cause of formation of an electric dipole moment), then it follows that the relative directions of **E** and **B** can be so chosen that A(**j**) leads preferentially to P and A(−**j**) leads preferentially to P*. Obviously, an equal mixture of the two precursor species results in a racemic mixture of P and P*. However, if one of the species, e.g., A(**j**), can be preselected by some mechanism, then asymmetric synthesis becomes possible, in principle. In many cases the degree of asymmetric synthesis would be reduced, in practice, by short magnetic moment relaxation times.

Examples of the second source of current density are (a) nonadiabatic current response to the applied fields and (b) nonadiabatic current response to intermolecular interaction, as with a bimolecular transition state. However, these are mentioned only as possible sources of transient currents in molecules. Whether or not they are capable of contributing significantly to asymmetric synthesis remains to be determined.

The above discussion has established the point that, in principle, it is possible for constant and uniform electric and magnetic fields to modify the kinetics of a prochiral reaction to permit asymmetric synthesis. The magnitude of this effect, of course, depends on the particular mechanism and system. However, to provide a basis for order of magnitude estimates, we have calculated the kinetic effect for transition states having electric and magnetic moments for the following set of parameters: electric field, 10^3 V/cm; magnetic field, 10^4 G; transition state electric dipole moment (μ), 1 eÅ; transition state magnetic moment (**m**), 1 B_μ. For parallel **E** and **B** and for parallel electric and magnetic moments, application of the Langevin equation gives the ratio of rate constants

$$k_P/k_{P^*} \approx \exp\left(\frac{1}{3}\frac{\mu \mathbf{E}}{kT}\frac{\mathbf{mB}}{kT}\right)$$

This corresponds to an enantiomeric excess of 0.3 ppm at 298 K, which suggests that for most systems the degree of asymmetric synthesis is expected to be very small.

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(1) This work was supported (in part) by Contract No. EY-76-S-05-2690 between the Division of Biomedical and Environmental Research of the Department of Energy and Florida State University.
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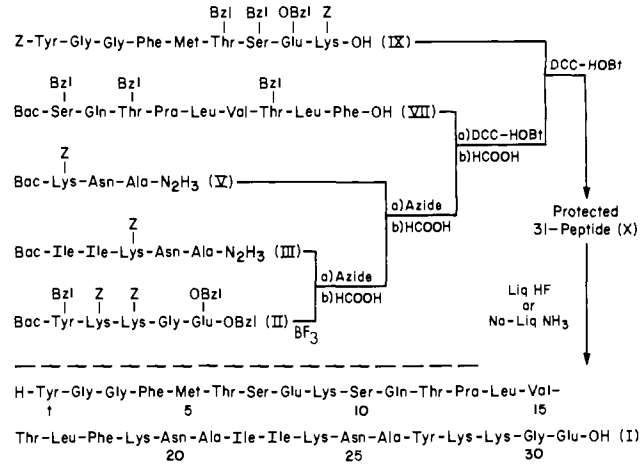
A Synthesis of Human β-Endorphin in Solution

Sir:

β-Endorphin (β-EP)[1] has been isolated from pituitary glands of several mammalian species including man, and characterized chemically and biologically. It is a 31-residue peptide whose amino acid sequence was noted to be identical with that of β-lipotropin-(61–91). β-EP possesses potent morphine-like analgesic activity by intracerebroventricular or intravenous injection in laboratory animals. Certain behavioral changes caused by β-EP in experimental animals are of considerable current interest; see ref 2 for a review. Several solid-phase syntheses of human β-EP have been reported.[3] This communication describes a solution synthesis of human β-EP (I) via segment condensation and maximum protection of side-chain functionalities by benzyl-type groups, as shown in Scheme I. Homogeneous β-EP, indistinguishable from authentic material[2,4] in physicochemical and biological characteristics, was obtained in a single reversed-phase preparative liquid chromatographic step after protecting group cleavage.

Scheme I. Synthetic Route to Human β-EP (I)



For the synthesis of the COOH-terminal-protected decapeptide Boc-Ile-Ile-Lys(Z)-Asn-Ala-Tyr(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, IV, the protected pentapeptide hydrazide III, in DMF, was converted[5] to the azide and coupled with the pentapeptide amine obtained from II by Boc group cleavage with BF_3–OEt_2 in AcOH.[6] Decapeptide IV had mp 239–241 °C dec, $[\alpha]^{25}_D$ −18.5° (*c* 0.99, Me_2SO), diagnostic $AAA^7.Asp_{1.0}$, $Glu_{1.0}$.[8] To prepare the 13-peptide, Boc-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, VI, the protected tripeptide azide prepared[5] from Boc-Lys(Z)-Asn-Ala-N_2H_3, V (mp 177–180 °C; $[\alpha]^{25}_D$ −14.9° (*c* 1, DMF)), was coupled in a DMF–Me_2SO solution (1 h at −15 °C and 4 days at 4 °C) with the decapeptide amine, resulting from treatment of IV with HCOOH for 3.5 h at 25 °C. The 13-peptide, VI, was obtained in 83% yield, mp 257–259 °C dec, $[\alpha]^{25}_D$ −24.8° (*c* 1, Me_2SO). The 22-peptide, Boc-Ser(Bzl)-Gln-Thr(Bzl)-Pro-Leu-Val-Thr(Bzl)-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, VIII (mp 283–290 °C dec; $[\alpha]^{25}_D$ −23.8° (*c* 0.49, Me_2SO); AAA, $Val_{0.98}$,$Gly_{1.00}$), was synthesized in 86% yield by dicyclohexyl carbodiimide-hydroxybenzotriazole (DCC–HOBt) mediated preactivation coupling[9] (2 hr at 0 °C, 3 days at 25 °C) of the protected nonapeptide acid VII (mp 205–207 °C, $[\alpha]^{25}_D$ −20.1° (*c* 1, DMF)) and the 13-peptide amine, prepared from VI by Boc group cleavage (3.5 h, 25 °C) with HCOOH. To prepare the final protected 31-peptide, Z-Tyr-Gly-Gly-Phe-Met-Thr(Bzl)-Ser(Bzl)-Glu(OBzl)-Lys(Z)-Ser(Bzl)-Gln-Thr(Bzl)-Pro-Leu-Val-Thr(Bzl)-Leu-Phe-Lys(Z)-Asn-Ala-Ile-Ile-Lys(Z)-Asn-Ala-Tyr-(Bzl)-Lys(Z)-Lys(Z)-Gly-Glu(OBzl)-OBzl, X (82%; mp 273–276 °C dec; $[\alpha]^{25}_D$ −36.4° (*c* 0.5, *i*-C_3F_6HOH); AAA, $Met_{1.1}$, $Val_{0.93}$), the DCC–HOBt preactivation coupling[9] (1 h at 0 °C, 3 days at 25 °C) of the protected nonapeptide acid IX (mp 227–231 °C dec, $[\alpha]^{25}_D$ −3.2° (*c* 1, DMF)) and the 22-peptide amine, produced from VIII by HCOOH treatment (3.5 h at 25 °C), had to be carried out in a 1:1 mixture of DMF and phenol[10] owing to the limited solubility of the 22-peptide

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amine.

Protecting groups were removed (a) by liquid HF,[11] yielding at best 13% β-EP, and (b) by sodium in liquid NH_3,[12] providing up to 27.5% β-EP. For the HF cleavage, compound X (153 mg, 30 μmol) was exposed for 30 min at 0 °C to anhydrous liquid HF–anisole–diethyl sulfide (6:1:2). Typical workup[13] yielded 100 mg (84%) of a white powder. Reversed-phase HPLC (76 mg, 0.9 × 500 mm Partisil ODS-2 column, Pyr–AcOH–CH_3CN–*i*-C_3H_7OH–H_2O (5.9:1.9:13:13:66.2, v/v), isocratic mode, *o*-phthalaldehyde-fluorescence monitoring) produced directly homogeneous β-EP, Ia (12.0 mg, 13.2% based on X[14]), besides a multitude of side-product peaks (total peptide recovery was 55 mg (61%)). For the Na–liquid NH_3 cleavage, a suspension of compound X (51 mg, 10 mol) in anhydrous refluxing liquid NH_3 (125 mL) was treated for 30 min with a 10-fold excess of Na (60 mg, 2.6 mmol). Addition of NH_4Cl (0.13 g, 2.5 mmol), evaporation, Sephadex G-15 desalting (0.5 M AcOH), and lyophilization yielded 27 mg (68.7%) of white powder. Reversed-phase HPLC, as above, produced β-EP, Ib (10.8 mg, 27.5%, of a total peptide recovery of 24 mg in a few peaks).

Physicochemical and biological properties of human β-endorphin samples Ia and Ib were identical and indistinguishable from authentic material: AAA, $Lys_{5.00}$, $Asp_{2.08}$, $Thr_{2.97}$, $Ser_{1.90}$, $Glu_{3.13}$, $Pro_{0.95}$, $Gly_{3.2}$, $Ala_{2.08}$, $Val_{0.95}$, $Met_{1.03}$, $Ile_{1.70}$ (72-h hydrolysis), $Leu_{2.05}$, $Tyr_{1.92}$, $Phe_{1.93}$; analytical HPLC (5 μ, 0.46 × 15 cm ES Industries C-8 column, system as above), single symmetrical peak, $K' = 2.8$. ORD and CD spectroscopy of I in CH_3OH–H_2O (3:1) produced spectra superimposable with authentic human β-EP:[2,4] $[\alpha]^{270}_{\lambda}$ −206° at 400 nm, −538° at 300 nm, −2190° at 240 nm. The opioid activity of synthetic human β-EP was identical with that of authentic material in the guinea pig ileum assay[15] (50% inhibition at 1.8×10^{-9} M) and was blocked by the specific opiate antagonist naloxone (complete reversal at 3.0×10^{-8} M).

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Supplementary Material Available: Elemental analysis of peptides II–X (1 page). Ordering information is given on any current masthead page.

References and Notes

(1) Abbreviations used: AAA, amino acid analysis; AcOH, acetic acid; Boc, *tert*-butyloxycarbonyl; Bzl, benzyl; DCC, dicyclohexylcarbodiimide; DMF, dimethylformamide; β-EP, β-endorphin; Et, ethyl; *i*-C_3F_6HOH, hexafluoroisopropyl alcohol; *i*-C_3H_7OH, isopropyl alcohol; HOBt, 1-hydroxybenzotriazole; HPLC, high performance liquid chromatography; OBzl, benzyl ester; Pyr, pyridine; Z, benzyloxycarbonyl.
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A Kinetic α-Deuterium Isotope Effect for the Binding of Purine Nucleosides to Calf Spleen Purine Nucleoside Phosphorylase. Evidence for Catalysis by Distortion

Sir:

Kinetic α-deuterium isotope effects, k_H/k_D, for the calf spleen purine nucleoside phosphorylase (E.C. 2.4.2.1) catalyzed phosphorolysis of adenosine at pH 7.2 and inosine at pH 7.5 are 1.047 ± 0.017 and 1.043 ± 0.004, respectively, Table I. As detailed below, these values suggest that binding of the nucleoside to the active site of the enzyme occurs with significant change of the geometry of these substrates at C-1′.

Kinetic α-deuterium isotope effects originate almost wholly from differences between the zero-point energies of the two isotopic species in the ground state and transition state resulting from the sensitivity of the α-hydrogen bending mode vibrations to isotopic substitution.[1] Thus, such isotope effects can provide information about changes in bonding which occur as the reactant proceeds from ground to transition state and have been successfully exploited in mechanistic studies of organic reactions[2] as well as enzymic ones.[3,4] In certain enzymatic cases, the α-deuterium effect reflects only binding of the substrate to the enzyme and thus may reveal vibrational perturbations at the isotopically substituted atom resulting from enzyme-induced substrate distortion.

The potential utility of kinetic α-deuterium isotope effects as probes of substrate distortion for enzymic reactions was investigated for calf spleen purine nucleoside phosphorylase (PNPase):[5]

$$\text{purine nucleoside} + P_i \rightleftharpoons \text{purine base} + \text{ribose 1-phosphate} \quad (1)$$

PNPases are specific for purine nucleosides having the β configuration at C-1′ and the reaction proceeds with inversion at this center. The nature of kinetic plots for all PNPases studied suggests a sequential rather than ping-pong mechanism; that is, catalysis occurs through a ternary complex of PNPase, nucleoside, and orthophosphate.[5]

Specifically, the reaction catalyzed by PNPase from calf spleen proceeds through an ordered Bi-Bi kinetic mechanism with nucleoside adding to the enzyme before phosphate.[6] Northrop has demonstrated that for enzymes having such a kinetic mechanism the observed kinetic isotope effect, determined by a competitive method, is for binding of the first

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