Synthesis of Novel 5′-Hydrogenphosphonothioate Derivatives of AZT, d4T and ddI

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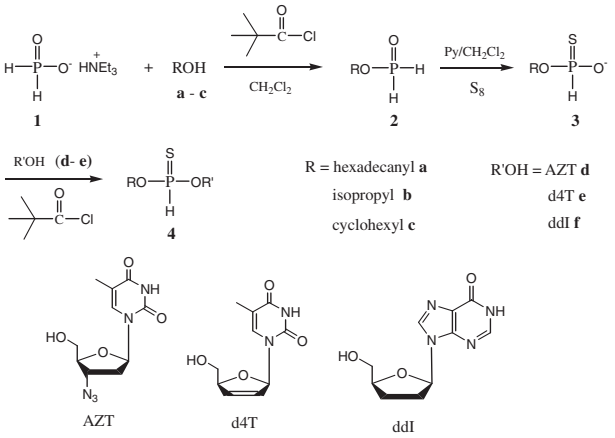
5′-hydrogenphosphonothioate derivatives of AZT, d4T, and ddI as anti-HIV prodrug candidates were synthesized in 61–76% yields under mild conditions through sequential one-pot reactions, i.e., couple of triethylammonium phosphinate with different alcohols in the presence of pivaloyl chloride (PV-Cl), following oxidation with elemental sulfur and further condensation with AZT, d4T, or ddI in the presence of PV-Cl.

3′-azido-3′-deoxythymidine (AZT) and other nucleoside analogs are antiviral drugs for treatment of AIDS.[1–8] The 5′-triphosphates (ddNTPs) may act as alternate substrates incorporated in the growing DNA chain, leading to termination of the newly synthesized viral nucleic acid.[9] However, in many cases the unnatural ddNs have poor affinity for nucleoside kinases,[10] and one possibility to improve the efficiency of ddNs could be to bypass the phosphorylation steps. Unfortunately, these polar nucleotides are not able to cross the cell membrane efficiently.[11] Hence, the strategies of temporarily masking the phosphate negative charges of nucleoside 5′-monophosphates (NMPs) with neutral substituents to prodrugs are used. Selectivity indexes of AZT 5′-cyclohexylphosphite and d4T 5′-isopropylphosphite are 19- and 6-fold more active when compared to these parent nucleosides, AZT and d4T, respectively.[12] Usually various phosphates modified by sulfur atom replacing the non-bridging oxygen atom show similar functions and certain specificity such as resistance to nuclease. In this paper, we synthesized a new kind of 5′-hydrogenphosphonothioate derivatives of AZT/d4T/ddI to search for more efficient compounds with low toxicity and high anti-HIV activity.

Stawinski et al. developed several convenient and efficient methods to H-phosphonothioates,[13–15] and this provided important references for preparation of the target products. 5′-hydrogenphosphonothioate derivatives of AZT, d4T, and ddI were synthesized as shown in Scheme 1. We explored the experimental conditions including solvents, sequence of added nucleoside and alcohol in the two condensations and amount of sulfur and pivaloyl chloride.

The optimum experimental conditions have been developed.[16] CH_2Cl_2 was chosen as solvent in the first coupling reaction. To a dichloromethane solution of triethylammonium phosphinate and one equivalent of alcohol, 1.5 equiv. of pivaloyl chloride was added at room temperature under nitrogen atmosphere. The reaction quantitatively yielded **2** within 10 min, i.e. alcohol was used up. Pyridine of twice volume relative to CH_2Cl_2 was added to the resulting solution, and then an equiv. of sulfur was added. **2** was quantitatively transferred to **3** in 30 min. Addition of pyridine aimed at improving dissolving power of sulfur, which could increase reaction efficiency. Finally, nucleoside and PV-Cl were added to the resulting solution,



Scheme 1. Synthetic route of 5′-hydrogenphosphonothioate derivatives.

and 10 min later the ^{31}P NMR showed that yield of target product **4** was more than 90%. Loss of yields was mainly caused by separation on silica gel column chromatography. The addition of an equiv of sulfur above instead of excess of sulfur in Ref. 15 aimed at avoiding further oxidation of **4**. So the three-step one-pot synthesis was completed smoothly. Table 1 shows the ^{31}P NMR, ESI–MS data and yields of compounds **4ad**–**4cf**.

In conclusion, new 5′-hydrogenphosphonothioate derivatives of AZT, d4T, and ddI were prepared in high yields under the mild reaction conditions, and the convenient and efficient one-pot method can generally be used to synthesis of other 5′-hydrogenphosphonothioate derivatives. Study on 5′-hydrogenphosphonothioate derivatives of AZT, d4T, and ddI is in progress.

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Table 1. The ^{31}P NMR, ESI–MS data and yields of **4ad**–**4cf**

NO.	^{31}P NMR /ppm	ESI–MS: $[M + Na]^+$ m/z	Yield
4ad	71.89, 72.29	594.4	73%
4ae	71.74, 72.09	551.3	65%
4af	71.85, 72.16	563.2	61%
4bd	69.56, 69.92	411.8	76%
4be	69.31, 69.77	368.7	67%
4bf	69.92, 69.51	380.8	61%
4cd	69.39, 69.77	451.9	74%
4ce	69.09, 69.52	408.8	71%
4cf	69.26, 69.54	421.9	67%

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16 General synthetic procedure: To triethylammonium phosphinate (**1**, 167 mg, 1 mmol) and ROH (1 mmol) in dry CH_2Cl_2 (2 mL) was added dropwise pivaloyl chloride (180 mg, 1.5 mmol) at room temperature under nitrogen atmosphere. About 10 min later, pyridine (4 mL) and sulfur (32 mg, 1 mmol) were sequentially added to the resulting solution. The oxidation with elemental sulfur completed within 30 min, and ^{31}P NMR showed almost quantitative formation of alkyl hydrogenphosphonothioate (**3a–c**). Without isolation, nucleoside (1 mmol, AZT, d4T, or ddI) and PV-Cl (180 mg, 1.5 mmol) were sequentially added to the above solution at room temperature, and the second condensation was finished in 10 min. The solvents were removed by rotary evaporation, and the residue was subjected to column chromatography (silica gel) to give target product **4**. For example *O*-hexadecanyl-5′-AZT-H-phosphonothioate (**4ad**), eluent: ethyl acetate: petroleum ether (20:15); Colorless oil; Yield: 73%. ^{31}P NMR ($CDCl_3$, 81 MHz): δ 71.89, 72.29; ^{1}H NMR ($CDCl_3$, 500 MHz): δ 9.60 (br, 1H, 3-H), 7.85, 7.83 (dd, 1H, $^1J_{P\text{-}H} = 654$ Hz, P-H), 7.37 (s, 1H, 6-H), 6.22 (m, 1H, 1'-H), 4.41–4.16 (m, 4H, 3'-H, 4'-H, 5'-CH_2), 4.10–4.06 (m, 2H, O$CH_2(CH_2)_{14}CH_3$), 2.50–2.33 (m, 2H, 2′-H), 1.95 (s, 1H, 5-CH_3), 1.70–1.66 (m, 2H, OCH_2CH_2-$(CH_2)_{13}CH_3$), 1.35–1.25 (br, 26H, $OCH_2CH_2(CH_2)_{13}CH_3$), 0.88 (t, 3H, $OCH_2CH_2(CH_2)_{13}CH_3$); ^{13}C NMR ($CDCl_3$, 125 MHz): δ 163.85 (C-4), 150.29 (C-2), 135.11 (C-6), 111.51 (C-5), 84.51 (C-1′), 82.19 (C-4′), 66.98 (C-5′), 64.37 ($OCH_2(CH_2)_{14}CH_3$), 60.13 (C-3′), 37.46 (C-2′), 31.83($OCH_2CH_2(CH_2)_{13}CH_3$), 30.19, 30.18–29.03, 25.44, 22.60 ($OCH_2CH_2(CH_2)_{13}CH_3$), 14.03 (5-CH_3), 12.48 ($OCH_2CH_2(CH_2)_{13}CH_3$); Positive ion ESI–MS: $[M + Na]^+$ m/z 594.4.

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