DOI: 10.1002/ejoc.200900536

An Efficient Synthesis of an Orthogonally Protected Aromatic Diamine as Scaffold for Tweezer Receptors with Two Different Arms

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Keywords: Protecting groups / Template synthesis / Peptides / Solid-phase synthesis / Receptors

The synthesis of a new versatile template, 3-(*N*-Boc-aminomethyl)-5-(*N*-Fmoc-aminomethyl)benzoic acid (**1**), was developed and is hereby reported. The key feature of the synthetic strategy is the selective mono reduction of a symmetric diazide using a biphasic reaction mixture. The mono reduction allows the sequential introduction of two orthogonal protecting groups – e.g., Fmoc and Boc. This building block can be used as a scaffold for the solid-phase synthesis of dipodal or cyclic peptidic receptors. It allows the preparation of tweezer receptors with unsymmetrically substituted side arms and therefore for the implementation of an enhanced structural and functional diversity in comparison to symmetric tweezers.

Introduction

The design of new sophisticated artificial receptors has led to the need for templates that are preorganized and yet flexible enough to allow for efficient binding of the substrate. If such a scaffold is equipped with a suitable anchor group it is possible to generate libraries of potential receptors on solid support using combinatorial methods.[1] Consequent screening then identifies the best receptors for a given substrate.

Synthetic host molecules with two arms, so-called tweezer receptors, have proven to feature selective substrate binding.[2] Additional interactions between host and guest often lead to superior results in comparison to their one armed analogs.[3] Thereby the template plays a decisive role in the binding behavior of the receptor: it defines its flexibility as well as the angle and distance between the two side chains.[3b,4] Both Boc- and Fmoc-protected derivatives of bis(aminomethyl)benzoic acid have shown to perform extraordinarily well as building blocks for the solid-phase synthesis of artificial peptidic and peptidomimetic hosts.[3b,5] However, most of the reported host systems are symmetric compounds with two identical side arms. In order to introduce a greater extent of functional and structural diversity the design of more elaborate receptors with two different arms is desirable. One of the few examples of unsymmetric tweezer receptors was recently presented by Kilburn et al.[6] A combinatorial library of receptors for a biological important tripeptide was synthesized using a guanidinium scaffold and screened on bead. Binding studies in aqueous medium but still on-bead revealed selective tripeptide binding with moderate affinities ($K \approx 10^3\ M^{-1}$). For a more pronounced use of unsymmetrical tweezers, additional scaffolds are needed which allow the introduction of two different side arms while keeping the synthetic effort as small as possible. We decided to develop a novel scaffold for unsymmetrical tweezer receptors. Therefore we designed and prepared the orthogonally protected template **1** (Figure 1) which allows the solid-phase peptide synthesis (SPPS) of artificial hosts with two different side arms. Other examples of orthogonally protected scaffolds (based on β-amino acids or pentaerythritol, for instance) have recently been reported e.g. by Heinonen or Madder, respectively.[7] A convenient synthesis of the dipodal scaffold **1** is reported herewith as well as the solid-phase synthesis of a first prototype of a tweezer receptor **11** with two different side arms.

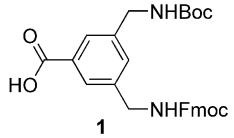

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Figure 1. Orthogonally protected template **1**.

Results and Discussion

Besides an acid group that can be attached to a resin, the newly developed template **1** incorporates each an Fmoc and a Boc protected amine – two orthogonal protecting groups that are well established in SPPS. The design therefore offers a synthetic strategy on solid support that follows the standard Fmoc procedure during the preparation of the first arm (with the need, however, to avoid Boc or *t*Bu

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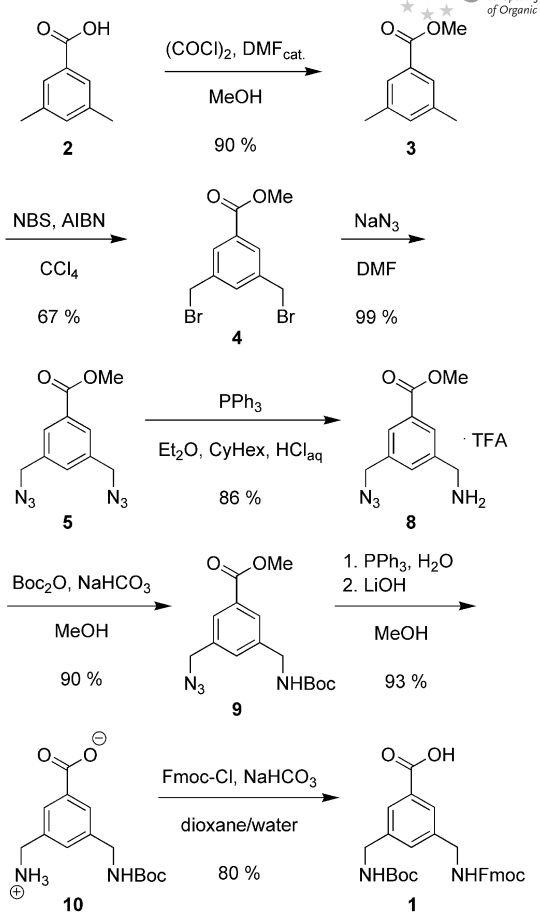
groups in the side chains of the amino acids in the first arm). After Boc deprotection the second side chain may then be synthesized, again, by the standard Fmoc procedure.

Scheme 1 describes the preparation of 3-(*N*-Boc-aminomethyl)-5-(*N*-Fmoc-aminomethyl)benzoic acid (**1**). The preparation of diazide **5** starting from **2** is known in literature and has been achieved accordingly. The synthesis could be carried out in batches of 5 grams with the commercially available 3,5-dimethylbenzoic acid (**2**) as starting material. Acid **2** was reacted to give the corresponding acyl chloride with oxalyl chloride and catalytic amounts of dimethylformamide (DMF).[8] In situ reaction with the solvent methanol gave the corresponding methyl ester **3**. The introduction of one bromine substituent to each methyl group (**4**) was carried out in a Wohl–Ziegler bromination with *N*-bromosuccinimide (NBS) and azobis(isobutyronitrile) (AIBN) in carbon tetrachloride.[9] The reported yield of 84% could not be reproduced. We obtained dibromide **4** in 67% yield after purification. Side products mainly consisted of starting material and the mono-brominated derivative. Increasing the amount of *N*-bromosuccinimide to more than 2.2 equiv. led to the formation of higher brominated products which complicated the purification of **4**. Elongation of the reaction time, exchange of the solvent to methyl formate and/or UV irradiation did not improve the yield. However, in the original literature procedure no data on the purity of the dibromide are provided. A moderate yield of a subsequent reaction using **4** as the starting material suggests that **4** was not completely pure, questioning the reported high yield.



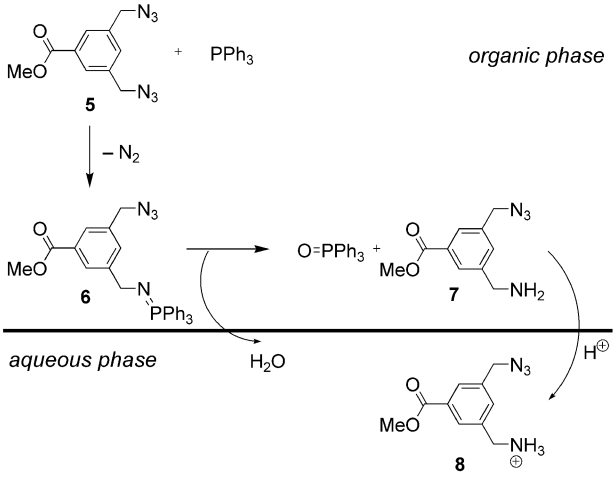
Scheme 1. Synthesis of the orthogonally protected template **1**.

The introduction of the amine functionalities was achieved by a classical Staudinger reaction. The bromines were quantitatively substituted by heating **4** with sodium azide in DMF to afford the diazide **5**, which can then be reduced to the corresponding amine with triphenylphosphane.[10] The complete reduction of the diazide with triphenylphosphane in methanol to the corresponding diamine works without problems (93% yield). However, any attempt to selectively functionalize one of the two amino groups failed.

Attempts to protect symmetric diamines with just one equivalent of protecting group are inefficient leading to product mixtures of unprotected, mono- and bis-protected product.[11] Sometimes the diprotected compound is even favoured due to solubility reasons. A simultaneous reaction with two different protecting groups is also possible but at best gives rise to a statistical distribution of products which often goes along with difficult purification steps. Both variants were tested on the diamine obtained after the double reduction of diazide **5** and did not lead to satisfying results.

Thus, in order to create an asymmetry we chose to carry out a selective mono reduction of the diazide **5** to the corresponding azidoamine **8**. This kind of reaction has been reported to the best of our knowledge so far only for two examples, one aliphatic and one polyethylene glycol spaced diazide.[12] These reductions were carried out with triphenylphosphane in a two-phase system composed of diethyl ether and an aqueous phase containing 5% HCl. The generation of the iminophosphorane intermediate **6** takes place in the organic phase while the hydrolysis takes place at the phase interface. The resulting amine **7** is immediately protonated by HCl and transferred as ammonium salt into the aqueous phase where it cannot be reduced further as the triphenylphosphane is only soluble in the organic phase (Scheme 2).

We could successfully adopt this procedure for the synthesis of diazide **5** (yield 61%) and even further improve the yield by addition of cyclohexane (CyHex) to the ether phase. This way the yield for the mono reduction increased to 86% (cf. 93% yield for the complete unselective reduction of both azide groups to the diamine). The addition of cyclohexane decreases the polarity of the organic layer favouring the phase transfer of the protonated amine and thus decreasing the amount of double reduction. The amine **8** was isolated as the trifluoracetic acid (TFA) salt by means of reversed-phase MPLC. The only side products were the diamine and compounds in which the methyl ester was hydrolyzed to the free carboxylic acid. Thus, the mono reduction of the diazide **5** in a biphasic reaction mixture provides an elegant and facile approach to a diamine scaffold in which the two amines can be individually functionalized further. The next step of the synthesis is the protection of

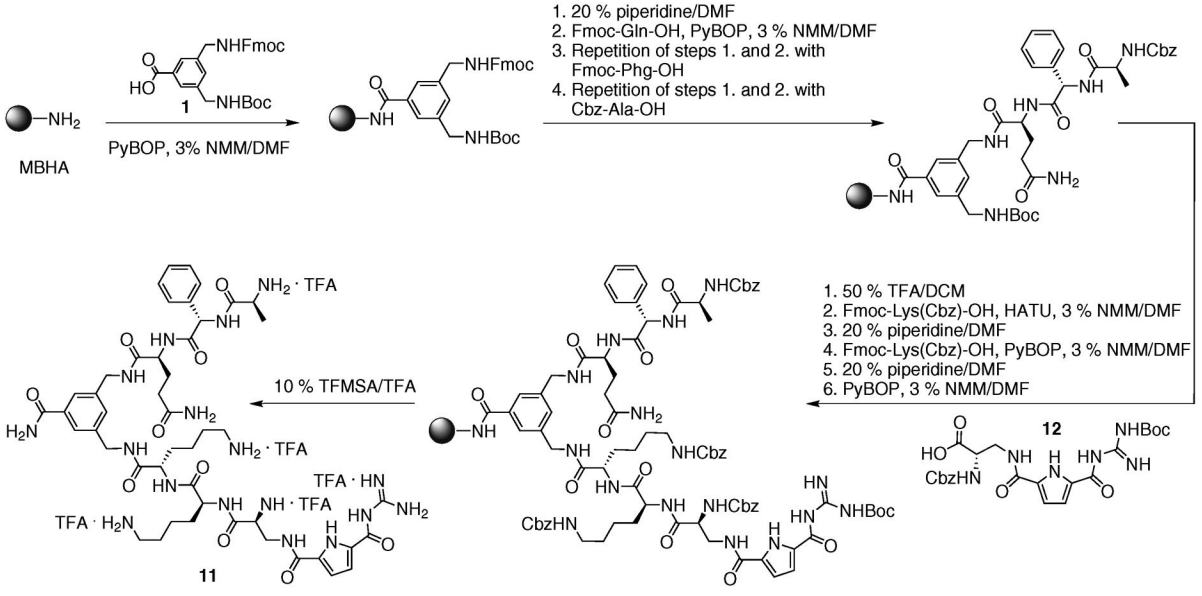


Scheme 2. Selective mono reduction of diazide **5** in a two-phase system consisting of an organic and an acidic aqueous phase.

the free amine in **8** with di-*tert*-butyl dicarbonate (Boc_2O) and sodium hydrogen carbonate under standard conditions in methanol to afford the Boc-protected product **9**. Reduction of the remaining azide function in **9** and saponification of the methyl ester were carried out in a one-pot synthesis. We abstained from a purification step after the reduction because the amine proved to be unstable during the column chromatography on silica. The resulting compound **10** was isolated via reversed-phase MPLC with 0.1% NEt_3 added to the solvent and obtained as zwitterion after lyophilisation. The last step is the protection of the amine with Fmoc-Cl and sodium hydrogen carbonate in dioxane/water to give the orthogonally protected template **1**. Approximately two grams of the product could easily be obtained from each batch after a final purification via flash column chromatography on silica gel.

As depicted in Scheme 3 this building block was then used for the solid-phase synthesis of the unsymmetric tweezer receptor **11**. The synthesis was carried out according to standard Fmoc protocol.[13] Completion of coupling and deprotection steps were monitored by the Kaiser test.[14] After swelling the MBHA resin (1.3 mmol/g) in dichloromethane (DCM), the template was attached with PyBOP as coupling reagent in 3% *N*-methylmorpholine (NMM)/DMF using 2.5 equiv. of each reactant. The coupling step was repeated with another equivalent of the reactants to assure complete conversion of all accessible amino groups on the resin. The resin was then treated with 10 equiv. of acetic anhydride and NMM in DMF to acetylate any remaining free amino groups. After deprotection of the Fmoc group with 20% piperidine/DMF the first arm – consisting of the three amino acids glutamine, phenylglycine and alanine – was synthesized. The deprotection time after the glutamine coupling was kept short in order to prevent pyroglutamate formation.[15] The last amino acid of the first arm carries a Cbz protecting group at the *N*-terminus which is stable under the conditions needed for the following Boc deprotection (50% TFA/DCM). The resin was then washed with DCM and 3% NMM/DMF to assure that no amino group is present as TFA salt which could lead to trifluoracetylation during the next coupling step. Afterwards lysine was attached to the template utilizing the more reactive HATU instead of PyBOP.[16] The coupling step was carried out twice in order to ensure quantitative conversion of the sterically demanding amine functionality of the template.

The next amino acid, again lysine, and the artificial arginine analog **12** were coupled following standard procedure again. Amino functions in the side chains were all protected with Cbz groups. **12** was incorporated into the receptor as an efficient binding motif for carboxylates. Its preparation and application have been described elsewhere.[17] After the



Scheme 3. Solid-phase peptide synthesis of the unsymmetric tweezer receptor **11**.

resin was thoroughly washed and dried the tweezer was cleaved from the solid support with 10% trifluormethanesulfonic acid (TFMSA)/TFA. After a first crude purification via reversed-phase MPLC with 0.1% TFA the receptor **11** could be obtained as TFA salt in 45% yield and with 85% purity according to HPLC analysis [Supelcosil™ LC-18 column (25 cm × 4.6 mm, 5 μm), solvent: 5 min water (0.1% TFA), then within 35 min to methanol (0.1% TFA), flow rate 1 mL/min, retention time T_R = 26.4 min, peak integration based on detection at 250 nm]. Hence, even though our synthesis incorporates a nonpeptidic template, two nonstandard amino acids (Phg and **12**) and an amino acid which often can cause problems in SPPS (glutamine)[18] the unsymmetrical tweezer **11** was obtained in good yields and purity. Also with respect to the efficient synthesis of **1**, this opens the way for a further exploration of this new tweezer class. Binding studies of the prototype **11** with anionic oligopeptides as guests are currently underway in our laboratory and will be reported in due time.

Conclusions

The new dipodal scaffold **1** could successfully be synthesized with an overall yield over seven steps of 34%. The key step is the selective mono reduction of a symmetric diazide taking advantage of the different solubility of starting material, reducing agent and mono reduction product in a biphasic reaction mixture. Our improved protocol might also be useful for other diazides and their conversion into orthogonally protected diamines. Template **1** can thus be easily synthesized in gram quantities starting from commercially available compounds. We could also show that **1** can be used in a modified SPPS Fmoc-protocol for the synthesis of unsymmetrical tweezers such as **11** with two different side arms.

Experimental Section

General Remarks: The starting materials and reagents were used as obtained from the commercial suppliers unless otherwise stated. Solvents were dried and distilled before use. All reactions were carried out in oven dried glassware. Lyophilization was carried out in an Alpha 1–4 2D plus freeze drying apparatus from Christ. Analytical TLC was carried out on SiO_2 aluminium foils 60 F254 from Merck and C18 SiO_2 aluminium foils ALUGRAM R*P*-18W/UV254 from Macherey–Nagel. Column chromatography was done on columns packed with ICN Silica with a spherical size of 32–63 μm from Biomedicals GmbH. Reversed-phase column chromatography was done with an Isco Inc. Combi Flash MPLC apparatus with RediSep C-18 reversed-phase columns. Analytical HPLC was done with a Dionex HPLC apparatus consisting of a P680 HPLC pump, an ASI-100 automated sample injector and a UVD 340U UV-detector with a Supelcosil™ LC-18 (25 cm × 4.6 mm, 5 μm) column. Commercially available HPLC grade solvents were used as eluents. The IR spectra were recorded on a FT-IR 1600 spectrometer from Perkin–Elmer. Bands were quoted in cm^{-1} and the following abbreviations are used: w weak, m medium, s strong, br. broad. ^1H and ^{13}C NMR spectra were recorded on an AVANCE 400 MHz and a DMX 600 MHz spectrometer from Bruker at ambient temperature. The chemical shifts are reported in parts per million (ppm) relative to the deuterated solvents $CDCl_3$ or [D_6]DMSO. The following abbreviations are used for peak multiplicities: s, singlet; d, doublet; t, triplet; m, multiplet; br, broad. Coupling constants (*J* values) are expressed in Hertz [Hz]. HR-ESI-mass spectra were received by using a Bruker MicroTOF focus. EI-mass spectra were obtained by using a Finnigan MAT 90. Melting points were obtained in open glass capillary tubes using an apparatus from Büchi and are quoted uncorrected.

Methyl 3,5-Dimethylbenzoate (3): To a solution of 3,5-dimethylbenzoic acid (5.00 g, 0.33 mol, 1 equiv.) in dry methanol (100 mL) a catalytic amount of dry dimethylformamide and oxalyl chloride (9.50 mL, 1.00 mol, 3 equiv.) were slowly added dropwise under argon atmosphere at 0 °C. After stirring for 5 h at 50 °C the solvent was evaporated under reduced pressure. The crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate, 1:1) to give the esterified product **3** as a white solid (4.92 g, 90%). R_f = 0.83 (SiO_2, hexane/ethyl acetate, 1:1), m.p. 30 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 3344 (w), 2975 (w), 2093 (m), 1714 (s), 1668 (m), 1604 (w), 1521 (m), 1436 (w), 1314 (s), 1220 (s), 1158 (m), 863 (w), 771 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 2.36 (s, 6 H, CH_3), 3.90 (s, 3 H, OMe), 7.18 (dd, $^4J_1 = {}^4J_2$ = 1.7 Hz, 1 H, CH_{ar}), 7.66 (d, ^4J = 1.7 Hz, 2 H, CH_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 21.1 (CH_3), 51.9 (OMe), 127.3 (CH_{ar}), 130.0 (C_q), 134.5 (CH_{ar}), 138.0 (C_q), 167.4 (CO_2) ppm. MS (pos. EI): *m*/*z* calculated for $C_{10}H_{12}O_2^{+\cdot}$ [$M^{+\cdot}$] 164.083; found 164.1.

Methyl 3,5-Bis(bromomethyl)benzoate (4): To a refluxing solution of **3** (5.20 g, 0.31 mol, 1 equiv.) in carbon tetrachloride (100 mL) *N*-bromosuccinimide (12.40 g, 0.70 mol, 2.2 equiv.) and a catalytic amount of 2,2′-azobisisobutyronitrile were added and stirred for 48 h. After cooling to room temperature the resulting precipitate was removed by filtration, washed with carbon tetrachloride and rejected. The yellow-orange filtrate was washed with a saturated aqueous solution of sodium hydrogen carbonate (50 mL) and brine (50 mL). The colorless organic phase was dried with sodium sulfate and the solvent was evaporated under reduced pressure. The raw product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate, 19:1) affording the dibrominated **4** as a white solid (6.83 g, 67%). R_f = 0.67 (SiO_2, hexane/ethyl acetate, 1:9), m.p. 99 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 2117 (w), 1723 (s), 1435 (m), 1315 (m), 1212 (s), 994 (m), 898 (w), 771 (m), 697 (s) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.93 (s, 3 H, OMe), 4.50 (s, 4 H, CH_2), 7.61 (dd, $^4J_1 = {}^4J_2$ = 1.7 Hz, 1 H, CH_{ar}), 7.99 (d, ^4J = 1.7 Hz, 2 H, CH_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 31.8 (CH_2), 52.4 (OMe), 130.0 (CH_{ar}), 131.4 (C_q), 133.8 (CH_{ar}), 138.9 (C_q), 165.9 (CO_2) ppm. MS (pos. EI): *m*/*z* calculated for $C_{10}H_{10}Br_2O_2^{+\cdot}$ [$M^{+\cdot}$] 319.904; found 319.9.

Methyl 3,5-Bis(azidomethyl)benzoate (5): A suspension of **4** (7.30 g, 22.65 mmol, 1 equiv.) and sodium azide (5.89 g, 90.76 mmol, 4 equiv.) in dimethylformamide (100 mL) was stirred at 65 °C for 3 h. The solution was diluted with water (300 mL) and extracted with ethyl acetate (5 × 200 mL). The combined organic phases were dried (Na_2SO_4) and the solvent was removed under reduced pressure. The brown, oily raw product was purified by flash column chromatography on silica gel (cyclohexane/ethyl acetate, 5:1) to give the yellowish, oily product **5** (5.52 g, 99%). R_f = 0.55 (SiO_2, hexane/ethyl acetate, 1:9), m.p. 99 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 2952 (w), 2091 (w), 1718 (s), 1607 (w), 1303 (m), 1216 (s), 1116 (m), 1007 (w), 860 (m), 765 (s), 718 (w), 610 (w) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 3.94 (s, 3 H, OMe), 4.43 (s, 4 H, CH_2), 7.48 (dd, $^4J_1 = {}^4J_2$ = 1.6 Hz, 1 H, CH_{ar}), 7.96 (d, ^4J = 1.6 Hz, 2 H, CH_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 52.4 (OMe), 54.1 (CH_2), 128.9 (CH_{ar}), 131.4 (C_q), 131.8 (CH_{ar}), 136.7 (C_q), 166.1 (CO_2) ppm. MS (pos. EI): *m*/*z* calculated for $C_{10}H_{10}N_6O_2^{+\cdot}$ [$M^{+\cdot}$] 246.087; found 246.1.

Methyl 3-(Aminomethyl)-5-(azidomethyl)benzoate, TFA Salt (8): To a solution of **5** (4.00 g, 16.24 mmol, 1 equiv.) in diethyl ether (120 mL) cyclohexane (40 mL) and 5% aqueous hydrochloric acid (160 mL) were added. The mixture was cooled to 0 °C, triphenylphosphane (4.26 g, 16.24 mmol, 1 equiv.) was added and the solution was stirred for 8 h at 0 °C and for 40 h at ambient temperature. The phases were separated and the organic layer was extracted once with 5% aqueous hydrochloric acid (80 mL). The combined aqueous phases were freeze dried in vacuo. The raw product was purified by MPLC on C18 reversed-phase silica gel (20% water/methanol to 40% water/methanol in 40 min, 0.1% TFA) to give **8** as white solid (4.43 g, 86%). R_f = 0.66 (C18 RP SiO_2, water/methanol, 1:1 + 0.1% TFA), m.p. 138 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 2969 (br. w), 2098 (w), 1710 (m), 1671 (s), 1423 (w), 1313 (w), 1237 (m), 1176 (s), 889 (w), 836 (w), 779 (m), 722 (m) cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 3.89 (s, 3 H, OMe), 4.15 (s, 2 H, CH_2), 4.61 (s, 2 H, CH_2), 7.73 (dd, $^4J_1 = {}^4J_2$ = 1.6 Hz, 1 H, CH_{ar}), 7.97 (dd, ^4J = 1.6 Hz, 1 H, CH_{ar}), 8.26 (dd, ^4J = 1.6 Hz, 1 H, CH_{ar}), 8.39 (br. s, 3 H, NH_3^+) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 41.6 (CH_2), 52.2 (OMe), 52.6 (CH_2), 128.7 (CH_{ar}), 129.3 (CH_{ar}), 130.2 (C_q), 133.5 (CH_{ar}), 135.2 (C_q), 137.0 (C_q), 165.5 (CO_2) ppm. HR-MS (pos. ESI): *m/z* calculated for $C_{10}H_{13}N_4O_2^+$ [M + H$^+$ – TFA] 221.1033; found 221.1033.

Methyl 3-(*N*-Boc-aminomethyl)-5-(azidomethyl)benzoate (9): Sodium hydrogen carbonate (0.54 g, 6.43 mmol, 1.2 equiv.) and **8** (1.70 g, 5.36 mmol, 1 equiv.) were dissolved in water/methanol (5:1, 50 mL) and Boc anhydride (1.40 g, 6.43 mmol, 1 equiv.) was added. After stirring for 20 h water (100 mL) was added and the mixture was extracted with ethyl acetate (3 × 75 mL). The combined organic phases were dried with sodium sulfate and the solvent was removed under reduced pressure. The raw product was purified by flash column chromatography on silica gel (cyclohexane/ethyl acetate, 7:3) to afford **9** as white solid (1.85 g, 90%). R_f = 0.84 (SiO_2, hexane/ethyl acetate, 1:1), m.p. 64–66 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 3343 (w), 2984 (w), 2092 (m), 1713 (s), 1675 (m), 1520 (m), 1435 (w), 1276 (m), 1222 (s), 1156 (s), 1122 (m), 929 (w), 862 (m), 773 (m), 706 (m), 617 (m) cm^{-1}. ^1H NMR (400 MHz, $CDCl_3$): δ = 1.47 (s, 9 H, *t*Bu), 3.93 (s, 3 H, OMe), 4.38 (d, ^3J = 5.6 Hz, 2 H, CH_2), 4.40 (s, 2 H, CH_2), 7.45 (s, 1 H, CH_{ar}), 7.89 (s, 1 H, CH_{ar}), 7.93 (s, 1 H, CH_{ar}) ppm. ^{13}C NMR (100 MHz, $CDCl_3$): δ = 28.3 (Boc-CH_3), 44.1 (CH_2), 52.3 (OMe), 54.2 (CH_2), 79.8 (Boc-C_q), 128.1 (CH_{ar}), 128.2 (CH_{ar}), 131.1 (C_q), 131.3 (CH_{ar}), 136.3 (C_q), 140.4 (C_q), 155.8 ($NHCO_2$), 166.4 (CO_2) ppm. HR-MS (pos. ESI): *m/z* calculated for $C_{15}H_{20}N_4O_4Na^+$ [M + Na$^+$] 343.1377; found 343.1383.

3-(*N*-Boc-aminomethyl)-5-(aminomethyl)benzoic Acid (10): To a solution of **9** (1.72 g, 5.37 mmol, 1 equiv.) in methanol/water (10:1, 30 mL) triphenylphosphane (1.41 g, 5.37 mmol, 1 equiv.) was added. After stirring for 20 h lithium hydroxide (1.29 g, 53.69 mmol, 10 equiv.) and water (27 mL) were added and the mixture was stirred for additional 24 h. The solvent was concentrated to a few mL under reduced pressure, water (50 mL) was added and the resulting suspension was extracted with ethyl acetate (50 mL). The aqueous phase was freeze dried in vacuo. The raw product was purified by MPLC on C18 reversed-phase silica gel (water to 40% water/methanol in 30 min, 0.1% NEt_3) to give **10** as white solid (1.40 g, 93%). R_f = 0.68 (C18 RP SiO_2, water/methanol, 1:1 + 0.1% TFA), m.p. > 250 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 3344 (m), 2979 (w), 2093 (m), 1713 (s), 1676 (s), 1520 (s), 1276 (m), 1222 (s), 1157 (s), 863 (m), 775 (m) cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 1.40 (s, 9 H, *t*Bu), 3.69 (s, 2 H, CH_2), 4.10 (d, ^3J = 5.9 Hz, 2 H, CH_2), 7.12 (s, 1 H, CH_{ar}), 7.32 (t, ^3J = 5.7 Hz, 1 H, NH), 7.61 (s, 1 H, CH_{ar}), 7.68 (s, 1 H, CH_{ar}) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 28.2 (Boc-CH_3), 43.5 (CH_2), 45.0 (CH_2), 77.6 (Boc-C_q), 126.2 (CH_{ar}), 126.8 (CH_{ar}), 126.9 (CH_{ar}), 138.7 (C_q), 139.4 (C_q), 155.7 ($NHCO_2$) ppm; the COOH signal could not be detected. HR-MS (pos. ESI): *m/z* calculated for $C_{14}H_{20}N_2O_4Na^+$ [M + Na$^+$] 303.1315; found 303.1315.

3-(*N*-Boc-aminomethyl)-5-(*N*-Fmoc-aminomethyl)benzoic Acid (1): Fmoc-Cl was recrystalized from toluene/cyclohexane. To a solution of **10** (1.38 g, 4.92 mmol, 1 equiv.) and sodium hydrogen carbonate (1.12 g, 13.29 mmol, 2.7 equiv.) in dioxane/water (5:2, 70 mL) Fmoc-Cl (1.91 g, 7.38 mmol, 1.5 equiv.) was added and the mixture was stirred for 5 h. Water (80 mL) was added and the pH was adjusted to 6 with 5% aqueous hydrochloric acid. The resulting suspension was extracted with ethyl acetate (5 × 250 mL). The combined organic phases were dried with sodium sulfate and the solvent was removed under reduced pressure. The raw product was purified by flash chromatography on silica gel (ethyl acetate, then ethyl acetate/methanol, 9:1) to give **1** as white solid (1.91 g, 80%). R_f = 0.58 (SiO_2, ethyl acetate), m.p. 184–185 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 3340 (w), 2366 (m), 1688 (s), 1540 (m), 1476 (w), 1250 (m), 1162 (w), 1055 (w), 783 (w) cm^{-1}. ^1H NMR (400 MHz, [D$_6$]DMSO): δ = 1.39 (s, 9 H, *t*Bu), 4.15 (d, ^3J = 6.0 Hz, 2 H, CH_2), 4.20–4.25 (m, 3 H, Fmoc-CH + CH_2), 4.31 (d, ^3J = 7.0 Hz, 2 H, Fmoc-CH_2), 7.28–7.45 (m, 6 H, 4 × Fmoc-CH_{ar} + CH_{ar} + NH), 7.68–7.76 (m, 4 H, Fmoc-CH_{ar}), 7.88 (d, ^3J = 7.5 Hz, 2 H, CH_{ar}), 7.94 (dd, $^3J_1 = {}^3J_2$ = 6.1 Hz, 1 H, NH) ppm. ^{13}C NMR (100 MHz, [D$_6$]DMSO): δ = 28.1 (Boc-CH_3), 43.1 (CH_2), 43.6 (CH_2), 46.6 (Fmoc-CH), 65.5 (Fmoc-CH_2), 77.8 (Boc-C_q), 119.9 (CH_{ar}), 120.0 (CH_{ar}), 121.3 (C_q), 125.1 (CH_{ar}), 126.3 (CH_{ar}), 126.3 (CH_{ar}), 127.0 (CH_{ar}), 127.2 (CH_{ar}), 127.5 (CH_{ar}), 128.8 (CH_{ar}), 139.7 (C_q), 140.2 (C_q), 140.6 (C_q), 143.8 (C_q), 155.7 ($NHCO_2$), 156.2 ($NHCO_2$) ppm; the COOH signal could not be detected. HR-MS (pos. ESI): *m/z* calculated for $C_{29}H_{30}N_2O_6Na^+$ [M + Na$^+$] 525.1996; found 525.1997.

Solid-Phase Peptide Synthesis of the Receptor (11): The reaction was carried out in a flask equipped with a frit on a Heidolph Rotamax 120 shaker. MBHA resin (58 mg, 1.3 mmol/g) was swollen in DCM (5 mL) for 1 h and washed with DMF (3 × 5 mL). The template **1** (95 mg, 0.19 mmol, 2.5 equiv.) was attached to the resin by shaking for 5 h under argon atmosphere with PyBOP (98 mg, 0.19 mmol, 2.5 equiv.) in 3% NMM/DMF (5 mL) and consequent washing with DMF (3 × 5 mL). The coupling and washing steps were repeated with 1 equiv. PyBOP (39 mg, 0.08 mmol) and **1** (38 mg, 0.08 mmol). Afterwards the resin was treated with acetic anhydride (71 μL, 0.75 mmol, 10 equiv.) and NMM (83 μL, 0.75 mmol, 10 equiv.) in DMF (5 mL) for one hour. Fmoc deprotection was achieved by shaking the resin in 20% piperidine/DMF (5 mL) for 15 min twice followed by washing with DMF (6 × 5 mL). Fmoc-Gln-OH (69 mg, 0.19 mmol, 2.5 equiv.), Fmoc-Phg-OH (70 mg, 0.19 mmol, 2.5 equiv.) and Cbz-Ala-OH (42 mg, 0.19 mmol, 2.5 equiv.) were coupled in the same manner without repetition of the coupling and washing steps. The duration of the Fmoc deprotection step of Fmoc-Gln-OH was reduced to two times 10 min. Boc deprotection of the template was achieved by shaking the resin in 50% TFA/DCM (5 mL) for 15 min twice followed by washing with DCM (3 × 5 mL) and 3% NMM/DMF (3 × 5 mL). Then Fmoc-Lys(Cbz)-OH (95 mg, 0.19 mmol, 2.5 equiv.) was coupled with HATU (69 mg, 0.18 mmol, 2.4 equiv.) in the same manner as described above. The coupling was carried out twice. Then the second Fmoc-Lys(Cbz)-OH (95 mg, 0.19 mmol, 2.5 equiv.) and **12** (97 mg, 0.19 mmol, 2.5 equiv.) were coupled with PyBOP again. The resin was washed with DCM (3 × 5 mL), methanol (3 × 5 mL) and DCM (3 × 5 mL) and dried under reduced pressure for one hour. In order to cleave the product the resin was shaken for 3 h in 10% TFMSA/TFA (5 mL) and washed with TFA (5 mL). The red-brown filtrate was collected and

concentrated in high vacuum at room temperature. Diethyl ether (20 mL) was added and the resulting suspension was centrifuged. The supernatant solvent was decanted and the solid was washed with diethyl ether and centrifuged again. The raw product was dissolved in little methanol, water (30 mL) and TFA (1 mL) were added and the mixture was freeze dried in vacuo. The resulting solid was purified by MPLC on C18 reversed-phase silica gel (water to methanol in 45 min, 0.1% TFA) to give **11** as voluminous white solid (51 mg, 45%). HPLC: 5 min water (0.1% TFA), then in 35 min to methanol (0.1% TFA), 1 mL/ min, T_R = 26.4 min (250 nm), m.p. 245 °C. FT-IR (KBr disk): $\tilde{\nu}$ = 3291 (w), 1661 (s), 1536 (s), 1429 (m), 1282 (w), 1197 (m), 1130 (s), 986 (s), 721 (w), 610 (m) cm^{-1}. ^1H NMR (600 MHz, [D$_6$]DMSO): δ = 1.25–1.41 (m, 8 H), 1.47–1.57 (m, 5 H), 1.58–1.64 (m, 1 H), 1.66–1.77 (m, 2 H), 1.79–1.93 (m, 2 H), 1.98 (t, ^3J = 8.3 Hz, 1 H), 2.12 (t, ^3J = 7.9 Hz, 1 H), 2.70–2.79 (m, 4 H, CH_2), 3.95–4.09 (m, 2 H), 4.19–4.37 (m, 7 H), 5.58 (d, ^3J = 7.9 Hz, 1 H, CH), 6.76–6.84 (m, 1 H), 6.86–6.91 (m, 1 H), 7.17–7.21 (m, 1 H), 7.23 (s, 1 H), 7.25–7.38 (m, 5 H), 7.42 (t, ^3J = 7.4 Hz, 2 H), 7.63 (s, 2 H), 7.92–8.01 (m, 1 H), 8.13 (br. s, 3 H, NH_3^+), 8.32–8.36 (m, 1 H), 8.38 (br. s, 2 H, NH_2), 8.42 (t, ^3J = 5.8 Hz, 1 H), 8.52–8.62 (m, 3 H), 8.63–8.67 (m, 1 H), 8.74 (d, ^3J = 7.2 Hz, 1 H, NH), 8.77 (d, ^3J = 7.6 Hz, 1 H, NH), 8.85 (br. s, 2 H, NH_2), 8.97 (d, ^3J = 7.5 Hz, 1 H, NH), 9.14 (d, ^3J = 8.1 Hz, 1 H, NH), 11.64 (br. s, 1 H, NH), 12.30 (s, 1 H, NH) ppm. ^{13}C NMR (150 MHz, [D$_6$]DMSO): δ = 17.2 (CH_3), 22.0 (CH_2), 22.3 (CH_2), 26.4 (CH_2), 26.6 (CH_2), 27.8 (CH2), 27.9 (CH_2), 31.0 (CH_2), 31.1 (CH_2), 38.5 (CH_2), 44.7 (CH_2), 44.8 (CH_2), 47.8 (CH), 51.8 (CH), 52.5 (CH), 52.7 (CH), 55.7 (CH), 56.0 (CH), 112.7 (CH_{ar}), 115.4 (CH_{ar}), 124.6 (CH_{ar}), 126.6 (CH_{ar}), 127.4 (CH_{ar}), 127.7 (CH_{ar}), 128.3 (CH_{ar}), 128.4 (CH_{ar}), 128.8 (CH_{ar}), 132.1 (CO), 134.3 (CO), 137.6 (CO), 138.4 (CO), 139.1 (CO), 139.4 (CO), 155.3 (CO), 158.3 (CO), 158.6 (CO), 159.9 (CO), 167.0 (CO), 167.8 (CO), 167.9 (CO), 169.3 (CO), 170.8 (CO), 171.5 (CO), 173.6 (CO) ppm. HR-MS (MALDI-TOF): *m/z* calculated for $C_{47}H_{70}N_{17}O_{10}^+$ [M – 5 TFA + H^+] 1032.548; found 1032.556.

Acknowledgments

Financial support of our work from the Deutsche Forschungsgemeinschaft (DFG) and the Fonds der Chemischen Industrie is gratefully acknowledged. H. Y. K. would like to thank the Studienstiftung des Deutschen Volkes for a PhD scholarship.

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Received: May 14, 2009
Published Online: July 27, 2009

