

Scalable Synthesis of Anomerically Pure Orthogonal-Protected GlcN₃ and GalN₃ from D-Glucosamine

Emil Glibstrup and Christian Marcus Pedersen*

Department of Chemistry, University of Copenhagen, Universitetsparken 5, 2100 Copenhagen Ø, Denmark

(5) Supporting Information



ABSTRACT: An improved and scalable synthesis of orthogonally protected D-glucosamine and D-galactosamine building blocks from inexpensive D-glucosamine has been developed. The key reaction is an inversion/migration step providing access to a fully orthogonal protecting group pattern, which is required for microbial oligosaccharide synthesis. The method can be carried out on a multigram scale as several of the reactions can be purified by crystallization to give anomerically pure products.

nterest in the synthesis of microbial glycoconjugates and l oligosaccharides has increased dramatically during the past decade.¹ Besides the common monosaccharide building blocks, these natural products also contain rare carbohydrates, which are less available and require elaborate synthesis. The D-glucosamine and D-galactosamine moieties are found throughout glycospace, and easy access to substantial quantities of these building blocks is therefore of great importance.³ Several methods have been developed for the synthesis of galactosamine derivatives from cheaper starting materials. An early example was the azidonitration protocol on the corresponding D-galactal by Lemieux and Ratcliffe in 1979, which has the disadvantage of giving a mixture of 1,2 isomers.⁴ This area has recently been reviewed.⁵ Several methods have been refined in terms of orthogonal protective group patterns for their use in synthesis of microbial oligosaccharides. Despite these achievements, the synthesis of D-galactosamine derivates remains a bottleneck and suffers from lengthy reaction sequences, harsh reaction conditions, modest yields, and anomeric mixtures, which cannot effectively be separated by chromatography. As part of our ongoing work toward the total synthesis of teichoic acids, easy access to multiple grams of orthogonally protected D-galactosamine derivatives was required. The requirements for the building blocks were orthogonal access to the 3-, 4-, or 6-OH and, having an anomeric protecting group able to tolerate the protection group manipulations, required in a multistep synthesis. A cornerstone in the protective group strategy would be the use of azide as a protective group for the amine functionality, since this would offer a unique reactivity; i.e., it would be stable under acidic and basic conditions but could be selectively reduced under various conditions. The azido moiety is furthermore not participating in glycosylations, and hence, both anomers can be obtained from the same donor depending on the exact reaction conditions.⁶ The stability and versatility of the anomeric protecting group is also important, and the use of thiophenyl has proved highly efficient in this regard. β -Thiomannosides

have earlier been used as a precursor for 2-azido glucose derivatives,⁸ wherein a nucleophilic displacement of a 2-OTf with the azide ion yields the protected glucosamine derivative (Scheme 1a). The starting material for this route is, however,

Scheme 1. Previous Strategies for Obtaining Protected D-Glucosamine and D-Galactosamine Derivatives



still a four-step procedure from commercially available D-mannose.⁹ D-Glucosamine, on the other hand, is an ideal starting material for this synthesis as it is readily available and inexpensive in contrast to its C4-epimer D-galactosamine.¹⁰

Conversion of glucosamine derivatives to the corresponding galactosamine has been performed via inversion of the 4-OH: Using the Lattrell–Dax^{8c,11} inversion with nitrite substituting a triflate^{8c,d,12} or by a nucleophilic displacement with acetate¹³ or benzoate¹⁴ (Scheme 1b). However, these reactions usually require elevated temperatures. The guidelines for substitution of glycoside nonanomeric triflates with external nucleophiles have

Received: July 30, 2016

recently been updated.¹⁵ A third option is anchimeric assistance leading to migration of an ester protecting group from the 3-O by substituting the 4-OTf of a glucopyranoside, causing inversion of stereochemistry; this is usually seen for pivaloyl esters¹⁶ but has also been observed for the acetyl group¹⁷ (Scheme 1c). However, pivaloyl is not an attractive protective group as it cannot be removed selectively and under mild conditions. It has only been observed for the benzoyl protecting group in special cases using highly reactive 2,6-dideoxy sugars (Scheme 1d).¹⁸ With only a few acetyl examples and no relevant benzoyl examples, the most suitable ester protecting group for total synthesis purposes, we were prompted to investigate this migration/inversion reaction as a means to obtain fully orthogonally protected D-galactosamine derivatives. Starting from the inexpensive D-glucosamine-HCl, the amine functionality was azidated using freshly prepared TfN₃, followed by peracetylation, as described by Yan et al.¹⁹ providing 1 in 95% yield (Scheme 2). The subsequent



Tf ₂ O NaN ₃		
он ру	OAc	OAc
HO-CO i)TfN3CuSO4 Et3	N Aco	PhSH ACO
⊖ NH ₃ [™] OH ii) Ac ₂ O Py	N ₃ "OA	NCBF3 OEt2 N3 SPh
či 🕀	1 (95%, 2 steps) 2 (76% (87% BRSM))
	α:β 1:1.7	α:β 1:0.4
		α cryst (80% of all α)

substitution with thiophenol, using an excess of $BF_3 \cdot OEt_2$ (10 equiv) and keeping the reaction time at 2 d, led to a 75% yield of 2 along with some starting material 1 corresponding to an 87% yield based on recovered starting material (BRSM). After recovery of starting material and removal of excess thiophenol by short-plug SiO₂ column chromatography, 80% of the α -anomer could be crystallized from the α/β mixture, allowing for much simpler purification and characterization in the later stages of the synthesis. The crystals formed were of such quality that a crystal structure of the α -anomer could be obtained (Figure S1). The α anomer has previously been crystallized, albeit with no information on the starting α/β ratio and actual quantity crystallized.²⁰ Zemplén conditions (cat. NaOMe in MeOH) provided the deacetylated product 3 in 99% yield. With this common building block in hand, the installation of the orthogonal protecting groups began. The 4,6-O-benzylidene was easily installed using benzaldehyde dimethylacetal and catalytic camphorsulfonic acid (CSA) in MeCN to yield 4 in 94% yield (Scheme 3). If a mixture of anomers was used, the pure α or β -anomer could be crystallized out at this stage. Installing the 2-naphthylmethylidene using the 2-naphthylaldehyde directly along with VO(OTf)₂ as both Lewis acid and hygroscopic reagent, as described by Chen et al.,²¹ did not yield more than 15-29% of the desired acetal protected sugar. Preparing the dimethyl acetal of 2-naphthylaldehyde in 94% yield, according to a literature procedure,²² allowed the same conditions as the benzylidene protection, which gave a 86% yield of 5. Anomeric mixtures of the aryl acetal protected sugars were also possible to separate by SiO₂ column chromatography. To investigate the migration of the benzoyl group, it was installed at the free 3-OH using BzCl. Excess BzCl could easily be removed by quenching the reaction with (N,N-dimethylamino)propylamine (DMAPA) followed by acidic aqueous workup, as described by Andersen et al.²³ which produced pure 6, 7, 8, and 9 in 96%, 99%, 97%, and 90% yield, respectively, without the need for chromatography (Scheme 3). Opening the 4,6-acetal to yield the 4-OH 10, 11,





and 12 was first attempted on the 2-naphthylmethylidene to give the 2-naphthylmethyl ether (Nap) as an orthogonal 6-O protecting group, which can selectively be removed using CAN or DDQ or mildly by cat. HCl in hexafluoroisopropanol (HFIP).²⁴ Opening of the acetal using BF₃·OEt₂, instead of the more common TFA, was done to avoid acid catalyzed loss of the acetal. The initial results were promising with 84% yield on a 0.1 mmol scale, but upon scale-up the yield dropped to 54% with 35% of the corresponding 4,6-diol isolated as byproduct. Cooling the reaction to -78 °C and slowly reaching 5 °C over 3-4 h increased the yield of **10** to 95% on a 1 mmol scale (Scheme 3). The same procedure was used for the β -anomers. However, they seemed to perform slightly worse than the α -anomer and gave 11 in 80% yield. The opening of the 4,6-O-benzylidene giving a more permanent protecting group; i.e., the 6-OBn in 12 proceeded in 78% yield. A simple removal of the acetal protecting group, using *p*-toluenesulfonic acid ($TsOH \cdot H_2O$) in CH₂Cl₂/MeOH 1:1, gave the 4,6-diol 13 and 14 in 91% and 95% vield, respectively. These could in turn be selectively protected as the 6-OTBDPS ethers 15 and 16 in 99% and 97% yield, respectively (Scheme 3). The stage was now set for investigation of our proposed inversion/migration sequence (Table 1). The initial conditions reported in the literature^{16e} (entry 1) gave low yields, although TLC analysis showed mainly desired product, alongside a significant baseline spot. The conditions used for the acetyl migration in the literature^{17b} were also tested (entry 2). This, however, gave a very complex mixture as judged by TLC, and no HRMS traces of product could be observed. A reaction performed solely with pyridine as solvent (entry 3), gave a low yield as a mixture of the 3-OBz and 4-OBz. Analysis of the crude reaction mixture by NMR and HRMS suggested the presence of a pyridinium sugar. The substitution of carbohydrate triflates by pyridine is known under similar conditions.²⁵ To reduce the nucleophilicity of the base, pyridine was replaced by 2,6-lutidine (entry 4), but no conversion of the triflate was observed. Returning to pyridine but decreasing the volume of pyridine as to only have a large excess (entries 5-7), was attempted but still baseline material was detected. Switching pyridine to a smaller excess of DMAP caused the reaction mixture to become biphasic upon addition of H₂O. Therefore, a solvent change to THF in the inversion step was considered necessary. The reaction was performed at a lower temperature in the hope of avoiding the nucleophilic nature of DMAP (entry 8); this, however, did not improve the yield. Instead, a mixture of 3- and 4-OBz-protected

Table 1.	Intramolecular	Inversion/Migration	on of the Benzo	vl Protecting Gr	oup from C3 to C4 ^a
I ubic I.	munulioiccului	mit croion/ migraci	on or the Denizo	yr i roteeting or	

	10: X=H, Y=SPh, Pg 11: X=SPh, Y=H, Pg 15: X=SPh, Y=H, Pg 16: X=H, Y=SPh, Pg 12: X=SPh, Y=H, Pg	= Nap = Nap = TBDPS HO = TBDPS BZO = Bn	$ \begin{array}{c} DPg & i) \\ O \\ O \\ V_{3} \end{array} X \begin{array}{c} 0 \\ 0 \\ ii \end{array} $	Tf ₂ O, py C, CH ₂ Cl ₂ O, conditions	BzO OPg HO O X N _{3 Y}	17: X=H, Y=SPh, Pg = Nap 18: X=SPh, Y=H, Pg = Nap 19: X=SPh, Y=H, Pg = TBDPS 20: X=H, Y=SPh, Pg = TBDPS 21: X=SPh, Y=H, Pg = Bn	6
entry	starting material	solvent	temp (°C)	time (d)	additives	product	yield (%)
1	HO BzO N ₃ 11	CH ₂ Cl ₂ :Py 1:1	60	overnight	-	BzO ONap HO N ₃ SPh N ₃ 18	41
2	HO BZO N ₃ SPh	MeCN	reflux	3 h	-	BZO OTBDPS HO O 20 N ₃ SPh	no product
3	HO BZO N ₃ SPh	Py	60	overnight	-	BzO ONap HO Na 17 N ₃ SPh	45 ^b
4	10	2,6-lutidine ^c	100	3	-	17	no conversion
5	10	CH_2Cl_2	reflux	overnight	Ру	17	52 ^b
6	HO BZO N ₃ 15	CH_2Cl_2	reflux	overnight	Ру	BZO OTBDPS HO O SPh N ₃ 19	54
7	16	CH_2Cl_2	reflux	overnight	Ру	20	56
8	16	THF	40	7	DMAP	20	45 ^b
9	10	THF	40	1.5	Ру	17	46
10	11	THF	40	1.5	Ру	18	58
11	15	THF	40	1.5	Ру	19	57
12	16	THF	40	1.5	Ру	20	54
13	HO BZO N ₃ 12	THF	40	1.5	Ру	BzO OBn HO O SPh N ₃ 21	74

^{*a*}Standard conditions: (i) 2 equiv of Tf_2O , 10 equiv of Py, concentration: 0.2 M. (ii) Conditions described in the table; for further details, see the Supporting Information. ^{*b*}A mixture of the 3-OBz and 4-OBz products was obtained. ^{*c*}2,6-lutidine used instead of Py in (i).

GalN₃ derivatives, along with HRMS traces of the DMAP substituted sugar, was obtained. Returning once again to pyridine, at lower temperature (40 °C) in THF (entry 9–13), generally increased yields, but reaction times were extended from overnight to 1.5 d. These results suggested that a nucleophilic catalyst was necessary when the intramolecular substitution has to take place with the less nucleophilic benzoyl group, as suggested by the pK_a of the corresponding acids, i.e., 5.03, 4.76, and 4.2 for the pivalic acid, acetic acid, and benzoic acid, respectively. The lack of conversion when using the non-nucleophilic 2,6-lutidine hinted toward a mechanism (Scheme 4), wherein the pyridine assists in forming the tetrahedral





intermediate, which then undergoes hydrolysis. The hydrolysis forms the axial benzoyl ester, as the equatorial alcohol is the better leaving group.²⁶ One application for these building blocks would be to use them directly as acceptors in glycosylations. However, the axial electronically deactivating benzoyl ester along with the electron-withdrawing azide at C-2 could prove a

с

challenge. The 6-OTBDPS, in 19 and 20, further adds to make this a very bulky glycosyl acceptor. We therefore set out to perform representative glycosylations resembling the terminal disaccharide of the teichoic acid from S. pneumonia.²⁷ As donors we therefore used the known perbenzylated GalN₃ trichloracetimidate, 22,²⁸ along with the 6-OTBDPS version, 23,²⁵ which could of course also be synthesized from our building blocks. The two donors could be α -selectively coupled with both acceptors, 17 and 20, following literature procedure for similar donor systems,²⁸ but unoptimized for these more challenging acceptors. The glycosylations were performed at room temperature to achieve high α -selectivity with catalytic TfOH and 1.2 equiv of acceptor. Coupling donor 22 and 23 with acceptor 20 gave only the α -coupled dissacharide 24 and 25 in 47% and 36% yield, respectively (Scheme 5). Substantial recovery of the acceptors, 31% and 50%, was observed. Glycosylations using the same conditions with acceptor 17 and donor 22 and 23 also yielded only α -coupled product 26 and 27 in 41% and 46%, respectively (Scheme 5). Again, recovered acceptor was observed, this time in 10% and 30% yield, respectively. We only achieved modest yields using these unoptimized conditions, but compared to recent literature with similar donors and less deactivated acceptors these yields and selectivities were respectable.^{28,30}

In conclusion, we have improved the synthesis of fully orthogonal protected D-glucosamine derivatives to give 47–59% overall yield in seven or eight steps starting from inexpensive Dglucosamine hydrochloride. A major advantage is the possibility

Scheme 5. Glycosylations Using 17 and 20 as Acceptors



to purify the products by crystallizations at several stages, and hence, the procedure is scalable. Furthermore, access to the corresponding D-galactosamine derivatives was achieved via an intramolecular migration/inversion using a benzoyl ester as the migrating group, ensuring orthogonality and sufficient stability for complex oligosaccharide synthesis. Both are key building blocks in oligosaccharide synthesis, and we have shown how they can be used directly as acceptors in glycosylations with α selectivity.

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b02241.

Crystal structure of the α -anomer of 2 (CCDC no. 1477833) (CIF)

Experimental procedures, characterization data, crystallographic information, and NMR spectra (PDF)

AUTHOR INFORMATION

Corresponding Author

*E-mail: cmp@chem.ku.dk.

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENTS

We thank CHEM, UCPH, for funding and Rikke Munch Gelardi, CHEM, UCPH, for solving the X-ray crystallographic data.

REFERENCES

(1) Dube, D. H.; Champasa, K.; Wang, B. Chem. Commun. 2011, 47, 87–101.

(2) (a) Adibekian, A.; Stallforth, P.; Hecht, M.-L.; Werz, D. B.; Gagneux, P.; Seeberger, P. H. *Chem. Sci.* **2011**, *2*, 337–344. (b) Werz, D. B.; Ranzinger, R.; Herget, S.; Adibekian, A.; von der Lieth, C.-W.; Seeberger, P. H. *ACS Chem. Biol.* **2007**, *2*, 685–691.

(3) Moran, A. P.; Holst, O.; Brennan, P. J.; von Itzstein, M. Part I: Microbial Glycolipids, Glycoproteins and Glycopolymers. In *Microbial Glycobiology: Structures, Relevance and Applications*; Academic Press: London, 2009; pp 3–231.

(4) Lemieux, R. U.; Ratcliffe, R. M. Can. J. Chem. 1979, 57, 1244-1251.

(5) Mirabella, S.; Cardona, F.; Goti, A. Org. Biomol. Chem. 2016, 14, 5186–5204.

(6) Enugala, R.; Carvalho, L. C. R.; Dias Pires, M. J.; Marques, M. M. B. *Chem. - Asian J.* **2012**, *7*, 2482–2501.

(7) Codée, J. D. C.; Litjens, R. E. J. N.; van den Bos, L. J.; Overkleeft, H. S.; van der Marel, G. A. *Chem. Soc. Rev.* **2005**, *34*, 769–782.

(8) (a) Pozsgay, V. J. Org. Chem. **1999**, 64, 7277–7280. (b) Pozsgay, V. J. Carbohydr. Chem. **2001**, 20, 659–665. (c) Emmadi, M.; Kulkarni, S. S. J. Org. Chem. **2011**, 76, 4703–4709. (d) Emmadi, M.; Kulkarni, S. S. Org. Biomol. Chem. **2013**, 11, 4825–4830.

(9) Pedretti, V.; Veyrières, A.; Sinaÿ, P. *Tetrahedron* **1990**, *46*, 77–88. (10) A price comparison in February 2016 showed galactosamine hydrochloride to be 27–49 times more expensive.

(11) (a) Lattrell, R.; Lohaus, G. *Justus Liebigs Ann. Chem.* **1974**, 1974, 901–920. (b) Albert, R.; Dax, K.; Link, R. W.; Stütz, A. E. *Carbohydr. Res.* **1983**, *118*, C5–C6.

(12) Hederos, M.; Konradsson, P. J. Carbohydr. Chem. 2005, 24, 297–320.

(13) Gross, P. H.; Du Bois, F.; Jeanloz, R. W. Carbohydr. Res. 1967, 4, 244–248.

(14) (a) Nashed, M. A.; El-Sokkary, R. I.; Rateb, L. *Carbohydr. Res.* **1984**, *131*, 47–52. (b) McGeary, R. P.; Wright, K.; Toth, I. J. Org. Chem. **2001**, *66*, 5102–5105.

(15) Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A. Org. Lett. **2014**, *16*, 4838–4841. Hale, K. J.; Hough, L.; Manaviazar, S.; Calabrese, A. Org. Lett. **2015**, *17*, 1738–1741.

(16) (a) Lay, L.; Nicotra, F.; Panza, L.; Russo, G. *Helv. Chim. Acta* **1994**, 77, 509–514. (b) Belot, F.; Jacquinet, J.-C. *Carbohydr. Res.* **1996**, 290, 79–86. (c) Rochepeau-Jobron, L.; Jacquinet, J.-C. *Carbohydr. Res.* **1997**, 305, 181–191. (d) Cai, Y.; Ling, C.-C.; Bundle, D. R. *J. Org. Chem.* **2009**, 74, 580–589. (e) Feng, J.; Ling, C.-C. *Carbohydr. Res.* **2010**, 345, 2450–2457. (f) Rasmussen, M. R.; Marqvorsen, M. H. S.; Kristensen, S. K.; Jensen, H. H. *J. Org. Chem.* **2014**, 79, 11011–11019.

(17) (a) Takeda, Y.; Horito, S. Carbohydr. Res. 2005, 340, 211–220.
(b) Behera, A.; Emmadi, M.; Kulkarni, S. S. RSC Adv. 2014, 4, 58573–58580.

(18) (a) Binkley, R. W.; Sivik, M. R. J. Org. Chem. **1986**, 51, 2619–2621. (b) Binkley, R. W.; Abdulaziz, M. A. J. Org. Chem. **1987**, 52, 4713–

4717. (c) Binkley, R. W. J. Org. Chem. 1991, 56, 3892-3896.

(19) Yan, R.-B.; Yang, F.; Wu, Y.; Zhang, L.-H.; Ye, X.-S. *Tetrahedron Lett.* **2005**, *46*, 8993–8995.

(20) Gammon, D. W.; Steenkamp, D. J.; Mavumengwana, V.; Marakalala, M. J.; Mudzunga, T. T.; Hunter, R.; Munyololo, M. *Bioorg. Med. Chem.* **2010**, *18*, 2501–2514.

(21) Chen, C.-T.; Weng, S.-S.; Kao, J.-Q.; Lin, C.-C.; Jan, M.-D. Org. Lett. 2005, 7, 3343–3346.

(22) Szabó, Z. B.; Borbás, A.; Bajza, I.; Lipták, A. Tetrahedron: Asymmetry 2005, 16, 83–95.

(23) Andersen, S. M.; Heuckendorff, M.; Jensen, H. H. Org. Lett. 2015, 17, 944–947.

(24) Volbeda, A. G.; Kistemaker, H. A. V.; Overkleeft, H. S.; van der Marel, G. A.; Filippov, D. V.; Codée, J. D. C. *J. Org. Chem.* **2015**, *80*, 8796–8806.

(25) El Nemr, A.; Tsuchiya, T. Carbohydr. Res. 2001, 330, 205-214.

(26) Pedersen, C. M.; Olsen, J.; Brka, A. B.; Bols, M. Chem. - Eur. J. 2011, 17, 7080–7086.

(27) Gisch, N.; Kohler, T.; Ulmer, A. J.; Müthing, J.; Pribyl, T.; Fischer, K.; Lindner, B.; Hammerschmidt, S.; Zähringer, U. J. Biol. Chem. **2013**, 288, 15654–15667.

(28) Kalikanda, J.; Li, Z. J. Org. Chem. 2011, 76, 5207-5218.

(29) Dere, R. T.; Kumar, A.; Kumar, V.; Zhu, X.; Schmidt, R. R. J. Org. Chem. 2011, 76, 7539–7545.

(30) van der Es, D.; Groenia, N. A.; Laverde, D.; Overkleeft, H. S.; Huebner, J.; van der Marel, G.; Codée, J. D. C. *Bioorg. Med. Chem.* **2016**, *24*, 3893–3907.