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Spinosyn G: Proof of Structure by Semisynthesis

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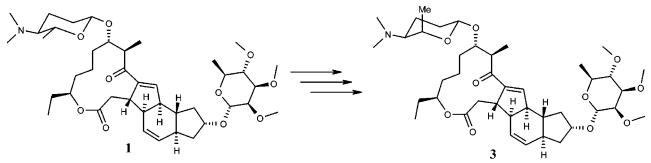

Spinosyn G was isolated in the late 1980s as a minor component from the broth of our potent, fermentation-derived insecticide spinosad. Its structure was then tentatively identified as 5″-epispinosyn A (**3**) on the basis of ^{1}H and ^{13}C NMR data, but the 4″-epi compound **4** could not be conclusively ruled out with the data available. Described herein are unambiguous syntheses of both **3** and **4**, whereby **3** was proved identical to the natural product. Compound **4** was prepared from intact spinosyn A by a novel F-TEDA-promoted oxidative deamination to the 4″-ketone **5**, stereoselective reduction to the equatorial alcohol **6**, and nitrogen incorporation via the axial azide **7**. Compound **3** was obtained by coupling spinosyn A 17-pseudoaglycone (**9**) with the *N*-protected dihydropyran **16** derived from methyl L-ossaminide (**14**). This gave an ~2:1 mixture of anomeric products **17** with the desired equatorial glycoside predominating, which was then converted to **3** by *N*-deprotection and methylation.

Introduction

2,3,6-Trideoxy-4-aminoglycosides are relatively rare in the natural product literature, with two families of compounds dominating the landscape. As forosamine, the glycosides appear in spiramycins[1a] and related compounds (chimeramycins,[1b] and shengjimycins[1c]) which consist of a 16-macrocyclic lactone also substituted with a disaccharide. The spinosyns[2] also predominately consist of a macrocyclic core, substituted with a forosamine and a permethylated rhamnose unit. Even more rare than forosamine is the epimeric glycoside subunit ossamine, which has only been reported in a very few natural products. Initially reported as the amino-sugar fragment derived by hydrolysis of the cytotoxic agent ossamycin[3a] during the 1960s—a compound whose total structure was not determined until the 1990s[3b]—this unit has only since appeared as part of dunaimycin D2S[4] and proposed as part of spinosyn G,[2b] the subject of this report.

Spinosyn G was isolated in the late 1980s as a minor component from the broth of the potent, fermentation-derived insecticide Spinosad, a ~90:10 mixture of spinosyns A (**1**) and D (**2**). Its structure was tentatively assigned as 5″-epispinosyn A (**3**) on the basis of ^{1}H and ^{13}C NMR data, but the 4″-epi compound **4** could not be conclusively ruled out with the data available. In both, the 1″-anomeric linkage was equatorial, the same as in A. G thus presented an anomaly, as the rest of the more than 24 natural spinosyns characterized to date differed from A only in the methylation patterns of the amine and hydroxyl groups or in the extent of C-methylation of the nucleus.

If spinosyn G were indeed the 5″-epi compound **3** then the sugar was of the L-configuration and G represented the only naturally occurring spinosyn with an L-sugar at C-17, in this case, L-ossamine.

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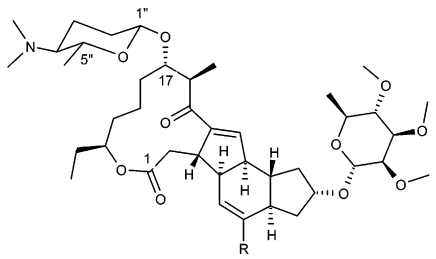
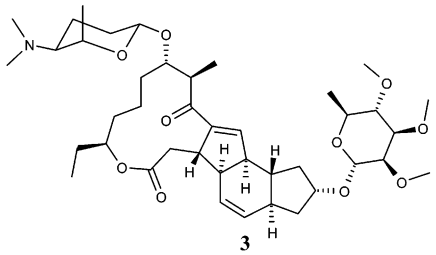


1 R=H (spinosyn A); **2** R=Me (spinosyn D)



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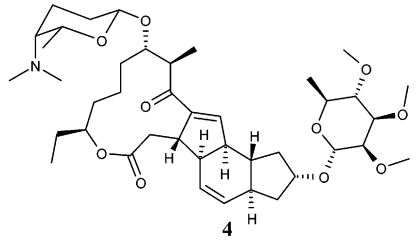

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Described herein are some more recent NMR analysis and unambiguous syntheses of both **3** and **4**, which proved **3** to be the structure of the natural product.

Results and Discussion

Synthesis of 4″-Epispinosyn A (4). It was decided to first attempt the synthesis of **4** as it was felt that, on the basis of the wealth of synthetic information accumulated from our spinosyn synthetic analogue program, it might be the more readily available of the two compounds, resulting directly from intact spinosyn A by oxidative deamination to the 4″-ketone **5**. Indeed, **5** had been observed, albeit in low yields, when spinosyn A had been treated with a variety of oxidizing reagents. Additionally, a practical synthesis of **5**, amenable to the preparation of gram quantities, would be a boon to our analogue program, allowing the preparation of novel 4″-substituted analogues.

Unfortunately, a viable, high-yield synthesis of **5** from **1** proved elusive, though enough material was isolated from several attempts at same to carry on to the preparation of **4**. This synthesis is outlined in Scheme 1. Treatment of **1** with 1-chloromethyl-4-fluorotriethylenediamine tetrafluoroborate (Selectfluor, F-TEDA) in acetonitrile−water gave ketone **5** by a novel Polonovsky-like oxidative deamination in low yield (18−30%). Stereo- and chemoselective reduction of this with lithium tri-*tert*-butoxyaluminohydride gave the equatorial alcohol **6** which was converted to the axial azide **7** by mesylation and subsequent reaction with sodium azide. Chemoselective azide reduction with stannous chloride in methanol gave the amine **8** which was converted to **4** by permethylation with iodomethane to the quarternary amine iodide and demethylation.

The F-TEDA-mediated oxidative deamination of **1** to **5** deserves mention. Attempts at "normal" Polonovsky deamination[5] via the *N*-oxide of **1** were unsuccessful, with Cope elimination to the olefin the major reaction. The F-TEDA oxidative deamination was discovered serendipitously, when in connection with other work fluorination of **1** was attempted. Presumably, the tertiary amine is *N*-fluorinated and oxidized under these conditions to an imine, which is then quickly hydrolyzed to the ketone. The reaction of tertiary amines with halogens and oxidizing reagents to give imines or iminium ions is well-known.[6] It was later found that conversion to **5** could be improved by using a two-step process. Thus, treatment of spinosyn A with 1.25 equiv of F-TEDA and excess pyridine in dry acetonitrile, isolation of the intermediate product *N*-demethyl spinosyn A tetrafluoroborate salt (90%), and subsequent treatment with an additional 1.25 equiv of F-TEDA and pyridine and finally with ion-exchange resin in aqueous methanol resulted in isolation of **5** in 63% overall yield.

The NMR spectra of **4** were clearly different from that of spinosyn G, adding substance to the tentative assignment of spinosyn G as the 5″-epi compound **3**. At this time, a greater sample of the naturally occurring factor G was available, and so further NMR experiments were undertaken to confirm this assignment. In particular, 2D gradient-enhanced rotating frame NOESY (gROESY)[7] experiments were run, revealing a strong cross-peak between 1″ and 6″, indicating the axial nature of the 5″-methyl group. These cross-peaks had been difficult to detect in earlier experiments due to the lack of intensity of NOE cross-peaks or the presence of noise in the spectra acquired without gradient selection. With the structure now seemingly confirmed as the 5″ epimer of spinosyn A, attention now focused on an unambiguous synthesis of **3**.

Synthetic Strategy to 5″-Epispinosyn A (3). It was envisioned that **3** could be obtained by coupling of a suitably protected and activated L-ossamine derivative with spinosyn A 17-pseudoaglycone (**9**, 17-Pseudoaglycone),[2] as shown in Scheme 2. A potential major limitation to this approach was the understanding that C-2 unsubstituted sugars gave predominantly α (axial) coupled products in glycosidic coupling reactions; G had a *β*-glycosidic linkage. In earlier work from these laboratories[8a] and in the reported total syntheses of **1** by Evans[8b] and Paquette,[8c] the coupling of forosamine derivatives with 17-Pseudoaglycone or close analogues had given predominately (66 to >90%) α-coupled products.

Synthesis of the L-Ossaminyl Coupling Partner. It was decided at the outset to prepare *N*-demethyl-*N*-

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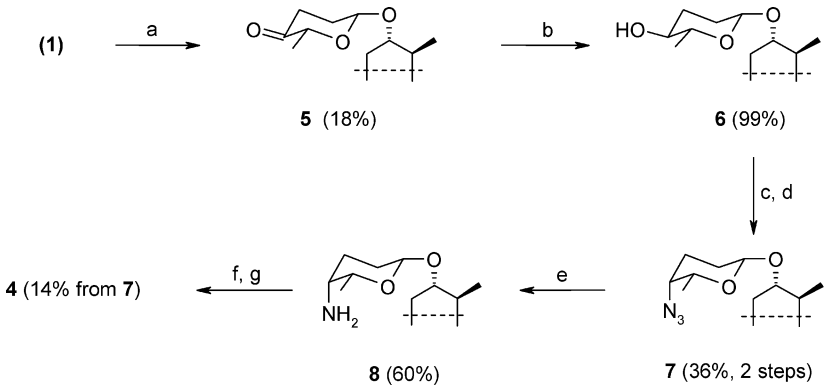
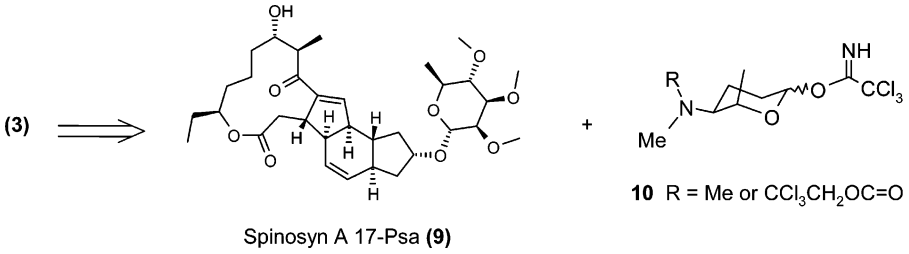
SCHEME 1. Synthesis of 4″-Epispinosyn A (4)[a]

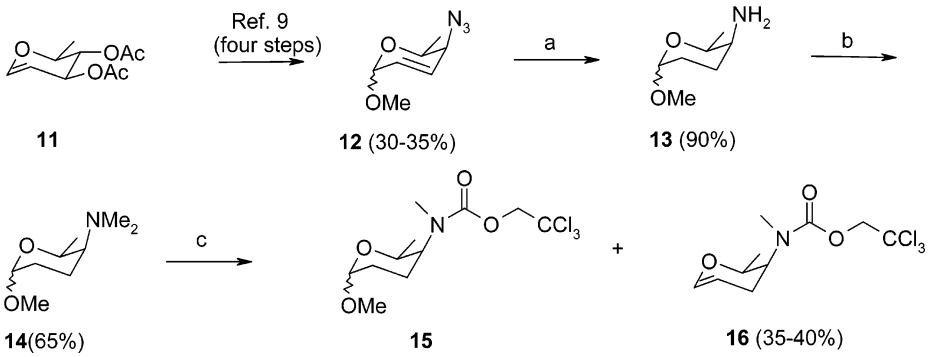


[a] Reagents and conditions: (a) F-TEDA, MeCN−H_2O, 0 °C; (b) Li(*t*-BuO)$_3$AlH, Et_2O, 0 °C; (c) $MeSO_2Cl$, (*i*-Pr)$_2$NEt (DIPEA), CH_2Cl_2, 0 °C; (d) NaN_3, DMF, 120 °C; (e) $SnCl_2$, MeOH, rt; (f) MeI (xs), MeCN, reflux; (g) *m*-xylene, reflux.

SCHEME 2. Retrosynthetic Plan for Compound 3



SCHEME 3. Synthesis of the L-Ossaminyl Coupling Partner[a]



[a] Reagents and conditions: (a) H_2, 45 psi, $Pd(OH)_2$ on C, EtOH, rt; (b) formalin (37%), MeOH, reflux, 1 h, then $NaBH_4$; (c) TROC-Cl, K_2CO_3, toluene, reflux.

trichloroethoxycarbonyl-1-*O*-trichloroacetimidoyl-L-ossamine [**10**, R = trichloroethoxycarbonyl (TROC), Scheme 2] and use this as the coupling partner with the pseudoaglycone. This derivative was chosen over the acetimidate derived from natural ossamine (**10**, R = Me) because of the anticipated ease of coupling of a nonbasic amide derivative. Indeed, this strategy had worked well previously in a preparation of 9-*O*-forosaminyl spinosyn A.[8a] Once coupled, simple *N*-protecting group removal followed by *N*-methylation would give the targeted 5″-epi compound (**3**).

Methyl L-ossaminide (**14**) was prepared from 3,4-di-*O*-acetyl-6-deoxy-L-glucal (**11**) by slight modification of the literature procedures[9] (Scheme 3). As per ref 9, glucal **11** was converted to azide **12** possessing the desired axial *N*-configuration. In our hands, conversion of **12** to **14** by a two-step hydrogenation/reductive amination procedure consistently gave better yields than the one-step literature conversion.

Reaction of methyl ossaminide (**14**) with TROC-Cl and K_2CO_3 in benzene at reflux afforded a 65% yield of the desired *N*-demethyl-*N*-TROC ossaminide **15** along with 15−20% of dihydropyran **16** (Scheme 3), formed by loss of methanol from **15** under the reaction conditions. The unexpected formation of **16** proved to be a boon to the synthesis of **3**, for the glycosidic coupling of dihydropyranyl "sugars" is well documented in the literature obviating the need to prepare **10**. The yield of **16** was subsequently raised to 35−40% by conducting the reaction in refluxing toluene in the presence of 3 equiv of K_2CO_3.

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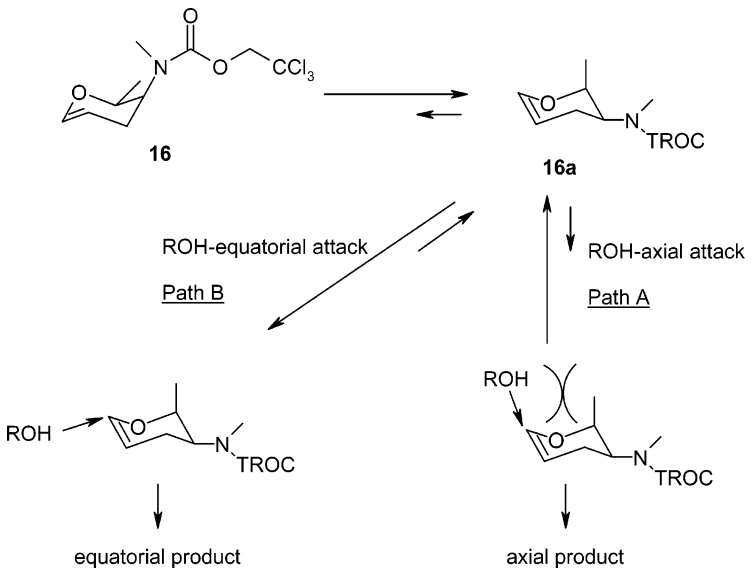
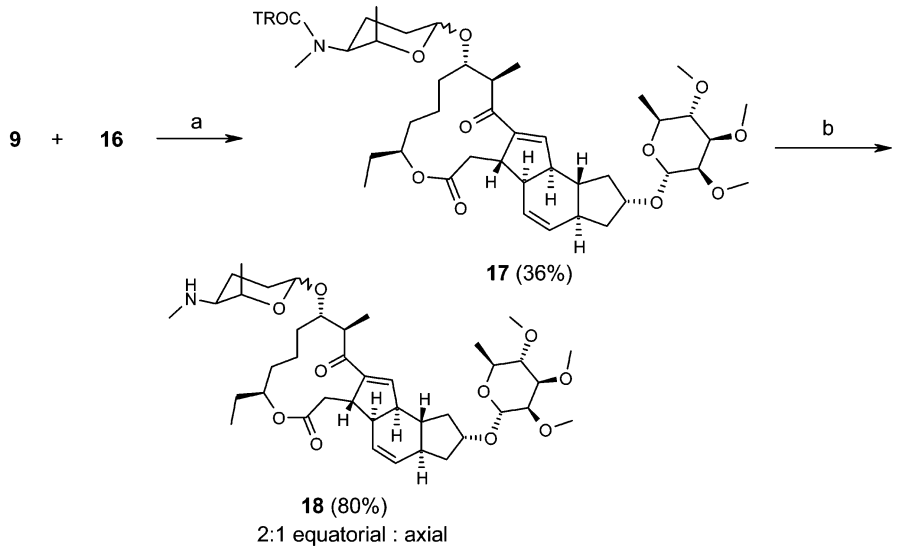


FIGURE 1. Transition states for axial vs equatorial attack on **16**.

SCHEME 4. Synthesis of 18[a]



[a] Reagents and conditions: (a) PPTS, BF_3OEt_2, 1,2-dichloroethane, rt; (b) Zn, 1M NH_4OAc, THF-MeOH, rt.

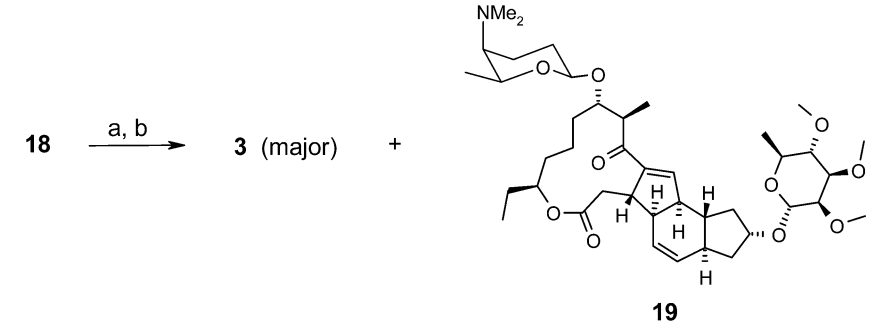
Coupling of Dihydropyran 16 with 17-Pseudoaglycone (9). It was envisaged that coupling of dihydropyran **16** with **9** would give a preponderance of the desired equatorial coupled product (in this case α product as **16** is derived from an L-sugar) based on the putative transition states (Figure 1) for axial and equatorial attack and the fair assumption that the sterically large *N*-TROC-*N*-methyl group of **16** would prefer that configuration in which it was equatorial (or pseudoequatorial), thus forcing the 5-methyl group to be axial (**16a**). Axial attack by the pseudoaglycone (path A) would be impeded by a severe 1,3 steric interaction with the axial methyl group. Equatorial attack by the pseudoaglycone (path B) is not impeded and should be favored.

This indeed was found to be the case. Coupling of **16** with 1 equiv of **9** in dichloroethane with a mixture of $BF_3 \cdot Et_2O$ and pyridinium *p*-toluenesulfonate as catalyst gave a 36% yield of coupled products (**17**, Scheme 4). The composition of this mixture was difficult to determine by NMR due to the presence of the TROC protecting group because the resonance pattern of its CH_2 moiety overlapped the key 1″-H resonance. This mixture was treated with zinc, and the resulting mixture of deprotected amines (**18**) was shown by NMR to be an ~2:1 anomeric mixture at C-1″ with the desired equatorial (*β*) glycoside predominating.

Completion of the Synthesis of 5″-Epispinosyn A (3). Treatment of the anomeric mixture of amines **18** with 2.5 equiv of MeI in THF in the presence of Hunig's base gave a mixture of the two expected tertiary amines in 49% yield. These were easily separated by silica gel chromatography. The major of these, the α (L-sugar nomenclature) equatorial coupled product, was shown by NMR and LC analysis to be identical in all respects to natural spinosyn G (Supporting Information).

It is interesting that the minor coupled product (**19**) also possessed an equatorial C-1″ glycosidic linkage (Scheme 5). Apparently, axial coupling induces a severe

SCHEME 5. Completion of the Synthesis of 3[a]



[a] Reagents and conditions: (a) MeI (2.5 equiv), DIPEA, THF, rt; (b) silica gel chromatography.

1,3-diaxial steric interaction between the C-5″ methyl group, and the bulky pseudoaglycone forces the sugar to ring flip, this putting the 4″-dimethylamino group in the axial configuration and the methyl into the equatorial position. NMR was used to confirm this geometry and was also able to distinguish **19** unambiguously from 4″-epispinosyn A (**4**). Compound **19** was thus β-L-ossaminylspinosyn A 17-pseudoaglycone and **4**, β-D-ossaminylspinosyn A 17-pseudoaglycone.

Experimental Section

4″-Ketospinosyn A (5). A stirred solution of **1** (10 g, 13.6 mmol) and pyridine (3.5 g, 44 mmol) in acetonitrile (100 mL) was cooled to 5 °C and treated with F-TEDA (6 g, 17 mmol), in portions over 1 h. After addition was complete, the reaction was allowed to stir for an additional 1 h and then concentrated in vacuo. HCl (1 N, 50 mL) and ether (100 mL) were added, and the solution was stirred for 1 h, during which time a fine precipitate formed. Filtration and air-drying gave 9.9 g (90%) of *N*-demethylspinosyn A tetrafluoroborate as an off-white powder. To a solution of this (6.4 g, 8 mmol) in dry acetonitrile (100 mL) was added pyridine (1.4 g, 17.6 mmol). The solution was cooled to 5 °C and F-TEDA (3.75 g, 10.6 mmol) added in portions over 40 min. After addition was complete, the solution was allowed to stir for an additional 2 h whereupon the solvent was removed in vacuo and the residual oil partitioned between ether (70 mL) and water (50 mL). The layers were separated, and the organic layer was washed with 0.1 N HCl (2 × 50 mL) and with saturated $NaHCO_3$ solution (50 mL). Drying and concentration gave an oil which was dissolved in MeOH (50 mL) and water (10 mL). The solution was stirred and treated with Dowex resin (H^+ form, 5 g) at ambient temperature for 4 h and the solution filtered and concentrated. Silica gel chromatography (50:50 ethyl acetate/hexane) yielded **5** (4 g, 63% total yield from **1**) as a white powder: ^{1}H NMR ($CDCl_3$) δ 6.73 (br s, 1H), 4.94 (dd, 1H, J = 8.0, 3.0 Hz), 3.98 (q, 1H, J = 6.8 Hz), 3.68 (m, 1H), 3.50 (s, 3H), 3.44 (s, 6H), 1.26 (d, 3H, J = 6.8 Hz), 1.23 (d, 3H, J = 6.6 Hz), 1.15 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 208.8, 202.8, 172.6, 147.7, 144.1, 129.4, 128.8, 101.0, 95.5, 82.2, 81.1, 81.0, 77.6, 76.7, 76.1, 75.8, 67.8, 60.9, 58.9, 57.6, 49.4, 47.5, 47.3, 46.0, 41.3, 41.0, 37.3, 36.2, 34.8, 34.2, 34.1, 30.1, 28.2, 21.1, 17.6, 16.3, 15.4, 9.2.

(4*S*)-4″-Desdimethylamino-4″-hydroxyspinosyn A (6). Lithium tri-*tert*-butoxyaluminohydride (610 mg, 2.33 mmol) was added in one portion to a cold (0 °C), well-stirred, N_2-blanketed solution of **5** (415 mg, 0.59 mmol) in dry Et_2O (20 mL) and dry dioxane (4 mL). After 10 min, brine (10 mL) was slowly added, followed by Et_2O (100 mL), and this mixture was treated with 2 N aq NaOH (100 mL). The phases were separated, and the ethereal layer was washed with 10% $KHCO_3$ (2 × 25 mL), dried (K_2CO_3), and concentrated leaving pure **6** as a white powder which was dried in vacuo at room temperature (414 mg, 99%): ^{1}H NMR ($CDCl_3$) δ 6.72 (br s, 1H), 4.41 (d, 1H, J = 8.6 Hz), 3.59 (m, 1H), 3.49 (s, 3H), 3.43 (s, 6H), 1.22 (d, 6H, J = 6.5 Hz), 1.12 (d, 3H, J = 6.6 Hz), 0.77 (t, 3H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 203.0, 172.6, 147.7, 144.2, 129.3, 128.8, 103.1, 95.3, 82.2, 81.0, 80.7, 76.6, 75.9, 75.8, 71.1, 67.8, 60.8, 58.9, 57.6, 49.3, 47.5, 47.4, 45.8, 41.4, 41.0, 37.2, 36.1, 34.1, 33.9, 31.3, 31.0, 30.5, 29.9, 28.2, 21.4, 17.9, 17.6, 16.0, 9.2.

(4*S*)-4″-Desdimethylamino-4″-methanesulfonyloxyspinosyn A (6a). Methanesulfonyl chloride (1.80 mL) was added dropwise during 10 min to a cold (0 °C), well-stirred, N_2-blanketed solution of **6** (414 mg, 0.59 mmol) in dry CH_2Cl_2 (10 mL) containing pyridine (4 mL) and DIPEA (2 mL). This mixture was stirred at 0 °C for 1 h, then Et_2O (100 mL) and brine (10 mL) were added, and this mixture was stirred at room temperature for 15 min. After dilution with H_2O (100 mL), the phases were separated and the organic layer was washed with 10% $KHCO_3$ (4 × 25 mL), dried (K_2CO_3), and concentrated. The residue was purified by flash silica gel chromatography with 15% Et_2O in CH_2Cl_2 affording **6a** as a white powder (418 mg, 91%): ^{1}H NMR ($CDCl_3$) δ 6.73 (br s, 1H), 4.48 (d, 1H, J = 9.0 Hz), 4.20 (ddd, 1H, J = 10.7, 9.6, 5.0 Hz), 3.51 (s, 3H), 3.45 (s, 6H), 2.98 (s, 3H), 1.25 (d, 3H, J = 6.0 Hz), 1.23 (d, 3H, J = 6.0 Hz), 1.13 (d, 3H, J = 6.8 Hz), 0.77 (t, 3H, J = 7.4 Hz); ^{13}C NMR ($CDCl_3$) δ 202.8, 172.6, 147.7, 144.1, 129.4, 128.8, 102.7, 95.4, 82.2, 81.0, 80.9, 79.9, 77.6, 76.5, 76.0, 72.7, 67.8, 65.7, 60.8, 58.9, 57.6, 49.3, 47.5, 47.4, 45.9, 41.4, 41.0, 38.5, 37.2, 36.1, 34.1, 30.1, 29.9, 29.0, 28.2, 21.3, 17.9, 17.6, 16.0, 9.2.

(4*R*)-Desdimethylamino-4″-azidospinosyn A (7). Sodium azide (3.10 g) was added to a solution of **6a** (171 mg, 0.22 mmol) in dry DMF and the resulting mixture heated, with stirring under N_2, to 120 °C and kept there for 1 h. The mixture was then cooled to room temperature, and Et_2O (100 mL) and H_2O (80 mL) were added. The phases were separated, and the organic layer was washed with 10% $KHCO_3$ solution, dried (K_2CO_3), and concentrated at reduced pressure. The residue was purified by reversed-phase HPLC over a C-18 column with 8% H_2O in MeOH as eluent to give **7** as a white powder (62 mg, 39%): ^{1}H NMR ($CDCl_3$) δ 6.73 (br s, 1H), 4.43 (dd, J = 8.0, 3.4 Hz), 3.60 (m, 2H), 3.51 (s, 3H), 3.45 (s, 3H), 3.44 (s, 3H), 1.23 (d, 3H, J = 6.1 Hz), 1.20 (d, 3H, J = 6.3 Hz), 1.13 (d, 3H, J = 6.8 Hz), 0.76 (t, 3H, J = 7.6 Hz); ^{13}C NMR ($CDCl_3$) δ 203.0, 172.6, 147.7, 144.2, 129.3, 128.8, 103.4, 95.4, 82.2, 81.0, 80.5, 77.6, 76.7, 76.0, 72.6, 67.8, 60.8, 58.9, 58.7, 57.6, 49.3, 47.5, 47.4, 45.9, 41.4, 41.0, 37.2, 36.1, 34.1, 34.0, 29.8, 28.3, 27.2, 25.5, 21.5, 17.8, 17.6, 15.9, 9.1; MS (CI) *m/z* 747 (M + NH_4^+).

4″-Epispinosyn A (4). To a solution of **7** (51 mg, 0.07 mmol) in MeOH (4.0 mL) was added excess $SnCl_2 \cdot 2\ H_2O$ (140 mg) in one portion. This mixture was stirred at room temperature under N_2 for 20 h, and then the MeOH was removed at reduced pressure. The residue was dissolved in 1:1 EtOAc−Et_2O (100 mL) and this solution washed with aq NH_4OH (20 mL) and dried (K_2CO_3). Concentration left a colorless glassy solid (38 mg) which by NMR analysis was a mixture of amine **8** (60%)

and unreacted **7** (40%). This mixture was carried on without separation. Some ^{13}C NMR ($CDCl_3$) signals ascribable to **8**: δ 203.1, 172.8, 147.4, 144.5, 129.4, 128.8, 103.8, 48.1, 35.0, 30.7, 25.0, 15.7, 9.2.

The mixture of **7** and **8** (38 mg) was dissolved in MeCN (5 mL), excess iodomethane (3.5 mL) added, and the solution heated at reflux, with stirring under N_2 for 2 h. The solvent and excess iodomethane were removed, and the residue was dissolved in *m*-xylene (10 mL). This solution was heated at reflux for 1 h with periodic removal of a small amount (~0.5−1 mL) of the condensate through distillation (5 times during the 1 h). After cooling, the solvent was removed at reduced pressure and the residue purified by flash silica gel chromatography giving **4** (7.0 mg, 14% from **7**) as a colorless glass: ^1H NMR ($CDCl_3$) δ 6.75 (br s, 1H), 5.84 (br d, 1H, J = 9.8 Hz), 5.78 (ddd, 1H, J = 9.8, 2.5, 2.5 Hz), 4.82 (d, 1H, J = 1.4 Hz), 4.65 (m, 1H), 4.56 (br d, 1H, J = 10.0 Hz), 4.28 (m, 1H), 3.81 (m, 1H), 3.62 (m, 1H), 3.51 (s, 3H), 3.45 (s, 6H), 2.60 (s, 6H), 1.43 (d, 3H, J = 6.4 Hz), 1.23 (d, 3H, J = 6.2 Hz), 1.13 (d, 3H, J = 6.8 Hz), 0.80 (t, 3H, J = 7.5 Hz); ^{13}C NMR ($CDCl_3$) δ 202.6, 172.5, 147.7, 144.2, 129.3, 128.7, 102.9, 95.4, 82.2, 81.0, 80.4, 77.7, 76.8, 76.0, 72.8, 67.9, 61.0, 59.5, 59.0, 57.7, 49.4, 47.7, 47.6, 45.9, 43.7, 41.5, 41.2, 37.4, 36.3, 34.1, 29.9, 28.4, 27.9, 21.8, 20.5, 19.2, 17.8, 15.9, 9.3; MS (ESI) *m/z* 732 (M + H).

Spinosyn A 17-Pseudoaglycone (9). To a mechanically stirred suspension of spinosyn A (**1**, 21.9 g, 0.03 mol) in water (450 mL) was added 1 N aq H_2SO_4 (60 mL) in one portion. The resulting solution was heated under N_2 to 80−90 °C and kept at that temperature range for 24 h. During this time, **9** separated and the resulting suspension became quite thick. The mixture was then cooled to rt and filtered and the collected **9** washed with water (3 × 50 mL). The solid product was then dissolved in CH_2Cl_2 (500 mL) and the solution washed with brine (100 mL), dried (K_2CO_3), and evaporated at reduced pressure. The crude product was purified by flash silica gel chromatography with 30% hexane in EtOAc to give **9** (15.9 g, 90%) as a white powder: ^1H NMR ($CDCl_3$) δ 6.78 (br s, 1H), 5.89 (br d, 1H, J = 9.8 Hz), 5.80 (ddd, 1H, J = 9.8, 2.5, 2.5 Hz), 4.86 (d, 1H, J = 1.4 Hz), 4.70 (m,1), 4.32 (m, 1), 3.70 (m, 1H), 3.55 (s, 3H), 3.40 (s, 6H), 1.27 (d,3H, J = 6.2 Hz), 1.21 (d, 3H, J = 6.8 Hz), 0.92 (m, 1H), 0.82 (t, 3H, J = 7.5 Hz); MS (EI) *m/z* 591 (M + H).

Methyl 4-Amino-2,3,4,6-tetradeoxy-L-*threo*-hexopyranoside (13). A solution of the 4-azide **12**[9] (2.0 g, 11.8 mmol) as an anomeric mixture, in absolute EtOH (50 mL), was hydrogenated over Pearlman's catalyst [20% $Pd(OH)_2$/C, 1.0 g] at 45 psi for 5 h. The catalyst was filtered (Celite) and washed with EtOH (25 mL). The combined filtrate and wash was concentrated at reduced pressure and ambient temperature leaving the crude amine **13** (1.5 g) as a colorless, gelatinous oil which was used immediately and without further purification. α-Anomer: ^{1}HNMR ($CDCl_3$) δ 4.68 (d,1H, J = 1.9 Hz), 3.98 (dq, 1H, J = 6.5, 1.6 Hz), 3.36 (s, 3H), 2.74 (br s, 1H), 2.1−1.5 (m, 6H), 1.17 (d, 3H, J = 6.5 Hz); MS *m/z* 145 (M +, 5), 114 (M − OCH_3, 35), 89 (100).

Methyl L-Ossaminide (14). To a stirred solution of the above amine **13** (1.5 g, 0.01 mol) in reagent grade MeOH (35 mL) was added 37% aq CH_2O solution (10.2 mL), and the resulting solution was heated at reflux for 1 h. The mixture was then cooled to 0−5 °C, and sodium $NaBH_4$ (1.37 g, 0.036 mol) was added in portions during 5 min. The cooling bath was removed after 20 min, and the reaction was allowed to stir at rt for 18 h. The solvent was then removed at reduced pressure and the residue extracted with CH_2Cl_2 (3 × 30 mL portions, each time decanting the extract from the tarry insolubles). The combined extracts were then washed with brine (5 mL) and dried ($MgSO_4$). Concentration left 1.3 g of product which was purified by flash chromatography over silica (75 mL) using 10% MeOH in CH_2Cl_2 as eluent to give 0.65 g (38%) methyl l-ossaminide (**14**) as a colorless oil as an ~5:1 α/β anomeric mixture. α-Anomer:^{1}H NMR ($CDCl_3$) δ 4.66 (dd, 1H, J = 6.1, 3.1 Hz), 4.25 (dq, 1H, J = 6.5, 4.6 Hz), 3.40 (s, 3H), 2.35 (m, 1H), 2.30 (s, 6H), 2.30 (m, 1H), 1.93−1.82 (m, 2H),1.75 (m, 1H), 1.55 (m, 1H), 1.28 (d, 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) δ 97.5, 69.2, 61.4, 55.2, 43.5, 28.9, 19.0, 15.0; MS *m/z* 173 (M^+, 10), 142 (10), 84 (20), 71 (100).

Methyl *N*-Demethyl-*N*-trichloroethoxycarbonyl-L-ossaminide (15) and 4-(*N*-Methyl-*N*-trichloroethoxycarbonylamino)-1,2,3,4,6-pentadeoxy-L-*threo*-hexenopyranose (16). A solution of methyl ossaminide (**14**) (1.6 g, 9.24 mmol) in toluene (60 mL) was heated at reflux in a round-bottom flask equipped with a Dean−Stark water separator for 20 min to remove any water. The solution was then cooled to room temperature, and trichloroethylchloroformate (4.0 mL) and powdered, anhydrous K_2CO_3 (1.6 g, 11.5 mmol) were added. The resulting mixture was heated with stirring at reflux for 20 h. The mixture was then cooled to rt and filtered, and the collected salts were washed with EtOAc (25 mL). The combined filtrate and wash was washed with satd Na_2CO_3 solution (2 × 50 mL) and brine (25 mL) and dried ($MgSO_4$). Concentration left 5.0 g of crude product containing excess chloroformate which was flash chromatographed over silica (200 mL) with 15% EtOAc in hexane as eluent. After a forerun of 150 mL, 20 mL fractions were collected. Pure dihydropyran **16** (1.0 g) as a colorless oil was obtained from fractions 6−8. Pure ossaminide **15** (0.6 g), also a colorless oil, was obtained from fractions 9−12. Data for **16**: ^1H NMR ($CDCl_3$, 600 MHz) δ 6.45 (br s, 1H), 4.87 (m, 2H), 4.72 (m, 1H), 4.54 and 4.48 (br d, total 1H, J = 8.6 Hz), 4.10 (m, 1H), 3.10 and 3.05 (s, total 3H), 2.65 and 2.05 (m, total 2H), 1.30 and 1.25 (d, total 3H, J = 6.5 Hz); ^{13}C NMR ($CDCl_3$) 155.9, 145.2, 101.2, 75.7, 73.9, 51.5, 31.3, 30.7, 24.9, 17.4. Data for **15** (major α-anomer): ^1H NMR ($CDCl_3$, 600 MHz) δ 4.75 (m, 3H), 4.15 (m, 1H), 4.15 (s, 3H), 3.20 (m,1H), 3.00 and 3.08(s, total 3H), 2.4−1.7 (m, 4H), 1.30 (d, 3H, J = 6.5 Hz).

***N*-Demethyl-*N*-trichloroethoxycarbonyl-L-ossaminyl Spinosyn A 17-Pseudoaglycone (17).** Crushed 5 Å molecular sieves were added to a solution of dihydropyran **16** (0.2 g, 0.66 mmol) and spinosyn A 17-pseudoaglycone (**9**, 0.39 g, 0.66 mmol) in reagent grade 1,2-dichloroethane (10 mL). To this stirred mixture was added PPTS (33 mg, ~20 mol %) at rt. After 1 h, the mixture was heated to reflux. After 4 h, the mixture was cooled, and $BF_3{\cdot}Et_2O$ (25 μL, ~0.19 mmol) was added. The mixture was again heated to reflux, kept at reflux for 2 h, and then recooled to room temperature, 25 μL of additional $BF_3{\cdot}Et_2O$ was added, and the mixture was allowed stir at room temperature. After 18 h, 25 μL of additional $BF_3{\cdot}Et_2O$ was added and the mixture allowed stir an additional 24 h. The mixture was then diluted with CH_2Cl_2 (20 mL) and filtered (Celite). The collected solids were washed with more CH_2Cl_2 (10 mL), and the combined filtrate and wash was washed with satd $NaHCO_3$ (2 × 10 mL) and then brine (10 mL) and dried ($MgSO_4$). Concentration left 0.5 g of crude product which was purified by flash silica chromatography (50 mL silica) using 4.5% MeOH in CH_2Cl_2 to give 0.21 g (36%) of coupled product as a thick viscous oil as a 2:1 β/α anomeric mixture (this ratio determined after removal of the protecting TROC group, below). **17**: ^1H NMR ($CDCl_3$) δ 6.78 (br s, 1H), 5.88 (m, 1H), 5.78 (m, 1H), 4.90−4.62 (m, 6H), 4.30 (m, 1H), 3.56 (s, 3H), 3.50 (3, 6H), 1.30 (d, 3H, J = 6.2 Hz), 1.18 and 1.12 (d, total 3H, J = 6.8 Hz).

***N*-Demethyl-L-ossaminyl Spinosyn A 17-Pseudoaglycone (18).** To a well-stirred solution of the mixture of coupled products **17** (0.2 g, 0.22 mmol) in THF (6.0 mL) was added 1 M aq NH_4OAc solution (1.2 mL), followed by more THF (3.0 mL) and MeOH (1.5 mL). To this homogeneous solution was added freshly activated[10] zinc dust (1.0 g) and the resulting mixture stirred for 3.5 h at ambient temperature. The mixture was then filtered (Celite), the collected solids were washed with 1:1 THF−MeOH (25 mL), and the combined filtrate and wash was concentrated to ~15% volume at reduced pressure and

(10) Fieser, L. F.; Fieser, M. *Reagents for Organic Synthesis*; John Wiley and Sons: New York, 1968; Vol. 1, p 1276.

25−30 °C. The residue was basified with satd $NaHCO_3$ to ~pH 9 and then extracted with CH_2Cl_2 (3 × 15 mL). The combined organic extracts were then washed with brine (10 mL) and dried ($MgSO_4$). Concentration left 0.12 g of crude deprotected product which was purified by silica chromatography with 5% MeOH in CH_2Cl_2 as eluent to give 66 mg (42%) of **18** as a colorless foam as a 2:1 β/α anomeric mixture: ^{1}H NMR ($CDCl_3$, 600 MHz) δ 6.78 (s, 1H, H-13), 4.85 (m, H-1, H1″), 4.68 (m, 1H, H-21), 4.33 (m, 1H, H-9), 4.15 (m, H-5β), 3.92 (m, H-17α), 3.70 (m, H-17β), 3.74 (m, H-5α), 2.45 and 2.42 (s, NCH_3).

Spinosyn G (3) and β-L-Ossaminyl Spinosyn A 17 Pseudoaglycone (19). To a solution of the mixture of anomeric products **18** (60 mg, 0.084 mmol) in anhydrous THF (2 mL) were added MeI (10.4 μL, ~0.168 mmol) and DIPEA (44 μL, ~0.25 mmol), and this solution was stirred at room temperature for 20 h and then diluted with Et_2O (15 mL), washed with brine (5 mL), and dried ($MgSO_4$). Concentration left 60 mg of crude methylated product which was purified by radial chromatography over one 2.0 mm silica chromatotron plate using 9% MeOH in CH_2Cl_2 and 30% MeOH in CH_2Cl_2 as eluent, with the 30% eluent started after the first (major) product band (spinosyn G) had come off. This afforded spinosyn G (α-L-ossaminyl spinosyn A 17 pseudoaglycone (**3**), 20 mg, 33%) as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 6.79 (br s, 1H, 13), 5.89 (br d, 1H, 6), 5.80 (ddd, 1H, 5), 4.87 (d, 1H, 1′), 4.78 (dd, 1H, 1″), 4.69 (m, 1H, 21), 4.32 (m, 1H, 9), 4.29 (dq, 1H, 5″), 3.64 (ddd, 1H, 17), 3.58 (s, 3H, 4′-OMe), 3.56 (dq, 1H, 5′), 3.51 (s, 3′-OMe) 3.50 (s, 3H, 2′-OMe), 3.30 (dq, 1H16), 3.13 (dd, 1H, 4′), 3.11 (dd, 1H, 2), 3.03 (m, 1H, 3), 2.89 (m, 1H, 12), 2.42 (dd, 1H, 2), 2.35 (m, 1H, 4″), 2.29 (s, 6H, NMe_2), 2.27 (m, 1H, 10), 2.18 (m, 1H, 7), 1.30 (d, 3H6″), 1.27 (d, 3H, 6″), 1.20 (d, 3H, 24), 0.91 (dddd, 1H, 11), 0.83 (t, 3H, 23). ^{13}C NMR ($CDCl_3$) δ 203.2, 172.9, 147.7, 144.4, 129.7, 129.4, 99.0, 95.2, 82.7, 81.9, 81.5, 78.2, 77.2, 76.7, 76.6, 70.3, 68.4, 62.4, 61.3, 59.4, 58.1, 49.9, 48.0, 47.7, 46.7, 43.8, 41.9, 41.6, 37.8, 36.7, 34.9, 34.8, 31.0, 29.8, 28.6, 21.3, 20.2, 18.2, 17.4, 14.7, 9.8. Also isolated was the β-L-ossaminyl isomer **19** (6 mg, 10%) also as a colorless foam: ^{1}H NMR ($CDCl_3$) δ 6.81 (br s, 1H, 13), 5.89 (br d, 1H, 6), 5.80 (ddd, 1H, 5), 4.86 (d, 1H, 1′), 4.68 (m, 1H, 21), 4.56 (dd, 1H, 1″), 4.33 (ddd, 1H, 9), 3.95 (ddd, 1H, 17), 3.78 (dq, 1H, 5″), 3.58 (s, 3H, 4′-OMe), 3.52 (s, 3H, 2′-OMe), 3.51 (s, 3H, 3′-OMe), 3.30 (dq, 1H, 16), 3.13 (t, 1H, 4′), 3.11 (dd, 1H, 2), 3.07 (m, 1H, 3), 2.90 (dddd, 1H, 12), 2.47 (dd, 1H, 2), 2.43 (s, 6H, NMe_2), 2.32 (m, 1H, 4″), 2.26 (m, 1H, 10), 2.21 (m, 1H, 2″), 2.16 (m, 1H, 7), 1.94 dd, 1H, 8), 1.35 (d, 3H, 6″), 1.31 (d, 3H, 6′), 1.29 (s, 3H, 24), 0.91 (dddd,1H, 11), 0.84 (t, 3H, 23); ^{13}C NMR ($CDCl_3$) δ 203.7, 172.9, 147.6, 144.2, 129.7, 129.5, 99.2, 95.9, 82.7, 81.5, 78.2, 77.9, 77.2, 76.6, 74.6, 68.4, 61.3, 59.6, 59.4, 58.1, 50.0, 48.1, 46.9, 46.7, 44.1, 41.8, 41.5, 37.8, 36.8, 35.0, 32.4, 31.2, 30.2, 28.4, 20.2, 20.1, 18.3, 18.2, 18.1, 9.8.

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Note Added after ASAP Publication. The reaction conditions for Scheme 3 were mistakenly included in Scheme 2 and those for Scheme 4 included with Scheme 3 in the version published ASAP February 11, 2005; the corrected version was published February 15, 2005.

Supporting Information Available: NMR spectra for **1**, **3**, **4**, and **19**. This material is available free of charge via the Internet at http://pubs.acs.org.

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