VOL. 53 NO. 2, FEB. 2000 THE JOURNAL OF ANTIBIOTICS pp. 171 — 178

Synthesis and Insecticidal Activity of Spinosyn Analogs Functionally Altered at the 2′-, 3′- and 4′-Positions of the Rhamnose Moiety

LAWRENCE C. CREEMER*,[a], HERBERT A. KIRST[a], JONATHAN W. PASCHAL[b] and THOMAS V. WORDEN[c]

[a] Elanco Animal Health, Research and Development,
P. O. Box 708, 2001 West Main Street, Greenfield, Indiana 46140
[b] Lilly Research Laboratories, Lilly Corporate Center,
Indianapolis, Indiana 46285
[c] Dow AgroSciences Discovery and Field Research,
Indianapolis, Indiana 46268

(Received for publication October 25, 1999)

In an effort to increase the insecticidal activity of the spinosyn family of naturally occurring macrolides, the 2′-, 3′- and 4′-*O*-desmethyl-*O*-acetyl analogs and the 2′-, 3′-, and 4′-*O*-desmethoxy analogs have been synthesized. These analogs were prepared synthetically from the minor spinosyn factors H, J, K, L and Q either *via* direct acylation of the corresponding factor or deoxygenation of an intermediate xanthate. The acylated analogs were all more potent insecticides against *Heliothis virescens* larvae than their respective parent factors, but not as potent as spinosyns A or D. The deoxy analogs were also more potent insecticides than their respective parent factors. The 2′-desmethoxy analogs showed, for the first time, analogs with insecticidal potency against *H. virescens* greater than that of spinosyns A and D, indicating that polarity is not well tolerated in the rhamnose moiety of spinosyn A.

The spinosyns are a family of macrolide natural products produced by the soil microorganism *Saccharopolyspora spinosa*.[1] Several of the spinosyn factors have shown extremely potent levels of insecticidal activity against *Lepidoptera* species.[2] The new commercial insecticide Tracer™ (Dow AgroSciences) is composed of a mixture of spinosyns A (**1**) and D (**2**) and is useful against many crop pests such as tobacco budworm.[3] During strain selection studies of *S. spinosa*, designed to improve the fermentation yield of spinosyns A and D, several strains were also found which gave increased yields of minor family members.[4~7] These mutant strains allowed these factors to be produced in synthetically useful quantities.

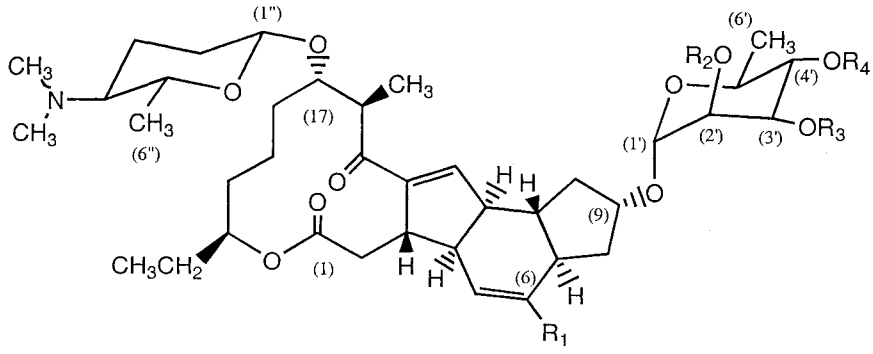
In an effort to increase biological potency, broaden the insecticidal spectrum and extend the duration of activity over that of spinosyn A, semi-synthetic studies were undertaken using minor factors such as spinosyns H (**3**), J (**4**), K (**5**), L (**6**), O (**7**) and Q (**8**) as starting points for modifications.[2] The free hydroxyl substituent at the 2′-, 3′-, or 4′-position of these factors provided a useful handle for synthetic manipulations of the rhamnose moiety, a critical feature not present in the rhamnose moiety of spinosyns A and D.

Results

The 2′-, 3′-, and 4′-keto derivatives have proved to be useful and versatile synthetic intermediates. As previously reported, spinosyn J (**4**) and spinosyn L (**6**) were oxidized, using *N*-chlorosuccinimide, a dialkylsulfide and triethylamine, to their corresponding 3′-keto analogs.[8] Subsequent β-elimination of the resulting 3′-keto-sugar yielded the 9-pseudoaglycones of spinosyn A and spinosyn D respectively. The 9-pseudoaglycone of spinosyn D was further hydrolyzed under mild acidic conditions that permitted formation of the strong-acid-sensitive aglycone of spinosyn D.[8]

Spinosyns H (**3**) and K (**5**) were each oxidized under similar conditions to yield the corresponding 2′-keto analog (**9**) and the 4′-keto analog (**10**), respectively. When **9** was treated with anhydrous K_2CO_3 in methanol, β-elimination of the 4′-methoxy group occurred giving the α,β-unsaturated-keto-rhamnosyl analog (**11**). **10** was reductively

Fig. 1. The structure of spinosyns A and D as well as spinosyn minor factors H, J, K, L, O, and Q, altered at the rhamnose sugar.



		R1	R2	R3	R4
1	**Spinosyn A**	H	CH_3	CH_3	CH_3
2	**Spinosyn D**	CH_3	CH_3	CH_3	CH_3
3	**Spinosyn H**	H	H	CH_3	CH_3
4	**Spinosyn J**	H	CH_3	H	CH_3
5	**Spinosyn K**	H	CH_3	CH_3	H
6	**Spinosyn L**	CH_3	CH_3	H	CH_3
7	**Spinosyn O**	CH_3	CH_3	CH_3	H
8	**Spinosyn Q**	CH_3	H	CH_3	CH_3

aminated with ammonium acetate and sodium cyanoborohydride in methanol and yielded the 4′-axial amino analog (**12**).

Replacement of the 2′-, 3′- and 4′-methoxy groups found in spinosyn A (**1**) with hydroxyl groups, as found in spinosyns H (**3**), J (**4**), K (**5**), led to a substantial loss of insecticidal activity which was especially acute for the 3′-*O*-demethylation.[3] Therefore, a limited SAR was conducted around the 2′-, 3′-, and 4′-substituents of both spinosyn A (**1**) and spinosyn D (**2**) to explore further the relationship of polarity to insecticidal activity.

Spinosyns H (**3**), J (**4**), and K (**5**) were individually dissolved in anhydrous pyridine and treated with acetic anhydride at room temperature, giving rise to the corresponding 2′-, 3′-, and 4′-*O*-acetyl analogs **13**, **14**, and **15** respectively. These analogs showed increased insecticidal activity against neonate *Heliothis virescens* (tobacco budworm) when compared to their respective unacylated parent molecules. This increased activity over the free hydroxyl analogs strongly suggests that polar groups are not well tolerated in the rhamnose portion of spinosyn A (**1**). Although these new analogs showed significant increases in insecticidal activity over their parent molecules, they were all less active than **1**. To see if this loss of activity was due to steric effects in the larger acetyl group, the deoxy analogs **19**, **20**, and **21** were subsequently synthesized.

Spinosyns H (**3**), J (**4**), and K (**5**) were each individually dissolved in anhydrous THF with a catalytic amount of imidazole and deprotonated with sodium hydride. The xanthates **16**, **17**, and **18** were formed by condensation of the resulting alkoxide with carbon disulfide, followed by methylation with methyl iodide. The corresponding xanthates, which showed some instability, were then each treated in refluxing toluene with tributyltin hydride and catalytic AIBN, giving the desired deoxy analogs. The insecticidal activity of **19**, **20**, and **21** was much greater than **3**, **4**, and **5**, respectively. Furthermore, the activity of 3′-deoxy spinosyn J (**20**) was approximately the same as spinosyn A (**1**), and the activity of 2′-deoxy spinosyn H (**19**) was slightly greater than that of spinosyn A.

Since the 6-methyl analogs spinosyn L (**6**), spinosyn O (**7**), and spinosyn Q (**8**) were slightly more potent than spinosyns J (**4**), K (**5**), and H (**3**),[2] it was proposed that deoxy derivatives of **6** and **8** in the spinosyn D series might have even greater insecticidal activity than the 2′-deoxy

Fig. 2. Oxidation of spinosyns H and K and subsequent formation of the 2′-keto-3′,4′-unsaturated derivative of spinosyn A and the 4′-desmethoxy-4′-β-amino-spinosyn A analog.

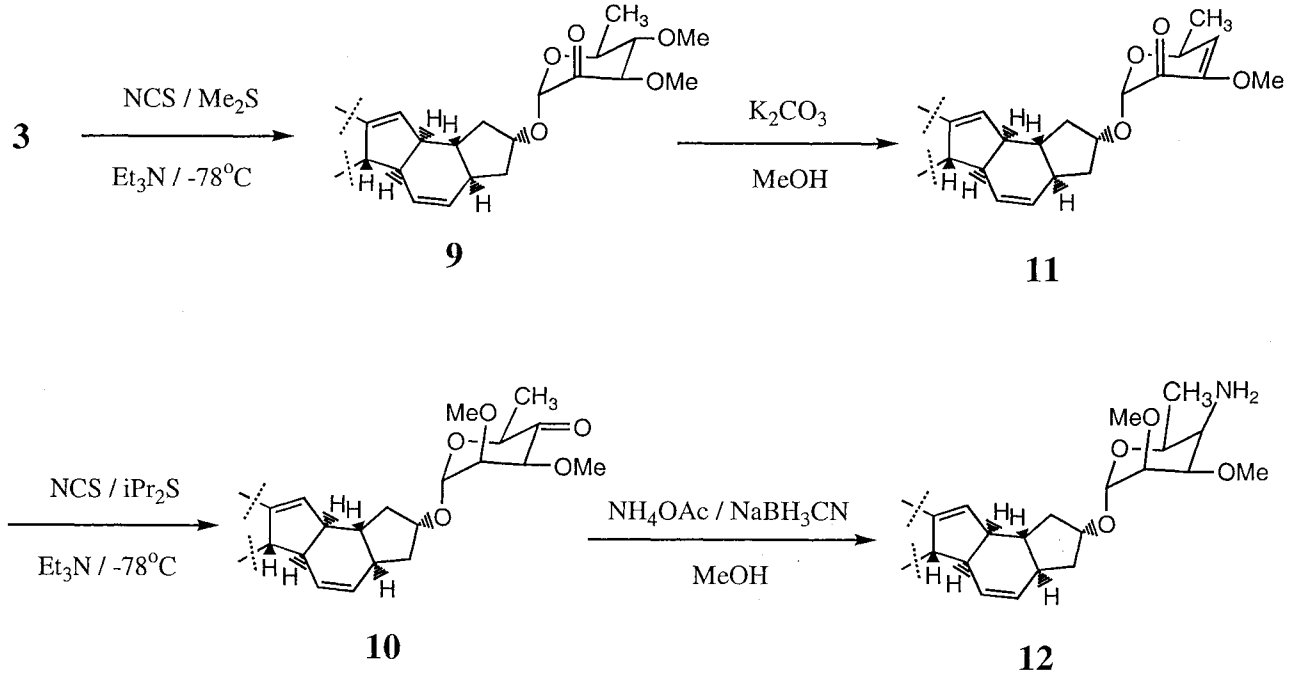
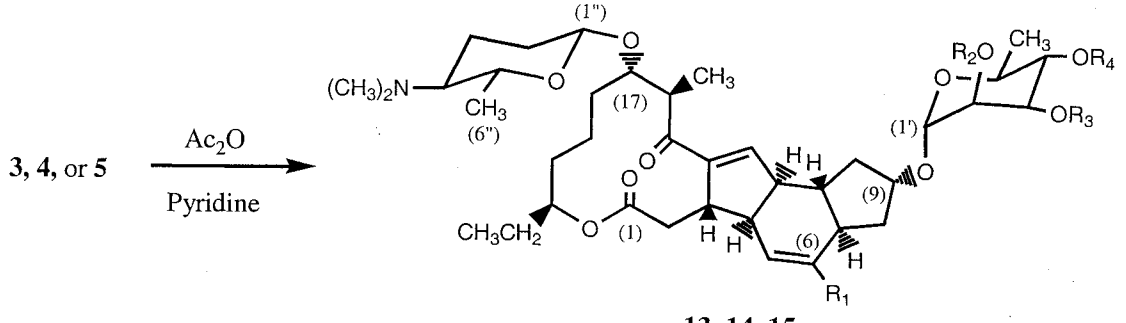


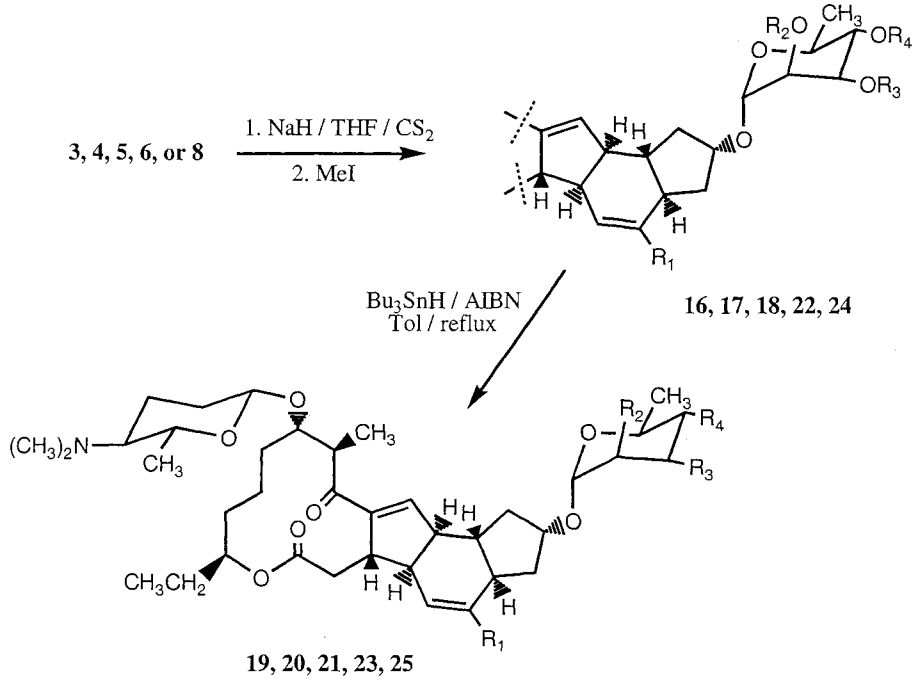
Fig. 3. Acylation of spinosyns H, J, and K.



	R1	R2	R3	R4
13	H	$COCH_3$	CH_3	CH_3
14	H	CH_3	$COCH_3$	CH_3
15	H	CH_3	CH_3	$COCH_3$

derivative (**19**) and 3′-deoxy derivative (**20**) as previously outlined in the spinosyn A series. Therefore, 2′-deoxy spinosyn Q (**23**) and 3′-deoxy spinosyn L (**25**) were synthesized *via* the intermediate xanthates **22** and **24**. However, the insecticidal activity of both **23** and **25** were found to be of similar potency as 2′-deoxy spinosyn H (**19**). While 2′-deoxy spinosyn Q (**23**) and 3′-deoxy spinosyn L (**25**) had a significant increase in insecticidal activity over spinosyn D (**2**), they were of similar activity to 2′-deoxy spinosyn H (**19**) and of 3′-deoxy spinosyn J (**20**) and

Fig. 4. Xanthate formation and deoxygenation of spinosyns H, J, K, L, and Q.



	R1	R2	R3	R4
16	H	CS_2CH_3	CH_3	CH_3
17	H	CH_3	CS_2CH_3	CH_3
18	H	CH_3	CH_3	CS_2CH_3
19	H	H	OCH_3	OCH_3
20	H	OCH_3	H	OCH_3
21	H	OCH_3	OCH_3	H
22	CH_3	CS_2CH_3	CH_3	CH_3
23	CH_3	H	OCH_3	OCH_3
24	CH_3	CH_3	CS_2CH_3	CH_3
25	CH_3	OCH_3	H	OCH_3

therefore, only slightly more potent than spinosyn A (**1**).

The small increase in potency of analogs **19**, **23** and **25** over spinosyn A (**1**) represent the first synthetic modifications of the spinosyn structure which have led to improvements in the insecticidal activity against tobacco budworm. These results suggested that other modifications of the rhamnose moiety may lead to analogs having useful increases in bio-activity over the naturally occurring spinosyn factors.

Experimental

General Methods

NMR spectra were determined on either a Bruker AMX-500 NMR spectrometer or a General Electric QE300 NMR spectrometer. Mass spectra were obtained on a Finnigan MAT 731 mass spectrometer interfaced to a Finnigan MAT SS-200 data system. Infrared spectra were determined on a Nicolet 510P spectrometer. UV spectra were measured on a Shimadzu UV-2101 PC Spectrophotometer. The preparation of spinosyns H, J, K, L, O, and Q were carried out in the Lilly fermentation facilities at Indianapolis, IN.,

USA. All other reagents were obtained from Aldrich Chemical Co., Milwaukee, WI., USA.

Table 1. The insecticidal activity of spinosyn natural factors and analogs *vs.* neonate *Heliothis virescens* larvae (tobacco budworm) as determine by drench application.[a]

Compound	*H. virescens* activity LD_{50} (ppm)
1	0.31
2	0.8
3	3.2
4	>64
5	3.5
6	26
7	1.4
8	0.39
11	35
12	>64
13	1.2
14	33
15	1.3
19	0.23
20	0.36
21	4.1
23	0.23
25	0.27

[a] The *H. virescens* drench test was performed as described in reference 3.

Synthesis of 2′-keto Derivative of Spinosyn A (**9**)

N-Chlorosuccinimide (1.16 g, 8.7 mmol) was suspended in 32 ml of dichloromethane and cooled to −78°C under nitrogen. Dimethyl sulfide (760 μl, 10.3 mmol) was then added, and the mixture was stirred at −78°C. After 0.5 hour, spinosyn H (2.01 g, 2.78 mmol), dissolved in 21 ml of dichloromethane, was added slowly. When the addition was complete, the solution was stirred at −78°C for 4.0 hours. Triethylamine (1.34 ml, 9.6 mmol) was then added, and the solution was warmed to room temperature. The solvent was then evaporated at room temperature under reduced pressure. The residue was semi-purified by silica gel chromatography eluting with 10% methanol in dichloromethane to give **9** as a white solid (2.68 g). This product was used without further purification. FD-MS *m/z*, 716 (MH^+), 715 (M^+); IR ($CHCl_3$) cm^{-1}, 3409, 3024, 2938, 2877, 1780, 1754, 1721, 1658, 1455, 1432, 1345, 1290, 1160, 1071, 1037, 989, 901.

Synthesis of 4′-keto Derivative of Spinosyn A (**10**)

N-Chlorosuccinimide (861.6 mg, 6.45 mmol) was suspended in 20 ml of dichloromethane and cooled to −78°C under nitrogen. Diisopropyl sulfide (1.0 ml, 6.88 mmol) was then added, and the mixture was stirred at −78°C. After 0.5 hour, spinosyn K (1.48 g, 2.07 mmol) dissolved in 9 ml of dichloromethane was added slowly. When the addition was complete, the solution was stirred at −78°C for 2.0 hours. Triethylamine (900 μl, 6.46 mmol) was then added, and the solution was warmed to room

Table 2. The ^1H NMR assignments of the rhamnose associated protons for several new 2′-, 3′-, and 4′-substituted analogs of spinosyn A (**1**) and spinosyn D (**2**) run in $CDCl_3$ (unless otherwise noted), and reported from internal TMS. Assignments have been made by comparison to spinosyns A and D.

Compound	Proton position and chemical shift in ppm from internal TMS											
	1′	2′	3′	4′	5′	6′	2′-OMe	3′-OMe	4′-OMe	2′-OAc	3′-OAc	4′-OAc
11	4.96	—	—	5.75	4.87	1.47	—	3.67	—	—	—	—
12[a]	5.03	3.58	3.57	3.66	4.17	1.37	3.50	3.47	—	—	—	—
13	4.47	5.18	3.2[b]	3.12	3.6[b]	1.2[b]	—	3.58	3.43	2.16	—	—
14	4.82	3.6[b]	5.13	3.26	3.6[b]	1.31	3.48	—	3.51	—	2.17	—
15	4.90	3.5[b]	3.5[b]	5.05	3.7[b]	1.20	3.53	3.46	—	—	—	2.11
19	4.88	NA[c]	3.5[b]	2.77	3.6[b]	1.33	—	3.48	3.60	—	—	—
20	4.73	3.5[b]	NA[c]	3.18	3.7[b]	1.29	3.43	—	3.41	—	—	—
21	4.95	3.5[b]	3.5[b]	NA[c]	3.8[b]	1.23	3.54	3.43	—	—	—	—
23	4.88	NA[c]	3.5[b]	2.80	3.6[b]	1.29	—	3.47	3.60	—	—	—
25	4.72	3.5[b]	NA[c]	3.13	3.7[b]	1.30	3.43	—	3.40	—	—	—

[a] Solvent is acetone-d_6.
[b] Approximate shift due to several coincidental peaks.
[c] Not assigned.

temperature, giving a red color. This solution was diluted with fresh dichloromethane and washed with 0.1 N HCl. The organic layer was then washed with brine, dried ($MgSO_4$) and evaporated at room temperature under reduced pressure. The crude product was semi-purified by silica gel chromatography eluting with 5% methanol in dichloromethane to give **10** as an off white semi-solid (1.48 g). This product was used without further purification. FD-MS *m/z*, 715 (M^+); IR ($CHCl_3$) cm^{-1}, 3022, 2975, 1745, 1714, 1658, 1604, 1458, 1372, 1223, 1161, 1123, 1047.

Synthesis of 2′-keto-3′,4′-Unsaturated Derivative of Spinosyn A (**11**)

To a solution of **9** (104 mg, 0.14 mmol) in methanol (7 ml), anhydrous potassium carbonate (162.9 mg, 1.18 mmol) was added and the mixture stirred at room temperature for 4 hours. The mixture was then diluted with water and extracted with ethyl acetate. The ethyl acetate was washed with brine, dried with magnesium sulfate, and evaporated at room temperature under reduced pressure to give **11** as a colorless glass (61.3 mg, 64% yield). The crude product could be purified by silica gel chromatography eluting with 5% methanol in dichloromethane, and then further purified by reverse phase HPLC eluting with methanol/acetonitrile using a 0.5% ammonium acetate buffer. FD-MS *m/z*, 684 (MH^+), 683 (M^+); UV λ_{max} nm (ε) 249.4 (14,363); IR ($CHCl_3$) cm^{-1}, 3456, 3025, 2975, 1713, 1603, 1234, 1044; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{39}H_{57}NO_9$: C 68.50, H 8.40, N 2.05
Found: C 68.20, H 8.52, N 2.23

Synthesis of 4′-Desmethoxy-4′-β-amino-spinosyn A (**12**)

To a solution of **10** (115.3 mg, 0.16 mmol) in methanol (2 ml), ammonium acetate (149.3 mg, 1.9 mmol) and sodium cyanoborohydride (10 mg, 0.16 mmol) were added and the mixture stirred at room temperature for 7 hours. The mixture was then diluted 1 N HCl and washed with diethyl ether. The aqueous was then basified with 5 N NaOH and, after saturation with NaCl, was extracted with fresh diethyl ether. The ether was dried with $MgSO_4$, and then evaporated at room temperature under reduced pressure. The crude product was purified by silica gel chromatography eluting with 10% methanol in dichloromethane going to 50% methanol in dichoromethane in a step-wise gradient. This gave **12** (23.3 mg, 20% yield) as a colorless glass. FD-MS *m/z*, 717 (MH^+), 716 (M^+); UV λ_{max} nm (ε) 244 (9,647); IR ($CHCl_3$) cm^{-1}, 2973, 1713, 1658, 1373, 1234, 1161, 1064; ^1H NMR (acetone-d_6) see Table 2.

Exact Mass Calcd for $C_{40}H_{65}N_2O_9$: 717.4690
Found: FAB-MS *m/z* 717.4692

Synthesis of 2′-*O*-Desmethyl-2′-*O*-acetyl-spinosyn A (**13**)

The reaction was run as with **14**, starting with spinosyn H (211.9 mg, 0.29 mmol) and giving **13** (175.2 mg, 80% yield) as a colorless glass. FD-MS *m/z*, 759 (M^+); UV λ_{max} nm (ε) 244 (10,835); IR ($CHCl_3$) cm^{-1}, 3021, 2987, 1714, 1658, 1456, 1373, 1240, 1060, 989, 903; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{42}H_{65}NO_{11}$: C 66.38, H 8.62, N 1.84
Found: C 66.39, H 8.73, N 1.89

Synthesis of 3′-*O*-Desmethyl-3′-*O*-acetyl-spinosyn A (**14**)

Spinosyn J (207.9 mg, 0.29 mmol) was dissolved in 2 ml of dry pyridine and acetic anhydride (140 μl, 1.5 mmol) was added to this solution. The mixture was then stirred at room temperature for 24 hours, and evaporated at room temperature under reduced pressure. The residue was purified by silica gel chromatography eluting with 7% methanol in dichloromethane to give **14** (148.8 mg, 68% yield) as a colorless glass. FD-MS *m/z*, 760 (MH^+), 759 (M^+); UV λ_{max} nm (ε) 244 (10,506); IR ($CHCl_3$) cm^{-1}, 2974, 2937, 1714, 1374, 1239, 1211, 1208, 1162, 1123, 1103, 1063, 1040; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{42}H_{65}NO_{11}$: C 66.38, H 8.62, N 1.84
Found: C 66.18, H 8.59, N 1.67

Synthesis of 4′-*O*-Desmethyl-4′-*O*-acetyl-spinosyn A (**15**)

The reaction was run as with **14**, starting with spinosyn K (207.0 mg, 0.29 mmol) and giving **15** (204.9 mg, 93% yield) as a white glass. FD-MS *m/z*, 760 (MH^+), 759 (M^+); UV λ_{max} nm (ε) 244 (10,556); IR ($CHCl_3$) cm^{-1}, 3019, 2937, 1738, 1658, 1604, 1457, 1374, 1237, 1219, 1119, 1038; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{42}H_{65}NO_{11}$: C 66.38, H 8.62, N 1.84
Found: C 66.13, H 8.54, N 1.77

Synthesis of 2′-*O*-Desmethyl-2′-methyl Xanthate of Spinosyn A (**16**)

The reaction was run as with **18**, starting with spinosyn H (204.0 mg, 0.28 mmol) and giving **16** (218.8 mg, 97% yield) as an unstable yellow glass which was used without further purification. FD-MS *m/z*, 810 ($M+3H^+$), 809 ($M+2H^+$), 808 (MH^+); UV λ_{max} nm (ε) 280 (11,698), 230 (12,922); IR ($CHCl_3$) cm^{-1}, 2936, 1713, 1658, 1456, 1372, 1213, 1203, 1073, 989.

Synthesis of 3′-*O*-Desmethyl-3′-methyl Xanthate of Spinosyn A (**17**)

The reaction was run as with **18**, starting with spinosyn J

(207.1 mg, 0.29 mmol) and giving **17** (224.0 mg, 96% yield) as an unstable yellow glass which was used without further purification. FD-MS *m/z*, 810 ($M+3H^+$), 809 ($M+2H^+$), 808 (MH^+); UV λ_{max} nm (ε) 278 (11,404), 234 (12,875); IR ($CHCl_3$) cm^{-1}, 2935, 1713, 1658, 1456, 1372, 1225, 1208, 1164, 1102, 1064, 989.

Synthesis of 4′-*O*-Desmethyl-4′-methyl Xanthate of Spinosyn A (**18**)

Spinosyn K (201.0 mg, 0.28 mmol), along with a catalytic amount of imidazole, was dissolved in 2 ml of anhydrous THF (2 ml), and this solution was stirred at room temperature under nitrogen. Sodium hydride (50% in mineral oil; 25 mg, 0.52 mmol), carbon disulfide (90 μl, 1.5 mmol) and methyl iodide (90 μl, 1.44 mmol) was then added to the solution. When all the reagents were added the mixture was stirred at room temperature for 1 hour then acetic acid (90 μl) was added, and the mixture was poured into saturated $NaHCO_3$. This biphasic mixture was then extracted with dichloromethane. The organic layer was then washed with brine, dried ($MgSO_4$) and evaporated at room temperature under reduced pressure. This gave **18** (218.1 mg, 97% yield) as an unstable yellow glass which was used without further purification. FD-MS *m/z*, 810 ($M+3H^+$), 809 ($M+2H^+$), 808 (MH^+); UV λ_{max} nm (ε) 278 (10,462), 234 (13,587); IR ($CHCl_3$) cm^{-1}, 2935, 1713, 1658, 1457, 1373, 1219, 1164, 1060, 989.

Synthesis of 2′-Desmethoxy Spinosyn A (Deoxy Spinosyn H; **19**)

To a solution of **16** (1.15 g, 1.4 mmol) in anhydrous toluene (50 ml), fresh tributyltin hydride (750 μl, 2.8 mmol) and AIBN (30 mg) were added and the mixture was heated to refluxed for 2.5 hours. After this time, more AIBN (20.5 mg) was added and refluxing was continued for an additional 5 hours. The mixture was then cooled to room temperature and stirred for 11.5 hours. The solvent was then evaporated at room temperature under reduced pressure to a small volume and the residue was purified by silica gel chromatography, eluting with 3.5% methanol in dichloromethane. This gave **19** (821.5 mg, 84% yield) as a colorless glass. FD-MS *m/z*, 702 (MH^+), 701 (M^+); UV λ_{max} nm (ε) 244 (8,193); IR ($CHCl_3$) cm^{-1}, 2936, 1713, 1233, 1213, 1064; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{40}H_{63}NO_9$: C 68.43, H 9.05, N 2.00
Found: C 68.19, H 9.15, N 1.87

Synthesis of 3′-Desmethoxy Spinosyn A (Deoxy Spinosyn J; **20**)

To a solution of **17** (849.1 mg, 1.05 mmol) in anhydrous toluene (50 ml), fresh tributyltin hydride (500 μl, 1.6 mmol) and AIBN (10.2 mg) were added and the mixture was heated to reflux for 8 hours. An additional 32.8 mg of AIBN was then added, and refluxing was continued for another 2 hours. The mixture was then cooled to room temperature, and after sitting at approximately 5°C for 48 hours, the solvent was evaporated at room temperature under reduced pressure to a small volume. The residue was purified by silica gel chromatography, eluting with 3.5% methanol in dichloromethane. This gave **17** (557.0 mg, 76% yield) as a colorless glass. FD-MS *m/z*, 703 ($M+2H^+$), 702 (MH^+), 701 (M^+); UV λ_{max} nm (ε) 244 (8,539); IR ($CHCl_3$) cm^{-1}, 2932, 1713, 1658, 1223, 1208, 1062; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{40}H_{63}NO_9$: C 68.43, H 9.05, N 2.00
Found: C 68.20, H 9.30, N 1.74

Synthesis of 4′-Desmethoxy Spinosyn A (Deoxy spinosyn K; **21**)

To a solution of **18** (182.5 mg, 0.23 mmol) in anhydrous toluene (10 ml), tributyltin hydride (93 μl, 0.35 mmol) and a catalytic amount of AIBN were added and the mixture was heated to reflux for 8 hours. Then an additional 100 μl of tributyltin hydride was added, and refluxing was continued for another 12 hours. The mixture was then cooled to room temperature and stirred for 5 hours. The solvent was then evaporated at room temperature under reduced pressure to a small volume. The residue was purified by silica gel chromatography eluting with 80% ethyl acetate in hexane. This gave **21** (59.9 mg, 37% yield) as a colorless glass. FD-MS *m/z*, 703 ($M+2H^+$), 702 (MH^+); UV λ_{max} nm (ε) 244 (9,912); IR ($CHCl_3$) cm^{-1}, 3022, 2974, 1713, 1658, 1457, 1373, 1234, 1215, 1049; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{40}H_{63}NO_9$: C 68.43, H 9.05, N 2.00
Found: C 68.36, H 8.75, N 1.24

Synthesis of 2′-*O*-Desmethyl-2′-methyl Xanthate of Spinosyn D (**22**)

The reaction was run as with **18** (with omission of imidazole), starting with spinosyn Q (1.00 g, 1.4 mmol) and giving **22** (1.13 g, 98% yield) as an unstable yellow glass which was used without further purification. FD-MS *m/z*, 824 ($M+3H^+$), 823 ($M+2H^+$), 822 (MH^+); UV λ_{max} nm (ε) 280 (11,611), 232 (13,396); IR ($CHCl_3$) cm^{-1}, 2972, 2936, 1713, 1658, 1456, 1373, 1232, 1222, 1209, 1207, 1164, 1127, 1073, 989, 970.

Synthesis of 2′-Desmethoxy Spinosyn D (Deoxy Spinosyn Q; **23**)

To a solution of **22** (1.08 g, 1.3 mmol) in anhydrous

toluene (50 ml), fresh tributyltin hydride (750 μl, 2.8 mmol) and AIBN (47.5 mg) were added and the mixture was heated to reflux for 2.5 hours. The mixture was then cooled to room temperature and stirred for 20 hours. The solvent was then evaporated at room temperature under reduced pressure. The residue was purified by silica gel chromatography eluting with 3.5% methanol in dichloromethane. This gave **23** (912.9 mg, 98% yield) as a colorless glass. FD-MS *m/z*, 717 ($M+2H^+$), 716 (MH^+); UV λ_{max} nm (ε) 244 (10,631); IR ($CHCl_3$) cm^{-1}, 3008, 2972, 2937, 1713, 1658, 1456, 1373, 1233, 1161, 1125, 1098, 1070; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{41}H_{65}NO_9$: C 68.78, H 9.15, N 1.96
Found: C 67.49, H 8.92, N 2.04

Synthesis of 3′-*O*-Desmethyl-3′-methyl Xanthate of Spinosyn D (**24**)

Spinosyn L (1.65 g, 2.2 mmol) was dissolved in anhydrous THF (50 ml) and cooled in an ice bath under nitrogen. To this solution was added imidazole (21.2 mg, 0.3 mmol), followed by sodium hydride (60% in mineral oil; 105.0 mg, 2.6 mmol), carbon disulfide (700 μl, 11.64 mmol) and then methyl iodide (700 μl, 11.1 mmol). This mixture was stirred for 1 hour at room temperature. A slight amount of additional NaH was added and the mixture was stirred for another hour, poured into saturated ammonium chloride and extracted with dichloromethane. The organic layer was then dried with K_2CO_3) and evaporated at room temperature under reduced pressure. This gave **24** (1.86 g, 100% yield) as an unstable yellow solid which was used without further purification. FD-MS *m/z*, 822 (MH^+), 821 (M^+).

Synthesis of 3′-Desmethoxy Spinosyn D (Deoxy Spinosyn L; **25**)

The reaction was run as with **23**, starting with **24** (1.85 g, 2.25 mmol) and giving **25** (1.16 g, 74% yield) as a colorless glass. FD-MS *m/z*, 717 ($M+2H^+$), 716 (MH^+); UV λ_{max} nm (ε) 251 (4,826); IR ($CHCl_3$) cm^{-1}, 3018, 3009, 2972, 2936, 2830, 1713, 1658, 1456, 1373, 1258, 1232, 1153, 1124, 1098, 1066, 1050, 1016, 989; ^1H NMR ($CDCl_3$) see Table 2.

Anal Calcd for $C_{41}H_{65}NO_9$: C 68.78, H 9.15, N 1.96
Found: C 68.97, H 9.03, N 1.93

Acknowledgments

The authors wish to thank TOM ELZEY for assistance in acquiring NMR spectra, JAN TURNER, MARY HUBER and CHRISTINE BROUGHTON for strain selections, LAVERNE BOECK, PATRICK BAKER and the Eli Lilly Natural Products Division pilot plants for fermentation and isolation of starting materials.

References

1) MERTZ, F. R. & R. C. YAO: *Saccharopolyspora spinosa* sp. nov. isolated from soil collected in a sugar mill rum still. Int. J. Sys. Bact. 40: 34, 1990
2) SPARKS, T. C.; G. D. THOMPSON, H. A. KIRST, M. B. HERTLEIN, J. S. MYNDERSE, J. R. TURNER & T. V. WORDEN: Fermentation-derived Insect Control Agents: The Spinosyns. Meth. Biotech. Vol 5: Biopesticides: Use and Delivery. p. 171, *Eds.*, HALL, F. R. and MENN, J. J., Humana Press Inc., Totowa, N.J.
3) SPARKS, T. C.; G. D. THOMPSON, H. A. KIRST, M. B. HERTLEIN, L. L. LARSON, T. V. WORDEN & S. T. THIBAULT: Biological activity of the spinosyns, new fermentation derived insect control agents, on tobacco budworm (*Lepidoptera: Noctuidae*) larvae. J. Econ. Entomol. 91 (6): 1277, 1998
4) MYNDERSE, J. S.; J. W. MARTIN, J. R. TURNER, L. C. CREEMER, H. A. KIRST, M. C. BROUGHTON & M. L. B. HUBER: A83543 compounds and processes for production thereof. U.S. Pat. 5, 202, 242, 1993
5) TURNER, J. R.; M. L. B. HUBER, M. C. BROUGHTON, J. S. MYNDERSE & J. W. MARTIN: A83543A compounds; factors Q, R, S, and T. U.S. Pat. 5, 767, 253, 1998
6) MYNDERSE, J. S.; M. C. BROUGHTON, W. M. NAKATSUKASA, J. A. MABE, J. R. TURNER, L. C. CREEMER, M. L. B. HUBER, H. A. KIRST & J. W. MARTIN: A83543 compounds and processes for production thereof. U.S. Pat. 5, 840, 861, 1998
7) BOECK, L. D.; E. H. CHIO, T. E. EATON, O. W. Jr. GODFREY, K. H. MICHEL, W. M. NAKATSUKASA & R. C. YAO: Insecticide and miticide A83543 compounds and their method of production by fermentation. U.S. Pat. 5, 496, 931, 1996
8) CREEMER, L. C.; H. A. KIRST & J. W. PASCHAL: Conversion of spinosyn A and spinosyn D to their respective 9- and 17-pseudoaglycones and their aglycones. J. Antibiotics 51: 795, 1998