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Peptide modification by introduction of α-trifluoromethyl substituted amino acids

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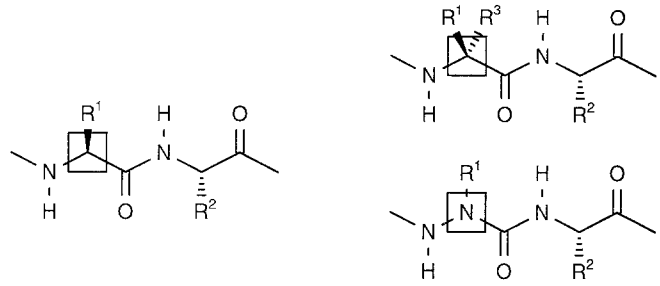
Summary. Methodology for the synthesis and incorporation of α-trifluoromethyl substituted amino acids into N- and C-terminal position of peptides is described. The incorporation of α-trifluoromethyl substituted amino acids into strategical positions of peptides enhances proteolytic stability and lipophilicity. Furthermore, it improves transport rates in vivo and permeability through certain body barriers.

Keywords: Amino acids – Methyl 2-carbamoylimino-3,3,3-trifluoropyruvate – Trifluoromethyl substituted 2,5-dioxopiperazines – 4-Trifluoromethyl N-carboxy anhydrides – 4-Trifluoromethyl-4H-oxazolones-5 – Ugi reaction

Transport rates of peptides through membranes *in vivo* are known to be enhanced by increasing the lipophilicity. The site specific incorporation of highly lipophilic amino acids and amino acid analogues into biologically active peptides seems to be a major aim in modern peptide chemistry. This strategy often is combined with the development of non-natural amino acids and amino acid analogues as highly specific enzyme inhibitors.

Modification of peptides can for instance be achieved via incorporation of α,α-disubstituted amino acids or aza analogues of amino acids.

α,α-Dialkyl amino acids frequently induce conformational restrictions on the peptide chain and promote helical secondary structures in peptides (Marshall et al., 1988). Incorporation of α-trifluoromethyl substituted amino acids (TFM amino acids) into biologically active peptides should doubtlessly result in severe conformational restrictions and highly increased lipophilicity, as the CF_3 group is probably one of the most lipophilic substituents known. The hydrogen/



fluorine exchange is assumed to be nearly isosteric (van der Waals-radii: H 120 pm; F 135 pm). Nevertheless, it was found that the van der Waals-volumes of a CF_3 group (0.0426 nm^3) and of a CH_3 group (0.0168 nm^3) differ significantly.

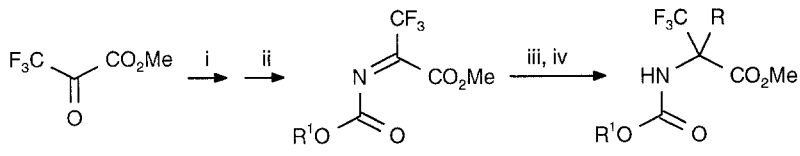
A CF_3 group exerts considerable polarization effects on the neighboring substitutents (pK_a values: Ala-CO_2H: 2.34; TFMAla-CO_2H: 1.98; Ala-NH_2: 9.87; TFMAla-NH_2: 5.91 (Kobzev et al., 1989)). This is expected to affect the hydrolytic stability of TFM amino acids containing peptides, *i.e.* degradation by peptidases should be retarded. Consequently, the presence of TFM amino acids in peptide drugs should result in higher metabolic stability.

In spite of the lipophilic character of the CF_3 group, the fluorine atoms can also act as electron donors in hydrogen bonding.

TFM amino acids on their own show a broad spectrum of biological activity. Besides their antibacterial and sometimes antihypertensive properties, they gained attention as potent suicide inhibitors of pyridoxalphosphate dependent enzymes (transaminases, decarboxylases) (Welch, 1987; Mann, 1987).

Synthesis of α-TFM amino acids

Several somewhat complementary preparative routes towards α-TFM amino acids **2** have been developed (Kukhar' et al., 1990; Burger et al., 1990; Burger and Sewald, 1990; Burger et al., 1991b). The most general synthesis proceeds via amidoalkylation of carbon nucleophiles with methyl 2-carbamoylimino-3,3,3-trifluoropyruvate **1**, an electrophilic synthon for TFM amino acids:

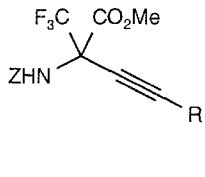


1 **2**

i) R^1OCONH_2; ii) $(CF_3CO)_2O$, pyridine; iii) RMgX; iv) H_3O^+

More complex TFM amino acids can be synthesized *via* transformation of functional groups present in the side chain, *e.g.* isoxazolyl- (**4**), pyrazolyl- (**5**), triazolyl-TFMGly **6** from alkynyl-TFMGly **3** (Sewald and Burger, 1992); TFMAsp (**7**, n = 1), TFMGlu (**7**, n = 2), TFMAad (**7**, n = 3) (Burger and Gaa,

1990); derivatives of TFMGly and TFMAla with organometallic moieties in the side chain like **8** (Sewald et al., 1993); ω-diorganophosphinoyl substituted TFM amino acids **9** (Burger et al., 1991a); etc.



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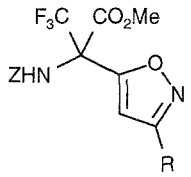

R= Aryl, Br

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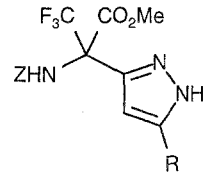
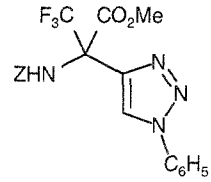


R= H, CO_2Et

5



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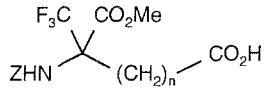
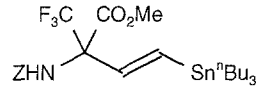


n= 1-4

7



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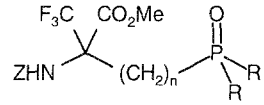
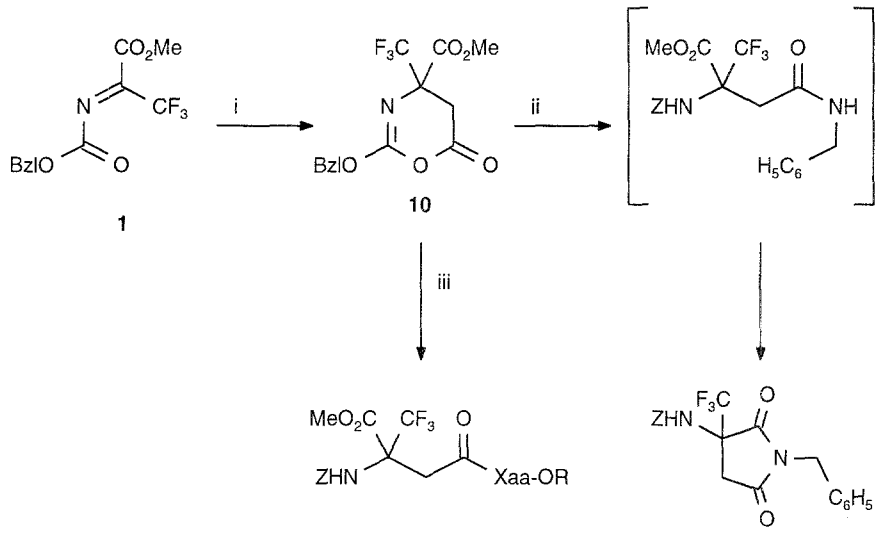


n= 1-4; R= CH_3, C_6H_5

9

The cyclic anhydride **10**, which is a ω-activated synthon for TFM-Asp, can be used for the synthesis of TFM isoaspartylpeptides **11** (Sewald et al., 1992).



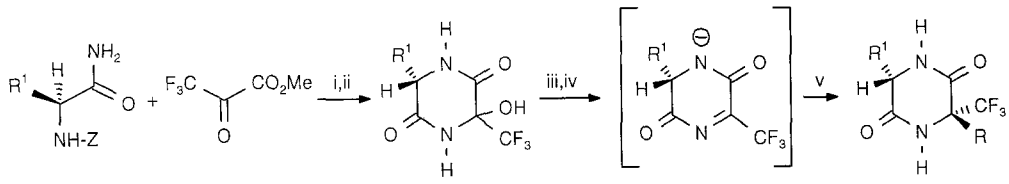
1 **10** **11** Z-TFMAsp(Xaa-OR)-OR **12**

i) CH_3COCl, NEt_3; ii) $C_6H_5CH_2NH_2$; iii) H-Xaa-OR

The formation of succinimides from **11**, which frequently is observed with isoaspartyl peptides, does not occur in the presence of bulky C-terminal amino acid side chains. The fully protected or preferrably the N-deprotected

diastereoisomeric isoaspartyl peptides **11** can be separated by flash chromatography.

Until now, nearly all synthetic routes to optically pure TFM amino acids rely on resolution by chemical or biochemical means. A promising strategy for the diastereoselective synthesis of TFM amino acids being investigated by us proceeds via amidoalkylation of carbon nucleophiles with *in situ* formed cyclic homochiral acyl imines **14**. The corresponding dioxopiperazines (DOP) **15** are formed with good stereoselectivity.



13 **14** **15**

i) r.t.; ii) H_2/Pd/C; iii) $(CF_3CO)_2O$; iv) RMgX; v) H_3O^+

Possible epimerization of the leucine part of this mixed DOP should be prevented by deprotonation of the leucine NH. *Via* selective chemical or enzymatic hydrolysis, linear dipeptides containing TFM amino acids in C-terminal position should be obtained.

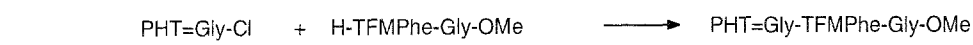
Peptide synthesis with TFM amino acids

Protective group strategy

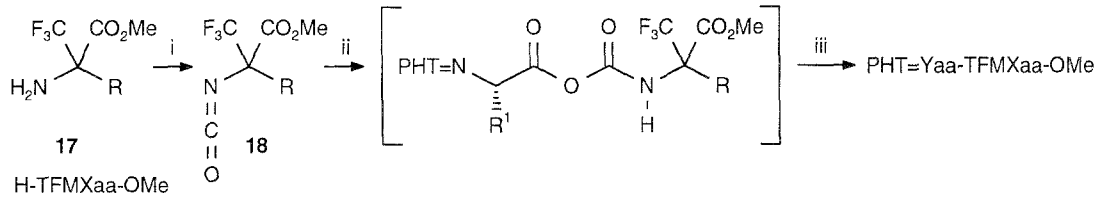
TFM amino acids with orthogonal protective groups (BOC/Z and OMe) are obtained using **1** as electrophilic synthon. Alkaline hydrolysis with 1N KOH/methanol 1:1 (v/v) gives the free carboxylic acids BOC-TFMXaa-OH or Z-TFMXaa-OH **16**. Hydrogenolytic or acidolytic cleavage of the Z or BOC group yields H-TFMXaa-OMe **17**. However, the presence of the electron-withdrawing CF_3 substituent in α-position exerts considerable electronical (pK_a, *vide supra*) and sterical effects on the reactivity of both the carboxylic and the amino group. The low basicity of the amino group in **17** prevents formation of dioxopiperazines; esters of TFM amino acids can even be distilled without oligomerization or decomposition.

Amino group activation

Therefore, the activation of the amino group of TFM amino acids is very difficult to achieve. Until now, satisfactory results have only been obtained with H-TFMAla-OMe (with mixed anhydrides or Fmoc-Yaa-Cl). For the bulkier TFM amino acids, all classical methods fail or result in substantial epimerization of the non-fluorinated amino acid. Peptide bond formation under quite drastic reaction conditions is useful only with substrates where epimerization is not possible.



In some cases, dipeptides with C-terminal TFM amino acids can be obtained on reaction of PHT = Yaa-OH with isocyanates **18** derived from TFM amino acids.

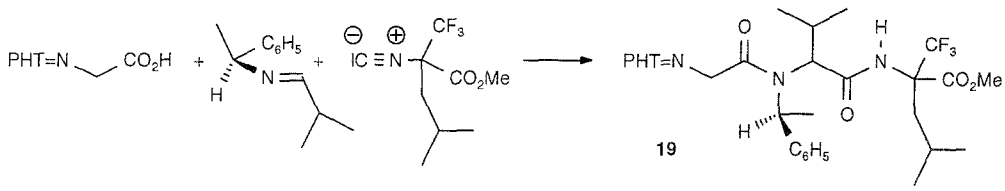


i) CCl_3OCOCl; ii) PHT = Yaa-OH; iii) $-CO_2$

Isocyanates derived from amino acids are valuable components for the synthesis of azapeptides, which are obtained in good yields on reaction of the isocyanate with amino acid hydrazides.

Azatripeptides with TFM amino acids in N-terminal (H-TFMXaa-Agly-Yaa-OR), C-terminal (Z-Xaa-Agly-TFMYaa-OMe) or in both positions (H-TFMXaa-Agly-TFMYaa-OMe) can be synthesized.

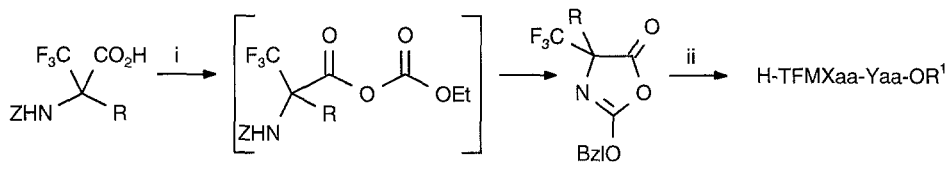
Similarly, TFM amino acids can subsequently be N-formylated and dehydrated to give the corresponding isonitrile, which can be used for the synthesis of tripeptides **19** with C-terminal TFM amino acids *via* Ugi reaction.



A promising strategy seems to be the selective cleavage of the DOP **15** to the dipeptide with the TFM amino acid as C-terminus, which is currently under investigation. Further N-terminal chain elongation is expected to be unproblematic.

Carboxylic group activation

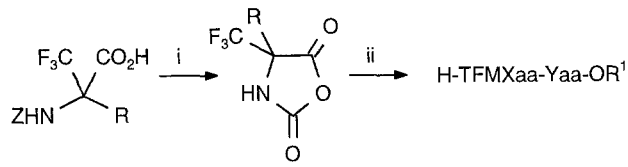
Carboxylic group activation is achieved via formation of mixed anhydrides with alkyl chloroformates or of Leuchs anhydrides (N-carboxy anhydrides, NCA). The mixed anhydrides formed primarily cyclize spontaneously to the surprisingly stable oxazolones **20**, which are also formed on treatment of Z-TFMXaa-OH with DCCI (even in presence of HOBt) or diphenylchlorophosphate/base. Epimerization on the stage of the oxazolone, however, is not a problem with α,α-disubstituted amino acids, as there is no α-proton. Formation of dipeptides Z-TFMXaa-Yaa-OR occurs upon ring opening of the oxazolones **20** with amino acid esters H-Yaa-OR.



16 Z-TFMXaa-OH 20

i) EtOCOCl, base; ii) H-Yaa-OR[1]

The NCA derivatives **21** of TFM amino acids are obtained in very good yields on heating Z-TFMXaa-OH with PCl_5, diphosgene or thionyl chloride.



16 Z-TFMXaa-OH 21

i) $SOCl_2$, ΔT; ii) H-Yaa-OR[1]

The major disadvantage of the NCA method in classical peptide chemistry is their high tendency towards oligomerization, because the amino group of the peptide formed during the reaction can compete with the amino acid ester component. This problem does not arise on ring opening of NCAs derived from TFM amino acids due to the low pK_a of the newly formed amino function. The NCA derivatives **21** can subsequently be deprotonated and alkylated, providing an efficient route to N-alkyl TFM amino acids.

Hydrolytic and proteolytic stability of Z-TFMXaa-OMe Protease catalyzed peptide synthesis with TFM amino acids

Z-TFMGly-OMe (methyl N-benzyloxycarbonyl-3,3,3-trifluoroalaninate) is very unstable at pH > 7. The presence of an α-proton severely destabilizes the CF_3 group and leads to sequential base-catalyzed HF-elimination. All other TFM amino acids are lacking an α-proton and are therefore stable towards base. Their rate of alkaline ester hydrolysis (pH 9) is decreased considerably. After 20 min at pH 9, only 4% of Z-TFMPhe-OMe are hydrolyzed to the acid, whereas Z-Phe-OMe is hydrolyzed completely after 5 min under the same conditions. This shows that chemical hydrolysis is slowed down by approximately factor 12 upon introduction of a CF_3 group in α-position. Proteases like subtilisin, chymotrypsin or papain accept TFM amino acids only to a very limited extent. Z-TFMGly-OMe is hydrolyzed by these enzymes. Both the hydrolysis rate and the turnover decreases in the row Z-TFMGly-OMe > Z-TFMAla-OMe > Z-TFMPhe-OMe. The latter amino acid is not turned over at all. These data exclude the application of enzyme catalyzed peptide synthesis to TFM amino

acids. However, some dipeptide esters with N-terminal TFM amino acid are accepted as substrates by proteolytic enzymes. H-TFMPhg-Phe-OMe is converted by chymotrypsin or substilisin within 20 min to the tripeptide H-TFMPhg-Phe-Leu-NH_2 in the presence of H-Leu-NH_2 (Burger et al., 1993).

The sequence -Phe-Xaa-Gly-Leu-Met-NH_2 is found at the C-terminus of Tachykinines. In various pentapeptides with this sequence, the biological activity of the Tachykinines is retained. Therefore, several pentapeptides H-TFMXaa-Phe-Gly-Leu-Met-NH_2 (substance P analogues) were synthesized by a combination of enzymatic and chemical methods for evaluation of their biological activity.

Acknowledgements

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