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A practical synthesis of peptide mimetics via the solid-phase Petasis reaction

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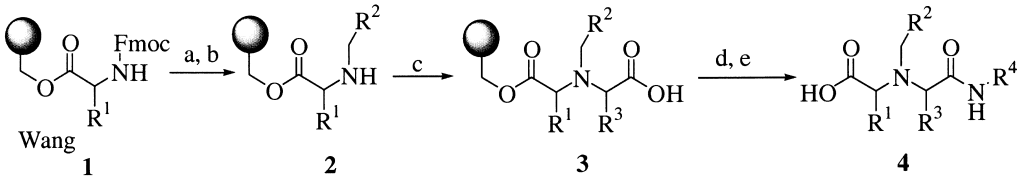
Abstract

The boronic acid Mannich reaction (Petasis reaction) is demonstrated on a solid support. Peptide mimetics are formed from *N*-alkylated amino acid resin esters, glyoxylic acid and boronic acids.

Keywords: solid-phase synthesis; glyoxylic acid; Mannich reaction; boronic acid; combinatorial chemistry.

We have been interested for some time in using parallel synthesis to prepare small molecule peptide mimetics for high-throughput screening. Of the numerous syntheses of peptide mimetics,[1] multi-component reactions[2] are particularly valuble for their simplicity, speed, and the opportunities they present to provide a densely functionalized central fragment.

We wish to report a solid-phase route to peptide mimetics utilizing a boronic acid Mannich reaction (Petasis reaction)[3] (Scheme 1). The Petasis reaction is a three-component condensation reaction between an activated carbonyl, an amine, and a boronic acid. We recognized this reaction as a convenient way to make three-component Ugi products (Fig. 1) without having to deal with isocyanides[4] and their associated limitations.



Scheme 1. General route to peptide mimetics. *Reagents and conditions*: (a) 25% Pip/DMF; (b) R^2CHO, 1% AcOH/TMOF, $NaCNBH_3$; (c) $R^3B(OH)_2$, glyoxylic acid, DCM; (d) DIC, HOBt, R^4NH_2; (e) 95:5 TFA:H_2O

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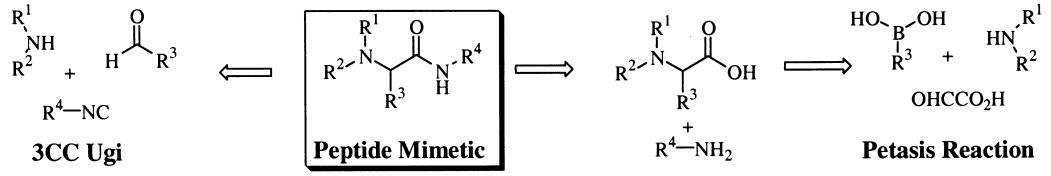


Figure 1. Complementary routes to peptide mimetics

Starting from Fmoc-protected amino acid resin esters and, following standard Fmoc removal conditions,[5] the amine is reductively alkylated[6] to provide **2**. The secondary amine is then treated with glyoxylic acid and a boronic acid in the key carbon–carbon bond-forming step.[7] We have found that dichloromethane gives the desired resin swelling characteristics and solubility/reactivity of the solutes. Under typical experimental conditions, the boronic acids are often not fully soluble but over time most dissolve completely. It is important to thoroughly wash the resin with a variety of solvents (especially protic solvents) following the Petasis reaction to ensure complete removal of all reaction components. We did not observe appreciable diastereoselectivity in this process[8] even though significant asymmetric induction has been reported for other chiral amines[3b] in solution. The resulting carboxylic acid (**3**) is then treated with DIC/HOBt and an amine to provide the final substituent. The Petasis chemistry in the solid-phase seems to tolerate a host of aryl boronic acids, although pyridine-3-boronic acid showed no reaction. A small selection of products synthesized via this method is illustrated in Table 1.

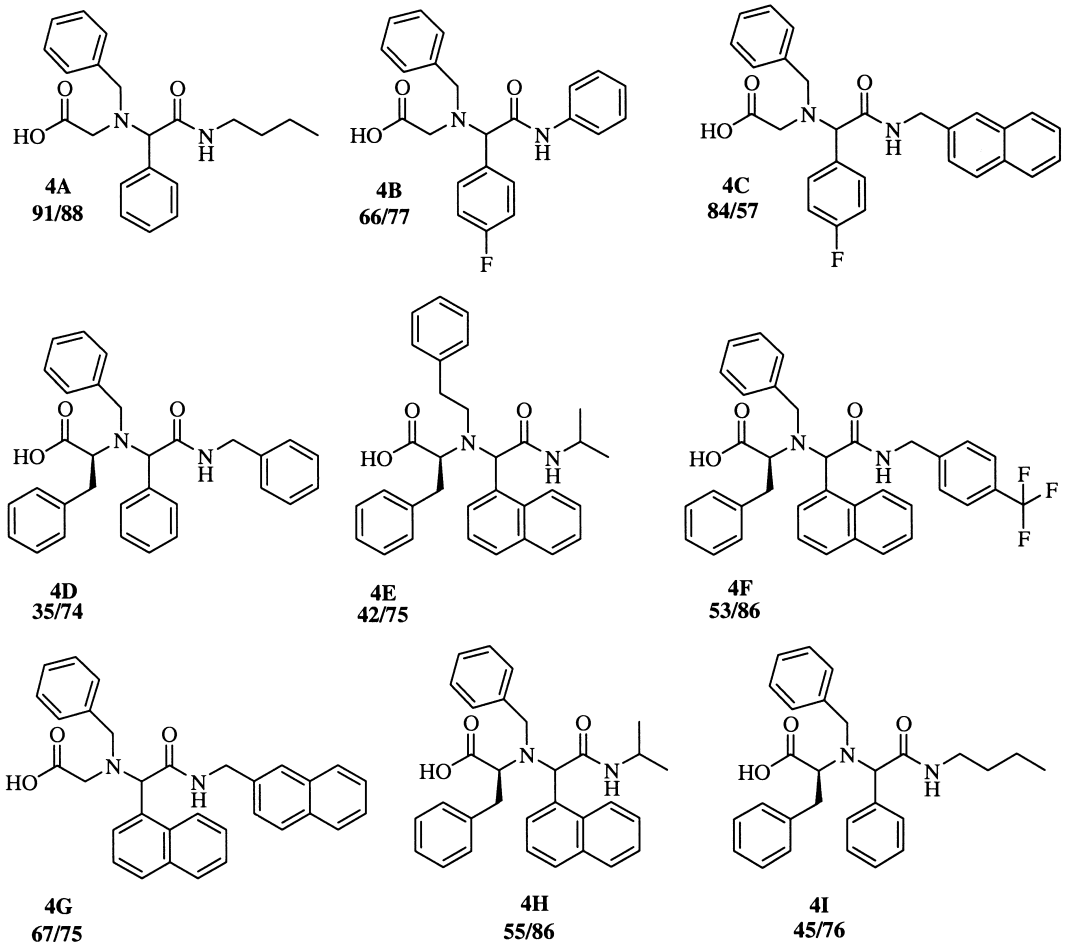
The chiral integrity of the products were tested by synthesis of compound *rac*-**4I** from DL-phenylalanine. The resulting product mixture was separated by chiral HPLC[9] to cleanly resolve four enantiomerically pure diastereomers. Product **4I** from L-phenylalanine was subjected to the same chiral HPLC conditions to identify possible epimerization; some epimerization had occurred (5–10%). The starting α-amino acid was shown to be optically pure following cleavage from the resin and chiral HPLC analysis. Therefore, the observed epimerization is a consequence of the reductive alkylation/Petasis chemistry or the inherent instability of the product mixtures.[10] We are currently looking at methodology to minimize this problem and the results of these efforts will be discussed in future communications.

The presented protocol is readily amenable to high-throughput organic synthesis and has been used in 96-well plate format (Robbins Flexchem® Reactor Blocks) to synthesize libraries of peptide mimetic analogs. This chemistry is capable of incorporating four pieces of chemical diversity which significantly increases the potential size of the virtual library. Use of a multicomponent reaction as the key synthetic transformation allows construction of the desired peptide mimetics in only five steps. The product purity[11] is routinely greater than 75% with synthetic yields averaging more than 90% per synthetic step.

In summary, the solid phase synthesis of peptide mimetics utilizing the Petasis reaction was developed. The chemistry is mild in nature and utilizes starting materials which are commercially available and readily abundant. The presented protocol is simple, efficient and has the ability to quickly deliver large numbers of pharmaceutically relevant compounds for high-throughput screening.

Typical experimental procedures: Wang resin ester **2A** and its free acid (TFA salt): Fmoc-glycine loaded Wang resin (1.00 g, 0.44 mmol/g, Advanced ChemTech) was rinsed three times with dichloromethane (DCM) then treated with a 25% solution of piperidine in dimethylformamide

Table 1
Representative products and results. Crude yields (%) and purities (%, LC/MS with ELSD)



(DMF) for 45 min. The resin was filtered and washed several times with DMF then alternating DCM and methanol (MeOH) multiple times followed by trimethylorthoformate (TMOF) three times. The following reductive alkylation procedure was modified from that reported by Campbell et al.[5b] The resin was swelled in TMOF (10 mL) and to this was added benzaldehyde (0.412 mL, 8 mmol) and the resin was agitated for 1 h. The resin was filtered and rinsed three times with TMOF before adding a solution of sodium cyanoborohydride (0.496 g, 8 mmol.) in 1% AcOH/TMOF. The mixture was agitated for 15 min (during which time the reaction vessel was periodically vented), filtered and washed multiple times with alternating DCM and MeOH. Approximately half of the resin (0.22 mmol) was treated with 95% TFA/H_2O for 1 h. The resin was filtered and washed several times with MeOH. The filtrate and washings were combined and evaporated to dryness. Following co-evaporation of the resulting oil with chloroform (CHL), the product was vacuum dried to yield the free acid of **2A** as a white solid 60 mg (98%). ^{1}H NMR (300 MHz, CD_3OD, δ) 7.60–7.40 (m, 5H), 4.30 (s, 2H), 3.70 (s, 2H). $^{13}C\{^1H\}$ (150 MHz, $CDCl_3$, δ) 162.7, 131.0, 130.5, 129.8, 129.4, 51.1, 47.9. MS (DCI): m/z 166 $[M+H]^+$. Compound **4A** (TFA salt): The

remaining resin (0.22 mmol) was swelled in DCM (10 mL) and to this was added phenylboronic acid (0.244 g, 2 mmol) and glyoxylic acid (0.184 g, 2 mmol). The reaction was agitated for 18 h at room temperature, filtered and rinsed three times with DCM. The reaction was repeated for an additional 60 h. The resin was filtered and washed with DCM and MeOH several times in an alternating fashion. The resin was swelled in DMF (10 mL) and to this was added hydroxybenzotriazole (HOBt) (0.306 g, 2 mmol) followed by diisopropylcarbodiimide (DIC) (0.313 mL, 2 mmol). This reaction mixture was agitated for 1 h at room temperature before filtering and rinsing three times with DMF. To the resin was added DMF (5 mL) followed by *n*-butylamine (0.195 mL, 2 mmol) and the reaction was agitated for 16 h. The resin was filtered and washed five times with DMF followed by alternating DCM/MeOH washes. The resin was subjected to a cleavage cocktail of 95% TFA/H_2O for 1 h. The resin was filtered and washed several times with MeOH. The filtrate and washings were combined and evaporated to dryness. Following co-evaporation of the resulting white oil with CHL, the product was vacuum dried for 60 h to yield **4A** as a white solid 90.9 mg (88.3%). ^{1}H NMR (300 MHz, CD_3OD, δ) 7.52–7.35 (m, 10H), 4.95 (s, 1H), 4.14 (d, J_{ab} = 12 Hz, 2H), 4.02 (d, J_{ab} = 12 Hz, 2H), 3.38 (d, J_{ab} = 17.8 Hz, 2H), 3.27 (td, J = 6.8, 2.2 Hz, 2H), 3.14 (d, J_{ab} = 17.8 Hz, 2H), 1.56–1.45 (m, 2H), 1.38–1.25 (m, 2H). ^{13}C{^{1}H} (150 MHz, CD_3OD, δ) 172.4, 171.5, 135.8, 135.0, 129.9, 129.6, 129.1, 128.9, 128.8, 128.4, 70.4, 56.9, 50.1, 39.2, 31.4, 20.0, 13.0. MS (DCI): m/z 355 $[M+H]^+$.

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7. The mechanism of this reaction has not been fully elaborated. For a brief discussion see Ref 3a.
8. In most cases diastereomers could be separated by reverse-phase HPLC and rarely showed greater than 1:1 selectivity. One possible explanation for this is that the amines in the above examples did not contain the (*S*)-2-phenylglycinol substitution demonstrated by Petasis to increase diastereomeric ratios (see Ref. 3b).
9. Column: Chiralcel OD-R, 4.6×150 mm; mobile phase: 78% methanol:22% water:0.1% formic acid (v/v/v); flow rate: 1 mL/min; UV detection at 210 nm.
10. The configurationally labile intermediate in the Petasis sequence may be the imino ester. Related imino acid analogs have been shown to be configurationally labile under acid. See Lubell, W. D., et al. *J. Org. Chem*. **1996**, *61*, 9447.
11. Products analyzed for purity by LC/MS with ELSD and UV detection at 220 nm.