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Synthesis and hydrolysis studies of a peptide containing the reactive triad of serine proteases with an associated linker to a dye on a solid phase support †

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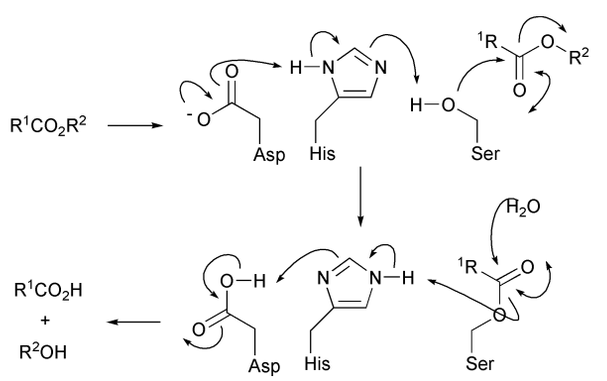
The synthesis of a Tentagel®-supported peptide incorporating the reactive triad of serine, histidine and aspartic acid, found within serine protease enzymes, is described.

Introduction

Serine protease enzymes are characterised by the presence of a uniquely reactive serine side chain. Its reactivity is associated with the interaction of the side chains of three amino acid residues: aspartic acid, histidine and serine. This is commonly referred to as the 'charge relay system' or 'reactive triad' within the enzyme (Scheme 1).[1] A number of peptide-based synthetic catalysts, both synthetic and based on antibodies, have been prepared in an attempt to mimic the method by which serine proteases work.[2] However to date none of these systems have proven capable of reproducing the high rate enhancements and stereoselectivity achieved by enzymes. In this paper, we describe the synthesis of a model system to probe the capacity for a simple linear peptide, incorporating the reactive triad, to cleave ester bonds *via* intramolecular hydrolysis. The model consists of a highly coloured red azo dye, disperse red, connected to a supported peptide through a glycol linker and the ester to be hydrolysed (Fig. 1). We anticipate that the model can potentially be utilised to analyse a combinatorial library of beads. Using this technique, peptide sequences with hydrolytic activity would be identified by observing the loss of colour from the individual beads. In this way, we reasoned that we should be able to identify the optimum length of linker for intramolecular cleavage of



Scheme 1 Reactive triad in serine protease enzymes.

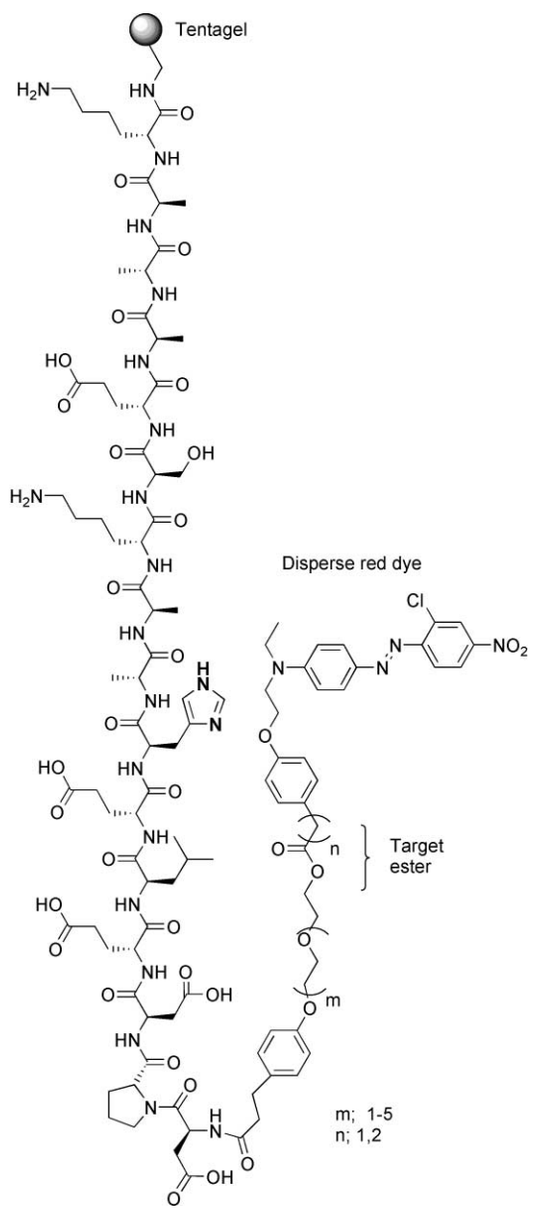


Fig. 1 Structure of synthetic esterase model **1**.

† Electronic supplementary information (ESI) available: Description of preliminary qualitative hydrolysis studies using the materials prepared and described in the main paper. See http://www.rsc.org/suppdata/ob/b3/b302239k/

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the ester bond. With this information in hand, an investigation could be carried out into enantioselective ester hydrolysis.

Results and discussion

Initially the sixteen residue peptide NH_2–Asp–Pro–**Asp**–Glu–Leu–Glu–**His**–Ala–Ala–Lys–**Ser**–Glu–Ala–Ala–Ala–Lys–OH (DP**D**ELE**H**AAKS**S**EAAAK) was selected for incorporation into our protease model **1**. This peptide is a modified version of a sequence reported to form an α-helical structure stabilised by salt bridges and helix-promoting alanine residues. This peptide has been demonstrated to hydrolyse DNA when covalently linked to a rhodium intercalator in the presence of zinc.[3] On this basis it seemed that this sequence could represent a minimal protease model. The histidine residue at position six was replaced with a serine residue with the intention that, should the peptide form the predicted α-helical structure, the three key residues would be aligned in a favourable conformation for co-operative catalysis of the hydrolysis of an ester bond.

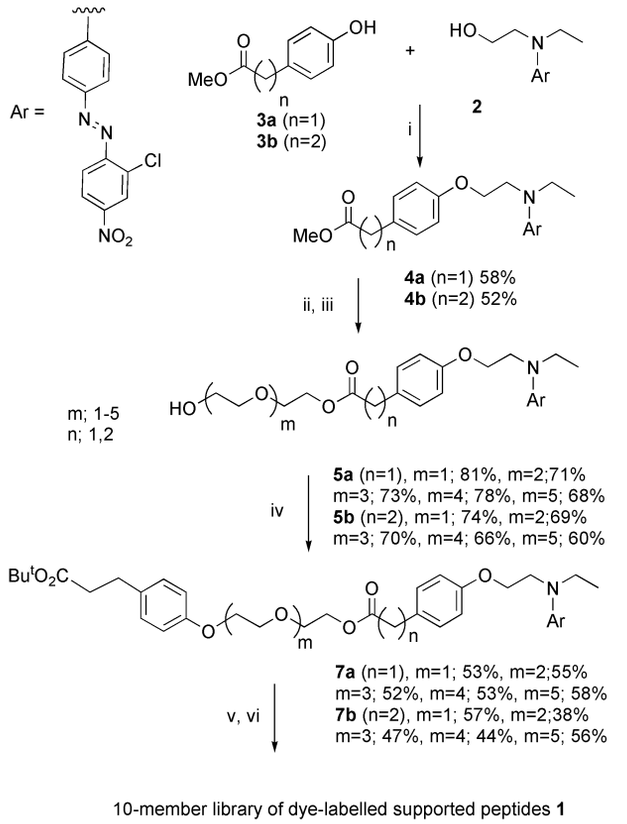
Tentagel® was selected as the support in this application for its favourable swelling properties in a range of solvents, high loading and mechanical stability. In addition to the Tentagel®, *ca.* 10% of a Rink acid-labile linker was employed in the solid phase synthesis. This was employed in order to permit analysis of the material on the beads using ESI-mass spectrometry, and would serve to follow the progress of the functionalisation process at several stages (see below).

The Fmoc-protected amino acids were purchased from Novabiochem with side chains protected, where applicable, with acid-labile groups. Couplings were mediated by benzotriazole-1-yloxy-tris(pyrrolidino)phosphonium hexafluorophosphate (PyBop), *N*-hydroxybenzotriazole (HOBt) and diisopropylethylamine (DIPEA or Hunig's base) in DMF. Double couplings were used for all residues and complete conversion was deduced by Kaiser, 2,4,6-trinitrobenzenesulfonic acid (TNBS) and Chloroanil resin tests. Removal of the amino Fmoc protection was effected with washes of 20% piperidine in DMF. Partial cleavage of a portion of the peptide with concomitant deprotection of its side chains was effected with 2.5% triisopropylsilane (TIS) in trifluoroacetic acid. The filtrate was analysed by ESI-MS and exhibited the expected peptide.

The strongly red coloured and readily available azo dye, disperse red, was chosen as our visual indicator. Five lengths of linker and two simple esters were examined. The correct choice of linker length was crucial. If too short, the serine hydroxy group would not be able to reach the ester to effect the desired intramolecular hydrolysis. However, if the length was too long then it is likely that intermolecular hydrolysis would compete. The parallel synthesis of ten dye-linkers was performed using a common reaction sequence in moderate overall yield to eventually produce ten synthetic esterase systems upon coupling to the peptide and side-chain deprotection (Scheme 2).

Initially, the primary alcohol of disperse red **2** was coupled with the hydroxyphenyl of **3a/b** *via* Mitsunobu alkylation to give **4a/b**. Saponification with lithium hydroxide afforded the respective acids, which were again coupled *via* Mitsunobu alkylation to a range of glycols to yield **5a/b** (five derivatives of each) in reasonable yields. Previous attempts to oxidise the primary alcohol of disperse red (in order to perform reductive amination) and displacement of its sulfonates had proved troublesome. An acidic functionality was therefore introduced *via* Mitsunobu alkylation with the hydroxyphenyl of the tertiary butyl ester **6** to give esters **7a/b** (a total of 10 derivatives).

Following deprotection with TFA, the individual ester derivatives **7a/b** were efficiently coupled to the bead-supported peptide using a combination of PyBOP and HOBt. Deep red-coloured beads were quickly afforded indicating a high level of loading (Fig. 2 illustrates the contrast between functionalised and unfunctionalised beads). Partial cleavage of the peptide during the acidic side-chain deprotection stage allowed tandem



Scheme 2 *Reagents and conditions:* i) DEAD, Ph_3P, toluene, 80 °C, 1 h; ii) LiOH, THF, H_2O, rt, 3 h; iii) DEAD, PH_3P, DCM, rt, 3 h; iv) *o*-(OH)$C_6H_4CH_2CH_2CO_2t$Bu **6**, DEAD, Ph_3P, toluene, 80 °C, 1 h; v) TFA, TIS, DCM, 5 min, rt; vi) Tentagel®-supported peptide, PyBOP, HOBt, DIPEA, DMF, rt, 1 h.

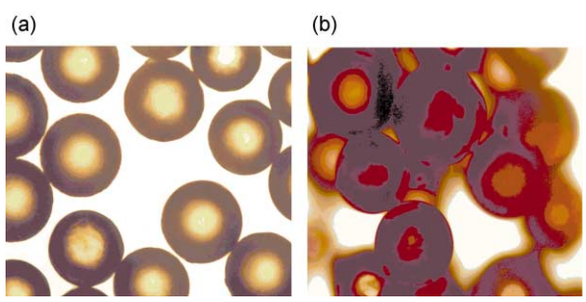


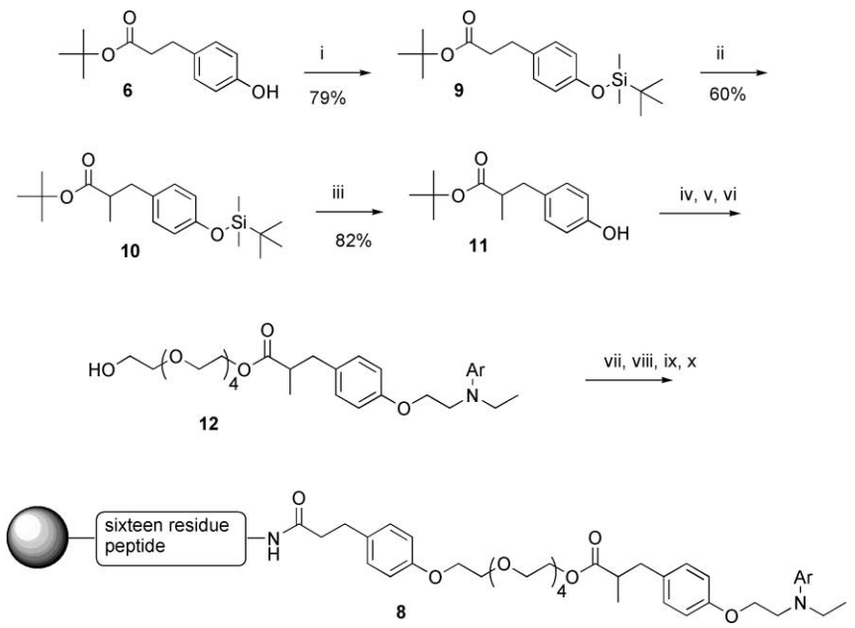
Fig. 2 a) unfunctionalised beads, b) functionalised beads.

ESI-MS analysis of the crude peptide–dye-linker derivatives to be completed, indicating that the coupling had been realised successfully.

In an alternative approach, the free amine of the supported peptide was functionalised with 4-hydroxyphenylacetic acid to terminal hydroxyphenyl functionality. This was used to attach the dye linkers **5a/b** *via* Mitsunobu alkylation. Unfortunately the level of loading was extremely low giving light red beads whereas in contrast the identical coupling with Tentagel® bearing the same terminal hydroxyphenyl functionality but no peptide, quickly yielded dark red beads, indicating a far higher level of loading (Fig. 2).

In order to investigate if it was possible to observe if enantioselective control was being expressed during any hydrolysis process, we prepared another supported peptidic DL_{52} system, *i.e.* **8** in which a chiral centre was incorporated α- to the carbonyl group on the ester being hydrolysed (Scheme 3). It was envisaged that any enantioselective control during the hydrolysis would be evaluated *via* chiral HPLC analysis.

The synthesis of the racemic chiral glycol dye-linker **8** was initially undertaken by protecting the hydroxyphenyl group of **6** with *tert*-butyldimethylsilyl chloride to give **9** in 79% yield.



Scheme 3 *Reagents and conditions:* i) TBDMSCl (1.1 eq.), Et_3N (1.5 eq.), DMAP (catalytic), DCM, rt, 8 h; ii) LDA (1.1 eq.), HMPA (10 eq.), MeI (10 eq.), THF, −78 °C, 6 h, rt, 12 h; iii) TBAF (1.0 eq.), THF, rt, 20 min; iv) DEAD (1.0 eq.), PPh_3 (1.0 eq.), disperse red (1.0 eq.), DCM, rt, 3 h, 33%; v) TIS : TFA, 1 : 49, DCM, rt, 10 min, 81%; vi) Pentaglycol (1.3 eq.), DEAD (1.1 eq.), PPh_3 (1.1 eq.), DCM, rt, 4 h, 56%; vii) DEAD (1.1 eq.), PPh_3 (1.1 eq.), *p*-(OH)$C_6H_4CH_2CH_2CO_2t$Bu **6** (1.0 eq.), DCM, rt, 2 h, 59%; viii) TIS : TFA, 1 : 49, DCM, rt, 10 min, 79%; ix) Tentagel®-supported peptide (1.0 eq.), **12** (2.5 eq.), PyBOP (2.5 eq.), HOBt (2.5 eq.), DIPEA (5.0 eq.), DMF, rt, 2 h; x) TIS : TFA, 1 : 49, rt, 1 h.

Formation of the enolate of **9** *via* deprotonation α- to the carbonyl group with LDA at −78 °C, followed by quenching at −78 °C with a ten fold excess of methyl iodide in the presence of HMPA afforded **10** in 60% yield. The HMPA was required to reduce unwanted side products resulting from polyalkylation of the enolate intermediate, and di-carbon and oxygen alkylation during the methyl iodide quenching. The silyl protecting group of **10** was then removed with TBAF in THF. The deprotection was rapid and full conversion was observed within 3 minutes to give **11** in 82% yield.

The coupling of **11** to disperse red was successfully achieved *via* Mitsunobu alkylation using DCM as solvent. Full conversion was observed within 3 hours to give the adduct in 33% yield. We found that a major side product was the dimer of disperse red. The removal of the *tert*-butyl group was achieved in 81% yield by dissolving the substrate in a minimum of DCM and direct delivery to a solution of TIS : TFA, 1 : 49. It was found if a higher concentration of TIS was present or too much DCM was used to dissolve the substrate, efficient deprotection was difficult to achieve. The acid was then converted to the ester of pentaglycol to give **12** in 56% yield.

The coupling of **12** to **6** was also achieved *via* Mitsunobu alkylation in a moderate yield of 59% using DCM as the solvent and the final deprotection was again achieved by dissolving the substrate in a minimum of dichloromethane followed by addition to a solution of TIS : TFA, 1 : 49. The removal of the *tert*-butyl group gave the acid in 79% yield. The acid was coupled to the Tentagel®-supported protected peptide using PyBOP. The solvent of choice was DMF and an excellent level of loading was achieved after gentle stirring at rt for 2 h to give a jet black resin. Side chain deprotection was afforded by gently stirring the supported peptide (9 mg) at rt for 1 h in a solution of TIS : TFA (1 : 49). After subjecting the resin to washes with a series of solvents including TFA : methanol : dichloromethane (1 : 10 : 39), the resin was found to retain a high level of loading, giving the supported deprotected peptidic system **8** as a jet black resin (Scheme 3).

Although we have not yet systematically investigated quantitatively the use of any of our peptides in the intramolecular ester hydrolysis process, a preliminary qualitative investigation has been completed. This is described in the supporting data. †

In conclusion, we have completed the synthesis of a bead-supported peptide–linker–ester–dye conjugate which provides a basis for the study of intramolecular cleavage of the ester by the peptide sequence. This lays the foundations for a possible combinatorial chemistry study for the optimisation of this reaction through the production of a large peptide library. We are continuing our studies in this area.

Experimental

General

All air and moisture sensitive reactions were performed under an atmosphere of dry nitrogen in flame dried glassware. All anhydrous solvents were used as supplied by Romil in HyDry™ bottles except dimethylsulfoxide and dimethylformamide which were supplied by Aldrich in Sure Seal bottles. Commercially available starting materials were used without further purification unless otherwise stated. Petroleum ether refers to that fraction which boils in the range 40–60 °C and $MgSO_4$ was used as the drying agent for solutions.

The reactions were monitored by TLC using aluminium backed silica gel (F_{254}) plates, pre-coated with a layer of silica (Merck), visualised by UV_{254nm} and then 2,4-dinitrophenolhydrazine, phosphomolybdic acid, potassium permanganate or ninhydrin solution. All organic layers were removed by rotary evaporation on a Buchi rotary evaporator, the final traces of solvents being removed on a static oil pump (0.2 mbar). Column chromatography was carried out on silica gel 60 (40–63 μm).

Melting points were measured on a Stuart Scientific SMP1 instrument and are uncorrected. Infrared spectra (IR) were recorded on a Perkin-Elmer 1310 FTIR spectrometer between sodium chloride plates. Nuclear magnetic resonance spectra (NMR) were recorded on either a Bruker AC 250 MHz or 300 MHz spectrometer. The chemical shift values were quoted in values of ppm relative to the standard tetramethylsilane (TMS) for ^{1}H NMR and to the centre line of the chloroform triplet, δ 77.0, for ^{13}C NMR. The peak multiplicities were specified as singlet (s), doublet (d), doublet-doublet (dd), triplet (t), quartet (q) or quintet (quint) and coupling constants (*J*) quoted

in Hz. Mass spectra (MS) were obtained from the EPSRC Mass Spectrometry Service Centre, Swansea, as were high resolution determinations unless indicated, then mass spectra were obtained with a Kratos analytical MS80 RFAO spectrometer. Elemental analyses were performed with a Carlo Erba 1106 elemental analyser. Reverse phase HPLC was performed with a C_{18} Anachem ODS2 analytical column or a C_{18} technology ODS semi-preparative column using a Krontron HPLC pump 422 and 332 UV detector. The absorbance was measured at 254 nm.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]acetic acid methyl ester, 4a

To a solution of methyl-4-hydroxyphenyl acetate (4.8 g, 29 mmol), triphenylphosphine (7.6 g, 29 mmol) and diethyl azodicarboxylate (4.6 mL, 29 mmol) in toluene (350 mL) at 80 °C was added disperse red (5 g, 14.3 mmol, 0.5 eq.). The reaction mixture was stirred at 80 °C for 30 minutes and then cooled to room temperature. This was then concentrated at 50 °C under reduced pressure, then firstly crystallised and secondly recrystallised from ethyl acetate–hexane to give **4a** as dark purple fine needles (4.18 g, 8.41 mmol, 58%). Mp 109–110 °C (ethyl acetate–hexane); (Found: C, 60.50; H, 5.09; N, 11.28. $C_{25}H_{25}ClN_4O_5$ requires C, 60.42; H, 5.07; N, 11.27%); ν_{max}(Nujol)/cm^{-1} 1737, 1602, 1512, 1339; δ_H(300 MHz; $CDCl_3$) 8.33 (1 H, d, *J* 2.5, ArC**H**), 8.09 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.91 (2 H, d, *J* 9.2, ArC**H**), 7.74 (1 H, d, *J* 9.0, ArC**H**), 7.19 (2 H, d, *J* 8.8, ArC**H**), 6.84 (2 H, d, *J* 8.8, ArC**H**), 6.78 (2 H, d, *J* 9.2, ArC**H**), 4.16 (2 H, t, *J* 5.8, ArOC**H**$_2$CH$_2$), 3.83 (2 H, t, *J* 5.8, ArOCH$_2$C**H**$_2$), 3.67 (3 H, s, CO_2**Me**), 3.60 (2 H, q, *J* 7.1, C**H**$_2$Me), 3.56 (2 H, s, ArC**H**$_2$), 1.28 (3 H, t, *J* 7.1, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 172.7 (C*i*), 158.0 (C*i*), 153.4 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 130.8 (2 CH), 127.4 (CH), 127.1 (C*i*), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 115.0 (2 CH), 111.9 (2 CH), 65.7 (CH_2), 52.4 (CH_3), 50.3 (CH_2), 46.7 (CH_2), 40.6 (CH_2), 12.7 (CH_3); *m*/*z* (EI) 496 (M^+), 498 (M^+ + 2); (CI) 497 (MH^+), 499 (MH^+ + 2); COSY and HMQC spectra both exhibited a good correlation with the proposed structure.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]acetic acid

To a solution of the protected disperse red derivative **4a** (1.08 g, 2.17 mmol) in tetrahydrofuran (150 mL) and water (10 mL) was added lithium hydroxide monohydrate (182 mg, 4.34 mmol, 2.0 eq.). The reaction mixture was stirred at 45 °C for 30 minutes after which time TLC (ethyl acetate–hexane, 4 : 1) indicated that the starting material had been consumed. This was then cooled to room temperature, concentrated at 30 °C under reduced pressure, diluted with dichloromethane (40 mL), washed with HCl (2 M, 3 × 30 mL), water (3 × 30 mL), dried ($MgSO_4$) and concentrated *in vacuo* to give the acid as a red powder (828 mg, 1.71 mmol, 79%). Mp 192–193 °C (ethyl acetate–hexane); (Found: C, 59.43; H, 4.82; N, 11.65. $C_{24}H_{23}ClN_4O_5$ requires C, 59.69; H, 4.80; N, 11.60%); ν_{max}(Nujol)/cm^{-1} 1695, 1601, 1511, 1337; δ_H(300 MHz; $CDCl_3$) 12.26 (1 H, br s, CO_2**H**), 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.20 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.84 (2 H, d, *J* 9.3, ArC**H**), 7.74 (1 H, d, *J* 9.0, ArC**H**), 7.17 (2 H, d, *J* 8.7, ArC**H**), 6.94 (2 H, d, *J* 9.3, ArC**H**), 6.88 (2 H, d, *J* 8.7, ArC**H**), 4.17 (2 H, t, *J* 5.4, ArOC**H**$_2$CH$_2$), 3.86 (2 H, t, *J* 5.4, ArOCH$_2$C**H**$_2$), 3.62 (2 H, q, *J* 7.0, C**H**$_2$Me), 3.48 (2 H, s, ArC**H**$_2$), 1.19 (3 H, t, *J* 7.0, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 157.4 (C*i*), 152.6 (C*i*), 152.5 (C*i*), 147.0 (C*i*), 143.7 (C*i*), 132.7 (C*i*), 130.8 (2 CH), 127.7 (C*i*), 127.1 (CH), 126.0 (CH), 123.6 (CH), 118.3 (2 CH), 114.5 (2 CH), 112.2 (2 CH), 65.7 (CH_2), 49.6 (CH_2), 45.8 (CH_2), 40.1 (CH_2), 12.4 (CH_3); *m*/*z* (EI) 482 (M^+), 484 (M^+ + 2); (CI) 483 (MH^+), 485 (MH^+ + 2); COSY and HMQC spectra both exhibited a good correlation with the proposed structure.

3-(4-Hydroxyphenyl)propionic acid methyl ester 3b

To a solution of 3-(4-hydroxyphenyl)propionic acid (1.0 g, 6.0 mmol) dissolved in methanol (100 mL), 4 drops of concentrated sulfuric acid were carefully added. The reaction mixture was refluxed for 3 hours after which TLC (ethyl acetate–hexane, 2 : 3) indicated complete conversion of the starting material. The reaction mixture was allowed to cool to room temperature and the methanol removed under reduced pressure. The product was firstly precipitated from diethyl ether and hexane at −78 °C and then further purified by careful recrystallisation at −20 °C from diethyl ether, cyclohexane and hexane to give white needles (594 mg, 3.3 mmol, 55%). Alternatively the product could be isolated *via* reduced pressure distillation on a larger scale at 148 °C (0.2 bar) to give a comparative yield, (66%). Isolation of the product *via* flash column chromatography (ethyl acetate–hexane, 1 : 4) gave an improved yield of 94%. Mp 36–37 °C (diethyl ether–cyclohexane); (Found: C, 67.00; H, 6.77. $C_{10}H_{12}O_3$ requires C, 66.65; H, 6.71%); ν_{max}(Nujol)/cm^{-1} 3398, 1715, 1614; δ_H(300 MHz; $CDCl_3$) 7.03 (2 H, d, *J* 8.6, ArC**H**), 6.75 (2 H, d, *J* 8.6, ArC**H**), 6.14 (1 H, s, ArO**H**), 3.67 (3 H, s, CO_2**Me**), 2.87 (2 H, t, *J* 7.8, ArCH$_2$C**H**$_2$), 2.60 (2 H, t, *J* 7.8, ArC**H**$_2$CH$_2$); δ_C(300 MHz; $CDCl_3$) 174.7 (C*i*), 154.7 (C*i*), 132.6 (C*i*), 129.8 (2 CH), 115.8 (2 CH), 52.3 (CH_3), 36.5 (CH_2), 30.5 (CH_2); *m*/*z* (EI) 180 (M^+); (CI) 181 (MH^+), 198 (MNH_4^+); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid methyl ester, 4b

To a solution of **3b** (3 g, 16.7 mmol), triphenylphosphine (4.37 g, 16.7 mmol) and diethyl azodicarboxylate (2.59 mL, 16.7 mmol) in toluene (250 mL) at 80 °C was added disperse red (5.2 g, 15.0 mmol, 0.9 eq.). The reaction mixture was stirred at 80 °C for 6 hours after which time TLC (ethyl acetate–hexane, 4 : 1) indicated that the disperse red had been entirely consumed. The toluene was then removed at 50 °C under reduced pressure and the product isolated by flash column chromatography (ethyl acetate–hexane, 2 : 3) to give **4b** as a purple powder (4.43 g, 8.67 mmol, 52%). Mp 116–118 °C (ethyl acetate–hexane); (Found: C, 61.09; H, 5.32; N, 10.92. $C_{26}H_{27}ClN_4O_5$ requires C, 61.11; H, 5.33; N, 10.96%); ν_{max}(Nujol)/cm^{-1} 1734, 1599, 1512, 1336; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.4, ArC**H**), 8.13 (1 H, dd, *J* 8.9, 2.4, ArC**H**), 7.94 (2 H, d, *J* 8.9, ArC**H**), 7.77 (1 H, d, *J* 8.9, ArC**H**), 7.11 (2 H, d, *J* 8.9, ArC**H**), 6.81 (4 H, d, *J* 8.9, ArC**H**), 4.17 (2 H, t, *J* 5.7, ArOC**H**$_2$CH$_2$), 3.85 (2 H, t, *J* 5.7, ArOCH$_2$C**H**$_2$), 3.72–3.59 (5 H, m, CO_2**Me,** C**H**$_2$Me), 2.89 (2 H, t, *J* 7.6, CH$_2$C**H**$_2$CO$_2$), 2.59 (2 H, t, *J* 7.6, C**H**$_2$CH$_2$CO$_2$), 1.29 (3 H, t, *J* 7.1, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 156.8 (C*i*), 153.0 (C*i*), 151.6 (C*i*), 147.0 (C*i*), 144.3 (C*i*), 133.9 (C*i*), 133.2 (C*i*), 129.3 (CH), 126.9 (CH), 126.0 (CH), 122.6 (CH), 118.0 (CH), 114.4 (CH), 111.5 (CH), 65.2 (CH_2), 51.6 (CH_3), 50.0 (CH_2), 46.3 (CH_2), 35.9 (CH_2), 30.0 (CH_2), 12.3 (CH_3); *m*/*z* (EI) 510 (M^+), 512 (M^+ + 2); (CI) 511 (MH^+), 513 (MH^+ + 2); COSY and HMQC spectra both exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid

To a solution of **4b** (2 g, 3.92 mmol) in tetrahydrofuran (150 mL) and water (5 mL) was added lithium hydroxide monohydrate (247 mg, 5.88 mmol, 1.5 eq.). The reaction mixture was stirred at room temperature for 12 hours and then 40 °C for 3 hours after which TLC (ethyl acetate–hexane, 4 : 1) indicated that the starting material had been consumed. This was then allowed to cool to room temperature, concentrated at 30 °C under reduced pressure, diluted with dichloromethane (40 mL), washed with HCl (2 M, 3 × 30 mL) and water

(3 × 30 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The product was eluted by flash column chromatography (ethyl acetate–hexane, 3 : 2) to give a dark red oil which solidified on standing (1.46 g, 2.94 mmol, 75%). A sample of this solid was recrystallised from hexane–EtOAc. Mp 177–179 °C (ethyl acetate–hexane); (Found: C, 60.33; H, 5.08; N, 11.13. $C_{25}H_{25}ClN_4O_5$ requires C, 60.42; H, 5.07; N, 11.27%); ν_{max}(Nujol)/cm^{-1} 1698, 1603, 1511, 1341; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.18 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.80 (2 H, d, *J* 9.2, ArC**H**), 7.73 (1 H, d, *J* 9.0, ArC**H**), 7.06 (2 H, d, *J* 8.6, ArC**H**), 6.91 (2 H, d, *J* 9.2, ArC**H**), 6.78 (2 H, d, *J* 8.6, ArC**H**), 4.10 (2 H, t, *J* 5.4, ArOC$\mathbf{H}_2$$CH_2$), 3.81 (2 H, t, *J* 5.4, ArOCH_2C$\mathbf{H}_2$), 3.56 (2 H, q, *J* 7.0, C$\mathbf{H}_2$Me), 2.68 (2 H, t, *J* 7.5, CH_2C$\mathbf{H}_2$$CO_2$), 2.41 (2 H, t, *J* 7.5, C$\mathbf{H}_2$$CH_2CO_2$), 1.14 (3 H, t, *J* 7.0, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 174.3 (C*i*), 156.8 (C*i*), 152.7 (C*i*), 152.5 (C*i*), 147.1 (C*i*), 143.7 (C*i*), 133.9 (C*i*), 132.7 (C*i*), 129.6 (2 CH), 127.1 (CH), 126.1 (CH), 123.7 (CH), 118.4 (2 CH), 114.5 (2 CH), 112.3 (2 CH), 65.7 (CH_2), 49.6 (CH_2), 45.8 (CH_2), 36.6 (CH_2), 30.2 (CH_2), 12.4 (CH_3); *m*/*z* (CI) 497 (MH^+), 499 (MH^+ + 2); COSY spectra exhibited a good correlation with the proposed structure.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]acetic acid 2-(2-hydroxyethoxy)ethyl ester, 5a, *m* = 1 (DL21)

To a solution of **4a** (200 mg, 0.41 mmol), triphenylphosphine (162 mg, 0.62 mmol, 1.5 eq.) and diethyl azodicarboxylate (108 mg, 0.62 mmol, 1.5 eq.) in toluene (20 mL), diethylene glycol (66 mg, 0.62 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5a**, *m* = 1 as a dark red oil (190 mg, 0.33 mmol, 81%). ν_{max}(Nujol)/cm^{-1} 3451, 1731, 1599, 1514, 1335; δ_H(300 MHz; $CDCl_3$) 8.38 (1 H, d, *J* 2.4, ArC**H**), 8.14 (1 H, dd, *J* 9.0, 2.4, ArC**H**), 7.95 (2 H, d, *J* 9.0, ArC**H**), 7.78 (1 H, d, *J* 9.0, ArC**H**), 7.21 (2 H, d, *J* 9.3, ArC**H**), 6.85 (2 H, d, *J* 9.0, ArC**H**), 6.82 (2 H, d, *J* 9.3, ArC**H**), 4.26 (2 H, t, *J* 4.7, C$\mathbf{H}_2$), 4.18 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$$CH_2$), 3.86 (2 H, t, *J* 5.6, ArOCH_2C$\mathbf{H}_2$), 3.72–3.67 (4 H, m, C$\mathbf{H}_2$), 3.64–3.54 (6 H, m, C$\mathbf{H}_2$), 1.95 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.0, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 172.2 (C*i*), 158.0 (C*i*), 153.5 (C*i*), 152.0 (C*i*), 147.5 (C*i*), 144.8 (C*i*), 134.3 (C*i*), 130.8 (2 CH), 127.4 (CH), 127.0 (C*i*), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 112.0 (2 CH), 72.7 (CH_2), 69.4 (CH_2), 65.7 (CH_2), 64.2 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.8 (CH_2), 40.7 (CH_2), 12.7 (CH_3); *m*/*z* (CI) 571 (MH^+), 573 (MH^+ + 2) (Found: MH^+, 571.1947. $C_{28}H_{31}{}^{35}ClN_4O_7$ requires MH^+, 571.1960); HMQC spectra exhibited a good correlation with the proposed structure.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]acetic acid 2-[2-(2-hydroxyethoxy)ethoxy]ethyl ester, 5a *m* = 2 (DL31)

To a solution of **4a** (200 mg, 0.41 mmol), triphenylphosphine (162 mg, 0.62 mmol, 1.5 eq.) and diethyl azodicarboxylate (108 mg, 0.62 mmol, 1.5 eq.) in toluene (20 mL) was added triethylene glycol (93 mg, 0.62 mmol, 1.5 eq.). The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5a**, *m* = 2 as a dark red oil (180 mg, 0.29 mmol, 71%). ν_{max}(Nujol)/cm^{-1} 3439, 1736, 1599, 1513, 1336; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.93 (2 H, d, *J* 9.2, ArC**H**), 7.76 (1 H, d, *J* 9.0, ArC**H**), 7.20 (2 H, d, *J* 8.7, ArC**H**), 6.84 (2 H, d, *J* 8.7, ArC**H**), 6.81 (2 H, d, *J* 9.2, ArC**H**), 4.25 (2 H, t, *J* 4.7, C$\mathbf{H}_2$), 4.17 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$$CH_2$), 3.85 (2 H, t, *J* 5.7, ArOCH_2C$\mathbf{H}_2$), 3.74–3.68 (4 H, m, C$\mathbf{H}_2$), 3.66–3.58 (10 H, m, C$\mathbf{H}_2$), 2.41 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.0, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 172.3 (C*i*), 158.0 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 130.9 (2 CH), 127.4 (CH), 127.0 (C*i*), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 111.9 (2 CH), 72.9 (CH_2), 70.9 (CH_2), 70.7 (CH_2), 69.5 (CH_2), 65.7 (CH_2), 64.2 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 40.6 (CH_2), 12.7 (CH_3); *m*/*z* (CI) 615 (MH^+), 617 (MH^+ + 2) (Found: M^+, 614.2140. $C_{30}H_{35}{}^{35}ClN_4O_8$ requires M^+, 614.2143); HMQC spectra exhibited a good correlation with the proposed structure.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}ethoxy)-phenyl]acetic acid 2-{2-[2-(2-hydroxyethoxy)ethoxy]ethoxy}-ethyl ester, 5a, *m* = 3 (DL41)

To a solution of **4a** (200 mg, 0.41 mmol), triphenylphosphine (162 mg, 0.62 mmol, 1.5 eq.) and diethyl azodicarboxylate (108 mg, 0.62 mmol, 1.5 eq.) in toluene (20 mL) was added tetraethylene glycol (120 mg, 0.62 mmol, 1.5 eq.). The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5a**, *m* = 3 as a dark red oil (198 mg, 0.30 mmol, 73%). ν_{max}(Nujol)/cm^{-1} 3441, 1737, 1600, 1514, 1337; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.14 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.94 (2 H, d, *J* 9.1, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.20 (2 H, d, *J* 8.6, ArC**H**), 6.84 (2 H, d, *J* 8.6, ArC**H**), 6.81 (2 H, d, *J* 9.1, ArC**H**), 4.25 (2 H, t, *J* 4.8, C$\mathbf{H}_2$), 4.18 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$$CH_2$), 3.86 (2 H, t, *J* 5.7, ArOCH_2C$\mathbf{H}_2$), 3.73–3.59 (18 H, m, C$\mathbf{H}_2$), 2.46 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.1, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 172.2 (C*i*), 157.9 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.8 (C*i*), 134.3 (C*i*), 130.9 (2 CH), 127.4 (CH), 127.1 (C*i*), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 112.0 (2 CH), 72.9 (CH_2), 71.0 (CH_2), 70.9 (2 CH_2), 70.7 (CH_2), 69.5 (CH_2), 65.7 (CH_2), 64.3 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 40.6 (CH_2), 12.7 (CH_3); *m*/*z* (EI) 658 (M^+), 660 (M^+ + 2); (CI) 659 (MH^+), 661 (MH^+ + 2) (Found: M^+, 658.2413. $C_{32}H_{39}{}^{35}ClN_4O_9$ requires M^+, 658.2406); HMQC spectra exhibited a good correlation with the proposed structure.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]acetic acid 2-(2-{2-[2-(2-hydroxyethoxy)ethoxy]-ethoxy}ethoxy)ethyl ester, 5a, *m* = 4 (DL51)

To a solution of **4a** (200 mg, 0.41 mmol), triphenylphosphine (162 mg, 0.62 mmol, 1.5 eq.) and diethyl azodicarboxylate (108 mg, 0.62 mmol, 1.5 eq.) in toluene (20 mL) was added pentaethylene glycol (148 mg, 0.62 mmol, 1.5 eq.). The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5a**, *m* = 4 as a dark red oil (225 mg, 0.32 mmol, 78%). (Found: C, 57.89; H, 6.17; N, 7.91. $C_{34}H_{43}ClN_4O_{10}$ requires C, 58.07; H, 6.16; N, 7.97%); ν_{max}(Nujol)/cm^{-1} 3466, 1731, 1599, 1518, 1336; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.94 (2 H, d, *J* 9.3, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.21 (2 H, d, *J* 8.8, ArC**H**), 6.84 (2 H, d, *J* 8.8, ArC**H**), 6.81 (2 H, d, *J* 9.3, ArC**H**), 4.24 (2 H, t, *J* 4.8, C$\mathbf{H}_2$), 4.17 (2 H, t, *J* 5.8, ArOC$\mathbf{H}_2$$CH_2$), 3.85 (2 H, t, *J* 5.8, ArOCH_2C$\mathbf{H}_2$), 3.73–3.58 (22 H, m, C$\mathbf{H}_2$), 2.66 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.1, CH_2**Me**); δ_C(300 MHz; $CDCl_3$) 172.2 (C*i*), 157.9 (C*i*), 153.5 (C*i*), 152.1

(C*i*), 147.5 (C*i*), 144.8 (C*i*), 134.3 (C*i*), 130.9 (2 CH), 127.4 (CH), 127.1 (C*i*), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 112.0 (2 CH), 72.9 (CH_2), 70.9 ($5CH_2$), 70.7 (CH_2), 69.4 (CH_2), 65.7 (CH_2), 64.3 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 40.6 (CH_2), 12.7 (CH_3); *m*/*z* (EI) 702 (M^+), 704 (M^++2); (CI) 703 (MH^+), 705 (MH^++2); HMQC spectra exhibited a good correlation with the proposed structure.

[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]acetic acid 2-[2-(2-{2-[2-(2-hydroxyethoxy)-ethoxy]ethoxy}ethoxy)ethoxy]ethyl ester, 5a, *m* = 5 (DL61)

To a solution of **4a** (200 mg, 0.41 mmol), triphenylphosphine (162 mg, 0.62 mmol, 1.5 eq.) and diethyl azodicarboxylate (108 mg, 0.62 mmol, 1.5 eq.) in toluene (20 mL) was added hexaethylene glycol (175 mg, 0.62 mmol, 1.5 eq.). The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5a**, *m* = 5 as a dark red oil (209 mg, 0.28 mmol, 68%). ν_{max}(Nujol)/cm^{-1} 3463, 1731, 1598, 1513, 1337; δ_H(300 MHz; $CDCl_3$) 8.36 (1 H, d, *J* 2.5, ArC**H**), 8.12 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.93 (2 H, d, *J* 9.2, ArC**H**), 7.76 (1 H, d, *J* 9.0, ArC**H**), 7.20 (2 H, d, *J* 8.7, ArC**H**), 6.84 (2 H, d, *J* 8.7, ArC**H**), 6.81 (2 H, d, *J* 9.2, ArC**H**), 4.24 (2 H, t, *J* 4.8, C$\mathbf{H}_2$), 4.17 (2 H, t, *J* 5.8, ArOC$\mathbf{H}_2$CH$_2$), 3.85 (2H, t, *J* 5.8, ArOCH$_2$C$\mathbf{H}_2$), 3.73–3.58 (26 H, m, C$\mathbf{H}_2$), 2.76 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); δ_C(300 MHz; $CDCl_3$) 172.2 (C*i*), 157.9 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 130.9 (2 CH), 127.4 (CH), 127.0 (C*i*), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 111.9 (2 CH), 72.9 (CH_2), 71.0 (CH_2), 70.9 ($6CH_2$), 70.7 (CH_2), 69.4 (CH_2), 65.7 (CH_2), 64.4 (CH_2), 62.1 (CH_2), 50.3 (CH_2), 46.7 (CH_2), 40.6 (CH_2), 12.7 (CH_3); *m*/*z* (EI) 746 (M^+), 748 (M^+ + 2); (CI) 747 (MH^+), 749 (MH^+ + 2) (Found: M^+, 746.2914. $C_{36}H_{47}{}^{35}ClN_4O_{11}$ requires M^+, 746.2930); HMQC spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-(2-hydroxyethoxy)ethyl ester, 5b, *m* = 1 (DL22)

To a solution of **4b** (200 mg, 0.40 mmol), triphenylphosphine (158 mg, 0.60 mmol, 1.5 eq.) and diethyl azodicarboxylate (105 mg, 0.60 mmol, 1.5 eq.) in dichloromethane (20 mL) diethylene glycol (64 mg, 0.60 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 3 hours at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5b**, *m* = 1 as a dark red oil (174 mg, 0.30 mmol, 74%). ν_{max}(Nujol)/cm^{-1} 3443, 1731, 1599, 1513, 1336; δ_H(300 MHz; $CDCl_3$) 8.36 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.93 (2 H, d, *J* 9.1, ArC**H**), 7.76 (1 H, d, *J* 9.0, ArC**H**), 7.11 (2 H, d, *J* 8.5, ArC**H**), 6.81 (2 H, d, *J* 8.5, ArC**H**), 6.80 (2 H, d, *J* 9.1, ArC**H**), 4.23 (2 H, t, *J* 4.6, C$\mathbf{H}_2$), 4.16 (2 H, t, *J* 5.6, ArOC$\mathbf{H}_2$CH$_2$), 3.84 (2 H, t, *J* 5.6, ArOCH$_2$C$\mathbf{H}_2$), 3.73–3.55 (8 H, m, C$\mathbf{H}_2$), 2.89 (2 H, t, *J* 7.6, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.63 (2 H, t, *J* 7.6, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.12 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.0, CH$_2$**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.5 (C*i*), 129.8 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.8 (2 CH), 111.9 (2 CH), 72.7 (CH_2), 69.5 (CH_2), 65.7 (CH_2), 63.9 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 36.4 (CH_2), 30.4 (CH_2), 12.7 (CH_3); *m*/*z* (CI) 585 (MH^+), 587 (MH^+ + 2) (Found: MH^+, 585.2113. $C_{29}H_{33}{}^{35}ClN_4O_7$ requires MH^+, 585.2116).

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-[2-(2-hydroxyethoxy)ethoxy]-ethyl ester, 5b, *m* = 2 (DL32)

To a solution of **4b** (200 mg, 0.40 mmol), triphenylphosphine (158 mg, 0.60 mmol, 1.5 eq.) and diethyl azodicarboxylate (104 mg, 0.60 mmol, 1.5 eq.) in toluene (20 mL), triethylene glycol (91 mg, 0.60 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5b**, *m* = 2 as a dark red oil (175 mg, 0.28 mmol, 69%). ν_{max}(Nujol)/cm^{-1} 3456, 1736, 1599, 1514, 1337; δ_H(300 MHz; $CDCl_3$) 8.36 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.93 (2 H, d, *J* 8.9, ArC**H**), 7.76 (1 H, d, *J* 9.0, ArC**H**), 7.12 (2 H, d, *J* 8.9, ArC**H**), 6.81 (4 H, d, *J* 8.9, ArC**H**), 4.23 (2 H, t, *J* 4.7, C$\mathbf{H}_2$), 4.16 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 3.85 (2 H, t, *J* 5.7, ArOCH$_2$C$\mathbf{H}_2$), 3.73–3.58 (12 H, m, C$\mathbf{H}_2$), 2.89 (2 H, t, *J* 7.6, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.63 (2 H, t, *J* 7.6, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.41 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.0, CH$_2$**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.6 (C*i*), 129.8 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.8 (2 CH), 112.0 (2 CH), 72.9 (CH_2), 70.9 (CH_2), 70.7 (CH_2), 69.5 (CH_2), 65.7 (CH_2), 63.8 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 36.4 (CH_2), 30.4 (CH_2), 12.7 (CH_3); *m*/*z* (EI) 628 (M^+), 630 (M^+ + 2); (CI) 629 (MH^+), 631 (MH^+ + 2) (Found: M^+, 628.2296. $C_{31}H_{37}{}^{35}ClN_4O_8$ requires M^+, 628.2300).

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-{2-[2-(2-hydroxyethoxy)ethoxy]-ethoxy}ethyl ester, 5b, *m* = 3 (DL42)

To a solution of **4b** (200 mg, 0.40 mmol), triphenylphosphine (158 mg, 0.60 mmol, 1.5 eq.) and diethyl azodicarboxylate (104 mg, 0.60 mmol, 1.5 eq.) in toluene (20 mL) tetraethylene glycol (117 mg, 0.60 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5b**, *m* = 3 as a dark red oil (190 mg, 0.28 mmol, 70%). ν_{max}(Nujol)/cm^{-1} 3457, 1731, 1599, 1519, 1337; δ_H(300 MHz; $CDCl_3$) 8.35 (1 H, d, *J* 2.5, ArC**H**), 8.11 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.92 (2 H, d, *J* 9.2, ArC**H**), 7.76 (1 H, d, *J* 9.0, ArC**H**), 7.12 (2 H, d, *J* 8.5, ArC**H**), 6.81 (2 H, d, *J* 8.5, ArC**H**), 6.80 (2 H, d, *J* 9.2, ArC**H**), 4.23 (2 H, t, *J* 4.7, C$\mathbf{H}_2$), 4.16 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 3.84 (2 H, t, *J* 5.7, ArOCH$_2$C$\mathbf{H}_2$), 3.73–3.58 (16 H, m, C$\mathbf{H}_2$), 2.89 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.62 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.61 (1 H, s, O**H**), 1.29 (3 H, t, *J* 7.0, CH$_2$**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 157.3 (C*i*), 153.4 (C*i*), 152.1 (C*i*), 147.4 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.6 (C*i*), 129.8 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.8 (2 CH), 111.9 (2 CH), 72.9 (CH_2), 71.0 (CH_2), 70.9 ($2CH_2$), 70.7 (CH_2), 69.5 (CH_2), 65.7 (CH_2), 63.9 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 36.4 (CH_2), 30.4 (CH_2), 12.7 (CH_3); *m*/*z* (EI) 672 (M^+), 674 (M^+ + 2); (CI) 673 (MH^+), 675 (MH^+ + 2) (Found: M^+, 672.2553. $C_{33}H_{41}{}^{35}ClN_4O_9$ requires M^+, 672.2562).

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-(2-{2-[2-(2-hydroxyethoxy)-ethoxy]ethoxy}ethoxy)ethyl ester, 5b, *m* = 4 (DL52)

To a solution of **4b** (200 mg, 0.40 mmol), triphenylphosphine (158 mg, 0.60 mmol, 1.5 eq.) and diethyl azodicarboxylate (104 mg, 0.60 mmol, 1.5 eq.) in toluene (20 mL), pentaethylene glycol (144 mg, 0.60 mmol, 1.5 eq.) was added. The reaction

mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5b**, $m = 4$ as a dark red oil (190 mg, 0.26 mmol, 66%). ν_{max}(Nujol)/cm^{-1} 3477, 1731, 1599, 1519, 1337; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.1, 2.5, ArC**H**), 7.93 (2 H, d, *J* 8.8, ArC**H**), 7.77 (1 H, d, *J* 9.1, ArC**H**), 7.12 (2 H, d, *J* 8.8, ArC**H**), 6.81 (4 H, d, *J* 8.8, ArC**H**), 4.22 (2 H, t, *J* 4.8, C**H**$_2$), 4.16 (2 H, t, *J* 5.8, ArOC**H**$_2$CH$_2$), 3.85 (2 H, t, *J* 5.8, ArOCH$_2$C**H**$_2$), 3.73–3.58 (20 H, m, C**H**$_2$), 2.89 (2 H, t, *J* 7.7, CH$_2$C**H**$_2$CO$_2$), 2.68 (1 H, s, O**H**), 2.62 (2 H, t, *J* 7.7, C**H**$_2$CH$_2$CO$_2$), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.6 (C*i*), 129.8 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.8 (2 CH), 111.9 (2 CH), 72.9 (CH$_2$), 70.9 (5CH$_2$), 70.7 (CH$_2$), 69.5 (CH$_2$), 65.7 (CH$_2$), 64.0 (CH$_2$), 62.1 (CH$_2$), 50.4 (CH$_2$), 46.7 (CH$_2$), 36.4 (CH$_2$), 30.4 (CH$_2$), 12.7 (CH$_3$); *m*/*z* (CI) 717 (MH$^+$), 719 (MH$^+$ + 2) (Found: MH$^+$, 717.2897. $C_{35}H_{43}{}^{35}ClN_4O_{10}$ requires MH$^+$, 717.2902).

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-[2-(2-{2-[2-(2-hydroxyethoxy)-ethoxy]ethoxy}ethoxy)ethoxy]ethyl ester, 5b, $m = 5$ (DL62)

To a solution of **4b** (200 mg, 0.40 mmol), triphenylphosphine (158 mg, 0.60 mmol, 1.5 eq.) and diethyl azodicarboxylate (104 mg, 0.60 mmol, 1.5 eq.) in toluene (20 mL), hexaethylene glycol (170 mg, 0.60 mmol, 1.5 eq.) was added. The reaction mixture was stirred for 5 days at room temperature after which TLC (methanol–ethyl acetate, 1 : 9) indicated that the acid had been entirely consumed. The toluene was removed at 50 °C under reduced pressure and the product eluted by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **5b**, $m = 5$ as a dark red oil (184 mg, 0.24 mmol, 60%). ν_{max}(Nujol)/cm^{-1} 3455, 1732, 1600, 1519, 1337; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.3, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.3, ArC**H**), 7.94 (2 H, d, *J* 8.9, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.12 (2 H, d, *J* 8.9, ArC**H**), 6.81 (4 H, d, *J* 8.9, ArC**H**), 4.22 (2 H, t, *J* 4.7, C**H**$_2$), 4.17 (2 H, t, *J* 5.7, ArOC**H**$_2$CH$_2$), 3.85 (2 H, t, *J* 5.7, ArOCH$_2$C**H**$_2$), 3.73–3.58 (24 H, m, C**H**$_2$Me), 2.89 (2 H, t, *J* 7.7, CH$_2$C**H**$_2$CO$_2$), 2.79 (1 H, s, O**H**), 2.62 (2 H, t, *J* 7.7, C**H**$_2$CH$_2$CO$_2$), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); δ_C(300 MHz; $CDCl_3$) 173.3 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.6 (C*i*), 129.8 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.8 (2 CH), 111.9 (2 CH), 73.0 (CH$_2$), 71.0 (CH$_2$), 70.9 (6CH$_2$), 70.7 (CH$_2$), 69.5 (CH$_2$), 65.7 (CH$_2$), 64.0 (CH$_2$), 62.1 (CH$_2$), 50.4 (CH$_2$), 46.7 (CH$_2$), 36.4 (CH$_2$), 30.4 (CH$_2$), 12.7 (CH$_3$); *m*/*z* (CI) 761 (MH$^+$), 763 (MH$^+$ + 2) (Found: MH$^+$, 761.3165. $C_{37}H_{49}{}^{35}ClN_4O_{11}$ requires MH$^+$, 761.3165).

3-(4-Hydroxyphenyl)propionic acid *tert*-butyl ester, 6

To a solution of 3-(4-hydroxyphenyl)propionic acid (3.32 g, 20 mmol) in DMF (20 mL) was carefully added carbonyldiimidazole (3.24 g, 20 mmol). The reaction mixture was stirred at 40 °C for 2 hours. DBU (6.08 mL, 40 mmol, 2 eq.) and *tert*-butanol (3.70 mL, 50 mmol, 2.5 eq.) were then added and the reaction mixture stirred at 65 °C for 2 days after which time TLC (ethyl acetate–hexane, 1 : 4) indicated that the starting material had been consumed. The reaction mixture was cooled to room temperature, water (40 mL) added and the product extracted with diethyl ether (3 × 20 mL). The organic fraction was dried ($MgSO_4$), concentrated under reduced pressure and the product isolated by flash column chromatography (ethyl acetate–hexane, 1 : 4) to give **6** as a clear colourless oil (2.89 g, 13 mmol, 65%). ν_{max}(Nujol)/cm^{-1} 3400, 1700, 1601, 1516; δ_H(300 MHz; $CDCl_3$) 7.05 (1 H, s, ArO**H**), 7.01 (2 H, d, *J* 8.5, ArC**H**), 6.74 (2 H, d, *J* 8.5, ArC**H**), 2.82 (2 H, t, *J* 7.6, ArCH$_2$C**H**$_2$), 2.51 (2 H, t, *J* 7.6, ArC**H**$_2$CH$_2$), 1.41 (9 H, s, C*i***Me**$_3$); δ_C(300 MHz; $CDCl_3$) 174.0 (C*i*), 154.9 (C*i*), 132.4 (C*i*), 129.8 (2 CH), 115.8 (2 CH), 81.5 (C*i*), 38.0 (CH$_2$), 30.7 (CH$_2$), 15.5 (CH$_3$); *m*/*z* (CI) 240 (MNH$_4^+$) (Found: MNH$_4^+$, 240.1597. $C_{13}H_{18}O_3$ requires MNH$_4^+$, 240.1600); COSY spectra exhibited a good correlation with the proposed structure.

3-{4-[2-(2-{2-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethyl-amino}ethoxy)phenyl]acetoxy}ethoxy)ethoxy]phenyl}propionic acid *tert*-butyl ester, 7a, $m = 1$

To a solution of **5a**, m = 1 (144 mg, 0.25 mmol), triphenylphosphine (98 mg, 0.38 mmol, 1.5 eq.) and diethyl azodicarboxylate (66 mg, 0.38 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (111 mg, 0.50 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7a**, m = 1 as a dark red oil (102 mg, 0.13 mmol, 53%). ν_{max}(Nujol)/cm^{-1} 1729, 1600, 1513, 1340; δ_H(300 MHz; $CDCl_3$) 8.39 (1 H, d, *J* 2.5, ArC**H**), 8.15 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.95 (2 H, d, *J* 9.2, ArC**H**), 7.78 (1 H, d, *J* 9.0, ArC**H**), 7.19 (2 H, d, *J* 8.7, ArC**H**), 7.10 (2 H, d, *J* 8.7, ArC**H**), 6.84–6.79 (6 H, m, ArC**H**), 4.27 (2 H, t, *J* 4.8, ArOC**H**$_2$CH$_2$), 4.16 (2 H, t, *J* 5.8, ArOCH$_2$CH$_2$), 4.07 (2 H, t, *J* 4.8, OCH$_2$C**H**$_2$O$_2$C), 3.87–3.74 (6 H, m, C**H**$_2$), 3.62 (2 H, q, *J* 7.2, C**H**$_2$Me), 3.58 (2 H, s, ArC**H**$_2$), 2.84 (2 H, t, *J* 7.6, CH$_2$C**H**$_2$CO$_2$), 2.50 (2 H, t, *J* 7.6, C**H**$_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i***Me**$_3$), 1.29 (3 H, t, *J* 7.2, CH$_2$**Me**); *m*/*z* (FAB) 774 (MH$^+$), 797 (MNa$^+$) (Found: MH$^+$, 775.3120. $C_{41}H_{47}{}^{35}ClN_4O_9$ requires MH$^+$, 775.3110); COSY spectra exhibited a good correlation with the proposed structure.

3-(4-{2-[2-(2-{2-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]-ethylamino}ethoxy)phenyl]acetoxy}ethoxy)ethoxy]ethoxy}-phenyl)propionic acid *tert*-butyl ester, 7a, $m = 2$

To a solution of **5a**, m = 2 (168 mg, 0.27 mmol), triphenylphosphine (108 mg, 0.41 mmol, 1.5 eq.) and diethyl azodicarboxylate (71 mg, 0.41 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (121 mg, 0.55 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7a**, m = 2 as a dark red oil (123 mg, 0.15 mmol, 55%). ν_{max}(Nujol)/cm^{-1} 1735, 1601, 1514, 1340; δ_H(300 MHz; $CDCl_3$) 8.39 (1 H, d, *J* 2.5, ArC**H**), 8.15 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.95 (2 H, d, *J* 9.2, ArC**H**), 7.78 (1 H, d, *J* 9.0, ArC**H**), 7.19 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.7, ArC**H**), 6.85–6.79 (6 H, m, ArC**H**), 4.24 (2 H, t, *J* 5.3, ArOC**H**$_2$CH$_2$), 4.17 (2 H, t, *J* 5.7, ArOC**H**$_2$CH$_2$), 4.09 (2 H, t, *J* 5.1, OCH$_2$C**H**$_2$O$_2$C), 3.87–3.81 (4 H, m, CH$_2$), 3.71–3.61 (8 H, m, C**H**$_2$), 3.58 (2 H, s, ArC**H**$_2$), 2.83 (2 H, t, *J* 7.7, CH$_2$C**H**$_2$CO$_2$), 2.49 (2 H, t, *J* 7.7, C**H**$_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i***Me**$_3$), 1.30 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 819 (MH$^+$), 841 (MNa$^+$) (Found: MNa$^+$, 841.3181. $C_{43}H_{51}{}^{35}ClN_4O_{10}$ requires MNa$^+$, 841.3191); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{2-[2-(2-{2-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)-phenyl]-ethylamino}ethoxy)phenyl]acetoxy}ethoxy)ethoxy]-ethoxy}ethoxy)phenyl]propionic acid *tert*-butyl ester, 7a, $m = 3$

To a solution of **5a**, m = 3 (300 mg, 0.46 mmol), triphenylphosphine (179 mg, 0.68 mmol, 1.5 eq.) and diethyl azodicarb-

oxylate (120 mg, 0.68 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (202 mg, 0.91 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7a**, m = 3 as a dark red oil (204 mg, 0.24 mmol, 52%). ν_{max}(Nujol)/cm^{-1} 1733, 1601, 1513, 1266; δ_H(300 MHz; $CDCl_3$) 8.38 (1 H, d, *J* 2.4, ArC**H**), 8.14 (1 H, dd, *J* 8.9, 2.4, ArC**H**), 7.94 (2 H, d, *J* 9.0, ArC**H**), 7.78 (1 H, d, *J* 8.9, ArC**H**), 7.19 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.7, ArC**H**), 6.86–6.79 (6 H, m, ArC**H**), 4.24 (2 H, t, *J* 5.3, ArOC$\mathbf{H}_2$CH$_2$), 4.17 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.09 (2 H, t, *J* 4.9, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.87–3.56 (16 H, m, C$\mathbf{H}_2$), 2.83 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.49 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.30 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 863 (MH$^+$), 885 (MNa$^+$) (Found: MH$^+$, 863.3619. $C_{45}H_{55}{}^{35}ClN_4O_{11}$ requires MH$^+$, 863.3634); COSY spectra exhibited a good correlation with the proposed structure.

3-{4-[2-(2-{2-[2-(2-{2-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)-phenyl]ethylamino}ethoxy)phenyl]acetoxy}ethoxy)ethoxy]-ethoxy}ethoxy)ethoxy]phenyl}propionic acid *tert*-butyl ester, 7a, $m = 4$

To a solution of **5a**, m = 4 (280 mg, 0.40 mmol), triphenylphosphine (108 mg, 0.60 mmol, 1.5 eq.) and diethyl azodicarboxylate (104 mg, 0.60 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (177 mg, 0.80 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7a**, m = 4 as a dark red oil (187 mg, 0.21 mmol, 53%). ν_{max}(Nujol)/cm^{-1} 1730, 1601, 1513, 1340; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.4, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.4, ArC**H**), 7.94 (2 H, d, *J* 9.2, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.20 (2 H, d, *J* 8.9, ArC**H**), 7.09 (2 H, d, *J* 8.7, ArC**H**), 6.86–6.79 (6 H, m, ArC**H**), 4.23 (2 H, t, *J* 4.8, ArOC$\mathbf{H}_2$CH$_2$), 4.17 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.09 (2 H, t, *J* 5.0, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.87–3.81 (4 H, m, C$\mathbf{H}_2$), 3.73–3.58 (18 H, m, C$\mathbf{H}_2$), 2.83 (2 H, t, *J* 7.8, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.49 (2 H, t, *J* 7.8, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 907 (MH$^+$), 929 (MNa$^+$) (Found: MH$^+$, 907.3904. $C_{47}H_{59}{}^{35}ClN_4O_{12}$ requires MH$^+$, 907.3896); COSY spectra exhibited a good correlation with the proposed structure.

3-(4-{2-[2-(2-{2-[2-(2-{2-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)-phenyl]ethylamino}ethoxy)phenyl]acetoxy}ethoxy)ethoxy]-ethoxy}ethoxy)ethoxy]ethoxy}phenyl)propionic acid *tert*-butyl ester, 7a, $m = 5$

To a solution of **5a**, m = 5 (245 mg, 0.33 mmol), triphenylphosphine (129 mg, 0.49 mmol, 1.5 eq.) and diethyl azodicarboxylate (86 mg, 0.49 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (146 mg, 0.66 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7a**, m = 5 as a dark red oil (184 mg, 0.19 mmol, 58%). ν_{max}(Nujol)/cm^{-1} 1731, 1601, 1514, 1340; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.4, ArC**H**), 8.13 (1 H, dd, *J* 9.1, 2.4, ArC**H**), 7.94 (2 H, d, *J* 9.2, ArC**H**), 7.77 (1 H, d, *J* 9.1, ArC**H**), 7.19 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.7, ArC**H**), 6.86–6.79 (6 H, m, ArC**H**), 4.23 (2 H, t, *J* 4.8, ArOC$\mathbf{H}_2$CH$_2$), 4.17 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.09 (2 H, t, *J* 4.9, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.87–3.81 (4 H, m, C$\mathbf{H}_2$), 3.73–3.55 (22 H, m, C$\mathbf{H}_2$), 2.83 (2 H, t, *J* 7.8, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.49 (2 H, t, *J* 7.8, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 951 (MH$^+$), 973 (MNa$^+$) (Found: MH$^+$, 951.4167. $C_{49}H_{63}{}^{35}ClN_4O_{13}$ requires MH$^+$, 951.4158); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-{2-[4-(2-*tert*-butoxycarbonyl-ethyl)phenoxy]ethoxy}ethyl ester, 7b, $m = 1$

To a solution of **5b**, m = 1 (178 mg, 0.30 mmol), triphenylphosphine (120 mg, 0.46 mmol, 1.5 eq.) and diethyl azodicarboxylate (78 mg, 0.45 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (135 mg, 0.61 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7b**, m = 1 as a dark red oil (138 mg, 0.18 mmol, 57%). ν_{max}(Nujol)/cm^{-1} 1735, 1600, 1513, 1339; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.4, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.4, ArC**H**), 7.94 (2 H, d, *J* 9.4, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.11 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.9, ArC**H**), 6.83–6.78 (6 H, m, ArC**H**), 4.24 (2 H, t, *J* 4.7, ArOC$\mathbf{H}_2$CH$_2$), 4.16 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.08 (2 H, t, *J* 4.8, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.83 (2 H, t, *J* 5.7, ArOCH$_2$C$\mathbf{H}_2$), 3.81 (2 H, t, *J* 4.7, ArO-CH$_2$C$\mathbf{H}_2$), 3.74 (2 H, t, *J* 4.8, OC$\mathbf{H}_2$CH$_2$O$_2$C), 3.61 (2 H, q, *J* 7.1, C$\mathbf{H}_2$Me), 2.88 (2 H, t, *J* 7.8, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.84 (2 H, t, *J* 7.8, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.61 (2 H, t, *J* 7.8, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.49 (2 H, t, *J* 7.8, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.28 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 789 (MH$^+$), 811 (MNa$^+$) (Found: MH$^+$, 789.3252. $C_{42}H_{49}{}^{35}ClN_4O_9$ requires MH$^+$, 789.3266); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-(2-{2-[4-(2-*tert*-butoxycarbonyl-ethyl)phenoxy]ethoxy}ethoxy)ethyl ester, 7b, $m = 2$

To a solution of **5b** m = 2 (767 mg, 1.22 mmol), triphenylphosphine (480 mg, 1.83 mmol, 1.5 eq.) and diethyl azodicarboxylate (318 mg, 1.83 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (542 mg, 2.44 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7b**, m = 2 as a dark red oil (386 mg, 0.46 mmol, 38%). ν_{max}(Nujol)/cm^{-1} 1736, 1600, 1512, 1339; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.93 (2 H, d, *J* 9.4, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.11 (2 H, d, *J* 8.9, ArC**H**), 7.09 (2 H, d, *J* 8.9, ArC**H**), 6.83–6.79 (6 H, m, ArC**H**), 4.24 (2 H, t, *J* 4.7, ArOC$\mathbf{H}_2$CH$_2$), 4.16 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.09 (2 H, t, *J* 4.9, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.86–3.81 (4 H, m, C$\mathbf{H}_2$), 3.75–3.58 (8 H, m, C$\mathbf{H}_2$), 2.88 (2 H, t, *J* 7.8, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.83 (2 H, t, *J* 7.8, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.61 (2 H, t, *J* 7.8, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.49 (2 H, t, *J* 7.8, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.29 (3 H, t, *J* 7.0, CH$_2$**Me**); *m*/*z* (FAB) 833 (MH$^+$), 855 (MNa$^+$) (Found: MH$^+$, 833.3507. $C_{44}H_{53}{}^{35}ClN_4O_{10}$ requires MH$^+$, 833.3528); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-[2-(2-{2-[4-(2-*tert*-butoxycarbonylethyl)phenoxy]ethoxy}ethoxy)ethoxy]ethyl ester, 7b, *m* = 3

To a solution of **5b**, *m* = 3 (470 mg, 0.70 mmol), triphenylphosphine (275 mg, 1.05 mmol, 1.5 eq.) and diethyl azodicarboxylate (183 mg, 1.05 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (311 mg, 1.40 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7b**, *m* = 3 as a dark red oil (291 mg, 0.33 mmol, 47%). ν_{max}(Nujol)/cm^{-1} 1728, 1600, 1513, 1340; δ_H(300 MHz; $CDCl_3$) 8.34 (1 H, d, *J* 2.5, ArC**H**), 8.10 (1 H, dd, *J* 8.9, 2.5, ArC**H**), 7.92 (2 H, d, *J* 9.2, ArC**H**), 7.75 (1 H, d, *J* 8.9, ArC**H**), 7.11 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.7, ArC**H**), 6.84–6.77 (6 H, m, ArC**H**), 4.21 (2 H, t, *J* 4.8, ArOC$\mathbf{H}_2$CH$_2$), 4.15 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.08 (2 H, t, *J* 4.9, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.85–3.80 (4 H, m, C$\mathbf{H}_2$), 3.73–3.57 (12 H, m, C$\mathbf{H}_2$), 2.88 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.83 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.61 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.49 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.28 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 877 (MH$^+$), 899 (MNa$^+$) (Found: MH$^+$, 877.3802. $C_{46}H_{57}{}^{35}ClN_4O_{11}$ requires MH$^+$, 877.3790); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-{2-[2-(2-{2-[4-(2-*tert*-butoxycarbonylethyl)phenoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethyl ester, 7b, *m* = 4

To a solution of **5b**, *m* = 4 (420 mg, 0.59 mmol), triphenylphosphine (231 mg, 0.88 mmol, 1.5 eq.) and diethyl azodicarboxylate (154 mg, 0.89 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (260 mg, 1.17 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7b**, *m* = 4 as a dark red oil (235 mg, 0.26 mmol, 44%). ν_{max}(Nujol)/cm^{-1} 1727, 1601, 1513, 1340; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.94 (2 H, d, *J* 9.2, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.11 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.7, ArC**H**), 6.84–6.79 (6 H, m, ArC**H**), 4.21 (2 H, t, *J* 4.8, ArOC$\mathbf{H}_2$CH$_2$), 4.16 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.09 (2 H, t, *J* 4.9, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.86–3.81 (4 H, m, C$\mathbf{H}_2$), 3.73–3.56 (16 H, m, C$\mathbf{H}_2$), 2.89 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.83 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.61 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.49 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.41 (9 H, s, C*i*$\mathbf{Me}_3$), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 921 (MH$^+$), 943 (MNa$^+$) (Found: MH$^+$, 921.4048. $C_{48}H_{61}{}^{35}ClN_4O_{12}$ requires MH$^+$, 921.4053); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]propionic acid 2-(2-{2-[2-(2-{2-[4-(2-*tert*-butoxycarbonylethyl)phenoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethoxy)-ethyl ester, 7b, *m* = 5

To a solution of **5b**, *m* = 5 (767 mg, 1.01 mmol), triphenylphosphine (397 mg, 1.51 mmol, 1.5 eq.) and diethyl azodicarboxylate (264 mg, 1.51 mmol, 1.5 eq.) in dichloromethane (5 mL) was added *tert*-butyl ester **6** (448 mg, 2.02 mmol, 2.0 eq.). The reaction mixture was stirred at room temperature for 5 hours after which time TLC (methanol–ethyl acetate, 1 : 9) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated by gradient flash column chromatography (ethyl acetate–hexane, 4 : 1; methanol–ethyl acetate, 1 : 9) to give **7b**, *m* = 5 as a dark red oil (549 mg, 0.57 mmol, 56%). ν_{max}(Nujol)/cm^{-1} 1727, 1601, 1513, 1340; δ_H(300 MHz; $CDCl_3$) 8.37 (1 H, d, *J* 2.5, ArC**H**), 8.13 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.94 (2 H, d, *J* 9.2, ArC**H**), 7.77 (1 H, d, *J* 9.0, ArC**H**), 7.11 (2 H, d, *J* 8.7, ArC**H**), 7.09 (2 H, d, *J* 8.9, ArC**H**), 6.83–6.79 (6 H, m, ArC**H**), 4.21 (2 H, t, *J* 4.8, ArOC$\mathbf{H}_2$CH$_2$), 4.16 (2 H, t, *J* 5.7, ArOC$\mathbf{H}_2$CH$_2$), 4.09 (2 H, t, *J* 4.9, OCH$_2$C$\mathbf{H}_2$O$_2$C), 3.86–3.81 (4 H, m, C$\mathbf{H}_2$), 3.73–3.56 (20 H, m, C$\mathbf{H}_2$), 2.89 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.83 (2 H, t, *J* 7.7, CH$_2$C$\mathbf{H}_2$CO$_2$), 2.61 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 2.49 (2 H, t, *J* 7.7, C$\mathbf{H}_2$CH$_2$CO$_2$), 1.31 (9 H, s, C*i*$\mathbf{Me}_3$), 1.29 (3 H, t, *J* 7.1, CH$_2$**Me**); *m*/*z* (FAB) 965 (MH$^+$), 987 (MNa$^+$) (Found: MH$^+$, 965.4323. $C_{50}H_{65}{}^{35}ClN_4O_{13}$ requires MH$^+$, 965.4315); COSY spectra exhibited a good correlation with the proposed structure.

General procedure for deprotection of 7a, *m* = 1–5 and 7b, *m* = 1–5 to carboxylic acids

To a solution of TIS : TFA, 1 : 49 (10 mL) was added a solution of **7a**, *m* = 1 (200 mg, 258 μmol) in dichloromethane (1.5 mL). The reaction mixture was stirred at room temperature for 10 minutes after which time TLC (ethyl acetate–hexane, 3 : 2) indicated that the starting material had been consumed. The reaction mixture was concentrated under reduced pressure and the product isolated *via* flash column chromatography to give the carboxylic acid as a red oil (158 mg, 220 μmol, 85%). The acid derivative was then coupled directly to the supported protected peptide as described below.

Synthesis of the protected and deprotected 16 residue peptide on Tentagel® and the cleaved peptide

The 16 residue peptide was sequentially synthesised *via* a conventional manual solid phase Fmoc–TFA strategy on Tentagel® polymer support. The peptide's sequence was NH_2–Asp–Pro–**Asp**–Glu–Leu–Glu–**His**–Ala–Ala–Lys–**Ser**–Glu–Ala–Ala–Ala–Lys–OH, where L-lysine was the first amino acid attached, and L-aspartic acid was the last. The peptide was prepared on a mixture of 90% Tentagel® and 10% acid labile Rink linker.

The amino acids were all Fmoc protected at the amino terminal, leaving the acid terminal unprotected. Additional protecting groups were used for the side chains if necessary. These were:

a) *t*-Butyl for aspartic acid, glutamic acid and serine.
b) *t*-Butyl carbamate for lysine.
c) Trityl for histidine.

The coupling reagent of choice was PyBOP, utilised with DIPEA and HOBt in DMF. 2 g of Tentagel® with a loading of 0.60 mmol g^{-1} and 200 mg of Tentagel® Rink linker with a loading of 0.40 mmol g^{-1} was used, giving a mixture with a loading of 0.58 mmol g^{-1}.

The general amino acid coupling and Fmoc deprotection procedures were:

a) The Tentagel® resin (1.0 eq.) was swollen for 30 minutes in DMF (15 mL) in a peptide flask designed for nitrogen bubbling and suction. Gentle mixing was afforded by bubbling nitrogen through the suspension. The protected amino acid, (2.5 eq.), PyBOP (2.5 eq.), HOBt (2.5 eq.) and DIPEA (5 eq.) were then added and the mixing continued by bubbling nitrogen through the solution for 3 hours. The solvent was then removed *via* filtration and the support washed with DMF (2 × 20 mL), dichloromethane (2 × 20 mL), methanol (2 × 20 mL) and DMF (1 × 20 mL). The conversion was verified by a negative Kaiser and TNBS test. A double coupling was undertaken if necessary.

b) The resin functionalised with an Fmoc protected amino acid was gently mixed in a solution of piperidine–DMF, 1 : 4, by bubbling nitrogen through for 20 minutes. The solution was then removed by filtration and the resin washed with DMF (2 × 20 mL). The Kaiser and TNBS tests indicated successful deprotection by yielding a positive result.

Upon coupling the last amino acid residue and removal of its Fmoc protection, the bulk resin was washed with DMF (2 × 20 mL), dichloromethane (2 × 20 mL) and methanol (2 × 20 mL) and dried under reduced pressure to give peptide on Tentagel® as a pale yellow resin (3.8 g, 71%). The yield was based on the expected increase of the resin's weight.

Removal of the peptide's side chain protecting groups and partial cleavage of the peptide (*i.e.* from the 10% acid-cleavable Rink linker) was performed on a small sample (200 mg) by gently bubbling nitrogen through a suspension of the resin in a solution of triisopropylsilane–TFA, 1 : 49 (2 mL) for 1 hour. The solution was removed by filtration and the filtrate was collected and dried under reduced pressure to give an off white powder (6 mg). *m*/*z* (FAB) 1681 (M^+) (Found (ES) 561.2787 (MH_3^{3+}), 841.4056 (MH_2^{2+}), $C_{70}H_{114}N_{21}O_{27}$ requires (ES) 561.2809 (MH_3^{3+}), 841. 4175 (MH_2^{2+})).

The supported deprotected peptide was washed with DMF, dichloromethane and methanol, and dried under reduced pressure to give a pale yellow resin (160 mg).

Individual attachment of the dye-linkers 7a, *m* = 1–5 and 7b, *m* = 1–5 to the supported 16 residue peptide

General preparation for attaching the ten dye-linkers to the terminal amino group of the peptide, followed by deprotection and partial cleavage of these systems from the polymer supported was:

a) To a suspension of the peptide on Tentagel® (25 mg) in DMF (1 mL) were added the dye-linker (20 mg, 21.0–27.9 μmol, excess), PyBOP (30 mg, 57.7 μmol, excess), HOBt (30 mg, 222 μmol, excess) and DIPEA (3 drops, ~30 mg, 233 μmol, excess) and gently mixed by bubbling nitrogen through at room temperature for 3 hours. The resin was then isolated by filtration and washed with DMF (2 × 10 mL), dichloromethane (2 × 10 mL), methanol (2 × 10 mL) and dried under reduced pressure to give the supported protected peptidyl dye-linker system as a black resin (26 mg).

b) The protected peptide dye-linker system (20 mg) was suspended in triisopropylsilane–TFA, 1 : 49 (3 mL) and gently mixed by bubbling nitrogen through at room temperature for 2 hours. The resin was isolated by filtration, washed with methanol (2 × 10 mL), dichloromethane (2 × 10 mL) and dried under reduced pressure to give the supported unprotected peptide dye-linker system as a black resin (18 mg). The filtrate was dried under reduced pressure to give a red powder (2 mg), the result of cleavage of the 10% Rink linker. The peptide dye-linker systems were identified from their filtrates by low resolution electrospray mass spectrometry performed by the EPSRC Swansea Mass Spectrometry Service as summarised below.

1, *n* = 1, *m* = 1; *m*/*z* (ES) 795 (MH_3^{3+}), 1191 (MH_2^{2+}), for $C_{107}H_{150}ClN_{25}O_{35}$.

1, *n* = 1, *m* = 2; *m*/*z* (ES) 809 (MH_3^{3+}), 1214 (MH_2^{2+}), for $C_{109}H_{154}ClN_{25}O_{36}$.

1, *n* = 1, *m* = 3; *m*/*z* (ES) 824 (MH_3^{3+}), 1236 (MH_2^{2+}), for $C_{111}H_{158}ClN_{25}O_{37}$.

1, *n* = 1, *m* = 4; *m*/*z* (ES) 839 (MH_3^{3+}), 1258 (MH_2^{2+}), for $C_{113}H_{162}ClN_{25}O_{38}$.

1, *n* = 1, *m* = 5; *m*/*z* (ES) 854 (MH_3^{3+}), 1280 (MH_2^{2+}), for $C_{115}H_{166}ClN_{25}O_{39}$.

1, *n* = 2, *m* = 1; *m*/*z* (ES) 780 (MH_3^{3+}), 1199 (MH_2^{2+}), for $C_{108}H_{152}ClN_{25}O_{35}$.

1, *n* = 2, *m* = 2; *m*/*z* (ES) 814 (MH_3^{3+}), 1221 (MH_2^{2+}), for $C_{110}H_{156}ClN_{25}O_{36}$.

1, *n* = 2, *m* = 3; *m*/*z* (ES) 829 (MH_3^{3+}), 1243 (MH_2^{2+}), for $C_{112}H_{160}ClN_{25}O_{37}$.

1, *n* = 2, *m* = 4; *m*/*z* (ES) 829 (MH_3^{3+}), 1243 (MH_2^{2+}), for $C_{114}H_{164}ClN_{25}O_{38}$.

1, *n* = 2, *m* = 5; *m*/*z* (ES) 858 (MH_3^{3+}), 1287 (MH_2^{2+}), for $C_{116}H_{168}ClN_{25}O_{39}$.

3-[4-(*tert*-Butyldimethylsilanyloxy)phenyl]propionic acid *tert*-butyl ester, 9[4]

To a solution of **6** (8.0 g, 36.0 mmol) in dichloromethane (100 ml) was added dimethylaminopyridine (1–2 crystals), triethylamine (7.80 ml, 54.1 mmol, 1.5 eq.) and *tert*-butyldimethylsilyl chloride (5.95 g, 39.6 mmol, 1.1 eq.). The reaction was stirred at room temperature for 8 hours after which time TLC (ethyl acetate–hexane, 1 : 9, and phosphomolybdic acid (PMA) dip to visualise) indicated that the starting material had been consumed. The reaction mixture was washed with water (3 × 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The product was isolated with flash column chromatography (ethyl acetate–hexane, 1 : 9) to give **9** as a clear colourless oil (9.60 g, 28.2 mmol, 79%). ν_{max}(Nujol)/cm^{-1} 1731, 1600, 1511; δ_H(300 MHz; $CDCl_3$) 7.06 (2 H, d, *J* 8.5, ArC**H**), 6.77 (2 H, d, *J* 8.5, ArC**H**), 2.86 (2 H, t, *J* 7.7, $ArCH_2C\mathbf{H_2}$), 2.52 (2 H, t, *J* 7.7, $ArC\mathbf{H_2}CH_2$), 1.43 (9 H, s, C*i***Me**$_3$), 1.00 (9 H, s, SiC*i***Me**$_2$), 0.20 (6 H, s, Si**Me**); δ_C(300 MHz; $CDCl_3$) 172.7 (C*i*), 154.3 (C*i*), 133.8 (C*i*), 129.6 (2 CH), 120.3 (2 CH), 80.6 (C*i*), 37.7 (CH_2), 30.8 (CH_2), 28.5 (CH_3), 26.1 (CH_3), 18.6 (C*i*), −4.0 (CH_3); *m*/*z* (EI) 336 (M^+); (CI) 354 (MNH_4^+); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(*tert*-Butyldimethylsilanyloxy)phenyl]-2-methylpropionic acid *tert*-butyl ester, 10[5]

A solution of **9** (4.5 g, 13.4 mmol) in tetrahydrofuran (25 ml) was cooled to −78 °C and stirred for 30 minutes. To this methyl iodide (8.2 ml, 132.4 mmol, 10 eq.), HMPA (16.2 ml, 132.4 mmol, 10 eq.) and LDA (2 M solution in THF), (7.3 ml, 14.6 mmol, 1.1 eq.) were added and stirred at −78 °C for 6 hours. The reaction mixture was then stirred at room temperature overnight after which time TLC (diethyl ether–hexane, 1 : 9) indicated that the starting material had been consumed. The excess reagents and solvent were carefully removed under reduced pressure and the product isolated *via* flash column chromatography (ethyl acetate–hexane, 1 : 9) to give **10** as a clear colourless oil (2.8 g, 8.0 mmol, 60%). ν_{max}(Nujol)/cm^{-1} 1729, 1600, 1510; δ_H(300 MHz; $CDCl_3$) 7.04 (2 H, d, *J* 8.4, ArC**H**), 6.76 (2 H, d, *J* 8.4, ArC**H**), 2.94–2.83 (1 H, m, $ArCH_2C\mathbf{H}$), 2.64–2.49 (2 H, m, $ArC\mathbf{H_2}CH$), 1.39 (9 H, s, C*i***Me**$_3$), 1.12 (3 H, d, *J* 6.6, CH**Me**), 1.00 (9 H, s, SiC*i***Me**$_3$), 0.20 (6 H, s, Si**Me**$_2$); δ_C(300 MHz; $CDCl_3$) 172.7 (C*i*), 154.3 (C*i*), 133.8 (C*i*), 129.6 (2 CH), 120.3 (2 CH), 80.6 (C*i*), 42.9 (CH), 38.0 (CH_2), 28.5 (CH_3), 26.1 (CH_3), 16.5 (CH_3), 18.6 (C*i*), −4.0 (CH_3); *m*/*z* (EI) 350 (M^+); (CI) 368 (MNH_4^+) (Found: MNH_4^+, 368.2622. $C_{20}H_{34}O_3Si$ requires MNH_4^+, 368.2621). COSY spectra exhibited a good correlation with the proposed structure.

3-(4-Hydroxyphenyl)-2-methylpropionic acid *tert*-butyl ester, 11[4]

A solution of **10** (1.5 g, 4.29 mmol) in tetrahydrofuran (20 ml) was cooled to −10 °C to which TBAF (1.34 g, 4.24 mmol) was quickly added. The reaction mixture was stirred at room temperature for 20 minutes after which time TLC (ethyl acetate–hexane, 1 : 4 and PMA dip to visualise) indicated that the starting material had been consumed. The reaction mixture was diluted with diethyl ether (40 ml), washed with water

(3 × 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The product was isolated *via* flash column chromatography (ethyl acetate–hexane, 1 : 4) to give **11** as a clear colourless oil (830 mg, 3.52 mmol, 82%). ν_{max}(Nujol)/cm^{-1} 3392, 1728, 1600, 1516; δ_H(300 MHz; $CDCl_3$) 7.00 (2 H, d, *J* 8.5, ArC**H**), 6.72 (2 H, d, *J* 8.5, ArC**H**), 6.53 (1 H, s, ArO**H**), 2.89–2.80 (1 H, m, $ArCH_2C$**H**), 2.64–2.49 (2 H, m, ArC**H**$_2CH$), 1.38 (9 H, s, C*i***Me**$_3$), 1.11 (3 H, d, *J* 6.6, CH**Me**); δ_C(300 MHz; $CDCl_3$) 177.0 (C*i*), 154.3 (C*i*), 132.6 (C*i*), 131.5 (2 CH), 115.7 (2 CH), 81.0 (C*i*), 43.2 (CH), 39.4 (CH_2), 28.5 (CH_3), 17.4 (CH_3); *m*/*z* (EI) 236 (M^+); (CI) 254 (MNH_4^+); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]-2-methylpropionic acid *tert*-butyl ester[6]

To a solution of **11** (660 mg, 2.80 mmol) in dichloromethane (10 ml) was added triphenylphosphine (734 mg, 2.80 mmol), diethyl azodicarboxylate (0.44 ml, 2.80 mmol) and disperse red (974 mg, 2.80 mmol). The reaction mixture was stirred at room temperature for 3 hours after which time TLC (ethyl acetate–hexane, 1 : 4) indicated that the starting material had been consumed. The dichloromethane was removed under reduced pressure and the product isolated *via* flash column chromatography to give the product as a dark red oil (514 mg, 0.91 mmol, 33%). ν_{max}(Nujol)/cm^{-1}, 1724, 1601, 1514, 1337; δ_H(300 MHz; $CDCl_3$) 8.35 (1 H, d, *J* 2.5, ArC**H**), 8.11 (1 H, dd, *J* 8.9, 2.5, ArC**H**), 7.92 (2 H, d, *J* 9.0, ArC**H**), 7.75 (1 H, d, *J* 8.9, ArC**H**), 7.08 (2 H, d, *J* 9.0, ArC**H**), 6.79 (4 H, d, *J* 9.0, ArC**H**), 4.16 (2 H, t, *J* 5.8, $ArOC$**H**$_2CH_2$), 3.84 (2 H, t, *J* 5.8, $ArOCH_2C$**H**$_2$), 3.61 (2 H, q, *J* 7.1, C**H**$_2$Me), 2.94–2.80 (1 H, m, $ArCH_2C$**H**), 2.63–2.47 (2 H, m, ArC**H**$_2CH$), 1.41 (9 H, s, C*i***Me**$_3$), 1.28 (3 H, t, *J* 7.1, CH_2**Me**), 1.09 (3 H, d, *J* 6.6, CH**Me**); δ_C(300 MHz; $CDCl_3$) 176.0 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.2 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 132.8 (C*i*), 130.5 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.6 (2 CH), 111.9 (2 CH), 81.5 (C*i*), 65.7 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 42.9 (CH), 39.3 (CH_2), 28.4 (CH_3), 17.3 (CH_3), 12.7 (CH_3); *m*/*z* (EI) 566 (M^+), 568 ($M^+ + 2$); (CI) 567 (MH^+), 569 ($MH^+ + 2$); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]-2-methylpropionic acid[7]

To a solution of TIS : TFA, 1 : 49 (5 ml) was added a solution of the *t*-butyl ester from the previous reaction (354 mg, 0.63 mmol) in dichloromethane (1 ml). The reaction mixture was stirred at room temperature for 10 minutes after which time TLC (ethyl acetate–hexane, 2 : 3) indicated that the starting material had been consumed. The reaction mixture was concentrated under reduced pressure and the product isolated *via* flash column chromatography to give the acid as a red oil (258 mg, 0.51 mmol, 81%). ν_{max}(Nujol)/cm^{-1} 1707, 1601, 1515, 1339; δ_H(300 MHz; $CDCl_3$) 8.35 (1 H, d, *J* 2.5, ArC**H**), 8.11 (1 H, dd, *J* 8.9, 2.5, ArC**H**), 7.92 (2 H, d, *J* 9.0, ArC**H**), 7.75 (1 H, d, *J* 8.9, ArC**H**), 7.08 (2 H, d, *J* 9.0, ArC**H**), 6.79 (4 H, d, *J* 9.0, ArC**H**), 4.16 (2 H, t, *J* 5.8, $ArOC$**H**$_2CH_2$), 3.85 (2 H, t, *J* 5.8, $ArOCH_2C$**H**$_2$), 3.61 (2 H, q, *J* 7.1, C**H**$_2$Me), 3.01–2.93 (1 H, m, $ArCH_2C$**H**), 2.74–2.58 (2 H, m, ArC**H**$_2CH$), 1.29 (3 H, t, *J* 7.1, CH_2**Me**), 1.15 (3 H, d, *J* 6.6, CH**Me**); δ_C(300 MHz; $CDCl_3$) 176.0 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.2 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 132.8 (C*i*), 130.5 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (CH), 115.7 (CH), 114.7 (2 CH), 111.9 (2 CH), 65.7 (CH_2), 50.3 (CH_2), 46.7 (CH_2), 41.8 (CH), 38.9 (CH_2), 16.9 (CH_3), 12.7 (CH_3); *m*/*z* (EI) 510 (M^+), 512 ($M^+ + 2$); (CI) 511 (MH^+), 513 ($MH^+ + 2$) (Found MH^+, 511.1751. $C_{26}H_{27}{}^{35}ClN_4O_5$ requires MH^+, 511.1748); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]-2-methylpropionic acid 2-(2-{2-[2-(2-hydroxy-ethoxy)ethoxy]ethoxy}ethoxy)ethyl ester, 12[6]

To a solution of acid from the previous step (200 mg, 0.39 mmol) in dichloromethane (10 ml) was added triphenylphosphine (113 mg, 0.43 mmol, 1.1 eq.), diethyl azodicarboxylate (75 mg, 0.43 mmol, 1.1 eq.) and pentaglycol (0.11 ml, 0.51 mmol, 1.3 eq.). The reaction mixture was stirred at room temperature for 4 hours after which time TLC indicated that the starting material had been consumed. The reaction mixture was diluted with further dichloromethane (20 ml), washed with water (3 × 20 ml), dried ($MgSO_4$) and concentrated under reduced pressure. The product was isolated *via* flash column chromatography to give **12** as a red oil (160 mg, 0.22 mmol, 56%). ν_{max}(Nujol)/cm^{-1} 1731, 1600, 1514, 1337; δ_H(300 MHz; $CDCl_3$) 8.39 (1 H, d, *J* 2.4, ArC**H**), 8.15 (1 H, dd, *J* 8.9, 2.4, ArC**H**), 7.95 (2 H, d, *J* 9.3, ArC**H**), 7.78 (1 H, d, *J* 8.9, ArC**H**), 7.08 (2 H, d, *J* 8.7, ArC**H**), 6.81 (2 H, d, *J* 9.3, ArC**H**), 6.79 (2 H, d, *J* 8.7, ArC**H**), 4.24–4.15 (4 H, m, $ArOC$**H**$_2C$**H**$_2$), 3.86 (2 H, t, *J* 5.8, HOC**H**$_2CH_2$), 3.73–3.58 (20 H, m, C**H**$_2$), 2.99–2.93 (1 H, m, $ArCH_2C$**H**), 2.75–2.58 (2 H, m, ArC**H**$_2CH$), 1.30 (3 H, t, *J* 7.1, CH_2**Me**), 1.13 (3 H, d, *J* 6.8, CH**Me**); δ_C(300 MHz; $CDCl_3$) 176.4 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 132.4 (C*i*), 130.5 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.6 (2 CH), 111.9 (2 CH), 72.9 (CH_2), 71.0 (4 CH_2), 70.7 (2 CH_2), 69.5 (CH_2), 65.7 (CH_2), 63.8 (CH_2), 62.1 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 41.9 (CH), 39.1 (CH_2), 17.0 (CH_3), 12.7 (CH_3); *m*/*z* (FAB) 753 (MNa^+), 755 ($MNaH^+ + 2$) (Found: MH^+, 731.3053. $C_{36}H_{47}{}^{35}ClN_4O_{10}$ requires MH^+, 731.3059); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]-2-methylpropionic acid 2-{2-[2-(2-{2-[4-(2-*tert*-butoxycarbonylethyl)phenoxy]ethoxy}ethoxy)ethoxy]ethoxy}-ethyl ester[6]

To a solution of **12** (105 mg, 144 μmol) in dichloromethane was added triphenylphosphine (41.4 mg, 158 μmol, 1.1 eq.), diethyl azodicarboxylate (28 mg, 158 μmol, 1.1 eq.) and **6** (32 mg, 144 μmol). The reaction mixture was stirred at room temperature for 2 hours after which time TLC (ethyl acetate–hexane, 1 : 4) indicated that the starting material had been consumed. The reaction mixture was concentrated under reduced pressure and the product isolated *via* flash column chromatography (ethyl acetate–hexane, 1 : 4) to give the ester (79 mg, 85 μmol, 59%); δ_H(300 MHz; $CDCl_3$) 8.38 (1 H, d, *J* 2.5, ArC**H**), 8.14 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.95 (2 H, d, *J* 9.2, ArC**H**), 7.78 (1 H, d, *J* 9.0, ArC**H**), 7.09 (2 H, d, *J* 8.5, ArC**H**), 7.07 (2 H, d, *J* 8.7, ArC**H**), 6.82 (2 H, d, *J* 8.7, ArC**H**), 6.80 (2 H, d, *J* 9.2, ArC**H**), 6.78 (2 H, d, *J* 8.5, ArC**H**), 4.24–4.14 (4 H, m, $ArOC$**H**$_2C$**H**$_2$), 4.09 (2 H, t, *J* 4.9, HOC**H**$_2CH_2$), 3.87–3.81 (4 H, m, C**H**$_2$), 3.76–3.59 (16 H, m, C**H**$_2$), 2.99–2.92 (1 H, m, $ArCH_2C$**H**), 2.83 (2 H, t, *J* 7.8, $ArCH_2C$**H**$_2$), 2.75–2.57 (2 H, m, ArC**H**$_2CH$), 2.49 (2 H, t, *J* 7.8, ArC**H**$_2CH_2$), 1.41 (9 H, s, C*i***Me**$_3$), 1.29 (3 H, t, *J* 7.0, CH_2**Me**), 1.13 (3 H, d, *J* 6.8, CH**Me**); δ_C(300 MHz; $CDCl_3$) 176.4 (C*i*), 172.8 (C*i*), 164.0 (C*i*), 157.6 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.5 (C*i*), 132.4 (C*i*), 130.5 (2 CH), 129.6 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 114.6 (2 CH), 111.9 (2 CH), 80.7 (CH_2), 71.2 (CH_2), 71.0 ($3CH_2$), 70.2 (CH_2), 69.5 (CH_2), 67.8 (CH_2), 65.7 (CH_2), 63.8 (CH_2), 62.7 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 41.9 (CH), 39.1 (CH_2), 37.7 (CH_2), 30.7 (CH_2), 28.5 (CH), 17.1 (CH_3), 12.7 (CH_3); *m*/*z* (FAB) 957 (MNa^+), 959 ($MNa^+ + 2$) (Found: MH^+, 935.4216. $C_{49}H_{63}{}^{35}ClN_4O_{12}$ requires MH^+, 935.4209); COSY spectra exhibited a good correlation with the proposed structure.

3-[4-(2-{[4-(2-Chloro-4-nitrophenylazo)phenyl]ethylamino}-ethoxy)phenyl]-2-methylpropionic acid 2-{2-[2-(2-{2-[4-(2-carboxyethyl)phenoxy]ethoxy}ethoxy)ethoxy]ethoxy}ethyl ester[7]

To a solution of TIS : TFA, 1 : 49 (3 ml) was added a solution of ester from the previous step (20 mg, 21 μmol) in dichloromethane (0.5 ml). The reaction mixture was stirred at room temperature for 10 minutes after which time TLC (ethyl acetate–hexane, 2 : 3) indicated that the starting material had been consumed. The reaction mixture was concentrated under reduced pressure and the product isolated *via* flash column chromatography to give the acid as a red oil (14.5 mg, 16.5 μmol, 79%); δ_H(300 MHz; $CDCl_3$) 8.38 (1 H, d, *J* 2.5, ArC**H**), 8.14 (1 H, dd, *J* 9.0, 2.5, ArC**H**), 7.95 (2 H, d, *J* 9.2, ArC**H**), 7.78 (1 H, d, *J* 9.0, ArC**H**), 7.09 (2 H, d, *J* 8.5, ArC**H**), 7.07 (2 H, d, *J* 8.7, ArC**H**), 6.82 (2 H, d, *J* 8.7, ArC**H**), 6.80 (2 H, d, *J* 9.2, ArC**H**), 7.68 (2 H, d, *J* 8.5, ArC**H**), 4.24–4.14 (4 H, m, ArOC$\mathbf{H_2}$C$\mathbf{H_2}$), 4.09 (2 H, t, *J* 4.9, HOC$\mathbf{H_2}$C$\mathbf{H_2}$), 3.87–3.81 (4 H, m, C$\mathbf{H_2}$), 3.76–3.59 (16 H, m, C$\mathbf{H_2}$), 2.99–2.92 (1 H, m, ArCH$_2$C**H**), 2.83 (2 H, t, *J* 7.8, ArC$\mathbf{H_2}$C$\mathbf{H_2}$), 2.75–2.57 (2 H, m, ArC$\mathbf{H_2}$CH), 2.49 (2 H, t, *J* 7.8, ArC$\mathbf{H_2}$C$\mathbf{H_2}$), 1.29 (3 H, t, *J* 7.0, C$\mathbf{H_2}$**Me**), 1.11 (3 H, d, *J* 6.8, CH**Me**); δ_C(300 MHz; $CDCl_3$) 176.4 (C*i*), 172.8 (C*i*), 157.6 (C*i*), 157.3 (C*i*), 153.5 (C*i*), 152.1 (C*i*), 147.5 (C*i*), 144.7 (C*i*), 134.3 (C*i*), 133.5 (C*i*), 132.4 (C*i*), 130.5 (2 CH), 129.6 (2 CH), 127.4 (CH), 126.4 (CH), 123.0 (CH), 118.4 (2 CH), 114.9 (2 CH), 114.6 (2 CH), 111.9 (2 CH), 80.7 (CH_2), 71.2 (CH_2), 71.0 (3CH_2), 70.2 (CH_2), 69.5 (CH_2), 67.8 (CH_2), 65.7 (CH_2), 63.8 (CH_2), 62.7 (CH_2), 50.4 (CH_2), 46.7 (CH_2), 41.9 (CH), 39.1 (CH_2), 37.7 (CH_2), 30.7 (CH_2), 17.1 (CH_3), 12.7 (CH_3); *m*/*z* (FAB) 901 (MNa^+), 903 (MNa^+ + 2) (Found: MH^+, 879.8577. $C_{45}H_{55}{}^{35}ClN_4O_{12}$ requires MH^+, 879.3583); COSY spectra exhibited a good correlation with the proposed structure.

Synthesis of the peptidyl chiral dye-linker systems, 8

The method for attaching the chiral dye-linker to the terminal amino group of the peptide, followed by deprotection and partial cleavage of the system from the polymer support was as follows: to a suspension of the peptide on Tentagel® (10 mg) in dimethylformamide (1 ml) was added the dye-linker from the previous experiment (10 mg, 11.4 μmol, excess), PyBOP (30 mg, 57.7 μmol, excess), HOBt (30 mg, 222 μmol, excess) and DIPEA (3 drops, ~30 mg, 233 μmol, excess) and gently mixed by bubbling nitrogen through at room temperature for 3 hours. The resin was then isolated by filtration and washed with dimethylformamide (2 × 10 ml), dichloromethane (2 × 10 ml), methanol (2 × 10 ml) and dried under reduced pressure to give a black resin (11 mg). This was suspended in triisopropylsilane : TFA, 1 : 49 (3 ml) and gently mixed by bubbling nitrogen through at room temperature for 2 hours. The resin was isolated by filtration, washed with methanol (2 × 10 ml), dichloromethane (2 × 10 ml) and dried under reduced pressure to give the supported unprotected peptide chiral dye-linker system **8** as a black resin (7 mg). The filtrate was dried under reduced pressure to give a red powder (1 mg), the result of cleavage of the 10% Rink resin.

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