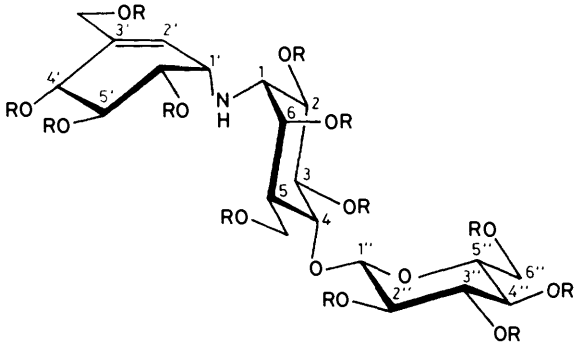
Synthetic Studies on Antibiotic Validamycins. Part 12.[1,2] Total Synthesis of (+)-Validamycin B and (+)-Validoxylamine B

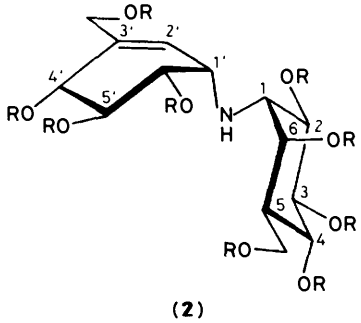
Seiichiro Ogawa,* Yasunobu Miyamoto, and (in part) Taisuke Nose
Department of Applied Chemistry, Faculty of Science and Technology, Keio University, Hiyoshi, Yokohama, 223 Japan

The first complete synthesis of validamycin B (**1a**) and validoxylamine B (**2a**) is reported; coupling of the epoxide (**12**) and the partially protected derivative (**16**) of (+)-valienamine, followed by deprotection, gives (**1a**), which was identified from the ^{1}H n.m.r. spectrum of its totally *O*-acetylated derivative (**1b**). Alternatively, coupling of the epoxide (**24**) with the amine (**16**) affords compound (**25**), the structure of which can be established by converting (**25**) into validoxylamine B nona-*O*-acetate (**2b**). The appropriately protected amine (**29**) derived from (**25**) is glycosylated, followed by deprotection and acetylation, to give (**1b**).

Validamycin B is one of the components of the antibiotic validamycin complex,[3] and shows growth inhibition activity against sheath blight disease which affects rice plants. Since validamycin A was first isolated as the major and most active compound from the fermentation broth of *Streptomyces hygroscopicus* var. *limoneus* by Iwasa *et al.*,[4] in 1970, other minor validamycins B, C, D, E, F, and G,[5] and validoxylamines A, B, and G[5] have been isolated and characterised successfully. Validamycins B and G are somewhat different in structure from the others, containing hydroxyvalidamine and valiolamine, respectively, instead of validamine, as a saturated branched chain aminocyclitol moiety. Validamycin A,[2,6] and racemic validoxylamine A[7,8] and B[8,9] have been synthesized so far. We now describe in detail the first total synthesis of (+)-validamycin B (**1a**) and (+)-validoxylamine B (**2a**).



(**1**)



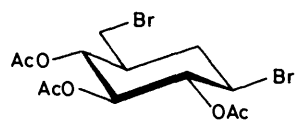
(**2**)

(**1**)
a; R = H (validamycin B)
b; R = Ac

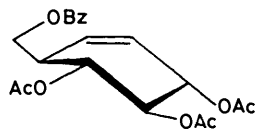
(**2**)
a; R = H (validoxylamine B)
b; R = Ac

Our common synthetic strategy for construction of the pseudo-di- and tri-saccharide structures linked by way of an imino group is a facile coupling of the amino and epoxy derivatives, the hydroxy functions of which are protected appropriately as benzyl ether groups. The present synthesis comprises two approaches successfully carried out: one is a coupling of the β-D-glucopyranoside derivative of cyclohexene oxide and the protected (+)-valienamine, and the other a glycosylation of the protected derivative of validoxylamine B, obtained by a coupling of the protected cyclohexene oxide and the protected (+)-valienamine.

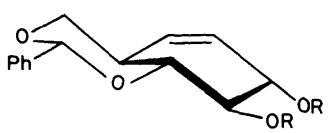
First, treatment of (1*R*)-(1,3/2,4)-1,2,3-triacetoxy-4-bromo-6-bromomethylcyclohexane (**3**)[10] with sodium benzoate and lithium bromide in *N*,*N*-dimethylformamide (DMF) at 105 °C gave the crystalline benzoate (**4**) (28%), the structure of which was confirmed by comparison with its racemic modification.[11]



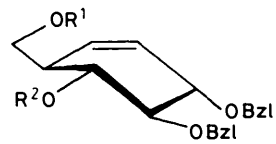
(**3**)



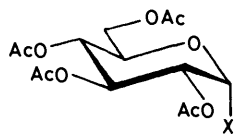
(**4**)



(**5**) R = H
(**6**) R = Bzl



(**7**) $R^1 = R^2 = H$
(**8**) R^1 = Ac, R^2 = H
(**9**) $R^1 = R^2$ = Ac

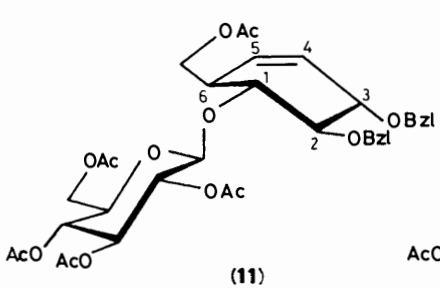


(**10a**) X = Cl
(**10b**) X = Br

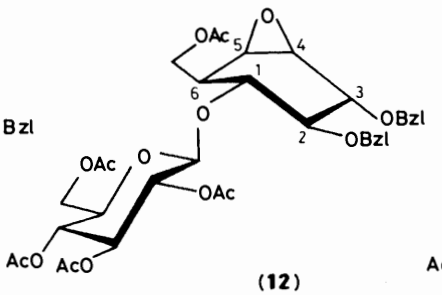
Bz = PhCO, Bzl = $PhCH_2$

Compound (**4**) was *O*-deacylated with methanolic sodium methoxide in methanol, and the resulting tetrol was treated with α,α-dimethoxytoluene in DMF in the presence of toluene-*p*-sulphonic acid (PTSA) to afford the benzylidene acetal (**5**) (96% overall yield). Benzylation of (**5**) with benzyl bromide and sodium hydride in DMF gave the dibenzyl ether (**6**) (92%), which was then *O*-debenzylidenated with aqueous acetic acid to give the diol (**7**) (95%). Selective protection of the primary hydroxy group of (**7**) was effected by treatment with acetyl chloride and imidazole[12] to provide the 7-acetate (**8**) (88%), along with the 4,7-diacetate (**9**) (8%).

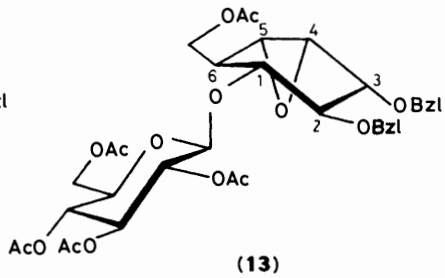
Condensation of the aglycone (**8**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl chloride (**10a**) in dry dichloromethane in the presence of silver trifluoromethanesulphonate and 1,1,3,3-tetramethylurea at 40 °C for 4 h afforded the β-glucoside (**11**) (87%), the structure of which was supported by ^{1}H n.m.r. spectral results. Epoxidation of (**11**) with *m*-chloroperbenzoic acid (MCPBA) in 1,2-dichloroethane and phosphate buffer solution (pH 8) at 50 °C for 22 h gave the crystalline epoxides (**12**) (70%) and (**13**) (14%). The structures of (**12**) and (**13**) were tentatively assigned on the basis of previous results for epoxidation of the related olefins.[11,13]



(**11**)

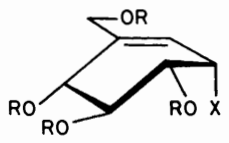


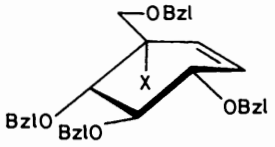
(**12**)



(**13**)

On the other hand, the amino synthon, tetra-*O*-benzyl-(+)-valienamine (**16**) was prepared in the following sequence. The readily available (1*S*)-(1,3,6/2)-1,2,3-triacetoxy-4-acetoxymethyl-6-azidocyclohex-4-ene (**14**)[14] was *O*-deacetylated with methanolic sodium methoxide and then benzylated in the usual way to produce a mixture of the azides (**15**) and (**18**). Without





(**14**) R = Ac, X = N_3
(**15**) R = Bzl, X = N_3
(**16**) R = Bzl, X = NH_2
(**17**) R = Bzl, X = NHAc

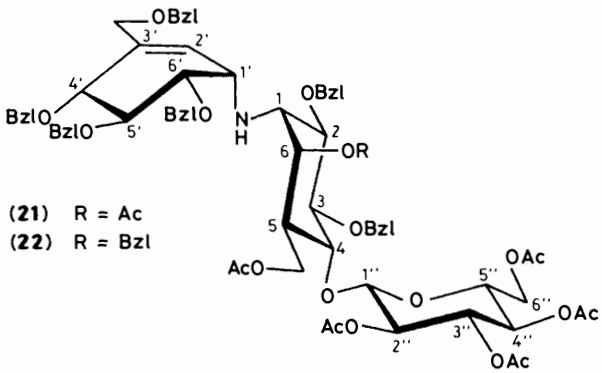
(**18**) X = N_3
(**19**) X = NH_2
(**20**) X = NHAc

isolation, they were selectively reduced with hydrogen sulphide in aqueous pyridine to give the amines (**16**) (65%) and (**19**) (12%), which were characterised as the *N*-acetates (**17**) and (**20**). The ^{1}H n.m.r. spectrum supported the proposed structure of the minor component (**18**) which was considered to be formed *via* a sigmatropic rearrangement of the allylic azido group.[15]

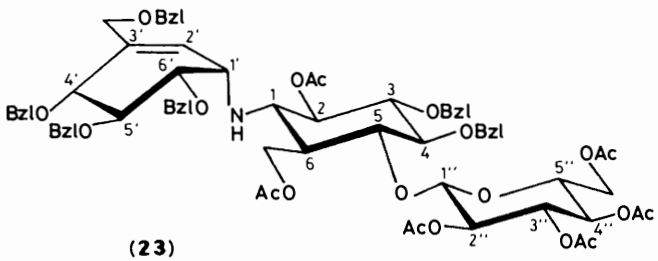
Coupling of the epoxide (**12**) with the amine (**16**) was conducted in propan-2-ol in a sealed tube at 120 °C for 125 h to afford two protected pseudotrisaccharides (**21**) (47%) and (**23**) (30%). Compound (**21**) was shown to be the desired product resulting from diaxial cleavage of the epoxide function, on the basis of its ^{1}H n.m.r. spectrum, which contained a triplet (δ 3.20, *J* 4 Hz) attributable to the equatorial 1-H: in (**23**), the signal due to the axial 1-H appeared as a triplet (δ 2.81, *J* 10 Hz). Removal of the protecting groups of (**21**) with sodium in liquid ammonia at −78 °C gave validamycin B (**1a**), which was identical with an authentic sample on t.l.c.,[3b] and further characterised by conversion into the undeca-acetate (**1b**), the ^{1}H n.m.r. results (Table) for which were superimposable on those of an authentic sample.*

Alternatively, the other complete synthesis *via* glycosidation of the protected derivative (**29**) of validoxylamine B was elaborated.

Epoxidation of the olefin (**6**) with MCPBA in the presence of a buffer solution gave a single epoxide (**24**) (84%) the ^{1}H n.m.r. spectrum of which supported the proposed structure. Similar coupling of (**24**) with the amine (**16**) in propan-2-ol at 120 °C afforded preferentially the desired amine (**25**) (75%), along with compound (**27**) (15%). The identity of the amine (**25**) was supported by ^{1}H n.m.r. spectral results and finally established by converting the compound into validoxylamine B (**2a**) and its nona-acetate (**2b**): the ^{1}H n.m.r. spectral results of the latter (Table) were identical with those of an authentic sample.* Compound (**25**) was further benzylated to the heptabenzyl ether



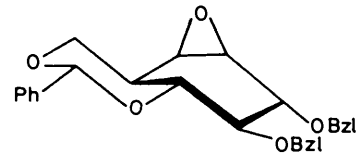
(**21**) R = Ac
(**22**) R = Bzl



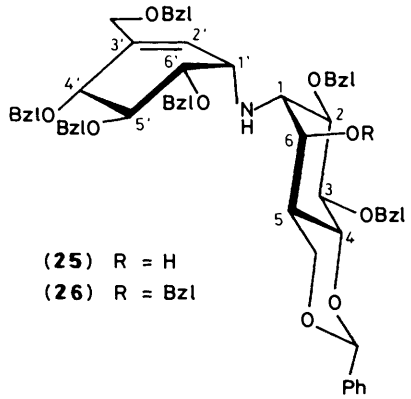
(**23**)

(**26**) (78%), which was *O*-debenzylidenated to yield the diol (**28**) (93%). Selective acetylation of the latter afforded the acetate (**29**) (84%) and the diacetate (**30**) (7%).

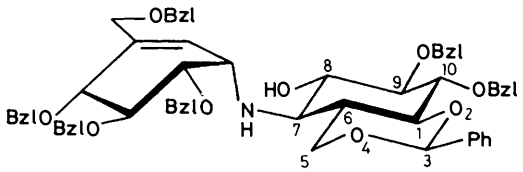
*^{1}H N.m.r. results for the totally *O*-acetylated derivatives of validamycin B and validoxylamine B have kindly been provided by Dr. S. Horii, to whom our thanks are due.



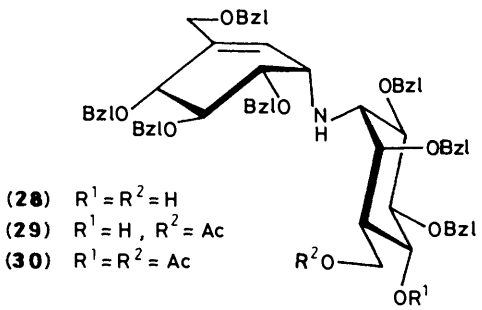
(24)



(25) R = H
(26) R = Bzl



(27)



(28) $R^1 = R^2 = H$
(29) $R^1 = H$, $R^2 = Ac$
(30) $R^1 = R^2 = Ac$

Condensation of the acetate (**29**) with 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**10b**)* under conditions similar to those used in the preparation of compound (**21**) produced the protected derivative (**22**) of validamycin B (42%), which was deprotonated and acetylated to give the dodeca-acetate (**1b**) (51%).

Experimental

M.p.s were determined on a MEL-TEMP capillary melting point apparatus and are uncorrected. N.m.r. spectra were measured in deuteriochloroform solution with a Varian EM-390 (90 MHz) or JEOL FX-400 (400 MHz) instrument. Optical rotations were measured with a Jasco DIP-4 instrument. T.l.c. was performed by use of silica gel 60 F-254 (E. Merck, Darmstadt). The silica gel used for column chromatography was Wakogel C-300 (Wako Co., Osaka, Japan; 300 Mesh).

* The chloride (**10a**) is stable and, after the reaction, the excess of (**10a**) was easily separable during purification. However, in this case, the coupling by use of (**10a**) did not proceed smoothly.

Table. ^{1}H N.m.r. spectral data[a] (400 MHz; $CDCl_3$) for compounds (**1b**) and (**2b**)

	Chemical shifts (δ)			Coupling constants (Hz)	
Proton	(**1b**)	(**2b**)	*J*	(**1b**)	(**2b**)
1	3.22 (t)[b]	3.31 (t)[b]	1,2	5.1	3.9
2	5.12 (dd)	5.20 (dd)	2,3	10.3	10.7
3	5.31 (dd)	5.34 (dd)	3,4	8.8	9.3
4	3.78 (dd)	5.21 (dd)	4,5	10.8	11.7
5	2.66 (m)	2.74 (m)	5,6	3	3
6	5.13 (dd)	5.18 (dd)	1,6	3.9	3.4
7a	4.16 (dd)	3.94 (dd)	5,7a	9.8	4.4
7b	4.30 (dd)	4.21 (dd)	5,7b	8.8	3.9
1′	3.59 (dd)	3.58 (m)	7a,7b	10.7	11.2
2′	5.97 (br d)	5.99 (dd)	1′,2′	4.9	4.9
4′	5.51 (d)	5.50 (br d)	4′,5′	6.2	6.2
5′	5.41 (dd)	5.42 (dd)	5′,6′	9.7	9.9
6′	4.98 (dd)	5.02 (dd)	1′,6′	4.4	4.6
7′a	4.38 (d)	4.38 (d)	7′a,7′b	13.0	13.2
7′b	4.66 (br d)	4.65 (d)	1″,2″	8.1	
1″	4.50 (d)		2″,3″	9.3	
2″	4.95 (dd)		3″,4″	9.3	
3″	5.15 (t)		4″,5″	9.3	
4″	5.10 (t)		5″,6″a	2.3	
5″	3.68 (ddd)		5″,6″b	3.7	
6″a	4.04 (dd)		6″a,6″b	12.5	
6″b	4.44 (dd)				
$COCH_3$	1.99	2.02			
	2.01	2.04			
	2.03	2.05			
	2.05	2.06			
	2.06	2.07(2)[c]			
	2.07(3)[c]	2.08			
	2.075	2.12			
	2.08	2.13			
	2.11				
	2.12				

[a] Values given for coupling constants are of first-order spectra. [b] Splitting patterns are recorded in appearance. [c] Values in parentheses show number of acetoxy groups.

(1S)-(1,3/2,4)-1,2,3-*Triacetoxy*-4-*benzoyloxymethylcyclohex*-5-*ene* (**4**).—A mixture of (1*R*)-(1,3/2,4,6)-1,2,3-triacetoxy-4-bromo-6-bromomethylcyclohexane (**3**) (6.45 g, 15 mmol), sodium benzoate (6.5 g, 45 mmol), lithium bromide monohydrate (3.15 g, 30 mmol), and dry DMF (100 ml) was stirred at 105 °C for 30 h, and then concentrated. The residue was taken up in ethyl acetate (250 ml), and the solution was washed with water and dried. Evaporation of the solvent gave a syrup, which was crystallised from ethanol to give the benzoate (**4**) (1.66 g, 28.4%) as prisms, m.p. 113—114 °C (Found: C, 61.4; H, 5.7. $C_{20}H_{22}O_8$ requires C, 61.5; H, 5.7%); $[\alpha]_D^{23}$ +129° (*c* 1.5 in $CHCl_3$); δ_H(90 MHz; $CDCl_3$) (*inter alia*) 2.01, 2.02, and 2.05 (total 9 H, 3 s, 3 $COCH_3$), 2.79—3.08 (1 H, m, 4-H), 4.19 (1 H, dd, *J* 5, J_{gem} 10.5 Hz, 7-H), 4.47 (1 H, dd, *J* 4.5, J_{gem} 10.5 Hz, 7′-H), and 7.35—7.78 (5 H, m, Ph).

(1R,3R,6R,9S,10S)-9,10-*Dihydroxy*-3-*phenyl*-2,4-*dioxabicyclo*[4.4.0]*dec*-7-*ene* (**5**).—The benzoate (**4**) (1.0 g, 2.6 mmol) was treated with 1M methanolic sodium methoxide (4 ml) in methanol (10 ml) at 0 °C for 1 h after which the mixture was neutralised with Amberlite IR-120B (H^+) resin and concen-

trated to give the tetrol (0.49 g, quantitative). Without purification, the tetrol was treated with α,α-dimethoxytoluene (0.50 ml, 3.3 mmol) and PTSA (5 mg) in dry DMF (10 ml) at 60 °C for 4.5 h under reduced pressure (15—20 mmHg). After treatment with sodium hydrogen carbonate, the mixture was concentrated, and the residue was chromatographed on a silica gel column (10 g), with methanol–chloroform (1:10, v/v) as eluant, to give the benzylidene acetal (**5**) (0.61 g, 96%) as feathers, m.p. 165—167 °C (from EtOH) (Found: C, 67.45; H, 6.5. $C_{14}H_{16}O_4$ requires C, 67.7; H, 6.5%); $[\alpha]_D^{21}$ +28° (*c* 2.5 in EtOH).

(1R,3R,6R,9S,10S)-9,10-*Dibenzyloxy*-3-*phenyl*-2,4-*dioxabicyclo*[4.4.0]*dec*-7-*ene* (**6**).—50% Sodium hydride (0.62 g, 13 mmol) was added to a solution of the diol (**5**) (0.80 g, 3.2 mmol) in dry DMF (18 ml), and the mixture was stirred at 0 °C for 30 min. Benzyl bromide (1.53 ml, 12.9 mmol) was then added dropwise to the cooled mixture which was then stirred for 1.5 h. After addition of methanol, the mixture was diluted with EtOAc (150 ml), and the solution was washed with water, dried, and concentrated. The product was chromatographed on a silica gel column (40 g), with EtOAc–hexane (1:10, v/v) as eluant, to give the dibenzyl ether (**6**) (1.27 g, 92%) as a syrup (Found: C, 78.2; H, 6.6. $C_{28}H_{28}O_4$ requires C, 78.5; H, 6.6%); $[\alpha]_D^{20}$ +34° (*c* 2.6 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 2.47—2.90 (1 H, m, 6-H), 3.60 (1 H, t, *J* 11 Hz, 5-H_{ax}), 3.78 (1 H, t, *J* 9.5 Hz, 10-H), 3.97 (1 H, t, *J* 9.5 Hz, 1-H), 4.25 (1 H, dd, J_{gem} 11, 4.5 Hz, 5-H_{eq}), 4.16—4.40 (1 H, m, 9-H), 4.65—5.10 (4 H, m, 2 CH_2Ph), 5.35 (1 H, dt, J_{cis} 10.5, 1.5 Hz, 8-H), 5.61 (1 H, s, 3-H), 5.73 (1 H, dt, J_{cis} 10.5, 3 Hz, 7-H), and 7.15—7.62 (15 H, m, 3 Ph).

(1S)-(1,3/2,4)-1,2-*Dibenzyloxy*-3-*hydroxy*-4-*hydroxymethylcyclohex*-5-*ene* (**7**).—Compound (**6**) (0.91 g, 2.1 mmol) was treated with aqueous 80% acetic acid (20 ml) at 40 °C for 1.5 h. The mixture was concentrated and the residue was crystallised from ethanol to give the diol (**7**) (0.69 g, 95%) as plates, m.p. 124—125 °C (Found: C, 74.4; H, 7.1. $C_{21}H_{24}O_4$ requires C, 74.1; H, 7.1%); $[\alpha]_D^{23}$ +147° (*c* 1.1 in acetone).

(1S)-(1,3/2,4)-4-*Acetoxymethyl*-1,2-*dibenzyloxy*-3-*hydroxycyclohex*-5-*ene* (**8**) and 3-*Acetoxy*-4-*acetoxymethyl*-1,2-*dibenzyloxycyclohex*-5-*ene* (**9**).—Acetyl chloride (0.24 ml, 3.4 mmol) was added dropwise at 0 °C to a solution of imidazole (0.47 g, 6.8 mmol) in $CHCl_3$ (5 ml). The precipitate was filtered off and washed with $CHCl_3$ (1 ml) and the diol (**7**) (0.58 g, 1.7 mmol) in $CHCl_3$ (10 ml) was added to the filtrate. The mixture was then refluxed for 44 h, diluted with $CHCl_3$ (50 ml), washed with water, dried, and concentrated. The product was chromatographed on a silica gel column (15 g), with butan-2-one–toluene (1:3, v/v) as eluant, to give, first, the diacetate (**9**) (60 mg, 8.2%) as a syrup (Found: C, 70.8; H, 6.7. $C_{25}H_{28}O_6$ requires C, 70.7; H, 6.7%); $[\alpha]_D^{25}$ +61° (*c* 0.42 in $CHCl_3$); δ_H(90 MHz; $CDCl_3$) (*inter alia*) 1.94 and 2.01 (each 3 H, 2 s, 2 $COCH_3$), 2.50—2.83 (1 H, m, 4-H), 3.62—4.33 (4 H, m, 1,2,7,7′-H), 4.57—4.93 (4 H, m, 2 CH_2Ph), 5.15 (1 H, t, *J* 9.5 Hz, 3-H), 5.54 (1 H, dt, J_{cis} 10, 2.5 Hz, 6-H), 5.78 (1 H, dt, J_{cis} 10, 2 Hz, 5-H), and 7.10—7.43 (10 H, m, 2 Ph).

The second fraction gave the monoacetate (**8**) (0.57 g, 88%) as a syrup (Found: C, 72.6; H, 6.8. $C_{23}H_{26}O_5$ requires C, 72.2; H, 6.85%); $[\alpha]_D^{25}$ +124° (*c* 2.5 in $CHCl_3$); δ_H(90 MHz; $CDCl_3$) (*inter alia*) 2.01 (3 H, s, $COCH_3$), 2.40—2.63 (1 H, m, 4-H), 2.80 (1 H, br s, OH), 4.11 (1 H, dd, J_{gem} 10.5, 5.5 Hz, 7-H), 4.05—4.31 (1 H, m, 1-H), 4.33 (1 H, dd, J_{gem} 10.5, 4.5 Hz, 7′-H), 4.57—5.07 (4 H, m, 2 CH_2Ph), 5.56 (1 H, dt, J_{cis} 9, 3 Hz, 6-H), 5.75 (1 H, dt, J_{cis} 9, 2 Hz, 5-H), and 7.18—7.43 (10 H, m, 2 Ph).

Condensation of the Hydroxy Compound (**8**) *with* 2,3,4,6-*Tetra*-O-*acetyl*-α-D-*glucopyranosyl Chloride* (**10a**).—Silver trifluoromethanesulphonate (1.05 g, 4.1 mmol) and 1,1,3,3-tetramethylurea (0.98 ml, 8.2 mmol) were added under argon to a stirred mixture of compound (**8**) (1.04 g, 2.7 mmol) in dry dichloromethane (15 ml); a solution of the halide (**10a**) (2.98 g, 8.1 mmol) in dry dichloromethane (10 ml) was then added dropwise at room temperature. After the mixture had been stirred at 40 °C for 4 h in the dark, it was neutralised with 10% triethylamine–chloroform and filtered. The filtrate was concentrated and the residue was chromatographed on a silica gel column (100 g), with EtOAc-hexane (2:5, v/v) as eluant, to give [(1*R*)-(1,3/2,6)-6-acetoxymethyl-2,3-dibenzyloxycyclohex-4-enyl]-2,3,4,6-tetra-*O*-acetyl-β-D-glucopyranoside (**11**) (1,69 g, 87%) as silky needles, m.p. 112—112.5 °C (from EtOH) (Found: C, 62.5; H, 6.1. $C_{37}H_{44}O_{14}$ requires C, 62.35; H, 6.2%); $[\alpha]_D^{19}$ + 85° (*c* 1.1 in $CHCL_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.95, 1.98, 2.03, and 2.05 (15 H, 4 s, 5 $COCH_3$), 2.50—2.84 (1 H, m, 6-H), 5.52 (1 H, dt, J_{cis} 10.5, 4.5 Hz, 4-H), 5.73 (1 H, dt, J_{cis} 10.5, 3 Hz, 5-H), and 7.13—7.47 (10 H, m, 2 Ph).

[(1R)-(1,3/2,4,5,6)- (**12**) *and* (1,3,4,5/2,6)-6-*Acetoxymethyl*-2,3-*dibenzyloxy*-4,5-*epoxycyclohexyl*]-2,3,4,6-*tetra*-O-*acetyl*-β-D-*glucopyranoside* (**13**).—A mixture of the olefin (**11**) (0.82 g, 1.2 mmol), MCPBA (0.42 g, 1.7 mmol), aqueous 1M Na_2HPO_4 (12 ml), aqueous 1M NaH_2PO_4 (12 ml), and 1,2-dichloroethane (20 ml) was stirred vigorously at 50 °C for 22 h. The mixture was diluted with $CHCl_3$ (80 ml), washed with water, dried, and concentrated. The product was chromatographed on a silica gel column (30 g), with EtOAc–hexane (1:2, v/v) as eluant, to give, first, the epoxide (**12**) (0.58 g, 70%) as silky needles, m.p. 157.5—159 °C (from EtOH) (Found: C, 60.9; H, 5.8. $C_{38}H_{44}O_{15}$ requires C, 61.0; H, 6.1%); $[\alpha]_D^{20}$ +51° (*c* 1.4 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.93, 1.97, 2.07, and 2.09 (15 H, 4 s, 5 $COCH_3$), and 7.13—7.40 (10 H, m, 2 Ph).

The second fraction gave the epoxide (**13**) (0.12 g, 14%) as needles, m.p. 141—142 °C (from EtOH) (Found: C, 61.3; H, 6.1%); $[\alpha]_D^{22}$ +18° (*c* 0.6 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.93, 1.98, 2.07, and 2.11 (15 H, 4 s, 5 $COCH_3$), and 7.10—7.41 (10 H, m, 2 Ph).

(1R)-(1,2,4/3)-2,3,4-*Tribenzyloxy*-5-*benzyloxymethylcyclohex*-5-*enylamine* (**16**) *and* (1S)-(1,2,4/3)-2,3,4-*Tribenzyloxy*-1-C-(*benzyloxymethyl*)*cyclohex*-5-*enylamine* (**19**).—(1*S*)-(1,3,6/2)-1,2,3-Triacetoxy-4-acetoxymethyl-6-azidocyclohex-4-ene (**14**) (0.80 g, 2.2 mmol) was treated with 1M methanolic sodium methoxide (4 ml) at room temperature for 3 h. The mixture was neutralised with Amberlite IR 120 (H^+) resin and then concentrated to give a crude hydroxy compound which, without purification, was treated with 50% NaH (0.83 g, 17 mmol) and benzyl bromide (2.1 ml, 17 mmol) in dry DMF (20 ml) at room temperature for 2 h. The mixture was diluted with EtOAc (200 ml), washed with water, dried, and concentrated. The product was eluted from a silica gel column (10 g), with EtOAc–hexane (1:10, v/v) as eluant, to give a syrupy mixture (1.0 g) of the tetrabenzyl ethers (**15**) and (**18**). H_2S Gas was bubbled into the mixture of compounds in pyridine–water (20 ml, 3:1, v/v) at room temperature for 3 h, after which the mixture was stirred for a further 15 h. The mixture was worked up and the crude product chromatographed on a silica gel column (30 g), with butan-2-one–toluene (1:3, v/v) as eluant, to give, first, the amine (**19**) (0.14 g, 12%) as a syrup (Found: C, 76.9; H, 6.8; N, 2.4. $C_{35}H_{37}NO_4 \cdot 0.5H_2CO_3 \cdot H_2O$ requires C, 77.2; H, 7.0; N, 2.6%); $[\alpha]_D^{22}$ +44° (*c* 2.5 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.61 (2 H, br s, NH_2), 3.21 and 3.35 (each 1 H, each d, J_{gem} 9 Hz, CH_2O), 4.40—5.06 (8 H, m, 4 CH_2Ph), 5.60 (1 H, dd, J_{cis} 10, 1.5 Hz, 5-H), 5.77 (1 H, dd, J_{cis} 10, 1.5 Hz, 6-H), and 7.10—7.50 (20 H, m, 4 Ph).

The second fraction gave the amine (**16**) (0.76 g, 65%) as a syrup (Found: C, 78.7; H, 6.9; N, 2.6. $C_{35}H_{37}NO_4$ requires C, 78.5; H, 7.0; N, 2.6%); $[\alpha]_D^{22}$ +4.2° (*c* 0.7 in $CHCl_3$); δ_H(90 MHz,

$CDCl_3$) (*inter alia*) 1.60 (2 H, br s, NH_2), 4.33—5.00 (8 H, m, 4 CH_2Ph), 5.83 (1 H, br d, *J* 4.5 Hz, 6-H), and 7.13—7.40 (20 H, m, 4 Ph).

(1R)-(1,2,4/3)-1-*Acetamido*-2,3,4-*tribenzyloxy*-5-*benzyloxymethylcyclohex*-5-*ene* (**17**).—The amine (**16**) (89 mg, 0.17 mmol) was acetylated with acetic anhydride and pyridine to give the *N*-acetate (**17**) (89 mg, 93%) as needles, m.p. 123—124 °C (from EtOH) (Found: C, 76.7; H, 6.8; N, 2.3. $C_{37}H_{39}NO_5$ requires C, 76.9; H, 6.8; N, 2.4%); $[\alpha]_D^{24}$ +25° (*c* 1.2 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.90 (3 H, s, $COCH_3$), 4.36—4.73 (8 H, m, 4 CH_2Ph), 5.74 (1 H, br d, *J* 4.5 Hz, 6-H), 5.56—5.80 (1 H, m, NH), and 7.07—7.33 (20 H, m, 4 Ph).

(1R)-(1,2,4/3)-1-*Acetamido*-2,3,4-*tribenzyloxy*-1-C-(*benzyloxymethyl*)*cyclohex*-5-*ene* (**20**).—The amine (**19**) (77 mg, 0.14 mmol) was acetylated to give the *N*-acetate (**20**) (78 mg, 94%) as needles, m.p. 104—105 °C (from EtOH) (Found: C, 76.9; H, 6.8; N, 2.3. $C_{37}H_{39}NO_5$ requires C, 76.9; H, 6.8; N, 2.4%); $[\alpha]_D^{22}$ +59° (*c* 2.1 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.87 (3 H, s, $COCH_3$), 3.45 and 3.85 (each 1 H, each d, *J* 8.7 Hz, CH_2O), 4.24—4.98 (8 H, m, 4 CH_2Ph), 5.83 (1 H, dd, J_{cis} 10.5, 2.3 Hz, 5-H), 6.06 (1 H, s, NH), 6.47 (1 H, br d, J_{cis} 10.5 Hz, 6-H), and 7.02—7.44 (20 H, m, 4 Ph).

6,7,2″,3″,4″,6″-*Hexa*-O-*acetyl*-2,3,4′,5′,6′,7′-*hexa*-O-*benzylvalidamycin B* (**21**) *and* (1S)-(1,3,5/2,4,6)-5-O-(2,3,4,6-*Tetra*-O-*acetyl*-β-D-*glucopyranosyl*)-2-*acetoxy*-6-*acetoxymethyl*-3,4-*dibenzyloxy*-N-[(1S)-(1,4,6/5)-4,5,6-*tribenzyloxy*-3-*benzyloxymethylcyclohex*-2-*enyl*]*cyclohexylamine* (**23**).—A mixture of the epoxide (**12**) (0.41 g, 0.56 mmol) and the amine (**16**) (0.30 g, 0.56 mmol) in propan-2-ol (2 ml) was heated in a sealed tube at 120 °C for 125 h. The mixture was concentrated and the residue was treated with acetic anhydride (5 ml) in pyridine (5 ml) at room temperature overnight. After work-up, the products were chromatographed on a silica gel column (20 g), with butan-2-one–toluene (1:7, v/v) as eluant, to give, first [R_F 0.71 in butan-2-one–toluene (2:5, v/v)], the amine (**23**) (0.22 g, 30%) as a syrup (Found: C, 67.7; H, 6.3; N, 1.3. $C_{74}H_{83}NO_{20}$ requires C, 68.0; H, 6.4; N, 1.1%); $[\alpha]_D^{22}$ −5.7° (*c* 2.1 in $CHCl_3$); δ_H(200 MHz, $CDCl_3$) (*inter alia*) 1.86, 1.92, 1.96, 1.98, 1.99, and 2.01 (each 3 H, 6 s, 6 $COCH_3$), 2.81 (1 H, t, *J* 10 Hz, 1-H), 5.74 (1 H, d, *J* 4.5 Hz, 2′-H), and 7.12—7.39 (35 H, m, 7 Ph).

The second fraction (R_F 0.62) gave the protected validamycin B (**21**) (0.345 g, 47%) as a syrup (Found: C, 67.2; H, 6.5; N, 1.1. $C_{74}H_{83}NO_{20}\cdot H_2O$ requires C, 67.1; H, 6.5; N, 1.1%); $[\alpha]_D^{22}$ +26° (*c* 1.5 in $CHCl_3$); δ_H(200 MHz, $CDCl_3$) (*inter alia*) 1.83, 1.95, 1.96, 1.98, 2.00, and 2.07 (each 3 H, 6 s, 6 $COCH_3$), 2.79—2.98 (1 H, m, 5-H), 3.20 (1 H, t, *J* 4 Hz, 1-H), 3.37 (1 H, t, *J* 4.5 Hz, 1′-H), 3.53 (1 H, dd, *J* 8, 4.5 Hz, 6′-H), 5.32 (1 H, t, *J* 4 Hz, 6-H), 5.89 (1 H, d, *J* 4.5 Hz, 2′-H), and 7.10—7.41 (35 H, m, 7 Ph).

Validamycin B (**1a**).—Compound (**21**) (0.30 g, 0.23 mmol) in dry THF (5 ml) was added to a liquid ammonia (30 ml) solution of sodium (0.30 g, 0.13 mmol) at −78 °C, and the mixture was stirred at the same temperature for 3 h. Further sodium (0.20 g, 8.7 mmol) was added at intervals of 3 h for 6 h. After addition of an excess of ammonium chloride, the mixture was kept at room temperature and then concentrated. T.l.c. indicated the formation of a single compound identical with validamycin B (**1a**) in propanol–acetic acid–water (4:1:1, v/v). The amine was acetylated with acetic anhydride in pyridine and the product was purified on a silica gel column (15 g), with butan-2-one–toluene (1:2, v/v) as eluant, to give the dodeca-acetate (**1b**) (0.105 g, 45%) as a syrup (Found: C, 51.75; H, 5.65; N, 1.6. $C_{44}H_{59}NO_{26}$ requires C, 51.9; H, 5.8; N, 1.4%); $[\alpha]_D^{23}$ +39° (*c* 1 in $CHCl_3$); δ_H(400 MHz, $CDCl_3$) data shown in the Table.

(1R,2S,5R,7R,8R,9R,10R)-8,9-*Dibenzyloxy*-5-*phenyl*-4,6,11-*trioxatricyclo*[8.1.0.0^{2,7}]*undecane* (**24**).—MCPBA (0.65 g, *ca.* 2.7 mmol), aqueous 1M Na_2HPO_4 (15 ml), and aqueous 1M NaH_2PO_4 were added to a solution of the olefin (**6**) (0.95 g, 2.2 mmol) in 1,2-dichloroethane (20 ml) and the mixture was vigorously stirred at 50 °C for 23 h. It was then diluted with $CHCl_3$ (50 ml), and the organic layer washed with water, dried, and concentrated. The product was eluted from a silica gel column (20 g), with EtOAc–hexane (1:10, v/v) as eluant, to give the epoxide (**24**) (0.83 g, 84%) as crystals, m.p. 108—109 °C (from EtOH) (Found: C, 75.3; H, 6.4. $C_{28}H_{28}O_5$ requires C, 75.65; H, 6.35%); $[\alpha]_D^{21}$ +8.8° (*c* 1.1 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 2.32 (1 H, td, *J* 11, 4.5, 4.5 Hz, 2-H), 3.00 (1 H, br d, *J* 4.5 Hz, 10-H), 3.11 (1 H, br d, *J* 4.5 Hz, 1-H), 3.96 (1 H, t, J_{gem} 11 Hz, 3-H_{ax}), 3.66—4.10 (2 H, m, 8-H, 9-H), 4.31 (1 H, dd, J_{gem} 11, 4.5 Hz, 3-H_{eq}), 4.50—5.17 (4 H, m, 2 CH_2Ph), 5.56 (1 H, s, 5-H), and 7.10—7.60 (15 H, m, 3 Ph).

2,3,4′,5′,6′,7′-*Hexa*-O-*benzyl*-4,7-O-*benzylidenevalidoxylamine B* (**25**) *and* (1S,3S,6S,7R,8S,9S,10R)-9,10-*Dibenzyloxy*-7-[(1S)-(1,4,6/5)-4,5,6-*tribenzyloxy*-3-*benzyloxymethylcyclohex*-2-*enylamino*]-8-*hydroxy*-3-*phenyl*-2,4-*dioxabicyclo*[4.4.0]-*decane* (**27**).—A mixture of the amine (**16**) (0.23 g, 0.43 mmol) and the epoxide (**24**) (0.21 g, 0.47 mmol) in propan-2-ol (1 ml) was heated in a sealed tube at 120 °C for 110 h. The mixture was then concentrated and the residue chromatographed on a silica gel column (25 g), with butan-2-one–toluene (1:15, v/v) as eluant, to give, first, the amine (**27**) (61 mg, 15%) as a syrup (Found: C, 76.95; H, 6.6; N, 1.4. $C_{63}H_{65}NO_9$ requires C, 77.2; H, 6.7; N, 1.4%); $[\alpha]_D^{23}$ +13° (*c* 1.5 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 2.30—2.54 (1 H, m, 6-H), 5.38 (1 H, s, 3-H), 5.92 (1 H, d, *J* 4.5 Hz, 2′-H), and 7.13—7.70 (35 H, m, 7 Ph).

The second fraction gave the amine (**25**) (0.31 g, 75%) as a syrup (Found: C, 76.9; H, 6.8; N, 1.4. $C_{63}H_{65}NO_9$ requires C, 77.2; H, 6.7; N, 1.4%); $[\alpha]_D^{23}$ +46° (*c* 2.5 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.70 (1 H, br s, OH), 2.35—2.57 (1 H, m, 5-H), 3.01—3.18 (1 H, m, 1-H), 3.20—3.38 (1 H, m, 1′-H), 5.54 (1 H, s, CHPh), 5.84 (1 H, d, *J* 4.5 Hz, 2′-H), and 7.17—7.73 (35 H, m, 7 Ph).

2,3,6,4′,5′,6′,7′-*Hepta*-O-*benzyl*-4,7-O-*benzylidenevalidoxylamine B* (**26**).—Sodium hydride (20 mg, 0.42 mmol) was added to a solution of the alcohol (**25**) (0.21 g, 0.21 mmol) in DMF (4 ml) at 0 °C, and the mixture was stirred for 0.5 h. Benzyl bromide (0.05 ml, 0.42 mmol) was added to the mixture which was then stirred at room temperature for 3 h. It was then diluted with EtOAc (30 ml), washed with water, dried, and concentrated. The residue was chromatographed on a silica gel column (10 g), with EtOAc–hexane (1:9, v/v) as eluant, to give the heptabenzyl ether (**26**) (0.174 g, 78%) as a syrup (Found: C, 78.25; H, 6.7; N, 1.2. $C_{70}H_{71}NO_9$ requires C, 78.55; H, 6.7; N, 1.1%); $[\alpha]_D^{23}$ +35° (*c* 1.4 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 2.30—2.60 (1 H, m, 5-H), 4.40—4.86 (14 H, m, 7 CH_2Ph), 5.53 (1 H, s, CHPh), 5.81 (1 H, d, *J* 4.5 Hz, 2′-H), and 7.10—7.50 (40 H, m, 8 Ph).

Validoxylamine B (**2a**).—Compound (**26**) (241 mg, 0.23 mmol), deprotected as described in the preparation of compound (**1b**) from (**21**), gave the amine (**2a**), which was identical with an authentic sample on t.l.c. in butanol–ethanol–water (3:2:2, v/v). This compound was then acetylated to give the nona-acetate (**2b**) (70 mg, 43%) as a syrup (Found: C, 52.65; H, 5.7; N, 2.2. $C_{32}H_{43}NO_{18}$ requires C, 52.6; H, 5.9; N, 1.9%); $[\alpha]_D^{20}$ +79° (*c* 1.8 in $CHCl_3$); δ_H(400 MHz, $CDCl_3$) data shown in the Table.

2,3,6,4′,5′,6′,7′-*Hepta*-O-*benzylvalidoxylamine B* (**28**).—Compound (**26**) (174 mg, 0.16 mmol) was treated with aqueous 80% acetic acid (7 ml) at 55 °C for 23 h. The mixture was concentrated and co-evaporated with EtOH. The residue was purified on a silica gel column (10 g), with EtOAc–hexane (1:2, v/v) as eluant, to give the heptabenzyl ether (**28**) (149 mg, 93%) as a syrup (Found: C, 77.3; H, 7.0; N, 1.2. $C_{63}H_{67}NO_9$ requires C, 77.0; H, 6.9; N, 1.4%); $[\alpha]_D^{23}$ +48° (*c* 1.6 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.50—1.90 (1 H, m, OH), 2.10—2.46 (1 H, m, 5-H), 2.40—2.67 (1 H, m, OH), 4.43—5.10 (14 H, m, 7 CH_2Ph), 5.83 (1 H, d, *J* 4.5 Hz, 2′-H), and 7.06—7.46 (35 H, m, 7 Ph).

7-O-*Acetyl*- (**29**) *and* 4,7-*Di*-O-*acetyl*-2,3,6,4′,5′,6′,7′-*hepta*-O-*benzylvalidoxylamine B* (**30**).—Acetyl chloride (0.016 ml, 0.23 mmol) was added dropwise to a solution of imidazole (31 mg, 0.45 mmol) in $CHCl_3$ (1 ml) at 0 °C. The insoluble material was filtered off and washed with $CHCl_3$ (1 ml). The imidazole solution was added to a solution of the diol (**28**) (0.15 g, 0.15 mmol) in $CHCl_3$ (5 ml) and the mixture was refluxed for 25 h. It was then diluted with $CHCl_3$ (10 ml), washed with water, dried, and concentrated. The product was chromatographed on a silica gel column (8 g), with EtOAc–hexane (2:11, v/v) as eluant, to give, first, the diacetate (**30**) (11 mg, 6.6%) as a syrup (Found: C, 75.2; H, 6.8; N, 1.2. $C_{67}H_{71}NO_{11}$ requires C, 75.5; H, 6.7; N, 1.3%); $[\alpha]_D^{23}$ +35° (*c* 1 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.78 and 1.90 (each 3 H, 2 s, 2 $COCH_3$), 2.33—2.73 (1 H, m, 5-H), 4.26—5.00 (14 H, m, 7 CH_2Ph), 5.17 (1 H, dd, *J* 12, 9.7 Hz, 4-H), 5.78 (1 H, br d, *J* 4.5 Hz, 2′-H), and 7.05—7.43 (35 H, m, 7 Ph).

The second fraction gave the monoacetate (**29**) (130 mg, 84%) as a syrup (Found: C, 76.0; H, 6.7; N, 1.4. $C_{65}H_{69}NO_{10}$ requires C, 76.2; H, 6.8; N, 1.4%); $[\alpha]_D^{23}$ +36° (*c* 1.5 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.79 (3 H, s, $COCH_3$), 2.07—2.66 (2 H, m, OH and 5-H), 4.20—5.03 (14 H, m, 7 CH_2Ph), 5.78 (1 H, br d, *J* 4.5 Hz, 2′-H), and 7.02—7.42 (35 H, m, 7 Ph).

7,2″,3″,4″,6″-*Penta*-O-*acetyl*-2,3,6,4′,5′,6′,7′-*hepta*-O-*benzylvalidamycin B* (**22**).—A solution of 2,3,4,6-tetra-*O*-acetyl-α-D-glucopyranosyl bromide (**10b**) (254 mg, 0.62 mmol) in dry 1,2-dichloroethane (2 ml) was added dropwise at room temperature to a mixture of the alcohol (**29**) (158 mg, 0.15 mmol), silver trifluoromethanesulphonate (99 mg, 0.39 mmol), 1,1,3,3-tetramethylurea (0.075 ml, 0.63 mmol), and powdered molecular sieve 4A (0.2 g) in dry 1,2-dichloroethane (3 ml) and the mixture was stirred at 60 °C for 30 h in the dark. The mixture was neutralised with 10% Et_3N in $CHCl_3$ and insoluble material was filtered off. The filtrate was concentrated and the residue was chromatographed on a silica gel column (20 g), with EtOAc–hexane (1:2, v/v) as eluant, to give, first, the diacetate (**30**) (19 mg, 12%). The second fraction gave the alcohol (**29**) (57 mg, 36%). The third fraction gave the validamycin **B** derivative (**22**) (88 mg, 42%) as a syrup (Found: C, 69.6; H, 6.4; N, 1.0. $C_{79}H_{87}NO_{19}$ requires C, 70.05; H, 6.5; N, 1.0%); $[\alpha]_D^{27}$ +27° (*c* 1.1 in $CHCl_3$); δ_H(90 MHz, $CDCl_3$) (*inter alia*) 1.93, 1.94, 1.97, and 2.02 (total 15 H, 4 s, 5 $COCH_3$), 2.48—2.88 (1 H, m, 5-H), 5.96 (1 H, br d, *J* 4.5 Hz, 2′-H), and 7.35—7.57 (35 H, m, 7 Ph).

Compound (**22**) (64 mg, 0.047 mmol) was deprotected similarly and then acetylated to give compound (**1b**) (25 mg, 51%) as a syrup, identical with the compound derived from (**21**) in all respects.

Acknowledgements

We express our sincere thanks to Mr. Akio Takahashi and Mr. Hisao Arita for elemental analyses, and to Dr. Noritaka Chida for measurement of the 400 MHz ^1H n.m.r. spectra. We are also grateful to Dr. Satoshi Horii (Takeda Chemical Industries Ltd., Osaka, Japan) for providing authentic samples of validoxylamine B and validamycin B, and their ^1H n.m.r. data.

References

1 Preliminary communication: S. Ogawa and Y. Miyamoto, *Chem. Commun.*, 1987, 1843
2 Part 11, S. Ogawa, T. Nose, T. Ogawa, T. Toyokuni, Y. Iwasawa, and T. Suami, *J. Chem. Soc., Perkin Trans. 1*, 1985, 2368.
3 (*a*) T. Iwasa, E. Higashide, H. Yamamoto, and M. Shibata, *J. Antibiot.*, 1971, **24**, 107; (*b*) S. Horii, Y. Kameda, and K. Kawahara, *ibid.*, 1972, **25**, 48.
4 T. Iwasa, H. Yamamoto, and M. Shibata, *J. Antibiot.*, 1970, **23**, 595.
5 S. Horii, H. Fukase, Y. Kameda, N. Asano, T. Yamaguchi, and K. Matsui, *J. Antibiot.*, 1986, **39**, 1491.
6 S. Ogawa, T. Ogawa, T. Nose, T. Toyokuni, Y. Iwasawa, and T. Suami, *Chem. Lett.*, 1983, 921.
7 S. Ogawa, T. Ogawa, N. Chida, T. Toyokuni, and T. Suami, *Chem. Lett.*, 1982, 749.
8 S. Ogawa, T. Ogawa, Y. Iwasawa, N. Chida, T. Toyokuni, and T. Suami, *J. Org. Chem.*, 1984, **49**, 2594.
9 S. Ogawa, T. Toyokuni, Y. Iwasawa, Y. Abe, and T. Suami, *Chem. Lett.*, 1982, 279.
10 S. Ogawa, Y. Iwasawa, T. Nose, T. Suami, S. Ohba, M. Ito, and Y. Saito, *J. Chem. Soc., Perkin Trans. 1*, 1985, 903.
11 S. Ogawa, N. Chida, and T. Suami, *Chem. Lett.*, 1980, 1559; *J. Org. Chem.*, 1983, **48**, 1203.
12 J. Vernon, S. Roseman, and Y. C. Lee, *Carbohydr. Res.*, 1980, **82**, 56.
13 S. Ogawa, H. Ito, T. Ogawa, S. Iwasaki, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 2319.
14 S. Ogawa, Y. Shibata, T. Nose, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1985, **58**, 3387.
15 J. Bovin, M. Pais, and C. Monneret, *Carbohydr. Res.*, 1980, **79**, 193; H. Paulsen and F. R. Heiker, *ibid.*, 1982, **102**, 103; T. Toyokuni, S. Ogawa, and T. Suami, *Bull. Chem. Soc. Jpn.*, 1983, **56**, 1161.

Received 25*th February* 1988; *Paper* 8/00038G