Tetrahedron Letters 54 (2013) 1566–1568

Contents lists available at SciVerse ScienceDirect

Tetrahedron Letters

journal homepage: www.elsevier.com/locate/tetlet

Efficient methods for the synthesis of α-aminophosphonate fluoroalkyl esters

Marcin Skoreński, Józef Oleksyszyn, Marcin Sieńczyk *

Division of Medicinal Chemistry and Microbiology, Faculty of Chemistry, Wrocław University of Technology, Wybrzeże Wyspiańskiego 27, 50-370 Wrocław, Poland

ARTICLE INFO

Article history:
Received 19 October 2012
Revised 2 January 2013
Accepted 10 January 2013
Available online 16 January 2013

Keywords:
Bis(2,2,2-trifluoroethyl) phosphonic esters
Aminophosphonates
α-Amidoalkylation reaction
Serine protease inhibitors

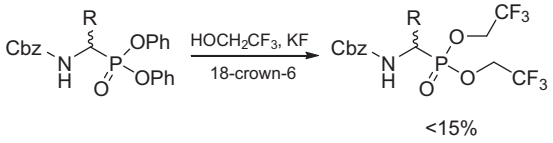
ABSTRACT

Two novel synthetic methods for the preparation of bis(trifluoroethyl) esters of α-aminophosphonic acids are presented. Preliminary results on the application of the compounds synthesized as inhibitors of serine proteases are also reported. Structures originating from α-aminoalkylphosphonate diaryl esters represent a novel class of serine protease inactivators.

© 2013 Elsevier Ltd. All rights reserved.

Diaryl esters of α-aminoalkylphosphonate acids and their peptidyl derivatives are well known inhibitors of serine proteases.[1,2] The mechanism of their action involves the nucleophilic attack of catalytic serine on the inhibitor phosphorus atom. Recent studies have focused on balancing the electrophilic potential of the phosphorus atom through the introduction of different substituents onto the ester ring structure.[3–6] This has led to the development of inhibitors displaying superior selectivity and specificity of action toward particular serine proteases.[7] In addition, this class of compounds is relatively easy to synthesize through α-amidoalkylation of a triaryl phosphite with benzyl carbamate and an appropriate aldehyde.[8] Despite the great potential and utility of α-aminoalkylphosphonate diaryl esters,[7,9] a major disadvantage is their poor solubility in aqueous media which limits their practical application in biological systems. One strategy to at least partially overcome such a limitation is the incorporation of solubility-enhancing groups into the inhibitor structure.[10]

Herein, we present a slightly different approach. We have replaced the aryl ester rings with 2,2,2-trifluoroethyl esters. This modification resulted in increased water solubility of the synthesized derivatives in comparison to their parent compounds and, more importantly, due to the presence of the fluorine atoms within the ethyl esters, the electrophilicity of the phosphorus atom was high enough to be susceptible to nucleophilic attack of a protease catalytic serine residue. Although the preliminary results indicated a decrease in inhibitory potency in comparison to aromatic esters, nevertheless, bis(2,2,2-trifluoro)ethyl esters of α-aminophosphonic acids represent an interesting class of irreversible serine protease inhibitors.



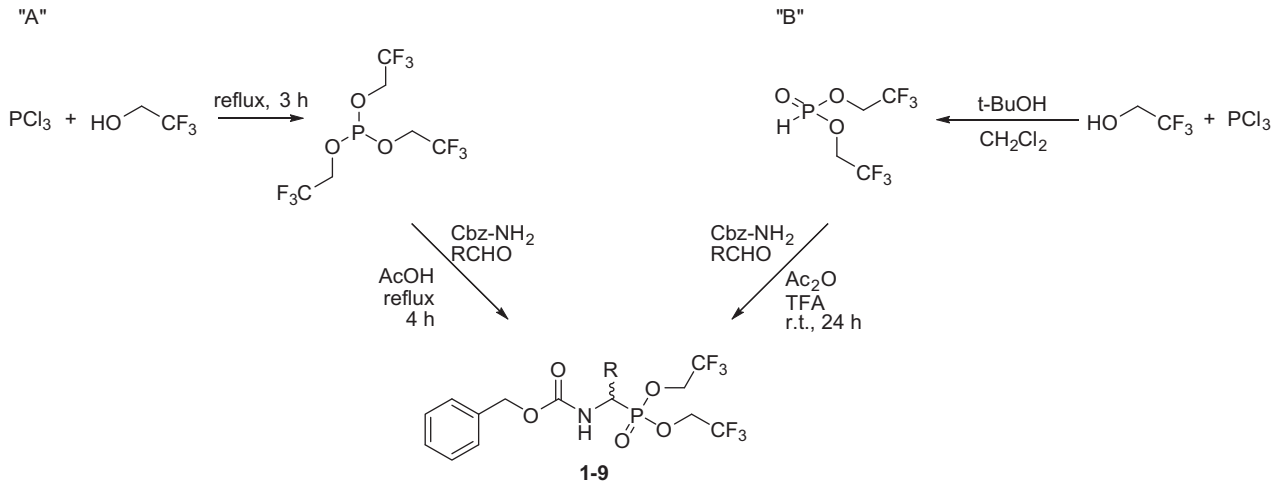
Scheme 1. Synthesis of α-aminoalkylphosphonate bis(2,2,2-trifluoroethyl) esters through diphenyl ester transesterification.[11,12]

The previously reported synthetic method for the preparation of α-aminoalkylphosphonate bis(2,2,2-trifluoroethyl) esters employed the transesterification of α-aminoalkylphosphonate diphenyl esters[11] (Scheme 1), which was based on the Szewczyk procedure.[12] Unfortunately, we have found this method inadequate due to the low synthetic yields (<15%), difficulties in final product purification, and the necessity to use a large excess of fluorinated alcohols.

In the present Letter, we describe two novel methods for α-aminoalkylphosphonate bis(2,2,2-trifluoroethyl) ester synthesis (Scheme 2). The first approach (Scheme 2, Route A) employs amidoalkylation of tris(2,2,2-trifluoroethyl) phosphite with benzyl carbamate and an aldehyde. Briefly, tris(2,2,2-trifluoroethyl) phosphite was prepared from phosphorus trichloride and 2,2,2-trifluoroethanol under reflux,[13] and was used directly in the amidoalkylation reaction with benzyl carbamate and an aldehyde in acetic acid. This is the first example of Cbz-protected α-aminoalkylphosphonate dialkyl ester synthesis via trialkyl phosphite amidoalkylation, the reaction characteristic for diaryl ester derivative preparation, which was initially postulated by Birum when 2-chloroethyl phosphite underwent amidoalkylation with urea and an appropriate aldehyde.[14] The second approach (Scheme

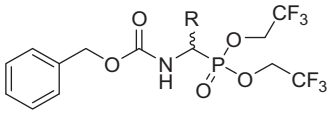
* Corresponding author. Tel.: +48 71 320 2439; fax: +48 71 320 2427.
E-mail address: marcin.sienczyk@pwr.wroc.pl (M. Sieńczyk).

0040-4039/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.tetlet.2013.01.039



Scheme 2. Synthesis of α-aminoalkylphosphonate bis(trifluoroethyl) esters.

Table 1
Synthesis of α-aminoalkylphosphonate bis(trifluoroethyl) esters



Compound	R	Isolated yield (%)	
		Route A	Route B
1	$-CH_2Ph$	18	31
2	$-CH_3$	11	25
3	$-CH(CH_3)_2$	28	31
4	$-CH_2CH(CH_3)_2$	36	57
5	$-CH(CH_3)CH_2CH_3$	31	41
6	$-(CH_2)_2SCH_3$	12	22
7	$-CH_2CH_2Ph$	31	56
8	$-CH_2CH_3$	16	29
9	$-CH_2CH_2CH_3$	40	45

Table 2
The solubility of α-aminoalkylphosphonate bis(trifluoroethyl) esters and their corresponding diphenyl parent compounds in PBS and human plasma

Compound	Solubility (mM)	
	PBS, pH 7.4	Human plasma
1	1.9	1.9
1DPP[a]	0.13	0.13
2	1.1	1.1
2DPP[a]	0.15	0.15
3	0.9	0.9
3DPP[a]	0.2	0.2
4	5.1	5.1
4DPP[a]	0.3	0.3
5	3.2	5.4
5DPP[a]	0.6	0.6
6	0.9	0.9
6DPP[a]	0.3	0.3
7	1.8	1.8
7DPP[a]	0.09	0.09
8	5.4	5.4
8DPP[a]	0.9	0.9
9	5.3	5.3
9DPP[a]	0.14	0.14
11	1.2	1.2
11DPP[a]	0.4	0.4

[a] DPP—corresponding diphenyl ester analogue.

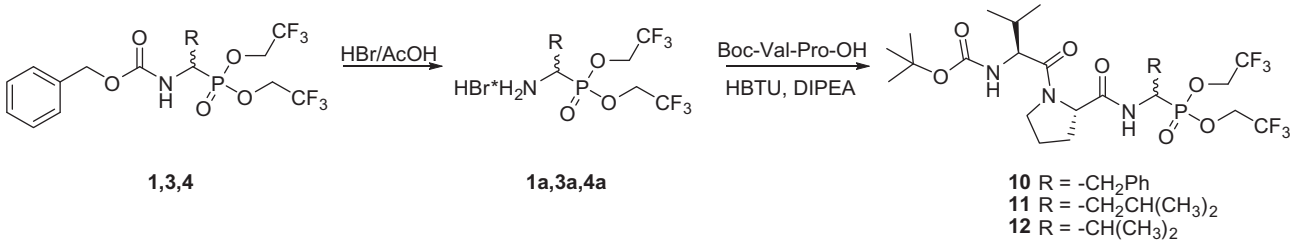
2, Route B) relies on the classical method used for α-aminoalkylphosphonate dialkyl ester synthesis.[15] Thus, phosphonic dichloride was initially prepared from phosphorus trichloride in the presence of *tert*-butyl alcohol, which was then reacted with 2,2,2-trifluoroethanol to give bis(2,2,2-trifluoroethyl) phosphite.[16] Next, bis(2,2,2-trifluoroethyl) phosphite was dissolved in acetic anhydride followed by the addition of benzyl carbamate, trifluoroacetic acid, and an aldehyde.

Applying both methods, we synthesized a series of Cbz-protected phosphonic analogues of natural and unnatural amino acids in reasonable yields (Table 1). When the synthesis followed Route B, slightly higher yields were noted ranging from 22% [Cbz-Met-$^{P}(OCH_2CF_3)_2$, **6**] to 57% [Cbz-Leu$^{P}(OCH_2CF_3)_2$, **4**].

To examine the solubility of the resulting compounds, either in PBS buffer or human plasma, we applied a spectrophotometric assay as described previously (Table 2).[4] Briefly, a serial dilution of the compounds under analysis was prepared in PBS or freshly isolated human plasma and the absorbance at 620 nm was measured with reference to the medium used in the assay. In order to determine the increase in solubility of the fluoroalkylphosphonate, the solubility of the corresponding phosphonic diphenyl esters (**1–9 DPP**, Table 2) was examined in parallel. As shown, the solubility of the fluoroalkyl phosphonic inhibitors was significantly increased in comparison to the parent aromatic structures, both in PBS and plasma, ranging from 3-times (**6** vs **6DPP**) to 38-times (**9** vs **9DPP**).

In order to synthesize peptidyl derivatives of bis(2,2,2-trifluoroethyl) esters of α-aminophosphonic acids, the Cbz-protecting group was first removed using a 33% solution of HBr in acetic acid and the resulting hydrobromide salt of the α-aminophosphonate bis(2,2,2-trifluoroethyl) ester was coupled to Boc-Val-Pro-OH using HBTU in the presence of DIPEA as a coupling reagent (Scheme 3).

The inhibitory activity of the compounds synthesized was evaluated using chymotrypsin as the model enzyme. The rates of chymotrypsin inhibition (3 nM, Calbiochem) were measured in 100 mM HEPES, 500 mM NaCl, and pH 7.5 containing 9% DMSO using a fluorogenic substrate (Suc-Ala-Ala-Pro-Phe-AMC, 5 μM, Ex. 350 nm, Em. 460 nm). The calculated Michaelis constant (K_M) was 70 μM. The observed rate of inhibition was determined by the progress curve method as described previously.[17,18] For compounds that showed >50% of chymotrypsin inhibition at a



Scheme 3. Synthesis of α-aminoalkylphosphonate bis(trifluoroethyl) ester peptidyl derivatives.

Table 3
Inhibition of chymotrypsin by α-aminoalkylphosphonate bis(trifluoroethyl) esters and their peptidyl derivatives

Compound		Chymotrypsin	
		K_i (μM)	k_2/K_i ($M^{-1}s^{-1}$)
1	Cbz-Phe^P(OCH_2CF_3)$_2$	770	4.50
4	Cbz-Leu^P(OCH_2CF_3)$_2$	353	6.86
10	Boc-Val-Pro-Phe^P(OCH_2CF_3)$_2$	54	11.09
11	Boc-Val-Pro-Leu^P(OCH_2CF_3)$_2$	67	9.75
12	Boc-Val-Pro-Val^P(OCH_2CF_3)$_2$	NI	NI

NI—no inhibition was observed after 30 min of compound incubation with chymotrypsin at 37 °C.

500 μM concentration, we examined the kinetic parameters (k_{obs}, K_i, and k_2/K_i). The preliminary data (Table 3) indicate that all the inhibitors displayed lower potency of action against chymotrypsin, in comparison to α-aminophosphonate diaryl esters, where the highest activity was shown by Boc-Val-Pro-Leu^P(OCH_2CF_3)$_2$ (**11**) with a k_2/K_i value of 9.75 M^{-1} s^{-1} (K_i = 67 μM). The obtained data fit the irreversible model of inhibition, although the activity of the synthesized compounds was weaker than the corresponding diphenyl esters.[1,2] The pK_a values of the leaving groups after nucleophilic attack by the serine hydroxyl group on the inhibitor phosphorus atom could provide an insight into one possible explanation. The pK_a of phenol (9.95) is lower than the pK_a of trifluoroethanol (12.46); thus, phosphonic diphenyl esters could be more susceptible to nucleophilic attack from serine than the corresponding phosphonic fluoroalkyl esters. Nevertheless, they can still react with the protease active site nucleophile. In addition, a flat and rigid phenyl moiety could fit better into the chymotrypsin S1′ pocket driven by hydrophobic forces.

Nevertheless, the most interesting features of this class of inhibitors are their solubility and irreversible mode of action. Moreover, small alkyl esters can be accommodated into the proteases with small S1′ binding pockets, and where bulky aryl esters may have limited access. Further studies on α-aminophosphonates containing various fluorinated alkyl ester groups as serine proteases inhibitors are now in progress.

In summary, we have reported efficient methods for the synthesis of bis(2,2,2-trifluoroethyl) esters of α-aminophosphonic acids and their peptidyl derivatives. In addition, we have evaluated their ability to block irreversibly the proteolytic activity of chymotrypsin.

Acknowledgment

This project was financed by the Wroclaw University of Technology Statute Funds S10156/Z0313.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.tetlet.2013.01.039.

References and notes

1. Oleksyszyn, J.; Powers, J. C. *Biochemistry* **1991**, *30*, 485–493.
2. Pietrusewicz, E.; Sieńczyk, M.; Oleksyszyn, J. *J. Enzyme Inhib. Med. Chem.* **2009**, *24*, 1229–1236.
3. Oleksyszyn, J.; Powers, J. C. *Biochem. Biophys. Res. Commun.* **1989**, *161*, 143–149.
4. Sieńczyk, M.; Lesner, A.; Wysocka, M.; Legowska, A.; Pietrusewicz, E.; Rolka, K.; Oleksyszyn, J. *Bioorg. Med. Chem.* **2008**, *16*, 8863–8867.
5. Brown, C. M.; Ray, M.; Eroy-Reveles, A. A.; Egea, P.; Tajon, C.; Craik, C. S. *Chem. Biol.* **2011**, *18*, 48–57.
6. Boduszek, B.; Oleksyszyn, J.; Kam, C. M.; Selzler, J.; Smith, R. E.; Powers, J. C. *J. Med. Chem.* **1994**, *37*, 3969–3976.
7. Sieńczyk, M.; Oleksyszyn, J. *Curr. Med. Chem.* **2009**, *16*, 1673–1687.
8. Oleksyszyn, J.; Subotkowska, L.; Mastalerz, P. *Synthesis* **1979**, 985–986.
9. Zou, F.; Schmon, M.; Sieńczyk, M.; Grzywa, R.; Palesch, D.; Boehm, B. O.; Sun, Z. L.; Watts, C.; Schirmbeck, R.; Burster, T. *Anal. Biochem.* **2012**, *421*, 667–672.
10. Winiarski, L.; Oleksyszyn, J.; Sieńczyk, M. *J. Med. Chem.* **2012**, *55*, 6541–6553.
11. Powers, J. C.; Boduszek, B.; Oleksyszyn, J. U.S. Patent 5,686,419, 1997; *Chem. Abstr.* **1998**, *128*, 3887s.
12. Szewczyk, J.; Lejczak, B.; Kafarski, P. *Synthesis* **1982**, 409–412.
13. Krogh, L. C.; Reid, T. S.; Brown, H. A. *J. Org. Chem.* **1954**, *19*, 1124–1126.
14. Birum, G. H. *J. Org. Chem.* **1974**, *39*, 209–213.
15. Dmitriev, M. E.; Rossinets, E. A.; Ragulin, V. V. *Russ. J. Gen. Chem.* **2011**, *81*, 1092–1104.
16. Timperley, C. M.; Arbon, R. E.; Saunders, S. A. *J. Fluorine Chem.* **2002**, *113*, 65–78.
17. Burchacka, E.; Walczak, M.; Sieńczyk, M.; Dubin, G.; Zdżalik, M.; Potempa, J.; Oleksyszyn, J. *Bioorg. Med. Chem. Lett.* **2012**, *22*, 5574–5578.
18. Knight, C. G. In *Proteinase Inhibitors*; Barett, A. J., Salvesen, G., Eds.; Elsevier: Amsterdam, 1986; pp 23–51.