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Graphical Abstract

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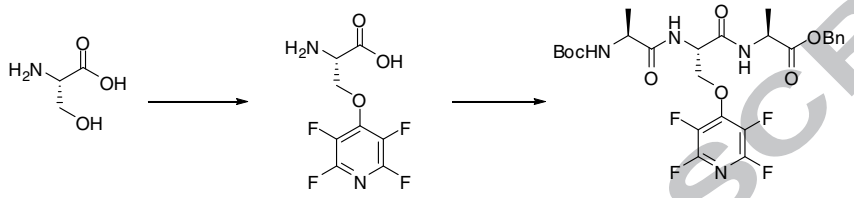

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Synthesis of a novel tetrafluoropyridine-containing amino acid and tripeptide

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ABSTRACT

The synthesis of a novel fluoropyridine-containing amino acid from pentafluoropyridine by a nucleophilic substitution process is reported. The orthogonal protecting groups can be manipulated and the fluoropyridine amino acid incorporated into a tripeptide.

A significant hurdle in the potential clinical application of peptide-based drugs is their inherent *in vivo* susceptibility towards enzymatic degradation.[1] Several different strategies have been developed in an effort to overcome this problem, with one of the most commonly used methods involving cyclisation of amino acid side chains to prepare cyclic peptides.[2,3] The resulting cyclic peptides have been shown, in many cases, to have both enhanced stability and biological properties.[4] Backbone cyclisation can be achieved using proteinogenic amino acids, for example, by forming an amide bond between lysine and glutamic acid residues.[5] Non-proteinogenic amino acids, such allylglycine (**1**) and propargylglycine (**2**), which can be used to form cyclic peptides via ring-closing metathesis or click chemistry respectively, can also be exploited.[6,7] The applications of **1** and **2** offer several advantages over the formation of backbone amide bridges derived from naturally occurring amino acids. In particular, the reactive functionality present in **1** and **2**, that permits the formation of cyclic peptides is orthogonal to standard peptide coupling conditions and thus there is no need to utilize a protecting group strategy. In designing new non-proteinogenic amino acids that can be utilized to prepare backbone cyclized peptides, a suitably reactive functional group must be present within the side chains. The functional group that is selected must be stable to typical protection/coupling/deprotection reaction conditions common in peptide chemistry and offer functionality that has a different reactivity profile, 'orthogonal reactivity', to these processes. This would enable, for example, cyclisation to be carried out at any stage of a multi-step peptide coupling sequence on demand, leaving the growing peptide backbone structurally intact.

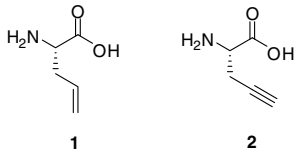

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Figure 1. Structures of non-proteinogenic amino acids commonly used to prepare cyclic peptides.

Perfluoroheteroaromatic systems are very reactive towards nucleophilic attack due to the presence of several highly-electron withdrawing fluorine atoms attached to the heterocyclic ring and many processes involving reactions of, for example, pentafluoropyridine, with a wide range of nucleophiles have been developed.[8,9] Reactions of oxygen- and nitrogen-centered nucleophiles with pentafluoropyridine (**3**) occur, in general, regioselectively at the 4-positon, *para* to the ring nitrogen, to give 4-substituted derivatives which, in turn, may react sequentially with further nucleophilic species to give, in general, 2,4- and 2,4,6-polysubstituted systems, regioselectively (Scheme

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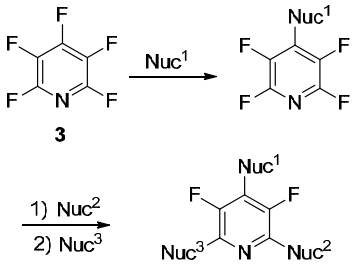
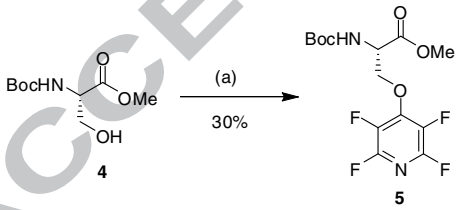
1). This reactivity profile has been utilised recently for the synthesis of a wide range of macrocycles,[10,11] fused ring systems,[12] polyfunctional heterocycles[13,14] and glycosyl donors[15] derived from the pentafluoropyridine scaffold in convenient, high yielding, regioselective processes.



Scheme 1. Regioselective sequential S_NAr reactions of pentafluoropyridine

Given this reactivity profile (Scheme 1), we were eager to investigate if we could access a fluoropyridine-containing amino acid that could be incorporated into linear peptide chains, because, in principle, subsequent reaction with nucleophilic sites on adjacent amino acid side chains could be utilized to prepare novel cyclic peptides. As part of a wider developing research programme in fluorinated peptide systems, we now report reactions between pentafluoropyridine and the amino acid serine, and the subsequent use of the tetrafluorinated residue for the synthesis of a representative tripeptide as the first stage in the synthesis of cyclic peptide derivatives.

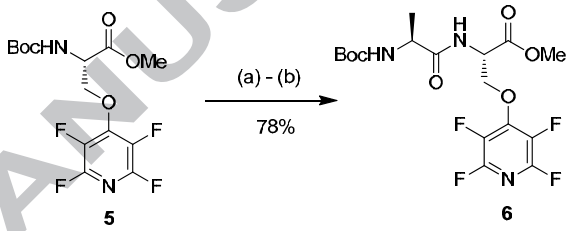
Our aim was to prepare the desired building block with orthogonal protecting groups so that further synthetic manipulations could be carried out selectively on either the amine or acid functionality. Thus, initially, we chose to investigate Boc-Ser-OMe (**4**) as a nucleophile as it can easily be prepared on a large scale. The reaction of **4** with pentafluoropyridine (2.0 eq.) in the presence of potassium carbonate (1.0 eq.) afforded **5** in moderate 30% yield (Scheme 2).



Scheme 2. Synthesis of fluoropyridine amino acid **5**. Reagents and conditions: (a) pentafluoropyridine, K_2CO_3, MeCN, rt, 20 h.

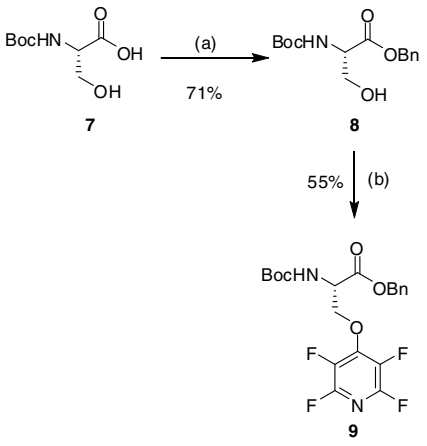
Despite the low yield, the reaction proceeded cleanly and ^{1}H NMR analysis of the crude reaction mixture showed that only starting material **4** and product **5** were present, and these could be easily separated by column chromatography. ^{19}F NMR analysis of **5**, in comparison to literature data,[13] confirmed that nucleophilic substitution had occurred as expected at the 4-positon, *para* to the ring nitrogen. Further, investigation of the reaction conditions showed that increased yields of 45-55% could be obtained if an excess of pentafluorpyridine (4 equiv.) was used.[16] Prolonged reaction times (>72 h) at room temperature, and the use of heating did not improve the recovered yield of **5**.

With **5** in hand we next sought to confirm that the Boc and methyl ester protecting groups could be removed selectively, and that **5** would tolerate standard peptide coupling conditions. To this end, **5** was treated with TFA/ CH_2Cl_2 (1:1, rt, 4 h) and the resulting TFA salt obtained was immediately coupled to Boc-Ala-OH via PyBOP-mediated amide bond formation to give the di-peptide **6** in an excellent yield (78%) (Scheme 3). The fact that **6** was isolated in a good yield confirmed that the fluoropyridyl group was stable to both the acidic conditions required for removal of the Boc group and also the PyBop mediated-peptide coupling conditions used. Furthermore, both ^{1}H and ^{13}C NMR spectra gave no indication of the presence of diastereoisomers indicating that the formation of **5** does not affect the stereochemistry at the alpha-carbon of the amino acid substrate. Attempts to remove the methyl ester protecting group from **6** using standard reaction conditions (e.g. aqueous base / MeOH, rt) proved to be problematic and several degradation products were observed.



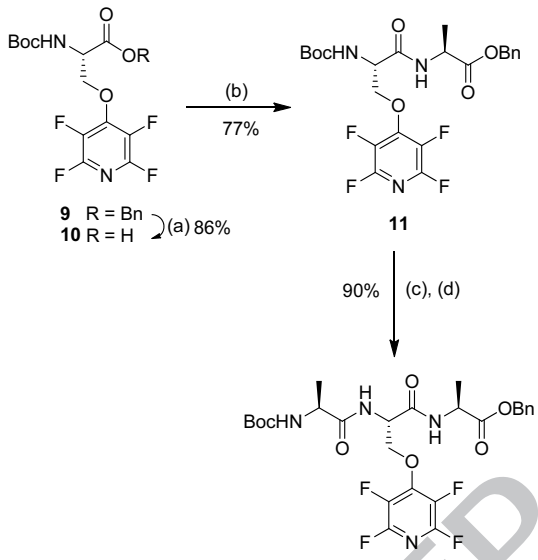
Scheme 3. Synthesis of dipeptide **6**. Reagents and conditions: (a) TFA, CH_2Cl_2, rt, 4 h (b) Boc-Ala-OH, PyBop, NMM, CH_2Cl_2, rt, 18 h.

Given the instability of **6** to basic hydrolysis of the methyl ester, an alternative protecting group strategy was investigated. In order to retain protecting group orthogonality, we chose to investigate the suitability of the benzyl ester protecting group. Boc-Ser-OBn (**8**), which can be prepared in excellent yield (71%) from commercially available Boc-Ser-OH (**7**), was reacted with pentafluoropyridine utilizing the reaction conditions previously developed for the preparation of **5**. After purification by column chromatography, amide **9**[17] was obtained in moderate yield (55%) (Scheme 4). Again ^{19}F NMR analysis of **9** confirmed that the nucleophilic substitution had occurred, as expected, at the 4-position, *para* to the ring nitrogen [δ -89.84 (2F, m, F-2), -158.22 (2F, m, F-3)].



Scheme 4. Synthesis of fluoropyridine amino acid **9**. Reagents and conditions: (a) BnBr, $NaHCO_3$, H_2O, EtOAc, rt, 16 h, (b) pentafluoropyridine, K_2CO_3, MeCN, rt, 20 h.

With **9** in hand we sought to extend the peptide chain in both directions and prepare a model tripeptide. Firstly, removal of the benzyl ester was achieved via Pd/carbon-catalyzed hydrogenation in an excellent yield (86%). PyBop-mediated coupling of the resultant free acid **10** to NH_2-Ala-OBn afforded the dipeptide **11** in excellent yield (77%). Finally, removal of the Boc protecting group under acidic conditions and subsequent coupling to Boc-Ala-OH afforded the tripeptide **12**,[18] demonstrating that the tetrafluoropyridine side-group was able to tolerate a typical peptide coupling reaction protocol (Scheme 5).



Scheme 5. Synthesis of a fluoropyridine-containing tripeptide **12**. Reagents and conditions: (a) H_2, Pd/carbon, rt (b) NH_2-Ala-OBn, PyBop, NMM, CH_2Cl_2, rt, 18 h (c) TFA, CH_2Cl_2, rt, 4 h (d) Boc-Ala-OH, PyBop, NMM, CH_2Cl_2, rt, 18 h.

In conclusion, this work describes the synthesis of two orthogonally protected amino acids that contain a tetrafluoropyridine functionality on the side chain (**5** and **9**). The tetrafluoropyridine group was found to be stable to manipulations of both the Boc and Bn protecting groups, but the attempted removal of a methyl ester resulted in degradation of the starting material. Incorporation of the tetrafluoropyridine amino acid **9** into a test tripeptide demonstrated the stability of the tetrafluoropyridine amino acids to standard peptide coupling reaction conditions. Efforts are now underway to investigate the potential applications of **9** in the synthesis of backbone-cyclized peptides.

Acknowledgments

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Supplementary Material

Supplementary data associated with compounds **5**, **9**, and **12** can be found, in the online version.

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16. Preparation of **5**: Pentafluoropyridine (**3**) (0.7 mL, 6.2 mmol) was added via a syringe to a stirred solution of BocNH-Ser-OMe (0.34 g, 1.5 mmol) and K_2CO_3 (0.20 g, 1.5 mmol) in MeCN (30 mL). The mixture was stirred at rt for 20 h. Filtration followed by removal of the solvent under reduced pressure yielded the crude product, which was purified by column chromatography (hexane-EtOAc, 9:1) to give **5** as a white powder (0.27 g, 48%). R*f* = 0.5 (hexane – EtOAc, 2:1); $[\alpha]_D^{23.9}$ = +26.90 (*c* 1.0, $CHCl_3$); m.p. 40-42 °C; IR ν_{max}/cm^{-1} 1644, 1678, 1730, 2992, 3362; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s, C(C$\underline{H}_3$)$_3$), 3.81 (3H, s, OC$\underline{H}_3$), 4.68 (1H, m, α-C$\underline{H}$CH$_2$OArF), 4.80 (2H, m, β-C$\underline{H}_2$OArF), 5.48 (1H, d, *J* 7.4 Hz, BocN$\underline{H}$); ^{13}C NMR (176 MHz, $CDCl_3$) δ 28.2 (3C, s, C($\underline{C}$H$_3$)$_3$), 53.1, 54.0, 74.4 (1C, s, $\underline{C}$H$_2$), 81.5, 135.0 (2C, dm, $^1J_{CF}$ 257 Hz, C-3), 144.1 (2C, dm, $^1J_{CF}$ 243 Hz, C-2), 146.8 (1C, m, C-4), 154.8, 169.3; ^{19}F NMR δ (376 MHz, $CDCl_3$) -89.81 (2F, m, F-2), -158.23 (2F, m, F-3); *m/z* (ESI$^+$) 391.09 ([M + Na]$^+$, 100%); HRMS (ESI$^+$) $C_{14}H_{16}N_2O_5F_4Na^+$ ([M + Na]$^+$); requires 391.0893; found 391.0893.
17. Preparation of **9**: To a stirred solution of BocNH-Ser-OBn (**8**) (1.20 g, 4.3 mmol) and K_2CO_3 (0.60 g, 4.3 mmol) in MeCN (40 mL) was added pentafluoropyridine (**3**) (1.9 mL, 17.2 mmol). The mixture was stirred at rt for 20 h before the solution was filtered and the solvent removed under reduced pressure. Purification by column chromatography (hexane-EtOAc, 9:1) gave **9** (0.95 g, 55%); as a white powder. R*f* = 0.23 (hexane – EtOAc, 9:1); m.p. 50-51 °C; $[\alpha]_D^{29.8}$ = +7.22 (*c* 1.0, $CHCl_3$); IR ν_{max}/cm^{-1} 1681, 1728, 2984, 3356; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.44 (9H, s, C(C$\underline{H}_3$)$_3$), 4.71 (1H, m, α-C$\underline{H}$CH$_2$OArF), 4.81 (2H, m, β-C$\underline{H}_2$OArF), 5.23 (2H, dd, *J* 32.7, 12.0 Hz, C$\underline{H}_2$Ph), 5.56 (1H, d, *J* 7.8 Hz, BocN$\underline{H}$), 7.31 (5H, m, Ph); ^{13}C NMR (176 MHz, $CDCl_3$) δ 27.2 (3C, s, C($\underline{C}$H$_3$)$_3$), 53.1, 66.9, 73.4, 79.7, 127.0, 127.9, 132.08 – 135.5 (2C, dm, $^2J_{CF}$ 257 Hz, C-3), 133.8, 143.0 (1C, dm, J_{CF} 242.4 Hz, C-2), 145.6 (1C, m, C-1), 154.1, 167.7; ^{19}F NMR (376 MHz, $CDCl_3$) δ -89.84 (2F, m, F-2), -158.22 (2F, m, F-3); *m/z* (ESI$^+$) 467.8 ([M + Na]$^+$, 100%); HRMS (ESI$^+$) $C_{20}H_{20}N_2O_5F_4Na^+$ ([M + Na]$^+$); requires 467.1206; found 467.1194.
18. Preparation of **12** from dipeptide **11**: **Boc Deprotection (i):** TFA (xs, 4 mL) was carefully syringed into a solution of BocNH-SerArF-Ala-OBn (**11**) (0.12 g, 0.23 mmol) in CH_2Cl_2 (4 mL) and left to stir for 4 h.

Et_2O was added and subsequently evaporated (4 x 30 mL) to remove excess TFA. **Peptide Coupling (ii):** The deprotected dipeptide was dissolved in CH_2Cl_2 (10 mL) and stirred at room temperature. To this solution BocNH-Ala-OH (0.044 g, 0.23 mmol) and PyBOP (0.12 g, 0.23 mmol) were added. NMM (0.08 mL, 0.69 mmol) was slowly added and the mixture was left to stir for 18 h at rt. The solvent was evaporated and the crude material was purified by column chromatography (hexane-EtOAc, 7:3). The product BocNH-SerArF-Ala-OBn (**11**) was isolated (0.13 g, 90%); as a white powder. R*f* = 0.61 (hexane-EtOAc, 1:1); IR ν_{max}/cm^{-1} 1477, 1509, 1643, 2954, 2998, 3304; ^{1}H NMR (600 MHz, $CDCl_3$) δ 1.37 (3H, d, *J* 7.1 Hz, CH_3), 1.40 (9H, s, $C(C\underline{H}_3)_3$), 1.42 (3H, d, *J* 7.2 Hz, CH_3), 4.15 (1H, m, α-CH), 4.54 - 4.58 (2H, m, α-CH, β-C<u>H</u>OArF), 4.89 (1H, m, α-C<u>H</u>CH_2OArF), 4.93 (1H, d, *J* = 6.5 Hz, N<u>H</u>Boc), 5.02 – 5.07 (1H, m, β-C<u>H</u>OArF), 5.07 – 5.17 (2H, m, $C\underline{H}_2$Ph), 7.22 (1H, d, *J* = 7.5 Hz, NH), 7.27 – 7.33 (5H, m, Ph); ^{13}C NMR (151 MHz, $CDCl_3$) δ 17.7, 28.1, 29.7, 48.7, 50.9, 52.5 , 67.1, 73.5, 80.8, 128.0, 128.3, 128.5, 134.8 (2C, dm, $^1J_{CF}$ 257 Hz, C-3), 135.2, 142.9 – 145.0 (2C, dm, J_{CF} 242.4 Hz, C-2), 146.5 (1C, m, C-1), 155.9, 167.6, 171.9, 172.9; ^{19}F NMR (376 MHz, $CDCl_3$) δ -89.84 (2F, m, F-2), -158.27 (2F, m, F-3); *m/z* (ESI^+) 609.3 ($[M + Na]^+$, 100%); HRMS (ESI^+) $C_{26}H_{30}N_4O_7F_4Na^+$ ($[M + Na]^+$); requires 609.1948; found 609.1948;