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Report

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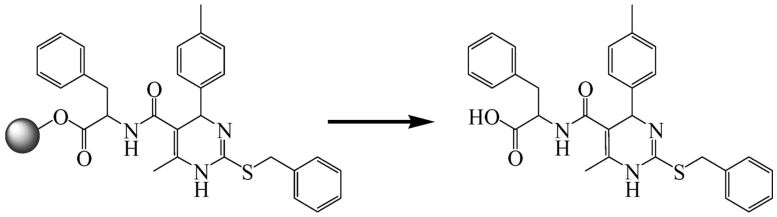

5a **6a**

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Reports

Solid-Phase Synthesis of α-(2-(Benzylthio)-1,4-dihydro-6-methyl-4-*p*-tolylpyrimidine-5-carboxamido) Acids: a New Strategy To Create Diversity in Heterocyclic Scaffolds

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Combinatorial chemistry has become an increasingly important tool for developing new candidate drugs for fighting diseases. The most widely used strategy for library preparation is solid-phase organic synthesis (SPOS), with a large number of reactions having been successfully established on solid support.

In the past 10 years, interest in the Biginelli three-component reaction[1,2] has increased rapidly[3−8] because of the diverse range of pharmacological properties exhibited by some Biginelli compounds,[9−14] such as calcium channel modulation,[10] vasorelaxant activity,[11] α_{1a}-adrenergic receptor antagonism,[12] neuropeptide Y (NPY) antagonism,[13] and antimitotic activity,[14] yet only limited dihydropyrimidine libraries have been reported.[7c,d,8a] Study on solid-phase Biginelli reaction also remains very unexplored.[7,8] Because of the biological significance of the Biginelli-type compounds and our interest in solid-phase synthesis of biologically active compounds to modulate the HIV-1 replication cycle,[15,16] we have designed different pathways for building combinatorial libraries containing a dihydropyrimidine scaffold. One pathway involves introducing amino acids into this core scaffold structure. We reasoned that incorporation of amino acids should not only increase the water solubility of compounds, but should also increase library diversity, including the diversity of biological properties. Amino acids between the core structure and solid support can also act as linkers and spacers that may facilitate the solid-phase cyclocondensation reaction. Herein, we present the first solid-phase synthesis protocol of dihydropyrimidines bearing an amino acid at the 5-position in very high yields and purity suitable for preparation of large libraries.

Among a wide selection of resins, we utilized Wang resin, which is a common and cost-effective resin for solid-phase peptide synthesis and solid-phase organic synthesis. *N*-Fmoc-protected α-L-phenylalanine (Fmoc-Phe-OH, **1a**), *p*-tolualdehyde, and 2-benzyl-2-thiopseudourea hydrochloride were selected for optimization of solid-phase Biginelli reaction conditions (Scheme 1). Direct cleavage ^{1}H NMR determination[17] was used to follow the reaction. The amino acid was linked to the solid support by standard DIC/DMAP method. The Fmoc group was deprotected by 20% piperidine in DMF to afford phenylalanine attached resin **2a**. The 1,3-diketone fragment was then linked to **2a** by acetoacetylation of the N-terminal end of the resin bound amino acid with diketene, which was introduced to the resin/DCM slurry very slowly under shaking in a dropwise manner. The reaction was kept at 0 °C for 30 min and then allowed to shake at room temperature for 4 h. A Kaiser test showed complete disappearance of the free amine group, resulting in the formation of resin-bound acetoacetamide derivative **3a**. About 10 mg of the beads **3a** was treated with TFA/$CDCl_3$ (1:1) solution containing hexamethyldisiloxane (HMDSO, 0.74 mM, 100 μL) at room temperature for 3 h in a NMR tube and then was diluted with DMSO-d_6 (600 μL). The direct cleavage ^{1}H NMR spectra demonstrated that clean reactions occurred in the first three steps.

Knoevenagel condensation[18] of polymer-bound β-acetoacetamido acid **3a** with 10 equiv of *p*-tolualdehyde in the presence of piperidinium acetate (1 equiv) in anhydrous toluene or 1,2-dichlorobenzene at 80 °C for 4 h produced desired polymer bound enones **4a**. Compared to the impurities generated in the absence of molecular sieves, the reaction led to quantitative conversion and significantly high purity in the presence of the water absorbent according to the direct cleavage ^{1}H NMR spectrum and LC/MS spectra of this NMR solution. The ^{1}H NMR spectrum indicated the formation of both trans and cis isomers in approximately 1:1 ratio. The resulting immobilized material, composed of a mixture of two isomers, was used directly for the next step of the reaction. Therefore, enone-bound resin **4a** was swollen in anhydrous NMP and mixed with 2-benzyl-2-thiopseudourea hydrochloride (5 equiv), cesium carbonate (6 equiv), and a few grains of molecular sieves (4 Å) as a water absorbent. The reaction was carried out at 80 °C for 24 h under nitrogen atmosphere to give resin-bound 1,4-dihydropyrimidine **5a**, in which the α-L-phenylalanine was linked to the 5-position via a carboxamido bond. Again, direct cleavage ^{1}H NMR spectrum (Figure 1), and LC/MS spectra of this NMR solution not only confirmed the expected structure of **6a**, but also indicated that the former five-step reactions are almost clean. In our case of solid-phase Biginelli reaction, two diastereomers were formed because of the existence of α-L-phenylalanine. The reaction did not show diastereoselectivity at the 4-position chiral center, and both diastereomers had been formed in approximately equal amounts, as

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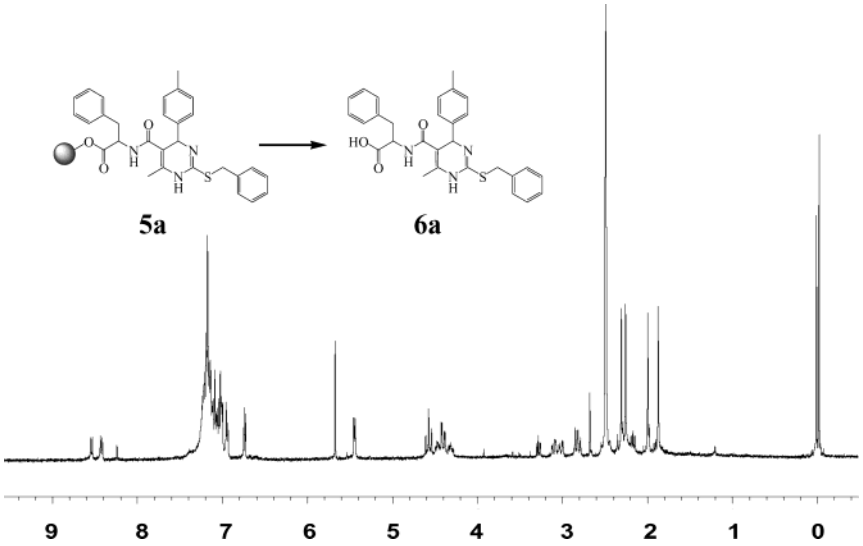
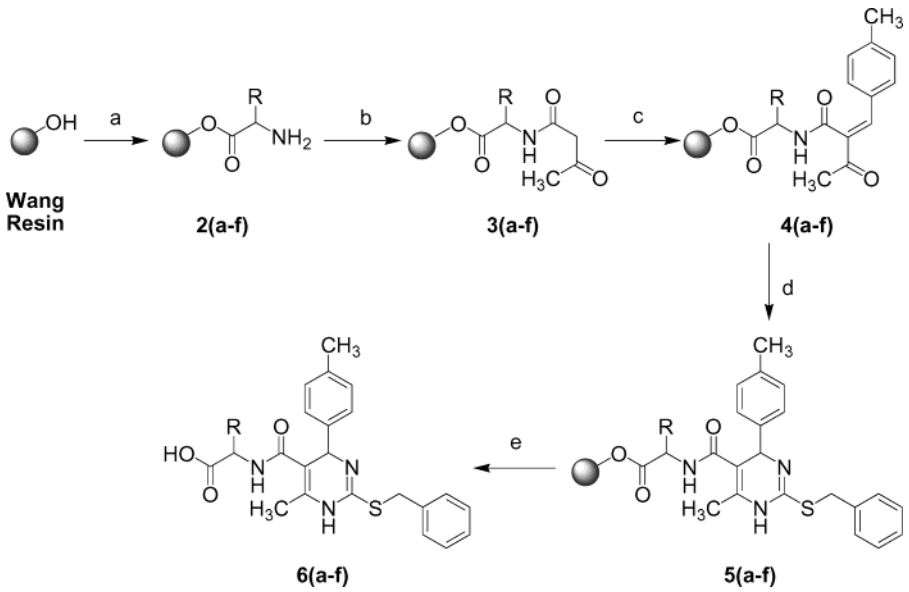


Figure 1. ^{1}H NMR spectrum of direct cleavage **5a** → **6a**, diluted with DMSO-d_6.

Scheme 1[a]



[a] (a) (i) Fmoc-NHCH(R)COOH **1**(**a**−**f**) (5 equiv, 1 M, DMF), DIC (5 equiv), DMAP (0.1 equiv, 0.1 M, DMF), room temp, 12 h. Repeat once. (ii) 20% piperidine, DMF, room temp, 15 min. Repeat twice. (b) diketene (10 equiv), DMAP (0.1 equiv, 0.1 M, DCM), 0 °C, 30 min, room temp, 4 h. (c) *p*-Tolualdehyde (10 equiv), piperidine (0.5 equiv, 1 M, toluene), AcOH (0.5 equiv, 1 M, toluene), molecular sieves, toluene, 80 °C, 4 h. (d) *S*-Benzylisothiourea hydrochloride (5 equiv), Cs_2CO_3 (6 equiv), NMP, 80 °C, 24 h. (e) TFA/H_2O/TIPS (95:2.5:2.5), room temp, 3 h.

demonstrated in the ^{1}H NMR spectrum of **6a**. Thus, the 6-methyl and 4-*p*-tolyl methyl groups are observed as two pairs of singlets at δ 1.85/2.00 (3H) and 2.05/2.10 ppm (3H). We collected the product as a mixture of two diastereomers without further separation. There were no other major impurity signals in the direct cleavage ^{1}H NMR spectrum except the reference peaks of HMDSO at δ ~0 ppm (2 s) and some solvent signals.

The above optimized reaction conditions were then applied to solid-phase parallel synthesis of six different amino acids carrying a side chain that included positively charged arginine and lysine, negatively charged glutamic acid, heterocycle containing histidine, hydroxyl bearing serine, and phenylalanine. Fmoc-protected amino acids Fmoc-Phe-OH (**1a**), Fmoc-Arg(pbf)-OH (**1b**), Fmoc-Lys(BOC)-OH (**1c**), Fmoc-His(Trt)-OH (**1d**), Fmoc-Glu(^{t}Bu)-OH (**1e**), and Fmoc-Ser(^{t}Bu)-OH (**1f**) were used for the parallel synthesis. Fmoc-Phe-OH (**1a**) was used as a referential control. All six Fmoc-protected amino acids **1**(**a**−**f**) were initially coupled to Wang resin utilizing the DIC/DMAP procedure to give resin-bound amino acids **2**(**a**−**f**). Cleavage of the Fmoc protection group and acetoacetylation of the free amine group with diketene led to resins **3**(**a**−**f**). The following solid-phase Knoevenagel condensation with *p*-tolualdehyde afforded polymer-bound enones **4**(**a**−**f**) as mixtures of trans/cis isomers. Finally, in all six cases, solid-phase Atwal-modified Biginelli cyclocondensation led to almost quantitative formation of polymer-bound α-(2-(benzylthio)-1,4-dihydro-6-methyl-4-*p*-tolylpyrimidine-5-carboxamido) acid diastereomers **5**(**a**−**f**). Each step of the reaction sequence was followed by direct cleavage ^{1}H NMR determination of ~10 mg of washed and dried resin. This solution was also analyzed by LC/MS (see Supporting Information). All ^{1}H NMR spectra showed that the side-chain protection groups were also removed during

the cleavage stage for direct cleavage ^{1}H NMR except resins **2a**−**5a** for the phenylalanine analogues. The arginine-bound resins **2b**−**5b** generally gave rise to slightly complicated ^{1}H NMR spectra, potentially because of slight decomposition under heating conditions. The selected protection groups (pbf, BOC, Trt, and ^{t}Bu ester) were stable under the conditions applied in these experiments. All resulting compounds in each step also seemed quite stable to the cleavage conditions. Finally, 100 mg of beads **5**(**a**−**f**) was treated with a solution of 95% TFA/2.5% H_2O/2.5% TIPS in a peptide synthesis tube at room temperature for 3 h. Evaporating solvents, washing the residue with diethyl ether, and lyophilization gave desired products **6**(**a**−**f**) as white or pale yellow solids in 54.8−76.4% of isolated yields and ∼95% purity. All the structures were confirmed by ^{1}H NMR and high resolution mass spectra (HRMS) (see Supporting Information). The two diastereomers were formed in all six cases in a ratio of approximately 1:1, as shown in ^{1}H NMR spectra for 6-CH_3, 4-CH, and 4-tolyl methyl groups of the dihydropyrimidine ring. To date, a critical point for Biginelli reaction is the resolution of enantiomers by tedious semipreparative chiral HPLC or biocatalytic methods.[19] Our strategy, using pure amino acid enantiomers to introduce a novel chiral center to the Biginelli dihydropyrimidine scaffold, afforded two diastereomers. Although we did not separate the two diastereomers, their separation should be quite straightforward using reversed-phase HPLC.

In conclusion, we have demonstrated a six-step method for solid-phase parallel synthesis of 5-amino acid-substituted 4-aryl-2-benzylthio-6-methyl-1,4-dihydropyrimidines. Each step of the reaction gave excellent conversions and very high purity. This procedure provides an easy methodology for preparing libraries of amino acid-substituted Biginelli compounds that are potentially bioactive and, therefore, will be important for both biological and medicinal applications.

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Supporting Information Available. Experimental procedures, direct-cleavage ^{1}H NMR, and LC/MS spectra for resin bound compunds **3**(**a**−**f**), **4**(**a**−**f**) and **5**(**a**−**f**). ^{1}H NMR and HRMS spectra of **6**(**a**−**f**). This material is available free of charge via the Internet at http://pubs.acs.org.

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