Table I. Electrochemical Data for $Na^+$ Effect on Nitroaromatics

| compd | couple[a] | $Na^+$/L ratio[b] | $E_p^{red}$, V | $E_p^{ox}$, V | $E^{\circ\prime}$, V[c] |
|---|---|---|---|---|---|
| 1 | A | 0 | −1.36 | −1.21 | −1.28 |
| | A | 1/2 | −1.38 | −1.18 | −1.28 |
| | A | 1 | | | |
| | B | 0 | | | |
| | B | 1/2 | −1.17 | −1.04 | −1.11 |
| | B | 1 | −1.22 | −1.00 | −1.11 |
| 2 | C | 0 | −1.36 | −1.22 | −1.29 |
| | C | 1/2 | −1.37 | −1.21 | −1.29 |
| | C | 1 | −1.37 | −1.21 | −1.29 |
| | D | 0 | | | |
| | D | 1/2 | −1.20 | −0.87 | |
| | D | 1 | −1.20 | −0.78 | |
| 3 | | 0 | −1.28 | −1.15 | −1.22 |
| | | 1/2 | −1.28 | −0.99 | |
| 4 | | 0 | −1.29 | −1.17 | −1.23 |
| | | 1/2 | −1.33 | −1.17 | −1.25 |

[a] See figure. [b] L = nitroaromatic (ligand). [c] Diffusion coefficients for reduced and oxidized forms were assumed to be equal.

were done with a Princeton Applied Research apparatus (Models 173 and 176) and recorded on an H-P 7045A $x$–$y$ recorder.

The cyclic voltammograms (cvs) for **1** and 2-nitroanisole (**2**) in the presence and absence of $Na^+$ are shown in Figures 1 and 2. The data for **1**, **2**, **3**, and 4-nitroanisole (**4**) are summarized in Table I. The quasi-reversible, one-electron redox couples for **1** and **2** (A and C, respectively) exhibit virtually identical $E^{\circ\prime}$ values (see figures) indicating that $CH_3O$– or crown-$CH_2O$– has a similar effect on the nitroaromatic nucleus. When $NaClO_4$ (0.5 equiv) is added to solutions of **1** or **2**, a new redox couple (quasi-reversible for **1**/B; irreversible for **2**/D, see figure) appears in each case. Couple A disappears when a full equivalent of $Na^+$ is added to **1** and only couple B is observed, with an enhanced current. The oxidation peak of D moves to more positive potential when **1** equiv of $Na^+$ is added to **2**, the reduction potential does not change, and C is not dissipated. In the absence of $Na^+$, the single redox couples observed for **3** and **4** are nearly identical. The similarity continues when 0.5 equiv of $Na^+$ is present in solutions of **3** or **4**. No new redox couple is observed in either case although additional $Na^+$ increases irreversibility (see table).

The results described clearly suggest an intramolecular interaction between the macroring-bound cation and the reduced nitroaromatic side arm of **1**. The intramolecularity of this interaction is clear from two lines of evidence. First, **3**, which has all the structural elements of **1**, behaves differently because the nitro group is inappropriately situated. Second, the significant intermolecular interaction between $Na^+$ and $\mathbf{2}^-$ (a surprising, but not difficult to rationalize, observation), which leads to electrochemically irreversible behavior, contrasts nicely with the weaker interaction observed between $Na^+$ and $\mathbf{1}^-$. The latter process is quasi-reversible, indicative of kinetically fast electrode processes for couple B. This probably results from the ready availability of macroring-bound $Na^+$ to intramolecularly ion pair with the reduced nitroaromatic side arm.

The electrochemically enhanced binding constant, $K_s$, for $\mathbf{1}^-$ with $Na^+$ can be assessed by a simple, thermochemical cycle since the $E^{\circ\prime}$ values are known and $Ks_{1\cdot Na^+}$ has been measured by previously described methods.[1,8] Redox couples A and B correspond to eq 2 and 1, respectively. The difference (eq 2 − eq 1 = eq 3) gives $K_{ee}$ for electron exchange between $\mathbf{1}^-$ and $\mathbf{1}\cdot Na^+$.

$$\mathbf{1}\cdot Na^+ + e \rightarrow \mathbf{1}\cdot Na \quad (1)$$

$$\mathbf{1}^- \rightarrow \mathbf{1} + e \quad (2)$$

$$\mathbf{1}\cdot Na^+ + \mathbf{1}^- \rightarrow \mathbf{1}\cdot Na + \mathbf{1} \quad (3)$$

$K_{ee}$, when multiplied by $Ks_{1\cdot Na^+}$, gives the stability constant for the $Na^+\cdot\mathbf{1}^-$ complex. From the $E^{\circ\prime}$ values, the electrochemically reduced ligand binds $Na^+$ 750 times more strongly than does the neutral ligand. Therefore, log $Ks_{1\cdot Na}$ = 6.33.

To our knowledge, this is the first time an intramolecular complex between $Na^+$ and a radical anion crown ether ligand has been detected. This is also the first direct evidence for enhanced binding by an electrochemically reduced macrocyclic ligand. The latter is especially important since it represents a new switching mechanism for lariat ether complexes. The generality of these observations and this approach, especially the potential for reversible, electrochemically switched cation transport, are currently under investigation.

**Acknowledgment.** We warmly thank J. R. Beadle, H. Durst, M .B. Goli, and W. Maldonado for preparation of various samples. We gratefully acknowledge support of this work by the N.S.F. (to L.E. CHE-79-15201), the U.S. Army (to H.D.), the N.I.H. (to A.K. GM-08819, to L.E. RR-8102, to G.W.G. GM-29150 and GM-31846), and W. R. Grace and Co., Inc.

**Registry No.** **1**, 87453-20-1; $Na^+\mathbf{1}^-$, 87453-19-8; **2**, 91-23-6; **3**, 87453-21-2; **4**, 100-17-4; Na, 7440-23-5; 2-hydroxymethyl-15-crown-5 anion, 87453-22-3; 1-chloro-2-nitrobenzene, 88-73-3.

(8) Gokel, G. W.; Goli, D. M.; Minganti, C.; Echegoyen, L. *J. Am. Chem. Soc.*, in press.

## Glutathiohydroxyacetone: $^1H$ NMR Determination of the Stereochemistry of Proton Exchange by Glyoxalase I. Evidence for a *cis*-Enediol Intermediate Based on Mirror-Image Catalysis

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*Received September 3, 1982*

We have recently reported that (glutathiomethyl)glyoxal (**1**) is an inverse substrate for glyoxalase I [(*S*)-lactoylglutathione methylglyoxal-lyase (isomerizing) EC 4.4.1.5; GX I].[2] The reaction is characterized by a loss of specificity for glutathione in thioester **3** formation *via* thiohemiacetal **2** and by the production of the *S* isomer of **3** (Scheme I). We have termed the effect "mirror-image catalysis". The key implication of this finding is the uncoupling of the two functions of glutathione: activation of the C-1 proton by thiohemiacetal formation and binding of the thiohemiacetal to the enzyme. Since the role of RSH in the inverse processing of **1** is essentially activation and not binding, one might intuit that the increased acidity of the C-1 proton could be accomplished by a variety of structures that are intrinsically more stable and better defined than the stereochemically ambiguous thiohemiacetal **2**. To this end, we have found that glutathiohydroxyacetone **6**[2] undergoes a stereospecific GX I catalyzed exchange of one hydroxymethyl proton and have established the absolute stereochemistry by the unambiguous synthesis of both monodeuterated diastereomers of **6**. The analysis relies on the nonequivalence of the hydroxymethyl proton resonances by 500-MHz $^1H$ NMR. The results strongly suggest the selective processing of one of the diastereomeric thiohemiacetals and are consistent with the intermediacy of a *cis*-enediol.

The synthesis of **6** was accomplished by the reaction of chlorohydroxyacetone[3] and glutathione (GSH) under aqueous conditions (Scheme II).[4] At 500 MHz the hydroxymethyl protons

(1) American Cancer Society Faculty Research Awardee (1983–1988).

(2) Kozarich, J. W.; Chari, R. V. J. *J. Am. Chem. Soc.* **1982**, *104*, 2655. The proper (current *Chemical Abstracts* Index) name for **1** is *N*-[*N*-L-γ-glutamyl-*S*-(2,3-dioxopropyl)-L-cysteinyl]glycine and for **6**, *N*-[*N*-L-γ-glutamyl-*S*-(3-hydroxy-2-oxopropyl)-L-cysteinyl]glycine.

(3) Chari, R. V. J.; Kozarich, J. W. *J. Org. Chem.* **1982**, *47*, 2355.

(4) In a typical reaction, chlorohydroxyacetone (15 μmol) and GSH (15 μmol) were added to $^2H_2O$ (>95%) containing 0.2 M potassium phosphate (pD 7.2; 0.6-mL total volume). Formation of **6** was monitored at 293 nm ($\epsilon_{293} \cong 200\ M^{-1}\ cm^{-1}$) and was complete in 15 min.

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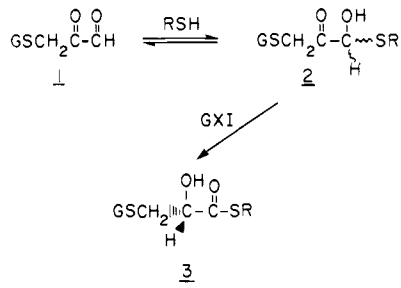
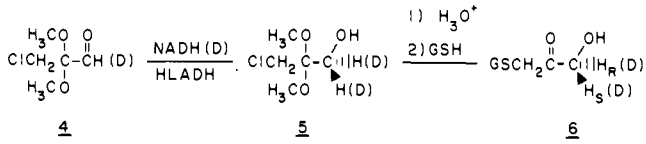
Scheme I




Scheme II




afforded an AB quartet with an apparent $\Delta\nu$ of 7.7 Hz (0.015 ppm) (Figure 1A).[5] Incubation of **6** with GX I (yeast, Sigma grade X) in $^2H_2O$ resulted in a decrease in the AB quartet intensity with the concomitant appearance of a broadened triplet ($J$ = 2.7 Hz) slightly upfield (0.015 ppm) (Figure 1B).[6] Prolonged incubation of **6** in $^2H_2O$ without enzyme resulted in the appearance of two triplets of equal intensity ($\Delta\nu$ = 7.7 Hz) (Figure 1C), indicating a random chemical exchange.[7]

These observations provide direct evidence for the stereospecificity of proton exchange in **6** by GX I and confirm our prediction on the generality of proton activation implicit in mirror-image catalysis. Both monodeuterated enantiomers were synthesized by exploitation of the broad specificity of horse liver alcohol dehydrogenase (EC 1.1.1.1).[8] Thus, each enantiomer of **5** was prepared enzymatically using the appropriately deuterated **4**[4] or reduced nicotinamide cofactor[9] and with the knowledge that the pro *R* hydrogen (deuterium) is derived from cofactor.[10] Acid-catalyzed deketalization and reaction with GSH[2] afforded a monodeuterated **6** of known chirality. The 500-MHz NMR spectra of the diastereomers show unequivocally that GX I catalyzes the exchange of the *pro-S* proton (Figure 2).

The major significance of this finding lies in the recognition of the hydroxymethyl protons of **6** as positionally equivalent to the two possible proton configurations of thiohemiacetal **2**. Exchange of the *pro-S* proton of **6**, therefore, may be related to proton abstraction from the *R* isomer of thiohemiacetal **2**.[11] Moreover,

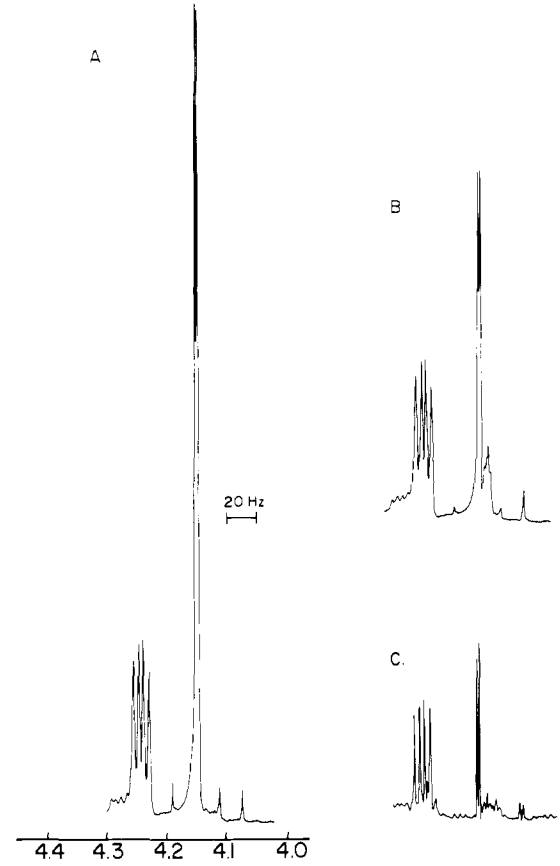



**Figure 1.** 500-MHz $^1H$ NMR ($^2H_2O$) spectra of the hydroxymethyl region of glutathiohydroxyacetone **6**: (A) prior to addition of GX I; (B) after 12 h in the presence of GX I (5 units); (C) after approximately 1 week without enzyme. The downfield quartet is the cysteine $\alpha$H. Concentration of **6** was 15 mM. Chemical shifts are standarized to an HDO resonance of 4.7 ppm.

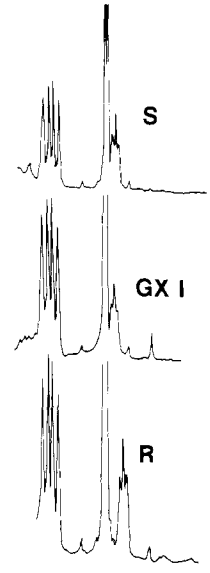



**Figure 2.** 500-MHz NMR ($^2H_2O$) spectra of unambiguously synthesized *R* and *S* deuterated isomers of **6** compared to the spectrum of GX I catalyzed exchange. Each synthetic sample was doped with diprotio material for reference.

the known formation of the *S* isomer of **3** in the inverse reaction fixes the stereochemistry of proton addition at the keto group. Given a suprafacial proton transfer as most reasonable, the geometry of the enediol intermediate is thus fixed as cis. The normal GX I reaction, then, requires the (*S*)-thiohemiacetal to afford (*R*)-hydroxy acid via the cis intermediate.

(5) **6**: $^1H$ NMR ($^2H_2O$) $\delta$ 4.24 (dd, 1 H, $J$ = 4.9, 8.8 Hz, cys $\alpha$H), 4.140 (d, 1 H, $J$ = 19.2), 4.155 (d, 1 H, $J$ = 19.2 Hz), 3.43 (m, 5 H), 2.71 (dd, 1 H, $J$ = 4.9, 14.1 Hz), 2.55 (dd, 1 H, $J$ = 8.8, 14.1 Hz), 2.20 (m, 2 H), 1.82 (m, 2 H). The multiplet at $\delta$ 3.43 contains resonances for the $\alpha$-proton of glutamate, the methylene of glycine, and the methylene between the keto and alkylthio groups. The latter methylene undergoes a rapid chemical exchange ($t_{1/2} \cong$ 4 h: Guth, J. J.; Gross, R. L.; Carson, F. W. *J. Org. Chem.* **1982**, *47*, 2666). This exchange has no effect on our analysis. The AB quartet is centered at $\delta$ 4.15.

(6) The rate constant for solvent exchange is approximately 1 $s^{-1}$. This value is similar to the solvent exchange of (*S*)-D-lactoylglutathione by GX I (Sellin, S.; Rosevear, P. R.; Mannervik, B.; Mildvan, A. S. *J. Biol. Chem.* **1982**, *257*, 10023).

(7) The overall decrease in peak area is expected since chemical exchange also produces dideuterio material. The upfield shifts are consistent with monodeuteration (Tiers, G. Van D. *J. Chem. Phys.* **1958**, *29*, 963. Gutowsky, H. S. *Ibid.* **1959**, *31*, 1683).

(8) Sund, H.; Theorell, H. In "The Enzymes", 2nd ed.; Boyer, P. D., Lardy, H., Myrback, K., Eds.; Academic Press: New York, 1963; Vol. 7, p 25.

(9) Fisher, H. F.; Cohn, E. E.; Vennesland, B.; Westheimer, F. H. *J. Biol. Chem.* **1953**, *202*, 687. Loesche, W.; Wenz, I.; Till, U.; Petermann, H.; Horn, A. *Methods Enzymol.* **1980**, *66*, 11.

(10) Levy, H. R.; Vennesland, B. *J. Biol. Chem.* **1957**, *228*, 85.

(11) This, of course, assumes that the hydroxyl group of **6** resides in the same position as the hydroxyl of **2**. The methoxy analogue of **6** is not a substrate for proton exchange by GX I, excluding the possibility of stereochemical infidelity by hydroxyl binding to the thiol site. Our argument is further strengthened by the fact that the (*R*)-thiohemiacetal is configurationally equivalent to the D-hydroxy acid product formed in the normal reaction.

In conclusion, "mirror-image catalysis" provides a method for deducing the absolute stereochemical course of the GX I reaction. The establishment of a single stereochemical outcome at both carbon positions strongly suggests that the recently observed nonstereospecific thiohemiacetal utilization[12] by GX I is not a property of the initial proton abstraction but is most likely due to an enzyme-catalyzed epimerization of the wrong isomer by an addition–elimination mechanism prior to abstraction. Finally, the results highlight the utility of high-field NMR in distinguishing marginally nonequivalent protons.

**Acknowledgment.** We thank the National Institutes of Health (GM 29204 and GM 31879) for support of this research. The Brüker WM 500-MHz NMR spectrometer is supported in part by a grant from the National Science Foundation (CHE-791620). We express our gratitude to Peter Demou for his expert technical assistance and Professor John Gerlt for valuable discussions. R.V.J.C. is a Yale-Celanese Fellow.

(12) Griffis, C. E. F.; Ong, L. H.; Buettner, L.; Creighton, D. J. *Biochemistry* **1983**, *22*, 2945.

## Synthesis and Characterization of [7]Circulene[1]

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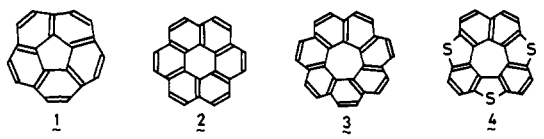
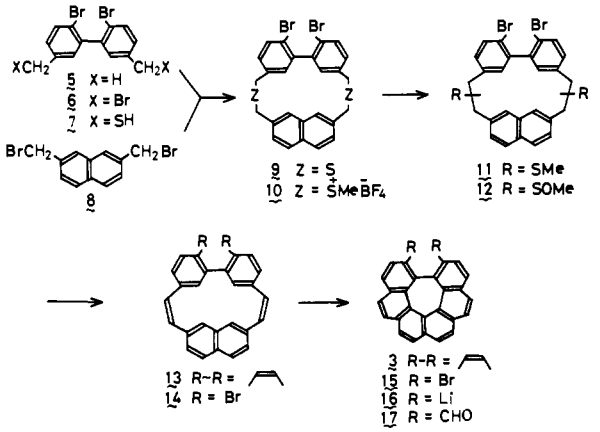
Lawton's papers describing his elegant synthetic approach[2] to the bowl-shaped[3] [5]circuene (**1**) ("corannulene", the next lower



**1** **2** **3** **4**

homologue of [6]circulene (coronene) (**2**)) have provoked considerable interest in the preparation of [7]circulene (**3**), the next higher homologue of [6]circulene.[4]

In this communication, we report our successful synthesis of [7]circulene (**3**) and an X-ray analysis, which has revealed its




(1) Presented in part at the 47th Annual Meeting of the Chemical Society of Japan, April 1983, Kyoto.

(2) Barth, W. E.; Lawton, R. G. *J. Am. Chem. Soc.* **1966**, *88*, 380–381.

(3) Barth, W. E.; Lawton, R. G. *J. Am. Chem. Soc.* **1971**, *93*, 1730–1745.

(4) A heterocyclic [7]circulene analogue **4** incorporating three thiophene rings has been reported: Dopper, J. H.; Wynberg, H. *Tetrahedron Lett.* **1979**, 763–766; *J. Org. Chem.* **1975**, *40*, 1957–1966.

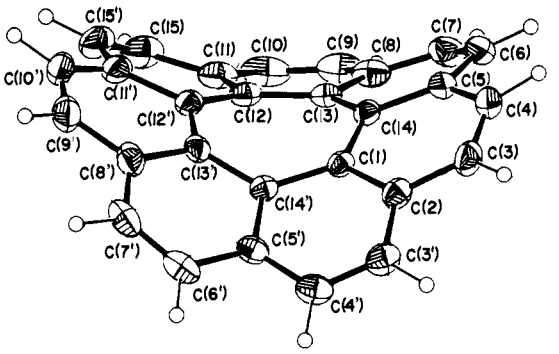



**Figure 1.** Molecular structure of **3**. Non-hydrogen atoms are drawn by the thermal ellipsoids at the 30% probability level. Hydrogen atoms are shown by the spheres corresponding to the artifical isotropic temperature factor of 1.0 Å$^{-2}$.

saddle-shaped structure as expected from the examination of the molecular model.

We reasoned that Reiss's attempted photocyclization[5] of the cyclophane intermediate **13** directly into **3** failed because of the inherent rigid structure of **13**, incorporating both naphthalene and phenanthrene moieties. We directed our efforts to a two-stage synthetic approach that involves (1) photocyclization of the more flexible biphenylnaphthalene cyclophane **14** to the 1,16-dehydrohexahelicene **15** and (2) modification of the side chains to secure the missing benzene ring to complete the synthesis.

N-Bromosuccinimide bromination of 2,2′-dibromo-5,5′-dimethylbiphenyl (**5**)[6] afforded the bis(bromomethyl) derivative **6**, which was converted into the bis(mercaptomethyl)biphenyl **7** by routine procedures. The coupling of **7** and 2,7-bis(bromomethyl)naphthalene (**8**) was carried out in dimethylformamide with cesium carbonate[8] to give the dithiacyclophane **9**,[9] mp 173–174 °C (56% yield). Reaction of **9** with dimethoxycarbonium fluoroborate in dichloromethane yielded the disulfonium salt **10** whose Stevens rearrangement mediated by sodium hydride provided a 67% yield of the bis(sulfide) **11**, an oil. Oxidation of **11** with *m*-chloroperbenzoic acid gave the bis(sulfoxide) **12** whose pyrolysis at 300 °C (0.001 mm) produced the unsaturated cyclophane **14**, pale orange needles, mp 213–215 °C (63% yield from **11**). A cyclohexane solution of **14** containing a trace amount of iodine was irradiated with a high-pressure mercury lamp[10] for 1 h to afford 1,16-dehydro-2,15-dibromohexahelicene (**15**):[11] mp 299–301 °C (47% yield); mass spectrum, *m/e* 484, M$^+$; $^1$H NMR ($CDCl_3$) $\delta$ 7.82–8.66 (multiplet); UV (cyclohexane) $\lambda_{max}$ (log $\epsilon$), 245 (4.80), 270 (sh, 4.72), 277 (4.73), 312 (4.48), 326 (4.40). Lithiation of the dibromide **15** with *n*-BuLi in tetrahydrofuran and formylation of the resulting dilithio derivative **16** with dimethylformamide gave the dialdehyde **17**: pale yellow prisms, mp 303–305 °C (54% yield); mass spectrum, *m/e* 382, M$^+$; $^1$H NMR ($CDCl_3$) $\delta$ 2.02 (s, 2 H), 7.78–8.45 (m, 12 H). Intramolecular reductive coupling[13] of the dialdehyde **17** with $LiAlH_4$ and titanium trichloride in dimethoxyethane completed the outer perimeter of [7]circulene (**3**): yellow plates, mp 295–296 °C (35% yield); mass spectrum, *m/e* 350 M$^+$; $^1$H NMR is characteristic in its single sharp peak at $\delta$ 7.45, and the $^{13}$C NMR exhibiting

(5) Jessup, P. J.; Reiss, J. A. *Aust. J. Chem.* **1977**, *30*, 851–857.

(6) 2,2′-Dibromo-5,5′-dimethylbiphenyl (**5**), mp 109–110 °C, was prepared from 5,5′-dimethyl-2,2′-dinitrobiphenyl[7] via 2,2′-diamino-5,5′-dimethylbiphenyl.

(7) Ullman, F.; Frentzel, L. *Chem. Ber.* **1905**, *38*, 725–729.

(8) Buter, J.; Kellogg, R. M. *J. Chem. Soc., Chem. Commun.* **1980**, 466–467.

(9) Satisfactory analytical and spectroscopic data have been obtained for all new compounds.

(10) Halos, EH-300, Eikosha Co., Osaka, Japan.

(11) Optical resolution of the dibromide **15** by means of HPLC employing (+)-poly(triphenylmethyl methacrylate)[12] gave (−)-**15**, $[\alpha]^{22}_D$ −505° (*c* 1.36 × 10$^{-3}$, MeOH).

(12) Yuki, H.; Okamoto, Y.; Okamoto, I. *J. Am. Chem. Soc.* **1980**, *102*, 6356–6358. Okamoto, Y.; Honda, S.; Okamoto, I.; Yuki, H. *Ibid.* **1981**, *103*, 6971–6973.

(13) McMurry, J. E.; Kess, K. L. *J. Org. Chem.* **1977**, *42*, 2655–2656. Baumstark, A. L.; Closkey, C. J.; Witt, K. E. *Ibid.* **1978**, *43*, 3609–3611.

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