Full Papers

Purification, Structure Determination, and Antimicrobial Activity of Neutramycins B−G

Edmund I. Graziani,* Cassia R. Overk, and Guy T. Carter

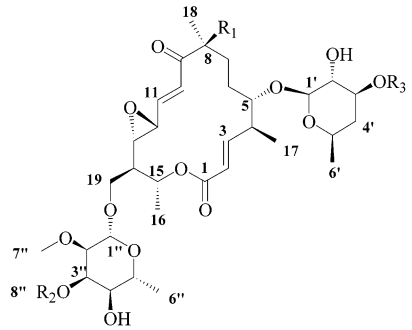
Department of Chemical and Screening Sciences, Wyeth Research, 401 N. Middletown Road, Pearl River, New York 10965

Received April 14, 2003

Neutramycins B−G were purified from a historical sample of neutramycin in our antibiotic collection. The structures of the compounds were solved by 2D NMR spectroscopic analysis. Four of the compounds (**2**−**5**) are probable biosynthetic intermediates or shunt metabolites of neutramycin biosynthesis, while two (**6**, **7**) are likely to be degradation products. Only one intermediate (**5**) showed weak Gram-positive activity.

In the course of work involving antibiotics from our historical collection, we evaluated the purity of a sample of the bacterial metabolite neutramycin (**1**) that had been prepared in 1965. It was immediately apparent that the sample was a mixture of a number of compounds related to neutramycin by analysis of the resultant LC/MS trace. We herein report the isolation, structure determination, and antimicrobial activity of six new neutramycin analogues, B through G (**2**−**7**, Figure 1).

Historically, the preparation of multigram quantities of neutramycin was accomplished by researchers at American Cyanamid via large-scale fermentation of a high-producing strain, followed by extraction, precipitation, and multiple rounds of recrystallization. The original isolation of neutramycin from culture broths of *Streptomyces luteoverticillatus* (Shinobu 1956, ATCC 23933) involved chloroform extraction and subsequent washing with dilute sodium bicarbonate, hydrochloric acid, and water.[1] In the course of evaluating a number of high-producing strains, considerable quantities of a major, inactive neutramycin analogue were obtained. Attempts to purify this inactive fraction by chromatography over Celite and recrystallization failed. The remainder of this material was deposited in the Lederle antibiotic collection, where it remained from 1965 until the present time.[2] It speaks to the tremendous advances in separation science that the sample succumbed quickly to reversed-phase chromatography, and all seven compounds were isolated. Similarly, the structure elucidation of each compound proceeded in a straightforward manner via analysis of the high-field NMR data.



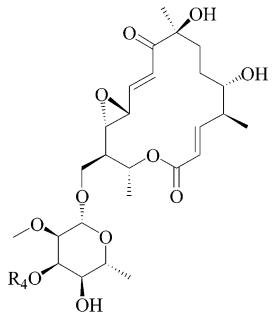
(1) neutramycin A: R_1= OH $R_2 = CH_3$ $R_3 = CH_3$

(2) neutramycin B: R_1= OH $R_2 = CH_3$ R_3 = H

(3) neutramycin C: R_1= OH R_2 = H R_3 = H

(4) neutramycin D: R_1= H R_2 = H R_3 = H

(5) neutramycin E: R_1= H $R_2 = CH_3$ R_3 = H



(6) neutramycin F: $R_4 = CH_3$

(7) neutramycin G: R_4 = H

Figure 1. Structures of Neutramycins B (**2**) through G (**7**).

Results and Discussion

The HRFTMS data for neutramycin B (**2**) yielded a molecular formula of $C_{33}H_{52}O_{14}$ (found 673.34304 ($M + H^+$), calcd 673.34353, Δ = 0.48 mmu). An initial survey of the ^{1}H NMR spectrum of **2** indicated that the two double bonds at C2,3 and C10,11 were intact, as evidenced by the set of two doublets and doublets of doublets at δ 6.98, 6.57, 6.44, and 6.02 ppm. HMBC correlations from these resonances into two carbonyl carbons at δ 167.4 and 201.7 ppm confirmed the presence of both the lactone and ketone functionalities, respectively. Both of these carbonyl moieties are conjugated to the double bonds, as required by the standard enone UV absorbance spectrum shown by neutramycin and all its congeners. One of the olefinic reso-

* Corresponding author. E-mail: graziaei@wyeth.com. Tel: 845-602-2876. Fax: 845-602-5687.

10.1021/np0301691 CCC: $25.00
Published on Web 08/15/2003

nances (δ 6.44 ppm, H11) also showed a strong COSY correlation to a resonance at δ 3.41 ppm, which in turn showed weak coupling to a proton at δ 3.07 ppm. The multiplicity edited (ME)HSQC spectrum[3] of **2** showed that these two proton resonances correlated to carbons at δ 59.8 and 60.0 ppm, respectively, consistent with an epoxide functionality. The resonance assigned to H13 showed an additional COSY correlation to a multiplet at δ 1.42 ppm that could be assigned to H14. This in turn showed a COSY correlation to a signal at δ 5.40 ppm. The only other COSY correlation to this signal at δ 5.40 ppm was from the methyl doublet at δ 1.34 ppm, confirming that the signal at δ 5.40 ppm was H15 and the methyl was H16. The resonance at δ 1.42 ppm (H14) also showed weak COSY correlations to both methylene protons at δ 4.11 and 3.68 ppm, which were assigned to H19. MEHSQC confirmed that the chemical shift of this methylene (δ 68.0 ppm) was consistent with this assignment to the only oxygen-bearing methylene carbon in the compound. Thus, the spin system from H10 through H16 and encompassing H19 is intact, as in the structure for neutramycin.

The other "half" of the aglycone of **2**, from C1 to C9, was also straightforward to assign, on the basis of the spin system from H2 through H8 and including H17 and H18. HMBC correlations from the methyl doublet at δ 1.16 ppm into carbons at δ 41.8, 82.9, and 152.4 ppm identified H17, since the carbon resonance at δ 152.4 could be assigned to C3 from the MEHSQC spectrum. Similarly, MEHSQC allowed for the assignment of the other two HMBC correlations from Me17 as C4 (δ 41.8 ppm, H4: δ 2.67 ppm) and C5 (δ 82.9 ppm, H5: δ 3.50 ppm). The proton resonance for H5 at δ 3.50 ppm showed COSY correlations to H4 (δ 2.67 ppm) as well as a signal at δ 2.00 ppm. The MEHSQC spectrum of **2** identified this resonance as one of two methylene protons attached to a carbon resonance at δ 28.2 ppm, which could therefore be assigned to C6. The other H6 proton at δ 0.73 ppm showed COSY correlations back to its geminal partner at δ 2.00 ppm as well as to an almost overlapping resonance at δ 1.95 ppm. Again, MEHSQC confirmed that there was an additional methylene carbon at δ 31.9 ppm that correlated with proton resonances at δ 1.95 and 1.92 ppm; this was assigned to C7. This assignment was confirmed by the HMBC correlations from the methyl singlet at δ 1.40 ppm to a carbon at δ 31.9 ppm (C7) as well as to a quaternary carbon at δ 79.3 ppm. These correlations allow for the assignment of the methyl at δ 1.40 ppm to C18 and confirms the attachment of a hydroxyl group, as in neutramycin A, at C8 (δ 79.3 ppm). Last, the macrocyclic nature of the aglycone is confirmed by the observation of an additional HMBC correlation from Me18 at δ 1.40 ppm to the carbonyl resonance at 201.7 ppm that had already been assigned to C9, as well as the observed HMBC correlation from H15 (δ 5.40 ppm) to the carbonyl of the lactone at δ 167.4 ppm (C1).

The observation of two proton resonances at δ 4.22 and 4.58 ppm that correlated to carbons at δ 106.0 and 101.9 ppm, respectively, was strongly suggestive of anomeric carbons and indicated that the compound contained two sugars, as in the parent compound, **1**. The anomeric proton at δ 4.58 ppm showed a COSY correlation to a resonance at δ 3.09 ppm that was itself correlated to a carbon at δ 82.8 ppm in the MEHSQC spectrum. This carbon resonance at δ 82.2 ppm showed an HMBC correlation from the OMe singlet at δ 3.54 ppm (Me7″), indicating that this is C2″ of the mycinose sugar of neutramycin. From H2″ (δ 3.09 ppm) it was possible to trace the entire spin system via COSY, beginning with H3″ at δ 3.78 ppm (whose associated carbon at δ 81.4 also showed an HMBC correlation from the other OMe singlet at δ 3.58 ppm, Me8″). H3″ also showed a weak COSY correlation to a resonance at δ 3.18 ppm (H4″, C4″: δ 74.6 ppm), which in turn coupled to a resonance at δ 3.66 ppm corresponding to H5″ (C5″: δ 71.1 ppm). The COSY spectrum of **2** showed that this resonance at δ 3.66 ppm correlated to a methyl doublet at δ 1.23 ppm (Me6″, C6″: δ 18.0 ppm), confirming the presence of an intact mycinose sugar on neutramycin B (**2**). The point of attachment of the mycinose across the oxygen on C19 was confirmed by the observation of an HMBC correlation between H1″ (δ 4.58 ppm) and C19 (δ 68.0 ppm).

The other anomeric proton at δ 4.22 ppm also showed a COSY correlation to a resonance at δ 3.09 ppm. However, it was possible through MEHSQC to distinguish this resonance as H2′ (^{1}H: δ 3.09; ^{13}C: 77.1) from the overlapping resonance belonging to H2″ (^{1}H: δ 3.09; ^{13}C: 82.8) as well as through the HMBC correlations from these carbons. It was possible to distinguish a COSY correlation from H2′ to a proton resonance at δ 3.53 ppm (H3′, C3′: δ 72.3 ppm), which in turn showed COSY correlations to two protons at δ 1.91 and 1.27 ppm. Analysis of the MEHSQC spectrum showed that these two resonances were methylene protons attached to a carbon at δ 42.1 ppm. One of the methylene protons at δ 1.27 showed an additional COSY correlation to a proton resonance at δ 3.57 ppm, which was in turn coupled to a methyl doublet at δ 1.21 ppm in the COSY spectrum. These assignments for H4′ through 6′ were confirmed by the observation of HMBC correlations from the methyl protons (Me6′, δ 1.21 ppm) to both C4′ (δ 42.1 ppm) and C5′ (δ 68.8 ppm). The absence of a methyl group on the oxygen of C3′, in contrast to neutramycin (**1**), was deduced from (i) a cursory examination of the ^{1}H NMR spectrum of **2** indicating that there were only two OMe singlets; (ii) the chemical shift of C3′, δ 72.3 ppm, being more appropriate for a hydroxyl-bearing carbon as compared to the expected shift for a methyl ether; (iii) the requirements of the molecular formula. It was therefore straightforward to conclude that **2** is in fact 3′-desmethylneutramycin, and the point of attachment of the 4,6-dideoxyxylohexose at the oxygen on C5 was further confirmed by an HMBC correlation between the anomeric proton H1′ at δ 4.22 ppm and the carbon resonance at C5 (δ 82.9 ppm).

With the complete NMR assignments for neutramycin B (**2**) in hand, it was possible to determine the structures of the remaining neutramycin analogues; the salient details for the structural assignments will be presented here. Neutramycin C (**3**) gave a molecular ion at $m/z = 659.32841$ ($M + H^+$), yielding a molecular formula of $C_{32}H_{50}O_{14}$ (calcd 659.32787, $\Delta = 0.54$ mmu). This formula required a formal deficit of C_2H_4 or, more likely, the loss of two methyl groups. Preliminary analysis of the ^{1}H NMR spectrum of **3** indicated that there was only one OMe present in the compound, as evidenced by the solitary methyl singlet at δ 3.52 ppm. After assigning the aglycone core of the compound by arguments similar to those detailed above, it became clear that the structural differences from neutramycin lay in the sugar moieties of the compound. From the anomeric proton H1″ (δ 4.62 ppm) was observed a COSY correlation to a resonance at δ 3.04 ppm that was assigned to H2″; supporting this assignment was the observed HMBC correlation from the OMe singlet at δ 3.52 ppm to the carbon attached to this resonance at δ 3.04 (H2″, ^{13}C: δ 81.6 ppm). The H2″ proton at δ 3.04 ppm showed an additional COSY correlation to a resonance at δ 4.15 ppm that was assigned to H3″. In contrast to

Table 1. Selected Physicochemical Properties of Neutramycins B though G (**2**−**7**)

	2	**3**	**4**
appearance	white solid	white solid	white solid
molecular formula	$C_{33}H_{52}O_{14}$	$C_{32}H_{50}O_{14}$	$C_{32}H_{50}O_{13}$
molecular weight	672	658	642
HRFTMS	673.34304 (M + H^+)	659.32841 (M + H^+)	643.33203 (M + H^+)
found:	673.34353	659.32787	643.33296
calcd:	(Δ = 0.48 mmu)	(Δ = 0.54 mmu)	(Δ = 0.93 mmu)
UV λ_{max} (nm) in MeOH	218, 248 (sh)	214, 244 (sh)	216, 244 (sh)
IR ν_{max} in KBr (cm^{-1})	3444, 2934, 1717, 1631, 1170, 1082, 1033	3440, 2932, 1713, 1664, 1631, 1173, 1078, 1031	3435, 2930, 1712, 1690, 1626, 1176, 1079, 983
$[\alpha]_D$ in MeOH (conc)	−35 (0.13%)	−138 (0.05%)	−163 (0.065%)

	5	**6**	**7**
appearance	white solid	white solid	white solid
molecular formula	$C_{33}H_{52}O_{13}$	$C_{27}H_{42}O_{11}$	$C_{26}H_{40}O_{11}$
molecular weight	656	542	528
HRFTMS	657.34713 (M + H^+)	543.28023 (M + H^+)	529.26344 (M + H^+)
found:	657.34861	543.28053	529.26488
calcd:	(Δ = 1.48 mmu)	(Δ = 0.30 mmu)	(Δ = 1.40 mmu)
UV λ_{max} in MeOH	216, 244 (sh)	218, 244 (sh)	218, 246 (sh)
IR ν_{max} in KBr (cm^{-1})	3436, 2973, 2934, 1717, 1689, 1629, 1175, 1081, 1066, 984	3447, 2970, 2931, 1714, 1689, 1630, 1229, 1167, 1084, 1033, 980	3434, 2975, 2931, 1715, 1654, 1631, 1453, 1350, 1230, 1172, 1081, 1033, 980
$[\alpha]_D$ in MeOH (conc)	−28 (0.06%)	−26 (0.09%)	−42 (0.05%)

neutramycin B (**2**), the ^{13}C chemical shift for C3″, as identified from the MEHSQC spectrum, was δ 70.4 ppm, suggesting that C3″ bears a hydroxyl and not a methyl ether. The remainder of the assignments for this sugar were made by similar arguments, and therefore neutramycin C (**3**) bears a javose at C19. The NMR assignments for the other sugar at C5 were identical to those for neutramycin B (**2**), thus accounting for the other absent methyl group (relative to neutramycin A).

Neutramycin D (**4**) gave a molecular ion at m/z = 643.33203 (M + H^+), yielding a molecular formula of $C_{32}H_{50}O_{13}$ (calcd 643.33296, Δ = 0.93 mmu). Examination of the ^1H NMR spectrum of **4** showed a solitary OMe singlet at δ 3.52 ppm, and NMR structural assignments confirmed that the C3″ and C3′ methyls were missing, as per compound **3**. Additionally, careful analysis of the ^1H NMR spectrum showed that the methyl singlet at δ 1.40 ppm present in the spectra of **2** and **3** was missing and, moreover, that there was a new methyl doublet at δ 1.18 ppm. This immediately suggested that the hydroxyl group at C8 was missing, as required by the molecular formula, effectively making C18 into a methyl doublet, where it had previously been a singlet in the parent compound. A COSY correlation from H5 (δ 3.46 ppm) to a resonance at δ 1.70 ppm identified one of the methylene protons on C6. MEHSQC confirmed that the resonance at δ 1.70 ppm was attached to a methylene carbon at δ 33.4 ppm and that its geminal partner was at δ 1.00 ppm. This H6 proton at δ 1.00 ppm also showed a COSY correlation to resonances at δ 1.80 and 1.63 ppm that were identified as the H7 methylene protons attached to a carbon at δ 26.8 ppm. The proton at δ 1.63 ppm showed an additional COSY correlation to a resonance at δ 2.25 ppm, which in turn correlated to a methyl doublet at δ 1.18 ppm. The assignment of H8 and H18 was further confirmed by the observation of HMBC correlations from the methyl doublet at δ 1.18 ppm to carbons at δ 50.0 (C8), 26.8 (C7), and 204.5 ppm (C9).

Neutramycin E (**5**) gave a molecular ion at m/z = 657.34713 (M + H^+), yielding a molecular formula of $C_{33}H_{52}O_{13}$ (calcd 673.34861, Δ = 1.47 mmu). Examination of the 2D NMR data for **5** indicated that the C3′ methyl was absent, as in compounds **2**−**4**, by similar arguments. Similarly, the spin system from H5 could be traced to a methine resonance at δ 2.26 ppm (H8), which correlated in the COSY spectrum with a methyl doublet at δ 1.18 ppm (Me18), indicating that the C8 hydroxyl was absent as in neutramycin D (**4**). However, the presence of two OMe singlets in the ^1H spectrum of **5** suggested that the mycinose sugar was intact in neutramycin E (**5**). This assignment was confirmed by the observation of HMBC correlations from the OMe resonances at δ 3.54 ppm and 3.57 ppm into signals at δ 3.08 and 3.77 ppm, respectively. Additional COSY correlations from the resonance at δ 3.08 ppm to the anomeric proton at δ 4.57 ppm allowed for the assignment of this resonance to H2″, and the expected correlations from the proton at δ 3.57 to H4″ (δ 3.17 ppm) assigned this peak to H3″.

Mass spectral data for neutramycins F (**6**) and G (**7**) indicated that these two neutramycins were considerably smaller than the other neutramycins isolated from this sample. The molecular formulas obtained for these compounds (see Table 1) from the HRFTMS data suggested a formal loss of $C_6H_{10}O_3$ for the former and $C_7H_{12}O_3$ for the latter, relative to neutramycin B (**2**). This difference in mass was highly suggestive of a loss of one of the sugars, in particular the 3′-*O*-desmethylchalcose (4,6-dideoxyxylohexose) attached across C5 of the parent aglycone. The presence of an unmodified aglycone and an intact mycinose sugar on neutramycin F (**6**) was confirmed by NMR assignments (see Table 2), and the presence of a free hydroxyl group at C5 (and the absence of a glycoside linkage at this position) was further confirmed by (i) the absence of all NMR signals for the 4,6-dideoxyxylohexose moiety and (ii) the change in chemical shift for the C5 resonance in **6** (^{13}C: δ 73.5 ppm) compared to **2** (^{13}C: δ 82.9 ppm). By a similar analysis, neutramycin G (**7**) was determined to be the deglycosylated analogue of neutramycin C (**3**); namely, the compound consists of an unmodified neutramycin aglycone and a javose (8″-*O*-desmethylmycinose) as the C19 glycoside, while the 4,6-dideoxyxylohexose (3′-*O*-desmethylchalcose) is absent.

The absolute stereochemistry of the neutramycins presented here is inferred from a recent study on the absolute stereochemistry of the related compound chalcomycin,[4] in which the authors present a unified stereochemical model for all 16-membered-ring macrolides that complements

Table 2. ^{1}H NMR Data for Neutramycins B−G (**2**−**7**), 400 MHz, CD_3OD

	2	**3**	**4**	**5**	**6**	**7**
2	6.02 d 15.5	6.02 d 15.4	6.00 d 15.5	6.00 d 15.5	6.03 d 15.4	6.01 d 15.5
3	6.57	6.57	6.58	6.58	6.56	6.55
	dd 15.5, 10.5	dd 15.4, 10.6	dd 15.5, 10.7	dd 15.5, 10.7	dd 15.4, 10.6	dd 15.5, 10.6
4	2.67 m	2.65 m	2.63 m	2.64 m	2.49 m	2.47 m
5	3.50 m	3.49 m	3.46 m	3.45 m	3.48	3.47 m
					ddd 10.3, 4.4, 2.3	
6	2.00 m	2.00 m	1.70 m	1.70 m	1.71 m	1.71 m
	0.73 m	0.72 m	1.00 m	1.00 m	0.79 m	0.79 m
7	1.95 m	1.90 m	1.80 m	1.81 m	1.93 m	1.94 m
	1.92 m	1.26 m	1.63 m	1.63 m	1.83 m	1.80 m
8			2.25 m	2.26 m		
10	6.98 d 15.3	6.98 d 15.3	6.92 d 15.4	6.91 d 15.4	7.01 d 15.4	6.99 d 15.3
11	6.44	6.43	6.32	6.31	6.43	6.46
	dd 15.3,9.3	dd 15.3, 9.4	dd 15.4, 9.4	dd 15.4, 9.5	dd 15.4, 9.3	dd 15.3, 9.5
12	3.41	3.40	3.40	3.39	3.41	3.41
	dd 9.4, 1.9	dd 9.4, 1.5	dd 9.4, 1.8	dd 9.5, 1.8	dd 9.3, 1.9	dd 9.5, 1.9
13	3.07 m	3.06 m	3.04	3.03	3.05	3.07
			dd 7.9, 1.8	dd 9.3, 1.8	dd 9.3, 1.9	dd 9.4, 1.9
14	1.42 m	1.42 m	1.42 m	1.42 m	1.42 m	1.41 m
15	5.40 m	5.40 m	5.40 m	5.41 m	5.40 m	5.40 m
16	1.34 d 6.4, 3H	1.33 d 6.3 3H	1.33 d 6.2 3H	1.33 d 6.4 3H	1.33 d 6.4 3H	1.33 d 6.3 3H
17	1.16 d 6.4, 3H	1.15 d 6.5 3H	1.16 d 6.7 3H	1.16 d 6.6 3H	1.09 d 6.5 3H	1.09 d 6.5 3H
18	1.40 s 3H	1.39 s 3H	1.18 d 7.2 3H	1.18 d 7.1 3H	1.41 s 3H	1.42 s 3H
19	4.11	4.12	4.12	4.11	4.12	4.13
	dd 10.1, 2.8	dd 10.1, 2.8	dd 10.2, 2.9	dd 10.2, 2.8	dd 10.2, 2.8	dd 10.1, 3.0
	3.68	3.69	3.68	3.67	3.68	3.69
	dd 10.1, 3.0	dd 10.1, 3.0	dd 10.2, 2.9	dd 10.2, 3.1	dd 10.2, 3.0	dd 10.1, 2.9
1′	4.22 d 7.7	4.22 d 7.6	4.22 d 7.8	4.22 d 7.8		
2′	3.09 m	3.08 m	3.08 m	3.08 m		
3′	3.53 m	3.53 m	3.53 m	3.53 m		
4′	1.91 m	1.90 m	1.91	1.91		
	1.27	1.28 m	ddd 12.5, 5.1, 1.5	ddd 12.5, 5.0, 1.7		
	dd 11.7, 1.1		1.28	1.28 m		
			d 12.5			
5′	3.57 m	3.58 m	3.57	3.57 m		
			dd 6.1, 1.5			
6′	1.21 d 6.2	1.20 d 6.2	1.19 d 6.0	1.18 d 6.1		
1″	4.58 d 8.0	4.62 d 7.8	4.62 d 7.9	4.57 d 8.0	4.58 d 8.0	4.62 d 7.9
2″	3.09	3.04 m	3.03	3.08	3.09	3.03
					dd 8.0, 2.9	dd 7.9, 2.9
	dd 8.0, 2.8		dd 7.9, 2.9	dd 8.0, 2.8		
3″	3.78 t 2.8	4.15 t 2.7	4.16 t 2.9	3.77 t 2.8	3.77 t 2.9	4.16 t 2.9
4″	3.18	3.16	3.16	3.17	3.17	3.16
					dd 9.6, 2.9	dd 9.5, 2.9
	dd 9.6, 2.8	dd 9.6, 2.7	dd 9.5, 2.9	dd 9.4, 2.8		
5″	3.66	3.72	3.71	3.65 m	3.65	3.72
	dd 9.6, 6.2	dd 9.6, 6.3	dd 9.6, 6.2		dd 9.6, 6.4	dd 9.5, 6.2
6″	1.23 d 6.2 3H	1.24 d 6.5 3H	1.24 d 6.2 3H	1.22 d 6.2 3H	1.25 d 6.2 3H	1.24 d 6.2 3H
7″	3.54 s 3H	3.52 s 3H	3.52 s 3H	3.54 s 3H	3.54 s 3H	3.52 s 3H
8″	3.58 s 3H			3.57 s 3H	3.57 s 3H	

Celmer's original model.[5,6] Similarly, the relative configuration of C8 in neutramycins D (**4**) and E (**5**) is consistent with hydroxylation of the glycosylated polyketide synthase (PKS) product with retention of configuration as predicted in the stereochemical model[4] as well as literature precedent.[7,8] It should be noted that recent reports[9] in an ongoing series exploring the biochemical underpinnings of Celmer's rules lend additional support to the unified stereochemical model.

The structures of neutramycins B (**2**) through E (**5**), consisting as they do of desmethyl and deoxy versions of neutramycin, are consistent with the expected course of transformations predicted for the biosynthesis of type II polyketides. By analogy with the biosynthesis of the highly related mycinamicin compounds from *Micromonospora griseorubida*[10] it is reasonable to assume that the product of the neutramycin PKS is subsequently oxidized at C19 (also by analogy with tylosin biosynthesis[11]) and then glycosylated at the hydroxyls on C5 and C19. The isolation of **2**−**5** suggests that the final steps in the biosynthesis of neutramycin involve (i) methylation at the C3″ oxygen to convert javose into mycinose; (ii) hydroxylation at C8, presumably by a cytochrome P450 monooxygenase, and (iii) methylation at the C3′ oxygen to convert 4,6-dideoxyxylohexose into chalcose. The order in which these steps occur, however, cannot be determined from the intermediates isolated, since either **3** or **5** must be a shunt product, although it is likely that methylation at the C3′ hydroxyl is the final step in the biosynthesis of neutramycin. It is also probable that neutramycins F (**6**) and G (**7**) are degradation products of neutramycins B (**2**) and C (**3**), respectively. Multiple sampling of the complex consistently gave the same chromatographic profile described in the Experimental Section, and we therefore speculate that the formation of artifacts **6** and **7** occurred during the original processing of the large-scale fermentation at the time of production, as opposed to in situ degradation of the sample over the past 37 years.

Neutramycins B through G were inactive against a number of Gram-positive and Gram-negative strains in both agar disk diffusion assays and determinations of minimum inhibitory concentrations,[12] with MICs greater

Table 3. ^{13}C NMR Data for Neutramycins B−G (**2**−**7**), 100 MHz, CD_3OD

	2	3	4	5	6	7
1	167.4	167.4	167.6	167.1	167.5	167.3
2	122.0	121.9	122.3	122.0	122.2	121.8
3	152.4	152.5	152.7	152.3	153.3	153.0
4	41.8	41.7	42.0	41.6	42.5	42.2
5	82.9	82.9	84.0	83.7	73.5	73.4
6	28.2	31.7	33.4	33.0	29.4	29.0
7	31.9	31.9	26.8	26.5	31.6	31.4
8	79.3	79.3	50.0	49.7	79.2	78.9
9	201.7	201.8	204.5	204.1	201.8	201.5
10	127.3	127.2	128.3	127.9	127.7	127.4
11	146.1	146.1	145.3	144.8	146.5	146.3
12	59.8	59.2	60.5	59.8	60.3	59.9
13	60.0	59.9	60.4	59.8	60.4	60.1
14	50.8	50.5	50.8	50.5	50.9	50.6
15	69.7	69.9	70.2	69.7	70.0	69.8
16	18.5	18.5	18.7	18.5	18.6	18.4
17	18.5	18.4	18.6	18.1	18.6	18.4
18	26.3	26.3	17.6	17.5	26.4	26.2
19	68.0	68.1	68.4	67.9	68.3	68.0
1′	106.0	105.9	106.5	106.1		
2′	77.1	77.1	77.0	46.9		
3′	72.3	72.2	72.5	72.0		
4′	42.1	42.0	42.2	41.9		
5′	68.8	69.2	69.0	68.6		
6′	21.3	21.3	21.5	21.2		
1″	101.9	101.6	102.1	102.0	102.4	101.6
2″	82.8	81.6	81.9	82.6	83.0	81.7
3″	81.4	70.4	70.7	81.1	81.6	70.3
4″	74.6	74.3	74.4	74.4	74.8	74.2
5″	71.1	70.5	70.7	70.8	71.3	70.4
6″	18.0	18.0	18.22	18.0	18.2	18.0
7″	59.3	58.8	58.9	59.2	59.6	58.6
8″	62.1			61.9	62.3	

than 128 μg/mL. The only exception was neutramycin E (**5**), which showed slight activity (MIC = 32 μg/mL) against *Staphylococcus aureus* (ATCC 6538P). These results are consistent with the observation[13] that small changes in the sugar attached to a macrolide can dramatically reduce biological activity such that many intermediates of macrolide antibiotic biosynthesis either are devoid of biological activity or show greatly reduced antimicrobial activity. In particular, this dependence of antimicrobial activity upon glycosylation, methylation, and hydroxylation has been well-studied in compounds related to neutramycin, notably leucomycin[14] and tylosin.[15]

Experimental Section

General Experimental Procedures. NMR spectra were recorded on a Bruker Avance DPX-400 spectrometer using a 3 mm broadband detect probe; HRFTMS spectra were obtained using a Bruker Daltonics 7 T system. Preparative chromatography was accomplished on a Varian HPLC system equipped with dual Dynamax SD-300 pumps and a Prostar diode array detector (DAD) under the control of Varian Star software. Analytical chromatography was carried out using an Agilent HP1100 HPLC system equipped with a DAD using Chemstation software. All HPLC solvents were EM Omnisolv quality and used without further purification. Water used in chromatography was doubly distilled using a Millipore Milli-Q purifier.

Purification of Neutramycins B−G (2−7) from LL4266C-50A. A sample of neutramycin complex (100 mg) was dissolved in methanol (1.0 mL), injected onto a YMC ODS-A HPLC column (20 × 250 mm), and eluted in a gradient of 48% acetonitrile/water to 65% acetonitrile/water at 16 mL/min over 55 min, to yield a mixture of neutramycins C (**3**) and G (**7**) (t_R = 13.5−15.7 min), neutramycin F (**6**) (t_R = 23.5 min, 21.2 mg), neutramycin B (**2**) (t_R = 26.7 min, 24.2 mg), neutramycin D (**4**) (t_R = 35.3 min, 10.1 mg), and a mixture of neutramycins A (**1**) and E (**5**) (t_R = 47.0 min).

The crude mixture of neutramycins C (**3**) and G (**7**) was further separated by HPLC (YMC ODS-A, 10 × 250 mm), eluting with 1:1 methanol/water at 2.5 mL/min to yield pure neutramycin G (**7**, 5.3 mg) and neutramycin C (**3**, 8.4 mg). The crude mixture of neutramycins A (**1**) and E (**5**) was further separated by HPLC (YMC ODS-A, 10 × 250 mm), eluting with 65% methanol/water at 2.5 mL/min to yield pure neutramycin E (**5**, 13.9 mg).

Physicochemical properties of compounds are summarized in Table 1; ^1H and ^{13}C NMR data are summarized in Tables 2 and 3, respectively.

Acknowledgment. We would like to thank Xidong Feng for HRFTMS measurements, Andrew Schork for obtaining IR spectra and optical rotations, and Daniel Arias for antimicrobial assays. In addition, we would like to thank M. Abou-Gharbia for support of CO through a Wyeth Research Internship.

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NP0301691