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A VERSATILE SYNTHESIS OF PEPTIDYL FLUOROMETHYL KETONES

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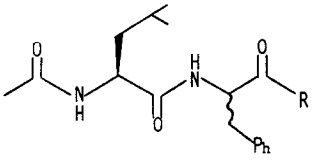
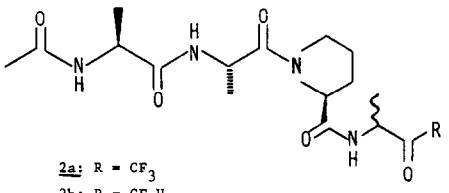
SUMMARY: A versatile synthesis of peptidyl fluoromethyl ketones with potential as serine protease inhibitors is described.

The dramatic effect of the incorporation of a fluoromethyl ketone[1] moiety into substrate analogues of hydrolytic enzymes has been highlighted by the development of several extremely potent inhibitors.[2] Peptidyl fluoromethyl ketones such as 1 and 2 directed against the serine proteases chymotrypsin and elastase, respectively, also represented attractive synthetic targets. The biological importance of small synthetic inhibitors of serine proteases is well-established.[3] Numerous synthetic approaches including a modified Dakin West procedure[4] or the use of fluoromethyl carbanions[5] met with limited success. In the course of the preliminary work it became increasingly apparent that no general methods for the synthesis of these peptide analogues were known.



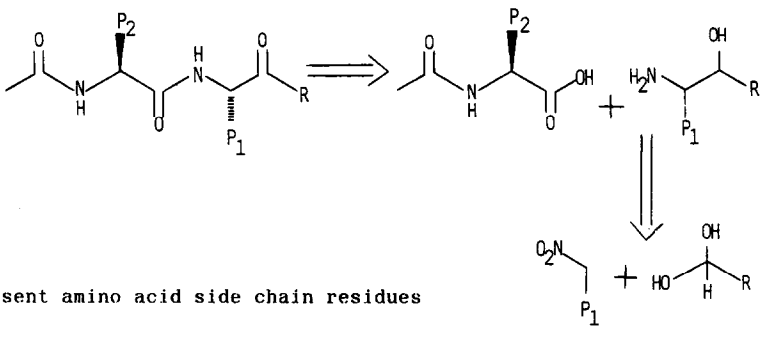
1a: R = CF_3
1b: R = CF_2H
1c: R = CFH_2



2a: R = CF_3
2b: R = CF_2H
2c: R = CFH_2

A versatile synthesis involves the recognition of β-amino alcohols as the basic building blocks of peptides with the carboxyl terminus replaced by a fluoromethyl ketone. This is illustrated retrosynthetically in Scheme 1.

SCHEME 1

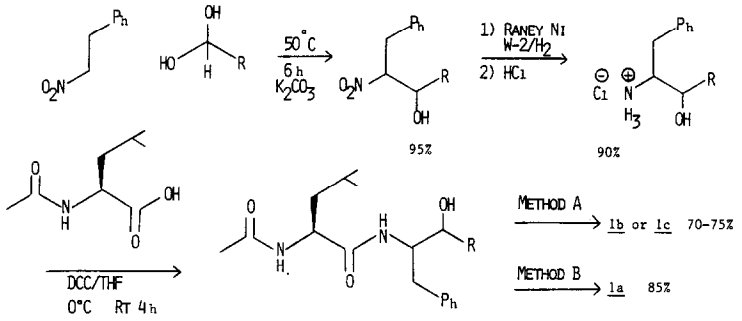


P_1 and P_2 represent amino acid side chain residues

These amino alcohols are readily obtained from the potassium carbonate catalysed reaction of the corresponding nitroalkane with the appropriate fluorinated aldehyde hydrate or hemi-acetal.[6] The choice of nitroalkane defines the first amino acid residue in the peptide; thus 2-nitrophenyl ethane would be used if phenylalanine were the desired residue. Reduction of the β-nitro alcohol in a Parr shaker under 50 psi of hydrogen pressure with Raney nickel (W-2) as catalyst, followed by treatment with concentrated hydrochloric acid affords the β-amino alcohol hydrochloride as a crystalline product which is a mixture of DL, threo and erythro, diastereomers.[7] These two steps routinely proceed in an overall yield of over 85% regardless of the aldehyde component used.

Peptide coupling with the appropriate N-blocked peptide is carried out under standard conditions in anhydrous tetrahydrofuran using N-methylmorpholine (1 equiv.) to liberate the free amine from its hydrochloride and 1,3-dicyclohexylcarbodiimide (1 equiv.) to activate the acid component.[8] This sequence is outlined in Scheme 2.

SCHEME 2



1a: R = CF_3
1b: R = CF_2H
1c: R = CFH_2

The final step in the synthesis is the oxidation of the fluoromethyl carbinols. While the difluoro- and monofluoromethyl compounds oxidize cleanly utilizing a Sarett oxidation[9] - Method A - the corresponding trifluoromethyl compound proved to be exceedingly resistant to oxidation under a variety of conditions.[10] This alcohol was finally oxidized in good yield (85%) under basic aqueous conditions (0.3N sodium hydroxide) with 1.2 equivalents of potassium permanganate (0.5h, 25°), Method B.[11]

Diagnostic physical data for 1a, 1b, and 1c [12] as unseparated mixtures of diastereomers.

Compound	^{19}F NMR (280 MHz) (shift in ppm)*	Mass Spectrum (EI-20eV)
1a	(acetone/D_2O) δ:-82.2(s)	372(M+), 329(M-$COCH_3$),316, 275(M-$COCF_3$).
1b	(acetone/D_2O) δ:-130.5 (dd, J=282 and 54.8Hz), -135.9(dd,J=282 and 57Hz)	354(M+), 310, 275(M-$COCF_2H$) 255.
1c	(acetone) δ:-231.0(t,J= 46Hz), -230.6(t,J=46Hz)	336(M+), 303(M-CFH_2), 275 (M-$COCFH_2$), 260, 243.

* ^{19}F NMR shifts are reported relative to $CFCl_3$ set at 0 ppm.

The preparation of these peptidyl fluoromethyl ketones represents a standard protocol for the synthesis of many analogous compounds which can be targeted against specific serine proteases by the selection of an appropriate nitroalkane and suitably protected peptide component.[13]

Acknowledgements

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REFERENCES & FOOTNOTES

1. The term "fluoromethyl ketone" is applied generally to mono-, di, and trifluoromethyl ketones.

2. M.H. Gelb, J.P. Svaren, and R.H. Abeles Biochemistry 24, 1813 (1985).

3. For examples see a) "Proteases and Biological Control", Eds. E. Reich, D.B. Rifkin, and E.S. Shaw. Cold Spring Harbor Conferences on Cell Proliferation, Vol. 2. 1975. b) "Proteinase Inhibitors; Medical and Biological Aspects" Eds. N. Katumuna, H. Umezawa, and H. Holzer, Springer Verlag, 1983.

4. Although treatment of N-blocked amino acids with acetic anhydride and pyridine results in good yields of the corresponding methyl ketones, similar treatment with trifluoroacetic anhydride (in the presence or absence of catalytic 4-dimethylaminopyridine) affords only ketone products with the unsubstituted amino acid glycine. E.J. Bourne, J. Burdon, V.C.R. McLoughlin, and J.C. Tatlow J. Chem. Soc. 1771 (1961).

5. Because of the instability of CF_3Li and CF_3MgI we have found the corresponding zinc reagent CF_3ZnI useful in the preparation of simple trifluoromethyl ketones (T. Kitazume, and N. Ishikawa Chem. Lett. 1679 (1981)). However, the use of such organometallic reagents is problematic in the preparation of peptidyl products.

6. D.J. Cook, O.R. Pierce, and E.T. McBee J. Am. Chem. Soc. 76, 83 (1954).

7. At this time, the diastereomers have not been separated or resolved, however, in order to obtain optically pure peptides, the stage of the β-amino alcohol would be an appropriate point at which to resolve the intermediate since protocols for the resolution of numerous β-amino alcohols are available. "Optical Resolution Procedures for Chemical Compounds; Vol. 1, Amines and Related Compounds", P. Newman, Published by Optical Resolution Center, N.Y. 1978.

8. The yield in the case of the trifluoromethyl compound is slightly lower than that for the di- and monofluoromethyl alcohols. This is due to the reactivity of the secondary alcohol (pK_a 12.4) causing side reactions in the peptide coupling.

9. G.I. Poos, G.E. Arth, R.E. Beyler, and L.H. Sarett J. Am. Chem. Soc. 75, 422 (1953). The Sarett oxidation is a chromium trioxide/pyridine oxidation with the active complex generated in situ. In this case double the normal equivalents of pyridine and chromium trioxide and longer reaction times (i.e., 2h, 25°) are necessary to ensure complete oxidation.

10. The only literature procedures available for the oxidation of trifluoromethyl carbinols employ very harsh conditions which are unsuitable for these peptidyl compounds. For examples see: "Chemistry of Organic Fluorine Compounds", M. Hudlicky, 2nd edition, Ellis Horwood Ltd., 1976, p. 208-213.

11. If optically active materials were subjected to the oxidation procedure, the following points are pertinent. Method A has been frequently used to oxidize α-chiral aldehydes without racemization, and therefore should proceed in a similar manner for the preparation of optically active mono- and difluoromethyl ketones. In the oxidation of the trifluoromethyl compounds (Method B) since the oxidation is carred out under aqueous conditions, it is hoped that the ketone in its hydrated form will not be prone to racemization, even in base.

12. Both ^{1}H NMR and ^{13}C NMR were obtained for all intermediates and final products, all were completely consistent with the designated structures.

^{13}C NMR (75.5 MH_3) proton decoupled, of final products.

1a (Acetone-d_6)

δ:22.4,23.4,25.5,34.5,41.4,52.8,57.0,94.4(q,J=28.8Hz), 123.0(q,J=270Hz),127.2, 129.2,130.3,139.5,171.2,175.3.

1b (Acetone-d_6 + 20% D_2O)

δ:22.4,23.0,23.1,25.5,34.8,41.4,53.5,55.0,94.6(t,J=25Hz),115.4(t,J=245Hz),127.3,129.3,130.7, 139.5,173.3,174.2

1c ($CDCl_3$)

δ:22.3,22.6,22.9,24.5,36.1,36.4,41.0,51.3,51.7,56.3,84.27(d,J=185.3Hz),84.41(d,J=183.8Hz), 127.2,128.7,129.2,135.7,136.1,170.6,170.8,172.9,173.2,203.9(d,J=17.9Hz).

13. Compounds 1a, 1b, and 1c were all specific inhibitors for bovine chymotrypsin, as were compounds 2a and 2b towards porcine pancreatic elastase. Full details of these results will be reported elsewhere.

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