Synthesis and Phosphorylating Properties of Hydroxyamino Acid Phosphoramidites

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The preparation of *N*-(benzyloxycarbonyl)-*O*-[(benzyloxy)(diisopropylamino)phosphino]serine [-threonine, -tyrosine and -hydroxyproline] benzyl esters using the versatile phosphitylating reagent benzyl *N,N,N',N'*-tetraisopropylphosphorodiamidite [benzyloxybis(diisopropylamino)phosphine] is described. The application of the benzyl (hydroxyamino acid) phosphoramidites is illustrated in the synthesis of several phosphate diesters.

Phosphate diesters are essential structural elements of nucleic acids i.e. DNA and RNA. Apart from this, it is well established that formation of phosphate diesters in proteins is a reversible post-translational modification. The latter process may result in nucleoproteins[1] in which the hydroxy groups of the L-amino acids serine, threonine or tyrosine are covalently attached via a phosphate diester bond to the 5'-end of the nucleic acids. Recently, the presence of a phosphate diester between a serine residue and a threonine residue in the protein *Azotobacter* flavodoxin[2] has been proposed. It has further been suggested that this unusual linkage retains or stabilizes the three-dimensional structure of the protein[3] in a similar fashion as the well-known disulfide bridge.[4] Another well-known post-translational modification is the reversible formation of phosphate monoesters of hydroxyamino acids in proteins.[5]

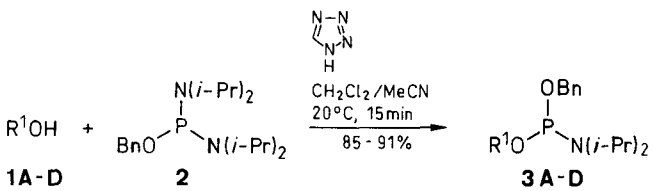
In order to get a deeper insight into the biological and chemical properties of biomolecules containing phosphate mono- and diesters, the availability of well-defined model compounds is of great significance.[6]

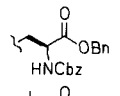
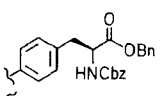
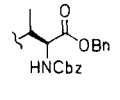
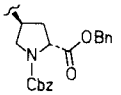
At present several phosphorylation methods for the synthesis of phosphopeptides[7] and nucleopeptides[8] in solution as well as on solid support have been developed. In a preliminary paper[9] we showed that seryl threonyl phosphate (**6Ab**) could be prepared using the bifunctional phosphitylating reagent benzyloxybis(diisopropylamino)phosphine.[10]

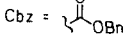
We here report in full that the latter phosphoramidite approach is not only suitable for the introduction of a phosphate diester union between a hydroxyamino acid and a nucleoside, i.e. **6Ae**, or ethanolamine, i.e. **6Af**, individual hydroxyamino acids (i.e. **6Ab, 6Ac, 6Da** and **6Bg**), but also for the preparation of phosphate monoesters of hydroxyamino acids (i.e. **6Ad, 6Cd** and **6Dd**).

The synthetic route to the benzyl *N,N*-diisopropylphosphoramidites of the hydroxyamino acids serine, threonine, tyrosine and hydroxyproline is depicted in Scheme 1. Thus, the *N*-(benzyloxycarbonyl)hydroxyamino acid benzyl esters **1A–D**[11] were phosphitylated with benzyloxybis(diisopropylamino)phosphine **(2)**[10] in the presence of 1*H*-tetrazole to give the phosphoramidites **3A–D**. Work up and purification by silica gel column chromatography afforded the homogeneous phosphoramidites **3A–D**, as evidenced by TLC analysis and ^{31}P NMR spectroscopy, in high yield (Table 1). The resulting phosphoramidites **3A–D** can be stored for several weeks at $-20\,^{\circ}C$ without any detectable decomposition.

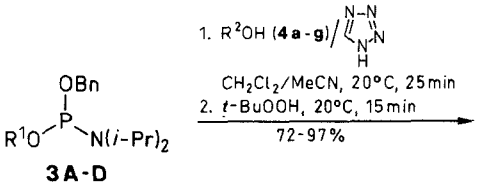
The successful synthesis of the phosphoramidites **3A–D** urged us to explore their phosphitylating properties. To this end, *O*-[(benzyloxy)(diisopropylamino)phosphino]-serine **3A** was employed for the synthesis of fully protected seryl threonyl phosphate **5Ab**. Thus, 1*H*-tetrazole mediated coupling of **3A** (Scheme 2) with *N*-(benzyloxycarbonyl)threonine benzyl ester (**4b** or **1B**) resulted in the rapid formation of an intermediate phosphite triester.

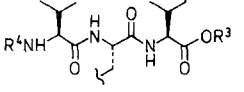


1, 3	R^1	1, 3	R^1
A		C	
B		D	

Cbz = 

Scheme 1



4-6	R^2	4-6	R^2
a	1A	e	
b	1B	f	
c	1C	g	
d	Bn		

for **4**, **5**: R^3 = Bn, R^4 = Cbz, R^5 = Ac, for **6**: R^3 = R^4 = R^5 = H

Scheme 2

Table 1. Preparation of Phosphoramidites **3** and Benzyl (Hydroxyamine Acid) Phosphate Triesters **5**

Reactants	Product	Yield (%)	R_f[a]	^{31}P NMR[b] δ
1A + 2	**3A**	88[c]	0.48 (A)	149.23/149.96
1B + 2	**3B**	91[c]	0.53 (A)	148.41/147.84
1C + 2	**3C**	86[c]	0.35 (A)	146.54
1D + 2	**3D**	85[c]	0.40 (A)	148.05/148.16/148.26
3A + 4b	**5Ab**	90[d]	0.34 (B)	−1.56/−1.58
3A + 4c	**5Ac**	75[d]	0.16 (C)	−6.07
3A + 4d	**5Ad**	75[d]	0.37 (B)	−0.54
3A + 4e	**5Ae**	95[d]	0.38 (D)	−0.62
3A + 4f	**5Af**	89[d]	0.38 (B)	−0.47
3B + 4g	**5Bg**	87[d]	0.29/0.43 (C)	−1.45/−2.15
3C + 4a	**5Ca**	85[d]	0.16 (C)	−6.06
3C + 4d	**5Cd**	72[d]	0.34 (C)	−6.03
3D + 4a	**5Da**	97[d]	0.24 (B)	−1.49
3D + 4d	**5Dd**	94[d]	0.34 (B)	−1.27

[a] TLC solvent systems; A: Et_3N/hexane/EtOAc (1:4:35); B: hexane/EtOAc (2:3); C: hexane/EtOAc (3:7); D: acetone/CH_2Cl_2 (1:4).
[b] Spectra were recorded in CH_2Cl_2 with 85% H_3PO_4 as external standard.
[c] Based on **1**.
[d] Based on **3**.

Subsequent in situ oxidation of the latter with *tert*-butyl hydroperoxide[12] afforded, as monitored by ^{31}P NMR spectroscopy, the phosphate triester **5Ab** (as a pair of diastereoisomers) without concomitant formation of other phosphate containing products. Work up and purification by silica gel column chromatography, gave the homogeneous phosphate triester **5Ab** in excellent yield (Table 1).

The scope of the reaction was further extended by the synthesis of fully protected phosphoserine **5Ad**, seryl thymidinylate **5Ae** and aminoethyl seryl phosphate **5Af**. In a similar fashion, as mentioned above for compound **5Ab**, coupling of **3A** with benzyl alcohol **(4d)**, 3-*O*-acetyldeoxythymidine **(4e)** and 2-(benzyloxycarbonylamino)ethanol **(4f)**, gave fully protected **5Ad, 5Ae** and **5Af**, respectively. In addition, more hindered alcohols could also be effectively coupled via the same two-step phosphoramidite method. For instance, phosphinothreonine **3B** reacted smoothly with N_α-(benzyloxycarbonyl)valylserylisoleucyl $C_{\alpha-1}$-benzyl ester **4g** to give, after oxidation, fully protected valylserylisoleucyl threonyl phosphate **5Bg** in an excellent yield.

The versatility of the amidite approach was further illustrated by the preparation of nonaliphatic phosphate esters e. g. seryl tyrosyl phosphate **5Ca**, phosphotyrosine **5Cd**, hydroxyprolyl seryl phosphate **5Da** and phosphohydroxyproline **5Dd** starting with the respective tyrosine and hydroxyproline phosphoramidites **3C** and **3D**.

Hydrogenolysis of the benzyloxycarbonyl and benzyl protecting groups from **5** was easily effected over palladium on activated charcoal. The resulting completely deblocked phosphate esters **6** ($R^3 = R^4 = H$) were converted into the corresponding sodium salts by ion-exchange column chromatography (SP Sephadex C25), to give the homogeneous compounds **6** in high yield. Finally, nucleopeptide **5Ae** was fully deprotected by mild basic hydrolysis (triethylamine/water)[14] and purified by anion-exchange column chromatography to afford homogeneous **6Ae** ($R^3 = R^4 = R^5 = H$). Yields and NMR data pertaining to compounds **6** are summarized in Table 2.

In conclusion, the results presented in this paper indicate that the phosphitylating reagent **2** proves to be a valuable tool in the preparation of the benzyl (hydroxyamino acid) phosphoramidites **3A–D** which, in turn, can be used in the synthesis of biologically important model compounds containing either a phosphomono- or diester of a hydroxyamino acid. For instance, aminoethyl seryl phosphate ester **6Af** has been found in earthworm tissue.[13]

MeCN, CH_2Cl_2 and dioxane were dried by refluxing with CaH_2 (5 g/L), distilled and stored over molecular sieves (4 Å). Dioxane was redistilled prior to use from $LiAlH_4$ (5 g/L). Et_2O was distilled from P_2O_5 and stored over Na wire. BnOH was distilled prior to use. Et_3N was distilled from KOH. Et_3NHCO_3 buffer (TEAB, 2 M): a mixture of freshly distilled Et_3N (825 mL) and H_2O (2175 mL) was saturated with CO_2 gas at 0 °C until pH 7.0. 1*H*-Tetrazole was purchased from Janssen (Belgium). Compounds **1A–D** were purchased from Novabiochem (Switzerland). Pd–C (10%) was purchased from Fluka (Switzerland). 3′*O*-Acetyl-2′-deoxythymidine,[15] 2-(benzyloxycarbonylamino)ethanol[16] and N_α-(benzyloxycarbonyl)valylserylisoleucyl $C_{\alpha-1}$-benzyl ester[17] were prepared according published procedures. TLC analysis was performed on Schleicher & Schüll (Germany) DC Fertigfolien F 1500 LS 254. Short column chromatography was performed on Merck Kieselgel 60 (230–400 mesh, ASTM). SP Sephadex C25 and DEAE Sephadex A25 were purchased from Pharmacia (Sweden). ^1H NMR spectra were recorded on a Bruker WM-300 spectrometer, equipped with an ASPECT-2000 computer operating in the Fourier transform mode at 300 MHz. ^{13}C and ^{31}P NMR spectra were recorded on a Jeol JNM-FX 200 spectrometer on line with a JEC 980B computer at 50.1 and 80.7 MHz, respectively. FPLC analysis was carried out on a Pharmacia LCC-500 liquid chromatograph using a Mono Q HR (5/5) column. Gradient elution was performed at 20 °C, by building up a gradient, starting with buffer A (0.05 M NaH_2PO_4, pH 6.0) and applying buffer B (0.05 M NaH_2PO_4, 1.2 M, NaCl, pH 6.0) with a flow rate of 2.0 mL/min.

Benzyloxybis(diisopropylamino)phosphine (2):
A solution of BnOH (1.1 mL, 10 mmol) and Et_3N (1.4 mL, 10 mmol) in Et_2O (25 mL) was added dropwise to a cooled (0 °C) solution of chlorobis(*N*,*N*-diisopropyl)phosphoramidite (2.7 g, 10 mmol) in Et_2O (25 mL). The solution was stirred for 1 h at 20 °C and the salts are removed by filtration. The filtrate was concentrated in vacuo and stored at −20 °C.
^{31}P NMR (CH_2Cl_2): δ = 123.3.

***N*-(Benzyloxycarbonyl)-*O*-[(benzyloxy)(diisopropylamino)phosphino]hydroxyamino Acid Benzyl Esters (Phosphoramidites) 3; General Procedure:**
A solution of **2** (3.6 mL, 1.0 M) in CH_2Cl_2 was added to amino acid **1** (3.0 mmol) in CH_2Cl_2 (5 mL), which had been dried by repeated coevaporation with dioxane (2 × 10 mL). To the stirred solution was added a solution of 1*H*-tetrazole (6.0 mL, 0.5 M) in MeCN. The mixture was stirred for 15 min at 20 °C. After the addition of 2 M TEAB (5 mL), the mixture was diluted with CH_2Cl_2 (100 mL) and washed with H_2O (5 mL), 1 M TEAB (20 mL) and H_2O (20 mL). The organic phase was dried ($MgSO_4$) and concentrated in vacuo. Silica gel column chromatography (40 g, elution: hexane/EtOAc/Et_3N, 37:2:1 to 35:4:1, v/v) of the crude product afforded the pure phosphoramidite **3**, as an oil.

Table 2. Relevant Data of Hydroxyamino Acid Phosphate Ester and Diesters **6** ($R^3 = R^4 = R^5 = H$)

Product	Yield[a] (%)	Molecular Formula	^{31}P NMR[b,c] δ	^{1}H NMR[c] δ	^{13}C NMR (50.3 MHz, D_2O) δ, J (Hz)
6Ab	90	$C_7H_{16}N_2NaO_8P$ (310.2)	−1.28	1.42 [d, Hγ (Thr), $J_{H\gamma,H\beta} = 6.6$]; 3.70 [dd, Hα (Thr), $J_{H\alpha,H\beta} = 3.3$, $J_{H\alpha,P} = 1.9$]; 3.95 [m, Hα (Ser), $J_{H\alpha,H\beta a} = 6.3$, $J_{H\alpha,H\beta b} = 3.6$, $J_{H\alpha,P} = 1.2$]; 4.14 [m, Hβa (Ser), $J_{H\beta a,H\beta b} = 11.3$, $J_{H\beta a,P} = 5.0$]; 4.22 [m, Hβb (Ser), $J_{H\beta b,P} = 5.1$]; 4.74 [m, Hβ (Thr), $J_{H\beta,P} = 7.4$]	19.4 [Cγ (Thr)]; 55.7 [d, Cα (Ser), $J_{C\alpha,P} = 8.8$]; 60.1 [d, Cα (Thr), $J_{C\alpha,P} = 8.8$]; 64.8 [d, Cβ (Ser), $J_{C\beta,P} = 4.4$]; 72.5 [d, Cβ (Thr), $J_{C\beta,P} = 4.4$]; 172.1, 172.8 [C=O (Ser and Thr)]
6Ac/Ca	88	$C_{12}H_{18}N_2NaO_8P$ (372.25)	−4.08	3.04 [dd, Hβa (Tyr), $J_{H\beta a,H\alpha} = 8.4$, $J_{H\beta a,H\beta b} = 14.6$]; 3.25 [dd, Hβb (Tyr), $J_{H\beta b,H\alpha} = 4.9$]; 3.93 [dd, Hα (Tyr)]; 3.97 [m, Hα (Ser), $J_{H\alpha,H\beta a} = 4.6$, $J_{H\alpha,H\beta b} = 3.9$, $J_{H\alpha,P} = 1.9$]; 4.30 [m, Hβa (Ser), $J_{H\beta a,H\beta b} = 11.4$, $J_{H\beta a,P} = 4.9$]; 4.35 [m, Hβb (Ser), $J_{H\beta b,P} = 5.7$]; 7.14 [dd, 2 × Hε (Tyr), $J_{H\varepsilon,H\delta} = 8.7$, $J_{H\varepsilon,P} = 1.3$]; 7.26 [d, 2 × Hδ (Tyr)]	36.5 [Cβ (Tyr)]; 55.7 [d, Cα (Ser), $J_{C\alpha,P} = 8.8$]; 56.9 [Cα (Tyr)]; 65.4 [d, Cβ (Ser), $J_{C\beta,P} = 4.4$]; 121.6 [d, Cε (Tyr), $J_{C\varepsilon,P} = 4.4$]; 131.5 [Cδ (Tyr)]; 132.0 [Cq (Tyr); 174.8, 172.0 [C=O (Ser and Tyr)]
6Ad	80	$C_3H_6NNaO_6P$ (206.0)	2.83	3.94 (dd, Hα, $J_{H\alpha,H\beta a} = 6.2$, $J_{H\alpha,H\beta b} = 3.5$); 4.10 (m, Hβa, $J_{H\beta a,H\beta b} = 11.6$, $J_{H\beta a,P} = 6.1$); 4.19 (m, Hβb, $J_{H\beta b,P} = 3.5$)	55.9 (d, Cα, $J_{C\alpha,P} = 9.2$); 64.4 (d, Cβ, $J_{C\beta,P} = 4.4$); 172.4 (C=O)
6Ae	60	$C_{13}H_{20}N_3NaO_{10}P$ (432.3)	0.14	1.89 [t, CH_3 (dT)], $J_{CH_3,H6} = 1.2$); 2.35 [m, H2′, H2″ (dT), $J_{H2',H1'} = 6.9$, $J_{H2',H3'} = 4.6$]; 3.95 [ddd, Hα (Ser), $J_{H\alpha,H\beta a} = 3.7$, $J_{H\alpha,H\beta b} = 5.0$, $J_{H\alpha,P} = 1.5$]; 4.04 [m, H5′ (dT), $J_{H5',H5''} = 11.8$, $J_{H5',P} = 4.2$]; 4.10 [m, H5″ (dT), $J_{H5'',P} = 3.1$]; 4.21 [m, Hβa (Ser), $J_{H\beta a,H\beta b} = 11.3$, $J_{H\beta a,P} = 5.0$]; 4.26 [m, Hβb (Ser), $J_{H\beta b,P} = 4.5$]; 4.56 [m, H3′ (dT), $J_{H3',H4'} = 1.8$]; 6.33 [t, H1′ (dT)]; 7.68 [d, H6 (dT)]	12.2 [CH_3 (dT)]; 39.2 [C2′ (dT)]; 55.5 [d, Cα (Ser), $J_{C\alpha,P} = 4.4$]; 64.8 [d, C5′ (dT), $J_{C5',P} = 9.8$]; 65.8 [d, Cβ (Ser), $J_{C\beta,P} = 5.9$]; 71.5 [C1′ (dT)]; 85.6 [d, C4′ (dT), $J_{C4',P} = 4.4$]; 85.9 [C3′ (dT)]; 112.2 [C5 (dT)]; 137.9 [C6 (dT)]; 152.3 [C=O (dT)]; 167.1 [C=O (dT)]; 171.8 [C=O (Ser)]
6Af	93	$C_5H_{14}N_2NaO_6P$ (252.1)	0.03	3.27 [m, H2 (EA), $J_{H2,H1} = 5.1$, $J_{H2,P} = 1.1$]; 3.99 [m, Hα (Ser), $J_{H\alpha,H\beta a} = 3.1$, $J_{H\alpha,H\beta b} = 4.4$, $J_{H\alpha,P} = 1.9$]; 4.08 [m, H1 (EA), $J_{H1,P} = 4.4$]; 4.23 [m, Hβa (Ser), $J_{H\beta a,H\beta b} = 11.4$, $J_{H\beta a,P} = 3.1$]; 4.30 (m, Hβb (Ser), $J_{H\beta,P} = 4.4$]	40.4 [C2 (EA), $J_{C2,P} = 7.3$]; 55.2 [Cα (Ser), $J_{C\alpha,P} = 8.8$]; 62.5 [C1 (EA), $J_{C1,P} = 4.4$]; 64.8 [Cβ (Ser)]; 172.0 (C=O)
6Bg	70	$C_{18}H_{36}N_4NaO_{10}P$ (522.5)	−1.00	0.88 [t, Hδ (Ile), $J_{H\delta,H\gamma(CH_2)} = 7.4$]; 0.90 [d, Hγ ($CH_3$) (Ile), $J_{H\gamma,H\beta} = 6.9$]; 1.06 [d, Hγ (Val), $J_{H\gamma,H\beta} = 6.9$]; 1.45 [d, Hγ (Thr), $J_{H\gamma,H\beta} = 6.6$]; 1.14 [m, Hγ (CH_2a)]; 1.41 [m, Hγ (CH_2b)]; 1.85 [m, Hβ (Ile), $J_{H\beta,H\alpha} = 6.1$]; 2.28 [m, Hβ (Val), $J_{H\beta,H\alpha} = 6.3$]; 3.72 [dd, Hα (Thr), $J_{H\alpha,H\beta} = 4.2$, $J_{H\alpha,P} = 1.6$]; 3.94 [d, Hα (Val)]; 4.09 [dd, Hβa (Ser), $J_{H\beta a,H\beta b} = 10.9$, $J_{H\beta a,H\alpha} = 7.2$, $J_{H\beta a,P} = 6.9$]; 4.14 [d, Hα (Ile)]; 4.17 [m, Hβb (Ser), $J_{H\beta b,H\alpha} = 4.6$, $J_{H\beta b,P} = 4.6$]; 4.72 [m, Hβ (Thr), $J_{H\beta,P} = 6.8$]; 4.77 [dd, Hα (Ser)]	11.4 [Cγ (Ile)]; 15.9 [Cδ (Ile)]; 17.6 and 18.4 [Cγ (Val)]; 19.2 [Cγ (Thr)]; 25.2 [Cγ (Ile)]; 30.7 [Cβ (Val)]; 37.8 [Cβ (Ile)]; 54.8 [d, Cα (Ser), $J_{C\alpha,P} = 8.8$]; 59.3 [Cα (Ile)]; 60.1 [d, Cα (Thr), $J_{C\alpha,P} = 7.3$]; 60.8 [Cα (Val)]; 64.9 [d, Cβ (Ser), $J_{C\beta,P} = 5.8$]; 72.3 [d, Cβ (Thr), $J_{C\beta,P} = 5.8$]; 182.1–170.1 (4 × C=O, Ile, Ser, Thr and Val)
6Cd	71	$C_9H_{11}NNaO_6P$ (283.15)	0.70	3.01 (dd, Hβa, $J_{H\beta a,H\beta b} = 14.7$, $J_{H\beta a,H\alpha} = 4.7$); 3.27 (dd, Hβb, $J_{H\beta b,H\alpha} = 8.7$); 3.94 (dd, Hα); 7.16 (dd, Hε, $J_{H\varepsilon,H\delta} = 8.7$, $J_{H\varepsilon,P} = 1.0$); 7.22 (d, Hδ)	36.4 (Cβ); 56.8 (Cα); 121.7 (d, Cε, $J_{C\varepsilon,P} = 4.4$); 131.3 (Cδ); 152.3 (Cq); 174.8 (C=O)
6Da	95	$C_8H_{15}N_2NaO_8P$ (321.2)	−0.98	2.22 [m, Hβa (Hypro), $J_{H\beta a,H\beta b} = 14.5$, $J_{H\beta a,H\alpha} = 10.5$, $J_{H\beta a,H\gamma} = 3.8$, $J_{H\beta a,P} < 1$]; 2.66 [bdd, Hβb (Hypro), $J_{H\beta b,H\alpha} = 7.9$, $J_{H\beta b,H\gamma} < 1$, $J_{H\beta b,P} < 1$]; 3.53 [bdd, Hδa (Hypro), $J_{H\delta a,H\delta b} = 13.0$, $J_{H\delta a,H\gamma} = 3.3$); 3.60 [m, Hδb (Hypro), $J_{H\delta b,H\gamma} < 1$]; 4.00 [m, Hα (Ser), $J_{H\alpha,H\beta a} = 3.4$, $J_{H\alpha,H\beta b} = 4.4$, $J_{H\alpha,P} < 1$]; 4.24 [m, Hβa (Ser), $J_{H\beta a,H\beta b} = 11.4$, $J_{H\beta a,P} = 4.1$]; 4.27 [m, Hβb (Ser), $J_{H\beta b,P} = 5.4$]; 4.39 [dd, Hα (Hypro)]; 4.96 [quint, Hγ (Hypro), $J_{H\gamma,P} = 3.6$]	37.1 [Cβ (Hypro), $J_{C\beta,P} = 2.9$]; 52.9 [Cδ (Hypro), $J_{C\delta,P} = 4.4$]; 54.9 [Cα (Ser)]; 59.9 [Cα (Hypro)]; 64.5 (Cβ (Ser), $J_{C\beta,P} = 4.5$]; 75.6 [Cγ (Hypro), $J_{C\gamma,P} = 4.4$]; 171.4 [C=O (Ser)]; 173.9 [C=O (Hypro)]
6Dd	85	$C_5H_9NNaO_6P$ (233.1)	−0.05	2.19 (m, Hβa, $J_{H\beta a,H\beta b} = 14.5$, $J_{H\beta a,H\alpha} = 10.5$, $J_{H\beta,H\gamma} = 4.0$, $J_{H\beta a,P} = 1.2$); 2.64 (m, Hβb, $J_{H\beta b,H\alpha} = 7.9$, $J_{H\beta,H\gamma} = 1.0$, $J_{H\beta b,P} = 1.7$); 3.52 (m, Hδa, $J_{H\delta a,H\delta b} = 12.7$, $J_{H\delta a,H\gamma} = 3.4$, $J_{H\delta a,P} = 1.0$); 3.59 (m, Hδb, $J_{H\delta b,H\gamma} = 1.2$, $J_{H\delta b,P} < 1$); 4.38 (dd, Hα); 4.96 (m, Hγ, $J_{H\gamma,P} = 3.8$)	36.7 (Cβ, $J_{C\beta,P} = 3.1$); 53.1 (Cδ, $J_{C\delta,P} = 5.4$); 59.0 (Cα); 74.6 (Cγ, $J_{C\gamma,P} = 5.4$); 172.3 (C=O)

[a] Based on **5**.
[b] With 85% H_3PO_4 as external standard.
[c] Spectra were recorded in D_2O (pD 7.0)

Benzyl (Hydroxyamino Acid) Phosphate Triesters 5; General Procedure:
A solution of 1*H*-tetrazole (5.5 mL, 0.5 M) in MeCN was added to a solution of **3** (1.0 mmol) and the appropriate alcohol **4** (1.0 mmol, except **4d**: 5.0 mmol), which had been dried by repeated coevaporation with dioxane or MeCN (2 × 5 mL), in CH_2Cl_2 (2.5 mL). The mixture was stirred for 25 min at 20 °C. Subsequently *t*-BuOOH (0.5 mL, 4.0 mmol) was added and the mixture was stirred for another 15 min at 20 °C. The mixture was diluted with CH_2Cl_2 (50 mL) and washed with brine (2 × 10 mL). The organic layer was dried ($MgSO_4$) and concentrated in vacuo. Silica gel column chromatography (12 g, elution: hexane/EtOAc, 3:2 to 2:3, v/v; except **5Ag**: acetone/CH_2Cl_2, 1:9 to 1:4, v/v) afforded pure **5**, as a colorless oil.

Amino Acid Phosphate Esters and Diesters 6; General Procedure:
The benzyl phosphate **5** (150–200 mg) was dissolved in *i*-PrOH/H_2O/AcOH (10.0 mL, 6:3:1, v/v) and Pd–C (100 mg, 10%) was added. The solution was placed under an H_2 atmosphere (500 KPa) for 4–6 h. The catalyst was removed by filtration and rinsed with *i*-PrOH/H_2O (100 mL, 1:3 to 1:10, v/v). The filtrate and washings were concentrated in vacuo and lyophilized (3 × 10 mL). The product was passed over a SP Sephadex C25 (Na^+ form) column and lyophilized.

Seryl (Valylserylisoleucyl) Phosphate 6Ag:
The phosphate triester **5Ag** (150 mg) was hydrogenated as described above. After lyophilization the diester was dissolved in H_2O/MeOH (2.0 mL, 1:1, v/v) and Et_3N (0.09 mL) was added. After 3 h at 0 °C the solution was neutralized by the addition of AcOH. The solvents were evaporated and the residue was lyophilized (3 × 5.0 mL). The crude product was purified by DEAE Sephadex A25 anion exchange chromatography (25 cm, 0.05 M to 1.0 M TEAB in 24 h). Finally the phosphate diester **6Ag** was passed over a SP Sephadex C25 (Na^+ form) column and lyophilized.

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