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Synthesis of Phosphonopeptides as Thrombin Inhibitors[1]

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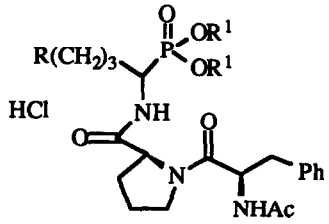
Abstract: Synthesis of phosphonopeptides **1** and their inhibitory activity of thrombin are described.

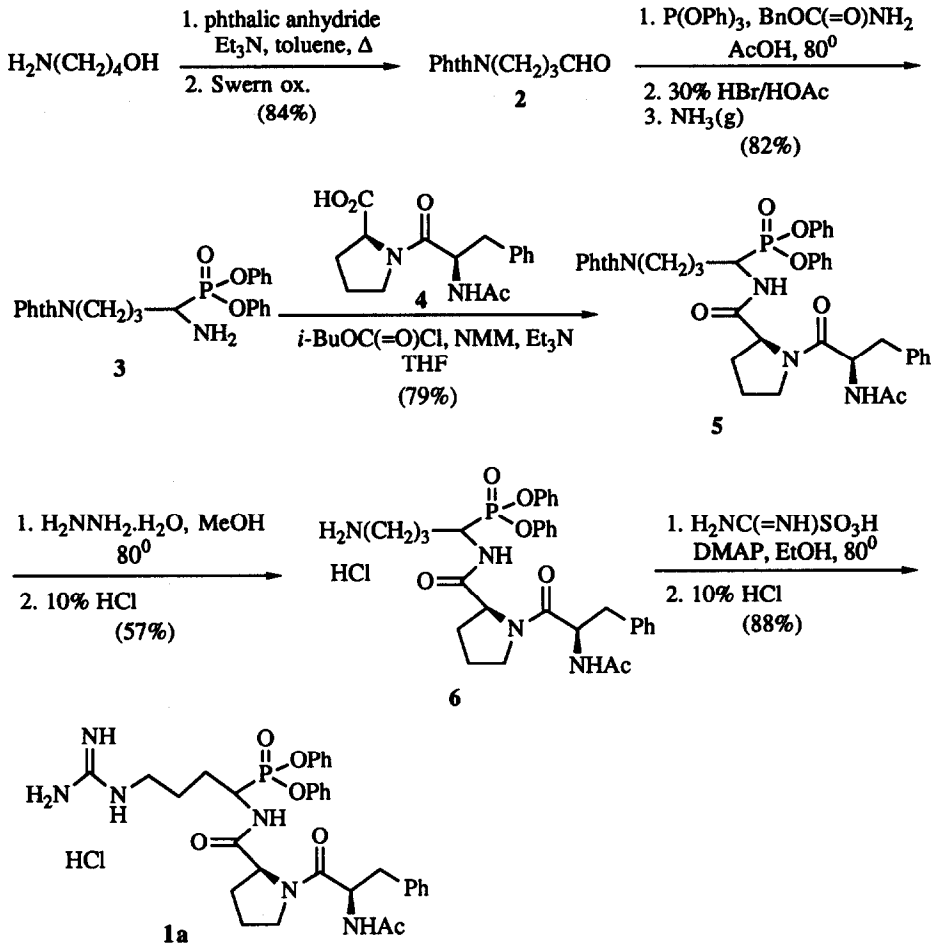
Thrombin, a serine protease in the blood coagulation cascade, catalyzes the conversion of fibrinogen to fibrin clots and activates factor XIII, which, in turn, cross-links and stabilizes fibrin clots.[2] Numerous thrombin inhibitors have been designed and synthesized as potential anticoagulants.[3] A recent report[4] describing the synthesis of novel phosphonopeptides as a new class of thrombin inhibitors prompts us to disclose our efforts in this area. Herein we report the syntheses of phosphonates **1**, analogs of Kettner's potent boronic acid thrombin inhibitor (Ac-(D)Phe-Pro-boroArg-OH)[3], and their inhibitory activity of thrombin.



1

a. R = $H_2NC(=NH)NH$, R^1 = Ph
b. R = H_2NCH_2, R^1 = Ph
c. R = $H_2NC(=NH)NH$, R^1 = Me
d. R = H_2NCH_2, R^1 = H

The synthesis of **1a** is shown in the scheme. Azeotropic refluxing of 4-amino-1-butanol and phthalic anhydride in toluene in the presence of triethylamine gave the protected amino alcohol, $PhthN(CH_2)_4OH$, which was subjected to the Swern oxidation (1. $(COCl)_2$, DMSO, CH_2Cl_2, -78^0; 2. Et_3N, r.t.) to afford aldehyde **2** (84% yield) as a white solid, mp 66-68^0. Compound **2** was converted into crystalline solid amino phosphonate **3** (mp 87-89^0) in 82% yield by the literature procedure[5]: 1. $(PhO)_3P$, $BnOC(=O)NH_2$, AcOH, 80^0; 2. 30% HBr/HOAc; and 3. NH_3(g). Coupling of **3** with dipeptide **4**[3] by the mixed anhydride method (*i*-$BuOC(=O)Cl$, 4-methylmorpholine, Et_3N, THF, r.t.) furnished **5** in 79% yield after column chromatography on silica gel. The two diastereomers were separated by HPLC to give **5A**, $[\alpha]_D^{25}$= -58.27^0 (c= 0.544, CH_2Cl_2), ^1H NMR($CDCl_3$): δ 1.90 (s, $NHC(=O)CH_3$); and **5B**, $[\alpha]_D^{25}$= -63.02^0 (c= 0.53, CH_2Cl_2), ^1H NMR($CDCl_3$): δ 1.73 (s, $NHC(=O)CH_3$).

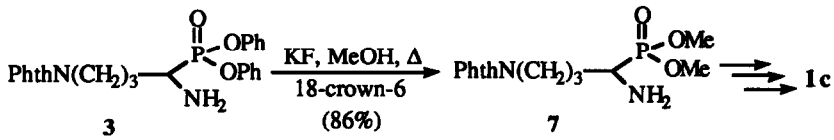


Scheme

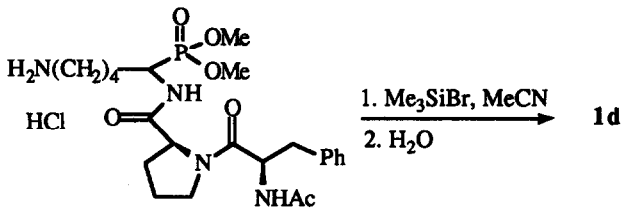
Alternatively, the two enantiomers of compound **3** could be separated by chiral column to afford **3A**, $[\alpha]_D^{25}$= +6.22^0 (c= 0.386, CH_2Cl_2) and **3B**, $[\alpha]_D^{25}$= -10.82^0 (c= 0.388, CH_2Cl_2). Coupling of **3A** and **3B** with **4** resulted the formation of **5A** and **5B**, respectively. Treatment of **5A** and **5B** with hydrazine monohydrate in methanol at 80^0 for 3h yielded the free amine which were isolated as their hydrochloride salts **6A**, MS(FAB): m/z 607 (M^++1) and **6B**, MS(FAB): m/z 607 (M^++1), respectively. Finally, guanidinylation was carried out by using the Mosher's method[6] to afford amorphous solids **1aA**, MS(FAB): m/z 649 (M^++1) and **1aB**, MS(FAB): m/z 649 (M^++1).

Starting from 5-amino-1-pentanol, compounds **1bA** and **1bB** were similarly prepared by using the above procedures for preparation of **6A** and **6B**. To synthesize **1c**, compound **3** was treated with KF (8.5 equiv) in refluxing methanol containing catalytic amount of 18-crown-6[7] to give **7** (86% yield), which was then converted

into **1c** by a similar procedure described in the above scheme except the hydrazine monohydrate reaction was done at room temperature. At 80^0 hydrazine displaced one of the phosphonate methoxy group. Compound **1d**, MS(FAB): m/z 469 (M^++1), was prepared by treatment of **8** (synthesized from 5-amino-1-pentanol) with Me_3SiBr in MeCN followed by aqueous hydrolysis.[8]



3 7



8

The inhibitory activity of thrombin was determined by using S-2238 (H-(D)Phe-Pip-Arg-*p*NA) chromogenic assay.[9] The results are shown in the Table. Like the previously reported,[4] these compounds show slow-binding behavior. For example, compound **1aB** has ca. 90 fold higher potency after 10 min incubation of enzyme and inhibitor before addition of substrate. Longer preincubation time does not seem to change the IC_{50} values. Surprisingly, our compounds are less potent than the previously reported compounds.[4]

Table: Inhibitory Activity of Thrombin

Compound	IC_{50} (μM)	
	A	B
1aA	3	25
1aB	1	90
1bA	8	125
1bB	6.5	12.5
1c	I	I
1d	I	I

A: with 10 min preincubation; B: without preincubation; I: inactive

In conclusion, among the phosphonates prepared diphenylphosphonates are the best thrombin inhibitors, whereas dimethylphosphonates and free phosphonic acids are inactive. Generally, phosphonates are less effective than boronic acids as thrombin inhibitors.[10] Unlike boronic acid, the absolute configuration of the carbon bearing the phosphonate does not seem to affect the inhibitory activity.

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9. The IC_{50} (inhibitor concetration required to inhibit 50% of the thrombin activity) was measured with preincubation of the inhibitor and thrombin at 37^0 for 10 min. The free enzyme activity was measured spectrophotometrically (405 nm) by following the release of *p*-nitroaniline.
10. The IC_{50} of Ac-(D)Phe-Pro-boroArg-OH against thrombin is 7 nM.

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