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A new efficient synthesis of the immunosuppressive agent (±)-15-deoxyspergualin

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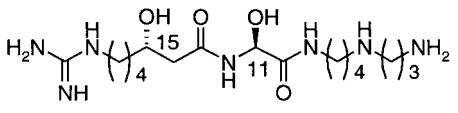
Abstract—(±)-15-Deoxyspergualin is a new promising immunosuppressive agent, recently marketed in Japan for the control of corticoresistant acute renal graft rejection. We describe here a nine-step synthesis starting from 7-bromoheptanenitrile and N^1,N^4-bis(benzyloxycarbonyl)spermidine, suitable for the production of multigram quantities of this unstable highly polar compound with an 18% overall yield. Furthermore, it opens the way for the synthesis of α-hydroxyglycine analogues. © 2001 Elsevier Science Ltd. All rights reserved.

1. Introduction

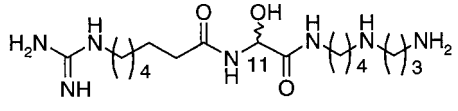
(±)-15-Deoxyspergualin (DSG) **1** has aroused considerable interest[1] as an immunosuppressive agent in transplantation. This synthetic derivative of the natural antitumor and immunosuppressive antibiotic spergualin **2** (Fig. 1) is usually found more effective than the popular immunosuppressants Cyclosporin A, FK 506 or Rapamycin,[2] and routinely elicits fewer side effects and a different mechanism of action.[3] (±)-DSG has a rather unstable peptidomimetic[4] structure containing an asymmetric carbon. Although in vivo experiments clearly showed that (−)-DSG is the only immunosuppressive enantiomer and that both isomers contribute at least to the acute toxicity of the compound,[4,5] most of the biological, pharmacological and clinical data have been obtained with the more readily available racemic trishydrochloride DSG. Furthermore, the racemic form (Spanidin®) received marketing approval in Japan for the treatment of corticoresistant acute renal graft rejection episodes. We have been involved for some years in a structure–activity relationship study in this series in order to better define its structural requirements and to design more stable analogues.[6] To evaluate the potency of our compounds in several pharmacological experiments, we needed multigram quantities of (±)-DSG as reference. DSG consists of a rather unstable α-hydroxyglycine central part, connecting two highly polar moieties: guanidinoheptanoic acid and spermidine (Fig. 1). Owing to the unusual hemiaminal structure of the α-hydroxyglycine unit, DSG hydrolyses gradually, in basic or acidic aqueous solution, into 7-guanidinoheptanamide and hydrated glyoxylspermidine.[1b,7] These different properties have made the synthesis and the purification of this hygroscopic unstable salt derivative a real challenge.

2. Results and discussion

The synthesis of (±)-DSG was first described in a low overall yield (3.5%) from 7-aminoheptanoic acid through condensation of a glyoxylspermidine derivative with 7-guanidinoheptylamide.[5a,8] This synthesis involves amino and guanidino intermediates simply protected as their hydrochloride salts. The drawback of this strategy is the purification, which requires the use of ion exchange chromatography at each step of the synthesis, making the scale up difficult. A more elegant synthesis of (±)-DSG in a 7.2% overall yield from non commercially available 7-aminoheptylamide used condensation of Boc-protected 7-guanidinoheptylamide with methyl-2-hydroxy-2-methoxy acetate for the synthesis of the α-hydroxyglycine central part.[9] This protective group strategy avoids the use of ion exchange chromatography for the purification of the intermediates, but the condensation step could not be improved by heating due to the thermal instability of the



(-) Spergualin **2**



(±)-15-Deoxyspergualin **1**

Figure 1.

Keywords: α-hydroxyglycine; polyamine; deoxyspergualin; immunosuppressive agent.

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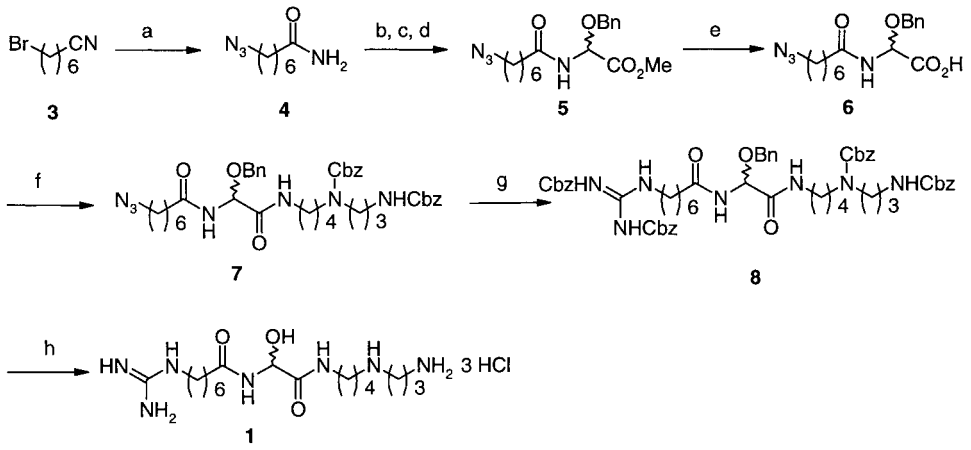

Scheme 1. *Reagents and reaction conditions*: (a) see Ref. 10a, 62%; (b) (MeO)(HO)CHCO$_2$Me, CH_2Cl_2, 40°C, 24 h, molecular sieves 4 Å; (c) $SOCl_2$, CH_2Cl_2, 40°C, 2 h; (d) benzyl alcohol, Et_3N, CH_2Cl_2, rt, 24 h, 67% overall from **4**; (e) 1N NaOH, DME, rt, 1.5 h; (f) $H_2N(CH_2)_4N(Cbz)(CH_2)_3NHCbz$ (**9**), DCC, HOBT, CH_2Cl_2, rt, 48 h, 72% overall from **5**; (g) (i) Ph_3P, H_2O, THF, 65°C, overnight; (ii) CbzN=C(SMe)NHCbz (**10**), THF, rt, 3 h, 79% overall from **7**; (h) (i) $Pd(OH)_2/C$ (20%), MeOH/AcOH, H_2 1 atm, (ii) 1N HCl pH 3, 0°C, and lyophilization, 2 cycles, 77%.

Boc-protected intermediates. Furthermore, in this synthesis, the hemiaminal hydroxyl had to be protected by a silyl ether group in order to avoid a retro reaction during the coupling of Boc-protected spermidine with the glyoxylic ester. The final acidic deprotection step required a TFA treatment, which led to the trifluoro acetate salt of DSG in moderate yield. Ion exchange chromatography followed by a desalting chromatography were necessary to obtain the drug as its tris-hydrochoride salt. Moreover, the first synthesis of the optically active (−)-DSG involved several steps with many difficult purifications and could not be adapted for our purpose.[5a] However, we have recently described[10] an enantioselective synthesis of (−)-DSG, with a good overall yield, using only benzyl type protective group strategy for the three different functional groups of the molecule and introducing the guanidino function in the last step before deprotection. This approach made the synthesis more efficient and the purification of the intermediates easier, but we still required ion exchange chromatography for the obtention of the final tris-hydrochloride salt. However, we felt that we could use a similar strategy for a multigram synthesis of the racemic form and that the final deprotective step could be modified in order to obtain the hydrochloride salt without using ion exchange chromatography.

7-Azidoheptylamide **4** was obtained from 7-bromoheptanenitrile **3** in 62% yield as described previously (Scheme 1).[10a] This amide was then converted in a three-step one-pot process into the desired α-benzyloxy glycine derivative **5**. The first step consisted of a condensation with methyl-2-hydroxy-2-methoxy acetate, trapping the formed methanol with molecular sieves.[11] The resulting α-hydroxyglycine was converted in situ into a labile α-chloroglycine intermediate by treatment with thionyl chloride at 40°C. The absence of Boc-protected guanidine group allowed us to use these rather acidic but efficient conditions for the condensation step and allowed warming to further improve the yield. The chloro compound was then treated with benzyl alcohol in presence of triethylamine to afford the α-benzyloxy derivative in 67% overall yield for the three steps. It favorably compares with the 29% yield reported using the Boc protective group strategy for a similar sequence.[9] Saponification of **5** gave the corresponding acid **6** which could be either purified or used directly in the following step. Coupling of N^1,N^4-bis(benzyloxycarbonyl)spermidine[12] with **6** using the usual DCC/HOBT methodology provided the polyamine derivative **7**. Conversion of **7** to **8** was made in a two-step one-pot process. First the azido function was reduced without affecting the benzyloxy and the benzyloxycarbonyl protective groups using Ph_3P/H_2O.[13] The primary amine was then reacted in situ with *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea[14] to give **8** in 79% yield. The product could be purified by a simple crystallization. The high purity of this intermediate made it possible to avoid chromatography for purification. Final deprotection was accomplished using Perlman's catalyst in 1N AcOH in methanol under 1 atm of hydrogen. We did not attempt to carry out the deprotection in the presence of hydrochloric acid because of the instability of the product in acid and the difficulty of adjusting the pH of the solution. In such conditions, the α-hydroxyglycine moiety could either be hydrolyzed or converted to its corresponding methyl ether. At this stage, we found that the so formed tris-acetate derivative could be easily converted to its corresponding tris-hydrochloride using a simple salt exchange procedure. It simply consists of adjusting a cold aqueous solution of the tris-acetate derivative to pH 3 with hydrochloric acid solution, followed by lyophilization. The salt exchange could be followed by ^{1}H NMR spectroscopy checking the disappearance of the methylic protons of acetic acid. Two cycles of this simple procedure enabled a complete salt exchange and gave the tris-hydrochloride salt of (±)-DSG with high purity in 77% yield.

3. Conclusion

The present synthesis affords an easy access to racemic DSG which is the prototype of a new family of immunosuppressor agents[5b,6,15] from 7-bromoheptanenitrile in a nine-step process and an 18% overall yield. Furthermore, it opens new possibilities for the synthesis of analogues modified on the polyamine and/or on the guanidine moieties.

4. Experimental

4.1. General

All chemicals were purchased from commercial sources and used without further treatment except 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. 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.

4.1.1. Methyl [(7-azidoheptanoyl)amino](benzyloxy)-acetate (5). A solution of **4** (9.74 g, 52.2 mmol) and methyl-2-hydroxy-2-methoxy acetate (6.8 mL, 68.7 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 40 g of 4 Å molecular sieves. After cooling, the Soxhlet apparatus was replaced by a reflux condenser. Thionyl chloride (5.4 mL, 74.4 mmol) was added and the resulting mixture was refluxed for 2 h. The reaction mixture was concentrated under reduced pressure. The crude chloroglycine derivative was dissolved in 100 mL of CH_2Cl_2, then benzyl alcohol (7.1 mL, 68.7 mmol) and triethylamine (9.55 mL, 68.7 mmol) in 50 mL of CH_2Cl_2 were added dropwise for 24 h at room temperature. The reaction mixture was then washed with 1N HCl (100 mL) and brine (100 mL). The organic layer was dried ($MgSO_4$), filtered and concentrated under reduced pressure, and the compound was purified by flash chromatography on silica gel (hexane–*i*-PrOH, 9:1) to give 13.4 g (67%) of **5** as a colorless oil. IR (neat) 1660, 1750, 2100, 2930, 2970 cm^{-1}; ^1H NMR ($CDCl_3$) δ 1.34–1.4 (m, 4H), 1.55–1.7 (m, 4H), 2.3 (t, J=7.5 Hz, 2H), 3.26 (t, J=6.6 Hz, 2H), 3.8 (s, 3H), 4.67–4.76 (m, 2H), 5.74 (d, J=7.8 Hz, 1H), 6.5 (d, J=7.8 Hz, 1H), 7.26–7.37 (m, 5H); ^{13}C NMR (CD_3CN) δ 25.39, 26.55, 28.78, 35.93, 51.51, 52.63, 70.26, 77.23, 128.26, 128.47, 128.80, 138.17, 168.88, 173.88 (2); Anal. calcd for $C_{17}H_{24}N_4O_4$: C, 58.61; H, 6.94; N, 16.08. Found: C, 58.75; H, 6.97; N, 16.22.

4.1.2. Benzyl 3-{(4-{[[(7-azidoheptanoyl)amino](benzyloxy)acetyl]amino}butyl)[(benzyloxy)carbonyl]amino} propyl carbamate (7). A mixture of 1N NaOH (37 mL, 37 mmol) and **5** (10.7 g, 30.6 mmol) in 200 mL of DME was stirred for 1.5 h at room temperature, then diluted with brine (100 mL) and Et_2O (200 mL). The aqueous phase was acidified to pH 2 with 1N HCl, extracted with Et_2O (3×100 mL) and the organic phases were dried (Na_2SO_4) and concentrated to yield 10.2 g (100%) of the crude acid. This product was dissolved in CH_2Cl_2 (200 mL), and hydroxybenzotriazole (4.13 g, 30.6 mmol) followed by DCC (7.06 g, 34.2 mmol) were added. After stirring at room temperature for 30 min, N^1,N^4-bis(benzyloxycarbonyl)spermidine[12] **9** (14.18 g, 34.2 mmol) in CH_2Cl_2 (100mL) was added. The mixture was stirred at room temperature for 20 h and concentrated under reduced pressure. EtOAc (100 mL) was added and the insoluble dicyclohexyl urea was eliminated by filtration and washed with EtOAc (50 mL). The filtrate was washed with saturated aqueous $NaHCO_3$, brine, and the organic layer was dried ($MgSO_4$). Concentration under reduced pressure of the organic layer and flash chromatography on silica gel (EtOAc–*i*-Pr_2O, 8:2) gave **7** (17.5 g, 72%) as an oil; R_f: 0.38 (EtOAc–*i*-Pr_2O, 8:2); IR ($CHCl_3$) 1680, 2100 cm^{-1}; ^1H NMR (CD_3OD) δ 1.3–1.73 (m, 14H), 2.25 (t, J=7.2 Hz, 2H), 3–3.3 (m, 10H), 4.55–4.67 (m, 2H), 5.04 (s, 2H), 5.07 (s, 2H), 5.52 (s, 1H), 7.2–7.4 (m, 15H); ^{13}C NMR (CD_3CN) δ 25.49, 26.56, 28.81, 36.07, 38.35, 44.76, 51.50, 62.21, 66.92, 70.22, 78.06, 128.01, 128.16, 128.18, 128.28, 128.41, 128.77, 137.83, 137.94, 138.34, 156.80, 168.04, 174.33; Anal. calcd for $C_{39}H_{51}N_7O_7$: C, 64.18; H, 7.04; N, 13.44. Found: C, 64.55; H, 7.09; N, 13.35.

4.1.3. Benzyl 21-[(benzyloxy)carbonyl]amino-11-(benzyloxy)-4-[(benzyloxy)carbonyl]-10,13,23-trioxo-25-phenyl-24-oxa-4,9,12,20,22-pentaazapentacos-21-en-1-yl carbamate (8). A mixture of triphenyl phosphine (6.75 g, 25.7 mmol), **7** (17 g, 21.4 mmol) and water (0.46 mL, 25.7 mmol) in THF (200 mL) was heated at reflux overnight. After cooling at room temperature, *N*,*N*′-bis(benzyloxycarbonyl)-*S*-methylisothiourea **10** (9.21 g, 25.7 mmol) was added and the mixture was stirred for 6 h at room temperature. Concentration of the mixture gave a crude product, which was purified by chromatography on silica gel (EtOAc). **8** (17.5 g, 79%) was obtained as a white solid by crystallization from Et_2O and recrystallization from MEK–*i*-Pr_2O, 7:10; mp 58–64°C; R_f: 0.43 (EtOAc–*i*-Pr_2O, 8:2); IR (KBr) 1640, 1690, 1710, 1730, 2830, 3020, 3060, 3300 cm^{-1}; ^1H NMR (d_6-DMSO) δ 1.23–1.64 (m, 14H), 2.16–2.18 (m, 2H), 2.96–2.98 (m, 2H), 3–3.4 (m, 8H), 4.44–4.54 (m, 2H), 4.99–5.20 (4s, 8H), 5.46 (d, J=9 Hz, 1H), 7.2–7.4 (m, 26H), 8.08 (brt, 1H), 8.38 (brt, 1H), 8.59 (d, J=9 Hz, 1H), 11.57 (s, 1H); ^{13}C NMR (CD_3CN) δ 25.62, 26.73, 28.99, 36.21, 39.99, 41.22, 66.31, 67.02, 68.45, 70.33, 78.17, 128.09, 128.26, 128.35, 128.38, 128.41, 128.50, 128.61, 128.87, 129.00, 129.11, 129.19, 135.90, 137.87, 137.93, 138.03, 138.43, 154.08, 156.58, 156.89, 164.36, 168.14, 174.43; Anal. calcd for $C_{56}H_{67}N_7O_{11}$: C, 66.32; H, 6.66; N, 9.67. Found: C, 66.21; H, 6.65; N, 9.69.

4.1.4. 7-{[Amino(imino)methyl]amino}-*N*-[2-({4-[(3-aminopropyl)amino]butyl}amino)-1-hydroxy-2-oxoethyl]heptanamide tris-hydrochloride (1). A flask containing a solution of **8** (3 g, 2.96 mmol) in 1N AcOH in methanol (10 mL) was purged with nitrogen. To this solution were added 300 mg (50 wt%) of palladium hydroxide (Pearlman's catalyst, 20% on carbon/50% H_2O). The mixture was stirred for 8 h under 1 atm of hydrogen at rt. The mixture was purged with N_2 and filtered. The filtrate was purged with N_2, 300 mg (50 wt%) of Pearlman's catalyst were 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 tris-acetate form of the compound was transformed into its tris-hydrochloride form by the following procedure. The powder was dissolved in H_2O (40 mL) and the pH of the solution was adjusted to 3 by a

slow addition of 1N HCl at 0°C and the solution was lyophilised. This procedure was repeated to give the tris-hydrochloride **1** as a hygroscopic white powder (1.14 g, 77%); R_f: 0.6 (R.P.18, CH_3CN–H_2O–TFA, 3:6.5:0.5); ^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; calcd for $(M+H)^+=C_{18}H_{38}N_7O_3$: 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).

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