

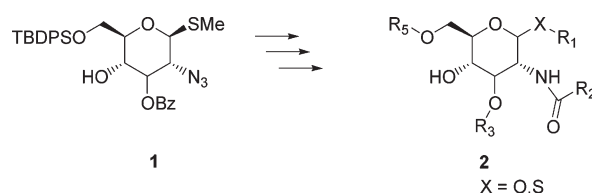
A Versatile Synthetic Approach toward Diversity Libraries using Monosaccharide Scaffolds

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The pyranose scaffold is unique in its ability to position pharmacophore substituents in various ways in 3D space, and unique pharmacophore scanning libraries could be envisaged that focus on scanning topography rather than diversity in the type of substituents. Approaches have been described that make use of amine and acid functionalities on the pyranose scaffolds to append substituents, and this has enabled the generation of libraries of significant structural diversity. Our general aim was to generate libraries of pyranose-based drug-like mimetics, where the substituents are held close to the scaffold, in order to obtain molecules with better defined positions for the pharmacophore substituents. Here we describe the development of a versatile synthetic route toward peptide mimetics build on 2-amino pyranose scaffolds. The method allows introduction of a wide range of substituent types, it is regio- and stereospecific, and the later diversity steps are performed on solid phase. Further, the same process was applied on glucose and allose scaffolds, in the exemplified cases, and is likely adaptable to other pyranose building blocks. The methods developed in this work give access to molecules that position the three selected binding elements in various 3D orientations on a pyranose scaffold and have been applied for the production of a systematically diverse library of several hundred monosaccharide-based mimetics.

Introduction

The pyranose scaffold offers unique opportunities for drug discovery in terms of positioning pharmacophore groups in 3D space. The presence of five functionalized chiral centers provides chemically accessible attachment points to append target-binding substituents. Altering a chiral center or position of one substituent results in a different spatial presentation of the binding elements, on the same scaffold.

The principle of using the monosaccharide scaffold for peptide-mimetic design was pioneered by Hirschmann and collaborators¹ in the early 1990s and has been exploited over the past 2 decades.^{2,3} Several groups have developed solid-phase and solution-phase approaches in the development of monosaccharide-based drug discovery libraries.^{4,5}

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For some time now we have been interested in evaluating the use of the pyranose scaffold in creating a systematic diversity library of drug-like peptide mimetics, by building on its unique 3D characteristics and by combining systematic geometrical diversity with systematic functional diversity.⁶ Previously described methods do not serve our purpose, as we wish to generate diverse libraries of products that are more drug-like and more rigid, i.e., not using amino acids as the substituents themselves⁵ and not using carbamates as linkage functionalities to append the substituents to the core scaffold.⁴ Screening of such libraries would not only deliver pharmacophore information in terms of a combination of binding elements (substituent motif) and their relative positioning in space (topography, 3D-presentation) but would also help identify drug-like hits that can directly enter lead optimization programs. The methods described in this paper have been applied in the production of such a geometrically diverse library containing several hundred monosaccharide-based mimetics of one substituent motif, which helped identify hits for many G-protein-coupled receptors recognizing that motif. The library and the biological results will be described elsewhere. Here we wish to disclose the synthetic details that allowed the library production.

Our aim was to establish a versatile synthetic route toward peptide mimetics **2**, where 3 out of R1–R5 carry “binding” substituents. The synthetic process should allow for multiple substitution patterns on multiple scaffolds to be generated simultaneously in one library and should allow a broad range of substituents to be included in the library. The synthetic route would have the following properties: (a) compatible to solid-phase library production; (b) versatile, chemical route compatible with a wide range of substituents; (c) enabling the positioning of three pharmacophore substituents close to the core scaffold; (d) convergent—creating diversity at the later stages in the synthetic process.

The most challenging of these with regards to the monosaccharide scaffold is to maintain versatility in a regiospecific synthetic route, given the five functionalized centers. In this paper we report the development of a versatile and practical solid-phase synthetic route applied on two monosaccharide scaffolds, which has enabled us to generate libraries of high geometrical and functional diversity.

For a multistep solid-phase library process to be successful, each step in the synthesis needs to proceed in high yields with high purity, without premature cleavage of intermediates off the resin. Further, as we are aiming to apply the strategy in the development of diversity libraries, synthetic versatility is critical for success. We opted to use 2-amino-glycopyranosides as the core building blocks. The use of the amine functionality at position 2, rather than the typical oxygen, simplifies the protecting group strategy and allows for using the high-yielding and versatile amide bond formation at one position of the scaffold. Second, we planned to use ether bonds to link substituents at oxygen positions, rather than the carbamate bond used in other strategies,^{4,7,8} in order to increase drug-likeness in the design of our

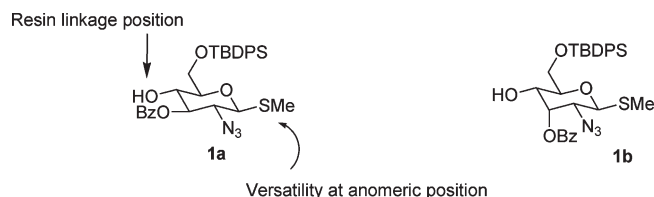


FIGURE 1. Protected building blocks as starting materials; TBDPS = *tert*-butyldiphenylsilyl.

molecules. This requires nucleophilic substitutions and strong basic reaction conditions, and therefore ether bonds will need to be established prior to amide bond formation and the amine protecting group will need to be stable to the strong basic alkylating conditions. The carbamate-based protecting groups are therefore precluded; however, the azide protecting group is practical and suitable for this purpose. Further, a resin-linkage position and functionality needs to be selected on the core scaffold for resin attachment. The cleavage chemistry for this linkage position should be robust (independent of selection of substituents) and highly selective and should allow removal of secondary protecting groups on the substituents. The *p*-methoxybenzyl ether link⁹ represents a highly inert bond, which is labile only to oxidizing or acidic conditions. Alcohols are loaded using the readily available trichloroacetimidate resin (TCA resin) and typical TCA-donor promoters. As we aim to introduce three binding elements on a scaffold with four attachment positions (not counting the resin-linkage position), we plan to cap the remaining attachment position with a methyl group.

The building blocks **1a** and **1b** (Figure 1) are suited to our needs.¹⁰ The thio-glycoside is stable to many reaction conditions but at the same time is an excellent and versatile donor,¹¹ which enables introduction of a variety of anomeric substituents.

Results and Discussion

Step 1. Anomeric Substituent and Loading on Resin. We opted to introduce the anomeric substituents prior to loading on resin. This avoids the issue of achieving high stereoselectivity and yield for an on-resin glycosylation. In Scheme 1, the glycosyl donor **1a** was reacted with 2-phenyl-ethanol **3** using the thiophilic reagent dimethyl(methylthio)sulfonium triflate (DMTST) as the glycosyl promoter, in the presence of molecular sieves. The glycosylation gave anomers **4a** and **4b** in a 1:1 anomeric ratio, which were readily separated on standard column chromatography.

An excess of the α -anomer **4a** (3 equiv to the resin-bound trichloro acetimidate) was loaded onto the resin via the 4-hydroxy position using $\text{BF}_3 \cdot \text{Et}_2\text{O}$ as promoter. After completion, a resin loading was determined by HPLC analysis of a cleavage solution from a known weight of resin **5a** against a reference standard curve for compound **4a**. The

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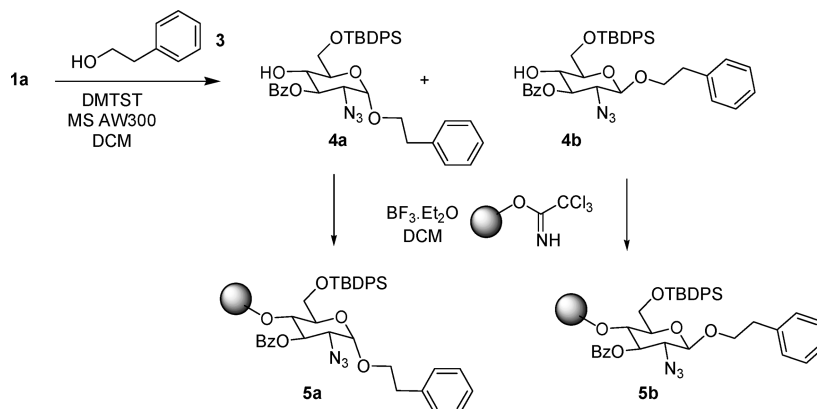
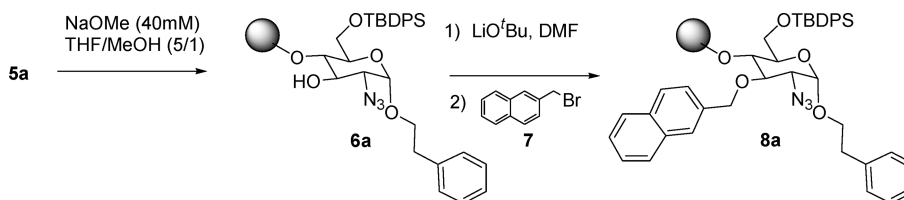
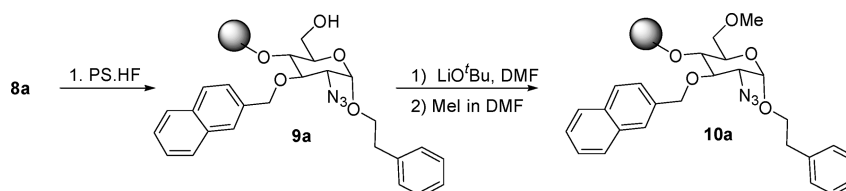
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SCHEME 1. Glycosylation and Resin Loading Steps

SCHEME 2. Introduction of R₃SCHEME 3. Introduction of R₅

excess α -glucopyranoside **4a** could be recovered from the DCM solution through a simple washing process with brine, and the recovered glucopyranoside **4a** could be reused in additional loading reaction. Similar loading of the β -anomer **4b** provided resin **5b**.

Step 2. Introduction of R₃. To introduce the R₃ substituent (scheme 2), the resin **5a** was first subjected to Zemplén O-deacylation conditions for removal of the benzoate protecting group.¹² Subsequent alkylation of the free hydroxyl group in product **6a** with 2-bromomethyl naphthalene **7** was examined using various reagents and reaction conditions.

In our initial attempts we used KO^tBu as a base but could not achieve alkylation in good yield (maximum of 25%). Although different solvents (THF, DMF), additives (crown ether, TBAI), concentrations, and order of addition of reagents were tested, the yield of the alkylation remained lower than 25%. Subsequently we found that if LiCl was added to the reaction, the yields improved dramatically (up to 90% after 48 h alkylation). One possible explanation for this observation is that a smaller Li⁺ cation may be able to stabilize the alkoxide better in a polystyrene resin environment.

Best results were obtained when the resin was pretreated with a LiO^tBu/DMF solution for 20 min, then drained, and immediately treated with bromide in DMF. This reaction

sequence was repeated 3 times, to drive the reaction to product **8a** to completion.

Step 3. Introduction of R₅ (Scheme 3). Removal of the *tert*-butyldiphenylsilyl (TBDPS) protecting group to the free alcohol **9a** can be achieved using HF-pyridine solution. However, as we were planning to adapt the solid-phase chemistry to library synthesis using ceramic 2D bar-coded mini-Kans, we decided to look for a different method that is less prone to etching. For this reason we prepared a proton sponge/HF salt (PS·HF) by simply mixing an HF-pyridine solution with a THF solution of proton sponge. The PS·HF salt precipitates out as a white solid and can be weighed out to create accurate concentrations of the HF salt for deprotection. We established suitable conditions for complete and clean removal of the TBDPS group, using a PS·HF solution (0.5N in DMF with 5% acetic acid) at 65 °C overnight. These conditions do not etch glass or ceramic 2D barcodes and thus reactions can be carried out in standard glassware. The PS·HF salt and its use in halogen exchange reactions have been described earlier,¹³ though to our knowledge its use in removing silyl-based protecting groups has not been reported before.

Following complete removal of TBDPS, the alkylation process was carried out as described using MeI as the halide to generate the intermediate **10a**.

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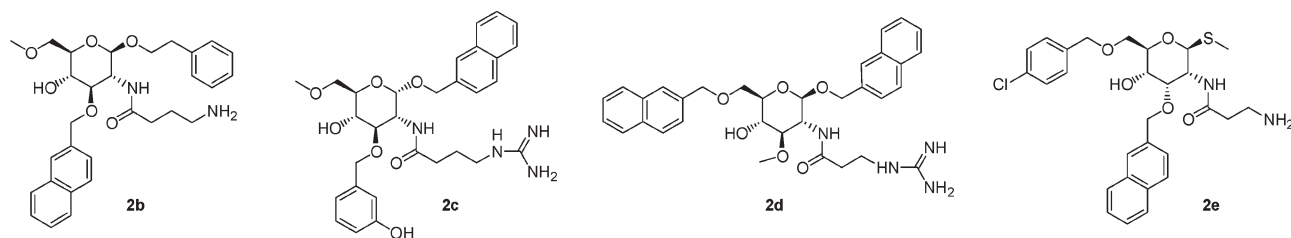
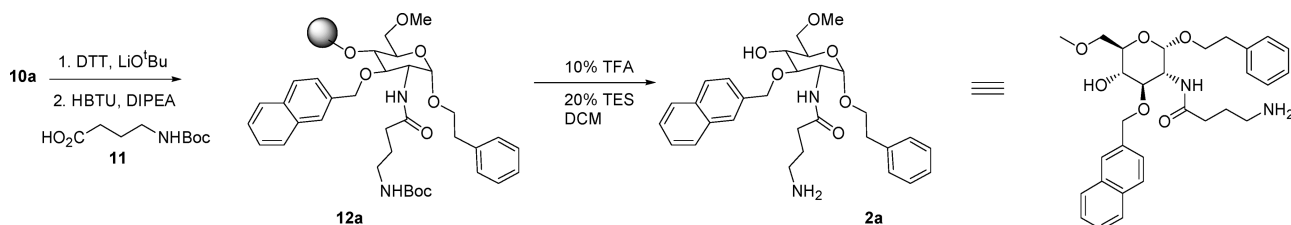
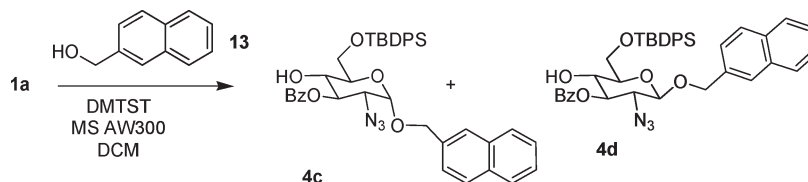


FIGURE 2. Representative examples of final compounds.

SCHEME 4. Introduction of R₂



SCHEME 5. Production of Naphtyl Glycosides 4c and 4d



Step 4. Introduction of R₂ and Cleavage off Resin (Scheme 4). The azide function at position 2 of compound **10a** was reduced to the free amine using DTT under basic conditions. The resulting amine was then acylated using HBTU preactivated acid **11** in DMF, to form product **12a**.

Finally, the product **2a** was cleaved off the resin using a 10% TFA solution in DCM, with 20% triethylsilane as scavenger.

Representative Examples. Having established a functional solid-phase approach, we wanted to evaluate the robustness of this approach by further synthesizing additional examples **2b–e** (figure 2).

Product **2b** is the β -anomer of compound **2a** and demonstrates anomeric integrity in the process. Product **2c** carries other amino acid side chain substituents (guanidine and phenol) and serves to demonstrate the functional versatility of the approach. Compounds **2d** and **2e** have substitution patterns different from that of **2a** and help demonstrate the ability to create structurally diverse molecules. Compound **2e** further illustrates the application on the allose scaffold and the stability of the thiomethyl anomeric substituent to the synthetic approach. The solid-phase synthesis and list of reagents for products **2a–e** are summarized in Scheme 6 and Table 1.

The synthesis of compound **2b** starts off with the β -anomer **4b** and follows the identical synthetic route as for the α -anomer **4a**. The product **2b** is obtained in good yield and high purity following this solid-phase process. The results demonstrate that the anomeric substituent is stable to the acidic cleavage conditions.

The synthetic route toward guanidines **2c** and **2d** starts off with a glycosylation of the glucose building block **1a** with naphthyl-2-methanol **13** (Scheme 5).

Separation of anomers **4c** and **4d** and loading on resin provides starting resins **5** for the solid-phase synthetic process (Scheme 6) using reactants as outlined in Table 1. Specifically for compound **2c**, the phenol functionality is introduced through alkylation with (3-*tert*-butoxy-phenyl)-methyl bromide **17**, prepared from commercially available (3-*tert*-butoxy-phenyl)methanol. The *tert*-butyl protecting group is effectively removed under the TFA cleavage conditions to produce the free phenol **2c**.

Fmoc-protected amino acids **20** and **19** were used for both target compounds **2c** and **2d**, respectively. Following acylation, the Fmoc protecting group was removed by treatment with piperidine. The guanidine functionality was then introduced on solid phase¹⁴ from the free amine **14** with 3,5-dimethyl-1-pyrazolylformamidinium nitrate (DMPFN) **15** to afford the resin-bound guanidinylated compounds **16**. TFA-induced cleavage and HPLC purification provided the fully unprotected guanidines **2c** and **2d**.

Synthesis of the allose molecule **2e** proceeds in the same way as for the glucose products, following loading of the allose building block **1b** on resin. Loading and introduction of the R₃ substituent is slower at the axial allose position, when compared to the equatorial glucose position but still proceeds to completion after 3–5 alkylation cycles.

Experimental Section

Glycosylation of Glc-SMe with Phenyl Ethanol 3. Building block **1a** (Glc-SMe) (40.0 gr, 0.069 mol) and phenyl ethanol **3** (41.5 mL, 0.346 mol, 5 equiv) were dissolved in dry DCM

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SCHEME 6. Synthetic Route toward Representative Examples 2a–e

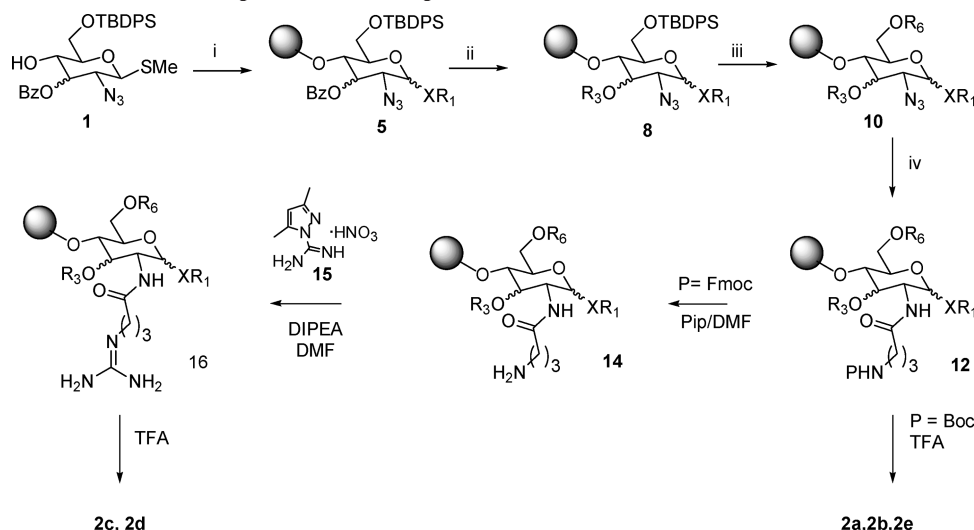
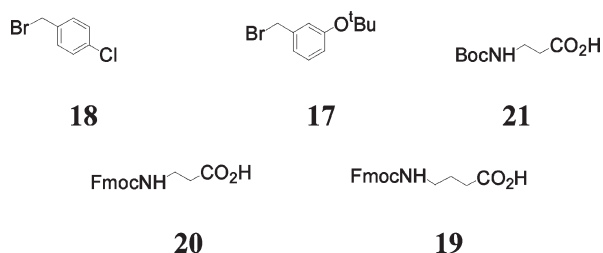


TABLE 1. Reactants per Synthetic Steps 1–4 for Products 2a–e

step ^a	reactant	representative examples				
		2a	2b	2c	2d	2e
1	BB ^b	1a	1a	1a	1a	1b
	R-OH	3	3	13	13	
	product ^c	4a	4b	4c	4d	1b
	RL ^d	0.45	0.37	0.35	0.37	0.32
2	R-Br	7	7	17	MeI	7
3	R-Br	MeI	MeI	MeI	8	18
4	R-CO ₂ H	11	11	20	19	21

^aRefers to the synthetic steps 1–4 in the process as described in the text. ^bBB means building block. ^cRefers to the product from the solution-phase glycosylation (see Schemes 1 and 5). ^dResin loading of the BB, expressed in mmol/gr.



(640 mL), and the mixture was stirred over molecular sieves (AW300, previously dried at 300 °C) under N₂. After 1 h a solution of DMTST (44.7 gr, 0.173 mol, 2.5 equiv) in dry DCM (346 mL) was added to the building block **1a** and alcohol mixture and stirred under N₂ at rt; evolution of reaction was checked by TLC and LC/MS. After complete reaction of starting material (1–3 h), the reaction mixture was filtered on Celite and washed with DCM (1 L). The organic phase was washed with a saturated NaHCO₃ solution (3 × 1.6 L). The aqueous phase was re-extracted with DCM (200 mL). The combined organic phases were dried (MgSO₄), filtered, and evaporated under reduced pressure to afford a crude residue, which contained desired α/β anomers in both TBDPS-protected and unprotected form. To reprotect the 6-position with the TBDPS group, this crude material was treated with imidazole (3.83 gr, 0.056 mol) and TBDPS-Cl (8.65 mL, 0.034 mol) in dry DMF (50 mL). The mixture was stirred under N₂ at rt for 2 h. The reaction mixture was diluted with EtOAc (500 mL), and the

solution was washed with distilled water (500 mL). The water layer was re-extracted with DCM (3 × 100 mL). All organic layers are combined, dried (MgSO₄), filtered, and evaporated to dryness. The anomers **4a** and **4b** were separated and purified directly from this residue by FC (PE/EtOAc 10:1 to 1:10).

(2-Phenethyl)-2-azido-3-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-1-(2-phenethyl)-α-D-glucopyranoside (**4a**). After FC purification, the title compound was obtained as a white solid (17.6 gr, 39%); *R*_f (PE/DCM 1:5) = 0.59; ¹H NMR (400 MHz, CDCl₃) δ 1.06 (s, 9H, CH₃), 2.94–3.03 (m, 2H, OCH₂-CH₂Ar), 3.32 (ddd, 1H, *J*_{H₂,H₃} = 10.6 Hz, H-2), 3.41–3.46 (ddd, 1H, H-5), 3.73–3.90 (m, 4H, OCH₂(B)), H-4, H-6a, H-6b), 3.90 (m, 1H, OCH₂(A)), 5.00 (d, 1H, *J*_{H₁,H₂} = 3.3 Hz, H-1), 5.54 (dd, 1H, *J*_{H₃,H₄} = 9.4 Hz, H-3), 7.18–8.13 (m, 20 H, H_{Ar}); ¹³C NMR (100.6 MHz, CDCl₃) δ 19.3, 26.8, 36.0, 61.1, 63.8, 68.7, 70.8, 71.7, 74.0, 97.6, 126.4, 127.69, 127.71, 128.42, 128.43, 129.1, 129.4, 129.78, 129.79, 130.0, 133.08, 133.09, 133.4, 135.59, 135.62, 138.4, 167.0. MS (ES) *m/z* = 674.1 [M + Na]; HRMS (ESI, pos mode) *m/z* [M + Na]⁺ calcd for C₃₇H₄₁N₃O₆Si 674.2657; found 674.2651.

(2-Phenethyl)-2-azido-3-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-1-(2-phenethyl)-β-D-glucopyranoside (**4b**). After FC purification, the title compound was obtained as a white solid (14.4 gr, 32%); *R*_f (PE/DCM 1:5) = 0.43; ¹H NMR (400 MHz, CDCl₃) δ 1.06 (s, 9H, CH₃), 2.99 (t, 2H, *J* = 7.2 Hz, OCH₂CH₂Ar), 3.47 (ddd, 1H, H-5), 3.58 (ddd, 1H, *J*_{H₁,H₂} = 8.0 Hz, *J*_{H₂,H₃} = 10.2 Hz, H-2), 3.77–3.83 (m, 1H, OCH₂(B)), 3.86 (t, 1H, H-4), 3.96 (d, 2H, *J* = 4.6 Hz, H-6a, H-6b), 4.10–4.16 (m, 1H, OCH₂(A)), 4.46 (d, 1H, H-1), 5.07 (dd, 1H, *J*_{H₃,H₄} = 9.4 Hz, H-3), 7.18–8.13 (m, 20 H, H_{Ar}); ¹³C NMR (100.6 MHz, CDCl₃) δ 19.3, 26.8, 36.2, 64.0, 64.2, 70.80, 70.88, 75.40, 76.45, 101.9, 126.4, 127.76, 127.80, 128.42, 128.50, 128.90, 129.29, 129.86, 130.04, 132.88, 132.99, 133.55, 135.57, 135.66, 138.19, 166.9; MS (ES) *m/z* [M + Na] = 674.1; HRMS (ESI, pos mode) *m/z* [M + Na]⁺ calcd for C₃₇H₄₁N₃O₆Si 674.2657; found 674.2655.

Glycosylation of Glc-SMe with 2-Naphthalene Methanol. Glc-SMe (**1a**) (37.0 g, 64.0 mmol) and 2-naphthalene methanol (40.5 g, 256.2 mmol) were used in the glycosylation as described above. The crude material was purified by FC using EtOAc/PE (1:19–1:9) as eluent to give the α-anomer **4c** (12.12 g, 27%) and the β-anomer **4d** (8.32 g, 19%).

(2-Naphthylmethyl)-2-azido-3-*O*-benzoyl-6-*O*-(*tert*-butyldiphenylsilyl)-2-deoxy-1-α-D-glucopyranoside (**4c**). After FC purification, title compound was obtained as a white solid (12.12 g,

27%); R_f TLC (DCM) = 0.78; ^1H NMR (400 MHz, CDCl_3) δ 1.10 (s, 9H, CH_3), 3.41 (dd, 1H, $J_{\text{H}_2,\text{H}_3}$ = 10.6 Hz, H-2), 3.87 (t, 1H, $J_{\text{H}_4,\text{H}_5}$ = 9.2 Hz, H-4), 3.90–3.99 (m, 3H, H-5, H-6a, H-6b), 4.76 (d, 1H, $\text{OCH}_2(\text{B})$), 4.94 (d, 1H, $J_{\text{A,B}}$ = -11.9 Hz, $\text{OCH}_2(\text{A})$), 5.11 (d, 1H, $J_{\text{H}_1,\text{H}_2}$ = 3.5 Hz, H-1), 5.66 (dd, 1H, $J_{\text{H}_3,\text{H}_4}$ = 8.9 Hz, H-3), 7.36–8.14 (m, 22H, H_{Ar}); ^{13}C NMR (100.6 MHz, CDCl_3) δ 19.3, 26.9, 61.2, 63.9, 69.4, 70.9, 72.1, 74.3, 96.4, 125.8, 126.1, 126.2, 127.2, 127.70, 127.74, 127.76, 127.96, 128.39, 128.45, 129.32, 129.8, 130.0, 133.09, 133.12, 133.16, 133.18, 133.5, 133.8, 135.62, 135.67, 167.1; MS (ES) m/z 710.1 [M + Na]; HRMS (ESI, pos mode) m/z [M + Na] $^+$ calcd for $\text{C}_{40}\text{H}_{41}\text{N}_3\text{O}_6\text{Si}$ 710.2657; found 710.2649.

(2'-Naphthylmethyl)-2-azido-3-O-benzoyl-6-O-(tert-butylidiphenyl)-2-deoxy-1- β -D-glucopyranoside (**4d**). After FC purification, the title compound was obtained as a white solid (8.32 g, 19%); R_f TLC (DCM) = 0.67; ^1H NMR (400 MHz, CDCl_3) δ 1.10 (s, 9H, CH_3), 3.50 (ddd, 1H, H-5), 3.69 (dd, 1H, $J_{\text{H}_2,\text{H}_3}$ = 10.2 Hz, H-2), 3.89 (t, 1H, $J_{\text{H}_4,\text{H}_5}$ = 9.9 Hz, H-4), 3.99–4.05 (m, 2H, H-6a, H-6b), 4.56 (d, 1H, $J_{\text{H}_1,\text{H}_2}$ = 8.0 Hz, H-1), 4.86 (d, 1H, $\text{OCH}_2(\text{B})$), 5.06 (dd, 1H, $J_{\text{H}_3,\text{H}_4}$ = 9.4 Hz, H-3), 5.10 (d, 1H, $J_{\text{A,B}}$ = -11.9 Hz, $\text{OCH}_2(\text{A})$), 7.37–8.13 (m, 22H, H_{Ar}); ^{13}C NMR (100.6 MHz, CDCl_3) δ 19.3, 26.9, 64.05, 64.14, 70.76, 70.99, 75.6, 76.6, 100.3, 125.8, 126.1, 126.2, 127.0, 127.70, 127.77, 127.81, 127.95, 128.37, 128.48, 129.22, 129.87, 129.88, 130.0, 132.9, 133.07, 133.15, 133.22, 133.6, 133.97, 135.6, 135.7, 167.0; MS (ES) m/z 710.1 [M + Na]; HRMS (ESI, pos mode) m/z [M + Na] $^+$ calcd for $\text{C}_{40}\text{H}_{41}\text{N}_3\text{O}_6\text{Si}$ 710.2657; found 710.2661.

General Solid-Phase Procedures. Resin Loading. TCA-Wang resin (1 g, 1.08 mmol/g) and glycosides **1b** and **4a–d** (3 mmol) were dried under high vacuum over KOH overnight before use. In the glovebox, each glycoside was dissolved in anhydrous DCM (8 mL). Then the resin was washed with dry THF (10 mL) followed by dry DCM ($\times 2$, each 10 mL), the glycoside solution was added to the resin, and the mixture was shaken for 2 min. $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.3 mmol) was then added, and the mixture was shaken for 10 min. After that, additional $\text{BF}_3 \cdot \text{Et}_2\text{O}$ (0.3 mmol) was added, and the resin mixture was shaken for 10 min, drained, and washed with dry DCM ($\times 5$, each 10 mL) to recover the glycoside. The combined DCM with unreacted glycoside was washed with saturated NaHCO_3 ($\times 3$, each 20 mL) solution, dried (MgSO_4), and evaporated under reduced pressure. Finally the resin was washed with dry THF ($\times 4$, each 10 mL) and dry DCM ($\times 4$, each 10 mL) and dried under high vacuum overnight.

Resin Loading Determination. The glycoside-resin was dried under high vacuum for a minimum of 16 h. Anhydrous DCM/triethylsilane (TES)/trifluoroacetic acid (TFA) (7:2:1) was prepared under N_2 . About 70 mg of the dried loaded resin was then weighed into a 4.5 mL reaction tube, and the cleavage solution (1 mL) was added. After the top of the tube was sealed with a white plastic cap, the cleavage reaction was left at rt for 2–3 h, then filtered, and washed with DCM ($\times 4$, each 2 mL), and the filtrates were combined and diluted to a known volume. The concentration of the desired glycoside was then determined using HPLC/ C^{18} chromatography comparison against a standard curve obtained from five known glycoside concentrations (ranging from 0.06 to 1.2 mg/mL).

Benzoate Removal at O-3. NaOMe solution (40 mM in THF/MeOH 5:1) was added to the resin (80 mg) under N_2 and was gently shaken overnight (vortex). The reaction solution was filtered, and the resin was washed with THF/AcOH/MeOH (8:1:1, $\times 3$, each 4 mL), THF ($\times 3$, each 4 mL), and DCM ($\times 3$, each 4 mL) and dried on high vacuum overnight.

Alkylation (O-3 and O-6). A solution of LiO t Bu in dry DMF (0.25 M) was prepared, as well as a solution of alkylating agent in dry DMF (0.25 M). The resin was washed with dry DMF (4 mL) and then treated with the LiO t Bu solution (2.5 mL) for 10 min at room temperature. The LiO t Bu solution was removed, and the alkylating agent solution (2.5 mL) added. The reaction

was gently shaken for 20 min at rt, and the resin was filtered off. This stepwise process of LiO t Bu and alkylating agent treatment was performed 5 times, using fresh LiO t Bu solution and fresh alkylating agent solution. The resin was finally washed with DMF ($\times 3$, each 4 mL), MeOH ($\times 3$, each 4 mL), and DCM ($\times 3$, each 4 mL).

Preparation of PS·HF reagent. Proton sponge (4.8 g; 22.4 mmol) was dissolved in THF (30 mL) in a plastic vessel. HF·pyridine solution was slowly added dropwise until no precipitation occurred (ca. 1.2 mL). The precipitated white solid was filtered under N_2 , washed with THF ($\times 2$, each 5 mL) and ether ($\times 3$, each 10 mL), and dried in the vacuum oven over P_2O_5 for 24 h (5.03 g; 96%). This material was prepared fresh each time and used without further purification in the TBDPS deprotection step.

TBDPS Deprotection at O-6. A solution of 0.5 M PS·HF and AcOH 5% in dry DMF (5 mL) was added to the resin and stirred and heated at 65 °C for 24 h. After cooling down, the solution was aspirated, and the resin was washed with DMF ($\times 3$, each 4 mL), THF ($\times 3$, each 4 mL), and DCM ($\times 3$, each 4 mL).

Azide Reduction. A dry DMF solution of 0.2 M LiO t Bu and 0.2 M DTT was prepared, the solution (5 mL) was added to the resin, and the resin was gently shaken for 24 h (vortex) at rt. The solution was then aspirated, and the resin was washed with DMF ($\times 3$, each 4 mL), MeOH ($\times 3$, each 4 mL), and DCM ($\times 3$, each 4 mL) and dried under N_2 .

Acylation at N₂. A solution of HBTU 0.5 M in dry DMF was prepared. The acid (2 mmol) was dissolved in dry DMF (4 mL), and HBTU solution (4 mL) was added. DIPEA (0.7 mL, 4 mmol). The resulting mixture was swirled and allowed to stand for 10 min. The solution was added to the resin at room temperature and was gently shaken for 24 h. The solution was then aspirated and the resin washed with DMF ($\times 3$, each 4 mL), MeOH ($\times 3$, each 4 mL) and DCM ($\times 3$, each 4 mL) and dried under N_2 .

Fmoc Deprotection. (Only for products **2c** and **2d**.) A solution of piperidine in dry DMF (1:5; 5 mL) was added to the resin. The mixture was gently shaken (vortex) for 2 h at rt. The solution was then aspirated, and the resin was washed with DMF ($\times 3$, each 4 mL), MeOH ($\times 3$, each 4 mL), and DCM ($\times 3$, each 4 mL) and dried under N_2 .

Formation of Guanidine. (Only for products **2c** and **2d**.) A solution of 0.25 M DMPFN **15** and 1 M DIPEA in dry DMF was added to the resin. The mixture was stirred and heated at 65 °C overnight. After cooling down, the solution was aspirated, and the resin was washed with DMF, MeOH, and DCM (3 times each).

Cleavage off Resin. The resin was treated with cleavage solution (TFA/TES/dry DCM 10/20/70, 1 mL) for 3 h at rt. The cleavage solution was then drained, and the resin was washed with THF, DCM, and THF (1 mL each). The solvents were evaporated to obtain crude cleavage product.

NH₃ Treatment. The crude cleavage product from the cleavage step was treated with freshly prepared saturated ammonia solution in MeOH (2 mL). The solution was gently shaken and left to stand for 2 h at room temperature. The solution was evaporated, and crude samples were purified by LCMS on a standard C^{18} column using gradient programs of acetonitrile and water.

(2-Phenethyl)-2-(4'-aminobutyrylamido)-2-deoxy-6-O-methyl-3-O-(2'-naphthylmethyl)- α -D-glucopyranoside (**2a**). Resin loaded glycoside **4a** (80 mg resin, 0.45 mmol/g) was carried through the solid-phase synthetic process. The title product was isolated by HPLC purification and MS detection as a white solid (8.7 mg, 46% over 7 steps): ^1H NMR (400 MHz, CDCl_3) δ 1.45–1.56 (m, 2H, $\text{NHCOCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$), 1.79–1.92 (m, 2H, $\text{NHCOCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$), 2.51 (t, 2H, J = 6.9 Hz, $\text{NHCOCH}_2\text{CH}_2\text{CH}_2\text{NH}_2$), 2.85 (dd, 2H, J = 6.7 Hz, $\text{OCH}_2\text{CH}_2\text{Ph}$), 3.39 (s, 3H,

OCH₃), 3.53–3.62 (m, 5H, H₃/H₅/H₆ and one H from OCH₂CH₂Ph), 3.73 (dd, 1H, $J_{H_3,H_4} = 8.8$ Hz, H₄), 3.95 (dt, 1H, $J = 9.7$ Hz, $J = 5.8$ Hz, one H from OCH₂CH₂Ph), 4.23 (ddd, 1H, $J = 10.5$ Hz, $J = 9.4$ Hz, $J = 3.6$ Hz, H₂), 4.70 (d, 1H, $J = 3.6$ Hz, H₁), 4.83 (d, 1H, $J = 11.9$ Hz, one H from OCH₂Ph), 4.89 (d, 1H, $J = 11.9$ Hz, one H from OCH₂Ph), 5.43 (d, 1H, $J = 9.4$ Hz, NH), 7.13–7.26 (m, 5H, H_{Ar}), 7.42–7.49 (m, 3H, H_{Ar}), 7.77–7.83 (m, 4H, H_{Ar}); ¹³C NMR (100.6 MHz, CDCl₃) δ 29.2, 34.2, 36.1, 41.7, 51.8, 59.7, 68.5, 70.4, 71.8, 72.8, 73.6, 80.0, 98.0, 126.3, 126.5, 126.6, 126.9, 127.0, 127.9, 128.1, 128.4, 128.6, 129.1, 133.2, 133.5, 136.1, 139.2, 172.6; HRMS (ESI, pos mode) m/z [M + H]⁺ calcd for C₃₀H₃₉N₂O₆ 523.2803; found 523.2804.

(2'-Phenethyl)-2-(4'-aminobutyrylamido)-2-deoxy-6-O-methyl-3-O-(2'-naphthylmethyl)-β-D-glucopyranoside (2b). Resin loaded glycoside **4b** (80 mg resin, 0.37 mmol/g) was carried through the solid-phase synthetic process. The title product was isolated by HPLC purification and MS detection as a white solid (4.3 mg, 28% over 7 steps): ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.56–1.77 (m, 2H, NHCOCH₂CH₂CH₂NH₂), 1.99–2.15 (m, 2H, NHCOCH₂CH₂CH₂NH₂), 2.72–2.95 (m, 2H, NHCOCH₂CH₂CH₂NH₂), 2.78 (t, $J = 6.3$ Hz, 2H, OCH₂CH₂Ph), 3.29 (s, 3H, OCH₃), 3.30–3.37 (m, 2H, H₄, H_{6b}), 3.45–3.52 (m, 2H, H-3, H-6a), 3.57–3.69 (m, 3H, H-2, H-5, OCH₂(B)CH₂-Ph), 3.88 (m, 1H, 1H, OCH₂(A)CH₂-Ph), 4.41 (d, 1H, $J_{H_1,H_2} = 8.4$ Hz, H-1), 4.73 (d, 1H, $J_{A,B} = -11.8$ Hz, 1H, OCH₂(B)-Naph), 4.92 (d, 1H, OCH₂(A)-Naph), 5.43 (m, 1H, OH-4), 6.69 (bs, 3H, NH₃), 7.83 (d, 1H, $J_{NH,H-2} = 8.4$ Hz, C-2NH), 7.16–8.00 (m, 12H, H_{Ar}); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 25.98 (CH₂CH₂CH₂), 33.3 (COCH₂), 35.3 (CH₂CH₂Ph), 40.3 (H₂NCH₂), 58.4 (OCH₃), 68.9 (OCH₂CH₂Ph), 70.1 (C-4/C-5*), 71.5 (C-6), 73.2 (OCH₂Nap), 75.4 (C-4/C-5*), 82.4 (C-3), 100.6 (H-1), 124.1, 125.5, 125.87, 125.88, 125.89, 125.90, 127.3, 127.4, 127.5, 128.00, 128.02, 128.70, 128.72, 132.2, 132.6, 136.6, 138.7 (all aromat. C), 156.9 (TFA), 171.4 (NHCO); HRMS (ESI, pos mode) m/z [M + H]⁺ calcd for C₃₀H₃₉N₂O₆ 523.2803; found 523.2803.

(2'-Naphthylmethyl)-2-deoxy-2-(4'-guanidinobutyrylamido)-3-O-(3'-hydroxybenzyl)-6-O-methyl-α-D-glucopyranoside (2c). Resin loaded glycoside **4c** (80 mg resin, 0.35 mmol/g) was carried through the solid-phase synthetic process. The title product was isolated by HPLC purification and MS detection as a white solid (11.0 mg, 58% over 9 steps): ¹H NMR (400 MHz, DMSO-*d*₆) δ 1.61–1.68 (m, 2H, NHCOCH₂CH₂CH₂NH₂), 2.20 (t, 2H, $J = 7.2$ Hz, NHCOCH₂CH₂CH₂NH₂), 3.02–3.10 (dq, 2H, NHCOCH₂CH₂CH₂NH₂), 3.31 (s, 3H, OCH₃), 3.29 (s, 3H, OCH₃), 3.34–3.39 (m, 1H, H₄), 3.52 (dd, 1H, $J_{H_5,H_6} = 5.8$ Hz, $J_{H_{6a},H_{6b}} = -10.7$ Hz, H-6), 3.58–3.67 (m, 2H, H-3, H-6), 3.72 (ddd, 1H, H-5), 3.86–3.92 (m, 1H, H-2), 4.57 (d, 1H, $J_{A,B} = -11.2$ Hz, OCH₂(B)-Ar¹), 4.63 (d, 1H, $J_{A,B} = -12.5$ Hz, OCH₂(B)-Ar²), 4.73 (d, 1H, OCH₂(A)-Ar¹), 4.75 (d, 1H, $J_{H_2} = 3.5$ Hz, H-1), 4.84 (d, 1H, OCH₂(A)-Ar²), 5.42 (d, 1H, $J_{OH,H-4} = 7.1$ Hz, OH-4), 6.63–7.95 (m, 12H, 11 aromat. H, NH=C), 7.68 (bs, 3H, NH₃), 8.15 (d, 1H, $J_{NH,H-2} = 8.7$ Hz, C-2NH), 9.29 (s, 1H, OH-Ar); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 24.6 (CH₂CH₂CH₂), 31.7 (COCH₂), 40.3 (H₂NCH₂), 52.3 (C-2), 58.4 (OCH₃), 68.2 (OCH₂Ar²), 70.4 (C-4), 71.4 (C-6), 71.7 (C-5), 73.4 (OCH₂Ar¹), 79.3 (C-3), 96.1 (C-1), 113.8, 114.2, 117.8, 125.83, 125.86, 126.1, 126.2, 127.45, 127.51, 127.7, 128.7, 132.4, 132.6, 135.1, 140.6, (all aromat. C), 156.7, 157.0 (TFA + TFA-salt), 158.21, 158.45, (guanidine C), 171.7 (NHCO); HRMS

(ESI, pos mode) m/z [M + H]⁺ calcd for C₃₀H₃₉N₄O₇ 567.2813; found 567.2810.

(2'-Naphthylmethyl)-2-deoxy-2-(3'-guanidinopropionylamido)-3-O-methyl-6-O-(2'-naphthylmethyl)-β-D-glucopyranoside (2d). Resin loaded glycoside **4d** (80 mg resin, 0.37 mmol/g) was carried through the solid-phase synthetic process. The title product was isolated by HPLC purification and MS detection as a white solid (11.3 mg, 54% over 9 steps): ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.40 (dq, 2H, COCH₂), 3.19 (t, 1H, $J_{H_4,H_5} = 9.3$ Hz, H-5), 3.26–3.43 (m, 7H, OCH₃, H-3, H-4, CH₂NH₂), 3.64–3.72 (m, 2H, H-2, H-6), 3.85 (d, 1H, $J_{H-6a,H-6b} = -10.5$ Hz, H-6), 4.47 (d, 1H, $J_{H_1,H_2} = 8.4$ Hz, H-1), 4.72 (d, 3H, OCH₂(A,B)Nap1, OCH₂(B)Nap2), 4.91 (d, 1H, $J_{A,B} = -12.7$ Hz, OCH₂(A)Nap2), 5.36 (d, 1H, $J_{OH,H-4} = 6.3$ Hz, OH-4), 7.40–7.94 (m, 15H, aromat. H, NH=C), 7.63 (t, 1H, $J_{CH_2,NH} = 5.3$ Hz, CH₂NH), 8.15 (d, 1H, $J_{NH,H_2} = 9.1$ Hz, CONH); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 34.6, (COCH₂), 37.0 (H₂NCH₂), 53.9 (C-2), 59.1 (OCH₃), 69.478 (C-6), 69.482 (C-4), 69.6 (OCH₂Nap), 72.3 (OCH₂Nap), 75.6 (C-3), 83.3 (C-5), 100.1 (C-1), 125.57, 125.61, 125.68, 125.70, 125.76, 126.0, 126.03, 127.43, 127.43, 127.52, 127.53, 127.64, 127.67, 132.30, 132.32, 132.60, 132.70, 135.36, 136.21 (all aromat. C), 156.8 (TFA), 158.18, 158.43, (guanidine C), 169.94 (CONH); HRMS (ESI, pos mode) m/z [M + H]⁺ calcd for C₃₃H₃₉N₄O₆ 587.2864; found 587.2865.

Methyl [2-(3'-Aminopropionylamido)-6-O-(4'-chlorobenzyl)-2-deoxy-3-(2'-naphthylmethyl)-1-thio-β-D-allopyranose] (2e). Resin loaded building block **1b** (80 mg resin, 0.32 mmol/g) was carried through the solid-phase synthetic process. The title product was isolated by HPLC purification and MS detection as a white solid (4.1 mg, 29% over 7 steps): ¹H NMR (400 MHz, DMSO-*d*₆) δ 2.04 (s, 3H, SCH₃), 2.37–2.52 (2 × td, under DMSO peak, NHCOCH₂CH₂NH₂), 2.86 (dd, $J = 7.0$ Hz, 2H, NHCOCH₂CH₂NH₂), 3.54–3.59 (m, 2H, H₄/H_{6a}), 3.70–3.76 (m, 1H, $J_{H_{6a},H_{6b}} = -11.0$ Hz, $J_{H_5,H_{6b}} = 1.3$ Hz, H_{6b}), 3.82–3.90 (m, 3H, H₂/H₃/H₅), 4.52 (s, 2H, OCH₂Ph₄Cl), 4.62 (d, $J = 10.3$ Hz, 1H, H₁), 4.83 (d, $J = 11.8$ Hz, 1H, one H from OCH₂Nap), 4.98 (d, $J = 11.8$ Hz, 1H, one H from OCH₂Nap), 5.33 (bs, 1H, OH, exchanged in D₂O), 7.34–7.40 (m, 4H, aromat. H), 7.46–7.50 (m, 2H, aromat. H), 7.62 (dd, 1H, $J = 8.2$ Hz, $J = 1.5$ Hz, aromat. H), 7.86–7.90 (m, 4H, aromat. H), 8.20 (d, 1H, $J = 8.8$ Hz, NH, exchanged with D₂O); ¹³C NMR (100.6 MHz, DMSO-*d*₆) δ 11.8, 32.7, 36.0, 51.8, 68.8, 70.6, 72.1, 75.2, 76.9, 78.7, 81.1, 126.4, 126.7, 126.8, 126.9, 127.0, 128.2, 128.4, 128.9, 129.8, 132.5, 133.1, 133.4, 137.2, 138.4, 169.6; HRMS (ESI, pos mode) m/z [M + H]⁺ calcd for C₂₈H₃₄ClN₂O₅S 545.1871; found 545.1867.

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Supporting Information Available: Experimental details for the synthesis of (3-*tert*-butoxy-phenyl)methyl bromide **17**, and NMR spectral data (¹H, ¹³C and COSY) for compounds **4a–d** and **2a–e**. This material is available free of charge via the Internet at <http://pubs.acs.org>.