Carbohydrate Research, 60 (1978) 275–288

A NEW METHOD FOR SELECTIVE *N*-ACYLATION OF AMINOGLYCOSIDE ANTIBIOTICS

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(Received January 26th, 1977; accepted for publication in revised form, March 28th, 1977)

ABSTRACT

Per-*N*-formylation of aminoglycoside (aminocyclitol) antibiotics followed by mild hydrolysis with aqueous ammonia gave mono-*N*-deformylated derivatives. Each positional isomer of the mono-*N*-deformylated derivatives thus obtained was separated by column chromatography on Amberlite CG-50 (NH_4^+). Acylation of mono-*N*-deformylated derivatives gave the corresponding mono-*N*-acylated derivatives. The *N*-formyl groups of the mono-*N*-acylates were removed by the treatment with dilute aqueous hydrazine acetate, whereas the newly introduced *N*-acyl group was stable under these conditions. The 1-*N*-formyl group of the deoxystreptamine moiety of per-*N*-formylated aminoglycoside antibiotics containing neamine (or 3′-deoxyneamine) is more readily deformylated than the 3-*N*-formyl group. In this report, isolation and structural-elucidation studies, including ^{13}C-n.m.r. spectral assignments, of positional isomers of tri-*N*-formyl derivatives of xylostasin (**1**), 3′-deoxyxylostasin (**2**), kanamycin A (**3**), and neamine (**4**) are described. This selective *N*-acylation provides a useful method for the preparation of 1-*N*-modified derivatives, and the synthesis of 3′-deoxybutirosin A (**2f**) from **2** is described in detail as an example.

INTRODUCTION

On the basis of the structure–activity relationships of butirosins and their deacylated derivatives, many semisynthetic aminoglycoside (aminocyclitol) antibiotics acylated with (*S*)-4-amino-2-hydroxybutanoic acid (or an analog thereof) at the 1-amino group of the deoxystreptamine moiety have been synthesized[1]. Some of these new semisynthetic aminoglycoside antibiotics display improved antibiotic activities, compared with the parent antibiotics.

In the present studies, all of the amino groups of the aminoglycoside antibiotics examined, namely xylostasin[2] (**1**), 3′-deoxyxylostasin (**2**), kanamycin A (**3**), and neamine (**4**), were first protected with the *N*-formyl group and then the per-*N*-formylated derivatives were hydrolyzed with aqueous ammonia to give partially deformylated derivatives, including mono-*N*-deformylated derivatives. A desired

positional isomer from among the mono-*N*-deformylated derivatives was then chromatographically isolated and the free amino group acylated with an appropriate acid, and the remaining formyl groups were then removed to give a desired mono-*N*-acyl antibiotic.

As an amino-protecting group, the formyl group shows the following advantages: (*i*) *N*-formyl groups are readily removed by treatment with dilute, aqueous hydrazine acetate (pH 6.0)[3] and a selectively substituted *N*-acyl group, such as the (*S*)-4-amino-2-hydroxybutanoyl group is stable under these conditions, (*ii*) formamido groups are stable under the purification and the selective *N*-acylation processes, and (*iii*) all of the *N*-formyl derivatives are water-soluble, so that ion-exchange chromatography may be used for separation and purification of the partially deformylated derivatives; furthermore, this method is suited for large-scale preparation.

RESULTS AND DISCUSSION

Tetra-*N*-formylxylostasin (**1e**), 3′-deoxy-tetra-*N*-formylxylostasin (**2e**), tetra-*N*-formylkanamycin A (**3e**), and tetra-*N*-formylneamine (**4e**) were partially deformylated by treatment with 10% aqueous ammonia for 5 days at room temperature, and the mixture of partially deformylated derivatives was chromatographed on a column of Amberlite CG-50 (NH_4^+). When the column was developed with water, the unreacted tetra-*N*-formyl derivative was first eluted, and then four tri-*N*-formyl positional-isomers were eluted. The tri-*N*-formyl derivatives were designated as compounds **1a**, **1b**, **1c**, and **1d** for the tri-*N*-formyl xylostasins; compounds **2a**, **2b**, **2c**, and **2d** for the 3′-deoxy-tri-*N*-formylxylostasins, compounds **3a**, **3b**, **3c**, and **3d** for the tri-*N*-formylkanamycins A; and compounds **4a**, **4b**, **4c**, and **4d** for the tri-*N*-formylneamines, in their order of elution, respectively. The R_F values on t.l.c. (silica gel), and optical rotations of these compounds are summarized in Tables I and II, respectively.

The position of the free amino group of each isomer was determined as follows: When the tri-*N*-formyl derivatives **4a** and **4b**, for example, were treated with 1-fluoro-2,4-dinitrobenzene and then the resulting *N*-(2,4-dinitrophenyl) derivatives hydrolyzed

TABLE I

T.L.C. ANALYSIS OF THE TETRA- AND TRI-N-FORMYL DERIVATIVES OF **1**, **2**, **3** AND **4**

Compound	R_F[a]	*Compound*	R_F[a]	*Compound*	R_F[b]	*Compound*	R_F[b]
1a	0.58	**2a**	0.78	**3a**	0.62	**4a**	0.66
1b	0.47	**2b**	0.68	**3b**	0.52	**4b**	0.60
1c	0.71	**2c**	0.80	**3c**	0.46	**4c**	0.42
1d	0.52	**2d**	0.74	**3d**	0.20	**4d**	0.25
1e	0.87	**2e**	0.89	**3e**	0.70	**4e**	0.76

[a]Solvent *A*. [b]Solvent *B*.

TABLE II

SPECIFIC OPTICAL ROTATIONS OF THE TETRA- AND TRI-*N*-FORMYL DERIVATIVES OF **1**, **2**, **3**, AND **4**

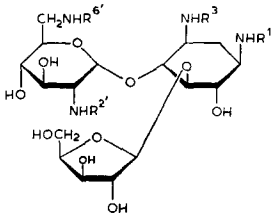
Compound	$[\alpha]_D$[a]	*Compound*	$[\alpha]_D$	*Compound*	$[\alpha]_D$	*Compound*	$[\alpha]_D$
1a	+52.5°	**2a**	+54.4°	**3a**	+118.3°	**4a**	+109.0°
1b	+ 7.0°	**2b**	+ 5.9°	**3b**	+123.2°	**4b**	+ 55.7°
1c	+26.2°	**2c**	+24.2°	**3c**	+101.8°	**4c**	+ 82.4°
1d	+38.5°	**2d**	+32.4°	**3d**	+120.8°	**4d**	+107.7°
1e	+27.1°	**2e**	+28.5°	**3e**	+110.8°	**4e**	+ 95.4°

[a] *c* 1, in water.

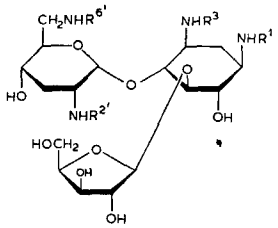
with acid, the *N*-(2,4-dinitrophenyl) derivative of **4a** gave mono-*N*-(2,4-dinitrophenyl)-deoxystreptamine, which was characterized as the tetraacetate; $[\alpha]_D$ −31.4° (*c* 1, chloroform). This mono-*N*-(2,4-dinitrophenyl)deoxystreptamine was identical with that derived from per-*N*-(2,4-dinitrophenyl)butirosin A. On the other hand, the 2,4-dinitrophenyl derivative of **4b** gave 2-deoxy-1-*N*-(2,4-dinitrophenyl)streptamine, which was characterized as the tetraacetate; $[\alpha]_D$ +29.8° (*c* 1, chloroform). By treating the tri-*N*-formyl derivatives **1a**, **1b**, **2a**, **2b**, **3a**, and **3b** as just described, the tri-*N*-formyl derivatives **1a**, **2a**, and **3a** gave 2-deoxy-3-*N*-(2,4-dinitrophenyl)streptamine, and the tri-*N*-formyl derivatives **1b**, **2b**, and **3b** gave 2-deoxy-1-*N*-(2,4-dinitrophenyl)streptamine. Consequently, **1a**, **2a**, and **4a** were identified as the 1,2′,6′-tri-*N*-formyl derivative; **3a**, as the 1,6′,3″-tri-*N*-formyl derivative; **1b**, **2b**, and **4b** as the 3,2′,6′-tri-*N*- formyl derivative; and **3b** as the 3,6′,3″-tri-*N*-formyl derivative.

The structures of **1b**, **2b**, **3b**, and **4b** were further confirmed by the following reactions. The tri-*N*-formyl derivatives **1b**, **2b**, **3b**, and **4b** were acylated with the *N*-hydroxysuccinimide ester of (*S*)-2-hydroxy-4-phthalimidobutanoic acid in *N*,*N*-dimethylformamide, and the protecting groups were removed by treatment with aqueous hydrazine acetate to give compounds **1f**, **2f**, **3f**, and **4f**, respectively. These (*S*)-4-amino-2-hydroxybutanoyl derivatives (**1f**, **2f**, **3f**, and **4f**) were identical with butirosin A[1] (**1f**), 3′-deoxybutirosin A (**2f**), BB-K8[1] (**3f**), and 1-*N*-(*S*)-4-amino-2-hydroxybutanoyl neamine[1] (**4f**), and showed improved antibiotic activities in comparison with the corresponding parent antibiotics. The structure of **2f**, a new antibiotic, was confirmed by synthesizing it by an unequivocal route, as shown in the following paper[4].

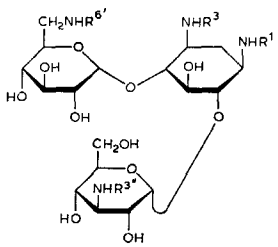
The structures of the tri-*N*-formyl derivatives **1d**, **2d**, **3d**, and **4d** were identified synthetically as follows: As the 6′-amino group is the sole $-CH_2NH_2$ group and the most reactive amino group in the molecule[5], the 6′-amino group was first protected by the benzyloxycarbonyl (Z) group, and then the other amino groups were formylated. After the removal of the Z group by catalytic hydrogenolysis (palladium black), the tri-*N*-formyl derivatives were obtained. Consequently, **1d**, **2d**, and **4d** were identified as the 1,3,2′-tri-*N*-formyl derivative, and **3d** as the 1,3,3″-tri-*N*-formyl derivative.



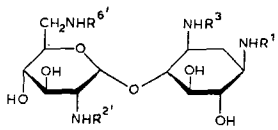
1 $R^1, R^3, R^{2'}, R^{6'}$ = H (xylostasin)
1a $R^1, R^{2'}, R^{6'}$ = CHO; R^3 = H
1b $R^3, R^{2'}, R^{6'}$ = CHO; R^1 = H
1c $R^1, R^3, R^{6'}$ = CHO; $R^{2'}$ = H
1d $R^1, R^3, R^{2'}$ = CHO; $R^{6'}$ = H
1e $R^1, R^3, R^{2'}, R^{6'}$ = CHO
1f R^1 = AHB; $R^3, R^{2'}, R^{6'}$ = H



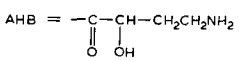
2 $R^1, R^3, R^{2'}, R^{6'}$ = H (3'-deoxyxylostasin)
2a $R^1, R^{2'}, R^{6'}$ = CHO; R^3 = H
2b $R^3, R^{2'}, R^{6'}$ = CHO; R^1 = H
2c $R^1, R^3, R^{6'}$ = CHO; $R^{2'}$ = H
2d $R^1, R^3, R^{2'}$ = CHO; $R^{6'}$ = H
2e $R^1, R^3, R^{2'}, R^{6'}$ = CHO
2f R^1 = AHB; $R^3, R^{2'}, R^{6'}$ = H



3 $R^1, R^3, R^{6'}, R^{3''}$ = H (kanamycin A)
3a $R^1, R^{6'}, R^{3''}$ = CHO; R^3 = H
3b $R^3, R^{6'}, R^{3''}$ = CHO; R^1 = H
3c $R^1, R^3, R^{6'}$ = CHO; $R^{3''}$ = H
3d $R^1, R^3, R^{3''}$ = CHO; $R^{6'}$ = H
3e $R^1, R^3, R^{6'}, R^{3''}$ = CHO
3f R^1 = AHB; $R^3, R^{6'}, R^{3''}$ = H



4 $R^1, R^3, R^{2'}, R^{6'}$ = H (neamine)
4a $R^1, R^{2'}, R^{6'}$ = CHO; R^3 = H
4b $R^3, R^{2'}, R^{6'}$ = CHO; R^1 = H
4c $R^1, R^3, R^{6'}$ = CHO; $R^{2'}$ = H
4d $R^1, R^3, R^{2'}$ = CHO; $R^{6'}$ = H
4e $R^1, R^3, R^{2'}, R^{6'}$ = CHO
4f R^1 = AHB; $R^3, R^{2'}, R^{6'}$ = H



In the ^{1}H-n.m.r. spectra of the *N*-formyl derivatives, the resonances of the formyl-group protons were, unfortunately, complex, probably because of the existence of rotational isomers, and therefore, they were not diagnostic for each positional isomer.

In the ^{13}C-n.m.r. spectra (Tables III, IV, and V) of **1a–e**, **2a–e**, and **3a–e**, the existence of rotational isomers was also observed. The ^{13}C n.m.r. spectrum of each tetra-*N*-formyl derivative **1e**, **2e**, and **3e** showed four pairs of resonances corresponding to three CH carbon atoms (α-CH) and one CH_2 carbon atom (α-CH_2) to which formamido groups were attached (C-1, 3, 2′, and 6′ of the tetra-*N*-formyl derivatives **1e** and **2e**; C-1, 3, 3″, and 6′ of the tetra-*N*-formyl derivative **3e**). The observed chemical-shift differences for these α-CH and α-CH_2 groups are 4–5 p.p.m., and are attributable to the shift differences corresponding to α-carbon atoms cisoid and

TABLE III

^{13}C CHEMICAL-SHIFT ASSIGNMENTS FOR THE TRI-*N*-FORMYL POSITIONAL ISOMERS AND THE TETRA-*N*-FORMYL DERIVATIVE OF **1**

Carbon atom	**1e**	**1b**	**1a**	**1c**	**1d**
1	49.1 (53.5)[a]	50.9	49.3 (53.9)	49.2 (53.5)	49.1 (53.6)
2	33.1 (33.7)	34.7 (35.1)	34.6 (35.0)	33.1 (33.8)	33.1 (33.6)
3	48.0 (52.7)	48.3 (53.4)	51.2	48.8 (52.8)	48.0 (53.0)
2′	53.0 (58.0)	53.0 (58.1)	53.2 (58.3)	56.1	53.0 (58.1)
6′	39.1 (43.2)	39.1 (43.1)	39.5 (43.4)	39.3 (43.2)	42.0
$R^{6'}$-CHO	165.3 (169.1)	165.4 (169.0)	165.3 (169.0)	165.3 (169.0)	
$R^{2'}$-CHO	165.3 (168.2)	165.4 (168.2)	165.3 (168.2)		165.2 (168.3)
R^{3}-CHO	164.8 (168.0)	164.7 (168.0)		164.6 (167.9)	164.8 (168.1)
R^{1}-CHO	164.8 (167.9)		164.8 (167.9)	164.8 (167.9)	164.8 (167.9)

[a]The figures in parentheses show chemical shifts of minor rotational isomers.

TABLE IV

^{13}C CHEMICAL-SHIFT ASSIGNMENT FOR THE TRI-N-FORMYL POSITIONAL ISOMERS AND THE TETRA-N-FORMYL DERIVATIVE OF **2**

Carbon	**2e**	**2b**	**2a**	**2c**	**2d**
1	49.1 (53.6)[a]	51.0	49.3 (53.9)	49.8 (53.6)	49.1 (53.6)
2	33.1 (33.6)	34.1 (34.5)	34.6 (35.1)	33.1 (33.8)	33.2 (33.6)
3	48.1 (53.1)	48.2 (53.3)	51.3	47.9 (52.8)	48.0 (53.1)
2′	47.0 (51.7)	47.1 (51.7)	47.2 (51.9)	49.3	47.0 (51.5)
3′	32.7 (33.6)	32.7 (33.5)	32.7 (33.8)	34.2	32.8 (33.6)
6′	39.2 (43.2)	39.2 (43.2)	39.5 (43.5)	39.4 (43.4)	41.7
$R^{6'}$-CHO	165.3 (168.9)	165.3 (168.9)	165.2 (168.9)	165.3 (168.9)	
$R^{2'}$-CHO	164.3 (167.6)	164.3 (167.6)	164.5 (167.5)		164.3 (167.6)
R^{3}-CHO	164.8 (168.1)	164.8 (168.1)		164.6 (167.9)	164.8 (168.2)
R^{1}-CHO	164.8 (167.9)		164.8 (167.9)	164.8 (167.9)	164.8 (167.9)

[a]The figures in parentheses show chemical shifts of minor rotational isomers.

transoid to the formyl hydrogen atom[6,7]. When a formamido group is selectively deformylated, the pair of signals for the α-carbon atom coalesces to a single signal, and this spectral change is diagnostically valuable in the structure analysis of positional isomers of the *N*-formyl derivatives.

The observed chemical-shift differences for the β-methylene groups (C-2) in the deoxystreptamine moiety and the β-methylene group (C-3′) in the 3-deoxyneosamine C moiety are in the range of 0.4–0.9 and 0.8–1.1 p.p.m., respectively. These pairs also are diagnostic for the positional isomers of the *N*-formyl derivatives.

TABLE V

^{13}C CHEMICAL-SHIFT ASSIGNMENT FOR THE TRI-*N*-FORMYL POSITIONAL ISOMERS AND THE TETRA-*N*-FORMYL DERIVATIVE OF **3**

Carbon	**3e**	**3b**	**3a**	**3c**	**3d**
1	48.7 (53.6)[a]	51.0	48.8 (53.7)	48.7 (53.6)	48.7 (53.6)
2	33.5 (34.4)	34.7 (35.5)	35.0 (35.8)	33.5 (34.4)	33.6 (34.4)
3	47.5 (52.3)	47.8 (52.6)	49.7	47.6 (52.3)	47.4 (52.3)
3″	53.6 (58.4)	53.9 (58.8)	53.8 (58.4)	54.9	53.6 (58.4)
6′	39.0 (43.3)	39.0 (43.3)	39.5 (43.5)	39.0 (43.3)	41.9
$R^{6'}$-CHO	165.3 (168.9)	165.3 (168.9)	165.2 (168.9)	165.3 (168.9)	
$R^{3''}$-CHO	165.9 (168.7)	165.9 (168.6)	165.9 (168.6)		165.9 (168.7)
R^{3}-CHO	164.6 (167.7)	164.6 (167.7)		164.6 (167.8)	164.6 (167.8)
R^{1}-CHO	164.9 (168.1)		164.9 (168.1)	164.9 (168.1)	164.9 (168.7)

[a]The figures in parentheses show chemical shifts of minor rotational isomers.

The pair of ^{13}C signals corresponding to C-2 coalesces to a single peak by deformylation of both formamido groups at C-1 and C-3. When either of the formamido groups at C-1 and C-3 was deformylated, the pair of resonances still remained, but the relative peak-intensities changes (the upper-field signal became stronger than the lower).

The chemical-shift difference between the two C-O resonances of each formamido group is 3.1–3.7 p.p.m., which is in good agreement with the difference reported for *N*-methylformamide[6]. However, these signals are not suited for diagnosis, because of crowding and overlapping of the four pairs of signals.

The position of the free amino group was further confirmed by the difference of the β-carbon shift between a primary amine and its protonated salt[8]. The result of this diagnostic test confirms that the tri-*N*-formyl derivatives **1c**, **2c**, and **3c** are the 1,3,6′-tri-*N*-formyl derivatives (Table VI).

Positional isomers of tri-*N*-formyl derivatives of **1**, **2**, **3**, and **4** were obtained by alkaline hydrolysis of the corresponding tetra-*N*-formyl derivative in the ratios shown in Table VII. The favored deformylation of the formamide group at C-1 in comparison with that at C-3 may suggest appreciable spatial interaction between the formamido groups at C-6′ and C-3. The 3-formamido group of **3e** was hydrolyzed at a rate higher than those of **1e**, **2e**, and **4e**, as shown in Table VII. This might be due to the fact that the 2″- and 6″-hydroxyl groups of the 3-deoxy-3-formamido-D-glucose moiety of **3e** have a steric effect on the 1-formamido group; moreover, the substituent at C-2′ that interacts with the 3-formamido group is a hydroxyl group in the case of **3e** instead of the bulkier formamido group. Alkaline hydrolysis of the formamido group at C-2′ of neamine-type antibiotics was also retarded by the steric effect of the xylose substituent at O-5 and the neighboring hydroxyl-group at C-3′.

TABLE VI

THE EFFECT OF PROTONATION ON ^{13}C CHEMICAL-SHIFTS OF THE TRI-*N*-FORMYL DERIVATIVES **1c**, **2c**, AND **3c**

Carbon	**1c**		**2c**		*Carbon*	**3c**	
	2'-NH₂	*2'-NH₃*	*2'-NH₂*	*2'-NH₃*		*3''-NH₂*	*3''-NH₃*
1	49.2 (53.5)[a]	49.2 (53.2)	49.8 (53.6)	49.2 (53.6)	1	48.7 (53.6)	48.6 (53.3)
2	33.1 (33.8)	33.0 (33.6)	33.1 (33.8)	33.0 (33.7)	2	33.5 (34.4)	33.5 (34.3)
3	48.8 (52.8)	47.6 (52.1)	47.9 (52.8)	47.7 (52.5)	3	47.6 (52.3)	47.6 (43.3)
4	77.9	78.4	77.7	78.1	4	83.0	83.4
5	85.7	87.7	86.5	87.8	5	76.0	76.0
6	75.1	75.0	75.2	75.0	6	81.3	81.5
1'	99.0	95.9	97.5	94.3	5'	72.6	72.6
2'	56.1	55.0	49.3	47.7	6'	39.0 (43.3)	39.1 (43.3)
3'	73.5	69.9	34.2	31.0	1''	100.7	100.9
4'	71.5	71.4	66.6	65.4	2''	72.0	68.9
5'	71.9	71.8	72.2	72.4	3''	54.9	56.2
6'	39.3 (43.2)	38.9 (42.9)	39.4 (43.4)	39.4 (43.0)	4''	69.7	66.5

[a]The figures in parentheses show chemical shifts of minor rotational isomers.

TABLE VII

RATIOS OF THE TRI-*N*-FORMYL POSITIONAL ISOMERS[a] OBTAINED BY PARTIAL, ALKALINE HYDROLYSES OF THE TETRA-*N*-FORMYL DERIVATIVES

Starting compound	*Compound deformylated at*		
	1-NH₂	*3-NH₂*	*2'- or 3''-NH₂*
1e	1 (**1b**)[b]	0.06 (**1a**)	0.27 (**1c**)
2e	1 (**2b**)	0.03 (**2a**)	0.34 (**2c**)
3e	1 (**3b**)	0.14 (**3a**)	0.73 (**3c**)
4e	1 (**4b**)	0.05 (**4a**)	0.55 (**4c**)

[a]The yields of **1d**, **2d**, **3d**, and **4d** were not determined quantitatively. [b]Parenthetical symbols show the positional isomers of the tri-*N*-formyl derivatives.

Steric interference in the vicinity of the amino group at C-3 of the neamine moiety was also observed in the following experiment. When neamine was treated with an excess of methyl trifluoroacetate in methanol, the main tris(trifluoroacetate) obtained was the 1,2',6'-tris(*N*-trifluoroacetyl) derivative, and the proportion of other positional isomers was negligible. The position of the unsubstituted amino group of the main product was determined as follows. The tris(*N*-trifluoroacetyl) derivative was acetylated with acetic anhydride in pyridine, and then the trifluoroacetyl groups were removed by treatment with methanolic ammonia to give a mono-*N*-acetyl-neamine; $[\alpha]_D$ +70.1° (*c* 1, water). The mono-*N*-acetyl derivative was treated with

1-fluoro-2,4-dinitrobenzene, and then the *N*-(2,4-dinitrophenyl) derivative was hydrolyzed to give 2-deoxy-1-*N*-(2,4-dinitrophenyl)streptamine, which was identified as its tetraacetate; $[\alpha]_D$ +29.0° (*c*, 1, chloroform).

Although the yield of each positional isomer of the tri-*N*-formyl derivatives is low in one experimental run, one of the advantages of this selective *N*-acylation method is that such byproducts as the undesirable positional isomers of the tri-*N*-formyl derivatives, as well as the di- and mono-*N*-formyl derivatives produced in the deformylation reaction, may be recycled readily for use as starting materials (as described in the Experimental section), and consequently the desired product may be obtained in good yield.

Although this selective *N*-acylation method is especially suited for the preparation of 1-*N*-modified derivatives, it is also feasible to prepare the 2′-*N*-modified derivatives of neamine-containing antibiotics and 3″-*N*-modified derivatives of kanamycin A by this method.

EXPERIMENTAL

General methods. — Optical rotations were measured with a Perkin–Elmer model 141 polarimeter at 23 ± 2°. P.m.r. spectra were recorded on a Varian HA-100 instrument, and chemical shifts are reported in p.p.m. from tetramethylsilane and coupling constants in Hz. I.r. spectra were obtained with a Jasco IRA-1 spectrophotometer. ^{13}C-N.m.r. spectra were recorded on a Varian XL-100 spectrometer operating at 25.2 MHz in the Fourier-transform mode in D_2O solutions at 34° with the use of 1,4-dioxane as internal reference. Chemical shifts are expressed in p.p.m. downfield from tetramethylsilane. Mass spectra were recorded with a JEOL O1SC type spectrometer, using the direct-insertion technique and an ionizing voltage of 70 eV. T.l.c. was performed on glass plates precoated with silica gel (Merck) with the solvent systems specified; the following solvent systems were put to frequent use: 10:10:0.5:10 1-propanol–pyridine–acetic acid–water (solvent *A*), 1:1:1 methanol–pyridine–0.4% aqueous ammonia (solvent *B*), 2:1:1 chloroform–methanol–17% aqueous ammonia (solvent *C*), 3:4:4 chloroform–methanol–28% aqueous ammonia, upper layer (solvent *D*), and 5:3 15% (w/v) sodium chloride solution–methanol (solvent *E*). Spots were detected by spraying the plates with 5% ethanolic sulfuric acid containing 0.2% (w/v) of naphthoresorcinol and then heating. Unless otherwise indicated, the compositions of solvent mixtures are expressed in volume %.

*6,2″-Di-*O*-acetyl-tetra-*N*-(benzyloxy)carbonyl-3″,5″-*O*-cyclohexylidene-3′-*O*-tosylxylostasin* (**5**). — This 3′-*O*-sulfonate **5** was synthesized from xylostasin (**1**) by a method virtually identical with that described for a synthesis of 6,5″-di-*O*-acetyl-tetra-*N*-(benzyloxy)carbonyl-2″,3″-*O*-cyclohexylidene-3′-*O*-tosylribostamycin from ribostamycin by Ikeda *et al.*[9]; $[\alpha]_D^{25}$ −9.1° (*c* 1, chloroform), p.m.r. data (chloroform-*d*): δ 2.30 (3H, s, C*H*$_3$-Ph-), 2.00 (6H, s, 2 acetyl), 1.2–1.7 (10H, m, cyclohexylidene).

Anal. Calc. for $C_{66}H_{76}N_4O_{22}S$: C, 60.54; H, 5.85; N, 4.28; S, 2.45. Found: C, 60.81; H, 6.06; N, 4.25; S, 2.63.

*6,2″-Di-*O*-acetyl-tetra-*N*-benzyloxycarbonyl-3″,5″-*O*-cyclohexylidene-3′-deoxy-xylostasin* (**6**). — The 3′-*O*-sulfonate **5** (2.3 g) and sodium iodide (23 g) were dissolved in acetone (200 ml) and the solution was heated for 48 h at 110° in a sealed tube. Insoluble material was filtered off and the filtrate was evaporated to dryness. The residue was partitioned between chloroform and water. The organic phase was washed successively with saturated sodium thiosulfate solution and water, and evaporated to dryness. The residue was dissolved in toluene (50 ml), and α,α′-azobis-(isobutyronitrile) (20 mg) and tributylstannane (1.0 ml) were added to the solution. The solution was refluxed for 3.5 h under nitrogen. The mixture was evaporated and the residue chromatographed on a column of silica gel with 4:1 chloroform–ethyl acetate to give the 3′-deoxygenated derivative **6** (0.8 g, 40%), $[\alpha]_D^{24}$ −25.9° (*c* 1, chloroform).

Anal. Calc. for $C_{59}H_{70}N_4O_{19}$: C, 62.20; H, 6.19; N, 4.92. Found: C, 62.55; H, 6.25; N, 4.64.

3′-Deoxyxylostasin (**2**). — The 3′-deoxygenated derivative **6** (0.4 g) was dissolved in ammonia-saturated methanol (50 ml) and the solution was kept overnight at room temperature. The mixture was evaporated to dryness and the residue was dissolved in 70% aqueous acetic acid, and the solution was stirred for 2 h at 50°. Palladium black was then added and the mixture was subjected to catalytic hydrogenolysis for 3 h. The catalyst was filtered off and the filtrate was evaporated to dryness. The residue was adsorbed on a column of CM-Sephadex (NH_4^+), and the column was eluted with 0.3% aqueous ammonia. The eluate was concentrated and lyophilized to give **2**; yield 140 mg (77%); R_F 0.58 (solvent *D*, compare xylostasin, R_F 0.54), m.p. 171–173° (decomp.), $[\alpha]_D^{23}$ +27.4° (*c* 1, water); p.m.r. data (D_2O): δ 5.51 (1H, d, *J* 3.5, H-1′), 5.38 (1H, d, *J* < 1, H-1″), 2.0–2.3 (2H, m, H-2eq and H-3′eq), 1.75 (1H, q, *J* 12, H-3′ax), 1.31 (1H, q, *J* 13, H-2ax).

Anal. Calc. for $C_{17}H_{34}N_4O_9 \cdot H_2O$: C, 44.73; H, 7.95; N, 12.27. Found: C, 44.50; H, 7.90; N, 12.11.

*3′-Deoxy-tetra-*N*-formylxylostasin* (**2e**). — *A.* A solution of *p*-nitrophenyl formate (10 g) and **2** (4.38 g) in 50% aqueous 1,4-dioxane (150 ml) was stirred for 16 h at room temperature, and then evaporated. A solution of the residue in water (20 ml) was washed with ethyl acetate. The aqueous phase was applied to a column of Amberlite CG-50 (H^+, 250 ml), and the column was eluted with water. The eluate was concentrated and lyophilized to give **2e** (4.74 g, 86%).

B. To a suspension of **2** (8.77 g) in *N,N*-dimethylformamide (50 ml), acetic formic anhydride (20 ml) was added with stirring and cooling in ice–water. The mixture was further stirred for 16 h at room temperature. The resulting, clear solution was evaporated to an oil, and the 3′-deoxy-per-*N,O*-formylxylostasin (13.7 g) was precipitated by addition of ethyl acetate; ν_{max}^{KBr} 1660 (amide, type I band) and 1720–1730 (ester C=O) cm^{-1}. The per-*N,O*-formyl derivative was dissolved in 1% aqueous ammonia (500 ml), and the solution was kept for 30 min at room temperature. The

mixture was adjusted to pH 6 with acetic acid, and passed through a column of activated carbon (250 ml). After washing with water and 10% aqueous methanol, the column was eluted with 50% aqueous methanol. The effluent was concentrated and lyophilized to give **2e** (10.5 g, 95%); p.m.r. data (D_2O): δ 8.26 and 8.08 (minor rotational-isomer peak) (total 4H, 4 formyl), 5.54 (1H, d, J 3.5, H-1'), 5.41 (1H, d, $J < 1$, H-1''), 1.85–2.25 (2H, m, H-2ex and H-3'ex), and 1.2–2.0 (2H, m, H-2ax and H-3'ax). The R_F, $[\alpha]_D$, and ^{13}C n.m.r. data are shown in Tables I, II, and IV, respectively.

3'-Deoxy-1,2',6'-tri-N-*formylxylostasin* (**2a**), *3'-deoxy-3,2',6'-tri*-N-*formylxylostasin* (**2b**), *3'-deoxy-1,3,6'-tri*-N-*formylxylostasin* (**2c**), *and 3'-deoxy-1,3,2'-tri*-N-*formylxylostasin* (**2d**). — A solution of the tetra-*N*-formyl derivative (**2e**) (10.0 g) in 10% aqueous ammonia (1 liter) was kept for 5 days at room temperature and then evaporated. The residue was dissolved in water (50 ml) and adsorbed onto a column of Amberlite CG-50 (NH_4^+, 850 ml). When the column was developed with water, a mixture of the unreacted tetra-*N*-formyl derivative **2e** and ammonium formate was eluted first, and then the tri-*N*-formyl derivatives **2a**, **2b**, and **2c** were eluted in this order. The fractions were monitored by t.l.c. (Table I), and concentrated and lyophilized to yield 35 mg (0.4%) of **2a**, 1.282 g (14%) of **2b**, and 404 mg (4%) of **2c**, respectively. After elution with water, the tri-*N*-formyl derivative **2d** was eluted with 0.1% aqueous ammonia. Crude **2d** was purified by preparative t.l.c. on silica gel (solvent *A*) to obtain the sample for analytical use, because **2d** overlapped somewhat the di-*N*-formyl derivatives in the chromatography on Amberlite CG-50. Derivatives further deformylated were eluted with 2% aqueous ammonia. The mixture of **2e** and ammonium formate was passed through a carbon column (100 ml). After washing with water and 10% aqueous methanol, the column was eluted with 50% aqueous methanol. The effluent was concentrated and lyophilized to recover pure **2e** (2.93 g). The fractions containing the di-*N*-formyl derivatives and the further deformylated compounds were evaporated. To a suspension of the resulting residue (3.55 g) in *N,N*-dimethylformamide (10 ml), acetic formic anhydride (2.5 ml) was added under stirring and cooling in an ice–water bath, and then the mixture was stirred for 16 h to give 3'-deoxy-per-*N,O*-formylxylostasin (4.26 g). The 3'-deoxy-per-*N,O*-formylxylostasin was dissolved in 10% aqueous ammonia (400 ml), and the solution was kept for 5 days at room temperature. The second crop (480 mg) of the tri-*N*-formyl derivative **2b** was obtained by a chromatographic method similar to that already described. The R_F, $[\alpha]_D$ and ^{13}C n.m.r. data of these tri-*N*-formyl derivatives are summarized in Tables I, II, and IV, respectively.

Tetra-N-*formylxylostasin* (**1e**), *tetra*-N-*formylkanamycin A* (**3e**), *tetra*-N-*formylneamine* (**4e**), *tri*-N-*formylxylostasins* (**1a**, **1b**, **1c**, **1d**), *tri*-N-*formylkanamycins A* (**3a**, **3b**, **3c**, **3d**), *and tri*-N-*formylneamines* (**4a**, **4b**, **4c**, **4d**). — Compounds **1a–e**, **3a–e**, and **4a–e** were prepared from **1**, **3**, and **4**, respectively, as described for compounds **2a–e**.

The yields of **1b**, **3b**, and **4b** were 26%, 15%, and 20% from the corresponding

tetra-*N*-formyl derivatives, respectively (compare Table VII). The physicochemical properties are summarized in Tables I–VI.

1-N-[(S)-*4-amino-2-hydroxybutanol*]-*3'-deoxyxylostasin* (*3'-deoxybutirosin A*, **2f**). — A solution of the 3,2',6'-tri-*N*-formyl derivative **2b** (1.0 g) and the *N*-hydroxysuccinimide ester of (*S*)-2-hydroxy-4-phthalimidobutanoic acid (800 mg) in *N*,*N*-dimethylformamide (20 ml) was kept overnight at room temperature. After concentration of the mixture, ethyl acetate was added to precipitate the crude 1-*N*-[(*S*)-2-hydroxy-4-phthalimidobutanyl] derivative. The precipitate was dissolved in 10% hydrazine hydrate (50 ml), and then the solution was adjusted to pH 6 with acetic acid and boiled for 6 h under reflux. The mixture was refrigerated overnight and the resulting, insoluble crystalline material was filtered off. The filtrate was diluted with water (100 ml) and passed through a column of Amberlite CG-50 resin (NH_4^+, 130 ml). After washing with water and 0.4% aqueous ammonia, the column was eluted with 0.8% aqueous ammonia. The fractions containing **2f** were collected, concentrated, and lyophilized to yield 614 mg (60%) of **2f**; $[\alpha]_D^{25}$ +20.7° (*c* 1, water); R_F 0.31 (solvent *C*), R_F 0.48 (solvent *E*).

Anal. Calc. for $C_{21}H_{41}N_5O_{11} \cdot 2H_2O$: C, 43.82; H, 7.88; N, 12.17. Found: C, 43.85; H, 7.98; N, 12.06.

1-N-[(S)-*4-amino-2-hydroxybutanoyl*]*xylostasin* (*butirosin A*, **1f**). — Compound **1f** (780 mg, 76%) was prepared from the 3,2',6'-tri-*N*-formyl derivative **1b** (1.0 g) as described for **2f**; $[\alpha]_D^{24}$ +23.7° (*c* 1, water), lit.[10] $[\alpha]_D^{25}$ +26.0° (*c* 1.46, water); R_F 0.32 (solvent *C*), R_F 0.32 (solvent *E*).

Anal. Calc. for $C_{21}H_{41}N_5O_{12} \cdot H_2O$: C, 43.97; H, 7.56; N, 12.21. Found: C, 43.81; H, 7.68; N, 12.13.

1-N-[(S)-*4-amino-2-hydroxybutanoyl*]*kanamycin A* (*BB-K8*, **3f**). — Compound **3f** (720 mg, 66%) was prepared from the 3,6',3''-tri-*N*-formyl derivative **3b** (1.0 g) as described for **2f**; $[\alpha]_D^{22}$ +101° (*c* 1, water), lit.[5] $[\alpha]_D^{23}$ +99° (*c* 1, water); R_F 0.33 (solvent *E*, compare kanamycin A R_F 0.41), R_F 0.22 (solvent *D*; compare kanamycin A, R_F 0.50).

Anal. Calc. for $C_{22}H_{43}N_5O_{13} \cdot 2H_2O$: C, 42.51; H, 7.62; N, 11.27. Found: C, 42.48; H, 7.62; N, 11.26.

6'-N-(*Benzyloxy*)*carbonyl-3'-deoxyxylostasin* (**7**). — To a solution of **2** (4.38 g) in 50% aqueous tetrahydrofuran (100 ml), a solution of benzyl *p*-nitrophenyl carbonate (3.2 g) in tetrahydrofuran (20 ml) was added with stirring and cooling in an ice–water bath. The solution was stirred overnight at room temperature, and then evaporated. The residue was extracted with water (50 ml) and the mixture was filtered to remove insoluble material. The filtrate was adsorbed on a column of Amberlite CG-50 resin (NH_4^+, 250 ml). The column was washed with water and then eluted with 0.2% aqueous ammonia to give compound **7**; yield 2.44 g (43%), $[\alpha]_D^{22}$ +24.6° (*c* 1, water); R_F 0.31 (105:45:50 1-propanol–ethyl acetate–28% ammonia water); p.m.r. data (D_2O): δ 7.52 (5H, s, *Ph*-CH_2-), 5.20 (3H, PH-CH_2- and H-1'', overlapped), 5.33 (1H, d, *J* 3.5, H-1'), 1.8–2.3 (2H, m, H-2eq and H-3'eq), 1.74 (1H, q, *J* 12, H-3'ax), 1.18 (1H, q, *J* 13, H-2ax).

Anal. Calc. for $C_{25}H_{40}N_4O_{11} \cdot 2H_2O$: C, 49.33; H, 7.29; N, 9.21. Found: C, 49.21; H, 7.05; N, 9.35.

6'-N-(*benzyloxy*)*carbonyl-3'-deoxy-1,3,2'-tri*-N-*formylxylostasin* (**8**). — *p*-Nitrophenyl formate (4.6 g) and compound **7** (2.3 g) were dissolved in 1:1 *N,N*-dimethylformamide–water (100 ml) and the solution was stirred overnight at room temperature. The mixture was evaporated, and the residue was partitioned between water and ethyl acetate. The aqueous layer was concentrated to 10 ml and the concentrate was applied to a column of Amberlite CG-50 resin (H^+, 250 ml), which was eluted with water. The appropriate fractions were concentrated and lyophilized to give the tri-*N*-formyl derivative **8**; yield 2.4 g (91%), $[\alpha]_D^{22}$ +25.5° (*c* 1, water); p.m.r. data (D_2O): δ 8.14, 8.07, and 8.00 (total 3H, 3 formyl), 7.46 (5H, s, *Ph*-CH_2-), 5.17 (2H, s, Ph-CH_2-), 5.38 (1H, d, *J* 3.5, H-1'), 5.18 (1H, d, $J < 1$, H-1''), 1.9–2.2 (2H, m, H-2eq and H-3'eq), and 1.3–1.9 (2H, m, H-2ax and H-3'ax).

Anal. Calc. for $C_{28}H_{40}N_4O_{14} \cdot H_2O$: C, 49.84; H, 6.28; N, 8.30. Found: C, 49.88; H, 6.14; N, 8.40.

3'-Deoxy-1,3,2'-tri-N-*formylxylostasin* (**2d**) *from* **8**. — To a solution of compound **8** (2.0 g) in 1:0.1:2 methanol–acetic acid–water (100 ml), palladium black (200 mg) was added. The mixture was hydrogenated for 3 h at room temperature in a stream of hydrogen. The catalyst was filtered off and washed with 1% aqueous acetic acid (100 ml). The filtrate and the washings were combined and concentrated to dryness. The residue was chromatographed on Amberlite CG-50 resin (NH_4^+, 250 ml). The column was washed with water and eluted with 0.1% aqueous ammonia to give the 1,3,2'-tri-*N*-formyl derivative **2d** (1.21 g, 76%).

3-N-*Acetyl-4,5,6-tri*-O-*acetyl-2-deoxy-1*-N-(*2,4-dinitrophenyl*)*streptamine* (**9**). — *A. From 3,2',6'-tri*-N-*formylneamine* (**4b**). To a solution of the tri-*N*-formyl derivative **4b** (200 mg) in 50% aqueous ethanol (50 mg), sodium hydrogencarbonate (200 mg) and 1-fluoro-2,4-dinitrobenzene (200 mg) were added. After stirring overnight at room temperature, the mixture was neutralized with M hydrochloric acid, and evaporated. The residue was partitioned between water and ethyl acetate. The aqueous layer was concentrated to 5 ml, and applied to a column of Amberlite CG-50 resin (H^+, 130 ml). After washing with water, the column was eluted with 1% aqueous ethanol. The eluate was concentrated and lyophilized to give 1-*N*-(2,4-dinitrophenyl)-3,2',6'-tri-*N*-formylneamine (238 mg, 85%) as a yellowish powder. $[\alpha]_D^{22}$ +91.6° (*c*, 1, water); R_F 0.53 (4:1:1 1-propanol–acetic acid–water).

Anal. Calc. for $C_{21}H_{28}N_6O_{13} \cdot H_2O$: C, 42.71; H, 5.12; N, 14.23. Found: C, 42.31; H, 5.06; N, 14.12.

The mono-*N*-(2,4-dinitrophenyl)-tri-*N*-formyl derivative was dissolved in 6M hydrochloric acid (50 ml) and then the solution was boiled for 10 h under reflux. The hydrolyzate was evaporated, pyridine (20 ml) and acetic anhydride (10 ml) were added to the residue, and the mixture was kept overnight at room temperature. The solution was evaporated and the residue was chromatographed on a column of silica gel (100 ml) with 1:1 chloroform–ethyl acetate. The tetraacetate **9** was obtained as yellow needles; yield 36 mg, (42%); $[\alpha]_D^{23}$ +29.8° (*c* 1, chloroform), p.m.r. data

(chloroform-*d*): δ 9.05 (1H, d, *J* 2.0, aromatic H), 8.55 (1H, d, *J* 8.5, aromatic H), 8.25 (1H, dd, *J* 2.0 and 8.5 aromatic H), 7.17 (1H, d, *J* 9.5, -N*H*- at C-3), 6.26 (1H, d, *J* 9.5, -N*H*- at C-1), 4.9–5.4 (3H, m, H-4,5,6), 3.9–4.5 (2H, m, H-1,3), 2.2–2.7 (1H, m, H-2eq), 1.7–2.1 (1H, m, H-2ax), 2.09, 2.00, 1.95, and 1.85 (3H × 4, 4s, 4 acetyl).

Anal. Calc. for $C_{20}H_{24}N_4O_{11}$: C, 48.39; H, 4.87; N, 11.29. Found: C, 48.30; H, 4.82; N, 10.97.

B. Via 1,2′,6′-tris(N-*trifluoroacetyl*)*neamine.* Methyl trifluoroacetate (5.0 ml) and **4** (2.0 g) in methanol (50 ml) were stirred for 24 h at room temperature, and the mixture was evaporated to dryness. The residue was chromatographed on a column of silica gel with 4:1 ethyl acetate–acetone. The fractions were monitored by t.l.c. (5:1 ethyl acetate–ethanol) and those containing the component having R_F 0.17 were combined and evaporated. The resulting tris(*N*-trifluoroacetyl)neamine (1.0 g) was acetylated conventionally with pyridine and acetic anhydride for 24 h at room temperature. The mixture was evaporated, methanol was added to the residue, the solution was evaporated again, and the residue was chromatographed on a column of silica gel. The column was eluted with 1:1 chloroform–ethyl acetate and the appropriate fractions containing the component having R_F 0.31 (t.l.c., 1:2 chloroform–ethyl acetate) were concentrated to dryness to give the peracetate. The peracetate (1.1 g) was dissolved in methanolic ammonia. The solution was stirred for 20 h at room temperature and then evaporated. The residue was chromatographed on a column of Dowex-1 × 2 (OH^-) resin with water to give a mono-*N*-acetylneamine, $[\alpha]_D^{22}$ +70.1° (*c* 1, water), p.m.r. data (D_2O): δ 2.14 (3H, s, acetyl); mass spectrum: *m/e* 365 (M^+ +1), 364 (M^+), 233.

To a solution of the mono-*N*-acetylneamine (440 mg) in 50% aqueous ethanol (50 ml), sodium hydrogencarbonate (500 mg) and 1-fluoro-2,4-dinitrobenzene (500 mg) was added, and the mixture was stirred overnight at room temperature. The mixture was neutralized with M hydrochloric acid and evaporated. The residue was extracted with 2:1 benzene–acetone and the extract was applied to a column of silica gel. The column was eluted with 2:1 benzene–acetone to give the per(2,4-dinitrophenyl)derivative. By hydrolyzing this product as in the foregoing experiment 2-deoxy-1-*N*-(2,4-dinitrophenyl)streptamine was obtained and characterized as its tetraacetate (294 mg).

1-N-*acetyl-4,5,6-tri*-O-*acetyl-2-deoxy-3*-N-(*2,4-dinitrophenyl*)*streptamine* (**10**). — *A. From butirosin A* (**1f**). To a solution of **1f** (1.0 g) in 50% aqueous ethanol (120 ml), sodium hydrogencarbonate (1.1 g) and 1-fluoro-2,4-dinitrobenzene (2.4 g) were added, and the mixture was stirred for 24 h at room temperature. The mixture was neutralized with M hydrochloric acid and evaporated. The residue was extracted with 1:1 benzene–acetone and the extracts were applied to a column of silica gel (200 ml), and the column was eluted with 1:1 benzene–acetone to give tetrakis(*N*-2,4-dinitrophenyl)butirosin A. The residue was dissolved in 1:1 concentrated hydrochloric acid–1,4-dioxane, and then the solution was boiled for 8 h under reflux. The mixture was evaporated and the residue was chromatographed on a column of silica

gel (100 ml) with 75% aqueous methanol. The resulting 2-deoxy-3-*N*-(2,4-dinitrophenyl)streptamine was acetylated conventionally with acetic anhydride–pyridine to give the tetraacetate **10** (171 mg, 19% from **1f**), $[\alpha]_D^{22}$ −30.8° (*c* 1, chloroform).

Anal. Calc. for $C_{20}H_{24}N_4O_{11}$: C, 48.39; H, 4.87; N, 11.29. Found: C, 48.46; H, 4.88; N, 10.93.

*B. From 1,2′,6′-tri-*N*-formylneamine* (**4a**). By treating the tri-*N*-formyl derivative **4a** (200 mg) with 1-fluoro-2,4-dinitrobenzene as described for the positional isomer **4b**, 3-*N*-(2,4-dinitrophenyl)-1,2′,6′-tri-*N*-formylneamine (250 mg, 89%) was obtained; $[\alpha]_D^{22}$ +74.4° (*c* 1, water); R_F 0.55 (4:1:1 1-propanol–acetic acid–water).

Anal. Calc. for $C_{21}H_{28}N_6O_{13} \cdot 2H_2O$: C, 41.45; H, 5.30; N, 13.81. Found: C, 41.34; H, 4.82; N, 14.04.

This mono-*N*-(2,4-dinitrophenyl)-tri-*N*-formyl derivative (100 mg) was hydrolyzed with 6M hydrochloric acid and the product acetylated to give **10** (41 mg, 47%), $[\alpha]_D^{23}$ −31.4° (*c* 1, chloroform) as described for the preparation of the enantiomer **9**.

ACKNOWLEDGMENTS

We thank Drs. S. Tatsuoka, E. Ohmura, K. Mizuno, and M. Yoneda, Central Research Division of our company for their support and encouragement throughout this study. Grateful acknowledgment is extended to Mr. E. Mizuta for operating the ^{13}C-n.m.r. spectrometer and for helpful advice and discussions, and to Messrs. Y. Yasumura, K. Kawahara, and T. Matsuyama for their technical assistance.

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