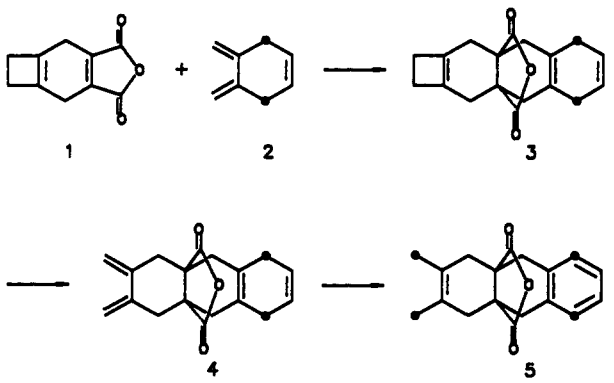
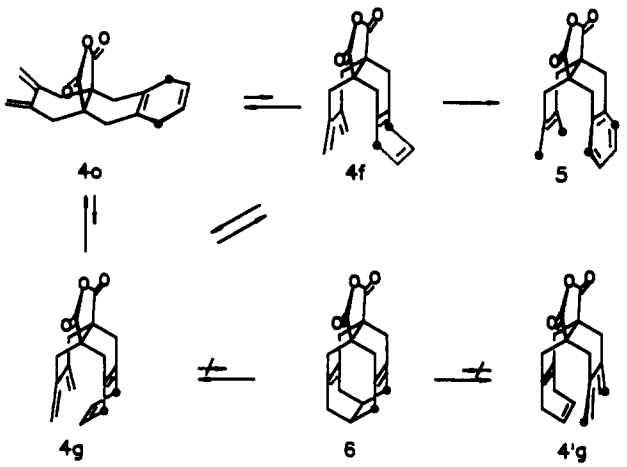
Scheme I[a]



[a] **2**-d_2–**4**-d_2: Each of the marked positions is substituted by one deuterium. **5**-d_2: Any two of the marked positions, separated by σ_v, are substituted by one deuterium. **2**-d_4–**4**-d_4: Each of the marked positions is substituted by two deuteriums. **5**-d_4: Each of the marked positions is substituted by one deuterium.

Scheme II[a]



[a] **4**-d_4, **6**-d_4: Each of the marked positions is substituted by two deuteriums. **5**-d_4: Each of the marked positions is substituted by one deuterium.

bicyclo[4.2.0]octa-1(6),3-diene, which after flash vacuum pyrolysis led to both stereoisomers of **2**-d_2.

Samples of **3**, **3**-d_2, and **3**-d_4 were heated in degassed 0.023 M benzene solution at 160.7 °C for 17.5 h and analyzed by ^{1}H NMR spectroscopy. From the conversion of **4** (84%) and **4**-d_4 (44%), the primary kinetic isotope effect for the dyotropic migration of two deuterium atoms, $k_{2H}/k_{2D} = 3.16$ (±0.16), is obtained directly. **4**-d_2, however, resulting from **2**-d_2 via **3**-d_2, is a 1:2:1 mixture of three stereoisomers with respect to the orientation of the deuterium atoms. Only in the main isomer with trans deuteriums do H and D migrate, whereas the other two isomers behave as **4** or **4**-d_4, neglecting secondary isotope effects. The measured conversion of **4**-d_2 (67%) had to be corrected for this reason as well as for partial deuteration (82% d_2) to obtain the actual conversion of *trans*-**4**-d_2 (66.4%). From the latter is derived the isotope effect for the dyotropic migration of H and D, $k_{2H}/k_{HD} = 1.68$ (±0.08).

In a synchronous migration, the lower zero point energies of two breaking C–D bonds add in raising the activation energy, i.e., the isotope effects are squares: $k_{2H}/k_{HD} = (k_{2H}/k_{2D})^{1/2} = 1.78$ (±0.05). In a stepwise process, on the other hand, the two isotope effects are related by eq 2,[9] which links $k_{2H}/k_{2D} = 3.16$ (±0.16) with $k_{2H}/k_{HD} = 1.52$ (±0.08).

$$k_{2H}/k_{HD} = 2k_{2H}/(k_{2H} + k_{2D}) \quad (2)$$

(8) Kariv-Miller, E.; Swenson, K. E.; Lehman, G. K.; Andruzzi, R. *J. Org. Chem.* **1985**, *50*, 556.
(9) Taagepera, M.; Thornton, E. R. *J. Am. Chem. Soc.* **1972**, *94*, 1168.

A distinction between the two mechanisms using the double-labeling experiment is not possible because of the error involved in measuring the small isotope effect. An argument for a concerted process is the enthalpy of formation of the diradical intermediate, obtained by addition of increments,[10] which lies 35.5 kcal/mol above that of the reactant **4f** and 7 kcal/mol above that of the transition state. Acid catalysis of the reaction is ruled out by the observation that a 3-fold increase in the surface of the reaction vessel by the addition of glass chips did not affect the rate.

Another possible fate of **4** in the folded conformation **4g** would be closure to the tetrahydro[4]beltene **6**.[11] Although this compound was shown by force field calculations[5] to be 10 kcal/mol more stable than **4g**, it is not produced in the thermolysis reaction. The use of **4**-d_4 allows one to decide whether the failure to observe **6** has a thermodynamic or kinetic origin: Due to the C_{2v} symmetry of **6**, its occurrence even as a minor equilibrium component would transfer the label into the methylene groups of **4'g**-d_4. Since no deuterium could be detected by ^{1}H NMR spectroscopy in the methylene groups of recovered **4**-d_4, the closure of **4** to **6** must be foiled for kinetic reasons.

Acknowledgment. We gratefully acknowledge the Fonds der Chemischen Industrie for support of this work.

Registry No. 1, 138090-41-2; **2**, 54290-41-4; *cis*-**2**-d_2, 138090-46-7; *trans*-**2**-d_2, 138090-47-8; **2**-d_4, 138090-45-6; **3**, 138090-42-3; **3**-d_2, 138090-48-9; **3**-d_4, 138090-49-0; **4**, 138090-43-4; **4**-d_2, 138090-51-4; **4**-d_4, 138090-50-3; **5**, 138090-44-5; D_2, 7782-39-0.

(10) Benson, S. W. *Thermochemical Kinetics*, 2nd ed.; Wiley-Interscience: New York, 1979; pp 272–284.
(11) For the beltene nomenclature, see: Alder, R. W.; Sessions, R. B. *J. Chem. Soc., Perkin Trans. 2* **1985**, 1849.

Preassociating α-Nucleophiles[1]

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The Ohio State University
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Received October 11, 1991

Research on cyclodextrin (CD) transacylase mimics has been among the most fruitful in the artificial enzyme field. While most proteases function efficiently at pH 7.4, βCD itself is well-known to be inert at this pH; rather, it reacts rapidly with esters only when its secondary hydroxyl groups (pK_a 12.1) have begun to deprotonate.[4] Thus, the synthesis of synthetic transacylases with reactivity at neutral pH presents itself as an important goal of practical significance. Toward this end, CDs have been prepared bearing imidazole as a group with reactivity at pH 7;[5] pendant coordination complexes have likewise been employed.[6] However,

(1) This paper is dedicated to Professor Ronald C. D. Breslow on the occasion of his 60th birthday.
(2) On faculty leave from Ohio Wesleyan University, Delaware, OH.
(3) NSF/OSU Young Science Scholars Program participant, Summer 1989.
(4) VanEtten, R. L.; Clowes, G. A.; Sebastian, J. F.; Bender, M. L. *J. Am. Chem. Soc.* **1967**, *89*, 3253.
(5) (a) Cramer, F.; Mackensen, G. *Angew. Chem.* **1966**, *78*, 641. (b) Iwakura, Y.; Uno, K.; Toda, F.; Onozuka, S.; Hattori, K.; Bender, M. L. *J. Am. Chem. Soc.* **1975**, *97*, 4432. (c) Breslow, R.; Doherty, J. B.; Guillot, G.; Lipsey, C. *J. Am. Chem. Soc.* **1978**, *100*, 3227. (d) Ikeda, T.; Kojin, R.; Yoon, C.-J.; Ikeda, H.; Iijima, M.; Hattori, K.; Toda, F. *J. Inclusion Phenom.* **1984**, *2*, 669.
(6) (a) Breslow, R.; Overman, L. E. *J. Am. Chem. Soc.* **1970**, *92*, 1075. (b) Akkaya, E. U.; Czarnik, A. W. *J. Am. Chem. Soc.* **1988**, *110*, 8553. (c) Breslow, R.; Singh, S. *Bioorg. Chem.* **1988**, *16*, 408. (d) Rosenthal, M. I.; Czarnik, A. W. *J. Inclusion Phenom. Mol. Recognit. Chem.* **1991**, *10*, 119.

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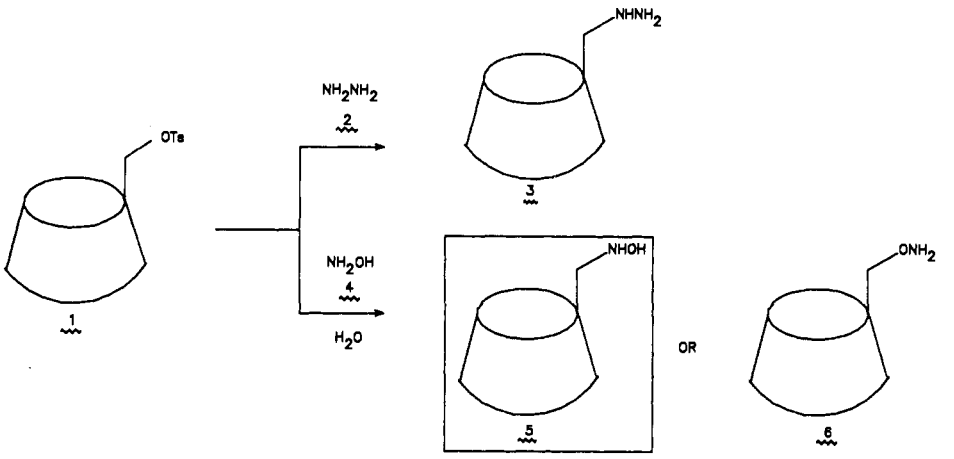
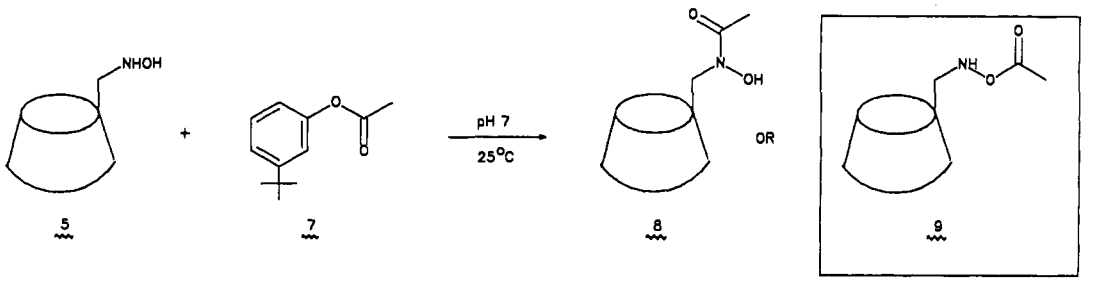
Scheme I



Scheme II



as potential pendant groups, α-nucleophiles such as hydrazine or hydroxylamine offer unique properties. (1) In solution, α-nucleophiles show enhanced reactivity toward acyl transfer as compared to isosteric alcohols or amines.[7] (2) Despite their greater reactivity toward acyl compounds, hydroxylamine (pK_a 5.97) and hydrazine (pK_a 8.0) are less basic than isosteric amines (pK_a 9–10) and thus exist in a reactive form near neutral pH. (3) Both hydroxylamine and hydrazine transacylate alkyl esters and amides. (4) Because they are physically small, pendant α-nucleophiles would necessarily reside proximal to the CD binding cavity. We now report on the syntheses, characterizations, and reactivities of βCDNHNH$_2$ and βCDNHOH.

Reaction of βCD-1°-tosylate (**1**) in anhydrous hydrazine (**2**) at room temperature for 4 h, followed by precipitation from EtOH, gave the crude product (**3**) (Scheme I). Physically entrained NH_2NH_2[8] was removed by reprecipitation from EtOH (5×), which gave **3** in 60% yield. In an analogous manner, reaction of **1** with a 6% aqueous solution of hydroxylamine (**4**) at 90 °C for 3 h, followed by multiple reprecipitation from EtOH, gave **5** in 86% yield. While either the N- or the O-alkylation product might have been formed, catalytic hydrogenation, which yielded βCDNH$_2$ and not βCD itself, confirmed the former. Notably for an unsymmetrically substituted CD derivative, **5** yields colorless plates (dec 207–210 °C) from water.[9]

Both βCDNHNH$_2$ and βCDNHOH are acylated rapidly by *p*-nitrophenyl acetate (pNPA) with saturation behavior. The reaction of pNPA (0.05 mM) fully complexed to **5** (10 mM) at pH 7.0 and 25 °C is faster than that with an equal concentration of CH_3NHOH (k_2 = 1.0 M^{-1} s^{-1}), demonstrating an effective RNHOH concentration of 37 mM. βCDNHOH is acylated as efficiently at pH 7.0 as at pH 9.5; furthermore, the rate of acyl transfer is 1500 times faster than that afforded using equimolar βCD, which is not reactive under neutral conditions (Figure 1).

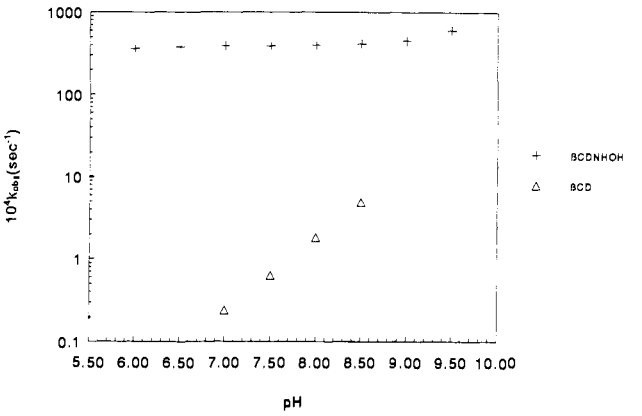


Figure 1. Reaction of pNPA with βCDs: effect of pH on k_{obsd} (various buffers, 0.1 M).

As shown in Scheme II, βCDNHOH binds and is acylated by a less activated ester (**7**) at pH 7.0 and 25 °C. The intracomplex reaction ([**5**] = 20 mM; [**7**] = 0.82 mM) occurs with a half-life of 7.5 min; a reference reaction (minimal DMSO added for solubility) with CH_3NHOH and βCD shows $t_{1/2}$ = 4.8 h, while the hydrolysis of **7** without added βCDNHOH occurs to less than 5% after 7 days. Once again, either N- or O-acylation, leading to **8** or **9**, is possible. The ^{1}H NMR spectrum of the acylation product in DMSO-d_6 reveals a one-proton, D_2O-exchangeable triplet at δ 7.65. Decoupling experiments demonstrate one-bond coupling to a single, diastereotopic H-6 proton (δ 3.07) on the modified CD residue, which permits assignment as an NH proton, and thus an unambiguous assignment of the acyl enzyme mimic as **9**. Because *O*-acylhydroxylamines hydrolyze more rapidly than structurally related esters, the deacylation kinetics of **9** are currently under investigation.

(7) Jencks, W. P.; Carriuolo, J. *J. Am. Chem. Soc.* **1960**, *82*, 1778.

(8) The 1:1 hydrazone between acetone and hydrazine yields methyl singlets at 1.72 and 1.81 ppm (D_2O); the 2:1 bis(hydrazone) yields methyl singlets at 1.68 and 1.91 ppm. The 1:1 hydrazone between acetone and βCDNHNH$_2$ yields somewhat broadened methyl singlets at 1.74 and 1.83 ppm. Likewise, the 1:1 oxime between acetone and hydroxylamine yields methyl singlets at 1.75 and 1.79 ppm; βCDNHOH does not form an oxime with acetone.

(9) Full synthetic details, with characterization data, are included in the supplementary material.

Acknowledgment. This work was supported by a grant from the Office of Naval Research. FT-NMR spectra were obtained with equipment funded in part by NIH Grant 1 S10 RR01458-01A1. L.E.F. acknowledges a faculty summer fellowship from the donors of the Petroleum Research Fund, administered by the American Chemical Society. A.W.C. thanks the A. P. Sloan and Dreyfus Foundations for support in the form of fellowships and Eli Lilly and Co. for support in the form of a granteeship.

Supplementary Material Available: Experimental details for the syntheses of **3** and **5** (4 pages). Ordering information is given on any current masthead page.

Synthesis of Diphthamide: The Target of Diphtheria Toxin Catalyzed ADP-Ribosylation in Protein Synthesis Elongation Factor 2

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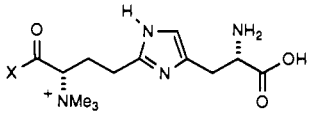
Received November 20, 1991

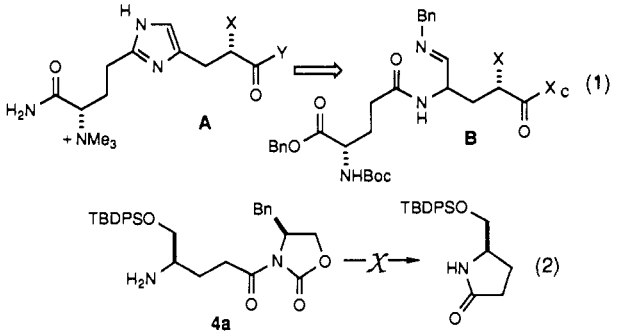
Diphtheria toxin (DT) expresses its cytotoxicity by inhibiting protein synthesis. Mechanistically, this toxin effects a single ADP-ribosylation of the critical enzyme, protein synthesis elongation factors 2 (EF-2), at a unique amino acid residue, thus terminating the translocation step of translation.[1] The gross structure of this targeted amino acid constituent of EF-2, initially referred to as amino acid X and later as diphthamide, was proposed by Bodley and co-workers from NMR and mass spectral studies of the hydrolysis products from ADP-ribosylated EF-2, ribosyldiphthamide and diphthine.[2] Biosynthetic labeling experiments support the proposed structure and reveal that the side chain of this elaborated histidine derivative is derived from methionine.[3] Diphthamide is the most complex posttranslationally modified amino acid known to date.



Diphthamide X = NH_2
Diphthine X = OH

The purpose of this communication is to describe the first syntheses of diphthamide and diphthine, which was prepared for direct comparison to the natural amino acid. A synthesis plan was developed which united the two carboxylic acid side chains prior to the construction of the imidazole nucleus through intermediates such as B (eq 1).[4] This plan afforded the flexibility of introducing the second amino-bearing stereocenter (X = H or NH_2) either before or after the imidazole construction.

The synthesis was initiated from D-pyroglutamic acid ethyl ester (**1**),[5] which was transformed to the *tert*-butyldiphenylsilyl-protected (TBDPS-protected) imide **2** in 77% overall yield (Scheme I). Peroxide-mediated hydrolysis of **2**[6] followed by its subsequent mixed anhydride acylation[7] with 4(*S*)-benzyloxazolidone[8] afforded imide **4** in 89% yield. After removal of the *N*-Boc protecting group, amine **4a** was acylated with mixed anhydride **6**,[9] derived from L-glutamic acid, to provide **7** in 89% yield. This transformation is noteworthy in that the potentially damaging intramolecular acylation of **4a** was not observed (eq 2).



In preparation for the construction of the imidazole nucleus, **7** was desilylated (HF–pyr, 13 h, 25 °C), the derived primary alcohol was transformed to the aldehyde,[10] and the *N*-benzylimine B (X = H) was formed (1.0 equiv of $BnNH_2$, $MgSO_4$, CH_2Cl_2, 1 h, 25 °C) without purification of intermediates. Cyclocondensation of this intermediate to imidazole **8** was effected by a modified Lee reaction (1.5 equiv of Ph_3P, 1.5 equiv of C_2Cl_6, 3.0 equiv of Et_3N, MeCN, 14 h, 35 °C) in an overall yield of 70% from **7**.[11] Our chiral enolate azidation methodology[12] was then employed to incorporate the α-amino moiety with the requisite (*S*) configuration. Unfortunately, the diastereoselection in the azidation of imide **8** was only moderate (76:24); nonetheless, the desired diastereomer **9** was isolated in 62% yield. Reduced diastereoselectivity was also observed in the analogous azidation of the C_2 unsubstituted imidazole imide, which was further transformed to L-histidine.[13] It is tentatively concluded that the imidazole moiety in these reactions is partially disrupting the chelated enolate and thus the reaction diastereoselectivity.

As a consequence of our collaborative interest in evaluating diphthamide amides as substrates for diphtheria toxin catalyzed ribosylation,[14] we selected *N*-acetyldiphthamide methyl ester **13** as the first target for synthesis. Treatment of **9** with thiolacetic acid (neat, 4 h, 25 °C)[15] afforded the *N*-acetamide, which was transformed to **10** via Boc removal (TFA) and reductive methylation (CH_2O, $NaBH_3CN$) in 86% overall yield. In the final steps of the synthesis, it was found that the benzyl ester in **10** could

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(2) Purification and properties: (a) Bodley, J. W.; Dunlop, P. C.; Van Ness, B. G. *Methods Enzymol.* **1984**, *106*, 378–387. (b) Van Ness, B. G.; Howard, J. B.; Bodley, J. W. *J. Biol. Chem.* **1980**, *255*, 10717–10720. Structure: (c) Van Ness, B. G.; Howard, J. B.; Bodley, J. W. *J. Biol. Chem.* **1980**, *255*, 10710–10716. (d) Bodley, J. W.; Upham, R.; Crow, F. R.; Tomer, K. B.; Gross, M. L. *Arch. Biochem. Biophys.* **1984**, *230*, 590–593. Recent review: Bodley, J. W.; Veldman, S. A. In *ADP-Ribosylating Toxins and G Proteins: Insights into Signal Transduction*; Moss, J., Vaughan, M., Eds.; American Society for Microbiology: Washington, DC, 1990; Chapter 2 and references therein.

(3) (a) Dunlop, P. C.; Bodley, J. W. *J. Biol. Chem.* **1983**, *258*, 4754–4758. (b) Chen, J.-Y. C.; Bodley, J. W. *J. Biol. Chem.* **1988**, *263*, 11692–11696. (c) Moehring, J. M.; Moehring, T. J. *Methods Enzymol.* **1984**, *106*, 388–395. (d) Moehring, T. J.; Danley, D. E.; Moehring, J. M. *Mol. Cell. Biol.* **1984**, *4*, 642–650.

(4) Preliminary studies in this laboratory demonstrated that diphthamide is not amenable to synthesis via conventional nucleophilic substitution at the C-2 position of the imidazole due to the poor nucleophilicity of the C-2 anion.

(5) (a) Silverman, R. B.; Levy, M. A. *J. Org. Chem.* **1980**, *45*, 815–818. (b) Amstutz, R.; Ringdahl, B.; Karlén, B.; Roch, M.; Jenden, D. J. *J. Med. Chem.* **1985**, *28*, 1760–1765.

(6) Evans, D. A.; Britton, T. C.; Ellman, J. A. *Tetrahedron Lett.* **1987**, *28*, 6141–6144.

(7) For a representative procedure for this reaction, see: Evans, D. A.; Ellman, J. A. *J. Am. Chem. Soc.* **1989**, *111*, 1063–1072.

(8) Gage, J. R.; Evans, D. A. *Org. Synth.* **1989**, *68*, 77–82.

(9) Intermediate **5** is commercially available from BaChem or may be synthesized according to the following procedure: Pawelczak, K.; Krzyzanowski, L.; Rzcszotarska, B. *Org. Prep. Proced. Int.* **1985**, *17*, 416–419.

(10) Mancuso, A. J.; Swern, D. *Synthesis* **1981**, 165–185. This oxidation was carried out with diisopropylethylamine.

(11) The supplementary material should be consulted for a detailed description of this procedure. For a similar α-acylamino ketimine cyclization, see: Engel, N.; Steglich, W. *Liebigs Ann. Chem.* **1978**, 1916–1927.

(12) Evans, D. A.; Britton, T. C.; Ellman, J. A.; Dorow, R. L. *J. Am. Chem. Soc.* **1990**, *112*, 4011–4030.

(13) The stereochemistry was assigned by comparison of the MTPA amides of both diastereomers with those derived from L-His and by analogy to the sense of induction seen in ref 12.

(14) This project is part of an ongoing collaboration with John Collier and co-workers at the Harvard Medical School.

(15) Rosen, T.; Lico, I. M.; Chu, D. T. W. *J. Org. Chem.* **1988**, *53*, 1580–1582.

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