THE SYNTHESES AND BIOLOGICAL PROPERTIES OF 1-*N*-(*S*-4-AMINO-2-HYDROXYBUTYRYL)-GENTAMICIN B AND 1-*N*-(*S*-3-AMINO-2-HYDROXYPROPIONYL)-GENTAMICIN B*

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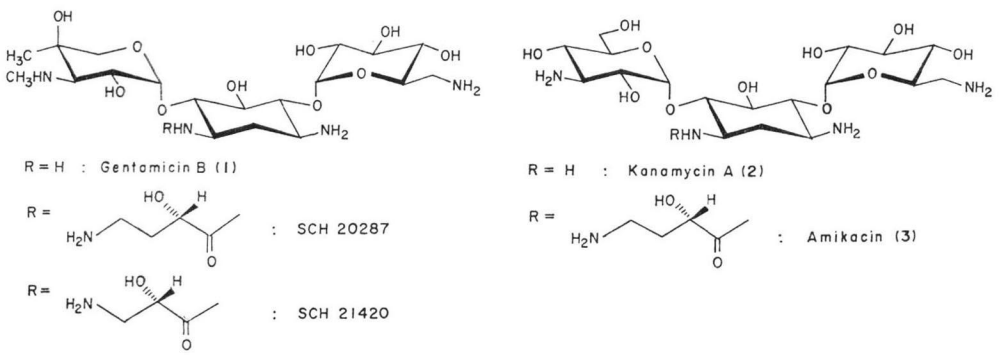
The syntheses of 1-*N*-(*S*-4-amino-2-hydroxybutyryl)-gentamicin B and 1-*N*-(*S*-3-amino-2-hydroxypropionyl)-gentamicin B, designated Sch 20287 and Sch 21420, respectively, by procedures similar to those developed by KAWAGUCHI and co-workers for the transformation of kanamycin A to amikacin are described. The *in vitro* microbiological properties of Sch 20287 and Sch 21420 are compared with amikacin, gentamicin and tobramycin.

The syntheses and preliminary *in vitro* microbiological properties of 1-*N*-(*S*-4-amino-2-hydroxybutyryl)- and 1-*N*-(*S*-3-amino-2-hydroxypropionyl)-derivatives of gentamicin C_1[1] and gentamicin C_{1a}[2] were published from our laboratory. In this paper we present the syntheses of the analogous gentamicin B derivatives and discuss their microbiological properties.

Gentamicin B is 4-*O*-(6-amino-6-deoxy-α-D-glucopyranosyl)-garamine (**1**)[3] and is coproduced in the gentamicin fermentation[4]. Its close structural relationship with kanamycin A (**2**) prompted us to synthesize the 1-*N*-(*S*-4-amino-2-hydroxybutyryl)- and 1-*N*-(*S*-3-amino-2-hydroxypropionyl)- derivatives (Sch 20287 and Sch 21420, respectively) to compare their biological properties with amikacin, the 1-*N*-(*S*-4-amino-2-hydroxybutyryl)- derivative of kanamycin A (**3**)[5,6].

As illustrated in Scheme 1, the conversion of gentamicin B (**1**) to Sch 20287 and Sch 21420 was accomplished following the procedure of KAWAGUCHI and co-workers[5] for the synthesis of amikacin (**3**) from kanamycin A (**2**). The condensation of 6′-*N*-*tert*-butoxycarbonyl-gentamicin B (**4**) with *N*-(*S*-4-benzyloxycarbonylamino-2-hydroxybutyryloxy)-succinimide[1,2,5] afforded a mixture of products from which the desired 1-*N*-(*S*-4-benzyloxycarbonylamino-2-hydroxybutyryl)-6′-*N*-*tert*-butoxycarbonyl



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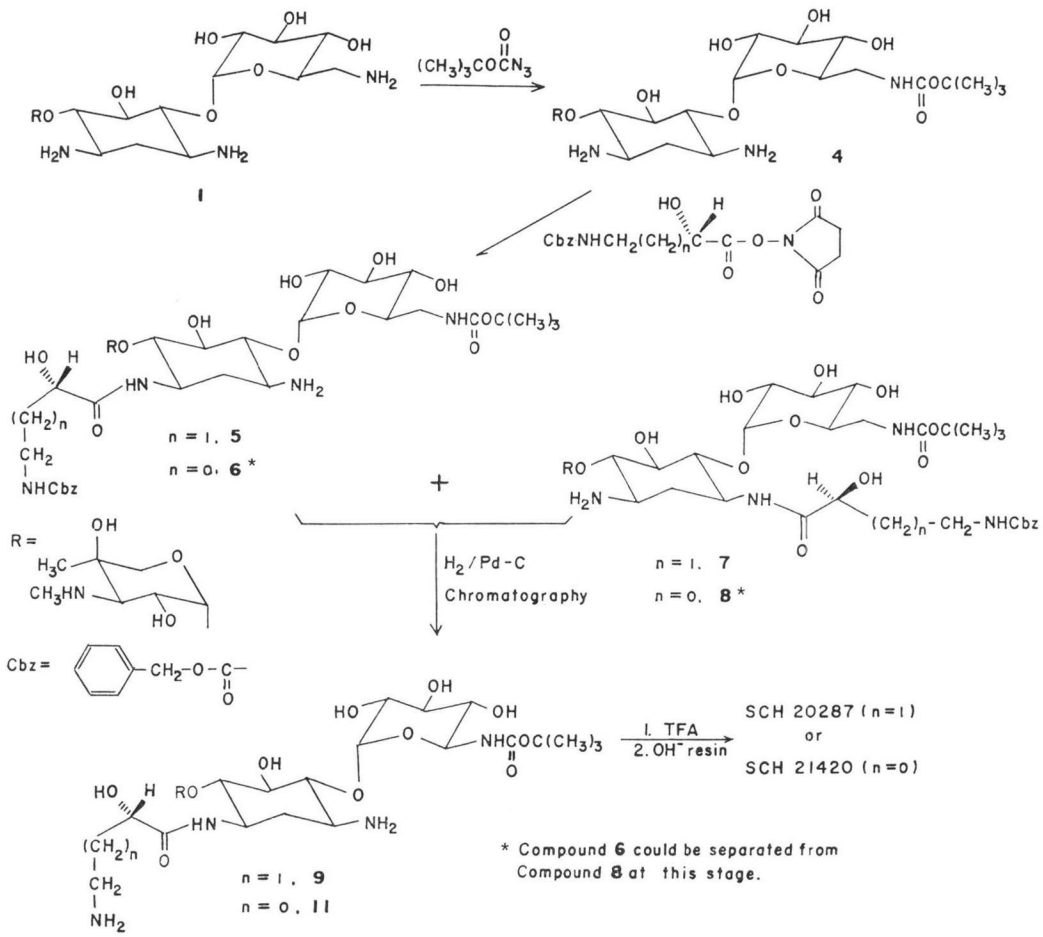
Scheme 1.



gentamicin B (**5**) was isolated by chromatography as a mixture with its 3-isomer (**7**). Similar results were obtained when compound (**4**) was condensed with *N*-(*S*-3-benzyloxycarbonylamino-2-hydroxypropionyloxy)-succinimide[1,2]. However, in this case, the desired condensation product (**6**) could be separated from its **3** isomer (**8**). After removal of the benzyloxycarbonyl group by catalytic hydrogenation, the 1-*N*-(*S*-4-amino-2-hydroxybutyryl)-6′-*N*-*tert*-butoxycarbonyl-gentamicin B (**9**) could be separated from the 3-isomer (**10**) by column chromatography. Catalytic hydrogenation of compound **6** afforded compound (**11**), the analogous 1-*N*-propionyl derivative. Removal of the *tert*-butoxycarbonyl protecting groups of (**9**) and (**11**) with trifluoroacetic acid and deionization led to the desired compounds, Sch 20287 and Sch 21420, respectively.

Confirmation of the structures of Sch 20287 and Sch 21420 was obtained from a study of the proton magnetic resonance and proton-decoupled carbon-13 magnetic resonance spectra of these two compounds and amikacin in basic and acidic media. In Table 1, the carbon-13 chemical shifts of Sch 20287 and Sch 21420 and amikacin are given along with those of gentamicin B (**1**) and kanamycin A (**2**)[7,8]. The pmr parameters are given in the experimental section and are self-explanatory. A comparison of the ^{13}C chemical shifts of C6 in kanamycin A (88.5 ppm) and amikacin (81.3 ppm) indicate a shielding of 7.2 ppm in the latter caused by attachment of the *S*-4-amino-2-hydroxybutyryl side chain

to the amino group at the 1-position of kanamycin A. However, no significant change in the resonance positions of C2 (35.8 ppm) and C4 (86.8 ppm) takes place. Furthermore, as shown in Table 1, upon acidification, C6 of kanamycin A (88.5 ppm) undergoes a shielding of 4 ppm due to protonation of the 1-amino group and C2 (35.8 ppm) experiences an upfield shift of nearly twice that magnitude (7.6 ppm) due to protonation of both the 1- and the 3- amino groups on adjacent carbon atoms. In contrast to C6, the C4 nucleus of kanamycin A (86.8 ppm) experiences a much larger upfield shift on protonation, the magnitude of which is nearly twice that of C6 although there is only one amino group in the β-position. Also notable is the fact that C1′ of kanamycin A, which does not have an amino group in the β-position, undergoes an upfield shift of 3.2 ppm on protonation of the amino groups elsewhere in the molecule. Consistent with the structure, protonation of amikacin causes an upfield shift of C2 of only 4.2 ppm as compared to protonation of kanamycin A which causes an upfield shift of C2 of 7.6 ppm indicating substitution at one of the deoxystreptamine nitrogens by the acyl side chain. The fact that C6 of amikacin undergoes no acid shift and C4 still maintains the unexpectedly large shielding (7.8 ppm) on protonation found in kanamycin A confirms that the acyl side chain in amikacin is at

Table 1. Carbon-13 chemical shifts of kanamycin A, amikacin, gentamicin B, Sch 21420 and Sch 20287

Carbon nucleus	Kanamycin A			Amikacin			Gentamicin B			Sch 21420			Sch 20287		
	pD 9.5	2	Δ	>9.0	<3	Δ	9.5	4	Δ	9.5	~3	Δ	~9	~2	Δ
1	51.2	50.6		50.4	49.5		51.4	50.6		50.2	49.4		50.2	49.5	
2	35.8	28.2	−7.6	35.0	30.8	−4.2	36.2	28.3	−7.9	35.2	30.8	−4.4	35.0	30.9	−4.1
3	49.6	48.4		49.4	48.6		49.7	48.4		49.4	48.8		49.4	48.8	
4	86.8	78.8	−8.0	87.7	79.9	−7.8	87.7	79.0	−8.7	87.5	79.8	−7.7	86.8	79.9	−6.9
5	74.9	73.4		75.4	73.2		74.9	73.1		75.3	73.3		75.2	73.4	
6	88.5	84.5	−4.0	81.3	81.2	0	87.7	84.6	−3.1	80.6	80.9	0	80.7	80.7	0
1′	99.6	96.4	−3.2	99.2	96.3	−2.9	100.1	96.6	−3.5	99.7	96.6	−3.1	99.3	96.6	−2.7
2′	71.9	71.7		72.5	72.8		72.6	71.7		72.9	71.7		72.6	71.7	
3′	73.5	72.9		73.8	71.7		73.7	73.0		73.7	73.1		73.6	73.1	
4′	71.9	71.6		71.9	71.7		71.9	71.6		71.9	71.1		71.9	71.7	
5′	73.0	69.4	−3.6	73.8	69.5	−4.3	72.8	69.5	−3.3	72.9	69.4	−3.5	72.6	69.5	−3.1
6′	41.9	41.2		42.3	41.1		42.1	41.1		42.2	41.2		42.0	41.2	
1″	100.2	101.2		100.4	98.8		101.3	102.0		100.3	99.2		100.0	98.9	
2″	72.5	68.9	−3.6	72.7	68.8	−3.9	69.7	67.2	−2.5	69.1	66.9	−2.2	69.1	66.9	−2.2
3″	55.1	55.7		54.9	56.1		64.4	64.2		64.2	65.2		64.5	65.3	
4″	70.0	66.3	−3.7	70.2	66.4	−3.8	72.6	70.8	−1.8	72.7	70.7	−2.0	72.1	70.8	−1.3
5″	72.5	72.9		72.9	72.8		68.5	68.6		68.4	68.5		68.5	67.8	
6″	61.1	60.9		61.2	60.6										
N-Me							37.4	35.3		37.6	35.9		37.2	35.9	
C-Me							22.5	21.7		22.4	21.8		22.3	21.8	
1‴				177.5	176.5					175.8	173.8		176.5		
2‴				70.7	70.5					73.3	67.7	−5.6	70.5	70.5	
3‴				36.9	31.5	−5.4				44.5	42.6		37.2	31.6	−5.6
4‴				38.0	37.7								37.7	37.8	

the 1-position. As in kanamycin A, the "non β-amino-protonation shielding" of C1′ is still observed in amikacin. Furthermore, like in kanamycin A, the nuclei C5′, C2″, and C4″ of amikacin show the expected upfield shifts on protonation confirming the presence of amino groups at C6′ and C3″.

It was apparent, therefore, that a similar study with gentamicin B, Sch 21420 and Sch 20287 would allow unequivocal assignments of structures to the latter two derivatives. Comparison of ^{13}C chemical shifts of Sch 21420 and Sch 20287 with those of the parent compound, gentamicin B (**1**)[9~11] at acidic and basic pD's clearly established their structures (Table 1). Thus, the C6 resonance in Sch 21420 and Sch 20287 was shifted 7.1 ppm and 7.0 ppm, respectively, to higher field relative to the corresponding signal in gentamicin B (87.7 ppm). Protonation of these two compounds showed no change in the chemical shift of C6, and C2 experienced an upfield shift of only 4.4 ppm and 4.1 ppm, respectively. Again, C4 showed the unusually large shielding observed in these types of structures on protonation. These results and the fact that the chemical shifts of the nuclei of the two aminosugar residues in Sch 21420 and Sch 20287 were identical, within experimental error, with the corresponding nuclei of gentamicin B at both acidic and basic pD's established that the acyl side chain in these two compounds was located at the 1-position.

Biology

The *in vitro* microbiological properties of Sch 20287 and Sch 21420 are summarized in Tables 2~4. The minimum inhibitory concentrations (MIC's) were determined against a number of Gram-positive and Gram-negative organisms and the median values are reported in the Tables. As seen in Table 2, against gentamicin-sensitive strains which include several strains resistant to neomycin and kanamycin mediated by phosphorylating enzymes [APH(3′)], both Sch 21420 and Sch 20287 are active and their potencies are very similar and parallel those of amikacin. Except against sensitive strains of *Providencia*

Table 2. The activities of Sch 21420 and Sch 20287 against gentamicin sensitive strains*

Organism	No. of strains tested	Median MIC (mcg/ml 24 hours)				
		Gentamicin	Tobramycin	Amikacin	Sch 21420	Sch 20287
Enterobacter	5	0.25	0.5	1	0.5	0.5
Escherichia coli	6	0.25	0.25	0.5	0.25	0.125
Pseudomonas aeruginosa	17	2	1	4	8	8
Providencia	9	4	2	0.5	0.5	0.5
Serratia	4	0.5	1	2	1	2
Proteus	9	0.25	0.25	0.5	0.5	0.5

* Includes many strains resistant to neomycin and kanamycin *via* phosphorylating enzymes [APH (3′)].

Table 3. The activities of Sch 21420 and Sch 20287 against adenylylating strains

Organism	No. of strains tested	Inactivation mechanism	Median MIC (mcg/ml 24 hours)				
			Genta-micin	Tobra-mycin	Amikacin	Sch 21420	Sch 20287
Enterobacter	3	ANT (2″)	32	128	1	0.5	0.5
Escherichia coli	6	ANT (2″)	32	64	2	0.5	0.5
Klebsiella	14	ANT (2″)	64	64	1	0.5	0.5
Pseudomonas aeruginosa	6	ANT (2″)	128	64	2	2	4
Serratia	8	ANT (2″)	32	32	2	1	2
Staphylococcus	5	ANT (4′)	0.5	>64	32	32	—

Table 4. The activities of Sch 21420 and Sch 20287 against acetylating strains

Organism	No. of strains tested	Inactivation mechanism	Median MIC (mcg/ml 24 hours)				
			Genta-micin	Tobra-mycin	Amikacin	Sch 21420	Sch 20287
Pseudomonas aeruginosa	14	AAC (3)-I	64	2	2	2	4
Providencia	9	AAC (2′)	64	—	2	4	—
Proteus rettgeri	7	AAC (2′)	64	—	8	8	—
Serratia	4	AAC (6′)-I	4	—	32	16	—
Pseudomonas aeruginosa	9	AAC (6′)-II[13]	128	—	4	8	—

and *Escherichia coli*, gentamicin is more potent than either Sch 21420 or Sch 20287. While it is equipotent with the latter two compounds against sensitive *E. coli*, gentamicin is considerably less potent against sensitive *Providencia*.

The activities of Sch 21420 and Sch 20287 against organisms known to inactivate aminoglycoside antibiotics by adenylylation at the 2″- position are compared with those of gentamicin and amikacin in Table 3. Sch 20287 appeared to be slightly less potent than Sch 21420 against *Pseudomonas aeruginosa* and *Serratia* but equipotent against *Enterobacter*, *E. coli* and *Klebsiella*. Sch 21420 was more potent than amikacin against strains of *Enterobacter, E coli, Klebsiella* and *Serratia*, and equipotent against *P. aeruginosa*. Neither Sch 21420 nor amikacin was active against gentamicin-sensitive kanamycin-resistant *Staphylococcus* containing [ANT (4′)] mode of inactivation.

Finally, Sch 21420 and amikacin had identical spectra of activity against gentamicin-resistant Gram-negative organisms containing various aminoglycoside *N*-acetylating enzymes. These results are shown in Table 4.

In potency, Sch 21420 was equi-active or slightly more potent than amikacin against strains of *Proteus rettgeri* and *Serratia* containing *N*-acetylating enzymes. Against strains of *P. aeruginosa* containing *N*-acetylating enzymes, Sch 21420 was equal to, or slightly less active than amikacin. Against *Providencia* strains possessing [AAC (2′)] enzymes, amikacin was slightly more active.

Detailed biology of Sch 21420 is described in the accompanying paper[12].

Experimental

Details of experimental techniques have been published in our earlier papers[1,8].

6′-*N*-*tert*-Butoxycarbonylgentamicin B (4)

To a stirred solution of gentamicin B (**1**) (1 g, 2.07 mmol) in 50% aqueous methanol (30 ml) at 5°C was added *tert*-butoxycarbonyl azide (0.297 g, 2.26 mmol) in a dropwise manner. Triethylamine (0.186 ml) was added next and the solution stirred for 18 hours at room temperature. The solution was concentrated to dryness *in vacuo* and the residue chromatographed on a column of silica gel (60~200 mesh) (100 g) using the lower phase of a solvent mixture consisting of chloroform, methanol and ammonium hydroxide (2 : 1 : 1) as the eluant. Work-up of the homogeneous fractions (tube Nos. 180~230) in the usual manner gave 0.36 g of pure **4** (30%) as lyophilized powder, $[\alpha]_D^{26}+124.3°$ (*c* 1, methanol).

PMR (D_2O) δ1.21 (3H, s, C-C$\underline{H}_3$), 1.42 (9H, s, *t*-butyl), 2.55 (3H, s, N-C$\underline{H}_3$), 5.06 (1H, d, $J_{1'',2''}$ =4.5 Hz, H-1″), 5.21 (1H, d, $J_{1',2'}$=3.0 Hz, H-1′).

Anal. Calcd. for $C_{24}H_{46}N_4O_{12}\cdot H_2CO_3$: C, 46.57; H, 7.51; N, 8.69%.
Found: C, 46.80; H, 7.82; N, 8.54%.

The mass spectrum of a sample of **4** which had been per-*N*-acetylated and treated with trifluoroacetic acid to de-*N*- protect the *tert*-butoxycarbonyl group followed by de-ionization showed a strong

peak at *m/e* 162 for the 6-amino-6-deoxy-glucosyl cation.

1-*N*-(*S*-4-Amino-2-hydroxybutyryl)-gentamicin B (Sch 20287)

To a stirred solution of 6′-*N*-*tert*-butoxycarbonylgentamicin B (**4**) (2.34 g, 4 mmol) in water (10 ml) was added methanol (30 ml) and the mixture was cooled to about 5°C. A solution of *N*-(*S*-4-benzyloxycarbonylamino-2-hydroxybutyryloxy)-succinimide[1,2] (2.71 g, 7.75 mmol) in dimethyl formamide (12 ml) was added dropwise. After 5 hours at 5°C, the solution was concentrated to dryness *in vacuo* and the residue chromatographed on silica gel (60～200 mesh, 250 g) using a solvent mixture composed of chloroform, methanol and ammonium hydroxide (30 : 10 : 1) as the eluant. Fractions 691～1200 were pooled, concentrated and dried to give 1.99 g of mixture of 1-*N*- and 3-*N*-(*S*-4-benzyloxycarbonylamino-2-hydroxy)-6′-*N*-*tert*-butoxycarbonylgentamicin B (**5** and **7**). The mixture containing **5** and **7** was dissolved in water (120 ml) and methanol (48 ml), and hydrogenated in the presence of 5% palladium-on-carbon catalyst (0.3 g) at 50 p.s.i for 3 hours. The catalyst was removed by filtration and the filtrate which showed two spots on TLC examination was concentrated to dryness (1.7 g) and the residue chromatographed on 100 g silica gel using a solvent system composed of chloroform, methanol, ammonium hydroxide (2 : 1 : 0.35). Fractions 130～220 gave, upon work-up as above, 1.052 g of 1-*N*-(*S*-4-amino-2-hydroxybutyryl)-6′-*N*-*tert*-butoxycarbonylgentamicin B (**9**) which was de-*N*-protected by dissolving in 4 ml of trifluoroacetic acid for 5 minutes and precipitating with excess ether. The solid was isolated by filtration, washed with ether and de-ionized with Amberlite IRA-401S OH^- ion-exchange resin. The aqueous solution containing the free base was lyophilized to give pure 1-*N*-(*S*-4-amino-2-hydroxybutyryl)-gentamicin B (Sch 20287) (0.2 g) as judged by TLC (chloroform - methanol - ammonium hydroxide, 3 : 4 : 2), $[\alpha]_D^{26}+107°$ (*c* 0.3, water).

PMR(D_2O) of the sulfate salt: δ 1.3 (3H, s, C-CH_3), 2.9 (3H, s, N-CH_3), 4.28 (1H, dd, J=9.0 Hz and 4.0 Hz, H2‴), 5.16 (1H, d, J=3.5 Hz, H-1″), 5.12 (1H, d, J=3.0 Hz, H-1′).

Anal. Calcd. for $C_{23}H_{45}N_5O_{12}\cdot H_2CO_3\cdot 2.5H_2O$: C, 41.73; H, 7.58; N, 10.14%.
Found: C, 41.62; H, 7.42; N, 10.28%.

Fractions 100～125 contained the 3-*N*-isomer of **9** which when processed as above gave biologically inactive material

1-*N*-(*S*-3-Amino-2-hydroxypropionyl)-gentamicin B (Sch 21420)

This compound was prepared from **4** in a manner similar to that described above for Sch 20287. Thus, 6′-*N*-*tert*-butoxycarbonylgentamicin B (**4**) (2.34 g, 4.02 mmol) was treated with *N*-(*S*-3-benzyloxycarbonylamino-2-hydroxypropionyloxy)-succinimide (2.6 g, 7.75 mmol) to obtain a crude product which was chromatographed on silica gel (250 g) using chloroform-methanol-ammonium hydroxide (30 : 10 : 1) as the eluant. Work-up of fractions 420～735 as in the above experiment gave 0.913 g of pure 1-*N*-(*S*-3-benzyloxycarbonylamino-2-hydroxypropionyl)-6′-*N*-*tert*-butoxycarbonylgentamicin B (**6**) which was hydrogenated and the product de-*N*-protected with trifluoroacetic acid as described above to obtain the desired 1-*N*-(*S*-3-amino-2-hydroxypropionyl)-gentamicin B as the trifluoroacetic acid salt (0.86 g). Further purification and deionization on a column of silica gel (25 g) using chloroform - methanol - ammonium hydroxide (1 : 1.5 : 1) as eluant gave pure Sch 21420 (0.352 g) which was converted into the sulfate salt in the usual manner, $[\alpha]_D^{26}+110.9°$ (*c* 1, water). PMR (D_2O)δ 1.35 ppm (3H, s, C-CH_3), 2.89 (3H, s, N-CH_3), 4.47 (1H, dd, J=4.5 Hz, 7.5 Hz, H2‴), 5.13 (1H, d, J=3.5 Hz, H-1″), 5.61 (1H, d, J=3.0 Hz, H-1′).

Anal. Calcd. for $C_{22}H_{43}N_5O_{12}\cdot 2H_2SO_4\cdot H_2O$: C, 33.72; H, 6.30; N, 8.94%.
Found: C, 33.56; H, 6.56; N, 8.73%.

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