CO$); 60.10 (<u>C</u>H, Val); 59.35 ($NH\underline{C}(CH_2)_3$) 52.06 (<u>C</u>H, Leu); 50.21 (<u>C</u>H, Asp); 45.55 ($NH\underline{C}H_2CH_2$); 40.27 ($\underline{C}H_2$, Leu); 38.89, 38.79 ($NH\underline{C}H_2\underline{C}H_2NH$); 36.50 ($COCH_2\underline{C}H_2CO$); 35.50 ($\underline{C}H_2$, Asp); 30.32 ($\underline{C}H(CH_3)_2$, Val); 26.92 ($CH_2\underline{C}H_2CH_2$); 24.23 ($\underline{C}H(CH_3)_2$, Leu); 21.59, 21.39, 18.80, 17.78 ($\underline{C}H_3$).

m/z (MALDI-TOF): 3076.77 ($M+H^+$).

RP-HPLC (Method 1, λ= 220): 8.92 mins.

Resin (loading, 3.28 mmol/g, 200 mg) afforded 50 mg of the title compound in 26% yield.

Synthesis of [4-(3-*o*-Tolyl-ureido)-phenyl]-acetic acid (26).[85]



To a suspension of 4-aminophenylacetic acid (2.84 g, 18.80 mmol) in DMF (7.50 mL) was added dropwise *o*-tolyl isocyanate (2.50 g, 18.80 mmol). The reaction mixture was stirred for six hrs at 50°C. It was allowed to cool and poured into ether (100 mL) with stirring. The precipitate was filtered off and washed with, acetonitrile, ethyl acetate and finally ether (each 20 mL) then dried under high vacuum affording the title compound 3.74g as a pale brown powder in 70 % yield.

Experimental data in accordance with literature.[85]

TLC: Ethyl acetate/Hexane (1:1) R_f = 0.27.

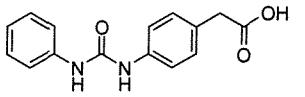
δ_H (300 MHz, $(CD_3)_2SO$): 12.29 (1H, br, s, O<u>H</u>); 9.01 (1H, s, N<u>H</u>); 7.91 (1H, s, N<u>H</u>) 7.88 (1H, d, *J* 8, *Ar*C^6H); 7.43 (2H, d, *J* 8 Ar$C\underline{H}^{3,5}$); 7.19-7.10 (4H, m, *Ar*$C^{3,5}$H, Ar$C^{2,6}\underline{H}$); 6.94 (1H, dd, *J* 8, *Ar*C^5H); 3.51 (2H, s, $C\underline{H}_2CO_2H$); 2.25 (3H, s, *Ar*$C\underline{H}_3$).

δ_C (75 MHz, $(CD_3)_2SO$): 173.05 ($\underline{C}O_2H$); 152.73 (NH$\underline{C}$ONH); 138.49, 137.53 ($Ar\underline{C}^1$NH, Ar$\underline{C}^4$NH); 130.26 ($Ar\underline{C}^5$); 129.83 (Ar$\underline{C}^{2,\ 6}$H); 128.31 (Ar$\underline{C}^1$); 127.44 ($Ar\underline{C}^6CH_3$); 126.24 ($Ar\underline{C}^3$H); 122.66 ($Ar\underline{C}^4$H); 120.97 (Ar$\underline{C}^{3,\ 5}$H); 118.10 ($Ar\underline{C}^1$H); 40.1 ($\underline{C}H_2CO_2H$); 17.9 ($\underline{C}H_3$).

m/z (ES+): 285.20 ($M+H^+$ 100%); 307.20 ($M+Na^+$ 70%).

IR (ν) cm^{-1}: 3277 (NH) (s); 2967 (OH) (s); 1700 (CO) (s).

Synthesis of [4-(3-phenyl-ureido)-phenyl]-acetic acid (28).[85]



To a suspension of 4-aminophenylacetic acid (2g, 14.60 mmol) in DMF (6 mL) was added phenyl isocyanate (1.74 g, 14.58 mmol) dropwise. The reaction mixture was allowed to stir for six hours and then poured into ether (100 mL) with stirring providing the title 3.94 g compound as a white powder in 75% yield. Experimental data in accordance with literature.[85]

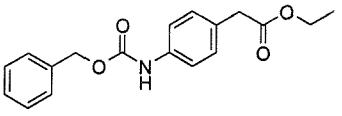
TLC: Ethyl acetate/Hexane (1:1) $R_f = 0.26$.

δ_H (300 MHz, $(CD_3)_2SO$): 9.85 (1H, br s, O$\underline{H}$); 8.62 (1H, s, N$\underline{H}$); 8.61 (1H, s, N$\underline{H}$); 7.43 (2H, d, *J* 8, $Ar\underline{C}^{2,\ 6}$H); 7.37 (2H, d, *J* 8, Ar$\underline{C}^{3,\ 5}$H); 7.26 (2H, t, *J* 8, $Ar\underline{C}^{3,\ 5}$H); 7.15 (2H, d, *J* 8, Ar$\underline{C}^{2,\ 6}$H); 6.95 (1H, t, *J* 7, $Ar\underline{C}^4$H); 3.48 (2H, s, $C\underline{H}_2CO_2H$)

δ_C (75 MHz, $(CD_3)_2SO$): 173.05 ($\underline{C}O_2H$); 152.62 (NH$\underline{C}$ONH); 139.81, 138.30 ($Ar\underline{C}^1$, Ar$\underline{C}^4$); 129.78 (Ar$\underline{C}^{2,\ 6}$H); 128.86 ($Ar\underline{C}^{3,\ 5}$H); 128.43 (Ar$\underline{C}^1$); 121.84 ($Ar\underline{C}^4$H); 118.22 ($Ar\underline{C}^{2,\ 6}$H, Ar$\underline{C}^{3,\ 5}$H); 40.00 ($\underline{C}H_2CO_2H$).

m/z (ES+): 271.00 ($M+H^+$ 80%); 293.00 ($M+Na^+$ 50%); 309.00 ($M+K^+$ 40%).

Synthesis of (4-Benzyloxycarbonylamino-phenyl)-acetic acid ethyl ester (31).[85]



To a solution of ethyl 4-aminophenylacetate (2g, 11 mmol) in DCM (5 mL) were added benzyloxycarbonyl *N*-hydroxysuccinimide ester (2.6 g, 10.4 mmol) and Et_3N (2.45g, 24.1 mmol). The reaction mixture was stirred for 6 hrs and then concentrated *in vacuo* to give a viscous liquid which was dissolved in ethyl acetate (50 mL) washed with citric acid (3 x 50 mL), H_2O (3 x 50 mL), saturated $NaHCO_3$ (2 x 50 mL), and brine, dried

(Na_2SO_4) and concentrated *in vacuo*. The residue was recrystallised from ethyl acetate/hexane to afford the title compound 2.4g, as a brown solid in 70% yield. Experimental data in accordance with literature.[85]

TLC: Ethyl acetate/Hexane (1:1) $R_f = 0.51$.

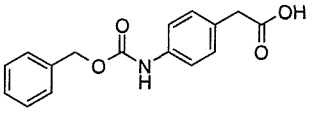
δ_H (300 MHz, $(CD_3)_2SO$): 9.76 (1H, s, NH); 7.48-7.29 (7H, m, *Ar*CH, ArCH); 7.17 (2H, d, *J* 8, $ArC^{2,6}H$); 5.14 (2H, s, CH_2OCO); 4.04 (2H, q, *J* 7, $COOCH_2CH_3$); 3.57 (2H, s, $CH_2CO_2CH_2CH_3$); 1.16 (3H, t, *J* 7, CH_3).

δ_C (75 MHz, $(CD_3)_2SO$): 171.36 (CO_2Et); 153.45 ($NHCO_2CH_2$); 137.89 (ArC^1); 136.7 (ArC^4); 129.72 ($ArC^{2,6}H$,); 128.53 (*Ar*CH); 128.46 (CCH_2CO_2Et); 128.18 (*Ar*CH); 128.12 (*Ar*CH); 118.23 ($ArC^{3,5}H$); 65.78 (CH_2OCONH); 60.27 (OCH_2CH_3); 30.76 (CH_2CO_2Et); 14.14 (CH_3).

m/z (ES+): 317.30 ($M+H^+$ 25%); 331.30 ($M+ NH_4^+$ 100%); 352.30 ($M+Na^+$ 20%); 352.30 ($M+K^+$ 25%).

IR (ν) cm^{-1}: 3326 (NH) (s); 2988 (CH_2) (w); 1719 (CO) (s).

Synthesis of (4-Benzyloxycarbonylamino-phenyl)-acetic acid (32).[85]



To a solution of (4-Benzyloxycarbonylamino-phenyl)-acetic acid ethyl ester (2g, 6.37 mmol) in ethanol (30 mL) was added 1N NaOH (30 mL) and the reaction mixture was stirred overnight. It was concentrated *in vacuo*, then diluted with H_2O (30 mL) and acidified to pH 2 (1 N HCl). The resulting white precipitate was filtered and washed with 10% citric acid and dried to provide the title compound as a white powder (60%, 1.81g). Experimental data in accordance with literature.[85]

TLC: Ethyl acetate/Hexane (1:1) $R_f = 0.30$

δ_H (300 MHz, $(CD_3)_2SO$): 12.30 (1H, br, s, OH); 9.74 (1H, s, NH); 7.5-7.3 (7H, m, *Ar*CH, ArCH); 7.16 (2H, d, *J* 8, $ArC^{2,6}H$); 5.14 (2H, s, CH_2OCO); 3.48 (2H, s, CH_2CO_2H).

δ_C (75 MHz, $(CD_3)_2SO$): 172.95 (CO_2H); 153.46 (CO_2NH); 137.69 9 (ArC^1); 136.71 (ArC^4); 129.78 ($ArC^{2,6}H$); 129.08 (ArC^1) 128.53 ($ArC^{3,6}H$); 128.19 ($ArC^{2,6}H$); 128.12 (ArC^4H); 118.17 ($ArC^{3,5}H$); 65.77 (CH_2)CH_2); 42.88 (CH_2CO_2H).

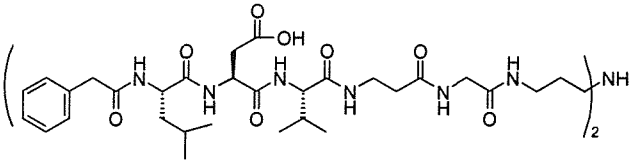
m/z (ES+): 324.10 ($M+K^+$ 100%).

IR (ν) cm^{-1}: 3330 (NH) (s); 2945 (OH) (s); 1702 (CO) (s).

General procedure for the Synthesis of compounds (34)-(41).

Generation [0.0] dendrimer resin **(10)** (200 mg, 0.933 mmol/g) was swollen for 30 minutes in DCM (5 mL). Fmoc amino acids (5eq), with reference to the theoretical number of free amine groups on the resin, were activated with HOBt, (5eq), DIC (5eq), in DCM (5 mL). The activated mixture was then added to the resin and shaken for 2-3 hrs. Valine (5 eq) was coupled using PyBOp (4.9 eq), HOBt (5 eq), DIPEA (10 eq). Fmoc groups were removed by shaking two times 30 mins with 20% piperidine in DMF. Final compounds were cleaved from the resin using the cleavage mixture of TFA/thioanisole/phenol /H_2O/EDT, 82.5 %/5%/5%/5%/2.5% for 2 hrs. The cleavage mixture solvents were removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of TFA, then precipitated in ether and centrifuged. The pellets produced were washed again with ether and finally dried *in vacuo.*

Synthesis of N^1, N^9-*bis*-phenyl-acetyl-LDVβAG)-1,5,9-triazanonane (37).



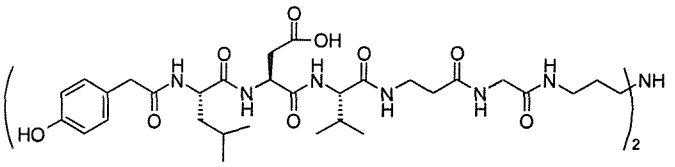
δ_H (400 MHz, $(CD_3)_2SO$): 8.50 (2H, d *J* 9 NH, Asp); 8.40 (1H, br s, NH); 8.35 (4H, d, *J* 9 NH, Leu, Gly); 8.28 (2H, m, NH, βAa); 8.05 (3H, m, $NHCH_2CH_2CH_2NH$); 7.50 (2H, d, *J* 9 NH, Val); 7.38 (10H, m, ArCH); 4.67 (2H, dd, *J* 8, 7, CH, Asp); 4.41 (2H, m, CH, Leu); 4.18 (2H, m, CH, Val); 3.75 (4H, d, *J* 5, CH_2, Gly); 3.55-3.30 (4H, m, $COCH_2CH_2NH$); 3.35-3.20 (4H, m, $NHCH_2CH_2CH_2NH$); 2.98 (4H, m, $NHCH_2CH_2CH_2NH$); 2.90 (2H, dd, J_{AX} 17, J_{BX} 6, CHH, Asp); 2.65 (2H, dd, J_{BX} 17, J_{BA} 6, CHH, Asp); 2.43 (4H, m, $COCH_2CH_2NH$); 2.01 (2H, m, $CH(CH_3)_2$, Val); 1.82 (4H, m, $NHCH_2CH_2CH_2NH$); 1.68 (2H, m, $CH(CH3)_2$, Leu); 1.56 (4H, m, CH_2CH, Leu); 0.96-0.87 (24H, m, CH_3).

δ_C (100 MHz, $(CD_3)_2SO$): 172.92, 172.32, 171.25, 170.90, 170.58, 170.53, 169.97 (CO); 158.87 (ArC^1); 129.42 ($ArC^{2,6}H$); 128.56 ($ArC^{3,5}H$) 126.70 (ArC^3H); 58.01 (CH, Val); 51.36 (CH, Leu); 49.90 (CH, Asp); 45.12 ($NHCH_2CH_2CH_2NH$); 42.64

((NHCH$_2$CH$_2$CH$_2$NH); 42.47 (COCH$_2$CH$_2$NH); 36.07, 35.79, 35.66, 35.61 (CH$_2$, Leu, COCH$_2$CH$_2$NH, NHCH$_2$CH$_2$CH$_2$NH, CHCH$_2$, Asp); 31.12 (COCH$_2$CCH); 26.46 (CH(CH$_3$)$_2$, Val); 24.60 (CH(CH$_3$)$_2$, Leu); 23.41, 21.95, 19.51, 18.15 (CH$_3$).

Resin (200 mg, loading, 0.40 mmol/g) afforded 41 mg of the title compound in 40% yield.

Synthesis of N^1, N^9-*bis*-(4-hydroxy-phenyl) acetyl-LDVβAG)-1,5,9-triazanonane (39).



δ_H (400 MHz, D_2O): 7.11 (4H, d, *J* 9, ArC$^{2,\ 6}$H); 6.81 (4H, d, *J* 9, ArC$^{3,\ 5}$H); 4.66 (2H, m, CH, Asp); 4.24 (2H, m, CH, Leu); 3.93 (2H, d, *J* 7, CH, Val); 3.79 (4H, s, CH$_2$, Gly); 3.50 (4H, s, ArCH$_2$); 3.43 (COCH$_2$CH$_2$NH); 3.26 (4H, m, CH$_2$CH$_2$CH$_2$NH); 2.96 (4H, t, *J* 8, NHCH$_2$CH$_2$CH$_2$NH); 2.89 (2H, dd, J_{AX} 17, J_{AB} 7, CHH, Asp); 2.75 (2H, dd, J_{BX} 17, J_{BA} 7, CHH, Asp); 2.48, (4H, m, COCH$_2$CH$_2$NH); 1.95-1.8 (6H, m, CH(CH$_3$)$_2$, Val, NHCH$_2$CH$_2$CH$_2$NH); 1.60-1.45 (6H, CH(CH$_3$)$_2$ Leu, CH$_2$, Leu); 0.85 (6H, CH$_3$); 0.81-0.77 (6H, CH$_3$).

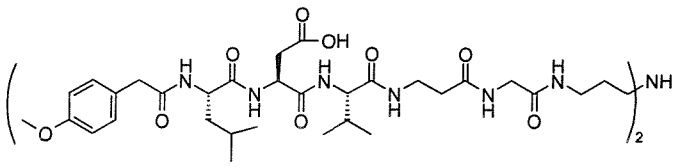
δ_C (100 MHz, D_2O): 173.41, 173.06, 172.94, 172.51, 171.41, 170.23, 152.94 (CO); 128.87 (ArC$^{2,\ 6}$H); 113.99 (ArC$^{3,\ 5}$H); 58.20 (CH, Val); 51.10 (CH, Leu); 48.38 (CH, Asp); 43.35 (NHCH$_2$CH$_2$CH$_2$NH); 41.07 (CH$_2$, Gly); 39.48 (NHCOCH$_2$C); 38.06 (CH(CH$_3$)$_2$ Leu, CH$_2$ Leu); 34.40 (CH$_2$CH$_2$CH$_2$NH); 33.41, 33.25 (NHCH$_2$CH$_2$CH$_2$NH); 28.15 (CH(CH$_3$)$_2$, Val, CH$_2$); 23.95, 22.68 (CH(CH$_3$)$_2$, Val); 20.33, 19.09, 16.65, 15.88 (CH$_3$).

m/z (MALDI-TOF): 1310.20 M^+ 100%.

RP-HPLC (Method 2, λ= 220): 10.98 mins.

Resin (loading, 0.39 mmol/g, 200 mg) afforded 32 mg of the title compound in 30% yield.

Synthesis of N^1, N^9-*bis*-(4-methoxyoxy-phenyl) acetyl-LDVβAG)-1,5,9-triazanonane (40).



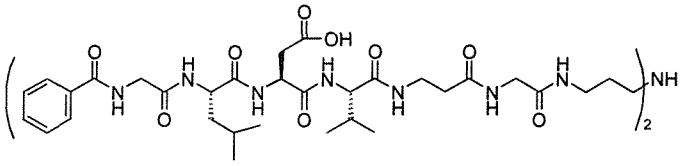
δ_H (400 MHz, $(CD_3)_2SO$): 8.50 (2H, m, NH, Asp); 8.30 (4H, m, NH, Leu, Gly); 8.05 (2H, m, NH, βAla); 7.50 (2H, m, NH, Val); 7.26 (4H, d, *J* 9, $ArC^{2,6}H$); 6.94 (4H, d, *J* 9, $ArC^{3,5}H$); 4.65 (2H, t, *J* 7, CH, Asp); 4.36 (2H, m, CH, Leu); 4.15 (2H, d, *J* 6, CH, Val); 3.77 (6H, s, OCH_3); 3.75 (2H, s, CH_2, Gly); 3.63 (4H, s, $COCH_2C$); 3.37-3.31 (4H, m, $CH_2NHCOCH_2CH_2NH$); 3.24 (4H, br t, *J* 7, $NHCH_2CH_2CH_2NH$); 2.96 (4H, t, *J* 8, $NHCH_2CH_2CH_2NH$); 2.85 (2H, dd, J_{AX}, 17, J_{AB} 7, CHH, Asp); 2.61 (2H, dd, J_{BX}, 17, J_{BA} 7, CHH, Asp); 2.44-2.41 (4H, t, *J* 7, $NHCOCH_2CH_2NH$); 2.00-1.98 (2H, m, $CH(CH_3)_2$, Val); 1.84-1.80 (4H, m, $NHCH_2CH_2CH_2NH$); 1.68-1.56 (2H, m, $CH(CH_3)_2$, Leu); 1.63-1.51 (4H, m, CH_2, Leu); 0.95-0.94 (12H, d, *J* 7, CH_3, Leu); 0.90-0.85 (12H, m, CH_3, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 172.34, 171.81, 170.88, 170.59, 170.04, 169.46 (CO); 157.73 (ArC^4); 129.86 ($ArC^{2,6}$); 128.03 (C^1); 113.47 ($ArC^{3,5}H$); 57.47 (CH, Val); 54.88 (OCH_3); 50.87 (CH, Leu); 50.78 (CH, Asp); 49.21 (CH_2, Gly); 44.38 ($NHCH_2CH_2CH_2NH$); 41.95 ($NHCH_2CH_2CH_2NH$); 40.93 ($COCH_2CH_2NH$); 35.38 ($COCH_2C$); 35.20 (CH_2, Asp); 35.11 ($COCH_2CH_2NH$); 34.98 (CH_2, Leu); 30.40 ($CH(CH_3)_2$, Leu); 25.73 ($NHCH_2CH_2CH_2NH$); 24.03 ($CH(CH_3)_2$, Val); 22.80, 21.36, 18.91, 17.56 (CH_3).

m/z (MALDI-TOF): 1338.41 100% M^+.

Resin (loading, 0.39 mmol/g, 200 mg) afforded 40 mg of the title compound in 38% yield.

Synthesis of N^1, N^9-*bis*-benzoylamino-acetyl-LDVβAG)-1,5,9-triazanonane (38).



δ_H (400 MHz, $(CD_3)_2SO$): 8.79 (2H, m, $COCH_2NHCO$); 8.43 (2H, m, NH, Asp); 8.35 (1H, br s, NH); 8.21 (4H, m, NH, Gly, Leu); 8.02 (2H, m, NH, βAla); 8.0-7.91 (6H, m, $ArC^{2,6}H$, NH, $NHCH_2CH_2CH_2NH$); 7.6 (2H, m, $ArC^{3,5}H$); 7.53 (4H, t, *J* 8, ArC^4H); 7.41 (2H, d, 9, NH, Val); 4.64 (2H, dd, *J* 8, 7, CH, Asp); 4.39 (2H, dd, *J* 8, CH, Leu);

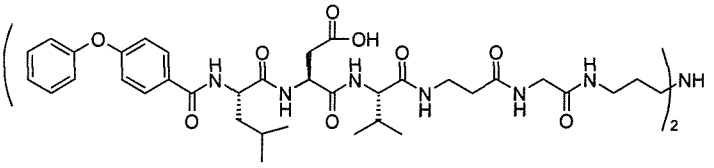
4.10 (2H, dd, *J* 9, C<u>H</u>, Val); 3.97 (4H, dd, *J* 6, 6, COC<u>H</u>$_2$NH); 3.70 (4H, d, *J* 6, C<u>H</u>$_2$, Gly); 3.31 (4H, m, COCH$_2$C<u>H</u>$_2$NH); 3.20 (4H, m, NHCH$_2$CH$_2$C<u>H</u>$_2$NH); 2.93 (4H, br m, NHC<u>H</u>$_2$CH$_2$CH$_2$NH); 2.84 (2H, dd, J_{AX} 17, J_{AB} 8, C<u>H</u>H, Asp); 2.6 (2H, J_{BX} 17, J_{BA} 8, CH<u>H</u>, Asp); 2.37 (4H, t, *J* 8 COC<u>H</u>$_2$CH$_2$NH); 1.95 (2H, m, C<u>H</u>(CH$_3$)$_2$, Val); 1.77 (4H, m, NHCH$_2$C<u>H</u>$_2$CH$_2$NH); 1.68 (2H, m, C<u>H</u>(CH$_3$)$_2$, Leu); 1.52 (4H, dd, *J* 7.53, 7.03 C<u>H</u>$_2$, Leu); 0.8 (12H, m, C<u>H</u>$_3$); 0.6 (12H, m, C<u>H</u>$_3$).

δ_C (100 MHz, (CD$_3$)$_2$SO): 172.82, 172.28, 171.24, 170.88, 170.59, 169.97, 169.50, 167.07 (<u>C</u>O); 134.35 (Ar<u>C</u>^{1}); 131.82 (Ar<u>C</u>^{4}H); 128.73 (Ar<u>C</u>$^{3,\ 5}$H); 127.75 (Ar<u>C</u>$^{2,\ 6}$H); 58.03 (<u>C</u>H, Val); 51.46 (<u>C</u>H, Leu); 50.02 (<u>C</u>H, Asp); 45.12 (<u>C</u>H$_2$, Gly); 43.10 (<u>C</u>H$_2$, Leu); 41.50 (NHCH$_2$CH$_2$<u>C</u>H$_2$NH) 36.07 (NH<u>C</u>H$_2$CH$_2$CH$_2$NH); 35.83 (COCH$_2$<u>C</u>H$_2$NH); 35.61, (<u>C</u>H$_2$, Asp); 31.05 (NHCH$_2$<u>C</u>H$_2$CH$_2$NH); 26.46 (CO<u>C</u>H$_2$CH$_2$NH); 24.56 (<u>C</u>H(CH$_3$)$_2$, Val); 23.49 (<u>C</u>H(CH$_3$)$_2$, Leu); 22.00, 19.45 (<u>C</u>H$_3$, Val); 18.15, 15.12 (<u>C</u>H$_3$, Leu).

m/z (MALDI-TOF): 1386.57 [M+Na].$^+$

Resin (loading, 0.39 mmol/g, 200 mg) afforded 71 mg of the title compound in 66%.

Synthesis of *N*^{1}, *N*^{9}-*bis*-(4-phenoxy-benzyl-LDVβAG)-1,5,9-triazanonane (41).



δ_H (300 MHz, (CD$_3$)$_2$SO): 8.52-8.50 (2H, m, N<u>H</u>, Asp, Leu); 8.44 (1H, br s, N<u>H</u>); 8.26 (2H, m, N<u>H</u>, Gly): 8.08 (4H, m, N<u>H</u>, βAa, , N<u>H</u>CH$_2$CH$_2$CH$_2$NH); 8.05 (4H, d, *J* 9, ArC$^{2,\ 6}$<u>H</u>); 7.53 (6H, m, N<u>H</u>, *Ar*C$^{3,\ 5}$<u>H</u>); 7.31 (2H, t, *J* 8, *Ar*C^4<u>H</u>); 7.18 (4H, *J* 8, ArC$^{3,\ 5}$<u>H</u>); 7.14 (4H, d, *J* 9, *Ar*C$^{2,\ 6}$<u>H</u>); 4.70 (2H, dd, *J* 7, C<u>H</u>, Asp); 4.62 (2H, m, C<u>H</u>, Leu); 4.17 (2H, dd, *J* 6, 7, C<u>H</u>, Val); 3.75 (4H, d, *J* 4 C<u>H</u>$_2$, Gly); 3.38 (4H, m, COCH$_2$C<u>H</u>$_2$NH); 3.25 (4H, m, NHCH$_2$CH$_2$C<u>H</u>$_2$NH); 2.98 (4H, br m, NHC<u>H</u>$_2$CH$_2$CH$_2$NH); 2.87 (2H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.65 (2H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.44 (4H, t, *J* 7, COC<u>H</u>$_2$CH$_2$NH); 2.02 (2H, m, C<u>H</u>(CH$_3$)$_2$, Val); 1.81 (8H, m, NHCH$_2$C<u>H</u>$_2$CH$_2$NH, CH$_2$, Leu); 1.62 (2H, m, C<u>H</u>(CH$_3$)$_2$, Val); 0.99 (12H, m, C<u>H</u>$_3$); 0.88 (12H, m, 7.00 C<u>H</u>$_3$).

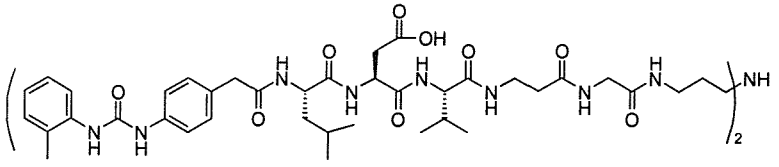
δ_C (75 MHz, (CD$_3$)$_2$SO): 173.13, 172.42, 171.28, 170.93, 170.64, 169.98, 166.16 (<u>C</u>O); 159.91, 156.19, (*Ar*<u>C</u>^{1}O<u>C</u>^{4}Ar); 130.65, (Ar<u>C</u>$^{2,\ 6}$H); 130.23 (Ar<u>C</u>^{1}) 124.64 (*Ar*<u>C</u>$^{3,\ 5}$H);

119.77 (*Ar*C$^{2,\ 6}$H, ArC$^{3,\ 5}$H); 117.81 (*Ar*C^{4}H); 58.05 (CH, Val); 52.32 (CH, Leu); 49.92 (CH, Asp); 45.12 (CH_2, Gly); 42.65 (CH_2, Leu); 40.86 ($NHCH_2CH_2CH_2NH$); 36.67, 36.06, 35.80, 35.66, 35.61 ($NHCH_2CH_2CH_2NH$, $NHCH_2CH_2CH_2NH$, $COCH_2CH_2NH$, CH_2, Asp); 31.07 ($CH(CH_3)_3$, Val) 26.44 ($NHCH_2CH_2CH_2NH$); 24.84, ($CH(CH_3)_3$, Leu); 23.44, 21.80 (CH_3, Leu); 19.50, 18.15 (CH_3, Val).

m/z (MALDI-TOF): 1456.68 $(M+Na)^+$

Resin (loading, 0.38 mmol/g, 200 mg) afforded 35 mg of the title compound in 30% yield.

Synthesis of N^1, N^9-*bis*-[4-(3-*o*-Tolyl-ureido)-phenyl]-acetyl-LDVβAG)-1,5,9-triazanonane (36).



δ_H (300 MHz, $(CD_3)_2SO$): 9.09 (2H, s, NH); 8.50 (2H, d, *J* 8, NH, Asp); 8.40 (1H, br s, NH); 8.3 (4H, d, *J* 8, NH, Leu, Gly); 8.09-8.00 (2H, m, NH, βAla); 8.00 (2H, s, NH); 7.93 (2H, d, *J* 8, *Ar*C^{2}H); 7.52 (2H, d, NH, Val); 7.48 (4H, d, *J* 9, ArC$^{3,\ 5}$H); 7.28 (8H, m, *Ar*CH, ArCH); 7.04 (2H, t, *J* 8 *Ar*C^{4}H) 4.68 (1H, m, CH, Asp); 4.42 (1H, m, CH, Leu); 4.18 (1H, m, CH, Val); 3.80 (2H, d, *J* 4, CH_2, Gly); 3.48 (2H, s, CH_2): 3.45-3.29 (4H, m, $COCH_2CH_2NH$); 3.26 (4H, m, $NHCH_2CH_2CH_2NH$); 2.97 (4H, br m, $NHCH_2CH_2CH_2NH$); 2.85 (1H, dd, J_{AX} 17, J_{AB} 7, CHH, Asp); 2.62 (2H, dd, J_{BA} 17, J_{BX} 7, CHH, Asp); 2.43 (4H, br t, *J* 8, $COCH_2CH_2NH$); 2.34 (6H, s, $NHCCCH_3$); 2.02 (2H, m, $CH(CH_3)_2$, Val); 1.82 (4H, m, $NHCH_2CH_2CH_2NH$); 1.69 (2H, m, $CH(CH_3)_2$, Leu); 1.55 (4H, m, CH_2, Leu); 0.96 (12H, d, *J* 7, CH_3); 0.98 (12H, m, CH_3).

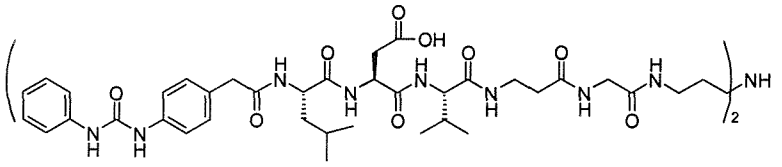
δ_C (75 MHz, $(CD_3)_2SO$): 172.96, 172.34, 171.26, 170.91, 170.81, 170.59, 169.98, (CO); 153.14 (NHCONH); 130.60 (*Ar*C^1); 130.04 (ArC^4); 129.78 (ArC$^{2,\ 6}$H); 127.94 (*Ar*C^6); 126.56 (*Ar*C^{5}H), 123.05, 121.48, 119.30, 118.37, 115.66 (*Ar*CH, ArCH); 58.04 (CH, Val); 51.38 (CH, Leu); 49.91 (CH, Asp); 45.12 ($(NHCH_2CH_2CH_2NH$); 42.65 (CH_2, Gly); 41.82(CH_2, Leu); 41.65 ($NHCH_2CH_2CH_2NH$) 40.59 ($NHCH_2CH_2CO$); 36.06 (CH_2COOH); 35.79 ($NHCH_2CH_2CO$); 35.66 ($NHCH_2CH_2CH_2NH$) 31.11 ($CH(CH_3)_2$,

Val); 31.11 ($\underline{C}H_2$, Leu); 26.47 ($NHCH_2\underline{C}H_2CH_2NH$); 24.61 ($\underline{C}H(CH_3)_2$, Leu); 23.41, 21.99 ($\underline{C}H_3$, Leu); 18.30, 18.16 ($\underline{C}H_3$, Val) 15.59 ($Ar\underline{C}H_3$).

m/z (MALDI-TOF): 1575.96 ($M+H^+$ 100%); 1597.93 ($M+Na^+$ 30%); 1613.87 ($M+K^+$ 20%).

Resin (loading, 0.37 mmol/g, 200 mg) afforded 48 mg of the title compound in 41% yield.

Synthesis of N^1, N^9-*bis*-[4-(3-phenyl-ureido)-phenyl]-acetic acid-LDVβAG)-1,5,9-triazanonane (35).



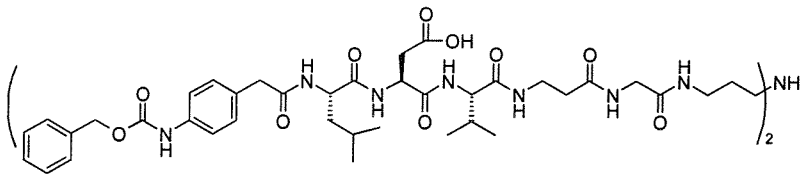
δ_H (300 MHz, $(CD_3)_2SO$): 8.63 (2H, s, N$\underline{H}$); 8.59 (2H, s, N$\underline{H}$) 8.33 (2H, d, *J* 8, N$\underline{H}$, Asp); 8.10 (4H, m, N$\underline{H}$, Gly, Leu); 7.89 (4H, m, $N\underline{H}CH_2CH_2CH_2NH$, βAla); 7.38 (6H, m, $Ar\underline{C}^{2,\ 6}\underline{H}$, N$\underline{H}$, Val); 7.30 (4H, d, *J* 9, $Ar\underline{C}^{3,\ 5}\underline{H}$); 7.21 (4H, dd, *J* 8, 9, $Ar\underline{C}^{3,\ 5}\underline{H}$); 7.08 (4H, d, *J* 9, $Ar\underline{C}^{2,\ 6}\underline{H}$); 6.89 (2H, dd, *J* 7, 8, $Ar\underline{C}^4\underline{H}$); 4.50 (2H, m, C$\underline{H}$, Asp); 4.24 (2H, m, C$\underline{H}$, Leu); 4.00 (2H, m, C$\underline{H}$, Val); 3.58 (4H, d, *J* 6 $C\underline{H}_2$, Gly); 3.40-3.10 (8H, m, $COC\underline{H}_2CCH$, $COCH_2C\underline{H}_2NH$); 3.08 (4H, m, $NHC\underline{H}_2CH_2CH_2NH$); 2.80 (4H, m, $NHCH_2CH_2C\underline{H}_2NH$); 2.69 (2H, dd, J_{AX} 17, J_{AB} 7, C$\underline{H}$H, Asp); 2.44 (2H, dd, J_{BX} 17, J_{BA} 7, CH$\underline{H}$, Asp); 2.26 (4H, m, $COC\underline{H}_2CH_2NH$); 1.84 (2H, m, C$\underline{H}$, Val); 1.65 (4H, m, $NHCH_2C\underline{H}_2CH_2NH$); 1.52 (2H, m, C$\underline{H}$, Leu); 1.37 (4H, m, $C\underline{H}_2$, Leu); 0.80-0.70 (24H, m, $C\underline{H}_3$, Leu, Val).

δ_C (75 MHz, $(CD_3)_2SO$): 172.94, 172.30, 171.31, 170.90, 170.78, 170.68, 169.97, ($\underline{C}O$); 153.01 ($NH\underline{C}ONH$); 140.23 ($Ar\underline{C}^1$); 138.46 ($Ar\underline{C}^4$); 130.15 ($Ar\underline{C}^1$); 129.74 ($Ar\underline{C}^{2,\ 6}H$) 129.20 ($Ar\underline{C}^{3,\ 5}H$); 122.17 ($Ar\underline{C}^4H$); 118.59 ($Ar\underline{C}^{2,\ 6}H$); 118.53 ($Ar\underline{C}^{3,\ 5}H$); 58.03 ($\underline{C}H$, Val); 51.36 ($\underline{C}H$, Leu); 49.89 ($\underline{C}H$, Asp); 45.10 ($\underline{C}H_2$, Gly); 42.66 ($\underline{C}H_2$, Leu); 41.82 ($NH\underline{C}H_2CH_2CH_2NH$); 41.66 ($CO\underline{C}H_2C$); 36.07 ($NHCH_2CH_2\underline{C}H_2NH$); 35.90 ($COCH_2\underline{C}H_2NH$); 35.64 ($\underline{C}H_2$, Asp); 31.10 ($NHCH_2\underline{C}H_2CH_2NH$); 26.47 ($CO\underline{C}H_2CH_2NH$); 25.0 ($\underline{C}H$, Val); 24.61, 23.43, 19.53, 18.18 ($\underline{C}H_3$).

m/z (MALDI-TOF): 1546.72 ($M+H^+$ 50%); 1568.71 ($M+Na^+$ 100%); 1585.72 ($M+K^+$ 50%); 1606.65 ($M+K^++Na^+$ 48%).

Resin (loading, 0.38 mmol/g, 200 mg) afforded 50 mg of the title compound in 42% yield.

Synthesis of N^1, N^9-*bis*-[(4-Benzyloxycarbonylamino-phenyl)-acetyl -LDVβAG)-1,5,9-triazanonane (34).



δ_H (400 MHz, $(CD_3)_2SO$): 9.80 (2H, s, NH); 8.50 (3H, m, NH, Asp, NH); 8.28 (4H, NH, m, Gly, Leu); 8.05 (4H, m, NH, βala, $NHCH_2CH_2CH_2NH$); 7.50 (10H, m, *Ar*CH, NH Val); 7.25 (2H, d, *J* 9, *Ar*CH); 7.10 (2H, d, *J* 8, $ArC^{3,}$ ^5H); 6.76 (2H, d, *J* 8, $ArC^{2,}$ ^6H); 5.24 (4H, s, $ArCH_2OCO$); 4.67 (2H, m, CH, Asp); 4.40 (2H, br m, CH, Leu); 4.18 (2H, m, CH, Val); 3.75 (4H, d, *J* 5 CH_2, Gly); 3.59-3.30 (8H, m, $COCH_2CH_2NH$, $ArCH_2CO$) 3.28-3.20 (4H, m, $NHCH_2CH_2CH_2NH$); 2.98 (4H, br m, $NHCH_2CH_2CH_2NH$); 2.84 (2H, J_{AX} 16, J_{AB} 6 CHH, Asp); 2.60 (2H, dd J_{BX} 16, J_{BA} 6 CHH, Asp) 2.43, (4H, m, $COCH_2CH_2NH$); 2.01 (2H, m, $CH(CH_3)_2$, Val); 1.83 (4H, m, $NHCH_2CH_2CH_2NH$); 1.68 (2H, m, $CH(CH_3)_2$, Leu); 1.54 (4H, m, CH_2, Leu); 0.96-0.86 (24H, m, CH_3)

δ_C (100 MHz, $(CD_3)_2SO$): 172.94, 172.32, 171.24, 170.90, 170.70, 170.58, 169.94, 153.00 (CO); 138.00 (*Ar*C^1); 137.11 (ArC^4); 130.84 (ArC^1); 130.05 ($ArC^{2,}$ ^6H); 129.72 ($ArC^{3,}$ ^5H); 128.88 ($ArC^{2,}$ ^6H); 128.49 ($ArC^{2,}$ ^6H); 118.53 ($ArC^{3,}$ ^5H); 66.10 (*Ar*CH_2OCO); 58.02 (CH, Val); 51.33 (CH, Leu); 49.90 (CH, Asp); 45.11 (CH_2, Gly); 42.63 ($NHCH_2CH_2CH_2NH$); 41.65 ($NHCH_2CH_2CH_2NH$); 36.08 (CH_2, Leu); 35.79, 35.66 35.59 ($COCH_2CH_2NH$, $COCH_2CH_2NH$, CH_2, Asp); 31.11 ($CH(CH_3)_2$, Val); 26.43 ($NHCH_2CH_2CH_2NH$,); 24.00 ($CH(CH_3)_2$, Leu); 23.42, 21.98, 19.51, 18.15 (CH_3).

m/z (MALDI-TOF): 1577.83 (M+H 100%); 1599.83 ($M+Na^+$ 20%); 1615.83 ($M+K^+$ 10%).

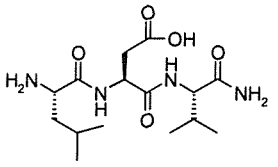
Resin (loading, 0.37 mmol/g, 200 mg) afforded 38 mg of the title compound in 32% yield.

Synthesis of monovalent LDV containing compounds.

Compounds **(42)** and **(52)** were synthesised on TentaGel resin derivatised with Rink linker.

Tentagel resin (1g, 0.29 mmol/g) was swollen for 10 mins in DCM before the addition of Fmoc Rink linker (0.31g, 0.58 mmol, 2 eq) which had been activated for 10 minutes with DIC (0.10 ml, 0.64 mmol, 2.20 eq) HOBt (0.086g, 0.64 mmol, 2.20 eq) and shaken overnight. Fmoc deprotection of the protected linker allowed peptide synthesis to begin using standard Fmoc chemistry using the ninhydrin test to confirm completion of reaction. Valine (5 eq) was coupled using PyBOp (4.9 eq), HOBt (5 eq), DIPEA (10 eq). The final compounds were cleaved from the resin using the cleavage mixture of TFA/thioanisole/phenol/H_2O/EDT, 82.5%/5%/5%/5%/2.5%. The cleavage mixture solvents were then removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of acetic acid, then precipitated in ether and centrifuged. The pellets produced were rinsed again with ether and finally dried *in vacuo.*

Synthesis of LDV-NH_2 (42).



δ_H (400 MHz, D_2O): 4.70 (1H, t, *J* 7, C<u>H</u>, Asp); 4.01 (1H, d, *J* 6, C<u>H</u>, Val); 3.89 (1H, t, *J* 7, C<u>H</u>, Leu); 3.58 (2H, br s, N<u>H</u>$_2$); 2.75 (1H, dd, J_{AX} 16, J_{AB} 6, C<u>H</u>H Asp); 2.60 (1H, dd, J_{BX} 16, J_{BA} 6 CH<u>H</u>, Asp); 2.00 (1H, m, C<u>H</u>$(CH_3)_2$, Val); 1.60 (2H, m, C<u>H</u>$_2$, Leu); 1.52 (1H, m, C<u>H</u>$(CH_3)_2$, Leu); 0.84-0.74 (12H, m, C<u>H</u>$_3$ Leu, Val).

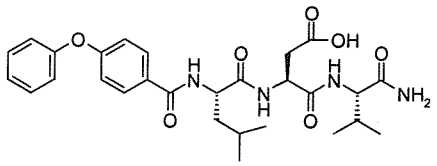
δ_C (100 MHz, D_2O): 176.20, 175.80, 172.66, 170.39 (<u>C</u>O); 59.48 (<u>C</u>H, Asp); 52.03 (<u>C</u>H, Leu); 50.89 (<u>C</u>H, Val); 40.18 (<u>C</u>H$_2$, Asp); 36.97 (<u>C</u>H$_2$, Leu); 30.10 (<u>C</u>H$(CH_3)_2$, Val); 24.18 (C<u>H</u>$(CH_3)_2$, Leu); 21.82, 21.52 (<u>C</u>H$_3$, Val); 18.72, 17.50 (<u>C</u>H$_3$, Leu).

m/z (ES+): 345.40 ($M+H^+$ 100%).

RP-HPLC: (Method 1, λ= 220) 7.74 mins.

Resin (loading 0.24mmol/g, 600 mg) afforded 31 mg of the title compound in 62 % yield.

Synthesis of 4-phenoxy-benzyl-LDV-NH_2 (52).



δ_H (400 MHz, $(CD_3)_2SO$): 8.58-8.48 (4H, m, NH Asp, Leu); 8.05 (2H, d, *J* 9, $ArC^{2,6}H$); 7.55 (2H, t, *J* 7, $ArC^{3,5}H$); 7.47 (2H, d, *J* 9, NH, Val); 7.42 (2H, s, NH_2); 7.32 (1H, t, *J* 7, ArC^4H); 7.22-7.11 (4H, m, $ArC^{2,6}H$, $ArC^{3,5}H$); 4.76-4.68 (1H, m, CH, Asp); 4.67-4.58 (1H, m, CH, Leu); 4.24-4.16 (1H, m, CH, Val); 2.88 (1H, dd, J_{AX} 17, J_{AB} 7, CHH, Asp); 2.65(1H, dd, J_{BX} 17, J_{BA} 7, CHH, Asp); 2.14-2.02 (1H, m, CH, Val); 1.87-1.73 (2H, m, CH_2, Leu); 1.65-1.57 (1H, m, CH, Leu); 1.03-0.85 (12H, m, CH_3, Val, Leu).

δ_C (100 MHz, $(CD_3)_2SO$): 173.08, 172.98, 172.40, 170.61, 166.12 (CO); 159.89 (ArC^4); 156.21 (ArC^1); 130.67 ($ArC^{2,6}H$); 130.24 ($ArC^{3,5}H$); 129.28 (ArC^1); 124.66 (ArC^4H); 119.79 ($ArC^{3,5}H$); 117.83 ($ArC^{2,6}H$); 57.70 (CH Val); 52.27 (CH, Leu); 49.95 (CH, Asp); 35.86 (CH_2, Leu); 30.93 (CH_2, Asp); 24.85 (CH, Val); 23.48 (CH, Leu); 21.84, 19.67 (CH_3, Leu); 17.91, 15.61 (CH_3, Val).

m/z (ES+): 563.40 ($M+Na^+$ 100%).

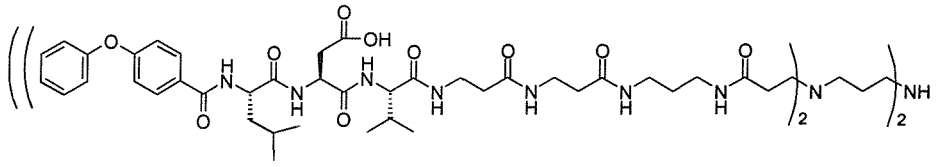
HR-ES+: ($M+Na^+$) $C_{28}H_{36}N_4O_7$ 563.2481 (calc), 563.24762 (found).

Resin (loading 0.23 mmol/g, 200 mg) afforded 16 mg of the title compound in 62% yield.

General procedure for synthesis of optimised LDV ligand dendrimer conjugates.

Generation [0.0], [1.0] [2.0] dendrimer resins (200 mg) were swollen for 30 minutes in DCM. Fmoc amino acids (5eq), with reference to the theoretical number of free amine groups on the resin, were activated with HOBt, (5eq), DIC (5eq), in DCM. The activated mixture was then added to the resin and shaken for 2-3 hrs. Valine (5 eq) was coupled using PyBOp (4.9 eq), HOBt (5 eq), DIPEA (10 eq). Fmoc groups were removed by shaking two times 30 mins with 20% piperidine in DMF. Final compounds were cleaved from the resin using the cleavage mixture of TFA/thioanisole/phenol/H_2O/EDT, 82.50 %/5%/5%/5%/2.50%. The cleavage mixture solvents were then removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of TFA, then precipitated in ether and centrifuged. The pellets produced were rinsed again with ether and finally dried *in vacuo.*

Synthesis of 4-phenoxy-benzoic acid-LDVβAβA-Gen [1.0] dendrimer conjugate (43).



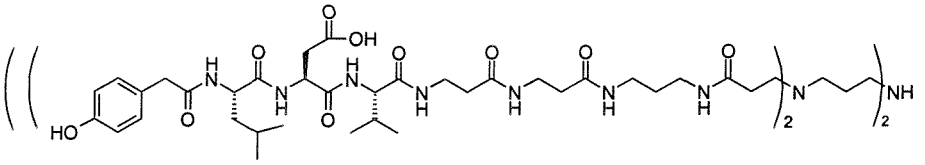
δ_H (400 MHz, $(CD_3)_2SO$): 8.55 (8H, m, NH Asp, Leu); 8.30-8.20 (1H, br s, NH); 8.05 (8H, d, *J* 8, $ArC^{2,6}H$); 8.04-7.95 (16H, m, NH, βAla, $NHCH_2CH_2CH_2$); 7.52 (8H, t, *J* 8, $ArC^{3,5}H$); 7.48 (4H, d, *J* 9, NH, Val); 7.30 (4H, dd, *J* 7 ArC^4H); 7.18 (8H, d, *J* 7, $ArC^{3,5}H$); 7.14 (8H, d, *J* 9, $ArC^{2,6}H$); 4.54-4.52 (4H, m, CH, Asp); 4.51-4.48 (4H, m, CH, Leu); 4.25-4.20 (4H, m, CH, Val); 3.50-3.10 (32H, m, CH_2NH, $NHCH_2CH_2CO$, $COCH_2CH_2NH$); 2.87 (4H, dd, J_{AX} 17, J_{AB} 6, CHH, Asp); 2.73 (8H, br m, $COCH_2CH_2NH$); 2.62 (4H, dd, J_{BX} 17, J_{BA} 6, CHH, Asp); 2.35-2.25 (16H, m, $NHCH_2CH_2CO$); 2.12 (4H, br s, CH_2 Gen [0.0]); 2.02 (4H, m, CH, Val); 1.80-1.70 (12H, m, CH_2, CH_2, Leu); 1.62-1.55 (12H, $NHCH_2CH_2CH_2NH$, CH, Leu); 1.00 (24H, dd, *J* 6, CH_3, Val); 0.88 (24H, dd, *J* 7, CH_3, Leu).

δ_C (100 MHz, $(CD_3)_2SO$): 174.13, 173.41, 171.87, 171.79, 171.62, 171.59, 170.50, 167.17 (CO); 160.91 (ArC^4); 157.21 (ArC^1); 131.68 ($ArC^{2,6}H$); 131.24 ($ArC^{3,5}H$); 130.27 (ArC^1); 125.66 (ArC^4H); 120.79 ($ArC^{2,6}H$); 118.83 ($ArC^{3,5}H$); 59.03 (CH, Val); 53.33 (CH, Leu); 50.93 (CH, Asp); 41.88, 37.94, 37.71, 36.94, 36.85, 36.77, 36.68 (CH_2); 32.10 ($CH(CH_3)_2$, Val); 30.44 (CH_2, βAla); 25.85 ($CH(CH_3)_2$, Leu); 24.47 22.83 (CH_3, Leu); 20.52 19.17 (CH_3, Val).

m/z (MALDI-TOF): 3307.70 ($M+H^+$ 100%).

Resin (loading 0.56 mmol/g, 200 mg) afforded 95 mg of the title compound in 25% yield.

Synthesis of (4-hydroxy-phenyl)-acetyl-LDVβAβA-Gen [1.0] dendrimer conjugate (44).

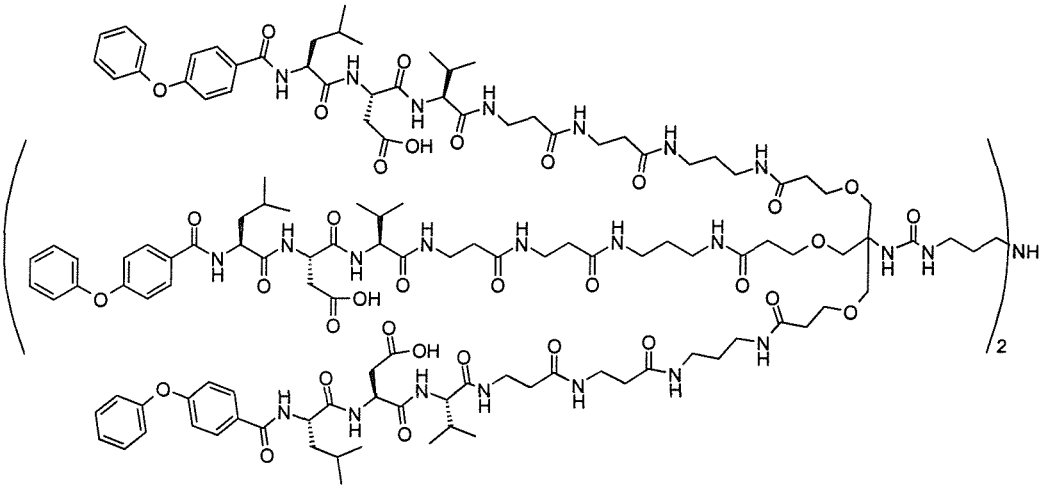


δ_H (400 MHz, $(CD_3)_2SO$): 8.52-8.37 (8H, m, N<u>H</u>, Asp, N<u>H</u>CH_2); 8.30-8.20 (8H, m, N<u>H</u>, Leu, N<u>H</u>$CH_2\beta$Ala); 8.10-7.90 (12H, m, N<u>H</u>CH_2); 7.50 (4H, d, *J* 9 N<u>H</u> Val); 7.13 (8H, d, *J* 9, Ar$C^{2,}$ ^{6}<u>H</u>); 6.77 (8H, d, *J* 9, Ar$C^{3,}$ ^{5}<u>H</u>); 4.70-4.62 (4H, m, C<u>H</u>, Asp); 4.46-4.36 (4H, m, C<u>H</u>, Leu); 4.20-4.13 (4H, m, C<u>H</u>, Val); 3.50-3.10 (40H, m, C<u>H_2</u>); 2.90 (4H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.78-2.65 (8H, m, C<u>H_2</u>, βAla); 2.60 (4H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.38-2.25 (16H, m, C<u>H_2</u>CH_2CO); 2.21-2.10 (4H, m, C<u>H_2</u>); 2.05-1.98 (4H, m, C<u>H</u>, Val); 1.96-1.88 (6H, m, C<u>H_2</u>); 1.70-1.60 (12H, C<u>H_2</u>, C<u>H</u>, Val); 1.58-1.47 (8H, m, C<u>H_2</u>, Leu); 1.53 (48H, m, C<u>H_3</u>).

δ_C (100 MHz, $(CD_3)_2SO$): 171.21, 170.55, 169.34, 169.08, 169.02, 168.81, 167.69 (<u>C</u>O); 154.50 (ArC^4); 128.65, 125.12 (Ar<u>C</u>^{1}); 113.61 (<u>C</u>H); 63.58, 56.26, 49.60 (<u>C</u>H); 48.14 (<u>C</u>H_2); 39.88 (<u>C</u>H_2); 35.16, 34.92, 34.16, 34.01, 33.90 (<u>C</u>H_2); 29.34, 27.65 (<u>C</u>H); 22.82 (<u>C</u>H_2); 21.64, 20.21, 17.73, 16.39 (<u>C</u>H_3).

Resin (loading, 0.58 mmol/g, 300 mg) afforded 114 mg of the title compound in 21% yield.

Synthesis of 4-phenoxy-benzoic acid-LDVβAβA-Gen [1.0] AB_3 type dendrimer conjugate (45).



δ_H (400 MHz, $(CD_3)_2SO$): 8.59 (12H, N<u>H</u> Leu, N<u>H</u>, Asp); 8.04 (12H, d, *J* 8, Ar$C^{2,}$ ^{6}<u>H</u>); 8.01-7.91 (12H, m, N<u>H</u>$CH_2CH_2CH_2$N<u>H</u>); 7.56-7.48 (18H, m, *Ar*$C^{3,}$ ^{5}<u>H</u>, N<u>H</u>, Val); 7.31 (6H, t, *J* 7, *Ar*C^4<u>H</u>); 7.18 (12H, d, *J* 9, Ar$C^{3,}$ ^{5}<u>H</u>); 7.14 (12H, d, *J* 9, *Ar*$C^{2,}$ ^{6}<u>H</u>); 4.72-4.69 (6H, m, C<u>H</u>, Asp); 4.65-4.60 (6H, m, C<u>H</u>, Leu); 4.20-4.12 (6H, m, C<u>H</u>, Val); 3.74-3.66 (24H, m, $OCH_2C\underline{H_2}CO$, $C\underline{H_2}OCH_2CO$); 3.45-3.26 (24H, m, $NHC\underline{H_2}$, β Ala); 3.20-3.08 (28H, m, $NHC\underline{H_2}CH_2C\underline{H_2}NH$ Gen [1.0], $NHC\underline{H_2}CH_2$ Gen [0.0]); 3.04-2.95 (4H, m,

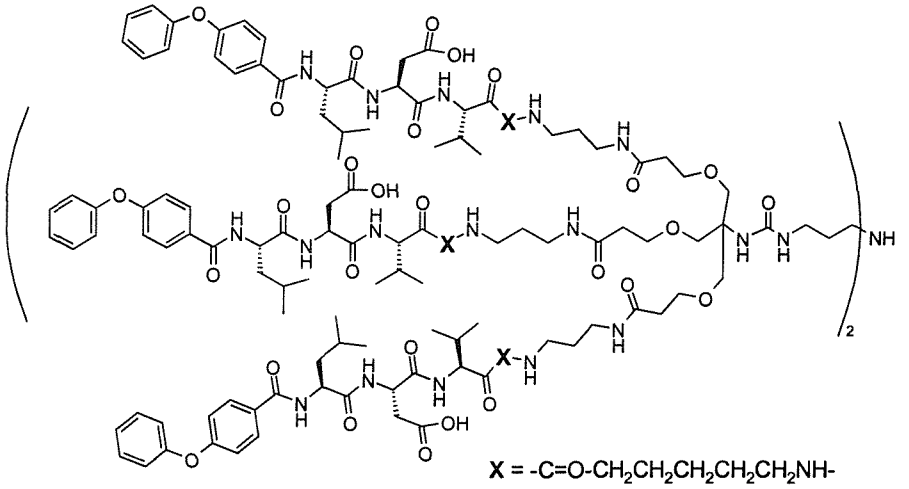
$NHCH_2CH_2C\underline{H}_2$); 2.87 (6H, dd, J_{AX} 17, J_{AB} 6, C$\underline{H}$H Asp); 2.64 (6H, dd, J_{BX} 17, J_{BA} 6, CH$\underline{H}$ Asp); 2.44-2.37 (12H, m, $COC\underline{H}_2CH_2$); 2.36-2.28 (24H, m, $COC\underline{H}_2CH_2NH$, β Ala); 2.07-1.98 (6H, m, $C\underline{H}C(CH_3)_3$, Val); 1.86-1.73 (10H, m, $CH_2C\underline{H}_2CH_2$ Gen [0.0], C$\underline{H}$, Leu); 1.68-1.57 (18H, m, $CH_2C\underline{H}_2CH_2$, C$\underline{H}$, Leu); 1.20-0.84 (72H, m, CH_3 Leu, $\underline{C}H_3$, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 173.31, 172.40, 170.84, 170.75, 170.58, 166.12, ($\underline{C}O$); 159.90 (Ar$\underline{C}^4$); 156.20 (*Ar*$\underline{C}^1$); 130.66 (*Ar*$\underline{C}^{3,\ 5}$H); 130.23 (Ar$\underline{C}^{2,\ 6}$H); 129.27 (Ar$\underline{C}^1$); 124.65 (*Ar*$\underline{C}^4$H); 119.78 (*Ar*$\underline{C}^{2,\ 6}$H); 117.82 (Ar$\underline{C}^{3,\ 5}$H); 67.84 (Ar$\underline{C}H_2OCH_2$); 65.34 ($ArCH_2O\underline{C}H_2$); 58.00 ($\underline{C}$H, Val); 52.26 ($\underline{C}$H, Leu); 49.91 ($\underline{C}$H, Asp); 36.78, 36.52 ($NH\underline{C}H_2CH_2\underline{C}H_2$); 35.91 ($\underline{C}H_2$, Leu); 35.83 ($COCH_2\underline{C}H_2NH$); 35.75 ($\underline{C}H_2$, Asp); 35.65 ($CO\underline{C}H_2CH_2NH$, $CCH_2OCH_2\underline{C}H_2$); 31.11; ($\underline{C}H(CH_3)_3$, Val); 29.64 ($NHCH_2\underline{C}H_2CH_2$); 24.85 ($\underline{C}H(CH_3)_3$, Leu); 23.47, 21.83 ($\underline{C}H_3$, Leu); 19.51 18.17 ($\underline{C}H_3$, Val).

m/z (MALDI-TOF): 5187.78 (M^+).

Resin (loading, 0.66 mmol/g, 300 mg) afforded 150 mg of the title compound in 14% yield.

Synthesis of 4-phenoxy-benzyl-LDV∈Ahx- Gen [1.0] AB_3 type dendrimer conjugate (46).



δ_H (400 MHz, $(CD_3)_2SO$): 8.53 (6H, d, *J* 8, N$\underline{H}$, Leu); 8.50 (6H, d, *J* 7, N$\underline{H}$, Asp); 8.04 (12H, d, *J* 8, Ar$C^{2,\ 6}\underline{H}$); 8.01-7.91 (12H, m, $N\underline{H}CH_2CH_2CH_2N\underline{H}$); 7.56-7.48 (12H, m, *Ar*$C^{3,\ 5}\underline{H}$); 7.31 (6H, t, *J* 7, *Ar*$C^4\underline{H}$); 7.18 (12H, d, *J* 8, Ar$C^{3,\ 5}\underline{H}$); 7.14 (12H, d, *J* 8, *Ar*$C^{1,\ 5}\underline{H}$); 4.76-4.67 (6H, m, C$\underline{H}$, Asp); 4.65-4.57 (6H, m, C$\underline{H}$, Leu); 4.22-4.14 (6H, m, C$\underline{H}$, Val); 3.70-3.65 (24H, m, $OCH_2\underline{C}H_2CO$, $\underline{C}H_2OCH_2CO$); 3.23-3.03 (40H, m, $NHC\underline{H}_2CH_2C\underline{H}_2NH$ Gen [1.0], $NHCH_2CH_2CH_2$ Gen [0.0], ($(CH_2)_4C\underline{H}_2NH$); 3.02-2.93

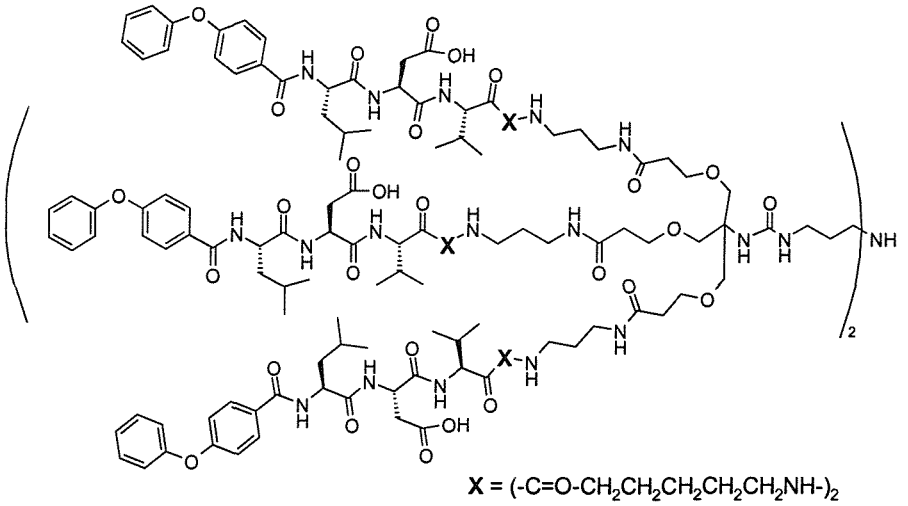
(4H, m, $NHCH_2CH_2CH_2$ Gen [0.0]); 2.87 (6H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.64 (dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.44-2.83 (12H, m, $COCH_2CH_2$); 2.28-2.13 (12H, m, CH_2); 2.09-1.98 (6H, C<u>H</u>, Val); 1.86-1.74 (16H, m, $CH_2CH_2CH_2$ Gen [0.0], CH_2, Leu); 1.68-1.55 (28H, m, $NHCH_2CH_2CH_2NH$, $CH_2CH_2CH_2$, CH_2, Leu); 1.53-1.43 (12H, m, CH_2); 1.38-1.28 (12H, m, CH_2); 1.04-0.94 (36H, m, CH_3, Leu); 0.92-0.84 (36H, m, CH_3, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 173.09, 172.53, 172.53, 172.39, 170.65, 170.59, 170.51 (CO); 159.90 (ArC^4); 156.20 (*Ar*C^1); 130.66, 130.23 (ArC^{2,6}H); 129.27 (ArC^1); 124.65, 119.78, 117.83 (ArCH); 69.75, 67.84 (CH_2); 58.05, 52.29, 49.94 (CH); 40.87, 38.82, 36.85, 36.76, 36.53, 35.83 (CH_2); 31.13 (CH); 29.76, 29.22, 26.55, 25.46 (CH_2); 24.84 (CH); 23.46, 21.83 (CH_3, Leu); 19.56, 18.17 (CH_3, Val).

m/z (MALDI-TOF): 5014.26 (M^+ 100%).

Resin (loading, 0.66 mmol/g, 240 mg) afforded 85 mg of the title compound in 10% yield.

4-phenoxy-benzyl-LDV∈Ahx∈Ahx-Gen [1.0] AB_3 type dendrimer conjugate (47).



δ_H (400 MHz, $(CD_3)_2SO$): 8.53 (6H, d, *J* 8, N<u>H</u>, Leu); 8.50 (6H, d, *J* 8, N<u>H</u>, Asp); 8.04 (12H, d, *J* 9, ArC^{2,6}<u>H</u>); 8.01-7.91 (12H, m, N<u>H</u>$CH_2CH_2CH_2$N<u>H</u>); 7.56-7.48 (12H, m, *Ar*C^{3,5}<u>H</u>); 7.31 (6H, t, *J* 7, *Ar*C^4<u>H</u>); 7.18 (12H, d, *J* 9, ArC^{3,5}H); 7.14 (12H, d, *J* 9, *Ar*C^{2,6}<u>H</u>); 4.76-4.67 (6H, m, C<u>H</u>, Asp); 4.65-4.57 (6H, m, C<u>H</u>, Leu); 4.22-4.14 (6H, m, C<u>H</u>, Val); 3.7-3.65 (24H, m, OCH_2CH_2CO, CH_2OCH_2CO); 3.23-3.02 (50H, $NHCH_2CH_2CH_2NH$ Gen [1.0], $NHCH_2CH_2CH_2$ Gen [0.0], $(CH_2)_4CH_2NH$); 3.02-2.93 (4H, m, $NHCH_2CH_2CH_2$ Gen [0.0]); 2.87 (6H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.64 (6H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.44-2.83 (12H, m, $COCH_2CH_2$); 2.20-2.10 (24H, m,

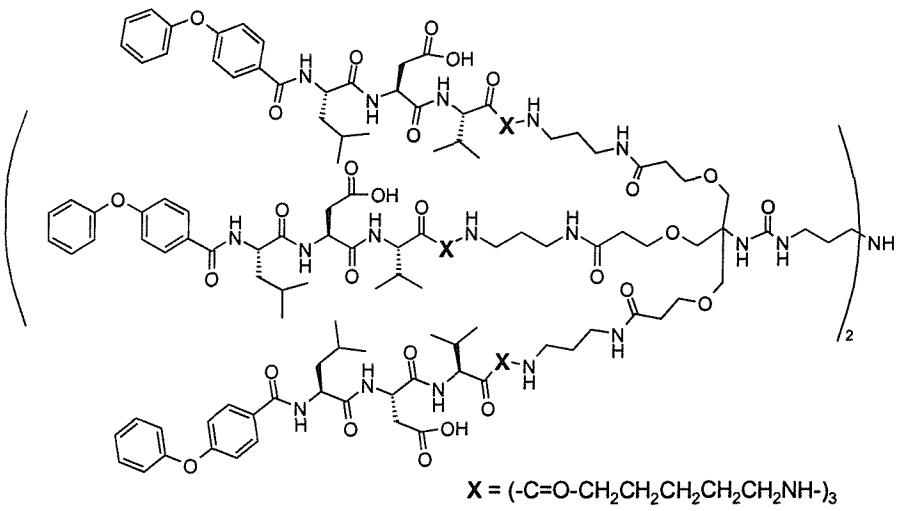
$C\underline{H}_2$); 2.09-1.98 (6H, C<u>H</u>, Val); 1.86-1.74 (16H, m, $CH_2C\underline{H}_2CH_2$ Gen [0.0], $C\underline{H}_2$ Leu); 1.69-1.53 (56H, $NHCH_2C\underline{H}_2CH_2NH$, $CH_2C\underline{H}_2CH_2$, $C\underline{H}_2$, Leu); 1.53-1.43 (24H, m, $C\underline{H}_2$); 1.38-1.28 (24H, m, $C\underline{H}_2$); 1.04-0.94 (36H, m, $C\underline{H}_3$, Leu); 0.92-0.84 (36H, m, $C\underline{H}_3$, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 174.65, 174.11, 173.95, 173.88, 172.21, 172.16, 172.09, 167.71 (<u>C</u>O); 161.46 (Ar<u>C</u>^{4}); 157.76 (*Ar*C^1); 132.2 (*Ar*<u>C</u>$^{2,\ 4}$H); 131.80 (Ar<u>C</u>$^{3,\ 5}$H); 130.84 (Ar<u>C</u>^{5}); 126.22, 121.35, 119.39 (<u>C</u>H); 69.73, 67.84 ($\underline{C}H_2$); 59.62, 53.86, 51.51 (<u>C</u>H); 42.44, 40.35, 38.41, 38.31, 38.10, 37.41 ($\underline{C}H_2$); 31.12 (<u>C</u>H); 29.77, 29.42, 29.21, 26.55, 25.48 ($\underline{C}H_2$); 24.84 (<u>C</u>H); 23.46, 21.82 ($\underline{C}H_3$, Leu); 19.56, 18.17 ($\underline{C}H_3$, Val).

m/z (MALDI-TOF): 5687.00 (calc) 5685.72 (found).

Resin (loading, 0.61 mmol/g, 245mg) afforded 102 mg of the title compound in 12% yield.

4-phenoxy-benzyl-LDV∈Ahx∈Ahx∈Ahx-Gen [1.0] AB_3 type dendrimer conjugate (48).



δ_H (400 MHz, $(CD_3)_2SO$): 8.53 (6H, d, *J* 8, N<u>H</u>, Leu); 8.50 (6H, d, *J* 8, N<u>H</u>, Asp); 8.04 (12H, d, *J* 9, ArC$^{2,\ 6}$<u>H</u>): 8.01-7.91 (12H, m, $N\underline{H}CH_2CH_2CH_2N\underline{H}$); 7.56-7.48 (12H, m, *Ar*C$^{3,\ 5}$<u>H</u>); 7.31 (6H, t, *J* 7, *Ar*C^4<u>H</u>); 7.18 (12H, d, *J* 9, ArC$^{3,\ 5}$<u>H</u>); 7.14 (12H, d, *J* 9, *Ar*C$^{2,\ 6}$<u>H</u>); 4.76-4.67 (6H, m, C<u>H</u>, Asp); 4.65-4.57 (6H, m, C<u>H</u>, Leu); 4.22-4.14 (6H, m, C<u>H</u>, Val); 3.70-3.65 (24H, m, $OC\underline{H}_2CH_2CO$, $C\underline{H}_2OCH_2CO$); 3.23-3.02 (40H, $C\underline{H}_2NH$); 3.02-2.93 (4H, m, $NHC\underline{H}_2$); 2.87 (6H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.64 (6H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.44-2.83 (12H, m, $COCH_2C\underline{H}_2$); 2.20-2.10 (36H, m, $C\underline{H}_2$); 2.09-1.98 (C<u>H</u>, Val); 1.86-1.74 (16H, m, $CH_2C\underline{H}_2CH_2$, $C\underline{H}_2$, Leu); 1.70-1.50 (58H, m,

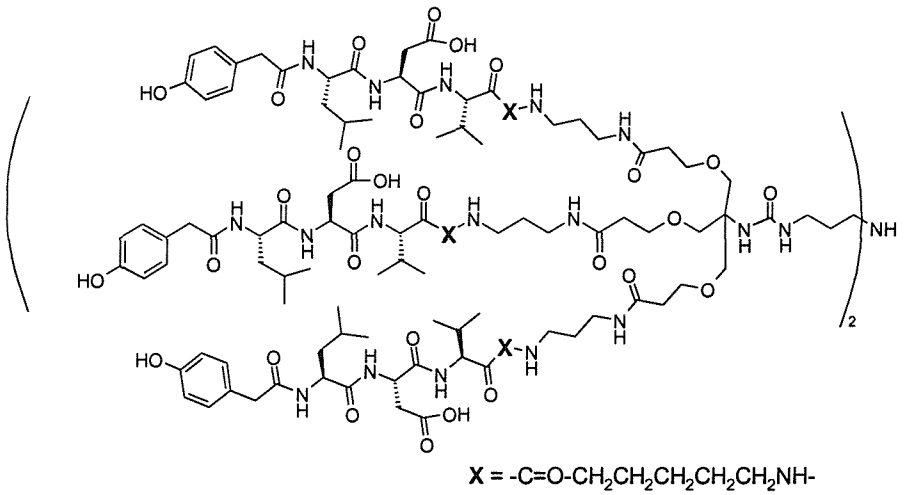
$NHCH_2C\underline{H}_2CH_2NH$, $CH_2C\underline{H}_2CH_2$, C<u>H</u>, Leu); 1.49-1.39 (44H, m, C<u>H</u>$_2$); 1.35-1.22 (44H, m, C<u>H</u>$_2$); 1.04-0.94 (36H, m, C<u>H</u>$_3$, Leu); 0.92-0.84 (36H, m, C<u>H</u>$_3$, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 173.06, 172.52, 172.36, 172.28, 170.62, 170.57, 170.50, 166.12 (<u>C</u>O); 159.88 (Ar<u>C</u>^{4}); 156.18 (*Ar*<u>C</u>^{1}); 130.64, 130.21, 132.23, 131.80 (Ar<u>C</u>H); 129.25 (Ar<u>C</u>^{1}); 124.62, 119.76, 117.80 (<u>C</u>H); 67.82, 65.33 (<u>C</u>H$_2$); 58.03, 52.27, 49.92 (<u>C</u>H); 40.87, 38.78, 36.85, 36.75, 35.84 (<u>C</u>H$_2$); 31.12 (<u>C</u>H$(CH_3)_2$, Val); 29.77, 29.42, 29.21, 26.60, 25.50 (<u>C</u>H$_2$); 24.84 (<u>C</u>H$(CH_3)_2$, Leu); 23.46, 21.82, 19.57, 18.16 (<u>C</u>H$_3$, Val, Leu).

m/z (MALDI-TOF): 6365.00 (calc) 6371.95 (found).

Resin (loading, 0.58 mmol/g, 250 mg) afforded 100 mg of the title compound in 10% yield.

Synthesis of (4-hydroxy-phenyl)-acetyl-LDV∈Ahx-Gen [1.0] AB_3 type dendrimer conjugate (49).



δ_H (400 MHz, $(CD_3)_2SO$): 8.48 (6H, d, *J* 8, N<u>H</u>, Asp); 8.21 (6H, d, *J* 8, N<u>H</u>, Leu); 8.00-7.90 (12H, br m, $N\underline{H}CH_2CH_2CH_2N\underline{H}$); 7.89-7.81 (4H, br m, N<u>H</u> Gen [0.0]); 7.49 (6H, d, *J* 9 N<u>H</u> Val); 7.13 (12H, d, *J* 9, Ar$C^{2,\ 6}$<u>H</u>); 6.77 (12H, d, *J* 9, Ar$C^{3,\ 5}$<u>H</u>); 4.70-4.63 (6H, m, C<u>H</u>, Asp); 4.45-4.35 (6H, m, C<u>H</u>, Asp); 4.20-4.12 (6H, m, C<u>H</u>, Val); 3.80-3.65 (24H, m, $OCH_2C\underline{H}_2CO$, $OC\underline{H}_2CH_2CO$); 3.22-3.03 (40H, $NHC\underline{H}_2$); 3.04-2.92 (4H, m, $NHCH_2CH_2C\underline{H}_2$ Gen [0.0]); 2.84 (6H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.62 (6H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.45-2.35 (12H, m, $COC\underline{H}_2CH_2$); 2.19-2.10 (20H, m, C<u>H</u>$_2$); 2.05-1.95 (6H, m, C<u>H</u>$(CH_3)_2$, Val); 1.84-1.73 (4H, m, $CH_2C\underline{H}_2NH$ Gen [0.0]); 1.70-1.43

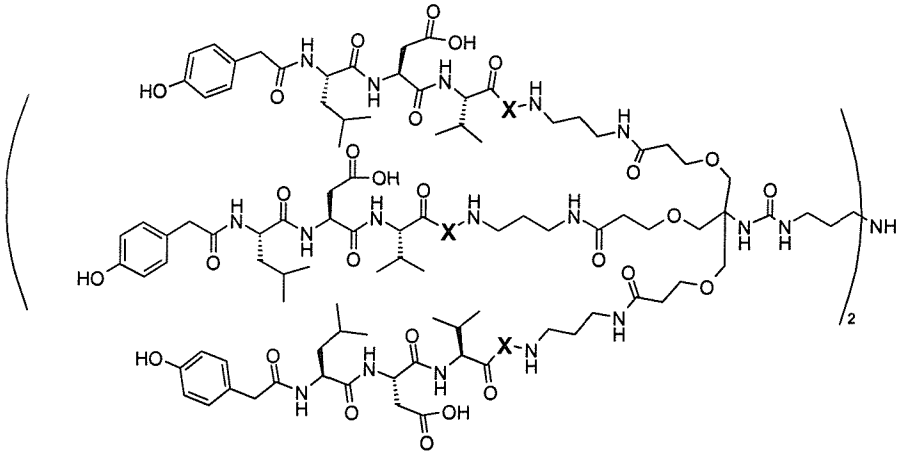
(58H, $CH_2C\underline{H}_2CH_2$ Gen [0.0], [1.0], $CH_2C\underline{H}_2CH_2CH_2C\underline{H}_2$, $C\underline{H}_2$ Leu, C<u>H</u>, Leu); 1.39-1.30 (12H, m, $CH_2CH_2C\underline{H}_2CH_2C\underline{H}_2$); 0.95-0.75 (72H, m, $C\underline{H}_3$, Leu, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 171.79, 171.40, 171.15, 169.93, 169.49, 169.38 (<u>C</u>O); 155.10 (Ar<u>C</u>^{4}); 129.17 (Ar<u>C</u>$^{2,\ 6}$H); 125.76 (Ar<u>C</u>^{1}); 114.22 (Ar<u>C</u>$^{3,\ 5}$H); 69.09, 68.61 ($C\underline{C}H_2OCH_2$); 66.70 ($CCH_2O\underline{C}H_2$); 55.90 (<u>C</u>H, Val); 50.20 (<u>C</u>H, Leu); 48.78 (<u>C</u>H, Asp); 37.67 35.71 35.61 34.68 ($NH\underline{C}H_2$, $\underline{C}H_2$, Asp, $\underline{C}H_2$, Leu); 30.01 (<u>C</u>H, Val); 28.61 28.07 25.40 24.31 ($\underline{C}H_2$); 23.45 (<u>C</u>H, Leu); 22.26 20.84 ($\underline{C}H_3$, Leu); 18.40 17.01 ($\underline{C}H_3$, Val).

m/z (MALDI-TOF): 4637.00 (calc) 4641.15 (found).

Resin (loading, 0.69 mmol/g, 100 mg) afforded 39 mg of the title compound in 12% yield.

Synthesis of (4-hydroxy-phenyl)-acetyl-LDV∈Ahx∈Ahx-Gen [1.0] AB_3 type dendrimer conjugate (50).



X = (-C=O-$CH_2CH_2CH_2CH_2CH_2NH$-)$_2$

δ_H (400 MHz, $(CD_3)_2SO$): 9.30 (6H, br s, O<u>H</u>); 8.48 (6H, d, *J* 8, N<u>H</u>, Asp); 8.21 (6H, d, *J* 8, N<u>H</u>, Leu); 8.0-7.90 (24H, br m, $N\underline{H}CH_2CH_2CH_2N\underline{H}$); 7.89-7.81 (4H, br m, N<u>H</u> Gen [0.0]); 7.49 (6H, *J* 9, N<u>H</u>, Val); 7.13 (12H, d, *J* 9, Ar$C^{2,\ 6}$<u>H</u>); 6.77 (12H, d, *J* 9, Ar$C^{3,\ 5}$<u>H</u>); 4.70-4.63 (6H, m, C<u>H</u>, Asp); 4.45-4.35 (6H, m, C<u>H</u>, Asp); 4.20-4.12 (6H, m, C<u>H</u>, Val); 3.80-3.65 (24H, m, $OC\underline{H}_2CH_2CO$, $OCH_2C\underline{H}_2CO$); 3.22-3.03 (56H, m, $C\underline{H}_2NH$); 2.84 (6H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.62 (6H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.45-2.35 (12H, m, $COC\underline{H}_2CH_2$); 2.20-2.08 (24H, m, $(CH_2)_4C\underline{H}_2CO$); 2.05-1.95 (6H, m,

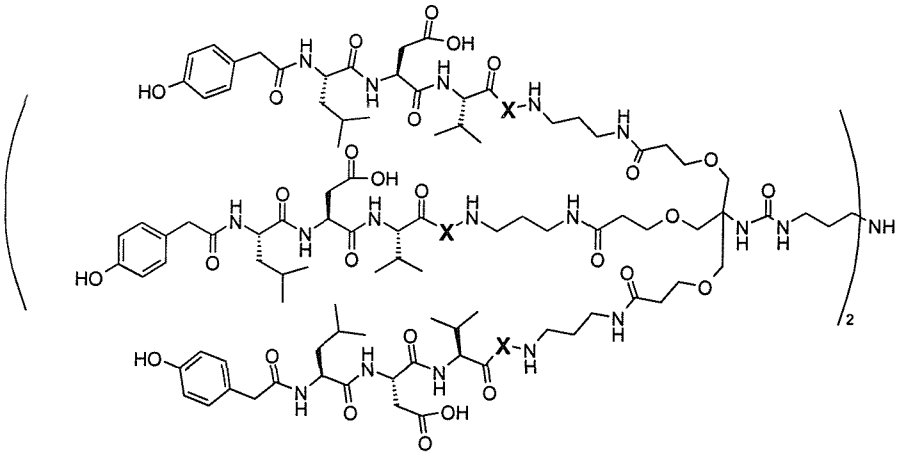
C<u>H</u>(CH$_3$)$_2$, Val); 1.80-1.40 (82H, CH$_2$C<u>H$_2$</u>CH$_2$, CH$_2$C<u>H$_2$</u>CH$_2$CH$_2$C<u>H$_2$</u>, C<u>H$_2$</u>, Leu, C<u>H</u>, Leu); 1.39-1.30 (CH$_2$CH$_2$C<u>H$_2$</u>CH$_2$CH$_2$); 0.95-0.75 (72H, m, C<u>H$_3$</u>, Leu).

δ_C (100 MHz, (CD$_3$)$_2$SO): 172.79, 172.40, 172.16, 170.93, 170.48, 170.39 (<u>C</u>O); 156.10 (Ar<u>C</u>^{4}); 130.18 (Ar<u>C</u>$^{2,\ 6}$H); 126.76 (Ar<u>C</u>^{1}); 115.22 (Ar<u>C</u>$^{3,\ 5}$H); 70.09, 69.81 (C<u>C</u>H$_2$OCH$_2$); 67.69 (CCH$_2$O<u>C</u>H$_2$); 57.90 (<u>C</u>H, Val); 51.19 (<u>C</u>H, Leu); 49.79 (<u>C</u>H, Asp); 41.49 (<u>C</u>); 38.64, 36.70, 36.60, 35.67 (NH<u>C</u>H$_2$, <u>C</u>H$_2$, Asp, <u>C</u>H$_2$, Leu); 29.27 (<u>C</u>H(CH$_3$)$_2$, Val); 29.06, 26.46, 26.39, 25.35 (<u>C</u>H$_2$); 24.44 (<u>C</u>H(CH$_3$)$_2$, Leu); 23.26, 21.84 (<u>C</u>H$_3$, Leu); 19.41, 18.01 (<u>C</u>H$_3$,Val).

m/z (MALDI-TOF): 5321.25 (M$^+$ 100%).

Resin (loading, 0.65 mmol/g, 130 mg) afforded 58 mg of the title compound in 12% yield.

Synthesis of (4-hydroxy-phenyl)-acetyl-LDV∈Ahx∈Ahx∈Ahx-Gen [1.0] AB$_3$ type dendrimer conjugate (51).



X = (-C=O-CH$_2$CH$_2$CH$_2$CH$_2$CH$_2$NH-)$_3$

δ_H (400 MHz, (CD$_3$)$_2$SO): 9.30 (6H, br s, O<u>H</u>); 8.48 (6H, d, *J* 8, N<u>H</u>, Asp); 8.21 (6H, d, *J* 8, N<u>H</u>, Leu); 8.00-7.90 (24H, br m, N<u>H</u>CH$_2$CH$_2$CH$_2$N<u>H</u>); 7.89-7.81 (4H, br m, N<u>H</u> Gen [0.0]); 7.49 (6H, d, *J* 9, N<u>H</u>, Val); 7.13 (12H, d, *J* 9, ArC$^{2,\ 6}$<u>H</u>); 6.77 (12H, d, *J* 9 ArC$^{3,\ 5}$<u>H</u>); 4.70-4.63 (6H, m, C<u>H</u>, Asp); 4.45-4.35 (6H, m, C<u>H</u>, Asp); 4.20-4.12 (6H, m, C<u>H</u>, Val); 3.80-3.65 (24H, m, OC<u>H$_2$</u>CH$_2$CO, OCH$_2$C<u>H$_2$</u>CO); 3.25-2.94 (80H, m, NHC<u>H$_2$</u>); 2.84 (6H, dd, J_{AX} 17, J_{AB} 7, C<u>H</u>H, Asp); 2.62 (6H, dd, J_{BX} 17, J_{BA} 7, CH<u>H</u>, Asp); 2.45-2.35 (12H, m, COCH$_2$C<u>H$_2$</u>); 2.20-2.08 (36H, m, (CH$_2$)$_4$)C<u>H$_2$</u>CO); 2.07-1.95 (6H, m, C<u>H</u>(CH$_3$)$_2$, Val); 1.85-1.40 (106H, m, CH$_2$C<u>H$_2$</u>CH$_2$, CH$_2$C<u>H$_2$</u>CH$_2$C<u>H$_2$</u>CH$_2$, C<u>H$_2$</u>,

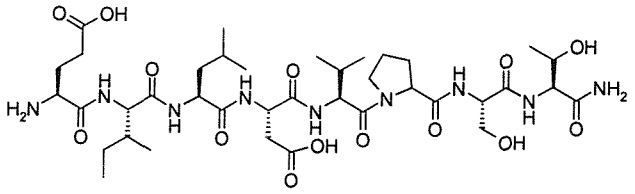
Leu, C<u>H</u>$(CH_3)_2$, Leu); 1.38-1.25 (36H, m, $CH_2CH_2C\underline{H}_2CH_2CH_2$): 0.95-0.75 (72H, m, C<u>H</u>$_3$, Leu, Val).

δ_C (100 MHz, $(CD_3)_2SO$): 172.75, 172.37, 172.13, 170.90, 170.44, 170.35 (<u>C</u>O); 156.05 (Ar<u>C</u>^{4}); 130.14 (Ar<u>C</u>$^{2,\ 6}$H); 126.72 (Ar<u>C</u>^{1}); 115.16 (Ar<u>C</u>$^{3,\ 5}$H); 67.65 ($\underline{C}H_2$); 57.87, 51.16, 49.74 (<u>C</u>H); 41.45, 38.60, 36.56, 35.64 ($\underline{C}H_2$); 31.16 (<u>C</u>H); 29.58, 29.23, 29.01, 26.41, 26.35, 25.32 ($\underline{C}H_2$); 24.41 (<u>C</u>H); 23.22, 21.80, 19.37, 17.97 (<u>C</u>H$_3$ Val, Leu).

m/z (MALDI-TOF): 5993.00 (calc) 5993.26 (found).

Resin (loading, 0.60 mmol/g, 100 mg) afforded 31 mg of the title compound in 8% yield.

Synthesis of EILDVPST-NH_2 (55).



Resin aminomethyl (2.66g) (loading 1.13 mmol/g) Novabiochem 200-400 mesh functionalised with rink linker was suspended in DCM for 30 mins. Fmoc amino acids (5eq), with reference to the theoretical number of free amine groups on the resin, were activated with HOBt, (5eq), DIC (5eq), in DCM. Valine (5 eq) as before was coupled using PyBOp (4.9 eq), HOBt (5 eq), DIPEA (10 eq). The activated mixture was then added to the resin and shaken for 2-3 hrs. Fmoc groups were removed by shaking two times 30 mins with 20% piperidine in DMF. The compound was then cleaved from the resin using two cycles of 90 % TFA with 1 drop of water as a scavenger for 2 hours. The cleavage mixture solvents were then removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of TFA, then precipitated in ether and centrifuged. The pellet produced were rinsed again with ether and finally dried *in vacuo*.

δ_H (400 MHz, $(CD_3)_2SO$): 8.60-7.50 (7H, m, N<u>H</u>); 7.30 (2H, s, N<u>H</u>$_2$, Glu); 7.15 (2H, s, N<u>H</u>$_2$, Thr); 4.70-3.50 (11H, m, C<u>H</u>, Asp, Val, Pro, Leu, Thr, CHC<u>H</u>(OH)(CH_3), Thr, C<u>H</u>$_2$ Ser, C<u>H</u>, Glu, C<u>H</u>$_2$, Pro); 2.77 (1H, dd, J_{AX} 17, J_{AB} 8, C<u>H</u>H, Asp); 2.60 (1H, m, CH<u>H</u>, Asp); 2.42-2.32 (1H, m, CHC<u>H</u>$(CH_2)(CH_3)$, Ileu); 2.21-1.42 (11H, m, C<u>H</u>$_2$, Leu, C<u>H</u>, Leu, C<u>H</u>$_2$, Glu, C<u>H</u>$_2$, Pro); 1.25-1.05 (5H, m, C<u>H</u>$_2$, Ileu, C<u>H</u>$_3$, Thr); 1.03-0.74 (18H, m, C<u>H</u>$_3$, Leu, Val, Ileu).

δ_C (100 MHz, $(CD_3)_2SO$): 173.79 172.58 172.46 172.34 172.04 170.84 170.67 170.53 169.90 168.30 (CO); 66.34 (CH, Thr); 61.88 (CH_2OH, Ser); 59.69 ($CHCH_3(OH)$, Thr); 58.44 (CH, Val); 57.49 (CH, Ileu); 55.75 (CH, Pro, Glu); 51.75 ($CHCH_2OH$, Ser); 51.05 (CH, Leu); 49.66 (CH, Asp); 47.50 (CH_2, Leu); 41.49 (CH_2, Leu); 36.84 ($CHCHCH_3(CH_2CH_3)$, Ileu); 35.98 (CH_2, Asp); 30.71 ($CH(CH_3)_2$, Val); 29.50, 29.40 ($CH_2CH_2CO_2H$, Glu); 27.14 ($CHCHCH_3(CH_2CH_3)$, Ileu); 24.90, 24.83 (CH_2, Pro); 24.56 ($CH(CH_3)_2$, Leu); 23.51, 21.64, 20.63, 19.64, 18.26, 15.72, 11.33 (CH_3).

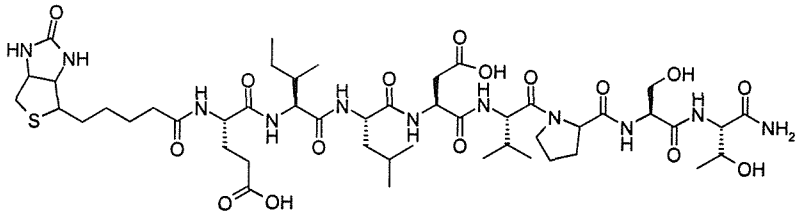
RP-HPLC: (Method 1, ELS) 6.71 mins.

m/z (MALDI-TOF): 872.52 (M^+ 100%) 894.54 ($M+Na^+$ 48%); 910.57 ($M+K^+$ 30%).

HR-m/z (ES+): ($M+H^+$) $C_{38}H_{65}N_9O_{14}$ 872.4730 (calc) 872.4723 (found).

Resin (loading, 0.44 mmol/g, 500 mg) afforded 102 mg of the title compound in 53% yield.

Synthesis of Biotinylated-EILDVPST-NH_2 (56).



NH_2-EILDVPST resin (1.26 g) was suspended in DMSO for 30 minutes before the addition of activated solution of d-biotin (sigma), DIC and HOBt (5 eq with respect to free amine) in DMSO and shaken overnight. The compound was then cleaved from the resin using two cycles of 90 % TFA with 1 drop of water as a scavenger for 2 hours. The cleavage mixture solvents were then removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of TFA, then precipitated in ether and centrifuged. The pellet produced were rinsed again with ether and finally dried *in vacuo*.

RP-HPLC: (Method 1, λ= 220) 7.20 mins.

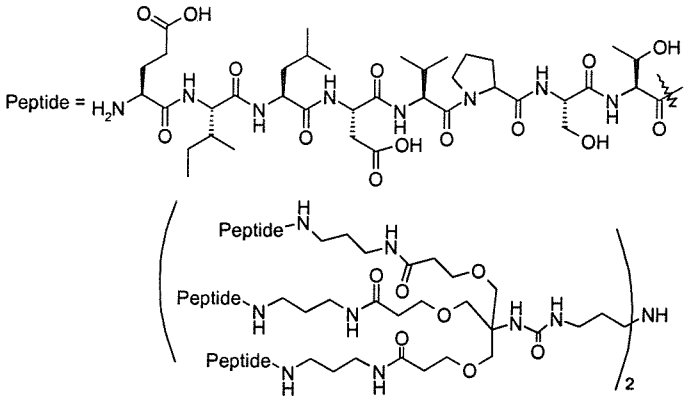
m/z (MALDI-TOF): 1098.87 ($M+H^+$ 100%).

Resin (loading, 0.40 mmol/g, 500 mg) afforded 132 mg of the title compound in 60% yield.

General procedure for the synthesis of EILDVPST-AB_3 type dendrimer conjugates (53), (54).

Dendrimer resin was suspended in DCM for 30 mins. Fmoc amino acids (5eq), with reference to the theoretical number of free amine groups on the resin, were activated with HOBt and DIC (5eq) with reference to the theoretical number of free amine groups on the resin in DCM. Valine (5 eq) as before was coupled using PyBOp, (4.9 eq), HOBt (5 eq), DIPEA (10 eq). The activated mixture was then added to the resin and shaken for 2-3 hrs. Fmoc groups were removed by shaking two times 30 mins with 20% piperidine in DMF. The compound was then cleaved from the resin using 90 % TFA with 1 drop of water as a scavenger for 2 hours. The cleavage mixture solvents were then removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of TFA, then precipitated in ether and centrifuged. The pellet produced were rinsed again with ether and finally dried *in vacuo.*

Synthesis of NH_2-EILDVPST-Gen [1.0]-AB3 type dendrimer conjugate (53).



δ_H (400 MHz, $(CD_3)_2SO$): 8.60-7.60 (54H, m, N<u>H</u>); 4.70-4.60 (6H, m, C<u>H</u>, Asp); 4.59-4.30 (12H, m, C<u>H</u>, Val, Pro, Leu, Thr); 4.20 (6H, m, CHC<u>H</u>(OH)(CH_3), Thr); 4.01-3.91 (12H, m, C<u>H_2</u>, Ser); 3.80-3.70 (18H, m, C<u>H</u>, Glu, CH_2, Pro); 3.69-3.50 (24H, m, $COCH_2CH_2OC\underline{H}_2$, $COCH_2C\underline{H}_2OCH_2$); 3.30-2.90 (32H, m, C<u>H_2</u>NH); 2.77 (6H, dd, J_{AX} 17, J_{AB} 8, C<u>H</u>H, Asp); 2.65-2.50 (6H, m, C<u>H</u>H, Asp); 2.46-2.27 (12H, m, $COC\underline{H}_2CH_2OCH_2$); 2.23-2.11 (6H, m, C<u>H</u>, Val); 2.10-1.42 (88H, m, $CH_2C\underline{H}_2CH_2$, C<u>H_2</u>, Leu, C<u>H</u>, Leu, C<u>H</u>, Ileu, C<u>H_2</u>, Glu, C<u>H_2</u>, Pro); 1.25-1.05 (30H, m, C<u>H_2</u>, Ileu, C<u>H_3</u>, Thr): 1.03-0.74 (108H, m, C<u>H_3</u>, Leu, Val, Ileu).

δ_C (100 MHz, DMSO-d_6): 173.80, 172.46, 172.03, 170.84, 170.76, 170.59, 170.24, 169.93, 168.31, 158.92, 158.57 (<u>C</u>O); 69.87 ($C\underline{C}H_2OCH_2$); 67.82 ($CCH_2O\underline{C}H_2$); 66.52

(CH, Thr); 61.88 ($\underline{C}H_2OH$, Ser); 59.68 ($\underline{C}H)OH(CH_3)$, Thr); 58.73 (CH, Val); 57.48 (CH, ILeu); 55.82 (CH, Pro, Glu); 55.63 (C); 51.74 (CH, Ser); 51.05 (CH Leu); 49.64 (CH, Asp); 47.54 ($\underline{C}H_2$, Pro); 41.46 ($\underline{C}H_2$, Leu); 37.02 ($CH\underline{C}HCH_3(CH_2CH_3)$, Ileu); 36.84, 36.65 ($\underline{C}H_2$, Pro); 36.03 ($\underline{C}H_2$); 30.70 ($\underline{C}H(CH_3)_2$, Val); 29.40 ($\underline{C}H_2\underline{C}H_2CO_2H$); 27.14 ($CHCHCH_3(\underline{C}H_2CH_3)$, Ileu); 24.83 ($\underline{C}H_2$, Pro); 24.54 ($\underline{C}H(CH_3)_2$, Leu); 23.49, 21.64, 20.47, 19.57, 18.33, 15.71, 11.31 ($\underline{C}H_3$).

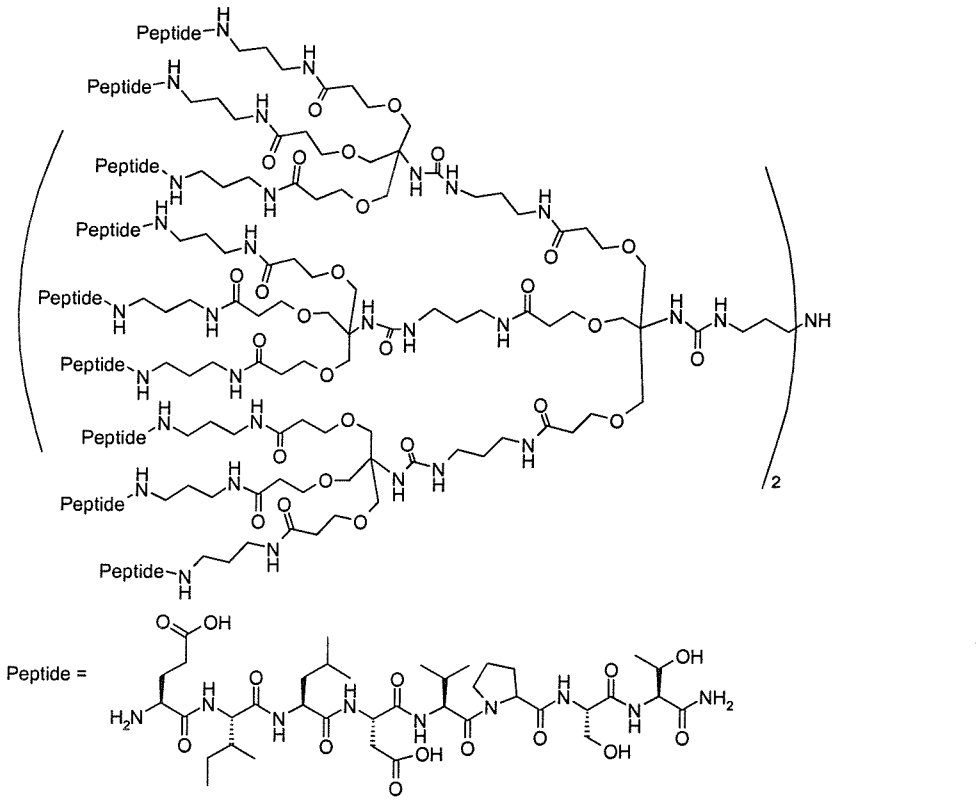
δ_C (100 MHz, $(CD_3)_2SO$); 174.69, 173.34, 172.92, 171.73, 171.65, 171.47, 171.13, 170.82, 169.21, 159.77, 159.42 ($\underline{C}O$); 70.62 ($C\underline{C}H_2OCH_2$); 68.73 ($CCH_2O\underline{C}H_2$); 67.41 (CH, Thr); 62.76 ($\underline{C}H_2OH$, Ser); 60.58 ($\underline{C}H)OH(CH_3)$, Thr); 59.89 (C); 59.63 (CH, Val); 58.38 (CH, Ileu); 56.71 (CH, Pro, Glu); 52.64 (CH, Ser); 51.95 (CH, Leu); 50.53 (CH, Asp); 48.45 ($\underline{C}H_2$, Pro); 42.37 ($\underline{C}H_2$, Leu); 37.02 ($CH\underline{C}HCH_3(CH_2CH_3)$, Ileu); 36.84 36.66 36.50, 36.01 ($\underline{C}H_2$); 31.60 ($\underline{C}H(CH_3)_2$, Val); 30.30 ($\underline{C}H_2\underline{C}H_2CO_2H$); 28.04 ($CHCHCH_3(\underline{C}H_2CH_3)$, Ileu); 25.72 ($\underline{C}H_2$, Pro); 25.44 ($\underline{C}H(CH_3)_2$, Leu); 24.39 22.54 21.39 20.46 19.22 16.60 12.21 ($\underline{C}H_3$).

RP-HPLC: (Method 2, ELS) 9.21 mins.

m/z (MALDI-TOF): 6330.41 ($M+6H^+$ 100%).

Resin (loading, 0.53 mmol/g, 700 mg) afforded 280 mg of the title compound in 21% yield.

Synthesis of NH_2-EILDVPST-Gen [2.0]-AB_3 type dendrimer conjugate (54).



δ_H (400 MHz, $(CD_3)_2SO$): 8.60-7.60 (162H, m, NH); 4.70-4.60 (18H, m, CH, Asp); 4.59-4.30 (72H, m, CH, Val, Pro, Leu, Thr); 4.20 (18H, m, $CHCH(OH)(CH_3)$, Thr); 4.01-3.91 (36H, m, CH_2, Ser); 3.80-3.70 (54H, m, CH, Glu, CH_2, Pro); 3.69-3.50 (96H, m, $COCH_2CH_2OCH_2$, $COCH_2CH_2OCH_2$); 3.30-2.90 (104H, m, CH_2NH); 2.77 (18H, dd, J_{AX} 17, J_{AB} 8, CHH, Asp); 2.65-2.50 (18H, m, CHH, Asp); 2.46-2.27 (48H, m, $COCH_2CH_2OCH_2$); 2.23-2.11 (18H, m, CH, Val); 2.10-1.42 (264H, m, $CH_2CH_2CH_2$, CH_2, Leu, CH, Leu, CH, Ileu, CH_2, Glu, CH_2, Pro); 1.25-1.05 (90H, m, CH_2, Ileu, CH_3, Thr): 1.03-0.74 (324H, m, CH_3, Leu, Val, Ileu).

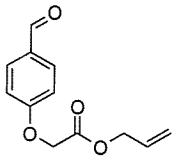
δ_C (100 MHz, $(CD_3)_2SO$): 173.80, 172.46, 172.03, 170.84, 170.76, 170.59, 170.24, 169.93, 168.31, 158.92, 158.57 (CO); 69.87 (CCH_2OCH_2); 67.82 (CCH_2OCH_2); 66.52 (CH, Thr); 61.88 (CH_2OH, Ser); 59.68 ($CH)OH(CH_3)$, Thr); 58.73 (CH, Val); 57.48 (CH, Ileu); 55.82 (CH, Pro, Glu); 55.63 (C); 51.74 (CH, Ser); 51.05 (CH, Leu); 49.64 (CH, Asp); 47.54 (CH_2, Pro); 41.46 (CH_2, Leu); 37.02 ($CHCHCH_3(CH_2CH_3)$, Ileu); 36.84, 36.65 (CH_2, Pro); 36.03 (CH_2); 30.70 ($CH(CH_3)_2$, Val); 29.40 ($CH_2CH_2CO_2H$); 27.14 ($CHCHCH_3(CH_2CH_3)$, Ileu); 24.83 (CH_2, Pro); 24.54 ($CH(CH_3)_2$, Leu); 23.49, 21.64, 20.47, 19.57, 18.33, 15.71, 11.31 (CH_3).

RP-HPLC: (Method 2, ELS) 9.39 mins.

m/z: Molecular ion was not observed.

Resin (loading, 0.77 mmol/g, 100 mg) afforded 80 mg of the title compound in 5% yield.

Synthesis of (4-Formyl-phenoxy)-acetic acid allyl ester (63).[187]



4-Hydroxybenzaldehyde, (18.36g, 150.44 mmol) K_2CO_3 (27g, 195.36 mmol), and KI (2.49g 15 mmol) were dissolved in acetonitrile (400 mL) and the mixture stirred for 15 minutes. To this was added, drop-wise, allyl chloroacetate (22.68 mL, 195.39 mmol). The mixture was allowed to reflux overnight and the salts removed after cooling by filtration and the solid washed with acetonitrile. Acetonitrile was removed *in vacuo* and the crude product was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate, (3:1) to afford the title compound 28.14g as a pale yellow oil in 85 % yield. Experimental data in accordance with the literature.[187]

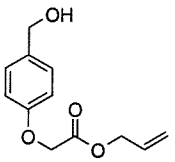
TLC: Ethylacetate/Hexane (3:1) $R_f = 0.42$

δ_H (300 MHz, $CDCl_3$): 9.90 (1H, s, C<u>H</u>O); 7.82 (2H, d, *J* 9, Ar$C^{3,}$ ^{5}<u>H</u>); 7.01 (2H, d, *J* 9, $C^{2,}$ ^{6}<u>H</u>); 5.92 (1H, ddt, *J* 17, 10, 6, $OCH_2C\underline{H}=CH_2$); 5.34 (1H, dd, *J* 17, 2 $OCH_2CH=C\underline{H}_{trans}$); 5.32-5.28 (1H, dd, *J* 10, 2, $OCH_2CH=C\underline{H}_{cis}$); 4.71 (2H, s, $OC\underline{H}_2CO$); 4.69 (2H, d, *J* 6, $C\underline{H}_2CH=CH_2$).

δ_C (75 MHz, $CDCl_3$): 190.86 (<u>C</u>HO); 167.90 (<u>C</u>O_2); 162.67 (Ar<u>C</u>^{1}); 132.10 (<u>C</u>^{3,} ^{5}H); 131.33 (<u>C</u>H=CH_2); 130.87 (Ar<u>C</u>^{4}); 119.50 (CH=<u>C</u>H_2); 115.02 (Ar<u>C</u>^{2,} ^{6}H); 66.21 (OCH_2CO); 65.21 ($CO_2CH_2CH=CH_2$).

IR (ν) cm^{-1}: 1757 (CO, ester); 1686 (CHO).

Synthesis of (4-Hydroxymethyl-phenoxy)-acetic acid allyl ester (64).[187]



(4-Formyl-phenoxy)-acetic acid allyl ester (16.96g, 77 mmol), and a trace of bromocresol green (5 mg) were dissolved in THF/Water (3:1, 320ml: 80 ml), and sodium cyanoborohydride (5.31g, 84 mmol) was added, this caused the solution to turn a deep blue. 2N HCL in THF/water was added drop-wise until the pH reached 4 (solution was a yellow colour). The solution was then stirred for a further 3 hours and

monitored by TLC. Drops of HCL were added periodically to maintain the pH at 4. When the reaction had reached completion the reaction mixture was saturated with NaCl, and the THF removed *in vacuo*. The aqueous phase was extracted with ethyl acetate (3 x 100 mL). The combined extracts were dried over magnesium sulphate and evaporated *in vacuo*. The crude product was purified by column chromatography on SiO_2 eluting with hexane/ethyl acetate (2:1) affording the title compound as a white solid 13.68g, in 80% yield. Experimental data in accordance with the literature.[187]

TLC: Hexane/Ethyl acetate (2:1) Rf = 0.20

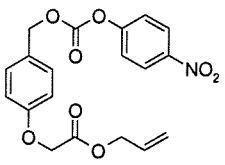
δ_H (300 MHz, $CDCl_3$): 7.28 (2H, d, *J* 9, $ArC^{3,5}\underline{H}$); 6.89 (2H, d, *J* 9, $ArC^{2,6}\underline{H}$); 5.93 (1H, ddt, *J* 17, 10, 6, $C\underline{H}=CH_2$); 5.34 (1H, dd, *J* 17, 2, $CH=C\underline{H}_{trans}$); 5.28 (1H, dd, *J* 10, 2 $CH=C\underline{H}_{cis}$); 4.70 (2H, dd, *J* 6, 2, $OC\underline{H}_2CH=CH_2$); 4.65 (2H, s, $OC\underline{H}_2CO$); 4.59 (2H, d, *J*, $ArC\underline{H}_2OH$); 1.95 (1H, s, $O\underline{H}$).

δ_C (75 MHz, $CDCl_3$): 168.79 ($\underline{C}O_2$); 157.46 ($Ar\underline{C}^1$); 134.46 ($CH_2\underline{C}H=CH_2$); 131.52 ($Ar\underline{C}^4$); 128.79 ($Ar\underline{C}^{3,5}$); 119.34 ($CH_2CH=\underline{C}H_2$); 114.85 ($Ar\underline{C}^{2,6}H$); 66.04 ($O\underline{C}H_2CO$); 65.50 ($O\underline{C}H_2CH=CH_2$); 64.96 ($\underline{C}H_2OH$).

MP: 31-35°C.

IR (ν) cm^{-1}: 3387 (OH) (s); 2932, 2902 (C=CH) (w); 1759 (CO) (s).

Synthesis of [4-(4-Nitro-phenylcarbonyloxymethyl)-phenoxy]-acetic acid allyl ester (65).[187]



(4-Hydroxymethyl-phenoxy)-acetic acid allyl ester (5.31g, 23.9 mmol) was dissolved in dichloromethane (55 mL), pyridine (2.12 mL, 26.23 mmol) and cooled in an ice bath. A solution of 4-nitrophenylchloroformate was added dropwise over 30 mins with stirring and cooling was maintained for a further 3 hours and stirred overnight. The reaction mixture was poured into water (100 mL) and the aqueous phase was extracted with DCM (3 x 50 mL) the organic extracts were combined, dried over $MgSO_4$ and the solvent removed *in vacuo*. The crude product was purified by column chromatography on SiO_2 eluting with hexane/ethylacetate (2:1) to afford the title compound 8.33g as a pale yellow oil in 90% yield. Experimental data in accordance with the literature.[187]

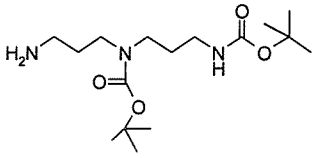
TLC: Hexane/Ethylacetate (2:1) Rf = 0.40

δ_H (300 MHz, $CDCl_3$): 8.27 (2H, d, *J* 9, *Ar*$C^{3,}$ ^{5}H); 7.43-7.35 (4H, m, *Ar*$C^{2,}$ ^{6}H, Ar$C^{3,}$ ^{5}H); 6.95 (2H, d, *J* 8, Ar$C^{2,6}$); 5.94 (1H, ddt, *J* 17, 10, 6, C<u>H</u>=CH_2); 5.36 (1H, dd, *J* 17, 2, OCH_2CH=C<u>H</u>$_{2trans}$); 5.29 (1H, dd, *J* 10, 2, OCH_2CH=C<u>H</u>$_{2cis}$) 5.24 (2H, s, Ar<u>C</u>H_2-O-CO); 4.72 (2H, d, *J* 6, <u>C</u>H_2CH=CH_2); 4.70 (2H, s, <u>C</u>H_2CO).

δ_C (75 MHz, $CDCl_3$): 168.55 (<u>C</u>O_2CH_2CH=CH_2); 158.52 (Ar<u>C</u>^{4}); 155.67 (*Ar*<u>C</u>^{1}); 152.60 (O<u>C</u>O_2); 145.51 (*Ar*<u>C</u>^{4}); 131.45 (Ar<u>C</u>^{4}); 130.89 (<u>C</u>H=CH_2); 127.61 (Ar<u>C</u>^{3,} ^{5}H); 125.44 (*Ar*<u>C</u>^{3,} ^{5}H); 121.95 (*Ar*<u>C</u>^{2,} ^{6}H); 119.44 (Ar<u>C</u>^{2,} ^{6}H); 115.00 (CH=<u>C</u>H_2); 70.83 (<u>C</u>CH_2O); 66.14 (O<u>C</u>H_2CO); 65.37 (O<u>C</u>H_2CH=CH_2).

IR (ν) cm^{-1}: 3117, 3089 (C=CH) (w); 1751, 1727 (CO) (s).

Synthesis of *N*^{5}-*N*^{9}-*bis*-(butyloxycarbonyl)-1,5,9 triazanonane (58).



To 1,5,9 triazanonane (6 mL, 42.80 mmol) was added dropwise a solution of ethyltrifluoroacetate (5.09 mL, 42.80 mmol) in methanol (36 mL) at -78°C for one hour and then allowed to warm to 0°C. After stirring for four hours a solution of Boc-anhydride (28g, 128.40 mmol mmol) in methanol (55 mL) was added drop-wise at 0°C. The reaction mixture was allowed to warm to room temperature and left stirring overnight. The solvent was removed *in vacuo* and methanol (200 mL) and water (50 mL) were added followed by potassium carbonate (11.20g). After stirring overnight at room temperature the methanol was evaporated *in vacuo* and the crude product was purified by column chromatography on SiO_2 eluting with DCM/MeOH/NH_4OH (70:10:1) to afford the title compound 4.25g, as a colourless yellow oil in 30% yield.

TLC: DCM/MeOH/NH_4OH (70:10:1) R_f = 0.30.

δ_H (300 MHz, $CDCl_3$): 3.18 (4H, m, <u>C</u>H_2N(Boc)<u>C</u>H_2); 3.04 (2H, m, <u>C</u>H_2NHBoc); 2.62 (2H, t, *J* 9, NH_2<u>C</u>H_2); 2.00 (2H, s, <u>N</u>H_2); 1.60 (4H, m, CH_2<u>C</u>H_2CH_2); 1.38 (9H, s, *tert*-<u>Bu</u>); 1.35 (9H, s, *tert*-<u>Bu</u>).

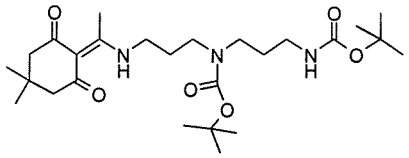
δ_C (75 MHz, $CDCl_3$): 156.43 (<u>C</u>O); 80.00 (<u>C</u> *tert*-Bu); 44.76, 44.04 (<u>C</u>H_2NH<u>C</u>H_2); 39.73 (<u>C</u>H_2NH-Boc); 39.33 (<u>C</u>H_2NH_2); 32.79 (NH_2CH_2<u>C</u>H_2); 31.52 (<u>C</u>H_2CH_2NH-Boc); 28.82 (<u>C</u>H_3 *tert*-<u>Bu</u>).

m/s (ES+): 332.30 (M+H 100%).

HR (ES+): (M+H$^+$) $C_{16}H_{33}N_3O_4$ 332.2550 (calc) 332.2543 (found).

IR: (υ) cm^{-1}: 2974 (NH) (m), 1571 (CO).

Synthesis of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene) ethyl- N^5N^9-*bis*-(butyloxycarbonyl)- 1,5,9 triazanonane (59).



To a solution of N^5-N^9-*bis*-(butyloxycarbonyl)-1,5,9 triazanonane (3.69g, 11.00 mmol) in DCM (25 mL) and triethylamine (1.84 mL, 1.2 eq) was added drop wise a solution of 2-acetyldimedone (2.23g, 1.1 eq) and the solution stirred overnight. Acetic acid (1 ml) in water (25 mL) was added. The organic layer was washed twice with a saturated solution of sodium carbonate then dried over sodium sulphate, filtered and solvent removed *in vacuo* to afford the title compound 5.23g a yellow foam in 96% yield.

TLC: (DCM/MeOH 9:1) $R_f = 0.67$.

δ_H (300 MHz, $CDCl_3$): 13.47 (1H, br s, N<u>H</u> Dde); 3.33 (2H, m, $CH_2CH_2C\underline{H}_2NH$-Boc); 3.21 (4H, m, $C\underline{H}_2N(Boc)C\underline{H}_2$); 3.03 (2H, m, $C\underline{H}_2NH$-Dde); 2.49 (3H, s, $C\underline{H}_3$-Dde); 2.29 (4H, s, $C\underline{H}_2$-Dde); 1.86 (2H, t, *J* 8, $CH_2C\underline{H}_2CH_2$); 1.52 (2H, m, $CH_2C\underline{H}_2CH_2$); 1.39 (18H, s, $C\underline{H}_3$-*tert*-Bu); 0.97 (6H, s, $C\underline{H}_3$-Dde).

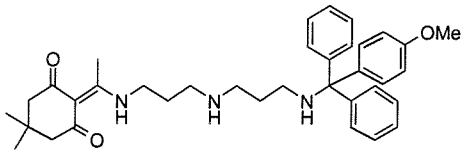
δ_C (75 MHz, $CDCl_3$): 198.00 (CO, Dde); 174.34 ($NH(CH_3)\underline{C}$=C-Dde); 173.70, 156.18 (<u>C</u>O, Boc); 108.04 (($\underline{C}$=CNH(CH_3), Dde); 80.25, 79.17 ($\underline{C}(CH_3)_3$); 53.60 ($\underline{C}H_2$, Dde); 44.35 ($\underline{C}H_2$); 41.13 ($\underline{C}H_2$); 37.50 ($\underline{C}H_2$); 30.24, 28.57 ($C(\underline{C}H_3)_2$, Dde); ($\underline{C}H_3$-*tert*-<u>Bu</u>); 28.38 ($\underline{C}H_3$, Dde); 18.05 ($\underline{C}(CH_3)_2$, Dde).

m/z (ES+): 496.50 (M+H$^+$ 35%); 518.50 (M+Na$^+$ 100%).

(HR-ES+): (M+H$^+$) $C_{26}H_{45}N_3O_6$ 496.3387 (calc) 496.3387 (found).

IR: (υ) cm^{-1}: 2958, 2932 (CH) (m); 1686 (CO) (s).

Synthesis of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-methoxy trityl-1,5,9 triazanonane (60).



N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene) ethyl- N^5N^9-*bis*-(butyloxycarbonyl)- 1,5,9 triazanonane (1.65g, 3.3 mmol) was dissolved in MeOH (10 mL) and acetyl chloride (4.73 mL, 20 eq) was added drop wise at 0°C and the resulting solution was allowed to warm up to room temperature. After stirring for 2hr the solvent was removed *in vacuo* and the crude product was dried under high vacuum. The resulting dry material was dissolved in DCM (15 mL) and triethylamine (1.37 mL, 3 eq) added at 0°C. The solution turned deep red on the drop-wise addition of Mmt-Cl (1 eq, 3.3 mmol) in DCM (5 mL) this then became yellow. After 3 hrs the salt formed was filtered off and solvent removed *in vacuo*. The crude product was purified by column chromatography on SiO_2 eluting with DCM/MeOH (9:1) to afford the title compound 0.73g a yellow powder in 40 % yield.

TLC: DCM/MeOH (9:1) $R_f = 0.32$.

δ_H (300 MHz, $CDCl_3$): 7.34 (4H, d, *J* 9 $ArC^{3,5}\underline{H}$, Mmt); 7.30-7.15 (6H, m, C<u>H</u>, Mmt); 7.10 (2H, t, *J* 8, $ArC^4\underline{H}$, Mmt); 6.74 (2H, d, *J* 9, $ArC^{3,5}\underline{H}$); 3.69 (3H, s, $C\underline{H}_3O$, Mmt); 3.41-3.40 (2H, m, $C\underline{H}_2NH$-Dde); 2.82-2.68 (4H, m, $CH_2C\underline{H}_2CH_2NH$-Dde); 2.44 (3H, s, $C\underline{H}_3$-Dde); 2.27 (4H, s, $C\underline{H}_2$-Dde); 2.22 (2H, t, *J* 6, $C\underline{H}_2$); 1.93 (2H, t, *J* 7, $C\underline{H}_2$); 1.76 (2H, t, *J* 7, $C\underline{H}_2$); 0.90 (6H, s, CH_3-Dde).

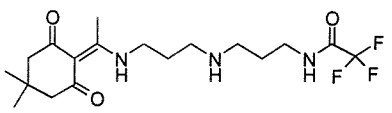
δ_C (75 MHz, $CDCl_3$): 199.00 (CO, Dde); 174.16 (C=<u>C</u>-NH(CH_3)-Dde); 158.40 (<u>C</u>-OMe); 146.29 (CH<u>C</u>-C-NH); 138.13 (OMeCCHCH<u>C</u>); 130.17, 128.88, 128.30, 128.15, 126.81, 113.61 (<u>C</u>H-Mmt); 108.41 (<u>C</u>=C); 70.99 (NH<u>C</u>-Mmt); 55.61 ($O\underline{C}H_3$); 53.26 ($\underline{C}H_2$); 48.57 ($\underline{C}H_2NH\underline{C}H_2$); 46.44 ($\underline{C}H_2$NH-Dde); 42.43 ($\underline{C}H_2$NH-Mmt); 41.41 ($CH_2\underline{C}H_2CH_2$); 30.48 ($C\underline{C}H_3)_2$-Dde); 29.07 (C=CNH-($\underline{C}H_3$)-Dde); 18.30 ($\underline{C}CH_3)_2$-Dde).

m/z (ES+): 568.40 ($M+H^+$ 100%).

(HR-ES+): ($M+Na^+$) $C_{36}H_{45}N_3O_3$ 590.3358 (calc) 590.3353 (found).

IR: (υ) cm^{-1}: 2948 (NH) (m); 2612, 2489 (C=CH) (w); 1570 (CO) (s).

Synthesis of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-1,5,9 triazanonane (63).



To a stirred solution of 1,5,9 triazanonane (5.85g, 44.58 mmol) in MeOH (30 mL) was added drop-wise a solution of ethyl trifluoroacetate (1eq, 5.30 mL, 44.58 mmol) in methanol (20 mL) at -78°C over 1 hr. The reaction mixture was allowed to warm to 0°C and stirred for 4 hours. The solvent was co-distilled with DCM to remove trace amounts of methanol. The crude product was then dissolved in DCM (50 mL) and 2-acetyldimedone (9.75g, 53.50 mmol) was added at room temperature and the mixture was stirred overnight. The solvent was then removed *in vacuo* and the crude product was purified by column chromatography on SiO_2 eluting with DCM/MeOH (9:1). To afford the title compound 8.88g as a yellow oil in 51% yield.

TLC: DCM/MeOH (9:1) Rf = 0.26.

δ_H (300 MHz, $CDCl_3$): 5.28 (1H, s, N<u>H</u>); 3.55-3.42 (4H, m, C<u>H</u>$_2$NH-TFA, C<u>H</u>$_2$NH-Dde); 2.81-2.60 (4H, m, C<u>H</u>$_2$NH); 2.53 (3H, s, C<u>H</u>$_3$); 2.32 (4H, s, C<u>H</u>$_2$-Dde); 1.90-1.70 (4H, m, $CH_2C\underline{H}_2CH_2$); 0.99 (6H, s, C<u>H</u>$_3$).

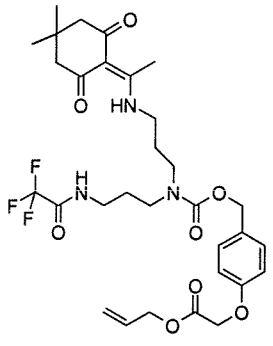
δ_C (100 MHz, $CDCl_3$): 198.31 (<u>C</u>O, Dde); 173.98 (C=<u>C</u>NH(CH_3)-Dde); 158.17, 157.80, 157.44, 157.07 (NH<u>C</u>OCF_3); 120.75, 117.89, 115.03, 112.17 (<u>C</u>F_3); 108.38 (<u>C</u>=CNH(CH_3)-Dde); 53.21 (<u>C</u>H_2-Dde); 48.35 (<u>C</u>H_2NH-Dde); 47.08, 46.92 ($CH_2\underline{C}H_2NH$); 41.65, 41.43 ($CH_2\underline{C}H_2CH_2$); 39.59 (<u>C</u>H_2NH-TFA); 30.44 (<u>C</u>(CH_3)$_2$-Dde); 29.63, 28.62 (<u>C</u>H_2); 28.60 ((<u>C</u>H_3)$_2$); 27.59 (<u>C</u>H_2); 18.22 (<u>C</u>H_3-=-Dde).

m/z (ES+): 392.10 ($M+H^+$ 100%). 460.20 ($M+3Na^+$ 20%).

(HR-ES+): ($M+H^+$) $C_{18}H_{28}N_3O_3F_3$ 392.2162 (calc); 392.2156 (found).

IR (υ) cm^{-1}: 2956 (NH) (m); 2868 (C=CH(CH_3) (m) 1561 (CO).

Synthesis of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyallylacetate)-1,5,9 triazanonane (66).



To a solution of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-1,5,9 triazanonane (1.93g, 4.93 mmol) and triethylamine (2.06 mL, 3 eq) in DMF (10 mL) 0°C was added [4-(4-Nitro-phenylcarbonyloxymethyl)-phenoxy]-acetic acid allyl ester (1.9g, 4.93 mmol) drop-wise and then allowed to stir overnight at room temperature. The solution was then extracted using H_20 (20 mL) and ethyl acetate (100 mL). Solvent was then removed *in vacuo* and the crude product was purified by column chromatography on SiO_2 eluting with ethylacetate/hexane (7:5) with 1% Et_3N. Affording title compound 1.45g as a colourless oil in 46 % yield.

TLC: DCM/MeOH (20:1) $R_f = 0.30$

δ_H (400 MHz, $CDCl_3$): 8.00 (1H, br s, NH-TFA); 7.29 (2H, d, *J* 9, $ArC^{3,5}H$); 6.88 (2H, d, *J* 9, $ArC^{2,6}H$); 6.08-5.83 (1H, ddt, *J* 17, 10, 6, $CH{=}CH_2$); 5.34 (1H, dd, *J* 17, 2, $OCH_2CH{=}CH_{trans}$); 5.28 (1H, dd, *J* 10, 2, $OCH_2CH{=}CH_{cis}$); 5.09 (2H, s, CCH_2O); 4.71 (2H, d, *J* 6, $CH_2{=}CHCH_2O$); 4.67 (2H, s, OCH_2CO); 3.5-3.23 (8H, m, CH_2); 2.50 (3H, s, CH_3); 2.36 (4H, s, CH_2-Dde); 2.00-1.80 (2H, m, CH_2); 1.75-1.65 (2H, m, CH_2); 1.03 (6H, s, CH_3).

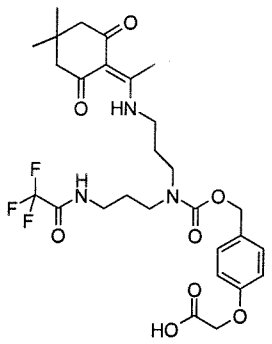
δ_C (100 MHz, $CDCl_3$): 198.42 (CO, Dde); 174.07 (C=C-NH-Dde); 168.82 (CH_2COOCH_2); 158.34 (CON); 157.93, 157.57 (CF_3); 131.79 (ArC^1); 130.56 ($ArC^{3,5}H$); 129.81 (ArC^4); 119.57 ($CH_2{=}CH$); 117.80 ($CH{=}CH_2$); 115.17 ($ArC^{2,6}H$); 108.40 ($C{=}(CH_3)C$-NH-Dde); 67.86 (OCH_2CO); 66.30 ($CH_2{=}CHCH_2O$): 65.66 (CCH_2O): 53.21 (CH_2, Dde); 44.28 (CH_2); 41.07 (CH_2); 36.38 (CH_2); 30.49 ($C(CH_3)_2$-Dde); ($C(CH_3)_2$-Dde); 18.27 (CH_3-=-Dde).

m/z (ES+): 640.40 ($M+H^+$ 100 %); 662.30 ($M+Na^+$ 35%).

(HR-ES+): ($M+Na^+$) $C_{31}H_{40}F_3N_3O_8$ 662.2665 (calc) 662.2659 (found).

IR (υ) cm^{-1}: 2953 (NH) (m); 2869 (C=CH) (w); 1758, 1718 (CO) (s).

Synthesis of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyacetic acid)-1,5,9 triazanonane (67).



To a solution of N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyallylacetate)-1,5,9 triazanonane (0.90g, 1.40 mmol) and pyrrolidine (0.11 mL, 1.40 mmol) in THF (10 mL) was added solid supported Pd (0) catalyst,[176] which was cut in 4 pieces. The reaction was then allowed to reflux overnight. The supported catalyst was filtered off and the solvent was removed. The crude compound was then re-dissolved in dioxane (5 mL) and water (5 mL) and acidified with a 1M solution of $KHSO_4$ until the solution became cloudy. The organics were then extracted from the water using ethylacetate (100 mL), washed with brine (50 mL), dried with anhydrous magnesium sulphate and the solvent removed *in vacuo*. The title compound 0.59g as thick yellow oil, which did not require any further purification in 70% yield.

TLC: DCM/MeOH (2:1) $R_f = 0.23$.

δ_H (400 MHz, $CDCl_3$): 8.0 (1H, br s, O<u>H</u>); 7.20 (2H, d, *J* 9, $ArC^{3,5}$<u>H</u>); 6.87 (2H, d, *J* 9, $ArC^{2,6}$<u>H</u>); 5.00 (2H, s, $CC\underline{H}_2O$); 4.58 (2H, s, $OC\underline{H}_2CO_2H$); 3.40-3.12 (6H, m, $C\underline{H}_2$); 2.30 (4H, s, $C\underline{H}_2$-Dde); 2.25 (3H, s, $C\underline{H}_3$); 1.85-1.70 (2H, m, $CH_2C\underline{H}_2CH_2$); 1.70-1.6 (2H, m, $CH_2C\underline{H}_2CH_2$); 0.95 (6H, s, $CC\underline{H}_3$-Dde).

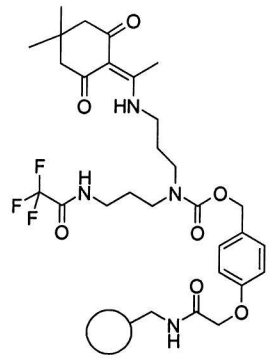
δ_C (100 MHz, $CDCl_3$): 198.90 (<u>C</u>O Dde); 174.43 (<u>C</u>O_2H); 171.14 (<u>C</u>O TFA, O-<u>C</u>O-N); 158.35 (<u>C</u>-OCH_2); 157.97 (C=<u>C</u>NH(CH_3); 157.61 (N<u>C</u>O-O); 130.59 ($ArC^{3,5}$<u>H</u>); 129.45 (ArC^4); 118.00 (<u>C</u>F_3); 114.95 ($ArC^{2,6}$<u>H</u>); 108.23 (<u>C</u>=C-Dde); 67.93 (O<u>C</u>H_2CO); 65.56 (<u>C</u>H_2CO); 52.87 (<u>C</u>H_2CO-Dde); 44.43, 44.23 (N<u>C</u>H_2); 41.30 (<u>C</u>H_2NH-Dde); 36.32 (<u>C</u>H_2NH-TFA); 30.60 (<u>C</u>(CH_3)$_2$-Dde); 30.18 (<u>C</u>H_2CH_2NH-TFA); 28.58 (C(<u>C</u>H_3)$_2$-Dde); 27.28 (<u>C</u>H_2CH_2NH-Dde); 18.33 (<u>C</u>H_3-C=C-Dde).

m/z (ES+): 600.40 ($M+H^+$ 45%); 622.30 ($M+Na^+$ 100%).

IR (υ) cm^{-1}: 2953 (NH) (m); 2869 (CH) (w); 1758, 1718, 1570 (CO) (s).

(HR-ES+): ($M+H^+$); $C_{28}H_{36}N_3O_8F_3$ 600.2533 (calc) 600.2527 (found).

Synthesis of resin bound N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (68).

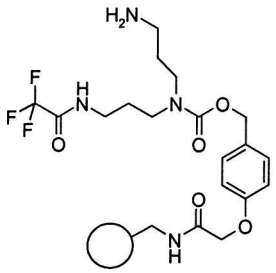


N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyacetic acid)-1,5,9 triazanonane in DCM (300 mg, 1.50 mmol, 3eq to free amine) was coupled to TentaGel resin (1.50g, 0.29 mmol/g) using standard coupling conditions DIC (0.23 mL, 1.50 mmol, 3 eq), HOBt (0.20g, 1.50 mmol, 3 eq) overnight at RT. The resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). Completion of the reaction was checked by qualitative ninhydrin test. The compound was cleaved from the resin with 90% TFA/DCM (5 mL), 1 drop of water as a scavenger.

RP-HPLC (Method 1, ELS): 6.93 mins.

m/z (ES+): 392.20 ($M+H^+$ 100%).

Synthesis of resin bound N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (69).



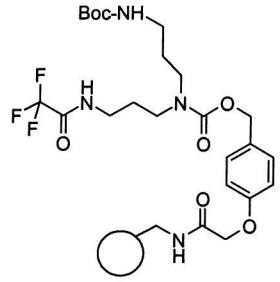
Resin bound N^1-(4, 4-dimethyl-2, 6-dioxocyclohexlidene)ethyl-N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane **(68)** (100 mg, 0.25 mmol/g) was suspended in DMF (5 mL) for 30 mins before the addition of 5% N_2H_4 in DMF (5 mL). The resin was then shaken for 90 mins at RT. After deprotection the resin was washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). The completion of the

reaction was checked by HPLC and qualitative ninhydrin test. The compound was cleaved from the resin with 90% TFA/DCM (5 mL), 1 drop of water as a scavenger.

RP-HPLC (Method 1, ELS): 2.21 mins.

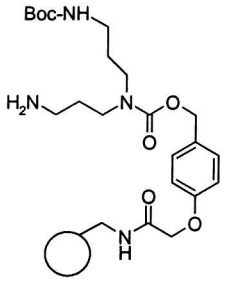
m/z (ES+): ($M+H^+$ 100%) 228.00.

Synthesis of resin bound N^1-Boc- N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (70).



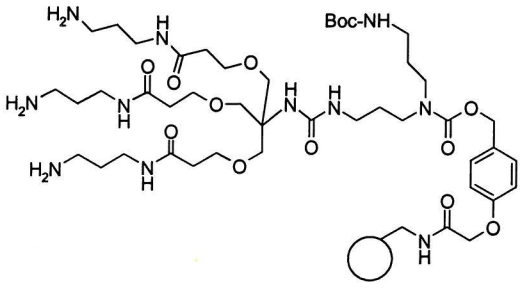
Resin bound N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (69) (100 mg, 0.26 mmol/g) was suspended in dioxane (5 mL) for 30 mins before addition of Boc_2O (16 mg, 3 eq free amine) DIPEA (3 μL, 1 eq) in dioxane (5 mL). The resin was then shaken overnight and the following day was washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). The completion of the reaction was checked and qualitative ninhydrin test.

Synthesis of resin bound N^1-Boc-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (71).



Resin bound N^1-Boc- N^9-trifluoroacetyl-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane **(70)** (400 mg, 0.25 mmol/g) was suspended in THF (5 mL) for 30 mins before addition of a 1:1 mixture of 1M KOH and (THF/MeOH (3:1)) (8 mL) the resin was then shaken for 2 hrs at RT on a rotating wheel. The completion of the reaction was checked by HPLC and qualitative ninhydrin test.

Synthesis of resin bound N^1-Boc- N^9-Gen [1.0] AB_3 type dendrimer-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (72).

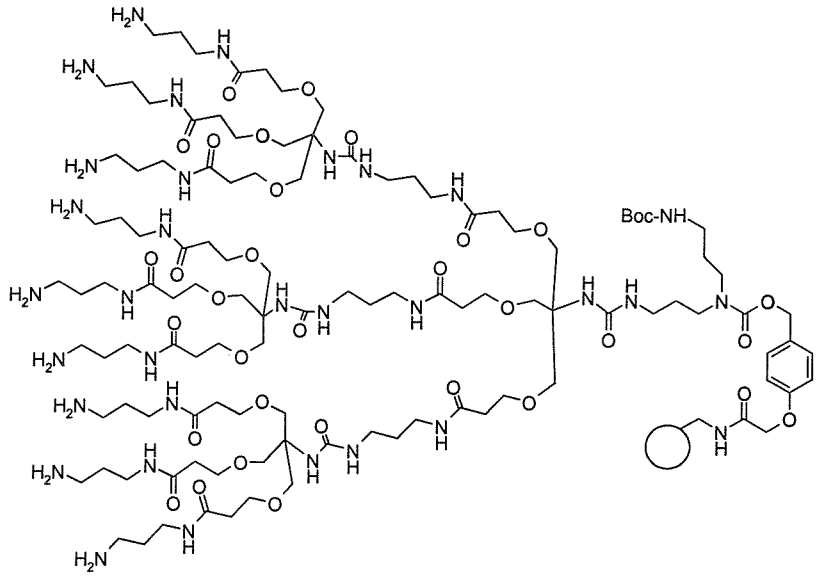


Resin bound N^1-Boc-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane **(71)** (100 mg, 0.26 mmol/g) was suspended in DCM (5 mL) for 30 mins before addition of a solution of 3-[2-Isocyanato-3-(2-methoxycarbonyl-ethoxy)-2-(2-methoxycarbonyl-ethoxymethyl)-propoxy]-propionic acid methyl ester (42 mg, 0.10 mmol, 4 eq to resin loading), DIPEA, (0.018 mL, 0.10 mmol, 5 eq to resin loading), DMAP (catalytic) in DCM (5 mL). The resin was shaken overnight at RT. The resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). Completion of the reaction was checked by qualitative ninhydrin test. Resin bound N^1-Boc- N^9-Gen [0.5] AB_3 type dendrimer-N^5-((4-hydroxymethylphenoxy)-allylacetate)oxycarbonyl norspermidine (200 mg) was suspended in DMSO (5 mL) for 30 mins before addition of a solution of 1, 3 diamino propane (2 mL, 23.2 mmol, 500 eq free amine) and DMSO (1 mL, 0.5 eq v/v). The resin was then shaken for 3 days at RT. Then washed with DMSO before the addition of a fresh solution of 1, 3 diaminopropane (2 mL, 23.20 mmol, 500 eq free amine and DMSO (1 mL, 0.5 eq v/v). After a further 3 days reaction the resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). Reaction completion was checked by taking 10 mg of resin and coupling (4-hydroxy-phenyl)-acetic acid with DIC HOBt (5 eq resin loading) for 2 hrs at RT. Completion of the reaction was checked by qualitative ninhydrin test. After washing as before the derivatised compound was cleaved from the resin with 90% TFA/DCM, 1 drop of water as a scavenger and analysed by RP-HPLC.

m/z (MALDI-TOF): 1065.83 ($M+H^+$), 1087.84 ($M+Na^+$).

IR (υ) cm^{-1}: 2863 (NH) (s); 1678 (CO) (m).

Synthesis of resin bound N^1-Boc- N^9-Gen [2.0] AB_3 type dendrimer-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane (73).

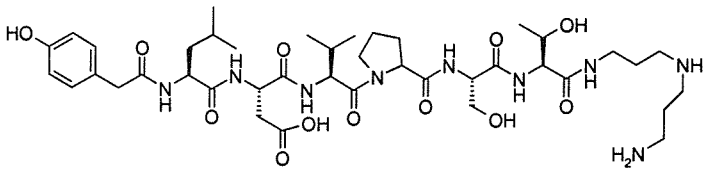


Resin bound N^1-Boc- N^9-Gen [1.0] AB_3 type dendrimer-N^5-((4-Benzyloxycarbonyl-1-oxyamide)-1,5,9 triazanonane **(72)** (250 mg, 0.23 mmol/g) was suspended in DCM (5 mL) for 30 mins before addition of a solution of 3-[2-Isocyanato-3-(2-methoxycarbonyl-ethoxy)-2-(2-methoxycarbonyl-ethoxymethyl)-propoxy]-propionic acid methyl ester (0.34g, 5 eq to resin loading), DIPEA (0.14, 5 eq to resin loading), DMAP (catalytic) in DCM (5 mL). The resin was shaken overnight at RT. The resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). Completion of the reaction was checked by qualitative ninhydrin test.Gen [1.5] AB_3 type dendrimer resin was suspended in DMSO (5 mL) for 30 mins before addition of a solution of 1, 3 diaminopropane (3.7 mL, 44.10 mmol, 250 eq reactive sites) and DMSO (2 mL, 0.5 eq v/v). The resin was then shaken for 4 days at RT. Then washed with DMSO before the addition of a fresh solution of 1, 3 diaminopropane (3.70 mL 44.10 mmol, 250 eq free amine) and DMSO (2 mL, 0.5 eq v/v). After a further 4 days reaction the resin was then washed with DCM, DMF, MeOH and ether, (each 3 x 10 mL). Reaction completion was checked by taking 10 mg of resin and coupling (4-hydroxy-phenyl)-acetic acid with DIC HOBt (5 eq resin loading) for 2 hrs at RT. Completion of the reaction was checked by qualitative ninhydrin test. After washing as before the derivatised compound was cleaved from the resin with 90% TFA/DCM, 1 drop of water as a scavenger and analysed by RP-HPLC. M/z: Molecular ion was not observed.

General method for the coupling of (4-Hydroxy-phenyl)-acetaldehyde-LDVPST to dendrimeric resin.

Generation [0.0], [1.0] and [2.0] AB_3 type dendrimer resins **(71)**, **(72)** and **(73)** (200 mg) were swollen for 30 minutes in DCM (5 mL). Fmoc amino acids (5 eq), with reference to the theoretical number of free amine groups on the resin, were activated with HOBt, (5 eq), DIC (5eq), in DCM. The activated mixture was then added to the resin and shaken for 2-3 hrs. Valine (5 eq) was coupled using PyBOp (4.90 eq), HOBt (5 eq), DIPEA (10 eq). Fmoc groups were removed by shaking two times for 30 mins with 20% piperidine in DMF. Final compounds were cleaved from the resin with 90 % TFA with 1 drop of water as a scavenger for 2 hours. The cleavage mixture solvents were then removed under vacuum and the crude compound was worked up by dissolving in the minimum amount of TFA, then precipitated in ether and centrifuged. The pellet produced were rinsed again with ether and finally dried *in vacuo.*

Synthesis of N^9-(4-hydroxy-phenyl)-acetic acid-LDVPST-1,5,9 triazanonane) (78).



δ_H (400 MHz, DMSO-d_6): 9.31 (2H, br s, NH_2); 8.66 (1H, br s, OH); 8.44 (1H, d, *J* 8, NH, Asp); 8.24 (1H, d, *J* 7, NH, Val); 8.20 (1H, d, *J* 8, NH, Leu); 7.98 (1H, br s, OH); 7.95-7.86 (1H, m, $NHCH_2$); 7.70-7.57 (2H, m, NH, Thr, Ser); 7.14 (2H, d, *J* 9, $ArC^{2,6}H$); 6.78 (2H, d, *J* 9, $ArC^{3,5}H$); 5.27 (1H, br s, OH); 5.40 (1H, br s, OH); 4.70-4.60 (1H, m, CH, Asp); 4.55-4.50 (1H, m, CH, Val); 4.45-4.35 (3H, m, CH, Ser, CH, Pro, CH, Leu); 4.25-4.15 (2H, m, $CHCH(OH)(CH_3)$, $CHCH(OH)(CH_3)$, Thr); 3.85-3.75 (2H, m, CH_2, Ser); 3.14-2.92 (6H, m, CH_2N); 2.80 (1H, dd, J_{AX} 17, J_{AB} 8, CHH, Asp);); 2.60-2.55 (3H, m, $COCH_2CCH$, CHH, Asp); 2.25-1.48 (12H, m, CH, Val, CH_2, CH, Leu, $CH_2CH_2CH_2$); 1.23-1.15 (3H, m, CH_3, Thr); 1.07-0.80 (12H, m, CH_3, Val, Leu).

δ_C (100 MHz, DMSO-d_6): 172.18, 170.95, 170.58 158.36, 156.25 (CO); 149.87 (ArC^4); 130.31 ($ArC^{2,6}H$); 126.93 (ArC^1); 115.37 ($ArC^{3,5}H$); 66.46 (CH, Thr); 61.78 (CH_2OH, Ser); 59.76 (CH, Val); 58.97 (CH, Thr); 55.91 (CH, ser); 55.74 (CH, Leu); 51.35 (CH, Pro); 49.71 (CH, Asp); 45.03 (CH_2NH); 44.47 (CH_2, Pro); 43.04 (CH_2, Leu); 41.67,

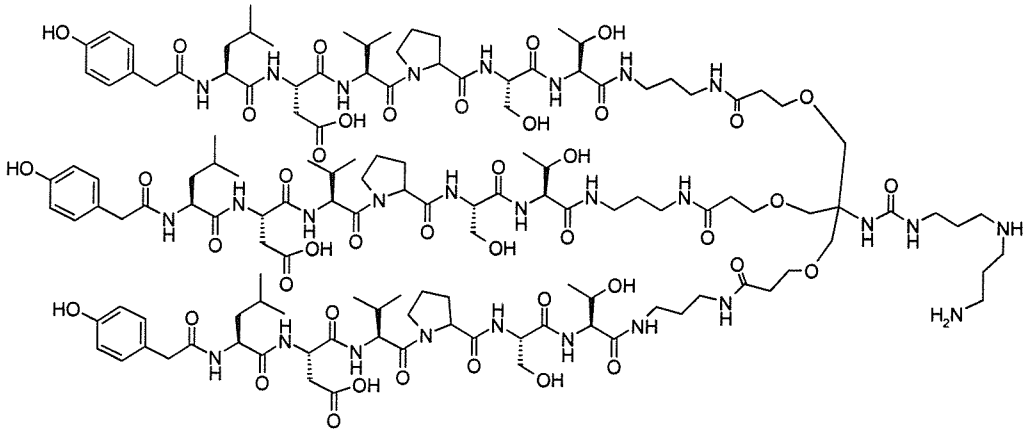
41.56 ($\underline{C}H_2NH$); 36.65 ($CO\underline{C}H_2CCH$); 36.17 ($\underline{C}H_2$, Asp); 35.88 ($\underline{C}H_2CH_2NH_2$); 30.62 ($\underline{C}HCH_3)_2$, Val); 29.54 ($NHCH_2\underline{C}H_2CH_2NH$); 26.30, 24.93 ($\underline{C}H_2$, Pro); 24.60 ($\underline{C}H$, Leu); 23.45 21.95 20.58 19.54 18.36 ($\underline{C}H_3$, Leu, Val, Thr).

RP-HPLC (Method 2, ELS): 7.91 mins.

m/z (HR-ES+); ($M+H^+$) $C_{41}H_{67}N_9O_{12}$ 877.4909 (calc); 878.4981 (found).

Resin (loading 0.21 mmol/g, 200 mg) afforded 12 mg of title compound in 30% isolated yield.

Synthesis of N^9-(4-hydroxy-phenyl)-acetic acid-LDVPST-Gen [1.0] AB_3 type dendrimer-1,5,9 triazanonane) (79).



δ_H (400 MHz, DMSO-d_6): δ_H (400 MHz, DMSO-d_6): 8.44 (3H, d, *J* 8, N$\underline{H}$, Asp); 8.25-8.15 (6H, m, N$\underline{H}$, Leu, Val); 8.00-7.90 (6H, m, N$\underline{H}CH_2$); 7.75-7.70 (3H, N$\underline{H}CH_2$); 7.65-7.55 (6H, m, N$\underline{H}$, Thr, Ser); 7.14 (6H, d, *J* 9, $ArC^{2,\ 6}\underline{H}$); 6.78 (6H, d, *J* 9, $ArC^{3,\ 5}\underline{H}$); 4.70-4.60 (3H, m, C$\underline{H}$, Asp); 4.55-4.50 (3H, m, C$\underline{H}$, Val); 4.45-4.35 (9H, m, C$\underline{H}$, Ser, C$\underline{H}$, Pro, C$\underline{H}$, Leu); 4.25-4.15 (6H, m, $CHC\underline{H}(OH)(CH_3)$, $C\underline{H}CH(OH)(CH_3)$, Thr); 3.85-3.75 (6H, m, $C\underline{H}_2$, Ser); 3.70-3.65 (6H, m, $COCH_2C\underline{H}_2OCH_2$); 3.62 (6H, s, $COCH_2CH_2OC\underline{H}_2$); 3.25-2.90 (20H, m, $C\underline{H}_2NH$); 2.80 (3H, dd, J_{AX} 17, J_{AB} 8, C$\underline{H}$H, Asp); 2.60-2.55 (9H, m, $COC\underline{H}_2CCH$, CH$\underline{H}$, Asp); 2.45-2.35 (6H, m, $COC\underline{H}_2CH_2OCH_2$); 2.20-2.15 (3H, m, $C\underline{H}(CH_3)_2$, Val); 2.10-1. (31H, m, $CH_2C\underline{H}_2CH_2$, $C\underline{H}_2$ Leu, C$\underline{H}$, Leu, $C\underline{H}_2$, Pro); 1.15 (9H, d, *J* 6, $C\underline{H}_3$, Thr); 1.05-0.85 (36H, m, $C\underline{H}_3$, Val, Leu).

δ_C (100 MHz, DMSO-d_6): 172.86, 172.18, 171.05, 170.77, 170.58, 170.27, 169.93 ($\underline{C}O$); 158.90 ($\underline{C}OCH_2Ar$); 158.54, ($Ar\underline{C}^4$); 156.25 ($NH\underline{C}ONH$); 130.32 ($Ar\underline{C}^{2,\ 6}H$); 126.93 ($Ar\underline{C}^1$); 115.37 ($Ar\underline{C}^{3,\ 5}H$); 70.23 ($C\underline{C}H_2OCH_2$); 67.87 ($CCH_2O\underline{C}H_2$); 66.46

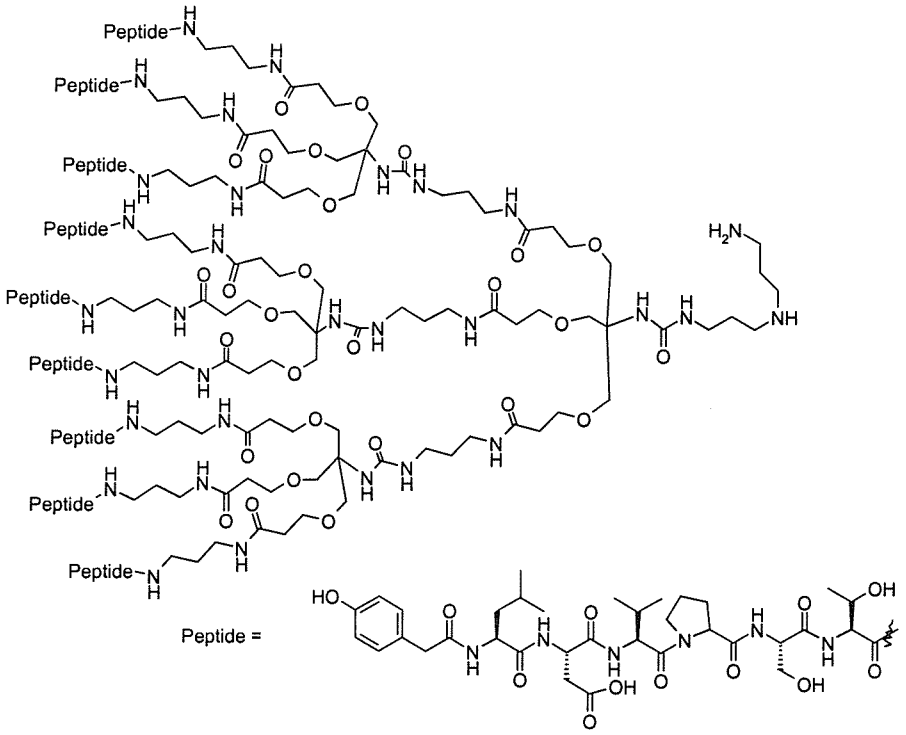
(CH, Thr); 61.83 ($\underline{C}H_2$, Ser); 59.69 (CH, Val); 58.78 (CH_3-CH-OH, Thr); 55.86 (CH, Ser); 55.67 (CH, Leu); 51.29 (CH, Pro); 49.71 (CH, Asp); 47.55 ($\underline{C}H_2NH$) 41.61, 36.66, 35.92 ($\underline{C}H_2$); 30.65 $(\underline{C}HCH_3)_2$, Val); 29.45 ($NHCH_2\underline{C}H_2CH_2NH$); 24.92 ($\underline{C}H_2$, Pro); 24.60 (CH, Leu); 23.45, 21.94, 20.55, 19.54, 18.35 ($\underline{C}H_3$, Leu, Val, Thr).

RP-HPLC (Method 2, ELS): 10.75 mins.

m/z (MALDI-TOF); 2901.52 (calc) 2902.05 ($M+H^+$ 100%); 2924.48 ($M+Na^+$ 80%); 2940.21 ($M+K^+$ 30%) (found).

Resin (loading 0.42 mmol/g, 130 mg) afforded 32 mg of title compound in 20% crude yield.

Synthesis of N^9-(4-hydoxy-phenyl)-acetic acid-LDVPST-Gen [2.0] AB_3 type dendrimer-1,5,9 triazanonane) (80).



δ_H (400 MHz, DMSO-d_6): 8.44 (9H, d, J 8, N$\underline{H}$, Asp); 8.25-8.15 (18H, m, N$\underline{H}$, Leu, Val); 8.00-7.90 (18H, m, N$\underline{H}CH_2$); 7.75-7.70 (9H, N$\underline{H}CH_2$); 7.65-7.55 (18H, m, N$\underline{H}$, Thr, Ser); 7.14 (18H, d, J 9, $ArC^{2,6}\underline{H}$); 6.78 (18H, d, J 9, $ArC^{3,5}\underline{H}$); 4.70-4.60 (9H, m, C$\underline{H}$, Asp); 4.55-4.50 (9H, m, C$\underline{H}$, Val); 4.45-4.35 (27H, m, C$\underline{H}$, Ser, C$\underline{H}$, Pro, C$\underline{H}$, Leu); 4.25-4.15 (18H, m, CHC$\underline{H}$(OH)(CH_3), C$\underline{H}$CH(OH)(CH_3), Thr); 3.85-3.75 (18H, m, $C\underline{H}_2$, Ser); 3.70-3.65 (18H, m, $COCH_2C\underline{H}_2OCH_2$); 3.62 (18H, s, $COCH_2CH_2OC\underline{H}_2$); 3.25-2.90 (56H, m, $C\underline{H}_2NH$); 2.80 (9H, dd, J_{AX} 17, J_{AB} 8, C$\underline{H}$H, Asp); 2.60-2.55 (27H,

m, $COC\underline{H}_2CCH$, $CH\underline{H}$, Asp); 2.45-2.35 (18H, m, $COC\underline{H}_2CH_2OCH_2$); 2.20-2.15 (9H, m, $C\underline{H}(CH_3)_2$, Val); 2.10-1.48 (88H, m, $C\underline{H}_2$ Leu, $C\underline{H}$, Leu, $C\underline{H}_2$, Pro, $CH_2C\underline{H}_2CH_2$); 1.15 (27H, d, *J* 6, $C\underline{H}_3$, Thr); 1.05-0.85 (108H, m, $C\underline{H}_3$, Val, Leu).

δ_C (100 MHz, DMSO-d_6): 172.85, 172.44, 172.17, 171.05, 170.76, 170.58, 170.25, 169.93 ($\underline{C}O$); 158.92 ($\underline{C}OCH_2C$); 158.55 ($Ar\underline{C}^4$); 156.24 ($NH\underline{C}ONH$); 130.32 ($Ar\underline{C}^{2,6}H$); 126.93 ($Ar\underline{C}^1$); 115.37 ($Ar\underline{C}^{3,5}H$); 70.23 ($C\underline{C}H_2OCH_2$); 67.83 ($CCH_2O\underline{C}H_2$); 66.48 ($\underline{C}H$, Thr); 61.87 ($\underline{C}H_2$, Ser); 59.69 ($\underline{C}H$, Val); 58.92 (CH_3-$\underline{C}H$-OH, Thr); 55.86 ($\underline{C}H$, Ser); 55.67 ($\underline{C}H$, Leu); 51.27 ($\underline{C}H$, Pro); 49.71 ($\underline{C}H$, Asp); 47.53 ($\underline{C}H_2NH$); 41.67 ($\underline{C}H_2$); 37.02 36.66 36.50 35.91 ($\underline{C}H_2$); 30.66 ($\underline{C}HCH_3)_2$, Val); 29.45 ($NHCH_2\underline{C}H_2CH_2NH$); 24.92 ($\underline{C}H_2$, Pro); 24.60 ($\underline{C}HCH_3)_2$, Leu); 23.44, 21.94, 20.53, 19.54, 18.33 ($\underline{C}H_3$, Leu Val, Thr).

RP-HPLC (Method 2, ELS): 12.62 mins.

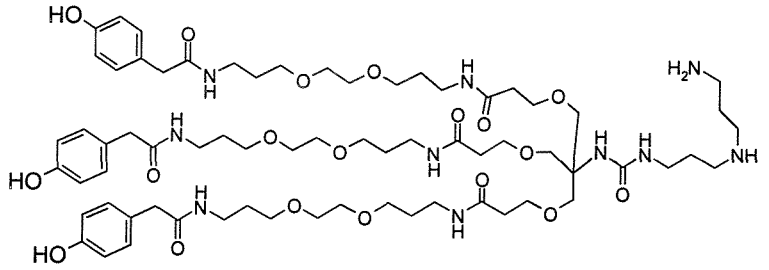
m/z (MALDI-TOF); 8969 (calc) 8960.60 (100% Found).

Resin (loading 0.63 mmol/g, 270 mg) afforded 100 mg of title compound in 7% crude yield.

Synthesis of AB_3-Peptide-Dendrimer Conjugates (75)-(77)

Dendrimer resin **(74)** was suspended in DMSO for 30 mins before addition of a solution of 7, 10-trioxa-1, 13-tridecanediamine as the diamine (250 eq reactive sites) (0.5 eq v/v). The resin was shaken for 3 days at RT. The resin was washed with DMSO (3 x 10 mL) before the addition of a fresh solution of 7, 10-trioxa-1, 13-tridecanediamine as the diamine (250 eq reactive sites) and DMSO (0.5 eq v/v). After a further 3 days reaction the resin was then washed with DCM, DMF, MeOH and ether, (each 3 x10 mL). Reaction completion was checked by taking 10 mg of resin and coupling (4-hydroxy-phenyl)-acetic acid with DIC HOBt (5 eq resin loading) for 2 hrs at RT. Completion of the reaction was checked by qualitative ninhydrin test. After washing as before the derivatised compound was cleaved from the resin with 90% TFA/DCM, 1 drop of water as a scavenger and analysed by RP-HPLC.

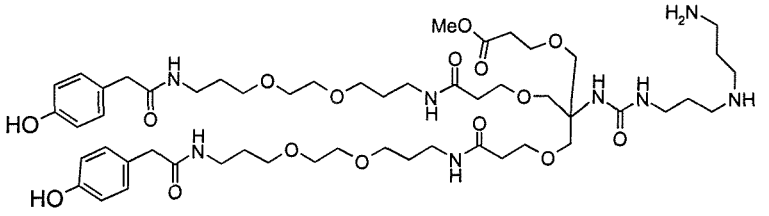
Product (75).



m/z (MALDI-TOF); 1504.17 (M^+ 50%); 1527.27 ($M+Na^+$ 100%).

RP-HPLC (Method 2, ELS): 9.22 mins.

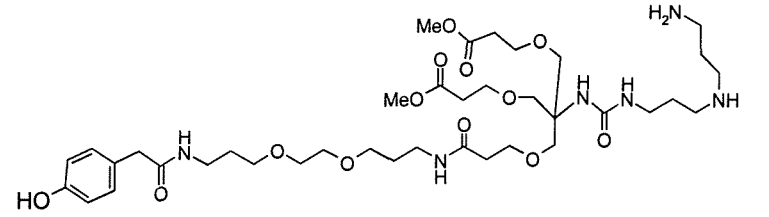
Product (76).



m/z (MALDI-TOF); 1203.66 (M+Na+ 100%).

RP-HPLC (Method 2, ELS): 8.83 mins.

Product (77).



m/z (MALDI-TOF); 859.51 ($M+H^+$ 100%); 881.57 ($M+Na^+$ 70%); 897.59 ($M+K^+$ 25%).

RP-HPLC (method 2, ELS): 8.52 mins.

Chapter 6.2 Biological Experimental

General procedure for screening Peptide-Dendrimer conjugates.

A375M melanoma cells (CRL-1619, American Tissue Culture Collection, Manassas, US). Cells were plated at 2.5 × 10^4 cells/100μL medium/well into 96 well plates. Twenty-four hours later media was gently removed and 100μL of 0.25% glutaraldehyde was added to each well for 30 minutes. This was subsequently removed and 100 μL PBS/0.05% w/v azide added to each well for storage at 4 °C. After washing three times in PBS containing 0.05% v/v Tween 20 (the wash buffer) the plate was blocked with 10% w/v BSA in PBS for 1 hour at 37°C, 200 μL/well. After a washing step, biotinylated EILDVPST (100 μL/well in duplicates) was added in the presence and absence of the competing peptide /dendrimer conjugate at a molar excess. The plate was incubated at 37°C for 1 hour. After thoroughly washing off the unbound biotinylated peptide Neutravidin Horseradish peroxidase (2.0 μg/mL, 100 μL/well, Pierce Chester, UK) was added to each well and incubated for 1 hour at 37°C. After washing off unbound enzyme 100 μL of the substrate *o*-phenylenediamine dihydrochloride (OPD) (Sigma, Poole, Dorset, UK) was added to all wells and the plate incubated for 40 minutes at room temperature in the dark before measuring the absorbance at 450nm.
Note non water-soluble compounds were dissolved in 10% DMSO/PBS.

Reagents:

Cell culture medium-RPMI 1640 containing glutamax, penstrep and 10 % v/v FCS
Diluted trypsin- 1 part trypsin/EDTA 1 (Cibco 25300-05 Trypsin 0.05%, EDTA 0.02 %) 3 parts Versene (Gibco 15040-033, EDTA 0.02%).
"Superblock"- Pierce (37515). 10% w/v BSA in PBS. Or prepare 10% w/v BSA (sigma) in PBS.
PBS/TWEEN 0.05% v/v. 1L PBS + 0.5 mL TWEEN 20.
PBS/BSA 1 % w/v (e.g. 90 ml PBS + 10 mL super block) (Peptide diluent).
Phosphate citrate (0.05M). 1 tablet (sigma P4809) + 100 mL distilled water.

One 10 mg tablet of o-phenylenediamine (OPD), dihydrochloride (sigma, P8287) was dissolved in 25 mL of the 0.05M phoshate Citrate pH 5.0 solution and 10 µL of 30% v/v hydrogen peroxide (sigma, H1099), immediately before use.

Neutravidin, Horseradish peroxidase conjugated (Pierce, #31001). The lyophilized powder (2 mg) was dissolved in 0.4 mL distilled water and diluted further to 2ml in PBS. The solutions were then stored in 50 µL aliquots at - 20°C. This stock solution is 1mg/ mL, and on the day of screen 40 µL of the frozen stock was taken and added to 20 mL PBS/TWEEN to make 2 µL/mL.

Chapter 3: Screening of LDV-Dendrimer Conjugates

Compound (20)

Concentration (µM)	Wells	Values	Mean Value	Std.Dev	Corrected
1000.00	A8	1.05	0.94	0.16	16.9
	A9	0.82			
100.00	B8	1.30	1.27	0.05	3.70
	B9	1.24			
50.00	C8	1.36	1.40	0.07	4.60
	C9	1.45			
25.00	D8	1.41	1.42	0.01	0.90
	D9	1.43			
12.50	E8	1.50	1.44	0.09	6.00
	E9	1.38			
6.25	F8	1.44	1.40	0.07	4.70
	F9	1.35			
3.12	G8	1.53	1.40	0.18	12.50
	G9	1.28			
0.31	H8	1.33	1.40	0.06	4.10
	H9	1.41			

Compound (21)

Concentration (μM)	Wells	Values	Mean Value	Std.Dev	Corrected
1000.00	A10	0.70	0.83	0.18	21.30
	A11	0.96			
100.00	B10	1.13	1.16	0.04	3.50
	B11	1.19			
50.00	C10	1.25	1.31	0.09	6.60
	C11	1.37			
25.00	D10	1.19	1.27	0.11	8.80
	D11	1.37			
12.50	E10	1.41	1.40	0.03	2.40
	E11	1.37			
6.25	F10	1.35	1.40	0.08	5.60
	F11	1.46			
3.12	G10	1.26	1.33	0.09	7.10
	G11	1.40			
0.31	H10	1.27	1.31	0.06	4.40
	H11	1.35			

Compound (22)

Concentration (μM)	Wells	Values	Mean Value	Std.Dev	Corrected
1000.00	A8	0.53	0.56	0.04	7.50
	A9	0.59			
100.00	B8	0.84	0.88	0.06	6.20
	B9	1.92			
50.00	C8	1.04	1.06	0.02	2.10
	C9	1.07			
25.00	D8	1.99	1.05	0.09	8.60
	D9	1.12			
12.50	E8	1.12	1.14	0.04	3.30

	E9	1.20			
6.25	F8	1.15	1.16	0.01	0.90
	F9	1.17			
3.12	G8	1.11	1.10	0.02	1.80
	G9	1.09			
0.31	H8	1.09	1.10	0.02	1.50
	H9	1.11			

Compound (23)

Concentration (μM)	Wells	Values	Mean Value	Std.Dev	Corrected
1000.00	A10	0.74	0.79	0.06	7.70
	A11	0.83			
100.00	B10	0.89	0.98	0.12	12.60
	B11	1.06			
50.00	C10	1.22	1.22	0.01	0.60
	C11	1.21			
25.00	D10	1.02	1.07	0.06	5.70
	D11	1.11			
12.50	E10	1.25	1.20	0.07	5.60
	E11	1.15			
6.25	F10	1.13	1.16	0.04	3.30
	F11	1.18			
3.12	G10	1.07	1.17	0.15	12.60
	G11	1.27			
0.31	H10	1.09	1.06	0.05	4.90
	H11	1.02			

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Concentration (μM)	Wells	Values	Mean Value	Std.Dev	Corrected
1000.00	A6	0.15	0.15	0.00	1.20
	A7	0.15			

100.00	B6 B7	0.25 0.25	0.25	0.00	1.20
50.00	C6 C7	0.32 0.31	0.31	0.00	1.20
25.00	D6 D7	0.39 0.38	0.39	0.00	1.70
12.50	E6 E7	0.66 0.74	0.70	0.06	8.00
6.25	F6 F7	0.87 1.03	0.95	0.11	11.80
3.12	G6 G7	1.19 1.12	1.16	0.05	4.30
0.31	H6 H7	1.01 1.07	1.08	0.02	1.80

Controls

Wells	Values	Mean Value
A2	0.15	0.15
B2	0.13	
C2	0.17	
D2	0.16	
E2	0.17	
F2	0.14	
G2	0.17	
H2	0.12	
A12	0.21	0.20
B12	0.20	
C12	0.19	
D12	0.19	
E12	0.19	
F12	0.20	
G12	0.19	

H12	0.19	

Table Corresponding to (Figure 38)

Sample	Concentration (μM)	Values	Mean Value	Std.Dev	Corrected Value
Ant 3697	1000.00	0.15 0.15	0.15	0.00	0.00
Compound (20)	1000.00	1.05 0.82	0.94	0.16	0.75
Compound (21)	1000.00	0.70 0.96	0.83	0.18	0.65
Compound (22)	1000.00	0.53 0.59	0.56	0.04	0.40
Compound (23)	1000.00	0.74 0.83	0.79	0.06	0.63
Biotinylated EILDVPST-NH_2 (1)	1000.00	1.33 1.44 1.41	1.40.	0.06	1.21
Biotinylated EILDVPST-NH_2 (2)	1000.00	0.90 0.94 1.013	0.95	0.06	0.79

	Mean Value
Control 1	0.18
Control 2	0.15

Corrected abs = mean value – control mean value

%Competition = 1- Corrected abs for competition

Corrected abs for b-EILDVPST

3.5: Screening of Capped Divalent Peptide-Dendrimer Conjugates

Plate 1

Compound (34)

Concentration (μM)	Wells	Value
1000.00	A8	0.20
100.00	B8	0.52
50.00	C8	0.47
25.00	D8	0.42
12.50	E8	0.52
6.25	F8	0.69
3.13	G8	0.98
0.31	H8	1.12

Compound (35)

Concentration (μM)	Wells	Value
1000.00	A7	0.17
100.00	B7	0.22
50.00	C7	0.40
25.00	D7	0.45
12.50	E7	0.64
6.25	F7	0.82
3.13	G7	1.05
0.31	H7	1.01

Compound (36)

Concentration (μM)	Wells	Value
1000.00	A6	0.21
100.00	B6	0.31
50.00	C6	0.54

25.00	D6	0.60
12.50	E6	0.76
6.25	F6	0.81
3.13	G6	0.99
0.31	H6	0.93

Compound (38)

Concentration (μM)	Wells	Value
1000.00	A12	0.32
100.00	B12	0.31
50.00	C12	0.48
25.00	D12	0.55
12.50	E12	0.70
6.25	F12	0.90
3.13	G12	1.15
0.31	H12	1.09

Compound (39)

Concentration (μM)	Wells	Value
1000.00	A9	0.26
100.00	B9	0.37
50.00	C9	0.65
25.00	D9	0.63
12.50	E9	0.80
6.25	F9	0.92
3.13	G9	1.08
0.31	H9	1.09

Compound (40)

Concentration (μM)	Wells	Value
1000.00	A10	0.48
100.00	B10	0.27
50.00	C10	0.38
25.00	D10	0.40
12.50	E10	0.52
6.25	F10	0.75
3.13	G10	0.95
0.31	H10	1.17

Compound (41)

Concentration (μM)	Wells	Value
1000.00	A11	0.19
100.00	B11	0.24
50.00	C11	0.30
25.00	D11	0.33
12.50	E11	0.38
6.25	F11	0.46
3.13	G11	0.84
0.31	H11	1.03

Compound (42)

Concentration (μM)	Wells	Value
1000.00	A5	0.38
100.00	B5	0.60
50.00	C5	0.74
25.00	D5	0.66
12.50	E5	0.72
6.25	F5	0.89
3.13	G5	0.97

0.31	H5	0.82

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Concentration (μM)	Wells	Value
1000.00	A4	0.26
100.00	B4	0.22
50.00	C4	0.33
25.00	D4	0.37
12.50	E4	0.47
6.25	F4	0.58
3.13	G4	0.70
0.31	H4	0.77

Biotinylated-EILDVPST

Concentration (μM)	Wells	Values	Mean	Std.Dev	CV%
200.00	A2 A3	0.10 1.04	1.02	0.03	3.30
100.00	B2 B3	0.67 0.69	0.68	0.02	2.50
50.00	C2 C3	0.41 0.47	0.44	0.04	9.40
25.00	D2 D3	0.31 0.34	0.32	0.02	5.30
12.50	E2 E3	0.18 0.19	0.18	0.01	5.00
6.25	F2 F3	0.18 0.17	0.18	0.01	5.40
3.13	G2 G3	0.17 0.16	0.17	0.08	3.90
1.56	H2	0.13	0.14	0.02	11.50

	H3	0.15			

Control

Wells	values	Mean value
A1	0.01	0.09
B1	0.09	
C1	0.10	
D1	0.07	

3.7: Screening of Optimised Ligand-Dendrimer Conjugates (39), (41), (43)-(45), (52)

Compound (39)

Concentration (μM)	Wells	Value
100.00	A6	0.44
20.00	B6	0.92
4.00	C6	0.91
0.80	D6	0.88
0.16	E6	0.94
0.03	F6	1.06
0.01	G6	1.19
0.00	H6	1.17

Compound (41)

Concentration (μM)	Wells	Value
100.00	A5	0.42
20.00	B5	0.80
4.00	C5	0.84
0.80	D5	0.91

0.16	E5	0.91
0.03	F5	1.12
0.01	G5	1.03
0.00	H5	1.07

Compound (43)

Concentration (μM)	Wells	Value
100.00	A7	0.41
20.00	B7	0.85
4.00	C7	0.94
0.80	D7	0.92
0.16	E7	0.99
0.03	F7	0.94
0.01	G7	1.14
0.00	H7	1.17

Compound (44)

Concentration (μM)	Wells	Value
100.00	A8	0.35
20.00	B8	0.79
4.00	C8	0.98
0.80	D8	0.98
0.16	E8	0.97
0.03	F8	1.07
0.01	G8	1.14
0.00	H8	1.22

Compound (45)

Concentration (μM)	Wells	Value
100.00	A9	0.33

20.00	B9	0.75
4.00	C9	0.89
0.80	D9	0.93
0.16	E9	1.04
0.03	F9	1.04
0.01	G9	1.12
0.00	H9	1.24

Compound (52)

Concentration (μM)	Wells	Value
100.00	A11	0.43
20.00	B11	0.95
4.00	C11	1.09
0.80	D11	1.08
0.16	E11	1.09
0.03	F11	1.13
0.01	G11	1.20
0.00	H11	1.10

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Concentration (μM)	Wells	Value
100.00	A4	0.25
20.00	B4	0.49
4.00	C4	0.65
0.80	D4	0.74
0.16	E4	0.92
0.03	F4	0.96
0.01	G4	1.04
0.00	H4	1.18

Standards (biotinylatesd EILDVPST)

Concentration (μM)	Wells	Values	Mean	Std.Dev	CV%
400.00	A2	1.14	1.06	0.11	10.30
	A3	0.99			
200.00	B2	1.03	0.93	0.15	16.30
	B3	0.82			
100.00	C2	0.79	0.72	0.10	13.60
	C3	0.65			
50.00	D2	0.55	0.52	0.05	8.70
	D3	0.48			
25.00	E2	0.41	0.40	0.02	5.20
	E3	0.38			
12.50	F2	0.31	0.31	0.00	0.40
	F3	0.31			
6.25	G2	0.26	0.25	0.00	1.60
	G3	0.25			
3.13	H2	0.24	0.26	0.03	12.90
	H3	0.29			

Control

Wells	Values	Mean value
E1	0.15	0.16
F1	0.17	
G1	0.17	
H1	0.17	

3.7: Screening of Optimised Ligand-Dendrimer Conjugates (39), (46)-(50).

Compound (39)

Concentration (μM)	Wells	Value
100.00	A6	0.56
20.00	B6	1.03
4.00	C6	1.07
0.80	D6	0.99
0.16	E6	0.94
0.03	F6	1.05
0.01	G6	1.17
0.00	H6	1.08

Compound (46)

Concentration (μM)	Wells	Value
100.00	A8	0.53
20.00	B8	1.08
4.00	C8	1.02
0.80	D8	1.01
0.16	E8	0.99
0.03	F8	1.08
0.01	G8	1.09
0.00	H8	1.20

Compound (47)

Concentration (μM)	Wells	Value
100.00	A1	0.55
20.00	B1	0.85
4.00	C1	0.90
0.80	D1	0.96
0.16	E1	0.98
0.03	F1	1.09
0.01	G1	1.17

0.00	H1	1.02

Compound (48)

Concentration (μM)	Wells	Value
100.00	A9	0.46
20.00	B9	0.89
4.00	C9	1.02
0.80	D9	1.03
0.16	E9	1.02
0.03	F9	1.02
0.01	G9	1.10
0.00	H9	1.03

Compound (49)

Concentration (μM)	Wells	Value
100.00	A10	0.39
20.00	B10	0.86
4.00	C10	1.00
0.80	D10	1.03
0.16	E10	1.05
0.03	F10	1.09
0.01	G10	1.08
0.00	H10	1.05

Compound (50)

Concentration (μM)	Wells	Value
100.00	A11	0.42
20.00	B11	0.84
4.00	C11	1.01
0.80	D11	1.05

0.16	E11	1.07
0.03	F11	1.07
0.01	G11	1.09
0.00	H11	1.09

Compound (51)

Concentration (μM)	Wells	Value
100.00	A12	0.50
20.00	B12	0.91
4.00	C12	1.06
0.80	D12	1.05
0.16	E12	1.01
0.03	F12	1.00
0.01	G12	0.96
0.00	H12	1.02

Ant 35

Concentration (μM)	Wells	Value
100.00	A4	0.37
20.00	B4	0.61
4.00	C4	0.72
0.80	D4	0.86
0.16	E4	0.90
0.03	F4	1.03
0.01	G4	1.13
0.00	H4	1.00

Standards biotinylated EILDVPST

Concentration (μM)	Wells	Values	Mean Value	Std.Dev	CV%
400.00	A2	1.09	1.12	0.06	4.90
	A3	1.16			
200.00	B2	1.16	1.14	0.04	3.40
	B3	1.11			
100.00	C2	0.71	0.75	0.05	7.00
	C3	0.79			
50.00	D2	0.58	0.57	0.02	3.30
	D3	0.56			
25.00	E2	0.43	0.41	0.04	9.10
	E3	0.38			
12.50	F2	0.39	0.35	0.05	14.50
	F3	0.32			
6.25	G2	0.33	0.33	0.01	3.40
	G3	0.32			
3.13	H2	0.19	0.22	0.03	13.80
	H3	0.24			

Control

Wells	Values	Mean
E1	0.19	0.19
F1	0.20	
G1	0.20	
H1	0.17	

3.8: Screening of EILDVPST-Dendrimer Conjugates (53)-(55).

Compound (53)

Concentration (mM)	Wells	Values	Mean	Std.Dev	CV%
2.3	A1	0.24	0.24	0.03	12.10

	A2	0.26			
	A3	0.21			
1.150	B1	0.22	0.26	0.04	14.70
	B2	0.27			
	B3	0.29			
0.58	C1	0.48	0.49	0.01	2.80
	C2	0.49			
	C3	0.51			
0.29	D1	0.56	0.56	0.04	7.70
	D2	0.60			
	D3	0.51			
0.14	E1	0.51	0.56	0.06	10.80
	E2	0.63			
	E3	0.55			
0.07	F1	0.51	0.56	0.06	10.80
	F2	0.63			
	F3	0.55			

Compound (54)

Concentration (mM)	Wells	Values	Mean	Std.Dev	CV%
0.59	A9	0.38	0.39	0.01	2.90
		0.39			
		0.40			
0.30	B9	0.53	0.52	0.03	5.80
		0.54			
		0.48			
0.15	C9	0.64	0.63	0.01	2.30
		0.62			
		0.62			
0.07	D9	0.59	0.58	0.02	3.00
		0.56			

		0.60			
0.04	E9	0.59 0.65 0.55	0.60	0.05	8.60
0.02	F9	0.57 0.58 0.59	0.58	0.01	1.80
0.01	G9	0.59 0.66 0.61	0.62	0.04	5.70
0.01	H9	0.72 0.70 0.75	0.72	0.02	3.40

Compound (55)

Concentration (mM)	Wells	Values	Mean	Std.Dev	CV%
18.10	A7 A8	0.22 0.25	0.23	0.02	8.40
9.05	B7 B8	0.38 0.36	0.37	0.02	4.50
4.53	C7 C8	0.58 0.57	0.57	0.01	1.40
2.26	D7 D8	0.65 0.64	0.65	0.01	1.70
1.13	E7 E8	0.56 0.57	0.57	0.00	0.70
0.57	F7 F8	0.53 0.53	0.53	0.00	0.30

Biotinylated-EILDVPST

Wells	Values	Mean

A10	0.71	0.68
B10	0.74	
C10	0.73	
D10	0.58	
E10	0.67	
F10	0.64	
G10	0.67	
H10	0.73	

Chapter 4 Screening of Peptide-Dendrimer Conjugates (55), (79)-(81)

Compound (55)

Concentration (mM)	Wells	Values	Mean	Std.Dev	CV%
11.00	A8	0.69	0.67	0.05	7.00
	A9	0.62			
	A10	0.71			
5.50	B8	0.99	0.91	0.08	14.70
	B9	0.89			
	B10	0.84			
2.75	C8	1.16	1.15	0.03	2.20
	C9	1.16			
	C10	1.12			
1.38	D8	1.12	1.18	0.07	6.00
	D9	1.17			
	D10	1.25			
0.69	E8	1.26	1.37	0.10	7.20
	E9	1.38			
	E10	1.46			
0.34	F8	1.30	1.35	0.05	3.40
	F9	1.39			
	F10	1.37			
0.17	G8	1.42	1.29	0.11	8.70

	G9	1.21			
	G10	1.25			
0.09	H8	1.29	1.28	0.03	2.20
	H9	1.25			
	H10	1.30			

Compound (78)

Concentration (mM)	Wells	Values
5.46	A7	0.95
2.73	B7	1.26
1.37	C7	1.23
0.68	D7	0.10
0.34	E7	1.26
0.17	F7	1.37
0.09	G7	1.23
0.04	H7	1.23

Compound (79)

Concentration (mM)	Wells	Values	Mean	Std.Dev	CV%
3.70	A4	0.31	0.35	0.03	9.50
	A5	0.37			
	A6	0.37			
1.88	B4	0.38	0.45	0.06	14.20
	B5	0.49			
	B6	0.49			
0.94	C4	0.60	0.66	0.07	10.30
	C5	0.64			
	C6	0.74			
0.47	D4	1.04	1.04	0.06	5.40

	D5	0.98			
	D6	1.09			
0.23	E4	1.20	1.31	0.09	6.90
	E5	1.36			
	E6	1.36			
0.12	F4	1.36	1.37	0.06	4.60
	F5	1.43			
	F6	1.31			
0.06	G4	1.38	1.39	0.16	11.70
	G5	1.55			
	G6	1.23			
0.03	H4	1.49	1.49	0.06	4.10
	H5	1.42			
	H6	1.54			

Compound (80)

Concentration (mM)	Wells	Values	Mean	Std.Dev	CV%
1.39	A1	0.74	0.77	0.03	3.60
	A2	0.79			
	A3	0.77			
0.70	B1	0.90	0.75	0.14	18.30
	B2	0.64			
	B3	0.70			
0.35	C1	0.85	0.76	0.08	10.30
	C2	0.75			
	C3	0.70			
0.17	D1	1.13	1.13	0.05	4.20
	D2	1.17			
	D3	1.08			
0.09	E1	1.24	1.15	0.08	7.30
	E2	1.08			

	E3	1.12			
0.04	F1	1.05	1.29	0.21	16.20
	F2	1.44			
	F3	1.39			
0.02	G1	1.09	1.14	0.0.09	7.70
	G2	1.08			
	G3	1.24			
0.01	H1	1.19	1.36	0.23	17.00
	H2	1.26			
	H3	1.62			

Chapter 5.4: Preparation of Peptide-Dendrimer-DNA conjugates

Template DNA

3 X 100 μL of stock DNA template (70 BP) were freeze-dried.

Each vial contained 0.90 nM = 20.50 μg (stock conc is 205 μg/ mL).

Stock solutions of coupling reagents.

PyBOP 10 mM solution

DIPEA 10 mM solution

HOBt 10 mM solution

The stock solutions were then diluted 1:50.

Stock solutions of Peptide-Dendrimers.

Compound **(78)** 2.73 mM

Compound **(79)** 0.41 mM

Compound **(80)** 0.50 mM

Compound **(78)** was diluted 1:8; compound **(79)** and **(80)** 1:2.

The coupling reagents (0.20 mM, 5.00 μL) were added to the freeze-dried templates and activated for five minutes before the addition of the peptide-dendrimers. The reaction was allowed to proceed for 3 hours and the samples were then freeze-dried. Next day the residue was dissolved in 12 μL of buffer from spin columns (Microspin^{TM} G-50 columns Amersham Pharmacia biotech 27-5330-01). The columns were prepared according to the manufacturers instructions and then the reaction product was applied to the column carefully and centrifuged for 1 minute at 5000 rpm. The liquid pushed through the column was collected and freeze-dried.

The amount of DNA-Peptide –dendrimer conjugate was estimated from the following procedure and equation.

O.D = c x ε

Or 1 O.D = 33 μg/mL

ε for each base can be calculated from multiplying the following numbers by the number of times the base appears in the oligo.

dGTP = 11.70 mL/μM

dCTP = 7.30mL/μM

dATP = 15.40 mL/μM

dTTP = 8.80 mL/μM

Concentration of

(81): 0.000596 μmol/mL or 14.85 μg /mL

(82): 0.000519 μmol/mL or 13.00 μg/mL

(83): 0.000487 μmol/mL or 12.20 μg/mL.

5.5: General procedure for performing the affinity assay.

A375M melanoma cells (CRL-1619, American Tissue Culture Collection, Manassas, US). Cells were plated at 2.5 × 10^4 cells/100μL medium/well into 96 well plates. Twenty-four hours later the media was gently removed and 100μL of 0.25%

glutaraldehyde was added to each well for 30 minutes. This was subsequently removed and 100 μL PBS/0.05% w/v azide added to each well for storage at 4 °C. After washing three times in PBS containing 0.05% v/v Tween 20 (the wash buffer) the plate was blocked with 10% w/v BSA in PBS for 1 hour at 37°C, 200 μL/well. After a washing step, peptide-dendrimer-DNA conjugate (30 μL/well) was added to the cells. The plate was incubated at 37°C for 1 hour. After thoroughly washing off the unbound peptide-dendrimer-DNA conjugate sterilised water 10 μL was added to the wells which were then heated to 70°C for twenty minutes and then scraped and sucked up with a pipetter and the scraped cells were placed in an PCR eppendorf tube. PCR was then performed.

General procedure for performing the affinity assay and then competing off bound peptide-dendrimer-DNA conjugate.

A375M melanoma cells (CRL-1619, American Tissue Culture Collection, Manassas, US). Cells were plated at 2.5×10^4 cells/100μL medium/well into 96 well plates. Twenty-four hours later media was gently removed and 100μL of 0.25% glutaraldehyde was added to each well for 30 minutes. This was subsequently removed and 100 μL PBS/0.05% w/v azide added to each well for storage at 4 °C. After washing three times in PBS containing 0.05% v/v Tween 20 (the wash buffer) the plate was blocked with 10% w/v BSA in PBS for 1 hour at 37°C, 200 μL/well. After a washing step, peptide-dendrimer-DNA conjugate (30 μL/well) were added to the cells of 3 wells. The plate was incubated at 37°C for 1 hour. After thoroughly washing off the unbound peptide-dendrimer-DNA conjugate sterilised water 10 μL was added to 2 of the wells and to the third an excess (27 μM) solution of peptide-dendrimer conjugate **(82)** (30 μL) was added and the cells then incubated for a further 1hour. After thoroughly washing off the unbound peptide-dendrimer conjugate by pipetting the PBS/Tween into the affected well only, sterilised water 10 μL was then added to this well and plate was then heated to 70°C for twenty minutes and the cells in the test wells are scraped and sucked up with a pipetter and the scraped cells placed in an PCR eppendorf tube. PCR was then performed.

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