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Facile Synthesis of Saponins Containing 2,3-Branched Oligosaccharides by Using Partially Protected Glycosyl Donors

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Abstract: Two natural saponins **1** and **2**, isolated from *Solanum indicum* L., and containing 2,3-branched sugar moieties, have been efficiently synthesized. Partially protected monosaccharide and disaccharide donors were used to facilitate target synthesis. Stereo factors were critical in incorporating 2,3-branched sugars on steroid aglycones. Saponin **1** was synthesized in five steps and 30% overall yield, while saponin **2** was obtained using six straightforward sequential reactions in 31% overall yield. Saponin **2** shows promising cytotoxic activity toward human hepatocellular carcinoma BEL-7402 with an IC_{50} of <6 μg/mL.

Saponins constitute a structurally and biologically diverse class of molecules that are widely distributed in terrestrial plants and in certain marine organisms.[1] Most traditional Chinese medicines contain saponins as major components and, thus, represent glycoconjugate templates in drug design and development.[2] Dioscin (diosgenyl 2,4-di-*O*-α-L-rhamnopyranosyl-*β*-D-glucopyranoside), Pa (diosgenyl α-L-rhamnopyranosyl-(1→2)-[α-L-arabinofuranosyl-(1→4)]-*β*-D-glucopyranoside), methyl protodioscin (3-*O*-(2,4-di-*O*-α-L-rhamnopyranosyl-*β*-D-glucopyranosyl)-26-*O*-(*β*-D-glucopyranosyl)-22-methoxy-25(*R*)-furost-5-ene-3*β*,26-diol), and balanitin 7 (diosgenyl α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)-*β*-D-glucopyranosyl-(1→4]-*β*-D-glucopyranoside) are all clinically recognized for their cardiovascular, antifungal, and antitumor activities.[3] Both the carbohydrate and steroid components of saponins are structurally essential for their bioactivities.[4] For example, removal of ester-linked tetrasaccharide from julibroside, a bioactive saponin isolated from *Albizia julibrissin*, dramatically decreases its cytotoxicity against KB cells,[5] and the rhamnose moiety of solamargine (solasodinyl 2,4-di-*O*-α-L-rhamnopyranosyl-*β*-D-glucopyranoside) plays a crucial role in triggering cell death by apoptosis.[6] The great difficulty associated with purification of closely related saponins from natural sources makes chemical synthesis the most realistic approach for obtaining homogeneous saponins. Significant effort has been directed toward the development of novel, powerful glycosylation reactions or strategies to access defined glycoconjugates.[7] Natural saponins containing 2,3-branched carbohydrate moieties are typically assembled in two ways. The first strategy is to couple a 4,6-benzylidenated reducing end sugar unit to C-3 of the steroid or triterpene aglycone. Protection group manipulation (2-OH/3-OH), followed by glycosylation (3-OH/2-OH), deprotection (2-OH/3-OH), and a second glycosylation (2-OH/3-OH), furnishes the natural saponin having a 2,3-dibranched sugar structure.[8] The disadvantages of this strategy are that it is a lengthy and low-efficiency synthesis. More importantly, it is sometimes problematic to remove the 2-acyl protecting group prior to a second glycosylation step.[9] In the second strategy, a 2,3-branched oligosaccharide is first prepared and used to glycosylate the aglycone in the final step. Unfortunately, the final glycosylation step often affords an α,*β* mixture due to the absence of C-2 neighboring group participation.[10,5b] We recently described a new strategy for preparing natural saponins having 2,4-branched oligosaccharides that relies on partially protected glycosyl donors.[11] We report here the extension of this strategy to the facile synthesis of natural saponins containing 2,3-branched oligosaccharides, diosgenyl 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[*β*-D-glucopyranosyl-(1→3)]-*β*-D-galactopyranoside} (**1**) and diosgenyl 3-*O*-{α-L-rhamnopyranosyl-(1→2)-[*β*-D-xylopyranosyl-(1→3)]-*β*-D-galactopyranoside} (**2**). These saponins had previously been extracted from *Solanum indicum* L.,[12] a traditional Chinese medicinal plant that has long been used for its antiinflam-

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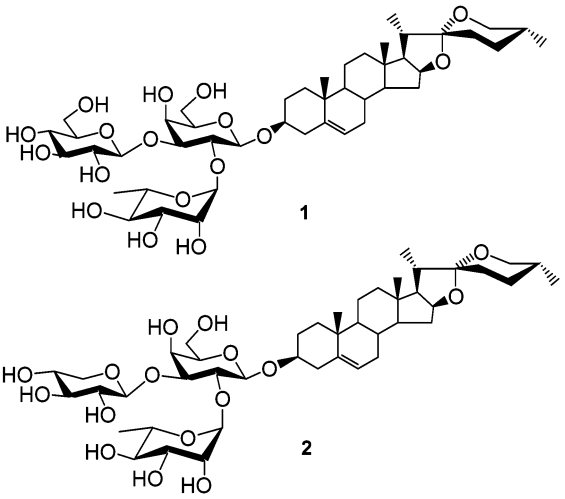
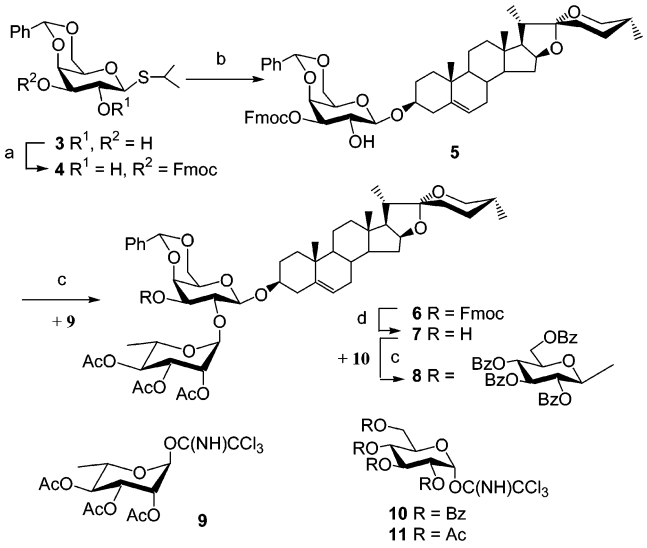


FIGURE 1. Structure of saponin target compounds **1** and **2**.

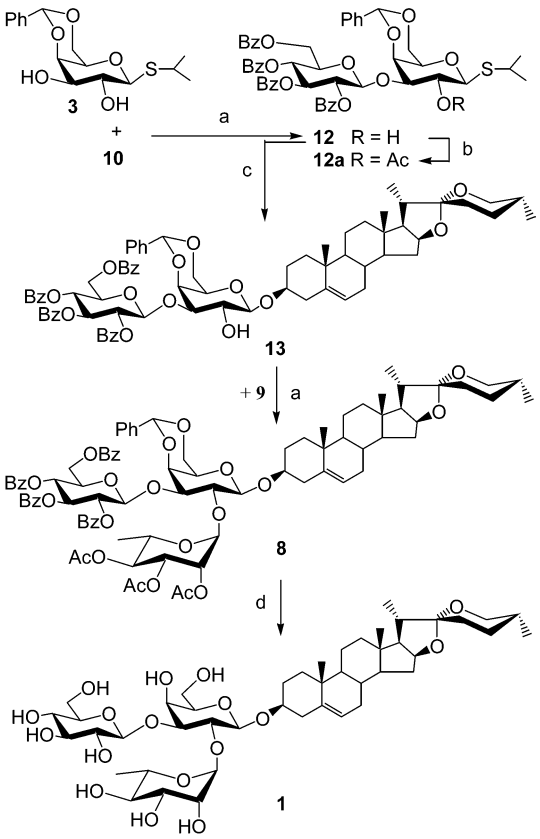
SCHEME 1. Attempted Synthesis of Saponin 1[a]



[a] Reagents and conditions: (a) FmocCl, Pyr, DMAP, 0 °C to room temperature, 87%. (b) Diosgenin, NIS, TMSOTf, CH_2Cl_2, −42 °C, 75%. (c) TMSOTf, CH_2Cl_2, 0 °C, 88% for **6**; 27% for **8**. (d) Et_3N, 85% from **5**.

matory activity.In our previous study,[11] we showed that direct coupling of isopropyl 4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside (**3**) with diosgenin gave complicated mixtures. Thus, in our first attempt to synthesize **1**, the 3-OH of **3** was regioselectively protected by 9-fluorenylmethyloxycarbonyl (Fmoc) using FmocCl in pyridine, yielding partially protected donor **4**,[13] which was used to glycosylate diosgenin affording saponin derivative **5** (Scheme 1) in 75% yield. Glycosylation of **5** with rhamnosyl imidate **9** gave disaccharide glycoside **6**, and Et_3N-promoted removal of Fmoc furnished acceptor **7** in one-pot (85% for two steps). In this straightforward strategy, the final glycosylation of saponin derivative **7** with

SCHEME 2. Successful Synthesis of Saponin 1[a]



[a] Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 86% for **12**; 84% for **8**. (b) Ac_2O, Pyr. (c) Diosgenin, NIS, TMSOTf, CH_2Cl_2, −42 °C, 63%. (d) Aqueous 80% AcOH, then aqueous 1 N NaOH, MeOH/CH_2Cl_2 2:1, 81%.

glucosyl imidate **10** in CH_2Cl_2 with trimethylsilyl trifluoromethanesulfonate (TMSOTf) promotion was problematic. The crude protected saponin **8** was obtained in a low yield (∼27%). MS analysis showed the presence of **8**, but a sufficiently pure sample for NMR analysis could not be obtained. A detailed evaluation of this reaction suggested that the 3-OH of the galactosyl moiety was encircled by the 4,6-benzylidene group and the fully protected rhamnosyl residue, making the 3-OH group sterically inaccessible for glycosylation. To circumvent this problem, fully benzoylated donor **10** was replaced with a smaller acetylated glucosyl imidate **11**; however, this reaction was sluggish, indicating that Scheme 1 was an impractical route to target saponin.

We next turned our attention to an alternative strategy for the efficient synthesis of natural saponin **1** utilizing a partially protected disaccharide donor **12** (Scheme 2). To these ends, sugar diol **3** was glycosylated with imidate donor **10** in anhydrous CH_2Cl_2 at 0 °C using TMSOTf catalyst, regioselectively affording the 1→3-linked disaccharide **12** in 86% yield.[14] To confirm its structure, compound **12** was acetylated with Ac_2O in pyridine to generate **12a**. The ^1H NMR spectra of **12a** showed an H-2 signal (δ 5.31 ppm) shifted downfield from the corresponding H-2 signal of **12** (δ 3.90 ppm). Glycosylation of diosgenin with partially protected disaccharide

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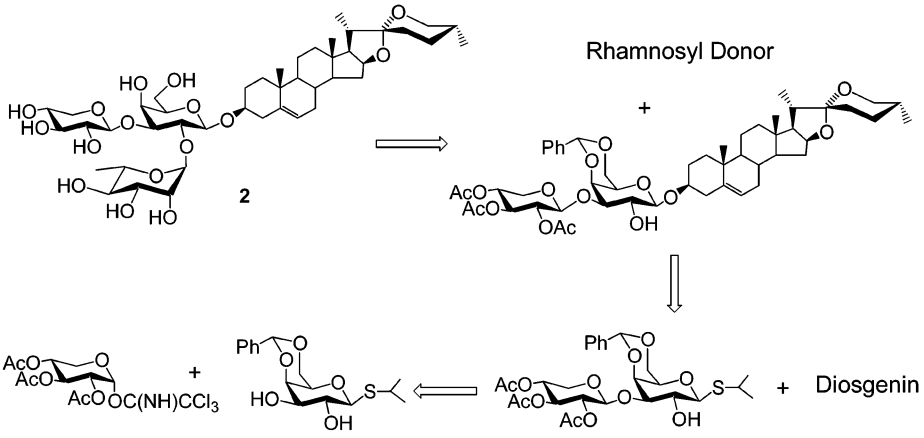
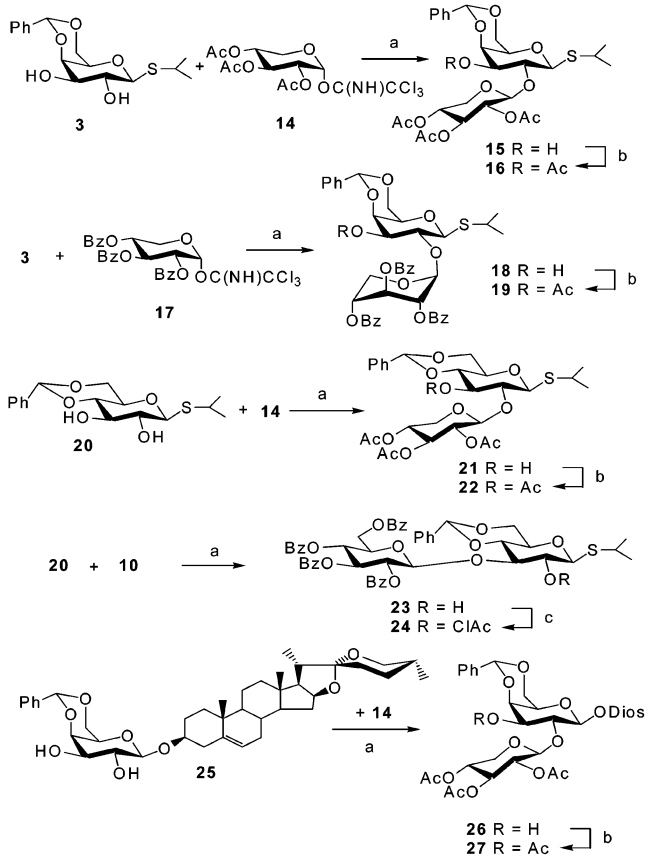
SCHEME 3. Retrosynthetic Analysis of Saponin 2



donor **12** in dry CH_2Cl_2 at 0 °C, under the promotion of *N*-iodosuccinimide (NIS) and TMSOTf, afforded **13** in 63% yield. Introduction of the rhamnosyl moiety **9** into **13** under standard glycosylation conditions afforded the protected saponin derivative **8**. Treatment of **8** in aqueous 80% acetic acid and 1 N NaOH gave the natural saponin **1** in five steps and a 30% overall yield.

With the successful completion of the synthesis of saponin **1**, our attention was turned to the synthesis of natural product **2**, which we thought could be prepared in a similar way (Scheme 3). Surprisingly, when galactose diol **3** was glycosylated with acetylated xylopyranosyl imidate **14** (Scheme 4), the 1→2-linked disaccharide **15** was obtained as the major product (54%), together with the 2,3-dixylosylated trisaccharide (35%) and recovered starting material **3**. To establish the linkage position, **15** was acetylated with acetic anhydride in pyridine to give **16**. The ^{1}H NMR spectrum of **16** showed the H-2 at δ 4.16 ppm and the H-3 at δ 4.93 ppm, confirming the presence of a 1→2 glycosidic bond in **15**. The desired 1→3-linked disaccharide could not be obtained as a major product by changing solvent, catalyst, or reaction temperature. Curious about this regioselectivity, we undertook a detailed investigation of the scope of this reaction (Scheme 4). Glycosylation of galactosyl diol **3** with benzoylated xylopyranosyl imidate **17** afforded isopropyl 2,3,4-tri-*O*-benzoyl-β-D-(1→2)-4,6-*O*-benzylidene-1-thio-β-D-galactopyranoside **18** in 65% yield. The fully assigned ^{1}H NMR spectrum of the acetylated derivative **19** confirmed its 1→2 linkage. Interestingly, the xylosyl residues in **18** and **19** adopt a $^1C_4$ conformation, instead of the typical $^4C_1$ form. This conformational assignment was supported by the small coupling constants observed for **19** ($J_{1,2} = 3.9$, $J_{2,3} = J_{3,4} = 5.7$, $J_{4,5a} = 4.2$, $J_{4,5b} = 3.4$ Hz). When glucopyranosyl 2,3-diol **20** was glycosylated with xylosyl donor **14**, a 1→2-linked disaccharide **21** (48%) was again isolated. In contrast, glucosyl donor **10** predominantly afforded the β-(1→3)-linked disaccharide **23** (83%), as supported by ^{1}H NMR spectra analysis of its chloroacetylated derivative **24**. Xylosylation of the diosgenyl galactopyranoside **25** also afforded the undesired β-(1→2) product **26**, confirming that this was an unsuccessful strategy for synthesizing target **2**. Realizing that regio- and stereochemical control is critical in synthesis of natural saponins and inspired by the observation that glucosyl and xylosyl donors show different reactivity in

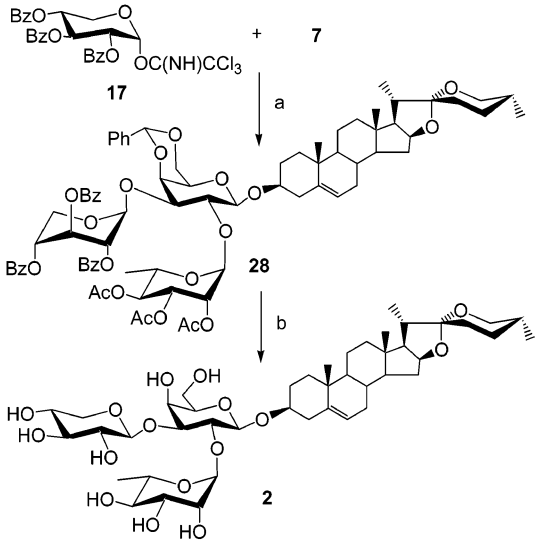
SCHEME 4. Studies on Regioselective Glycosylation[a]



[a] Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 54% for **15**; 65% for **18**; 48% for **21**; 83% for **23**; 51% for **26**. (b) Ac_2O, Pyr. (c) ClAcCl, Pyr.

glycosylation (as observed in Scheme 4, **20** + **14** and **20** + **10**), we attempted to xylosylate the 3-position of **7**, despite our previous observation that incorporation of **10** into **7** had failed (Scheme 1). Thus, the acetyl-protected xylosyl imidate **14** was initially used to glycosylate the disaccharide glycosyl acceptor **7**. Only trace amounts of product were obtained, and the rapid consumption of **14** was observed by TLC. In contrast, glycosylation of acceptor **7** with benzoylated donor **17**, carried out in dry CH_2Cl_2 with catalytic TMSOTf, afforded the protected

SCHEME 5. Total Synthesis of Natural Saponin 2[a]



[a] Reagents and conditions: (a) TMSOTf, CH_2Cl_2, 0 °C, 78%. (b) Aqueous 80% AcOH, then aqueous 1 N NaOH, MeOH/CH_2Cl_2 2:1, 85%.

saponin derivative **28** in 78% isolated yield. The small proton coupling constants of the xylosyl residue ($J_{1,2}$ < 1.0, $J_{2,3} = J_{3,4} = 3.2$, $J_{4,5a}$ < 1.0, $J_{4,5b} = 2.1$ Hz) of saponin derivative **28** shows that benzoylated xylose residue takes the $^1C_4$ conformation instead of typical $^4C_1$ form. The xylosyl C-1 signal appears at δ 100.03 ppm in the ^{13}C NMR spectra, confirming its β-linkage to the galactosyl residue. Deprotection of **28** using aqueous 80% acetic acid, followed by deacylation with 1 N NaOH furnished natural saponin **2** in six steps from commercially available isopropyl β-D-1-thiogalactopyranoside (IPTG) and in a 31% overall yield. A doublet, corresponding to xylosyl H-1, at δ 5.05 ppm (J = 7.5 Hz) in ^1H NMR spectra indicates the expected xylosyl $^4C_1$ conformation in natural product **2**. The cytotoxicity of compounds **1** and **2** was examined using the procedure of Connolly et al.[15] Saponin **2** showed good activity (IC_{50} < 6 μg/mL) toward human hepatocellular carcinoma BEL-7402 cells. The combinatorial synthesis of analogues of **2** is currently under investigation by our group.

In conclusion, an efficient and practical method has been developed for the synthesis of saponins with 2,3-branched oligosaccharide moieties. The key to this approach is the use of partially unprotected thioglycoside donors, resulting in significantly simplified protecting group manipulation and oligosaccharide assembly. The results of the present investigation should be of value in the synthesis of 2,3-branched natural steroidal glycosides.[16] Importantly, the application of this method in combinatorial chemistry may generate an efficient entry into libraries of more complex glycoconjugates.[17]

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Experimental Section

Diosgenyl 3-*O*-Fluorenylmethoxycarbonyl-4,6-di-*O*-benzylidene-β-D-galactopyranoside (5). To a solution of compound **4** (1.1 g, 2.01 mmol) and diosgenin (833 mg, 2.01 mmol) in anhydrous CH_2Cl_2 (10 mL) were added NIS (497 mg, 2.21 mmol) and a catalytic amount of TMSOTf (36 μL, 0.2 mmol) at −42 °C under N_2 protection. The mixture was stirred under these conditions for 45 min, at the end of which time TLC indicated that the reaction was complete. The reaction mixture was neutralized with Et_3N and concentrated to dryness. The residue was subjected to column chromatography on a silica gel column with 5:1 petroleum ether/EtOAc as the eluent to give **5** as a white solid (1.34 g, 75%); $[\alpha]^{20}_D$ −34° (*c* 1.3, $CHCl_3$). Selected ^1H NMR ($CDCl_3$): δ 0.79 (d, 6 H, J = 6.2 Hz), 0.89−0.96 (m, 2 H), 0.97 (d, 3 H, J = 6.9 Hz), 1.03 (s, 3 H), 1.04−2.38 (m, 22 H), 3.37 (t, 1 H, J = 10.9 Hz), 3.46−3.50 (m, 2 H), 3.55−3.66 (m, 1 H), 4.04−4.09 (m, 2 H), 4.27−4.34 (m, 2 H), 4.35−4.50 (m, 5 H), 4.75 (dd, 1 H, J = 10.2, 3.7 Hz), 5.34 (br d, 1 H, J = 5.6 Hz), 5.51 (s, 1 H), 7.42−8.11 (13 H, *Ph*). Anal. Calcd for $C_{55}H_{66}O_{10}$: C, 74.47; H, 7.50. Found: C, 74.69; H, 7.41.

Diosgenyl 2,3,4,6-Tetra-*O*-benzoyl-β-D-glucopyranosyl-(1→3)-4,6-di-*O*-benzylidene-β-D-galactopyranoside (13). To a solution of compound **12** (452 mg, 0.50 mmol) and diosgenin (207 mg, 0.50 mmol) in anhydrous dichloromethane (8 mL) were added NIS (124 mg, 0.55 mmol) and a catalytic amount of TMSOTf (11 μL, 0.06 mmol) at −42 °C under N_2 protection. The reaction mixture was stirred under these conditions for 45 min, at the end of which time TLC indicated the completion of the reaction. The mixture was then neutralized with Et_3N and concentrated. The residue was subjected to column chromatography on a silica gel column with 3:1 petroleum ether/EtOAc as the eluent to give **13** as a white solid (391 mg, 63%); $[\alpha]^{20}_D$ −24° (*c* 1, $CHCl_3$). Selected ^1H NMR ($CDCl_3$): δ 0.79 (d, 6 H, J = 6.3 Hz), 0.97 (d, 3 H, J = 6.9 Hz), 1.00 (s, 3 H), 3.23 (br s, 1 H), 3.37 (t, 1 H, J = 10.9 Hz), 3.45−3.57 (m, 2 H), 3.74 (dd, 1 H, J = 9.6, 3.3 Hz), 3.81−3.88 (m, 2 H), 4.15−4.22 (m, 3 H), 4.33 (d, 1 H, J = 7.6 Hz), 4.39 (q, 1 H, J = 7.5 Hz), 4.53 (dd, 1 H, J = 12.2, 5.3 Hz), 4.68 (dd, 1 H, J = 12.2, 3.0 Hz), 5.32 (d, 1 H, J = 5.0 Hz), 5.38 (d, 1 H, J = 7.9 Hz), 5.40 (s, 1 H), 5.57 (dd, 1 H, J = 9.6, 7.9 Hz), 5.69 (t, 1 H, J = 9.6 Hz), 5.91 (t, 1 H, J = 9.6 Hz), 7.25−8.05 (m, 25 H). Anal. Calcd for $C_{74}H_{82}O_{17}$: C, 71.48; H, 6.65. Found: C, 71.69; H, 6.48.

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Supporting Information Available: Preparation and physical data for compounds **3**−**8**, **12**, **12a**, **13**, **1**, **16**, **19**, **24**, **25**, **27**, **28**, and **2**. This material is available free of charge via the Internet at http://pubs.acs.org.

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