

Fluorine containing amino acids: Synthesis and peptide coupling of amino acids containing the all-*cis* tetrafluorocyclohexyl motif

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Abstract: A synthesis of two (*S*)-phenylalanine derivatives is described which have the all-*cis*, 2,3,5,6-tetrafluorocyclohexyl motif attached to the aromatic ring at the *meta* and *para* positions; the *para* substituted isomer is elaborated into illustrative dipeptides *via* the free amine and carboxylate respectively demonstrating its utility as a novel amino acid for peptide synthesis and offering a vehicle for incorporation of this unique and facially polarized ring system into bioactive compounds.

Our laboratory has developed an interest in preparing selectively fluorinated cyclohexane rings, with no more than one fluorine on each carbon and where at least two of the C-F bond lie 1,3-diaxial to each other.¹ Such a diaxial arrangement generates two parallel C-F bonds, and as a consequence of the high polarity of this bond, the arrangement differentially polarises the two faces of the cyclohexane. This creates a large molecular dipole moment and offers unique polar hydrophobic properties to the motif. In order to hold this conformation in the ground state it has been necessary to prepare cyclohexanes with at least four C-F bonds and all with the same 'up' stereochemistry. This was first illustrated by the synthesis of tetrafluorocyclohexanes **1**^{1a} and **2**.^{1b} for these compounds there are always four fluorines on one face of the ring, with two C-F bonds axial and the other two equatorial, even after a ring flip.

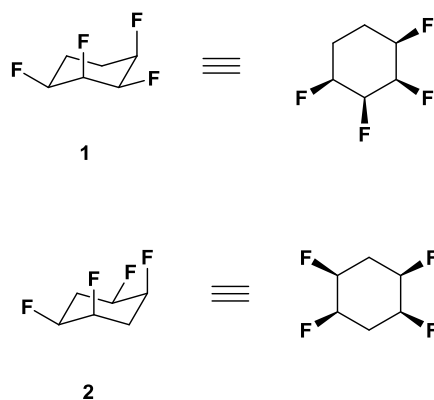
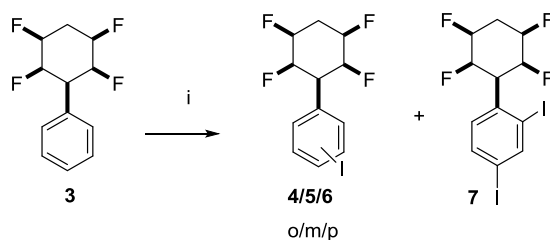


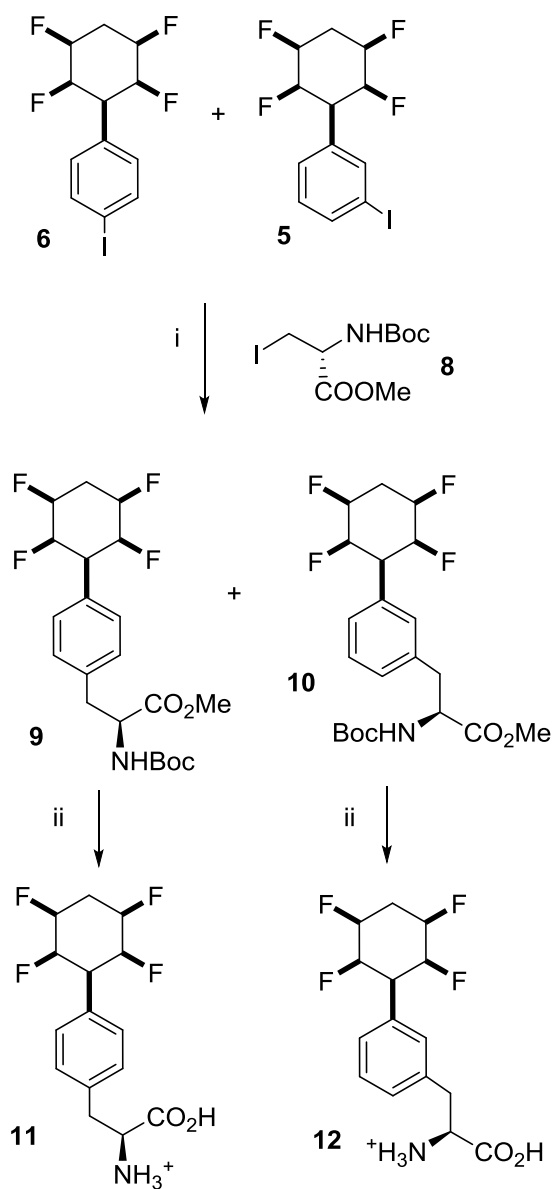
Fig. 1 All-*cis* tetrafluorocyclohexane isomers **1** and **2**.

Of note is the high molecular dipole moments of **1** and **2** (4.9 D and 5.2D respectively)^{1a,1b}. Crystallography reveals that these molecules have a tendency to stack one on top of another consistent with electrostatic compensation in the solid state. In solution the axial hydrogens on the non-fluorous face of the rings display anisotropic shifts by ¹H-NMR when the solvent is changed from dichloromethane to toluene, consistent with the aromatic solvent associating with the electropositive face of the cyclohexane. The interesting properties of these ring systems have challenged us to find relatively accessible building blocks such that they can be incorporated as a substituent in a building block for utility in a diversity of research programmes. To that end we recently described a method for the preparative synthesis of all-*cis* 3-phenyl-1,2,4,5-tetrafluorocyclohexane **3**.^{1c,1d} This compound is amenable to a range of electrophilic aromatic substitution reactions, and some of these transformations have recently been described.^{1c} Selectively fluorinated amino acids have been the focus of much attention² and we were attracted by the prospect of placing this motif into an amino acid as a building block for its inclusion into designer peptides. In this *Communication* we report the synthesis of the new amino acids (*S*)-**11** and (*S*)-**12** carrying the all-*cis* 1,2,4,5,-tetrafluorocyclohexane ring system. One of these (*S*)-phenylalanine derivatives **11** is differentially protected for regioselective elaboration at the carboxy and N-termini to generate dipetides with (*S*)-phenylalanine, and also an amide to isopenicillin-N is also prepared. Our approach to the amino acids came from an initial iodination reaction of **3** in a periodic acid protocol³. This resulted in a mixture of *ortho/meta/para* isomers **4**, **5** and **6** and diiodo **7** in a ratio of 1:5:15:4 respectively.



Scheme 1 Products of the iodination of **3**. i. $\text{HIO}_4 \cdot 2\text{H}_2\text{O}$ (50 %), AcOH , H_2SO_4 , I_2 , H_2O , 70°C 24 h, 92 %.

Chromatography readily removed the minor *ortho* isomer **4** and the diiodo product **7** respectively from the major *meta/para* products **5/6**, however these latter two isomers were proved inseparable. Iodides **5** and **6** were taken as a mixture (ratio 1:3 respectively) and were subjected to Jackson's methodology⁴⁻⁶ for amino acid synthesis *via* Zn homoenolate coupling with the protected (*R*)-iodoalanine **8**^{5,6} as illustrated in Scheme 2. This resulted in an excellent conversion to the fully protected amino acid isomers **9** and **10**, which were readily separated from each other by chromatography. When treated separately, deprotection⁷ of the isomers gave the individual free amino acids (*S*)-**11** and (*S*)-**12**. A crystal of (*S*)-**11** proved amenable to X-ray analysis and the structure is shown in Figure 2. The structure has the expected 1,3-diaxial arrangement of two C-F bonds, which imparts polarity to this ring motif.



Scheme 2 Synthesis of amino acids **11** and **12**: i. Zn, I₂, DMF, **8**, Pd₂(dba)₃, SPhos, 85 %; ii. 6M HCl in 1,4-dioxane, 70 °C, 48 h, 100%.

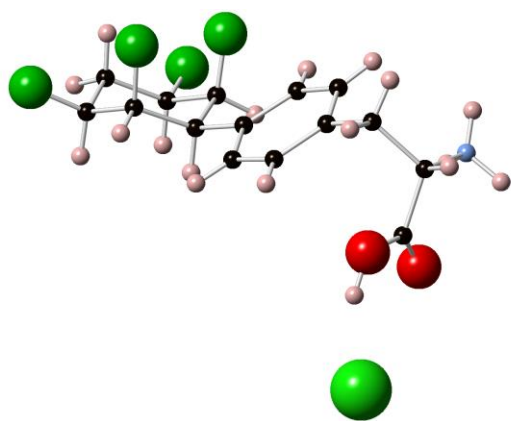
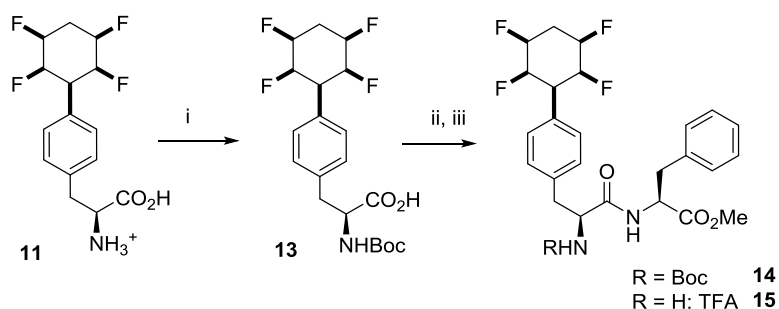


Figure 2 X-Ray structure of amino acid (*S*)-**11** as a HCl salt. (Structure data deposited at the Cambridge Crystallographic Data Centre, CCDC No = 1057152).

In order to explore further elaboration of a tetrafluorocyclohexyl amino acid, peptide coupling of the hydrochloride salt of (*S*)-**11** via the carboxylate, was explored. Thus *N*-Boc protection using di-*t*-butyldicarbonate in THF gave *N*-Boc **13**⁸ which could be coupled with L-phenylalanine-OMe⁹ to generate dipeptide **14** in good yield.

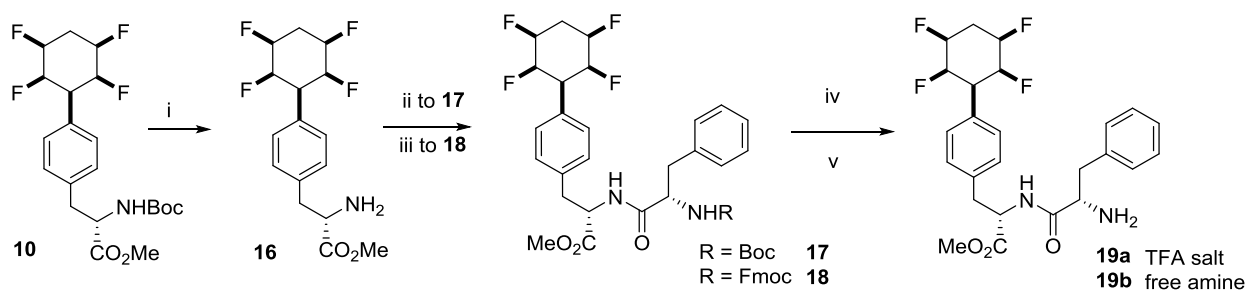


Scheme 3 C-Terminal coupling to generate dipeptide **14** and **15** i. NaHCO₃, THF/H₂O (1:1), (Boc)₂O, rt, overnight 93 %; ii. EDCI, HOBT, NMM, DMF, rt, overnight 87 %; iii. TFA, DCM (1:4), rt, 4h, 94 %.

N-Boc deprotection with trifluoroacetic acid¹⁰ resulted in the dipeptide methyl ester **15** as its trifluoroacetate salt. This sequence is summarized in Scheme 3.

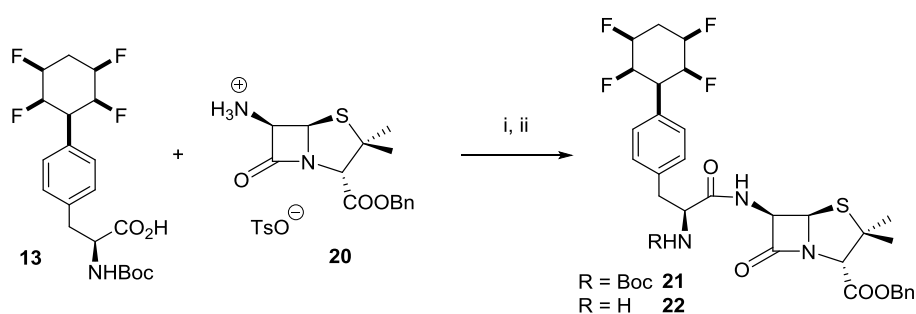
Complementary *N*-terminal coupling was achieved by selective deprotection^{11,12} of amino acid **10** as illustrated in Scheme 4. Treatment of **10** with 4 M HCl in ethyl acetate triggered *N*-Boc deprotection to afford the methyl ester amine hydrochloride salt **16** in quantitative yield. This intermediate was then coupled with either *N*-Boc-L-Phe-OH⁹ or *N*-Fmoc L-Phe-OH¹³ to generate dipeptides **17** and **18** in good yields. Deprotection of **17** with TFA afforded dipeptide

19a as its trifluoroacetate salt, or Fmoc deprotection of **18** with 10 % diethylamine¹⁴ in DMF or (20 % v/v) piperidine¹⁵ in DMF to afford the free NH₂ dipeptide of **19b**.



Scheme 4: *N*-terminal coupling of **16** to generate dipeptide **19**; i. 4 M HCl, EtOAc (1:1), rt, overnight, 100%. ; ii. HOBt, HBTU, NMM, 6h, rt, 81 %; iii. EDCI, HOBt, DMF, DIPEA, rt, overnight, 92 %; iv. **17** TFA, DCM (1:4), rt, 4h, 89 %; v. **18** DEA, DMF, rt, 2h, 94 %, or piperidine in DMF (20% v/v), rt, 2 h, 97%.

To illustrate a level of elaboration of this amino acid moiety, a penicillin derivative was prepared as illustrated in Scheme 5. Thus coupling⁹ of *N*-Boc amino acid **13** with 6-aminopenicillanic acid (APA) benzyl ester **20**¹⁶, gave penicillin dipeptide **21** which could be partially deprotected (4 M HCl in dioxane)¹⁷ to give the novel amino acid acylated penicillin **22** as its hydrochloride salt.



Scheme 5 Preparation of *N*-acylated penicillin derivatives **21** and **22**. i. EDCI, HOBt, DMF, NMM, rt, overnight, 81 %; ii. 4 M HCl, 1,4-Dioxane (1:1), 1 h, 83%.

The tetrafluorocyclohexyl moiety is resistant to acidic conditions and transformations using trifluoroacetic acid and concentrated mineral acid are compatible with the ring system. The ring is also compatible with organic base deprotections such as a 10% diethylamine. However attempts

to mediate aqueous base hydrolysis of the ester moiety of **14** resulted in defluorination and complex product mixtures. Thus elaborations of protecting groups under those conditions are limiting. In conclusion, we have demonstrated the conversion of the tetrafluorophenylcyclohexane **3** into the amino acids **11** and **12** using Jackson's organozinc protocol. These are elaborated derivatives of L-phenylalanine, and present an unexplored polar hydrophobic moiety in an amino acid, amenable to inclusion in peptides. The amino acids were coupled to (*S*)-phenylalanine through both its *C* and *N* terminus, and one was coupled to isopenicillin-*N* to demonstrate an elaboration to a relevant bioactive compound class. The tetrafluorocyclohexane moiety is compatible with acid mediated deprotection strategies, and organic bases, however the ring system is incompatible with aqueous base.

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References

- (a) A. J. Durie, A. M. Z. Slawin, T. Lebl, P. Kirsch, D. O'Hagan, *Chem Commun.*, 2011, **47**, 8265. (b) A. J. Durie, A. M. Z. Slawin, T. Lebl, P. Kirsch, D. O'Hagan, *Chem. Commun.*, 2012, **48**, 9643. (c) A. J. Durie, T. Fujiwara, N. Al-Maharik, A. M. Z. Slawin, D. O'Hagan, *J. Org. Chem.*, 2014, **79**, 8228. (d) A. J. Durie, T. Fujiwara, R. Cormanich, M. Bühl, A. M. Z. Slawin, D. O'Hagan, *Chem. Eur. J.*, 2014, **20**, 6259.
- (a) V. Asante, J. Mortier, G. Wolber, B. Koksich, *Amino Acids*, 2014, **46**, 2733. (b) L. Hunter, S. Butler, S. B. Ludbrock, *Org. Biol. Chem.*, 2012, **10**, 8911. (c) X-L. Qiu, F-L. Qing, *Eur. J. Org. Chem.*, 2011, 3261. (d) Y-L. Liu, T-D. Shi, F. Zhou, X-L. Zhao, X. Wang, J. Zhou, *Org. Lett.*, 2011, **13**, 3826.
- B. A. Hathaway, K. L. White, and M. E. McGill, *Synth. Commun.*, 2007, **37**, 3855.
- R. F. W. Jackson, D. Turner, H. M. Block, *Synlett.*, 1997, **7**, 789.
- A. J. Ross, H. L. Lang, R. F. W. Jackson, *J. Org. Chem.*, 2010, **75**, 245.
- A. J. Ross, F. Dreiocker, M. Schäfer, J. Oomens, A. J. H. M. Meijer, B. T. Pickup, R. F. W. Jackson, *J. Org. Chem.*, 2011, **76**, 1727.
- C. W. Barfoot, J. E. Harvey, M. N. Kenworthy, J. P. Kilburn, M. Ahmed, R. J. K. Taylor, *Tetrahedron*, 2005, **61**, 3403.

8. D. M. Shendage, R. Fröhlich, G. Haufe, *Org. Lett.*, 2004, **6**, 3675.
9. C. Crauste, M. Froeyen, J. Anne, P. Herdewijn, *Eur. J. Org. Chem.*, 2011, 3437.
10. G. W. Anderson, F. M. Callahan, *J. Am. Chem. Soc.*, 1960, **82**, 3359.
11. R. B. Merrifield, *J. Am. Chem. Soc.*, 1963, **85**, 2149.
12. R. B. Merrifield, *Adv. Enzymol.*, 1969, **32**, 221.
13. L. Carpino, H. Imazumi, A. El-Faham, F. J. Ferrer, C. Zhang, Y. Lee, B. M. Foxman, P. Henklein, C. Hanay, C. Mügge, H. Wenschuh, J. Klose, M. Beyermann, M. Bienert, *Angew.Chem. Int. Ed.*, 2002, **41**, 441.
14. C. D. Chang, M. Waki, M. Ahmad, J. Meienhofer, E.O. Lundell, J. D. Haug, *Int. J. Peptide Protein Res.*, 1980, **15**, 59-66.
15. F. Albericio, E. Nicolás, J. Rizo, E. Ruiz-Gayo, E. Pedroso, E. Giralt, *Synthesis*, 1990, 119.
16. M.S. Manhas, K. Gala, S. S. Bari, A. K. Bose, *Synthesis*, 1983, 549-552.
17. G. Han, M. Tamaki, V. J. Hruby, *J. Peptide Res.*, 2001, **58**, 338.