Supported Oligosaccharides

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Efficient Polymer-Assisted Strategy for the Deprotection of Protected Oligosaccharides**

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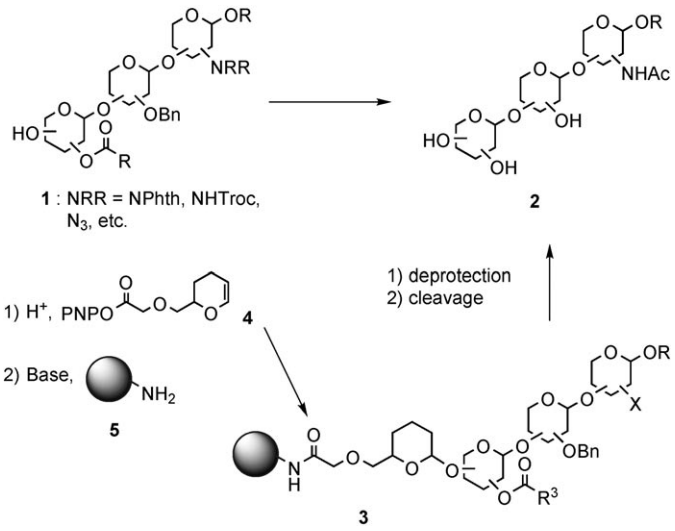
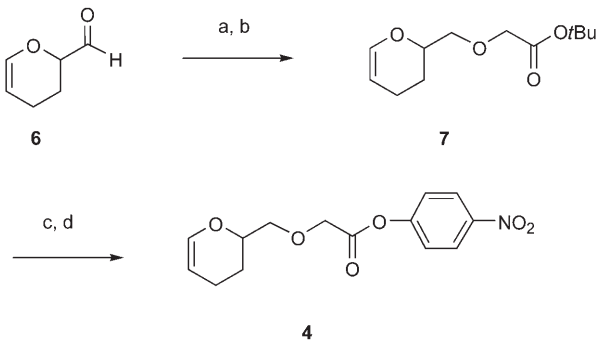
Oligosaccharides play important roles in cell-surface events through carbohydrate–protein and carbohydrate–carbohydrate interactions.[1] The chemical synthesis of structurally defined oligosaccharides would be highly desirable in structure–activity studies because oligosaccharides from natural sources can be produced in only limited qualities. Recent progress in oligosaccharide synthesis has resulted in a number of new and efficient glycosidation methodologies, which are amenable to the synthesis of protected oligosaccharides **1** by standardized and routine protocols.[2] However, deprotection of the protected oligosaccharides **1**→**2**, including the cleavage of various O-protecting groups and the replacement of N-protecting groups with *N*-acetyl groups is difficult to achieve by standardized protocols (Scheme 1). The complete deprotection of protected oligosaccharides frequently requires careful selection of the reaction solvents to prevent the partially deprotected intermediates from precipitating. Herein, we describe an efficient method for the deprotection of protected oligosaccharides based on a polymer-assisted strategy and its application to the synthesis of dimeric and trimeric Lewis X derivatives.

Our polymer-assisted strategy for the deprotection of protected oligosaccharides **1** is illustrated in Scheme 1. The solid-supported protected oligosaccharide **3** linked through a tetrahydropyranyl (THP) linker was designed as a key intermediate. The solid-supported complex oligosaccharides



Scheme 1. Polymer-assisted strategy for deprotection of the protected oligosaccharides **1**. Bn = benzyl, PNP = *para*-nitrophenyl, Phth = phthalyl, Troc = 2,2,2-trichloroethoxycarbonyl.

would smoothly undergo deprotection because they are aggregated to only a very limited extent. A Birch reduction was adapted for removing the solid-supported benzyl ethers and esters on **3**.[3] The THP linker would survive deprotection reactions and can be cleaved under mildly acidic conditions to release the fully deprotected oligosaccharide **2** without anomerization or cleavage of the glycosidic bonds.[4,5] The ease of handling of solid-supported compounds would be effective not only for the high-speed synthesis of a single target oligosaccharide but also for the deprotection of a protected oligosaccharide library.[6] The polymer-supported protected oligosaccharide **3** can be prepared using the following methodology: 1) acetal formation of the protected saccharides **1** with prelinker **4** containing a dihydropyranyl (DHP) moiety and an activated ester and 2) subsequent



Scheme 2. Reagents and conditions: a) $LiAlH_4$, THF; b) *tert*-butyl bromoacetate, NaH, DMF; c) 1 N aq. NaOH, dioxane; d) 4-nitrophenol, EDCI, DIEA, CH_2Cl_2, 42% from **6**. DMF = *N*,*N*-dimethylformamide, EDCI = 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide, DIEA = diisopropylethylamine.

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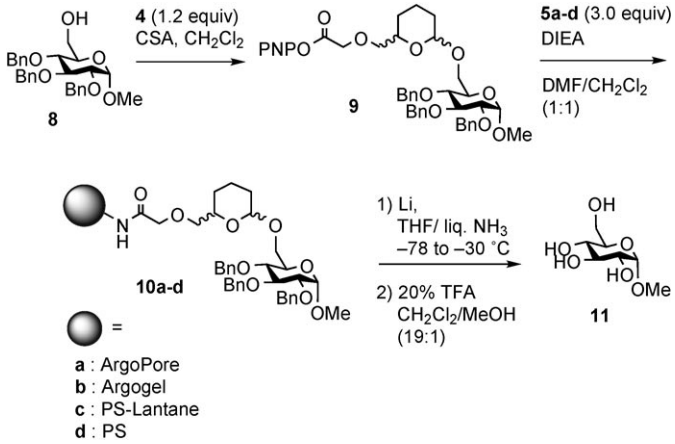
Table 1: Deprotection of the *O*-benzyl-protected glucose derivative **8** based on a polymer-assisted strategy.[a]



Entry	**10**	Loading yield (**8**→**10**) [%][b]	Conv. [%][c]	Purity of **11** [%][c]	Overall yield (**8**→**11**) [%]
1	**10a**	99	>99	>99	91
2	**10b**	97	59	58	–
3	**10c**	99	trace	trace	–
3	**10d**	93	3	3	–

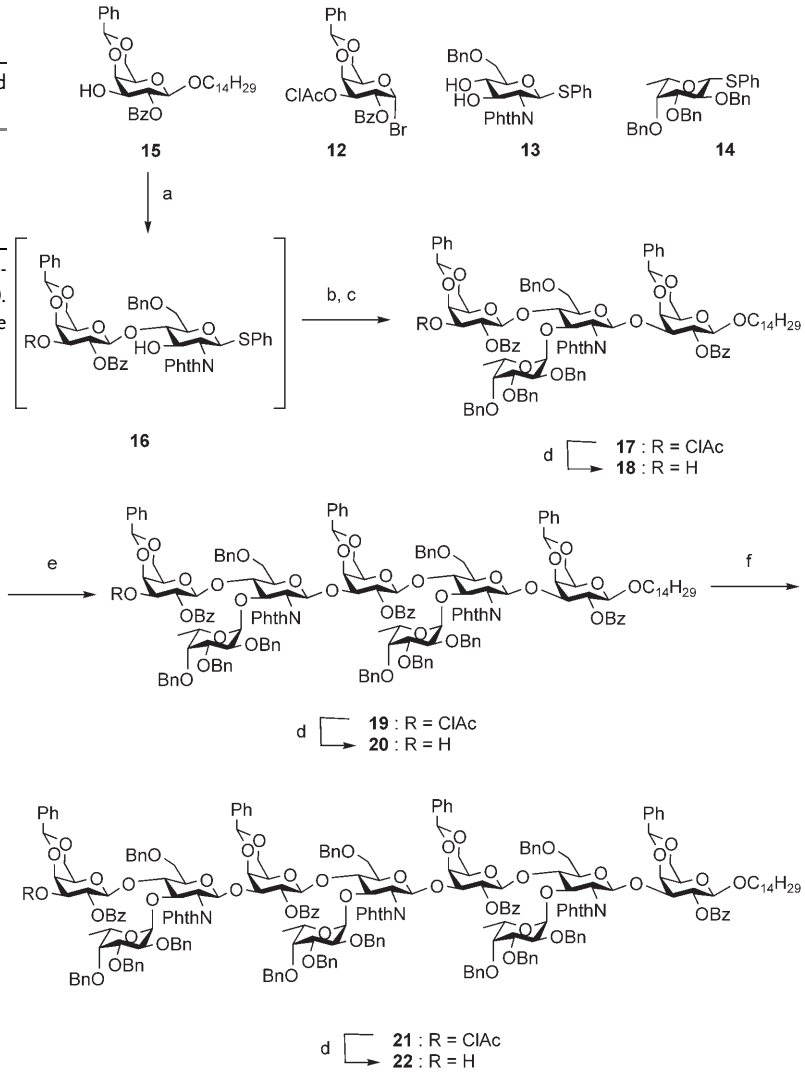
[a] CSA = (+)-camphorsulfonic acid, PS = polystrene, TFA = trifluoroacetic acid. [b] The yield was estimated by the release of **8** from **10**. [c] The conversion and purity were estimated by HPLC analysis of the cleaved materials using ELSD.

amidation of the resulting activated ester with the solid-supported amines **5** to give **3**, irreversibly. The irreversible loading reaction would enable the complete immobilization of the protected oligosaccharides **1**.[7]

The preparation of prelinker **4** is outlined in Scheme 2. The reduction of aldehyde **6** followed by alkylation of the resulting alcohol with *tert*-butyl α-bromoacetate provided the *tert*-butyl ester **7**. Hydrolysis of the *tert*-butyl ester **7** under basic conditions followed by esterification with 4-nitrophenol afforded prelinker **4** in 42% overall yield from **6**.

We first examined the polymer-assisted deprotection of methyl 2,3,4-*O*-tribenzylglucoside (**8**) using the solid supports **5a–d** (Table 1). Treatment of **8** with 1.2 equiv of prelinker **4** under acidic conditions provided the protected glucoside **9**, which contains an activated ester, as a mixture of diastereomers. The subsequent amidation of **9** with 3.0 equiv of the solid-supported amines **5a–d** afforded the solid-supported protected glucoses **10a–d**. The yields of **10a–d** based on **8**, estimated by cleavage of **8** from **10**, were excellent. The removal of the benzyl ethers was achieved by treatment of the solid-supported protected glucose derivatives **10a–d** with lithium in liquid ammonia and tetrahydrofuran at −30 °C for 1 h.

Exposure of the solid-supported glucose derivatives to mildly acidic conditions permitted the products to be released. An HPLC analysis of the released products using an evaporative light-scattering detector (ELSD) revealed that only the benzyl ethers supported on the ArgoPore resin underwent complete reductive cleavage under the Birch reduction conditions (Table 1, entry 1). These results suggest that the substrates on the surface of the polymer might selectively undergo Birch reduction because the solution of liquid ammonia in THF did not cause the hydrophobic polymers to swell. Further purification of the product

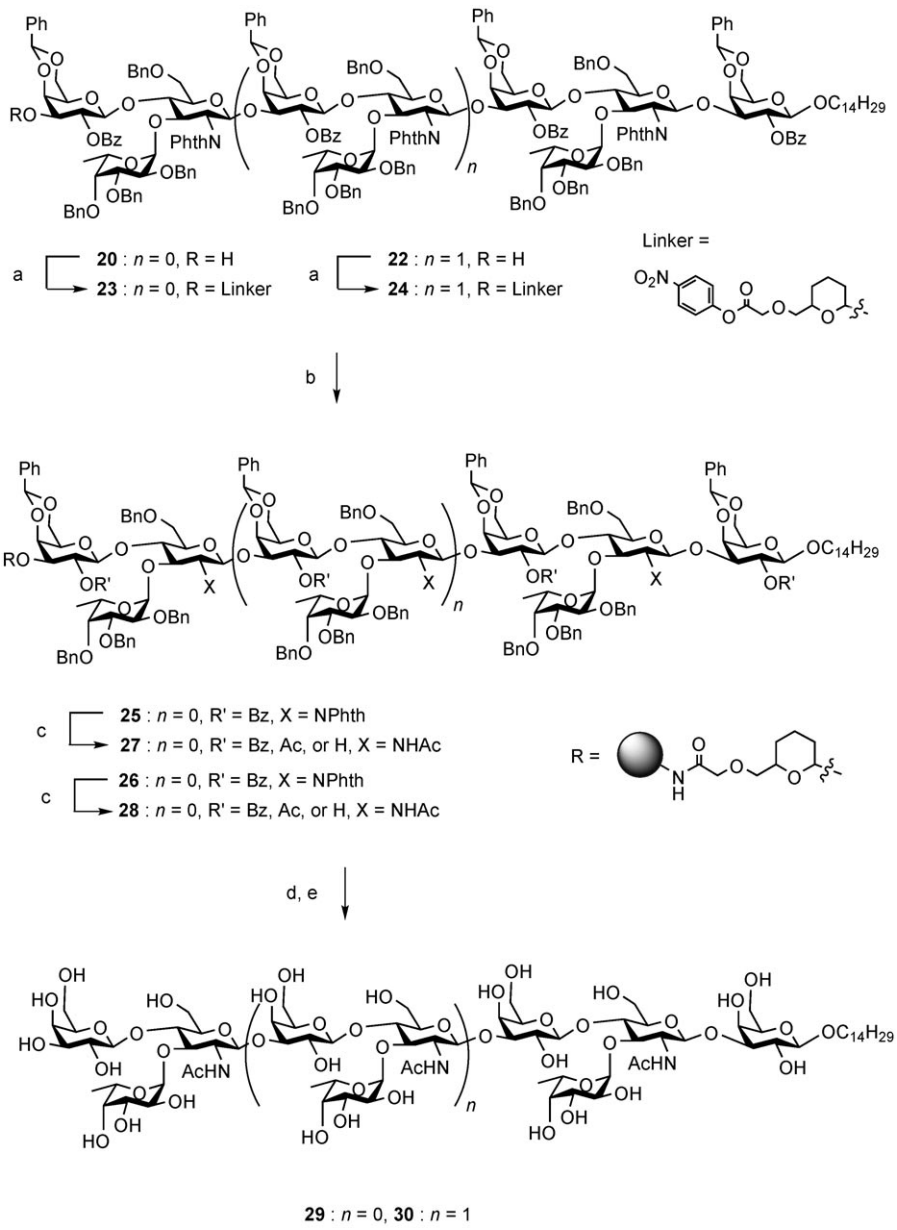


Scheme 3. Reagents and conditions: a) **12** (1.8 equiv), **13** (1.5 equiv), AgOTf (1.8 equiv), CH_2Cl_2, 4-Å M.S., −20 °C; b) **15** (1.0 equiv), NIS (1.5 equiv), −10 °C; c) **14** (5.0 equiv), NIS (5.5 equiv), 77% based on **15;** d) thiourea, 2,6-lutidine, DMF, 99% for **18**, 92% for **20**, 70% for **22**; e) **12** (1.8 equiv), **13** (1.5 equiv), AgOTf (1.5 equiv), CH_2Cl_2, 4-Å M.S., −20 °C, then **18** (1.0 equiv), NIS (1.5 equiv), −10 °C, then, **14** (5.0 equiv), NIS (5.5 equiv), 56% based on **18**; f) **12** (1.8 equiv), **13** (1.5 equiv), AgOTf (1.8 equiv), CH_2Cl_2, 4-Å M.S., −20 °C, then **20** (1.0 equiv), NIS (1.5 equiv), −10 °C, then, **14** (10 equiv), NIS (10.5 equiv), 44% based on **20**. Bz = benzoyl, M.S. = molecular sieves, NIS = *N*-iodosuccinimide, Tf = trifluoromethanesulfonyl.

released from the ArgoPore resin provided α-methyl glucoside **11** in 91% yield based on **8** (Table 1, entry 1). These results indicate that the substrate on the ArgoPore resin was not released during the Birch reduction. To the best of our knowledge, this is the first report on an application of a Birch reduction to solid-phase synthesis.

We next synthesized the trimeric and dimeric Lewis X derivatives **29** and **30** by a one-pot glycosylation and the polymer-assisted deprotection procedure (Scheme 3). The hepta- and decasaccharides **29** and **30** are important tumor-associated antigens[8] and have served as effective synthetic targets for demonstrating the feasibility of new methodologies owing to the complexity of their branched structure, which contains both α- and β-glycosidic linkages.[9–11]

A one-pot glycosylation was used to prepare the protected hepta- and decasaccharides **20** and **22**, respectively (Scheme 3).[12] The chemo-, stereo-, and regioselective glycosylation of glucosamine **13** at the C4 hydroxy group with the galactosyl bromide **12** provided the glycosylated thioglycoside **16**. Subsequent activation of thioglycoside **16** to couple with alkyl galactoside **15** at the 3-position, followed by the α-selective glycosylation of the remaining hydroxy group at the C3 position with thiofucoside **14** provided tetrasaccharide **17** in 77% overall yield based on **15**. Removal of the chloroacetyl group at the C3 position on **17** provided alcohol **18** in 99% yield, which was used as an acceptor in the next one-pot glycosylation. A one-pot glycosylation using these three building blocks—**12**, **13**, and **14**—and acceptor **18** provided heptasaccharide **19** in 57% yield based on **18**. Subsequent deprotection of the C3 protecting group gave the heptasaccharide acceptor **20** in 92% yield. A one-pot glycosylation using the three building blocks **12**, **13**, and **14** and heptasaccharide **20** afforded the protected decasaccharide **21** in 44% yield based on **20**. Removal of the chloroacetyl protecting group gave the decasaccharide **22**, containing a hydroxy group, in 70% yield.



Scheme 4. Reagents and conditions: a) **4** (3 equiv), CSA, CH_2Cl_2, room temperature; b) aminomethyl ArgoPore **5a** (10 equiv), DIEA, DMF, room temperature, 12 h; c) $NH_2NH_2{\cdot}H_2O$/EtOH (1:5), reflux, 6 h, then AcOH, DIC, DIEA, room temperature, 6 h; d) lithium, THF, liq. NH_3, −78 °C, 1.5 h, reflux, 1 h, then MeOH, 12 h; (l); e) TFA, MeOH/CH_2Cl_2 (1:19), 58% yield for **20**→**29**, 58% yield for **22**→**30**. DIC = 1,3-diisopropylcarbodiimide.

Deprotection of the protected trimeric and dimeric Lewis X epitopes **20** and **22** based on the polymer-supported strategy was examined (Scheme 4). The treatment of **20** and **22** with 3 equiv of prelinker **4** in the presence of CSA at room temperature provided the protected oligosaccharides **23** and **24**, which contain an activated ester. After removal of the remaining prelinker **4** by flash column chromatography, the activated esters **23** and **24** were reacted with 10 equiv of the solid-supported amine **5a** in DMF under basic conditions for 12 h to give the solid-supported protected oligosaccharides **25** and **26**, respectively. The phthalyl groups of **25** and **26** were removed by treatment with $NH_2NH_2{\cdot}H_2O$/EtOH (1:5) at reflux for 6 h.[13] Acetylation of the resulting amines provided the solid-supported acetamides **27** and **28**, respectively. Oligosaccharides **27** and **28** were then subjected to Birch reduction conditions and then cleaved from the resin under mildly acidic conditions. ^{1}H NMR spectra of the released products revealed that most of the solid-supported benzyl esters on the complex oligosaccharides smoothly underwent cleavage in the Birch reduction. Purification of the released

products afforded the fully deprotected trimeric and dimeric Lewis X epitopes **29** and **30**, each in 58% overall yield, based on **20** and **22**, respectively. Although the loading and release of these complex protected oligosaccharides would be difficult to achieve without loss of material, this method should be of practical use for the synthesis of oligosaccharide libraries.

In conclusion, an efficient polymer-assisted method for the deprotection of protected oligosaccharides has been demonstrated. The prelinker **4** composed of a DHP unit and an activated ester was effective not only for the loading of the protected oligosaccharides from the solution but also for the release of the fully deprotected oligosaccharide from the resin. ArgoPore was the best support for the cleavage of solid-supported benzyl ethers, which was achieved using Birch reduction conditions.

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[13] Treatment of **10a** with $NH_2NH_2{\cdot}H_2O$/EtOH (1:5) at reflux for 12 h did not reduce the loading amount of the solid-supported **10a**. These results indicated that the THP linker could be stable during cleavage of the phthalyl groups.

