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Solid Phase Synthesis of C-Terminal Boronic Acid Peptides

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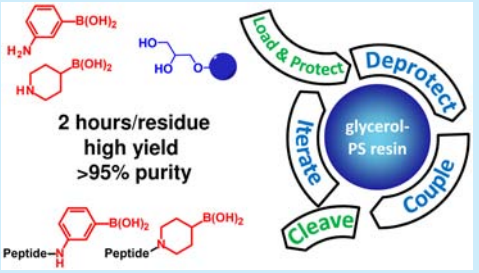
Supporting Information

ABSTRACT: Peptides and peptidomimetics with a C-terminal boronic acid group have prolific applications in numerous fields of research, but their synthetic accessibility remains problematic. A convenient, high yield synthesis of peptide-boronic acids on a solid support is described here, using commercially available 1-glycerol polystyrene resin. The method is compatible with Fmoc chemistry and offers a versatile approach to aryl and alkyl aminoboronic acids without additional purification steps.



Peptide-boronic acids (PBAs) are attracting rapidly growing interest in biomedical research, proteomics, nanotechnology, material science, organic electronics, analytical, and medicinal chemistry. In drug discovery, introduction of a boronic acid group into peptides and peptidomimetics yielded covalent-reversible protease inhibitors, with a >10 000-fold affinity increase relative to the parent peptide.[1] Bortezomib is a peptide-boronic acid that was the first proteasome inhibitor in clinical practice.[2] In contrast, PBAs with C-terminal aryl boronic acids cover an even broader range of applications. In addition to their use as enzyme inhibitors in medicinal chemistry,[1a,3] these peptidic and peptidomimetic compounds have been reported and patented for numerous applications such as molecular recognition, sensors, and material science.[4] PBAs have been recently described for the fabrication of antibody microarrays,[5] which can be also based on glyco-gold nanoparticles.[6] With respect to molecular recognition, PBAs have been used for protein tagging,[7] as bioconjugates for protein immobilization,[8] as artificial receptors,[9] and for fluorescent[10] and electrochemiluminescent detection.[11] PBAs allow the traceless labeling of glycoproteins to study their interactions,[12] the development of electroconductive molecules for nanotechnology,[13] and glucose biosensors[14] and have been described as membrane carriers for sugars.[15] The latter application was expanded into a class of polymers that can be functionalized for therapeutic or diagnostic purposes,[16] such as target-selective membrane fusion nanovesicles for drug delivery[17] or target-selective photodegradation,[18] in addition to their ability to form nanofibers and smart organogels.[19]

Despite countless applications, the synthetic routes toward PBAs remain inefficient and underdeveloped. Apart from branched peptide boronic acids that can be synthesized using common resins and conditions of solid-phase peptide synthesis (SPPS),[20] only solution-phase syntheses of peptidic derivatives with C-terminal boronic acid electrophiles have been reported. Simple coupling reactions, otherwise handled with ease and efficiency in SPPS, become problematic in solution. Composition and length of the sequence remain limited, and automation or expansion into multipurpose combinatorial libraries is difficult. Problems are encountered during the coupling and the final deprotection step of the boronate-pinanediol ester, in particular for nonpolar peptide sequences. These products are extremely difficult to separate from the phenyl boronic acid that is used as a deprotection−transesterification reagent.

Syntheses on solid support offer numerous advantages,[21] and SPPS became the standard synthetic approach toward peptides and peptidomimetics since it was first established by Merrifield.[22] Our aim is to bring the advantages of solid-phase synthesis to the field of PBAs by establishing a method that allows rapid and efficient access to this important compound class.

Although the main concepts of SPPS and diol−boronic acid interactions are well-studied, the synthesis of peptides on solid support via boronic acid ester anchoring was not described before.

Two special polystyrene polymers were described previously for the immobilization of small-molecular organoboronic acids: The 2-methyl-2-(hydroxymethyl)-1,3-propanediol polystyrene (MHMP-PS)[23] and the *N,N*-diethanolaminomethyl polystyrene (DEAM-PS) resin.[24] The application of these polymers was not extended toward Fmoc SPPS, probably because of expected incompatibilities due to the requirement for fast coupling and cleavage steps or hygroscopic solvents (DMF, NMP) in SPPS. The MHMP-PS resin showed slow boronic acid attachment (16 h, reflux) and cleavage (48 h),[23a] and DEAM-PS resin was relatively sensitive toward water and alcohols (cleavage in <1 min using H_2O/THF 5:95).[24b]

Solid-support synthesis of peptide-boronic acids has been limited to two examples with restricted applicability. In one example, a glutaric acid linker was coupled to a resin in order to

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create an inverse SPPS, thus allowing the attachment of a C-terminal aminoboronic acid pinanediol ester.[25] However, inverse SPPS is highly prone to epimerization and this approach lacks the possibility of variation in the first residue, which is restricted to a diacid moiety. In the second example, the α-aminoboronic acid is synthetically protected with a diol-containing citronellic acid,[26] which provides an attachment point to MBHA amide resin.[27] Along with synthetic difficulties, the exchange of pinanediol by the suggested diol linker results in a lack of stereochemical orientation in the side chain. Other disadvantages of this method are the low yield resulting from the inefficient coupling of the protected building block, and the expected contamination of the product because the linker and the peptide are both cleaved under acidic conditions.[27]

The solid-phase synthesis of peptide-boronic acids described here was performed by loading aryl or alkyl aminoboronic acids on commonly available and inexpensive 1-glycerol Merrifield resin, performing standard Fmoc SPPS, and finally releasing the PBAs from the resin.

1-Glycerol Merrifield resin (Figure 1) was originally developed for the protection, isolation, and derivatization of

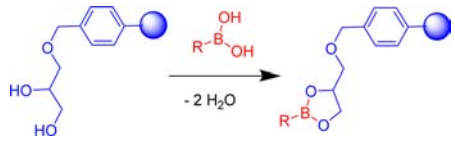


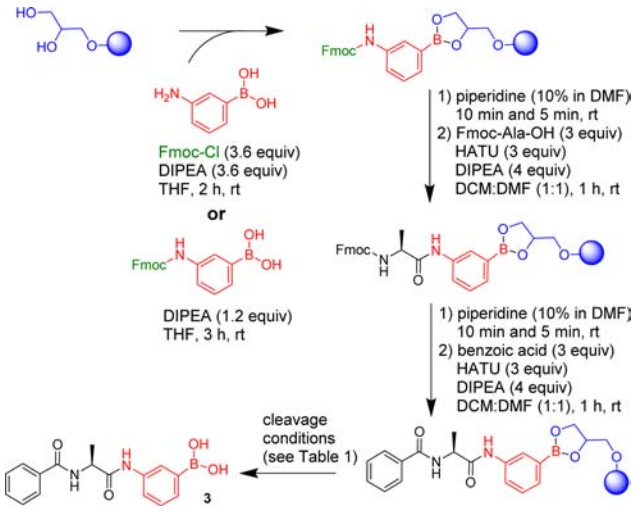
Figure 1. 1-Glycerol Merrifield solid support for boronic acids and the respective boronic acid−diol complex.

aldehydes and ketones.[28] It was dismissed as unsuitable for boronic acid immobilization in former publications because of (allegedly) low coupling efficiency−a problem that can, as shown below, easily be handled via the coupling conditions. The hydrolytic stability of boronic acid loaded 1-glycerol Merrifield resin is significant. The resin has comparable loading capacity (0.6 mmol/g) to the widely used Rink amide and Wang resins. Furthermore, the glycerol linker is traceless[29] and stable under SPPS cleavage conditions and, thereby, avoids the generation of impurities in the crude product, which may pose problems for product purification. The method allows construction of the sequence from the C toward the N terminus, providing an advantage over the epimerization−prone inverse SPPS[30] that proceeds in the N → C direction.

3-Aminophenylboronic acid and 4-piperidineboronic acid pinacol ester, which were previously reported as fragments of PBAs used as enzyme inhibitors[1a,31] or for other applications, were employed as starting materials. Deprotection of the pinacol ester is achieved by transesterification with phenylboronic acid (0.95 equiv) in a biphasic system of 0.1 M HCl/acetonitrile and hexane for 24 h, yielding the free 4-piperidineboronic acid.[32]

The formation of boronic acid esters with diols is favored at neutral to basic pH in a nonaqueous medium, whereas dissociation is favored in an aqueous acidic medium.[33] Attachment of boronic acid to a diol under neutral conditions was reported to require long reaction times on the order of 16−18 h.[23a] Since the binding kinetics of boronic acids to diols are faster at basic pH, our strategy was to optimize the loading step by adding 1 equiv of DIPEA,[33a] thereby decreasing the reaction time to 2−3 h. To avoid the dimerization of aminoboronic acid during the loading step, the free amino group was protected with an Fmoc-protection group. This protection can be performed as a separate step before loading or, more conveniently, in a one-pot sequence with the loading step (see Scheme 1). Protection was verified by releasing the Fmoc-

Scheme 1. Synthetic Procedure Exemplified by Aryl Boronic Acid Peptide 3



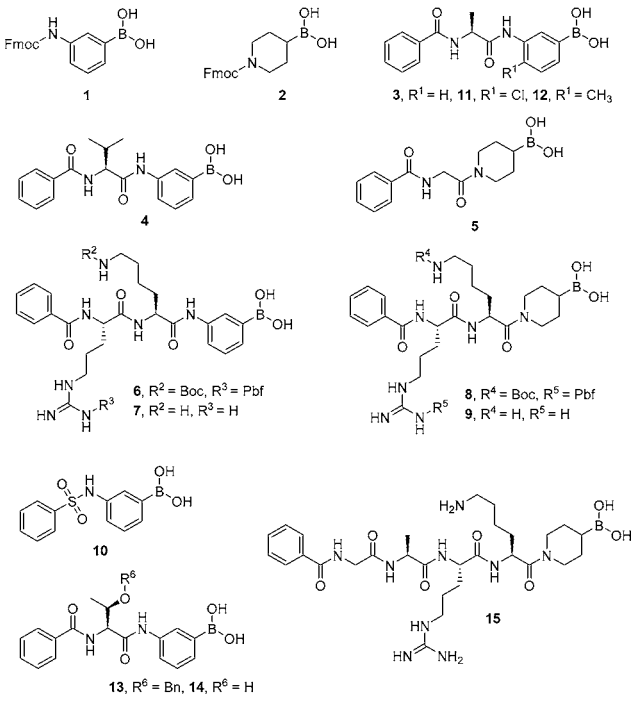


Figure 2. Structures of the boronic acids (**1**−**15**).

aminoboronic acids **1** and **2** (Figure 2) with THF/TFA/H_2O (70:20:10) (condition A; see Table 1). For aryl aminoboronic acids, both approaches generated high loading efficiencies and purities. For alkyl aminoboronic acid, the one-pot strategy was preferred to avoid the difficult purification of the Fmoc-aminoboronic acid.

After the loading step, further extension of the sequence proceeded by standard Fmoc-protocol for SPPS.[34] Fmoc-deprotection was performed using 10% piperidine in DMF,[35] and coupling of amino acids or N-terminal acids (3 equiv) was

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Table 1. Resin Cleavage Conditions with Yield and Purity of the Crude Products 1−15

	cleavage conditions	yield (%)	purity (%)
1	A. THF/TFA/H_2O (70:20:10)	99	>95
2	A. THF/TFA/H_2O (70:20:10)	84	>95
3	B. THF/H_2O (90:10)	90	>95
	C. dioxane/H_2O (85:15)	90	>95
	D. TFA/H_2O (10:90)	98	>95
	E. THF/TFA/H_2O (80:10:10)	98	>95
	A. THF/TFA/H_2O (70:20:10)	96	>95
	F. THF/TFA/H_2O (50:40:10)	95	>95
	G. THF/AcOH/H_2O (70:20:10)	98	>95
	H. THF/1 M HCl (70:30)	96	>95
	I. dioxane/TFA/H_2O (70:20:10)	98	>95
	J. MeCN/TFA/H_2O (70:20:10)	62	>95
	K. THF/H_2O (90:10) and 0.9 equiv of $PhB(OH)_2$	95	80
4	A. THF/TFA/H_2O (70:20:10)	96	>95
	J. MeCN/TFA/H_2O (70:20:10)	59	>95
5	A. THF/TFA/H_2O (70:20:10)	76	>95
6	B. THF/H_2O (90:10)	60	>95
	K. THF/H_2O (90:10) and 0.9 equiv of $PhB(OH)_2$	65	77
7	L. TFA/H_2O/TIPS (95:4:1)	94	>95
8	B. THF/H_2O (90:10)	49	>95
	C. dioxane/H_2O (85:15)	50	>95
9	L. TFA/H_2O/TIPS (95:4:1)	62	78
	B. THF/H_2O (90:10) followed by L. TFA/H_2O/TIPS (95:4:1)	49	>95
10	A. THF/TFA/H_2O (70:20:10)	96	>95
	H. THF/1 M HCl (70:30)	95	>95
	J. MeCN/TFA/H_2O (70:20:10)	69	>95
11	C. dioxane/H_2O (85:15)	81	93
12	C. dioxane/H_2O (85:15)	87	>95
13	C. dioxane/H_2O (85:15)	77	>95
14	C. dioxane/H_2O (85:15) followed by reduction	94	88
15	B. THF/H_2O (90:10) followed by L. TFA/H_2O/TIPS (95:4:1)	46	94

performed, with HATU (3 equiv) and DIPEA (4 equiv) in DCM/DMF (1:1) for 1 h. The benzenesulfonyl chloride (4 equiv) was coupled with NMM (6 equiv) in DCM for 3 h.[36] The entire procedure was performed in a syringe equipped with a frit. All described steps were carried out at room temperature without the need for an inert atmosphere or dry solvents, except for dry THF in the loading step.

Model compounds, inspired by reported PBAs, were used to assess the method (Figure 2), and a large variety of cleavage conditions were evaluated (Table 1, A−L). The reaction time was 3 h (except condition L for 2 h) with 1 mL of reagent per 50 mg of resin.

Although the PBA **3** could be released from the resin under mild nonacidic conditions (B and C), the influence of acidic conditions (A, D−J) was also evaluated which mostly enhance the yield of this step. A lower yield was observed for condition J (acetonitrile). However, the use of dioxane or acetonitrile instead of THF allows direct lyophilization after dilution with water. The purity of **3** depends on the coupling efficiency during synthesis and not on the cleavage cocktail used for conditions A−J (see Table 1). In comparison to condition A, the addition of phenylboronic acid (condition K) as a transesterification−deprotection reagent moderately increased the yield but resulted in impurities of the final product. For alkyl aminoboronic acids (**2**, **5**), the yields were lower, probably due to their higher pK_a in relation to aryl boronic acids.[37]

In addition to PBAs (**3**−**5**), the present method was assessed on several model compounds (**6**−**15**). Basic peptide sequences were chosen that have considerable relevance in medicinal chemistry, particularly for protease inhibitors.[34,38] These target structures posed an additional, potential difficulty in that a Pbf protecting group needs to be removed from the arginine side chain. In the absence of acid, or with 10% TFA, the fully protected peptide **6** could be obtained. The lower yield may be due to steric hindrance by the bulky protecting groups. At 20% TFA, about 9% of Boc-deprotection resulted after 3 h. Quantitative removal of the Boc and Pbf groups was achieved in 3 h with 95% TFA (condition L), yielding the fully deprotected peptide **7**.

Compound **9** was contaminated by impurities incorporating the sequence (Bz-Arg-Lys) in the case of direct cleavage and deprotection (condition L). The contaminant was probably built at free resin positions that were not linked to the aminoboronic acid. The contaminant was not formed in the synthesis of the analogous aryl boronic acid **7** using condition L, probably due to the higher efficiency of the loading step.

Removal of the contaminant can easily be performed not only by preparative HPLC but also by nonacidic cleavage conditions that are selective for the boronic acid−diol linkage and yield pure **8**. The latter can be deprotected (L, 2 h, then precipitation with ether) to yield pure **9**. As evidenced by compound **10**, the method can be expanded toward peptidosulfonamides. In addition, electron-deficient or -rich arylboronic acids were used as building blocks to synthesize **11** and **12**. The deprotection of other orthogonal protection groups (e.g., benzyl, **13**) can be easily achieved by hydrogenation with palladium on charcoal, providing **14** in high yield and purity. Similarly, peptides such as **15** can be obtained.

In conclusion, solid-phase peptide boronic acid synthesis (SPPBS) on glycerol-Merrifield resin is a fast and elegant method that allows straightforward access to PBAs. It does not require uncommon or expensive resins, can be performed under standard conditions, and is efficient in yield and time. Tedious intermediate or final purification steps are not required. The method was assessed on representative aryl and alkyl aminoboronic acids and under various cleavage conditions. It is compatible with Fmoc SPPS chemistry and can be expanded toward peptidosulfonamides. Orthogonally protected intermediates may be isolated and represent a straightforward avenue toward further functionalization of the C-terminal boronic acid moiety by the Suzuki, Chan−Lam, or Petasis coupling reactions, thereby opening a multitude of structural variations in chemical biology, medicinal chemistry, and materials science.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b00625.

Full synthetic procedures, NMR spectra, HPLC chromatograms, and MS spectra (PDF)

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Notes

The authors declare no competing financial interest.

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