

molecule", camphor, where  $\Delta\nu/\nu \approx 10^{-8}$  was achieved.<sup>[9]</sup> Even if more recent techniques could provide higher resolving power,<sup>[10]</sup> a full rotational analysis for the IR spectrum of camphor would be more difficult than for fluorooxirane, and the experiment itself does not provide a value for  $\Delta_{\text{pv}}E$ . The data calculated here are also sufficient for an estimate of the equilibrium constant for racemization<sup>[28]</sup> from Equations (9) and (10) where  $x$  and  $y$  explicitly show the deviation from unity of the prefactor and the exponential factor.

$$K_{\text{eq}}^{R,S} = \frac{q_R}{q_S} \exp(-\Delta_r H_0^0/RT) = \frac{q_{\text{vib},R} q_{\text{rot},R}}{q_{\text{vib},S} q_{\text{rot},S}} \exp(-\Delta_r H_0^0/RT) \quad (9)$$

$$\approx (1+x)(1+y) \quad (10)$$

Within the SHAA,<sup>[20,21]</sup> the equilibrium constant for racemization at 300 K is  $K_{\text{eq}}^{R,S} \approx 1 + 8.20 \times 10^{-16}$  with  $x \approx -1.01 \times 10^{-17}$  and  $y \approx 8.30 \times 10^{-16}$ . These values have been obtained with perturbation theory and high precision arithmetic (MAPLE V).<sup>[29]</sup> To our knowledge, this is the first time that these contributions have been explicitly estimated, although it should be understood that the harmonic approximation is not quite adequate.<sup>[28]</sup>

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- [1] J. H. van't Hoff in *La Chimie dans l'Espace* (Ed.: P. M. Bazendijk), Rotterdam, **1887**.
- [2] W. J. Hehre, L. Radom, P. von R. Schleyer, J. A. Pople, *Ab initio Molecular Orbital Theory*, Wiley, New York, **1986**.
- [3] M. Quack, *Angew. Chem.* **1989**, *101*, 588–604; *Angew. Chem. Int. Ed. Engl.* **1989**, *28*, 571–586.
- [4] M. Quack, *Chem. Phys. Lett.* **1986**, *132*, 147–153.
- [5] A. L. Barra, J. B. Robert, L. Wiesenfeld, *Europhys. Lett.* **1988**, *5*, 217–222.
- [6] A. Bauder, A. Beil, D. Luckhaus, F. Müller, M. Quack, *J. Chem. Phys.* **1997**, *106*, 7558–7570.
- [7] V. Letokhov, *Phys. Lett. A* **1975**, *53*, 275–276.
- [8] O. Kompanets, A. Kukudzhanov, V. Letokhov, L. Gervits, *Opt. Commun.* **1976**, *19*, 414–416.
- [9] E. Arimondo, P. Glorieux, T. Oka, *Opt. Commun.* **1977**, *23*, 369–372.
- [10] C. Daussey, T. Marrel, A. Amy-Klein, C. Nguyen, C. Bordé, C. Chardonnet, *Phys. Rev. Lett.* **1999**, *83*, 1554–1557.
- [11] R. Compton, unpublished work, cited in R. F. Service, *Science* **1999**, *286*, 1282–1283.
- [12] "Chemical Evolution: Physics of the Origin and Evolution of Life": A. Bakasov, T. K. Ha, M. Quack in *Proc. 4th Trieste Conf. 1995* (Eds.: J. Chela-Flores, F. Raulin), Kluwer, Netherlands, **1996**, pp. 287–296.
- [13] A. Bakasov, T. K. Ha, M. Quack, *J. Chem. Phys.* **1998**, *109*, 7263–7285.
- [14] R. A. Hegstrom, D. W. Rein, P. G. H. Sandars, *J. Chem. Phys.* **1980**, *73*, 2329–2341.
- [15] S. F. Mason, G. E. Tranter, *Mol. Phys.* **1984**, *53*, 1091–1111.
- [16] P. Lazzeretti, R. Zanasi, *Chem. Phys. Lett.* **1997**, *279*, 349–354.
- [17] A. Bakasov, M. Quack, *Chem. Phys. Lett.* **1999**, *303*, 547–557.
- [18] J. K. Laerdahl, P. Schwerdtfeger, *Phys. Rev. A* **1999**, *60*, 4439–4453.
- [19] R. Berger, M. Quack, *J. Chem. Phys.* **2000**, *112*, 3148–3158.
- [20] M. Quack, J. Stohner, *Phys. Rev. Lett.* **2000**, *84*, 3807–3810.
- [21] M. Quack, J. Stohner, *Z. Phys. Chem.* **2000**, *214*, 675–703.
- [22] J. Laerdahl, P. Schwerdtfeger, H. Quiney, *Phys. Rev. Lett.* **2000**, *84*, 3811–3814.
- [23] A. Beil, D. Luckhaus, R. Marquardt, M. Quack, *J. Chem. Soc. Faraday Discuss.* **1994**, *99*, 49–76.

- [24] H. Hollenstein, D. Luckhaus, J. Pochert, M. Quack, G. Seyfang, *Angew. Chem.* **1997**, *109*, 136–138; *Angew. Chem. Int. Ed. Engl.* **1997**, *36*, 140–143.
- [25] H. Gross, G. Grassi, M. Quack, *Chem. Eur. J.* **1998**, *4*, 441–448.
- [26] H. Sengstschnid, A. Bauder, D. Luckhaus, M. Quack, unpublished results.
- [27] M. Quack, *Nova Acta Leopoldina* **1999**, *81*, 137–173.
- [28] M. Quack, J. Stohner, *Chirality*, **2001**, in press.
- [29] B. Char, K. Geddes, G. Gonnet, B. Leong, M. Monagan, S. Watt, MAPLE V, Waterloo Maple Software, Waterloo, Ontario, **1990**.

## Synthesis of Conformationally Locked Carbohydrates: A Skew-Boat Conformation of L-Iduronic Acid Governs the Antithrombotic Activity of Heparin

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The conformational flexibility<sup>[1]</sup> of L-iduronic acid, a typical monosaccharide component of heparin, most probably explains its remarkable protein adaptability and resulting diverse biological activities.<sup>[2,3]</sup> The analysis of this feature has been the matter of a long controversy,<sup>[4]</sup> which not surprisingly originates from the complexity of the heparin primary structure. A major breakthrough in heparinology has been the identification,<sup>[5,6]</sup> followed by the total synthesis,<sup>[7,8]</sup> of a well-defined pentasaccharide sequence inserted into the heparin chain, which specifically binds to antithrombin (AT)—a physiological inhibitor of activated blood coagulation factors—and amplifies its action. This is the molecular origin of the anticoagulant and antithrombotic activities of heparin.

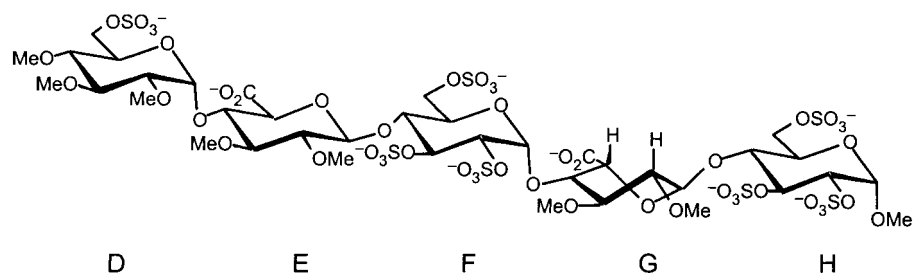
The <sup>1</sup>H NMR spectroscopic data of this original synthetic pentasaccharide in aqueous solution were easily extracted and

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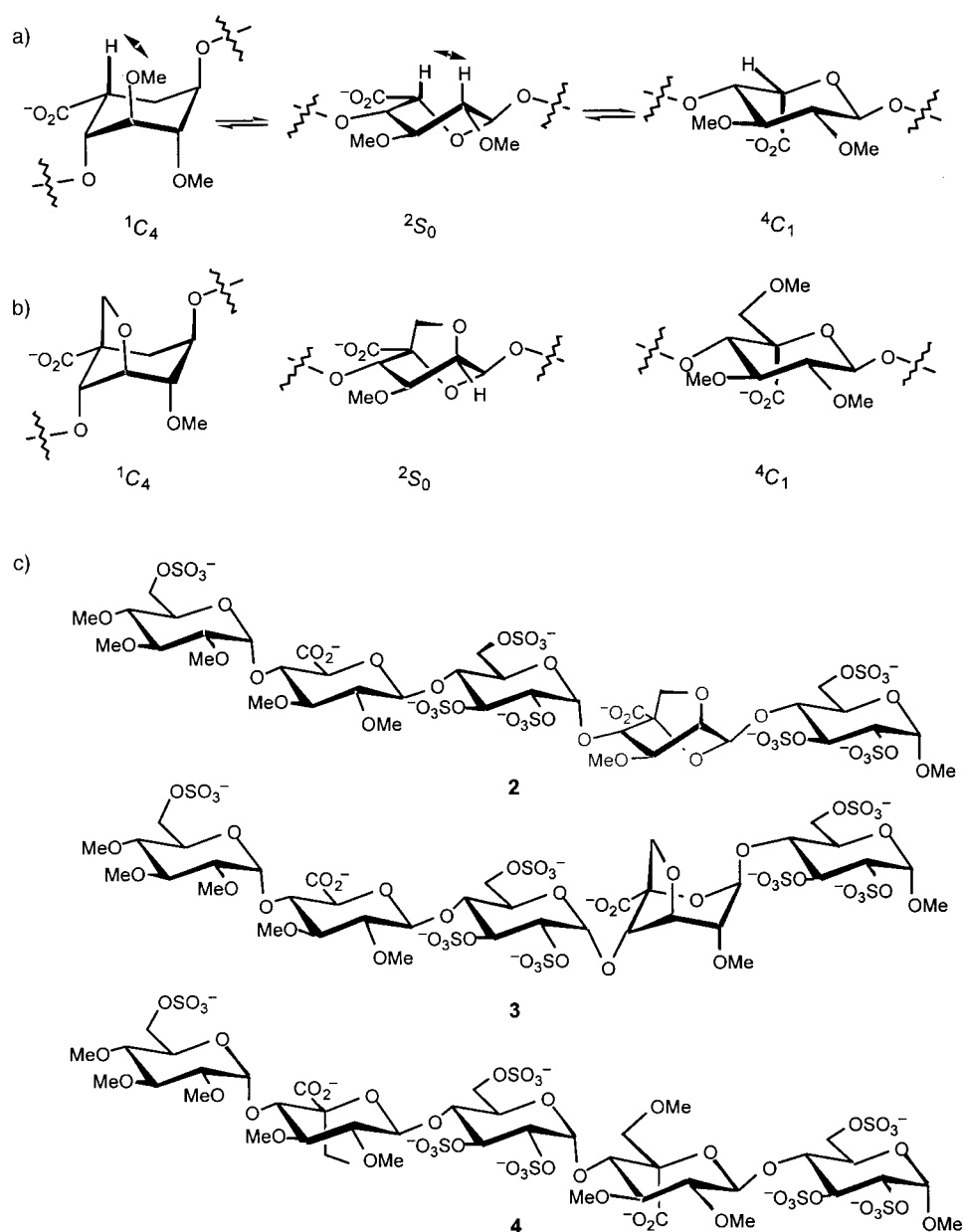
were best explained by taking into account the participation of the unusual  $^2S_0$  skew-boat conformer in addition to the classical  $^4C_1$  and  $^1C_4$  chair forms of the single L-iduronic acid component.<sup>[9]</sup> This was confirmed by force field studies and energy computations.<sup>[10]</sup> The  $^2S_0$  conformer was shown to be the major contributor to the conformational equilibrium of L-iduronic acid in this pentasaccharide.<sup>[11]</sup>

In order to establish the relationship between conformation and AT activity, we performed total syntheses of three pentasaccharides in which the single L-iduronic residue is conformationally locked, either in the  $^1C_4$ ,  $^4C_1$ , or  $^2S_0$  form. The known<sup>[12]</sup> synthetic methylated pentasaccharide **1** (Scheme 1) was chosen as the reference compound for several reasons. Firstly, it binds strongly to AT and selectively inhibits the blood coagulation factor -Xa; secondly, the presence both of methoxy groups in place of free hydroxyl functions and of O- in place of N-sulfonates largely simplifies the corresponding synthetic approach. Lastly, the hydroxyl group at the C-2 position of L-iduronic acid unit G in **1** is methylated and not sulfated. Among the various sulfate groups present in the original pentasaccharide, the one on the C-2 atom of L-iduronic acid was found not to be essential for activity. This auspicious feature is critical, inasmuch as it is a prerequisite for the synthesis of a locked  $^2S_0$  skew-boat conformer.

In the conformational formula of **1** shown in Scheme 1 the L-iduronate unit G is represented exclusively for the sake of simplicity in its  $^2S_0$  form. There is actually an equilibrium in aqueous solution between the three conformers:  $^1C_4 \rightleftharpoons ^2S_0 \rightleftharpoons ^4C_1$  (Scheme 2a). As indicated in



Scheme 1. The synthetic pentasaccharide **1** selected as the reference compound in this work.



Scheme 2. a) The three most energetically stable conformers of the methylated iduronic acid unit G of the biologically active pentasaccharide **1**. b) The three conformationally locked L-iduronic acid mimics, which have been synthesized and inserted in **1** in place of the unit G. c) The three resulting synthetic analogues of the biologically active pentasaccharide **1** in which the L-iduronic acid G unit is unambiguously locked in either the  $^1C_4$ ,  $^2S_0$ , or  $^4C_1$  form, as shown in (b).

Scheme 2b, we have locked the L-iduronate ring G in one of these conformations, and the three corresponding pentasaccharides **2–4** (Scheme 2c) have been synthesized.

The  $^1C_4$  and  $^4C_1$  chair forms are well-defined conformers which are rather easy to lock. The challenging chemical question is how to isolate the  $^2S_0$  form from the pseudorotational itinerary of the pyranoid ring.<sup>[13]</sup> We reasoned that a covalent connection between carbon atoms C-2 and C-5 would necessarily confine this itinerary to the section  $^2S_0 \rightleftharpoons ^{2,5}B \rightleftharpoons ^5S_1$ . More precisely, a one-atom bridge

would freeze the unwanted  ${}^{2,5}B$  conformation, whereas a more flexible two-atom bridge would probably allow the equilibrium between the three forms, and a shift toward the more stable skew-boat form. In the case of L-iduronic acid, this equilibrium would be shifted to the wanted and favorable  ${}^2S_0$ , wherein the substituents are equatorially and isoclinally oriented (see Scheme 2b).

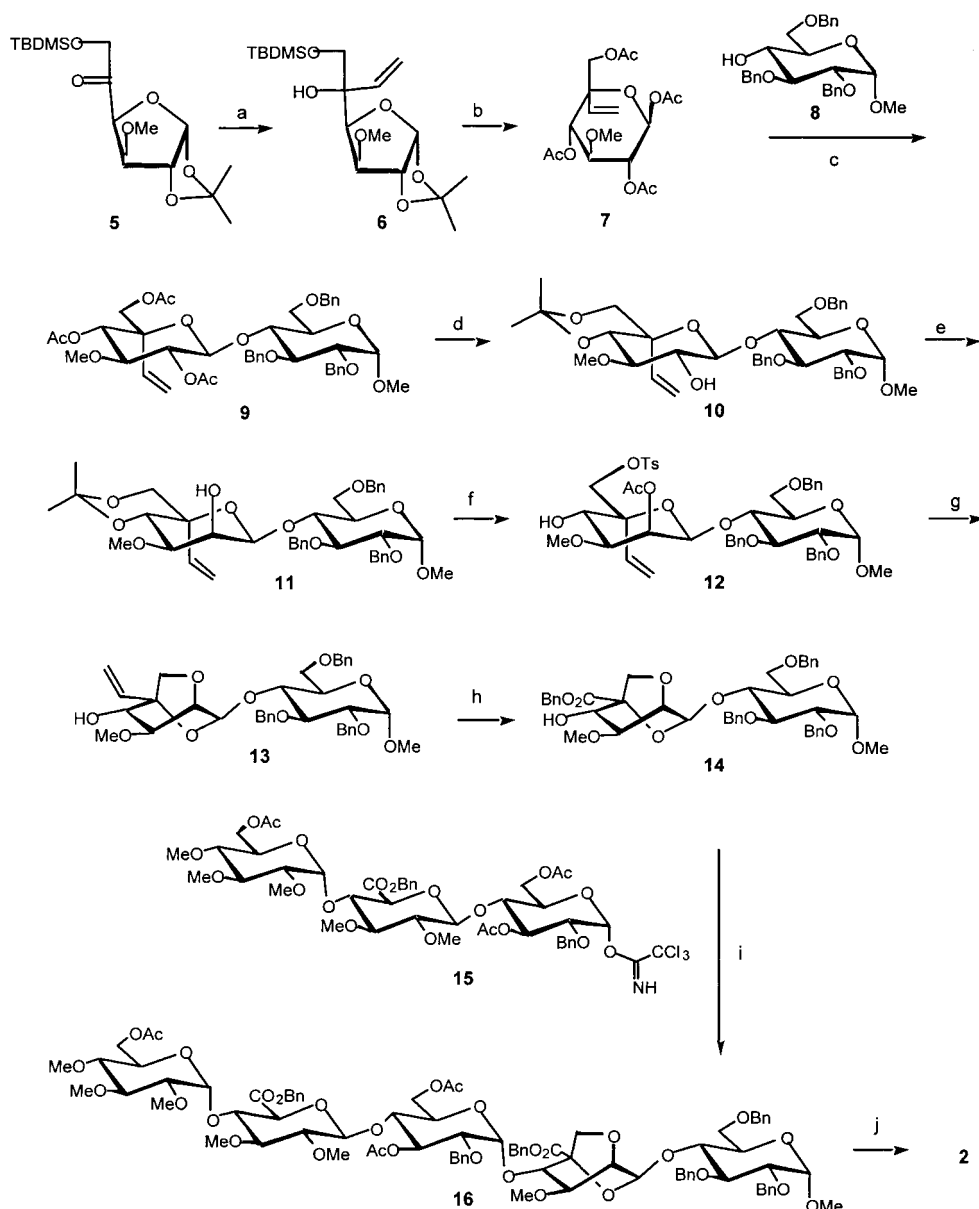
In order to bridge the C-2 and C-5 positions, we first synthesized a protected disaccharide **9** (Scheme 3). The easily available<sup>[14]</sup> ketone **5** was transformed in three steps into the

tetraacetate **7**. The first step, the face-selective addition of vinyl magnesium bromide, is explained by a chelation of the magnesium to the ring oxygen atom of **5**. The  $\beta$ -acetate **7**, whose structure has been confirmed by NMR spectroscopy, is well suited<sup>[15]</sup> for selective 1,2-transglycosylation of the known<sup>[16]</sup> alcohol **8**. The derivative **9** was then easily transformed (see Scheme 3) into the key alcohol **14**, which is a locked protected equivalent of the G-H section of the pentasaccharide **1**. The known<sup>[17]</sup> trisaccharidic imidate **15**, which has successfully been used for the synthesis of the

parent active pentasaccharide **1**, was then condensed with the alcohol **14** to afford the protected pentasaccharide **16**. A sequence of well-established reactions<sup>[18]</sup> then gave the pentasaccharide **2**. Comparison of the  ${}^1H$  NMR coupling constants of the locked L-iduronic acid residue in **2** ( $J_{1,2} = 1.3$ ,  $J_{2,3} = 1.4$ ,  $J_{3,4} = 2.7$ ,  $J_{2,4} = 0.5$  Hz) with those reported by Köll et al.<sup>[19]</sup> for methyl-2,6-anhydro- $\beta$ -D-mannopyranoside derivatives that exist in the  ${}^2S_0$  conformation ( $J_{1,2} = 1.2$ – $1.4$ ,  $J_{2,3} = 1.2$ – $2.4$ ,  $J_{3,4} = 3.0$ – $3.4$ ,  $J_{2,4} = 0.7$  Hz) confirms that our derivative adopts a similar conformation. Much to our delight, the  ${}^2S_0$ -locked pentasaccharide **2** was able to strongly activate AT with respect to factor Xa (Table 1).

As shown schematically in Scheme 4, a pivotal compound of type **9** has also been used to construct  ${}^1C_4$ - and  ${}^4C_1$ -locked blocks, which were then transformed into the two pentasaccharides **3** and **4**.<sup>[20, 21]</sup> A pentasaccharide similar to **3** has already been synthesized,<sup>[22]</sup> but it bears a sulfate group at the C-2 position of L-iduronic acid so its properties cannot be directly compared with those of our locked compounds to assess the influence of the conformation of L-iduronic acid on the biological properties. It should be noted that the  ${}^4C_1$  chair conformation of the G unit of **4** is ensured by the methoxymethyl group attached to the C-5 atom, which replaces the hydrogen atom of L-iduronic acid (Scheme 2b).

As shown in Table 1, the two pentasaccharides **3** and **4**, which contain the G unit locked in the  ${}^4C_1$  and  ${}^1C_4$  conformations, respectively, only very slightly potentiate

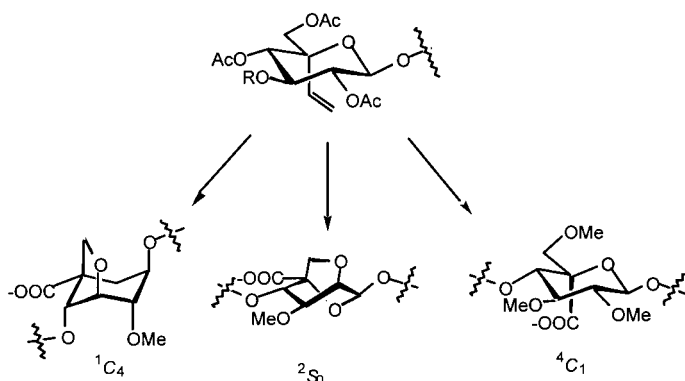


Scheme 3. General strategy used to synthesize the pentasaccharide **2**. a)  $CH_2=CHMgBr$ , THF,  $0^\circ C$ , 1 h, 70%; b) 1. IR-120  $H^+$  ion-exchange resin,  $H_2O$ ,  $80^\circ C$ , 6 h; 2.  $Ac_2O$ , pyridine, RT, 16 h, 75% (two steps); c) **8**, TMSOTf,  $CH_2Cl_2$ ,  $-78^\circ C$ , 2 h, 85%; d) 1. MeONa, MeOH,  $0^\circ C$  then RT, 3 h; 2.  $(CH_3O)_2C(CH_3)_2$ , *p*-TsOH, acetone, RT, 16 h, 70% (two steps); e) 1.  $(COCl)_2$ , DMSO,  $CH_2Cl_2$ ,  $-78^\circ C$ , 45 min; 2.  $LiEt_3BH$ , THF,  $-78^\circ C$  then RT, 1 h, 70% (two steps); f) 1.  $Ac_2O$ , pyridine, RT, 3 h; 2.  $AcOH$ ,  $60^\circ C$ , 2 h, 70% (two steps); 3. TsCl, pyridine, RT, 3 h, 80%; g) NaOH, EtOH,  $70^\circ C$ , 3 h, 70%; h) 1.  $O_3$ ,  $CH_2Cl_2$ ,  $-78^\circ C$ ,  $Me_2S$ , then 2-methyl-2-butene, *t*BuOH,  $H_2O$ ,  $NaH_2PO_4$ ,  $NaClO_2$ , RT, 16 h; 2. BnBr,  $Bu_4NI$ ,  $KHCO_3$ , DMF, RT, 5 h, 80% (three steps); i) TBDMSOTf,  $CH_2Cl_2$ ,  $Et_3O$ ,  $-20^\circ C$ , 30 min, 67%; j) 1.  $H_2$ , Pd/C, AcOH,  $40^\circ C$ , 12 h; 2. NaOH,  $H_2O$ ,  $55^\circ C$ , 3 h, 86% (two steps); 3.  $Et_3N \cdot SO_3$ , DMF,  $55^\circ C$ , 18.5 h, 85%. Bn = benzyl, DMF = dimethylformamide, DMSO = dimethyl sulfoxide, TBDMS = *tert*-butyldimethylsilyl, Tf = triflate = trifluoromethanesulfonyl, TMS = trimethylsilyl, Ts = tosyl = toluene-4-sulfonyl.

Table 1. Activity *a* against factor Xa by the pentasaccharides **1**–**4** and the reference compound **17**.

| Compound                 | <i>a</i> [u mg <sup>−1</sup> ] <sup>[c]</sup> |
|--------------------------|-----------------------------------------------|
| <b>1</b> <sup>[a]</sup>  | 1208 ± 63                                     |
| <b>2</b>                 | 1073 ± 61                                     |
| <b>3</b>                 | 115 ± 3                                       |
| <b>4</b>                 | 43 ± 3                                        |
| <b>17</b> <sup>[b]</sup> | 1345 ± 65                                     |

[a] The activity against factor Xa of the genuine nonmethylated synthetic pentasaccharide is 1013 ± 52 u mg<sup>−1</sup>. [b] Compound **17** is a synthetic nonlocked reference analogue of **1** in which the H-5 atom of the D-glucuronic acid unit E has been replaced by an ethyl group. [c] The values are the mean activities against factor Xa (*n* = 3). Human factor Xa (71 nkat per vial), antithrombin, and S-2222 substrate (Bz-Ile-Glu-Gly-Arg-pNA) were from Chromogenix (Mölndal, Sweden). The activity was determined in buffer by an amidolytic method adapted from Teien and Lie.<sup>[29]</sup> For an accurate comparison, compound concentrations were determined by <sup>1</sup>H NMR spectroscopy with reference to an internal standard. u = the inhibitory effect, in the same assay, of one unit of the IVth International heparin standard, Bz = benzoyl.



Scheme 4. Our synthetic strategy is based on the construction of a key structural element in which the C-5 atom is harnessed with two appendages, an equatorially oriented protected hydroxymethyl group and an axially oriented vinyl group. Through classical manipulations, this architecture can be selectively converted into one of the three conformers.

the inhibition of the blood coagulation protease factor Xa. This work unambiguously demonstrates for the first time that the AT-bound L-iduronic acid unit G adopts the unusual <sup>2</sup>S<sub>0</sub> conformation and clearly explains how the unique conformational behavior of L-iduronic acid translates in terms of biological activity.

It is likely that the accessibility of the three conformers is the basis for the high versatility of glycosaminoglycans (GAGs) that contain L-iduronic acid for binding to basic sites of interacting proteins. Although the <sup>2</sup>S<sub>0</sub> skew-boat conformer nicely governs the antithrombotic activity of heparin, it could very well be that the chair forms are critical in other situations, such as high affinity for fibroblast growth factors.<sup>[23]</sup> The availability of the three synthetic locked conformers offers a unique opportunity to replace any flexible L-iduronic acid residue in a GAG chain by a conformationally defined counterpart, thus providing a direct tool for general exploration of the relationship between conformational flexibility and biological properties of GAGs.

As laid out by Barton,<sup>[24]</sup> a conformational analysis of cyclic molecules is critical for the understanding of their biological action. We have demonstrated here that the conformational

changes of the pyranose ring have important significance in the biology of heparin. Other recent studies have also emphasized the importance of boat or skew-boat conformers in biology.<sup>[25–28]</sup> We believe that the importance of the conformational flexibility of carbohydrates is now very clear and is going to emerge as a key feature in this field of science.

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- [1] B. Casu, M. Petitou, A. Provasoli, P. Sinaÿ, *Trends Biochem. Sci.* **1988**, 13, 221–225.
- [2] U. Lindahl, D. A. Lane, *Heparin*, Edward Arnold, London, **1989**.
- [3] H. E. Conrad, *Heparin-Binding Proteins*, Academic Press, New York, **1998**.
- [4] B. Casu, J. Choay, D. R. Ferro, G. Gatti, J.-C. Jacquinet, M. Petitou, A. Provasoli, M. Ragazzi, P. Sinaÿ, G. Torri, *Nature* **1986**, 322, 215.
- [5] J. Choay, J.-C. Lormeau, M. Petitou, P. Sinaÿ, J. Fareed, *Ann. NY Acad. Sci.* **1981**, 370, 644–649.
- [6] L. Thunberg, G. Bäckström, U. Lindahl, *Carbohydr. Res.* **1982**, 100, 393–410.
- [7] J. Choay, M. Petitou, J.-C. Lormeau, P. Sinaÿ, B. Casu, G. Gatti, *Biochem. Biophys. Res. Commun.* **1983**, 116, 492–499.
- [8] P. Sinaÿ, J.-C. Jacquinet, M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, G. Torri, *Carbohydr. Res.* **1984**, 132, C5–C9.
- [9] G. Torri, B. Casu, G. Gatti, M. Petitou, J. Choay, J.-C. Jacquinet, P. Sinaÿ, *Biochem. Biophys. Res. Commun.* **1985**, 128, 134–140.
- [10] M. Ragazzi, D. R. Ferro, A. Provasoli, *J. Comput. Chem.* **1986**, 7, 105–112.
- [11] D. R. Ferro, A. Provasoli, M. Ragazzi, G. Torri, B. Casu, G. Gatti, J.-C. Jacquinet, P. Sinaÿ, M. Petitou, J. Choay, *J. Am. Chem. Soc.* **1986**, 108, 6773–6778.
- [12] P. Westerduin, C. A. A. van Boeckel, J. E. M. Basten, M. A. Broekhoven, H. Lucas, A. Rood, H. van der Heijden, R. G. M. van Amsterdam, T. G. van Dinther, D. G. Meuleman, A. Visser, G. M. T. Vogel, J. B. L. Damm, G. T. Overklist, *Bioorg. Med. Chem.* **1994**, 2, 1267–1283.
- [13] J. F. Stoddart, *Stereochemistry of Carbohydrates*, Wiley, New York, **1971**, p. 57.
- [14] M. K. Gurjar, S. K. Das, U. K. Saha, *Tetrahedron Lett.* **1994**, 35, 2241–2244.
- [15] H. Paulsen, T. Hasenkamp, M. Paal, *Carbohydr. Res.* **1985**, 144, 45–55.
- [16] J. M. Küster, I. Dyong, *Liebigs Ann. Chem.* **1975**, 2179–2189.
- [17] H. van der Heijden, T. Geertsens, M. Pennekamp, R. Willems, D. J. Vermaas, P. Westerduin, *Abstr. Pap. 9th Europ. Carbohydr. Symp. (Utrecht)* **1997**, p. 154.
- [18] M. Petitou, P. Duchaussoy, I. Lederman, J. Choay, J.-C. Jacquinet, P. Sinaÿ, G. Torri, *Carbohydr. Res.* **1987**, 167, 67–75.
- [19] P. Köll, F. S. Tayman, K. Heyns, *Chem. Ber.* **1979**, 112, 2305–2313.
- [20] A full account of the syntheses of **3** and **4** will be separately described elsewhere.
- [21] In the case of the pentasaccharide **4**, the glucuronic acid unit E has also been modified, with the H-5 atom being replaced by an ethyl group. This is a direct consequence of the selected converging strategy that has been selected for the sake of simplicity. We have, however, shown that replacement of the H-5 atom by an ethyl group in compound **1** does not affect its biological activity (see Table 1).
- [22] N. Sakairi, J. E. M. Basten, G. A. van der Marel, C. A. A. van Boeckel, J. H. van Boom, *Chem. Eur. J.* **1996**, 2, 1007–1013.
- [23] J. Kovensky, P. Duchaussoy, F. Bono, M. Salmivirta, P. Sizun, J.-M. Herbert, M. Petitou, P. Sinaÿ, *Biorg. Med. Chem.* **1999**, 1567–1580.
- [24] D. H. R. Barton, *Science* **1970**, 169, 539–544.
- [25] P. E. Marszalek, A. F. Oberhauser, Y.-P. Pang, J. M. Fernandez, *Nature* **1998**, 396, 661–664.
- [26] P. E. Marszalek, Y.-P. Pang, H. Li, J. E. Yazal, A. F. Oberhauser, J. M. Fernandez, *Proc. Natl. Acad. Sci. USA* **1999**, 96, 7894–7898.
- [27] E. Sabini, G. Sulzenbacher, M. Dauter, Z. Dauter, P. L. Jorgensen, M. Schülein, C. Dupont, G. J. Davies, K. S. Wilson, *Chem. Biol.* **1999**, 6, 483–492.
- [28] G. J. Davies, L. Mackenzie, A. Varrot, M. Dauter, M. Brzozowski, M. Schülein, S. Withers, *Biochemistry* **1998**, 37, 11707–11713.
- [29] A. N. Teien, M. Lie, *Thromb. Res.* **1977**, 10, 399–410.