Pergamon

Tetrahedron Letters 43 (2002) 5281–5283

TETRAHEDRON LETTERS

Novel approach to the synthesis of AZT 5′-*O*-hydrogen phospholipids

Qiang Xiao,[a] Jing Sun,[b] Yong Ju,[a,*] Yu-fen Zhao[a,*] and Yu-xin Cui[b]

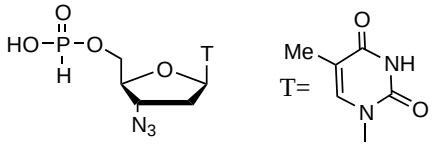
[a]*The Key Laboratory of Bioorganic Phosphorus Chemistry, Ministry of Education, Department of Chemistry, School of Life Sciences and Engineering, Tsinghua University, Beijing 100084, PR China*

[b]*National Research Laboratory of Natural and Biomimetic Drugs, School of Pharmaceutical Sciences, Peking University, Beijing 100083, PR China*

Received 28 March 2002; revised 15 May 2002; accepted 31 May 2002

Abstract—A series of AZT 5′-*O*-hydrogen phospholipids were synthesized by a tandem transesterification of diphenyl phosphite with AZT and a long-chain alcohol. The method possesses the merits of ease of operation and high yields. It can also be extended to synthesize other biological phospholipids.

The development of effective antiviral therapy for the treatment of individuals infected with human immunodeficiency virus (HIV) presents a unique challenge. Nucleosides and nucleotides have demonstrated widespread utility as antiviral and anticancer therapeutics.[1] One of them is 3′-azido-2′, 3′-dideoxythymidine (AZT), which was the first clinically approved drug against HIV infection, despite its undesirable side effects, such as bone marrow suppression, myopathy and hepatic abnormalities.[2] In order to reduce its toxicity and increase the anti-HIV activity, a lot of work has been reported aimed at developing 5′-*O*-ester prodrugs of AZT.[3] The 5′-hydrogen phosphate of AZT (PZT, Scheme 1), which is much less toxic than AZT (CC_{50} values of 2.5 mM and 210 μM for PZT and AZT, respectively), is one of the most significant compounds. The overall selectivity (SI) for PZT (CC_{50}/IC_{50}) is superior to that of AZT,[4] and now PZT is currently in Phase I clinical trials.[5] PZT, unlike highly acidic nucleoside 5′-phosphonates, which cannot enter the cell, may penetrate the cell membrane due to its weakly acidic undissociated nature. Encouraged by the results with PZT, 5′-*O*-hydrogen phospholipids were synthesized in a convenient manner in order to: (1) increase transmembrane transport characteristics; (2) determine their antiviral effects and (3) further investigate the intracellular metabolism of 5′-*O*-hydrogen phosphonates, which may be different from that of AZT.[6]



PZT

Scheme 1.

Two methods can be used to synthesize AZT 5′-*O*-hydrogen phospholipids. The first uses the phosphoramidate strategy, which has been extensively developed for the synthesis of oglionucleotides.[7] Using this methodology, phosphoramidate diesters of phosphites were synthesized and then hydrolyzed under the activation of 1*H*-tetrazole in the presence of water. The second is the *H*-phosphonate method.[8] In this method, it is necessary to prepare *H*-monophosphonate and then couple to an alcohol in the presence of a condensing reagent. Both of these methods suffer from the disadvantage of laborious synthetic procedures and chromatographic purification. In order to obtain multigram quantities of AZT 5′-*O*-hydrogen phospholipids, we describe, herein, a mild and one-pot synthesis.

Diphenyl phosphite (DPP) is a commercially available, inexpensive phosphorylation reagent, which undergoes fast transesterification with alcohols in pyridine to afford mixtures of the corresponding dialkyl and alkyl

Keywords: phosphorylation; transesterification; *H*-phosphonate; AZT; phospholipid.

* Corresponding authors. Tel.: +86-10-62772259; fax: +86-10-62781695; e-mail: juyong@tsinghua.edu.cn; yfzhao@tsinghua.edu.cn

0040-4039/02/$ - see front matter
PII: S0040-4039(02)01045-6

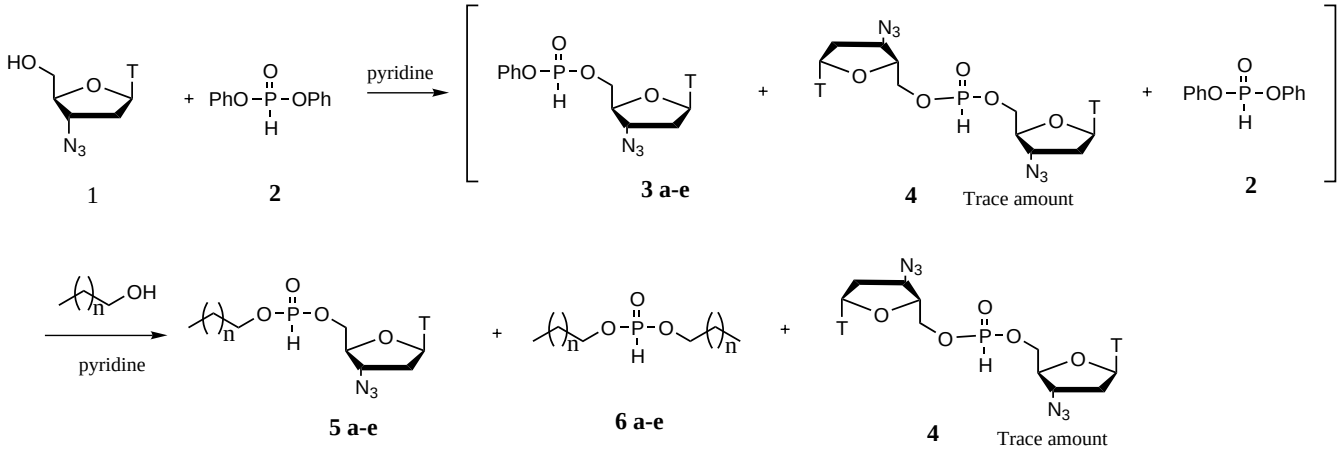
phenyl *H*-phosphonates.[9] In order to obtain monophenyl *H*-phosphonate only, a large excess of DPP was needed to obviate the formation of dialkyl *H*-phosphonates.[10] Later research showed that the amount of DPP can be greatly reduced if DPP was added to the desired monohydroxylic nucleoside very slowly.[11] Inspired by these results, we intended to synthesize the target phospholipids with a tandem transesterification reaction.

Phospholipid **5d** was selected to check the viability of this method. Under an argon atmosphere, a solution of AZT (1 mmol) in 10 ml dry pyridine was added to a stirred solution of DPP (2 mmol) in 10 ml dry pyridine over 1 hour. After 15 min, the ^{31}P NMR spectrum showed four new signals other than that for DPP (δ_P ca. 1.7 ppm). Two main peaks at 6.75 and 5.98 ppm are the signals of a pair of diastereoisomers of phenyl *H*-phosphonate **3d** due to the chirality of AZT. The small peak at 14.48 ppm is the symmetric *H*-phosphonate **4**. Then, hexadecanol (3 mmol) was added in one portion. After 1 hour, the ^{31}P NMR showed that the signals at 6.75 and 5.98 ppm had disappeared and a further three new signals had appeared. The signals at 9.83 and 9.11 ppm correspond to the target phospholipid **5d** and the signal at 8.65 ppm is the signal due to symmetric *H*-phosphonate **6d**, which was identified by comparison with an authentic sample (Scheme 2). After purification, the phospholipid **5d** was obtained in 87% yield[12] based on the quantity of AZT. Using the same method, the other phospholipids were also synthesized in good yields (Table 1). It is noteworthy to mention that these 5′-*O*-hydrogen phospholipids are also important reaction intermediates: (1) they can be used to synthesize other phosphoramidate prodrugs of AZT by the Todd reaction[13], and (2) they can be oxidized by iodine, sulfur or selenium to obtain phosphonate, phosphorothioate and phosphoroselenoate derivatives of AZT respectively.[14]

In summary, AZT 5′-*O*-hydrogen phospholipids were synthesized in an easy one-pot reaction and high yields. The extension of this methodology to other hydrogen phospholipids should prove to be very useful for the development of phospholipid prodrugs of nucleosides such as d4T and ddI. This methodology can also be used to synthesize phospholipids of carbohydrate and other biological molecules. Further efforts in this direction and the assessment of biological activities against HIV are in progress.

Acknowledgements

The authors would like to acknowledge financial support from the National Natural Science Foundation of China (No. 20132020, No. 20175026, No. 29672022 and No. 20172033), the Ministry of Science and Technol-



Scheme 2.

Table 1. Phospholipids **5a**–**e** prepared using the tandem transesterification reaction

Compds.	*n*	^{31}P NMR[a]		ESI MS			Yield (%)[c]
		^{31}P NMR (δ ppm)	$^{1}J_{P-H}$[b]	$(M+H)^+$	$(M+Na)^+$	$(M+K)^+$	
5a	8	9.19, 8.62	696	472	494	510	90
5b	10	9.20, 8.63	697	500	522	538	85
5c	12	9.28, 8.76	695	528	550	566	85
5d	14	9.28, 8.69	698	556	578	594	87
5e	16	9.39, 8.84	697	584	606	622	84

[a] The values were determined in $CDCl_3$ using a Bruker AMP 200 at 81 Hz (85% H_3PO_4 as internal standard).
[b] In Hz.
[c] Based on the reactant AZT.

ogy, the Chinese Ministry of Education and Tsinghua University.

References

1. Jones, R. J.; Bischofberger, N. *Antivir. Res.* **1995**, *27*, 1.
2. Moore, K. H. P.; Raasch, Rh.; Brouwer, K. L. R.; Opheim, K.; Cheeseman, S. H.; Eyster, E.; Lemon, S. M.; Vanderhorst, C. M. *Antimicrob. Agents Chemother.* **1995**, *39*, 2732.
3. Parang, K.; Wiebe, L. I.; Knaus, E. E. *Curr. Med. Chem.* **2000**, *7*, 995.
4. (a) Machado, J.; Salomon, H.; Oliveira, M. *Nucleos. Nucleot.* **1999**, *18*, 901; (b) Tarussova, N. B.; Kukhanova, M. K.; Krayevsky, A. A.; Karamov, E. K.; Lukashov, V. V.; Kornilayeva, G. B.; Rodina, M. A.; Galegov, G. A. *Nucleos. Nucleot.* **1991**, *10*, 351.
5. Yurin, O.; Kravtchenko, A.; Serebrovskaya, L.; Golochvastova, E.; Burova, N.; Voronin, E.; Pokrovsky, V. *AIDS* **1998**, *12*, 240.
6. Boal, J. H.; Iyer, R. P.; Egan, W. *Nucleos. Nucleot.* **1993**, *12*, 1075.
7. Beaucage, S. L.; Iyer, R. P. *Tetrahedron* **1993**, *49*, 6123.
8. Stawinski, J. In *Handbook of Organophosphorus Chemistry*; Engel, R., Ed.; Marcel Dekker: New York, 1992; pp. 377–434.
9. Kers, A.; Kers, I.; Stawinski, J.; Sobkowski, M.; Kraszewski, A. *Synthesis* **1995**, 427.
10. Jankowska, J.; Sobkowski, M.; Stawinski, J.; Kraszewski, A. *Tetrahedron Lett.* **1994**, *35*, 3355.
11. Iyer, V. V.; Griesgraber, G. W.; Radmer, M. R.; McIntee, E. J.; Wagner, C. R. *J. Med. Chem.* **1999**, *42*, 5188.
12. Characteristics are given for a representative compound **5d**: colorless oil; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 9.47 (br, 1H, H-3), 6.86, 6.85* (d, 1H, $^{1}J_{P-H}=705$ Hz, P-H), 7.32, 7.29* (s, 1H, H-6), 6.14, 6.13* (t, 1H, $J_{1',2'}=6.3$ Hz, H-1′), 3.97–4.33 (m, 6H, CH_2-5′, H-4′, H-3′, $HOCH_2(CH_2)_{14}CH_3$), 2.34 (m, 2H, H-2′), 1.87 (s, 3H, CH_3-5), 1.64 (m, 2H, $HOCH_2CH_2(CH_2)_{13}CH_3$), 1.14–1.18 (br, 26H, $H\text{-}OCH_2CH_2(CH_2)_{13}CH_3$), 0.81 (t, 3H, $J=6.6$ Hz, $HOCH_2CH_2(CH_2)_{13}CH_3$); ^{13}C NMR (75 MHz): 169.66 (C-4), 150.47, 150.30* (C-2), 135.47, 135.37* (C-6), 111.49, 111.43* (C-5), 84.88 (C-4′), 82.17, 82.08* (C-1′), 66.71, 66.64* (C-5′, $^{2}J=3.1$ Hz), 64.22 (C-3′), 59.96 ($CH_3(CH_2)_{14}CH_2O$), 37.32, 37.27* (C-2′), 31.83 ($CH_3(CH_2)_{13}CH_2CH_2O$), 30.34, 30.26* (1C), 29.13–29.47 (br, 10C), 25.41, 25.37* (1C), 22.60 (1C), 14.03 ($CH_3(CH_2)_{14}CH_2O$), 12.37 (CH_3-5); ESI MS (+): m/z 556 $(M+H)^+$, 578 $(M+Na)^+$, 594 $(M+K)^+$; HRMS (ESI): found 556.3253, $C_{26}H_{47}N_5O_6P$ $(M+H)^+$ requires 556.3258.
13. Ji, C. J.; Xue, C. B.; Zeng, J. N.; Zhao, Y. F. *Synthesis* **1988**, 444.
14. Lindh, I.; Stawinski, J. *J. Org. Chem.* **1989**, *54*, 1338.