Easy Preparation of Bioactive Peptides from the Novel N^{α}-Trifluoroethyl Amino Acids

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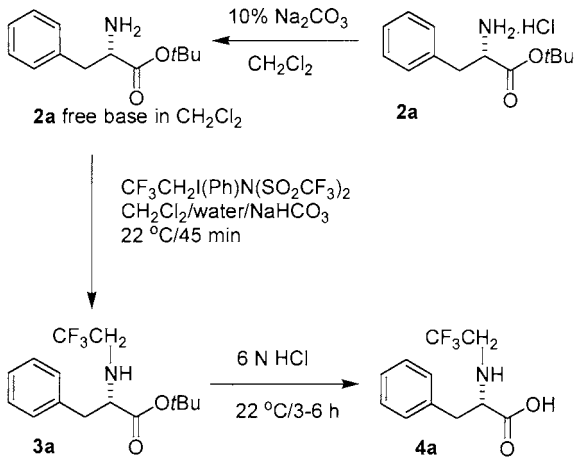
N^{α}-Trifluoroethyl amino acids have been prepared for the first time and unexpectedly found to behave as conventionally *N*-protected amino acids. Novel unnatural peptides are easily prepared in high yields without racemization. The observed chemistry results from steric factors as well as from the acidity of the CF_3CH_2NH group, according to comparative experiments. Of ten dipeptides that were tested for antitumor activity, CF_3CH_2–L-Tyr–L-Ile–O*t*Bu was the most active.

We wish to report a direct synthesis of unprecedented fluoroalkyl-substituted molecules from the common amino acids.

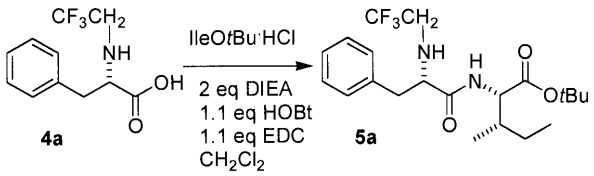
We reported recently the first rapid alkylations of the side chains of cysteine, glutathione, and N^{α}-protected lysine in aqueous media at ambient temperature,[1] consisting of the transfer of a 2,2,2-trifluoroethyl group from the iodonium salt $(CF_3SO_2)_2NI(Ph)CH_2CF_3$ (**1**). Because large amounts of compound **1** are prepared easily,[2] we have further studied the potential of **1** as a discovery tool for novel bioactive substances.[1,3]

We now report that amino acid esters are alkylated at the α-nitrogen by **1** under convenient two-phase conditions (CH_2Cl_2/water/$NaHCO_3$). Scheme 1 shows the simple preparation of the representative products N^{α}-2,2,2-trifluoroethyl-phenylalanine (**4a**), -tyrosine (**4b)** and -valine (**4c**).



Scheme 1. Preparation of the title amino acids.

Initially, investigating CF_3CH_2–L-PheO*t*Bu **3a** as a model, we hoped to form a peptide bond to the α-nitrogen but we had no success. We supposed that the α-nitrogen had become essentially non-nucleophilic. Such a degree of deactivation is the purpose of protecting groups in peptide synthesis.[4] On this basis we reasoned that standard peptide synthesis might be applicable with the free acids **4**. This proved to be true. Standard coupling conditions gave dipeptides **5a–l** (Scheme 2, Table 1) in excellent yields.[6] The commercially available amino acid *t*-butyl esters **2a-c** are the most convenient starting materials for preparing **4**. Because *t*-butyl esters are especially stable to bases, but readily hydrolyzed by dilute acids, the direct procedure of Scheme 1 becomes possible.[5] The analytically pure amino acid products **4** are obtained by simple partitioning between organic and aqueous phase. Chromatographic isolation of the intermediate esters **3a-c** is possible but unnecessary.



Scheme 2. Typical reaction of an N^{α}-2,2,2-trifluoroethyl-amino acid under standard peptide coupling conditions.

Table 1. Dipeptides[a] from **4a, 4b and 4c**

Trifluoroethyl amino acid	Second component	Product	Y./ %
4a	L-IleO*t*Bu	**5a**	64
4a	L-AlaOMe	**5b**	64-95[b,c]
4a	D-AlaOMe	**5c**	62
4a	D,L-AlaOMe	**5d**	24[d]
4a	L-ValOMe	**5e**	73-90[c]
4a	D,L-ValOMe	**5f**	88
4b	L-AlaO*t*Bu	**5g**	74
4b	L-LeuO*t*Bu	**5h**	95
4b	L-IleO*t*Bu	**5i**	95
4b	L-Asp(OAll)OAll	**5j**	98
4b	L-Glu(OAll)OAll	**5k**	97
4c	L-PheO*t*Bu	**5l**	95

[a]All reactions on a 0.50 mmol scale, with 1.1 eq HOBt, 1.1 eq EDC, 2 eq DIEA unless otherwise indicated. [b]55% using DCC in place of EDC. [c]4 sets of conditions, see text. [d]Water/$NaHCO_3$ in place of DIEA.

Possible racemization of the amino acid that undergoes the coupling is a major issue of peptide chemistry.[4,7] The method of *N*-protection can be one of the factors in racemization. Therefore we investigated the eight combinations (four preparations each of **5b** and **5e**) of **3a** with L-AlaOMe and L-ValOMe as the incoming amino acids, CH_2Cl_2 or DMF as the solvent, and 1 or 2 eq of DIEA. The methyl esters of L-Ala and L-Val and the corresponding D- and D,L- forms are commercially available, so we could use **5d** and **5f** as NMR references. Undesired diastereomers were not detected in any run by 500 MHz ^1H and 470 MHz ^{19}F NMR. The best conditions from this screening experiment were used to prepare dipeptides from N^{α}-

trifluoroethyltyrosine (**4b**) or -valine (**4c**) and a second amino acid having the practical[4] *t*-butyl or allyl ester *O*-protection. All the dipeptides so prepared were essentially pure as obtained from workup.

To determine if the electron-withdrawing character of the CF_3CH_2 group could explain our findings, firstly we measured pK_2 of the water-soluble CF_3CH_2–Gly–OH·HCl by titration. We found that pK_2 of trifluoroethyl glycine is only slightly lower than pK_a of trifluoroethylamine, as shown in Figure 1. However, trifluoroethylamine and Z-PheOH gave under the same coupling conditions of Scheme 2 a 75% yield of the crystalline amide Z-Phe–$NHCH_2CF_3$. The importance of steric factors was confirmed when, still under the same conditions, no product was formed from Z-PheOH and secondary *N*-2,2,2-trifluoroethyl amines such as $CF_3CH_2NHCH(CH_3)Ph$ and $CF_3CH_2NH(CH_2)_2Ph$.

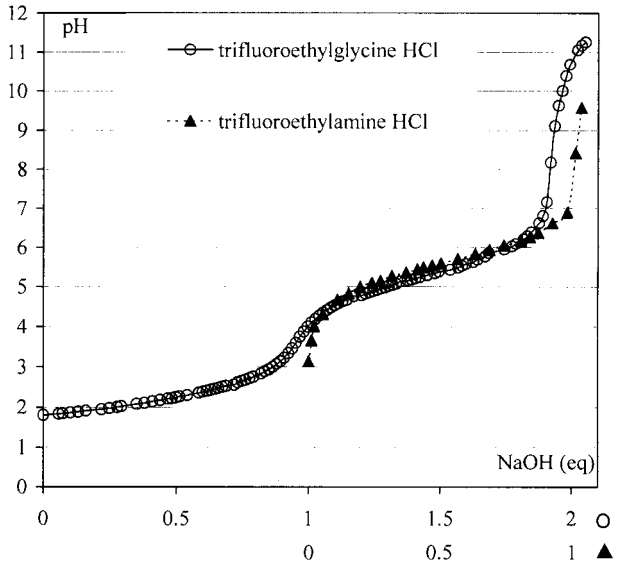


Figure 1. Titration of $CF_3CH_2NHCH_2COOH·HCl$ (pK_1 = 2.2, pK_2 = 5.3) and $CF_3CH_2NH_2·HCl$ (pK_a = 5.6) with NaOH in water.

Protecting groups such as Boc, Z, arenesulfonyl and formyl occur in synthetic bioactive peptides and are evaluated as structural units in pharmaceutics design.[8] While structure–activity relationship is a very complex subject, a simple chemical function of such "protection" is to retard or prevent metabolic deactivation by oxidative dealkylation. That is precisely the known utility of a fluoroalkyl residue.[9]

Assays for anticancer activity were carried out at NIH/NCI[10] on the compounds in Table 1. CF_3CH_2–L-Phe–L-IleO*t*Bu (**5a**), CF_3CH_2–L-Tyr–L-LeuO*t*Bu (**5h**) and CF_3CH_2–L-Tyr–L-IleO*t*Bu (**5i**) reduced cancer cell growth. Compound **5i** killed cancer cells at 10^{-5} M concentration. These results from a very small pool of new substances warrant further synthetic work.

In summary, preparative amounts of the novel type of amino acids represented by **4a–c** are available easily. Most importantly, they undergo standard peptide chemistry. This unexpected property of **4** affords very large numbers of potentially bioactive fluoroalkylated substances possessing very lipophilic moieties whose in vivo stability is also anticipated.

The financial support of this research by the National Science Foundation is gratefully acknowledged.

References and Notes

1 D. D. DesMarteau and V. Montanari, *Chem. Commun.*, **1998**, 2241.
2 Following Ref. 1 on a larger scale, $HN(SO_2CF_3)_2$ (64 mmol), $CF_3CH_2I(OCOCF_3)_2$ (61 mmol) and benzene (70 mmol) reacted in CFC 113 (50 mL) during 22 h. Evaporating the volatiles, stirring the residue with ice, filtering and freeze-drying yielded **1** as a powder (30.7 g, 54 mmol, 89%). Crystallization (CH_2Cl_2, 4 mL/g, –20 °C) gave 27.3 g (79% overall) of **1** as transparent prisms, mp 77–79 °C, dec. 105–120 °C (TGA, 5 °C/min).
3 Fluoroalkylation reactions by $CF_3CH_2I(Ph)OSO_2CF_3$ (**6**) were first reported in detail by Umemoto and Gotoh: T. Umemoto and Y. Gotoh, *Bull Chem Soc. Jpn.*, **60**, 3307 (1987); T. Umemoto, *Chem. Rev.*, **96**, 1757 (1996). The preparation of **1** is similar to that of **6**, with $HN(SO_2CF_3)_2$ in place of triflic acid, but **1** requires more strictly anhydrous conditions until workup. Compound **1** extends the reactions of **6** to aqueous systems. The reason of the much greater stability of **1** to water relative to **6** is not yet clear; a crystallographic investigation is in progress: D. D. DesMarteau, W. T. Pennington, and V. Montanari, *J. Mol. Struct.*, in press.
4 "Chemistry and Biochemistry of the Amino Acids," ed. by G. C. Barrett, Chapman and Hall, London (1985); "The Peptides: Analysis, Synthesis, Biology,"ed. by E. Gross and J. Meienhofer, Academic Press, London (1979), Vol. 1.
5 Typical procedure for **4**: Phenylalanine *t*-butyl ester hydrochloride (**2a**) (9.85 mmol) was suspended in 75 mL CH_2Cl_2. Water (75 mL) and Na_2CO_3 (7 g) were added and the mixture stirred for 30 min. The clear organic layer was separated. $NaHCO_3$ (11.9 mmol), water (70 mL) and **1** (10.62 mmol) were added with stirring at 20 °C. After 45 min the CH_2Cl_2 phase was separated and washed with 3 × 100 mL water. It was then stirred at 20 °C with 2 × 150 mL 6M HCl for 3 h. The combined acid solutions were dried to constant weight, yielding 2.30 g (77%) of crystalline **4a** hydrochloride monohydrate, mp 159–160 °C. Similarly were prepared **4b** hydrochloride (78%), mp 203–204 °C, and **4c** (95%), mp 166–167 °C. The composition of **4a–c** was established by the correct elemental analyses of the bulk products.
6 Typical procedure for **5**: CF_3CH_2–L-PheOH·HCl·H_2O (**4a**) (0.50 mmol), L-AlaOMe·HCl (0.50 mmol), HOBt·H_2O (0.55 mmol) and EDC (0.55 mmol) were suspended in CH_2Cl_2 (5 mL) at 5 °C. DIEA (175 μL, 1.0 mmol) was added rapidly by syringe. The reaction was run for 1 h at 5 °C, then for 3 h at 22 °C. The reaction mixture was diluted to 50 mL with CH_2Cl_2, and washed with 0.1 M $NaHCO_3$ (50 mL), 0.5 M HCl (50 mL), and water (2 × 50 mL). Drying on Na_2SO_4, evaporating, and pumping at 0.05 mmHg gave **5a** (158 mg, 95%) as a white powder, mp 73–76 °C.
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10 Details on the in vitro anticancer testing protocols are available at http://dtp.nci.nih.gov.