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An efficient route from trifluoroacetates to water soluble free amines using Diaion® HP-20

Short Communication

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Summary. A series of polybasic lysine and ornithine derivatives were synthesised as trifluoroacetate salts. Attempts to prepare their free amines according to standard methodology were not successful due to the excellent water solubility of these compounds. Free amines were however efficiently obtained if the column with Diaion® HP-20 adsorbent was loaded with the trifluoroacetate and 1% $NaHCO_3$ aq was passed, followed by elution of free amine with methanol.

Keywords: Amino acid – Peptide – Diaion® HP-20

Introduction

Isolation of bestatin a potent aminopeptidase inhibitor and its numerous biological activities have triggered extensive research in the area of low molecular weight peptide molecules (Leung et al., 2000). A variety of peptide and peptidomimetic compounds have been synthesised and tested as protease inhibitors. Many of them have proven to be potent and selective slowing or halting disease progression. Thus, there exists continuous interest in synthetic procedures giving easy access to this type of molecules. The majority of these compounds are prepared from their BOC protected analogues, typically by treatment with trifluoroacetic acid, and isolated as trifluoroacetates. In this communication we describe the methodology of handling water soluble polybasic compounds of peptide character so that they can be efficiently transformed into the free-base form.

Materials and methods

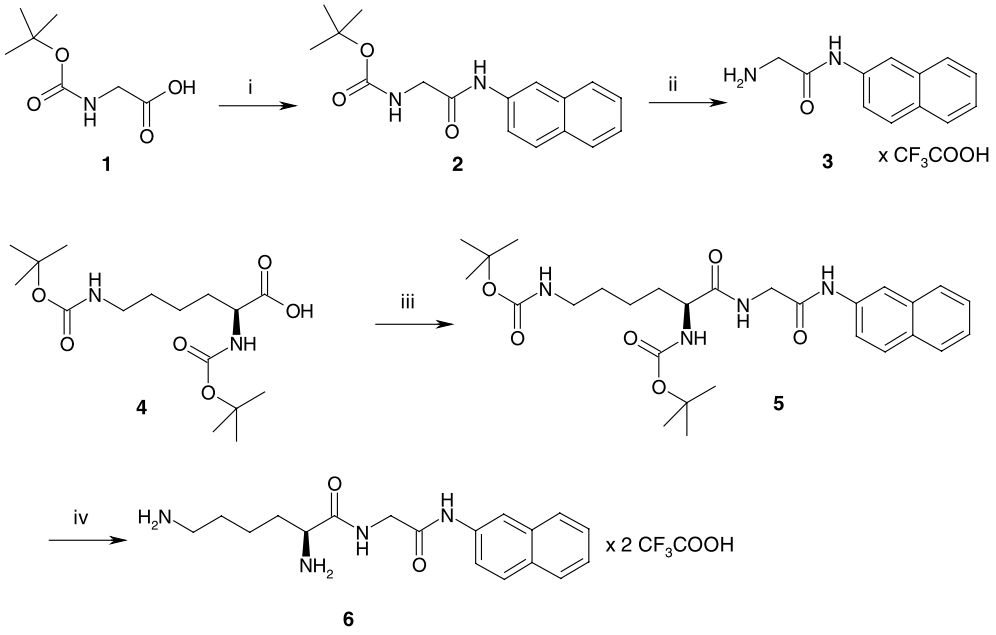
Aminoacids and other reagents were from Aldrich or Fluka. Diaion® HP-20 was purchased from Supelco. Purification of BOC protected products was accomplished using silica gel flash column chromatography (Merck 60). NMR and MS analyses were done on Mercury 400BB and Mariner machines, respectively.

Chemistry: A representative example of the synthesis of trifluoroacetate salt **6** from N-BOC glycine, 2-naphthylamine and N_α,N_ε bis-BOC (L) lysine as is outlined in Scheme 1. Other compounds were similarly prepared except that the starting materials were as appropriate: N-BOC-(D,L)-alanine, benzylamine and N_α,N_γ-bis-BOC-(L)-ornithine.

Typical procedure for transformation of trifluoroacetate salts to free amines: A 50 mL column was filled with fully hydrated Diaion® HP-20 adsorbent. The column was loaded with an aqueous solution of (L) lysyl-glycine-naphtyl-2-amide trifluoroacetate (**6**) (300 mg, 0.53 mmole in 5 mL H_2O) and 1% $NaHCO_3$ solution (200 mL) was passed through the column followed with 200 mL of water (no leakage of the peptide was observed up to this point) and finally with methanol:water (4:1 vol) until TLC ($EtOH:NH_3aq. = 4:1$, $R_f = 0.25$) revealed that all desired material was eluted. Throughout the process a rate of 2 bed volumes per hour was maintained. Appropriate methanol fractions were evaporated to give the amine (**14**) (148 mg, 0.45 mmole, 85%) as white solid. (Fluorine assay for all obtained compounds was below 0.5%).

Results and discussion

A number of polybasic peptides were synthesised as trifluoroacetate salts. Their spectral characteristics are collected in Table 1. Once the trifluoroacetates are obtained and the need arises to prepare free amines or different salt forms problems are encountered. Intuitively, one would consider dissolving the trifluoroacetate salt in water, neutralizing it with the solution of sodium bicarbonate and extracting the free amine with suitable organic solvent. It has however proved futile due to the excellent water solubility of the obtained amines. Such experiments were conducted using compounds **6**, **8**, **11** and **13** but HPLC anal-



Scheme 1. Reagents: (i) 2-naphthylamine, DCC, CH_2Cl_2, (rt/18 h); (ii) CF_3COOH (rt/0.5 h); (iii) Et_3N, ClCOOEt, CH_2Cl_2 (Ar, 0°C, 2 h), **3**, Et_3N, CH_2Cl_2 (0° to rt/1 h); (iv) CF_3COOH, (rt/0.5 h)

Table 1. Synthesis of trifluoroacetates

Entry	Compound	Yield[a]	Spectral characteristic ^{1}H NMR all in DMSO-d_6 expressed as δ
1	(L) Lysyl-glycine-naphthyl-2-amide trifluoroacetate (**6**)	28%	^{1}H NMR 1.37–1.47 (m, 2H), 1.54–1.61 (m, 2H), 1.75–1.79 (m, 2H), 2.79 (t, J = 7.8 Hz, 2H), 3.89 (t, J = 6.3 Hz, 1H), 4.02 (dd, J = 16.6, 5.6 Hz, 1H), 4.13 (dd, J = 16.6, 5.9 Hz, 1H), 7.41 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.48 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.60 (dd, J = 8.8, 2.0 Hz, 1H), 7.80–7.89 (m, 6H), 8.21 (br s, 3H), 8.26 (s, 1H), 8.90 (t, J = 5.8, 1H) 10.32 (s, 1H) MS (ESI) $C_{18}H_{24}N_4O_2$ found $(M+H)^+ = 329$ MSHR calc. 329.1972, found 329.1990
2	(L) Lysyl-(DL) alanine-naphthyl-2-amide trifluoroacetate (**7**)	27%	^{1}H NMR 1.39–1.43 (m, 5H), 1.54–1.61 (m, 2H), 1.73–1.79 (m, 2H), 2.78 (s, 2H), 3.83–3.90 (m, 1H), 4.51–4.62 (m, 1H), 7.38–7.49 (m, 2H), 7.59–7.65 (m, 1H), 7.79–7.95 (m, 6H), 8.22 (br s, 3H), 8.27 (s, 1H), 8.85 (d, J = 7.0 Hz, 0.5H, one diastereoisomer), 8.93 (d, J = 7.0 Hz, 0.5H, second diastereoisomer) 10.35 (s, 0.5H, one diastereoisomer), 10.38 (s, 0.5H, second diastereoisomer) MS (ESI) $C_{19}H_{26}N_4O_2$ found $(M+H)^+ = 343$ MSHR calc. 343.2129, found 343.2145
3	(L) Lysyl-glycine-benzylamide trifluoroacetate (**8**)	28%	^{1}H NMR 1.32–1.41 (m, 2H), 1.51–1.58 (m, 2H), 1.70–1.76 (m, 2H), 2.75 (s, 2H), 3.84 (d, J = 5.6 Hz, 2H), 4.25–4.35 (m, 1H), 7.21–7.33 (m, 5H), 7.90 (br s, 3H), 8.24 (br s, 3H), 8.52 (t, J = 5.9 Hz, 1H), 8.81 (t, J = 5.8 Hz, 1H) MS (EI) $C_{15}H_{24}N_4O_2$ found $(M+H)^+ = 293$ MSHR calc. 293.1972, found 293.1983
4	(L) Lysyl-(D,L) alanine-benzylamide trifluoroacetate (**9**)	34%	^{1}H NMR 1.27–1.30 (m, 3H) 1.31–1.41 (m, 2H), 1.49–1.58 (m, 2H), 1.69–1.75 (m, 2H), 2.73 (s, 2H), 3.81 (s, 1H), 4.25–4.30 (m, 2H), 4.33–4.42 (m, 1H), 7.21–7.30 (m, 5H), 7.87 (br s, 3H), 8.20 (br s, 3H), 8.54–8.59 (m, 1H), 8.69 (d, J = 7.3 Hz, 0.5H, one diastereoisomer) 8.77 (d, J = 7.3 Hz, 0.5H, another diastereoisomer) MS (ESI) $C_{16}H_{26}N_4O_2$ found $(M+H)^+ = 307$ MSHR calc. 307.2143, found 307.2129

(*continued*)

Table 1 (*continued*)

Entry	Compound	Yield[a]	Spectral characteristic ^{1}H NMR all in DMSO-d_6 expressed as δ
5	(L) Ornithyl-glycine-naphthyl-2-amide trifluoroacetate (**10**)	31%	^{1}H NMR 1.63–1.71 (m, 2H), 1.77–1.84 (m, 2H), 2.84 (t, J = 7.6 Hz, 2H), 3.93 (t, J = 6.3 Hz, 1H), 4.03–4.14 (m, 2H), 7.41 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.47 (ddd, J = 8.1, 6.9, 1.2 Hz, 1H), 7.60 (dd, J = 9.0, 2.0 Hz, 1H), 7.78–7.89 (m, 6H), 8.21 (br s, 3H), 8.26 (s, 1H), 8.92 (t, J = 5.8, 1H) 10.37 (s, 1H) MS (ESI) $C_{17}H_{22}N_4O_2$ found $(M+H)^+ = 315$ MSHR calc. 315.1816, found 315.1834
6	(L) Ornithyl-(D,L) alanine-naphthyl-2-amide trifluoroacetate (**11**)	31%	^{1}H NMR 1.40 (t, J = 6.9 Hz, 3H), 1.60–1.74 (m, 2H), 1.75–1.85 (m, 2H), 2.82–2.86 (m, 2H), 3.89–3.94 (m, 1H), 4.53–4.64 (m, 1H), 7.38–7.49 (m, 2H), 7.60–7.66 (m, 1H), 7.79–7.88 (m, 6H), 8.12 (br s, 3H), 8.29 (s, 1H), 8.86 (d, J = 7.0 Hz, 0.5H, one diastereoisomer), 8.96 (d, J = 7.0 Hz, 0.5H, second diastereoisomer) 10.39 (s, 0.5H, one diastereoisomer), 10.41 (s, 0.5H, second diastereoisomer) MS (ESI) $C_{18}H_{24}N_4O_2$ found $(M+H)^+ = 329$ MSHR calc. 329.1972, found 329.1986
7	(L) Ornithyl-glycine-benzylamide trifluoroacetate (**12**)	36%	^{1}H NMR 1.58–1.66 (m, 2H), 1.73–1.80 (m, 2H), 2.81 (t, J = 7.3 Hz, 2H), 3.73–3.93 (m, 3H), 4.25–4.36 (m, 2H), 7.21–7.34 (m, 5H), 7.93 (br s, 3H), 8.24 (br s, 3H), 8.54 (t, J = 6.0, 1H), 8.83 (t, J = 5.6, 1H) MS (ESI) $C_{14}H_{22}N_4O_2$ found $(M+H)^+ = 279$ MSHR calc. 279.1816, found 279.1821
8	(L) Ornithyl-(D,L)-alanine-benzylamide trifluoroacetate (**13**)	34%	^{1}H NMR 1.29 (dd, J = 7.0, 5.0 Hz, 3H), 1.55–1.65 (m, 2H), 1.71–1.79 (m, 2H), 2.80 (s, 2H), 3.86 (dd, J = 8.2, 6.0 Hz, 1H), 4.23–4.44 (m, 3H), 7.22–7.34 (m, 5H), 7.90 (br s, 3H), 8.25 (br s, 3H), 8.59 (q, J = 6.0 Hz, 1H), 8.69 (d, J = 7.1 Hz, 0.5H, one diastereoisomer), 8.80 (d, J = 7.7 Hz, 0.5H, second diastereoisomer) MS (ESI) $C_{15}H_{24}N_4O_2$ found $(M+H)^+ = 293$ MSHR calc. 293.1972, found 293.1979

[a] Yield of four steps according to Scheme 1, based on N-BOC glycine or N-BOC (D,L) alanine

ysis revealed that in all cases the amine remains entirely in water, none is extracted to ethyl acetate or dichloromethane. Somewhat better results are obtained if the neutralized water solution is evaporated to dryness and the solid material is extracted with ethyl acetate, but it is far from being efficient.

To overcome this problem and obtain free amines from trifluoroacetate salts we thought of taking advantage of known desalting properties of Diaion® HP-20. Aromatic type adsorbents based on crosslinked polystyrenic matrix are widely used in different industrial and research fields, extraction of antibiotic intermediates from fermentation broth, separation of peptides and food additives etc. However when an aqueous solution of a trifluoroacetate salt e.g. **6** or **13** is applied on a column filled with HP-20 and an ample amount of water is passed in order to remove trifluoroacetic acid the desired product, eluted with methanol/water mixture, contains the same amount of trifluoroacetic acid as the initial salt. Free amine can however be obtained if a 1% solution of sodium bicarbonate is passed through HP-20 filled column prior to elution with organic solvent. As shown in Table 2 free amines of all

Table 2. Synthesis of free amines from trifluoroacetates

Entry	Compound	m.p.	Yield
1	(L) Lysyl-glycine-naphthyl-2-amide (**14**)	88–90°C	85%
2	(L) Lysyl-(D,L) alanine-naphthyl-2-amide (**15**)	72–74°C	87%
3	(L) Lysyl-glycine-benzylamide (**16**)	oil	90%
4	(L) Lysyl-(D,L) alanine-benzylamide (**17**)	oil	89%
5	(L) Ornithyl-glycine-naphthyl-2-amide (**18**)	74–76°C	90%
6	(L) Ornithyl-(D,L) alanine-naphthyl-2-amide (**19**)	84–86°C	84%
7	(L) Ornithyl-glycine-benzylamide (**20**)	oil	93%
8	(L) Ornithyl-(D,L)-alanine-benzylamide (**21**)	oil	86%

studied compounds could be obtained efficiently under mild conditions (Table 2).

Diaion® HP-20 adsorbent can be easily regenerated by washing with methanol and water. In our hands the same portion was used 15 times without loss of separation properties.

In order to expand the scope of this methodology selected ester type compounds were prepared i.e. (L) ornithyl-glycine-ethyl ester trifluoroacetate and (L) ornithyl-glycine-benzyl ester trifluoroacetate and their behavior in contact with HP-20 examined. Surprisingly, these salts were easily eluted with water and thus the procedure could not be used to prepare free amines.

Reference

Leung D, Abbenante G, Fairlie D (2000) Protease inhibitors: current status and future prospects. J Med Chem 43: 305 and the references cited therein

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