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2-(3-Aminopropyl)-4-pentenoic acid as a bio-compatible/cleavable linker for solid-phase organic synthesis

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Abstract—2-(3-Aminopropyl)-4-pentenoic acid lithium salt (**1**) was prepared and used as a bio-compatible, cleavable linker in solid-phase organic synthesis. The products were released from solid support through cycloelimination.

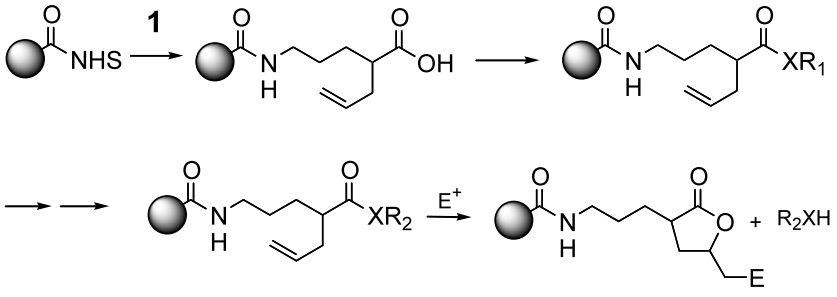
With the advances of combinatorial chemistry and solid-phase organic synthesis, numerous small molecule libraries have been generated.[1–3] An inherent part of a solid-phase synthesis is the choice of the linker, which should be stable under the reaction conditions and allow the product to be cleaved from the linker, with mild reagents, which can readily be removed. A number of linkers have been developed for solid-phase synthesis.[4,5] Normally the cleavage conditions are either acid/base, or light irradiation/fluoride ion, depending on the kind of linker used.

Our goal is to use combinatorial chemistry to develop small molecule affinity ligands for biomolecule separation. Libraries are generated on solid-phase and screened for affinity ligands directly on solid support, such as Agarose, which is the most widely used media for bio-separation. We required a linker which was sufficiently stable under a variety of reaction and screening conditions, biologically compatible (have minimal interference with screening targets), and one from which the ligands could be cleaved under mild conditions to monitor ligand incorporation.

4-Pentenoic acid has been widely used as a protecting group.[6,7] Based on this, we designed (Scheme 1) 2-(3-aminopropyl)-4-pentenoic acid lithium salt (**1**) as a new core reagent for building our linker. The non-aromatic, straight-chain short carbon-branched linker has minimal non-specific interference with the target protein, which permits us to screen the libraries directly on the beads. Herein we report our preliminary results on the synthesis of **1** and its use in solid-phase organic synthesis.

The synthesis of 2-(3-aminopropyl)-4-pentenoic acid lithium salt (**1**) is outlined in Scheme 2.

In a typical experiment, diethyl allylmalonate was slowly added to a suspension of 1.1 equiv. of sodium hydride in DMF and stirred at rt for 5 min. *N*-(3-Bromopropyl)phthalimide dissolved in DMF was added



Scheme 1. Design strategy of the new linker.

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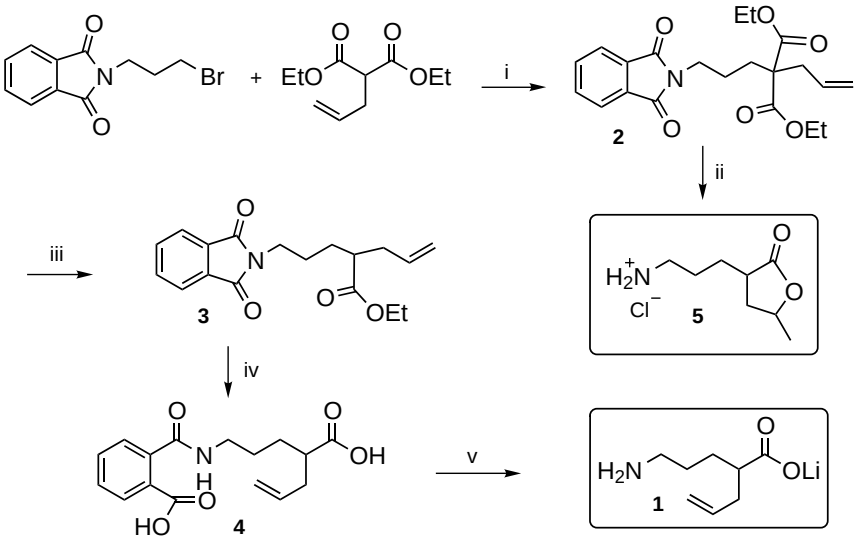
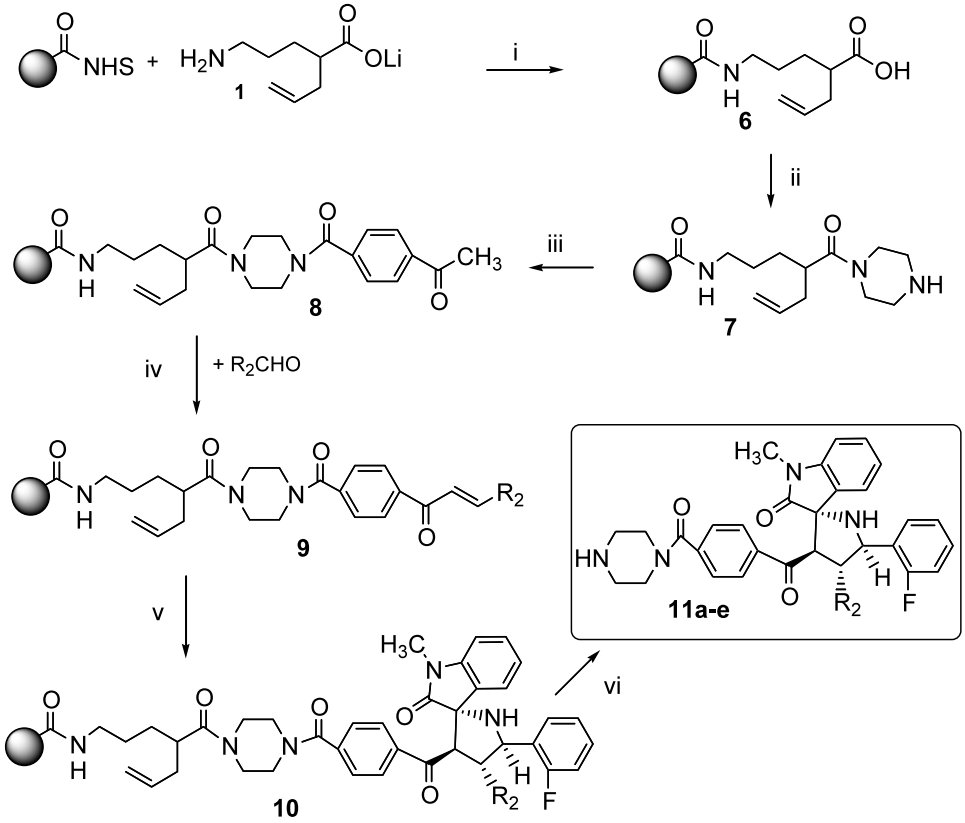


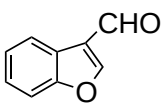
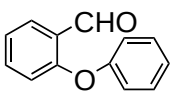
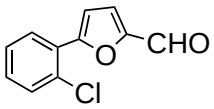
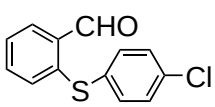
Scheme 2. The synthesis of 2-(3-aminopropyl)-4-pentenoic acid lithium salt. *Reagents and conditions*: (i) NaH/DMF, 60°C 24 h; (ii) 6N HCl, refluxing for 48 h; (iii) LiBr, H_2O, DMF refluxing 40 h; (iv) NaOH aq.; (v) (a) H_2NNH_2, (b) LiOH.

and the reaction mixture was stirred at 60°C overnight. The solution was slowly poured into 3 volumes of ice-water to produce **2** as a precipitate. After filtration and washing with water, HPLC showed that **2** was pure and it was used in the next step without further purification. Attempts to deprotect, hydrolyze and decarboxylate compound **2** by refluxing in 6 M HCl[8] led to the formation of **5** instead of **1**. Decarboxylation was achieved by refluxing **2** in DMF with 1 equiv. of LiBr and 2 equiv. of water for 40 h.[9] Compound **3** was converted to **4** by refluxing for 1 h with 2 equiv. NaOH (1 M aq.) in DMF, followed by 1 h in water. After



Scheme 3. Using a linker derived from **1** for solid-phase synthesis of a spiropyrrolidinone library. *Reagents and conditions*: (i) (a) 2 equiv. of **1** (corresponding to the loadings on solid-phase) in DMF, rt, 24 h, (b) 0.25 M HCl/DMF 30 min; (ii) 5 equiv. NHS and DIC in DMF, rt 24 h then 5 equiv. piperazine, rt 24 h; (iii) 3 equiv. HBTU, 3 equiv. *p*-acetylbenzoic acid and 10 equiv. NMM, DMF, rt 5 h; (iv) 5 equiv. aldehydes, 1 equiv. MeONa in EtOH, rt, 60 h; (v) 5 equiv. *N*-methylisatin and 2-(2-fluorophenyl)glycine in dioxane/water (5/1), rt, 60 h; (vi) 0.1 M iodine in THF/water (4/1), rt, 1 h.

Table 1. Aldehydes used in the spiropyrrolidinone library and molecular weights of product **11**

	a	b	c	d	e
R_2CHO					
Molecule weight **11**	606	628	680	688	730

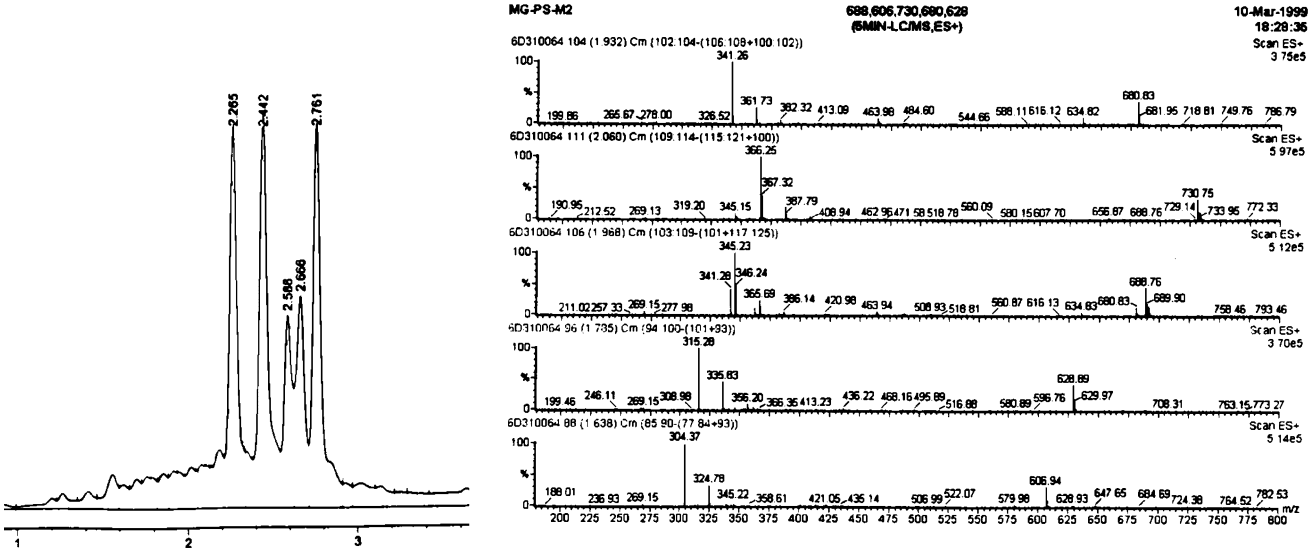


Figure 1. HPLC and LC-MS of **11** after cleavage.

removing the volatiles, 5 equiv. of hydrazine was added to the residue, and the mixture was suspended in absolute ethanol and heated at reflux overnight. Upon cooling, most of the by-product (phthalhydrazide) precipitated and was removed by filtration. To the filtrate was added 2 equiv. LiOH, and the solution was concentrated to provide lithium salt **1**[10] which was used without further purification for solid-phase synthesis.

Compound **1** was coupled to solid-phase (both Agarose[11] and Polystyrene[11]) using an NHS activated carboxyl group strategy and converted to free acid by treating with 0.25 M HCl/DMF.[12] A small spiropyrrolidinone library[13] was generated as shown in Scheme 3.

Thus, after standard amide bond formation (i, ii, iii; **6** to **8**), compound **8** was divided into five portions and treated with five different aldehydes (Table 1) to form chalcones **9**. The five portions were then pooled and treated with *N*-methylisatin and 2-(2-fluorophenyl)glycine to give **10** (the assignment of stereochemistry was based on an analogous system that had been reported in the literature[13]). After cleavage with iodine/water/THF, spiropyrrolidinone **11** were released and excess iodine was reduced with sodium sulfite. HPLC showed five peaks for the five compounds in the mixture. LC-MS gave a correct molecule weight for each compound (Fig. 1).

In conclusion, we used a simple and efficient route to make 2-(3-aminopropyl)-4-pentenoic acid lithium salt (**1**) and used it as a bio-compatible, cleavable linker for solid-phase organic synthesis.

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10. Compounds **2**–**5** all gave correct molecule weight on LC-MS. Compound **1** was analyzed by LC-MS and NMR. It contains about 10% phthalhydrazide but this does not affect its use as a linker.
11. NHS Sephose was supplied by Amersham Pharmacia Biotech; carboxypolystyrene was purchased from Nova Biochem.
12. 0.25 M HCl/DMF was made by mixing 1 ml 4N HCl aq. with 15 ml DMF.
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