SYNTHESIS AND PROPERTIES OF N-, O-, AND S-PHOSPHO DERIVATIVES OF AMINO ACIDS, PEPTIDES, AND PROTEINS

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I. INTRODUCTION

In recent years it has become evident that phosphoproteins play a central role in enzyme regulation in biological systems.[1-3] Efforts (including our own) to phosphorylate proteins by chemical methods are increasing in number and frequency. The most recent reviews on phosphoproteins, however, cover only enzymic methods.[4,5] Nonenzymic phosphorylation of amino acids was last reviewed by Chabrier and Carayon-Gentil[6] in 1962, and of peptides by Mellander[7] in 1963 and Österberg[8] in 1966. The present review covers the literature on chemical (i.e., nonenzymic) phosphorylation of amino acids, peptides, and proteins from the earliest papers through 1982. To keep the length within bounds, only 20 or so α-amino acids that are commonly found in proteins are covered. Other interesting compounds, such as *N*-phospho-β-alanine or *O*-phosphoethanolamine, had to be excluded. The same criteria apply to the peptides. A further restriction, which applies specifically to naturally occurring phosphoamino acids (those of cysteine, serine, threonine, histidine, lysine, arginine, and tyrosine), is that only data for the synthetic compounds are reported. Within these criteria, any omissions are unintentional.

The literature on chemical phosphorylation dates to 1901, when Bechhold treated egg albumin with phosphorus oxychloride (method 1e) and obtained a product regarded to be a true ester of phosphoric acid.[9] Amino acids were first phosphorylated in 1914[10] (see also References 11 and 12a), and peptides in 1945.[13] Extensive investigations were carried out by Fölsch[14-24] on the N- and O-protected phosphoamino acids and peptides and by Avaeva and colleagues[22,25-39] and Katchalsky et al.[40-47] on the anhydrides of these substances, but the literature is noted more for its diversity than for its dominance by any single investigator or group of investigators.

Several methods are now available for the synthesis of the phosphoamino acids and peptides, and increasingly sophisticated methods have been developed for their separation and characterization. These trends are expected to continue. Most of the phosphoproteins described in this review were prepared prior to 1970, and are inadequately characterized; many will undoubtedly be reexamined with the aid of new techniques such as ^{31}P NMR, HPLC, and amino acid sequencing. We anticipate that this field will continue to grow as both chemical and enzymic methods of phosphorylation are developed for modifying the properties of amino acids, peptides, and proteins.

II. SYNTHETIC METHODS

The methods described in this section are classified by reagent rather the product. In some cases, it will be noted that the same reagent can be used to phosphorylate NH groups, OH (or SH) groups, or both depending on the substrate and the reaction conditions. In general,

alkaline conditions favor the N- and S-phospho derivatives and acid conditions the O-phospho derivatives.

A. From Phosphorus Oxychloride

Phosphorus oxychloride reacts with amines in aqueous solution in the presence of excess magnesium oxide to give phosphoramidic acids in the form of magnesium salts (method 1a):

$$2\ RNH_2 + 2\ POCl_3 + 5\ MgO \rightarrow 2\ RNHP(O)O_2Mg + 3\ MgCl_2 + H_2O \qquad (1a)$$

Method 1a[10]

A mixture of DL-alanine (1.8 g), magnesium oxide (10 g), and water (150 g) is stirred for 1 hr at room temperature, cooled in an ice bath, and treated dropwise with a solution of phosphorus oxychloride (6.1 g) in carbon tetrachloride (50 mℓ) over a 4-hr period with stirring. After stirring another hour, the mixture is filtered, neutralized carefully with dilute acetic acid, treated with 3 volumes ethanol, allowed to stand several hours and then filtered. The product is rinsed with 80% ethanol until chloride-free and reprecipitated from water with ethanol, giving 0.4 g (12%) of the magnesium salt of *N*-phospho-DL-alanine.

This method has been applied to the amino acids glycine,[10,48] alanine,[10,48] glutamic acid,[48-51] leucine,[48] phenylalanine,[48] and tyrosine,[10] and to the peptides diglycine[48] and triglycine.[52] Tyrosine is phosphorylated at both NH_2 and OH. The yield is about 50% for glycine but much lower for the other amino acids.

Somewhat better results are obtained by adding phosphorus oxychloride drop by drop to an ice-cold solution of the amine at pH 11 or above (method 1b):

$$RNH_2 + POCl_3 + 5\ NaOH \rightarrow RNHP(O)(ONa)_2 + 3\ NaCl + 3\ H_2O \qquad (1b)$$

This method has been applied to the amino acids alanine,[53] cysteine,[54] histidine,[53,55-57] lysine,[58-60] and arginine.[60-63] Cysteine is phosphorylate at both NH_2 and SH, histidine at NH_2 and NH, and lysine at one or both NH_2, depending on the conditions. The copper chelates of lysine and arginine give products in which the terminal NH_2 group is phosphorylated exclusively.[60]

Phosphorus oxychloride reacts readily with alcohols to form alkyl phosphorodichloridates, which can be subsequently hydrolyzed to alkyl dihydrogen phosphates (method 1c). Alternatively, phosphorus oxychloride can be partially hydrolyzed to an unstable reactive intermediate, phosphorochloridic acid, prior to the introduction of the alcohol (method 1d). Both methods have been applied to the proteins and their derivatives.

$$\begin{array}{ccc} POCl_3 & \xrightarrow[-HCl]{ROH} & ROP(O)Cl_2 \\ \Big\downarrow {\scriptstyle H_2O,\ -HCl} & & \Big\downarrow {\scriptstyle H_2O,\ -HCl} \\ ClP(O)(OH)_2 & \xrightarrow[-HCl]{ROH} & ROP(O)(OH)_2 \end{array} \qquad (1c,\ 1d)$$

Method 1c has been applied to herring sperm clupeine; the protamine, in the form of its capronate salt for enhanced solubility, is phosphorylated with phosphorus oxychloride in trimethyl phosphate for 2 to 6 days at room temperature, quenched with water, and hydrolyzed with 6 *N* hydrochloric acid to split off the acid-labile phosphate groups.[64]

Method 1d has been applied to the amino acids serine,[65,66] threonine,[23,66] hydroxyproline and tyrosine,[66] and also to 2-[^{2}H]-serine[67,68] and [^{32}P]-serine.[65] The procedure for DL-serine

is described in detail by Neuhaus and Korkes.[65] Typical conditions are 2 hr at 60°C in 85% phosphoric acid[66] or in the absence of a solvent,[65] followed by quenching with water and brief hydrolysis with acid to break down any polyphosphates formed. Yields are 77 to 96%.

Phosphorylation of the alcohol groups of amino acids, peptides, and proteins is often carried out in the presence of a base such as sodium hydroxide (method 1e):

$$ROH + POCl_3 + 5\ NaOH \rightarrow ROP(O)(ONa)_2 + 3\ NaCl + 3\ H_2O \qquad (1e)$$

Since the conditions are the same as those of method 1b, this reaction must be followed by brief hydrolysis with acid to cleave the N-phospho groups that are formed. The following procedure is typical:

Method 1e[69]

Caseinogen (18 g, containing 0.80% P) is dissolved in sufficient sodium hydroxide solution to give a neutral solution, and the volume is adjusted to 250 mℓ. The solution is cooled to <5°C and treated dropwise over a 9-hr period with rapid stirring with phosphorus oxychloride (50 g) in carbon tetrachloride (200 mℓ), sodium hydroxide being added as needed to maintain a faintly alkaline reaction. The aqueous layer is separated, treated with sufficient 2 *N* hydrochloric acid (59.5 mℓ) to precipitate the protein, and filtered. The product is purified to constant P/N ratio by repeated (5- to 6-fold) precipitation from 0.1 *N* sodium hydroxide solution with the equivalent amount of 0.2 *N* hydrochloric acid, and is then washed with water, alcohol, and dried *in vacuo* giving a 61% yield of phosphocaseinogen. Analysis: P, 1.77; N, 13.53; P/N ratio 0.130.

Method 1e has been applied to human serum albumin,[70,71] globulin,[70] hemoglobin,[71] globin[71] and [^{32}P]-protein;[72] to bovine lactalbumin,[13] β-lactoglobulin,[73] type II hemoglobin,[58] casein,[10] dephosphorylated casein[69,70] and caseinogen;[69] to horse serum albumin[74] and globulin;[69] to chicken egg albumin;[9,75] and to silk fibroin peptone, Witte peptone, and blood globulin.[10] In modified form, with pyridine or magnesium oxide as the base, it has also been applied to serine,[76] tyrosine,[14,77] 3,5-diiodotyrosine and thyroxine,[78] and to peptides of tyrosine with glycine.[14]

Phosphorylation of cysteine by method 1e gives N,S-diphosphocysteine, which can be isolated as the calcium salt. When this is acidified to pH 3, the N-P bond is cleaved and *S*-phosphocysteine precipitates out.[54]

B. From Dialkyl or Diaryl Phosphorochloridates

These reagents react with primary amines to give dialkyl or diaryl phosphoramidates (method 2a):

$$2\ RNH_2 + (R'O)_2P(O)Cl \rightarrow RNHP(O)(OR')_2 + RNH_2{\cdot}HCl \qquad (2a)$$

Since the products are acid-sensitive, it is necessary to protect any carboxyl groups that may be present. This method has been applied to esters of glycine,[79-83] serine,[79,84] threonine,[79] tyrosine,[81] glycylglycine,[81] glycyltyrosine,[81] and [^{32}P]-glycine[85] and to an amide of glycine.[79] Yields are good (70 to 80%), but half of the amine is used up in salt formation.

The procedure is improved if a tertiary amine, such as triethylamine, is added as an acid acceptor (method 2b):

$$RNH_2 + (R'O)_2P(O)Cl + R_3N \rightarrow RNHP(O)(OR')_2 + R_3N{\cdot}HCl \qquad (2b)$$

Method 2b[86]

To a suspension of amino acid ester hydrochloride (1 mol) in chloroform at 0°C is added triethylamine (2 mol), followed by slow addition, with stirring, of dibenzyl phosphorochloridate (1 mol). After the addition is completed, the reaction mixture is taken out of the cooling bath and stirring continued for 30 min. Next day, the precipitate

is filtered off and the filtrate is washed successively with water, 1 *N* HCl, 10% $NaHCO_3$, and water, then dried over anhydrous sodium sulfate. The solvent is removed under reduced pressure and the residue, after further purification, is recrystallized. The products are soluble in organic solvents but insoluble in water.

In some cases, better yields are obtained if the amino acid ester is liberated from its hydrochloride with ammonia prior to the reaction.[86,87] The reaction is catalyzed by dimethylformamide.[88] Triethylamine may be replaced by pyridine[79] or aqueous bicarbonate,[89] but aqueous reagents should be avoided because the products may be hydrolyzed under these conditions to amine salts of dialkyl or diaryl phosphates.[90]

This method has been applied to esters of glycine,[80,86-89,91-93] alanine,[86,87,91,92] cysteine,[84,87] serine,[86,87] aspartic acid,[92,94] threonine,[87] glutamic acid,[49,87,89,92] valine,[86-88] histidine,[86] cystine,[87] leucine,[86-88,92] lysine,[87] arginine,[87,95] phenylalanine,[86-89,92,96] tyrosine,[79,86,87] tryptophan,[86] glycylalanine,[96] alanylalanine,[96] alanylarginine,[253] isoleucylalanine,[96] leucylarginine,[253] leucylphenylalanine,[96] phenylalanylarginine,[97] tyrosylarginine,[97] and leucyltryptophan;[96] to amides of glycine,[87] alanine,[87] and leucine;[96] and even to glycine itself.[98] Although some of these compounds contain free OH, SH, and other NH groups, all but four are phosphorylated exclusively at the α-NH_2 position. Cysteine and tyrosine are phosphorylated at NH_2 with one equivalent of reagent and at NH_2 and SH (or OH) with two equivalents; lysine and arginine are phosphorylated at both α-NH_2 and terminal NH_2.

Dialkyl and diaryl phosphorochloridates are also capable of reacting with alcohols in the presence of tertiary amines to give the fully substituted esters (method 2c):

$$ROH + (R'O)_2P(O)Cl + R_3N \rightarrow ROP(O)(OR')_2 + R_3N{\cdot}HCl \qquad (2c)$$

Since these reagents attack amino groups, amino acids must be blocked with N-protecting groups that can be later removed, such as the carbobenzoxy group, $-O_2CCH_2Ph$. The reaction of diphenyl phosphorochloridate with amino acids protected at both NH_2 and CO_2H is best carried out in pyridine solution with pyridine acting as acid acceptor and solvent; triethylamine in chloroform gives poor results.[87,99]

Method 2c[100]

N-Carbobenzoxy-DL-serine ethyl ester (8.0 g), dry pyridine (100 mℓ), and diphenyl phosphorochloridate (9.0 g) are mixed. After being kept at 0°C for 12 hr, the mixture is diluted with chloroform (100 mℓ) and washed with dilute hydrochloric acid and with water. Evaporation and recrystallization from ether/light petroleum gives the diphenyl phosphate as needles (13.7 g, 85%), mp 40 to 41°C.

Dibenzyl phosphorochloridate requires a reaction temperature well below 0°C, owing to the sensitivity of the benzyl groups.[17] If the reaction mixture is allowed to stand too long before work-up small amounts (<5%) of the pyrophosphate ROP(O) (OR')OP(O)(OR')$_2$ are formed as byproducts.[22] Di-*p*-nitrobenzyl phosphorochloridate, on the other hand, is too unreactive; a modified method has been developed for this reagent that employs an imidazole intermediate.[99] Diisopropyl phosphorofluoridate, in contrast to the chloridate, is unreactive.[79]

This method has been applied to esters of D-, L-, and DL-serine,[15,19,25,99-101] threonine,[100] serylglycine,[14,16,17,100] glycylserine,[16,17,23,99] serylalanine,[23,99] serylserine,[17] aspartylserine,[23] serylaspartic acid,[17] glutamylserine,[17,23] serylglutamic acid,[17,100] serylhistidine,[23] leucylserine,[17] isoleucylserine,[23] serylleucine,[17,18] lysylserine,[23] seryllysine,[21] glycylserylglycine,[16] aspartylserylglycine,[23,102] glutamylserylglycin,[23] aspartylserylalanine,[23] glutamylserylalanine,[23] leucylglycylserine,[23] lysylserylglycine,[20,103] aspartylserylglutamic acid,[23,102] 2-[^{14}C]-serylglycine[24] and 3-[^{14}C]-glycylserine,[24] and to amides of serine.[25]

Dialkyl and diaryl phosphorochloridates react with carboxylic acids in the presence of tertiary amines[92] or with the silver salts of carboxylic acids[41] to give mixed anhydrides (method 2d):

$$RCO_2Ag + (R'O)_2P(O)Cl \rightarrow RCO_2P(O)(OR')_2 + AgCl \quad (2d)$$

Method 2d[41]

To a water-free carbon tetrachloride solution of dibenzyl phosphorochloridate, prepared from 8.6 g of dibenzyl phosphite, is added 12 g of the silver salt of *N*-carbobenzoxyleucine. The reaction mixture is agitated for 2 hr at room temperature by a stream of dry nitrogen. After the mixture has stood overnight, the silver salts are removed by filtration and the carbon tetrachloride by distillation under reduced pressure. The *N*-carbobenzoxyleucine dibenzyl phosphate remains as a viscous oil.

Since the products react readily with amines (see Section III.A.3), it is necessary to block any NH groups by suitable protecting groups that can be later removed. The mixed anhydrides are usually clear, heavy, highly hygroscopic oils. Amino acids that have been phosphorylated in this manner are glycine,[40,44,104] alanine,[40] aspartic acid,[41] glutamic acid,[41] and leucine.[41] Negative: *N*-phthaloylglycine.[105]

Finally, dialkyl phosphorochloridates react with dialkyl phosphates to form unsymmetrical tetraalkyl pyrophosphates (method 2e). Typical reaction conditions are 3 hr in acetone at −40°C with pyridine as the base.

$$(RO)_2P(O)Cl + (R'O)_2P(O)OH + R_3N \rightarrow (RO)_2P(O)OP(O)(OR')_2 + R_3N{\cdot}HCl \quad (2e)$$

This reaction has been applied to O-phospho derivatives of serine[29,31] and glycylserine.[29]

C. From Phosphinothioyl Chlorides

Diphenyl phosphinothioyl chloride reacts with primary amines under Schotten-Baumann conditions to give diphenyl phosphinothioic amides (method 3):

$$Ph_2P(S)Cl + RNH_2 + NaOH \rightarrow Ph_2P(S)NHR + NaCl + H_2O \quad (3)$$

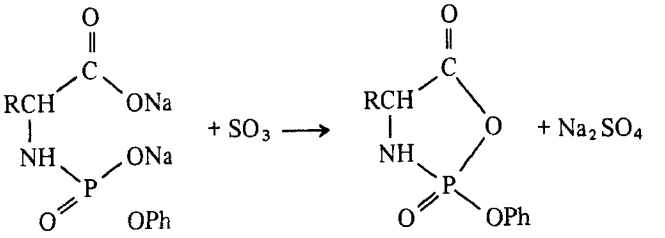
This method, unlike methods 2a and b, can be applied to the amino acids themselves since unprotected hydroxyl or carboxyl groups do not interfere. The reaction is carried out under ambient conditions in water with sodium hydroxide or in aqueous dioxane with triethylamine. Amino acids that have been phosphorylated in this manner are glycine, alanine, cysteine, serine, aspartic acid, threonine, proline, glutamic acid, valine, methionine, leucine, isoleucine, arginine, phenylalanine, and tyrosine.[106,107] Serine and threonine give monosubstitution products, cysteine a disubstitution product, and tyrosine gives both.

D. From Phenyl Phosphorodichloridate

Phenyl phosphorodichloridate reacts with primary amines in aqueous solution in the presence of barium hydroxide to give phenyl hydrogen phosphoramidates in the form of barium salts (method 4a):

$$2\,RNH_2 + 2\,PhOP(O)Cl_2 + 3\,Ba(OH)_2 \rightarrow [RNHP(O)(OPh)O]_2Ba + 2\,BaCl_2 + 4\,H_2O \quad (4a)$$

This method has been applied to the amino acids glycine, alanine, phenylalanine, valine, and leucine.[108] Negative: glycine, glutamic acid, arginine, and leucylglycylglycine.[109] Yields are 60 to 84%. On treatment with a solution of sulfur trioxide in phosphorus oxychloride, the products are converted to inner anhydrides (method 4b):



(4b)

Yields are low (25 to 39%), as the products are very moisture-sensitive and difficult to separate from byproducts.[108]

Phenyl phosphorodichloridate reacts with alcohols to form alkyl phenyl phosphorochloridates, ROP(O)(OPh)Cl. If the reaction is carried out in an aqueous medium,[109,110] or if the chloridate is subsequently hydrolyzed,[101] the product is an alkyl phenyl phosphate (method 4c). This method has been used to phosphorylate the amino acids serine[101,109] and hydroxyproline[109] and the proteins gelatin and pepsin.[110]

$$ROP(O)(OPh)Cl + H_2O \rightarrow ROP(O)(OPh)OH + HCl \quad (4c)$$

If the chloridate is treated with an alcohol, the product is a dialkyl phenyl phosphate (method 4d). This method has been used to phosphorylate serine.[26,111]

$$ROP(O)(OPh)Cl + R'OH + R_3N \rightarrow ROP(O)(OPh)OR' + R_3N{\cdot}HCl \quad (4d)$$

The phenyl groups may be removed from the amino acid derivatives by acid hydrolysis[109] or by catalytic hydrogenolysis,[111] but hydrolyze spontaneously off the proteins.[110]

E. From Dialkyl Phosphates

Sodio diethyl phosphite reacts with N-bromoamines giving the N-phosphoramidates (method 5a):

$$RNHBr + (EtO)_2PONa \rightarrow RNHP(O)(OEt)_2 + NaBr \quad (5a)$$

This reaction has been applied to esters of alanine and glutamic acid. No details are given for this reaction, nor for the subsequent cleavage of the ester groups.[112]

Silver salts of dialkyl phosphates react with alkyl halides to give the triesters (method 5b). This reaction has been used to prepare several O-phosphoserine derivatives.[101,102,113]

$$RBr + (R'O)_2P(O)OAg \rightarrow (RO)(R'O)_2PO + AgBr \quad (5b)$$

Reaction of the silver salts with acid chlorides gives the acyl phosphates (method 5c):

$$RCOCl + (R'O)_2P(O)OAg \rightarrow RCO_2P(O)(OR')_2 + AgCl \quad (5c)$$

The products are mixed anhydrides of carboxylic and phosphoric acids, and are powerful acylating agents. Product of this type, which are also accessible by method 2d, have been prepared from derivatives of glycine where the N-protecting group is phthaloyl,[105] carbobenzoxy,[105] or azido.[114] The reaction is carried out at room temperature in dry benzene.

F. From Monoalkyl Phosphates

Monoalkyl phosphates react with primary amines in the presence of a dehydrating agent

such as *N,N'*-dicyclohexylcarbodiimide (DCCI) to give alkyl hydrogen phosphoramidates (method 6a). This method has been used to prepare N-phospho derivatives of valine, methionine, and leucine.[115] The alkyl group, R' = 2-cyanoethyl, can be removed by mild hydrolysis with alkali.

$$RNH_2 + R'OP(O)(OH)_2 \xrightarrow{DCCI} RNHP(O)(OR')OH + H_2O \quad (6a)$$

This reaction can also be used to prepare O-phospho derivatives of serine that contain appropriate masking groups (method 6b). Serine esters whose NH_2 group is protected by the phthaloyl function tend to undergo β-elimination during the phosphorylation, but good results are obtained with the *t*-butoxycarbonyl function.[113] The reaction is carried out in pyridine solution over a 40-hr period at 0°C.

$$ROH + R'OP(O)(OH)_2 \xrightarrow{DCCI} ROP(O)(OR')OH + H_2O \quad (6b)$$

The products react further in the presence of DCCI to form pyrophosphates (method 6c). This reaction, which has been applied to esters of serine[25,29,31,116] and glycylserine,[23,27,29,116] is complete in 1.5 hr at 20°C.

$$2\ ROP(O)(OR')OH \xrightarrow{DCCI} [ROP(O)OR']_2O + H_2O \quad (6c)$$

The silver salt of phenyl dihydrogen phosphate reacts with acid chlorides to give mixed anhydrides (method 6d), which are capable of acylating primary amines such as glycine.[104] This reaction has been used to prepare peptides of glycine with itself or with tryptophan.

$$PhOP(O)(OAg)_2 + RCl \rightarrow PhOP(O)(OR)OAg + AgCl \quad (6d)$$

G. From Phosphoric Acid

Phosphoric acid may be "activated" for phosphorylation by reaction with trichloroacetonitrile in the presence of a tertiary amine (method 7a):

$$ROH + H_3PO_4 + CCl_3CN \xrightarrow{R_3N} ROP(O)(OH)_2 + CCl0_3CONH_2 \quad (7a)$$

This method has been applied to the salmon sperm protein protamine, both unlabeled and [^{32}P]-labeled.[117] Other activating agents are known, but have not been used for this purpose.

Phosphoserine has been identified as one of the products of the synthesis of amino acids under prebiotic conditions (pH 5.5, 35 days, 105°C) (method 7b). The synthetic mixture was comprised of formaldehyde, hydroxylamine, and various salts including dibasic potassium phosphate.[118]

Silver dihydrogen phosphate, prepared *in situ* from Ag_3PO_4 (1 part) and H_3PO_4 (2 parts), reacts with acid chlorides to give acyl phosphates (method 7c). An 80% yield of the aspartic acid derivative is obtained by this method.[119]

$$RCOCl + AgOP(O)(OH)_2 \rightarrow RCO_2P(O)(OH)_2 + AgCl \quad (7c)$$

H. From Polyphosphoric Acid

This reagent is a partial anhydride of orthophosphoric acid of indeterminate structure, consisting of linear, branched, and cyclic chains of -P(O)O- units. When treated with an alcohol, the P-O-P bonds are cleaved giving equal parts of phosphate ester and phosphoric acid (method 8a):

$$n\ ROH + H[-OP(O)(OH)OP(O)(OH)-]_nOH \rightarrow n\ ROP(O)(OH)_2 + n\ H_3PO_4 \quad (8a)$$

The polyphosphoric acid reagent may be prepared by heating 85% phosphoric acid to 350°C or above,[120] but is more commonly prepared by dissolving phosphorus pentoxide in 85% phosphoric acid.[121,122] The optimum time and temperature of reaction vary from one substrate to another; 20 min at 70°C is sufficient for an amino acid such as serine,[122] and 3 days at room temperature for a protein such as sericin.[121] A brief aftertreatment with hot 2 *N* HCl is necessary to hydrolyze any residual di- or triphosphate.

Amino acids that have been phosphorylated in this manner are serine,[76,120,122-125] threonine,[123,126] hydroxyproline,[127] and tyrosine,[123,128] and related substances such as serine esters[129] and anhydrides.[76] Proteins that have been phosphorylated in this manner are bovine serum albumin, crystalline egg albumin, ovomucoid, silk fibroin, sericin, gluten, gliadin, edestin, gramicidin, gelatin, γ-globulin, globin, isinglass, and insulin.[121] Little or no phosphorylation occurs with cysteine,[121] hydroxyaspartic acid,[123,127] hydroxyglutamic acid,[127] glycyltyrosine,[14] polyglutamic acid,[121] polyglutamine,[121] polyglycine,[121] or tyrosine/formaldehyde polymer.[121]

No esterification occurs if the phosphoric acid is strictly anhydride-free. Analysis of serine or threonine after hydrolysis with 6 *N* hydrochloric acid in the presence of phosphoric acid shows no O-phosphate, although sulfuric acid under the same conditions produces some O-sulfate.[130]

The cyclic phosphates $[-P(O)(ONa)O-]_n$ ($n = 3$ or 4) react with valine to give the N-phospho derivative in 3 to 22% yield after several weeks at room temperature (method 8b). The reaction is faster at 70°C but the yields are lower.[131-132a]

$$n\ RNH_2 + [-P(O)(ONa)O-]_n \rightarrow n\ RNHP(O)(ONa)OH \quad (8b)$$

Lysine reads with sodium trimetaphosphate ($n = 3$) in 2 hr at 12 and 30°C to give N_ϵ-phospholysine. Histidine, arginine, and tyrosine are not phosphorylated under these conditions, but serine and threonine react to give O-phospho derivatives. This method has been applied to soy protein.[133]

I. From Alkyl Meta- and Pyrophosphates

Ethyl metaphosphate, a product of the reaction of diethyl ether with phosphorus pentoxide, is a sirupy fluid, soluble in halogenated solvents such as chloroform and insoluble in ether. It reacts with alcohols to give alkyl dihydrogen phosphates (method 8c), but attempts to O-phosphorylate serine[12,76] or tyrosine[134] by this method were unsuccessful. Likewise, use of the reagent to n-phosphorylate alanine,[12] aspartic acid,[12] glutamic acid,[12] valine[12], or leucine[11,12] has been reported, but the reaction with alanine could not be verified[134] and the others remain in doubt.

$$2\ ROH + EtPO_2 \rightarrow ROP(O)(OH)_2 + EtOR \quad (8c)$$

Tetraalkyl pyrophosphates react with primary amines to give dialkyl phosphoramidates. If a tertiary amine is present, the reaction proceeds as follows (method 8d):

$$RNH_2 + [(RO)_2P(O)]_2O + R_3N \rightarrow RNHP(O)(O)_2 + [R_3NH][OP(O)(OR)_2] \quad (8d)$$

Glycine esters have been phosphorylated in this manner, but the products are difficult to separate from the starting materials.[79,80,103]

Tetraalkyl pyrophosphates react with esters of serine in the presence of imidazole to give the O-phospho derivatives in 37 to 40% yield (method 8e). No details are available.[102]

$$ROH + [(R'O)_2P(O)]_2O + R_3N \rightarrow ROP(O)(OR')_2 + [R_3NH][OP(O)(OR')_2] \quad (8e)$$

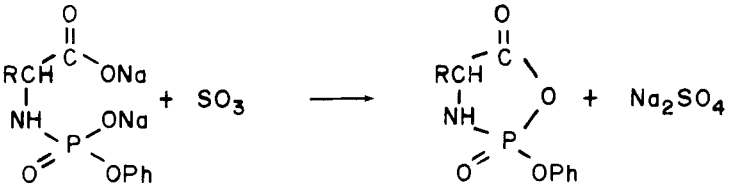
J. From Phosphoramidates

Potassium hydrogen phosphoramidate, prepared by the hydrolysis of diphenyl phosphoramidate with potassium hydroxide, is a water-soluble salt that is stable in alkaline solution but unstable in acid. It reacts with the ring nitrogens of histidine to form π- and τ-phospho derivatives (method 9a):

$$R_2NH + NH_2P(O)(OH)OK \rightarrow R_2NP(O)(OH)OK + NH_3 \quad (9a)$$

Under certain conditions, it also phosphorylates the α-amino group of histidine and other amino acids. Some amino acids and peptides that are phosphorylated by this method are glycine,[135] histidine,[56,57,136-138] and glycylglycine.[135] Proteins that are phosphorylated by this method are histone 4,[139,140] bovine myelin basic protein,[139] protein HPr,[141] phosphoramidate hexose transferase,[142] and insulin.[136] In some cases, [^{32}P]-phosphoramidate has been used.[139,140,142]

Phosphoramidate is most effective at pH 7 to 8, where most amines are still positively charged. As the pH is increased the amines become reactive, but the phosphoramidate is converted to the unreactive dianion.[135,136] This problem can be overcome by substituting an imidazole group for the NH_2 group of the phosphoramidate. Because of resonance stabilization, the imidazolides are effective over a broad range of alkalinity (method 9b):[136]



(9b)

Method 9b[143]

A solution of diphosphoimidazole in water (70 mℓ), prepared from the calcium salt (2 g) by treatment with Na-Dowex® (50 g), is adjusted to pH 11. Glycine (2 g) is added, and if necessary the pH is readjusted to 11. The solution is heated to 60°C for 30 min. After cooling, the solution is acidified with 60% perchloric acid to pH ~7.5, treated with barium chloride (0.15 g) to precipitate inorganic phosphate, and then acidified to pH 6.2 and treated with barium chloride (1 g) and ethanol (1/4 v:v) to precipitate the product. The yield of *N*-phosphoglycine is about 1 g.

This method has been applied to glycine,[143,144] alanine,[136,143,145] cysteine,[143] serine,[136,143,145] proline,[136,145] methionine,[143] histidine,[136,143-145] cystine,[143] tyrosine,[136,143,145] and tryptophan.[136,143,145] Yields are 20 to 50%. If the separation of the product from the *N*-phosphoimidazole is a problem, other similar phosphorylating agents are available.[144,146] Hydroxyl, carboxyl, and sulfhydryl groups do not interfere.

N_ω-Phosphoarginine may be prepared by the reaction of ornithine with *O*-methylisoureidophosphonate (method 9c). The yield is 75%, together with about 10% of the $N_\alpha N_\omega$-diphospho compound. The formation of the latter can be suppressed by converting the ornithine to its Cu(II) chelate.[95]

$$RNH_2 + MeOC(=NH)NHP(O)(OH)_2 \rightarrow RNHC(=NH)NHP(O)(OH)_2 + MeOH \quad (9c)$$

III. CHEMICAL REACTIONS

A. The Phospho Group

1. Hydrolysis

By far the most important chemical reaction of the compounds of this review is hydrolysis — especially hydrolysis in the presence of acid, base, or metal ion catalysts.

a. N-Phospho Derivatives

The N-phospho derivatives of the amino acids are, in general, stable in neutral or alkaline solution and unstable in acid. The rate of hydrolysis of *N*-phosphoglycine at 25°C, measured by the decrease in optical density at 224 nm is 0.059 sec^{-1} at pH 3.0.[48] The heat of hydrolysis is 7500 cal/mol, somewhat smaller than that for the high-energy P-N bond of *N*-phosphocreatine (ΔH = 11,000 cal/mol).[48] The hydrolysis is catalyzed by molybdate[48] and by lanthanide ions.[147]

$$RNHP(O)(OH)_2\ H_2O + \rightarrow RNH_2 + H_3PO_4$$

Rates of hydrolysis have also been reported for unsubstituted N-phospho derivatives of alanine,[48,53] glutamic acid,[48,51,147] and histidine.[57] All obey first-order kinetics except α,π,τ-triphosphohistidine.[57]

N-Phosphoglycine does not give a positive color test with ninhydrin at pH 7.5, even after several minutes at room temperature.[81] At 100°C, the N-phosphoamino acids develop purple colors, but at a slower rate than the free amino acids.[48]

Compounds in which the N-phospho group is attached to NH groups other than the α-amino group, such as histidine,[53,55-57,136,137,144,148,149] lysine,[58,139,144,149] or arginine,[61,63,95] are much more stable to hydrolysis than those described above, though still acid-sensitive. N_τ-Phosphohistidine, for example, is stable to 100°C in alkaline solution.[136] Rates of hydrolysis have been reported for N_π-, N_τ-, and $N_\pi N_\tau$-diphosphohistidine,[56,57,136,137] N_ϵ-phospholysine[58] and N_ω-phosphoarginine.[61]

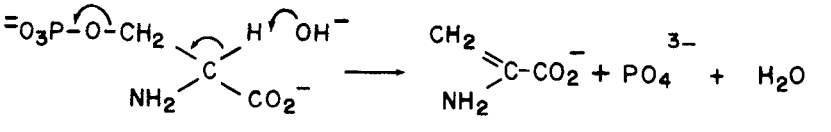
In contrast, N-phospho derivatives of amino acid esters[86,137] and amides[96] are stable to acid, as are amino acid derivatives in which both POH groups are esterified.[92]

b. O-Phospho Derivatives

The O-phospho derivatives of the hydroxyamino acids are, in general, stable in neutral or acid solution but unstable in alkaline solution. In the presence of alkali, *O*-phosphoserine readily decomposes to ammonia, pyruvic acid, and orthophosphoric acid:

$$(HO)_2P(O)OCH_2CH\begin{matrix}NH_2\\CO_2H\end{matrix} + H_2O \xrightarrow{OH^-} H_3PO_4 + CH_3COCO_2H + NH_3$$

Tracer studies with $H_2{}^{18}O$ prove that the cleavage occurs at the C-O ester bond.[67] A mechanism compatible with this cleavage is β-elimination of the phosphate residue, leaving dehydroalanine which subsequently hydrolyzes to ammonia and pyruvic acid:



The rate of hydrolysis of *O*-phosphoserine, measured by colorimetric estimation of phosphate by the molybdate method, is 0.040 sec^{-1} at pH 9.6 and 100°C. The rate is first order, and is practically constant over the pH range 7 to 13.5 but increases with hydroxide ion

concentration at pH >14. Serine itself is slowly deaminated under these conditions, but the rate is less than 5% of that for *O*-phosphoserine.[67]

Hydrolysis of *O*-phosphoserine in strongly acid solution proceeds by P-O ester bond cleavage, giving serine and orthophosphoric acid:

$$(HO)_2P(O)OCH_2CH\begin{matrix}NH_2\\CO_2H\end{matrix} + H_2O \xrightarrow{H^+} HOCH_2CH\begin{matrix}NH_2\\CO_2H\end{matrix} + H_3PO_4$$

In 6 *N* hydrochloric acid at 110°C, the rate of hydrolysis is 0.183 hr^{-1}.[149a] The rate is first order, and is almost independent of pH.[67] In the pH range 0 to 7, both mechanisms apply and the products are mixtures of hydrolysis and elimination products.[67]

The behavior of *O*-phosphoserine in 6 *N* HCl at 100°C is important because these conditions are often used for determining the amino acid composition of proteins. Upon prolonged heating (usually 22 hr), some deamination of serine occurs. The presence of an O-phospho group triples the rate of deamination, making the customary correction of 10 to 17% for the loss of serine inadequate.[150] The influence of neighboring amino acid residues in the O-phosphopeptides and -proteins is another complicating factor.[149a]

Rates of hydrolysis under a variety of conditions have been reported for unsubstituted O-phospho derivatives of serine,[38,39,67,123,149a-153] threonine,[123,149a,151] hydroxyproline[123] and tyrosine.[123] Hydrolysis of *O*-phosphothreonine under alkaline conditions yield α-ketobutyric acid,[149a] whereas *O*-phosphotyrosine yields tyrosine.[14,154]

The O-phosphoanhydrides of the amino acids (aminoacyl phosphates) undergo a variety of reactions in water. Depending on the structure of the compound and the reaction conditions, the anhydrides may undergo hydrolysis, polymerization, or deamination.

β-Aspartyl phosphate and γ-glutamyl phosphate are hydrolyzed, in part, to the respective amino acids.[41] First-order rate constants have been determined for β-aspartyl phosphate over a broad pH range. The rate of hydrolysis, which roughly parallels that for acetyl phosphate, increases sharply below pH 3 and above pH 13.[119]

$$(HO)_2(O)O_2C(CH_2)_nCH\begin{matrix}NH_2\\CO_2H\end{matrix} + H_2O \rightarrow HO_2C(CH_2)_nCH\begin{matrix}NH_2\\CO_2H\end{matrix} + H_3PO_4$$

The O-phosphoanhydrides of the monobasic amino acids (glycine, alanine, proline, and leucine) undergo polymerization at pH 6 or above. This reaction is discussed in Section III.A.3.

All of the compounds undergo deamination in the pH range 3 to 9, giving the corresponding hydroxyacids:

$$NH_2CHRCO_2P(O)(OH)_2 + 2\ H_2O \rightarrow HOCHRCO_2H + NH_3 + H_3PO_4$$

The yield is never less than 30%, and can be increased to 100% by means of a catalyst such as palladium on charcoal.[43] Alanyl and β-aspartyl phosphate yield lactic acid and malic acid, respectively;[43] glycyl phosphate dimerizes to aspartyl diphosphate, which then undergoes further deamination to malic acid.[44] Unsaturated intermediates can be detected during these reactions, suggesting a deamination/hydration mechanism.[43]

Pyrophosphate monoesters of the type ROP_2O_7, where R is a serine or substituted serine residue, are stable to water over the pH range 3 to 11 but hydrolyze in acid solution to the monophosphates:

$$\begin{matrix}RO\\HO\end{matrix}\!\!>P(O)OP(O)\begin{matrix}OH\\OH\end{matrix} + H_2O \xrightarrow{HCl} ROP(O)(OH)_2 + H_3PO_4$$

The rate constant for the hydrolysis of DL-serine-*O*-pyrophosphate is 0.00024 min^{-1} in 1 *N* HCl at 22°C. Only in 2 *N* HCl at 50°C do small amounts of serine appear.[30] Rate constants have been reported for pyrophosphate monoesters of serine,[30] *N*-benzoylserine methylamide,[36] and glycylserine.[30] All are first order, and are catalyzed in neutral or alkaline solution by metal ions such as Th, La, or Ce.[30,36]

Acidolysis of the pyrophosphate monoesters with carboxylic acids such as acetic acid or glycine results in transphosphorylation:

$$\begin{matrix} RO \\ HO \end{matrix}\!\!\diagdown\!\!\diagup P(O)OP(O)\!\!\diagdown\!\!\diagup \begin{matrix} OH \\ OH \end{matrix} + R'CO_2H \rightarrow ROP(O)(OH)_2 + R'CO_2P(O)(OH)_2$$

The maximum yield of acyl phosphate, measured by the hydroxamic acid method (see Section III.A.3), is 4 to 22%. The reaction is catalyzed by Be(II) ion.[28]

Symmetrical pyrophosphate diesters of the type $(RO)_2P_2O_6$, where R is a serine or substituted serine residue, are hydrolyzed by acid, but not as rapidly as the monoesters. Those which contain free NH_2 groups also hydrolyze readily at pH > 8.5. The products in both cases are the monophosphates:

$$\begin{matrix} RO \\ HO \end{matrix}\!\!\diagdown\!\!\diagup P(O)OP(O)\!\!\diagdown\!\!\diagup \begin{matrix} OR \\ OH \end{matrix} + H_2O \rightarrow 2\ ROP(O)(OH)_2$$

Rate constants, all first order, have been reported for pyrophosphate diesters of serine,[32,35] *N*-acetylserine[35] and glycylserine.[32]

Symmetrical pyrophosphate tetraesters of the type $[RO(R'O)P(O)]_2O$, where R is a substituted serine residue and R′ is benzyl, are hydrolyzed in aqueous acetone at 20°C:

$$\begin{matrix} RO \\ PhCH_2O \end{matrix}\!\!\diagdown\!\!\diagup P(O)OP(O)\!\!\diagdown\!\!\diagup \begin{matrix} OR \\ OCH_2Ph \end{matrix} + H_2O \rightarrow 2\ \begin{matrix} RO \\ PhCH_2O \end{matrix}\!\!\diagdown\!\!\diagup P(O)OH$$

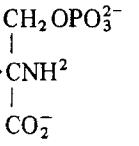
Rate constants have been reported for pyrophosphate tetraesters of *N*-carbobenzoxyserine benzyl ester and *N*-benzoylserine methylamide. The rates are first order at pH 7, but at pH 8.5 the rates are zero order and the reactions are complicated by β-elimination.[25]

c. S-Phospho Derivatives

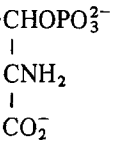
S-Phosphocysteine is stable in neutral or alkaline solution and in acid solution to pH 3, but decomposes in 1 *N* perchloric acid at room temperature with the liberation of ammonia, thiophosphate, and (presumably) pyruvic acid.[54] In alkaline solution, at 94°C, the products are cysteine and phosphate.[54] Evidently *S*-phosphocysteine, unlike *O*-phosphoserine, undergoes elimination in acid and hydrolysis in base.

2. Radiolysis

Upon exposure to a ^{60}Co source, phosphoserine undergoes radiation-induced cleavage in aqueous solution with the liberation of inorganic phosphate.[155] Attack is by the hydroxyl radical (OH) rather than e^-_{aq} or H because the rate of cleavage is increased by nitrous oxide (an e^-_{aq} scavenger) and decreased by methanol.[155] The radical formed depends on the pH. In alkaline solution, OH abstracts an α-hydrogen giving radical A which rapidly eliminates H_3PO_4. In neutral solution, H_α is deactivated by the protonated amino group; abstraction of a β-hydrogen by OH gives radical B which is somewhat stable and probably decomposes by disproportionation rather than elimination.[156]



A



B

3. Aminolysis

The O-phospho derivative of *N*-benzoylserine methylamide reacts with methylamine under strongly alkaline conditions (pH 12.6) with cleavage of the C-O bond:

$$(HO)_2P(O)OCH_2CH\begin{matrix}\nearrow NHCOPh\\ \searrow CONHMe\end{matrix} + MeNH_2 \rightarrow H_3PO_4$$

$$+ MeNHCH_2CH\begin{matrix}\nearrow NHCOPh\\ \searrow CONHMe\end{matrix}$$

Some P-O bond cleavage occurs if the pH is lowered to 12. Similar reactions take place with the hydroxylamines H_2NOH, MeNHOH, Me_2NOH, and H_2NOMe. The rate constants are all second order. The serine-containing products have not been identified, but based on the reaction rate the O-substituted products are favored.[33]

The *sym.*-pyrophosphate diester derivative of *N*-benzoylserine methylamide undergoes the same reactions, eliminating pyrophosphoric acid instead of phosphoric acid,[34] but if the phosphoryl acid groups are esterified,[25] or the carboxyl groups are free,[35] P-O bond cleavage occurs instead. The reaction with aniline is typical (method 10a):

$$[(RO)_2P(O)]_2O + PhNH_2 \rightarrow (RO)_2P(O)NHPh + [RNH_3][OP(O)(OR)_2] \quad (10a)$$

Aminoacyl phosphates react with hydroxylamine to form hydroxamic acids.[41] The reaction is quantitative at pH 8, and can be used for colorimetric determination of the anhydride content.[119] Alternatively, the anhydride is treated with ethanol, precipitating the phosphate salt of the ester which is then treated with hydroxylamine and analyzed.[41]

$$RCO_2P(O)(OH)_2 + H_2NOH \rightarrow RCONHOH + H_3PO_4$$

The anhydrides react with aqueous ammonia to give amides. Glutamine and asparagine are formed by this method in over 90% yield.[41]

$$RCO_2P(O)(OH)_2 + NH_3 \rightarrow RCONH_2 + H_3PO_4$$

Similarly, reaction with amino acids gives peptides.[41] This reaction is the basis of the self-polymerization that occurs spontaneously when the anhydrides are dissolved in water at pH 7 to 11. The phosphoanhydride reacts with free amino acid liberated by hydrolysis, forming a peptide; propagation proceeds by stepwise addition of the phosphoanhydride to the growing peptide chain, and proceeds until the phosphoanhydride is exhausted.[45]

$$NH_2CHRCO_2H + NH_2CHRCO_2P(O)(OH)_2 \rightarrow NH_2CHRCONHCHRCO_2H$$

$$+ H_3PO_4 \rightarrow \rightarrow H[NHCHRCO]_nOH + n\ H_3PO_4$$

Anhydrides whose homopolymerization has been studied are those of glycine,[40,44] alanine,[40,47] proline,[46] and leucine.[41] The anhydrides of the dibasic amino acids, aspartic acid

and glutamic acid, do not polymerize readily under these conditions,[41] through they react with other amino acids such as glycine.[44]

Dipeptides of glycine, alanine, and serine are obtained in yields of up to 36% when the amino acids are treated with polyphosphoric acid or trimetaphosphate under neutral or slightly alkaline conditions. The aminoacyl phosphates are probably formed *in situ* as labile intermediates.[52,125]

The monoesters and diesters of the phosphoanhydrides, prepared by methods 2d, 5c, or 6d likewise undergo aminolysis with loss of the phosphate ester group (methods 10 b, c):

$$RCO_2P(O)(OR')OH + R''NH_2 \rightarrow RCONHR'' + R'OP(O)(OH)_2 \quad (10b)$$

$$RCO_2P(O)(OR')_2 + R''NH_2 \rightarrow RCONHR'' + (R'O)_2P(O)OH \quad (10c)$$

These reactions provide a useful method for peptide synthesis if the amine employed is a free amino acid. Peptide derivatives that have been prepared in this manner are glycylglycine,[104] phenylalanylglycine,[92] phenylalanylleucine,[92] glycyltryptophan,[104] leucylphenylalanine,[92] and glycylglycyltryptophan.[104] Yields are 70 to 80% if R′ = phenyl and 52 to 62% if R′ = ethyl or isopropyl. Cleavage in the opposite sense to give RCO_2H and $R''NHP(O)(OR')_2$ occurs to an extent of only 0.5 to 2%, except for glycine which gives up to 20% of the phosphoramidate.[92]

4. Transfer Reactions

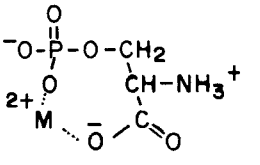
Migration of the phosphoryl group from nitrogen to oxygen occurs during hydrolysis of the ester groups of *N*-phosphoserine and -threonine with boiling hydrochloric acid (method 10d).[84] Efforts to isolate the intermediate O-phospho triesters were unsuccessful. The *N*-phosphocysteine analog is hydrolyzed to cysteine under these conditions.[84] No migration occurs with *N*-phosphoserine itself; the products are completely hydrolyzed.[136]

Transfer of the unsubstituted N-phospho group occurs fairly readily at pH 8.5 between the N_π and N_τ ring nitrogens of *N*-phosphohistidine, and between these compounds and histidine or N_α-acetylhistidine. The N_π position in the mono- and diphosphohistidines is more labile because of the protonation on N_α.[57]

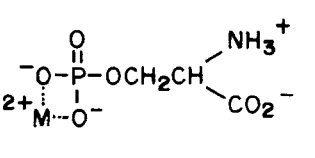
The O-phosphoanhydride of leucine is capable of phosphorylating AMP to ADP, and even to a slight extent to ATP.[42]

5. Metal Ion Complexes

Depending on the pH and the particular metal ion, *O*-phosphoserine forms binary complexes of the type ML, MHL, ML_2, MHL_2, or ML_3 with metal (II) ions, where M = metal and L = ligand,[157] and ternary complexes of the type MAL or MAHL with metal (II) ions and other ligands, where A = histamine, 1,10-phenanthroline, or α,α′-bipyridyl.[158] The extent of complexing with the phospho group can be determined by potentiometric titration, and verified in some cases by the broadening of the ^{31}P NMR signal. The evidence suggests the following structures for the ML and MHL complexes:[157]



ML



MHL

Table 1
METAL ION COMPLEXES FOR WHICH STABILITY CONSTANTS HAVE BEEN DETERMINED

Metal	O-Phospho ligand
Mg(II)	Serine,[124,157,159-161] threonine,[157] serylglycine,[162] glycylserine,[162] serylglutamic acid,[162] seryllysine,[162] glycylserylglycine[162]
Ca(II)	Serine,[124,157,159,160] threonine,[157] serylglycine,[162] glycylserine,[162] serylglutamic acid,[162] seryllysine,[162] glycylserylglycine[162]
Sr(II)	Serylglycine[7]
Mn(II)	Serine,[126,157] threonine,[157] serylglycine,[162] serylglutamic acid,[162] seryllysine,[162] glycylserylglycine[162]
Fe(III)	Serine[126]
Co(II)	Serine,[157,158] threonine[157]
Ni(II)	Serine,[157-159] threonine[157]
Cu(II)	Serine,[126,157,158] threonine,[157] serylglycine,[163] serylglutamic acid[164]
Zn(II)	Serine,[157,158] threonine[157]

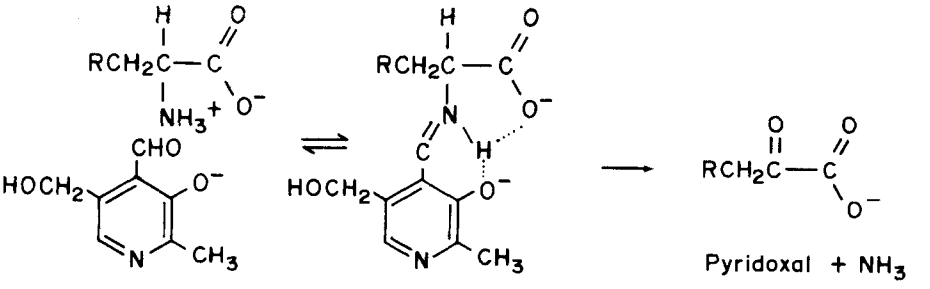
Stability constants have been reported for the complexes listed in Table 1.

These equilibrium studies are supplemented by a kinetic study of the formation of Ni(II) and Co(II) complexes of *O*-phosphoserine, measured by the temperature-jump method,[165] and by a theoretical study of the binding of Mg(II) and Ca(II) to *O*-phosphoserine, calculated by the *ab initio* SCF method.[166]

6. Reaction with Pyridoxal and other Aldehydes

The interaction of serine, pyridoxal, and their O-phosphates with metal ions have been studied extensively in connection with the role these substances play in enzymic processes.

Pyridoxal catalyzes the β-elimination of phosphate ion from *O*-phosphoserine. Labilization of the α-proton of the serine moiety is promoted by the ability of the Schiff base to accommodate the liberated electron pair in its conjugated π-bond system. The maximum rate is at pH 9, where the Schiff base species is in a monoprotonated form:[167]



Acid dissociation constants for the Schiff base and rate constants for the reaction and for each of the molecular species in solution are available.[167,168]

The elimination of phosphate ion from *O*-phosphoserine-pyridoxal Schiff base is strongly catalyzed by metal ions such as Mn(II), Fe(II), Fe(III), Cu(II), Zn(II), Al(III), and Ga(III).[167-172] Ni(II) is not a catalyst unless Mn(II) is also present.[172] Similar reactions occur with *O*-phosphothreonine, but the rates are slower.[170,173]

Pyruvate is also liberated from serine by pyridoxal, but pyridoxal-5′-phosphate liberates hydroxypyruvate.[174] If the reaction of *O*-phosphoserine with pyridoxal or pyridoxal-5′-phosphate and metal ions is carried out in the presence of sodium sulfide or sodium sulfite, the

product is cysteine or cysteic acid, respectively.[175] The pyridoxal can be replaced by pyruvate, provided that the metal ion is Cu(II).[176]

The N-benzylidene derivative of *O*-phosphoserine, prepared from *N*-benzylideneserine by method 1e, yields *N*-phosphoserine upon acid hydrolysis. The low yield is attributed to the formation of a cyclic oxazolidine.[76]

o-Phthalaldehyde is used to derivatize N- and O-phosphoamino acids for HPLC.[139,177]

7. Hydrogenolysis of Phenyl and Benzyl Protecting Groups

This method, coupled with method 2, is widely used for the synthesis of N-, O-, and S-phosphoamino acids and their derivatives. Phenyl and benzyl groups are easily removed by catalytic hydrogenolysis with palladium or platinum catalysts. N-phosphoamino acids, for example, may be prepared by hydrogenolysis of dibenzyl phosphoramidates over a palladium on charcoal catalyst (method 11a):

$$RNHP(O)(OCH_2Ph)_2 + 2\ H_2 \xrightarrow{Pd} RNHP(O)(OH)_2 + 2\ PhCH_3 \qquad (11a)$$

Method 11a[86]

"One gram of dibenzylphosphoryl amino acid ester is subjected to hydrogenolysis in dry methanol in the presence of about 0.04 g of palladium oxide. After the reaction has been completed (in 1 hour), the catalyst is filtered off and the solvent removed under reduced pressure in a water-bath of about 50°. The residue crystallizes on cooling and is recrystallized from methanol and ether."

This method has been used to prepare N-phospho derivatives of glycine,[49,81,87,92] aspartic acid,[94] glutamic acid,[49] leucine,[96] arginine,[95] phenylalanine,[86,87,96] tyrosine,[81] tryptophan,[86] glycylglycine,[81] alanylalanine,[96] isoleucylalanine,[96] glycyltyrosine,[81] leucylphenylalanine,[96] and leucyltryptophan,[96] but fails with some N-phospho derivatives of glycine ,[86] alanine,[86,87] serine,[87] glutamic acid,[87] and tyrosine.[87] Yields of 72 to 78% are common.

If the ultimate product is a free N-phosphoamino acid, two procedures are available. The triester can be hydrogenated in alcoholic sodium hydroxide to allow for saponification of the carboxyl ester,[81] or the triester can be prepared from the amino acid benzyl ester to allow for hydrogenolysis of all three ester groups. The yields by the second procedure, however, are poor because the free carboxyl group facilitates intramolecular degradation of the product.[88]

Method 11a is also applicable to N-phospho derivatives containing *p*-iodobenzyl[81] or *p*-nitrobenzyl[81,94,95] groups.

O-Phosphoamino acids and esters are similarly prepared by hydrogenolysis of the dibenzyl phosphate esters over a palladium on charcoal catalyst (method 11b). This method has been applied to O-phospho derivatives of serine[29,31,99,102,116] and their peptides,[14,17,20-24,29,99,116] including some monobenzyl esters.[21,99]

$$ROP(O)(OCH_2Ph)_2 + 2\ H_2 \xrightarrow{Pd} ROP(O)(OH)_2 + 2\ PhCH_3 \qquad (11b)$$

O-Phosphoamino acid anhydrides may be prepared by hydrogenolysis of the dibenzyl esters over a palladium on charcoal catalyst (method 11c). This method has been applied to O-phospho anhydrides of glycine,[114] alanine,[114] aspartic acid,[119] and phenylalanine.[114]

$$RCO_2P(O)(OCH_2Ph)_2 + 2\ H_2 \xrightarrow{Pd} RCO_2P(O)(OH)_2 + 2\ PhCH_3 \qquad (11c)$$

Phenyl protecting groups are stable to palladium catalysts, but may be removed by catalytic hydrogenolysis over Adams's platinum catalyst. The reaction takes longer and may be conducted in stages (methods 11d,e):

$$ROP(O)(OPh)_2 + 4\ h_2 \xrightarrow{Pt} ROP(O)(OPh)OH + C_6H_{12} \qquad (11d)$$

$$ROP(O)(OPh)OH + 4\ H_2 \xrightarrow{Pt} ROP(O)(OH)_2 + C_2H_{12} \quad (11e)$$

This method has been used to prepare N-phospho derivatives of glutamic acid[89] and O-phospho derivatives of serine[15,19,100,101,111] and their peptides.[16-18] A preliminary treatment with palladium catalyst is often carried out to remove the carboxyl- or amino-protecting groups,[15-18,101,111] but some difficulties with this procedure have been noted.[17,103]

8. Hydrolysis of Alkyl and Aryl Protecting Groups

This method, coupled with method 2, is sometimes used for the synthesis of N- and O-phosphoamino acids and their derivatives, but is not as general as method 11 since the outcome tends to vary with the nature of the R and R′ groups and the reaction conditions.

Saponification of diphenyl phosphoramidates with alkali (sodium, potassium, or barium hydroxide) results in stepwise removal of the phenyl groups, together with any carboxy-protecting ester group (methods 12a,b). No P-N bond cleavage occurs, provided the conditions are sufficiently alkaline:

$$RNHP(O)(OR')_2 + NaOH \rightarrow RNHP(O)(OR')ONa + R'OH \quad (12a)$$

$$RNHP(O)(OR')ONa + NaOH \rightarrow RNHP(O)(ONa)_2 + R'OH \quad (12b)$$

Method 12a[81]

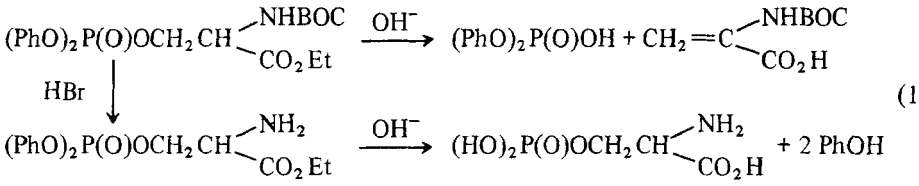
A suspension of 0.01 mol of the glycine derivative (R = CH_2CO_2Me, R′ = Ph) and 6.7 g of barium hydroxide octahydrate in 60 mℓ of water is shaken for 4 hr. Carbon dioxide is then bubbled through the mixture and the precipitated barium carbonate filtered. The product crystallizes as the dihydrate upon the addition of 60 mℓ of methanol to the filtrate; yield 70%.

Method 12b[81]

A solution of 4 g (0.01 mol) of the dihydrate from 12a and 2 g of barium hydroxide octahydrate in 70 mℓ of water is heated at 100°C for 30 min under nitrogen. After the mixture has been cooled, the amorphous precipitate is collected and washed with dilute aqueous barium hydroxide and methanol; yield, 3.2 g (88%). The precipitate (0.71 g, 0.001 mol) is shaken for 10 min with 7 mℓ of 30% acetic acid and then washed successively with cold water, 50% ethanol and anhydrous ethanol; yield of crystalline *N*-phosphoglycine barium salt, 84%.

These methods have been applied to derivatives of glycine,[81,93] leucine,[96] tyrosine,[81] glycyltyrosine,[81] and [^{32}P]-glycine.[85] The potential risk of racemization of the amino acid moiety has been noted[81] but not verified. Alkyl and benzyl esters of the N-phosphoamino acids are stable to alkali, and carboxyl ester groups may be saponified selectively in their presence (see method 14c). Migration of the phosphoryl group from nitrogen to oxygen occurs during the hydrolysis of the diisopropyl esters of *N*-phosphoserine and -threonine with hydrochloric acid (see Section III.A.4).

Saponification of the diphenyl esters of *O*-phosphoserine and -threonine with 1 *N* sodium hydroxide at room temperature yields the free O-phosphoamino acids, provided that the N-protecting group is removed first. Fortunately, both phenyl groups are liberated, for the products undergo β-elimination on heating in alkali and the phosphate group is lost. The N-carbobenzoxy derivatives eliminate diphenyl phosphate even at room temperature (method 12c):



(12c)

This method has been applied to O-phospho derivatives of serine, threonine, serylglycine, and serylglutamic acid. Yields range from 10 to 33%.[100]

Dibenzyl esters of *O*-phosphoserine and its dipeptides lose one of the two benzyl groups when treated with sodium iodide in acetone at reflux (method 12d).[21,23,25,27,29,99,102] This reaction is unique to tertiary phosphate esters and leaves N- and O-protecting groups untouched:

$$ROP(O)(OCH_2Ph)_2 + NaI \rightarrow ROP(O)(OCH_2Ph)ONa + PhCH_2I \qquad (12d)$$

Dibenzyl esters of O-phosphoamino acid anhydrides are debenzylated, together with the amino protecting groups, by dry hydrogen bromide in carbon tetrachloride (method 12e).

$$RCO_2P(O)(OCH_2Ph)_2 + 2\ HBr \rightarrow RCO_2P(O)(OH)_2 + 2\ PhCH_2Br \qquad (12e)$$

This method has been applied to O-phospho anhydrides of glycine,[40,44] alanine,[40] aspartic acid,[41] glutamic acid,[41] and leucine.[41]

B. The Amino Group

1. N-Aryl Derivatives

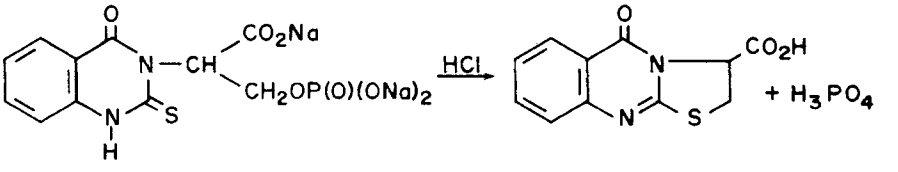
The reaction of amino groups with fluorodinitrobenzene has been used to distinguish the N-phospholysines prepared by method 1b. N_ϵ-Phospholysine gives N_α-DNP-lysine in 80% yield after mild acid treatment to cleave the P-N bond; conversely, N_α-phospholysine gives N_ϵ-DNP-lysine.[58,60] Reactions with *O*-phosphoserine and its peptides are said to proceed without difficulty, but no details are given.[23]

2. N-Acyl Derivatives

Acetylation of *O*-phosphoserine or -threonine with acetic anhydride in the presence of sodium hydroxide yields the N-acetyl derivatives (method 13a).[35,178] N_τ-Phosphohistidine, in the form of its calcium salt, yields the N_α-acetyl derivative.[137] The N-formyl derivative of *O*-phosphotyrosine, prepared from *N*-formyltyrosine by method 1e, is hydrolyzed to *O*-phosphotyrosine by boiling 2 *N* hydrochloric acid, but this method is unsatisfactory for *O*-phosphoserine.[77] The N-trifluoroacetyl derivatives of 3,5-diiodo-*O*-phosphotyrosine and *O*-phosphothyroxine esters are hydrolyzed, together with the ester groups, by mild alkali (method 13b).[78]

A considerable number of N-carbobenzoxy (BOC) derivatives have been prepared, mostly by method 2c. Attempts to react BOC chloride with *O*-phosphoserine or -tyrosine failed.[14,23] BOC serves as a protective group for the NH_2 function and is readily removed, together with any other benzyl groups, by catalytic hydrogenolysis (method 13c).[14-23,26,95,99,101,104,111] It is also cleaved by hydrolysis with hydrobromic acid[26,100] or phosphonium iodide[14] (method 13d).

O-Phosphoserine reacts with *o*-carbomethoxyphenyl isothiocyanate in the presence of sodium hydroxide giving the hydroquinazoline in 69% yield (method 13e). The latter, upon acidification, undergoes ring closure with loss of the phosphate group. Serine and cysteine react similarly giving the same final product.[179]



The N-trimethylsilyl derivatives of *O*-phosphoserine and -threonine are useful for the characterization of these compounds by GC-MS.[180] The derivatives are prepared by treating the O-phosphoamino acids with bis(trimethylsilyl)acetamide or bis(trimethylsilyl)trifluoroacetamide in acetonitrile for several hours at room temperature (method 13f):

$$(HO)_2P(O)OCHRCH\begin{matrix}NH_2\\CO_2H\end{matrix} + 4\ MeCON(SiMe_3)_2 \rightarrow$$
$$(Me_3SiO)_2P(O)OCHRCH\begin{matrix}NHSiMe_3\\CO_2SiMe_3\end{matrix} + 4\ MeCONH_2 \qquad (13f)$$

C. The Carboxyl Group

The carboxyl group of *O*-phosphoserine is esterified selectively by thionyl chloride in methanol (method 14a). The yield is 100% for the DL-isomer and 74% for the L-isomer.[23] The method, however, fails to give a pure benzyl ester.[23] Methanol alone does not esterify *O*-phosphoserine, but *O*-phosphoserylglutamic acid is less resistant.[21]

Esters of the N- and O-phosphoamino acids are usually prepared from the amino acid esters by methods 2a, b, or c. Benzyl esters are preferred if the ultimate products are the free N- and O-phosphoamino acids, for the benzyl groups are readily removed, together with any other benzyl groups present, by catalytic hydrogenolysis over palladium on charcoal,[14-18,20-23,80,81,87,88,92,94-96,99,101,102,114,119] platinum oxide,[16,100] or both[101,111] (method 14b). N-Phosphoamino acids sometimes suffer P-N scission during the hydrogenolysis.[87,88,92] Attempts to remove the benzyl group by reduction with sodium in liquid ammonia were unsuccessful.[14,87]

Alkyl groups may also be removed from alkyl esters of the N- and O-phosphoamino acids by mild alkaline hydrolysis (method 14c). This method is usually applied to methyl or ethyl esters[14,78,80,81,92,100,106,107] (see also References 86 and 89), and occasionally to benzyl esters.[88,99] The method is selective for the carboxyl ester, provided that the N- or O-phosphoryl ester is dialkyl or dibenzyl. Diphenyl esters are partially hydrolyzed under these conditions (see method 12a).

If the phosphate ester is base-sensitive but stable to acid, the carboxyl ester group may be removed by hydrolysis with strong acids such as hydrochloric acid[84] or hydrobromic acid[40,41,44] (method 14d). Weak acids such as acetic acid are ineffective.[88]

Unsubstituted amides of N- and O-phosphoamino acids may be prepared by phosphorylation of the amino acid amides (methods 2a-c), by catalytic reduction of the phosphorylated amino acid azides over Raney nickel (method 14e),[114] or by ammonolysis of the phosphorylated amino acid esters (method 14f).[83,87,98] The P-ester groups in the last are not converted to amides, but are prone to transesterify.[87] Hydrazinolysis of the phosphorylated amino acid esters yields the corresponding hydrazides, which can be converted to anilides via the azides (method 14g).[92]

The phospho group can be used as an amino-protecting group in peptide synthesis. Coupling of two amino acids, each protected with suitable blocking groups, is accomplished either by the mixed phosphoric anhydride route (see Section III.A.3) or by treatment of the mixture with *N,N'*-dicyclohexylcarbodiimide (method 14h).[92,107] Yields for the latter method are 73 to 91%.

$$RCO_2H + R'NH_2 + (C_6H_{11}N{=})_2C \rightarrow RCONHR' + (C_6H_{11}NH)_2CO \qquad (14h)$$

The protecting groups are subsequently removed by hydrolysis with hydrobromic acid or by catalytic hydrogenolysis, leaving the dipeptide phosphorus-free.[92] Coupling by the mixed anhydride route with carboxylic rather than phosphoric anhydrides is successful only with glycine and the yields are low.[92]

D. The Hydroxyl Group

The alcoholic hydroxyl group of *N*-phosphoserine derivatives can be benzylated with benzyl bromide and sodium hydride in 61% yield (method 15a).[107] A protecting *t*-butyl group, if present, can be removed by treatment with trifluoracetic acid in 91% yield (method 15b).[107]

IV. PHYSICAL PROPERTIES

A. Colligative Properties

1. Melting Point (mp)

Many of the compounds listed at the end of this review are well-defined crystalline compounds with sharp melting points, but the unsubstituted N- and O-phospho amino acids and peptides tend to melt with decomposition at temperatures that vary appreciably with the rate of heating.[15] Some crystallize as hydrates that retain water tenaciously. *O*- Phospho-DL-serine, for example, crystallizes from water as a hydrate that decomposes when dried at 120°C, but can be prepared in anhydrous form by precipitation from aqueous solution with ethanol and ether.[15] The powder and crystal X-ray diffraction patterns of the two forms are different.[15,181,182] Hydration of the calcium salt of *O*-phosphoserine has been studied by thermogravimetry (TGA).[183]

2. Boiling Point (bp)

Boiling points have been reported for a few N-phospho derivatives of glycine[79,80,83,91,98,146] and alanine,[91] but the majority of the compounds are too thermally unstable to be distilled even under reduced pressure. *O*-Phosphoserine and -threonine have been converted to their thermally stable N,O,O,O-tetrakis(trimethylsilyl) derivatives for characterization by GC-MS.[180]

3. Sedimentation Coefficient

Sedimentation coefficients have been determined for phosphorylated human serum albumin, hemoglobin and globin,[71] and horse serum albumin.[74] The values are all identical to those of the starting proteins.

4. Viscosity

The viscosity of crystalline egg albumin[75] and horse serum albumin[74] increases with the extent of phosphorylation, and is somewhat dependent on pH and salt concentration. The original viscosity (fluidity) is not completely restored upon dephosphorylation.[74,75]

5. Density

Densities have been determined for a few N-phospho derivatives of glycine.[83,98]

B. Optical Properties

1. Specific Rotation $[\alpha]_D$

Specific rotations have been reported for the majority of the compounds of this review other than those based on glycine or DL-amino acids. Except for *O*-phospho-D-serine[15] and *O*-phospho-D-seryl-L-leucine,[18] all of the compounds in the following list have the L configuration.

N-Phospho: alanine,[106,107] cysteine,[107] serine,[107] aspartic acid,[107] threonine,[107] proline,[106,107] glutamic acid,[107] valine,[106,107] methionine,[107] leucine,[106] isoleucine,[107] arginine,[107] phenylalanine,[88,107] tyrosine,[107] alanylarginine,[97] phenylalanylglycine,[92] leucylarginine,[97] phenylalanylarginine,[97] tyrosylarginine,[97] and prolylleucylglycine.[107]

O-Phospho: serine,[15,23,99,100,111,127] threonine,[23] hydroxyproline,[123] tyrosine,[14,77,123] various

dipeptides of serine[17,21,23,100,107] and tyrosine,[14,107] and some tripeptides of serine[20,23,102] and tyrosine.[14]

S-Phospho: cysteine.[107]

The optical activity of compounds that possess free NH_2, CO_2H, or PO_3H_2 groups is pH-dependent. This creates a problem if the compounds are sensitive to acid or base. Unsubstituted O-phospho compounds can be measured in strongly acidic media such as 2 *N* HCl, but N-phospho compounds are too unstable. Many of the N-phospho derivatives listed above contain a free CO_2H group, but none contain a free PO_3H_2 group.

Resolution of a DL isomer into its optical antipodes has been accomplished in one instance. L-Phosphoserine, $[\alpha]_D^{25}$ + 16.3°, was prepared from DL-phosphoserine by fractional precipitation of its brucine salt.[127]

The use of alkali with amino acid derivatives is often avoided because of racemization and consequent loss of optical activity, but the only verified instance involving a phospho derivative is the racemization of threonine, which occurs when *O*-phospho-L-threonine is hydrolyzed in 5 *M* HCl.[23]

2. Circular Dichroism (CD)

Comparison of the CD spectra of β-lactoglobulin before and after phosphorylation shows some permanent change in the structure of the protein but little loss of α-helical structure (16 vs. 18%).[73] Phosphorylation of clupeine raises its α-helical content to 50%, an effect ascribed to the solvent.[64]

3. Refractive Index (n_D)

Refractive index values have been reported for some N-phospho derivatives of glycine[80,83,91,98] and alanine,[91] and for some O-phospho derivatives of glycylserine and serylglycine.[16] All but two are full esters. Phosphorylated gelatin exhibits flow birefringence in acid solution, though gelatin itself does not.[110]

C. Spectroscopic Methods

Spectroscopic methods that have been employed for product identification are infrared (IR), ultraviolet (UV), nuclear magnetic resonance (NMR), mass spectrometry (MS), electron spin resonance (ESR), and X-ray, including XPS. The ESR spectra are discussed elsewhere (III.A.2) in connection with the radiolysis of phosphoserine.

1. Infrared Spectra (IR)

IR spectra have been reported for N-phospho derivatives of glycine,[88,91,144,146,184] alanine,[91,184] serine,[144] threonine,[184] glutamic acid,[51,184] valine,[91,131,132,184] histidine,[144] cystine,[91,184] leucine,[184] lysine,[144,184] arginine,[184] phenylalanine,[91,184] tyrosine,[184] tryptophan,[91,184] and glycyltyrosine;[184] and for O-phospho derivatives of serine,[15,23,111,184] tyrosine,[128,184] glycylserine,[16] glycylserylglycine,[16] and aspartylserylglycine.[23]

The N-phosphoramidic acids and their esters have characteristic absorption bands in the 10.62 to 10.80 and 11.38 to 11.54 μm regions, respectively, tentatively assigned to the N-P bond. These bands serve to distinguish the N-phosphoamino acids, which crystallize as hydrates, from the isomeric phosphate salts.[91]

2. Ultraviolet Spectra (UV)

The usefulness of UV is limited to amino acids that are aromatic or have aromatic substituents. The former group comprises N-phospho derivatives of histidine[57,137] and phenylalanine,[184] O-phospho derivatives of tyrosine,[128,154,185] and phospho derivatives of the proteins hemoglobin[71] and human serum protein.[71] The latter group comprises N-phospho derivatives of glycine[93] and O-phospho derivatives of serine,[100,102] threonine,[100] serylglycine,[100] serylglutamic acid,[100] aspartylserylglycine,[102] and aspartylserylglutamic acid.[102]

UV spectra are useful not only for characterization but also for measuring rates of hydrolysis of the N-P bond in glycine[93] or histidine[57,137] derivatives and of base-catalyzed β-elimination in serine[99,100,102,167,168] or threonine[100,173] derivatives.

The fluorescence of tyrosine under UV excitation is quenched by alkali at pH 9.7, owing to dissociation of the phenolic OH, but in *O*-phosphotyrosine quenching does not occur until the pH reaches 14.[154] Fluorescence is also used for the detection of N- and O-phosphoamino acids in HPLC.[139,177]

3. Nuclear Magnetic Resonance Spectra (NMR)

^{1}H NMR spectra have been reported for N-phospho derivatives of glycine,[49,93,144,146] serine,[144] histidine,[137,141,144] leucine,[96] lysine,[144] phenylalanine,[96,184] glycylalanine,[96] alanylalanine,[96] isoleucylalanine,[96] leucylphenylalanine,[96] and leucyltryptophan,[96] for O-phospho derivatives of serine,[167,168,178,186-189] threonine,[190,191] and for the phosphoprotein HPr.[141]

^{31}P NMR spectra have been reported for N-phospho derivatives of histidine[140,141,192] and arginine,[193] for O-phospho derivatives of serine[64,74,157,158,194-198] and threonine,[195] and for the phosphoproteins clupeine,[64] β-lactoglobulin,[73] protein HPr,[141] and histone 4.[140]

^{13}C NMR spectra have been reported for O-phospho derivatives of serine[178,199] and threonine.[190] In addition, some salts of *O*-phosphoserine have been examined by ^{23}Na or ^{113}Cd NMR spectroscopy.[200,201]

With few exceptions, the NMR spectra listed above share a common feature: a sensitivity to pH with respect to both chemical shift and coupling constant. The exceptions are those compounds that do not possess a free P-OH group. For *O*-phosphoserine, the ^{31}P chemical shift changes from +0.1 ppm to +4.0 ppm as the pH increases from 3 to 9.[195] The magnitude of these changes is due to conformational changes caused by the electrostatic interaction between the amino and phosphate groups.[178] The acid dissociation constants (pK_a) for *O*-phosphoserine, based on ^{13}C NMR measurements, are 2.3 (CO_2H), 6.5 (POH), and 9.9 (NH_2), in good agreement with titration data.[199] Similar measurements on ^{1}H NMR and ^{31}P NMR spectra have been used to calculate the acidities of the C_2 and C_4 imidazole protons in N-phospho histidines.[141]

^{31}P NMR chemical shielding tensors have been measured for *O*-phosphoserine in powder[197,202] or single crystal[198] form. The principal elements for the latter are −48, −2 and 51 ppm. The analysis of the ^{1}H NMR spectra of *O*-phosphoserine and -threonine, which contain protons that are strongly coupled to the phosphorus and to each other, is solved by heteronuclear two-dimensional subspectral analysis.[189,191]

4. Mass Spectra (MS)

Mass spectra have been reported for a few N-phospho derivatives of glycine[93,146] and O-phospho derivatives of serine and threonine.[180] MS is used for molecular weight determination[93,146] and for measuring the relative abundance of oxygen isotopes after a hydrolysis in ^{18}O-enriched acetate buffer,[93] and GC-MS for characterizing the O-phospho compounds in the form of trimethylsilyl derivatives.[180]

5. X-Ray Spectra

X-ray crystallographic data have been reported for *O*-phospho-D-, DL-, and L-serine[15,181,182,198,203] and *O*-phospho-DL-threonine.[126] All crystallize in the orthorhombic system (space group $P2_12_12_1$, Z = 4) except *O*-phospho-DL-serine, which crystallizes with a mole of water in rectangular prisms (space group C2/c, Z = 8). The molecules exist as zwitterions in which the amino groups are protonated by the phosphate groups and the carboxyl groups are not ionized.

The P_{2p} electron binding energies for *O*-phosphoserine and *O*-phosphothreonine, measured by X-ray photoelectron spectroscopy (XPS), are 133.5 and 133.7 eV, respectively.[204] This method holds little promise for distinguishing phosphorus atoms in biological systems.[204]

D. Chromatographic Methods

Chromatographic methods that have been employed for product separation and identification are paper (PC), thin layer (TLC), ion exchange (IEC), high performance liquid (HPLC), gel filtration (GFC), and charge transfer (CT). Countercurrent distribution methods are also listed. Preparative methods in this group are scarce.

1. Paper Chromatography (PC)

R_f values have been reported for N-phospho derivatives of glycine,[52,86,93,143] alanine,[86,136,143] cysteine,[143] serine,[86,136,143] valine,[131,132] methionine,[143] histidine,[136,143] cystine,[143] arginine,[62,95] phenylalanine,[86] tyrosine,[86,143] tryptophan,[86,143] and peptides of glycine;[52] for O-phospho derivatives of serine,[15,17,19,23,29,33,35,65,99,102,103,111,116,124,205-210] threonine,[23,206-209] tyrosine,[86,185] and serine-containing dipeptides[14,16,17,21-23,29,116] and tripeptides;[16,20,23] and for the O-phosphoanhydrides of aspartic acid[119] and leucine.[42]

Some useful solvent mixtures are *n*-butanol/acetic acid/water (40/10/50) for descending PC and phenol/water (80/20) for ascending PC.[21] The paper is sprayed with ninhydrin for NH_2 and ammonium molybdate for phosphate.

2. Thin Layer Chromatography (TLC)

R_f data have been reported for N-phospho derivatives of glycine,[106,144] alanine,[106,107] cysteine,[107] serine,[107,144] aspartic acid,[107] threonine,[107] proline,[106,107] glutamic acid,[50,51,107] valine,[106,107] methionine,[107] histidine,[144] leucine,[106] isoleucine,[107] lysine,[133,144] arginine,[107] phenylalanine,[107] and tyrosine,[107] and for O-phospho derivatives of serine,[113,133,211-214] threonine,[133,211,214] and tyrosine.[214]

Some useful solvent mixtures are 6.5:3.5 ethanol/water,[144] 1% formic acid,[211] and 1:2:1:1 acetic acid/*n*-propanol/water/phenol.[213] Detection is usually accomplished by spraying with ninhydrin for NH_2 and acid molybdate for phosphate. Some two-dimensional systems have been described.[212,214]

3. Ion Exchange Chromatography (IEC)

Retention times have been reported for N-phospho derivatives of valine,[131,132] histidine,[57,137,215] lysine,[58,139,215] and arginine;[62,63,139] and for O-phospho derivatives of serine,[31,116,216-227] threonine,[126,216,218,220,221,224,225,227] hydroxyproline,[216,221] hydroxylysine,[221] tyrosine,[128,216] and serine-containing dipeptides[17,21-23,29,220] and tripeptides.[20,23] All of these compounds contain unsubstituted $P(O)(OH)_2$ groups, and most also contain unsubstituted CO_2H groups.

O-Phosphoserine and -threonine elute with cysteic acid in automated amino acid analyzers such as the Beckman-Spinco® 120 or the Technicon® TSM.[219,221-223,226] To separate the O-phosphoamino acids from each other it is necessary to lengthen the column[227] or to use a different type of resin. Cation exchange resins such as Dowex® 50-X8 may be used if the eluent is strongly acidic,[218] but better separations are achieved with anion exchange resins such as Dowex® 1-X8 and acetate or formate buffers.[216,224,225] Dowex® 1-X2 is preferred for the O-phospho peptides.[220]

The N-phosphoamino acids, which are acid-sensitive, are purified by anion exchange chromatography on resins such as Dowex® 1 with salt[63,137] or potassium carbonate[57,58] gradient eluents.

Some preparative-scale methods have been reported.[57,137,220]

4. High Performance Liquid Chromatography (HPLC)

An HPLC method has been developed for the separation of both acid-stable and acid-labile phosphoamino acids on a single-anion exchange column, Chromex® DA-X12-11.[139] *N*-Phosphoarginine and -lysine are separated by means of a low-ionic-strength KH_2PO_4 buffer

at pH 7.5, and *N*-phosphohistidine, *O*-phosphoserine, and *O*-phosphothreonine by means of a high-ionic-strength KH_2PO_4 buffer at pH 6.3. The compounds are detected fluorometrically after derivatization with *o*-phthalaldehyde.[60,139,140] Precolumn derivatization has been employed for amino acid mixtures containing *O*-phosphoserine.[177]

5. Gel Filtration Chromatography (GFC)

The phospho derivatives of the proteins clupeine Z,[64] clupeine YI,[64] and β-lactoglobulin[73] have been purified by GFC on Sephadex® C-25 or G-25, employing gradient elution with sodium chloride. The method is useful not only for separating phosphoproteins from low-molecular-weight byproducts, but also for separating products of differing degrees of phosphorylation. Thus, clupeine Z, which contains three serine residues per mole, can be fractionated after phosphorylation into products containing one, two, and three phosphate residue per mole.[64]

GFC is also useful for purifying low-molecular-weight compounds such as *N*-phosphoglutamic acid.[49,51]

6. Charge Transfer Chromatography (CT)

The behavior of serine and phosphoserine toward CT chromatography on acriflavin-modified Sephadex® G-25 has been reported. Retention is slight with phosphoserine and negligible with serine, compared to nucleotides such as AMP.[228]

7. Countercurrent Distribution

For compounds that resist purification by classical methods, countercurrent distribution has proved to be useful. Phosphorylated derivatives of serine, aspartylserylglycine, and aspartylserylglutamic acid have been purified by 50 to 100 transfers with solvent systems such as chloroform/*n*-hexane/methanol/water (31:30:40:10) or methanol/water/ether (2:3:5).[102]

E. Electrophoretic Methods

1. Paper Electrophoresis (PE)

Electrophoretic mobilities have been reported for N-phospho derivatives of glycine and its peptides,[52] glutamic acid,[51] and histidine,[57,137,141] and for O-phospho derivatives of serine,[25,26,29,100,102,206,229,230] threonine,[206,230] tyrosine,[128,185] and glycylserine.[27,29] All but two of these compounds contain unsubstituted $P(O)(OH)_2$ groups, and most also contain unsubstituted CO_2H groups.

Typical conditions for electrophoresis are pH 2.2 (formic acid buffer) for the O-phospho derivatives[206,230] and pH 8.0 (ammonium acetate buffer) for the N-phospho derivatives.[137] Spots are detected by ninhydrin (NH_2), acid molybdate (PO_3H_2) or Pauly reagent (imidazole). Movement is toward the anode, with one exception,[100] and is retarded by Co(II) because of complexing.[137]

2. Thin-Layer Electrophoresis (TLE)

Electrophoresis on thin-layer plates has been used to separate *N*-phosphoglutamic acid from its hydrolysis products[50] and the O-phospho derivatives of serine, threonine, and tyrosine from each other.[214] A two-dimensional system has been described.[214]

3. Gel Electrophoresis

Electrophoretic methods are useful for determining the homogeneity of phosphorylated proteins. Gel electrophoresis has been applied to phospho derivatives of human serum protein and hemoglobin,[71] β-lactoglobulin,[73] horse serum albumin,[74] histone 4,[140] soy protein,[133] protein HP_1,[141] and insulin.[136] In one instance, measurement of electrophoretic mobility vs. pH was used to establish the isoelectric point at pH 3.9, in agreement with solubility data.[74]

F. Acid Dissociation Constants (pK_a)

Acid dissociation constants have been reported for N-phospho derivatives of glycine,[80,93] histidine,[137] and lysine,[59] and for O-phospho derivatives of serine,[19,65,100,124,157-161,167,168,231,232] threonine,[157,173,190] and serine-containing dipeptides[19,21] and tripeptides.[19]

The unsubstituted O-phosphoamino acids and peptides contain four ionizable hydrogens, but only three of these are measurable by direct titration. The pK_a values for *O*-phospho-DL-serine, for example, are 2.11 (CO_2H), 5.62 (PO_3H^-), and 9.72 (NH_3^+) by potentiometric titration,[157] or 2.3, 6.5, and 9.9, respectively, by ^{13}C NMR.[199] The first POH group is too strongly acidic to be measured by either of these methods. It can, however, be determined by differential spectrophotometry, using 2,6-dinitrophenol as an indicator. This method gives pK_a values of 0.72 (PO_3H_2) and 2.14 (CO_2H) for the two strongest groups.[232]

Only two of the three ionizable hydrogens in monoesters of *O*-phosphoserine and its peptides can be measured by direct titration.[19]

The pK_a values of DL-serine itself are 2.12 (CO_2H) and 9.02 (NH_3^+). Comparison with the data given above shows that phosphorylation increases the basicity of the amino group but has no effect on the carboxyl group. The increase in basicity of the amino group is matched by an increase in the acidity of the PO_3H^- group relative to other O-phosphate monoesters.[19]

The significance of the pK values of synthetic O-phosphopeptides in relation to enzyme action of biological systems has been discussed at length.[19,21]

V. USES

Derivatives of *N*-phosphoaspartic acid and their salts are useful for treating psychic and physic asthenia.[233] Salts of N_π-phosphohistidine and N_ϵ-phospholysine are useful as medicaments for fatigue and as cardiotonics.[59,138] The LD_{50} values for N_ϵ-phospholysine and $N_\alpha N_\epsilon$-diphospholysine in mice are 2430 and 622 mg/kg, respectively.[59] A subcutaneous dose of 5 mg/kg of the triethyl ester of *N*-phosphoglycine is lethal to white mice.[82]

O-Phosphoserine is useful as a stabilizer for bleach-fix solutions in color photography.[234] Its calcium salt is useful for the treatment of phosphocalcium deficiency.[235] The silver salts of O-phosphoserine and -threonine are useful against dermatosis, e.g., eczema.[236] Salts of *O*-phosphoserine and diisopropylamine have anti-Parkinson's activity and low side effects.[237]

VI. LIST OF COMPOUNDS

All phospho compounds are listed under the amino acid, peptide, or protein from which they are derived. Amino acids are listed in numerical order according to molecular formula, starting with glycine and ending with thyroxine. The L-isomer is assumed unless stated otherwise in the original paper. These criteria also apply to the peptide derivatives, which follow the amino acids. The protein derivatives, which follow the peptides, are grouped by species.

A. Amino Acid Derivatives

Glycine

N-Phospho Derivatives

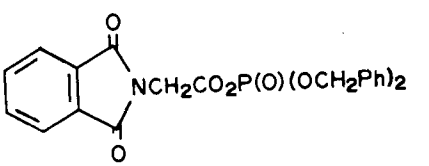
$(HO)_2P(O)NHCH_2CO_2H$ 1a,[10,48] 9a,[135] 9b,[143,144] 11a,[49,81,87] 12b.[81] Hygroscopic solid mp ~ 115°C d., IR,[144] PC,[52,143] TLC,[144] ^1H NMR,[49,144] paper electrophoresis,[52] ninhydrin reaction.[81] Mg salt, wh amorph pw,[10] gran so;[48] Ca salt, 3:2;[48] Ba salt, 1:1, cr,[81,144] 3:2, amorph.[81,143]

–,[^{32}P] labeled 2a/12ab. K salt.[85]

$(MeO)_2P(O)NHCH_2CONH_2$ 14f. Mp 112—3°C.[83]
$(MeO)_2P(O)NHCH_2CO_2Et$ 2a,[83] 7c.[146] Mp 55—6°C,[83] 56°C,[146] IR, ^1H NMR.[146]
$(EtO)_2P(O)NHCH_2CO_2H$ 2b,[98] 14b, 14c. [80] Oil, n_D^{20} 1.4510,[98] d_4^{20} 1.2579,[98] pK_a 3.85.[80] Ba salt, 1:2, wh so;[80] guanidine salt, 1:1 mp 159—60°C.[80]
$(EtO)_2P(O)NHCH_2CONH_2$ 14f. Mp 73—6°C d.[83]
$(ETO)_2P(O)NHCH_2CONHOH$ 14f. Visc oil, n_D^{20} 1.4455, d_4^{20} 1.1840.[98]
$(ETO)_2P(O)NHCH_2CONHMe$ 14f. Visc liq, bp 180°C d (1 mm), n_D [20] 1.4695, d_4 [20] 1.2126.[98]
$PhOP(O)NHCH_2C(O)O$ 4b. Ppt.[108]
$HO(PhO)P(O)NHCH_2CO_2H$ 4a,[108,109] 11a,[92] 12a.[81,93] IR,[184] ^1H NMR, UV, PC, pK_a 1.9, 4.12.[93] Na salt, 2:1, sm cubes;[108] K salt, 2:1, ppt;[93] Ba salt, 1:1 dihydrate, cr.[81,108]
$(EtO)_2P(S)NHCH_2CO_2Et$ 2a. Bp 116°C (1 mm), n_D^{20} 1.4720, d_4^{20} 1.1451.[82]
$(EtO)_2P(O)NHCH_2CO_2Et$ 2a,[80,82] 2b,[91] 8d.[79,80] Bp 123—8°C (0.3 mm),[80] 126—32°C (0.5 mm),[91] 135.5°C (1 mm),[82] n_D^{20} 1.4390,[82] n_D^{25} 1.4340,[91] n_D^{30} 1.4338,[80] d_4^{20} 1.1495, IR,[91,184] L_D 5 mg/kg.[82]
$(i\text{-}PrO)_2P(O)NHCH_2CO_2H$ 14b, 14c. Col visc oil.[80] Guanidine salt, 1:1, mp 167—8.5°C.[80]
$(PrO)_2P(O)NHCH_2CONH_2$ 14f. Mp 68—73°C. d.[83]
$(i\text{-}PrO)_2P(O)NHCH_2CONH_2$ 2a,[79] 14f.[83] Fine nd, mp 81—4°C,[79] 91—4°C.[83]
$(i\text{-}PrO)_2P(O)NHCH_2CO_2Me$ 2a. Bp 114—20°C (0.1—0.2 mm), n_D^{27} 1.4314.[80]
$(PrO)_2P(O)NHCH_2CO_2Et$ 2a. Bp 141—2°C (1 mm), n_D^{20} 1.4375, d_4^{20} 1.0926.[83]
$(i\text{-}PrO)_2P(O)NHCH_2CO_2Et$ 2a.[79,83] Mp 28—29°C,[79] bp 115—28°C (0.5 mm),[79] 129—30°C (1 mm),[83] n_D^{20} 1.4332, n_4^{20} 1.0856.[83]
$(BuO)_2P(O)NHCH_2CO_2H$ 2b,[98] 14c.[80] Oil, n_D^{20} 1.4500, d_4^{20} 1.0888.[98] Guanidine salt, 1:1, mp 156.5—7°C.[80]
$(BuO)_2P(O)NHCH_2CONH_2$ 14f. Mp 77—9°C.[83]
$(i\text{-}BuO)_2P(O)NHCH_2CONH_2$ 14f. Mp 93—5°C.[83]
$PhP(O)(OMe)NHCH_2CO_2Et$ 7c. Col oil, bp 190°C (0.03 mm), IR, ^1H NMR, MS.[146]
$(BuO)_2P(O)NHCH_2CO_2Me$ 2a. Bp 145—7°C (0.15 mm), n_D^{26} 1.4392.[80]
$(BuO)_2P(O)NHCH_2CO_2Et$ 2a. Bp 160—0.5°C (1 mm), n_D^{20} 1.4408, d_4^{20} 1.0660.[83]
$(i\text{-}BuO)_2P(O)NHCH_2CO_2Et$ 2a. Bp 145—6°C (1 mm), n_D^{20} 1.4375, d_4^{20} 1.0578.[83]
$(EtO)_2P(O)NHCH_2CO_2CH_2Ph$ 2b. Oil.[80]
$Ph_2P(S)NHCH_2CO_2H$ 3, 14c. Mp 118—9°C, TLC. Dicyclohexylamine salt, so.[106]
$Ph_2P(O)NHCH_2CO_2H$ 14c. Mp 129—30°C.[106]
$(PhO)_2P(O)NHCH_2CO_2H$ 14b. Col. gum, IR.[88]
$(PhO)_2P(O)NHCH_2CO_2Me$ 2a.[81] Mp 93°C,[81] IR.[184]
$(i\text{-}PrO)_2P(O)NHCH_2CO_2CH_2Ph$ 2b. Ye. oil.[80]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH_2CO_2H$ 14c. Mp >115°C, dec. 175—8°C.[81]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH_2CO_2H$ 14c.[81,88] Mp 145—7°C d.,[88] 149°C.[81]
$Ph_2P(O)NHCH_2CO_2Et$ 3,[106] 7c.[146] Mp 83—4°C,[146] 96—7°C,[106] IR, ^1H NMR, MS.[146]
$(PhO)_2P(O)NHCH_2CO_2Et$ 2b.[89,93] Mp 76—7°C, [93] 77—8°C,[89] MS.[93]
$(PhCH_2O)_2P(O)NHCH_2CONH_2$ 2b.[87] Mp 103—4.5°C,[87] IR.[91]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH_2CO_2Me$ 2a.[81] Mp 124—5°C,[81] IR.[184]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH_2CO_2Me$ 2a.[81] Mp 89°C,[81] IR.[184]
$(p\text{-}NO_2C_6H_4O)_2P(O)NHCH_2CO_2Bu\text{-}t$ 2b. Mp 113°C.[88]
$(PhCH_2O)_2P(O)NHCH_2CO_2Et$ 2b.[86] Mp 43—5°C, PC,[86] IR.[91,184]
$(PhO)_2P(O)NHCH_2CO_2CH_2Ph$ 2b.[88,92] Mp 60—1°C.[88]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH_2CO_2CH_2Ph$ 2a.[81] Mp 89°C,[81] IR.[184]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH_2CO_2CH_2Ph$ 2b,[88] 8d.[103] Mp 110—1°C,[88] 111—2°C.[103]
$(PhCH_2O)_2P(O)NHCH_2CO_2CH_2Ph$ 2b. Waxy so., mp 143—4°C,[87] ^1H NMR.[49]

O-Phospho Derivatives

$NH_2CH_2CO_2P(O)(OH)_2$ 11c,[114] 12e.[40,44] Heavy oil.[40,44] Ag salt, 2:1, mp 254—8°C;[114] Ba salt, 1:1, silky nd.[114]
$NHCH_2CO_2P(O)OPh$ See above.
$PhCH_2O_2CNHCH_2CO_2P(O)(OPh)OH$ 2d. Ag salt, so.[104]
$NH_2CH_2CO_2P(O)(OCH_2Ph)_2$ 14e. Pr, mp 62°C.[114]



5c. Nd., mp 63-5°C.[105]

$PhCH_2O_2CNHCH_2CO_2P(O)(OCH_2Ph)_2$ 2d,[40,44] 5c.[105] Nd, mp 76.5—7.5°C.[105]

L-Alanine

N-Phospho Derivatives

$(HO)_2P(O)NHCHMeCO_2H$ 5a,[112] 8c,[12,134] 9b.[136,143,145] PC.[136,143] Ba salt, 2:3.[112]
$Ph_2P(S)NHCHMeCO_2H$ 3.[106,107] Mp 120°C, $[\alpha]_D$ −13.7° (EtOH), TLC.[107]
Dicyclohexylamine salt, mp 177—8°C, $[\alpha]_D$ −3.7° (EtOH), TLC.[106]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCHMeCO_2H$ 14c. Mp 60—2°C.[92]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCHMeCO_2Me$ 2b. Mp 88—9°C.[92]

O-Phospho Derivatives

$NH_2CHMeCO_2P(O)(OH)_2$ 12e.[40]
$PhCH_2O_2CNHCHMeCO_2P(O)(OCH_2Ph)_2$ 2d.[40]

DL-Alanine

N-Phospho Derivatives

$(HO)_2P(O)NHCHMeCO_2H$ 1a,[10,48] 1b.[53] Mg salt, 3:2.[10,48]
$(MeO)_2P(O)NHCHMeCONH_2$ 14f. Mp 111—2°C.[87]
$(EtO)_2P(O)NHCHMeCO_2Me$ 2b.[91] Bp 118—9°C (0.5 mm),[91] n_D^{25} 1.4332,[91] IR.[91,184]
$PhOP(O)NHCHMeC(O)O$ 4b. Ppt.[108]
$HO(PhO)P(O)NHCHMeCO_2H$ 4a. Na salt, 2:1, cr; Ba salt, 1:1, cr.[108]
$(PhCH_2O)_2P(O)NHCHMeCONH_2$ 2b.[87] Mp 97—9°C,[87] IR.[91]
$(PhCH_2O)_2P(O)NHCHMeCO_2Me$ 2b.[86] Mp 40—1°C, PC,[86] IR.[184]
$(PhCH_2O)_2P(O)NHCHMeCO_2CH_2Ph$ 2b. Visc oil.[87]

O-Phospho Derivatives

$NH_2CHMeCO_2P(O)(OH)_2$ 11c. Ag salt, 2:1, mp 295—300°C; Ba salt, 1:1, cr pw.[114]
$NHCHMeCO_2P(O)OPh$ See above.
$NH_2CHMeCO_2P(O)(OCH_2Ph)_2$ 14e. Pa ye oil.[114]

L-Cysteine

N-Phospho Derivatives

$(HO)_2P(O)NHCH(CH_2SH)CO_2H$ 9b. PC.[143]
$(HO)_2P(O)NHCH(CO_2H)CH_2SP(O)(OH)_2$ 1be. Ca salt.[54]
$(i\text{-}PrO)_2P(O)NHCH(CH_2SH)CO_2Me$ 2b. Mp ~22°C.[84]
$Ph_2P(S)NHCH(CO_2H)CH_2SCH_2Ph$ 3. Dicyclohexylamine salt, mp 170—1°C, $[\alpha]_D$ +22.5° (MeOH), TLC.[107]
$Ph_2P(S)NHCH(CO_2H)CH_2SP(S)Ph_2$ 3. Cyclohexylamine salt, mp 141—4°C, $[\alpha]_D$ + 7.5° (MeOH) , TLC.[107]
$(PhCH_2O)_2P(O)NHCH(CO_2Me)CH_2SP(O)(OCH_2Ph)_2$ 2b. Ye sirup.[87]

S-Phospho Derivatives

$(HO)_2P(O)SCH_2CH(NH_2)CO_2H$ 1e. Solid.[54]
$(HO)_2P(O)SCH_2CH(CO_2H)NHP(O)(OH)_2$ See above.
$Ph_2P(S)SCH_2CH(CO_2H)NHP(S)Ph_2$ See above.
$(PhCH_2O)_2P(O)SCH_2CH(CO_2Me)NHP(O)(OCH_2Ph)_2$ See above.

D-Serine

$(HO)_2P(O)OCH_2CH(NH_2)CO_2H$ 11de.[15] Mp 170—3°C d,$[\alpha]_D^{21}$ −7.0° (H_2O), −15.6° (HCl), IR, X-ray.[15] Na, Mg, Ca salts, *ab initio* SCF study.[166,238]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 2c. Mp 52—3°C, $[\alpha]_D^{18}$ +3.7° (EtOH), −18.3° ($CHCl_3$).[15]

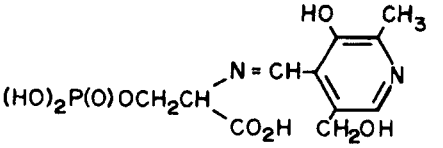
L-Serine

N-Phospho Derivatives

$(HO)_2P(O)NHCH(CH_2OH)CO_2H$ 9b.[136,143-145] IR,[144] ^1H NMR,[144] PC,[136,143] TLC.[144] Ba salt, 1:1.[144]
$Ph_2P(S)NHCH(CH_2OH)CO_2H$ 3. Dicyclohexylamine salt, mp 157—8°C, $[\alpha]_D$ −5.0° (EtOH), TLC.[107]
$Ph_2P(S)NHCH(CO_2H)CH_2OCH_2Ph$ 15a. Dicyclohexylamine salt, mp 138—9°C, $[\alpha]_D$ +5.0° (EtOH), TLC.[107]

O-Phospho Derivatives

$(HO)_2P(O)OCH_2CH(NH_2)CO_2H$ 1d,[66] 4c,[109] 7b,[118] 8a,[120,122,123] 8b,[133] 8c,[12,76] 11b,[99] 11de.[15,100] Pl, mp 165—6°C d,[123] 168—72°C d,[15] 169—70°C,[99] 175—6°C d;[100] use of EDTA in recryst;[239] $[\alpha]_D^{19}$ +7.4° (H_2O),[15] +12° (HCl),[100] $[\alpha]_D^{21}$ +16.2° (HCl),[15] $[\alpha]_D^{22}$ +17.5° (HCl),[99] $[\alpha]_D^{25}$ +16.3° (HCl);[127] pK_a 0.72, 2.14 (25°, KNO_3) by diff'l spect.,[232] 2.04—2.17, 5.771, 9.653—5 (KNO_3, 37°C) by pot. titr.;[160] IR,[15] ^1H NMR,[186,187,189] ^{31}P NMR,[64,73,197,198,202] ESR,[156] X-ray cryst.,[15,182,198,253] PC,[15,205-208] TLC,[133,211-213] IEC,[216-227] HPLC,[139,177] CT,[228] paper electrophoresis.[206,229,230] Na salt, ^{23}Na NMR,[200] *ab initio* SCF;[238] Mg salt, equil. consts.,[160] *ab initio* SCF;[166] Ca salt, 1:2, wh powder,[235] equil consts,[160] *ab initio* SCF,[166] TGA;[183] Ba salt,[123,205] 1:1, small plates,[109] $[\alpha]_D^{19}$ +5.5°,[100] $[\alpha]_D^{25}$ +9.4°;[127] Ln(III) complexes, ^1H NMR;[186,187] Ag salt, 2:1, regular snowwhite cr;[236] Cd salt, ^{113}Cd NMR;[201] Pb salt, 1:1;[123] brucine salt, 1:1, s 100°C, d ~130°C.[127]
$(HO)_2P(O)CH_2CH(NH_2)CO_2Me$ 8a,[129] 14a.[23] Mp 167°C d, $[\alpha]_D^{25}$ +12.0° (HCl), PC.[23] Ba salt, 1:2.[129]
$(HO)_2P(O)OCH_2CH(NHAc)CO_2H$ 13a. ^1H NMR, ^{13}C NMR.[178] Hydrolysis.[38]
$HO(NH_2CH_2CH_2O)P(O)OCH_2CH(NH_2)CO_2H$ 4d. 11e. Mp 139—41°C d, $[\alpha]_D^{23.5}$ −15.0° (H_2O), IR, PC.[111]
$HOP(O)[OCH_2CH(NH_2)CO_2H]_2$ 4d, 11e. Glassy, mp 125°C d, $[\alpha]_D^{23.5}$ −11.6° (H_2O), IR, PC.[111]
$[HOP(O)OCH_2CH(NH_2)CO_2H]_2O$ 6c/11b.[116] Pyridine salt, PC.[35,116]
$HO(PhO)P(O)OCH_2CH(NH_2)CO_2H$ 4c. Oil.[109]
$HO(PhO)P(O)OCH_2CH(NH_2)CO_2Me$ 13c,d. Colorless oil, paper electrophoresis. HBr salt.[26]
$[HOP(O)OCH_2CH(NHAc)CO_2H]_2O$ 13a. Pyridine salt, PC.[35]
$(HO)_2P(O)OCH_2CH(NHCOPh)CONHMe$ 11b.[116] Pyridine salt, mp 145—52°C d,[33] 145—57°C d,[116] PC.[33,116] −, [^{32}P]-labeled.[37]



From *O*-phospho-L-serine and pyridoxal.[167,168] pK_a 6.43, 9.80,[167] ^1H NMR.[167,168]

$(HO)_2P(O)OP(O)(OH)OCH_2CH(NHCOPh)CONHMe$ 2e/11b, 6c/11b. IEC.[31] −, [^{32}P]-labeled. From $H_3{}^{32}PO_4$ and the anhydride of $(PhO)_2P(O)Cl$ and $(HO)_2P(O)OCH_2CH(NHCOPh)CONHMe$.[31]
$PhOP(O)[OCH_2CH(NH_2)CO_2Me]_2$ 13d. Paper electrophoresis.[26]
$(PhO)_2P(O)OCH_2CH(NH_2)CO_2Et$ 13d. HBr salt, nd, mp 67—8°C, UV.[100]
$HO(PhCH_2O)P(O)OCH_2CH(NHCOPh)CONHMe$ 10a, 12d. Mp 108—10°C. Na salt, mp 171—6°C; aniline salt, mp 136—6.5°C.[25]
$[HOP(O)OCH_2CH(NHCOPh)CONHMe]_2O$ 6c. PC, IEC.[116]
$PhCH_2O(PhNH)P(O)OCH_2CH(NHCOPh)CONHMe$ 10a. Paper electrophoresis.[25]
$HO(PhCH_2O)P(O)OCH_2(NHCO_2CH_2Ph)CO_2CH_2Ph$ 12d. Mp 108°C. Na salt, cr.[25]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2Et$ 2c. Mp 39—40°C, $[\alpha]_D^{20}$ −1°, UV.[100]
$(PhCH_2O)_2P(O)OCH_2CH(NHCOPh)CONHMe$ 2c. Mp 90—2°C.[25]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 2c. Mp 53—3.5°C, $[\alpha]_D^{18}$ −3.8° (EtOH), +18.0° ($CHCl_3$).[15]
$PhOP(O)[OCH_2CH(NHCO_2CH_2Ph)CO_2Me]_2$ 4d. Visc bright yellow oil, paper electrophoresis.[26]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 2c. Mp 64—7°C, $[\alpha]_D^{24}$ −6.9° (AcOH).[99]
$(PhCH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 2c. Mp 74°C.[25]
$[PhCH_2OP(O)OCH_2CH(NHCOPh)CO_2CH_2Ph]_2O$ 6c.[25]
$[PhCH_2OP(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph]_2O$ 6c. Mp 95°C.[25]

DL-Serine

N-Phospho Derivatives

$(i\text{-}PrO)_2P(O)NHCH(CH_2OH)CO_2Me$ 2a.[79,84,218] Waxy so, mp 47—50°C,[218] 48—50°C[84]
$(PhCH_2O)_2P(O)NHCH(CH_2OH)CO_2Me$ 2b.[87] Visc sirup,[87] PC.[86]

O-Phospho Derivatives

$(HO)_2P(O)OCH_2CH(NH_2)CO_2H$ 1c,[65] 1d,[65,67,68] 1e,[76] 7b,[118] 8a,[76] 10d,[84,218] 11b,[99] 11de,[15,100] 11e,[101] 12c.[100] Pr, mp 163—4°C d,[100] 164—5°C,[101] d 165—6°C,[65] 166°C,[218] 166—7°C,[84,99] 167—70°C d.[15] IR;[184] ^{1}H NMR, [188] ^{13}C NMR,[199] ^{31}P NMR;[157,158,194-196] X-ray;[181] XPS P_{2p} 133.5 eV;[204] PC;[65,99,124,209,210] TLC;[214] pK_a 2.08, 5.64, 9.74 (KCl, 25°C)[124; see also19,65,100,157-159,161,231] paper electrophoresis;[100] TLE;[214] solubility;[210] catalyst poisoning by, in C, H microanal.[240] Hydrate, mp 153—6°C d, IR, X-ray, PC.[15] Na salt, solubility, PC;[210] K salt, stability consts.[161] Mg salt, stability consts;[124,157,159,161] Ca salt, solubility,[210] PC,[210] stability consts;[124,157,159] Ba salt, 1:1;[76,100] Mn(II) salt, stability consts;[124,157] Fe(III) salt, stability consts;[124] Co(II) salt, ^{31}P NMR,[157] stability consts,[157,158] kinetics;[165] Ni(II) salt, ^{31}P NMR,[157] stability consts,[157-159] kinetics;[165] Cu(II) salt, ^{31}P NMR,[157,158] stability consts;[124,157,158] Zn(II) salt, stability consts;[157,158] Pb salt;[100] brucine salt, 2:1.[127]

–, 2-[^{2}H]-labeled 1d.[67,68] Mp 185°C.[67]

–, [^{32}P]-labeled 1d.[65]

$(HO)_2P(O)OP(O)(OH)OCH_2CH(NH_2)CO_2H$ 2e/11b, 6c/11b. Solid white foam, PC, IEC, paper electrophoresis.[29]

$(HO)_2P(O)OCH_2CH(NH_2)CO_2Me$ 11de,[19] 14a.[23] Mp 173—6°C d,[19] 198°C d,[23] IR,[23] PC,[19,23] pK_a 5.33, 7.83 (KCl, 25°C).[19]

$(HO)_2P(O)OCH_2CH(NH_2)CO_2Et$ 11de. Mp 170—1°C, paper electrophoresis.[100]

$HO(NH_2CH_2CH_2O)P(O)OCH_2CH(NH_2)CO_2H$ 4d, 11b. Mp 180—1°C d, PC.[111]

$HOP(O)[OCH_2CH(NH_2)CO_2H]_2$ 4d, 11b. Mp 120—1°C d, IR, PC.[111]

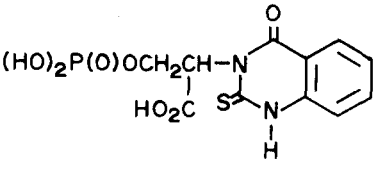
$[HOP(O)OCH_2CH(NH_2)CO_2H]_2O$ PC, paper electrophoresis.[29]

$HO(BuO)P(O)OCH_2CH(NH_2)CO_2H$ 11b. Oil, PC, countercurrent distribution, paper electrophoresis. Hg salt, 1:1, mp 180—2°C d.[102]

$HO(PhO)P(O)OCH_2CH(NH_2)CO_2H$ 3b,[103] 11d.[15,101] Mp 163—5°C, d,[15] 167—8°C,[101] IR.[15] PC,[15,103] pK_a 2.13, 8.79 (KCl, 25° C).[19]

$(HO)_2P(O)OCH_2CH(N{=}CHPh)CO_2H$ 1e. Mg salt, 3: 2.[76]

$HO(PhO)P(O)OCH_2CH(NH_2)CO_2Me$ 11d. Mp 162—3°C d, PC.[19]



13e. Na salt.[179]

$(PhO)_2P(O)OCH_2CH(NH_2)CO_2H$ 13c.[15,17] Mp 129—30°C d,[15] 130— 1°C d,[17] IR,[15] PC.[15,17]

$(Me_3SiO)_2P(O)OCH_2CH(NHSiMe_3)CO_2SiMe_3$ 13f. GC/MS.[180]

$(PhO)_2P(O)OCH_2CH(NH_2)CO_2Et$ 13d. HCl salt, mp 99—100°C; HBr, salt, nd, mp 63—4°C.[100]

$HO(PhCH_2O)P(O)OCH_2CH(NHCO_2Bu\text{-}t)CO_2Bu\text{-}t$ 6b. Col oil. Cyclohexylamine salt, 1:1, mp 173—5°C, TLC; Ba salt, 1:2, powder, mp 118—20°C; Ag salt, 1:1, col powder, mp 24—5°C.[113]

$HO(PhO)P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 4c. K salt, mp 184.5°C; Ag salt, wh powder, softens at 40°C.[101]

$HO(p\text{-}NO_2C_6H_4CH_2O)P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 12d.[99] Mp 83—5°C,[99] countercurrent distribution.[102] Na salt, mp 215°C;[99] Ca salt;[99] Ba salt;[99] Ag salt, mp 134—7°C.[102]

$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2Et$ 2c. Nd, mp 40—1°C, UV.[100]

$HO(PhCH_2O)P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 12d. Mp 105—6°C d. Na salt, mp 257—60°C.[102]

$BuO(p\text{-}NO_2C_6H_4CH_2O)P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 5b. Waxy, mp <40°C, countercurrent distribution, UV.[102]

$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 2c.[15,100,101] Mp 47—8°C,[15] 49—50°C,[100] UV.[100]

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 2c.[99] 8e.[102] Mp 108-10°C,[99] 109—10°C,[102] UV.[102]

$(PhCH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CO_2CH_2Ph$ 9c. Mp 62—4°C, UV.[102]

L-Aspartic Acid

N-Phospho Derivatives

$(HO)_2P(O)NHCH(CO_2H)CH_2CO_2H$ 8c,[12] 11a.[94] Li, Na, K salts, 3:1; Mg, Ca salts, 2:1; ethylenediamine salt, 2:1, mp 100—10°C d; lysine salt, 2:1, mp >260°C; ornithine salt, 2:1, mp 212°C d; arginine salt, 2:1, mp 220°C d.[94]

$Ph_2P(S)NHCH(CO_2H)CH_2CO_2CH_2Ph$ 3. Dicyclohexylamine salt, mp 156—7°C, $[\alpha]_D$ −13.7° (EtOH), TLC.[107]

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(CO_2CH_2Ph)CH_2CO_2CH_2Ph$ 2b. Mp 98°C,[94] 101°C.[92]

$(PhCH_2O)_2P(O)NHCH(CO_2CH_2Ph)CH_2CO_2CH_2Ph$ 2b. Mp 46—7°C.[94]

O-Phospho Derivatives

$NH_2CH(CO_2H)CH_2CO_2P(O)(OH)_2$ 11c/14b,[119] 12e.[41] Oil.[41]
$PhCH_2O_2CNHCH(CO_2CH_2Ph)CH_2CO_2P(O)(OH)_2$ 7c. Oil.[119]
$PhCH_2O_2CNHCH(CO_2\ CH_2Ph)CH_2CO_2P(O)(OCH_2Ph)_2$ 2d. Heavy yellowish oil.[41]

L-Threonine

N-Phospho Derivatives

$Ph_2P(S)NHCH(CHMeOH)CO_2H$ 3. Dicyclohexylamine salt, mp 147—9°C, $[\alpha]_D$ −10.0° (EtOH), TLC.[107]

O-Phospho Derivatives

$(HO)_2P(O)OCHMeCH(NH_2)CO_2H$ 1d,[23] 8b.[133] Mp 189°C d,[23] $[\alpha]_D^{27}$ −7.9° (H_2O), −2.0° (HCl),[23] PC,[23,206-208] TLC,[133,211] IEC,[216,218,221,224,225,227] paper electrophoresis.[206,230] Ag salt, 2:1, ppt.[236]

DL-Threonine

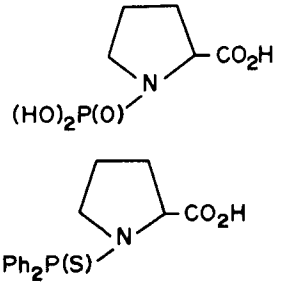
N-Phospho Derivatives

$(i\text{-}PrO)_2P(O)NHCH(CHOHMe)CO_2Me$ 2a.[79,218] Waxy so, mp 54—6°C,[79] 59—61°C.[218]
$(PhCH_2O)_2P(O)NHCH(CHOHMe)CO_2Me$ 2b.[87] Waxy so, mp 52—4°C,[87] IR.[184]

O-Phospho Derivatives

$(HO)_2P(O)OCHMeCH(NH_2)CO_2H$ 1d,[66] 8a,[123,126] 10d,[84,218] 12c.[100] Pr, mp 150-2°C d,[100] 169°C d,[123] 184°C,[84,218] 194°C d,[126] ^1H NMR,[190,191] ^{13}C NMR,[190] ^{31}P NMR,[195] X-ray,[126] XPS P_{2p} 133.7 eV,[204] PC,[209] TLC,[214] IEC,[126,220] HPLC,[139] pK_a 2.25, 5.83, 9.67 (KNO_3, 25°C)[157]; see also Ref. 190, TLE.[214] Ca(II), Mg(II), Mn(II), Co(II), Ni(II), Cu(II), and Zn(II) salts, stability constants;[157] Ba salt, 1:1;[100,123] Pb salt, 1:1.[123]
$(HO)_2P(O)OCHMeCH(NHAc)CO_2H$ 13a. ^1H NMR, ^{13}C NMR.[178]
$(Me_3SiO)_2P(O)OCHMeCH(NHSiMe_3)CO_2SiMe_3$ 13f. GC/MS.[180]
$(PhO)_2P(O)OCHMeCH(NH_2)CO_2Et$ 13d. HBr salt, mp 88—9°C, UV.[100]
$(PhO)_2P(O)OCHMeCH(NHCO_2Ph)CO_2Et$ 2c. Mp 56—7°C, UV.[100]

L-Proline

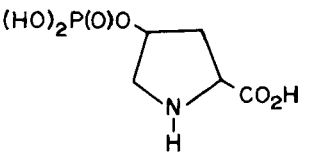


9b.[136,145]

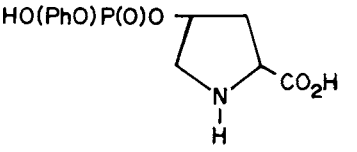
3.[106,107] Mp 128—30°C, $[\alpha]_D$ −15.0° (EtOH), TLC.[107]
Dicyclohexylamine salt, mp 194—5°C, $[\alpha]_D$ −40.0° (EtOH), TLC.[106]

L-Hydroxyproline

1d,[66] 4c,[109] 8a.[127] Nd, mp 130—1°C,[123] $[\alpha]_D$ −28.76° (H_2O),[123] IEC.[216,221] Hydrate, mp 115°C.[123] Ba salt, 1:1, small white plates;[109,123] Pb salt.[123]



4c. Oil.[109]



L-Glutamic acid

N-Phospho Derivatives

$(HO)_2P(O)NHCH(CO_2H)CH_2CH_2CO_2H$ 1a,[48-50] 2b/11a,[49] 5a,[112] 8c.[12] TLC,[50] thin layer electrophoresis,[50] GFC,[49] DEAE cellulose chromatography.[49] Mg salt, 2:1;[48] Ba salt, 2:3.[112]
$(MeO)_2P(O)NHCH(CONH_2)CH_2CH_2CONH_2$ 14f.[87] Mp 117—20°C d,[87] IR.[184]
$(HO)_2P(O)NHCH(CO_2Et)CH_2CH_2CO_2Et$ 11de. Na salt, 2:1, ppt.[89]
$(PhO)_2P(O)NHCH(CO_2Et)CH_2CH_2CO_2Et$ 2b. Mp 73.5—74°C.[89]
$(PhCH_2O)_2P(O)NHCH(CO_2Me)CH_2CH_2CO_2Me$ 2b. Visc oil.[87]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(CO_2Et)CH_2CH_2CO_2Et$ 2b. Mp 94°C.[92]
$Ph_2P(S)NHCH(CO_2H)CH_2CH_2CO_2CH_2Ph$ 3. *t*-Butylamine salt, mp 94—8°C, $[\alpha]_D$ +10.0° (EtOH), TLC.[107]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(CO_2CH_2Ph)CH_2CH_2CO_2CH_2Ph$ 2b. Mp 84°C.[92]
$(PhCH_2O)_2P(O)NHCH(CO_2CH_2Ph)CH_2CH_2CO_2CH_2Ph$ 2b.[87] Waxy so, mp 45—7°C,[87] IR.[184]

O-Phospho Derivatives

$NH_2CH(CO_2H)CH_2CH_2CO_2P(O)(OH)_2$ 12e. Heavy oil.[41]
$PhCH_2O_2CNHCH(CO_2CH_2Ph)CH_2CH_2CO_2P(O)(OCH_2Ph)_2$ 2d. Heavy oil.[41]

DL-Glutamic Acid

$(HO)_2P(O)NHCH(CO_2H)CH_2CH_2CO_2H$ 1a. Mg salt, wh powder, IR, TLC, GFC, paper electrophoresis.[51]

L-Valine

$(HO)_2P(O)NHCH(Pr\text{-}i)CO_2H$ 6a,[115] 8b,[131-132a] 8c.[12] IR, PC, IEC.[131,132] Li salt.[115]
$Ph_2P(S)NHCH(Pr\text{-}i)CO_2H$ 3.[106,107] Mp 112—4°C, $[\alpha]_D$ −17.5° (EtOH), TLC.[107] Dicyclohexylamine salt, mp 149—51°C, $[\alpha]_D$ −10.0° (EtOH), TLC.[106]

DL-Valine

N-Phospho Derivatives

$Ph\overline{OP(O)NHCH(Pr\text{-}i)C(O)O}$ 4b. Ppt.[108]
$HO(PhO)P(O)NHCH(Pr\text{-}i)CO_2H$ 4a. Na salt, 2:1, cr; Ba salt, 1:1, cr.[108]
$(PhCH_2O)_2P(O)NHCH(Pr\text{-}i)CO_2Me$ 2b.[86,87] Waxy so, mp 39—41°C,[87] IR.[91,184]
$(PhO)_2P(O)NHCH(Pr\text{-}i)CO_2CH_2Ph$ 2b. Mp 44—8°C.[88]

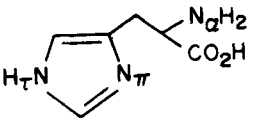
O-Phospho Derivatives

$N\overline{HCH(Pr\text{-}i)CO_2P(O)OPh}$ See above.

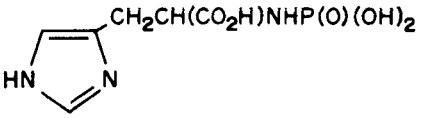
L-Methionine

$(HO)_2P(O)NHCH(CO_2H)CH_2CH_2SMe$ 6a,[115] 9b.[143] PC.[143] Li salt.[115]
$Ph_2P(S)NHCH(CO_2H)CH_2CH_2SMe$ 3. Dicyclohexylamine salt, mp 145—6°C, $[\alpha]_D$ −1.2° (MeOH), TLC.[107]

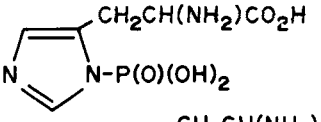
L-Histidine



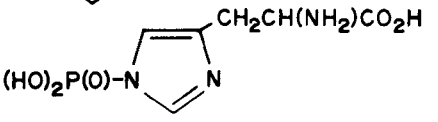
N_π and N_τ are N_3 and N_1 in the chemical literature, and N_1 and N_3 in the older biochemical literature.



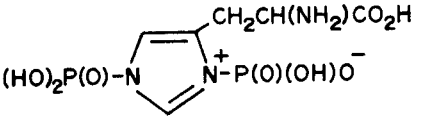
9a,[136] 9b.[136,143-145] IR,[144] ^{1}H NMR,[144] PC,[136,143] TLC.[144] Ca salt;[136,143] Ba salt.[144]



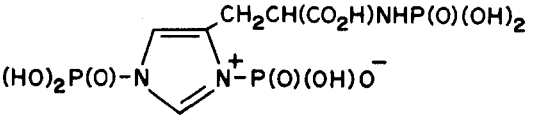
9a.[57,137] UV,[56,57] ^{1}H NMR,[141] ^{31}P NMR,[140,141] PC,[56] IEC,[56,57,137,215] paper electrophoresis.[56,57,137,141] Na salt; K salt; Ca salt, ppt.[57]



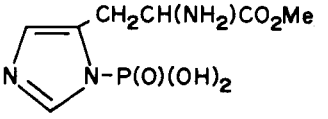
1b,[53,56] 9a.[56,137,138] UV,[56,57,137] ^{1}H NMR,[137,141] ^{31}P NMR,[140,141,192] pK_a 6.4, 9.5 (H_2O, 25°C)[137] PC,[56] IEC,[56,137,215] HPLC,[139,140] paper electrophoresis.[56,137,141] Li salt, amorph;[137] K salt;[137] Mg salt; 3:2, wh cr;[138] Ca salt, 3:2, wh cr;[56,138] Ba salt, 3:2, wh so.[138]



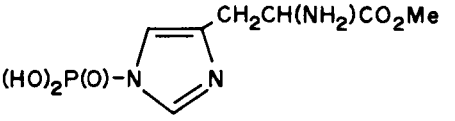
1a,[57] 9a.[137] UV,[56,137] ^{1}H NMR,[141] ^{31}P NMR,[141] PC,[56] IEC,[56,137] paper electrophoresis.[56,137,141] Na salt, Ca salt.[57]



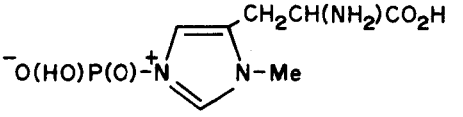
1b, 9a. UV, IEC, paper electrophoresis. Na salt, Ca salt.[57]



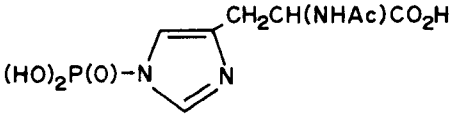
9a. Paper electrophoresis.[137]



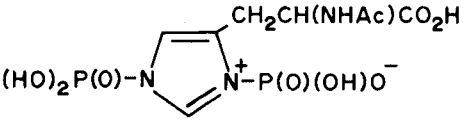
9a. Paper electrophoresis.[137]



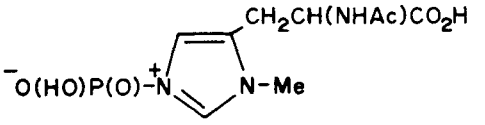
9a. Paper electrophoresis.[137]



1b,[57] 13.[137] UV, IEC.[57] Ca salt, ppt.[137]

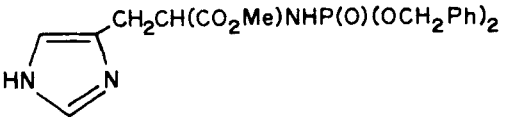


1b. UV, IEC.[57]



9a; from N_α-acetyl-N_τ-phosphohistidine, MeI and $KHCO_3$. IEC, paper electrophoresis.[137]

DL-Histidine



2b. Visc liquid.[86]

L-Cystine

$(HO)_2P(O)NHCH(CO_2H)CH_2SSCH_2CH(CO_2H)NHP(O)(OH)_2$ 9b. PC.[143]
$(PhCH_2O)_2P(O)NHCH(CO_2Me)CH_2SSCH_2CH(CO_2Me)NHP(O)(OCH_2Ph)_2$ 2b.[87] Mp 96—100°C,[87] IR.[91,184]

L-Leucine

N-Phospho Derivatives

$(HO)_2P(O)NHCH(Bu\text{-}i)CO_2H$ 1a,[48] 6a,[115] 8c.[11,12] Li salt;[115] Mg salt.[48]
$(HO)_2P(O)NHCH(Bu\text{-}i)CONH_2$ 11a. K salt, 2:1, cr, ^1H NMR.[96]
$HO(MeO)P(O)NHCH(Bu\text{-}i)CONH_2$ 12a. K salt, 1:1, cr, ^1H NMR.[96]
$Me_2P(S)NHCH(Bu\text{-}i)CO_2H$ Dicyclohexylamine salt, IR.[184]
$MeO(PhO)P(O)NHCH(Bu\text{-}i)CONH_2$ 2b. Mp 128—30°C, ^1H NMR.[96]
$Ph_2P(S)NHCH(Bu\text{-}i)CO_2H$ 3.[106,107] $[\alpha]_D$ −17.5° (EtOH), TLC.[107] Dicyclohexylamine salt, mp 137—8°C, $[\alpha]_D$ −15.0° (EtOH), TLC.[106]
$(p\text{-}BrC_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CO_2H$ 14c. Mp 81°C.[92]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CO_2H$ 14c. Sirup.[92]
$(PhCH_2O)_2P(O)NHCH(Bu\text{-}i)CONH_2$ 2b. Mp 116—7°C, ^1H NMR.[96]
$(p\text{-}BrC_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CO_2Me$ 2b. Sirup.[92]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CO_2Me$ 2b. Mp 48°C.[92]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CO_2Me$ 2b. Mp 75—6°C.[92]

O-Phospho Derivatives

$NH_2CH(Bu\text{-}i)CO_2P(O)(OH)_2$ 12e. Heavy oil.[41]
$PhCH_2O_2NHCH(Bu\text{-}i)CO_2P(O)(OCH_2Ph)_2$ 2d. Visc oil.[41]

DL-Leucine

N-Phospho Derivatives

PhOP(O)NHCH(Bu-i)C(O)O 4b. Ppt.[108]
$HO(PhO)P(O)NHCH(Bu\text{-}i)CO_2H$ 4a. Na salt, 2:1, cr; Ba salt, 1:1, wh so.[108]
$(PhCH_2O)_2P(O)NHCH(Bu\text{-}i)CO_2Me$ 2b.[86,87] Mp 45—6°C,[87] IR.[184]
$(PhO)_2P(O)NHCH(Bu\text{-}i)CO_2CH_2Ph$ 2b. Mp 52—3°C.[88]

O-Phospho Derivatives

NHCH(Bu-i)CO_2P(O)OPh See above.

L-Isoleucine

$Ph_2P(S)NHCH(Bu\text{-}s)CO_2H$ 3. Cyclohexylamine salt, mp 181—3°C, $[\alpha]_D$ −7.5°, TLC.[107]

L-Lysine

$NH_2CH(CO_2H)(CH_2)_4NHP(O)(OH)_2$ 1a,[59] 1b,[58,60] 8br.[133] Ppt, pK_a 5.0,[59] TLC,[133] IEC,[58,139,215] HPLC.[60,139] Li salt, 3:1, LD_{50} 2,430 mg/kg;[59] Na salt;[58] Mg salt, ppt.[58]
$(HO)_2P(O)NHCH(CO_2H)(CH_2)_4NHP(O)(OH)_2$ 1b. Li salt, 5:1, ppt, LD_{50} 622 mg/kg.[59]
$PhCH_2O_2CNHCH(CO_2H)(CH_2)_4NHP(O)(OH)_2$ 9b. IR, ^1H NMR, TLC. Ba salt.[144]
$(PhCH_2O)_2P(O)NHCH(CO_2Me)(CH_2)_4NHP(O)(OCH_2Ph)_2$ 2b.[87] Visc yel sirup, so when cooled,[87] IR.[184]

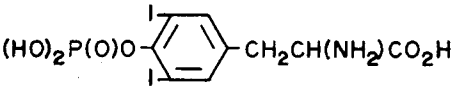
L-Hydroxylysine

$(HO)_2P(O)OCH(CH_2NH_2)CH_2CH_2CH(NH_2)CO_2H$ IEC.[221]

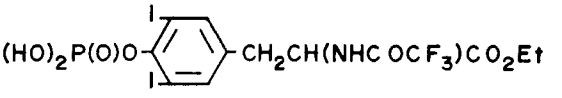
L-Arginine

$(HO)_2P(O)NHCH(CO_2H)(CH_2)_3NHC(=NH)NH_2$ 1b.[63]
$NH_2CH(CO_2H)(CH_2)_3NHC(=NH)NHP(O)(OH)_2$ 1b,[60-63] 9c,[95] 13c.[95] Cr, mp 175—80°C,[95] ^{31}P NMR δ3.6 ppm,[193] IEC,[62,63,139] PC,[62,95] HPLC.[60,139] Li salt, 2:1, mp 180°C;[62] Ca salt;[61] Ba salt, 1:1,[63] 1:2;[95] Cu salt, ppt.[95]
$(HO)_2P(O)NHCH(CO_2H)(CH_2)_3NHC(=NH)NHP(O)(OH)_2$ 1b.[63]
$Ph_2P(S)NHCH(CO_2H)(CH_2)_3NHC(=NH)NHNO_2$ 3. Mp 129—32°C $[\alpha]_D$ +2.5° (MeOH), TLC.[107] Dicyclohexylamine salt, mp 159—61°C, $[\alpha]_D$ + 5.0° (MeOH).[107]
$Ph_2P(S)NHCH(CO_2H)(CH_2)_3NHC(=NH)NHSO_3C_6H_4Me$-p 3. Mp 199—201°C, $[\alpha]_D$ +4.9° (DMF), TLC.[107]
$PhCH_2O_2CNHCH(CO_2CH_2Ph)(CH_2)_3NHC(=NH)NHP(O)(OCH_2C_6H_4NO_2$-p)OH 11a. Mp 180°C.[95]
$PhCH_2O_2CNHCH(CO_2CH_2Ph)(CH_2)_3NHC(=NH)NHP(O)(OCH_2C_6H_4NO_2$-p)$_2$ 2b. Glassy so, PC.[95]
$(PhCH_2O)_2P(O)NHCH(CO_2Me)(CH_2)_3NHC(=NH)NHP(O)(OCH_2Ph)_2$ 2b.[87] Mp 91—3°C,[87] IR.[184]

3,5-Diiodo-L-tyrosine



13b/14c. Colorless cr, mp 216°C d.[78]



1e. White cr, mp 185—7°C d.[78]

L-Phenylalanine

$(HO)_2P(O)NHCH(CH_2Ph)CO_2H$ 1a,[48] 11a.[96] ^1H NMR.[96] K salt, 3:1,[96] Mg salt.[48]
$Me_2P(S)NHCH(CH_2Ph)CO_2H$ Dicyclohexylamine salt, IR.[184]
$Ph_2P(S)NHCH(CH_2Ph)CO_2H$ 3. Dicyclohexylamine salt, mp 190—1°C, $[\alpha]_D$ +8.7° (EtOH), TLC.[107]
(*p*-$NO_2C_6H_4CH_2O)_2P(O)NHCH(CH_2Ph)CO_2H$ 14c. Mp 127—8°C.[92]
(*p*-$NO_2C_6H_4CH_2O)_2P(O)NHCH(CH_2Ph)CO_2Me$ 2b. Mp 111°C.[92]
$(PhO)_2P(O)NHCH(CH_2Ph)CO_2CH_2Ph$ 2b.[88,92] Mp 86°C,[88] 90°C,[92] $[\alpha]_D^{21}$ −5.2° (CCl_4).[88]
$(PhCH_2O)_2P(O)NHCH(CH_2Ph)CO_2CH_2Ph$ 2b. Mp 88—90°C, ^1H NMR.[96]

DL-Phenylalanine

N-Phospho Derivatives

$(HO)_2P(O)NHCH(CH_2Ph)CO_2H$ 11a.[87] Mp 163—4°C d,[87] IR.[91,184]
$(HO)_2P(O)NHCH(CH_2Ph)CO_2Me$ 11a.[86] Mp 143—5°C,[86] IR,[91,184] UV.[184]
$(MeO)_2P(O)NHCH(CH_2Ph)CONH_2$ 14f. Mp 148—9°C.[87]
$PhOP(O)NHCH(CH_2Ph)C(O)O$ 4b. Ppt.[108]
$HO(PhO)P(O)NHCH(CH_2Ph)CO_2H$ 4a. Na salt, 2:1, cr; Ba salt, 1:1, wh so.[108]
$(PhO)_2P(O)NHCH(CH_2Ph)CO_2Et$ 2b. Mp 78—9°C.[89]
$(PhCH_2O)_2P(O)NHCH(CH_2Ph)CO_2Me$ 2b.[86] Mp 82—3°C,[86] IR,[91,184] UV,[184] PC.[86]
$(PhO)_2P(O)NHCH(CH_2Ph)CO_2CH_2Ph$ 2b. Mp 90—1°C.[88]
$(PhCH_2O)_2P(O)NHCH(CH_2Ph)CO_2CH_2Ph$ 2b.[87] Mp 67—9°C,[87] IR,[91,184] UV,[184] ^1H NMR.[184]

O-Phospho Derivatives

$NH_2CH(CH_2Ph)CO_2P(O)(OH)_2$ 11c. Ba salt, 1:1, silky cr; Ag salt, 2:1, mp >320°C.[114]
$\overline{NHCH(CH_2Ph)CO_2P(O)OPh}$ See above.
$NH_2CH(CH_2Ph)CO_2P(O)OCH_2Ph)_2$ 14e. Mp 104—6°C d.[114]

L-Tyrosine

N-Phospho Derivatives

$(HO)_2P(O)NHCH(CO_2H)CH_2C_6H_4OH$-p 9b,[136,143,145] 11a/14c.[81] PC.[143] Ba salt, 3:2, ppt.[81]
$(HO)_2P(O)NHCH(CO_2H)CH_2C_6H_4[OP(O)(OH)_2]$-p 1a. Mg salt, 1:1, wh powder.[10]
$[(HO)_2P(O)]_2NCH(CO_2H)CH_2C_6H_4[OP(O)(OH)_2]$-p 1e/14c. Solid.[14]
$HO(PhO)P(O)NHCH(CO_2H)CH_2C_6H_4OH$-p 12a.[81] IR.[184] Ba salt, pr.[81]
$Ph_2P(S)NHCH(CO_2H)CH_2C_6H_4OH$-p 3, 14c. Dicyclohexylamine salt, mp 192—3°C, $[\alpha]_D$ −1.2° (MeOH), TLC.[107]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH(CO_2H)CH_2C_6H_4OH$-p 14c. Mp >80—5°C.[81]
$Ph_2P(S)NHCH(CO_2Et)CH_2C_6H_4OH$-p 3. Mp 93—8°C, $[\alpha]_D$ −32.5° (EtOH).[107]
$(PhO)_2P(O)NHCH(CO_2Et)CH_2C_6H_6OH$-p 2a. Mp 93—4°C.[81]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH(CO_2Et)CH_2C_6H_4OH$-p 2a. Mp 143°C.[81]
$(PhCH_2O)_2P(O)NHCH(CO_2Et)CH_2C_6H_4OH$-p 2b.[87] Mp 104—5°C,[87] IR.[184]
$Ph_2P(S)NHCH(CO_2H)CH_2C_6H_4(OCH_2Ph)$-p 3. Dicyclohexylamine salt, mp 182—4°C, $[\alpha]_D$ +15.0° (MeOH), TLC.[107]
$(PhCH_2O)_2P(O)NHCH(CO_2CH_2Ph)CH_2C_6H_4OH$-p 2b.[87] Mp 54—5°C,[87] IR.[184]
$Ph_2P(S)NHCH(CO_2H)CH_2C_6H_4[OP(S)Ph_2]$-p 3. Mp 117—24°C, $[\alpha]_D$ −17.5° (EtOH), TLC.[107]
$Ph_2P(S)NHCH(CO_2Et)CH_2C_6H_4[OP(S)Ph_2]$-p 3. Amorph wh powder, $[\alpha]_D$ −10.0° (EtOH).[107]
$(PhCH_2O)_2P(O)NHCH(CO_2Me)CH_2C_6H_4[OP(O)(OCH_2Ph)_2]$-p 2b. PC.[86]
$(PhCH_2O)_2P(O)NHCH(CO_2Et)CH_2C_6H_4[OP(O)(OCH_2Ph)_2]$-p 2b.[87] Ye waxy so, mp 95—7°C,[87] IR.[184]

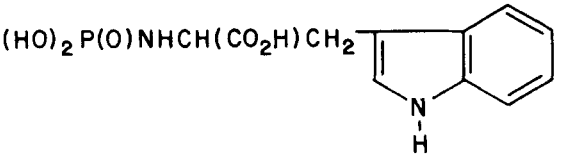
O-Phospho Derivatives

p-$[(HO)_2P(O)O]C_6H_4CH_2CH(NH_2)CO_2H$ 1e/14c,[14] 8a,[123,128] 8c,[134] 13b,[77] 13d/14c.[14] Pl, mp 225°C,[123] 227°C,[14] 246—7°C,[128] $[\alpha]_D$ −9.19° (HCl),[123] $[\alpha]_D^{20}$ −8.8° (HCl),[14] IR,[128] UV,[128,154,185] PC,[185] TLC,[214] IEC,[128,216] paper electrophoresis[128,185] TLE.[214] Ca salt, 1:1, ppt;[123] Ba salt, 1:1, ppt;[77,123] Pb salt, 1:1, ppt.[77,123]
p-$[(HO)_2P(O)O]C_6H_4CH_2CH(CO_2H)NHP(O)(OH)_2$ See above.
p-$[(HO)_2P(O)O]C_6H_4CH_2(CO_2H)N[P(O)(OH)_2]_2$ See above.
p-$[(HO)_2P(O)O]C_6H_4CH_2CH(CO_2H)NHCHO$ 1e. Mg salt, 1:1, ppt.[77]
p-$[Ph_2P(S)O]C_6H_4CH_2CH(NH_2)CO_2Et$ By HCl cleavage of the O,N-diphospho derivative. HCl salt, mp 137—41°C, $[\alpha]_D$ +22.5° (EtOH), TLC.[107]
p-$[Ph_2P(S)O]C_6H_4CH_2CH(CO_2H)NHP(S)Ph_2$ See above.
p-$[Ph_2P(S)O]C_6H_4CH_2CH(CO_2Et)NHP(S)Ph_2$ See above.
p-$[(PhCH_2O)_2P(O)O]C_6H_4CH_2CH(CO_2Me)NHP(O)(OCH_2Ph)_2$ See above.
p-$[(PhCH_2O)_2P(O)O]C_6H_4CH_2CH(CO_2Et)NHP(O)OCH_2Ph)_2$ See above.

DL-Tyrosine

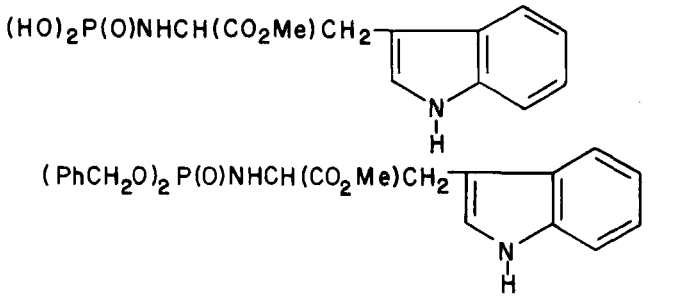
p-$[(HO)_2P(O)O]C_6H_4CH_2CH(NH_2)CO_2H$ 1d.[66]

L-Tryptophan



9b.[136,143,145] PC.[143]

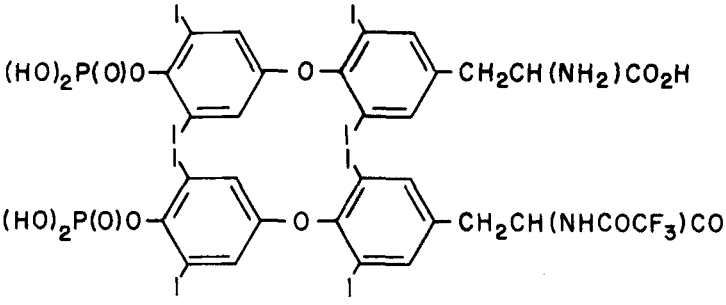
DL-Tryptophan



11a.[86] Mp 130—2°C,[86] IR.[91,184]

2b.[86] Mp 104.5—5°C,[86] IR,[91,184] PC.[86]

L-Thyroxine



13b/14c. Colorless ppt, mp 212—4°C d.[78]

1e. Colorless cr, mp 194—6°C d.[78]

B. Dipeptide Derivatives

Glycylglycine

$(HO)_2P(O)NHCH_2CONHCH_2CO_2H$ 1a,[48] 9a,[135] 11a/14c.[81] PC, paper electrophoresis.[52] Na salt;[52] Mg salt,[48] Ba salt, 3:2, so.[81]

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH_2CONHCH_2CO_2Et$ 2a. Mp 112—3°C.[81]

Glycyl-L-Alanine

$(PhCH_2O)P(O)NHCH_2CONHCHMeCO_2CH_2Ph$ 2b. Mp 73—5°C, ^1H NMR.[96]

L-Serylglycine

$(HO)_2P(O)OCH_2CH(NH_2)CONH^{14}CH_2CO_2H$ 2c/11b.[24]

DL-Serylglycine

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH_2CO_2H$ 2c/11b,[14] 3b,[17] 11de,[16] 12c.[100] Cr, mp 150—4°C d,[100] 178°C,[14] 189—92°C d,[16] pK_a (KCl, 25°C) 3.13, 5.41, 8.01,[19,162] PC,[14,16,162] IEC,[220] IR.[16] Metal(II) complexes: Mg,[162] Ca,[162] Sr,[7] Mn,[162] Cu.[163]

$HO(PhO)P(O)OCH_2CH(NH_2)CONHCH_2CO_2H$ 11d.[16] Mp 207—9°C d,[16] pK_a (KCl, 25°C) 3.18, 6.95,[19] PC.[16]

$(PhO)_2P(O)OCH_2CH(NH_2)CONHCH_2CO_2Et$ 13d. HBr salt, mp 129—30°C.[100]

$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH_2CO_2Et$ 2c. Oil, UV.[100]

$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH_2CO_2CH_2Ph$ 2c. Col oil, n_D^{21} 1.5642.[16]

$(PhCH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH_2CO_2CH_2Ph$ 2c. Mp 104—5°C.[17]

Glycyl-L-Serine

$NH_2CH_2CONHCH(CO_2H)^{14}CH_2OP(O)(OH)_2$ 2c/11b.[24]
$[NH_2CH_2CONHCH(CO_2H)CH_2OP(O)OH]_2O$ 6c/11b. PC.[116]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)(OCH_2Ph)OH$ 12d.[23,27] Mp 121—2°C,[27] 129°C,[23] PC,[23] paper electrophoresis.[27] Sodium salt, 1:1, white cryst, mp 178°C.[23]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)OCH_2Ph)_2$ 2c. Mp 79—80°C, PC.[23]
$[PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)OCH_2Ph]_2O$ 6c.[23,27] White solid. PC,[23] paper electrophoresis.[27]

Glycyl-DL-Serine

$NH_2CH_2CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 11b,[17,99] 11de.[16] Mp 198—201°C,[99] 201—4°C d,[16] pK_a (KCl, 25°C) 2.90, 6.02, 8.43,[19] IR,[16] PC,[16,29] IEC,[220] paper electrophoresis.[29] Metal(II) complexes: Ca, Mg.[162]
$NH_2CH_2CONHCH(CO_2H)CH_2OP(O)(OH)OP(O)(OH)_2$ 2e/11b, 6c/11b. Solid white foam, PC, IEC, paper electrophoresis.[29]
$[NH_2CH_2CONHCH(CO_2H)CH_2OP(O)OH]_2O$ PC, paper electrophoresis.[29]
$NH_2CH_2CONHCH(CO_2H)CH_2OP(O)(OPh)OH$ 11d.[16] Mp 176—8°C d,[16] pK_a (KCl, 25°C) 2.96, 8.07,[19] PC.[16]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)(OCH_2C_6H_4NO_2\text{-}p)OH$ 12d. Mp 136°C d.[102]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)(OCH_2Ph)OH$ 12d. Mp 121—2°C.[29]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)(OPh)_2$ 2c. Col oil, n_D^{20} 1.5620.[16]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)(OCH_2C_6H_4NO_2\text{-}p)_2$ 2c. Mp 116—8°C.[99]
$PhCH_2O_2CNHCH_2CONHCH(CO_2CH_2Ph)CH_2OP(O)(OCH_2Ph)_2$ 2c. Mp 81—2°C.[17]

L-Alanyl-L-Alanine

$(HO)_2P(O)NHCHMeCONHCHMeCO_2H$ 11a. K salt, 3:1, ^1H NMR.[96]
$(PhCH_2O)_2P(O)NHCHMeCONHCHMeCO_2CH_2Ph$ 2b. Mp 102—3°C, ^1H NMR.[96]

L-Seryl-L-Alanine

$(HO)_2P(O)OCH_2CH(NH_2)CONHCHMeCO_2H$ 2c,11b,[23] 11b.[99] Cr, $[\alpha]_D^{26}$ −16.5° (HCl), PC.[23] Hydrate, mp 170°C d.[99]
$HO(p\text{-}NO_2C_6H_4CH_2O)P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCHMeCO_2CH_2Ph$ 12d. Mp 159—60°C.[102]
$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCHMeCO_2CH_2Ph$ 2c. Mp 81—3°C.[99]

L-Seryl-L-Serine

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 11de.[17] $[\alpha]_D^{21}$ +8.2° (HCl), PC,[17] IEC.[17,220]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)CH_2OP(O)(OPh)_2$ 2c. Oil.[17]

L-Aspartyl-L-Serine

$HO_2CCH_2CH(NH_2)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 2c/11b. $[\alpha]_D^{27}$ +21.6° (HCl), PC, IEC.[23]
$HO_2CCH_2CH(NH_2)CONHCH(CO_2H)CH_2OP(O)(OH)OP(O)(OH)_2$ 11b. Wh so, PC, IEC.[22]

L-Seryl-L-Aspartic Acid

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH(CO_2H)CH_2CO_2H$ 11de.[17] $[\alpha]_D^{21}$ −2.2°C (HCl), PC,[17] IEC.[17,220]
$HO(PhO)P(O)OCH_2CH(NH_2)CONHCH(CO_2H)CH_2CO_2H$ 11d. Mp 167—71°C, PC, IEC.[17]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)CH_2CO_2CH_2Ph$ 2c. Mp 62—3°C.[17]

L-Glutamyl-L-Serine

$HO_2CCH_2CH_2CH(NH_2)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 11de.[17] $[\alpha]_D^{21}$ +23.3° (HCl),[17] PC.[17,23]
$HO_2CCH(NH_2)CH_2CH_2CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 2c/11b. $[\alpha]_D^{27}$ +24.7° (HCl), IEC.[23]
$HO_2CCH(NH_2)CH_2CH_2CONHCH(CO_2H)CH_2OP(O)(OH)OP(O)(OH)_2$ 11b. Wh so, PC, IEC.[22]
$PhCH_2O_2CCH_2CH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)CH_2OP(O)(OPh)_2$ 2c. Mp 75—6°C.[17]

L-Seryl-L-Glutamic Acid

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH(CO_2H)CH_2CH_2CO_2H$ 3de/11b,[17] 12c.[100] Mp 145—7°C,[100] sint ~145°C,[17] $[\alpha]_D^{21}$ −9.5° (HCl),[17] pK_a (KCl, 25°C) 3.02, 4.39, 5.69, 8.25,[21] PC,[17,21] IEC.[17,21,220] Ba salt,[17,100] $[\alpha]_D^{22}$ −1.3° (HCl);[100] brucine salt, mp 160—2°,[100] 171—3°C d.[17] Metal(II) complexes: Mg, Ca, Mn,[162] Cu.[164]

$HO(PhO)P(O)OCH_2CH(NH_2)CONHCH(CO_2H)CH_2CH_2CO_2H$ 11d. Mp 174—8°C d, $[\alpha]_D^{21}$ −7.6° (HCl), PC, IEC.[17]

$(PhO)_2P(O)OCH_2CH(NH_2)CONHCH(CO_2H)CH_2CH_2CO_2H$ 13c. Mp 182—5°C d, PC.[17]

$(PhO)_2P(O)OCH_2CH(NH_2)CONHCH(CO_2Et)CH_2CH_2CO_2Et$ 13d. HBr salt, sirup, UV.[100]

$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2Et)CH_2CH_2CO_2Et$ 2c. Oil, UV.[100]

$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)CH_2CH_2CO_2CH_2Ph$ 2c. Oil.[17]

$(PhCH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)CH_2CH_2CO_2CH_2Ph$ 2c. Oil.[17]

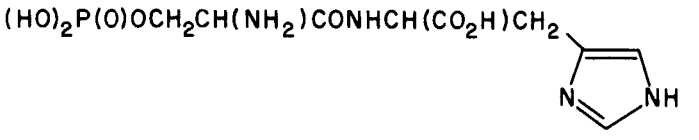
L-Leucylglycine

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CONHCH_2CO_2CH_2Ph$ 14h. Mp 134—6°C.[92]

Glycyl-L-Leucine

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH_2CONH(Bu\text{-}i)CO_2CH_2Ph$ 14h. Mp 87—8°C.[92]

L-Seryl-L-Histidine



2c/11b. $[\alpha]_D^{27}$ +9.4° (HCl), PC, IEC.[23]

L-Isoleucyl-L-Alanine

$(HO)_2P(O)NHCH(Bu\text{-}s)CONHCHMeCO_2H$ 11a. K salt, 3:1, ^1H NMR.[96]

$(PhCH_2O)_2P(O)NHCH(Bu\text{-}s)CONHCHMeCO_2CH_2Ph$ 2b. Mp 143—5°C, ^1H NMR.[96]

L-Alanyl-L-Leucine

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCHMeCONHCH(Bu\text{-}i)CONHNH_2$ 14g. Mp 202°C.[92]

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCHMeCONHCH(Bu\text{-}i)CO_2Me$ 14h. Mp 134°C.[92]

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCHMeCONHCH(Bu\text{-}i)CONHPh$ 14g. Mp 185°C.[92]

L-Leucyl-D-Serine

$NH_2CH(Bu\text{-}i)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ —.[7]

L-Leucyl-L-Serine

$NH_2CH(Bu\text{-}i)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 11de.[17] $[\alpha]_D^{21}$ +24.5°,[17] PC,[17] IEC.[220]

$NH_2CH(Bu\text{-}i)CONHCH(CO_2H)CH_2OP(O)(OH)OP(O)(OH)_2$ 11b. Wh so, PC, IEC.[22]

$PhCH_2O_2CNHCH(Bu\text{-}i)CONHCH(CO_2CH_2Ph)CH_2OP(O)(OPh)_2$ 2c. Oil.[17]

L-Isoleucyl-L-Serine

$NH_2CH(Bu\text{-}s)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 2c/11b. $[\alpha]_D^{27}$ +30.5° (HCl), PC.[23]

D-Seryl-L-Leucine

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH(Bu\text{-}i)CO_2H$ 11de. Mp 138—40°C d, $[\alpha]_D^{21}$ −28.1° (HCl).[18]

$HO(PhO)P(O)OCH_2CH(NH_2)CONHCH(Bu\text{-}i)CO_2H$ 11d. Mp 213—5°C d.[18]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(Bu\text{-}i)CO_2CH_2Ph$ 2c. Oil.[18]

L-Seryl-L-Leucine

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH(Bu\text{-}i)CO_2H$ 11b, 11de.[17] Mp 161—4°C d,[19] $[\alpha]_D^{21}$ −16.0° (HCl),[17] pK_a (KCl, 25°C) 3.11, 5.47, 8.26,[19] PC,[17] IEC.[220]
$(HO)_2P(O)OP(O)(OH)OCH_2CH(NH_2)CONHCH(Bu\text{-}i)CO_2H$ 11b. Wh so, PC, IEC.[22]
$HO(PhO)P(O)OCH_2CH(NH_2)CONHCH(Bu\text{-}i)CO_2H$ 11d.[17] Hydrate, mp 202—4°C d,[17] pK_a (KCl, 25°C) 3.16, 7.12,[19] PC.[17]
$(PhO)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(Bu\text{-}i)CO_2CH_2Ph$ 2c. Oil.[17]
$(PhCH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(Bu\text{-}i)CO_2CH_2Ph$ 2c. Oil.[17]

L-Lysyl-L-Serine

$NH_2(CH_2)_4CH(NH_2)CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 2c/11b. $[\alpha]_D^{25}$ +25.2° (HCl), PC, IEC.[23]

L-Seryl-L-Lysine

$(HO)_2P(O)OCH_2CH(NH_2)CONHCH(CO_2H)(CH_2)_4NH_2$ 11b. pK_a (KCl, 25°C) 2.98, 5.34, 7.58, 11.05, IEC. Monoformate, $[\alpha]_D^{19}$ −4.7° (HCl), PC; dihydrochloride, hygr so, PC.[21] Metal(II) complexes: Mg, Ca, Mn.[162]
$HO(PhCH_2O)P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)(CH_2)_4NHCO_2CH_2Ph$ 12d. Oil.[21]
$(PhCH_2O)_2P(O)OCH_2CH(NHCO_2CH_2Ph)CONHCH(CO_2CH_2Ph)(CH_2)_4NHCO_2CH_2Ph$ 2c. Mp 92—3°C.[21]

L-Alanyl-L-Arginine

$(PhCH_2O)_2P(O)NHCHMeCONHCH(CO_2Me)(CH_2)_3NHC(=NNO_2)NH_2$ 2b. Amor., $[\alpha]_D^{24}$ −18.4° (MeOH)[97]

L-Phenylalanylglycine

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(CH_2Ph)CONHCH_2CO_2CH_2Ph$ 10c, 14h. Mp 135—6°C, $[\alpha]_D^{20}$ −8.2° ($CHCl_3$).[92]

L-Tyrosylglycine

$p\text{-}[(HO)_2P(O)O]C_6H_4CH_2CH(NH_2)CONHCH_2CO_2H$ 13d. Mp 178°C d, $[\alpha]_D^{23}$ −20.0° (H_2SO_4).[14]
$p\text{-}[(HO)_2P(O)O]C_6H_4CH_2CH(NHCO_2CH_2Ph)CONHCH_2CO_2H$ 1e/14c. Amorph so. Pb salt, 1:1.[14]

Glycyl-L-Tyrosine

N-Phospho Derivatives

$(HO)_2P(O)NHCH_2CONHCH(CO_2H)CH_2C_6H_4OH\text{-}p$ 11a/14c. Ba salt, 3:2.[81]
$HO(PhO)P(O)NHCH_2CONHCH(CO_2H)CH_2C_6H_4OH\text{-}p$ 12a. Ba salt, ppt.[81]
$(PhO)_2P(O)NHCH_2CONHCH(CO_2Et)CH_2C_6H_4OH\text{-}p$ 2a. Mp 123—4°.[81]
$(p\text{-}IC_6H_4CH_2O)_2P(O)NHCH_2CONHCH(CO_2Et)CH_2C_6H_4OH\text{-}p$ 2a. Mp 127—8°C,[81] IR.[184]

O-Phospho Derivatives

$NH_2CH_2CONHCH(CO_2H)CH_2C_6H_4[OP(O)(OH)_2]\text{-}p$ Cr powder, mp 224—5°C d, $[\alpha]_D^{20}$ +27.9° (H_2SO_4.)[14]
$PhCH_2O_2CNHCH_2CONHCH(CO_2H)CH_2C_6H_4[OP(O)(OH)_2]\text{-}p$ 1e/14c. Ba salt, 1:1, flakes.[14]

L-Alanyl-L-Phenylalanine

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCHMeCONH(CH_2Ph)CO_2Me$ 14h. Mp 152°C.[92]

L-Seryl-L-Tyrosine

$PhCH_2O_2CNHCH(CH_2OH)CONHCH(CO_2Et)CH_2C_6H_4[OP(S)Ph_2]\text{-}p$ 15b. Oil, $[\alpha]_D$ −5.0° (EtOH).[107]
$PhCH_2O_2CNHCH(CH_2OBu\text{-}t)CONHCH(CO_2Et)CH_2C_6H_4[OP(S)Ph_2]\text{-}p$ 14h. Oil $[\alpha]_D$ +30.0°.[107]

L-Leucyl-L-Arginine

$(PhCH_2O)_2P(O)NHCH(Bu\text{-}i)CONHCH(CO_2Me)(CH_2)_3NHC(=NNO_2)NH_2$ 2b. Mp 134—6°C, $[\alpha]_D^{24}$ −16.6° (MeOH).[97]

L-Phenylalanyl-L-Leucine

$(p\text{-}NO_2C_6H_4CH_2O)_2P(O)NHCH(CH_2Ph)CONHCH(Bu\text{-}i)CO_2CH_2Ph$ 10c, 14h. Mp 108°C.[92]

L-Leucyl-L-Phenylalanine

$(HO)_2P(O)NHCH(Bu\text{-}i)CONHCH(CH_2Ph)CO_2H$ 11a. K salt, 3:1, ^1H NMR.[96]
$(p\text{-}BrC_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CONHCH(CH_2Ph)CONHNH_2$ 14g. Mp 196°C.[92]
$(p\text{-}BrC_6H_4CH_2O)_2P(O)NHCH(Bu\text{-}i)CONHCH(CH_2Ph)CO_2Me$ 10c, 14h. Mp 118°C.[92]
$(PhCH_2O)_2P(O)NHCH(Bu\text{-}i)CONHCH(CH_2Ph)CO_2CH_2Ph$ 2b. Mp 100—1°C, ^1H NMR.[96]

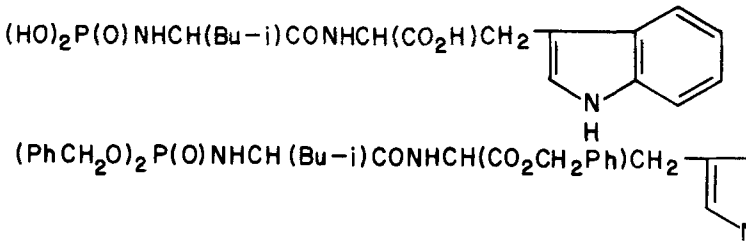
L-Phenylalanyl-L-Arginine

$(PhCH_2O)_2P(O)NHCH(CH_2Ph)CONHCH(CO_2Me)(CH_2)_3NHC(=NNO_2)NH_2$ 2b. Amor., $[\alpha]_D^{24}$ −5.6° (MeOH)[97]

L-Tyrosyl-L-Arginine

$(PhCH_2O)_2P(O)NHCH[CH_2C_6H_4(OCH_2Ph)\text{-}p]CONHCH(CO_2Me)(CH_2)_3NHC(=NNO_2)NH_2$ 2b. Amor., $[\alpha]_D^{24}$ −1.5° (MeOH).[97]

L-Leucyl-L-Tryptophan



11a. K salt, 2:1, ^1H NMR.[96]

2b. Mp 120—2°C, ^1H NMR.[96]

C. Tripeptide Derivatives

Triglycine

$(HO)_2P(O)[NHCH_2CO]_3OH$ 1a. PC, paper electrophoresis. Na salt, Mg salt.[52]

Glycyl-DL-Serylglycine

$NH_2CH_2CONHCH[CH_2OP(O)(OH)_2]CONHCH_2CO_2H$ 11de.[16] Mp 220—3°C d,[16] pK_a (KCl, 25°C) 3.29, 5.76, 8.23,[19] PC,[16] IR.[16] Metal(II) complexes: Mg, Ca, Mn.[162]
$NH_2CH_2CONHCH[CH_2OP(O)(OPh)OH]CONHCH_2CO_2H$ 11d. Mp 198—202°C d, PC.[16]
$PhCH_2O_2CNHCH_2CONHCH[CH_2OP(O)(OPh)_2]CONHCH_2CO_2CH_2Ph$ 2c. Mp 85—6°C.[16]

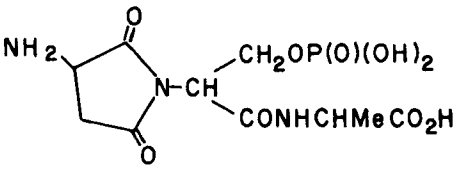
L-Aspartyl-L-Serylglycine

$HO_2CCH_2CH(NH_2)CONHCH[CH_2OP(O)(OH)_2]CONHCH_2CO_2H$ 2c/11b. $[\alpha]_D^{26}$ −4.3° (HCl), PC, IEC.[23]
$PhCH_2O_2CCH_2CH(NHCO_2CH_2Ph)CONHCH[CH_2OP(O)(OCH_2C_6H_4NO_2\text{-}p)_2]CONHCH_2CO_2CH_2Ph$ 2b. Mp 69—72°C, $[\alpha]_D^{25}$ −20° ($CHCl_3$), countercurrent distribution, UV.[102]

L-Glutamyl-L-Serylglycine

$HO_2CCH_2CH_2CH(NH_2)CONHCH[CH_2OP(O)(OH)_2]CONHCH_2CO_2H$ 2c/11b. $[\alpha]_D^{27}$ +11.5° (HCl), PC, IEC.[23]

L-Aspartyl-L-Seryl-L-Alanine



2b/11b. $[\alpha]_D^{26}$ −40.3° (HCl), IR, PC, IEC.[23]

$HO_2CCH_2CH(NH_2)CONHCH[CH_2OP(O)(OH)_2]CONHCHMeCO_2H$ 2c,11b. $[\alpha]_D^{26}$ −17.3° (HCl), IR, PC, IEC.[23]

L-Glutamyl-L-Seryl-L-Alanine

$HO_2CCH_2CH_2CH(NH_2)CONHCH[CH_2OP(O)(OH)_2]CONHCHMeCO_2H$ 2c/11b. $[\alpha]_D^{25}$ −4.5° (HCl), PC, IEC.[23]

L-Leucylglycyl-L-Serine

$NH_2CH(Bu\text{-}i)CONHCH_2CONHCH(CO_2H)CH_2OP(O)(OH)_2$ 2c, 11b. Cr, $[\alpha]_D^{27}$ +40.6° (HCl), PC.[23]

L-Lysyl-L-Serylglycine

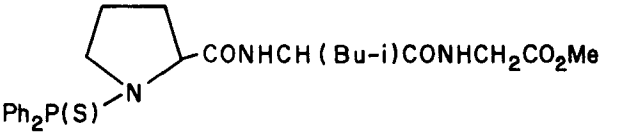
$NH_2(CH_2)_4CH(NHCO_2Et)CONHCH[CH_2OP(O)OH)_2]CONHCH_2CO_2H$ 2c/11b. $[\alpha]_D^{20}$ −23.6° (H_2O), PC, IEC.[20]

L-Aspartyl-L-Seryl-L-Glutamic Acid

$HO_2CCH_2(NH_2)CONHCH[CH_2OP(O)(OH)_2]CONHCH(CO_2H)CH_2CH_2CO_2H$ 2c/11b. $[\alpha]_D^{26}$ −11.2° (HCl), PC, IEC.[23]

$PhCH_2O_2CCH_2CH(NHCO_2CH_2Ph)CONHCH[CH_2OP(O)(OCH_2C_6H_4NO_2\text{-}p)_2]CONHCH(CO_2CH_2Ph)CH_2\text{-}CH_2CO_2CH_2Ph$ 2c. Mp 62—3°C, $[\alpha]_D^{25}$ −41° (AcOH), countercurrent distribution, UV.[102]

L-Prolyl-L-Leucylglycine



15b. Cr, mp 143—4°C, $[\alpha]_D$ −70.0° (EtOH).[107]

L-Tyrosylglycylglycine

p-$[(HO)_2P(O)O]C_6H_4CH_2CH(NH_2)CONHCH_2CONHCH_2CO_2H$ 13d. Cr, mp 182°C, $[\alpha]_D^{23}$ +7.5° (H_2SO_4).[14]

p-$[(HO)_2P(O)O]C_6H_4CH_2CH(NHCO_2CH_2Ph)CONHCH_2CONHCH_2CO_2H$ 1e/14c. Amorph so. Pb salt, 1:1.[14]

Glycyl-L-Tyrosylglycine

$NH_2CH_2CONHCH[CH_2C_6H_4OP(O)(OH)_2\text{-}p]CONHCH_2CO_2H$ 13d. Cr, mp 198°C d, $[\alpha]_D^{23}$ +8.0° (H_2SO_4).[14]

$PhCH_2O_2CNHCH_2CONHCH[CH_2C_6H_4OP(O)(OH)_2\text{-}p]CONHCH_2CO_2H$ 1e/14c. Amorph so. Pb salt, 1:1.[14]

D. Protein Derivatives

Human Protein

Serum albumin 1e.[70,71] Sedimentation coefficient, UV, electrophoresis.[71]
Serum globulin 1e.[70]
Hemoglobin 1e. Sedimentation coefficient, UV, electrophoresis.[71]
Globin 1e. Sedimentation coefficient.[71]
Serum [^{32}P]-protein 1e.[72]

Bovine Protein

Serum albumin 8a.[121]
Hemoglobin, type II 1e.[58]
Lactalbumin 1e.[13]
β-Lactoglobulin 1e. ^{31}P NMR, CD, GFC, gel electrophoresis.[73]
Casein 1e.[10]
Casein, dephosphorylated 1e.[69,70]
Caseinogen 1e.[69]
Histone 4 9a.[139,140] ^{31}P NMR, gel electrophoresis.[140]
Myelin basic protein 9a.[139]

Horse Protein

Serum albumin 1e. Sedimentation coefficient, viscosity, electrophoresis.[74]
Serum globulin 1e.[69]

Chicken Protein

Crystalline egg albumin 1e,[9,75] 8a.[121] Viscosity.[75]
Ovomucoid 8a.[121]

Silkworm Protein

Silk fibroin 8a.[121]
Silk fibroin peptone 1e.[10]
Sericin 8a.[121]

Herring Protein

Clupeine 1c. CD ^{31}P NMR.[64]
Clupeine YI 1c. GFC.[64]
Clupeine Z lc. GFC.[64]

Salmon Protein

Sperm protamine 7a,[117]
-, [^{33}P]-labeled 7a.[117]

Plant Protein

Gluten 8a.[121]
Gliadin 8a.[121]
Soy protein 8b.[133]
Edestin 8a.[121]

Bacterial Protein

Gramicidin 8a.[121]
Phosphoramidate hexose transferase *(Escherichia coli)* 9a.[142]
Protein HPr *(Staphylococcus aureus)* 9a. ^{1}H NMR, ^{31}P NMR, gel electrophoresis.[141]

Other Proteins (Sources Unidentified)

Gelatin 4c,[110] 8a.[121] Viscosity, flow birefringence.[110]
γ-Globulin 8a.[121]
Globin 8a.[121]
Insulin 8a,[121] 9a.[136] Electrophoresis.[136]
Witte peptone 1e.[10]
Blood globulin 1e.[10]
Isinglass 8a.[121]
Pepsin 4c.[110]

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