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Synthesis of a Tripodal Scaffold for Solid Phase Synthesis of Artificial Receptors

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A convergent synthesis for the preparation of a new orthogonally protected tripodal scaffold has been developed. The scaffold was successfully coupled to a Tentagel solid support and further derivatised at the three attachment points demonstrating its possible use for combinatorial chemistry.

Introduction

The design of new artificial receptors requires suitable scaffolds that allow incorporation of various chemical functionalities and at the same time preorganise the different built-in residues. By equipping such a scaffold with an anchor point, allowing attachment to a solid support, combinatorial methods can be used to generate libraries of possible artificial receptor candidates. Screening for affinity towards particular ligands can then enable identification of a synthetic receptor. Earlier developed tripodal scaffolds are mostly based on a rigid skeleton that imposes a specific orientation on the attached functionalities.[1] Only a few flexible tripods have been created to date, among which the pentaerithryltetramine scaffold developed by Virta.[2]

Our group has also made a contribution in this area using the flexibility of the pentaerythritol skeleton. In an attempt to design a non-rigid, yet conformationally constrained template molecule, three aromatic residues were introduced.[3] Molcular modelling studies suggested a preferred parallel orientation for the three benzylic chains in **1** (see Figure 1). However, when using scaffold **1** for the construction of multipodal peptides, various problems arose due to the presence of benzylic ether bonds. Moreover, hydrolysis of ester linkages between the scaffold and peptide chains was observed.[4] With these problems in mind we now wish to report on the convergent synthesis of the racemic tripodal scaffold **2**, omitting sensitive functionalities and providing amino groups as anchoring points. Molecular modelling showed that also in this case the arms are organized in a parallel way.[5] Next to comparable interactions between the aromatic moieties, extra stabilisation is achieved by intramolecular H-bonding as shown in Figure 2.

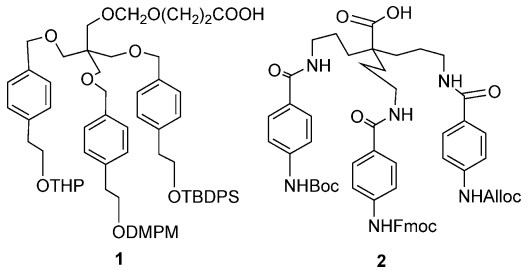

Figure 1. Structure of previously developed scaffold **1** and structure of the new tripodal scaffold **2**.

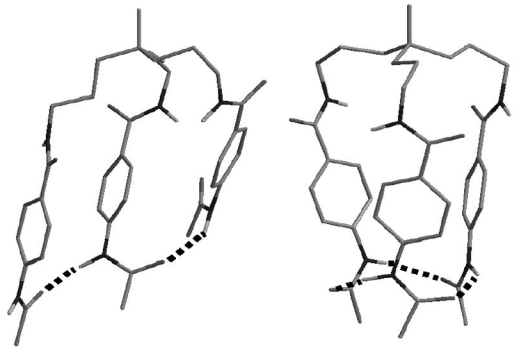

Figure 2. Results of molecular modelling studies on a simplified version of **2**.[5] Two energetically favoured conformations in H_2O are shown. The dotted lines represent intramolecular H-bonding.

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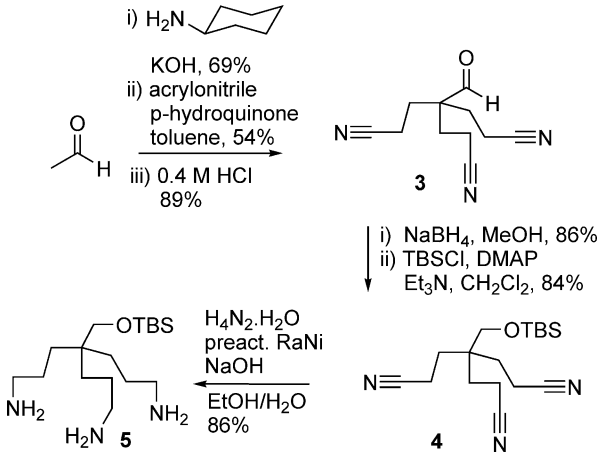
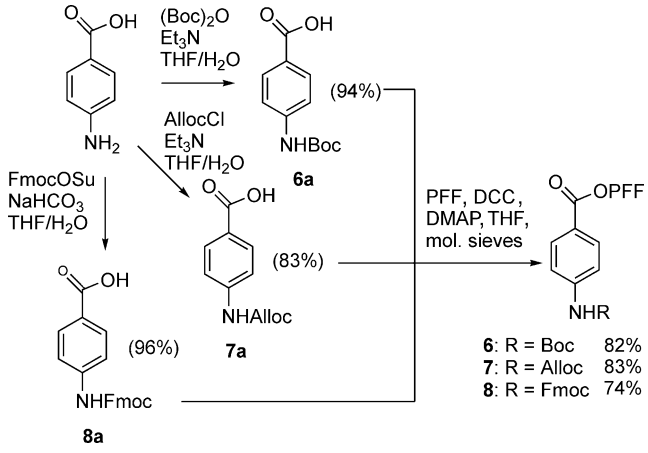
Results and Discussion

Scaffold **2** is characterized by a central core **5** (Scheme 1) easily synthesized via a previously described method.[6] Although according to this procedure, complete nitrile reduction should be achieved using hydrazine in the presence of preactivated Raney-Ni catalyst, TLC-monitoring showed the reduction not to be fully complete, with some mono- and di-nitriles still present. Changing the reaction variables and the type of catalyst did not result in a significant improvement. However, the crude product could be used as such and the impurities could be removed on a later stage in the synthesis.

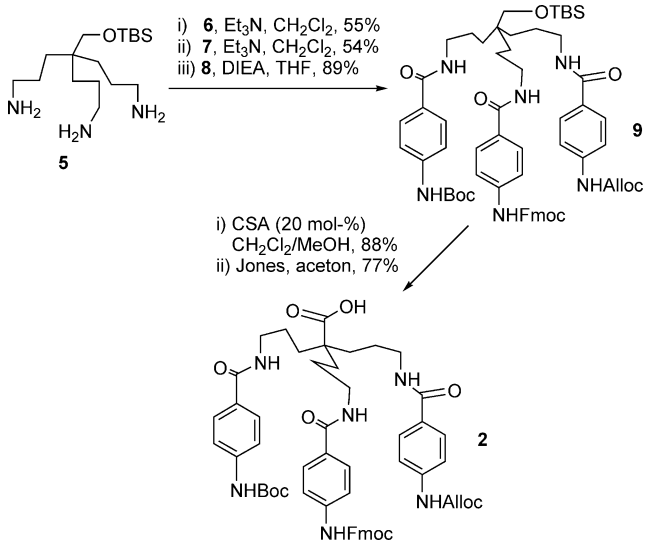
The three different aromatic moieties were synthesized starting from 4-aminobenzoic acid (Scheme 2). After protection of the amine,[7] the carboxylic acids were activated as the pentafluorophenyl ester derivatives **6**, **7** and **8** for further coupling with **5**.



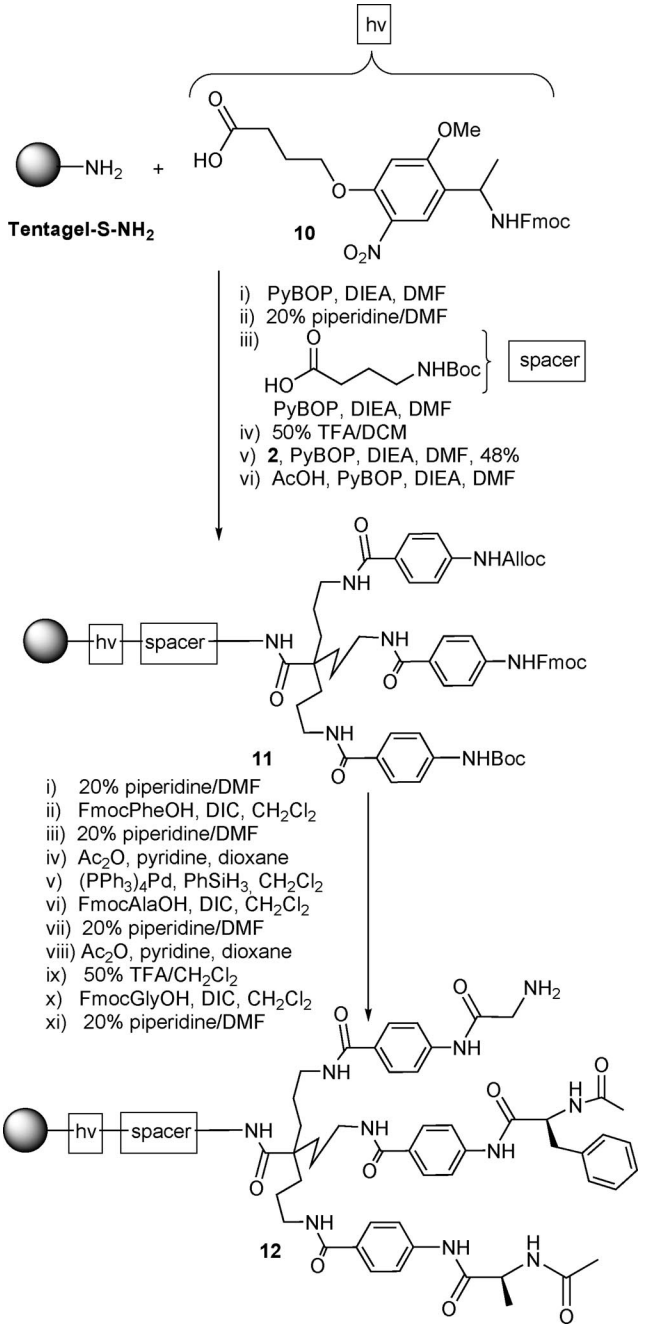
Scheme 1. Synthesis of the core.



Scheme 2. Synthesis of the orthogonally protected legs.



Scheme 3. Assembly of scaffold **2**.



Scheme 4. Solid-phase synthesis of a possible library member.

To maximize the yield of monoderivatisation in the first step, three equiv. of triamine **5** were used and activated **6** was added over a period of 8 h (Scheme 3). Separation of the monopodal product from the excess starting material and the side products was achieved via chromatography using a CH_2Cl_2/MeOH/NH_3 mixture. The two other aromatic residues, **7** and **8**, were coupled in a similar fashion with comparable yields.

Deprotection of the silyl ether was achieved with catalytic amounts of (±)-CSA and only a very small amount of Boc deprotection (< 1%) was observed. Oxidation to the carboxylic acid **2** was carried out using Jones reagent. Gram quantities of **2** are easily available via this synthetic route.

In order to facilitate monitoring of reactions on solid support, a photocleavable linker **10** was used which releases the product upon irradiation at 365 nm.[8] Only small amounts of resin are needed (typically 1 mg or less) and the solution obtained upon irradiation can be directly analyzed by LC-MS. Using **10**, in some cases yellow coloration of the solid support was observed, lowering the sensitivity of the TNBS-test.[9] This problem could be circumvented by using the NF31-test which has a higher sensitivity.[10] Boc-γ-aminobutyric acid was used as a spacer to minimize steric hindrance when performing coupling reactions to the secondary amine of **10**. It was noted that the use of Fmoc-γ-aminobutyric acid led to undesired alkylation of the spacer upon Fmoc deprotection with piperidine.

Following Boc deprotection, coupling to the solid support was first tested using a threefold excess of **2** in the presence of PyBOP (3 equiv.) and DIEA (9 equiv.) yielding a loading of 0.077 mmol g^{-1} (maximum theoretical loading is 0.164 mmol g^{-1}). LC control of the reaction mixture showed that a small amount of Fmoc-deprotected compound was present. In an attempt to improve coupling yields, raising the reaction temperature to 50 °C or performing a double coupling only resulted in a slightly higher loading of 0.084 mmol g^{-1} (51%). Moreover, increased Fmoc deprotection was observed. The use of alternative coupling reagents such as DIC with HOAt, although avoiding Fmoc deprotection, gave much lower loading values (0.059 mmol g^{-1}). Finally, optimized conditions were found using the PyBOP/DIEA protocol at room temp. with only 1.5 equiv. of **2**. A loading of 0.084 mmol g^{-1} was achieved. Under these conditions only 12% Fmoc deprotection was observed. The remaining free aliphatic spacer amines were selectively capped with AcOH/PyBOP/DIEA while less then 2% of deprotected **2** was acylated.

After attaching **2** to the solid support, deprotection of the different carbamate groups gives rise to arylamines with a low nucleophilicity. Activating 12 equiv. of the desired amino acid in the presence of 6 equiv. DIC for 30 min at 0 °C in CH_2Cl_2[11] generates the symmetrical anhydrides that gave a clean coupling.[12] Next to performing the NF31 test,[13] completeness of the reaction was checked via ES-MS (after photolytic cleavage) and the coupling was repeated when necessary.

The Fmoc group was removed first where after Fmoc-PheOH was coupled, Fmoc-deprotected and capped. A tandem-deprotection with the ternary system Pd^0/$PhSiH_3$/$(FmocAla)_2O$ gave incomplete deprotection and coupling. However, after Alloc deprotection with Pd^0 as a catalyst and $PhSiH_3$ as scavenger, FmocAlaOH was attached, deprotected and capped[14] (see Scheme 4).

Finally, the Boc group was deprotected with 50% TFA/CH_2Cl_2. FmocGlyOH was coupled and deprotected to give **12** in good purity as judged from LC (Figure 3) and ES-MS (Figure 4) analysis. It has been further shown that the order of aniline deprotection for the Boc and Alloc groups can be switched. We realize that including the Boc group excludes the use of functionalized Fmoc/*t*Bu amino acids in the previous strand(s). This can be circumvented by first deprotecting the Boc group of **11** and then coupling a *N*-α-ivDde-protected amino acid, after which the current Scheme can be applied in a Fmoc/*t*Bu protocol with functionalized amino acids.[15]

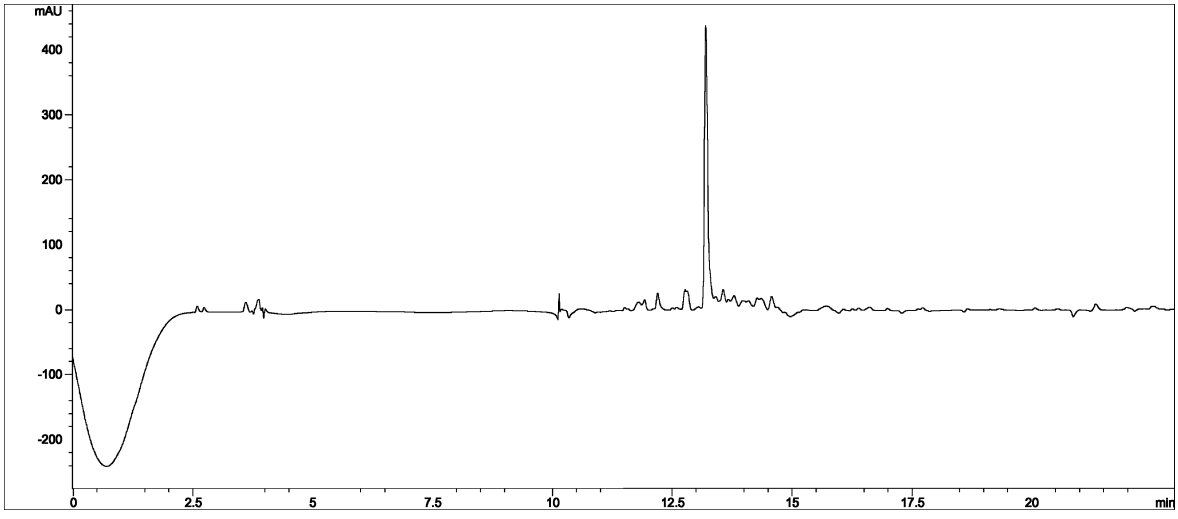


Figure 3. LC at 214 nm of the crude tripodal peptide obtained after photolysis of **12**.

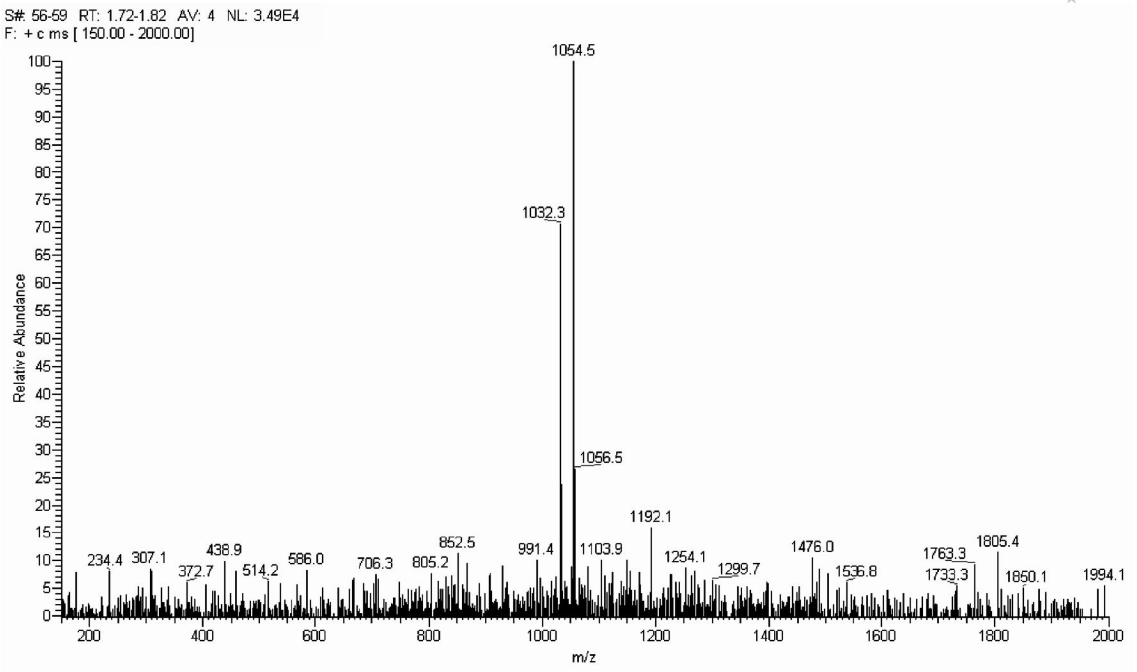


Figure 4. ES-MS$^+$ of the crude tripodal peptide obtained after photolysis of **12**.

Conclusions

In conclusion an efficient route towards orthogonally protected tripodal scaffold 2 has been developed. A particular feature of the new template molecule is its highly flexible, though conformationally preorganisable nature. Immobilisation onto a solid support and further derivatisation of the different attachement points has been achieved. The development of artificial receptors using **2** is currently under investigation.

Experimental Section

General Methods: All solid-phase reactions on at most 20 mg of resin were performed in polypropylene Chromabond columns of 1 mL with a polyethylene frit, closed at the bottom with a B7 septum from Aldrich. Solid-phase reactions on a larger scale were performed in a peptide vessel protected against light with aluminium foil and comprising a sintered glass funnel and a 3-way stopcock for easy filtration and washing. All solution phase reactions were conducted under an inert atmosphere of argon gas in oven dried glassware. The reactions were monitored by thin layer chromatography (TLC) using SIL G-25 UV_{254} pre-coated silica gel plates (0.25 mm thickness). The TLC plates were visualized using an anisaldehyde (5% anisaldehyde in ethanol with 1% sulfuric acid) or a PMA (5% phosphomolybdic acid in ethanol) solution. Flash column chromatography was performed using BIOSOLVE silica gel (0.063–0.200 mm particle size). NMR spectra were recorded at 500 MHz or 300 MHz for proton and at 125 MHz or 75 MHz for carbon nuclei in [D]chloroform, [D_6]DMSO, [D_4]MeOD or [D_6]-aceton. Chemical shifts are reported in units of parts per million (ppm), referenced relative to the residual ^{1}H or ^{13}C peaks of the used solvent as internal standards ([D]chloroform: ^{1}H 7.26 and ^{13}C 77.16; [D_6]DMSO: ^{1}H 2.50 and ^{13}C 39.52; [D_4]MeOD: ^{1}H 3.31 and ^{13}C 49.00; [D_6]aceton: ^{1}H 2.05 and ^{13}C 29.84 and 206.26). The following abbreviations were used to explain the multiplicities: s singlet, d doublet, t triplet, q quadruplet, m multiplet, br. broad. Infrared spectra (IR) were recorded on a Perkin–Elmer 1600 series FTIR spectrometer and reported in wave numbers (cm^{-1}). Samples were prepared as a thin films (neat) on KBr plates. Elemental analyses were performed by SIARE at the Université Pierre & Marie Curie, Paris, France. High-Resolution Mass Spectra (HRMS) were recorded by Amberlab at Ghent University on a Thermo Finnigan MAT95XP-Trap tandem mass spectrometer or by the Rega institute at the University of Leuven on a ThermoFinnigan LCQ MSn ion trap. Low Resolution Mass Spectra were recorded with an atmospheric pressure electrospray-ionisation (ESI) Hewlett–Packard 5988 A mass spectrometer. HPLC analyses were performed on an Agilent 1100 Series instrument with a Phenomenex Luna C18(2) column (250 × 4.6 mm, 5μ at 35 °C) using a flow rate of 1 mL/min and with the following solvent systems: 0.1% TFA in H_2O (A) and MeCN (B). Unless otherwise stated the column was flushed for 3 min with 100% A, then a gradient from 0 to 100% B over 15 min was used, followed by 5 min of flushing with 100% B. Photolyses were carried out with a 4 W Bioblock Scientific compact UV lamp set at 365 nm. Melting point ranges were determined with an Electrothermal 9100 melting point apparatus.

Materials: All amino acids and the solid support Tentagel-S-NH_2 were purchased from NovaBiochem. DMF extra dry was purchased from Aldrich. DMF peptide grade was purchased from Biosolve. All chemicals were purchased and used without any further purification, except tetrahydrofuran (THF), which was distilled from Na/benzophenone prior to use and dichloromethane, which was distilled from CaH_2.

Triamine 5: In a two-necked flask of 500 mL, **4** (22.0 g, 68.9 mmol) was dissolved in 250 mL of pure ethanol and 13.2 mL of water. To this mixture, NaOH (10.6 g, 26.0 mmol) was added, after which the mixture was cooled down to 0 °C. After stirring for half an hour, hydrazine monohydrate (26.4 mL, 544 mmol) was added. In the meantime a Raney-Ni slurry (6.2 g, 53 mmol, Merck, 50% active

catalyst in water) was washed thoroughly with water and pure ethanol in a little flask. This suspension was added to the reaction mixture over a period of 2 h, after which the solution was allowed to warm up to room temp. After 1 h of stirring, the reaction mixture was refluxed during 2 h. The reaction mixture was filtered off over celite and the filtrate was concentrated under reduced pressure. Toluene was added to the residue to precipitate the NaOH. The base was filltered off and the filtrate was evaporated under reduced pressure. This action was repeated until no more precipitation was formed. A light yellow oil was obtained (19.6 g, 59.2 mmol, 86%). ^{1}H NMR (300 MHz, $CDCl_3$): δ = 3.12 (s, 2 H), 3.10 (br. s, 6 H), 2.64 (t, J = 6.8 Hz, 6 H), 1.34 (m, 6 H), 1.15 (m, 6 H), 0.84 (s, 9 H), 0.03 (s, 6 H) ppm. ^{13}C + APT (75 MHz, $CDCl_3$): δ = 66.4 (CH_2), 43.0 (CH_2), 39.3 (C), 31.1 (CH_2), 27.4 (CH_2), 25.8 (CH_3), 18.1 (C), –5.7 (CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3358 (s), 2929 (s), 2857 (m) 1568 (m), 1472 (m), 1386 (w), 1328 (m) cm^{-1}. LRMS (ESI$^+$): 332.3 [MH$^+$]

Synthesis of the Orthogonally Protected Scaffold Legs

4-(*tert*-Butoxycarbonylamino)benzoic Acid (6a): See ref.[7]

Pentafluorophenyl 4-(*tert*-Butoxycarbonylamino)benzoate (6): To a solution of **6a** (10.0 g, 42.0 mmol) in THF (65 mL) was added pentafluorophenol (9.37 g, 50.4 mmol), DMAP (260 mg, 2.11 mmol) and molecular sieves. After stirring the solution for 10 min at 0 °C the coupling reagent DCC (10.5 g, 50.4 mmol) was added and the ice-bath was allowed to warm up to room temp. After 18 h the reaction mixture was filtered off and the filtrate was concentrated under reduced pressure. The resulting orange precipitate was chromatographed with pentane/CH_2Cl_2 (1:1) to give **6** (13.9 g, 34.4 mmol, 82%) as a white powder; m.p. 124–126 °C. ^{1}H NMR (COSY, 300 MHz, $CDCl_3$): δ = 8.12 (ddd, J = 8.4/2.4/1.8 Hz, 2 H), 7.54 (ddd, J = 9.3/2.3/1.9 Hz, 2 H), 6.77 (br. s, 1 H), 1.52 (s, 9 H). ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ = 162.1 (C), 151.9 (C), 144.4 (C), 132.2 (CH), 120.7 (C), 117.6 (CH), 81.7 (C), 28.2 (CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3355 (w), 1758 (s), 1736 (m) cm^{-1}. LRMS (ESI$^-$): m/z = 402.7 [M – H]$^-$. $C_{18}H_{14}F_5NO_4$ (403.08): calcd. C 53.61, H 3.50, N 3.47; found C 53.71, H 3.67, N 3.30.

4-(Allyloxycarbonylamino)benzoic Acid (7a): In a 2 L flask, 4-aminobenzoic acid (1 g, 7.29 mmol) was dissolved in 10 mL of dioxane and 10 mL of water. DIEA (2.6 mL, 14.9 mmol) and $NaHCO_3$ (1.8 g, 21.8 mmol) were added. Finally, allyl chloroformate (0.78 mL, 7.33 mmol) was added and the reaction is stirred overnight. A 1 N HCl solution was added drop wise until there was no more precipitate forming. The white powder was filtered off and dried with P_2O_5 under reduced pressure to provide **7a** (1.35 g, 6.10 mmol, 83%); m.p. 199–201 °C. ^{1}H NMR (COSY, 500 MHz, [D$_6$]DMSO): δ = 10.07 (br. s, 1 H), 7.83 (ddd, J = 9.3/2.3/1.8 Hz, 2 H), 7.74 (ddd, J = 9.3/2.3/1.9 Hz, 2 H), 5.95 (ddt, J = 17.3/10.5/5.5 Hz, 1 H), 5.35 (ddd, J = 17.3/3.3/1.6, 1 H), 5.21 (ddd, J = 10.4/2.9/1.3 Hz, 1 H), 4.58 (ddd, J = 5.5/1.3/1.3 Hz, 2 H). ^{13}C NMR (APT, 75 MHz, [D$_6$]acetone): δ = 167.3 (C), 153.9 (C), 144.5 (C), 133.9 (CH), 131.6 (CH), 125.3 (C), 118.3 (CH), 117.9 (CH_2), 66.0 (CH_2) ppm. IR (KBr): $\tilde{\nu}$ = 3334, 1702, 1685, 1600, 1532, 1315, 1230 cm^{-1}. LRMS (ESI$^-$): m/z = 220.2 [M – H]$^-$. $C_{11}H_{11}NO_4$ (221.07): calcd. C 59.73, H 5.01, N 6.33; found C 59.43, H 4.95, N 6.58.

Pentafluorophenyl 4-(Allyloxycarbonylamino)benzoate (7): To a solution of **7a** (19.2 g, 83.6 mmol) in THF (225 mL) was added pentafluorophenol (19.2 g, 104.5 mmol), DMAP (480 mg, 3.93 mmol) and molecular sieves. After stirring the solution for 10 min at 0 °C the coupling reagent DCC (21.6 g, 104.5 mmol) was added and the ice-bath is allowed to melt. After 18 h the reaction mixture was filtered off and the filtrate was concentrated under reduced pressure. The resulting powder was chromatographed with pentane/EtOAc (9:1) to give **7** (26.8 g, 69.4 mmol, 83%) as a white powder; m.p. 137–139 °C. ^{1}H NMR (COSY, 300 MHz, $CDCl_3$): δ = 8.17 (ddd, J = 9.4/2.4/2.0 Hz, 2 H), 7.75 (ddd, J = 9.4/2.4/2.0 Hz, 2 H), 5.97 (ddt, J = 17.3/10.5/5.7 Hz, 1 H), 5.40 (ddd, J = 17.1/2.9/1.5, 1 H), 5.30 (ddd, J = 10.4/2.4/1.2 Hz, 1 H), 4.70 (ddd, J = 5.9/1.3/1.3 Hz, 2 H). ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ = 162.1 (C), 152.6 (C), 143.9 (C), 132.3 (CH), 131.9 (CH), 121.3 (C), 118.3 (CH_2), 117.9 (CH), 66.4 (CH_2) ppm. IR (KBr): $\tilde{\nu}$ = 3355 (w), 1759 (s) cm^{-1}. LRMS (ESI$^-$): m/z = 386.1 [M – H]$^-$. $C_{17}H_{10}F_5NO_4$ (387.05): calcd. C 52.73, H 2.60, N 3.62; found C 52.65, H 2.78, N 3.45.

4-(Fluorenylmethoxycarbonylamino)benzoic Acid (8a): In a 5 L flask, 4-aminobenzoic acid (40.7 g, 0.297 mol) was dissolved in 810 mL water. $NaHCO_3$ (68.0 g, 0.809 mol) was then added, followed by the addition of 810 mL of dioxane and FmocOSu (100 g, 0.297 mol). After stirring for 4 h, 400 mL water was added. The solution was stirred for another 44 h and was then acidified with 1 N HCl solution until pH 3 was reached. The white precipitate was filtered off an dried with P_2O_5 under vacuum (102.9 g, 0.286 mol, 96%); m.p. 276–278 °C. ^{1}H NMR (COSY, 500 MHz, [D$_6$]DMSO): δ = 10.0 (br. s, 1 H), 7.91 (d, J = 7.5 Hz, 2 H), 7.84 (d, J = 8.1 Hz, 2 H), 7.75 (d, J = 7.5 Hz, 2 H), 7.54 (br. s, 2 H), 7.43 (dd, J = 7.4/7.4 Hz, 2 H), 7.35 (ddd, J = 7.4/7.4/0.9 Hz, 2 H), 4.53 (d, J = 6.4 Hz, 2 H), 4.33 (t, J = 6.4 Hz, 1 H). ^{13}C NMR (APT, 75 MHz, [D$_6$]DMSO): δ = 166.9 (C), 153.2 (C), 143.7 (C), 143.2 (C), 140.8 (C), 130.4 (CH), 127.7 (CH), 127.2 (CH), 125.2 (CH), 125.1 (C), 120.2 (CH), 117.4 (CH), 65.8 (CH_2), 46.5 (CH) ppm. IR (KBr): $\tilde{\nu}$ = 3342 (w), 1711 (s), 1678 (m), 1529 (s), 1238 (s), 739 (s) cm^{-1}. LRMS (ESI$^-$): m/z = 358.5 [M – H]$^-$. $C_{22}H_{17}NO_4$ (359.12): calcd. C 73.53, H 4.77, N 3.90; found C 73.40, H 4.93, N 3.93.

Pentafluorophenyl 4-[(9*H*-Fluoren-9-yl)methoxycarbonylamino]benzoate (8): To a solution of **8a** (10.0 g, 27.8 mmol, 1 equiv.) in THF (45 mL) was added pentafluorophenol (7.23 g, 38.9 mmol), DMAP (173 mg, 1.40 mmol) and molecular sieves. After stirring the solution for 10 min at 0 °C, the coupling reagent DCC (8.11 g, 38.9 mmol) was added and the ice-bath was allowed warm until room temp. After 18 h the reaction mixture was filtered off and the filtrate was concentrated under reduced pressure. The resulting light-yellow precipitate was recrystallized from EtOAc. Then the precipitate was taken into THF and refluxed. When everything was dissolved, the solution was slowly cooled in an ice-bath. The white precipitate was filtered off and the filtrate was evaporated to give **8** (10.8 g, 20.6 mmol, 74%) as a white powder; m.p. 194–196 °C. ^{1}H NMR (COSY, 300 MHz, $CDCl_3$): δ = 8.14 (ddd, J = 8.8/2.4/1.8 Hz, 2 H), 7.80 (d, J = 7.5 Hz, 2 H), 7.63 (d, J = 7.5 Hz, 2 H), 7.55 (d, J = 8.6 Hz, 2 H), 7.44 (dd, J = 7.2/7.2 Hz, 2 H), 7.35 (ddd, J = 7.5/7.5/1.1 Hz, 2 H), 6.90 (s, 1 H), 4.62 (d, J = 6.4 Hz, 2 H), 4.28 (t, J = 6.4 Hz, 1 H) ppm. ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ = 162.1 (C), 152.8 (C), 143.7 (C), 143.5 (C), 141.4 (C), 132.3 (CH), 128.0 (CH), 127.2 (CH), 124.8 (CH), 121.3 (C), 120.2 (CH), 117.9 (CH), 67.2 (CH_2), 47.0 (CH) ppm. IR (KBr): $\tilde{\nu}$ = 3343 (w), 1755 (s), 1708 (m) cm^{-1}. LRMS (ESI$^+$): m/z = 590.4 [M + MeCN + H]$^+$.

Synthesis of Silyl Ether 9: i) To a solution of **5** (11.182 g, 33.7 mmol) in CH_2Cl_2 (70 mL) was added Et_3N (1.475 mL, 10.5 mmol) and molecular sieves. To the resulting mixture, a solution of the activated ester **6** (4.247 g, 10.5 mmol) in CH_2Cl_2 (60 mL) was slowly added via the syringe pump over a period of 8 h. The solution was then stirred overnight and afterwards concentrated under reduced pressure. The residual oil was chromatographed with gradient elution where the amount of MeOH(NH_3)

in CH_2Cl_2 was varied from 5–25%. The desired diamine was obtained as a light-yellow foam (3.182 g, 55%); m.p. 66–68 °C. Finally the column was washed with pure MeOH(NH_3) to recover the excess starting material (6.512 g or 55%). ^{1}H NMR (COSY, 500 MHz, $CDCl_3$): δ = 8.26 (br. s, 1 H), 7.63 (d, J = 8.8 Hz, 2 H), 7.36 (d, J = 8.6 Hz, 2 H), 6.77 (t, J = 5.6 Hz, 1 H), 3.25 (m, 2 H), 3.16 (s, 2 H), 2.53 (t, J = 6.6 Hz, 4 H), 1.43–1.36 (m, 11 H), 1.28–1.05 (m, 10 H), 0.77 (s, 9 H), –0.10 (s, 6 H). ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ = 167.1 (C), 152.9 (C), 142.0 (C), 128.5 (C), 128.0 (CH), 117.6 (CH), 80.3 (C), 66.4 (CH_2), 42.9 (CH_2), 40.7 (CH_2), 39.2 (C), 31.1 (CH_2), 28.3 (CH_3), 27.1 (CH_2), 25.8 (CH_3), 23.4 (CH_2), 18.1 (C), –5.6 (CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3304 (w), 1755 (s), 1708 (m) cm^{-1}. LRMS (ESI$^+$): m/z = 551.3 [M + H]$^+$. HRMS (ESI$^+$): calcd. for [$C_{29}H_{54}N_4O_4Si$ + H]$^+$: 551.39924, found 551.39871.

ii) To a solution of the diamine (6.176 g, 11.2 mmol) in CH_2Cl_2 (60 mL) was added Et_3N (0.72 mL, 5.1 mmol) and molecular sieves. To the resulting mixture, a solution of activated ester **7** (1.974 g, 5.1 mmol) in THF (14 mL) was slowly added via the syringe pump over a period of 8 h. The solution was then stirred overnight and afterwards concentrated under reduced pressure. The residual oil was chromatographed with gradient elution where the amount of MeOH(NH_3) in CH_2Cl_2 was varied from 3% to 10%. The desired mono-amine was obtained as a light-yellow foam with a 54% (2.091 g) yield; m.p. 113–115 °C. ^{1}H NMR (COSY, 300 MHz, $CDCl_3$): δ = 7.78 (br. s, 1 H) 7.73 (d, J = 8.6 Hz, 2 H), 7.70 (d, J = 8.6 Hz, 2 H), 7.42 (d, J = 8.3 Hz, 2 H), 7.38 (d, J = 8.6 Hz, 2 H), 715 (br. s, 1 H), 6.64 (br. s, 1 H), 6.57 (br. s, 1 H), 5.96 (ddt, J = 17.2/10.5/5.7 Hz, 1 H), 5.36 (ddd, J = 17.2/2.9/1.5 Hz, 1 H), 5.26 (ddd, J = 10.3/2.4/1.3 Hz, 1 H), 4.66 (ddd, J = 5.6/1.3/1.3 Hz, 2 H), 3.38–3.31 (m, 4 H), 3.22 (s, 2 H), 2.62 (t, J = 6.8 Hz, 2 H), 1.53–1.43 (m, 13 H), 1.35–1.18 (m, 8 H), 0.84 (s, 9 H), –0.03 (s, 6 H). ^{13}C NMR (APT, 75 MHz, $CDCl_3$): δ = 167.2 (C), 167.1 (C), 153.2 (C), 152.6 (C), 141.5 (C), 141.0 (C), 132.3 (CH), 129.3 (C), 128.8 (C), 128.1 (CH), 128.0 (CH), 118.4 (CH_2),118.2 (CH), 118.0 (CH), 80.9 (C), 66.6 (CH_2), 66.0 (CH_2), 42.9 (CH_2), 40.8 (CH_2), 39.4 (C), 31.5 (CH_2), 30.8 (CH_2 or C), 28.3 (CH_3), 27.1 (CH_2), 25.8 (CH_3), 23.3 (CH_2), 18.1 (C), –5.6 (CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3304 (w), 1755 (s), 1708 (m) cm^{-1}. LRMS (ESI$^+$): m/z = 755.0 [M + H]$^+$. HRMS (ESI$^+$): calcd. for [$C_{29}H_{54}N_4O_4Si$ + H]$^+$: 754.45695, found 754.45493.

iii) To a solution of the mono-amine (2.09 g, 2.77 mmol) in THF (34 mL) was added DIEA (0.48 mL, 2.76 mmol) and **8** (2.90 g, 5.52 mmol). The solution was then stirred overnight and afterwards concentrated under reduced pressure. The residual yellow foam was chromatographed with gradient elution where the amount of MeOH in CH_2Cl_2 was varied from 2% to 4%. In this way the product **9** was collected as a white powder (2.70 g, 2.47 mmol, 89%); m.p. 134–136 °C. ^{1}H NMR (COSY, 300 MHz, $CDCl_3$): δ = 7.81 (br. s, 1 H), 7.74 (d, J = 7.7 Hz, 2 H), 7.72–7.65 (m, 6 H), 7.63 (br. s, 1 H), 7.59 (d, J = 7.5 Hz, 2 H), 7.45–7.30 (m, 8 H), 7.26 (ddd, J = 7.4/7.4/0.9 Hz, 2 H), 7.15 (br. s, 1 H), 6.95 (br. s, 2 H), 6.86 (t, J = 5.5 Hz, 1 H), 5.86 (ddt, J = 17.2/10.6/5.7 Hz, 1 H), 5.28 (ddd, J = 17.2/3.0/1.5 Hz, 1 H), 5.18 (J = 10.4/2.5/1.2 Hz, 1 H), 4.58 (ddd, J = 5.7/1.4/1.4 Hz, 2 H), 4.46 (d, J = 6.6 Hz, 2 H), 4.21 (t, J = 6.9 Hz, 1 H), 3.26 (br. s, 6 H), 3.13 (s, 2 H), 1.46 (s, 9 H), 1.36 (br. s, 6 H), 1.09 (br. s, 6 H), 0.77 (s, 9 H), –0.08 (s, 6 H). ^{13}C (APT, 75 MHz, $CDCl_3$): δ = 167.4 (C), 153.5 (C), 152.6 (C), 143.7 (C), 141.5 (C), 141.3 (C), 141.0 (C), 132.3 (CH), 129.1 (C), 128.6 (C), 128.1 (CH), 127.9 (CH), 127.2 (CH), 125.0 (CH), 120.1 (CH), 118.4 (C), 118.4 (CH), 118.3 (CH), 118.1 (CH), 81.0 (C), 67.1 (CH_2), 66.6 (CH_2), 65.6 (CH_2), 47.0 (CH), 40.8 (CH_2), 39.3 (C), 30.8 (CH_2), 28.3 (CH_3), 25.8 (CH_3), 23.1 (CH_2), 18.1 (C), –5.63 (CH_3) ppm. IR (KBr): $\tilde{\nu}$ = 3304 (w), 1755 (s), 1708 (m) cm^{-1}. LRMS (ESI$^+$): m/z = 1095.5 [M + H]$^+$. HRMS (ESI$^+$): calcd. for [$C_{62}H_{78}N_6O_{10}Si$ + H]$^+$: 1095.56215, found 1095.56582.

Synthesis of Carboxylic Acid 2: i) The silyl ether **9** (2.95 g, 2.70 mmol) was dissolved in a 1:1 mixture of CH_2Cl_2 (45 mL) and MeOH (45 mL). Then (±)-CSA (125 mg, 0.539 mmol, 20 mol-%) was added and the reaction mixture is stirred overnight. The reaction was monitored by reversed-phase HPLC at 262 nm and if there was more than 6% starting material, then an extra portion (±)-CSA (31 mg, 0.13 mmol, 5 mol-%) was added. After another 4 h the mixture is concentrated under reduced pressure in the presence of silica gel. The light-yellow powder is chromatographed with a gradient elution where the amount of MeOH is varied from 5 to 6% to give the alcohol (2.33 g, 88%); m.p. 153–155 °C. ^{1}H NMR (COSY, 500 MHz, MeOD): δ = 7.81 (d, J = 7.5 Hz, 2 H), 7.75–7.65 (m, 8 H), 7.56–7.42 (m, 6 H), 7.38 (dd, J = 7.4/7.4 Hz, 2 H), 7.29 (ddd, J = 7.4/7.4/0.9 Hz, 2 H), 5.96 (ddt, J = 17.2/10.6/5.5 Hz, 1 H), 5.34 (ddd, J = 17.2/3.1/1.6 Hz, 1 H), 5.20 (ddd, J = 10.5/2.6/1.5 Hz, 1 H), 4.60 (ddd, J = 5.7/1.4/1.4 Hz, 2 H), 4.48 (d, J = 6.4 Hz, 2 H), 4.27 (t, J = 6.6 Hz, 1 H), 3.30 (m, 8 H), 1.59–1.50 (m, 6 H), 1.48 (s, 9 H), 1.33–1.22 (m, 6 H) ppm. ^{13}C NMR (APT, 75 MHz, MeOD): δ = 169.8 (C), 155.5 (C), 155.3 (C), 154.8 (C), 145.2 (C), 143.9 (C), 143.5 (C), 143.4 (C), 142.7 (C), 134.1 (CH), 129.8 (C), 129.3 (C), 129.2 (CH), 129.1 (CH), 128.9 (CH), 128.2 (CH), 126.2 (CH), 121.0 (CH), 119.1 (CH), 119.0 (CH), 118.9 (CH), 118.0 (C), 81.7 (C), 67.9 (CH_2), 66.6 (CH_2), 48.4 (CH), 41.7 (CH_2), 40.3 (C), 32.0 (CH_2), 28.7 (CH_3) 24.2 (CH_2) ppm. IR (KBr): $\tilde{\nu}$ = 3304 (w), 1755 (s), 1708 (m) cm^{-1}. LRMS (ESI$^+$): m/z = 981.4 [M + H]$^+$. HRMS (ESI$^+$): calcd. for [$C_{56}H_{64}N_6O_{10}$ + Na]$^+$: 1003.4559, found 1003.4576.

ii) The alcohol (4.35 g, 4.43 mmol) was dissolved in acetone (28 mL) and the solution was cooled in an ice bath. Jones reagent was prepared by dissolving CrO_3 (4.01 g, 40.1 mmol) in a mixture of acetone (3.4 mL) and water (11.6 mL). The freshly prepared Jones reagent (6.7 mL, 2.67 M) was added slowly to the reagent mixture in two portions. After one hour another 1.7 mL Jones reagent (1 equiv.) was added. The reaction was stirred for another 2 h and then 2-propanol (10 mL) was added. The mixture was extracted twice with diethyl ether and 6 times with EtOAc, where after the organic phase was concentrated under reduced pressure in the presence of silica gel (20 g). The yellow powder was chromatographed with gradient elution where the percentage of MeOH in CH_2Cl_2 was varied from 5 to 15%) to give carboxylic acid **2** (3.39 g, 77%); m.p. 155–157 °C. ^{1}H NMR (COSY, 300 MHz, [D_6]DMSO): δ = 12.17 (br. s, 1 H), 9.96 (s, 1 H), 9.91 (s, 1 H), 9.56 (s, 1 H), 8.33 (br. s, 3 H), 7.96 (d, J = 7.4 Hz, 2 H), 7.80–7.68 (m, 8 H), 7.55–7.45 (m, 6 H), 7.41 (dd, J = 7.4/7.4 Hz, 2 H), 7.33 (ddd, J = 7.4/7.4/1.1 Hz, 2 H), 5.97 (ddt, J = 17.0/10.4/5.5 Hz, 1 H), 5.36 (ddd, J = 17.2/3.3/1.7 Hz, 1 H), 5.23 (ddd, J = 10.4/2.8/1.4 Hz, 1 H), 4.61 (ddd, J = 5.4/1.3/1.3 Hz, 2 H), 4.49 (d, 6.9 Hz, 2 H), 4.31 (t, 6.9 Hz, 1 H), 3.15 (s, 6 H), 1.51–1.41 (m, 6 H), 1.40–1.28 (s, 15 H), 1.40–1.28 (s, 6 H). ^{13}C (APT, 75 MHz, MeOD): δ = 180.4 (C), 169.7 (C), 155.5 (C), 154.8 (C), 145.2 (C), 144.0 (C), 143.5 (C), 143.4 (C), 142.7 (C), 134.1 (CH), 129.7 (C), 129.2 (CH), 128.9 (CH), 128.2 (CH), 126.2 (CH), 121.0 (CH), 119.1 (CH), 119.0 (CH), 118.8 (CH), 118.0 (C), 81.2 (C), 67.9 (CH_2), 66.6 (CH_2), 48.4 (CH), 41.2 (CH_2), 32.9 (CH_2), 28.7 (CH_3), 25.5 (CH_2) ppm. IR (KBr): 3304 (w), 1755 (s), 1708 (m) cm^{-1}. LRMS (ESI$^-$): m/z = 992.9 [M – H]$^-$. HRMS (ESI$^+$): calcd. for [$C_{56}H_{62}N_6O_{11}$ + H]$^+$: 995.4537, found 995.4549.

Solid-Phase Syntheses: After each step the resin was washed with three times DMF/MeOH/CH_2Cl_2 successively, unless differently

described. For the coupling reactions, the amount of solvent added was such that a concentration of ca. 0.1 M of coupling species was achieved. The loading was determined by treating an amount of resin with a 20% piperidine/DMF solution for 20 min. Then the absorbance of the piperidine-fulvene adduct was measured at 300 nm. From a correlation curve, the concentration of the adduct was retrieved from which the proper loading could be calculated.

Fmoc Deprotection: To the resin was added a 20% piperidine/DMF solution (10 mL/g resin) and the resin was shaken for 1 min. The solution was drained and the resin was washed. This step was repeated for 5 and 8 min.

Boc Deprotection: To the resin was added a 50% TFA/CH_2Cl_2 solution (10 mL/g resin) and the suspension was shaken for 5 min. The solution was drained and the resin was washed three times with CH_2Cl_2. This step was repeated once for a period of 25 min and afterwards the washing included CH_2Cl_2/10% Et_3N in CH_2Cl_2/MeOH/CH_2Cl_2.

Alloc Deprotection: To the resin was added $PhSiH_3$ (25 equiv.) in CH_2Cl_2 after which the $(PPh_3)_4Pd$ catalyst was added (10 mol-%). The suspension was shaken for 10 min. After draining the resin is washed with CH_2Cl_2/MeOH/CH_2Cl_2.

Phtotocleavage of the Resin: A small amount of resin (1 mg or less) was suspended in 100 μL of CH_3CN in a small glass tube. The tube was placed under UV light of 365 nm and after 3 h the solution was analysed via LC, ES-MS or LC-MS. When the amount of resin was increased, the irradiation time could be decreased enabling a fast analysis.

Synthesis of the Photocleavable Linker 10: See ref.[8]

Coupling of the Photocleavable Linker 10 to Tentagel-NH_2: Tentagel-NH_2 (539 mg, 135 μmol) was swollen in DMF (5.3 mL) and then the solvent was drained. The photocleavable linker **10** (210 mg, 404 μmol) was dissolved in 4 mL DMF and the solution was added to the pre-swollen resin. The coupling reagent PyBOP (520 mg, 404 μmol) and the base DIEA (145 μL, 808 μmol) were added. The suspension was shaken for 3 h. After draining, the resin was washed. The NF31 test was used to monitor the completeness of the reaction. By measuring the UV absorbance of the piperidine–fulvene adduct at 300 nm, the loading was determined to be 0.21 mmol g^{-1}.

Coupling of the Spacer Boc-GABA: First, the resin with the photocleavable linker (3 g, 0.75 mmol) was Fmoc-deprotected. Then the spacer Boc-GABA (475 mg, 2.25 mmol) and the coupling reagent PyBOP (1.17 g, 2.25 μmol) were added to the pre-swollen resin. DMF was added and the suspension was shaken until the reagents were dissolved. The base DIEA (785 μL, 4.5 mmol) was added where after the reaction vessel was shaken for 3 h. After draining and washing the resin, approximately 1 mg of the beads were subjected to a NF31 test which was negative.

Coupling of 1 to the Solid Anchor to Form Construct 11: The resin (14 mg, 3 μmol) was Boc-deprotected. A solution of **2** (4.5 mg, 4.5 μmol) and PyBOP (2.3 mg, 4.5 μmol) in 0.15 mL DMF was prepared. When homogeneous, the solution was transferred to the pre-swollen resin and after adding DIEA (2.4 μL, 13.5 μmol) the suspension was shaken for 18 h. The resin was drained and washed thoroughly. The TNBS test turned the beads dark-yellow and with NF31 we got red beads. The remaining free spacer-amino groups were capped for 25 min with a 0.1 M solution of AcOH and PyBOP in the presence of 2 equiv. of DIEA. The resin was drained and washed after which the capping was repeated for 20 min. The ninhydrin test was negative. The loading was 0.074 mmol/g and the amount of Fmoc deprotection is 12%. Thus the total loading was 0.084 mmol/g which correlated (maximal theoretical loading is 0.164 mmol g^{-1}) to a yield of 51%. LRMS (ESI^+, after photocleavage in CH_3CN): *m/z* = 1023 [M – *t*Bu + H]$^+$, 1079 [M + H]$^+$, 1101.5 [M + Na]$^+$. HRMS (ESI^+): calcd. for the Fmoc-deprotected **11**: $[C_{45}H_{60}N_8O_9 + H]^+$: 857.45556, found 857.45427

Supporting Information (see also the footnote on the first page of this article): Copies of ^1H and ^{13}C spectra of compounds **2**, **5**, **6**, **7**, **8**, **9** and intermediates in Scheme 3.

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