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Supporting Information

Sugar/Steroid/Sugar Conjugates: Sensitivity of Lipid Binding to Sugar Structure

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Experimental Section

General Methods. All reagents were purchased from Sigma-Aldrich with the exception of 5-α-androstane-3-β, 17β diol which was purchased from Steraloids. Solvents were dried over 4 Å molecular sieves. TLC was performed on Whatman normal phase silica plates and observed by UV or iodine visualization. Melting points were determined with a Thomas Hoover capillary melting point apparatus and were uncorrected. Yields were not optimized. Solvent A = 2/1 hexane/ethyl acetate; Solvent B = 7/1 hexane/ethyl acetate. ^{1}H NMR and ^{13}C NMR spectra were recorded on a Varian INOVA 300 or 400 MHz spectrophotometer. Mass Spectral data were obtained from the Emory University Mass Spectrometry Center; for this we acknowledge the use of Shared Instrumentation provided by grants from the NIH and the NSF. Elemental analyses were performed by Atlantic Microlab, Norcross, GA.

Synthesis

The procedures used and described below were adapted from a report by Deng et. al.[1]

1,2,3,4,6-Penta-*O*-benzoyl-α,β-D-glucopyranoside To 20.06g (0.11 mol) glucose stirring in 240 mL pyridine for 20 min was added 80 mL (0.67 mol) benzoyl chloride. The maroon solution was stirred in an oil bath at 60-65 ºC for an hour. The resulting suspension was quenched with 10 mL deionized water and allowed to sit at room temperature for 10 min after which 100 mL water was added. The mixture was then poured into 2L ice water and allowed to sit for an hour. The white precipitate was filtered and recrystallized repeatedly from

methanol and acetone to yield 44.07g (62.89 mmol, 57%) white solid, R_f = 0.56 (solvent A), which sintered at 158-161 ºC and melted at 176 ºC. lit.[2] 190-191 ºC. The protected sugar was carried on to the next step without further purification.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.28-8.06 (m, 25H 5xPh), 6.32 (d, 1H, *J* = 7.8 Hz), 6.06 (t, 1H, *J* = 9.6 Hz), 5.81-5.91 (q, 2H, *J* = 9.3), 4.70-4.65 (dd, 1H, *J* = 12.3, 2.7 Hz), 4.56-4.50 (dd, 1H, *J* = 12.3, 4.8), 4.39-4.51 (m, 1H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.34, 165.90, 165.35, 164.82, 134.06, 133.75, 133.68, 133.57, 133.31, 130.43, 130.05, 129.76, 128.94, 128.74, 128.68, 128.57, 92.92, 73.39, 73.04, 71.06, 69.28, 62.89.

2,3,4,6-Tetra-*O*-benzoyl-α,β-D-glucopyranose 30.01g (42.83 mmol) penta-*O*-benzoyl protected glucose was stirred in 100 mL CH_2Cl_2 and 20 mL HBr/HOAc (30%) at room temperature, forming a clear orange-yellow solution after 15 min. After 3 h, the solution was washed twice with 40 mL saturated $NaHCO_3$ and NaCl solutions and finally with 20 mL purified water. The resulting yellow oil was dried to an off- white solid overnight, then dissolved in 80 mL acetone and 4 mL pure water. The solution was treated with 6.04g (21.90 mmol) Ag_2CO_3 for 2h, and filtered over celite for a quantitative yield of the tetra benzoyl derivative, R_f = 0.36 (solvent A).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.37-8.06 (m, 20H 4xPh), 6.26-6.33 (m, 1H), 5.73-5.79 (m, 1H), 5.32-5.35 (dd, 1H, *J* = 10, 4 Hz), 4.65-4.71 (m, 2H), 4.43-4.59 (m, 2H).

^{13}C NMR (100 MHz, $CDCl_3$) δ 166.07, 134.17, 133.66, 133.34, 130.40, 130.13, 130.03, 129.94, 129.90, 129.34, 129.13, 129.00, 128.91, 128.66, 128.62, 128.56, 128.62, 90.68, 72.42, 71.02, 70.31, 69.62, 67.99, 63.04.

2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl trichloroacetimidate To a flask containing 5.30g (8.88 mmol) tetra-*O*-benzoyl glucopyranose, 50 mL sieve-dried CH_2Cl_2 and 5.00g (36.18 mmol) K_2CO_3, was added 5mL (49.86 mmol) Cl_3CCN. The mixture was stirred at room temperature for 62 h under argon. Column chromatography with solvent A as eluent (R_f = 0.64), followed by recrystallization from ethyl ether and petroleum ether isolated 3.44g (52%) product.

^1H NMR (300 MHz, $CDCl_3$) δ 8.64 (s, 1H, NH), 7.27-8.06 (m, 20 H 4xPh), 6.26 (t, 1H, *J* = 9.9 Hz), 5.68-5.77 (m, 1H), 5.30-5.34 (dd, 1H, *J* = 3.6, 10.5 Hz), 4.69-4.63 (m, 2H), 4.41-4.48 (dd, 2H, *J* = 4.8, 8.4 Hz).

FAB-HRMS $(M + Li)^+$: Calc. for 746.0939. Found: 746.0928.

Androstanyl bis β-D-glucopyranoside, 1 0.211g (0.72 mmol) androstane was added to a flask containing 1.23g (1.66 mmol) glucopyranosyl trichloroacetimidate, 0.55g 4 Å molecular sieves and 20 mL CH_2Cl_2. The mixture was stirred at ambient temperature under N_2 for 15 min and 3.5 mL of a 0.5M TMSOTf/CH_2Cl_2 solution was then added. After stirring for 3 h at ambient temperature, the reaction was quenched with the addition of 0.25 mL (1.79 mmol) Et_3N. The mixture was filtered over a cotton plug, dried and purified by column chromatography with solvent B (R_f = 0.49, solvent A). Multiple recrystallizations from ether and hexane yielded 0.37g (36%) protected steroidal glycoside as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.29-8.03 (m, 40H 8xPh), 5.90 (t, 2H, J = 9.6 Hz), 5.61-5.64 (m, 2H), 5.46-5.54 (m, 2H), 4.94 (d, 1H, J = 8 Hz), 4.86 (d, 1H, J = 7.6 Hz), 4.50-4.65 (m, 4H), 4.11-4.18 (m, 2H), 3.58 (t, 2H, J = 8.8 Hz), 0.4-2.18 (m, 28H, androstane peaks, including 2 Me singlets at 0.55 and 0.60).

FAB-LRMS $(M + Li)^+$: 1455.9.

Deprotection of the above product was achieved under Zémplen conditions[3] by stirring in 50 mL methanol with 2 mL 0.5M NaOMe/MeOH overnight. As the deprotection progressed, a clear solution was formed, from which white precipitate later fell out. 0.15g sugar-steroid conjugate **1** was collected in quantitative yield from the protected precursor.

^{1}H NMR (400 MHz, DMSO) δ 4.85-4.89 (dd, 4H, J = 4, 11.2 Hz), 4.38-4.45 (m, 2H), 4.21(d, 1H, J = 7.6 Hz), 4.15 (d, 1H, J = 7.6 Hz), 3.55-3.66 (m, 2H), 3.01-3.10 (m, 4H), 2.87-2.92 (m, 2H), 0.57-1.89 (m, 28H, androstane peaks, including 2 Me singlets at 0.73 and 0.76).

^{13}C NMR (100 MHz, DMSO) δ 103.06, 100.55, 87.00, 76.85, 76.77, 76.24, 73.72, 73.50, 70.13, 61.13, 53.87, 50.46, 44.11, 42.77, 40.14, 38.88, 37.13, 36.62, 35.30, 34.94, 34.03, 31.28, 29.09, 28.47, 28.31, 22.92, 20.38, 12.10, 11.49.

Anal. Calc. for $C_{31}H_{52}O_{12}$ + 1.5 mol H_2O: C, 57.84; H, 8.61; O, 33.55. Found: C, 57.83; H, 8.17; O, 33.44.

1,2,3,4,6-Penta-*O*-benzoyl-α,β-D-galactopyranoside The reaction was carried out as described above for the glucose analog, using 20.11g (0.11 mol) galactose, 240 mL pyridine and 80 mL (0.67 mol) benzoyl chloride. Aqueous work-up led to an orange gum which was recrystallized multiple times from

methanol and acetone to isolate 14.60g (20.84 mmol, 18%) benzoyl protected galactoside, R_f = 0.57 (solvent A).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.10-8.04 (m, 25H 5xPh), 6.86 (d, 1H, *J* = 4.8 Hz), 6.31 (t, 1H, *J* = 7.2 Hz), 5.89-5.93(m, 2H), 4.67-4.84 (m, 3H).

^{13}C NMR (100 MHz, $CDCl_3$): 166.13, 165.64, 165.16, 133.85, 133.80, 133.66, 133.32, 133.29, 130.14, 130.08, 129.91, 129.67, 128.86, 128.66, 128.52, 128.41, 94.28, 79.71, 76.26, 73.95, 70.48, 63.25.

2,3,4,6-Tetra-*O*-benzoyl-α,β-D-galactopyranoside Anomeric deprotection of 10.04g (14.33 mmol) of the penta-*O*-benzoyl galactoside was achieved beginning by treatment with 5 mL HBr/HOAc (30%) in 25 mL dry CH_2Cl_2 for an hour. After washing (conc $NaHCO_3$, NaCl and water as previously described) and drying, the white solid was stirred in 45 mL acetone, 2 mL purified water and 2.01g (7.29 mmol) Ag_2CO_3 for 1.5h. Filtering over a pad of celite and drying *in vacuo* yielded a quantitative amount of the tetra-*O*-benzoyl product as a white solid, mp 171-174 ºC, R_f = 0.3 (solvent A).

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.29-8.11 (m, 20H 4xPh), 6.06-6.10 9 (m, 2 H), 5.71-5.73 (m, 1H), 5.65 (d, 1H, *J* = 5.1 Hz), 4.85-4.88 (m, 1H), 4.68-4.77 (m, 2H).

2,3,4,6-Tetra-*O*-benzoyl-β-D-galactopyranosyl trichloroacetimidate 2.98g (4.99 mmol) Tetra-*O*-benzoyl galactopyranoside in 50 mL CH_2Cl_2 was stirred at ambient temperature with 2.91g (10.55 mmol) K_2CO_3 and 3 mL (29.92 mmol) Cl_3CCN under argon for 40 h. After filtering over celite with CH_2Cl_2, the solvent was removed and the resulting solid dried under vacuum. Column chromatography with solvent B (R_f = 0.67 in solvent A) followed by

recrystallization from ethyl ether and petroleum ether revealed 2.45g (66%) galactose product mp 140-141 ºC.

^{1}H NMR (300 MHz, $CDCl_3$) δ 8.74 (s, 1H, NH), 7.28-8.11 (m, 20 H, 4xPh), 6.71 (m, 1H), 6.12-6.17 (m, 2H), 5.77-5.79 (m, 1H), 4.86-4.88 (m, 1H), 4.77-4.79 (m, 2H).

^{13}C NMR (75 MHz, $CDCl_3$) δ 166.23, 165.94, 165.73, 160.54, 133.91, 133.78, 133.52, 133.31, 130.25, 130.13, 129.93, 129.52, 129.00, 128.69, 128.62, 128.56, 128.41, 126.67, 105.54, 103.13, 84.80, 84.09, 80.98, 70.24, 63.63, 63.29.

FAB-HRMS $(M + Li)^+$: Calc. for 746.0939. Found: 746.0961.

Androstanyl bis β-D-galactopyranoside, 2 2.39g (3.2 mmol)Tetra-*O*-benzoyl galactosyl trichloroacetimidate, 20 mL sieve-dried CH_2Cl_2, 1.04g 4 Å molecular sieves and 0.40g (1.37 mmol) androstane were stirred for 15 min under argon at room temperature. 6.2 mL of a 0.5M TMSOTf/CH_2Cl_2 solution was introduced, inducing an immediate clearing of the cloudy suspension, to a blush-colored solution. The reaction was quenched by the addition of 0.25 mL (1.79 mmol) Et_3N after stirring for 2 h at ambient temperature. The solution was filtered over a cotton plug with CH_2Cl_2 to remove sieves, followed by removal of the solvent by rotary evaporation and drying *in vacuo*. The orange hued-residue was partitioned between water and ether, and hexane was added to the ethereal solution to precipitate the desired product. Purification was achieved by multiple recrystallizations from ether and petroleum ether.

^{1}H NMR (300 MHz, $CDCl_3$) δ 7.25-8.09 (m, 40H 8xPh), 6.05-6.07 (m, 2H), 5.60-5.71 (m, 2H), 5.36-5.46 (m, 2H), 4.72-4.78 (m, 4H), 4.60-4.67 (m, 2H), 3.61 (t,

2H, J = 8.7 Hz), 0.58-2.01 (m, 28H, androstane peaks, including 2 Me singlets at 0.81 and 0.78).

The benzoyl groups were removed by stirring the protected precursor with 2 mL 0.5M NaOMe/MeOH solution in methanol. Neutralizing with mixed bed ion exchange resin, followed by filtering over charcoal, drying, recrystallization from methanol and acetone and finally heating in hexane, revealed 0.30g (38% over two steps) **2**, mp> 200 ºC.

^{1}H NMR (400 MHz, pyr.-d_5) δ 5.07-5.00 (m, 4H), 4.82-4.99 (m, 4H), 4.58-4.60 (m, 2H), 4.40-4.47 (m, 4H), 3.72-3.81 (m, 2H), 0.45-2.18 (m, 28H, androstane peaks, including 2 Me singlets at 0.67 and 0.84).

^{13}C NMR (100 MHz, pyr-d_5) δ 98.23, 87.64, 84.59, 83.98, 79.14, 78.91, 76.35, 72.99, 65.06, 51.04, 44.85, 43.34, 37.92, 37.31, 35.87, 35.50, 34.89, 31.89, 29.90, 29.00, 23.76, 21.22, 12.25.

FAB-HRMS $(M + Li)^+$: Calc. for 623.3619. Found: 623.3588

Anal. Calc. for $C_{31}H_{52}O_{12}$ + 2 mol H_2O: C, 57.02; H, 8.65; O, 34.33. Found: C, 57.38; H, 8.27; O, 34.57.

1,2,3,4,6-Penta-*O*-benzoyl-α,β-D-mannopyranoside To a solution of 20.02g (0.11 mol) mannose in 240 mL pyridine stirring over an ice-bath was added 80 mL (0.67 mol) benzoyl chloride. Stirring was continued overnight as the reaction mixture warmed to room temperature with the warming of the ice-bath. The mixture was poured into 2L ice water and the resulting oily residue was recrystallized multiple times from acetone and methanol to isolate 14.18g (18 %) fully protected mannosyl derivative, mp 156-158 ºC.

FAB-LRMS $(M + Li)^+$: 1455.9.

2,3,4,6-Tetra-*O*-benzoyl-α,β-D-mannopyranoside To 29.62g (42.27 mmol) benzoyl protected mannose was added 60 mL CH_2Cl_2 and 15 mL HBr/HOAc. The solution was stirred for 2 h at room temperature, then washed with saturated $NaHCO_3$, aqueous saturated NaCl and distilled water. Removal of the solvent followed by drying under vacuum yielded the crude product as 21.73g orange solid which was carried on to the next step. The hydrolysis was performed in 120 mL acetone and 6 mL Milli-Q water with 6.00g (21.76 mmol) Ag_2CO_3. After two hours of stirring at room temperature, the suspension was filtered over a pad of celite and the solvent removed. Purification by column chromatography with solvent B (R_f = 0.44 in solvent A) and recrystallization from ethyl ether and petroleum ether afforded 16.62g (66 % over 2 steps) tetra-*O*-benzoyl derivative, mp 171-174 ºC (lit.[2] 181 ºC).

^1H NMR (400 MHz, $CDCl_3$) δ 7.24-8.12 (m, 20H 4xPh), 6.17 (t, 1H, *J* = 10.4 Hz), 6.06 (d, 1H, *J* = 3.2 Hz), 5.73-5.74 (m, 1H), 5.53 (s, 1H), 4.74-4.78 (dd, 2H, *J* = 8.7, 12.4 Hz), 4.65-4.69 (m, 1H), 4.42-4.46 (dd, 1H, *J* = 3.6, 12.4 Hz,).

^{13}C NMR (100 MHz, $CDCl_3$) δ 133.68, 133.42, 133.32, 130.03, 129.98, 129.26, 128.79, 128.68, 128.53, 92.58, 70.99, 69.94, 69.12, 67.03, 62.91.

FAB-HRMS $(M + Li)^+$: Calc. for 603.1843. Found: 603.1849.

2,3,4,6-Tetra-*O*-benzoyl-α-D-mannopyranosyl trichloroacetimidate

8.21g (13.76 mmol) Tetra-*O*-benzoyl mannose, 7.76g (56.15 mmol) K_2CO_3, 50 mL CH_2Cl_2 and 7.8 mL (77.79 mmol) Cl_3CCN were stirred at room temperature under N_2 for 3 days. K_2CO_3 was filtered off, and the solvent removed to reveal an

orange solid. Column chromatography with solvent B (R_f = 0.52 in solvent A) isolated 1.89g (19 %) mannosyl trichloroacetimidate.

Androstanyl bis α-D-mannopyranoside, 3 To 1.89g (2.55 mmol) of the above trichloroacetimidate was added 0.31g (1.06 mmol) androstane, 0.51g 4 Å molecular sieves and 20 mL CH_2Cl_2. After stirring under nitrogen for 15 mins, 4 mL of a 0.5M solution of TMSOTf was introduced and stirring continued for 3 h. The reaction was then quenched with 0.25 mL (1.19 mmol) triethylamine, and stirred for 5 min. After filtering over a cotton plug, the crude product was purified by column chromatography using solvent B (R_f = 0.42 in solvent A) and recrystallized until pure by TLC to isolate 0.21g (15 %) product. Immediate deprotection was achieved by treating in 0.5M NaOMe/MeOH solution in methanol overnight. The product was then recrystallized from acetone and methanol to reveal 75mg (84%) **3**.

^{1}H NMR (400 MHz, pyr-d_5) δ 4.95-4.97 (m, 2H), 4.61-4.69 (m, 8H), 4.45-4.48 (m, 4H), 3.87-3.95 (m, 2H), 0.41-1.91 (m, 28H, androstane peaks, including 2 Me singlets at 0.69 and 0.77).

^{13}C NMR (100 MHz, pyr-d_5) δ 99.77, 83.86, 76.26, 75.69, 73.51, 69.94, 63.88, 54.88, 51.30, 45.39, 43.12, 36.82, 36.13, 35.67, 32.06, 29.17, 28.37, 27.31, 23.98, 21.32, 12.69, 12.43.

Anal. Calc. for $C_{31}H_{52}O_{12}$ + 0.5 mol H_20: C 59.50; H 8.54; O 31.96. Found: C 59.73; H 8.52; O 31.82.

Cholesteryl β-D-galactoside, 4 1.01g molecular sieves and 1.01g (2.61 mmol) cholesterol were added to a flask containing 2.55g tetra-*O*-benzoyl galactopyranosyl trichloroacetimidate dissolved in 30 mL CH_2Cl_2. The mixture was stirred at room temperature under N_2 for 15 min, after which 5 mL 0.5M TMSOTf in CH_2Cl_2 solution was added. After 2 hours, the green mixture was filtered over a cotton plug and the solvent subsequently removed. After drying, the residue was recrystallized from ether and hexane. The resulting white solid was triturated with acetone to remove the acetone-insoluble pellets. Recrystallization from ether and methanol yielded 2.02g (75%) product as a white solid, mp 159-160 ºC.

^{1}H NMR (400 MHz, $CDCl_3$) 7.29-8.09 (m, 20H, 4xBz), 6.06 (m, 1H), 5.62 (d, 1H, J = 4.8 Hz), 5.46 (d, 2H, J = 8.8), 5.35 (m, 1H), 4.67-4.78 (m, 3H), 3.48-3.58 (m, 1H), 0.68-2.41 (m, 43H, cholesterol peaks, including singlets at 0.68, 0.99 and sharp doublets at 0.88, J = 2 Hz and 0.87, J = 1.6 Hz)

FAB-LRMS: 971.8 $(M + Li)^+$

The benzoyl groups were removed by adding 100 mL methanol and 0.5M 2 mL NaOMe/MeOH solution to 1.60g (1.66 mmol) protected adduct dissolved in 25 mL $CHCl_3$ and stirring overnight at ambient temperature. The residual solid was filtered off and the filtrate was stirred with mixed bed ion exchange resin overnight. Rotary evaporation of the solvent after filtering off the resin followed by recrystallization twice from acetone yielded 0.85g (75%) **4** as a shiny crystalline white solid.

^{1}H NMR (400 MHz, CD_3OD) δ 5.35 (m, 1H), 4.99 (d, 1H, J = 1.6 Hz), 3.99-3.92 (m, 1H), 3.89-3.90 (m, 2H), 3.70-3.67 (m, 1H), 3.59 (d, 2 H, J = 5.6 Hz), 3.41-3.47 (m, 1H), 3.28-3.30 (m, 4H), 0.68-2.37 (m, 43H, cholesterol peaks, including singlets at 0.71, 1.01 and sharp doublets at 0.86, J = 1.6 Hz and 0.88, J = 1.6 Hz)

^{13}C NMR (100 MHz, CD_3OD) δ 84.05, 83.82, 78.71, 72.55, 64.73, 58.33, 57.71, 51.89, 41.31, 40.85, 38.72, 37.29, 33.40, 33.21, 31.01, 29.48, 29.32, 25.09, 23.34, 23.09, 22.32, 19.98, 19.38, 12.45.

Anal. Calc. For $C_{33}H_{56}O_6$: C 72.22; H 10.28; O 17.49. Found: C 72.23, H 10.35, O 17.70.

Differential Scanning Calorimetry (DSC).

A. Sample Preparation. Thin films of the appropriate amounts of lipid and additive were prepared by drying chloroform solutions in a glass vial under a steady stream of N_2. The films were then dried under vacuum for at least 16 h. Hydration of the mixed films was achieved by adding Milli-Q purified water to afford 5mg / 2mL suspensions. Heating with a heat gun and manual agitation led to opaque suspensions, which were subsequently mixed by vortex twice, for 1 min with a Scientific Products Deluxe Mixer. The suspensions were then sonicated in a water bath for an hour at 70-85 ºC using a Swen Sonic Corporation sonicating device, then placed in a –20 ºC freezer. After thawing, the mixtures were frozen again and then thawed for analysis. 500 µL Milli-Q water was placed in the reference cell and samples were manually shaken before 500

μL aliquots were injected into the 3 sample cells. Fresh samples were used for duplicate evaluations to ensure the reproducibility of obtained results.

B. Instrumentation. A Hart Scientific differential scanning calorimeter was used for calorimetric measurements. Each of 4 metallic ampules or cells was cleaned by rinsing with solvents in the order: Milli-Q water, acetone, ethanol and methanol; caps were cleaned by rinsing with Milli-Q water and ethanol only, as the other solvents disturbed the rubber gaskets. Ampules and caps were dried in a 110 °C oven for at least 12 hours and cooled to room temperature immediately before use. Scans were run with a 5 ºC/h heating rate under a steady stream of argon. The scans included a 30 min hold period at 20 ºC, a scan-up to 60 ºC to obtain endothermic peaks, a 10 min hold at 60 ºC followed by a scan-down to 20 ºC for exothermic evaluations.

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