[Chem. Pharm. Bull. 21(11)2507—2510(1973)]

UDC 547.466.1.057 : 547.831.04

Studies on Peptides. XXXIX.[1-3] N-Isobutoxycarbonyl-2-alkoxy-1,2-dihydroquinoline Derivatives, as Coupling Reagents in Peptide Synthesis

Yoshiaki Kiso, Yoshiyuki Kai and Haruaki Yajima

Faculty of Pharmaceutical Sciences, Kyoto University[4]

(Received March 22, 1973)

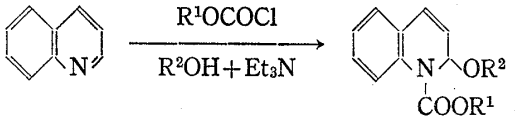
N-Isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline and two other analogous derivatives, N-isobutoxycarbonyl-2-methoxy-1,2-dihydroquinoline and N-isobutoxycarbonyl-2-ethoxy-1,2-dihydroquinoline, have been used as coupling reagents for peptide synthesis.

A pseudo base, N-ethoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (EEDQ) was introduced by Belleau and Malek[5] in 1968 as a coupling reagent for peptide synthesis. Recently we have presented data to suggest that this new reagent might be suitable for condensing acylpeptides to peptides anchored on copolymer.[6] The use of this compound, instead of dicyclohexylcarbodiimide, was also recommended by Sipos and Gaston[7] for the stepwise elongation procedure in the solid phase peptide synthesis. More recently, incorporation of EEDQ into an insoluble polymeric form was described by Brown and Williams.[8]

Belleau and Malek[5] offered the experimental evidence that the mechanism of carboxyl group activation by EEDQ involves the transient formation of a mixed carbonic carboxylic anhydride intermediate. Informations now available in the mixed anhydride procedure in peptide synthesis indicate that besides the nucleophilicity of the amino component, the steric environment of the anhydride plays the major role in determining the direction of its aminolysis.[9] For the suppression of the side reaction, *e.g.*, the urethan-formation, a mixed carbonic carboxylic anhydride with bulky alkyl groups, which are electron releasing part of the molecule, is considered to be favorable. For this reason, isobutyl chloroformate[10] is a reagent commonly recommended for the peptide synthesis. With this consideration, we have now prepared N-isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ) and two other analogous derivatives: N-isobutoxycarbonyl-2-methoxy-1,2-dihydroquinoline (IMDQ) and N-isobutoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (IEDQ). They will give, with a carboxylic component, an isobutoxy carboxylic anhydride as an intermediate in the peptide forming reaction.

1) Part XXXVIII: H. Yajima, K. Kitagawa, and T. Segawa, *Chem. Pharm. Bull.* (Tokyo), **21**, 2500 (1973).
2) The preliminary communication of this paper has appeared in *Chem. Commun.*, **1972**, 942.
3) Abbreviation used are those recommended by IUPAC-IUBC on Biochemistry Nomenclature: *Biochem.*, **5**, 2485 (1966); **6**, 362 (1967). Z=benzyloxycarbonyl, Z(OMe)=*p*-methoxybenzyloxycarbonyl, Bzl=benzyl, ONP=*p*-nitrophenyl ester, OPCP=pentachlorophenyl ester, OSU=N-hydroxysuccinimide ester.
4) Location: *Sakyo-ku, Kyoto.*
5) B. Belleau, R. Martel, G. Lacasse, M. Menard, N.L. Weinberg, and Y.G. Perron, *J. Am. Chem. Soc.*, **90**, 832 (1968); B. Belleau and G. Malek, *ibid.*, **90**, 1651 (1968).
6) H. Yajima and H. Kawatani, *Chem. Pharm. Bull.* (Tokyo), **19**, 1905 (1971).
7) F. Sipos and D.W. Gaston, *Synthesis*, **1971**, 321 [*C.A.* **75**, 98806P (1971)].
8) J. Brown and R.E. Williams, *Can. J. Chem.*, **49**, 3765 (1971).
9) Th. Wieland and H. Bernhard, *Ann. Chem.*, **572**, 190 (1951); R.A. Biossonnas, *Helv. Chim. Acta*, **34**, 847 (1951); H. Yajima, N. Mizokami, Y. Okada, and K. Kawasaki, *Chem. Pharm. Bull.* (Tokyo), **17**, 1958 (1969) and see also the review article: N.F. Albertson, "Organic Reactions," Col. Vol. XII, John Wiley & Sons, New York, 1962 p. 157.
10) J.R. Vaughan, Jr., *J. Am. Chem. Soc.*, **73**, 3547 (1951); F.E. King, J.W.C. Lewis, R. Wade and W.A. Swindi, *J. Chem. Soc.*, **1957**, 873.

According to the procedure described in the preparation of EEDQ,[11] IIDQ was prepared by direct action of isobutyl chloroformate and quinoline in isobutyl alcohol containing triethylamine (Chart 1). The product was obtained as a distilable liquid. Data of elemental analysis, infrared (IR) and nuclear magnetic resonance (NMR) spectra supported the empirical formula of the desired compound. When this reaction was performed in either methanol or ethyl alcohol, the corresponding 2-methoxy (IMDQ) or 2-ethoxy derivatives (IEDQ) was



	R^1	R^2
EEDQ	$-C_2H_5$	$-C_2H_5$
IIDQ	$-CH_2-CH(CH_3)_2$	$-CH_2-CH(CH_3)_2$
IMDQ	$-CH_2-CH(CH_3)_2$	$-CH_3$
IEDQ	$-CH_2-CH(CH_3)_2$	$-C_2H_5$

Chart 1. Preparation of N-Isobutoxycarbonyl-2-alkoxy-1,2-dihydroquinoline Derivatives

obtained as liquid. As a model experiment, the couplimg reaction of Z-Ala-OH with H-Tyr-OMe by one of these three reagents was performed in dimethylformamide. When the reaction was followed by thin-layer chromatography, IIDQ seems to be the most promising one in regards to its reactivity. Higher yield of Z-Ala-Tyr-OMe was obtained by IIDQ compared to the others. With this reagent, Z-Gln-Thr-OMe and Z(OMe)-Asn-Ala-OMe were further prepared. IR spectra of these products exhibited no observable CN bond indicating that no dehydration of the amide groups of these amino acids occurred during the peptide bond forming step by this reagent. Z-Ser-Ala-OMe was also prepared in satisfactory yield without masking the hydroxyl group of serine. As pointed out by Belleau and Malek,[5] in any case, a proposed intermediate, *i.e.*, a mixed anhydride derived from a N-protected amino acid by IIDQ, could not be isolated. However, when IIDQ was allowed to react with N-hydroxybenzotriazole, as an acid component, a mixed anhydride, isobutoxycarbonyl N-hydroxybenzotriazole ester, was isolated in crystalline form (Chart 2). This seems to offer the direct evidence that the peptide bond forming reaction by pseudo bases proceeds indeed through a mixed anhydride as an intermediate.

Since IIDQ has a tendency to react with acidic components, preparation of the active ester of N-protected amino acids by this reagent is not always successful. For example, preparation of the *p*-nitrophenyl ester of Z-Phe-OH offers no difficulty as in the case of dicyclohexylcarbodiimide (DCC) (Chart 2). However preparation of the active ester with more acidic phenols, such as pentachlorophenyl, was unsatisfactory, presumably due to the competitive isobutoxycarbonyl anhydride formation with phenols and N-protected amino acids. Situation was the same in the preparation of the N-hydroxysuccinimide ester of Z-Phe-OH.

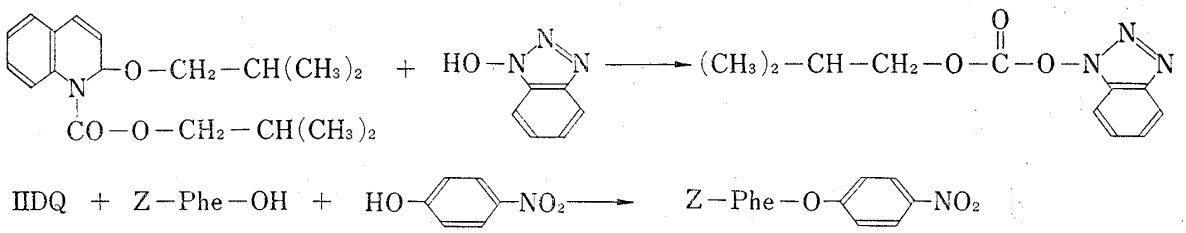


Chart 2. Reaction of IIDQ with Hydroxy Compounds

Next, application of IIDQ for the solid phase peptide synthesis was examined. The condensation reaction of Z(OMe)-Asp(OBzl)-Phe-Cys(Bzl)-Leu-Glu(OBzl)-Pro-OH (3 equimole.)

11) Reagents for Organic Synthesis, Vol. II, eds. M. Fieser and L.F. Fieser, Wiley Interscience, New York, 1969, p. 191.

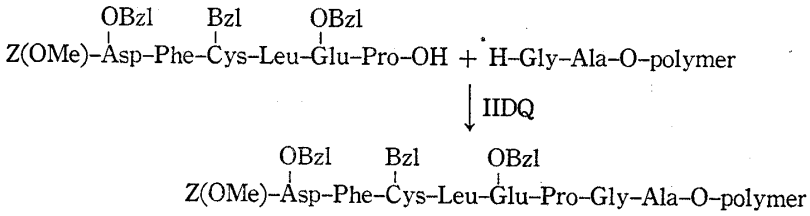


Chart 3. Application of IIDQ to the Solid Phase Synthesis

with H-Gly-Ala-resin (1 equimole.)[12] by IIDQ (3 equimole.) was performed in dimethylformamide for 24 hr (Chart 3). A part of the resin was removed and submitted to acid hydrolysis. Amino acid analysis indicated that the reaction proceeded slowly on the resin. Nearly quantitative coupling was achieved after 48 hr's reaction, with the peptide, resin and IIDQ in molar ratios of 4: 1: 4. Thus IIDQ seems to be an useful reagent, like EEDQ,[6] for the condensation of peptide fragments on polymer support.

Belleau and Malek[5] demonstrated by the Young test that EEDQ has an unique property to suppress the rate of racemization during the amide forming reaction. For the racemization test, we have applied the system of Bodanszky and Conklin.[13] Ac-Ile-OH was coupled with H-Gly-OMe by IIDQ in dimethylformamide at 20° for 18 hr and the racemate, *allo*-D-Ile-OH, was determined, after acid hydrolysis, by Spackman-Stein-Moore method[14] of quantitative amino acid analysis. Under these conditions, IIDQ gave a racemate in 4.1% while DCC gave 27.4%. Thus it appears that IIDQ, as EEDQ, is a good racemization depressant in peptide synthesis.

An easy procedure for the preparation of IIDQ will enhance the usefulness of this reagent in peptide synthesis.

Experimental

N-Isobutoxycarbonyl-2-isobutoxy-1,2-dihydroquinoline (IIDQ)——Isobutyl chloroformate (14.3 ml, 0.11M) was added to a solution of quinoline (11.7 ml, 0.1M) in dry ether (60 ml) at −5° with stirring. To this solution, a mixture of isobutyl alcohol (18.3 ml, 0.2M) and triethylamine (15.5 ml, 0.11M) was added at −5° over a period of 30 min. After stirring for an additional 1 hr, the solution was filtered, condensed and the residue was distilled under vacuum; bp 144—145°/0.5 mmHg, yield 16.4 g (55%). IR $\nu_{max}^{CHCl_3}$ 1705 cm^{-1}, NMR ($CDCl_3$) δ: 0.84(m, 4-CH_3), 1.80 (m, 2-CH–), 3.33 (d, J=6 Hz, –O–C<u>H</u>$_2$–CH–), 4.07 (d, J=6 Hz, –CO_2–C<u>H</u>$_2$–CH–), 5.9—7.8 (m, 7H). *Anal.* Calcd. for $C_{18}H_{25}O_3N$: C, 71.26; H, 8.31; N, 4.62. Found: C, 71.18; H, 8.45; N, 4.82.

N-Isobutoxycarbonyl-2-methoxy-1,2-dihydroquinoline (IMDQ)——In the above experiment, isobutyl alcohol was replaced by MeOH (8.1 ml, 0.2M). The reaction and the purification were performed in essentially the same manner as described above; bp 147—148°/0.8 mmHg, yield 13.0 g (50%). IR $\nu_{max}^{CHCl_3}$ 1705 cm^{-1}. NMR ($CDCl_3$) δ: 0.93 (d, J=6 Hz, –CH(C<u>H</u>$_3$)$_2$), 2.0 (m, –CH–), 3.30 (s, –OCH_3), 4.02 (d, J=6 Hz, –CH_2–), 5.9—7.8 (m, 7H). *Anal.* Calcd. for $C_{15}H_{19}O_3N$: C, 68.94; H, 7.33; N, 5.36. Found: C, 69.20; H, 7.70; N, 5.27.

N-Isobutoxycarbonyl-2-ethoxy-1,2-dihydroquinoline (IEDQ)——The reaction was performed as described above using EtOH (9.2 ml, 0.2M) as an alcohol component, bp 143—144°/0.2 mmHg, yield 15.0 g (55%). IR $\nu_{max}^{CHCl_3}$ 1705 cm^{-1}. NMR ($CDCl_3$) δ: 1.08 (m, 3-CH_3), 1.95 (m, –CH–), 3.65 (q, J=7 Hz, –O–C<u>H</u>$_2$–CH_3), 4.07 (d, J=7 Hz, –CO_2–CH_2), 5.95—7.8 (m, 7H). *Anal.* Calcd. for $C_{16}H_{21}O_3N$: C, 69.79; H, 7.69; N, 5.09. Found: C, 69.91; H, 7.70; N, 5.17.

Synthesis of Model Peptides——Four dipeptide derivatives were prepared. An equimolar mixture (5 mM each) of the carboxyl component, the amino component and a coupling reagent in dimethylformamide (17 ml) was stirred at room temperature for 24 hr. After evaporation of the solvent, the residue was dissolved in AcOEt, which was washed with 1N $NaHCO_3$, 10% citric acid and H_2O, dried over Na_2SO_4 and then evaporated. Each product was recrystallized from AcOEt or from a mixture of MeOH and AcOEt. The results are listed in Table I.

12) Y. Kiso and H. Yajima to be published.
13) M. Bodanszky and L.E. Conklin, *Chem. Commun.*, **1967**, 773.
14) D.H. Spackman, W.H. Stein, and S. Moore, *Anal. Chem.*, **30**, 1190 (1958).

Table I. Model Peptides prepared by IIDQ

	Yield (%)		$[\alpha]_D^{26}$	Found (%)			Formula	Required (%)		
				C	H	N		C	H	N
Z-Ala-Tyr-OMe[a]	88[b]	122—123	−8.5° (MeOH)	62.64	6.12	6.83	$C_{21}H_{24}O_6N$	62.99	6.04	7.00
Z-Gln-Thr-OMe[c]	84	162—165	+2.1° (DMF)	54.42	6.20	10.55	$C_{18}H_{25}O_7N_3$	54.68	6.37	10.63
Z-Ser-Ala-OMe[d]	80	113—114	−31.0° (MeOH)	55.45	6.25	8.37	$C_{15}H_{20}O_6N_2$	55.55	6.22	8.64
Z(OMe)-Asn-Ala-OMe	95	172—174	−8.5° (MeOH)	53.23	6.36	11.01	$C_{17}H_{23}O_7N_3$	53.54	6.08	11.02

a) lit.[6] yield 70% by EEDQ, mp 118—120°, $[\alpha]_D^{24}$ −8.8° in MeOH. lit.[15] mp 121—122°
b) yield 79% by IMDQ and 77% by IEDQ
c) lit.[6] yield 64% by EEDQ, mp 164—167°, $[\alpha]_D^{28}$ +1.9° in DMF
d) lit.[6] yield 58% by EEDQ, mp 112—114°, $[\alpha]_D^{26}$ −30.8° in MeOH. lit.[16] mp 113—114°

Reaction of IIDQ with N-Hydroxybenzotriazole——To a solution of IIDQ (1.52 g) in AcOEt (5 ml), N-hydroxybenzotriazole (0.68 g) was added and the mixture was stirred at room temperature for 48 hr. After evaporation of the solvent, the residue was treated with ether to afford the solid which was recrystallized from ether; yield 0.70 g (55%), mp 96—97°, IR $\nu_{max}^{CHCl_3}$ 1755 cm^{-1} (-O-CO-O-), NMR ($CDCl_3$), δ: 1.08 (d, J=6 Hz, 2-CH_3), 2.05 (m, -CH-), 4.34 (d, J=6 Hz, -CH_2-) 7.35—8.3 (m, 4H). *Anal.* Calcd. for $C_{11}H_{13}O_3N_3$: C, 56.16; H, 5.57; N, 17.86. Found: C, 56.21; H, 5.52; N, 17.71.

Preparation of the Active Ester with IIDQ——Z-Phe-ONP: To a solution of Z-Phe-OH (1.50 g) and *p*-nitrophenol (0.77 g) in tetrahydrofuran (30 ml), IIDQ (1.70 g) was added and the mixture was stirred at room temperature for 6 hr. After evaporation of the solvent, EtOH was added to the residue to give the solid, which was washed with petroleum ether and recrystallized from tetrahydrofuran and EtOH; yield 1.57 g (75%), mp 125—126° (lit.[17] mp 126—127.5°). *Anal.* Calcd. for $C_{23}H_{20}O_6N_2$: C, 65.71; H, 4.80; N, 6.66. Found: C, 66.01; H, 4.70; N, 6.77.

Z-Phe-OPCP: The reaction was performed in essentially the same manner as described above; yield 16%. mp 154—156° (lit.[18] mp 158°). *Anal.* Calcd. for $C_{23}H_{16}O_4NCl_5$: C, 50.63; H, 3.21; N, 2.60. Found: C, 50.44; H, 2.94; N, 2.56.

Z-Phe-OSU: A similar reaction was performed, but the isolation of the crystalline compound has failed. (lit.[19] mp 140—140.5°).

Application of IIDQ to the Condensation of Peptide Fragments on the Polymer Support——Coupling reaction between H-Gly-Ala-resin (300 mg, the peptide content, 0.03 mM attached to a copolymer of styrene and 2% divinylbenzene) and Z(OMe)-Asp(OBzl)-Phe-Cys(Bzl)-Leu-Glu(OBzl)-Pro-OH[12] (105 mg, 0.09 mM) was performed in dimethylformamide by IIDQ (54 mg, 0.09 mM). The mixture was shaken at room temperature, meanwhile a small amount of the resin was dispatched after 2 hr, 6 hr and 24 hr and these were submitted for acid hydrolysis. The ratios of Phe/Gly were 26%, 42%, and 78% respectively. Under identical conditions, EEDQ gave a coupling yield in 15% after 2 hr and 59% after 24 hr. When the reaction was performed in molar ratios of the carboxyl component: IIDQ: the amino component=4: 4: 1 (0.03 mM) at room temperature for 48 hr, amino acid ratios in an acid hydrolysate of the peptide resin were $Asp_{0.90}$ $Phe_{0.94}$ $Leu_{0.89}$ $Glu_{0.92}$ $Pro_{0.95}$ $Gly_{1.00}$ $Ala_{1.05}$.

Racemization Test——Two parallel experiments were performed in essentially the same manner as described previously.[1] To a mixture of Ac-L-Ile-OH (346 mg) and H-Gly-OMe (prepared from 251 mg of the hydrochloride and 0.28 ml of triethylamine) in DMF (10 ml), DCC (412 mg) or IIDQ (660 mg) was added and each solution, after stirring at 20° for 18 hr, was condensed *in vacuo*. The residue was dissolved in AcOEt, which was washed with 0.1N HCl, 5% Na_2CO_3 and H_2O and then evaporated. The resulting solid, after drying *in vacuo* overnight, was submitted to the acid hydrolysis (with 6N HCl, at 110° for 20 hr). Each hydrolysate was applied to a long column for the acid and neutral amino acid analysis. Under these conditions, IIDQ gave a racemate 4.1%, while DCC gave 27.4%.

Acknowledgement The authors wish to express their sincere appreciation to D.T.Y. Liu of Brookhaven National Laboratory, U.S.A., for his advice in preparation of this manuscript.

15) K. Jost, V. Debabov, H. Nesvadba, and J. Rudinger, *Collection Czech. Chem. Commun.*, **29**, 419 (1964).
16) J.S. Fruton, *J. Biol. Chem.*, **146**, 463 (1942).
17) M. Goodman and K.C. Stuben, *J. Am. Chem. Soc.*, **81**, 3980 (1959).
18) J. Pless and R.A. Boissonnas, *Helv. Chim. Acta*, **46**, 1609 (1963).
19) G.W. Anderson, J.E. Zimmerman, and F.M. Callahan, *J. Am. Chem. Soc.*, **86**, 1839 (1964).