hydrogen chloride gas for 1 h. Excess reagent and solvent were rotoevaporated to give the desired product as a white powder.

**Reductive Alkylation of Asperlicin (1). Preparation of 7-[[1-Ethyl-2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-1*H*-imidazo[1,2-*a*]indol-9-yl]methyl]-6,7,7a,8-tetrahydroquinazolino[3,2-*a*][1,4]benzodiazepine-5,13-dione (7).** Sodium cyanoborohydride (158 mg, 2.52 mmol) was added at room temperature to a solution of 10 mL of glacial acetic acid containing 450 mg (0.84 mmol) of 1. After 2 h, 3 equiv more of sodium cyanoborohydride was added and the reaction mixture was stirred at 55 °C for 14 h. The reaction mixture was cooled and poured into 150 mL of water. The resulting precipitate was collected, washed with water, and dried. The analytical sample was obtained as a white solid via silica gel chromatography ($CHCl_3$–EtOH, 95:5) followed by trituration with ether.

**Preparation of 6,7,7a,8-Tetrahydro-7-[[2,3,9,9a-tetrahydro-9-hydroxy-2-(2-methylpropyl)-3-oxo-1-(3-phenylpropyl)imidazo[1,2-*a*]indol-9-yl]methyl]quinazolino[3,2-*a*][1,4]benzodiazepine-5,13-dione (8).** Asperlicin (1; 535 mg, 1.0 mmol) was dissolved in 10 mL of glacial acetic acid. 3-Phenylpropionaldehyde (400 mg, 3.0 mmol) and sodium cyanoborohydride (504 mg, 8.0 mmol) were then added to this mixture. The reaction was stirred at room temperature for 60 h and worked up as in the preparation of 7.

**Acylation of the Tertiary Hydroxyl Group. Preparation of Phenylmethyl [2,3,9,9a-Tetrahydro-2-(2-methylpropyl)-3-oxo-1-[(phenylmethoxy)carbonyl]-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino[3,2-*a*][1,4]benzodiazepin-7-yl)-methyl]-1*H*-imidazo[1,2-*a*]indol-9-yl]butanedioic Acid Ester (18).** To 1.53 g (2.3 mmol) of 6 in 10 mL of methylene chloride was added in succession benzyl succinate half-acid ester (718 mg, 3.45 mmol), 4-(dimethylamino)pyridine[11] (421 mg, 3.45 mmol), and a 1 M solution of dicyclohexylcarbodiimide in methylene chloride (3.45 mL, 3.45 mmol). The reaction mixture was protected from moisture and stirred at room temperature overnight. The reaction mixture was filtered, diluted to 250 mL with methylene chloride, and washed with 5% citric acid solution, 50% sodium bicarbonate solution, and brine. The dried ($MgSO_4$) organic phase was rotoevaporated to give an amorphous solid that was chromatographed on silica gel (hexane–ethyl acetate, 7:3) to yield the analytical sample.

**Preparation of Mono[2,3,9,9a-Tetrahydro-2-(2-methylpropyl)-3-oxo-9-[(5,6,7,13-tetrahydro-5,13-dioxoquinazolino-[3,2-*a*][1,4]benzodizaepin-7-yl)methyl]-1*H*-imidazo[1,2-*a*]-indol-9-yl] Butanedioic Acid Ester (20).** A solution of 18 (300 mg, 0.35 mmol) in 75 mL of ethanol was treated with 50 mg of 10% palladium/carbon catalyst and hydrogenated at 50 psi at 23 °C for 4.5 h. Catalyst and solvent were removed to give an oil that was purified by PLC ($CHCl_3$–EtOH, 9:1) to afford the analytical sample as an off-white solid.

**Cholecystokinin Receptor Binding Method. Pancreas.** CCK-33 was radiolabeled with $^{125}I$ Bolton Hunter reagent (2000 Ci/mmol) as described by Sankaran.[16] Rat pancreatic receptor binding was performed according to Innis and Snyder[17] with the minor modification of adding the additional protease inhibitors, (phenylmethyl)sulfonyl fluoride and *o*-phenanthroline, which have no effect on the [$^{125}I$]-CCK receptor binding assay.

**Brain.** CCK-33 was radiolabeled and the binding to guinea pig cortical membranes was performed according to Saito.[18] In both pancreas and brain binding assays several concentrations of the test compounds were examined in triplicate and $IC_{50}$ values determined by regression analysis.

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**Registry No.** 1, 93413-04-8; 4, 103303-31-7; 5, 102743-49-7; 6, 102743-51-1; 7, 103303-32-8; 8, 102743-52-2; 9, 103241-31-2; 10, 103241-32-3; 11, 102743-57-7; 12, 102743-56-6; 13, 103022-89-5; 14, 102996-16-7; 14 (free base), 103303-33-9; 15, 102996-15-6; 16, 102996-17-8; 17, 102996-18-9; 18, 103241-33-4; 19, 103241-34-5; 20, 102996-22-5; (L)-*p*-$HO_2CCH(NHBoc)CH_2C_6H_4OCH_2Ph$, 2130-96-3; (L)-$HO_2CCH(NHBoc)(CH_2)_4NHCO_2CH_2Ph$, 2389-45-9; (L)-$HO_2CCH(NHBoc)(CH_2)_3NHCO_2CH_2Ph$, 2480-93-5; 3-phenyl propanal, 104-53-0; monobenzyl succinate, 103-40-2; cholecystokinin, 9011-97-6.

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# Cardiac Glycosides. 7. Sugar Stereochemistry and Cardiac Glycoside Activity

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Digitoxigenin α-L-, β-L-, α-D-, and β-D-glucosides; α-L-, β-L-, α-D-, and β-D-mannosides; and α-L- and β-L-rhamnosides were stereoselectively synthesized from the corresponding sugar tetrabenzyl trichloroacetimidates. The $Na^+,K^+$-ATPase receptor inhibitory activities of these glycosides (as a measure of receptor binding) were compared with those of digitoxigenin, digitoxigenin 6′-hydroxy-β-D-digitoxoside, digitoxigenin β-D-galactoside, and digitoxigenin β-D-digitoxoside. The observed activities reveal that a given sugar substituent may have a role in binding of some glycoside stereoisomers, but not others. With α-L- and possibly β-L-rhamnosides, the 5′-$CH_3$ and 4′-OH appear to have a predominant role in binding to the $Na^+,K^+$-ATPase receptor. Addition of a 6′-OH to form the corresponding mannosides dramatically disrupts the effect of both the 5′-$CH_3$ and 4′-OH in prompting receptor binding of the α-L isomer. However, with the β-L isomer, some influence of 4′-OH, 3′-OH, and 2′-OH binding remains. With β-D-glycosides, binding via the "5′-$CH_3$ site" appears to be of little importance and addition of a 6′-OH diminishes activity only slightly. With these β-D-glycosides, an equatorial 4′-OH, axial 3′-OH, and equatorial 2′-OH groups appear to contribute to binding.

Digitoxin, digoxin, and most other naturally occurring active cardiac glycosides have sugars with β-D stereochemistry. The notable exception is ouabain, an α-L-rhamnoside. In spite of this remarkably different stereochemistry, ouabain is very potent. Thus, there has been considerable interest in trying to understand the stereochemical preferences of the sugar binding site on the cardiac glycoside receptor $Na^+,K^+$-ATPase.[1-6] Brown and

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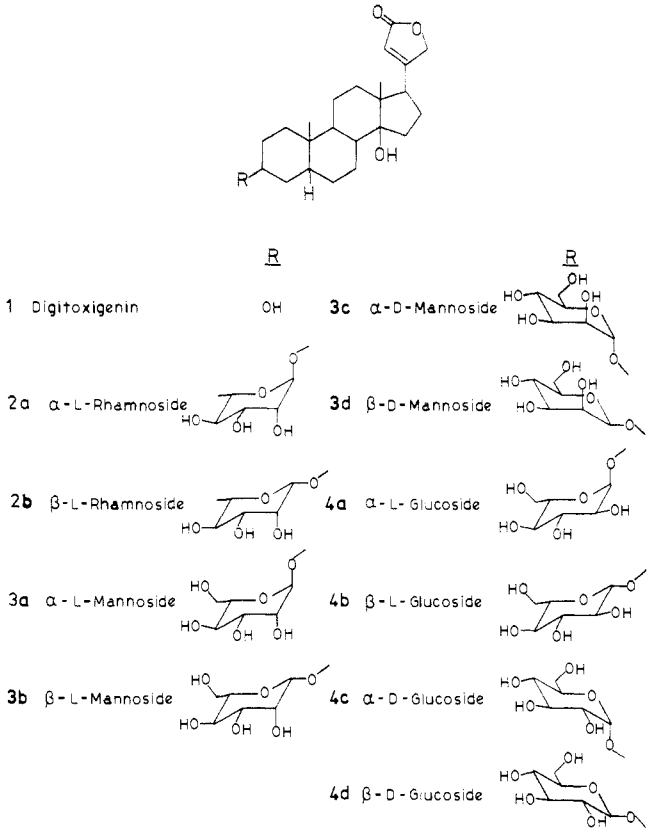
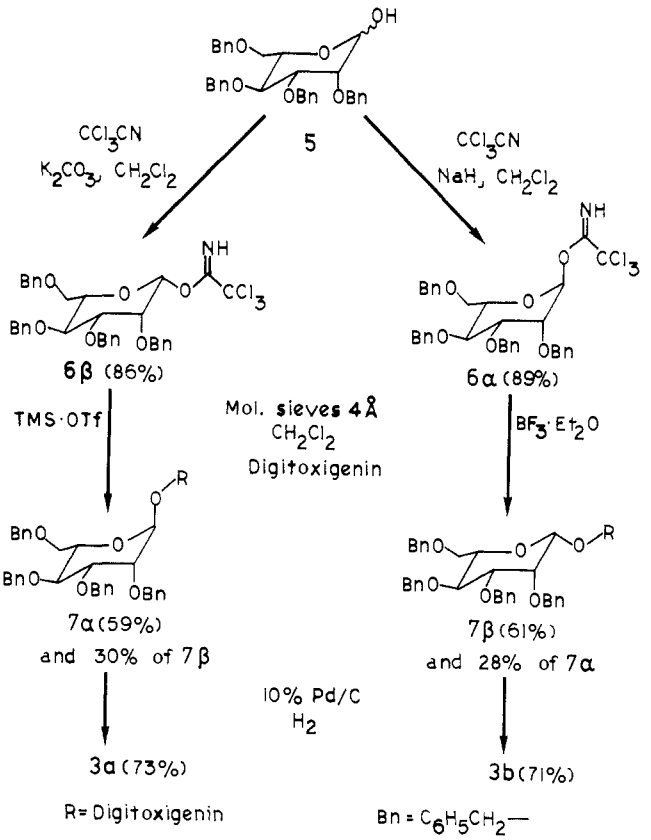
**Chart I**



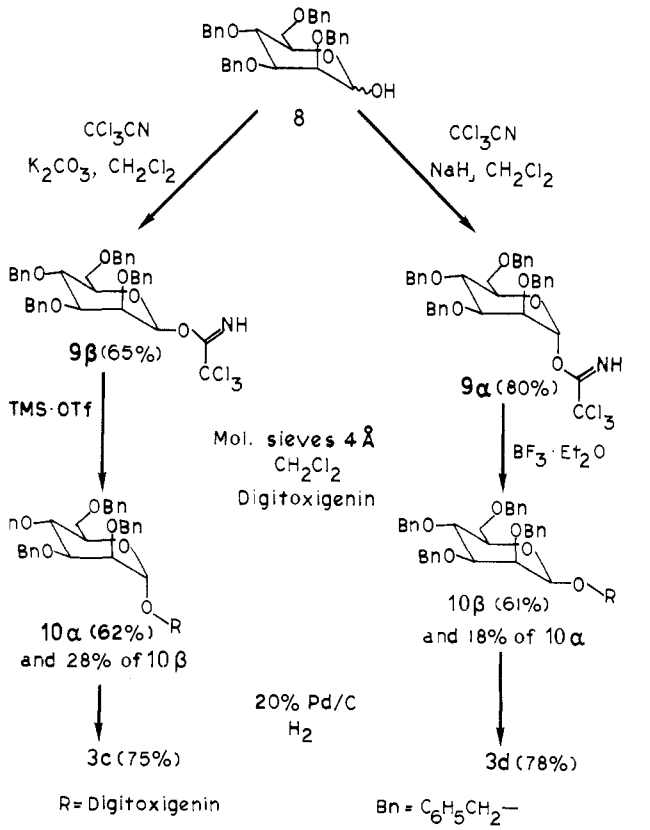

Thomas,[4] for example, found that digitoxigenin α-L-rhamnoside (**2a**) was much more active than the β-D-glucoside **4d**. However, as can be seen in Chart I, these sugars differ not only in stereochemistry but in structure as well. This raises the question whether the observed differences in biological activity were the result of stereochemical or structural differences—or both.

To approach this question, we developed a synthetic approach to stereoselectively make cardiac glycosides of all four possible stereochemistries.[7] The β-D-, α-D-, β-L-, and α-L-glucosides **4a–d** were used as representative models. In the present study, the four possible mannosides **3a–d** were also synthesized, along with α-L-rhamnoside **2a** and β-L-rhamnoside **2b**. Since mannose is the 2′-hydroxy epimer of glucose, and rhamnose is 6′-deoxymannose, there

**Scheme I**




**Scheme II**




was therefore a systematic and stepwise change of structure from **2a** to **3a** to **4a** and from **2b** to **3b** to **4b**. These 10 compounds were then evaluated for their ability to inhibit hog kidney $Na^+,K^+$-ATPase, a receptor system we have previously shown to be very useful in studying the mo-

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lecular pharmacology of cardioactive steroids.[8]

## Results and Discussion

**Chemistry.** In a preceding paper,[7] we reported that α-L- and α-D-glucosides **4a** and **4c** could be stereoselectively synthesized from their kinetically produced tetrabenzyl β-trichloroacetimidates. The β-L- and β-D-glucosides **4b** and **4d** were made from their thermodynamically produced tetrabenzyl α-trichloroacetimidates. With $BF_3$–$Et_2O$ or trimethylsilyl trifluoromethanesulfonate ($Me_3Si$-OTf) catalysis, glycosylation with digitoxigenin (**1**) proceeded in 3 h at 30 °C for the β-glucosides **4b** and **4d** and in 30 min for the α-glucosides **4a** and **4c**. (Halide ion catalysis[7,9] was slightly more stereoselective but required 14 days reaction and still resulted in much unreacted digitoxigenin.)

The trichloroacetimidate approach was repeated to make the α-L- and β-L-mannosides **3a** and **3b** as shown in Scheme I and the α-D- and β-D-mannosides **3c** and **3d** as shown in Scheme II. 2,3,4,6-Tetra-*O*-benzyl-L-mannopyranoside (**5**) was obtained in 90% overall yield by conventional procedures from L-mannose by methylation (anhydrous methanol, acid catalysis, 2 h reflux[10]), benzylation with sodium hydride and benzyl chloride in $Me_2SO$,[7,11] and demethylation (80% acetic acid and 1 N HCl, refluxing for 8 h). 2,3,4,6-Tetra-*O*-benzyl-D-mannopyranoside (**8**) was prepared analogously from D-mannose.

Reaction of **5** and **8** under kinetic conditions (anhydrous $K_2CO_3$, 6 h, room temperature) gave the β-trichloroacetimidates **6β** and **9β** in 86% and 65% yields. Reaction under thermodynamic conditions (NaH, 1 h, room temperature) gave the α-trichloroacetimidates **6α** and **9α** in 89% and 80% yields. With the imidates of the glucosides, $^1H$ NMR—and most important, the chemical shift of the C1′-H and $J_{1',2'}$—could easily distinguish the β-imidates from the α-imidates.[7] However, in the mannosides the 2′-OH is axial, so the dihedral angles between the C1′ and C2′ protons are similar and the C1′ protons for all the imidates appear at 6.35–6.38 ppm.

The α-trichloroacetimidates **6α** and **9α** react smoothly with digitoxigenin (**1**) with $BF_3$–$Et_2O$ at −20 °C for 2 h followed by 1 h at 0 °C. The isolated yields of the β-L- and β-D-tetrabenzylmannosides **7β** and **10β** after flash chromatography were 60–61%, with an additional 18–28% of the α-anomers **7α** and **10α**. With $Me_3Si$-OTf catalysis at −10 °C for 15–30 min, the α-L- and α-D-tetrabenzylmannosides **7α** and **10α** were obtained in similar yields with 20–30% of the anomeric **7β** and **10β**. The reaction periods, temperature, and ratio of catalyst to digitoxigenin were found to be very important, just as with the glucosides.[7]

$^{13}C$ NMR of C1′, C2′, C3′, and C5′ was very useful in characterization and differentiation of the α- vs. β-glucosides.[7] With the mannosides, the shifts for C3′ and C5′ were distinct for the α and β anomers as shown in Table I. Further, with both the glucosides and mannosides, the change in chemical shift of the C3, C2, and C1 carbons relative to digitoxigenin for the α and β anomers was also consistent. This observed change in chemical shift has been widely used to confirm the anomeric stereochemistry of glycosides.[12] The shifts were derived as $\Delta A$ = δ(digitoxigenin mannoside) − δ(digitoxigenin) and are shown in Table II. For the mannosides, the difference ($\Delta A$) of C3 of the β-mannosides vs. α-mannosides—about 2 ppm—is slightly less than with the glucosides—about 3 ppm.[7]

Debenzylation of the tetra-*O*-benzylmannosides **7α**, **7β**, **10α**, and **10β** to yield **3a**, **3b**, **3c**, and **3d** in 70–78% yield was achieved by catalytic hydrogenolysis over freshly prepared 20% palladium on charcoal (3:1 MeOH–EtOAc, room temperature, atmosphere pressure, 2 h). With L-glucosides, 40% palladium on charcoal was required,[7] reflecting perhaps a greater steric inaccessibility of the 2′ equatorial benzyl.

The α-L-rhamnoside **2a** and the β-L-rhamnoside **2b** were synthesized in the same way, starting from 2,3,4-tri-*O*-benzyl-α-L-rhamnose (**11**) via imidates **12β** and **12α** as shown in Scheme III. The $^{13}C$ NMR shifts for the two tribenzyl-L-rhamnosides (Table III) are consistent with those of the mannosides. A smaller difference in $\Delta A$ values (Table IV) of the β- vs. α-L-rhamnosides was noted than

**Table I.** $^{13}C$ Chemical Shifts of D-Tetrabenzylmannopyranosides, L-Tetrabenzylmannopyranosides, and Digitoxigenin[a]

| | 10β | 10α | 7β | 7α | digitoxigenin (1) |
|---|---|---|---|---|---|
| C-1′ | 99.79 | 95.79 | 99.89 | 95.81 | |
| C-2′ | 74.18 | 72.61 | 73.72 | 73.72 | |
| C-3′ | 77.11 | 73.17 | 77.08 | 73.02 | |
| C-4′ | 71.43 | 71.53 | 71.43 | 71.68 | |
| C-5′ | 78.72 | 75.39 | 78.07 | 75.39 | |
| C-6′ | 69.88 | 69.28 | 69.96 | 69.16 | |
| C-1 | 30.40 | 29.93 | 30.32 | 30.13 | 29.62 |
| C-2 | 29.77 | 29.54 | 29.69 | 29.34 | 27.85 |
| C-3 | 73.98 | 71.91 | 73.79 | 72.11 | 66.75 |
| C-4 | 35.18 | 35.03 | 35.23 | 34.92 | 33.28 |
| C-5 | 36.71 | 36.45 | 36.63 | 36.22 | 35.97 |
| C-6 | 26.70 | 26.31 | 26.49 | 26.40 | 26.47 |
| C-7 | 21.35 | 23.61 | 21.20 | 20.95 | 21.14 |
| C-8 | 41.80 | 41.63 | 41.83 | 41.45 | 41.73 |
| C-9 | 35.77 | 35.47 | 35.74 | 35.40 | 35.46 |
| C-10 | 35.26 | 35.19 | 33.10 | 32.81 | 35.37 |
| C-11 | 21.30 | 21.09 | 21.28 | 23.53 | 21.32 |
| C-12 | 39.12 | 39.85 | 40.02 | 39.69 | 39.99 |
| C-13 | 49.65 | 49.48 | 49.61 | 49.42 | 49.60 |
| C-14 | 85.49 | 85.32 | 85.51 | 85.12 | 85.45 |
| C-15 | 33.06 | 32.97 | 31.98 | 29.29 | 33.06 |
| C-16 | 26.92 | 26.72 | 29.88 | 26.66 | 26.88 |
| C-17 | 50.91 | 50.82 | 50.96 | 50.68 | 50.94 |
| C-18 | 15.76 | 15.63 | 15.78 | 15.53 | 15.76 |
| C-19 | 23.62 | 23.76 | 23.87 | 23.78 | 23.70 |
| C-20 | 174.85 | 174.69 | 174.57 | 174.78 | 174.94 |
| C-21 | 73.71 | 73.37 | 73.63 | 74.98 | 73.54 |
| C-22 | 117.55 | 117.44 | 117.03 | 117.23 | 117.52 |
| C-23 | 174.64 | 174.49 | 174.71 | 174.90 | 174.90 |

[a] In ppm downfield from $Me_4Si$ in $CDCl_3$.

**Table II.** $^{13}C$ Glycosidation Shifts ($\Delta A$, ppm; $\Delta\delta$ = δ(digitoxigenin mannoside) − δ(digitoxigenin))[a]

| | $C^\alpha$ (C3) ($\Delta\delta A$) | $C^\beta$ (C2) ($\Delta\delta A$) | $C^\lambda$ (C1) ($\Delta\delta A$) |
|---|---|---|---|
| **10β** | 73.98 (+7.23) | 29.77 (+1.92) | 30.40 (+0.78) |
| **10α** | 71.91 (+5.16) | 29.54 (+1.69) | 30.03 (+0.41) |
| **7β** | 73.79 (+7.04) | 29.69 (+1.84) | 30.32 (+0.70) |
| **7α** | 72.11 (+5.36) | 29.34 (+1.49) | 30.13 (+0.51) |

[a] Methods reported by Kasai et al.[12]

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Table III. $^{13}C$ Chemical Shifts ($\delta$) of the L-Tribenzylrhamnosides **2b**, **2a**, and Digitoxigenin (1)[a]

| | 2b | 2a | digitoxigenin (1) |
|---|---|---|---|
| C-1′ | 100.05 | 96.29 | |
| C-2′ | 73.71 | 72.50 | |
| C-3′ | 82.96 | 80.93 | |
| C-4′ | 71.76 | 71.82 | |
| C-5′ | 74.97 | 68.37 | |
| C-6′ | 18.12 | 18.06 | |
| C-1 | 30.48 | 30.29 | 29.62 |
| C-2 | 26.63 | 26.69 | 27.85 |
| C-3 | 73.23 | 71.84 | 66.75 |
| C-4 | 33.33 | 29.62 | 33.28 |
| C-5 | 36.79 | 36.49 | 35.97 |
| C-6 | 24.80 | 26.53 | 26.47 |
| C-7 | 21.35 | 21.22 | 21.14 |
| C-8 | 42.10 | 41.92 | 41.73 |
| C-9 | 35.97 | 35.78 | 35.46 |
| C-10 | 35.43 | 35.24 | 35.37 |
| C-11 | 21.46 | 21.42 | 21.32 |
| C-12 | 40.27 | 40.11 | 39.99 |
| C-13 | 49.82 | 49.70 | 49.60 |
| C-14 | 85.86 | 85.60 | 85.45 |
| C-15 | 32.19 | 33.21 | 33.06 |
| C-16 | 27.04 | 26.98 | 26.88 |
| C-17 | 51.25 | 51.05 | 50.94 |
| C-18 | 15.87 | 15.80 | 15.76 |
| C-19 | 23.97 | 23.79 | 23.70 |
| C-20 | 174.56 | 174.69 | 174.94 |
| C-21 | 73.71 | 74.16 | 73.54 |
| C-22 | 118.12 | 117.76 | 117.52 |
| C-23 | 175.17 | 174.85 | 174.90 |

[a] In ppm downfield from $Me_4Si$ in $CDCl_3$.

Table IV. $^{13}C$ Glycosidation Shifts ($\Delta A$, ppm; $\Delta A$ = $\delta$(digitoxigenin rhamnoside) − $\delta$(digitoxigenin))[a]

| | $C^{\alpha}$ (C3), ($\Delta\delta A$) | $C^{\beta}$ (C2) ($\Delta\delta A$) | $C^{\lambda}$ (C1) ($\Delta\delta A$) |
|---|---|---|---|
| β-L (**2b**) | 72.23 (+6.48) | 26.63 (−1.22) | 30.48 (+0.86) |
| α-L (**2a**) | 71.84 (+5.09) | 26.69 (−1.16) | 30.29 (+0.67) |

[a] Methods reported by Kasai et al.[12]

with the mannosides or glucosides.[7]

An additional method recently reported by Mukaiyama and co-workers[13] for synthesizing 1,2-trans glycosides was also investigated. They found that 3,5-dinitro-2-pyridyl-2,3,4-tetra-*O*-benzyl-α-D-glucopyranoside reacts with alcohols in the presence of anhydrous $ZnCl_2$ or $BF_3$–$Et_2O$. As also shown in Scheme III, reaction of **11** with 2-chloro-3,5-dinitropyridine in the presence of 2,6-lutidine, KF, and 18-crown-6 ether for 30 min resulted in a 90% yield of 1-*O*-(3,5-dinitro-2-pyridyl)-2,3,4-tri-*O*-benzyl-L-rhamnose (**13**). Reaction of **13** with digitoxigenin ($BF_3$–$Et_2O$ catalysis, −40 °C) followed by flash chromatography resulted in 44% of tribenzyl-β-L-rhamnoside **14β** and 11% of tribenzyl-α-L-rhamnoside (**14α**—yields very similar to those obtained via imidate **12α**). Catalysis with anhydrous $ZnCl_2$ at room temperature resulted in 14β-OH dehydration.

**Biology.** Hog kidney outer medulla $Na^+,K^+$-ATPase (EC. 3.6.1.3) was used as we have previously described[8] to determine the inhibitory activity of digitoxigenin, the glucosides **4a–d**, the four mannosides **3a–d**, and two rhamnosides **2a,b**. The resulting $I_{50}$ values (concentration required for 50% inhibition of the $Na^+,K^+$-ATPase under conditions of near-equilibrium binding of the drugs to the enzyme) are shown in Table V.

Table V. Hog Kidney $Na^+,K^+$-Dependent ATPase Inhibition

| analogue | $I_{50}$, M | approx rel act. |
|---|---|---|
| digitoxigenin (1) | $1.2 \times 10^{-7}$ | 1 |
| α-L-rhamnoside (**2a**) | $6.79 \times 10^{-9}$ | 18 |
| β-L-rhamnoside (**2b**) | $4.79 \times 10^{-9}$ | 25 |
| α-L-mannoside (**3a**) | $8.7 \times 10^{-8}$ | 1.4 |
| β-L-mannoside (**3b**) | $1.78 \times 10^{-8}$ | 7 |
| α-D-mannoside (**3c**) | $1.29 \times 10^{-7}$ | 1 |
| β-D-mannoside (**3d**) | $3.89 \times 10^{-8}$ | 3 |
| α-L-glucoside (**4a**) | $6.03 \times 10^{-8}$ | 2 |
| β-L-glucoside (**4b**) | $1.60 \times 10^{-8}$ | 7 |
| α-D-glucoside (**4c**) | $9.33 \times 10^{-8}$ | 1.1 |
| β-D-glucoside (**4d**) | $1.05 \times 10^{-8}$ | 11 |
| β-D-galactoside[a] | $6.45 \times 10^{-8}$ | 2 |
| β-D-digitoxoside[a] | $7.04 \times 10^{-9}$ | 17 |
| 6-hydroxy-β-D-digitoxoside[a] | $1.07 \times 10^{-8}$ | 11 |
| 2-deoxy-β-D-glucoside[a] | $2.82 \times 10^{-8}$ | 5 |

[a] Data from Fullerton et al.[3b]

## Discussion

Previous studies on the roles of sugar structure on the activity of cardiac glycosides have compared a limited number of glycosides with particular sugar stereochemistries.

For example, studies with β-D-glucosides, galactosides, and digitoxosides;[2-4,6] and with α-L-rhamnoside analogues[2,4-6] have suggested an important role for an equatorial 4′-OH and, to a lesser extent, an axial 3′-OH.

Removal of the 6′-OH improved activity in the one β-D-glycoside pair examined (β-D-digitoxoside vs. 6′-hydroxy-β-D-digitoxoside).[3] Brown and co-workers[4] made a similar observation after comparing the activities of digitoxigenin β-D-glucoside and galactoside with those of two α-L-glycosides lacking the 6′-OH (digitoxigenin α-L-rhamnoside and -thevetoside). They concluded that the 5′-$CH_3$ was probably assisting binding to the receptor (or alternatively, that the 6′-OH was interfering with binding).[4] Using gomphoside as a rigid model for conformational studies, Chiu and Watson[5] reached a similar conclusion about the role of the 5′-$CH_3$ in binding to the $Na^+,K^+$-ATPase receptor. Most importantly, they have proposed that the sugar binding region in the receptor has two major binding sites. One site was envisioned for the 5′-$CH_3$ and the other for any sugar OH that in an energetically feasible conformation can occupy the 3′-OH or 4′-OH of a glycoside with flexible C3–O–C1′ bonds.

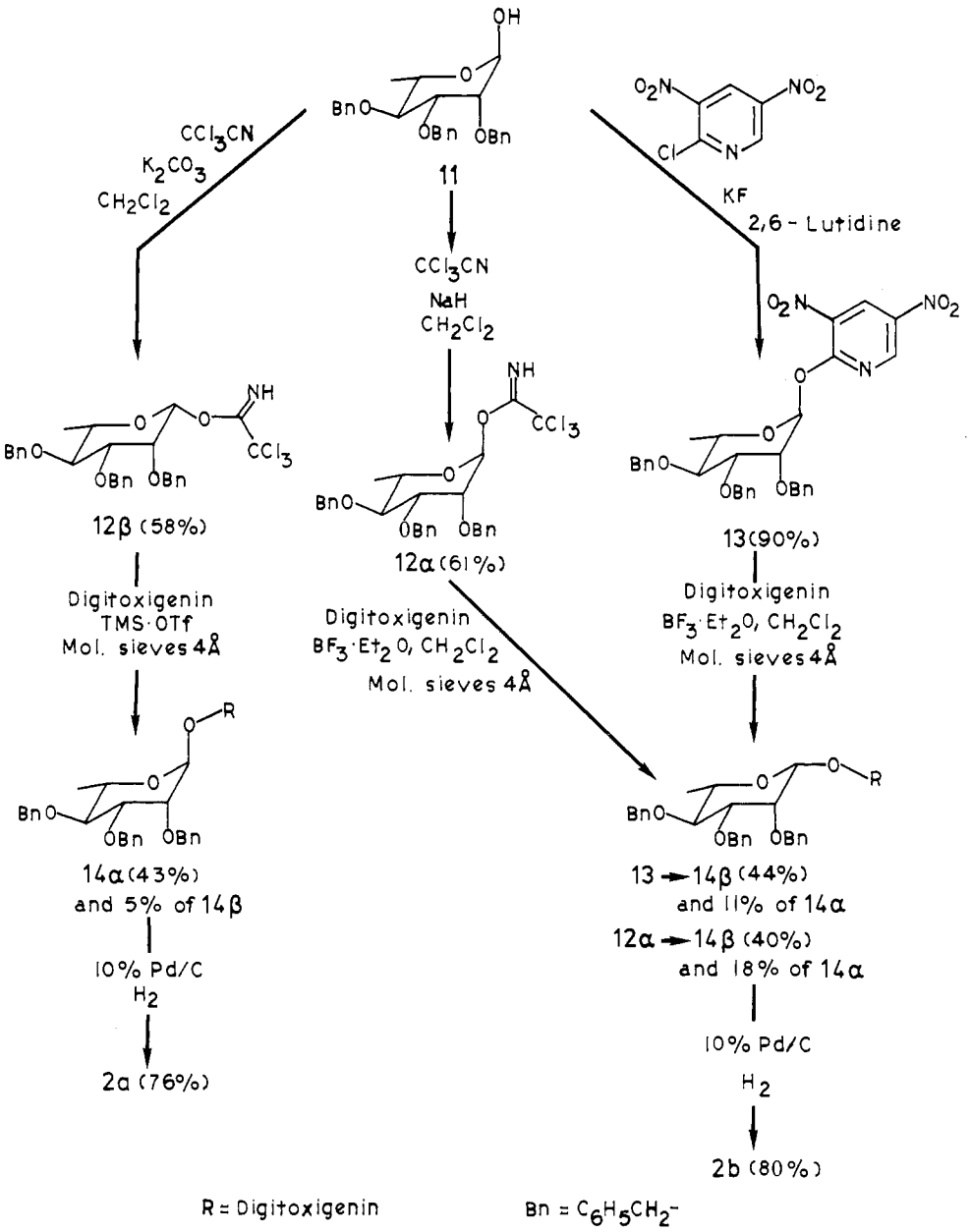
The role of the 2′-OH has also been studied with incomplete sets of glycosides. Kihara and co-workers[3] found that digitoxigenin β-D-glucoside (with 2′-OH equatorial) had about twice the activity of the 2′-deoxy-β-D-glucoside. In contrast, Brown and Thomas found that with α-L-rhamnosides (2′-OH axial) and α-L-thevetosides (2′-OH equatorial) the 2′-OH does not have an important role in binding. However, if the 2′-OH is blocked as an acetyl, stability of the drug–receptor complex was apparently reduced.[4]

The data in Table V are consistent with the following preliminary model for interaction of cardiac glycoside sugars with the $Na^+,K^+$-ATPase receptor.

**α-L-Glycosides.** Excellent binding is achieved with the 4′ equatorial OH and 5′-$CH_3$ of digitoxigenin α-L-rhamnoside (**2a**). (In studies with gomphoside and **2a**, Chiu and Watson[5] found that the 5′-$CH_3$'s could come within 1 Å of each other and the 4′-OH of **2a** and 3′-OH of gomphoside within 2.5 Å of each other.) Addition of the 6′-OH in α-L-mannoside (**3a**) dramatically disrupts hydrophobic binding at the "5′-$CH_3$ site". The significant

(13) (a) Mukaiyama, T.; Murai, Y.; Shoda, S. *Chem. Lett.* **1981**, 431–432. (b) Mukaiyama, T.; Shoda, S. *Ibid.* **1979**, 847–848.
(14) Still, W. C.; Kahn, M.; Mitra, A. *J. Org. Chem.* **1978**, *43*, 2923–2925.
(15) Glaudemans, C. P.; Fletcher, H. G., Jr. *Methods Carbohydr. Chem.* **1972**, *6*, 373–376.

**Scheme III**




drop in activity in **3a** would be consistent with (1) a resulting conformational change that moves the 4′-OH from a position of good binding to a position of poor binding and (2) the 2′-OH and 3′-OH groups having little or no role in receptor binding since α-L-mannoside (**3a**) and α-L-glucoside (**4a**) are only moderately more active than digitoxigenin. Changing the axial 2′-OH of **3a** to equatorial in α-L-glucoside (**4a**) has no effect at all, consistent with the previous observations with α-L-rhamnosides and -thevetosides.[4]

**β-L-Glycosides.** The good activity of β-L-rhamnoside **2b**, i.e. slightly better than α-L-rhamnoside **2a**, can be explained by (1) binding by the 4′-OH and 5′-$CH_3$ being about as good in β-L-rhamnoside **2b** as in α-L-rhamnoside **2a** or (2) binding by the 4′-OH, 3′-OH, and 2′-OH in **2b**, but the 4′-OH and 5′-$CH_3$ in **2a**. As with the α-L-glycosides, moving the 2′-OH from axial in **3b** to equatorial in **4b** has little effect with these β-L-glycosides.

**β-D-Glycosides.** Our previous studies with digitoxigenin β-D-digitoxoside and digitoxigenin 6′-hydroxy-β-D-digitoxoside seemed to show that the 5′-$CH_3$ may have little role in binding. A small drop in activity upon adding the 5′-OH was observed. It will be important to synthesize additional 6′-hydroxy-β-D-digitoxosides to confirm the observation. Moving the 3′-OH from axial in the 6′-hydroxy-β-D-digitoxoside to equatorial as in the 2′-deoxy-β-D-glucoside results in a reduction by half in the observed $I_{50}$ for $Na^+,K^+$-ATPase inhibition.[3] Addition of the equatorial 2′-OH to make β-D-glucoside **4d** restores activity to the same level as seen with the 6′-hydroxy-β-D-digitoxoside. Finally, moving the 2′-OH to axial in β-D-mannoside **3d** the activity is equivalent to not having any 2′-OH at all. Moving the 4′-OH from equatorial to axial (in β-D-galactose) significantly reduces activity. Thus, these results are consistent with a stereochemical preference for the 3′-OH being axial and the 4′-OH being equatorial for maximal binding of β-D sugars as we have previously proposed.[3b]

**α-D-Glycosides.** More α-D-glycosides certainly need to be examined. As can be seen in Table V, α-D-glucoside **4c** and α-D-mannoside **3c** are the least active of all the glycosides studied, being little more active than digitoxigenin itself.

Thus, the above preliminary binding model for α-L-glycosides is similar to the gomphoside model proposed by Chiu and Watson. However, an important difference

is that we believe that the 5′-$CH_3$ may not be significantly involved with binding of D-sugar glycosides. Additional glycosides, including 2′,3′-dideoxyrhamnosides, have been synthesized to further test both models.[16] X-ray crystallographic and conformational energy studies are in progress to answer two fundamental questions: (1) are the allowed conformations for the D-glycosides dissimilar from those of the L-glycosides; (2) can the 4′-OH groups occupy similar positions for all glycoside types (α-L, β-L, α-D, β-D).

## Experimental Section

Melting points were determined with a Thomas-Hoover melting point apparatus and are uncorrected. Elemental analyses were performed by MHW Laboratories, Phoenix, AZ. The 80-MHz $^1H$ NMR and 400-MHz $^1H$ and $^{13}C$ spectra were taken at the Oregon State University NMR Spectroscopy Laboratory, Department of Chemistry. The 400-MHz spectra were taken on a Bruker AM 400 spectrometer. IR spectra were run as KBr pellets on a Beckman Model Acculab 7 spectrophotometer. Optical rotations in methanol were taken on a Perkin-Elmer 141 polarimeter. Thin-layer chromatography was performed on 0.25-mm EM silica gel 60 F-254 glass plates by techniques and solvent combinations as we have previously described.[1,3,7] Flash chromatography[14] as we have previously reported[3] used silica gel 60, 230–400 mesh (EM Merck), in a column 4 cm in diameter and 20 cm in height, with a combination of $CH_2Cl_2$ and EtOAc (10:1) as solvent.

**2,3,4,6-Tetra-*O*-benzyl-α,β-L-mannoside (5).** Methyl-L-mannopyranoside was prepared from L-mannopyranoside (mixed anomers) following the method of Levene and Muskat:[10] $^1H$ NMR ($D_2O$) δ 3.8 (3 H, s, OMe). Benzylation using NaH–benzyl chloride–$Me_2SO$ at room temperature, following the method of Iwashige and Saeki[11] gave methyltetrabenzyl-L-mannopyranoside in almost quantitative yield. It was hydrolyzed[15] by 80% HOAc and $H_2SO_4$ at reflux for 10 h. After usual workup and chromatographic purification, 2,3,4,6-tetra-*O*-benzyl-L-mannopyranose (**5**) was obtained as an oil (90% overall): $[\alpha]^{20}_D$ −21.14° (*c* 0.1561, $CHCl_3$); $^1H$ NMR ($CDCl_3$) δ 7.3 (20 H, m, Ph), 4.58 (1 H, d, *J* = 3.5 Hz, C-1′ H), 4.5 (8 H, d, *J* = 7 Hz, $CH_2Ph$).

**2,3,4,6-Tetra-*O*-benzyl-α-L-mannopyranose 1-*O*-Trichloroacetimidate (6α) (Scheme I).** To a stirred suspension of NaH (0.2 g, 5.88 mmol) in $CH_2Cl_2$ (10 mL) was added a solution of **5** (3.5 g, 6.5 mmol) in $CH_2Cl_2$ (50 mL) at room temperature. The suspension was stirred for 10 min, and then trichloroacetonitrile (8 mL) was added dropwise. The resultant mixture was stirred for 1 h and filtered through Celite. Evaporation of the filtrate gave a crude oil (4.05 g, 89%), which was used without further purification: $^1H$ NMR ($CDCl_3$) δ 8.5 (1 H, s, C=NH), 7.25 (20 H, m, Ph), 6.3 (1 H, d, C-1 H), 4.8 (8 H, m, $CH_2Ph$).

**(3β,5β,14β,17β)-3-[(2,3,4,6-Tetra-*O*-benzyl-β-L-mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (7β).** To a stirred solution of digitoxigenin (1.5 g, 4 mmol) in $CH_2Cl_2$ (100 mL) was added 4-Å molecular sieves and a solution of **6α** (4.05 g, 5.97 mmol) in $CH_2Cl_2$ (50 mL). The mixture was stirred at 22 °C for 10 min and then was cooled to −20 °C. A solution of $BF_3$–$Et_2O$ (0.3 mL in 1 mL of $CH_2Cl_2$) was allowed to stir for 2 h at −20 °C and then poured in ice $H_2O$ (200 mL). The aqueous layer was extracted with $CH_2Cl_2$ (200 mL × 2). The organic layer was washed with $H_2O$ (200 mL), with 5% $NaHCO_3$ (100 mL), and again with $H_2O$ (100 mL). The $CH_2Cl_2$ extract was dried ($MgSO_4$) and then rotaevaporated to get crude oil (5.55 g). The crude oil was purified by flash chromatography ($SiO_2$, 10% $Et_2O$–$CH_2Cl_2$) to afford 1.23 g (61%) of **7β** as an homogeneous oil: $^1H$ NMR ($CDCl_3$) δ 7.2 (20 H, m, Ph), 5.82 (1 H, br s, C-22 H), 5.0 (2 H, d, *J* = 18, 1.5 Hz, C-21 H), 4.6 (8 H, m, $CH_2Ph$), 4.2 (1 H, br s, C-1′ H), 0.98 (6 H, s, C-18 and C-19 $CH_3$); $^{13}C$ NMR ($CDCl_3$) δ 99.98 (C-1′), 78.66 (C-2′), 82.69 (C-3′), 75.92 (C-4′), 75.06 (C-5′), 71.43 (C-3).

In addition, 0.525 g (28%) of α-L anomer (**7α**) (spectral data of **7α** are identical with those reported below) was also obtained.

**(3β,5β,14β,17β)-3-[(β-L-Mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (3b).** In 50 mL of EtOAc was dissolved 0.05 g (0.72 mmol) of **7β**. The solution was hydrogenated over 20% Pd/C, freshly prepared by activating 1.0 g of charcoal with 24 mL of 5% $PdCl_2$ in MeOH (50 mL). The activated charcoal was transferred with MeOH (30 mL) to the solution of **7β** at ambient temperature and atmospheric pressure. The theoretical amount of $H_2$ was absorbed in 2 h. The catalyst was removed by filtration, washed with MeOH, and then evaporated to afford 0.278 g (71%) of **3b** after purification with short-column chromatography ($SiO_2$, 7.5% EtOH–$CH_2Cl_2$): mp 250–251 °C (EtOH–EtOAc); $[\alpha]^{20}_D$ +38.16° (*c* 0.126, MeOH); UV $\lambda_{max}$ 217 nm (log ε 4.18); IR (KBr) $\gamma_{max}$ 3500 (OH), 2900 (CH), 1720 (C=O), 1615 (C=C) $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$) δ 5.0 (1 H, br s, C-22 H), 5.01 (2 H, d, *J* = 18, 2 Hz, C-21 H), 4.2 (1 H, br s, C-1′ H), 1.01 (3 H, s, C-18 $CH_3$), 0.85 (3 H, s, C-19 $CH_3$). Anal. ($C_{29}H_{44}O_9 \cdot H_2O$) C, H.

**2,3,4,6-Tetra-*O*-benzyl-β-L-mannopyranose 1-*O*-Trichloroacetimidate (6β).** To a suspension of anhydrous $K_2CO_3$ (2.0 g) in $CH_2Cl_2$ (10 mL) was added a solution of **5** (1.8 g, 3.33 mmol) at room temperature. The mixture was stirred at room temperature for 10 min, and then trichloroacetonitrile (2.0 mL) was added dropwise. The resultant mixture was stirred for an additional 8 h at 22 °C and then filtered through Celite. The filtrate was rotaevaporated to get homogeneous oil: yield 1.9 g (86%) of **6β**; $^1H$ NMR ($CDCl_3$) δ 8.5 (1 H, s, C=NH), 7.3 (20 H, m, Ph), 6.35 (1 H, d, *J* = 2 Hz, C-1′ H), 4.7 (8 H, m, $CH_2Ph$).

**(3β,5β,14β,17β)-3-[(2,3,4,6-Tetra-*O*-benzyl-α-L-mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (7α).** To a stirred solution of digitoxigenin (1.24 g, 3.33 mmol) in $CH_2Cl_2$ (50 mL) was added 4-Å molecular sieves and a solution of **6β** (1.9 g, 2.8 mmol) in $CH_2Cl_2$ (20 mL). The mixture was stirred at 22 °C for 10 min and then was cooled to −10 °C. $Me_3Si$-OTf (0.10 mL) was added dropwise over a period of 5 min. The reaction mixture was allowed to stir for an additional 30 min and then was poured in ice-cold $H_2O$ (200 mL) containing 50 mL of 5% $NaHCO_3$. The organic layer was evaporated to get crude oil that was purified by flash chromatography ($SiO_2$, 10% $Et_2O$–$CH_2Cl_2$) to afford 0.980 g (59%) of **7α** as a homogeneous oil: $^1H$ NMR ($CDCl_3$) δ 7.25 (20 H, m, Ph), 5.85 (1 H, br s, C-22 H), 4.9 (2 H, d, *J* = 18, 2 Hz, C-21 H), 4.6 (8 H, m, $CH_2Ph$), 4.15 (1 H, br s, C-1′ H), 0.95 (6 H, s, C-18 and C-19 $CH_3$); $^{13}C$ NMR ($CDCl_3$) δ 95.81 (C-1′), 73.35 (C-2′), 73.02 (C-3′), 71.68 (C-4′), 75.39 (C-5′), 72.11 (C-3).

In addition, 0.58 g (30%) of β-L anomer **7β** (spectral data are identical with those reported for **7β** above) and digitoxigenin (0.480 g) were also recovered.

**(3β,5β,14β,17β)-3-[(α-L-Mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (3a).** In 50 mL of EtOAc was dissolved 0.65 g (0.72 mmol) of **7α**. The solution was hydrogenated over 20% Pd/C, freshly prepared by activating 1.0 g of charcoal with 24 mL of 5% $PdCl_2$ in MeOH (50 mL). The activated charcoal was transferred with 30 mL of MeOH to the solution of **7α** at room temperature and atmospheric pressure. The theoretical amount of $H_2$ was absorbed in 3 h. The catalyst was removed by filtration, washed with MeOH, and then evaporated to afford 0.284 g (73%) of **3a** after purification with short-column chromatography ($SiO_2$, 10% EtOH–$CH_2Cl_2$): mp 242–245 °C; $[\alpha]^{20}_D$ −33.4° (*c* 0.096, MeOH); UV $\lambda_{max}$ 217 nm (log ε 4.20); IR (KBr) $\gamma_{max}$ 3500 (OH), 1730 (C=O), 1610 (C=C) $cm^{-1}$; $^1H$ NMR (MeOH-$d_4$) δ 5.82 (1 H, br s, C-22 H), 4.98 (2 H, d, *J* = 18, 1.5 Hz, C-21 H), 4.0 (1 H, br s, C-1′ H), 0.95 (3 H, s, C-18 $CH_3$), 0.89 (3 H, s, C-19 $CH_3$). Anal. ($C_{29}H_{44}O_9 \cdot 1.5H_2O$) C, H.

**2,3,4,6-Tetra-*O*-benzyl-α,β-D-mannopyranose (8).** Methyl-α,β-D-mannopyranoside was prepared from D-mannose following the method of Levene and Muskat[10] as white crystalline (granular) solid: yield 80%; mp 188–190 °C; $^1H$ NMR ($D_2O$) δ 3.85 (3 H, s, OMe).

The methyl-D-mannoside was benzylated following the method of Iwashige and Saeki[11] using NaH–benzyl chloride–$Me_2SO$ at room temperature for 10 h. After the usual workup the crude benzylated product was purified by column chromatography ($SiO_2$, 5% EtOAc–$C_6H_6$). The pure tetrabenzylmethyl-D-mannopyranoside was hydrolyzed by 80% HOAc–2 N $H_2SO_4$ at 90 °C for 8 h. After the usual workup 2,3,4,6-tetra-*O*-benzyl-D-mannopyranose (**8**) was obtained as a homogeneous oil (60%

(16) Fullerton, D. S.; Rathore, H.; Tjaharyanto, D.; From, A. H. L.; Griffin, J. F.; Ahmed, K. Paper presented at the 191st Annual Meeting of the American Chemical Society, New York, NY, April 1986; American Chemical Society: Washington, DC, 1986; MEDI 69.

overall): $^1$H NMR ($CDCl_3$) δ 7.2 (20 H, m, Ph), 4.7 (1 H, d, $J$ = 8 Hz, C-1′ H), 4.5 (8 H, d, $J$ = 7 Hz, $CH_2Ph$).

**2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranose 1-*O*-Trichloroacetimidate (9α).** To a stirred suspension of NaH (0.033 g, 1.25 mmol) in $CH_2Cl_2$ (10 mL) was added a solution of **8** (1.0 g, 1.85 mmol) in $CH_2Cl_2$ (10 mL) at room temperature. The suspension was stirred for 10 min, and then trichloroacetonitrile (1.0 mL) was added dropwise. The resultant mixture was stirred for 1 h at room temperature and then filtered through Celite. Evaporation of filtrate gave a crude oil that was used without further purification: yield 0.84 g (80%) of **9α**; $^1$H NMR ($CDCl_3$) δ 8.55 (1 H, s, C=NH), 7.25 (20 H, m, Ph), 6.35 (1 H, d, C-1′ H), 4.7 (8 H, m, $CH_2Ph$).

**(3β,5β,14β,17β)-3-[(2,3,4,6-Tetra-*O*-benzyl-β-D-mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (10β).** To a stirred mixture of digitoxigenin (1.6 g, 4.34 mmol) in $CH_2Cl_2$ (90 mL) was added a solution of **9α** (3.864 g, 5.64 mmol) in $CH_2Cl_2$ (20 mL). The mixture was stirred at room temperature for 10 min and was then cooled to −20 °C. A solution of $BF_3$–$Et_2O$ (0.10 mL) in $CH_2Cl_2$ (1 mL) was then added dropwise. The mixture was stirred at −20 °C for 2 h and then allowed to warm at room temperature (1 h). The reaction mixture was poured into a mixture of 5% $NaHCO_3$–ice-cold $H_2O$ (200 mL each). The quenched reaction mixture was extracted with $CH_2Cl_2$ (100 mL × 2). The $CH_2Cl_2$ extract was washed with $H_2O$ (100 mL), dried ($MgSO_4$), and evaporated to get an oil (4.492 g, 87%). The crude oil was purified via flash chromatography ($SiO_2$, eluent 7.5% $Et_2O$–$CH_2Cl_2$) to yield 3.2 g (61%) of **10β**: $^1$H NMR ($CDCl_3$) δ 7.3 (20 H, m, Ph) 5.9 (1 H, s, C-22 H), 4.95 (2 H, dd, J = 18, 2 H, C-21 H), 4.0 (1 H, br s, C-3 H), 0.98 (3 H, s, C-18 $CH_3$), 0.89 (3 H, s, C-19 $CH_3$); $^{13}$C NMR ($CDCl_3$) δ 99.79 (C-1′), 74.18 (C-2′), 77.11 (C-3′), 71.43 (C-4′), 78.22 (C-5′).

In addition 0.92 (18%) of α-D anomer **10α** (spectral data are identical with those reported for **10α** below) and digitoxigenin (0.1 g) were also recovered.

**(3β,5β,14β,17β)-3-[(β-D-Mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (3d).** In 250 mL of EtOAc was dissolved 3.0 g (3.285 mmol) of **10β**. The solution was hydrogenated over 20% Pd/C, freshly prepared by activating 4.6 g of charcoal with 55 mL of 5% $PdCl_2$ in MeOH (250 mL). The activated charcoal was transferred with 300 mL of MeOH to the solution of **10β** at room temperature and atmospheric pressure. The theoretical amount of $H_2$ was absorbed in 2 h. The catalyst was removed by filtration, washed with MeOH, and then rotaevaporated to afford 1.52 g (78%) of pure **3d** after purification with short-column chromatography ($SiO_2$, 10% EtOH–$CH_2Cl_2$): mp 274–276 °C dec; $[\alpha]^{20}_D$ −8.93 (*c* 0.0896, MeOH); UV $\lambda_{max}$ (MeOH) 217 nm (log ε 4.2); IR (KBr) 3490 (OH), 2900 (CH), 1740 (C=CC=O), 1620 (C=C) cm$^{-1}$; $^1$H NMR (MeOH-$d_4$) δ 5.85 (1 H, s, C-22 H), 5.0 (2 H, dd, $J$ = 18, 2 H, C-21 H), 4.15 (1 H, d, $J$ = 3.5 Hz, C-1′ H), 0.98 (3 H, s, C-18 $CH_3$), 0.89 (3 H, s, C-19 $CH_3$). Anal. ($C_{29}H_{44}O_9 \cdot H_2O$) C, H.

**2,3,4,6-Tetra-*O*-benzyl-β-D-mannopyranose 1-*O*-Trichloroacetimidate (9β).** To a stirred suspension of anhydrous potassium carbonate (3.0 g, 28 mmol) in $CH_2Cl_2$ (10 mL) was added a solution of **8** (2.7 g, 5 mmol) at room temperature. The suspension was stirred for 30 min at room temperature, and then trichloroacetonitrile (3.0 mL) was added dropwise. The resultant mixture was allowed to stir at room temperature for 6 h and then filtered through Celite. The filtrate was evaporated to give a crude oil. This crude oil was used without further purification: yield 3.342 g (65%) of **9β**; $^1$H NMR ($CDCl_3$) δ 8.54 (1 H, s, C=NH), 7.28 (20 H, m, Ph), 6.38 (1 H, d, $J$ = 3 Hz, C-1′), 4.72 (8 H, m, $CH_2Ph$).

**(3β,5β,14β,17β)-3β-[(2,3,4,6-Tetra-*O*-benzyl-α-D-mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (10α).** To a stirred mixture of digitoxigenin (2.4 g, 7.31 mmol) and molecular sieves (4 Å) in $CH_2Cl_2$ (70 mL) was added a solution of **9β** (3.342 g, 4.9 mmol) in $CH_2Cl_2$ (20 mL) at −10 °C. This resultant mixture was stirred for 10 min after which $Me_3Si$-OTf (0.33 mL, 1.6 mmol) was added slowly. The resultant solution was allowed to stir for 15 min at −10 °C and then at 0 °C for 10 min. The reaction mixture was added to a mixture of 5% $NaHCO_3$ (200 mL) and cold $H_2O$ (200 mL). The quenched reaction mixture was extracted with $CH_2Cl_2$ (100 mL × 2). The combined extract was washed twice with 100-mL portions of $H_2O$ and then dried ($MgSO_4$). Evaporation of the solvent gave crude oil (4.998 g, 97%). The crude oil was purified by flash chromatography ($SiO_2$, eluent 7.5% $Et_2O$–$CH_2Cl_2$) to give 3.194 g (62%) of **10α** as a homogeneous oil: $^1$H NMR ($CDCl_3$), δ 7.3 (20 H, m, Ph), 5.9 (1 H, s, C-22 H), 4.95 (2 H, dd, $J$ = 18.2 Hz, C-21 H), 3.8 (1 H, br s, C-3 H), 0.95 (3 H, s, C-18 $CH_3$), 0.92 (3 H, s, C-19 $CH_3$); $^{13}$C NMR ($CDCl_3$) δ 95.79 (C-1′), 72.61 (C-2′), 73.17 (C-3′), 71.53 (C-4′), 75.39 (C-5′), 71.91 (C-3).

In addition, 1.44 g (28%) of β-D anomer (**10β**) was also obtained (spectral data of **10β** are identical with those reported above).

**(3β,5β,14β,17β)-3-[(α-D-Mannopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (3c).** In 200 mL EtOAc was dissolved 3.0 g (3.285 mmol) of **10α**. The solution was hydrogenated over 20% Pd/C, freshly prepared by activating 4.6 g of charcoal with 55 mL of 5% $PdCl_2$ in 250 mL of MeOH. The activated charcoal was transferred with 300 mL of MeOH to the solution of **10α** at room temperature and atmospheric pressure. The theoretical amount of $H_2$ was absorbed in 2 h. The catalyst was removed by filtration, washed with MeOH, and then evaporated to afford 1.39 g (75%) of pure **3c** after purification with short-column chromatography ($SiO_2$, 10% EtOH–$CH_2Cl_2$): mp 250–252 °C; $[\alpha]^{23}_D$ +28.57° (*c* 0.1365, MeOH); UV $\lambda_{max}$ (MeOH) 217 nm (log ε 4.18); IR (KBr) 3500 (OH), 2945 (CH), 1750 (C=O), 1615 (C=C) cm$^{-1}$; $^1$H NMR (MeOH-$d_4$) δ 5.85 (1 H, s, C-22 H), 5.0 (2 H, dd, $J$ = 18, 2 Hz, C-21 H), 4.25 (1 H, d, $J$ = 3.5 Hz, C-1′ H), 0.98 (6 H, s, C-18 and C-19 $CH_3$). Anal. ($C_{29}H_{44}O_9$) C, H.

**2,3,4-Tri-*O*-benzyl-α-L-rhamnopyranose (11).** Methyl-α-L-rhamnopyranoside was prepared from α-L-rhamnose following the method of Levene and Muskat[10] as an oil. The methyl-α-L-rhamnopyranoside was benzylated following the method of Iwashige and Saeki[11] using KOH–benzyl chloride–DMF at room temperature for 7 h. After the usual workup the crude benzylated sugar was purified by column chromatography on silica gel (5% EtOAc in $C_6H_6$). The pure benzylated methyl rhamnoside was hydrolyzed with 80% HOAc–2 N HCl at 90 °C for 5 h. After the usual workup, the oil so obtained was crystallized by trituration with petroleum $Et_2O$ (30–60 °C). The crude crystalline solid was recrystallized with $Et_2O$–petroleum $Et_2O$ to get white needles of **11**: yield 85%; mp 89–90 °C; $^1$H NMR ($CDCl_3$) δ 7.3 (15 H, s, Ph) 5.0 (1 H, d, $J$ = 4 Hz, C-1′ H), 4.6 (6 H, m, $CH_2Ph$), 1.3 (3 H, d, $J$ = 6 Hz, C-5′ $CH_3$). Anal. ($C_{27}H_{30}O_5$) C, H.

**3,5-Dinitro-2-pyridyl-2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose (13).** To a stirred mixture of 2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose (**11**; 2.2 g, 5 mmol), anhydrous potassium fluoride (1.0 g), 18-crown-6 ether (2.6 g), and 2,6-lutidine (1.75 mL) in $CH_2Cl_2$ (30 mL) was added dropwise a solution of 2-chloro-3,5-dinitropyridine (1.22 g, 6.0 mmol) in $CH_2Cl_2$ (10 mL). The resultant deep red solution was stirred for 30 min at room temperature. The reaction mixture was then evaporated to dryness. The residue was dissolved in a 1:1 mixture of benzene and $CH_2Cl_2$ and flash chromatographed ($SiO_2$, eluent $C_6H_6$–*n*-hexane) to get pure **13** as a yellow oil: yield 2.9 g (90%); IR (NaCl) $\gamma_{max}$ 1608 (Ph), 1536 and 1340 ($NO_2$) cm$^{-1}$; $^1$H NMR ($CDCl_3$) δ 9.1 (1 H, d, $J$ = 4.5 Hz, ring proton H4), 8.9 (1 H, d, $J$ = 4 Hz, ring proton H6), 7.3 (15 H, br s, Ph), 6.5 (1 H, d, $J$ = 3.5 Hz, C-1′ H), 4.9 (6 H, m, $CH_2Ph$).

**2,3,4-Tri-*O*-benzyl-α-L-rhamnopyranose 1-*O*-Trichloroacetimidate (12α).** To a stirred suspension of NaH (0.18 g, 6.25 mmol) in $CH_2Cl_2$ (25 mL) was added a solution of **11** (2.2 g, 5.0 mmol) in $CH_2Cl_2$ (25 mL) at room temperature. The suspension was stirred for 15 min, and then trichloroacetonitrile (3.0 mL) was slowly added. The resultant mixture was stirred for 3 h at room temperature and filtered through Celite. Evaporation of the filtrate gave a crude oil that was purified rapidly via flash chromatography over silica gel (elution with 15% $Et_2O$–petroleum ether 30–60 °C), to yield 1.735 g (61%) of **12α** as an oil: IR (NaCl) 1670 (C=N), 3325 (NH) cm$^{-1}$; $^1$H NMR δ 6.24 (1 H, d, $J_{1,2}$ = 1.3 Hz, C-1′ H), 8.5 (1 H, s, C=NH), 1.35 (3 H, d, $J$ = 6.8 Hz, C-5′ $CH_3$).

**(3β,5β,14β,17β)-3-(2,3,4,6-Tri-*O*-benzyl-β-L-rhamnopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (14β). (a) From Dinitropyridyl Derivative 13.** 3,5-Dinitro-2-pyridyl-2,3,4-tri-*O*-benzyl-α-L-rhamnopyranose (**13**; 2.643 g, 4.4 mmol) was dissolved in $CH_2Cl_2$ (30 mL) at room temperature. To this was added a solution of digitoxigenin (1.1 g, 2.94 mmol) in $CH_2Cl_2$ (90 mL) slowly. The mixture was cooled to −40 °C, and then a

solution of $BF_3$·$Et_2O$ (0.3 mL) in $CH_2Cl_2$ (1 mL) was added dropwise. The mixture was allowed to stir for 20 min and then was poured in cold 10% $NaHCO_3$ solution (250 mL). The alkaline aqueous layer was extracted with $CH_2Cl_2$ (100 mL × 2). The $CH_2Cl_2$ extract was dried ($MgSO_4$), filtered, and then evaporated to produce an oil (1.635 g, 58.86%). The oil was purified by flash chromatography ($SiO_2$, eluent 5% $Et_2O$ in $CH_2Cl_2$) to yield 1.026 g (44%) of **14β** as an oil: $^1H$ NMR ($CDCl_3$) δ 7.25 (15 H, m, Ph), 5.82 (1 H, br s, C-22 H), 4.85 and 5.1 (3 H, dd, $J$ = 18, 1.5 Hz, C-21 and C-20 H), 4.65 (6 H, d, $J$ = 9 Hz, $CH_2Ph$), 3.7 (1 H, m, C-3 H), 1.29 (3 H, d, $J$ = 6 Hz, C-5′ $CH_3$), 0.80 and 0.85 (6 H, s, C-18 and C-19 $CH_3$); $^{13}C$ NMR ($CDCl_3$) δ 100.05 (C-1′), 82.97 (C-3′), 74.16 (C-2′), 74.95 (C-5′), 71.76 (C-4′), 18.13 (C-6 $CH_3$), 72.23 (C-3).

In addition, 0.262 g (11%) of α-L anomer **14α** (spectral data were identical with those reported for **14α** below) and digitoxigenin (0.25 g, 0.53 mmol) were also recovered.

**(b) From Imidate 12α.** To a stirred mixture of digitoxigenin (0.85 g, 2.26 mmol) and 4-Å molecular sieves in $CH_2Cl_2$ (80 mL) was added a solution of **12α** (1.7 g, 2.64 mmol) in $CH_2Cl_2$ (20 mL) at −30 °C. The solution was stirred for 10 min at −30 °C after which $BF_3$–$Et_2O$ (0.096 g, 0.083 mL, 0.67 mmol) in $CH_2Cl_2$ (1.0 mL) was slowly added. The resulting solution was allowed to stir at −30 °C for 1 h and then added to ice $H_2O$ (200 mL). The quenched reaction was extracted with $CH_2Cl_2$ (2 × 100 mL). The combined extract was washed twice with 50-mL portions of 5% aqueous $NaHCO_3$ and once with 100 mL of $H_2O$, dried ($MgSO_4$), and concentrated to an oil. This crude oil was purified via flash chromatography on silica gel (1:10 $Et_2O$–$CH_2Cl_2$) to yield 0.713 g (40%) of **14β** as an amorphous powder. The spectral data were identical with those reported for **14β** above.

In addition, 0.31 g (18%) of α-L anomer **14α** (spectral data identical with those reported for **14α** below) and 0.20 g (0.52 mmol) of digitoxigenin were also recovered.

**(3β,5β,14β,17β)-3-[(β-L-Rhamnopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (2b).** In 40 mL of EtOAc was dissolved 1.73 g (2.12 mmol) of **14β**. The solution was hydrogenated over 10% Pd/C, freshly prepared by activating 2.0 g of charcoal with 12.0 mL of 5% $PdCl_2$ in 150 mL of MeOH. The activated charcoal was transferred with 100 mL of MeOH to the solution of **14β** at room temperature and atmospheric pressure. The theoretical amount of $H_2$ was absrobed in 1 h. The catalyst was removed by filtration and washed with MeOH and the solvent evaporated to afford 0.905 (80%) of pure **2b** after short-column chromatography ($SiO_2$, 10% EtOH–$CH_2Cl_2$): mp 225–226 °C; UV $\lambda_{max}$ (MeOH) 217 nm (log ε 4.22); IR (KBr) $\gamma_{max}$ 3500 (OH), 1720 (C=O); $^1H$ NMR (MeOH-$d_4$) δ 5.82 (1 H, br s, C-22 H), 4.9 (2 H, qd $J_{21,21'}$ = 18 Hz, $J_{21,22}$ = 2 Hz, C-21 H); 4.3 (1 H, d, $J_{1',2'}$ = 3.5 Hz, C-1′ H), 4.04 (1 H, m, C-3 H), 1.25 (3 H, d, $J$ = 6.5 Hz, C-5′ $CH_3$), 0.89 (3 H, s, C-19 $CH_3$), 0.82 (3 H, s, C-18 $CH_3$); $[\alpha]^{23}_D$ +44.379° (c 0.055, MeOH). Anal. ($C_{29}H_{44}O_8$) C, H.

**2,3,4-Tri-O-benzyl-β-L-rhamnopyranose 1-O-Trichloroacetimidate (12β).** To a stirred suspension of anhydrous potassium carbonate (3.0 g, 29 mmol) in $CH_2Cl_2$ (20 mL) was added a solution of **11** (2.7 g, 5.55 mmol) in $CH_2Cl_2$ (10 mL) at room temperature. The suspension was stirred for 20 min, and then trichloroacetonitrile (3.0 mL) was slowly added. The resultant mixture was stirred for 6 h at room temperature and filtered through Celite. Evaporation of the filtrate gave a crude oil that was purified rapidly via flash chromatography over silica gel (10% $Et_2O$–*n*-hexane) to yield 1.925 g (58%) of **12β** as an oil: IR (NaCl) 1670 (C=N), 3325 (NH) $cm^{-1}$; $^1H$ NMR ($CDCl_3$) δ 8.50 (1 H, s, C=NH), 6.24 (1 H, d, $J_{1',2'}$ = 1.3 Hz, C-1′ H), 1.35 (3 H, d, $J$ = 6.8 Hz, C-5′ $CH_3$).

**(3β,5β,14β,17β)-3-[(2,3,4-Tri-O-benzyl-α-L-rhamnopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (14α).** To a stirred mixture of digitoxigenin (0.85 g, 2.27 mmol) and 4-Å molecular sieves in $CH_2Cl_2$ (80 mL) was added a solution of **12β** (1.8 g, 3.0 mmol) in $CH_2Cl_2$ (20 mL) at −10 °C. The solution was stirred for 15 min after which $Me_3Si$-OTf (0.3 mL, 1.4 mmol) in $CH_2Cl_2$ (2 mL) was slowly added. The resulting solution was allowed to stir at −10 °C for 15 min and at 0 °C for an additional 15 min. The reaction mixture was added to ice $H_2O$ (200 mL). The quenched reaction mixture was extracted with $CH_2Cl_2$ (2 × 100 mL). The combined extract was washed twice with 50-mL portions of 5% aqueous $NaHCO_3$ and once with 100 mL of $H_2O$, dried over $MgSO_4$, and concentrated to an oil. The crude oil was purified by flash chromatography on silica gel (10% $Et_2O$–$CH_2Cl_2$) to yield 0.800 g (43%) of **14α** as a white crystalline solid: mp 117–119 °C; $^1H$ NMR ($CDCl_3$) δ 7.2 (15 H, br s, Ph), 5.8 (1 H, br s, C-22 H), 4.90 (2 H, qd, $J$ = 17.5, 1.3 Hz, C-21 H), 3.85 (1 H, m, C-3 H), 3.6 (1 H, m, C-3′ H), 1.23 (3 H, d, $J$ = 6 Hz, C-5′ $CH_3$), 0.94 (3 H, s, C-19 $CH_3$), 0.88 (3 H, s, C-18 $CH_3$); $^{13}C$ NMR ($CDCl_3$) δ 96.29 (C-1′), 80.94 (C-3′), 72.51 (C-5′), 71.82 (C-4′), 68.38 (C-2′), 18.06 (C-6′), 73.05 (C-3). Anal. ($C_{50}H_{62}O_8$) C, H.

In addition, 0.085 g (5%) of β-L anomer **14β** (spectral data were identical with those reported for **14β** above) and digitoxigenin (0.210 g) were also recovered.

**(3β,5β,14β,17β)-3-[(α-L-Rhamnopyranosyl)oxy]-14-hydroxycard-20(22)-enolide (2a).** A 0.511-g (0.656 mmol) portion of **14α** was hydrogenated with 0.7 g of carbon and 16.0 mL of 5% $PdCl_2$, following the procedure used for **14β** above: yield of **2a** 0.248 g (76%); UV $\lambda_{max}$ (MeOH) 217 nm (log ε 4.18); $[\alpha]^{23}_D$ −16.41° (c 0.055, MeOH). Other spectral data are identical with those reported for **2b** above. Anal. ($C_{29}H_{44}O_8$) C, H.

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