prolyl isomerization[21,22] and C-N bond rotation in DMA.[15] With these interactions, the bond between Xaa and Pro will have lost resonance stabilization and double bond character. The energy barrier to rotation about this bond will be much lower than rotation about an amide bond and, thus, FKBP will have effected catalysis.

This mechanistic hypothesis has value in that is explains the perplexing observation noted above that the secondary deuterium isotope effect for enzymic prolyl isomerization (k_H/k_D = 1.13 for cyclophilin[6,17] and k_H/k_D = 1.12 for FKBP, Dr. D. Livingston, Vertex Inc., personal communication) is unaccountably larger than the isotope effect for nonenzymic isomerization (k_H/k_D = 1.05[6,16,17]). The hydrophobic environment in which the amide bond finds itself in the enzyme active site will stabilize a transition state with less polar character than the transition state for reaction in solution. As we discussed above, this will magnify the enzymic isotope effect.[27]

Registry No. CyP, 95076-93-0; Suc-Ala-Gly-Pro-Phe-pNA, 128802-77-7; Suc-Ala-Ala-Pro-Phe-pNA, 70967-97-4; Suc-Ala-Val-Pro-Phe-pNA, 95192-38-4; Suc-Ala-Ile-Pro-Phe-pNA, 128802-79-9; Suc-Ala-Nle-Pro-Phe-pNA, 128802-72-2; Suc-Ala-Leu-Pro-Phe-pNA, 128802-78-8; Suc-Ala-Phe-Pro-Phe-pNA, 128802-73-3; Suc-Ala-Trp-Pro-Phe-pNA, 128822-32-2; Suc-Ala-His-Pro-Phe-pNA, 128802-75-5; Suc-Ala-Lys-Pro-Phe-pNA, 128802-74-4; Suc-Ala-Glu-Pro-Phe-pNA, 128802-76-6.

Stereospecific Synthesis of Aryl β-Glucosides: An Application to the Synthesis of a Prototype Corresponding to the Aryloxy Carbohydrate Domain of Vancomycin

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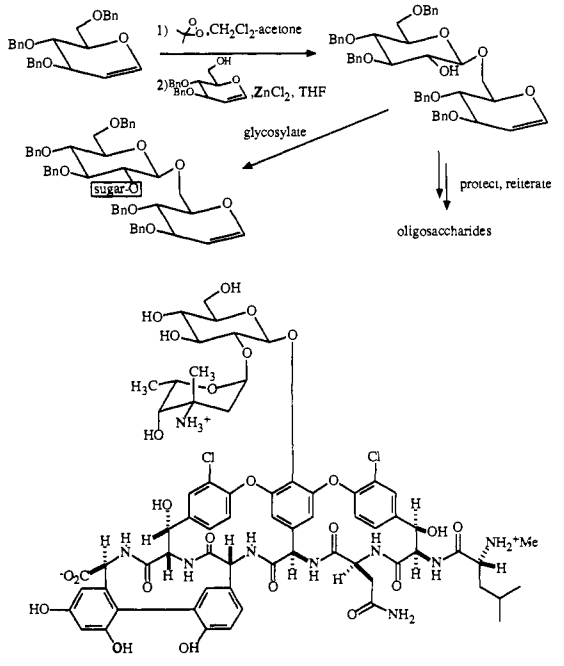
Contribution from the Sterling Chemistry Laboratory, Yale University, New Haven, Connecticut 06525. *Received July 15, 1991*

Abstract: The reaction of 3,4,6-tri-O-benzyl-D-glucal (**2**) with 3,3-dimethyldioxirane gives rise to the 2α,3α-oxirane with high stereoselection. Reaction of this compound with various phenols under alkaline conditions affords aryl 2α-hydroxy-β-glycosides (**3a–d**). Oxidative coupling (I^+) of these compounds with glycals followed by deiodination gives rise to aryloxydisaccharides (**6a** and **6b**). In addition, proton-mediated coupling of **3a** and **3b** with vancosamine-derived glycal **8** affords **10a** and **10b**, which simulate the aryloxy carbohydrate domain of vancomycin.

Previous reports from these laboratories described the synthesis of various β-glycosides from the Lewis acid catalyzed addition of aliphatic alcohols to 1,2-anhydrosugars[1,2] (Scheme I). The anhydrosugars employed were readily prepared in situ from the stereoselective oxidation of the appropriate glycals using 3,3-dimethyldioxirane.[3] The simple two-step procedure provided high yields of β-glycosides bearing a unique hydroxyl group at C-2. Through the use of glycals as glycosyl acceptors, the method lends itself to ready reiteration and thereby provides rapid access to relatively complex oligosaccharides. Noteworthy is the fact that the method is ideally suited for the synthesis of complex glycosides in which the C-2 position is glycosylated (see Scheme I).

We had hoped to extend this chemistry to the synthesis of aryl β-glucosides. A focusing goal of the investigation was that of assembling a realistic prototype of the aryloxy carbohydrate domain of vancomycin (**1**).[4] In this naturally occurring glycopeptide antibiotic, the central phenolic ring of the peptide-based aglycone is attached through a β-linkage to a glucosyl residue which is, in turn, attached through a (2–1)-α-linkage to the 3-aminosugar, vancosamine. Given the remarkable advances which have been

Scheme I



recently registered by the Evans school[5] toward the syntheses of the complex aglycone sector of vancomycin and related com-

(1) (a) Halcomb, R. L.; Danishefsky, S. J. *J. Am. Chem. Soc.* **1989**, *111*, 6661. (b) Gordon, D. M.; Danishefsky, S. J. *Carbohydr. Res.* **1990**, *206*, 361.

(2) For earlier work describing the preparation of 1,2-anhydrosugars and their use in the synthesis of glycosides, see: (a) Brigl, P. Z. *Z. Physiol. Chem.* **1922**, *122*, 245. (b) Lemieux, R. U. *Can. J. Chem.* **1953**, *31*, 949. (c) Lemieux, R. U.; Bauer, H. F. *Can. J. Chem.* **1954**, *32*, 340. (d) Lemieux, R. U.; Huber, G. *J. Am. Chem. Soc.* **1956**, *78*, 4117. (e) Lemieux, R. U.; Howard, J. *Methods Carbohydr. Chem.* **1963**, *2*, 400. (f) Sondheimer, S. J.; Yamaguchi, H.; Schuerch, C. *Carbohydr. Res.* **1979**, *74*, 327. (g) Yamaguchi, H.; Schuerch, C. *Carbohydr. Res.* **1980**, *81*, 192.

(3) Murry, R. W.; Jeyaraman, R. *J. Org. Chem.* **1985**, *50*, 2847.

(4) Isolation: (a) McCormick, M. H.; Stark, W. M.; Pittenger, G. F.; Pittenger, R. C.; McGuire, G. M. *Antibiot. Annu.* **1955–1956**, 606. Structure determination: (b) Sheldrake, G. M.; Jones, P. G.; Kennard, O.; Williams, D. H.; Smith, G. A. *Nature* **1978**, *271*, 223. (c) Williamson, M. P.; Williams, D. H. *J. Am. Chem. Soc.* **1981**, *103*, 6580. (d) Harris, C. M.; Kopecka, H.; Harris, T. M. *J. Am. Chem. Soc.* **1983**, *105*, 6915.

(5) (a) Evans, D. A.; Ellman, J. A.; DeVries, K. M. *J. Am. Chem. Soc.* **1989**, *111*, 8912. (b) Evans, D. A.; Weber, A. E.; Britton, T. C.; Sjogren, E. B. In *Peptides: Chemistry and Biology*, Proceedings of the 10th American Peptide Symposium; Marshall, G. R., Ed.; ESCOM Science Publishers: Leiden, The Netherlands, 1988; pp 143–149. (c) Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063.

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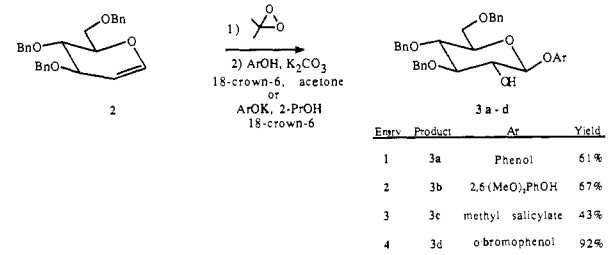
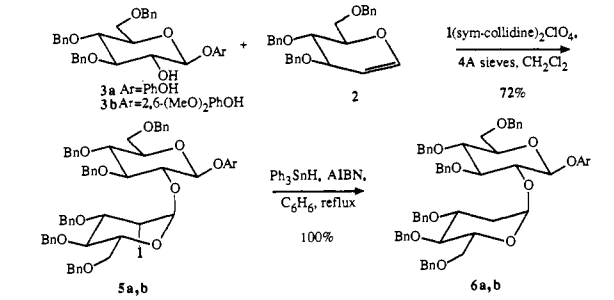
Scheme II



Entry	Product	Ar	Yield
1	3a	Phenol	61%
2	3b	2,6-$(MeO)_2$PhOH	67%
3	3c	methyl salicylate	43%
4	3d	o-bromophenol	92%

Scheme III



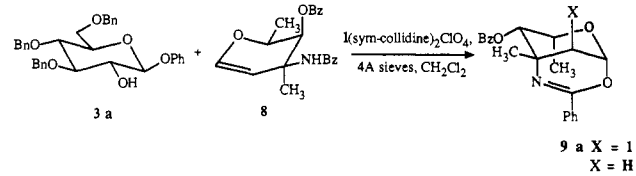
pounds, consideration of strategies for the synthesis of the carbohydrate segment and its attachment to an aglycone equivalent seemed to be timely.

We began by investigating the possibility that an aryl β-glucoside such as **3a** could be prepared from the reaction of a phenol with a 1α,2α-anhydrohexose. Hopefully, a subsequent glycosylation at the free C-2 hydroxyl group with a suitably protected vancosamine-based glycal might be accomplished either through oxidative or nonoxidative means.

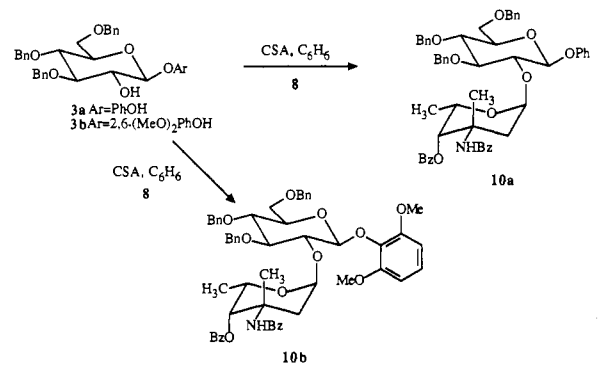
Under the Lewis acidic conditions previously described[1] (ROH, $ZnCl_2$, THF or CH_2Cl_2, 0 °C to room temperature), reaction of 1,2-anhydro-3,4,6-tri-O-benzyl-α-D-glucose with phenol gave a 1.8:1 ratio (NMR) of the phenyl α- and β-glucosides, respectively. These isomeric compounds were identified through conversion to the respective C-2 acetates and comparison of their ^{1}H NMR coupling constants of the relevant signals; the major isomer gave $J_{1,2}$ = 3.8 Hz and $J_{2,3}$ = 11 Hz, consistent with an α-glucoside in the $^4C_1$ chair conformation, while the minor isomer gave $J_{1,2}$ = 8.8 Hz and $J_{2,3}$ = 8.0 Hz, consistent with a slightly twisted chair conformation of an equatorial-substituted glucoside. Apparently, phenol itself is too weakly nucleophilic to bring about the direct displacement of the epoxide such as is observed using aliphatic alcohols. The lack of stereoselectivity in the glycosylation reaction is surely due to an S_N1-like component to the reaction. Accordingly, a variety of phenolic salts investigated in the hope that their increased nucleophilicity might result in clean inversion of configuration at the anomeric carbon.

The best and most reproducible results were found using the potassium salts of phenols in polar solvents. Reaction of potassium phenoxide (generated with KH) in 2-propanol with the 1α,2α-anhydrosugar gave a 70% yield of phenyl β-glucoside accompanied by only a small amount of propyl glucoside (<8%). A second set of conditions, advantageous in cases where the potassium phenoxide salts could not be crystallized, involved addition of the 1α,2α-anhydrosugar to a refluxing solution of the aryl alcohol, K_2CO_3, and catalytic amounts of 18-crown-6 in dry acetone. In no case was the isomeric α-anomer detected by TLC in either the crude reaction mixtures or during chromatographic purification of products. A limited series of glycosylations using various aryl alcohols is shown in Scheme II. As can be seen, the reaction appears to be quite general. Particularly noteworthy is the fact that 2,6-dimethoxyphenol (entry 2), a sterically encumbered aryl alcohol which approximates, in a formal sense, the steric environment of the aglycone of vancomycin, is smoothly glycosylated in good yield using this procedure. That the weakly nucleophilic methyl salicylate (entry 3) is also successfully glycosylated is an encouraging result, which might prove useful for the synthesis of some members of the TPI series of phosphodiesterase inhibitors.[6]

Scheme IV



Scheme V



Having demonstrated that the desired aryl β-glucosides can be readily obtained, we investigated the possibility that they might function as glycosyl acceptors with the uniquely distinguished free hydroxyl at C-2 in some variation of a halonium ion induced coupling process[7] (Scheme III). In the event, reaction of aryl glucosides **3a** and **3b** with 3,4,6-tri-O-benzyl-D-glucal (**2**) in methylene chloride in the presence of I(*sym*-collidine)$_2ClO_4$ and 4-Å molecular sieves provided high yields of disaccharides **5a** and **5b**, respectively, as the sole isolable products. Deiodination was smoothly effected using triphenyltin hydride and catalytic AIBN in refluxing benzene[8] to provide the respective 2′-deoxydisaccharides (**6a** and **6b**) in near-quantitative yields. That the configuration at the newly formed anomeric center was in each case α was evident from the chemical shift and coupling constant of the proton at C-1′ ($J_{1',2ax'}$ = 2.6 and 2.2 Hz for **6a** and **6b**, respectively).

We next attempted to extend this finding to the synthesis of compound **10b**, which approximates the aryloxy carbohydrate domain of vancomycin. The requisite vancosamine-based glycal **8** was obtained simply and efficiently from the known methyl α-vancosamide glycoside **7**.[9] This latter compound was available through degradation of vancomycin[9] and was effectively converted (in quantitative yield) to the respective glycal **8** through the action of camphorsulfonic acid in hot benzene. This constitutes a simple yet significant improvement over the previously described methods for synthesizing systems such as **8**.[10-13d]

(6) Yaginuma, S.; Awata, M.; Takada, M.; Kinoshita, K. Jpn. Kokai 215551, Sept. 22, 1987.

(7) (a) Lemieux, R. U. *Can. J. Chem.* **1964**, *42*, 1417. (b) Lemieux, R. U.; Fraser-Reid, B. *Can. J. Chem.* **1964**, *42*, 532; **1964**, *42*, 539; **1965**, *43*, 1460. (c) Lemiex, R. U.; Morgan, A. R. *Can. J. Chem.* **1965**, *43*, 2190. (d) Thiem, J.; Karl, H.; Schwenter, J. *Synthesis* **1978**, 696. (e) Thiem, J.; Elvers, J. J. *Chem. Ber.* **1979**, *112*, 818. (f) Thiem, J.; Ossowski, P. J. *Carbohydr. Chem.* **1984**, *3*, 287. (g) Thiem, J.; Prahst, A.; Wendt, T. *Liebigs Ann. Chem.* **1986**, 1044. (h) Thiem, J. In *Trends in Synthetic Carbohydrate Chemistry*; Horton, D., Hawkins, L. D., McGarvey, G. J., Eds.; ACS Symposium Series 386; American Chemical Society: Washington, DC, 1989; Chapter 8.

(8) See: (a) Horton, D.; Priebe, W.; Sznaidman, M. *Carbohydr. Res.* **1989**, 149. (b) Thiem, J.; Klaffke, W. *J. Org. Chem.* **1989**, *54*, 2006.

(9) Johnson, A. W.; Smith, R. M.; Guthrie, R. D. *J. Chem. Soc., Perkin Trans. 1* **1972**, 2153.

(10) Tatsuta, K.; Fujimoto, K.; Kinoshita, M.; Umezawa, S. *Carbohydr. Res.* **1977**, *54*, 85.

(11) See: (a) Nicolaou, K. C.; Chakraborty, T. K.; Ogawa, Y.; Daines, R. A.; Simpkins, N. S.; Furst, G. T. *J. Am. Chem. Soc.* **1988**, *110*, 4660. (b) Nicolaou, K. C.; Daines, R. A.; Ogawa, Y.; Chakraborty, T. K. *J. Am. Chem. Soc.* **1988**, *110*, 4696.

With vancosamide glycal **8** in hand, we first attempted to perform an oxidative (I^+) coupling with our simplest aryl β-glycoside, **3a**. Accordingly, an equimolar solution of glycal **8** with aryl glycoside **3a** in methylene chloride was treated first with dry powdered 4-Å molecular sieves and then with I(*sym*-collidine)$_2$ClO$_4$. Much to our surprise, compound **3a** was completely recovered. The glycal, however, had reacted with the iodonium reagent to form a chromatographically stable product, whose spectral data were consistent with its being the bridged bicyclic structure **9** (Scheme IV).[11,12]

Although systems such as **9a** have proven to be useful for α-specific proton-assisted glycosylations,[11b] in the case at hand a simpler possibility was reduced to practice. Thus, reaction of a benzene solution of the phenyl β-glucoside **3a** with an excess of glycal **8** in the presence of camphorsulfonic acid[13] gave a nearly quantitative yield (on the basis of consumed phenyl glucoside) of the desired disaccharide **10a**. Again, the fact that the newly created center was exclusively in the α-configuration was evident from ^{1}H NMR data ($J_{1',2a'}$ = 4.4 Hz). Similarly, the analogous reaction using 2,6-dimethoxyphenyl β-glucoside **3b**, which approximates, in a somewhat closer sense, the steric environment of the glycosylated phenolic system present in vancomycin, gave a reasonable yield of the desired disaccharide **10b** (51% plus 48% recovered aryl glucoside) (Scheme V).

In summary, we have developed new and useful methods for the stereospecific generation of aryl β-glucosides. We have demonstrated their usefulness as glycosyl acceptors in the synthesis of several disaccharides, including one which models the system found in the carbohydrate portion of vancomycin. Further studies on the scope and limitations of this new glycosylation reaction will be reported in due course.

Experimental Section

Phenyl 3,4,6-Tri-O-benzyl-β-D-glucopyranoside (3a). Dimethyldioxirane[3] in acetone (ca. 0.08 M, 6.25 mL) was added dropwise to a solution of 3,4,6-tri-O-benzyl-D-glucal (208 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 10 min, the solution was evaporated with a stream of dry N_2 and then dried in vacuo to afford the respective α-1,2-anhydrosugar. This material was dissolved in acetone (4 mL) and added to a refluxing solution of phenol (235 mg, 2.5 mmol, 5 equiv), K_2CO_3 (690 mg, 5 mmol, 10 equiv), and 18-crown-6 (ca. 2 mg) in acetone (10 mL). The mixture was then refluxed under N_2 for 4 h, cooled, filtered, and concentrated. The residue was dissolved in EtOAc (75 mL), washed twice with 50-mL portions of 1 N NaOH, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The resulting tan oil was chromatographed over silica gel (eluted with 15% EtOAc in hexanes) to give 160 mg (61%) of **3a** as white solids. Recrystallization from EtOAc–hexanes gave white needles: mp 100–101 °C; $[\alpha]^{23}_D$ = −22.48° (*c* 1.01, $CHCl_3$); IR ($CHCl_3$) 3580, 3050, 2885, 1600, 1495, 1220, 1105, 1072 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.02–7.39 (m, 20 H, ArH), 4.83–4.97 (m, 4 H), 4.48–4.61 (m, 3 H), 3.63–3.84 (m, 6 H), 2.40 (d, 1 H, *J* = 2.4 Hz, OH); FABLRMS (NOBA + NaI) *m/e* (relative intensity) 550 (11.2), 549 (21.6), 307 (100.0), 289 (57.9), 181 (93.9), 176 (64.8), 167 (22.3), 166 (24.4), 165 (27.1); FABHRMS calcd for $C_{33}H_{34}NaO_6$ 549.2256, found 549.2290. Anal. Calcd for $C_{33}H_{34}O_6$: C, 75.26; H, 6.51. Found: C, 75.26; H, 6.60.

2′,6′-Dimethoxyphenyl 3,4,6-Tri-O-benzyl-β-D-glucopyranoside (3b). Dimethyldioxirane[3] in acetone (ca. 0.08 M, 6.25 mL) was added dropwise to a solution of 3,4,6-tri-O-benzyl-D-glucal (208 mg, 0.5 mmol) in CH_2Cl_2 (5 mL) at 0 °C. After 10 min, the solution was evaporated with a stream of dry N_2 and then dried in vacuo to afford the respective α-1,2-anhydrosugar. To this material was added solid potassium 2,6-dimethoxyphenoxide (140 mg, 0.730 mmol, 1.45 equiv) and dry 2-propanal (20 mL). The resulting heterogeneous mixture was stirred overnight at room temperature under an atmosphere of N_2, whereupon most of the solids dissolved and the mixture darkened considerably. The mixture was then concentrated, diluted with water (50 mL), and extracted with 3 × 35 mL of EtOAc. The combined extracts were washed twice with 50-mL portions of 1 N NaOH, washed with brine, dried over $MgSO_4$, filtered, and concentrated. The residue was chromatographed over silica gel (eluted with 20% EtOAc in hexanes) to give 195 mg (67%) of **3b** as white solids. Recrystallization from EtOAc–hexanes gave small white rosettes: mp 112 °C; $[\alpha]^{23}_D$ = −14.31° (*c* 1.02, $CHCl_3$); IR ($CHCl_3$) 3450, 3015, 2900, 1600, 1480, 1260, 1120 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.15–7.42 (m, 15 H, ArH), 7.04 (t, 1 H, *J* = 8.4 Hz, ArH), 6.58 (d, 2 H, *J* = 8.4 Hz, ArH) 5.06 (d, 1 H, *J* = 11.2 Hz), 4.83 (t, 2 H, *J* = 11.2 Hz), 4.52–4.58 (m, 4 H), 3.82 (s, 6 H, ArOMe), 3.45–3.93 (m, 7 H); FABLRMS (NOBA + NaI) *m/e* (relative intensity) 610 (7.0), 609 (15.2), 325 (15.4), 307 (49.5), 289 (25.8), 245 (18.5), 182 (17.4), 181 (100.0), 167 (18.0), 166 (12.9), 165 (18.4); FABHRMS calcd for $C_{35}H_{38}NaO_8$ 609.2467, found 609.2478. Anal. Calcd for $C_{35}H_{38}O_8$: C, 71.65; H, 6.53. Found: C, 71.45; H, 6.48.

2′-Carbomethoxyphenyl 3,4,6-Tri-O-benzyl-β-D-glucopyranoside (3c). This compound was prepared in 43% yield from 3,4,6-tri-O-benzyl-D-glucal (**2**) as described above for the phenyl glycoside **3a**. Data for **3c**: $[\alpha]^{23}_D$ = −35.58° (*c* 0.98, $CHCl_3$); IR ($CHCl_3$) 3200–3500, 3010, 2940, 1715, 1600, 1440, 1455, 1260, 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.84 (d, 1 H, *J* = 7.2 Hz, ArH), 7.14–7.48 (m, 18 H, ArH), 5.15 (d, 1 H, *J* = 11.2 Hz), 5.01 (br s, 1 H), 4.92 (d, 1 H, *J* = 10.8 Hz), 4.84 (d, 1 H, *J* = 11.2 Hz) 4.73 (d, 1 H, *J* = 7.8 Hz, H1), 4.55–4.65 (m, 3 H), 3.93 (s, 3 H, $ArCO_2Me$), 3.69–3.93 (m, 5 H), 1.62 (br s, 1 H, OH); FABLRMS (NOBA + NaI) *m/e* (relative intensity) 608 (3.5), 607 (11.0), 553 (4.1), 460 (7.9), 433 (12.2), 325 (14.6), 308 (20.3), 307 (82.6), 289 (54.7), 245 (18.3), 217 (14.8), 181 (100), 165 (36.2); FABHRMS calcd for $C_{35}H_{36}O_8Na$ 607.2308, found 607.2335.

2′-Bromophenyl 3,4,6-Tri-O-benzyl-β-D-glucopyranoside (3d). This compound was prepared in 92% yield from 3,4,6-tri-O-benzyl-D-glucal (**2**) as described above for the phenyl glycoside **3a**. Data for **3d**: mp 80 °C; $[\alpha]^{23}_D$ = −52.12° (*c* 1.21, $CHCl_3$); IR ($CHCl_3$) 3200–3600, 3010, 2935, 1475, 1450, 1250, 1070 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.54 (dd, 1 H, *J* = 1.0, 7.7 Hz, ArH), 7.20–7.44 (m, 17 H, ArH), 6.97 (dt, *J* = 1.8, 8.3 Hz, ArH), 5.04 (d, 1 H, *J* = 11.2 Hz), 4.88 (m, 2 H), 4.79 (d, 1 H, *J* = 7.7 Hz, H1), 4.53–4.65 (m, 3 H), 3.95 (br t, 1 H, *J* = 7.3 Hz), 3.67–3.84 (m, 5 H), 2.75 (br s, 1 H, OH); FABLRMS (NOBA + NaI) *m/e* (relative intensity) 629 (8.6), 628 (3.6), 627 (8.9), 605 (3.2), 460 (4.8), 434 (4.4), 433 (12.5), 329 (18.7), 325 (16.0), 307 (72.2), 289 (43.8), 245 (31.6), 217 (14.0), 181 (100), 176 (59.5); FABHRMS calcd for $C_{33}H_{33}O_6BrNa$ 627.1359, found 627.1345.

Phenyl 2-O-Acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranoside (4a). A solution of phenyl glycoside **3a** (15.0 mg, 0.029 mmol) in pyridine (0.5 mL) at 0 °C was treated with Ac_2O (13.5 μL, 14.6 mg, 0.143 mmol, 5 equiv) and 4-(dimethylamino)pyridine (ca. 1 mg). The mixture was slowly allowed to warm to room temperature over a period of 5 h and then was concentrated and chromatographed over silica gel (eluted with 10% EtOAc–hexanes) to give 16.0 mg (99%) of **4a** as white solids. Recrystallization from Et_2O–pentane gave long white needles: mp 90–91 °C; $[\alpha]^{23}_D$ = +4.90° (*c* 0.96, $CHCl_3$); IR ($CHCl_3$) 3020, 2880, 1750, 1600, 1495, 1380, 1220, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 6.98–7.34 (m, 20 H, ArH), 5.26 (dd, 1 H, *J* = 8.8, 8.0 Hz, H2), 4.94 (d, 1 H, *J* = 8.0 Hz, H1), 4.81 (m, 2 H), 4.69 (d, 1 H, *J* = 11.4 Hz), 4.49–4.62 (m, 3 H), 3.62–3.82 (m, 5 H), 1.96 (s, 3 H, OAc).

2′,6′-Dimethoxyphenyl 2-O-Acetyl-3,4,6-tri-O-benzyl-β-D-glucopyranoside (4b). This compound was prepared in 88% yield from **3b** as described above for the acetate **4a** and was isolated as a colorless oil: $[\alpha]^{23}_D$ = +10.14° (*c* 1.08, $CHCl_3$); IR ($CHCl_3$) 3020, 2960, 1700, 1600, 1500, 1485, 1265, 1125, 1075 cm^{-1}; ^{1}H NMR ($CDCl_3$) δ 7.18–7.34 (m, 15 H, ArH), 6.99 (t, 1 H, *J* = 8.4 Hz, ArH), 6.54 (d, 2 H, *J* = 8.4 Hz, ArH), 5.31 (dd, 1 H, *J* = 8.2, 7.7 Hz, H2), 4.94 (d, 1 H, *J* = 7.7 Hz, H1), 4.48–4.82 (m, 6 H), 3.76 (s, 6 H, ArOMe), 3.67–3.82 (m, 4 H), 3.47–3.49 (m, 1 H), 1.97 (s, 3 H, OAc).

Phenyl O-(2-Deoxy-2-iodo-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-β-D-glucopyranoside (5a). A solution of phenyl glycoside **3a** (25 mg, 0.048 mmol, 1.1 equiv) and 3,4,6-tri-O-benzyl-D-glucal (**2**, 18.8 mg, 0.045 mmol) in CH_2Cl_2 (1.2 mL) was stirred for 30 min at room temperature in the presence of powdered 4-Å molecular sieves (10 mg). The mixture was then cooled to 0 °C and I(*sym*-collidine)$_2$ClO$_4$[7c] (40 mg, 0.068 mmol, 1.5 equiv) was added in one portion. The mixture was allowed to warm to ambient temperature over 5 h and was then filtered. The filtrate was washed with 10% aqueous $Na_2S_2O_3$ and water and then dried over $MgSO_4$. Filtration and concentration left an oil, which was chromatographed over silica gel (eluted with 12.5% EtOAc–hexanes) to give 36.5 mg (76%) of **5a** as a colorless oil: $[\alpha]^{23}_D$

(12) While the structure of compound **9** was not rigorously proven, it is further supported by chemical means. Thus, deiodination of **9a** through the action of triphenyltin hydride afforded a crude and hydrolytically unstable product, formulated as **9b**. Upon reaction of **9b** with **3a** in the presence of camphorsulfonic acid, compound **10a** was obtained. While this route was not followed synthetically (since the pathway via glycal **8** is much more straightforward), it does add strong chemical backing for the assignment.

(13) (a) Daniels, P. J. L.; Mallams, A. K.; Wright, J. J. *J. Chem. Soc., Chem. Commun.* **1973**, 675. (b) Arcamone, F.; Bargiotti, A.; Casinelli, G.; Redaelli, S.; Hanessian, S.; DiMarco, A.; Casazza, A. M.; Dasdia, T.; Necco, A.; Reggiani, P.; Supino, R. *J. Med. Chem.* **1976**, *19*, 733. (c) Yasumori, T.; Sato, K.; Hashimoto, H.; Yoshimura, J. *Bull. Chem. Soc. Jpn.* **1984**, *57*, 2538. (d) Thang, T. T.; Imbach, J. L.; Fizames, C.; Lavelle, F.; Ponsinet, G.; Olesker, A.; Lukacs, G. *Carbohydr. Res.* **1985**, *135*, 241. (e) Wiesner, K.; Tsai, T. Y. R.; Jin, H. *Helv. Chim. Acta* **1985**, *68*, 300.

= +15.20° (c 1.00, $CHCl_3$); IR ($CHCl_3$) 3010, 2860, 1600, 1495, 1450, 1220, 1065 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.04–7.42 (m, 35 H, ArH), 5.95 (br s, 1 H, H1′), 4.48–4.87 (m, 11 H), 4.41 (t, 2 H, J = 11.2 Hz), 4.32 (d, 1 H, J = 12.1 Hz), 3.99–4.01 (m, 2 H), 3.90 (t, 1 H, J = 8.4 Hz), 3.35–3.79 (m, 7 H), 3.20 (m, 1 H); FABLRMS (NOBA + NaI) m/e (relative intensity) 1092 (2.3), 1091 (3.5), 271 (8.5), 239 (8.2), 197 (6.9), 182 (16.8), 181 (100.0), 154 (16.5); FABHRMS calcd for $C_{60}H_{61}INaO_{10}$ 1091.3211, found 1091.3143.

Phenyl O-(2-Deoxy-3,4,6-tri-O-benzyl-α-D-glucopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-β-D-glucopyranoside (6a). A solution of disaccharide **5a** (25 mg, 0.023 mmol), triphenyltin hydride (24.7 mg, 0.070 mmol, 3 equiv), and AIBN (ca. 1 mg) was refluxed in benzene (2 mL) for 30 min. The mixture was then cooled, concentrated, and chromatographed over silica gel (eluted with 15% EtOAc–hexanes) to give 21.0 mg (95%) of **6a** as a clear oil: $[\alpha]^{25}_D$ = +28.20° (c 1.11, $CHCl_3$); IR ($CHCl_3$) 3010, 2870, 1600, 1500, 1460, 1220, 1110, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 6.99–7.36 (m, 35 H, ArH), 5.68 (d, 1 H, J = 2.6 Hz, H1′), 4.94 (d, 1 H, J = 7.8 Hz), 4.71–4.89 (m, 4 H), 4.45–4.59 (m, 7 H), 4.31 (d, 1 H, J = 12.1 Hz), 3.90–3.97 (m, 3 H), 3.55–3.79 (m, 6 H), 3.37 (m, 2 H), 2.27 (dd, 1 H, J = 5.1, 12.7 Hz, eq H2′), 1.72 (ddd, 1 H, J = 2.6, 12.7, 12.7 Hz, ax H2′); FABLRMS (NOBA + NaI) m/e (relative intensity) 966 (2.1), 965 (3.5), 309 (13.1), 245 (12.5), 217 (12.2), 182 (17.4), 181 (100.0), 179 (10.0), 154 (7.3); FABHRMS calcd for $C_{60}H_{62}NaO_{10}$ 965.4244, found 965.4276.

2′,6′-Dimethoxyphenyl O-(2-Deoxy-2-iodo-3,4,6-tri-O-benzyl-α-D-mannopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-β-D-glucopyranoside (5b). This compound was prepared in 64% yield from glycoside **3b** and 3,4,6-tri-O-benzyl-D-glucal (**2**) as described above for compound **5a** and was isolated as a colorless oil following chromatography over silica gel (eluted with 17.5% EtOAc–hexanes): $[\alpha]^{23}_D$ = +34.74° (c 1.14, $CHCl_3$); IR ($CHCl_3$) 3020, 2940, 2860, 1600, 1495, 1480, 1260, 1125 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.00–7.35 (m, 31 H, ArH), 6.57 (d, 2 H, J = 8.4 Hz, ArH), 6.07 (brs, 1 H, H1′), 4.67–4.90 (m, 8 H), 4.36–4.58 (m, 6 H), 4.16 (m, 1 H), 3.99 (m, 2 H), 3.79 (s, 6 H, ArOMe), 3.32–3.72 (m, 8 H); FABLRMS (NOBA + NaI) m/e (relative intensity) 1153 (5.4), 1152 (11.6), 1151 (16.0), 239 (8.2), 197 (6.4), 182 (16.1), 181 (100.0), 154 (31.7), 153 (20.5); FABHRMS calcd for $C_{62}H_{65}NaIO_{12}$ 1151.3422, found 1151.3430.

2′,6′-Dimethoxyphenyl O-(2-Deoxy-3,4,6-tri-O-benzyl-α-D-glucopyranosyl-(1→2)-3,4,6-tri-O-benzyl-β-D-glucopyranoside (6b). A solution of disaccharide **5b** (25 mg, 0.022 mmol), triphenyltin hydride (23.3 mg, 0.066 mmol, 3 equiv), and AIBN (ca. 1 mg) was refluxed in benzene (2 mL) for 30 min. The mixture was then cooled, concentrated, and chromatographed over silica gel (eluted with 20% EtOAc–hexanes) to give 22.0 mg (99%) of **6b** as a clear oil: $[\alpha]^{25}_D$ = +50.29° (c 1.05, $CHCl_3$); IR ($CHCl_3$) 3020, 3000, 2920, 2850, 1595, 1490, 1470, 1250, 1110, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 7.02–7.66 (m, 31 H, ArH), 6.56 (d, 2 H, J = 8.4 Hz, ArH), 5.83 (d, 1 H, J = 2.2 Hz, H1′), 4.81–4.98 (m, 5 H), 4.69 (br s, 2 H), 4.44–4.61 (m, 5 H), 4.35 (d, 1 H, J = 12.1 Hz), 3.97–4.12 (m, 3 H), 3.78 (s, 6 H, ArOMe), 3.34–3.71 (m, 8 H), 2.39 (dd, 1 H, J = 5.0, 12.7 Hz, eq H2′), 1.71 (ddd, 1 H, J = 2.2, 9.1, 12.7 Hz, ax H2′); FABLRMS (NOBA + NaI) m/e (relative intensity) 1027 (6.7), 1026 (18.4), 1025 (27.4), 245 (14.6), 217 (12.6), 182 (18.9), 181 (100.0), 167 (12.1), 155 (24.9), 154 (43.6), 153 (28.2); FABHRMS calcd for $C_{62}H_{66}NaO_{12}$ 1025.4455, found 1025.4487.

Methyl 3-Benzamido-4-O-benzoyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranoside (7). The basic procedure of Johnson et al.[9] was followed. Benzoyl chloride (10 mL) was added dropwise to a suspension of vancomycin hydrochloride (5.5 g) in pyridine (25 mL) at 0 °C. The heterogeneous mixture was allowed to warm to room temperature and was then stirred for 60 h, upon which time the mixture darkened considerably and became homogeneous. The solution was then cooled to 0 °C and methanol (50 mL) was added dropwise. After stirring for 1 h, the solution was evaporated under reduced pressure, and the resulting residue was triturated with Et_2O until the gummy material solidified. The resulting solids were washed with additional Et_2O and air dried to give about 22 g of crude benoylated vancomycin.

The aforementioned solids were dissolved in methanolic HCl (1.5 N, 35 mL), and the solution was heated to reflux for 12 h, then cooled, evaporated under reduced pressure, and partitioned between water and EtOAc. The aqueous layer was extracted with two more portions of EtOAc, and the combined organics were washed with brine, dried over $MgSO_4$, filtered, and concentrated. The resulting heavy yellow oil was passed through a column of silica gel using Et_2O elution. Concentration of the eluates left a clear colorless oil containing traces of methyl benzoate, partially benzoylated glucosides, and the desired α- and β-vancosamine derivatives. The α-anomer (423 mg) was isolated by chromatography over silica gel (eluted with 30% EtOAc–hexanes) and crystallized from EtOAc–hexanes: mp 167–168 °C (lit.[9] mp 168–169 °C); $[\alpha]^{23}_D$ = −189.13° (c 1.04, MeOH) (lit.[9] $[\alpha]^{22}_D$ = −199° (c 0.1, MeOH)); IR ($CHCl_3$) 3400, 3020, 2945, 1705, 1670, 1610, 1585, 1535, 1280, 1135, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.13 (dd, 2 H, J = 1.6, 5.4 Hz, ArH), 7.23–7.62 (m, 8 H, ArH), 6.67 (br s, 1 H, NHBz), 5.12 (s, 1 H, H4), 4.89 (d, 1 H, J = 4.2 Hz, H1), 4.31 (q, 1 H, J = 6.4 Hz, H5), 3.30 (s, 3 H, OMe), 2.81 (d, 1 H, J = 13.5 Hz, eq H2), 2.18 (dd, 1 H, J = 4.2, 13.5 Hz, ax H2), 1.88 (s, 3 H, C3-Me), 1.24 (d, 3 H, J = 6.4 Hz, H6).

Fractions containing the β-anomer and partially benzoylated glucosides were combined and concentrated to give 492 mg of clear oil. Selective hydrolysis of the former was accomplished by heating at 80 °C for 12 h in 0.05 M H_2SO_4 in dioxane–water (7:3, 30 mL). After cooling, the solution was extracted three times with EtOAc, and the extracts were washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. Chromatography over silica gel (eluted with 50% EtOAc–hexanes) gave crude α,β-*N*,*O*-dibenzoylvancosamides (285 mg) in addition to unhydrolyzed methyl 3,4,6-tribenzoylglucopyranosides. The crude vancosamide thus obtained was dissolved in methanol (12 mL) and treated with methanolic HCl (50 μL of 1.5 N). After stirring for 8 h, this mixture was concentrated under reduced pressure, diluted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, concentrated, and crystallized to give an additional 316 mg of pure **7**.

1,5-Anhydro-3-benzamido-4-O-benzoyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hex-1-enitol (8). Compound **7** (100 mg, 0.26 mmol) and camphorsulfonic acid (5 mg, cat.) were dissolved in benzene (20 mL) and heated to reflux for 12 h with the azeotropic removal of methanol. Upon cooling, the mixture was washed with saturated aqueous $NaHCO_3$, water, and brine, dried over Na_2SO_4, filtered, and concentrated to give a clean oil. This material was passed through a plug of silica gel (20% EtOAc–hexanes elution) and the eluate evaporated to give 92 mg (100%) of **8** as a clear colorless oil: $[\alpha]^{23}_D$ = −32.38° (c 1.22, $CHCl_3$); IR ($CHCl_3$) 3440, 3010, 2990, 1730, 1670, 1605, 1585, 1515, 1490, 1285, 1260, 1075 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.03 (d, 2 H, J = 7.4 Hz, ArH), 7.19–7.56 (m, 8 H, ArH), 6.44, (d, 1 H, J = 6.4 Hz, H1), 6.32 (br s, 1 H, NHBz), 5.55 (s, 1 H, H4), 5.25 (dd, 1 H, J = 1.9, 6.4 Hz, H2), 4.40 (q, 1 H, J = 6.6 Hz, H5), 1.84 (s, 3 H, C3-Me), 1.33 (d, 3 H, J = 6.6 Hz, H6); FABLRMS (NOBA + NaI) m/e (relative intensity) 353 (28.1), 352 (95.4), 307 (100), 289 (53.2), 220 (45.8), 205 (24.9), 165 (17.9); FABHRMS calcd for $C_{21}H_{22}NO_4$ 352.1550, found 352.1553.

Bicyclic Iodide 9a. This compound was isolated in 92% yield from the attempted iodonium-induced coupling reaction of glycal **8** with aryl glucoside **3a** as described above for the preparation of disaccharide **5a**: $[\alpha]^{25}_D$ = −20.9° (c 0.95, $CHCl_3$); IR ($CHCl_3$) 3010, 2975, 2960, 1715, 1655, 1450, 1295, 1275, 1180, 1120, 1075, 1035, 945 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 (d, 2 H, J = 7.2 Hz, ArH), 8.03 (d, 2 H, J = 6.8 Hz, ArH), 7.40–7.64 (m, 6 H, ArH), 5.80 (d, 1 H, J = 1.8 Hz, H1), 5.77 (d, 1 H, J = 7.5 Hz, H4), 4.80 (dq, 1 H, J = 7.5, 7.3 Hz, H5), 4.36 (d, 1 H, J = 1.8 Hz, H2), 1.60 (s, 3 H, CH_3), 1.17 (d, 3 H, J = 7.3 Hz, CH_3); MS (CI) m/e (relative intensity) 478 (5.6), 352 (7.3), 231 (3.4), 105 (12.0), 89 (100); HRMS (CI) calcd for $C_{21}H_{21}NO_4I$ 478.0517, found 478.0539.

Phenyl O-(3-Benzamido-4-O-benzoyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-β-D-glucopyranoside (10a). A solution of phenyl glycoside **3a** (25.0 mg, 0.048 mmol) and vancosamide glycal **8** (25.0 mg, 0.071 mmol, 1.5 equiv) in benzene (2.5 mL) was treated at room temperature with camphorsulfonic acid (2.2 mg, 0.01 mmol, 0.2 equiv). After 10 h, additional vancosamide glycal **8** (10 mg, 0.6 equiv) was added, and stirring was continued at room temperature for an additional 6 h. The reaction mixture was then diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, water, and brine. The solution was dried over $MgSO_4$, filtered, and concentrated, and the resulting clear colorless oil was chromatographed over silica gel (using 15–40% EtOAc–hexanes elution) to provide recovered **3a** (9.0 mg, 36% recovery) and the desired disaccharide **10a** (26.5 mg, 64%): $[\alpha]^{25}_D$ = −58.29° (c 0.82, $CHCl_3$); IR ($CHCl_3$) 3380, 3010, 2935, 2860, 1695, 1660, 1590, 1530, 1495, 1270, 1210, 1070 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.14 (d, 2 H, J = 7.7 Hz, ArH), 7.00–7.62 (m, 28 H, ArH), 6.78 (br s, 1 H, NHBz), 5.54 (d, 1 H, J = 4.4 Hz, H1′), 5.07 (s, 1 H, H4′), 5.02 (dd, 1 H, J = 7.8, 9.3 Hz), 4.78–4.97 (m, 4 H), 4.78 (q, 1 H, J = 6.4 Hz, H5′), 4.48–4.59 (m, 3 H), 4.06 (t, 1 H, J = 7.8 Hz), 3.65–3.84 (m, 4 H), 2.88 (d, 1 H, J = 14.1 Hz, eq H2′), 2.12 (dd, 1 H, J = 4.4, 14.1 Hz, ax H2′), 1.79 (s, 3 H, C3′-Me), 1.29 (d, 3 H, J = 6.4 Hz, H6′); FABLRMS (NOBA + NaI) m/e (relative intensity) 878 (8.5), 353 (27.5), 352 (100), 307 (17.9), 289 (9.7), 231 (19.2), 181 (13.2), 165 (6.2); FABHRMS calcd for $C_{54}H_{56}NO_{10}$ 878.3906, found 878.3871. Anal. Calcd for $C_{54}H_{55}NO_{10}$: C, 73.87; H, 6.31; N, 1.60. Found: C, 73.63; H, 6.10; N, 1.52.

2′,6′-Dimethoxyphenyl O-(3-Benzamido-4-O-benzoyl-2,3,6-trideoxy-3-*C*-methyl-α-L-*lyxo*-hexopyranosyl)-(1→2)-3,4,6-tri-O-benzyl-β-D-glucopyranoside (10b). A solution of 2,6-dimethoxyphenyl glycoside **3b** (28.0 mg, 0.048 mmol) and vancosamide glycal **8** (25.0 mg, 0.071 mmol, 1.5 equiv) in benzene (2.5 mL) was treated at room temperature with

camphorsulfonic acid (2.2 mg, 0.01 mmol, 0.2 equiv). After 10 h, additional vancosamide glycal **8** (10 mg, 0.6 equiv) was added, and stirring was continued at room temperature for an additional 6 h. The reaction mixture was then diluted with EtOAc and washed with saturated aqueous $NaHCO_3$, water, and brine. The solution was dried over $MgSO_4$, filtered, and concentrated, and the resulting clear colorless oil was chromatographed over silica gel (using 17.5–40% EtOAc–hexanes elution) to provide recovered **3b** (13.5 mg, 48% recovery) and the desired disaccharide **10b** (23.0 mg, 51%): $[\alpha]^{25}_D = -61.73°$ (*c* 0.81, $CHCl_3$); IR ($CHCl_3$) 3380, 3000, 2940, 1700, 1660, 1600, 1490, 1475, 1300, 1280, 1260, 1120, 1060 cm^{-1}; ^1H NMR ($CDCl_3$) δ 8.12 (d, 2 H, *J* = 7.3 Hz, ArH), 7.17–7.60 (m, 23 H, ArH), 7.02 (t, 1 H, *J* = 8.4 Hz, ArH), 6.78 (br s, 1 H, NHBz), 6.57 (d, 2 H, *J* = 8.4 Hz, ArH), 5.47 (d, 1 H, *J* = 3.9 Hz, H1′), 5.15 (d, 1 H, *J* = 7.7 Hz), 5.02 (s, 1 H, H4′), 4.90 (br s, 2 H), 4.76–4.84 (m, 3 H), 4.59 (d, 1 H, *J* = 11.0 Hz), 4.47 (m, 2 H), 4.02 (t, 1 H, *J* = 8.0 Hz), 3.78 (s, 6 H, ArOMe), 3.64–3.82 (m, 3 H), 3.40 (m, 1 H), 2.92 (d, 1 H, *J* = 13.7 Hz, eq H2′), 2.12 (dd, 1 H, *J* = 3.9, 13.7 Hz, ax H2′), 2.05 (s, 3 H, C3′-Me), 1.07 (d, 3 H, *J* = 6.4 Hz, H6′); FABLRMS (NOBA + NaI) *m/e* (relative intensity) 938 (2.8), 784 (4.6), 353 (27.0), 352 (100), 307 (8.2), 289 (6.0), 231 (17.0), 230 (4.4), 181 (11.5), 167 (4.6), 165 (5.5); FABHRMS calcd for $C_{56}H_{60}NO_{12}$ 938.4117, found 938.4084. Anal. Calcd for $C_{56}H_{59}NO_{12}$: C, 71.70; H, 6.34; N, 1.49. Found: C, 71.43; H, 6.06; N, 1.23.

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Registry No. 1, 1404-90-6; **1**·*x*HCl, 1404-93-9; **2**, 55628-54-1; **3a**, 135192-39-1; **3b**, 135192-43-7; **3c**, 138925-03-8; **3d**, 138925-04-9; **4a**, 138925-14-1; **4b**, 138925-15-2; **5a**, 138925-05-0; **5b**, 138925-06-1; **6a**, 138925-07-2; **6b**, 138925-08-3; **7**, 37091-13-7; **7** 1-demethyl derivative, 138925-16-3; **7** 1-demethyl derivative β-isomer, 138925-17-4; **8**, 138925-09-4; **9a** (X = H), 138925-11-8; **10a**, 138925-12-9; **10b**, 138925-13-0; 2,6-$(MeO)_2$PhOH, 91-10-1; phenol, 108-95-2; methyl salicylate, 119-36-8; *o*-bromophenol, 95-56-7; 3,3-dimethyldioxirane, 74087-85-7.

Epibatidine: A Novel (Chloropyridyl)azabicycloheptane with Potent Analgesic Activity from an Ecuadoran Poison Frog

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Abstract: A potent non-opioid analgesic, epibatidine, has been isolated from skins of the Ecuadoran poison frog, *Epipedobates tricolor*, and its structure determined by MS, IR, and ^1H NMR analyses as *exo*-2-(6-chloro-3-pyridyl)-7-azabicyclo[2.2.1]heptane. It represents a unique new class of alkaloids.

Skin extracts from an Ecuadoran poison frog, *Epipedobates tricolor*, of the family Dendrobatidae yielded a number of alkaloids, including the major alkaloid pumiliotoxin **251D**, whose indolizidine structure was revealed by X-ray crystallographic analysis.[1] These frogs had been obtained primarily because of the presence of a trace alkaloid in the skin extract that caused a Straub-tail response[2] when injected in mice.[3,4] The Straub-tail reaction initially served as an assay for this trace alkaloid during purification. Gas chromatographic–mass spectral analysis of fractions obtained by chromatography of alkaloids from *Epipedobates tricolor* on a silica column (see ref 1 for details) indicated that the trace alkaloid causing the Straub-tail reaction had molecular ions (208, 210) and several pairs of fragments all in a 3:1 ratio, indicating the presence of chlorine.[5] High-resolution mass measurements (Table I) established the formula for the *m/z* 208

Table I. High-Resolution Mass Measurements of Epibatidine

obsd	percent of base peak	calcd for	error (mmu) (calcd − obsd)
210.0764	4.4	$C_{11}H_{13}N_2{}^{37}Cl$	−2.9
208.0769	15.5	$C_{11}H_{13}N_2{}^{35}Cl$	−0.15
181.0259	~1	$C_9H_8N_2{}^{37}Cl$	+8.6
179.0362	2.7	$C_9H_8N_2{}^{35}Cl$	+1.4
150.1289[a]	3.3	$C_{10}H_{16}N$	−3.3
142.0245	2.7	$C_7H_7N^{37}Cl$	−0.9
141.0287	3.4	$C_7H_8N^{35}Cl$	+6.8
140.0263	9.2	$C_7H_7N^{35}Cl$	+0.4
124.1148[a]	3.6	$C_8H_{14}N$	+2.2
83.0749	2.1	C_5H_9N	−1.6
69.0489	100	C_4H_7N	+8.9

[a] Probably due to an impurity. The data indicate a much higher hydrogen to carbon ratio than that found in the parent ions.

(1) Daly, J. W.; Tokuyama, T.; Fujiwara, T.; Highet, R. J.; Karle, I. L. *J. Am. Chem. Soc.* **1980**, *102*, 830–836. From 750 skins, a total of 21 mg of the major alkaloid pumiliotoxin **251D** was isolated. The trace alkaloid that is the subject of the present paper is present at levels of at least 20-fold less than pumiliotoxin **251D**.

(2) A Straub-tail reaction is characteristic of opiate alkaloids and has been used as an assay for opiate agonists and antagonists (Aceto, M. D.; McKean, D. B.; Pearl, J. *Br. J. Pharmacol.* **1969**, *36*, 225–239). Unlike the Straub-tail reaction caused by morphine and other opiates, the reaction caused by the frog alkaloid was not reversed by naloxone (see Table II).

(3) Daly, J. W.; Brown, G. B.; Mensah-Dwumah, M.; Myers, C. W. *Toxicon* **1978**, *16*, 163–188.

(4) Daly, J. W.; Myers, C. W.; Whittaker, N. *Toxicon* **1987**, *25*, 1023–1095.

(5) Since a chloro substituent had not been previously seen in any of the more than 200 dendrobatid alkaloids, we considered it possible that a chlorine had been introduced into the **208/210** alkaloid from HCl during the usual preparation of alkaloid fractions from methanolic skin extracts (partition into $CHCl_3$, extraction into 0.1 N HCl, basification, and re-extraction into $CHCl_3$). Consequently, a separate extract was partitioned using CH_2Br_2 and HBr. The chlorine-containing **208/210** alkaloid was still obtained. It was also present in the methanol extracts of skin; thus, chlorine is not artifactually incorporated during isolation.

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