this factor when calculated for the ellipsoidal models given in Table II differs by less than 0.02% from the $P(90)$ for spheres having the same dissymmetry.

It is difficult to compare our results with some of the earlier studies of human serum lipoproteins. On the basis of ultracentrifugal and diffusion measurements Pedersen[6] obtained the value 2.6×10^6 for what he assumed to be the "hydrated" molecular weight of "X-protein." His estimate assumes that the partial specific volume of the sedimenting unit[22] (which may consist of protein plus associated water and salt) is independent of the salt concentration. This assumption is particularly critical in calculating a molecular weight since the partial specific volume was determined to be 0.97 and an error of only 0.01 in this figure would give an error of about 30% in the molecular weight calculated from the usual Svedberg equation. The light scattering method is, of course, free of this difficulty. In addition more recent ultracentrifugal studies[4,7] have shown that Pedersen's "X-protein" probably consisted of molecules with flotation constants ranging at least from S_f^0 4 to S_f^0 10. Without more information one can only say that there is no necessary disagreement between our results and Pedersen's. Oncley, Scatchard and Brown[24] have obtained an anhydrous molecular weight of 1.3×10^6 for β_1-lipoprotein, a lipoprotein whose ultracentrifugal characteristics are similar to our $S_f^0 \sim 6$ fraction. However, the degree of ultracentrifugal homogeneity of this preparation has not yet been reported, and as the preparation of β_1-lipoprotein involved ethanol fractionation and dialysis (the latter having been shown to effect irreversible changes in serum lipoproteins)[25] a comparison of our results and those of Oncley, Scatchard and Brown appears unwarranted.

Lindgren[7] has calculated minimum molecular weights for some lipoprotein fractions using ultracentrifugal data and assuming the molecules to be spherical. This method of calculation applied to our S_f^0 8.1 and S_f^0 6.4 fractions gave molecular weights which were 10% smaller than those calculated from the light scattering data.

Acknowledgments.—It is a pleasure to thank Professor John W. Gofman for his interest in and encouragement of this work. We also wish to thank Professor Howard K. Schachman and Dr. Verne Schumaker for helpful criticism of the manuscript.

(24) J. L. Oncley, G. Scatchard and A. Brown, *J. Phys. Colloid Chem.*, **51**, 184 (1947).

(25) B. R. Ray, R. O. Davisson and H. L. Crespi, *ibid.*, **58**, 841 (1954).

BERKELEY, CAL.

[CONTRIBUTION FROM THE MEDICINAL CHEMICAL RESEARCH SECTION, RESEARCH DIVISION, AMERICAN CYANAMID COMPANY]

The Use of Phosphorous Acid Chlorides in Peptide Synthesis[1]

BY RICHARD W. YOUNG, KATHRYN H. WOOD, R. JANICE JOYCE AND GEORGE W. ANDERSON

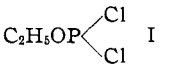
RECEIVED SEPTEMBER 2, 1955

The preparation of peptide derivatives using ethyl dichlorophosphite and ethylene chlorophosphite under varying conditions is reported. The best conditions involve the addition of the chlorophosphite to a suspension or solution of the N-acylamino acid or peptide and amino acid or peptide ester in diethyl phosphite containing triethylamine. The novel use of a trialkyl phosphite as an acid-acceptor in these peptide-forming reactions is described. The methods have been investigated for extent of racemization.

In previous papers of this series the use of diethyl chlorophosphite,[2,3] *o*-phenylene chlorophosphite[2,3] and tetraethyl pyrophosphite[4] for the synthesis of peptides was reported. Since these publications appeared, several reports on the use of these reagents for the synthesis of peptides of asparagine and glutamine,[5] arginine[6] and of oxytocin[7] have been published.

In this paper we wish to report the use of some other chlorophosphites and a study of various conditions under which the reagents may be employed for peptide synthesis.

Continuing the investigation of simple reactive halophosphites, ethyl dichlorophosphite (I)[8] was prepared. This reagent may be prepared in large



I

quantities from ethanol and phosphorus trichloride without using a tertiary base. Both halogens in I are reactive, the compound being both more stable (thermally) and reactive than diethyl chlorophosphite. The reagent may be employed exactly as are the monochlorophosphites; *i.e.*, I may react first with the acylated amino acid to form the "mixed anhydride" II which then may be used to acylate the amino ester (equation 1), or the amino ester may first be converted to the "phosphite amide" III which then may be treated with the

(1) Fourth paper in a series on phosphorus derivatives. Portions of this paper were presented at the September, 1951, Meeting of the American Chemical Society. In order to maintain consistent nomenclature with previous papers in this series, the currently accepted names for phosphorus compounds (*Chem. Eng. News*, **30**, 4515 (1952)) are not being employed.

(2) G. W. Anderson, J. Blodinger, R. W. Young and A. D. Welcher, THIS JOURNAL, **74**, 5304 (1952).

(3) G. W. Anderson and R. W. Young, *ibid.*, **74**, 5307 (1952).

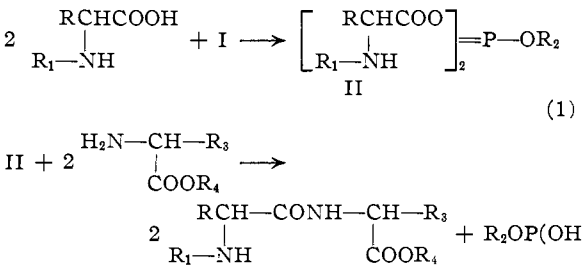
(4) G. W. Anderson, J. Blodinger and A. D. Welcher, *ibid.*, **74**, 5309 (1952).

(5) H. K. Miller and H. Waelsch, *Arch. Biochem. Biophys.*, **35**, 176 (1952); S. J. Leach and H. Lindley, *Aus. J. Chem.*, **7**, 173 (1954); A. Miller, A. Niedle and H. Waelsch, *Arch. Biochem. Biophys.*, **56**, 11 (1955).

(6) G. W. Anderson, THIS JOURNAL, **75**, 6081 (1953).

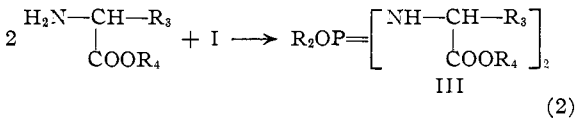
(7) V. duVigneaud, C. Ressler, J. M. Swan, C. W. Roberts and P. G. Katsoyannis, *ibid.*, **76**, 3115 (1954).

(8) N. Menschutkin, *Ann.*, **139**, 343 (1866).

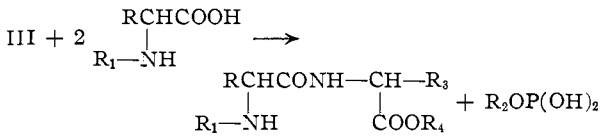


(1)

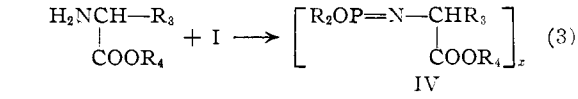
acylated amino acid (equation 2). The latter procedure may be modified by using a 1:1 ratio of I to



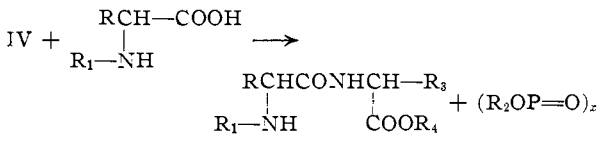
(2)



amino acid ester to form the presumed "phosphite imide" (IV) (equation 3).



(3)



After this work was complete, Goldschmidt and Obermeier[9] reported the use of I and phenyl dichlorophosphite employing similar procedures. In general, the yields reported by these workers exceeded those obtained in our own investigation.

Previous work in peptides has indicated that the solvent problem is one of the major difficulties to be overcome in developing good methods of peptide synthesis. The use of diethyl phosphite, a remarkable solvent, in the pyrophosphite procedure[4] offers promise as a versatile solvent for higher peptides. This was demonstrated dramatically in the synthesis of the protected nonapeptide precursor of oxytocin by duVigneaud and co-workers.[7]

In an effort to adapt this solvent to chlorophosphite reactions, several interesting modifications and useful procedures were developed. At first, efforts were made to avoid the use of organic bases in the procedures and, for this reason, the first reactions were performed in trialkyl phosphites. As has been known for a long time, trialkyl phosphites react readily with hydrogen halides to form alkyl halides and dialkyl phosphites[10] (equation 4).

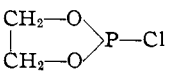
$$(RO)_3P + HX \longrightarrow (RO)_2POH + RX \qquad (4)$$

Taking advantage of this fact, the peptide-forming reactions were performed by the addition of chlorophosphite to a suspension of the N-acyl aminoacid and amino acid ester in a trialkyl phosphite (equation 5), this procedure being the "standard" for pyrophosphite reactions.

(5)

In this manner, the hydrogen chloride evolved in the initial reaction is converted to inert, neutral substances which do not interfere with the isolation procedure.

In addition to this procedure, an equally satisfactory procedure using diethyl phosphite as solvent and triethylamine as base was used. In these reactions ethylene chlorophosphite and ethyl di-



chlorophosphite proved to be the reagents of choice.

Experimental[11,12]

Phosphite Reagents. Ethyl Dichlorophosphite (EDCP).[8]—Absolute alcohol (167 g., 3.63 moles) was added over an hour to 360 cc. (566 g., 3.99 moles, 10% excess) of phosphorus trichloride which was being stirred mechanically and cooled in an ice-water bath. Fumes of hydrogen chloride were evolved during the addition and were conducted to the back of the hood. Within a few minutes after the addition, the mixture cleared of fumes and the dropping funnel and fume outlet were replaced by Drierite drying tubes. The mixture was stirred for 16 hours at room temperature. The product was then distilled *in vacuo* (*ca.* 40–50°) to obtain one large fraction. Redistillation at atmospheric pressure, using a Vigreux column, yielded 42% of theory. The refractive index at 25° ranged from 1.4643 (second fraction, b.p. 115–116°) to 1.4622 (third fraction, b.p. 117–118°).

Ethylene chlorophosphite (ECP) was prepared according to the procedure of Lucas[13] in 70–90% yield. Diethyl phosphite (DEP) and **triethyl phosphite** (TEP) were purchased from the Virginia-Carolina Chemical Corporation and were redistilled.

Trimethyl Phosphite (TMP).—A solution of 125 cc. of methanol and 425 cc. of triethylamine was dissolved in 2 l. of absolute ether and 86 cc. of phosphorus trichloride in 500 cc. of ether was added dropwise with cooling over a period of 1 hour. After this time the amine hydrochloride was filtered off, the cake being washed with 2 × 500 cc. of absolute ether. The ether was removed rapidly and the residue was distilled *in vacuo*, collecting the entire fraction in a Dry Ice–acetone cooled flask. Redistillation at atmospheric pressure through a 10-cm. jacketed Vigreux column gave 57% of trimethyl phosphite, b.p. 110–112°, $n^{25}D$ 1.4055, d^{25} 1.028, lit.[14] b.p. 111–112°, $n^{20}D$ 1.4095.

Triallyl Phosphite (TAP).—In a 5-l. 3-necked flask fitted with a powerful Hershberg stirrer, 87.1 g. (1.5 moles) of freshly distilled allyl alcohol was dissolved in 2.5 l. of anhydrous ether containing 160 g. of triethylamine. The solution was cooled to 0° and a solution of 68 g. of phosphorus trichloride in 450 cc. of anhydrous ether was added dropwise over a period of 1 hour. Stirring for an additional hour was followed by filtration of the precipitated triethylamine hydrochloride (aided by Hyflo). The ether was removed by distillation at atmospheric pressure and the residue was rapidly distilled at 0.75 mm. This distillate was carefully fractionated at 0.25 mm. to give 67.3 g. (67%) of

(9) S. Goldschmidt and F. Obermeier, *Ann.*, **588**, 24 (1954).

(10) G. M. Kosolapoff, "Organophosphorus Compounds," John Wiley and Sons, Inc., New York, N. Y., 1950, p. 188.

(11) All melting points are corrected and were taken on a Fisher-Johns melting point block. We are indebted to Dr J. A. Kuck and the staff of the Microanalytical Laboratory for the analyses.

(12) The abbreviations used in the tables are those of R. Brand (B. Erlanger and E. Brand, THIS JOURNAL, **73**, 3508 (1951)).

(13) H. J. Lucas, F. W. Mitchell and C. N. Scully, *ibid.*, **72**, 5491 (1950).

(14) Reference 10, p. 203.

TABLE I

THE PREPARATION OF PEPTIDES USING ETHYL DICHLOROPHOSPHITE

No.	Product	Yield[a] (%) by procedure Anhydride	Amide	Imide	M.p., °C. (cor.)	$[\alpha]^{25}D$	Recrystallization solvent
1	Z·Gly-Phe·OEt (DL)	91[b]	58	..	88–89	.....	50% EtOH
2	Z·Gly-Tyr·OEt (L)	44[c]	31[c]	..	125–127	+19.2° (*c* 3, EtOH)	50% EtOH
3	Z·Leu-Gly·OMe (L)	40[d]	21[d]	..	92–94	−26.9° (*c* 5, MeOH)	50% MeOH
4	Z·Leu-Gly·OEt (L)	42[e]	..	..	99–102	−27.2° (*c* 5, EtOH)	EtOAc–pet. ether
5	Z·Val-Gly·OEt (DL)[f]	86	36	33	148.5–149	.....	EtOAc
6	Z·Phe-Gly·OEt (L)	65[g]	..	..	110–111	−16.9° (*c* 5, EtOH)	EtOAc–pet. ether
7	Pht·Gly-Gly·OEt	62[h]	..	39[i]	192–193	.....	$CHCl_3$
8	Pht·Gly-Leu·OEt (L)[j]	91	72	76	143–144	−29.0° (*c* 2, EtOH)	50% EtOH
9	Z·Gly-Leu-Leu·OMe (LL)	60[k]	..	..	133–134	−47.4° (*c* 2, MeOH)	EtOAc–pet. ether
10	Z·Gly-Phe-Gly·OEt (DL)	75[l]	..	46[i]	132–133	.....	50% EtOH
11	Z·Gly-Phe-Gly·OEt (L)[m]	71	45	..	117–118	−12.0° (*c* 2, EtOH)	EtOAc–pet. ether
12	Z·Gly-Leu-Gly·OMe (L)[n]	64	51	41[o]	132–133	−35.5° (*c* 5, MeOH)	EtOAc–pet. ether

[a] The yields are those obtained after crystallization to the specified melting point. [b] Distilled H·Phe·OEt (DL) was used. H. Neurath, E. Elkins and S. Kaufman, *J. Biol. Chem.*, **170**, 221 (1947), give m.p. 90–91°. [c] H·Tyr·OEt was used. 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). [d] Distilled H·Gly·OMe was used. *Anal.* Calcd. for $C_{17}H_{24}N_2O_5$: C, 60.7; H, 7.2; N, 8.3. Found: C, 60.6; H, 7.3; N, 8.4. [e] Distilled H·Gly·OEt was used. Footnote *c* gives m.p. 104–105°, $[\alpha]^{24}D$ −25.6° (*c* 5, EtOH). [f] Distilled H·Gly·OEt was used. Reference 3 gives m.p. 148.5–149.5°. [g] Distilled H·Gly·OMe was used. J. R. Vaughan, THIS JOURNAL, **74**, 6137 (1952), gives m.p. 109–110°, $[\alpha]^{25}D$ −17.3° (*c* 2, EtOH). [h] R. A. Boissonnas, *Helv. Chim. Acta*, **34**, 874 (1951), gives m.p. 194–195°. [i] Distilled H·Gly·OEt was used. [j] Footnote *c* gives m.p. 143–145°, $[\alpha]^{24}D$ −28.5° (*c* 2, EtOH). [k] Prepared from Z·Gly-Leu·OH (L) and distilled H·Leu·OMe (L). J. S. Fruton, *et al.*, *J. Biol. Chem.*, **188**, 251 (1951), report m.p. 131–132°. [l] Prepared from Z·Gly-Phe·OH (DL) and H·Gly·OEt·HCl. Footnote *c* gives m.p. 131–132° (cor.). [m] This compound was prepared from Z·Gly-Phe·OH (L) and H·Gly·OEt·HCl and was isolated according to the procedure described in reference 3. No DL-peptide was isolated. [n] Reference 3 reports m.p. 132–133°, $[\alpha]^{24}D$ −34.9° (*c* 5, EtOH). [o] Slightly inferior material, m.p. 128–130°, $[\alpha]^{25}D$ −33.7°, was obtained in this procedure.

triallyl phosphite, b.p. 52–54°, $n^{25}D$ 1.4556, $d^{25}D$ 0.997, M_R calcd., 55.38; found, 55.02.

Anal. Calcd. for $C_9H_{15}O_3P$: C, 53.5; H, 7.5; P, 15.3. Found: C, 52.5; H, 7.6; P, 15.9.

Procedures for Table I. Anhydride Procedure.—An N-acyl amino or peptide acid (0.01 mole) is dissolved in 30 cc. of benzene containing triethylamine (0.01 mole) and to the solution there is added ethyl dichlorophosphite (0.005 mole) in 10 cc. of benzene. Following filtration of the amine hydrochloride, which is obtained in quantitative yield, the amino acid ester is added and the solution is held at reflux for 15 minutes. When an amino acid ester hydrochloride is used, it is added to the anhydride solution as a suspension in 25 cc. of solvent to which sufficient triethylamine has been added to convert to the free base. When this procedure is followed, the reaction mixture is best allowed to remain at room temperature for several hours or overnight. Following completion of the reaction, the mixture is washed with 10 cc. of water and 15 cc. of saturated sodium bicarbonate. The benzene solution is dried over sodium sulfate and concentrated *in vacuo* or in a stream of air. This procedure often gives a solid which may be purified by crystallization.

Amide Procedure.—The amino acid ester (0.01 mole) is dissolved in 50 cc. of benzene containing triethylamine (0.01 mole). To the solution (or suspension, if an amino acid ester hydrochloride is used) there is added, with shaking, ethyl dichlorophosphite (0.005 mole) in 10 cc. of solvent. After removing triethylamine hydrochloride, the resultant solution is heated at reflux for 30 minutes with the N-acyl amino or peptide acid (0.01 mole). After cooling, the product is isolated as in the anhydride procedure.

Procedure for Table II.—Z·Gly·OH (2.09 g., 0.010 mole) and H·Phe·OEt·HCl (DL) (2.30 g., 0.010 mole) were suspended in the trialkyl phosphite and 0.7 cc. (0.011 equiv.) of ethyl dichlorophosphite was added. The mixture was heated on a steam-bath; vigorous bubbling occurred during the first 5 minutes of reaction. At the end of the heating period, 40 cc. of water was added to precipitate the peptide (the solution is acidic at this point) and the product was washed on the funnel with 20 cc. of 3% sodium bicarbonate solution followed by 2 × 5 cc. of water. The solid was dried and the m.p. and yield recorded. While it is recognized that these yields are not those of the pure compound, they are probably more significant on a relative basis than yields obtained if the compound had been crystallized to a constant melting point. In view of the fact that these experiments were designed to discover the best combination of conditions, the error introduced by the small amounts of impurities probably is not significant.

TABLE II

THE PREPARATION OF Z·Gly·Phe·OEt (DL) USING ETHYL DICHLOROPHOSPHITE IN TRIALKYL PHOSPHITES

Expt. no.	Solvent	Time of heating, min.	Yield, %	M.p.[f] (uncor.), °C.
1	5 cc. TEP	15	69	86–93
2	5 cc. TEP	30	85[b]	85–91
3	5 cc. TEP[a]	30	64	85–90
4	5 cc. TEP[b]	15	82	86–90
5	5 cc. TEP	41 hr. at 25°	17	90–92
6	10 cc. TEP	15	72	83–90
7	10 cc. TEP	30	87	86–91
8	10 cc. TEP	45	88	84–90
9	10 cc. TEP	60	89	85–90
10	10 cc. TEP[a]	15	64	83–89
11	10 cc. TEP[a]	30	75	82–91
12	5 cc. TMP	15	96	88–91
13	5 cc. TMP	30	95	89–91
14	5 cc. TAP	15	92	86–89
15	5 cc. TAP[c]	15	94	89–92
16	5 cc. TAP[d]	20	93	87–89
17	5 cc. TAP + 5 cc. DEP	5	75	82–90
18	5 cc. TAP + 5 cc. DEP	15	89	88–91
19	10 cc. TEP + 5 cc. DEP[b]	15	87	89–91
20	10 cc. TEP[e]	30	22	85–90

[a] In these experiments an excess (0.015 equiv.) of ethyl dichlorophosphite was used. [b] Z·Gly·OH was added after 30 minutes of heating the chlorophosphite with H·Phe·OEt·HCl (DL) in TEP. [c] Ethyl dichlorophosphite was added in three portions at one-minute intervals. [d] Z·Gly·OH was added after heating H·Phe·OEt·HCl (DL) in TAP for 9 minutes with ethyl dichlorophosphite. [e] Control reaction, no chlorophosphite was used. [f] Footnote *b*, Table I, report m.p. 90–91° for the pure peptide.

Procedure for Table III.—A mixture of 0.01 mole of N-acylamino acid and of amino acid ester hydrochloride was suspended in 5 cc. of trimethyl phosphite, and to this sus-

TABLE III

THE PREPARATION OF PEPTIDES USING ETHYL DICHLOROPHOSPHITE IN TRIALKYL PHOSPHITES

No.	Product	Solvent	Crude yield, %	M.p., °C.	Pure yield, %	M.p. (cor.), °C.	Optical rotation $[\alpha]^{25}D$
1	Z·Phe-Gly·OEt (DL)[a]	5 cc. TMP	89	95–99	75	97.5–99	.....
2	Pht·Phe-Gly·OEt (L)[b]	5 cc. TMP	92	154–157	79	159–160	−141° (*c* 2, EtOH)
3	Z·Gly-Gly·OEt[c]	50 cc. TMP	76	...	36	79–81	.....
4	Z·Phe-Glu·$(OEt)_2$ (LL)[d]	5 cc. TMP	73	110	25	126–127	−11.4° (*c* 2, EtOH)
5	Z·Gly-Leu-Gly·OMe (DL)[e]	5 cc. TMP	67	88–103	..	...	.....
6	Pht·Gly-Phe·OEt (DL)[f]	5 cc. TMP	98	148–152	..	...	.....
7	Pht·Gly-Gly·OEt[g]	5 cc. TMP	98	195.5–197	..	...	.....
8	Z·Phe-Gly·OEt (L)[h]	10 cc. TMP	83	103–111	73	110–113	−16.6° (*c* 2, EtOH)
9	Pht·Gly-Leu·OEt (L)[i]	5 cc. TMP	94 (89)[j]	143–145	..	...	−29.9° (*c* 3, EtOH)
10	Z·Gly-Tyr·OEt (L)[k]	10 cc. TMP	91	122–126.5	85	125.5–126.5	+18.7° (*c* 3, EtOH)

[a] *Anal.* Calcd. for $C_{21}H_{24}N_2O_5$: C, 65.6; H, 6.29; N, 7.3. Found: C, 65.8; H, 6.43; N, 7.4. [b] J. C. Sheehan, *et al.*, [THIS JOURNAL, **74**, 3822 (1952)] give m.p. 160.6–161.4°, $[\alpha]^{26}D$ 146° (*c* 1, EtOH) for the peptide prepared from Pht·Phe·Cl (L). [c] This reaction was carried out on 0.10 mole scale. O. Süs, *Ann.*, **572**, 105 (1951), reports m.p. 82.5–83°. [d] J. Fruton and M. Bergmann [*J. Biol. Chem.*, **127**, 627 (1939)] report m.p. 115° (crude) prepared *via* the azide procedure; no rotation was given. [e] Prepared on 0.005 mole scale from Z·Gly-Leu·OH(DL) and H·Gly·OMe·HCl. Reference 2 gives m.p. 107–108°. [f] J. R. Vaughan and R. L. Osato [THIS JOURNAL, **73**, 5553 (1951)] give m.p. 149–150°. [g] Footnote *h*, Table I, gives m.p. 194–195°. The same yield was obtained using either H·Gly·OEt·HCl or H·Gly·OEt·HBr. A control reaction using no chlorophosphite and heating for 53 minutes gave a 38% yield. [h] Footnote *g*, Table I, gives m.p. 109–110°, $[\alpha]^{25}D$ −17.3° (*c* 2, EtOH). [i] Footnote *c*, Table I, gives m.p. 143–145°, $[\alpha]^{24}D$ −28.5° (*c* 2, EtOH). [j] TEP (5 cc.) was used. [k] Footnote *g*, Table I, gives m.p. 124–125°, $[\alpha]^{24}D$ +19.8° (*c* 5, EtOH). In this reaction two equivalents of ethyl dichlorophosphite were used to protect the phenolic hydroxyl. Using one equivalent, a 60% yield was obtained.

pension there was added 0.0055 mole of ethyl dichlorophosphite. The resulting suspension was heated on the steam-bath for 15 minutes, producing a clear solution. The solution was diluted with 40 cc. of water and chilled causing crystallization. The peptide was filtered off and washed successively with 5 cc. of water, 20 cc. of half-saturated sodium bicarbonate and 2 × 5 cc. of water. After drying to constant weight the peptide derivative was crystallized.

Procedure for Table IV.—The peptide was prepared on 0.01 mole scale from Z·Gly-Phe·OH (L) and H·Gly·OEt·HCl using the procedure for Table III except as noted. The peptide mixture was isolated according to the procedure described in reference 2. It is noteworthy that recovered Z·Gly-Phe·OH was always partially racemized.

TABLE IV

THE PREPARATION OF Z·Gly-Phe-Gly·OEt (L) USING ETHYL DICHLOROPHOSPHITE IN TRIALKYL PHOSPHITES

Solvent	5 cc. TMP	10 cc. TAP	10 cc. TMC[c]
Conditions	15 min. on steam-bath	2 min. at 100°[b]	15 min. on steam-bath
Total yield, %	*a*	96	85
DL yield, %	20	17	None
M.p., °C.	128–130	132–133	
L yield, %	31	32	79[d]
M.p., °C.	113–115	117–118	119–121
$[\alpha]^{25}D$ (*c* 2, EtOH)	−11.7°	−11.6°	−12.3°

[a] After dilution of the reaction mixture with water only an oil was obtained. This was taken up in the ethyl acetate, and the solution dried and concentrated. The crude product at this point was dissolved in 100 cc. of alcohol. [b] The suspension was warmed to 100° before ethyl dichlorophosphite was added. [c] Prepared from Z·Gly·OH and H·Phe-Gly·OEt·HBr (L). [d] Yield after one recrystallization from ethyl acetate–petroleum ether.

Reactions in Diethyl Phosphite. Procedure (Tables V–VIII).—A suspension of 0.010 mole of N-acylamino or peptide acid and 0.010 mole of amino acid or peptide ester hydrochloride in diethyl phosphite (usually 7 cc./0.010 mole) containing triethylamine (0.022 mole) was treated with 0.011 equivalent of chlorophosphite. The suspension was heated on the steam-bath, preferably with stirring, for 15 to 30 minutes. The product was worked up exactly as described for the trialkyl phosphite reactions.

Tables V and VI illustrate the preparation of two model peptides using this procedure and some simple modifications. Table VII illustrates the preparation of various peptides using the standard procedure described. The racemization study is reported in Table VIII using the standard procedure and some modifications thereof.

TABLE V

THE PREPARATION OF Z·Gly-Phe·OEt (DL) USING CHLOROPHOSPHITES IN DIETHYL PHOSPHITE CONTAINING TRIETHYLAMINE

Solvent	Chlorophosphite	Yield, %	M.p., °C.[d]
7 cc. DEP[a]	ECP	95	87–88
7 cc. DEP	ECP	88	87–89.5
7 cc. DEP	ECP[b]	89	87–88
7 cc. DEP	ECP[c]	91	...
7 cc. DEP + 7 cc. TMP	EDCP	93	86–91
21 cc. DEP + 7 cc. TMP	EDCP	75	86–91

[a] Distilled H·Phe·OEt (DL) was used. [b] The amide procedure was used. [c] The anhydride procedure was used. [d] Footnote *b*, Table I, gives m.p. 89–90°.

TABLE VI

THE PREPARATION OF Z·Gly-Tyr·OEt (L) USING CHLOROPHOSPHITES WITH TRIETHYLAMINE IN DIETHYL PHOSPHITE

Solvent	Chlorophosphite	Yield, %	M.p., °C.[f]
7 cc. DEP	ECP	66–73	123–125
10 cc. DEP containing 0.022 mole of Na	ECP	43[a]	119–125
7 cc. DEP + 2.5 cc. TMP	ECP	74	123–124
15 cc. DEP[e]	ECP	79	122–127
7 cc. DEP[b]	ECP	91[c]	119–121
14 cc. DEP	ECP	87	112–115
21 cc. DEP + 7 cc. TMP	EDCP	31	124–127
21 cc. DEP + 7 cc. TMP[d]	EDCP	78	123.5–126

[a] No triethylamine was used in this reaction. After two recrystallizations the yield was 37%. [b] A 100% excess of ethylene chlorophosphite and triethylamine was used. [c] After recrystallization from 50% ethanol the yield was 70%, m.p. 123–125°. [d] Two equivalents of chlorophosphite and triethylamine were used. [e] The anhydride procedure was used. [f] All samples had $[\alpha]^{25}D$ +19.0 ± 0.5° (*c* 3, EtOH). Footnote *c*, Table I, reports m.p. 124–125°, $[\alpha]^{25}D$ +19.8° (*c* 5, EtOH).

TABLE VII

THE PREPARATION OF PEPTIDES USING CHLOROPHOSPHITES IN DIETHYL PHOSPHITE CONTAINING TRIETHYLAMINE

No.	Product	Chloro-phos-phite	Crude yield, %	M.p., °C.	Pure yield, %	M.p., °C.	Recrystallization solvent	$[\alpha]^{23-25}D$
1	Z·Gly-Gly-Phe·OEt (DL)[a]	EDCP	96	75–80	78	78–80	50% EtOH	.....
2	Z·Gly-Phe-Gly·OEt (DL)[b]	EDCP	78	122–128	55	129–131.5	EtOAc	.....
3	Z·Tyr-Gly-Gly·OEt (L)[c]	ECP	71	143–155	44	162–164	50% EtOH	Not measured
4	Z·Leu-Tyr·OEt (LL)[d]	ECP	68	102–105	62	112–115	50% EtOH	−16.4° (*c* 5, EtOH)
5	Z·Gly-Leu-Gly·OMe (L)[e]	ECP	74	114–119	39	131.5–132	30% EtOH	−34.9° (*c* 5, EtOH)
6	Z·Gly-Leu-Gly·OEt (L)[f]	ECP	88	100–105	54	108–108.5	30% EtOH	−28.6° (*c* 3, EtOAc)
7	Z·Leu-Gly·OEt (L)[g]	ECP	85	98–99.5	74	102–103	50% EtOH	−26.7° (*c* 5, EtOH)
8	Pht·Gly-Leu·OEt (L)[h]	EDCP	69	145–146	..	...	50% EtOH	−28.7° (*c* 3, EtOH)
9	Z·Arg-Leu·OMe·HCl (LL)[i]	ECP	64	86–90	40	92–94	10% MeOH[j]	−21.6° (*c* 2, MeOH)
10	Z·Phe-Gly·OEt (L)[k]	EDCP	76	101–106	62	109–110.5	EtOAc	−15.9° (*c* 2, EtOH)
11	Z·Phe-Gly·OBz (L)[l]	EDCP	72	120–135	45	137–139	EtOAc	− 8.4° (*c* 2, HOAc)

[a] From Z·Gly·OH and H·Gly-Phe·OEt·HBr (DL). J. R. Vaughan [THIS JOURNAL, **73**, 1389 (1951)] gives m.p. 80–82° for the monohydrate. [b] From Z·Gly-Phe·OH (DL) and H·Gly·OEt·HCl. In this experiment, 7 cc. of TMP was also used. Similar yields were obtained both by the amide and the anhydride procedure and by the anhydride procedure allowing the reaction to stir for 24 hours at room temperature. Footnote *c*, Table I, gives a m.p. 131–132°. [c] Prepared from Z·Tyr·OH (L) and H·Gly-Gly·OEt (HCl). Using a 100% excess of ECP gave a poorer product. The yield using EDCP was 22% after several recrystallizations. M. Bergmann and J. S. Fruton [*J. Biol. Chem.*, **118**, 405 (1937)] give m.p. 165°. [d] Footnote *c*, Table I, gives m.p. 116–118°, $[\alpha]^{24}D$ −14.9° (*c* 10, EtOH). [e] From Z·Gly-Leu·OH (L) and H·Gly·OMe·HCl. Two recrystallizations were required to obtain this m.p. and rotation. Reference 3 reports m.p. 132–133°, $[\alpha]^{24}D$ −34.9° (*c* 5, EtOH). [f] From Z·Gly-Leu·OH (L) and H·Gly-OEt·HCl. Three crystallizations were required to obtain the final pure product. [g] M. A. Nyman and R. M. Herbst [*J. Org. Chem.*, **15**, 108 (1950)] report m.p. 99°, $[\alpha]^{25}D$ −26.8° (*c* 2.6, EtOH). [h] The same yield was obtained when 7 cc. of TMP was used in addition to DEP. Footnote *c*, Table I, gives m.p. 143–145°, $[\alpha]^{25}D$ −28.5° (*c* 2, EtOH). [i] Obtained as the monohydrate. *Anal.* Calcd.: C, 51.5; H, 7.4; N, 14.3. Found: C, 51.0; H, 7.4; N, 13.8. [j] Recrystallized by dissolving in minimum quantity of methanol and diluting with water containing hydrochloric acid. [k] Footnote *g*, Table I, gives m.p. 109–110°, $[\alpha]^{25}D$ −17.3° (*c* 2, EtOH). [l] Prepared from Z·Phe·OH (L) and H·Gly·OBz·$C_6H_5SO_3H$ [H. K. Miller and H. Waelsch, THIS JOURNAL, **74**, 1092 (1952)]. J. R. Vaughan and J. A. Eichler [*ibid.*, **75**, 5556 (1953)] report m.p. 135.5–137.5°, $[\alpha]^{24}D$ −9.2° (*c* 2, HOAc).

TABLE VIII

THE PREPARATION OF Z·Gly-Phe-Gly·OEt (L) USING CHLOROPHOSPHITES IN DIETHYL PHOSPHITE AND TRIETHYLAMINE

In a control reaction, Z·Gly-Phe·OH (L), $[\alpha]^{25}D$ +39.0° (*c* 5, EtOH), was heated in diethyl phosphite with triethylamine hydrochloride and was recovered essentially unchanged, $[\alpha]^{25}D$ +37.8° (*c* 5, EtOH).

No.	Solvent	Chloro-phos-phite	Crude yield, %	%DL and m.p., °C.	%L and m.p., °C.[i]
1	7 cc. DEP	ECP	91	5, 131–132	74, 117–118.5
2	7 cc. DEP[a]	ECP	82	22, 131–132	42, 115–117
3	14 cc. DEP	ECP	89	2, 130–131	72, 116–117.5
4	7 cc. DEP	ECP[h]	94	8, 131.5–132	74, 116–118
5	7 cc. DEP[b]	ECP	83	13, 131–132	53, 115–116
6	7 cc. DEP[c]	ECP	85	14, 131–132	52, 114–116
7	7 cc. DEP + 7.4 cc. TMP	ECP	94	4, 132–133.5	85, 119–121
8	7 cc. DEP	ECP[d]	95	8, 131–132	80, 115–118
9	7 cc. DEP	ECP[i]	93	18, 131.5–132	56, 115–117
10	7 cc. DEP	ECP[e]	74	None	70, 116–118
11	21 cc. DEP	EDCP	85	21, 131.5–133	40, 116–118[f]
12	21 cc. DEP	EDCP[g]	71	None	64, 118.5–120

[a] A 100% excess of ethylene chlorophosphite was used without stirring. [b] Amide procedure. [c] Anhydride procedure. [d] Ethylene chlorophosphite was dissolved in 2.72 g. of trimethyl phosphite, the resulting solution being added to the other ingredients in one portion. [e] Prepared from Z·Gly·OH and H·Phe-Gly·OEt·HBr (L). After one recrystallization from 50% ethyl acetate–petroleum ether the compound had m.p. 118–119°, $[\alpha]^{24}D$ −11.9° (*c* 3, EtOH). [f] Recrystallization from ethyl acetate–petroleum ether gave a 32% yield, m.p. 118–119.5°, $[\alpha]^{24}D$ −11.4° (*c* 3), EtOH. [g] Prepared from Z·Gly·OH and H·Phe-Gly·OEt·HBr (L) using the standard procedure. [h] The chlorophosphite was added at 0°. [i] The chlorophosphite was added at 90°. [j] The rotations were all $[\alpha]^{24}D$ −12 ± 1° (*c* 3, EtOH).

Carbobenzoxyglycyl-L-leucine.—This compound was prepared by saponification of Z·Gly-Leu·OMe (L) and Z·Gly-Leu·OEt (L) using 1.1 equivalents of 1 *N* NaOH in ethanol. The oily esters were prepared by the anhydride procedure in benzene using the ester hydrochlorides and triethylamine. Over-all yields of 60–70% were obtained.

It is noteworthy that when prepared by saponification of the methyl ester, Z·Gly-Leu·OH (L) was obtained, m.p. 100–101°, $[\alpha]^{25}D$ −9.5° (*c* 5, EtOH). When prepared from the oily ethyl ester an isomorph, m.p. 143–144°, $[\alpha]^{23}D$ −9.2° (*c* 2, EtOH), was obtained. Both materials gave the same neutral equivalent and the higher melting isomer was converted to the lower melting material by crystallization from ethyl acetate–petroleum ether using a seed of the 100° isomer.

This observation resolves the m.p. discrepancy reported for the compound in the literature. Stahmann, Fruton and Bergmann[15] report m.p. 141–142°, $[\alpha]^{22}D$ −10.3° (*c* 5, EtOH) for Z·Gly-Leu·OH (L) prepared by saponification of the oily ethyl ester. Smith and Brown[16] prepared the enantiomer Z·Gly-Leu·OH (D) by saponification of the oily D-methyl ester and found m.p. 101–102°.

Discussion

The results reported in Table I summarize the use of ethyl dichlorophosphite in an inert solvent.

(15) M. A. Stahmann, J. S. Fruton and M. Bergmann, *J. Biol. Chem.*, **164**, 753 (1946).

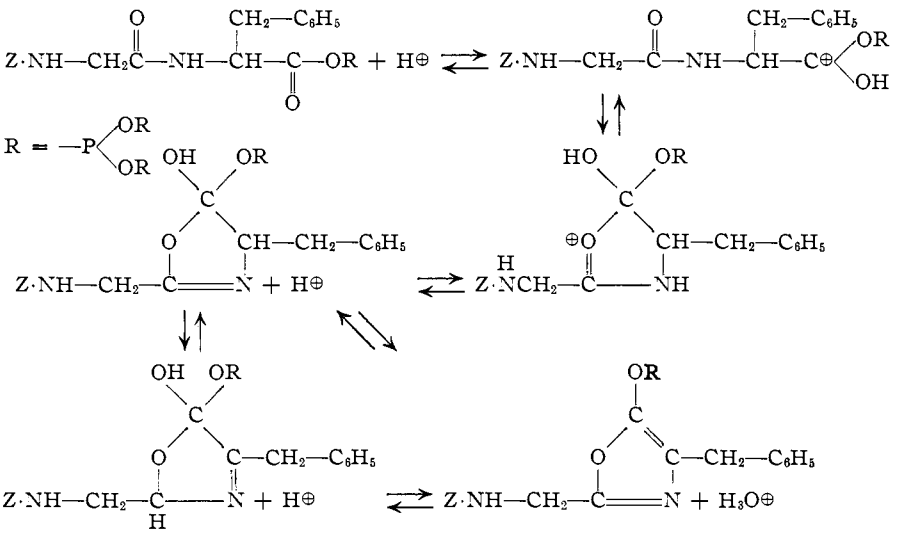
(16) C. S. Smith and A. E. Brown, THIS JOURNAL, **63**, 2605 (1941).

As has been observed before, using diethyl chlorophosphite[2,3] the yields are superior by the anhydride procedure. No apparent racemization was detected in the preparation of Z·Gly-Phe-Gly·OEt (L) from Z·Gly-Phe·OH (L) or in the preparation of Z·Gly-Leu-Gly·OMe (L) from Z·Gly-Leu·OH (L). The serious drawback to this procedure is the inadequacy of the solvents toluene and benzene when higher, less soluble peptide derivatives are used.

In search of the best combination of conditions and reagents, a large series of reactions was run in various trialkyl phosphites. Some of these are summarized in Table II. The yields using triallyl and trimethyl phosphites were superior to those obtained with triethyl phosphite, and the best procedure (no. 12) was used for the preparation of several peptides in Table III. In general, the yields are excellent and the technique is simple and easy to use. The facile hydrolysis of trialkyl phosphites to water-soluble substances make isolation by simple dilution with water very attractive. The method is not without disadvantages, however, and as illustrated in Table IV, racemization was observed in the preparation of Z·Gly-Phe-Gly·OEt (L) from Z·Gly-Phe·OH (L). However, this peptide was prepared in excellent yield using these conditions when Z·Gly·OH and H·Phe-Gly·OEt·HBr (L) were used, indicating that the peptide itself is stable under the reaction conditions. The best general procedure for preparing peptides using chlorophosphite reagents involves the use of diethyl phosphite as a solvent. In Tables V and VI is reported the preparation of two model peptides using this solvent. As was found with tetraethyl pyrophosphite,[4] any of the three procedures, amide, anhydride or standard, may be used. The standard procedure is the simplest and most satisfactory. Only small quantities of solvent are used and the reaction proceeds rapidly. The presence of triethylamine hydrochloride does not affect the yields.

In most of the reactions in diethyl phosphite, ethylene chlorophosphite was used. This reagent is very stable and is prepared from ethylene glycol in excellent yield. In its reactions it behaves like diethyl and *o*-phenylene chlorophosphite and gives generally good yields. The by-products from its hydrolysis are water soluble and easily removed. Like the other monohalophosphites, ECP is less reactive than the dichlorophosphites and is thus slightly easier to handle. In general, the yields using ECP and EDCP are equivalent. In Table VII the preparation of several peptides using these reagents is reported. Of particular interest in Table VII are the preparations of compound 9, Z·Arg-Leu·OMe·HCl (LL), and compound 11, Z·Phe-Gly·OBz (L). The latter compound was prepared using the benzenesulfonic acid salt of the amino ester directly in the reaction mixture.

An extensive study of conditions for the preparation of Z·Gly-Phe-Gly·OEt (L) was made and some racemization was observed when the dipeptide acid was used, although under favorable conditions this could be reduced to less than 10%. This phenomenon of racemization when using the dipeptide acid has been observed using phosphite reagents only when reactions were performed in diethyl- or trimethylphosphite in the presence of hydrogen chloride, either free or bound as the hydrochloride. No racemization is observed in using dipeptide acids in the absence of hydrogen chloride, as in pyrophosphite reactions or with chlorophosphites when triethylamine in inert solvents like benzene and toluene is used. In these solvents, the acid is effectively removed from the solution. It is possible that racemization occurs *via* the formation of an oxazolidine salt which destroys the configuration about the asymmetric carbon atoms



The N-acyl dipeptide acid is racemized only when the phosphite reagents are used. In a control reaction, omitting chlorophosphite, the acid was recovered unchanged. Thus the mixed anhydride must be involved. This concept is supported to some extent by the observation of O'Brien and Niemann[17] that certain α-acylamino acids may be converted to oxazolonium ions in acetic anhydride.

STAMFORD, CONNECTICUT

(17) J. L. O'Brien and C. Niemann, THIS JOURNAL, **72**, 5348 (1950).