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

Changqing Lu and Darryl D. DesMarteau*

Received (in Cambridge, UK) 16th August 2007, Accepted 25th September 2007
First published as an Advance Article on the web 18th October 2007
DOI: 10.1039/b712617d

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.

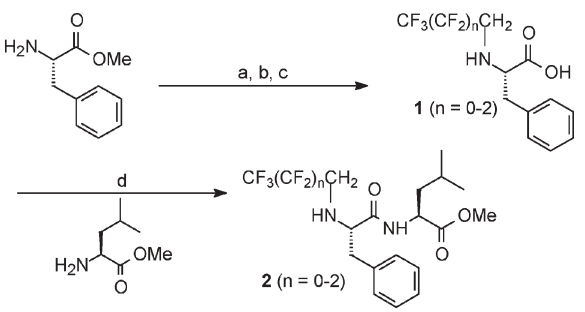
To solve the above racemization problem, we reasoned that the *C*-terminal elongation should be carried out before the formation of the trifluoroethylated peptide bond; if the more reactive acid chloride was used instead of acid fluoride, the deprotonation of the trifluoroethylated α-amino group of the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

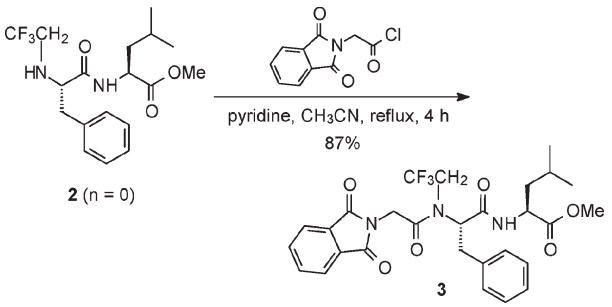
In different solvents, both the chemical shifts and the ratio of the two isomers of **3** changed dramatically. In less polar solvent $CDCl_3$, the chemical shifts of the two isomers shifted upfield with isomer **B** predominating, whereas in the more polar solvent



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).

Department of Chemistry, Clemson University, Clemson, SC 29634, USA. E-mail: fluorin@clemson.edu; Fax: +1 864 656 2545; Tel: +1 864 656 1251

† 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



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

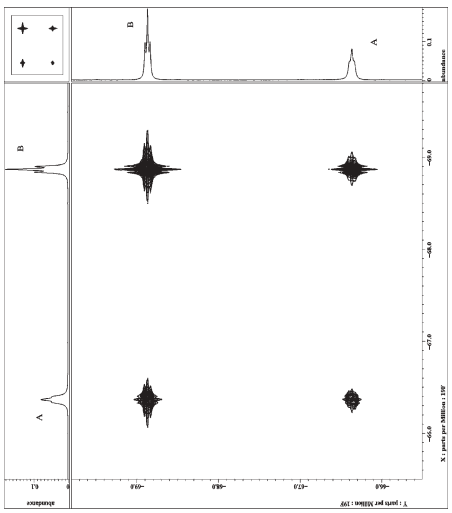


Fig. 1 ^{19}F NOESY spectrum of **3** (282.78 MHz, CD_3CN, 21 °C).

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]

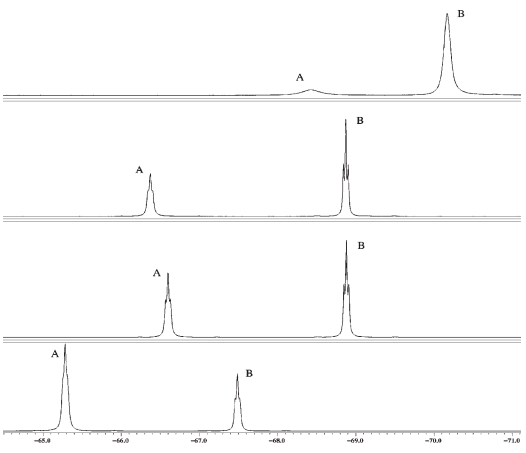
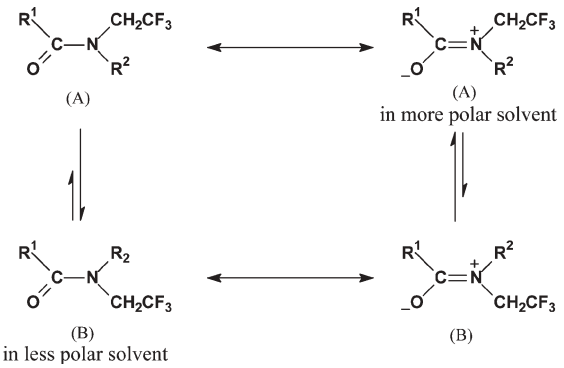


Fig. 2 ^{19}F NMR spectra of **3** in different solvents (282.78 MHz, 21 °C, from top to bottom: $CDCl_3$, CD_3CN, acetone-d_6, and DMSO-d_6).

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,

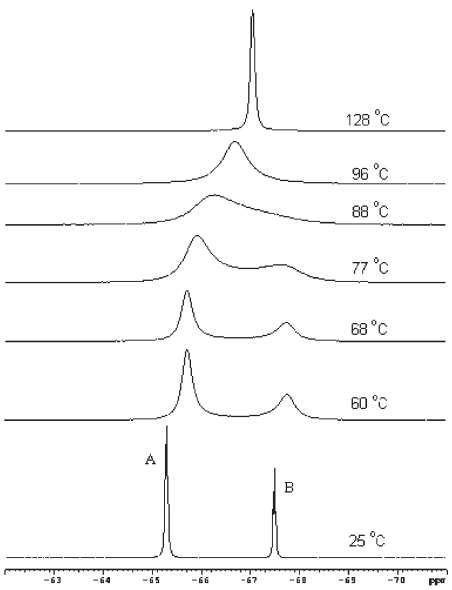
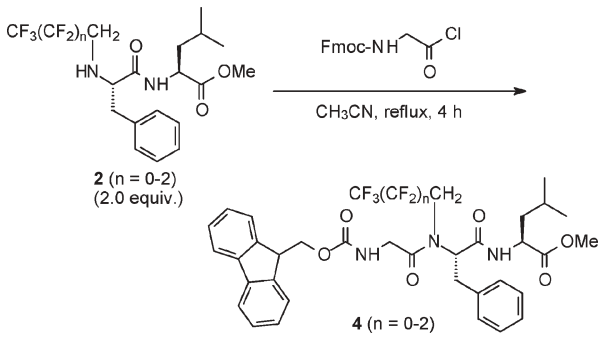
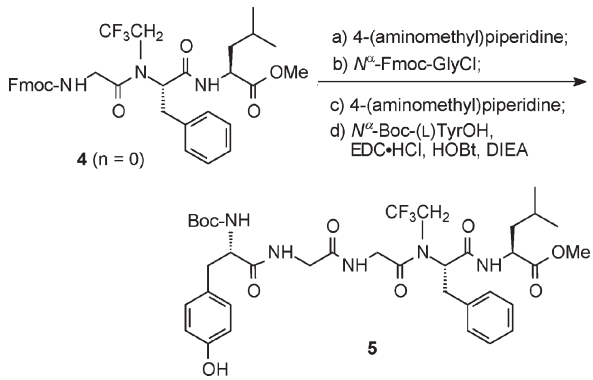


Fig. 3 Temperature effect on the exchange between two isomers of **3** in DMSO-d_6 shown by ^{19}F NMR spectroscopy (282.38 MHz).



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.

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

Notes and references

1 (*a*) R. J. Simon, R. S. Kania, R. N. Zuckermann, V. D. Huebner, D. A. Jewell, S. Banville, S. Ng, L. Wang, S. Rosenberg, C. K. Marlowe, D. C. Spellmeyer, R. Tan, A. D. Frankel, D. V. Santi, F. E. Cohen and P. A. Bartlett, *Proc. Natl. Acad. Sci. U. S. A.*, 1992, **89**, 9367; (*b*) H. Kessler, *Angew. Chem., Int. Ed. Engl.*, 1993, **32**, 543.

2 (*a*) M. Goodman and M. Fried, *J. Am. Chem. Soc.*, 1967, **89**, 1264; (*b*) J. E. Mark and M. Goodman, *J. Am. Chem. Soc.*, 1967, **89**, 1267; (*c*) W. L. Cody, J. X. He, M. D. Reily, S. J. Haleen, D. M. Walker, E. L. Reyner, B. H. Stewart and A. M. Doherty, *J. Med. Chem.*, 1997, **40**, 2228; (*d*) F. Haviv, J. Henkin, D. M. Kalvin and M. F. Bradley, *US Patent* 6777535 B1, 2004; (*e*) M. Teixidó, F. Albericio and E. Giralt, *J. Pept. Res.*, 2005, **65**, 153; (*f*) S. Zhang, S. Prabpai, P. Kongsaeree and P. I. Arvidsson, *Chem. Commun.*, 2006, 497.

3 For a review see: L. Aurelio, R. T. C. Brownlee and A. B. Hughes, *Chem. Rev.*, 2004, **104**, 5823.

4 (*a*) D. Ben-Ishai, *J. Am. Chem. Soc.*, 1957, **79**, 5736; (*b*) R. M. Freidinger, J. S. Hinkle, D. S. Perlow and B. H. Arison, *J. Org. Chem.*, 1983, **48**, 77; (*c*) S. Zhang, T. Govender, T. Norström and P. I. Arvidsson, *J. Org. Chem.*, 2005, **70**, 6918.

5 (*a*) D. H. Coy, S. J. Hocart and Y. Sasaki, *Tetrahedron*, 1988, **44**, 835; (*b*) N. j. Ede, K. H. Ang, I. W. James and A. M. Bray, *Tetrahedron Lett.*, 1996, **37**, 9097; (*c*) A. F. Abdel-Magid, K. G. Carson, B. D. Harrisk, C. A. Maryanoff and R. D. Shah, *J. Org. Chem.*, 1996, **61**, 3849; (*d*) A. K. Szardenings, T. S. Burkoth, G. C. Look and D. A. Campbell, *J. Org. Chem.*, 1996, **61**, 6720; (*e*) A. Nefzi, J. M. Ostresh and R. A. Houghten, *Tetrahedron Lett.*, 1997, **38**, 4943; (*f*) J. Offer, *Tetrahedron Lett.*, 1997, **38**, 9047.

6 (*a*) T. Fukuyama, C.-K. Jow and M. Chueng, *Tetrahedron Lett.*, 1995, **36**, 6373; (*b*) S. C. Miller and T. S. Scanlan, *J. Am. Chem. Soc.*, 1997, **119**, 2301.

7 (*a*) K. Wisniewski and A. S. Kolodziejczyk, *Tetrahedron Lett.*, 1997, **38**, 483; (*b*) J. F. Reichwein and R. M. J. Liskamp, *Tetrahedron Lett.*, 1998, **39**, 1243; (*c*) D. T. S. Rijkers, J. W. M. Höppener, G. Posthuma, C. J. M. Lips and R. M. J. Liskamp, *Chem.–Eur. J.*, 2002, **8**, 4285.

8 (*a*) F. Falb, T. Yechezkel, Y. Salitra and C. Gilon, *J. Pept. Res.*, 1999, **53**, 507; (*b*) B. Thern, J. Rudolph and G. Jung, *Angew. Chem., Int. Ed.*, 2002, **41**, 2307; (*c*) B. Thern, J. Rudolph and G. Jung, *Tetrahedron Lett.*, 2002, **43**, 5013; (*d*) N. Sewald, *Angew. Chem., Int. Ed.*, 2002, **41**, 4661.

9 (*a*) M. E. Bellemann, G. Brix, U. Haberkorn, H. J. Ostertag and W. J. Lorenz, *IEEE Trans. Nucl. Sci.*, 1994, **41**, 2856; (*b*) G. Papeo, P. Giordano, M. G. Brasca, F. Buzzo, D. Caronni, F. Ciprandi, N. Mongelli, M. Veronesi, A. Vulpetti and C. Dalvit, *J. Am. Chem. Soc.*, 2007, **129**, 5665; (*c*) K. Bruus-Jensen, T. Poethko, M. Schottelius, A. Hauser, M. Schwaiger and H.-J. Wester, *Nucl. Med. Biol.*, 2006, **33**, 173; (*d*) E. Kresnik, P. Mikosch, H.-J. Gallowitsch, S. Kohlfürst, I. Igerc and P. Lind, *PET Clinics*, 2006, **1**, 153.

10 (*a*) E. T. McBee, W. F. Marzluff and O. R. Pierce, *J. Am. Chem. Soc.*, 1952, **74**, 444; (*b*) C. W. Roberts, E. T. McBee and C. E. Hathaway, *J. Org. Chem.*, 1956, **21**, 1369; (*c*) P. Ballinger and F. A. Long, *J. Am. Chem. Soc.*, 1959, **81**, 1050; (*d*) A. Leo, C. Hansch and D. Elkins, *Chem. Rev.*, 1971, **71**, 525; (*e*) B. E. Smart, *J. Fluorine Chem.*, 2001, **109**, 3.

11 (*a*) D. D. DesMarteau and V. Montanari, *Chem. Commun.*, 1998, **20**, 2241; (*b*) D. D. DesMarteau and V. Montanari, *Chem. Lett.*, 2000, **29**, 1052; (*c*) D. D. DesMarteau and V. Montanari, *J. Fluorine Chem.*, 2001, **109**, 19.

12 D. D. DesMarteau and C. Lu, *J. Fluorine Chem.*, 2007, **128**, 1326.

13 D. D. DesMarteau and C. Lu, *Tetrahedron Lett.*, 2006, **47**, 561.

14 C. Lu and D. D. DesMarteau, *J. Fluorine Chem.*, 2007, **128**, 832.

15 (*a*) J. C. Sheehan, D. W. Chapman and R. W. Roth, *J. Am. Chem. Soc.*, 1952, **74**, 3822; (*b*) H. Tsubouchi, K. Tsuji and H. Ishikawa, *Synlett*, 1994, 63; (*c*) L. A. Carpino, B. J. Cohen, K. E. Stephens, Jr., S. Y. Sadat-Aalaee, J.-H. Tien and D. C. Langridge, *J. Org. Chem.*, 1986, **51**, 3734; (*d*) L. A. Carpino, M. Beyermann, H. Wenschuh and M. Bienert, *Acc. Chem. Res.*, 1996, **29**, 268.

16 L. A. LaPlanche and M. T. Rogers, *J. Am. Chem. Soc.*, 1963, **85**, 3728.

17 D. H. Williams and I. Fleming, *Spectroscopic Methods in Organic Chemistry*, McGraw-Hill, London, 5th edn, 1995, pp. 104–105.

