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A mild method for the synthesis of a novel dehydrobutyrine-containing amino acid☆

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ABSTRACT

Dehydrobutyrine is an amino acid that is present in a range of peptide natural products. Reaction of pentafluoropyridine with threonine and subsequent E1cb-type elimination allowed the preparation of novel dehydrobutyrine-containing amino acids under mild conditions.

1. Introduction

Dehydroamino acids such as dehydroalanine (Dha, **1**) and dehydrobutyrines (Dhb, **2a** and **2b**) are naturally occurring amino acids that are present in a range of peptides[1] including antimicrobial peptides of the lantibiotic family.[2] In lantibiotics such as nisin A, dehydroalanine (**1**) and dehydrobutyrine (**2a** and **2b**) react via Michael addition with cysteine residues to form lanthionine and methyl lanthionine thio-ether bridges, respectively.[2d] In nature the site-specific introduction of (**1**) and (**2**) into peptide chains arises due to enzymatic post-translational modifications, in which either serine or threonine residues are dehydrated by phosphorylation followed by β-elimination.[3] Recently, dehydroamino acids have also served to provide synthetic handles, enabling a highly useful route for tagging both short peptides and proteins.[4] The formation of dehydrolanine (**1**) can be readily achieved from cysteine both as the free amino acid[5] and within larger protein structures via a variety of reaction conditions.[4b,6] The synthesis of both dehydrobutyrine isomers (**2a** and **2b**) is possible from threonine using a range of reaction conditions.[7] Often the formation of **2a** or **2b** from threonine requires harsh reaction conditions, and generally involves electron-withdrawing substituents, e.g., Boc or nosyl, at the amino group of the amino acid to increase the acidity of the α-H.[8] Dehydrobutyrines can also be stereospecifically prepared by oxidative elimination of phenyl-selenocysteines. However, this requires several synthetic transformations from commercially available threonine prior to the elimination step[9] (Fig. 1).

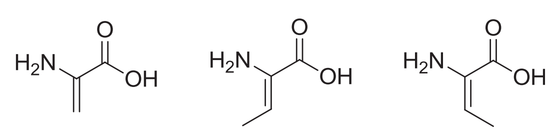

Fig. 1. Dehydroalanine (**1**) and dehydrobutyrines (**2a**) and (**2b**).

Herein, we report a mild one-pot method for the synthesis of a novel dehydrobutyrine-containing amino acid.

2. Results and discussion

Recently we have shown that pentafluoropyridine (**3**) reacts with orthogonally protected serine derivatives (e.g., **4** and **5**) as a route to generating novel peptide building blocks (**6** and **7**) (Scheme 1).[10] As part of this work, it was observed that under certain reaction conditions, typically an excess of base, dehydroalanine derivatives (**8** and **9**) were also formed as by-products. The formation of **8** and **9** arises due to abstraction of the acidic α-H and elimination of the stable 4-(tetrafluoropyridine)-oxide leaving group (**10**). In an effort to expand and exploit this observation to access a range of novel dehydrobutyrines potentially

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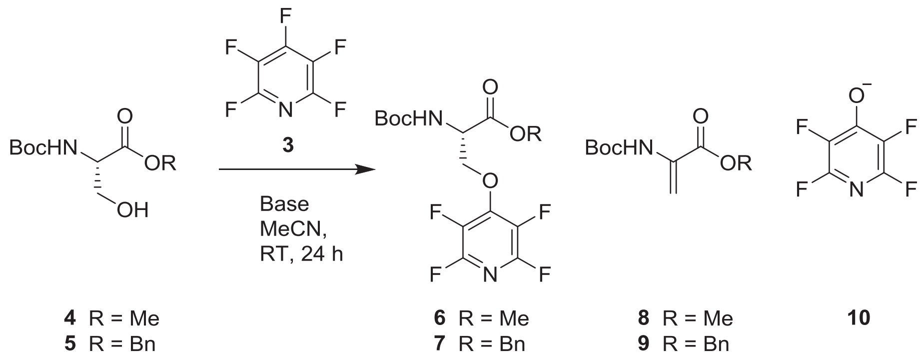
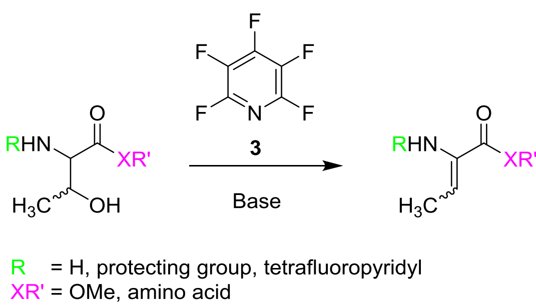


Scheme 1. Elimination to afford dehydroalanines as a by-product.

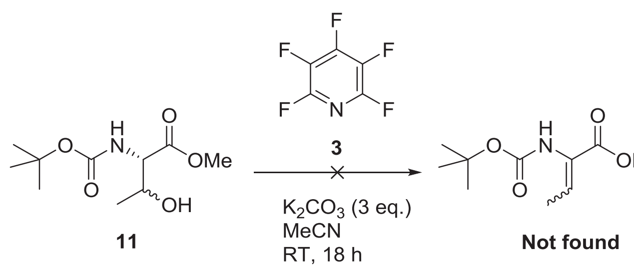
useful in peptide chemistry, we investigated the reaction between pentafluoropyridine (**3**) and various threonine derivatives (Scheme 2).



Scheme 2. General scheme for conversion of threonine derivatives in to dehydrobutyrines.

The treatment of Boc–Thr–OMe (**11**) with **3** (2 molar equiv) in the presence of K_2CO_3 (3 molar equiv) at room temperature did not yield any elimination products (Scheme 3).

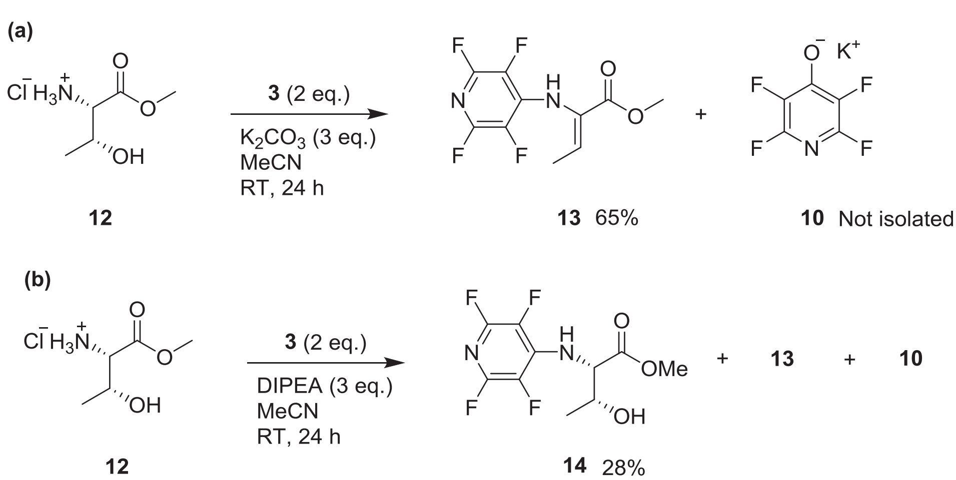
It was envisaged that the introduction of a more electron-withdrawing group on the amine, e.g., 4-tetrafluoropyridyl rather than Boc, would make the α-hydrogen more acidic and thereby promote the desired elimination. As such, under the above conditions, the reaction between mono-protected threonine methyl ester **12** and pentafluoropyridine (**3**) afforded the single elimination product 4-(*N*-tetrafluoropyridine)-Dhb-OMe (**13**) exclusively and salt **10** (Scheme 4a). ^{1}H NMR analysis of the crude reaction mixture showed no signals in the α-H region (δ 4.00–6.00) and a quartet at δ 6.76–6.85 (*J*=7.2 Hz), which was consistent with the formation of



Scheme 3. Reaction of Boc–Thr–OMe (**11**) with pentafluoropyridine (**3**).

a derivative of **2**.[7] Purification involved an initial filtration step to remove K_2CO_3 from the mixture, after which the dehydrobutyrine **13**, which was found to be stable on silica, could be easily separated from the leaving group anion **10** by column chromatography. Initially, **13** was obtained as a white crystalline solid in a modest 25% yield. In an attempt to account for the lower than expected recovered mass, the filtration step was replaced with partitioning between $CHCl_3$ and water, affording pure product **13**. By modification of the work up conditions the isolated yield was considerably improved to 65% and avoided the necessity for column chromatography.

Replacement of K_2CO_3 with *N*,*N*-diisopropylethylamine (DIPEA), afforded two products, which were distinguishable by ^{1}H and ^{19}F NMR analysis of the crude reaction mixture (Scheme 4b). Following isolation, the products were identified as *N*-substituted tetrafluoropyridine-L-threonine methyl ester (**14**, 28%) and dehydrobutyrine **13**. It therefore appeared that under these conditions the reaction failed to reach completion and that compound **14**,



Scheme 4. Reaction of L-threonine methyl ester (**12**) with pentafluoropyridine (**3**).

arising by substitution on the nitrogen of the threonine prior to the substitution on the β-hydroxyl group, is an intermediate required for the transformation into dehydrobutyrine **13**. It was considered that this may be present due to loss of volatile pyridine **3** during the reaction. Returning to using potassium carbonate as the base, but this time adding a total of 6 equiv of pyridine **3** in aliquots of 2 equiv/day over 3 days did not improve the conversion to product **13**. However, under these reaction conditions the putative intermediate **15** was formed. This prompted the suggestion that the elimination mechanism proceeded via disubstituted threonine methyl ester intermediate **15**; the structure of which was confirmed by X-ray crystallography (Fig. 2). Moreover, the X-ray structure of intermediate **15** clearly shows that in the crystal form at least, the α-H and the leaving group have the requisite antiperiplanar relationship needed to undergo elimination.

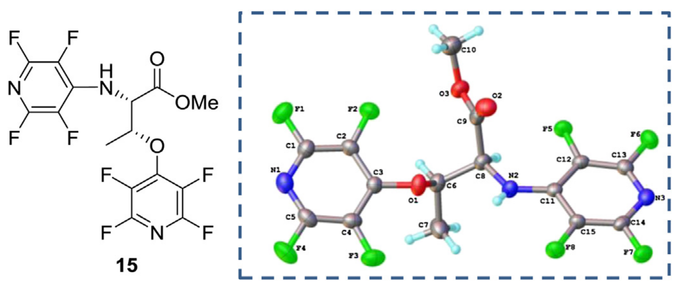


Fig. 2. Structure and X-ray structure of isolated intermediate **15**.

The mechanism of the formation of dehydrobutyrine **13** is, therefore, hypothesised to proceed by sequential substitution reactions of pentafluoropyridines; by reaction of first the nitrogen nucleophile to give intermediate **14** and then the β-oxygen nucleophile, affording intermediate **15** followed by elimination (Scheme 5). Whilst the first addition probably occurs rapidly, the second seems to follow rather more slowly, given the isolation of intermediate **15**, reflecting the lower nucleophilicity of O compared to N and steric effects. The elimination step would be expected to ensue relatively quickly once the leaving group has been installed, due to ready deprotonation of the acidic α-hydrogen present in intermediate **15**.[11]

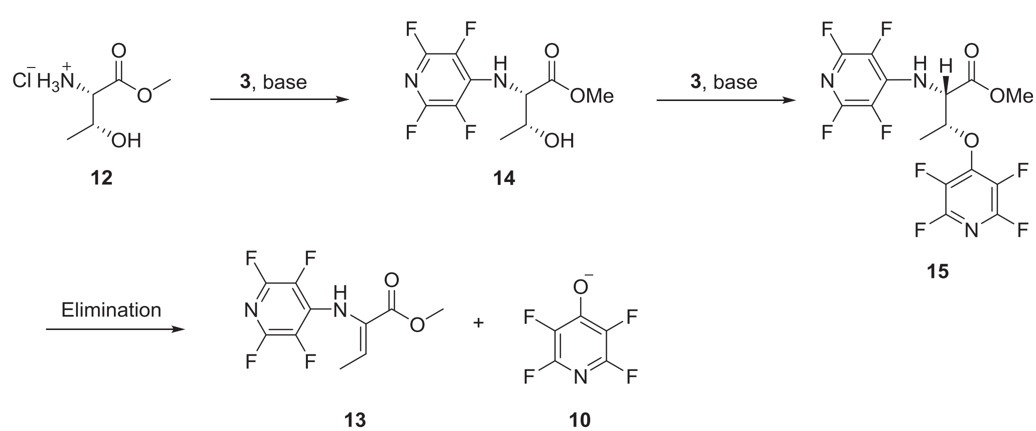
the predicted stereochemistry of dehydrobutyrine **13** was further provided by single crystal *X*-ray crystallography, which clearly shows the *Z*-configuration of the alkene (Fig. 3).

The mechanism for the formation of the alkene products was initially predicted to proceed via a concerted E2 elimination of the alkoxide from the doubly substituted fluoropyridine adduct **15**. This is reported to be the process by which dehydroalanines may be prepared synthetically.[7] However, the additional α-C anion stabilising effects of flanking electron-withdrawing groups in intermediate **15**, i.e., a methyl ester and a 4-tetrafluoropyridylamine group, not only increases the rate of elimination but may also serve to favour a possible E1cb mechanism. E1 elimination was considered to be improbable due to the required formation of a relatively unstable 2° carbocation at the β-position.

In order to define the exact mechanism by which elimination takes place, an experiment involving L-*allo*-threonine methyl ester (**16**) was carried out. Fig. 4 shows that in the case of the E2 mechanism, the geometry of the resulting alkene is influenced by the configuration of the starting threonine derivative. Enantiomers, e.g., L-Thr–OMe and D-*allo*-Thr–OMe are expected to give rise to the same alkene, whereas diastereomers, e.g., L-*allo*-Thr–OMe and D-Thr–OMe should afford the opposite alkene geometry when the required antiperiplanar arrangement is adopted with respect to leaving group and the α-H. The result of a concerted E2 elimination from the *N*,*O*-disubstituted adduct would be predicted in the case of L-*allo*-Thr–OMe to afford the *E*-alkene as shown by the Newman projections in Fig. 4.

An elimination reaction was therefore performed on L-*allo*-Thr–OMe (**16**) using the same reaction conditions that converted the diastereomer, L-Thr–OMe (**12**), in to the *Z*-isomer (**13**). Three compounds were isolated from the product mixture: a dehydrobuytrine, the *N*-substituted adduct (**17**) and the *N*,*O*-disubstituted adduct (**18**) (Scheme 6). Surprisingly, the only dehydrobutyrine product obtained was confirmed to be the *Z*-isomer of dehydrobutyrine **13** by ^{1}H NOESY NMR analysis and X-ray crystallography. This result ruled out the anticipated E2 elimination mechanism, which would have generated the *E*-isomer of dehydrobutyrine **13**.

The findings from the elimination reaction involving compound **16** are, however, consistent with the E1cb mechanism shown in Scheme 7. Removal of the acidic α-H would afford a relatively sta-



Scheme 5. Formation of dehydrobutyrine from L-threonine methyl ester.

In order to determine, which geometric isomer (**13**) was obtained from the elimination reaction a ^{1}H NOESY NMR experiment was performed. This showed a correlation between the amino group NH and the β-CH_3, whilst no coupling was visible with the β-H, indicating that only the *Z*-isomer was formed. Confirmation of

bilised carbanion, leading to a mixture of products arising from the *anti*-elimination from diastereomers L-*allo*-Thr and D-*allo*-Thr if an E2 elimination mechanism predominated (Fig. 4). Alternatively, the observed outcome may be explained by resonance stabilisation by the carbonyl group (well documented for E1cb), which affords

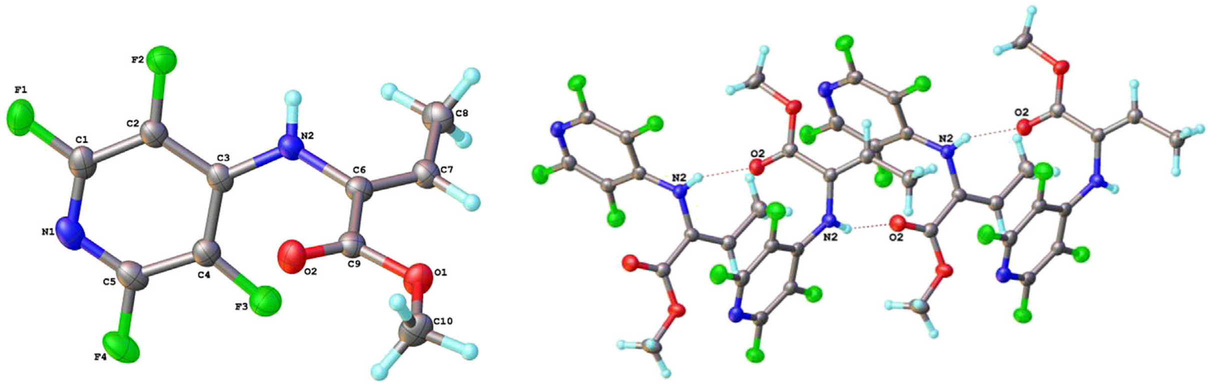


Fig. 3. Molecular structure showing 50% probability anisotropic displacement ellipsoids (*left*) and crystal packing arrangement (*right*) of dehydrobutyrine **13**.

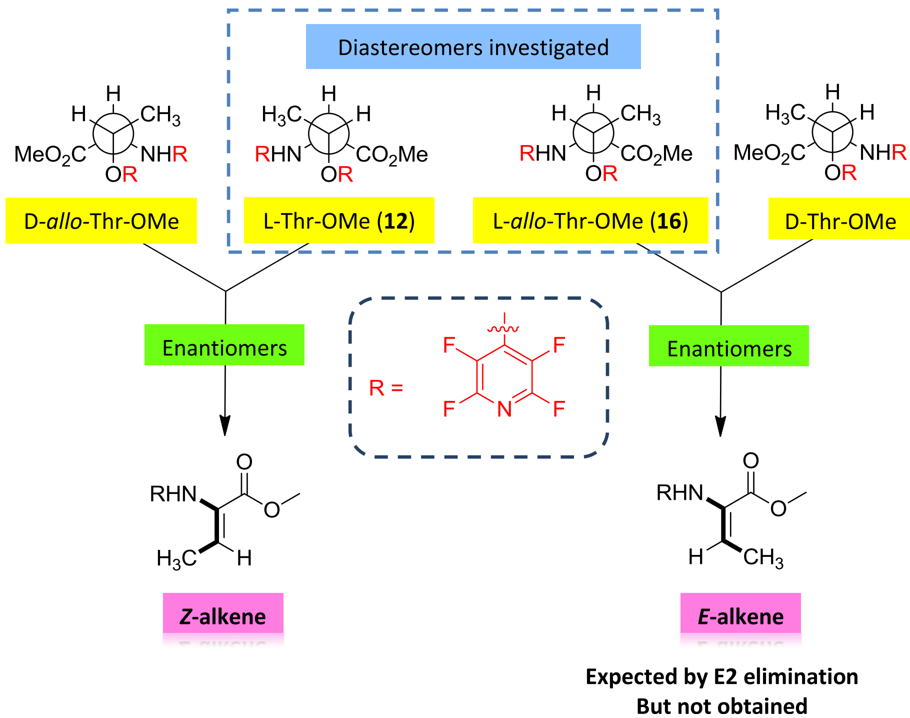
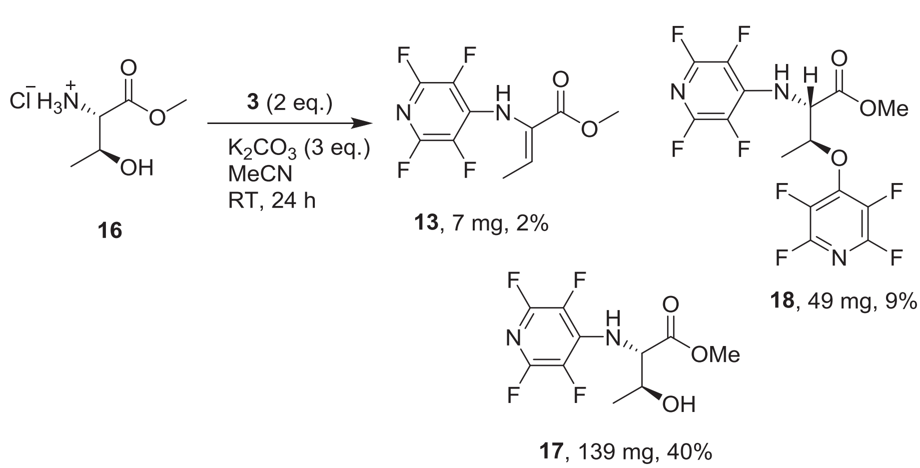
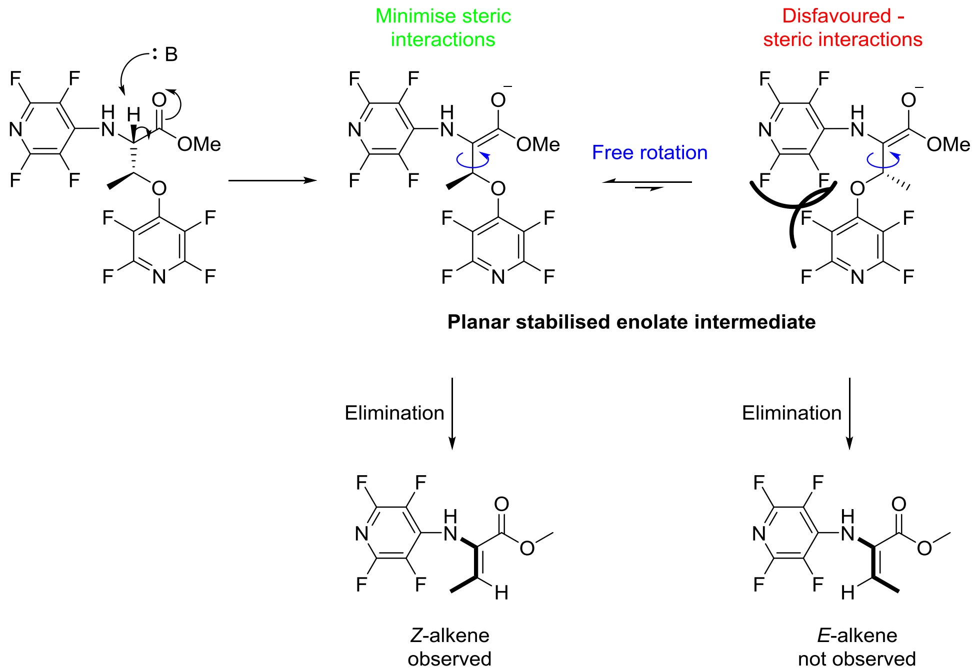


Fig. 4. Newman projections of the enantiomers and diastereomers of threonine methyl ester in configuration for E2 elimination and the expected stereochemical outcome.



Scheme 6. Reaction of L-*allo*-threonine methyl ester (**16**) with pentafluoropyridine (**3**).



Scheme 7. Proposed explanation for observed *Z*-selective alkene formation by E1cb mechanism.

a planar system about the α-C, whilst free rotation is permitted about the C_α–C_β bond. Minimisation of steric interactions between bulky fluoropyridine groups situates the β-CH_3 *syn* to the NHAr group and subsequent elimination affords the *Z*-isomer (Scheme 7).

3. Conclusions

The reaction of pentafluoropyridine with Boc–Thr–OMe (**11**) in the presence of a base was not found to generate any elimination products. However, the reaction of mono-protected threonine (methyl ester, **12**) under the same reaction conditions allowed the stereo-selective preparation of a novel dehydrobutyrine-containing amino acid (**13**). The yield for the formation of dehydrobutyrine **13** could be increased to 65% by simple modification of the work up procedure. The elimination pathway was investigated and the reaction is proposed to proceed via an E1cb-type mechanism, which is enabled by the presence of an *O*-fluoropyridyl leaving group. Dehydrobutyrine **13** offers a unique opportunity for the dual tagging of peptides (i.e., reaction on the fluoropyridine ring and Michael addition) and potential applications in this area are currently under investigation.

4. Experimental

4.1. General

NMR spectra were collected using Bruker Avance 400 MHz, Varian VNMRS 600 MHz and a Varian VNMRS 700 MHz spectrometers. multiplicities; s=singlet, d=doublet, dd=doublet of doublets, m=multiplet, t=triplet, q=quartet, qd=quartet of doublets, p=pentet, br s=broad singlet. Chemical shifts are reported in parts per million (ppm) and are referenced to residual solvent peaks; $CHCl_3$ (^{1}H 7.26 ppm, ^{13}C 77.2 ppm) and DMSO (^{1}H 2.50 ppm, ^{13}C 39.5 ppm). *J* couplings are measured in hertz (Hz). Reactions were monitored by TLC using Merck precoated silica gel plates. Column chromatography was performed using silica gel with the solvent system indicated. All reported yields refer to the isolated yield and the product purity was estimated to be >95% by ^{1}H NMR. IR spectra were recorded on a Perkin Elmer Spectrum RX1 fitted with an ATR attachment. IR absorptions reported are in cm^{-1}. L-*allo*-Threonine methyl ester hydrochloride was purchased from Bachem. All other chemicals were purchased from Sigma Aldrich. Mass spectra were collected on a Waters TQD mass spectrometer and accurate mass spectra were collected on a Waters LCT Premier XE mass spectrometer. Optical rotations were measured with a Jasco P-1020 polarimeter using $CHCl_3$ as solvent. *ortho* (*o*) and *meta* (*m*) are with respect to nitrogen in the ring.

4.2. General procedures

4.2.1. General procedure A: reaction of threonine derivative with pentafluoropyridine (PFP) in the presence of potassium carbonate. PFP (2 equiv) was added to a stirred suspension of amino acid starting material (200 mg, 1 equiv), potassium carbonate (3 equiv) and acetonitrile (8 mL) and left to stir at room temperature for 18 h and purified as indicated below.

4.2.1.1. Purification procedure A: filtration work up method. The reaction mixture was filtered and washed with acetonitrile (2×10 mL). The filtrate was concentrated in vacuo to afford the crude product, which was purified by column chromatography on silica (20% ethyl acetate, 80% hexane).

4.2.1.2. Purification procedure B: extraction work up method.- Acetonitrile was evaporated from the reaction mixture under reduced pressure and the resulting crude mixture was partitioned between chloroform (15 mL) and water (15 mL). The aqueous layer was washed twice with chloroform (2×10 mL) and the combined organic extracts were dried ($MgSO_4$). Volatiles were removed in vacuo to give a crude mixture, which was purified by column chromatography on silica.

4.3. *N*-(2,3,5,6-Tetrafluoropyridine)-dehydrobutyrine methyl ester (13)

General reaction procedure A was followed using threonine methyl ester hydrochloride (**12**) (200 mg, 1.24 mmol), PFP (0.27 mL, 2.48 mmol) and potassium carbonate (513 mg, 3.71 mmol).

Purification procedure B without the column chromatography step to give **13** as a white crystalline solid (200 mg, 65%).

Note: According to *general procedure A and purification procedure A*; employing L-*allo*-threonine methyl ester (0.20 g, 1.24 mmol), the same product (**13**) was also obtained as a white crystalline solid (7 mg, 2%). Mp 107–109 °C; ν_{max}/cm^{-1} 1481, 1702 (C=O ester), 2922 (CH_3), 3325 (NH); $[\alpha]_D^{24.0}$ −5.3; δ_H (600 MHz, DMSO-d_6) 1.84 (3H, d, *J* 7.2 Hz, CH_3), 3.69 (3H, s, OCH_3), 6.81 (1H, q, *J* 7.2 Hz, H-β), 8.87 (1H, s, NH); δ_F (376 MHz, DMSO-d_6) −162.22 (2 F, m, *m*-F), −95.94 (2 F, m, *o*-F); δ_C (176 MHz, DMSO-d_6) 13.5 (CH_3), 52.3 (OCH_3), 128.8 (*C*-α), 131.2 (2C, m, C-3), 134.4 (*C*-β), 137.2 (1C, m, C-4), 143.5 (2C, m, C-2), 164.0 (*C*OOCH$_3$); HRMS (ESI$^-$) $C_{10}H_7F_4N_2O_2^-$ ([M−H]$^-$); requires 263.0444; found 263.0450.

4.4. *N*-(2,3,5,6-Tetrafluoropyridine)-threonine methyl ester (14)

PFP (0.27 mL, 2.48 mmol) was added to a stirred suspension of L-threonine methyl ester hydrochloride (**12**) (200 mg, 1.24 mmol), DIPEA (0.65 mL, 3.72 mmol) and acetonitrile (8 mL) and left to stir at room temperature overnight. Acetonitrile was removed in vacuo and the resulting yellow oil was purified by column chromatography on silica (25% ethyl acetate, 75% hexane) to give **14** as a colourless oil (100 mg, 28%). ν_{max}/cm^{-1}; 1479, 1648, 1739 (C=O ester), 2981 (CH_3), 3400 (br, OH and secondary NH); $[\alpha]_D^{24.3}$ −57.4; δ_H (400 MHz, $CDCl_3$) 1.38 (3H, d, *J* 6.0 Hz, CH_3), 2.16 (1H, br s, OH), 3.80 (3H, s, OCH_3), 4.48–4.53 (2H, m, H-α and H-β), 5.32–5.41 (1H, m, NH); δ_F (376 MHz, $CDCl_3$) −162.61 (2F, m, *m*-F), −93.34 (2F, m, *o*-F); δ_C (176 MHz, $CDCl_3$) 20.3 (CH_3), 53.1 (OCH_3), 61.5 (*C*−H), 67.8 (*C*−H), 131.5 (2C, m, C-3), 137.5 (1C, m, C-4), 144.2 (2C, m, C-2), 171.6 (*C*OOCH$_3$); HRMS (ESI$^-$) $C_{10}H_{11}F_4N_2O_3^+$ ([M+H]$^+$); requires 283.0706; found 283.0710.

4.5. *N*-(2,3,5,6-Tetrafluoropyridine)-threonine-*O*-(2,3,5,6-tetrafluoropyridine)-methyl ester (15)

PFP (0.27 mL, 2.48 mmol) was added to a stirred suspension of L-threonine methyl ester hydrochloride (**12**) (200 mg, 1.24 mmol) and potassium carbonate (513 mg, 3.71 mmol) in acetonitrile (8 mL) and stirred at room temperature overnight. Additional PFP (0.27 mL, 2.48 mmol) was added to the reaction mixture and stirred for a further 18 h. A final portion of PFP (0.27 mL, 2.48 mmol) was added to the reaction mixture and left to stir for 18 h. *Purification procedure A* was followed to give intermediate **15** as a white solid (37 mg, 7%). Mp 65–66 °C; ν_{max}/cm^{-1} 1475, 1641, 1757 (C=O ester), 2975 (CH_3), 3315 (secondary NH); $[\alpha]_D^{24.4}$ +7.4; δ_H (700 MHz, $CDCl_3$) 1.59 (3H, d, *J* 6.4 Hz, CH_3), 3.80 (3H, s, OCH_3), 4.77 (1H, dd, *J* 10.7, 2.2 Hz, H-α), 5.32 (1H, d, *J* 10.7 Hz, NH), 5.42 (1H, qd, *J* 6.4, 2.2 Hz, H-β); δ_F (564 MHz, $CDCl_3$) −161.93 (2F, m, *m*-F), −157.16 (2F, m, *m*-F), −92.37 (2F, m, *o*-F), −88.79 (2F, m, *o*-F); δ_C (151 MHz, $CDCl_3$) 17.4 (CH_3), 53.5 (OCH_3), 60.7 (*C*−H), 81.1 (*C*−H), 132.0 (2C, m, C-3 Ar−N), 136.1 (2C, m, C-3 Ar−O), 137.0 (1C, m, C4 Ar−N), 144.1 (4C, m, C-2 Ar−O, C-2 Ar−N), 145.1 (1C, m, C4 Ar−O), 169.6 (*C*OOCH$_3$); HRMS (ESI$^-$) $C_{15}H_{10}F_8N_3O_3^+$ ([M+H]$^+$); requires 432.0594; found 432.0574.

4.6. *N*-(2,3,5,6-Tetrafluoropyridine)-*allo*-threonine methyl ester (17) and *N*-(2,3,5,6-tetrafluoropyridine)-*allo*-L-threonine-*O*-(2,3,5,6-tetrafluoro pyridine)-methyl ester (18)

General reaction procedure A was followed using L-*allo*-threonine methyl ester hydrochloride (**16**) (200 mg, 1.24 mmol), PFP (0.27 mL, 2.48 mmol) and potassium carbonate (513 mg, 3.71 mmol). *Purification procedure A* was followed to give **17** as a colourless oil (139 mg, 50%). ν_{max}/cm^{-1} 1478, 1648, 1738 (C=O ester), 2959 (CH_3), 3386 (br OH and secondary NH); $[\alpha]_D^{21.6}$ −14.4; δ_H (600 MHz, $CDCl_3$) 1.35 (3H, d, *J* 6.6 Hz, CH_3), 2.20 (1H, br s, OH), 3.81 (3H, s, OCH_3), 4.20–4.26 (1H, m, H-β), 4.58 (1H, dd, *J* 9.1, 3.7 Hz, H-α), 5.50 (1H, d, *J* 9.1 Hz, NH); δ_F (564 MHz, $CDCl_3$) −162.24 (2F, m, *m*-F), −93.11 (2F, m, *o*-F); δ_C (151 MHz, $CDCl_3$) 20.0 (CH_3), 53.2 (OCH_3), 61.6 (*C*−H), 69.4 (*C*−H), 131.6 (2C, m, C-3), 136.5 (1C, m, C-4), 144.3 (2C, m, C-2), 170.7 (*C*OOCH$_3$); HRMS (ESI$^+$) $C_{10}H_{11}F_4N_2O_3^+$ ([M−H]$^+$); requires 283.0706; found 283.0709.

And **18**, as a white solid (49 mg, 9%). Mp 54–55 °C; ν_{max}/cm^{-1} 1467, 1647, 1726 (C=O ester), 3320 (secondary NH); $[\alpha]_D^{24.2}$ +36.4; δ_H (700 MHz, $CDCl_3$) 1.57 (3H, d, *J* 6.5 Hz, CH_3), 3.92 (3H, s, OCH_3), 4.95 (1H, dd, *J* 8.6, 2.9, H-α), 5.16 (1H, qd, *J* 6.5, 2.9, H-β), 5.49 (1H, d, *J* 8.6, NH); δ_F (376 MHz, $CDCl_3$) −162.43 (2H, m, *m*-F), −157.64 (2H, m, *m*-F), −92.16 (2H, m, *o*-F), −88.97 (2H, m, *o*-F); δ_C (176 MHz, $CDCl_3$) 16.5 (CH_3), 53.4 (OCH_3), 60.2 (C-α), 81.7 (C-β), 131.8 (2C, m, C-3 Ar−N), 135.6 (2C, dm, J_{CF} 264 Hz, C-3 Ar−O), 135.8 (1C, m, C4 Ar−N), 144.1 (4C, m, C-2 Ar−O, C-2 Ar-N), 145.4 (1C, m, C4 Ar−O), 168.3 (*C*OOCH$_3$); HRMS (ESI$^-$) $C_{15}H_8F_8N_3O_3^-$ ([M−H]$^-$); requires 430.0438; found 430.0443.

4.7. X-ray crystallography

The X-ray single crystal data have been collected at 120(1) K on Agilent Gemini S-Ultra (**13**) and Bruker SMART CCD 6000 (**15**) diffractometers equipped with Cryostream (Oxford Cryosystems) open-flow nitrogen cryostats. Both structures were solved by direct method and refined by full-matrix least squares on F^2 for all data using Olex2[12] and SHELXTL[13] software. All non-hydrogen atoms were refined anisotropically, hydrogen atoms in both structures were located at the difference Fourier maps and refined isotropically, except those at C(10) methyl group in the structure **15**, which were refined in riding mode. Crystallographic data for the structure have been deposited with the Cambridge Crystallographic Data Centre as supplementary publication CCDC-972263–972264.

*4.7.1. Crystal data for **13***. $C_{10}H_8F_4N_2O_2$, M=264.18, monoclinic, space group $P2_1/c$, a=8.5991(4), b=8.3095(3), c=15.9343(10) Å, β=103.386(6)°, U=1107.63(10) Å^3, F(000)=536.0, Z=4, D_c=1.584 mg m^{-3}, μ=0.155 mm^{-1}. 12,463 reflections, 2673 unique data (R_{merg}=0.0854). Final $wR_2(F^2)$=0.01251 for all data (195 refined parameters), conventional $R_1(F)$=0.0527 for 1985 reflections with $I\geq 2\sigma$, GOF=1.074.

*4.7.2. Crystal data for **15***. $C_{15}H_9F_8N_3O_3$, M=431.25, orthorhombic, space group $P2_12_12$, a=9.5212(12), b=9.6957(12), c=17.731(2) Å, U=1636.9(4) Å^3, F(000)=864.0, Z=4, D_c=1.750 mg m^{-3}, μ=0.182 mm^{-1}. 25,278 reflections, 3966 unique data (R_{merg}=0.0669). Final $wR_2(F^2)$=0.1076 for all data (287 refined parameters), conventional $R_1(F)$=0.0409 for 2649 reflections with $I\geq 2\sigma$, GOF=0.990.

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