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SYNTHESIS OF OPTICALLY PURE ENKEPHALIN ANALOG, [D-Ala^2, Leu^5-ol]ENKEPHALIN, USING CHIRAL β-LACTAMS AS SYNTHETIC INTERMEDIATES

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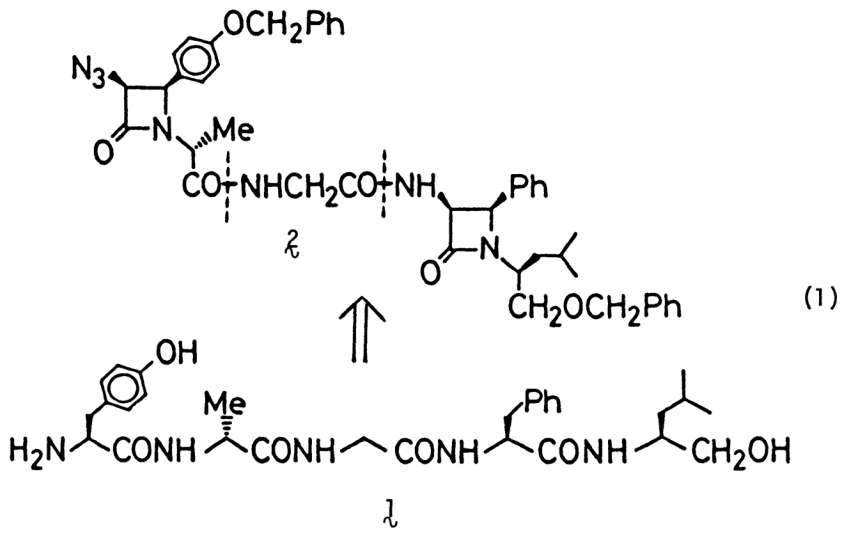
Optically pure pentapeptide, L-Tyr-D-Ala-Gly-L-Phe-L-Leu-ol (1) which is an analog of opioid hormone, enkephalin, was successfully synthesized by using β-lactam building blocks as chiral synthons of peptide units.

Recently, we have developed an entirely new method for peptide synthesis via β-lactam intermediates,[1-5] which is of considerable advantage to the manipulation of peptides because of the high solubility of the β-lactam intermediates in usual organic solvents and a good performance in chromatography on silica gel. In the preceding paper,[5] we reported a successful application of the β-lactam method to the synthesis of [Leu^5]enkephalin t-butyl ester which was, however, racemic except leucine residue. Now, we describe here the synthesis of optically pure [D-Ala^2, Leu^5-ol]enkephalin (1) which is a potent long-lasting analog of enkephalin,[6] an opioid hormone, by using chiral β-lactam building blocks.

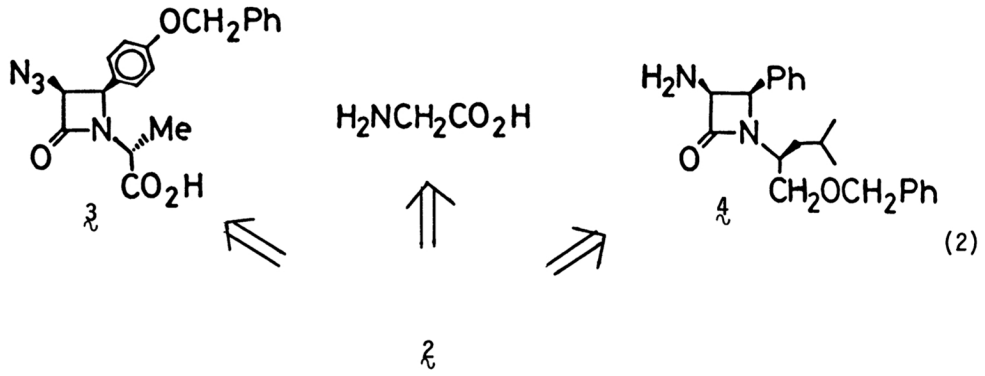
As the final precursor of [D-Ala^2, Leu^5-ol]enkephalin (1) we planned, based on the retrosynthetic scheme (eq. 1), to synthesize a chiral bis-β-lactam (2) which would readily be converted to 1 by hydrogenolysis on palladium.[1-5]



(1)

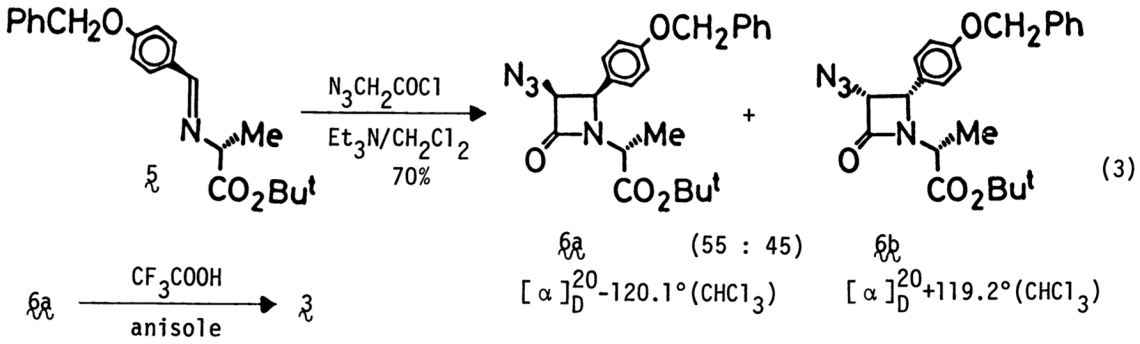
2 1

The bis-β-lactam (2) consists of three units, i.e., the β-lactam (3) which is a synthon of Tyr-(D)-Ala, glycine, and the β-lactam (4) which is a synthon of Phe-Leu-ol (eq. 2).



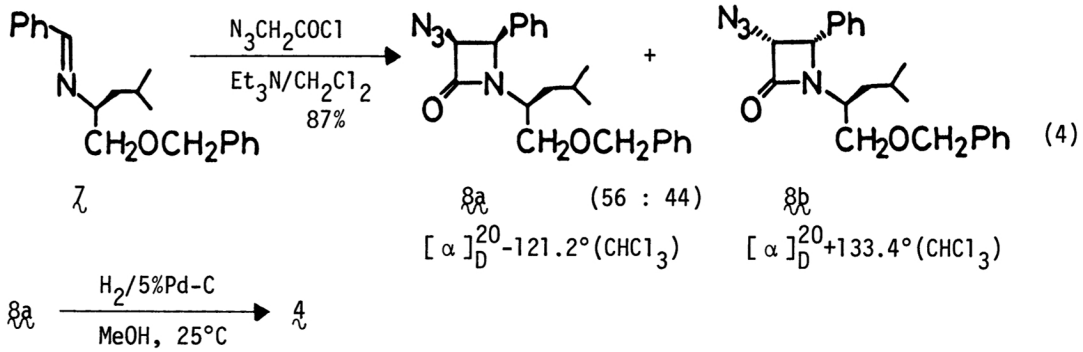
3 2 4 (2)

The β-lactam (3) was prepared via the in situ azidoketene addition to t-butyl 4-benzyloxybenzylidene-(D)-alaninate (5) followed by HPLC separation of two diastereomers (6a and 6b)[7] on silica gel (n-hexane/AcOEt=2/1) and deprotection of 6a (eq. 3).



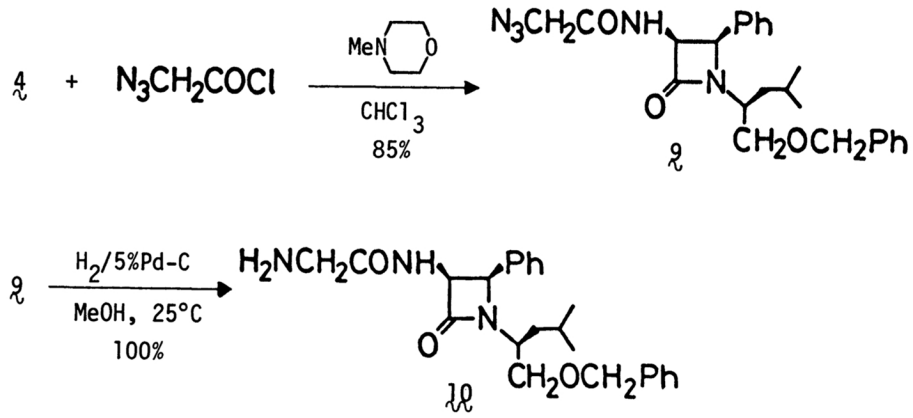
5 (3)

In a similar manner, the β-lactam (4) was prepared from benzylideneleucinol benzyl ether (7) and azidoacetyl chloride followed by HPLC separation of two diastereomers (8a and 8b)[8] on silica gel (n-hexane/AcOEt=4/1), and reduction of the azide group of 8a (eq. 4).



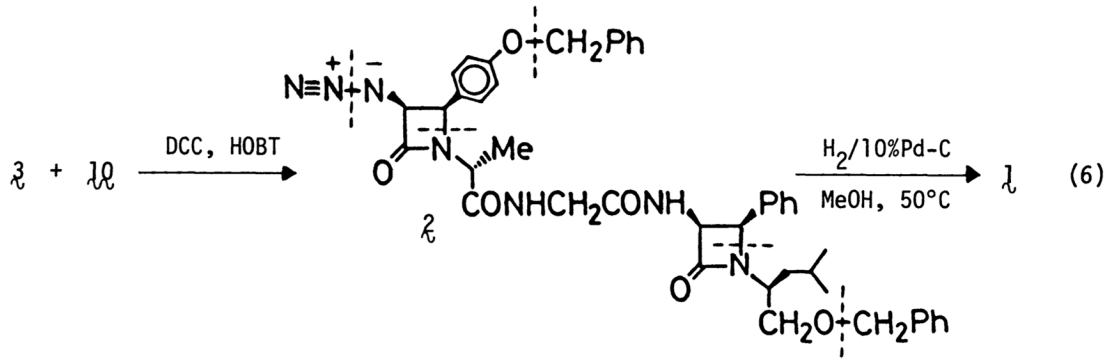
7 (4)

We employed azidoacetyl chloride as glycine synthon. Thus, the β-lactam (4) was azidoacetylated to give the azidoacetyl-β-lactam (9), which was transformed to the tripeptide synthon (10) by the reduction of azide moiety of 9 (eq. 5).



(5)

The coupling of Tyr-(D)-Ala synthon (3) and Gly-Phe-Leu-ol synthon (10) by using dicyclohexylcarbodiimide(DCC) and 1-hydroxybenzotriazole(HOBT) in dimethylformamide gave the bis-β-lactam (2),[9] which is the planned final precursor of 1, in 84% yield after purification on silica gel column (AcOEt). Then, the pentapeptide synthon (2) was submitted to hydrogenolysis on 10% Pd-C in methanol at 50°C to give 1[10] in 85% yield through the reductive cleavage of two β-lactam rings and the deprotection of two hydroxy groups and an azide group all at once (eq. 6).



(6)

It is noteworthy that the β-lactam ring of 3 acts not only as tyrosine synthon but also as excellent protecting group of D-alanine. According to the established rationale of the mechanism of racemization during peptide synthesis, the formation of oxazolone using an acylamino proton or an alkoxycarbonylamino proton is crucial,[11] which is more or less inevitable as far as ordinary protecting groups are employed. However, in the Tyr-(D)-Ala synthon (3), two amino protons of (D)-alanine are protected by the β-lactam ring; the racemization at chiral center cannot take place via oxazolone formation. Actually, no racemization was detected during the DCC-HOBT coupling of 3 and 10. This must be another advantageous feature of the β-lactam method.

As we have already disclosed, β-lactam building blocks exhibited good solubility toward usual organic solvents, readily purified on usual silica gel column, and fully characterized by spectroscopic analyses. In addition to these, i) the β-lactam building blocks, 6b and 8b, which were not used in the present synthesis, act as the chiral synthons of (D)-Tyr-(D)-Ala and (D)-Phe-Leu-ol, respectively: These are useful for the synthesis of other analogs, and ii) a variety of aromatic substituents, e.g., p-fluorophenyl, 3,4-dihydroxyphenyl, indole, furan, and pyrrole, etc., can be introduced to the β-lactam building blocks simply by using the corresponding aromatic aldehydes. These characteristics together with the stereochemical stability of β-lactam building blocks may provide a powerful device for the synthesis of various physiologically active oligopeptides.

References

1. I. Ojima, S. Suga, and R. Abe, Chem. Lett., 1980, 853.
2. I. Ojima, S. Suga, and R. Abe, Tetrahedron Lett., 21, 3907 (1980).
3. N. Hatanaka and I. Ojima, Chem. Lett., 1980, 231.
4. N. Hatanaka and I. Ojima, J. Chem. Soc., Chem. Comm., 1981, 346.
5. N. Hatanaka, R. Abe, and I. Ojima, Chem. Lett., 1981, 1297.
6. As for the design of enkephalin analogs having higher potency and longer duration of opioid activity, see J. S. Morley, Ann. Rev. Pharmacol. Toxicol., 20, 81 (1980); C. R. Beddell, R. B. Clark, G. W. Hardy, L. A. Lowe, F. B. Utaba, J. R. Vane, S. Wilkinson, K. J. Chang, P. Cuatrecasas, and R. J. Miller, Proc. Royal Soc. London, 198, 249 (1977), and references therein.
7. The absolute configurations of 6a and 6b were unambiguously determined on the basis of NMR and HPLC analyses by the comparison of the two diastereomers of Tyr-(D)-Ala-O^{t}Bu which were obtained by the hydrogenolysis of 6a and 6b following our method,[1-5] with the authentically prepared (L)-Tyr-(D)-Ala-O^{t}Bu.
8. The absolute configurations of 8a and 8b were unambiguously determined in a manner similar to the case of 6a and 6b on the basis of NMR and HPLC analyses by using authentically prepared (L)-Phe-(L)-Leu-ol; It was found that 8a gave (L)-Phe-(L)Leu-ol and 8b gave (D)-Phe-(L)-Leu-ol by hydrogenolysis.[1-5]
9. Hygroscopic colorless solid; NMR($CDCl_3$) δ 0.92 (d, J=5.5Hz, 3H), 0.94 (d, J=5.5Hz, 3H), 1.07 (d, J=7Hz, 3H), 1.14-2.05 (m, 3H), 3.14-3.42 (m, 2H), 3.56 (d, J=6Hz, 2H), 3.80 (m, 1H), 4.31 (q, J=7Hz, 1H), 4.24 (s, 2H), 4.95 (d, J=5Hz, 1H), 5.01 (ABq, J=5Hz, 2H), 5.03 (s, 2H), 5.41 (d of d, J=5Hz, 9Hz, 1H), 6.85-7.50 (m, 21H); IR(KBr) 3320 (νNH), 2110 (νN_3), 1750 (νC=O), 1660 (Amide I), 1540 (Amide II) cm^{-1}; Anal. Calcd. for $C_{43}H_{47}N_7O_6.3/2H_2O$; C, 65.80; H, 6.42; N, 12.49. Found: C, 65.58; H, 6.23; N, 12.38.; $[\alpha]_D^{20}$ -55.3° (c=1.07, $CHCl_3$).
10. Extremely hygroscopic colorless solid; IR(KBr) 3300 (νNH, νOH), 1655 (Amide I), 1540 (Amide II) cm^{-1}; Anal. Calcd. for $C_{29}H_{41}N_5O_6.5H_2O$: C, 53.94; H, 7.90; N, 10.84. Found: C, 53.75; H, 7.47; N, 10.44.; $[\alpha]_D^{20}$ +15.8° (c=0.91, MeOH). The product was also identified by comparing the HPLC chromatogram with that of authentically prepared HCl.H-(L)-Tyr-(D)-Ala-Gly-(L)-Phe-(L)-Leu-ol. HPLC analysis was carried out using a column packed with TOYO SODA LS 410K (ODS SIL) and MeOH-H_2O.
11. e.g., M. Bodanszky in " The Peptides", Vol. 1, ed. by E. Gross and J. Meienhofer, Academic Press, New York, 1979, pp 152-156.

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