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Rapid generation of *cis*-constrained norstatine analogs using a $TMSN_3$-modified Passerini MCC/*N*-capping strategy†

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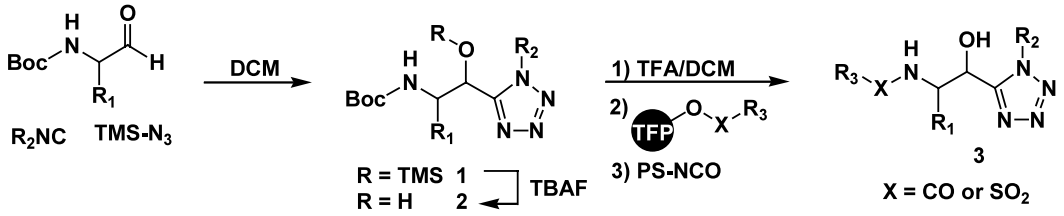
Abstract—A novel application of the $TMSN_3$-modified Passerini three-component reaction is disclosed for the rapid solution phase synthesis of *cis* constrained norstatine mimetic libraries. The reaction of an *N*-BOC-α-amino aldehyde, an isocyanide and trimethylsilylazide in dichloromethane, followed by deprotection and *N*-capping with TFP esters affords *cis* constrained norstatine mimetics. This efficient protocol, producing products with three diversity points, can be used to generate arrays of biologically relevant small molecules for protease targeted screening.

Aspartyl proteases are involved in a number of biological processes, including the progression of a variety of diseases,[1] and are therefore important therapeutic targets. These enzymes catalyze amide bond hydrolysis, a process which proceeds via a well known tetrahedral intermediate. Inhibitors of aspartyl proteases utilize a secondary alcohol as a stable mimetic of the tetrahedral intermediate, and common amide isosteres include hydroxyethylamine, hydroxyethylene, and hydroxymethylene. Several of these classes of compounds have proven amenable to the synthesis of libraries, leading to the discovery of a number of potent protease inhibitors.[2]

In the design of peptidomimetics it is often possible to enhance pharmacological properties of a molecule by replacing amides with amide isosteres.[3] Common replacements include *trans* olefins to mimic *trans* amides, and 1,5-disubstituted tetrazoles to mimic *cis* amides.[4] While amides generally prefer the *trans* conformation, proline and other *N*-alkylated amides show enhanced preference for the *cis* conformation. Biological activity of analogs with a tetrazole replacement provide strong evidence for the role of the *cis* amide conformation in receptor recognition, and hence the constraint is valuable in assessing the nature of enzyme–substrate interactions.

Abell has reported a combination of the above strategies, producing a tetrazole-based, *cis* constrained norstatine isostere as a new class of HIV-1 protease inhibitor.[5] Although these inhibitors are quite interesting for protease exploration, the linear synthesis is lengthy and not well suited for the production of arrays. We herein report the facile synthesis of analogous *cis* constrained norstatine mimetics. A $TMSN_3$-modified Passerini[6] reaction, combining *N*-BOC-α-amino aldehydes, $TMSN_3$, and isonitriles, gives a mixture of tetrazoles **1** and **2** in moderate to high yield (Scheme 1). The condensation products are then deprotected and *N*-capped with polymer-bound tetrafluorophenol[7] (TFP) esters to produce the con-



Scheme 1.

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† This article is dedicated to my daughter, Madison Lee Nixey.

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strained isostere **3** with three points of diversity. This methodology represents a significantly shortened route to this class of peptide mimetic and further illustrates the strength of multi-component condensation (MCC) methodology.

The use of azides in the Passerini reaction was first reported in 1961.[8] In a slight modification of Ugi's original procedure, $TMSN_3$ was employed as the azide source since it is less toxic/explosive than several commercially available alternatives and the byproduct, methoxytrimethylsilane, is volatile. A side product of further note was the TMS ether **1**, observed as up to 40% of the crude mixture by HPLC (UV 215 nm). The ether was readily hydrolyzed to the alcohol **2** with TBAF, or alternatively, the BOC and TMS groups can be removed concomitantly by treatment with TFA. The reaction proved general for a variety of isocyanides and *N*-BOC-α-amino aldehydes,[9] tolerating a range of functionalities. Following removal of the BOC group with TFA and treatment of the resulting salt with macroporous polystyrene-bound carbonate (MP-carbonate), the deprotected Passerini product was dissolved in DMF and added to various TFP esters. The slurry was then heated to give the desired amides and sulfonamides in good isolated yields.[10] A selection of both the initial condensation products and final *N*-capped norstatine mimetics are shown in Fig. 1, along with their corresponding yields.

Expanding to array synthesis, 80 resin bound TFP esters were added to a 96 well filter plate and the plate was assembled into a reaction frame. A solution of the free amine in DMF was pipetted into the plate, and the

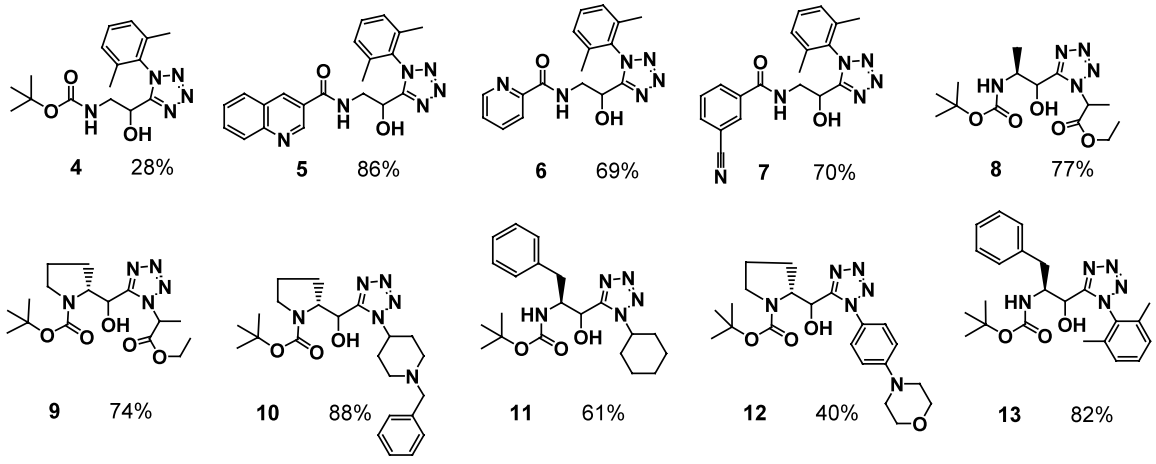
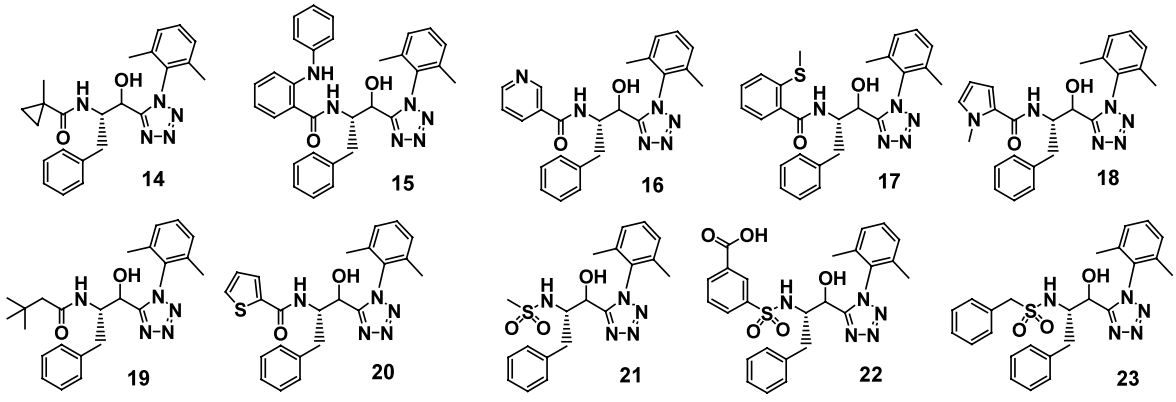


Figure 1.

Table 1.



Cmpd #	*A*%[a]	*A*%[b]	MH^+	Cmpd #	*A*%[a]	*A*%[b]	MH^+
14	73	83	406	**19**	30	69	422
15	71	76	519	**20**	36	77	434
16	68	77	429	**21**	56	84	402
17	69	80	474	**22**	67	79	508
18	66	80	431	**23**	74	83	478

[a] *A*% by LC/MS.[12]
[b] *A*% following PS-NCO scavenging.

plate was capped and heated in a shaker oven. The slurries were then filtered into a collection plate and evaporated. Final compound purities were improved (13% on average) by scavenging the unreacted amine with PS-NCO.[11] Several examples, obtained from reaction of TFP esters with the deprotected analog of compound **13**, are shown below. Area % (*A*%) data for crude and scavenged mixtures are listed in Table 1.

In summary, a novel solution phase procedure for the preparation of *cis* constrained norstatine mimetics has been reported. With final products containing three points of diversity and a facile and rapid production protocol, access to thousands of diverse analogues with the aforementioned core structure is now feasible.

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9. Preparation of compound **4**: Solutions of *N*-(*tert*-butoxycarbonyl)glycinal (0.1 M, 10 mL in DCM), 2,6-dimethylphenylisocyanide (0.1 M, 10 mL in DCM) and $TMSN_3$ (0.1 M, 10 mL in DCM) were added to a round bottom flask and stirred at rt for 18 h. The solution was concentrated and the resulting oil was fractionated by flash-column chromatography (1% MeOH/chloroform) to yield **4** as a white solid (93 mg, 28% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 7.37 (1H, dd, $J=7.5$, 7.5 Hz), 7.22 (1H, d, $J=7.5$ Hz), 7.20 (1H, d, $J=7.5$ Hz), 5.33 (1H, m), 4.78 (1H, m), 3.75 (2H, m), 2.00 (3H, s), 1.91 (3H, s), 1.39 (9H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.5, 155.5, 136.4, 135.1, 132.2, 128.8, 128.6, 80.6, 64.4, 44.6, 28.2, 17.5, 17.3. HRMS: MH^+ theoretical value 334.1874; actual value 334.1876. dM/M = 0.60 ppm.
10. Preparation of compound **5**: Compound **4** (12 mg, 0.036 mmol) was deprotected by reaction with 50% TFA/DCM (1 mL) for 5 min. The solution was concentrated and the resulting oil was dissolved in DCM, MP-carbonate (3.15 mmol/g, 50 mg) was added, and the mixture was shaken for 16 h, filtered, and concentrated. The oil was dissolved in DMF (1.2 mL) and was added to polymer bound quinoline-3-carboxylate TFP ester (54 mg, 0.83 mmol/g, 0.045 mmol). The slurry was heated at 60°C for 16 h, filtered, and the DMF was removed in vacuo. The resulting oil was purified by preparative HPLC to give **5** as an off-white solid (12 mg, 86% yield). ^{1}H NMR (400 MHz, $CDCl_3$): δ 9.85 (1H, s), 9.35 (1H, s), 9.12 (1H, m), 8.40 (1H, d, $J=8.5$ Hz), 8.18 (1H, d, $J=8.5$ Hz), 8.08 (1H, dd, $J=8.5$, 8.5 Hz), 7.89 (1H, dd, $J=8.5$, 8.5 Hz), 7.35 (1H, dd, $J=7.5$, 7.5 Hz), 7.21 (1H, d, $J=7.5$ Hz), 7.19 (1H, d, $J=7.5$ Hz), 4.98 (1H, m), 4.00 (2H, m), 1.99 (3H, s), 1.90 (3H, s). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.7, 144.7, 144.3, 140.0, 136.0, 135.8, 135.6, 131.5, 131.2, 130.4, 129.8, 129.0, 128.9, 128.0, 122.3, 62.8, 44.6, 17.3. HRMS: MH^+ theoretical value 389.1726; actual value 389.1735. dM/M = 2.3 ppm.
11. Production of an 80-member array was successfully completed using a 96 well filter plate encapsulated in a reaction frame assembly. A slurry of TFP resins (20 μmol), DMF (350 μL) and the free amine (18 μmol) was heated at 60°C for 18 h, cooled, and the mixture was then filtered into a collection plate and the solvent was evaporated in vacuo at 65°C. Scavenging with PS-NCO (1 equiv., 24 h) was performed to remove any remaining free amine. The secondary alcohol present in the products showed little reactivity towards the polymer bound isocyanate under the reaction conditions.
12. LC/MS analysis was performed using a C18 Hypersil BDS 3μ 2.1×50 mm column with a mobile phase of 0.1% TFA in CH_3CN/H_2O, gradient from 10% CH_3CN to 100% over 15 min. The HPLC was interfaced with an APCI probe, and detection was performed at UV 215 nm.