Pergamon

Tetrahedron: *Asymmetry* 11 (2000) 3665–3669

TETRAHEDRON: *ASYMMETRY*

Efficient synthesis of (*S*)-(−)- and (*R*)-(+)-enantiomers of 15-deoxyspergualin (15-DSG)

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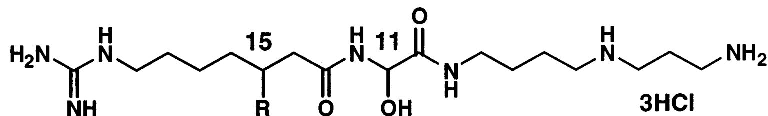
Received 14 August 2000; accepted 17 August 2000

Abstract

An efficient synthesis of the (*S*)-(−)- and (*R*)-(+)-enantiomers of 15-deoxyspergualin (15-DSG) is reported. The synthesis involves preparative HPLC separation and subsequent hydrogenolysis of two diastereoisomers **10a** and **10b** of fully protected 15-DSG penultimates. Alternatively, diastereomerically pure amide **10b** (97% de) was also prepared from acid **8b** (97% de), which was obtained via crystallization of a 1:1 diastereomeric mixture of **8a** and **8b**. © 2000 Elsevier Science Ltd. All rights reserved.

15-Deoxyspergualin trihydrochloride (15-DSG) is a potent immunosuppressive agent, which has been used in the racemic form to treat acute renal graft rejection episodes in Japan since 1994.[1] The (*S*)-(−)-enantiomer of 15-DSG was initially derived from spergualin isolated from the culture filtrates of bacillus laterosporus.[1a,2] (*S*)-(−)-15-Deoxyspergualin was found to have stronger antitumor activity against mouse leukemia L-1210 than the natural spergualin.[2c,d] In both compounds, the (*S*)-(−)-enantiomers are more potent than the (*R*)-(+)-enantiomers. For example, antibacterial and antitumor activity of the racemic mixture of spergualin is about half of that of the natural (*S*)-spergualin.[2c] The (*S*)-(−)-enantiomer of 15-DSG has strong antitumor activity against mouse leukemia L1210, while the (*R*)-(+)-enantiomer is almost inactive.[3]



R = OH, Spergualin
R = H, 15-DSG

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PII: S0957-4166(00)00350-5

Synthesis of racemic 15-DSG was reported by Umeda,[2d] Bergeron[4] and Dischio.[5] The key step in the racemic synthesis is the condensation of an amide with an aldehyde to construct the C-11 acylaminol.

Asymmetric synthesis of chiral 15-DSG is nontrivial due to chemical instability of the C-11 acylaminol. A relatively low ee in the product indicates that partial racemization at C-11 may have occurred in the original synthesis of (*S*)-(−)-15-DSG from natural spergualin.[2c] An alternative approach based on enzymatic resolution of a stable *N*-(7-guanidoheptanoyl)-α-alkoxyglycine intermediate is applicable only to small scale preparation of optically active 15-DSG.[3a]

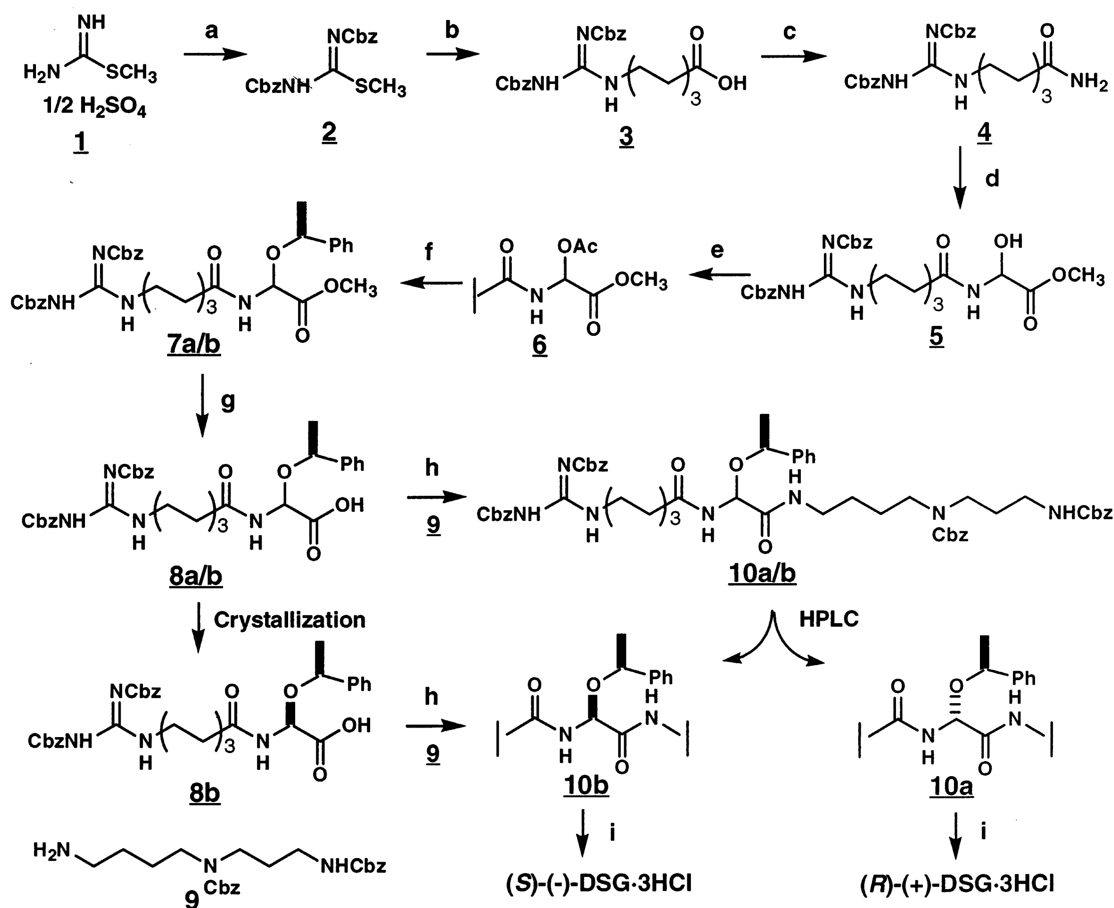
Herein, we report an efficient route for the preparation of both the (*S*)-(−)- and (*R*)-(+)-enantiomers of 15-DSG.[3b] This route involves separation of the diastereoisomeric ethers **10a** and **10b** derived from (*R*)-α-methylbenzyl alcohol by preparative HPLC. Alternatively, crystallization of the precursor acids **8a** and **8b** afforded diastereomerically pure **8b**, which was subsequently converted to **10b** without epimerization.

The overall synthesis is outlined in Scheme 1. The sulfate salt of 2-methyl-2-thiopseudourea **1** was reacted with excess CbzCl to afford **2** which, on reaction with 7-aminoheptanoic acid in the presence of $NaHCO_3$, gave *N*,*N*-(di-Cbz-guanidino)heptanoic acid **3**. Reaction of **3** with ammonia via the *N*-hydroxysuccinimide ester produced amide **4** in 87% crystallized yield. Amide **4** was heated with 1.4 equivalents of methyl glyxolate at 45°C to afford racemic amino acetal **5** in 85% crystallized yield.

Attempted conversion of **5**–**7** directly with α-methylbenzyl alcohol under various acidic conditions (conc. H_2SO_4 or CSA, 4 Å MS) was unsuccessful as the Cbz groups did not survive the acidic conditions. A successful route involved conversion of alcohol **5** to the acetate **6** with acetic anhydride and pyridine. Substitution of acetate **6** with (*R*)-α-methylbenzyl alcohol was performed in the presence of a Lewis acid and Hunig's base. Various Lewis acids were screened and $EtAlCl_2$ was found to be the most effective for this transformation. The Hunig's base was added to ensure a neutral or slightly basic reaction condition, thus suppressing cleavage of the acid labile Cbz groups. The acetate formation step and the substitution reaction were carried out in the same reaction flask with an overall yield of 74%.[6]

The product from the above substitution reaction was a mixture of the diastereomers **7a** and **7b** in approximately 1:1 ratio. Attempted separation of methyl ester **7a** from **7b** by crystallization in various organic solvents was unsuccessful. It was difficult to separate them by normal phase chromatography. Hence, saponification of the mixture to acids **8a** and **8b** followed by coupling to di-CBZ-spermidine **9**[3] afforded tetra-CBZ-11-(*R*)-α-methylbenzyloxyl-DSG diastereomers **10a** and **10b**. These fully protected DSG penultimates were separated effectively by preparative HPLC.[7]

Alternatively, a crystallization protocol to separate **8b** from **8a** was developed. The diastereomeric acids **8a** and **8b** (1:1 mixture) on crystallization from toluene at rt produced **8b** in 40% yield (50% maximum). The ratio of isomer **8b** to **8a** in the crystallized material was 96:4 (^1H NMR), while the ratio in the mother liquor was 20:80. A second crystallization from toluene increased the diastereomeric ratio to 98.5:1.5 with 94% recovery. It was observed that isomer **8a** was not as stable as isomer **8b**, with epimerization of **8a** at C-11 slowly occurring at rt. For example, the **8a** enriched mother liquor became a 1:1 mixture of **8a** and **8b** after storage at rt for about a week. The configuration of diastereomer **8b** was confirmed by conversion to **10b** and ultimately to the (*S*)-(−)-enantiomer of 15-DSG.



Scheme 1. Reagents and conditions: **a** CbzCl, 1N NaOH, CH_2Cl_2, H_2O, rt, 2 days, 97%; **b** 7-aminoheptanoic acid, $NaHCO_3$, THF, H_2O, 65°C, 6 h, 81%; **c** (i) NHS, DCC, CH_3CN, THF, rt, 16 h and (ii) NH_3, DMF, rt, 2 h, 87%; **d** methyl glyxolate, THF, 40°C, 20 h, 89%; **e** Ac_2O, pyridine, CH_2Cl_2; **f** *R*-α-methyl benzyl alcohol, $EtAlCl_2$, Hunig's base, CH_2Cl_2, 0°C to rt, 10 h, 74% two steps; **g** 0.1N LiOH, THF, H_2O, rt, 2 h, 93%; **h** PPA, TEA, CH_2Cl_2, 0°C, 1 h, 94%; **i** (i) H_2, Pd/C; $Pd(OH)_2$/C, 2N AcOH/MeOH, rt, 20 h, (ii) CM-Sephadex C-25 and (iii) Sephadex LH-20

Cleavage of all protecting groups of the penultimates **10a** and **10b** was accomplished by mild hydrogenolysis. Since 15-DSG is unstable in its free amine form, the hydrogenolysis was performed under acidic conditions. Interestingly, complete racemization occurred when hydrogenolysis was performed in 1N hydrochloric acid and methanol with the best result being obtained in acetic acid and methanol. To prevent epimerization during hydrogenolysis, the concentration of acetic acid in methanol was maintained at 2N or less. Under the acetic acid conditions, the Pearlman catalyst was used and the Cbz groups were easily cleaved in about 3 h at rt, while cleavage of the α-methyl benzyl protecting group was slower.

The crude tri-acetate salt from the hydrogenolysis reaction was converted to the tri-hydrochloride salt on a CM-Sephadex C25 column using aqueous sodium chloride as the mobile phase. The resulting tri-hydrochloride salt was contaminated with sodium chloride and was desalted on a CM-Sephadex LH20 column. After desalting, a pure final drug substance was obtained. The optical rotation of purified (*S*)-(−)-15-DSG tri-hydrochloride {$[\alpha]_D = -13.7$ (*c* 1.0,

H_2O)} compared well with the literature value ($[\alpha]_D = -14.1$, (*c* 1.0, H_2O)}.[8] The (+)-enantiomer of 15-DSG was prepared from **10b** separately following the same procedure.

In summary, a new and practical route for the synthesis of both enantiomers of chiral 15-DSG was established. The synthesis involves ten steps with an overall yield of ~25%. Key steps are: (1) protection of the C-11 hydroxy group as α-methyl benzyl ether, (2) subsequent separation of the resulting penultimates as a 1:1 diastereomeric mixture by preparative chromatography or by crystallization and (3) removal of the protecting group by mild hydrogenolysis without racemization.

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6. Preparation of 7-[*N*,*N*′-bis(benzyloxycarbonyl)guanidino]heptanoyl-(*RS*)-2-[(*R*)-*sec*-phenethoxy]glycine methyl ester **7**: Compound **5** (100 g, 171 mmol) was dissolved in CH_2Cl_2 in a 5 L three-necked round bottom flask, equipped with a mechanical stirrer, an argon line and a 250 mL addition funnel. The solution was cooled to 0°C (internal) with an ice bath. Anhydrous pyridine (225 mL) was added dropwise through the addition funnel. The internal temperature remained below 3°C during the course of the addition. Neat acetic anhydride (130 mL, 1.493 mmol) was added dropwise. The resulting reaction mixture was warmed to rt and stirred for 1 h. The reaction was complete as indicated by TLC. The yellowish reaction mixture was cooled to 5°C and quenched with dropwise addition of anhydrous MeOH (55 mL, 1.453 mmol). After 15 min of stirring at 5°C, the reaction mixture was evaporated to an oily residue. Anhydrous toluene (500 mL) was added to the residue and was removed on a rotovap. The addition and removal of anhydrous toluene were repeated three more times until ^1H NMR did not detect any pyridine in the residue. The resulting yellowish oil was re-dissolved in CH_2Cl_2 (2 L). The solution was cooled to −78°C and $EtAlCl_2$ (460.8 mL, 1.0 M in hexane, 460.8 mmol) was added dropwise through an addition funnel under argon. After 10 min of stirring, a mixture of neat *R*-α-methylbenzyl alcohol (33.2 mL, 276.4 mmol) and diisopropylethylamine (48.2 mL, 276.4 mmol) was added dropwise. The reaction mixture was warmed to rt and stirred for 16 h. The reaction was complete as indicated by TLC. It was cooled to −78°C and quenched with slow addition of Rochelle salt solution. The mixture was warmed up to 0°C and stirred for 3 h. The cold mixture was filtered through a pad of Celite. The filtrate was washed with Rochell salt solution (3×500 mL), dried over Na_2SO_4 and concentrated to give 150 g of crude oil, which was passed through a pad of silica gel (300 g, 23–40 mm), eluted with 33–50% EtOAc in hexanes. The solvents were evaporated to give 104 g of spectroscopically pure methyl ester **7** as a thick oil in 74% yield.
7. Chromatographic conditions for the separation of **10a** and **10b**: HPLC solid support: Porasil, Prepak (50 mm, 125 Å), column size: 80 mm (d)×400 mm (l), mobile phase: 80–100% EtOAc in hexanes, sample load: 15 g in 50 mL of EtOAc/hexanes (4/1) and 10 mL of CH_2Cl_2 per injection, flow rate: 350 mL/min, detection: 254 nm

UV, fractions: 500 ml each, collected: 8–9 fractions for the first diastereoisomer **10a** and 14–15 for the second diastereoisomer **10b**. The contaminated fractions 10–11 for **10a** and 12–13 for **10b** (analyzed by TLC) were combined, evaporated and re-injected.

8. The ee's of DSG·3AcOH and DSG·3HCl were determined by HPLC analysis of the diastereomeric GITC (2,3,4,6-tetra-*O*-acetyl-β-D-glycopyranosyl isothiocyanate) derivative according to the published procedure.[3a]