Report

Solid-Phase Synthesis of C-Terminal Peptide Hydroxamic Acids

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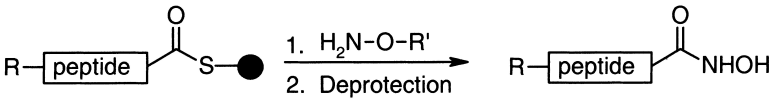

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Reports

Solid-Phase Synthesis of C-Terminal Peptide Hydroxamic Acids

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Hydroxamic acids are strong metal ion chelators and represent a wide spectrum of bioactive agents with antibacterial, antifungal, and anticancer properties.[1] In particular, they have been identified as potent inhibitors of matrix metalloproteinases (MMPs),[2] a family of zinc-dependent endoproteinases involved in extracellular matrix remodeling. Hydroxamic acids have generally been synthesized in solution from nitro compounds[3] or by acylation of hydroxylamine and its derivatives with activated carboxylic acids.[4] Recently, solid-phase synthesis has become an important tool for production of combinatorial libraries and lead generation,[5] and there have been several reports describing solid-phase syntheses of hydroxamic acid derivatives.[6] In principle, hydroxamic acids may be obtained by direct cleavage of resin-bound esters with hydroxylamine derivatives; however, it has been reported that this approach does not give reproducible results.[6a,g] Therefore, solid-phase synthesis of hydroxamic acid derivatives generally involves either immobilization of the hydroxylamine group through a special N- or O-linkage or formation of a protected resin-bound hydroxamate. In the current report, we describe a facile approach for the synthesis of peptide hydroxamic acids based on cleavage of resin-bound thioesters. During the preparation of this manuscript, Muir et al. described preliminary results of thioester resin cleavage by different nucleophiles including hydroxylamine.[7]

The thioester linkage, originally introduced by Hojo and Aimoto,[8] has served as an important tethering group in synthetic peptide and protein chemistry, useful for fragment coupling,[9] chemical ligation,[10] and formation of peptide dendrimers[11] and cyclic peptides.[12] Applications of the thioester linkage in solid-phase synthesis of small molecules have also been reported.[13] Since the thioester linkage is susceptible to nucleophilic attack but stable to TFA treatment during solid-phase peptide synthesis (using standard Boc protection), we envisioned that hydroxylamine derivatives could directly cleave resin-bound peptide thioesters to form the corresponding peptide hydroxamates.

Peptide chains were assembled on polystyrene resins bearing the 3-mercaptopropionyl[14] or 2-mercaptoacetyl[15] moiety and TentaGel[16] derivatized with 3-mercaptopropionic acid (see Scheme 1).[12b] The thiol group is nucleophilic enough to undergo thioesterification with Boc-amino acids preactivated by PyAOP and DIEA. After attachment of the first Boc-amino acid to the alkylthiol resin **1**,[12b] assembly of the peptide sequence was achieved by standard solid-phase peptide synthesis methodology[17] using preactivated Boc-amino acids (Boc-amino acid:PyAOP:DIEA, 1:1:1.5). Removal of the N-terminal Boc protecting group and subsequent neutralization with 5% DIEA in DMF, or acetylation of the N-terminus by Ac_2O/DIEA/DMF (1:1:8), resulted in peptide thioester resins **2** and **3**, respectively. Resins **2** and **3** were then subjected to nucleophilic cleavage by hydroxylamines. Several commercially available hydroxylamine derivatives were used to determine optimal cleavage conditions, including the following: 50% aqueous hydroxylamine, $NH_2OH\cdot HCl$ (neutralized by 1 equiv of DIEA), *O*-trityl hydroxylamine, *O*-benzyl hydroxylamine, and *O*-trimethylsilyl hydroxylamine. When the peptide thioesters were linked to TentaGel resins, the aqueous hydroxylamine caused severe hydrolysis (>30%, according to HPLC and LC/MS analyses), in addition to hydroxylaminolysis. This is due to the high susceptibility of the thioester linkage to basic aqueous conditions. Using polystyrene-based resins and nonaqueous reaction conditions, our results indicate that $NH_2OH\cdot HCl$ neutralized by 1 equiv of base cleaves the thioester linkage in DMF, which is consistent with the results of Muir et al.[7] However, $NH_2OH\cdot HCl$ has poor solubility in most organic solvents except for DMF, which brought difficulties to the workup after cleavage due to its high boiling point. Cleavage by trityl protected hydroxylamine was sluggish, which we attribute to steric hindrance. While both *O*-benzyl hydroxylamine and *O*-trimethylsilyl hydroxylamine cleaved the thioester linkage, the latter has the advantage of a more convenient O-deprotection. Therefore, *O*-trimethylsilyl hydroxylamine was used for cleavage of peptide thioester resins **2** and **3**. In a representative reaction, 30 equiv of *O*-trimethylsilyl hydroxylamine in dry THF or toluene displaced the thioester linker within 24 h at 25 °C. After cleavage, the TMS protecting group was cleanly removed by 5% TFA in CH_2Cl_2. The cleavage was essentially quantitative, as demonstrated by a negative ninhydrin test for the resin after cleavage of compounds **4.1** to **4.7**. A library of 17 peptide hydroxamic acids was synthesized with good to excellent purity (Table 1).

These results indicate that this methodology affords efficient preparation of peptide hydroxamic acids under mild conditions. Moreover, it does not require additional synthesis of a special linker between the peptide and the resin since commercially available thiol-containing resins can be used directly. An advantage of using *O*-trimethylsilyl hydroxyl-

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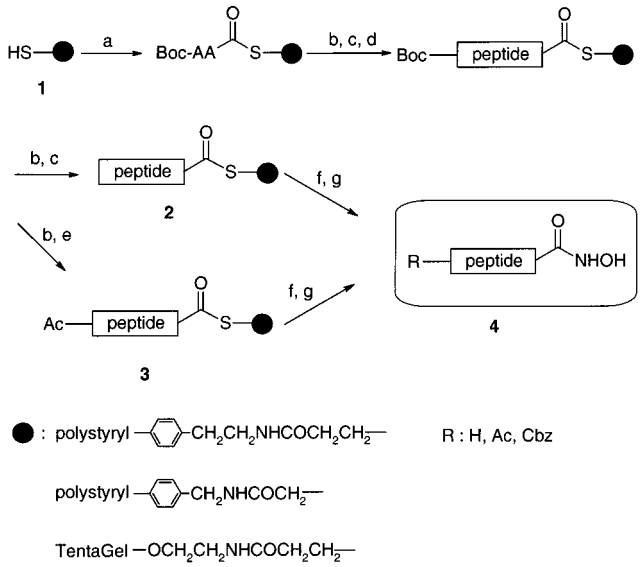
Scheme 1. Synthesis of Peptide Hydroxamic Acids Using Thioester Resin[a]



[a] Reagents: (a) Boc-amino acid/DIEA/PyAOP, DMF, 2 h; (b) 50% TFA/DCM, 20 min; (c) 5% DIEA/DMF/DCM, 2 × 2 min; (d) SPPS; (e)Ac_2O/DIEA (1:1), DMF, 20 min; (f) NH_2OTMS (30 equiv), dry toluene, 16-24 h; (g) 5% TFA/DCM, 10 min.

Table 1. Peptide Hydroxamic Acids Synthesized on Thioester Resins

entry	peptide hydroxamic acid	purity (%)[a]	mol wt calcd	mol wt found[b]
4.1	H-Gly-Leu-Phe-Ala-NHOH	90	421.5	421.4
4.2	H-Pro-Gly-Leu-Phe-Ala-NHOH	85	518.6	518.3
4.3	H-Met-Gly-Leu-Phe-Ala-NHOH	84	552.7	552.0
4.4	H-Trp-Gly-Leu-Phe-Ala-NHOH	80	607.7	607.5
4.5	H-His-Gly-Leu-Phe-Ala-NHOH	81	558.6	558.3
4.6	H-Lys(2-Cl-Z)-Gly-Leu-Phe-Ala-NHOH	82	718.2	717.7
4.7	H-D-Ala-Asn-Leu-Phe-Ala-NHOH	80	549.6	549.1
4.8	Ac-Gly-Leu-Phe-Ala-NHOH	100	463.5	463.3
4.9	Ac-D-Ala-Gly-Leu-Phe-Ala-NHOH	84	534.6	534.1
4.10	Ac-Val-Gly-Leu-Phe-Ala-NHOH	86	562.6	562.4
4.11	Ac-His-Gly-Leu-Phe-Ala-NHOH	86	600.7	600.7
4.12	Ac-D-Ala-Asn-Leu-Phe-Ala-NHOH	80	591.6	591.0
4.13	Ac-Trp-Pro-Gly-Val-Gly-NHOH	80	571.6	571.0
4.14	Ac-Lys(2-Cl-Z)-Gly-Leu-Phe-Ala-NHOH	80	760.2	759.6
4.15	Ac-Asp(OBzl)-Gly-Leu-Phe-Ala-NHOH	80	668.6	668.2
4.16	Cbz-Ala-Gly-Leu-Phe-Ala-NHOH	86	626.7	626.5
4.17	Cbz-Ala-Pro-Gly-Val-Gly-NHOH	86	548.5	548.2

[a] Determined by HPLC analysis of the crude product at $\lambda = 220$ nm with a gradient of 10% to 100% solvent B (solvent A: 0.05% TFA in water; solvent B: 0.05% TFA in acetonitrile) over a period of 15 min. Quantitative cleavage from the resin is assumed. [b] Obtained on an HP1100 LC/ESI-MS or a PE Biosystem Voyager MALDI-TOF MS.

amine over aqueous hydroxylamine[6g] is selective cleavage of the thioester linkage in the presence of esters (Table 1, entry **4.15**). In conclusion, we have developed a simple strategy based on a thioester linkage for solid-phase synthesis of peptide and non-peptide hydroxamic acids, which facilitates the generation of metalloenzyme inhibitor combinatorial libraries. We note that this approach is also applicable to generation of other C-terminal modified peptide libraries.

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Supporting Information Available. General procedure for peptide hydroxamate synthesis. This material is available free of charge via the Internet at http://pubs.acs.org.

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