in a chelated complex with a hydrogen at C_{11} properly aligned for syn elimination (to provide the trans allylic ether). Elimination toward the methoxyl would be disfavored since the carbon–hydrogen bond at C_9 cannot achieve syn-coplanarity with the C_{10}–copper bond in the chelated complex. Indeed, the fragmentation of the hydroperoxide that lacks this methoxyl substituent (**6c′**, MeO is replaced by H) resulted in the formation of all four possible olefin-containing macrolides.[17]

The conversion of octalin **1** into the 14-membered macrolide **2** provides an indication of the regio- and stereocontrolling elements required for the implementation of this new strategy for macrolide synthesis. To reach the intended goal of complex macrolide synthesis, we are in need of methods for the stereocontrolled synthesis of $\Delta^{9,10}$-octalin systems. Studies that pertain to this issue are currently under way and will be reported in due course.

Supplementary Material Available: Experimental procedures and spectroscopic data for all new compounds, as well as crystallographic data for compound **2b**[12] (22 pages). Ordering information is given on any current masthead page.

(17) Four macrolides are produced in a 12:2:1:1 ratio. On hydrogenation (H₂, Pd/C, EtOAc) a single saturated macrolide was obtained, indicating the fragmentation products are olefin isomers.

Synchrotron Light Source Applied to Measuring the Vacuum Ultraviolet Circular Dichroism of Heparin

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Heparin[2] is a glycosaminoglycan widely used therapeutically as an anticoagulant and antilipemic agent. It is a linear alternating copolymer of a hexosamine and uronic acid, with a predominant repeating disaccharide unit (Figure 1) of 2-sulfamino-2-deoxy-α-D-glucopyranosyl-6-sulfate and α-L-idopyranosyluronic acid 2-sulfate with both linkages being (1→4). Substantial microheterogeneity exists, the major features of which include the occurrence of β-D-glucopyranosyluronic acid as a minor uronic acid component, the occurrence of 2-acetamido-2-deoxy-α-D-glucopyranose as a minor hexosamine component, incomplete sulfation, with the number of sulfate groups per disaccharide ranging from approximately 2.0 to 2.5, and molecular weight polydispersity, with mean molecular weights in the range of 8000 to 15 000.

Circular dichroism (CD), applied to saccharides, has been shown to be sensitive to anomeric configuration, linkage type, and
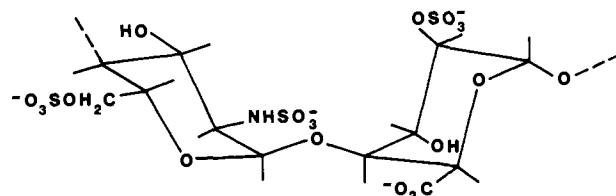
(1) (a) State University of New York. (b) National Institute of Medical Research. (c) Brookhaven National Laboratory.
(2) Casu, B. *Adv. Carbohydr. Chem. Biochem.*, in press.

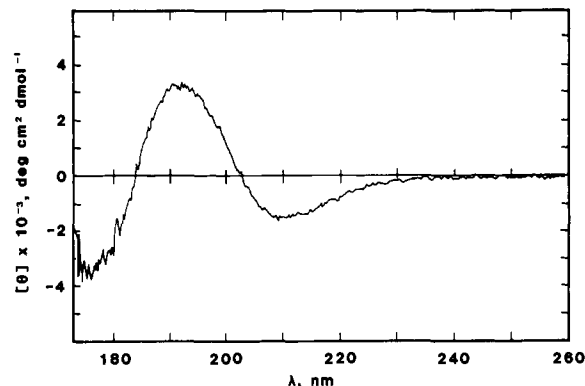**Figure 1.** Dominant repeating disaccharide unit in heparin.

Figure 2. Circular dichroism of heparin (D₂O, 25.5 mg/mL, 0.050-mm pathlength). Molar ellipticity, [θ], is given per disaccharide.

Table I. CD Summary of Heparin and Chondroitinsulfate

| compound | [θ],[a] 10³ deg cm² dmol⁻¹ | | | ref |
	n–π*	π–π*	175 nm	
heparin	−1.58	+3.37	−3.47	present work
	−1.9	+2.8		9[b]
	−2.74	+3.48		10[c]
	−1.45	+2.8		10[c]
	−1.28	+2.58		13[d]
	−1.8	+3.28		13[d]
chondroitin	−6.5	−2.5	−8.0	4
	−6.8			10
	−6.7			14, 15[e]
chondroitin-6-sulfate	−6.8	−2.5	−4.6	4
	−5.9			10
	−6.78			16[f]
	−11.2	−7.1		13

[a] Molar ellipticity, per disaccharide. [b] Boyd and Williamson[9] report molar ellipticities per tetrasaccharide; their values are reduced by 1/2 for proper comparison. [c] Park and Chakrabarti[10] measured two heparins from different sources. See also ref 11 and 12. [d] Stone[13] measured two heparins differing in source. [e] Values reported in ref 14 are corrected in ref 15. [f] Eyring and Yang[16] report molar ellipticities per monomer molecular weight; their values are doubled for proper comparison.

orientation of substituents on the sugar ring.[3] Extension of CD measurements to the vacuum ultraviolet region allows ring transitions to be observed directly. The vacuum ultraviolet circular dichroism (VUCD) of chondroitin and chondroitin-6-sulfate[4] has previously been measured and is of particular relevance to the present study. This report represents the first measurement of saccharide CD using synchrotron radiation[5] as a light source and the first observation of a 175-nm CD band in glycosaminoglycans.

The heparin sample (pig mucosa, Na salt, Sigma) contained 17.1% water (carbon analysis) and 2.28 sulfate groups per disaccharide (sulfur analysis) (disaccharide mol wt 578). Spectra were obtained on two instruments. One[5-7] uses radiation from

(3) Morris, E. R.; Frangou, S. A. In "Techniques in Carbohydrate Metabolism"; Elsevier: London, 1981; B308, 109–160.
(4) Stipanovic, A. J.; Stevens, E. S. *Biopolymers* **1981**, *20*, 1565–1573.
(5) Sutherland, J. C.; Desmond, E. J.; Takacs, P. Z. *Nucl. Instrum. Methods* **1980**, *172*, 195–199.
(6) Sutherland, J. C.; Griffin, K. P.; Keck, P. C.; Takacs, P. Z. *Proc. Natl. Acad. Sci. U.S.A.* **1981**, *78*, 4801–4804.
(7) Sutherland, J. C.; Griffin, K. P. *Biopolymers* **1983**, *22*, 1445–1448.

port U9B of the National Synchrotron Light Source (NSLS) at Brookhaven National Laboratory (BNL). The other[8] is of generally similar design except for its use of a conventional deuterium discharge Hinteregger light source. The instruments gave similar spectra except that the signal-to-noise ratio is significantly better with the synchrotron light source.

Figure 2 is a direct tracing of the spectrum obtained at BNL. There is a negative band at 208 nm with a molar ellipticity $[\theta]$ = -1.58×10^3 deg cm^2 dmol^{-1}, a positive band at 190 nm with $[\theta]$ = $+3.37 \times 10^3$ deg cm^2 dmol^{-1}, and a negative band at 175 nm with $[\theta]$ = -3.47×10^3 deg cm^2 dmol^{-1}. The CD of heparins above 185 nm has been measured previously[9-13] (Table I). Sample heterogeneity (see above) is the most likely source of the relatively wide range of values observed.

The molar ellipticity of heparin at 210 nm is substantially less than the sum of monomeric constituents, the molar ellipticity of the iduronic acid moiety alone being -5.43×10^3 deg cm^2 dmol^{-1}.[17] The source of this nonadditivity at 210 nm may lie in a difference in position of equilibrium between the two ring conformations of the iduronic acid moiety. In the $^1C_4$ (L) ring conformation the carboxyl group is located equatorially and the four oxygen atoms axially; in the $^4C_1$ conformation the situation is reversed. Proton and carbon-13 NMR evidence[18] indicates that the $^1C_4$ conformation, or a slightly distorted form of it, occurs in solutions of heparin, and the $^1C_4$ form is also favored in methyl-L-idopyranuronosides. However, as Morris et al.[17] have pointed out, even a small proportion of methyl idopyranuronosides in the $^4C_1$ form might contribute an inordinately large CD to the monomer spectrum if that form is intensely optically active by virtue of the axial position of the carboxyl chromophore.

The positive dichroism near 190 nm originates in the $\pi-\pi^*$ transitions of the uronic acid carboxyl chromophore and the hexosamine acetamido and sulfamino chromophores.[13] A correlation between positive 190-nm CD and (1→4)-linked amino sugars has been noted before.[13,15,19]

The negative CD at 175 nm is the first case of a discrete CD band being observed in aqueous solutions of a glycosaminoglycan below 180 nm. Stipanovic and Stevens[4] measured the VUCD of chondroitin and chondroitin-6-sulfate in aqueous solution and observed increasing negative ellipticity below 190 nm to the limits of those measurements near 170 nm. Hyaluronic acid measurements have been reported to 180 nm.[15,20]

Spectra of glycopyranoses,[21] glycopyranosides,[22] and cyclic ethers[23] provide evidence for an optically active transition near 175 nm associated with the unsubstituted sugar ring. The partial correlation of the sign of the 175-nm CD with anomeric configuration in glycopyranosides[22] implicates the linkage oxygen as one important determinant of CD, but its role could be either as the

chromophore itself or as a dominant perturber of the actual chromophore (e.g., the ring oxygen).

In glycosaminoglycans, high-energy transitions of the substituent acetamido and carboxyl groups are also potential sources of optical activity of 175 nm. Assignment of the 175-nm dichroism in glycosaminoglycans will therefore be difficult because of the large number of electronic transitions which are potential sources of dichroism. Correlation of CD sign and magnitude with structural features, based on an accumulation of VUCD data, can be expected to help the development of proper assignments.

Comparing the 175-nm dichroism of heparin with that of chondroitins (Table I) indicates that the major source of the 175-nm dichroism may not be a substituent group, since the sign of CD does not correlate with hexosaminidic linkage, as does the $\pi-\pi^*$ transition at 190 nm (see above). If the sign and magnitude of the 175-nm band proves to have its parentage largely in the ring and linkage oxygen chromophores, that band would then be an important spectroscopic indicator of glycosaminoglycan conformation in solution.

Oxidation of Red Ferryl [(Fe^{IV}O)$^{2+}$] Porphyrin Complexes to Green Ferryl [(Fe^{IV}O)$^{2+}$] Porphyrin Radical Complexes

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Ferryl (Fe^{IV}O)$^{2+}$ porphyrin complexes are widely accepted as important constituents of the reactive forms of a variety of heme proteins including the peroxidases[1] and cytochromes P-450.[2] Model compounds for these two different oxidation states have been prepared as transient intermediates. Six-coordinate complexes, BP(Fe^{IV}O) (B = *N*-methylimidazole, P = porphyrin dianion), have been prepared by reacting PFe^{II} with dioxygen (at -80 °C) to produce PFe^{III}OOFe^{III}P followed by treatment with B.[3] These have physical properties that match those of the reactive intermediate, II, of horseradish peroxidase.[3-6] A related red, five-coordinate complex, TMP(Fe^{IV}O), (TMP = dianion of tetramesitylporphyrin) is formed when the corresponding peroxo complex TMPFe^{III}OOFe^{III}TMP is warmed to -30 °C.[7] The reaction between *m*-chloroperoxybenzoic acid and TMPFe^{III}Cl at -78 °C produces the more highly oxidized green intermediate (TMP·)(Fe^{IV}O)X (X = monoanion) which has been formulated as containing a porphyrin π radical.[8] This green compound has been proposed as a model for horseradish peroxidase, I. While

(8) Pysh (Stevens), E. S. *Annu. Rev. Biophys. Bioeng.* **1976,** *5,* 63-75.

(9) Boyd, J.; Williamson, F. B. *J. Mol. Biol.* **1980,** *137,* 175-190.

(10) Park, J. W.; Chakrabarti, B. *Biochim. Biophys. Acta* **1978,** *544,* 667-675.

(11) Chakrabarti, B.; Park, J. W. *Crit. Rev. Biochem.* **1980,** *8,* 225-313.

(12) Park, J. W.; Chakrabarti, B. *Biochem. Biophys. Res. Commun.* **1977,** *78,* 604-610.

(13) Stone, A. L. *Biopolymers* **1971,** *10,* 739-751.

(14) Cowman, M. K.; Balazs, E. A.; Bergmann, C. W.; Meyer, K. *Biochemistry* **1981,** *20,* 1379-1385.

(15) Cowman, M. K.; Bush, C. A.; Balazs, E. A. *Biopolymers* **1983,** *22,* 1319-1334.

(16) Eyring, E. J.; Yang, J. T. *Biopolymers* **1968,** *6,* 691-701.

(17) Morris, E. R.; Rees, D. A.; Sanderson, G. R.; Thom, D. *J. Chem. Soc., Perkin Trans. 2* **1975,** 1418-1425.

(18) Gatti, G.; Casu, B.; Hamer, G. K.; Perkin, A. S. *Macromolecules* **1979,** *12,* 1001-1007.

(19) Coduti, P. L.; Gordon, E. C.; Bush, C. A. *Anal. Biochem.* **1977,** *78,* 9-20.

(20) Buffington, L. A.; Pysh (Stevens), E. S.; Chakrabarti, B.; Balazs, E. A. *J. Am. Chem. Soc.* **1977,** *99,* 1730-1734.

(21) Nelson, R. G.; Johnson, W. C., Jr. *J. Am. Chem. Soc.* **1976,** *98,* 4290-4295.

(22) Nelson, R. G.; Johnson, W. C., Jr. *J. Am. Chem. Soc.* **1976,** *98,* 4296-4301.

(23) Bertucci, C.; Lazzaroni, R.; Johnson, W. C., Jr. *Carbohydr. Res.* **1984,** *133,* 152-156.

(1) Hewson, W. D.; Hager, L. P. *Porphyrins* **1979,** *7,* 295.

(2) Griffin, B. W.; Peterson, J. A.; Estabrook, R. W. *Porphyrins* **1979,** *7,* 333.

(3) Chin, D. H.; Balch, A. L.; La Mar, G. N.; Balch, A. L. *J. Am. Chem. Soc.* **1980,** *102,* 4344, 5945.

(4) La Mar, G. N.; de Ropp, J. S.; Latos-Grazynski, L.; Balch, A. L.; Johnson, R. B.; Smith, K. M.; Parish, D. W.; Cheng, R. J. *J. Am. Chem. Soc.* **1983,** *105,* 782.

(5) Simonneaux, G.; Scholz, W. F.; Reed, C. A.; Lang, G. *Biochim. Biophys. Acta* **1982,** *716,* 1.

(6) Penner-Hahn, J. E.; McMurry, T. J.; Renner, M.; Latos-Grazynski, L.; Eble, K. S.; Davis, I. M.; Balch, A. L.; Groves, J. T.; Dawson, J. H.; Hodgson, K. O. *J. Biol. Chem.* **1983,** *258,* 12761.

(7) Balch, A. L.; Chan, Y. W.; Cheng, R. J.; La Mar, G. N.; Latos-Grazynski, L.; Renner, M. W. *J. Am. Chem. Soc.* **1984,** *106,* 7779.

(8) Groves, J. T.; Haushalter, R. C.; Nakamura, M.; Nemo, T. E.; Evans, B. J. *J. Am. Chem. Soc.* **1981,** *103,* 2884.