Polymer – Resin Hybrid Capture – Release Strategy for Rapid Oligosaccharide Construction

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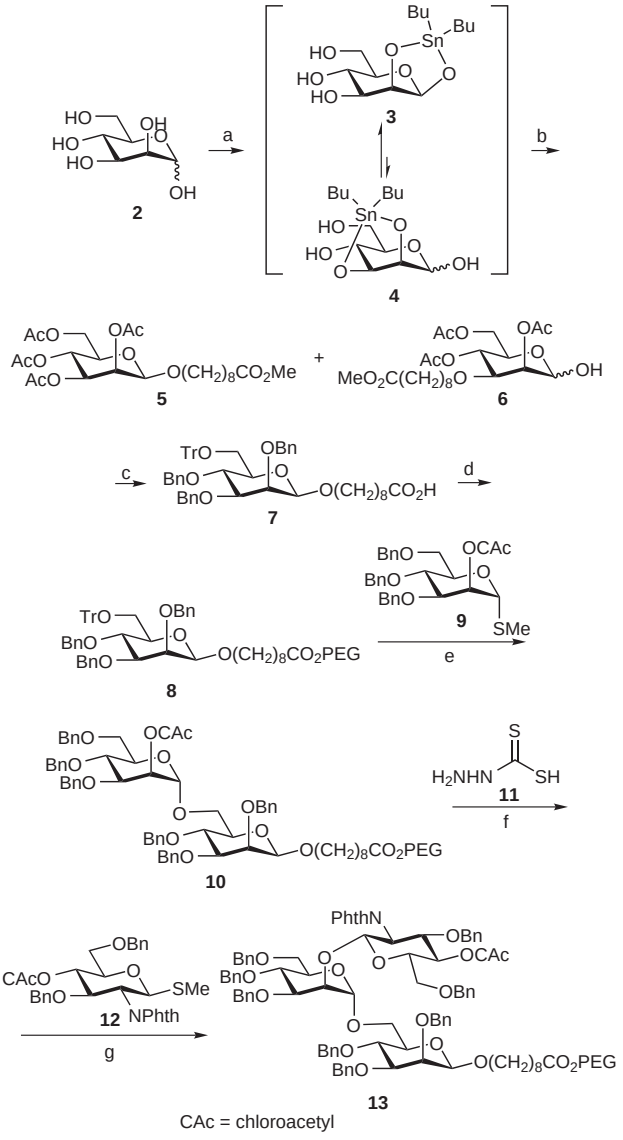
Abstract: The polymer-resin hybrid type capture-release purification strategy for oligosaccharide synthesis was renewed as more rapid and straightforward manner. The substrate for *N*-acetylglucosaminyltransferase V trisaccharide was synthesized rapidly on PEG (poly (ethylene glycol) methyl ether) and purified by use of the strategy.

Key words: capture-release purification strategy, carbohydrates, glycosylations, oligosaccharides, polymer-supported synthesis

Solid-phase synthesis has been a central issue in carbohydrate chemistry due to its potential in speeding up oligosaccharide synthesis and possible extension to combinatorial chemistry.[1] However, its generality has been still limited, because 1) resin-bound substrates have a compromised reactivity and 2) practical method for the real-time reaction monitoring has been lacking. We recently reported a novel strategy that removes these difficulties, while preserving the major advantage of polymer-supported synthesis.[2] It exploited a soluble support, namely low molecular weight poly(ethylene) glycol methyl ether (PEG), which functions as a 'tag' due to its extra high polarity in silica gel column chromatography purification. Real-time monitoring of glycosylation and selective deprotection was realized by MALDI-TOF MS and color test, respectively. It was further refined by resin-aided capture-release purification, which discriminates successfully coupled product from all other compounds. This strategy makes it possible to purify the desired glycoside without cleaving from polymer support. Here we report the renewed version of capture-release purification strategy, that was applied to the synthesis of trisaccharide **1** (Figure 1), which is demonstrated as a hydrophobic tagged substrate for *N*-acetylglucosaminyltransferase V by Hindsgaul et al.[3] A strong correlation between an increase in the activity of this enzyme and metastatic potential of several cancer cell lines has been reported.[4]

In the previous report,[2b,c] the capture-release isolation was performed after each glycosylation reaction using Fmoc cysteine bound resin [Scheme 2, i)]. Now, this strategy was further simplified as follows; after glycosylation the



Scheme 1 *Reagents and conditions*: (a) Bu_2SnO, MeOH; (b) i) $TfO(CH_2)_8CO_2Me$, TBAF, MS 4 Å, CH_3CN; ii) Ac_2O, pyridine; iii) piperidine, THF, **5** 28%, **6** 8% (4 steps); (c) i) NaOMe, MeOH; ii) TrCl, DMAP, pyridine, 87%; iii) BnBr, NaH, DMF; iv) 6 M KOH, MeOH, 82% (2 steps); (d) 2,4,6-trichlorobenzoyl chloride, Et_3N, THF then PEGOH, DMAP, toluene, 87%; (e) **9**, NIS, TfOH, MS 4 Å, CH_2Cl_2; (f) i) TFA, Et_3SiH, CH_2Cl_2; ii) Ac_2O, *i*-Pr_2NEt, CH_2Cl_2 (for capping); (iii) **11**, CH_3CN; (g) **12**, NIS, TfOH, MS 4 Å, CH_2Cl_2.

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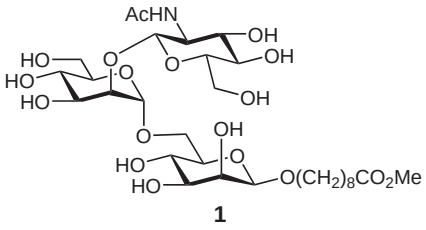

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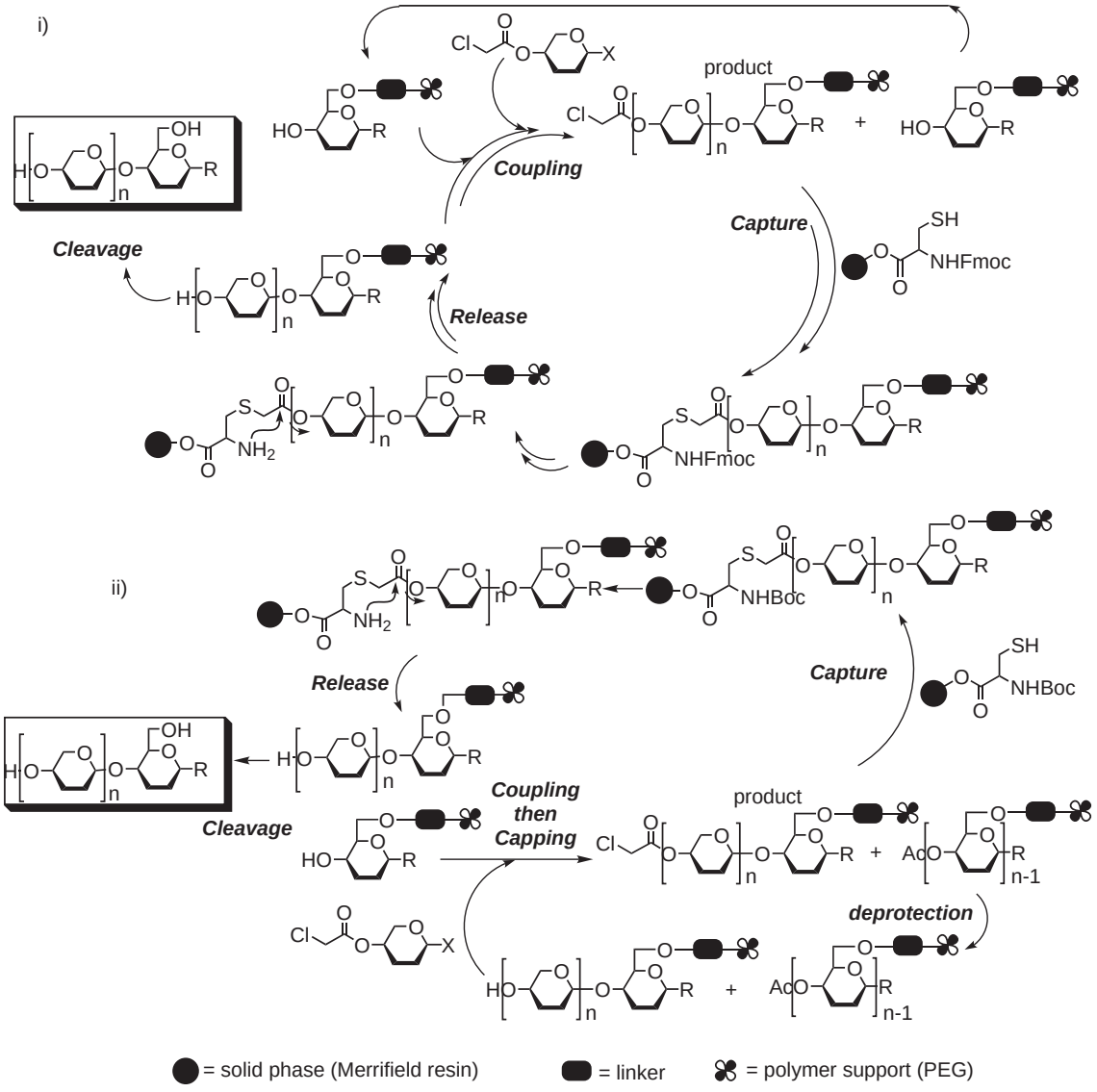
Figure 1 Hydrophobic tagged substrate for GnT-V.

unreacted hydroxyl group was capped by Ac_2O and the separation of the desired oligosaccharide was achieved by using resin-supported Boc cysteine only after final glycosylation reaction [Scheme 2, ii)].

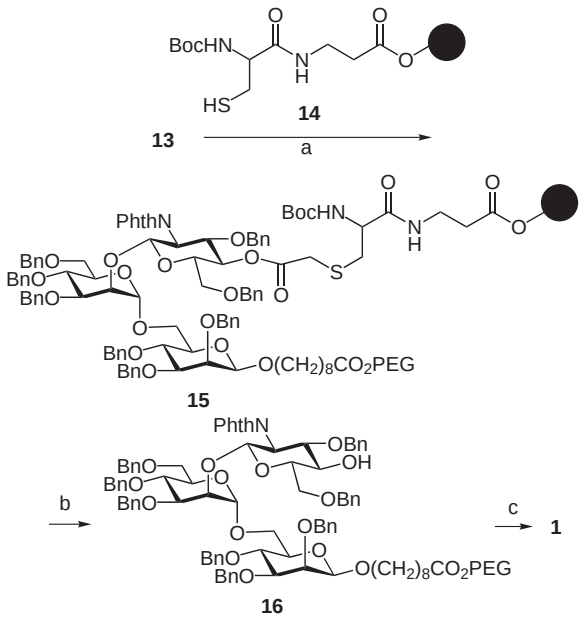
As the first step of our work, preparation of β-mannoside carrying hydrophobic linker was achieved based on Kováč's stannylene acetal protocol.[5,6] After isolation of the β-mannoside as tetraacetate **5**, the acetyl groups were removed under alkaline conditions and the primary alcohol was protected as trityl ether. After protection of residual hydroxy groups as benzyl ether,[7] the ester was hydrolyzed to acid **7**. Instrallation of poly(ethylene glycol) methyl ether (Ave. M. W. 750, PEG)[8] was performed by Yamaguchi's method to give **8** in 87% yield.[9] PEG-bound trityl ether **8** was directly submitted to the glycosylation reaction with thioglycoside **9** by use of NIS-TfOH as an activator.[10] Progress of the reaction was monitored by MALDI-TOF MS as previously reported.[11] Although the glycosylation reaction proceeded in quite high yield, in order to cap any remained acceptor, the mixture was treated successively with TFA–Et_3SiH (to deprotect remaining Tr) and Ac_2O. Subsequent chloroacetyl deprotection with hydrazinedithiocarbonate (HDTC) **11**[12] was monitored by *p*-(nitrobenzyl) pyridine color test method in a real-time manner.[2a] From the color test and ^1H NMR



Scheme 2 Concept of polymer-resin hybrid capture-release strategy for rapid oligosaccharide synthesis: i) capture-release purification cycle after each glycosylation reaction; ii) capture-release purification strategy only after final glycosylation reaction.

analysis, it was revealed that the deprotection of chloroacetyl group was completed within 10 minutes. Then the disaccharide acceptor was subjected to the glycosylation using **12** as a donor.[13]

As shown in Scheme 3, having completed the synthesis of trisaccharide **13** on the polymer support, the capture-release purification using resin bound Boc protected cysteine was examined. The desired PEG supported trisaccharide **13** was captured by 5.0 equivalents of polystyrene resin bound Boc-Cys derivative **14** in the presence of *i*-Pr_2NEt.[14] After filtration of the resins and washing, the trisaccharide product was released by successive treatment with TFA (to remove Boc) and piperidine (to generate free NH_2). The compound **16** was obtained successfully in a substantially pure form in 47% yield from **8.**[15]



Scheme 3 *Reagents and conditions:* (a) *i*-Pr_2NEt, CH_3CN, CH_2Cl_2; (b) i) TFA, CH_2Cl_2; ii) 10% piperidine, CH_2Cl_2; (c) i) 1 M KOH, EtOH, benzene; ii) ethylenediamine, 1-BuOH, then Ac_2O, pyridine, 91% (2 steps); iii) $TMSCHN_2$, MeOH, benzene (85%); iv) H_2, 20% $Pd(OH)_2$, HOAc, MeOH (88%).

Trisaccharide was then cleaved from PEG under alkaline conditions, and deprotected. Thus, the phthalimide group was converted to acetamide and the acid was esterified by (trimethylsilyl)diazomethane.[16] Finally, the benzyl groups were removed under catalytic hydrogenation conditions to afford **1** in 88% yield.[17]

In summary, we demonstrate the utility of the refined capture-release strategy by successfully synthesizing trisaccharide **1** on polymer support rapidly in a pure form.

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(6) (a) Separation of β-mannoside and 3-O-alkylated regioisomer was difficult at this stage. For easier separation, the remained hydroxy groups were acetylated and the anomeric acetyl group of the regio isomer was removed by piperidine. The linkage of stereochemistry of anomeric position of **6** was confirmed by ^1H NMR analysis (J_{CH} = 156.6 Hz in C_6D_6) and NOE experiment between H-1 and H-5. (b) Bock, K.; Pedersen, C. *J. Chem. Soc., Perkin Trans. 2* **1974**, 293.

(7) During the benzylation, the methyl group was changed to benzyl ether (7.9%) and carboxylic acid (9.5%). As the separation of methyl ester and benzyl ester was difficult, the mixture of the ester was hydrolyzed.

(8) Previously, the average molecular weight 550 PEG was used as a polymer support and it works as 'tag' efficiently. However, in this case, its molecular weight is not sufficient for the 'tag' for the purification because of lack of enough polarity.

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(11) Although the characteristic 'mountain' like shape of the spectra of monosaccharide and disaccharide were not completely separated, the monitoring of progress of the reaction was possible. After the reaction, quite high purity of PEG-bound disaccharide was confirmed by ^1H NMR spectroscopy.

(12) Van Boeckel, C. A. A.; Beetz, T. *Tetrahedron Lett.* **1983**, *24*, 3775.

(13) The ratio of α:β at the newly formed anomeric carbon is 1:9.9.

(14) Compound **14** was prepared from Boc-β-Ala-Merrifield resin (0.66 mmol/g) in 2 steps. i) TFA, CH_2Cl_2, then Et_3N; ii) Boc-*S*-*tert*-butylmercapto-L-cysteine, HOBt, *N*, *N*′-

diisopropylcarbodiimide, DMF. After peptide bond formation, ninhydrin test showed >99% yield.
(15) After releasing step, further purification of the released product was not necessary in the case of Boc group protected cysteine, compared to the Fmoc version.
(16) The acetyl group of glucosamine was deprotected under esterification by use of(trimethylsilyl)diazomethane.
(17) (a) ^{1}H NMR (500 MHz, D_2O): δ = 4.71 (br s, 1 H, H-1′), 4.60 (s, 1 H, H-1), 4.37 (d, 1 H, *J* = 8.7 Hz, H-1″), 3.78 (m, 1 H, H-2′), 3.78–3.74 (m, 2 H, H-2, H-6a), 3.72–3.69 (m, 2 H, H-6a′, H-6a″), 3.67–3.62 (m, 2 H, H-3′, CH_2O), 3.58–3.50 (m, 2 H, H-6b, H-6b″), 3.48–3.39 (m 9 H, H-2″, H-3, H-4, H-5′, H-6b′, OMe, OCH_2), 3.35 (dd, *J* = 8.3, 10.6 Hz, 1 H, H-3″), 3.32–3.20 (m, 4 H, H-4′, H-4″, H-5, H-5″), 2.18 (dd, *J* = 7.4, 7.4 Hz, 2 H), 1.85 (s, 3 H), 1.40–1.39 (m, 4 H), 1.10 (m, 8 H). ^{13}C NMR (125 MHz, D_2O): δ = 178.1, 175.0, 100.1, 99.7, 96.9, 76.4, 76.0, 74.6, 73.5, 73.4, 73.0, 70.7, 70.3, 70.1, 69.8, 67.5, 66.8, 66.2, 61.8, 60.8, 55.5, 52.2, 33.9, 28.8–28.2, 25.2, 24.5, 22.5. $[\alpha]_D^{25}$ –18.4 (*c* 0.32, H_2O). (b) Tahir, S. H.; Hindsgaul, O. *Can. J. Chem.* **1986**, *64*, 1771.