An 'Active Ester'-type Mixed Anhydride Method for Peptide Synthesis. Use of the New Reagent, Norborn-5-ene-2,3-dicarboximido Diphenyl Phosphate (NDPP)

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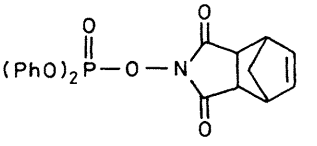
Summary Norborn-5-ene-2,3-dicarboximido diphenyl phosphate (NDPP) is a convenient activating reagent for peptide synthesis.

MIXED anhydrides of carboxylic acids with phosphoric acids play an important role in the biosynthesis of proteins, and a large number of coupling procedures using this type of compound[1] have been devised for biomimetic peptide syntheses. Of these procedures, only that using succinimido diphenyl phosphate[2] involved an 'active ester'-type phosphate. However, a major side-reaction takes place involving attack of amine at the succinimide carbonyl group when *N*-hydroxysuccinimide esters are used,[3] and *N*-hydroxynorborn-5-ene-2,3-dicarboximide esters with their rigid ring systems are preferable.[4] We therefore prepared norborn-5-ene-2,3-dicarboximido diphenyl phosphate (NDPP) and now report a convenient method for peptide synthesis using this new reagent.

NDPP (m.p. 128—129 °C) can be readily prepared in >70% yield by the reaction of diphenyl phosphorochloridate with *N*-hydroxynorborn-5-ene-2,3-dicarboximide (HONB) in



NDPP

the presence of triethylamine in methylene chloride at room temperature.†

A wide variety of modes of reaction are available to NDPP depending on the co-reactant and reaction conditions. These include reactions in which NDPP was used (i) for activation of the carboxy component before addition of the amine component, (ii) as a direct coupling reagent for peptide synthesis, and (iii) for 'active ester' synthesis.

Method (i) is a preactivation method. A mixture of Z(OMe)-Gly‡ (1 equiv.), triethylamine (1 equiv.), and NDPP (1 equiv.) in acetonitrile was stirred at room temperature. After confirmation of the disappearance of Z(OMe)-Gly by t.l.c. (usual reaction time, 2—3 h), a solution of Ala (1·1

† Satisfactory elemental analyses were obtained for all new compounds.

‡ Abbreviations used are those recommended by IUPAC-IUB Commission on Biochemical Nomenclature: *J. Biol. Chem.*, 1972, **247**, 977. Z(OMe) = *p*-methoxybenzyloxycarbonyl, OBzl = benzyl ester.

equiv) and triethylamine (1·1 equiv) in water was added to the preactivated mixture Stirring of the mixture at room temperature for 1 h gave Z(OMe)-Gly-Ala[5] (m p 142—144 °C) in 74% yield

Similarly, to a preactivated mixture of Z(OMe)-Gly (1 equiv), triethylamine (1 equiv) and NDPP (1 equiv) in acetonitrile was added a solution of Ala-Phe-OBzl CF_3CO_2H (1 equiv) and triethylamine in dimethylformamide Z(OMe)-Gly-Ala-Phe-OBzl (m p 104 °C) was obtained in 85% yield With the same method, Z(OMe)-Gly-D-Ala-Phe-OBzl (m p 133—135 °C) was obtained in 88% yield

Method (ii) is a direct coupling method To a stirred mixture of Z(OMe)-Ala (1 equiv), Phe-OBzl (1 equiv), and NDPP (1 equiv) in methylene chloride was added triethylamine (1 equiv) at 0 °C The mixture was stirred at 0 °C for 30 min and at room temperature overnight, and Z(OMe)-Ala-Phe-OBzl (m p 112 °C) was obtained in 92% yield With the same method, Z(OMe)-D-Ala-Phe-OBzl (m p 118 °C) was obtained in 89% yield

In order to examine the usefulness of this reagent for fragment condensaton in peptide synthesis, we directly condensed Z(OMe)-Gly-Ala with Phe-OBzl using NDPP in the presence of triethylamine The molar ratio of Z(OMe)-Gly-Ala-Phe-OBzl to Z(OMe)-Gly-D-Ala-Phe-OBzl was 98 0 · 2 0 in methylene chloride, 97 1 : 2 9 in ethyl acetate, and 97 0 3 0 in acetonitrile §

In method (iii) an active ester, Z(OMe)-Gly-ONB (m p 90—92 °C) was obtained from 1 mol equiv each of Z(OMe)-Gly, NDPP, and triethylamine in 95% yield

This method seems to be superior to the usual mixed anhydride method using isobutyl chloroformate[6] since the reaction can be conveniently performed at room temperature and in aqueous solution, and followed by t l c and also since it is possible to synthesize peptides using NDPP as a direct coupling reagent

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§ For the racemization test, (M Satomi and Y Kiso to be published) reversed-phase high performance liquid chromatography was used μBondapak C_{18} 0·39 × 30 cm methanol–water (57 43), 1 ml/min, 280 nm Retention volume Z(OMe) Gly-Ala Phe OBzl, 45 ml, Z(OMe) Gly-D-Ala-Phe-OBzl, 50 ml

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