ELSEVIER

www.elsevier.nl/locate/carres

Carbohydrate Research 328 (2000) 445–448

CARBOHYDRATE RESEARCH

Rapid Communication

Rapid access to uronic acid-based mimetics of Kdn2en from D-glucurono-6,3-lactone

Pas Florio [a], Robin J. Thomson [a,b], Mark von Itzstein [a,b,*]

[a] *Department of Medicinal Chemistry, Monash University (Parkville Campus), 381 Royal Parade, Parkville, Vic. 3052, Australia*

[b] *Centre for Biomolecular Science and Drug Discovery, Griffith University (Gold Coast Campus), PMB 50 Gold Coast Mail Centre, Qld 9726, Australia*

Received 22 June 2000; accepted 26 July 2000

Abstract

A concise route to novel mimetics of Kdn2en, based on Δ^4-uronic acids, from D-glucurono-6,3-lactone is presented. Uronic acid-based mimetics in which an aliphatic ether (*O*-glycoside), a thioether (*S*-glycoside), or acetamide takes the place of the natural C-6 glycerol sidechain of the sialic acid were synthesized from the key intermediate, methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide. © 2000 Elsevier Science Ltd. All rights reserved.

Keywords: Δ^4-Uronic acids; Kdn2en mimetics; Thioglycoside

Glycoconjugates containing the deaminated sialic acid, 3-deoxy-D-*glycero*-D-*galacto*-non-2-ulopyranosonic acid (Kdn), are found in both vertebrates and invertebrates [1]. The expression of Kdn in some mammalian systems has been found to be developmentally regulated [2–4], while both free Kdn [5] and α-(2→8)-linked Kdn-polymers [3], have been found to represent oncodevelopmental antigens for certain cancers. Consequently the investigation of both the chemistry and biology of Kdn and Kdn-recognizing proteins is of great interest.

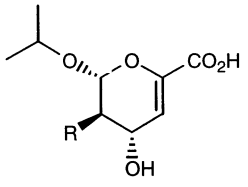
As part of our continuing research into sialic acid chemistry and biochemistry, we have been interested in developing a family of sialylmimetics that are readily accessible and that have the potential for relatively facile functional group modification. We have previously reported [6] the syntheses of the isopropyl Δ^4-pyranosiduronic acids **1** and **2** as mimetics of the 2,3-unsaturated sialic acids 2,6-anhydro-3-deoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (Kdn2en, **3**) and 5-acetamido-2,6-anhydro-3,5-dideoxy-D-*glycero*-D-*galacto*-non-2-enoic acid (Neu5Ac2en, **4**), respectively, where the C-6 glycerol side chain is replaced by a simple aliphatic ether. The interesting biological activities of these mimetics as sialidase inhibitors, and our desire to investigate the structural requirements of Kdn-recognising proteins has led us to pursue the synthesis of a range of mimetics related to **1**, including compounds with sulfur (thioglycosides) and nitrogen (glycosyl amides) at the anomeric position. Thioglycosides represent a

* Corresponding author. Tel.: +61-7-55948232; fax: +61-7-55948098.

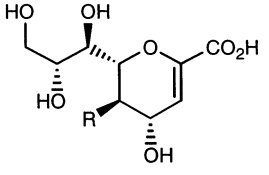
E-mail address: m.vonitzstein@mailbox.gu.edu.au (M. von Itzstein).

PII: S0008-6215(00)00228-7

potentially more metabolically stable glycoside [7], and depending upon the aglycon, may be more robust towards acid hydrolysis [8] than the corresponding *O*-glycosides. A glycosyl amine provides ready access to a range of relatively stable acylated species at the anomeric position. Both these alternate forms of the sialylmimetic **1** allow for the introduction of significant structural variation in the moiety that replaces the C-6 glycerol side chain of the sialic acids.

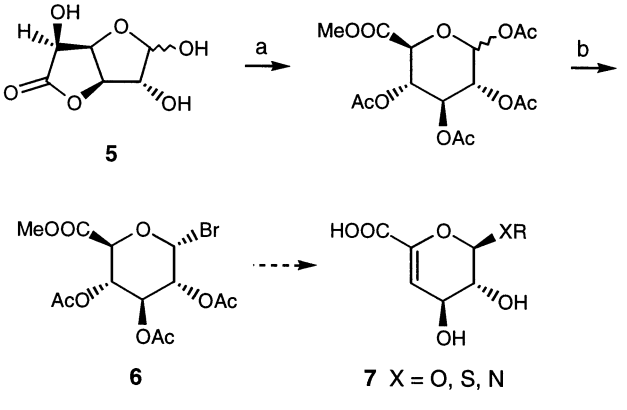


1 R = OH
2 R = NHAc



3 R = OH KDN2en
4 R = NHAc Neu5Ac2en

The previously reported syntheses [6] of the uronic acid derivatives **1** and **2** were via selective Pt(0) oxidation of the primary hydroxyl group of the corresponding isopropyl β-D-glucopyranosides. For the development of a wider range of Δ^4-uronic acid-based mimetics, including those containing a thioglycoside that are not readily accessible via the Pt(0) oxidative pathway [9], an approach in which the aglycon is introduced directly into the glucuronate was sought. This would avoid taking the aglycon through the oxidation and esterification steps of the previous pathway. A synthetic approach starting from D-glucurono-6,3-lactone (**5**), to compounds of the general structure **7**[1], fulfills this criterion. The key intermediate is the α-glucuronyl bromide, methyl 2,3,4-tri-*O*-acetyl-α-D-glucopyranosyluronate bromide (**6**) [10], which can be readily prepared in three steps from lactone **5** (Scheme 1).

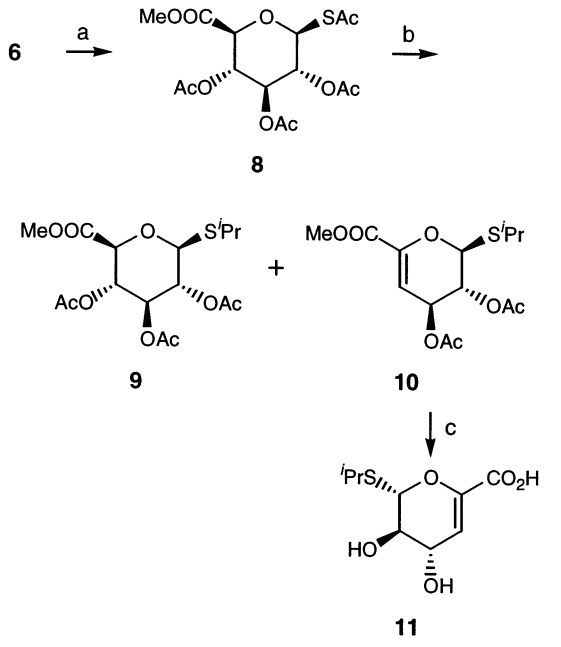


Scheme 1. Reagents and conditions: (a) i. NaOMe, MeOH, rt, 6 h; ii. Ac_2O, $HClO_4$, 4 °C–rt, overnight; (b) HBr, AcOH, 5 °C, overnight.

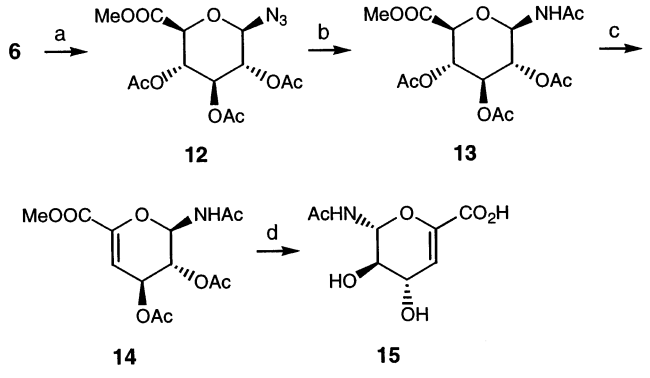
The glucuronyl bromide **6** is a convenient starting material for either *O*- or *S*-glucuronide synthesis. Accordingly, **1** could be synthesized in just three steps from the bromide **6** (glycosidation, β-elimination and saponification) in a moderate 38% yield, and in 27% overall yield from D-glucurono-6,3-lactone (**5**). Glycosidation of **6** with 2-propanol, carried out using Ag_2CO_3 as catalyst in the presence of 3 Å molecular sieves, proceeded to give the β anomer exclusively in excellent (85%) yield. While the overall yield for the synthesis of **1** was comparable to that obtained (30%) in nine steps from D-glucose via the previous pathway [6], the ease of conversion (two steps) to the target compound **1** after glycosidation (compared with six steps in the previous pathway) more than compensates for the moderate yield obtained.

The introduction of the aglycon moiety directly into the glucuronate is particularly attractive for the synthesis of 1-thiopyranosiduronates not readily accessible via the oxidative pathway. Alkyl 1-thioglucuronides have previously been prepared from the α-glucuronyl bromide **6** in moderate yields, either by reaction with a potassium thiolate formed in methanol at low temperature [11] or by synthesis and subsequent thermal rearrangement of *O*-alkyl *S*-glucuronyl xanthates [12]. In this work, we required a versatile entry point into a range of thioglucuronides and for this chose the glucuronyl β-thiolacetate derivative **8**. This compound has the potential for the thiol functionality to be selectively unmasked for coupling to a range of alkyl halides, as has previously been reported for a number of anomeric thioacetylated carbohydrates [13–15]. The β-thiolacetate derivative **8** could be prepared in 78% yield by reaction of the α-glucuronyl bromide **6** with KSAc in acetone (Scheme 2). Selective *S*-deacetylation of **8** using Et_2NH [13] and subsequent reaction with 2-bromopropane produced a mixture of the 1-thio-β-D-glucuronide **9** and the corresponding eliminated derivative **10** after

[1] All new compounds were fully characterized and gave satisfactory spectral and analytical data.



Scheme 2. Reagents and conditions: (a) KSAc, acetone, rt, overnight; (b) Et_2NH, DMF, $(CH_3)_2CHBr$, rt, 3 h; (c) NaOH, aq MeOH, rt, 2 h.



Scheme 3. Reagents and conditions: (a) NaN_3, TBAHS, CH_2Cl_2, satd aq $NaHCO_3$, rt, 2 h; (b) i. Pd/C, H_2, EtOAc, rt, overnight; ii. Ac_2O, pyr, rt, overnight; (c) DBU, $CHCl_3$, 65 °C, 2 h; (d) NaOH, aq MeOH, rt, 2 h.

workup. Purification by chromatography gave **9** and **10** (ca. 1:4 ratio) in moderate yield. Saponification of **10** produced the isopropyl Δ^4-pyranosiduronic acid **11**, which is the 1-thio-analogue of **1**.

In addition to the syntheses of the *O*- and *S*-glucuronides described above, this approach allows access to a series of *N*-acyl-(Δ^4-uronic acid)-1-yl amines such as **15**, which are mimetics of **3** in which an acylamino group replaces the C-6 glycerol side-chain. To this end, the β-glucuronyl azide **12** [16] was prepared in high yield (92%) by reaction of the α-glucuronyl bromide **6** with sodium azide under phase-transfer conditions [17] (Scheme 3). The azido group of **12** was readily reduced by hydrogenation in the presence of Pd/C. For the preparation of the acetylamino derivative **15**, acetylation of the resultant glucuronyl amine gave the *N*-acetyl-β-D-glucuronyl amine **13** in good yield (89% from **12**). Subsequent β-elimination using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) gave the desired *N*-acetyl-(Δ^4-uronate)-1-yl amine **14** (59% yield), which was then saponified to give **15**.

In conclusion, the approach described herein to Δ^4-uronic acids starting from D-glucurono-6,3-lactone **5**, provides ready access to a number of analogues of **1**. Such analogues will provide information on the substructural requirements of a range of Kdn-recognising proteins. For example, in a preliminary screen against a Kdn sialidase[2], compounds **1**, **11**, and **15** produced moderate inhibition (less than 30%, compared with 78% by Kdn2en **3**) of the hydrolysis of a Kdn glycoside. Synthesis of a range of analogues of **1** and their biological evaluation is continuing.

Acknowledgements

The authors thank Professor Yu-Teh Li (Tulane) for biological evaluation of the compounds prepared in this paper. We also thank Dr Milton Kiefel for his helpful suggestions during the course of this work, and gratefully acknowledge the financial support of the Australian Research Council, and the Australian Government for an Australian Postgraduate Award to PF.

References

[1] (a) R. Schauer, J.P. Kamerling, Chemistry, Biochemistry and Biology of Sialic Acids, in J. Montreuil, J.F.G. Vliegenthart, H. Schachter (Eds.), *Glycoproteins II*, Elsevier, Amsterdam, 1997, pp. 243–402. (b) A. Rosenberg (Ed.), *Biology of the Sialic Acids*, Plenum, New York, 1995.

[2] M.A. Campanero-Rhodes, D. Solís, E. Carrera, M.J. de la Cruz, T. Díaz-Mauriño, *Glycobiology*, 9 (1999) 527–532.

[2] Biological data provided by Professor Y.-T. Li (Tulane University). Full details of biological evaluation will be presented in a subsequent publication.

[3] B. Qu, M. Ziak, C. Zuber, J. Roth, *Proc. Natl. Acad. Sci. USA*, 93 (1996) 8995–8998.
[4] M. Ziak, B. Qu, X. Zuo, C. Zuber, A. Kanamori, K. Kitajima, S. Inoue, Y. Inoue, J. Roth, *Proc. Natl. Acad. Sci. USA*, 93 (1996) 2759–2763.
[5] S. Inoue, S-L. Lin, T. Chang, S-H. Wu, C-W. Yao, T-Y. Chu, F.A. Troy, Y. Inoue, *J. Biol. Chem.*, 273 (1998) 27199–27204.
[6] P. Florio, R.J. Thomson, A. Alafaci, S. Abo, M. von Itzstein, *Bioorg. Med. Chem. Lett.*, 9 (1999) 2065–2068.
[7] See for example: J.C. Wilson, M.J. Kiefel, D.I. Angus, M. von Itzstein, *Org. Lett.*, 1 (1999) 443–446.
[8] M.D. Saunders, T.E. Timell, *Carbohydr. Res.*, 6 (1968) 121–124.
[9] C.A. Marsh, Chemistry of D-Glucuronic Acid and its Glycosides, in G.J. Dutton (Ed.), *Glucuronic Acid. Free and Combined*, Academic Press, New York, 1966, pp. 3–136.
[10] G.N. Bollenback, J.W. Long, D.G. Benjamin, J.A. Lindquist, *J. Am. Chem. Soc.*, 77 (1955) 3310–3315.
[11] B. Helferich, D. Türk, F. Stoeber, *Chem. Ber.*, 89 (1956) 2220–2224.
[12] M. Sakata, M. Haga, S. Tejima, *Carbohydr. Res.*, 13 (1970) 379–390.
[13] S. Bennett, M. von Itzstein, M.J. Kiefel, *Carbohydr. Res.*, 259 (1994) 293–299.
[14] W.K.C. Park, S.J. Meunier, D. Zanini, R. Roy, *Carbohydr. Lett.*, 1 (1995) 179–184.
[15] H. Driguez, *Top. Curr. Chem.*, 187 (1997) 85–116, and references therein.
[16] (a) Y. Nitta, M. Kunanari, T. Kondo, *Yakugaku Zasshii*, 81 (1961) 1189–1194; *Chem. Abstr.*, 56 (1962) 6077I; (b) Z. Györgydeák, J. Thiem, *Carbohydr. Res.*, 268 (1995) 85–92.
[17] F.D. Tropper, F.O. Andersson, S. Braun, R. Roy, *Synthesis*, (1992) 618–620.