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Synthesis and properties of several isomers of the cardioactive steroid ouabain

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Abstract—The ouabain isomers **1**, **6**, **20**, **21**, and **22** have been synthesized and shown to be readily separable by HPLC, which indicates that they do not correspond to a putative endogenous ouabain-like cardioregulator. 11-*epi*-Ouabain (**1**) is of interest as a safer alternative to ouabain.

This letter describes synthetic studies on the synthesis of new cardioactive steroid derivatives aimed at the discovery of molecules that might be safer than ouabain and digoxin, which are currently among the mainstays for the medical management of heart disease, including congestive heart failure. These compounds are also relevant to the existence of an endogenous factor suggested to be an isomer of ouabain.

Congestive heart failure is an important cause of death in elderly humans. It is a progressive disease that is associated with reduced cardiac output, excess peripheral vasoconstriction, and salt and water retention due to impaired kidney function.[1,2] It is treated by medication with angiotension receptor blockers, ACE inhibitors, vasodilators, aldosterone antagonists, and also the steroidal Na^+–K^+–ATPase (Na^+/K^+ pump) inhibitors digoxin and ouabain.[1,2] There are two subunits (α and β) and multiple isoforms of Na^+–K^+–ATPase (four isoforms on the α-subunit and three isoforms of the β-subunit are now known). Cardiac tissue contains the α1 and α3 isoforms and there is good evidence that the α1-isoform is associated with contractile tissue. Digoxin and ouabain bind to the intracellular side of the transmembrane α1-subunit. The cardioactive natural plant steroids[3] increase the force of cardiac muscle contraction (positive inotropic effect) by maintaining higher intracellular Na^+ levels, which leads to higher Ca^{+2} levels and increased muscle contractility in the heart.[4,5]

The cardioactive steroid glycosides, although valuable for the treatment of heart failure, have a narrow therapeutic window. Their use can lead to death from cardiac arrhythmia and disturbances of atrio-ventricular contraction, as well as other less serious side effects (e.g., gastrointestinal disorders, neurological effects, anorexia). Because patients must be monitored very carefully, there is a great need for safer versions of digoxin or ouabain. Fortunately, presently available methods allow much easier biological evaluation of safety and selectivity than has been possible previously.

This study was aimed at the synthesis of isomers of ouabain for several reasons. First, ouabain is one of the most potent and rapidly acting cardioactive steroids with a satisfactory half-life in vivo (ca. 20 h). Second, it had been reported that the mammalian hypothalamus produces an isomer of ouabain that might be an endogenous regulator of cardiac function and that is ca. 10^3 times potent.[6,7] Finally, there is a simple and commercially available test (dog arrhythmia model) for determining the therapeutic index of cardioactive steroids, allowing a straightforward screen for safety relative to ouabain or digoxin.

The first isomer of ouabain that we synthesized was the C-11 diastereomer, 11-*epi*-ouabain (**1**). An effective synthesis of **1** is outlined in Scheme 1. A solution of ouabain octahydrate (Sigma) in acetone (1.25 g/100 mL) was

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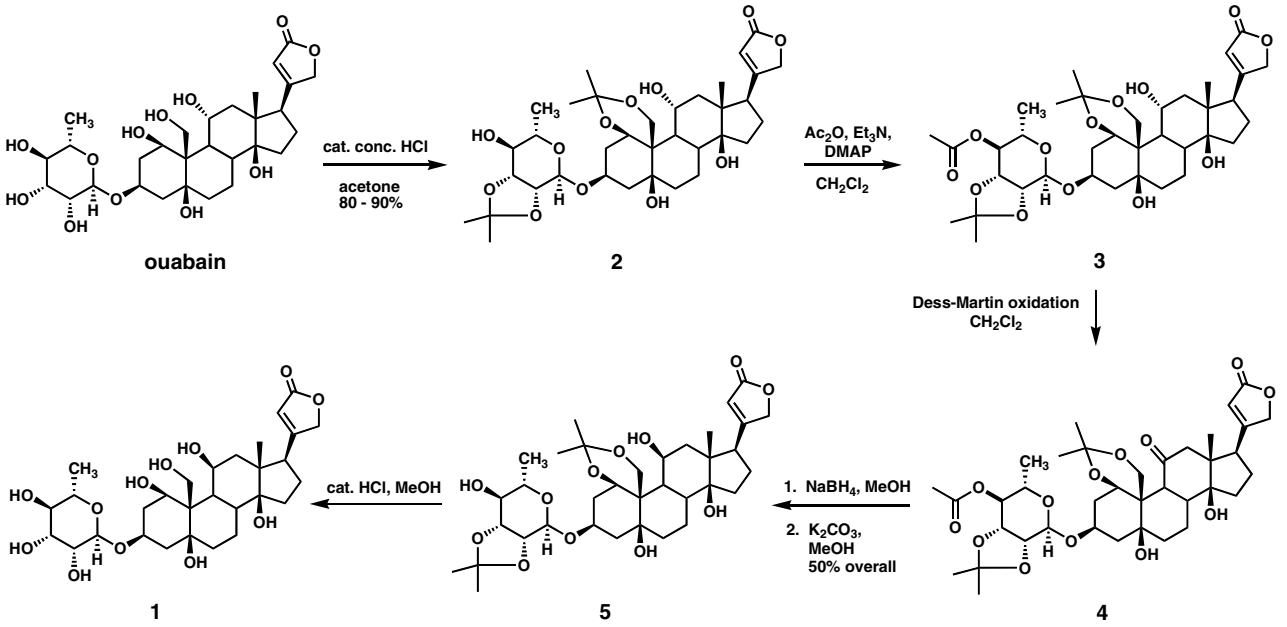


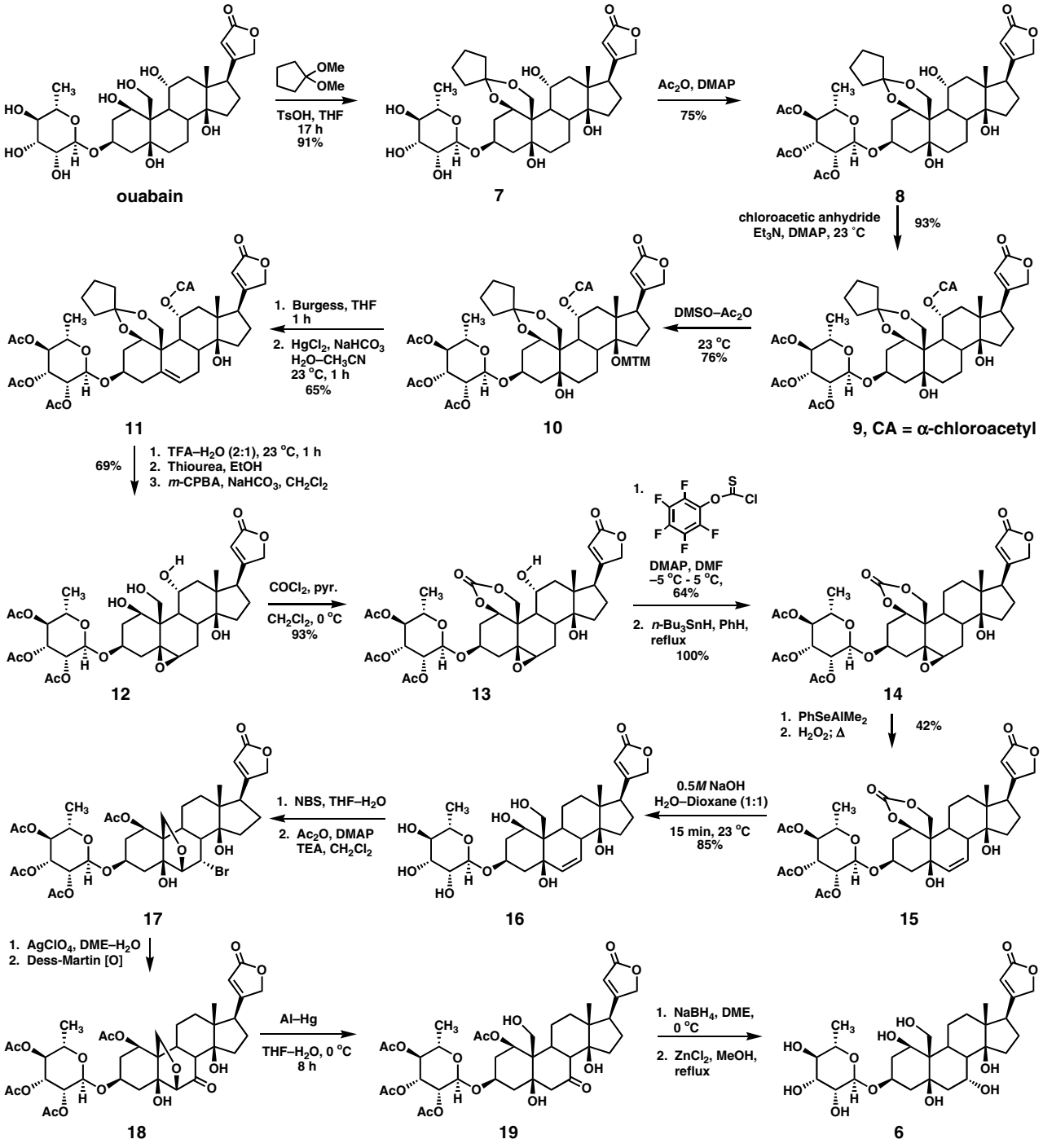
Scheme 1.

treated with 12 N hydrochloric acid (0.85 mL/100 mL of acetone) and allowed to react at 25 °C for 2 h. Neutralization (Et_3N), removal of acetone and flash chromatography on silica gel (5% MeOH–CH_2Cl_2) gave the bis-acetonide **2** in 89% yield. Acetylation of **2** (Ac_2O), Et_3N, 4-dimethylaminopyridine (DMAP) in CH_2Cl_2 at 0 °C (using TLC analysis at 5 min intervals to follow the formation of monoacetate and avoid further acetylation) gave the monoacetate **3** in 86% yield. Oxidation of **3** with the Dess–Martin periodinane reagent in CH_2Cl_2 at 25 °C produced the 11-keto steroid **4** in 85% yield. Reduction of **4** with sodium borohydride in methanol at 23 °C for 20 min followed by removal of solvent and chromatography gave the 11β-hydroxy steroid **5**. Finally, removal of the acetonide protecting groups using 4 N-hydrochloric acid in MeOH (0.8 mL/100 mL of MeOH) at 25 °C for 2 h, provided after column chromatography on silica gel (4:1 EtOH–H_2O), 11-*epi*-ouabain (**1**) in 80% yield.

11-*epi*-Ouabain (**1**) was found to be of comparable potency to ouabain but to be superior with respect to therapeutic index (TI) as determined from quadruplicate in vivo assays with the dog model. The TI for **1** was found to be at least 4, as compared to 2 for ouabain, that is an increase of safety by a factor of at least 2.[8] It was clear that **1** could be ruled out as a possible structure for the mammalian hypothalamic isomer of ouabain[6] since it had a much longer retention time on reverse-phase HPLC analysis (C_{18}-column at 23 °C using 90% H_2O–10% CH_3CN–0.1% CF_3CO_2H) than ouabain (26.7 min vs 14.7 min).

Another synthesis of **1** from ouabain was briefly examined to find a simpler route, but without positive results. Thus, although acetylation of ouabain gave good yields of a pentaacetate that could be oxidized to an 11-ketone, sodium borohydride reduction of this pentaacetoxy ketone was very slow and led to a complex mixture of very stable borate complexes from concurrent deacetylation and complexation.

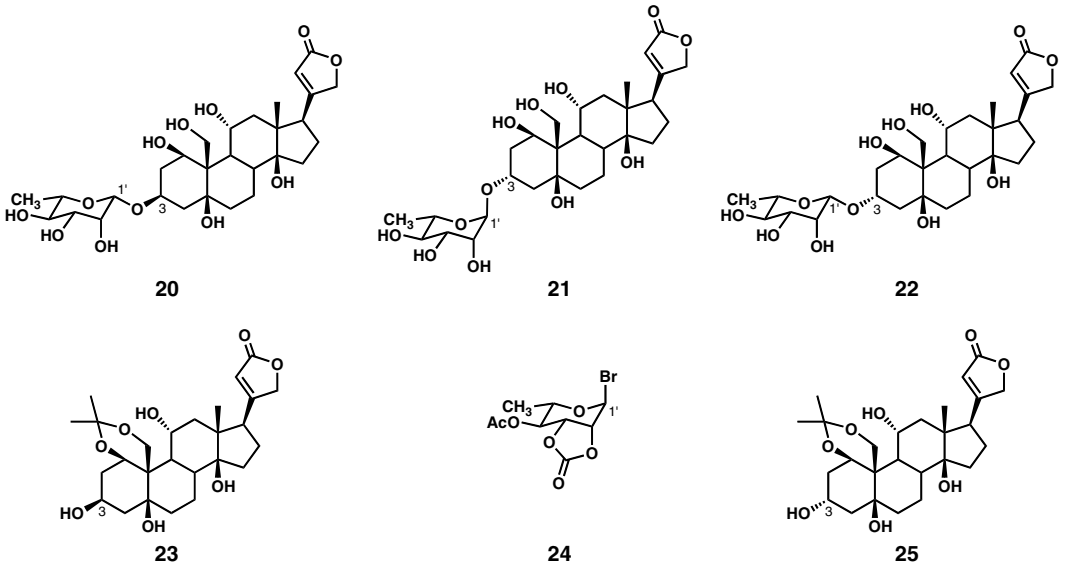
Encouraged by the promising biological profile of 11-*epi*-ouabain (**1**), we undertook the synthesis of another isomer that might be identical to the hypothalamic-derived compound,[6] specifically the regioisomer 11-desoxy-7α-hydroxyouabain (**6**). It was thought that this structure might explain the CD data measured for benzoylated hypothalamic factor.[6c] The synthesis of **6** was accomplished successfully by the multistep pathway shown in Scheme 2. The C(1)- and C(19)-hydroxyls of ouabain (dried at 100 °C in vacuo) were selectively protected by reaction with 1,1-dimethoxycyclopentane (1.1 equiv) in THF with tosic acid (0.17 equiv) as catalysts at 23 °C for 16 h to give the monoketal **7** (91%). Selective acetylation of the three rhamnose hydroxyls occurred at 23 °C in 2 h (reaction monitored by TLC) using Ac_2O, DMAP in CH_2Cl_2 to generate **8**, which was then chloracetylated to form **9** (23 °C, 30 min) efficiently. The 14β-hydroxyl group of **9** was selectively protected as the methylthiomethyl (MTM) ether by reaction with acetic anhydride-dimethylsulfoxide (DMSO)[9] at 23 °C for 24 h to afford **10**. Activation of the 5β-hydroxyl group of **10** using Burgess's reagent[10] in dry THF at 23 °C for 1 h effected elimination to the 5,6-olefin, which after MTM cleavage led to **11**. Acidic hydrolysis of **11**, dechloroacetylation using thiourea and $NaHCO_3$ in EtOH at 23 °C for 16 h, and epoxidation furnished the 5,6-epoxide **12**. The C(1)- and C(19)-hydroxyl groups were protected as the cyclic carbonate ester **13** and the C(11)-hydroxyl was removed by Barton deoxygenation of the C(11)-pentafluorophenylthionocarbonate mixed ester using Bu_3SnH, which provided the 11-deoxy-5,6β-epoxide **14**. Treatment of **14** with dimethylaluminophenylselenide[11] in CH_2Cl_2 at −78 to 0 °C resulted in selective epoxide cleavage to form a 5β-hydroxy-6α-phen-



Scheme 2.

ylselino derivative, which, after oxidation with H_2O_2–H_2O–CH_2Cl_2 at 0 °C for 30 min (vigorous stirring), isolation and heating in $ClCH_2CH_2Cl$ at 80 °C for 1 h afforded the 6,7-olefinic steroid **15**. Deacetylation of **15** gave the heptaol **16**, which upon bromoether formation and reacetylation yielded bromo ether **17**. Replacement of bromine in **17** by hydroxyl was accomplished by the sequence: (1) Dess–Martin oxidation with periodinane, which afforded the 7-ketosteroid **18**, (2) reductive cleavage of the C(6)–O bond, and (3) carbonyl reduction with sodium borohydride in dimethoxyethane and deacetylation with zinc chloride in methanol, which provided the desired 11α-deoxy-7α-hydroxy regioisomer of ouabain **6**.[12] Comparison of C_{18}-reverse-phase HPLC retention times of **6** and ouabain revealed a substantial difference using 15:85 CH_3CN–H_2O as an eluent (ouabain, 10.8 min; **6**, 12.1 min), clear evidence that the 'hypothalamic factor' is not the ouabain regioisomer **6**. Because of this fact and the limited supply of **6** further biological evaluation of **6** was not pursued.

Three other isomers of ouabain were prepared and evaluated, the stereoisomers **20**, **21**, and **22** (see Scheme 3). Reaction of ouabain octahydrate with acetone–12 N



Scheme 3.

hydrochloric acid (0.4 mL/100 mL acetone) at 23 °C for 10 days provided a precipitate of the 1,19-acetonide of the aglycone of ouabain **23**. Reaction of **23** with the acetate-cyclic carbonate ester of rhamnosyl-1-bromide (**24**)[13] (2 equiv) and mercuric cyanide (1.3 equiv) in CH_2Cl_2 at reflux for 30 h followed by the addition of 2 more equiv of **24** and 30 h heating at reflux afforded, after extractive isolation and preparative TLC, two C-1′ diastereomeric glycosides in a ratio of 2.5:1. Methanolysis of the major coupling product in MeOH–4 N hydrochloric acid (1000:1) at 25 °C for 2 h afforded the 1′-diastereomer of ouabain (**20**).[14] Methanolysis of the minor coupling product formed ouabain cleanly.

The aglycone **23** was transformed into the C(3)-diastereomer **25** by the sequence (1) oxidation with 1.2 equiv of periodinane in CH_2Cl_2 at 0 °C for 1 h and 23 °C for 1 h and (2) reduction with $NaBH_4$ at 23 °C. Coupling of **25** with the protected rhamnosyl bromide **24**, as described above for **23** + **24**, afforded a mixture of C(1)′-diastereomeric coupling products, which after chromatographic separation and acid-catalyzed methanolysis afforded the stereoisomers of ouabain **21**[15] and **22**.[16]

HPLC analysis and comparison of **20**, **21**, and **22** with ouabain revealed the following retention times under identical conditions [C_{18}-ODS reversed-phase column (250 × 4.6 mm), 90% H_2O, 10% CH_3CN, 0.1% CF_3CO_2H, 23 °C, flow rate 1.5 mL/min]: ouabain, 14.7 min; **20**, 19.5 min; **21**, 34.2 min; **22**, 39.3 min. Thus, none of the stereoisomers correspond to the chromatographic data reported[6] for the hypothalamic factor. Further, the radio-ouabain displacement assay using purified kidney Na^+–K^+–ATPase (Sigma) yielded the following IC_{50} values (nM): ouabain 16.1; **20**, 278; **21**, 220; **22**, 446.[17]

A number of conclusions can be drawn from the present investigation of the synthesis and properties of the new isomers of ouabain reported here. First, none of these isomers (**1**, **6**, **20**, **21**, or **22**) match the HPLC data reported[6] for the endogenous mammalian hypothalamic Na^+–K^+–ATPase inhibitor. In fact, the large differences in retention time of these isomers lead to skepticism that an isomer of ouabain would not differ from ouabain in reverse-phase HPLC analysis. Furthermore, in addition to the ouabain isomers reported here, we have also synthesized and studied a number of others (to be reported separately) including the following: (1) the regioisomer of ouabain in which rhamnose is attached to the C(19)-hydroxyl, (2) the C(4)-diastereomer of ouabain, (3) 1-deoxy-2β-hydroxyouabain, and (4) 2β-hydroxy-5-deoxyouabain. Each of these isomers was readily distinguished from ouabain by reversed-phase HPLC analysis, which adds to the doubt that there is an endogenous inhibitor of Na^+–K^+–ATPase that is chromatographically identical with ouabain. The most recent studies of 'endogenous' ouabain-like inhibitor of Na^+–K^+–ATPase[18,19] also failed to lend support to the original claim.[6]

Another significant result to emerge from this work is the finding that 11-*epi*-ouabain is a good inhibitor of Na^+–K^+–ATPase with a therapeutic index that is more than twice as large, and thus is a potentially useful new cardenolide.

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5. The use of foxglove extract, which contains digitalis (a mixture of cardioactive steroidal glycosides) was introduced by William Withering in 1775 for treatment of heart

failure (dropsy) long before the age of modern therapy or Na^+–K^+–ATPase.

6. (a) Hallaq, H. A.; Haupert, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1989**, *86*, 10080–10084; (b) Tymiak, A. A.; Norman, J. A.; Bolgar, M.; DiDonato, G. C.; Lee, H.; Parker, W. L.; Lo, L.-C.; Berova, N.; Nakanishi, K.; Haber, E.; Haupert, G. T. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 8189–8193; (c) Zhao, N.; Lo, L.-C.; Berova, N.; Nakanishi, K.; Tymiak, A.; Ludens, J. H.; Haupert, G. T., Jr. *Biochemistry* **1995**, *34*, 9893–9896.
7. This compound could only be obtained in microscopic amount (reported[6] as 1 μg from 5 kg of bovine hypothalamus). The chief characterization data were mass spectrum, HPLC data, and CD data on a benzoylated sample. Although the mass spectrum and HPLC retention times was reported to be the same as for ouabain, the CD spectrum of a benzoylated sample was reported to be different.
8. These tests were performed by Sierra Biomedical, Inc., San Diego, CA.
9. See: Yamada, K.; Kato, K.; Nagase, H.; Hirata, Y. *Tetrahedron Lett.* **1976**, 65–66.
10. Burgess, E. M.; Penton, H. R.; Taylor, E. A. *J. Org. Chem.* **1973**, *38*, 26–31.
11. Prepared from a 1:1 mixture of phenylselenol and trimethylaluminum in 1:1 toluene–CH_2Cl_2.
12. Physical data for compound **6** were as follows: ^{1}H NMR (CD_3OD–D_2O (v/v 1:1)) δ 5.92 (1H, s), 4.93 (1H, d, $J = 18$ Hz), 4.86 (1H, dd, $J = 18$ Hz), 4.71 (1H, s), 4.19 (1H, m), 4.06 (1H, d, $J = 12$ Hz), 4.05 (1H, m), 3.99 (1H, d, $J = 12$ Hz), 3.75 (1H, m), 3.66 (1H, m), 3.63 (1H, m), 3.50 (1H, s), 3.29 (1H, t, $J = 10$ Hz), 2.76 (1H, m), 1.15 (3H, d, $J = 6$ Hz), 0.80 (3H, s), 2.6–1.2 (16H, m); MS (FAB) *m/z* 585 (M^++H), 461, 369, 277; HRMS (FAB) *m/z* 585.2899 (M^++H) (calcd for $C_{29}H_{45}O_{12}$ 585.2911).
13. Backinowski, L. V.; Balan, N. F.; Shashkov, A. S.; Kochetkov, N. K. *Carbohydr. Res.* **1980**, *84*, 225–235.
14. Physical data for **20**: $[\alpha]_D^{23}$ +13.2 (*c* 0.50, H_2O); IR (neat): 3100–3500, 2933, 2887, 1740, 1623, 1446, 1442, 1438, 1419, 1399, 1374, 1346, 1326, 1321, 1178, 1120, 1061, 1024 cm^{-1}; ^{1}H NMR (CD_3OD–$CDCl_3$ (2:1), 500 MHz): δ 0.93 (s, 3H), 1.30 (d, $J = 6.0$ Hz, 3H), 1.15–1.40 (m, 4H), 1.42–2.20 (m, 12H), 2.85–2.92 (m, 1H), 3.18–3.25 (m, 1H), 3.41 (dd, $J = 9.2$, 3.3 Hz, 1H), 3.85 (d, $J = 3.3$ Hz, 1H), 4.07 (d, $J = 11.8$ Hz, 1H), 4.16 (br s, 1H), 4.22 (s, 1H), 4.40 (d, $J = 11.8$ Hz, 1H), 4.62 (s, 1H), 4.73 (br s, 2H and OH), 4.87 (dd, $J = 18.4$, 1.5 Hz, 1H), 5.00 (dd, $J = 18.4$, 1.5 Hz, 1H), 5.88 (s, 1H); ^{13}C NMR (CD_3OD–$CDCl_3$ (2:1), 100 MHz): δ 17.51 (CH_3), 17.96 (CH_3), 24.02 (CH_2), 24.57 (CH_2), 31.46 (CH_2), 33.43 (CH_2), 37.05 (CH_2), 38.30 (CH_2), 40.90 (CH), 48.06 (CH), 48.36 (C), 49.85 (CH_2), 50.59 (C), 51.36 (CH), 61.28 (CH_2), 68.69 (CH), 71.33 (CH), 72.22 (CH), 73.24 (CH), 73.43 (CH), 73.58 (C), 74.51 (CH), 74.91 (CH_2), 75.85 (CH), 85.16 (C), 99.17 (CH), 117.88 (CH), 176.74 (C), 176.86 (C); MS (*m/z*, relative intensity): 607 (M^++Na, 10), 585 (M^++H, 16), 553 (12); 461 (10), 369 (27), 299 (20), 277 (90), 207 (100); exact mass calculated for $C_{29}H_{45}O_{12}$ (M^++H): 585.2911; found 585.2916.
15. Physical data for **21**: $[\alpha]_D^{23}$ −11.43 (*c* 0.07, H_2O); IR (neat): 3100–3500, 2940, 2880, 1739, 1451, 1149, 1047 cm^{-1}; ^{1}H NMR (CD_3OD–$CDCl_3$ (2:1); 500 MHz): δ 1.12 (s, 3H), 1.25 (d, $J = 6.2$ Hz, 3H), 1.20–1.35 (m, 2H), 1.40–1.60 (m, 3H), 1.68–1.90 (m, 4H), 1.93–2.03 (m, 3H), 2.08–2.20 (m, 4H), 2.74–2.82 (m, 1H), 3.36 (t, $J = 9.4$ Hz, 1H), 3.70 (dd, $J = 9.5$, 3.5 Hz, 1H), 3.72 (d, $J = 11.7$ Hz, 1H), 3.76 (dd, $J = 3.3$, 1.7 Hz, 1H), 4.00 (d, $J = 2.0$ Hz, 1H), 4.03 (d, $J = 11.7$ Hz, 1H), 4.18 (s, 1H), 4.39 (s, 1H), 4.42 (s, 1H), 4.84 (d, $J = 1.4$ Hz, 1H), 4.87 (dd, $J = 18.4$, 1.5 Hz, 1H), 5.01 (dd, $J = 18.4$, 1.5 Hz, 1H), 5.87 (s, 1H); ^{13}C NMR (CD_3OD–$CDCl_3$ (2:1), 100 MHz): δ 17.91 (CH_3), 19.40 (CH_3), 23.79 (CH_2), 27.59 (CH_2), 33.08 (CH_2), 33.24 (CH_2), 34.32 (CH_2), 34.59 (CH_2), 35.99 (CH), 44.65 (CH), 46.78 (CH_2), 47.30 (C), 50.32 (C), 52.26 (CH), 58.93 (CH_2), 66.37 (CH), 69.00 (CH), 69.62 (CH), 70.76 (CH), 71.97 (two of CH), 73.86 (CH), 75.05 (CH_2), 78.36 (C), 86.57 (C), 98.73 (CH), 117.94 (CH), 176.84 (C), 177.50 (C); MS (*m/z*, relative intensity): 585 (M^++H, 10), 553 (6), 461 (9), 369 (27), 277 (100), 207 (27); exact mass calculated for $C_{29}H_{45}O_{12}$ (M^++H): 585.2911; found 585.2905.
16. Physical data for **22**: $[\alpha]_D^{23}$ +24.17 (*c* 0.12, H_2O); IR (neat): 3100–3500, 2980, 2880, 1733, 1663, 1648, 1633, 1451, 1422, 1413, 1399, 1335, 1097, 1059, 1019, 1015 cm^{-1}; ^{1}H NMR (CD_3OD–$CDCl_3$ (2:1), 500 MHz): δ 1.12 (s, 3H), 1.29 (d, $J = 6.0$ Hz, 3H), 1.20–1.35 (m, 2H), 1.45–1.60 (m, 3H), 1.72–1.90 (m, 5H), 1.95–2.05 (m, 2H), 2.08–2.35 (m, 4H), 2.76–2.82 (m, 1H), 3.20–3.28 (m, 1H), 3.42 (dd, $J = 9.2$, 3.3 Hz, 1H), 3.87 (d, $J = 3.1$ Hz, 1H), 3.98 (d, $J = 2.1$ Hz, 1H), 4.03 (d, $J = 11.8$ Hz, 1H), 4.29 (s, 1H), 4.35 (s, 1H), 4.40 (d, $J = 11.8$ Hz, 1H), 4.63 (s, 1H), 4.78 (br s, 1H and OH), 4.86 (dd, $J = 18.6$, 1.5 Hz, 1H), 5.00 (dd, $J = 18.6$, 1.5 Hz, 1H), 5.86 (d, $J = 1.5$ Hz, 1H); ^{13}C NMR (CD_3OD–$CDCl_3$ (2:1), 100 MHz): δ 17.92 (CH_3), 19.33 (CH_3), 23.87 (CH_2), 27.46 (CH_2), 30.62 (CH_2), 33.19 (CH_2), 35.47 (CH_2), 35.88 (CH), 36.72 (CH_2), 44.68 (CH), 46.70 (CH_2), 47.30 (C), 50.21 (C), 52.18 (CH), 58.73 (CH_2), 66.14 (CH), 68.88 (CH), 71.98 (CH), 73.20 (CH), 73.29 (CH), 74.38 (CH), 74.76 (C), 74.93 (CH_2), 78.80 (CH), 86.45 (C), 99.10 (CH), 117.93 (C), 176.71 (C), 177.19 (CH).
17. We are grateful to Dr. Albert L. Rauch of Pfizer Inc., Groton, MA for carrying out the assays and for these data.
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