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PAPER

Synthesis and evaluation of fluorogenic 2-amino-1,8-naphthyridine derivatives for the detection of bacteria†

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Several novel fluorogenic *N*-aminoacylnaphthyridine substrates were synthesized in good yield and tested for their ability to detect pathogenic bacteria in agar-based cell culture. Simple 2-*N*-(β-alanyl)amino-5,7-dialkylnaphthyridine substrates were selectively hydrolysed by β-alanylaminopeptidase expressing bacteria, but were subject to diffusion in the agar medium. Diffusion was reduced in the 2-*N*-(β-alanyl) amino-7-alkylnaphthyridine substrates with longer alkyl chains, but inhibition of growth was increased. 2-*N*-(β-Alanyl)amino-7-octylnaphthyridine inhibited the growth of all species tested, except for strains resistant to colistin/polymyxin, providing a rationale for the development of substrates for the selective detection of drug resistant species in clinical samples.

1 Introduction

The number of multidrug resistant nosocomial bacterial species is continually increasing, thus diagnosis and treatment of infections caused by these 'superbugs' provides a significant challenge. As early isolation and treatment of infected patients is essential, there is an emerging need for rapid detection and identification of pathogenic bacteria.[1] One method for the identification of specific bacterial species involves the use of chromogenic or fluorogenic substrates,[2] which offers rapid, simple and reliable screening of clinical samples and is relatively inexpensive, requiring no specialized analytical equipment. These tests are ideally based on an off-to-on switching mechanism, Fig. 1. The 'switched-off' substrate is represented by a chromophore or fluorophore attached to a targeting molecule. The chromophore or fluorophore provides a means for visualization, whilst the targeting molecule makes the substrate specific for a certain enzymatic activity. Covalent bonding between the two units results in enzyme specificity and quenching of the colour or fluorescence.

Often the targeting molecule is an amino acid or sugar, which is susceptible to enzymatic cleavage from the chromophore/fluorophore. Less commonly, a redox active residue, such as a nitro group, may be employed to take advantage of oxidoreductase activity. If a sufficient quantity of the molecule is taken into the bacterial cells and a specific enzyme is present, the colour or fluorescence is 'switched-on' when the bond between the targeting molecule and the visualizing unit is broken, allowing extended conjugation throughout the molecule.

Chromogenic substrates allow the rapid and easy identification of microorganisms in clinical or food samples. Such substrates, when utilised in a suitable cell culture medium, can reduce the need for subcultures and further biochemical tests. For example, we have previously shown that β-alanine-1-pentylresorufamine (β-Ala-1-PRF) **1**, Scheme 1, can be used to distinguish between *Pseudomonas aeruginosa* and other non-fermenting bacteria.[3] β-Alanylaminopeptidase activity, which results in the cleavage of the pale yellow coloured β-alanine-1-PRF **1** to release the red/purple 1-PRF **2**, is present in all *Pseudomonas aeruginosa* strains, but not in *Burkholderia gladioli*, *Acinetobacter* sp.,

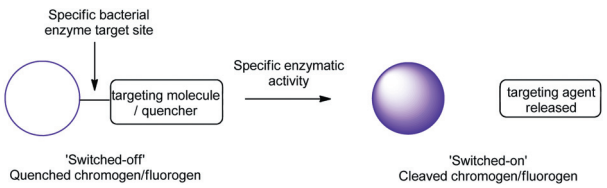

Fig. 1 Off-to-on switching mechanism for the identification of specific bacterial species.

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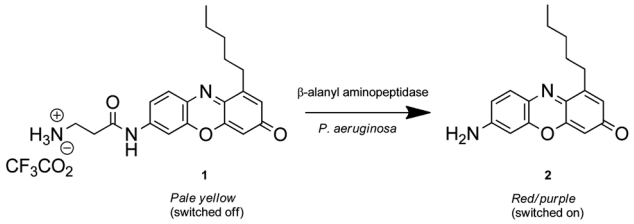
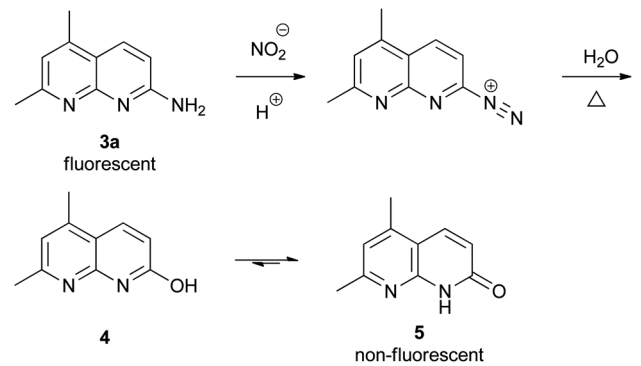


Scheme 1 Specific bacterial aminopeptidase action visualised by the release of the highly coloured chromophore, 1-pentylresorufamine **2**.[3]

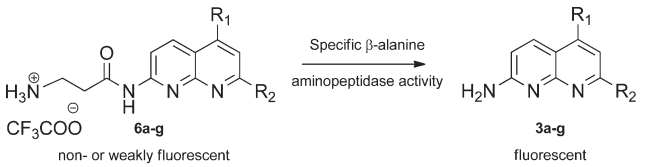


Scheme 2 Quantitative analysis of nitrite by 2-amino-5,7-dimethylnaphthyridine **3a**.[4]

Stenotrophomonas maltophilia, *Brevundimonas* sp. or *Chryseobacterium meningosepticum*.

The disadvantage of detection techniques that rely on the visualization of colour, even with significant colour changes such as those seen in the example in Scheme 1, is the requirement for at least 24 hours of incubation for conclusive results. Since the detection of fluorescence is inherently more sensitive than the perception of colour change, a smaller degree of enzymatic cleavage of the substrate is required, which would improve upon existing chromogenic sensors for these strains, in terms of both rapidity and sensitivity. Some fluorescent probes already exist as commercial products, but there are few amino substituted fluorogens that would enable the detection of bacterial aminopeptidases. The aim of the current work was the synthesis of fluorogenic substrates that would allow the specific detection of commonly encountered multidrug resistant bacteria through their aminopeptidase activity. As *P. aeruginosa* is a common nosocomial pathogen that requires improved detection for rapid treatment, β-alanine was chosen as the reactable moiety in order to target β-alanylaminopeptidase, an unusual aminopeptidase with strong expression and activity in *P. aeruginosa*, for selective cleavage of the fluorogenic substrate.[3]

The 2-amino-1,8-naphthyridines are fluorescent molecules with an emission wavelength between 350–490 nm in solution[4–9] and have potential in the detection of chemical and biological species. 2-Amino-5,7-dimethylnaphthyridine **3a** has been proposed as a fluorescent reagent for the detection of nitrite[4] under acidic conditions, Scheme 2; the fluorescence of **3a** was quenched by nitrite in a linear relationship through diazotization and reaction with water to form 2-hydroxy-5,7-dimethyl-1,8-naphthyridine **4**, which tautomerises to the corresponding 1,8-naphthyrid-2-one **5** with loss of fluorescence.



	a	b	c	d	e	f	g
R_1	Me	Et	CF_3	pentyn-1-yl	octyn-1-yl	pentyl	octyl
R_2	Me	Et	CF_3	H	H	H	H

Scheme 3 Detection of *Pseudomonas aeruginosa* by the β-alanine aminopeptidase activity on fluorogenic 2-*N*-(β-alanyl)amino-1,8-naphthyridines **6a–g** producing the fluorescent 2-amino-1,8-naphthyridine derivatives **3a–g**.

Amino acid derivatives of aminonaphthyridines have previously been reported by Nakatani *et al.* and studied as DNA sequence dependent binding probes using their hydrogen bonding and accepting patterns as imprints for the specific recognition of particular sequences.[5] Investigations in this area are still of interest, particularly with a view to improving selectivity; for example, increasing the binding constants of H-bond recognition by the use of a polyhedral oligomeric silsesquioxane core.[6] Although the naphthyridine structure has received attention as a possible scaffold for novel antibacterial molecules, the aminonaphthyridines have not previously been studied in the context of bacterial detection. The inherent fluorescence of the 2-amino-1,8-naphthyridines, and the potential for *N*-acylation to cause quenching due to a reduction of electron delocalization throughout the π-system, offers an opportunity to evaluate these structures for their potential in detecting specific bacterial aminopeptidase activity. Analogous to the quenched chromogenic substrate β-alanine-1-pentylresorufamine (β-Ala-1-PRF) **1** and the corresponding highly coloured 7-aminoresorufamine **2**, we report here the preparation of novel fluorogenic 2-*N*-(β-alanyl)amino-1,8-naphthyridines **6a–g**, Scheme 3, and their first evaluation as potential bacterial detection agents.

To investigate the requirements for binding to bacteria and the effect of substituents on the fluorescence, the substituents R_1 and R_2 were varied; simple symmetrical alkyl substituted naphthyridines **3a,b**,[11,12] which have previously been reported, served as the initial scaffolds. The bis(trifluoromethyl) analogue **3c** was expected to have increased lipophilicity and fluorescence, as is observed for other fluorinated fluorogens.[10] As we have already observed[3] that aliphatic substituents result in better adhesion to the bacterial colonies through an increase in the lipophilicity of the chromophores, the pentynyl **3d**, octynyl **3e**, pentyl **3f**, and octyl **3g** analogues were also synthesized.

2 Results and discussion

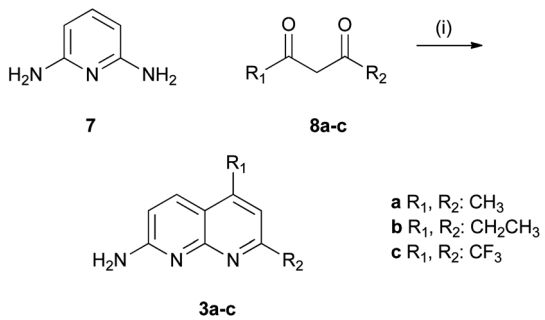
2.1 Chemistry

Synthesis of the 2-aminonaphthyridine substrates **6a–g** was achieved in 2 steps: the synthesis of the 2-aminonaphthyridine fluorogen and the coupling to the amino acid.

2.1.1 Preparation of 2-amino-1,8-naphthyridines. Two different strategies were used for the synthesis of the fluorogenic

substituted 2-amino-1,8-naphthyridines **3a–g**. For 5,7-disubstituted aminonaphthyridines **3a–c**, an adapted Combes quinoline synthesis was employed,[11–14] using 2,6-diaminopyridine **7** and the appropriate diketone **8a–c**, Scheme 4. In order to introduce a chlorine atom into these systems (to allow for further substitution of the naphthyridine core), 2-amino-7-hydroxy-1,8-naphthyridine **9** was prepared by the condensation of 2,6-diaminopyridine **7** and malic acid **10**, Scheme 5.[11] Before undertaking any reactions on the hydroxyl group, the amino functionality of 2-amino-7-hydroxy-1,8-naphthyridine **9** was first protected by *N*-acetylation,[11] to give the amide **11**. Chlorination using $POCl_3$ gave **12**, and the possibility of further structural elaboration involving the chloro group.[11]

Among the many possibilities offered by cross coupling strategies, a Sonogashira-type reaction was chosen to give the alkyne-substituted amide derivatives, **13a** and **13b**, with the aim of improving adherence to the bacterial cell wall. The unsaturated derivatives were also reduced, using 10% Pd/C catalyst and hydrogen, to give the alkyl substituted analogues **14a,b**. In the case of **13b**, the level of reduction appeared to be very sensitive to the activity of the catalyst and the reaction time, as long reaction times resulted in over-reduction to the corresponding dihydronaphthyridine **14c**. To avoid this over-reduction, the reaction was stopped at an early stage and **14b** was used without further purification as a mixture with the partially unsaturated derivative. Hydrolysis of the acetamido groups of **13a,b** and **14a,b** gave the fluorophores **3d,e** and **3f,g**, respectively.

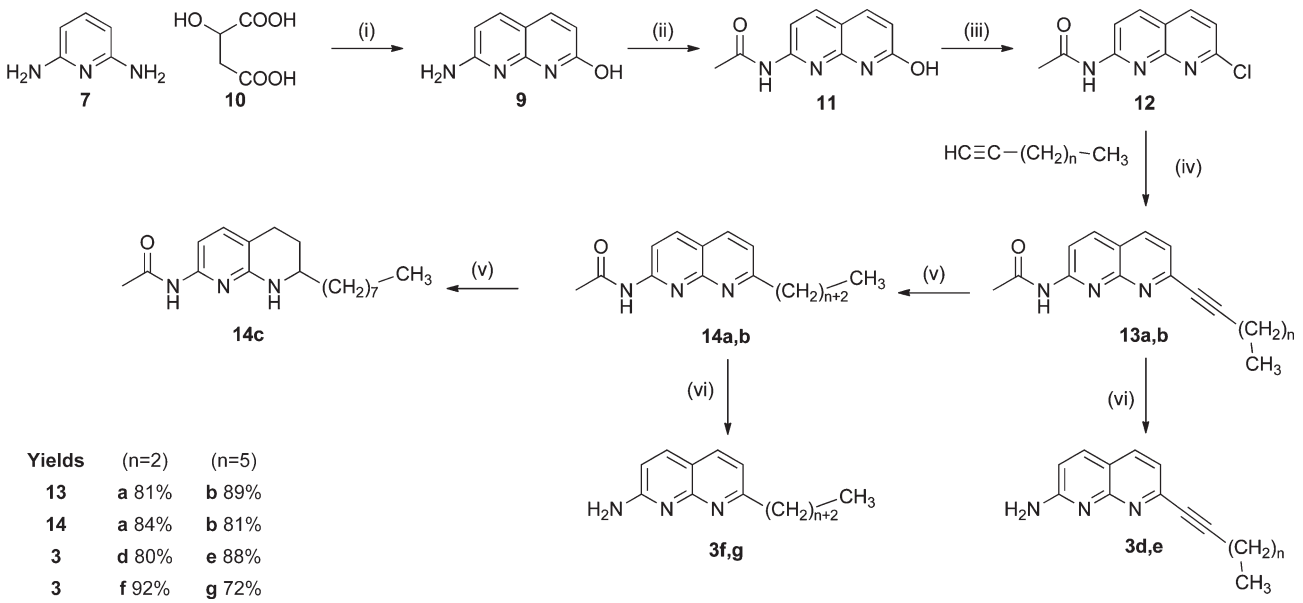


Scheme 4 Synthesis of symmetrical 2-amino-5,7-disubstituted-1,8-naphthyridines **3a–c**.[11–13] Reagents and conditions: (i) H_3PO_4, reflux.

2.1.2 Amide bond formation. Each of the aminonaphthyridines **3a–g** could be readily converted into the aminoacyl derived substrate **6a–g** for a specific bacterial aminopeptidase, through coupling to a protected amino acid, such as ^{t}Boc-β-alanine, followed by deprotection. The aminoacylation of the 2-aminonaphthyridines **3a–g** with ^{t}Boc-β-Ala-OH **17** was facilitated through the stable pentafluorophenyl ester **19**,[5] formed from ^{t}Boc-β-Ala-OH **17** and pentafluorophenol **20** with dicyclohexylcarbodiimide as the dehydrating agent. Reaction of the ^{t}Boc-β-alanylpentafluorophenol ester **19** with the 2-amino-1,8-naphthyridine derivatives **3a–g** provided the protected aminoacyl-substituted naphthyridines **21a–g** in good yield, Table 1. Deprotection gave the 2-*N*-(β-alanyl)aminonaphthyridines, **6a–g** in moderate to good yield, for microbiological evaluation, Scheme 6 and Table 1.

2.2 Fluorescent properties

The fluorescence spectra of the compounds prepared were obtained, as the project uses off-to-on fluorescence (before and after enzymatic activity) for bacterial detection. Practical evaluation of clinical samples is based on the visual inspection of agar plates, upon irradiation with UV light at 365 nm. Solutions of enzyme substrates **6** and fluorogens **3** were visually observed under UV light, followed by recording of the emission spectra at

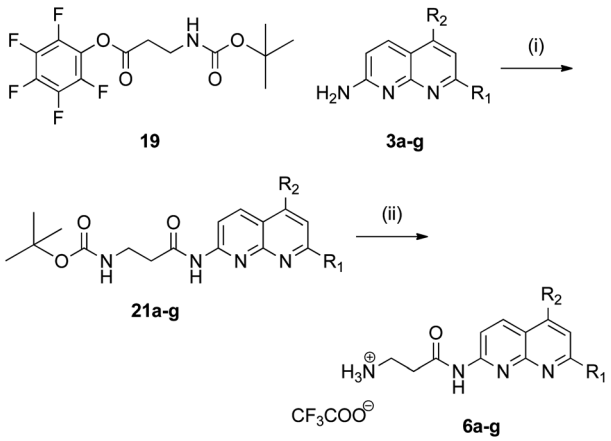


Yields	(n=2)	(n=5)
13	a 81%	b 89%
14	a 84%	b 81%
3	d 80%	e 88%
3	f 92%	g 72%

Scheme 5 Synthesis of 7-alkynyl and 7-alkylsubstituted naphthyridines **3d–g**. Reagents and conditions: (i) conc. H_2SO_4, 110 °C, 3 h; (ii) Ac_2O, reflux, 3 h; (iii) $POCl_3$, 95 °C, 2 h; (iv) $Pd(Ph_3)_2Cl_2$, CuI, Et_3N, DMF, N_2, 80 °C; (v) H_2, 10 mol% 10 w/w% Pd on charcoal, MeOH; (vi) NaOH, MeOH.

Table 1 Yields of (i) ^{t}Boc protected *N*-aminoacylnaphthyridines **21a–g** and (ii) final *N*-aminoacylnaphthyridine substrates **6a–g**

Starting amines	R_1	R_2	(i) Protected fluorogens	Yield (%)	(ii) *N*-Aminoacyl naphthyridines	Yield (%)
3a	Me	Me	**21a**	86	**6a**	83
3b	Et	Et	**21b**	62	**6b**	76
3c	CF_3	CF_3	**21c**	31	**6c**	84
3d	1-Pentynyl	H	**21d**	86	**6d**	77
3e	1-Octynyl	H	**21e**	94	**6e**	87
3f	1-Pentyl	H	**21f**	89	**6f**	65
3g	1-Octyl	H	**21g**	54	**6g**	59



Scheme 6 Peptide bond formation and deprotection to provide fluorogenic *N*-aminoacylnaphthyridine substrates **6a–g**. Reagents and conditions: (i) DIPEA, DMF, 40 °C; (ii) TFA.

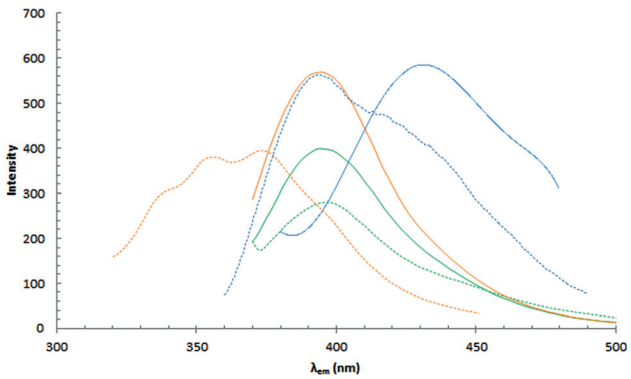


Fig. 2 Emission spectra of **3a** (green solid line), **6a** (green dashed line), **3b** (orange solid line), **6b** (orange dashed line), **3c** (blue solid line) and **6c** (blue dashed line) (8×10^{-8} M in MeOH–H_2O) (intensities are shown in arbitrary units).

fixed excitation wavelength (350 nm), Fig. 2 and 3. The emission spectra supported the conclusions reached from the observation of the solutions under UV; the bigger the increase in the intensity of emitted light caused by specific enzymatic activity, the more reliable the detection. In the case of **3a**, **3c** and **3d**, the presence of the aminoacyl moiety was found to have insufficient effect on the emission to distinguish the acylated form **3a,c,d** from the amine **6a,c,d**. As expected, the emission intensity slightly increased across the CH_3 (**3a**) < CH_2CH_3 (**3b**) < CF_3

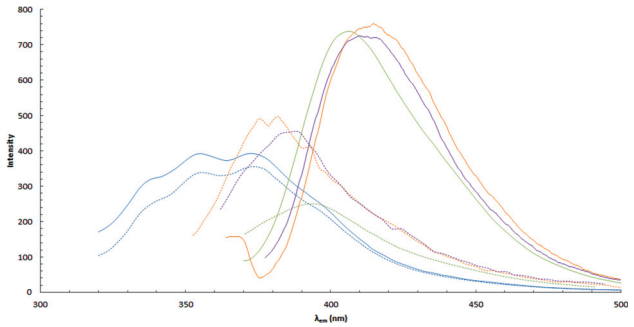


Fig. 3 Emission spectra of **3d** (blue solid line), **6d** (blue dashed line), **3e** (green solid line), **6e** (green dashed line), **3f** (orange solid line), **6f** (orange dashed line), **3g** (purple solid line), **6g** (purple dashed line) (8×10^{-8} M in MeOH–H_2O) (intensities are shown in arbitrary units).

(**3c**) disubstituted derivatives, presumably due to the increasing electronegativity of the substituents, thus promoting a greater delocalization effect.

When comparing the naphthyridines bearing longer alkynyl **6d**, **6e** and alkyl **6f**, **6g** substituents, an increase in the emission intensity and wavelength was noticeable, perhaps through the positive inductive effect of the substituents. This feature is beneficial as the emission of the released fluorophores must be differentiated from the background fluorescence of standard endogenous media ingredients. The largest difference in fluorescence intensities between a 2-*N*-(β-alanyl)aminonaphthyridine substrate and its corresponding fluorophore was observed for **3e** and **6e**.

It has been reported previously that naphthyridines can form complexes with certain transition metal and s-block ions, depending on the substituents on the donor naphthyridines and favourable acceptor ion sizes;[15,16] upon binding to these ions, the fluorescent properties of the naphthyridines can significantly change. Taking into account possible toxicity to bacterial cells and convenience for use in a detection plate, fluorescent emissions in the presence of Fe(III), Mg(II) and Ca(II) were also assessed for each derivative, using ion concentrations in the range of practical standards on agar plates (1.79×10^{-3} M).[17]

Once again, the octynyl substituted naphthyridines **3e** and **6e** showed the most desirable properties; complexation to Mg(II) and Ca(II), in particular, resulted in an increase in the intensity of the fluorescence, which would be advantageous for bacterial differentiation, Fig. 4. The presence of Fe(III) quenched light emission in each case, presumably due to its paramagnetic properties. Thus, octynyl substrate **6e** and its corresponding

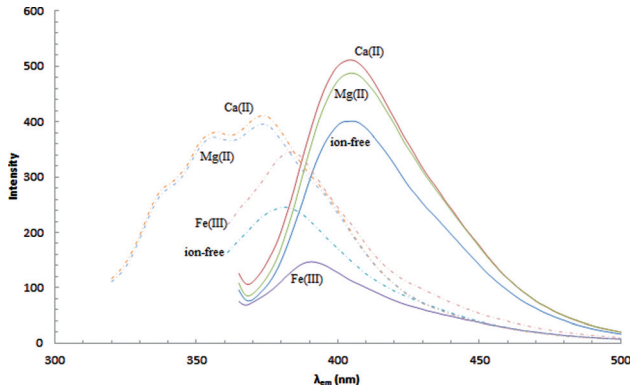


Fig. 4 Emission spectra of **3e** (continuous line) and **6e** (dotted line) in the presence of chosen ions (0.8×10^{-6} M **3e**/**6e** in MeOH–H_2O; 2×10^{-4} M Ca(II)/Mg(II)/Fe(III) concentration, respectively) (intensities are shown in arbitrary units).

fluorophore **3e** were established as most suitable for the aims of this project. In samples containing Mg(II) and Ca(II) ions, the intensity of the fluorescence was increased, as was the wavelength of the emitted light, the latter not significantly.

2.3 Biological evaluation

The substrates incorporated in a commercially available Columbia medium were subjected to microbiological evaluation against a range of clinically relevant microorganisms.

All of the substrates were tested on ten Gram negative (Table 2 entries 1–10), eight Gram positive (Table 2 entries 11–18) microorganisms and two yeasts (Table 2 entries 19 and 20). The results are shown in Table 2 and Fig. 5. The intended specificity was achieved by substrates **6a**, **6b**, **6c**, and **6f**, as these derivatives were hydrolyzed only by strains possessing β-alanylaminopeptidase activity. Under long-wave UV light, the colonies of strains hydrolyzing the substrates are highly fluorescent in comparison to the negative ones and the dark blue background of the medium. Substrate **6d**, with a pentynyl substituent, was hydrolyzed by all of the Gram negative bacteria, making it unsuitable for use to distinguish β-alanylaminopeptidase activity, as this enzyme is only expressed in selected Gram negative bacteria, indicating non-specific substrate hydrolysis. In such detection methods, fluorogens that do not inhibit bacterial growth are generally preferred and substrates **6a–d** and **6f** were non-growth inhibitory. However in the presence of substrates **6e** and **6g** (with longer saturated side chains) only *Burkholderia cepacia* and *Morganella morganii* grew, indicating greater toxicity, perhaps due to the disruption of the bacterial cell membrane through interaction of the long chain alkyl groups of **3e** and **3g**. The ability of the three colistin/polymyxin resistant bacterial strains, *B. cepacia*, *M. morganii* and *Providencia rettgeri*, to survive on the plates containing **6g**, Fig. 5, lends support to this argument—these strains are resistant to antibacterial agents known to disrupt the integrity of the cell wall.[18] These latter substrates, **6e** and **6g**, offer potential as selective inhibitors of bacterial growth—an alternative method for bacterial identification.

Upon hydrolysis of the substrates **6a–g**, the release of the fluorophores gave purple fluorescence (374–406 nm) with some diffusion away from bacterial colonies. Adherence to the bacterial colonies, another desirable property for a detection method, was improved with the introduction of more lipophilic substrates. The individual colonies highlighted by the hydrolysis of substrate **6f** testify to the localization of the fluorogen **3f** once released; by comparison, the fluorescence released by hydrolysis of **6a** and **6b** diffused across the plate, preventing visualization of individual colonies, Fig. 5.

3 Conclusion

Novel fluorescent 2-amino-1,8-naphthyridines **3a–g** were synthesized, using simple procedures, in reliably good yields from commercially available, inexpensive starting materials. These amines were then converted into their β-alanyl analogues **6a–g** which were subjected to biological evaluation to assess their potential for use in agar plate based diagnostic methods. It was established that **6a–c** and **6f** are specific substrates for β-alanylaminopeptidase, releasing the expected fluorogens. Diffusion of the fluorescent dyes was decreased by the use of more lipophilic substrates, as in **6c–g**, but this resulted in a loss of specificity for **6d**, or an increase in toxicity. The growth inhibitory effect of substrates **6e** and **6g** against many bacteria, and their detection of colistin/polymyxin resistant bacterial strains, support the case for development of agents for selective detection of these strains.

4 Experimental

4.1 Chemistry

4.1.1 General. NMR spectra were obtained on a Bruker Ultrashield 300 spectrometer (at 300 MHz for ^{1}H and at 75 MHz for ^{13}C spectra). The chemical shifts are shown in ppm downfield from tetramethylsilane, using residual chloroform (δ = 7.26 in ^{1}H NMR) or the middle peak of the $CDCl_3$ carbon triplet (δ = 77.23 in ^{13}C NMR) as an internal standard. Melting points were obtained using a Reichart-Kofler hot-stage microscope apparatus and are uncorrected. Infrared spectra were recorded using a Perkin Elmer Spectrum BX FT-IR instrument. Low resolution mass spectra were recorded on a Bruker Esquire 3000plus analyser using electrospray source in positive ion mode. High resolution mass spectra were obtained on a LTQ Orbitrap XL instrument in nanospray ionization mode. Elemental analyses were carried out using an Exeter Analytical CE-440 Elemental Analyzer. Excitation and emission spectra were recorded on a Perkin Elmer LS50B Luminescence Spectrometer. All commercially available reagents and solvents were obtained from Sigma-Aldrich, Alfa-Aesar, Fisher Scientific and Riedel-de-Haan and were used without any further purification. Thin layer chromatography was carried out on Merck silica gel plates (60F-254).

4.1.2 Preparation of 2-amino-5,7-disubstituted-1,8-naphthyridines 3a–c by condensation

4.1.2.1 2-Amino-5,7-dimethyl-1,8-naphthyridine ***3a***[12] A mixture of 2,4-pentanedione **8a** (0.50 mL, 4.85 mmol) and 2,6-diaminopyridine **7** (0.50 g, 4.58 mmol) in phosphoric acid (2.50 mL) were heated at reflux for 2 hours. After cooling to room temperature, the resulting mixture was poured over ice and neutralized with 10% aqueous NaOH solution; the solid formed was

Table 2 Microbiological evaluation of the bacterial detection and identification ability and fluorogenic properties of the substrates **6a–g**

		Control		**6a**		**6b**		**6c**		**6d**		**6e**		**6f**		**6g**	
	Microbes[a]	Growth	Fl.	Growth	Fl.	Growth	Fl.	Growth	Fl.	Growth	Fl.	Growth	Fl.	Growth	Fl.	Growth	Fl.
1	*Eschericia coli*	++	–	++	–	++	–	+	–	++	+/–	–	n/a	+	–	–	n/a
2	*Serratia marcescens*	++	–	++	+/– [D]	++	+/– [D]	+	+	++	+/–	–	n/a	+	+/–	–	n/a
3	*Pseudomonas aeruginosa*	++	–	++	+/– [D]	++	+/– [D]	+	+	++	+/–	–	n/a	+	+/–	–	n/a
4	*Burkholderia cepacia*	+	–	++	+/– [D]	++	+/– [D]	+	+	++	+/–	++	+	+	+/–	+	–
5	*Yersinia enterocolitica*	++	–	+	–	+	–	++	–	+	+/–	–	n/a	+	–	–	n/a
6	*Salmonella typhimurium*	++	–	++	–	++	–	+	–	++	–	–	n/a	+	–	–	n/a
7	*Citrobacter freundii*	++	–	++	–	++	–	+	–	++	+/–	–	n/a	+	–	–	n/a
8	*Morganella morganii*	++	–	++	–	++	–	+	–	++	+/–	+	n/a	+	+/–	+	–
9	*Enterobacter cloacae*	++	–	++	–	++	–	++	–	++	+/–	–	n/a	+	+/–	–	n/a
10	*Providencia rettgeri*	++	–	+	–	Traces	–	+	–	+	+/–	–	n/a	+	—	+	–
11	*Bacillus subtilis*	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
12	*Enterococcus faecalis*	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
13	*Enterococcus faecium*	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
14	*Streptococcus epidermidis*	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
15	*Staphylococcus aureus*	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
16	MRSA[b]	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
17	*Streptococcus pyogenes*	+	–	+	–	+	–	+	–	+	–	–	n/a	+	–	–	n/a
18	*Listeria monocytogenes*	+	–	+	–	+	–	+	–	+	–	–	n/a –	+	–	–	n/a
19	*Candida albicans*	+/–	–	+/–	–	+/–	–	+	–	+/–	–	–	n/a	+	–	–	n/a
20	*Candida glabrata*	Traces	–	Traces	–	Traces	–	Traces	–	Traces	–	–	n/a	Traces	–	–	n/a
	Background fluorescence	—	None	–	–	–	–	–	+/–	–	+/–	–	+/–	–	–	–	–

Fl.: fluorescence; ++ very good/strong; + medium; – none/weak; [D] diffusion of fluorescence in the medium;[a] Microbes 1–10: Gram negative bacteria; 11–18: Gram positive bacteria; 19 and 20: yeasts. [b] MRSA: methicillin resistant *Staphylococcus aureus*.

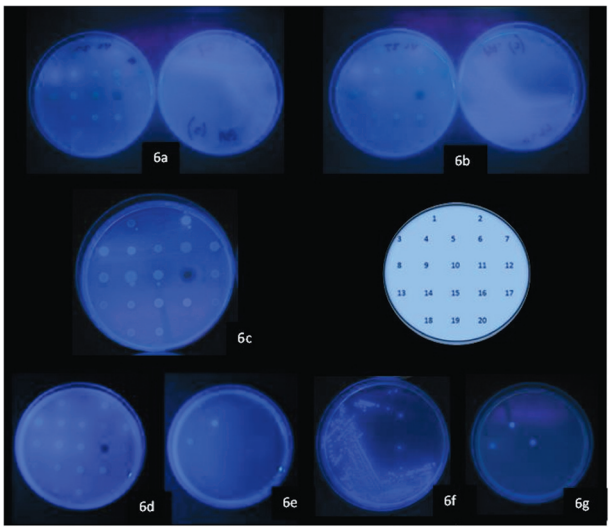


Fig. 5 Fluorescence visible after the culture of various bacterial species with fluorogenic substrates **6a–g** (λ_{ex} = 365 nm). Dotted cultures correspond to the numbered plate; numbers on the agar plates correspond to the entries in Table 2. Streaked cultures are *P. aeruginosa* samples.

collected by vacuum filtration. The desired product **3a** was obtained (0.33 g, 1.94 mmol, 40%) as a white solid after purification by column chromatography (10% methanol, 90% dichloromethane) followed by recrystallization from water; mp 226–227 °C [lit.[12] mp 216–218 °C]; ν_{max}/cm^{-1} 3245, 3179 (NH), 1588; ^1H NMR (300 MHz, $CDCl_3$) δ_H 2.54 (3H, s, C5-CH_3), 2.62 (3H, s, C7-CH_3), 4.95 (2H, br s, NH_2), 6.69 (1H, d, J = 8.7 Hz, CH-3), 6.91 (1H, s, CH-6), 8.01 (1H, d, J = 8.7 Hz, CH-4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 17.9 (CH_3, C5-CH_3), 25.3 (CH_3, C7-CH_3), 110.7 (CH-3), 115.2 (quat., C-4a), 120.1 (CH-6), 134.5 (CH-4), 145.4 (quat., C-5), 156.2 (quat., C-2), 158.7 (quat., C-8a), 161.7 (quat., C-7); MS (ESI) m/z 174.0 (MH^+).

4.1.2.2 2-Amino-5,7-diethyl-1,8-naphthyridine ***3b***[11] A mixture of 3,5-heptanedione **8b** (20.00 mL, 0.15 mol) and 2,6-diaminopyridine **7** (15.00 g, 0.14 mol) were heated in phosphoric acid for 2 hours. The mixture was poured into ice and neutralized with 28% NH_4OH. After filtration, the precipitated solid was continuously extracted with dichloromethane. The solvent was removed *in vacuo* and the crude product was purified by column chromatography (10% methanol, 90% dichloromethane). Recrystallization from ethyl acetate gave a pale yellow solid **3b** (1.66 g, 8.28 mmol, 6%); mp 192–195 °C [lit.[11] mp 187–190 °C]; [Found: C, 71.44; H, 7.45; N, 20.70. $C_{12}H_{15}N_3$ requires C, 71.61; H, 7.51; N, 20.88%]; ν_{max}/cm^{-1} 3252, 3122 (NH), 1582; ^1H NMR (300 MHz, DMSO-d_6) δ_H 1.20–1.29 (6H, m, 2 × CH_3), 2.77 (2H, q, J = 7.5 Hz, C7-CH_2), 2.89 (2H, q, J = 7.5 Hz, C5-CH_2), 6.64 (2H, s, NH_2), 6.79 (1H, d, J = 9.0 Hz, CH-3), 6.91 (1H, s, CH-6), 8.07 (1H, d, J = 9.0 Hz, CH-4); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.9 (C7-CH_3), 15.1 (C5-CH_3), 24.4 (C5-CH_2), 31.8 (C7-CH_2), 112.1 (CH-3), 113.8 (quat., C-4a), 116.1 (CH-6), 133.9 (CH-4), 150.8 (quat., C-5), 157.1 (quat., C-8a), 160.7 (quat., C-2), 165.2 (quat., C-7); MS (ESI) m/z 202.1 (MH^+).

4.1.2.3 2-Amino-5,7-di(trifluoromethyl)-1,8-naphthyridine ***3c***[13] A mixture of 2,6-diaminopyridine **7** (4.90 g, 45.00 mmol), 1,1,1,5,5,5-hexafluoropentane-2,4-dione **8c** (10.00 g, 48.00 mmol) and 85% phosphoric acid (25 mL) was stirred at 120 °C overnight. After standing at room temperature for 6 hours, the reaction mixture was poured into ice water and 10% NaOH was added until pH 7 was reached. The white precipitate obtained was filtered and dried over PCl_5 to yield **3c** (6.30 g, 22.00 mmol, 50%); mp 211–212 °C [lit.[13] mp 204–206 °C]; [Found: C, 42.47; H, 1.88; N, 14.93. $C_{10}H_5F_6N_3$ requires C, 42.72; H, 1.79; N, 14.95%]; ν_{max}/cm^{-1} 3335 (NH), 1642, 1113 (C-F); ^1H NMR (300 MHz, DMSO-d_6) δ_H 7.18 (1H, d, J = 9.3 Hz, CH-3), 7.56 (2H, br s, NH_2), 7.81 (1H, s, CH-6), 8.16 (1H, dd, J = 9.3 and 1.8 Hz, CH-4); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 109.4 (CH, m, J = 2.4 Hz, CH-6), 114.3 (quat., C-4a), 118.7 (CH-3), 121.4 (CF_3, q, J = 273 Hz, C7-CF_3), 123.2 (CF_3, q, J = 274 Hz, C5-CF_3), 133.0 (CH-4), 135.8 (quat., q, J = 32.1 Hz, C-5), 148.3 (quat., q, J = 34.5 Hz, C-7), 157.5 (quat., C-8a), 162.3 (quat., C-2); MS (ESI) m/z 282.0 (MH^+).

4.1.3 Preparation of key intermediate 2-acetylamino-7-chloro-1,8-naphthyridine 12

4.1.3.1 2-Amino-7-hydroxy-1,8-naphthyridine ***9***[19] Compound **9** was prepared by the previously published method (96%); mp >300 °C (deg.) [lit.[19] mp >350 °C]; ν_{max}/cm^{-1} 3377, 3157 (NH), 1615; ^1H NMR (300 MHz, DMSO-d_6) δ_H 6.11 (1H, d, J = 9.3 Hz, CH), 6.36 (1H, d, J = 8.4 Hz, CH), 6.94 (2H, br s, NH_2), 7.64 (2H, m, 2 × CH), 11.79 (1H, br s, OH); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 105.4 (quat.), 105.5 (CH), 115.4 (CH), 137.7 (CH), 140.0 (CH), 150.9 (quat.), 160.9 (quat.), 164.1 (quat.).

4.1.3.2 2-Acetylamino-7-hydroxy-1,8-naphthyridine ***11***[11] Compound **9** was converted into **11** using the known method to give a yellow powder (80%); mp >300 °C [lit. mp ~300 °C]; ν_{max}/cm^{-1} 3171 (broad NH and/or OH), 1653 (C═O), 1583 (amide I), 1522 (amide II); ^1H NMR (300 MHz, TFA-d_1) δ_H 3.30 (3H, s, CH_3), 7.78 (1H, d, J = 9.6 Hz, CH-3), 8.19 (1H, d, J = 8.7 Hz, CH-6), 8.93 (1H, d, J = 9.6 Hz, CH-4), 9.42 (1H, d, J = 8.7 Hz, CH-5); ^{13}C NMR (75 MHz, TFA-d_1) δ_C 22.6 (CH_3), 109.7 (CH-6), 113.2 (quat., C-4a), 121.9 (CH-3), 139.8 (quat., C-8a), 140.6 (CH-4), 146.9 (CH-5), 147.7 (quat., C-7), 164.2 (quat., C-2), 177.2 (quat., C═O); MS (ESI) m/z 204.0 (MH^+), 226.0 (MNa^+).

4.1.3.3 2-Acetylamino-7-chloro-1,8-naphthyridine ***12***[11] A mixture of naphthyridine **11** (1.00 g, 0.05 mol) and $POCl_3$ (17.50 mL) was heated at 95 °C for 1.5 hours, in the presence of a catalytic amount of DMF. The resulting solution was cooled to room temperature and poured carefully into ice water. The solution was made basic (pH 8) with 10% NaOH resulting in formation of a brown precipitate. The solid was collected by vacuum filtration, air dried and continuously extracted with chloroform for 12 hours. The water layer was extracted with chloroform. The solvent of the combined organic layers was removed by vacuum filtration, and the crude product was purified by column chromatography to give the title compound **12** after recrystallization from ethyl acetate (3.30 g, 15.00 mmol, 30%); mp 238–241 °C [lit. mp 250–252 °C]; [Found: C, 54.66; H, 3.74; N, 18.99. $C_{10}H_8ClN_3O$ requires C, 54.19; H 3.64; N, 18.96%]; ν_{max}/cm^{-1} 3189 (NH), 1694 (C═O), 1603 (amide I),

1484 (amide II); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ_H 2.19 (3H, s, CH_3), 7.56 (1H, d, J = 8.4 Hz, CH-6), 8.39–8.48 (3H, m, CH-3 and CH-4 and CH-5), 11.11 (1H, br s, NH); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ_C 24.7 (CH_3), 115.6 (CH), 119.4 (quat.), 121.9 (CH-6), 140.1 (CH), 140.8 (CH), 153.2 (quat.), 154.6 (quat.), 155.6 (quat.), 170.8 (quat., C═O); MS (ESI) m/z 222.0 (MH^+), 244.0 (MNa^+).

4.1.4 General procedure for the synthesis of 2-acetylamino-7-(alk-1-yn-1-yl)-1,8-naphthyridine 13. To a solution of 2-acetylamino-7-chloro-1,8-naphthyridine **12** in anhydrous DMF, an excess of Et_3N was added, followed by 4 mol% $Pd(PPh_3)_2Cl_2$ and 4 mol% CuI under N_2 at room temperature. The resulting mixture was stirred for 15 minutes at 80 °C. One equivalent of 1-alkyne in DMF was then added dropwise. The resulting solution was stirred at 80 °C for 24 hours and then the volatiles were removed under reduced pressure. The residue was dissolved in dichloromethane and passed through a short plug of Celite which was washed with ethyl acetate. The organic phase was evaporated to dryness and the residue purified by column chromatography (50% petroleum ether, 50% ethyl acetate) to give **13**.

*4.1.4.1 2-Acetylamino-7-(pent-1-yn-1-yl)-1,8-naphthyridine **13a**.* From the reaction of 2-acetylamino-7-chloro-1,8-naphthyridine **12** (0.20 g, 0.90 mmol), Et_3N (1.90 mL), $Pd(PPh_3)_2Cl_2$ (25 mg, 0.04 mmol), CuI (7 mg, 0.04 mmol) and 1-pentyne (61 mg, 0.90 mmol), after column chromatography (33% petroleum ether, 66% ethyl acetate) and recrystallization, **13a** was isolated as a white powder (0.19 g, 0.73 mmol, 81%); mp 182–184 °C; [Found: C, 70.91; H, 6.03; N, 16.61. $C_{15}H_{15}N_3O$ requires C, 71.13; H, 5.97; N, 16.59%]; ν_{max}/cm^{-1} 2957 (broad NH), 2217 (C≡C), 1702 (C═O), 1596 (amide I), 1499 (amide II); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 1.12 (3H, t, J = 7.2 Hz, CH_3-5′), 1.73 (2H, sextet, J = 7.2 Hz, CH_2-4′), 2.37 (3H, s, CH_3CO), 2.51 (2H, t, J = 7.2 Hz, CH_2-3′), 7.47 (1H, d, J = 8.7 Hz, CH-6), 8.07 (1H, d, J = 8.7 Hz, CH-5), 8.19 (1H, d, J = 8.7 Hz, CH-4), 8.55 (1H, d, J = 8.7 Hz, CH-3), 9.71 (1H, br s, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.6 (CH_3, C-5′), 21.5 (CH_2, C-3′), 21.8 (CH_2, C-4′), 25.1 (CH_3, CH_3CO), 81.2 (quat., C-1′), 93.8 (quat., C-2′), 115.7 (CH-3), 119.5 (quat., C-4a), 124.1 (CH-6), 136.4 (CH-5), 138.9 (CH-4), 147.5 (quat., C-7), 154.6 (2 × quat., C-2 and C-8a), 170.3 (quat., C═O); MS (ESI) m/z 254.1 (MH^+), 276.1 (MNa^+).

*4.1.4.2 2-Acetylamino-7-(oct-1-yn-1-yl)-1,8-naphthyridine **13b**.* Using 2-acetylamino-7-chloro-1,8-naphthyridine **12** (1.00 g, 4.50 mmol), Et_3N (9.40 mL), $Pd(PPh_3)_2Cl_2$ (0.15 g, 0.22 mmol), CuI (42 mg, 0.22 mmol) and 1-octyne (0.49 g, 4.50 mmol), **13b** was obtained (1.18 g, 4.00 mmol, 89% yield); mp 148–150 °C; ν_{max}/cm^{-1} 3327, 2928 (NH), 2226 (C≡C), 1679 (C═O), 1597 (amide I), 1503 (amide II); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.83 (3H, t, J = 6.9 Hz, CH_3-8′), 1.25 (4H, m, CH_2-6′ and 7′), 1.42 (2H, quint., J = 6.9 Hz, CH_2-5′), 1.59 (2H, quint., J = 6.9 Hz, CH_2-4′), 2.27 (3H, s, CH_3CO), 2.42 (2H, t, J = 6.9 Hz, CH_2-3′), 7.42 (1H, d, J = 8.4 Hz, CH-6), 8.02 (1H, d, J = 8.1 Hz, CH-5), 8.14 (1H, d, J = 8.8 Hz, CH-4), 8.51 (1H, d, J = 8.1 Hz, CH-3), 9.82 (1H, broad, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.9 (CH_3, C-8′), 19.4 (CH_2, C-3′), 22.4 (CH_2, C-7′), 24.9 (CH_3, CH_3CO), 28.1 (CH_2, C-4′), 28.5 (CH_2, C-5′), 31.2 (CH_2, C-6′), 80.9 (quat., C-1′), 93.7 (quat., C-2′), 115.3 (CH-3), 119.3 (quat., C-4a), 123.9 (CH-6), 136.2 (CH-5), 138.7 (CH-4), 147.4 (quat., C-7), 154.3 (quat., C-2), 154.5 (quat., C-8a), 169.9 (C═O); MS (ESI) m/z 296.2 (MH^+), 318.2 (MNa^+); HRMS (NSI) calcd for $(C_{18}H_{22}N_3O)^+$ 296.1757, found 296.1756.

4.1.5 General procedure for the reduction of 2-acetylamino-7-(alk-1-yn-1-yl)-1,8-naphthyridine 13. The catalyst, 10 mol% (10 w/w%) Pd on charcoal, was added to a solution of 2-acetylamino-7-(alk-1-yn-1-yl)-1,8-naphthyridine **13** in MeOH and the mixture was stirred under H_2 (1 atm) at ambient temperature. After H_2 absorption was complete, the catalyst was removed by filtration and the filtrate was concentrated to dryness. Trituration with diethylether gave the saturated product **14** as a solid.

*4.1.5.1 2-Acetylamino-7-pentyl-1,8-naphthyridine **14a**.* 2-Acetylamino-7-(pent-1-yn-1-yl)-1,8-naphthyridine **13a** (1.26 g, 5.00 mmol) was reduced to give the product as a yellow solid after trituration with diethyl ether (1.07 g, 4.20 mmol, 84%); mp 127–130 °C; [Found: C, 69.65; H, 7.40; N, 16.25. $C_{15}H_{19}N_3O$ requires C, 70.01; H, 7.44; N, 16.33%]; ν_{max}/cm^{-1} 3176, 2926 (NH), 1701 (C═O), 1605 (amide I), 1503 (amide II); ^{1}H NMR (300 MHz, DMSO-d$_6$) δ_H 0.88 (3H, t, J = 6.9 Hz, CH_3-5′), 1.33 (4H, m, CH_2-3′ and 4′), 1.79 (2H, quint., J = 7.5 Hz, CH_2-2′), 2.18 (3H, s, CH_3CO), 2.92 (2H, t, J = 7.5 Hz, CH_2-1′), 7.40 (1H, d, J = 8.1 Hz, CH-6), 8.24 (1H, d, J = 8.1 Hz, CH-5), 8.31 (1H, d, J = 8.7 Hz, CH-3), 8.36 (1H, d, J = 8.7 Hz, CH-4), 10.98 (1H, s, NH); ^{13}C NMR (75 MHz, DMSO-d$_6$) δ_C 13.9 (CH_3, C-5′), 21.9 (CH_2, C-4′), 24.1 (CH_3, CH_3CO), 28.1 (CH_2, C-2′), 30.9 (CH_2, C-3′), 38.1 (CH_2, C-1′), 113.8 (CH-3), 118.1 (quat., C-4a), 120.7 (CH-6), 136.7 (CH-5), 139.1 (CH-4), 153.9 (quat., C-2), 154.4 (quat., C-8a), 165.9 (quat., C-7), 169.9 (quat., C═O); MS (ESI) m/z 258.1 (MH^+).

*4.1.5.2 2-Acetylamino-7-octyl-1,8-naphthyridine **14b**.* 2-Acetylamino-7-(oct-1-yn-1-yl)-1,8-naphthyridine **13b** (0.91 g, 3.10 mmol) was reduced by the method above using 10% Pd on charcoal (0.66 g). The white product **14b** was obtained as a mixture after trituration with diethyl ether (0.75 g, 2.51 mmol, 81%), and was used without further purification; mp 86–88 °C; [Found: C, 72.36; H, 8.03; N, 13.97. $C_{18}H_{25}N_3O$ requires C, 72.21; H, 8.42; N, 14.03%]; ν_{max}/cm^{-1} 3189, 2925 (NH), 1697 (C═O), 1603 (amide I), 1500 (amide II); MS (ESI) m/z 298.2, 300.2 (MH^+).

*4.1.5.3 2-Acetylamino-7-octyl-5,6-dihydro-1,8-naphthyridine **14c**.* **13b** (0.81 g, 2.76 mmol) was reduced using 10% Pd/C (0.7 g) in MeOH–EtOAc mixture under 1.8 bar H_2 pressure overnight. The catalyst was removed by filtration through Celite and washing with MeOH. After evaporation of the solvent, the crude product was purified by gradient column chromatography (20% ethyl acetate, 80% petroleum ether to 50% ethyl acetate, 50% petroleum ether) to obtain a pale yellow sticky solid (0.60 g, 1.98 mmol, 71%); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.83 (3H, t, CH_3), 1.27–1.82 (14H, m, 7 × CH_2), 1.90–1.96 (2H, m, CH_2), 2.11 (3H, s, CH_3CO), 2.65–2.69 (2H, m, CH_2), 3.31–3.39 (1H, m, CH), 4.59 (1H, br, NH), 7.15 (1H, d, J = 8.1 Hz, CH), 7.32 (1H, br, CH), 7.67 (1H, br, C(O)NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.9 (CH_3), 22.5 (CH_2), 24.5 (CH_3, CH_3CO), 24.9 (CH_2), 25.5 (CH_2), 27.4 (CH_2), 29.1 (CH_2), 29.4 (CH_2), 29.5 (CH_2), 31.7 (CH_2), 36.4 (CH_2), 51.4 (CH), 101.9 (CH), 111.8 (quat.), 138.1 (CH), 147.8 (quat.), 154.4 (quat.), 167.9 (quat.), 168.0 (quat., C═O); MS (ESI) m/z 304.5 (MH^+).

4.1.6 General procedure for the synthesis of 2-amino-7-(alk-1-yn-1-yl)-1,8-naphthyridine 3d–g. The *N*-acetyl protected aminonaphthyridines **13**, **14** were stirred in MeOH with an excess of 1 M NaOH for 3–5 hours at room temperature. After concentration under vacuum, the residue was taken up into ethyl acetate and extracted with water. The organic layer was dried over $MgSO_4$ and evaporated to dryness. The isolated material was recrystallized from ethyl acetate and petrol.

*4.1.6.1 2-Amino-7-(pent-1-yn-1-yl)-1,8-naphthyridine **3d**.* The deprotection of 2-acetylamino-7-(pent-1-yn-1-yl)-1,8-naphthyridine **13a** (0.14 g, 0.55 mmol) gave **3d** as a white powder after recrystallization (0.09 g, 0.44 mmol, 80%); mp 162–164 °C; ν_{max}/cm^{-1} 3468, 3117 (NH), 2223 (C≡C); ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.05 (3H, t, J = 7.2 Hz, CH_3-5′), 1.66 (2H, sextet, J = 7.2 Hz, CH_2-4′), 2.44 (2H, t, J = 7.2 Hz, CH_2-3′), 5.85 (2H, br s, NH_2), 6.80 (1H, d, J = 8.7 Hz, CH-3), 7.19 (1H, d, J = 8.1 Hz, CH-6), 7.72 (1H, d, J = 8.7 Hz, CH-4), 7.78 (1H, d, J = 8.1 Hz, CH-5); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.6 (CH_3, C-5′), 21.5 (CH_2, C-3′), 21.9 (CH_2, C-4′), 81.4 (quat., C-1′), 92.4 (quat., C-2′), 113.1 (CH-3), 116.5 (quat., C-4a), 121.5 (CH-6), 136.2 (CH-5), 137.7 (CH-4), 146.2 (quat., C-7), 156.1 (quat., C-8a), 160.1 (quat., C-2); MS (ESI) *m/z* 212.1 (MH^+); HRMS (NSI) calcd for $(C_{13}H_{14}N_3)^+$ 212.1182, found 212.1181.

*4.1.6.2 2-Amino-7-(oct-1-yn-1-yl)-1,8-naphthyridine **3e**.* 2-Acetylamino-7-(oct-1-yn-1-yl)-1,8-naphthyridine **13b** (1.47 g, 5.00 mmol) was deprotected using the method above and resulted in **3e** as a white powder (1.10 g, 4.40 mmol, 88%); mp 112–114 °C; [Found: C, 75.81; H, 7.59; N, 16.70. $C_{16}H_{19}N_3$ requires C, 75.85; H, 7.56; N, 16.59%]; ν_{max}/cm^{-1} 3479, 3282, 3117, 2959 (NH), 2221(C≡C), 1628, 1388; ^1H NMR (300 MHz, $CDCl_3$) δ_H 0.89 (3H, t, J = 6.9 Hz, CH_3-8′), 1.30 (4H, m, CH_2-6′ and 7′), 1.47 (2H, quint., J = 6.9 Hz, CH_2-5′), 1.64 (2H, quint., J = 6.9 Hz, CH_2-4′), 2.52 (2H, t, J = 6.9 Hz, CH_2-3′), 6.56 (2H, br, NH_2), 7.01 (1H, d, J = 8.7 Hz, CH-3), 7.18 (1H, d, J = 7.8 Hz, CH-6), 7.74 (2H, m, CH-5 and 4); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.1 (CH_3, C-8′), 19.6 (CH_2, C-3′), 22.6 (CH_2, C-7′), 28.4 (CH_2, C-6′), 28.7 (CH_2, C-5′), 31.4 (CH_2, C-4′), 80.7 (quat., C-1′), 93.8 (quat., C-2′), 113.9 (CH-3), 116.3 (quat., C-4a), 121.7 (CH-6), 136.3 (CH-5), 137.8 (CH-4), 145.9 (quat., C-7), 151.3 (quat., C-8a), 159.9 (quat., C-2); MS (ESI) *m/z* 254.1 (MH^+).

*4.1.6.3 2-Amino-7-(pentyl)-1,8-naphthyridine **3f**.* 2-Acetylamino-7-(pentyl)-1,8-naphthyridine **14a** (0.56 g, 2.20 mmol) was deprotected, resulting in **3f** as a white powder (0.43 g, 2.00 mmol, 92%); mp 111–113 °C; ν_{max}/cm^{-1} 3146, 2923 (NH), 1595, 1509, 1381; ^1H NMR (300 MHz, $CDCl_3$) δ_H 0.87 (3H, t, J = 7.2 Hz, CH_3-5′), 1.33–1.38 (4H, m, CH_2-4′ and 3′), 1.80 (2H, quint., J = 7.5 Hz, CH_2-2′), 2.90 (2H, t, J = 7.5 Hz, CH_2-1′), 6.50 (2H, br, NH_2), 6.94 (1H, d, J = 8.7 Hz, CH-3), 7.07 (1H, d, J = 8.1 Hz, CH-6), 7.76–7.82 (2H, m, CH-4 and 5); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 14.0 (CH_3, C-5′), 22.6 (CH_2, C-4′), 29.5 (CH_2, C-2′), 31.6 (CH_2, C-3′), 38.8 (CH_2, C-1′), 112.6 (CH-3), 115.3 (quat., C-4a), 118.7 (CH-6), 136.5 (CH-5), 138.3 (CH-4), 154.7 (quat., C-8a), 159.4 (quat., C-2), 166.2 (quat., C-7); MS (ESI) *m/z* 216.2 (MH^+); HRMS (NSI) calcd for $(C_{13}H_{18}N_3)^+$ 216.1495, found 216.1491.

*4.1.6.4 2-Amino-7-(octyl)-1,8-naphthyridine **3g**.* The deprotection of **14b** (0.21 g, 0.70 mmol) gave **3g** as a yellow oil, which was purified by trituration with diethyl ether (0.13 g, 0.50 mmol, 72%); HRMS (NSI) calcd for $(C_{16}H_{24}N_3)^+$ 258.1965, found 258.1964; crude **3g** was used directly for the preparation of **6g**.

4.1.7 Preparation of activated ester ^{t}butyl-2-((perfluorophenoxy)carbonyl)ethylcarbamate 19.[5] ^{t}Boc-β-alanine (1.89 g, 10.00 mmol) and pentafluorophenol (2.00 g, 11.00 mmol) were dissolved in ethyl acetate and cooled in an ice bath. To the stirred solution, *N*,*N*-dicyclohexyl carbodiimide (2.30 g, 11.00 mmol) was added. After 1 hour at 0 °C, the precipitated *N*,*N*-dicyclohexylurea was collected by filtration. The filtrate was concentrated *in vacuo* and the resulting white solid was treated with petroleum ether. After standing at low temperature, colourless crystals were collected by filtration (3.00 g, 8.50 mmol, 85%); mp 62–64 °C; ν_{max}/cm^{-1} 2357, 1789, 1681 (C═O), 1517; ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.49 (9H, s, $(CH_3)_3C$), 2.96 (2H, t, J = 6.3 Hz, CH_2CO_2), 3.56 (2H, q, J = 6.3 Hz, CH_2NH), 5.03 (1H, br s, NH); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 28.3 ($(CH_3)_3C$), 33.9 (CH_2, d, J = 8.8 Hz, CH_2CO_2), 36.1 (CH_2, CH_2NH), 79.8 (quat., $C(CH_3)_3$), 124.9 (quat., m, C_{Ar}), 137.9 (quat., m, C_{Ar}), 141.3 (quat., m, C_{Ar}), 142.9 (quat., m, C_{Ar}), 155.8 (quat., $NHCO_2$), 168.4 (quat., CH_2CO_2).

4.1.8 General procedure for the coupling of the substituted 2-amino-1,8-naphthyridine 3 and ^{t}Boc-β-alanine. To a solution of the activated ester **19** in dry DMF, one equivalent of substituted 2-amino-1,8-naphthyridine **3** and 1 equivalent of *N*,*N*-diisopropylethylamine were added. The mixture was stirred at 40 °C for 24 hours. The solvent was evaporated to dryness and the crude residue was purified by silica gel column chromatography. Recrystallization from ethyl acetate and petroleum ether gave the protected amino acid derivatives **21** as a solid.

*4.1.8.1 2-N-(N′-(^{t}Butoxycarbonyl)-β-alanyl)-amino-5,7-dimethyl-1,8-naphthyridine **21a**[5]* By the reaction of activated ester **19** (1.00 g, 2.80 mmol), 2-amino-5,7-dimethyl-1,8-naphthyridine **3a** (0.48 g, 2.80 mmol) and *N*,*N*-diisopropylethylamine (0.48 mL, 2.80 mmol) the title product was obtained as a white solid (0.83 g, 2.40 mmol, 86%); mp 205–208 °C; [Found: C, 62.76; H, 7.07; N, 16.06. $C_{18}H_{24}N_4O_3$ requires C, 62.77; H, 7.02; N, 16.27%]; ν_{max}/cm^{-1} 3206, 3121, 2927 (NH), 1678 (C═O), 1599 (amide I), 1508 (amide II); ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.43 (9H, s, $(CH_3)_3C$), 2.63 (3H, s, CH_3), 2.67 (3H, s, CH_3), 2.80 (2H, br, CH_2-2′), 3.53 (2H, br, CH_2-3′), 5.44 (1H, br s, $NHCO_2$), 7.08 (1H, s, CH-6), 8.28 (1H, d, J = 9.0 Hz, CH-3), 8.43 (1H, d, J = 9.0 Hz, CH-4), 10.1 (1H, br s, NHCO); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 18.0 (CH_3), 25.4 (CH_3), 28.5 ($(CH_3)_3C$), 36.3 (CH_2, C-2′ or 3′), 37.4 (CH_2, C-3′ or 2′), 79.3 (quat., $C(CH_3)_3$), 114.0 (CH-3), 118.6 (quat., C-4a), 122.4 (CH-6), 135.6 (CH-4), 145.3 (quat., C-5), 153.4 (quat., C-2), 154.4 (quat., C═O, ^{t}Boc), 155.9 (quat., C-8a), 162.9 (quat., C-7), 171.8 (quat., C-1′); MS (ESI) *m/z* 345.2 (MH^+), 367.2 (MNa^+).

*4.1.8.2 2-N-(N′-(^{t}Butoxycarbonyl)-β-alanyl)-amino-5,7-diethyl-1,8-naphthyridine **21b**.* By the reaction of activated ester **19** (1.00 g, 2.80 mmol), 2-amino-5,7-diethyl-1,8-naphthyridine **3b** (0.56 g, 2.80 mmol) and *N*,*N*-diisopropylethylamine (0.48 mL, 2.80 mmol), the title product was obtained as a white solid (0.64 g, 1.70 mmol, 62%); mp 183–186 °C; ν_{max}/cm^{-1} 3359, 3201, 2967 (NH), 1677 (C═O), 1594 (amide I), 1507 (amide II); ^1H NMR (300 MHz, $CDCl_3$) δ_H 1.28–1.35 (6H, m, 2 ×

CH_3), 1.36 (9H, s, $(CH_3)_3C$), 2.69 (2H, t, J = 6.0 Hz, CH_2-2′), 2.87–3.02 (4H, m, 2 × CH_2), 3.46 (2H, br, CH_2-3′), 5.25 (1H, br s, $NHCO_2$), 7.07 (1H, s, CH-6), 8.28 (1H, d, J = 9.0 Hz, CH-3), 8.36 (1H, d, J = 9.0 Hz, CH-4), 9.32 (1H, br s, NHCO); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.4 (CH_3), 14.3 (CH_3), 24.7 (CH_2), 28.4 (($CH_3)_3C$), 32.2 (CH_2), 36.3 (CH_2, C-3′), 37.5 (CH_2, C-2′), 79.4 (quat., $C(CH_3)_3$), 113.9 (CH-3), 118.0 (quat., C-4a), 119.4 (CH-6), 135.3 (CH-4), 151.3 (quat., C-5), 154.6 (quat., C-2 or C-8a), 155.9 (2 × quat., C-2 or C-8a and C═O, ^{t}Boc), 167.9 (quat., C-7), 171.5 (quat., C-1′); MS (ESI) m/z 373.3 (MH^+), 395.3 (MNa^+); HRMS (NSI) calcd for $(C_{20}H_{29}O_3N_4)^+$ 373.2234, found 373.2233.

*4.1.8.3 2-N-(N′-(^{t}Butoxycarbonyl)-β-alanyl)-amino-5,7-ditrifluoromethyl-1,8-naphthyridine **21c**.* From the reaction of 2-amino-(5,7-ditrifluoromethyl-1,8-naphthyridine) **3c** (2.00 g, 7.10 mmol), ^{t}Boc-β-alanylpentafluorophenol ester **19** (2.80 g, 7.90 mmol) and *N*,*N*-diisopropylethylamine (2.23 mL, 12.90 mmol), the title compound was obtained after gradient column chromatography (66.6% petroleum ether, 33.3% ethyl acetate) giving a yellow oil. Upon trituration with diethyl ether, the product **21c** was obtained as a white powder (1.00 g, 2.20 mmol, 31%); mp 127–128 °C; ν_{max}/cm^{-1} 3342 (NH), 1689 (C═O), 1675 (C═O), 1587 (amide I), 1512 (amide II), 1271, 1140 (C–F); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.37 (9H, s, $(CH_3)_3C$), 2.67 (2H, t, J = 6.6 Hz, CH_2-2′), 3.28 (2H, q, J = 6.6 Hz, CH_2-3′), 6.79 (1H, br s, $NHCO_2$), 8.26 (1H, s, CH-6), 8.65 (1H, d, J = 9.3 Hz, CH-3), 8.71 (1H, d, J = 9.3 Hz, CH-4), 11.41 (1H, br s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 28.7 (($CH_3)_3C$), 36.6 (CH_2), 37.47 (CH_2), 78.1 (quat., $C(CH_3)_3$), 113.9 (CH-6), 116.9 (quat.), 119.6 (CH-3), 121.7 (CF_3, q, J = 274 Hz), 122.9 (CF_3, q, J = 273 Hz), 135.7 (CH-4), 136.8 (quat., q, J = 32.55 Hz, C-5 or 7), 149.6 (quat., q, J = 35.25, C-5 or 7), 154.8 (quat.), 155.9 (quat.), 156.7 (quat., C═O), 172.4 (quat., C═O); HRMS (NSI) calcd for $(C_{18}H_{19}N_4O_3F_6)^+$ 453.1356, found 453.1355.

*4.1.8.4 2-N-(N′-(^{t}Butoxycarbonyl)-β-alanyl)-amino-7-(pent-1-yn-1-yl)-1,8-naphthyridine **21d**.* By the reaction of 2-amino-7-(pent-1-yn-1-yl)-1,8-naphthyridine **3d** (0.10 g, 0.47 mmol) and the activated ester **19** (0.17 g, 0.47 mmol), the title compound **21d** was obtained as a yellow solid (0.15 g, 0.40 mmol, 86%); mp 149–151 °C; [Found: C, 65.81; H, 6.80; N, 14.57. $C_{21}H_{26}N_4O_3$ requires C, 65.95; H, 6.85; N, 14.65%]; ν_{max}/cm^{-1} 3210, 3120, 2966, 2933 (NH), 2227 (C≡C), 1700 (C═O), 1684 (C═O), 1597 (amide I), 1498 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.09 (3H, t, J = 7.5 Hz, CH_3-5″), 1.42 (9H, s, $(CH_3)_3C$), 1.68 (2H, sextet, J = 7.1 Hz, CH_2-4″), 2.56 (2H, t, J = 7.1 Hz, CH_2-3″), 2.69 (2H, t, J = 6.9 Hz, CH_2-2′), 3.31 (2H, q, J = 6.9 Hz, CH_2-3′), 6.87 (1H, br t, $NHCO_2$), 7.56 (1H, d, J = 8.1 Hz, CH-3), 8.37 (1H, d, J = 8.1 Hz, CH-4), 8.42 (2H, 2 × d, J = 8.7 Hz, CH-5 and CH-6), 11.04 (1H, s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.8 (CH_3, C-5″), 21.0 (CH_2, C-4″), 21.8 (CH_2, C-3″), 28.7 (($CH_3)_3C$), 36.7 (CH_2, C-3′), 37.3 (CH_2, C-2′), 78.1 (quat., $C(CH_3)_3$), 82.1 (quat., C-1″), 93.2 (quat., C-2″), 115.7 (CH-3), 119.6 (quat., C-4a), 124.1 (CH-6), 137.8 (CH-5), 139.6 (CH-4), 146.8 (quat., C-7), 155.0 (quat., C-2), 155.2 (quat., C-8a), 155.9 (quat., C═O, ^{t}Boc), 171.9 (quat., C-1′); MS (ESI) m/z 383.2 (MH^+), 405.2 (MNa^+).

*4.1.8.5 2-N-(N′-(^{t}Butoxycarbonyl)-β-alanyl)-amino-(7-oct-1-yn-1-yl)-1,8-naphthyridine **21e**.* The reaction of 2-amino-7-(oct-1-yn-1-yl)-1,8-naphthyridine **3e** (0.50 g, 1.97 mmol) and activated ester **19** (0.70 g, 1.97 mmol) resulted in a pale yellow solid after recrystallization from ethyl acetate (0.79 g, 1.85 mmol, 94%); mp 123–125 °C; ν_{max}/cm^{-1} 3369, 2928 (NH), 2225 (C≡C), 1702 (C═O), 1675 (C═O), 1596 (amide I), 1499 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 0.89 (3H, t, J = 6.9 Hz, CH_3-8″), 1.33 (4H, m, CH_2-5″ and 6″), 1.38 (9H, s, $(CH_3)_3C$), 1.46 (2H, m, CH_2-7″), 1.62 (2H, m, CH_2-4″), 2.53 (2H, m, CH_2-3″), 2.65 (2H, t, J = 6.6 Hz, CH_2-2′), 3.28 (2H, q, J = 6.6 Hz, CH_2-3′), 6.83 (1H, br, $NHCO_2$), 7.51 (1H, d, J = 8.1 Hz, CH-3), 8.31–8.42 (3H, m, CH-4, 5 and 6), 10.99 (1H, br s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 14.4 (CH_3, C-8″), 19.1 (CH_2, C-3″), 22.5 (CH_2, C-7″), 28.3 (CH_2, C-5″), 28.5 (CH_2, C-4″), 28.7 (($CH_3)_3C$), 31.2 (CH_2, C-6″), 36.8 (CH_2, C-3′), 37.3 (CH_2, C-2′), 78.1 (quat., $C(CH_3)_3$), 81.9 (quat., C-1″), 93.4 (quat., C-2″), 115.7 (CH-3), 119.6 (quat., C-4a), 124.1 (CH-6), 137.8 (CH-5), 139.6 (CH-4), 146.8 (quat., C-7), 155.0 (quat., C-2 or C-8a), 155.2 (quat., C-2 or C-8a), 156.0 (quat., C═O, ^{t}Boc), 171.9 (quat., C-1′); HRMS (NSI) calcd for $(C_{24}H_{33}O_3N_4)^+$ 425.2547, found 425.2547.

*4.1.8.6 2-N-(N′-(^{t}Butoxycarbonyl)-β-alanyl)-amino-7-pentyl-1,8-naphthyridine **21f**.* Starting with 2-amino-7-pentyl-1,8-naphthyridine **3f** (0.35 g, 1.63 mmol), activated ester **19** (0.58 g, 1.63 mmol) and *N*,*N*-diisopropylethylamine (0.28 mL, 1.63 mmol), the title compound was obtained, after purification, as a white solid (0.56 g, 1.45 mmol, 89%); mp 59–61 °C; ν_{max}/cm^{-1} 3372, 2925 (NH), 1709 (C═O), 1675 (C═O), 1606 (amide I), 1506 (amide II); ^{1}H NMR (300 MHz, $CDCl_3$) δ_H 0.88 (3H, t, J = 7.2 Hz, CH_3-5″), 1.25–1.40 (4H, m, CH_2-4″ and 3″), 1.43 (9H, s, $(CH_3)_3C$), 1.84–1.88 (2H, m, CH_2-2″), 2.76 (2H, t, J = 6.0 Hz, CH_2-2′), 3.02 (2H, t, J = 7.8 Hz, CH_2-1″), 3.53 (2H, q, J = 6.0 Hz, CH_2-3′), 5.17 (1H, br, $NHCO_2$), 7.33 (1H, d, J = 8.1 Hz, CH-6), 8.08 (1H, d, J = 8.1 Hz, CH-5), 8.18 (1H, d, J = 9.0 Hz, CH-3), 8.46 (1H, d, J = 9.0 Hz, CH-4), 9.54 (1H, br, NHCO); ^{13}C NMR (75 MHz, $CDCl_3$) δ_C 13.9 (CH_3, C-5″), 22.5 (CH_2, C-4″), 28.4 (($CH_3)_3C$), 29.1 (CH_2, C-2″), 31.6 (CH_2, C-3″), 36.7 (CH_2, C-3′), 37.8 (CH_2, C-2′), 38.8 (CH_2, C-1″), 79.5 (quat., $C(CH_3)_3$), 114.6 (CH-3), 118.8 (quat., C-4a), 121.5 (CH-6), 137.1 (CH-5), 139.7 (CH-4), 153.1 (quat., C-2), 153.3 (quat., C-8a), 155.9 (quat., C═O, ^{t}Boc), 167.5 (quat., C-7), 172.5 (quat., C-1′); MS (ESI) m/z 387.3 (MH^+), 409.3 (MNa^+); HRMS (NSI) calcd for $(C_{21}H_{31}O_3N_4)^+$ 387.2391, found 387.2384.

4.1.9 General procedure for the preparation of the substituted 3-amino-*N*-(1,8-naphthyridin-2-yl)propanamide trifluoroacetate 22. To the ^{t}Boc protected derivatives **21** (1.00 mmol), neat TFA (8 mL) was added. After stirring at room temperature for an hour, the reaction mixture was concentrated under reduced pressure and the residue was taken up into methanol. The solid crude product was obtained by trituration with diethyl ether at low temperature.

*4.1.9.1 3-Amino-N-(5,7-dimethyl-1,8-naphthyridin-2-yl)propanamide trifluoroacetate **6a**.* Deprotection of derivative **21a** (0.34 g, 1.00 mmol) resulted in the title compound **6a** (0.30 g, 0.83 mmol, 83%) after recrystallization from isopropyl alcohol; mp 238–239 °C; [Found: C, 49.97; H, 4.69; N, 15.52.

$C_{15}H_{17}F_3N_4O_3$ requires C, 50.28; H, 4.78; N, 15.64%]; ν_{max}/cm^{-1} 3263, 3084 (NH), 1674 (C═O), 1596 (amide I), 1508 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 2.63 (3H, s, CH_3), 2.65 (3H, s, CH_3), 2.9 (2H, t, J = 6.9 Hz, CH_2-2′), 3.16 (2H, br, CH_2-3′), 7.3 (1H, s, CH-6), 7.93 (3H, br s, NH_3^+), 8.3 (1H, d, J = 9.0 Hz, CH-2), 8.54 (1H, d, J = 9.0 Hz, CH-3), 11.18 (1H, br s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 18.1 (CH_3), 25.2 (CH_3), 34.10 (CH_2, C-2′), 35.14 (CH_2, C-3′), 114.1 (CH-3), 118.5 (quat., C-4a), 122.5 (CH-6), 136.6 (CH-4), 146.9 (quat., C-5), 154.0 (quat., C-2 or C-8a), 154.3 (quat., C-2 or C-8a), 162.3 (quat., C-7), 170.6 (quat., C═O); MS (ESI) m/z 245.3 (MH^+), 267.0 (MNa^+).

4.1.9.2 3-Amino-N-(5,7-diethyl-1,8-naphthyridin-2-yl)propanamide trifluoroacetate ***6b***. Deprotection of derivative **21b** (0.37 g, 1.00 mmol) resulted in the title compound **6b** as a yellow solid after recrystallization from isopropyl alcohol (0.29 g, 0.76 mmol, 76%); mp 227–229 °C; ν_{max}/cm^{-1} 3253, 2970 (NH), 1677 (C═O), 1597 (amide I), 1510 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 1.19–1.26 (6H, m, 2 × CH_3), 2.76–2.86 (4H, m, 2 × CH_2), 2.97 (2H, q, J = 7.5 Hz, CH_2), 3.06 (2H, t, J = 6.9 Hz, CH_2-3′), 7.19 (1H, s, CH-6), 7.77 (3H, br, NH_3^+), 8.21 (1H, d, J = 9.0 Hz, CH-3), 8.49 (1H, d, J = 9.0 Hz, CH-4), 11.11 (1H, br s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.3 (CH_3), 14.7 (CH_3), 24.4 (CH_2), 31.53 (CH_2), 34.1 (CH_2, C-2′), 35.1 (CH_2, C-3′), 114.1 (CH-3), 117.8 (quat., C-4a), 119.6 (CH-6), 136.2 (CH-4), 152.3 (quat., C-2 or C-8a), 153.9 (quat., C-5), 154.6 (quat., C-2 or C-8a), 166.9 (quat., C-7), 170.5 (quat., C═O); MS (ESI) m/z 273.1 (MH^+), 295.1 (MNa^+); HRMS (NSI) calcd for $(C_{15}H_{21}N_4O)^+$ 273.1710, found 273.1714.

4.1.9.3 3-Amino-N-(5,7-ditrifluoromethyl-1,8-naphthyridin-2-yl) propanamide trifluoroacetate ***6c***. By the deprotection of compound **21c** (0.50 g, 1.10 mmol) the title compound **6c** was obtained as a white powder after trituration with diethyl ether (0.43 g, 0.92 mmol, 84%); mp 162–169 °C; ν_{max}/cm^{-1} 3075 (NH), 1673 (C═O), 1585 (amide I), 1512 (amide II), 1156 (broad C-F); ^{1}H NMR (300 MHz, D_2O) δ_H 3.06 (2H, t, J = 6.3 Hz, CH_2), 3.42 (2H, t, J = 6.3 Hz, CH_2), 8.25 (1H, s, CH-6), 8.31 (1H, d, J = 9.6 Hz, CH-3), 8.58 (1H, dd, J = 1.5 and 9.3 Hz, CH-4); ^{13}C NMR (75 MHz, D_2O) δ_C 33.4 (CH_2), 35.1 (CH_2), 114.9 (m, CH-6), 116.5 (quat., q, J = 273 Hz, CF_3), 117.5 (quat.), 118.8 (CH-3), 120.5 (quat., q, J = 273 Hz, CF_3), 136.3 (CH-4), 137.9 (quat., q, J = 33 Hz, C-5 or 7), 150.0 (quat., q, J = 35 Hz, C-5 or 7), 153.8 (quat.), 154.9 (quat.), 171.8 (C═O); MS (ESI) m/z 353.1 (MH^+), 375.1 (MNa^+); HRMS (NSI) calcd for $(C_{13}H_{11}N_4OF_6)^+$ 353.0832, found 353.0832.

4.1.9.4 3-Amino-N-(7-(pent-1-yn-1-yl)-1,8-naphthyridin-2-yl) propanamide trifluoroacetate ***6d***. By the deprotection of **21d** (1.24 g, 3.25 mmol) the title compound **6d** was obtained as a yellow solid (0.99 g, 2.51 mmol, 77%); mp 203–206 °C; [Found: C, 50.23; H, 4.40; N, 12.71. $(C_{16}H_{19}N_4O)_2$ $(CF_3COO)_3$ requires C, 50.39; H, 4.23; N, 12.37%]; ν_{max}/cm^{-1} 3229, 3063 (NH), 2231 (C≡C), 1672 (C═O), 1597 (amide I), 1502 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 0.96 (3H, t, J = 7.2 Hz, CH_3-5″), 1.54 (2H, sextet, J = 7.2 Hz, CH_2-4″), 2.41 (2H, t, J = 7.2 Hz, CH_2-3″), 2.82 (2H, t, J = 6.3 Hz, CH_2-2′), 3.06 (2H, br t, J = 6.3 Hz, CH_2-3′), 7.45 (1H, d, J = 8.1 Hz, CH-6), 7.78 (3H, br, NH_3^+), 8.22–8.27 (2H, m, CH-3 and 5), 8.35 (1H, d, J = 9.0 Hz, CH-4), 11.15 (1H, br s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.8 (CH_3-5″), 21.0 (CH_2-3″), 21.8 (CH_2-4″), 34.2 (CH_2-2′), 35.1 (CH_2-3′), 82.0 (quat., C-1″), 93.4 (quat., C-2″), 115.6 (CH-3), 119.7 (quat., C-4a), 124.3 (CH-6), 137.9 (CH-5), 139.9 (CH-4), 146.9 (quat., C-7), 154.9 (quat., C-2), 155.0 (quat., C-8a), 170.8 (quat., C═O); MS (ESI) m/z 283.1 (MH^+); HRMS (NSI) calcd for $(C_{16}H_{19}N_4O)^+$ 283.1553, found 283.1557.

4.1.9.5 3-Amino-N-(7-(oct-1-yn-1-yl)-1,8-naphthyridin-2-yl)propanamide trifluoroacetate ***6e***. Deprotection of derivative **21e** (0.52 g, 1.23 mmol) resulted in the title compound **6e** as a brown solid (0.47 g, 1.07 mmol, 87%); mp 189–191 °C; ν_{max}/cm^{-1} 3235, 2924 (NH), 2224 (C≡C), 1672 (C═O), 1596 (amide I), 1503 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 0.89 (3H, t, J = 6.9 Hz, CH_3-8″), 1.33 (4H, m, CH_2-6″ and 7″), 1.47 (2H, m, CH_2-4″ or 5″), 1.60 (2H, m, CH_2-4″ or 5″), 2.53 (2H, t, J = 6.9 Hz, CH_2-3″), 2.91 (2H, t, J = 6.6 Hz, CH_2-2′), 3.15 (2H, broad, CH_2-3′), 7.51 (1H, d, J = 8.1 Hz, CH-6), 7.87 (3H, broad, NH_3^+), 8.33 (2H, m, CH-3 and 5), 8.42 (1H, d, J = 9.0 Hz, CH-4), 11.24 (1H, s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.9 (CH_3), 18.6 (CH_2, C-3″), 21.9 (CH_2, C-6″ or 7″), 27.7 (CH_2, C-4″ or 5″), 27.9 (CH_2, C-4″ or 5″), 30.7 (CH_2, C-7″ or 6″), 33.7 (CH_2, C-2′), 34.6 (CH_2, C-3′), 81.4 (quat., C-1″), 93.1 (quat., C-2″), 115.1 (CH-3), 119.2 (quat., C-4a), 123.8 (CH-6), 137.4 (CH-5), 139.4 (CH-4), 146.4 (quat., C-7), 154.4 (quat., C-2), 154.5 (quat., C-8a), 170.3 (quat., C═O); MS (ESI) m/z 325.2 (MH^+), 347.2 (MNa^+); HRMS (NSI) calcd for $(C_{19}H_{25}N_4O)^+$ 325.2023, found 325.2022.

4.1.9.6 3-Amino-N-(7-pentyl-1,8-naphthyridin-2-yl)propanamide trifluoroacetate ***6f***. The deprotection of **21f** (0.34 g, 0.88 mmol) resulted in the title compound **6f** as a white solid (0.23 g, 0.57 mmol, 65%); mp 185–188 °C; [Found: C, 53.88; H, 5.87; N, 13.88. $C_{18}H_{23}F_3N_4O_3$ requires C, 53.99; H, 5.79; N, 13.99%]; ν_{max}/cm^{-1} 3232, 2934 (NH), 1673 (C═O), 1610 (amide I), 1506 (amide II); ^{1}H NMR (300 MHz, DMSO-d_6) δ_H 0.87 (3H, t, J = 6.6 Hz, CH_3-5″), 1.30–1.35 (4H, m, CH_2-3″ and 4″), 1.79 (2H, quint., J = 7.2 Hz, CH_2-2″), 2.85–2.94 (4H, m, CH_2-1″ and 3′), 3.14 (2H, t, J = 6.6 Hz, CH_2-2′), 7.42 (1H, d, J = 8.4 Hz, CH-6), 7.82 (3H, br, NH_3^+), 8.25–8.30 (2H, m, CH-3 and 5), 8.38 (1H, d, J = 8.7 Hz, CH-4), 11.2 (1H, s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 13.8 (CH_3, C-5″), 21.9 (CH_2, C-4″), 28.1 (CH_2, C-2″), 30.9 (CH_2, C-3″), 33.6 (CH_2, C-2′), 34.7 (CH_2, C-3′), 38.1 (CH_2, C-1″), 113.8 (CH-3), 118.3 (quat., C-4a), 120.9 (CH-6), 136.8 (CH-5), 139.3 (CH-4), 153.6 (quat., C-2 or 8a), 154.3 (quat., C-2 or 8a), 166.2 (quat., C-7), 170.0 (C═O); MS (ESI) m/z 287.3 (MH^+).

4.1.9.7 3-Amino-N-(7-octyl-1,8-naphthyridin-2-yl)propanamide trifluoroacetate ***6g***. Amine **3g** (0.11g, 0.43 mmol) was dissolved in DMF, *N*,*N*-diisopropylethylamine (0.074 mL, 0.43 mmol) was added and the reaction mixture was stirred at 40 °C. The reaction was quenched with water and extraction with ethyl acetate was carried out. The organic layer was dried over Na_2SO_4, and the solvent was evaporated. The crude 2-*N*-(*N*′-(^{t}butoxycarbonyl)-β-alanyl)-amino-7-octyl-1,8-naphthyridine **21g** was obtained as a yellow oil (0.10 g, 0.23 mmol, 54%) and used without further purification. Deprotection of **21g** resulted in title compound **6g** (0.06 g, 0.14 mmol, 59%) as a white powder; mp 216–220 °C; [Found: C, 56.65; H, 6.66; N, 12.55. $C_{21}H_{29}F_3N_4O_3$ requires C, 57.00; H, 6.61; N, 12.66%]; ν_{max}/cm^{-1} 3244, 2927 (NH), 1674 (C═O), 1609 (amide I), 1507 (amide II); ^{1}H NMR (300 MHz,

DMSO-d_6) δ_H 0.87 (3H, m, CH_3-8″), 1.10–1.34 (10H, m, 5 × CH_2), 1.79–1.83 (2H, m, CH_2-2″), 2.78 (2H, t, J = 6.4 Hz, CH_2-2′), 2.94 (2H, t, J = 7.5 Hz, CH_2-1″), 3.09 (2H, m, CH_2-3′), 7.43 (1H, d, J = 8.2 Hz, CH_{Ar}), 7.81 (3H, NH_3^+) 8.28 (1H, d, J = 8.2 Hz, CH_{Ar}), 8.33 (1H, d, J = 8.8 Hz, CH_{Ar}), 8.41 (1H, d, J = 8.8 Hz, CH_{Ar}), 11.2 (1H, s, NHCO); ^{13}C NMR (75 MHz, DMSO-d_6) δ_C 14.4 (CH_3, C-8″), 22.5 (CH_2), 28.9 (CH_2), 29.1 (CH_2), 29.2 (CH_2), 29.3 (CH_2), 31.7 (CH_2), 36.2 (2 × CH_2, C-2′ and 3′), 38.6 (CH_2), 114.3 (CH-3), 118.6 (quat., C-4a), 121.3 (CH-6), 137.3 (CH-4 or 5), 139.7 (CH-4 or 5), 154.2 (quat., C-2 or 8a), 155.0 (quat., C-2 or 8a), 166.6 (quat., C-7), 171.3 (C═O); MS (ESI) m/z 329.3 (MH^+), 351.3 (MNa^+); HRMS (NSI) calcd for $(C_{19}H_{29}N_4O)^+$ 329.2336, found 329.2340.

4.2 Biological testing

4.2.1 Preparation of culture media containing substrates 6a–g. 1 L of Columbia agar was prepared as follows; 41 g of Columbia agar (Oxoid Basingstoke, UK) was dissolved by boiling in distilled water (1 L). The solution was then sterilized by autoclaving at 116 °C for 20 min and left to cool at 50 °C. 10 mg of each substrate **6a–g** to be tested was initially dissolved in 200 µl DMSO and this was added to Columbia agar (100 mL) and poured into sterile Petri dishes to give a final concentration of 100 mg L^{-1}. Columbia agar plus DMSO was used as a growth control. Solidified plates were surface dried in a warm air cabinet for 5 min.

4.2.2 Microbial suspension preparation. Microbial reference strains were obtained from either the National Collection of Type Cultures (NCTC) or the National Collection of Pathogenic Fungi (NCPF) which are both located at the Central Health Protection Agency Laboratory, Colindale, UK or the American Type Culture Collection (ATCC), Manassas, USA. The strains were *Escherichia coli* NCTC 10418, *Serratia marcescens* NCTC 10211, *Pseudomonas aeruginosa* NCTC 10662, *Yersinia enterocolitica* NCTC 11176, *Salmonella typhimurium* NCTC 74, *Citrobacter freundii* NCTC 9750, *M. morganii* NCTC 235, *Enterobacter cloacae* NCTC 11936, *P. rettgeri* NCTC 7475, *Bacillus subtilis* NCTC 10400, *Enterococcus faecails* NCTC 775, *Enterococcus faecium* NCTC 7171. *Staphylococcus epidermidis* NCTC 11047, *Staphylococcus aureus* NCTC 6571, *Staphylococcus aureus* (MRSA) NCTC 11939, *Streptococcus pyogenes* NCTC 8306, *Listeria monocytogenes* NCTC 11994, *Candida albicans* ATCC 90028 and *C. glabrata* NCPF 3943. The 20 test microorganisms were maintained on Columbia agar.

4.2.3 Multipoint inoculation. Colonies of each microbial strain were harvested using a loop from overnight cultures on Columbia agar. These were suspended in sterile deionized water to a suspension equivalent to 0.5 McFarland units using a densitometer. 100 µl of this suspension was pipetted into the corresponding wells of a multipoint inoculation device. Each set of plates received 1 µl of bacterial suspension, giving 1.5 × 10^5 organisms per spot on each inoculation. Twenty strains were inoculated per plate and the plates were incubated for 18 h in air at 37 °C.

4.2.4 Activity determination. After incubation, the activity of the microorganisms with the test substrates was determined by observing the plates under UV irradiation at 365 nm and comparing with the substrate-free control. The presence of fluorescence emission was considered as positive evidence for the hydrolysis of the substrate by β-alanylaminopeptidase.

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