JOC The Journal of Organic Chemistry

Article

Synthesis and Fluorescent Properties of #-Pyridyl #-Amino Acids

Alexander H. Harkiss, Jonathan D. Bell, Astrid Knuhtsen, Andrew G. Jamieson, and Andrew Sutherland

J. Org. Chem., **Just Accepted Manuscript** • DOI: 10.1021/acs.joc.9b00036 • Publication Date (Web): 06 Feb 2019

ACS Publications

is published by the American Chemical Society. 1155 Sixteenth Street N.W., Washington, DC 20036

Synthesis and Fluorescent Properties of β-Pyridyl α-Amino Acids

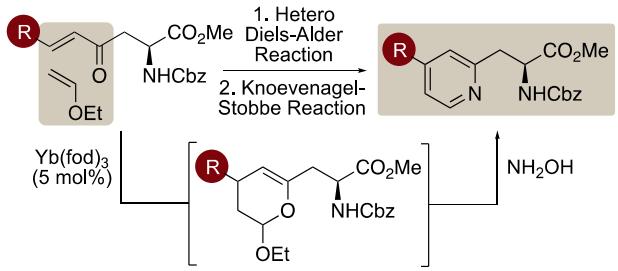
*Alexander H. Harkiss, Jonathan D. Bell, Astrid Knuhtsen, Andrew G. Jamieson and Andrew Sutherland**

WestCHEM, School of Chemistry, The Joseph Black Building, University of Glasgow, Glasgow G12 8QQ, United Kingdom.

Andrew.Sutherland@glasgow.ac.uk

RECEIVED DATE (to be automatically inserted after your manuscript is accepted if required according to the journal that you are submitting your paper to)

Table of Contents Graphic:



Abstract: The preparation of a new class of β-pyridyl α-amino acid is described using a highly regioselective, ytterbium-catalyzed hetero Diels-Alder reaction of enones with vinyl ethers followed by a modified Knoevenagel-Stobbe reaction as the key heterocycle forming steps. Investigation of the properties and applications of these amino acids showed that they could be utilized in solid phase peptide synthesis for the preparation of a biologically relevant hexapeptide, while pyridines bearing electron-rich substituents exhibited strongly fluorescent properties with high quantum yields and MegaStokes shifts. A solvatochromic study with the most fluorogenic amino acid, a *p*-methoxyphenyl analogue revealed that this charge-transfer based chromophore is highly sensitive to solvent polarity with a bathochromic shift of 115 nm on changing from THF to phosphate-buffered saline.

INTRODUCTION

α-Amino acids are the fundamental building blocks of life, serving as the basic structural components of all peptides and proteins.[1] They also play a key role in enzyme mechanisms and in signal induction pathways. With continued developments in biomedical and life sciences, proteinogenic and non-proteinogenic α-amino acids are used in a wide variety of applications, such as probes to study biological mechanism and function, the investigation of bioactive protein conformations and in the discovery of new pharmacologically active compounds.[1,2] In the field of organic chemistry, amino acids are used as chiral building blocks in total synthesis and as chiral ligands and auxiliaries for the development of new asymmetric methods.[3] This importance of α-amino acids in a range of scientific fields continues to drive development of new synthetic methods for their preparation.

Recently, attention has focused on the synthesis and application of unnatural α-amino acids with heteroaryl containing side-chains.[2,4,5] α-Amino acids bearing pyridyl moieties are of particular interest due to the improved medicinal chemistry properties imparted by this heterocycle. For example, the pyridine analogue of tyrosine has potent antibiotic and antitumour properties,[6] while the incorporation of 3-pyridylalanine into the gonadotropin-releasing hormone antagonist cetrorelix resulted in improved pharmacokinetic properties and receptor binding.[7] These types of properties have compelled the development of new syntheses of β-pyridyl α-amino acids.[8] The Brimble research group described the asymmetric synthesis of L-2-pyridylalanine using a modified Mitsunobu-Tsunoda reaction (Figure 1a).[8h] Treatment of the Belokon nickel complex[9] with cyanomethylene tributylphosphorane and 2-pyridinemethanol gave after hydrolysis, the corresponding amino acid in high overall yield and excellent enantiomeric excess. A number of chemoenzymatic approaches have also been reported,[10] such as the *Anabaena variabilis* phenylalanine ammonia lyase (*Av*PAL) catalyzed ammonolysis of cinnamates (Figure 1b).[11] Using *E.* coli BL21(DE3) whole cells expressing *Av*PAL and a high concentration of ammonia to drive the ammonolysis reaction gave L-2-pyridylalanine in 76% ee. A general synthesis of β-pyridyl α-amino acids was recently reported by the Jui group using a photoredox catalyzed radical

conjugate addition of halogenated pyridines with dehydroalanines (Figure 1c).[12] An asymmetric version of this process was also described using a chiral *tert*-butyl oxazolidinone dehydroalanine derivative.

We were interested in developing a new general approach for the synthesis of optically active β-pyridyl α-amino acids. In particular, the aim of the project was the preparation of α-amino acids bearing conjugated 4-arylpyridyl side-chains with the potential to display fluorescent properties and, that could be easily incorporated into peptides for biological imaging applications. Here we report a two-stage strategy utilizing a highly regioselective, lanthanide-catalyzed hetero Diels-Alder reaction of enone-derived α-amino acids with ethyl vinyl ether, followed by a modified Knoevenagel-Stobbe reaction of the resulting dihydropyran with hydroxylamine (Figure 1d). The utility of these amino acids for solid phase peptide synthesis (SPPS) and analysis of their photophysical properties is also described.

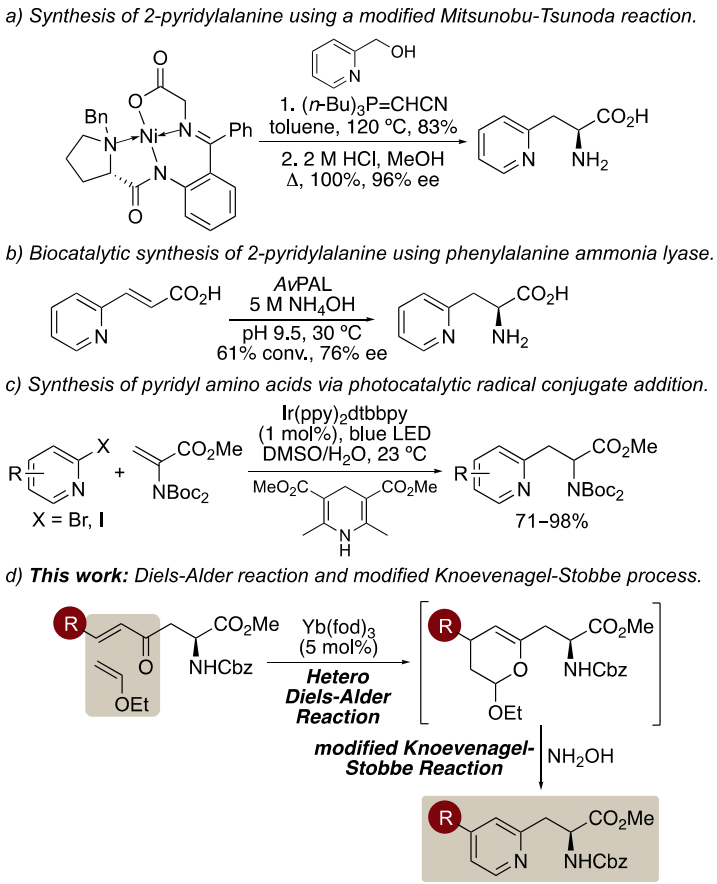
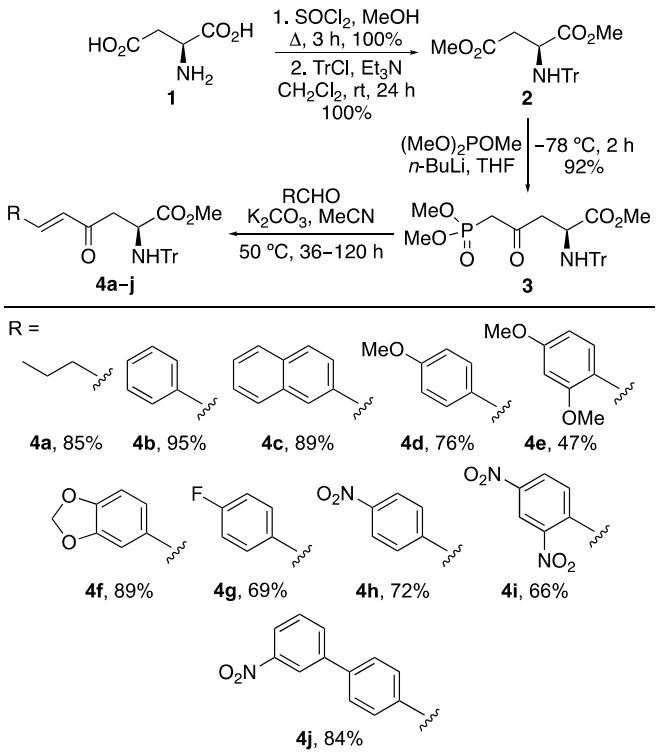


Figure 1. Selective synthetic methods for the preparation of β-(2-pyridyl)-α-amino acids.

RESULTS AND DISCUSSION

The first stage of the synthesis of the β-pyridyl α-amino acids involved the preparation of suitably protected enone-derived α-amino acids (Scheme 1). Initially, *N*-trityl protected phosphonate ester **3** was prepared in three-steps (92% overall yield) from L-aspartic acid **1**.[13] Following protection of the amino and carboxylic acid groups, the key step involved the highly regioselective reaction of the anion of dimethyl methylphosphonate with the sterically accessible β-methyl ester of **2** to give phosphonate ester **3**. Horner-Wadsworth-Emmons reaction of **3** with a wide range of aldehydes under mild conditions, gave enones **4a**–**j**, exclusively as the *E*-isomers.[14,15] The focus of this project was to prepare aryl substituted pyridyl side-chains and therefore, benzaldehyde derivatives were mainly used to explore the scope of this reaction. As expected, the majority of these gave high yields of the *E*-enones (66–95%), although highly electron-rich aldehydes required longer reaction times (120 h) and gave more modest yields (e.g. **4e**).

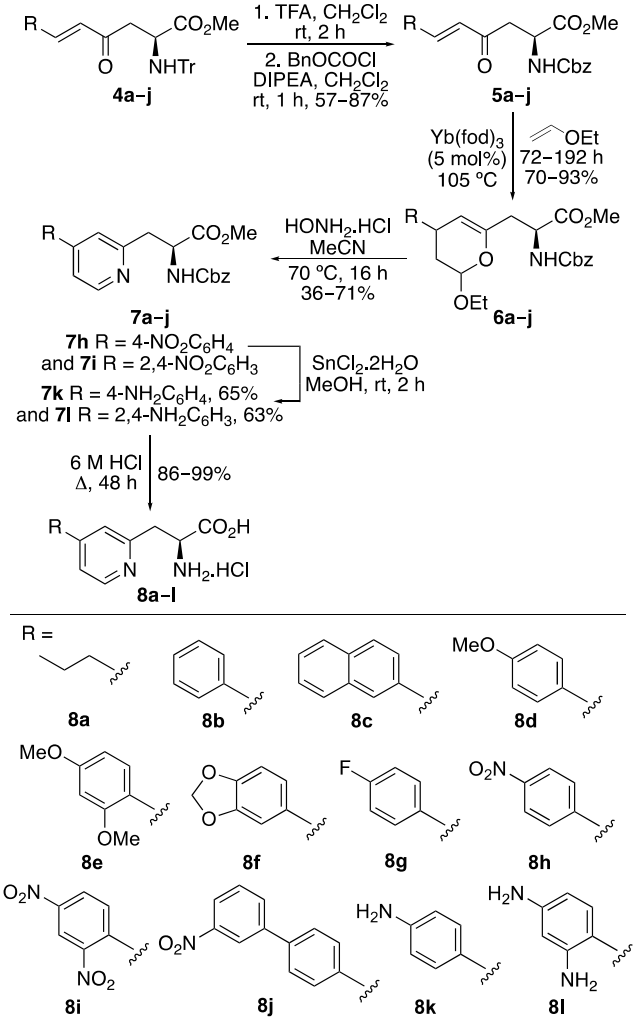
Scheme 1. Synthesis of Enone-Derived α-Amino Acids 4a–j[a]



[a]Isolated yields are shown.

The enone-derived α-amino acids were then investigated as substrates for the key two-stage pyridine-forming process (Scheme 2). The first-step involved an inverse electron-demand hetero Diels-Alder reaction with ethyl vinyl ether. Using *N*-trityl-protected enone **4b** and conditions first reported by Danishefsky and co-workers,[16] with $Yb(fod)_3$ as a Lewis acid catalyst (5 mol%) gave none of the resulting dihydropyran cycloaddition product. Previous work by us has shown that reactions of the enone moiety of amino acids such as **4a**–**j** are restricted by the steric bulk of the *N*-trityl protecting group.[5] Therefore, having used the trityl protecting group to conduct the regioselective functionalization of the side-chain ester of **2**, this was removed and replaced with a smaller *N*-protecting group (Cbz) using a two-step *N*-deprotection/reprotection strategy, under standard conditions. Diels-Alder reaction of the resulting Cbz-protected enone-derived α-amino acids **5a**–**j** with ethyl vinyl ether, in the presence of $Yb(fod)_3$ (5 mol%) proceeded smoothly and gave dihydropyrans **6a**–**j**, as single regioisomers in 70–93% yields. Ciufolini and co-workers have shown that dihydropyrans of this type are latent forms of 1,5-dicarbonyl compounds and can be converted to pyridines by reaction with hydroxylamine via a modified Knoevenagel-Stobbe reaction.[17,18] Treatment of dihydropyrans **6a**–**j** with hydroxylamine hydrochloride at 70 °C allowed clean conversion of all structural analogues to the corresponding pyridines **7a**–**j**. In general, this gave the majority of products in good yields (58–71%). While the three nitro analogues **6h**, **6i** and **6j** were also cleanly converted to the pyridines, challenging isolation and purification of these highly polar compounds resulted in more modest yields (36–40%). To expand the range of substituted pyridyl side-chains, two nitro-analogues were subjected to a chemoselective reduction using tin dichloride, which allowed access to amino-analogues **7k** and **7l**.[19] Finally, various deprotection strategies to access the parent amino acids were explored, however, direct acid-mediated removal of both the amine and carboxylic acid protecting groups was found to be the most efficient approach, giving all 12 structural analogues **8a**–**l** in high yields (86–99%).

Scheme 2. Synthesis of β-Pyridyl α-Amino Acids 8a–l[a]

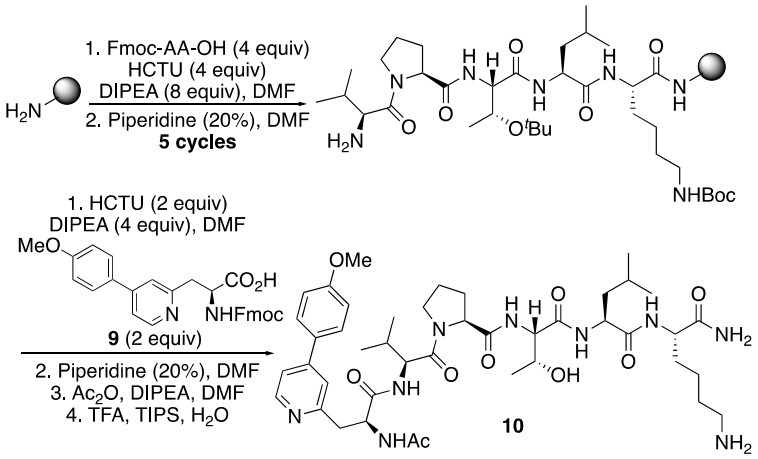


[a]Isolated yields are shown.

Having developed a general and effective route to β-pyridyl α-amino acids, the potential of these for various applications was explored. Initially, a study was conducted to demonstrate that these could be easily incorporated into peptides using routine Fmoc-based SPPS methodology. A pentapeptide, Val-Pro-Thr-Leu-Lys based on the Bax-binding domain of Ku70, a multifunctional protein involved in DNA repair and cell-death regulation was chosen as a model substrate.[20] Matsuyama and co-workers have previously shown that this pentapeptide and similar analogues have low cytotoxicity and are highly effective at penetrating living cells.[21] The Val-Pro-Thr-Leu-Lys pentapeptide was prepared in a microwave assisted

peptide synthesizer using Rink Amide ChemMatrix® resin as the polymer support[22] and routine SPPS methodology (Scheme 3). On coupling Fmoc-L-Lys(Boc)-OH onto the polymer support using HCTU activation, successive rounds of piperidine-mediated *N*-deprotection and coupling using the next amino acid gave the pentapeptide. Following a subsequent N-terminal Fmoc-deprotection, the Fmoc-protected version of β-pyridyl α-amino acid **8d**, compound **9** was coupled onto the polymer-supported pentapeptide.[23] After a final Fmoc-deprotection step, the N-terminus was acetyl capped and a TFA cleavage cocktail that included triisopropylsilane (TIPS) as a cation scavenger was used to remove the side-chain protecting groups and release the peptide from the polymer support. The resulting hexapeptide, **10** was purified by reverse phase-HPLC (>95% purity) and characterized by high-resolution electrospray ionization mass spectrometry (see Supporting Information for data).

Scheme 3. SPPS of a β-Pyridyl α-Amino Acid Containing Hexapeptide



As the α-amino acids contained π-deficient pyridine moieties, it was proposed that electron-rich conjugating groups may confer fluorescent properties through a charge-transfer (push-pull) mechanism.[24] Accordingly, the optical properties of β-pyridyl α-amino acids **8a**–**l** were measured. As expected, pyridines with non-conjugating or electron-deficient substituents showed weak fluorescence, while compounds with electron-rich substituents showed strong fluorescence (Figure 2b and Table 1). In

general, these amino acids showed emission maxima from 366 to 460 nm, with all compounds displaying large Stokes shifts. Amino acids **8c** and **8d** displayed particularly interesting optical properties. Naphthalene analogue **8c** gave a red-shifted emission spectrum with the main band at 452 nm and a MegaStokes shift of 193 nm. As well as the strongest fluorescence, 4-methoxyphenyl analogue **8d** displayed the highest quantum yield (0.46) and highest value of brightness (11923 cm^{-1} M^{-1}).

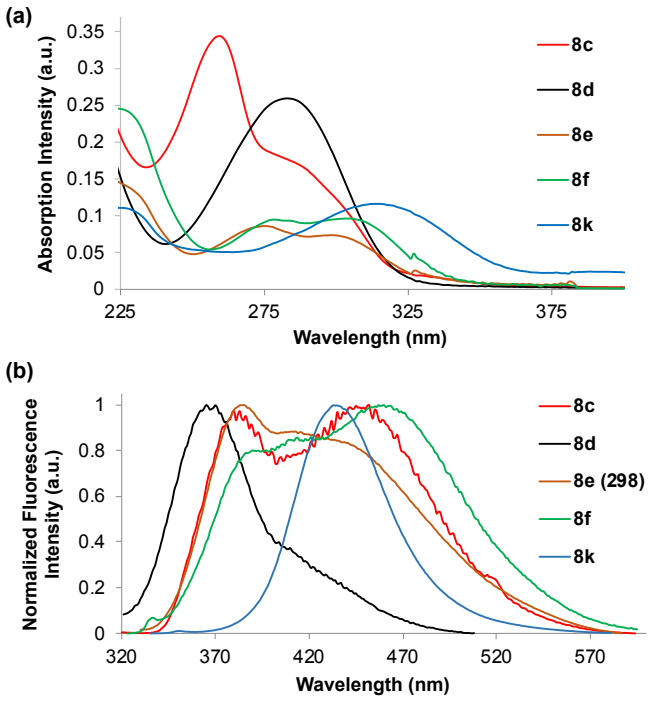


Figure 2. (a) Absorption spectra of amino acids **8c–8f** and **8k** recorded at 1×10^{-5} M in methanol. (b) Emission spectra of **8c** (0.5×10^{-5} M), **8d** (1×10^{-7} M) and **8e**, **8f** and **8g** (1×10^{-5} M), recorded in methanol.

Table 1. Photophysical data of pyridyl-derived α-amino acids 8c–f and 8k.

Amino acid	λ_{Abs} (nm)[a]	ε (cm^{-1} M^{-1})	λ_{Em} (nm)[a]	QY	Brightness (cm^{-1} M^{-1})
8c	259	34360	380, *452*[b]	0.18	6185
8d	283	25920	366[c]	0.46	11923
8e	275, *298*	8600	384, *413*	0.19	1634
8f	303	9620	460	0.06	577
8k	315	11600	433	0.04	464

Spectra were recorded in methanol at: [a] 1×10^{-5} M; [b] 0.5×10^{-5} M; [c] 1×10^{-7} M.

A solvatochromic study with **8d** was next performed to further explore the charge transfer properties of this β-pyridyl α-amino acid. The absorption maxima (278–280 nm) in a range of solvents were found to be independent of polarity, indicating negligible intramolecular interaction between the electron-rich methoxyphenyl and electron-deficient pyridine moieties in the ground state (see Supporting Information). In contrast, the emission maxima were found to be highly dependent on solvent polarity (Figure 3). In THF, a Stokes shift of 28 nm and an emission maximum of 308 nm was observed, while in phosphate-buffered saline (PBS), the emission maximum was found at 423 nm, with a Stokes shift of 143 nm. Such strong solvatochromism, which can be attributed to the stabilization of the highly polar excited-state charge transfer character of **8d** by polar solvents, is expected for compounds that undergo an internal charge transfer upon excitation.[25] These results show that **8d** is highly sensitive to the polarity of its surroundings, with fluorescence emission in the visible region under aqueous conditions (PBS).

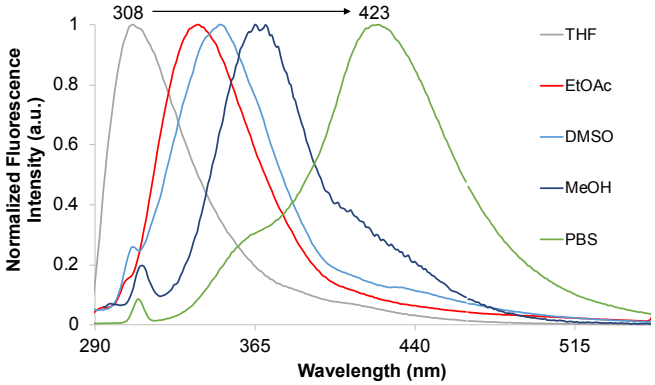


Figure 3. Emission spectra of **8d** in various solvents. All spectra were recorded using a concentration of 1×10^{-5} M, except for methanol which was recorded using a concentration of 1×10^{-7} M.

CONCLUSIONS

In summary, a new class of β-pyridyl α-amino acid has been synthesized, introducing side-chain diversity via a Horner-Wadsworth-Emmons reaction and using a highly regioselective lanthanide-catalyzed hetero Diels-Alder reaction and a modified Knoevenagel-Stobbe condensation to form the key pyridine ring-system. Despite the relatively small size of the amino acid side-chain, the electron-rich 4-arylpyridine chromophores were found to have strong charge-transfer based fluorescence, large Stokes shifts and high quantum yields. A solvatochromic study of the highly fluorogenic amino acid **8d** showed this compound to be particularly sensitive to the polarity of its surrounding with markedly different emission wavelengths in various solvents. Environment-sensitive amino acid **8d** was also directly incorporated into a biologically relevant peptide as part of a SPPS process. Work is underway on the preparation of further analogues in which the optical properties of these α-amino acids will be further tuned for potential spectroscopic and microscopic applications.

EXPERIMENTAL SECTION

The synthesis of dimethyl (2*S*)-2-aminobutandioate hydrochloride and, compounds **2**, **3**, **4a**–**4d**, **4g**–**4h**, **4j**, **5b**–**5d**, **5h** and **5j** has been previously described in the literature.[5,13,14a,14c,26] All reagents and starting

materials were obtained from commercial sources and used as received unless otherwise stated. Dry solvents were purified using a solvent purification system. Brine refers to a saturated solution of sodium chloride. All reactions were performed in oven-dried glassware under an atmosphere of argon unless otherwise stated. Flash column chromatography was carried out using silica gel (40–63 μm) and neutral aluminium oxide (50–200 μm). Aluminium-backed plates pre-coated with silica gel 60 (UV_{254}) were used for thin layer chromatography and were visualized under ultraviolet light and by staining with $KMnO_4$ or ninhydrin. ^1H NMR spectra were recorded on an NMR spectrometer at 400 or 500 MHz and data are reported as follows: chemical shift in ppm relative to tetramethylsilane or the solvent as the internal standard ($CDCl_3$, δ 7.26 ppm), multiplicity (s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet or overlap of nonequivalent resonances, integration). $^{13}C\{^1H\}$ NMR spectra were recorded on an NMR spectrometer at 101 or 126 MHz and data are reported as follows: chemical shift in ppm relative to tetramethylsilane or the solvent as internal standard ($CDCl_3$, δ 77.0 ppm), multiplicity with respect to hydrogen (deduced from DEPT experiments, C, CH, CH_2 or CH_3). IR spectra were recorded on a FTIR spectrometer; wavenumbers are indicated in cm^{-1}. Mass spectra were recorded using electron impact or electrospray ionization techniques. HRMS spectra were recorded using a dual-focusing magnetic analyzer mass spectrometer. Melting points are uncorrected. Optical rotations were determined as solutions irradiating with the sodium D line (λ = 589 nm) using a polarimeter. $[\alpha]_D$ values are given in units 10^{-1} deg cm^2 g^{-1}. Fluorescence spectra were recorded on a spectrofluorophotometer. Emission data were measured using an excitation slit width of 3 nm and emission slit width of 5 nm. Quantum yield data were measured using anthracene and L-tryptophan as standard references.

Methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (4e). Methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**3**) (1.11 g, 2.24 mmol) was dissolved in acetonitrile (24 mL) and potassium carbonate (0.370 g, 2.68 mmol) was added. The mixture was stirred at room temperature for 0.5 h followed by addition of 2,4-dimethoxybenzaldehyde (0.743 g, 4.48 mmol). The temperature was increased to 50 °C and the mixture was stirred for 120 h. Once the reaction was

complete, the solution was concentrated *in vacuo*, redissolved in ethyl acetate (40 mL) and washed with water (2 × 30 mL) and brine (30 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography on silica gel eluting with 30% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4e**) (0.564 g, 47%) as a yellow foam. Mp 66–70 °C; R_f 0.15 (30% ethyl acetate in petroleum ether); IR (neat) 2948, 1737, 1599, 1266, 1210, 1160, 1028, 734, 706 cm^{-1}; $[\alpha]_D^{26}$ +52.3 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.83 (dd, *J* = 14.9, 6.8 Hz, 1H), 2.87–2.95 (m, 2H), 3.25 (s, 3H), 3.74–3.82 (m, 1H), 3.83 (s, 3H), 3.84 (s, 3H), 6.45 (d, *J* = 2.3 Hz, 1H), 6.51 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.69 (d, *J* = 16.3 Hz, 1H), 7.12–7.27 (m, 9H), 7.44–7.54 (m, 7H), 7.82 (d, *J* = 16.3 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 45.3 (CH_2), 51.8 (CH_3), 53.9 (CH), 55.5 (CH_3), 55.5 (CH_3), 71.3 (C), 98.4 (CH), 105.5 (CH), 116.5 (C), 124.8 (CH), 126.4 (3 × CH), 127.8 (6 × CH), 128.9 (6 × CH), 130.2 (CH), 138.8 (CH), 145.9 (3 × C), 160.1 (C), 163.2 (C), 174.6 (C), 198.0 (C); MS (ESI) *m/z* 558 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{34}H_{33}NNaO_5$ ($M+Na^+$) 558.2251, found 558.2227.

Methyl (2*S*,5*E*)-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (4f). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4e**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**3**) (0.215 g, 0.434 mmol) and piperonal (0.130 g, 0.868 mmol) for 120 h. Purification by flash column chromatography on silica gel eluting with 20% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**4f**) (0.201 g, 89%) as a yellow oil. R_f 0.12 (20% ethyl acetate in petroleum ether); IR (neat) 3055, 2898, 1734, 1594, 1489, 1447, 1254, 1035, 705 cm^{-1}; $[\alpha]_D^{26}$ +49.2 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 2.75 (dd, *J* = 15.1, 6.8 Hz, 1H), 2.84–2.95 (m, 2H), 3.27 (s, 3H), 3.78 (dt, *J* = 9.3, 6.8 Hz, 1H), 5.98 (s, 2H), 6.52 (d, *J* = 16.1 Hz, 1H), 6.81 (d, *J* = 7.9 Hz, 1H), 6.97–7.04 (m, 2H), 7.13–7.29 (m, 9H), 7.39 (d, *J* = 16.1 Hz, 1H), 7.47–7.53 (m, 6H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 45.8 (CH_2), 51.9 (CH_3), 53.8 (CH), 71.3 (C), 101.6 (CH_2), 106.6 (CH), 108.6 (CH), 124.5 (CH), 125.0 (CH), 126.5 (3 × CH), 127.9 (6

× CH), 128.1 (C), 128.8 (6 × CH), 143.1 (CH), 145.8 (3 × C), 148.5 (C), 150.0 (C), 174.5 (C), 197.2 (C); MS (ESI) *m/z* 542 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{33}H_{29}NNaO_5$ ($M+Na^+$) 542.1938, found 542.1915.

Methyl (2*S*,5*E*)-6-(2',4'-dinitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (4i). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4e**) using methyl (2*S*)-5-(dimethoxyphosphoryl)-4-oxo-2-(tritylamino)pentanoate (**3**) (1.06 g, 2.15 mmol) and 2,4-dinitrobenzaldehyde (0.842 g, 4.29 mmol) for 1 h. Purification by flash column chromatography on silica gel eluting with 60% diethyl ether in petroleum ether (40–60) gave methyl (2*S*,5*E*)-6-(2',4'-dinitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4i**) (0.801 g, 66%) as an orange solid. Mp 58–61 °C; R_f 0.25 (60% diethyl ether in petroleum ether); IR (neat) 3057, 2952, 1734, 1596, 1531, 1448, 1266, 834, 706 cm^{-1}; $[\alpha]_D^{26}$ +37.7 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 2.79 (dd, J = 15.8, 6.8 Hz, 1H), 2.89 (dd, J = 15.8, 5.0 Hz, 1H), 2.96 (br s, 1H), 3.35 (s, 3H), 3.78–3.84 (m, 1H), 6.60 (d, J = 16.1 Hz, 1H), 7.15–7.28 (m, 9H), 7.43–7.52 (m, 6H), 7.78 (d, J = 8.6 Hz, 1H), 7.86 (d, J = 16.1 Hz, 1H), 8.46 (dd, J = 8.6, 2.3 Hz, 1H), 8.90 (d, J = 2.3 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 45.5 (CH_2), 52.1 (CH_3), 53.5 (CH), 71.3 (C), 120.7 (CH), 126.6 (3 × CH), 127.7 (CH), 128.0 (6 × CH), 128.8 (6 × CH), 130.6 (CH), 133.4 (CH), 136.0 (CH), 136.6 (C), 145.6 (3 × C), 148.1 (C), 148.2 (C), 174.1 (C), 196.5 (C); MS (ESI) *m/z* 588 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{32}H_{27}N_3NaO_7$ ($M+Na^+$) 588.1741, found 588.1714.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (5a). To a solution of methyl (2*S*,5*E*)-2-(tritylamino)-4-oxonon-5-enoate (**4a**) (0.398 g, 0.902 mmol) in dichloromethane (10 mL) was added trifluoroacetic acid (0.138 mL, 1.80 mmol). The reaction mixture was stirred at room temperature for 2 h before concentrating *in vacuo*. The resulting residue was dissolved in chloroform (5 mL) and petroleum ether (40–60) was added until an orange oil formed, at which point the solvent was decanted off. The resulting oil was dissolved in dichloromethane (10 mL) and *N*,*N*'-diisopropylethylamine (0.397 mL, 2.27 mmol) was added, followed by benzyl chloroformate (0.200 mL, 1.36 mmol). The reaction

mixture was stirred at room temperature for 1 h, before diluting with water (30 mL). The mixture was then extracted with dichloromethane (3 × 15 mL), dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography on silica gel eluting with 30% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**5a**) (0.201 g, 67%) as a white solid. Mp 40–42 °C; R_f 0.29 (30% ethyl acetate in petroleum ether); IR (neat) 3352, 2959, 1723, 1627, 1503, 1209, 1061, 979, 737, 698 cm^{-1}; $[\alpha]_D^{27}$ +23.7 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 0.94 (t, *J* = 7.4 Hz, 3H), 1.44–1.55 (m, 2H), 2.20 (qd, *J* = 7.2, 1.4 Hz, 2H), 3.11 (dd, *J* = 18.0, 4.2 Hz, 1H), 3.34 (dd, *J* = 18.0, 4.2 Hz, 1H), 3.73 (s, 3H), 4.62 (dt, *J* = 8.5, 4.2 Hz, 1H), 5.11 (s, 2H), 5.79 (d, *J* = 8.5 Hz, 1H), 6.06 (dt, *J* = 15.8, 1.4 Hz, 1H), 6.86 (dt, *J* = 15.8, 7.2 Hz, 1H), 7.28–7.39 (m, 5H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 13.8 (CH_3), 21.4 (CH_2), 34.7 (CH_2), 41.7 (CH_2), 50.1 (CH), 52.8 (CH_3), 67.2 (CH_2), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 130.1 (CH), 136.4 (C), 149.4 (CH), 156.2 (C), 171.8 (C), 197.8 (C); MS (ESI) *m/z* 356 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{18}H_{23}NNaO_5$ ($M+Na^+$) 356.1468, found 356.1455.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dimethoxyphenyl)-4-oxohex-5-enoate (5e). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**5a**) using methyl (2*S*,5*E*)-6-(2',4'-dimethoxyphenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4e**) (0.550 g, 1.03 mmol). Purification by flash column chromatography on silica gel eluting with 40% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dimethoxyphenyl)-4-oxohex-5-enoate (**5e**) (0.168 g, 70%) as a yellow oil. R_f 0.16 (40% ethyl acetate in petroleum ether); IR (neat) 3362, 2949, 1722, 1600, 1504, 1210, 1028 cm^{-1}; $[\alpha]_D^{26}$ +19.6 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.23 (dd, *J* = 17.9, 4.2 Hz, 1H), 3.47 (dd, *J* = 17.9, 4.2 Hz, 1H), 3.73 (s, 3H), 3.83 (s, 3H), 3.85 (s, 3H), 4.67 (dt, *J* = 8.7, 4.2 Hz, 1H), 5.11 (s, 2H), 5.91 (d, *J* = 8.7 Hz, 1H), 6.44 (d, *J* = 2.3 Hz, 1H), 6.50 (dd, *J* = 8.6, 2.3 Hz, 1H), 6.66 (d, *J* = 16.4 Hz, 1H), 7.27–7.39 (m, 5H), 7.45 (d, *J* = 8.6 Hz, 1H), 7.84 (d, *J* = 16.4 Hz, 1H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 41.8 (CH_2), 50.2 (CH), 52.6 (CH_3), 55.5 (CH_3), 55.5 (CH_3), 66.9

(CH_2), 98.4 (CH), 105.6 (CH), 116.1 (C), 123.6 (CH), 128.0 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 130.3 (CH), 136.3 (C), 139.4 (CH), 156.1 (C), 160.1 (C), 163.4 (C), 171.8 (C), 197.8 (C); MS (ESI) *m/z* 450 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{23}H_{25}NNaO_7$ ($M+Na^+$) 450.1523, found 450.1503.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxohex-5-enoate (5f). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**5a**) using methyl (2*S*,5*E*)-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxo-2-(tritylamino)hex-5-enoate (**4f**) (1.51 g, 2.91 mmol). Purification by flash column chromatography on silica gel eluting with 40% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxohex-5-enoate (**5f**) (0.972 g, 81%) as a yellow oil. R_f 0.20 (40% ethyl acetate in petroleum ether); IR (neat) 3369, 2953, 1721, 1503, 1490, 1447, 1253, 1035, 929, 737, 698 cm^{-1}; $[\alpha]_D^{29}$ +28.6 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.21 (dd, *J* = 17.9, 4.2 Hz, 1H), 3.43 (dd, *J* = 17.9, 4.2 Hz, 1H), 3.74 (s, 3H), 4.67 (dt, *J* = 8.6, 4.2 Hz, 1H), 5.11 (s, 2H), 5.88 (d, *J* = 8.6 Hz, 1H), 6.01 (s, 2H), 6.52 (d, *J* = 16.1 Hz, 1H), 6.81 (d, *J* = 8.1 Hz, 1H), 6.99–7.05 (m, 2H), 7.28–7.38 (m, 5H), 7.46 (d, *J* = 16.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 42.3 (CH_2), 50.1 (CH), 52.7 (CH_3), 67.0 (CH_2), 101.7 (CH_2), 106.6 (CH), 108.7 (CH), 123.5 (CH), 125.3 (CH), 128.0 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 128.5 (C), 136.3 (C), 143.9 (CH), 148.5 (C), 150.2 (C), 156.1 (C), 171.7 (C), 197.2 (C); MS (ESI) *m/z* 434 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{22}H_{21}NNaO_7$ ($M+Na^+$) 434.1210, found 434.1192.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-fluorophenyl)-4-oxohex-5-enoate (5g). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**5a**) using methyl (2*S*,5*E*)-6-(4-fluorophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4g**) (0.367 g, 0.744 mmol). Purification by flash column chromatography on silica gel eluting with 30% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-fluorophenyl)-4-oxohex-5-enoate (**5g**) (0.174 g, 57%) as a white solid. Mp 66–69 °C; R_f 0.20 (30% ethyl acetate in petroleum ether); IR (neat) 3333, 2951, 1748, 1730,

1685, 1508, 1236, 1161, 981, 829, 698 cm^{-1}; $[\alpha]_D^{27}$ +26.5 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.24 (dd, *J* = 17.9, 4.2 Hz, 1H), 3.46 (dd, *J* = 17.9, 4.2 Hz, 1H), 3.75 (s, 3H), 4.68 (dt, *J* = 8.5, 4.2 Hz, 1H), 5.12 (s, 2H), 5.84 (d, *J* = 8.5 Hz, 1H), 6.62 (d, *J* = 16.2 Hz, 1H), 7.06–7.13 (m, 2H), 7.28–7.39 (m, 5H), 7.49–7.58 (m, 3H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 42.5 (CH_2), 50.2 (CH), 52.9 (CH_3), 67.2 (CH_2), 116.4 (d, $^2J_{C\text{-}F}$ 22.0 Hz, 2 × CH), 125.3 (CH), 128.2 (2 × CH), 128.3 (CH), 128.7 (2 × CH), 130.5 (C), 130.5 (d, $^3J_{C\text{-}F}$ 8.5 Hz, 2 × CH), 136.4 (C), 142.9 (CH), 156.2 (C), 164.4 (d, $^1J_{C\text{-}F}$ 252.6 Hz, C), 171.7 (C), 197.4 (C); MS (ESI) *m/z* 408 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{21}H_{20}FNNaO_5$ ($M+Na^+$) 408.1218, found 408.1201.

Methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dinitrophenyl)-4-oxohex-5-enoate (5i). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**5a**) using methyl (2*S*,5*E*)-6-(2',4'-dinitrophenyl)-4-oxo-2-(tritylamino)hex-5-enoate (**4i**) (0.620 g, 1.10 mmol). Purification by flash column chromatography on silica gel eluting with 40% ethyl acetate in petroleum ether (40–60) gave methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dinitrophenyl)-4-oxohex-5-enoate (**5i**) (0.293 g, 59%) as a brown solid. Mp 88–91 °C; R_f 0.15 (40% ethyl acetate in petroleum ether); IR (neat) 3382, 2955, 1720, 1597, 1528, 1343, 1211, 835, 736 cm^{-1}; $[\alpha]_D^{27}$ +19.7 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.34 (dd, *J* = 18.1, 4.3 Hz, 1H), 3.49 (dd, *J* = 18.1, 4.3 Hz, 1H), 3.76 (s, 3H), 4.74 (dt, *J* = 8.5, 4.3 Hz, 1H), 5.10 (s, 2H), 5.91 (d, *J* = 8.5 Hz, 1H), 6.70 (d, *J* = 16.1 Hz, 1H), 7.26–7.36 (m, 5H), 7.85 (d, *J* = 8.6 Hz, 1H), 8.01 (d, *J* = 16.1 Hz, 1H), 8.49 (dd, *J* = 8.6, 2.3 Hz, 1H), 8.89 (d, *J* = 2.3 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 42.7 (CH_2), 49.9 (CH), 52.9 (CH_3), 67.0 (CH_2), 120.7 (CH), 127.8 (CH), 128.0 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 130.7 (CH), 132.4 (CH), 136.1 (C), 136.4 (C), 136.9 (CH), 148.1 (C), 148.2 (C), 156.0 (C), 171.3 (C), 196.5 (C); MS (ESI) *m/z* 480 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{21}H_{19}N_3NaO_9$ ($M+Na^+$) 480.1014, found 480.0997.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (7b). To a solution of methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**5b**) (0.186 g,

0.506 mmol) in ethyl vinyl ether (5 mL) was added tris(6,6,7,7,8,8,8-heptafluoro-2,2-dimethyl-3,5-octanedionato)ytterbium (0.0269 g, 0.0253 mmol). The reaction tube was then purged with argon, sealed and the reaction mixture was stirred at 110 °C for 96 h. The mixture was then allowed to cool to room temperature and concentrated *in vacuo*. The reaction mixture was washed through a silica plug eluting with 20% ethyl acetate in petroleum ether (40–60) and gave dihydropyran **6b** (0.208 g, 93%). Dihydropyran **6b** (0.208 g, 0.476 mmol) was then added to a solution of hydroxylamine hydrochloride (0.166 g, 2.38 mmol) in acetonitrile (5 mL). The reaction mixture was stirred at 70 °C for 16 h and then concentrated *in vacuo*. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) (0.131 g, 71%) as a yellow oil. R_f 0.79 (1% methanol in dichloromethane); IR (neat) 3346, 2951, 1719, 1506, 1212, 1061, 764, 697 cm^{-1}; $[\alpha]_D^{22}$ +27.6 (*c* 1.1, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.36 (dd, J = 14.9, 5.2 Hz, 1H), 3.44 (dd, J = 14.9, 5.2 Hz, 1H), 3.70 (s, 3H), 4.81 (dt, J = 8.3, 5.2 Hz, 1H), 5.10 (d, J = 12.4 Hz, 1H), 5.13 (d, J = 12.4 Hz, 1H), 6.35 (d, J = 8.3 Hz, 1H), 7.29–7.37 (m, 7H), 7.42–7.50 (m, 3H), 7.56–7.63 (m, 2H), 8.51 (d, J = 5.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.2 (CH_2), 52.5 (CH_3), 53.6 (CH), 67.0 (CH_2), 120.1 (CH), 121.8 (CH), 127.1 (2 × CH), 128.2 (3 × CH), 128.6 (2 × CH), 129.2 (2 × CH), 129.2 (CH), 136.6 (C), 138.1 (C), 149.3 (C), 149.7 (CH), 156.2 (C), 157.6 (C), 172.2 (C); MS (ESI) *m/z* 413 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{23}H_{22}N_2NaO_4$ ($M+Na^+$) 413.1472, found 413.1461.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-propylpyridin-2'-yl)propanoate (7a). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxonon-5-enoate (**5a**) (0.103 g, 0.309 mmol) for 96 h. This gave dihydropyran **6a** (0.108 g, 86%). Dihydropyran **6a** (0.107 g, 0.264 mmol) was then treated with hydroxylamine hydrochloride (0.0917 g, 1.32 mmol) in acetonitrile (3 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in

dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-propylpyridin-2'-yl)propanoate (**7a**) (0.054 g, 58%) as a pale yellow oil. R_f 0.85 (1% methanol in dichloromethane on alumina); IR (neat) 3344, 2959, 1721, 1606, 1506, 1209, 1055, 738, 697 cm^{-1}; $[\alpha]_D^{22}$ +18.0 (*c* 0.7, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 0.92 (t, *J* = 7.3 Hz, 3H), 1.57–1.67 (m, 2H), 2.52 (t, *J* = 7.3 Hz, 2H), 3.23 (dd, *J* = 14.8, 5.2 Hz, 1H), 3.32 (dd, *J* = 14.8, 5.2 Hz, 1H), 3.67 (s, 3H), 4.74 (dt, *J* = 8.2, 5.2 Hz, 1H), 5.08 (d, *J* = 12.4 Hz, 1H), 5.12 (d, *J* = 12.4 Hz, 1H), 6.38 (d, *J* = 8.2 Hz, 1H), 6.91–6.97 (m, 2H), 7.28–7.37 (m, 5H), 8.33 (d, *J* = 5.0 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 13.8 (CH_3), 23.5 (CH_2), 37.3 (CH_2), 38.9 (CH_2), 52.4 (CH_3), 53.6 (CH), 66.9 (CH_2), 122.3 (CH), 124.0 (CH), 128.2 (2 × CH), 128.2 (CH), 128.6 (2 × CH), 136.6 (C), 149.1 (CH), 152.3 (C), 156.2 (C), 156.9 (C), 172.2 (C); MS (EI) *m/z* 356 (M^+, 46), 297 (36), 253 (22), 189 (48), 135 (95), 91 (100); HRMS (EI) calcd for $C_{20}H_{24}N_2O_4$ (M^+) 356.1736, found 356.1729.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoate (7c). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(naphthalen-2'-yl)-4-oxohex-5-enoate (**5c**) (0.110 g, 0.264 mmol) for 72 h. This gave dihydropyran **6c** (0.129 g, 83%). Dihydropyran **6c** (0.129 g, 0.264 mmol) was then reacted with hydroxylamine hydrochloride (0.0917 g, 1.32 mmol) in acetonitrile (3 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoate (**7c**) (0.056 g, 48%) as a yellow solid. Mp 56–60 °C; R_f 0.84 (1% methanol in dichloromethane); IR (neat) 3346, 3054, 2951, 1717, 1597, 1504, 1437, 1210, 1056, 1028, 816, 734 cm^{-1}; $[\alpha]_D^{25}$ +26.1 (*c* 0.9, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.39 (dd, *J* = 15.0, 5.2 Hz, 1H), 3.48 (dd, *J* = 15.0, 5.2 Hz, 1H), 3.71 (s, 3H), 4.84 (dt, *J* = 8.4, 5.2 Hz, 1H), 5.10 (d, *J* = 12.4 Hz, 1H), 5.13 (d, *J* = 12.4 Hz, 1H), 6.38 (d, *J* = 8.4 Hz, 1H), 7.27–7.37 (m, 5H), 7.45–7.49 (m, 2H), 7.52–7.57 (m, 2H), 7.70 (dd, *J* = 8.6, 1.5 Hz, 1H), 7.86–7.92 (m, 2H), 7.94 (d, *J* = 8.6 Hz, 1H), 8.07 (br s, 1H), 8.55 (d, *J* = 5.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.3 (CH_2), 52.6 (CH_3), 53.6 (CH), 67.1 (CH_2), 120.3

(CH), 121.9 (CH), 124.7 (CH), 126.6 (CH), 126.9 (CH), 127.0 (CH), 127.9 (CH), 128.2 (3 × CH), 128.6 (CH), 128.6 (2 × CH), 129.1 (CH), 133.6 (C), 133.6 (C), 135.4 (C), 136.5 (C), 149.2 (C), 149.8 (CH), 156.3 (C), 157.7 (C), 172.3 (C); MS (EI) *m/z* 440 (M^+, 38), 381 (17), 332 (45), 305 (43), 273 (76), 245 (96), 219 (98), 189 (24), 91 (100); HRMS (EI) calcd for $C_{27}H_{24}N_2O_4$ (M^+) 440.1736, found 440.1738.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoate (7d). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-methoxyphenyl)-4-oxohex-5-enoate (**5d**) (0.602 g, 1.52 mmol) for 168 h. This gave dihydropyran **6d** (0.595 g, 83%). Dihydropyran **6d** (0.563 g, 1.20 mmol) was then reacted with hydroxylamine hydrochloride (0.416 g, 6.00 mmol) in acetonitrile (12 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade), eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoate (**7d**) (0.242 g, 48%) as a yellow oil. R_f 0.81 (1% methanol in dichloromethane); IR (neat) 3341, 2953, 1718, 1603, 1516, 1251, 1180, 1026, 826, 698 cm^{-1}; $[\alpha]_D^{25}$ +23.0 (*c* 0.8, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.33 (dd, *J* 14.9, 5.2 Hz, 1H), 3.42 (dd, *J* = 14.9, 5.2 Hz, 1H), 3.70 (s, 3H), 3.86 (s, 3H), 4.80 (dt, *J* = 8.2, 5.2 Hz, 1H), 5.09 (d, *J* = 12.3 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 6.36 (d, *J* = 8.2 Hz, 1H), 6.99 (d, *J* = 8.7 Hz, 2H), 7.29–7.35 (m, 7H), 7.55 (d, *J* = 8.7 Hz, 2H), 8.47 (d, *J* = 5.2 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.2 (CH_2), 52.5 (CH_3), 53.6 (CH), 55.6 (CH_3), 67.0 (CH_2), 114.7 (2 × CH_2), 119.6 (CH), 121.2 (CH), 128.2 (3 × CH), 128.3 (2 × CH), 128.6 (2 × CH), 130.4 (C), 136.6 (C), 148.8 (C), 149.6 (CH), 156.3 (C), 157.5 (C), 160.8 (C), 172.3 (C); MS (ESI) *m/z* 443 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{24}H_{24}N_2NaO_5$ ($M+Na^+$) 443.1577, found 443.1556.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoate (7e). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dimethoxyphenyl)-4-oxohex-5-enoate (**5e**) (0.140 g,

0.327 mmol) for 192 h. This gave dihydropyran **6e** (0.136 g, 83%). Dihydropyran **6e** (0.126 g, 0.252 mmol) was then treated with hydroxylamine hydrochloride (0.0876 g, 1.26 mmol) in acetonitrile (5 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoate (**7e**) (0.065 g, 57%) as a pale yellow oil. R_f 0.78 (1% methanol in dichloromethane on alumina); IR (neat) 3343, 2951, 1719, 1604, 1508, 1207, 1028, 737, 698 cm^{-1}; $[\alpha]_D^{27}$ +22.9 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.30 (dd, *J* = 14.8, 5.2 Hz, 1H), 3.40 (dd, *J* = 14.8, 5.2 Hz, 1H), 3.70 (s, 3H), 3.79 (s, 3H), 3.85 (s, 3H), 4.77 (dt, *J* = 8.2, 5.2 Hz, 1H), 5.09 (d, *J* = 12.6 Hz, 1H), 5.12 (d, *J* = 12.6 Hz, 1H), 6.39 (d, *J* = 8.2 Hz, 1H), 6.52–6.60 (m, 2H), 7.22–7.38 (m, 8H), 8.44 (d, *J* = 5.2 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.0 (CH_2), 52.3 (CH_3), 53.5 (CH), 55.5 (CH_3), 55.5 (CH_3), 66.9 (CH_2), 99.1 (CH), 105.0 (CH), 120.3 (C), 122.4 (CH), 124.0 (CH), 128.0 (CH), 128.1 (2 × CH), 128.5 (2 × CH), 131.1 (CH), 136.4 (C), 146.8 (C), 148.8 (CH), 156.1 (C), 156.4 (C), 157.7 (C), 161.5 (C), 172.2 (C); MS (ESI) *m/z* 451 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{25}H_{27}N_2O_6$ ($M+H^+$) 451.1840, found 451.1846.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoate (7f). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2*H*-1',3'-benzodioxol-5'-yl)-4-oxohex-5-enoate (**5f**) (0.972 g, 2.36 mmol) for 168 h. This gave dihydropyran **6f** (0.990 g, 87%). Dihydropyran **6f** (0.990 g, 2.05 mmol) was then treated with hydroxylamine hydrochloride (0.711 g, 10.2 mmol) in acetonitrile (20 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoate (**7f**) (0.530 g, 59%) as an orange oil. R_f 0.80 (1% methanol in dichloromethane on alumina); IR (neat) 3358, 2953, 1721, 1603, 1506, 1476, 1236, 1040, 808 cm^{-1}; $[\alpha]_D^{27}$ +28.0 (*c* 1.1, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.35 (dd, *J* = 15.0, 5.1 Hz, 1H),

3.44 (dd, J = 15.0, 5.1 Hz, 1H), 3.72 (s, 3H), 4.82 (dt, J = 8.2, 5.1 Hz, 1H), 5.11 (d, J = 12.5 Hz, 1H), 5.15 (d, J = 12.5 Hz, 1H), 6.05 (s, 2H), 6.35 (d, J = 8.2 Hz, 1H), 6.92 (d, J = 8.0 Hz, 1H), 7.09 (d, J = 2.0 Hz, 1H), 7.12 (dd, J = 8.0, 2.0 Hz, 1H), 7.26–7.40 (m, 7H), 8.49 (d, J = 5.2 Hz, 1H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 39.1 (CH_2), 52.4 (CH_3), 53.4 (CH), 66.9 (CH_2), 101.5 (CH_2), 107.2 (CH), 108.9 (CH), 119.6 (CH), 121.0 (CH), 121.2 (CH), 128.1 (2 × CH), 128.5 (3 × CH), 132.1 (C), 136.4 (C), 148.5 (C), 148.6 (C), 148.7 (C), 149.5 (CH), 156.1 (C), 157.4 (C), 172.1 (C); MS (ESI) *m/z* 457 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{24}H_{22}N_2NaO_6$ ($M+Na^+$) 457.1370, found 457.1355.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoate (7g). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-4-oxo-6-phenylhex-5-enoate (**5g**) (0.126 g, 0.310 mmol) for 96 h. This gave dihydropyran **6g** (0.0980 g, 70%). Dihydropyran **6g** (0.0872 g, 0.191 mmol) was then treated with hydroxylamine hydrochloride (0.0616 g, 0.955 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoate (**7g**) (0.046 g, 60%) as a white solid. Mp 48–52 °C; R_f 0.79 (1% methanol in dichloromethane); IR (neat) 3346, 2952, 1719, 1606, 1513, 1223, 1059, 827 cm^{-1}; $[\alpha]_D^{22}$ +25.5 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.35 (dd, J = 15.0, 5.2 Hz, 1H), 3.44 (dd, J = 15.0, 5.2 Hz, 1H), 3.70 (s, 3H), 4.81 (dt, J = 8.3, 5.2 Hz, 1H), 5.09 (d, J = 12.3 Hz, 1H), 5.13 (d, J = 12.3 Hz, 1H), 6.31 (d, J = 8.3 Hz, 1H), 7.13–7.19 (m, 2H), 7.28–7.37 (m, 7H), 7.53–7.60 (m, 2H), 8.51 (d, J = 5.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.2 (CH_2), 52.5 (CH_3), 53.5 (CH), 67.1 (CH_2), 116.3 (d, $^2J_{C\text{-}F}$ = 21.8 Hz, 2 × CH), 120.0 (CH), 121.6 (CH), 128.2 (3 × CH), 128.6 (2 × CH), 128.9 (d, $^3J_{C\text{-}F}$ = 8.3 Hz, 2 × CH), 134.3 (d, $^4J_{C\text{-}F}$ = 3.2 Hz, C), 136.5 (C), 148.2 (C), 149.8 (CH), 156.2 (C), 157.8 (C), 163.6 (d, $^1J_{C\text{-}F}$ = 249.4 Hz, C), 172.2 (C); MS (ESI) *m/z* 431 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{23}H_{21}FN_2NaO_4$ ($M+Na^+$) 431.1378, found 431.1364.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (7h). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(4'-nitrophenyl)-4-oxohex-5-enoate (**5h**) (0.104 g, 0.252 mmol) for 96 h. This gave dihydropyran **6h** (0.097 g, 79%). Dihydropyran **6h** (0.0968 g, 0.199 mmol) was then reacted with hydroxylamine hydrochloride (0.0691 g, 0.995 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (**7h**) (0.035 g, 40%) as a yellow solid. Mp 79–82 °C; R_f 0.79 (1% methanol in dichloromethane); IR (neat) 3350, 2952, 1719, 1595, 1346, 1265, 1211, 1176, 1108, 1051, 735 cm^{-1}; $[\alpha]_D^{25}$ +25.7 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.40 (dd, *J* = 15.1, 5.1 Hz, 1H), 3.48 (dd, *J* = 15.1, 5.1 Hz, 1H), 3.72 (s, 3H), 4.84 (dt, *J* = 8.4, 5.1 Hz, 1H), 5.09 (d, *J* = 12.3 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 6.21 (d, *J* = 8.4 Hz, 1H), 7.28–7.39 (m, 7H), 7.74 (d, *J* = 8.7 Hz, 2H), 8.33 (d, *J* = 8.7 Hz, 2H), 8.59 (d, *J* = 5.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.2 (CH_2), 52.5 (CH_3), 53.3 (CH), 67.0 (CH_2), 120.0 (CH), 121.8 (CH), 124.4 (2 × CH), 128.1 (2 × CH), 128.1 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 136.3 (C), 144.4 (C), 146.7 (C), 148.3 (C), 150.0 (CH), 156.0 (C), 158.2 (C), 172.0 (C); MS (ESI) *m/z* 434 ($[M-H]^-$, 100); HRMS (ESI) calcd for $C_{23}H_{20}N_3O_6$ ($[M-H]^-$) 434.1358, found 434.1347.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (7i). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(2',4'-dinitrophenyl)-4-oxohex-5-enoate (**5i**) (0.198 g, 0.432 mmol) for 168 h. This gave dihydropyran **6i** (0.193 g, 84%). Dihydropyran **6i** (0.193 g, 0.365 mmol) was then treated with hydroxylamine hydrochloride (0.127 g, 1.83 mmol) in acetonitrile (5 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1%

methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (**7i**) (0.068 g, 38%) as an orange oil. R_f 0.87 (1% methanol in dichloromethane on alumina); IR (neat) 3364, 2953, 1721, 1599, 1532, 1346, 1211, 833, 741 cm^{-1}; $[\alpha]_D^{27}$ +19.5 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.38 (dd, *J* = 15.1, 5.2 Hz, 1H), 3.45 (dd, *J* = 15.1, 5.2 Hz, 1H), 3.72 (s, 3H), 4.79–4.86 (m, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 6.16 (d, *J* = 8.3 Hz, 1H), 7.07–7.11 (m, 2H), 7.27–7.39 (m, 5H), 7.59 (d, *J* = 8.4 Hz, 1H), 8.50 (dd, *J* = 8.4, 2.1 Hz, 1H), 8.60 (d, *J* = 5.7 Hz, 1H), 8.83 (d, *J* = 2.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.2 (CH_2), 52.6 (CH_3), 53.1 (CH), 67.0 (CH_2), 120.2 (CH), 120.4 (CH), 122.2 (CH), 127.1 (CH), 128.2 (2 × CH), 128.2 (CH), 128.5 (2 × CH), 132.9 (CH), 136.3 (C), 139.6 (C), 144.2 (C), 147.8 (C), 148.5 (C), 149.7 (CH), 156.0 (C), 158.1 (C), 171.8 (C); MS (ESI) *m/z* 503 ($M+Na^+$, 100); HRMS (ESI) calcd for $C_{23}H_{20}N_4NaO_8$ ($M+Na^+$) 503.1173, found 503.1154.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoate (7j). Dihydropyran formation was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) using methyl (2*S*,5*E*)-2-[(benzyloxycarbonyl)amino]-6-(3''-nitrobiphen-4'-yl)-4-oxohex-5-enoate (**5j**) (0.103 g, 0.211 mmol) for 96 h. This gave dihydropyran **6j** (0.095 g, 86%). Dihydropyran **6j** (0.100 g, 0.191 mmol) was then treated with hydroxylamine hydrochloride (0.0664 g, 0.955 mmol) in acetonitrile (2 mL) at 70 °C for 16 h. Purification by flash column chromatography on neutral alumina (Brockmann V grade) eluting with 1% methanol in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoate (**7j**) (0.035 g, 36%) as an off-white solid. Mp 48–50 °C; R_f 0.70 (1% methanol in dichloromethane on alumina); IR (neat) 3340, 2951, 1719, 1602, 1519, 1349, 1212, 1062, 730 cm^{-1}; $[\alpha]_D^{24}$ +22.1 (*c* 0.6, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.38 (dd, *J* = 14.9, 5.2 Hz, 1H), 3.47 (dd, *J* = 14.9, 5.2 Hz, 1H), 3.72 (s, 3H), 4.83 (dt, *J* = 8.3, 5.2 Hz, 1H), 5.10 (d, *J* = 12.3 Hz, 1H), 5.13 (d, *J* = 12.3 Hz, 1H), 6.34 (d, *J* = 8.3 Hz, 1H), 7.27–7.43 (m, 7H), 7.65 (t, *J* = 8.0 Hz, 1H), 7.71–7.78 (m, 4H), 7.96 (ddd, *J* = 8.0, 1.9, 1.0 Hz, 1H), 8.24 (ddd, *J* = 8.0, 1.9, 1.0 Hz, 1H), 8.49

(t, J = 1.9 Hz, 1H), 8.55 (d, J = 5.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.2 (CH_2), 52.6 (CH_3), 53.5 (CH), 67.0 (CH_2), 120.0 (CH), 121.6 (CH), 122.0 (CH), 122.6 (CH), 127.9 (2 × CH), 128.0 (2 × CH), 128.2 (2 × CH), 128.2 (2 × CH), 128.6 (CH), 130.0 (CH), 133.0 (CH), 136.5 (C), 138.3 (C), 139.5 (C), 142.0 (C), 148.2 (C), 149.0 (CH), 149.9 (C), 156.2 (C), 157.8 (C), 172.2 (C); MS (EI) *m/z* 511 (M^+, 61), 452 (18), 376 (25), 344 (45), 290 (73), 279 (100), 244 (16), 169 (14), 108 (71), 79 (62); HRMS (EI) calcd for $C_{29}H_{25}N_3O_6$ (M^+) 511.1743, found 511.1747.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (7k). Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (**7h**) (0.0206 g, 0.0473 mmol) was dissolved in anhydrous methanol (2 mL) and tin(II) chloride dihydrate (0.0530 g, 0.235 mmol) was added. The reaction mixture was stirred at room temperature for 2 h. Once the reaction was complete, the solution was diluted with ethyl acetate (15 mL) and washed with saturated potassium fluoride solution (3 × 10 mL) and brine (10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography on silica gel eluting with 40% ethyl acetate/1% triethylamine in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (**7k**) (0.0127 g, 65%) as a yellow oil. R_f 0.24 (40% ethyl acetate/1% triethylamine in dichloromethane); IR (neat) 3364, 2947, 1721, 1597, 1520, 1211, 1057, 826 cm^{-1}; $[\alpha]_D^{29}$ +25.6 (*c* 0.5, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.31 (dd, J = 14.8, 5.2 Hz, 1H), 3.41 (dd, J = 14.8, 5.2 Hz, 1H), 3.69 (s, 3H), 3.89 (br s, 2H), 4.78 (dt, J = 8.3, 5.2 Hz, 1H), 5.09 (d, J = 12.4 Hz, 1H), 5.12 (d, J = 12.4 Hz, 1H), 6.40 (d, J = 8.3 Hz, 1H), 6.74 (d, J = 8.6 Hz, 2H), 7.27–7.37 (m, 7H), 7.44 (d, J = 8.6 Hz, 2H), 8.42 (d, J = 5.2 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 38.7 (CH_2), 52.4 (CH_3), 53.5 (CH), 66.9 (CH_2), 115.3 (2 × CH), 119.0 (CH), 120.6 (CH), 127.2 (C), 128.1 (2 × CH), 128.1 (CH), 128.1 (2 × CH), 128.5 (2 × CH), 136.4 (C), 147.9 (C), 148.7 (CH), 149.4 (C), 156.2 (C), 156.8 (C), 172.1 (C); MS (ESI) *m/z* 406 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{23}H_{24}N_3O_4$ ($M+H^+$) 406.1761, found 406.1745.

Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoate (7l). The reaction was carried out according to the above procedure for the synthesis of methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (**7k**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (**7i**) (0.359 g, 0.0747 mmol). Purification by flash column chromatography on silica gel eluting with 50% ethyl acetate/1% triethylamine in dichloromethane gave methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoate (**7l**) (0.0198 g, 63%) as a yellow oil. R_f 0.13 (50% ethyl acetate/1% triethylamine in dichloromethane); IR (neat) 3365, 2952, 1713, 1599, 1515, 1266, 1213, 1055, 698 cm^{-1}; $[\alpha]_D^{26}$ +15.8 (*c* 1.0, $CHCl_3$); ^1H NMR (500 MHz, $CDCl_3$) δ 3.30 (dd, *J* = 14.7, 5.3 Hz, 1H), 3.35 (dd, *J* = 14.7, 5.3 Hz, 1H), 3.71 (s, 3H), 4.78 (dt, *J* = 8.3, 5.3 Hz, 1H), 5.07 (d, *J* = 12.3 Hz, 1H), 5.11 (d, *J* = 12.3 Hz, 1H), 6.06 (d, *J* = 2.2 Hz, 1H), 6.18 (dd, *J* = 8.2, 2.2 Hz, 1H), 6.30 (d, *J* = 8.3 Hz, 1H), 6.91 (d, *J* = 8.2 Hz, 1H), 7.20–7.36 (m, 7H), 8.45 (d, *J* = 5.1 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, $CDCl_3$) δ 39.1 (CH_2), 52.4 (CH_3), 53.5 (CH), 66.9 (CH_2), 101.8 (CH), 106.6 (CH), 115.2 (C), 122.0 (CH), 123.6 (CH), 128.1 (2 × CH), 128.1 (CH), 128.5 (2 × CH), 131.3 (CH), 136.4 (C), 144.7 (C), 148.2 (C), 148.6 (C), 149.5 (CH), 156.1 (C), 157.1 (C), 172.2 (C); MS (ESI) *m/z* 421 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{23}H_{25}N_4O_4$ ($M+H^+$) 421.1870, found 421.1853.

(2*S*)-2-Amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (8b). Methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-phenylpyridin-2'-yl)propanoate (**7b**) (0.064 g, 0.16 mmol) was suspended in 6 M hydrochloric acid (5 mL) and heated under reflux for 48 h. The mixture was cooled to room temperature and concentrated *in vacuo*. Trituration with diethyl ether (5 mL) gave a brown solid. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) (0.045 g, 99%) as an off-white solid. Mp 110–113 °C; IR (neat) 3376, 2932, 1634, 1479, 1393, 1024, 844, 766 cm^{-1}; $[\alpha]_D^{22}$ +28.6 (*c* 1.1, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 3.73–3.84 (m, 2H), 4.76 (dd, *J* = 8.4, 6.5 Hz, 1H), 7.62–7.67 (m, 3H), 8.00–8.07 (m, 2H), 8.32 (dd, *J* = 6.3, 1.7 Hz, 1H), 8.50 (d, *J* = 1.7 Hz, 1H), 8.82 (d, *J* = 6.3 Hz, 1H); $^{13}C\{^1H\}$ NMR

(126 MHz, CD_3OD) δ 35.0 (CH_2), 52.9 (CH), 124.3 (CH), 126.6 (CH), 129.3 (2 × CH), 130.9 (2 × CH), 133.3 (CH), 135.8 (C), 143.1 (CH), 152.3 (C), 159.4 (C), 169.8 (C); MS (ESI) *m/z* 277 ([M−H]$^-$, 100); HRMS (ESI) calcd for $C_{14}H_{14}{}^{35}ClN_2O_2$ ([M−H]$^-$) 277.0749, found 277.0744.

(2*S*)-2-Amino-3-(4'-propylpyridin-2'-yl]propanoic acid hydrochloride (8a). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-(4'-propylpyridin-2'-yl]propanoate (**7a**) (0.023 g, 0.065 mmol) for 48 h. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-(4'-propylpyridin-2'-yl]propanoic acid hydrochloride (**8a**) (0.015 g, 97%) as an off-white solid. Mp 112–116 °C; IR (neat) 3360, 2924, 1636, 1506, 1227, 1063, 820 cm^{-1}; $[\alpha]_D^{22}$ +27.5 (*c* 0.9, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 1.03 (t, *J* = 7.3 Hz, 3H), 1.77–1.86 (m, 2H), 2.94 (t, *J* = 7.3 Hz, 2H), 3.66 (dd, *J* = 14.8, 8.9 Hz, 1H), 3.72 (dd, *J* = 14.8, 6.2 Hz, 1H), 4.65 (dd, *J* = 8.9, 6.2 Hz, 1H), 7.89 (d, *J* = 5.9 Hz, 1H), 8.05 (s, 1H), 8.69 (d, *J* = 5.9 Hz, 1H); ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 13.9 (CH_3), 24.1 (CH_2), 34.7 (CH_2), 38.9 (CH_2), 52.8 (CH), 127.3 (CH), 129.5 (CH), 142.1 (CH), 151.4 (C), 166.6 (C), 169.7 (C); MS (ESI) *m/z* 243 ([M−H]$^-$, 100); HRMS (ESI) calcd for $C_{11}H_{16}{}^{35}ClN_2O_2$ ([M−H]$^-$) 243.0906, found 243.0902.

(2*S*)-2-Amino-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoic acid hydrochloride (8c). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoate (**7c**) (0.026 g, 0.059 mmol) for 48 h. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-[4'-(naphthalen-2''-yl)pyridin-2'-yl]propanoic acid hydrochloride (**8c**) (0.019 g, 94%) as a light yellow solid. Mp 120–124 °C; IR (neat) 3338, 2924, 1740, 1622, 1456, 1366, 1229, 818, 754 cm^{-1}; $[\alpha]_D^{21}$ +35.9 (*c* 0.9, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.72–3.82 (m, 2H), 4.76 (br t, *J* = 7.3 Hz, 1H), 7.59–7.68 (m, 2H), 7.97 (d, *J* = 7.8 Hz, 1H), 8.03–8.13 (m, 3H), 8.38 (br d, *J* = 4.8 Hz, 1H), 8.56 (br s, 1H), 8.61 (br s, 1H), 8.81 (d, *J* = 4.8 Hz, 1H); ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 35.3 (CH_2), 53.0

(CH), 124.1 (CH), 125.1 (CH), 126.3 (CH), 128.4 (CH), 128.9 (CH), 129.6 (CH), 130.2 (CH), 130.3 (CH), 130.8 (CH), 133.1 (C), 134.8 (C), 136.2 (C), 143.8 (CH), 152.8 (C), 158.4 (C), 170.0 (C); MS (ESI) *m/z* 327 ([M−H]$^-$, 100); HRMS (ESI) calcd for $C_{18}H_{16}{}^{35}ClN_2O_2$ ([M−H]$^-$) 327.0906, found 327.0898.

(2*S*)-2-Amino-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (8d). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoate (**7d**) (0.061 g, 0.15 mmol) for 48 h. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8d**) (0.046 g, 99%) as a colorless solid. Mp 165–167 °C; IR (neat) 3340, 2926, 1738, 1370, 1225, 824 cm^{-1}; $[\alpha]_D{}^{21}$ +34.0 (*c* 0.8, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.70 (dd, *J* = 15.0, 9.5 Hz, 1H), 3.74 (dd, *J* = 15.0, 7.3 Hz, 1H), 3.91 (s, 3H), 4.68–4.74 (m, 1H), 7.17 (d, *J* = 8.9 Hz, 2H), 8.03 (d, *J* = 8.9 Hz, 2H), 8.22 (dd, *J* = 6.3, 1.3 Hz, 1H), 8.39 (d, *J* = 1.3 Hz, 1H), 8.70 (d, *J* = 6.3 Hz, 1H); ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 35.1 (CH_2), 52.9 (CH), 56.2 (CH_3), 116.4 (2 × CH), 122.8 (CH), 125.0 (CH), 127.6 (C), 131.1 (2 × CH), 143.1 (CH), 152.1 (C), 158.3 (C), 164.8 (C), 170.0 (C); MS (ESI) *m/z* 307 ([M−H]$^-$, 100); HRMS (ESI) calcd for $C_{15}H_{16}{}^{35}ClN_2O_3$ ([M−H]$^-$) 307.0855, found 307.0847.

(2*S*)-2-Amino-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (8e). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoate (**7e**) (0.047 g, 0.11 mmol) for 16 h. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-[4'-(2'',4''-dimethoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8e**) (0.038 g, 95%) as a light yellow solid. Mp 180–183 °C; IR (neat) 3410, 2932, 1744, 1597, 1211, 1018, 833 cm^{-1}; $[\alpha]_D{}^{27}$ +10.1 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 3.67–3.81 (m, 2H), 3.91 (s, 3H), 3.95 (s, 3H), 4.63–4.74 (m, 1H), 6.71–6.82 (m, 2H), 7.71 (d, *J* = 8.3 Hz, 1H), 8.26 (d, *J* = 6.1 Hz, 1H), 8.36 (br s,

1H), 8.67 (d, J = 6.1 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (101 MHz, CD_3OD) δ 34.9 (CH_2), 53.1 (CH), 56.4 (CH_3), 56.6 (CH_3), 100.0 (CH), 108.0 (CH), 117.4 (C), 126.1 (CH), 128.0 (CH), 133.8 (CH), 141.7 (CH), 150.7 (C), 158.1 (C), 160.9 (C), 166.1 (C), 169.9 (C); MS (ESI) *m/z* 303 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{16}H_{19}N_2O_4$ ($M+H^+$) 303.1339, found 303.1338.

(2*S*)-2-Amino-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid hydrochloride (8f). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoate (**7f**) (0.24 g, 0.56 mmol) for 16 h. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-[4'-(2*H*-1'',3''-benzodioxol-5''-yl)pyridin-2'-yl]propanoic acid hydrochloride (**8f**) (0.18 g, 99%) as a light yellow solid. Mp 197–200 °C; IR (neat) 3410, 2808, 1736, 1612, 1474, 1242, 1034, 810 cm^{-1}; $[\alpha]_D^{27}$ +38.5 (*c* 0.1, MeOH); ^{1}H NMR (400 MHz, CD_3OD) δ 3.69 (dd, J = 12.8, 5.7 Hz, 1H), 3.74 (dd, J = 12.8, 5.7 Hz, 1H), 4.71 (t, J = 5.7 Hz, 1H), 6.12 (s, 2H), 7.07 (d, J = 8.2 Hz, 1H), 7.55 (d, J = 1.9 Hz, 1H), 7.63 (dd, J = 8.2, 1.9 Hz, 1H), 8.20 (dd, J = 6.4, 1.9 Hz, 1H), 8.35 (d, J = 1.9 Hz, 1H), 8.71 (d, J = 6.4 Hz, 1H); $^{13}C\{^{1}H\}$ NMR (101 MHz, CD_3OD) δ 35.1 (CH_2), 52.9 (CH), 103.9 (CH_2), 108.8 (CH), 110.4 (CH), 123.4 (CH), 125.0 (CH), 125.5 (CH), 129.5 (C), 142.9 (CH), 150.9 (C), 152.0 (C), 153.1 (C), 158.7 (C), 169.9 (C); MS (ESI) *m/z* 287 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{15}H_{15}N_2O_4$ ($M+H^+$) 287.1026, found 287.1029.

(2*S*)-2-Amino-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoic acid hydrochloride (8g). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoate (**7g**) (0.014 g, 0.034 mmol) for 48 h. Purification by recrystallization from a mixture of methanol and diethyl ether gave (2*S*)-2-amino-3-[4'-(4''-fluorophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8g**) (0.010 g, 99%) as a yellow solid. Mp 80–84 °C; IR (neat) 3360, 2920, 1603, 1516, 1236, 1163, 827 cm^{-1}; $[\alpha]_D^{25}$ +30.8 (*c* 1.0,

MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.72 (dd, J = 11.0, 7.2 Hz, 1H), 3.76 (dd, J = 11.0, 7.2 Hz, 1H), 4.72 (t, J = 7.2 Hz, 1H), 7.38 (t, J = 8.8 Hz, 2H), 8.07 (dd, J = 8.8, 5.1 Hz, 2H), 8.22 (dd, J = 6.1, 1.1 Hz, 1H), 8.38 (br s, 1H), 8.78 (d, J = 6.1 Hz, 1H); ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 35.3 (CH_2), 52.9 (CH), 117.8 (d, $^2J_{C\text{-}F}$ = 22.3 Hz, 2 × CH), 123.8 (CH), 125.9 (CH), 131.7 (d, $^3J_{C\text{-}F}$ = 9.1 Hz, 2 × CH), 132.5 (d, $^4J_{C\text{-}F}$ = 3.0 Hz, C), 144.1 (CH), 153.1 (C), 157.2 (C), 166.5 (d, $^1J_{C\text{-}F}$ = 252.3 Hz, C), 170.0 (C); MS (ESI) *m/z* 283 (M+Na$^+$, 100); HRMS (ESI) calcd for $C_{14}H_{13}FN_2NaO_2$ (M+Na$^+$) 283.0853, found 283.0843.

(2*S*)-2-Amino-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (8h). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoate (**7h**) (0.026 g, 0.060 mmol) for 48 h. Purification by recrystallization from methanol gave (2*S*)-2-amino-3-[4'-(4''-nitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8h**) (0.017 g, 86%) as an off-white solid. Mp 140–143 °C; IR (neat) 3221, 2854, 1719, 1636, 1530, 1349, 1263, 1246, 839 cm^{-1}; $[\alpha]_D^{25}$ +31.9 (*c* 0.9, MeOH); ^{1}H NMR (500 MHz, CD_3OD) δ 3.78 (d, J = 6.5 Hz, 2H), 4.75 (t, J = 6.5 Hz, 1H), 8.22 (d, J = 8.5 Hz, 2H), 8.30 (d, J = 5.7 Hz, 1H), 8.46 (d, J = 8.5 Hz, 2H), 8.48 (br s, 1H), 8.89 (d, J = 5.7 Hz, 1H); ^{13}C{^{1}H} NMR (126 MHz, CD_3OD) δ 35.4 (CH_2), 52.9 (CH), 124.7 (CH), 125.6 (2 × CH), 126.9 (CH), 130.6 (2 × CH), 142.4 (C), 144.8 (CH), 151.0 (C), 153.9 (C), 155.7 (C), 170.0 (C); MS (ESI) *m/z* 322 ([M−H]$^-$, 100); HRMS (ESI) calcd for $C_{14}H_{13}{}^{35}ClN_3O_4$ ([M−H]$^-$) 322.0600, found 322.0593.

(2*S*)-2-Amino-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (8i). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoate (**7i**) (0.024 g, 0.049 mmol) for 48 h. Purification by recrystallization from methanol gave (2*S*)-2-amino-3-[4'-(2'',4''-dinitrophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8i**) (0.018 g, 97%) as a light yellow solid. Mp

130–134 °C; IR (neat) 3402, 3039, 1744, 1597, 1528, 1342, 1080, 833, 741 cm^{-1}; $[\alpha]_D^{26}$ +9.3 (*c* 1.0, MeOH); ^1H NMR (500 MHz, CD_3OD) δ 3.55 (dd, *J* = 16.3, 7.1 Hz, 1H), 3.62 (dd, *J* = 16.3, 5.4 Hz, 1H), 4.57 (dd, *J* = 7.1, 5.4 Hz, 1H), 7.53 (dd, *J* = 5.3, 1.6 Hz, 1H), 7.63 (br s, 1H), 7.84 (d, *J* = 8.4 Hz, 1H), 8.64 (dd, *J* = 8.4, 2.3 Hz, 1H), 8.71 (d, *J* = 5.3 Hz, 1H), 8.95 (d, *J* = 2.3 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, CD_3OD) δ 36.5 (CH_2), 52.9 (CH), 121.4 (CH), 123.6 (CH), 124.9 (CH), 128.7 (CH), 128.7 (CH), 134.5 (CH), 139.9 (C), 149.1 (C), 149.7 (C), 149.8 (C), 157.0 (C), 170.9 (C); MS (ESI) *m/z* 333 ([MH–HCl]$^+$, 100); HRMS (ESI) calcd for $C_{14}H_{13}N_4O_6$ ([MH–HCl]$^+$) 333.0830, found 333.0822.

(2*S*)-2-Amino-3-{4'-[4''(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoic acid hydrochloride (8j). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-{4'-[4''-(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoate (**7j**) (0.024 g, 0.047 mmol) for 48 h. Purification by recrystallization from methanol gave (2*S*)-2-amino-3-{4'-[4''(3'''-nitrophenyl)phenyl]pyridin-2'-yl}propanoic acid hydrochloride (**8j**) (0.018 g, 98%) as a light yellow solid. Mp 140–142 °C; IR (neat) 3414, 2922, 1634, 1522, 1348, 806, 748 cm^{-1}; $[\alpha]_D^{27}$ +14.1 (*c* 0.1, MeOH); ^1H NMR (400 MHz, CD_3OD) δ 3.72–3.83 (m, 2H), 4.72–4.80 (m, 1H), 7.77 (t, *J* = 7.8 Hz, 1H), 7.87–8.38 (m, 7H), 8.47–8.60 (m, 2H), 8.83 (s, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, CD_3OD) δ 35.3 (CH_2), 53.0 (CH), 122.8 (CH), 124.0 (CH), 124.1 (CH), 126.3 (CH), 129.6 (2 × CH), 130.2 (2 × CH), 131.6 (CH), 134.4 (CH), 136.0 (C), 142.5 (C), 143.4 (C), 144.0 (CH), 150.4 (C), 153.0 (C), 157.9 (C), 170.0 (C); MS (ESI) *m/z* 398 ([M−H]$^-$, 100); HRMS (ESI) calcd for $C_{20}H_{17}{}^{35}ClN_3O_4$ ([M−H]$^-$) 398.0913, found 398.0899.

(2*S*)-2-Amino-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (8k). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(4''-aminophenyl)pyridin-2'-yl]propanoate (**7k**) (0.014 g, 0.034 mmol) for 48 h. Purification by recrystallization from methanol gave (2*S*)-2-amino-3-[4'-(4''-

aminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8k**) (0.010 g, 98%) as an off-white solid. Mp 151–154 °C; IR (neat) 3379, 2832, 1744, 1628, 1589, 1474, 1312, 818 cm^{-1}; $[\alpha]_D^{27}$ +34.9 (*c* 0.1, MeOH); ^1H NMR (400 MHz, CD_3OD) δ 3.70 (d, *J* = 7.3 Hz, 2H), 4.69 (t, *J* = 7.3 Hz, 1H), 7.30 (d, *J* = 8.7 Hz, 2H), 8.04 (d, *J* = 8.7 Hz, 2H), 8.15 (dd, *J* = 6.3, 1.7 Hz, 1H), 8.33 (d, *J* = 1.7 Hz, 1H), 8.68 (d, *J* = 6.3 Hz, 1H); $^{13}C\{^1H\}$ NMR (101 MHz, CD_3OD) δ 35.3 (CH_2), 53.0 (CH), 121.5 (2 × CH), 122.6 (CH), 124.7 (CH), 131.1 (C), 131.1 (2 × CH and C), 143.8 (CH), 152.6 (C), 157.1 (C), 170.1 (C); MS (ESI) *m/z* 258 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{14}H_{16}N_3O_2$ ($M+H^+$) 258.1237, found 258.1236.

(2*S*)-2-Amino-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (8l). The reaction was carried out according to the above procedure for the synthesis of (2*S*)-2-amino-3-(4'-phenylpyridin-2'-yl)propanoic acid hydrochloride (**8b**) using methyl (2*S*)-2-[(benzyloxycarbonyl)amino]-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoate (**7l**) (0.016 g, 0.039 mmol) for 48 h. Purification by recrystallization from methanol gave (2*S*)-2-amino-3-[4'-(2'',4''-diaminophenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8l**) (0.011 g, 95%) as an off-white solid. Mp 159–162 °C; IR (neat) 3333, 2846, 1744, 1620, 1474, 1234, 1072, 841 cm^{-1}; $[\alpha]_D^{26}$ +15.7 (*c* 1.0, MeOH); ^1H NMR (400 MHz, CD_3OD) δ 3.75 (dd, *J* = 16.0, 7.3 Hz, 1H), 3.79 (dd, *J* = 16.0, 7.3 Hz, 1H), 4.72 (t, *J* = 7.3 Hz, 1H), 6.94 (dd, *J* = 8.3, 1.9 Hz, 1H), 7.04 (d, *J* = 1.9 Hz, 1H), 7.51 (d, *J* = 8.3 Hz, 1H), 8.16 (br d, *J* = 6.0 Hz, 1H), 8.35 (br s, 1H), 8.82 (d, *J* = 6.0 Hz, 1H); $^{13}C\{^1H\}$ NMR (126 MHz, CD_3OD) δ 35.0 (CH_2), 52.9 (CH), 112.2 (CH), 114.0 (CH), 122.6 (C), 127.0 (CH), 129.2 (CH), 133.8 (CH), 137.1 (C), 143.2 (CH), 145.9 (C), 152.2 (C), 158.6 (C), 169.8 (C); MS (ESI) *m/z* 273 ($[MH–HCl]^+$, 100); HRMS (ESI) calcd for $C_{14}H_{17}N_4O_2$ ($[MH–HCl]^+$) 273.1346, found 273.1346.

(2*S*)-2-[(9*H*-Fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (9). (2*S*)-2-Amino-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid hydrochloride (**8d**) (0.026 g, 0.086 mmol) was dissolved in 1,4-dioxane (1.5 mL) followed by 10% sodium carbonate solution (1.5 mL), *N*,*N*-diisopropylethylamine (0.016 mL, 0.094 mmol) and 9-fluorenylmethoxycarbonyl chloride (0.024 g, 0.094 mmol). The reaction mixture was stirred at room

temperature for 18 h. The solution was concentrated *in vacuo*. The resulting residue was redissolved in water (5 mL) and acidified to pH 1 with 2 M hydrochloric acid (10 mL). The aqueous solution was washed with dichloromethane (3 × 10 mL) and the organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. Purification by flash column chromatography on silica gel eluting with 3% methanol/1% acetic acid in dichloromethane gave (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (**9**) (0.017 g, 40%) as a brown solid. Mp 90–93 °C; R_f 0.09 (3% methanol/1% acetic acid in dichloromethane); IR (neat) 3326, 2936, 1717, 1603, 1518, 1252, 1182, 1047, 827, 740 cm^{-1}; $[\alpha]_D^{21}$ +60.1 (*c* 1.0, $CHCl_3$); ^1H NMR (400 MHz, $CDCl_3$) δ 3.37–3.56 (m, 2H), 3.87 (s, 3H), 4.24 (t, *J* = 7.3 Hz, 1H), 4.39 (d, *J* = 7.3 Hz, 2H), 4.47–4.57 (m, 1H), 6.31 (d, *J* = 3.4 Hz, 1H), 7.00 (d, *J* = 8.6 Hz, 2H), 7.32 (t, *J* = 7.4 Hz, 2H), 7.41 (t, *J* = 7.4 Hz, 2H), 7.55 (d, *J* = 5.6 Hz, 1H), 7.58–7.67 (m, 5H), 7.77 (d, *J* = 7.4 Hz, 2H), 8.49 (d, *J* = 5.6 Hz, 1H); $^{13}C\{^1H\}$ NMR (101 MHz, $CDCl_3$) δ 38.4 (CH_2), 47.2 (CH), 53.2 (CH), 55.5 (CH_3), 67.2 (CH_2), 114.9 (2 × CH), 120.0 (2 × CH), 120.3 (CH), 122.6 (CH), 125.2 (2 × CH), 127.2 (2 × CH), 127.8 (2 × CH), 128.6 (2 × CH), 141.3 (2 × C), 143.8 (2 × C), 143.9 (C), 145.6 (CH), 152.0 (C), 155.8 (C), 156.1 (C), 161.5 (C), 172.8 (C); MS (ESI) *m/z* 495 ($M+H^+$, 100); HRMS (ESI) calcd for $C_{30}H_{27}N_2O_5$ ($M+H^+$) 495.1914, found 495.1901.

Synthesis of Hexapeptide 10. The pentapeptide was synthesized on a peptide synthesizer using an Fmoc/^{t}Bu protecting group strategy on a 0.1 mmol synthetic scale using Rink Amide ChemMatrix® resin. The resin bound peptide was synthesized by first loading Fmoc-Lys(Boc)-OH to the resin and by introducing the amino acids (4 equivalents) successively with a combination of 0.5 M 2-(6-chloro-1-*H*-benzotriazole-1-yl)-1,1,3,3-tetramethylaminium hexafluorophosphate (HCTU) in DMF (4 equivalents) and 2 M diisopropylethylamine in NMP (8 equivalents). Fmoc groups were removed using 20% piperidine in DMF. The resin bound pentapeptide (0.0889 g, 0.0300 mmol) was treated with 20% piperidine in DMF (2 mL) and shaken for 0.25 h. The solution was filtered and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin bound peptide was suspended in DMF (2 mL) followed by addition of (2*S*)-2-[(9*H*-fluoren-9-ylmethoxycarbonyl)amino]-3-

[4'-(4''-methoxyphenyl)pyridin-2'-yl]propanoic acid (**9**) (0.0297 g, 0.0600 mmol), diisopropylethylamine (0.0209 mL, 0.120 mmol), 0.5 M HCTU in DMF (0.120 mL, 0.0600 mmol). The mixture was shaken for 4 h. The solution was filtered and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin bound peptide was treated with 20% piperidine in DMF (2 mL) and shaken for 0.25 h. The solution was filtered and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin bound peptide was suspended in DMF (2 mL) followed by sequential addition of acetic anhydride (0.190 mL, 2.01 mmol) and diisopropylethylamine (0.190 mL, 1.09 mmol). The mixture was shaken for 1 h. The solution was filtered and the resin bound peptide was washed with dichloromethane (3 × 2 mL), isopropanol (3 × 2 mL) and DMF (3 × 2 mL). The resin bound peptide was treated with trifluoroacetic acid/water/triisopropylsilane (2 mL, 95:2.5:2.5) and shaken for 2 h. The reaction mixture was filtered and the solution was evaporated using a stream of nitrogen. Peptide **10** was precipitated from the resulting residue using a solution of ice-cold diethyl ether (2 mL) and centrifuged at 3700 rpm for 5 minutes. The precipitate was washed with ice-cold diethyl ether (3 × 2 mL). Peptide **10** was purified on a reverse-phase HPLC system equipped with a UV-Vis detector (monitoring at 214 nm and 280 nm), using a C18, 5 μm, 250 × 21.2 mm column. Gradients were run using a solvent system consisting of A (H_2O + 0.1% TFA) and B (MeCN + 0.1% TFA), and collected fractions were lyophilized on a freeze dryer. Pure peptide **10** was analyzed on a reverse-phase HPLC (RP-HPLC) system equipped with a UV-Vis detector (monitoring at 214 nm and 280 nm) using a 5 μm, peptide XB-C18, 150 × 4.6 mm column at a flow rate of 1 mL/minute. RP-HPLC gradients were run using a solvent system consisting of solution A (5% MeCN in H_2O + 0.1% TFA) and B (5% H_2O in MeCN + 0.1% TFA). Two gradients were used to characterize peptide **10**; a gradient from 0–100% solution B over 20 minutes (Supporting information, Figure S1) and a gradient from 0–100% solution B over 50 minutes (Supporting information, Figure S2). Analytical RP-HPLC data is reported as column retention time (t_R) in minutes (Supporting information, Table S1). High resolution mass spectrometry (HRMS) was performed on a microTOF-Q mass spectrometer (ESI^+). HRMS data are reported as mass to charge ratio (*m/z*) = observed/MW.

SUPPORTING INFORMATION AVAILABLE. Analysis data for protein **10**, the absorption and emission spectra for amino acids **8c**–**8f** and **8k** and, ^{1}H and ^{13}C NMR spectra for all novel compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

ACKNOWLEDGEMENT. Financial support from EPSRC (studentships to A.H.H., EP/K503058/1 and J.D.B., EP/N509176/1), GlaxoSmithKline Ltd and the University of Glasgow is gratefully acknowledged.

REFERENCES:

(1) For reviews see: (a) Wagner, I.; Musso, H. New Naturally Occurring Amino Acids. *Angew. Chem. Int. Ed. Engl*. **1983**, *22*, 816–828. (b) Greenstein, J. P.; Winitz, M. Chemistry of the Amino Acids; Krieger, R. E., FL **1984**, Vols. 1–3; (c) Barrett, G. E., Ed. Chemistry and Biochemistry of the Amino Acids, Chapman and Hall: London, **1985**; (d) Breslow, R.; Chmielewski, J.; Foley, D.; Johnson, B.; Kumabe, N.; Varney, M.; Mehra, R. Optically Active Amino Acid Synthesis by Artificial Transaminase Enzymes, *Tetrahedron* **1988**, *44*, 5515–5524.

(2) (a) Ohfune, Y. Stereoselective Routes Towards the Synthesis of Unusual Amino Acids. *Acc. Chem. Res*. **1992**, *25*, 360–366. (b) Dougherty, D. A. Unnatural Amino Acids as Probes of Protein Structure and Function. *Curr. Opin. Chem. Biol*. **2000**, *4*, 645–652. (c) Vogt, H.; Bräse, S. Recent Approaches Towards the Asymmetric Synthesis of α,α-Disubstituted α-Amino Acids. *Org. Biomol. Chem*. **2007**, *5*, 406–430. (d) Smits, R.; Cadicamo, C. D.; Burger, K.; Koksch, B. Synthetic Strategies to α-Trifluoromethyl and α-Difluoromethyl Substituted α-Amino Acids. *Chem. Soc. Rev*. **2008**, *37*, 1727–1739. (e) Mortensen, M.; Husmann, R.; Veri, E.; Bolm, C. Synthesis and Applications of Silicon-Containing α-Amino Acids. *Chem. Soc. Rev*. **2009**, *38*, 1002–1010. (f) Lang, K.; Chin, J.

W. Cellular Incorporation of Unnatural Amino Acids and Bioorthogonal Labeling of Proteins. *Chem. Rev*. **2014**, *114*, 4764–4806.

(3) For example, see: (a) Easton, C. J. Free-Radical Reactions in the Synthesis of α-Amino Acids and Derivatives. *Chem. Rev*. **1997**, *97*, 53–82. (b) Nájera, C.; Sansano, J. M. Catalytic Asymmetric Synthesis of α-Amino Acids. *Chem. Rev*. **2007**, *107*, 4584–4671. (c) Noisier, A. F. M.; Brimble, M. A. C-H Functionalization in the Synthesis of Amino Acids and Peptides. *Chem. Rev*. **2014**, *114*, 8775–8806.

(4) (a) Kolar, P.; Petrič, A.; Tišler, M. Heteroarylalanines. *J. Heterocyclic Chem*. **1997**, *34*, 1067–1098. (b) Singh, P.; Samanta, K.; Das, S. K.; Panda, G. Amino Acid Chirons: A Tool for Asymmetric Synthesis of Heterocycles. *Org. Biomol. Chem*. **2014**, *12*, 6297–6339.

(5) Gilfillan, L.; Artschwager, R.; Harkiss, A. H.; Liskamp, R. M. J.; Sutherland, A. Synthesis of Pyrazole Containing α-Amino Acids via a Highly Regioselective Condensation/aza-Michael Reaction of β-Aryl α,β-Unsaturated Ketones. *Org. Biomol. Chem*. **2015**, *13*, 4514–4523.

(6) (a) Inouye, S.; Shomura, T.; Tsuruoka, T.; Ogawa, Y.; Watanabe, H.; Yoshida, J.; Niida, T. L-β-(5-Hydroxy-2-pyridyl)-alanine and L-β-(3-Hydroxyureido)-alanine from Streptomyces. *Chem. Pharm. Bull*. **1975**, *23*, 2669–2677. (b) Izawa, M.; Takayama, S.; Shindo-Okada, N.; Doi, S.; Kimura, M.; Katsuki, M.; Nishimura, S. Inhibition of Chemical Carcinogenesis *In Vivo* by Azatyrosine. *Cancer Res*. **1992**, *52*, 1628–1630.

(7) Beckers, T.; Bernd, M.; Kutscher, B.; Kühne, R.; Hoffmann, S.; Reissmann, T. Structure-Function Studies of Linear and Cyclized Peptide Antagonists of the GnRH Receptor. *Biochem. Biophys. Res. Commun*. **2001**, *289*, 653–663.

(8) (a) Jackson, R. F. W.; Wythes, M. J.; Wood, A. Synthesis of Enantiomerically Pure Protected β-Aryl Alanines. *Tetrahedron Lett*. **1989**, *30*, 5941–5944. (b) Bozell, J. J.; Vogt C. E.; Gozum, J. Transition-Metal-Assisted Asymmetric Synthesis of Amino Acids Analogues. A New Synthesis of Optically Pure D- and L-Pyridylalanines. *J. Org. Chem*. **1991**, *56*, 2584–2587. (c) Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J.; Tang, L. T. The Synthesis of Novel Heterocyclic Substituted α-Amino Acids; Further Exploitation of α-amino Acid Alkynyl Ketones. *J. Chem. Soc*., *Perkin Trans. 1* **2000**, 303–305. (d) Adlington, R. M.; Baldwin, J. E.; Catterick, D.; Pritchard, G. J.; Tang, L. T. The Synthesis of Novel Heterocyclic Substituted α-Amino Acids; Further Exploitation of α-Amino acid Alkynyl Ketones as Reactive Substrates. *J. Chem. Soc*., *Perkin Trans. 1* **2000**, 2311–2316. (e) Adamczyk, M.; Akireddy, S. R.; Reddy, R. E. Enantioselective Synthesis of (2-Pyridyl)alanines via Catalytic Hydrogenation and Application to the Synthesis of L-Azatyrosine. *Org. Lett*. **2001**, *3*, 3157–3159. (f) Tabanella, S.; Valancogne, I.; Jackson, R. F. W. Preparation of Enantiomerically Pure Pyridyl Amino Acids from Serine. *Org. Biomol. Chem*. **2003**, *1*, 4254–4261. (g) Dondoni, A.; Massi, A.; Minghini, E.; Sabbatini, S.; Bertolasi, V. Model Studies Toward the Synthesis of Dihydropyrimidinyl and Pyridyl α-Amino Acids via Three-Component Biginelli and Hantzsch Cyclocondensations. *J. Org. Chem*. **2003**, *68*, 6172–6183. (h) Noisier, A. F. M.; Harris, C. S.; Brimble, M. A. Novel Preparation of Chiral α-Amino Acids Using the Mitsunobu-Tsunoda Reaction. *Chem. Commun*. **2013**, *49*, 7744–7746.

(9) Belokon, Y. N.; Bulychev, A. G.; Vitt, S. V.; Struchov, Y. T.; Batsanov, A. S.; Timofeeva, T. V.; Tsyryapkin, V. A.; Ryzhov, M. G.; Lysova, L. A.; Bakhmutov, V. I.; Belikov, V. M. General Method of Diastereo- and Enantioselective Synthesis of β-Hydroxy-α-Amino Acids by Condensation of Aldehydes and Ketones with Glycine. *J. Am. Chem. Soc*. **1985**, *107*, 4252–4259.

(10) Busca, P.; Paradisi, F.; Moynihan, E.; Maguire, A. R.; Engel, P. C. Enantioselective Synthesis of Non-Natural Amino Acids Using Phenylalanine Dehydrogenases Modified by Site-Directed Mutagenesis. *Org. Biomol. Chem*. **2004**, *2*, 2684–2691.

(11) (a) Lovelock, S. L.; Lloyd, R. C.; Turner, N. J. Phenylalanine Ammonia Lyase Catalyzed Synthesis of Amino Acids by an MIO-Cofactor Independent Pathway. *Angew. Chem. Int. Ed*. **2014**, *53*, 4652–4656. (b) Lovelock, S. L.; Turner, N. J. Bacterial *Anabaena variabilis* Phenylalanine Ammonia Lyase: A Biocatalyst with Broad Substrate Specificity. *Bioorg. Med. Chem*. **2014**, *22*, 5555–5557.

(12) Aycock, R. A.; Vogt, D. B.; Jui, N. T. A Practical and Scalable System for Heteroaryl Amino Acid Synthesis. *Chem. Sci*. **2017**, *8*, 7998–8003.

(13) Rudisill, D. E.; Whitten, J. P. Synthesis of (*R*)-4-Oxo-5-phosphononorvaline, an *N*-Methyl-D-aspartic Acid Receptor Selective β-Keto Phosphonate. *Synthesis* **1994**, 851–854.

(14) (a) Fowler, L. S.; Ellis, D.; Sutherland, A. Synthesis of Fluorescent Enone Derived α-Amino Acids. *Org. Biomol. Chem*. **2009**, *7*, 4309–4316. (b) Fowler, L. S.; Thomas, L. H.; Ellis, D.; Sutherland, A. A One-Pot, Reductive Amination/6-*endo-trig* Cyclization for the Stereoselective Synthesis of 6-Substituted-4-oxopipecolic Acids. *Chem. Commun*. **2011**, *47*, 6569–6571. (c) Daly, M.; Cant, A. A.; Fowler, L. S.; Simpson, G. L.; Senn, H. M.; Sutherland, A. Switching the Stereochemical Outcome of 6-*Endo*-*Trig* Cyclizations; Synthesis of 2,6-*Cis*-6-Substituted 4-Oxopipecolic Acids. *J. Org. Chem*. **2012**, *77*, 10001–10009.

(15) Gosselin, F.; Lubell, W. D. An Olefination Entry for the Synthesis of Enantiopure α,ω-Diaminodicarboxylates and Azabicyclo[*X.Y.*0]alkane Amino Acids. *J. Org. Chem*. **1998**, *63*, 7463–7471.

(16) Danishefsky, S.; Bednarski, M. Lathanide Catalysis of Cycloadditions of Heterodienes with Enol Ethers. *Tetrahedron Lett*. **1984**, *25*, 721–724.

(17) (a) Ciufolini, M. A.; Byrne, N. E. Modified Knoevenagel-Stobbe Preparation of Substituted Pyridines: A New Approach to Streptonigrinoids. *J. Chem. Soc*., *Chem. Commun*. **1988**, 1230–1231. (b) Ciufolini, M. A.; Byrne, N. E. The Total Synthesis of Cystodytins. *J. Am. Chem. Soc*. **1991**, *113*, 8016–8024. (c) Ciufolini, M. A.; Bishop, M. J. Studies Towards Streptonigrinoids: Formal Total Synthesis of Lavendamycin Methyl Ester. *J. Chem. Soc*., *Chem. Commun*. **1993**, 1463–1464.

(18) (a) Stobbe, H. Bz-Tetra Hydro-Quinonoline Derivative from a Semi Cyclic 1,5 Diketone of Hexa-Methylene Series. *Ber*. **1902**, *35*, 3978–3891. (b) Striegler, C. Semicyclical 1,5-Diketone Through the Addition of Cyclopentanon on Piperonylidenacetophenon and on Anisylidenacetophenon. *J. prakt. Chem*. **1912**, *86*, 241–250.

(19) Sloan, N. L.; Luthra, S. K.; McRobbie, G.; Pimlott, S. L.; Sutherland, A. A One-Pot Radioiodination of Aryl Amines via Stable Diazonium Salts: Preparation of ^{125}I-Imaging Agents. *Chem. Commun*. **2017**, *53*, 11008–11011.

(20) Downs, J. A.; Jackson, S. P. A Means to a DNA End: The Many Roles of Ku. *Nat. Rev. Mol. Cell. Biol*. **2004**, *5*, 367–378.

(21) (a) Gomez, J. A.; Gama, V.; Yoshida, T.; Sun, W.; Hayes, P.; Leskov, K.; Boothman, D.; Matsuyama, S. Bax-Inhibiting Peptides Derived from Ku70 and Cell-Penetrating Pentapeptides. *Biochem. Soc. Trans*. **2007**, *35*, 797–801. (b) Gomez, J. A.; Chen, J.; Ngo, J.; Hajkova, D.; Yeh, I.-J.; Gama, V.; Miyagi, M.; Matsuyama, S. Cell-Penetrating Penta-Peptides (CPP5s): Measurement of Cell Entry and Protein-Transduction Activity. *Pharmaceuticals* **2010**, *3*, 3594–3613.

(22) Bacsa, B.; Bősze, S.; Kappe, C. O. Direct Solid-Phase Synthesis of the β-Amyloid (1–42) Peptide Using Controlled Microwave Heating. *J. Org. Chem*. **2010**, *75*, 2103–2106.

(23) Due to restrictive amounts of Fmoc-derivative **9**, SPPS coupling with the pentapeptide was conducted manually using two equivalents of the amino acid.

(24) For recent reviews of fluorescent amino acids, see: (a) Krueger, A. T.; Imperiali, B. Fluorescent Amino Acids: Modular Building Blocks for the Assembly of New Tools for Chemical Biology. *ChemBioChem*. **2013**, *14*, 788–799. (b) Kubota, R.; Hamachi, I. Protein Recognition using Synthetic Small-Molecular Binders Toward Optical Protein Sensing *In Vitro* and in Live Cells. *Chem. Soc. Rev*. **2015**, *44*, 4454–4471. (c) Harkiss, A. H.; Sutherland, A. Recent Advances in the Synthesis and Application of Fluorescent α-Amino Acids. *Org. Biomol. Chem*. **2016**, *14*, 8911–8921.

(25) (a) Bordeau, G.; Lartia, R.; Metge, G.; Fiorini-Debuisschert, C.; Charra, F.; Teulade-Fichou, M.-P. Trinaphthylamines as Robust Organic Materials for Two-Photon-Induced Fluorescence. *J. Am. Chem. Soc*. **2008**, *130*, 16836–16837. (b) Achelle, S.; Nouira, I.; Pfaffinger, B.; Ramondenc, Y.; Plé, N.; Rodríguez-López, J. V-Shaped 4,6-Bis(arylvinyl)pyrimidine Oligomers: Synthesis and Optical Properties. *J. Org. Chem*. **2009**, *74*, 3711–3717.

(26) Gmeiner, P.; Feldman, P. L.; Chu-Moyer, M. Y.; Rapoport, H. An Efficient and Practical Total Synthesis of (+)-Vincamine from L-Aspartic Acid. *J. Org. Chem.* **1990**, *55*, 3068–3074.