Pergamon

Tetrahedron Letters 41 (2000) 4329–4333

TETRAHEDRON LETTERS

A novel 4,5-dibromooctane-1,8-diol linker for solid-phase oligosaccharide synthesis

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Received 29 February 2000; accepted 13 April 2000

Abstract

A novel 4,5-dibromooctane-1,8-diol linker was applied to the solid support preparation of a β-(1→6) trisaccharide employing electrophilic activation of thioethyl glycoside building blocks. Debromination of the resin-bound linker-double bond could effectively be carried out by elimination, followed by olefin cross-metathesis to reveal the desired trimeric *n*-pentenyl glycoside. High-resolution Magic Angle Spinning NMR spectroscopy was used as an analytical tool for the monitoring and development of the solid-phase reactions.

Keywords: solid-phase synthesis; thioglycosides; carbohydrates; linker.

The importance of oligosaccharides in a multitude of biological processes[1] has sparked the interest of biologists and chemists alike. While the need for chemically defined oligosaccharides has steadily increased in recent years, the synthesis and purification of these molecules remains challenging and is carried out by a few specialized laboratories. Oligonucleotides[2] and oligopeptides[3] are now routinely prepared on automated synthesizers, providing pure substances in a rapid and efficient manner. Solid-phase oligosaccharide synthesis holds the potential to secure the necessary substrates for biochemical and biophysical studies.

A number of different approaches to solid-phase oligosaccharide synthesis involving a variety of glycosylation agents such as sulfoxides,[4] 1,2-anhydrosugars,[5] *n*-pentenyl glycosides,[6] glycosyl trichloroacetimidates,[7] thioglycosides,[8] and phosphates[9] have been explored. The connection of the first sugar to the polymeric support via a linker is of crucial importance in terms of the synthetic strategy and ultimately the success of the synthesis. The linker has to be completely stable under the reaction conditions but should be cleavable under selective and mild conditions at the end of the synthesis.

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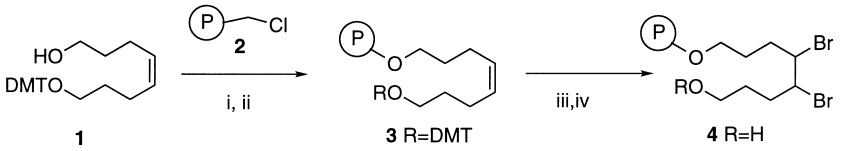
0040-4039/00/$ - see front matter
PII: S0040-4039(00)00663-8

A variety of groups including silanes,[10] thioethers,[8a] benzylidene acetals,[11] succinamides,[12] photolabile esters,[6,8b,13] *p*-acylaminobenzyl esters,[14] branched alkenes,[15] and tris(alkoxy)benzyl amines (BAL)[16] have been employed to anchor the growing oligosaccharide to the solid support. Most of these linkers interfere with some common activation or deprotection conditions, thus limiting the versatility and flexibility in synthetic planning.

Recently, we introduced a novel linker concept for the solid-support synthesis of oligosaccharides.[9] This 4-octene-1,8-diol (Scheme 1) can be cleaved by olefin cross-metathesis. The linker proved to be acid and base stable, and performed extremely well with glycosyl trichloroacetimidate and glycosyl phosphate building blocks. Two classes of versatile glycosylating agents, thioglycosides[17] and *n*-pentenyl glycosides[18] (NPG) require strongly electrophilic activators, such as *N*-iodosuccinimide (NIS) and trimethylsilyl triflate (TMSOTf). These conditions are incompatible with linkers containing olefinic double bonds. A universally applicable linker that is inert to the wide range of glycosylation and deprotection conditions employed in oligosaccharide synthesis would be most useful. Here we introduce a 4,5-dibromooctane-1,8-diol linker that makes the synthetic utility of the octenediol linker concept available to syntheses using NPG and thioglycoside building blocks. A trisaccharide was prepared using the new linker.



Scheme 1. Synthesis of the dibrominated octanediol linker. P = Merrifield' s resin. (i) NaH, DMF. (ii) NaH, MeOH. (iii) LiBr, $CuBr_2$, MeCN, THF, 90%. (iv) Cl_2 HCCOOH, CH_2Cl_2, quant.

Initially, a reliable sequence for the installation of the dibromooctanediol (DBOD) linker was developed. Reaction of mono-protected octenediol **1** resulted in efficient functionalization of Merrifield's resin **2** as described previously[9] followed by the capping of unreacted resin with methanol (Scheme 1). Dibromination[19] of linker **3** using $CuBr_2$ and LiBr in acetonitrile/THF, followed by exposure of the free hydroxyl group under acidic conditions proceeded smoothly to furnish resin-bound dibromooctanediol **4**. High-resolution magic angle spinning NMR spectroscopy (HR-MAS NMR)[20] indicated complete conversion of the alkene as judged by disappearance of the olefinic proton signals (~5.4 ppm in the ^{1}H NMR; Fig. 1a and b).

The stability of the new linker to electrophilic activation was first evaluated using a *n*-pentenyl mannoside donor (Scheme 2). Linker **4** was reacted with mannosyl donor **5** upon activation by NIS and TMSOTf to produce support-bound monosaccharide **6** in 73% yield.

Next, the use of thioglycoside donors for coupling on the DBOD linker was studied (Scheme 3). Resin-bound acceptor **4** was reacted with thioethyl donor **7** in the presence of NIS and TMSOTf to yield glucoside **8**. Deprotection of the 6-*O*-acetate proceeded smoothly using guanidine in MeOH/THF, to afford acceptor **9**.[7c,21] The use of stronger bases such as NaOMe resulted in side products caused by bromide elimination as determined by HR-MAS. The acetates could be removed in the presence of the dibromide by action of hydrogen chloride in 1,4-dioxane/methanol. Iteration of the coupling and deprotection sequence produced trisaccharide **12**. All intermediates were examined by HR-MAS to confirm the formation of the desired linkages.

After completion of the trisaccharide a two step cleavage protocol was developed. Reductive debromination[22] of the linker was achieved with tetrabutylammonium iodide (TBAI) in 4-butanone/

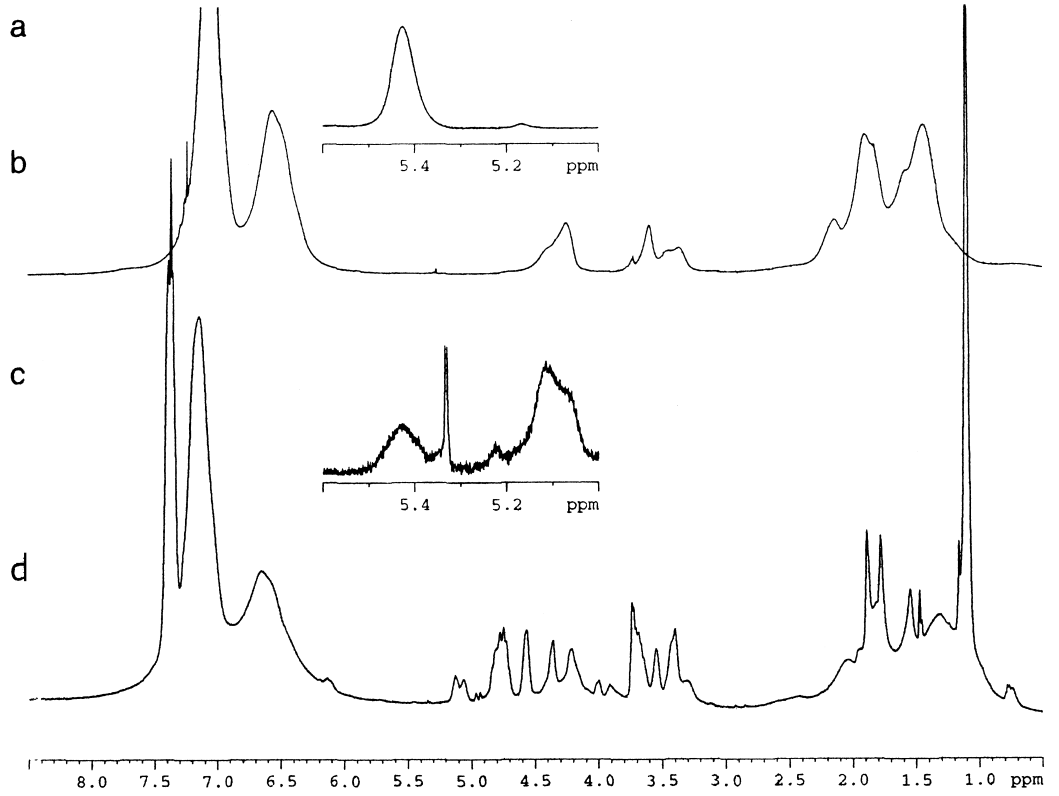
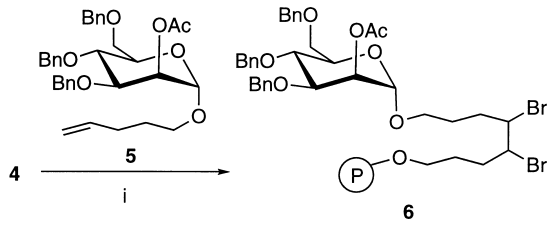


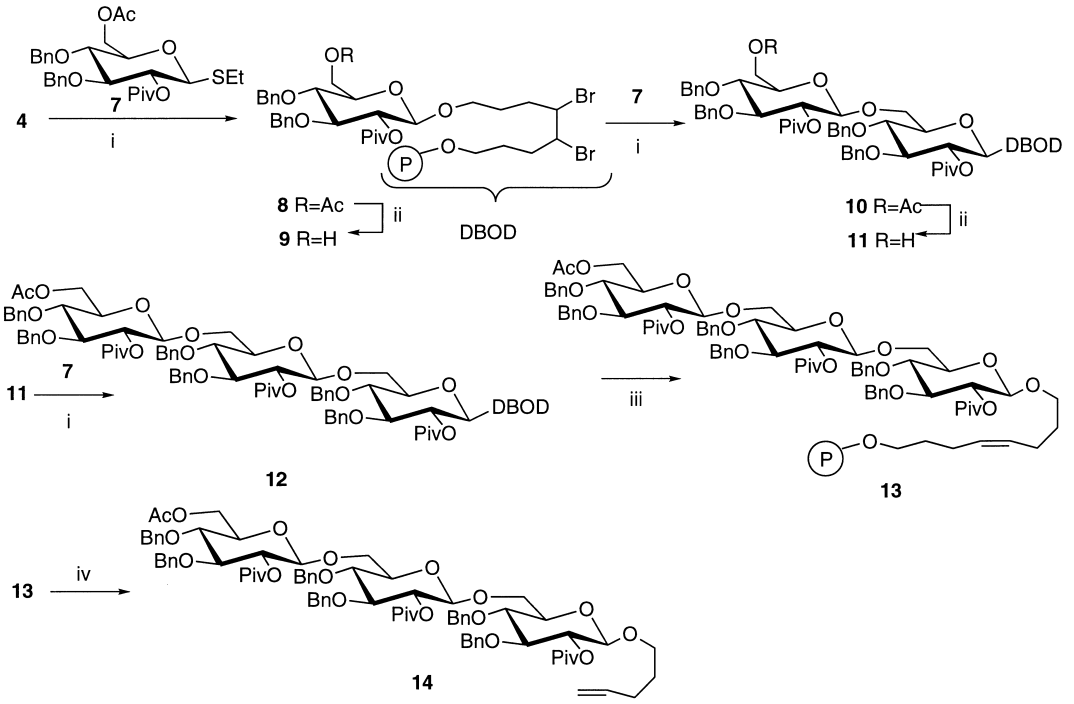
Figure 1. Illustration of the masking and unmasking of the olefinic double bond. HR-MAS NMR spectra of **3** (a), **4** (b), **13** (c), and **10** (d)

1,4-dioxane to yield octenediol-linked trisaccharide **13** as unambiguously confirmed by HR-MAS NMR (Fig. 1c). TBAI was found to perform better in this reaction than sodium iodide due to its considerably higher solubility. Cleavage from the solid support using 20 mol% of Grubbs' catalyst under an atmosphere of ethylene afforded the fully protected *n*-pentenyl glycoside **14** in 9% overall yield from **3** (77% per step over 9 steps).[†]

[†] Experimental procedures: (a) Bromination of the linker: functionalized resin **3** (100 mg, 0.065 mmol) was swollen in THF/MeCN. $CuBr_2$ (0.65 mmol) and LiBr (1.3 mmol) were added and the reaction mixture was shaken for 48 h. After washing, the resulting resin was repeatedly treated with 2% dichloroacetic acid, washed and dried to yield **4**. (b) Glycosylation: functionalized resin **4** (207 mg, 0.124 mmol) was swollen in CH_2Cl_2 and activated 4 Å molecular sieves (207 mg) and NIS (1.12 mmol) were added. Thioethyl donor **7** (0.372 mmol) was added as a solution in CH_2Cl_2 (2 mL) and the reaction mixture was cooled to 0°C. TMSOTf (0.186 mmol) was added and the reaction shaken for 3 h. The resin was washed and dried to yield monosaccharide **8**. (c) Deacetylation: resin-bound monosaccharide **8** (249 mg, 0.107 mmol) was swollen in THF. Guanidine (1.22 mmol) was added and the reaction shaken for 16 h. The resin was washed and dried to yield monosaccharide acceptor **9**. (d) Debromination of linker: resin bound trisaccharide **12** (247 mg, 0.084 mmol) was swollen in 4-butanone/1-4 dioxane (2:1, 3 mL). TBAI (2.11 mmol) was added and the reaction was shaken for 48 h at 95°C. The resin was washed and dried to yield trisaccharide **13**. (d) Cleavage from resin: resin-bound trisaccharide **13** (145 mg, 0.049 mmol) was swollen in CH_2Cl_2, Grubbs' catalyst (9.89 μmol) was added and the reaction was stirred for 36 h under an atmosphere of ethylene. Evaporation of the solvent in vacuo followed by silica gel chromatography yielded trisaccharide **14**.



Scheme 2. Glycosylation using a *n*-pentenyl donor. (i) NIS, TMSOTf, CH_2Cl_2, Et_2O, 3 h



Scheme 3. Synthesis of trisaccharide **13** on the DBOD linker. (i) NIS, TMSOTf, 4 Å molec. sieves, CH_2Cl_2, Et_2O, 3 h. (ii) Guanidine, MeOH, THF, 16 h. (iii) TBAI, 4-butanone, 1-4 dioxane, 95°C, 48 h. (iv) Grubbs' catalyst, CH_2Cl_2, ethylene, 36 h

In summary, we have introduced a 4,5-dibromooctane-1,8-diol linker that can be used in solid-phase oligosaccharide synthesis with *n*-pentenyl glycosides and thioethyl glycosyl donors. This linker, together with the octenediol linker we developed earlier constitutes a universal linker concept compatible with a wide range of activation and deprotection conditions. To demonstrate the utility of the DBOD linker, a trisaccharide was constructed in high yield using thioethyl donors. These studies underscored the value of HR-MAS NMR as a non-destructive analytical tool to monitor and develop solid-phase reactions.‡

‡ Financial support from the ACS-Petroleum Research Fund (ACS-PRF# 34649-G1), the Greenslaw Scholarship Fund (Fellowship for L.G.M.), and the DAAD (NATO Fellowship for W.-C.H.) is gratefully acknowledged. This work made use of MRSEC Shared Facilities supported by the NSF (Award DMR-9400334). Funding of the NMR facility at the Francis Bitter Magnet Laboratory was provided by the NIH (Grant RR-00995). We thank Dr. Susan S. Pochapsky for helpful discussions and assistance with the HR-MAS NMR.

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