Amino Acids (2015) 47:779–785
DOI 10.1007/s00726-014-1908-7

ORIGINAL ARTICLE

A direct method for the synthesis of orthogonally protected furyl- and thienyl- amino acids

Alex S. Hudson · Laurent Caron · Neil Colgin · Steven L. Cobb

Received: 2 July 2014 / Accepted: 19 December 2014 / Published online: 13 January 2015

Abstract The synthesis of unnatural amino acids plays a key part in expanding the potential application of peptide-based drugs and in the total synthesis of peptide natural products. Herein, we report a direct method for the synthesis of orthogonally protected 5-membered heteroaromatic amino acids.

Keywords Heteroaromatic amino acids · Negishi cross-coupling · Furyl-alanine · Thienyl-alanine

Introduction

The formation of novel amino acids is of considerable importance both in terms of enabling the synthesis of naturally occurring peptides and in providing a means by which to modulate peptide properties (Zhang et al. 2013; Lu and Freeland 2006; Perdih and Dolenc 2011). The formation of novel aromatic amino acids via palladium catalysed cross-coupling reactions of orthogonally protected iodoalanines has been well documented in the literature. (Jackson et al. 1992, 2004; Rodriguez et al. 2003; Oswald et al. 2008; Wang et al. 2010) In particular this approach has been successfully utilised to prepare a wide range of phenylalanine derivatives (Deboves et al. 2001; Colgin et al. 2011; Ross et al. 2010, 2011). This strategy can also be used to access heteroaromatic amino acids such as pyridyl (Walker et al. 1997; Tabanella and Valancogne 2003), purinyl (Capek et al. 2004), indolyl (Tanaka et al. 2011) and bicyclic isoxazolyl (Clausen et al. 2009). However, examples of either furyl-alanine (**1a** and **2a**) or thienyl-alanine amino acids (**1b** and **2b**) produced via this route are not well documented (Fig. 1). To the best of our knowledge only two examples exist: The synthesis of 2-thienyl-alanine (**1b**) in a 10 % yield (Jackson et al. 1989) and 4-phosphothiophen-2-yl alanine in a 59 % yield under optimised conditions (Lilley et al. 2014).

5-membered heteroaromatic amino acids such as **1** and **2** are frequently found in peptide natural products. For example, the 2-furyl moiety (**1a**) is contained in the natural peptide γ-glutamyl-2-furyl-alanine (**3**) (Hilker et al. 2010) and the 3-furyl moiety (**2a**) in Rhizonin (**4**) (Partida-Martinez et al. 2007) (Fig. 2). γ-Glutamyl-2-furyl-alanine (**3**) is produced by leaf beetle larvae as a chemical defence/feeding deterrent against insects and Rhizonin (**4**) is hepatotoxic cyclo-peptide produced by the fungal strain *Rhizopus microsporus*.

The chemical synthesis of **3** and **4** relies on having ready access to suitable 5-membered heteroaromatic amino acid building blocks. Herein, we report the preparation of five orthogonally protected 5-membered heteroaromatic amino acids. The key synthetic step in their preparation involves the Negishi-coupling reaction of an orthogonally protected iodoalanine with various halogenated 5-membered heterocycles.

Materials and methods

IR spectra were recorded on a Perkin Elmer Spectrum RX1 fitted with an ATR attachment. ^{1}H NMR spectra were

Handling Editor: P. Meffre.

Electronic supplementary material The online version of this article (doi:10.1007/s00726-014-1908-7) contains supplementary material, which is available to authorized users.

A. S. Hudson · S. L. Cobb (✉)
Department of Chemistry, Durham University, South Road, Durham DH1 3LE, UK
e-mail: s.l.cobb@durham.ac.uk

L. Caron · N. Colgin
Cambridge Research Biochemicals Ltd, Billingham, Cleveland TS23 4AZ, UK

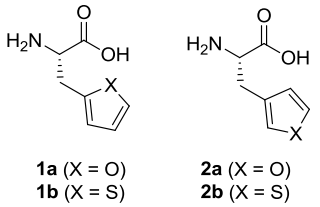


Fig. 1 Chemical structures of furyl-alanine (**1a** and **2a**) and thienyl-alanine (**1b** and **2b**)

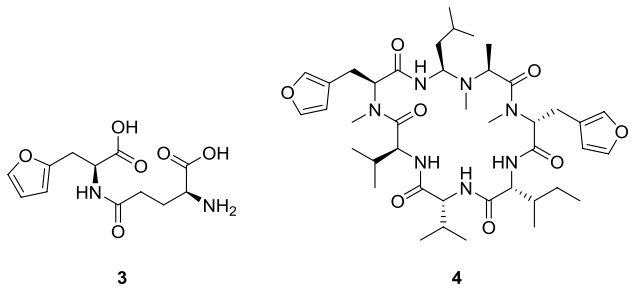


Fig. 2 Peptide natural products that contain a furyl-alanine residue

recorded at 400 MHz using a Bruker Avance 400 MHz and ^{13}C NMR spectra at 100 MHz with a Bruker Avance. Chemical shifts are reported in ppm and are referenced to residual solvent peaks; $CHCl_3$ (^{1}H 7.26 ppm, ^{13}C 77.0 ppm). J couplings are measured in Hertz (Hz). All reactions were monitored by T.L.C. using Merck precoated silica gel plates, Column chromatography was performed using silica gel (40–60 mM) using the solvent system indicated. Zinc dust (particle size <10 μM) was purchased from Sigma-Aldrich®. All reported yields refer to the isolated yield and the product purity was estimated to be >95 % by ^{1}H NMR. Mass spectra were collected on a Waters TQD mass spectrometer and accurate mass spectra were collected on a Waters LCT Premier XE mass spectrometer. High pressure liquid chromatography was performed on an analytical Varian LC with a diode array detector. Optical rotations were measured with a Jasco P-1020 polarimeter.

Synthesis of orthogonally protected heteroaromatic amino acids **13–16**, **21** general procedures

Reaction conditions A

Acid washed zinc dust (2.43 mmol, 4.00 equiv.) was heated under vacuum at 100 °C for 30 min whilst vigorously stirring. The zinc dust was cooled to 70 °C and placed under a positive pressure of argon, anhydrous DMF (0.5 mL) and I_2 (cat ~0.015 g) were added and the light grey suspension stirred for a further 20 min. The reaction mixture was cooled to 50 °C at point which iodoalanine (**7** or **19**) (0.61 mmol, 1.00 equiv.) was dissolved in DMF (0.5 mL) and added. Stirring under argon was continued for another 20 min followed by addition of the halo-aromatic (**8–12**) (0.61 mmol, 1.00 equiv.), Tris(dibenzylideneacetone)dipalladium ($Pd_2(dba)_3$) (0.02 mmol, 0.03 equiv.) and tri(*o*-tolyl) phosphine (P(*o*-tol)$_3$) (0.07 mmol, 0.10 equiv.). The reaction mixture was left to stir under argon at 50 °C for 5 h, then overnight at room temperature. The cooled reaction mixture was purified via column chromatography without workup (SiO_2; 80/20 hexane/EtOAc → 100 % EtOAc and if impure a second column was run using SiO_2; 100 % CH_2Cl_2).

Reaction conditions B

This follows the same reaction procedure as above: procedure **A**, however, 2-dicyclohexylphosphino-2′,6′-dimethoxybiphenyl (SPhos). is used as a replacement phosphine ligand to P(*o*-tol)$_3$ in the same molar equivalents. The reaction mixture was purified as outlined in Procedure A.

Reaction conditions C

Acid washed zinc dust (2.43 mmol, 4.00 equiv.) was heated under vacuum at 100 °C for 30 min whilst vigorously stirring. The reaction mixture was cooled to 50 °C and placed under a positive pressure of argon, anhydrous DMF (0.5 mL) and I_2 (cat. ~0.015 g) were added and the light grey suspension stirred for a further 20 min. The reaction mixture was cooled to room temperature at which point iodoalanine (**7** or **19**) (0.61 mmol, 1.00 equiv.) was dissolved in DMF (0.5 mL) and added. Stirring under argon was continued for another twenty mins and the halo-aromatic (**8–12**) (0.61 mmol, 1.00 equiv), $Pd_2(dba)_3$ (0.02 mmol, 0.03 equiv.) and SPhos (0.07 mmol, 0.10 equiv.) were added. The reaction mixture was left to stir overnight at room temperature under an argon atmosphere. The reaction mixture was purified as outlined in Procedure A.

*Fmoc-β-(2-furyl)-Ala-O^{t}Bu (**13**)*

The general procedure for palladium catalysed Negishi cross-coupling reaction conditions **A** was followed (0.61 mmol of **7**). **13** obtained as a yellow crystalline solid, 11 % (0.07 mmol). ν_{max}(solid)/cm^{-1} 2928 (w, C–H), 1702 (s bd, C = O); $[\alpha]_{27}^{D}$ −5.6° (c 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 1.47 (9H, s, CO_2^tBu), 3.20 (2H, d, *J* 5.2, β-CH_2), 4.24 (1H, app. t, Fmoc-C**H**CH_2), 4.38 (2H, m, Fmoc-CHC**H**$_2$), 4.55 (1H, dt, *J* 5.2, 8.0, α-CH), 5.50 (1H, bd, *J* 8.0, NH), 6.09 (1H, d, *J* 2.8, furan-CH), 6.31 (1H, m, furan-CH), 7.30–7.34 (2H, m, Fmoc-ArH), 7.32 (1H, m, furan-CH), 7.39–7.43 (2H, m, Fmoc-ArH), 7.60 (2H, bd, *J* 7.4, Fmoc-ArH), 7.77 (2H, d, *J* 7.4, Fmoc-ArH); δ_C (100 MHz; $CDCl_3$) 170.3, 155.7, 150.7, 144.0, 142.1, 141.4, 127.8,

127.2, 125.3, 120.1, 110.5, 108.1, 82.6, 67.2, 53.6, 47.3, 31.2, 28.0; HRMS *m/z* (ES^+) 456.1784 (M^+ + Na. $C_{26}H_{27}NO_5Na$ requires 456.1787). General procedure for palladium catalysed Negishi cross-coupling reaction conditions **B** was followed; Yellow crystalline solid, 42 % (0.26 mmol).

*Fmoc-β-(3-furyl)-Ala-O^{t}Bu (**14**)*

The general procedure for palladium catalysed Negishi cross-coupling reaction conditions **B** was followed (0.61 mmol of **7**). **14** was obtained as a colourless oil, 27 % (0.17 mmol). ν_{max}(solid)/cm^{-1} 3342 (w bd, N–H), 2979 (w, C–H), 1706 (s bd, C = O); $[\alpha]_{28}^{p}$ −6.8° (c 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 1.46 (9H, s, CO_2^tBu), 2.95 (2H, m, β-CH_2), 4.43 (1H, m, Fmoc-C**H**CH_2), 4.42 (2H, m, Fmoc-CHC**H**$_2$), 4.51 (1H, m, α-CH), 5.35 (1H, bs, NH), 6.24 (1H, m, furan-CH), 7.23 (1H, m, furan-CH), 7.30–7.34 (2H, m, Fmoc-ArH), 7.37 (1H, m, furan-CH), 7.39–7.43 (2H, m, Fmoc-ArH), 7.57–7.60 (2H, m, Fmoc-ArH), 7.78 (2H, m, Fmoc-ArH); δ_C (100 MHz; $CDCl_3$) 170.6, 155.8, 144.1, 143.1, 141.5, 140.6, 127.9, 127.2, 125.2, 120.2, 119.2, 111.5, 82.6, 67.1, 54.4, 47.4, 31.0, 28.2; HRMS *m/z* (ES^+) 456.1785 (M^+ + Na. $C_{26}H_{27}NO_5Na$ requires 456.1787).

*Fmoc-β-(2-thienyl)-Ala-O^{t}Bu (**15**)*

The general procedure for palladium catalysed Negishi cross-coupling reaction conditions **B** was followed with 2-iodothiophene (0.61 mmol of **7**). **15** was obtained as a yellow oil, 26 % (0.16 mmol). ν_{max}(solid)/cm^{-1} 3342 (w bd, N–H), 2977 (w, C–H), 1710 (s bd, C = O); $[\alpha]_{27}^{p}$ 15.0° (c 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 1.48 (9H, s, CO_2^tBu), 3.39 (2H, m, β-CH_2) 4.26 (1H, t, *J* 7.2, Fmoc-C**H**CH_2), 4.35 (1H, dd, 7.2, 10.4, Fmoc-CHC**H**$_2$), 4.48 (1H, dd, 7.2, 10.4, Fmoc-CHC**H**$_2$), 4.58 (1H, m, α-CH), 5.90 (1H, bd, *J* 8.0, NH), 6.83 (1H, d, *J* 3.0 thiophene-CH), 6.96 (1H, dd, *J* 3.0, 5.0, thiophene-CH), 7.20 (1H, d, *J* 5.0, thiophene-CH), 7.32–7.36 (2H, m, Fmoc-ArH), 7.40–7.44 (2H, m, Fmoc-ArH), 7.61–7.64 (2H, m, Fmoc-ArH), 7.78 (2H, bd, *J* 7.6, Fmoc-ArH); δ_C (100 MHz; $CDCl_3$) 170.0, 155.7, 144.0, 141.4, 137.6, 127.8, 127.1, 127.0, 126.9, 125.2, 124.8, 120.1, 83.8, 67.1, 55.0, 47.3, 32.5, 28.1; HRMS *m/z* (ES^+) 472.1562 (M^+ + Na. $C_{26}H_{27}NO_4SNa$ requires 472.1559).

General procedure for palladium catalysed Negishi cross-coupling reaction conditions **B** was followed with 2-bromothiophene; Yellow oil, 24 % (0.15 mmol).

General procedure for palladium catalysed Negishi cross-coupling reaction conditions **C** with 2-iodothiophene starting material was followed; Yellow oil, 22 % (0.13 mmol).

*Fmoc-β-(3-thienyl)-Ala-O^{t}Bu (**16**)*

The general procedure for palladium catalysed Negishi cross-coupling reaction conditions **B** was followed (0.61 mmol of **7**). **16** was obtained yellow oil, 20 % (0.12 mmol). ν_{max}(solid)/cm^{-1} 3342 (w bd, N–H), 2976 (w, C–H), 1707 (s bd, C = O); $[\alpha]_{28}^{p}$ −18.1° (c 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 1.45 (9H, s, CO_2tBu), 3.15 (2H, m, β-CH_2) 4.23 (1H, m, Fmoc-C**H**CH_2), 4.30–4.57 (3H, m, Fmoc-CHC**H**$_2$, α-CH), 5.35 (1H, bs, NH), 6.92 (1H, d, *J* 4.0, thiophene-CH), 6.98 (1H, m, thiophene-CH), 7.26 (1H, m, thiophene-CH), 7.29–7.35 (2H, m, Fmoc-ArH), 7.39–7.44 (2H, m, Fmoc-ArH), 7.58–7.61 (2H, m Fmoc-ArH) 7.77 (2H, d, *J* 7.6, Fmoc-ArH); δ_C (100 MHz; $CDCl_3$) 170.7, 155.7, 144.0, 141.4, 136.3, 128.7, 127.8, 127.2, 125.8, 125.2, 122.9, 120.1, 82.5, 67.0, 54.8, 47.3, 32.9, 28.0; HRMS *m/z* (ES^+) 472.1563 (M^+ + Na. $C_{26}H_{27}NO_4SNa$ requires 472.1559).

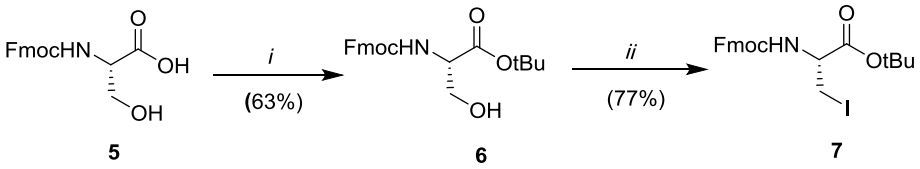
*Boc- β-(2-furyl)-Ala-OBn (**20**)*

The general procedure for palladium catalysed Negishi cross-Coupling reaction conditions **B** was followed (0.65 mmol of **19**). **20** was obtained yellow oil, 86 % (0.56 mmol). $[\alpha]_{28}^{p}$ −7.0° (c 1.0, $CHCl_3$); δ_H (400 MHz; $CDCl_3$) 1.43 (9H, s, BocH), 3.14 (2H, m, β-CH_2), 4.61 (1H, m, α-CH), 5.46 (2H, m, CH_2Ar), 5.95 (1H, d, *J* 3.2, furan-CH), 6.24 (1H, dd, *J* 2.0, 3.2, furan-CH), 7.27 (1H, d, *J*, 2.0 Hz, furan-CH), 7.31–7.40 (5H, m, ArH); δ_C (100 MHz; $CDCl_3$) 171.4, 155.2, 150.4, 142.2, 135.4, 128.6, 128.5, 110.4, 108.0, 80.1, 67.3, 52.9, 31.0 28.4, 18.6; HRMS *m/z* (ES^+) 368.1473 (M^+ + Na. $C_{19}H_{23}NO_5Na$ requires 368.1474).

*Z-Glu-(-β-(2-Furyl)-Ala-OBn)-OBn (**21**)*

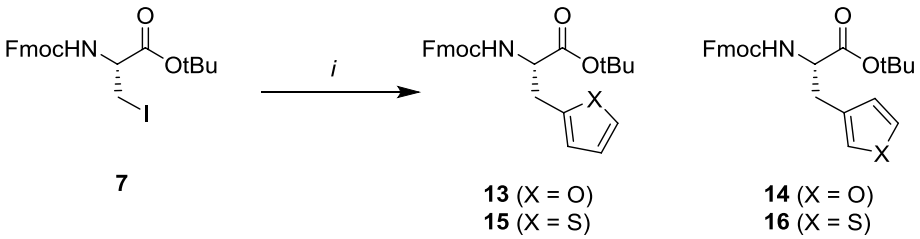
Boc-β-(2-furyl)-Ala-OBn **20** (0.14 mmol, 1.00 equiv.) was stirred in TFA: DCM 1:4 (10 mL) until the Boc deprotection was seen to be complete by TLC. The reaction mixture was then evaporated under reduced pressure to dryness. The resulting yellow powder was dissolved in DCM (15 mL). Z-Glu-OBn (0.14 mmol, 1.00 equiv.), PyBOP® (0.14 mmol, 1.00 equiv.) DIPEA (3.00 equiv.) were added and the reaction mixture was stirred overnight at room temperature. The reaction mixture was evaporated under reduced pressure and purified via column chromatography (SiO_2; 95/5 % hexane/EtOAc → 40/60 % hexane/EtOAc) to yield **21**; White powder, 78 % (0.11 mmol). δ_H (400 MHz; $CDCl_3$) 1.90–2.34 (4H, m, β-CH and γ-CH_2), 3.18 (2H, m, β-CH_2), 4.46 (1H, m, α-CH) 4.87 (1H, m, α-CH), 5.08–5.28 (6H, m, CH_2Ar), 5.62 (1H, d, *J* 8.0, NH), 5.94 (1H, d, *J* 2.8, furan-CH) 6.19 (1H, t, *J* 2.8, furan-CH), 6.28 (1H, d, *J* 7.2, NH), 7.21 (1H, m, furan-CH), 7.27–7.41

Scheme 1 Synthesis of orthogonally protected iodoalanine **7**



i = EtOAc, TBTA, 20h, r.t.;
ii = CH_2Cl_2, I_2, PPh_3, imidazole, CH_2Cl_2, 20h, r.t.

Scheme 2 Synthesis of orthogonally protected heteroaromatic amino acids **13–16**



i = DMF, Pd^0, Phosphine ligand, halo-aromatic, 5h, 50 °C, r.t. overnight

(15H, m, ArH); δ_C (100 MHz; $CDCl_3$) 171.0, 150.3, 142.3, 136.3. 135.3, 128.8, 128.7, 128.6, 128.5, 128.4, 128.3, 128.2, 110.4, 108.2, 67.6, 67.5, 67.2, 53.6, 51.8, 48.4, 32.2, 30.6, 28.4, 18.5; HRMS *m/z* (ES^+) 599.2387 (M^+ + H. $C_{34}H_{34}N_2O_8$ requires 599.2393).

Results and discussion

In the synthesis of amino acids via the Negishi coupling of orthogonally protected iodoalanines, a Boc/Bn protecting group strategy is most commonly adopted. It was our aim to prepare amino acids that would be compatible with Fmoc solid phase peptide synthesis (SPPS) and as such an Fmoc/^{t}Bu protecting group strategy was adopted. It should be noted that there are only a few examples of novel amino acids being prepared via the Negishi coupling of orthogonally protected iodoalanines in which Fmoc has been employed as the protecting group (Eerland and Hedberg 2012).

The known orthogonally protected iodoalanine (**7**) was prepared on a multi-gram scale in two steps from commercially available Fmoc-L-serine (**5**) as shown in Scheme 1. The first step in the synthesis involved protection of the carboxylic acid as the *tert*-butyl ester and this was achieved in good yield using *tert*-butyl 2,2,2-trichloroacetimidate (TBTA) (Cobb and Vederas 2007). The conversion of the serine alkyl alcohol (β-OH) to an alkyl iodide was readily achieved in excellent yield using I_2, PPh_3 and imidazole (Arndt et al. 2001).

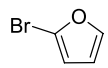
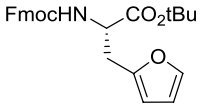
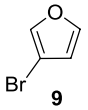
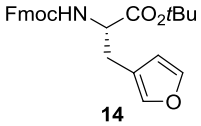
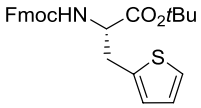
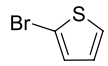
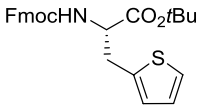
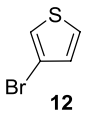
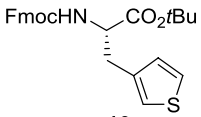
With the orthogonally protected iodoalanine (**7**) in hand, we attempted to carry out Negishi cross-coupling reactions (Scheme 2) with various halogenated 5-membered heteroaromatics (Table 1, Entries 1–5).

The initial coupling conditions employed (Table 1, Conditions A) only yielded the furan derivative **13** (Table 1, Entry 1) in low yield (11 %). The expected products for Entries 2–5 (Table 1, **14–16**) were visible in the mass spectrum obtained from the crude reaction mixtures but no products could be isolated by column chromatography. The lack of product formation in Entries 2–5 (Table 1) was in part due to the formation of Fmoc-Ala-O*t*Bu. The formation of the unwanted alanine side product in this type of reaction arises from protonation of the organozinc intermediate and is well documented in the literature (Jackson et al. 1998).

To obtain an improved synthesis of **13** and isolable amounts of products **14–16,** we looked to alter the reaction conditions. The bulky biaryl ligand, SPhos has literature precedence for improving Pd cross-coupling reactions, as seen by Buchwald's group in Suzuki–Miyaura reactions (Barder et al. 2005; Martin et al. 2007) and by Jackson's group in Negishi type cross-coupling reactions (Ross et al. 2010). Specifically of interest to our work, Jackson's group showed that in the Negishi type cross-coupling of iodoalanines switching from P(*o*-tolyl)$_3$ to SPhos could significant increase reaction yields (Ross et al. 2010).

In addition to altering the phosphine ligand, temperature, reaction times and solvent were also investigated. The final conditions used in the reaction [Table 1, conditions (B)] gave greatly improved but less than optimal yields. The lower yields achieved are believed to have arisen due to instability of the Fmoc protecting group under the reaction condition as this has been previous documented (Ross

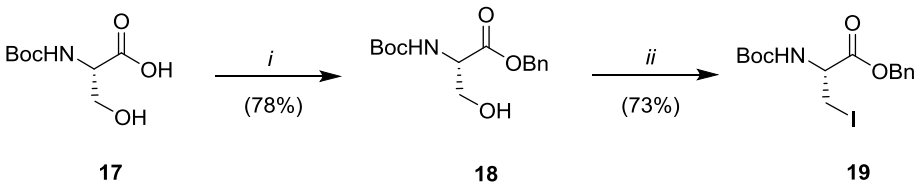
Table 1 Synthesis of 5-membered heteroaromatic amino acids

Entry	Halo-aromatic Starting Material	Product	Conditions (A) Yields (%)[b]	Conditions (B) Yields (%)[b]	Conditions (C) Yields (%)[b]
1	 **8**	 **13**	11	42	-
2	 **9**	 **14**	0	27	-
3	 **10**	 **15**	0	26	22
4	 **11**	 **15**	0	24	-
5	 **12**	 **16**	0	24	-

[a] Reaction conditions; (A) Zn dust (4.00 equiv.), vacuum 100 °C, 20 min; I_2, under argon, DMF, 70 °C, 20 min; Fmoc-AlaI-O*t*Bu (1.00 eq), $Pd_2(dba)_3$) (0.03 equiv.), P(*o*-tol)$_3$ (0.10 equiv.), under argon, DMF, 50 °C, 5 h, RT, 18 h. (B) Zn dust (4.00 equiv.), vacuum 100 °C, 30 min; I_2, under argon, DMF, 70 °C, 20 min; Fmoc-AlaI-O*t*Bu (1.00 equiv.), $Pd_2(dba)_3$) (0.03 equiv.), SPhos (0.10 equiv.), under argon, DMF, 50 °C, 5 h, RT, 18 h. (C) Zn dust (4.00 equiv.), vacuum 100 °C, 30 min; I_2, under argon, DMF, 50 °C, 20 min; Fmoc-AlaI-O*t*Bu (1.00 equiv.), $Pd_2(dba)_3$) (0.03 equiv.), SPhos (0.10 equiv.), under argon, DMF, RT, 24 h

[b] All yields given are isolated yields

Scheme 3 Synthesis of orthogonally protected iodoalanine **19**

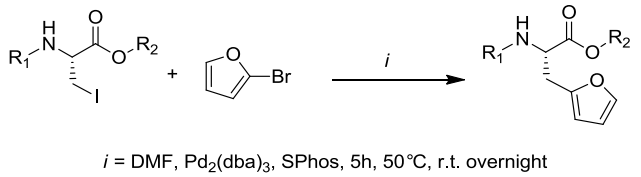


i = DMF, BnBr, K_2CO_3, 20h, r.t.
ii = CH_2Cl_2, imidazole, I_2 , PPh_3, 20h, r.t.

et al. 2010). This hypothesis was also investigated further using Boc-iodoalanine-OBn (**19**) as an alternative staring material in the couplings. Orthogonally protected **19** was accessed from cheap and readily available Boc-L-serine (**17**) on a multi-gram scale using the process described in Scheme 3.

Scheme 4 shows the synthesis of Boc-β-(2-furyl)-Ala-OBn (**20**) and Fmoc-β-(2-furyl)-Ala-O*t*Bu (**13**) from their respective iodoalanine pre-cursors via a Pd catalysed Negishi cross-coupling. As Table 2 highlights when the orthogonally protecting groups are altered there is a significant increase in the yield of the product obtained. It was also noted that the reaction was much cleaner and that the unwanted side-products such as the alanine derivative were suppressed to a much greater extent.

As previously discussed, furyl-alanines (**1a** and **2a**) can be found in peptide natural products (Partida-Martinez et al. 2007; Hilker et al. 2010) but the stability of these amino acids in synthetic procedures has been reported to be poor under acidic conditions (Schulz et al. 2004). To investigate this further, the TFA deprotection of Boc-β-(2-furyl)-Ala (**20**) and its subsequent incorporation into a di-peptide (**21**) was undertaken (Scheme 5). In our hands the furan moiety present in **20** proved stable to the acidic conditions employed and the desired di-peptide (**21**) was prepared in good yield. **21** is an intermediate in the synthesis of the natural product γ-glutamly-2-furyl-alanine (**3**) and various attempts were made to carry out the global deprotection of **21** to give **3**. Under all conditions, hydrogenation of **21** yielded a complex mixture of inseparable products with

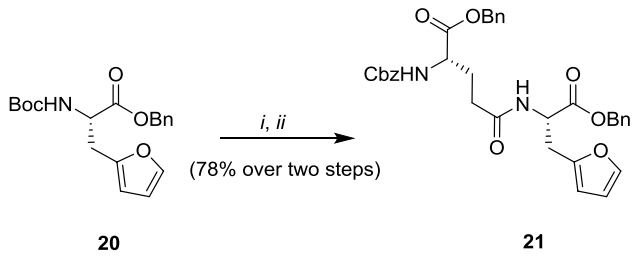


i = DMF, $Pd_2(dba)_3$, SPhos, 5h, 50°C, r.t. overnight

Scheme 4 Synthesis of orthogonally protected 2-furyl amino acids **13** and **20**

Table 2 Yields of orthogonally protected 2-furyl amino acids

R_1	R_2	Product	Isolated yields (%)
Fmoc	O*t*Bu	**13**	42
Boc	OBn	**20**	86



20 **21**

i = CH_2Cl_2, TFA 30min, r.t.
ii = CH_2Cl_2, PyBOP, Z-Glu-OBn, DIPEA, 20h, r.t.

Scheme 5 Synthesis of protected di-peptide (**21**), a precursor to γ-glutamyl-2-furyl-alanine (**3**)

the desired product **3** being identified only as a potential product in analysis of the crude reaction mixture by mass spectrometry.

Conclusions

Five orthogonally protected 5-membered heteroaromatic amino acids have been synthesised (**13–16**, **20**) via the Negishi cross-coupling of protected iodoalanines. The SPhos ligand was favoured in the Negishi cross-coupling reaction and Fmoc/OtBu protected furyl-alanines (**13** and **14**) and thienyl-alanines (**15** and **16**) were prepared in low to moderate yields (20–42 %). Despite the lower reaction yields, the synthetic process presented herein to access **13–16** has advantages in that it is; (a) shorter than other published routes (Hilker et al. 2010) (b) generates SPPS compatible amino acids and (c) it is amenable to scale up. Application of a Boc/OBn protecting group strategy does lead to an enhanced yield (86 %) in the Negishi cross-coupling reaction but the reaction conditions required to remove the benzyl ester were found to be incompatible with the furan ring. Amino acids **13–16** are now being investigated as key building blocks in the on resin synthesis of **3** and various analogues.

Acknowledgments This work was supported by the Engineering and Physical Science Research Council (ASH) and Cambridge Research Biochemicals Ltd.

Conflict of interest The authors have no conflicts of interest.

References

Barder TE, Walker SD, Martinelli JR, Buchwald SL (2005) New catalysts for Suzuki-Miyaura coupling processes: scope and studies of the effect of ligand structure. J Am Chem Soc 127:4685–4696

Capek P, Pohl R, Hocek M (2004) A facile and efficient synthesis of (Purin-6-yl)alanines. J Org Chem 69:7985–7988

Clausen RP, Naur P, Kristensen AS, Greenwood JR, Strange M, Brauner-Osborne H, Jensen AA, Nielsen AST, Geneser U, Ringgaard LM, Nielsen B, Pickering DS, Brehm L, Gajhede M, Krogsgaard-Larsen P, Kastrup JS (2009) The glutamate receptor GluR5 agonist (S)-2-amino-3-(3-hydroxy-7,8-dihydro-6H-cyclohepta[d]isoxazol-4-yl)propionic acid and the 8-methyl analogue: synthesis, molecular pharmacology, and biostructural characterization. J Med Chem 52:4911–4922

Cobb SL, Vederas JC (2007) A concise stereoselective synthesis of orthogonally protected lanthionine and β-ethyllanthionine. Org Biomol Chem 5:1031–1038

Colgin N, Flinn T, Cobb SL (2011) Synthesis and properties of MIDA boronate containing aromatic amino acids: new peptide building blocks. Org Biomol Chem 9:1864–1870

Deboves HJC, Montalbetti CAGN, Jackson RFW (2001) Direct synthesis of Fmoc-protected amino acids using organozinc chemistry: application to olymethoxylated phenylalanines and 4-oxoamino acids. J Chem Soc, Perkin Trans 1(16):1876–1884

Eerland MF, Hedberg C (2012) Design and synthesis of an Fmoc-SPPS-compatible amino acid building block mimicking the transition state of phosphohistidine phosphatase. J Org Chem 17:2047–2052

Hilker M, Hberlein C, Trauer U, Hilker Monika, Hberlein Christopher, Trauer Ute, Bünnige M, Vicentini M, Schulz S (2010) How to spoil the taste of insect prey? A novel feeding deterrent against ants released by larvae of the alder leaf beetle, *Agelastica alni*. ChemBioChem 11:1720–1726

Jackson RFW, Wythes MJ, Wood A (1989) Synthesis of enantiomerically pure protected β-aryl alanines. Tetrahedron Lett 30:5941–5944

Jackson RFW, Wishart N, Wood A, James K, Wythes JM (1992) Preparation of enantiomerically pure protected 4-oxo α-amino acids and 3-aryl α-amino acids from serine. J Org Chem 57:3397–3404

Jackson RFW, Moore RJ, Dexter CS, Elliot J, Mowbray CE (1998) Concise synthesis of enantiomerically pure phenylalanine, homophenylalanine, and bishomophenylalanine derivatives using organozinc chemistry: NMR studies of amino acid-derived organozinc reagents. J Org Chem 63:7875–7884

Jackson RFW, Rilatt I, Murray PJ (2004) Direct synthesis of unprotected phenols using palladium-catalysed cross coupling reactions of functionalised organozinc reagents. Org Biomol Chem 2(110):113

Lilley M, Mambwe B, Jackson RFW, Muimo R (2014) 4-phosphotheiopheny-2-yl alanine: a new 5-membered analogue of phosphotyrosine. Chem Comm 50:9343–9345

Lu Y, Freeland S (2006) On the evolution of the standard amino-acid alphabet. Genome Biol 7:102

Martin R, Buchwald SL (2008) Palladium-catalyzed Suzuki-Miyaura cross-coupling reactions employing dialkylbiaryl phosphine ligands. Acc Chem Res 41:1461–1473

Oswald CL, Carrillo-Márquez T, Caggiano L, Jackson RFW (2008) Negishi cross-coupling reactions of α-amino acid-derived organozinc reagents and aromatic bromides. Tetrahedron 64:681–687

Partida-Martinez LP, de Looß CF, Ishida K, Ishida M, Roth M, Buder K, Hertweck C (2007) Rhizonin, the first mycotoxin isolated from the zygomycota, is not a fungal metabolite but is produced by bacterial endosymbionts. Appl Environ Microbiol 73:793–797

Perdih A, Dolenc MS (2011) Recent advances in the synthesis of unnatural alpha-amino acids—an updated version. Curr Org Chem 15:3750–3799

Rodriguez A, Millar DD, Jackson RFW (2003) Combined application of organozinc chemistry and one-pot hydroboration–Suzuki coupling to the synthesis of amino acids. Org Biomol Chem 1:973977

Ross AJ, Lang HL, Jackson RFW (2010) Much improved conditions for the Negishi cross-coupling of iodoalanine derived zinc reagents with aryl halides. J Org Chem 75:245–248

Ross AJ, Dreiocker F, Schäfer M, Oomens J, Meijer AJ, Pickup BT, Jackson RFW (2011) Evidence for the role of tetramethylethylenediamine in aqueous Negishi cross-coupling: synthesis of nonproteinogenic phenylalanine derivatives on water. J Org Chem 76:1727–1734

Sabine A, Ulrich E, Bäurle S, Friedrich T, Grubert L, Koert U (2001) Quinone-annonaceous acetogenins: synthesis and complex I inhibition studies of a new class of natural product hybrids. Chem Eur J 7:993–1005

Schulz A, Busmann A, Kluver E, Schnebel M, Adermann K (2004) Stability and cleavage conditions of (2-Furyl)-L-alanine-containing peptides. Protein Pept Lett 11:601–606

Tabanella S, Valancogne Jackson RFW (2003) Preparation of enantiomerically pure pyridyl amino acids from serine. Org Biomol Chem 1:4254–4261

Tanaka M, Hikawa H, Yokoyama Y (2011) Convenient synthesis of chiral tryptophan derivatives using Negishi cross-coupling. Tetrahedron 67:5897–5901

Walker MA, Kaplita PK, Chen T, King DH (1997) Synthesis of all three regioisomers of pyridylalanine. Synlett 2:169–170

Wang L, Qu W, Lieberman B, Ploessel K, HF Funk (2010) Synthesis and in vitro evaluation of ^{18}F labelled tyrosine derivatives as potential positron emission tomography (PET) imaging agents. Bioorg Med Chem Lett 20:3483–3485

Zhang WH, Otting G, Jackson CJ (2013) Protein engineering with unnatural amino acids. Curr Oppin Chem Biol 23:581–587