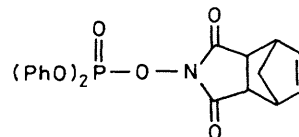


An 'Active Ester'-type Mixed Anhydride Method for Peptide Synthesis. Use of the New Reagent, Norborn-5-ene-2,3-dicarboximido Diphenyl Phosphate (NDPP)

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



NDPP

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 compound¹ have been devised for biomimetic peptide syntheses. Of these procedures, only that using succinimido diphenyl phosphate² 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

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-Ala⁵ (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 CF₃CO₂H (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 condensation 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 chloroformate⁶ 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. μ Bondapak C₁₈ 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.

¹ J. H. Jones 'The Peptides,' Vol. 1, eds E. Gross and J. Meienhofer, Academic Press, New York, 1979, p. 75.

² H. Ogura, S. Nagai, and K. Takeda *Tetrahedron Lett.* 1980, **21**, 1467.

³ J. Šavrdá *J. Org. Chem.* 1977, **42**, 3199.

⁴ M. Fujino, S. Kobayashi, M. Obayashi, T. Fukuda, S. Shinagawa, and O. Nishimura *Chem. Pharm. Bull.* 1974, **22**, 1857.

⁵ H. Yajima, Y. Kiso, and K. Kitagawa *Chem. Pharm. Bull.* 1974, **22**, 1079.

⁶ J. R. Vaughan, Jr. and R. L. Osato, *J. Am. Chem. Soc.* 1952, **74**, 676.