Table I presents the results from variation of cation (Li^+ and $^+NMe_4$),[10] alkylating agent (methyl and ethyl, various leaving groups), and solvent (ether, HMPA, THF, dichloromethane). In this series, with an oxidative isolation procedure,[15] the formation of the ketone is taken as a measure of alkylation at iron while the benzoate derivatives are suggested to arise from oxidation of the alkylidene form (e.g., **1**). Ether and dichloromethane both tend to favor reaction at iron, but addition of HMPA to an ether solution (compare entries 1, 2, and 4) strongly favors O-alkylation (alkylidene formation). The change from lithium cation to the tetramethylammonium ion (entry 3 vs. 4) again changes the selectivity toward O-alkylation. There is a strong dependence on the size of the alkylating agent, with the larger group (Et) favoring O-alkylation (entries 1 and 5, 3 and 6).[16] The soft iodide leaving group produces only the ketone (alkylation at iron) even in the presence of HMPA, while the relatively unreactive *p*-toluenesulfonate leaving group gives predominately O-alkylation (at low conversion, entry 7). Overall, these results give a picture entirely consistent with the familiar chemistry of enolate anions and allow a choice of conditions to favor either ketone products or alkylidene-iron complexes.[17]

We have used the optimum conditions (ethyl fluorosulfonate, ether-HMPA) to generate the complex **1** (64-72% yields), as well as new examples with R_2 = *t*-Bu (**7**), *n*-Bu (**8**), and CH_3 (**9**). The complexes are deep red oils with characteristic IR absorptions and other spectral data.[18] A typical procedure follows. To a solution of $Fe(CO)_5$ (5.88 g, 30.0 mmol) in ether (160 mL) was added dropwise PhLi (33 mmol in cyclohexane solution) at −78 °C under argon. The solution was allowed to warm to O °C over ca. 2 h, HMPA (40 mL) was added, and the deep brown solution was returned to −78 °C. Ethyl fluorosulfonate (14.5 mL, 150 mmol) was added dropwise with vigorous stirring. After being stirred at −78 °C for 4 h, the mixture was warmed slowly to 25 °C and partitioned between hexane and saturated aqueous sodium bicarbonate. After the usual washing, drying, and concentrating of the hexane solution, the residue was chromatographed (silica gel, hexane, under argon) to give 6.53 g (72%) of tetracarbonyl(ethoxyphenylmethylidene)iron, **1** (R_1 = Et, R_2 = Ph).[18,19]

Preliminary tests of the reactivity of **1** suggest some differences with the Cr analogue. Reaction of **1** with excess ethyl vinyl ether under 55 psi of CO at 50 °C for 1.0 h gave a mixture from which the major product (76% yield after isolation) has been shown to be **10**.[20] While reactions of an alkylidene-tantalum complex with alkenes is known to give products of the type represented by **10**,[21] this pathway is not significant with the chromium series and has not been tested previously with iron. The mechanisms and scope of the reactions of alkynes and alkenes with the alkylidene iron complexes are under study.

Acknowledgment. We are pleased to acknowledge financial support from the National Science Foundation (CHE-82-04339) and helpful discussions with Professors A. Mayr and J. Schwartz and J. W. Herndon at Princeton.

(15) Several techniques have been used to oxidize chromium derivatives such as **2**, replacing the carbon-chromium bond with a carbon-oxygen double bond: (a) Fischer, E. O.; Riedmüller, R. *Chem. Ber.* **1974**, *107*, 915. (b) Casey, C. P.; Burkhardt, T. J.; Bunnell, C. A.; Calabrese, J. C. *J. Am. Chem. Soc.* **1977**, *99*, 2127. We are employing a procedure previously used to detach 1,3-dienes from $Fe(CO)_3$: Emerson, G. F.; Mahler, J. F.; Kochbar, R.; Pettit, R. *J. Org. Chem.* **1964**, *29*, 3620. The reaction mixture was diluted with acetone at 25 °C, and a GC standard (*p*-chloroacetophenone) was added. Then solid ferric chloride was added in small portions until gas evolution ceased. The resulting mixture was partitioned between ether and saturated aqueous sodium carbonate solution; the ether solution was washed repeatedly with brine and concentrated carefully at reduced pressure. GC analysis for methyl and ethyl benzoates and acetophenone was calibrated with pure samples of the products.

(16) The effect of steric size in the alkylating agent for Me, Et, and *i*-Pr is observed in alkylation of β-dicarbonyl compounds: (a) Chatterjee, A.; Banerjee, E.; Banerje, S. *Tetrahedron Lett.* **1965**, 3851. (b) Yoffe, S. T.; Vatsuro, K. V.; Kugutcheva, E. E.; Kabachnik, M. I. *Ibid.* **1965**, 593. (c) House, H. O. "Modern Synthetic Reactions", 2nd ed.; Benjamin: New York, 1972; pp 526-527.

(17) It is of interest to see if the reactivity of the chromium acylate anions can be manipulated in the same way, to develop a chromium analogue of Collman's reaction. In a preliminary study, we find that acylate salt **2** (R_1 = Li) reacts with excess allyl iodide in a mixture of THF/HMPA to give phenyl propenyl ketones (mixture of α,β and β,γ isomers) in 17% yield. Similarly, benzyl iodide reacts slowly with **2** (R_1 = Li) to give phenyl benzyl ketone (deoxybenzoin) in 12% yield. These "soft" alkylating agents appear to enhance the tendency toward alkylation at the metal, but the process seems unlikely to be preparatively useful.

(18) **1**:[5,10] ^1H NMR (acetone-d_6) δ 7.50 (s, 5 H), 5.21 (q, J = 7.2 Hz, 2 H), 1.69 (t, J = 7.2 Hz, 3 H); IR[6] (hexane) 2055, 1988, 1962, 1945 cm^{-1}; MS, calcd 301.9878, found 301.9882. **7**: ^1H NMR δ 5.30 (q, J = 7.2 Hz, 2 H), 1.67 (t, J = 7.2 Hz, 3 H), 1.34 (s, 9 H); IR 2053, 1986, [1954 and 1941 maxima on one broad band] cm^{-1}; MS, calcd 282.0191, found 282.0196. **8**: ^1H NMR δ 5.17 (q, J = 7.2 Hz, 2 H), 3.43 (t, J = 7.5 Hz, 2 H), 1.63 (t, J = 7.2 Hz, 3 H), 1.85-1.04 (m, 4 H), 0.92 (t, J = 6.4 Hz, 3 H); IR 2056, 1992, [1960 and 1950 maxima on one broad band] cm^{-1}; MS, m/e 282 (P, 4.5% of base at 85), 254 (14) 226 (3.4), 198 (19), 170 (10), 142 (21), 114 (31), 99 (21), 85 (100). **9**: ^1H NMR δ 5.04 (q, J = 7.2 Hz, 2 H), 3.17 (s, 3 H), 163 (t, J = 7.2 Hz, 3 H); IR 2056, 1995, 1961, 1948 cm^{-1}. **9** was too unstable to obtain meaningful composition data.

(19) The complexes **1** and **7**, derived from phenyllithium and *tert*-butyllithium, respectively, are relatively air stable at 25 °C; they can be handled as neat oils and in solution without special precautions. However, the complexes **8** and **9** are considerably more sensitive to oxidation and have been manipulated in an argon atmosphere. In general, the hexane and aqueous solutions used in the extraction procedure were saturated with argon by bubbling the gas into the liquid for a few minutes. No effort was made to rigorously exclude oxygen during the isolation procedures.

(20) **10**: The product is homogeneous as judged by ^{13}C NMR and analytical chromatographic data; however, we have not established whether it is the *E* or *Z* isomer; ^1H NMR (acetone-d_6) δ 7.61-7.24 (m, 5 H), 5.46 (t, 1 H, J = 6.9 Hz), 4.19 (d, 2 H, J = 6.9 Hz), 3.69 (q, 2 H, J = 7.5 Hz), 3.48 (q, 2 H, J = 7.5 Hz), 1.23 (t, 3 H, J = 7.5 Hz), 1.15 (5, 3 H, J = 7.5 Hz); IR (neat) 1664 (s), 1079 (vs) cm^{-1}; MS, m/e 206 (P, 9.7% of base at 105), 177 (6.1), 161 (23), 133 (20), 115 (10), 105 (100), 91 (9.0), 77 (51).

(21) The formation of **10** can be rationalized by rearrangement of an initial ferrocyclobutane species, following the reactivity pattern of alkylidene niobium and tantalum complexes: McLain, S. M.; Wood, C. D.; Schrock, R. R. *J. Am. Chem. Soc.* **1977**, *95*, 3519-3520.

Design of a Peptide Hormone: Synthesis and Characterization of a Model Peptide with Calcitonin-like Activity

Gregory R. Moe,† Richard J. Miller,‡ and E. T. Kaiser*

Departments of Chemistry and Pharmacology
The University of Chicago, Chicago, Illinois 60637
Laboratory of Bioorganic Chemistry and Biochemistry
The Rockefeller University, New York, New York 10021

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Calcitonin is a peptide hormone produced by the parafollicular cells, which are scattered throughout the thyroid in mammals but which constitute a distinct organ, the ultimobranchial body, in lower animals. The common form of the hormone consists of 32 amino acids, has a disulfide bridge between the cysteine residues at positions 1 and 7, and ends at the carboxyl terminus with the amide of proline (Figure 1).

While calcitonins from at least nine different species have been sequenced and characterized biologically and a number of synthetic analogues have been studied, few clear correlations have been made between structure and biological activity. From studies of peptide models of apolipoprotein A-I, the bee venom toxin melittin, and most recently, the peptide hormone β-endorphin the importance of amphiphilic helical regions to the biological activities of a variety of peptides and proteins that interact with lipid and/or protein surfaces has been shown.[1] When the amino acid sequences of natural variants of calcitonin in the region from residues 8 to 22 were viewed as axial projections of α-helices (as in Figure 1B),

* To whom correspondence should be addressed at The Rockefeller University.
† Department of Chemistry, The University of Chicago.
‡ Department of Pharmacology, The University of Chicago.

(1) Taylor, John W.; Osterman, David G.; Miller, Richard J.; Kaiser, E. T. *J. Am. Chem. Soc.* **1981**, *103*, 6965.

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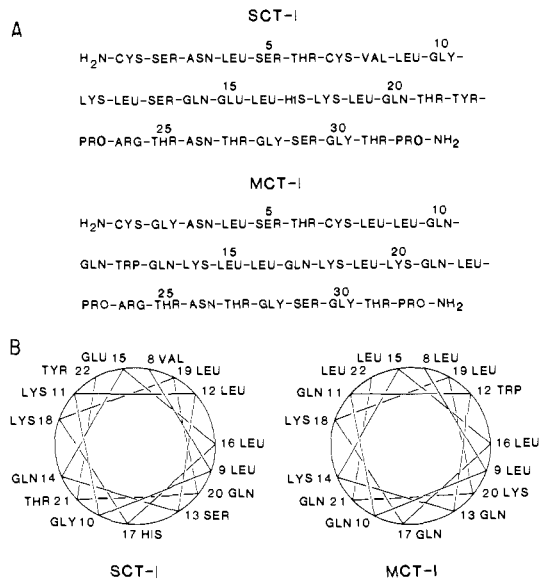

Figure 1. (A) Amino acid sequences of SCT-I and MCT-I. (B) Axial projection of the α-helical region (residues 8–22) of SCT-I and MCT-I.

it was readily apparent that the segregation of residues into opposing hydrophilic and hydrophobic faces of cylindrical segments was a feature common to all of them. Furthermore, there appeared to be a correlation between the relative amphiphilic α-helical character in the region from residues 8 to 22 and the biological activities of the calcitonins. On the basis of these observations, we propose a model for calcitonin structure in which there are three structural regions: a "loop" involving the disulfide bridge between cysteine residues 1 and 7, an amphiphilic α-helical segment beginning at residue 8 and ending at position 22, and a hydrophilic random coil sequence from residue 23 to residue 32.

To test the importance of amphiphilic α-helical structure to the biological activity of the calcitonins, we have synthesized a model peptide (MCT-I, Figure 1) and characterized its chemical and biological properties, using salmon calcitonin I (SCT-I), the naturally occurring calcitonin most active and most stable to enzymatic degradation, as the standard for comparison. In the region from residues 8 to 22 the model peptide was designed to have as little sequence homology as possible to any of the natural calcitonins, while retaining the same balance of charge and hydrophobicity to hydrophilicity as SCT-I. In addition, amino acids having a high helix-forming potential were chosen to constitute the helical region of MCT-I.[2] Thus, leucines were selected as the hydrophobic residues, glutamines as neutral hydrophilic residues, and lysines as basic hydrophilic residues. In the remainder of the MCT-I molecule, residues 1–7 correspond to the sequence from residues 1 to 7 of human calcitonin, and residues 23–32 correspond to the equivalent region of SCT-I. Because of its possible usefulness in future fluorescence studies, Trp was placed in the middle of the hydrophobic face of the helical region.

MCT-I was synthesized by the solid-phase method and purified to homogeneity by ion-exchange chromatography on CM-Sephadex C-25 and reverse-phase HPLC on a Waters C_{18} semipreparative column.[3] The peptide was judged to be pure on the basis of the observation at 230 nm of a single symmetrical peak when the peptide was eluted from a Waters C_{18} reverse-phase column using a gradient from 20% to 50% CH_3CN as the eluting solvent and from its amino acid analysis after hydrolysis with 5.5 M HCl. The amino acid analysis was as follows: Arg, 1.11 (1); Asp, 2.01 (2); Cys, 2.09 (2); Glu, 5.00 (5); Gly, 3.02 (3); Leu, 7.14 (7); Lys, 2.94 (3); Pro, 1.63 (2); Ser, 1.61 (2); Thr, 3.5 (4).

The circular dichroism (CD) spectra of MCT-1 and SCT-I from 250 to 205 nm show minima at 222 and 208 nm, characteristic of α-helical structure. For MCT-1, the mean residue molar ellipticity at 222 nm, $[\theta]_{222}$, was −7800 deg cm^2/dmol (10^{-4} M peptide, 0.02 M sodium phosphate buffer, 0.16 M KCl, pH 7.4), from which the α-helicity was estimated to be 30%.[4] The value of $[\theta]_{222}$ does not change over a range of concentration of MCT-I from 10^{-7} to 10^{-4} M, provided that binding to glass is prevented by pretreatment of the spectrometer cell with polyethylene glycol (Mr 15K–20K). This suggests strongly that MCT-I remains monomeric over the concentration range employed, a conclusion supported by the measurement of a molecular weight of about 4500 at a concentration of 10^{-4} M MCT-I by means of ultracentrifugation using of Beckman Spinco Airfuge.[5] Similarly, the value of $[\theta]_{222}$ for solutions of SCT-I over the same concentration range and under the same conditions also remains constant at −4600 deg cm^2/dmol, leading to an estimate of 20% α-helix for this peptide. In 50% trifluoroethanol, a structure promoting solvent, both MCT-I and SCT-I were estimated to be 50% α-helical at a concentration of 5×10^{-5} M, as was found by Brewer et al.[6] for porcine calcitonin (PCT) in 50% 2-chloroethanol.

At the air–water interface, MCT-I and SCT-I form insoluble monolayers when spread from concentrated solutions in 0.01 M HCl. The force–area (π–A) curves between 5 and 12 dyn/cm are described by the equation $\pi[A - A_0(1 - \kappa\pi)] = nRT$, where κ is the compressibility and A_0 is the limiting molecular area extrapolated to zero surface pressure. The parameters calculated

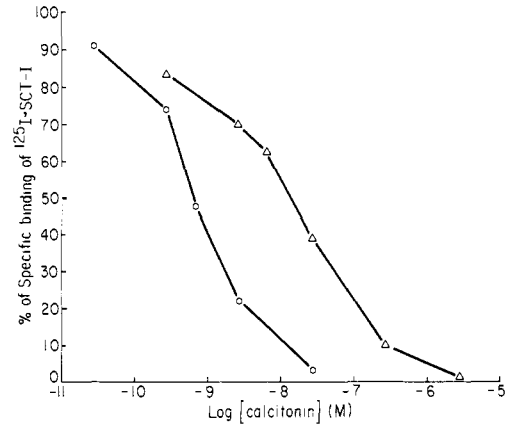

Figure 2. Competitive inhibition of ^{125}I–SCT-I binding to brain particulate fraction by SCT-I (○) and MCT-I (△). Each point represents the mean of three triplicate determinations.

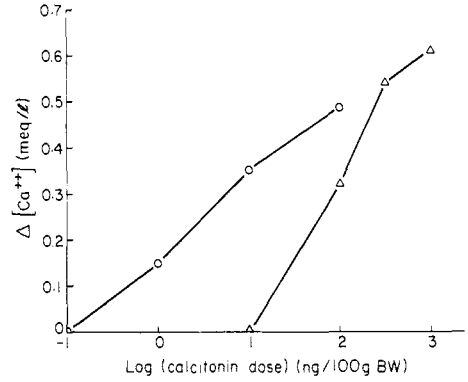

Figure 3. The log dose–response curves for SCT-I (○) and MCT-I (△). Each point represents the differences between the average serum $[Ca^{2+}]$ for rats (20 used) given only saline and the average for those given a particular dose of either MCT-I (15 rats per point) or SCT-I (5 rats per point).

(2) Chou, P. Y.; Fasman, G. D. *Annu. Rev. Biochem.* **1978**, *47*, 251.
(3) The procedures for the synthesis and purification of MCT-I will be published later.
(4) Morrisett, J. D.; Davis, J. S. K.; Pownall, H. J.; Gotto, A. M. *Biochemistry* **1973**, *12*, 1290.
(5) Pollet, R. J.; Haase, B. A.; Standaert, M. L. *J. Biol. Chem.* **1979**, *254*, 30.
(6) Brewer, H. B.; Edelhoch H. *J. Biol. Chem.* **1970**, *245*, 2402.

for the two peptides were very similar, κ = 0.016 cm/dyn for MCT-I and 0.02 cm/dyn for SCT-I, while A_0 = 362 Å^2 for the model peptide and A_0 = 322 Å^2 for the natural analogue. However, the collapse pressure of 24 dyn/cm found for the monolayer of MCT-I was much higher than the value of 14 dyn/cm observed for SCT-I.

In order to study the receptor binding properties of both peptides, ^{125}I-SCT-I was prepared by the method of Hunter and Greenwood.[7] The iodinated hormone was purified by ion-exchange chromatography on SP-Sephadex C-25. Competitive binding experiments with rat brain homogenates were carried out as described by Nakamuta et al.[8] The binding curves obtained (Figure 2) gave IC_{50} values for SCT-I of about 1 nM, in agreement with the value reported earlier,[8] and 20 nM for MCT-I, which compares to the value of 17 nM found for PCT.[8]

The biological potency of MCT-I was assessed in vivo by the method of Kumar et al.[9] The dose-response curve in Figure 3 summarizes the results. As with the binding studies, MCT-I is about 10-fold less potent than SCT-I, or approximately as active as PCT, the most potent mammalian analogue.

Although the sequence of amino acids in MCT-I differs from that in SCT-I from positions 8 to 22, the model peptide exhibited chemical and biological properties similar to those of the natural hormone. Like SCT-I, MCT-I was monomeric in aqueous solution. The model peptide showed somewhat more α-helical character than the natural one did under these conditions, and at the air–water interface, an amphiphilic environment, it formed a much more stable monolayer than did SCT-I. Moreover, MCT-I displaced a specifically bound ligand from calcitonin receptors in vitro and effected a potent hypocalcemic response in the rat bioassay. Taken together, these results provide strong evidence that the region from residues 8 to 22 of the calcitonins has a primarily structural role, providing an amphiphilic surface in the α-helical conformation for binding interactions with its receptor. Further studies are now in progress to determine what structural features might be altered to provide calcitonins with enhanced biological activity.

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(7) Hunter, W. M.; Greenwood, F. C. *Nature* (London) **1962**, *194*, 495.
(8) Nakamuta, H.; Furukawa, S.; Koida, M.; Yajima, H.; Orlowski, R. C.; Schlueter, R. *Jpn. J. Pharmacol.* **1981**, *31*, 53.
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Jump Rope Enantiomerization of 1,5-Naphthalenophanes as a Probe of Polymethylene Chain Conformational Dynamics

Moon Ho Chang and Dennis A. Dougherty*

Contribution No. 6788 from the Crellin Laboratory of Chemistry California Institute of Technology Pasadena, California 91125

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Interest in the structures, properties, and conformational dynamics of polymethylene chains remains intense because of the crucial role of such structures in biological membranes and related bilayer systems, micelles, and synthetic polymers.[1] A wide variety of techniques has been applied to probe chain dynamics, but relatively few studies involving the direct observation of an unambiguously defined conformational process involving a polymethylene chain have been reported.[1b,c] In the present work, we use dynamic NMR spectroscopy (DNMR) to study the interconversion of the enantiomers of 1,5-naphthalenophanes **1**. Such

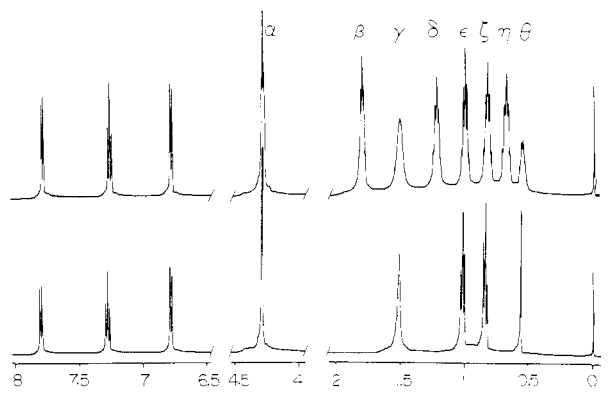
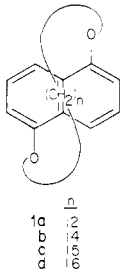


Figure 1. 500-MHz ^{1}H NMR spectra of **1c** (top) and **2** (bottom) at 49 °C in CCl_4 + 10% $CDCl_3$ (Me_4Si). Decoupling experiments on **1c** and the labeling pattern in **2** unambiguously led to the assignments shown.



	n
a	12
b	14
c	15
d	16

a process involves substantial conformational changes along the aliphatic chain of **1** and can thus provide a general method for probing substituent, solvent, and conformational effects in polymethylene chains.

Structures such as **1** are chiral, having at most C_2 symmetry. All methylene groups when n is even and all but the central methylene group when n is odd consist of a diastereotopic pair of protons. The enantiomers can interconvert by simply moving the polymethylene chain around to the other face of the naphthalene system[1c] in what has been termed[2] a "jump rope" reaction. This process also interconverts the diastereotopic protons of each methylene group, thus providing a potential DNMR probe of the enantiomerization.

Both CPK molecular models and molecular mechanics calculations[3] indicate that structures **1a–d** are essentially strain free and possess many low-energy conformations with varying numbers and locations of gauche interactions. These conformations are expected to interconvert rapidly on the NMR time scale by C–C bond rotations over relatively small barriers. We anticipated that in the enantiomerization transition state such torsions would be severely restricted, and the chain would adopt a relatively extended conformation in order to pass around the naphthalene system. If this were the case, the enantiomerization would provide a direct

(1) (a) For a recent review, see: Winnik, M. A. *Chem. Rev.* **1981**, *81*, 491–524. (b) For studies of large ring cycloalkanes, see: Dale, J. *Top. Stereochem.* **1976**, *9*, 199–270. Anet, F. A. L.; Rawdah, T. N. *J. Am. Chem. Soc.* **1978**, *100*, 7166–7171; 7810–7814. (c) The process studied in the present work is, of course, precisely analogous to the enantiomerization of *trans*-cycloalkenes. See, for example, Binsch, G.; Roberts, J. D. *J. Am. Chem. Soc.* **1965**, *87*, 5157–5162. For studies on systems related to **1**, see: Whitesides, G. M.; Pawson, B. A.; Cope, A. C. *Ibid.* **1968**, *90*, 639–644. Brown, H. S.; Muenchausen, C. P.; Sousa, L. R. *J. Org. Chem.* **1980**, *45*, 1682–1686. Whitlock, B. J.; Whitlock, H. W. *J. Am. Chem. Soc.* **1983**, *105*, 838–844.
(2) Marshall, J. A. *Acc. Chem. Res.* **1980**, *13*, 213–218.
(3) Details will be provided in the full account of this work. The program BIGSTRN (Andose, J. D.; et al. *QCPE* **1979**, *11*, 348) was used. See: Mislow, K.; Dougherty, D. A.; Hounshell, W. D. *Bull. Soc. Chim. Belg.* **1978**, *87*, 555–572.

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