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Conjugation of amino acid *O*-methyl esters with AZT-5′-*O*-phosphorothioate and phosphorodithioate

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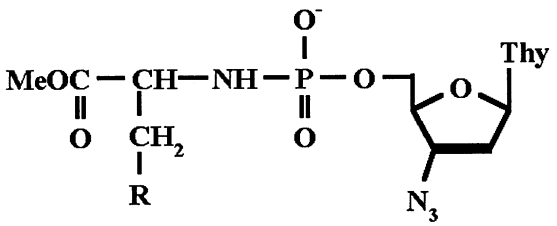
Abstract

Based upon 1,3,2-oxathia(-dithia)phospholane chemistry, 5′-*O*-derivatisation of AZT with the *O*-methyl esters of L-phenylalanine and L-tryptophan was performed and the corresponding 5′-aminoacidophosphoramidothioates or phosphoramidodithioates of AZT were obtained in satisfactory yield.

Keywords: AZT; *N*-phosphorothioylated amino acid; oxathia(-dithia)phospholane ring-opening condensation.

Since the discovery of the therapeutic efficacy of 3′-azido-3′-deoxythymidine (AZT) against human immunodeficiency virus (HIV),[1] extensive research towards AZT-derivatisation enhancing antiviral activity has been carried out in numerous laboratories.[2] It has been reported recently that 5′-*O*-conjugation of 3′-azido-3′-deoxythymidine (AZT) with *N*-phosphorylated aromatic amino acids provides the corresponding 3′-azido-3′-deoxythymidine-5′-aminoacidophosphoramidates (**1**) which exhibit many-fold higher activity against HIV-1 replication in PBMCs cells than the parent AZT.[3a] It has also been shown, that due to their negative charge, compounds **1** are considerably more water soluble and stable in human blood plasma. As an amino acid, L-tryptophan, was found to be the best choice: in vitro, compound **1b** was 8-fold more active when compared to free AZT, without any symptoms of toxicity.[3b]



1a: R = Phenyl
1b: R = 3-Indolyl

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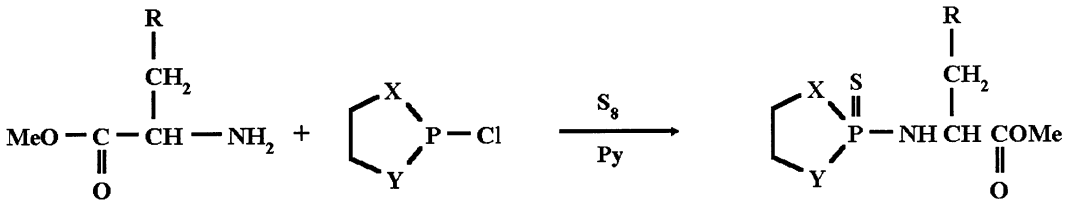
In these studies, all compounds of type **1** described were obtained using phosphoramidite methodology.[4] Their low toxicity and enhanced anti-HIV activity prompted us to search for more efficient synthetic routes providing compounds of type **1** and also their phosphorothio- or phosphorodithioate analogues. To reach this goal we adapted methodology that is based upon the oxathiaphospholane chemistry developed in this laboratory.[5]

Although that approach had been designed for the stereocontrolled synthesis of oligo(nucleoside phosphorothioate)s, it has been independently applied to the synthesis of N3′→P5′-dinucleotides[6] and *O*-alkyl-*N*-alkyl phosphoramidothioates.[7] The synthetic strategy presented in this communication consisted of two steps: (i) the simple derivatisation of the corresponding amino acid-*O*-methyl esters (**2**) either with 2-chloro-1,3,2-oxathiaphospholane (**3**) or 2-chloro-1,3,2-dithiaphospholane (**4**) (Scheme 1) and (ii) the reaction of resulting *N*-phosphorothioylated amino acid esters **5** or **6** with AZT (Scheme 2).

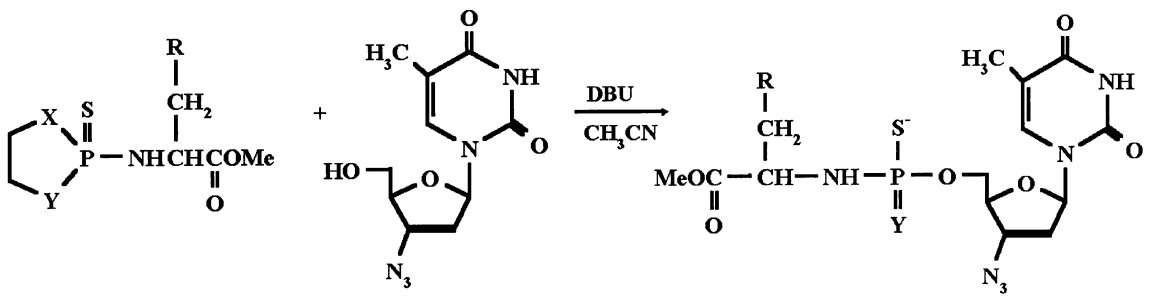


2a: R = Phenyl
2b: R = 3-Indolyl

3: X = S, Y = O
4: X = S, Y = S

5a: X = S, Y = O, R = Phenyl
5b: X = S, Y = O, R = 3-Indolyl
6a: X = S, Y = S, R = Phenyl
6b: X = S, Y = S, R = 3-Indolyl

Scheme 1.



5a: X = S, Y = O, R = Phenyl
5b: X = S, Y = O, R = 3-Indolyl
6a: X = S, Y = S, R = Phenyl
6b: X = S, Y = S, R = 3-Indolyl

7a: Y = O, R = Phenyl
7b: Y = O, R = 3-Indolyl
8a: Y = S, R = Phenyl
8b: Y = S, R = 3-Indolyl

Scheme 2.

Accordingly the *O*-methyl esters of L-phenylalanine (**2a**) or L-tryptophan (**2b**) were treated with an equimolar amount of 2-chloro-1,3,2-oxathia(dithia-)phospholane (**3** or **4**) in pyridine solution in the presence of elemental sulphur. These reactions were nearly quantitative and resulted in the formation of 2-*N*-(carbomethoxyaminoacido)-[2-thiono-1,3,2-oxathia(1,3,2-dithia)phospholanes] (**5** or **6**). Compounds **5**–**6** were isolated from the reaction mixture by silica

gel column chromatography[†] and characterised by ^{1}H NMR, ^{31}P NMR and FAB-MS analysis (Table 1).[‡] Condensation of **5** or **6** with an equimolar amount of AZT in the presence of DBU occurred smoothly providing compounds **7**–**8** in high yield.[§]

Table 1
Physicochemical characteristics of compounds **1** and **5**–**8**

Comp. no.	^{31}P NMR [ppm] Chemical shifts (H_3PO_4)	FAB-MS (M − 1) m/z	Yield [%]
5a	96.50, 95.59[a]	316	89
5b	96.54, 95.52[b]	355	75
6a	101.02[a]	332	84
6b	101.04[a]	371	72
7a	58.51, 58.24[b]	523	87
7b	58.50, 58.43[a]	562	92
8a	103.21[a]	539	95
8b	103.48[a]	578	93
1a	7.01[c]	507	80
1b	7.20[c]	546	82

[a] NMR spectra were determined in $CDCl_3$.
[b] NMR spectra were determined in CD_3CN.
[c] NMR spectra were determined in CD_3OD.

Due to the asymmetry of the phosphorus atom, compounds **7** consisted of diastereomeric mixtures and can be separated into diastereomerically pure species by means of HPLC. Compounds **8** are *P*-achiral. Their physicochemical characteristics are also presented in Table 1. Moreover, compounds **7** dissolved in a solution of OXONE® (potassium peroxymonosulfate, buffered solution, pH 6.5) underwent conversion into compounds **1**, described earlier, in high yield. It should be emphasised that the present methodology allowed us to obtain not only the known compounds **1**, but also a new class of phosphorothio- and phosphorodithio-analogues (compounds **7** and **8**). Since the chromatographic resolution of diastereomeric mixtures of compounds **7** is feasible, antiviral activity of the pure species and compounds **8** is under investigation. The synthetic strategy presented in this paper, in comparison with the previously described one based upon phosphoramidite methodology, is more efficient and does not require the use of an excess of amino acid.

[†] As an eluent chloroform:hexane (7:3) was used.
[‡] ^{1}H NMR data were in accordance with literature[2] and are not included in this communication.
[§] The synthesis of compound **7b**: compound **5b** (0.178 g, 0.5 mmol) was dissolved in dry acetonitrile (2 ml) and to this solution was added a solution of 3′-azido-3′-deoxythymidine (0.134 g, 0.5 mmol) and DBU (0.083 g, 0.55 mmol) in dry acetonitrile (3 ml). After 3 h the reaction mixture was concentrated under reduced pressure, and the crude product was purified by silica gel column chromatography (0→15% methanol in chloroform) to provide **7b** in 92% yield. Separation into diastereomerically pure **7** was performed by HPLC.

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References

1. Yarchoan, R.; Weinhold, K. J.; Lyerly, H. K.; Gelman, E.; Blum, R. M.; Shearer, G. M.; Mitsuya, H.; Collins, J. M.; Meyers, C. E.; Klecker, R. W.; Markham, P. D.; Durack, D. T.; Nussinoff-Lehrmann, S.; Barry, D. W.; Fischl, M. A.; Gallo, R. C.; Bolognesi, D. P.; Border, S. *Lancet* **1986**, *1*, 575–585.
2. (a) Aggarwal, S. K.; Gogu, S. R.; Rangan, S. R. S.; Agrawal, K. C. *J. Med. Chem.* **1990**, *33*, 1505–1510. (b) Henin, Y.; Gouyette, C.; Schwartz, O.; Debouzy, J.; Neumann, J.; Huynh-Dinh, T. *J. Med. Chem.* **1991**, *34*, 1830–1837. (c) Piantadosi, C.; Marasco, C. J.; Morris-Natschke, S. L.; Meyer, K. L.; Gumus, F.; Surles, J. L.; Ishaq, K. H.; Kucera, S. L.; Iyer, N.; Wallen, C. N.; Piantadosi, S.; Modest, E. J. *J. Med. Chem.* **1991**, *34*, 1408–1414.
3. (a) McIntee, E. J.; Remmel, R. P.; Schinazi, R. F.; Abraham, T. W.; Wagner, C. R. *J. Med. Chem.* **1997**, *40*, 3323–3331. (b) Wagner, C. R.; McIntee, E. J.; Schinazi, R. F.; Abraham, T. W. *Bioorg. Med. Chem. Lett.* **1995**, *5*, 1819–1824.
4. Abraham, T. W.; Wagner, C. R. *Nucleosides Nucleotides* **1994**, *13*, 1891–1903.
5. Stec, W. J.; Grajkowski, A.; Karwowski, B.; Kobylanska, A.; Koziolkiewicz, M.; Misiura, K.; Okruszek, A.; Wilk, A.; Guga, P.; Boczkowska, M. *J. Am. Chem. Soc.* **1995**, *117*, 12019–12029.
6. Baraniak, J.; Korczynski, D.; Stec, W. J. *J. Org. Chem.* **1999**, *64*, 4233–4536.
7. Uznanski, B.; Grajkowski, A.; Krzyzanowska, B.; Kazmierkowska, A.; Stec, W. J.; Wieczorek, M. W.; Blaszczyk, J. *J. Am. Chem. Soc.* **1992**, *114*, 10197–109202.