Bioorganic & Medicinal Chemistry 22 (2014) 1250–1261

Contents lists available at ScienceDirect

Bioorganic & Medicinal Chemistry

journal homepage: www.elsevier.com/locate/bmc

Synthesis of 2-arylbenzothiazole derivatives and their application in bacterial detection

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ARTICLE INFO

Article history:
Received 21 November 2013
Revised 6 January 2014
Accepted 8 January 2014
Available online 15 January 2014

Keywords:
Fluorogenic substrates
Aminopeptidase
Esterase
Phosphatase
β-Galactosidase
Bacteria detection

ABSTRACT

A series of 2-arylbenzothiazole derivatives have been prepared as fluorogenic enzyme substrates in order to detect aminopeptidase, esterase, phosphatase and β-galactosidase activity in clinically important Gram-negative and Gram-positive bacteria. Substrates were incorporated into an agar-based culture medium and this allowed growth of intensely fluorescent bacterial colonies based on hydrolysis by specific enzymes. Substrate **20** targeted L-alanine aminopeptidase activity and was hydrolysed exclusively by a range of Gram-negative bacteria and inhibited the growth of a range of Gram-positive bacteria. Substrate **19a** targeted β-alanyl aminopeptidase activity and generated fluorescent colonies of selected Gram-negative species including *Pseudomonas aeruginosa*. Substrate **21b** targeted C8-esterase activity and resulted in strongly fluorescent colonies of selected species known to harbour such enzyme activity (e.g., *Salmonella* and *Pseudomonas*). Most Gram-negative species produced colonies with an intense blue fluorescence due to hydrolysis of phosphatase substrates **24a–c** and substrate **24c** was also hydrolysed by strains of *Staphylococcus aureus*. Compounds **26b** and **26c** targeted β-galactosidase activity and generated strongly fluorescent colonies with coliform bacteria that produced this enzyme (e.g., *Escherichia coli*).

1. Introduction

The use and application of enzymatic substrates as tools for the detection and identification of bacteria is a subject of intense academic and commercial interest.[1,2] One strategy for detecting and identifying bacteria is to allow the bacteria to grow in the presence of an enzymatic substrate which is transformed by bacterial enzymes into a product that can readily be detected. The formation of fluorescent heterocycles from bacterial transformations of heterocyclic substrates has been frequently used for this purpose as outlined in Scheme 1. Thus, enzyme induced hydrolysis of weakly fluorescent amino acid **3**, ester **4**, phosphate **5** and sugar **6** derivatives by aminopeptidase, esterase, phosphatase and glycosidase enzymes, respectively liberates either fluorescent amines **1** or phenols **2**. The generation of fluorescence (and also colour) from the hydrolytic enzyme activities depicted in Scheme 1 has found use in numerous commercially available protocols for bacterial detection.[1]

Enzyme substrates derived from *O*-substituted 2-(2-hydroxyphenyl)heterocycles **7** have been reported and these compounds have found application in the detection of phosphatase and other enzymatic activities (Fig. 1).[3,4] The core 2-(2-hydroxyphenyl) heterocycles **7** are highly fluorescent in comparison to their *O*-substituted derivatives and this has been rationalised by an excited-state intramolecular proton transfer (ESIPT) process. In our recent work in this area,[5,6] we prepared a series of L-alanyl and β-alanyl aminopeptidase substrates from heterocycles **8** and observed that these substrates were hydrolysed by some bacteria liberating the corresponding fluorescent amines **8**. During the course of this work we also observed that the 2-(4-hydroxyphenyl)- and 2-(4-aminophenyl)benzothiazoles **9a** and **10a**, respectively appeared to be relatively good fluorophores. We were therefore interested in discovering whether enzyme substrates based upon these structures might also be useful for the detection of bacterial enzyme activity. In this paper we report the synthesis and evaluation of aminopeptidase substrates derived from the amines **9a** and **9b** and also esterase, phosphatase and glycosidase substrates derived from the phenols **10a–10c**.

2. Synthesis of substrates

The parent amine **9a**[7] and phenol **10a**[8] have been reported previously and the synthetic routes used for the preparation of the other amine **9b** and phenols **10b** and **10c** are shown in Schemes 2 and 3, respectively. Thus, reaction of 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride with 4-nitrobenzaldehyde afforded the dihydro derivative **11** which was subsequently

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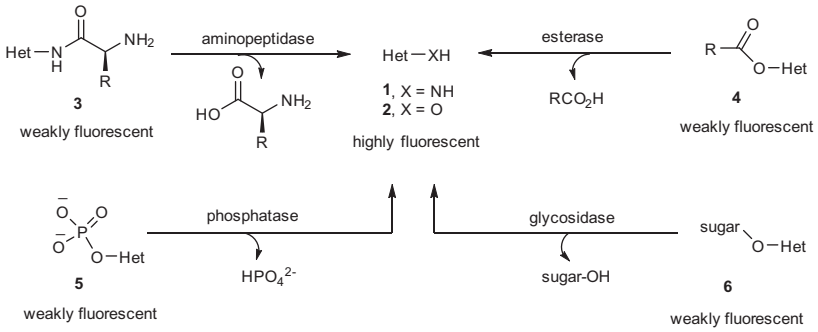
http://dx.doi.org/10.1016/j.bmc.2014.01.013



Scheme 1. Generation of fluorescence from hydrolytic enzymatic activity (Het = heterocycle).

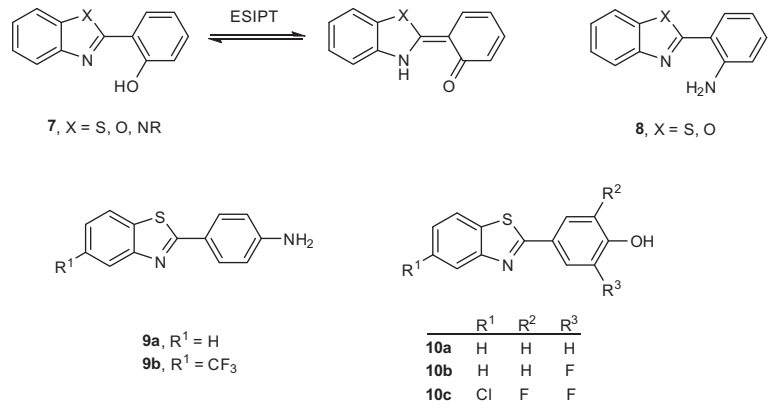
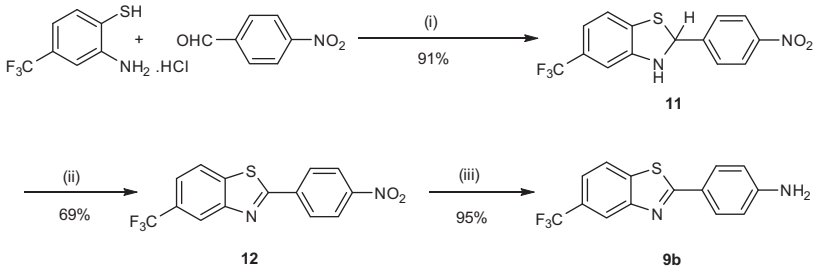
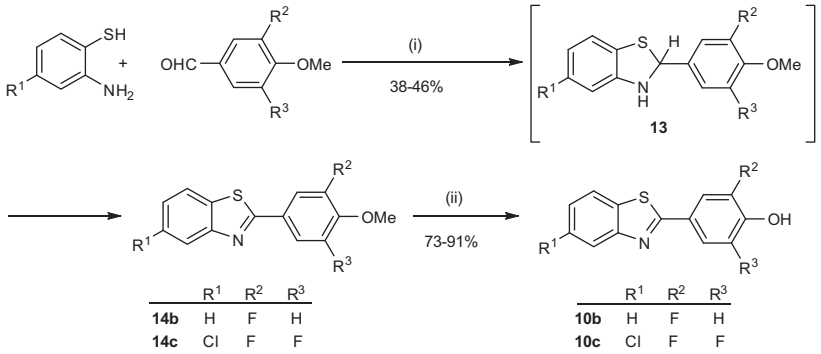


Figure 1. The structures of fluorescent heterocycles relevant to this paper.



Scheme 2. Synthesis of 2-(4-aminophenyl)-5-(trifluoromethyl)benzothiazole **9b**. Reagents and conditions: (i) $NaHCO_3$, EtOH, rt, 2 h; (ii) 2,3,5,6-tetrachloro-1,4-benzoquinone (*p*-chloranil), CH_2Cl_2, rt, 20 h; (iii) $SnCl_2{\cdot}2H_2O$, EtOH, reflux, 2 h.



Scheme 3. Synthesis of the phenols **10b** and **10c**. Reagents and conditions: (i) EtOH, rt then *p*-chloranil, CH_2Cl_2, rt; (ii) pyridinium hydrobromide, melt.

oxidised using *p*-chloranil giving the nitro-compound **12**. Reduction of compound **12** with tin(II) chloride dihydrate gave the required amine **9b**.

3-Fluoro-4-methoxybenzaldehyde[9] was reacted with 2-aminothiophenol and the resulting dihydro-intermediate **13** was not isolated but was oxidised with *p*-chloranil giving the methoxy-derivative **14b** (Scheme 3). Heating this compound with pyridinium hydrobromide in the melt afforded the required phenol **10b**. Compounds **14c** and **10c** were prepared using a similar procedure.

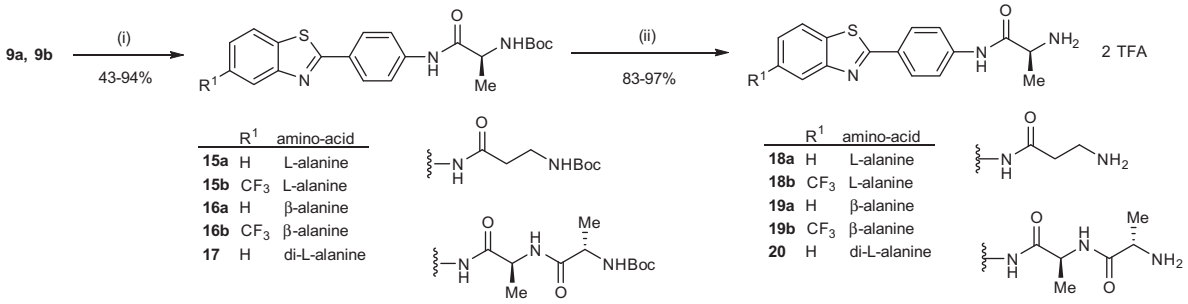
Amine **9a** was coupled to *t*-Boc-protected L-alanine, β-alanine and di-L-alanine giving the amino acid derivatives **15a**, **16a** and **17**, respectively as shown in Scheme 4. Removal of the *t*-Boc-groups from these compounds yielding the required substrates **18a**, **19a** and **20** as their bis-trifluoroacetate salts was achieved using trifluoroacetic acid. Similarly prepared from amine **9b** were the *t*-Boc-protected trifluoromethylated derivatives **15b** and **16b** from which the substrates **18b** and **19b** were obtained.

A series of esterase, phosphatase and glycosidase substrates have been prepared from phenols **10a–c** as outlined in Scheme 5. Thus, treatment of the phenols **10a–c** with octanoyl chloride in the presence of triethylamine afforded the corresponding octanoate derivatives **21a–c**, respectively. The nonate derivative, compound **22b**, was also prepared from phenol **10b** using a similar procedure. The benzyl phosphates **23a–c** were synthesised from phenols **10a–c**, respectively following a literature method.[10] Debenzylation of these compounds giving the required substrates **24a–c** was achieved by treatment with trifluoroacetic acid. Phenols **10a–c** were reacted with 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide using Koenigs–Knorr methodology giving the acylated β-galactoside derivatives **25a–c**, respectively. Deacylation of these compounds was achieved by treatment with methanolic sodium methoxide solution which afforded the β-galactoside substrates **26a–c**.

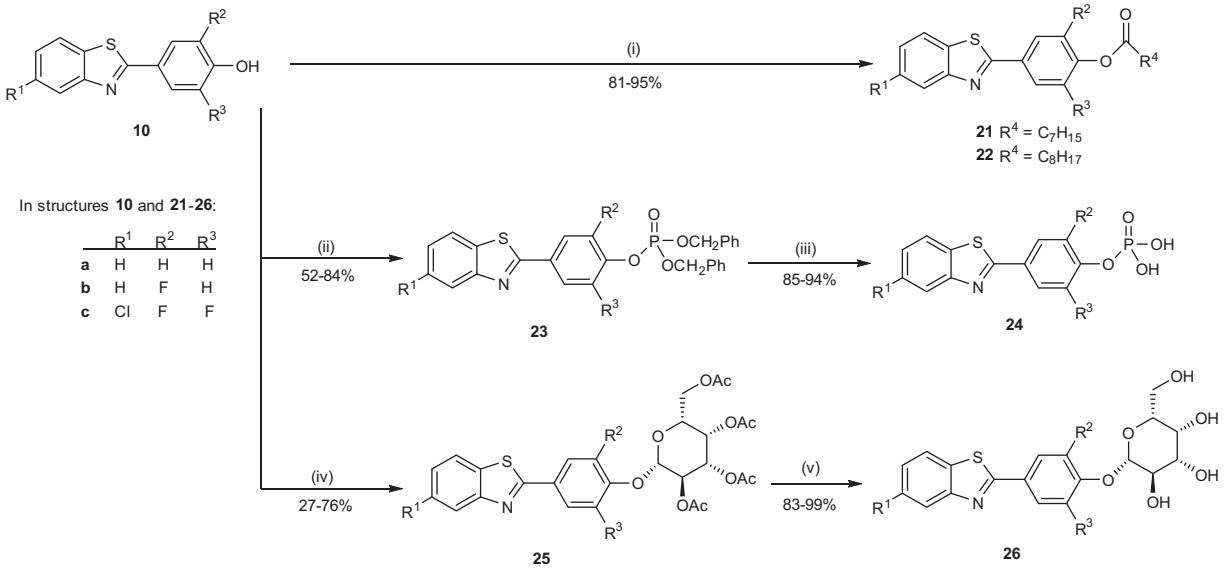
3. Evaluation of substrates

Bacterial colonies were grown on Columbia agar plates in the presence of a substrate. Plates were incubated (18 h) at 37 °C before being viewed in order to assess colony growth and fluorescence in comparison with control plates which did not contain the substrate. Ideally, when fluorescent colonies are generated by enzymatic hydrolysis of the substrate, the fluorescence should be localised within the bacterial colonies. However, in some cases the fluorophore does diffuse noticeably into the media.

L-Alanyl aminopeptidase activity has been used to differentiate between Gram-negative bacteria and Gram-positive bacteria and β-alanyl aminopeptidase activity has been used for specific detection of *Pseudomonas* spp., a common respiratory pathogen associated with cystic fibrosis.[11,12] The five substrates listed in Table 1



Scheme 4. Synthesis of aminopeptidase substrates. Reagents and conditions: *t*-Boc-amino acid, 1-ethyl-3-(3-diethylamino)propylcarbodiimide hydrochloride (EDAC), 1-hydroxybenzotriazole, CH_2Cl_2, rt; (ii) CF_3CO_2H, rt.



Scheme 5. Synthesis of esterase, phosphatase and glycosidase substrates. Reagents and conditions: (i) RCOCl, Et_3N, CH_2Cl_2, rt; (ii) $HP(O)(OCH_2Ph)_2$, CCl_4, CH_3CN, *i*-Pr_2NEt, 4-dimethylaminopyridine (cat.), -10 °C; (iii) trifluoroacetic acid, rt; (iv) CH_2Cl_2, 2,6-lutidine, Ag_2CO_3, 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide, rt; (v), NaOMe, MeOH, rt.

were evaluated for aminopeptidase activity against six Gram-negative and six Gram-positive bacteria. The substrates caused growth inhibition of all Gram-positive species. It is evident from the data presented in Table 1 that the trifluoromethylated substrates **18b** and **19b** are also inhibitory towards most of the Gram-negative bacteria. In cases where bacterial growth had occurred, the generation of fluorescence was generally weak, or at best, moderate. Bacterial growth was generally good in the presence of the L-alanyl aminopeptidase substrate **18a** (with the exception of *Escherichia coli* and *Salmonella typhimurium*) and moderately fluorescent colonies were produced. These results are broadly similar in terms of organism growth and the intensity of fluorescence to those of the L-alanyl substrate derived from heterocycle **8** (X = S) reported previously by us.[5] Based on previous work,[13] the di-L-alanyl substrate **20** was expected to be less inhibitory than the mono-L-alanyl substrate **18a**, but in fact growth was only moderate. Blue fluorescent colonies were produced with substrate **20**, with *Salmonella typhimurium* and *Enterobacter cloacae* colonies showing particularly intense fluorescence. With the exception of *Escherichia coli* and *Salmonella typhimurium*, the other Gram-negative bacteria listed in Table 1 all grew well in the presence of the β-alanyl substrate **19a** but only two bacteria, *Serratia marcescens* and *Pseudomonas aeruginosa*, produced fluorescent colonies, as expected.[11] The β-alanyl substrate derived from heterocycle **8** (X = S) was more inhibitory than substrate **19a** but gave more intensely fluorescent colonies.[5]

As a consequence of the trifluoromethyl-substituent in substrates **18b** and **19b** inhibiting bacterial growth, this group was replaced by a chlorine atom located at the 5-postion of the benzothiazole ring in some esterase, phosphatase and β-galactosidase substrates. Additionally, up to two fluorine atoms were located *ortho* to the phenolic hydroxyl group in order to produce a series of substrates with increasingly acidic hydroxyl groups. Thus, it was anticipated that the fluorinated substrates might give

Table 1
Fluorescent colonies produced by various bacteria in the presence of the aminopeptidase substrates

Substrate	18a		18b		19a		19b		20	
	Growth[a]	Fluorescence[b,c]	Growth[a]	Fluorescence[b]	Growth[a]	Fluorescence[b]	Growth[a]	Fluorescence[b]	Growth[a]	Fluorescence[b,c]
Gram-negative organisms[d]										
Escherichia coli NCTC 10418	NG	NF	NG	NF	NG	NF	NG	NF	NG	NF
Serratia marcescens NCTC 10211	++	+Blue	+	+Purple/Blue	++	+Blue	NG	NF	+	+Blue
Pseudomonas aeruginosa NCTC 10662	+	+Blue	+	+Purple/Blue	++	+Blue	+/−	+/− Purple/Blue	+/−	+/− Blue
Salmonella typhimurium NCTC 74	Trace	+Blue	NG	NF	NG	NF	NG	NF	+	++Blue
Morganella morganii WS 462403	++	+Blue	+	+Purple/Blue	++	NF	+	+/− Purple/Blue	+	+Blue
Enterobacter cloacae NCTC 11936	++	+Blue	+	+Purple/Blue	++	NF	NG	NF	+	++Blue
Providencia rettgeri NCTC 7475	++	+Blue	NG	NF	++	NF	+	NF	+	+Blue

[a] ++ strong growth, + moderate growth, +/− weak growth, NG no significant growth.
[b] ++ strong fluorescence, + moderate fluorescence, +/− weak fluorescence, NF no significant fluorescence. Substrate concentration = 100 mg L^{-1}.
[c] There was noticeable diffusion of the fluorophore into the medium.
[d] The following Gram-positive bacteria did not grow on the media and failed to give fluorescent colonies with any of the substrates: *Staphylococcus epidermidis* NCTC 11047, *Staphylococcus aureus* NCTC 6571, *Staphylococcus aureus* (MRSA) NCTC 11939, *Enterococcus faecalis* NCTC 775, *Enterococcus faecium* NCTC 7171, *Streptococcus pyogenes* NCTC 8306, *Listeria monocytogenes* NCTC 11994.

Table 2
Fluorescent colonies produced by various bacteria in the presence of the esterase substrates

Substrate	21a		21b		21c		22b	
	Growth[a]	Fluorescence[b,c]	Growth[a]	Fluorescence[b,c]	Growth[a]	Fluorescence	Growth[a]	Fluorescence[b,c]
Gram-negative organisms								
Escherichia coli NCTC 10418	++	NF	++	NF	++	See footnote d	++	NF
Serratia marcescens NCTC 10211	++	+Blue	++	++ Purple	++		++	+Purple
Pseudomonas aeruginosa NCTC 10662	++	+ Blue	++	++ Purple	++		++	+Purple
Salmonella typhimurium NCTC 74	++	+/− Blue	++	++ Purple	++		++	+Purple
Enterobacter cloacae NCTC 11936	++	NF	++	NF	++		++	NF
Providencia rettgeri NCTC 7475	++	NF	++	NF	++		++	NF
Gram-positive organisms[e]								
Staphylococcus epidermidis NCTC 11047	+	+/− Blue	+	++ Purple	+		+	+Purple
Staphylococcus aureus NCTC 6571	+	+ Blue	+	++ Purple	+		+	+ Purple
Staphylococcus aureus (MRSA) NCTC 11939	+	+ Blue	+	++ Purple	+		+	+Purple

[a] ++ strong growth, + moderate growth, +/− weak growth, NG no significant growth.
[b] ++ strong fluorescence, + moderate fluorescence, +/− weak fluorescence, NF no significant fluorescence. Substrate concentration = 100 mg L^{-1}.
[c] There was noticeable diffusion of the fluorophore into the medium.
[d] A strong blue fluorescent background was produced in all plates.
[e] The following Gram-positive bacteria did not give fluorescent colonies with any of the substrates: *Enterococcus faecalis* NCTC 775, *Enterococcus faecium* NCTC 7171, *Streptococcus pyogenes* NCTC 8306, *Listeria monocytogenes* NCTC 11994. Growth of these bacteria was moderate.

improved fluorescent characteristics as they were expected to be more ionised in agar media than their non-fluorinated counterparts.

Salmonella is frequently detected in culture media by utilising a C8 esterase substrate (usually in combination with other substrates, inhibitors of other bacteria and other additives) because few other species of *Enterobacteriaceae* produce a C8 esterase.[14] The C8 esterase substrates **21a–c** and the C9 substrate **22b** were therefore examined (Table 2). With this series of substrates, strong growth was evident for all of the Gram-negative bacteria and moderate growth was apparent for the Gram-positive bacteria. Compound **21c** was ineffective as a substrate because an intense blue background fluorescence was produced. This was attributed to a facile non-enzymatic hydrolysis of this substrate as a consequence of the phenolic moiety being a relatively good leaving group due to the presence of the two fluorine atoms. In cases where fluorescence was generated from the substrate, compound **21b** produced strongly fluorescent purple colonies (including *Salmonella typhimurium*) whereas the intensity of fluorescence for the other two substrates **21a** and **22b** was moderate at best.

Phosphatase substrates are typically targeted at the detection of *Staphylococcus* spp.[15] Particularly important is the detection of *S. aureus*, the most common cause of wound infections. Several chromogenic media are commercially available that utilise a phosphatase substrate, usually in combination with complementary glycosidase substrates.[16] All three phosphatase substrates **24a–c** gave good growth and strongly fluorescent colonies with Gram-negative bacteria (Table 3). With Gram-positive bacteria, growth of all bacteria was moderate with all substrates. Substrate **24a** gave only weakly fluorescent colonies with *S. aureus* and no fluorescence was observed with other Gram-positive bacteria. In contrast, substrates **24b** and **24c** both produced fluorescent colonies with all the Gram-positive bacteria listed in Table 3 and particularly striking was the production of very intense blue-coloured colonies with *S. aureus* from substrate **24c**.

In order to evaluate the effectiveness of the β-galactosidase substrates **26a–c**, these substrates were incubated in the presence of isopropyl-β-D-1-thiogalactopyranoside, a promoter of β-galactosidase activity.[17] β-Galactosidase activity is typically used for the identification of coliforms (e.g. *E. coli*, *E. cloacae*, *S. marcesens*) that

Table 3
Fluorescent colonies produced by various bacteria in the presence of the phosphatase substrates

Substrate	24a		24b		24c	
	Growth[a]	Fluorescence[b]	Growth[a]	Fluorescence[b,c]	Growth[a]	Fluorescence[b,c]
Gram-negative organisms						
Escherichia coli NCTC 10418	++	++ Blue	++	++ Blue	++	++ Blue
Serratia marcescens NCTC 10211	++	++ Blue	++	++ Blue	++	++ Blue
Pseudomonas aeruginosa NCTC 10662	++	+ Blue	++	+ Blue	++	++ Blue
Salmonella typhimurium NCTC 74	++	++ Blue	++	++ Blue	++	++ Blue
Enterobacter cloacae NCTC 11936	++	++ Blue	++	++ Blue	++	++ Blue
Providencia rettgeri NCTC 7475	++	++ Blue	++	++ Blue	++	++ Blue
Gram-positive organisms						
Staphylococcus epidermidis NCTC 11047	+	NF	+	+/− Blue	+	+/− Blue
Staphylococcus aureus NCTC 6571	+	+/− Blue	+	+/− Blue	+	++ Blue
Staphylococcus aureus (MRSA) NCTC 11939	+	+/− Blue	+	+ Blue	+	++ Blue
Enterococcus faecalis NCTC 775	+	NF	+	+/− Blue	+	+/− Blue
Enterococcus faecium NCTC 7171	+	NF	+	+/− Blue	+	+/− Blue
Streptococcus pyogenes NCTC 8306	+/−	NF	+	+ Blue	+	+/− Blue
Listeria monocytogenes NCTC 11994	+	NF	+	+/− Blue	+	+/− Blue

[a] ++ strong growth, + moderate growth, +/− weak growth, NG no significant growth.
[b] ++ strong fluorescence, + moderate fluorescence, +/− weak fluorescence, NF no significant fluorescence. Substrate concentration = 100 mg L^{-1}.
[c] There was noticeable diffusion of the fluorophore into the medium.

Table 4
Fluorescent colonies produced by various bacteria in the presence of the β-galactosidase substrates

Substrate	26a		26b		26c	
	Growth[a]	Fluorescence[b,c]	Growth[a]	Fluorescence[b,c]	Growth[a]	Fluorescence[b,c]
Gram-negative organisms						
Escherichia coli NCTC 10418	+	+ Purple	+	++ Purple	++	++ Blue
Serratia marcescens NCTC 10211	+	+Purple	+	++ Purple	++	++ Blue
Pseudomonas aeruginosa NCTC 10662	+	NF	+	NF	++	+/− Blue
Salmonella typhimurium NCTC 74	+	NF	+	NF	++	+/− Blue
Enterobacter cloacae NCTC 11936	+	+Purple	+	++ Purple	++	++ Blue
Providencia rettgeri NCTC 7475	+	NF	+	NF	++	+/− Blue
Gram-positive organisms						
Staphylococcus epidermidis NCTC 11047	+	NF	+	+/− Purple	+	Trace Blue
Staphylococcus aureus NCTC 6571	+	NF	+	+/− Purple	+	+Blue
Staphylococcus aureus (MRSA) NCTC 11939	+	NF	+	+/− Purple	+	+Blue
Enterococcus faecalis NCTC 775	+	+/− Purple	+	+Purple	+	Trace Blue
Enterococcus faecium NCTC 7171	+	+/− Purple	+	+Purple	+	Trace Blue
Streptococcus pyogenes NCTC 8306	+	NF	+	+/− Purple	+	Trace Blue
Listeria monocytogenes NCTC 11994	+	NF	+	NF	+	Trace Blue

[a] ++ strong growth, + moderate growth, +/− weak growth, NG no significant growth. IPTG (30 mg L^{-1}) was added to the media to promote β-galactosidase activity.
[b] ++ strong fluorescence, + moderate fluorescence, +/− weak fluorescence, NF no significant fluorescence. Substrate concentration = 100 mg L^{-1}.
[c] There was noticeable diffusion of the fluorophore into the medium.

are often found as a result of faecal contamination of water supplies.[1] There are numerous commercial products which contain a β-galactosidase substrate indicating the importance of such compounds in diagnostic microbiology.[1] Moderate growth was observed for all of the bacteria listed in Table 4 in the presence of substrates **26a** and **26b**. Only five of the bacteria produced weakly-moderately intense purple-coloured fluorescent colonies with substrate **26a**. However, substrate **26b** showed a broader spectrum of activity and three coliform species, *E. coli*, *E. cloacae* and *S. marcesens*, produced strongly fluorescent colonies. Similarly, substrate **26c** gave strongly fluorescent blue-coloured colonies with these three species and bacterial growth was stronger with this substrate compared to substrate **26b**.

The heterocycles **21b**, **24c** and **26b**, which have emerged as promising substrates for the detection of esterase, phosphatase and β-galactosidase activities, respectively in bacteria, have been compared with their corresponding 4-methylumbelliferone (4-MU) substrates (Figs. 2–4). All three substrates produced a deeper blue fluorescence than their 4-MU analogues. Additionally, significantly less diffusion of the substrates into the media was

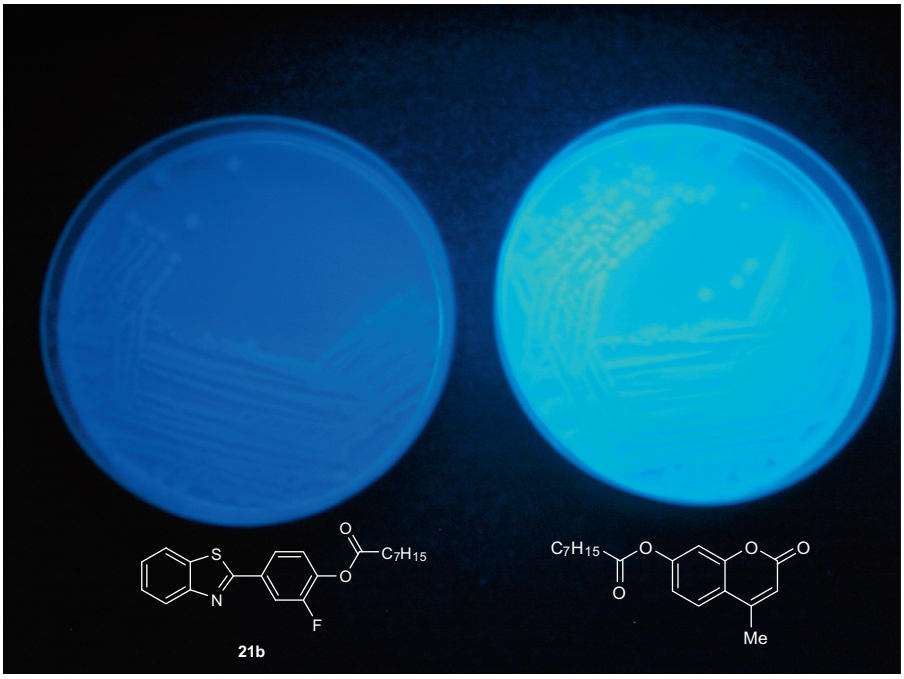


Figure 2. Substrate **21b** and its 4-MU analogue for *Salmonella typhimurium* detection (concentration of each substrate was 0.05 mmol L^{-1}).

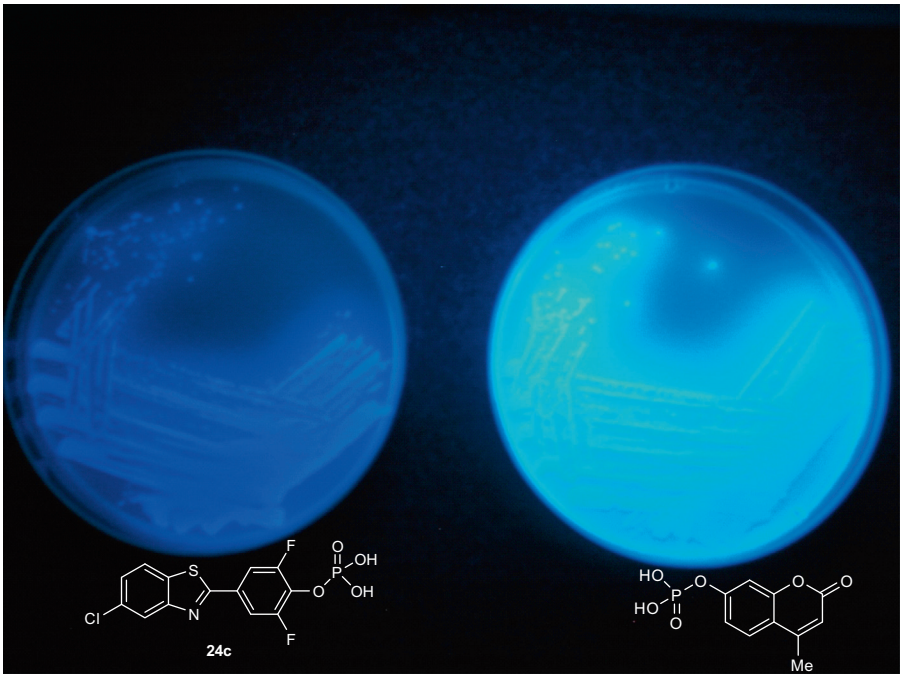


Figure 3. Substrate **24c** and its 4-MU analogue for *S. aureus* detection (concentration of each substrate was 0.05 mmol L^{-1}).

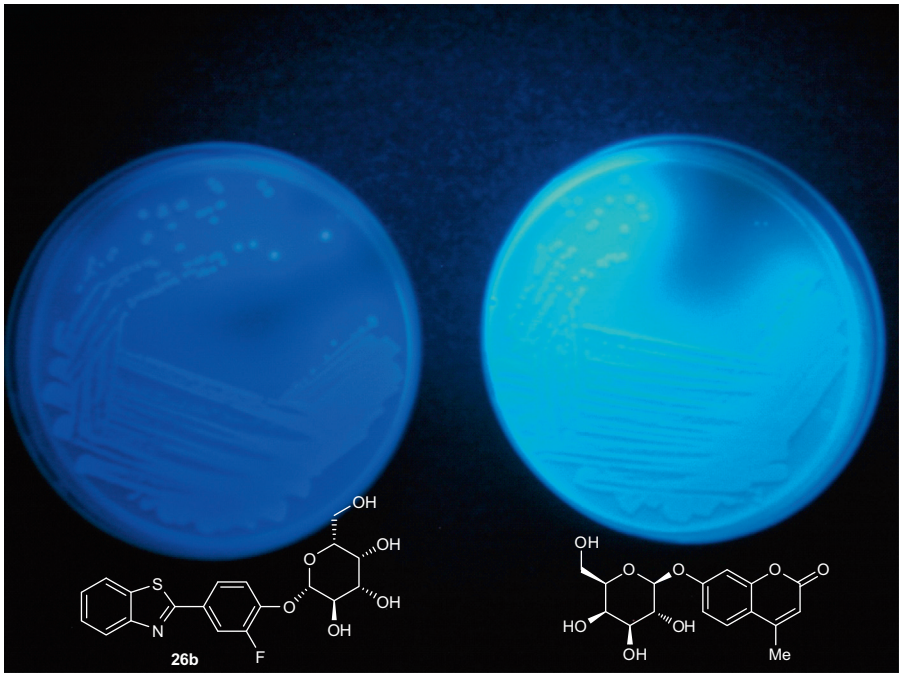


Figure 4. Substrate **26b** and its 4-MU analogue for *E. coli* detection (concentration of each substrate was 0.05 mmol L^{-1}).

noticeable for the substrates **21b**, **24c** and **26b** compared with their 4-MU counter-parts.

4. Conclusions

A series of novel fluorogenic enzymatic substrates have been successfully prepared for the purpose of detecting bacterial activity. The L-alanyl substrates **18a** and **20** can differentiate between Gram-negative and Gram-positive bacteria but these compounds offer no significant advantage over existing L-alanyl substrates. Of particular interest are substrates for the detection of *Salmonella typhimurium*, *Staphylococcus aureus* and coliforms for which compounds **21b**, **24c** and **26b**/**c**, respectively are well suited.

5. Experimental

^{1}H NMR spectra (270 or 400 MHz) and ^{13}C NMR spectra (68 or 100 MHz) were recorded on a Jeol EX270 or Jeol ECS400 instrument. High resolution mass spectrometry (HRMS) was performed by the EPSRC mass spectrometry service. Infrared spectra were obtained via a diamond anvil sample cell using a Perkin Elmer 1000 spectrometer. Melting points are reported uncorrected as determined on a Stuart SMP 1 melting point apparatus. Thin layer chromatography was performed on Merck plastic foil plates pre-coated with silica gel 60 F_{254}. Merck silica gel 60 was used for column chromatography.

5.1. Synthetic work

5.1.1. 2-(4-Nitrophenyl)-5-(trifluoromethyl)-1,2-dihydrobenzothiazole 11

A mixture of 4-nitrobenzaldehyde (0.66 g, 4.40 mmol), 2-amino-4-(trifluoromethyl)benzenethiol hydrochloride (1.00 g, 4.40 mmol) and $NaHCO_3$ (0.74 g, 8.80 mmol) in EtOH (50 mL) was stirred (2 h) at room temperature under a nitrogen atmosphere. The mixture was filtered and water was slowly added to the filtrate until crystals began to form. The solid was collected and dried under vacuum giving compound **11** (1.31 g, 91%) as bright yellow crystals, mp 121–122 °C; δ_H (270 MHz; $CDCl_3$) 4.70 (1H, d, J = 2.7 Hz, NH), 6.52 (1H, d, J = 2.7 Hz, CH), 6.87 (1H, d, J = 1.2 Hz, Ar-H), 6.99–7.10 (2H, m, Ar-H), 7.65 (2H, d, J = 8.7 Hz, Ar-H), 8.19 (2H, d, J = 8.7 Hz, Ar-H); δ_C (68 MHz; $CDCl_3$) 68.7, 106.0 (q, J = 3.6 Hz), 118.1 (q, J = 4.2 Hz), 121.5, 124.3 (q, J = 272.0 Hz), 124.3, 127.4, 128.5 (q, J = 32.7 Hz), 130.4, 146.3, 148.1, 148.7; IR ν_{max} cm^{-1}: 840, 859, 870, 1161, 1238, 1324, 1451, 1514, 1581, 1604, 3076, 3347.

5.1.2. 2-(4-Nitrophenyl)-5-(trifluoromethyl)benzothiazole 12

A mixture of the dihydrobenzothiazole derivative **11** (1.31 g, 4.0 mmol) and *p*-chloranil (0.98 g, 4.0 mmol) in CH_2Cl_2 (200 mL) was stirred (20 h) at room temperature. The resulting mixture was filtered and the filtrate was washed with 10% aqueous NaOH solution, dried (Na_2SO_4) and evaporated. The crude product was purified by recrystallisation from MeOH giving compound **12** (0.89 g, 69%) as orange needles, mp 167–168 °C; HRMS (+NSI) found: MH^+, 325.0255. Calcd for $C_{14}H_8F_3N_2O_2S$: MH, 325.0253; δ_H (270 MHz; $CDCl_3$) 7.67 (1H, dd, J = 1.7, 8.4 Hz, Ar-H), 8.06 (1H, d, J = 8.4 Hz, Ar-H), 8.28 (2H, d, J = 8.9 Hz, Ar-H), 8.33–8.38 (3H, m, Ar-H); δ_C (68 MHz; $CDCl_3$) 121.2 (q, J = 4.2 Hz), 122.6 (q, J = 3.6 Hz), 124.1 (q, J = 272.0 Hz), 124.5, 128.5, 129.7 (q, J = 33.2 Hz), 130.6, 138.5, 138.8, 149.4, 153.7, 167.0; IR ν_{max} cm^{-1}: 685, 817, 851, 1144, 1264, 1335, 1526, 1597.

5.1.3. 2-(4-Aminophenyl)-5-(trifluoromethyl)benzothiazole 9b

A stirred solution of nitro-compound **12** (0.50 g, 1.5 mmol) and tin(II) chloride dihydrate (1.74 g, 7.7 mmol) in EtOH (50 mL) was heated (2 h) at reflux. The mixture was allowed to cool to room temperature and poured onto ice. The pH was made slightly basic by the addition of 5% aqueous sodium hydrogen carbonate solution. The mixture was extracted with ethyl acetate (3 × 50 mL) and the combined organic layers were washed with brine, dried (Na_2SO_4) and evaporated giving compound **9b** (0.42 g, 95%) as an orange solid, mp 185–186 °C; HRMS (+NSI) found: MH^+, 295.0515. Calcd for $C_{14}H_{10}F_3N_2S$: MH, 295.0511; δ_H (270 MHz; $CDCl_3$) 4.00 (2H, br s, NH_2), 6.68 (2H, d, J = 8.7 Hz, Ar-H), 7.48 (1H, dd, J = 1.5, 8.2 Hz, Ar-H), 7.83 (2H, d, J = 8.7 Hz, Ar-H), 7.89

(1H, d, J = 8.2 Hz, Ar-H), 8.17 (1H, d, J = 1.5 Hz, Ar-H); δ_C (68 MHz; $CDCl_3$) 114.8, 119.6 (q, J = 4.2 Hz), 120.8 (q, J = 3.6 Hz), 122.0, 123.3, 124.4 (q, J = 272.0 Hz), 128.6 (q, J = 32.7 Hz), 129.5, 138.1, 149.9, 154.0, 170.6; IR ν_{max} cm^{-1}: 700, 710, 735, 1108, 125, 1412, 1470, 1603, 3226, 3330.

5.1.4. 2-(3-Fluoro-4-methoxyphenyl)benzothiazole 14b

A mixture of 2-aminothiophenol (0.69 mL, 6.45 mmol) and 3-fluoro-4-methoxybenzaldehyde (0.99 g, 6.42 mmol) were stirred in EtOH (15 mL) at room temperature (2 h) before cooling in an ice-water bath. The resulting light coloured precipitate was filtered and washed with ice-cold EtOH. The filtered material was dissolved in CH_2Cl_2 (10 mL) and *p*-chloranil (1.58 g, 6.43 mmol) was added with stirring at room temperature. After 19 h the mixture was filtered through a shallow bed of Celite and the residue rinsed with CH_2Cl_2 (20 mL). The filtrate was washed with aqueous NaOH (1 M, 20 mL) and then brine (20 mL), dried ($MgSO_4$) and evaporated yielding compound **14b** (0.76 g, 46%) as a light brown powder. The compound was used without further purification. An analytical portion was recrystallised from aqueous EtOH giving lilac-coloured needles, mp 115 °C; HRMS (+NSI) found: MH^+, 260.0543. Calcd for $C_{14}H_{11}FNOS$: MH, 260.0540; δ_H (400 MHz; $CDCl_3$) 3.95 (3H, s, OCH_3), 7.03 (1H, t, J = 8.2 Hz, Ar-H), 7.36 (1H, ddd, J = 0.9, 7.6, 8.2 Hz, Ar-H), 7.47 (1H, ddd, J = 0.9, 7.6, 8.2 Hz, Ar-H), 7.77–7.80 (1H, m, Ar-H), 7.83–7.88 (2H, m, Ar-H), 8.02 (1H, d, J = 8.2 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 56.4, 113.3, 115.2 (d, J = 21.1 Hz), 121.7, 123.1, 124.0 (d, J = 2.9 Hz), 125.2, 126.5, 126.9 (d, J = 6.9 Hz), 135.0, 150.2 (d, J = 11.5 Hz), 152.5 (d, J = 246.8 Hz), 154.2, 166.6; IR ν_{max}/cm^{-1} 719, 748, 860, 1000, 1116, 1267, 1278, 1493, 1616.

5.1.5. 2-(3,5-Difluoro-4-methoxyphenyl)-5-chlorobenzothiazole 14c

3,5-Difluoro-4-methoxybenzaldehyde[9] (2.38 g, 13.83 mmol) was dissolved in EtOH (40 mL) and 2-amino-4-chlorothiophenol (2.24 g, 14.03 mmol) was added with stirring at room temperature. After 18 h solvent was evaporated and replaced by CH_2Cl_2 (40 mL). *p*-chloranil (3.41 g, 13.87 mmol) was added and the mixture was stirred (20 h) at room temperature. The mixture was filtered through a bed of Celite and the residue rinsed by CH_2Cl_2 (50 mL). The filtrate was washed sequentially with aqueous NaOH (1 M, 2 × 50 mL), water (50 mL) and brine (50 mL), dried ($MgSO_4$) and evaporated. The crude product was purified by column chromatography over silica gel (eluent: heptane/EtOAc, 10:1) giving compound **14c** (1.65 g, 38%). An analytical portion was recrystallised from aqueous EtOH giving white crystals, mp 161–162 °C; HRMS (+NSI) found: MH^+, 312.0055. Calcd for $C_{14}H_9{}^{35}ClF_2NOS$: MH, 312.0056; δ_H (400 MHz; $CDCl_3$) 4.10 (3H, s, CH_3), 7.38 (1H, dd, J = 2.1, 8.7 Hz, Ar-H), 7.63 (2H, d, J = 9.2 Hz, Ar-H), 7.80 (1H, d, J = 8.7 Hz, Ar-H), 8.03 (1H, d, J = 2.1 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 61.9, 111.7 (dd, J = 17.3, 7.7 Hz), 122.4, 123.3, 126.2, 127.7 (t, J = 9.6 Hz), 132.7, 133.4, 139.1 (t, J = 13.4 Hz), 154.8, 155.6 (dd, J = 6.7, 250.2 Hz), 166.9; IR ν_{max}/cm^{-1} 750, 804, 871, 944, 1032, 1281, 1343, 1426, 1440, 1486, 2951, 3086.

5.1.6. 2-(3-Fluoro-4-hydroxyphenyl)benzothiazole 10b

Compound **14b** (0.75 g, 2.89 mmol) was mixed with pyridine hydrobromide (3.70 g, 23.12 mmol) and heated under an argon atmosphere until melting had occurred. After 1.5 h the mixture was cooled and ice (50 g) was added with stirring. The resulting slurry was extracted into EtOAc (3 × 50 mL) and the combined organic layers washed with brine (50 mL), dried ($MgSO_4$) and evaporated giving compound **10b** (0.65 g, 91%) as light brown crystals, mp 222–223 °C; HRMS (+NSI) found: MH^+, 246.0387. Calcd for $C_{13}H_9FNOS$: MH, 246.0383; δ_H (400 MHz; DMSO-d_6) 7.08 (1H, t, J = 8.5 Hz, Ar-H), 7.36 (1H, ddd, J = 1.2, 7.8, 8.0 Hz, Ar-H), 7.46 (1H, ddd, J = 1.4, 7.8, 8.0 Hz, Ar-H), 7.69 (1H, dd, J = 1.6, 8.5 Hz, Ar-H), 7.81 (1H, dd, J = 1.6, 11.9 Hz, Ar-H), 7.95 (1H, d, J = 8.0 Hz, Ar-H), 8.03 (1H, d, J = 8.0 Hz, Ar-H), 10.69 (1H, s, OH); δ_C (100 MHz; DMSO-d_6) 115.2 (d, J = 20.1 Hz), 118.9 (d, J = 2.9 Hz), 122.7, 123.0, 124.9 (d, J = 2.9 Hz), 125.0 (d, J = 6.7 Hz), 125.7, 127.1, 134.8, 148.7 (d, J = 12.5 Hz), 151.6 (d, J = 243.5 Hz), 154.1, 166.8 (d, J = 2.4 Hz); IR ν_{max}/cm^{-1} 754, 1209, 1263, 1301, 1434, 2200–3100 (broad).

5.1.7. 2-(3,5-Difluoro-4-hydroxyphenyl)-5-chlorobenzothiazole 10c

Using a similar procedure to that described above for the synthesis of compound **10b**, compound **14c** (3.83 g, 12.29 mmol) gave compound **10c** (2.67 g, 73%) as a light brown powder, mp 240–242 °C; HRMS (+NSI) found: MH^+, 297.9895. Calcd for $C_{13}H_7{}^{35}ClF_2NOS$: MH, 297.9899; δ_H (400 MHz; DMSO-d_6) 7.39 (1H, dd, J = 1.8, 8.7 Hz, Ar-H), 7.63 (2H, dd, J = 1.4, 7.3 Hz, Ar-H), 7.96 (1H, d, J = 1.8 Hz, Ar-H), 8.05 (1H, d, J = 8.7 Hz, Ar-H), 11.13 (1H, br s, OH); δ_C (100 MHz; DMSO-d_6) 111.4 (dd, J = 16.3, 8.2 Hz), 122.5, 123.3 (t, J = 9.1 Hz), 124.2, 126.0, 131.9, 133.8, 137.7 (t, J = 16.8 Hz), 152.8 (dd, J = 7.7, 243.5 Hz), 154.7, 167.9; IR ν_{max}/cm^{-1} 748, 754, 797, 856, 1017, 1261, 1333, 1434, 1485, 2200–3200 (broad).

5.1.8. General procedure for the preparation of the Boc-protected derivatives 15–17

To a stirred solution of the amine **9a** or **9b** (1 equiv) in CH_2Cl_2 was added 1-ethyl-3-(3-dimethylamino)propylcarbodiimide·HCl (EDAC) (0.6 equiv), 1-hydroxybenzotriazole (HOBt) (0.6 equiv) and the relevant *t*-Boc protected amino acid (0.6 equiv). The mixture was allowed to stir at room temperature (24 h) and a further 0.6 equiv of each reagent was added. The mixture was then allowed to stir at room temperature (4 days) and the solvent was evaporated giving the crude product.

5.1.8.1. 2-[4-(*t*-Boc-L-alanylamino)phenyl]benzothiazole 15a. Compound **15a** was synthesized from amine **9a** (0.50 g, 2.21 mmol), EDAC·HCl (0.46 g, 2.44 mmol), HOBt (0.32 g, 2.44 mmol), and *t*-Boc-L-alanine (0.42 g, 2.22 mmol) in CH_2Cl_2 (50 mL) following the general procedure described above. The crude product was purified by column chromatography [eluent: CH_2Cl_2 changing to CH_2Cl_2/MeOH (97:3)] giving compound **15a** (75%) as pale green crystals, mp 197–198 °C; HRMS (+NSI) found: MH^+, 398.1530. Calcd for $C_{21}H_{24}N_3O_3S$: MH, 398.1533; δ_H (270 MHz; DMSO-d_6) 1.27 (3H, d, J = 7.2 Hz, CH_3), 1.37 (9H, s, *t*-Bu), 4.12 (1H, td, J = 7.2, 7.2 Hz, CH), 7.20 (1H, d, J = 7.2 Hz, NH), 7.40–7.45 (1H, m, Ar-H), 7.49–7.55 (1H, m, Ar-H), 7.80 (2H, d, J = 8.7 Hz, Ar-H), 8.00–8.03 (1H, m, Ar-H), 8.05 (2H, d, J = 8.7 Hz, Ar-H), 8.11 (1H, ddd, J = 0.7, 1.5, 7.9 Hz, Ar-H), 10.29 (1H, s, NH); δ_C (68 MHz; DMSO-d_6) 18.5, 28.8, 51.2, 78.7, 119.9, 122.8, 123.1, 125.8, 127.1, 128.1, 128.6, 134.9, 142.5, 154.2, 155.8, 167.5, 173.0; IR: ν_{max} cm^{-1}: 724, 752, 834, 1155, 1248, 1314, 1367, 1455, 1515, 1672, 2988, 3327.

5.1.8.2. 2-[4-(*t*-Boc-L-alanylamino)phenyl]-5-trifluoromethylbenzothiazole 15b. Compound **15b** was synthesized from amine **9b** (0.50 g, 1.70 mmol), EDAC·HCl (0.40 g, 2.04 mmol), HOBt (0.28 g, 2.04 mmol), and *t*-Boc-L-alanine (0.38 g, 2.04 mmol) in CH_2Cl_2 (50 mL) following the general procedure described above. The crude product was purified by recrystallisation from MeOH/H_2O giving compound **15b** (43%) as pale green crystals, mp 217–219 °C; HRMS (+NSI) found: MH^+, 466.1402. Calcd for $C_{22}H_{23}F_3N_3O_3S$: MH, 466.1407; δ_H (270 MHz; DMSO-d_6) 1.27 (3H, d, J = 7.2 Hz, CH_3), 1.37 (9H, s, *t*-Bu), 4.13 (1H, td, J = 7.2, 7.2 Hz, CH), 7.19 (1H, d, J = 7.2 Hz, NH), 7.75 (1H, d, J = 8.7 Hz, Ar-H), 7.83 (2H, d, J = 8.7 Hz, Ar-H), 8.08 (2H, d, J = 8.7 Hz, Ar-H), 8.45–8.39 (2H, m, Ar-H), 10.34

(1H, s, NH); IR ν_{max} cm^{-1}: 669, 819, 834, 1119, 1147, 1250, 1318, 1338, 1406, 1515, 1673, 2985, 3334.

5.1.8.3. 2-[4-(*t*-Boc-β-alanylamino)phenyl]benzothiazole 16a. Compound **16a** was synthesized from amine **9a** (0.39 g, 1.72 mmol), EDAC·HCl (0.40 g, 2.06 mmol), HOBt (0.28 g, 2.06 mmol) and *t*-Boc-β-alanine (0.38 g, 2.06 mmol) in CH_2Cl_2 (50 mL) following the general procedure described above. The crude product was purified by column chromatography (eluent; CH_2Cl_2/MeOH, 9:1) giving compound **16a** (94%) as pale green crystals, mp 171–173 °C; HRMS (+NSI) found: MH^+, 398.1535. Calcd for $C_{21}H_{24}N_3O_3S$: MH, 398.1533; δ_H (270 MHz, DMSO-d_6) 1.31 (9H, s, *t*-Bu), 2.44–2.49 (2H, m, CH_2), 3.18 (2H, td, *J* = 5.4, 6.7 Hz, CH_2), 6.87 (1H, t, *J* = 5.4 Hz, NH), 7.34–7.39 (1H, m, Ar-H), 7.43–7.49 (1H, m, Ar-H), 7.74 (2H, d, *J* = 8.7 Hz, Ar-H), 7.94–7.96 (1H, m, Ar-H), 7.98 (2H, d, *J* = 8.7 Hz, Ar-H), 8.05 (1H, ddd, *J* = 0.5, 0.7, 7.9 Hz, Ar-H), 10.24 (1H, br s, NH); δ_C (68 MHz; DMSO-d_6) 28.8, 37.0, 37.5, 78.2, 119.8, 122.8, 123.1, 125.8, 127.1, 128.0, 128.6, 134.8, 142.6, 154.2, 156.1, 167.6, 170.5; IR ν_{max} cm^{-1}: 724, 751, 832, 1163, 1227, 1247, 1406, 1480, 1590, 1683, 2985, 3360.

5.1.8.4. 2-[4-(*t*-Boc-β-alanylamino)phenyl]-5-trifluoromethylbenzothiazole 16b. Compound **16b** was synthesized from amine **9b** (0.5 g, 1.70 mmol), EDAC·HCl (0.40 g, 2.04 mmol), HOBt (0.28 g, 2.04 mmol) and *t*-Boc-β-alanine (0.38 g, 2.04 mmol) in CH_2Cl_2 (50 mL) following the general procedure described above. The crude product was purified by recrystallisation from MeOH/CH_2Cl_2 giving compound **16b** (40%) as pale green crystals, mp 197–198 °C; HRMS (+NSI) found: MH^+, 466.1391. Calcd for $C_{22}H_{23}F_3N_4O_4S$: MH, 466.1407; δ_H (270 MHz; DMSO-d_6) 1.32 (9H, s, *t*-Bu), 2.49–2.53 (2H, m, CH_2), 3.23 (2H, td, *J* = 5.7, 6.6, 6.3 Hz, CH_2), 6.92 (1H, t, *J* = 5.7 Hz, NH), 7.73 (1H, dd, *J* = 1.2, 8.7 Hz, Ar-H), 7.81 (2H, d, *J* = 8.7 Hz, Ar-H), 8.05 (2H, d, *J* = 8.7 Hz, Ar-H), 8.34–8.35 (1H, m, Ar-H), 8.37 (1H, d, *J* = 8.7 Hz, Ar-H), 10.34 (1H, s, NH); δ_C (68 MHz; DMSO-d_6) 28.8, 37.0, 37.5, 78.2, 119.7 (q, *J* = 4.2 Hz), 119.8, 121.7 (q, *J* = 4.2 Hz), 124.3, 124.9 (q, *J* = 272.0 Hz), 127.3, 128.0 (q, *J* = 31.7 Hz), 128.9, 139.0, 143.2, 153.8, 156.1, 170.3, 170.6; IR ν_{max} cm^{-1}: 669, 819, 831, 1119, 1146, 1248, 1334, 1406, 1517, 1681, 2926, 3358.

5.1.8.5. 2-[4-(*t*-Boc-L-alanyl-L-alanylamino)phenyl]benzothiazole 17. Compound **17** was synthesized from amine **9a** (0.5 g, 2.21 mmol), EDAC·HCl (0.50 g, 2.64 mmol), HOBt (0.36 g, 2.64 mmol), and *t*-Boc-L-alanine-L-alanine (0.68 g, 2.64 mmol) in CH_2Cl_2 (50 mL) following the general procedure described above. The crude product was purified by recrystallisation from MeOH/CH_2Cl_2 (with charcoal) giving compound **17** (66%) as white crystals, mp 265–266 °C; HRMS (+NSI) found: MH^+, 469.1893. Calcd for $C_{24}H_{29}N_4O_4S$: MH, 469.1904; δ_H (270 MHz; DMSO-d_6) 1.19 (3H, d, *J* = 7.2 Hz, CH_3), 1.33 (3H, d, *J* = 6.9 Hz, CH_3), 1.38 (9H, s, *t*-Bu), 3.95–4.06 (1H, m, CH), 4.37–4.48 (1H, m, CH), 6.95 (1H, d, *J* = 7.2 Hz, NH); 7.43 (1H, ddd, *J* = 1.2, 7.2, 7.9 Hz, Ar-H), 7.53 (1H, ddd, *J* = 1.2, 7.2, 8.3 Hz, Ar-H), 7.80 (2H, d, *J* = 8.7 Hz, Ar-H), 8.00–8.07 (4H, m, 3× Ar-H, 1× NH), 8.12 (1H, ddd, *J* = 0.5, 1.2, 7.9 Hz, Ar-H), 10.27 (1H, s, NH); IR ν_{max} cm^{-1}: 724, 752, 834, 1162, 1227, 1251, 1366, 1480, 1520, 1652, 2988, 3295.

5.1.9. General procedure for the preparation of the amino acid derivatives 18–20

A mixture of the appropriate *t*-Boc-protected derivative in trifluoroacetic acid (TFA) was stirred at room temperature for 24 h. The excess trifluoroacetic acid was removed under reduced pressure and bis-TFA salt obtained was dried under vacuum. No further purification was required.

5.1.9.1. 2-[4-(L-Alanylamino)phenyl]benzothiazole bis TFA salt 18a. Compound **15a** (0.50 g, 1.30 mmol) in TFA (5 mL) gave compound **18a** (0.66 g, 97%) as a pale green solid, mp 112 °C; HRMS (ESI) found: MH^+, 298.1005. Calcd for $C_{16}H_{16}N_3OS$: MH, 298.1009; δ_H (270 MHz; DMSO-d_6) 1.47 (3H, d, *J* = 6.9 Hz, CH_3), 4.02–4.08 (1H, m, CH), 7.44 (1H, ddd, *J* = 1.2, 6.9, 7.4 Hz, Ar-H), 7.53 (1H, ddd, *J* = 1.2, 6.9, 7.7 Hz, Ar-H), 7.80 (2H, d, *J* = 8.7 Hz, Ar-H), 8.02 (1H, ddd, *J* = 0.5, 1.2, 7.4 Hz, Ar-H), 8.10 (2H, d, *J* = 8.7 Hz, Ar-H), 8.11–8.12 (1H, m, Ar-H), 8.25 (3H, d, *J* = 5.7 Hz, NH_3^+), 10.77 (1H, s, NH); δ_C (68 MHz; DMSO-d_6) 17.6, 49.7, 120.3, 122.8, 123.2, 125.9, 127.1, 128.7, 128.9, 134.9, 141.5, 154.2, 167.3, 169.3; IR ν_{max} cm^{-1}: 702, 721, 760, 1127, 1314, 1445, 1524, 1667, 2400–3000, 2920, 3358.

5.1.9.2. 2-[4-(L-Alanylamino)phenyl]-5-trifluoromethylbenzothiazole bis TFA salt 18b. Compound **15b** (0.30 g, 0.65 mmol) in TFA (5 mL) gave compound **18b** as a pale orange solid, (0.37 g, 96%), mp 205–207 °C; HRMS (+NSI) found: MH^+, 366.0888. Calcd for $C_{17}H_{15}F_3N_3OS$: MH, 366.0882; δ_H (270 MHz; DMSO-d_6) 1.48 (3H, d, *J* = 6.7 Hz, CH_3), 4.09 (1H, q, *J* = 6.7 Hz, CH), 7.75 (1H, d, *J* = 8.4 Hz, Ar-H), 7.84 (2H, d, *J* = 8.7 Hz, Ar-H), 8.13 (2H, d, *J* = 8.7 Hz, Ar-H), 8.31–8.39 (5H, m, 2× Ar-H, NH_3^+), 10.83 (1H, s, NH); δ_C (68 MHz; DMSO-d_6) 17.6, 49.7, 119.9 (q, *J* = 4.2 Hz), 120.3, 121.9 (q, *J* = 3.6 Hz), 124.3, 124.9 (q, *J* = 272.5 Hz), 128.1 (q, *J* = 32.2 Hz), 128.3, 129.0, 139.0, 142.1, 153.8, 169.4, 170.0; IR ν_{max} cm^{-1}: 673, 724, 802, 1122, 1313, 1333, 1485, 1544, 1672, 2700–3200, 2917, 3305.

5.1.9.3. 2-[4-(β-Alanylamino)phenyl]benzothiazole bis TFA salt 19a. Compound **16a** (0.50 g, 1.30 mmol) in TFA (5 mL) gave compound **19a** as a pale green solid (0.63 g, 92%), mp 137 °C; HRMS (+NSI) found: MH^+, 298.1008. Calcd for $C_{16}H_{16}N_3OS$: MH, 298.1009; δ_H (270 MHz, DMSO-d_6) 2.76 (2H, t, *J* = 6.7 Hz, CH_2), 3.06–3.18 (2H, m, CH_2), 7.39–7.46 (1H, m, Ar-H), 7.49–7.55 (1H, m, Ar-H), 7.80 (2H, d, *J* = 8.7 Hz, Ar-H), 8.01 (1H, ddd, *J* = 0.5, 1.2, 7.9 Hz, Ar-H), 8.06 (2H, d, *J* = 8.7 Hz, Ar-H), 8.11 (1H, ddd, *J* = 0.5, 1.0, 7.7 Hz, Ar-H), 10.52 (1H, s, NH); δ_C (68 MHz, DMSO-d_6) 34.0, 35.4, 119.9, 122.8, 123.1, 125.8, 127.2, 128.3, 128.6, 134.9, 142.3, 154.2, 167.5, 169.4; IR ν_{max} cm^{-1}: 725, 753, 827, 1073, 1311, 1481, 1538, 1661, 2700–3100, 3053, 3342.

5.1.9.4. 2-[4-(β-Alanylamino)phenyl]-5-trifluoromethylbenzothiazole bis TFA salt 19b. Compound **16b** (0.30 g, 0.58 mmol) in TFA (5 mL) gave compound **19b** as cream crystals (0.33 g, 96%), mp 229–230 °C; HRMS (+NSI) found: MH^+, 366.0883. Calcd for $C_{17}H_{15}F_3ON_3S$: MH, 366.0882; δ_H (270 MHz; DMSO-d_6) 2.78 (2H, t, *J* = 6.4 Hz, CH_2), 3.13 (2H, t, *J* = 6.4 Hz, CH_2), 7.71 (1H, dd, *J* = 1.2, 8.4 Hz, Ar-H), 7.82 (2H, d, *J* = 8.7 Hz, Ar-H), 7.99 (3H, br s, NH_3^+), 8.06 (2H, d, *J* = 8.7 Hz, Ar-H), 8.30 (1H, d, *J* = 1.2 Hz, Ar-H), 8.33 (1H, d, *J* = 8.4 Hz, Ar-H), 10.61 (1H, s, NH); δ_C (68 MHz; DMSO-d_6) 34.0, 35.4, 119.7 (q, *J* = 4.2 Hz), 119.9, 121.7 (q, *J* = 4.2 Hz), 124.2, 124.8 (q, *J* = 272.0 Hz), 127.8, 128.0 (q, *J* = 31.7 Hz), 128.8, 139.0, 142.8, 153.8, 169.5, 170.1; IR: ν_{max} cm^{-1}: 654, 723, 796, 1135, 1329, 1409, 1520, 1669, 2600–3000, 2922, 3323.

5.1.9.5. 2-[4-(L-Alanyl-L-alanylamino)phenyl]benzothiazole 20. Compound **17** (0.50 g, 1.07 mmol) in TFA (5 mL) gave compound **20** as off-white crystals (0.53 g, 83%), mp 241–242 °C; HRMS (+NSI) found: MH^+, 369.1383. Calcd for $C_{19}H_{21}N_4O_2S$: MH, 369.1380; δ_H (270 MHz; DMSO-d_6) 1.37 (3H, d, *J* = 7.2 Hz, CH_3), 1.38 (3H, d, *J* = 6.7 Hz, CH_3), 3.91 (1H, q, *J* = 6.7 Hz, CH), 4.50 (1H, dq, *J* = 6.9, 7.2 Hz, CH), 7.42 (1H, ddd, *J* = 1.0, 7.2, 8.0 Hz, Ar-H), 7.52 (1H, ddd, *J* = 1.0, 7.0, 8.0 Hz, Ar-H), 7.81 (2H, d, *J* = 8.7 Hz, Ar-H), 8.01 (1H, ddd, *J* = 0.5, 1.0, 7.2 Hz, Ar-H), 8.05 (2H, d,

J = 8.7 Hz, Ar-H), 8.10 (1H, ddd, J = 0.5, 1.0, 7.0 Hz, Ar-H), 8.19 (3H, br s, NH_3^+), 8.81 (1H, d, J = 6.9 Hz, NH), 10.49 (1H, s, NH); δ_C (68 MHz; DMSO-d_6) 17.7, 18.4, 48.6, 50.0, 120.0, 122.8, 123.2, 125.9, 127.2, 128.3, 128.6, 134.8, 142.3, 154.2, 167.5, 170.0, 171.8; IR ν_{max} cm^{-1}: 699, 722, 756, 1117, 1382, 1484, 1538, 1651, 2700–3100, 2986, 3282.

5.1.10. General procedure for the preparation of the esters 21 and 22

The procedure described below for the synthesis of compound **21a** is typical. The other esters were prepared similarly.

5.1.10.1. 4-(Benzothiazol-2-yl)-2-phenyl octanoate 21a. A solution of octanoyl chloride (0.21 g, 1.32 mmol) in CH_2Cl_2 (10 mL) was added drop-wise to a stirred solution of compound **10a** (0.30 g, 1.32 mmol) and Et_3N (0.27 g, 2.64 mmol) in CH_2Cl_2 (10 mL). The mixture was allowed to stir (1 h) at room temperature. The mixture was neutralized by the addition of dilute aqueous HCl solution and then water (10 mL) was added. The mixture was extracted with CH_2Cl_2 (2 × 10 mL) and the combined organic extracts were dried ($MgSO_4$) and evaporated yielding the crude product. The crude product was purified by recrystallisation from CH_2Cl_2/MeOH giving compound **21a** as a cream solid, (0.38 g, 81%), mp 88–89 °C; HRMS (+NSI) found: MH^+, 354.1518. Calcd for $C_{21}H_{23}NO_2S$: MH, 354.1522; δ_H (270 MHz; $CDCl_3$) 0.89 (3H, t, J = 6.4 Hz, CH_3), 1.30–1.36 (8H, m, 4× CH_2), 1.76 (2H, tt, J = 6.9, 7.3 Hz, CH_2), 2.57 (2H, t, J = 7.30 Hz, CH_2), 7.22 (2H, d, J = 8.7 Hz, Ar-H), 7.33–7.39 (1H, m, Ar-H), 7.44–7.50 (1H, m, Ar-H), 7.87 (1H, dd, J = 1.2, 7.9 Hz, Ar-H), 8.05 (1H, dd, J = 1.2, 8.2 Hz, Ar-H), 8.09 (2H, d, J = 8.7 Hz, Ar-H); δ_C (68 MHz; $CDCl_3$) 14.2, 22.7, 25.0, 29.0, 29.1, 31.7, 34.5, 121.7, 122.4, 123.3, 125.3, 126.5, 128.8, 131.3, 135.2, 152.9, 154.2, 167.1, 172.0; IR ν_{max} cm^{-1}: 690, 724, 755, 1112, 1164, 1410, 1465, 1478, 1606, 1749, 2922.

5.1.10.2. 4-(Benzothiazol-2-yl)-2-fluorophenyl octanoate 21b. Compound **10b** (277 mg, 90%) was obtained as a waxy yellow solid, mp 77–78 °C; HRMS (+NSI) found: MH^+, 372.1424. Calcd for $C_{21}H_{23}FNO_2S$: MH, 372.1428; δ_H (400 MHz; $CDCl_3$) 0.90 (3H, t, J = 6.9 Hz, CH_3), 1.24–1.46 (8H, m, 4× CH_2), 1.78 (2H, quintet, J = 7.8 Hz, βCH_2), 2.62 (2H, t, J = 7.8 Hz, αCH_2), 7.22–7.26 (1H, m, Ar-H), 7.39 (1H, ddd, J = 0.9, 7.7, 8.2 Hz, Ar-H), 7.49 (1H, ddd, J = 0.9, 7.7, 8.2 Hz, Ar-H), 7.82–7.84 (1H, m, Ar-H), 7.89 (1H, d, J = 8.2 Hz Ar-H), 7.93 (1H, dd, J = 2.3, 11.0 Hz, Ar-H), 8.06 (1H, d, J = 8.2 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 14.2, 22.7, 25.0, 29.0, 29.1, 31.7, 34.0, 115.8 (d, J = 20.1 Hz), 121.8, 123.5, 123.8 (d, J = 2.9 Hz), 124.5, 125.6, 126.6, 132.9 (d, J = 7.7 Hz), 135.2, 140.4 (d, J = 13.4 Hz), 154.1, 154.4 (d, J = 250.2 Hz), 165.1, 171.0; IR ν_{max}/cm^{-1} 726, 760, 890, 1108, 1125, 1269, 1430, 1770, 2847, 2918.

5.1.10.3. 4-(5-Chlorobenzo[d]thiazol-2-yl)-2,6-difluorophenyl octanoate 21c. Compound **21c** (137 mg, 95%) was obtained as a waxy light-brown solid, mp 66–67 °C; HRMS (+NSI) found: MH^+, 424.0946. Calcd for $C_{21}H_{21}{}^{35}ClF_2NO_2S$: MH, 424.0944; δ_H (400 MHz; $CDCl_3$) 0.84–0.91 (3H, t, CH_3), 1.25–1.46 (8H, m, 4× CH_2), 1.79 (2H, quintet, J = 7.6 Hz, CH), 2.66 (2H, t, J = 7.6 Hz, CH_2), 7.37 (1H, dd, J = 2.3, 8.7 Hz, Ar-H), 7.67 (2H, d, J = 8.2 Hz, Ar-H), 7.79 (1H, d, J = 8.7 Hz, Ar-H), 8.02 (1H, d, J = 2.3 Hz, Ar-H); IR ν_{max}/cm^{-1} 805, 846, 879, 906, 1027, 1043, 1092, 1431, 1488, 1777, 2856, 2925, 3027. The ^{1}H-NMR spectrum indicated that a small quantity of an aliphatic impurity was present.

5.1.10.4. 4-(Benzothiazol-2-yl)-2-fluorophenyl nonanoate 22b. Compound **22b** (297 mg, 92%) was prepared using nonanoyl chloride and was obtained as a waxy cream solid, mp 67–68 °C; HRMS (+NSI) found: MH^+, 386.1586. Calcd for $C_{22}H_{25}FNO_2S$: MH, 386.1585; δ_H (400 MHz; $CDCl_3$) 0.89 (3H, t, J = 7.3 Hz, CH_3), 1.46–1.24 (10H, m, 5× CH_2), 1.78 (2H, quintet, J = 7.3 Hz, βCH_2), 2.62 (2H, td, J = 7.3 Hz, αCH_2), 7.22–7.25 (1H, m, 1H, Ar-H), 7.39 (1H, ddd, J = 0.9, 6.9, 8.2 Hz, Ar-H), 7.49 (1H, ddd, J = 1.4, 6.9, 8.5 Hz, Ar-H), 7.82–7.84 (1H, m, Ar-H), 7.89 (1H, d, J = 8.5 Hz, Ar-H), 7.95 (1H, dd, J = 2.3, 10.5 Hz, Ar-H), 8.06 (1H, d, J = 8.2 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 14.2, 22.8, 25.0, 29.1, 29.2, 29.3, 31.9, 34.0, 115.8 (d, J = 21.1 Hz), 121.8, 123.5, 123.8 (d, J = 3.8 Hz), 124.5, 125.6, 126.6, 132.9 (d, J = 7.7 Hz), 135.2, 140.4 (d, J = 13.4 Hz), 154.1, 154.4 (d, J = 251.1 Hz), 165.7, 171.0; IR ν_{max}/cm^{-1} 730, 755, 878, 994, 1118, 1134, 1270, 1432, 1775, 2847, 2914.

5.1.11. General procedure for the synthesis of the dibenzyl phosphates 23

To a stirred solution of the appropriate phenol (1 equiv) in anhydrous CH_3CN, at −10 °C, was added CCl_4 (5 equiv), *N*,*N*-diisopropylethylamine (DIPEA) (2.1 equiv), and *N*,*N*-dimethylaminopyridine (DMAP) (0.1 equiv). The mixture was allowed to stir (1 min) before dibenzyl phosphite (1.45 equiv) was added drop-wise while keeping the temperature of the reaction mixture below −10 °C. The reaction was allowed to stir (1.5 h) and 0.5 M aq KH_2PO_4 (32 mL per 100 mL CH_3CN) was then added. The mixture allowed to warm to room temperature and was extracted with EtOAc (3 × 20 mL). The combined organic extracts were washed successively with water (30 mL) and brine (30 mL), dried ($NaSO_4$) and evaporated.

5.1.11.1. 4-(Benzothiazol-2-yl)phenyl-1-dibenzylphosphate 23a. Compound **23a** was synthesized from compound **10a** (0.30 g, 1.32 mmol), CCl_4 (1.02 g, 6.6 mmol), DIPEA (0.36 g, 2.77 mmol), DMAP (0.16 g, 0.13 mmol), and dibenzyl phosphite (0.50 g, 1.91 mmol) in acetonitrile (20 mL) following the general procedure described above. Yield (0.54 g, 84%), mp 110–112 °C; HRMS (+NSI) found: MH^+, 488.1085. Calcd for $C_{27}H_{23}NO_4PS$: MH, 488.1080; δ_H (270 MHz; DMSO-d_6) 5.08 (4H, d, J = 8.7 Hz, 2× CH_2), 7.15 (2H, d, J = 8.4 Hz, Ar-H), 7.21–7.26 (10H, m, Ar-H), 7.27–7.32 (1H, m, Ar-H), 7.40 (1H, ddd, J = 1.2, 7.2, 8.2 Hz, Ar-H), 7.80 (1H, ddd, J = 0.5, 1.2, 7.9 Hz, Ar-H), 7.92 (2H, d, J = 8.4 Hz, Ar-H), 7.97 (1H, ddd, J = 0.5, 1.2, 8.2 Hz, Ar-H); δ_C (68 MHz; DMSO-d_6) 70.3 (d, J = 5.7 Hz), 120.8 (d, J = 5.2 Hz), 122.0, 123.2, 125.6, 126.7, 128.3, 128.8, 129.0, 129.1, 130.6, 135.1, 135.4 (d, J = 6.8 Hz), 152.7 (d, J = 6.8 Hz), 154.1, 166.6; IR ν_{max} cm^{-1}: 696, 731, 744, 1013, 1168, 1208, 1274, 1436, 1479, 1606, 3059.

5.1.11.2. 4-(Benzothiazol-2-yl)-2-fluorophenyl-1-dibenzylphosphate 23b. Compound **23b** was synthesized from compound **10b** (490 mg, 2.00 mmol), CCl_4 (969 μL, 10.04 mmol), DIPEA (418 μL, 2.40 mmol), DMAP (a few crystals), and dibenzyl phosphite (944 μL, 4.27 mmol) in CH_3CN (25 mL) following the general procedure described above. The crude product was purified by column chromatography (eluent: CH_2Cl_2 changing to EtOAc/CH_2Cl_2, 1:10) yielding compound **23b** (674 mg, 67%) as a pale yellow solid, mp 96–97 °C; HRMS (+NSI) found: MH^+, 506.0980. Calcd for $C_{27}H_{21}FNO_4PS$: MH, 506.0986; δ_H (400 MHz; $CDCl_3$) 5.18 (4H, d, J = 8.7 Hz, 2× CH_2), 7.37 (1H, d, J = 9.2 Hz, Ar-H), 7.33–7.40 (10H, m, Ar-H), 7.40 (1H, m, Ar-H), 7.50 (1H, ddd, J = 1.4, 7.8, 8.2 Hz, Ar-H), 7.72 (1H, d, J = 8.2 Hz, Ar-H), 7.87–7.90 (2H, m, Ar-H), 8.06 (1H, d, J = 8.2 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 70.6 (d, J = 5.8 Hz), 116.0 (d, J = 20.1 Hz), 121.8, 122.9 (d, J = 2.9 Hz), 123.5, 123.8 (d, J = 1.9 Hz), 125.7, 126.7, 128.2, 128.7, 128.9, 131.9 (d, J = 7.2 Hz), 135.2 (d, J = 6.7 Hz), 135.3, 140.3 (dd, J = 6.7, 12.5 Hz), 154.1,

153.7 (dd, J = 5.8, 251.1 Hz), 165.6; IR ν_{max}/cm^{-1} 694, 732, 868, 894, 930, 994, 1021, 1270, 1490.

5.1.11.3. 4-(5-Chlorobenzothiazol-2-yl)-2,6-difluorophenyl-1-dibenzyl phosphate 23c. Compound **23c** was prepared in a similar manner to compound **23b**. The crude product was purified was by column chromatography (eluent: CH_2Cl_2) yielding compound **23c** (583 mg, 52%) as a cream solid, mp 106–107 °C; HRMS (+NSI) found: MH^+, 558.0497. Calcd 558.0502 for $C_{27}H_{20}{}^{35}ClF_2NO_4PS$: MH, 558.0502; δ_H (400 MHz; $CDCl_3$) 5.26 (4H, dd, J = 1.8, 7.3 Hz, 2× CH_2), 7.34–7.40 (11H, m, Ar-H), 7.65 (2H, d, J = 8.2 Hz, Ar-H), 7.77 (1H, d, J = 8.7 Hz, Ar-H), 8.02 (1H, d, J = 1.8 Hz, Ar-H); δ_C (100 MHz; $CDCl_3$) 70.7 (d, J = 5.8 Hz), 111.6 (dd, J = 5.8, 18.2 Hz), 122.6, 123.5, 126.5, 128.2, 128.7, 128.9, 130.0 (td, J = 7.7, 16.3 Hz), 131.0 (t, J = 8.6 Hz), 132.9, 133.4, 135.2 (d, J = 6.7 Hz), 154.7, 155.2 (dt, J = 3.4, 252.1 Hz), 166.1; ν_{max}/cm^{-1} 696, 734, 912, 1016, 1283, 1432, 1487.

5.1.12. General procedure for preparing the phosphate derivatives 24

TFA was added drop-wise to the appropriate compound **23** and the mixture was stirred (4–24 h) at room temperature. The mixture was evaporated and the resulting TFA salt was dried under vacuum in a desiccator. No further purification was required.

5.1.12.1. 4-(Benzothiazol-2-yl)phenyl-1-dihydrogen phosphate 24a. Following the general procedure described above, compound **23a** (0.50 g, 0.62 mmol) in TFA (5 mL) (24 h) gave compound **24a** as an off-white solid (0.18 g, 94%), mp 235–236 °C; HRMS (+NSI) found: MH^+, 308.0144. Calcd for $C_{13}H_{11}NO_4PS$: MH, 308.0141; δ_H (270 MHz; DMSO-d_6) 7.36 (2H, d, J = 8.7 Hz, Ar-H), 7.44 (1H, ddd, J = 1.2, 7.2, 7.9 Hz, Ar-H), 7.53 (1H, ddd, J = 1.2, 7.2, 7.9 Hz, Ar-H), 8.04 (1H, ddd, J = 0.5, 1.2, 7.9 Hz, Ar-H), 8.08 (2H, d, J = 8.7 Hz, Ar-H), 8.11–8.14 (1H, m, Ar-H); δ_C (68 MHz; DMSO-d_6) 121.4 (d, J = 5.2 Hz), 122.9, 123.3, 126.0, 127.2, 129.1, 129.3, 135.0, 154.2, 154.6 (d, J = 6.2 Hz), 167.2; IR ν_{max} cm^{-1}: 689, 726, 755, 963, 1186, 1253, 1444, 1505, 1604, 2100–2700.

5.1.12.2. 4-(Benzothiazol-2-yl)-2-fluorophenyl-1-dihydrogen phosphate 24b. Following the general procedure described above, compound **23b** (193 mg, 0.38 mmol) in TFA (5 mL) (4 h) yielded compound **24b** (121 mg, 97%) as a pale yellow powder after evaporation of the solvent and trituration of the residue with ether, mp 189–190 °C; HRMS found: MH^+, 326.0051. Calcd for $C_{13}H_{10}FNO_4PS$: MH, 326.0047; δ_H (400 MHz; DMSO-d_6) 7.43 (1H, m, Ar-H), 7.52 (1H, ddd, J = 0.9, 7.4, 8.2 Hz, Ar-H), 7.56 (1H, dd, J = 8.2, 8.2 Hz, Ar-H), 7.88 (1H, d, J = 8.7 Hz, Ar-H), 7.96 (1H, dd, J = 1.4, 11.5 Hz, Ar-H), 8.02 (1H, d, J = 8.0 Hz, Ar-H), 8.12 (1H, d, J = 8.0 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 115.6 (d, J = 20.1 Hz), 123.0, 123.5, 123.4 (d, J = 3.8 Hz), 124.4 (d, J = 1.9 Hz), 126.2, 127.3, 130.1 (d, J = 5.8 Hz), 135.2, 142.2 (dd, J = 5.8, 11.5 Hz), 153.8 (dd, J = 7.2, 247.8 Hz), 153.9, 166.0; ν_{max}/cm^{-1} 712, 751, 824, 881, 913, 966, 1135, 1293, 1448, 1510, 1613, 2500–3200.

5.1.12.3. 4-(5-Chlorobenzothiazol-2-yl)-2,6-difluorophenyl-1-dihydrogen phosphate 24c. Following the general procedure described above, compound **23c** (192 mg, 0.34 mmol) in TFA (5 mL) (4 h) yielded compound **24c** (111 mg, 85%) as a pale yellow powder after evaporation of the solvent and trituration of the residue with ether, mp 197 °C; HRMS (+NSI) found: MH^+, 377.9568. Calcd for $C_{13}H_7{}^{35}ClF_2NO_4PS$: MH, 377.9563; δ_H (400 MHz; DMSO-d_6) 6.05 (br s, 2× OH), 7.51 (1H, dd, J = 1.8, 8.5 Hz, Ar-H), 7.87 (2H, d, J = 8.2 Hz, Ar-H), 8.11 (1H, d, J = 1.8 Hz, Ar-H), 8.18 (1H, d, J = 8.5 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 111.9 (dd, J = 6.7, 18.2 Hz), 123.0, 124.6, 126.6, 129.7 (t, J = 10.5 Hz), 131.4 (td, J = 7.7, 16.3 Hz), 132.2, 134.3, 154.6, 155.8 (dd, J = 4.8, 255.0 Hz), 167.3; ν_{max}/cm^{-1} 808, 906, 962, 1037, 1346, 1436, 1434, 2200–3200.

5.1.13. General procedure for the preparation of the acylated sugars 25

The procedure described below for the synthesis of compound **25a** is typical. Compounds **25b** and **25c** were prepared similarly.

5.1.13.1. 4-(Benzothiazol-2-yl)-1-(*tetra*-*O*-acetyl-β-D-galactosyl)phenol 25a. A mixture of compound **10a** (0.46 g, 2.02 mmol) and 2,6-lutidine (0.23 mL, 1.97 mmol) in dry CH_2Cl_2 (10 mL) was stirred at room temperature ($CaCl_2$ guard tube). After 10 min Ag_2CO_3 (0.55 g, 1.99 mmol) and additional 2,6-lutidine (0.23 mL, 1.97 mmol) were added and the vessel was covered to exclude light. After a further 15 min 2,3,4,6-tetra-*O*-acetyl-α-D-galactosyl bromide (0.82 g, 1.99 mmol) was added and stirring continued (60 h) in the dark. The reaction mixture was filtered through a shallow bed of silica and the residue rinsed with CH_2Cl_2 (10 mL). The filtrate was dried ($MgSO_4$) and evaporated. The residue was recrystallised from aqueous EtOH yielding compound **25a** (0.62 g, 56%) as pale pink needles, mp 147–149 °C; HRMS (+NSI) found: MH^+, 558.1419. Calcd for $C_{27}H_{28}NO_{10}S$: MH, 558.1428; δ_H (400 MHz; DMSO-d_6) 1.92 (3H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$), 2.02 (3H, s, $COCH_3$), 2.12 (3H, s, $COCH_3$), 4.09 (2H, d, J = 6.2 Hz, CH), 4.46 (1H, dd, J = 6.2, 6.2 Hz, CH), 5.30–5.20 (2H, m, 2× CH), 5.34 (1H, d, J = 7.3 Hz, CH), 5.61 (1H, d, J = 7.3 Hz, anomeric CH), 7.14 (2H, d, J = 9.2 Hz, Ar-H), 7.41 (1H, ddd, J = 1.4, 6.9, 7.9 Hz, Ar-H), 7.50 (1H, ddd, J = 1.4, 6.9, 7.9 Hz, Ar-H), 8.00 (1H, d, J = 7.9 Hz, Ar-H), 8.05 (2H, d, J = 9.2 Hz, Ar-H), 8.09 (1H, d, J = 7.9 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 20.9, 21.0, 21.1, 61.9, 67.7, 68.8, 70.6, 71.1, 97.7, 117.5, 122.8, 123.2, 125.9, 127.2, 128.1, 129.5, 134.9, 154.1, 159.2, 167.2, 169.8, 170.1, 170.4, 170.5; ν_{max}/cm^{-1} 758, 832, 1044, 1210, 1740.

5.1.13.2. 4-(Benzothiazol-2-yl)-2-fluoro-1-(*tetra*-*O*-acetyl-β-D-galactosyl)phenol 25b. Compound **25b** (880 mg, 76%) was obtained as pink needles, 139–140 °C after recrystallisation form from aqueous EtOH. HRMS (+NSI) found: MH^+, 576.1334. Calcd for $C_{27}H_{27}FNO_{10}S$: MH, 576.1331; δ_H (400 MHz; DMSO-d_6) 1.90 (3H, s, $COCH_3$), 1.97 (3H, s, $COCH_3$), 2.00 (3H, s, $COCH_3$), 2.11 (3H, s, $COCH_3$), 4.07 (2H, d, J = 6.0 Hz, CH), 4.42 (1H, dd, J = 6.0, 6.0 Hz, C), 5.22–5.28 (2H, m, 2× CH), 7.34–7.40 (2H, m, Ar-H), 7.47 (1H, ddd, J = 1.4, 7.8, 8.1 Hz, Ar-H), 7.82 (1H, d, J = 9.2 Hz, Ar-H), 7.87 (1H, dd, J = 1.8, 11.5 Hz, Ar-H), 7.96 (1H, d, J = 8.1 Hz, Ar-H), 8.02 (1H, d, J = 8.1 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 20.8, 20.9, 21.0, 61.8, 67.6, 68.6, 70.5, 71.3, 98.9, 115.6 (d, J = 21.1 Hz), 119.1, 122.9, 123.4, 124.7, 126.2, 127.3, 129.1 (d, J = 6.7 Hz), 135.1, 146.9 (d, J = 10.5 Hz), 152.6 (d, J = 247.3 Hz), 153.9, 166.0, 169.7, 170.1, 170.4, 170.5; ν_{max}/cm^{-1} 758, 1014, 1047, 1210, 1738.

5.1.13.3. 4-(5-Chlorobenzothiazol-2-yl)-2,6-difluoro-1-(*tetra*-*O*-acetyl-β-D-galactosyl)phenol 25c. Compound **25c** (340 mg, 27%) was obtained as light orange crystals, 179–181 °C; HRMS (+NSI) found: MH^+, 628.0851. Calcd for $C_{27}H_{25}{}^{35}ClF_2NO_{10}S$: MH, 628.0850; δ_H (400 MHz; DMSO-d_6) 1.90 (3H, s, Ac-CH_3), 1.92 (3H, s, $COCH_3$), 2.06 (3H, s, $COCH_3$), 2.14 (3H, s, 3H, $COCH_3$), 3.98–4.11 (2H, m, 2× CH), 4.28 (1H, dd, J = 6.4, 6.4 Hz, CH), 5.19–5.31 (3H, m, 3× CH), 5.37 (1H, d, J = 7.8 Hz, anomeric CH), 7.50 (1H, dd, J = 1.8, 8.7 Hz, Ar-H), 7.88 (2H, d, J = 8.2 Hz, 2H, Ar-H), 8.12 (1H, d, J = 1.8 Hz, Ar-H), 8.15 (1H, d, J = 8.7 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 20.9, 21.0, 61.1, 67.5, 69.2, 70.4, 71.2, 102.4, 112.1 (dd, J = 7.7, 18.2 Hz), 123.0, 124.6, 126.7, 130.2 (t, J = 9.6 Hz), 132.2, 134.3, 135.3 (t, J = 14.9 Hz), 154.6, 155.9 (dd, J = 4.8, 250.2 Hz),

167.2, 169.8, 170.0, 170.3, 170.5; ν_{max}/cm^{-1} 923, 1027, 1065, 1212, 1368, 1434, 1486, 1745.

5.1.14. General procedure for preparation of the galactose derivatives 26

The procedure described below for the synthesis of compound **26a** is typical. Compounds **26b** and **26c** were prepared similarly.

5.1.14.1. 4-(Benzothiazol-2-yl)-1-(β-D-galactosyl)phenol 26a. Compound **26a** (0.33 g, 0.59 mmol) and sodium methoxide (0.64 g, 11.85 mmol) were added to LC-grade methanol (15 mL) and stirred (70 h) at room temperature. The mixture was cooled in an ice-water bath the precipitate was collected yielding compound **26a** (0.19 g, 83%) as a white powder, >180 °C (decomp.); HRMS (+NSI) found: MH^+, 390.1009. Calcd for $C_{19}H_{20}NO_6S$: MH, 390.1011; δ_H (400 MHz; DMSO-d_6) 3.28–3.61 (5H, m, 5× CH), 3.69 (1H, d, J = 2.8 Hz, CH), 4.61 (1H, br s, OH), 4.72 (1H, br s, OH), 4.93 (1H, d, J = 7.8 Hz, anomeric CH), 4.99 (1H, br s, OH), 5.28 (1H, br s, OH), 7.16 (2H, d, J = 8.7 Hz, Ar-H), 7.40 (1H, ddd, J = 1.4, 6.9, 8.0 Hz, Ar-H), 7.49 (1H, ddd, J = 1.4, 6.9, 8.1 Hz, Ar-H), 7.97–8.01 (3H, m, Ar-H), 8.08 (1H, d, J = 8.0 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 60.8, 68.6, 70.7, 73.8, 76.2, 101.1, 117.4, 122.8, 123.1, 125.7, 127.0, 127.1, 129.3, 134.8, 154.2, 160.4, 167.5; ν_{max}/cm^{-1} 757, 825, 1035, 1064, 1078, 3000–3500.

5.1.14.2. 4-(Benzothiazol-2-yl)-2-fluoro-1-(β-D-galactosyl)phenol 26b. Compound **26b** (0.35 g, 99%) was obtained as a white powder, >195 °C (decomp.); HRMS (+NSI) found: MH^+, 408.0912. Calcd for $C_{19}H_{19}FNO_6S$: MH, 408.0912; δ_H (400 MHz; DMSO-d_6) 3.40–3.55 (2H, m, 2× CH), 3.60–3.64 (3H, m, 3× CH), 3.70 (1H, d, J = 2.8 Hz, CH), 4.62 (1H, br s, –OH), 4.71 (1H, br s, OH), 4.98 (1H, br s, OH), 5.04 (1H, d, J = 7.8 Hz, anomeric CH), 5.33 (1H, br s, OH), 7.38–7.43 (2H, m, Ar-H), 7.50 (1H, ddd, J = 1.4, 7.8, 8.1 Hz, Ar-H), 7.79–7.82 (1H, m, Ar-H), 7.90 (1H, dd, J = 1.8, 11.5 Hz, Ar-H), 8.00 (1H, d, J = 8.1 Hz, Ar-H), 8.09 (1H, dd, J = 1.4, 8.1 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 60.8, 68.6, 70.6, 73.8, 76.3, 101.3, 115.1 (d, J = 21.1 Hz), 118.1, 122.9, 123.3, 124.6, 126.0, 127.3, 127.5 (d, J = 6.7 Hz), 135.0, 148.0 (d, J = 10.5 Hz), 152.3 (d, J = 246.8 Hz), 154.0, 166.3; ν_{max}/cm^{-1} 751, 1027, 1052, 1072, 1225, 1278, 2800–3500.

5.1.14.3. 4-(5-Chlorobenzothiazol-2-yl)-2,6-difluoro-1-(β-D-galactosyl)phenol 26c. Compound **26c** (89 mg, 97%) was obtained as a white powder, >200 °C (decomp.); HRMS (+NSI) Found 297.9903. Shows breakdown of product to starting compound **10c** (Calcd 297.9903 for $C_{13}H_7{}^{35}ClF_2NOS$, $[M+H]^+$); MS (+ESI) m/z 482.16 ($[M+Na]^+$; 100%); δ_H (400 MHz; DMSO-d_6) 3.36–3.41 (3H, m, 3× CH), 3.48–3.57 (2H, m, 2× CH), 3.66 (1H, br d, J = 2.8 Hz, OH), 4.56 (1H, br s, OH), 4.61 (1H, br s, OH), 4.92 (2H, d, J = 7.8 Hz, CH_2), 5.34 (1H, br s, OH), 7.50 (1H, dd, J = 2.1, 8.5 Hz, Ar-H), 7.81 (2H, d, J = 8.7 Hz, Ar-H), 8.10 (1H, d, J = 2.1 Hz, Ar-H), 8.18 (1H, d, J = 8.5 Hz, Ar-H); δ_C (100 MHz; DMSO-d_6) 60.5, 68.3, 71.5, 73.5, 76.5, 104.8, 112.0, 122.9, 124.6, 126.5, 128.6, 132.2, 134.2, 135.9 (t, J = 12.5 Hz), 154.7, 155.8 (dd, J = 5.8, 248.7 Hz), 167.4; ν_{max}/cm^{-1} 878, 1027, 1078, 1246, 1347, 1426, 3100–3500.

5.2. Microbiological work

5.2.1. Agar plate preparation

Each substrate (10 mg) was dissolved in 1-methyl-2-pyrrolidone (0.2 mL) and added to molten Columbia agar (100 mL) (Oxoid, Basingstoke) at 50 °C to a final concentration of 100 mg L^{-1}. Agar plates were then prepared and dried to remove excess moisture. Bacterial strains (obtained from the National Collection of Type Cultures, London, UK) were sub-cultured onto Columbia agar. Colonies of each strain were sampled using a loop and suspended in 0.85% sterile physiological saline to generate a suspension equivalent to 10^8 colony forming units per mL using a densitometer. Each agar plate was then inoculated with 10 μL of this suspension and spread to obtain single colonies. Plates were incubated at 37 °C in air for 24 h. For the evaluation of the β-galactosidase substrates, IPTG (3 mg) in sterile deionised water (1 mL) was also added to the molten agar.

Acknowlegements

We thank bioMérieux SA for generous financial support and the EPSRC Mass Spectrometry Centre, Swansea, for high resolution mass spectra.

References and notes

1. (a) Orenga, S.; James, A. L.; Manfi, M.; Perry, J. D.; Pincus, D. H. *J. Microbiol. Methods* **2009**, *79*, 139; (b) Manafi, M. *Int. J. Food Microbiol.* **2000**, *60*, 205; (c) Haughland, R. P. *Handbook of Fluorescent Probes and Research Products*, 9th ed.; Molecular Probes: Eugene, OR, 2002.
2. Reymond, J.-L.; Fluxà, V. S.; Maillard, N. *Chem. Commun.* **2009**, 34.
3. Haughland, R. P.; Zhang, Y. -z.; Yue, S. T.; Terpetschnig, E.; Olsen, N. A.; Naleway, J. J.; Larison, K. D.; Huang, Z. US Patent 5,316,906, 1994.
4. (a) Kim, T.-I.; Kang, H. J.; Han, G.; Chung, S. J.; Kim, Y. *Chem. Commun.* **2009**, 5895; (b) Otsubo, T.; Minami, A.; Fujii, H.; Taguchi, R.; Takahashi, T.; Suzuki, T.; Teraoka, F.; Ikeda, K. *Bioorg. Med. Chem Lett.* **2013**, *23*, 2245.
5. Cellier, M.; Fabrega, O. J.; Fazackerley, E.; James, A. L.; Orenga, S.; Perry, J. D.; Salwatura, V. L.; Stanforth, S. P. *Bioorg. Med. Chem.* **2011**, *19*, 2903.
6. Fabrega, O.; James, A.; Salwatura, V. L.; Orenga, S.; Stanforth, S. P. Patent WO 152306, 2008.
7. Shi, D.-F.; Bradshaw, T. D.; Wrigley, S.; McCall, C. J.; Lelieveld, P.; Fichtner, I.; Stevens, M. F. G. *J. Med. Chem.* **1996**, *39*, 3375.
8. Wang, Y.; Jin, K. L.; Myers, L. R.; Glover, S. A.; Novak, M. *J. Org. Chem.* **2009**, *74*, 4463.
9. Lawrence, N. J.; Hepworth, L. A.; Rennison, D.; McGown, A. T.; Hadfield, J. A. *J. Fluorine Chem.* **2003**, *123*, 101.
10. Silverberg, L. J.; Dillon, J. L.; Vemishetti, P. *Tetrahedron Lett.* **1996**, *37*, 771.
11. Zaytsev, A. V.; Anderson, R. J.; Bedernjak, A.; Groundwater, P. W.; Huang, Y.; Perry, J. D.; Orenga, S.; Roger-Dalbert, C.; James, A. L. *Org. Biomol. Chem.* **2008**, *6*, 682.
12. Carlone, G. M.; Valadez, M. J.; Pickett, M. J. *J. Clin. Microbiol.* **1982**, *16*, 1157.
13. Anderson, R. J.; Groundwater, P. W.; Huang, Y.; James, A. L.; Orenga, S.; Rigby, A.; Roger-Dalbert, C.; Perry, J. D. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 832.
14. Cooke, V. M.; Miles, R. J.; Price, R. G.; Richardson, A. C. *Appl. Environ. Microbiol.* **1999**, *65*, 807.
15. Janda, W. M.; Ristow, K.; Novak, D. *J. Clin. Microbiol.* **1994**, *32*, 2056.
16. Gaillot, O.; Wetsch, M.; Fortineau, N.; Berche, P. *J. Clin. Microbiol.* **2000**, *38*, 1587; Perry, J. D.; Rennison, C.; Butterworth, L. A.; Hopley, A. L. J.; Gould, F. K. *J. Clin. Microbiol.* **2003**, *41*, 5695.
17. Jacob, F.; Monod, J. J. *Mol. Biol.* **1961**, *3*, 318.