[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES, AMERICAN CYANAMID COMPANY]

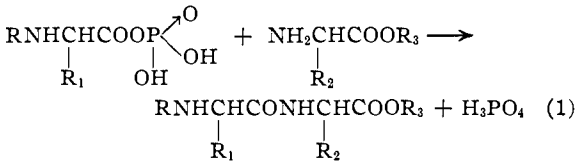
The Use of Diester Chlorophosphites in Peptide Syntheses. Mixed Anhydrides[1]

BY GEORGE W. ANDERSON AND RICHARD W. YOUNG

RECEIVED MARCH 3, 1952

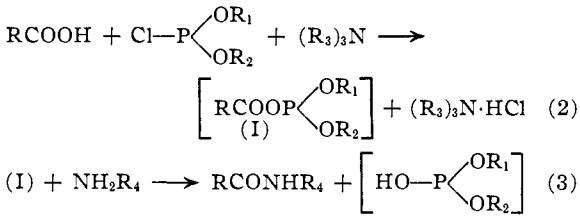
N-Acyl peptide esters have been synthesized by the reaction of diester chlorophosphites with N-acylamino- and peptide acids, followed by reaction with amino esters. The procedure is simple, rapid and gives good yields, with no apparent racemization of optically active derivatives. The preparation of several simple amides is also reported.

The concept of the role of energy-rich phosphoric acid derivatives in biological syntheses, and particularly the suggestion by Lipmann[2] that acyl phosphates derived from amino acids might react with amino groups of other amino acid derivatives in biological systems to form peptide links (equation 1) led to the development of analogous peptide

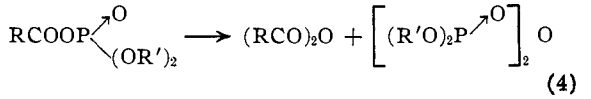


syntheses by Chantrenne,[3] who used carbobenzoxyglycylphenylphosphate, and Sheehan and Frank,[4] who used acyl dibenzylphosphates. Although of theoretical interest, both of these synthetic methods involve a difficult preparation of the mixed anhydrides through acylamino acid chlorides. Since the chlorides themselves can be used for coupling with amino acid salts or esters,[5] it appears unlikely that phosphate anhydrides, prepared by these procedures at least, will have wide utility.

In contrast, mixed anhydrides of phosphorous acid diesters[6] with amino acid derivatives are readily prepared from the chlorophosphites, and are conveniently reacted *in situ* with amino acid esters or peptide esters to form peptide derivatives in good yields (equations 2 and 3). No effort has been made to establish definitely the structure of the phosphite by-products; these compounds do not interfere with isolation of the products.

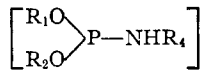


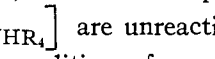
The problem of rigorously establishing the structure of the mixed phosphite anhydrides is difficult; no crystalline anhydride has been isolated, and attempted distillation, even of the lower homologs, has resulted in extensive decomposition. Sheehan and Frank[4] obtained phthaloylglycyl dibenzyl phosphate as a crystalline solid, and showed that it decomposed to the symmetrical anhydrides on standing, on attempted crystallization from benzene, or in the presence of triethylamine (equation 4). If an analogous decomposition occurs with



(4)

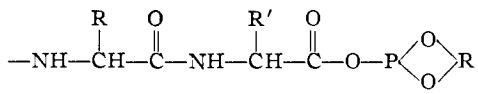
phosphite anhydrides, the symmetrical phosphite anhydride formed $[(RO)_2P\text{-}O\text{-}P(OR)_2]$ can react with the amine added to form a phosphite amide



which in turn can react with the carboxylic acid formed from the reaction of $(RCO)_2O$ with the amine, thereby forming the amide $RCONHR_4$.[7] Thus, the net results are the same as from equations 2 and 3. This would not be true for the phosphate reactions, since diester phosphate amides  are unreactive to carboxylic acids under the conditions of equations 2 and 3.[7]

The observation that a quantitative yield of $R_3N\cdot HCl$ is obtained in the reaction of equation 2 indicates that the mixed anhydride is formed, at least as a transitory intermediate. The failure to isolate benzoic anhydride after the mixed anhydride between *o*-phenylene chlorophosphite and benzoic acid was allowed to stand in the absence of amine suggests that disproportionation had not occurred. However, the mixed anhydride between *o*-phenylene chlorophosphite and phthaloylglycine on standing deposited 20% of the symmetrical anhydride of phthaloylglycine.

The possibility for racemization when an anhydride is prepared from an N-acyl dipeptide such as carbobenzoxyglycyl-L-leucine has been investigated. Formation of the mixed anhydride (II)



II

might facilitate nucleophilic displacement on the adjacent hydrogen atom, or azlactone formation[8] could occur. In either case the product could be racemized. However, both carbobenzoxyglycyl-L-

(1) Second paper in a series on phosphorus derivatives. See THIS JOURNAL, **74**, 5304 (1952), for the first paper; also presented in part at the September, 1951, Meeting of the American Chemical Society.

(2) F. Lipmann, *Advances in Enzymology*, **1**, 99 (1941).

(3) H. Chantrenne, *Nature*, **164**, 576 (1949); *Biochem. et Biophys. Acta*, **4**, 484 (1950).

(4) J. C. Sheehan and V. S. Frank, THIS JOURNAL, **72**, 1312 (1950).

(5) This reaction is summarized in a review article by J. S. Fruton, *Advances in Protein Chem.*, **5**, 1 (1949).

(6) For convenience, these will be called mixed phosphite anhydrides.

(7) See first paper in this series, THIS JOURNAL, **74**, 5304 (1952).

(8) An acylamino azlactone would be formed, but this would probably racemize as readily as an azlactone formed from a simple α-acylamino acid (*cf.* H. Carter, *Org. Reactions*, **3**, 198 (1946)).

TABLE I

THE PREPARATION OF N-ACYLPEPTIDE ESTERS

No.	Product[a]	Yield, %	M.p., °C. (cor.)	Recrystallization solvent	$[\alpha]^{25}D$
1	Z·Gly-Phe·OEt(DL)[b]	85	89–90	50% EtOH	.....
2	Z·Gly-Gly·OEt[c]	83[d]	86–87	50% EtOH	.....
3	Z·Leu-Gly·OEt(L)[e]	80[f]	104–105	50% EtOH	−26.4° (*c*, 5, EtOH)
4	Z·Gly-Gly-Gly·OEt[g,h]	43	165–167	50% EtOH	.....
5	Z·Gly-Tyr·OEt(L)[i]	36	122–124	60% EtOH	+19.8 (*c*, 5, EtOH)
6	Z_2-Lys-Gly·OEt(L)[j]	92	88–89	EtOH	−12.1 (*c*, 5, EtOH)
7	Z·Val-Gly·OEt(DL)[k]	82	148.5–149.5	EtOAc	.....
8	Z·Phe-Gly·OEt(L)[l]	53	109–110	EtOAc–pet. ether	−16.0 (*c*, 2, EtOH)
9	Z·Gly-Leu-Gly·OMe(DL)[m]	25	107–108	EtOAc–pet. ether	.....
10	Z·Gly-Leu-Leu·OMe(L)[n]	50	131–132	EtOAc–pet. ether	−47.3 (*c*, 2, MeOH)

[a] The following abbreviations are used (*cf.* B. F. Erlanger and E. Brand, THIS JOURNAL, **73**, 3508 (1951)): Z = carbobenzoxy, $C_6H_5CH_2OCO$; Ph = phthaloyl; peptide linkage indicated by hyphen (-); Et = C_2H_5; configuration follows compound in parentheses; amino acids are denoted by the first three letters of name. [b] H. Neurath, *et al.*, *J. Biol. Chem.*, **170**, 222 (1947), report m.p. 90–91°. [c] O. Süs, *Ann.*, **572**, 105 (1951), reports m.p. 82.5–83°. [d] When triethylamine hydrochloride was not removed the yield dropped to 50%. [e] M. A. Nyman and R. M. Herbst, *J. Org. Chem.*, **15**, 108 (1950), report m.p. 99° and $[\alpha]^{25}D$ −26.8° (*c*, 2.6, EtOH). [f] Dioxane as a solvent gave a 60% yield. [g] J. S. Fruton, *et al.*, *J. Biol. Chem.*, **173**, 467 (1948), reported m.p. 165°. The compound was prepared from Z·Gly·OH and H·Gly-Gly·OEt. [h] The amino ester was prepared by dissolving it in chloroform containing triethylamine and precipitating triethylamine hydrochloride with benzene. [i] J. R. Vaughan and R. L. Osato, THIS JOURNAL, **74**, 676 (1952), give m.p. 124–125°, $[\alpha]^{24}D$ +19.8° (*c*, 5, EtOH). [j] Ref. *i* gives m.p. 92–93°, $[\alpha]^{24}D$ −12.0° (*c*, 4, EtOH). [k] The reaction mixture was allowed to stand at 25° for 18 hours instead of being heated. R. Kuhn and H. W. Ruelius, *Ber.*, **85**, 38 (1952), give m.p. 144–145°. [l] M. Bergmann and J. S. Fruton, *J. Biol. Chem.*, **118**, 414 (1937), give m.p. 111°. [m] Prepared from Z·Gly-Leu·OH(DL) (ref. *k*) and distilled H·Gly·OMe. *Anal.* Calcd. for $C_{19}H_{27}N_3O_6$: N, 10.7. Found: N, 10.7, 10.7. [n] Prepared from Z·Gly-Leu·OH(L) (M. A. Stahman, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **164**, 759 (1946)) and H·Leu·OMe·HCl(L) (S. Simmonds, J. I. Harris and J. S. Fruton, *ibid.*, **188**, 260 (1951)), give m.p. 131–132°).

leucine and carbobenzoxyglycyl-L-phenylalanine were coupled with a glycine ester in good yield without racemization.

Both diethyl[9] and *o*-phenylene chlorophosphite[10] have been used and no significant differences were noted. Because of the stability and ease of preparation of *o*-phenylene chlorophosphite, it has been preferred. Although other solvents have been used, *e.g.*, dioxane and chloroform, the yields obtained were not generally as satisfactory as when benzene or toluene were employed.

Experimental[11]

The N-acylamino acid or peptide (0.01 mole) was dissolved in 50 cc. of benzene or toluene (dried by azeotroping) containing an equivalent of triethylamine. One equivalent of the chlorophosphite in 10 cc. of solvent was added to the solution, with sufficient cooling to maintain the temperature below 25°. The precipitated triethylamine hydrochloride was removed by filtration and the filtrate was treated with an amino acid or peptide ester.[12] Following 15 minutes of warming on the steam-bath, the solution was washed successively with 10 cc. of water, 25 cc. of half-saturated sodium bicarbonate, and finally with 10 cc. of water. After drying the organic layer over sodium sulfate, the solvent was removed *in vacuo* or in a stream of air, and the resultant solid or oil was crystallized from an appropriate solvent.

Table I illustrates the results of the general procedure for preparing peptide derivatives, and Table II shows the preparation of some simple amide derivatives. Compounds 18 and 19, not included in the tables, illustrate the preparation of two optically active tripeptides from an N-acyl dipeptide acid.

18. **Methyl Carbobenzoxyglycyl-L-leucylglycinate.**[13]—The mixed anhydride between carbobenzoxyglycyl-L-leucine and *o*-phenylene chlorophosphite was prepared by adding the chlorophosphite in 10 cc. of toluene to a solution of the triethylammonium salt in 25 cc. of toluene. To the solution of the anhydride there was added an equivalent of distilled methyl glycinate.[14] After the reaction was completed, 50 cc. of ethyl acetate was added to facilitate working up by the general method. The oil resulting after concentration was dissolved in 25 cc. of ethyl acetate and made cloudy with 100 cc. of petroleum ether. The results are summarized in Table III.

Recrystallization of the combined samples gave a constant rotation $[\alpha]^{25}D$ −36.1° (*c* 5, methanol), m.p. 132–133°, after two crystallizations from ethyl acetate–petroleum ether.

TABLE II

PREPARATION OF HYDRAZIDES AND AMIDES

No.	Product	Yield, %	M.p., °C. (cor.)	Recrystallization solvent
11	Z·Gly-Leu·Anilide(L)[a]	40–50[b]	137–138	EtOAc–pet. ether
12	Z·Gly-Leu·Anilide(DL)[c]	38	153–154	EtOAc–pet. ether
13	Z·Gly·Anilide[d]	85–95[e]	144–145	50% EtOH
14	Z_2·Lys·$NHNH_2$(L)[f]	92	160–161	EtOH
15	Z·Phe·$NHNH_2$(L)[g]	87[h]	167–168	None
16	Salicyl-*p*-toluide[i]	66	154–155	MeOH
17	Adipic acid dianilide[j]	83[k]	245–246	None

[a] *Anal.* Calcd. for $C_{22}H_{27}N_3O_4$: C, 66.5; H, 6.9; N, 10.6. Found: C, 66.3; H, 6.8; N, 10.8, $[\alpha]^{25}D$ −43.8° (*c* 3, EtOAc). [b] These yields were obtained when 11 was prepared from Z·Gly-Leu·OH(L) and aniline. A slightly lower yield was obtained when 11 was prepared from Z·Gly·OH and H-Leu·Anilide·HCl(L), but the compounds were identical in all respects. [c] *Anal.* Calcd. for $C_{22}H_{27}N_3O_4$: N, 10.6. Found: N, 10.6. Prepared from Z·Gly-Leu·OH(DL) and aniline. [d] M. Bergmann and H. Fraenkel-Conrat, *J. Biol. Chem.*, **119**, 707 (1937), report m.p. 144°. [e] The lower yield was obtained when an excess of chlorophosphite was used. [f] M. Bergmann, L. Zervas and J. P. Greenstein, *Ber.*, **65**, 1692 (1932), give m.p. 159°. [g] J. I. Harris and T. S. Work, *Biochem. J.*, **46**, 196 (1950), report m.p. 168°. [h] The reaction was carried out in dioxane and the product was isolated by dilution of the reaction mixture with water causing crystallization. [i] L. Anschütz, *Ann.*, **439**, 265 (1924), report m.p. 155.5–156.5°. [j] J. W. Hill, THIS JOURNAL, **52**, 4110 (1930), reported m.p. 240–241°. [k] The reaction was conducted in dioxane and the product crystallized when the solution was cooled.

(9) H. G. Cook, *et al.*, *J. Chem. Soc.*, 2921 (1949).

(10) L. Anschütz, *Ber.*, **76**, 222 (1943).

(11) All melting points are corrected and were taken on a Fisher-Johns block. Microanalyses were obtained under the direction of J. A. Kuck of these laboratories.

(12) These esters may be conveniently prepared from the hydrohalides by shaking a suspension with triethylamine in 25 cc. of benzene and filtering after the character of the precipitate has changed.

(13) M. Bergmann, L. Zervas and J. S. Fruton, *J. Biol. Chem.*, **111**, 239 (1935), give m.p. 131°.

(14) Prepared by the procedure of M. Frankel and E. Katchalski, THIS JOURNAL, **64**, 2264 (1942).

TABLE III

Conditions for coupling	Yield, %	M.p., °C.	$[\alpha]^{25}D$ (*c* 5, methanol)
(1) 60° for 5 minutes	84	130–132	−35.1°
(2) 25° for 48 hours	79	132–133	−36.1
(3)[a] 90° for 10 minutes	75	131.5–132.5	−35.5

[a] Triethylamine hydrochloride was not removed.

19. **Ethyl Carbobenzoxyglycyl-L-phenylalanylglycinate.**[15]—Carbobenzoxyglycyl-L-phenylalanine[16] (1.78 g.) was dissolved in 50 cc. of benzene containing 0.6 g. of triethylamine. To this solution 0.88 g. of *o*-phenylene chlorophosphite was added and, after filtration, the solution was refluxed for 15 minutes with 0.5 g. of distilled ethyl glycinate.[14] The solution was cooled, 25 cc. of ethyl acetate was added to facilitate separation, and the organic layer was washed as usual. The residual oil after concentration was dissolved in ethanol to make a 2% solution and was seeded with *rac*-19. After storage at 5° for 2 hours, nothing crystallized and the solution was again concentrated. The residue rapidly solidified upon the addition of anhydrous ether (10 cc.) and 2.03 g. (92.5%) of 19 was obtained; m.p. 115–118°, $[\alpha]^{25}D$ −11.5° (*c* 2, ethanol). Recrystallization from 20 cc. of ethyl acetate–petroleum ether gave 1.85 g. (84%), m.p. 116–118°, $[\alpha]^{25}D$ −12.0° (*c* 2, ethanol). Subsequent recrystallization did not change the rotation. This work-up procedure permits the separation of any racemic tripeptide from the mixture.

Stability of Mixed Anhydrides.—Benzoic acid (2.44 g.) was dissolved in 50 cc. of benzene containing 2.02 g. of triethylamine; the solution then was treated with 3.5 g. of *o*-phenylene chlorophosphite. This solution was allowed to remain for 24 hours at 35°, after which time it was washed with 50 cc. of saturated sodium bicarbonate solution. Acidification of the bicarbonate extract afforded 2.0 g. (83%) of benzoic acid.

Benzoic anhydride (2.26 g.) when dissolved in 50 cc. of benzene and treated with 50 cc. of saturated sodium bicarbonate was quite stable, only 0.05 g. (2%) of benzoic acid being isolated. On the basis of this experiment, not more than 17% of the symmetrical anhydride could have been formed.

The mixed anhydride between phthaloylglycine and *o*-phenylene chlorophosphite, after standing for 2 days at 30°, deposited 20% of the symmetrical anhydride; m.p. 230–233°, reported[4] 240–241°.

STAMFORD, CONNECTICUT

(15) Paper 3 in this series, THIS JOURNAL, **74**, 5309 (1952), reports m.p. 117–118°, $[\alpha]^{25}D$ −12.3° (*c* 2, ethanol).

(16) K. Hofmann and M. Bergmann, *J. Biol. Chem.*, **134**, 225 (1940).

[CONTRIBUTION FROM THE CHEMOTHERAPY DIVISION, STAMFORD RESEARCH LABORATORIES, AMERICAN CYANAMID COMPANY]

Tetraethyl Pyrophosphite as a Reagent for Peptide Syntheses[1]

BY GEORGE W. ANDERSON, JACK BLODINGER AND ALICE D. WELCHER

RECEIVED MARCH 3, 1952

Tetraethyl pyrophosphite has been found to be a useful reagent for making peptide derivatives. It is most conveniently used by adding it to a mixture of the amine (amino acid or peptide ester) and the acid (carbobenzoxy- or phthaloylamino acid or peptide), but may first react with either. No racemization of optically active compounds has been observed when one of the reactants is the acylamino acid. The synthesis of ethyl carbobenzoxyglycyl-L-phenylalanylglycinate from the acyldipeptide and ethyl glycinate was found to give partial racemization under certain conditions.

In the first two papers of this series,[1] it was shown that a diester chlorophosphite such as diethyl chlorophosphite can be used as reagent for forming a peptide link by reaction with either the amino function or the carboxylic function to form a phosphite amide or a phosphite anhydride, and this in turn reacts with the other function. We have now found that tetraethyl pyrophosphite can be used in place of the chlorophosphite with certain advantages. The reactions as written for the pyrophosphite are

(A) $(C_2H_5O)_2POP(OC_2H_5)_2 + NH_2CHCOOR_1 \longrightarrow$

$(C_2H_5O)_2PNHCHCOOR_1 + (C_2H_5O)_2POH$

I II

$R_2CHCOOH + (I) \longrightarrow R_2CHCONHCHCOOR_1 + (II)$

(B) $(C_2H_5O)_2POP(OC_2H_5)_2 + R_2CHCOOH \longrightarrow$

$[R_2CHCOOP(OC_2H_5)_2] + (II)$

III

(1) Third paper in a series on phosphorus derivatives. See THIS JOURNAL, **74**, 5304 (1952) for previous papers; also presented in part at the September, 1951, Meeting of the American Chemical Society.

(III) + $NH_2CHCOOR_1 \longrightarrow$

$R_2CHCONHCHCOOR_1 + (II)$

For convenience, (A) will be called the "amide" procedure and (B) the "anhydride" procedure. It has been found most convenient to add the reagent to a mixture of the acid and the amine, as indicated by Equation (C).

(C) $R_2CHCOOH + NH_2CHCOOR_1 +$

$(C_2H_5O)_2POP(OC_2H_5)_2 \longrightarrow$

$R_2CHCONHCHCOOR_1 + 2\,(II)$

This will be called the "standard" procedure. The validity of the first equations of (A) and (B) when tetraethyl pyrophosphite is used was shown by characterization of both products of a reaction using the (A) procedure and of diethyl phosphite from a (B) reaction. In the latter case, the anhydride was indirectly characterized by reaction with aniline to form a known anilide (see Experimental). When an amine hydrochloride is used, it is usually advantageous to add an equivalent of triethylamine, although this is not necessary.