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Preparation of Peptide Thioacids using the Kaiser Oxime Resin

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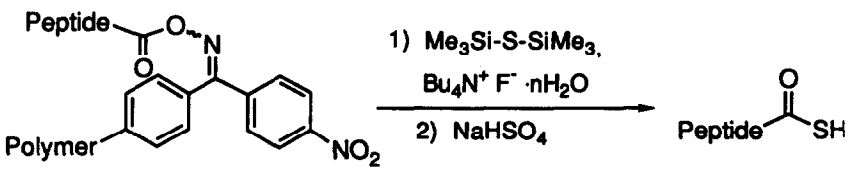
Abstract: Peptide C-terminal thioacids are readily prepared with protecting groups intact by cleavage of peptides from Kaiser's oxime ester resin by treatment with hexamethyldisilathiane / tetrabutylammonium fluoride. Such thioacids are useful for peptide fragment couplings.

Thioacids are among the most versatile of functional groups at the C-terminus of peptides. They tend to be more soluble than their oxygen counterparts, and may be activated for peptide coupling in a variety of ways[1]: carbodiimide-induced coupling proceeds with less racemization than for oxygen acids,[1] excess silver ion in the presence of N-hydroxysuccinimide has been used for peptide fragment coupling,[2,3] as has 2-pyridyl disulfide.[1] Amino thioacids may also be used as active esters for papain-mediated condensations,[4] as well as in the novel construction of backbone-engineered proteins by alkylation of sulfur.[5] We have recently demonstrated highly selective acylations of thiol-bearing amines at low concentration in the presence of a large excess of other amines by using silver ion under specific conditions.[6] Clearly, peptidyl thioacids have significant utility.

The problem is that preparation of peptides with C-terminal thioacid groups is more difficult than is preparation of ordinary peptides. The thioacids are prepared without racemization by reaction of a p-nitrophenyl ester[2] or an N-hydroxysuccinimide ester[1,4,7] of a protected amino acid with a salt of H_2S. Thiocarboxylate displacement of a benzhydryl halide from a linker, followed by attachment of linker to a solid support, yields the preferred resin for peptide thioacid synthesis.[1,7] The difficulty lies in the need to prepare a different resin through this route for each different amino acid, demonstrated to date only for glycine and leucine.

The oxime resin developed in Kaiser's laboratory[8,9] has proven useful for synthesis of short peptides that may be cleaved from the resin with a range of groups at the C-terminus, and with sidechain protecting groups intact.[10,11] We decided to try to prepare thioacids from acylated oxime resin.

There are two strategies by which a moderately active peptidyl ester such as an O-acyl oxime may be converted to a thioacid. Either a H_2S equivalent may be used to nucleophilically cleave the ester directly to a thioacid, or the amine of an unprotected thioamino acid may be used to cleave the ester, yielding a peptide thioacid of one amino acid more. The second strategy has close precedent, in that tetrabutylammonium salts of amino acids do react with peptidyl oxime resin to yield the desired peptide free acids.[12] The direct cleavage with a H_2S equivalent was more appealing, however, as it obviated the need to prepare a different reagent for each amino acid desired at the C-terminus of a peptide; we investigated this approach.



Our intial studies employed the simple benzophenone oxime ester of t-BOC-L-alanine as a model. Reaction of this material with H_2S/diisopropylethylamine or Na_2S in DMF or other aprotic solvents in the presence or absence of dimethylaminopyridine led to little or no reaction over the course of several hours. Partially aqueous solvents led to hydrolysis. Rapid reaction was ultimately achieved using the lithium trimethylsilyl sulfide in THF reagent developed by Kraus.[13] An even milder procedure was also found to be

effective: commercially available hexamethyldisilathiane reacts with the oxime ester in the presence of tetrabutylammonium fluoride to yield the desired thioacid on workup. The reactions go rapidly at room temperature, producing the desired thiopeptides in excellent yields. This process is perhaps driven by a hard/soft acid/base exchange as seen in the reaction of phenyl esters with trimethylsilyl imidazole to produce the acyl imidazolides.[14]

We employed this new reaction to prepare the peptide thioacids shown in Table 1, using the Kaiser resin.

Table 1

Peptide	Yield	Area %[a]
BOC-Phe-SH	73	81
BOC-Ala-SH	67	79
BOC-Ser(OBn)-SH	73	82
BOC-Asp(OBn)-SH	78	94
BOC-Pro-Ser(OBn)-SH	77	61
BOC-Asp(OBn)-Ala-SH	66	61
BOC-Phe-Asp(OBn)-SH	81	67
BOC-Glu(OBn)-Ser(OBn)-SH	80	63

[a] HPLC (C-18, 1:1 CH_3CN/H_2O, 0.1% TFA, 220 nm detection) area % of crude peptide trace that corresponds to desired product.

Typical reaction conditions: To a solution of hexamethyldisilathiane (0.056mmol) in 0.5mL THF at 0°C under N_2, tetrabutylammonium fluoride (0.056mmol) is added as a 1M solution in THF. The blue solution is allowed to come to room temperature over the course of 1 hour, at which time it is added by syringe to resin-bound peptide (0.011mmol) swollen in 1 mL THF. The mixture is stirred for 30 minutes at room temperature, then the solid is filtered off and rinsed thoroughly with THF. The THF extract is dissolved in 5mL ethyl acetate, washed repeatedly with 0.1 N $NaHSO_4$ to remove tetrabutylammonium, once with saturated NaCl to remove excess water, dried over Na_2SO_4, filtered, and rotary evaporated to dryness. Analytical and preparative HPLC showed the reactions proceeded cleanly. All products gave 300 MHz ^1H NMR, IR, and MS spectra (NH_3 CI, negative ion, m-1) consistent with their structures. The major impurity observed is the oxygen acid, which is readily removed by HPLC, and which does not interfere in reactions of the thioacids, not even DCC coupling.[1] Neither thioacids nor byproduct oxygen acids are detectably racemized (<1%).[15]

In summary, we have developed a new procedure for the preparation of peptide C-terminal thioacids that is quite efficient. In contrast to previous methods,[1,7] it may be carried out using commerically available reagents, and yields thioacids of whatever amino acid is desired at the C-terminus. The advantages of peptide thioacids for fragment couplings[1,4-7] may now lead to their wider application.

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