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Synthesis of *N*-Hydroxy Peptides: Chemical Ligation of *O*-Acyl Hydroxamic Acids

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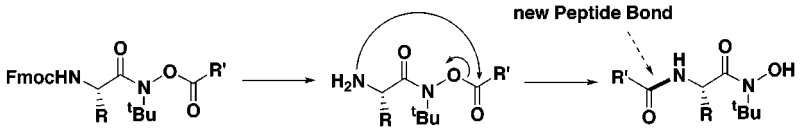
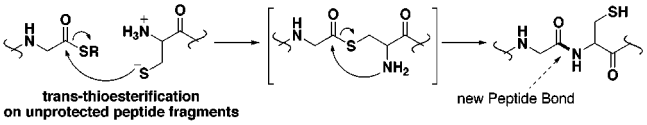
ABSTRACT



A novel chemical ligation process is described that results in the construction of *N*-hydroxy peptides.

Hydroxamic acids have received much attention as biologically active compounds such as ACE,[1] lipoxygenase,[2] peptidase,[3] and PDE inhibitors,[4] chelating agents,[5] and anticancer,[6] antiarthritic,[7] and antimalarial[8] agents. While working on *O*-acyl hydroxamic amino acids, we discovered a new variation on chemical ligation that results in the formation of a new peptide bond and leaves a residual hydroxamic acid in the peptide chain. Previous chemical ligation methods include the elegant work by Kent[9] based on a cysteine-mediated rearrangement (Scheme 1), the cysteine disulfide work by Kemp,[10] the pseudoproline methods of Tam,[11] the oxime method as exemplified by Rose,[12] and the 6-nitro-2-hydroxybenzyl approach by Meutermans and Smythe.[13] Chemical ligation has been utilized as a key tool for syntheses of many biologically crucial compounds such as lipopeptides,[14] proteins,[15] DNA strands

Scheme 1. Cysteine-Based Native Chemical Ligation



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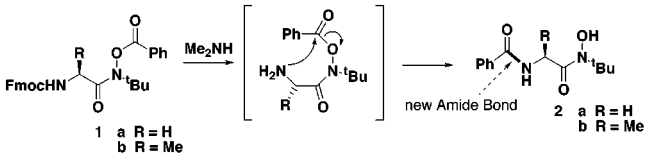
and templates,[16] oligonucleotides,[17] enzyme inhibitors,[18] and HIV targets[19] and for solid-phase syntheses of unprotected peptides[20] and glycopeptides.[21]

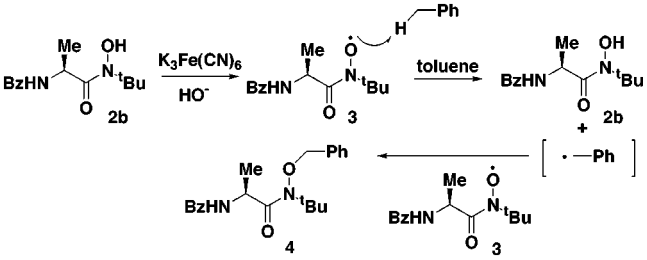
The *O*-acyl hydroxamic acid substrates **1** were prepared by converting Fmoc-glycine or Fmoc-alanine into the stable acid chlorides with oxalyl chloride.[22] Coupling to *O*-benzoyl *N*-*tert*-butyl hydroxylamine with pyridine in refluxing benzene gave the *O*-protected hydroxamic acid derivatives **1** (Gly 82%, Ala 96% yields). Removal of the Fmoc group with dimethylamine resulted in rearrangement through a six-membered transition state (Scheme 2).[23] The identity of the

Scheme 2. Hydroxamic Acid Based Chemical Ligation



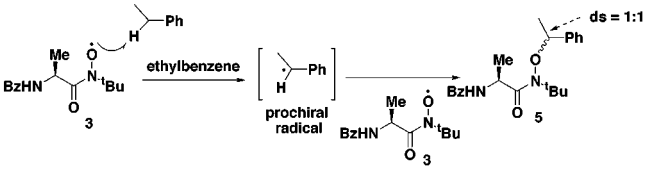
resultant *N*-acyl amino hydroxamic acids **2** (Gly 88%, Ala 83% yields) was confirmed both by IR (peaks at 3200 and 1634 cm^{-1}) and by visualization of the TLC by acidic ferric chloride.[24] Mild oxidation of **2b** with saturated aqueous potassium ferricyanide solution gave acyl nitroxide **3**, isolated in 73% yield as a deep blue solid. Treatment of **3** with warm toluene resulted in the formation of a mixture of the parent hydroxamic acid **2b** and the corresponding *O*-benzyl hydroxamic acid **4** (33% yield) (Scheme 3). Mechanistically,

Scheme 3. Formation and Trapping of Acyl Nitroxide **3**



acyl nitroxide **3** abstracts a benzylic hydrogen[25] from toluene, forming 1 equiv of hydroxamic acid **2b** and 1 equiv of benzyl radical. The benzyl radical is scavenged by unreacted persistent acyl nitroxide **3** in a coupling reaction. In conjunction with stereoselectivity studies in our laboratories on the coupling of prochiral radicals with chiral nitroxides,[26] acyl nitroxide **3** was trapped with 1-phenethyl radical generated from ethylbenzene both at room temperature and at −78 °C (Scheme 4). No stereoselectivity was observed; *O*-alkyl

Scheme 4. Nondiastereoselective Coupling of Optically Active Acyl Nitroxide **3** with a Prochiral Radical



hydroxamic acid **5** was produced as a 1:1 mixture of diastereoisomers, reflecting the conformational mobility of acyclic acyl nitroxide **3**.

The *O* to *N* acyl rearrangement process was then extended to the formation of new peptide bonds. *O*-Acylation of *tert*-butylhydroxylamine with *N*-acetyl glycine or alanine **6** under coupling conditions (EDCI, CH_2Cl_2) yielded hydroxylamine esters **7** (Table 1). A small amount of DMF was added when necessary to achieve solubility of all reactants. No products resulting from *N*-acylation of the *tert*-butylhydroxylamine were observed. Treatment of hydroxylamine esters **7** with

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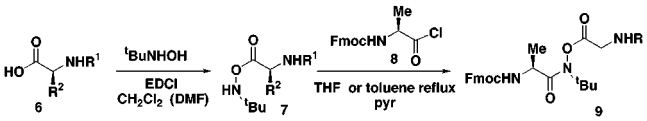
Table 1. Preparation of *O*-Acyl Hydroxamic Acid Peptide Substrates

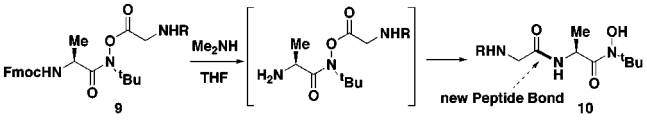


compd	R^1	R^2	% yield
7a	Ac	H	42
7b	BzGly	H	71
7c	BocGly	H	75
7d	ZLeu	H	62
7e	Bz	H	84
7f	Bz	Me	63
7g	ZGly	Me	95
9a	Ac	H	49
9d	Bz	H	24
9e	ZLeu	H	27

Fmoc-amino acid chlorides **8** gave *N*-acylated products **9**. This reaction tended to be sluggish due to the sterically hindered neopentyl hydroxylamine nitrogen in **7** and thus required use of the preformed acid chlorides, and for particularly hindered cases (**9d** and **9e**), the use of toluene at reflux in place of THF.

The key ligation step was effected by deprotection of the Fmoc group under mild conditions (Table 2). Thus, brief exposure of ester **9** to dimethylamine in THF gave immediate formation of rearranged product **10**. In the preparation of **10a**, we found that the *N*-hydroxy peptide was easily purified by removal of the dibenzofulvene byproduct using trituration of the residue with benzene followed by evaporation. Thus, the ligation of Ac-glycine from *O*-acyl hydroxamic acid **9a** gave Ac-Gly-Ala-hydroxamic acid **10a** in 75% isolated yield following column chromatography, and ligation of the dipeptide *Z*-Leu-Gly fragment gave *Z*-Leu-Gly-Ala-hydroxamic acid **10e** in 24% isolated yield (purification by flash column followed by HPLC). This hydroxamic acid based rearrangement, like the cysteine rearrangement, is effective due to the good leaving group properties of the hydroxamic acid conjugate base, similar to that of thiolate. The pK_a values of alkyl hydroxamic acids[27] are found at approximately 9.4, whereas the thiol of cysteine has a pK_a of 8.3.

Table 2. Ligation To Form Di- and Tripeptides



compd	R	% yield
10a	Ac	75
10d	Bz	70
10e	ZLeu	24

We have been working on extending this methodology to the formation of longer peptides in which the *tert*-butyl amino group of precursor **7** is an *N*-hydroxy amino acid, derived by the oxaziridine route.[28]

The resulting *N*-hydroxy peptides available by this new ligation method are expected to be valuable as peptidomimetics and as metalloenzyme inhibitors by virtue of their strong metal chelation properties. This new ligation methodology adds to the existing arsenal of mild experimental conditions for peptide bond formation.

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Supporting Information Available: Experimental procedures with spectral data for compounds **1a** **1b**, **2a**, **2b**, **3**, **4**, **5**, **7a**−**f**, **9a**, and **10a**. This material is available free of charge via the Internet at http://pubs.acs.org.

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