mild reduction to the labile Co(II) oxidation state. Secondly, Co(III) significantly activates the carboxyl group toward addition of an amino acid or peptide. A 10^4–10^6 enhancement in the rate of the coupling relative to the uncoordinated amino acid ester is involved,[11] and this allows for very short reaction times. Also the relatively mild reaction conditions suggest that more labile side-chain protecting groups may well be used. Further, the asymmetric nature of the $Co(N_4)$ group, its orange color (ϵ_{480} = 100–150 mol^{-1} dm^3 cm^{-1}), and its water solubility provide other features which differ from those usually encountered in peptide synthesis.

Cobalt(III)-Mediated Peptide Synthesis. 2. Synthesis of Tetrapeptides and [Leu^5]enkephalin

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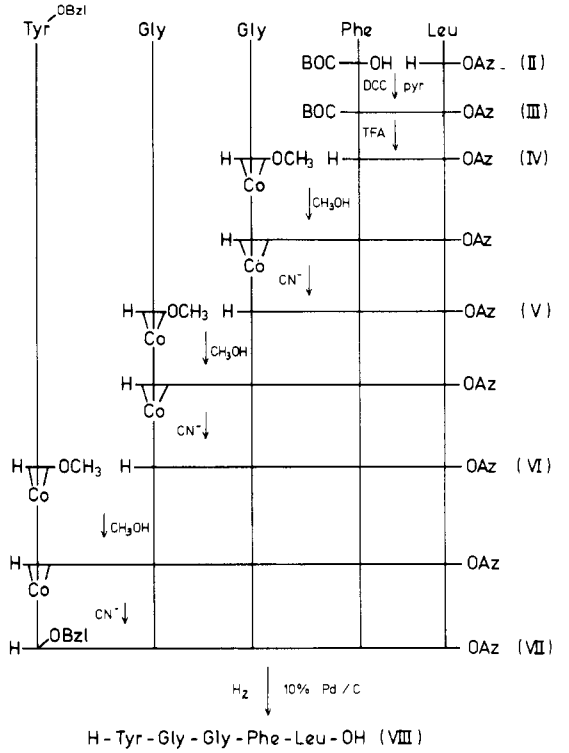
The preceding communication outlined the cobalt(III)-mediated synthesis of dipeptides.[1] We now report on the applicability of the method to larger peptides. The new feature here is the sequential removal of the cobalt(III) moiety, isolation of the growing peptide fragment, and its subsequent reuse to extend the amino acid chain. Two complementary isolation procedures are described. The first utilizes zinc amalgam reduction of $[Co(en)_2(peptide\text{-}OR)]^{3+}$ followed by isolation of the peptide-OR by gel filtration. The stepwise synthesis of Ala-Gly-Phe-Phe-OBzl and Leu-Ala-Gly-Gly-OEt starting from the C-terminal amino acid esters exemplifies this approach. The second method uses the lipophilic and chromogenic *p*-[*p*-(dimethylamino)phenylazo]benzyl C-terminal protecting group (Az-ester) recently devised in one of our laboratories.[2] Following displacement of the cobalt moiety with CN^-, the peptide ester intermediate is isolated by organic solvent extraction and silica gel chromatography.

In the synthesis of the tetrapeptide ester H-Ala-Gly-Phe-Phe-OBzl the following final coupling is representative of the first approach. To Gly-Phe-Phe-OBzl·CH_3COOH (15.8 μmol) in dry Me_2SO (0.2 cm^3) was added 1 equiv of Et_3N followed by $[Co(en)_2(Ala\text{-}OMe)](CF_3SO_3)_3$ (52.7 μmol), and the mixture was stirred for 20 min under anhydrous conditions (reaction 1).

$$\text{Gly-Phe-Phe-OBzl} + [Co(en)_2(\text{Ala-OMe})]^{3+} \rightarrow [Co(en)_2(\text{Ala-Gly-Phe-Phe-OBzl})]^{3+} + \text{MeOH} \quad (1)$$

Then HOAc (0.5 cm^3) and H_2O (4 cm^3) were added to quench the reaction, and the two orange cobalt(III) products[3] were separated by ion exchange chromatography (SP-C25 Sephadex, 8 cm × 1 cm). The coordinated ester hydrolysis product $[Co(en)_2(Ala)]^{2+}$ was eluted with 0.2 mol dm^{-3} of pyridinium acetate and the 3+ peptide-OR complex (reaction 1) with 1.0 mol dm^{-3} of pyridinium acetate. The latter eluate was reduced to a volume of 10 cm^3 under reduced pressure and freeze-dried when it yielded 12.4 mg (14 μmol, 88%) of $[Co(en)_2\text{Ala-Gly-Phe-Phe-OBzl}](CH_3COO)_3$.

Scheme I



The peptide was quantitatively recovered from the complex following Hg/Zn reduction (reaction 2). To the complex (12.4 mg) in water (1.0 cm^3) adjusted to pH 1.0 (HCl_{aq}) was added zinc amalgam (2%, 5 cm^3) and the mixture was stirred vigorously for 5 min. The tetrapeptide was isolated from inorganic salts and ethylenediamine by gel filtration chromatography (Bio-Gel P-2, 70 × 2.5 cm, 2 mol dm^{-3} HOAc). It was shown to be homogeneous by thin-layer chromatography and amino acid analysis.[4] Yields of peptides by this procedure are generally good (>90%) but were sometimes lower,[5] and further experimentation in this area is being undertaken. Confirmation of the presence of benzyl and ethyl ester protecting groups following the reduction step is sometimes possible by high-resolution mass spectrometry.[6]

$$[Co(en)_2(\text{Ala-Gly-Phe-Phe-OBzl})]^{3+} \xrightarrow{\text{Hg/Zn}} Co_{aq}^{2+} + \text{H-Ala-Gly-Phe-Phe-OBzl} + 2enH_2^{2+} \quad (2)$$

The synthesis of [Leu^5]enkephalin is shown diagrammatically in Scheme I and is representative of the second approach. A conventional coupling was used for the first two amino acid units, Boc-Phe-OH and H-Leu-OAz.[7] The next coupling using the

(1) See C. R. Clark, R. F. Tasker, D. A. Buckingham, D. R. Knighton, D. R. K. Harding, and W. S. Hancock, *J. Am. Chem. Soc.*, preceding paper in this issue.

(2) G. D. Reynolds, D. R. K. Harding, and W. S. Hancock, *Int. J. Pept. Protein Chem.*, **17**, 231 (1981).

(3) The excess Δ,Δ-$[Co(en)_2(Ala\text{-}OMe)]^{3+}$ ion is rapidly hydrolyzed to $[Co(en)_2(Ala)]^{2+}$ during workup. Reaction rates in water: Δ form, $t_{1/2}$ = 76 s; Λ form, $t_{1/2}$ = 57 s [25 °C, I = 1.0 ($NaClO_4$)]. D. A. Buckingham, C. R. Clark, and R. F. Tasker, unpublished results.

(4) TLC, silica gel; methanol, R_f = 0.62; acetone, R_f = 0.55; 9:1 acetic acid–methanol, R_f = 0.22; 2:2:1 butanol–pyridine–water, R_f = 0.84. A sample hydrolyzed with 6 N HCl at 110 °C, 22 h, gave on amino acid analysis Ala 1.04, Gly 1.00, Phe 1.96.

(5) Very hydrophobic peptides such as Gly-Phe-Phe-OBzl are retarded when chromatographed on Bio-Gel P-2, presumably due to hydrophobic interactions between the solute and the support. The separation of such peptides from the other byproducts was difficult and resulted in lower yields (67% for Gly-Phe-Phe-OBzl).

(6) Ala-Gly-Phe-Phe-OBzl was not sufficiently volatile to give the characteristic parent ion by high-resolution mass spectroscopy. But Gly-Phe-Phe-OBzl gave *m/e* 459 and Ala-Gly-Gly-OEt gave *m/e* 231.

(7) Coupling times vary depending on the amino acid side chain and/or ester grouping. Generally more bulky side chains result in slower rates; e.g., coupling rates (mol^{-1} dm^3 s^{-1}) are 4.75 for $[Co(en)_2(\text{Gly-OMe})]^{3+}$ + Phe-OEt, 1.86 for $[Co(en)_2(\text{Val-OMe})]^{3+}$ + Phe-OEt (both in CH_3CN), 14 for $[Co(en)_2(\text{Gly-OC}_3H_7)]^{3+}$ + Gly-OEt, and 216 for $[Co(en)_2(\text{Gly-OC}_3H_7)]^{3+}$ + Val-OEt (both in Me_2SO) at 25 °C. Improved methods for the coupling of the more bulky amino acid residues will be described in detail later.

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Co(III) method was as follows. To a stirred solution of H-Phe-Leu-OAz (0.5 g) in dry MeOH (3 cm^3) was added 1.0 g of $[Co(en)_2(Gly\text{-}OCH_3)](CF_3SO_3)_3$ in 0.1-g aliquots. The reaction was followed by TLC (silica gel, ethanol); after 15 min all of the H-Phe-Leu-OAz had reacted. The red-orange solution was diluted with water (3 cm^3), NaCN (0.1 g) was added, and the solution was stirred for 5 min. H-Gly-Phe-Leu-OAz which had been displaced from the complex (reaction 3) was isolated by extraction

$$[Co(en)_2(Gly\text{-}Phe\text{-}Leu\text{-}OAz)]^{3+} + CN^- \text{ (excess)} \rightarrow CoCN_6^{3-} + {}^+H_2\text{-}Gly\text{-}Phe\text{-}Leu\text{-}OAz + 2enH_2^{2+} \quad (3)$$

into $CHCl_3$ and chromatography on silica gel. Subsequent couplings were carried out in a similar manner.[8] All peptide intermediates were shown to be homogeneous by TLC, spectral techniques (IR and ^1H NMR), and amino acid analysis. Catalytic hydrogenation[9] of the protected intermediate VII (Scheme I) produced [Leu^5]enkephalin in 24% overall yield. This was shown to be homogeneous by TLC and HPLC;[10] the amino acid composition was $Gly_{2.1}Leu_{1.0}Phe_{1.0}Tyr_{0.95}$, and it was shown to be identical with the product obtained by an alternative solution synthesis.[2]

(8) Percentage yields of the various intermediate peptides were as follows: III, 75%; IV, 95%; V, 82%; VI, 75%; VII, 55%. Yields were estimated by wt % recovery following silica gel chromatography and recrystallization. Amino acid analysis was carried out at each stage. VII exhibited poor solubility in the cyanide displacement reaction (despite the addition of 2 cm^3 of Me_2SO), and this is probably responsible for the lower recovery in this case.

(9) A solution of protected peptide (VII, 0.05 g) and 10% Pd/C (0.03 g) in methanol (20 mL) was shaken with hydrogen at 50 psi for 10 h. Following removal of catalyst and solvent, the product was chromatographed on Biogel P-2 (2 mol dm^{-3} of HOAc eluant).

(10) M. T. W. Hearn, C. A. Bishop, W. S. Hancock, D. R. K. Harding, and G. D. Reynolds, *J. Liq. Chromatogr.*, **2**, 1 (1979).

Total Synthesis of FK-156 Isolated from a *Streptomyces* as an Immunostimulating Peptide: Application of a Novel Copper Chelate Amino Protection

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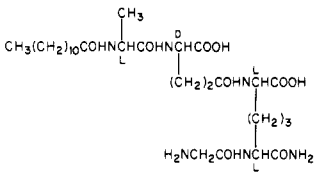
In recent years, considerable attention has been focused on the peptidoglycan fragments of bacterial cell walls because of their unique immunostimulating activity.[1] Recently, Imanaka et al.[2] isolated FK-156 as a metabolite possessing an activity similar to that of peptidoglycans from *Streptomyces olivaceogriseus* sp. nov.[3]

(1) For reviews, see, e.g.: (a) Chedid, L.; Lederer, E. *Biochem. Pharmacol.* **1978**, *27*, 2183. (b) Duker, P.; Tarcsay, L.; Baschang, G. *Annu. Rep. Med. Chem.* **1979**, *14*, 146. (c) Lederer, E. *J. Med. Chem.* **1980**, *23*, 819.

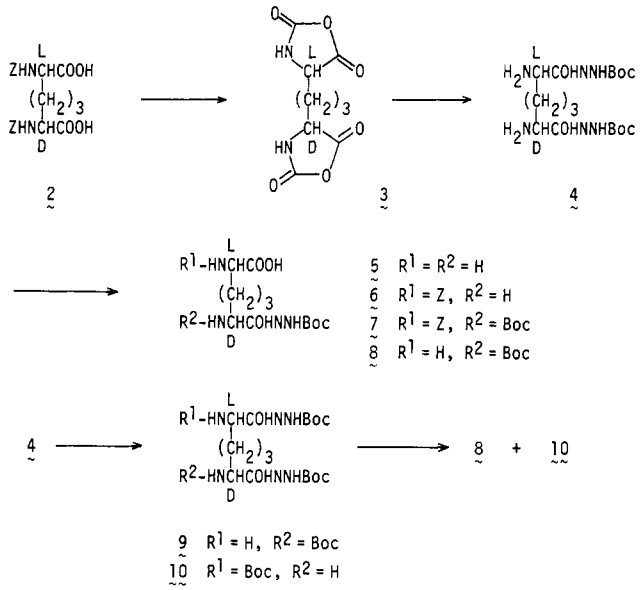
(2) Gotoh, T.; Kuroda, Y.; Okuhara, M.; Tanaka, H.; Nishiura, T.; Kohsaka, M.; Aoki, H.; Imanaka, H. "Abstract", *21st Interscience Conference Antimicrobial Agents and Chemotherapy*, Chicago, IL, 1981; in press.

(3) Migliore-Samour et al. have also reported an adjuvant activity of the peptidolipid i prepared by lauroylation of a cell-wall tetrapeptide isolated from *Streptomyces stimulosus*: Migliore-Samour, D.; Bauchaudon, J.; Floc'h, F.; Zerial, A.; Ninet, L.; Werner, G. H.; Jollès, P. *C. R. Hebd. Seances Acad. Sci., Ser. D* **1979**, *289*, 473. *Life Sci.* **1980**, *26*, 889.

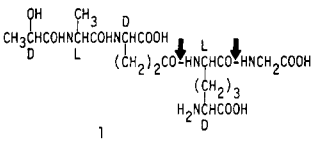


i

Scheme I



and proposed its structure to be **1**. Herein we report the total synthesis of **1** which finally confirmed the proposed structure.



The key in the synthesis of **1** is to create the peptide bonds at the positions marked by arrows with proper differentiation between the two pairs of the amino acid functions in *meso*-2,2′-diaminopimelic acid. This differentiation was cleanly performed by a sequence of reactions involving, as key steps, an enzyme-mediated asymmetric hydrolysis (**4** → **5**) followed by a selective carbobenzyloxylation using a copper chelate procedure (**5** → **6**).

The synthetically available di-Z-*meso*-2,2′-diaminopimelic acid (**2**)[4,5] was treated with PCl_5 (2.2 equiv) in CH_2Cl_2 (Scheme I) (0 °C → reflux, 1 h) to form the crystalline bis(*N*-carboxyanhydride) **3** [mp >250 °C; IR (Nujol) 1840, 1765 cm^{-1}] in 95% yield, which was subsequently allowed to react with *tert*-butyl carbazate as follows: A solution of **3** in MeCN and a solution of *tert*-butyl carbazate (2.0 equiv) and oxalic acid dihydrate (2.0 equiv) in MeOH were mixed at room temperature and vigorously stirred for 30 min to provide an 86% yield of the bis(Boc-hydrazide) **4**[6] [dioxalate; mp 130–135 °C dec; R_f = 0.39 (A)[8]].

Enzymatic hydrolysis of **4** to **5** was accomplished by using an aminopeptidase produced by *Streptomyces sapporonensis*.[9] After conversion to the free base, **4** was dissolved in Tris buffer ($^1/_{20}$ M, pH 7.5), and a 0.25 M solution was incubated at 37 °C in the presence of a crude powder of the enzyme [activity, 5.9 units/mg (protein);[10] application quantity, 100 units/g (sub-

(4) Wade, R.; Birnbaum, S. M.; Winitz, M.; Koegel, R. J.; Greenstein, J. P. *J. Am. Chem. Soc.* **1957**, *79*, 648.

(5) Work, E.; Birnbaum, S. M.; Winitz, M.; Greenstein, J. P. *J. Am. Chem. Soc.* **1955**, *77*, 1916.

(6) This compound **4** had been prepared, by Bricas et al.,[7] from **2** via coupling with *tert*-butyl carbazate by a mixed anhydride method using *i*-BuOCOCl followed by hydrogenolysis (lit. mp 128–130 °C). As compared to this, our method is apparently superior because of the simplicity in synthetic manipulations and the adaptability to a large scale preparation of **4**.

(7) Dezélée, P.; Bricas, E. *Bull. Soc. Chim. Biol.* **1967**, *44*, 1579.

(8) Analytical TLC was performed with silica gel 60-F_{254} (E. Merck AG) using the following solvent systems: (A) *n*-BuOH–AcOH–H_2O (4:1:5, upper phase); (B) *n*-BuOH–AcOH–H_2O (2:1:1); (C) AcOEt–AcOH (10:1); (D) *n*-PrOH–H_2O (3:2).

(9) Isolation and characterization of this enzyme will be reported by Imanaka et al. in due course.

(10) One unit was defined as the quantity capable of hydrolyzing 1.0 μmol of L-leucine *p*-nitroaniline per min at pH 7.0 at 37 °C.

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