ORGANIC LETTERS

2009
Vol. 11, No. 20
4528−4531

Scalable Synthesis of L-Iduronic Acid Derivatives via Stereocontrolled Cyanohydrin Reaction for Synthesis of Heparin-Related Disaccharides

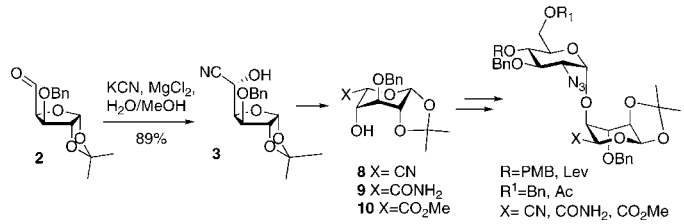
Steen Uldall Hansen,[*,†] Marek Baráth,[‡,†] Bader A. B. Salameh,[§,†] Robin G. Pritchard,[†] William T. Stimpson,[†] John M. Gardiner,[*,†] and Gordon C. Jayson[||]

School of Chemistry and Manchester Interdisciplinary Biocentre, The University of Manchester, 131 Princess Street, Manchester M1 7DN, U.K., and Cancer Research U.K. and University of Manchester Department of Medical Oncology, Christie Hospital, Wilmslow Road, Manchester M20 4BX, United Kingdom

gardiner@manchester.ac.uk.

Received July 27, 2009

ABSTRACT



L-Ido cyanohydrin 3 was prepared from diacetone-D-glucose in four steps and 76% overall yield and 90% de via cyanohydrin reaction of aldehyde 2. This process can be scaled to provide >1 mol of pure L-ido cyanohydrin 3. Cyanohydrin 3 was elaborated to 1,2-isopropylidine-protected L-ido nitrile (8), iduronic amide 9, and known carboxy ester 10. Coupling of 8 and 9 with glucosamine donors leads to new types (6-cyano and 6-carboxamide) of heparin-related disaccharides.

Heparin/heparan sulfates (HS) are linear glycosaminoglycan (GAG) polysaccharides, consisting of alternating *N*-glucosamino and hexuronic acid residues. They play critical roles through binding to a wide range of enzymes, growth factors, and other proteins regulating diverse biological effects, with sulfation at several possible sites being central to biological recognition and effect.[1] These include fibroblast growth factors (FGFs). The complex heterogeneity of natural HS oligosaccharide sources means that defining details of the roles and effects of specific HS sequences in relation to specific FGFs[2] (of which more than 20 are known) needs to be addressed by specific synthesis of a diversity of structurally defined HS sequences. Access to pure natural sequences and mimetics also offers prospects for providing designed sequences for specific therapeutic applications.[3] In natural HS sequences,

[†] The University of Manchester.
[‡] Current address: Institute of Chemistry, Centre of Glycomics, Slovak Academy of Sciences, Dubravska Cesta 9, 845 38 Bratislava, Slovak Republic.
[§] Current address: Chemistry Department, The Hashemite University, P.O. Box 150459, Zarqa 13115, Jordan.
[||] Cancer Research U.K. and University of Manchester Department of Medical Oncology.

(1) (a) Bishop, J.; Schuksz, M; Esko, J. D *Nature* **2007**, *446*, 1030–1037. (b) Tumova, S.; Woods, A.; Couchman, J. R. *Int. J. Biochem. Cell Biol.* **2000**, *32*, 269–288. (c) Sasisekharan, R.; Shriver, Z.; Venkataraman, G.; Narayanasami, U. *Nat. Rev. Cancer* **2002**, *2*, 521–528.

(2) (a) Pellegrini, L. *Curr. Opin. Struct. Biol.* **2001**, 629–634. (b) Hung, K. W.; Kurnar, T. K. S.; Kathir, K. M.; Xu, P.; Ni, F.; Ji, H. H. *Biochemistry* **2005**, *44*, 15787–15798. (c) Olsen, S. K.; Ibrahimi, O. A.; Raucci, A.; Zhang, F. *Proc. Natl. Acad. Sci.* **2004**, *101*, 935–941.

10.1021/ol901723m CCC: $40.75
Published on Web 09/18/2009

the uronic acid component is either D-glucuronic or L-iduronic acid, which differ only in their C5-configuration. Various biological studies indicate that sequences containing domains of L-iduronic components modulate effects on a number of FGFs. Synthetic access to disaccharide components of such oligosaccharides is thus of considerable interest, as they are key precursors to the synthesis of longer H/HS-like sequences.

Of the uronic acid components, L-iduronic acid provides a key challenge to effective synthesis, as this sugar is not readily available. Thus, efficient synthesis of L-iduronic acid derivatives is critical to provision of structurally defined saccharides for evaluation, and scalability is important for any future therapeutic potential.

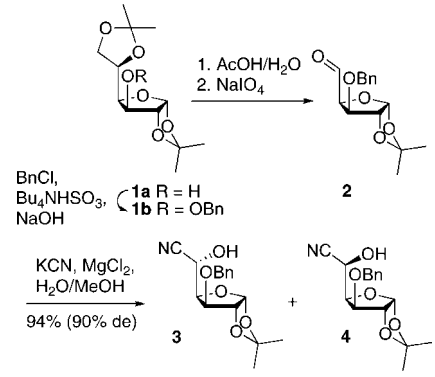
A number of previous syntheses of L-iduronic acid and derivatives have been described,[4] involving obtention of the C5 L-configuration by diastereomer separation[4a−c] or inversion of the C5 stereochemistry of D-sugar precursors,[4d−i] in some cases with subsequent oxidation of intermediate L-idose derivatives.[4j−l] Low temperature addition of tris(phenylthio)methyllithium (a carboxylate surrogate)[5] to **2** has also provided stereoselective introduction of the C5 stereocenter.[6] Oxidation of L-iditose components in oligosaccharides has also been employed.[7]

We report here our approach to stereocontrolled introduction of the key C5 L-ido strereochemistry, through reaction at ambient temperature, under air and without the need for anhydrous conditions, using inexpensive reagents. The key stereochemical step involves purification by crystallization and is thus designed to enable large-scale synthesis, facilitating access to larger amounts of various L-iduronate targets. Scalability underpins the potential for viable development of L-ido-containing heparan-related saccharides for clinical evaluations.

The cyanohydrin reaction has been used in carbohydrate homologation chemistry[8] (originally reported by Kiliani[8a]) and, while high yielding, proceeds with low diastereoselectivity in most cases, though its utility has been applied to synthesis of isotopically labeled sugars.[8b]

The diastereoselectivity of the cyanohydrin reaction of **2** has previously been reported to be poor (typically a 1:1 mixture).[9] While addition of appropriate dialkylhydrazone reagents has provided a stereoselective entry to the D-gluco cyanohydrin (**4**), in two steps from **2**,[10] there is no prior stereoselective access to **3**. We thus undertook an reinvestigation of the conditions for this cyanohydrin reaction of aldehyde **2**, with a view to developing a more diastereoselective and scalable process.

Scheme 1. Stereocontrolled Cyanohydrin Synthesis



We found the cyanohydrin reaction to be fast (complete in <30 min) with or without additives, but that diastereocontrol, as previously reported,[9] is very low at short reaction times. However, following the reaction over longer periods showed that, in reactions with added magnesium chloride, the de increases[11] and after 5 days affords 90% de in favor of the L-ido configuration (Scheme 1 and Table 1a). During this reaction increasing precipitation was observed. This outcome is rationalized as a combination of cyanohydrin epimer equilibration (slightly basic reaction conditions) and the preferential crystallization of the L-ido configuration diastereomer **3** from the reaction solution,[12] driving equilibrium toward the L-ido product. Pure **3** is then isolated in high yields after recrystallization.

Analysis of the effect of substrate concentration on the diastereoselectivity (using 1.1 equiv of $MgCl_2$ and KCN, 16 h time point, where optimum de = 82%), illustrates that the de is also related to a critical concentration limit, below which the de drops markedly (Table 1b).

(3) Cole, C.; Jayson, G. C. *Expert Opin. Biol. Ther.* **2008**, *8*, 351–362.

(4) (a) Rochepeau-Jobron, L; Jacquinet, J.-C. *Carbohydr. Res.* **1997**, *303*, 395–406. (b) Hinou, H.; Kuorsawa, H.; Matsuoka, K.; Terunuma, D.; Kuzuhara, H. *Tetrahedron Lett.* **1999**, *40*, 1501–1504. (c) Schell, P.; Orgueira, H. A.; Roehrig, S.; Seeberger, P. H. *Tetrahedron Lett.* **2001**, *42*, 3811–3814. (d) Orgueira, H. A.; Bartolozzi, A.; Schell, P.; Litjens, R. E. J. N.; Palmacci, E. R.; Seeberger, P. H. *Chem.—Eur. J.* **2003**, *9*, 140–169. (e) Ke, W.; Whitfield, D. M.; Gill, M.; Larocque, S.; Yu, S. H. *Tetrahedron Lett.* **2003**, *44*, 7767–7770. (f) Ojeda, R.; de Paz, J. L.; Martín-Lomas, M.; Lassaletta, J. M. *Synlett* **1999**, 1316–1318. (g) Jacquinet, J-C; Petitoi, M; Duchaussoy, P.; Lederman, I.; Choay, J.; Torri, G.; Sinay, P. *Carbohydr. Res.* **1984**, *130*, 221–241. (h) Yu, H. N.; Furukawa, J.-i.; Ikeda, T.; Wong, C. H. *Orglett* **2004**, *6*, 723–726. Via C6 bromo: (i) Blanc-Muesser, M.; Defaye, J.; Horton, D.; Tsai, J.-H. *Methods Carbohydr. Chem.* **1980**, *8*, 177–183. (j) Tatai, J.; Osztrovszky, G.; Kajtár-Peredy, M.; Fügedi, P. *Carbohydr. Res.* **2007**, *343*, 596–606. (k) Codee, J. D. C.; Stubba, B.; Schiattarella, M.; Overkleeft, H. S.; van Boeckel, C. A.; van Boom, J. H.; van der Marel, G. A. *J. Am. Chem. Soc.* **2005**, *127*, 3767–3773. (l) Davis, N. J.; Flitsch, S. L. *Tetrahedron Lett.* **1993**, *34*, 1181–1184.

(5) Seebach, D. *Angew. Chem., Int. Ed. Engl.* **1967**, *6*, 442–443.

(6) (a) Lohman, G. J. S.; Hunt, D. K.; Hogermeier, J. A; Seeberger, P. *J. Org. Chem.* **2003**, *68*, 7559–7561. (b) Gavard, O.; Hersant, Y. I.; Alais, J.; Duverger, V.; Dilhas, A.; Bascou, A.; Bonnaffé, D. *Eur. J. Org. Chem.* **2003**, 3603–3620. (c) Lubineau, A.; Gavard, O.; Alais, J.; Bonnaffé, D. *Tetrahedron Lett.* **2000**, *41*, 307–311.

(7) Polat, T.; Wong, C.-H. *J. Am. Chem. Soc.* **2007**, *127*, 12795–12800.

(8) (a) Kiliani, H. *Ber.* **1887**, *20*, 339. (b) Synder, J. R.; Serianni, A. S. *Carbohydr. Res.* **1987**, *163*, 169–188. (c) Synder, J. R.; Serianni, A. S. *J. Org. Chem.* **1986**, *51*, 2694–2702.

(9) (a) Wolfrom, M. L.; Thomas, G. H. S. *Methods Carbohydr. Chem.* **1963**, *2*, 32–4. (b) Shafizadeh, F.; Wolfrom, M. L. *J. Am. Chem. Soc.* **1955**, *77*, 2568–2569. (c) Hudson, C. S. *J. Am. Chem. Soc.* **1951**, *73*, 4498–4499. Idogluco system gave 1:1 ratio under all conditions: (d) Rauter, A.; Figueiredo, J.; Ismael, I.; Pais, M. *J. Carbohydr. Chem.* **1987**, *6*, 259–272.

(10) (a) Fernández, R.; Martín-Zamora, E.; Pareja, C.; Lassaletta, J. M. *J. Org. Chem.* **2001**, *66*, 5201–5207. (b) Fernández, R.; Martín-Zamora, E.; Pareja, C.; Lassaletta, J. M. *Tetrahedron Lett.* **1996**, *375*787–5790.

(11) A graphical representation is available in the Supporting Information.

(12) Solubilities in 1:1 MeOH/H_2O: **3**, 0.9 mg/mL, and **4**, 1.9 mg/mL.

Table 1. Effects of (a) Reaction Time and (b) Substrate Concentration on Diastereoselectivity of Cyanohydrin Reaction of **2**[a]

(a) reaction time[a]				(b) substrate concentration[b,c]		
entry	time/h	yield/%	de	*C*/M of 2	yield/%	de
1	0.3	97	9	0.5	93	83
2	1	94	28	0.27	94	82
3	2	94	69	0.17	91	75
4	4	96	74	0.06	96	36
5	16	94	82			
6	48	94	87			
7	120	93	90			

[a] All reactions using 1.1 equiv of KCN and $MgCl_2$. [b] 0.27 M of **2**. [c] Time = 16 h.

To determine the scope of additives in synthesis of **3**, evaluation of other metal salts and alternative magnesium salts ($MgSO_4$ and $MgBr_2$) was undertaken (Table 2). Several magnesium salts gave high diastereoselectivity, but magnesium chloride had the advantage of a superior overall yield, with the optimum combination of isolated yield and de obtained using 1.1 equiv of $MgCl_2$.

Table 2. Effects of Metal Salt on Diastereoselectivity of Cyanohydrin Reaction of **2**[a]

metal salt	equiv	yield (%)	de
none	n/a	64	33
$MgCl_2$	0.55	80	82
$MgCl_2$	1.1	94	87
$MgBr_2$	1.1	77	82
$MgSO_4$	1.1	76	83

[a] Reaction time = 48 h.

The L-ido configuration of **3** was unambiguously established by an X-ray structure determination (Figure 1).

The overall yield from diacetone-D-glucose (**1a**) to pure L-ido cyanohydrin **3** was 76% (four steps). Moreover, the residue in solution after recrystallization (a mixture of L-ido and D-gluco cyanohydrins (**3** and **4**)) could be re-equilibrated by stirring in 1:1 methanol−water with 0.1 equiv of potassium cyanide and magnesium chloride for 5 days, re-establishing 90% de of L-ido over D-gluco. The mild conditions allied with purification of **3** by crystallization enabled scale up to >1 mol scale. This offers significant process cost advantages over prior homologations of **2**.

L-Ido cyanohydrin **3** was elaborated to several different L-iduronic acid derivatives, as acceptors for glycosidic couplings. The 5-membered ring cyanohydrin **3** was first converted into the 1,2-isopropylidine-protected pyranose **8** using a modified version of methodology for the analogous carboxy ester system.[6a] Thus, removal of the isopropylidene from **3** by treatment with aqueous TFA afforded deprotected crystalline cyano sugar **5** in 84% yield (Scheme 2). The 1,2-isopropylidene was then reformed using excess 2-methoxypropene and CSA, conditions leading preferentially to the 1,2-isoproylidine-protected pyranoside ring system. The primary product was in fact **7**, which contains an open acetal attached to the 4-position. Product **7** was isolated in 62% yield, along with 18% of the desired *O*-4-deprotected pyranoside **8**. This reaction proceeds via tris-acetal-protected intermediate **6**,[14] which could be isolated when the reaction was run for 2 h at 0 °C.

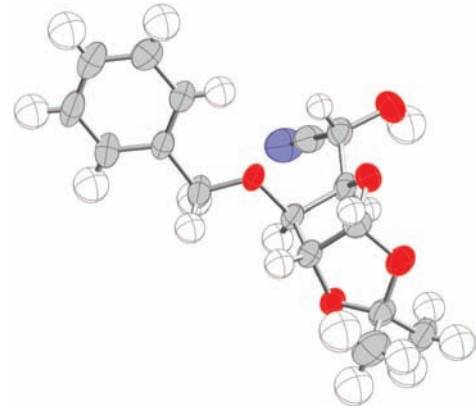
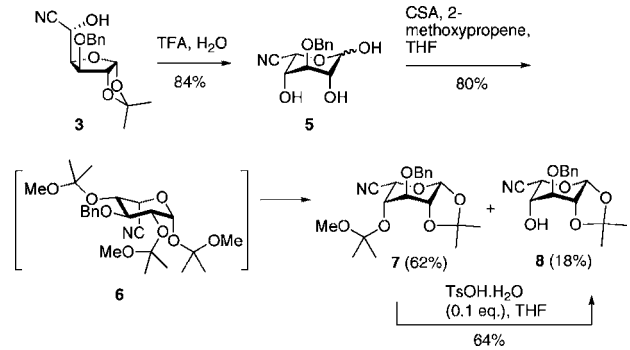


Figure 1. X-ray structure of L-ido configuration cyanohydrin **3**.[13]

Scheme 2. Conversion of L-Ido Cyanohydrin **3** into L-Ido Cyanopyranoside **8**[a]



[a] Note: *O*-4 deprotection of **7** also leads to 12% of starting furanoside **3**.

The *O*-4 acetal was selectively removed to convert **7** into **8**, using 0.1 equiv of *p*-TSA as under these conditions **8** is

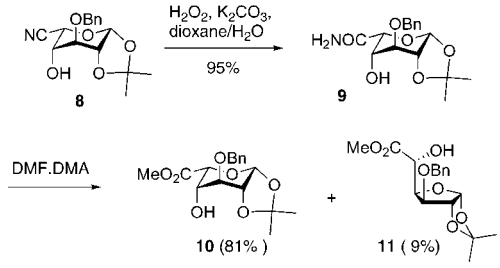
(13) Data deposited with the Cambridge Crystallographic Database and allocated the deposition no. CCDC 736690.

(14) NMR data indicate **6** is $4C_1$: J_{H2-H3} = 9.8 Hz for **6**, while H-4 in **7**, for example, shows only J < 5 Hz.

only slowly converted back to the furanose **3**, maximizing overall conversion of **5** to **8**.[15]

The nitrile **8** was converted into the (crystalline) L-ido amide **9** in excellent yield using hydrogen peroxide and potassium carbonate (Scheme 3). This primary amide was treated with dimethylformamide dimethyl acetal in dry methanol[16] to facilitate the direct conversion to the known methyl ester **10** in 81% yield (with concomitant formation of 9% of the furanoside **11**, separable from **10**.)

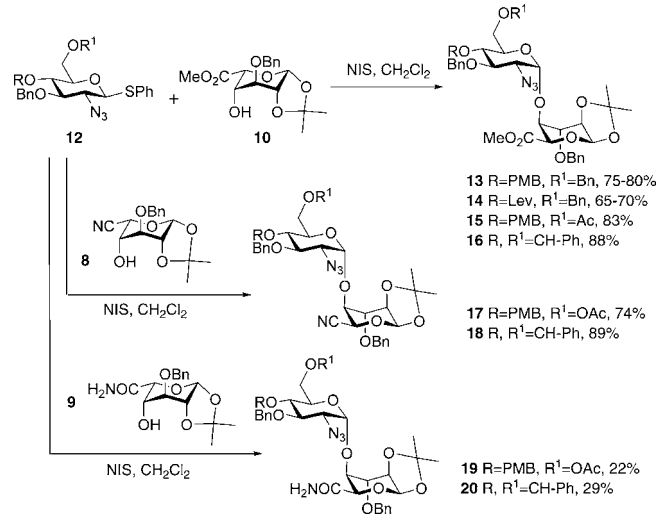
Scheme 3. Conversion of Idonitrile **8** to Iduronamide and Iduronic Ester Derivatives



The ester **10** is known to be an excellent acceptor for the synthesis of heparin-like disaccharides.[4d,k,17] In our hands, acceptor **10** was thus employed for synthesis of heparin-related disaccharide units **13**−**16**, providing good yields and selectivities against variations in nature of the *O*-4 and *O*-6 protecting groups of donor **12**,[4d,18] thereby affording useful intermediates with different alternatives to diverge to disaccharides for further homologation (Scheme 4).

The utility of 6-cyano- or 6-carboxamide-bearing acceptors (in general) is essentially unexplored in oligosaccharide synthesis. (Both cyano and amido L-ido intermediates **8** and **9** are novel.) We thus decided to evaluate coupling of these acceptors with the 2-azido gluco thioglycoside donors **12**, which had, as anticipated, performed well with the 6-carbomethoxy ido acceptor **10**. Thioglycoside donors **12** coupled with nitrile **8** to provide a high yield of the α-linked disaccharides **17** and **18**. The amide **9** proved to be a poorer acceptor, affording amide-containing α-linked disaccharides **19** and **20** but isolated in lower yields.[19] Notwithstanding lower yields using amide **9**, this establishes a route to novel 6-modified heparin disaccharides **13** and **15**−**20**. Yields for glycosylations of **8**−**10** using 4,6-*O*-benzylidine protected donor **12** [R = R^1 = CHPh] were very similar to those for donors having preinstalled differentiations of *O*-4/*O*-6. However, anomeric selectivities proved lower, with anomeric ratios (α/β) for **16** (7:2), **18** (3:2), and **20** (5:1).

Scheme 4. L-Ido Disaccharide Syntheses



In conclusion, a new scalable approach to the established valuable L-iduronic acid building block (**10**) for heparan sulfate syntheses has been developed. The route to the key precursor L-ido cyanohydrin **3** is highly stereoselective and efficient and offers significant advantages on a larger scale as it involves no column chromatography, proceeds at ambient temperature, has no requirement for anhydrous conditions, and utilizes very inexpensive reagents affording a highly crystalline product in a single step. In addition to conversion of cyanohydrin **3** to a known L-iduronic ester acceptor derivative **10**, novel nitrile and primary amide L-iduronic intermediates **8** and **9** were also prepared and evaluated as acceptors in glycoside couplings, yielding novel 6-cyano and 6-carboxamide L-ido disaccharide analogues. Further elaborations of **3** into various heparan-sulfate related oligosaccharides, and the uses of novel disaccharides, will be reported elsewhere.

Acknowledgment. The Holt Foundation, CRUK (C2075/A9106 and C2075/A5048), and MRC (G0601746) are thanked for project funding, and EPSRC is thanked for NMR instrumentation (GR/L52246).

Supporting Information Available: Experimental and copies of spectral data for compounds **3**, **7**−**10**, **13**, and **15**−**20**. This material is available free of charge via the Internet at http://pubs.acs.org.

OL901723M

(15) Increasing acid concentration in the first step (to effect in situ removal of the *O*-4 acetal of **7**) was ineffective.

(16) Anelli, P.; Brocchetta, M.; Palano, D.; Visigalli, M. *Tetrahedron Lett.* **1997**, *38*, 2367–2368.

(17) (a) Lohman, G.; Seeberger, P. *J. Org. Chem.* **2004**, *69*, 4081–4093. (b) Orgueira, H.; Bartolozzi, A.; Schell, P.; Seeberger, P. *Angew. Chem., Int. Ed.* **2002**, *41*, 2128–2131.

(18) **12** (R = PMB, R^1 = Ac): (a) Karst, N.; Islam, T.; Avci, F.; Linhardt, R. *Tetrahedron Lett.* **2004**, *45*, 6433–6437 **12** (R = R^1 = CHPh): (b) Martin-Lomas, M.; Flores-Mosquera, M.; Chiara, J. *Eur. J. Org. Chem.* **2000**, *154*, 7–1562. Codee, J. D. C.; Litjens, R.; den Heeten, R.; Overkleeft, H.; van Boom, J. H.; van der Marel, G. A *Orglett* **2003**, 1519–1522 **12** (R = PMB, R^1 = Bn): (c) Lindberg, J.; Ohberg, L.; Garegg, P. J.; Konradsson, P. *Tetrahedron Lett.* **2002**, *58*, 1387–1398.

(19) The same reactivity trends were evident using corresponding trichloroacetimidate 2-azido gluco donors.