JOURNAL OF combinatorial CHEMISTRY

Report

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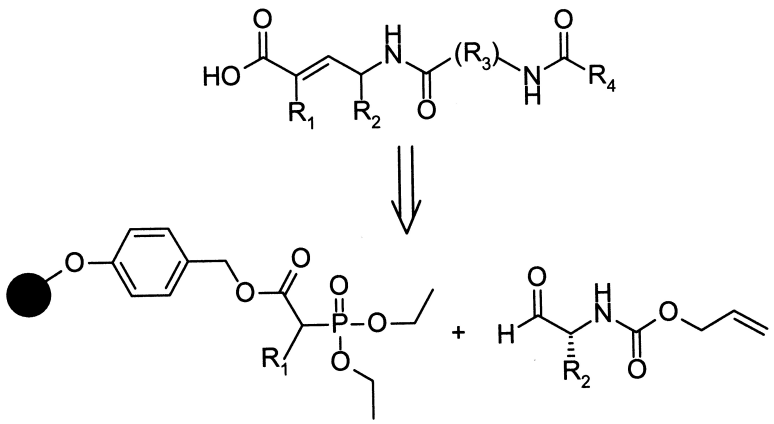
J. Comb. Chem., **2000**, 2 (6), 600-603• DOI: 10.1021/cc0000444 • Publication Date (Web): 21 October 2000



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Parallel Solid-Phase Synthesis of Peptidyl Michael Acceptors

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Received June 2, 2000

Introduction

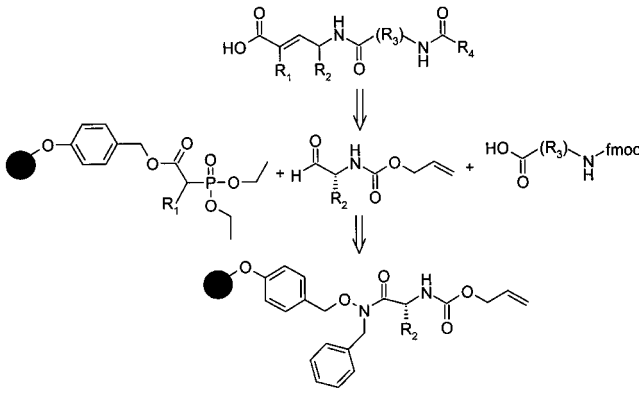
Cysteine proteases are a family of important enzymes that play a role in a number of biological phenomena including regulation of intercellular calcium levels (Calpain),[1] regulation of apoptosis (Caspases),[2] viral replication (human rhinovirus proteases),[3] as well as inflammation and antigen presentation (Cathepsins).[1] Chemical libraries of potential inhibitors are important tools to help identify selective inhibitors and establish structure−activity relationships within families of these proteases.

Recently, it has been disclosed that peptidyl Michael acceptors based on vinylogous amino acids inactivate cysteine proteases selectively and specifically.[3b] In light of this finding, a chemical library of peptidyl Michael acceptors would be highly desirable as potential tools to understand these proteases. Therefore we developed a synthetic strategy targeting a library of peptidyl Michael acceptors with four points of diversity. The envisioned route requires a resin bound phosphonoester, custom peptide aldehydes, and conformationally constrained peptidomimetics (Scheme 1 [retrosynthesis]) as building blocks. The key step in this synthetic sequence is a supported Horner−Emmons[4] reaction. It was desired to use a wide variety of N-protected amino acid aldehydes in the Horner−Emmons condensation. However, these materials are not readily commercially available. Thus, as a strategy, these aldehydes were produced in a parallel fashion from the resin bound Weinreb amides[5] of the readily available Fmoc-amino acids. The use of the resin bound Weinreb amide for parallel aldehyde generation offers several advantages over solution-phase synthesis. Among these advantages is the facile generation of a wide variety of aldehydes from almost any carboxylic acid. Also, the supported synthesis allows for the generation of a diverse number of products in parallel over a number of synthetic steps without laborious extractive workup procedures and chromatographic purification. Furthermore, this approach allows for the facile change from the readily available Fmoc-protecting group[6] to the more desirable Alloc-protecting group[7] for the amino acid nitrogen.

Results and Discussion

A series of small optimization libraries were generated around the designed synthetic route. These small focused

Scheme 1



Scheme 2

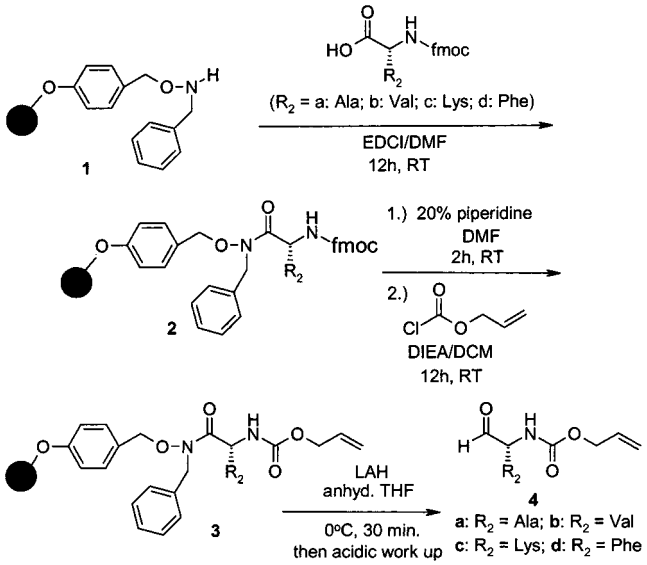
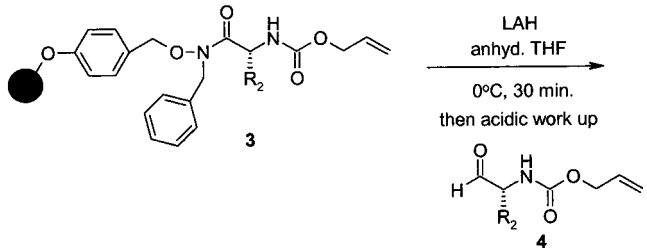


Table 1. Yields and Purities of Peptide Aldehydes



Compound	R2	% Yield[a]	Purity[b]
4a	CH_3 (Ala)	20	95
4b	$CH(CH_3)_2$ (Val)	12	90
4c	$(CH_2)_4NH_2$ (Lys)	23	85
4d	CH_2Ph (Phe)	13	95

[a] Crude yield. [b] Purity estimated by ^1H NMR.

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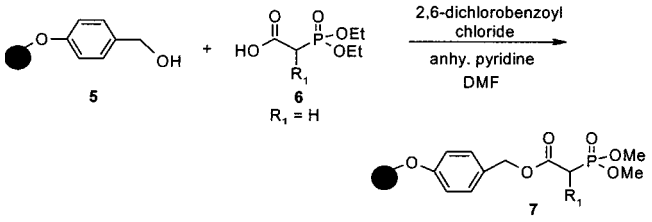
10.1021/cc0000444 CCC: $19.00
Published on Web 10/21/2000

Scheme 3



Scheme 4

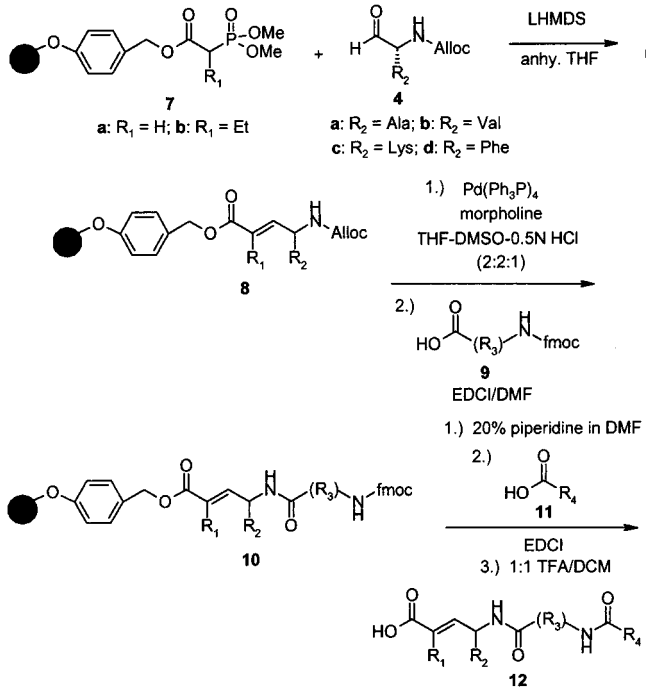
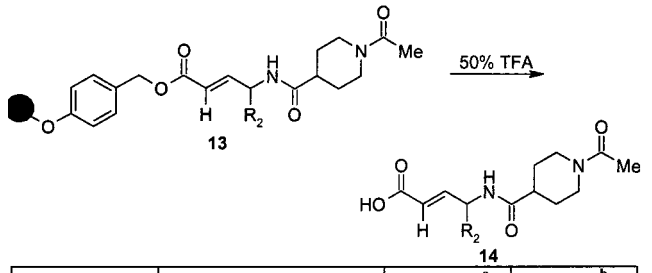


Table 2. Validation of R_2 Group: Horner−Emmons Reaction of Peptide Aldehydes



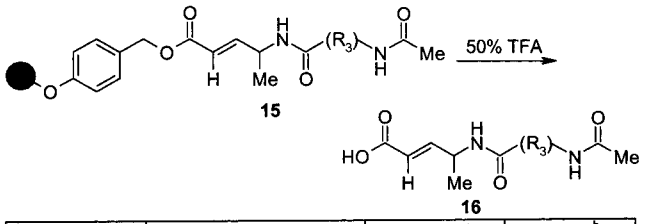
Compound	R2	% Yield[a]	Purity[b]
14a	CH_3 (Ala)	75	89
14b	$CH(CH_3)_2$ (Val)	92	92
14c	$(CH_2)_4NH_2$ (Lys)	87	76
14d	CH_2Ph (Phe)	81	99

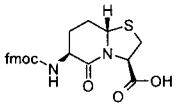
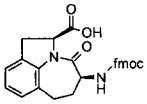
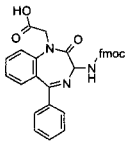
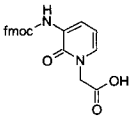
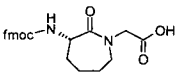
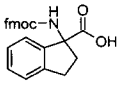
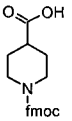
[a] Crude yield. [b] Purity estimated by HPLC (A% by ELS).

libraries served two purposes: (1) to verify the designed synthetic approach and (2) to confirm the validity of the individual reactants. The results of this exercise are discussed below.

To validate the feasibility of the designed synthetic route, a series of peptide aldehydes were required. The initial goal was to synthesize a library of peptide aldehydes from

Table 3. Validation of R_3: Peptidomimetic Coupling Reaction



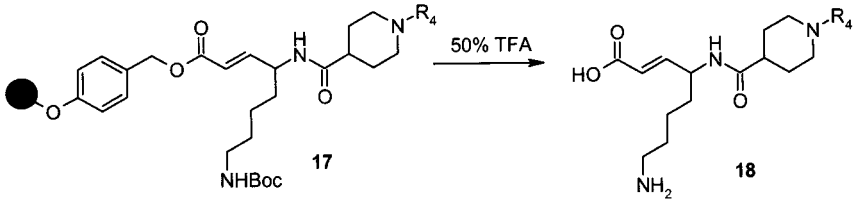
Compound	R3	% Yield[a]	Purity[b]
16a	 **9a**	68	65
16b	 **9b**	62	97
16c	 **9c**	71	95
16d	 **9d**	75	90
16e	 **9e**	78	90
16f	 **9f**	63	75
14a	 **9g**	75	80

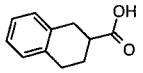
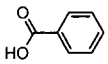
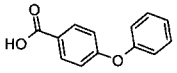
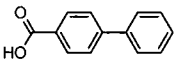
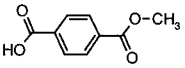
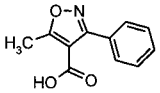
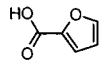
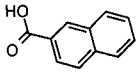
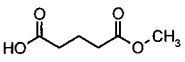
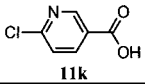
[a] Crude yield. [b] Purity estimated by HPLC (A% by ELS).

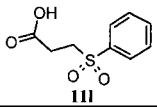
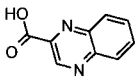
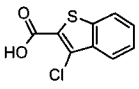
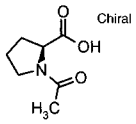
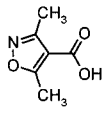
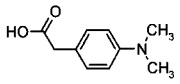
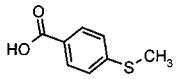
supported Weinreb amide.[8] This was accomplished via the intermediate benzyl alkylated Wang-O-hydroxylamine resin **1**.

The first step involved coupling Fmoc-protected amino acids[9] to the N-benzylated hydroxylamine resin (**1**) using EDCI to give the amides **2** (Scheme 2 [synthesis of aldehydes on solid-support]). Since it was foreseen that the aldehydes would be subjected to Horner−Emmons reaction conditions, the base-sensitive Fmoc-protecting group was replaced by the more robust Alloc-protecting group. First the Fmoc group was removed by treatment with 20% piperidine in DMF. At this point loading was determined by Fmoc cleavage quan-

Table 4. Validation of R_4: Acid Coupling Reaction



Compound	R4	% Yield[a]	Purity[b]
18a	 11a	81	76
18b	 11b	78	79
18c	 11c	68	90
18d	 11d	72	84
18e	 11e	85	75
18f	 11f	65	75
18g	 11g	70	77
18h	 11h	78	76
18i	 11i	69	92
18j	 11j	65	94
18k	 11k	75	99

Compound	R4	% Yield[a]	Purity[b]
18l	 11l	71	85
18m	 11m	67	99
18n	 11n	84	95
18o	 11o	77	78
18p	 11p	70	82
18q	 11q	66	98
18r	 11r	84	95
18s	 11s	80	75
18t	 11t	76	96

titation.[10] The resulting resin bound amine was treated with allylchloroformate and *N*,*N*-diisopropylethylamine.

The required peptide aldehydes were cleanly generated according to the protocol previously developed in our laboratory.[5c] The conditions require treating the N-alkylated resin **3** in THF at 0 °C with LAH for 30 min followed by workup with aqueous potassium hydrogensulfate and saturated Rochelle salt. The reaction mixture was dried with anhydrous sodium sulfate and then filtered through a short plug of silica gel to afford the aldehydes **4** in good purities. The purity range was estimated to be between 85% and 95% as determined by ^{1}H NMR (Table 1 [yields and purities of peptide aldehydes]). This method readily provided up to 20 new aldehydes on a 300−500 mg scale.[11]

The resin bound phosphonoester utilized in the Horner−Emmons reaction was prepared from Wang resin[4a] (Scheme 3 [synthesis of phosphonoester resins]). Diethylphosphonoacetic acid was loaded onto Wang resin to give the phosphonoester solid support (**7**: R_1 = H) that would serve as the starting resin for the main library. With both the peptidyl aldehydes and phosphonoester resin in hand, the critical supported Horner−Emmons reaction was ready to be tested (Scheme 4 [synthesis of peptidyl Michael acceptors]). This was carried out under anhydrous conditions using

LHMDS as base. To validate the synthetic approach, a small library utilizing aldehydes **4a**−**d** was generated. Since the supported Horner−Emmons reaction is sensitive to moisture and purity of the aldehydes, the results of this mini-library served as a good validation of the aldehyde synthesis. The aldehydes reacted well as demonstrated by synthesizing a small library, and the substituted olefins (**14**) were produced in good yields and purities (Table 2 [yields and purities of R_2]). It was noted that of the five peptide aldehydes attempted, the *tert*-butyl-protected aspartic acid gave poor results. Upon cleavage, ^{1}H NMR evidence suggested that the carboxylic acid group undergoes an intramolecular cyclization with the olefin to generate a lactone instead of the desired Michael acceptor.

The Alloc group was removed by mild treatment with palladium catalyst. The third point of diversity, the constrained peptidomimetic, was introduced. The conformationally constrained peptidomimetics (**9**) were reacted with the resin bound amine using EDCI. These were also validated by the generation of an optimization library. Good yields and purities resulted upon cleavage of the mini-optimization library with TFA (Table 3 [yields and purities of R_3]).

After removing the Fmoc group, the final point of diversity was attached. This was carried out by reacting various carboxylic acids (**11**) with resin bound amine using EDCI as the coupling reagent. Cleavage with 1:1 TFA/DCM furnished the final desired products. The mini-optimization library gave good yields and purities (Table 4 [yields and purities of R_4]).

Conclusion

A general solid-phase approach for the efficient parallel synthesis of peptidyl Michael acceptors has been developed. This method furnishes a number of potential inhibitors of cysteine proteases. The approach features the efficient synthesis of peptide aldehydes in parallel from resin bound Weinreb amides. The quick and easy conversion of the Fmoc-amino acids to the desired N-Alloc-protected amino aldehydes allowed for the rapid validation of the chemical sequence. This approach worked very well to rapidly survey the initial chemistry on resin on a 20 mg to 1 g scale. Future work in our laboratory will focus on library production.

Supporting Information Available. Experimental Section and ^{1}H NMR spectra for compounds **4a**−**d**, **14a**−**d**, **16a**−**f**, and **18a**−**k**. This material is available free of charge via the Internet at http://pubs.acs.org.

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(6) (a) Atherton, E.; Logan, C. J.; Sheppard, R. C. *J. Chem. Soc., Part 1* **1981**, 538. (b) Carpino, L. A.; Mansour, E. M. E.; Knapczyk, J. *J. Org. Chem.* **1983**, *48*, 666. (c) *Novabiochem Catalog & Peptide Synthesis Handbook*, 1999; p S52.

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(8) Evidence indicated that the amino acid aldehydes underwent racemization. The extent of epimerization was observed to be about 50% by LC/MS in the final cleaved products.

(9) A wide variety of Fmoc-protected amino acids are readily available at a cheaper cost than the ultimately desired N-Alloc analogues.

(10) Bunin, B. A. In *The Combinatorial Index*; Academic Press: San Diego, CA, 1998; p 219.

(11) A variety of conditions for the reductive cleavage were investigated in an effort to improve the yield of aldehydes. The conditions presented in the text are fairly optimized to give the cleanest possible recovery of the desired aldehydes with minimal post-cleavage workup. Raising the temperature of reaction led to significant over-reduction. Other reducing agents gave inferior results. Acid cleavage of the resins after treatment with LAH indicated that a small amount of hydroxylamine remained attached to the resin. However, a second treatment with LAH led to the recovery of only a small amount of aldehyde of significantly lower purity than the premiere treatment.

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