Facile Synthesis of 3β-Cholesterol *H*-Phosphonates

Qiang Xiao, Jing Sun,† Yong Ju,* Yufen Zhao,* and Yuxin Cui†
The Key Laboratory of Bioorganic Phosphorus Chemistry, Ministry of Education, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing 100084, P. R. China
†*National Research Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100083, P. R. China*

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A series of *H*-phosphonates of 3β-cholesterol, which are convenient precursors of various 3β-cholesterol phosphates, were synthesized in one pot by a tandem transesterification of diphenyl phosphite (DPP) with 3β-cholesterol and hydroxyl compounds.

Steroid phosphates are important biological molecules.[1] It was reported that some steroid phosphate can significantly augment lymphocyte response to phytohemagglutinin, and enhance the production of interleukin-2 and interleukin-3-like activity, interferon and tumor necrosis factor by human mononuclear cells in vitro. According to in vivo experiments, these steroid derivatives were found to induce a rise in blood glucose and an increase in leucocytes when injected into mice.[1b]

A number of literatures have reported to phosphorylate the bioactive steroids. The earliest example was the phosphorylation of cholesterol with $POCl_3$,[2] which was lack of sufficiently effective techniques for purification.[3] The phosphoryamidite method had been used to construct the phosphodiester of 7β-hydroxyl cholesterol and carbohydrate.[4] Later, *H*-phosphonate method was also used to synthesize hydrophilic phosphodiester derivatives between 25-hydroxyl cholesterol and different carbohydrates.[5] These methods were multi-step synthesis with low overall yields.

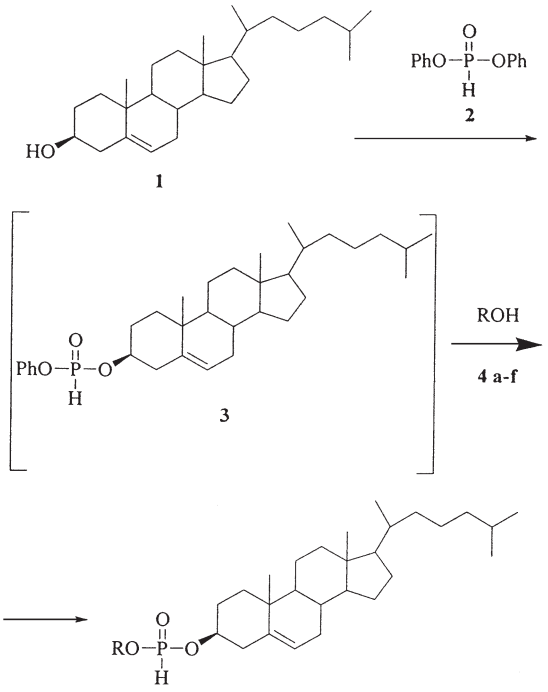
To study the biological activities of steroid phosphate derivatives, we involved in the preparation of phosphodiesters consisted of different steroids and various biological molecules. *H*-Phosphonates of steroids were chosen as the first target molecules. The related interesting steroid derivatives can be easily obtained from the corresponding *H*-phosphonate, such as phosphates, phosphorothionates, phosphoroselenoates by oxidation[6] and phosphoramidates conjugated with amino acid or peptide by Atherton-Todd reaction.[7] Thus, a great choice of molecule diversity can be achieved.

In general, there were two pathways to synthesize these kinds of *H*-phosphonates. The first is phosphoramidite strategy, which has been developed for the synthesis of oligonucleotides. Using this approach, phosphoramidite diesters of phosphite were synthesized and then hydrolyzed with a weak acid such as 1*H*-tetrazole.[8] The second method called *H*-phosphonate approach, which involves firstly the preparation of *H*-monophosphonate and then couples with an alcohol in the presence of a condensing reagent such as pivaloyl chloride.[9] Both of them were laborious. For large-scale synthesis of steroid *H*-phosphonates and further to study the bioactivity of their derivatives, we developed a one-pot strategy.

Diphenyl phosphite (DPP) **2**, a commercially available and inexpensive phosphorylation reagent, undergoes fast transesterification with various alcohols in pyridine to yield mixtures of double-exchange and mono-exchange *H*-phosphonates.[10] Owing to lack of selectivity, it has limited scope in synthesis of asymmetry *H*-phosphonate. Nevertheless, our previous research on the transesterification reaction between DPP and 3β-cholesterol had shown that mono-phenyl *H*-phosphonate transesterification product could be obtained in high selectivity. This experimental might be explained by the steric hindrance demand of the 3β-cholesterol. Inspired by this phenomenon, we intended to synthesize the *H*-phosphonates of steroids by a tandem transesterification reaction (Scheme 1). Herein, we report to synthesize various *H*-phosphonates of 3β-cholesterol, which serve as a model of steroids.

For the initial investigations into this approach, it was decided to synthesize *H*-phosphonate **5a**. Under an argon atmosphere, 3β-cholesterol (1 mmol) in 2 mL pyridine was added to DPP (1 mmol) in 3 mL pyridine with stirring for 0.5 h, the mono-phenyl cholesterol phosphite was obtained in high selectivity (90% selectivity judged from ^{31}P NMR). And then hexadecanol (1 mmol) was added. After 3 h, the target *H*-phosphonate **5a** was obtained in 81% yield.[11]

Thereby, the assumed method could be valuable for synth-



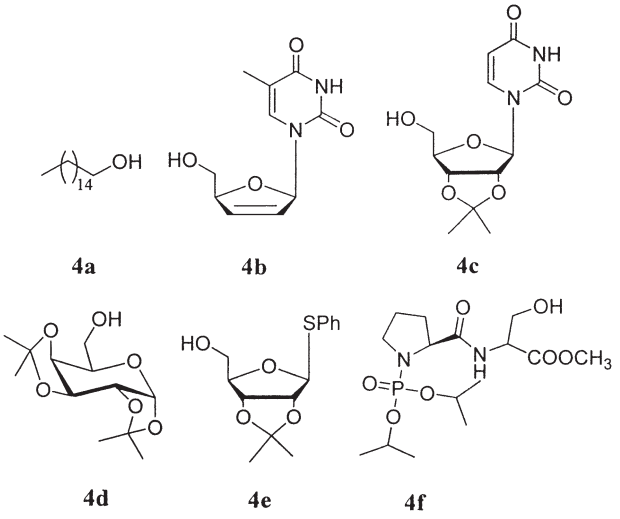
Scheme 1. Synthesis of 3β-cholesterol *H*-phosphonate by transesterification.

esis of these kinds of compounds. Using the same procedure, two *H*-phosphonate **5b** and **5c** conjugates of nucleosides **4b** and **4c** (Scheme 2) were synthesized in good yields (Table 1). In these kinds of analogues, steroids can be chosen for their lipophilic character and their lymphocyte membrane affinity[12] and its ability to reduce CD4 receptor activity at the cell surface. Hence, they could increase the bioactivity of the nucleosides.[13]

Using the transesterification approach, the steroid *H*-phosphonates **5d** and **5e** conjugated with carbohydrate **4d** and **4e** (Scheme 2) were also synthesized in reasonable yields (Table 1). The carbohydrate moiety can modify the physicochemical properties of these lipophilic steroids. It can not only increase the water solubility of steroid drugs but also permit a targeting of these drugs to a specific organ.[4]

In order to further confirm the scope of this methodology, phosphoryl dipeptide **4f** (Scheme 2) was chosen to synthesize the *H*-phosphonate conjugates with peptides. The corresponding *H*-phosphonate was obtained in 70% yield (Table 1).

In conclusion, the one-pot transesterification method can be used to synthesize various *H*-phosphonate conjugates of 3β-cholesterol. The extension of the approach should prove to be an economic method to develop various phosphate conjugates of steroids. Further efforts are in progress.



Scheme 2. The alcohol moieties used in transesterification reaction.

Table 1. *H*-phosphonates of 3β-cholesterol prepared using tandem transesterification reaction

Entry	Compds.	^{31}P NMR[a]		Yield
		δ ppm	$^{1}J_{P\text{-}H}$[b]	
1	**5a**	6.61	692	81%
2	**5b**	7.33, 6.93	700	83%
3	**5c**	7.46, 7.07	707	85%
4	**5d**	7.73, 7.01	703	75%
5	**5e**	7.63, 7.03	712	73%
6	**5f**	5.17, 7.48, 7.12	702	70%

[a]The values were determined in $CDCl_3$ using a Bruker ACP 200 at 81 MHz (85% H_3PO_4 as internal standard). [b]In Hz.

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11 Spectra data of the *H*-phosphonates **5a–f**: Compound **5a**: 6.84 (d, 1H, $^{1}J_{P\text{-}H} = 693$ Hz, P-H), 5.38 (s, 1H, H-6), 4.28 (br, 1H, H-3), 4.05 (t, 2H, $J = 7.5$ Hz, $OCH_2(CH_2)_{14}CH_3$), 2.43 (m, 2H, H-7), 0.67 (s, 3H, CH_3-18); ESI-MS (+): 697 $(M+Na)^+$; Compound **5b**: 8.84 (br, 1H, NH), 7.34, 7.29* (d, 1H, $J = 1$ Hz, H-6′), 6.90, 6.87* (1H, d, $^{1}J_{P\text{-}H} = 700$ Hz, P-H), 7.04 (m, 1H, H-1″), 6.34 (m, 1H, H-2″), 5.94 (m, 1H, H-3″), 5.38 (m, 1H, H-6), 5.01 (br, 1H, H-4″), 4.30 (m, 3H, H-5″, H-3), 2.41 (m, 2H, H-7), 0.67 (s, 3H, CH_3-18); ESI-MS (+): 679 $(M+Na)^+$; Compound **5c**: 9.35 (br, 1H, NH, H-3′), 7.35, 7.33* (d, 1H, $J = 8.5$ Hz, H-6′), 6.90, 6.89* (d, 1H, $J_{P\text{-}H} = 706$ Hz, P-H), 5.74 (d, 1H, $J = 8.5$ Hz, H-5′), 5.72 (d, 1H, $J = 2.5$ Hz, H-1″), 5.38 (m, 1H, H-6), 4.97 (m, 1H, H-2″), 4.87 (m, 1H, H-3"), 4.26–4.36 (m, 4H, H-5″, H-4″, H-3), 2.43 (m, 2H, H-7), 0.67 (s, 3H, CH_3-18); ESI-MS (+): 639 $(M+Na)^+$; Compound **5d**: 6.86, 6.84* (d, 1H, $J_{P\text{-}H} = 704$ Hz, P-H), 5.47, 5.46* (d, 1H, $J = 3.75$ Hz, H-1′), 5.31 (d, 1H, $J = 1.5$ Hz, H-6), 4.56 (m, 1H, H-3′), 4.26 (m, 1H, H-2′), 4.15–4.23 (m, 4H, H-6′, H-4′,H-3), 3.99 (m, 1H, H-5′), 2.38 (m, 2H, H-7), 0.67 (s, 3H, CH_3-18); ESI-MS (+): 715 $(M+Na)^+$; Compound **5e**: 7.24-7.21 (m, 5H, H-Ar.), 6.94, 6.88* (d, 1H, $J_{P\text{-}H} = 710$ Hz, P-H), 5.57, 5.54* (d, 1H, $J = 2.0$ Hz, H-1′), 5.36 (br, 1H, H-6), 4.74 (m, 2H, H-2′, H-3′), 4.19–4.40 (m, 4H, H-5′, H-4′, H-3), 2.41 (m, 2H, H-7), 0.67 (s, 3H, CH_3-18); ESI-MS (+): 737 $(M+Na)^+$; Compound **5f**: 6.90, 6.85* (d, 1H, $J_{P\text{-}H} = 703$ Hz, P-H), 5.37 (br, 1H, H-6), 4.57 (m, 1H, $(CH_3)_3CHO$), 4.86 (m, 1H, $(CH_3)_3CHO$), 4.24 (m, 1H, H-3), 3.90–4.10 (m, 4H), 3.68 (s, 3H, OCH_3), 2.38 (m, 2H, H-7), 0.67 (s, 3H, CH_3-18); ESI-MS (+): 835 $(M+Na)^+$.
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