Published in final edited form as:

Angew Chem Int Ed Engl. 2010 April 12; 49(17): 3049–3052. doi:10.1002/anie.200907178.

Efficient, Highly Stereoselective Synthesis of α -Keto-deoxy-D-glycero-D-galacto-nonulosonic Acid (KDN) Glycosides by Means of the 4,5-O-Carbonate Protecting Group

David Crich^{*} and Chandrasekhar Navuluri

Department of Chemistry, Wayne State University, 5101 Cass Avenue, Detroit, MI 48202, USA

Abstract

A 1-adamantyl thioglycoside derivative of KDN, derived from *N*-acetylneuraminic acid, carrying a 3,4-*O*-carbonate protecting group is a highly efficient and α -selective KDN donor on activation with NIS and TfOH in dichloromethane and acetonitrile at -78°C . Glycosylations conducted with this protecting group do not suffer from competing glycal formation. Seven examples are given, including the use of galactose 3- and 6-hydroxy groups.

Keywords

Glycosylation; Sialic Acid; Carbonate; Thioglycoside; Stereoselectivity

2-Keto-deoxy-D-glycero-D-galacto-nonulosonic acid (KDN) is a member of the sialic acid family of carbohydrates that are commonly found at the non-reducing terminus of cell surface glycans and in the form of homopolymers.[1] KDN and its glycosides have been long known in marine organisms and have more recently been detected in humans thanks to improved analytical techniques opening the way to potential applications as markers of disease states.[2] The minute quantities of these materials available by isolation, and their microheterogeneous nature, points to a strong need for efficient, versatile methods for the synthesis of homogeneous substances by enzymatic[3] or, as described here, chemical methods.

The chemical synthesis of KDN glycosides presents many of the same problems as that of the neuraminic acid glycosides, but has been much less extensively investigated.[4] With this in mind, and building on the recent successes of the Takahashi,[5] De Meo,[6] and our[7] laboratories on the use of 4-*O*,5-*N*-oxazolidinone-protected neuraminic acid donors, [8,9] we have prepared a novel 4,5-*O*-carbonyl-protected KDN donor and report here on its application in highly efficient and selective α -glycosylations.

We began with a modification of the Zbiral synthesis of KDN from peracetyl *N*-acetylneuraminic acid methyl ester,[10] which on nitrosylation with nitrosyl tetrafluoroborate gave the *N*-nitrosyl-*N*-acetyl neuraminic acid derivative **1** essentially quantitatively. Exposure to sodium isopropoxide and then acetic acid gave the KDN

^{*}Prof. Dr. D. Crich, Centre de Recherche de Gif, Institut de Chimie des, Substances Naturelles, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette, France, Fax: (+33) 1 69 07 77 52 dcrich@icsn.cnrs-gif.fr.

Dedicated to Professor Jan Roček

Supporting information for this article is available on the WWW under <http://www.angewandte.org> or from the author.

derivative **2** after an ozonolytic[11] work-up in 51% yield (Scheme 1). Conversion to the adamantanyl thioglycoside, selected because of the anticipated ease of activation at $-78\text{ }^{\circ}\text{C}$, [7b,c] was achieved under standard conditions and gave a separable mixture of the two anomers **3** in excellent yield. Saponification of each anomer individually gave the corresponding pentaols that were immediately protected as the 8,9-*O*-acetonides **4**. The optimum conditions found for the installation of the 4-*O*-carbonate group involved reaction with 4-nitrophenyl carbonate and Hunig's base, and gave **5** in high yield for both anomers. Cleavage of the acetonide with HCl in THF followed by peracetylation then gave the desired donors **6** (Scheme 1).

With two anomeric donors in hand we proceeded to examine their coupling reactions with a variety of acceptor alcohols, with activation by *N*-iodosuccinimide (NIS) and trifluoromethanesulfonic acid (TfOH) in a mixture of acetonitrile and dichloromethane at $-78\text{ }^{\circ}\text{C}$ leading to the results depicted in Table 1.

The results laid out in Table 1, entries 1–7 show uniformly high yield and excellent α -selectivity for reactions conducted with activation by the NIS/TfOH combination in a 2:1 dichloromethane acetonitrile mixture.[12] Comparison of entries 1 and 2 of Table 1 reveals that neither the efficiency nor the anomeric selectivity is dependent on the configuration of the donor and consequently all subsequent work was conducted with the more abundant β -isomer. Table 1, entry 3 illustrates the successful application of this chemistry to a tertiary alcohol, while entries 4 and 5 demonstrate applicability to the important galactopyranose 6-OH in the presence of two different protecting group arrays. Table 1, entries 6 and 7 are directed at the glycosylation of the galactopyranose 3-OH, in the presence of the 4-OH and with the 4-OH protected in the form of a benzyl ether. Comparison of Table 1, entries 5 and 8 reveals that while the selectivity remains high in the absence of acetonitrile, the presence of this solvent is certainly advantageous. Finally, entries 9 and 10 of Table 1 indicate that moderate selectivities are possible with other means of activation, notably the combinations of diphenyl sulfoxide and BSP with TiF_2O . [13] Interestingly, the coupling reactions illustrated in Table 1 appeared to be devoid of the usual type of byproduct in sialidation chemistry; namely the elimination product. The synthesis of an authentic sample of this glycal (Scheme 2, **19**), by elimination of the glycosyl sulfoxide, enabled to confirm this observation. Indeed, inspection of the crude reaction mixtures of the examples from the experiments presented in Table 1 reveals the major byproduct to be the hemiacetal resulting from hydrolysis of the donor.

To probe the influence of the cyclic carbonate protecting group on the glycosylation reactions a smaller series of coupling reactions was also conducted with the peracetylated donors **3** as set out in Table 2. With primary acceptors these couplings gave good albeit reduced α -selectivities, but suffered from considerably lower yields owing to the formation of the glycal **20** as a significant byproduct in every case. However, with the one secondary acceptor assayed both the yield and selectivity were considerably reduced from those observed in the carbonate series.

Finally, cleavage of the carbonate group along with that of the acetate esters was achieved in essentially quantitative yield under standard Zemplen deacetylation conditions (Table 3).

Clearly, as with the oxazolidinone and *N*-acetyl oxazolidinone groups in the neuraminic acid series, the 4,5-*O*-carbonate protecting group confers distinct advantages on the KDN donors **6a** and **6b**. These advantages include excellent α -selectivity and the virtual absence of glycal formation. While the underlying reasons for these improved properties are unclear at present and are the subject of ongoing investigations, we suggest the increased dipole moment of the cyclic carbonate group, with respect to two individual esters, renders it more electron-

withdrawing and likely stabilizes any intermediate adducts with acetonitrile (Fig. 1).[14] In this manner, the transition state for displacement of the acetonitrile by the acceptor alcohol will be tighter, that is to say, will have greater S_N2 character.[15] Stabilizing the acetonitrile adduct will also have the effect of reducing the overall positive charge on the anomeric carbon in the course of the glycosylation reaction and so of limiting the elimination reaction.

Experimental Section

Standard Protocol for Glycosylation with Donor 6 α or 6 β

A mixture of donor (0.1 mmol), acceptor (0.11 mmol), and powdered AW-300 molecular sieves (2 g/mmol of donor) dissolved in CH₂Cl₂/CH₃CN (2:1, 2 mL) was stirred for 2 h at room temperature then was cooled to -78 °C and was treated with NIS (0.12 mmol), and one drop of TfOH (2–3 μ L, 0.02 mmol). The resulting mixture was stirred for 1 h, at -78 °C, then was quenched by addition of diisopropylethylamine (18 μ L, 0.1 mmol), and warmed to room temperature. The molecular sieves were filtered off and the organic layer was washed with 20% aqueous Na₂S₂O₃, brine, dried over Na₂SO₄ and concentrated. The crude mixture was purified by flash chromatography eluting with ethyl acetate/hexanes.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

We thank the NIH (GM62160) for partial support of this work.

References

1. a) Varki A. *Glycobiol.* 1993; 3:97–130. b) Schauer R. *Adv. Carbohydr. Chem. Biochem.* 1982; 40:131–234. [PubMed: 6762816]
2. a) Inoue S, Kitajima J. *Glycoconj. J.* 2006; 23:277–290. [PubMed: 16897172] b) Inoue S, Poongodi GL, Suresh N, Chang T, Inoue Y. *Glycoconj. J.* 2006; 23:401–410. [PubMed: 16897181]
3. a) Kajihara Y, Akai S, Nakagawa T, Sato R, Ebata T, Kodama H, Sato K-i. *Carbohydr. Res.* 1999; 315:137–141. [PubMed: 10385976] b) Blixt O, Paulson JC. *Adv. Synth. Catal.* 2006; 345:687–690.
4. a) Chen C-C, Ress D, Linhardt RJ. *ACS Symposium Series.* 2005; 896:53–80. b) Boons, G-J.; Demchenko, AV. *Carbohydrate-Based Drug Discovery.* Wong, C-H., editor. Vol. 1. Weinheim: WILEY-VCH; 2003. p. 55–102. c) Kiso, M.; Ishida, H.; Ito, H. *Carbohydrates in Chemistry and Biology.* Ernst, B.; Hart, GW.; Sinaÿ, P., editors. Vol. 1. Weinheim: WILEY-VCH; 2000. p. 345–365. d) Ando H. *Trends Glycosci. Glycotech.* 2008; 20:141–158.
5. a) Tanaka H, Nishiura Y, Takahashi T. *J. Am. Chem. Soc.* 2006; 128:7124–7125. [PubMed: 16734441] b) Tanaka H, Nishiura Y, Takahashi T. *J. Org. Chem.* 2009; 74:4383–4386. [PubMed: 19413275]
6. a) Farris MD, De Meo C. *Tetrahedron Lett.* 2007; 48:1225–1227. b) De Meo C, Farris M, Ginder N, Gulley B, Priyadarshani U, Woods M. *Eur. J. Org. Chem.* 2008; 3673–3677.
7. a) Crich D, Li W. *J. Org. Chem.* 2007; 72:2387–2391. [PubMed: 17338570] b) Crich D, Li W. *J. Org. Chem.* 2007; 72:7794–7797. [PubMed: 17824651] c) Crich D, Wu B. *Org. Lett.* 2008; 10:4033–4035. [PubMed: 18715011]
8. For subsequent applications of this protecting system see a) Liang F-F, Chen L, Xing G-W. *Synlett.* 2009:425–428. b) Hamashina S, Sato K-I, Ito Y, Yamaguchi Y. *Eur. J. Org. Chem.* 2009:4215–4220.
9. For reviews on the effect of cyclic protecting groups in carbohydrate chemistry see: a) Litjens REJN, van den Bos LJ, Codée JDC, Overkleeft HS, van der Marel G. *Carbohydr. Res.* 2007; 342:419–429. [PubMed: 17007825] b) Imamura A, Ando H, Ishida H, Kiso M. *Heterocycles.* 2008; 76:883–908.

10. Schreiner E, Zbiral E. Liebigs Ann. Chem. 1990:581–586.
11. The ozonolytic work up, which cleaves the byproducts arising from elimination rather than substitution of the *N*-acetyl group, greatly facilitates isolation of **2**.
12. Anomeric stereochemistry was determined by measure of the C1-H3ax $^3J_{CH}$ coupling constants:
(a) Hori H, Nakajima T, Nishida Y, Ohru H, Meguro H. Tetrahedron Lett. 1988; 29:6317–6320.
(b) Prytulla S, Lauterwein J, Klessinger M, Thiem J. Carbohydr. Res. 1991; 215:345–349.
13. For the activation of thiosialosides with sulfoxides and Tf₂O see: Crich D, Li W. Org. Lett. 2006; 8:959–962. [PubMed: 16494484]
14. For the acetonitrile effect in glycosylation see: a) Pougny J-R, Sinaÿ P. Tetrahedron Lett. 1976; 17:4073–4076. b) Schmidt RR, Rücker E. Tetrahedron Lett. 1980; 21:1421–1424. c) Murase T, Kameyama A, Kartha KPR, Ishida H, Kiso M, Hasegawa A. J. Carbohydr. Chem. 1989; 8:265–283. d) Ratcliffe AJ, Fraser-Reid B. J. Chem. Soc., Perkin Trans. 1990; 1:747–750. e) Braccini J, Derouet C, Esnault J, de Penhoat CH, Mallet J-M, Michon V, Sinaÿ P. Carbohydr. Res. 1993; 246:23–41. f) Schmidt RR, Behrendt M, Toepfer A. Synlett. 1990:694–696.
15. In support of this hypothesis we recall the advantages of a 3,4-*O*-carbonate group in mannopyranosyl donors and the demonstrated stabilizing effect exerted on the intermediate mannosyl triflates. a) Crich D, Vinod AU, Picione J. J. Org. Chem. 2003; 68:8453–8458. [PubMed: 14575470] b) Crich D, Jayalath P. J. Org. Chem. 2005; 70:7252–7259. [PubMed: 16122245]

Figure 1.
Hypothetical Intermediate Stabilized by the Presence of the Cyclic Carbonate

Donor synthesis. Ada = 1-adamantanyl, pTSA = *para*-toluenesulfonic acid.

Donor synthesis. Ada = 1-adamantanyl, pTSA = *para*-toluenesulfonic acid.

Scheme 2.

Synthesis of an authentic sample of glycal. *m*CPBA = *meta*-chloroperoxybenzoic acid.

Table 1

Glycosylation with Donors **6 α** and **6 β**

Entry	Donor	Acceptor	Product	Yield, %
1[a]	6α			86%, α
2[a]	6β			81%, α
3[a]	6β			86%, α
4[a]	6β			84%, α
5[a]	6β			82%, α

Entry	Donor	Acceptor	Product	Yield, %
6[a]	6 β			81%, α
7[a]	6 β			80%, α
8[b]	6 β			84%, 13
9[c]	6 β			78%, 9:
10[d]	6 β			73%, (7

[a] Reaction conducted in a 2:1 mixture of dichloromethane and acetonitrile at -78 °C with activation by NIS and TfOH.

[b] Reaction conducted in dichloromethane at -78 °C with activation by NIS and TfOH.

[c] Reaction conducted in a 2:1 mixture of dichloromethane and acetonitrile at -78 °C with activation by diphenyl sulfoxide and trifluoromethanesulfonic anhydride (Tf₂O).

[d] Reaction conducted in a 2:1 mixture of dichloromethane and acetonitrile at -78 °C with activation by 1-benzenesulfinyl piperidine (BSP) and Tf₂O.

Table 2

Glycosylation with Donors **3 α** and **3 β**

Donor, Acceptor	Product	Yield, α : β ratio	Yield of Glycal 20
3α, 10	 21	60%, 12:1	29%
3β, 10	 21	60%, 12:1	28%
3β, 7	 22	62%, 13:1	26%
3β, 11	 23	52% 1.6:1	34%

Table 3

Deprotection of Carbonate Protected Saccharides

$$\begin{array}{c}
 \text{R}^1-\text{O}-\text{C}(=\text{O})-\text{O}-\text{R}^2 \\
 \text{R}^1-\text{O}-\text{C}(=\text{O})-\text{O}-\text{R}^2 \\
 \text{R}^1-\text{O}-\text{C}(=\text{O})-\text{O}-\text{R}^2
 \end{array}
 \xrightarrow[\text{4. 90\%}]{\text{1. NaOMe, MeOH, 0\%}}
 \begin{array}{c}
 \text{R}^1-\text{O}-\text{C}(=\text{O})-\text{O}-\text{R}^2 \\
 \text{R}^1-\text{O}-\text{C}(=\text{O})-\text{O}-\text{R}^2 \\
 \text{R}^1-\text{O}-\text{C}(=\text{O})-\text{O}-\text{R}^2
 \end{array}$$

Substrate	Product
13	<p>24</p>
14	<p>25</p>
15	<p>26</p>
16	<p>27</p>
17	<p>28</p>
18	<p>29</p>