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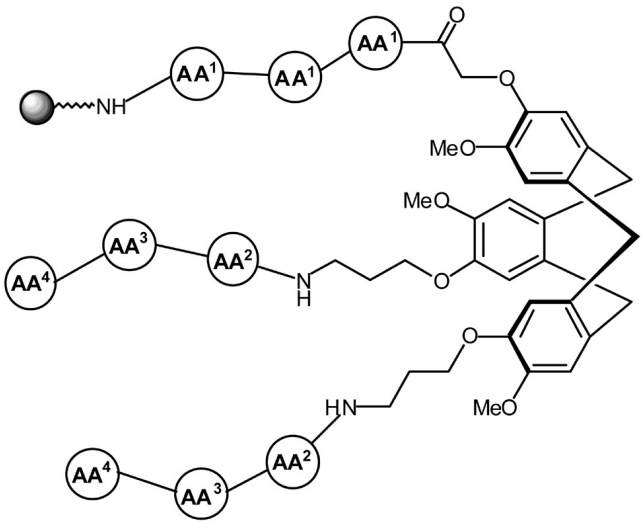
2197 member-library



AA^1 = Ala

AA^2, **AA**^{3}, **AA**^{4} = Gly, Ala, Val, Leu, Ser, Tyr, Phe, Hys, Pro, Asp, Glu, Gln, Lys

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Approaches to the Solid Phase of a Cyclotriveratrylene Scaffold-Based Tripodal Library as Potential Artificial Receptors

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Two versatile tripodal cyclotriveratrylene (CTV)-based scaffolds (**7** and **9**) have been prepared for the solid phase construction of libraries of tripodal artificial synthetic receptors. A 2197-member library of CTV-based tripodal receptor molecules, **20**{*1*−*13*,*1*−*13*,*1*−*13*}, was prepared on the solid phase using split−mix synthesis. The CTV-based receptors contain three peptide arms; one of them is attached to the solid phase and is different from the other two identical peptide arms.

Introduction

In recent years, significant progress has been made in the area of synthetic or artificial receptors containing two binding arms, which have been often designated as tweezer-like synthetic receptors.[1,2] To increase diversity, possibly affinity, selectivity, and other properties, we are interested in the development of artificial receptors containing three binding arms, that is, tripodal molecules containing ultimately three different sites of interaction. Still, the number of suitable molecules to serve as suitable scaffolds for attachment of three binding sites is very limited. Noteworthy examples in the literature include steroids,[3] diverse macrocycles,[4] Kemp's triacid,[5] amidopyridine,[6] and hexasubstituted benzenes.[7]

We have been particularly interested in the triazacyclophane (TAC) scaffold and the cyclotriveratrylene (CTV) scaffold. Recently, we have developed an efficient synthesis for the former TAC scaffold and libraries employing this scaffold.[8] This led to the construction of a selectively deprotectable TAC scaffold, allowing the introduction of three different functional groups and the preparation and screening of libraries of synthetic tripodal receptor molecules with three different amino acid or peptide arms.[8b−c] A CTV-scaffold-based library synthesized in solution was also described by us,[9] and the successful use of this scaffold in the construction of triple helical collagen mimics clearly demonstrates the capability of this scaffold to position functional groups in space.[10] Here, we describe the preparation of two versatile CTV-scaffold derivatives for the solid phase construction of libraries of tripodal artificial synthetic receptors. As an illustration of the usefulness, a 2197-member library was prepared.

Results and Discussion

Since the CTVscaffold has three possible sites of attachment, in order to use this scaffold in solid phase synthesis, one site has to be reserved for linking it to a solid phase resin. It was decided to convert this site for the time being to an "invariant arm". More or less arbitrarily the Ala-Ala-Ala sequence was chosen for this arm. Alanine represents the simplest (chiral) amino acid and is also not bulky. In addition, it was decided not to include functional amino acids in this "linkage" arm but to include them in the two other arms to obtain a "general" library. Future opportunities for the linkage arm include the use of more rigid spacers[11] and functional-group-containing spacers for focused libraries.

Synthesis of the $(Boc)_2$CTV-Scaffold Derivative (7). The C_3 hinge part of the CTV-scaffold derivatives, CTV (**1**), was synthesized in three steps on a multigram scale starting from vanillyl alcohol.[12] When the condensation step was performed using the ionic liquid N_{6444} imide as a solvent,[13] comparable yields of **1** were obtained in a more "green" procedure.

The first step in the synthesis of Boc-protected CTV scaffold (**5**), being compatible with Fmoc chemistry on the solid phase and functionalized with a carboxylic acid moiety to allow attachment to resin beads, was monoalkylation of **1** with benzyl bromoacetate to **2** (Scheme 1). Because of the presence of three equivalent hydroxy groups, mono-, di-, and trialkylated CTV derivatives (**2**, **3**, and **4**, respectively) were always obtained, and the desired monoalkylated **2** required tedious purification. Optimal reaction conditions were the use of 1.1 equiv of benzyl bromoacetate and 1 equiv of cesium carbonate in DMF. The desired monoalkylated compound **2** was obtained in 40% yield, together with dialkylated derivative **3** (20%), traces of the trialkylated derivative **4** (1%), and starting material (5%). Treatment of **2** with 1-bromo-3-*N*-Boc-propylamine gave CTV derivative **5** in 80% yield. Hydrogenation of **5** afforded the di-Boc-protected CTV scaffold **7** (98%) (Scheme 1).

Although this derivative is excellently suitable for solid-phase synthesis of libraries consisting of (a) members not having acid-labile functional groups in the linkage arms and (b) members irreversibly attached to the solid phase resin, a Fmoc derivative is clearly needed as a "super" amino acid derivative to allow all possible amino acids to be included

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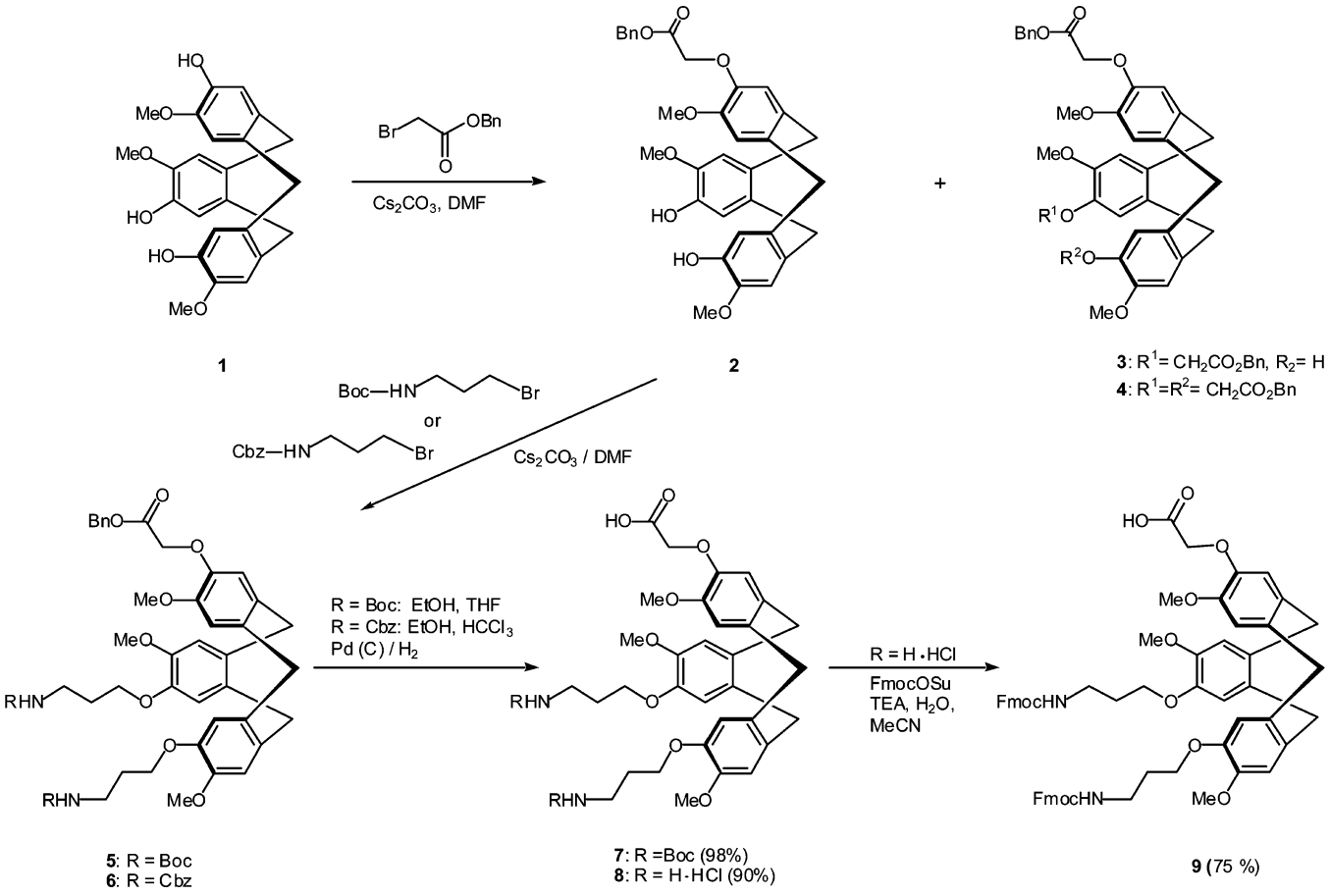
Scheme 1. Synthesis of $(Boc)_2$-CTV−OH (**7**) and $(Fmoc)_2$-CTV−OH (**9**)



in the linkage arm and to prevent premature cleavage from the resin when an acid-labile solid-phase resin linker is used.

Therefore, a convenient di-Fmoc-protected CTV scaffold (**9**) compatible with the acid-labile Argogel Rink resin was synthesized. Thus, treatment of **2**[9] with 1-bromo-3-*N*-Cbz-propylamine gave **6** in 86% yield. Hydrogenolysis of **6** in EtOH/$CHCl_3$ (1:1) led to 90% of the hydrochloride salt **8**, which was reacted with Fmoc-OSu to give **9** in 75% yield (Scheme 1).

Solid-Phase Synthesis of CTV-Based Receptor 14. To evaluate if $(Boc)_2$CTV-scaffold derivative (**7**) can, indeed, be used in a continuous solid-phase synthesis procedure of CTV-based tripodal receptor molecules, **14** was chosen as a test case (Scheme 2). Although the amino acid sequence was somewhat arbitrarily, deliberately bulky amino acids were included to determine the completeness of the couplings as well as functional amino acids. Since the $(Boc)_2$CTV scaffold was used in this example in the linkage arms, amino acid residues without protecting groups were introduced (vide supra).

The solid-phase route of receptor **14** is depicted in Scheme 2: First, construction of the peptide linkage arm on the solid phase by three subsequent BOP coupling steps[14] and Fmoc-deprotection steps of Fmoc-L-Ala−OH, Fmoc-L-Phe−OH, and Fmoc-L-Leu−OH on Argogel-OH resin loaded with glycine (**10**) to give **11**. Introduction of a Fmoc-glycine residue to Argogel-OH resin enables a reliable determination of the loading of the resin. Unreacted OH groups of **11** on the resin were capped by acetylation. This was followed by Fmoc deprotection and attachment of $(Boc)_2$CTV scaffold **7** to the linkage arm to give **12**. Next, the two identical tripeptide binding arms were introduced after removal of the Boc groups in **12**, followed by three subsequent BOP coupling steps and Fmoc-deprotection steps involving Fmoc-L-Ser(^{t}Bu)−OH, Fmoc-L-Lys(Boc)−OH, and Boc-L-Val−OH to afford the Boc-protected CTV-based receptor on the solid phase, that is, **13**. Finally, transesterification with NaCN in methanol afforded protected receptor **14** in an overall yield for the last nine steps of 75%. This corresponds to an average of 96% per step. When **14** was analyzed by HPLC, two peaks of equal intensity at 25.9 and 26.2 min were found (Figure 1). The CTV core exists as a racemic mixture of M−(−) or P−(+)[15,16] enantiomers; therefore, the two peaks found in

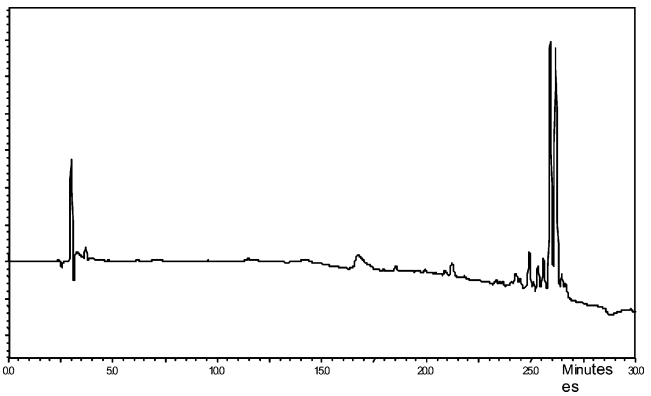
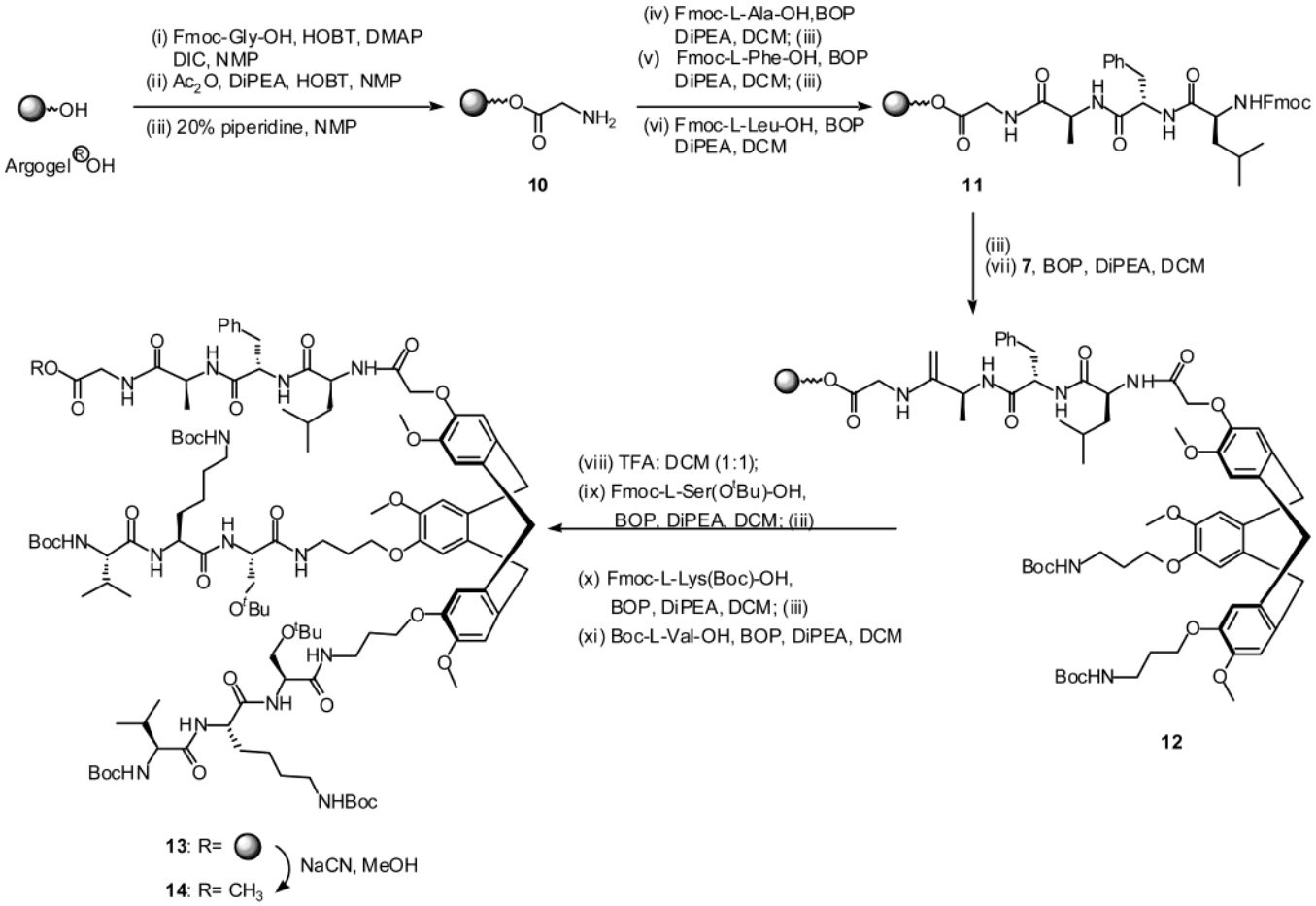


Figure 1. HPLC pattern of synthetic receptor **14** (crude product) showing the presence of both diastereomers.

Scheme 2. Solid Phase Synthesis of CTV-Based Receptor (**14**)



HPLC probably correspond to both diastereomers. ES-MS of both diastereomers of **14** clearly showed the presence of the $[M + 2Na]^{2+}$ peak at $m/z = 1084.85$.

Solid-Phase Synthesis of a CTV Library. Having shown that solid-phase synthesis of a CTV tripod receptor went very well, a CTV-based receptor molecules library was now designed as a "one-bead−one-compound" split−mix[17] library. To facilitate future screening experiments, the CTV-based receptors were covalently bound to Argogel-NH_2 resin. The strategy for the preparation of the CTV-based library was identical to the one used before for the construction of the CTV-based receptor **14**, except for the final cleavage of the receptors from the solid phase. A trialanine sequence was chosen as a simple, hydrophobic, and not sterically encumbered sequence for the peptidic arm connected to the solid phase. In the future, diversity can be introduced in this first peptidic arm by variation of this sequence. The construction of the first peptidic arm consisted of three subsequent BOP coupling/Fmoc deprotection of Fmoc-L-Ala−OH to Argogel-NH_2 (Scheme 3). Then, the $(Boc)_2$CTV scaffold **7** was coupled, and the Boc groups were cleaved from **15**, resulting in **16** (Scheme 3). A 2197-member library of Fmoc-protected CTV-based **20**{*1*−*13*,*1*−*13*,*1*−*13*} was prepared by 3-fold coupling of 13 Fmoc-protected amino acids, **17**{*1*−*13*}, Fmoc-Gly−OH, Fmoc-L-Ala−OH, Fmoc-L-Val−OH, Fmoc-L-Leu−OH, Fmoc-L-Ser(^{t}Bu)−OH, Fmoc-L-Asp(O^{t}Bu)−OH, Fmoc-L-Glu(O^{t}Bu)−OH, Fmoc-L-Gln(Trt)−OH, Fmoc-L-Lys(Boc)−OH, Fmoc-L-Phe−OH, Fmoc-L-Tyr(^{t}Bu)−OH, Fmoc-L-His(Trt)−OH, and Fmoc-L-Pro−OH, to both free amine groups of **16** and using the split-and-mix strategy (Scheme 3). Finally, Fmoc deprotection followed by Boc, *tert*-butyl, and trityl cleavage of the relevant amino acid side chains generated the resin-bound fully deprotected CTV-based receptors library, **20**{*1*−*13*,*1*−*13*,*1*−*13*}(Scheme 3).

Conclusions

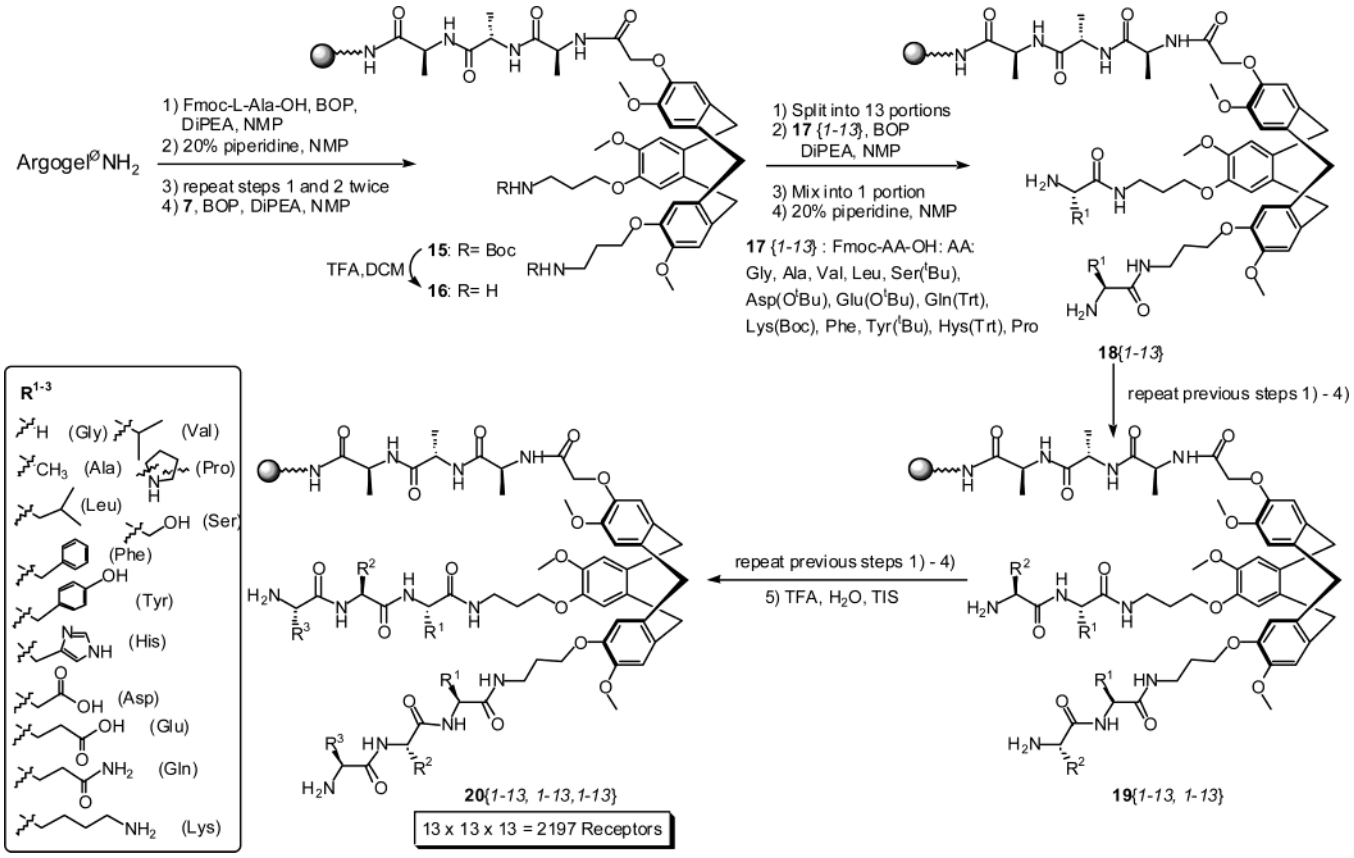
We have shown that the cyclotriveratrylene (CTV) scaffold is a versatile scaffold for the preparation of tripodal synthetic receptor molecules, and this was exemplified by the preparation of an arbitrary synthetic receptor containing functional amino acid residues. Furthermore, the scaffold was also successfully used in the split−mix synthesis of a library of CTV-containing solid-phase bound synthetic receptors containing 2197 members.

An especially interesting feature of this scaffold is the possibility to orient the peptidic arms in a parallel way, which might favor binding of ligands and already has proven to be useful for alignment of peptidic sequences, as was recently shown in the preparation of collagen mimics.[10] The selective functionalization of the CTV will also enable expansion of the diversity of these CTV receptors, which is underway. Finally, screening experiments using these libraries will be reported soon.

Experimental Section

General. All the reagents were purchased from commercial sources and used without further purification. Ar-

Scheme 3. Split−Mix Synthesis of 2197-Member CTV-Based Receptor Library



gogel-NH_2 (0.40 mmol/g, average bead diameter 178 μm) and Argogel-OH (0.48 mmol/g, average bead diameter 154 μm) resins were purchased from Argonaut Technologies, Inc. Argogel-OH resin was dried over P_2O_5. Protected amino acids were purchased from Alexis Corporations (Läufelfingen, Switzerland) and Advanced Chemtech Europe (Machelen, Belgium). All reactions on the solid phase were performed in standard glassware or polyethylene glycol (PE) syringes with PE frits. Peptide grade solvents dried on molecular sieves were used for reactions and resin washing. The capping agent used was a mixture of acetic anhydride (42 mL), DiPEA (2.18 mL), HOBT (0.23 g), and NMP (100 mL). Kaiser and bromophenol blue (BPB) tests[18,19] were used for detection of primary amines on the solid phase. Analytical thin-layer chromatography (TLC) was performed on Merck precoated silica gel 60 F_{254} (0.25 mm) plates. Spots were visualized with UV light, ninhydrine, or Cl_2−TDM.[20] Column chromatography was performed using Merck Kieselgel 60 (40−63 μm). ^{1}H NMR and ^{13}C NMR were obtained on a Varian 300 MHz spectrometer. Chemical shifts are given in parts-per-million (ppm) with respect to the internal standard TMS for ^{1}H NMR. ^{13}C NMR spectra were recorded using the attached proton test (APT) pulse sequence. Elemental analyses were performed at Kolbe Mikroanalytisches Laboratorium (Mülheim an der Ruhr, Germany), and the determined values are within 0.4% of theory. ES-MS experiments were performed on a Shimadzu LCMS QP8000 system. HPLC analyses were performed on a Shimadzu 10Avp (Class VP) using a PL-ELS-1000 detector and UV detector operating at 220 and 254 nm. An Adsorbophere C8, 90 Å, (4.6 × 250 mm, 5 μm) was used; flow rate was 1 mL/min; two mobile phases (mobile phase A, 99.9% water, 0.1% TFA; mobile phase B, 5% water, 95% acetonitrile, 0.085% TFA) were used with a standard protocol, 0% B for 5 min then 100% B in 20 min, 100% B for 5 min then 0% B in 5 min, and reequilibration at 0%B for 5 min.

Synthesis of [2-*O*-(Benzyloxycarbonyl)methyl]-CTV (2), [2,7-di-*O*-(Benzyloxycarbonyl)methyl]-CTV (3) and [2,7,-12-tri-*O*-(Benzyloxycarbonyl)methyl]-CTV (4). To a solution of **1** (2.04 g, 5 mmol) in DMF (100 mL) was added Cs_2CO_3 (1.63 g, 5 mmol). After cooling to 0°C, a solution of benzyl bromoacetate (871 μL, 5.5 mmol) in DMF (1 mL), was added dropwise. The suspension was stirred overnight at room temperature. The solvent was evaporated, and DCM was added. The mixture was washed with $KHSO_4$ (1M), H_2O, and brine. Upon drying (Na_2SO_4) and evaporating the solvent, the crude product was subjected twice to column chromatography (DCM/methanol, 100:1, 50:1, 20:1 gradient and DCM/methanol, 10:1) to give:

2 (1.10 g, 40%) as a white foam. R_f = 0.37 (DCM/methanol, 10:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.29−7.17 (m, 5H, Ar−Bn), 6.88, 6.86, 6.81, 6.77, 6.67 (6s, 6H, Ar-CTV), 5.51, 5.47 (2s, 2H, OH), 5.16 (d, 1H, CH_{2a}Ph, $J_{a,b}$ = 12.1 Hz), 5.09 (d, 1H, CH_{2b}Ph), 4.70 (d, 1H, CH_{2a}CO, $J_{a,b}$ = 16.3 Hz), 4.65 (d, 3H, CH_{2ax}-CTV), 4.58 (d, 1H, CH_{2b}-CO), 3.81, 3.78 (2s, 9H, OCH_3), 3.48, 3.47, 3.40 (3d, 3H, CH_{2ec}-CTV, $J_{ec,ax}$ = 14.0 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.36 (CO_2Bn), 148.45, 145.71, 145.24, 145.15, 144.07, 143.99 (C-2,3,7,8,12,13-CTV), 135.07, 134.08, 132.42, 131.88, 131.21, 131.03 (Ar-*C*CH_2-CTV, Ar-*C*-Bn), 128.39, 128.26, 128.07 (Ar-*H*-Bn), 117.79, 115.47, 115.38, 113.83, 112.17, 112.02 (C-1,4,6,9,11,14-CTV), 67.42, 66.57 (OCH_2-

Ph, OCH_2CO), 55.97, 55.94, 55.88 (OCH_3), 36.15 (CH_2-CTV). ES-MS (m/z) = 579.30 (100%, $[M + Na]^+$). Anal. Calcd for $C_{33}H_{32}O_8$: C 71.21, H 5.79. Found: C 71.16, H 5.84.

3 (0.70 g, 20%) as a white foam. R_f = 0.56 (DCM/methanol, 10:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.30−7.17 (m, 10H, Ar−Bn), 6.87, 6.84, 6.83, 6.73, 6.68 (6s, 6H, Ar-CTV), 5.39 (s, 1H, OH), 5.16, 5.14 (2d, 2H, CH_{2a}Ph, $J_{a,b}$ = 12.1 Hz), 5.09 (d, 2H, CH_{2b}Ph), 4.71−4.65 (m, 3H, CH_{2ax}-CTV), 4.70, 4.66 (2d, 2H, $CH_{2a}CO$, $J_{a,b}$ = 16.5 Hz), 4.59, 4.53 (2d, 2H, $CH_{2b}CO$), 3.83, 3.81, 3.80 (3s, 9H, OCH_3), 3.51, 3.45, 3.43 (3d, 3H, CH_{2ec}-CTV, $J_{ec,ax}$ = 13.5 Hz). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.33, 169.28 (CO_2Bn), 148.50, 148.45, 145.70, 145.22, 144.06 (C-2,3,7,8,12,13-CTV), 135.06, 134.09, 133.97, 131.90, 131.70, 131.49, 131.08 (Ar-*C*CH_2CTV, Ar-*C*-Bn), 128.32, 128.26, 128.15, 128.06 (Ar-*H*-Bn), 117.69, 115.44, 113.74, 113.57, 111.98 (C-1,4,6,9,-11,14-CTV), 67.39, 67.33, 66.61, 66.57 (OCH_2Ph, OCH_2CO), 55.96, 55.87 (OCH_3), 36.20 (CH_2-CTV). ES-MS (m/z) = 727.45 (100%, $[M + Na]^+$). Anal. Calcd for $C_{42}H_{40}O_{10}$: C 71.58, H 5.72. Found: C 71.16, H 5.84.

4 (0.05 g, 1%) as a white foam. R_f = 0.80 (DCM/methanol, 10:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.37−7.16 (m, 15H, Ar−Bn), 6.84, 6.73 (2s, 6H, Ar-CTV), 5.13 (d, 3H, CH_{2a}-Ph, $J_{a,b}$ = 12.1 Hz), 5.08 (d, 3H, CH_{2b}Ph), 4.68 (d, 3H, CH_{2ax}-CTV, $J_{ax,ec}$ = 13.7 Hz), 4.67 (d, 3H, $CH_{2a}CO$, $J_{a,b}$ = 16.5 Hz), 4.52 (2d, 3H, $CH_{2b}CO$), 3.80 (s, 9H, OCH_3), 3.44 (d, 3H, CH_{2ec}-CTV). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.24 (CO_2Bn), 148.51, 145.75 (C-2,3,7,8,12,13-CTV), 135.06, 133.98, 131.35 (Ar-*C*CH_2-CTV, Ar-*C*-Bn), 128.41, 128.27, 128.10 (Ar-*H*-Bn), 117.66, 113.56 (C-1,4,6,9,11,14-CTV), 67.30, 66.58 (OCH_2Ph, OCH_2CO), 55.88 (OCH_3), 36.19 (CH_2-CTV). ES-MS (m/z) = 891.35 (28%, $[M + K]^+$), 875.45 (100%, $[M + Na]^+$), 853.35 (5%, $[M + H]^+$). Anal. Calcd for $C_{51}H_{48}O_{12}$: C 71.82, H 5.67. Found: C 71.79, H 5.74.

Starting material **1** (0.10 g, 5%) was also recovered.

Synthesis of [2-*O*-(Benzyloxycarbonyl)methyl-7,12-di-*O*-[*N*-*tert*-butyloxycarbonyl]propylamine]-CTV (5). To a solution of CTV derivative **2** (1.11 g, 2 mmol) in acetonitrile (40 mL), Cs_2CO_3 (1.95 g, 6 mmol) and the 1-bromo-3-*N*-(*tert*-butyloxycarbonyl)propylamine (1.43 g, 6 mmol) was added. After the suspension was stirred at room temperature overnight, the solvent was evaporated, and ethyl acetate was added. The mixture was washed with $KHSO_4$ (1 M), H_2O, and brine. After drying (Na_2SO_4) and evaporating the solvent, the crude was purified by column chromatography using DCM/ethyl acetate, 10:1 and 5:1, to yield **5** (1.30 g, 80%) as a white foam. R_f = 0.30 (DCM/ethyl acetate, 5:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.28−7.12 (m, 5H, Ar−Bn), 6.85, 6.81, 6.80, 6.77, 6.70 (5s, 6H, Ar-CTV), 5.41 (bs, 2H, NH), 5.16 (d, 1H, CH_{2a}Ph, $J_{a,b}$ = 12.2 Hz), 5.07 (d, 1H, CH_{2b}Ph), 4.73, 4.71 (2d, 3H, CH_{2ax}-CTV, $J_{ax,ec}$ = 13.4 Hz), 4.68 (d, 1H, $CH_{2a}CO$, $J_{a,b}$ = 16.2 Hz), 4.58 (d, 1H, CH_{2b}-CO), 4.11−3.86 (m, 4H, CH_2O), 3.82, 3.81, 3.77 (3s, 9H, OCH_3), 3.51, 3.50, 3.43 (3d, 3H, CH_{2ec}-CTV), 3.33−3.20 (m, 4H, CH_2N), 1.94−1.90 (m, 4H, CH_2), 1.41 (s, 18H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.15 (CO_2Bn), 155.98 (CONH), 148.35, 148.14, 146.69, 146.57, 145.78 (C-2,3,7,8,12,13-CTV), 134.97, 133.84, 132.45, 132.26, 131.80, 131.32 (Ar-*C*CH_2-CTV, Ar-*C*-Bn), 128.31, 128.11, 128.01 (Ar-*H*-Bn), 117.55, 115.07, 113.80, 113.13, 112.90 (C-1,4,6,9,11,14-CTV), 78.65 ($C(CH_3)_3$), 68.32, 67.18, 66.56 (OCH_2), 56.02, 55.84, 55.69 (OCH_3), 38.68 (NCH_2), 36.27, 36.12 (CH_2-CTV), 29.06 (CH_2), 28.06 ($C(CH_3)_3$). ES-MS (m/z) = 893.50 (100%, $[M + Na]^+$), 771.45 (11%, $[M - (Boc) + H]^+$), 715.75 (55%, $[M - (Boc, {}^tBu) + H]^+$). Anal. Calcd for $C_{49}H_{62}N_2O_{12}$: C 67.57, H 7.17; N 3.22. Found: C 67.38, H 7.12, N 3.15.

Synthesis of [2-*O*-(Benzyloxycarbonyl)methyl-7,12-di-*O*-[*N*-benzyloxycarbonyl]propylamine]-CTV (6). To a solution of CTV derivative **2** (1.11 g, 2 mmol) in DMF (40 mL), Cs_2CO_3 (1.95 g, 6 mmol) and 1-bromo-3-(*N*-benzyloxycarbonyl)propylamine (1.63 g, 6 mmol) was added. After the suspension was stirred at room temperature overnight, the solvent was evaporated, and ethyl acetate was added. The mixture was washed with $KHSO_4$ (1 M), H_2O, and brine. After drying (Na_2SO_4) and evaporating the solvent, the crude product was purified by column chromatography using DCM/methanol, 50:1, and **6** (1.61 g, 86%) was obtained as a white foam. R_f = 0.58 (DCM/methanol, 20:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.34−7.26, 7.19−7.12 (2m, 15H, Ar−Bn), 6.85, 6.81, 6.80, 6.77, 6.76, 6.68 (6s, 6H, Ar-CTV), 5.86 (m, 2H, NH), 5.18−5.04 (m, 6H, CH_2Ph), 4.73, 4.72 (2d, 3H, CH_{2ax}-CTV, $J_{ax,ec}$ = 13.6 Hz), 4.70 (d, 1H, $CH_{2a}CO$, $J_{a,b}$ = 16.5 Hz), 4.58 (d, 1H, $CH_{2b}CO$), 4.10−3.79 (m, 2H, CH_2O), 3.77, 3.69, 3.65 (3s, 9H, OCH_3), 3.58−3.32 (m, 5H, CH_2N, CH_{2ec}CTV), 1.95 (CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 169.26 (CO_2Bn), 156.53 (CONH), 148.45, 148.19, 146.57, 145.87 (C-2,3,7,8,12,13-CTV), 136.77, 135.07, 133.89, 132.63, 132.43, 131.87, 131.57 (Ar-*C*CH_2-CTV, Ar-*C*-Bn), 128.44, 128.41, 128.27, 128.16, 128.06, 127.95 (Ar-*H*-Bn), 117.66, 115.11, 113.83, 113.01, 112.83 (C-1,4,6,9,-11,14-CTV), 68.66, 67.28, 66.69, 66.45 (OCH_2), 56.11, 55.74, 55.61 (OCH_3), 39.59 (NCH_2), 36.42 (CH_2-CTV), 29.02 (CH_2). ES-MS (m/z) = 961.60 (100%, $[M + Na]^+$), 940.40 (80%, $[M + H]^+$). Anal. Calcd for $C_{55}H_{58}N_2O_{12}$: C 70.35, H 6.23, N 2.98. Found: C 70.25, H 6.11, N 2.88.

Synthesis of [2-*O*-Carboxymethyl-7,12-di-*O*-(*N*-*tert*-butyloxycarbonyl)propylamine]-CTV (7). To cooled a solution (0 °C) of CTV derivative **5** (1.11 g, 2 mmol) in ethanol/THF, 2:1 (120 mL), Pd charcoal (10%) (20% w/w) was added, and the suspension was stirred under H_2 pressure from a balloon at room temperature overnight. The reaction was filtered, and the solvent was evaporated to give **7** (1.53 g, 98%) as a white foam. R_f = 0.27 (DCM/methanol, 10:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 6.74, 6.71, 6.66, 6.50 (4s, 6H, Ar-CTV), 5.52, 5.44 (2bs, 2H, NH), 4.54 (m, 3H, CH_{2ax}-CTV), 4.32 (m, 2H, CH_2COOH), 4.04 (bs, 4H, CH_2O), 3.81, 3.78 (2s, 9H, OCH_3), 3.53, 3.43 (2bs, 3H, CH_{2ec}-CTV), 3.27 (m, 4H, CH_2N), 1.90 (m, 4H, CH_2), 1.42, 1.37 (2s, 18H, $C(CH_3)_3$). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 173.84 (COOH), 156.11, 156.08 (CONH), 148.17, 146.85, 146.68, 144.95 (C-2,3,7,8,12,13-CTV), 132.88, 132.44, 132.04, 131.88, 131.71, 131.50 (Ar-*C*CH_2-CTV), 115.15, 113.08, 112.42 (C-1,4,6,9,-11,14-CTV), 78.82, 78.71 ($C(CH_3)_3$), 68.38, 68.03 (OCH_2), 56.13, 55.81 (OCH_3), 38.67 (NCH_2), 36.25 (CH_2-CTV), 29.27 (CH_2), 28.42 ($C(CH_3)_3$). ES-MS (m/z) = 803.25

(100%, $[M + Na]^+$), 681.45 (31%, $[M - (Boc) + H]^+$), 625.30 (43%, $[M - (Boc - {}^tBu) + H]^+$, 581.50 (28%, $[M - (2Boc) + H]^+$). Anal. Calcd for $C_{42}H_{56}N_2O_{12}$: C 64.60, H 7.23, N 3.59. Found: C 64.52, H 7.22, N 3.65.

Synthesis of [2-*O*-Carboxymethyl-7,12-di-*O*-(*N*-fluoren-9-ylmethoxycarbonyl)propylamine]-CTV (9). To a cooled solution (0 °C) of CTV derivative **6** (1.11 g, 2 mmol) in ethanol/chloroform, 1:1 (120 mL), Pd charcoal (10%) (20% w/w) was added, and the suspension was stirred under H_2 pressure from a balloon at room temperature overnight. The reaction was filtered, and the solvent was evaporated to give the hydrochloride salt **8** (1.18 g, 90%) as a white foam. R_f = 0.16 (DCM/methanol, 5:1). ^{1}H NMR (DMSO, 300 MHz) δ: 8.04 (bs, 4H, NH_2), 7.09, 6.99, 6.97 (3s, 6H, Ar-CTV), 4.65 (d, 3H, CH_{2ax}-CTV, $J_{ax,ec}$ = 13.7 Hz), 4.63 (s, 2H, CH_2-CO), 4.06, 3.98 (m, 4H, CH_2O), 3.72 (s, 9H, OCH_3), 3.48 (d, 3H, CH_{2ec}-CTV), 2.89 (m, 4H, CH_2N), 1.95 (CH_2). ^{13}C NMR (DMSO, 75 MHz) δ: 170.38 (COOH), 147.82, 147.68, 147.45, 146.15, 145.98, 145.77 (C-2,3,7,8,12,13-CTV), 132.87, 132.77, 132.59, 132.06 (Ar-*C*CH_2-CTV), 115.48, 114.91, 114.12, 113.57 (C-1,4,6,9,11,14-CTV), 66.05, 65.95, 65.11 (OCH_2), 56.20, 55.85 (OCH_3), 38.36 (NCH_2), 36.59, 35.22 (CH_2-CTV), 26.89 (CH_2). ES-MS (*m*/*z*) = 1183.85 (36%, $[2M + Na]^+$), 603.95 (35%, $[M + Na]^+$), 581.55 (100%, $[M + H]^+$). The hydrochloride salt **8** (0.98 g, 1.5 mmol) was dissolved in water (7.5 mL) and TEA (627 μL, 4.5 mmol), and a solution of Fmoc-OSu (1.01 g, 3 mmol) in acetonitrile (7.5 mL) was added. The mixture was stirred at room temperature for 2 h, the solvent was evaporated, and ethyl acetate was added. The mixture was washed with $KHSO_4$ (1M), H_2O, and brine. After drying (Na_2SO_4) and evaporating the solvent, the crude product was purified by column chromatography (DCM/methanol, 50:1, 20:1) to give **9** (1.15 g, 75%) as a white foam. R_f = 0.61 (DCM/methanol, 5:1). ^{1}H NMR ($CDCl_3$, 300 MHz) δ: 7.67, 7.51 (2d, 8H, Ar-Fmoc, J = 7.4 Hz), 7.30, 7.19 (2t, 8H, Ar-Fmoc), 6.86, 6.77, 6.76, 6.75, 6.73, 6.72 (6s, 6H, Ar-CTV), 5.88 (bs, 2H, NH), 4.68, 4.66 (2d, 3H, CH_{2ax}-CTV, $J_{ax,ec}$ = 13.7 Hz), 4.57 (d, 1H, $CH_{2a}CO$, $J_{a,b}$ = 16.2 Hz), 4.47 (d, 1H, $CH_{2b}CO$), 4.32 (dd, 2H, CH_2-Fmoc, J_{gem} = 5.2 Hz, $J_{CH2a,CH} = J_{CH2b,CH}$ = 2.2 Hz), 4.05 (m, 4H, CH_2O), 3.93 (bs, 1H, CH-Fmoc), 3.75, 3.69, 3.66 (3s, 9H, OCH_3), 3.46 (d, 3H, CH_{2ec}-CTV), 3.33 (m, 4H, CH_2N), 1.92 (m, 4H, CH_2). ^{13}C NMR ($CDCl_3$, 75 MHz) δ: 170.45 (COOH), 156.58 (CONH), 148.36, 146.72, 143.98, 141.24 (C-2,3,7,8,12,13-CTV, Ar-*C*-Fmoc), 132.39, 131.08 (Ar-*H*-CTV), 127.58, 126.97, 125.02, 119.88 (Ar-*H*-Fmoc), 115.25, 113.14 (C-1,4,6,9,11,14-CTV), 68.73, 68.04, 66.42 (CH_2-Fmoc, OCH_2), 55.87 (OCH_3), 47.29 (CH-Fmoc), 39.52 (CH_2N), 36.45 (CH_2-CTV), 29.14 (CH_2). ES-MS (*m*/*z*) = 1047.95 (47%, $[M + Na]^+$), 1026.40 (100%, $[M + H]^+$). Anal. Calcd for $C_{62}H_{60}N_2O_{12}$: C 72.64, H 5.90; N 2.73. Found: C 72.52, H 6.04, N 2.80.

General Procedure for Coupling Fmoc/Boc-Amino Acids or (Boc)$_2$-CTV−OH (7) on the Solid Phase. The resin (1 equiv) was swollen in NMP (2 min), and after draining the solvent, BOP (4 equiv), the Fmoc/Boc-amino acid (4 eequivq) and NMP (15 mL/mmol) were added. The mixture was shaken until complete disolution, then DiPEA (8 equiv) was added. Additional coupling of two Fmoc/Boc-amino acids was carried out using a double amount of reagents. After 3 h of shaking, the resin was washed with NMP (5 × 2 min) and DCM (5 × 2 min). When (Boc)$_2$-CTV−OH derivative **7** was used, 2 equiv of **7**, 2 equiv of BOP, and 4 equiv of DiPEA were used, and the reaction was shaken at room temperature overnight. Negative Kaiser and BPB tests indicated completion of the coupling. The loading of the resin was determined by Fmoc-photometric quantification, after drying the resin under vacuum overnight.

General Procedure for N^{α}-Fmoc Deprotection. The N^{α}-Fmoc-protected resin was swollen in NMP (2 min), and after draining the solvent, the resin was shaken with 20% piperidine in NMP (3 × 10 mL/mmol, each 10 min). The resin was washed with NMP (5 × 2 min) and DCM (5 × 2 min). Positive Kaiser and BPB tests indicated the Fmoc deprotection.

Synthesis of the CTV-Based Receptor 14 on the Solid Phase. Argogel-OH (1 g, 0.48 mmol), was swollen in NMP (8 mL, 2 min). After draining off the solvent, HOBT monohydrate (0.58 g, 1.92 mmol), DMAP (58 mg, 0.48 mmol), Fmoc-Gly−OH (0.57 g, 1.92 mmol), and NMP (8 mL) were added. The suspension was shaken by bubbling nitrogen through the mixture until complete disolution, and then DIC (150 μL, 0.96 mmol) was added. After N_2 bubbling overnight, the resin was drained and washed with NMP (5 × 8 mL, each 2 min), DCM (5 × 8 mL, each 2 min) and Et_2O (5 × 8 mL, each 2 min) and dried under vacuum overnight (loading = 0.40 mmol/g). Next, the resin (1 g, 0.39 mmol) was swollen in NMP (7 mL, 2 min), and capping solution (8 mL) was added. After shaking for 1 h, the resin was washed with NMP (5 × 7 mL, each 2 min) and DCM (5 × 7 mL, each 2 min). Upon N^{α}-Fmoc deprotection of the resin, Fmoc-L-Ala−OH·H_2O (0.52 g, 1.56 mmol) was coupled to resin **10** (1 g, 0.39 mmol) following the general procedures described above. Then N^{α}-Fmoc deprotection, coupling of Fmoc-Phe−OH (0.60 g, 1,56 mmol), N^{α}-Fmoc deprotection and coupling of Fmoc-L-Leu−OH (0.55 g, 1.56 mmol) were performed to give resin **11** (loading = 0.34 mmol/g). After removal of N^{α}-Fmoc from **11** (234 mg, 0.079 mmol), (Boc)$_2$-CTV−OH scaffold (**7**) was attached to give **12** following the general procedure. Subsequently, resin **12** was swollen in DCM (7 mL, 2 min), the solvent was drained, and the resin was treated with 50% TFA in DCM (10 mL) for 45 min, followed by washing with NMP (3 × 7 mL, each 2 min), 25% DiPEA in NMP (4 × 7 mL, each 2 min), NMP (5 × 7 mL, each 2 min), and DCM (5 × 7 mL, each 2 min) to give positive Kaiser test and BPB tests. After coupling of Fmoc-L-Ser(But)−OH, N^{α}-Fmoc deprotection, coupling of Fmoc-L-Lys(Boc)−OH, N^{α}-Fmoc deprotection, and coupling of Boc-L-Val−OH, resin **13** was obtained. Finally, resin **13** was washed with methanol (4 × 7 mL, each 2 min), and the solvent was drained. Sodium cyanide (catalytic amount) and methanol (2 mL) were added, and the reaction mixture was shaken overnight. After filtration, collection of the filtrate of the resin gave, after evaporation, receptor **14** (126 mg, 75%) as a white solid. HPLC: Rt = 25.9 min (44%), Rt = 26.2 min (45%), ES-MS (*m*/*z*) = 1084.85 (100%, $[M + 2Na]^{2+}$), 1023.60 (29%, $[M - (Boc) + Na + H]^{2+}$), 996.05 (20%, $[M - (Boc - {}^tBu) + Na +$

H]$^{2+}$), 962.60 (24%, [M − (2Boc) + 2H]$^{2+}$), 934.41 (36%, [M − (3Boc) + 2Na]$^{2+}$).

Loading of the Resin Argogel-NH_2 (15). Fmoc-L-Ala−OH·H_2O (1.05 g, 3.2 mmol) was coupled to the Argogel-NH_2 resin (2 g, 0.8 mmol), and the N^{α}-Fmoc group was cleaved following the general procedure. Coupling of Fmoc-L-Ala−OH·H_2O was repeated twice, ending with resin-bound trialanine in a loading of 0.36 mmol/g. N^{α}-Fmoc deprotection and coupling of **7** (687 mg, 0.86 mmol) gave **15**. Treatment of **15** with 50% TFA in DCM (7 mL) for 45 min and further washing of the resin with NMP (3 × 7 mL, each 2 min), 25% DiPEA in NMP (4 × 7 mL, each 2 min), NMP (5 × 7 mL, each 2 min), and DCM (5 × 7 mL, each 2 min) led to resin **16**.

Preparation of Library 20{*1−13,1−13,1−13*}. Resin **16** was dried overnight under vacuum, divided into 13 equal portions (100 mg) in PE syringes with PE frits, each containing 0.036 mmol and, swollen in NMP (0.7 mL, 2 min). After draining the solvent, BOP (127 mg, 8 equiv), a different Fmoc-amino acid from **17**{*1−13*} (8 equiv) and NMP (0.7 mL) were added to each portion. The mixtures were shaken until all the reagents were dissolved, and DiPEA (98.4 μL, 16 equiv) was added to every reaction. The syringes were placed on a shaker overnight. The resin was washed with NMP (5 × 1 mL, each 2 min) and DCM (5 × 1 mL, each 2 min). After each reaction, the loading was determined by quantification of the Fmoc-cleavage product to give an average loading of 0.30 mmol/g. Then the content of the syringes was pooled and mixed in a reaction vessel washed with NMP (2 × 13 mL, each 2 min), and N^{α}-Fmoc was removed following the general procedure to give the library **18**{*1−13*}. Upon drying library **18**{*1−13*} overnight under vacuum, the split−mix procedure was repeated for the coupling of the second Fmoc-amino acid **17**{*1−13*}, followed by N^{α}-Fmoc deprotection to give library **19**{*1−13,1−13*}. The last split−mix cycle led to the attachment of the third Fmoc-amino acid **17**{*1−13*} to give the Fmoc-protected library of **20** {1−13, 1−13, 1−13}. The separate reactions (average loading = 0.25 mmol/g) were pooled and mixed for Na-deprotection. This was carried out using 80 mg (∼0.020 mmol) following the general procedure. After that, the library was treated with 0.5 mL of a mixture of TFA/H_2O/TIS, 95:2.5:2.5 (v/v/v) for 4 h and washed with NMP (3 × 2 mL, each 2 min), 25% DiPEA in NMP (4 × 2 mL, each 2 min), NMP (5 × 2 mL, each 2 min), and DCM (5 × 2 mL, each 2 min) to give library **20**{*1−13,1−13,1−13*}. Upon washing with dioxane (4 × 2 mL, each 2 min); dioxane/H_2O, 1:1 (4 × 2 mL, each 2 min); H_2O (4 × 2 mL, each 2 min); and Et_2O (4 × 2 mL, each 2 min), library **20**{*1−13,1−13,1−13*} was dried and stored under vacuum.

Abbreviations

Boc, *tert*-butoxycarbonyl; BOP, benzotriazol-*tris*-(dimethylamino)phosphonium hexafluorophosphate; ^{t}Bu, *tert*-butyl; Cbz, benzyloxycarbonyl; CTV, 2,7,12-trihydroxy-3,8,13-trimethoxy-10,15-dihydro-5*H*-tribenzo[*a*,*d*,*g*]cyclononene; DCM, dichloromethane; DIC, diisopropylcarbodiimide; DiPEA, *N*,*N*-diisopropyl-*N*-ethylamine; DMAP, *N*,*N*-(dimethylamino)pyridine; DMF, dimethylformamide; ES-MS, electrospray ionization mass spectrometry; Et_2O, diethyl ether; Fmoc, *N*-fluoren-9-ylmethoxycarbonyl; Fmoc-OSu, Fmoc-*N*-hydroxysuccinimide; HOBT, 1-hydroxybenzatriazol; NMP, *N*-methylpirrolidone; Rt, retention time; TEA, triethylamine; TFA, trifluoroacetic acid; TIS, triisopropylsilane.

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References and Notes

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