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Solid-phase synthesis of peptide aldehydes directly on acetal resin

Wu Yao and Hong Yan Xu*

Shanghai Institute of Organic Chemistry, Chinese Academy of Sciences, Shanghai 200032, China

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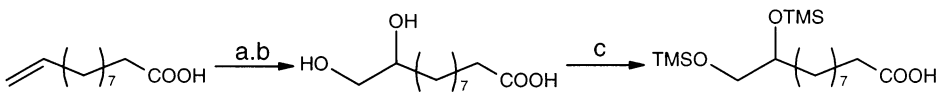
Abstract—A practical strategy for solid-phase synthesis of peptide aldehydes has been developed employing a 10,11-dihydroxyundecanoic acid (DHUA) functionalized resin. High loading levels are obtained for amino aldehydes as their respective acetals. After classical Fmoc SPPS, treatment of the derivatized resin with 95% TFA/H_2O affords peptide aldehydes in high yield and purity. © 2001 Elsevier Science Ltd. All rights reserved.

The field of drug discovery stands on the threshold of a new era in which more new synthetic molecules will be prepared over the next few years than have ever existed before. Preparation of such large numbers of small, organic molecules mainly relies on the new techniques, tools, and strategies of solid-phase organic synthesis.

Peptide C-terminal aldehydes (PAs) are an important class of transition-state analogs, which have attracted considerable attention since their initial discovery as natural products.[1] They have been found to be potential inhibitors of several classes of enzymes such as seryl and thiol proteases, prohormone convertases, cysteinyl protease, and aspartyl proteases.[2,3] Further, PAs can be used in pseudopeptide chemistry, particularly for the synthesis of reduced peptides, by fragment condensation.

Several methods for the solid-phase synthesis of peptide aldehydes have been reported.[4–9] Murphy[4] described a linker based on the semicarbazone derivative of a protected amino acid aldehyde. Linkers based on Weinreb amides have also found applications for PAs,[5,6] which release the aldehyde group by $LiAlH_4$ reduction. An alternative strategy, applicable to the solid-phase synthesis of PAs, uses ozonolysis of resin supported α,β-unsaturated δ-amino acids.[7,8] An additional method has engaged an intermediary oxazolidine.[9]

Our interest in developing a facile method for attaching amino aldehydes to a solid support, allowing subsequent classical solid-phase peptide synthesis (SPPS) and release as peptide aldehydes, has evolved from a desire to generate a series of tripeptide aldehydes rapidly. We settled on the following criteria for a good PAs linker: (i) it must be cost effective and easy to construct; (ii) it must be chemically stable in SPPS; (iii) it must give crude products with high purity, stereochemical integrity and high yield; (iv) it must be cleaved under mild conditions; (v) it must be time economical. It was reasoned that if an acetal could be prepared with a functional group such as a hydroxyl group or a carboxylic acid, to allow attachment to a solid support, it may be possible to use Fmoc strategy to elongate



1 **2** **3**

Scheme 1. (a) 30% H_2O_2, HCOOH; (b) 1N NaOH, 100°C; (c) TMSCl, Et_3N, CH_2Cl_2, 20°C.

Abbreviations: BOP, (1*H*-benzotriazol-1-yloxy)tris(dimethylamino)phosphonium hexafluorophosphate; DHUA, 10,11-dihydroxyundecanoic acid; DIEA, diisopropylethylamine; ESI, electron spraying ionization; Fmoc, fluoren-9-ylmethyloxycarbonyl; HOBt, 1-hydroxybenzotriazole; PAs, peptide aldehydes; SPPS, solid-phase peptide synthesis; TFA, trifluoroacetic acid; TMS, trimethylsilyl; TMSCl, trimethylsilyl chloride; TMSOTf, trimethylsilyl trifluoromethanesulfonate.

* Corresponding author. Fax: (86) (21) 64166128; e-mail: xhongyan@pub.sioc.ac.cn

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peptide chains and cleave the PAs from the resin with aqueous acid. Leznoff[10] and Metz[11] had separately developed these kinds of linkers for aromatic dialdehydes and aryl aldehydes. Herein, we describe such a linker for the solid-phase synthesis of PAs.

10,11-Dihydroxyundecanoic acid (DHUA) was chosen as the linker for the strategy. It can be easily synthesized from commercially available and inexpensive undecylenic acid.[12] In order to prepare the acetal under mild conditions and avoid racemization of the amino aldehydes to provide acetals of high purity, we selected alkyloxysilane with TMSOTf as the catalyst.[13] Therefore, DHUA was suspended in CH_2Cl_2 at 20°C under argon and 2.1 equiv. of TMSCl was added followed by 2.1 equiv. of Et_3N. After 4 h most of the white solid had dissolved, the reaction mixture was diluted with additional CH_2Cl_2, then washed with H_2O and dried with $MgSO_4$. Because the intermediate isolated is very sensitive, concentration under vacuum should be performed at 5°C and provides the bis-TMS ether as a viscous, colorless oil in high yield 95% (Scheme 1).

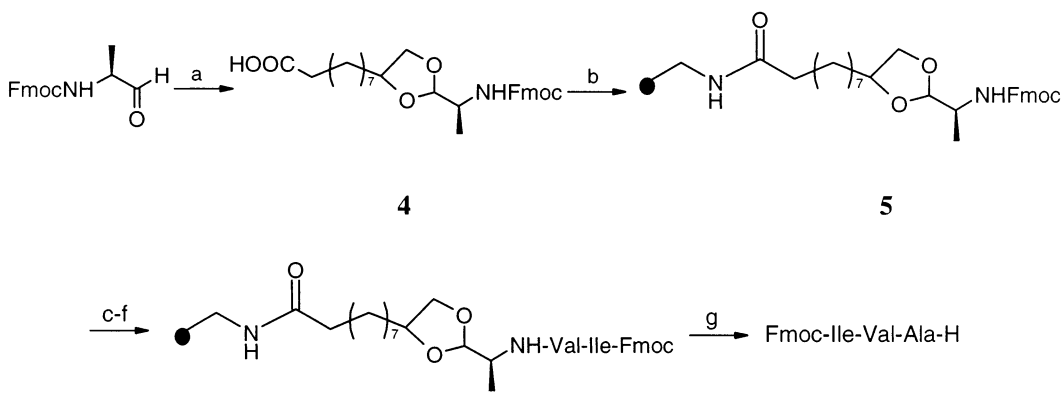
The intermediate was treated with Fmoc-Ala-H and, after 1 h, the mixture was washed with water and dried ($MgSO_4$) to give acetal **4** as a white solid (mp 245–246°C) in 78% yield after purification by flash chromatography on silica gel.[14] Acetal **4** was then coupled to the aminomethylated resin (1.1 mmol/g) using BOP, HOBt and DIEA in CH_2Cl_2 until a negative Kaiser test was observed. After deprotection of the N-terminal Fmoc, elongation by classical Fmoc SPPS provides the peptide acetal on the solid support (Scheme 2).

In order to optimize the cleavage step for release of the peptide aldehydes from the resin with aqueous acid, three parameters were investigated: acid (50% TFA/CH_2Cl_2 or 95% TFA/H_2O), temperature (50°C or room temperature), and reaction time (from 5 min to 12 h). Reaction mixtures from different conditions were studied by HPLC. It was observed that 50% TFA/CH_2Cl_2 resulted in only a little cleavage from the resin even after a long reaction time. At a higher reaction temperature (50°C), 10% more cleavage was observed than at room temperature after the same reaction time. Longer reaction times (12 h) resulted in more product formation (80%) than the shorter reaction times (20 min) at room temperature. With respect to yield and total reaction time, we selected treatment with 95% TFA/H_2O three times over 15 min, each at room temperature. Furthermore, this strategy was more applicable to combinatorial synthesis than others employing higher temperatures or longer reaction times. After evaporation of the TFA under vacuum, the combined solutions were dried by lyophilization to give peptide aldehydes as white powders in good yields and high purities.

It is well known that peptide aldehydes are reactive, sensitive and configurationally labile entities.[15] Indeed, racemization at the P_1-aldehyde center occurs rapidly under base-catalyzed conditions. In this work, an acid catalyzed hydrolysis reaction was employed in the final cleavage step. In order to check chiral integrity, we used 600 MHz NMR to analyze the crude product from Fmoc-Ile-Val-Ala-H and only one peak was found at δ 9.52 ppm showing no detectable racemization. When this product was left in $CDCl_3$ solution at room temperature for 6 days, the single peak changed into two peaks, which demonstrated that racemization had occurred. This experiment confirmed that our peptide aldehydes had retained their chiral integrity. Our findings are in accord with the disclosure by Martinez[6] regarding the NMR of peptide aldehydes. Fig. 1 shows the HPLC and ESI profiles of the crude peptide aldehyde, Fmoc-Ile-Val-Ala-H.

The utility of this method was demonstrated by the synthesis of a selection of tripeptide aldehydes, such as Fmoc-Ile-Ile-Ala-H, Fmoc-Gly-Ile-Ala-H and Fmoc-Leu-Gly-Leu-H with 91–95% yields and 75–89% purities. All products were characterized by LC–ESI.

In conclusion, a very simple and cost-effective strategy for the solid-phase synthesis of peptide aldehydes has been developed. This strategy does not involve oxidation or reduction procedures and makes it applicable to combinatorial chemistry, allowing rational investigation of structure–activity relationships for peptide analog based enzyme inhibitors.



Scheme 2. (a) **3**, TMSOTf, 0°C; (b) polymer–CH_2NH_2, BOP, HOBt, DIEA; (c) 20% piperidine/DMF; (d) Fmoc-Val-OH, BOP, HOBt, DIEA; (e) 20% piperidine/DMF; (f) Fmoc-Ile-OH, BOP, HOBt, DIEA; (g) 95% TFA/H_2O, 3×15 min.

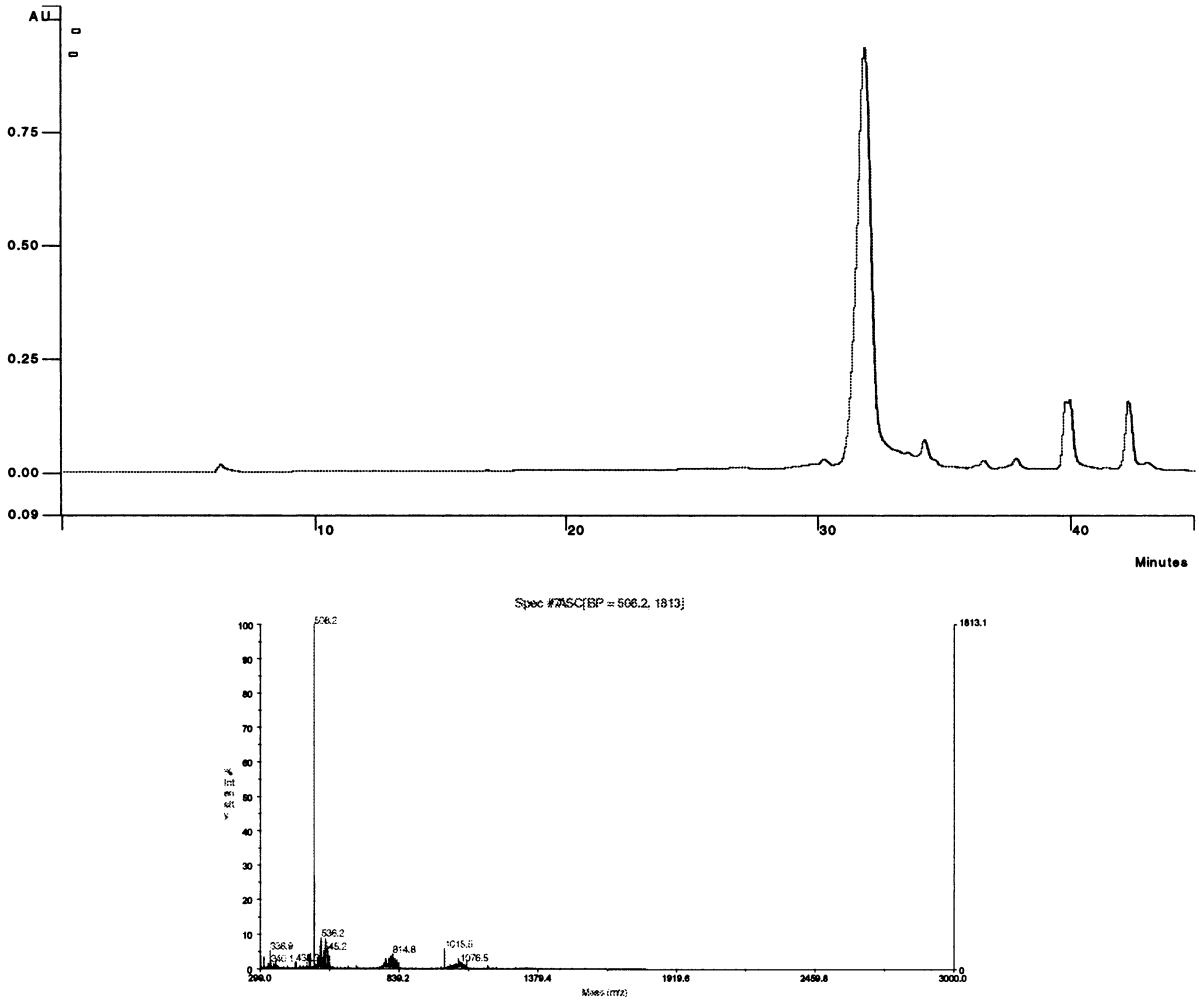


Figure 1. HPLC (265 nm) and ESI profiles of the crude peptide aldehyde, Fmoc-Ile-Val-Ala-H.

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14. Preparation of **4**. To 10,11-dihydroxyundecanoic acid (DHUA, 2.18 g, 10 mmol) suspended in CH_2Cl_2 (20 ml) at room temperature under argon was added TMSCl (2.1 equiv., 2.7 ml), followed by Et_3N (2.1 equiv., 2.9 ml). The reaction mixture was stirred (4 h), then quenched with H_2O, extracted with CH_2Cl_2, dried with $MgSO_4$ and concentrated (ice bath) to provide the TMS ether of DHUA **3** as a colorless oil in 90–97% yield. To Fmoc-Ala-H (590 mg, 2.0 mmol) dissolved in 15 ml of CH_2Cl_2 in a 50 ml three-neck flask at 0°C was added **3** (1.0 equiv., 724 mg), followed by 1 mol% TMSOTf (0.10 mmol, 20 μL). The reaction mixture was stirred at ice bath temperature for 1 h when TLC showed that the

reaction was complete. The reaction mixture was diluted with CH_2Cl_2 and washed successively with H_2O and brine, dried with $MgSO_4$, followed by purification with flash column chromatography to provide the white solid product **4** (770 mg, 78%). ^1H NMR (300 MHz, $CDCl_3$): 7.80–7.30 (m, 8H), 4.92 (d, $J=9.56$, 1H), 4.44 (m, 2H), 4.23 (m, 1H), 3.94 (m, 3H), 3.53 (m, 1H), 2.35 (m, 2H), 1.45 (m, 14H). Anal. calcd for $C_{27}H_{31}NO_6$: C, 70.31; H, 7.13; N, 2.82. Found: C, 70.14; H, 7.33; N, 2.78%.

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