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Solid-phase synthesis of a library based on biphenyl-containing trypsin-like serine protease inhibitors

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ABSTRACT

The solid-phase synthesis of a library based on an unusual biphenyl-containing trypsin-like serine protease inhibitor is described. Key to this effort was the synthesis of a highly functionalized aryl boronic acid reagent which required the development of a novel and efficient method to convert a triflate to a pinacolboronate in large scale.

The trypsin-like serine protease superfamily contains a number of drug discovery targets, including thrombin, elastase, urokinase, and coagulation factors VIIa, Xa, and XIa.[1] The therapeutic importance of this superfamily has prompted the discovery of a number of common pharmacophores, as shown in Figure 1. Chemotypes **1–3** are peptidomimetic inhibitors,[2–4] but structure **4** (IC_{50} 8 nM for factors VIIa, first disclosed by Ono Pharmaceuticals in 1999[5]) represents a structurally unique class of trypsin-like serine protease inhibitors due to its biphenyl scaffold, a 'privileged' structure found in 4.3% of all known drugs.[6] Our ongoing interest in members of the trypsin-like serine protease superfamily prompted us to embark on the solid-phase synthesis of a library of potential protease inhibitors related to chemotype **4**.

As reported by Kohrt et al.,[7] the synthesis of **4** was not described in detail in the original Ono disclosure. Accordingly, as shown in Scheme 1, we developed a solid-phase retrosynthesis of **5** with three points of diversity (R^1, R^2, R^3R^4). A Suzuki coupling was identified as the critical bond-forming step to permit disconnection of **5** to two components, pinacolboronate **6** and resin-bound amide **7**. Boronate **6** was envisioned to arise from triflate **8**, which in turn could arise from commercial available salicylic acid **9**. Resin-bound amide **7** could be disconnected into 2-halobenzoyl chloride **10** and resin-bound amine **11** which could arise from PL-FMP resin **12** and primary amine **13** via reductive amination.

Boronate **6** was synthesized as shown in Scheme 2. Compound **14**[8] was converted to the orthogonally protected diester **8** in excellent yield according Forsch and Rosowsky's procedure.[9] Miyaura et al. reported an efficient one-step procedure[10] for preparation of arylboronates from aryl triflates and bis(pinacolato)-diboron **15** utilizing a palladium-catalyzed coupling in the presence of a base. However, when we tried to convert triflate **8** to arylboronate **6** according Miyaura's standard conditions (dioxane, 80 °C, 1.1 equiv of diboron **15**, 1 equiv of triflate **8**, 3% equiv of Pd(dppf), 3% equiv of dppf, 3 equiv of KOAc, 20 h), only trace quantities of arylboronate **6** were obtained along with large amounts of unreacted triflate **8**. In order to make sufficient quantities of **6** for use in a large solid-phase library synthesis, further optimization was required.

After extensive experimentation, we found that by increasing the Pd(dppf) loading to 8% of the triflate, adding no external dppf, the reaction was complete after 2 h at 60–65 °C and product **6** was obtained in more than 90% yield. However, the purification of the product **6** was problematic- it could not be isolated by crystallization and had to be purified by flash chromatography. Although the R_f of **6** is 0.31 in DCM, the pinacolboronate rapidly hydrolyzed upon exposure to silica gel even when the silica was pre-treated with triethylamine. To prevent hydrolysis, a significantly more polar eluent (DCM/EtOAc = 7:3) was used to quickly flush the product from a column loaded with silica pre-treated with triethylamine. This purification protocol provided **6** contaminated with approximately 5% of diboronate **15**. This impure material worked very well for Suzuki couplings in solution, but the presence of diboronate **15**

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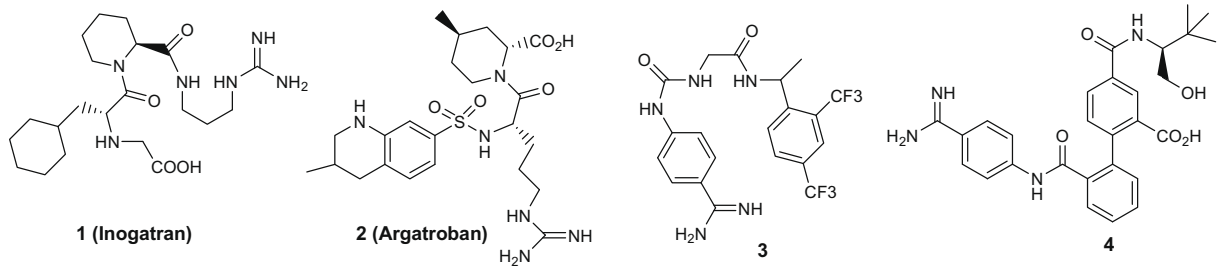
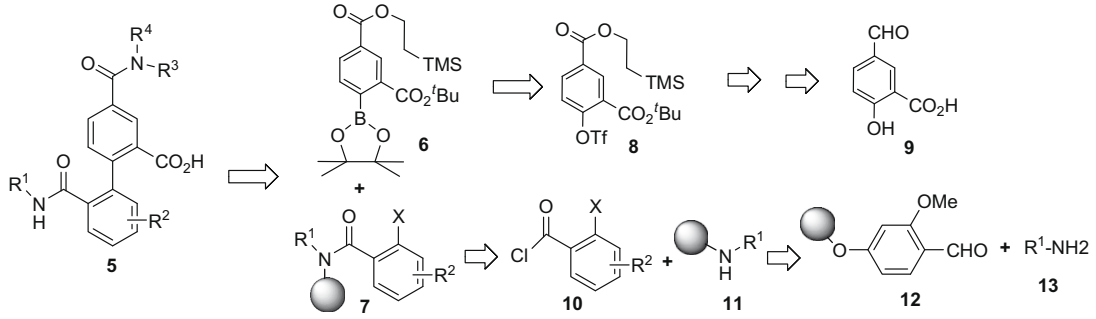
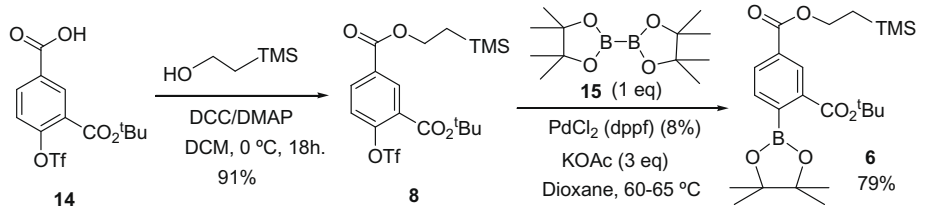


Figure 1. Representative trypsin-like serine protease inhibitors.



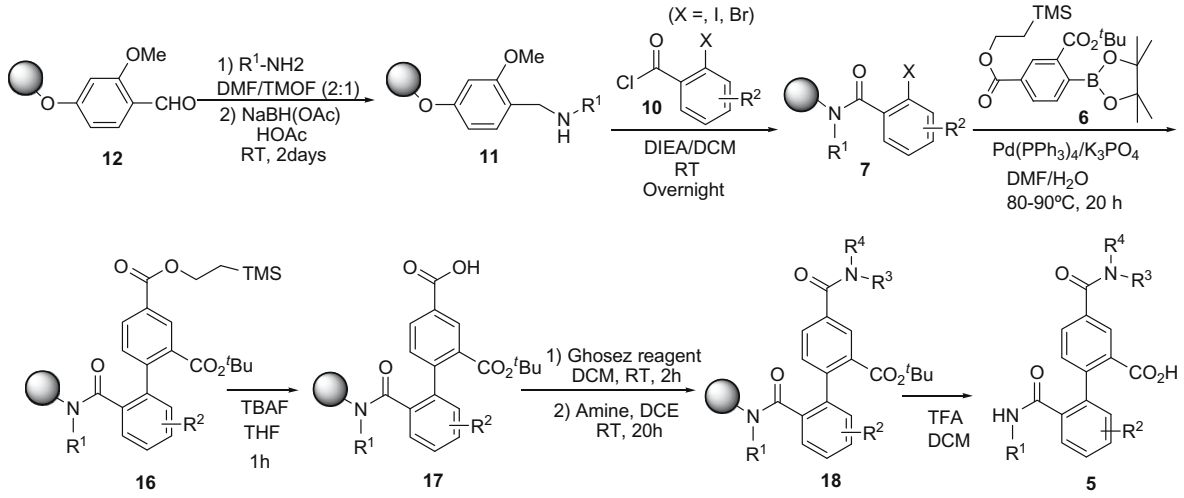
Scheme 1.



Scheme 2.

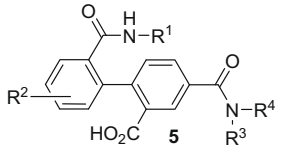
adversely affected the reaction on solid-phase because it was more reactive than **6** in the Suzuki reaction with the solid-supported aryl iodide. To overcome this issue, we reduced the diboron **15** to 1 equiv of the triflate and prolonged the reaction time to 20 h. Under these conditions, boronate **6** was obtained in 79% yield with no diboronate **15** contamination. A dimer byproduct (Suzuki coupling between starting material triflate **8** and product boronate **6**) was clearly seen by UV (16%), but the presence of the dimer had no effect on the solid-phase reaction because it did not react with the solid-supported aryl iodide. The optimized protocol[11] is very efficient and 180 g of the key intermediate **6** were prepared in several batches.

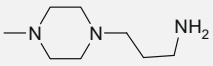
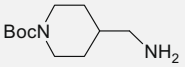
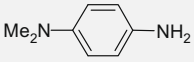
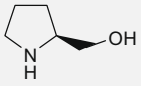
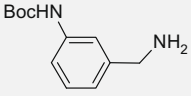
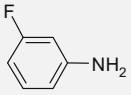
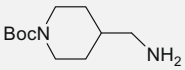
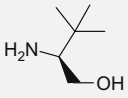
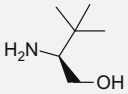
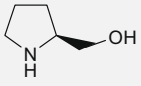
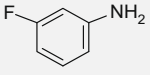
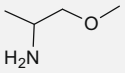
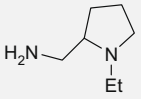
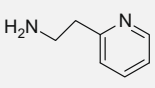
The solid-phase synthesis of **5** is shown in Scheme 3, starting with PL-FMP resin **12** loaded into Nanokans.[12] The first diversity



Scheme 3.

Table 1
Purity and yields of representative library products



Entry	R^1NH_2	R^2	NR^3R^4	Purity (%)	Yield (%)
1				100	66
2				100	52
3		H		100	50
4				100	53
5				97	79
6		H		95	65
7		5-MeO		97	43
8		4-Cl		97	34
9		4,5-Di-MeO		97	45
10		5-*i*PrO		93	32
11				99	81
12				98	66
13		4,5-Di-MeO		87	55
14				82	56
15				81	74

The Boc-group in these reagents falls off in cleavage. [14]

element (R^1) was introduced via reductive amination. Thus, PL-FMP resin in Nanokans (1.2 mmol/g, 8 mg/Nanokan) was treated with primary amine in DMF/TMOF for 4 h, followed by treatment with $NaBH(OAc)_3$ and HOAc for 2 days at room temperature to afford resin-bound amine **11**. The second diversity element (R^2) was introduced via coupling benzoyl chloride **10** to resin-bound amine **11** in the presence of DIEA in DCE. The biphenyl core **16** was constructed via Suzuki coupling between **7** and boronate **6** at 80–90 °C for 20 h. It was critically important to use degassed DMF and we found that aqueous potassium phosphate was optimal. The 2-(trimethylsilyl)ethyl ester was removed by treating **16** with TBAF in THF for 1 h to afford acid **17**. The third diversity element (NR^3R^4) was introduced via amide formation. Thus, dried resin-bound acid **17** was treated with 1-chloro-*N*,*N*,2-trimethyl-propenylamine (Ghosez reagent)[13] in DCM for 2 h, then washed with DCM and treated with amine in DCE for 20 h at rt. For amino alcohols, an alternative method was applied. Resin-bound acid **17** was treated with EDC, HOBt, DIEA and amino alcohol in DMA for 20 h. In either case, the final product **5** was cleaved from resin **18** with TFA/DCM (1:1) at rt for 2 h, then the resin was filtered off and solution was collected in 96-well plates via IRORI Nanokan system. Concentration of the filtrate afforded product **5** without further purification. The purity of library products was determined by HPLC at 254 nm and mass spectroscopy. Product samples which met our purity criteria (product peak $\geqslant$80% by HPLC at 254 nm, correct MS) were submitted. The quantity of products was determined by weight (most of library products were obtained in 1–3 mg per compound). The library size is more than 10,000 products and the more than 80% of library products were submitted. The purity and yields of some representative products are shown in Table 1.

Prior to embarking on the synthesis of a large library, Scheme 3 was validated with a wide variety of building blocks to determine best reagents and conditions. The ^1H and ^{13}C NMR of one product can be seen in Supplementary data. In the reductive amination, anilines with halides, –OMe, –SMe, $-NO_2$, –CN, amide, acetamide and alkylamines gave generally high yields (80–100%), some anilines with an tertiary amine gave lower yields (60–79%). In the Suzuki coupling, aromatic iodines, bromines with electron-withdrawing groups gave high yields (80–100%), but aromatic bromines with electrondonating groups gave lower yields (42–68%). In the amide formation via Ghosez reagent pathway, general amines gave high yields (80–100%), some amines with tertiary amine and pyridine ring gave mediate yields (68–79%), and amino alcohols gave low yields (42–70%). The yields from reagents amino alcohol were increased to 80–100% by a regular coupling with EDC, HOBt and DIEA in DCE. By reagent rehearsal, low yielding candidates were excluded, suitable building blocks were selected based on reliability and product diversity.

In summary, we have discovered a very unique and easy method to convert a triflate to a pinacolboronate in large scale. We also developed an efficient solid-phase chemistry for large library of biphenyl core with multiple functionalities.

Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmcl.2009.08.100.

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11. *General experimental procedure*: A mixture of triflate **8** (9.39 g,19.96 mmol), bis(pinacolato)diboron **15** (5.068 g,19.96 mmol), $PdCl_2$(dppf) (1.168 g, 1.596 mmol, 8%) and KOAc (6.855 g, 69.84 mmol) in degased 1,4-dioxane (100 mL) was heated at 60–65 °C for 20 h. After cooled down, dioxane was removed by Rotovap, then DCM and water were added to the crude mixture. Layers were separated and the aqueous layer was extracted by DCM one more time. The combined organic extract was washed once with water and dried over potassium carbonate. The crude product was purified by flash chromatography (DCM and ethyl acetate, 70:30) to give 8.37 g (93.5%, theoretical yield 8.949 g) of compound **6.** LCMS showed M+1 (449) peak with purity of 84% for the compound and peak 665 ($M_{dimer}+Na^+$) as the major impurity (16%). NMR (^1H, ^{13}C) can be seen in Supplementary data.
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