Solid-Phase Synthesis of Complex Oligosaccharides Using Azidoglucose as a Glycosyl Acceptor

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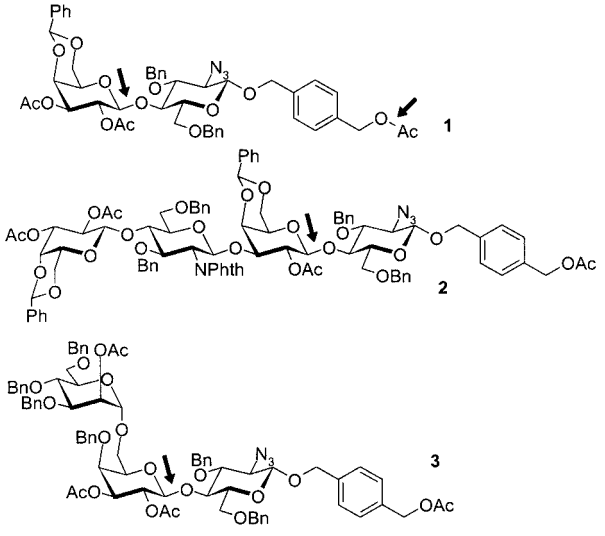
The solid phase synthesis of oligosaccharides **1**–**3** was performed on Merrifield resin. These syntheses are based on the hydroxymethylbenzyl benzoate spacer-linker system and, for regioselective chain extension, the use of *O*-glycosyl trichloroacetimidates as glycosyl donors containing temporary *O*-Fmoc protection. The important lactosamine-containing oligosaccharides **1**–**3** are accessible in excellent yields by using a 2-azidoglucose residue as the glucosamine building block adjacent to the spacer-linker system. Only standard amounts of the glycosyl donor are employed in this solid phase oligosaccharide synthesis and we did not have to resort to any capping procedures.

Introduction

Oligosaccharides, which play an important role in various biological processes, including inflammation, immune response, metastasis, and fertilization, have attracted a lot of interest in recent years[1−3] and, as a consequence, oligosaccharide synthesis has become an important task.[4−8] Recently, successful solid phase oligosaccharide syntheses (SPOS) have been developed by several research groups[9−20] because SPOS exhibits inherent advantages over solution phase synthesis, such as higher reaction yields because of the use of excess building blocks and/or reagents, shorter times for the completion of the syntheses, and more convenient purification procedures. In contrast to oligopeptides and oligonucleotides, which are routinely prepared on automated synthesizers, no general strategy has yet appeared for the efficient construction of various complex oligosaccharides on polymer supports, which, therefore, limits the use of automated synthesizers. To this end, quite a deal of fine tuning of the SPOS methodology is still required.

One of the problems that we encountered recently is the low reactivity of *N*-phthaloyl- or *N*-dimethylmaleoyl-protected, 4-*O*-unprotected glucosamine residues as acceptors, particularly when these residues are positioned next to the resin linkage. Therefore, in our previous approaches to the syntheses of *N*-glycan, lactosamine, and oligolactosamine oligosaccharides, we have introduced a novel capping procedure to overcome the low reactivity of this acceptor and, thus, to avoid the accumulation of undesired by-products.[21−23] Alternatively, a reactivity increase of the acceptor moiety can be envisaged by introducing the azido group as a latent amino functionality, because this group has proven, in solution phase oligosaccharide synthesis, to have great versatility and efficiency and it can be transformed readily into an *N*-acetylamino group.[24,25] Hence, we selected an azido group-containing glucosamine residue for the solid phase synthesis of target molecules **1**−**3** (Scheme 1), which are constituents of various oligosaccharide types.



Scheme 1. Target molecules **1**−**3**

Results and Discussion

Attachment of target molecules **1**−**3** to the Merrifield resin (for instance, to carboxylic acid **5**) will lead to intermediate **4a** (Scheme 2, which also exhibits the synthesis strategy).

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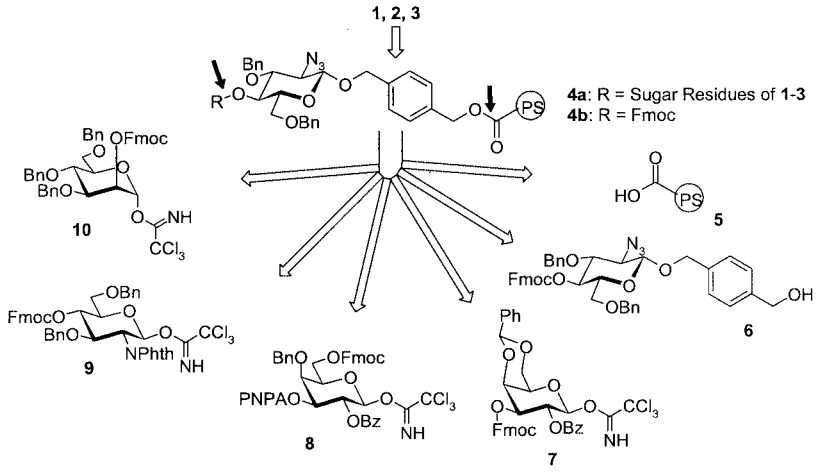
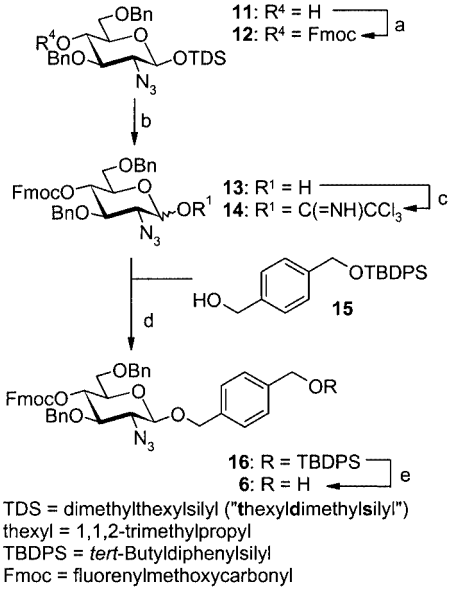


Scheme 2. Synthesis strategy and building blocks required for the synthesis of **1**−**3**

This strategy is based on our hydroxymethylbenzyl benzoate spacer-linker system[26] with **4b** as the decisive intermediate and *O*-Fmoc protected *O*-glycosyl trichloroacetimidates as the glycosyl donors; hence, we required building blocks **6**−**10** for the synthesis of **1**−**3**.

Synthesis of Building Blocks 6−10

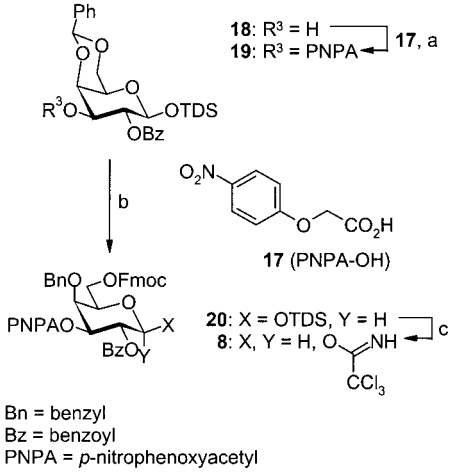
Building blocks **7**, **9**, and **10** are readily available by following previously reported procedures.[27] The sugar-linker building block **6** (Scheme 3) was prepared from the known 2-azido-3,6-di-*O*-benzyl-2-deoxy-glucose derivative **11**, which was obtained from D-glucosamine in five steps.[28]



Scheme 3. Synthesis of sugar-linker building block **6**; reagents and conditions: (a) FmocCl, pyridine, DMAP, 95%; (b) HF·pyridine, THF, 95%; (c) Cl_3CCN, NaH, 95%; (d) CH_2Cl_2/CH_3CN, 1:1, TMSOTf, 60%; (e) HF·pyridine, THF, 78%

The Fmoc group was introduced using Fmoc-Cl and a catalytic amount of Steglich's reagent (DMAP) in pyridine at room temperature (→**12**). 1-*O*-Desilylation using an excess of HF·pyridine complex in THF furnished 1-*O*-unprotected **13**, which we transformed subsequently into trichloroacetimidate **14** by treatment with trichloroacetonitrile in the presence of sodium hydride (0.1 equiv.). Glycosylation of linker **15**[29] in dichloromethane/acetonitrile, 1:1, in the presence of TMSOTf as catalyst, led, as a result of the nitrile effect,[30] to β-glycoside **16**, which we transformed into building block **6** by desilylation with an excess of HF·pyridine complex in THF.

The synthesis of the galactosyl donor **8** was based on the known galactoside **18** (Scheme 4).[27]



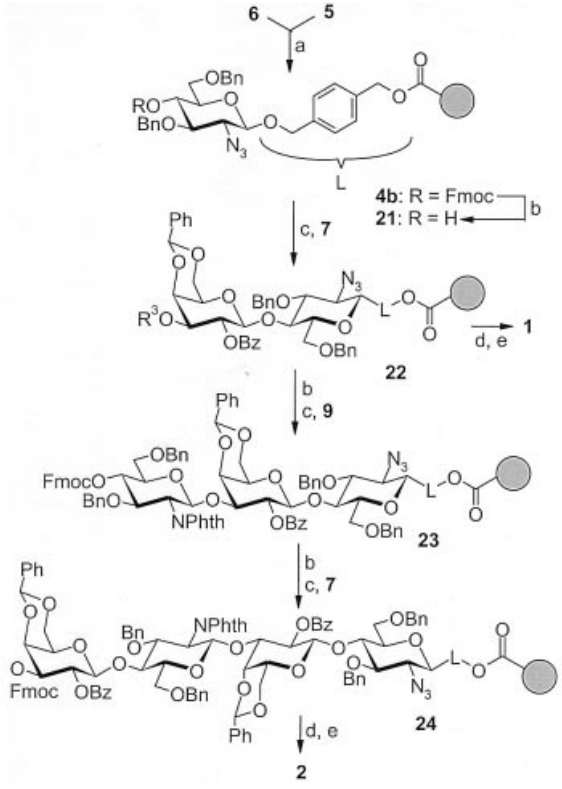
Scheme 4. Synthesis of building block **8**; reagents and conditions: (a) DCC, DMAP, CH_2Cl_2, quant.; (b) 1. BH_3·THF, Bu_2BOTf, 74%; 2. FmocCl, pyridine, 90%; (c) 1. HF·pyridine, THF, 98%; 2. CCl_3CN, HaH, 70%.

4-Nitrophenoxyacetic acid (PNPA-OH) **17** was obtained by following a published procedure.[31] Treatment of **17** and

18 with DCC as condensing agent and a catalytic amount of DMAP in dichloromethane afforded **19** in quantitative yield. Reductive opening of the 4,6-*O*-benzylidene group containing ring with borane in the presence of dibutylborane triflate[32] afforded the desired 6-*O*-unprotected intermediate, which on treatment with Fmoc-Cl in pyridine furnished the 1-*O*-silyl-protected glycoside **20** that carries two temporary protecting groups (Fmoc and PNPA) for orthogonal cleavage. Removal of the 1-*O*-silyl group using an excess of HF·pyridine complex in THF, and then reaction with trichloroacetonitrile in the presence of sodium hydride (0.1 equiv.) as base, gave the desired galactosyl donor **8**.

Solid Phase Synthesis of Target Molecules 1−3

The SPOS was initiated by attaching primary alcohol **6** to the solid support **5** through a condensation reaction with *N*,*N*-diisopropylcarbodiimide (DIC) and a catalytic amount of DMAP in dichloromethane; unchanged carboxylate functions were transformed into their methyl esters as described previously (Scheme 5).[27]

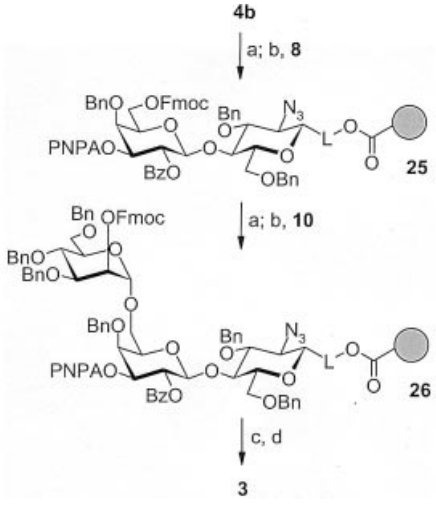


Scheme 5. Synthesis of oligosaccharides **1** and **2**; reagents and conditions: (a) 1. DIC, DMAP, CH_2Cl_2; 2. MeOH; (b); NEt_3/CH_2Cl_2, 8:1; (c) CH_2Cl_2, −40 °C, TMSOTf; (d) NaOMe/MeOH; (e) Ac_2O, pyridine.

We determined the loading of the resin **4b** to be 0.12 mmol/g resin after recycling and purification of the starting material **6**. Treatment of resin **4b** with triethylamine in dichloromethane afforded the 4-*O*-unprotected resin **21**, which we subjected to glycosylation with 3 equiv. of donor **7**[27] and TMSOTf (0.3 equiv.) as catalyst to furnish the resin-bound disaccharide **22**. Generally, we monitored the progress of the reaction by performing TLC and MALDI-TOF analyses of the crude cleavage product (NaOMe, CH_2Cl_2/MeOH) obtained from a small sample of resin (2 mg). Preparative cleavage of resin **22** and per-*O*-acetylation of the product afforded, after flash chromatography, the desired lactosamine disaccharide **1** in 81% yield from **6** (96% per step over five steps); this result suggests that resin **21**, which has a 2-azido group in the glucosamine residue, is an excellent acceptor.

A β-(1−3)-linked *N*-acetyllactosamine chain is a frequently occurring structural unit in, for instance, human milk. Therefore, we repeated the previous synthesis up to resin **22**, which contains one lactosamine residue and an Fmoc unit as a temporary protecting group at the 3b-*O* for chain extension. Treatment of resin **22** with triethylamine in dichloromethane (for selective Fmoc removal) and then glycosylation with glucosamine donor **9** in the presence of TMSOTf as catalyst afforded the trisaccharide resin **23**. After washing with dichloromethane/THF, 1:1, and then performing Fmoc removal and glycosylation with donor **7** as described before, we obtained tetrasaccharide resin **24**. A final preparative cleavage of resin **24** using NaOMe/MeOH, and then *O*-acetylation of the cleavage product using acetic anhydride in pyridine, afforded, after flash chromatography, target molecule **2** in a satisfactory yield of 21% from **6** (84% per step over nine steps). Investigations with the 2-azidoglucose donor **14**, rather than the 2-phthalimidoglucose donor **9**, led to higher product yields, but, to our surprise, the glycosylation step on the polymer support, although carried out in a 1:1-mixture of dichloromethane/acetonitrile at low temperature, was not fully β-selective.

For the synthesis of target molecule **3**, again we first treated resin **4b** with triethylamine to remove the 4-*O*-Fmoc group; the subsequent glycosylation with galactosyl donor **8** (3 equiv.) in the presence of TMSOTf (0.3 equiv.) as catalyst in dichloromethane at −20 °C furnished disaccharide resin **25**. The Fmoc group was then removed selectively in



Scheme 6. Synthesis of trisaccharide **3**; reagents and conditions: (a) NEt_3/CH_2Cl_2, 8:1; (b) TMSOTf, −20 °C, CH_2Cl_2; (c) NaOMe/MeOH; (d) Ac_2O, pyridine.

the presence of the PNPA group using triethylamine in dichloromethane. After washing with dichloromethane/THF, 1:1, glycosylation with the mannosyl donor **10**[26,27] afforded the trisaccharide resin **26**. A final preparative cleavage and *O*-acetylation, as described above, afforded, after flash chromatography, the trisaccharide **3** in 63% overall yield from **6** (95% per step over seven steps). Thus, with this addition to our previously introduced SPOS methodology, oligosaccharides containing glucosamine residues at their reducing end also can be obtained in excellent yields (Scheme 6).

Conclusion

The use of a 4-*O*-unprotected 2-azidoglucose acceptor, rather than a corresponding 2-phthalimido- or 2-dimethylmaleimido-glucose acceptor, close to the resin linkage greatly improved the SPOS results when applied to our previously introduced methodology. Hence, the amount of glycosyl donor required for high-yielding glycosylation is reduced and the capping of the unchanged acceptor after the glycosylation step, to reduce by-product formation and to ease product purification, is not required.

Experimental Section

General Remarks: Each solvent was purified and dried in the usual manner. All reactions were performed using dry solvents and under argon unless otherwise stated. TLC was performed on plastic plates of silica gel 60 F_{254}. Detection was achieved by treatment with a solution of ammonium molybdate (20 g) and cerium(IV) sulfate (0.4 g) in 10% H_2SO_4 (400 mL), or with 15% H_2SO_4, and then heating at 150 °C. Flash chromatography was carried out on silica gel (Baker 30−60 mm). Adsorption of crude reaction products was performed using silica gel (Baker 60−200 mm). Petroleum ether was used in the boiling range 35−70 °C; toluene, CH_2Cl_2, MeOH, and EtOAc were distilled. Optical rotations were determined at 21 °C using a Perkin−Elmer 241/MC polarimeter (1-dm cell). NMR spectra were recorded using Bruker 600 DRX instruments; tetramethylsilane was the internal standard. MS spectra were recorded using a MALDI-kompakt (Kratos) instrument operating in the positive mode; 2,5-dihydroxybenzoic acid in THF was the matrix. Microanalyses were performed in the microanalysis unit at the Fachbereich Chemie, Universität Konstanz.

Dimethylthexylsilyl 2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranoside (12): FmocCl (7.0 g, 27.5 mmol) and DMAP (24 mg, 0.2 mmol) were added at room temperature to a solution of **11**[28] (2.9 g, 5.5 mmol) in pyridine (20 mL). After 2 h, the mixture was concentrated at low pressure, and the residue purified by flash chromatography (petroleum ether/ethyl acetate, 20:1) to afford **12** (3.9 g, 95%) as a white foam. TLC (petroleum ether/ethyl acetate, 10:1): R_f = 0.33. $[\alpha]_D$ = −21.6 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.08, 0.11 (2 s, 6 H, 2 CH_3), 0.78−0.81 (m, 12 H, 4 CH_3), 1.57 [m, 1 H, C*H*(CH_3)$_2$], 3.3 (m, 2 H, 3-*H*, 2-*H*), 3.48−3.60 (m, 3 H, 5-*H*, 6-*H*, 6′-*H*), 4.18 (d, ^2J = 7.2 Hz, 2 H), 4.20 (m, 1 H), 4.33 (d, $J_{1,2}$ = 7.2 Hz, 1 H, 1-*H*), 4.40−4.57 (m, 3 H, 3/2 CH_2Ph), 4.67−4.74 (m, 2 H, 4-*H*, 1/2 CH_2Ph), 7.11−7.69 (m, 18 H, Ar) ppm. MALDI-MS: *m*/*z* = 772.5 [M + Na^+]. $C_{43}H_{51}N_3O_7Si$ (749.9): calcd. C 68.87, H 6.85, N 5.60; found C 68.87, H 6.86, N 5.60.

2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranose (13): HF·pyridine (7.0 mL, 50.7 mmol) was added dropwise at room temperature to a solution of **12** (3.8 g, 5.07 mmol) in dry THF (30 mL), and then the mixture was stirred overnight. The solution was diluted with ethyl acetate (50 mL), neutralized with solid calcium carbonate and water (1 mL), and poured into water (50 mL) and then the layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 4:1) to afford **13** (2.9 g, 95%) as a white foam. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.43. $[\alpha]_D$ = +5.9 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.18 (d, 1 H, OH), 3.39−3.62 (m, 4 H, 2-*H*, 5-*H*, 6-*H*, 6′-*H*), 4.08 (t, ^3J = 7.2, ^3J = 9.6 Hz, 1 H, 3-*H*), 4.18−4.34 (d, ^2J = 7.2 Hz, 2 H), 4.48 (s, 2 H, CH_2Ph), 4.57 (t, 1 H), 4.65 (d, ^2J = 9.3 Hz, 1 H, 1/2 CH_2Ph), 4.84 (dd, ^3J = 9.6, ^3J = 7.7 Hz, 1 H, 4-*H*), 5.3 (br., 1 H, 1-*H*), 7.18−7.75 (m, 18 H, Ar) ppm. MALDI-MS: *m*/*z* = 629.9 [M + Na^+]. $C_{35}H_{33}N_3O_7$ (607.7): calcd. C 69.18, H 5.48, N 6.91; found C 69.14, H 5.59, N 6.93.

2-Azido-*O*-[3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-α/β-D-glucopyranosyl] Trichloroacetimidate (14): Compound **13** (2.4 g, 3.95 mmol) was dissolved in a mixture of dichloromethane (10 mL) and Cl_3CCN (10 mL), and then sodium hydride (2 mg) was added at 0 °C. After 30 min, the mixture was neutralized with silica gel and concentrated at low pressure. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 4:1) to afford **14** (2.8 g, 95%) as a white foam. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.67 (β). $[\alpha]_D$ = −1.4 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.54−3.78 (m, 4 H, α/β, 2-*H*, 5-*H*, 6-*H*, 6′-*H*), 4.03−4.14 (m, 1 H, α/β, 3-*H*), 4.27−4.33 (m, 2 H), 4.45−4.49 (m, 2 H, CH_2Ph), 4.65−4.80 (m, 2 H, CH_2Ph), 4.93−5.00 (dd, ^3J = 9.3, ^3J = 9.6 Hz, 1 H, 4-*H*β), 5.08−5.17 (dd, ^3J = 9.5, ^3J = 10.0 Hz, 1 H, 4-*H*α), 5.63 (d, $J_{1,2}$ = 8.3 Hz, 1 H, 1-*H*β), 6.41 (d, $J_{1,2}$ = 3.5 Hz, 1 H, 1-*H*α), 7.06−7.75 (m, 18 H, Ar), 8.72 (s, 1 H, NHα), 8.75 (s, 1 H, NHβ) ppm. MALDI-MS: *m*/*z* = 629.3 [M − Cl_3CCN + Na^+]. $C_{37}H_{33}Cl_3N_4O_7$ (752.2): calcd. C 58.08, H 4.42, N 7.45; found C 58.02, H 4.11, N 7.55.

4-(*tert*-Butyldiphenylsilyloxymethyl)benzyl 2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranoside (16): A solution of **15**[29] (170 mg, 0.45 mmol) and **14** (444 mg, 0.5 mmol) in a mixture of CH_2Cl_2/CH_3CN, 1:1 (5 mL) was stirred in the presence of 4-Å molecular sieves under argon at room temperature for 5 min. TMSOTf (3 μL, 0.015 mmol) was slowly added dropwise and then the reaction mixture was stirred for 30 min. The solution was filtered and the filtrates were concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 6:1) to afford **16** (260 mg, 60%). TLC (petroleum ether/ethyl acetate, 4:1): R_f = 0.57. $[\alpha]_D$ = −21.2 (*c* = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 1.04 (s, 9 H, *t*Bu), 3.36−3.57 (m, 5 H), 4.09 (t, ^3J = 7.1 Hz, 1 H) 4.22−4.26 (dd, ^3J = 7.5 Hz, 2 H), 4.32 (d, $J_{1,2}$ = 7.7 Hz, 1 H, 1-*H*), 4.48 (s, 2 H, CH_2Ph), 4.56−4.66 (t, ^2J = 12.9 Hz, 2 H, CH_2Ph), 4.71 (s, 2 H, CH_2Ph), 4.75−4.90 (m, 3 H, 4-*H*, CH_2Ph), 7.14−7.72 (m, 32 H, Ar) ppm. MALDI-MS: *m*/*z* = 989.9 [M + Na^+]. $C_{59}H_{59}N_3O_8Si$ (966.2): calcd. C 73.34, H 6.15, N 4.35; found C 73.64, H 6.26, N 4.37.

4-(Hydroxymethyl)benzyl 2-Azido-3,6-di-*O*-benzyl-2-deoxy-4-*O*-(9-fluorenylmethoxycarbonyl)-β-D-glucopyranoside (6): HF·pyridine

(0.35 mL, 2.28 mmol) was added dropwise at room temperature to a solution of **16** (220 mg, 0.228 mmol) in dry THF (10 mL), and then the mixture was stirred overnight. The solution was diluted with ethyl acetate (10 mL), neutralized with solid calcium carbonate and water (0.5 mL), and then poured into water (10 mL); the combined organic layers were dried with magnesium sulfate and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to afford **6** (130 mg, 78%) as a white oil. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.25. $[\alpha]_D$ = −15.4 (c = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.32−3.56 (m, 5 H, 2-*H*, 3-*H*, 5-*H*, 6-*H*, 6′-*H*), 4.04 (t, ^3J = 7.1 Hz, 1 H), 4.22 (dd, ^3J = 7.6 Hz, 2 H), 4.27 (d, $J_{1,2}$ = 7.8 Hz, 1 H, 1-*H*), 4.45 (s, 2 H, CH_2Ph), 4.53−4.62 (m, 4 H, 2 CH_2Ph), 4.68−4.88 (m, 3 H, 4-*H*, CH_2Ph), 7.12, 7.69 (m, 22 H, Ar) ppm. MALDI-MS: m/z = 749 [M + Na^+]. $C_{43}H_{41}N_3O_8$ (726.9): calcd. C 70.09, H 5.70, N 5.75; found C 70.32, H 5.77, N 6.00.

Dimethylthexylsilyl 2-*O*-Benzoyl-4,6-*O*-benzylidene-3-*O*-(4-nitrophenoxyacetyl)-β-D-galactopyranoside (19): A solution of **18**[27] (3.0 g, 5.84 mmol) and **17**[31] (1.38 g, 7 mmol) in dry CH_2Cl_2 (20 mL) was treated with a catalytic amount of DMAP (70 mg, 0.58 mmol) and DCC (2.4 g, 11.7 mmol). After 2 h, the reaction mixture was concentrated at low pressure, and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to afford **19** (4.8 g, quant.) as a white foam. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.43. $[\alpha]_D$ = +57.7 (c = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.08−0.19 (2 s, 6 H, 2 CH_3), 0.71 (m, 12 H, 4 CH_3), 1.51 [m, 1 H, C*H*(CH_3)$_2$], 3.57 (s, 1 H, 5-*H*), 4.07 (m, 1 H, 6-*H*), 4.32 (d, ^2J = 11.9 Hz, 1 H, 6′-*H*), 4.45 (d, ^3J = 3.6 Hz, 1 H, 4-*H*), 4.56-4-72 (dd, ^2J = 16.6 Hz, 2 H, CH_2OPh), 4.93 (d, $J_{1,2}$ = 7.6 Hz, 1 H, 1-*H*), 5.26 (dd, ^3J = 3.7 Hz, 1 H, 3-*H*), 5.52 (s, 1 H, PhCH), 5.60−5.67 (dd, ^3J = 7.6 Hz, 1 H, 2-*H*), 6.6−8.0 (m, 14 H, Ar) ppm. MALDI-MS: m/z = 717.2 [M + Na^+]. $C_{36}H_{43}NO_{11}Si$ (693.7): calcd. C 62.33, H 6.24, N 2.02; found C 62.19, H 6.23, N 2.32.

Dimethylthexylsilyl 2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-3-*O*-(4-nitrophenoxyacetyl)-β-D-galactopyranoside (20): 19 (1.4 g, 2.0 mmol) was dissolved in dry dichloromethane (10 mL) under argon and then 1 M BH_3·THF (20 mL, 20 mmol) and 1 M dibutylborane triflate (2 mL, 2 mmol) were slowly added dropwise at 0 °C before the reaction mixture was stirred for 4 h. The solution was concentrated at low pressure, and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 3:1) to afford dimethylthexylsilyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-nitrophenoxyacetyl)-β-D-galactopyranoside (1.53 g, 74%) as a white oil. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.25. $[\alpha]_D$ = +11.9 (c = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.03, 0.13 (2 s, 6 H, 2 CH_3), 0.67−0.70 (m, 12 H, 4 CH_3), 1.47−1.53 [m 1 H, C*H*(CH_3)$_2$], 3.64−3.68 (m, 2 H, 5-*H*, 6-*H*), 3.86 (m, 1 H, 6′-*H*), 3.97 (d, ^3J = 3.2 Hz, 1 H, 4-*H*), 4.34−4.52 (dd, ^2J = 16.5 Hz, 2 H, CH_2Ph), 4.62−4.74 (dd, ^2J = 12.1 Hz, 2 H, CH_2OPh), 4.83 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1-*H*), 5.26 (dd, ^3J = 3.2 Hz, 1 H, 3-*H*), 5.57−5.64 (dd, ^3J = 7.5 Hz, 1 H, 2-*H*), 6.6−7.98 (m, 14 H, Ar) ppm. MALDI-MS: m/z = 719.3 [M + Na^+]. $C_{36}H_{45}NO_{11}Si$ (693.7): calcd. C 62.15, H 6.52, N 2.01; found C 62.10, H 6.61, N 2.23.

A catalytic amount of DMAP (3 mg, 0.02 mmol) and FmocCl (2.1 g, 8.2 mmol) were added at room temperature to a solution of dimethylthexylsilyl 2-*O*-benzoyl-4-*O*-benzyl-3-*O*-(4-nitrophenoxyacetyl)-β-D-galactopyranoside (1.43 g, 2.06 mmol) in pyridine (15 mL). After 2 h, the mixture was concentrated at low pressure and the residue was purified by flash chromatography (petroleum ether/ethyl acetate, 4:1) to afford **20** (1.7 g, 90%) as a white foam. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.43. $[\alpha]_D$ = +16.1 (c = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 0.01, 0.10 (2 s, 6 H, 2 CH_3), 0.63−067 (m, 12 H, 4 CH_3), 1.41−1.46 [m, 1 H, C*H*(CH_3)$_2$], 3.67−3.83 (t, ^3J = 6.5 Hz, 1 H, 5-*H*), 3.94 (d, ^3J = 2.9 Hz, 1 H, 4-*H*), 4.07−4.46 (m, 7 H), 4.64 (s, 2 H, CH_2OPh), 4.78 (d, $J_{1,2}$ = 7.5 Hz, 1 H, 1-*H*), 5.23 (dd, ^3J = 3.2 Hz, 1 H, 3-*H*), 5.4 (dd, ^3J = 7.5 Hz, 1 H, 2-*H*), 6.6−7.9 (m, 22 H, Ar) ppm. MALDI-MS: m/z = 940.6 [M + Na^+]. $C_{51}H_{55}NO_{13}Si$ (918.1): calcd. C 66.72, H 6.04, N 1.53; found C 66.44, H 5.89, N 1.60.

***O*-[2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-3-*O*-(4-nitrophenoxyacetyl)-α/β-D-galactopyranosyl] Trichloroacetimidate (8):** HF·pyridine (1.4 mL, 9 mmol) was added dropwise at room temperature to a solution of **20** (1.65 g, 1.8 mmol) in dry THF (20 mL), and then the mixture was stirred overnight. The solution was diluted with ethyl acetate (50 mL), neutralized with solid calcium carbonate and water (1 mL), and poured into water (50 mL) and then the layers were separated. The aqueous layer was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were dried over magnesium sulfate and concentrated in vacuo. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to afford 2-*O*-benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-3-*O*-(4-nitrophenoxyacetyl)-α/β-D-galactopyranose (1.38 g, 98%) as a white foam. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.36 (α). $[\alpha]_D$ = +61.5 (c = 1.0, $CHCl_3$). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 3.0 (s, 1 H, OH), 4.1−4.7 (m, 11 H), 5.50-5.57 (dd, ^3J = 3.5 Hz, 1 H, 2-*H*), 5.64 (m, 1 H, 1-*H*), 5.73−5.78 (dd, ^3J = 3.0 Hz, 1 H, 3-*H*), 6.6−8.1 (m, 22 H, Ar) ppm. MALDI-MS: m/z = 797.0 [M + Na^+]. $C_{43}H_{37}NO_{13}$ (775.8): calcd. C 66.57, H 4.81, N 1.81; found C 66.22, H 4.94, N 1.70.

2-*O*-Benzoyl-4-*O*-benzyl-6-*O*-(9-fluorenylmethoxycarbonyl)-3-*O*-(4-notrophenoxyacetyl)-α/β-D-galactopyranose (300 mg, 0.39 mmol) was dissolved in a mixture of dichloromethane (5 mL) and Cl_3CCN (5 mL) and then sodium hydride (2 mg) was added at 0 °C. After 0.5 h, the mixture was neutralized with silica gel and concentrated at low pressure. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 5:1) to afford **8** (250 mg, 70%) as a white foam. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.28 (α). ^{1}H NMR (250 MHz, $CDCl_3$): δ = 4.18 (s, 1 H, 4-*H*), 4.41−4.30 (m, 2 H, 6-*H*, 6′-*H*), 4.35−4.58 (m, 6 H), 4.73 (s, 2HCH_2OPh), 5.80 (m, 2 H, 2-*H*, 3-*H*), 6.66−6.71 (m, 3 H), 7.12−7.95 (m, 20 H, Ar), 8.53 (s, 1 H, NH) ppm. $C_{45}H_{37}Cl_2N_2O_{13}$ (920.3): calcd. C 58.73, H 4.05, N 3.04; found C 58.64, H 4.13, N 3.04.

4-(Polystyrene-divinylbenzene-carbonyloxymethyl)benzyl 2-Azido-3,6-di-*O*-benzyl-4-*O*-(9-fluorenylmethoxycarbonyl)-2-deoxy-β-D-glucopyranoside (4b): Carboxyl-PS-resin **5** (1.0 g, loading = 2 mmol/g) was swollen under argon in a mixture of primary alcohol **6** (100 mg, 0.137 mmol) and dichloromethane (6 mL) and then it was shaken for 10 min. DIC (1.6 mL, 10 mmol) and DMAP (24 mg, 0.2 mmol) were added to the resin solution, which was then shaken for 1 day. MeOH (0.5 mL) was added to the resin solution, which was then shaken for 24 h. The resin **4b** was filtered off, washed with CH_2Cl_2 (4 × 10 mL) and THF (4 × 10 mL), and dried under high vacuo (10 h, 60 °C oil bath). The loading of resin was determined to be 0.12 mmol/g by recycling the unchanged acceptor and also by a very clean preparative cleavage of NaOMe (4 equiv.) for 6.0 h.

General Procedure for Fmoc Deprotection (Procedure A): Fmoc deprotection was performed according to the procedure previously described.[27]

General Procedure for the Solid Phase Glycosylation (Procedure B): Solid phase glycosylation was performed according to the procedure previously described.[27]

General Procedure for Cleavage (Procedure C): Cleavage was performed according to the procedure previously described.[27]

Resin 21: The Fmoc group of **4b** was removed using procedure **A**.

Resin 22: Resin **21** was glycosylated with donor **7** according to procedure **B** using 0.3 equiv. of TMSOTf at −40 °C.

4-(Acetoxymethyl)benzyl 2,3-*O*-Di-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (1): Resin **22** (36 μL) was treated as described in procedure **C**. After removal of the solvents in vacuo, the crude cleavage residue was treated with Ac_2O (1 mL) and pyridine (2 mL) for 10 h. The resulting mixture was concentrated in vacuo and coevaporated twice with toluene. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to furnish **1** (25.6 mg, 81% overall yield) as an amorphous solid. TLC (petroleum ether/ethyl acetate, 2:1): R_f = 0.60. $[\alpha]_D$ = −3.4 (c = 1.7, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.92−2.03 (m, 9 H, 3 Ac), 3.04 (s, 1 H, 5b-*H*), 3.24 (d, ^3J = 8.0 Hz, 1 H, 5a-*H*), 3.32 (m, 1 H, 3a-*H*), 3.41 (m, 1 H, 2a-*H*), 3.67−3.74 (m, 2 H, 6a-*H*, 6′a-*H*), 3.82 (d, ^2J = 11.5 Hz, 1 H, 6b-*H*), 3.94 (t, ^3J = 9.1 Hz, 1 H, 4a-*H*), 4.12 (d, ^2J = 12.1 Hz, 1 H, 6′b-*H*), 4.20 (m, 1 H, 4b-*H*), 4.22 (d, ^2J = 8.1 Hz, 1 H, 1a-*H*), 4.41 (d, ^2J = 12.0 Hz, 1 H, 1/2 CH_2Ph), 4.53 (d, ^3J = 8.0 Hz, 1 H, 1b-*H*), 4.59 (d, ^2J = 12.1 Hz, 1 H, 1/2 CH_2Ph), 4.66−4.70 (m, 4 H, 3b-*H*, 3/2 CH_2Ph), 4.85 (d, ^2J = 12.1 Hz, 1 H, 1/2 CH_2Ph), 5.03 (m, 4 H, 2 CH_2Ph), 5.25 (m, 1 H, 2b-*H*), 5.38 (s, 1 H, CHPh), 7.14−740 (m, 19 H, Ar) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 66.1 (C-2a), 66.58 (C-5b), 67.56 (C-6a), 68.6 (C-6b), 69.4 (C-2b), 73.36 (C-4b), 75.2 (C-5a), 75.8 (C-3b), 77.1 (C-4a), 81.2 (C-3a), 100.56 (C-1a), 100.62 (C-1b), 101.03 (C−*C*HPh) ppm. MALDI-MS: m/z = 905.3 [M + Na^+]. $C_{47}H_{51}NO_{14}$ (881.9).

Resin 23: The Fmoc group of **22** was removed using procedure **A**. Chain elongation with donor **9** was then performed according to procedure **B** using 0.3 equiv. of TMSOTf at −40 °C.

Resin 24: The Fmoc group of **23** was removed using procedure **A**. Chain elongation with donor **7** was then performed according to procedure **B** using 0.3 equiv. of TMSOTf at −40 °C.

4-(Acetoxymethyl)benzyl 2,3-Di-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyranosyl-(1→4)-(3,6-di-*O*-benzyl-2-deoxy-2-phthalimido-β-D-glucopyranosyl-(1→3)-(2-*O*-acetyl-4,6-*O*-benzylidene-β-D-galactopyransyl)(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (2): Resin **24** (24 μL) was treated as described in procedure **C**. After removal of the solvents in vacuo, the crude cleavage residue was treated with Ac_2O (1 mL) and pyridine (2 mL) for 10 h. The resulting mixture was concentrated in vacuo and coevaporated twice with toluene. The residue was purified by HPLC (ethyl acetate/hexane, 6:5) to furnish **2** (8.3 mg, 21% overall yield) as an amorphous solid. TLC (petroleum ether/ethyl acetate, 1:1): R_f = 0.35. ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.86−2.10 (m, 12 H, 4 Ac), 3.03 (s, 1 H, 5d-*H*), 3.14 (s, 1 H, 5b-*H*), 3.19 (d, 1 H, 5c-*H*), 3.24 (t, ^3J = 9.3 Hz, 1 H, 3c-*H*), 3.40 (t, ^3J = 9.7 Hz, 1 H, 2c-*H*), 3.54 (dd, ^3J = 3.4 Hz, 1 H, 3d-*H*), 3.60 (m, 1 H, 5a-*H*), 3.70−3.87 (m, 8 H), 3.97 (t, ^3J = 9.1 Hz, 1 H, 4a-*H*), 4.15 (d, ^2J = 12.9 Hz, 1 H, 1/2 CH_2Ph), 4.17−4.26 (m, 5 H), 4.38 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1d-*H*), 4.47 (d, ^2J = 12.1 Hz, 1 H, 1/2 CH_2Ph), 4.51−4.65 (m, 5 H, 1a-*H*, 2 CH_2Ph), 4.68 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1b-*H*), 4.71-4-75 (dd, ^2J = 11.8 Hz, 2 H, CH_2Ph), 4.80 (dd, 1 H, 3b-*H*), 4.88 (d, ^2J = 12.0 Hz, 1 H, 1/2 CH_2Ph), 5.0−5.1 (m, 5 H, 2d-*H*, 2 CH_2Ph), 5.35 (t, ^3J = 10.3 Hz, 1 H, 2b-*H*), 5.43 (s, 1 H, CHPh), 5.46 (s, 1 H, CHPh), 6.9−7.41 (m, 38 H, Ar) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 63.9 (C-2a), 65.8 (C-2c), 65.9 (C-5b), 66.5 (C-5d), 67.3 (C-6c), 68.4 (C-6a, C-6d), 68.3 (C-6b), 69.2 (C-2b), 70.6 (C-2d), 71.9 (C-3b), 73.0 (C-4b), 75.0 (C-5a, C-5c), 75.6 (C-4d), 76.8 (C-4c), 77.0 (C-3d), 77.4 (C-4a), 81.1 (C-4a), 81.1 (C-3c), 82.6 (C-3a), 100.3 (C-1c), 100.7 (C-1d), 100.8 (C-1b), 102.7 (C-1a) ppm. MALDI-MS: m/z = 1669.7 [M + Na^+]. $C_{90}H_{92}N_4O_{26}$ (1645.7).

Resin 25: Resin **21** was glycosylated with donor **8** according to procedure **B** using 0.3 equiv. of TMSOTf at −20 °C.

Resin 26: The Fmoc group of **25** was removed using procedure **A**. Chain elongation with donor **10** was then performed according to procedure **B** using 0.3 equiv. of TMSOTf at −20 °C.

4-(Acetoxymethyl)benzyl 2-*O*-Acetyl-3,4,6-tri-*O*-benzyl-α-D-mannopyranosyl-(1→6)-(2,3-di-*O*-acetyl-4-*O*-benzyl-β-D-galactopyranosyl)(1→4)-2-azido-3,6-di-*O*-benzyl-2-deoxy-β-D-glucopyranoside (3): Resin **26** (24 μL) was treated as described in procedure **C**. After removal of the solvents in vacuo, the crude cleavage residue was treated with Ac_2O (1 mL) and pyridine (2 mL) for 10 h. The resulting mixture was concentrated in vacuo and coevaporated twice with toluene. The residue was purified by flash chromatography (petroleum ether/ethyl acetate, 2:1) to furnish **3** (20 mg, 63% overall yield) as an amorphous solid. TLC (petroleum ether/ethyl acetate, 1:1): R_f = 0.44. $[\alpha]_D$ = +1.1 (c = 1.0, $CHCl_3$). ^{1}H NMR (600 MHz, $CDCl_3$): δ = 1.98−2.16 (m, 12 H, 4 Ac), 3.26−3.32 (m, 3 H, 3a-*H*, 5c-*H*, 6b-*H*), 3.37 (m, 1 H, 5b-*H*), 3.44−3.49 (m, 2 H, 2a-*H*, 6a-*H*), 3.53 (m, 1 H, 6′b-*H*), 3.60 (d, ^3J = 9.7 Hz, 1 H, 5a-*H*), 3.71−3.73 (m, 3 H, 6′a-*H*, 6c-*H*, 6′c-*H*), 3.85 (m, 2 H, 3c-*H*, 4b-*H*), 3.93−3.95 (m, 2 H, 4a-*H*, 4c-*H*), 4.23 (d, $J_{1,2}$ = 8.1 Hz, 1 H, 1a-*H*), 4.38−4.50 (m, 5 H, 5/2 CH_2Ph), 4.52 (s, 1 H, 1c-*H*), 4.55 (d, $J_{1,2}$ = 8.0 Hz, 1 H, 1b-*H*), 4.61−4.66 (m, 4 H, 2 CH_2Ph), 4.72 (d, ^2J = 12.0 Hz, 1 H, 1/2 CH_2Ph), 4.79−4.93 (m, 5 H, 3b-*H*, 2 CH_2Ph), 5.09 (s, 2 H, CH_2Ph), 5.21 (s, 1 H, 2c-*H*), 5.30 (dd, ^3J = 8.3, ^3J = 10.1 Hz, 1 H, 2b-*H*), 7.16−7.35 (m, 34 H, Ar) ppm. ^{13}C NMR (150.9 MHz, $CDCl_3$): δ = 64.8 (C-6b), 65.4 (C-2a), 67.5 (C-6c), 68.1 (C-6a), 68.5 (C-2c), 70.3 (C- 2b), 71−4 (C-5a), 72.5 (C-5b), 73.8 (C-3b, C-4a), 73.9 (C-4b), 74.9 (C-5c), 75.7 (C-4c), 77.8 (C-3c), 81.1 (C-3a), 97.9 (C-1c), 100.0 (C-1b), 100.4 (C-1a) ppm. MALDI-MS: m/z = 1381.5 [M + Na^+]. $C_{76}H_{83}N_3O_{20}$ (1358.5).

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[1] A. Varki, *Glycobiology* **1993**, *3*, 97−130.
[2] R. A. Dwek, *Chem. Rev.* **1996**, *96*, 683−720.
[3] N. Sharon, H. Lis, in *Glycosciences − Status and Perspectives* (Eds.: H.-J. Gabius, S. Gabius), Chapman and Hall; Weinheim, **1997**, 113−162.
[4] R. R. Schmidt, *Angew. Chem.* **1986**, *98*, 213−236; *Angew. Chem. Int. Ed. Engl.* **1986**, *25*, 212−235.
[5] R. R. Schmidt, W. Kinzy, *Adv. Carbohydr. Chem. Biochem.* **1994**, *50*, 21−123.
[6] S. J. Danishefsky, M. T. Bilodeau, *Angew. Chem.* **1996**, *108*, 1483−1522; *Angew. Chem. Int. Ed. Engl.* **1996**, *35*, 1380−1419.
[7] P. J. Garegg, *Adv. Carbohydr. Chem. Biochem.* **1996**, *52*, 179−205.

[8] A. Demchenko, T. Stauch, G.-J. Boons, *Synlett* **1997**, 818−820.
[9] W. C. Haase, P. H. Seeberger, *Chem. Rev.* **2000**, *100*, 4349−4393.
[10] For the first investigation of solid phase oligosaccharide synthesis with an automated synthesizer, see: O. J. Plante, E. R. Palmacci, P. H. Seeberger, *Science* **2001**, *291*, 1523−1527.
[11] F. Roussel, M. Takhi, R. R. Schmidt, *J. Org. Chem.* **2001**, *66*, 8540−8548.
[12] F. Roussel, L. Knerr, M. Grathwohl, R. R. Schmidt, *Org. Lett.* **2000**, *2*, 3043−3046.
[13] F. Roussel, L. Knerr, R. R. Schmidt, *Eur. J. Org. Chem.* **2001**, 2066−2073.
[14] X. Wu, M. Grathwohl, R. R. Schmidt, *Org. Lett.* **2001**, *3*, 747−750.
[15] J. Rademann, A. Geyer, R. R. Schmidt, *Angew. Chem.* **1998**, *110*, 1309−1313; *Angew. Chem. Int. Ed.* **1998**, *37*, 1241−1245.
[16] J. Rademann, R. R. Schmidt, *J. Org. Chem.* **1997**, *62*, 3650−3653.
[17] T. Zhu, G.-J. Boons, *Chem. Eur. J.* **2001**, *7*, 2382−2389.
[18] T. Zhu, G.-J. Boons, *J. Am. Chem. Soc.* **2000**, *122*, 10222−10223.
[19] K. C. Nicolaou, N. Watanabe, J. Li, J. Pastor, N. Winssinger, *Angew. Chem.* **1998**, *110*, 1636−1638; *Angew. Chem. Int. Ed.* **1998**, *37*, 1559−1561.
[20] K. C. Nicolaou, N. Winssinger, J. Pastor, F. DeRoose, *J. Am. Chem. Soc.* **1997**, *119*, 449−450.
[21] H. Ando, S. Manabe, Y. Nakahara, Y. Ito, *Angew. Chem.* **2001**, *113*, 4861−4864; *Angew. Chem. Int. Ed.* **2001**, *40*, 4725−4728.
[22] E. R. Palmacci, M. C. Hewitt, P. H. Seeberger, *Angew. Chem.* **2001**, *113*, 4565−4569; *Angew. Chem. Int. Ed.* **2001**, *40*, 4433−4437.
[23] X. Wu, R. R. Schmidt, *J. Org. Chem.* **2003**, submitted.
[24] G. Hummel, R. R. Schmidt, *Tetrahedron Lett.* **1997**, *36*, 1173−1176.
[25] G. Hummel, Dissertation, University of Konstanz, **1998**.
[26] X. Wu, M. Grathwohl, R. R. Schmidt, *Angew. Chem.* **2002**, *114*, 4664−4668; *Angew. Chem. Int. Ed.* **2002**, *41*, 4489−4493.
[27] M. Grathwohl, R. R. Schmidt, *Synthesis* **2001**, 2263−2272.
[28] M. Grathwohl, Dissertation, University of Konstanz, Germany, **2001**.
[29] X. Wu, Dissertation, University of Konstanz, Germany, **2003**.
[30] For a paper in which the nitrile effect is employed for anomeric stereocontrol, see: R. R. Schmidt, M. Behrendt, A. Toepfer, *Synlett* **1990**, 694−697.
[31] E. T. Yamansorova, A. G. Kukovinets, O. S. Kukovinets, *Russ. J. Org. Chem.* **2001**, *37*, 246−255.
[32] L. Liang, T.-H. Chan, *Tetrahedron Lett.* **1998**, *39*, 355−358.

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