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Solid-phase oligosaccharide synthesis with tris(alkoxy)benzyl amine (BAL) safety-catch anchoring

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A tris(alkoxy)benzylamine (BAL) handle strategy was developed for safety-catch anchoring of D-glucosamine derivatives in solid-phase synthesis of oligosaccharides; the linkage between the BAL handle and the amine proved stable to conc. TFA and Lewis acids, but after *N*-acylation the amide could be released by treatment with TFA–H_2O (19 : 1).

Cell-surface oligosaccharides are involved in many biological recognition processes.[1] Also, free oligosaccharides can have biological effects such as in rhizobial lipochitin oligosaccharides which function as nodulation factors.[2] Amino sugars are found in many biologically important poly- and oligo-saccharides, *e.g.* in glycans of *O*- and *N*-glycoproteins and -peptides, lipochitin nodulation factors, and amino glycoside antibiotics such as streptomycin.

Solid-phase synthesis of oligosaccharides, when performed combinatorially, offers the prospect of easy access to very large numbers of well-defined oligosaccharides as tools for glycobiology. Despite Frechet and Schuerch's early pioneering work[3] and recent significant progress,[4] considerable developments are required before solid-phase oligosaccharide synthesis can be put to general use. Crucial to any solid-phase synthesis plan is the efficient and reliable anchoring of the first building block, the stability of the anchoring linkage to the synthesis conditions, and upon completion of the synthesis the efficient release under conditions compatible with the structural integrity of the final product. The most common cleavage reagent in solid-phase peptide synthesis is conc. TFA. Previously reported handles for anchoring of carbohydrates to solid supports have included esters,[4a] silyl ethers,[4b] sulfonyl esters,[4c] acetals[4d] and thioalkyls[4e] and ethers.[4f,g]

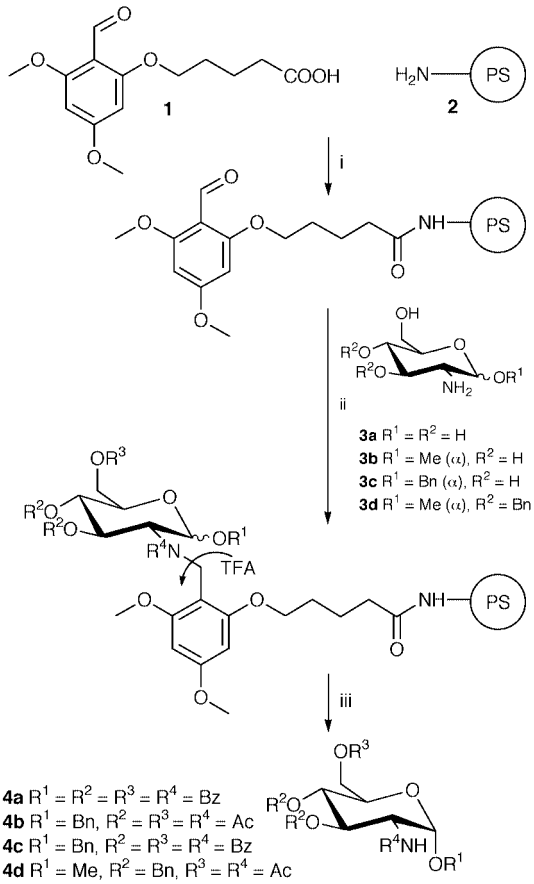
For the solid-phase synthesis of amino sugar containing oligosaccharides, we decided upon a strategy relying on release of final products by treatment with conc. TFA, with semi-permanent protecting groups to be removed in solution after purification and characterization.[5] The recently developed Backbone Amide Linker (BAL) handle strategy relies on anchoring of a peptide not through a carboxy group but through a backbone amide nitrogen leaving the *C*-terminus available for modifications.[6] Applied to anchoring of amino sugars, a BAL strategy would allow anchoring through an acetamido functionality leaving all hydroxy groups, including the anomeric, available for modification.

To investigate the anchoring concept, *o*-PALdehyde **1** was coupled to high-loading aminomethylated polystyrene **2** and unprotected D-glucosamine **3a** anchored through a BAL linkage by reductive amination in DMF–AcOH (99 : 1) in the presence of $NaBH_3CN$ (Scheme 1).† Perbenzoylation with BzCl followed by treatment with TFA–H_2O (19 : 1) yielded after column chromatography the α-pentabenzoate **4a** in 41% for a four step synthesis sequence. The presence of five benzoyl groups in the final product confirmed that anchoring had occurred through the amine rather than the putative *O*,*O*- or *O*,*N*-acetals.

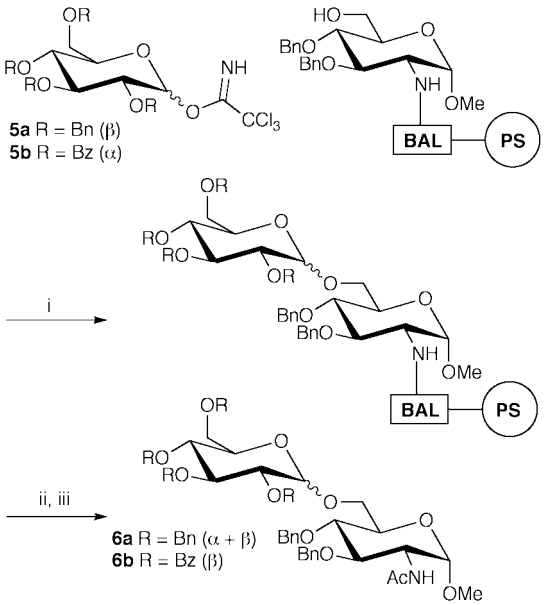
For fast and efficient quantification of resin-bound carbohydrates we used two techniques. In the first, UV-active amino sugar derivatives were released into solution with TFA–H_2O (19 : 1) followed by quantification with HPLC–UV from standard curves.

In the second technique, the resin-bound monosaccharide was treated with 4,4′-dimethoxytrityl chloride (DMTCl) to attach a DMT moiety at the primary 6-OH group. For quantification, the resin was exposed to TFA–MeCN (1 : 49) and released DMT estimated by UV. Quantification using DMT was performed on resin bound monosaccharides in order to determine the efficiency of the coupling of *o*-PALdehyde to the resin as well as the coupling of the amino sugar to the linker. The methyl and benzyl glycosides of D-glucosamine **3b** and **3c** were coupled with an efficiency of at least 95% (four steps from **2**, including DMT coupling and cleaving) independently of the resin loading. The solid-phase bound benzyl α-D-glucosaminide **3c** was then per-acylated with Ac_2O or BzCl. This yielded the fully acetylated and benzoylated products **4b** and **4c** in 91–96 and 82% yield, respectively, measured by HPLC standard curves.

Several solution-phase glycosylation methods have been adapted for solid-phase; they all require Lewis acids for activation.[4] While studying the use of trichloroacetimidates in



Scheme 1 *Reagents and conditions*: i, aminomethylated polystyrene **2**, **1** (2.0 equiv.), HBTU (1.9 equiv.), HOBt (2.0 equiv.), Pr^i_2NEt (3.9 equiv.), DMF, 10 h; ii, amino sugar (2.0 equiv.), $NaBH_3CN$ (10 equiv.), DMF–AcOH (99 : 1), 16 h, then protecting group chemistry including *N*-acylation; iii, TFA–H_2O (19 : 1), 30 min.



Scheme 2 *Reagents and conditions*: i, TMSOTf, CH_2Cl_2, 3 Å (Table 1); ii, Ac_2O–Py (2:1), 16 h; iii, TFA–H_2O (19:1), 30 min.

the presence of Lewis acid promoters for the glycosylation of BAL anchored D-glucosaminides, we realized that BAL anchoring could be operated in a safety-catch mode. The amine–BAL linkage proved stable to treatment with conc. TFA and more than stoichiometric amounts of Lewis acids such as $BF_3{\cdot}OEt_2$ and TMSOTf. After acylation to form the amide, the linkage became acid-labile and the carbohydrate could be released with conc. TFA.

For solid-phase glycosylations, we first synthesized the partially protected D-glucosamine derivative **3d**. Anchoring through a BAL handle to a polystyrene support was achieved as above, leaving the 6-OH free. In initial studies we used 2,3,4,6-tetra-*O*-benzyl-β-D-glucopyranosyl trichloroacetimidate **5a**[7] in the presence of $BF_3{\cdot}OEt_2$ or TMSOTf as promoters (Scheme 2). Solid-phase reactions are most conveniently carried out at room temperature, but this gave only low yields of the disaccharide. However, when performing the glycosylation at −50 °C the disaccharide **6a** was obtained in 35% yield as an α/β mixture (five steps from **2**).

When using the *less reactive* benzoyl protected glycosyl donor 2,3,4,6-tetra-*O*-benzoyl-α-D-glucopyranosyl trichloroacetimidate **5b** in the presence of TMSOTf, yields of up to 82% of the disaccharide **6b**[8] were obtained (five steps from **2**), leaving as little as 2% of the unglycosylated monosaccharide (Table1). The disaccharide was obtained with an α:β ratio of < 1:10, the high β-selectivity being due to the neighboring group participation of the benzoyl group. Stoichiometric and sub-stoichiometric amounts of Lewis acids were used, relative to the donor. We found that TMSOTf was superior to $BF_3{\cdot}OEt_2$ as promoter, and MAS NMR revealed that the secondary amine did *not* carry a TMS group after exposure to TMSOTf in glycosylations. The high yield of 82% for the five step synthesis of **6b** also indicated that release of the final product with TFA–H_2O was near quantitative.

Table 1 Reaction conditions for synthesis of **6b**

T/°C	**5b**/equiv.	TMSOTf/ equiv.	Yield of **6b** (%) (β/α)
Room temp.	5.0	1.0	0
Room temp.	5.0	2.0	76 (14)
Room temp.	5.0	5.0	74 (20)
Room temp.	5.0	10.0	64 (17)
Room temp.	10.0	2.0	82 (11)
Room temp.	10.0	5.0	82 (16)
Room temp.	10.0	10.0	67 (17)

The initial resin loading of the aminomethylated polystyrene **2** was 0.40 mmol g^{-1}, which dropped to 0.36 mmol g^{-1} after coupling of *o*-PALdehyde **1** to the resin, and 0.32 mmol g^{-1} after attaching the partly protected monosaccharide **3d**. With high-loading resin (1.20 mmol g^{-1}) glycosylation became much less efficient..

In conclusion, we have developed a new and efficient strategy for anchoring amino sugars through a BAL handle to a solid support in which the amino sugar is attached by an efficient reductive amination. BAL anchoring was operated in a safety-catch mode as BAL linked amines were stable to Lewis acids and conc. TFA, whereas the corresponding amides were released from the support with conc. TFA. This allowed the use of Lewis acids in excess in solid-phase glycosylations. Using this strategy, we synthesized 1-6 linked disaccharides in high yields. The safety-catch protocol for BAL anchoring should also be useful for other applications.

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Notes and references

† All solid-phase reactions were carried out in plastic syringes at room temperature, except for glycosylations at −50 °C, which were carried out in glass flasks. Syringes were fitted with a polypropylene filter, a Teflon valve in the bottom, and closed in the top with the syringe plunger. For glycosylations, the syringe was instead closed with a septum, after the solid reactants were placed in the syringe. It was then dried in a desiccator *in vacuo* with the Teflon valve open. The desiccator was opened under argon, the Teflon valve closed, and the solvent and Lewis acid added through the septum. After 18 h the resins were washed and dried; final products were released from the support with TFA–H_2O (19:1). Glycosylations were typically performed on a 5 μmol scale. Quantifications were done by HPLC–UV integration of peak areas and use of standard curves established from benzyl and benzoyl containing compounds. Benzyl groups were monitored at λ = 215 nm, and benzoyl groups at λ = 265 nm. At the 5 μmol scale and without purification, the structure of **6b** was established by MS, ^{1}H NMR and gCOSY spectroscopy (ref. 8).

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8 *Selected data* for **6b**: δ_H($CDCl_3$, 500 MHz) 8.01 (dd, *J* 1.3, 8.5, 2H), 7.90 (dd, *J* 1.3, 8.5, 4H), 7.82 (dd, *J* 1.7, 8.5, 2H), 7.54–7.48 (m, 2H), 7.44–7.41 (m, 2H), 7.38–7.22 (m, 16H), 7.14 (dd, *J* 1.7, 7.7, 2H), 5.91 (dd, *J* 9.8, 9.4, 1H), 5.69 (dd, *J* 9.8, 9.4, 1H), 5.59 (dd, *J* 9.8, 8.1, 1H), 5.21 (d, *J* 9.4, 1H), 4.91 (d, *J* 7.7, 1H), 4.71 (d, *J* 11.5, 1H), 4.63 (dd, *J* 12.0, 3.4, 1H), 4.57–4.53 (m, 2H), 4.52 (d, *J* 11.5, 1H), 4.48 (d, *J* 3.4, 1H), 4.39 (d, *J* 11.1, Hz, 1H), 4.19–4.13 (m, 3H), 3.75 –3.68 (m, 2H), 3.57 (dd, *J* 10.7, 9.0, 1H), 3.42 (dd, *J* 9.4, 9.0, 1H), 3.07 (s, 3H), 1.81 (s, 3H); LC-MS ($C_{57}H_{55}NO_{15}$) calc. [M+H$^+$], 994.4, [M − MeO$^-$], 962.4; found 994.9, 962.8.

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