Carbohydrate Research, 169 (1987) 241–246
Elsevier Science Publishers B.V., Amsterdam – Printed in The Netherlands

Note

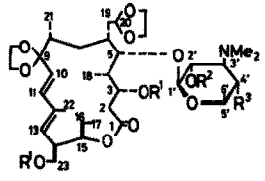
Synthesis of 5-*O*-(4-deoxy-4-fluoro-β-D-mycaminosyl)tylonolide*

SHUNJI KAGEYAMA, TOSHIHIKO ONODA, TSUTOMU TSUCHIYA†, SUMIO UMEZAWA, AND HAMAO UMEZAWA

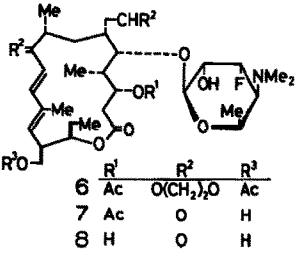
Institute of Bioorganic Chemistry, 1614 Ida, Nakaharaku, Kawasaki 211 (Japan)

(Received December 10th, 1986; accepted for publication in revised form, March 4th, 1987)

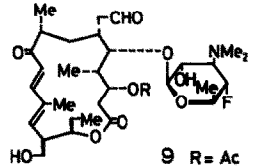
5-*O*-(β-D-Mycaminosyl)tylonolide[1] (MT) is a hydrolysis product of tylosin[2] and has respectively fairly strong and weak antibacterial activities against Gram-positive and Gram-negative bacteria; macrolide antibiotics are usually inactive against the latter. 5-*O*-(4-Deoxy-β-D-mycaminosyl)tylonolide[3] (DT, synthesized from MT) displays enhanced activities, as compared with MT, against both Gram-positive and -negative bacteria. We thus decided to introduce a fluorine atom in place of the 4′-hydroxy group of mycaminose. This fluorination is expected to produce a reverse effect to that of 4′-deoxygenation[3] in terms of electronegativity of the 3′-dimethylamino group, thus directly influencing the antibacterial activity.



	R^1	R^2	R^3
1	H	H	OH
2	Ac	Ac	OAc
3	Ac	H	OH
4	Ac	H	OSO_2CH_2Ph
5	Ac	H	F



	R^1	R^2	R^3
6	Ac	$O(CH_2)_2O$	Ac
7	Ac	O	H
8	H	O	H



9 R = Ac
10 R = H

*Dedicated to Dr. R. Stuart Tipson.
†Author for correspondence.

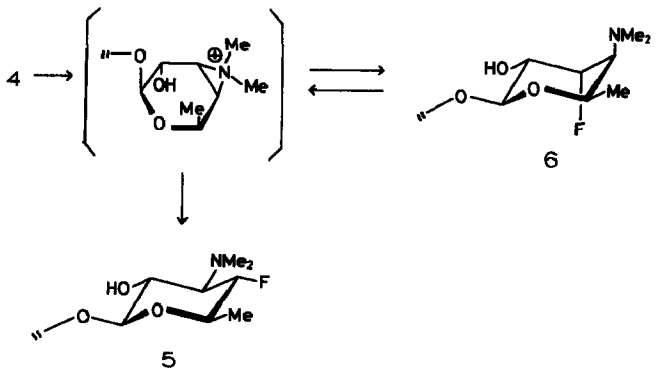
0008-6215/87/$ 03.50

RESULTS AND DISCUSSION

5-*O*-(β-D-Mycaminosyl)tylonolide 9,20-bis(ethylene acetal)[4] (**1**) was acetylated (to give the 3,23,2′,4′-tetraacetate, **2**), partially deacetylated (to give the 3,23-diacetate, **3**), and benzylsulfonylated as described[5] for demycarosyltylosin 20-(diethyl acetal), to give the 4′-*O*-sulfonyl derivative (**4**). Treatment of **4** with KHF_2 in *N,N*-dimethylformamide (DMF) gave a mixture of products from which the 3′-fluoro-4′-(dimethylamino) derivative **6** was isolated in moderate (56%) yield. This fluorination is presumed to occur through a 3′,4′-aziridinium intermediate, as proposed by Picq *et al.*[6,7], with *trans*-diaxial ring-opening. The structure of **6** was determined by ^{19}F- and ^{1}H-n.m.r. spectroscopy. The large (geminal) coupling constant[8] of $J_{H\text{-}3',F}$ (47.5 Hz) and the large and moderate coupling constants[8] of $J_{H\text{-}2',F}$ (29 Hz) and $J_{H\text{-}4',F}$ (15 Hz), respectively, indicate that the fluorine had been introduced axially at C-3′ (H-2′ and F are antiperiplanar, and H-4′ and F are gauche).

Acid treatment[9] of **6** gave the deacetalated 3-acetate (**7**). It is noteworthy that the 3-*O*-acetyl group of macrolactone ring resists hydrolysis, although more-forcing conditions also removed the acetyl group to give the free macrolide (**8**). Alkaline hydrolysis of **6**, followed by deacetalation, did not give **8** because of the formation of a 2,3-unsaturated compound[10].

Treatment of **4** with KHF_2 in ethylene glycol instead of DMF gave the desired 4′-fluoro derivative (**5**) with retention of configuration. The yield was, however, poor on account of the formation of several by-products, including **6**. This may, in part, be ascribed to the involvement of 2-hydroxyethoxy derivative(s), as reported by Evelyn and Hall[11]. The same treatment of **6** as just described also gave **5** (28%), although the yield was not improved. The fact that **5** is formed from **6** suggests that the same intermediate as described in the preparation of **6** (from **4**) is also involved in the conversion of **6** into **5** (Scheme 1). The structure of **5** was determined by n.m.r. spectroscopy. The large $J_{H\text{-}4',F}$ coupling-constant (50.5 Hz) indicates that the fluorine is present at C-4′, and the moderate-to-low values of $J_{H\text{-}3',F}$ (10 Hz) and $J_{H\text{-}5',F}$ (2.5 Hz) indicate, in conjunction with the $J_{2',3'}$, $J_{3',4'}$, and $J_{4',5'}$ values (all 10



Scheme 1.

TABLE I

ANTIBACTERIAL ACTIVITIES OF **9**, **10**, MT AND DT (μg/mL)

Test organisms[a]	**9**	**10**	*MT*	*DT*
Staphylococcus aureus 193	0.39	0.39	0.78	<0.2
Staphylococcus aureus 209P	0.39	0.39	0.78	<0.2
Staphylococcus aureus Smith	0.78	0.78	0.78	0.2
Micrococcus luteus PCI 1001	0.2	0.2	0.39	<0.2
Bacillus subtilis NRRL B-558	1.56	0.78	3.12	0.78
Corynebacterium bovis 1810	0.78	0.39	1.56	0.39
Escherichia coli NIHJ	6.25	3.12	6.25	1.56
Escherichia coli ML 1629	>100	50	100	12.5
Klebsiella pneumoniae PCI 602	1.56	1.56	1.56	0.78
Salmonella typhi T-63	50	25	50	12.5
Serratia marcescens	25	3.12	25	3.12
Pseudomonas aeruginosa A3	12.5	6.25	6.25	6.25

[a]Agar-dilution streak method (Mueller Hinton agar, 17 h, 37°).

Hz), that the fluorine is equatorially disposed. Acid treatment of **5** gave the deacetalated 3-acetate (**9**), and under stronger conditions, the free macrolide (**10**).

The antibacterial activities of **9** and **10** against twelve microorganisms are shown in Table I, alongside those of MT and DT. Compound **10** has enhanced activity in comparison to MT, but weaker activity than that of DT. The result suggests that the activity of MT is not necessarily proportional to the basicity of the 3′-dimethylamino group of the sugar portion. Other biochemical properties of **10** are now under study. Compound **8** is almost devoid of activity, indicating the importance of the 3′-dimethylamino group in the parent compound (MT).

EXPERIMENTAL

General. — Optical rotations were measured with a Perkin–Elmer 241 polarimeter. T.l.c. was performed on Kieselgel 60 F_{254} silica gel (Merck). The developing systems for t.l.c. of 10:1:0.1 and 20:1:0.1 $CHCl_3$–MeOH–29% aq. NH_3 are denoted as solvents *A* and *B*, respectively. Column chromatography was performed on Wakogel C-200 or Kieselgel 60. F.a.b.m.s. was performed on a Jeol JMS-DX 300 (HF) mass spectrometer, using xenon as the primary bombarding gas. N.m.r. spectra (^1H at 250 MHz, ^{19}F at 235.3 MHz) were recorded in the F.t. mode with a Bruker WM 250 spectrometer, the chemical shifts (δ in p.p.m.) being measured downfield from internal Me_4Si and Freon 11 ($CFCl_3$), respectively.

3,23-Di-O-acetyl-5-O-(2,4-di-O-acetyl-β-D-mycaminosyl)tylonolide 9,20-bis-(ethylene acetal) (**2**). — A solution of **1** (1.35 g, 2.0 mmol) in C_5H_5N (27 mL) was treated with Ac_2O (7.44 mL, 79 mmol) as described[5] (overnight, 50°) to give **2** as a solid; yield 1.59 g (95%). Column chromatography with 3:1 hexane–Me_2CO gave analytically pure **2**; $[\alpha]_D^{22}$ −29° (*c* 1, chloroform); ^1H-n.m.r. ($CDCl_3$): δ 2.05 (9 H) and 2.13 (3 H) (each s, Ac × 4).

Anal. Calc. for $C_{43}H_{67}NO_{16}$: C, 60.48; H, 7.91; N, 1.64. Found: C, 60.75; H, 8.06; N, 1.57.

*3,23-Di-*O*-acetyl-5-*O*-(β-*D*-mycaminosyl)tylonolide 9,20-bis(ethylene acetal)* (**3**). — A solution of **2** (893 mg, 1.05 mmol) in MeOH (20 mL) was heated overnight at 50° as described[5] to give **3** as a solid; yield 758 mg (94%). Column chromatography with 17:1:0.1 $CHCl_3$–MeOH–28% aq. NH_3 gave analytically pure **3**; $[\alpha]_D^{22}$ −30° (*c* 1, chloroform); ^{1}H-n.m.r. ($CDCl_3$): δ 2.05 and 2.09 (each s, 3 H, Ac × 2).

Anal. Calc. for $C_{39}H_{63}NO_{14}$: C, 60.84; H, 8.25; N, 1.82. Found: C, 61.07; H, 8.35; N, 1.74.

*3,23-Di-*O*-acetyl-5-*O*-(4-*O*-benzylsulfonyl-β-*D*-mycaminosyl)tylonolide 9,20-bis(ethylene acetal)* (**4**). — A solution of **3** (304 mg, 0.40 mmol) in C_5H_5N (6 mL) was treated with benzylsulfonyl chloride (115 mg, 0.6 mmol) as described[5] (4 h, −40°) to give **4** as an unstable solid; yield 352 mg (97%); t.l.c. (solvent *A*): R_F 0.9 (compare **3**: R_F 0.4).

*3,23-Di-*O*-acetyl-5-*O*-(4-deoxy-4-fluoro-β-*D*-mycaminosyl)tylonolide 9,20-bis(ethylene acetal)* (**5**). — A mixture of **4** (544 mg, 0.59 mmol) and KHF_2 (233 mg, 3.0 mmol) in ethylene glycol (8.3 mL; after distillation *in vacuo*, dried over 4 Å molecular sieves) was stirred for 1 h at 120°. T.l.c. (solvent *B*) showed several spots, having R_F 0.4 (major, **5**), 0.34 (slight, **6**), 0.23, 0.18 (major, a product formed from ethylene glycol?), and 0.16, with other trace spots. The mixture was poured into aq. saturated $NaHCO_3$ (100 mL), and the whole mixture was extracted with $CHCl_3$. The organic solution was washed with aq. saturated NaCl, dried ($MgSO_4$), and evaporated. Column chromatography of the syrup with 9:1 $CHCl_3$–Me_2CO gave **5** as a solid; yield 90.2 mg (20%), $[\alpha]_D^{22}$ −31° (*c* 0.9, chloroform); *m/z* 772 (M^+ + 1); ^{19}F-n.m.r. ($CDCl_3$): δ −194.5° (br dd); ^{1}H-n.m.r. ($CDCl_3$): δ 0.92 (t, 3 H, Me-17), 1.01 (d, 3 H, Me-18), 1.04 (d, 3 H, Me-21), 1.30 (d, 3 H, Me-6′), 1.74 (s, 3 H, Me-22), 2.05 and 2.09 (each s, 3 H, Ac × 2), 2.49 (s, 6 H, NMe_2), 2.60 (ddd, 1 H, H-3′), 2.91 (m, 1 H, H-14), 3.31 (dd, 1 H, H-2′), 3.45 (dq, 1 H, H-5′), 4.24 (dt, 1 H, H-4′), 4.28 (d, 1 H, H-1′), 4.83 (m, 1 H, H-15), 5.14 (sl. br d, 1 H, H-3), 5.42 (d, 1 H, H-13), 5.65 (d, 1 H, H-10), 6.57 (d, 1 H, H-11); $J_{1',2'}$ 7.5, $J_{2',3'} = J_{3',4'} = J_{4',5'} = 10$, $J_{3',F}$ 10, $J_{4',F}$ 50.5, and $J_{5',F}$ 2.5 Hz.

Anal. Calc. for $C_{39}H_{62}FNO_{13}$: C, 60.68; H, 8.10; N, 1.81; F, 2.46. Found: C, 60.97; H, 8.14; N, 1.70; F, 2.31.

*3,23-Di-*O*-acetyl-5-*O*-[3,4,6-trideoxy-3-fluoro-4-(dimethylamino)-β-*D*-gulo-pyranosyl]tylonolide 9,20-bis(ethylene acetal)* (**6**). — A mixture of **4** (369 mg, 0.40 mmol) and KHF_2 (158 mg, 2.0 mmol) in dry DMF was stirred for 1 h at 100° and then for 3 h at 120°. T.l.c. (solvent *B*) showed several spots having R_F 0.34 (**6**), 0.19 (trace), 0.17, and 0.15 (**3**) (compare **4**; R_F 0.85). Processing as described for **5**, but changing the solvent system for column chromatography to 5:1 $CHCl_3$–Me_2CO, gave **6** as a solid; yield 171 mg (56%); **3**, 43 mg; and the compound having R_F 0.17, 29 mg. Compound **6** had $[\alpha]_D^{22}$ −60° (*c* 1, $CHCl_3$); *m/z* 772 (M^+ + 1); ^{19}F-n.m.r. ($CDCl_3$): δ −201.8 (dq, *J* 15, 29, and 47.5 Hz), ^{1}H-n.m.r. ($CDCl_3$): δ 0.92 (t, 3 H, *J* 8 and 8 Hz, Me-17), 0.97 (d, 3 H, $J_{4,18}$ 7 Hz, Me-18), 1.01 (d, 3 H, $J_{8,21}$ 7 Hz,

Me-21), 1.28 (d, 3 H, $J_{5',6'}$ 6.6 Hz, Me-6'), 1.74 (s, 3 H, Me-22), 2.05 and 2.09 (each s, 3 H, Ac × 2), 2.45 (s, 6 H, NMe_2), 2.75 (dt, 1 H, H-4'), 2.91 (m, 1 H, H-14), 3.66 (ddd, 1 H, H-2'), ~4.1 (m, 2 H, H-23a,23b; confirmed by the shift-correlated 2D spectrum), 4.52 (d, 1 H, H-1'), 4.85 (m, 1 H, H-15), 5.06 (dt, 1 H, H-3'), 5.13 (m, 1 H, H-3), 5.42 (d, 1 H, $J_{13,14}$ 11 Hz, H-13), 5.68 (d, 1 H, $J_{10,11}$ 16 Hz, H-10), and 6.50 (d, 1 H, H-11); $J_{1',2'}$ 8, $J_{2',3'} = J_{3',4'} = J_{4',5'} = \sim 3$, $J_{2',F}$ 29, $J_{3',F}$ 47.5, and $J_{4',F}$ 15 Hz.

Anal. Calc. for $C_{39}H_{62}FNO_{13}$: C, 60.68; H, 8.10; N, 1.81; F, 2.46. Found: C, 60.68; H, 8.10; N, 1.77; F, 2.21.

3-O-Acetyl-5-O-[3,4,6-trideoxy-3-fluoro-4-(dimethylamino)-β-D-gulopyranosyl]tylonolide (**7**). — To a solution of **6** (30.6 mg, 0.04 mmol) in 50% aq. MeCN (1.2 mL) was added *p*-toluenesulfonic acid hydrate (46 mg, 0.24 mmol), and the solution was heated for 16 h at 50°. T.l.c. (solvent *A*) showed a spot of **7** at R_F 0.32 (compare **6**: R_F 0.62) plus weak spot at R_F 0.5. After neutralization with $NaHCO_3$, the mixture was extracted with $CHCl_3$. The organic solution was washed with saturated aqueous NaCl, dried ($MgSO_4$), and evaporated. Purification of the residue by column chromatography (solvent *B*) gave **7** as a solid; yield 23.3 mg (92%), $[\alpha]_D^{22}$ −25 (*c* 1.8, $CHCl_3$); *m/z* 642 ($M^+ + 1$); ^{19}F-n.m.r. ($CDCl_3$): δ −202.0 (dq), ^{1}H-n.m.r. ($CDCl_3$): δ 1.25 (d, 3 H, Me-6'), 2.14 (s, 3 H, AcO-3), 2.47 (s, 6 H, NMe_2), 2.76 (dt, 1 H, H-4'), 3.64 (ddd, 1 H, H-2'), 3.96 (m, 1 H, H-5'), 4.47 (d, 1 H, H-1'), 5.05 (dt, 1 H, H-3'), 5.19 (br d, 1 H, H-3), 5.92 (d, 1 H, H-13), 6.30 (d, 1 H, H-10), 7.41 (d, 1 H, H-11), and 9.65 (s, 1 H, H-20); $J_{1',2'}$ 8, $J_{2',3'} = J_{3',4'} = \sim 2$, $J_{4',5'}$ 3, $J_{2',F}$ ~30, $J_{3',F}$ 47, and $J_{4',F}$ 15 Hz.

Anal. Calc. for $C_{33}H_{52}FNO_{10}$: C, 61.76; H, 8.17; N, 2.18. Found: C, 62.10; H, 8.32; N, 1.96.

5-O-[3,4,6-Trideoxy-3-fluoro-4-(dimethylamino)-β-D-gulopyranosyl]tylonolide (**8**). — To a solution of **6** (50.6 mg, 0.066 mmol) in 50% aq. MeCN (2 mL) was added *p*-toluenesulfonic acid hydrate (150 mg, 0.79 mmol), and the solution was heated for 18 h at 75°. T.l.c. (solvent *A*) showed a several spots, including R_F 0.28 (major, **8**) and 0.3 (**7**). The same processing as described for **7** gave **8** as a solid; yield 15.6 mg (40%), $[\alpha]_D^{21}$ −50° (*c* 0.8, chloroform); *m/z* 600 ($M^+ + 1$); ^{19}F-n.m.r. ($CDCl_3$): δ −202.2 (dq), ^{1}H-n.m.r. ($CDCl_3$): δ 0.95 (t, 3 H, Me-17), 1.03 (d, 3 H, Me-18), 1.23 (d, 3 H, Me-21), 1.26 (d, 3 H, Me-6'), 1.83 (s, 3 H, Me-22), 2.47 (s, 6 H, NMe_2), 2.75 (br d, 1 H, H-4'), 2.93 (m, 1 H, H-14), 3.63 (ddd, 1 H, H-2'), 3.96 (m, 1 H, H-5'), 4.51 (d, 1 H, H-1'), 4.96 (dt, 1 H, H-15), 5.05 (br d, 1 H, H-3'), 5.86 (d, 1 H, $J_{13,14}$ 11 Hz, H-13), 6.30 (d, 1 H, $J_{10,11}$ 16 Hz, H-10), 7.33 (d, 1 H, H-11), and 9.71 (s, 1 H, H-20); $J_{1',2'}$ 8, $J_{2',3'} = J_{3',4'}$ ~2, $J_{4',5'}$ 3, $J_{2',F}$ ~28, $J_{3',F}$ 47, and $J_{4',F}$ 15 Hz.

Anal. Calc. for $C_{31}H_{50}FNO_9$: C, 62.08; H, 8.40; N, 2.34. Found: C, 62.35; H, 8.57; N, 2.14.

3-O-Acetyl-5-O-(4-deoxy-4-fluoro-β-D-mycaminosyl)tylonolide (**9**). — Compound **5** (19.6 mg, 0.025 mmol) was treated as described for **7**. Purification of the crude product by column chromatography with 30:1:0.1 $CHCl_3$–MeOH–28% aque-

ous NH_3 gave **9** as a solid; yield 14.2 mg (87%), $[\alpha]_D^{22}$ +2° (*c* 0.6, chloroform); *m/z* 642 (M^+ + 1); ^{19}F-n.m.r. ($CDCl_3$): δ −195.0 (dd), ^{1}H-n.m.r. ($CDCl_3$): δ 1.28 (d, 3 H, Me-6'), 2.12 (s, 3 H, AcO-3), 2.48 (s, 6 H, NMe_2), 2.58 (ddd, 1 H, H-3'), 3.22 (dd, 1 H, H-2'), 3.45 (dq, 1 H, H-5'), 4.25 (dt, 1 H, H-4'), 4.25 (d, 1 H, H-1'), 5.16 (d, 1 H, H-3), 5.91 (d, 1 H, H-13), 6.32 (d, 1 H, H-10), 7.43 (d, 1 H, H-11), and 9.63 (s, 1 H, H-20); $J_{1',2}$ 7, $J_{2',3'} = J_{3',4'} = J_{4',5'} = 10$, $J_{3',F}$ 10, $J_{4',F}$ 51, and $J_{5',F}$ 2.5 Hz.

Anal. Calc. for $C_{33}H_{52}FNO_{10}$: C, 61.76; H, 8.17; N, 2.18. Found: C, 61.71; H, 8.37; N, 1.94.

*5-O-(4-Deoxy-4-fluoro-β-*D*-mycaminosyl)tylonolide* (**10**). — Compound **5** (30.6 mg, 0.04 mmol) was treated as described for **8**. Purification of the crude product by column chromatography with 30:1 $CHCl_3$–MeOH gave **10** as a solid; yield 8.5 mg (36%), $[\alpha]_D^{22}$ −21° (*c* 0.9, $CHCl_3$); *m/z* 600 (M^+ + 1); ^{19}F-n.m.r. ($CDCl_3$): δ −195.0 (dd), ^{1}H-n.m.r. ($CDCl_3$): δ 0.95 (t, 3 H, Me-17), 1.07 (d, 3 H, Me-18), 1.21 (d, 3 H, Me-21), 1.28 (d, 3 H, Me-6'), 1.81 (s, 3 H, Me-22), 2.45 (s, 6 H, NMe_2), 2.59 (ddd, 1 H, H-3'), 3.23 (dd, 1 H, H-2'), 3.43 (dq, 1 H, H-5'), 4.25 (dt, 1 H, H-4'), 4.30 (d, 1 H, H-1'), 4.96 (m, 1 H, H-15), 5.88 (d, 1 H, H-13), 6.32 (d, 1 H, H-10), 7.35 (d, 1 H, H-11), and 9.70 (s, 1 H, H-20); $J_{1',2'}$ 7.5, $J_{2',3'} = J_{3',4'} = J_{4',5'} = 10$, $J_{3',F}$ 10, $J_{4',F}$ 50.5, and $J_{5',F}$ 2.5 Hz.

Anal. Calc. for $C_{31}H_{50}FNO_9$: C, 62.08; H, 8.40; N, 2.34. Found: C, 61.83; H, 8.44; N, 2.07.

ACKNOWLEDGMENTS

We are grateful to the members of the Physico-Analysis Center in Central Research Laboratories of Yamanouchi Pharmaceutical Co. Ltd. for measurements of f.a.b.m.s. and elemental analysis, to Dr. Masa Hamada of Institute of Microbial Chemistry for bioassay, to Miss Yoshiko Koyama of our Institute for measurements of n.m.r. spectra.

REFERENCES

1 R. B. Morin and M. Gorman, *Tetrahedron Lett.*, (1964) 2339–2345.
2 J. M. McGuire, W. S. Boniece, C. E. Higgens, M. M. Hoehn, W. M. Stark, J. Westhead, and R. N. Wolfe, *Antibiot. Chemother (1954–1968)*, 11 (1961) 320–327.
3 A. Tanaka, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiot.*, 34 (1981) 1374–1376.
4 S. Sakamoto, T. Tsuchiya, S. Umezawa, and H. Umezawa, *Bull. Chem. Soc. Jpn.*, 60 (1987) 1481–1488.
5 A. Tanaka, A. Watanabe, T. Tsuchiya, S. Umezawa, and H. Umezawa, *J. Antibiot.*, 34 (1981) 1381–1384.
6 D. Picq, D. Anker, C. Rousset, and A. Laurent, *Tetrahedron Lett.*, 24 (1983) 5619–5622.
7 D. Picq and D. Anker, *J. Carbohydr. Chem.*, 4 (1985) 113–123.
8 A. A. E. Penglis, *Adv. Carbohydr. Chem. Biochem.*, 38 (1981) 256–268.
9 A. Tanaka, A. Watanabe, R. Kobayashi, T. Tsuchiya, and S. Umezawa, *Bull. Chem. Soc. Jpn.*, 54 (1981) 3837–3845.
10 M. Muroi, M. Izawa, and T. Kishi, *Chem. Pharm. Bull.*, 24 (1976) 450–462.
11 L. Evelyn and L. D. Hall, *Carbohydr. Res.*, 47 (1976) 285–297.