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A concise, stereocontrolled synthesis of spectinomycin☆

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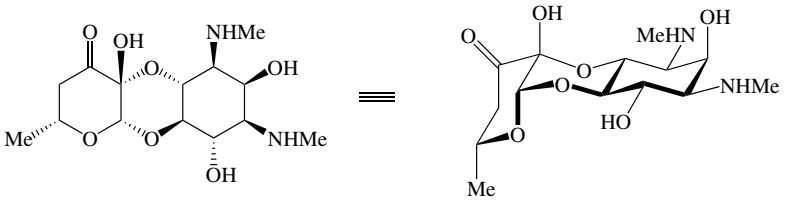
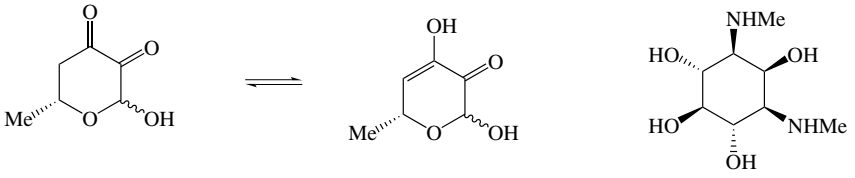
Abstract—A simple and concise synthesis of the antibiotic spectinomycin is described. The key step comprises a reaction cascade initiated by the β-selective 5-*O*-glycosylation of an *N*,*N*-blocked *myo*-1,3-inosadiamine **10** with a suitable actinospectosyl donor—the D-glucose-derived enol benzoate of 4,6-dideoxy-α-D-hex-3-enulosyl chloride **5**—which is spontaneously followed by regio- and stereospecific cyclohemiketalization and a 3-*O*→2-*O*-benzoyl group migration to directly elaborate the *cis–cisoid–trans*-fused pyran–dioxane–cyclohexane framework of **1**. The approach is flexible enough to be applied to other inosadiamines towards the generation of novel spectinomycin analogues.

1. Introduction

The broad spectrum antibiotic spectinomycin **1**[2] can be perceived, in formal terms, to be a pseudodisaccharide in which a tricarbonyl sugar, the 4,6-dideoxy-D-*glycero*-hexose-2,3-diulose **2** designated actinospectose, is fused to *N*,*N*-methylated 1,3-diamino-*myo*-inositol actinamine **3** by both a β-glycosidic and a hemiketal linkage to form a pyran–dioxane–cyclohexane system in *cis–cisoid–trans* arrangement. Whilst actinamine **3** can readily be secured by a variety of syntheses,[3] as well as by acid hydrolysis of spectinomycin,[2b] the actinospectose portion



Spectinomycin **1** *cis-cisoid-trans*



2 (actinospectose) **3** (actinamine)

☆ Part 39 of the series, Sugar-derived building blocks'. Part 38, see Ref. 1.

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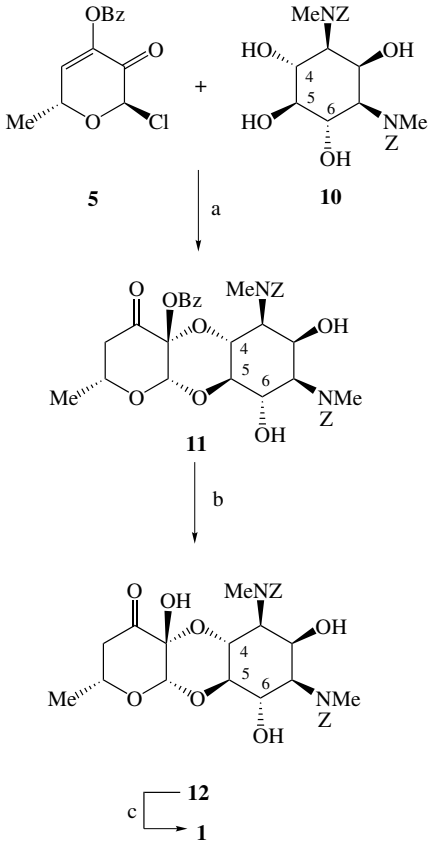
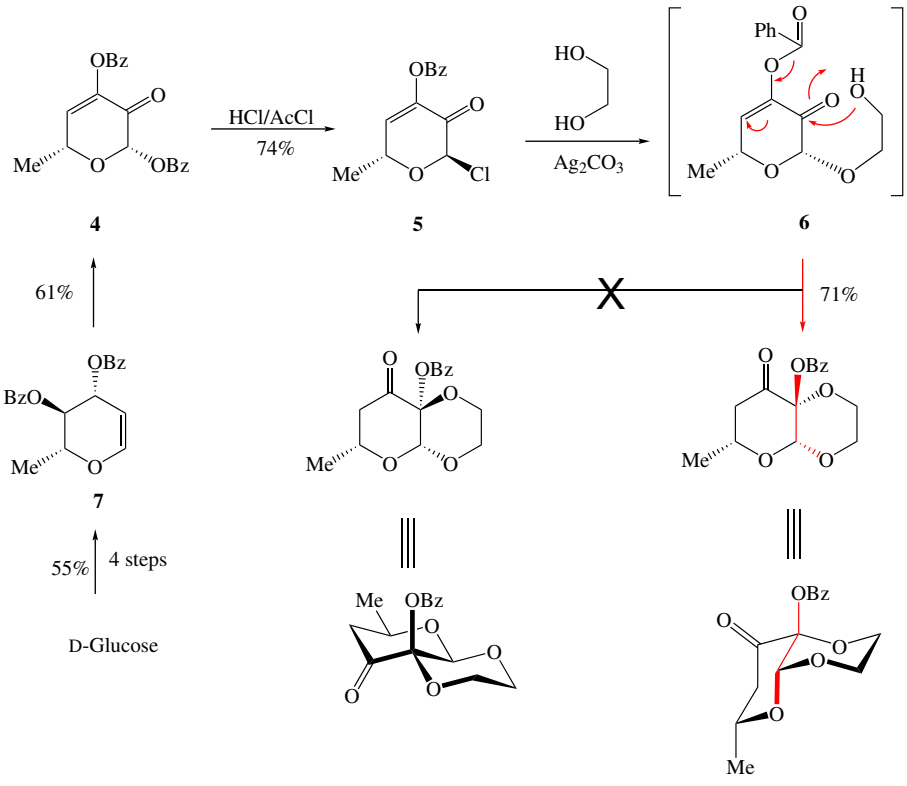
does not survive the harsh acidic conditions required for cleaving the bis-glycosidic linkage (6 M HCl, 6 h reflux) and is left as an intractable tar.

A number of actinospectose derivatives have been obtained in crystalline form by synthetic means, such as the β-dibenzoate of its enol form **4** and the respective α-chloride **5**, which can serve as an actinospectosyl donor substrate. Under Koenigs–Knorr conditions, it not only reacts with alcohols to smoothly give the respective β-glycosides,[4] but also with 1,2-diols in a unique cascade reaction: the essentially β-specific mono-*O*-glycosylation of one of the OH groups of glycol, for example, is spontaneously followed by cyclo-hemiketalization of the other with the 2-carbonyl, thereby generating a sterically favourable disposition for the ensuing 3-*O*→2-*O*-benzoyl migration (arrows in **6**), which liberates the 3-carbonyl group (Scheme 1). Of the two possible products **8** and **9**, only the *cis*-linked **9** is formed (TLC, ^{1}H NMR, 71% isol. yield[4]), obviously due to the favourable thermodynamics derived from the operation of two vicinal anomeric effects: the OH→C=O attack occurs from the lower (axial) face of the pyran ring to product **9**, in which electronic interactions are minimized by the *trans*-diaxial disposition of the pyranoid ring oxygen and the acetalic benzoyloxy group.[5]

The essentially complete stereoselection in this cyclo-hemiketal 'folding', followed by the benzoyl shift-induced liberation of the enolester-blocked carbonyl, can be similarly observed on reaction of **5** with (*R*,*R*)-1,2-cyclohexanediol[6] or the steroidal diol gomphogenine, which led to the first total synthesis of the gomphosides.[6] In analogous fashion,



Scheme 2. Reagents and conditions: (a) Ag aluminosilicate (1.2 equiv), toluene, 80 °C, 3 h, 51%; (b) K_2CO_3, MeOH, 25 °C, 10 min, 89%; (c) H_2, 5% Pd/C, *i*PrOH, 25 °C, 12 h, 90%, Z = benzyloxycarbonyl.



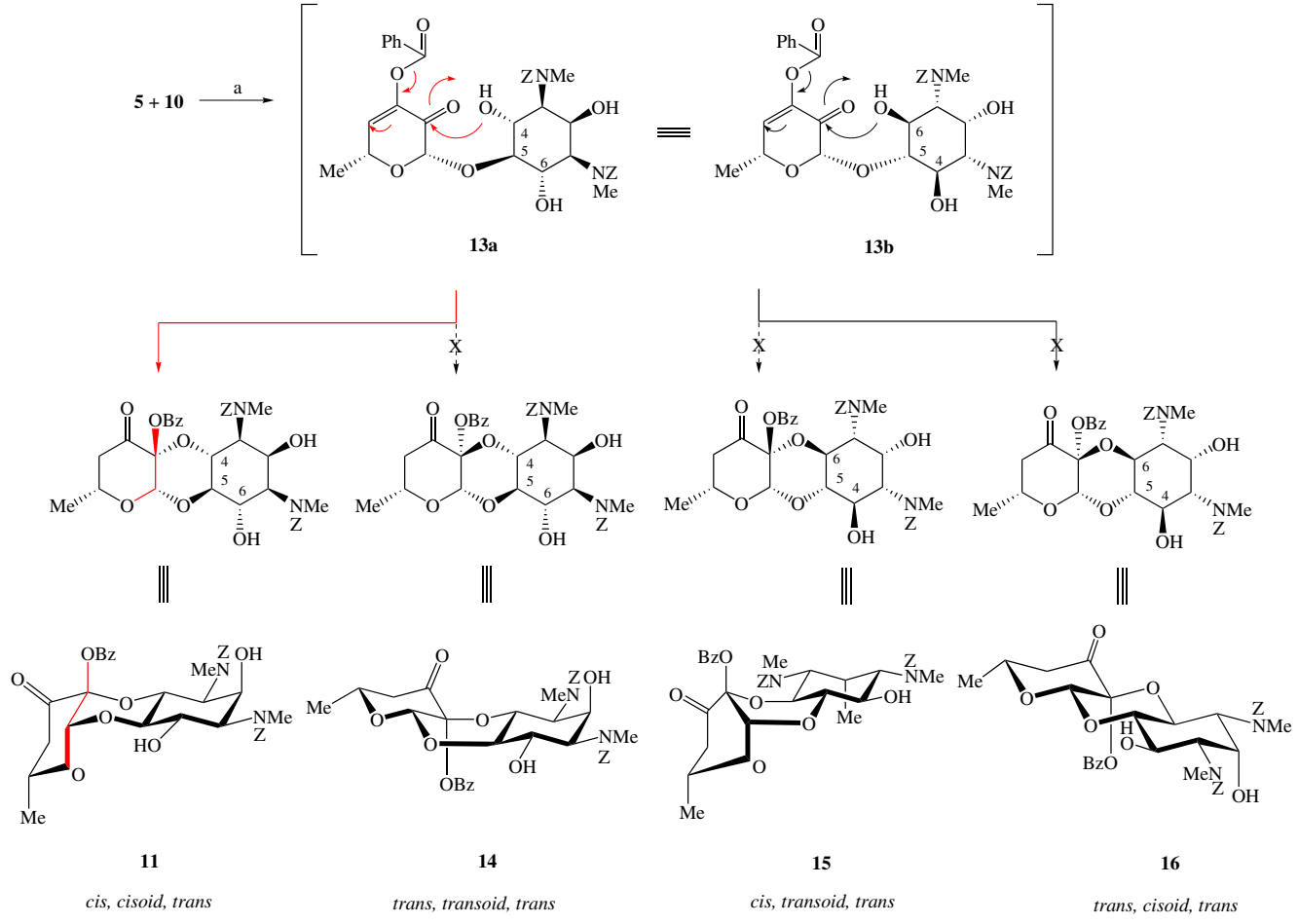
Scheme 1. Generation of actinospectosyl donor **5** from D-glucose and reaction with glycol.

coupling of the actinospectosyl donor **5** with a suitably blocked actinamine was surmised to entail a comparatively simple synthetic access to spectinomycin **1**, inasmuch as previous synthetic efforts required nine steps from L-glucose (overall yield 3%)[7] or 23 from D-glucose[8] (~1%[9]). Herein we report a new, 10-step sequence of **1** from D-glucose in 10.2% overall yield, which proceeds in a particularly concise manner and proceeds along lines possibly relevant to its biosynthesis.[10]

2. Results and discussion

After elaborating an improved procedure for the acquisition of actinospectosyl donor **5** from D-glucose—the three steps from hydroxyglucal ester **7**[11] were merged into one continuous operation—its glycosidation with the readily accessible[12] *N*,*N*-bis(benzyloxycarbonyl)actinamine **10** was probed with the reasonable[7,8] expectation that the bulky N-protecting groups would impede glycosylation at the vicinal hydroxyls through steric shielding. However, the acceptor reactivity of the 3-OH of **10** proved to be considerably lower than that in cyclohexane-1,2-diol or in gomphogenine—seemingly due to the high oxygen substitution in its cyclohexane ring—entailing sluggish glycosylations when promoted with Ag_2CO_3. After substantial experimentation with various insoluble silver catalysts—the more reactive soluble promoters, such as silver triflate, were excluded as they entail α-selectivity—silver alumosilicate[13] in THF/CH_2Cl_2 or in toluene was found to effect β-selective *O*-glycosylation of **10** with donor **5** to give **11** as the major product, isolable in 51% yield after the removal of 4-*O*- and 6-*O*-glycosylated analogues (ca. 5% each) by preparative HPLC (Scheme 2). The integrity of **11** to be the 2′-*O*-benzoyl derivative of *N*,*N*-bis-(benzyloxycarbonyl)spectinomycin in its *cis–cisoid–trans*-fused tricyclic framework clearly followed from its spectral data, its ready de-*O*-benzoylation to the known[8,14] Z-blocked **12** as well as from its hydrogenolysis to spectinomycin **1**, which was found to be identical to the natural product in all respects.

Whilst the regio- and β-selectivity of the glycosylation reaction is already remarkable, the stereocontrol exercised in the hemiketal folding of the glycosidulose intermediate is even more so as it leads from a product with two stereogenic centres to one with nine altogether: enjoying the thermodynamics derived from two anomeric effects and the propensity to form the sterically most favourable linear-fused tricycle, the glycosidulose-C=O in **13** is attacked with high, or exclusive, preference by the 5*R*-OH from the axial



Scheme 3. Unique stereocontrol in the intramolecular hemiketal folding of glycosidulose intermediate **13** towards the *cis*,*cisoid*,*trans*-fused framework of spectinomycin.

(lower) face of the pyran ring, elaborating **11** with a pyranoid ring oxygen and ketalic OBz in the favourable *trans*-diaxial disposition, as well as the chair conformations of the three rings (Scheme 3). In contrast, the alternate possibilities—4*R*-OH attack from the β-(equatorial) face (**13a**→**14**) or of the 6*S*-OH in the two conceivable steric modes (**13b**→**15** or **16**)—invariably lead to thermodynamically less stable products because the central dioxane ring is forced into a boat conformation (**14** and **15**), or unfavourable dipolar interactions are operative (**14** and **16**).

The ease with which the glycosidulose intermediate spontaneously 'folds' into the natural *cis–cisoid–trans* geometry can be taken as evidence for such a process to be operative in non-enzyme mediated fashion in the biosynthesis of spectinomycin from D-glucose,[15] with an unprotected form of glycosidulose **13** conceivably being the decisive intermediate.

3. Conclusion

In conclusion, the chemistry detailed herein defines a concise and general method for the construction of products with a *cis–cisoid–trans*-interconnected pyran–dioxane–cyclohexane framework, such as the antibiotic spectinomycin. The key feature of the methodology is the selective, insoluble silver salt-promoted mono-*O*-glycosylation of the central OH of the 1,2,3-triol segment of the actinamine aglycone by the glucose-derived 4,6-dideoxy-2-ketohexosyl donor—an approach that has enough flexibility to be applied to other inosadiamine aglycones towards the generation of novel spectinomycin analogues.

4. Experimental

4.1. General

Melting points, determined with a Bock hot-stage microscope, are uncorrected. Optical rotations were measured on a Perkin–Elmer 241 polarimeter at 20 °C using a cell of 1 dm path length; concentration (*c*) in g/100 mL and solvent are given in parentheses. ^{1}H and ^{13}C NMR spectra were recorded on a Bruker ARX-300 spectrometer in the solvents given. Mass spectra were acquired on Varian MAT 311 and MAT 212 spectrometers. Microanalyses were determined on a Perkin–Elmer 240 elemental analyzer. Analytical thin layer chromatography (TLC) was performed on precoated Merck plastic sheets (0.2 mm silica gel 60 F_{254}) with detection by UV (254 nm) and/or by spraying with H_2SO_4 (50%), and heating. Column and flash chromatography were carried out on Fluka silica gel 60 (70–230 mesh) using the specified eluents.

4.2. 1,3-Di-*O*-benzoyl-β-D-actinospectose 4 [*syn* 1,3-di-*O*-benzoyl-4,6-dideoxy-β-D-*glycero*-hex-3-enopyranos-2-ulose or (2*S*,6*R*)-2,4-bis(benzoyloxy)-6-methyl-2*H*-pyran-3(6*H*)-one]

Dry chlorine gas was slowly passed through a stirred and cooled (−20 °C) solution of 10.0 g (21.8 mmol) of 3,4-di-*O*-benzoyl-2-benzoyloxy-6-deoxy-D-glucal **7**[11] in toluene (200 mL) that contained 3 mL of water. Upon reaching a pale yellow solution (5–10 min), stirring was continued at room temperature (20 min), followed by evaporation to dryness in vacuo. The resulting amorphous residue, consisting of the anomeric ulose hydrates (mainly the β-anomer, $R_f = 0.12$–0.24 in $CHCl_3/CH_2Cl_2$ 5:1) and about 10% of the *manno*-1,2-dichloride ($R_f = 0.77$),[4] was directly subjected to elimination of benzoic acid by dissolution in benzene (200 mL), addition of $NaHCO_3$ (15 g) and water (5 mL) and heating at reflux with stirring for 1.5 h. After cooling, $MgSO_4$ was stirred into the mixture (30 min) and the inorganic salts were filtered off. Evaporation to dryness in vacuo followed by two co-evaporations from ethanol left a syrup, which crystallized from ethanol (30 mL) on standing overnight in a refrigerator: 3.95 g (51%) of enolone dibenzoate **4** as colourless needles; mp 82–83 °C, $[\alpha]_D^{22} = -89$ (*c* 1, $CHCl_3$), and ^{1}H NMR data corresponded to literature values.[4] A second crop (0.78 g, 10%) was secured from the mother liquor; the total yield for **7**→**4**: 4.73 g (61%).

4.3. 3-*O*-Benzoyl-α-D-actinospectosyl chloride 5 [*syn* 3-*O*-benzoyl-4,6-dideoxy-α-D-*glycero*-hex-3-enopyranosulosyl chloride and (2*R*,6*R*)-2-chloro-4-benzoyloxy-6-methyl-2*H*-pyran-3(6*H*)-one]

Enolone dibenzoate **4** (2.40 g, 6.8 mmol) was gradually added with stirring to 65 mL of acetyl chloride presaturated with HCl gas and the mixture was allowed to stand at room temperature for 12 h, followed by evaporation in vacuo. The resulting syrup was co-evaporated twice from benzene for removal of traces of acetyl chloride and subsequently crystallized by dissolution in ether and addition of *n*-pentane to turbidity. After standing at 0–5 °C for 1 day, the colourless crystals were collected: 1.15 g (63%) of **5**; mp 90–92 °C, $[\alpha]_D^{22} = +151$ (*c* 1, $CHCl_3$); ^{1}H NMR data corresponded to the literature values.[4] A second crop (600 mg, 11%) was similarly isolated from the mother liquor; total yield: 1.75 g (74%).

4.4. 2′-*O*-Benzoyl-*N*,*N*-bis(benzyloxycarbonyl)-spectinomycin 11

A slurry of *N*,*N*′-bis(benzyloxycarbonyl)-actinamine **10**[12] (145 mg, 0.3 mmol), silver alumosilicate catalyst[13] (180 mg, 0.6 mmol) and freshly desiccated molecular sieves 4 Å (1 g) in toluene (5 mL) was stirred at ambient temperature for 15 min. 3-*O*-Benzoyl-actinospectosyl chloride **5** (160 mg, 0.6 mmol) was then added, and the mixture refluxed for 2.5 h with the exclusion of light and moisture, whereafter **10** ($R_f = 0.01$, $CHCl_3$/acetone 9:1) had been fully consumed in favour of a major spot at $R_f = 0.15$ and two minor ones (ca. 5% each) at 0.20 and 0.23. Filtration through Kieselgur, washing with CH_2Cl_2 and evaporation of the combined filtrates to dryness in vacuo left a residue, which was subjected to HPLC (Lichrosorb RP-18, 5 μm, MeOH/water 60:40, 1 mL/min). Removal of the solvents in vacuo gave 108 mg (51%, based on **10**) of **11** as an amorphous solid; $[\alpha]_D^{20} = -5.3$ (*c* 0.9, $CHCl_3$). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.40 (d, $J_{5',6'}$, 3H, 6′-H_3), 2.58 (dd, $J_{4',4'}$ 14.2, $J_{4'e,5'}$ 1.7, 1H, 4′-He), 2.70 (dd, $J_{4',4'}$ 14.2, $J_{4'a,5'}$ 10.2, 1H, 4′-Ha), 3.05, 3.07 (two 3H-s, 2

NMe_2), 4.26 (m, 1H, 5′-H), 5.43 (s, 1H, 1′-H), 7.31 (4H-s, 2 BnCH_2), 7.4–8.0 (5H-m, Bz-H_5). Ms (FD, 25 mA): $m/z = 705$ (M^++1). Anal. Calcd for $C_{37}H_{40}N_2O_{12}$ (704.74): C, 63.06; H, 5.72. Found: C, 62.90; H, 5.74.

4.5. *N*,*N*-Bis(benzyloxycarbonyl)-spectinomycin 12

4.5.1. A. De-*O*-benzoylation of 11. K_2CO_3 (12 mg) was added to a methanolic solution of **23** (56 mg, 0.08 mmol, in 5 mL) and the mixture was stirred for 30 min at room temperature. Dilution with CH_2Cl_2 (50 mL), washing with 2 M HCl (20 mL) and water (2 × 20 mL), drying over Na_2SO_4 and removal of CH_2Cl_2 in vacuo left 49 mg (89%) of **12** as a colourless amorphous solid of $[\alpha]_D^{20} = -4.3$ (*c* 0.9, $CHCl_3$), indistinguishable spectroscopically (IR, ^{1}H NMR) and chromatographically ($CHCl_3$/MeOH 10:1, *n*-hexane/EtOAc 5:1, or EtOH) from a sample prepared[14] by carbobenzoxylation of commercial (Sigma) spectinomycin; lit.: $[\alpha]_D = -3$ ($CHCl_3$),[14] −4.6 (*c* 0.28, $CHCl_3$).[8]

4.5.2. B. Glycosylation of 10 with actinospectosyl chloride 5, followed by de-*O*-benzoylation. Reaction of **10** (320 mg, 1.2 mmol) with **5** (285 mg, 0.6 mmol) in toluene (15 mL) in the presence of silver alumosilicate[13] (360 mg, 1.2 mmol) and molecular sieves 4 Å (2 g) for 2.5 h at reflux, followed by filtration through Kieselgur, evaporation of the filtrate to dryness in vacuo and exposure of the syrupy residue of crude **11** to K_2CO_3 in MeOH for 30 min gave, upon workup as described under procedure A, crude **12** ($R_f = 0.36$ in $CHCl_3$/MeOH 9:1) with two minor, slightly faster spots (ca. 5% each). Purification by HPLC on Lichrosorb RP-18 (5 μm) with MeOH/water 60:40 at 1 mL/min and removal of the solvents of the appropriate fraction in vacuo afforded 208 mg of **12** (58%, based on **5**) as a colourless solid, identical in all respects with the product described under A.

4.6. Spectinomycin 1

A 100 mg portion of **12** was hydrogenolyzed over 5% Pd/C (100 mg) in 1:1 *i*PrOH/water (5 mL) and processed as described[8] to give, upon crystallization from aqueous acetone, 74 mg (90%) of **1** dihydrochloride pentahydrate as needles with mp 200–204 °C (dec) and $[\alpha]_D^{20} = +14.3$ (*c* 1.1, H_2O) {lit. mp 210 °C, $[\alpha]_D^{20} = +14.0$ (*c* 5, water);[16] mp 205–207 °C (dec), $[\alpha]_D = +14.8$ (*c* 0.42, H_2O)[8]}. IR and ^{1}H NMR data were identical to the natural product in all respects.

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