(−)-15-Deoxyspergualin: A New and Efficient Enantioselective Synthesis Which Allows the Definitive Assignment of the Absolute Configuration

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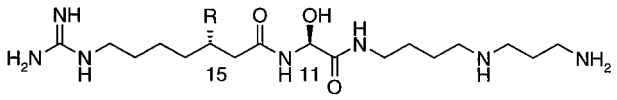
(±)-15-Deoxyspergualin (DSG) has recently been marketed in Japan for the control of corticoresistant acute renal graft rejection. A nine-step total synthesis of its eutomer ((−)-DSG) **2** has been developed starting from 7-bromoheptanenitrile **3** and N^1,N^4-bis(benzyloxycarbonyl)spermidine. The use of a chiral α-alkylbenzyl group to protect the hydroxyl of the α-hydroxyglycine moiety allowed its chromatographic resolution and afforded a practical access to **2** with a high optical purity and a 7% overall yield. Moreover, X-ray structure analysis of the key crystalline intermediate **7b** definitely confirmed the previously proposed absolute configuration of **2**.

(−)-Spergualin **1** is an antitumor antibiotic that was first isolated from the culture filtrates of the bacterium strain *Bacillus laterosporus* BMG 162-aF2.[1] This was characterized[2] as (−)-(15*S*)-1-amino-19-guanidino-11,15-dihydroxy-4,9,12-triazanonadecane-10,13-dione and finely synthesized by H. Umezawa et al. [3] in 1981. A subsequent antitumor structure−activity relationship study[4] has led to the isolation of the nor-15-hydroxy derivative (−)-15-Deoxyspergualin ((−)-DSG) **2**. This hemisynthetic product proved to be a very potent antitumor and immunosuppressor agent and represents the eutomer[5] of (±)-DSG. In 1994 the racemic form received a marketing approval in Japan for the treatment of corticoresistant acute renal graft rejection episodes. Two syntheses of racemic (±)-DSG[6a,b] have been described.The enantiomer **2** was obtained by hemisynthesis from (−)-Spergualin,[4] or by total synthesis via a 9-step process involving an enzymatic resolution.[7] Although the overall yield of this last sequence was very low (0.3%), it allowed the access to both (−)- and (+)-DSG. The (+)-isomer proved to be far less active,[4,7,8] demonstrating the crucial role of the stereochemistry at C11. Nevertheless, determination of the absolute configuration of the hydroxyglycine unit present in this compound represents a real challenge due to the difficulty in obtaining single crystals of the hygroscopic and easily hydrolyzable DSG. However, enzymatic resolution used for the synthesis[7] of **2** suggests that the C11 configuration could be *S*.



R = OH (-) Spergualin **1**

R = H (-) 15-Deoxyspergualin **2**

There is no general methodology available for the synthesis of optically active hydroxyglycine derivatives.[9] The described synthesis of **2** relies on an enzymatic resolution.[7] Unfortunately, this synthesis required the use of the rather uncommon and expensive carboxypeptidase P. Other hydrolytic enzymes have been used to resolve alkoxy glycine containing compounds, but they showed strict selectivity regarding the alkoxy group and limited examples of amino substituents have been tested.[10a−d] More recently, Patel et al.[10e] have described an elegant enzymatic acylative resolution of a protected *N*-(7-guanidinoheptanoyl)-α-hydroxy glycine ester with commercially available lipase. However, no synthesis of **2** has been described using this intermediate. A second type of resolution of this amino acid unit is given by the total synthesis of **1**[3] and other examples of peptides encompassing a hydroxyglycine. This moiety is introduced non stereoselectively in the peptidic sequence and the chirality brought by the other amino acids permits

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the resolution of the hydroxyglycine motif using preparative HPLC to separate the resulting diastereomers.[11] A third approach for the resolution of the hydroxyglycine unit consists of fractional crystallization of a mixture of diastereomeric salts or esters obtained respectively by reacting the racemic acid part with an optically active amine or alcohol.[10c,12,]

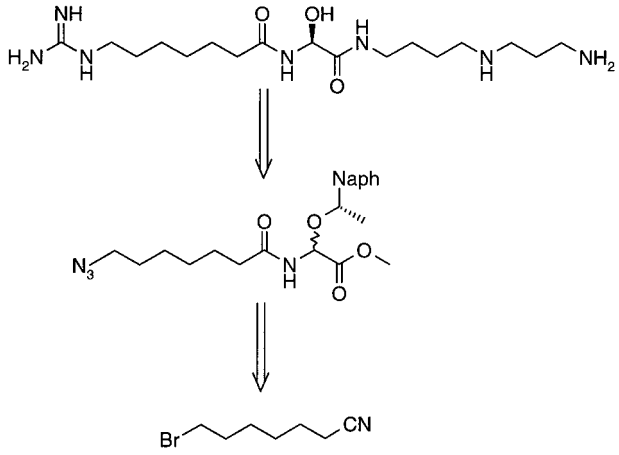
Results and Discussion

As part of a program aimed at discovering new and more potent immunosuppressors, we needed an efficient access to analogues of **2**. We wish to report here on a new total synthesis of **2** which also allows the definitive assignment of the absolute stereochemistry at C11 of **2** and (−)-Spergualin.

It utilizes a new approach involving the introduction of a chiral auxiliary directly on the hydroxyl group of the hydroxyglycine. To our knowledge, this methodology has never been explored.[13] This site of derivatization offers a priori two major advantages: (i) the introduced chirality is located as close as possible to the center to be resolved which is usually a favorable situation for separation by chromatography or crystallization, (ii) if the chiral auxiliary introduced can play the role at the same time of a protective group, it could alleviate the problem of the presence of the free hydroxyl group which makes such hemiaminal functionality rather sensitive toward hydrolysis and renders separation and purification uncertain. Some examples for the removal of O-benzyl protective group on α-benzyloxy glycine derivatives have already been described[7,10d] with retention of configuration. We therefore decided to use an optically active α-substituted benzylic alcohol as a mixed chiral auxiliary-protective group as depicted in the retrosynthetic analysis shown in Scheme 1. Moreover, to avoid the use of a highly unstable free amino α-alkoxy glycine,[14,15] we decided to build up the N-acylated α-hydroxy glycine unit using a properly substituted precursor amide. To facilitate purification of intermediates, to obtain the mildest conditions of deprotection compatible with the poor stability of **2**, and to shorten the synthesis as much as possible, we introduced benzyloxycarbonyl protecting groups on the guanidine moiety and on the amine so that all O and N protecting groups could be removed simultaneously in the final step.

α-Alkoxy glycine derivative syntheses have already been described in the literature starting from α-hydroxy

Scheme 1



glycine,[16,17b] α-thioalkyl glycine,[16b] α-acetoxy glycine,[17,11f] *N*-chloro glycine,[16a,14] or α-halogeno glycine derivatives.[18] We chose the latter for its efficiency whatever the alkoxy group is. Examples of α-chloroglycine derivatives used as starting materials were already described in the literature and are readily accessible in a few steps.[19−22]

Scheme 2 shows the pathway used to obtain **2**. Commercial 7-bromoheptanenitrile **3** was first hydrolyzed with concentrated aqueous HCl into the corresponding amide **4**.[23] Nucleophilic displacement of the bromide with sodium azide in DMSO at 80 °C[24] yielded **5** in which the azido function served as a protected primary amine needed for elaboration of the guanidine function. This latter functionality was introduced late in the synthesis, and in a protected form, to avoid difficulties associated with handling such a moiety. The amide **5** was then converted in a three-step one-pot process into the desired intermediate **7**. The first step consisted of a condensation with methyl 2-hydroxy-2-methoxyacetate. The formed methanol was trapped with molecular sieves.[22e] The so formed α-hydroxy glycine **6** could be isolated, or treated *in situ*, with thionyl chloride at 40 °C in order to form a labile α-chloroglycine intermediate. This was then treated with chiral benzylic alcohol in the presence of triethyl-

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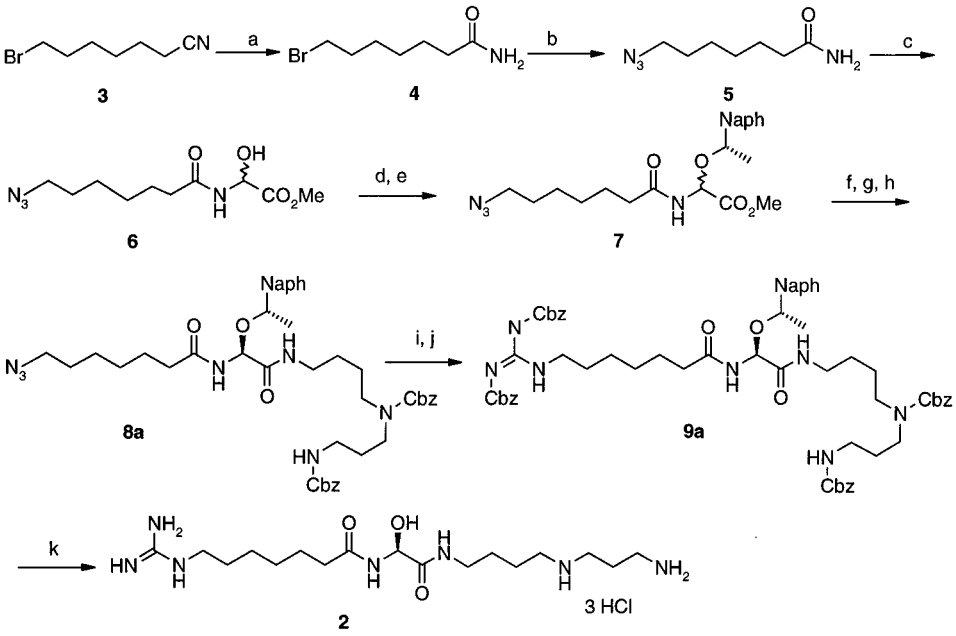
Scheme 2[a]



[a] Reagents and reaction conditions: (a) HCl conc, 12 h, rt, 95%; (b) NaN_3, DMSO, 80 °C, 3.5 h, 65%; (c) (MeO)(HO)$CHCO_2Me$, CH_2Cl_2, 40 °C, 24 h, molecular sieves 4 Å; (d) $SOCl_2$, CH_2Cl_2, 40 °C, 1.75 h; (e) (S)-2-NaphtCH(OH)Me, Et_3N, CH_2Cl_2, rt, 24 h, 62% overall from **5**; (f) NaOH 1 N, DME, rt, 1.5 h; (g) $H_2N(CH_2)_4N(Cbz)(CH_2)_3NHCbz$, DCC, HOBT, CH_2Cl_2, rt, 48 h, 48% overall from **7**; (h) flash chromatography; (i) Ph_3P, H_2O, THF, 65 °C, overnight; (j) CbzN=C(SMe)NHCbz, THF, rt, 3 h, 77% overall from **8a**; (k) (1) $Pd(OH)_2/C$ (20%), MeOH/AcOH, H_2 1 atm, 76%, (2) Sephadex C-25 and Sephadex LH-20.

amine. Different benzylic alcohols have been tested.[25] All of them gave about the same yield (50−70%) and none led to a significant induction.

Finally and despite the fact that there is no induction with this alcohol, we chose (S)-(−)-α-methyl-2-naphthalenemethanol as the most efficient auxiliary in term of ease of separation, cost, yield, and crystallinity of the resulting diastereomers **7** and **8**. The approximately 1:1 mixture of diastereomers **7** was obtained in an overall 62% yield starting from amide **5**. The synthesis of **2** was completed as follows. Saponification of **7** gave the corresponding acid, which was then coupled with the N^1,N^4-bis (benzyloxycarbonyl) spermidine[26] using the DCC/HOBT methodology to afford a mixture of the diastereomers **8a** and **8b**. Fortunately these diastereomers were easily separated by simple flash chromatography. Conversion of **8a** to **9a** was made in a one-pot two-step process. Among the numerous methods described[27] to convert an azido group in a primary amine, we chose the action of Ph_3P/H_2O[28] which allows a selective conversion in the presence of benzyloxy, and benzyloxycarbonyl groups. The so formed primary amine was then reacted in situ with *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea[29] to give **9a** in 77% yield. Final deprotection accomplished in one step using Pearlman's catalyst either in a H_2O/AcOH/THF mixture under 20 atm of hydrogen or in 1 N AcOH in methanol under 1 atm of hydrogen. It is worth mentioning that these experimental conditions provided triacetate **2** with high purity as an hygroscopic solid without concomitant reductive dehydroxylation as already described for related systems.[30] The compound was transformed into the corresponding tris hydrochloride salt and further purified as described in the literature.[6b]

Optical purity of **2** was measured[31] and proved to be ≥99.5%. This scheme is thus extremely efficient for the synthesis in 9 steps of (+)-DSG and **2** with an overall yield of 7.5% and an enantiomeric excess ≥99.5%.

Moreover, the intermediate diastereomers **7** were separated by preparative HPLC. Fortunately, one of the purified diastereomer, **7b** gave single crystals suitable for X-ray analysis.[32] The ORTEP view for **7b** is given in Supporting Information. The absolute configuration of the hetero-disubstitued carbon proved to be *R*. Compound **7a** has been further elaborated to **2** following the same pathway as previously described where the absolute configuration of the chiral center is preserved.[33] The absolute sterechemistry of (−)-Deoxyspergualin can thus be assigned definitively to the *S* configuration.

(25) Optically active benzylic alcohols, *S*-(−)-1-phenyl ethanol, *S*-(−)-phenyl-1-butanol, *S*-(−)-1-(1-naphthyl)ethanol, *S*-(−)-α-methyl-2-naphthalenemethanol, *S*-(+)-1-indanol, *R*-(−)-α-tetralol are commercially available. A slight induction (d.e. 30%) was observed with the latter two compounds as determined by NMR analysis.

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(33) We have checked that there was no epimerisation during saponification of **7a** by reesterification of the crude product with an ethereal diazomethane solution and analysis of the crude product by NMR. Attempts to purify and better characterize the acid intermediate revealed its instability.

Conclusion

It is desirable to develop and market new chiral drugs as single enantiomer, especially when only one enantiomer is pharmacologically active. The present synthesis affords an easy access to (−)-Deoxyspergualin which is the prototype of a new family of immunosuppressor agents[34] and therefore could help in the synthesis of even more potent optically active analogues.[13a] Furthermore, this work proves definitively the absolute *S* configuration of (−)-Deoxyspergualin and consequently that of the C11 of (−)-Spergualin. Although nothing is known to our knowledge about the biosynthesis of (−)-Spergualin, it is interesting to note that the α-hydroxy glycine absolute configuration is the same as that induced by peptidylglycine α-hydroxylating monooxygenase (PHM, EC 1.14.17.3), an enzyme responsible for the hydroxylation of the α-carbon of the carboxy terminal glycine of some peptides.[35] Finally, the approach followed here could be useful for the synthesis of other hydroxyglycine-containing molecules.

Experimental Section

General. All chemicals were purchased from commercial sources and used without further treatment excepted when specified. THF was freshly distilled from sodium benzophenone ketyl. Melting points were uncorrected. Proton magnetic resonance spectra were determined either at 250 or 300 MHz with TMS as internal standard. Carbon magnetic resonance spectra were recorded at 62.9 or 75 MHz. The chemical shifts are expressed in δ values relative to TMS. For spectra recorded in D_2O we used HOD (^{1}H δ 4.80) and dioxane (^{13}C δ 67.3) as internal standards. IR spectra were obtained on KBr, 3M Disposable IR card (type 61) or in solution in the specified solvent. R_f values were measured after thin-layer chromatography performed with precoated silica plates (Kieselgel 60 F_{254}, Merck). Elemental analyses were performed with an elemental analyzer Perkin-Elmer 2400 CHN. Preparative HPLC separation were done with laboratory-scale preparative HPLC, Prochrom Lab. LC 50 equipped with a thermostated (29 °C) dynamic axial column (diameter 5 cm) and using Matrex Amicon, spheric silica Si 100 15 SP (reference 84997; pore size, 100 Å; particle size, 15 μm; quantity, 250 g).

7-Bromoheptanamide (4). A mixture of 7-bromoheptanenitrile (25 g, 131 mmol) and 100 mL of concentrated HCl was stirred for 12 h at room temperature. The mixture was then poured into cold water (300 mL), and the white precipitate was collected by filtration, washed with water, dried, and crystallized from EtOAc−methylcyclohexane to yield **4** (26.2 g, 95%) as white crystals; R_f 0.32 (70:30, EtOAc/*i*-Pr_2O); mp 84 °C; IR (KBr) 1650, 3190, 3390 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.34−1.52 (m, 4H), 1.66 (m, 2H), 1.87 (m, 2H), 2.24 (t, J = 6 Hz, 2H), 3.41(t, J = 6 Hz, 2H), 5.5 (m, 1H), 5.7 (m, 1H); ^{13}C NMR (DMSO-d_6) δ 24.8, 27.2, 27.7, 32.0, 34.9, 35.0, 174.1. Anal. Calcd for $C_7H_{14}BrNO$: C, 40.40; H, 6.78; N, 6.73. Found: C, 40.60; H, 6.55; N, 6.54.

7-Azidoheptanamide (5). A mixture of **4** (26.2 g, 126 mmol) and sodium azide (16.4 g, 250 mmol) in DMSO (150 mL) was stirred at 80 °C for 3.5 h. The cooled mixture was poured into water and extracted with EtOAc. The organic layer was washed with brine, dried ($MgSO_4$), and concentrated to yield a crude product which was purified by crystallization from EtOAc/*i*-Pr_2O. **5** was obtained as white needles (14 g, 65%); R_f 0.38 (70:30, EtOAc: *i*-Pr_2O); mp 62 °C; IR(KBr) 1630, 1660, 2100, 3180, 3380 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.37−1.4 (m, 4H), 1.56−1.7 (m, 4H), 2.23 (t, J = 7.5 Hz, 2H), 3.27 (t, J = 9 Hz, 2H), 5.6 (m, 2H); ^{13}C (DMSO-d_6) δ 24.9, 25.9, 28.1, 28.2, 35.0, 50.6, 174.3. Anal. Calcd for $C_7H_{14}N_4O$: C, 49.39; H, 8.29; N, 32.92. Found: C, 49.54; H, 8.11; N, 32.96.

Acetic Acid, [(7-Azido-1-oxoheptyl)amino]hydroxy-, methyl ester (6). A solution of **5** (3 g, 17.6 mmol) and methyl 2-hydroxy-2-methoxy acetate (1.9 mL, 19.1 mmol) in 70 mL of CH_2Cl_2 was heated under reflux for 24 h in a flask connected to a Soxhlet apparatus filled with 20 g of 4 Å molecular sieves. The mixture was concentrated under reduced pressure and purified by flash chromatography on silica gel (EtOAc-cyclohexane, 4.5:5.5). Compound **6** was obtained as a glassy solid (3.17 g, 70%) after recrystallization from Et_2O; R_f 0,18 (EtOAc-cyclohexane, 40:60); mp 47 °C; IR(KBr) 1445, 1545, 1655, 1750, 2096, 2861, 3331 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35−1.42 (m, 4H), 1.57−1.68 (m, 4H), 2.26 (t, J = 7.2 Hz, 2H), 3.26(t, J = 6.8 Hz, 2H), 3.85 (s, 3H), 4.28 (d, J = 6 Hz, 1H), 5.59(dd, J = 6 Hz, J = 7 Hz, 1H), 6.73(d, J = 7 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 24.9, 26.3, 28.53, 28.57, 36.0, 51.27, 53.2, 71.9, 169.9, 173.9; Anal. Calcd for $C_{10}H_{18}N_4O_4$: C, 46.51; H, 6.98; N, 21.71. Found: C, 46.92; H, 6.75; N, 21.68.

Acetic acid, [(7-azido-1-oxoheptyl)amino] [1-(2-naphthalenyl)ethoxy]-, methyl ester, [*S*-(*R,*R**)] (7a) and Acetic acid, [(7-azido-1-oxoheptyl)amino] [1-(2-naphthalenyl)ethoxy]-, methyl ester, [*S*-(*R**,*S**)] (7b).** A solution of **5** (4.4 g, 26.1 mmol) and methyl 2-hydroxy-2-methoxy acetate (2.9 mL, 29.2 mmol) in 250 mL of CH_2Cl_2 was heated under reflux for 24 h in a flask connected to a Soxhlet apparatus filled with 30 g of 4 Å molecular sieves. After cooling, the Soxhlet apparatus was replaced by a reflux condenser. Thionyl chloride (2.29 mL, 31 mmol) was added, and the resulting mixture was refluxed for 1.75 h. The reaction mixture was concentrated under reduced pressure. The crude chloroglycine derivative was dissolved in 50 mL of CH_2Cl_2, treated dropwise with (*S*)-(−)-α-methyl-2-naphthalenemethanol (4.5 g, 26 mmol) and triethylamine (2.29 mL, 31 mmol) in 50 mL of CH_2Cl_2 for 24 h at room temperature. The reaction mixture was then washed with HCl 1 N (100 mL), brine (100 mL). The organic layer was dried ($MgSO_4$), concentrated under reduced pressure, and the compound was purified by flash chromatography on silica gel (hexane/*i*-PrOH, 9.5:0.5) to give 6.67 g (62%) of a mixture of **7a** and 7**b** which can be directly used in the next step or purified by preparative HPLC to separate **7a** and **7b**. From three successive injections of 1.5 g (0.5 g/mL), elution with CH_2Cl_2/EtOAc, 95:5 (*P*, 3 bar; flux, 120 mL/min) and after evaporation of solvent, 2.05 g of pure **7a** (t_r = 14 min), 1.41 g of pure **7b** (t_r = 16 min) and 1.04 g of a mixture were obtained.

Compound **7a** crystallized after extensive drying under vacuum. mp 32 °C; $[\alpha]^{25}_D$= −50° (*c* 0.94, $CHCl_3$); IR($CHCl_3$) 1680, 1745, 2100, 2920, 2970, 3000, 3420, 3620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.35−1.39 (m, 4H), 1.48 (d, J = 6.5 Hz, 3H), 1.54−1.72 (m, 4H), 2.2−2.3 (m, 2H), 3.26 (t, J = 6.8 Hz, 2H), 3.7 (s, 3H), 4.97 (q, J =6.5 Hz, 1H), 5.55 (d, J = 9.3 Hz, 1H), 6.52 (d, J = 9.3 Hz, 1H), 7.44−7.54 (m, 3H), 7.81−7.87 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 24.02, 25.06, 26.41, 28.65, 36.44, 51.32, 52.78, 57.08, 124.50, 125.93, 126.05, 126.11, 127.65, 128.11, 128.27, 133.2, 139.60, 168.85, 173.2. Anal. Calcd for $C_{22}H_{28}N_4O_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.40; H, 6.88; N, 13.50.

Compound **7b** was crystallized from Et_2O/petroleum ether at 20 °C and submitted to X-ray analysis. mp 52 °C; $[\alpha]^{25}_D$ = −92° (c 0.96, $CHCl_3$); IR($CHCl_3$) 1680, 1750, 2090, 2920, 2970, 3000, 3420, 3620 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 1.05−1.18 (m, 4H), 1.23−1.31 (m, 2H), 1.39−1.44 (m, 2H), 1.58 (d, J = 6.4 Hz, 3H), 1.69−1.79 (*A*B*X*, 1H), 1.87−1.94 (A*B*X, 1H), 3.16 (t, J = 7 Hz, 2H), 3.8 (s, 3H), 4.95 (q, J = 6.5 Hz, 1H), 5.86 (d, J = 9 Hz, 1H), 6.17 (d, J = 9 Hz, 1H), 7.42−7.5 (m, 3H), 7.71 (s,1H), 7.79−7.82 (m, 3H);^{13}C NMR ($CDCl_3$) δ 29.6, 24.5, 26.23, 28.45, 28.52, 36.0, 51.29, 52.86, 76.42, 77.95, 124.63, 124.11, 125.86, 126.16, 127.64, 127.91, 128.14, 132.91, 133.17, 140.96, 168.67, 172.72. Anal. Calcd for $C_{22}H_{28}N_4O_4$: C, 64.06; H, 6.84; N, 13.58. Found: C, 64.21; H, 6.87; N, 13.59.

2,6,11,14-Tetraazaheneicosanoic acid, 21-azido-13-[1-(2-naphthalenyl)ethoxy]-12,15-dioxo-6[(phenylmethoxy)-

(34) See ref 8. (b) See ref 13a. (c) Renaut, P.; Lebreton, L.; Dutartre, P.; Derrepas, P.; Samreth, S. Eur. Pat. Appl. EP 600762, Jun 8, 1994. (d) Lebreton, L.; Renaut, P.; Dumas C. Eur. Pat. Appl. EP 743300, Nov. 20, 1996.

(35) Kawahara, T.; Susuki, K.; Iwasaki, Y.; Shimoi, H.; Akita, M.; Moro-oka, Y.; Nishikawa, Y. *J. Chem. Soc., Chem. Commun.* **1992**, 625−626.

carbonyl]-, phenylmethyl ester, [*S*-(*R,*R**)] (8a) and 2,6,11,14-Tetraazaheneicosanoic acid, 21-azido-13-[1-(2-naphthalenyl)ethoxy]-12,15-dioxo-6[(phenylmethoxy)-carbonyl]-, phenylmethyl ester, [*S*-(*R**,*S**)] (8b).** A mixture of 1 N NaOH (1.45 mL, 1.45 mmol) and **7** (0.5 g, 1.21 mmol) in 10 mL of DME was stirred for 1.5 h at room temperature then diluted with water (20 mL) and acidified with HCl 1N to pH 2. The aqueous phase was extracted with EtOAc and the organic phases were dried (Na_2SO_4) and concentrated to yield 0.47 g (98%) of the crude diastereomeric mixture of acids. This mixture was dissolved in 10 mL of CH_2Cl_2 and hydroxybenzotriazole (0.16 g, 1.18 mmol) followed by DCC (0.27 g, 1.3 mmol) were added. After stirring at room temperature for 30 min N^1,N^4-bis (benzyloxycarbonyl) spermidine[26] (0.52 g, 1.26 mmol) was added, the mixture was stirred at room temperature for 48 h, washed with saturated aqueous $NaHCO_3$, brine, and the organic layer was dried ($MgSO_4$). Concentration of the organic phases gave the mixture of epimers which were separated by flash chromatography on silica gel (EtOAc/*i*-Pr_2O, 8.5:1.5). **8a** (0.45 g, 48%) was obtained as an oil; *Rf*: 0.63 (EtOAc/*i*-Pr_2O, 8:2); $[\alpha]^{22}_D = -40.7°$ (*c* 5.4, $CHCl_3$); IR ($CHCl_3$) 1680, 2100 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.1−1.6 (m, 17H), 2−2.2 (m, 2H), 2.85−3.17 (m, 8H), 3.3 (t, J = 6.7 Hz, 2H), 4.79 (q, J = 6 Hz, 1H), 5 (s, 2H), 5.04 (s, 2H), 5.17 (d, J = 9 Hz, 1H), 7.1−7.37 (m, 11H), 7.45−7.57 (m, 3H), 7.8 (s, 1H), 7.81−7.9 (m, 3H), 7.95−8.05 (m, 1H), 8.65, (d, J = 9 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 24.12, 25.07, 26.04, 26.42, 28.23, 28.32, 35.21, 38.2, 38.4, 50.75, 65.36, 66.18, 74.72, 76.15, 124.65, 125.47, 126.32, 127.50, 127.72, 127.92, 128.18, 128.53, 128.58, 132.79, 132.90, 137.26, 137.40, 140.61, 155.42, 156.2, 167.15, 173.48. Anal. Calcd for $C_{44}H_{55}N_7O_7$: C, 66.56; H, 6.98; N, 12.35. Found: C, 66.51; H, 7.00; N, 12.29.

8b (0.33 g, 35%) was obtained as an oil; $[\alpha]^{22}_D = -19.4°$ (c 1.1, $CHCl_3$); IR ($CHCl_3$) 1680, 2100 cm^{-1}; ^1H NMR (DMSO-d_6) δ 1.1−1.5 (m, 15H), 1.6−1.7 (m, 2H), 1.9−2.1 (m, 2H), 2.9−3 (m, 2H), 3.1−3.23 (m, 8H), 4.79 (q, J = 6.2 Hz, 1H), 4.99 (s, 2H), 5.05 (s, 2H), 5.56 (d, J = 9 Hz, 1H), 7.29−7.35 (m, 11H), 7.46−7.51 (m, 3H), 7.78 (s, 1H), 7.78−7.9 (m, 3H), 8.1−8.2 (m, 1H), 8.51 (d, J = 9 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 22.56, 24.54, 25.67, 26.18, 27.87, 27.9, 30.31, 34.74, 37.9, 38.19, 50.41, 65.07, 65.89, 74.76, 46.45, 124.11, 124.546, 125.549, 125.87, 127.21, 127.34, 127.43, 127.63, 128.23, 128.28, 132.23, 132.60, 136.99, 137.12, 141.1, 155.15, 155.98, 167.35, 172.88. Anal. Calcd for $C_{44}H_{55}N_7O_7$: C, 66.56; H, 6.98; N, 12.35. Found: C, 66.62; H, 7.07; N, 12.01.

2,4,12,15,20,24-Hexaazapentacos-2-enedioic acid, 13-[1-(2-naphthalenyl)ethoxy]-11,14-dioxo-20[(phenylmethoxy)-carbonyl]-3-[[(phenylmethoxy)carbonyl]amino]-, bis-(phenylmethyl) ester, [*S*-(*R,*R**)] (9a).** A mixture of triphenyl phosphine (0.149 g, 0.567 mmol), **8a** (0.450 g, 0.567 mmol) and 0.68 mL of water in THF (30 mL) was heated at reflux overnight. After cooling at room temperature, *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea (0.223 g, 0.623 mmol) was added and the mixture was stirred for 3 h at room temperature. Concentration of the mixture gave a crude product which was purified by chromatography on silica gel (EtOAc/*i*-Pr_2O, 5:5). **9a** (0.47 g, 77%) was obtained as a white solid by crystallization from Et_2O; mp 98 °C; *Rf* 0.59 (EtOAc/*i*-Pr_2O, 8:2), R_f 0.25 (hexane/*i*-PrOH, 9:1); $[\alpha]^{25}_D = -33.1°$ (*c* 1.0, $CHCl_3$); IR(KBr) 1640, 1690, 1710, 1730, 2830, 3020, 3060, 3300 cm^{-1}; ^1H NMR (CD_3CN) δ 1.29−1.57 (m,14H), 1.48 (d, J = 7.5 Hz, 3H), 2.17 (t, J = 7 Hz, 2H), 2.95−3 (m, 2H), 3.04−3.2 (m, 6H), 3.32 (m, 2H), 4.86 (q, J = 6 Hz, 1H), 5.01−5.17 (4s, 8H), 5.2 (d, J = 9 Hz, 1H), 5.7−5.9 (2m, 1H), 6.9−7.4 (m, 22H), 7.44−7.54 (m, 3H), 7.81−7.9 (m,4H), 8.26 (br t, 1H), 11.7-(s, 1H); ^{13}C NMR (CD_3CN) δ 24.24, 26.09, 27.16, 28.98, 29.39, 29.53, 36.66, 39.22, 41.67, 45.28, 66.74, 67.45, 67.72, 68.90, 76.39, 76.95, 125.44, 126.91, 127.06, 127.31, 128.57, 128.66, 128.72, 128.82, 128.86, 129.08, 129.32, 129.35, 129.43, 129.47, 129.58, 129.66, 134.14, 134.20, 138.34, 138.41, 141.24, 154.57, 157.09, 164.84, 168.77, 174.75. Anal. Calcd for $C_{61}H_{71}N_7O_{11}$: C, 67.94; H, 6.64; N, 9.09. Found: C, 67.68; H, 6.65; N, 9.06.

7-[(Aminoiminomethyl)amino]-*N*-[(1*S*)-2-[[4-[(3-amino-propyl)amino]butyl]amino]-1-hydroxy-2-oxoethyl]hep-tanamide (2). A flask containing a solution of **9a** (0.1 g, 0.093 mmol) in 1 N AcOH in methanol (10 mL) was purged with nitrogen. To this solution was added 50 mg (50 wt %) of palladium hydroxide (Pearlman's catalyst, 20% on carbon/50% H_2O). The mixture was stirred 8 h under 1 atm of hydrogen at room temperature. The mixture was purged with N_2 and filtered. The filtrate was purged with N_2, 50 mg (50 wt %) of Pearlman's catalyst was added again and the mixture was treated as above overnight. The mixture was purged with N_2, filtered and water was added. The solution was concentrated under reduced pressure and extracted three times with CH_2Cl_2. The aqueous phase was lyophilized. The resulting powder was dissolved in H_2O (10 mL) and lyophilized again to give the triacetate **2** as an hygroscopic white powder. The chemical purity was determined to be >98% by HPLC analysis (as described below). The product was then further purified and transformed to the tris(hydrochloride) following the literature protocol[6b] on CM Sephadex C-25 and Sephadex LH-20 columns. The freeze-dried product was obtained as an hygroscopic powder (30 mg, 68%); *Rf* 0.6 (R. P.18, 3:6.5:0.5, CH_3CN/H_2O/TFA); $[\alpha]^{22}_D = -14.5°$ (*c* 1, H_2O) [lit.[7] $[\alpha]^{21}_D = -14.3$ (*c* 1, H_2O)]; ^1H NMR (D_2O) δ 1.3−1.45 (m, 4H), 1.5−1.8 (m, 8H), 2−2.2 (m, 2H), 2.3 (t, J = 4.5 Hz, 2H), 3.05−3.25 (m, 8H), 3.27−3.3 (m, 2H), 5.45 (s, 1H); ^{13}C NMR (D_2O) δ 23.64, 24.46, 25.57, 26.22 (2C), 28.42, 28.47, 36.20, 37.28, 39.22, 41.81, 45.16, 48.08, 72.63, 157.39, 171.97, 178.08. HRMS found 388.3036 (C_{18} H_{38} N_7 O_3 (M+H) calcd 388.30361). The chemical purity was determined to be >98% by HPLC analysis (Inertsil OSD 2, Gl Sciences Inc.; Solvent A, water with 0.05% TFA; Solvent B, CH_3CN with 0.05% TFA: 2% Solvent B, 5 min, 2−80% Solvent B in 15 min, 1 mL/min, 30 °C). The enantiomeric purity of the product was determined to be ≥99.5% by HPLC column analysis (Chiralcel OD, Daicel Chemical Industries, Ltd.) following the literature protocol.[31]

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Supporting Information Available: Synthesis and characterization data of bis(7-bromoheptane)imide from 7-bromoheptanenitrile, ORTEP drawing, and X-ray structure data for the intermediate **7b** (8 pages). This material is contained in libraries on microfiche, immediately follows this article in the microfilm version of the journal, and can be ordered from the ACS; see any current masthead page for ordering information.

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