Synthesis of Novel Dimeric Steroidal-nucleoside Phosphoramidates

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(Received April 12, 2005; CL-050489)

Some dimeric steroidal conjugated with 3′-azido-3′-deoxythymidine (AZT) as potential membrane-soluble prodrugs of the phosphate forms were synthesized in good yields and the structures were confirmed on the basis of ^{1}H, ^{13}C, and ^{31}P NMR and ESI-MS.

3′-Azido-3′-deoxythymidine (AZT, Zidovudine, Retrovir) was the first chemical agent approved by the Food and Drug Administration (FDA) for the treatment of AIDS.[1] It can be converted into corresponding 5′-*O*-triphosphate, and it then may inhibit the replication of the virus by competitive inhibition of the viral reverse transcriptase (RT) and/or by incorporation and subsequent chain termination of the growing viral DNA strand.[2] However, there are some problems associated with AZT chemotherapy, such as bone marrow toxicity and suppression, low therapeutic index, low localization in brain and a short half time in blood.[3] Moreover, in many cases, the nucleoside analogues are poor substrates for cellular kinases.[4] In order to reduce the toxicity and increase the anti-HIV activity, a lot of works have been reported to develop the more efficacious and more selective derivatives of AZT.[5] The expected advantage of the AZT prodrugs can be many including, synergistic drug interactions, enhancement of AZT intracellular uptake, increasing of AZT brain delivery, bypass the first AZT phosphorylation step into the cells and a decreasing toxicity.[6]

Steroid phosphates are important biological molecules.[7] It was reported that some steroid phosphates 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.[8] Letsinger et al. have synthesized a series of 3′ and 5′ cholesteryl modified phosphodiester and phosphorothioate oligonucleotides,[9] and found that the striking feature of these compounds is the high reactivity; they exhibit as inhibitors of replication of HIV in tissue culture.[10] In the course of studies on biologically active steroids, Luu et al. prepared phosphodiesters between different steroids and monosaccharides in order to increase their water solubility to permit a targeting of the drug to a specific organ.[11–13]

Inspired by enhancing steroids lipophilicity, membrane penetration, lymphocyte membrane affinity and the ability of binding to low density lipoprotein,[9,10] we synthesized some steroidal *H*-phosphonates.[14,15]

Some polyamine analogues, with impressive in vitro antitumor effects,[16,17] were designed to enter the cell by the polyamine transports system,[18] and then disrupt the biosynthesis and metabolic interconversion of cellular polyamines. Differentially substituted symmetrical amine moieties, particularly piperazines, are found as either a key pharmacophoric element or an important structural scaffold in a large number of drugs and drug candidates that encompass a wide range of biochemical targets across all therapeutic areas.[19] As our continuous study of novel conjugates to search for bioactive prodrugs,[14,15,20,21,23] some novel steroidal bivalent ligand-based phosphoramidates conjugates with AZT were synthesized in a convenient way reported in this paper.

The target phosphoramidates **2** were obtained in high yield (Table 1) by the Atherton–Todd reaction[22] of *H*-phosphonate **1** with the corresponding amino acid methyl esters (Scheme 1). It is worth mentioning that in compound **2**, the linkage of bivalent ligand-based phosphoramidates is lysine methyl ester, and is also an analogue of nucleotide kinase inhibitor.[24,25] Lysine methyl ester derivative **2a** was obtained in the reaction of **1a** with L-lysine methyl ester dihydrochlorides (L-LysOMe 2HCl) in the presence of Et_3N and CCl_4 (Scheme 1). The end of the reaction was easy to follow by the observation of the disappear-

Table 1. ^{31}P NMR, ESI-MS data of the Synthesized Compounds

Compounds	^{31}P NMR	ESI-MS $[M + Na]^+$
2a	8.84, 8.71, 6.91, 6.75	1382
2b	8.87, 8.81, 6.92, 6.89	1579
2c	9.91, 9.05, 7.23, 7.05	1635
3a	8.95, 9.74	1282
3b	9.29, 9.93	1479
3c	8.98, 8.58	1507
4a	9.30, 9.82	1310
4b	9.07, 9.56	1534
4c	8.97, 8.43	1562

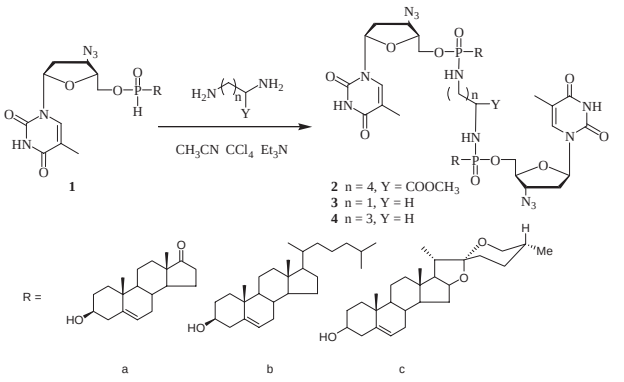

Figure 1. Synthetic route of steroidal phosphoramidate derivatives of **2a–2c**, **3a–3c**, and **4a–4c**.

ance of the ^{1}J coupling constant (710 Hz) of **1a** in the ^{31}P NMR spectrum (coupled mode). Because of the chirality at phosphorus of compound **2a**, they were mixtures of four diastereoisomers with ^{31}P NMR shifts at 8.84, 8.71, 6.91, and 6.75 ppm. Similar diasiastereoisomeric splittings were also observed in the H-decoupled ^{13}C and ^{1}H NMR spectrum. The structures of steroidal phosphoramidate conjugates with AZT were confirmed by the NMR, ESI-MS, and HRMS.[26]

In conclusion, the above protocol for the preparation of dimeric steroidal phosphoramidates represents a new, efficient, and general entry to this class of compounds. It makes use of readily available starting materials, involves mild and efficient chemical transformations. The extension of this methodology to other steroidal phosphoramidates can prove to be very useful for the development of phosphoramidate prodrugs of nucleosides, such as, d4T and ddI. This methodology can also be used to synthesis of phosphoramidates of carbohydrate and other biological molecules.

This work was supported by the NSF of China (No. 20172033) and the SRF for ROCS (Education Ministry of China).

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26 **2a**: As above the method, the corresponding *H*-phosphonate reacted with L-lysine to yield **2a** in 78% yield. ESI MS (+): m/z 1359 $[M + H]^+$, 1382 $[M + Na]^+$. HRMS (ESI) found: 1359.6310, $[C_{65}H_{92}N_{12}O_{16}P_2 + H]^+$ calcd: 1359.6304. ^{31}P NMR (121 MHz, δ): 8.84, 8.71, 6.91, and 6.75. ^{1}H NMR (300 MHz, $CDCl_3$, δ): 9.96 (br, 2H, H-3), 7.46–7.22 (m, 2H, H-6), 6.14–5.94 (m, 2H, H-1′), 5.33 (br, 2H, H-6″), 4.48–4.28 (m, 1H, H-α), 4.24–3.7 (m, 8H, H-3″, H-3′, H-5′), 3.97 (br, 2H, H-4′), 3.66 (d, 3H, $J = 2.07$ Hz, OCH_3), 2.89 (br, 2H, NH), 2.49–2.27 (m, 5H, H-2′, H-7″, H-β), 1.86 (s, 6H, 5-CH_3), 0.96 (s, 6H, CH_3-19), 0.81 (s, 6H, CH_3-18). ^{13}C NMR (150 MHz, $CDCl_3$, δ): 221.08 (C-17″), 173.71 ($COOCH_3$), 164.09 (C-4), 150.58 (C-2), 139.60, 139.46* (C-5″), 136.47, 136.27* (C-6), 122.53, 122.41 (C-6″), 111.35, 111.16* (C-5), 86.20, 86.14* (C-4′), 82.69, 82.64* (C-1′), 77.81, 77.69* (C-3″), 65.52, 65.34* (C-5′), 60.72, 60.59* (C-3′), 53.54 (C-α), 52.57 (OCH_3), 51.69 (C-14″), 50.09 (C-9″), 47.57 (C-13″), 41.16, 41.05* (C-ε), 40.11, 40.02* (C-4″), 37.01, 36.99* (C-1″), 36.89 (C-2′), 36.61 (C-10″), 35.89 (C-16″), 34.16, 34.09* (C-β), 31.47, 31.43 (C-7″, C-8″), 31.25, 31.21* (C-δ), 30.81 (C-2″), 29.78 (C-12″), 22.43 (C-γ), 21.92 (C-15″), 20.39 (C-11″), 19.36 (C-19″), 13.61 (C-18″), 12.61, 12.59* (5-CH_3).

Published on the web (Advance View) June 4, 2005; DOI 10.1246/cl.2005.944