Total Synthesis of Polyamine Toxin HO-416b and Agel-489 Using a 2-Nitrobenzenesulfonamide Strategy

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Total synthesis of spider toxins HO-416b (1) and Agel-489 (2) was accomplished using the 2-nitrobenzenesulfonamide (Ns) group as both a protecting and activating group. In this strategy, the C–N bonds were constructed by alkylation of sulfonamides with alkyl halides or Mitsunobu reaction with the corresponding alcohol. Beginning with monoprotection of the symmetrical diamine, the construction of the backbone from diamine 3 was efficiently accomplished in 7 steps for 14 and 9 steps for 29. Removal of the Ns group while the substrate was attached to a novel solid support enabled the efficient isolation of this highly polar compound.

Key words  2-nitrobenzenesulfonamide (Ns) group; protecting and activating group; alkylation; monoprotection; solid support

Polyamine toxins derived from spider venom have been shown to be specific glutamate receptor blockers.1) They are expected to be useful as tools for studying neurophysiology and as lead structures for pharmacological and agrochemical agents. Although many synthetic studies of these compounds have been reported,2) there are still few versatile syntheses of sequent material samples.3) Recently, we reported an efficient method for the construction of secondary amines using the Ns group as a protecting and activating group.4) We envisioned that this protocol would provide an efficient synthetic route to polyamine toxins. Described herein is our practical total synthesis of the polyamine spider toxin HO-416b (1)5) and Agel-489 (2).6)

Monoprotected diamines seemed to be ideal starting materials for incorporation into a polyamine chain. Selective protection and purification of diamines is reported to be difficult;7) however, the Ns group provided good results. Thus, treatment of 1,3-diaminopropane with 1,3-dibromopropane afforded bromide 7. Sulfonamide 9, readily obtained from 3-aminopropanol (8), was converted to the right-hand triamine 10 by treatment with 7 and Cs2CO3. The left-hand fragment 13 was obtained by condensation of 3-indoleacetic acid (12) and 4 under mixed-anhydride conditions. Although the two sides can be coupled under Mitsunobu conditions, we chose the conventional alkylation methods to simplify the purification of alkylation adduct 14. Conversion of alcohol 10 to the iodide 11 was performed by mesylation and iodide displacement. Upon treatment with 11 and Cs2CO3, the sulfonamide 13 underwent smooth alkylation to provide 14. Subsequent removal of the Boc group under acidic conditions gave the primary amine 15.

Solid phase supports have proven effective as tools for the isolation of highly polar compounds, making their use in our final deprotection attractive. Initial attempts to load 15 onto a commercially available 2-chlorotrityl chloride resin were inefficient. We thus planned to prepare the novel resin 16. This resin would be more reactive since a phenol unit separated the bulky polystyrene support from the reactive site and an haloxy group stabilized the trityl cation. Treatment of Merrifield resin with p-hydroxytrityl alcohol19) and K2CO3, followed by reaction with SOCl2, afforded the desired resin 16 (Chart 3). This resin could be recycled by treatment with SOCl2 : CH2Cl2 (1 : 9) after cleavage of the substrates.

Linkage of Ns-protected HO-416b 15 to the resin 16 was induced by i-Pr2NEt (Chart 4). Upon treatment of the resin with 2-mercaptoethanol and DBU, the Ns groups were removed.9) Cleavage from the resin under acidic conditions (1% TFA/CH2Cl2) and evaporation of the solvent provided 1 without the need for any chromatographic purification (Chart 4).1H- and 13C-NMR spectral data of 1 indicated the presence of highly pure material, and tandem FAB MS-MS spectroscopy also proved 1 was identical with naturally occurring HO-416b.

Next, we turned our attention to the synthesis of Agel-489...
(2), isolated from the venomous spider *Agelenopsis apearta*. The key structural feature of 2 is a hydroxylamine-containing polyamine chain. Because of the inherent instability of secondary hydroxylamines, we planned to generate this functionality late in our synthesis. Although several methods have been reported for the transformation,\textsuperscript{10} we planned to construct this group by oxidation of a 2-cyanoethylamine and subsequent elimination of acrylonitrile by retro-Michael re-
action. We would assemble the backbone 2 by coupling the cyanoethylamine 23 with the spermine derivative 27.

The left-hand fragment 23 was obtained by an efficient 6-step synthesis (Chart 5). Condensation of 3-indoleacetic acid (11) and diamine 21 under mixed-anhydride conditions provided the sulfonamide 17. Bromide 18, readily obtained from 3-bromopropanol, was converted to a precursor of sulfonamide 12 (Chart 6). Condensation of 3-indoleacetic acid with DEAD and triphenylphosphine provided amines 19, 20 and 21. Upon treatment with acrylonitrile, the amine 21 underwent smooth cyanoethylation. Removal of the TBS group gave the primary alcohol 23.

The protected spermine derivative 27 was also synthesized from the monoprotected diamine 3 (Chart 6). Selective alkylation of 6 with 1,3-dibromopropane afforded bromide 24. Sulfonamide 25, readily prepared from diamine 3, was converted to the tetraamine 26 by treatment with 24 in the presence of Cs₂CO₃. A change of protecting groups from Alloc to Ns provided sulfonamide 27.

Condensation of primary alcohol 23 and sulfonamide 27 was accomplished under Mitsunobu conditions. Treatment of 23 and 27 with DEAD and triphenylphosphine provided hexamine 28. Upon treatment of cyanoethylamine 28 with mCPBA, smooth oxidation and retro-Michael reaction gave hydroxy amine 29. Subsequent removal of the Boc group with TFA afforded the primary amine 30. A similar treatment to that used for the Ns deprotection of HO-416 provided the natural product 2 (Chart 7). 1H/13C NMR spectra and tandem FAB MS-MS spectroscopy data of synthetic 2 were identical with those of naturally occurring Agel-489.

In conclusion, utilizing the 2-nitrobenzenesulfonamide (Ns) group as both a protecting and activating group (Ns-strategy), the total synthesis of 1 was accomplished in 11 steps and 41% total yield, while the total synthesis of 2 was achieved in 12 steps and 31% yield. In both cases, efficient monoprotection of symmetrical diamines allowed very short syntheses. All C–N bond formations were accomplished in high yield by alkylation of 2-nitrobenzenesulfonamides. Finally, removal of the Ns group while the substrate was attached to a novel solid support enabled the efficient isolation of these highly polar compounds.

**Experimental**

**General Comments** IR spectra were recorded on a JASCO FT/IR-410 spectrophotometer. Nuclear magnetic resonance. 1H and 13C NMR spectra were taken on a JEOL JNM-LA400 spectrometer with tetramethylsilane (TMS) as the internal standard. Mass spectra (MS) and high resolution mass spectra (HRMS) were measured with a JEOL JMS-GCXmate instrument.

**N-(3-Aminopropan-1-yl)-2-nitrobenzenesulfonamide (3) (Large-Scale Preparation)** To a stirred solution of 55.6 g (75.0 mmol) of 1,3-diaminopropane in 11 of ethanol was slowly added 55.4 g (25.0 mmol) of 2-nitrobenzenesulfonyl chloride at 0 °C under an argon atmosphere. After 30 min, the reaction mixture was quenched with 1 N sodium ethoxide, filtered with celite, and concentrated in vacuo. Purification of the crude product by recrystallization with ethyl acetate in ether yielded sulfonamide 3 (33.0 g, 59%) as a yellow powder. IR (CHCl₃) cm⁻¹: 3308, 3094, 2936, 2867, 1734, 1592, 1540, 1466, 1440, 1369, 1335, 1242, 1163, 1127, 1092, 853, 782, 742; 1H-NMR (DMSO) δ: 1.46 (2H, tt, J = 6.8, 6.8 Hz), 2.85 (2H, t, J = 6.8 Hz), 4.25 (1H, bs), 7.80—7.87 (2H, m), 7.92—7.99 (2H, m), 13C-NMR (DMSO) δ: 27.0, 29.8, 40.8, 42.8, 124.2, 129.4, 132.5, 132.9, 133.8, 147.8; MS: m/z=260 (MH⁺); HRMS (C₁₀H₁₄N₃O₄S, MH⁺): Calcd for 260.0705. Found: 260.0701.

**N-(3-Aminopropan-1-yl)-2-nitrobenzenesulfonamide (3) (Small-Scale Preparation)** To a stirred solution of 2.64 g (30 mmol) of 1,3-diaminopropane in 50 ml of ethanol was slowly added 2.2 g (10 mmol) of 2-nitrobenzenesulfonyl chloride at 0 °C under an argon atmosphere. After 30 min, the reaction mixture was quenched with 1 N sodium ethoxide, filtered with celite, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel, eluted with a gradient of isoproplamine: methanol: dichloromethane (0:2:96 to 2:5.2:98) gave n-furished sulfonamide 3 (2.5 g, 87%) as a yellow powder.

**N-(4-Aminobutyl-1-yl)-2-nitrobenzenesulfonamide (4)** Similar treatment of 2.64 g (30 mmol) of 1,4-diaminobutane and 2.2 g (10 mmol) of 2-nitrobenzenesulfonyl chloride to the reaction of 1,3-diaminopropane provided sulfonamide 4 (2.10 g, 77%) as a yellow powder. IR (CHCl₃) δ: 3368, 3310, 3270, 3099, 2936, 2867, 1734, 1592, 1540, 1466, 1440, 1317, 1369, 1335, 1242, 1163, 1127, 1092, 853, 782, 742 cm⁻¹; 1H-NMR (DMSO) δ: 1.43 (2H, tt, J = 6.8, 6.8 Hz), 2.47 (2H, t, J = 6.8 Hz), 2.85 (2H, t, J = 6.8 Hz), 4.25 (1H, bs), 7.80—7.87 (2H, m), 7.92—7.98 (2H, m), 13C-NMR (DMSO) δ: 27.0, 29.8, 40.8, 42.8, 124.2, 129.4, 132.4, 133.2, 133.7, 147.8; MS: m/z=274 (47, MH⁺); HRMS (C₁₀H₁₄N₃O₄S, MH⁺): Calcd for 274.0862. Found: 274.0868.
To a stirred solution of 2.23 g (8.61 mmol) of diamine (17.3 mmol) of pyridine at 0 °C under an argon atmosphere. After 10 min, the reaction mixture was poured into water, and the aqueous layer was extracted thoroughly with dichloromethane (3 times). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel (20% ethyl acetate in hexane) yielded a yellow oil. IR (CHCl3): 3423, 3093, 2931, 1706, 1548, 1530, 1517, 1486, 1382, 1243, 1131, 1033, 1020, 934, 852, 773 cm⁻¹. MS: m/z = 458 (MH⁺), 430 (MH⁺). HRMS (C₁₀H₁₂N₃O₅S): Calcd for (C₁₀H₁₂N₃O₅S, M⁺): 458.0446. Found: 458.0446.}

**4-tert-Butoxy carbamoylaminopropan-1-yl-2-nitrobenzenesulfonamide**

To a stirred solution of 2.23 g (8.61 mmol) of diamine (17.3 mmol) of pyridine at 0 °C under an argon atmosphere. After 10 min, the reaction mixture was poured into water, and the aqueous layer was extracted thoroughly with dichloromethane (3 times). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel (20% ethyl acetate in hexane) yielded a yellow oil. IR (CHCl3): 3423, 3093, 2931, 1706, 1548, 1530, 1517, 1486, 1382, 1243, 1131, 1033, 1020, 934, 852, 773 cm⁻¹. MS: m/z = 458 (MH⁺), 430 (MH⁺). HRMS (C₁₀H₁₂N₃O₅S): Calcd for (C₁₀H₁₂N₃O₅S, M⁺): 458.0446. Found: 458.0446.
Purification of the crude product by column chromatography on silica gel (10% methanol in chloroform) yielded polyanine 15 (1.40 g, 96%) as a yellow powder. IR (CHCl₃): 2961, 1638, 1548, 1459, 1373, 1344, 1262, 1216, 1104, 1059, 941, 852, 750 cm⁻¹; ¹H-NMR (DMSO-δ): δ 1.28 (2H, m), 1.37 (2H, m), 1.59 (2H, m), 1.68 (2H, m), 1.78 (2H, m), 2.73 (2H, m), 2.98 (2H, m), 3.09–3.23 (6H, m), 3.47 (2H, s), 6.94 (1H, d, J = 7.7, 1.0 Hz), 7.03 (1H, d, J = 7.7, 1.0 Hz), 7.16 (1H, bs), 7.31 (1H, d, J = 8.1 Hz), 7.51 (1H, d, J = 7.7 Hz), 7.80–7.89 (12H, m), 8.31 (1H, s); ¹³C-NMR (DMSO-δ): δ 26.1, 26.2, 36.7, 34.6, 38.0, 44.8, 44.8, 47.1, 108.9, 111.3, 118.2, 118.6, 120.9, 123.7, 124.4, 124.5, 127.2, 129.6, 129.6, 131.4, 131.6, 132.1, 132.5, 132.6, 134.5, 134.7, 136.1, 147.4, 170.6; HRMS (C₉₀H₇₄N₄O₅S₂·M⁺): Calcd for 972.2690. Found: 972.2682.

4-Chlorophenylmethylphenoxybenzamidine Polystyrene Polymer (16) (1.144 g, 514.0 mg (1% cross-linked in DMSO)) (0.25 ml, 3.4 mmol) at room temperature for 5 min, the resin was filtered and washed with MeOH : CH₂Cl₂ (1 : 9). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel (10% methanol in chloroform) yielded indole (19.47 g, 83%) as a yellow oil. IR (CHCl₃): 3405, 2929, 2928, 1472, 1387, 1361, 1257, 1121, 1147, 1063, 1006, 952, 836, 777 cm⁻¹; ¹H-NMR (CDCl₃) δ: 0.01 (6H, s), 0.83 (9H, t), 1.97 (2H, t, J = 6.1, 6.1 Hz), 3.50 (2H, t, J = 6.1 Hz), 3.67 (2H, t, J = 6.1 Hz); ¹³C-NMR (CDCl₃) δ: –5.40, 18.3, 25.9, 30.7, 35.5, 60.4; MS: m/z = 254 (MH⁺); HRMS (C₁₇H₁₇BrO₂S·M⁺): Calcd for 253.0623. Found: 253.0620.

N-(7-Butylthiophen-2-yl)benzofuran-4-(2-nitrobenzenesulfonyl)-4-azahep-1-yl-1H-indole-3-acetamide (19) To a stirred solution of 3.10 g (7.45 mmol) of sulfonamide 17, 7.28 g (22.3 mmol) of cesium carbonate and 1.37 g of (3.73 mmol) n-tetrahydroammonium iodide in 10 ml of acetonitrile was added slowly 1.89 (8.20 mmol) of bromide 18 at 60 °C under an argon atmosphere. After 90 min, the reaction mixture was poured into water, and the aqueous layer was extracted thoroughly with ethyl acetate (3 ×). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel (70% ethyl acetate in hexane) yielded indole 19 (3.47 g, 80%) as a yellow oil. IR (CHCl₃): 3405, 2929, 2856, 1653, 1545, 1458, 1437, 1373, 1342, 1256, 1163, 1099, 1009, 957, 836, 777, 744 cm⁻¹; ¹H-NMR (CDCl₃) δ: 0.01 (6H, s), 0.85 (9H, t), 2.34–3.31 (4H, m), 3.12 (2H, t, J = 6.5 Hz), 3.24 (2H, t, J = 6.5 Hz), 3.29 (2H, t, J = 6.5 Hz), 3.51 (2H, t, J = 5.6 Hz), 3.75 (2H, s), 6.24 (1H, bs), 7.13 (1H, d, J = 7.7 Hz), 7.20 (1H, d, J = 7.7 Hz), 7.21 (1H, bs), 7.37 (1H, d, J = 7.6 Hz), 7.58 (1H, d, J = 7.7 Hz), 7.59–7.68 (4H, m), 8.29 (1H, bs); ¹³C-NMR (CDCl₃) δ: 57.87 (1H), 61.1, 110.4, 111.4, 118.6, 122.3, 124.0, 124.2, 130.4, 131.6, 133.3, 133.6, 136.9, 171.9; MS: m/z = 588 (M⁺); HRMS (C₉₀H₇₄N₁₂O₄S₂·M⁺): Calcd for 588.2438. Found: 588.2423.

N-(7-Butylthiophen-2-yl)benzofuran-4-(2-nitrobenzenesulfonyl)-4-azahep-1-yl-1H-indole-3-acetamide (20) To a stirred solution of 3.14 g (5.33 mmol) of indole 19 in 25 ml of dichloromethane were added 0.8 ml (5.86 mmol) of triethylamine, 1.3 ml (5.66 mmol) of di-t-butyldimethylsilylanilinium iodide (10% solution in DMAP) at room temperature under an argon atmosphere. After 1 h, the reaction mixture was poured into a solution of 1 N hydrochloric acid, and the aqueous layer was extracted thoroughly with dichloromethane (3 ×). The combined organic extracts were washed with brine, dried over anhydrous magnesium sulfate, filtered, and concentrated in vacuo to give indole 20 (3.60 g, 98%) as a yellow oil. IR (CHCl₃): 3298, 2930, 1734, 1654, 1545, 1437, 1308, 1257, 1227, 1159, 1087, 1016, 957, 836, 774, 747 cm⁻¹; ¹H-NMR (CDCl₃) δ: 0.01 (6H, s), 0.85 (9H, t), 1.64 (9H, s), 1.68–1.71 (4H, m), 3.16 (2H, t, J = 6.3 Hz), 3.28 (2H, t, J = 5.9 Hz), 3.30 (2H, t, J = 6.3 Hz), 3.52 (2H, t, J = 5.9 Hz), 3.68 (2H, bs), 6.20 (1H, bs), 7.25 (1H, d, J = 7.7 Hz), 7.27 (1H, bs), 7.34 (1H, d, J = 7.7 Hz), 7.54 (1H, d, J = 7.7 Hz), 7.57–7.90 (4H, m), 8.17 (8H, t); ¹³C-NMR (CDCl₃) δ: –5.36, 3.90, 18.3, 25.9, 27.2, 28.2, 31.4, 33.5, 36.0, 44.9, 45.2, 60.1, 83.8, 113.9, 115.4, 119.0, 122.9, 124.1, 124.8, 125.2, 130.5, 131.7, 133.5, 170.6; MS: m/z = 588 (MH⁺–H₂O·Boc); HRMS (C₉₀H₇₄N₁₂O₄S₂·M⁺): Calcd for 589.3040. Found: 589.3070.
To a stirred solution of 1.60 g (9.22 mmol) of triethylamine at 0 °C, 2.47 g (81%) as a yellow oil. IR (CHCl₃): 3297, 2930, 2857, 1732, 1649, 1547, 1453, 1369, 1308, 1256, 1227, 1158, 1086, 1016, 836, 774 cm⁻¹; ¹³C-NMR (CDCl₃) δ: 5.01 (6H, s), 0.85 (9H, s), 1.43 (2H, tt, J = 6.6, 6.6 Hz), 1.55 (2H, J = 6.3, 6.3 Hz), 1.67 (9H, s), 2.14 (2H, J = 6.3 Hz), 2.30 (2H, J = 6.5 Hz), 2.35 (2H, J = 6.6 Hz), 2.49 (2H, J = 6.5 Hz), 3.27 (2H, J = 6.3 Hz), 3.51 (2H, J = 6.6 Hz), 3.64 (2H, s), 6.13 (1H, bs), 7.25 (1H, J = 7.0 Hz), 7.34 (1H, J = 7.0 Hz), 7.51 (1H, J = 7.0 Hz), 7.54 (1H, s), 8.15 (1H, m); ¹³C-NMR (CDCl₃) δ: 5.58, 15.4, 17.7, 25.4, 26.1, 27.9, 27.1, 37.7, 49.0, 50.9, 60.1, 83.5, 113.5, 114.9, 118.6, 122.4, 124.4, 125.9, 149.0, 169.8; MS: m/z = 556 (M⁺); HRMS (C₂₁H₂₆N₄O₈Si) δ: Calculated for C₂₁H₂₆N₄O₈Si: M⁺ = 556.2675. Found: 557.2582.

5-[(2-Cyanoethyl)-4-hydroxy-4-azaoctan-1-yl]-1-tert-butoxycarbonyl-indole-3-acetamide (23) To a stirred solution of 1.95 g (3.95 mmol) of bromide 22 in 20 ml of acetonitrile was added 973 mg (17.1 mmol) of amonium fluoride at 60 °C under an argon atmosphere. After 24 h, the reaction mixture was poured into a solution of saturated sodium bicarbonate, and the aqueous layer was extracted thoroughly with dichloromethane (3×). The combined organic extracts were washed with brine, dried over magnesium sulfate, filtered, and concentrated in vacuo. Purification of the crude product by column chromatography on silica gel, eluted with a gradient of methanol in chloroform (10 to 30%) furnished alcohol 23 (1.47 g, 98%) as a yellow oil. IR (CHCl₃): 3298, 2934, 1731, 1654, 1540, 1453, 1369, 1308, 1257, 1227, 1158, 1086, 1017, 942, 856, 768, 748 cm⁻¹; ¹¹H-NMR (CDCl₃) δ: 0.01 (6H, s), 0.85 (9H, s), 1.32 (2H, bs, J = 8.6, 8.6 Hz), 2.60 (4H, t, J = 7.6 Hz), 2.61 (4H, s), 3.31 (2H, bs, J = 7.0 Hz), 3.32 (2H, si, J = 7.0 Hz), 3.65 (2H, s), 6.19 (1H, bs), 7.27 (1H, J = 7.6 Hz), 7.36 (1H, J = 7.6 Hz), 7.53 (1H, d, J = 7.6 Hz), 7.57 (1H, s), 8.16 (1H, bs); ¹³C-NMR (CDCl₃) δ: 15.7, 26.6, 28.3, 28.5, 33.4, 37.7, 49.3, 50.8, 52.3, 62.3, 84.1, 114.0, 115.5, 118.7, 119.1, 123.0, 125.0, 125.1, 130.0, 135.7, 149.6, 170.5; MS: m/z = 442 (M⁺); HRMS (C₂₁H₂₆N₄O₈Si): δ: Calculated for C₂₁H₂₆N₄O₈Si: M⁺ = 442.2685. Found: 443.2685.
product by column chromatography on silica gel, eluted with a gradient of ethyl acetate in hexane (80% to 100%), furnished polyamine 28 (587 mg, 90%) as a yellow powder. IR (CHCl₃): 2931, 1715, 1669, 1545, 1539, 1334, 1315, 1303, 1290, 1282, 1160, 1086, 852, 736 cm⁻¹; ¹H-NMR (CDCl₃): δ: 1.32 (2H, m), 1.43 (9H, s), 1.49 (2H, m), 1.84 (4H, m), 1.66 (9H, s), 1.68 (2H, m), 1.83 (2H, m), 2.26 (2H, t, J = 6.4 Hz), 2.32 (4H, t, J = 6.4 Hz), 2.55 (2H, t, J = 6.4 Hz), 3.11 (2H, m), 3.20—3.31 (14H, m), 3.64 (2H, s), 4.84 (1H, bs), 6.16 (1H, bs), 7.23 (1H, t, J = 7.5 Hz), 7.32 (1H, t, J = 7.5 Hz), 7.47—7.57 (5H, m), 7.67—7.71 (6H, m), 7.95—7.98 (3H, m), 8.14 (1H, bs); ¹³C-NMR (CDCl₃): δ: 16.5, 23.9, 25.1, 25.2, 27.9, 28.3, 28.5, 28.8, 30.4, 33.4, 37.9, 38.9, 45.2, 45.5, 46.5, 47.2, 47.4, 49.4, 50.6, 51.1, 114.2, 115.5, 119.2, 123.0, 124.2, 124.3, 124.4, 124.9, 125.1, 128.6, 128.7, 128.9, 130.1, 130.6, 130.8, 131.0, 132.0, 132.1, 132.2, 132.3, 132.9, 133.0, 133.2, 133.8, 143.1, 170.6; HRMS (C₅₄H₇₃N₁₀O₁₈S₃, MH⁺): Caled: for 1282.4583. Found: 1282.4578.

N-(20-tert-Butoxy carbonyl amino-4-hydroxy-8,12,17-tris-(2-nitrobenzenesulfonoyl)-1-triazacyclaoicosan-1-yl)-1-tert butyloxycarbonyl indole-3-acetamide (29) To a stirred solution of 220 mg (0.172 mmol) of polyamine 28 in 5 ml of dichloromethane was slowly added 30 mg of 29 (0.13 mmol) and 27.0 mg of amine (0.025 mmol) in 0.4 ml of dichloromethane. After shaking for 24 h, excess methanol (0.3 ml, 5.7 mmol) was added to the reaction mixture. The resin was filtered, washed with MeOH:THF (1:9), MeOH:CH₃Cl (1:9), and CH₂Cl₂, and dried in vacuo for 8 h to give the resin. To a suspension of the resulting resin in 1.5 ml of DMF was added excess 2-mercaptoethanol (0.053 ml, 0.75 mmol) and excess DBU (0.11 ml, 0.75 mmol) at room temperature under an argon atmosphere. After shaking for 26 h, the resin was filtered, washed with H₂O:THF (1:9), MeOH:CH₃Cl (1:9), and CH₂Cl₂, and dried in vacuo for 8 h to give the resin. To a mixture of the resulting resin in 2.5 ml of CH₂Cl₂ was added trifluoroacetic acid (30 µl, 0.36 mmol) at room temperature. After shaking for 5 min, the resin was filtered and washed with MeOH:CH₃Cl (1:1). The combined washings were evaporated and dried in vacuo to provide 2 (27.0 mg, 92%) as the TFA salt. ¹H-NMR (D₂O): δ: 1.72 (4H, m), 1.92—2.06 (8H, m), 3.02—3.17 (20H, m), 3.71 (2H, s), 7.13 (1H, dt, J = 7.3, 1.0 Hz), 7.22 (1H, dt, J = 7.3, 1.0 Hz), 7.29 (1H, s), 7.48 (1H, d, J = 8.0 Hz), 7.56 (1H, d, J = 7.3 Hz); ¹³C-NMR (D₂O): δ: 23.5, 24.5, 33.2, 36.8, 37.3, 45.2, 45.3, 47.8, 56.4, 57.4, 108.4, 112.8, 119.1, 120.3, 122.9, 126.0, 137.1, 176.8; HRMS (C₅₄H₇₃N₁₀O₁₈S₃, MH⁺): Caled: for 490.3869. Found: 490.3878; MS/MS (FAB) 474 (MH⁺—H₂O), 460 (MH⁺—CH₃N), 446 (MH⁺—C₅H₅N), 432 (MH⁺—C₅H₄N), 416 (M⁺—C₂H₃N₂), 402 (M⁺—C₂H₂N₃), 374 (M⁺—C₁H₁N₄), 360 (M⁺—C₁H₂N₅), 345 (M⁺—C₁H₃N₆), 317 (M⁺—C₁H₄N₇), 275 (M⁺—C₁H₅N₈), 258 (M⁺—C₁H₆N₉), 244 (M⁺—C₁H₇N₁₀), 215 (M⁺—C₁H₈N₁₁), 130 (C₆H₄N⁺).