concentrated. The solid product was purified by HPLC (μ-Porasil) to furnish 13 mg (90% overall) of desepoxyasperdiol as an oil: TLC R_f 0.15 (30% ethyl acetate in hexane); ^{1}H NMR (300 MHz) δ 5.46 (d, J = 8.7 Hz, 1 H), 5.02–4.81 (m, 2 H), 4.91 (br s, 1 H), 4.72 (br s, 1 H), 4.32 (dd, J = 8.7, 4.0 Hz, 1 H), 4.05 (AB q, J = 13.2 Hz, 2 H), 2.38–1.80 (m, 11 H), 1.72 (br s, 3 H), 1.56 (br s, 3 H), 1.49 (br s, 3 H), 1.61–1.20 (m, 4 H; includes two OH protons); ^{13}C NMR (75 MHz) δ 145.50, 138.98, 134.04, 133.54, 129.73, 125.55, 124.18, 113.71, 69.41, 65.75, 49.39, 40.21, 35.98, 28.16, 28.08, 24.54, 24.52, 23.19, 15.68, 15.32; IR ($CDCl_3$) 3344, 2938, 1662, 1638, 1441, 1367, 1251, 1050, 910 cm^{-1}; mass spectrum, m/e 305 (M^+ + 1), 304 (M^+), 286 ($M^+ - H_2O$), 274, 256, 244, 217, 204, 189, 175, 161, 149, 136, 123, 109, 100, 92, 81 (100%), 69; calcd for $C_{20}H_{32}O_2$ 304.2402, found 304.2409.

Acknowledgement. We thank the National Institutes of Health (Grant GM-30390) for generous financial support. NSF Grant CHE 81-00240 supported the purchase of the 300-MHz NMR spectrometer. We thank Professor Tadahiro Kato for providing spectra of racemic desepoxyasperdiol.

Studies on the Synthesis of Vitamin B_{12}. 4

Robert V. Stevens,† Normand Beaulieu, Wing Hong Chan, Andrej R. Daniewski, Takeshi Takeda, Adrian Waldner, Paul G. Williard,‡ and Ulrich Zutter*

Contribution from the Department of Chemistry and Biochemistry, University of California, Los Angeles, California 90024. *Received May 29, 1985*

Abstract: Chiral syntheses of the four precursors (**A1–D1**) to cobyric acid (**1**) and their assembly into the triisoxazole **4** are presented.

A novel strategy for the synthesis of cobyric acid (**1**) (Scheme I) was outlined in previous accounts from this laboratory.[1] The key feature of this design was the use of a triisoxazole scaffold (e.g., **3** or **4**) as a latent synthon for the crucial secocorrin intermediate **2**. This Cd complex **2** was used by the Eschenmoser group and undergoes a remarkably stereoselective photochemically induced A/D cycloisomerization.[2]

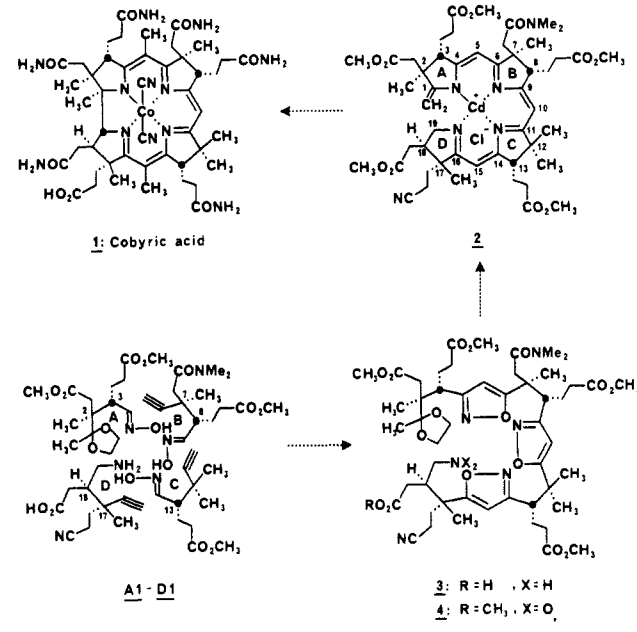
In an earlier report, an enantiospecific approach to the synthesis of four precursors (**A1–D1**) from dextro- and levorotatory camphor was described.[3] Unfortunately, with but one exception (shown in Scheme II), the Tanabe–Eschenmoser fragmentation (e.g., **6** → **C7**), which would have led to the four necessary acetylenes **A1–D1**, did not occur. Although cyclopentenone oxide **5** fragmented via the epoxyhydrazone **6** to the acetylenic aldehyde **C7**, the yield was modest and, as will be shown later, racemization took place.

"Nevertheless, the synthesis of the vitamin remained a dream unfulfilled, and as experiment after experiment failed, we thought seriously of abandoning our dream. However, rather than giving up we decided to undertake an entirely different approach".[4] Herein we describe the syntheses of the four precursors **A1–D1** in enantiomerically pure form and their assembly into the triisoxazole **4** via nitrile oxide cycloaddition methodology.

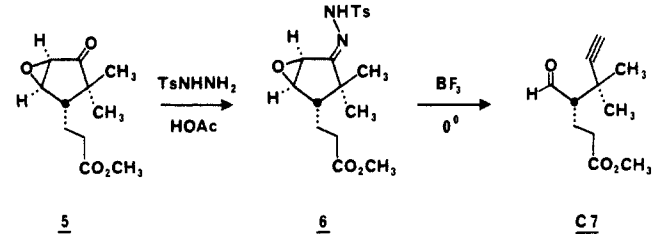
Synthesis of the A Ring. The first five steps of the synthesis remained unchanged from our previous approach[3] (Scheme III). Starting from (−)-camphor, the C-9 methyl group was functionalized via bromide **A3**[5,6] to the nitrile **A4**. Sodium borohydride reduction of the keto group gave predominantly the *exo*-alcohol **A5** that was subjected to oxidative fragmentation with ceric ammonium nitrate (CAN)[7] to afford cyclopentene **A6**. At this point, we were able to shorten the synthesis by a modified route and homologate the aldehyde side chain to the nitrile **A8**. This was accomplished by sodium borohydride reduction followed by tosylation and displacement of the tosylate with sodium cyanide in dimethylformamide (DMF). Oxidative ring opening with ozone afforded the crystalline keto aldehyde **A9** in 14% overall yield starting from (−)-camphor.

As shown in Scheme IV, reductive amination of **A9** with dimethylamine and sodium cyanoborohydride[8] gave the amino ketone **A10** in high yield. Oxidation with *m*-chloroperbenzoic acid (MCPBA) to the *N*-oxide **A11** followed by Cope elimination yielded the keto olefin **A12** which was protected as the ethylene

Scheme I



Scheme II

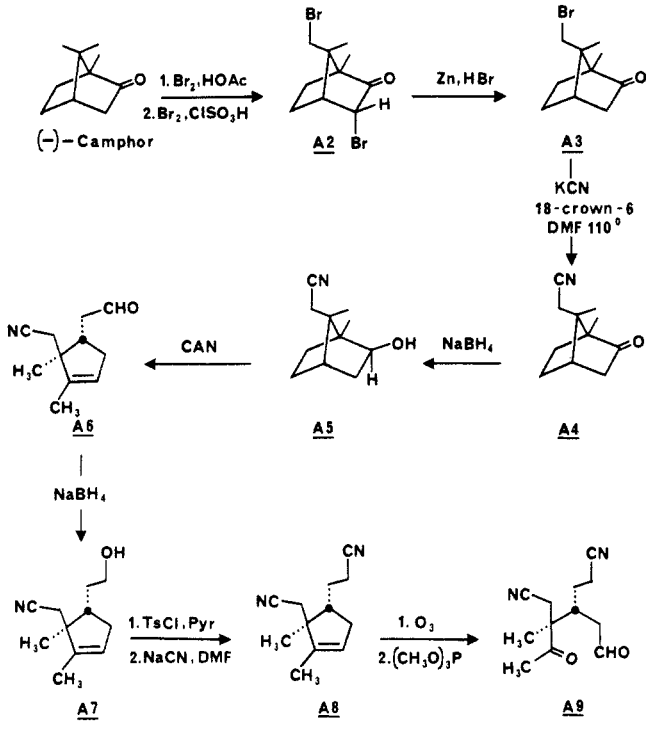


* Author to whom correspondence should be addressed: Cellulose Attisholz AG, CH-4708 Luterbach, Switzerland. Reprints can also be obtained from Prof. Michael E. Jung, UCLA.
† Deceased March 9, 1984.
‡ Department of Chemistry, Brown University, Providence, RI 02912.

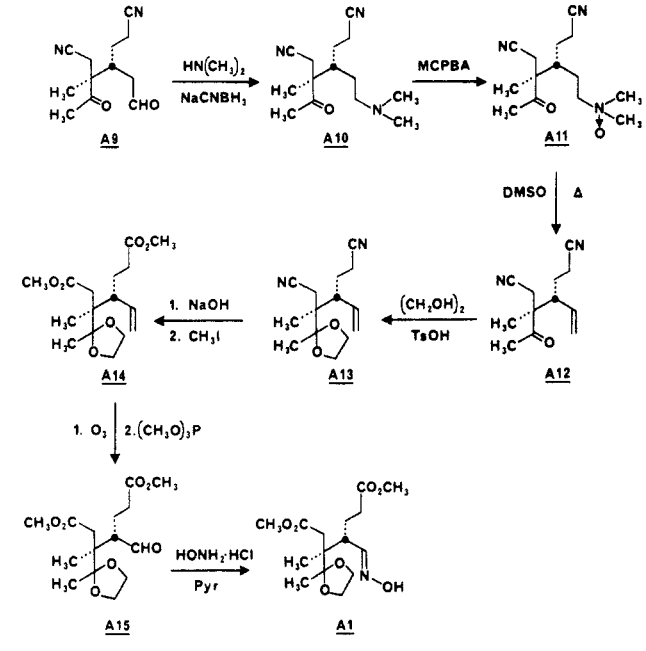
(1) Cf.: (a) Stevens, R. V.; Lapalme, R.; Fitzpatrick, J. M.; Germeraad, P. B.; Harrison, B. L. *J. Am. Chem. Soc.* **1976**, *98*, 6313. (b) Stevens, R. V.; Cherpeck, R. E.; Harrison, B. L.; Lai, J.; Lapalme, R. *Ibid.* **1976**, *98*, 6317. (c) Stevens, R. V. *Tetrahedron* **1976**, *32*, 1599. (d) Stevens, R. V. "Vitamin B-12. Proceedings of the Third European Symposium on Vitamin B-12 and Intrinsic Factors"; Zagalak, B.; Friedrich, W., Ed.; W. de Gryter: Berlin, 1979 and references cited therein.

0002-7863/86/1508-1039$01.50/0

Scheme III

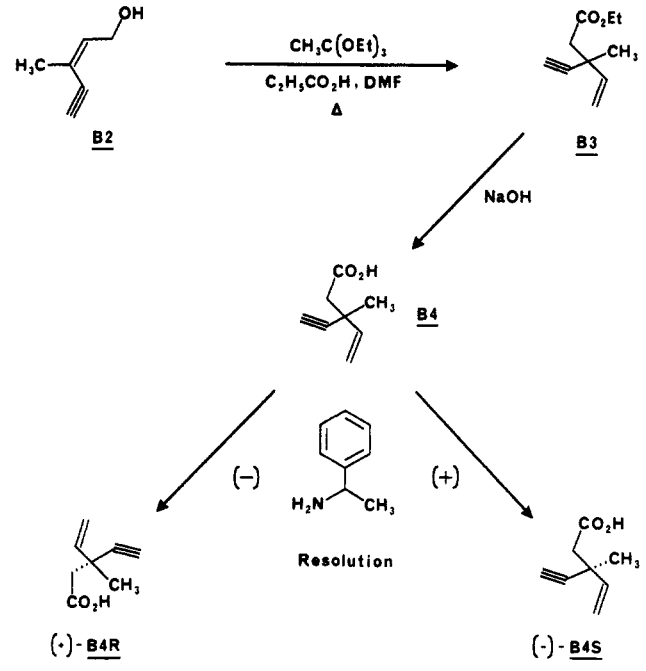


Scheme IV

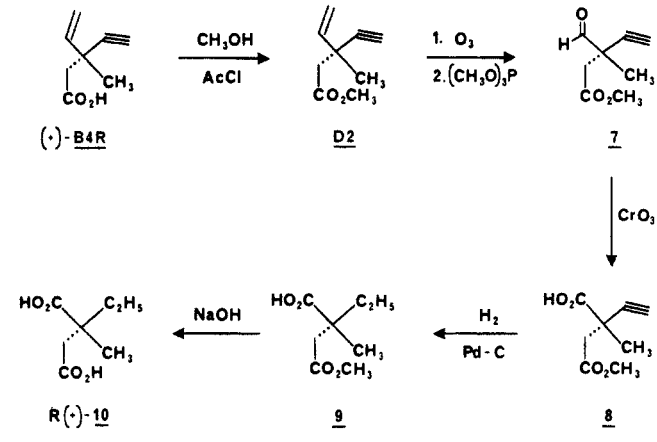


ketal **A13**. The dinitrile was then converted to the corresponding diester **A14** by basic hydrolysis and treatment with methyl iodide.

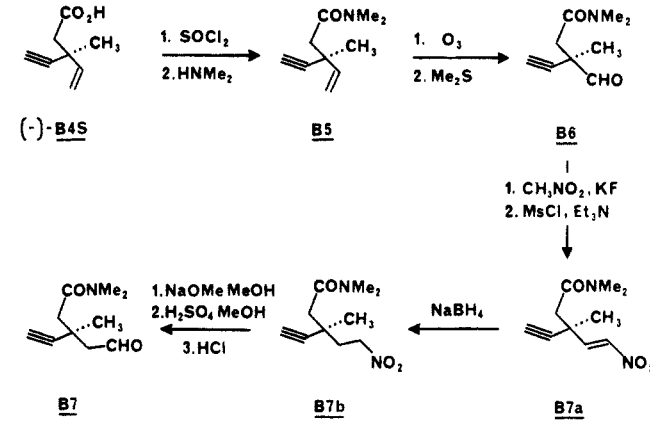
Scheme V



Scheme VI



Scheme VII



Ozonolysis of the olefin afforded the aldehyde **A15** which was finally treated with hydroxylamine hydrochloride in pyridine to give predominantly the *anti*-aldoxime **A1**. The overall yield from (−)-camphor to the A ring precursor **A1** was a satisfactory 7%.

Synthesis of the B and D Rings. Since the quaternary centers at C-7 in **B1** and C-17 in **D1** have similar substituents of opposite

(2) Eschenmoser, A. *Naturwissenschaften* **1974**, *61*, 513 and references cited therein.
(3) Stevens, R. V.; Chang, J. H.; Lapalme, R.; Schow, S.; Schlageter, M. G.; Shapiro, R.; Weller, H. N. *J. Am. Chem. Soc.* **1983**, *105*, 7719 and references cited therein.
(4) Quotation from R. V. Stevens' NSF proposal, Fall 1983.
(5) Kipping, F. S.; Pope, W. J. *J. Chem. Soc.* **1893**, *63*, 549, 577, 593.
(6) Meyer, W. L.; Lobo, A. P.; McCarty, R. N. *J. Org. Chem.* **1967**, *32*, 1754. Finch, A. M. T., Jr.; Vaughan, W. R. *J. Am. Chem. Soc.* **1961**, *91*, 416. Joshi, G. C.; Warnhoff, E. W. *J. Org. Chem.* **1972**, *37*, 2383. Corey, E. J.; Ohno, M.; Chow, S. W.; Scherrer, R. A. *J. Am. Chem. Soc.* **1959**, *81*, 6304. Eck, C. R.; Mills, R. W.; Money, T. *J. Chem. Soc., Perkin Trans. 1* **1975**, 251. Eck, C. R.; Hodgson, G. L.; McSweeney, D. F.; Mills, R. W.; Money, T. *Ibid.* **1974**, 1938.
(7) Tranhanovsky, W. S.; Fox, N. S. *J. Am. Chem. Soc.* **1974**, *96*, 7968 and references cited therein.
(8) Borch, R. F.; Bernstein, M. D.; Durst, H. D. *J. Am. Chem. Soc.* **1971**, *93*, 2897.

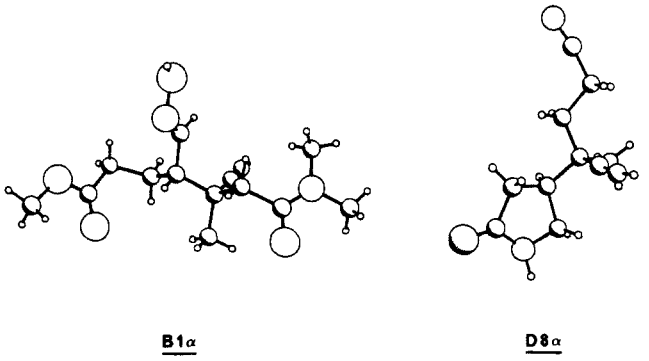
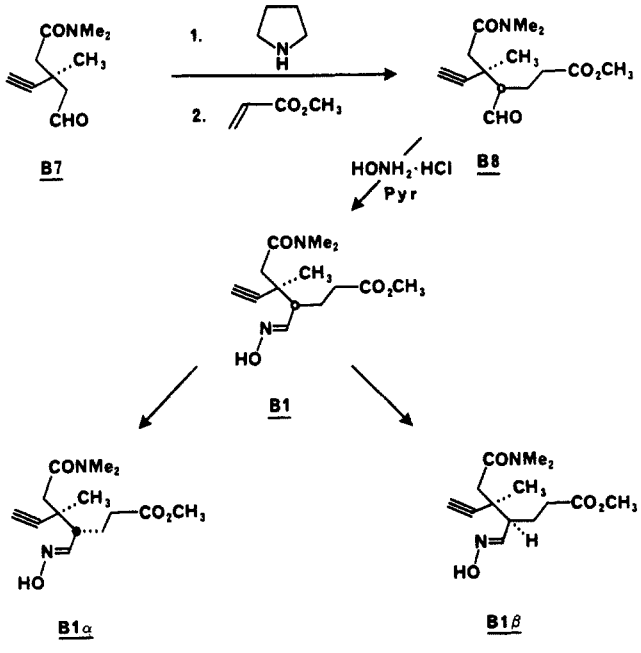


Figure 1.

Scheme VIII



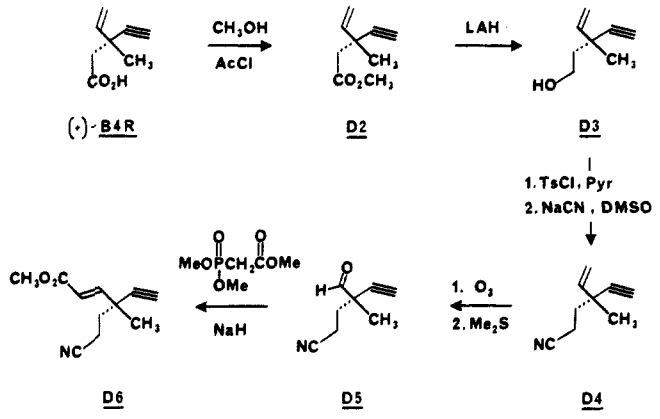
configuration, the enantiomers of acid **B4** (Scheme V) seemed to be appropriate as starting points for both syntheses. Claisen rearrangement of commercially available allylic alcohol **B2** followed by hydrolysis and resolution of the resultant acid **B4** with (+)- and (−)-α-phenylethylamine afforded (+)-**B4R** and (−)-**B4S**. The absolute configuration of the (+)-acid **B4R** was determined by its transformation to (+)-(*R*)-α-ethyl-α-methylsuccinic acid (**10**) of known chirality[9] as shown in Scheme VI.

The synthesis of the B ring was therefore continued by using the (−)-acid **B4S** which was converted via the amide **B5** to aldehyde **B6** (Scheme VII). Homologation to the aldehyde **B7** was accomplished by condensation with nitromethane followed by reduction of the double bond and Nef reaction[10] without purification of the intermediates.

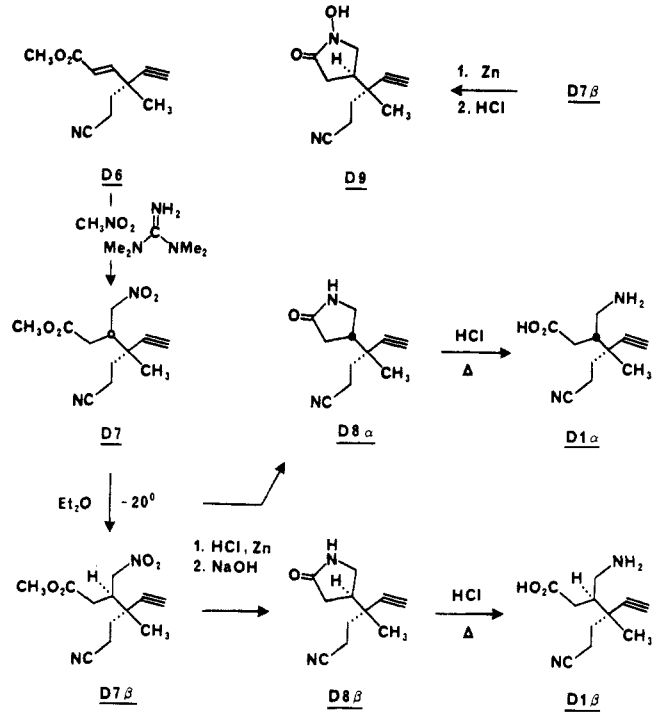
The C-8 propionate side chain was now incorporated via the enamine modification of the Michael addition to methyl acrylate (Scheme VIII). The resulting 1:1 mixture of the 8α and β diastereomers **B8** was converted directly to the aldoxime **B1** (13% overall yield from **B4S**). Due to the presence of both *syn*- and *anti*-oximes (*syn*/*anti*-oxime ratio ~1:3 by ^{1}H NMR), **B1** was a mixture of four isomers which at first did not crystallize. Finally, after months, the extremely viscous oxime crystallized, and we were gratified to isolate first the pure *anti*-aldoxime **B1α** and from the mother liquor its diastereomer **B1β**. The relative configuration at C-8 in **B1α** was confirmed via X-ray diffraction analysis as shown in Figure 1. Since it is known from the pioneering synthetic investigations of Eschenmoser and Woodward[2,11,12] that the

(9) Porath, J. *Ark. Kemi* **1951**, *3*, 163.
(10) Noland, W. E. *Chem. Rev.* **1955**, *55*, 137 and references cited therein.

Scheme IX



Scheme X

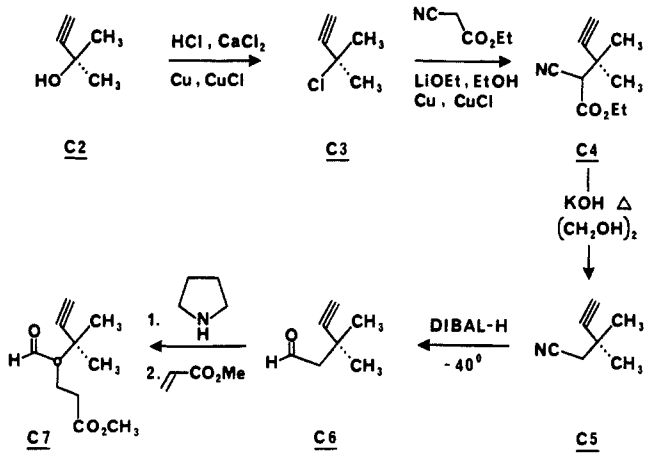


stereochemistry at C-8 can be adjusted later, both diastereomers were suitable for the synthesis of cobyric acid.

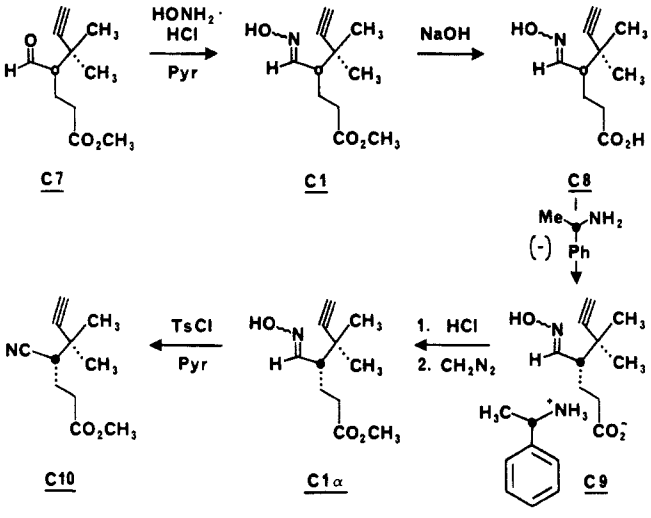
The synthesis of the D ring is shown in Schemes IX and X. Esterification of the (+)-acid **B4R** followed by lithium aluminum hydride (LAH) reduction provided alcohol **D3** which was converted to the nitrile **D4** via the tosylate. After oxidation with ozone to the aldehyde **D5**, the C-18 acetate side chain was established via Wittig–Horner reaction.[13] Introduction of the second chiral center (shown in Scheme X) was accomplished by Michael addition of nitromethane to the α,β-unsaturated ester **D6**,[14] resulting in a 2:1 mixture of the diastereomers **D7** (by ^{1}H NMR). The major diastereomer **D7β** was separated by one crystallization from ether and has, as will be seen later, the desired *R* configuration on both centers. In order to work out the reaction conditions to the desired target amino acid **D1β**, the mother liquor of the nitroester **D7β** (**D7β**:**D7α** ~1:2 by ^{1}H NMR) was used first. Reduction of the nitro group with zinc and hydrochloric acid (HCl) followed by basic workup gave the lactam **D8**. The enriched

(11) Woodward, R. B. *Pure Appl. Chem.* **1968**, *17*, 519; **1971**, *25*, 283; **1973**, *33*, 145 and references cited therein.
(12) For an account of this monumental achievement, see: Stevens, R. V. "Vitamin B-12"; Dolphin, D., Ed.; Wiley: New York, 1982; Vol. 1, Chapter 6.
(13) Wadsworth, W. S.; Emmons, W. D. *J. Am. Chem. Soc.* **1961**, *83*, 1733.
(14) Pollini, G. P.; Barco, A.; De Giuli, G. *Synthesis* **1972**, 44.

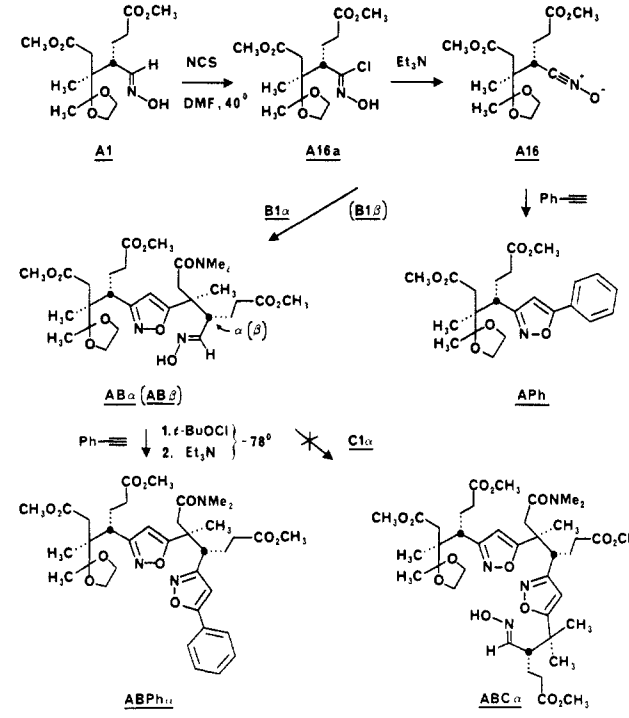
Scheme XI



Scheme XII



Scheme XIII



diastereomer **D8α** (mp 157–158 °C) was isolated in pure form by crystallization, and its X-ray structure revealed that the tertiary center in **D8α** has the *S* configuration (Figure 1). Acidic lactam opening afforded the extremely water soluble γ-amino acid **D1α**. D ring precursor **D1β** was finally obtained in the same way by opening lactam **D8β** (mp 115–117 °C). The overall yield from the acid **B4R** was 4%.

The reduction of the nitro ester **D7β** illustrates how the order of addition of reagents can affect the course of a reaction. If the zinc is added first followed by slow addition of HCl, the reduction stops at the hydroxylamine which cyclizes to the hydroxamic acid **D9**.

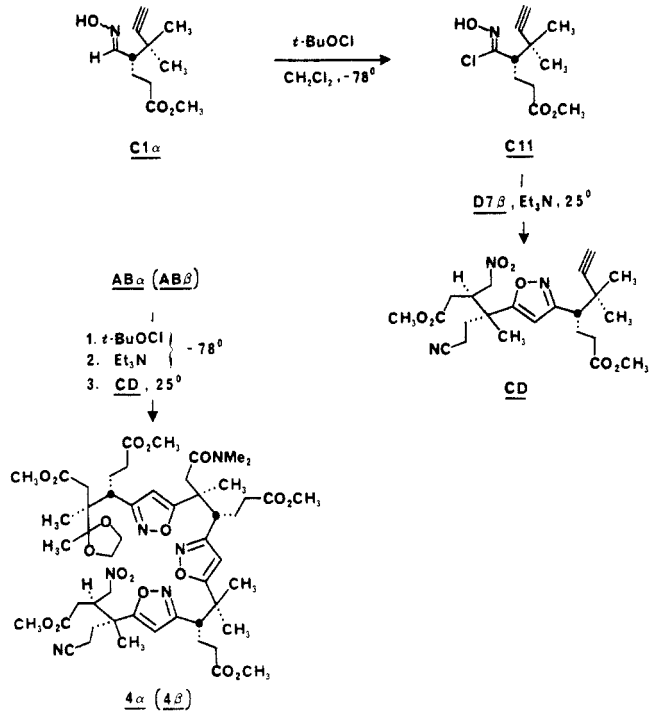
Synthesis of the C Ring. In our first approach to the synthesis of the four precursors **A1–D1**,[3] the C ring was the one exception which could be prepared via the originally planned fragmentation pathway (Scheme II). This quite long synthesis with its modest overall yield caused us to investigate an alternate method of preparing the acetylenic oxime **C1** (Scheme XI). Starting from commercially available alcohol **C2**, the chloride **C3** was prepared according to a procedure reported by Hennion.[15] The alkylation of **C3** with ethyl cyanoacetate and subsequent decarboethoxylation of **C4** as well as the reduction of nitrile **C5** to the aldehyde **C6** with diisobutylaluminum hydride (DIBAL-H) was employed earlier in our laboratories.[16,17] Introduction of the C-13 propionate side chain via enamine Michael addition afforded acetylenic aldehyde **C7** in 17% overall yield from alcohol **C2**. The second part of the C ring synthesis is shown in Scheme XII. Conversion of the aldehyde **C7** to the oxime **C1** followed by saponification afforded the racemic acid **C8** which was resolved with (−)-α-phenylethylamine. Since the recrystallization of the ammonium salt **C9** caused a change in the *syn*/*anti*-oxime ratio during the resolution, the optical rotation $[\alpha]^{25}_D$ of the oxime **C1α** was not an appropriate measure for its optical purity. Therefore, small samples of the different fractions of **C9** were converted to the nitrile **C10**, and its $[\alpha]^{25}_D$ was determined. The resolution was deemed complete as soon as further recrystallizations did not improve the optical purity of **C10** ($0° \geq [\alpha]^{25}_D \geq -65.7°$). Proof for the sufficient resolution (>95%) of the C ring precursor **C1α** is the fact (as will be seen later) that the combination of C with the D ring gave the "southern half" **CD** as a pure diastereomer. To determine the absolute configuration of **C10**, optically active aldehyde **C7**, synthesized from (−)-borneol via the fragmentation pathway (Scheme II), was converted to the nitrile **C10**. Its $[\alpha]^{25}_D$ −8.5° showed that the resolved oxime **C1α** has the desired *S* configuration. The smaller absolute specific rotation revealed that racemization occurred during the boron trifluoride catalyzed fragmentation (**6** → **C7**, Scheme II).

Synthesis of the "Northern Half" AB. Due to the bulky α substituents, nitrile oxide **A16** (Scheme XIII) proved to be less reactive than other tertiary examples and therefore more stable. Chlorination of the oxime **A1** with *N*-chlorosuccinimide (NCS) in DMF at 40 °C gave the hydroxamoyl chloride **A16a** which was converted to **A16** by elimination of HCl with triethylamine (Et_3N). The chromatographed nitrile oxide **A16** which was stable at room temperature for weeks showed a characteristic IR absorption at 2290 cm^{-1} and reacted with phenylacetylene as expected to give **APh**. The cycloaddition of **A16** to the sterically more hindered acetylene **B1α** (and **B1β**) required special conditions: high concentration of reactants (1.0–0.5 M), long reaction time (6 days), and warming. Under these conditions, the northern half **ABα** (and **ABβ**) was obtained in about 95% yield.

The initial plan was to construct the triisoxazole **3** according to the clockwise approach[1d] via the diisoxazole **ABCα**. In order to work out the conditions for the nitrile oxide generation, **ABα** was added to the more reactive phenylacetylene first. The method employed for **A16** utilizing NCS/DMF/Et_3N failed, and the *N*-bromosuccinimide/Et_3N/DMF (0 °C) procedure[16] gave only small amounts of diisoxazole **ABPhα**. As will be seen below, the instability of the hydroxamoyl chloride of **AB** was responsible for these unexpected difficulties. Therefore, a new method for the

(15) Hennion, G. F.; Boiselle, A. P. *J. Org. Chem.* **1961**, *26*, 725.
(16) Stevens, R. V.; Christensen, C. G.; Edmonson, W. L.; Kaplan, M.; Reid, E. B.; Wentland, M. P. *J. Am. Chem. Soc.* **1971**, *93*, 6629.
(17) Stevens, R. V.; Reid, E. B. *Tetrahedron Lett.* **1975**, 4193.

Scheme XIV



low-temperature chlorination of aldoximes was worked out. *tert*-Butyl hypochlorite (*t*-BuOCl)[18] in methylene chloride (CH_2Cl_2) proved to be the ideal reagent and reacted at −78 °C to give the hydroxamoyl chloride of **AB**. The ensuing elimination of HCl had to be carried out at low temperature (−78 °C); otherwise little or no nitrile oxide was formed. The presence and decay of the **AB** nitrile oxide was analyzed by IR spectroscopy at room temperature (the half-life of the nitrile oxide in the reaction mixture is about 1.5 days). Using this procedure, the diisoxazole **ABPhα** was isolated in about 60% yield. While the nitrile oxide of **ABα** could also be added to *tert*-butylethylene, many attempts to add it to the C ring acetylene **C1α** failed despite high concentration and large excess (4–20-fold) of the acetylene component. Besides the recovery of nearly all the C ring, medium-pressure liquid chromatography (MPLC) of the complicated mixture yielded unidentified high molecular compounds and very little (<5%), impure diisoxazole **ABCα** (by ^1H NMR and IR). Due to these difficulties, the plan to synthesize the triisoxazole **3** via the diisoxazole **ABCα** was abandoned in favor of the approach via the "northern half" and "southern half".

Synthesis of the "Southern Half" CD. We had planned originally to incorporate the D ring into the triisoxazole **3** and therefore into the "southern half" **CD** in the form of the amino acid **D1β**. The anticipation of troublesome isolations of the products from amino acid mixtures caused us to work with the γ-nitro ester **D7β** instead and to postpone the reduction of the nitro group.

Chlorination of the aldoxime **C1α** to give the hydroxamoyl chloride **C11** could be accomplished by either *t*-BuOCl/CH_2Cl_2 at −78 °C or NCS/DMF at 40 °C (Scheme XIV). In order to suppress the cycloaddition to itself, the nitrile oxide was generated with Et_3N in the presence of a 3-fold excess of **D7β** which could be recovered after the reaction by chromatography. The pure diastereomer **CD** (by ^1H and ^{13}C NMR) was isolated as an extremely viscous oil in 57% overall yield from the ammonium salt **C9**.

Linkage of the Northern with the Southern Half: Triisoxazole 4. The nitrile oxide of **ABα** (and **ABβ**) was generated as described above with *t*-BuOCl/Et_3N/CH_2Cl_2 at −78 °C and reacted with a 3-fold excess of the acetylene **CD** at room temperature for 2.5 days. The triisoxazole **4α** (and **4β**) was isolated by chromatography on silica with benzene–pyridine 9:1 and 7:1 as eluents in about 30% yield. The spectroscopic data, NMR (^1H and ^{13}C), IR, and fast atom bombardment MS (MH^+ 1071), confirmed the structure shown in Scheme XIV.

In conclusion, this report describes the successful construction of the triisoxazole skeleton **4** which incorporates all the appropriate functionality for conversion to the Eschenmoser intermediate **2**. The four precursors to this skeleton were synthesized in a concise and enantioselective manner and were then connected via a nitrile oxide cycloaddition onto an acetylenic moiety. Although it is as yet a "dream unfulfilled", very significant progress has been made toward this goal, and the feasibility of the approach has clearly been demonstrated. It is hoped that further studies will be undertaken.

Experimental Section

Melting points determined in glass capillaries and boiling points are uncorrected. Infrared (IR) spectra were obtained on a Beckman spectrophotometer IR-4210. ^1H NMR and ^{13}C NMR spectra were recorded on a Brucker WP 200 spectrometer at 200 and 50 MHz, respectively, using tetramethylsilane as an internal standard (chemical shifts (δ) are reported in parts per million (ppm) and the coupling constants (*J*) are given in hertz). High-resolution mass spectra were measured on an AET Kratos MS 902. Combustion analyses were done by Spang Microanalytical Laboratory, Eagle Harbor, MI. All chromatographies were done with Merck silica gel 60 (70–230 mesh).

3-[(1*R*,2*S*)-2-(Cyanomethyl)-2,3-dimethylcyclopent-3-en-1-yl]-propionitrile (A8). To the campholenaldehyde **A6** (17.7 g, 0.1 mol) in methanol (250 mL) $NaBH_4$ (3.8 g, 0.1 mol) was added at 0 °C over 20 min. Stirring was continued at 25 °C for 1 h, the mixture neutralized with AcOH (6 mL), and the solvent removed by rotary evaporation. The residue was treated with H_2O (150 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The extract was dried (Na_2SO_4), filtered, and evaporated to give the crude alcohol **A7**. It was dissolved in pyridine (200 mL), and tosyl chloride (38.0 g, 0.2 mol) in pyridine (100 mL) was added at 0 °C. The mixture was maintained at that temperature for 5 h and then at 25 °C for 2 h (at higher temperature, some chloride was formed). The excess of tosyl chloride was hydrolized with ice (100 g) and after stirring for 2 h, the mixture was poured into 4 N HCl (1.5 L). Extraction with ether (5 × 200 mL) afforded after drying (Na_2SO_4) and evaporation (VRE) the crude tosylate. It was dissolved in DMF (250 mL) containing pulverized NaCN (15 g, 0.3 mol), stirred at 20 °C overnight, and then poured into water (1.2 L). The precipitate was filtered and recrystallized from ether–hexane to give pure dinitrile **A8** (15.5 g, 82.4%): mp 58–59.5 °C; $[\alpha]^{25}_D$ −29.02°, $[\alpha]^{25}_{435}$ −56.33° (*c* 6.98, $CHCl_3$); IR (KBr) 3040 (H—C=), 2240 (C≡N), 1655 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 0.98 (s, 3 H, CH_3C), 1.50–2.60 (m, 10 H, CH_3C=C, $CH_2CHCH_2CH_2CN$), 2.39 (s, 2 H, CH_2CN), 5.42 (m, 1 H, =CH—); ^{13}C NMR ($CDCl_3$) δ 143.59, 124.26, 119.24, 117.80, 49.01, 46.29, 34.65, 26.82, 25.86, 17.85, 15.83, 12.14; MS (EI), *m/z* 188.1318, calcd for $C_{12}H_{16}N_2$ 188.1315.

(4*R*,5*S*)-5-(Cyanomethyl)-4-(formylmethyl)-5-methyl-6-oxoheptanenitrile (A9). The dicyanide **A8** (18.8 g, 0.1 mol) in CH_2Cl_2 (300 mL) and methanol (50 mL) was ozonized at −78 °C until the blue color appeared. The reaction mixture was swept with N_2 for 1 h, and then $P(OCH_3)_3$ (50 mL) was added. The mixture was allowed to warm up to 25 °C and stirred overnight. After removing the solvents, the $P(OCH_3)_3$, and the $PO(OCH_3)_3$ by rotary evaporation on the high vacuum, the oily residue was crystallized by adding ether (100 mL) to give the colorless keto aldehyde **A9** (19.8 g, 90%): mp 83–84 °C; $[\alpha]^{25}_D$ +4.88°, $[\alpha]^{25}_{435}$ +20.57° (*c* 5.22, $CHCl_3$); IR (KBr) 2840 and 2730 (CHO), 2240 and 2230 (2 × C≡N), 1720 and 1700 cm^{-1} (C=O, aldehyde and ketone); ^1H NMR ($CDCl_3$) δ 1.35 (s, 3 H, CH_3C), 1.40–1.75 (m, 2 H, CH_2C—CN), 2.30 (s, 3 H, CH_3CO), 2.25–3.00 (m, 7 H, 2 × CH_2CN, $CHCH_2CO$), 9.85 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 208.72 (C=O, ketone), 199.44 (CHO), 118.87 (CN), 117.79 (CN), 52.94, 44.29, 35.51, 27.67, 25.35, 21.97, 20.52, 15.97; MS (EI), *m/z* 177.1032 (M^+ − 43), calcd for $C_{10}H_{13}N_2O$ (M^+ − CH_3CO) 177.1092.

(4*R*,5*S*)-5-(Cyanomethyl)-4-[2-(dimethylamino)ethyl]-5-methyl-6-oxoheptanenitrile (A10). The keto aldehyde **A9** (22.0 g, 0.1 mol) was added to a mixture of dimethylamine hydrochloride (16.0 g, 0.196 mol), sodium acetate (13.0 g, 0.158 mol), and sodium cyanoborohydride (60%) (15.0 g, 0.143 mol) in methanol (500 mL). The mixture was stirred at 25 °C for 24 h while the pH was adjusted with AcOH to 7–8. Acetone (50 mL) was added to the mixture and then 5 N HCl until the pH was 2–1. The solvent was removed by rotary evaporation and the residue dissolved in water (150 mL) and extracted with ether (3 × 100 mL). The aqueous layer was alkalized with NaOH to liberate the amine and ex-

(18) Teeter, H. M.; Bell, E. W. "Organic Syntheses"; Wiley: New York, 1963; Collect. Vol. 4, p 125.

tracted again with ether (6 × 100 mL). The combined ether layers were dried (K_2CO_3) and the solvent evaporated (VRE) to give the amino ketone **A10** (24.0 g, 96.7%) which was used without further purification for the next step: IR (film) 2820 and 2770 (s, Bohlmann bands), 2240 (m, C≡N), 1705 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.40 (s, 3 H, CH_3), 2.22 (s, 6 H, $N(CH_3)_2$), 2.27 (s, 3 H, CH_3CO), 1.15–2.75 (m, 11 H).

(4*R*,5*S*)-5-(Cyanomethyl)-5-methyl-6-oxo-4-vinylheptanenitrile (A12). A solution of 80% *m*-chloroperbenzoic acid (13 g, 0.06 mol) in CH_2Cl_2 (100 mL) was added at 0 °C to the amino ketone **A10** (15.0 g, 0.06 mol) in CH_2Cl_2 (400 mL) and the reaction mixture was stirred for 1 h. Diazomethane in ether was added to transform the *m*-chlorobenzoic acid into the methyl ester, and the solvents were removed by rotary evaporation. The residue was triturated with pentane to wash out the methyl *m*-chlorobenzoate. The resultant crude *N*-oxide **A11** was dissolved in Me_2SO (150 mL) and benzene (80 mL), and the reaction mixture was heated—while benzene with some water and dimethylhydroxylamine was distilled off—until the temperature of the reaction mixture reached 110 °C. After cooling down, the mixture was poured into brine (500 mL) and extracted with ether (6 × 100 mL). After drying (Na_2SO_4) and evaporation (VRE), the residue was chromatographed on silica using ethyl acetate–hexane 1:2 as an eluent to afford the olefin **A12** (9.3 g, 75.6%) which crystallized upon standing: mp 45–46 °C; $[\alpha]^{25}_D$ +38.99°, $[\alpha]^{25}_{435}$ +128.44° (*c* 8.40, $CHCl_3$); IR (film) 3075 (w, H—C=), 2245 (m, C≡N), 1705 (s, C=O), 1635 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 1.40 (s, 3 H, CH_3C), 1.42–1.75 (m, 2 H, CH_2), 2.25 (s, 3 H, CH_3CO), 2.10–2.75 (m, 5 H, 2 × CH_2CN, CH), 5.25–5.65 (m, 3 H, CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 208.66 (C=O), 133.28, 122.54 (C=C), 118.77 (C≡N), 117.84 (C≡N), 52.45, 49.51, 25.83, 25.12, 22.25, 21.42, 15.61; MS (EI), *m/z* 204.1260, calcd for $C_{12}H_{16}N_2O$ 204.1264.

Ethylene Ketal of (4*R*,5*S*)-5-(Cyanomethyl)-5-methyl-6-oxo-4-vinylheptanenitrile (A13). The mixture of the keto olefin **A12** (5.1 g, 25 mmol), ethylene glycol (5 mL), benzene (400 mL), and TsOH (150 mg) was refluxed by using a Dean-Stark adapter for 20 h. After cooling, 1 mL of pyridine was added, and the reaction mixture was washed with brine (3 × 20 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated (VRE). Chromatography of the residue on silica (ethyl acetate–hexane 1:4) afforded the pure ketal **A13** (5.5 g, 90.6%) as a colorless oil: $[\alpha]^{25}_D$ −13.71°, $[\alpha]^{25}_{435}$ −26.04° (*c* 5.10, $CHCl_3$); IR (film) 3070 (w, H—C=), 2240 (m, C≡N), 1630 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 1.19 and 1.31 (s, each 3 H, 2 × CH_3), 1.40–2.60 (m, 7 H), 3.90–4.15 (m, 4 H, OCH_2CH_2O), 5.10–5.80 (m, 3 H, CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 135.98, 120.18, 119.46, 119.19, 112.77, 65.08, 63.53, 49.56, 46.02, 24.98, 21.76, 20.26, 19.39, 15.86; MS (EI), *m/z* 189.1016 (M^+ − 59), calcd for $C_{11}H_{13}N_2O$ (M^+ − CH_3 − CH_2CH_2O) 189.1029.

Ethylene Ketal of Methyl (4*R*,5*S*)-5-[(Methoxycarbonyl)methyl]-5-methyl-6-oxo-4-vinylheptanoate (A14). The mixture of the dinitrile **A13** (5.0 g, 20 mmol), NaOH (15.0 g), ethylene glycol (15 mL), H_2O (25 mL), and MeOH (50 mL) was heated, and MeOH was distilled off until the temperature of the mixture reached 110 °C. The mixture was refluxed for 75 h until no more ammonia could be detected with pH paper. The mixture was cooled down, DMF (150 mL) and methyl iodide (40 mL) were added, and the reaction mixture was stirred overnight at 25 °C. After the addition of water (400 mL), the product was extracted with CH_2Cl_2 (3 × 100 mL), the combined organic layers were dried (Na_2SO_4), and the solvents were removed by rotary evaporation. The residue was chromatographed on silica (ethyl acetate–hexane 1:4) to give the pure diester **A14** (5.0 g, 78.5%) as a colorless oil: $[\alpha]^{25}_D$ +0.98°, $[\alpha]^{25}_{435}$ +5.65° (*c*, 4.60, $CHCl_3$); IR (film) 3070 (w, H—C=), 1735 (s, C=O), 1635 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 1.16 and 1.27 (s, each 3 H, 2 × CH_3), 1.30–2.60 (m, 7 H), 3.62 and 3.65 (s, each 3 H, 2 × OCH_3), 3.70–4.05 (m, 4 H, OCH_2CH_2O), 4.90–5.70 (m, 3 H, CH=CH_2); ^{13}C NMR ($CDCl_3$) δ 174.34, 173.35 (2 × C=O), 138.47, 117.56 (C=C), 113.76 (O—C—O), 64.84, 63.29, 51.34, 51.13, 50.42, 47.79, 36.52, 32.58, 24.19, 21.11, 19.34; MS (EI), *m/z* 314.1724, calcd for $C_{16}H_{26}O_6$ 314.1729.

Ethylene Ketal of Methyl (4*S*,5*S*)-4-Formyl-5-[(methoxycarbonyl)-methyl]-5-methyl-6-oxoheptanoate (A15). The olefin **A14** (3.14 g, 10 mmol) in CH_2Cl_2/MeOH 1:1 (50 mL) was cooled to −78 °C. Ozone was bubbled into the solution through a glass tube at −78 °C until a blue color was detected (~1 h). The reaction mixture was swept with N_2 for 1 h and then $(CH_3O)_3P$ (10 mL) was added. The mixture was allowed to warm up to 25 °C and stirred overnight. After removing the solvents and the excess of $(CH_3O)_3P$ by rotary evaporation under high vacuum, the residue was chromatographed on silica (EtOAc/hexane 1:3) to afford the oily aldehyde **A15** (3.00 g, 95%): $[\alpha]^{25}_D$ +31.03°, $[\alpha]^{25}_{435}$ +72.91° (*c* 9.12, $CHCl_3$); IR (film) 2840 and 2740 (CHO), 1730 (C=O, ester), 1705 cm^{-1} (C=O, aldehyde); ^1H NMR ($CDCl_3$) δ 1.26 and 1.32 (s, each 3 H, 2 × CH_3), 1.70–2.15 (m, 2 H, CH_2), 2.31 (t, *J* = 7.7, 2 H, CH_2CO), 2.49 (s, 2 H, CH_2CO), 2.50–2.65 (m, 1 H, CH), 3.65 and 3.66 (s, each 3 H, 2 × OCH_3), 3.70–4.00 (m, 4 H, OCH_2CH_2O), 9.49 (d, *J* = 5.1, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 199.91 (CHO), 173.61 and 171.47 (C=O, ester), 112.87 (O—C—O), 64.92 and 63.33 (OCH_2CH_2O), 53.15 and 51.52 (2 × OCH_3), 48.87, 40.06, 31.98, 19.73, 1928, 18.48; MS (EI), *m/z* 301.1290 (M^+ − 15), calcd for $C_{14}H_{21}O_7$ (M^+ − CH_3) 301.1287.

Oxime A1. To $HONH_2$·HCl (0.87 g, 12.5 mmol) in pyridine (10 mL) was added the aldehyde **A15** (3.16 g, 10 mmol) in pyridine (5 mL) at 0 °C. The reaction mixture was stirred for 1 h at 0 °C and overnight at 25 °C. It was then diluted with H_2O (100 mL) and extracted with CH_2Cl_2 (3 × 100 mL). The organic layers were washed with H_2O (50 mL) and brine (25 mL) and dried (Na_2SO_4), and the solvent was removed by rotary evaporation. Filtration through silica with EtOAc/hexane 1:1 afforded predominantly (>95%, by ^1H NMR) the *anti*-oxime **A1** (3.2 g, 96%) as an extremely viscous oil: $[\alpha]^{25}_D$ +28.66°, $[\alpha]^{25}_{435}$ +57.42° (*c* 6.50, $CHCl_3$); IR (film) 3500–3200 (br, OH), 1735 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.15 and 1.29 (s, each 3 H, 2 × CH_3), 1.50–2.70 (m, 7 H), 3.64 and 3.66 (s, each 3 H, 2 × OCH_3), 3.80–4.00 (m, 4 H, OCH_2CH_2O), 7.32 (d, *J* = 9.2, 1 H, HC=N, *anti*-oxime), 7.70 (br s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 173.96 and 172.52 (C=O), 153.18 (C=N), 113.19 (O—C—O), 64.38 and 64.25 (OCH_2CH_2O), 51.58 and 51.42 (OCH_3), 47.94, 44.05, 38.40, 32.17, 23.05, 19.57, 19.06; MS (EI), *m/z* 282.1367 (M^+ − 49), calcd for $C_{14}H_{20}NO_5$ (M^+ − H_2O − OCH_3) 282.1342.

Ethyl 3-Methyl-3-vinyl-4-pentynoate (B3). In a 12 L flask with a Dean-Stark trap and a reflux condensor, freshly distilled *trans*-3-methyl-2-penten-4-yn-1-ol (**B2**) (577 g, 6 mol, Fluka), triethyl orthoacetate (3410 g, 21 mol), DMF (4.5 L), and propionic acid (42 g, 0.6 mol) were mixed and refluxed for 6 days. Every day, 300 mL solvent was distilled off and propionic acid (9 g, 0.12 mol) was added. After cooling down to 25 °C, water (6 L) was added and the upper layer was separated (the workup was done in three parts). The aqueous layer was extracted with ether (3 × 1.5 L) and the combined organic layers were washed with H_2O (2 × 1.5 L), saturated $NaHCO_3$ (600 mL), and brine (300 mL). After drying (Na_2SO_4), the ether was removed by rotary evaporation and the excess of triethyl orthoacetate was recovered by distillation through a 50-cm Vigreux column at reduced pressure (bp ~ 60 °C/30 torr). Then, the ethyl ester **B3** (704 g, 71%) was distilled over at 93–95 °C/30 torr: IR (film) 3290 (s, H—C≡), 3080 (w, H—C=), 2110 (w, C≡C), 1730 (s, C=O), 1635 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 1.26 (t, *J* = 7, 3 H, CH_3C—O), 1.44 (s, 3 H, CH_3), 2.34 (s, 1 H, HC≡), 2.54 (s, 2 H, CH_2), 4.15 (q, *J* = 7, 2 H, CH_2O), 5.11 (dd, J_1 = 10, J_2 = 1, H_a), 5.42 (dd, J_1 = 17, J_2 = 1, H_b), 5.87 (dd, J_1 = 17, J_2 = 10, H_c, CH_c=CH_aH_b); ^{13}C NMR ($CDCl_3$) δ 170.07 (C_1), 141.43 (—HC=), 113.73 (H_2C=), 86.56 (C_4), 71.75 (C_5), 60.36 (CH_2O), 46.19 (C_2), 36.71 (C_3), 27.61 (CH_3), 14.27 (CH_3C—O); MS (EI) *m/z* 166.0987, calcd for $C_{10}H_{14}O_2$ 166.0994.

3-Methyl-3-vinyl-4-pentynoic Acid (B4). NaOH (320 g, 8 mol) was dissolved in 30% ethanol–water (4 L) and added to the ester **B3** (665 g, 4 mol). After stirring at 25 °C overnight, the reaction mixture was diluted with H_2O (2 L) and extracted with ether (2 × 1 L). The aqueous solution was acidified with 2 N HCl (4.4 L) and extracted with ether (3 × 1 L). The extract was washed with water (2 × 400 mL) and brine (200 mL) and dried (Na_2SO_4), and the ether was removed by rotary evaporation. Distillation afforded the pure acid **B4** (515 g, 93%): bp 73–75 °C/0.2 torr; IR (film) 3400–2500 (br, OH), 3290 (s, H—C≡), 1705 (s, C=O), 1635 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 1.46 (s, 3 H, CH_3), 2.37 (s, 1 H, HC≡C), 2.60 (s, 2 H, CH_2), 5.13 (dd, J_1 = 10, J_2 = 1, H_a), 5.45 (dd, J_1 = 17, J_2 = 1, H_b), 5.88 (dd, J_1 = 17, J_2 = 10, H_c, CH_c=CH_aH_b), 10.75 (br s, 1 H, COOH); ^{13}C NMR ($CDCl_3$) δ 176.61 (C_1), 140.99 (—CH=), 114.12 (H_2C=), 86.25 (C_4), 71.95 (C_5), 46.03 (C_2), 36.42 (C_3), 27.52 (CH_3); MS (EI) *m/z* 138.0681, calcd for $C_8H_{10}O_2$ 138.0681.

Resolution of Racemic Acid B4 with (+)-α-Phenylethylamine: (−)-B4S. (+)-α-Phenylethylamine (606 g, 5 mol, Aldrich, $[\alpha]^{25}_D$ +38.1, neat) was added to the acid **B4** (691 g, 5 mol) in 2-propanol (7.8 L ≡ 6 mL per g of salt). The solution was allowed to cool down to 25 °C and then cooled overnight to −20 °C. The salt precipitate was filtered and dried. After *30* recrystallizations using the same ratio of solvent per gram of salt, the diastereomeric purity of the salt (188 g, 29%) was 97.5 ± 2% [the diastereomeric purity was calculated from the heights of the acid methyl singlets (200-MHz ^1H NMR) referring to the RS salt (δ 1.26) and its diastereomer (δ 1.29)]. The melting point rose only by 4 °C from 138–139 to 142–143 °C.

The diastereomeric salt (188 g, 0.725 mol) in 2 N HCl (1.45 L) was extracted with ether (4 × 150 mL). The combined ether layer was washed with brine (2 × 25 mL) and dried (Na_2SO_4), and the solvent was removed by rotary evaporation. Distillation afforded the (−)-acid **B4S** (99.2 g, 29%) in ~95% optical purity: bp 73–75 °C/0.2 torr; $[\alpha]^{25}_D$

−23.00°, $[\alpha]^{25}_{435}$ −48.42° (*c* 15.68, $CHCl_3$).

The water layer was basified carefully under stirring with NaOH (130 g) and the cold solution was extracted with ether (4 × 150 mL). The combined ether layers were washed with brine (2 × 25 mL) and dried (Na_2SO_4). After removing the solvent (VRE), the residue was distilled to give the recovered (+)-α-phenylethylamine (86.1 g, 98%): bp 84–85 °C/20 torr.

The same procedures were applied to recover the acid and the amine from the mother liquors.

Resolution of Racemic Acid B4 with (−)-α-Phenylethylamine: (+)-B4R. The acid **B4** (691 g, 5 mol) was resolved with (−)-α-phenylethylamine (606 g, 5 mol) to give the (+)-acid **B4R** (100.3 g, 29%) in ~95% optical purity (according to the procedure for (−)-B4S): $[\alpha]^{25}_D$ +23.08°, $[\alpha]^{25}_{435}$ +47.56° (*c* 10.84, $CHCl_3$).

(*S*)-*N*,*N*-Dimethyl-3-methyl-3-vinyl-4-pentynamide (B5). The (−)-acid **B4S** (138.2 g, 1 mol) and thionyl chloride (178.5 g, 1.5 mol) were refluxed until the gas evolution stopped (1 h at a bath temperature of 100 °C). After cooling, the excess of thionyl chloride was removed by rotary evaporation, and the crude acid chloride in absolute ether (500 mL) was added to dimethylamine (113 g, 2.5 mol) in absolute ether (500 mL) at −40 °C over 0.5 h. After stirring the reaction mixture for 1 h at 0 °C, the excess of the amine and its salt were extracted with 2 N HCl (500 mL). The aqueous layer was extracted with ether (2 × 500 mL) and the organic layers were washed with H_2O (200 mL) and brine (100 mL). After the combined ether layers (Na_2SO_4) were dried, the solvent was removed (VRE) and the residue distilled to give the amide **B5** (155 g, 94%) as a colorless oil: bp 69–70 °C/0.5 torr; $[\alpha]^{25}_D$ −12.27°, $[\alpha]^{25}_{435}$ −24.50° (*c* 14.31, $CHCl_3$); IR (film) 3290 and 3230 (m, HC≡), 3080 (w, HC=), 2100 (w, C≡C), 1640 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.49 (s, 3 H, CH_3C), 2.35 (s, 1 H, HC≡C), 2.60 (s, 2 H, CH_2), 2.95 and 3.05 (s, each 3 H, NMe_2), 5.09 (dd, J_1 = 10, J_2 = 1, H_a), 5.43 (dd, J_1 = 17, J_2 = 1, H_b), 5.96 (dd, J_1 = 17, J_2 = 10, H_c, $CH_c{=}CH_aH_b$); ^{13}C NMR ($CDCl_3$) δ 169.49 (C_1), 142.23 (—CH=), 113.09 (H_2C=), 87.46 (C_4), 71.50 (C_5), 43.79 (C_2), 38.27 and 37.15 (NMe_2), 35.40 (C_3), 27.87 (CH_3); MS (EI), *m*/*z* 165.1142, calcd for $C_{10}H_{15}NO$ 165.1154.

(*R*)-*N*,*N*-Dimethyl-3-formyl-3-methyl-4-pentynamide (B6). The olefin **B5** (41.3 g, 0.25 mol) in CH_2Cl_2 (500 mL) was cooled to −78 °C and ozone was bubbled into the solution through a glass tube at −78 °C until no starting material was detected by TLC (~10 h). The reaction mixture was swept with N_2 for 1 h and then dimethyl sulfide (100 mL) was added. The mixture was allowed to warm up to 25 °C and stirred overnight. After washing with H_2O (100 mL) and brine (50 mL), the solution was dried and the solvents were removed (VRE). Distillation of the residue afforded oily colorless aldehyde **B6** (35.0 g, 84%): bp 100–102 °C/0.5 torr; $[\alpha]^{25}_D$ −39.68°, $[\alpha]^{25}_{435}$ −96.37° (*c* 11.97, $CHCl_3$); IR (film) 3270 (s, HC≡), 2820 and 2720 (w, CHO), 2110 (C≡C), 1730 (s, C=O, aldehyde), 1640 cm^{-1} (s, C=O, amide); ^1H NMR ($CDCl_3$) δ 1.41 (s, 3 H, CH_3C), 2.36 (s, 1 H, HC≡C), 2.93 (s, 5 H, CH_2 + NMe), 3.02 (s, 3 H, NMe), 9.76 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 198.32 (CHO), 169.05 (C_1), 84.55 (C_4), 72.58 (C_5), 43.76 (C_3), 43.31 (C_2), 37.51 and 35.40 (NMe_2), 22.47 (CH_3); MS (EI), *m*/*z* 138.0918 (M^+ − 29), calcd for $C_8H_{12}NO$ (M^+ − CHO) 138.0918.

(*R*)-*N*,*N*-Dimethyl-3-(formylmethyl)-3-methyl-4-pentynamide (B7). The mixture of the aldehyde **B6** (83.6 g, 0.5 mol), 2-propanol (1 L), nitromethane (100 mL, 1.75 mol), and KF (14.5 g, 0.25 mol) was stirred at 25 °C for 1 day. After removing the solvent by rotary evaporation, water (250 mL) was added and the mixture was extracted with CH_2Cl_2 (3 × 500 mL). The combined organic layer was washed with brine (250 mL) and dried (Na_2SO_4) and the solvent was evaporated (VRE) to give the crude nitro alcohol (111.7 g, 98%) as a viscous orange oil.

It was dissolved in ethyl acetate (1 L), and methanesulfonyl chloride (68.7 g, 0.6 mol) was added all at once at 0 °C. Then triethylamine (101 g, 1 mol) was added under stirring over 20 min at 0 °C, and stirring at that temperature was continued for 2 h. After 2 N HCl (250 mL) was added, the water layer was extracted with EtOAc (2 × 250 mL), and the organic layers were washed with concentrated Na_2CO_3 (250 mL) and brine (100 mL). Drying (Na_2SO_4) and evaporation of the solvent (VRE) gave the crude nitro olefin **B7a** (101.0 g, 96%) which was used without further purification for the next step. To a solution of the nitro olefin **B7a** (101 g) in ethanol (500 mL) at 0 °C was added $NaBH_4$ (18.9 g, 0.5 mol) over 20 min. After stirring for 2 h at 0 °C, the alcohol was removed (VRE) and 2 N HCl (500 mL) was added carefully under cooling. The product was extracted with CH_2Cl_2 (3 × 500 mL) and the extracts were washed with brine (250 mL). The combined organic layers were dried (Na_2SO_4), and the CH_2Cl_2 was removed (VRE) to give the crude nitro compound **B7b** (89 g, 84%) which was dissolved in MeOH (250 mL) and added at 0 °C to a solution of sodium methoxide (11.5 g of dissolved Na, 0.5 mol) in MeOH (250 mL) over 15 min and the mixture was stirred for 0.5 h. This nitronate solution was added under stirring over 0.5 h to a −40 °C mixture of concentrated H_2SO_4 (100 mL) and MeOH (500 mL). During the addition, the mixture was kept at −40 °C and after additional stirring at −20 °C for 1 h, it was poured into CH_2Cl_2 (2.5 L). The organic layer was washed with cold water (1 L) and the water layer was extracted with CH_2Cl_2 (500 mL). The combined organic layers were dried (Na_2SO_4), and the solvent was removed (VRE) to afford the crude dimethyl acetal (70.5 g, 62%).

CH_2Cl_2 (500 mL) and 2 N HCl (500 mL) were added, and the mixture was stirred for 1 day at 25 °C. Then the water layer was extracted with CH_2Cl_2 (2 × 250 mL), and all three organic layers were washed with brine (100 mL), combined, and dried (Na_2SO_4). The solvent was removed by rotary evaporation and the residue chromatographed on silica (EtOAc–hexane 1:1) to afford the pure aldehyde **B7** (22.5 g, 25% overall from **B6**) as a colorless oil: $[\alpha]^{25}_D$ +7.21°, $[\alpha]^{25}_{435}$ +14.15° (neat); IR (film) 3260 (m, HC≡), 1715 (s, C=O, aldehyde), 1635 cm^{-1} (s, C=O, amide); ^1H NMR ($CDCl_3$) δ 1.48 (s, 3 H, CH_3), 2.29 (s, 1 H, HC≡C), 2.61 and 2.69 (AB, J_{AB} = 15, each 1 H, CH_2CON), 2.70 and 2.95 (ABX, J_{AB} = 16, J_{AX} = J_{BX} = 2, each 1 H, CH_2CHO), 2.95 and 3.06 (s, each 3 H, NMe_2), 9.89 (t, *J* = 2, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 201.44 (CHO), 169.52 (C_1), 88.13 (C_4), 70.73 (C_5), 52.54 (CCHO), 42.33 (C_2), 37.95 and 35.43 (NMe_2), 31.19 (C_3), 28.12 (CH_3); MS (EI) *m*/*z* 153.1155 (M^+ − 28), calcd for $C_9H_{15}NO$ (M^+ − CO) 153.1155.

Methyl (4*R*/*S*,5*S*)-5-[(Dimethylcarbamoyl)methyl]-4-formyl-5-methyl-6-heptynoate (B8). The mixture of the aldehyde **B7** (18.1 g, 0.1 mol) and pyrrolidine (10.7 g, 0.15 mol) in benzene (200 mL) was refluxed for 1 h with continuous removal of H_2O by a Dean-Stark trap. The benzene and the excess pyrrolidine were evaporated (VRE) to give the crude enamine which was dissolved in acetonitrile (200 mL). After the addition of methyl acrylate (17.2 g, 0.2 mol), the mixture was refluxed for 8 h and cooled, and 50% AcOH (100 mL) was added. The mixture was then refluxed again for 0.5 h, cooled, and poured into ice–water (500 mL). After extraction with CH_2Cl_2 (3 × 500 mL), the combined organic layers were washed with saturated $NaHCO_3$ (2 × 100 mL) and brine (100 mL) and dried over Na_2SO_4. The solvent was removed (VRE) and the residue chromatographed on silica (EtOAc–hexane 2:1) to give pure oily ester **B8** (21.5 g, 80%) as a 1:1 diastereomeric mixture: IR (film) 3270 (m, HC≡), 1735 (C=O, ester), 1720 (C=O, aldehyde), 1640 cm^{-1} (C=O, amide); ^1H NMR ($CDCl_3$) δ 1.43 and 1.47 (s, 3 H, CH_3C), 1.80–2.95 (m, 7 H), 2.33 and 2.35 (s, 1 H, HC≡C), 2.96, 3.04, 3.06 (3s, total 6 H, NMe_2), 3.67 (s, 3 H, OCH_3), 9.88 and 9.91 (d, *J* = 3, 1 H, CHO); MS (EI), *m*/*z* 252.1244 (M^+ − 15), calcd for $C_{13}H_{18}NO_4$ (M^+ − CH_3) 252.1236.

Oxime B1α and B1β. The aldehyde **B8** (13.36 g, 50 mmol, 1:1 mixture of diastereomers) in pyridine (10 mL) was added all at once to an ice-cold solution of $HONH_2{\cdot}HCl$ (4.34 g, 62.5 mmol) in pyridine (40 mL). The mixture was stirred at 0 °C for 1 h and then at 25 °C overnight. It was added to ice-cold 4 N HCl (150 mL) and extracted with CH_2Cl_2 (3 × 200 mL). The extract was washed with 2 N HCl, saturated $NaHCO_3$, and brine (50 mL) and dried (Na_2SO_4). Evaporation of the solvent (VRE) afforded the crude oxime **B1** (13.1 g, 93%) which was a 1:1 mixture of diastereomers with a *syn*/*anti*-oxime ratio of 1:3 (by ^1H NMR).

The extremely viscous **B1** was dissolved in ether (130 mL) and the oxime crystallized at −10 °C. Two additional recrystallizations from CH_2Cl_2/Et_2O yielded the *anti*-oxime **B1α** (3.94 g, 56%) as a pure diastereomer: mp 114–115 °C; $[\alpha]^{25}_D$ +45.46°, $[\alpha]^{25}_{435}$ +90.22 (*c* 5.35, $CHCl_3$); IR (KBr) 3400–3220 (s, OH), 3210 (s, HC≡), 2100 (w, C≡C), 1735 (C=O, ester), 1685 (w, C=N), 1615 cm^{-1} (s, C=O, amide); ^1H NMR ($CDCl_3$) δ 1.49 (s, 3 H, CH_3C), 1.75–2.75 (m, 7 H), 2.25 (s, 1 H, HC≡C), 2.95 and 3.03 (s, each 3 H, NMe_2), 3.67 (s, 3 H, CH_3O), 7.41 (d, *J* = 9, 1 H, CH=N), 8.27 (s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 173.67 (C=O, ester), 170.00 (C=O, amide), 152.03 (C=N), 86.95 (HC≡*C*), 71.66 (HC≡), 51.65 (OCH_3), 47.56, 41.24, 38.40, 36.52, 35.62, 31.95, 24.96, 23.01; MS (EI), *m*/*z* 265.1549 (M^+ − 17), calcd for $C_{14}H_{21}N_2O_3$ (M^+ − OH) 265.1553. The X-ray structure which shows the absolute configuration of **B1α** is plotted in Figure 1.[19]

The mother liquors of **B1α** were evaporated and redissolved in 10 part Et_2O, and the oxime **B1β** was crystallized at −10 °C. Two additional recrystallizations from CH_2Cl_2/Et_2O yielded the *anti*-oxime **B1β** (3.67 g, 52%) as a pure diastereomer (>95% *anti*-oxime by ^1H NMR): mp 82–83 °C; $[\alpha]^{25}_D$ +13.70°, $[\alpha]^{25}_{435}$ +27.62° (*c* 3.70, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.37 (s, 3 H, CH_3C), 1.70–2.80 (m, 7 H), 2.25 (s, 1 H, HC≡C), 2.96 and 3.06 (s, each 3 H, NMe_2), 3.67 (s, 3 H, CH_3O), 7.37 (d, *J* = 9, 1 H, CH=N), 7.88 (s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 173.77 (C=O, ester), 169.88 (C=O, amide), 151.71 (C=N), 87.43 (HC≡*C*),

(19) The final agreement factors for compounds **B1α** and **D8α** are R = 0.0558 and R_w = 0.0619 and R = 0.0972 and R_w = 0.0845, respectively. Complete details of the X-ray crystal structure analyses of these two compounds can be found in the supplementary material.

71.44 (HC≡), 51.61 (CH_3O), 47.11, 41.02, 38.37, 36.64, 35.62, 31.98, 24.67, 23.56.

Methyl (*R*)-3-Methyl-3-vinyl-4-pentynoate (D2). Acetyl chloride (25 mL) and methanol (500 mL) were mixed together at 0 °C, and then the (+)-acid **B4R** (69.1 g, 0.5 mol) was added. After the mixture was stirred at 25 °C overnight, the methanol was removed (VRE) and the residue dissolved in CH_2Cl_2 (500 mL). The solution was washed with H_2O, concentrated $NaHCO_3$, and brine (100 mL) and dried (Na_2SO_4). After removal of the CH_2Cl_2 by rotary evaporation, the residue was distilled under reduced pressure to give the pure methyl ester **D2** (71.5 g, 94%) as a colorless liquid: bp 79–80 °C/25 torr; $[\alpha]^{25}_D$ +20.9°, $[\alpha]^{25}_{435}$ +43.1° (*c* 10.90, $CHCl_3$); IR (film) 3300 (m, HC≡), 1735 (s, C=O), 1635 cm^{-1} (w, C=C); ^1H NMR ($CDCl_3$) δ 1.44 (s, 3 H, CH_3C), 2.36 (s, 1 H, HC≡C), 2.56 (s, 2 H, CH_2), 3.68 (s, 3 H, CH_3O), 5.11 (dd, J_1 = 10, J_2 = 1, H_a), 5.43 (dd, J_1 = 17, J_2 = 1, H_b), 5.87 (dd, J_1 = 17, J_2 = 10, H_c, CH_c=CH_aH_b); ^{13}C NMR ($CDCl_3$) δ 170.47 (C_1), 141.30 and 113.81 (C=C), 86.44 (C_4), 71.75 (C_5), 51.44 (CH_3O), 46.00 (C_2), 36.60 (C_3), 27.57 (CH_3); MS (EI), *m/z* 152.0834, calcd for $C_9H_{12}O_2$ 152.0838.

Methyl (*S*)-3-Formyl-3-methyl-4-pentynoate (7). The mixture of the olefin **D2** (0.91 g, 6 mmol) and MeOH/CH_2Cl_2 1:1 (15 mL) was ozonized at −78 °C until the blue appeared and then swept with N_2 for 0.5 h. After the addition of $(CH_3O)_3P$ (2 mL), the mixture was allowed to warm up to 25 °C and stirred overnight. Evaporation of the solvents and chromatography of the residue on silica with EtOAc–hexane 1:4 afforded 0.79 g (85%) of acetylenic aldehyde **7**: $[\alpha]^{25}_D$ +2.6°, $[\alpha]^{25}_{435}$ −2.0° (*c* 6.10, $CHCl_3$); IR (film) 3280 (m, HC≡), 1735 cm^{-1} (s, CHO and CO_2Me); ^1H NMR ($CDCl_3$) δ 1.42 (s, 3 H, CH_3); 2.42 (s, 1 H, HC≡C), 2.76 and 2.86 (AB, J_{AB} = 16, each 1 H, CH_2), 3.70 (s, 3 H, CH_3O), 9.64 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 197.20, 170.23, 82.70, 73.99, 51.90, 43.99, 41.46, 21.86; MS (EI), *m/z* 142.0633 (M^+ − 12), calcd for $C_7H_{10}O_3$ (M^+ − C) 142.0630.

(*S*)-2-Ethynyl-2-methylsuccinic Acid 4-Methyl Ester (8). To the aldehyde **7** (0.77 g, 5 mmol) in acetone (12.5 mL) Jones reagent was added dropwise at 25 °C until the orange remained for 0.5 h. The acetone was decanted and the green salt washed with acetone (2 × 5 mL). After evaporation of the combined organic layers, H_2O (5 mL) was added, and the product was extracted with CH_2Cl_2 (3 × 25 mL). Evaporation of the solvent and chromatography on silica with EtOAc–hexane 2:3 afforded 0.52 g (61%) of acid **8**: $[\alpha]^{25}_D$ +19.0°, $[\alpha]^{25}_{435}$ +36.45° (*c* 4.90, $CHCl_3$); IR (film) 3600–2600 (br, CO_2H), 3280 (m, HC≡), 1735 (s, C=O, ester), 1715 cm^{-1} (s, C=O, acid); ^1H NMR ($CDCl_3$) δ 1.62 (s, 3 H, CH_3), 2.37 (s, 1 H, HC≡C), 2.87 and 2.93 (AB, J_{AB} = 16.4, each 1 H, CH_2), 3.72 (s, 3 H, CH_3O); ^{13}C NMR ($CDCl_3$) δ 177.66, 170.23, 83.07, 71.80, 51.81, 43.41, 39.95, 25.43; MS (EI), *m/z* 170.0589 (M^+), calcd for $C_8H_{10}O_4$ 170.0579.

(*R*)-2-Ethyl-2-methylsuccinic Acid (10). The acetylenic acid **8** (0.26 g, 1.5 mmol) in MeOH (7.5 mL) was hydrogenated over 10% Pd–C at 1 atm until the theoretical amount of H_2 was consumed. The catalyst was filtered off, and the methanol was evaporated to give the crude ester **9** which was used without purification for the next step. NaOH (2 N) (7.5 mL) was added, and the mixture was stirred at 25 °C overnight. The solution was then acidified with 2 N HCl and extracted with EtOAc (3 × 15 mL). Evaporation of the solvent (VRE) and chromatography of the residue on silica (EtOAc–hexane 2:1) afforded 0.20 g (84%) diacid **10**. Crystallization from benzene–cyclohexane gave 0.16 g of optically active 2-ethyl-2-methylsuccinic acid: mp 63–64 °C; $[\alpha]^{25}_D$ +5.6° (*c* 5.40, $CHCl_3$); [lit.[9] (*S*)-acid mp 64.6–65.4 °C; $[\alpha]^{22}_D$ −5.9°); IR, ^1H, and ^{13}C NMR were identical with those of an authentic sample.

(*R*)-3-Methyl-3-vinyl-4-pentyn-1-ol (D3). At 0 °C, the ester **D2** (68.5 g, 0.45 mol) was added to a suspension of LAH (17.1 g, 0.45 mol) in absolute ether (450 mL) under stirring over 0.5 h. The reaction mixture was now refluxed for 2 h and then cooled below 0 °C with dry ice–acetone. HCl (6 N) (350 mL) was added at a rate that the temperature did not exceed 5 °C and then the ether layer was separated. The aqueous layer was extracted with ether (2 × 200 mL), and the combined organic layers were washed with 100 mL of H_2O, concentrated $NaHCO_3$, and brine. After drying (Na_2SO_4) and evaporation of the ether (VRE), distillation of the residue afforded the alcohol **D3** (52.4 g, 94%): bp 92 °C/25 torr; $[\alpha]^{25}_D$ +14.8°, $[\alpha]^{25}_{435}$ +28.6° (*c* 10.13, $CHCl_3$); IR (film) 3325 (br, OH), 3300 (s, HC≡), 3085 (w, HC=), 2110 (w, C≡C), 1640 cm^{-1} (m, C=C); ^1H NMR ($CDCl_3$) δ 1.33 (s, 3 H, CH_3), 1.65–1.95 (m, 2 H, CH_2), 1.98 (s, 1 H, OH), 2.38 (s, 1 H, HC≡C), 3.78 (t, J = 6.5, 2 H, CH_2O), 5.10 (dd, J_1 = 10, J_2 = 1.4, H_a), 5.43 (dd, J_1 = 17, J_2 = 1.4, H_b), 5.72 (dd, J_1 = 17, J_2 = 10, H_c, CH_c=CH_aH_b); ^{13}C NMR ($CDCl_3$) δ 142.52, 113.60 (C=C), 87.39 (C_4), 72.42 (C_5), 60.07 (C_1), 44.02 (C_2), 37.17 (C_3), 28.76 (CH_3); MS (EI), *m/z* 124.0887, calcd for $C_8H_{12}O$ 124.0888.

(*R*)-4-Methyl-4-vinyl-5-hexynonitrile (D4). *p*-Toluenesulfonyl chloride (95.4 g, 0.5 mol) was added in portions to the alcohol **D3** (49.7 g, 0.4 mol) in pyridine (200 mL) at 0 °C. After stirring at 25 °C overnight, ice (40 g) was added and the mixture stirred for 0.5 h. Then 6 N HCl (500 mL) was added under cooling and the aqueous layer was extracted with ether (3 × 200 mL). The combined organic layers were washed with 100 mL of 2 N HCl, concentrated $NaHCO_3$, and brine and dried (Na_2SO_4). Evaporation of the solvent afforded the oily tosylate (111.5 g, 100%) which was dissolved in Me_2SO (400 mL, dried over CaH_2 at 120 °C for 2 h and then distilled at 20 torr). NaCN (39.2 g, 0.8 mol, dried for 2 days at 90 °C under high vacuum) was added and the mixture stirred at 90 °C for 2 h. The dark-brown reaction mixture was cooled and poured into ice–water (800 g). The aqueous layer was extracted with ether (4 × 200 mL), and the combined ether layers were washed with brine (2 × 50 mL). After drying ($NaSO_4$), the solvent was evaporated (VRE) and the residue distilled to afford the colorless liquid nitrile **D4**: bp 98–99 °C/20 torr; $[\alpha]^{25}_D$ +38.9°, $[\alpha]^{25}_{435}$ +79.5° (*c* 9.42, $CHCl_3$); IR (film) 3290 (s, HC≡), 3080 (w, HC=), 2240 (m, C≡N), 2100 (w, C≡C), 1635 cm^{-1} (m, C=C); ^1H NMR ($CDCl_3$) δ 1.35 (s, 3 H, CH_3), 1.70–2.05 (m, 2 H, CH_2), 2.25–2.60 (m, 2 H, CH_2CN), 2.39 (s, 1 H, HC≡C), 5.18 (dd, J_{BX} = 9.5, J_{AX} = 1.8, H_X), 5.44 and 5.60 (ABX, J_{AB} = 16.9, J_{AX} = 1.8, J_{BX} = 9.5, H_A and H_B, CH_B=CH_AH_X); ^{13}C NMR ($CDCl_3$) δ 140.44 (—CH=), 119.74 (C_1), 115.61 (H_2C=), 85.22 (C_5), 73.54 (C_6), 38.69 (C_4), 37.09 (C_3), 28.18 (CH_3), 13.46 (C_2); MS (EI), *m/z* 133.0871, calcd for $C_9H_{11}N$ 133.0892.

(*S*)-4-Formyl-4-methyl-5-hexynonitrile (D5). The olefin **D4** (66.6 g, 0.5 mol) in CH_2Cl_2 (1000 mL) was ozonized at −78 °C until a light-blue color was detected (~10 h). The reaction mixture was swept with N_2 for 1 h, and then Me_2S (200 mL) was added. The mixture was allowed to come to 25 °C and stirred overnight. After washing with H_2O (2 × 200 mL) and brine (100 mL), the solution was dried (Na_2SO_4). Evaporation of the solvent (VRE) and distillation of the residue afforded the colorless liquid aldehyde **D5** (52.7 g, 78%): bp 65–67 °C/0.5 torr; $[\alpha]^{25}_D$ −93.8°, $[\alpha]^{25}_{435}$ −243.6° (*c* 14.12, $CHCl_3$); IR (film) 3280 (s, HC≡), 2800 and 2720 (w, CHO), 2250 (w, C≡N), 1730 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.39 (s, 3 H, CH_3), 1.78–1.98 and 2.10–2.30 (m, each 1 H, CH_2), 2.45–2.58 (m, 2 H, CH_2CN), 2.53 (s, 1 H, HC≡C), 9.47 (s, 1 H, CHO); ^{13}C NMR ($CDCl_3$) δ 196.38 (C=O), 119.06 (C_1), 80.95 (C_5), 76.16 (C_6), 46.28 (C_4), 30.90 (CH_3), 21.71 (C_3), 13.28 (C_2); MS (EI), *m/z* 107.0732 (M^+ − 28), calcd for C_7H_9N (M^+ − CO) 107.0735.

Methyl (*R*)-4-(2-Cyanoethyl)-4-methylhex-2(*E*)-en-5-ynoate (D6). In a three-necked flask with a mechanical stirrer, 50% NaH oil dispersion (26.4 g, 0.55 mol) was washed with pentane (3 × 100 mL), and dry DME (250 mL) was added. To this NaH suspension trimethylphosphonoacetate (100.2 g, 0.55 mol) in DME (125 mL) was added over 0.5 h at 0 °C. After stirring for 0.5 h, the aldehyde **D5** (67.6 g, 0.5 mol) in DME (125 mL) was added at a rate that the temperature did not exceed 30 °C and then the mixture was stirred for 4 h at 25 °C (meanwhile the initially insoluble salt gradually dissolved and the mixture turned brown). The reaction mixture was poured into ether (2.5 L) and extracted with saturated NH_4Cl (250 mL) and brine (3 × 250 mL). The solution was dried (Na_2SO_4), the ether evaporated (VRE), and the residue chromatographed on silica (EtOAc–hexane 1:5) to give the oily ester **D6** (60.9 g, 64%): $[\alpha]^{25}_D$ +13.3°, $[\alpha]^{25}_{435}$ +28.1° (*c* 8.80, $CHCl_3$); IR (film) 3280 (s, HC≡), 2240 (w, C≡N), 1725 (s, C=O), 1650 cm^{-1} (m, C=C); ^1H NMR ($CDCl_3$) δ 1.42 (s, 3 H, CH_3), 1.80–2.10 (m, 2 H, CH_2), 2.20–2.65 (m, 2 H, CH_2CN), 2.47 (s, 1 H, HC≡C), 3.76 (s, 3 H, CH_3O), 6.22 and 6.67 (d, J = 15.5, each 1 H, CH=CH); ^{13}C NMR ($CDCl_3$) δ 166.37 (C_1), 149.34 (C_3), 122.20 (C_2), 119.13 (CN), 83.71 (C_5), 74.62 (C_6), 51.79 (CH_3O), 38.18 (C_4), 36.76 (CH_2), 27.66 (CH_3), 13.60 (CH_2CN); MS (EI), *m/z* 176.0714 (M^+ − 15), calcd for $C_{10}H_{10}NO_2$ (M^+ − CH_3) 176.0712.

Methyl (3*R*,4*R*)-4-(2-Cyanoethyl)-4-methyl-3-(nitromethyl)-5-hexynoate (D7β). The mixture of the α,β-unsaturated ester **D6** (9.6 g, 50 mmol), tetramethylguanidine (2.30 g, 20 mmol), and nitromethane (20 mL, 0.375 mol) was stirred at 25 °C for 6 days. The mixture was then diluted with CH_2Cl_2 (250 mL), washed with 2 N HCl (50 mL) and brine (25 mL), and dried (Na_2SO_4). After evaporation of the solvent (VRE), the residue was chromatographed on silica (EtOAc–hexane 1:3) to give the nitro ester **D7** (8.0 g, 63%) as a mixture of two diastereomers (**D7β**/**D7α** ≃ 2:1 by ^1H NMR).

The viscous oily **D7** was dissolved in ether (40 mL) and cooled to −20 °C for 2 days to afford the pure crystalline diastereomer **D7β** (4.01 g, 50%): mp 46–48 °C; $[\alpha]^{25}_D$ −6.4°, $[\alpha]^{25}_{435}$ −24.4° (*c* 9.40, $CHCl_3$); IR (film) 3280 (m, HC≡), 2240 (w, C≡N), 1735 (s, C=O), 1555 cm^{-1} (NO_2); ^1H NMR ($CDCl_3$) δ 1.22 (s, 3 H, CH_3), 1.70–2.10 (m, 2 H, CH_2), 2.37 (s, 1 H, HC≡C), 2.40–2.75 (m, 4 H, CH_2CN and CH_2CO), 2.85–3.05 (m, 1 H, CH), 3.71 (s, 3 H, CH_3O), 4.48 and 4.76 (ABX, J_{AB} = 13.6, J_{AX} = 7.7, J_{BX} = 4.2, each 1 H, CH_2NO_2); ^{13}C NMR ($CDCl_3$) δ 171.66 (s, C_1), 119.19 (s, CN), 84.49 (d, C_5), 76.93 (t, C—NO_2), 74.19 (d, C_6), 52.28 (q, CH_3O), 41.05 (d, C_3), 37.67 (s, C_4), 34.44 (t, CH_2), 33.23 (t, *C*—CO), 22.59 (q, CH_3), 13.28 (t, *C*—CN); MS (EI), *m/z*

221.0924 ($M^+ - 31$), calcd for $C_{11}H_{13}N_2O_3$ ($M^+ - CH_3O$) 221.0927.

(*R*)-4-[(*R*)-3-Cyano-1-ethynyl-1-methylpropyl]-2-pyrrolidinone (D8β). To a mixture of concentrated HCl (40 mL) and methanol (40 mL) were added at the same time at 0 °C the nitro ester **D7β** (5.1 g, 20 mmol) in methanol (20 mL) and Zn powder (13.1 g, 0.2 mol) dropwise and in small portions, respectively, over 0.5 h. After the addition, the reaction mixture was stirred at 25 °C for 0.5 h and then filtered and treated with 6 N NaOH (400 mL). The clear solution was extracted with CH_2Cl_2 (3 × 200 mL), and the combined extracts were washed with brine (20 mL) and dried (Na_2SO_4). Evaporation of the solvent and crystallization of the residue from benzene (10 parts) gave the pure lactam **D8β** (2.86 g, 75%): mp 115–117 °C; $[\alpha]^{25}_D$ +40.2°, $[\alpha]^{25}_{435}$ +82.2° (*c* 2.72, $CHCl_3$); IR (KBr) 3260 (s, HC≡), 2240 (w, C≡N), 1710 and 1670 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.22 (s, 3 H, CH_3), 1.60–1.95 (m, 2 H, CH_2), 2.25–2.73 (m, 5 H, $CHCH_2CO$ and CH_2CN), 2.33 (s, 1 H, HC≡C), 3.42 (m, 2 H, CH_2N), 6.47 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 177.20 (C=O), 119.45 (CN), 84.46 and 74.22 (C≡C), 44.37, 43.83, 37.87, 34.89, 32.72, 23.67, 13.22; MS (EI), *m/z* 175.0870 ($M^+ - 15$), calcd for $C_{10}H_{11}N_2O$ ($M^+ - CH_3$) 175.0872.

(*S*)-4-[(*R*)-3-Cyano-1-ethynyl-1-methylpropyl]-2-pyrrolidinone (D8α). The mother liquor of **D7β** 5.1 g, 20 mmol, diastereomeric ration **D7β**/**D7α** ≃ 1:2 by ^1H NMR) was treated as described for **D8β** to afford 2.70 g of crude lactam as a diastereomeric mixture. The major diastereomer **D8α** was isolated by two crystallizations from benzene (the compound was dissolved in 50 parts benzene and the solution was concentrated to half of its volume by distilling off benzene) to give pure colorless **D8α** (1.41 g, 37%). A third crystallization afforded suitable crystals for an X-ray structure which is shown in Figure 1:[19] mp 157–158 °C; $[\alpha]^{25}_D$ +6.7°, $[\alpha]^{25}_{435}$ +9.9° (*c* 5.25, $CHCl_3$); IR (KBr) 3250 (s, HC≡), 3225 (m, NH), 2250 (w, C≡N), 1690 and 1665 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.20 (s, 3 H, CH_3), 1.60–1.79 and 1.82–2.02 (m, each 1 H, CH_2), 2.25–2.73 (m, 5 H, $CHCH_2CO$ and CH_2CN), 2.33 (s, 1 H, HC≡C), 3.42 (m, 2 H, CH_2N), 6.29 (br s, 1 H, NH); ^{13}C NMR ($CDCl_3$) δ 177.31 (C=O), 119.51 (CN), 84.34, 74.37, 44.31, 44.02, 37.95, 35.52, 32.92, 23.13, 13.17.

(3*R*,4*R*)-3-(Aminoethyl)-4-(2-cyanoethyl)-4-methyl-5-hexynoic Acid (D1β). The mixture of the lactam **D8β** (0.95 g, 5 mmol) and 1 N HCl (25 mL) was refluxed for 2 h. After the mixture cooled down to 25 °C **D8β** (0.47 g, 49%) was recovered by extraction with CH_2Cl_2 (3 × 50 mL). After neutralizing the water layer with 1 N NaOH (25 mL, pH ≃ 7), the water was removed by rotary evaporation on the high vacuum at 25 °C. Addition of benzene (100 mL) and evaporation (VRE) afforded a crystalline residue which was extracted 3 tims under stirring with absolute ethanol (25 mL) at 25 °C for 1 h. The combined, filtered extracts were concentrated at 25 °C (VRE) to about 5 mL and the amino acid crystallized at −20 °C to give colorless **D1β** (0.23 g, 44%): mp 122–124 °C; $[\alpha]^{25}_D$ −28.4°, $[\alpha]^{25}_{435}$ −58.7° (*c* 2.5, Me_2SO); IR (KBr) 3250 (s, HC≡), 3200–2400 (NH_2, OH), 2240 (w, C≡N), 1605 cm^{-1} (s, C=O); ^1H NMR (D_2O) δ 1.20 (s, 3 H, CH_3), 1.75–2.76 (m, 7 H), 2.77 (s, 1 H, HC≡C), 3.00 and 3.48 (ABX, J_{AB} = 13, J_{AX} = 8, J_{BX} = 3, each 1 H, CH_2N), 4.75 (s, ~4 H, HOD); ^{13}C NMR (D_2O) δ 183.09 (s, C_1), 124.61 (s, CN), 89.73 (s, C_5), 76.51 (d, C_6), 45.07 (t, CH_2N), 44.21 (d, C_3), 40.25 (s, C_4), 40.03 (t, CH_2), 36.13 (t, C_2), 24.10 (q, CH_3), 15.51 (t, CH_2CN); MS (EI), *m/z* 175.0879 ($M^+ - 33$), calcd for $C_{10}H_{11}N_2O$ ($M^+ - H_2O - CH_3$) 175.0872 (MS shows the same pattern as for the lactam **D8β**).

(3*S*,4*R*)-3-(Aminomethyl)-4-(2-cyanoethyl)-4-methyl-5-hexynoic Acid (D1α). The lactam **D8α** (0.95 g, 5 mmol) was treated and worked up as described for compound **D1β** to afford the crystalline amino acid **D1α** (0.22 g, 42%): mp 135–137 °C; IR (KBr) 3270 (m, HC≡), 3200–2400 (NH_2, OH), 2240 (w, C≡N), 1560 cm^{-1} (s, br, C=O); ^1H NMR (D_2O) δ 1.27 (s, 3 H, CH_3), 1.75–2.80 (m, 7 H), 2.75 (s, 1 H, HC≡C), 3.00 and 3.43 (ABX, J_{AB} = 13, J_{AX} = 8, J_{BX} = 3, each 1 H, CH_2N), 4.72 (s, ~18 H, HDO).

(*R*)-4-[(*R*)-3-Cyano-1-ethynyl-1-methylpropyl]-1-hydroxy-2-pyrrolidinone (D9). To the nitro ester **D8β** (2.52 g, 10 mmol) and Zn powder (3.9 g, 60 mmol) in MeOH (20 mL) 4 N HCl in MeOH (20 mL) was added under stirring over 0.5 h at 0 °C. The Zn was filtered off and washed with MeOH (5 mL). After evaporation of the MeOH (VRE), H_2O (10 mL) was added to the residue and the product was extracted with CH_2Cl_2 (3 × 10 mL). The combined extract was dried (Na_2SO_4) and the solvent removed by rotary evaporation. Crystallization from H_2O (10 mL) yielded the colorless hydroxamic acid **D9** (1.61 g, 78%): mp 120–122 °C, $[\alpha]^{25}_D$ −17.6°, $[\alpha]^{25}_{435}$ −36.4° (*c* 10.00, $CHCl_3$); IR (KBr) 3400 (br, OH), 3260 (s, HC≡), 2220 (w, C≡N), 1660 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.20 (s, 3 H, CH_3), 1.60–1.95 (m, 2 H, CH_2), 2.35 (s, 1 H, HC≡C), 2.35–2.75 (m, 5 H, CH_2CN and $CHCH_2CO$), 3.68 (m, 2 H, CH_2N), 10.00 (br s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 169.27 (C=O), 119.35 (C≡N), 84.08 and 74.53 (C≡C), 50.98, 38.27, 37.86, 34.73, 31.08, 23.05, 13.25; MS (EI), *m/z* 206.1052, calcd for $C_{11}H_{14}N_2O_2$ 206.1056. Anal.: C, 64.06; H, 6.85; N, 13.47%. Calcd for $C_{11}H_{14}N_2O_2$: C, 64.06; H, 6.84; N, 13.58%.

Ethyl 2-Cyano-3,3-dimethyl-4-pentynoate (C4). To 2.5 L of ethanol in a 5-L three-necked flask fitted with a reflux condenser, mechanical stirrer, and drying tube, Li wire (34.7 g, 5 mol) was added in portions at a rate that maintained gentle boiling. After all the Li was dissolved, the suspension was cooled to about 40 °C, ethyl cyanoacetate (566 g, 5 mol) was added, and the clear solution was cooled to 25 °C while stirring. Meanwhile, a saturated solution of CuCl in hot concentrated HCl was prepared and diluted with 5 vol of H_2O. The aqueous solutions were decanted from the white precipitate of CuCl which was rinsed twice with H_2O and then several times with absolute ethanol. The slurry of CuCl in EtOH (2.5 mL) and Cu powder (1.0 g) was added to the Li salt solution and then 3-chloro-3-methyl-1-butyne (**C3**)[5] (256 g, 2.5 mol) was added over 2 h at 30 °C (cooling). After additional stirring at room temperature for 2 h, the reaction mixture was poured into 1 N HCl (5 L) and extracted with ether (3 × 500 mL). The extracts were then washed with H_2O (2 × 500 mL), concentrated $NaHCO_3$, and brine (250 mL). The combined ether layers were dried (Na_2SO_4), the solvent was removed (VRE), and the residue was distilled through a Vigreux column to afford the cyano ester **C4** (246 g, 55%): bp 61–62 °C/0.3 torr; the ^1H NMR and IR spectra of this product were identical with those previously reported;[16] ^{13}C NMR ($CDCl_3$) δ 164.04 (C=O), 114.91, (CN), 85.88 (C_4), 71.81 (C_5), 62.79 (OCH_2), 48.64 (C_2), 34.00 (C_3), 27.61 and 27.10 (2 × CH_3), 14.05 (CH_3C—O); MS (EI), *m/z* 150.0550 ($M^+ - 29$), calcd for $C_8H_8NO_2$ ($M^+ - C_2H_5$).

Methyl 5,5-Dimethyl-4-formyl-6-heptynoate (C7). The mixture of 3,3-dimethyl-4-pentynal (**C6**)[16] (121.2 g, 1.1 mol) and pyrrolidine (117.3 g, 1.65 mol) in benzene (1.1 L) was refluxed for 2 h with continuous removal of H_2O by a Dean-Stark trap. The benzene and the excess pyrrolidine were removed by rotary evaporation to give the crude enamine (184 g, 102%) which was dissolved in acetonitrile (1.1 L). After the addition of methyl acrylate (189.5 g, 2.2 mol), the mixture was refluxed for 10 h and cooled, and 50% AcOH (550 mL) was added. The mixture was then refluxed again for 0.5 h, cooled, and poured into ice–water (2.2 L). After the extraction with ether (3 × 500 mL), the combined organic layers were washed with saturated $NaHCO_3$ (500 mL) and brine (200 mL) and dried over Na_2SO_4. Removal of the solvent (VRE) and distillation of the residue afforded the ester **C7** (176.5 g, 82%): bp 80–82 °C/0.4 torr; ^{13}C NMR ($CDCl_3$) δ 204.15 (CHO), 173.17 (C_1), 88.18 (C_6), 70.82 (C_7), 59.44, 51.59 (CH_3O), 32.06, 31.64, 27.84 and 27.21 (2 × CH_3), 20.34; ^1H NMR, IR, and MS spectra were identical with those previously reported.[3]

Acetylenic Oxime C1. The aldehyde **C7** (172.7 g, 0.88 mol) in pyridine (180 mL) was added all at once to an ice-cold solution of $HON{\cdot}H_2{\cdot}HCl$ (76.5 g, 1.1 mol) in pyridine (700 mL). The reaction mixture was stirred at 0 °C for 1 h and then at 25 °C overnight. After the addition of 4 N HCl (3.5 L), the aqueous layer was extracted with ether (3 × 500 mL). The combined extracts were washed with 2 N HCl, saturated $NaHCO_3$, and brine (200 mL) and dried over Na_2SO_4. Removal of the solvent (VRE) yielded the crude aldoxime **C1** (180.5 g, 97%) which was used without further purification for the next step: IR (film) 3400 (br, OH), 3280 (s, HC≡), 2100 (w, C≡C), 1735 (s, C=O), 1720 cm^{-1} (m, C=N); ^1H NMR ($CDCl_3$) δ 1.21 and 1.28 (s, each 3 H, $(CH_3)_2C$), 1.65–2.50 (m, 5 H, $CHCH_2CH_2CO$), 2.18 (s, 1 H, HC≡C), 3.67 (s, 3 H, CH_3O), 6.72 (d, *J* = 9, 1 H, CH=N, 10% *syn*-oxime), 7.35 (d, *J* = 9, 1 H, CH=N, 90% *anti*-oxime), 8.54 (s, 1 H, OH); MS (EI), *m/z* 196.0972 ($M^+ - 15$), calcd for $C_{10}H_{14}NO_3$ ($M^+ - CH_3$) 196.0974.

Oxime of 5,5-Dimethyl-4-formyl-6-heptynoic Acid (C8). The crude ester **C1** (137.3 g, 0.65 mol) was dissolved in a solution of NaOH (64 g, 1.6 mol) in ethanol (2 L) and H_2O (500 mL). After stirring overnight at 25 °C, the alcohol was removed by rotary evaporation, and the aqueous layer was extracted with ether (2 × 100 mL) and then acidified with 4 N HCl (650 mL). The acid was extracted with ether (3 × 300 mL) and the combined extracts were washed with brine (100 mL) and dried (Na_2SO_4). Evaporation of the solvent afforded the crude acid **C8** (122.5 g, 96%) as a viscous oil: IR (film) 3500–2500 (br, OH oxime and acid), 3280 (s, HC≡), 2100 (w, C≡C), 1710 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.21 and 1.28 (s, each 3 H, 2 × CH_3, *anti*-oxime), 1.22 and 1.31 (s, each 3 H, 2 × CH_3, *syn*-oxime), 1.70–3.25 (m, 5 H, $CHCH_2CH_2CO$), 2.20 (s, 1 H, HC≡C), 6.76 (d, *J* = 9, 1 H, CH=N, 30% *syn*-oxime), 7.39 (d, *J* = 9, 1 H, CH=N, 70% *anti*-oxime), 8.79 (br s, 2 H, CO_2H and NOH).

Resolution of Racemic Acid C8: Salt C9. To the crude acid **C8** (122.3 g, 0.62 mol) in ethanol (1500 mL) (−)-α-phenylethylamine (75 g, 0.62 mol) was added. The solution was cooled and kept at −20 °C for 1 day. The salt precipitate was filtered and dried and then redissolved in the minimum amount of refluxing ethanol (~10–15 mL per g of salt). After cooling to −20 °C for 1 day, fraction 2 was filtered and dried. This procedure was repeated 5 times to give fraction 7 (28.1 g, 28%). The

melting point rose from 158–168 °C (fraction 1) to 180–182 °C (fraction 7). Further crystallizations did not improve the diastereomeric purity of the salt **C9** (see under **C10**).

Methyl (*S*)-4-Cyano-5,5-dimethyl-6-heptynoate (C10). The salt **C9** of one fraction (0.32 g, 1 mmol) in 2 N HCl (10 mL) was extracted with ether (3 × 10 mL). The ether layers were washed with brine (2.5 mL), combined, and dried over Na_2SO_4. After treatment with CH_2N_2 as usual, the solvent was evaporated (VRE) and the residue dried on the high vacuum. The crude oxime ester in pyridine (3 mL) was cooled to 0 °C, and tosyl chloride (0.29 g, 1.5 mmol) in pyridine (2 mL) was added. After the mixture was stirred at 25 °C for 1 day, H_2O (1 mL) was added and stirring continued for 15 min. The reaction mixture was then poured into 4 N HCl (10 mL) and extracted with ether (3 × 10 mL). The organic layers were washed with brine (2 mL) and dried (Na_2SO_4). After removal of the solvent (VRE), the residue was chromatographed on silica (petroleum ether–ether 3:2) and then distilled in a Kugelrohr oven to afford pure nitrile **C10** (0.17 g, 88%): bp ~100 °C/0.5 torr; IR (film) 3280 (m, HC≡), 2240 (w, C≡N), 1735 cm^{-1} (s, C=O); ^1H NMR ($CDCl_3$) δ 1.40 (s, 6 H, $(CH_3)_2C$), 1.85–2.75 (m, 5 H, $CHCH_2CH_2CO$), 2.27 (s, 1 H, HC≡C), 3.71 (s, 3 H, CH_3O); ^{13}C NMR ($CDCl_3$) δ 172.64 (C=O), 119.31 (C≡N), 86.88 (C_6), 71.28 (C_7), 51.84 (CH_3O), 42.71, 33.68, 31.70, 27.97, 26.36, 23.80; MS (EI), *m/z* 193.1082, calcd for $C_{11}H_{15}NO_2$ 193.1103

fraction	$[\alpha]^{25}_D$	$[\alpha]^{25}_{435}$	*c* ($CHCl_3$)
4	−43.4°	−85.5°	10.80
6	−59.6°	−117.5°	7.78
7	−65.6°	−129.3°	11.07
10	−65.7°	−129.4°	8.29

Nitrile Oxide A16. NCS (1.47 g, 11 mmol) in absolute DMF (25 mL) was added to the aldoxime **A1** (3.31 g, 10 mmol) in DMF (50 mL) at 40 °C over 10 min. After additional stirring at 40 °C for 1 h (after about 10 min, the mixture turned green and an exothermic reaction occurred → cooling!), the reaction mixture was poured onto ice (200 g) and extracted with ether (3 × 200 mL). The ether layers were washed with ice-cold H_2O (2 × 50 mL) and brine (50 mL) and combined. After drying (Na_2SO_4), evaporation of the solvent (VRE) afforded 3.66 g (100%) of crude hydroxamoyl chloride **A16a** which was dissolved in ether (100 mL) and added at 0 °C over 20 min to Et_3N (2.0 g, 20 mmol) in ether (100 mL). The mixture was stirred at 25 °C for 0.5 h and then filtered. After removal of the solvent (VRE), the residue was chromatographed on silica (EtOAc–hexane 1:2) to afford pure oily nitrile oxide **A16** (2.49 g, 76%): $[\alpha]^{25}_D$ −19.8°, $[\alpha]^{25}_{435}$ −38.9° (*c* 6.05, $CHCl_3$); IR (film) 2290 (s, CNO), 1735 (C=O), 1375 cm^{-1} (N—O); ^1H NMR ($CDCl_3$) δ 1.26 and 1.33 (s, each 3 H, 2 × CH_3), 1.75–2.70 (m, 6 H, 3 × CH_2), 3.38 (dd, J_1 = 3, J_2 = 12, 1 H, CHCNO), 3.68 and 3.71 (s, each 3 H, 2 × CH_3O), 3.80–4.10 (m, 4 H, OCH_2CH_2O); ^{13}C NMR ($CDCl_3$) δ 172.84 and 171.63 (C=O), 112.32 (O—C—O), 64.57 (OCH_2CH_2O), 51.81 and 51.71 (CH_3O), 47.75, 38.02, 37.73, 32.43, 24.64, 20.30, 19.44, the nitrile oxide (CNO) could not be observed;[20] MS (EI), *m/z* 314.1242 (M^+ − 15), calcd for $C_{14}H_{20}NO_7$ (M^+ − CH_3) 314.1240.

Isoxazole APh. The mixture of the nitrile oxide **A16** (66 mg, 0.2 mmol), phenylacetylene (0.5 mL), and CH_2Cl_2 (1 mL) was stirred at 25 °C for 1 day. After evaporation of the solvents, the residue was chromatographed on silica with EtOAc–hexane 1:2 to give **APh** (60 mg, 70%) reaction occurred a viscous oil: IR (film) 1735 (s, C=O), 1610, 1590, 1570 cm^{-1} (w, C_6H_5 and isoxazole); ^1H NMR ($CDCl_3$) δ 1.21 and 1.34 (s, each 3 H, 2 × CH_3), 1.80–2.70 (m, 4 H, CH_2CH_2CO), 2.42 and 2.75 (AB, J_{AB} = 13, each 1 H, CH_2CO), 3.61 and 3.62 (s, each 3 H, 2 × CH_3O), 3.75–4.07 (m, 4 H, OCH_2CH_2O), 6.43 (s, 1 H, —CH=, isoxazole), 7.35–7.55 and 7.70–7.85 (m, 3 H + 2 H, C_6H_5); MS (EI), *m/z* 416.1707 (M^+ − 15), calcd for $C_{22}H_{26}NO_7$ (M^+ − CH_3) 416.1710.

Northern Half ABα. The oxime **B1α** (1.41 g, 5 mmol) and the nitrile oxide **A16** (3.29 g, 10 mmol) in $CHCl_3$ (10 mL) were kept at 40 °C for 6 days. The solvent was evaporated (VRE) and the residue chromatographed on silica with EtOAc–hexane 1:1, 4:1, and then 100% EtOAc to afford **ABα** (2.88 g, 94%) as an amorphous foam (1.40 g of **A16** was recovered): $[\alpha]^{25}_D$ −3.9°, $[\alpha]^{25}_{435}$ −6.5° (*c* 3.25, $CHCl_3$); IR ($CHCl_3$) 3500–3200 (br, OH), 1730 (s, C=O, ester), 1635 (m, C=O, amide), 1590 cm^{-1} (w, C=C, isoxazole); ^1H NMR ($CDCl_3$) δ 1.12 and 1.31 (s, each 3 H, 2 × CH_3, ring A), 1.56 (s, 3 H, CH_3, ring B), 1.60–3.20 (m, 14 H, 6 × CH_2 + 2 × CH, ring A + B), 2.83 and 2.89 (s, each 3 H, NMe_2), 3.62, 3.64 and 3.66 (s, each 3 H, 3 × CH_3O), 3.70–4.10 (m, 4 H, OCH_2CH_2O), 5.91 (s, 1 H, CH, isoxazole), 7.20 (d, *J* = 9, 1 H, CH=N, *anti*-oxime) 8.73 (s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 175.81 (C—O), isox.), 173.99, 173.52 and 173.51 (3 × C=O, ester), 169.52 (C=O, amide), 163.84 (C=N, isox.), 150.95 (C=N, oxime); 113.35 (O—C—O), 102.94 (CH, isox.), 64.92 and 63.07 (OCH_2CH_2O), 51.61 and 51.36 (3 × CH_3O), 48.17, 47.72, 43.16, 41.56, 40.19, 37.73, 36.23, 35.46, 32.43, 31.89, 24.61, 22.54, 21.51, 19.92, 18.90; MS (EI), *m/z* 611.3044, calcd for $C_{29}H_{45}N_3O_{11}$ 611.3056.

Northern Half ABβ. The procedure was the same as for the isoxazole **ABα** using the oxime **B1β** instead to yield **ABβ** (2.94 g, 96%): $[\alpha]^{25}_D$ +20.1°, $[\alpha]^{25}_{435}$ +41.8° (*c* 5.85, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.15 and 1.30 (s, each 3 H, 2 × CH_3, ring A), 1.56 (s, 3 H, CH_3, ring B), 1.60–3.20 (m, 14 H, 6 × CH_2 + 2 × CH, ring A + B), 2.83 and 2.91 (s, each 3 H, NMe_2), 3.608, 3.629, and 3.634 (s, each 3 H, 3 × CH_3O), 3.70–4.10 (m, 4 H, OCH_2CH_2O), 5.95 (s, 1 H, CH, isox.), 7.29 (d, *J* = 9, 1 H, CH=N, *anti*-oxime), 8.05 (s, 1 H, OH); ^{13}C NMR ($CDCl_3$) δ 175.78, 173.83, 173.26, 172.91, 169.75, 164.19, 150.34, 113.35, 103.29, 64.89, 63.17, 51.58, 51.49, 51.23, 48.13, 47.69, 43.06, 41.62, 40.67, 37.76, 36.64, 35.53, 32.27, 32.05, 24.80, 23.30, 21.51, 19.44, 19.02; MS and IR are identical with those of **ABα**.

Diisoxazole ABPhα. *t*-BuOCl (22 mg, 0.2 mmol) in CH_2Cl_2 (0.5 mL) was added at −78 °C to a solution of **ABα** (122 mg, 0.2 mmol) in CH_2Cl_2 (0.5 mL) over 20 min. After additional stirring at −78 °C for 1 h (the greenish color faded), Et_3N (40 mg, 0.4 mmol) in phenylacetylene (0.5 mL) was added at −78 °C over 0.5 h and stirring was continued at that temperature for 1 h. The cooling bath was removed and the mixture warmed up to 25 °C. The reaction was kept at room temperature overnight, and the solvents were then removed by rotary evaporation. Chromatography on silica with EtOAc–hexane 4:1 yielded pure foamy **ABPhα** (89 mg, 63%): IR (film) 1735 (s, C=O, ester), 1640 (s, C=O, amide), 1590 (w, C=C, isox.), 1570 cm^{-1} (w, C_6H_5); ^1H NMR ($CDCl_3$) δ 1.08 and 1.27 (s, each 3 H, 2 × CH_3, ring A), 1.70 (s, 3 H, CH_3, ring B), 1.70–3.20 (m, 14 H, 6 × CH_2 + 2 × CH, ring A + B), 2.84 and 2.89 (s, each 3 H, NMe_2), 3.52, 3.58 and 3.63 (s, each 3 H, 3 × CH_3O), 3.70–4.10 (m, 4 H, OCH_2CH_2O), 5.85 and 6.14 (s, each 1 H, 2 × CH, isox.), 7.45–7.55 and 7.75–7.85 (m, 3 H + 2 H, C_6H_5); ^{13}C NMR ($CDCl_3$) δ 175.78, 173.61, 173.35, 172.68, 169.68, 169.63, 164.19, 163.59, 130.10, 128.89*, 127.29, 125.79* (*superimposed C, C_6H_5), 113.31, 102.97, 99.78, 64.76, 63.20, 51.58, 51.36, 51.17, 48.07, 45.68, 42.80, 42.13, 41.69, 40.22, 37.67, 36.84, 35.46, 32.14 (2 superimposed C), 24.87, 24.10, 21.51, 21.10, 18.99; MS (EI), *m/z* 711, calcd for $C_{37}H_{49}N_3O_{11}$ 711.3369.

Southern Half CD. The salt **C9** (3.18 g, 10 mmol) in 2 N HCl (20 mL) was extracted with ether (3 × 50 mL), and the ether layers were washed with brine (10 mL). After drying (Na_2SO_4), the concentrated ethereal acid solution (~25 mL) was treated with CH_2N_2 in ether as usual to give after evaporation the ester **C1α** (2.10 g, 100%). *t*-BuOCl (1.09 g, 10 mmol) in CH_2Cl_2 (25 mL) was added to the oxime **C1α** in CH_2Cl_2 (25 mL) at −78 °C over 1 h. After additional stirring at that temperature for 1 h, the yellow-green solution was warmed up to 25 °C over 20 min (the solution turned blue) and the CH_2Cl_2 was evaporated (VRE, 30 °C) to afford the highly viscous hydroxamoyl chloride **C11**. It was dissolved in CH_2Cl_2 (20 mL) and added under stirring to the acetylene **D7β** (10.1 g, 40 mmol) and Et_3N (2.0 g, 20 mmol) in CH_2Cl_2 (20 mL) over 0.5 h. After reacting at 25 °C for 2 days, the solvent was evaporated and the residue chromatographed on silica with EtOAc–hexane 1:2 and 2:3 to give yellow oily **CD** (2.64 g, 57%; the unreacted nitro ester **D7β** was recovered quantitatively): $[\alpha]^{25}_D$ −45.5°, $[\alpha]^{25}_{435}$ −96.6° (*c* 3.32, $CHCl_3$); IR (film) 3280 (m, HC≡), 3120 (w, HC=), 2240 (w, C≡N), 1735 (s, C=O), 1585 (m, C=C, isox.), 1550 cm^{-1} (s, NO_2); ^1H NMR ($CDCl_3$) δ 1.12 (s, 3 H, CH_3, ring D), 1.37 (s, 6 H, 2 × CH_3, ring C), 1.90–2.80 (m, 11 H, 5 × CH_2 + CH), 2.26 (s, 1 H, HC≡C), 3.21 (m, 1 H, CH, ring D), 3.64 and 3.70 (s, each 3 H, 2 × CH_3O), 4.35 and 4.46 (ABX, J_{AB} = 13.7, J_{AX} = 4.5, J_{BX} = 7.3, each 1 H, CH_2NO_2), 6.33 (s, 1 H, CH, isox.); ^{13}C NMR ($CDCl_3$) δ 173.26, 172.97, 171.47, 164.42 (C=N, isox.), 118.68 (CN), 102.75 (CH, isox.), 89.09, 76.48, 70.77, 52.32 and 51.61 (CH_3O), 46.47, 41.62 (2C)*, 34.03, 33.71, 33.13, 31.98, 28.50, 28.15, 25.06, 19.09, 12.70,*DND-experiment[21] shows two superimposed signals; MS (EI), *m/z* 430.1968 (M^+ − 31), calcd for $C_{22}H_{28}N_3O_6$ (M^+ − CH_3O) 430.1979; MS (FAB), *m/z* 462 (MH^+).

Triisoxazole 4α. *t*-BuOCl (0.22 g, 2 mmol) in CH_2Cl_2 (5 mL) was added at −78 °C to **ABα** (1.22 g, 2 mmol) in CH_2Cl_2 (5 mL) over 0.5 h. After additional stirring at −78 °C for 1 h (the azure color faded), Et_3N (0.40 g, 4 mmol) in CH_2Cl_2 (2.5 mL) was added over 0.5 h (the light-blue turned yellow) and stirring at −78 °C continued for 1 h (the yellow faded). The mixture was warmed to room temperature over 15 min and then added to neat **CD** (3.69 g, 8 mmol). After stirring at 25

(20) Christl, M.; Warren, J. P.; Hawkins, B. L.; Roberts, J. D. *J. Am. Chem. Soc.* **1973**, *95*, 4392.

(21) DND: Delayed Noise Decoupling. Anet, F. A. L.; Jaffer, N.; Strouse, J. Abstract presented at the Experimental NMR Conference, Tallahassee, FL., 1980.

°C for 2.5 days the solvent was removed (VRE) and the residue chromatographed on silica with benzene–pyridine 9:1 and 7:1 to afford the triisoxazole **4α** (0.66 g, 31%) as an amorphous foam (the unconverted **CD** was recovered quantitatively). The minor impurities were removed by a second chromatography on silica with 100% EtOAc to give 0.51 g (24%) of **4α**: $[\alpha]^{25}_{D}$ −6.1°, $[\alpha]^{25}_{435}$ −16.6° (*c* 5.00, $CHCl_3$); IR ($CHCl_3$) 2240 (w, C≡N), 1730 (s, C=O, ester), 1635 (m, C=O, amide), 1585 (m, C=C, isox.), 1550 cm^{-1} (m, NO_2); ^1H NMR ($CDCl_3$) δ 1.11 (s, 3 H, CH_3, ring A), 1.29, 1.31, 1.33 (s, 6 H + 3 H + 3 H, 4 × CH_3, ring A, C, D), 1.65 (s, 3 H, CH_3 ring B), 1.70–3.45 (m, 26 H, 11 × CH_2 + 4 × CH), 2.84 and 2.92 (s, each 3 H, NMe_2), 3.60, 3.62, 3.64, 3.71 (s, total 15 H, 5 × CH_3O), 3.75–4.05 (m, 4 H, OCH_2CH_2O), 4.37 and 4.45 (ABX, J_{AB} = 14, J_{AX} = 7.5, J_{BX} = 4.5, each 1 H, CH_2NO_2), 5.72, 5.920, 5.925 (s, each 1 H, 3 × CH, isox.); ^{13}C NMR ($CDCl_3$) δ 177.82 and 175.78 (2 × C—O, isox.), 173.64, 173.29, 173.16, 173.00, 172.81, 171.50 (5 × C=O ester, C—O isox.), 169.36 (C=O, amide), 164.13, 163.52, 162.95 (3 × C=N, isox.), 118.71 (C≡N), 113.35 (O—C—O), 103.48, 102.97, 101.34 (3 × CH, isox.), 76.57 (CH_2NO_2), 64.86, 63.10 (OCH_2CH_2O), 52.28, 51.58 (intense, 2C), 51.42, 51.13 (5 × CH_3O), 48.07, 46.51, 45.80, 43.09, 41.59 (intense, 3C)*, 39.90, 38.94, 37.60, 36.52, 35.43, 33.74, 33.10, 32.30, 32.17, 32.05, 25.66, 24.83, 24.64, 24.23, 23.88, 21.90, 21.77, 18.93, 18.80, 12.70 (CH_2CN); MS (FAB), *m/z* 1071 (MH^+); *two of the three superimposed peaks at δ 41.59 also overlap in **CD** at δ 41.62, the third missing signal is only visible in the diastereomer **4β**. Anal.: C, 58.27; H, 6.83; N, 7.87. Calcd for $C_{52}H_{74}N_6O_{18}$: C, 58.31; H, 6.96; N, 7.85%.

Triisoxazole 4β. The procedure was the same as for the triisoxazole **4α** using **ABβ** instead to yield **4β** (0.53 g, 25%): $[\alpha]^{25}_{D}$ +3.4°, $[\alpha]^{25}_{435}$ +2.3° (*c* 2.82, $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.15 (s, 3 H, CH_3, ring A), 1.31, 1.32, 1.34, 1.37 (s, each 3 H, 4 × CH_3, ring A, C, D), 1.55 (s, 3 H, CH_3, ring B), 1.70–3.30 (m, 26 H, 11 × CH_2, 4 × CH), 2.78 and 2.87 (s, each 3 H, NMe_2), 3.60, 3.62, 3.63, 3.71 (s, total 15 H, 5 × CH_3O), 3.75–4.05 (m, 4 H, OCH_2CH_2O), 4.35 and 4.45 (ABX, J_{AB} = 14, J_{AX} = 7.5, J_{BX} = 4.5, each 1 H, CH_2NO_2), 5.88, 5.92, 6.03 (s, each 1 H, 3 × CH, isox.); ^{13}C NMR ($CDCl_3$) δ 178.14 and 175.72 (2 × C—O, isox.), 173.61, 173.35, 173.00, 172.90, 172.75, 171.50 (5 × C=O ester, C—O isox.), 169.40 (C=O, amide), 164.29, 163.46, 162.82 (3 × C=N, isox.), 118.71 (CN), 113.38 (O—C—O), 103.48 (br, 2C) and 101.50 (3 × CH, isox.), 76.45 (CH_2NO_2), 64.92 and 63.23, (OCH_2CH_2O), 52.25, 51.61, 51.52, 51.42, 51.13 (5 × CH_3O), 48.17, 46.51, 45.13, 43.12, 41.72 (intense, 2C), 41.59, 41.24, 39.13, 37.73, 36.68, 35.40, 33.71, 33.13, 32.33, 32.24, 32.08, 25.31, 24.93, 24.83, 24.51, 24.26, 21.55, 19.82, 19.06, 18.96, 12.70 (CH_2CN); IR and MS are identical with those of **4α**.

Acknowledgment. The financial support of the National Science Foundation (NSF CHE 78-27084 and CHE 81-15444) is gratefully acknowledged. N. B. is grateful for a NATO Science Fellowship with the Natural Sciences and Engineering Research Council of Canada. A. W. and U. Z. are grateful to the Stiftung für Stipendien auf dem Gebiete der Chemie and the Swiss National Science Foundation, respectively, for a fellowship.

Supplementary Material Available: Tables of final atomic coordinates, bond lengths and angles, and anisotropic thermal parameters along with a computer generated plot with atom labels (11 pages). Ordering information is given on any current masthead page.

Single-Crystal EXAFS of Nitrogenase

A. M. Flank,[†] M. Weininger,[‡] L. E. Mortenson,[§] and S. P. Cramer*[§]

Contribution from the Corporate Research Science Labs, Exxon Research and Engineering Company, Annandale, New Jersey 08801, LURE Batiment 209C, 91405 Orsay, France, and the Department of Biological Chemistry, Purdue University, West Lafayette, Indiana 47907.
Received November 19, 1984

Abstract: Single crystals of the nitrogenase Mo–Fe protein have been examined by polarized X-ray absorption spectroscopy. For different orientations, the Mo–Fe amplitude of the Mo K-edge EXAFS was found to change by a factor of 2.5, whereas the Mo–S component varied by only ±15%. The orientation dependence of the EXAFS spectra has been used to investigate the geometry and orientation of the Mo, Fe, S clusters within the Mo–Fe protein. This represents the first application of single-crystal EXAFS to an enzyme of unknown crystal structure. The orientation dependence for single crystals of the model compounds $(Ph_4P)_2[Cl_2FeS_2MoS_2FeCl_2]$ and $(Et_4N)_3[Fe_6Mo_2S_8(SEt)_9]$ was also examined to quantify the experimental precision of this technique. The analysis procedures overcame the difficulty of four molybdenum sites per unit cell by using the X-ray diffraction evidence for a crystallographic 2-fold axis and a molecular 2-fold axis. Given initial assumptions about the symmetry of the Mo, Fe, S clusters, as well as the orientation of one cluster with respect to the crystallographic axes, it was possible to calculate the expected EXAFS orientation dependence. The patterns for linear, bent, tetrahedral, and square-pyramidal symmetries in various orientations were then compared with the experimental spectra. It was found that the experimental data were not well simulated by clusters with a linear arrangement of Fe–Mo–Fe atoms, whereas trinuclear clusters with a Fe–Mo–Fe angle between 50° and 130° gave satisfactory agreement. Tetrahedral $MoFe_3$ and square-pyramidal $MoFe_4$ cluster symmetries also gave satisfactory simulations of the orientation dependence. Assuming a tetrahedral Mo–Fe geometry, the preferred orientation of the 3-fold axis of one of the Mo–Fe clusters was found to lie at an angle of 75 ± 10° from the crystallographic *a* axis and 215 ± 10 or 285 ± 10° from the *b* axis.

Most of the nitrogen fixation on earth is accomplished through the catalytic action of the enzyme nitrogenase.[1] This enzyme consists of two proteins, the Fe protein and the Mo–Fe protein. The Mo–Fe protein is an $\alpha_2\beta_2$ tetramer with a molecular weight of 220 000 that contains 2 molybdenums, 28–32 irons,[2,3] and approximately 30 acid labile sulfides.[4] An unusual molybdenum–iron–sulfur cluster, the iron–molybdenum cofactor or "FeMo-co", is thought to be at the catalytic site of this complex.[2]

The first technique that revealed information about the molybdenum site in nitrogenase was X-ray absorption spectroscopy.[5,6]

* To whom correspondence should be addressed.
[†] LURE.
[‡] Purdue University.
[§] Exxon Research.

(1) "Current Perspectives in Nitrogen Fixation"; Gibson, A. H., Newton, W. E., Eds.; Australian Academy of Sciences: Canberra, 1981.
(2) Shah, V. K.; Brill, W. J. *Proc. Natl. Acad. Sci. U.S.A.* **1977**, *74*, 3249–3253.
(3) Burgess, B. K.; Jacobs, D. R.; Stiefel, E. I. *Biochim. Biophys. Acta* **1980**, *614*, 196–209.
(4) Orme-Johnson, W. H.; Davis, L. C. "Iron-Sulfur Proteins"; Lovenberg, W., Eds.; Academic Press: New York, 1977; p 15.

0002-7863/86/1508-1049$01.50/0