Construction of *N*-1*H*,1*H*-perfluoroalkylated peptide bonds†

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The preparation of a variety of optically pure peptides containing an *N*-1*H*,1*H*-perfluoroalkyl label on a selected backbone amide bond is now possible.

Peptide based drugs suffer from their poor bioavailability and poor proteolytic stability. To overcome these disadvantages, several modifications have been developed, *e.g.* the introduction of unnatural amino acids and the use of peptidomimetics including peptoids.[1] Among these modifications, *N*-alkylation of amino acids and peptide bonds, especially *N*-methylation, has been pursued for decades and continues to be of great interest for many researchers.[2]

To obtain site-specific *N*-alkylation, an amino acid was *N*-alkylated, followed by the subsequent coupling reaction to form the desired peptide bond. To date, many methods,[3] *e.g.* oxazolidinone formation and reduction,[4] reductive amination,[5] temporary protection and activation followed by S_N2[6] or Mitsunobu reaction,[6a,7] *etc.*, have been developed for the *N*-alkylation of amino acids. The more effective coupling reagents, *e.g.* triphosgene, have been used in the coupling reactions of *N*-alkylated amino acids.[8]

Fluoroalkyl groups have certain unique properties. Once attached to biologically active compounds, fluorine(s) can be used as a marker or tracer of the substrates by ^{19}F NMR or ^{18}F PET study.[9] Electron withdrawing fluoroalkyl groups alter the electron density of the adjacent heteroatoms, and hence affect the pK_a value, hydrogen bonding, and lipophilicity of the substrates.[10] Therefore, the introduction of fluoroalkyl groups to the functionalities of amino acids and peptides has been investigated by us for a decade.[11]

In our recent research, the 1*H*,1*H*-trifluoroethyl group, CF_3CH_2–, was introduced onto the *N*-terminus of small peptides to increase the lipophilicity of the substrates.[12] The trifluoroethylated α-amino group did not show enough nucleophilicity towards either the activated carboxyl group in conventional linear peptide coupling reactions or an amino acid fluoride. However, the trifluoroethylated *N*-terminus of a linear dipeptide did undergo intramolecular cyclization reactions to form diketopiperazines.[13] Subsequently, the trifluoroethylated α-amino group of an amino acid ester was deprotonated using a strong base, *e.g.* NaH, and the resulting anionic intermediate exhibited enough nucleophilicity to couple with an N^{α}-phthaloyl protected amino acid fluoride to form a linear peptide bond.[14] However, because of the electronic effect of both the adjacent *N*-trifluoroethylated peptide bond and the activated carboxyl group, the α-carbon of the central amino acid was racemized to give a pair of diastereomers in the process of the *C*-terminal elongation with a third amino acid ester.[14] The phthaloyl protecting group employed in the latter work made deprotection for further elongation at the *N*-terminus difficult due to the formation of diketopiperazines.

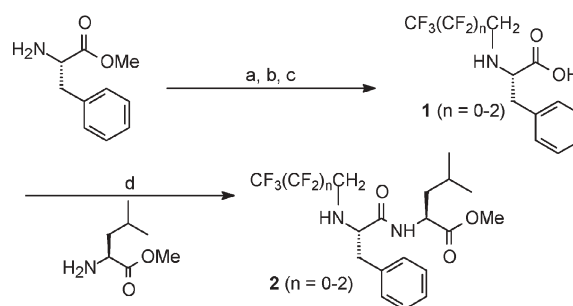
To solve the above racemization problem, we reasoned that the *C*-terminal elongation should be carried out before the formation of the trifluoroethylated peptide bond; if the more reactive acid chloride was used instead of acid fluoride, the deprotonation of the trifluoroethylated α-amino group of the central amino acid using a strong base could be avoided.

In this research, the trifluoroethyl group and its two homologues, $CF_3(CF_2)_nCH_2$– (n = 1, 2), were first introduced to the α-amino group of L-phenylalanine methyl ester using iodonium fluoroalkylating agents.[12] The intermediates **1** were then elongated at the *C*-terminus with a second amino acid ester, *e.g.* L-leucine methyl ester, to form the dipeptides **2**, Scheme 1.

The optically pure model peptide **3** was obtained by coupling dipeptide **2** (n = 0) with N^{α}-phthaloyl glycine acid chloride,[15] in the presence of pyridine as a catalyst and base, Scheme 2.

Introduction of the electron withdrawing trifluoroethyl group facilitates rotation around the corresponding peptide bond. The exchange between *cis* and *trans* isomers of the optically pure model peptide **3** was evidenced by the cross peaks in the ^{19}F NOESY spectrum shown in Fig. 1.

In different solvents, both the chemical shifts and the ratio of the two isomers of **3** changed dramatically. In less polar solvent $CDCl_3$, the chemical shifts of the two isomers shifted upfield with isomer **B** predominating, whereas in the more polar solvent



Scheme 1 *Reagents and conditions*: (a) $CF_3(CF_2)_nCH_2I(C_6H_5)$-$N(SO_2CF_3)_2$ (n = 0–2), $NaHCO_3$, CH_2Cl_2–H_2O, rt, 4 h; (b) 1.0 M NaOH, rt, 16 h, 40 h, and 64 h for n = 0–2; (c) 0 °C, conc. HCl to pH 4.5 (89%, 92%, and 83% for n = 0–2, 3 steps); (d) *N*-(3-dimethylaminopropyl)-*N′*-ethylcarbodiimide (EDC)·HCl, 1-hydroxybenzotriazole (HOBt), *N*,*N*-diisopropylethylamine (DIEA), CH_2Cl_2, 0 °C to rt, overnight (91%, 87%, and 81% for n = 0–2, respectively).

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† Electronic supplementary information (ESI) available: Experimental procedures for the syntheses of **2** (n = 0), **3**, **4** (n = 0) and **5**; NMR and HRMS spectra of **3**, **4**, and **5**. See DOI: 10.1039/b712617d

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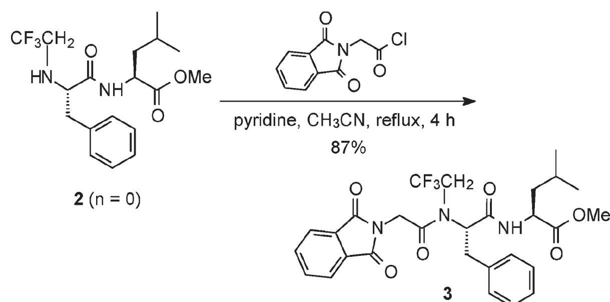


Scheme 2 Synthesis of optically pure model peptide **3**.

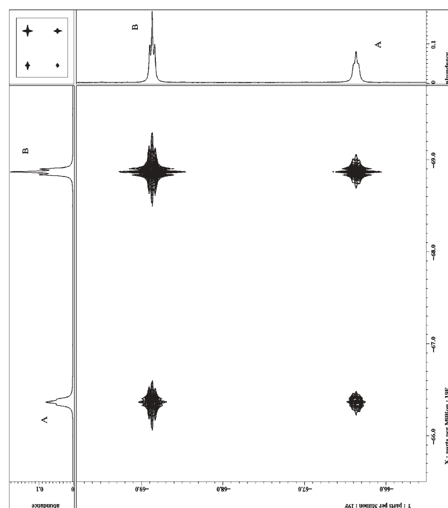


Fig. 1 ^{19}F NOESY spectrum of **3** (282.78 MHz, CD_3CN, 21 °C).

DMSO-d_6, the chemical shifts of the two isomers shifted downfield with isomer **A** predominating, Fig. 2 and Table 1.

More polar solvents favor the charged resonance structure of the *N*-trifluoroethylated peptide bond, Scheme 3. The partial formal positive charge on the nitrogen results in downfield chemical shifts of the two isomers of **3** in DMSO-d_6. The isomer with the CF_3CH_2– group *trans* to the carbonyl oxygen is assigned as isomer **A** with downfield chemical shift based on the fact that the larger electronic interaction is for the *trans* isomer.[16]

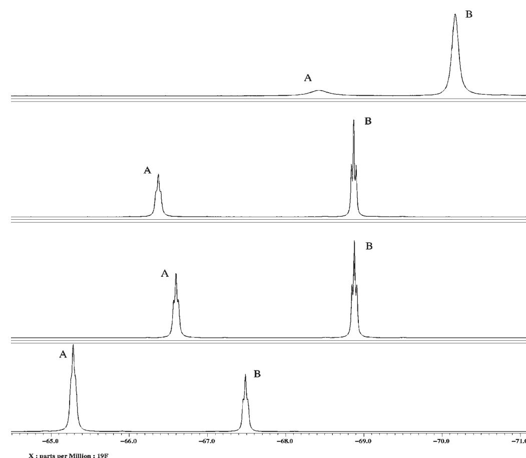
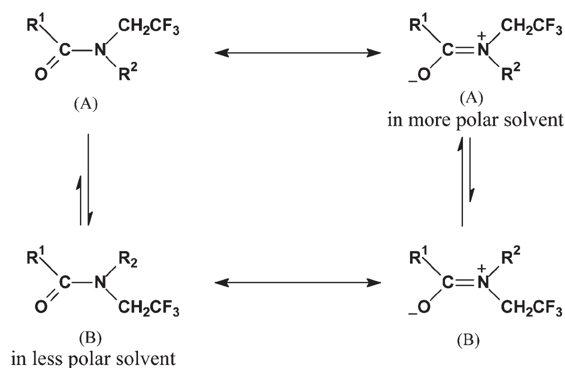


Fig. 2 ^{19}F NMR spectra of **3** in different solvents (282.78 MHz, 21 °C, from top to bottom: $CDCl_3$, CD_3CN, acetone-d_6, and DMSO-d_6).

Table 1 Chemical shifts and ratio of two isomers of **3** in different solvents[a]

Solvent	$CDCl_3$	CD_3CN	Acetone-d_6	DMSO-d_6
δ_F/ppm	−68.41, −70.17	−66.37, −68.87	−66.60, −68.88	−65.28, −67.49
A/B	0.18	0.62	0.77	1.89

[a] The ratio was determined by ^{19}F NMR spectroscopy, Fig. 2.



Scheme 3 Resonance structures and isomer exchanges of the *N*-trifluoroethylated peptide bond in different solvents.

The effect of temperature on the exchange rate between the *cis* and *trans* isomers of **3** is shown in Fig. 3. With increasing temperature, two well separated triplets eventually merged and became one broad singlet. In DMSO-d_6, the two peaks coalesced at 88 °C. The rate constant for the exchange and the free energy of activation for the rotation[17] around the *N*-trifluoroethylated peptide bond were calculated as 1.38×10^3 s^{-1} and 67.4 kJ mol^{-1}, respectively.

To enable the further elongation at the *N*-terminus of the model peptides, Fmoc[15c] was used as the protecting group in the syntheses of **4**. However, in the presence of pyridine as a catalyst and base, N^{α}-Fmoc–GlyCl underwent self-polymerization,

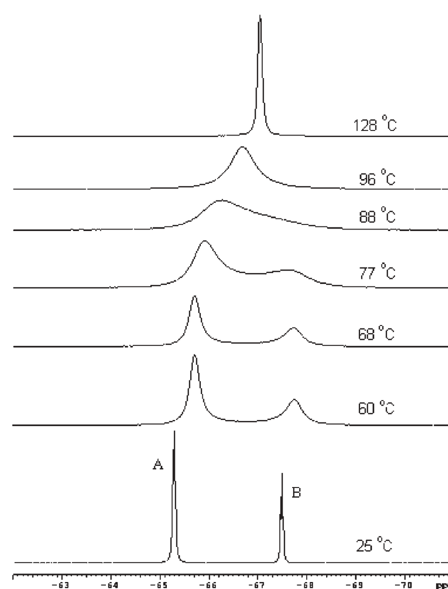
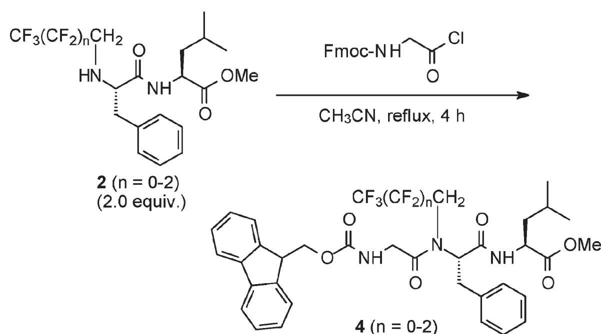
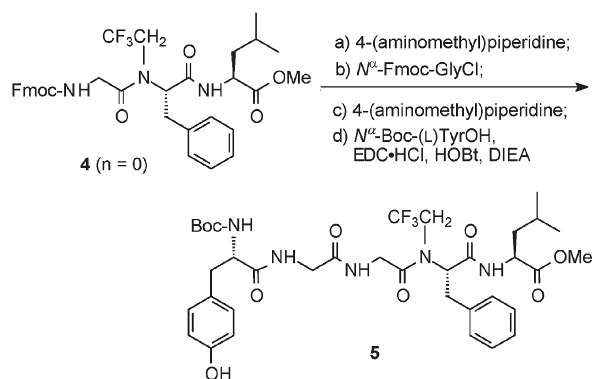


Fig. 3 Temperature effect on the exchange between two isomers of **3** in DMSO-d_6 shown by ^{19}F NMR spectroscopy (282.38 MHz).



Scheme 4 Syntheses of optically pure peptide building blocks **4** in the presence of excess **2** as a base (83%, 84%, and 82% for n = 0–2, respectively).



Scheme 5 Elongation of the peptide building block **4** (n = 0) into a pentapeptide **5** (27%, 4 steps).

especially at elevated temperatures, which compromised the coupling yield. To avoid using pyridine, dipeptides **2** were used in 2.0 equivalents in the coupling reactions to obtain optically pure peptide building blocks **4**, Scheme 4. Excess **2** was easily recovered by chromatography.

The optically pure peptide building blocks **4** all showed *cis* and *trans* isomers with similar solution dynamics to that of **3**.

In the further elongation at the *N*-terminus of peptide building block **4** (n = 0), the Fmoc protecting group was removed using 4-(aminomethyl)piperidine.[15c] Deprotected **4** (n = 0) was converted into an optically pure pentapeptide **5**, leucine enkephalin, containing an *N*-trifluoroethylated peptide bond in the selected position, Scheme 5.

In conclusion, the optically pure peptide building blocks **4** containing an *N*-1*H*,1*H*-perfluoroalkylated backbone amide bond have been synthesized by coupling the N^α-Fmoc-protected amino acid chloride with the excess of *N*-terminus 1*H*,1*H*-perfluoroalkylated peptide fragments. The coupling reaction is straightforward and no racemization is observed. Further elaboration of **4** into **5** clearly indicates the potential of this work for the generation of a variety of strategically labeled *N*-1*H*,1*H*-perfluoroalkyl peptides.

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