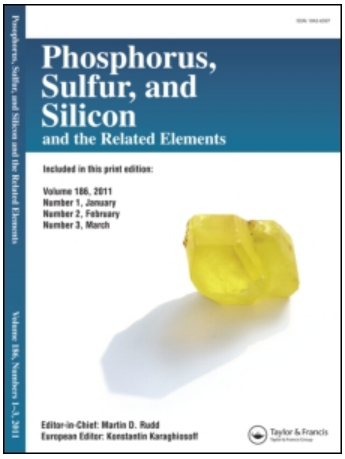


Phosphorus, Sulfur, and Silicon and the Related Elements
Publication details, including instructions for authors and subscription information:
http://www.informaworld.com/smpp/title~content=t713618290

Synthesis of Novel Steroidal Bioconjugates of Phospholipid with AZT

Pei-Yuan Jin[a]; Kai Liu[a]; Yong Ju[a]; Yu-Fen Zhao[a]
[a] Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Ministry of Education, Department of Chemistry, Tsinghua University, Beijing, P. R. China

To cite this Article Jin, Pei-Yuan , Liu, Kai , Ju, Yong and Zhao, Yu-Fen(2008) 'Synthesis of Novel Steroidal Bioconjugates of Phospholipid with AZT', Phosphorus, Sulfur, and Silicon and the Related Elements, 183: 2, 538 — 542
To link to this Article: DOI: 10.1080/10426500701764874
URL: http://dx.doi.org/10.1080/10426500701764874

Phosphorus, Sulfur, and Silicon, 183:538–542, 2008

ISSN: 1042-6507 print / 1563-5325 online
DOI: 10.1080/10426500701764874

Taylor & Francis
Taylor & Francis Group

Synthesis of Novel Steroidal Bioconjugates of Phospholipid with AZT

Pei-Yuan Jin, Kai Liu, Yong Ju, and Yu-Fen Zhao
Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Ministry of Education, Department of Chemistry, Tsinghua University, Beijing, P. R. China

Some novel asymmetry steroidal bioconjugates of phospholipid with AZT were synthesized by oxidation or conjugation with amines using Atherton–Todd reaction, and the structures were confirmed based on spectral methods. The result shown that some of bioconjugates possess activity against breast cancer MCF-7 in bioassay.

Keywords AZT; cytotoxic activity; phospholipids; steroidal bioconjugate

INTRODUCTION

Steroidal phosphates are important biological molecules. 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.[1] Some mono- and dimeric-steroidal conjugated with AZT as potential membrane-soluble prodrugs of the phosphate forms were synthesized in good yields.[2] 3′-Azido-3′-deoxythymidine (AZT, Zidovudine) is activated by phosphorylation in vivo and inhibits HIV replication by blocking a critical HIV enzyme.[3] The resistance of HIV-1 to AZT involves phosphorolytic excision of chain-terminating AZT-5′-monophosphate. Both pyrophosphate and ATP act as excision substrates in vitro, but the intracellular substrate used during replication of AZT-resistant HIV is still unknown.[4] The amount of azidothymidine-triphosphate (AZT-TP) was of the same order of magnitude as the intracellular concentrations of AZT-TP needed in blood cells for efficient treatment of

Project supported by the NSF of China (No. 20542004, 20772071) and SRFDP of Higher Education (No. 20050003104).

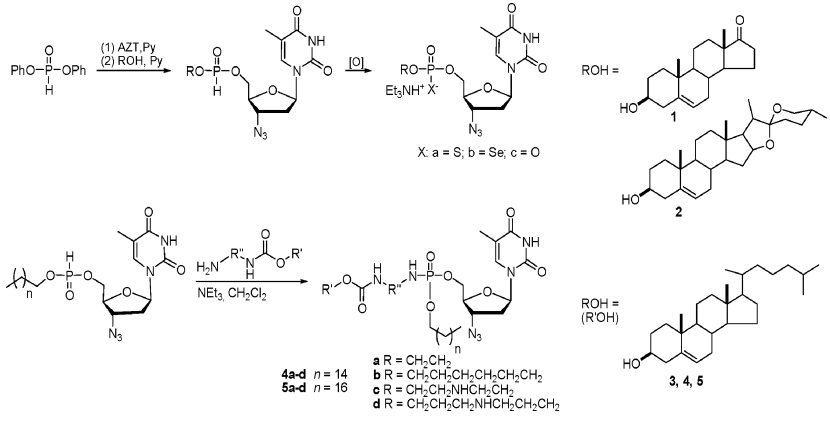
Address correspondence to Yong Ju, Key Laboratory of Bioorganic Phosphorus Chemistry & Chemical Biology, Ministry of Education, Department of Chemistry, Tsinghua University, Beijing 100084, P. R. China. E-mail: juyong@tsinghua.edu.cn

HIV-infected patients treated with AZT.[5] There are some major problems associated with AZT chemotherapy,[6] and many *H*-phosphonates of AZT have also been shown to possess high anti-HIV activity, such as PZT (5′-hydrogenphosphate of AZT).[7] These phospholipids were easily transformed to corresponding phosphates, phosphorothioates, and phosphoroselenoates or phosphoramidates by conjugated with amines.[8] The *H*-phosphonate can be oxidized by sulfur, selenium, or iodine to give out the phosphorothioate, phosphoroselenoate, and phosphate of carbohydrate respectively.[9] Nucleoside phosphoroselenoates and phosphorothioselenoates were synthesized via stereospecific selenization of the corresponding *H*-phosphonate and *H*-phosphonothioate diesters with the aid of new selenium-transfer reagent, 3*H*-1,2-benzothiaselenol-3-one.[10] Shishkin reported on the Atherton-Todd oxidative phosphorylation of hydroxy imidates and C-phosphorylated imidates.[11]

In our previous work, some novel symmetric phosphate, phosphorothioate, and phosphoroselenoate derivatives of AZT and D4T were synthesized.[8] Herein, the synthesis of novel asymmetric steroidal steroidal derivatives of phospholipid conjugated with AZT are described in this article.

RESULTS AND DISCUSSION

The key asymmetric *H*-phosphonates were synthesized by the established transesterification method using diphenyl phosphonate (DPP). Then they can be oxidized or using Atherton-Todd reaction to afford the corresponding phosphate, phosphorothioate, phosphoroselenoate, and phosphoramidate (Scheme 1).



SCHEME 1 Synthesis of steroidal derivatives of phospholipid conjugated with AZT.

TABLE I ^{31}P NMR and Yields of the Prepared Phospholipids

	^{31}P NMR	Yield[a] (%)		^{31}P NMR	Yield (%)
1a	56.47	84	**4a**	10.17	90
1b	50.87	88	**4b**	10.29	85
1c	−0.32	90	**4c**	10.34	92
2a	56.15	89	**4d**	10.45	91
2b	50.06	75	**5a**	10.16	88
2c	−0.34	86	**5b**	10.19, 10.24	87
3a	57.27	85	**5c**	9.60, 9.87	89
3b	52.15	78	**5d**	10.41, 10.60	92
3c	−0.29	87			

[a]Based on the reactant AZT.

At first, steroid (1 mmol) was added to a stirred solution of DPP (1 mmol) at 0°C in 30 min and stirring was continued for 5 h. Then, AZT (1 mmol) was added in one portion and the resulting solution was stirred overnight. The solvent was removed by co-distillation with toluene under reduced pressure. The reaction residue was purified on a silica gel column to afford *H*-phosphonates as colorless oils. The obtained *H*-phosphonates were oxidized using sulfur (S_8), selenium, or I_2 in pyridine/H_2O (49:1) respectively. The oxidizing reaction with sulfur (S_8) and I_2 was fast and completed in 30 min, while the reaction with suspension selenium was slower and completed by stirring for 12 h. The targeted phosphorothioates, phosphoroselenoates, and phosphates were obtained as solid after purification (Table I).

Many synthesized phosphoramidate prodrugs of AZT showed high anti-HIV activity. In order to determine whether the activity can be increased when a lipid group was introduced, the phosphoramidates prodrugs were also synthesized using a modified Atherton–Todd reaction in reasonable yields. A solution of *H*-phosphonate (1 mmol) was dropwise added to the solution of the corresponding amines (1.2 mmol) at 0°C. The mixture was stirred at rt. for 10 min, and then it was concentrated in vacuum below 40°C. The residue was purified on a silica gel column to yield the corresponding phosphoramidate.

CONCLUSION

Some novel asymmetry steroidal bioconjugates of phospholipid with AZT were easily prepared in fine yield by oxidation or the Atherton-Todd reaction. The steroidal phosphorothioate, phosphoroselenoate,

phosphate, and phosphoramidate derivatives were tested against breast cancer MCF-7 in vitro by the standard MTT method. Additional work is in progress.

EXPERIMENTAL

Diphenyl phosphonate (DPP) was purchased from Aldrich Company. Pyridine was dried over calcium hydride and distilled prior to use. NMR spectra were recorded on a spectrometer JEOL JNM–ECA300 and chemical shifts (δ) are given in ppm. ^{31}P NMR spectra were with 85% phosphoric acid ($\delta = 0.0$) as external standard. ^{1}H NMR spectra were with TMS as internal standard and coupling constants (J) are given in Hz. ESI mass spectra were acquired using a Bruker Esquire-LC ion trap mass spectrometer operated in positive ion mode or negative ion mode.

General Methods

Under argon atmosphere, the corresponding dried steroid (1 mmol) was added to a stirred solution of DPP (1 mmol) in 5 mL dry pyridine at 0°C, then AZT (267 mg, 1 mmol) was added in one portion after 5 h, and the resulting solution was stirred overnight. The solvent was removed by co-distillation with toluene under reduced pressure. After purification on a silica gel column (CH_2Cl_2:$CH_3OH = 40:1$), the *H*-phosphonates was obtained as colorless oils. Then sulfur or iodine (0.4 mmol) in pyridine/H_2O (49:1) (0.4 mmol) was added to the *H*-phosphonates (0.2 mmol) in pyridine/dioxane (1:1, 4 mL). The mixture was stirred for an additional 30 min. the reaction was stirred overnight after adding selenium (0.4 mmol) as using selenium. Triethylamine (1 mL) was added and the mixture was stirred for an additional 30 min when the reactions were over. The pyridine and excess triethylamine were removed by co-distillation with toluene under reduced pressure. The obtained residue was purified by silica gel chromatography (CH_2Cl_2:CH_3OH:$Et_3N = 200:5:1$) to give the target phosphorothioates, phosphoroselenoates, and phosphates. A solution of *H*-phosphonate (1 mmol) in CH_2Cl_2 (2 mL) was dropwise added to the solution of the corresponding amines (1.2 mmol) in Et_3N (0.5 mL) —CCl_4 (0.5 mL) —H_2O (0.5 mL) —MeCN (5 mL) at 0°C. The reaction mixture was stirred at rt. for 10 min and the solvent was concentrated in vacuum below 40°C. The residue was purified on a column (silica gel; CH_2Cl_2:$CH_3OH = 50:1$) to yield the corresponding phosphoramidate.

REFERENCES

[1] M. Sprecher, R. Breslow, R. Philosof-Oppenheimer, and E. Chavet, *Tetrahedron*, **55,** 5465 (1999).
[2] (a) S. H. Ji, Q. Xiao, Y. Ju, and Y. F. Zhao, *Chem. Lett.*, **34,** 944 (2005); (b) Q. Xiao, J. Sun, Y. Ju, Y. F. Zhao, and Y. X. Cui, *Chem. Lett*., **32,** 522 (2003).
[3] A. Yarnell, *Chem. Eng. News*, **83,** 48 (2005).
[4] S. Dharmasena, Z. Pongracz, E. Arnold, S. G. Sarafianos, and M. A. Parniak, *Biochemistry*, **46,** 828 (2007).
[5] H. Hillaireau, T. Le Doan, M. Appel, and P. Couvreur, *J. Controlled Release*, **116,** 346 (2006).
[6] K. Parang, L. I. Wiebe, and E. E. Knaus, *Curr. Med. Chem.*, **7,** 995 (2000).
[7] J. Machado, H. Salomon, M. Oliveira, C. Tsoukas, A. A. Krayevsky, and M. A. Wainberg, *Nucleosides Nucleotides*, **18,** 901 (1999).
[8] (a) C. J. Ji,; C. B. Xue, J. N. Zeng, and Y. F. Zhao, *Synthesis*, **6,** 444 (1988); (b) Q. Xiao, J. Sun, Q. Sun, Y. Ju, Y. F. Zhao, and Y. X. Cui, *Synthesis*, **1,** 107 (2003).
[9] I. Lindh and J. Stawinski, *J. Org. Chem.*, **54,** 1338 (1989).
[10] J. Stawinski and M. Thelin, *J. Org. Chem.*, **59,** 130 (1994).
[11] V. E. Shishkin, Y. M. Yukhno, S. V. Ufimtsev, and B. I. No, *Russ. J. Gen. Chem.*, **72,** 1820 (2002).