Efficient Methods for Glycosidations with Glycals – A Key Intermediate for the Synthesis of Mucin Core 1-Type *O*-Glycan

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Abstract: The use of glycals as acceptors in glycosylation reactions is hampered by their sensitivity to acids. We report here on the successful use of mild Lewis acid [$Sn(OTf)_2$] as catalyst for the glycosylation with *O*-glycosyl trichloroacetimidates and on the development of this method to construct a key intermediate for the synthesis of mucin type *O*-glycans. To this end, chemoselective nitration of O-glycosylated glycals, stereoselective threonine addition, and reduction of the nitro group to the amino group by an efficient procedure avoiding the use of an expensive catalyst was performed.

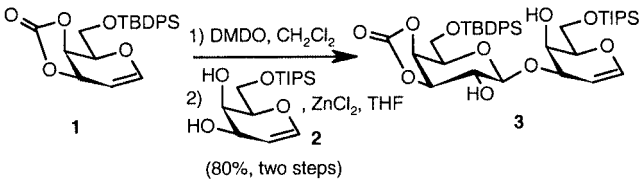
Key words: carbohydrates, glycols, glycosylation, Michael additions, glycopeptides, nitro reduction

Glycals, are among the most versatile chiral building blocks. Not surprisingly, glycals have been the subject of considerable interest in carbohydrate chemistry,[1] in oligosaccharide synthesis,[2] and in the development of combinatorial synthesis of oligosaccharide libraries.[3] Glycals as chiral building blocks, serve as precursors for a broad variety of optically active products.[4]

Application of glycals to the synthesis of the mucin class of glycopeptides has recently attracted our attention[11] because of their fundamental importance in biological processes such as cell-cell adhesion, cell growth, fertilization, parasitic infection and inflammation.[5–7]

All mucin core structures contain at the reducing end an *N*-acetylgalactosamine α-glycosidically linked to L-serine or L-threonine. We have already demonstrated that nitrogalactal concatenation is a well-suited tool for the synthesis of all members of the mucin family[8a–d] as well as for the synthesis of *S*-, *P*-, and *C*-glycosides[8e,f] and of nucleosides.[8g] Herein, we want to report on our studies to the synthesis of the important core 1 disaccharide β-D-Gal-(1→3)-D-GalNac-α-(1→*O*)Thr.

A wide range of glycosylation methods using glycals as donors has been developed,[9] employing 3,3-dimethyldioxirane (DMDO) for the activation of the double bond. This methodology was successfully used to prepare disaccharide **3** with the epoxide of glycal **1** as donor and glycal **2** as acceptor (Scheme 1).[10,11]



Scheme 1 Synthesis of Gal-β-(1→3)-Gal glycal using DMDO.

Only few examples of glycosylations using other activated donors and glycals as acceptors have been reported. In some cases, the use of trichloroacetimidate **4**[11,12] or fluoride **5**,[13,14] was described (Figure 1).

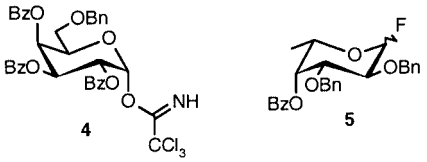
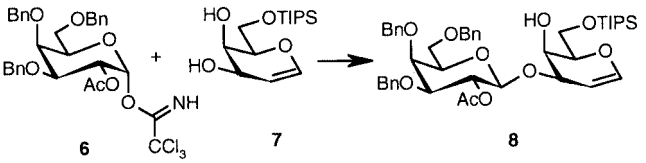


Figure 1

We focused our attention to the development of an efficient method using *O*-galactosyl trichloroacetimidate **6** in order to generate this linkage with the acid sensitive galactal **7**. A variety of Lewis acids were tested, some results are given in Table 1.



Scheme 2 Synthesis of Gal-β-(1→3)-Gal glycal.

Table 1 Glycosylation between **6** and **7**

Entry	Catalyst (%)	T (°C)	Yield (%) [a]
1	0.01 equiv TMSOTf	–78	–
2	0.01 equiv $BF_3{\cdot}OEt_2$	–78	–
3	0.5 equiv $ZnCl_2$	20	44
4	0.02 equiv $Sn(OTf)_2$	–20	75

[a] All reactions were carried out in CH_2Cl_2.

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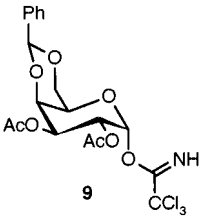
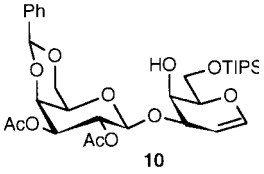
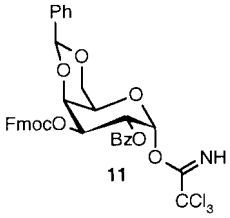
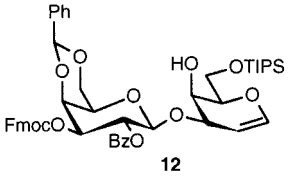
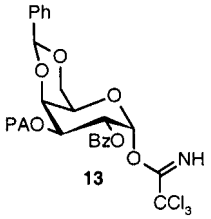
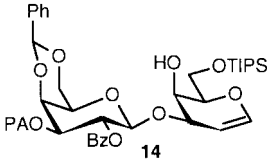
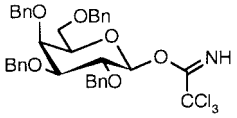
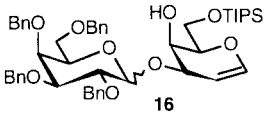
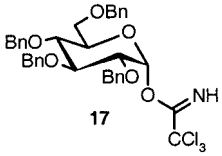
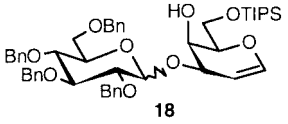
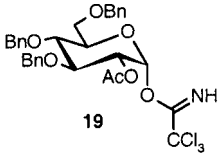
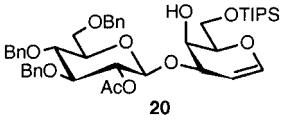
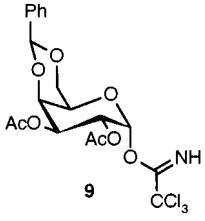
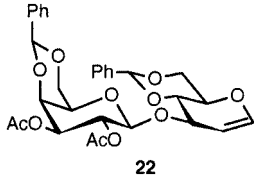
The most efficient Lewis acid to afford this linkage was found to be $Sn(OTf)_2$ (Table 1, entry 4). In the presence of catalytic amounts of $Sn(OTf)_2$, the reaction proceeded readily to afford the desired compound **8**[15,16] in good yield (Scheme 2).

In order to confirm that our method can be generalized, different *O*-glycosyl trichloroacetimidates and glycals were submitted to similar reaction conditions. The results are resumed in Table 2 (PA = phenoxyacetyl). All trichloroacetimidates, which were tested, furnished the expected

Table 2 Coupling Reactions of Imidate Glycosides Using $Sn(OTf)_2$ 0.1 M as Activators[a]

Donor	Acceptor	T (°C)	Compound	Yield (%)	Ratio (a:b)
 **9**	**7**	–20	 **10**	60	0:1
 **11**	**7**	–20	 **12**	60	0:1
 **13**	**7**	–20	 **14**	71	0:1
 **15**	**7**	r.t.	 **16**	61	2:1[b]
 **17**	**7**	r.t.	 **18**	60	1<15[b]
 **19**	**7**	40	 **20**	55	0:1
 **9**	**21**	r.t.	 **22**	55	0:1

[a] All reactions, unless otherwise stated, were conducted in CH_2Cl_2.
[b] Anomeric ratios were determined by isolated products.

disaccharides in good to excellent yield. With donors **15**, **17** and particularly **19**, an increase of the temperature[17] was necessary to speed up the reaction and generate the desired disaccharide. A similar strategy was applied when less reactive benzylidene glucal **21** was used as an acceptor.

With these disaccharides in hand, we were particularly interested if *O*-benzylidene protected glycals can be chemoselectively nitrated in the C-2 position. For this purpose, free hydroxy groups were first protected with an acetyl or benzyl group, and standard conditions[8a–g] for the nitration were then applied to get the corresponding nitrated compounds in good yields (Scheme 3).

As expected, this sequence of reactions furnished 2-nitro compounds in good yields. In the case of **12**,[16] the sensitivity of the Fmoc group under basic conditions resulted in its partial loss during the elimination step and therefore only 20% of desired product **24** was obtained. This result was unexpected because complete removal of the Fmoc group in the corresponding 1-O-silylated monosaccharide was only achieved after 16 hours,[18] however **24** was generated in only a few minutes. The successful nitration was confirmed in compounds **23**–**26** on the basis of their NMR spectral data. The shift of the ethylenic proton H-1 from δ = 6.5 ppm to δ = 8.2 ppm together with the downfield shifts of the signals for C-1 from δ = 143 ppm to δ = 154 ppm and C-2 from δ = 103 ppm to δ = 137 ppm clearly proved the structure of these compounds.

Having demonstrated the efficiency of this glycosylation and nitration method, we decided to improve the synthesis of the key intermediates **35** and **36**,[11] required for the formation of core 1 derived mucins or for antifreeze glycoproteins (Scheme 4).

To this end, compound **8** was converted into per-O-benzylated derivative **27** by the sequence desilylation, deacetylation, and perbenzylation. Next, glycal **27** underwent addition of acetyl nitrate and elimination of acetic acid to afford the corresponding nitroglycal **28**. This Michael-type acceptor was glycosylated under standard conditions[8a] with **29** or **30** to give the corresponding α-glycosides **31** and **32**, with 77% and 73% yield, respectively. The α-linkage was confirmed by the coupling constant $J_{1,2}$ = 4 Hz.

In order to avoid the use of the expensive reagent Raney-Ni T4 (Pt),[8a–g] usually employed for the reduction of the nitro group, a cheaper method using excess of zinc dust[19] and aqueous 1 N HCl in a solvent mixture ($HOAc/H_2O$) was explored. This method, which was first used to reduce hydroxylamines to the corresponding amino derivatives,[20] gave access to the desired amino derivatives to afford the acetamido glycosides **33** and **34**. Benzyl groups were exchanged for acetyl groups, and the Boc protection on the amino acid moiety of **33** was removed to afford **35**, an intermediate in the further synthesis of antifreeze glycoproteins. In addition, all protecting groups on the amino acid moiety of **34** were removed, and finally the Fmoc group was introduced to give known target building block **36**.[11,21]

In conclusion, an efficient and simple method for the glycosylation using glycals as acceptors is described. This way disaccharides were prepared, which can be used as key intermediates for the synthesis of various important classes of glycoconjugates. For instance, regioselective C-2 nitration, then stereoselective threonine addition, and development of a new, convenient procedure for nitro group reduction led to useful building blocks for *O*-glycopeptide synthesis.

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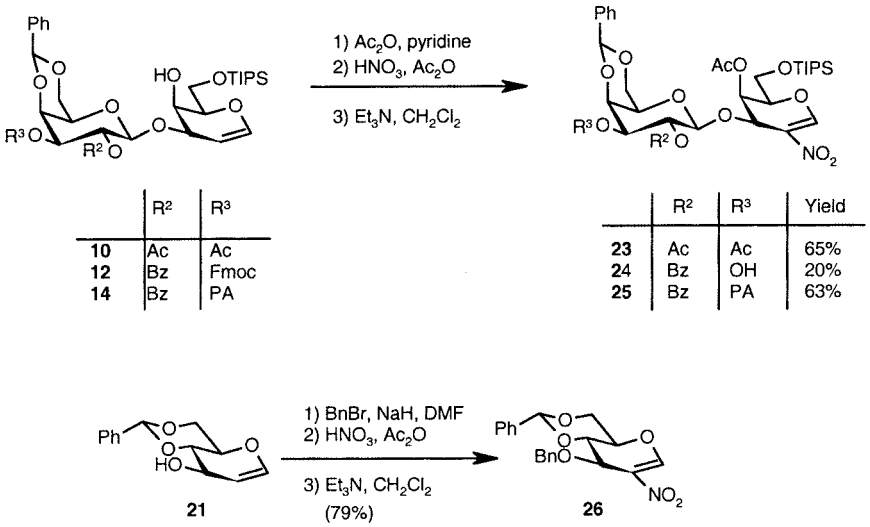
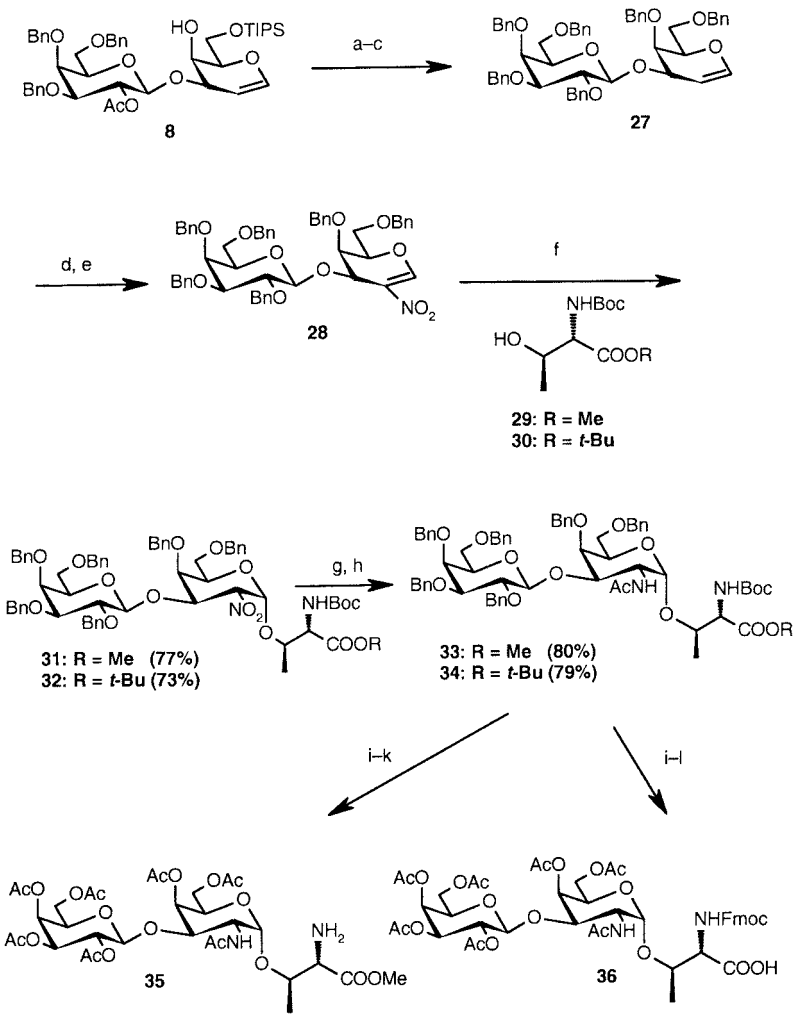


Scheme 3 Nitration of compounds containing a benzylidene acetal.



Scheme 4 Example of a synthesis of a core 1 building block used for glycopeptide synthesis. a) TBAF, THF; b) NaOMe, MeOH; c) BnBr, NaH, DMF, (61% three steps); d) HNO_3, Ac_2O; e) Et_3N, CH_2Cl_2, (75% two steps); f) *t*-BuOK, Toluene; g) Zn, HCl/HOAc/H_2O/THF; h) Ac_2O, pyridine; i) Pd/C, H_2, HOAc, MeOH; j) Ac_2O, pyridine, 71% (two steps); k) TFA, CH_2Cl_2, (90%); l) Fmoc-ONSu, $NaHCO_3$, MeCN, H_2O, 81%.

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(15) **General Procedure for the Glycosylation:** A mixture of trichloroacetimidate (4.2 mmol), and galactal (4.7 mmol) in anhyd CH_2Cl_2 (70 mL) was stirred for 1 h at r.t. under dry Ar,

then cooled to –20 °C. A solution of 0.1 M $Sn(OTf)_2$ in MeCN (0.42 mmol) was added, and the mixture was stirred for 10 min at –20 °C. Et_3N was added. Evaporation of the solvent and purification by chromatography on silica gel yielded the target disaccharide.

(16) NMR-physical data: **8** [δ (1-Hb) = 4.52, $J_{1,2}$ = 8.0 Hz); **10** {δ (1-Hb) = 4.70, $J_{1,2}$ = 7.9 Hz, $[\alpha]_D$ +2 (*c* 0.5, $CHCl_3$)}; **12** {δ (1-Hb) = 4.90, $J_{1,2}$ = 7.9 Hz, $[\alpha]_D$ +24 (*c* 1.6, $CHCl_3$)}; **14** {δ (1-Hb) = 4.88, $J_{1,2}$ = 8.0 Hz}; **16** {β: δ (1-Hb) = 4.51, $J_{1,2}$ = 7.3 Hz, α: δ (1-Hb) = 4.95, $J_{1,2}$ = 3.5 Hz}; **18** after debenzylation and peracetylation [δ (1-Hb) = 4.63, $J_{1,2}$ = 7.7 Hz]; **20** [δ (1-Hb) = 4.68, $J_{1,2}$ = 8.1 Hz]; **22** [δ (1-Hb) = 4.69, $J_{1,2}$ = 7.81 Hz); **31 [**δ (1-Ha) = 5.43, $J_{1,2}$ = 4.8 Hz, δ (1-Hb) = 4.77, $J_{1,2}$ = 7.9 Hz]; **32** [δ (1-Ha) 5.39, $J_{1,2}$ = 4.1 Hz, δ (1-Hb) 4.74, $J_{1,2}$ = 7.3 Hz]; **33** [δ (1-Ha) = 4.87, $J_{1,2}$ = 3.6 Hz]; **36** [δ (1-Ha) 4.85, $J_{1,2}$ = 3.6 Hz].

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(20) **General Procedure for the Reduction of the Nitro Group:** The nitro compound (0.98 mmol) was dissolved in a mixture of THF (75 mL), concd HCl (3 mL), HOAc (16 mL), H_2O (30 mL) and cooled to 0 °C. Zn dust (24 mmol) was added. After stirring for 2 h at 0 °C the Zn was filtrated, the reaction mixture was diluted with CH_2Cl_2, washed with H_2O, sat. aq $NaHCO_3$, and H_2O, and dried over anhyd $MgSO_4$. Evaporation of the solvents and conventional acetylation (Ac_2O in pyridine) of the residue followed by concentration of the mixture and purification by chromatography on silica gel afforded the corresponding *N*-acetamido glycoside.

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