Chemoselective Glycosylation Strategy for the Convergent Assembly of Phytoalexin-Elicitor Active Oligosaccharides and Their Photoreactive Derivatives

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Received May 21, 1999

A highly convergent route for the synthesis of branched glucosides having phytoalexin-elicitor activity has been developed. The readily available building blocks **2**, **3**, and **6** were used for the assembly of a core trisaccharide in two chemo- and regioselective glycosylations. Compound **7** was converted into the artificial spacer-containing glycosyl acceptor **9**. Regioselective condensation of fragment **7** with **9** led to the formation of fully protected hexasaccharide **14**. Deprotection of hexasaccharide **14** followed by the reaction with 4-azidosalicylate gave compound **1b**. It was established that compound **1b** is a photoreactive compound. Reaction time and conditions were established for photolysis of compound **1b** and labeling with radioactive iodine. A soybean root binding site for an elicitor-active hepta-β-glucoside bound compound **1b** with the same affinity as the underivatized hepta-β-glucoside.

Introduction

Oligosaccharides such as fungal β-glucans are regarded as essential players in the cellular communication between host plants and fungal pathogens. This communication is based on specific recognition of signaling or eliciting molecules at the plant cell surface, which requires the functional expression of a receptor capable of transmitting the signal to the inside of the cell. Subsequent transmission of the signal within the cell leads to the induced biosynthesis of antimicrobial phytoalexins and possibly activation of other plant defense responses. Specific binding proteins for a branched hepta-β-glucoside elicitor have been the subject of extensive studies in several laboratories, because this molecule was one of the first oligosaccharide signals to be purified to homogeneity and structurally characterized.[1] The existence of a specific plasma membrane binding site for this molecule has been established by binding studies that utilized, as the labeled ligand, the hepta-β-glucoside covalently coupled to iodinatable functional groups.[2] Binding of the radiolabeled hepta-β-glucoside to the root membranes from the soybean displays characteristics typical of physiological receptors: saturability, high affinity [apparent dissociation constant ($K_d \approx 1$ nm)], low abundance [binding site concentration ($B_{max} \approx 1$ pmol/mg protein)], and high degree of specificity.[2]

Previous efforts to purify the hepta-β-glucoside binding proteins from the mixture of proteins solubilized from soybean root membranes have primarily relied on affinity chromatography using a matrix carrying an immobilized mixture of elicitor-active glucan fragments.[3] However, it is not yet clear which of the proteins present in the affinity-purified fractions are responsible for elicitor-binding activity. Photoaffinity labeling is one method that has often been used to identify polypeptides carrying a specific binding site for a ligand of interest. Previously published photoaffinity labeling experiments carried out on soybean membrane extracts were performed with a photoreactive derivative prepared by a series of chemical modifications of the hepta-β-glucoside.[3a,b,4] The conversion of the arylamine to the azido derivative carries a great risk of affecting the integrity of the hepta-β-glucoside because of the exposure of the oligosaccharide to sulfuric acid. Therefore, we decided to synthesize a de novo photosensitive oligoglucoside ligand where the synthetic steps for introduction of the photosensitive moiety can be performed under mild and controlled reaction conditions.

We report here the successful synthesis of a hexa-β-glucoside (**1a**) mimicking the effect of the original hepta-β-glucoside and its conversion into a photoreactive 4-azidosalicylate derivative (**1b**). Additionally, we confirmed that the time and conditions for the photolysis of this aryl azido derivative were the same as for similar azido compounds. Moreover, we established the conditions for labeling of the 4-azidosalicylate moiety with radioactive iodine. Finally, we determined that these de novo oligoglucosides have the ability to competitively inhibit binding of radiolabeled hepta-β-glucoside to soybean root membrane proteins with a similar efficiency as the unlabeled hepta-β-glucoside. These controls are essential for the validation of the synthesis of these analogues and establish the conditions for performing the photoaffinity experiments that will identify the proteins carrying a specific binding site for the hepta-β-glucoside ligand.

Results and Discussion

The artificial spacer-containing derivative **1a** is a suitable derivative for the selective and controlled incor-

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(3) (a) Cosio, E. G.; Frey, T.; Ebel, J. *Eur. J. Biochem.* **1992**, *204*, 1115−1123. (b) Mithöfer, A.; Lottspeich, F.; Ebel, J. *FEBS Lett.* **1996**, *381*, 203−207. (c) Umemoto, N.; Kakitani, M.; Iwamatsu, A.; Yamaoka, N.; Ishida, I. *Proc. Natl. Acad. Sci. U.S.A.* **1997**, *94*, 1029−1034.

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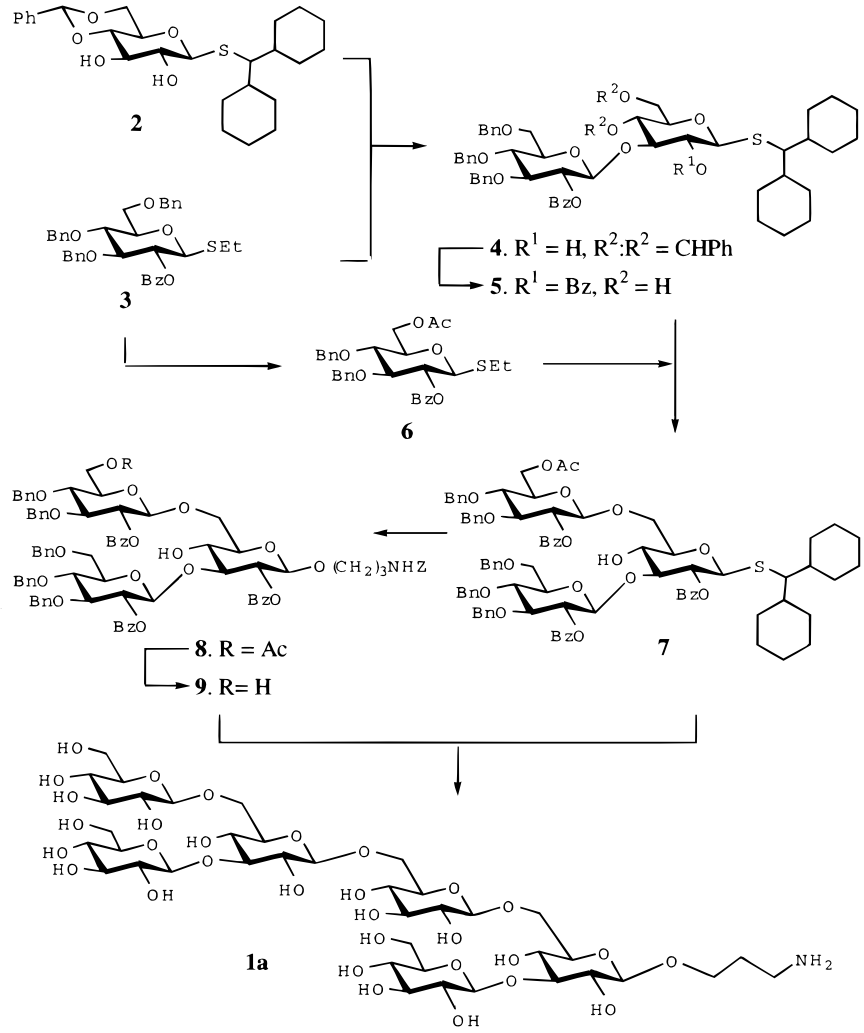
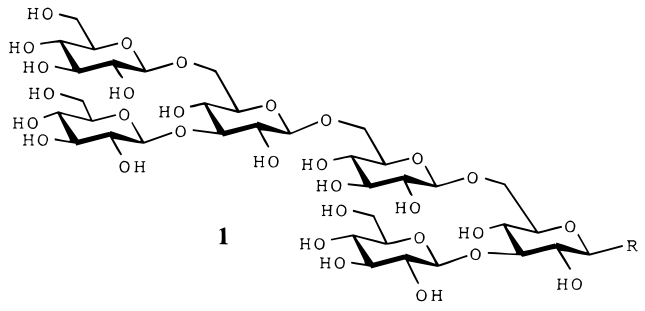
10.1021/jo990836o CCC: $18.00
Published on Web 09/25/1999

Scheme 1



poration of the photoreactive 4-azidosalicylate moiety to give the target compound **1b** (Figure 1).[5] We have designed a synthetic plan in which all acceptors and donors that are required for the preparation of target compound **1a** are obtained from key building blocks **2** and **3** (Scheme 1). Furthermore, the core trisaccharide **7** was used as the starting material for the preparation of the spacer-containing trisaccharide **9** but was also used for the extension of the latter derivative to give a hexasaccharide.

Thioglycosides were selected as glycosyl donors and acceptors. These sugar derivatives are stable to a wide range of reaction conditions commonly used in carbohydrate chemistry. Therefore, the anomeric thio alkyl group first can act as effective protecting group; however, at an appropriate stage they can readily be activated with a wide range of thiophilic reagents to give *O*-glycosides in high yields.



1a $R = O(CH_2)_3NH_2$ **1b** R =

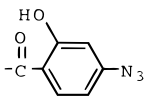


Figure 1.

Another attractive feature of thioglycosyl donors and acceptors is that they can be assembled to oligosaccharides by chemoselective glycosylation strategies. This approach is based on the observation that protecting-

(5) For other synthetic approaches leading to oligomeric β-glucosides see: (a) Ossowski, B. P.; Pilotti, Å; Garegg, P. J.; Lindberg, B. *Angew. Chem., Int. Ed. Engl.* **1983**, *10*, 793. (b) Ossowski, B. P.; Pilotti, Å; Garegg, P. J.; Lindberg, B. *J. Biol. Chem.* **1984**, *259*, 11337. (c) Fügedi, P.; Birberg, W.; Garegg, P. J.; Pilotti, Å *J. Carbohydr. Res.* **1987**, *164*, 297. (d) Fügedi, P.; Garegg, P. J.; Kvarnström, I.; Svansson, L. *Carbohydr. Chem.* **1988**, *7*, 389. (e) Birberg, W.; Fügedi, P.; Garegg, P. J.; Pilotti, Å *J. Carbohydr. Res.* **1989**, *8*, 47. (f) Hong, N.; Ogawa, T. *Tetrahedron Lett.* **1990**, *31*, 3179. (g) Lorentzen, J. P.; Helpap, B.; Oswald, L. *Angew. Chem., Int. Ed. Engl.* **1991**, *12*, 1681. (h) Verduyn, M.; Douwes, P. A. M.; van der Marel, G. A.; van Boom, J. H. *Tetrahedron* **1993**, *49*, 7301. (i) Hong, N.; Nakahara, Y.; Ogawa, T. *Proc. Jpn. Acad.* **1993**, *69B*, 55. (j) Yamada, H.; Harada, T.; Takahashi, T. *J. Am. Chem. Soc.* **1994**, *116*, 7919−7920. (k) Timmers, C. M.; Turner, J. J.; Ward, C. M.; van der Marel, G. A.; Kouwijzer, M. L. C. E.; Grootenhuis, P. D. J.; van Boom, J. H. *Chem. Eur. J.* **1997**, *3*, 920. (l) Nicolaou, K. C.; Winssinger, N.; Pastor, J.; Deroose, F. *J. Am. Chem. Soc.* **1997**, *119*, 449−550. (m) Nicolaou, K. C.; Watanabe, N.; Li, J.; Pastor, J.; Winssinger, N. *Angew. Chem., Int. Ed.* **1998**, *37*, 1559−1561.

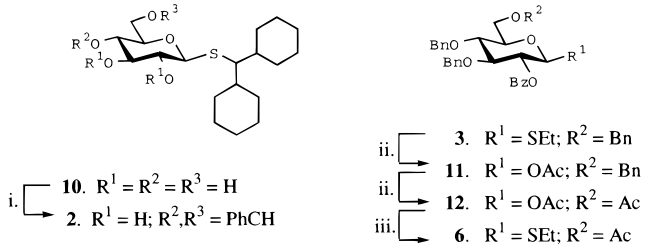
group patterns can control anomeric reactivities of thioglycosides. Thus, it is possible that a thioglycosyl donor can be coupled with a thioglycosyl acceptor of lower anomeric reactivity to give a well-defined product. In a subsequent glycosylation, the resulting product can act as a glycosyl donor and react with a thioglycosyl acceptor that has even a lower reactivity. This process can be repeated several times to give rather complex structures without the need for intermediate protecting-group chemistry. The attractiveness of this approach has been further enhanced by the creation of a database that makes it possible to predict the relative reactivities of a large number of *p*-methylphenyl thioglycosides.[6] In general, an electron-donating ether substituent at C-2 activates and an electron-withdrawing ester functionality deactivates the reactivity of an anomeric leaving group. Furthermore, cyclic acetals and ketals reduce anomeric reactivities, and thioglycosides protected with this type of protecting group have reactivities between that of acylated- and benzylated derivatives.

Control of the reactivity of thioglycosides by a C-2 protecting group imposes limitations because this functionality is a critical determinant of the anomeric selectivity of a glycosylation. To address this problem, we have introduced an approach whereby anomeric reactivities are controlled by the size of an alkyl thio leaving group.[7]

Target compound **1a** was assembled by a novel chemoselective glycosylation strategy whereby the reactivities of thioglycosyl donors and acceptors are controlled by a new combination of properly selected protecting groups and use of either an anomeric thioethyl or bulky dicyclohexylmethanethio group. We envisaged that the electronically deactivated (C-2 benzoyl) thioglycoside **3** is of higher reactivity than the sterically (bulky anomeric thio group) and torsionally (benzylidene group) deactivated thioglycoside **2**. Furthermore, it was expected that the bulky dicyclohexylmethane thio group would sterically block the C-2 hydroxyl. Thus, a chemo- and regioselective glycosylation of **2** with **3** should yield **4**. Disaccharide **4** can easily be transformed into glycosyl acceptor **5**, which will be glycosylated with donor **6** to give core trisaccharide **7**. In the latter reaction, we will take advantage of the lower reactivity of the sterically and electronically deactivated thioglycoside **5** compared to that of the electronically deactivated compound **6**. The well-known higher reactivity of a primary sugar hydroxyl over a secondary one is also exploited in this reaction.

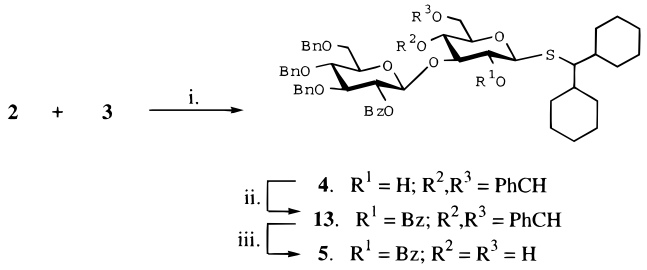
The synthesis of monosaccharide building blocks **2**, **3**, and **6** was undertaken as detailed in Scheme 2. Thioglycosyl acceptor **2** was obtained in 82% yield by the treatment of dicyclohexylmethyl 1-thio-*β*-D-glucopyranoside (**10**)[7] with benzaldehyde dimethyl acetal and a catalytic amount of camphorsulfonic acid (CSA) in DMF. Ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-*β*-D-glucopyranoside (**3**) was easily prepared by a known procedure.[8] Compound **3** was also the precursor for the synthesis of **6**. Thus, treatment of **3** with a catalytic amount of H_2SO_4 in $HOAc/Ac_2O$ (1/1, v/v)[9] resulted in acetolysis of the anomeric ethyl thio moiety and 6-*O*-benzyl group to give

Scheme 2[a]



[a] (i) Benzaldehyde dimethylacetal, CSA, DMF; (ii) H_2SO_4 (cat.), $HOAc/Ac_2O$ (1/1); (iii) EtSH, TMSOTf, CH_2Cl_2.

Scheme 3[a]



[a] (i) NIS, TMSOTf, CH_2Cl_2/Et_2O (1/1), −70 °C; (ii) BzCl, DMAP, py; (iii) TFA, H_2O, CH_3CN/CH_2Cl_2 (2/1).

12 in a yield of 54%. Compound **11** was isolated in high yield (80%) when the reaction time was shortened. This observation shows that the anomeric ethyl thio group is more acid labile than the benzyl ether at C-6. Reaction of **12** with ethanethiol in the presence of trimethylsilyl triflate gave the required glycosyl donor **6** (61%).

With significant quantities of building blocks in hand, the hexasaccharide was assembled. NIS/TMSOTf-mediated coupling of **2** with **3** in dichloromethane (DCM)/diethyl ether at −70 °C furnished disaccharide **4** in an acceptable 40% yield along with a regioisomer in which glycosylation had taken place at C-2 (15%) (Scheme 3). The two regioisomers could be separated by silica gel column chromatography. When the glycosylation was performed at room temperature, a loss of regioselectivity accompanied by the formation of a considerable amount of trisaccharide was observed. However, no side products resulting from activation of the bulky anomeric dicyclohexylmethane thio group could be detected. Benzoylation of **4** with benzoyl chloride and *N*,*N*-(dimethylamino)-pyridine in pyridine (→ **13**), followed by removal of the benzylidene group in DCM/acetonitrile with TFA/H_2O, yielded disaccharide acceptor **5** in an 87% overall yield. The ^1H NMR spectrum of **13** showed a strong downfield shift of H-2, demonstrating that the glycosylation had taken place at C-3.

The glycosyl donor **6** was used in a second NIS/TMSOTf-mediated chemo- and regioselective glycosylation with the acceptor **5** at −70 °C in DCM to afford key trisaccharide **7** in a 50% yield (Scheme 4). In this reaction, we exploit the fact that the electronically deactivated compound **6** has a significant higher anomeric reactivity than compound **5,** which is deactivated electronically by a C-2 benzoyl group and sterically by the bulky dicyclohexylmethane thio moiety. No side products resulting from glycosylation at C-4 of **5** could

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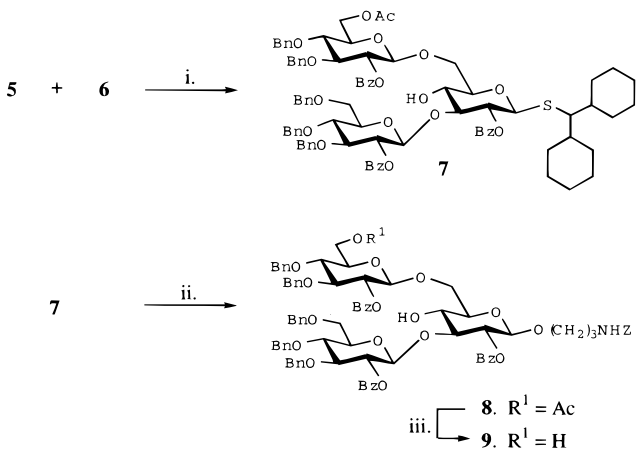
Scheme 4[a]



[a] (i) NIS, TMSOTf, CH_2Cl_2, −70 °C; (ii) NIS, TMSOTf, $HO(CH_2)_3$-NHZ; (iii) KO^{t}Bu, CH_2Cl_2/MeOH (9/1).

be detected. The ^{1}H NMR spectrum of **5** showed that the H-4′ coupled to a hydroxyl signal. The hydroxyl signal disappeared on exposure to D_2O. These experiments prove the regioselectivty of the glycosylation.

The 4-hydroxyl function of glycosyl donor **7** is significantly less reactive than primary hydroxyls as a result of the steric hindrance by the neighboring glycosyl residues and therefore was left unprotected. Condensation of **7** with 3-(benzyloxycarbonylamino)-1-propanol[10] in the presence of NIS/TMSOTf in DCM afforded the spacer-containing trisaccharide **8** in 74% yield. Next, we focused on the selective deacetylation of the 6′-OAc moiety of trisaccharide **8**. In general, *O*-acetyl groups are more labile than *O*-benzoyl groups under both acidic and basic reaction conditions. Critical to our studies was the observation that the acetyl group could be cleaved selectively in the presence of three benzoyl groups. Fortunately, selective deacetylation of **8** could easily be achieved by treatment with a catalytic amount of KO^{t}Bu in DCM\MeOH to afford glycosyl acceptor **9** in a 69% yield. It is useful to note that selectivity was maintained even when a relatively large amount of KO^{t}Bu was used (0.4 equiv, 5 min).

Regioselective NIS/TMSOTf-mediated condensation of **7** with **9** in DCM at room temperature resulted in the formation of hexamer **14** in an excellent 75% yield (Scheme 5). All glycosylations leading to target compound **1a** proceeded with exclusive β-anomeric stereoselectivity as a result of neighboring group participation of C-2 benzoyl groups.

Deprotection of oligomer **14** was accomplished by treatment with KO^{t}Bu in DCM/MeOH (→ **16**), followed by hydrogenation in 10% aqueous EtOH in the presence of $Pd(OAc)_2$ to give the deprotected substrate **1a** in 77% overall yield. The *O*-acetyl protecting group of **14** could selectively be deprotected by employing KO^{t}Bu in DCM\MeOH to give **15**. The structural identity of **1a** was proven by homo- and heteronuclear NMR spectroscopy and high-resolution MS. Finally, a photoreactive group was introduced by reaction of **1a** with succinimido 4-azidosalicylate[11] in triethylammonium bicarbonate buffer/DMF to furnish **1b** in a 52% yield.

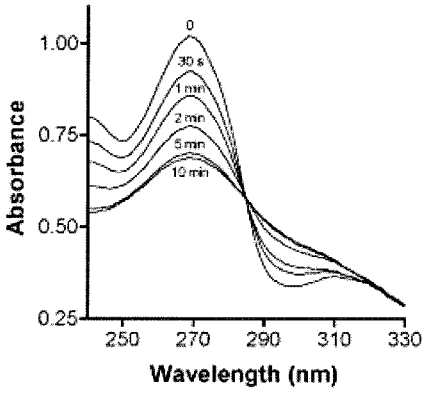
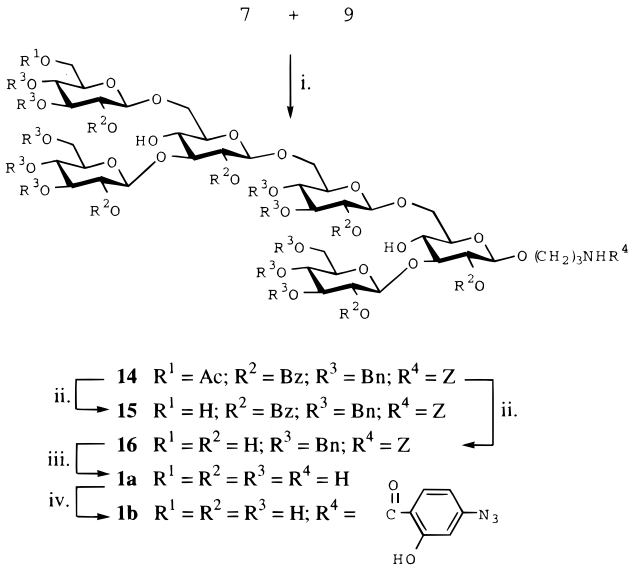


Figure 2. UV light-induced photodecomposition of hexaglucoside **1b**. The sample was dissolved in a 90% (v/v) ethanol solution and was irradiated for various periods of time (as indicated) with a UV light source as described in the Experimental Section.

Scheme 5[a]



[a] (i) NIS, TMSOTf, CH_2Cl_2; (ii) KO^{t}Bu, CH_2Cl_2/MeOH (9/1); (ii) $Pd(OAc)_2$, H_2(1 atm), EtOH, H_2O; (iv) triethylammonium bicarbonate buffer (1.0 M), *N*-hydroxysuccinimido-4-azidosalicylate, DMF.

Compound **1b** shows an absorption maximum at 269 nm (Figure 2), which is consistent with data from other compounds carrying a similar functionality.[11] Ultraviolet irradiation of compound **1b** induces a marked decrease in A_{269} after 30 s. The decrease in A_{269} reaches a maximum after 5 min of irradiation. Further irradiation for an additional 5 min only causes marginal diminution in A_{269}. These results were interpreted as evidence of the light-induced decomposition of the aryl azido function in hexaglucoside **1b** to give a nitrene species, which then undergoes further reactions.[12]

Further validation of the hexaglucosides **1a** and **1b** as valuable mimics of the hepta-β-glucoside elicitor involved testing the compounds as inducers of phytoalexin accumulation in a biological assay, as competitive inhibitors of the binding of the radiolabeled hepta-β-glucoside elicitor to soybean root membrane proteins, and as ligands in direct binding assays with the same proteins.

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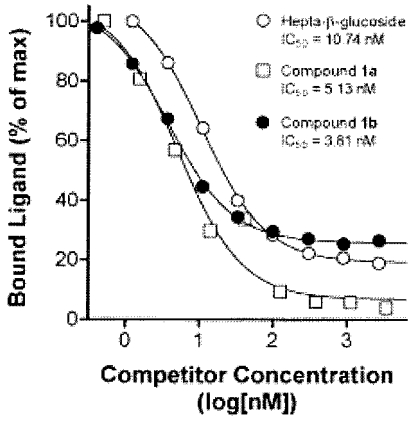

Figure 3. Competitive inhibition, by unlabeled oligoglucosides, of binding of ^{125}I-labeled tyraminylated hepta-β-glucoside elicitor to soybean root membranes. Radiolabeled tyraminylated hepta-β-glucoside (1 nM) was incubated with soybean membranes (0.1 mg of protein) in the presence of increasing amounts of hepta-β-glucoside (○), hexaglucoside **1a** (□), and hexaglucoside **1b** (●). The numbers in the legend refer to the relative binding activity (IC_{50}), which is defined as the concentration of an oligoglucoside required to give 50% inhibition of the binding of radiolabeled hepta-β-glucoside to its binding site on soybean root membranes. These values were obtained by a linear regression analysis of the central portion of the curves. Competitive binding assays were carried out as described in the Experimental Section. The amount of radiolabeled elicitor remaining bound to the membranes was normalized to the amount bound in the absence of any unlabeled oligoglucoside.

Hexaglucoside **1a** was an equally effective inducer of phytoalexin accumulation in soybean cotyledons as the hepta-β-glucoside elicitor (data not shown). Furthermore, both hexaglucosides **1a** and **1b** were as effective as the unmodified hepta-β-glucoside in ligand competition assay with soybean membranes (Figure 3). The IC_{50} values (defined in the figure legend) are 10.7, 5.1, and 3.8 nM for the hepta-β-glucoside elicitor, hexaglucosides **1a** and **1b** respectively. These data confirm the results from our previous structure−activity relationship studies where we showed that the hexaglucoside and heptaglucoside were equally biologically active. We had concluded then that the reducing end of the hepta-β-glucoside was not important for maximum biological activity; however, the three terminal nonreducing glucoses are essential for both the recognition by plant binding proteins and maximum biological activity.[2b,13]

Finally, a radioiodinated derivative of hexaglucoside **1b** was prepared and used in binding assays to demonstrate that this radioligand binds to soybean root membrane proteins as well as the radiolabeled hepta-β-glucoside used previously. Binding of radiolabeled **1b** could be competed by 98−99% with a 1000-fold excess of either unlabeled hepta-β-glucoside elicitor or unlabeled hexaglucoside **1b**. These data indicate that the soybean elicitor binding site has an affinity for the de novo hexaglucosides **1a** and **1b** very similar to the affinity for the hepta-β-glucoside.

Conclusions

The new methodology is a reliable approach for the construction of complex carbohydrates. Furthermore, the combination of regio- and chemoselective glycosylations were shown to be highly effective in the preparation of oligosaccharides. Optimal tuning of the substrates (by torsional, electronic, and anomeric sterical deactivation) combined with the differences in the reactivity of various unprotected hydroxyl functions led to the construction of branched hexasaccharide analogues in a highly convergent fashion.

Experimental Section

General Methods. Gravity column chromatography was performed on silica gel 60 (Merck, 70−230 mesh), and reactions were monitored by TLC on Kieselgel 60 F_{254} (Merck). Detection was effected by examination under UV light and by charring with a solution of 20% (v/v) sulfuric acid in methanol or with a molybdate solution (0.02 M solution of ammonium cerium(IV) sulfate dihydrate and ammonium molybdate(VI) tetrahydrate in aqueous 10% (v/v) H_2SO_4). Size exclusion column chromatography was performed on Sephadex LH-20 or LH-60 (both Pharmacia Biotech AB, Uppsala, Sweden) with dichloromethane/methanol (1/1, v/v) as the eluent or on Sephadex G-15 (Pharmacia Biotech AB, Uppsala, Sweden) and deionized water as the eluent. Extracts were evaporated under reduced pressure at 40 °C (bath). All solvents were distilled in the presence of appropriate drying agents; dichloromethane and toluene were distilled over P_2O_5 and stored over molecular sieves (4 Å). Diethyl ether was distilled over CaH_2, redistilled from $LiAlH_4$, and stored over sodium wire. *N*,*N*-Dimethylformamide was stirred with CaH_2 for 16 h, distilled under reduced pressure, and stored over molecular sieves (4 Å). Methanol was dried by refluxing with magnesium methoxide, distilled, and stored over molecular sieves (3 Å), and pyridine was dried by refluxing with CaH_2, then distilled, and stored over molecular sieves (4 Å).

Dicyclohexylmethyl 4,6-*O*-Benzylidene-1-thio-β-D-glucopyranoside (2). To a solution of dicyclohexylmethyl 1-thio-β-D-glucopyranoside **10** (3.0 g, 7.9 mmol) in DMF (25 mL) were added benzaldehyde dimethyl acetal (1.8 mL, 11.9 mmol) and camphorsulfonic acid (70 mg, 0.3 mmol). The solution was stirred for 18 h at room temperature under reduced pressure (20 mmHg). The reaction mixture was diluted with Et_2O (100 mL) and washed with aqueous 1 M $NaHCO_3$ (1 × 10 mL) and H_2O (3 × 15 mL). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo and coconcentrated with toluene (3 × 15 mL). The residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 95/5, v/v) to yield **2** as an amorphous white solid (3.0 g, 82%): R_f 0.55 (CH_2Cl_2/MeOH, 6/1, v/v); $[\alpha]^{25}_D$ −40.6 (*c* 1); FAB−MS *m*/*z* (%) 485 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 137.0, 129.3−126.4 (C_6H_5CH), 101.9 (C_6H_5CH), 89.2 (C-1), 80.4, 74.5 (2×), 70.4 (C-2,3,4,5), 68.6 (C-6), 61.2 (S*C*H), 41.1, 39.8, 31.8−26.5 (2 C_6H_{11}); ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.50−7.20 (m, 5H, C_6H_5CH), 5.54 (s, 1H, C_6H_5CH), 4.37 (d, 1H, H-1, $J_{1,2}$ 4.4 Hz), 4.30 (dd, 1H, H-6a, $J_{5,6a}$ 5.0, $J_{6a,6b}$ 10.3 Hz), 3.84 (dt, 1H, H-3, $J_{2,3}J_{3,4}$ 9.2 Hz), 3.78 (t, 1H, H-6b, $J_{5,6b}J_{6a,6b}$ 10.3 Hz), 3.60 (t, 1H, H-4, $J_{3,4}J_{4,5}$ 9.2 Hz), 3.50−3.41 (m, 2H, H-2,5), 2.71 (d, 1H, O*H*, *J* 1.8 Hz), 2.60 (d, 1H, O*H*, *J* 1.8 Hz), 2.45 (t, 1H, SC*H*, *J* 5.5 Hz), 2.00−0.90 (m, 22H, 2 C_6H_{11}); HR−FAB MS calcd for $C_{26}H_{38}NaO_5S$ 485.2338, found 485.2346.

Ethyl 6-*O*-Acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-β-D-glucopyranoside (6). Ethyl 2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-1-thio-β-D-glucopyranoside **3** (2.0 g, 3.3 mmol) was dissolved in HOAc/Ac_2O (1/1, v/v, 15 mL). A 2% (v/v) solution of H_2SO_4 in HOAc/Ac_2O (1/1, v/v, 0.2 mL) was added, and the solution was stirred for 24 h at room temperature. Next, the solution was diluted with CH_2Cl_2 (100 mL) and neutralized with aqueous NaOAc (10%, w/v). The organic phase was washed with H_2O (3 × 15 mL), dried ($MgSO_4$), and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography to give **12** as an amorphous white solid (1 g, 54%): R_f 0.41 (CH_2Cl_2/acetone, 98/2, v/v). Compound **12** (950 mg, 1.73 mmol) was dissolved in CH_2Cl_2

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(30 mL). EtSH (0.3 mL, 4.1 mmol) and TMSOTf (50 μL, 0.28 mmol) were added, and the solution was stirred for 18 h. Next, the solution was neutralized with TEA (0.1 mL), diluted with CH_2Cl_2 (100 mL), and washed with H_2O (3 × 15 mL). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 99/1, v/v) to give **6** as a colorless syrup (586 mg, 61%): R_f 0.38 (CH_2Cl_2/acetone, 99/1, v/v); FAB−MS *m/z* (%) 573 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 170.9 (CH_3CO), 165.5 (C_6H_5CO), 137.8−128.0 (2 $C_6H_5CH_2$, C_6H_5CO), 84.6, 83.9, 77.7, 77.4, 72.6 (C-1,2,3,4,5), 75.6, 75.4 (2 $C_6H_5CH_2$), 63.3 (C-6), 24.4 (SCH_2CH_3), 21.1 (CH_3CO), 15.2 (SCH_2CH_3); ^1H NMR ($CDCl_3$, 300 MHz) δ 8.07−7.10 (m, 15H, 2 $C_6H_5CH_2$, C_6H_5CO), 5.31 (t, 1H, H-2, $J_{1,2}J_{2,3}$ 9.6 Hz), 4.88−4.58 (m, 4H, 2 $C_6H_5CH_2$), 4.55 (d, 1H, H-1), 4.39 (dd, 1H, H-6a, $J_{5,6a}$ 1.8, $J_{6a,6b}$ 11.8 Hz), 4.22 (dd, 1H, H-6b, $J_{5,6b}$ 4.6 Hz), 3.87, 3.68 (2 t, 2H, H-3,4), 3.61 (m, 1H, H-5), 2.69 (m, 2H, SCH_2CH_3, *J* 7.4 Hz), 2.04 (s, 3H, CH_3CO), 1.23 (t, 3H, SCH_2CH_3); HR FAB−MS calcd for $C_{31}H_{34}NaO_7S$ 573.1923, found 573.1903.

Dicyclohexylmethyl 3-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (4). To a stirred mixture of compound **3** (244 mg, 0.41 mmol), compound **2** (236 mg, 0.51 mmol), and molecular sieves (4 Å, 2 g) in CH_2Cl_2/Et_2O (1/1, v/v, 6 mL) at −80 °C were added NIS (107 mg, 0.48 mmol) and TMSOTf (4 μL, 0.02 mmol). After stirring for 30 min at −70 °C, the reaction mixture was quenched with TEA (0.02 mL), diluted with CH_2Cl_2 (40 mL) and washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 99/1, v/v) to yield **4** as a white glass (161 mg, 40%): R_f 0.51 (CH_2Cl_2/acetone, 99/1, v/v); $[\alpha]^{25}_D$ +1.60 (*c* 1); FAB−MS *m/z* (%) 1021.5 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 165.7 (C_6H_5CO), 138.3−126.2 (3 $C_6H_5CH_2$, C_6H_5-CO, C_6H_5CH), 101.5 (C_6H_5CH), 101.1 (C-1′), 89.0 (C-1), 82.8, 81.8, 79.4, 77.8, 75.2, 74.6, 74.2, 70.8 (C-2,3,4,5,2′,3′,4′,5′), 75.0, 74.9, 73.6 (3 $C_6H_5CH_2$), 68.6(2x) (C-6,6′), 60.9 (SCH), 41.0, 39.9, 31.8−26.5 (2 C_6H_{11}); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.20−7.30 (m, 25H, 3 $C_6H_5CH_2$, C_6H_5CO, C_6H_5CH), 5.57 (s, 1H, C_6H_5CH), 5.43 (dd, 1H, H-2′, $J_{1',2'}$ 7.7, $J_{2',3'}$ 9.0 Hz), 5.05 (d, 1H, H-1′), 4.90−4.56 (m, 6H, 3 $C_6H_5CH_2$), 4.365 (d, 1H, H-1, $J_{1,2}$ 10.0 Hz), 4.357 (d, 1H, H-6′a, $J_{5',6'a}$ 5.0, $J_{6'a6'b}$ −10.3 Hz), 3.96−3.90 (m, 3H, H-3,4,3′), 3.83 (t, 1H, H-6′b, $J_{5'6'b}$ 10.0 Hz), 3.78 (t, 1H, H-4′, $J_{3'4'}J_{4'5'}$ 9.2 Hz), 3.75 (dd, 1H, H-6a, $J_{5,6a}$ 4.3, $J_{6a,6b}$ −11.0 Hz), 3.70 (dd, 1H, H-6b, $J_{5,6b}$ 2.0 Hz), 3.56 (m, 1H, H-5), 3.52 (m, 1H, H-2), 3.46 (dt, 1H, H-5′, $J_{4',5'}$ 9.2 Hz), 2.70 (d, 1H, O*H*, $J_{2,OH}$ 2.3 Hz), 2.49 (t, 1H, SC*H*, *J* 5.5 Hz), 2.00−1.10 (m, 22H, 2 C_6H_{11}); HR FAB−MS calcd for $C_{60}H_{70}NaO_{11}S$ 1021.4537, found 1021.4545.

Dicyclohexylmethyl 3-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-2-*O*-benzoyl-4,6-*O*-benzylidene-1-thio-β-D-glucopyranoside (13). To a stirred solution of compound **4** (2.1 g, 2.1 mmol) in pyridine (60 mL) were added DMAP (77 mg, 0.6 mmol) and BzCl (0.7 mL, 6.3 mmol). After stirring for 18 h at room temperature, the mixture was diluted with CH_2Cl_2 (60 mL) and washed with H_2O (3 × 15 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was co-concentrated with toluene, MeOH, and CH_2Cl_2, respectively (3 × 15 mL each). The residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 99/1, v/v) to yield **13** as a white glass (2.3 g, 98%): R_f 0.51 (CH_2Cl_2/acetone, 99/1, v/v); $[\alpha]^{25}_D$ +19.3 (*c* 1); FAB−MS *m/z* (%) 1125 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 164.9, 164.5 (2 C_6H_5CO), 138.5−127.8 (3 $C_6H_5CH_2$, 2 C_6H_5CO, C_6H_5CH), 101.4 (C_6H_5CH), 101.0 (C-1′), 88.2 (C-1), 83.0 (C-3′), 79.5 (C-4), 78.4 (C-3), 77.9 (C-4′), 75.3 (C-5′), 74.9, 74.6, 73.5 (3 $C_6H_5CH_2$), 74.0 (C-2′), 73.3 (C-2), 70.7 (C-5), 69.2 (C-6′), 68.6 (C-6), 62.0 (SCH), 40.8, 39.6, 31.7−26.1 (2 C_6H_{11}); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.10−7.00 (m, 30H, 3 $C_6H_5CH_2$, 2 C_6H_5CO, C_6H_5CH), 5.47 (s, 1H, C_6H_5CH), 5.23 (dd, 1H, H-2, $J_{1,2}$ 10.0, $J_{2,3}$ 8.6 Hz), 5.19 (dd, 1H, H-2′, $J_{1',2'}$ 7.7, $J_{2',3'}$ 8.6 Hz), 4.69−4.42 (m, 6H, 3 $C_6H_5CH_2$), 4.47 (d, 1H, H-1), 4.26 (dd, 1H, H-6a, $J_{5,6a}$ 5.0, $J_{6a,6b}$ −10.5 Hz), 4.19 (t, 1H, H-3, $J_{3,4}$ 8.6 Hz), 3.83 (t, 1H, H-4, $J_{4,5}$ 9.0 Hz), 3.62 (dd, 1H, H-6′a, $J_{5',6'a}$ 2.0, $J_{6'a,6'b}$ −11.0 Hz), 3.59 (t, 1H, H-3′), 3.53 (dd, 1H, H-6′b, $J_{5',6'b}$ 11.0 Hz), 3.40 (m, 1H, H-5), 3.37 (m, 1H, H-5′), 2.20 (t, 1H, SC*H*, *J* 5.9 Hz), 1.90−0.60 (m, 22H, 2 C_6H_{11}); HR FAB−MS calcd for $C_{67}H_{74}NaO_{12}S$ 1125.4799, found 1125.4763.

Dicyclohexylmethyl 3-*O*-(2-*O*-Benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)-2-*O*-benzoyl-1-thio-β-D-glucopyranoside (5). To a stirred solution of compound **13** (2.3 g, 2.1 mmol) in CH_3CN/CH_2Cl_2 (50 mL, 2/1, v/v) were added H_2O (0.3 mL) and TFA (0.2 mL). After stirring for 3 h at room temperature, the mixture was washed with aqueous 1 M $NaHCO_3$ (1 × 10 mL) and H_2O (3 × 10 mL). The organic layer was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 98/2, v/v) to give **5** as an amorphous white solid (1.9 g, 89%): R_f 0.41 (CH_2Cl_2/acetone, 95/5, v/v); $[\alpha]^{25}_D$ +30.3 (*c* 1); FAB−MS *m/z* (%) 1037.4 $[M + Na]^+$ (90); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.0, 164.8 (2 C_6H_5CO), 137.5−127.7 (3 $C_6H_5CH_2$, 2 C_6H_5CO), 101.2 (C-1′), 88.1 (C-1), 86.3 (C-3), 82.9 (C-3′), 79.6 (C-5), 77.7, 74.7, 70.3 (C-4,4′,5′), 75.1, 75.0, 73.7 (3 $C_6H_5CH_2$), 73.5 (C-2′), 72.0 (C-2), 69.0 (C-6′), 63.6 (C-6), 62.6 (SCH), 40.7, 39.2, 31.8−26.0 (2 C_6H_{11}); ^1H NMR ($CDCl_3$, 400 MHz) δ 7.70−6.90 (m, 25H, 3 $C_6H_5CH_2$, 2 C_6H_5CO), 5.21 (dd, 1H, H-2′, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.0 Hz), 5.08 (dd, 1H, H-2, $J_{1,2}$ 10.0, $J_{2,3}$ 9.0 Hz), 4.76−4.45 (m, 6H, 3 $C_6H_5CH_2$), 4.64 (d, 1H, H-1′), 4.36 (s br, 1H, O*H*-4), 4.35 (d, 1H, H-1), 3.89 (m br, 1H, H-6a), 3.79 (t, 1H, H-3, $J_{3,4}$ 9.0 Hz), 3.75−3.66 (m, 3H, H-6b,3′,6′a), 3.66−3.57 (m, 3H, H-4,4′,5′), 3.54 (dd, 1H, H-6′b, $J_{5',6'a}$ 6.0, $J_{6'a,6'b}$ −10.0 Hz), 3.35 (m, 1H, H-5), 2.12 (t br, 2H, SC*H*, O*H*-6), 1.90−0.45 (m, 22H, 2 C_6H_{11}); HR FAB−MS calcd for $C_{60}H_{70}NaO_{12}S$ 1037.4486, found 1037.4483.

Dicyclohexylmethyl 6-*O*-(6-*O*-Acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)]-2-*O*-benzoyl-1-thio-β-D-glucopyranoside (7). To a stirred mixture of ethyl 6-*O*-acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-1-thio-β-D-glucopyranoside **6** (373 mg, 0.68 mmol), compound **5** (630 mg, 0.62 mmol), and molecular sieves (4 Å, 3.5 g) in CH_2Cl_2 (20 mL) at −80 °C were added NIS (182 mg, 0.81 mmol) and TMSOTf (15 μL, 0.08 mmol). After stirring for 60 min at −70 °C, the reaction mixture was quenched with TEA (0.05 mL), diluted with CH_2Cl_2 (40 mL), and washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 98/2, v/v) to give **7** as a white glass (467 mg, 50%): R_f 0.59 (CH_2Cl_2/acetone, 95/5, v/v); $[\alpha]^{25}_D$ +24.1 (*c* 1); FAB−MS *m/z* (%) 1526 $[M + Na]^+$ (100);^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.8 (CH_3CO), 165.0, 164.7, 164.2 (3 C_6H_5CO), 137.8−127.6 (5 C_6H_5-CH_2, 3 C_6H_5CO), 101.2 (C-1′,1″), 87.3 (C-1), 85.9, 82.9 (C-3,3′), 83.1 (C-3″), 80.6 (C-5), 77.7(2x), 74.7, 73.0 (C-4′,5′,4″,5″), 75.11, 75.09, 75.0, 74.8, 73.7 (5 $C_6H_5CH_2$), 74.1 (C-2″), 73.4 (C-2′), 72.0 (C-2), 68.9 (C-6″), 68.74 (C-4), 68.67 (C-6), 63.1 (C-6′), 61.0 (SCH), 40.9, 39.6, 31.7−26.0 (2 C_6H_{11}), 20.9 (CH_3CO); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.10−7.00 (m, 40H, 5 $C_6H_5CH_2$, 3 C_6H_5-CO), 5.29 (dd, 1H, H-2″, $J_{1'',2''}$ 7.9, $J_{2'',3''}$ 9.2 Hz), 5.19 (dd, 1H, H-2′, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.0 Hz), 4.92 (dd, 1H, H-2, $J_{1,2}$ 10.0, $J_{2,3}$ 9.0 Hz), 4.87−4.47 (m, 10H, 5 $C_6H_5CH_2$), 4.81 (d, 1H, H-1″), 4.58 (d, 1H, H-1′), 4.38 (dd, 1H, H-6′a, $J_{5',6'a}$ 2.2, $J_{6'a,6'b}$ −11.9 Hz), 4.25 (dd, 1H, H-6′b, $J_{5',6'b}$ 5.0 Hz), 4.23 (d, 1H, H-1), 4.13 (d, 1H, O*H*-4, $J_{4,OH}$ 1.5 Hz), 4.10 (dd, 1H, H-6a, $J_{5,6a}$ 1.4, $J_{6a,6b}$ −12.5 Hz), 3.86 (t, 1H, H-3″, $J_{3'',4''}$ 9.0 Hz), 3.82 (dd, 1H, H-6b, $J_{5,6b}$ 6.6 Hz), 3.72−3.58 (m, 6H, H-3,3′,4′,5′,4″,5″), 3.53 (m, 1H, SC*H*), 2.07 (s, 3H, CH_3CO), 1.90−0.40 (m, 22H, 2 C_6H_{11}); HR FAB−MS calcd for $C_{89}H_{98}NaO_{19}S$ 1525.6321, found 1525.6306.

3-(Benzyloxycarbonylamino)-1-propyl 6-*O*-(6-*O*-Acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-β-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-β-D-glucopyranosyl)]-2-*O*-benzoyl-β-D-glucopyranoside (8). To a stirred mixture of 3-(benzyloxycarbonylamino)-1-propanol (21 mg, 0.10 mmol), compound **7** (111 mg, 0.07 mmol), and molecular sieves (4 Å, 0.4 g) in CH_2Cl_2 (3 mL) were added NIS (34 mg, 0.15 mmol)

and TMSOTf (3 μL, 0.02 mmol). After stirring for 3 min at room temperature, the reaction mixture was quenched with TEA (0.02 mL), diluted with CH_2Cl_2 (30 mL), and washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 95/5, v/v) to yield **8** as a white glass (82 mg, 74%): R_f 0.37 (CH_2Cl_2/acetone, 95/5, v/v); $[\alpha]^{25}_D$ +10.5 (*c* 1); FAB−MS *m*/*z* (%) 1522.8 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 170.7 (CH_3CO), 165.1, 164.7, 164.3 (3 C_6H_5CO), 156.3 ($C_6H_5CH_2OCO$), 137.7−127.6 (5 C_6H_5-CH_2, 3 C_6H_5CO, $C_6H_5CH_2OCO$), 101.3 (2×) (C-1′,1″), 100.6 (C-1), 85.1, 82.8, 77.7, 77.5, 74.7, 73.1 (C-3,4′,5′,3″,4″,5″), 82.8 (C-3′), 75.4 (C-5), 75.1 (2×), 75.0 (2×), 73.7 (5 $C_6H_5CH_2$), 73.8 (C-2′), 73.4 (C-2″), 72.3 (C-2), 69.3 (C-4), 69.1, 68.9 (C-6,6′), 66.2 (OCH_2, $C_6H_5CH_2OCO$), 63.0 (C-6″), 37.8 (CH_2NHZ), 29.2 ($CH_2CH_2CH_2$), 20.8 (CH_3CO); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.00−6.90 (m, 45H, 5 $C_6H_5CH_2$, 3 C_6H_5CO, $C_6H_5CH_2OCO$), 5.29 (dd, 1H, H-2′, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.1 Hz), 5.19 (dd, 1H, H-2″, $J_{1'',2''}$ 7.9, $J_{2'',3''}$ 9.1 Hz), 5.03 (s, 2H, $C_6H_5CH_2OCO$), 4.90 (m, 2H, CH_2NHZ, H-2), 4.85−4.47 (m, 10H, 5 $C_6H_5CH_2$), 4.62 (d br, 1H, H-1′), 4.58 (d br, 1H, H-1″), 4.37 (dd, 1H, H-6″a, $J_{5'',6''a}$ 2.0, $J_{6''a,6''b}$ −12.0 Hz), 4.27−4.18 (m, 4H, H-1,6a,6″b,O*H*-4), 3.81 (t, 1H, H-3′, $J_{3',4'}$ 9.1 Hz), 3.73−3.51 (m, 9H, H-3,-6b,4′,5′,6′a,6′b,3″,4″,5″), 3.46−3.36 (m, 2H, H-4,5), 3.33 (m, 1H, OCH_2), 3.08 (m, 1H, OCH_2), 2.85 (m, 2H, CH_2NHZ), 2.01 (s, 3H, CH_3CO), 1.28 (m, 2H, $CH_2CH_2CH_2$); HR FAB−MS calcd for $C_{87}H_{89}NNaO_{22}$ 1522.5774, found 1522.5746.

3-(Benzyloxycarbonylamino)-1-propyl 6-*O*-(2-*O*-Benzoyl-3,4-di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-2-*O*-benzoyl-*β*-D-glucopyranoside (9). To a stirred solution of compound **8** (290 mg, 0.20 mmol) in CH_2Cl_2/MeOH (3 mL, 9/1, v/v) was added KO^{t}Bu (9 mg, 0.08 mmol). After stirring for 4 min at room temperature, the reaction mixture was neutralized with camphorsulfonic acid and concentrated in vacuo. The residue was co-concentrated with CH_2Cl_2 (2 × 5 mL) and purified by silica gel column chromatography (CH_2Cl_2/acetone, 92/8, v/v) to yield **9** as a white glass (194 mg, 69%): R_f 0.35 (CH_2Cl_2/acetone, 91/9, v/v); $[\alpha]^{25}_D$ +6.60 (*c* 1); FAB−MS *m*/*z* (%) 1480.7 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 100 MHz) δ 165.1, 164.7, 164.3 (3 C_6H_5CO), 156.4 ($C_6H_5CH_2OCO$), 137.9−127.6 (5 C_6H_5-CH_2, 3 C_6H_5CO, $C_6H_5CH_2OCO$), 101.3 (2×) (C-1′,1″), 100.7 (C-1), 85.0, 82.8, 77.7 (2×), 75.6, 75.5, 74.7 (C-3,5,4′,5′,3″,4″,5″), 82.5 (C-3′), 75.1, 75.0 (2×), 74.7, 73.6 (5 $C_6H_5CH_2$), 73.8 (C-2′), 73.4 (C-2″), 72.3 (C-2), 69.1 (2×) (C-4,6), 68.9 (C-6′), 66.4 (OCH_2), 66.2 ($C_6H_5CH_2OCO$), 61.9 (C-6″), 37.8 (CH_2NHZ), 29.2 ($CH_2CH_2CH_2$); ^1H NMR ($CDCl_3$, 400 MHz) δ 8.00−6.90 (m, 45H, 5 $C_6H_5CH_2$, 3 C_6H_5CO, $C_6H_5CH_2OCO$), 5.26 (dd, 1H, H-2′, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.2 Hz), 5.21 (dd, 1H, H-2″, $J_{1'',2''}$ 7.9, $J_{2'',3''}$ 9.1 Hz), 5.03 (m, 2H, $C_6H_5CH_2OCO$), 4.94 (m, 2H, CH_2NHZ, H-2), 4.87−4.48 (m, 10H, 5 $C_6H_5CH_2$), 4.65 (d br, 1H, H-1′), 4.60 (d, 1H, H-1″), 4.33 (s br, 1H, O*H*-4), 4.24 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz), 4.22 (d br, 1H, H-6a, $J_{6a,6b}$ -11.0 Hz), 3.92−3.39 (m, 13H, H-3,4,5,6b,4′,5′,6′a,6′b,3″,4″,5″,6″b,OCH_2(1H)), 3.89 (d br, 1H, H-6″a, $J_{6''a,6''b}$ -11.0 Hz), 3.82 (t, 1H, H-3′, $J_{3',4'}$ 9.2 Hz), 3.12 (m, 1H, OCH_2), 2.86 (m, 2H, CH_2NHZ), 2.25 (m br, 1H, O*H*-6′), 1.32 (m, 2H, $CH_2CH_2CH_2$); HR FAB−MS calcd for $C_{85}H_{87}$-$NNaO_{21}$ 1480.5668, found 1480.5673.

3-(Benzyloxycarbonylamino)-1-propyl 6-*O*-(6-*O*-(6-*O*-(6-*O*-Acetyl-2-*O*-benzoyl-3,4-di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-2-*O*-benzoyl-*β*-D-glucopyranosyl)-2-*O*-benzoyl-3,4-di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-2-*O*-benzoyl-*β*-D-glucopyranoside (14). To a stirred mixture of compound **7** (240 mg, 0.16 mmol), compound **9** (194 mg, 0.13 mmol), and molecular sieves (4 Å, 1 g) in CH_2Cl_2 (20 mL) were added NIS (63 mg, 0.28 mmol) and TMSOTf (10 μL, 0.06 mmol). After stirring for 3 min at room temperature, the reaction mixture was quenched with TEA (0.03 mL), diluted with CH_2Cl_2 (40 mL) and washed with aqueous $Na_2S_2O_3$ (2 × 15 mL, 15%, w/v). The organic phase was dried ($MgSO_4$) and filtered. The filtrate was concentrated in vacuo, and the residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 98/2, v/v) to yield **14** as a white glass (274 mg, 75%): R_f 0.36 (CH_2Cl_2/acetone, 94/6, v/v); $[\alpha]^{25}_D$ +11.0 (*c* 1); FAB−MS *m*/*z* (%) 2771.8 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 170.7 (CH_3CO), 165.1−164.0 (6 C_6H_5CO), 156.3 ($C_6H_5CH_2OCO$), 138.1−127.3 (10 $C_6H_5CH_2$, 6 C_6H_5CO, $C_6H_5CH_2OCO$), 101.5 (C-1′), 101.41 (C-1‴), 101.36 (C-1⁗), 101.1 (C-1″), 100.9 (C-1‴), 100.6 (C-1), 85.3−82.8, 77.9−76.8, 75.4−73.0 (10 $C_6H_5CH_2$, C-3,5,-2′,3′,4′,5′,2″,3″,4″,5″,3‴, 4‴/5‴,5‴,2⁗,3⁗,4⁗,2⁗⁗,3⁗⁗,4⁗⁗,5⁗⁗), 72.5(2x) (C-2,2‴), 69.5 (C-4), 69.3 (C-6‴a), 69.2 (C-4‴/5‴), 68.9(2x) (C-6,6′), 68.7 (C-6⁗⁗), 67.3 (C-6″), 66.3 (OCH_2), 66.1 ($C_6H_5CH_2OCO$), 63.0 (C-6‴), 37.9 (CH_2NHZ), 29.2 (CH_2CH_2-CH_2), 20.9 (CH_3CO); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.00−6.90 (m, 85H, 10 $C_6H_5CH_2$, 6 C_6H_5CO, $C_6H_5CH_2OCO$), 5.30 (dd, 1H, H-2⁗⁗, $J_{1'''',2''''}$ 7.9, $J_{2'''',3''''}$ 9.5 Hz), 5.26 (dd, 1H, H-2‴, $J_{1''',2'''}$ 8.0, $J_{2''',3'''}$ 9.2 Hz), 5.21 (dd, 1H, H-2⁗⁗, $J_{1'''',2''''}$ 8.0, $J_{2'''',3''''}$ 9.2 Hz), 5.10 (dd, 1H, H-2″, $J_{1'',2''}$ 8.0, $J_{2'',3''}$ 9.2 Hz), 5.02 (pt, 1H, H-2‴, $J_{1''',2'''}$ 7.9, $J_{2''',3'''}$ 8.6 Hz), 5.02, 4.99 (2 d, 2H, $C_6H_5CH_2OCO$, *J* 12.5 Hz), 4.91 (pt, 1H, H-2, $J_{1,2}J_{2,3}$ 8.8 Hz), 4.87 (m, 1H, CH_2NHZ), 4.82−4.38 (m, 18H, 9 $C_6H_5CH_2$), 4.64 (d, 1H, H-1⁗⁗), 4.62 (d, 1H, H-1′), 4.58 (d, 1H, H-1⁗⁗), 4.40 (d, 1H, H-1″), 4.33 (dd, 1H, H-6⁗⁗a, $J_{5'''',6''''a}$ 2.2, $J_{6''''a,6''''b}$ −11.9 Hz), 4.31 (d, 1H, H-1‴), 4.22 (dd, 1H, H-6⁗⁗b, $J_{5'''',6''''b}$ 5.0 Hz), 4.20 (s br, 1H, O*H*-4⁗⁗), 4.18 (d br, 1H, H-1), 4.17−4.05 (m, 4H, $C_6H_5CH_2$, O*H*-4, H-6‴a), 4.07 (d br, 1H, H-6a, $J_{6a,6b}$ −10.5 Hz), 3.83 (t, 1H, H-3⁗⁗, $J_{3'''',4''''}$ 9.0 Hz), 3.76 (t, 1H, H-3′, $J_{3',4'}$ 9.0 Hz), 3.75−3.26 (m, 16H, H-3,4″,5′,6′a,6′b,3‴,6‴a,3⁗⁗,5⁗⁗,6⁗⁗b,4⁗⁗,3⁗⁗⁗,4⁗⁗⁗,-5⁗⁗⁗,6⁗⁗⁗a, OCH_2(1H)), 3.16 (m, 1H, H-5″), 3.03 (m, 1H, OCH_2), 2.83 (m, 2H, CH_2NHZ), 2.01 (s, 3H, CH_3CO), 1.27 (m, 2H, $CH_2CH_2CH_2$); HR FAB−MS calcd for $C_{161}H_{161}NNaO_{40}$ 2771.0493, found 2771.0473.

3-(Benzyloxycarbonylamino)-1-propyl 6-*O*-(6-*O*-(6-*O*-(2-*O*-Benzoyl-3,4-di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-2-*O*-benzoyl-*β*-D-glucopyranosyl)-2-*O*-benzoyl-3,4-di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(2-*O*-benzoyl-3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-2-*O*-benzoyl-*β*-D-glucopyrano- side (15). To a stirred solution of compound **14** (163 mg, 0.06 mmol) in CH_2Cl_2/MeOH (7 mL, 9/1, v/v) was added a 0.1 M solution of KO^{t}Bu in CH_2Cl_2/MeOH (0.1 mL, 1/1, v/v). After stirring for 30 min at room temperature, the reaction mixture was neutralized with camphorsulfonic acid and concentrated in vacuo. The residue was co-concentrated with CH_2Cl_2 (2 × 5 mL) and purified by silica gel column chromatography (CH_2Cl_2/acetone, 94/6, v/v) to give **15** as a white glass (85 mg, 53%): R_f 0.49 (CH_2Cl_2/acetone, 90/10, v/v); $[\alpha]^{25}_D$ +2.80 (*c* 1); FAB−MS *m*/*z* (%) 2729.9 $[M + Na]^+$ (100); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 165.1−164.0 (6 C_6H_5CO), 156.4 (C_6H_5-CH_2OCO), 138.0−126.8 (10 $C_6H_5CH_2$, 6 C_6H_5CO, $C_6H_5CH_2$-OCO), 101.9 (C-1⁗⁗), 101.4 (2×) (C-1″,1⁗⁗⁗), 101.1 (2×) (C-1′,1‴), 100.6 (C-1), 85.3−73.5 (10 $C_6H_5CH_2$, C-3,5,2′,3′,4′,-5′,2″,3″,4″,5″,3‴,4‴/5‴,5‴,2⁗⁗,3⁗⁗,4⁗⁗,2⁗⁗⁗,3⁗⁗⁗,4⁗⁗⁗,5⁗⁗⁗), 72.5 (2×) (C-2,2⁗⁗), 69.6 (C-4), 69.5 (C-6⁗⁗), 69.1 (C-4⁗⁗/5⁗⁗⁗), 68.9 (2×) (C-6,6′), 68.7 (C-6⁗⁗⁗), 67.7 (C-6′), 66.3 (OCH_2), 66.1 ($C_6H_5CH_2OCO$), 37.9 (CH_2NHZ), 29.2 ($CH_2CH_2CH_2$); ^1H NMR ($CDCl_3$, 500 MHz) δ 8.00−6.90 (m, 85H, 10 $C_6H_5CH_2$, 6 C_6H_5-CO, $C_6H_5CH_2OCO$), 5.30 (dd, 1H, H-2⁗⁗⁗, $J_{1'''',2''''}$ 8.0, $J_{2'''',3''''}$ 9.2 Hz), 5.26 (dd, 1H, H-2⁗⁗⁗, $J_{1'''',2''''}$ 8.0, $J_{2'''',3''''}$ 9.2 Hz), 5.22 (dd, 1H, H-2″, $J_{1'',2''}$ 8.0, $J_{2'',3''}$ 9.2 Hz), 5.13 (dd, 1H, H-2′, $J_{1',2'}$ 7.9, $J_{2',3'}$ 9.2 Hz), 5.022 (dd, 1H, H-2⁗⁗, $J_{1''',2'''}$ 8.0, $J_{2''',3'''}$ 9.2 Hz), 5.019, 4.98 (2 d, 2H, $C_6H_5CH_2OCO$, *J* −12.0 Hz), 4.93 (m, 2H, H-2, CH_2NHZ), 4.85−4.38 (m, 18H, 9 $C_6H_5CH_2$), 4.653 (d, 1H, H-1⁗⁗⁗), 4.645 (d, 1H, H-1⁗⁗⁗), 4.60 (d, 1H, H-1″), 4.43 (d br, 1H, H-1′), 4.35 (d, 1H, H-1‴), 4.32 (s br, 1H, O*H*-4⁗⁗), 4.25, 4.178 (2 d, 2H, $C_6H_5CH_2$, *J* −11.0 Hz), 4.21 (s br, 1H, O*H*-4), 4.180 (d, 1H, H-1, $J_{1,2}$ 8.0 Hz), 4.08 (d br, 1H, H-6‴a, $J_{6'''a,6'''b}$ −11.0 Hz), 4.07 (d br, 1H, H-6a, $J_{6a,6b}$ −10.0 Hz), 3.87 (dd, 1H, H-6⁗⁗⁗a, $J_{5'''',6''''a}$ 2.5, $J_{6''''a,6''''b}$ −12.0 Hz), 3.85 (t, 1H, H-3⁗⁗⁗, $J_{3'''',4''''}$ 9.2 Hz), 3.77 (t br, 1H, H-3⁗⁗⁗, $J_{3'''',4''''}$ 9.2 Hz), 3.75−3.55 (m, 16H, H-3,3′,6′a,3″,4″,5″,6″a,3‴,5‴,6‴b,4⁗⁗⁗,6⁗⁗⁗b,4⁗⁗⁗⁗,-5⁗⁗⁗⁗,6⁗⁗⁗⁗a,6⁗⁗⁗⁗b), 3.53 (dd, 1H, H-6″b, $J_{5'',6''b}$ 6.1, $J_{6''a,6''b}$ −10.1 Hz), 3.48−3.28 (m, 8H, H-4,5,6b,4′,6′b,4‴,5⁗⁗⁗⁗,OCH_2(1H)), 3.23 (m, 1H, H-5′), 3.05 (m, 1H, OCH_2), 2.84 (m, 2H, CH_2NHZ), 2.58 (s br, 1H, O*H*-6⁗⁗⁗⁗), 1.29 (m, 2H, $CH_2CH_2CH_2$); HR FAB−MS calcd for $C_{159}H_{159}NNaO_{39}$ 2729.0387, found 2729.0436.

3-(Benzyloxycarbonylamino)-1-propyl 6-*O*-(6-*O*-(6-*O*-(3,4-Di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-*β*-D-glucopyranosyl)-3,4-di-*O*-benzyl-*β*-D-glucopyranosyl)-3-*O*-[(3,4,6-tri-*O*-benzyl-*β*-D-glucopyranosyl)]-*β*-D-glucopyranoside (16). To a solution of **14** (56 mg, 0.02 mmol) in CH_2Cl_2/MeOH (9/1, v/v, 2 mL) was added KO^{t}Bu (3 mg, 0.03 mmol). The solution was stirred for 18 h at room temperature. After neutralization with camphorsulfonic acid the mixture was concentrated in vacuo. The residue was purified by silica gel column chromatography (CH_2Cl_2/acetone, 7/3, v/v) followed by size exclusion column chromatography (LH-20, CH_2Cl_2/MeOH, 1/1, v/v) to give **16** as a white glass (36 mg, 85%): R_f 0.56 (CH_2Cl_2/acetone, 7/3, v/v); $[\alpha]^{25}_D$ −8.90 (*c* 1); FAB−MS *m*/*z* (%) 2106 $[M + Na]^+$ (100); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.40−7.00 (m, 55H, 10 C_6H_5-CH_2, $C_6H_5CH_2OCO$), 5.09, 5.03 (2 d, 2H, $C_6H_5CH_2OCO$), 4.98−4.31 (m, 20H, 10 $C_6H_5CH_2$), 4.46−4.28 (m, 6H, 6 H-1), 4.16−3.19 (m, 27 H, CH_2NHZ(1H), OCH_2, 6 H-3,4,5,6), 3.64−3.46 (m, 6H, 6 H-2), 3.24 (m, 1H, CH_2NHZ(1H)), 1.75 (m, 2H, CH_2-CH_2CH_2); HR FAB−MS calcd for $C_{117}H_{135}NNaO_{33}$ 2104.8814, found 2104.8771.

3-Amino-1-propyl 6-*O*-(6-*O*-(6-*O*-(*β*-D-Glucopyranosyl)-3-*O*-[(*β*-D-glucopyranosyl)]-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)-3-*O*-[(*β*-D-glucopyranosyl)]-*β*-D-glucopyranoside (3-Amino-1-propyl 3^2,3^4-Di-*β*-D-glucopyranosyl-gentiotetraoside) (1a). Compound **16** (36 mg, 0.02 mmol) was dissolved in 10% (v/v) aqueous EtOH (2 mL) and treated with hydrogen at 1 atm in the presence of $Pd(OAc)_2$ (20 mg, 0.09 mmol). After stirring for 18 h at room temperature, the solution was filtered through Celite. The filtrate was concentrated, and the residue was purified over Sephadex G-15. The final solution was freeze-dried to give **1a** as a colorless fluffy glass (16 mg, 90%): R_f 0.70 (EtOH/*t*-BuOH/H_2O, 2/5/4, v/v/v); FAB−MS *m*/*z* (%) 1048.5 $[M]^+$ (100); ^{13}C NMR (D_2O, 125 MHz) δ 105.5, 105.4, 105.3, 104.6 (6 C-1), 86.9, 78.6, 78.5, 78.2, 78.1, 77.4, 77.2, 77.03, 76.96, 76.03, 75.98, 75.7, 75.6, 75.3, 75.24, 72.17, 72.1, 72.0, 70.6 (6 C-2,3,4,5), 71.5, 71.4, 71.2, 63.3 (3×) (6 C-6), 70.5 (OCH_2), 40.1 (CH_2NH_2), 29.2 ($CH_2CH_2CH_2$); ^{1}H NMR (D_2O, 500 MHz) δ 4.67−4.62 (2H) and 4.48−4.39 (4H) (2 m, 6H, 6 H-1), 4.12 (m, 3H) and 3.83 (d, 3H) (6 H, 6 H-6a), 3.95 (m, 1H, OCH_2), 3.82−3.72 (m, 4H) and 3.65−3.60 (m, 3H) (2 m, 7H, OCH_2(1H), 6 H-6b), 3.69−3.20 (m, 24H, 6 H-2,3,4,5), 3.07 (t, 2H, CH_2NH_2), 1.92 (m, 2H, $CH_2CH_2CH_2$); HR FAB−MS calcd for $C_{39}H_{69}NO_{31}$ 1048.3932, found 1048.3955.

***N*-(4-Azidosalicyloyl)-3-amino-1-propyl 6-*O*-(6-*O*-(6-*O*-(*β*-D-Glucopyranosyl)-3-*O*-[(*β*-D-glucopyranosyl)]-*β*-D-glucopyranosyl)-*β*-D-glucopyranosyl)-3-*O*-[(*β*-D-glucopyranosyl)]-*β*-D-glucopyranoside (*N*-(4-Azidosalicyloyl)-3-amino-1-propyl 3^2,3^4-Di-*β*-D-glucopyranosyl-gentiotetraoside) (1b).** To a solution of **1a** (5 mg, 5 μmol) in triethylammonium bicarbonate buffer (1 mL, 1.0 M, pH 8.5, Sigma) was added a soltion of *N*-hydroxysuccinimido-4-azidosalicylate (3 mg, 11 μmol) in DMF (1 mL). The mixture was stirred for 18 h in the dark at room temperature. The solvent was removed in vacuo, and the residue was purified over Sephadex G15 to yield **1b** as an amorphous white fluffy solid (3 mg, 52%): R_f 0.38 (*n*-BuOH/EtOH/H_2O, 2/1/1, v/v/v); FAB−MS *m*/*z* (%) 1231.4 $[M + Na]^+$ (100); 1247.4 $[M + K]^+$ (15); ^{1}H NMR (D_2O, 500 MHz) δ 7.73 (d, 1H, H_{Ar}, *J* 8.3 Hz), 6.35 (s, 1H, H_{Ar}), 6.30 (d, 1H, H_{Ar}), 4.10−3.20(m), 2.60(s) and 1.25(d) (44H, OCH_2, 6 H-1,2,3,4,5,6a,6b,), 2.45−2.25 (m, 2H, CH_2NH), 1.88 (m, 2H, $CH_2CH_2CH_2$).

Photoreactivity of Hexaglucoside 1b. The compound **1b** was dissolved in water to a final concentration of 0.33 mM and diluted 10-fold with ethanol. Absorbance was measured in the range 240−360 nm in a quartz cuvette. The quartz cuvette was placed at a distance of 10 cm from an UV lamp (UVGL-52, UVP products, CA) and irradiated with short-wave UV (252 nm) for 30 s. The absorbance of the solution was then scanned. The solution was then irradiated for an additional time period. After each irradiation period increment, the absorbance of the solution was rescanned. Control irradiation experiments were performed with 4-[*p*-azidosalicylamido] butylamine (ASBA; Pierce Chemicals, Rockford, IL). ASBA was also iodinated with nonradioactive iodine by using the procedure described below. Although a small absorbance shift was observed upon iodination, photosensitivity was not affected.

Radioiodination of Tyraminylated Hepta-*β*-glucoside and Hexaglucoside 1b. The tyramine conjugate of the hepta-*β*-glucoside elicitor was iodinated using a published protocol.[14] The protocol was slightly modified to use a molar ratio of iodine to tyramine conjugate of 5:2. Consequently, 400 ng of Iodogen (Pierce Chemicals, Rockford, IL) were used to coat the bottom of a glass test tube. This procedure typically yielded radioiodinated oligosaccharides with a specific radioactivity of ∼1000 Ci/mmol.

Hexaglucoside **1b** was iodinated according to a protocol modified from Chizzonite et al.[15] Radioactive iodine (1 mCi Na^{125}[I] in 10 μL; Amersham, Arlington Heights, IL) was added to 40 μL PBS (20 mM sodium phosphate, pH 7.2 containing 150 mM NaCl) in a prewetted 12 mm × 50 mm glass tube precoated with 50 μg Iodo-gen. The iodine was allowed to react for 6 min, while the tube was flicked every 30 s. The solution was then immediately transferred to a tube containing 500 pmol of hexaglucoside **1b** (in 1.5 μL). The solution was mixed every 30 s for 9 min, then applied to a Sephadex G-10 (Pharmacia) gel filtration column (0.5 cm × 15 cm), and eluted with bis-Tris buffer (25 mM, pH 7.0). The fractions corresponding to the void volume of the column and containing the radioactive oligosaccharide were pooled and used in binding assays.

Preparation of Soybean Root Membranes and Biological and Binding Assays. Foundation quality soybean (*Glycine max* L. cv. Williams 82) was obtained from Illinois Foundation Seeds, Inc. (Champaign, IL), and the seedlings were grown as described.[12] The biological assay used to determine the ability of the synthesized compounds to elicit the synthesis and accumulation of phytoalexins in soybean tissue was performed as described.[14] Briefly, cotyledons from young soybean plants were excised and surface-sterilized. A thin layer of tissue was cut from the bottom surface of each cotyledon with a razor blade. Samples (90 μL) were applied to the wound of each cotyledon, and the cotyledons were incubated in the dark at 26 °C for 20 h. The absorbance at 285 nm of wound-droplet solutions was measured, which is linearly correlated with the phytoalexin content. Crude total cellular membranes were prepared from the roots of 10-day-old soybean seedlings as described,[2b,2c] except that 25 mM bis-Tris, pH 7.0 (4 °C), containing 2 mM $MgCl_2$, 2 mM DTT, 5 mM ascorbic acid, 8% (w/w) sucrose, 10 mM KCl, 10 mM EDTA, 200 μM AEBSF, 10 μg/mL leupeptin, 1 μM pepstatin, and 1 μg/mL aprotinin was used as the homogenization buffer at a ratio of 2 mL per g (fresh weight) of roots.

The assays to determine binding of radiolabeled hepta-*β*-glucoside or hexaglucoside **1b** to intact membranes were carried out as described in 96-well microtiter plates,[2c] except that the binding assay buffer was modified (25 mm bis-Tris, pH 7.0 (4 °C), 10 mM $MgCl_2$, 2 mM dithiothreitol (DTT), 100 mM NaCl, 7.5 mM *β*-D-thioglucoside, and 7.5 mM D-gluconic acid lactone). The incubation time for the binding assays was 2 h. Competitive binding assay were carried out by mixing increasing amounts of competitive ligands (0.1−3000 times the amount of radioligand) with constant amount of radiolabeled hepta-*β*-glucoside and membrane proteins, and incubating for 4 h.

Acknowledgment. This work was supported by the University of Georgia Faculty start-up funds (to G.J.B.) and by a grant (to M.G.H.) from the U.S. National Science Foundation (MCB-9723685).

JO990836O

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