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ORGANOPHOSPHORUS ANALOGUES AND DERIVATIVES OF THE NATURAL L-AMINOCARBOXYLIC ACID AND PEPTIDES VII*. ENZYME SYNTHESIS OF PHOSPHA-C PEPTIDES

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Abstract — It is proved that the phospha-C peptides (with PO—NH instead of CO—NH bond) can be obtained by enzyme-catalyzed condensation of esters of alkylphosphonic and dialkylphosphinic acids with esters of L-α-aminocarboxylic acids Condensations of the natural phosphono-, methylphosphino esters **1**—**4** and the ethyl esters of glycine, L-alanine, L-methionine, and L-histidine were carried out in the presence of alkaline phosphatase (with the esters **1** and **2**) and phosphodiesterase I (with the esters **3** and **4**) to give the phospha-C peptides **5**—**20**, respectively The synthesis of the free dipeptides **21**—**36** was achieved by hydrolysis of the protecting groups using total bee venom A similar procedure applied to the dipeptides **37** and **38** (obtained by selective hydrolysis with the enzyme catalyst alkaline mesintericopeptidase of N-acetyl group of **9** and **17**) and the phosphino component **4** yielded the phospha-C tripeptides **39**—**42**, and with glycine anhydride to tetrapeptides **43** and **44**, respectively

The phospha-C peptides are bioactive compounds of considerable interest The first samples of this class were obtained by Imoto et al [1] and Martell et al [2] The main problem of their synthesis is the high instability of the PO—NH bond under conditions designed for the acid mediated liberation of the functional groups[2] The same difficulties have been encountered and cited by other authors[3] In particular, they have employed the DCC-method for condensation of the phenylalanine phosphonic analogue $AcNHCH(CH_2Ph)PO_3H_2$ and H—Phe—OEt Issleib et al [4] have succeeded in synthesis of free phospha-C peptides by condensation of the mono(trimethylsilyl)ester of 2-(benzyloxycarbonylamino)ethylphosphonic acid — $[PhCH_2OCONHCH_2CH_2P(O)(OH)(OSiMe_3)]$ and ethylisocyanoacetoacetate $[C{=}NCH_2COCH_2COOEt]$ followed by hydrogenation and hydrolysis of the trimethylsilyl protective group We have recently reported[5] the synthesis of the phospha-C peptide analogue of the tripeptide antibiotic "Bialaphos" $[H_2NCH(COOH)CH_2CH_2P(O)(CH_3)$-Ala-Ala-OH], where the liberation of the protected group was achieved by application of the enzymatic approach developed in our laboratory[6]

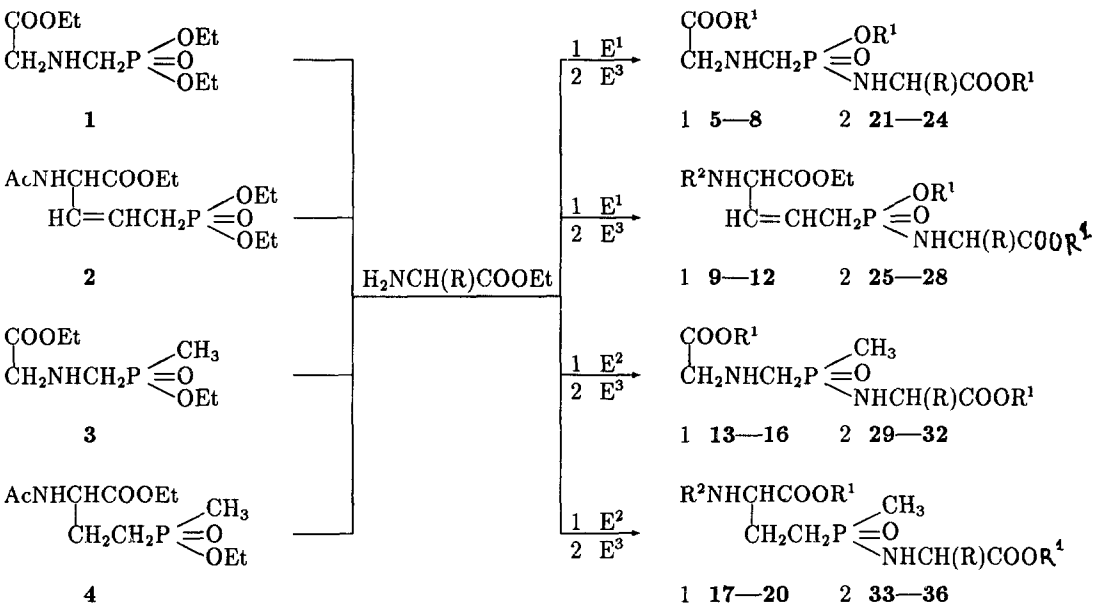
RESULTS AND DISCUSSIONS

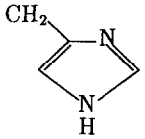
The enzyme synthesis was initially applied to N-phosphonomethylglycine triethyl ester (**1**)[7] and glycine ethyl ester with the enzyme phosphodiesterase I The components were mixed in water (pH 8 4) at 35 °C for 6 h and N-phosphonomethylglycine ethyl ester ($EtOCOCH_2NHCH_2P(O)(OH)_2$) and glycine anhydride were isolated The water medium was assumed to be responsible for the lack of condensation and was substituted by the alcoholic one *No enzyme-substrate interactions, neither condensation nor hydrolysis were* observed The aid of water (i—PrOH H_2O = 20 1, 0 1 mol of each component, 10 mg enzyme, 6 h, 35 °C)

gave rise to an inselective interaction, which resulted in the required product **5** in a yield of about 10 %, the free phosphono acid $H_2O_3PCH_2$—Gly—OEt, and the phosphono esters (i—PrO)(EtO)(O)PCH_2—Gly—OEt and (i—PrO)$_2$(O)PCH_2—Gly—OEt due to three enzyme-catalyzed interactions — condensation, hydrolysis, preesterification, respectively The substitution of the isopropyl alcohol with the ethanol did not increase the yield of the product **5**

The experiments, where the enzyme alkaline phosphatase was introduced, turned out to be more selective with respect to production of the phospha-C dipeptide **5** Under the same conditions **5** was isolated in a yield of 65 % and the coproducts as follows the combined phosphono diester (i—PrO)(EtO)(O)PCH_2—Gly—OEt in a yield of 20 % and the phosphono monoesters (i—PrO)$_2$(OH)(O)PCH_2—Gly—OEt, (EtO)(OH)(O)PCH_2—Gly—OEt in yields of about 5—6 %

SCHEME 1



R	Compound	R^1	Compound	R^2	Compound
H	**5, 9, 13, 17, 21, 25, 29, 33**	OEt	**5—20**	Ac	**9—12, 17—20**
CH_3	**6, 10, 14, 18, 22, 26, 30, 34**	H	**21—36**	H	**25—28, 33—36**
$CH_2CH_2SCH_3$	**7, 11, 15, 19, 23, 27, 31, 35**				
	**8, 12, 16, 20, 24, 28, 32, 36**				

E^1 — Alkaline phosphatase, E^2 — Phosphodiesterase I, E^3 — Bee venom

It seems the enzyme catalyzed preesterification provides the required conditions for the amide condensation Substitution of the solvent, for instance with dioxane, affords only one product — the phosphono monoester (EtO)(OH)(O)PCH_2—Gly—OEt The same compound was obtained from N-phosphonomethylglycine triethyl ester **1** in the presence of alkaline phosphatase in water (20 g of substrate, 10 mg enzyme, 600 ml buffer, pH 10 4, 6 h, 35 °C, practical quantitative yield) without a second amide component

The interaction of **1** with the ethyl esters of L-alanine, L-methionine, and L-histidine was carried out following a similar procedure to give the phospha-C peptides **6**, **7**, and **8** isolated in yields of 60, 66, and 72 %, respectively It is worth noting that no condensation with participation of the nitrogen atom of imidazole group was observed for the histidine interaction On the other hand, the condensation of imidazole and **1** resulted in the phosphamide (Imid)(EtO)(O)PCH_2—Gly—OEt Also the condensation in systems with molar ratio of **1** and the corresponding ethyl esters of L-ornithine, L-lysine, and L-arginine was found to affect only the α-amino group

For a better characterization of the structure-activity relationship the behaviour of the antibiotic Plumbemicine **2**[8] produced by *Streptomices plumbens strains* was studied The condensation products **9**—**12** were isolated under the same conditions as above in yields of 67, 65, 71, and 76 %, respectively, from **2** and the ethyl esters of glycine, L-alanine, L-methionine, and L-histidine using alkaline phosphatase The following compounds were obtained as reaction coproducts monophosphono- and ethyl(propyl)phosphono esters

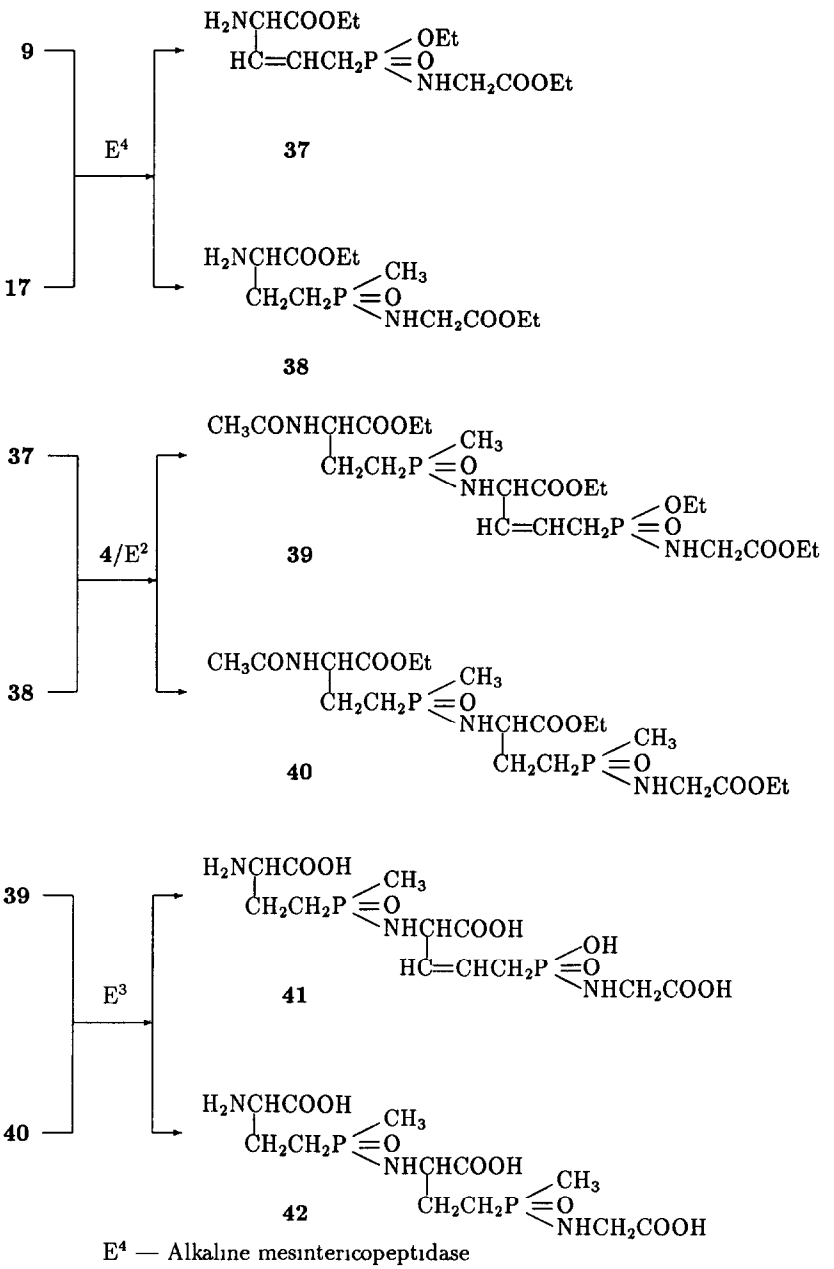
At the next step, the reaction of the methylphosphinic derivative of glycine **3** known for its herbicidal activity and the glycine ethyl ester was considered It was found that the enzyme alkaline phosphatase does not interact with the substrate **3** without regard to the medium — water or isopropyl alcohol/water By using the enzyme phosphodiesterase I in water (0 1 mol of both **3** and H—Gly—OEt, 10 mg of the enzyme, 600 ml of water, pH 8 4, 6 h, 35 °C) the methylphosphinic acid Me(OH)(O)PCH_2—Gly—OEt was isolated Substitution of the reaction medium with isopropyl alcohol/water afforded the phospha-C dipeptide **13** in a yield of 50 % and the following coproducts Me(i—PrO)(O)PCH_2—Gly—OEt and Me(OH)(O)PCH_2—Gly—OEt No traces of the starting compound **3** were detected

The condensation of the substrate **3** and the ethyl esters of L-alanine, L-methionine, and L-histidine was similarly carried out to give the phospha-C peptides **14**, **15**, and **16**, respectively in yields of between 50—55 %

The same procedure (amino components and phosphodiesterase I) applied to the natural antibiotic phosphinotricine **4**[11] gave the phospha-C dipeptides **17**—**20** in yields of between 60—68 %

The hydrolysis of the protecting groups of dipeptides **5**—**20** was achieved by employing total bee venom It was established that the enzyme provides simultaneous hydrolysis of N-acetylic, ethoxycarbonylic, and ethhoxyphosphinylic groups of the substrates **5**—**20** to the free phospha-C dipeptides **21**—**36** isolated in yields of between 80—95 % We note that the high-molecular weight components of a dialyzed or a ultrafiltrated bee venom do not exhibit any hydrolytic activity The attempts to reactivate the enzyme by mixing high- and low-molecular weight components failed also It seems that some molecular complexes responsible for the hydrolytic activity of the total bee venom are irreversibly destroyed by dialysis or ultrafiltration On the other hand, the enzymes alkaline phosphatase and phosphodiesterase I treated in the same way preserve their activity for at least twelve subsequent experiments

SCHEME 2



E⁴ — Alkaline mesintericopeptidase

To extend the scope of the enzyme catalyzed approach experiments aimed at phospha-C tripeptide synthesis were carried out The N-acetyl protecting groups encountered in phospha-C dipeptides **9** and **17**

were initially hydrolyzed by using the enzyme alkaline mesintericopeptidase (EC 3 4 4) It is well known that under certain conditions[12] the enzyme supports the hydrolysis of ethoxycarbonylic groups to give free acids, for instance H—Cys(O_2NH_2)—OEt to H—Cys(O_2NH_2)—OH We succeeded in isolation of the free (with respect to the aminogroups) dipeptides **37** and **38** in yields of 72 and 79 %, respectively, using the following conditions 20 g of the substrate **9** and **17**, respectively, 10 mg of enzyme, 600 ml water, pH 8 6, 25 °C, 6 h The protected phosphinotricine **4** was employed as a phosphino component

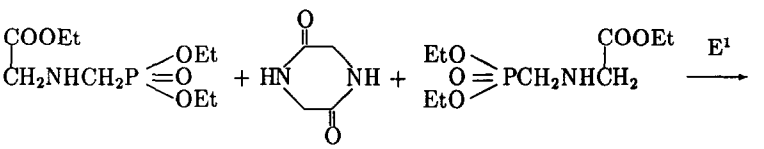
The phospha-C tripeptides **39** and **40** were obtained in a yield of about 70 % by using the enzyme catalyst phosphodiesterase I (0 1 mol of **9** and **17**, respectively, 10 mg of enzyme, medium of i—PrOH H_2O = 20 1, 6 h, 35 °C) The simultaneous hydrolysis of N-acetylic and ethoxycarbonylic groups was realized by using bee venom to give the corresponding free phospha-C tripeptides **41** and **42** in yields above 90 %

The attempts for enzyme-supported condensation of Z—Ala—Ala—OEt and the glycine phosphono analogue $AcNHCH_2P(O)(OEt)_2$ in the presence of alkaline phosphatase or phosphodiesterase I failed The only products obtained under the above conditions were mono- or diesters of acylaminomethylphosphonic acid

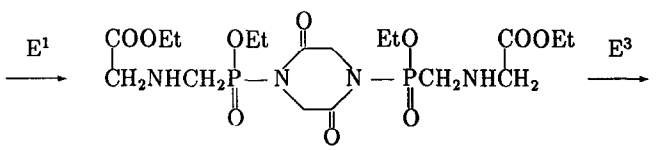
It was surprisingly found that a condensation of **1** and glycine anhydride takes place in presence of alkaline phosphatase to give the condensation product **43** isolated in a yield of 65 % The hydrolysis of the protecting groups was carried out by using total bee venom to afford the free tetrapeptide **44**

The results on condensation of **1**—**4** with mixed anhydrides of L-α-aminocarboxylic acids or with some cyclic peptides as well as the corresponding physiological data will be published elsewhere

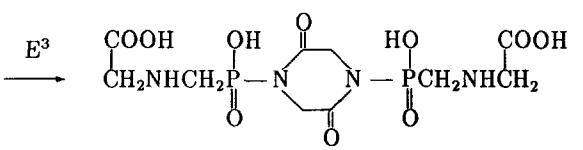
SCHEME 3



1 **1**



43



44

EXPERIMENTAL

1 General notes IR-spectra, elemental analysis, melting point, HPLC, and $[\alpha]_D^{20}$ were measu Perkin-Elmer instruments, Mass-spectra — on Varian, R_f-values, silica gel film "Merck", reager solvents from "Aldrich" and "Merk", enzymes and buffers from "Sigma", bee venom from "K Bulgaria

2 Synthesis of phospha-C peptides **5—20, 39, 42, 43** General procedure The phosphono **1—4** (0 1 mol), the ethyl esters of glycine, L-alanine, L-methionine, and L-histidine (0 1 mol), a enzymes alkaline phosphatase for the substrates **1, 2** and phosphodiesterase I for **3, 4** (25 mg), respe are mixed in medium of isopropyl alcohol (200 ml) and water (20 ml) and stirred at 35 °C for 6 h enzyme separation the reaction mixture is evaporated in vacuum (50 °C) to dryness and the res placed in a silica gel column (eluent ethyl acetate benzene = 9 1) The eluent is concentrated to n-Hexane is added until the solution becomes opaque and the corresponding products are filtered a h at -5 °C

The same procedure applied to **1** and the glycine anhydride and **37, 38, 4** gives **39, 41, 43,** tively

3 Enzyme catalyzed hydrolysis of the protected groups of **5—20, 39, 41, 44** General proce The esters **5—20, 39, 41, 44** (20 g) and a lyophilizated raw bee venom (20 mg) are suspended ir (600 ml, pH 7 8) and stirred at 39 °C for 6 h until the solution becomes homogeneous The reaction n is neutralized and concentrated in vacuum to an oily residue, which is then dissolved in hot ethanol cooling the products **21—36, 40, 42, 44** are filtered respectively

4 Synthesis of the amino components **37** and **38** The acetyl derivatives **9** and **17** (20 g) a enzyme alkaline mesintericopeptidase (10 mg) were stirred in water (600 ml, pH 7 9) at 25 °C f After concentration in vacuum the organic phase is extracted by ethyl acetate (3×100 ml) The dry c residue is concentrated to 50 ml and cooled n-Hexane is added until the solution becomes opaq the corresponding products **37** and **38** are filtered after 24 h at -5 °C

Table Physical and Spectral Data of Compounds **5—44** Prepared

No	M p (°C) (decomp)	Yield (%)	Molecular Formula	Required(Found)% C	H	N	IR (KBr) ν(cm^{-1})	MS (m$^+$/e)	R_f a)
5	121—124	64 5	$C_{11}H_{23}N_2O_6P$ (310 3)	42 58 (42 69	7 47 7 31	9 03 9 12)	1748, 1643, 1520, 1348, 1255, 1118, 946, 843, 756, 648	310	0 7(
6	111—114	59 3	$C_{12}H_{25}N_2O_6P$ (324 3)	44 44 (44 72	7 77 7 38	8 64 8 60)	1756, 1653, 1518, 1356, 1248, 1132, 965, 930, 811, 729	324	0 7'
7	92—95	66 2	$C_{14}H_{29}N_2O_6PS$ (384 4)	43 74 (43 82	7 60 7 33	7 29 7 35)	1732, 1680, 1630, 1575, 1342, 1256, 1152, 1068, 941, 856	384	0 7(
8	133—136	72 4	$C_{15}H_{28}N_4O_6P$ (391 4)	46 01 (46 22	7 21 7 14	14 31 14 15)	1762, 1675, 1628, 1592, 1530, 1368, 1249, 1138, 1102, 935, 848, 729, 642	391	0 5(
9	86—89	67 2	$C_{15}H_{27}N_2O_7P$ (378 4)	47 84 (47 56	7 19 7 38	7 40 7 51)	1752, 1705, 1672, 1612, 1548, 1320, 1226, 1084, 949, 862, 736, 618	378	0 7(
10	100—103	64 8	$C_{16}H_{29}N_2O_7P$ (392 4)	48 98 (48 69	7 45 7 72	7 14 7 18)	1760, 1710, 1666, 1630, 1555, 1312, 1240, 1056, 960, 858, 729, 623	392	0 6(
11	92—94	71 3	$C_{18}H_{33}N_2O_7PS$ (452 5)	47 78 (47 92	7 35 7 26	6 19 6 21)	1762, 1708, 1652, 1546, 1318, 1243, 1058, 964, 839, 721	453	0 5(

Table (continued)

No	M p (°C) (decomp)	Yield (%)	Molecular Formula	Required(Found)% C	H	N	IR (KBr) ν(cm^{-1})	MS (m$^+$/e)	R_f a)	$[\alpha]_D^{20}$ b)
12	134–136	75 8	$C_{19}H_{32}N_4O_7P$ (459 5)	49 67 (49 88	7 02 6 94	12.19 12 31)	1758, 1701, 1642, 1552, 1324, 1256, 1043, 968, 843, 718	459	0 44	+52 3
13	142–144	49 6	$C_{10}H_{21}N_2O_5P$ (280 3)	42 86 (42 95	7 55 7 34	9 99 10 07)	1742, 1700, 1632, 1601, 1572, 1530, 1412, 1336, 1305, 1252, 1111, 1093, 956, 838, 721, 652	280	0 78	
14	121–124	47 6	$C_{11}H_{23}N_2O_5P$ (294 3)	44 89 (45 03	7 88 7 56	9 52 9 38)	1738, 1712, 1628, 1611, 1563, 1534, 1415, 1352, 1311, 1262, 1120, 1082, 934, 842, 730, 650	294	0 75	+29 3
15	93–95	52 6	$C_{13}H_{27}N_2O_5PS$ (354 4)	44 06 (43 82	7 68 7 79	7 90 7 63)	1729, 1691, 1618, 1560, 1529, 1418, 1349, 1305, 1256, 1118, 1075, 929, 830, 806, 726, 653	354	0 70	+53 8
16	156–159	48 3	$C_{14}H_{26}N_4O_5P$ (361 4)	46 53 (46 48	7 25 7 41	15 50 15 26)	1733, 1686, 1615, 1538, 1492, 1406, 1356, 1310, 1261, 1126, 1089, 1005, 986, 930, 831, 792, 706	361	0 60	+44 3
17	129–131	62 3	$C_{13}H_{25}N_2O_6P$ (336 3)	46 42 (46 80	7 49 7 33	8 33 8 29)	1756, 1652, 1523, 1480, 1321, 1261, 1110, 930, 865, 793, 701, 652	336	0 82	+62 4
18	100–103	64 8	$C_{14}H_{27}N_2O_6P$ (350 3)	47 99 (48 11	7 77 7 62	7 99 8 18)	1750, 1648, 1525, 1472, 1318, 1272, 1106, 929, 860, 799, 700, 662	350	0 76	+52 4
19	86–89	60 2	$C_{16}H_{31}N_2O_6PS$ (410 5)	46 82 (46 98	7 61 7 48	6 82 6 80)	1744, 1639, 1521, 1480, 1321, 1265, 1100, 934, 850, 805, 705, 664	411	0 80	+48 6
20	150–153	66 3	$C_{17}H_{30}N_4O_6P$ (417 4)	48 91 (49 11	7 24 7 01	13 42 13 56)	1758, 1645, 1526, 1471, 1318, 1256, 1110, 946, 840, 800, 711, 648	417	0 66	+53 8
21	(~ 260)	89 3	$C_5H_{11}N_2O_6P$ (226 1)	26 56 (26 92	4 90 4 63	12 39 12 41)	3600–3300, 2850–2640, 1532, 1380, 1256, 938, 845, 705, 630	226	0 56	
22	(~ 255)	92 3	$C_6H_{13}N_2O_6P$ (240 2)	30 01 (30 26	5 45 5 11	11 67 11 56)	3600–3310, 2875–2640, 1540, 1384, 1264, 942, 850, 711, 638	240	0 50	+63 3
23	(~ 240)	90 3	$C_8H_{17}N_2O_6PS$ (300 3)	32 00 (32 18	5 71 5 55	9 33 9 46)	3600–3300, 2860–2645, 1529, 1396, 1258, 956, 836, 705, 645	300	0 42	+40 4
24	(~ 280)	97 3	$C_9H_{16}N_4O_6P$ (307 2)	35 51 (35 26	5 25 5 48	18 24 18 03)	3600–3305, 2855–2640, 1533, 1402, 1249, 948, 840, 711, 652	307	0 33	+44 2

Table (continued)

No	M p (°C) (decomp)	Yield (%)	Molecular Formula	Required(Found)% C	H	N	IR (KBr) ν(cm^{-1})	MS (m$^+$/e)	R_f a)
25	262–264	90 7	$C_7H_{13}N_2O_6P$ (252 2)	33 34 (33 62	5 19 5 01	11 11 11 38)	3600–3300, 2860–2650, 1520, 1503, 1456, 1402, 1253, 962, 831, 705, 648	252	0 72 +
26	251–254	86 3	$C_8H_{15}N_2O_6P$ (266 2)	36 09 (35 84	5 68 5 89	10 53 10 48)	3600–3310, 2855–2645, 1538, 1509, 1423, 1392, 1246, 958, 828, 692, 640	266	0 68 +
27	233–236	93 6	$C_{10}H_{19}N_2O_6PS$ (326 3)	36 81 (36 66	5 87 5 93	8 59 8 69)	3600–3300, 2850–2640, 1542, 1493, 1418, 1396, 1252, 969, 834, 685, 642	326	0 70 +
28	256–259	96 4	$C_{11}H_{18}N_4O_6P$ (333 3)	39 64 (40 00	5 44 5 18	16 81 16 92)	3600–3310, 2850–2645, 1536, 1499, 1403, 1383, 1249, 975, 853, 680, 648	333	0 60 +
29	(~ 260)	90 5	$C_6H_{13}N_2O_5P$ (224 2)	32 15 (32 36	5 84 5 62	12 50 12 88)	3600–3300, 1753, 1548, 1331, 1243, 1110, 992, 848, 726, 676, 603	224	0 77
30	(~ 250)	93 8	$C_7H_{15}N_2O_5P$ (238 2)	35 30 (35 21	6 35 6 46	11 78 11 88)	3600–3300, 1764, 1539, 1330, 1238, 1111, 986, 852, 730, 682, 605	238	0 71 +
31	(~ 260)	96 3	$C_9H_{19}N_2O_5PS$ (298 3)	36 24 (36 18	6 42 6 66	9 39 9 48)	3600–3300, 1752, 1555, 1326, 1246, 1103, 1005, 843, 756, 680, 611	298	0 72 +
32	(~ 280)	96 6	$C_{10}H_{18}N_4O_5P$ (305 2)	39 35 (39 61	5 94 5 76	18 36 18 52)	3600–3300, 1746, 1548, 1331, 1228, 1111, 1002, 856, 748, 692, 606	305	0 62 +
33	198–200	86 3	$C_7H_{15}N_2O_5P$ (238 2)	35 29 (35 61	6 35 6 12	11 76 11 52)	3600–3320, 1755, 1560, 1340, 1258, 1212, 1146, 1102, 1036, 948, 860, 721	238	0 76 +
34	179–181	93 4	$C_8H_{17}N_2O_5P$ (252 2)	38 09 (38 33	6 80 6 71	11 11 11 29)	3600–3315, 1750, 1553, 1349, 1256, 1160, 1153, 1100, 1043, 952, 855, 730	252	0 71 +
35	216–219	90 4	$C_{10}H_{21}N_2O_5P$ (312 3)	38 45 (38 66	6 78 6 42	8 97 9 11)	3600–3310, 1744, 1555, 1353, 1264, 1162, 1142, 1106, 1053, 943, 844, 713	312	0 66 +
36	248–251	94 6	$C_{11}H_{20}N_4O_5P$ (319 3)	41 38 (41 55	6 31 6 12	17 55 17 77)	3600–3300, 1752, 1546, 1346, 1253, 1178, 1152, 1112, 1043, 956, 839, 705	319	0 56 +
37	169–172 HCl salt	84 6	$C_{13}H_{25}N_2O_6P$ (336 3)	46 42 (46 58	7 49 7 31	8 33 8 47)	3000–2560 ($-NH_3^+Cl^-$), 1762, 1700, 1528, 1333, 1256, 1093, 955, 864, 720, 605	336	0 62 +
38	192–194 HCl salt	90 1	$C_{11}H_{23}N_2O_5P$ (294 3)	44 89 (45 03	7 88 7 52	9 52 9 50)	3010–2600 ($-NH_3^+Cl^-$), 1745, 1711, 1533, 1318, 1249, 1069, 948, 846, 751, 611	294	0 66 +

Table (continued)

No	M p (°C) (decomp)	Yield (%)	Molecular Formula	Required(Found)% C	H	N	IR (KBr) ν(cm^{-1})	MS (m$^+$/e)	R_f a)	$[\alpha]_D^{20}$ b)
39	oil	70 5	$C_{22}H_{41}N_3O_{10}P_2$ (569 5)	46 39 (46 51	7 26 7 12	7 38 7 41)	1684, 1645, 1583, 1552, 1503, 1428, 1325, 1255, 1127, 1036, 974, 814, 753	traces	0 73	+33 2
40	oil	68 3	$C_{20}H_{39}N_3O_9P_2$ (527 5)	45 54 (45 44	7 45 7 68	7 97 7 82)	1672, 1652, 1594, 1550, 1511, 1453, 1329, 1241, 1136, 1028, 981, 826, 745	traces	0 80	+28 3
41	(~ 240)	92 6	$C_{12}H_{23}N_3O_9P_2$ (415 3)	34 71 (34 86	5 58 5 35	10 12 10 18)	3500–3240, 2840–2460, 1762, 1748, 1562, 1498, 1444, 1332, 1259, 1003, 972, 846, 729, 643	415	0 38	+30 3
42	(~ 250)	94 3	$C_{12}H_{35}N_3O_8P_2$ (401 3)	35 91 (36 18	6 28 6 05	10 47 10 56)	3650–3300, 1750, 1704, 1652, 1566, 1515, 1430, 1350, 1246, 1018, 978, 846, 729, 649	401	0 33	+42 1
43	93–95	64 3	$C_{18}H_{38}N_4O_8P_2$ (500 5)	43 20 (43 53	7 65 7 60	11 20 11 31)	1748, 1528, 1440, 1380, 1246, 1112, 1079, 984, 844, 728	traces	0 66	
44	(~ 280)	89 3	$C_{10}H_{22}N_4O_8P_2$ (388 3)	30 93 (31 16	5 71 5 56	14 43 14 56)	3600–3310, 2840–2430, 1756, 1728, 1538, 1365, 1305, 1246, 1005, 974, 846, 728, 652	388	0 36	

a) Systems **A** $CHCl_3$ MeOH AcOEt = 9 2 1 for **5–20, 37–40, 43**
B DMF AcOH MeOH dioxane = 6 2 1 1 for **21–36, 41, 42, 44**

b) c = 0 1 (MeOH) for **5–20, 37–40**
c = 0 1 (NaOH) for **21–36, 41, 42**

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