Balhimycin, a New Glycopeptide Antibiotic with an Unusual Hydrated 3-Amino-4-oxoaldopyranose Sugar Moiety

Sugata Chatterjee,* Erra K. S. Vijayakumar, Suresh R. Nadkarni, Mahesh V. Patel, J. Blumbach, and Bimal N. Ganguli†

Microbiology Department, Research Centre, Hoechst India Limited, Bombay 400 080, India

H.-W. Fehlhaber,* H. Kogler, and L. Vertesy

Pharmaceutical Research, Hoechst AG, D-6230 Frankfurt, Germany

Received December 3, 1993 (Revised Manuscript Received March 28, 1994•)

Balhimycin (1), $C_{66}H_{73}Cl_2N_9O_{24}$, is a new glycopeptide antibiotic of the vancomycin class. It was isolated from the fermentation broth of an *Amycolatopsis* sp. Hoechst India Limited Y-86,21022. Its structure was elucidated on the basis of spectroscopic and chemical degradation studies. The aglycon is identical to that of vancomycin. The molecule contains two sugars, D-glucose and dehydrovancosamine. The latter unit remains as a hydrate in solution.

Infection due to methicillin-resistant *Staphylococcus aureus* (MRSA) is reported to be assuming menacing proportions in several infective conditions, e.g. postoperative wounds and burns, and is sometimes fatal.[1] The clinically used antibiotic to combat such infections is vancomycin, which is the first isolated member of a unique class of glycopeptides typified by an aglycon (**AGL**) consisting of a heptapeptide backbone comprised of two unusual aromatic amino acids, actinoidinic acid (**A**) and vancomycinic acid (**V**). The aglycon is usually glycosylated at the phenolic and/or the benzylic hydroxyls by neutral and basic sugar units. These compounds act on the bacterial cell wall and are reported to form a complex with L-lysyl-D-alanyl-D-alanine terminus of a muramylpentapeptide thereby interfering in the subsequent transpeptidase mediated cross linking of peptidoglycan chains required to form the three-dimensional network of the bacterial cell wall.[2] Vancomycin had moderate nephrotoxicity and is also reported to cause allergic reaction in patients.[3] These facts compounded with the emergence of vancomycin-resistant organisms[4] have led to an intensive search for new members of this class of antibiotics which should have higher potency, lesser toxicity, and superior pharmacokinetics.

In our screening program directed toward the discovery of new MRSA-active compounds, we isolated[5] a new glycopeptide antibiotic Balhimycin (1) from the fermentation broth of an *Amycolatopsis* sp. culture number Hoechst India Limited Y-8621022 collected from the Thamu forest of the Himalayas.[6] Balhimycin was obtained from the culture filtrate by successive chromatographies over Diaion HP-20 (aqueous MeOH eluant), Dowex (OAc^- form, water), and reverse-phase silica gel (50–70 μm C_{18}, CH_3CN/0.1% TFA in water) and isolated as the trifluoroacetate salt. The isolation was monitored by a bioassay model specifically designed for this class of glycopeptide antibiotics and involved an observance of reduction in antibacterial activity in the presence of *N*-acetyl-L-lysyl-D-alanyl-D-alanine.[7]

The molecular formula of balhimycin was determined to be $C_{66}H_{73}Cl_2N_9O_{24}$ by HRFAB MS (matrix NBA, internal reference PEG) ($(M+H)^+$: found m/z 1446.4204, calcd 1446.4205, monoisotopic mass). An alkali induced UV bathochromic shift (285 to 305 nm) indicated phenolic hydroxyls, while IR frequencies at 3500 and 1700 (broad) cm^{-1} revealed hydroxyl and carbonyl groups. Drastic hydrolysis (6 N HCl, 110 °C, 18 h) yielded aspartic acid and *N*-methylleucine (GC–MS of N-TFA/O-Me derivatives). Acid hydrolysis (2 N HCl, 100 °C, 2 h) liberated a carbohydrate unit which was identified as D-glucose by analysis (GC–MS, optical rotation) of the alditol acetate. This glucose could be cleaved under very mild conditions indicating its attachment to a phenolic OH group. Its location was established by a differential alkylation method.[8] Thus, 1 on sequential methylation (CH_3I/K_2CO_3), hydrolysis (1 N HCl, 95 °C, 1 H), ethylation (C_2H_5I/K_2CO_3), oxidative degradation ($KMnO_4$–NH_3), and methylation (CH_2N_2) produced the triester **v'** as a degradation product of the vancomycinic acid component. This established that glucose was attached to the phenolic OH group of the amino acid residue 4.[9]

Controlled hydrolysis (Figure 1) of balhimycin gave a pseudo-aglycon which was identified as deglucobalhimycin (2) (FAB-MS $(M+H)^+$ 1284). Both **1** and **2** gave well-resolved ^1H and ^{13}C NMR spectra in D_2O. Assignments of nearly all the proton signals could be done from the ^1H–^1H shift correlation spectra COSY.[10] Starting from the best dispersed spectrum, the protonated carbons were

† Departmental Head.

• Abstract published in *Advance ACS Abstracts*, May 15, 1994.

(1) Guenthner, S. H.; Wenzel, R. P. *Antimicrob. Agents Chemother.* **1984**, *26*, 268.

(2) Barna, J. C. J.; Williams, D. H. *Annu. Rev. Microbiol.* **1984**, *38*, 339.

(3) Rybak, M. J.; Albrecht, L. M.; Boike, S. C.; Chandrasekar, P. H. *J. Antimicrob. Chemother.* **1990**, *25*, 679.

(4) Courvalin, P. *Antimicrob. Agents Chemother.* **1990**, *34*, 2291.

(5) (a) Nadkarni, S. R.; Patel, M. V.; Chatterjee, Sugata; Vijayakumar, E. K. S.; Desikan, K. R.; Blumbach, J.; Ganguli, B. N.; Limbert, M. *J. Antibiot.* **1994**, *47*, 334. (b) European Patent No. 0468504 A1.

(6) The producing organism has been deposited at the German Collection of Microorganisms and Cell Cultures, Braunschweig, with the accession number DSM 5908.

(7) Rake, J. B.; Gerber, R.; Mehta, R. J.; Newmann, D. J.; Oh, Y. K.; Phelen, C.; Shearer, M. C.; Sitrin, R. D.; Nisbet, L. J. *J. Antibiot.* **1986**, *39*, 58.

(8) McGahren, W. J.; Martin, J. H.; Morton, G. O.; Hargreaves, K. T.; Leese, R. A.; Lovell, F. M.; Ellestad, G. A.; O'Brien, E.; Holker, J. S. E. *J. Am. Chem. Soc.* **1980**, *102*, 1671.

(9) The nomenclature for numbering the amino acid residues used in this article is in accordance with that used by D. H. Williams et al.; see Skelton, N. J.; Williams, D. H.; Rance, M. J.; Ruddock, J. C. *J. Chem. Soc., Perkin Trans. 1* **1990**, 77.

(10) Aue, W. P.; Barthold, E.; Ernst, R. R. *J. Chem. Phys.* **1976**, *64*, 2229.

0022-3263/94/1959-3480$04.50/0

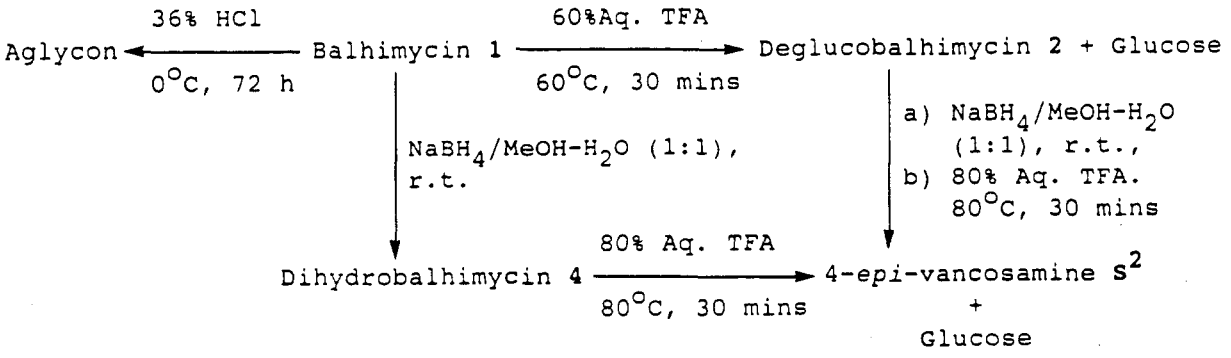
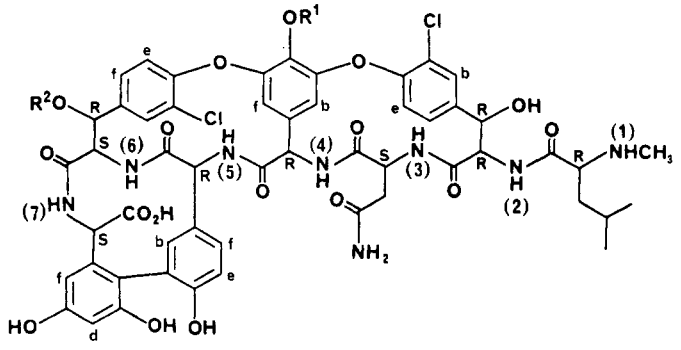
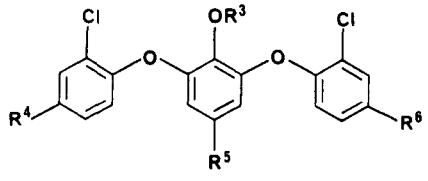


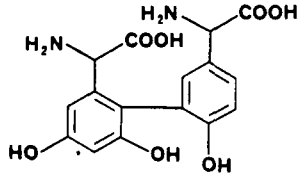
Figure 1. Structurally diagnostic chemical degradation reactions of balhimycin (1).

Chart 1



AGL : $R^1 = R^2 = H$
1 : R^1 = β-D-glucose; R^2 = s
1' : R^1 = β-D-glucose; R^2 = s'
2 : $R^1 = H$; R^2 = s or s'
3 : R^1 = β-D-glu-(2→1)-s^1; $R^2 = H$
4 : R^1 = β-D-glucose; R^2 = s^2
5 : R^1 = β-D-glucose; R^2 = s^3





(A)

V : $R^3 = H$; $R^4 = R^6$ = CH(OH)CH(NH$_2$)-COOH; R^5 = CH(NH$_2$)-COOH

V' : $R^3 = C_2H_5$; $R^4 = R^5 = R^6 = COOCH_3$

identified by analysis of the inverse-detected HMQC[11] spectrum. The quaternary carbons were assigned by interpretation of inverse-detected proton–carbon long-range correlation spectra HMBC[12] optimized for $^nJ_{CH}$ values of 6–8 Hz. Assignment of the amide protons was achieved by recording the COSY spectra in a 4:1 mixture of H_2O and D_2O. However the long-range HMBC experiments were not performed in this solvent because of significant reduction of the relaxation times in nondeuterated solvents. Attempts to assign the amide protons by recording the spectra of **1** and **2** in DMSO-d_6 were less successful since the overall quality of the spectra was poor due to severe line-broadening effects. Tables 1 and 2 summarize the ^1H and ^{13}C NMR spectra of balhimycin (**1**) and *deglucobalhimycin* (*2*).

Analysis of these spectral data gave an early indication of the presence of a second carbohydrate moiety in **1** which was retained in **2**. Thus **1** showed two anomeric sites: δ_C/δ_H 105.84/5.41 (d, J = 6.4 Hz) and δ_C/δ_H 94.00/5.00 (d, J = 3.4 Hz) corresponding to β-glucose and the second sugar unit, respectively, while **2** showed only this second anomeric site. In D_2O as the solvent, both **1** and **2** showed quaternary carbons at δ_C 94.29 and δ_C 94.27, respectively, belonging to this second sugar unit. The 2D NMR studies carried out on **1** and **2** established that the aglycon portion in both **1** and **2** were identical to that of vancomycin (**3**). This was further confirmed by HPLC, MS, and ^1H NMR comparison of an authentic sample of vancomycin aglycon[13] with that of a hydrolysis (36% HCl, 0 °C, 72 h) product of balhimycin (Figure 1). Eliminating the aglycon and the glucose portion from the molecular formula of balhimycin led us to an uncharacterized $C_7H_{10}NO_2$ moiety as representing the second sugar unit of balhimycin.

All attempts to liberate this unit from balhimycin using various conditions of hydrolysis were uniformly unsuccessful. Its anomeric proton showed COSY correlation to a CH_2 group (δ_C 38.23; δ_H 2.46 (dd, J = 11.5, 3.5 Hz), 2.33 (broad d, J = 11.5 Hz) indicating a 2-deoxy sugar. It had two methyl groups, a singlet at δ_H 1.68 (δ_C 21.91) and a doublet at δ_H 1.31, J = 6.3 Hz (δ_C 13.48) coupled to an oxymethine quartet (δ_C/δ_H 68.27/3.97). The consolidated

(11) (a) Muller, L. *J. Am. Chem. Soc.* **1979**, *101*, 4481. (b) Bax, A.; Subramanian, S. *J. Magn. Reson.* **1986**, *67*, 555.
(12) Bax, A.; Summers, M. F. *J. Am. Chem. Soc.* **1986**, *108*, 2093.
(13) Vancomycin aglycon was prepared following the procedure described in Marshall, F. J. *J. Med. Chem.* **1965**, *8*, 18.

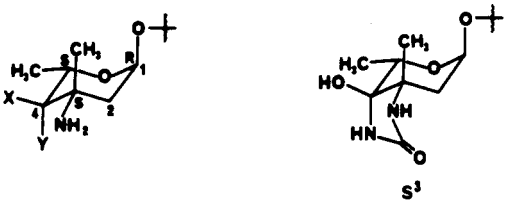
Table 1. ^{1}H NMR Data of Balhimycin (1), Deglucobalhimycin (2), and Dihydrobalhimycin (4)

proton	1[a]		2[b]	4[c]
1 (*N*-methylleucine)				
	4.20 (t, 6.4 Hz)	(3.05)	3.97	4.18
	1.77 (m)	(1.47)	1.68	1.77
	1.77 (m)	(1.45)	1.68	1.77
	1.66 (m)	(1.76)	1.61	1.65
	0.88 (d, 6.0 Hz)	(0.91)	0.86	0.87
	0.86 (d, 6.0 Hz)	(0.88)	0.84	0.85
N-Me	2.81 (s)	(2.31)	2.81	2.60
2				
NH	8.70	(7.80		
	5.44 (d, 5.3 Hz)	(4.83)	5.30	5.41
	5.50 (d, 5.3 Hz)	(5.16)	5.37	5.49
2b	7.36 (broad s)	(7.38)	7.32	7.31
2e	7.34 (d, 8.5 Hz)	(7.36)	7.36	7.36
2f	7.62 (d, 8.5 Hz)	(7.50)	7.62	7.60
3 (aspargine)				
NH	8.11	(8.75)		
	4.91 (t, 6.6 Hz)	(4.39)	4.87	4.91
	2.60 (dd, 14.4, 4.6 Hz)	(2.18)	2.70	2.58
	2.55 (dd, 14.4, 8.0 Hz)	(2.18)	2.66	2.55
NH	7.76 (d)	(7.26)		
NH	6.66 (broad)	(6.81)		
4				
NH	7.85	(8.16)		
	6.44 (broad s)	(5.74)	6.34	6.44
4b	5.52 (broad s)	(5.68)	5.48	5.53
4f	5.28 (broad s)	(5.22)	5.25	5.29
5				
NH	9.41	(8.61)		
	4.53 (s)	(4.51)	4.50	4.54
5b	7.09 (broad s)	(7.15)	7.08	7.11
5e	7.00 (d, 8.5 Hz)	(6.73)	6.98	6.98
5f	7.08 (d, 8.5 Hz)	(6.80)	7.06	7.07
6				
NH	7.65	(6.63)		
	4.30 (broad)	(4.32)	4.23	4.25
	5.40 (broad)	(5.28)	5.37	5.37
6b	7.69 (d, 1.2 Hz)	(7.89)	7.61	7.72
6e	5.15 (d, 8.0 Hz)	(5.40)	5.30	5.15
6f	6.89 (dd, 8.0, 1.2 Hz)	(7.34)	6.99	6.89
7				
NH	9.40	(8.49)		
	4.79 (s)	(4.48)	4.78	4.62
7d	6.57 (d, 2.4 Hz)	(6.39)	6.56	6.51
7f	6.45 (d, 2.4 Hz)	(6.31)	6.45	6.51
glucose				
1	5.41 (d, 6.4 Hz)	(5.41)		5.42
2	3.75 (m)	(3.41)		3.74
3	3.75 (m)	(3.30)		3.76
4	3.64 (m)	(3.25)		3.61
5	3.70 (dd, 12.0, 4.0 Hz)	(3.31)		3.70
6	3.62 (m)	(3.74)		3.64
	3.50 (dd, 12.0, 2.2 Hz)	(3.51)		3.49
vancosamine	4-oxo		4-oxo	4-*epi*
1	5.00 (d, 3.4 Hz)	(4.90)	4.96	4.99
2	2.46 (dd, 11.5, 3.5 Hz)	(2.48)	2.41	2.47
	2.33 (d, 11.5 Hz)	(2.16)	2.33	2.23
4				3.43
5	3.97 (q, 6.4 Hz)	(4.39)	3.97	3.78
6	1.31 (d, 6.3 Hz)	(1.22)	1.30	1.37
3-Me	1.68 (s)	(1.25)	1.68	1.61

[a] Recorded at 285 K in 4:1 H_2O–D_2O using 16 mg/mL concentration. Values in parentheses are those recorded in DMSO-d_6 at 320 K using a 28 mg/mL concentration. [b] Recorded at 325 K in D_2O using 25 mg/mL concentration. [c] Recorded at 300 K in D_2O using 18 mg/mL concentration.

spectral information led to the structure **S** which is a 4-dehydro derivative of vancosamine (S^1) or *epi*-vancosamine (S^2) present in such glycopeptides as vancomycin (3) and chloroorienticin B (4),[14] respectively.

The α-aminoketo structure **S** was in agreement with the results of $NaBH_4$ reduction of balhimycin (1) and deglucobalhimycin (2) (Figure 2). On reduction (5 equiv of $NaBH_4$, aqueous MeOH, room temperature, 3 h) followed by hydrolysis (Figure 1), both of them furnished 4-*epi*-vancosamine (S^2) (GC–MS, ^{1}H NMR), indicating that the parent ketosugar structure might be unstable to hydrolysis conditions and thus could not be detected from direct hydrolysis product. Furthermore, $NaBH_4$ reduction of 1 produced dihydrobalhimycin (4) which lacked the quaternary carbon at δ_C 94.29. Instead a new oxymethine carbon appeared at δ 76.44. The ^{1}H and ^{13}C NMR spectra of 4, assigned by 2D NMR measurements as well as chemical shift comparison, are listed in Tables 1 and 2, respectively. This product was found to be identical with an authentic sample of the known antibiotic chloroorienticin B (HPLC, superimposable ^{1}H NMR).



Despite these observations, the ketosugar structure was not in *total agreement* with the spectral features of 1 and 2. Thus, neither balhimycin nor its degluco derivative showed any carbonyl resonance above δ_C 180 in their ^{13}C NMR spectra as is expected out of a keto structure. Additionally, the quaternary carbon at δ_C 94.29 which showed no correlation remained to be interpreted. This intriguing puzzle could, however, be solved in a most simple manner. Thus the ^{13}C NMR spectrum of a thoroughly dried[15] sample of balhimycin recorded in DMSO-d_6 showed disappearance of the quaternary carbon at δ_C 94.29; instead a new signal at δ_C 211.47 corresponding to a typical ketone carbonyl emerged (Table 2). This finding could be rationalized by the hypothesis that *in aqueous solution balhimycin existed as a stable ketone hydrate* 1′ in which the second sugar had the geminal diol structure **S′**. The fact that FAB ionization of balhimycin gave exclusively the molecular ion of the ketone might be due to a facile gas-phase dehydration process. Under electrospray ionization (ESI) conditions where sample ionization is performed on a nebulized aqueous solution at atmospheric pressure, balhimycin displayed two protonated molecular ion peaks at average m/z 1448 and 1466 corresponding to the ketone and the hydrate, respectively. That the hydrated molecular ion is a real molecule and not a water "adduct" was established by the following findings: (i) the intensity ratio of the two molecular ion peaks (100:70) did not alter when the ESI cone voltage was raised; (ii) the ureido derivative 5[16] of balhimycin, which possesses a hemiaminal function instead of the keto group, gave only one molecular ion peak at m/z 1491 $(M + H)^+$. These results were indicative of an equilibrium between the keto

(14) Tsuji, N.; Kamigauchi, T.; Kobayashi, M.; Terui, Y. *J. Antibiot.* 1988, *31*, 1506.

(15) Balhimycin was dried with activated molecular sieves in solution in DMSO followed by evaporation of the solvent at 10^{-6} Torr. The process was repeated several times.

(16) Ureidobalhimycin was prepared by reacting balhimycin with aqueous KOCN.

Table 2. ^{13}C NMR data of Balhimycin (1), Deglucobalhimycin (2), and Dihydrobalhimycin (4)[a]

carbon	1[b]	2[c]	4[b]
1 (*N*-methylleucine)			
CO	172.28 (174.38)	171.79	172.25
	61.98 (62.18)	62.10	62.26
	40.48 (40.93)	40.41	40.42
	25.18 (24.05)	25.29	25.22
	23.74 (22.85)	23.61	23.37
	22.32 (22.14)	22.50	22.43
N-Me	33.20 (33.66)	33.17	33.28
2			
CO	170.18 (170.24)	170.39	170.12
	60.21 (58.19)	60.53	60.25
	72.53 (71.09)	72.45	72.75
2a	138.85 (139.62)	138.62	137.84
2b	131.51 (128.30)	131.19	131.41
2c	129.13 (127.47)	129.21	129.04
2d	151.44 (150.19)	151.63	151.45
2e	126.26 (124.21)	126.41	126.27
2f	128.36 (126.63)	128.12	128.59
3 (asparagine)			
CO	172.30 (166.49)	172.17	172.32
	53.87 (50.88)	53.70	53.85
	38.05 (42.92)	38.14	38.11
CO′	175.10 (170.95)	174.68	175.32
4			
CO	172.71 (169.15)	173.07	172.65
	55.80 (54.46)	55.63	55.91
4a	136.30 (133.83)	130.41	136.36
4b	106.09 (107.91)	105.64	106.16
4c	154.77 (151.39)	149.87	154.76
4d	134.89 (132.31)	135.01	134.87
4e	154.61 (150.95)	150.52	154.57
4f	105.41 (104.61)	105.00	105.53
5			
CO	172.92 (169.28)	172.82	173.86
	56.13 (53.53)	56.19	56.10
5a	126.82 (126.75)	126.95	126.76
5b	137.09 (135.50)	136.92	138.11
5c	122.68 (121.60)	122.52	123.12
5d	156.98 (154.93)	155.91	155.96
5e	119.71 (116.13)	119.73	119.72
5f	128.34 (125.38)	128.80	128.45
6			
CO	170.57 (167.08)	169.56	168.50
	63.54 (60.58)	63.41	63.53
	75.63 (74.37)	75.81	75.76
6a	137.11 (136.29)	136.16	137.25
6b	131.34 (128.73)	131.00	131.45
6c	128.45 (125.75)	128.35	128.42
6d	151.85 (149.24)	152.14	151.75
6e	124.80 (125.03)	124.94	124.70
6f	128.52 (123.41)	128.85	128.52
7			
CO	175.72 (171.97)	175.68	178.52
	58.60 (56.67)	58.61	60.87
7a	136.93 (137.50)	136.69	139.25
7b	119.00 (117.67)	119.04	119.14
7c	156.70 (156.21)	158.19	156.41
7d	104.86 (102.18)	104.86	104.32
7e	158.26 (156.90)	156.67	157.98
7f	108.94 (105.94)	108.93	109.63
glucose			
1	105.84 (102.54)		105.79
2	75.40 (74.17)		75.18
3	77.44 (76.54)		77.38
4	70.67 (69.99)		70.54
5	77.73 (77.39)		77.24
6	62.47 (61.08)		61.90
vancosamine	4-oxo	4-oxo	4-*epi*
1	94.00 (93.62)	93.99	94.14
2	38.23 (36.99)	38.61	39.78
3	60.18 (55.72)	60.20	57.92
4	94.29 (211.47)	94.27	76.44
5	68.27 (68.65)	68.22	67.92
6	13.48 (15.25)	13.51	18.88
3-Me	21.91 (26.15)	21.90	19.59

[a] The carbon multiplicities were determined from DEPT-135 spectra. [b] Recorded at 300 K in D_2O using a concentration of 16 mg/mL. Values in parentheses are those recorded at 320 K in DMSO-d_6 using a concentration of 28 mg/mL. [c] Recorded at 325 K in D_2O using a concentration of 25 mg/mL.

and hydrated forms of the 4-dehydrovancosamine unit in balhimycin.

The attachment of the dehydrovancosamine unit to the benzylic OH of the amino acid residue 6 was established from long-range ^{13}C–^{1}H (HMBC) as well as 2D NOESY correlation studies. Figure 2 illustrates the relevant interactionns in this segment of the molecule. The inter-residual HMBC correlations of the C-1/H-1 signals of the dehydrovancosamine unit with the H-6/C-6 of the aglycon unambiguiously established the location of this amino sugar. This was fully supported by the observed NOE interaction between the anomeric proton with the 6 benzylic proton.

The structure of balhimycin was thus established as represented by 1.

Balhimycin, as its various salts, exhibited *in vitro* and *in vivo* antibacterial activity against MRSA strains. The minimum inhibitory concentrations (MICs) varied from 0.05 to 0.8 μg/mL and were comparable with those of vancomycin. Against anaerobes, particularly *Clostridium* strains, balhimycin showed bactericidal activity superior to that of vancomycin. Balhimycin also showed better postantibiotic effects. Balhimycin is, to our knowledge, the first example of a glycopeptide antibiotic with a hydrated ketosugar moiety which offers a very useful chemical handle for semisynthetic modifications in this part of the molecule. We are currently delineating such scopes.

Experimental Section

Melting points are uncorrected. Mass spectra were obtained on VG-ZAB SEQ (FAB), VG B10-Q (ESI), and Kratos MS-80 (GC–MS) spectrometers. NMR spectra were recorded on a Bruker ARX 500 spectrometer using TSP (trimethylsilylpropionic acid sodium salt) as the internal reference. NOESY spectrum of balhimycin was recorded on a Varian VXR-300 spectrometer at 295 K using a concentration of 20 mg/mL in D_2O and a mixing time of 400–600 ms. HPLC analysis was carried out on a 10 μm ODS-Hypersil [4 × (30 + 250) mm] column using 40:60 CH_3CN–H_2O containing 0.1% TFA as the eluant with a flow rate of 1 mL/min and detection at 220 nm. Medium-pressure liquid chromatography (MPLC) was carried out in a glass column packed with 50–60 μm C_{18} silica gel using CH_3CN–H_2O containing 0.1% TFA mixtures as the eluants. Preparative TLC was carried out on SiO_2 gel plates (Article No. 13794, E. Merck).

Balhimycin (1). The isolation of balhimycin has been reported earlier.[5] Balhimycin trifluoroacetate salt was obtained as a water-soluble white powder: mp > 300 °C (dec); $[\alpha]_D$ −23.22° (*c* 5.0, H_2O); HPLC R_t 3.9 min. ^{1}H and ^{13}C NMR data are given in Tables 1 and 2.

Preparation of Deglucobalhimycin Trifluoroacetate (2). Balhimycin (0.1 g, 0.081 mmol) was heated with 4 mL of 60:40 TFA–H_2O at 60 °C for 30 min. The reaction mixture was neutralized with 2 N ammonia to pH 6. The crude product was purified by MPLC with a flow rate of 10 mL/min and detection at 220 nm. Deglucobalhimycin (2) eluted out with 35% CH_3CN in H_2O containing 0.1% TFA. Concentration under reduced pressure followed by lyophilization yielded 0.069 g (0.054 mmol) of 2 as a white powder: mp > 320 °C; $[\alpha]_D$ −45.55° (*c* 0.18, H_2O); HPLC R_t: 5.8 min. The ^{1}H and ^{13}C NMR data are listed in Tables 1 and 2.

Preparation of Triester V′ from 1. To a suspension of balhimycin (0.49 g, 0.4 mmol) in anhydrous MeOH (50 mL) and K_2CO_3 (0.625 g, 4.52 mmol), CH_3I (12.5 mL, 200 mmol) was added and the mixture was refluxed overnight at 70–75 °C. The solvent was evaporated under reduced pressure and the residue was washed with H_2O (2 × 15 mL). The methyl ether, thus obtained, was hydrolyzed with 1 N HCl for 1 h at 95 °C. The reaction mixture was neutralized to pH 6 with 2 N NaOH, centrifuged, and decanted. The residue was washed with H_2O (10 mL),

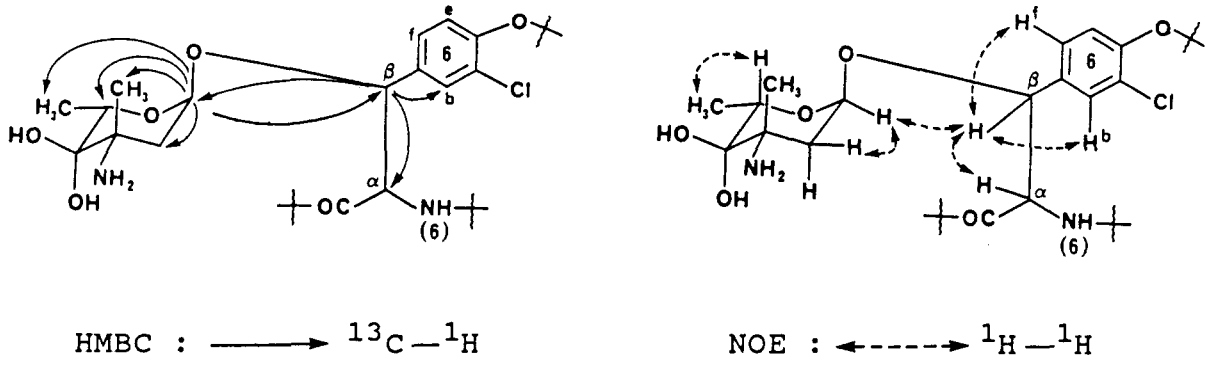


Figure 2. HMBC and NOE network establishing the attachment of the 4-dehydrovancosamine.

filtered, and dried in a desiccator over P_2O_5 under vacuum. The product obtained was dissolved in anhydrous MeOH (50 mL) and K_2CO_3 (0.625 g, 4.52 mmol) and C_2H_5I (12.5 mL, 156 mmol) were added. The reaction mixture was refluxed overnight at 70–75 °C. The solvent was then evaporated under vacuum and the residue was triturated with H_2O (2 × 10 mL), centrifuged, and dried over P_2O_5 to furnish 0.14 g of the differentially alkylated aglycon. This product was taken in H_2O (6 mL) to which $KMnO_4$ (0.814 g, 5.15 mmol) dissolved in 20 mL of H_2O and 2 M ammonia (3.1 mL) were added. The mixture was kept at 70–75 °C for 5 h, then filtered, adjusted to pH 6 with 3 N HCl, and extracted with ethyl acetate (3 × 50 mL). The combined extracts were dried over Na_2SO_4 and concentrated to dryness. The product was then esterified by treatment with CH_2N_2/ether–MeOH at 0 °C for 3 h. The crude triester (34 mg) was purified by preparative TLC (solvent for development: diisopropyl ether; solvent for elution: 20:80 MeOH–CH_2Cl_2) to afford 5 mg (0.01 mmol) of the pure trimethyl ester V′: mp 92–94 °C; DCI-MS: *m/z* 549 (MH^+). Anal. Calcd for $C_{26}H_{22}O_9Cl_2$: C, 56.93; H, 4.01; Cl, 12.95. Found: C, 56.80; H, 4.10; Cl 12.65. UV (MeOH): 256 nm; IR (KBr) cm^{-1} 1740, 1620, 1600 and 1580; ^1H NMR (300 MHz, $CDCl_3$ as solvent and internal reference) δ 8.17 (d, *J* = 1.6 Hz, 2H), 7.89 (dd, *J* = 8.6, 1.6 Hz, 2H), 7.61 (bs, 2H), 6.83 (d, *J* = 8.6 Hz, 2H), 4.05 (q, 7.0 Hz, 2H), 3.84 (s, 6H), 3.79 (s, 3H), 1.02 (t, *J* = 7.0 Hz, 3H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.25, 165.97, 157.21, 149.45, 147.24, 133.14, 130.41, 126.94, 126.64, 124.71, 120.04, 117.61, 71.14, 53.31, 53.18, and 16.16.

Reduction of Balhimycin. To balhimycin (0.1 g, 0.081 mmol) suspended in 1:1 MeOH–H_2O (20 mL) was added 7 equiv of $NaBH_4$ (20 mg, 0.51 mmol), and the mixture was stirred at room temperature for 3 h. AcOH (200 μL) was added to quench the excess borohydride. The product was purified by MPLC using increasing amounts of CH_3CN in H_2O containing 0.1% TFA as eluant with a flow rate of 10 mL/min and detection at 220 nm. Concentration under reduced pressure followed by lyophilization yielded 0.062 g (0.043 mmol) of dihydrobalhimycin. The ^1H and ^{13}C NMR spectral shifts are given in Tables 1 and 2.

Acknowledgment. We thank Shionogi Research Laboratories, Japan, for providing us with authentic samples of chloroorienticins. We also acknowledge the high-field NMR facilities at the Tata Institute of Fundamental Research (TIFR) and the Regional Sophisticated Instrumentation Centre (RSIC), Bombay. We thank Dr. R. V. Hosur of TIFR for some helpful discussions.