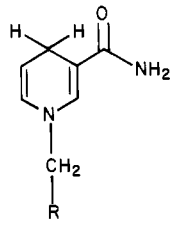

9, R = C_6H_5
10, R = CH_2CH_3
11, R = $(CH_2)_4CH_3$

and extrapolate to zero as the concentrations of flavin approaches zero.[14,15] These model rate constants are also shown in Table I. The data are consistent with the expected mechanism of oxidation: a bimolecular oxidation of nicotinamide by flavin followed by a fast reoxidation of the reduced flavin by oxygen.

As can be seen from the data presented in Table I, flavopapain **1** is capable of producing enzymatic rate enhancements of about 600-fold by using N^1-hexyldihydronicotinamide **11** as a substrate. The next best semisynthetic enzyme, flavopapain **4**, is capable of producing only an 18-fold enzymatic rate enhancement for the same substrate.[3] Catalysis by flavopapain **1** thus represents an improvement of well over an order of magnitude in the rate enhancement.

The new flavopapain **1** is also more selective than is either the isolated flavin **3** or the previously described flavopapain **4**. The selectivity of enzyme **1** is in complete accord with our postualated hydrophobic binding interaction. Since the largest kinetic differences among the substrates reside in the K_m term, we feel that the selectivity of this enzyme may be derived mainly from differences in the binding ability for the various substrates. The K_m and k_{cat} may not be kinetically simple, and therefore further explanation of the catalytic efficiency and selectivity of this enzyme must await future mechanistic study.

The kinetic parameters determined for flavopapain **1** compare favorably with those for many natural flavoenzymes which oxidize dihydropyridine nucleotides. Although out best value of k_{cat}/K_m for **1** is well below the $k_{cat}/K_m \simeq 10^8$ M^{-1} s^{-1} reported for bovine heart NADH dehydrogenase,[16] this is an unusually efficient catalyst and the rate parameter measured probably represents an upper limit for this type of dehydrogenation reaction. Values of k_{cat}/K_m of 3227 M^{-1} s^{-1} [17] and 610 M^{-1} s^{-1} [18] have been reported for the oxidation of NADH by old yellow enzyme. Using the more recently obtained value of 610 M^{-1} s^{-1}, this represents a catalytic rate enhancement of 1365-fold over the rate of oxidation of NADH by lumiflavin—a rate enhancement on the same order as that which we have obtained. Old yellow enzyme will also oxidize N^1-propyl-1,4-dihydronicotinamide **9** with k_{cat}/K_m = 20 300 M^{-1} s^{-1}; this represents a rate enhancement of about 200 over that measured using lumiflavin. For this substrate, at least, our semisynthetic enzyme, with a k_{cat}/K_m of 59 000 M^{-1} s^{-1} is the superior catalyst.

The comparison between old yellow enzyme and flavopapain may not be the best one to make because the in vivo function of old yellow enzyme, as well as the identity of its natural substrates, remain unknown. Consequently, we have sought other examples of flavoenzymes with which to compare the kinetic parameters of flavopapain. One such enzyme is melilotate hydroxylase, a microbial monooxygenase which uses NADH and O_2 as substrates to hydroxylate melilotate and thus produces 2,3-dihydroxy-β-phenylpropionate and water. Extensive kinetic studies have revealed that enzyme first binds melilotate to form an oxidized enzyme–substrate complex, which reacts very rapidly with NADH forming a ternary complex of reduced flavoenzyme, NAD^+, and melilotate with an observed second-order rate constant of 2.3 × 10^6 M^{-1} s^{-1}.[19] This value is approximately 4 times larger than the k_{cat}/K_m value for the reaction of flavopapain **1** with N^1-hexyldihydronicotinamide.[20] Another useful comparison is to the glucose oxidase from *Aspergillus niger*. Values of k_{cat}/K_m of about 10 500 M^{-1} s^{-1} have been determined for this enzyme[21] which is thought to operate via a kinetic scheme analogous to the one we have postulated for flavopapain **1**. Here, flavopapain **1** is more effective in catalyzing the oxidation of a good substrate by a factor of over 50-fold. Clearly, the k_{cat}/K_m values measured for our semisynthetic flavopapain **1** are of roughly the same magnitude as those observed for typical naturally occurring flavoenzymes.

Further work, directed at elucidating the mechanism and stereospecificity of this interesting semisynthetic flavopapain **1** is now in progress.

Acknowledgment. Support of this research by NSF Grant DAR 7910245 (E.T.K.) and USPHS Postdoctoral Fellowship GM-07766-03 (J.T.S.) is gratefully acknowledged.

(14) These results are obtained only when kinetics are measured in the presence of superoxide dismutase and catalase.
(15) Although the oxidation of dihydronicotinamide by flavins has been postulated to proceed via a charge-transfer complex, the dissociation constant for the complex of 0.1 M would mean that under the conditions of our experiments where [N^1-RNH] ≤ 2.5 × 10^{-4} M complex formation would be negligible and saturation would not be observed. See: Blankenhorn, G. *Biochemistry* **1975**, *14*, 3172-3176 and references therein.
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(18) See references in ref 13.
(19) Massey, V.; Hemmerich, P. *Enzymes, 3rd Ed.* **1975**, *12*, 217-221.
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A Strategy for the Synthesis of Cylindrical Macropolycyclic Hosts with Hydrophilic Interior Surfaces: Crown Ether Rings Fused by the Tetrahydroxymethylethylene (THYME) Unit[1]

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Host–guest chemistry,[2] the design, preparation, and study of organic compounds capable of molecular recognition in complexation, has recently become an exciting and rapidly growing area of research. Many elegant excursions into this field are described in the literature,[2-5] and great strides have been made toward development of methods for design and synthesis of host molecules and toward an understanding of the complexation process. In an effort to extend the host–guest frontier, we have begun a program directed toward the synthesis and study of cylindrical macropolycyclic hosts composed of crown ether rings fused by the tetrahydroxymethylethylene (THYME) unit. Consideration of prior art in the field and examination of CPK molecular models indicate that such hosts may have hydrophilic interior surfaces and, therefore, may show novel and interesting properties including useful catalytic activity. Herein, we report on successful completion of the first phase of this study: development of an efficient, regioselective strategy for synthesis of polycyclic cylindrical hosts possessing the THYME unit as the

[†] University of Colorado Chemistry X-ray facility.
(1) A preliminary account of some of this work was presented at the Second Symposium on Macrocyclic Compounds, Aug 14-16, 1978, at Brigham Young University, Provo, UT.
(2) (a) Cram, D. J.; Cram, J. M. *Science (Washington, D.C.)* **1974**, *183*, 803. (b) Cram, D. J.; Cram, J. M. *Acc. Chem. Res.* **1978**, *11*, 8-14.
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(5) (a) Bender, M. L.; Komiyama, M. "Cyclodextrin Chemistry"; Springer-Verlag: New York, 1977. (b) Bender, M. L.; Komiyama, M. "Bioorganic Chemistry", van Tamelen, E. E., Ed.; Academic Press: New York, 1977; Vol. 1, Chapter 2.

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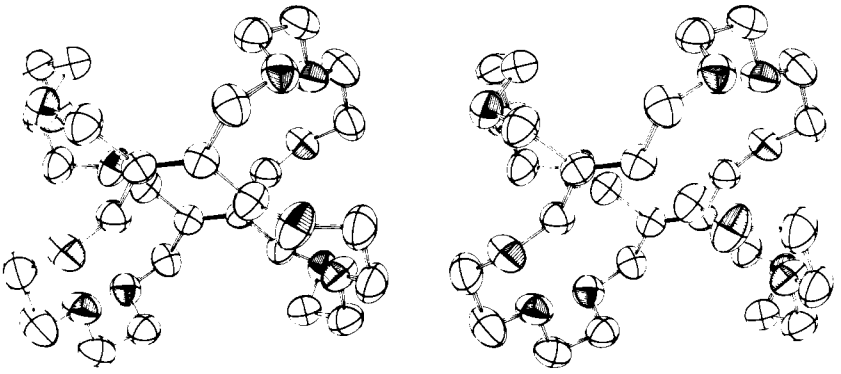
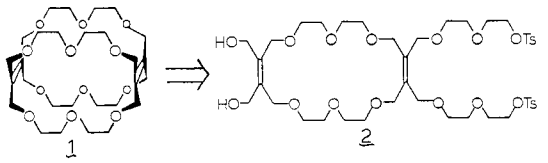


Figure 1. Three-dimensional view of cage 1 as determined by X-ray analysis. Molecule of toluene solvate is omitted.

ring fusion. The strategy is demonstrated with a total synthesis of the first THYME cage, cylindrical macrotricyclic polyether **1**.[6] The key step in our approach is illustrated in eq 1. The

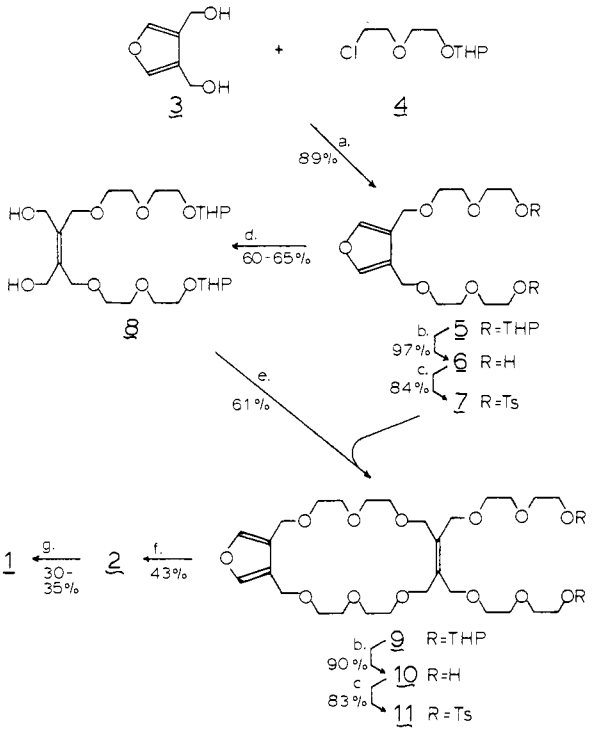


(1)

prototypical THYME cage **1** derives from high dilution cyclization of diol ditosylate **2**. The synthesis thus simplifies to development of methodology for construction of diol ditosylate **2**. To achieve this goal, several problems involving functional group manipulation must be addressed. Chiefly, a method for regioselective generation of free diol units of the THYME in a pairwise manner is required. The chemistry utilized for this operation must be tolerant of primary tosylate groupings and the THYME tetraether function. With respect to the latter, several preliminary experiments directed toward synthesis of cage **1** indicate limitations on possible solutions to the problem. Specifically, the following reaction conditions result in decomposition, probably including allylic ether cleavage, of THYME tetraethers: H_2, Pd/C, CH_3OH; Li, liquid NH_3, −78 °C, 5 min, ethylene bromide quench; BCl_3, CH_2Cl_2, −78 °C, 3 h; LAH, THF, room temperature, 2 h; CH_3Li, THF/ether, −20 °C, 1 h.

The successful strategy for preparation of diol ditosylates of type **2** and the total synthesis of THYME cage **1** are illustrated in Scheme I. Thus, Williamson etherification of readily available 3,4-bis(hydroxymethyl)furan[7] (**3**) with 2-[2-(2-chloroethoxy)-ethoxy]tetrahydro-2*H*-pyran[8] (**4**) affords the key "two-arm" THP ether **5**.[9] Conversion of THP ether **5** to ditosylate **7**[9] via diol **6**[9] is straightforward. After extensive experimentation, conditions for the crucial transformation of furan THP ether **5** to the THYME diether **8** were developed on the basis of an efficient electrochemical oxidation of furans reported by Magnusson.[11] Platinum anodic oxidation of furan **5** (100 mA, 6 V, lithium tetrafluoroborate) in aqueous acetonitrile containing sodium bicarbonate (argon ebullition) gives a labile bis-hemiacetal that rapidly polymerizes on standing. However, immediate addition of a trace of hydroquinone, removal of solvent at reduced pressure,

Scheme I



[a] (a) 4 equiv of **4**, NaH, DMF, room temperature 48; (b) Dowex 50W-X12, CH_3OH/CH_2Cl_2, reflux, 12 h; (c) TsCl/pyr, 0 °C, 12 h; (d) 1. Pt anode, CH_3CN/aqueous $NaHCO_3$, trace $LiBF_4$, argon ebullition, 2. $NaBH_4$, 50% $EtOH/H_2O$; (e) 0.01 M in DMF, 10 equiv by NaH, room temperature, 48h; (f) 1. same as (d), 2. $NaBH_4$, EtOH; (g) DMF, 20 equiv of NaH, slow addition over 12 h, final concentration 0.002 M.

and borohydride reduction in 50% aqueous ethanol affords the desired THYME diether **8**[9] in 60–65% overall yield from **5**. When THYME diether **8** is allowed to react with furan-ditosylate **7** under standard crown ether forming conditions, a good yield of the 22-crown-6 derivative **9**[9] is obtained. The synthetic strategy now requires acid-catalyzed deprotection of the THP alcohol moieties of THYME tetraether **9**. It was expected that such a tetraether function would be stable to mildly acidic conditions since the electron-withdrawing alkoxymethyl substituents should destabilize cationic intermediates derived from protonation on oxygen or carbon of the THYME unit. In fact, under the conditions utilized for the deprotection (50% methanol/dichloromethane, Dowex 50W-X12, reflux, 12 h) both the furan and THYME functions are stable, and diol **10**[9] is obtained in good yield. Conversion to ditosylate **11**[9] is straightforward.

At this stage the efficacy of our approach depends upon the stability of the tosylate groupings of furan-crown-ditosylate **11** under conditions required for THYME diol formation. In fact, platinum anodic oxidation of furan **11**, followed by borohydride

(6) The IUPAC name of THYME cage **1** is 4,7,10,15,18,21,24,27,30,33,36,39-dodecaoxatricyclo[11.9.9.9^{2,12}]tetraconta-1,12-diene.

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(9) All new compounds except THYME cage **1** were viscous oils and were purified by flash chromatography[10] on silica gel. All new compounds showed consistent ^1H and ^{13}CNMR spectra and IR spectra and were homogeneous by TLC and ^{13}C NMR spectroscopy. Compounds **5**, **8**, **9**, and **1** gave satisfactory combustion analyses. Apparently as a result of their extremely viscus nature, compounds **6**, **7**, **10**, and **11** could not be dried sufficiently for satisfactory combustion analysis.

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reduction of the crude bis-hemiacetal, affords the key diol-ditosylate **2**[9] with adequate efficiency, thereby paving the way for the crown ether forming macrocyclization reaction leading to THYME cage **1**. Gratifyingly, addition of a dry DMF solution of diol-ditosylate **2** to a suspension of oil-free sodium hydride in dry DMF over 12 h (final concentration 0.002 M) followed by removal of solvent at reduced pressure, aqueous workup (dichloromethane), and flash chromatography[10] on Woelm N III alumina gives the tricyclic THYME cage **1** (white microcrystalline solid, mp 118-123 °C, 33-35% yield) as the major product.

The physical and spectral properties of cage **1** are consistent with the high symmetry (D_{2h}) and tricyclic nature of the molecule. Cage **1** is soluble in water, dichloromethane, and chloroform. It is slightly soluble in toluene and insoluble in hexane. The mass spectrum of cage **1** shows an abundant molecular ion (16% of the base peak); the ^{1}H NMR spectrum shows two peaks [($CDCl_3$) δ 3.50-3.83 (br s, 2 H, OCH_2CH_2O), 4.13, 4.31 (ABq, 1 H, J = 11.8 Hz, allylic CH_2)], and the proton-decoupled ^{13}C NMR spectrum shows four unique carbon resonances [($CDCl_3$) δ 67.95, 69.80, 71.11, 136.58). Recrystallization of the chromatographed solid from toluene affords prisms of a 1:1 toluene solvate suitable for X-ray analysis. This solvate loses toluene slowly at room temperature and pressure and rapidly at reduced pressure. After removal of toluene in vacuo, the remaining solid exhibits a melting point identical with that of the chromatographed material. The assigned structure is unequivocally proven by single-crystal X-ray analysis of the toluene solvate[12] (Figure 1). As indicated by the extreme ease with which toluene is lost from this solvate, toluene molecules in the crystal are not associated with molecules of the cage. As expected, the free ligand adopts a conformation in the crystal effectively filling the cavity space. The atoms of the cage are located about a center of symmetry. The two planes defined by the THYME units are parallel but are not perpendicular to the plane defined by the four olefinic carbons. While all of the OCH_2CH_2O units are gauche, four of the eight allylic methylenes are pointing inside the cavity. This sort of behavior is common in crystalline free crown ethers[3] and is not indicative of a poor host.

In conclusion, the efficacy of an approach to the synthesis of novel cylindrical polyether hosts is proven with the directed total synthesis of THYME cage **1**. We hope that compound **1** and related hosts will prove interesting and useful as ion binders, hosts for organic species, and catalysts. Studies on the complexation properties of host **1** are in progress as are studies directed toward extension of the synthetic strategy to preparation of larger cylindrical cages and other topologically fascinating structures.

Acknowledgment is made to the donors of the Petroleum Research Fund, administered by the American Chemical Society, for partial support of this research, to the Research Corporation and the National Science Foundation (RIAS) for financial assistance, and the University of Colorado Computing Center for blocks of computer time. We thank Professor Donald J. Cram for many helpful discussions during the early phases of this work.

Supplementary Material Available: Tables of atomic positional and thermal parameters and observed and calculated structure factors (6 pages). Ordering information is given on any current masthead page.

(12) Crystals of cage **1** grown from toluene are triclinic, space group $P\bar{1}$—C_i^1 (no. 2) with a = 8.451 (4) Å, b = 10.722 (7) Å, c = 10.054 (6) Å, α = 93.05 (5)°, β = 102.59 (4)°, γ = 95.33° and U = 882.7 (8) Å^3. On the basis of density considerations (D_0 = 1.29, D_c = 1.26 g/cc) there is one molecule of cage and one molecule of toluene per unit cell. Three dimensional X-ray data were collected on a computer controlled Nicolet P$\bar{1}$ four-circle diffractometer by using graphite monochromated Mo K$\bar{\alpha}$ radiation and θ-2θ scans. Of the 1730 reflections measured up to 2θ = 40° 1091 were determined to be observed [$F_o^2 > 3.0\sigma(F_o^2)$]. The structure was solved by direct methods and was refined using full-matrix least-squares procedures. Hydrogen atoms, except the methyl hydrogens of the disordered toluene, were located and included in fixed idealized positions. The disordered toluene solvent was treated as a rigid group in refinement. All other nonhydrogen atoms were treated anisotropically. At convergence, the final residuals were R = 0.056 and wR = 0.070. Full details of the structural analysis will be reported.

Facile Carbon-Carbon Bond Rotation in Azaallyllithium Reagents

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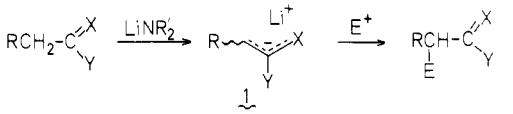
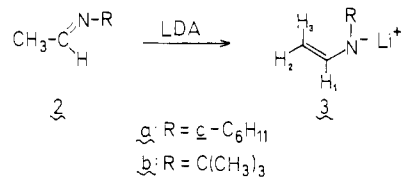
Stabilized carbanions (**1**), generally prepared by lithium dialkylamide deprotonation of carbonyl compounds and their derivatives, are an important and versatile class of reactive intermediates. Recent work has emphasized the stereoselectivity which



(1)

can be obtained in reactions employing these intermediates.[1] In particular, we and others have related the overall stereoselectivity observed in a two-step asymmetric synthesis involving the reaction sequence of eq 1 in part to the stereochemistry about the C_1–C_2 bond in **1**.[2] This of course assumes that the C_1–C_2 bond is "rigid" on the synthetic time scale. Previous studies[3] as well as the present study support this belief; however, the present study demonstrates the first unambiguous example of rapid C_1–C_2 bond rotation in an azaallyllithium reagent. These results clearly show that the utility of intermediates like **1** in stereoselective reactions may be determined not only by the stereoselectivity of their formation but also by the surprisingly low barrier to C_1–C_2 bond rotation.

Aldimines **2a** and **2b** were prepared by the reaction of acetaldehyde with the appropriate primary amine. Deprotonation of **2a** and **2b** with lithium diisopropylamide (LDA) in tetrahydrofuran (THF) produced the azaallyllithium reagents **3a** and **3b**, respectively. In both cases, the rotation about the C_1–C_2 bond was



readily observed by variable temperature ^{1}H NMR spectroscopy. At low temperature (0 °C), the formyl proton, H_1, appeared as a doublet of doublets (**3a**: δ 6.90, J_{cis} = 7.7 Hz, J_{trans} = 14.5 Hz; **3b**: δ 6.93, J_{cis} = 7.4 Hz, J_{trans} = 14.6 Hz). The ^{1}H NMR spectrum reported for **3a** at 25 °C in THF-d_8 is virtually identical with that which we observed.[3c] These vicinal coupling constants were similar to those reported for the lithium enolate of acetaldehyde[4] and the azaallyllithium reagents prepared from acetaldehyde dimethylhydrazone[3a] and *N*-isopropylacetaldimine.[5] However, warming solutions of **3a** and **3b** led to an unexpected, reversible change in the multiplicity of the H_1 signals. At 70 °C,

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