Letter

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Incorporation of Acid-Labile Masking Groups for the Traceless Synthesis of C-Terminal Peptide α-Ketoacids

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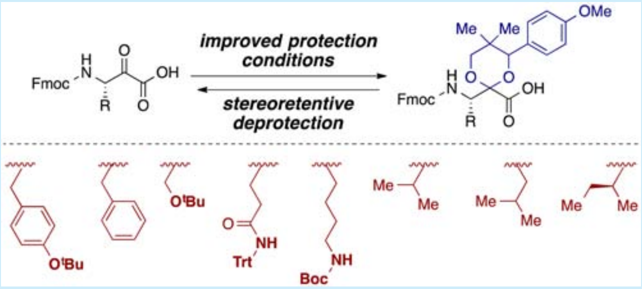
Supporting Information

ABSTRACT: An optimized protocol for the masking of α-ketoacids with acid-labile cyclic acetal protecting groups is reported. Unlike prior approaches, these new conditions allow the synthesis of protected α-ketoacids bearing aromatic, hindered alkyl, and protected polar side chains. Attachment to a Wang-type linker and solid support provides a resin that delivers fully unprotected C-terminal peptide α-ketoacids upon resin cleavage. These peptides are the key starting materials for chemical protein synthesis using the α-ketoacid−hydroxylamine ligation.

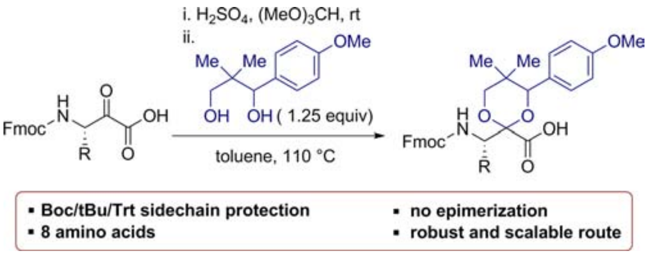


The α-ketoacid−hydroxylamine (KAHA) ligation makes possible the total synthesis of proteins by the combination of unprotected peptide segments under aqueous, acidic conditions.[1,2] This ligation, which employs two distinct functional groups, complements the well-established native chemical ligation (NCL)[3] and has been applied to the preparation of numerous proteins.[4,5] The KAHA ligation requires C-terminal peptide α-ketoacids. In addition to a two-step strategy via C-terminal cyanosulfurylides,[6] we recently reported the traceless preparation of C-terminal peptide α-ketoacids by Fmoc solid-phase peptide synthesis (Fmoc-SPPS).[7] This approach delivers fully unprotected, C-terminal α-ketoacids directly upon resin cleavage, is compatible with segments containing oxidation-sensitive residues (Cys, Met and Trp), and reduces the number of manipulations and purifications. We have used this traceless approach to α-ketoacids for the preparation of several proteins and for the direct formation of peptide macrocycles from the unpurified peptide segments.[8]

Although the traceless linker and α-ketoacid deprotection work extremely well, our existing approach to preparing the requisite protected α-ketoacid starting materials has so far been limited to nonbranched amino acids without acid-labile protecting groups, essentially limiting the choices to phenylalanine and leucine. The origin of this problem lies in the method for the introduction of the acid-labile cyclic acetal. Unlike classic acetal formation, the reaction most likely proceeds via an intermediate benzylic carbocation. This requires highly acidic conditions (in situ generated triflic acid), prohibiting the use of acid-labile side-chain protecting groups. To obtain the product in acceptable yields, high dilution and continuous slow addition of the bis-TMS protected diol protecting group are required.[9] This limits both the choice of substrates as well as the scalability of the process. In order to extend the KAHA ligation to a broader range of C-terminal α-ketoacids, we required an an alternative approach to the protected monomers.

In this report, we document an alternative, general approach to the protection of α-ketoacids for use in our traceless synthesis of C-terminal peptide α-ketoacids using Fmoc-SPPS. The key step involves transacetalization of α-ketoacid dimethyl acetals with benzylic diols under thermal conditions without additional Brønsted or Lewis acids (Scheme 1). The requisite dimethyl acetals are formed by treatment of the α-ketoacid with neat trimethyl orthoformate and sulfuric acid.[10] Importantly, acid-labile protecting groups commonly used in Fmoc SPPS (tBu/Boc/Trt) are tolerated under these conditions.

Scheme 1. Stereoretentive Protection of α-Ketoacids



As depicted in Scheme 2, α-ketoacids derived from commercially available Fmoc-protected amino acids were prepared using our reported method of sulfur ylide oxidation by Oxone. After acid-promoted dimethyl acetal formation, the protecting group was introduced by transacetalization under

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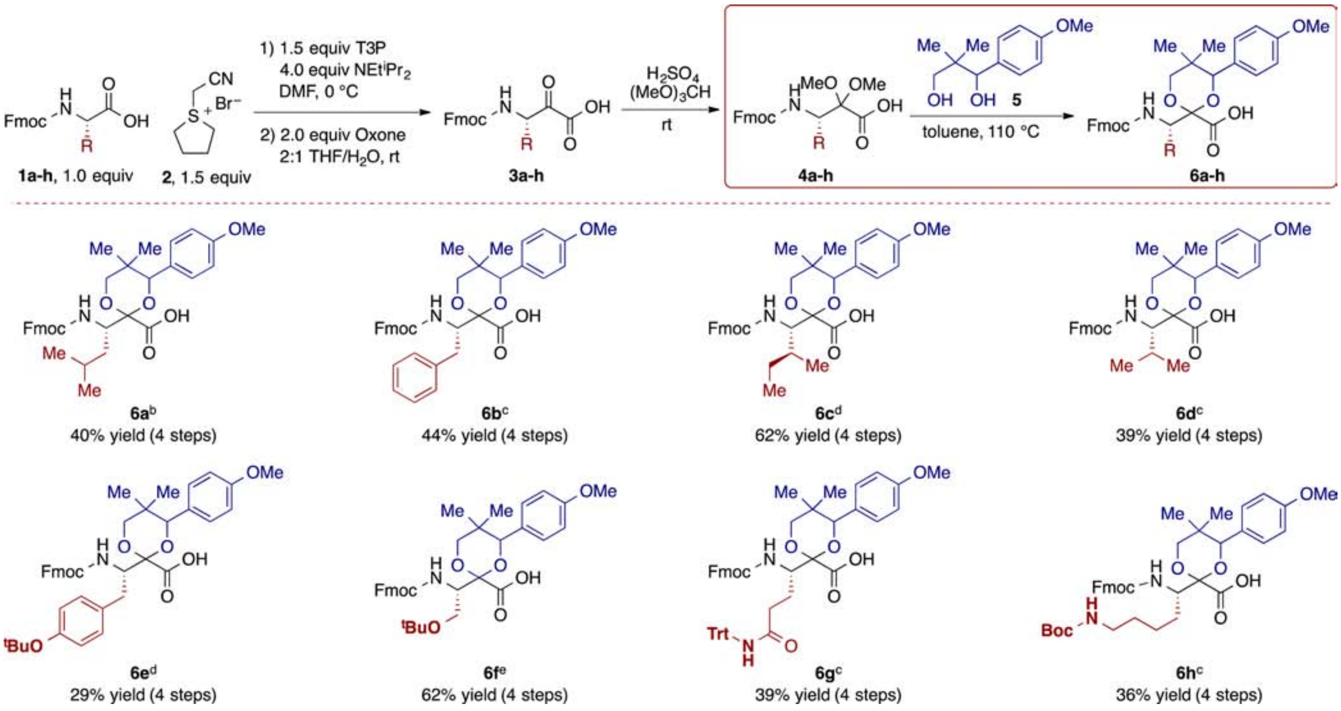
Scheme 2. Synthesis and Protection of Enantiopure α-Ketoacids[a]



[a] Yield refers to isolated yield of pure material starting from the corresponding Fmoc-protected amino acid; T3P = propylphosphonic anhydride. [b] 0.25 equiv of H_2SO_4 were used. [c] 1.0 equiv of H_2SO_4 were used. [d] 0.50 equiv of H_2SO_4 were used. [e] 0.45 equiv of H_2SO_4 were used.

thermal conditions in the presence of diol **5** in toluene.[11,12] This two-step protocol for the preparation of protected α-ketoacid is easily scalable using reaction concentrations >0.3 M from readily accessible starting materials and without the use of special techniques. One of our main concerns about this strategy was possible epimerization during dimethyl acetal formation and transacetalization. Satisfyingly, no epimerization was observed during any of these steps using our previously reported assay (see the Supporting Information).

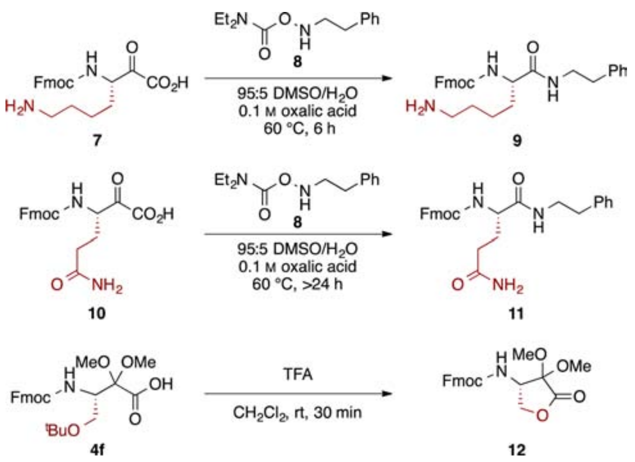
In addition to the preparation of the protected L-leucine **6a** and L-phenylalanine **6b**, α-ketoacids that we previously reported,[1,4] we were pleased to find that this protocol was applicable to a wide variety of other amino acid derivatives. The preparation of the protected valine **6d** proceeded in 39% yield over four steps. In contrast, our prior approach gave less than 10%. These conditions also worked very well for the related L-isoleucine derivative **6c**.

The major limitation of our prior approach was the incompatibility with amino acids bearing acid-labile side-chain protecting groups. We were therefore very pleased to find that these conditions were compatible with Boc-, tBu-, and even Trt protecting groups. In the case of Boc-protected substrates, such as L-lysine **6h**, 1 equiv of H_2SO_4 was required to complete the dimethyl acetal formation, but no significant amounts of side-chain-deprotected products or methyl esters were observed.

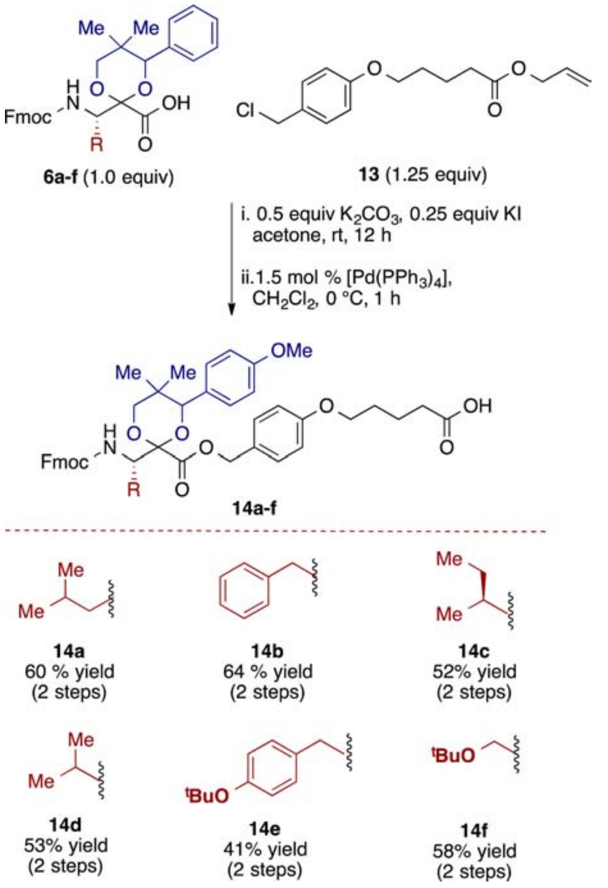
Fmoc-(*O*-^{t}Bu)-L-tyrosine (**6e**) and Fmoc-(*O*-^{t}Bu)-L-serine (**6f**), both bearing *tert*-butyl ether protecting groups, could be protected without any deprotection or ester formation. Even Fmoc-(*N*-Trt)-L-glutamine (**6g**), bearing the acid-sensitive trityl group, was cleanly prepared using 1 equiv of H_2SO_4. The transacetalization under the optimized conditions proceeded well in all cases to give the protected α-ketoacids.

As some of these α-ketoacids had not been previously prepared, we evaluated their reactivity in a KAHA ligation with phenethylhydroxylamine **8** (Scheme 3). Side chain unprotected lysine α-ketoacid **7** proved to be a good ligation partner with complete conversion after 6 h at 60 °C. Side chain unprotected L-glutamine α-ketoacid **10** reacted slowly (up to 24 h for complete conversion), possibly due to reversible attack of the primary amide at the α-keto carbonyl, making it a potential candidate for kinetically controlled multisegment ligations. Unfortunately, upon deprotection, the L-serine α-ketoacid **4f** cyclizes to form a 6-membered lactone with its side chain hydroxyl group under deprotection conditions (TFA) and is therefore not likely to be suitable as a C-terminal α-ketoacid.

Scheme 3. KAHA Ligations of New α-Ketoacids



Scheme 4. Improved Conditions for Linker Attachment

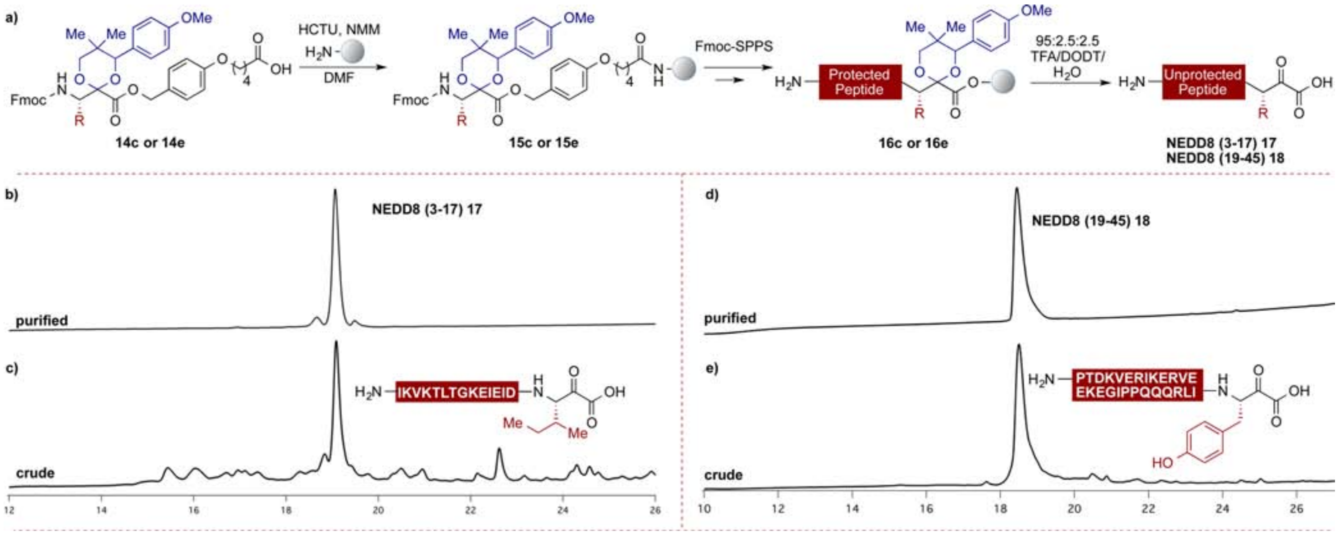


As part of the optimization of the synthesis of protected α-ketoacids, we also refined the conditions to introduce the linker.[13] Our previous strategy, which utilized a phase-transfer-catalyzed alkylation of the carboxylic acid, often resulted in a tedious chromatographic purification due to hydrolysis to give a benzylic alcohol that tended to coelute with the desired product. We were pleased to find that using 0.5 equiv of K_2CO_3 and 0.25 equiv of KI in dry acetone avoided both hydrolysis and Fmoc deprotection, a side reaction we had observed under other conditions (Scheme 4). When the reaction is performed in more concentrated solutions (1 M), completion could be observed within a few hours at room temperature, compared to overnight reaction at 50 °C for the phase-transfer conditions. Allyl ester deprotection with $Pd(PPh_3)_4$ and morpholine provided the protected α-ketoacids on a Wang-type linker, ready for attachment to the resin and Fmoc-SPPS.

With all monomers prepared in an easy and scalable manner, we demonstrated their usefulness by the preparation of α-ketoacid peptides with novel C-terminal residues by Fmoc-SPPS. For example, the protected α-ketoacids of L-isoleucine **14c** and L-tyrosine **14e** were loaded on Rink-amide resin using standard coupling conditions (HCTU, NMM in DMF, Scheme 5). Peptides of various lengths were assembled (from 17 to 26 amino acids) without any deviation from standard Fmoc-SPPS or unexpected difficulties. After cleavage from the resin by treatment with TFA, C-terminal α-ketoacid peptides could be isolated by preparative HPLC in good yield (see the Supporting Information for full details).

In summary, we have developed a new synthetic method for the masking of α-ketoacids with traceless protecting groups. This approach is compatible with acid-labile side chain protecting groups, which allowed us to prepare a spectrum of protected α-ketoacids, including those with sterically hindered aliphatic side chains and polar side chains bearing acid-labile protecting groups. In conjunction with improved conditions for linker attachment, these new protocols provide an expedient, scalable approach to C-terminal peptide α-ketoacids for KAHA ligation. Resins bearing these protected α-ketoacids can be stored for months and will soon be commercially available, thereby further facilitating the use of the KAHA ligation for protein synthesis.

Scheme 5. Solid Phase Peptide Synthesis of C-Terminal Peptide α-Ketoacids



[a] Immobilization of α-ketoacid monomers followed by Fmoc-SPPS and final cleavage from solid support and deprotection of the peptide with TFA. [b] Purified NEDD8 (3−17) **17**. [c] Crude NEDD8 (3−17) **17**. [d] Purified NEDD8 (19−45) **18**. [e] NEDD8 (19−45) **18**.

■ ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b01692.

Synthesis and characterization of all intermediates (PDF)

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Notes

The authors declare no competing financial interest.

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