1480, 1100–1300 cm^{-1}; ^{19}F NMR ($CDCl_3$) −10.8 (3 F, m), −10.0 (3 F, m), −5.2–2.8 (6 F, m) ppm; ^{1}H NMR ($CDCl_3$) 7.5 (s) ppm; mass spectrum, m/e 477 ($M^+ - N_2$); high-resolution mass spectrum, calcd for $C_{14}H_5F_{12}P_2N_3$ 476.970, found 476.969.

Irradiation of 7. After irradiation of **7** in *n*-pentane with a high-pressure mercury lamp for 4 h, the solvent was evaporated under vacuum. Bulb-to-bulb distillation [60–65 °C (5 mmHg)] of the residue gave 7-phenyl-1,3,4,6-tetrakis(trifluoromethyl)-2,5-diphospha-7-azatetracyclo[4.1.0.0^{2,4}.0^{3,5}]heptane (**9**) as a pale yellow oil (63% estimated by ^{19}F NMR): IR ($CHCl_3$) 1595, 1490, 1100–1300 cm^{-1}; ^{19}F NMR ($CDCl_3$) −7.2 (3 F, m), −6.0 (3 F, m), −2.8 (6 F, d, J_{PF} = 21.4 Hz) ppm; ^{1}H NMR ($CDCl_3$) 6.9–7.7 (aromatic) ppm; mass spectrum, m/e 477 (M^+); high-resolution mass spectrum, calcd for $C_{14}H_5F_{12}P_2N$ 476.970, found 476.969.

Thermolysis of 7. A solution of **7** in *n*-pentane was sealed in a Pyrex tube under vacuum and heated at 140 °C for 8 h. ^{19}F NMR spectrum of this mixture indicated the formation of **9** and **4**, the latter of which might be formed by retro 1,3-dipolar reaction.

1-Phenyl-4,5-bis(trifluoromethyl)-1,2,3-triazole (8). Compound **7** was chromatographed on silica gel TLC plate, using ether–*n*-pentane (1:6) as eluant. Extraction of the fluorescent zone by a UV lamp gave the triazole **8**.[11]

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Registry No. 2, 62218-18-2; **3**, 62218-19-3; **4**, 65114-90-1; **5**, 74930-70-4; **6**, 74930-71-5; **7**, 74930-72-6; **9**, 74930-73-7; 2,3-dimethyl-1,3-butadiene, 513-81-5; phenyl azide, 622-37-7; furan, 110-00-9.

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Isolation and Structure of the Novel Branched-Chain Amino Sugar Derived from Antibiotic A35512B[1]

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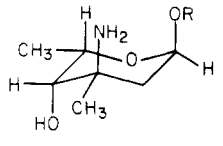
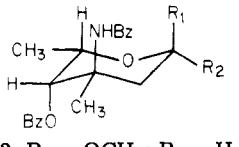
Methanolysis of glycopeptide antibiotic A35512B with 1.5 N HCl (methanol) gave a new 2,3,6-trideoxy amino sugar. The structure of this amino sugar was determined by NMR and CD techniques and shown to be 3-amino-2,3,6-trideoxy-3-*C*-methyl-L-*xylo*-hexopyranose, **1**. This structure determination was carried out on the *N,O*-dibenzoyl anomeric methyl glycosides **3** and **5**. Amino sugar **1** was shown to be the C-3 epimer of L-vancosamine.

A35512B is a new gram-positive antibiotic which was recently isolated by Michel and Shah from an actinomycete, *Streptomyces candidus*.[2] Comparative chromatographic behavior indicated that A35512B belonged to the glycopeptide family of antibiotics and therefore was related to vancomycin, ristocetin, avoparcin, and others.[3]

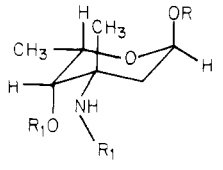
Johnson[4] and also Williams[5] reported that an amino sugar, L-vancosamine (**6**), was a constituent part of the glycopeptide vancomycin. Similarly, several other glycopeptides have since been shown to have an amino sugar as an integral part of their structure.[6–8] In this paper we report the isolation, structure, and stereochemistry of **2**,



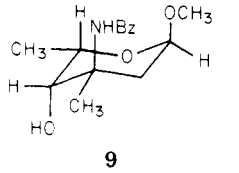
1, R = H
2, R = CH_3



3, R_1 = OCH_3; R_2 = H
4, R_1 = OH; R_2 = H
5, R_1 = H; R_2 = OCH_3



6, R = R_1 = H (L-vancosamine)
7, R = CH_3; R_1 = H
8, R = CH_3; R_1 = Bz



9

a novel amino sugar constituent of glycopeptide antibiotic A35512B.

Results and Discussion

Methanolysis of A35512B (1.5 N HCl, reflux 18 h) produced a peptide (aglycon) which precipitated from solution when the pH was adjusted to 7–8. The filtrate which contained the liberated neutral and basic fragments was passed through a cation-exchange column (NH_4^+). An aqueous wash removed the neutral methyl glycosides. A stepwise elution with NH_4OH solution (0.5–3 N NH_4OH) gave a product mixture which was purified further by cellulose column chromatography (*n*-butanol, saturated

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(2) K. Michel, R. M. Shah, and R. L. Hamill, "A35512, A Complex of New Antibacterial Antibiotics Produced by *Streptomyces candidus* II. Isolation and Characterization", 17th Conference on Antimicrobial Agents and Chemotherapy, New York, NY, Oct 14, 1977, Abstract 41.
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(8) J. J. Hlavka, P. Bitha, J. H. Boothe, and G. Morton, *Tetrahedron Lett.*, 175 (1974).
(9) See ref 4 and 6 for examples of the NMR properties and anomers of amino sugars of the general type studied here.

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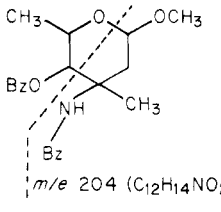
Table I. NMR Data of 3 (α-Anomer) and Relative Stereochemical Assignment Based on Spin-Decoupling Experiments

proton assignment	chemical shift, δ	multiplicity	coupling constant
C_1	5.00	d	$J_{1,2ax}$ = 3.5 Hz
C_2 (eq)	1.87	d	J_{2ax} = 14.5 Hz, $J_{1,2eq}$ = 1 Hz
C_2 (ax)	2.19	dd	$J_{1e,2a}$ = 3.5, J_{eq} = 14.5 Hz
C_3-Me	1.60	s	
C_4	5.93	(s)	(trace coupling to C-5)
C_5	4.33	qd	J = 6.5 Hz
C_6-Me	1.15	d	J = 6.5 Hz
NH	7.82		

with water) to produce **2** as a crystalline compound ($C_8H_{17}NO_3$, elemental analysis and mass spectral data). The basic compound **2** was dibenzoylated (C_6H_5COCl, pyridine) to give **3** and its anomer **5**. The empirical formulas for **2** and **3** were identical with those reported for methyl α-L-vancosaminide (**7**) and its corresponding dibenzoyl adduct **8**, respectively.[4] The mass spectrum of **2** (*m/e* 175 (M^+), 144, 126, 108) was essentially identical with that reported for **7**.[5] However, **3** and **8** differed significantly in their R_f values (TLC) which suggested that **2** was derived from a new amino sugar, **1**, which is an isomer of L-vancosamine (**6**). The nature of the structural differences between **1** and **6** was determined from the properties of benzoyl adducts **3** and **5** of the new amino sugar.

A35512B was benzoylated directly with pyridine–benzoyl chloride and the crude product was methanolyzed. The methanolysis products were purified on alumina (benzene–ethyl acetate) to give the two crystalline isomeric products **3** and 5 in high yield. The high-resolution mass spectral data for both of these products showed *m/e* 383 (M^+, $C_{22}H_{25}NO_5$) and 351 ($M^+ - CH_3OH$), 204 ($M^+ - C_{10}H_{11}O_3$). The empirical formula derived for the molecular ion was in complete agreement with that obtained by elemental analysis. The general characteristics of the mass spectrum of **3** and **5** were similar to those reported for methyl *N,O*-dibenzoyl-α-L-vancosaminide (**8**).[4] The fragmentation pattern of **3** (*m/e* 204, $C_{12}H_{14}NO_2$) is shown below and is typical of a 2,3,6-trideoxy hexopyranose methyl glycoside.[4]



m/e 204 ($C_{12}H_{14}NO_2$)

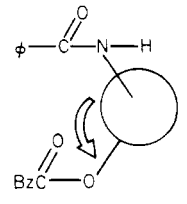
The NMR spectrum of **3** and the structural assignments derived from spin-decoupling experiments are shown in Table I.[9] The NMR spectrum of **5** showed the same general features as those obtained for **3** (see Experimental Section) but differed in the chemical shift and splitting patterns observed for the signals of protons at the C-1 and C-2 positions.

In the spectra of both **3** and **5** the protons at C-1 and C-2 appeared as an ABX system which allowed the structural assignment of the α-anomer **3** ($J_{1,2ax}$ = 3.5, $J_{1,2eq}$ ≤ 1 Hz) and the β-anomer **5** ($J_{1,2ax}$ = 10, $J_{1,2eq}$ = 2 Hz). The relative configurations represented by structures **3** and **5** are in agreement with the observed magnitudes of these coupling constants. Similar stereochemical assignments have been made on this basis with other 2,3,6-trideoxy sugars.[6,14] The spectrum of **3** showed the signal for the C-2eq proton (δ 1.87) at higher field than its C-2ax resonance (δ 2.19) while the corresponding resonances for the β-isomer **5** have the inverse relationship (H-2eq, δ 2.48; H-2ax, δ 1.90). The chemical shifts of the protons at C-2 are strongly influenced by the anisotropic properties of the neighboring benzamido and methoxyl functionalities. These effects at C-2 have also been discussed in structural studies of the ristosamine and vancosamine molecules.[4,6] These data are in general agreement with the conclusion that **3** and **5** are α and β-anomers of the dibenzoyl adduct of **2**.

The NMR spectrum of **3** revealed that it had the same functional groups as its vancosamine counterpart, **8**, but these differed in their chemical shift values. Spin-decoupling experiments showed that the protons attached to positions C-4, C-5, and C-6 have the same arrangement in both **3** and **8**. Since it was established that **3** bore only hydrogen at C-2, the only possible difference between **3** and **8** must lie in the configuration of the C-3 amino and methyl groups.

The correct assignment of the relative configuration of groups at C-3 was derived from a series of NOE (nuclear Overhauser effect) experiments on **3**. Irradiation of the C-3 methyl group (δ 1.60) caused a 7% NOE at the C-4 proton (δ 5.93) and no effect on the C-5 proton (δ 4.33) while irradiation of the amide NH proton (δ 7.82) caused a 7% NOE at the C-5 proton and no NOE at the C-4 proton (δ 5.93). These data were consistent with the assignment of the relative configuration shown in structure **3**.

The optical rotation of the α-anomer **3** was more negative than that of the β-anomer **5**. Therefore, according to Hudson's rules **3** and **5** must have the L configuration.[10] This conclusion was confirmed by CD studies. The CD spectra of **3** and **5** had negative Cotton effect minima at $\Delta\epsilon_{238}$ = −11.3 and $\Delta\epsilon_{238}$ = −7.09, respectively. That these bonds were due to dibenzoyl interaction (Davydov splitting) was demonstrated by their disappearance upon selective alkaline hydrolysis of the *O*-benzoyl group to give the monobenzoyl derivative **9**.[11] The Nakanishi dibenzoyl rule correctly predicts the observed negative chirality for **3** and **5** if the groups are oriented as shown below.



This chirality is only possible if these compounds are L sugar derivatives. Theoretical considerations predict no Davydov splitting between chromophores having a trans diaxial relationship (dihedral ∠ = 180°). The presence of steric factors apparently can produce enough conformational distortion away from the ideal trans diaxial orientation to allow the dibenzoyl interaction to occur, albeit at lowered intensity.[12] The weak interactions observed in the CD spectra of **3** and **5** are most likely the result of just such considerations. Similarly, the CD spectrum of **2** in Cupra A gave a very weak negative Cotton effect ($\Delta\epsilon_{580}$ = −0.015) which is of the expected sign and magnitude for

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an L-amino sugar bearing unfavorably situated trans diaxial ligands.[13]

Compounds **2**, **3**, and **5** were assigned the L configuration. Therefore, the amino sugar **1** is designated the structure 3-amino-2,3,6-trideoxy-3-C-methyl-L-*xylo*-hexopyranose. This sugar is a novel amino sugar related to L-vancosamine, differing from the latter in the configuration of groups at C-3, and is directly related to its 3-nitro-4-methoxylated analogue L-rubranitrose, recently reported by Mizak.[15]

Expermental Section

The following instruments were used in this study: ^1H NMR spectrometer, Varian HA100; ^{13}C NMR spectrometer, JEOL PFT 100; UV spectrophotometer, Cary 15; ORD and CD spectropolarimeter, Perkin Elmer 241 and Jasco J40AS; IR spectrophotometer, Beckman IR426; high-resolution mass spectrometer, Varian MAT731. Melting points were obtained on a Kohler hot stage and were uncorrected.

Amino Sugar from A35512B. A solution of 10 g of A35512B in 500 mL of 1.5 N methanolic HCl was refluxed for 18 h. After cooling, the reaction mixture was concentrated at 40 °C under reduced pressure to a syrup. The residue was dissolved in 25 mL of MeOH and poured into an aqueous suspension of Bio-Rad 1 × 4 (OH^-) resin (85 mL in volume) in 150 mL of H_2O. The resin was removed by filtration at pH 4.6 and the filtrate was adjusted to pH 6.8, filtered, and then adjusted to pH 8 (NaOH) to yield A35512B aglycon (isolated by filtration). The filtrate was then passed over an Amberlite 120 (NH_4^+) column (3 × 20 cm) and the column eluted with 1 L of H_2O. The eluate was collected and lyophilized to yield neutral sugars. The column was then eluted stepwise with 1 L of 0.5 N NH_4OH in 200-mL volumes, yielding 12 g of ninhydrin-positive material contaminated with inorganic salts. Elution with 500 mL of 0.15 N NH_4OH gave 0.5 g of a lyophilizate (pH adjusted to 6 with 3 N methanolic HCl before lyophilization). Mass spectral fragmentation showed peaks at *m/e* 175, 144, 126, 108, 100, 79, and 74. Field desorption mass spectrum (FDMS) showed ($M^+ + 1$) = 176. This material was also seen in the TLC of the fractions eluted with 0.5 N NH_4OH. These fractions were lyophilized and then deionized by being dissolved in 10% aqueous methanol with precipitation of the inorganics by acetone addition. The filtrate was concentrated to 10 mL and acetone precipitation was repeated. This filtrate was concentrated to dryness (624 mg), combined with the fraction eluted with 0.15 N NH_4OH, and chromatographed over cellulose (Schleicher and Schnell Grade 286, 50 g), eluting with *n*-butanol (saturated with H_2O). A fraction was eluted between 200 and 600 mL of solvent (464 mg) which was essentially a single spot on TLC R_f ~0.45 (cellulose–*n*-BuOH/pyridine/HOAc/H_2O, 15:10:3:12) (BPAW). This material was rechromatographed on 80 g of cellulose, using BPAW and collecting 10-mL fractions. Fractions 16–50 gave 360 mg of a yellow green oil which crystallized when triturated with $CHCl_3$ to give methyl 3-amino-2,3,6-trideoxy-3-C-methyl-α-L-*xylo*-hexopyranoside (**2**): mp 123–125 °C; NMR (D_2O) δ 5.33 (s, H-1), 2.27 (1 H, d, J = 1.5 Hz, H-2eq), 2.48 (1 H, q, J = 1.5, 3.5 H, H-2ax), 3.90 (s, H-4), 4.64 (1 H, 8, J = 7 Hz, H-5), 1.68 (3 H, d, J = 7 Hz, C-6), 1.80 (3 H, s, 3 Me), 3.81 (3 H, s, OCH_3); mass spectrum, *m/e* 175 (M^+), 144, 126, 108; $[\alpha]^{25}_D$ −52.4° (*c* 1, MeOH); $[\alpha]^{25}_{365}$ −157° (*c* 1, MeOH); CD (Cupra A) $\Delta\epsilon_{580}$ = −0.015, $\Delta\epsilon_{280}$ = +0.084. Anal. Calcd for $C_8H_{17}NO_3$·HCl·0.5H_2O: C, 43.54; H, 8.68; N, 6.35. Found: C, 43.24; H, 8.45; N, 5.94.

Benzoylation and Methanolysis of A35512B. A solution of A35512B (10 g) in pyridine (50 mL) was treated with benzoyl chloride (20 mL) and the reaction was allowed to stir overnight at room temperature. A 100-mL portion of MeOH was added and allowed to react for a short time and the reaction mixture was taken to dryness below 60 °C in vacuo. The residue was extracted by trituration with three 200-mL portions of ether to remove benzoic acid and methyl benzoate, leaving an insoluble white solid. This solid was dissolved in 1.5 N methanolic HCl, and the solution was refluxed for 7 h and then taken to dryness under reduced pressure. The product was partitioned between water and ethyl acetate. The ethyl acetate layer was concentrated to dryness to give 6.06 g of a white powder. This solid was extracted with three portions (400 mL) of ether to yield 3.92 g of ether-soluble material. The latter substance was chromatographed on 400 g of Grade I neutral alumina, beginning with petroleum ether and varying amounts of benzene. The benzene eluate contained 387 mg of a crystalline solid of methyl 3-benzamido-*O*-benzoyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside (**3**): mp 178–179.5 °C (petroleum ether–ether, 4:1); high-resolution mass spectrum, *m/e* 383 (M^+, calcd for $C_{22}H_{25}NO_5$ 383.1729, found 383.1732), 351 ($M^+ - CH_3OH$, calcd for $C_{21}H_{21}NO_4$ 351.1471, found 351.1474), 204 (calcd for $C_{12}H_{14}NO_2$ 204.1025, found 204.1025); UV (EtOH) 231/nm (ε 29 819), 270 (2000, sh), 280 (200); $[\alpha]^{25}_D$ −191° (*c* 1, MeOH); $[\alpha]^{25}_{365}$ −664.9° (*c* 1, MeOH); CD (MeOH) $\Delta\epsilon_{238.5}$ = −11.3 (θ = −23.3° M), $\Delta\epsilon_{215}$ = +1.3 (θ = +2.7° M). Anal. Calcd for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57; O, 20.86; N, 3.65. Found, C, 68.69; H, 6.55; O, 20.86; N, 3.98.

The column was then eluted with EtOAc–benzene mixtures. Fractions eluted with 4% EtOAc–benzene gave 36 mg of a crystalline solid, mp 166–171 °C (ether–petroleum ether, 1:4), which proved to be a mixture of **4** and **5**. EtOAc–benzene (10%) eluted 59 mg of crystalline methyl 3-benzamido-*O*-benzoyl-2,3,6-trideoxy-3-*C*-methyl-β-L-*xylo*-hexopyranoside (**5**): mp 171–174 °C (petroleum ether–ether, 4:1); $[\alpha]^{25}_D$ −91.5°; $[\alpha]^{25}_{365}$ −368.1° (*c* 1, MeOH); UV 231 nm (ε 26 420); IR (mull) 3260, 1710, 1700, 1625 cm^{-1}; NMR ($DCCl_3$) δ 4.72 (1 H, dd, J = 2, 10 Hz, H-1), 2.48 (1 H, d, $J_{1,2eq}$ = 1 Hz, J = 14 Hz, H-2eq), 1.90 (1 H, dd, J = 10, 14 Hz, H-2ax), 1.47 (3 H, s, C-3 Me), 5.76 (s, trace coupling to H-2eq and H-5, 4-H) 4.19 (1 H, qd, J = 1, 6 Hz, H-5), 1.21 (d, J = 6 Hz, C-6), 3.54 (3 H, s, OCH_3), 6.10 (1 H, s, NH). Anal. Calcd for $C_{22}H_{25}NO_5$: C, 68.91; H, 6.57: O, 20.86; N, 3.65. Found: C, 69.09; H, 6.37; O, 20.89; N, 3.53.

Further elution with 10% EtOAc–benzene gave 278 mg of a crystalline solid and an additional 198 mg of the same substance was eluted with 100% EtOAc to give 3-benzamido-*O*-benzoyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranose (**4**): mp 184–186 °C (EtOAc–petroleum ether, 1:3) (21% yield overall); mass spectrum, *m/e* (369 (M^+, calcd for $C_{21}H_{23}NO_5$ 369.1576, found 369.1566), 190 (calcd for $C_{11}H_{12}NO_2$ 190.0668, found 190.0872); $[\alpha]^{25}$ −165.2° (*c* 1, MeOH), $[\alpha]^{25}_{365}$ −595.1° (*c* 1, MeOH). Anal. Calcd for $C_{21}H_{23}O_5$: C, 68.28; H, 6.28; O, 21.65; N, 3.79. Found: C, 68.23; H, 6.42; O, 21.89; N, 3.76.

Partial Hydrolysis of 3. A solution of **3** (100 mg) in 10 mL of MeOH was treated with 10 mL of 1 N NaOH at room temperature. TLC (silica gel, 100% EtOAc) showed only a small amount of starting material remaining after 3 h. The reaction mixture was poured into 100 mL of H_2O and extracted three times with $CHCl_3$. The extract was washed with water and dried over Na_2SO_4. Evaporation of the solvent gave a residue which was chromatographed on alumina (neutral) with 100% benzene to give 5.3 mg of recovered starting material; elution with 30% EtOAc in benzene gave 42.5 mg of methyl 3-benzamido-2,3,6-trideoxy-3-*C*-methyl-α-L-*xylo*-hexopyranoside (**9**): mp 112–113 °C (petroleum ether–Et_2O); IR ($CHCl_3$) 1720, 1655 cm^{-1}; UV (MeOH) λ_{max} 226 nm (ε 9443); $[\alpha]^{25}_D$ −150° (*c* 2, MeOH); CD $\Delta\epsilon_{23-}$ = −1.2, $\Delta\epsilon_{275}$ = −0.2. Anal. Calcd for $C_{15}H_{21}NO_4$ H_2O: C, 60.66; H, 7.13; N, 4.72. Found: C, 60.96; H, 7.16; N, 4.81.

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Registry No. **1**, 74966-73-7; **2**, 74966-74-8; **3**, 75044-07-4; **4**, 74966-75-9; **5**, 75044-08-5; **9**, 74966-76-0; A35512B, 63849-30-9.

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