formation with octadecanoyl chloride (**20** → **21**, 97%), (iii) desilylation (**21** → **22**, 95%), and (iv) pivalate removal (**22** → **8**, 90%) proceeded smoothly, leading to globotriaosylceramide (Gb_3, **8**) in excellent overall yield. Synthetic Gb_3 (**8**) was identical with an authentic sample of this compound by the usual criteria.[20]

The described total synthesis of Gb_3 (**8**) demonstrates the power of the developed strategy for the synthesis of complex glycosphingolipids, confirms the assigned structure[16] to Gb_3 as **8**, and renders this glycosphingolipid readily available in pure form for further biological investigations. Further extensions of this strategy to the synthesis of more complex glycosphingolipids from the globo-, ganglio-, and lactoseries are currently in progress.

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Supplementary Material Available: Schemes with reagents and conditions for the synthesis of galactosylceramide (gal-cer) and lactosylceramide (lac-cer) and a listing of R_f, mp*, $[\alpha]_D$, IR, ^1H NMR, and ^{13}C** NMR data for the compounds **6****, **7***, **9**, **14**, **15****, **18**, **19**, **21**, gal-cer, lac-cer, and Gb_3 (**8**)** (8 pages). Ordering information is given on any current masthead page.

(20) For previous syntheses of the trisaccharide fragment of Gb_3 (**8**), see: (a) Jacquinet, J.-C.; Sinay, P. *Carbohydr. Res.* **1985**, *143*, 143. (b) Garregg, P. G.; Hultberg, H. *Carbohydr. Res.* **1982**, *110*, 261. (c) Cox, D. D.; Metzner, E. K.; Reist, E. J. *Carbohydr. Res.* **1978**, *63*, 139. (d) Dahmen, J.; Frejd, T.; Magnusson, G.; Noori, G.; Carlstrom, A.-S. *Carbohydr. Res.* **1984**, *127*, 15. (e) Paulsen, H.; Bunsch, A. *Carbohydr. Res.* **1982**, *100*, 143. For previous synthesis of Gb_3, albeit in low overall yield, see: (a) Koike, K.; Sugimoto, M.; Sato, S.; Ito, Y.; Nakahara, Y.; Ogawa, T. *Carbohydr. Res.* **1987**, *163*, 189. (b) Shapiro, D.; Acher, A. *J. Chem. Phys. Lipids* **1978**, *22*, 197. To the best of our knowledge, this is the first reported comparison of synthetic and natural samples of Gb_3 thus providing an unambiguous proof of the structural identify of this glycosphingolipid.

ENDOR Detection of Diastereomers Formed by Inclusion of a Prochiral Spin Probe into β-Cyclodextrin

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Molecular receptors which are capable of recognizing chiral guests attract much attention as sophisticated models of enzymes which have the capability of binding with such substances.[1] Cyclodextrin is a natural product which can recognize chiral molecules by producing diastereomeric pairs upon inclusion of components of racemates,[2-6] and induced circular dichroism can be observed when an achiral guest molecule is included.[7,8] A combination of the induced chirality in the guest molecule and the chirality of cyclodextrin itself should result in the formation of a diastereomeric mixture. This report deals with the detection of such diastereomers by ENDOR spectroscopy.

It has been reported that the ESR spectra of diphenylmethyl *tert*-butyl nitroxide (aminoxyl) (**1**) in aqueous solutions of β-cyclodextrin show two kinds of inclusion complexes which are assigned to bimodal inclusion, i.e., "*tert*-butyl-in" or "phenyl-in".[9] Both complexes have similar β-hydrogen and nitrogen hyperfine splittings (hfs). Because of the small differences in hfs, ENDOR spectroscopy was applied to obtain more accurate values for the hfs constants. ENDOR spectra were obtained by choosing a relatively pure ESR line in which the line intensity from one species is dominant. When the external field is fixed on the line which was previously assigned to the "phenyl-in" inclusion complex,

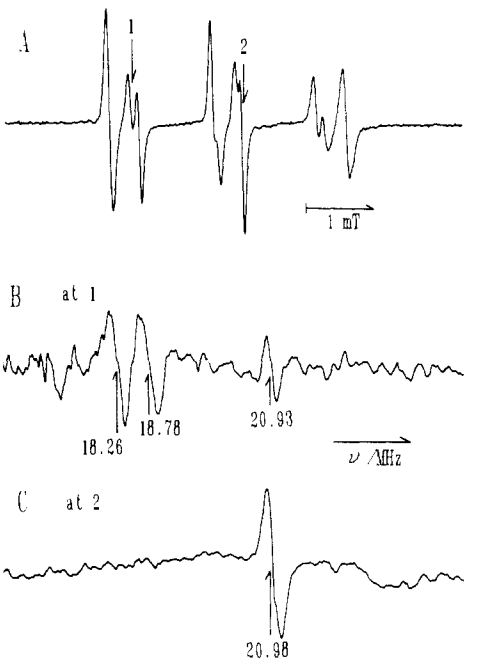
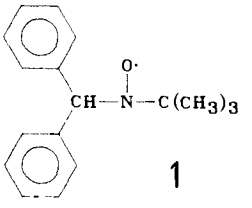


Figure 1. A: ESR spectrum of **1** in water in the presence of 1.0×10^{-2} M β-cyclodextrin at room temperature. Incident microwave power is 6 mW, and the modulation width is 0.0125 mT. B: Proton-ENDOR spectrum of **1** in water in the presence of 1.0×10^{-2} M β-cyclodextrin at 285 K. The external field is fixed at the position marked as **1** in Figure 1A. The incident microwave power is 100 mW, the rf power setting is 150 W, and the FM depth of rf is 100 kHz at the FM frequency of 12 kHz. The scan rate is 15 s over 10 MHz, and the signal is accumulated for 500 times. Free-proton frequency is 14.42 MHz. C: Proton-ENDOR spectrum of **1** in water in the presence of 1.0×10^{-2} M β-cyclodextrin at 285 K. The external field is fixed at the position marked as *2* in Figure 1A. The spectrometer settings are the same as Figure 1B, except the accumulation is 100 times. Free-proton frequency is 14.47 MHz.

Table I. Hyperfine Splitting Constants of β-Cyclodextrin Inclusion Complex of Nitroxide **1** in Water

	A_H,[a] mT	A_N,[b] mT
phenyl-in complex	0.311	1.565
	0.274	1.565
tert-butyl-in complex	0.465	1.541
1 in water	0.422[b]	1.590

[a] By ENDOR at 285 K, error is ±0.003 mT. [b] By ESR at room temperature, error is ±0.005 mT.



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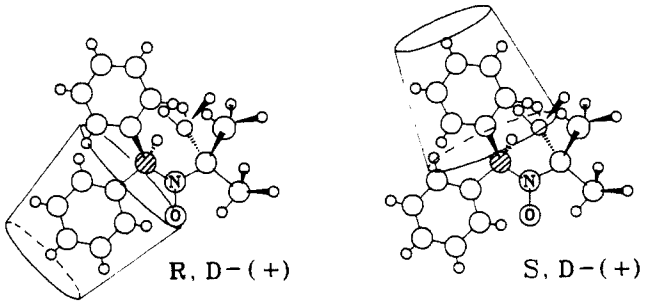

Figure 2. Schematic illustration which shows the formation of a diastereomeric pair by inclusion. R and S denote the stereochemical configuration of induced chiral center, and D-(+) denotes the configuration of naturally occurring β-cyclodextrin.

proton ENDOR spectra from the β-hydrogen are obtained as a doublet (Figure 1B). The hfs was analyzed to be 0.274 and 0.311 mT. In contrast proton-ENDOR spectra of the "*tert*-butyl-in" inclusion complex gives only a single line with an hfs of 0.465 mT. Nitrogen-ENDOR peaks were not observed under these conditions because the optimum temperature for the N-ENDOR line is expected to be higher than room temperature, and at these temperatures the equilibrium favors the free species. Also the proton-ENDOR spectrum of the free nitroxide **1** cannot be obtained at room temperature. Inclusion in cyclodextrin provides just enough restriction in tumbling motion for **1** so that an ENDOR signal can be obtained at the same temperature as used for the ESR experiments. Hfs constants obtained by ENDOR and ESR are listed in Table I.

The doublet structure near 18.5 MHz of the proton-ENDOR line of the "phenyl-in" inclusion complex is interpreted in terms of the formation of a diastereomeric mixture. The inclusion of one phenyl group in **1** changes the α-carbon into an asymmetric carbon. Since the β-cyclodextrin used is the D-(+) optical isomer, the combination with a newly formed chiral center in the spin probe can produce a diastereomeric mixture. The different configurations in the probe produced by β-cyclodextrin are schematically illustrated in Figure 2.

The inclusion of the phenyl group by either the narrow or wide end of β-cyclodextrin could give two different complexes. However, space filling models show that the inner diameter of the narrower end of β-cyclodextrin is approximately 4.5 Å which should not be wide enough to include either the phenyl group or the *tert*-butyl group.

The difference in hfs of diastereomeric nitroxides in which two asymmetric carbons exist on one side of the nitroxide function has been extensively studied.[10-12] The difference in β-hydrogen hfs in such cases is usually 0.07 to 0.09 mT compared to 0.04 mT for the present example. An estimation of the dihedral angle difference by the Heller–McConnell equation[13] gives 0.5°. Although the NMR spectrum of a solute–solvent diastereomeric pair is widely used[14] for the determination of optical purity, such examples in ESR spectroscopy are not common. Recently however, Stegmann et al.[15] observed a splitting of the proton-ENDOR line of a chiral phenoxyl radical in an optically active solvent. The formation of a solute–solvent diastereomeric pair is responsible for this separation, and the difference in the β-hydrogen hfs is again very small (0.03 mT). The interaction responsible for this separation is of the same type as in the present case.

Cyclodextrin solutions of **1** were prepared by the method described previously.[9] The solutions were investigated in a quartz flat cell, 5 mm wide and 0.3 mm inside diameter. ESR and ENDOR spectra were obtained by using a Bruker ER200D-SRC spectrometer with ENDOR accessory. Temperature was controlled with chilled nitrogen gas with a Bruker VT4100 variable temperature unit. The spectrometer settings were specified in the figure captions.

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The Resonance Raman Spectrum of Horseradish Peroxidase Compound I

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The oxidation of substrates by horseradish peroxidase proceeds via the formation of two key intermediates. Upon reaction with H_2O_2, the active site (histidyl imidazole ligated protoheme) undergoes a two-electron oxidation to form compound I (HRP-I), generally formulated as a ferryl (porphyrin π-cation radical). A subsequent one-electron reduction by substrate yields a ferrylporphyrin species, compound II (HRP-II).[1] The initially formed species (HRP-I) is but one example of what is believed to be a common intermediate in the enzymatic cycles of several important oxidative heme enzymes.[2] Thus, characterization of the structure and reactivity of these species is of intense current interest.[1-3]

Resonance Raman spectroscopy,[4] well-suited to the investigation of the active site structure of heme proteins and reactive intermediates, has been successfully applied to the study of the resting state[5] and the (ferrylporphyrin) HRP-II species.[6] Unfortunately, the inherent reactivity and photolability[1] of HRP-I have prevented unambiguous documentation of its RR spectrum. An early report of this spectrum[7a] was later shown to be attributable to a photogenerated mixture of the ferrous and HRP-II species.[7b] More recently, efforts were made to eliminate these problems by using relatively low (CW) laser powers with flowing samples[8] or short duration (10 ns) laser pulses.[9] However, the frequencies reported for HRP-I in these studies are apparently anomalous by comparison with the RR data obtained for metalloporphyrin π-cation radicals by Babcock's group[10,11] and ourselves.[12] Specifically,

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