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Masked side-chain aldehyde amino acids for solid-phase synthesis and ligation

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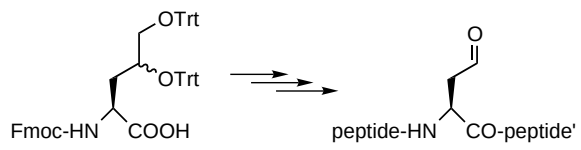
Abstract—The masked aldehyde amino acid Fmoc-Hyl(Boc-oxazolidine) **1**, has been synthesized from the parent amino acid in five steps (3 pots). The employed protection scheme renders **1** well suited for standard Fmoc-based solid-phase assembly of peptides and similar structures, including TFA-based deprotections. The resulting peptides possess a side-chain 1,2-amino alcohol, and post-TFA treatment, periodate oxidation of the unprotected peptide unmasks the aldehyde function. The given order of transformations circumvents the known, problematic release of reactive aldehydes in TFA solution. The post-TFA generated peptide aldehydes have been utilized in model chemo-selective ligations, with formation of hydrazone constructs. Additionally, Fmoc-Hyl(Alloc-oxazolidine) **10** was synthesized, and used for on-resin aldehyde generation and hydrazone transformation

The wide range of synthetic transformations that are available for the aldehyde function makes it an attractive stepping-stone in the construction of chemical diversity.[1] In the field of peptides, aldehyde groups are used for example as intermediates for oxime, hydrazone and thiazolidine-based chemo-selective ligation (coupling of unprotected peptides).[2,3] The most common methods for the introduction of aldehyde functions in peptide sequences rely on solid-phase coupling of protected or masked aldehyde building blocks on peptide amino groups, which are selectively accessible via the use of orthogonal protecting groups.[4,5] However, for general preparation of peptide aldehydes, we found it desirable to gain access to amino acid derivatives with protected or masked aldehydes built directly into the amino acid side-chains,[6,7] thus simplifying structural complexity and eliminating the need for multi-dimensional deprotection schemes.

The inherent reactivity of aldehydes is pronounced in TFA, which is the standard reagent for peptide deprotections and Fmoc-based solid-phase cleavage schemes. There are several examples of the TFA-promoted reactivity of peptide aldehydes, e.g. Pictet–Spengler condensations[8,9] and conversions of mercaptyl peptide aldehydes into macrocycles.[10] Also, TFA-promoted reactivity of the peptide backbone towards aldehyde groups has been described, and circumvented by use of amide backbone protection.[11] However, when isolation of the peptide aldehyde is desired, handling in strong TFA solutions does not appear attractive. Amino acids with e.g. acetal protected side-chain aldehydes[10] are therefore not generally suited for the preparation and isolation of peptide aldehydes.

In order to circumvent the problematic release of aldehydes in TFA solution, we found it desirable to mask side-chain aldehydes as 1,2-diols or 1,2-amino alcohols, liberating the aldehyde by mild post-TFA periodate oxidation.[12,13] Notably, oxidation sensitive residues such as tryptophan, methionine and cystine (but not cysteine) are compatible with the mild conditions typically used in periodate oxidations of peptide diols and amino alcohols (5–10 min, pH 7).[14]



Scheme 1. Fmoc-Adi(Trt)$_2$ and the derived peptide aldehyde.[15]

Abbreviations: Adi, L-2-amino-4,5-dihydroxy-pentanoic acid; Alloc, allyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; DNPH, 2,4-dinitrophenylhydrazine; EDTA, ethylenediaminetetraacetate; Fmoc, 9-fluoronylmethoxycarbonyl; Hyl, (*R*)-5-hydroxy-L-lysine; MALDI, matrix-assisted laser desorption ionization; OSu, N-oxy-succinimide; Trt, trityl, triphenylmethyl.

Keywords: peptide aldehyde; hyl; periodate; ligation.

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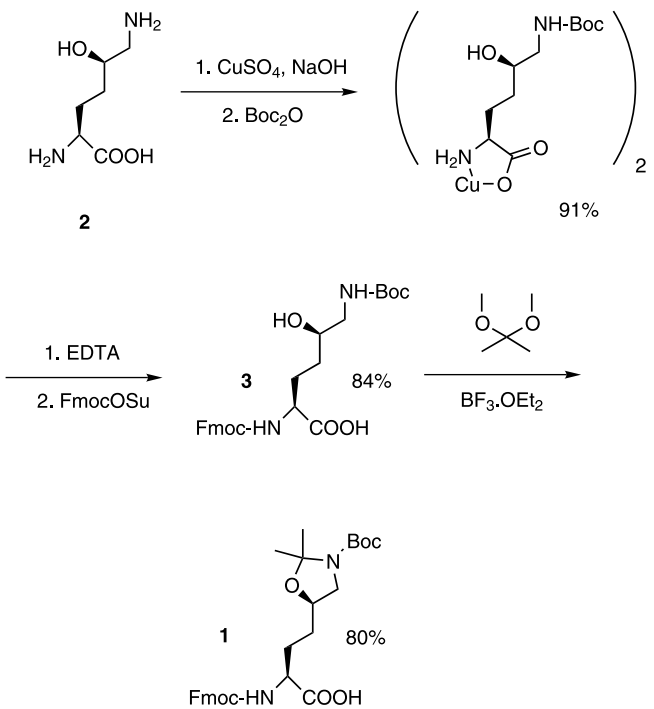
In compliance with the above specifications, we have recently described the synthesis and use of a protected side-chain diol amino acid, Fmoc-Adi(Trt)$_2$.[15] Unfortunately, the stereochemistry introduced at the Adi γ-carbon was difficult to control, and Adi is presently used as a diastereomeric mixture (8:2). Importantly, since the periodate oxidation eliminates the chirality in the Adi γ-carbon, the diastereomer problem is only present in passing (Scheme 1), but it is nevertheless a potential nuisance for peptide purifications at the diol stage, which is of course advisable due to the inherent reactivity of the aldehyde function.

(*R*)-5-Hydroxy-L-lysine (Hyl, **2**) is a natural compound, which can be isolated from collagen,[16–18] and **2** is commercially available at a reasonable cost (1 g, 212 USD; Fluka). Alternatively, **2** can be accessed by stereoselective synthesis (>ten steps).[19] Since **2** carries a stereochemically predefined 1,2-amino alcohol in the side-chain, we found **2** to be attractive as a masked aldehyde building block. The aldehyde potential of **2** has previously been noted,[20] but apparently never practiced in peptide synthesis, and suitably protected derivatives of **2** are not available. Kihlberg et al. have recently used **2** for the construction of glycopeptides, exploiting the 5-hydroxy group as a glycosylation anchor.[21,22] As depicted in Scheme 2, we extended Hyl chemistry by protecting the amino acid as the N^{α}-Fmoc-N^{ε}-Boc-oxazolidine derivative **1**, thereby allowing the use of **1** in standard Fmoc-based solid-phase synthesis with TFA-based cleavage, followed by mild periodate oxidation to the peptide aldehyde. *O*,*N*-Acetals, such as the oxazolidine used here, are robust as long as their *N*-atoms are



Scheme 2. Synthesis of Fmoc-Hyl(Boc-oxazolidine) **1**.

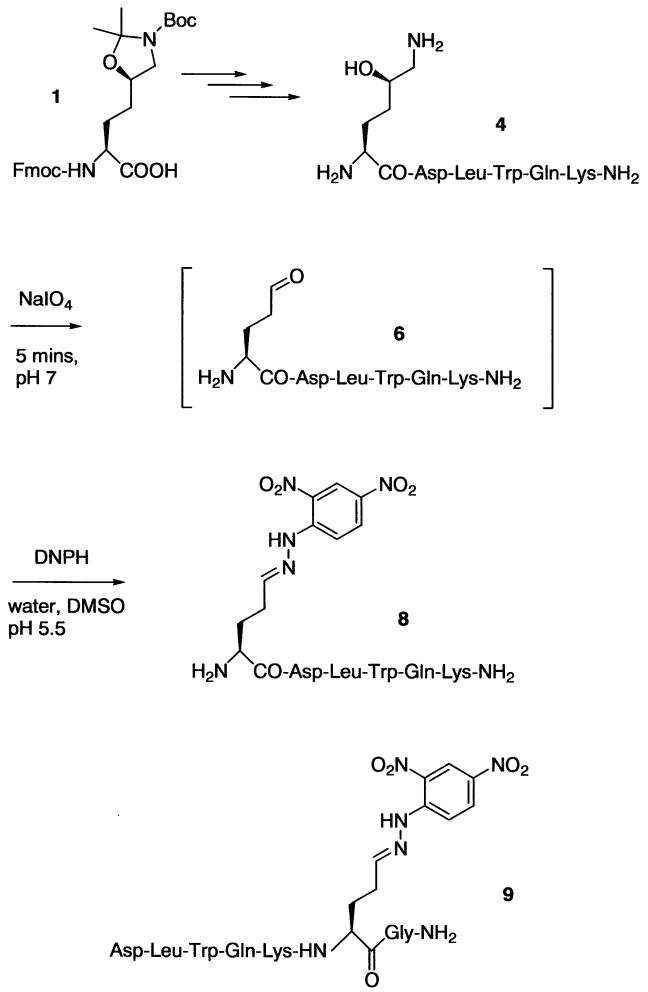
Boc-protected, but once the Boc-group is cleaved, the *O*,*N*-acetal hydrolyses easily.[23]

As depicted in Scheme 2, the α-amino acid function of **2** was initially protected by treatment with $CuSO_4 \cdot 5H_2O$ under basic, aqueous conditions. We found that $CuSO_4$ gave better reproducibility than the previously used, rather insoluble, mixed copper carbonate ($CuO \cdot CuCO_3$).[21,22] The generated copper complex was treated with Boc anhydride in dioxane, and the product was allowed to precipitate, typically over 3 days. Next, the copper complex was dissociated by treatment with an aqueous solution of EDTA,[24] and the liberated α-amine was protected by addition of Fmoc-OSu/Na_2CO_3 in dioxane/water. We found the use of EDTA for copper complex dissociation to be a convenient and effective alternative to the previously applied Na-Chelex column.[21,22] In order to avoid lactone formation in Fmoc-Hyl(Boc) **3**, the storage of **3** should be avoided, and hence **3** was soon treated with dimethoxypropane–$BF_3 \cdot OEt_2$ in acetone.[25] Product **1** was isolated upon work-up, via precipitation from ether/hexane. For a preparative procedure and NMR/MS-data, see note.[26]

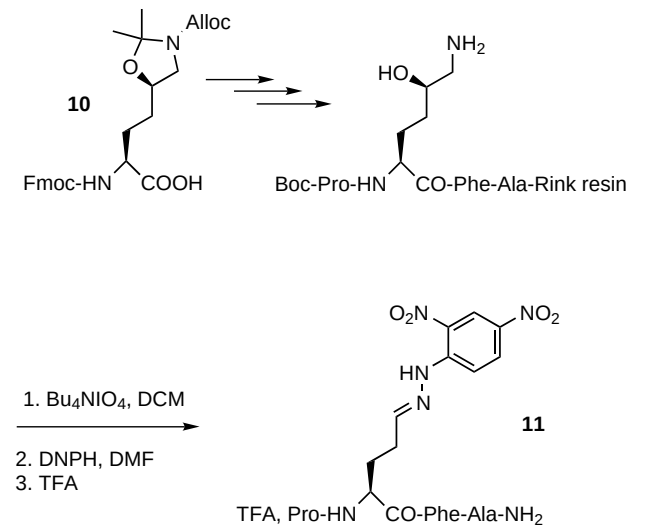
The masked aldehyde amino acid **1** was used in standard Fmoc-based solid-phase preparation of peptides Hyl-Asp-Leu-Trp-Gln-Lys-NH_2 **4** and Asp-Leu-Trp-Gln-Lys-Hyl-Gly-NH_2 **5**, yields 71 and 90% (based on resin loadings). Upon TFA-based cleavage, the 1,2-amino alcohol peptides **4** and **5** were converted to the peptide aldehydes **6** and **7** by treatment with $NaIO_4$ in DMSO–water (5 min, pH 7). Notably, MALDI-MS detected both **6** and **7** in their hydrated forms.[27] Peptide aldehydes/hydrates **6** and **7** were used in model ligation reactions with 2,4-dinitrophenylhydrazine, which proceeded as expected to give hydrazones **8** and **9** (Scheme 3), as characterized by MALDI-MS and HPLC (yields from **4**/**5**>60%).

Finally, Fmoc-Hyl(Alloc-oxazolidine) **10** (NMR-data in note)[28] was prepared analogously to **1** and used in solid-phase synthesis of Boc-Pro-Hyl(Alloc-oxazolidine)-Phe-Ala-Rink resin. The resin-bound peptide was Alloc-deprotected,[29] oxidized with tetrabutylammonium periodate[30] and reacted with DNPH. TFA treatment gave peptide **11** (yield 55%, Scheme 4).

In conclusion, the protected masked aldehyde amino acid **1** has been made available by a facile synthetic route, and proven useful in preparation of peptides with side-chain aldehydes, via mild post-TFA periodate oxidation. This method circumvents the otherwise problematic release of peptide aldehydes in TFA solution. Peptide aldehydes generated from **1** are useful in the formation of peptide ligation constructs and could find use as intermediates for other transformations, in the generation of peptide mimics, isosters and similar molecules. Additionally, Hyl-alloc-derivative **10** was prepared and used for on-resin oxidation and transformation.



Scheme 3. Peptide synthesis, oxidation and ligation.



Scheme 4. Use of Fmoc-Hyl(Alloc-oxazolidine) **10**.

Acknowledgements

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26. $(Hyl(Boc))_2Cu$: Hyl dihydrochloride hydrate (Fluka, 2.0 g, 7.9 mmol) was dissolved in water (12 ml) and treated with NaOH (0.95 g, 23.7 mmol), followed by $CuSO_4 \cdot 5H_2O$ (0.99 g, 3.95 mmol) in water (6 ml). The blue solution was heated to 80°C and allowed to cool slowly to room temperature. Boc anhydride (2.6 g, 15.8 mmol) in dioxane (15 ml) was added, and the mixture was stirred overnight. Additional Boc anhydride (0.86 g, 3.95 mmol) was added, and the mixture was stirred for 2

days. The dark blue crystals were collected by filtration, washed with water and ether, and dried in vacuo, to yield 2.08 g (91%).

Fmoc-Hyl(Boc)**3**: $(Hyl(Boc))_2Cu$ (0.436 g, 0.75 mmol) in water (8 ml) was stirred with a solution of Na_2EDTA (0.376 g, 1.13 mmol) and NaOH (90 mg, 2.25 mmol) in water (8 ml). The copper complex dissolved within 30 min. After 2 h, a solution of Fmoc-OSu (0.506 g, 1.13 mmol) in dioxane (10 ml) was added at ice bath temperature. Na_2CO_3 (80 mg, 0.75 mmol) was added, to give pH 8–9. The mixture was stirred overnight and monitored by TLC (ethyl acetate/cyclohexane/acetic acid, 23:7:3). The reaction mixture was washed with ether, acidified under cooling to pH 2–3 with 2 M HCl, and extracted with ethyl acetate (2×25 ml). The organic solution was washed with brine, dried over $MgSO_4$ and evaporated in vacuo to give 0.61 g (84%).

^{1}H NMR ($CDCl_3$, 400 MHz) δ: 7.74 (d, 2H, *J* 7.0, ArH), 7.56 (d, 2H, *J* 7.0, ArH), 7.37 (t, 2H, *J* 7.0, ArH), 7.28 (t, 2H, *J* 7.0, ArH), 5.93 (d, 1H, *J* 7.5, NH), 5.20 (bs, 1H, NH), 4.38 (m, 3H, $ArCH_2$+αCH), 4.19 (t, 1H, *J* 6.9, ArCH), 3.67 (m, 1H, CHO), 3.23 (m, 1H, CHN), 3.04 (m, 1H, C′HN), 2.03 (m, 1H, βCH), 1.79 (m, 1H, βC′H), 1.55 (m, 2H, γCH_2), 1.42 (s, 9H, ^{t}Bu). ESMS (*m*/*z*): 506.8 (M+Na), 385.5 (M+H-Boc); $C_{26}H_{32}N_2O_7$ requires 484.6.

Fmoc-Hyl(Boc-oxazolidine) **1**: Fmoc-Hyl(Boc) **3** (0.61 g, 1.25 mmol) in acetone (5 ml) and 2,2-dimethoxypropane (5 ml) was cooled with an ice-bath and treated with catalytic $BF_3{\cdot}OEt_2$ (25 μl). The reaction was monitored by TLC (THF/hexane/acetic acid, 10:20:1) and was complete within 1–2 h. Ethyl acetate was added (50 ml) and the solution was washed with water (2×) and brine, dried over $MgSO_4$ and evaporated in vacuo. The product was precipitated from cold ether/hexane, to give 0.53 g (80%).

^{1}H NMR (acetone-d_6) δ: 7.72 (d, 2H, *J* 7.0, ArH), 7.59 (t, 2H, *J* 7.2, ArH), 7.28 (t, 2H, *J* 6.7, ArH), 7.19 (t, 2H, *J* 6.8, ArH), 6.63 (d, 1H, NH), 4.22 (d, 2H, *J* 7.9, $ArCH_2$), 4.11 (m, 2H, ArCH+αCH), 3.98 (m, 1H, J_1 6.3, J_2 5.3, J_3 3.3, CHO), 3.55 (m, 1H, J_1 6.2, J_2 3.5, CHN), 2.90 (t, 1H, *J* 9.4, C′HN), 1.92 (m, 1H, βCH), 1.66 (m, 1H, βC′H), 1.62 (m, 2H, γCH_2), 1.37 (s, 3H, Me), 1.32 (bs, 12H, Me+^{t}Bu). ^{13}C NMR (acetone-d_6) δ: 173.0, 156.3, 152.2, 144.4, 141.4, 127.8, 127.2, 125.5, 120.1, 93.7, 80.4, 73.7, 66.4, 53.9, 51.0, 47.3, 29.8, 28.8, 28.2, 27.9. ESMS (*m*/*z*): 547.2 (M+Na), 425.2 (M+H-Boc); $C_{29}H_{36}N_2O_7$ requires 524.6.

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28. Fmoc-Hyl(Alloc-oxazolidine) **10**: Prepared analogously to **1**, using allyloxycarbonyl chloride. However, $(Hyl(Alloc))_2Cu$ is quite soluble in water/dioxane, so the crude solution of copper complex was used directly in the next steps. Fmoc-Hyl(Alloc-oxazolidine) **10** was purified by flash chromatography on silica, eluting with THF/hexane/acetic acid, 10:20:1. Yield 1.08 g (54%) overall from $Hyl{\cdot}2HCl{\cdot}H_2O$ (1.0 g, 3.95 mmol).

^{1}H NMR ($CDCl_3$): 7.77 (d, 2H, *J* 7.5, ArH), 7.59 (bd, 2H, ArH), 7.40 (t, 2H, *J* 7.5, ArH), 7.31 (t, 2H, *J* 7.6, ArH), 5.93 (m, 1H, J_1 5.4, J_2 1.5, CH=), 5.65 (d, 1H, *J* 7.7, NH), 5.24 (dd, 2H, J_1 17.2, J_2 10.3, CH_2=), 4.58 (m, 3H, OCH_2+αCH), 4.33 (d, 2H, $ArCH_2$), 4.22 (t, 2H, *J* 6.7, ArCH), 4.08 (m, 1H, CHO), 3.71 (m, 1H, CHN), 3.11 (m, 1H, C′HN), 2.06 (m, 1H, βCH), 1.84 (m, 1H, βC′H), 1.72 (m, 2H, γCH_2), 1.58 (s, 3H, Me), 1.50 (s, 3H, Me). ESMS (*m*/*z*): 530.8 (M+Na), 409.6 (M+H-Boc); $C_{28}H_{32}N_2O_7$ requires 508.6.

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