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Peptide Heterocycle Conjugates: A Diverted Edman Degradation Protocol for the Synthesis of N-Terminal 2-Iminohydantoins

Ghotas Evindar and Robert A. Batey*

Davenport Research Laboratories, Department of Chemistry, University of Toronto, 80 St. George Street, Toronto, Ontario, M5S 3H6, Canada

rbatey@chem.utoronto.ca

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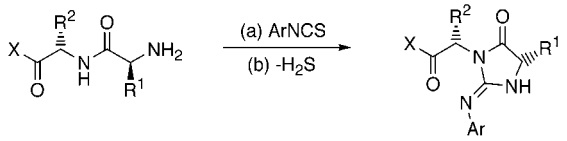
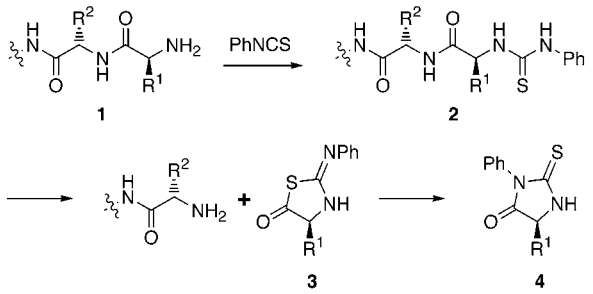
ABSTRACT



A modified Edman degradation procedure provides an effective means of introducing a heterocycle at the N-terminus of an α-amino acid amide or peptide. Reaction of a peptide with an isothiocyanate, followed by dehydrothiolative trapping of the intermediate thiourea, by intramolecular cyclization of the weakly nucleophilic adjacent amide nitrogen, generates an iminohydantoin. A solution-phase parallel synthesis of iminohydantoins and a polymer-supported synthesis of dipeptide- and tripeptide-derived iminohydantoins were also achieved.

The synthesis of modified peptides and peptidomimetics is an area of considerable interest for the development of biologically active compounds.[1,2] The incorporation or substitution of heterocyclic groups into peptidic ligands is one strategy for the development of small molecule peptidomimetic structures.[3] Novel transformations, particularly those that are compatible with existing solution and polymer-supported combinatorial procedures, are required to meet this goal. One venerable procedure in peptide chemistry is the Edman degradation reaction, which has been widely used for the sequence analysis of polypeptides and proteins.[4] In this process, the N-terminal amino group of **1** reacts with phenyl isothiocyanate to generate a thiourea **2**. Intramolecular cyclization of **2** then occurs via nucleophilic addition of the thio group to the adjacent amide carbonyl, generating phenylthiohydantoin **4** via a thiazolinone intermediate **3** (Scheme 1). The net result of this process is the removal of the N-terminal amino acid. Given the degradative nature of this procedure, it is not surprising that this process has not attracted synthetic interest. However, the initial thiourea

Scheme 1



(1) Following reviews give an overview of small biologically relevant heterocyclic pharmacophores: (a) Thompson, L. A.; Ellman, J. A. *Chem. Rev.* **1996**, *96*, 555−600. (b) Nefzi, A.; Ostresh, J. M.; Houghten, R. A. *Chem. Rev.* **1997**, *97*, 449−472. (c) Franzen, R. G. *J. Comb. Chem.* **2000**, *2*, 195−214.

(2) Reviews on peptidomimetics: (a) Wiley, R. A.; Rich, D. H. *Med. Res. Rev.* **1993**, *13*, 327−84. (b) Bursavich, M. G.; Rich, D. H. *J. Med. Chem.* **2002**, *45*, 541−58.

(3) See for example, the synthesis of peptidic triazines: Scharn, D.; Wenschuh, H.; Reineke, U.; Schneider-Mergener, J.; Germeroth, L. *J. Comb. Chem.* **2000**, *2*, 361−369.

(4) (a) Edman, P. *Acta Chem. Scand.* **1950**, *4*, 283−293. (b) Edman, P.; Begg, G. *Eur. J. Biochem.* **1967**, *1*, 80−91.

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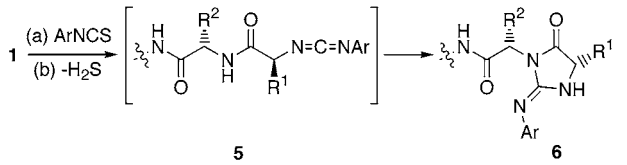
intermediate **2**, while prone to degradation, does offer the potential for further synthetic elaboration. We envisaged reversing the polarity of the system[5] by conversion of **2** to an electrophilic carbodiimide group **5**, through a dehydrothiolation reaction, and subsequent trapping by addition of the adjacent weakly nucleophilic amide nitrogen to generate an iminohydantoin (iminoimidazolinone) **6** (Scheme 2). This

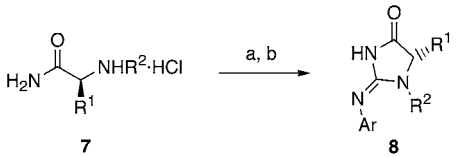
Scheme 2



would provide a method for the heterocyclic modification of the N-terminus of a peptide, with the simultaneous incorporation of an element of diversity originating from the isothiocyanate. We now disclose an operationally straightforward and high-yielding method for the formation of iminohydantoin **6** that requires minimal purification and is amenable to parallel synthesis and polymer-supported techniques.

As a model study, reaction of Leu-NH_2 hydrochloride **7** with phenyl isothiocyanate in the presence of triethylamine (TEA) gave the corresponding thiourea in quantitative yield in less than 10 min, which could then be converted to iminohydantoin **8** using several well-known dehydrothiolation agents: $HgCl_2$, HgO, $CuCl_2$, Ag_2O, AgO, Ag_2CO_3, EDCI, and Mukaiyama's reagent (Scheme 3).[6−14] Formation

Scheme 3[a]



[a] Key: (a) ArNCS, Amberlyst A21 (or Et_3N); (b) $HgCl_2$.

of **8** was achieved in high yields and purity using EDCI, Mukaiyama's reagent, Ag_2CO_3, and $HgCl_2$. For subsequent, solution-phase, proof of concept studies, $HgCl_2$ was used since reaction occurred in less than 4 h and the products did not require column chromatographic purification.

Although the mechanism for the reaction has not been experimentally confirmed, the intermediacy of a carbodiimide is likely. The formation of a limited series of iminohydantoins through a similar intramolecular cyclization of amides onto cyanamides and carbodiimides has been reported in the literature by Lempert and co-workers.[15] However, their approach is not general since it occurs via the initial ring-opening of 1-*tert*-butyl-3,3-diphenyl-aziridinone by cyanamides to form the cyclization precursors and thus relies on access to appropriately substituted aziridinone intermediates and monosubstituted cyanamides. Also, Houghten and co-workers have reported the solid-phase synthesis of iminoimidazolidines through the reaction of polymer-bound ethylenediamines with arylisothiocyanates and subsequent mercury(II)-promoted cyclization of an amine onto the intermediate thiourea.[16] In contrast to Houghten's method, our results demonstrate that a weakly nucleophilic amide **5** is suitable for cyclization (Scheme 2) and permits this process to be generally applicable to peptides, vide infra.[16]

The two-step reaction to generate iminohydantoins **8** from amino acid amides **7** was modified to a one-pot protocol by substituting the organic base TEA with a scavenger base that could be removed with the mercury byproducts. A number of weakly basic ion-exchange resins were tested, from which Amberlyst-A21 was found to be the optimal choice. An optimized set of conditions using the minimum amount of Amberlyst-A21 for the one-pot, two-step synthesis of **8** was then applied to the parallel synthesis of 28 iminohydantoins from four aryl isothiocyanates and seven amino acid amides (Table 1).[17]

The reactions were stirred for 1 h for the first step to allow reaction of the different amino acid amides, with varying solubilities, with phenyl isothiocyanate. To ensure complete reaction of the generated thioureas with $HgCl_2$, the reactions were stirred at room temperature for 12 h. The reactions were

(5) N-Terminal cyclization of peptides to hydantoins and thiohydantoins has been achieved by reaction of peptides with *N*,*N*′-carbonyldiimidazole and *N*,*N*′-thiocarbonyldiimidazole, respectively. Esser, F.; Roos, O. *Angew. Chem.* **1978**, *90*, 495−496; *Angew.* Chem., Int. Ed. Engl. **1978**, *17*, 467−468.

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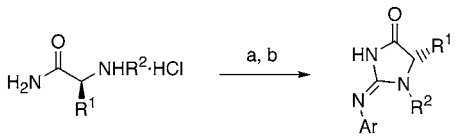
(16) Yu, Y.; Ostresh, J. M.; Houghten, R. A. *J. Org. Chem.* **2002**, *67*, 3138−3141. Houghten et al. recently demonstrated a resin-bound reaction in which Mukaiyama's reagent promotes the cyclization of an amide onto a thiourea group to form polymer-bound iminohydantoins, analogous to Scheme 3. This process was demonstrated using single amino acids coupled to *p*-methylbenzhydrylamine (MBHA) resin, providing the product **8** (or their tautomeric forms, 2-aminoimidazolidin-4-ones) following HF/anisole cleavage. See: Yu, Y.; Ostresh, J. M.; Houghten, R. A. *Tetrahedron* **2002**, *58*, 3349−3353.

(17) **General Synthetic Procedure.** To a mixture of the amino acid amide (0.38 mmol) and weakly basic Amberlyst-A21 ion-exchange resin (0.5 g) in MeCN (2.0 mL) was added the desired isothiocyanate (0.38 mmol), and the mixture was stirred at room temperature for 1 h. To the reaction was added $HgCl_2$ (0.42 mmol), and the mixture was then stirred overnight. The reaction mixture was diluted with EtOAc (2.0 mL) and filtered through a prepacked Celite column, using EtOAc (4 mL) to remove the Amberlyst-A21 resin and HgS byproduct. The solution was added to the Celite column and allowed to sit on the column for 1 min before applying suction to the column.

Table 1. Synthesis of 2-Iminohydantoins **8** from Amino Acid Amides[a] and Aryl Isothiocyanates Using Amberlyst A21[a]



7 **8**

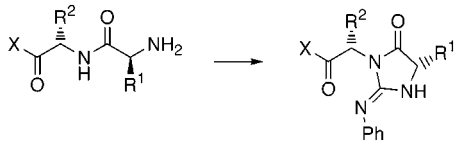
entry	amino acid amide	aryl-NCS	yield (%)	purity[c,d] (%)
1	Leu-NH_2	Ph	99	99 (>98)
2	Phe-NH_2	Ph	99	>99 (>98)
3	Tyr-NH_2	Ph	68	>99 (>98)
4	Met-NH_2	Ph	87	92 (97)
5	Glu(OBn)-NH_2	Ph	92	97 (96)
6	Trp-NH_2	Ph	80	>99 (97)
7[a]	Pro-NH_2	Ph	99	94 (>98)
8	Leu-NH_2	2-BrPh	99	98 (95)
9	Phe-NH_2	2-BrPh	98	97 (>98)
10	Tyr-NH_2	2-BrPh	76	95 (>98)
11	Met-NH_2	2-BrPh	95	94 (97)
12	Glu(OBn)-NH_2	2-BrPh	99	95 (94)
13	Trp-NH_2	2-BrPh	88	97 (97)
14[a]	Pro-NH_2	2-BrPh	96	90 (96)
15	Leu-NH_2	2-CH_3Ph	95	95 (>98)
16	Phe-NH_2	2-CH_3Ph	89	99 (>98)
17	Tyr-NH_2	2-CH_3Ph	81	92 (96)
18	Met-NH_2	2-CH_3Ph	84	93 (97)
19	Glu(OBn)-NH_2	2-CH_3Ph	87	97 (>98)
20	Trp-NH_2	2-CH_3Ph	72	96 (95)
21	Pro-NH_2	2-CH_3Ph	83	>98 (98)
22	Leu-NH_2	3,4,5-$(MeO)_3$Ph	95	>99 (>98)
23	Phe-NH_2	3,4,5-$(MeO)_3$Ph	91	>99 (>98)
24	Tyr-NH_2	3,4,5-$(MeO)_3$Ph	68	>99 (95)
25	Met-NH_2	3,4,5-$(MeO)_3$Ph	83	>99 (97)
26	Glu(OBn)-NH_2	3,4,5-$(MeO)_3$Ph	91	>99 (96)
27	Trp-NH_2	3,4,5-$(MeO)_3$Ph	71	99 (96)
28[a]	Pro-NH_2	3,4,5-$(MeO)_3$Ph	98	99 (>98)

[a] All amino acid amides were used as hydrochloride salts, except for proline amide. [b] Reactions were conducted with Amberlyst A21 at room temperature, as shown in Scheme 3, except for three of the proline amide reactions conducted at 50 °C (entries 7, 14, and 28). [c] Purity determined by HPLC (UV_{254}, Hewlett-Packard series 1100MSD electrospray ionization mass spectrometer). [d] Values in parentheses represent purities estimated by ^{1}H NMR.

diluted with an equal volume of EtOAc then filtered through a short plug of Celite. In each case, the yield and purity (by ^{1}H NMR and HPLC) of the products was high. No epimerization of product **8** from L-Phe-NH_2·HCl (Table 1, entry 2) was detected by chiral HPLC either under the reaction conditions or after storage for 1 year. A similar approach was envisaged for the synthesis of iminohydantoins from Pro-amide and aryl isothiocyanates, although clearly reaction cannot proceed through a carbodiimide intermediate in the case of proline amide. However, previous studies have demonstrated that mercury salts promote attack of nucleophiles on trisubstituted thioureas to generate aminotetrazoles.[18] Also, the key cyclization step in Houghten's synthesis of iminoimidazolidines, involved nucleophilic attack of secondary amines onto mercury activated trisubstituted thioureas.[16] Initial results for the reaction of Pro-amide with phenyl isothiocyanate were not encouraging, resulting in incomplete conversion to products at room temperature, but reaction with 2-methylphenyl isothiocyanate did provide products effectively (Table 1, entry 21). However, reaction at 50 °C provided a more general set of conditions for the formation of proline-based iminohydantoins (Table 1, entries 7, 14, and 28).

To test the feasibility of this method for the generation of iminohydantoins from peptides, several di- and tripeptide substrates **9** were synthesized in solution using standard peptide coupling methods and reacted, as above, with phenyl isothiocyanate in a one-pot reaction using $HgCl_2$ and TEA as the base (Table 2). After purification of the crude material by flash column chromatography, the desired 2-iminohydantoins **10** were obtained in 87 and 90% yields from Leu-

Table 2. Solution-Phase Synthesis of 2-Iminohydantoins **10** from Di-/Tripeptides and Phenyl Isothiocyanate

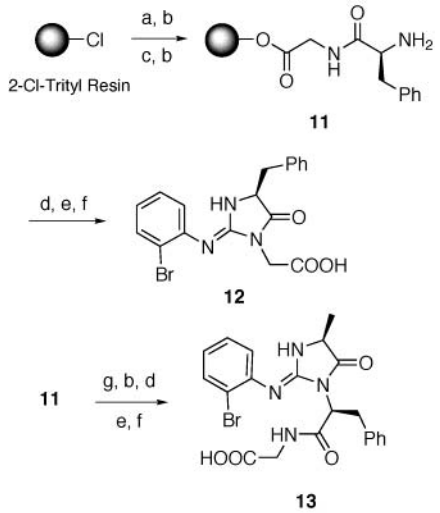


9 **10**

entry	R^1	R^2	X	yield (%)[a]
1	*sec*-Bu	Bn	O*t*Bu	87
2	Me	*sec*-Bu	O*t*Bu	90
3	Me	*sec*-Bu	Phe-O*t*Bu	83
4	Bn	Me	Leu-O*t*Bu	81

[a] Isolated yields after column chromatography.

Scheme 4[a]



[a] Key: (a) Fmoc-Gly, DIPEA, CH_2Cl_2, 2 h, then MeOH; (b) 20% piperidine in DMF; (c) Fmoc-Phe, DIC, HOBT, DIPEA; (d) 2-BrPh-NCS; (e) Mukaiyama's reagent, TEA; (f) 20% HFIP in CH_2Cl_2; (g) Fmoc-Ala, DIC, HOBT, DIPEA.

(18) Batey, R. A.; Powell, D. A. *Org. Lett.* **2000**, *2*, 3237−3240.

Phe-O*t*Bu and Ala-Leu-O*t*Bu, respectively (Table 2, entries 1 and 2). Similar results could be achieved using tripeptides, with the desired 2-iminohydantoins obtained in 83% yield from Ala-Leu-Phe-O*t*Bu and 81% from Phe-Ala-Leu-O*t*Bu (Table 2, entries 3 and 4). ^{1}H NMR study of the phenyl thiourea **2** formed from Ala-Leu-Phe-O*t*Bu (Table 2, entry 3) and phenyl isothiourea in CD_3CN confirmed the stability of **2** over a period of 16 h at room temperature and also in the presence of $Et_3N{\cdot}HCl$ (1 equiv) over a period of 3 days.

More significantly, the process is readily amenable to polymer-supported chemistry. For example, 2-chlorotrityl chloride polystyrene resin-supported multistep synthesis of **12** and **13** was achieved in ≥95% overall yields, with 93 and 91% purities, respectively, following cleavage from the resin (Scheme 4). Of particular note here is the use of Mukaiyama's reagent as the activator,[16] rather than $HgCl_2$, and the absence of column chromatographic purification of the products (cf. products shown in Table 2).

In conclusion, an efficient method for the synthesis of 2-iminohydantoins from commercially available amino acid amides and aryl isothiocyanates has been established, using a two-step, one-pot, parallel synthesis protocol employing a basic scavenging resin. The method is amenable to the solution-phase synthesis of 2-iminohydantoins from di- and tripeptides and adaptable to polymer-supported synthesis. This study clearly demonstrates the utility of amino acid- and peptide-derived thioureas for purposes other than Edman degradation. We anticipate that such methods will allow the synthesis of novel N-terminal modified peptides and are currently synthesizing a combinatorial library of peptide−iminohydantoin conjugates.

Acknowledgment. This work was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) and the Ontario Research and Development Challenge Fund (ORDCF). G.E. thanks the University of Toronto for fellowship support. R.A.B gratefully acknowledges the receipt of a Premier's Research Excellence Award. We thank Dr. A. B. Young for mass spectrometric analysis and Prof. Andrei Yudin for providing LCMS access.

Note Added after ASAP Posting. In the version posted ASAP March 19, 2003, a recent reference citation was omitted in ref 16. The corrected version was posted March 31, 2003.

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