ORGANIC LETTERS

2003
Vol. 5, No. 8
1309−1312

DISAL Glycosyl Donors for the Synthesis of a Linear Hexasaccharide under Mild Conditions

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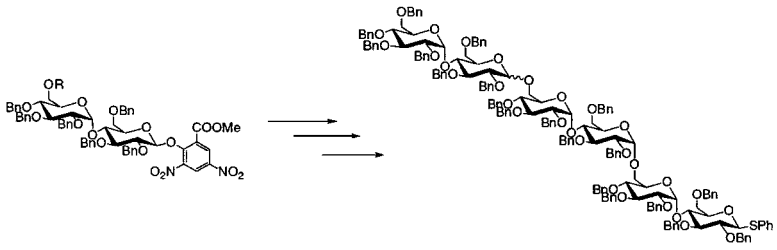
Received February 11, 2003

ABSTRACT



The new class of glycosyl donors with a methyl 3,5-dinitrosalicylate (DISAL) anomeric leaving group has proved efficient for glycosylation under strictly neutral, mildly basic, or mildly acidic conditions. Here, we report the synthesis of novel DISAL disaccharide glycosyl donors prepared by easy nucleophilic aromatic substitution. These DISAL donors proved efficient in the synthesis of a starch-related hexasaccharide under very mild conditions. Glycosylations proceeded with α-selectivity and were compatible with Trt protecting groups.

Cell-surface oligosaccharides are involved in many biological recognition processes including leukocyte−endothelial cell adhesion (leukocyte recruitment in inflamed tissue), bacterial and viral infection, and immunological recognition of tumor cells and foreign tissue in xenotransplantation.[1] Furthermore, free oligosaccharides can have biological effects such as the rhizobial lipochitin oligosaccharides, which function as nodulation factors.[2] Besides, well-defined linear and branched oligosaccharides are important substrates for biosynthesis and degradation studies.[3]

Established glycosylation methods, most notably those employing glycosyl bromides, fluorides, trichloroacetimidates, sulfoxides, and *n*-pentenyls, as well as thioglycosides and glycals, all require activation by a Lewis acid (e.g., AgOTf, BF_3·Et_2O, TMSOTf, or DMTST) for efficient glycosylation of aliphatic alcohols. Waldmann and coworkers have reported activation of glycosyl fluorides, trichloroacetimidates, and phosphites using the very mild

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10.1021/ol034242q CCC: $25.00
Published on Web 04/01/2003

Lewis acid $LiClO_4$, which can exert a "special salt effect".[4] Lubineau et al. have described LiOTf as an alternative to $LiClO_4$ as a promoter in these glycosylation reactions.[5]

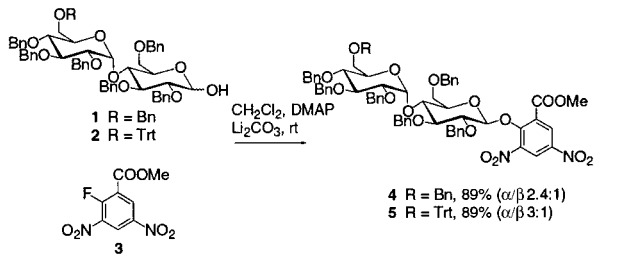
In three recent papers, we have described a new, efficient method for glycosylation under strictly neutral, mildly basic, or very mild acidic ($LiClO_4$) conditions.[6] In this glycosylation technique, the anomeric leaving group on benzyl- or benzoyl-protected donors is methyl 3,5-dinitrosalicylate (DISAL) or its para regioisomer. The potential of DISAL glycosyl donors was demonstrated in their successful application to solid-phase oligosaccharide synthesis. The carboxyl moiety in DISAL was furthermore utilized for linking two monosaccharides together, followed by intramolecular glycosylation in a 1,9-glycosyl shift to yield 1,4-linked disaccharides of gluco- and mannopyranosides, otherwise accessible only with considerable difficulty.

Here, we report the first synthesis of disaccharide DISAL glycosyl donors and their application for the synthesis of longer oligosaccharides, i.e., a starch-related hexasaccharide.

The disaccharide precursor Bn_4-α-Glc*p*-(1→4)-Bn_3-D-Glc*p*-OH (**1**) was readily available.[7] The second disaccharide precursor 6-*O*-Trt-Bn_3-α-D-Glc*p*-(1→4)-Bn_3-D-Glc*p*-OH (**2**) was obtained from phenyl 2,3,6-tri-*O*-benzyl-4-*O*-(2,3-di-*O*-benzyl-4,6-*O*-benzylidene-α-D-glucopyranosyl)-1-thio-*β*-D-glucopyranoside[8] via a regioselective reductive cleavage of the benzylidene acetal function with a $LiAlH_4-AlCl_3$ reagent[9] to give disaccharide acceptor **7**. Conventional tritylation of **7** followed by removal of the anomeric phenylthio group with NBS/aq acetone[10] gave the desired precursor **2**. The latter contained the very acid-labile Trt protecting group, which would allow extension at *O*-6 after the first glycosylation step (to form a tetrasaccharide). However, it is well-documented that both primary and secondary hydroxyls protected as their Trt ethers in some cases can be glycosylated with concomitant Trt cleavage.[11] Trityl protecting groups are labile to Lewis acids; for example, TMSOTf can be used for trityl deprotection. However, thioglycosides generally require soft Lewis acids for their activation, and it has been demonstrated that the Trt moiety can be stable to these glycosylation conditions; for example, activation of an "armed" Trt-protected thioglycoside with NIS/TfOH is compatible with Trt integrity.[12] It would thus be a test of the DISAL donor concept, whether it would be compatible with the very versatile and highly acid-labile Trt protecting group.

Benzyl-protected lactol **1** was reacted with 2-fluoro-3,5-dinitrobenzoic acid (DISAL-F, **3**) in the presence of 1,4-dimethylpiperazine (DMP) and Li_2CO_3 in CH_2Cl_2. This provided a (nonoptimized) 68% yield of the DISAL disaccharide **4** with an α/*β* ratio of 1:3.3, a *β*-selectivity which was lower than previously achieved for monosaccharides.[6] Substituting DMP for DMAP gave a yield of 89% with an α/*β* ratio of 2.4:1, in a faster reaction (Scheme 1). For the transformation of Trt-protected lactol **2** into the corresponding DISAL derivative **5**, we thus relied on the DMAP protocol, which after column chromatography of the reaction mixture using EtOAc−toluene (1:15) on dry silica gel gave an 89% yield with an α/*β* ratio of ∼3:1 (Scheme 1).

Scheme 1. Donor Synthesis



In previous DISAL studies, we established three sets of standard conditions for glycosylation with DISAL donors (1.5−3 equiv): (i) donor and acceptor in NMP at 40−60 °C; (ii) donor and acceptor in $(CH_2Cl)_2$, CH_2Cl_2, or CH_3NO_2 in the presence of $LiClO_4$ (2.0−3.7 equiv), in some cases also with Li_2CO_3 as an acid scavenger (2 equiv); (iii) donor and acceptor in $(CH_2Cl)_2$ or CH_2Cl_2 in the presence of $LiClO_4$ and Bu_4NI.

These operationally simple reactions were carried out in plastic vials. The reactions were analyzed by HPLC, and the

Table 1. Model Glycosylations Using Cyclohexanol as Model Acceptor[a]

entry	solvent	additives (equiv)	yield (α/*β*)[b]
1[c]	NMP		61% **6** (2.7:1)
2	CH_2Cl_2	$LiClO_4$ (2.5)	*d*
3	CH_2Cl_2	Bu_4NI, $LiClO_4$ (2.5)	87% **6** (26.5:1)
4	CH_3CN	Bu_4NI, $LiClO_4$ (2.5)	66% **6** (9.4:1)
5	CH_3NO_2	Bu_4NI, $LiClO_4$ (2.5)	67% **6** (11.8:1)
6	Et_2O	Bu_4NI, $LiClO_4$ (2.5)	40% **6** (8.9:1)
7	CH_3NO_2	Bu_4NI (2.5)	80% **6** (14.5:1)
8	CH_3NO_2	Bu_4NI (0.1)	*d*

[a] Reaction conditions were rt for 17 h, unless otherwise noted. [b] Yields were determined by HPLC (area at 215 nm). [c] Reaction conditions were 40 °C for 4 h. [d] Incomplete conversion.

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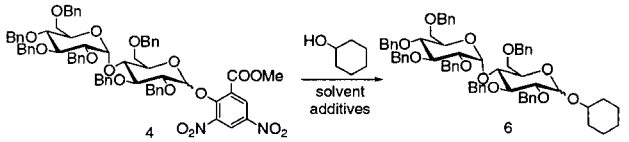
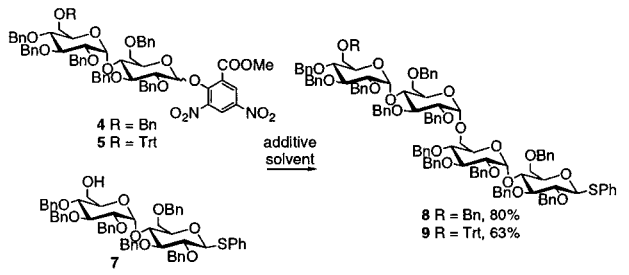
yields in Table 1 were based on integration of relevant peaks at 215 nm. The disaccharide donor proved efficient in these glycosylations. The highest yield and α/β ratio of cyclohexyl disaccharide **6** was obtained for glycosylations in CH_2Cl_2 in the presence of $LiClO_4$ and Bu_4NI (Scheme 2, Table 1, entry 3).

Scheme 2. Model Glycosylations Using Cyclohexanol as Acceptor



Glycosylation of disaccharide acceptor **7** with DISAL donor **4** to give the tetrasaccharide **8** proceeded under a variety of conditions (Scheme 3, Table 2). Good yields and

Scheme 3. 1,6-Glycosylations



the highest α/β ratio (pure α within our detection limit) was obtained in the presence of $LiClO_4$ and Bu_4NI in CH_2Cl_2 (rt) or CH_3NO_2 (5 °C). At elevated temperatures (50 °C in $(CH_2Cl)_2$), the glycosylation proceeded smoothly within 2 h and with an acceptable α/β ratio of 3:1 (Table 2, entries 1−4 versus 5).

Table 2. Glycosylations of Benzylated and Tritylated Donors Forming Tetrasaccharides

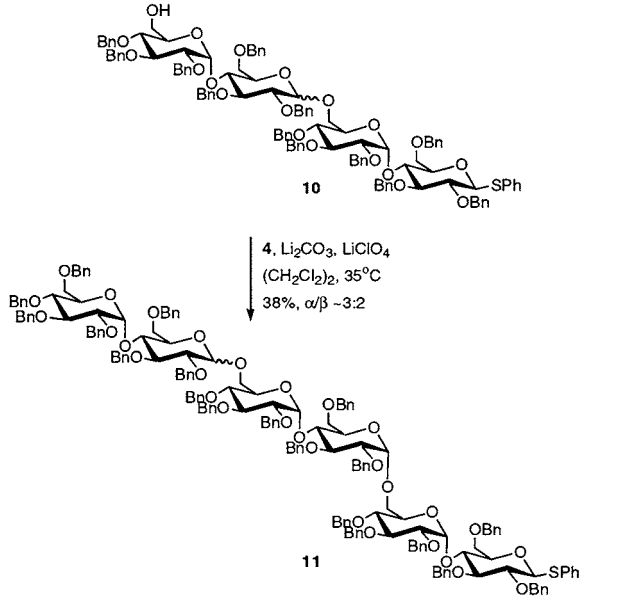
entry	donor	solvent	additives (equiv)	yield[a] (α/β)
1[b]	**4**	CH_2Cl_2	$LiClO_4$, Li_2CO_3, Bu_4NI (2.5)	48% **8** (>20:1)
2[b]	**4**	CH_2Cl_2	$LiClO_4$, Bu_4NI (2.5)	51% **8** (>20:1)
3[c]	**4**	CH_3NO_2	$LiClO_4$, Bu_4NI (2.5)	30% **8** (>20:1)
4[d]	**4**	CH_3NO_2	$LiClO_4$, Bu_4NI (2.5)	60% **8** (>20:1)
5[e]	**4**	$(CH_2Cl)_2$	$LiClO_4$ (2.5)	80% **8** (3:1)
6[d]	**5**	CH_3NO_2	$LiClO_4$, Li_2CO_3, Bu_4NI (4.0)	63% **9** (>20:1)
7[f]	**5**	$(CH_2Cl)_2$	$LiClO_4$, Li_2CO_3 (4.0)	*g* (3:2)

[a] Isolated yields. [b] Reaction conditions were rt for 5 days. [c] Reaction conditions were rt for 17 h. [d] Reaction conditions were 5 °C for 5 days. [e] Reaction conditions were 50 °C for 2 h. [f] Reaction conditions were 40 °C for 17 h. [g] The product was not isolated but taken on in the synthesis without purification. Estimated yield of glycosylation by HPLC analysis: 60%.

Glycosylation of **7** with 6-*O*-Trt-protected donor **5** in CH_3NO_2 in the presence of $LiClO_4$, Li_2CO_3, and Bu_4NI gave tetrasaccharide **9** with α selectivity in ~63% isolated yield after 5 days (Table 2, entry 6). A comparable yield but poorer diastereoselectivity was obtained much faster at elevated temperatures in $(CH_2Cl)_2$ with $LiClO_4$ and Li_2CO_3 (Table 2, entry 7). It was rewarding to see that the Trt protecting group was completely stable to the very mild conditions used in glycosylations with DISAL donors. Removal of the 6-*O*-Trt protecting group in **9** was performed directly on the crude product from the fast glycosylation reaction by applying mild acidic conditions (AcOH/H_2O/CH_3CN, 90:7:3) at 60 °C. This gave partially deprotected tetrasaccharide **10**, ready for further elongation, in 38% yield for the glycosylation and deprotection (two steps).

Detritylated tetrasaccharide **10** was glycosylated with disaccharide donor **4** using the optimized conditions derived from the synthesis of tetrasaccharides **8** and **9**: $LiClO_4$ and Li_2CO_3 in $(CH_2Cl)_2$ (Scheme 4). As expected, the reactivity

Scheme 4. Hexasaccharide Synthesis



of tetrasaccharide glycosyl acceptor **10** was lower than for disaccharide acceptor **4**, and a larger excess of donor and slightly elevated temperatures (35 °C) had to be applied.

Hexasaccharide **11** was isolated in 38% yield while 50% of the acceptor was recovered. This glycosylation gave an α/β ratio of 3:2. Higher temperatures (50 °C) led to fast destruction of the glycosyl donor. However, the much faster reaction at 50 °C gave an acceptable yield of 34% hexasaccharide **11**. In addition, the long reaction time caused a more complex reaction mixture by formation of more byproducts.

In conclusion, novel disaccharide DISAL glycosyl donors were prepared following procedures established for monosac-

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charides. The β-selectivity in DMP-promoted reactions to form DISAL glycosyl donors was lower than for monosaccharides; DMAP-promoted reactions gave, as expected, the DISAL donors with good α-selectivity. Two disaccharide DISAL glycosyl donors proved efficient for the glycosylation of model alcohol, cyclohexanol. Optimal glycosylations were at room temperature in the presence of Bu_4NI, and in some cases $LiClO_4$, in either CH_2Cl_2 or CH_3NO_2. These conditions were transferred to the synthesis of tetrasaccharides and a hexasaccharide. Glycosylations proceeded with good α-selectivity; however, yields decreased with increasing size of the glycosyl acceptor. This decrease in yield of the *inter*molecular glycosylation has often been observed and is probably due to the competing *intra*molecular elimination to form the glycal. Glycosylations with DISAL donors were compatible with the presence of thioglycosides and the very acid-labile Trt protecting group. These glycosylations were operationally simple and were carried out in standard plastic vials; purification of preparative reactions was achieved by preparative HPLC on a C_4 reversed-phase column after the reaction. However, further developments are required for this new methodology to give yields comparable with the best literature procedures for oligosaccharide synthesis. The mildness and efficiency of this glycosylation protocol should make it useful for glycosylation of a wide range of substrates, including in oligosaccharide syntheses.

Acknowledgment. We thank the Technical University of Denmark (L.P. and J.B.L.), The Danish National Research Foundation (K.L. and M.S.M.), and the LEO foundation (K.J.J.) for financial support of this work. We thank Professors John Nielsen and Birger L. Møller for their support of this work.

Supporting Information Available: General experimental procedures for synthesis of and analytical data for compounds **4**−**6** and **8**−**11**. NMR spectra of coumpounds **5**, **8**, and **11**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL034242Q