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A novel method for solid-phase synthesis of oligosaccharides using the *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) linker

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Abstract—The application of the *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde) linker for the solid-phase synthesis of oligosaccharides is described. The oligosaccharide products can be cleaved from the resin by hydrazine, ammonia or primary amines, but the linker is stable under the conditions of oligosaccharide synthesis. The first sugar can be attached to the resin linker via a vinylogous amide bond, or by ether linkage using a *p*-aminobenzyl alcohol converter. © 2001 Elsevier Science Ltd. All rights reserved.

Oligosaccharides constitute a major class of bioactive polymers and are implicated in numerous biomolecular processes,[1] and are considered to be potential therapeutics or targets for intervention in a variety of human diseases. However, their development as therapeutics has been hindered by the lack of appropriate technologies for their synthesis. Traditional solution-phase synthesis is time consuming, labour intensive and results in complex mixtures of oligomers, which are difficult to separate and purify. Hence, attention has been focused on solid-phase synthesis as a means to provide a facile and cost effective method of oligosaccharide synthesis. Unfortunately solid-phase synthesis of oligosaccharides can be problematic due to low coupling yields, inadequate stereocontrol, and the necessary employment of low loading resin to achieve significant coupling yields.[2]

As was recently noted, a particularly desirable goal is the development of generally applicable methods for the rapid assembly of oligosaccharides with a long-term view towards automation.[3] A critical element in the realisation of this goal is the nature of the linker between the solid support and the initial synthon. It is necessary that (1) the linker should be stable under glycosylation conditions and protecting group manipulations, and (2) the cleavage conditions should not effect the structure of the synthesised compounds. To date, solid-phase linkers have been developed from the protecting and activating groups that are used in carbohydrate synthesis. The sugar may be connected to the linker via an ether,[4] ester,[5] silyl,[6] alkyl thio-ether,[7] aryl thio-ether[8] or sulphonate linker.[9] A tris(alkoxy)benzyl amine anchoring was also reported recently.[10] Most of these linkers satisfy the above mentioned requirements,[11] but after cleavage the resin-linker is not reusable for further synthesis.[12]

We have reported a hydrazine-labile primary amino-protecting group, *N*-1-(4,4-dimethyl-2,6-dioxocyclohexylidene)ethyl (Dde), employed for solid-phase carbohydrate synthesis.[13] This Dde group has been used previously for protection of lysine side chains during solid-phase peptide synthesis (SPPS).[14] A Dde analogue was also used as a protecting group in combinatorial amine synthesis, again on a solid support.[15]

We report here the use of Dde analogue linkers for the solid-phase synthesis of oligosaccharides. Initially the dimedone based linker was coupled to the solid-phase. Dimedone was reacted with glutaric anhydride in the presence of triethylamine and dimethylaminopyridine in anhydrous dichloromethane to give vinylogous acid **1** (80% yield after crystallisation), which was subse-

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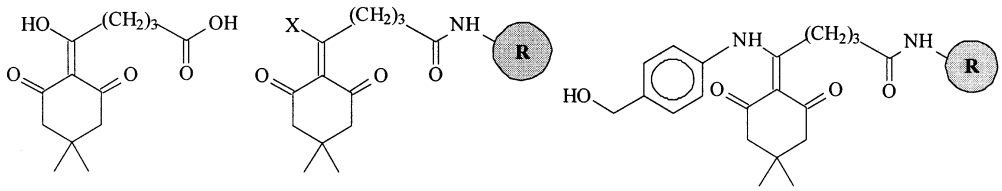
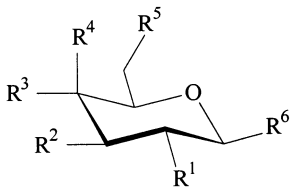


1 **2a** (X=OH) **3**

2b (X=NH_2)

Figure 1.

Table 1.



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	R^1	R^2	R^3	R^4	R^5	R^6
4a	NH_2	BnO	OH	H	BnO	BnO
4b	ClBnO	ClBnO	H	ClBnO	ClBnO	SMe
4c	ClBnO	ClBnO	H	ClBnO	ClBnO	α/β-$OCH_2C_6H_4NH_2$

quently coupled to Novabiochem 4-methylbenzhydrylamine (MBHA) resin (substitution: 0.7 mmol/g) to give compound **2a** (with higher than 99.5% coupling efficiency according to quantitative ninhydrin test). Resin-linker conjugate **2a** was then heated at reflux in THF/ethanol (1:1 v/v) with *p*-aminobenzyl alcohol (five-fold excess) to afford resin-linker-converter conjugate **3** (Fig. 1). Alternatively, glutaric acid was coupled to MBHA resin (0.42 mmol/g), in presence of 1.2 equiv. dicyclohexylcarbodiimide (DCC), then the intermediate was stirred with dimedone in the presence of DCC resulting in compound **2a**, which was reacted with *p*-aminobenzyl alcohol, resulting in compound **3**.

In an alternative strategy, protected aminosugar **4a** (Table 1) was stirred with acid **1** (1.2 equiv.) in ethanol in the presence of triethylamine (1 equiv.) at 60°C, overnight, to afford sugar-linker conjugate **5**. Conjugate **5** (Fig. 2) was then coupled to MBHA resin (0.7 mmol/g) to give resin bound sugar **6** (with higher than 99.5% coupling efficiency according to ninhydrin assay) (Fig. 3). Subsequent cleavage experiments indicated that no *O*-acylation had occurred.

Carbohydrates without free amino groups could be coupled to the resin-linker when a *p*-aminobenzyl alcohol 'converter' was built into the system. Resin-linker-converter conjugate **3** was glycosylated with *p*-chloro benzylated mono-saccharide donor **4b** (4 equiv.) in dichloromethane in the presence of dimethyl-

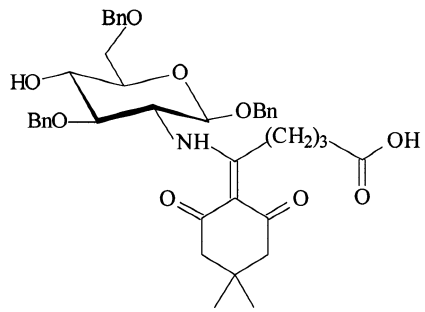

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Figure 2.

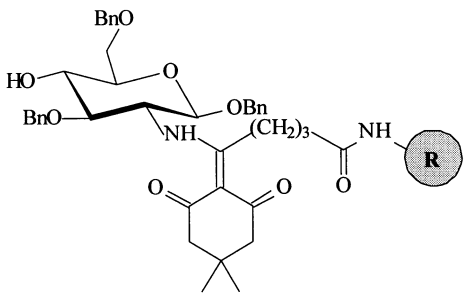

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Figure 3.

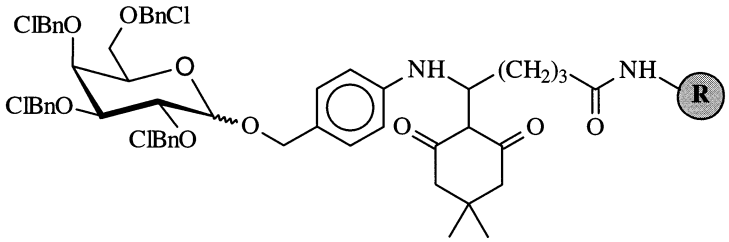

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Figure 4.

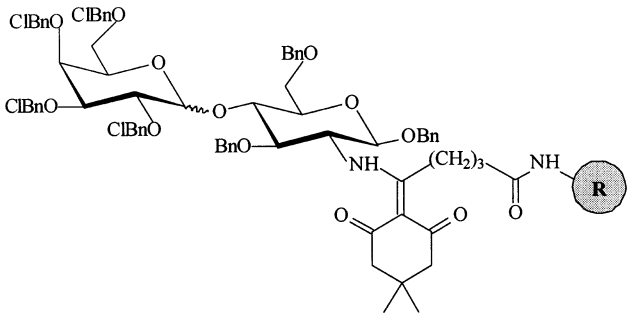

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Figure 5.

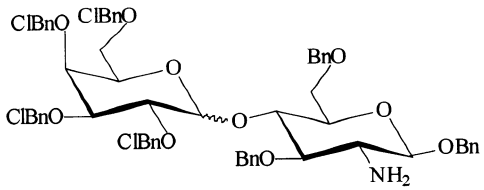

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Figure 6.

(methylthio)sulphonium triflate (4 equiv.) and 4 Å powdered molecular sieves, at ambient temperature to give resin bound sugar conjugate **7** (Fig. 4). Resin-sugar conjugate **7** was treated with 2% hydrazine hydrate in DMF for 2 hours to afford **4c** as an anomeric mixture (90% yield after chromatographic purification).

As a final proof of concept disaccharide **9** was synthesised. The 4-hydroxyl group of the resin-linker-saccharide conjugate **6** was glycosylated with thio-methyl glycoside **4b** (5 equiv.) in the presence of methyl trifluoromethanesulphonate (5 equiv.) and 4 Å powdered molecular sieves, at ambient temperature to provide disaccharide conjugate **8** (Fig. 5). The resin complex **8** was treated with 5% hydrazine hydrate in DMF for 4 h, to afford an anomeric mixture of disaccharides **9** (88% yield after chromatography, α/β; 17/3).[16](Fig. 6)

Quantitative yields employing ammonia solutions as the cleavage reagent could only be achieved after repeated cleavages. The initially formed resin-linker conjugate **2b** was then regenerated to give conjugate **2a** by treatment with tetrabutylammonium hydroxide in DMF. These preliminary results indicated that the resin could be successfully reused.

In summary the Dde based linkers were stable under carbohydrate reaction conditions. The cleavage of the Dde based linkers were carried out under mild conditions and did not adversely effect the nature of the synthesised carbohydrate.

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16. Analytical data for compound **9**: Gal: 5.58 (d, 1H, J_{1-2} = 3.6 Hz, H′-1), $GlcNH_2$: 4.33 (d, 1H, J_{1-2} = 7.8 Hz, H-1), 3.04 (dd, 1H, H-2), ES–MS: $C_{61}H_{61}Cl_4NO_{10}$ (1109.96) = 1110.94 (100), $[M+H]^+$.