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Solid-phase synthesis of C-terminal peptide aldehydes from amino acetals anchored to a backbone amide linker (BAL) handle

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Abstract

Peptide aldehydes were synthesized, starting from amino acetals, by a solid-phase backbone amide linker (BAL) strategy.

Keywords: peptide aldehydes; solid-phase synthesis; BAL; amino acetals.

C-Terminal peptide aldehydes are potential serine, cysteine, and aspartic protease inhibitors that have emerged as promising therapeutic agents for the treatment of, for example, viral infections such as HIV and human rhinovirus 3C.[1–4] While peptide aldehydes have been prepared previously by solution synthesis, combinatorial syntheses of such compounds are more likely facilitated by solid-phase strategies. Current methods include release from a Weinreb amide-based handle with $LiAlH_4$, from a semicarbazone handle with dilute acid, or from an olefinic linker by ozonolysis.[5–7] Recently, we reported the synthesis of a peptide aldehyde with a C-terminal glycinal residue, starting from 2,2-dimethoxyethylamine anchored to a backbone amide linker (BAL) handle.[8] Here we report on the extension of our rapid and efficient strategy to allow for the general synthesis of complex peptide aldehydes starting from amino acid-derived amino acetals.

Initial studies focused on peptide aldehydes with C-terminal alaninal (R = Me) or phenylalaninal (R = Bn). Amino acetals were derived from N^{α}-Z-amino acids by a four-step sequence (Scheme 1). First, the Weinreb amides were prepared and then reduced with $LiAlH_4$ at 0 to 25°C to give the corresponding aldehydes. Treatment with ethylene glycol in refluxing toluene, in the presence of a catalytic amount of TsOH, established the 1,3-dioxolane moiety, following which removal of the

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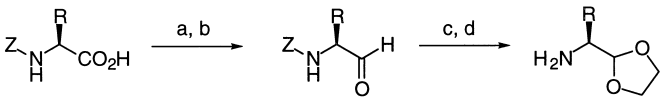
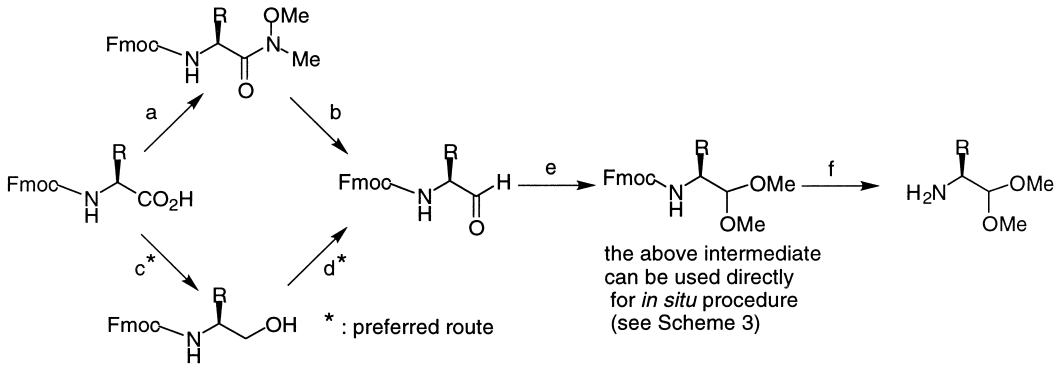
Z-protecting group with 1,4-cyclohexadiene in the presence of Pearlman's catalyst gave the desired amino acetals.[9]



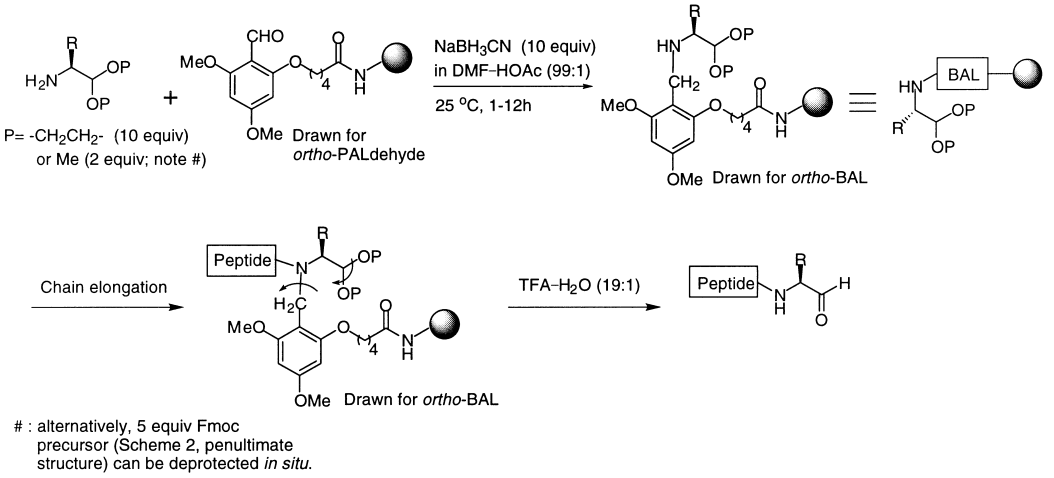
Scheme 1. Synthesis of amino 1,3-dioxolanes. Reagents and conditions: (a) HN(Me)OMe·HCl (1.5 equiv.), $^{i}Pr_2NEt$ (2.6 equiv.), HBTU (1.1 equiv.), HOBt (1.1 equiv.), DMF, 25°C, 2 h; R = Me, 90%; R = Bn, 95%; (b) $LiAlH_4$ (3.0 equiv.), THF, 0°C to 25°C, 0.5 h; R = Me, 70%; R = Bn, 90%; (c) $(CH_2OH)_2$ (10 equiv.), TsOH (0.1 equiv.), PhMe, reflux, 6 h; R = Me, 92%; R = Bn, 64%; (d) $Pd(OH)_2$ (1 equiv.), 1,4-cyclohexadiene (10 equiv.), EtOAc, 4 h; R = Me, 71%; R = Bn, 99%

Alternatively, N^{α}-Fmoc-amino acids were converted to the corresponding Weinreb amides which, upon treatment with $LiAlH_4$ at –78°C gave the N^{α}-Fmoc-protected amino aldehydes (Scheme 2).[10,11] DIBAL-H also proved efficient for the reduction. However, both reductive procedures suffered from partial cleavage of the Fmoc moiety under the reaction conditions, and the initial crude products were of low purity. In a superior approach, the N^{α}-Fmoc-amino acids were treated[12] with ^{i}BuOCOCl to form the corresponding mixed anhydrides, which were subsequently reduced with $NaBH_4$ to give the N^{α}-Fmoc-amino alcohols. Swern oxidation cleanly converted the alcohols to the aldehydes, i.e. N^{α}-Fmoc-Phe-H in 65% and N^{α}-Fmoc-Ala-H in 67% yield. Protection of the aldehyde moieties was then accomplished by treatment with trimethyl orthoformate in methanol, in the presence of a catalytic amount of TsOH at 25°C, to form the corresponding acetals under mild conditions (Scheme 2). Selective removal of the Fmoc group was difficult, with some methods giving little or no product.[13] The relatively best method was treatment of the acetals with 4 N aq. NaOH–MeOH–dioxane (1:9:30) for 10 min, which gave the expected amino acetals. Preparative HPLC immediately after Fmoc-removal from the Phe-derivative yielded 21% of phenylalaninal dimethyl acetal;[14] other purification procedures were inferior.



Scheme 2. Synthesis of N^{α}-Fmoc-amino aldehydes and amino dimethyl acetals. Reagents and conditions: (a) HN(Me)OMe·HCl (1.1 equiv.), Et_3N (2.1 equiv.), HBTU (1.0 equiv.), CH_2Cl_2, 25°C, 18 h; R = Me, 91%; R = Bn, 98%; (b) DIBAL-H (1.5 equiv.), CH_2Cl_2, –78°C, 1 h; R = Me, 30%; R = Bn, 49%; (c) NMM (1.0 equiv.), ^{i}BuOCOCl (1.0 equiv.), DME, –15°C, 1 min; then $NaBH_4$ (1.5 equiv.), H_2O, –15°C, 30 sec; R = Me, 91%; R = Bn, 94%; (d) $(COCl)_2$ (1.5 equiv.), DMSO (3.0 equiv.), NEt_3 (6.0 equiv.), CH_2Cl_2, –60°C, 1 h; R = Me, 67%; R = Bn, 65%; (e) $(MeO)_3CH$ (2.0 equiv.), TsOH (0.1 equiv.), MeOH, 25°C, 2 h; R = Me, 79%; R = Bn, 97%; (f) R = Me, DBU (4.0 equiv.), DMF, 25°C, 10 min (in situ procedure); R = Bn, 4 N aq NaOH–MeOH–dioxane (1:9:30), 10 min, 25°C, 21%

The methodologies described in the preceding paragraph gave amino 1,3-dioxolanes (Scheme 1) or amino dimethyl acetals (Scheme 2). These were coupled to PALdehyde-PS or PEG-PS resins by $NaBH_3CN$-promoted reductive aminations in DMF–HOAc (99:1) (Scheme 3). However, given the low yields and associated difficulties in the preparation of amino dimethyl acetals from their Fmoc precursors (Scheme 2), a simpler alternative in situ strategy was developed. Thus, the Fmoc-protected alaninal acetal was dissolved in DMF–DBU (9:1); after 10 min, HOAc was added, and the solution was transferred to PALdehyde resin together with $NaBH_3CN$ to complete the reductive amination in 1 h (Scheme 3).



Scheme 3. Solid-phase synthesis of peptide aldehydes

Peptide synthesis proceeded with acylation of the secondary BAL-linked amine using either Fmoc-amino acid as preformed symmetrical anhydrides in CH_2Cl_2 or the Fmoc-amino acid activated in situ by HATU/DIEA in CH_2Cl_2–DMF (9:1). Past this point, chain elongation followed standard Fmoc procedures. Treatment of completed peptidyl-resins with TFA:–H_2O (19:1) released the final products (Scheme 3). Concomitant cleavage of the acetal moiety to free the C-terminal aldehyde functionality was confirmed by MS. Although both the preformed and the in situ-generated amino acetals gave the expected peptide aldehydes, the in situ protocol gave simpler and more economical access to peptide aldehydes. Sequences synthesized by this strategy include: N^{α}-Fmoc-Ala-Ala-Pro-Ala-H, N^{α}-Fmoc-Asp-Phe-Val-Ala-H, N^{α}-Fmoc-Ala-Ala-Pro-Phe-H, and N^{α}-Fmoc-Glu-Val-Lys-Phe-H from dimethyl acetals and N^{α}-Fmoc-Asp-Phe-Val-Ala-H and N^{α}-Fmoc-Glu-Val-Val-Phe-H from 1,3-dioxolanes.[15] N^{α}-Fmoc-Asp-Phe-Val-Ala-H was also obtained when the completed dimethyl acetal peptidyl-resin was treated with TFA–thioanisole–ethanedithiol (EDT)–anisole (90:5:3:2; Reagent R). Interestingly, thioacetal adduct(s) of N^{α}-Fmoc-Ala-Ala-Pro-Phe-H with EDT were obtained upon treatment of the completed peptidyl-resin with TFA–EDT (19:1).[16]

To address the question of racemization[17] of the amino aldehyde moiety, the route to N^{α}-Fmoc-Glu-Val-Val-Phe-H, starting from Z-Phe-OH, was repeated starting from N^{α}-Z-D-Phe-OH to provide a reference to the corresponding diastereomeric Fmoc-protected peptide aldehyde. Starting with the same protected peptide-resins, final Fmoc deprotection steps were carried out on resin, followed by TFA cleavage, to provide the free peptides H-Glu-Val-Val-Phe-H and

H-Glu-Val-Val-D-Phe-H. These free peptides were resolved cleanly by HPLC; in the L-Phe-H peptide, 12% of the opposite diastereomer (D) was present, while in the D-Phe-H peptide, 26% of L was present. Further racemization[17] of the L-Phe-H peptide was not observed upon standing overnight in TFA.

In conclusion, we have developed a general strategy for the synthesis of C-terminal peptide aldehydes that relies on anchoring of amino acid-derived acetals through a BAL handle to a solid support. Final peptide aldehydes were released with TFA–H_2O (19:1), with concomitant cleavage of the acetal moiety. Extension of this approach to trifunctional N^{α}-Fmoc-amino acids with appropriate TFA-labile side-chain protecting groups would give precursors to peptides with C-terminal trifunctional aldehydes.

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9. The described conditions gave a good yield and clean product. More standard catalytic hydrogenolysis conditions gave lower yields, and showed unreacted starting material and/or the formation of by-products. For example, H_2 in the presence of Pd/C gave the desired product in only 22% yield after purification. H_2 in the presence of $Pd(OH)_2$ gave erratic results, with the range of product formed being 26–62%.
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12. Rodriguez, M.; Llinares, M.; Doulut, S.; Heitz, A.; Martinez, J. *Tetrahedron Lett.* **1991**, *32*, 923–926.
13. Other conditions tried include (with the associated problems): piperidine:DMF (1:4), difficult to remove piperidine; 5 equiv. aq. NaOH in THF for 3 h, incomplete reaction; 5 equiv. aq. NaOH in 1,4-dioxane for 3 h, solubility problems, difficult to reproduce, loss during work up; ammonium formate in the presence of Pd/C in MeOH–dioxane (1:1) under argon, no reaction after 1 day; DBU in EtOAc at 70°C for 3 h, amino acetal could not be isolated.
14. Optical rotation for the phenylalaninal dimethyl acetal was $[\alpha]_D$ –32.6 (*c* 1.5, MeOH, at 21°C); lit. $[\alpha]_D$ –27.2 (*c* 1.6, MeOH), Gacek, M.; Undheim, K. *Tetrahedron* **1974**, *30*, 4233–4237.
15. The purities (HPLC, 265/220 nm) and *m*/*z* of the Fmoc-protected crude peptide aldehydes were: (from dimethyl acetals) Glu-Val-Lys-Phe-H 50%, 728.6 $[M+H]^+$; Asp-Phe-Val-Ala-H, 54%, 657.4 $[M+H]^+$; Ala-Ala-Pro-Ala-H,

69%, 535.5 $[M+H]^+$; (from 1,3-dioxolanes) Asp-Phe-Val-Ala-H, 82%, 657.3 $[M+H]^+$; Glu-Val-Val-Phe-H, 78%; 699.2 $[M+H]^+$; Glu-Val-Val-D-Phe-H, 84%, 699.4 $[M+H]^+$; for unprotected peptide aldehydes H-Glu-Val-Val-Phe-H, 82%, 477.1 $[M+H]^+$; H-Glu-Val-Val-D-Phe-H, 58%, 477.1 $[M+H]^+$.

16. Thioacetal adduct(s) were the major product (44%), assigned to the hemimercaptal and/or dithioacetal structures, and only small amounts of the desired peptide aldehyde (21%) formed under these cleavage conditions (LC/MS calcd peptide aldehyde 610.28; found *m*/*z* 3.56 min: 743.2 $[M+EDT+K]^+$, 765.3 $[M+EDT+K+Na]^+$, and 687.2 $[M+EDT–H_2O+H]^+$; 3.86 min, 646.2 $[M+2H_2O]^+$). Compare this result to that from treatment with the EDT-containing cocktail Reagent R, where no thioacetal formed upon cleavage. We speculate that formation of the peptide aldehyde only upon cleavage with Reagent R could be due to residual water in the anisole or thioanisole used.
17. The final C-terminal peptide aldehydes, as well as the starting amino aldehydes, have been reported to racemize under conditions of silica gel column chromatography and HPLC (Fehrentz, J. A.; Paris, M.; Heitz, A.; Velek, J.; Liu C.-F.; Winternitz, F.; Martinez, J. *Tetrahedron Lett*. **1995**, *36*, 7871–7874; Ede, N. J.; Eagle, S. N.; Wickham, G.; Bray, A. M.; Warne, B.; Shoemaker, K.; Rosenberg, S. *J. Pept. Sci*. **2000**, *6*, 11–18). Experiments are currently under way to determine at which stage of the overall process our compounds racemize, and to develop protocols to maximize the chiral integrity of the final aldehyde products.