Chemical and Biomedical Motifs of the Reactions of Hydroxymethylphosphines with Amines, Amino Acids, and Model Peptides

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Abstract: The reactions of tris(hydroxymethyl)phosphine (THP, **1**), 1,2-bis(bis(hydroxymethyl)phosphino)-benzene (HMPB, **2**), and 1,2-bis(bis(hydroxymethyl)phosphino)ethane (HMPE, **3**) with various amines including amino acids and model peptides have been explored. The reactions of these multifunctional phosphines with excess amino acids unexpectedly produced monomeric products. The reaction of THP with excess glycine produced THP(glycine)$_3$ (**4**) in high yield. The reactions of HMPB with the secondary amines *N*-methylaniline and diethylamine produced the compounds HMPB(*N*-methylaniline)$_4$ (**5**) and HMPB(diethylamine)$_4$ (**6**), respectively. However, the reactions of HMPB and HMPE with excess glycine produced trans annular-bonded bicyclic compounds HMPB(glycine)$_2$ (**7**) and HMPE(glycine)$_2$ (**10**). The reactions of HMPB with excess alanine and glycylglycylglycine were also explored to determine the generality of the reactions and correspondingly yielded the novel heterocyclic compounds HMPB(alanine)$_2$ (**8**) and HMPB(gly-gly-gly)$_2$ (**9**), respectively. The products are oxidatively stable in air and under a wide pH range. All of the new compounds have been characterized by a combination of analytical and spectroscopic techniques, and the molecular structures of compounds **4**, **5**, **7**, and **10** have been confirmed by single-crystal X-ray diffraction studies.

Introduction

Chemical molecules that link two or more biologically useful amines, peptides, or proteins, under mild (and biologically benign) reaction conditions, are vital in a number of biomolecular structural motifs,[1,2] which include formulation of synthetic β turns and β sheets[3] and construction of three, four, or six helix bundles.[4−6] A number of chemical approaches to linking basic subunits of simple amines, amino acids, peptides, or other biomolecules (e.g., sugars) are currently being used for the formulation of larger biomolecular vectors.[1−7] In this context, the reaction of tris(hydroxymethyl)phosphine ($P(CH_2OH)_3$ (THP)) (Scheme 1) with primary amines, first reported by Daigle and co-workers, provides an excellent example of linking amine-containing compounds (e.g., amino acids, peptides, or other biomolecules) in a single but yet effective methodology.[8] Although the utility of the reaction outlined in Scheme 1 has been studied for cross-linking and immobilization of specific enzymes,[9,10] the vast scope that this reaction offers to potentially link various amino acids and peptides for applications in biomedical sciences is still untapped.

The Mannich-type condensation of CH_2OH groups (of THP) with NH_2 groups of amines (Scheme 1) may be used in the introduction of phosphine centers on peptide backbones. Phosphine-containing peptides present applications in a number of areas. Immobilization of phosphines on peptides would yield interesting peptide (or protein)−metal conjugates.[11] In fact, tailoring metal-binding sites in peptides and proteins have been

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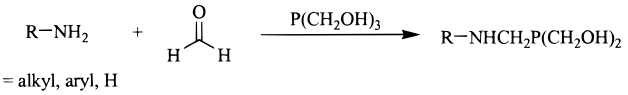
Scheme 1. Reaction of THP (**1**) with Amines



R = alkyl, aryl, H

shown to stabilize biologically active conformations, enhance structural integrity, and thereby promote new enzymatic activities.[12]

Since phosphines display versatile coordination chemistry with transition metals and radiometals, phosphine-containing peptides (or peptide-avid biomolecules) have gained importantance in the design and development of tumor-specific radiopharmaceuticals.[13−17] Despite the significant utility offered by phosphine-containing peptides (and proteins), synthetic strategies of producing such bioconjugates are still in infancy. The elegant work by Gilbertson and co-workers on the incorporation of aryl and cyclohexyl phosphines on specific peptides has provided impetus to this burgeoning field of chemical and biomedical sciences.[18]

From the aforementioned discussions, it is clear that the model reaction, outlined in Scheme 1, can be used in multiple applications in chemistry, biochemistry, and biomedical sciences. Therefore, a detailed understanding of the fundamental organic chemistry of the reactions of hydroxymethylphosphines with amines and amino acids will further aid the applications of these reactions in chemical and biomedical sciences. As part of our studies involving the development of new bioconjugates,[19] we have undertaken a systematic investigation of the reactions of (a) tris(hydroxymethyl)phosphine (THP, **1**), (b) 1,2-bis[bis(hydroxymethyl)phosphino]benzene (HMPB, **2**), and (c) 1,2-bis[bis(hydroxymethyl)phosphino]ethane (HMPE, **3**) with primary/secondary amines, amino acids, and model peptides. We, herein, report isolation and full characterization of phosphine−amine (or amino acid) and peptide conjugates and also X-ray structures of several of the amino acid−phosphine conjugates: (i) monophosphine (THP)−glycine conjugate, (ii) bisphosphine (HMPB)−*N*-methylaniline conjugate, (iii) bisphosphine (HMPB)−glycine conjugate, and (iv) bisphosphine (HMPE)−glycine conjugate. These are the first examples of structurally characterized hydroxymethyphosphine−amino acid linkages, and they provide, for the first time, definite evidence for the Mannich-type of addition of $-CH_2OH$ groups with $-NH_2$ groups as discussed in the following sections

Experimental Section

All reactions were carried out under purified nitrogen by standard Schlenk techniques. Solvents were purified and dried by standard methods and distilled under nitrogen prior to use. Glycine, alanine, and gly-gly-gly were obtained from Sigma Chemical Co. Diethylamine and *N*-methylaniline were obtained from Aldrich Chemical Co. All commercial reagents were used as received. The synthesis of THP (**1**),[20] HMPB (**2**),[21] and HMPE (**3**)[22] has been previously described. Waters Sep-Pak Vac C18 columns (10 g, 35 mL) were obtained from Fisher Scientific Co.

Nuclear magnetic resonance spectra were recorded on a Bruker ARX-300 spectrometer. The ^1H and ^{13}C chemical shifts are reported relative to an external standard of TMS, and ^{31}P NMR chemical shifts are reported to an external standard of 85% H_3PO_4.

High-pressure liquid chromatography (HPLC) analyses and separations were performed using a Waters 600 dual-pump system equipped with a 486 tunable absorbance detector and a 746 data module. Standard reverse phase HPLC separations were performed on a C18 column (Whatman Partisil 10 ODS-3 (9.5 × 500 mm)) using a gradient mobile phase with solvent A composed of 0.1% trifluoroacetic acid in 3:1 acetonitrile/water mixture and solvent B composed of 0.1% trifluoroacetic acid in water. The gradient used is as follows: 0−3 min 2% A, 98% B; 3−18 min linear gradient to 100% A; 18−20 min 100% A; 20−30 min linear gradient to 2% A, 98% B. The flow rate and wavelength were set to 4 mL/min and 254 nm; respectively. Mass spectral analyses were performed by the Washington University Resource for Biomedical and Bio-organic Mass Spectrometry, St. Louis, Missouri. Elemental analyses were performed by Oneida Research Services, Inc. Whitesboro, New York. Melting points were determined on Mel-Temp II apparatus and are uncorrected.

2-Di(carboxymethylaminomethyl)phosphanylmethylamino-acetic Acid (4). Tris(hydroxymethyl)phosphine (0.933 g, 7.52 mmol) in 10 mL of distilled water was added dropwise to glycine (0.282 g, 3.76 mmol) in water (10 mL) at 25 °C. The reaction was stirred under dry nitrogen for 3 h. The product was filtered off and dried in vacuo to give the analytically pure compound in 82% yield as a white solid. Anal. Calcd for $C_9H_{18}N_3O_6P\cdot H_2O$: C, 34.49; H, 6.44; N,13.42. Found: C, 32.75; H, 6.34; N, 13.14. HRFAB calcd for $[M + H]^+$ 295.0933, found 296.1011. Mp 202−204 °C dec. ^1H NMR (D_2O, NaOD): δ 2.70 (s, 6H, NCH_2COOH), 3.04 (s, 6H, PCH_2N). ^{13}C NMR (D_2O, NaOD): δ 44.55 (d, NCH_2COOH, $J = 5.21$ Hz), 53.50 (d, PCH_2N, $J = 9.96$ Hz), 179.14 (s). ^{31}P NMR (D_2O, NaOD): δ −38.1 (s).

1,2-Di[di(methylanilinomethyl)phosphanyl]benzene (5). *N*-methylaniline (0.453 g, 4.23 mmol) was added dropwise to 1,2-bis[bis(hydroxymethyl)phosphino]benzene (0.222 g, 0.850 mmol) in ethanol (5 mL) at 25 °C. The reaction was stirred under dry nitrogen for 1 h. The product was filtered off and dried in vacuo to give the analytically pure compound in 90% yield as a white solid. Anal. Calcd for $C_{38}H_{44}N_4P_2$: C, 73.76; H, 7.17; N, 9.05. Found: C, 73.60; H, 7.05; N, 9.02. HRFAB calcd for $C_{38}H_{44}N_4P_2$ $[M + H]^+$ 618.3041, found 619.3095. Mp 88−90 °C. ^1H NMR ($CDCl_3$): δ 2.76 (s, 12H, NCH_3), 3.83 (m, 8H, PCH_2N), 6.63−6.68 (m, 12H, $MeNC_6H_5$-*o*,*p*), 7.07−7.19 (m, 8H, $MeNC_6H_5$-*m*), 7.39−7.44 (m, 2H, PC_6H_4-*m*), 7.59−7.64 (m, 2H, PC_6H_4-*o*). ^{13}C ($CDCl_3$) NMR: δ 39.2 (virtual triplet, NCH_3, $J = 6.7$ Hz), 53.8 (virtual triplet, PCH_2N, $J = 9.1$ Hz), 113.4 (t, $J = 1.2$ Hz), 116.9 (s), 128.9 (s), 129.4 (s), 131.3 (t, $J = 3.8$ Hz), 143.9 (t, $J = 5.3$ Hz), 149.4 (s). ^{31}P ($CDCl_3$) NMR: δ −43.6 (s).

1,2-Di[di(ethylaminomethyl)phosphanyl]benzene (6). Diethylamine (3.00 g, 41.0 mmol) was added dropwise to 1,2-bis[bis(hydroxymethyl)phosphino]benzene (2.56 g, 9.77 mmol) in ethanol (10 mL) at 25 °C. The reaction was stirred under dry nitrogen for 1 h. The solvent and excess diethylamine were removed in vacuo to obtain a viscous oil. The residue was suspended in water (10 mL) and extracted with CH_2Cl_2 (3 × 10 mL). The combined organic fractions were concentrated to approximately 10 mL and purified on a silica gel column to give the analytically pure product in 62% yield. Anal. Calcd for

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Table 1. Crystal Data and Details of Data Collection for Compounds **4**, **5**, **7**, **10**

	4	**5**	**7**	**10**
formula	$C_9H_{21}N_3O_6P)(Cl)_3 \cdot H_2O$	$C_{38}H_{44}N_4P_2$	$(C_{14}H_{19}N_2O_4P_2)(Cl)$	$C_{10}H_{18}N_2O_4P_2$
space group	$P\bar{1}$	$P2/c$, No. 13	$C2/c$	$C2/c$
crystal system	triclinic	monoclinic	monoclinic	monoclinic
fw	422.63	618.73	376.71	292.21
a, Å	6.8627(8)	21.199(4)	13.573(1)	14.6181(11)
b, Å	9.354(2)	8.1123(7)	13.279(1)	8.3199(7)
c, Å	14.486(4)	20.854(3)	10.653(1)	11.8780(9)
α, deg	84.51(2)	90.0	90.0	90.0
β, deg	77.44(2)	105.510(10)	108.816(1)	115.8770(10)
γ, deg	86.25(2)	90.0	90.0	90.0
T, K	295(2)	295(2)	295(2)	295(2)
λ, X	1.54056	1.54056	0.71073	0.71073
Z	2	4	4	4
$F(000)$	443.89	1320	784	616
V, Å^3	902.5(3)	3455.7(9)	1817.4	1299.77(18)
ρ_{calcd}, g/cm^3	1.555	1.189	1.377	1.493
ρ_{obsd}, g/cm^3	not measured	not measured	not measured	not measured
μ, mm^{-1}	5.89	1.37	0.40	0.33
gof	2.85	2.43	1.64	2.12
R_F, R_w[a]	0.039, 0.073	0.47, 0.073	0.031, 0.055	0.042, 0.069

[a] $R_F = \sum(||F_o| - |F_c||)/\sum(|F_o|)$; $R_w = [\sum(w(|F_o| - |F_c|)^2)/\sum (w|F_o|)^2]^{1/2}$.

$C_{26}H_{52}N_4P_2$: C, 64.68; H, 10.86; N, 11.61. Found: C, 64.55; H, 10.78; N, 11.72. HRFAB calcd for $C_{26}H_{52}N_4P_2$ $[M + H]^+$ 482.3667, found 483.3745. ^{1}H NMR ($CDCl_3$): δ 0.92−1.01 (m, 24H, NCH_2CH_3), 2.49−2.81 (m, 16H, NCH_2CH_3), 3.02−3.14 (m, 8H, PCH_2N), 7.28−7.34 (m, 2H, PC6*H*4-*m*), 7.46−7.53 (m, 2H, PC_6H_4-*o*). ^{13}C ($CDCl_3$) NMR: δ 12.1 (s, NCH_2CH_3), 48.2 (s, NCH_2CH_3), 55.3 (s, PCH_2N), 128.6 (s), 131.1 (s), 144.9 (t, J = 7.1 Hz). ^{31}P ($CDCl_3$) NMR: δ −60.3 (s).

2-(13-Carboxymethyl-10,13-diaza-1,8-diphosphatricyclo[6.3.3.0^{2,7}]-tetradeca-2,4,6-triene-10-yl)acetic Acid (7). 1,2-Bis[bis(hydroxymethyl)phosphino]benzene (0.5007 g, 1.910 mmol) in water (5 mL) was added dropwise to glycine (0.7209 g, 9.603 mmol) also in water (10 mL) at 25 °C. The reaction mixture was continuously stirred under dry nitrogen for 1 h. The product was filtered off, washed with water, and dried in vacuo to give the analytically pure compound in 85% yield. Anal. Calcd for $C_{14}H_{18}N_2O_4P_2 \cdot H_2O$: C, 46.92; H, 5.63; N, 7.82. Found: C, 46.81; H, 5.48; N, 7.86. HRFAB calcd for $C_{14}H_{18}N_2O_4P_2$ 340.0742. Found $[M + H]^+$ 341.0820. Mp 210−212 °C. ^{1}H NMR (D_2O, NaOD): δ 2.82 (s, 4H, NCH_2COOH), 2.84−2.94 (m, 4H, PCH_2N), 3.21−3.27 (m, 4H, PCH_2N), 7.20−7.24 (m, 2H, PC_6H_4-*m*), 7.53−7.81 (m, 2H, PC_6H_4-*o*). ^{13}C NMR (D_2O, NaOD): δ 50.2 (d, PCH_2N, J = 15.1 Hz), 65.2 (s, NCH_2COOH), 130.5 (s), 137.8 (d, J = 52.8 Hz), 142.0 (s), 178.8 (s). ^{31}P NMR (D_2O, NaOD): δ −9.9 (s).

2-[13-(1-Carboxyethyl)-10,13-diaza-1,8-diphosphatricyclo[6.3.3.0^{2,7}]-tetradeca-2,4,6-trien-10-yl)propanoic Acid (8). 1,2-Bis[bis(hydroxymethyl)phosphino]benzene (0.8603 g, 3.282 mmol) in water (15 mL) was added dropwise to alanine (1.498 g, 16.81 mmol) also in water (15 mL) at 25 °C. The reaction was continuously stirred under dry nitrogen at room temperature for 1 h. The product was filtered off, washed with water, and dried in vacuo to give the analytically pure product in 78% yield. Anal. Calcd for $C_{16}H_{22}N_2O_4P_2 \cdot H_2O$: C, 49.73; H, 6.26; N, 7.25. Found: C, 50.02; H, 6.77; N, 7.55. HRFAB calcd for $C_{16}H_{22}N_2O_4P_2$ $[M + H]^+$ 368.1055, found 369.1133. Mp 208−210 °C. ^{1}H NMR (D_2O, NaOD): δ 0.83 (d, 6H, $NCHCH_3COOH$, J = 6.91 Hz), 2.73−2.82 (m, 4H, $NCHCH_3COOH$, PCH_2N), 2.91−3.07 (m, 2H, PCH_2N), 3.14−3.21 (m, 4H, PCH_2N), 7.12−7.15 (m, 2H, PC_6H_4-*m*), 7.44−7.52 (m, 2H, PC_6H_4-*o*). ^{13}C NMR (D_2O, NaOD): δ 15.1 (s, $NCHCH_3COOH$), 45.8 (br s), 48.8 (br s), 67.4 (s), 130.3 (d, J = 18.1 Hz), 137.9 (d, J = 51.3 Hz), 142.3 (s), 182.0 (s). ^{31}P NMR (D_2O, NaOD): δ −8.3 (s).

2-(13-Carboxymethylcarbamoylmethylcarbamoylmethyl-10,13-diaza-1,8-diphosphatricyclo[6.3.3.0^{2,7}]tetradeca-2,4,6-trien-10-yl-methylcarboxamidomethylcarboxamido)acetic Acid (9). 1,2-Bis[bis-(hydroxymethyl)phosphino]benzene (0.4370 g, 1.667 mmol) in water (5 mL) was added dropwise to gly-gly-gly (1.578 g, 8.340 mmol) also in water (100 mL) at 25 °C. After stirring for 3 h, the reaction mixture was concentrated and chromatographed by semipreparatory HPLC to give the analytically pure compound as a viscous oil in 52% yield. HRFAB calcd for $C_{22}H_{30}N_6O_8P_2$ $[M + Na]^+$ 568.1600, found 591.1495. ^{1}H NMR (D_2O, NaOD): δ 2.82 (m, 4H, PCH_2N), 3.16 (m, 4H, PCH_2N), 2.90−3.62 (m, 12H, NCH_2CO), 7.16−7.17 (br m, 2H, PC_6H_4-*m*), 7.44−7.52 (m, 2H, PC_6H_4-*o*). ^{13}C (D_2O, NaOD): δ 43.2 (br s), 44.6 (s), 50.9 (d, J = 15.1 Hz), 63.9 (s), 130.7 (d, J = 15.1 Hz), 138.4 (d, J = 52.8 Hz), 142.0 (br s), 170.6 (s) 174.1 (s) 176.5 (s), ^{31}P NMR (D_2O, NaOD): δ −8.6 (s).

2-(7-Carboxymethyl-3,7-diaza-1,5-diphosphabicyclo[3.3.2]dec-3-yl)acetic Acid (10). 1,2-Bis[bis(hydroxymethyl)phosphino]ethane (0.2800 g, 1.308 mmol) in water (5 mL) was added dropwise to glycine (0.5019 g, 6.686 mmol) also in water (5 mL) at 25 °C. After stirring for 3 h, the reaction mixture was concentrated and purified on a Water's Sep-Pak Vac column (10 g, 35 mL). The solvent was removed in vacuo and dried overnight in vacuo to give the compound in 78% yield. Anal. Calcd for $C_{10}H_{18}N_2O_4P_2 \cdot H_2O$: C, 38.43; H, 12.27; N, 8.97. Found: C, 38.12; H, 12.18; N, 8.82. HRFAB calcd for 292.0820, $[M + H]^+$ found 293.0820. Mp 185−190 °C dec. ^{1}H NMR (D_2O): δ 2.26 (m, 4H, PCH_2CH_2P, $^2J_{PC}$ = 6.00 Hz), 3.33−3.39 (m, 4H, PCH_2N), 3.52 (s, 4H, NCH_2COOH), 3.78−3.87 (m, 4H, PCH_2N). ^{13}C NMR (D_2O): δ 24.2−24.4 (m), 54.5 (d, J = 30.2 Hz), 62.7 (s), 173.2 (s). ^{31}P NMR (D_2O): δ −36.3 (s).

X-ray Data Collection and Process. The crystal data and details of data collection for complexes **4**, **5**, **7**, and **10** are given in Table 1. Clear, colorless crystals of **4** and **7** were obtained from methanol/hydrochloric acid at −20 °C. Clear, colorless crystals of **5** were obtained from ethyl acetate at −20 °C. Clear, colorless crystals of **10** were obtained from water/methanol at 25 °C. Intensity data for compounds **4** and **5** were collected on an Enraf-Nonius CAD-4 diffractometer with Cu Kα radiation and a graphite monochromator at 25 °C. Intensity data for compounds **7** and **10** were collected on a Siemens SMART CDD system using the omega scan mode. Data were collected for absorption using the program SADABS, which is based on the method of Blessing.[23] Crystal decay were negligible, and corrections were deemed unnecessary. The structures were solved by the Patterson method using SHELXS-86[24] and refined by the full-matrix least-squares method on F^2 using SHELXL-93.[25]

Results and Discussion

Conjugation of Glycine with Tris(hydroxymethyl)phosphine (1). The addition of THP (**1**) to 5−6-fold excess glycine in water (Scheme 2) produces a white precipitate (compound **4**) which has been found to be soluble in acidic or alkaline

(23) Blessing R. H. *Acta Crystallogr*. **1995**, *A51*, 33−38.
(24) Sheldrick, G. M. *Acta Crystallogr*. **1990**, *A46*, 467−473.
(25) Sheldrick, G. M. *SHELXL-93*; University of Gottingen: Germany, 1993.

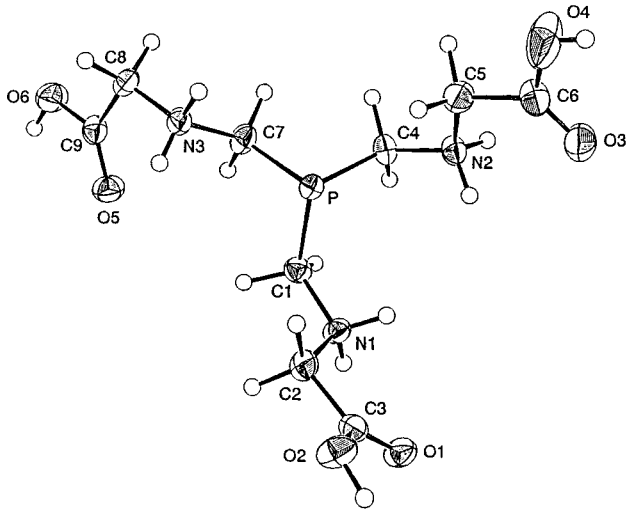


Figure 1. ORTEP drawing of **4** showing 50% probability ellipsoids.

Scheme 2. Synthesis of $P(CH_2NHCH_2COOH)_3$ (**4**)

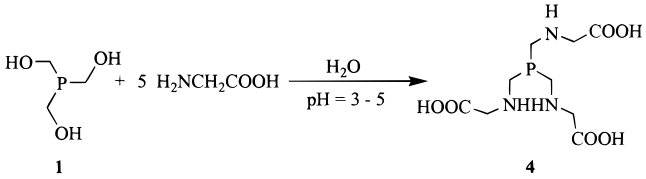


Table 2. Selected Bond Distances (Å) and Angles (deg) for **4**

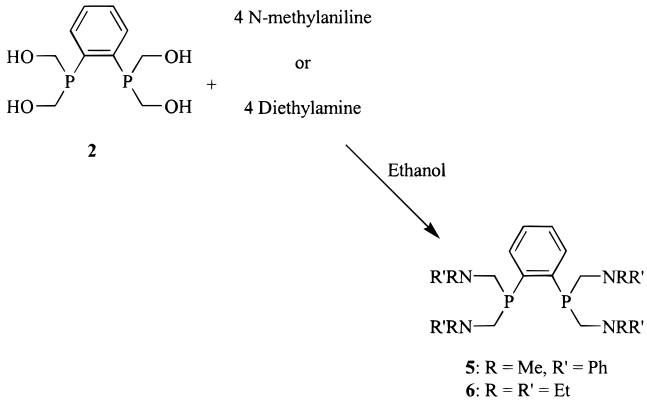
P−C1	1.863(2)	P−C4	1.870(2)
P−C7	1.866(2)		
C1−P−C4	102.07(11)	C1−P−C7	96.54(11)
C4−P−C7	95.52		

solutions, presumably as a result of the protonation of the amines or the deprotonation of the carboxylic acid, respectively. The high-resolution fast atom bombardment mass spectrum of compound **4** shows a molecular ion $[M + H]^+$ with a m/z = 296.1011. The ^{31}P NMR spectrum of compound **4** shows a single resonance upfield from THP (**1**) at −38.1 ppm ($\Delta\delta$ = −14.7). Both the ^1H and ^{13}C NMR spectra are consistent with the proposed structure.

Crystals of **4** suitable for X-ray diffraction analysis were obtained from a methanol/hydrochloric acid solution at −20 °C. The crystal data and details of data collection are listed in Table 1. The ORTEP diagram of compound **4** is shown in Figure 1. The selected bond distances and bond angles of complex **4** are listed in Table 2. Atomic coordinates and their equivalent isotropic displacement coefficients for **4** are included in the Supporting Information. The asymmetric unit for complex **4** consists of the cation $[P(CH_2NH_2COOH)_3]^{3+}$, three noncoordinating chloride counterions, and one water molecule. As revealed by the structure, all three of the hydroxymethyl groups of THP (**1**) reacted with glycine to give the fully substituted product. Growing the crystals in methanol/hydrochloric acid resulted in the protonation of the amines to give a cationic product. The P−C1, P−C4, and P−C7 bond distances are 1.863(2), 1.870(2), and 1.866(2) Å, respectively with an average of 1.866. The C1−P−C4, C1−P−C7, and C4−P−C7 bond angles are 102.1(1), 96.5(1), and 95.5(1)°, respectively.

Compound **4** represents a novel example of a tertiary phosphine functionalized with an amino acid moiety. The presence of three amino acids on **4** may aid in the development of large biomolecular vectors via the reaction with acid groups.

Scheme 3. Synthesis of $C_6H_4[P(CH_2NMePh)_2]_2$ (**5**) and $C_6H_4[P(CH_2NCH_2CH_3)_2]_2$ (**6**)



Additionally, the high oxidative stability and aqueous solubility of **4** will present new opportunities in the development of water-soluble transition metal/organometallic compounds with potential applications in biphasic catalysis.

Conjugation of Secondary Amines with 1,2-Bis[bis(hydroxymethyl)phosphino]benzene (2). The addition of HMPB (**2**) to the secondary amines, *N*-methylaniline and diethylamine, in ethanol produces compounds **5** and **6**, respectively (Scheme 3). The high-resolution fast atom bombardment mass spectra of compounds **5** and **6** show molecular ions $[M + H]^+$ with m/z = 619.3095 and 483.3745, respectively. The ^{31}P NMR spectrum of compound **5** shows an upfield resonance at −43.6 ppm ($\Delta\delta$ = −12.4), while the ^{31}P NMR spectrum of compound **6** shows an upfield resonance at −60.3 ppm ($\Delta\delta$ = −29.1). The ^1H NMR and ^{13}C NMR spectra for compounds **5** and **6** are consistent with the proposed structures.

Crystals of **5** suitable for X-ray diffraction analysis were obtained from an ethyl acetate solution at −20 °C. The crystal data and details of data collection are listed in Table 1. The ORTEP diagram of compound **5** is shown in Figure 2. The selected bond distances and bond angles of complex **5** are listed in Table 3. Atomic coordinates and their equivalent isotropic displacement coefficients for compound **5** are included in the Supporting Information. The asymmetric unit of the complex $[((C_6H_5)CH_3NCH_2)_2PC_6H_4P(CH_2NCH_3(C_6H_5))_2]$ (**5**) contains two crystallographically independent molecules. As revealed by the structure, all four of the hydroxymethyl groups of HMPB (**2**) reacted with *N*-methylaniline to give the fully substituted product. The bond distances for Pa−C1a, Pa−C4a, and Pa−C12a are 1.851(2), 1.877(3), and 1.874(2) Å, respectively, with an average bond distance of 1.867 Å. The bond distances for Pb−C1b, Pb−C4b, and Pb−C12b are 1.840(2), 1.866(3), and 1.879(3) Å, respectively, with an average of 1.862 Å. The bond angles C1a−Pa−C4a, C1a−Pa−C12a, and C4a−Pa−C12a are 100.5(1), 97.5(1), and 99.5(1)°, respectively. The bond angles C1b−Pb−C4b, C1b−Pb−C12b, and C4b−Pb−C12b are 101.7-(1), 97.1(1), and 99.9(1)°, respectively.

Conjugation of Glycine, Alanine, and Glyglyglycine with 1,2-Bis[bis(hydroxymethyl)phosphino]benzene (2) or 1,2-Bis-[bis(hydroxymethyl)phosphino]ethane (3). The addition of HMPB (**2**) to the amino acids glycine and alanine in water produces compounds **7** and **8**, respectively (Scheme 4). The high-resolution fast atom bombardment mass spectra of compounds **7** and **8** show molecular ions $[M + H]^+$ with a m/z = 341.0820 and 369.1133, respectively. The ^{31}P NMR spectrum of compound **7** shows a downfield resonance at −9.9 ppm ($\Delta\delta$ = 21.3), while the ^{31}P NMR spectrum of compound **8** shows a

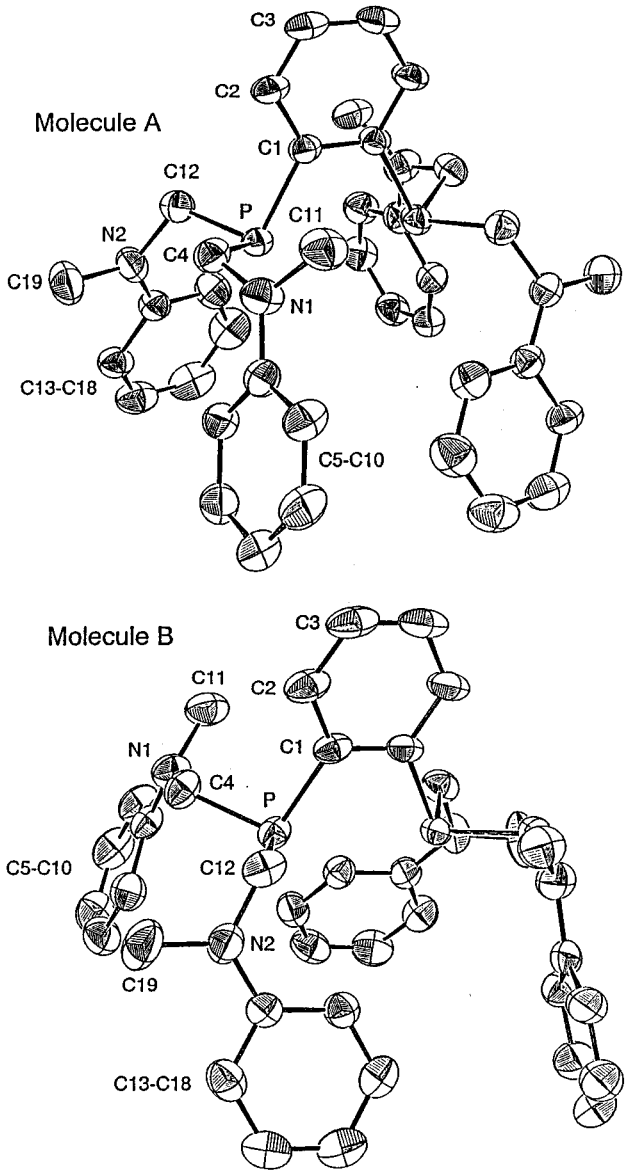


Figure 2. ORTEP drawings of the two crystallographically independent molecules **5A** and **5B** showing 50% probability ellipsoids.

Table 3. Selected Bond Distances (Å) and Angles (deg) for **5**

Pa−C1a	1.851(2)	Pb−C1b	1.840(2)
Pa−C4a	1.877(3)	Pb−C4b	1.866(3)
Pa−C12a	1.874(2)	Pb−C12b	1.879(3)
C1a−Pa−C4a	100.52(11)	C1a−Pa−C12a	97.49(10)
C4a−Pa−C12a	99.49(11)	C1b−Pb−C4b	101.67(11)
C1b−Pb−C12b	97.11(11)	C4b−Pb−C12b	99.94(12)

downfield resonance at −8.6 ppm ($\Delta\delta = 22.6$). The ^{1}H NMR and ^{13}C NMR spectra of **7** and **8** are consistent with the proposed structures.

Crystals of **7** suitable for X-ray diffraction analysis were obtained from a methanol/hydrochloric acid solution at −20 °C. The crystal data and details of data collection are listed in Table 1. The ORTEP diagram of compound **7** is shown in Figure 3. The selected bond distances and bond angles of complex **7** are listed in Table 4. Atomic coordinates and their equivalent isotropic displacement coefficients for compound **7** are included in the Supporting Information. The asymmetric unit for complex **7** consists of the cation $[C_{14}H_{19}N_2O_4P_2]^+$ and a noncoordinating chloride counterion. As revealed by the structure, all four of the hydroxymethyl groups of HMPB (**2**) reacted with 2 equiv

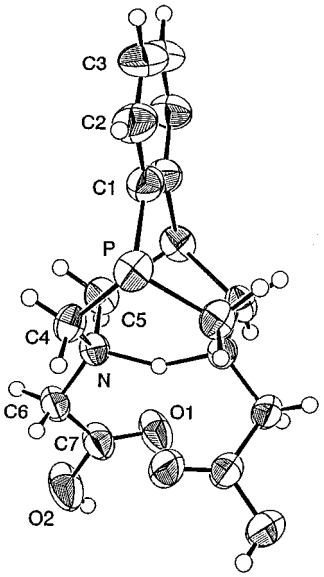


Figure 3. ORTEP drawing of compound **7** showing 50% probability ellipsoids.

Scheme 4. Synthesis of HMPB(Glycine)$_2$ (**7**), HMPB(Alanine)$_2$ (**8**), and HMPB(gly-gly-gly)$_2$ (**9**)

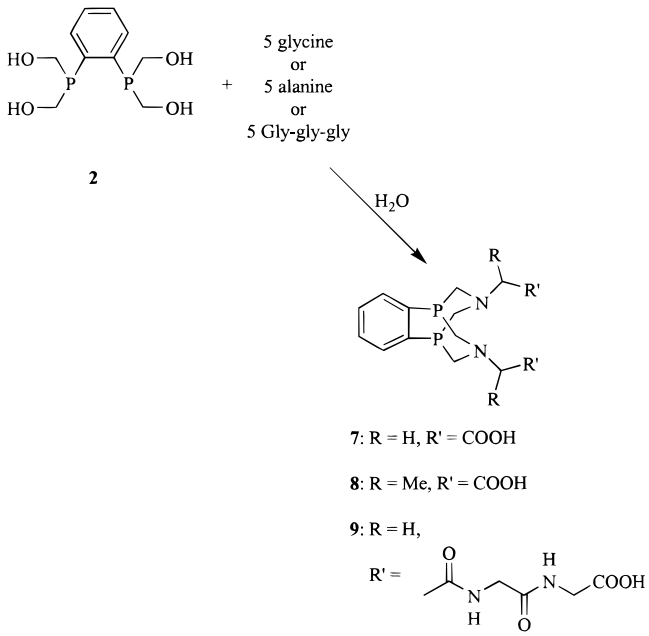


Table 4. Selected Bond Distances (Å) and Angles (deg) for **7**

P1−C1	1.822(2)	P1−C4	1.857(2)
P1−C5	1.852(2)		
C1−P1−C4	106.99(9)	C4−N−C5^a	113.6(2)
C4−P1−C5	102.32(9)	C4−N−C6	113.65(14)
C1−P1−C5	103.63(10)	C5^a−N−C6	110.7(2)
P1−C5−N^a	117.60	P1−C4−N	118.24(13)

^{a} Atom at $1 - x, y, {}^1/_2 - z$.

of glycine to give a tricyclic structure composed of a phenyl ring attached to two, heterocyclic seven-membered rings as shown above. An intriguing feature of the structure is that even though the crystals were obtained from relatively concentrated hydrochloric acid, the nitrogens are only hemiprotonated. The hydrogen atom has been refined to be equidistant between the two nitrogens. The bond distances P1−C1, P1−C4, and P1−C5 are 1.822(2), 1.857(2), and 1.852(2) Å, respectively with an average bond distance of 1.844 Å. The bond angles C1−

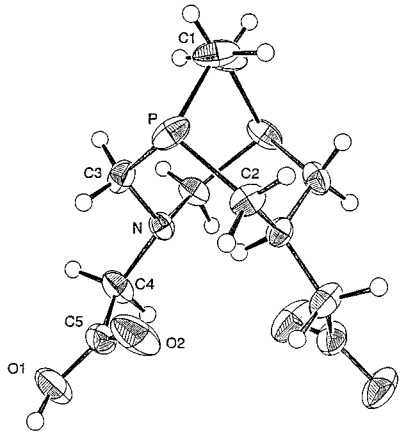
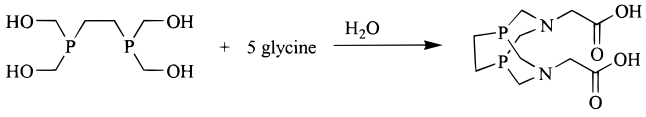


Figure 4. ORTEP drawing of compound **10** showing 50% probability ellipsoids.

Scheme 5. Synthesis of HMPE(Gly)$_2$ (**10**)



3 **10**

Table 5. Selected Bond Distances (Å) and Angles (deg) for **10**

P1−C1	1.846(2)	P1−C2	1.8531(14)
P1−C3	1.858(2)		
C1−P1−C2	103.86(8)	C2−N1−C3^a	113.75(11)
C2−P1−C3	102.56(7)	C2−N1−C4	113.31(11)
C1−P1−C3	104.54(8)	C3^a−N1−C4	111.47(12)
P1−C2−N1	116.36(9)	P1−C3−N1^a	118.62(10)

^{a} Atom at $1 - x, y, 1.5 - z$.

P1−C4, C1−P1−C5, and C4−P1−C5 are 107.0(1), 103.6(1), and 102.3(1)°, respectively. The bond angles C4−N−C5a, C4−N−C6, and C5a−N−C6 are 113.6(2), 113.6(1), and 110.7(2)°, respectively. The bond angles P1−C4−N and P1−C5−Na are 118.2(1) and 117.6(1)°, respectively.

The addition of HMPB (**2**) to excess glycylglyclyglycine in water followed by removal of the solvent produces a viscous oil as outlined in Scheme 4. The product (compound **9**) was purified by semipreparatory HPLC. The high-resolution fast atom bombardment mass spectrum shows a molecular ion $[M + Na]^+$ with a $m/z = 591.1495$. The ^{31}P NMR spectrum of **9** shows a single resonance downfield from HMPB at −8.6 ppm ($\Delta\delta = 22.6$). In addition, the ^{1}H NMR and ^{13}C NMR spectra for **9** are consistent with the proposed structure.

The addition of HMPE (**3**) to excess glycine produces compound **10** in near quantitative yields as outlined in Scheme 5. The high-resolution fast atom bombardment mass spectrum of **10** shows a molecular ion $[M + H]^+$ with a $m/z = 293.0820$. The ^{31}P NMR spectrum of compound 10 shows an upfield resonance at −36.3 ppm ($\Delta\delta = -11.2$). Furthermore, the ^{1}H and ^{13}C NMR spectra of **10** are consistent with the proposed structure.

Crystals of **10** suitable for X-ray diffraction analysis were obtained from a water/methanol mixture at 25 °C. The details of data collection are listed in Table 1. The ORTEP diagram of compound **10** is shown in Figure 4. The selected bond distances and bond angles of complex **10** are listed in Table 5. Atomic coordinates and their isotropic displacement coefficients for **10** are included in the Supporting Information. The asymmetric unit for complex **10** consists only of the complex $[C_{10}H_{18}N_2O_4P_2]$.

As revealed by the structure, all four of the hydroxymethyl groups of HMPE (**3**) react with 2 equiv of glycine to give the bicyclic compound. The bond distances P1−C1, P1−C2, and P1−C3 are 1.846(2), 1.853(1), and 1.858(2) Å, respectively, with an average bond distance of 1.852 Å. The bond angles C1−P1−C2, C1−P1−C3, and C2−P1−C3 are 103.9(1), 104.5-(1), and 102.6(1)°, respectively. The bond angles C2−N1−C3a and C2−N1−C4 are 113.8(1), and 113.3(1)°, respectively. The bond angle C3a−N1−C4 is 111.5(1)°. The bond angles P1−C2−N1 and P1−C3−N1a are 116.4(1) and 118.6(1)°, respectively.

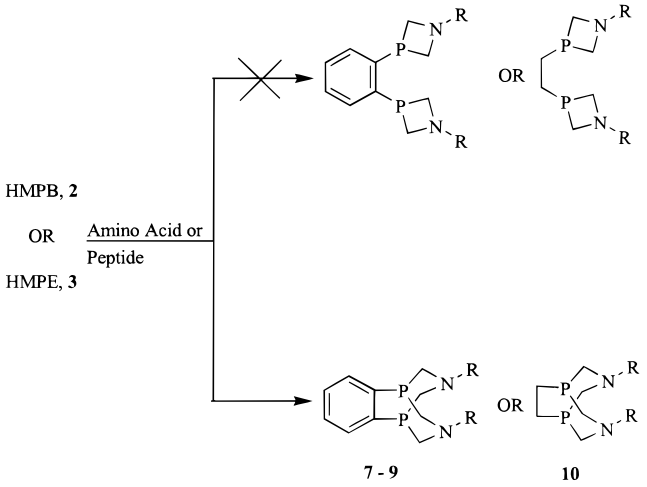
Tris(hydroxymethyl)phosphine (**1**) has been shown to react with ammonia, primary and secondary amines by the Mannich reaction, as shown in Scheme 1.[8,9,26,27] The reaction of THP with ammonia and primary amines generally leads to polymerization which was of interest in the 1960s as a method of producing flame-retardant textiles.[26] In addition, the reaction of THP with secondary amines to give monomeric products has also been explored.[8] Recently, there has been a renewed interest in the use of THP for the immobilization of enzymes.[9] However, the reaction of THP (**1**) with amino acids, peptides, and antibodies has not been explored. Thus, the ability of the hydroxymethyl groups to react with amines and leave the phosphines free for further coordination to transition metals may provide a novel method of producing site-directed radiopharmaceuticals.[13−17,19] Earlier work in our laboratory of conjugation of THP to antibodies and subsequently labeling with Tc-99m suggested that the idea was feasible.[28] Due to the difficulty of identifying the THP−antibody products, the reaction of THP with simple peptides and amino acids, as described above, was explored. The addition of THP to excess glycine leads primarily to the formation of a monomer in which all three of the hydroxymethyl groups react with a glycine molecule. Compound **4** is highly stable to oxidation in acidic and basic solution even though the phosphine is nucleophilic (as suggested by the ^{31}P NMR chemical shift). This represents the first example of an X-ray crystallographically characterized THP−amine conjugate.

When 5 or 6 equiv of peptides or amino acids were reacted with HMPB or HMPE, only 2 equiv of the amines were found to conjugate with the hydroxymethylphosphines. The degree of substitution for compounds **7**−**10** has been determined by elemental analyses, high-resolution mass spectroscopy, and integration of the protons in the ^{1}H NMR spectra. For each compound there are two possible isomers that fit the given data as shown in Scheme 6. Although unlikely due to ring strain, one possible isomer is where two hydroxymethyl groups from each phosphine reacts with an amino acid or peptide to form two four-membered rings (Scheme 6). The other possibility is where two hydroxymethyl groups from adjacent phosphines reacts with an amino acid to form two heterocyclic seven-membered rings. The final molecular structure of the compounds has been confirmed by X-ray diffraction analysis and proves to be the latter of the two possible isomers described above. Similar to compound **4**, compounds **7**−**10** are highly stable to oxidation in both acidic and alkaline media.

The reactions of HMPB (and HMPE) with primary amines, as depicted in Schemes 4−6, provide important information on the kinetic propensity of the reactivity of $-P(CH_2OH)_2$ groups with primary amines. It must be recognized that HMPB (**2**) (and HMPE (**3**)) possesses four $-CH_2OH$ groups (two on each phosphine center) and they could conceivably produce cross-

(26) Daigle, D. J.; Frank, A. W. *Tex. Res. J.* **1982**, *52*, 751−755.
(27) Frank, A. W.; Drake, G. L., Jr. *J. Org. Chem.* **1972**, *37*, 2752−2755.
(28) Berning, D. E.; Katti, K. V.; Volkert, W. A., unpublished results.

Scheme 6. Possible Isomers of the Reaction of HMPB (**2**) or HMPE (**3**) with Amino Acids or Peptides



linked polymers upon interactions with the N terminus of amino acids and peptides. However, the observation that these reactions strictly result in the formation of well-defined heterocyclic rings suggests a strong kinetic propensity to monomeric product formation. These are important results in the context of using the water-soluble HMPB (**2**) (and HMPE (**3**)) phosphines as synthons to modify the structures and properties of peptides (and proteins) wherein specific primary amines would interact to produce PCH_2N linkages without cross-linking the biomolecules.

Conclusions and Comments

The generality of reactions, outlined in Schemes 1−6, demonstrate that virtually any molecule with primary or secondary amines can be conjugated to other materials that contain one or more $P(CH_2OH)_n$ moieties. It must be recognized that although gluteraldehyde and other aldehydes are being routinely used for conjugation of bioactive amines, the products from such reactions lead to unstable imine (CH═NR) linkages and often require further reduction, under harsh conditions, to produce the more stable amines. In contrast, the reactions of hydroxymethylphosphines with amines result in stable amine (H_2C−NHR) linkages in a single reaction step in biologically benign media, (i.e., water). Reactions of amino functionalities, present on the backbone of biomolecules, with hydroxymethylphosphines functionalities may aid in altering the biochemical properties for potential use in protein/peptide structure modifications. The conjugation of amines, amino acids, and peptides with hydroxymethylphosphines, as described in Schemes 1−6, and the kinetic propensity of these reactions to produce well-defined singular chemical/biochemical species are, therefore, unique.

Acknowledgment. This work was supported by funds provided by the Department of Energy (Grant No. DEFGO289-ER60875), DuPont-Merck Pharmaceuticals, and the Departments of Chemistry, Radiology, and Research Reactor at the University of Missouri-Columbia. Partial funding of the X-ray diffractometer by the National Science Foundation, Grant No. CHE[90-11804], is gratefully acknowledged.

Supporting Information Available: Tables of crystal data, structure solution and refinement, atomic coordinates, bond lengths and angles, and anisotropic thermal parameters for **4**, **5**, **7**, and **10** (PDF). This material is available free of charge via the Internet at http://pubs.acs.org.

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