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Novel protocol for the solid-phase synthesis of peptidyl and peptidomimetic P_1-argininal derivatives

Daniel V. Siev, John A. Gaudette and J. Edward Semple *

Department of Medicinal Chemistry, Corvas International, Inc., 3030 Science Park Road, San Diego, CA 92121, USA

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Abstract

The design, synthesis and application of novel argininal aminals **1** tethered onto AM resin is described. Efficient solid-phase synthesis routes to a wide array of the title derivatives **2** have been implemented using this convenient technology. The resulting P_1-argininal targets serve as useful exploratory scaffolds for serine and cysteine protease inhibitor discovery.

The development of new combinatorial chemistry platforms is currently at the forefront of organic and peptide synthesis.[1] Such technology has greatly advanced the state of the art with regard to synthetic productivity and, coupled with automation methods, has enabled the generation and screening of large, structurally diverse collections of molecules. These emerging paradigms are finding widespread application in academic and industrial research laboratories by accelerating the discovery and development of novel classes of pharmaceutical agents.[2] We are actively engaged in the design and synthesis of potent, selective, and orally bioavailable serine protease inhibitors[3] and wish to report a convenient, general solid phase protocol for the production of peptidyl and peptidomimetic P_1-argininal derivatives.

Peptide aldehydes are useful as transition state analog (TSA) inhibitors of proteolytic enzymes. A number of solid phase synthetic approaches to these labile species have been disclosed which feature

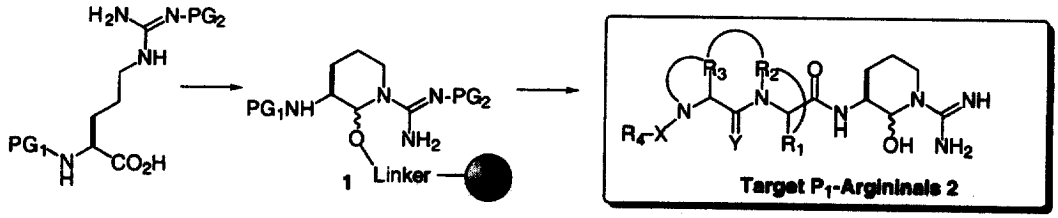

Figure 1. Strategy for the hydrolytic solid phase synthesis of P_1-argininal libraries. Curved lines delineate optional ring systems; X=direct link, CO, OCO, NHCO, or SO_2; Y=O or H_2; R_1–R_3=amino acid sidechains, may constitute cyclic arrays, R_4=optional amino terminus capping group

* Corresponding author.

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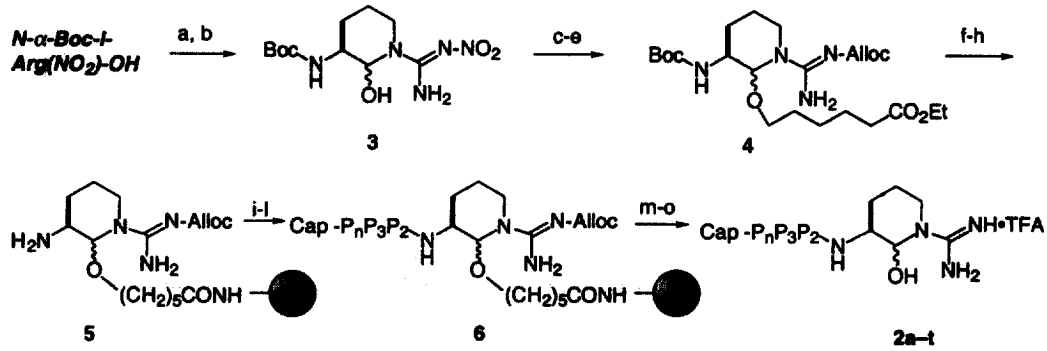
hydrolytic,[4a,b] oxidative[4c,d] or reductive[4e,f] strategies for generating and/or releasing the final target from the solid support. However, perusal of the reaction conditions employed for effecting target release indicated potential limitations in their scope. Recently, we described convenient solution phase protocols to novel antithrombotic P_1-argininal P_3–P_4 dipeptide surrogates utilizing either final-stage oxidative or hydrolytic steps.[5] We envisioned adaptation of our hydrolytic approach to the solid phase and report in this letter general new methodology which complements the existing techniques and appears to have broad scope for P_1-argininal synthesis.

Our global strategy is outlined in Fig. 1. Construction of suitably protected P_1-argininal precursors and linking of the hemiaminal moiety via a bifunctional tether to a suitable solid support provides key intermediate **1**. Chemical manipulations (deprotection, coupling, orthogonal side chain reactions, etc.) and final hydrolysis releases the fully elaborated target **2** from the resin matrix. Although our tethering moiety is attached to the resin via a typical amide bond, such a linker may formally be regarded as 'traceless' since after final cleavage of the hemiaminal function, no part of it remains in the final product.[6] Although we favor the production of relatively small, focused libraries using the parallel synthesis approach, in practice, either split-and-pool or parallel synthesis techniques may be used. This methodology has been successfully applied to the preparation of focused combinatorial libraries containing from 20 up to 2000 members. Examples of specific targets prepared by parallel techniques will be presented herein which feature a range of structural diversity.



Scheme 1. Reagents and conditions. (a) MeOMeNH, EDC, HOBt, NMM, CH_3CN, rt, 94–100%; (b) $LiAlH_4$, THF, −78°C, $KHSO_4$, H_2O, ~quant.; (c) 1: $HO(CH_2)_5CO_2Et$, HCl(cat.), CH_3CN; 2: Ac_2O, pyridine, CH_3CN, 95% overall; (d) H_2, Pd/C, EtOH, H_2O, HOAc, 40 psi, ~quant.; (e) Alloc-Cl, 1 N NaOH, CH_3CN, 88%; (f) 3 N LiOH, EtOH; HCl, ~quant.; (g) AM Resin, PyBOP, DIEA, DMF, ~quant. (Kaiser test); (h) TFA, thioanisole, CH_2Cl_2: 3, 1, 6, rt, 15–20 min, ~quant. (Kaiser test); (i) Fmoc-AA_n-OH, PyBOP, DIEA, DMF; (j) piperidine, DMF; (k) repeat steps i and j until P_n residue is attached; (l) optional deprotection and capping N-terminus, see text; (m) $(Ph_3P)_4Pd$(cat.), THF, DMSO, 0.5 N HCl, morpholine: 2, 2, 1, 5; (n) TFA, CH_2Cl_2, H_2O: 6, 3, 1; (o) optional HPLC purification

Our foray to the library targets proceeded through the key P_1-argininal resin-bound intermediate *N*-α-amino-N^g-Alloc-*l*-argininyl-cyclo-6-oxahexanoyl-AM-resin **5** as outlined in Scheme 1.[7] The argininal library members **2** prepared from **5** via parallel synthesis platforms used either segment or block coupling methods. Commercially available (Bachem) *N*-α-Boc-N^g-nitro-*l*-arginine was converted to the Weinreb amide and then reduced to the argininal **3** on a multi-gram scale by a modification of our recently described procedure.[5,8] Acid-catalyzed aminal formation with the preferred tethering reagent ethyl 6-hydroxyhexanoate, hydrogenolysis of the nitro-protecting group, and reprotection of the guanidino residue as the Alloc-derivative delivered **4** in high overall yield. Basic ester hydrolysis of **4** was followed by attachment of the linker through the carboxy terminus onto the aminomethylated polystyrene (AM) resin matrix and was achieved in essentially quantitative yield with the PyBOP reagent[9], reaction progress being monitored by ninhydrin (Kaiser) testing for free amino resin. Treatment of the resultant

Table 1
Peptidyl and peptidomimetic P_1-argininal derivatives **2a–t** produced via Scheme 1

Compd 2	Cap[1]	P_n	P_3	P_2	Mass Spec[2]	HPLC % Purity[3]	% Overall Yield
a	$BnSO_2$	none	3-aminoazepin-2-one	CH_2CO	481	98	42
b	i-Boc	none	d-Arg	l-Pro	512	96	55
c	3,4-$(OH)_2$-PhAc	none	none	*trans*-4-BnO-l-Pro	512	97	34
d	4-BuO-PhAc	none	none	*trans*-4-BnO-l-Pro	552	97	17
e	none	d-Arg	3-NH-Ph-CO		434	95	51
f	3,4-$(OH)_2$-PhAc	none	3-NH-Ph-CO		427	98	23
g	none	none	*trans*-3-NH-C6H10-CO		403	95	33
h	$BnSO_2$	none	3-NH-Ph-CO		432	98	29
i	$BnSO_2$	3-NH-Ph-CO		Gly	489	95	19
j	*trans*-3NH-C_6H_{10}-CO	3-NH-Ph-CO		Gly	460	95	20
k	4-BuO-PhAc	3-NH-Ph-CO		Gly	525	97	17
l	3,4-$(OH)_2$-PhAc	3-NH-Ph-CO		Gly	485	95	19
m	d-Arg	3-NH-Ph-CO		Gly	491	95	48
n	l-Arg	*trans*-4-$NHCH_2C_6H_{10}CO$		Gly	511	95	51
o	3,4-$(OH)_2$-PhAc	*trans*-4-$NHCH_2C_6H_{10}CO$		Gly	505	97	55
p	4-BuO-PhAc	*trans*-4-$NHCH_2C_6H_{10}CO$		Gly	545	98	53
q	*trans*-3NH-C_6H_{10}-CO	*trans*-4-$NHCH_2C_6H_{10}CO$		Gly	480	95	21
r	3,4-$(OH)_2$-PhAc	none	*trans*-4-$NHCH_2C_6H_{10}CO$		448	98	44
s	4-BuO-PhAc	none	*trans*-4-$NHCH_2C_6H_{10}CO$		488	90	17
t	l-Arg	none	*trans*-4-$NHCH_2C_6H_{10}CO$		454	95	83

[1]Cap = N-terminal amino capping group. [2]Low resolution mass spectra, value reported is for MH^+.
[3]RP-HPLC analysis performed using two independent gradients, water/CH_3CN with 0.1% TFA.

intermediate with a mixture of TFA and thioanisole provided the key resin-bound amine intermediate **5**. Several acylation reactions of **5** were successful, including iterative PyBOP-mediated peptide couplings with *N*-α-Fmoc-amino acids-piperidine deblocking protocols, to produce an amino-terminal P_3–P_2 intermediate that was optionally reductively alkylated or capped with acyl, carbamate, carbamoyl, or sulfonamide P_4-groups to generate the fully elaborated intermediate **6**. Palladium(0)-catalyzed removal of the N^g-Alloc moiety[10] was followed by acidic hydrolysis with a TFA, CH_2Cl_2, H_2O cocktail to cleave the aminal linker along with any acid-labile sidechain protecting groups. Either automated C18-RPHPLC or short C18-RP Varian Bond-Elut® columns effected final purification.

Hydrophobic peptide aldehyde derivatives are regarded as reactive, sensitive and configurationally labile entities.[8,11] Although an acid-catalyzed hydrolysis reaction is employed for the final cleavage step, it is important to underscore the utility of our procedure for the production of argininals, which are structurally unique since they exist almost entirely in hemiaminal and hydrate forms and are, therefore, moderately acid stable.[8] The breadth and scope of our method is further illustrated with the examples collected in Table 1. The targets were prepared employing either commercial materials or readily accessible intermediates. Typically, parallel SPS reactions on 14 to 44 mg of the resin-bound intermediate **5** (loading capacity of 0.62 mmol/g) were conducted in either Irori Kans® or Whatman minicolumn reactors, and delivered 0.2–3.5 mg quantities of final products **2** of good (90%) to excellent (97–98%) purity for in vitro screening. The unoptimized overall yields of **2** prepared by this route were satisfactory and ranged from 17–83%. Structural integrity and purity was confirmed by MS, NMR and RP-HPLC analysis.

In conclusion, a practical and efficient solid phase synthetic approach to peptidyl and peptidomimetic

argininal derivatives **2a–t** was developed from readily available precursors. Construction of the key resin-bound hemiaminal **5**, synthetic elaboration, and hydrolytic cleavage from the solid support led to the desired targets **2a–t** in satisfactory overall yields. Since P_1-aldehyde scaffolds are currently receiving considerable attention as small molecule drug leads in exploratory serine[3] and cysteine[12] 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 neutral 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.

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