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Novel Hydrazino-Carbonyl-Amino-Methylated Polystyrene (HCAM) Resin Methodology for the Synthesis of P_1-Aldehyde Protease Inhibitor Candidates

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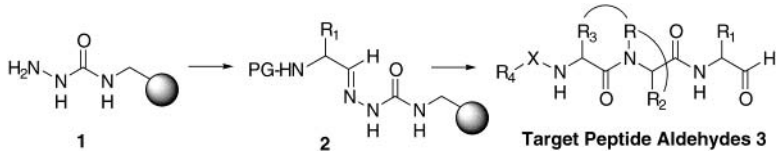
ABSTRACT



A new strategy for the synthesis of peptidyl and peptidomimetic P_1-aldehydes 3 on HCAM solid support is described. The appropriate C-terminal aldehyde precursors were prepared and anchored to a resin support via a semicarbazone linkage (HCAM resin). After synthetic elaboration, acidic hydrolysis efficiently delivered C-terminal target aldehydes 3a–h in good overall yields and in excellent purity.

Combinatorial technology is at the forefront of organic and medicinal chemistry and is receiving widespread attention as a powerful tool in drug discovery and optimization.[1] We recently described tethered semicarbazone[2] and aminal linker-based[3] methodologies for the construction of P_1-argininal libraries. In connection with our exploratory protease inhibitor platforms,[4] a novel and convenient protocol for the combinatorial production of peptidyl and peptidomimetic P_1-aldehyde (PA) libraries was sought. In addition to complementing our existing methods, the criteria established for products derived from this new protocol included (a) diversity at the P_1-position, (b) satisfactory overall yields, (c) high chemical purity, and (d) retention of chiral integrity of the labile P_1-aldehyde α-center. In this letter, we describe an efficient route to the title compounds **3** by application of a novel Hydrazino-Carbonyl-Amino-Methylated polystyrene resin (HCAM resin).[5]

PA derivatives serve as transition-state analogue (TSA) inhibitors of numerous classes of proteolytic enzymes and

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(5) Application of this technology to the synthesis of novel, potent P_1-argininal urokinase inhibitors was recently disclosed by Corvas: Weinhouse, M. I.; Roberts, C.; Cohen, C. R.; Bradbury, A. E.; Ma, M. G.; Dixon, S. A.; Nolan, T. G.; Tamura, S. Y.; Brunck, T. K. 217th National American Chemical Society Meeting, Anaheim, CA, March 21−25, 1999; MEDI.090.

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are of high current interest due to their potent biological activities.[4,6] A variety of solid-phase synthetic (SPS) approaches to PA's have been described.[7] Our HCAM technology features the simultaneous protection and resin anchoring of *N*-α-protected P_1-aldehyde synthons as their semicarbazone derivatives. In contrast to typical aldehyde-linker arrays,[1,2,3a,7] these are attached directly to the resin matrix without the agency of a tether. Subsequent SPS techniques then allow for the ready preparation of targeted PA combinatorial library members **3**.

Our global synthetic strategy is outlined in Figure 1. The

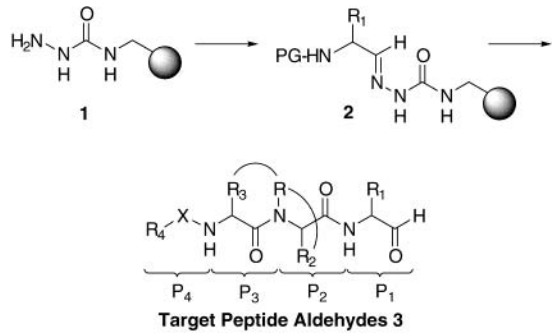
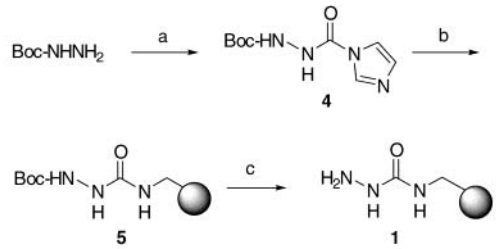


Figure 1. Strategy for the SPS of P_1-peptidyl aldehyde libraries **3** via application of the HCAM resin matrix **1**. Curved lines delineate optional ring systems. X = direct link, CO, OCO, NHCO, or SO_2.

HCAM resin **1** is prepared from commercial amino methylated polystyrene resin (AMPS resin) and condensed with an appropriate *N*-α-protected peptide aldehyde derivative to provide intermediate **2**. This simple but key step serves to (a) link the aldehyde to the solid phase via a short "traceless" tether[8] and (b) protect the aldehyde functionality as a stable, selectively cleavable semicarbazone species that preserves the chiral integrity of the original α-methine center due to attenuation of pK_a/reactivity.[2a,7a] Typical SPS chemical manipulations (deprotection, coupling, orthogonal side-chain reactions, etc.) on intermediate **2** followed by a final hydrolysis releases the elaborated targets **3** from the solid support. In practice, this methodology has proven to be of general scope and has facilitated the preparation of small, focused combinatorial libraries containing up to 500 members. Examples of specific targets prepared by parallel techniques will be presented herein which embrace a range of structural variety.

Complementary routes for the synthesis of HCAM resin **1** are shown in Scheme 1.[9] Our foray entailed reaction of

Scheme 1

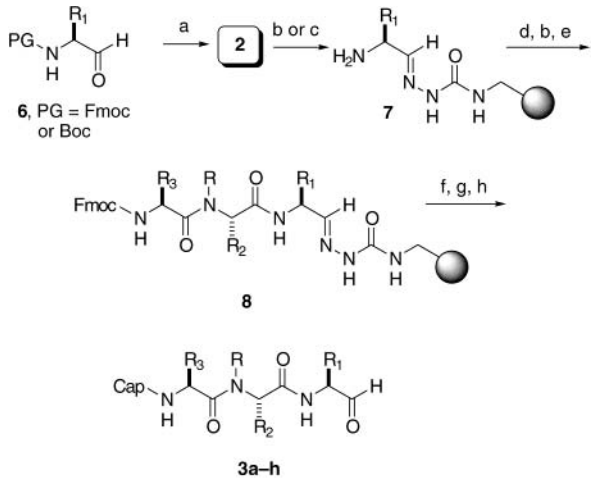


Reagents and conditions. (a) CDI, DMF, rt, 2.5 h; (b) AM resin, DMF, rt, 16 h; (c) 1. TFA, CH_2Cl_2:1, 1, rt, 20 min; 2. DIEA, CH_2Cl_2. *All yields ~quant.*

tert-butyl carbazate with 1,1′-carbonyldiimidazole in DMF to generate **4**,[2a] which underwent a smooth displacement reaction with AM resin to provide **5**. Intermediate **5** was then treated with trifluoroacetic acid to afford HCAM resin **1**.[2b] The Boc-protected HCAM resin intermediate **5** is a very stable, robust material that is well suited for combinatorial library production. It can be prepared on large scales (>100 g) and is stable upon storage for at least 9 months.

Application of HCAM resin **1** to the production of P_1-aldehyde libraries **3** is outlined in Scheme 2. The targets were

Scheme 2



Reagents and conditions. (a) **1**, CH_2Cl_2, rt, 10-18 h; (b) DMF, piperidine: 7,3, rt ,20-30 min; (c) TFA, CH_2Cl_2, thioanisole: 3, 6, 1, N_2 purge, rt, 25 min; (d) Fmoc-AA_n-OH, PyBOP, DIEA, DMF, rt, 16 h; (e) repeat steps d and b until P_3 (or P_n) residue is attached; (f) optional sidechain and/or N-deprotection and capping of N-terminus, see text; (g) TFA, CH_2Cl_2, H_2O: 8,1,1, rt, 1 h; (h) RP-HPLC purification.

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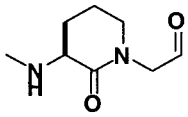
Table 1. Peptidyl and Peptidomimetic P_1-Aldehyde Derivatives **3a**−**h** Produced via Scheme 2

Compd. 3	Cap[a]	P_3	P_2	P_1	Mass Spec[b]	HPLC Purity[c] (%)	Overall Yield (%)
a	Cbz	homo-Glu	Sarc	(*S*)-Arg-al	507	97	37
b	$BnSO_2$			(*S*)-Arg-al	467	98	50
c	PhCO	Asp	nor-Val	(*S*)-Propargyl Gly-al	416	99	45
d	PhCO	Asp	nor-Val	(*S*)-Allyl Gly-al	418	99	42
e	PhCO	Asp	nor-Val	(*S*)-Cys(Me)-al	438	99	33
f	MeCO	Asp	nor-Val	(*S*)-Propargyl Gly-al	354	99	31
g	MeCO	Asp	nor-Val	(*S*)-Allyl Gly-al	356	99	40
h	MeCO	Asp	nor-Val	(*S*)-Cys(Me)-al	376	99	29

[a] Cap = N-terminal amino capping group. [b] Identity confirmed by low-resolution mass spectra, value reported is for MH^+. [c] RP-HPLC analysis performed using two independent gradients, water/CH_3CN with 0.1% TFA.

synthesized using either commercial materials or readily accessible intermediates. Although the scheme depicts products possessing the *all*-(*S*)-α-configuration, we have successfully applied this methodology to targets featuring (*R*)- or (*S*)-centers or various combinations thereof. Initial resin loading as well as coupling progress was monitored via the Kaiser (ninhydrin) test.[10] Reaction of **1** with representative hydrophobic or hydrophilic *N*-α-protected peptide aldehyde derivatives **6**[11,12] delivered semicarbazones **2** which were deprotected to afford the key resin-bound intermediates **7**. Several acylation reactions of **7** were successful, including iterative PyBOP-mediated peptide couplings via sequential *N*-α-Fmoc amino acid piperidine deblocking protocols, and produced the *N*-protected amino P_3−P_1 intermediates **8**. In turn, **8** could be optionally deprotected, reductively alkylated, or capped with acyl, carbamate, carbamoyl, or sulfonamide groups to generate the fully elaborated resin-bound intermediates.[13] Acidic hydrolysis with a TFA−H_2O−dichloromethane cocktail cleaved the semicarbazone linker along with any acid-labile side chain protecting groups. Semiautomated C18−RP HPLC or short C18−RP Varian Bond-Elut columns effected final purification of targets **3**.

Experimentally determined advantages of this method vs our previously described *trans*-1,4-disubstituted cyclohexanecarboxamide−semicarbazone protocol[2a] include more concise preparation of the key intermediates, direct attachment to the resin without the agency of a tether, and operationally simpler, cleaner and more rapid cleavage from the resin matrix that resulted in improved overall yields of the final targets **3**.

Peptide aldehyde derivatives are regarded as reactive, sensitive, and configurationally labile entities.[11,14] Indeed, racemization at the P_1-aldehyde center will occur rapidly under base-catalyzed conditions. In this work, an acid-catalyzed hydrolysis reaction is employed in the final semicarbazone cleavage step. It is important to underscore the utility of our procedure for the production of argininals (cf. **3a**,**b**), which are structurally unique since they exist in equilibrating hemiaminal and hydrate forms and are therefore moderately acid stable.[3] We were also delighted to observe that the hydrophobic P_1-aldehydes **3c**−**h** retained their chiral integrity, as determined by NMR and HPLC analysis and comparisons with authentic P_1-racemic samples. Our findings

(9) All new intermediates were characterized by full spectroscopic (IR, NMR, low/high resolution MS) data. Yields refer to spectroscopically and chromatographically homogeneous (≥95% by HPLC, TLC) materials.

(10) Coupling yields and loading of the resin were determined via the method of Kaiser et al.: Kaiser, E.; Colescott, R. L.; Bossinger, C. D.; Cook, P. I. *Anal. Biochem.* **1970**, *34*, 595.

(11) All *N*-protected α-aminoaldehydes were prepared by either $LiAlH_4$ reduction of the corresponding Weinreb amide or by Moffatt-type oxidation of the corresponding alcohol precursors: (a) Jurczak, J.; Golebiowski, A. *Chem. Rev.* **1989**, *89*, 149. (b) Ho, P. T.; Ngu, K. *J. Org. Chem.* **1993**, *58*, 2313. (c) Hyun, S. I.; Kim, Y. G. *Tetrahedron Lett.* **1998**, *39*, 4299.

(12) In this work, P_1-argininal targets **3a**,**b** were prepared via *N*-α-Fmoc-(or α-Boc)-ϵ,ω-$(Alloc)_2$-argininal precursors: (a) Loffet, A.; Zhang, H. X. *Int. J. Pept. Protein Res.* **1993**, *42*, 346. (b) Verdini, A. S.; Lucietto, P.; Fossati, G. L.; Giordani, C. *Tetrahedron Lett.* **1992**, *33*, 6541.

(13) For penultimate intermediates wherein a P_1-*N*-ϵ,ω-$(Alloc)_2$-argininal semicarbazone moiety was present, selective Pd(0)-catalyzed Alloc cleavage was employed, cf.: (a) Lloyd-Williams, P.; Jou, G.; Albericio, F.; Giralt, E. *Tetrahedron Lett.* **1991**, *32*, 4207. (b) Kunz, H.; Dombo, B. *Angew. Chem., Int. Ed. Engl.* **1988**, *27*, 711.

(14) Semple, J. E.; Rowley, D. C.; Brunck, T. K.; Uong, T. H.; Minami, N. K.; Owens, T. D.; Tamura, S. Y.; Goldman, E. A.; Siev, D. V.; Ardecky, R. J.; Carpenter, S. H.; Ge, Y.; Richard, B. M.; Nolan, T. G.; Håkanson, K.; Tulinsky, A.; Nutt, R. F.; Ripka, W. C. *J. Med. Chem.* **1996**, *39*, 4531.

are in accord with the recent disclosure by Ramage[7a] regarding configurational stability studies on peptide aldehydes. However, we note that the latter members must be stored as lyophilized powders at low temperatures (0−4 °C) and will indeed slowly racemize over the course of several months.

The breadth and scope of our new methodology is illustrated by examples **3a**−**h** collected in Table 1. The reactions were conducted in parallel mode using 50−60 mg of the resin-bound intermediate **2** (loading capacity of 0.63−0.73 mmol/g) in either Irori Kans or Whatman minicolumn reactors, and produced 9−13 mg quantities of the desired products **3a**−**h** for biological screening. These targets were produced in excellent (97−99%) purity for in vitro screening. The unoptimized overall yields of **3** prepared by this route were quite satisfactory for a nine-step process and ranged from 29 to 50%. Structural integrity and purity was confirmed by LR/HRMS, NMR, and RP-HPLC analysis.

In conclusion, the HCAM resin technology provides a practical, efficient and general platform for the construction of peptidyl and peptidomimetic P_1-aldehyde derivatives. Topographically, targets **3a**−**h** display a range of structural diversity typically required for small molecule protease inhibitor discovery programs. Construction of the key resin-bound semicarbazone **2**, synthetic elaboration, and hydrolytic cleavage from the solid support led to the desired targets **3** in satisfactory overall yields. Since P_1-aldehyde scaffolds are currently receiving considerable attention in exploratory serine[4] and cysteine[15] protease inhibitor programs, this new technology may find immediate applications therein for the rapid generation of novel manifolds as well as for lead development and SAR optimization. A large variety of novel permutations are possible. Extension and application to other classes of hydrophobic or hydrophilic P_1-carbonyl systems is envisioned. Novel protease inhibitor leads have emerged via this platform and are under active study in our laboratories.[16]

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(15) Review of cysteine protease inhibitors: Otto, H.-H.; Schirmeister, T. *Chem. Rev.* **1997**, *97*, 133.

(16) Parallel SPS of compounds **3a**−**h**: **Preparation of HCAM Resin 1.** To a suspension of 1,1′-carbonyldiimidazole (29.19 g, 180 mmol) in dimethylformamide (300 mL) was added portionwise *tert*-butyl carbazate (23.76 g, 180 mmol) while stirring at ambient temperature under a nitrogen atmosphere. The reaction was allowed to stir for 2.5 h and then mixed with commercial amino methylated polystyrene resin (30 g, 30 mmol). The suspension was shaken for 16 h at ambient temperature. The resin was filtered and washed alternately with CH_2Cl_2 and MeOH. The above resin was added to a solution of CH_2Cl_2/TFA (1/1) (300 mL) and thioanisole (10 mL). The mixture was purged slowly with a stream of nitrogen gas in a standard peptide synthesis flask at ambient temperature for 30 min. The deprotected resin was filtered, washed sequentially with CH_2Cl_2, CH_2Cl_2/DIEA (1/1), and CH_2Cl_2; washed alternately with MeOH and CH_2Cl_2; and dried to afford 30.64 g (loading capacity of 0.85 mmol/g) of HCAM resin **1**. **Example of Coupling and Deprotection on HCAM Resin.** *N*-α-Boc-allylglycinal (237 mg, 1.19 mmol, 1.4 equiv) was added to a suspension of HCAM resin **1** (1.00 g, 0.85 mmol) in CH_2Cl_2 (10 mL). The mixture was allowed to shake for 24 h at ambient temperature. The resin was filtered and washed with copious amounts of solvent (CH_2Cl_2, MeOH) to afford **2** (PG_1 = Boc, R = allyl). A solution of CH_2Cl_2/TFA/thioanisole (6/3/1) (15 mL) was added to the preceding resin intermediate (1.15 g, 0.73 mmol). The mixture was allowed to shake in a closed container at ambient temperature for 25 min. The deprotected resin was filtered, washed successively with CH_2Cl_2 and CH_2Cl_2/DIEA (9/1), alternately washed with CH_2Cl_2 and MeOH, and dried to afford 1.00 g (loading capacity of 0.79 mmol/g) of **8** (R_1 = allyl). **Elaboration to 9 and Resin Cleavage to Targets 3.** As outlined in Scheme 2, after application of standard iterative Fmoc-AA_n-OH SPS peptide couplings, deprotection, and N-terminus capping protocols, a solution of TFA/CH_2Cl_2/H_2O (8/1/1) or TFA/H_2O (9/1) (1.5 mL) was added to the elaborated resin (100 mg) in a Whatman minicolumn. The mixture was allowed to shake for 1 h at ambient temperature. The filtrate was collected and adjusted to pH = 3 with 6 M ammonium acetate (∼1.5 mL), and the resulting solution was purified through a miniprep RP-HPLC column. The combined fractions were lyophilized to afford the desired targets **3a**−**h** as colorless, amorphous solids, which were stored at 0−4 °C under a nitrogen atmosphere.