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Short Communication

The epimerization of peptide aldehydes – a systematic study

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Abstract: Peptide aldehydes are interesting targets as enzyme inhibitors, and can be used for pseudopeptide chemistry or ligation. However, they are known to be subjected to epimerization during synthesis or purification. By ^{1}H NMR, a model dipeptide aldehyde can be used to check the possible epimerization occurring during synthesis. Various purification methods were investigated, but none was free from epimerization. Copyright © 2006 European Peptide Society and John Wiley & Sons, Ltd.

Keywords: Weinreb amide; peptide aldehyde; epimerization; ^{1}H NMR

INTRODUCTION

Several peptide aldehydes have been found to be potent inhibitors of enzymes, including serine [1,2], cysteine [3,4] and aspartyl proteases [5,6] and prohormone convertases [7]. On the other hand, these peptide aldehydes can also be used in a wide range of chemistries including pseudopeptide bond formation, reduced peptide bond formation [8] or chemical ligation [9].

Various methods for the synthesis of peptide aldehydes have been described both in solution [3,10–15] and on solid support [16–28]. Whatever the chosen synthetic route, the peptide aldehydes obtained, commonly purified by reversed-phase HPLC, are partially epimerized [18]. We have previously reported that epimerization in a peptide aldehyde containing three residues will be indicated by an extra signal for the aldehyde proton in the NMR spectrum [11]. We have also previously studied the possible epimerization of a model tripeptide aldehyde, Boc-Phe-Val-Ala-H, synthesized by the solid phase approach and noted that this epimerization could be observed by ^{1}H NMR only in $CDCl_3$ and not in DMSO-d_6 [18]. To our knowledge, no systematic study has been done on this phenomenon. We describe in this paper the study of epimerization of dipeptide, tripeptide and tetrapeptide aldehyde models.

MATERIALS AND METHODS

General Procedure

As the reduction of Weinreb amide [29] has been well studied in our laboratory and as this amide can be used both as a *C*-terminal-protecting group during peptide elongation and as a precursor for the aldehyde function, we decided to perform the synthesis in solution, which allows the purification of all intermediates before reduction (Scheme 1). We considered our previous model tripeptide, Boc-Phe-Val-Ala-H, and we chose to add at the *N*-terminus Boc-Tyr(Bzl)-OH for its hydrophobic character allowing easy purification of the Weinreb precursor on a silica gel column. The syntheses were performed in parallel starting from L-Ala and D-Ala to get the authentic diastereoisomers LL, LD, LLL, LLD LLLL and LLLD corresponding to the di- tri- and tetrapeptides. After synthesis of the Weinreb amides and prior to reduction, compounds **3**, **5** and **7** were purified on a silica gel column and their purity was checked by ^{1}H NMR spectroscopy. These compounds were then reduced with $LiAlH_4$ at 0 °C in anhydrous THF as previously described [11] using $(4 + 2n)$ equivalents of hydride, n corresponding to the number of amide functions contained in the peptide sequence. The reaction was quenched after 15 min with aqueous $KHSO_4$ 1 M solution, and a classical workup was performed. After evaporation of the solvents, peptide aldehydes **4**, **6** and **8** were analyzed by ^{1}H NMR spectroscopy with $CDCl_3$ as solvent. In all cases only one peak corresponding to the aldehyde proton was observed, showing once again that the Weinreb amide reduction in these conditions is racemization-free.

We then studied the methods of purification to quantify the optical purity of the peptide aldehydes. So far, most of the described chemistry using peptide aldehydes used crude materials because no purification method of these aldehydes was reported without racemization of the *C*-terminal amino–aldehyde residue. Ho and Ngu proposed the purification of *N*-Fmoc-protected α-amino aldehydes on a silica gel column with 0.1% pyridine in the elution solvent system [30]. We tested this procedure with *N*-Boc and *N*-Z protected α-amino aldehydes and found it very convenient and free of racemization (data not reported). We decided to check the epimerization of our model peptide aldehydes **4**, **6** and **8** in silica gel chromatography purification conditions. We placed peptide aldehydes in various conditions: solvent in the presence of silica gel, solvent containing 0.1% pyridine in the presence of silica gel and solvent in the presence of

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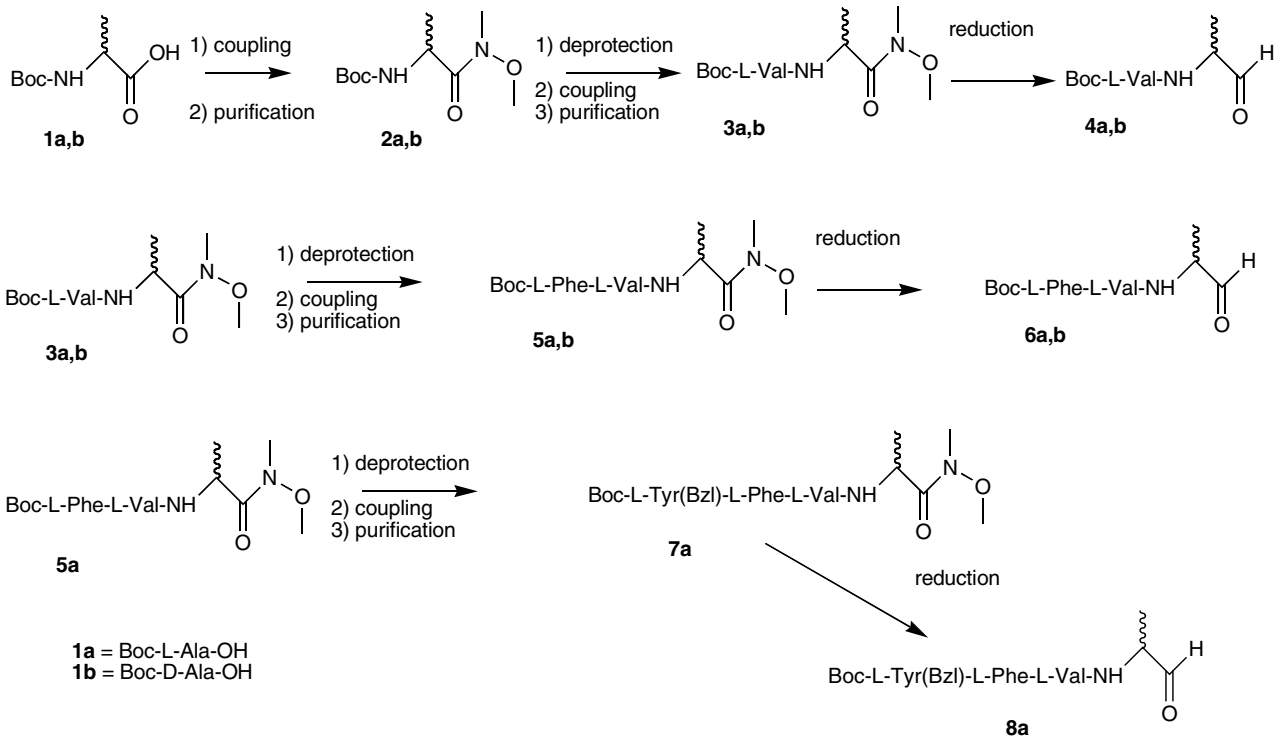

Scheme 1 Synthetic route to peptide aldehydes.

alumina, the last two conditions supposed to suppress the acid-catalyzed epimerization by inhibiting enolization of the aldehyde moiety. For these epimerization tests, the conditions were standardized. One mmole of peptide aldehyde was dissolved in a mixture of AcOEt/hexane (depending on the sequence length) with or without 0.1% pyridine, and then 1 g of the chromatographic medium (SiO_2, Al_2O_3) was added. The mixture was allowed to stand with stirring for 2 h. After filtration, the solvents were concentrated and compounds analyzed by ^1H NMR. NMR of crude samples were also reanalyzed after one week in $CDCl_3$ solution (last column in the Table 1) to check their stability in solution.

As can be seen in Table 1, which presents the results of this study, the three different conditions tested for purification were not free of epimerization, either at level of the dipeptide or at the levels of tri- and tetrapeptides. Whatever the purification system used (SiO_2, SiO_2 with pyridine or Al_2O_3), compounds **4**, **6** and **8** epimerized. No correlation could be found regarding the epimerization rate in the three purification systems. On the other hand, it could also be observed that the peptide aldehydes epimerized when they were stored in $CDCl_3$ solution (even on storage for only a week at room temperature).

In conclusion, we have demonstrated that epimerization of the Cα adjacent to the aldehyde function in peptide aldehydes can be easily checked by ^1H NMR in $CDCl_3$ in dipeptide, tripeptide and tetrapeptide aldehydes. No purification method of these peptide aldehydes was safe with respect to their optical purity. Even the capture–release procedure with threonyl resin [31] at room temperature resulted in 20% epimerization. The Weinreb amide approach in solution is a good alternative since the aldehyde precursor can be purified before reduction. However, it is not compatible with long and/or hydrophilic sequences or with Asp- or Glu-containing peptides when their side chains are protected as esters. The oxazolidine approach on solid support [20] seems to be a good compromise for long sequences, but apparently causes partial epimerization [31]. Even the backbone amide linker (BAL) strategy was found to be unsafe in this respect [32]. No safe method for the synthesis of long peptide aldehydes is available. The above described simple dipeptide model can be used to evaluate epimerization in peptide aldehyde synthesis.

Table 1 Determination of the epimerization of the *C*-terminal residue by ^1H NMR under various purification conditions and after storage of the crude peptide aldehyde in solution

Compounds	% D[a] crude, of reduction	% D[a] SiO_2	% D[a] SiO_2/π	% D[a] Al_2O_3	% D[a] after one week
4a	0	41	49	32	35
4b	100	71	68	57	83
6a	0	49	30	48	30
6b	100	75	59	52	68
8a	0	25	34	45	28

[a] Within the limits of ^1H NMR detection.

Materials

All reagents were of commercial quality. Solvents were dried and purified by standard methods. Analytical TLC was performed on aluminum sheets coated with a 0.2-mm layer of silica gel 60 F_{254}. Silica gel 60 (40–63 μm) was used for flash chromatography. ^1H NMR spectra were obtained at 300 K on a Brucker ACF 300 spectrophotometer in $CDCl_3$.

Chemical shifts were reported as δ-values (ppm) indirectly referenced to the solvent signal. Mass spectra were recorded on a Platform II (Micromass, Manchester, UK) quadrupole mass spectrometer operating at an ionization potential of 30 eV by positive electrospray (ES) fitted with an electrospray interface and coupled with an Alliance Waters LC. RP-HPLC analyses were performed on a RPC_{18} Chromolith Speedrod (4.6 mm × 50 mm) from Merck, with a flow rate of 5 ml/min and using the system Gold from Beckman with a 126-solvent module and a 168 detector. Solutions of 0.1% of TFA in H_2O (solvent A) and of 0.1% of TFA in CH_3CN (solvent B) were used as mobile phases. Gradients from A to B were performed in 3 min.

Amino-protected Weinreb Amides

Starting from Boc-Ala-OH, the corresponding Weinreb amide was obtained as described previously [33] with BOP as reagent and DIEA as base. The *N* -protecting group was removed with TFA/DCM (5/5) for 30 min at room temperature and after neutralization of the TFA salt, and the next *N*-protected amino acid was coupled under standard conditions using BOP as coupling reagent. These last two steps were repeated till the desired sequences were obtained. Before reduction, each Weinreb amide was purified by flash chromatography.

Boc-L-Ala-*N*(Me)OMe (2a)

^{1}H NMR: δ 1.10 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.32 (9H, s, CH_3 Boc); 3.06 (3H, s, N-CH_3); 3.68 (3H, s, N-OCH_3); 4.36 (1H, m, CH α-Ala); 6.97 (1H, d, $J = 8$ Hz, Boc-NH).

FAB-MS calcd for $C_{10}H_{20}N_2O_4$ 232.14, found $[M + H]^+$: 233.3; HPLC RT: 1.02 min.

Boc-L-Val-L-Ala-*N*(Me)OMe (3a)

^{1}H NMR: δ 0.90 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.96 (3H, d, $J = 9$ Hz, CH_3 γ-Val); 1.32 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.4 (9H, s, CH_3 Boc); 2.12 (1H, m, CH β-Val); 3.18 (3H, s, N-CH_3); 3.74 (3H, s, N-OCH_3), 3.94 (1H, m, CH α-Ala); 4.92 (1H, m, CH α-Val); 5.04 (1H, d, $J = 7$ Hz, Boc-NH); 6.63 (1H, d, $J = 7.45$ Hz, NH Ala).

FAB-MS calcd for $C_{15}H_{29}N_3O_5$ 331.21, found $[M + H]^+$: 332.3; HPLC RT: 1.25 min.

Boc-L-Val-D-Ala-*N*(Me)OMe (3b)

^{1}H NMR: δ 0.87 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.94 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.32 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.41 (9H, s, CH_3 Boc); 2.15 (1H, m, CH β-Val); 3.19 (3H, s, N-CH_3); 3.74 (3H, s, N-OCH_3), 3.98 (1H, m, CH α-Ala); 4.90 (1H, m, CH α-Val); 5.04 (1H, m, Boc-NH); 6.70 (1H, d, $J = 7$ Hz, NH Ala).

FAB-MS calcd for $C_{15}H_{29}N_3O_5$ 331.21, found $[M + H]^+$: 332.5; HPLC RT: 1.22 min.

Boc-L-Phe-L-Val-L-Ala-*N*(Me)OMe (5a)

^{1}H NMR: δ 0.82 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.86 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.30 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.36 (9H, s, CH_3 Boc); 2.03 (1H, m, CH β-Val); 3.06 (2H, m, CH_2 β-Phe); 3.20 (3H, s, N-CH_3); 3.76 (3H, s, N-OCH_3); 4.25 (1H, m, CH α-Ala); 4.38 (1H, m, CH α-Phe); 4.81 (1H, m, CH α-al); 5.10 (1H, m, Boc-NH); 6.63 (1H, m, NH Ala); 6.79 (1H, m, NH Val); 7.24 (5H, m, CH ar Phe).

FAB-MS calcd for $C_{24}H_{38}N_4O_6$ 478.28, found $[M + H]^+$: 479.4; HPLC RT: 1.53 min.

Boc-L-Phe-L-Val-D-Ala-*N*(Me)OMe (5b)

^{1}H NMR: δ0.85 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.87 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.30 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.36 (9H, s, CH_3 Boc); 2.10 (1H, m, CH β-Val); 3.01 (2H, m, CH_2 β-Phe); 3.17 (3H, s, N-CH_3); 3.74 (3H, s, N-OCH_3); 4.26 (2H, m, CH α-Ala + Phe); 4.80 (1H, m, CH α-Val); 5.08 (1H, m, Boc-NH); 6.62 (1H, m, NH Ala); 6.98 (1H, m, NH Val); 7.20 (5H, m, CH ar Phe).

FAB-MS calcd for $C_{24}H_{38}N_4O_6$ 478.28, found $[M + H]^+$: 479.2; HPLC RT: 1.55 min.

Boc-L-Tyr(Bzl)-L-Phe-L-Val-L-Ala-*N*(Me)OMe (7a)

^{1}H NMR: δ 0.83 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.85 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.22 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.31 (9H, s, CH_3 Boc); 2.01 (1H, m, CH β-Val); 3.03 (4H, m, CH_2 β-Phe + Tyr); 3.20 (3H, s, N-CH_3); 3.75 (3H, s, N-OCH_3); 4.38 (2H, m, CH α-Val + Phe); 4.65 (1H, m, CH α-Tyr); 4.90 (3H, m, CH α-Ala + CH_2-phenyl); 5.10 (1H, m, Boc-NH); 6.75 (1H, m, NH Ala or Val); 6.83 (2H, d, $J = 8$ Hz, CH ar Tyr); 6.95 (1H, m, NH Phe); 7.05 (2H, d, $J = 7$ Hz, CH ar Tyr); 7.25 (11H, m, CH ar Phe + phenyl, NH Ala or Val).

FAB-MS calcd for $C_{40}H_{53}N_5O_8$ 731.38, found $[M + H]^+$: 732.3; HPLC RT: 1.96 min.

Reduction of the Weinreb Amides

The Weinreb amides were dissolved in a minimum amount of anhydrous THF and the flask was placed in an ice bath. $LiAlH_4$ (powder $(4 + 2n)$ equivalents of hydride, n corresponding to the number of amide functions contained in the sequence) was added and allowed to react for 15 min. After quenching and a classical workup, crude peptide aldehydes were obtained and analyzed by LC/MS and ^{1}H NMR in $CDCl_3$.

Boc-L-Val-L-Ala-H (4a)

^{1}H NMR: δ 0.91 (3H, d, $J = 13$Hz, CH_3 γ-Val); 0.96 (3H, d, $J = 9$ Hz, CH_3 γ-Val); 1.33 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.4 (9H, s, CH_3 Boc); 2.10 (1H, m, CH β-Val); 3.87 (1H, m, CH α-Ala); 4.45 (1H, m, CH α-al); 5.10 (1H, m, Boc-NH); 6.7 (1H, d, $J = 7$ Hz, NH Ala); 9.52 (1H, s, CHO).

FAB-MS calcd for $C_{13}H_{24}N_2O_4$ 272.17, found $[M + H]^+$: 273.3.

Boc-L-Val-D-Ala-H (4b)

^{1}H NMR: δ 0.90 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.94 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.34 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.42 (9H, s, CH_3 Boc); 2.13 (1H, m, CH β-Val); 3.95 (1H, m, CH αAla); 4.47 (1H, m, CH α-Val); 5.04 (1H, m, Boc-NH); 6.66 (1H, d, NH Ala); 9.52 (1H, s, CHO).

FAB-MS calcd for $C_{13}H_{24}N_2O_4$272.17, found $[M + H]^+$: 273.3.

Boc-L-Phe-L-Val-L-Ala-H (6a)

^{1}H NMR: δ0.83 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.89 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.30 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.39 (9H, s, CH_3 Boc); 2.27 (1H, m, CH β-Val); 3.07 (2H, m, CH_2 β-Phe); 4.31 (2H, m, CH α-Ala + Phe); 3.73 (1H, m, CH α-al); 4.94 (1H, d, $J = 5$ Hz, Boc-NH); 6.51 (1H, d, $J = 7$ Hz, NH Ala); 6.80 (1H, d, $J = 5$ Hz, NH Val); 7.21 (5H, m, CH ar Phe); 9.47 (1H, s, CHO).

FAB-MS calcd for $C_{22}H_{33}N_3O_5$ 419.24, found $[M + H]^+$:420.5.

Boc-L-Phe-L-Val-D-Ala-H (6b)

^{1}H NMR: δ0.84 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 0.88 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.31 (3H, d, $J = 7$ Hz, CH_3 β-Ala); 1.37 (9H, s, CH_3 Boc); 2.24 (1H, m, CH β-Val); 3.08 (2H, m, CH_2 β-Phe); 4.33 (3H, m, CH α-Ala + Phe + Val); 4.97 (1H, d, $J = 6$ Hz, Boc-NH); 6.47 (1H, d, $J = 8$ Hz, NH Ala); 6.92 (1H, m, NH Val); 7.22 (5H, m, CH ar Phe); 9.46 (1H, s, CHO).

FAB-MS calcd for $C_{22}H_{33}N_3O_5$ 419.24, found $[M + H]^+$:420.3.

Boc-L-Tyr(Bzl)-L-Phe-L-Val-L-Ala-H (8a)

^{1}H NMR: δ0.83 (3H, d, $J = 7$ Hz, CH_3 γ-al); 0.86 (3H, d, $J = 7$ Hz, CH_3 γ-Val); 1.29 (3H, d, $J = 4$ Hz, CH_3 β-Ala); 1.32 (9H, s, CH_3 Boc); 2.25 (1H, m, CH β-Val); 2.90 (4H, m, CH_2 β-Phe + Tyr); 4.20 (1H, CH αPhe or Tyr); 4.32 (2H, CH α Ala + Val); 4.57 (1H, CH αPhe or Tyr); 4.83 (1H, m, NH-Boc); 6.62 (2H, m, NH Ala + Val); 7.21 (15H, CH ar Tyr + Phe + phenyl + NH Phe); 9.51 (1H, s, CHO).

FAB-MS calcd for $C_{38}H_{48}N_4O_7$ 672.35, found $[M + H]^+$:673.3.

Epimerization Studies

Peptide aldehydes (1 mmol) were placed in a round-bottom flask in the appropriate solvent mixture (10 ml, AcOEt/hexane with or without 0.1% pyridine), and 1 g of SiO_2 or Al_2O_3 was added under stirring. After 2 h, the solid support was eliminated by filtration, solvents were evaporated *in vaccuo* and the aldehydes were analyzed by ^{1}H NMR spectroscopy. The two aldehyde signals observed by ^{1}H NMR were magnified and plotted. Two copies of the spectra were taken and the peaks corresponding to the diastereomers were integrated by weighing. The observed percentage of epimerization was then obtained as an average of two integrations.

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