A model study directed towards the preparation of nucleopeptides via H-phosphonate intermediates

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ABSTRACT

The monofunctional phosphitylating reagents bis-(N,N-diethylamino)chlorophosphine and salicylchlorophosphine have been applied for the preparation of H-phosphonates of the amino acids serine, threonine and tyrosine. Experimental evidence showed that the latter reagent was less effective for the synthesis of a tyrosine H-phosphonate. The amino acids (peptide) H-phosphonates of serine or threonine proved to be suitable starting compounds for the formation of a phosphate diester bond with the 5'-OH of a d-nucleoside derivative using pivaloyl chloride as the activating reagent.

INTRODUCTION

Nucleoproteins are naturally occurring biopolymers in which the 5'-OH of nucleic acids (RNA or DNA) is covalently linked through a phosphodiester bond with the hydroxyl groups of the L-amino acids serine, threonine and/or tyrosine in proteins.

The preparation of small fragments of nucleoproteins (nucleopeptides), taken into consideration the impressive results so far obtained in nucleic acids and peptides chemistry, would be a straightforward procedure. Thus, the introduction of an intermediate phosphotriester linkage between a properly-protected oligonucleotide and peptide would afford a fully-protected oligonucleopeptide. Removal of all protective groups than gives the required compound. For example, recently we prepared the tyrosine-nucleopeptide \( \text{1} \) and the serine(threonine)-nucleopeptide \( \text{2a(b)} \) \(^5\) by using the hydroxylbenzotriazole activated reagents \( \text{3} \) and \( \text{4} \), respectively, for the formation of the internucleotide-(P-O)-amino acid phosphodiester bonds via phosphotriester intermediates.

We now report that the introduction of an internucleotide-(P-O)-amino acid phosphodiester linkage can also be accomplished via a H-phosphonate approach.
RESULTS AND DISCUSSION

In a preliminary paper we illustrated that the easily accessible phosphitylating reagents and proved to be very convenient for the preparation of 3′-H-phosphonates of properly-protected d-nucleosides. Coupling of the latter compounds with a free hydroxyl group, in the presence of pivaloyl chloride, gives an intermediate H-phosphonate-diester which is easily converted by oxidation into the corresponding phosphodiester function. Adoption of the H-phosphonate approach, originally devised for a solid-phase synthesis of DNA, to the formation of an internucleotide-(P-O)-amino acid phosphodiester bond, would be in several aspects superior over the phosphotriester approach. For instance, in an earlier study we demonstrated that the introduction of the particularly base-labile internucleotide-(P-O)-serine bond could be realized by selecting the 4-methylthiophenyl as a P(V)-protecting group [see fully-protected 2a; R°-SC₆H₅(CH₃)]. This group could be removed slowly but selectively under neutral conditions. However, subsequent deblocking (i.e., oximate anion assisted deblocking of the internucleotide P(V)-2-chlorophenyl protecting group from partially-protected 2a (R²=OH, R²=2-ClC₆H₄) was accompanied by degradation of the internucleotide-(P-O)-serine bond. With the
purpose in mind of developing a general and mild procedure for the formation of internucleotide-(P-0)-amino acid linkages, we expected to achieve our goal by exploring in detail the possible application of the H-phosphonate approach. To this end, we first prepared the amino acid H-phosphonates 9a, b and 15 using 5 and 6 as the phosphitylating reagents.

Thus, to a solution of the protected serine derivative 7a in dioxane and pyridine was added a slight excess of the monofunctional reagent 5. TLC-analysis as well as 31P-NMR spectroscopy revealed fast and complete conversion of 7a to intermediate 8a. The latter was treated immediately with an excess of dry acetic acid and left for 30 min at 20°C. Addition of water to the reaction mixture followed by work-up and purification afforded homogeneous H-phosphonate 9a in an excellent yield. The same three-step procedure (i.e., phosphitylation, acidolysis followed by hydrolysis) could also be applied successfully to the preparation of the similarly protected H-phosphonates of threonine (9b) and tyrosine (15). The identity and homogeneity of the three H-phosphonates thus obtained were unambiguously ascertained by 31P- and 13C-NMR spectroscopy.

A more convenient and less time-consuming approach to the three H-phosphonates 9a, b and 15 would be phosphitylation of the corresponding and similarly protected amino acids 7a, b and 11 with the recently by us introduced 6 reagent 6. Indeed, phosphitylation of the serine derivative 7a in dry pyridine with a small excess of reagent 6 for 10 min at 20°C showed, as evidenced by TLC-analysis and 31P-NMR spectroscopy, complete conversion of 7a to the phosphate-triester intermediate 10a. Addition of water to the reaction mixture followed by the same work-up and purification procedures, as mentioned before
for the isolation of 9a using 5 as the phosphitylating reagent, afforded the serine H-phosphonate 9a in an excellent yield. The same result was obtained by phosphitylation of the threonine derivative 7b with reagent 5. However, in the case of the tyrosine derivative 10 the two-step salicylchlorophosphine method (i.e., phosphitylation and subsequent hydrolysis) was not completely satisfactory: the tyrosine H-phosphonate 15 could only be isolated, despite many attempts, in a relatively low yield. The loss of 15 may be explained by assuming that the removal of the salicylic acid moiety from the proposed intermediate 14, formed in the first step of the hydrolysis of 13, is not completely selective. The above assumption was supported by the observation (TLC-analysis) that the phosphitylation of the tyrosine derivative 11 with 6 never went, in contrast with 7a and 7b, to completion. It was also established that the presence of starting product 11 in the reaction mixture was not due to rapid decomposition of the tyrosine H-phosphonate 15.

The formation of the required phosphodiester bond between the 5'-OH of the thymidine derivative 16 and the amino acid H-phosphonates 9a,b and 15 [(R benzyl(Bzl); R benzylxycarbonyl (Z)] was now examined following an approach recently developed for a solid-phase synthesis of DNA using properly-protected d-nucleoside 3'-H-phosphonates as the incoming nucleotides and pivaloyl chloride (PVC1) as the activating reagent. Thus, coupling of the thymidine derivative 16 with a slight deficiency of the protected serine 9a or threonine 9b, in the presence of PVC1, showed rapid formation (TLC-analysis) of the corresponding H-phosphonate-diesters 17a and 17b, respectively. However, work-up and purification of the products thus obtained
afforded homogeneous 17a,b in unacceptable low yields. With the intention of increasing the overall yield of the required phosphodiester derivatives 17c,d, we decided to convert the intermediate H-phosphonates 17a,b into the required products by in-situ oxidation with iodine immediately followed by hydrolysis with water. The latter procedure has the advantage that the intermediate formed H-phosphonate-diester functions will not be exposed to pyridine-water which may lead, as reported earlier7c, to rapid decomposition of H-phosphonate-diester functions. Following the above described sequential procedure (i.e., coupling, in-situ oxidation, hydrolysis) the nucleopeptide fragments 17c,d could be isolated in a yield of 80% (based on 9a,b). However, application of the same two-step procedure to the preparation of the tyrosine nucleopeptide 18b was less satisfactory. Addition of PVCl to a mixture of the tyrosine H-phosphonate 15 (R = Bzl; R2 = Z) and 16, gave, after in-situ oxidation of 18a and further processing, 18b in a yield of only 56% (based on 15). An explanation of this low yield could be that the intermediate H-phosphonate 18a containing an aryl-alkyl H-phosphonate-diester function is intrinsically less stable than the corresponding non-mixed H-phosphonate-diesters 17a,b. In this respect it is interesting to note that the introduction of a phosphodiester bond between the phenolic hydroxyl of tyrosine and the 5'-OH of an RNA dimer via a phosphotriester approach (i.e., preparation of 1 (R1 = R2 = R3 = R4 = H) from fully-protected 1 (R2 = 2-nitrophenylsulphenyl (NPS); R2 = 2-ClC6H4; R3 = THP) could be executed with a high degree of efficiency 4.

The positive results so far obtained in the preparation of the nucleopeptide fragments 17c,d, via the H-phosphonate approach, urged us to examine whether this procedure would also be successful for the formation of phosphodiester bonds between the tripeptides Nα-Z-Val-Ser(OH)-Ile-OBzl, Nα-Z-Val-Thr(Oh)-Ala-OBzl and 3'-O-acetyl-thymidine. Phosphitylation of the tripeptides, obtained by coupling (DCC/HOBT method11) the dipeptides Nα-Z-Val-Ser(OH)-OH12 and Nα-Z-Val-Thr(Oh)-OH12 with the benzylester of the amino acids iso-
leucine and alanine, using salicylchlorophosphine 6 gave, after work-up and purification, the corresponding H-phosphonates 19a,b in a good yield. Condensation of 19a,b thus obtained with 3'-O-acetyl-thymidine, in the presence of PVC1, afforded after usual work-up and purification, the corresponding nucleopeptide fragments 20c,d, the identity and homogeneity of which was corroborated by 31P- and 13C-NMR spectroscopy.

In conclusion the results of the present study show that: (a) bie-(N,N-diethylamino)chlorophosphine 5 is a convenient reagent for the preparation of H-phosphonates of serine, threonine and tyrosine; (b) the same holds, apart from tyrosine, for the reagent salicylchlorophosphine 6; (c) peptides containing H-phosphonates of serine and threonine can be coupled efficiently with the 5'-OH of a protected d-nucleoside. Furthermore, preliminary experiments indicated that the relatively ineffective phosphorylation of the phenolic hydroxyl of tyrosine with salicylchlorophosphine 6 may open the way to phosphorylate serine (threonine) selectively in peptides containing also a tyrosine moiety. At present we are studying in detail the preparation of naturally occurring nucleopeptides starting from properly-protected peptides as well as nucleic acid fragments using the H-phosphonate approach described in this paper.
EXPERIMENTAL 

General methods and materials

Dioxane and pyridine were dried by refluxing with CaH₂ (5 g/l) for 16 h and then distilled. Dioxane was redistilled from LiAlH₄ (5 g/l) and stored over molecular sieves 4Å. Pyridine was redistilled from p-toluenesulphonyl chloride (60 g/l) and stored over molecular sieves 4Å. Acetonitrile was dried by distillation first from P₂O₅ and then from CaH₂. Triethylammonium bicarbonate buffer (TEAB, 2 M) was prepared by saturation of a mixture of triethylamine (825 ml) and water (2175 ml) with carbon dioxide gas at 0°C until pH 7.0. Schleicher and Schüll DC Fertigfolien F-1500 LS 254 were used for TLC-analysis in the solvent system: A) CH₂Cl₂-EtOAc-Et₂N, 10:9:1, v/v; B) CH₂Cl₂-MeOH, 9:1, v/v; C) CH₂Cl₂-MeOH, 95:5, v/v). Short column chromatography was performed on silanized silica gel RP 2 (Merck) 70-230 mesh ASTM. The columns were eluted with ethylacetate applying a methanol gradient (0-20%). ³¹P- and ¹³C-NMR spectra were measured at 80.7 MHz and 50.1 MHz, respectively, using a JEOL JNM-FX 200 spectrometer. ³¹P-chemical shifts (δ) are given in ppm relative to 85% H₃PO₄ as an external standard. ¹³C-chemical shifts are given in ppm relative to tetramethylsilane as an internal standard.

The H-phosphonate monoesters 9a-b, 15, 19a-b and the phosphodiester derivatives 17c-d, 18b and 20c-d were, after purification by column chromatography, freeze-dried and converted into the corresponding sodium-salts by passing them (2 x) over a column of Dowex 50W (Na⁺-form) cation-exchange resin. The sodium-salts thus obtained were lyophilized and used for NMR-spectroscopic and elemental analysis.

Preparation of protected amino acids H-phosphonates 9a, 9b and 15 (R =NBzl; R²=Z) using bis-(N,N'-diethylamino)chlorophosphine 5.

To each of a stirred solution of 7a R=H, 7b R=CH₃ or 11 (1.0 mmol) in dioxane (5.0 ml) and pyridine (0.12 ml, 1.5 mmol) was added the phosphitylating reagent 5 (253 mg, 1.2 mmol). After 20 min, TLC (system A) indicated complete conversion of starting materials into the intermediates 8a, 8b and 12 [³¹P-NMR (dioxane): δ 120.1, 131.5 and 132.0, respectively]. Acidolysis of the intermediates with acetic acid (0.34 ml, 6.0 mmol) for 30 min, followed by addition of water (0.5 ml), afforded crude 9a, 9b and 15. The reaction mixtures were taken up in CH₂Cl₂ (150.0 ml) and washed with TEAB (1 M, 10.0 ml). The organic layers were concentrated and coevaporated with toluene (2 x 25.0 ml). The residues thus obtained were dissolved in a minimal amount of EtOAc and applied to a silanized silica gel column (20.0 g). The columns were eluted
with EtOAc and applying a methanol gradient (0-20%), to give the corresponding H-phosphonates \(9a\), \(9b\) and \(15\), respectively.

**Compound \(9a\) (\(R^1\)-NBzl; \(R^2\)-Z), yield 90%. \(Rf\) 0 (system A).**

\[3^1P\text{-NMR (CH}_2\text{Cl}_2/\text{MeOH)}: \delta 5.1 \text{ ppm } J_{P-H}, 630 \text{ Hz.} \]

\[^{13}C\text{-NMR (CDCl}_3/\text{CD}_3\text{OD): } \delta 170.5 (C=O, Ser); 157.4 (C=O, Z); 143.7-117.4 (aromatic carbons); 67.6, 66.3 and 64.0 (CH\text{\_2} of Z, 4-nitrobenzyl and BCH, Ser); 55.9 (aCH, Ser).\]


**Compound \(9b\) (\(R^1\)-NBzl; \(R^2\)-Z), yield 90%. \(Rf\) 0 (system A).**

\[3^1P\text{-NMR (CH}_2\text{Cl}_2/\text{MeOH)}: \delta 2.9 \text{ ppm } J_{P-H}, 627 \text{ Hz.} \]

\[^{13}C\text{-NMR (CDCl}_3/\text{CD}_3\text{OD): } \delta 171.1 (C=O, Thr); 158.3 (C=O, Z), 148.5-124.5 (aromatic carbons); 71.4 (BCH, Thr); 67.8 and 66.6 (CH\text{\_2} of Z and 4-nitrobenzyl); 60.7 (aCH, Thr); 20.3 (CH\text{\_3}, Thr).\]

**Anal. Calc. for C\(_{19}\)H\(_{20}\)O\(_7\)N\(_2\)PNa: P 6.53. Found P 6.45.**

**Compound \(15\) (\(R^1\)-NBzl; \(R^2\)-Z), yield 80%. \(Rf\) 0 (system A).**

\[3^1P\text{-NMR (CH}_2\text{Cl}_2/\text{MeOH)}: \delta 1.1 \text{ ppm } J_{P-H}, 640 \text{ Hz.} \]

\[^{13}C\text{-NMR (CDCl}_3/\text{CD}_3\text{OD): } \delta 172.3 (C=O, Tyr); 154.9 (C=O, Z); 151.5-121.2 (aromatic carbons); 67.2 and 65.8 (CH\text{\_2} of Z and 4-nitrobenzyl); 55.7 (aCH, Tyr); 37.3 (BCH, Tyr).\]

**Anal. Calc. for C\(_{24}\)H\(_{22}\)O\(_7\)N\(_2\)PNa: P 5.78. Found P 5.96.**

In the same way the protected H-phosphonates \(9a\), \(9b\) and \(15\) (\(R^1\)-Bzl; \(R^2\)-Z) were prepared. Analytical data (\(3^1P-\) and \(^{13}C\text{-NMR) were in complete accordance (apart from the \(^{13}C\)-resonances of the benzyl functions) with \(9a\), \(9b\) and \(15\) (\(R^1\)-NBzl; \(R^2\)-Z) obtained above.

**Preparation of protected amino acids H-phosphonates \(9a\), \(9b\) and \(15\) (\(R^1\)-NBzl; \(R^2\)-Z) using salicylchlorophosphine 6.**

The phosphorylating reagent 6 (243 mg, 1.2 mmol) was added separately to stirred solutions of \(7a\), \(7b\) and \(11\) (\(R^1\)-NBzl; \(R^2\)-Z, 1.0 mmol) in a mixture of dioxane (4.0 ml) and pyridine (1.0 ml). After 20 min, TLC-analysis (system A and B) indicated complete conversion of starting compounds into the intermediates 10a, 10b and 13, respectively. \[3^1P\text{-NMR (dioxane/pyridine): } 125.0 (10a), 127.0, 125.0 (10b) and 117.7, 117.4 (13).\]

Hydrolysis with water (0.5 ml), followed by the same work-up and purification procedure as described earlier, gave \(9a\) (90%), \(9b\) (90%) and \(15\) (65%) as a homogeneous oil.

**Synthesis of the nucleopeptide H-phosphonate \(17a\) (\(R^1\)-Bzl; \(R^2\)-H).**

Pivaloyl chloride (0.15 ml, 1.2 mmol) was added to a solution of \(9a\) (495 mg, 1.0 mmol) and 3'-O-tetrahydropyranylthymidine (326 mg, 1.0 mmol) in acetonitrile (2.5 ml) and pyridine (2.5 ml). TLC-analysis (system C), after 20 min, indicated complete conversion of starting compounds into crude \(17a\). The reaction mixture was taken up in CH\(_2\text{Cl}_2\) (75 ml) and washed with water (2 x 25 ml), dried over MgSO\(_4\) and concentrated (water-bath temperature below 30°C). The
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residue was coevaporated with toluene (2 x 25 ml), dissolved in CH₂Cl₂ (5 ml) and applied to a silica gel column (30.0 g). The column was eluted with CH₂Cl₂ applying a methanol gradient (0-10%), to give 17a in a yield of 40%.

³¹P-NMR (CH₂Cl₂): δ 9.8, 8.7 ppm, JP-H 720 Hz. ¹³C-NMR (CDCl₃/CD₃OD): δ 168.4 (C=O, Ser); 164.2 (C-4, T); 156.0 (C=O, Z); 150.3-127.2 (aromatic carbons, C-2 and C-6, T), 110.8 (C-5, T); 98.1 and 98.0 (THP); 85.0, 84.8, 84.6, 84.4, 82.5, 82.0 (THP, C-1' and C-4', T); 75.3, 75.1 and 75.0 (C-3', T); 67.3-61.7 (CH₂-Bzl, Z, ßCH₂ Ser and C-5', T); 55.9 and 54.0 (ßCH-Ser); 37.7 and 36.8 (C-2', T); 30.2, 24.7, 19.0 and 18.6 (THP); 13.3 and 12.2 (CH₃, T).

Synthesis of the phosphodiester derivatives 17c, 17d and 18b (R= Bzl; R = Z).

Pivaloyl chloride (0.15 ml, 1.2 mmol) was added to separate solutions of acetonitrile (2.5 ml) and pyridine (2.5 mmol) containing 9a, 9b and 15 (R= Bzl; R = Z: 0.9 mmol) as well as 3'-O-tetrahydropyranylthymidine (326 mg, 1.0 mmol). TLC-analysis (system C), after 20 min, indicated complete conversion of starting compounds into intermediates 17a, 17b and 18a. [³¹P-NMR (CH₂Cl₂) of 17a: δ 9.8, 8.7 ppm, JP-H 720 Hz]. The latter were oxidized with I₂ (700 mg, 2.76 mmol) followed, after 1 min, by the addition of water (0.2 ml). After 15 min, excess iodine was destroyed with sodium hydrogen sulphite (850 mg, 8.2 mmol) in water (1.0 ml) and the mixtures were taken up in CH₂Cl₂ (150.0 ml) and washed with TEAB (1 M, 10.0 ml). The organic layers were concentrated and coevaporated with toluene (2 x 25.0 ml). The residues were dissolved in CH₂Cl₂ and applied to separate silanized silica gel columns (30-40 g). The columns were eluted with CH₂Cl₂ applying a methanol gradient (0-20%). Repetition of the purification step gave homogeneous 17c, 17d and 18b.

Compound 17c (R= Bzl; R = Z), yield 88%. Rf 0 (system B). ³¹P-NMR (CH₂Cl₂/MeOH): δ -0.3 ppm. ¹³C-NMR (CDCl₃/CD₃OD): δ 169.3 (C=O, Ser); 164.1 (C-4, T); 156.1 (C=O, Z); 150.3-126.7 (aromatic carbons, C-2, T and C-6, T); 110.6, 109.7 (C-5, T); 97.9 and 97.6 (THP); 84.7-82.9 (THP, C-1', T and C-4', T); 76.0, 75.7 (C-3', T); 66.8-62.1 (CH₂, Z, CH₂, Bzl; ßCH₂ Ser and C-5', T); 54.6, 54.5 (ßCH, Ser); 37.8, 36.7 (C-2', T); 30.2, 30.1 (THP); 24.7 (THP); 19.0, 18.7 (THP); 14.4, 12.3 (CH₃, T).


Compound 17d (R= Bzl; R = Z), yield 80%. Rf 0 (system B). ³¹P-NMR (CH₂Cl₂/CH₃OH): δ -1.3 ppm. ¹³C-NMR (CDCl₃/CD₃OD): δ 169.7 (C=O, Thr); 163.9 (C-4, T); 156.3 (C=O, Z); 150.1-126.3 (aromatic carbons, C-2, T and C-6, T); 110.3, 109.4 (C-5, T); 97.5, 97.3 (THP); 84.5-82.8 (THP, C-1', T and C-4', T); 75.7, 75.5 (C-3', T); 71.9, 71.8 (ßCH, Thr); 66.6-61.7 (CH₂, Z, Bzl and C-5', T); 58.9, 58.8 (ßCH, Thr); 37.6, 36.5 (C-2', T); 30.0, 29.9, 24.5, 18.8 and 18.5
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(THP); 18.1, 14.3 and 12.2 (CH₃, Thr, T).


**Compound 18b (R'=Bzl; R''=Z), yield 56%.** Rf 0 (system B). ³¹P-NMR (CH₂Cl₂/CH₃OH): δ -6.5 ppm. ¹³C-NMR (CDCl₃/CD₃OD): δ 172.1 (C=O, Tyr); 165.2 (C-4, T); 156.9 (C=O, Z); 152.1-120.4 (aromatic carbons, C-2, T and C-6, T); 111.3 (C-5, T); 98.8, 98.4 (THP); 84.8, 84.7, 84.2 and 84.0 (THP, C-1', T and C-4', T); 77.3, 77.1 (C-3', T); 67.4-62.9 (CH₂, Z, Bzl and C-5', T); 55.9 (aCH, Tyr); 37.6, 37.4, 37.1 and 36.9 (ßCH₂, Tyr and C-2', T); 31.0, 25.6, 19.8 and 19.6 (THP); 15.2, 12.4 (CH₃, T).


**Synthesis of the tripeptide H-phosphonates 19a [R=H; R''=CH(CH₃)CH₂CH₃] and 19b (R'=CH₃).**

The title compounds were prepared by phosphorylation of the tripeptides Z-Val-Ser(OH)-Ile-OBzl or Z-Val-Thr(OH)-Ala-OBzl with salicylchlorophosphine 6. Further work-up and purification was performed in the same way as described earlier for the preparation of the H-phosphonates 9a,b using reagent 6. According to this procedure the H-phosphonates 19a (δ-³¹P: 5.5 ppm, JP-H 630 Hz) and 19b (δ-³¹P: 3.9 ppm, JP-H 630 Hz) were obtained in a yield of 62 and 67%, respectively.

¹³C-NMR (CDCl₃/CD₃OD) data of 19a: 171.8, 170.5 and 168.9 (3 x C=O, Val, Ser and Ile); 127.8-127.1 (aromatic carbons), 66.3 (CH₂, Z and Bzl); 66.1 (ßCH₂, Ser); 59.6, 56.3 and 53.5 (3 x aCH, Val, Ser and Ile); 36.8 (ßCH, Ile); 30.7 (ßCH₂, Val); 24.3 (γCH₂, Ile); 18.6, 16.7, 14.6 and 10.7 (4 x CH₃, Val and Ile).


¹³C-NMR (CDCl₃/CD₃OD) data of 19b: 171.9, 171.4, 168.7 (3 x C=O, Val, Thr and Ala); 156.3 (C=O, Z); 135.9-126.8 (aromatic carbons), 69.3, 69.2 (ßCH₂, Thr); 66.2, 66.0 and 65.9 (CH₂, Z and Bzl); 60.0, 57.5 and 57.4 (3 x aCH, Val, Thr and Ala); 30.2 (ßCH₂, Val); 18.3, 17.7, 16.7 and 16.2 (4 x CH₃, Val. Thr and Ala).


**Preparation of the nucleopeptides 20c and 20d.**

The nucleopeptides 20c and 20d were obtained by coupling 3'-O-acetyl-thymidine (283 mg, 1.0 mmol) with each of the tripeptide H-phosphonates 19a (545 mg, 0.9 mmol) and 19b (519 mg, 0.9 mmol) in the presence of pivaloyl chloride (0.15 ml, 1.2 mmol).

Nucleopeptide 20c, yield 80%. Rf 0 (system B). ³¹P-NMR (CH₂Cl₂/CH₃OH): δ 0.3 ppm. ¹³C-NMR (CDCl₃/CD₃OD): δ 172.1, 170.9, 170.1 and 169.0 (C=O, Val, Ser,
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Ile and Ac), 164.0 (C-4, T); 156.2 (C=O, Z); 150.5-127.4 (aromatic carbons, C-2, T and C-6, T); 110.0 (C-5, T); 83.3 (broad, C-1', T and C-4', T); 75.1, 75.0 (C-3', T); 66.3-63.2 (CH₂, Z, Br, CH₂, Ser and C-5', T); 59.7, 56.4 (3 x aCH, Ser, Val, Ile); 37.2 (CH₂, Ile); 31.1 (BCH, Val); 24.6 (γCH₂, Ile); 20.6-11.1 (6 x CH₃, Val, Ile, T and Ac).

Anal. Calc. for CₓHᵧOₜNₓPNa: C 54.12; H 5.87; P 3.41. Found: C 53.31; H 5.77; P 3.33.

Nucleotide 20d, yield 60%. Rf 0 (system B). 3¹P-NMR (CH₂Cl₂/CH₃OH): δ -2.9 ppm. C-NMR (CDCl₃/CD₃OD): δ 172.5, 171.9, 170.6 and 168.0 (C=O, Val, Thr, Ala and Ac); 164.3 (C-4, T); 156.6 (C-O, Z); 150.4-125.1 (aromatic carbons, C-2, T and C-6, T); 110.9 (C-5, T); 84.8, 83.2 (C-1', T and C-4', T); 74.8, 71.0 (βCH, Thr and C-3', T); 66.7-62.3 (CH₂, Z, Br and C-5', T); 60.2, 56.7 and 48.3 (3 x aCH, Val, Thr and Ala); 36.4 (C-2', T); 20.5-11.8 (6 x CH₃, Val, Thr, Ala, T and Ac).

Anal. Calc. for CₓHᵧOₜNₓPNa: C 53.12; H 5.66; P 3.52. Found: C 52.27; H 5.52; P 3.45.

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REFERENCES AND NOTES


14. The conditions required for the removal of the protecting groups used in this study are not completely compatible with the stability of the final products. For instance, basic hydrolysis of the acetyl group in 20c will also give rise to the concomitant formation (via 1,2-elimination of the phosphate function) of a dehydroalanine derivative. On the other hand, acidic hydrolysis of the THP group in 17c is a feasibility as shown before in the preparation of 2a and 2b. It is also not excluded that hydrogenolysis of the benzyl (Bzl) and benzyloxycarbonyl (Z) protecting groups in 17c can be performed without overreduction of the pyrimidine bases. The latter possibility is based on the results of a recent study of B.E. Watkins et al. [J. Am. Chem. Soc., 104, 5702 (1982)] which showed that N-Z and O-Bzl protected d-nucleosides could be unmasked selectively by transfer hydrogenolysis in the presence of the catalyst Pd black.