Oligosaccharide Synthesis on Controlled-Pore Glass as Solid Phase Material[1]

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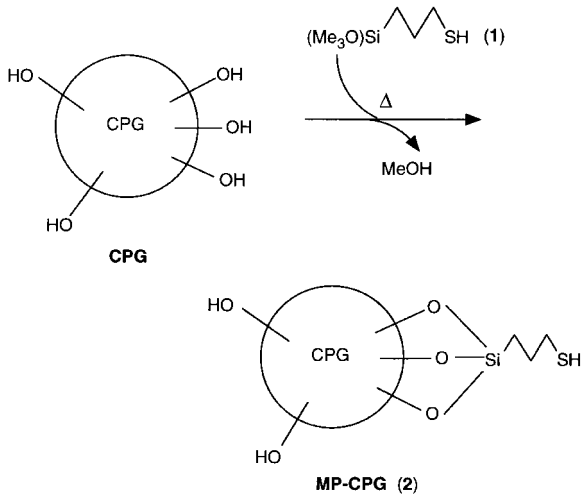
Abstract: Mercaptopropyl-functionalized controlled-pore glass (MP-CPG, **2**) was employed as a solid phase material for the synthesis of α(1-2)-connected trimannoside **16**. As a glycosyl donor *O*-(3,4,6-tri-*O*-benzyl-2-*O*-phenoxyacetyl-α-D-mannopyranosyl)trichloroacetimidate (**6**) was employed which was readily obtained from D-mannose. Glycosylation of **2** and of intermediates **9** and **13** was performed with excess **6** in CH_2Cl_2 at -40 °C in the presence of TMSOTf as the catalyst (→ **7, 11, 15**). For the removal of the temporary phenoxyacetyl (PA) protective group guanidine in DMF was used (→ **9, 13**). Cleavage of products **7, 9, 11, 13** and **15** from MP-CPG was carried out with N-bromosuccinimide (NBS) in THF/MeOH in the presence of 2,6-di-*tert*-butylpyridine (DTBP) affording **8, 10, 12, 14,** and methyl trimannoside **16**, respectively.

Progress has recently been made in solid phase supported oligosaccharide synthesis based on the use of the Merrifield resin.[2,3] In order to permit reactions, this resin requires swelling by the solvent. However, because resin swelling is highly solvent and temperature dependent, anomer control (and sometimes also regioselectivity) is often limited. Therefore, we investigated 'controlled-pore glass' (CPG) as a solid support, because it does not require swelling. Although this material is extensively employed in oligonucleotide synthesis,[4] the promises for its uselfulness in oligosaccharide synthesis were not high according to previous work;[2,5,6] the ubiquitous presence of hydroxy groups at the surface of this material and the sensitivity to aqueous base could be major drawbacks. Yet, CPG could be recently successfully employed as solid phase material for enzymatic glycosylation reactions.[7]

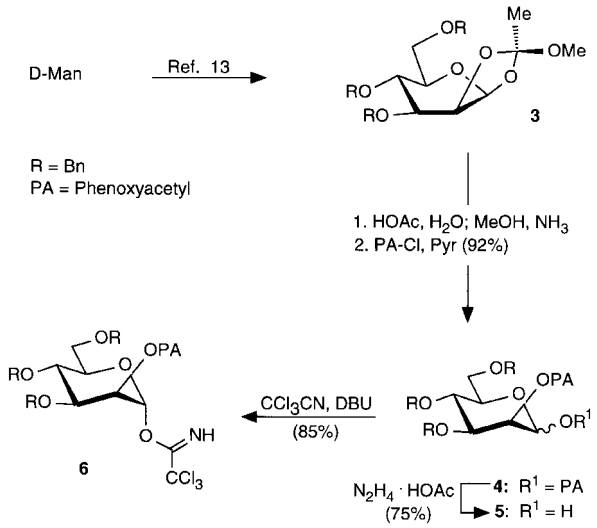
Our first goal was to find a linker system for ligation to CPG and for the attachment of the first sugar residue. In order to gain high loading of the CPG surface with the linker system, we selected 250 Å CPG.[8] After investigating various linker systems, 3-mercaptopropyl-trimethoxysilane[9] (**1**, Scheme 1) was chosen because it provides mercaptopropyl functionalized MP-CPG **2**.[10] Thus, a thioglycosidic linkage is available to the first sugar residue; it is compatible with the mild acid catalysis required for the *O*-glycosyl trichloroacetimidate methodology which will be used for further chain extension reactions;[3a] additionally, after completion of the reaction sequence at the polymer, it offers the possibility of selective cleavage by thiophilic reagents which do not affect *O*-glycosides.[3a] Loading of MP-CPG **2** was determined by reaction with dithiodinitrobenzoate (DTNB) following known procedures.[10,11] Thus, 0.3 mmol of free mercapto groups/g CPG was found as mean value on **2**. In order to remove traces of methanol, **2** had to be heated to 150 °C *in vacuo* for 6 h. (Monomethoxytritylation of this material with monomethoxytrityl (MMT) chloride, silylation of the remaining free hydroxyl groups, and then selective removal of the MMT groups did not result in higher glycosylation yields as later found).

For the glycosylation studies, α(1-2)-linked oligomannose was chosen as model, because this glycosidic connection is often found in nature and short oligosaccharides of this type have been already successfully synthesized on other supports.[3a] In order to ease cleavage of the temporary protective group at the 2-*O*-position of the required mannosyl donor, the phenoxyacetyl group was chosen (**6**, Scheme 2), because



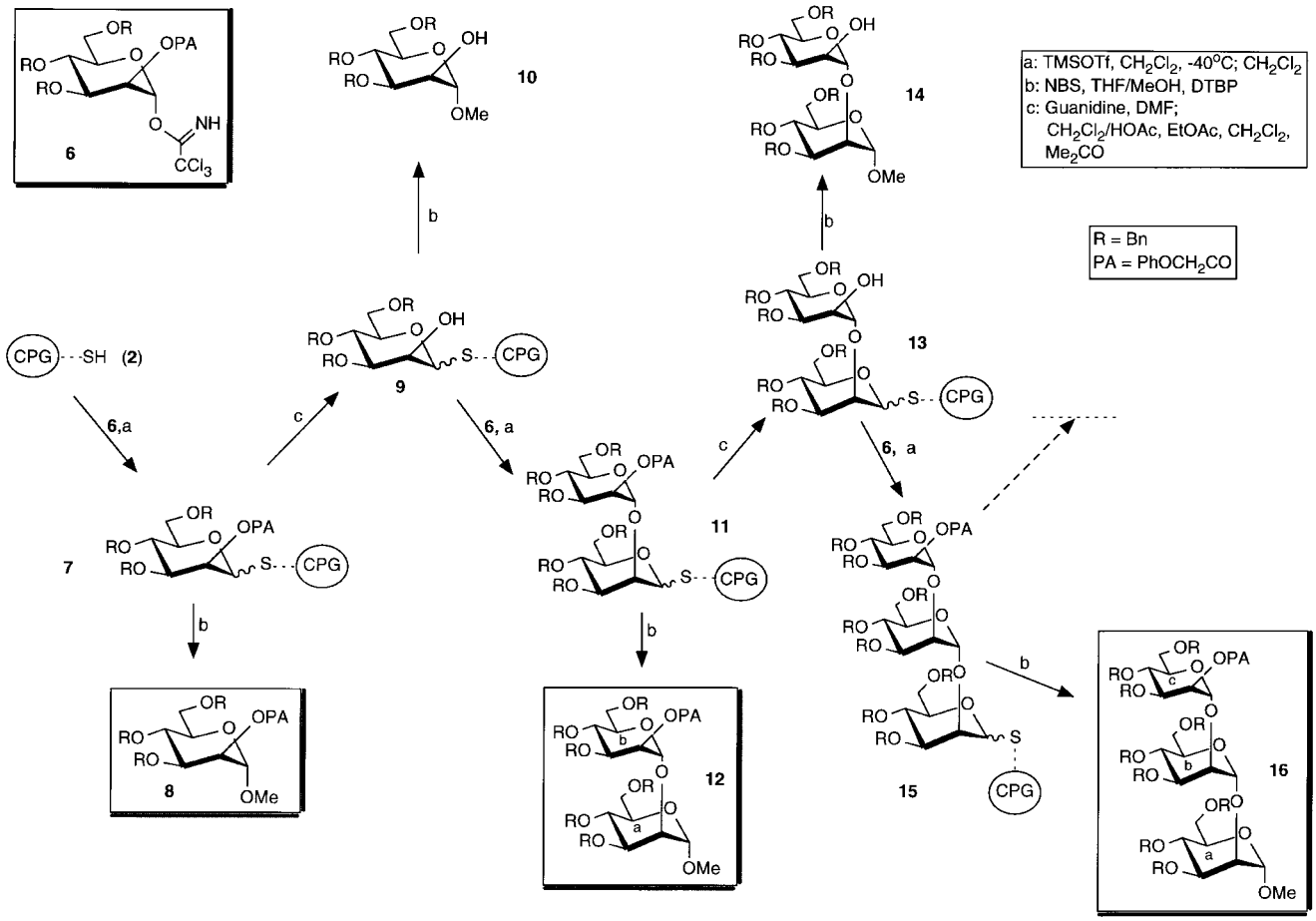
Scheme 1

alcoholysis or aminolysis of this group under mild basic conditions is much faster than the common *O*-acetyl protective group.[12] Therefore, from D-mannose via the known intermediate **3**[13] we synthesized the bis-phenoxyacetyl derivative **4** which is selectively 1-*O*-deacylated with hydrazinium acetate (→ **5**) and then transformed into trichloroacetimidate **6**[14] with trichloroacetonitrile in the presence of DBU.



Scheme 2

Linking the first mannosyl residue to the mercapto group of **2** with **6** as mannosyl donor was performed essentially under standard conditions; i.e. the reaction was carried out in CH_2Cl_2 as solvent at -40 °C with TMSOTf as catalyst.[15] In order to get complete glycosylation yielding **7** two times three equivalents of glycosyl donor **6** were employed. The reaction was followed by gravimetry and by MALDI-TOF analysis of small samples, from which the thiomannoside was cleaved with NBS in THF/MeOH in the presence of 2,6-di-*tert*-butylpyridine (DTBP); thus,



Scheme 3

methyl α-mannoside **8** was obtained and could be easily identified by comparison with independently obtained material.[16]

The temporary phenoxyacetyl group at 2-*O* of **7** was best cleaved with two times two equivalents of guanidine in DMF; after extensive washing with DMF and then with CH_2Cl_2/HOAc and with dry EtOAc, CH_2Cl_2, and acetone, evaporation *in vacuo* afforded 2-*O*-unprotected **9**.[17] Application of the cleavage procedure gave the corresponding methyl mannoside **10**,[16] thus confirming the successful 2-*O*-deprotection.

Chain extension with glycosyl donor **6** under the conditions described above gave disaccharide **11**;[15] the reaction was monitored by MALDI-TOF analysis and product formation was confirmed after cleavage also by ^{1}H NMR, thus proving the synthesis of methyl dimannoside **12**.[16,18] Removal of the phenoxyacetyl group, applying the same procedure as described above, gave 2β-*O*-unprotected **13**;[17] application of the cleavage process led to the corresponding dimannoside **14**.[16] In order to demonstrate the success with MP-CPG **2** as solid phase material, **13** was again glycosylated with **6,** thus affording **15**,[15] which was again characterized by cleavage with NBS/MeOH/DTBP and by MALDI-TOF analysis affording α(1-2)-linked trimannoside **16**[16,18] which contained small traces of **8** and **12**.

In conclusion, mercaptopropyl-functionalized CPG (MP-CPG, **2**) offers with reactive glycosyl donors and temporary protective groups, which can be removed under nonaqueous conditions, success in solid phase oligosaccharide synthesis.

References and Notes

(1) This work was supported by the Deutsche Forschungsgemeinschaft and the Fonds der Chemischen Industrie. – The helpful discussions with Dr. Rademann, J. are gratefully acknowledged.

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(8) Type 332, pore diameter 250 Å, particle size 20-45 µm, Fa. Grace GmbH (in der Hollerecke 1, D-67547 Worms); CPG with a pore diameter of 1500 Å was employed with similar success.

(9) Commercially available from Aldrich.

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(14) ^{1}H NMR data (250 MHz, $CDCl_3$): δ 3.70-4.06 (m, 5 H, H-3,4,5,6,6'), 4.41-4.79 [m, 8 H, 3 CH_2(Bn), CH_2(PA)], 5.62 (dd, 1 H, $^3J_{1,2}$ = 2.1, $^3J_{2,3}$ = 3.1 Hz, H-2), 6.33 (d, 1 H, H-1), 6.87-6.98 and 7.13-7.35 (m, 20 H, 3 Bn, PA), 8.7 (s, 1 H, NH).

(15) General procedure for the synthesis of **7**, **11**, and **15**: MP-CPG (**2**: 1.22 g, 0.366 mmol SH) was suspended in 6 mL of dry CH_2Cl_2 under an argon atmosphere and cooled to -45 °C in a three-mantle flask. Then **6** (800 mg, 1.098 mmol) in dry CH_2Cl_2 (2 mL) was added and the suspension was horizontally shaken (300 times/ min) for 10 min. The reaction was finally started by adding TMSOTf (30 µL, 0.16 mmol). After gentle shaking for 1 h the reaction mixture was brought to room temp. Then the product was isolated by filtration and subsequently washed with dry CH_2Cl_2 and dry acetone, 60 mL each. Finally the product was dried in a stream of dry argon and then *in vacuo* with gentle warming, affording **7** (1.3829 g; ~ 80%) as a slightly yellowish powder. In order to prevent the contamination of unreacted materials, each glycosylation reaction was usually performed twice, thus increasing the yield to over 95%. Similar results were obtained for the synthesis of **11** and **15**.

(16) General procedure for the synthesis of **8**, **10**, **12**, **14**, and **16**: Glycosylated MP-CPG is shaken with 3 equivalents of NBS in a 33:3:1 solution of dry THF, dry methanol and DTBP for 2 h. This mixture is suitable for MALDI-TOF analysis. For preparative cleavage triethylsilane is then added to remove excess NBS. The product is isolated by filtration, followed by washing the CPG with dry CH_2Cl_2 and dry acetone, 40 mL each. After evaporation of the solvent the product is pre-purified by column chromatography. Pure product is finally obtained by HPLC with a silica gel column and hexane/ethyl acetate as eluent.

(17) General procedure for the synthesis of **9** and **13**: Guanidine is prepared by reaction of guanidinium chloride (61.9 mg, 0.648 mmol) with sodium methoxide (35 mg, 0.648 mmol) in dry methanol under argon, followed by filtration and evaporation of the solvent. The guanidine is then dissolved in dry DMF (1 mL) and added to a suspension of **7** (1.330 g, 0.324 mmol) in dry DMF (5 mL). After shaking for 16 h the product is isolated by filtration, followed by washing with dry DMF and dry acetone, 20 mL each. The product is dried in a stream of dry argon and then *in vacuo* with gentle warming. To complete the reaction, the treatment with guanidine was usually repeated once more. For the final purification the product is washed with dry DMF (60 mL), a 10% solution of acetic acid in dry CH_2Cl_2 (20 mL), dry ethyl acetate (40 mL), dry CH_2Cl_2 (40 mL) and finally dry acetone (40 mL). Then the product is dried as described, affording **9** (1.2809 g) as a slightly yellow powder; yield: > 90%; MALDI-TOF analysis did not show any phenoxyacetylated material.

(18) NMR data: **12**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 3.23 (H_3C-O), 3.64 (4b-H), 3.68 (6b-H, 6b-H'), 3.69 (5a-H), 3.71 (6a-H, 6a-H'), 3.80 (4a-H), 3.87 (3a-H), 3.91 (5b-H), 3.96 (3b-H), 3.96 (2a-H), 4.39-4.84 [6 CH_2 (Bn)], 4.64 [CH_2 (PA)], 4.74 (1a-H), 5.07 (1b), 5.62 (2b). – ^{13}C NMR (150.9 MHz, $CDCl_3$): 54.88 [H_3C-O], 65.13 [CH_2 (PA)], 68.96 (C-6b), 69.25 (C-6a), 69.51 (C-2b), 71.67 (C-5a), 71.83 (C-5b), 72.09 (Bn), 72.14 (Bn), 73.32 (Bn), 73.41 (Bn), 74.25 (C-4b), 74.58 (C-4a), 74.65 (C-2a), 75.07 (2 Bn), 78.10 (C-3b), 79.77 (C-3a), 99.19 (C-1b), 99.65 (C-1a), 114.76-138.37 [Bn, PA], 157 (PA), 168.24 (C=O).
NMR data **16**: ^{1}H NMR (600 MHz, $CDCl_3$): δ 2.97 (5c-H), 3.07 (H_3C-O), 3.31 (3c-H), 3.49 (6c-H), 3.5 (4b-H), 3.54 (6b-H), 3.55 (6c-H') 3.57 (5b-H, 6a-H), 3.58 (5a-H), 3.62 (6b-H'), 3.66 (4c-H), 3.68 (4a-H), 3.76 (3a-H), 3.78 (3b-H), 3.8 (6a-H), 4.0 (2a-H), 4.2-4.7 [9 CH_2(Bn)], 4.32 (2b-H), 4.39 (1c-H), 4.62 [CH_2, (PA)], 4.79 (1a-H), 5.02 (1b-H), 5.45 (2c-H). – ^{13}C NMR (150.9 MHz, $CDCl_3$): 54.6 (CH_3O), 64.9 [CH_2(PA)], 68.9 (C-2c), 68.8 (C-4b), 69.2 (C-6b), 69.9 (C-6c), 70.9 (C-2b), 71.5 (C-6a), 74.0 (C-2a), 74.2 (C-4c), 74.9 (C-4a), 74.9 (C-5c), 77.5 (C-3b), 79.2 (C-3c), 80.3 (C-3a), 95.3 (C-1c), 99.2 (C-1b), 99.9 (C-1a), 114.5-157.6 (Bn, PA).