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Synthesis of N-Phosphoryl Branched Peptides

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Abstract: H-phosphonates were conveniently prepared by direct transesterification of diphenyl phosphite (DPP) with the corresponding alcohols, without further purification they were reacted with branched peptide methyl ester (L-Leu$_2$-L-LysOMe) through Atherton-Todd method, a series of different substituted alkyloxy (N-phosphoryl-L-Leu)$_2$-L-LysOMe were synthesized, and their structures were confirmed by ^{31}P NMR, ESI-MS, ^{1}H NMR, ^{13}C NMR, IR and elemental analysis. The approach possesses the advantages of easy operation, high yield and inexpensive phosphorylating reagent.

Keywords: N-Phosphoryl branched peptide, H-phosphonate, Atherton-Todd reaction, transesterification.

Phosphorus plays a crucial role in life chemistry. Some N-phosphoryl amino acids and N-phosphopeptides are of important biological activities and medicinal value. Previous researches in our lab have shown that some N-phosphoryl peptide methyl esters such as (N-diisopropyoxyl-L-Leu)$_2$-L-LysOMe[1,2] and (N-diisopropyoxyl-L-Phe)$_2$-L-LysOMe[2] exhibited inhibition activity on K562 cells proliferation, and we also found that diisopropyoxyl (DIPP-) and methyl ester group (-CO_2CH_3) were necessary for this activity[3]. In attempts to increase the transmembrane transport characteristics and improve the inhibition activity on K526 cells, here a series of substituted alkyloxy (N-phosphoryl-L-Leu)$_2$-L-LysOMe were synthesized through modified Atherton-Todd reaction[4] as shown in **Scheme 1**.

The symmetric H-phosphonates **3a-h** were prepared by direct transesterification of diphenyl phosphate (DPP) with the corresponding alcohols. Diphenyl phosphite, which is a commercially available and an inexpensive phosphorylation reagent, can undergo fast transesterification with various alcohols in dry pyridine to yield mixtures of the corresponding double-exchange and mono-exchange H-phosphonates[5]. Inspired by this observation, a new approach to symmetric H-phosphonate diesters was investigated by us[6] in our previous work, the symmetric H-phosphonates of the monohydroxylic compounds were synthesized. Following this method, the symmetric H-phosphonates

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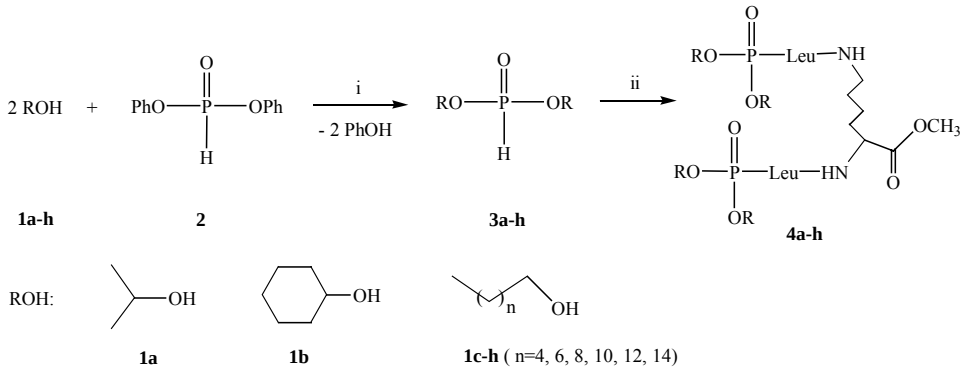
3a-h were conveniently prepared by direct transesterification. For example, DPP was treated with 2.1 equiv. of hexadecanol **1h** in anhydrous pyridine at room temperature. The reaction was monitored by ^{31}P NMR spectroscopy. After 1.5 h, the peak of DPP (signal δ_P=1.2 ppm) was quantitatively converted into a new peak (δ_P=8.5 ppm), which was assigned to the H-phosphonate **3h**. Other long-chain or cyclic alcohols were also tested, and the experimental results were excellent, their structures were determined by ESI-MS and ^{31}P NMR, the spectral data of symmetric H-phosphonates **3a-h** were shown in **Table 1**. After the pyridine was removed under reduced pressure, the products were obtained in purity of more than 95%, and without further purification they were directly conjugated with branched peptide methyl ester (L-Leu$_2$-L-LysOMe) using a modified Atherton-Todd reaction in reasonable yields.

Scheme 1



i) Anhydrous pyridine; ii) CCl_4/Et_3N/EtOH, (L-Leu)$_2$-L-LysOMe

Table 1 The spectral data of symmetric H-phosphonates **3a-h**[a]

Compd.	Alcohol	^{31}P NMR			ESI-MS (*m/z*)
		δ (ppm)	$^1J_{P-H}$ (Hz)	$^3J_{P-H}$ (Hz)	
3a	Isopropanol	5.3	689(d)	8.2(t)	167.0[M+H]$^+$, 189.0[M+Na]$^+$
3b	Cyclohexanol	5.3	684(d)	8.9(t)	247.1[M+H]$^+$, 269.1[M+Na]$^+$
3c	Hexyl alcohol	8.7	690(d)	8.2(m)	251.1[M+H]$^+$, 273.1[M+Na]$^+$
3d	Octanol	8.8	689(d)	8.1(m)	307.2[M+H]$^+$, 329.3[M+Na]$^+$
3e	Decyl alcohol	8.7	691(d)	8.3(m)	363.3[M+H]$^+$
3f	Dodecyl alcohol	8.7	691(d)	8.2(m)	419.4[M+H]$^+$
3g	Tetradecanol	8.9	692(d)	8.3(m)	475.6[M+H]$^+$
3h	Hexadecanol	8.9	687(d)	8.3(m)	531.7[M+H]$^+$, 553.6[M+Na]$^+$

[a] All reaction were performed in 2 mmol scale.

[b] The value were determined in pyridine using a Bruker AMP 200 at 81Hz.

Table 2 Physical constants of compounds **4a-h**

Compd.	Yield[a] (%)	m.p. (℃)	ESI-MS (*m/z*)	^{31}P NMR[b] (δ ppm)
4a	90	108-110	715.8$[M+H]^+$, 737.7$[M+Na]^+$	6.70
4b	85	81-83	876.2$[M+H]^+$, 898.0$[M+Na]^+$	6.89
4c	86	57-58	884.2$[M+H]^+$, 906.0$[M+Na]^+$	8.47
4d	88	58-59	996.2$[M+H]^+$, 1018.1$[M+Na]^+$	8.60
4e	88	[c]	1108.3$[M+H]^+$, 1130.1$[M+Na]^+$	8.71
4f	89	[c]	1220.3$[M+H]^+$, 1242.0$[M+Na]^+$	8.76
4g	90	[c]	1332.4$[M+H]^+$, 1354.3$[M+Na]^+$	8.74
4h	90	67-69	1444.8$[M+H]^+$, 1466.9$[M+Na]^+$	8.73

[a] Based on the reactant $(L\text{-}Leu)_2$-L-LysOMe.

[b] $CDCl_3$ as solvent.

[c] Off-white gum.

In Atherton-Todd reaction, a solution of H-phosphonate (2.1 mmol) in CCl_4 (2 mL) was added dropwise to the solution of branched peptide methyl ester $(L\text{-}Leu)_2$-L-LysOMe (1 mmol) in the mixture of Et_3N (1 mL) and EtOH (0.5 mL) at 0℃. The reaction mixture was stirred at room temperature for 30 min. Then the solvent was distilled off in vacuum below 40℃ and the residue was purified on a column of silica gel with CH_3Cl/MeOH (50:1) as eluent to yield compounds **4a-h**, their structures were confirmed by ESI-MS, ^{31}P **(Table 2)**, ^{1}H NMR, ^{13}C NMR, IR and elemental analysis[7].

In conclusion, the transesterification of alcohols with DPP is an effective method to prepare symmetric H-phoshonate diesters, and the mild conditions are compatible with the presence of sensitive protecting groups. In this paper, we employed this approach in the synthesis of N-phosphoryl branched peptide methyl esters and found it has the distinctive features of easy operation and high yield and inexpensive phosphorylating reagent. Investigation about the inhibition activities of the modified N-phosphoryl branched peptide methyl esters are currently under way in our laboratory, and the mechanism and structure-activity relationship remain to be further studied.

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7. ^{1}H NMR, ^{13}C NMR, IR and elemental analysis data of typical compound **4c**: ^{1}H NMR ($CDCl_3$, δ ppm): 0.90 (m, 24H, CH_3×4, CH_3×4 in Leu), 1.15-1.92 (m, 44H, CH_2×4×4, γ-CH_2, δ-CH_2, β-CH_2 in Lys and β-CH_2×2, γ-CH×2 in Leu), 3.22 (m, 2H, ε-CH_2 in Lys), 3.63-3.82 (m, 5H, α-CH×2 in Leu and OCH_3), 3.83-4.09 (m, 8H, CH_2O×4), 4.40-4.60 (m, 3H, NH×2 in Leu and α-CH in Lys), 7.49 (t, 1H, *J*=5.16 Hz, ε-NH in Lys), 7.59 (d, 1H, *J*=7.20 Hz,α-NH in Lys); ^{13}C NMR ($CDCl_3$, δ ppm): 13.96 (CH_3×4), 21.64, 21.75, 23.08, 23.12 (CH_3×4 in Leu), 21.80 (γ-CH_2 in Lys), 22.53 (($POCH_2(CH_2)_3$**C**H_2CH_3)×4), 24.35, 24.60 (γ-CH×2 in Leu), 25.25-31.18 (($POCH_2$(**C**H_2)$_2CH_2CH_2CH_3$)×4, β-CH_2, δ-CH_2 in Lys), 31.40 (($POCH_2(CH_2)_2$ **C**$H_2CH_2CH_3$)×4), 38.12 (ε-CH_2 in Lys), 43.17, 43.46 (β-CH_2×2 in Leu), 51.91, 52.00 (α-CH in Leu), 54.07, 54.21 (α-CH in Lys and OCH_3), 66.59 (CH_2O×4), 172.43, 174.09, 174.73 (C=O×3); IR (KBr, cm^{-1}): 3301 (N-H), 3181 (P-N), 2956 (-CH_3), 2927, 2855 (-CH_2-), 1746 (-CO_2-), 1656 (CO-N), 1545 (C-N), 1467 (-CH_2-), 1235-1221 (C-N, N-H), 1018 (P-O), 722 (-$(CH_2)_n$-, n>3); Anal. Calcd. for $C_{43}H_{88}N_4O_{10}P_2$ (%): C, 58.48; H, 10.04; N, 6.34; Found: C, 58.21; H, 10.07; N, 6.49.

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