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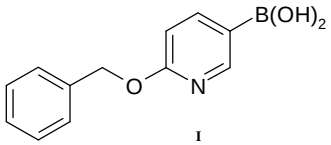
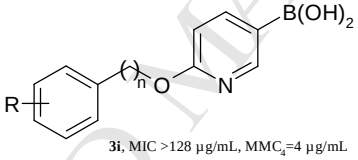
Boronic species as promising inhibitors of the *Staphylococcus aureus* NorA efflux pump: study of 6-substituted pyridine-3-boronic acid derivatives

Fanny Fontaine,[†,‡] Arnaud Héquet,[‡] Anne-Sophie Voisin-Chiret,[†,‡,*] Alexandre Bouillon,[∫] Aurélien Lesnard,[†,‡] Thierry Cresteil,[§] Claude Jolivalt,[‡,‼] Sylvain Rault[†,‡]

GRAPHICAL ABSTRACT



I
MIC >128 µg/mL
MMC_4=16 µg/mL



3i, MIC >128 µg/mL, MMC_4=4 µg/mL
3j, MIC=32µg/mL, MMC_4=4 µg/mL

Boronic species as promising inhibitors of the *Staphylococcus aureus* NorA efflux pump: study of 6-substituted pyridine-3-boronic acid derivatives

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KEYWORDS

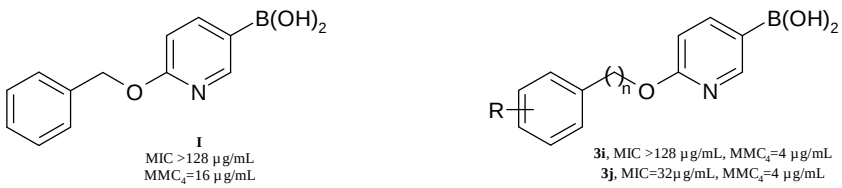
Boronic acids; Pyridine derivatives; Antimicrobial agents; Efflux pump inhibitors; *Staphylococcus aureus.*

HIGHLIGHTS

- A series of 6-(aryl)alkoxypyridine-3-boronic acids was designed and synthesized

- They were evaluated as efflux pump inhibitors against the *S. aureus* 1199B strain
- Compounds **3i** and **3j** potentiate the activity of ciprofloxacin by a 4-fold increase
- They represent a new chemotype for the development of compounds with improved potency

ABSTRACT. In response to the extensive use of antibiotics, bacteria have evolved numerous mechanisms of defense against antimicrobial agents. Among them, extrusion of the antimicrobial agents outside the bacterial cell through efflux pumps is a major cause of concern. At first limited to one or few structurally-related antibiotics, bacterial resistance have then progressed towards cross-resistance between different classes of antibiotics, leading to multidrug-resistant microorganisms. Emergence of these pathogens requires development of novel therapeutic strategies and inhibition of efflux pumps appears to be a promising strategy that could restore the potency of existing antibiotics. NorA is the most studied chromosomal efflux pump of *Staphylococcus aureus*; it is known to be implied in resistance of Methicillin-resistant *S. aureus* (MRSA) strains against a wide range of unrelated substrates, including hydrophilic fluoroquinolones. Starting from 6-benzyloxypyridine-3-boronic acid **I** that we previously identified as a potential inhibitor of the NorA efflux pump against the NorA-overexpressing *S. aureus* 1199B strain (SA1199B), we describe here the synthesis and biological evaluation of a series of 6-(aryl)alkoxypyridine-3-boronic acids. 6-(3-Phenylpropoxy)pyridine-3-boronic acid **3i** and 6-(4-phenylbutoxy)pyridine-3-boronic acid **3j** were found to potentiate ciprofloxacin activity by a 4-fold increase compared to the parent compound **I**. In addition, it has been shown that both compounds promote Ethidium Bromide (EtBr) accumulation in SA1199B, thus corroborating their potential mode of action as NorA inhibitors.



Graphical abstract

INTRODUCTION

Enhanced efflux through overexpressed pumps is an important mechanism of resistance by which bacteria efficiently extrude antimicrobial agents outside the cell [1,2,3]. Many of these transporters can export a broad range of structurally unrelated compounds leading to multidrug-resistant (MDR) microorganisms with cross-resistance between various antibiotics [4,5].

Structural pharmacomodulation of existing antibacterial classes is proving its limits in the quest for new antimicrobial agents [6]. The fight against resistance mechanisms, and particularly against efflux pumps, appears to be a promising approach that could restore the potency of existing antibiotics. This strategy relies on the combination between an efflux pump inhibitor (EPI) and the extruded antibiotic to prevent its efflux and recover its intracellular concentration [7,8]. Therefore, EPIs are expected to have added clinical benefits in increasing antibiotics potency, expanding their spectrum of activity and reducing the rate of resistance development [9].

Methicillin-resistant Staphylococcus aureus (MRSA) strains are of particular concern among MDR microorganism and are responsible for both community- and hospital-acquired infections [10]. NorA-overexpressing strains have been shown to be the most common among MRSA strains [11]. This pump belongs to the Major Facilitator Superfamily (MFS) and confers resistance to a wide range of unrelated substrates such as hydrophilic fluoroquinolones (ciprofloxacin, norfloxacin), biocides (acriflavine, cetrimide, benzalkonium chloride) and dyes (ethidium bromide) [12,13]. Over the past decade, a number of NorA inhibitors have been identified [14]. The alkaloid reserpine is commonly used as a positive control in pump inhibitory assays but it is not clinically relevant due to neurotoxicity at concentrations required for activity [15]. Other natural-based EPIs [16] include flavonolignan and flavone compounds [17,18], *N*-caffeoylphenalkylamide derivatives [19], piperine and piperine analogs [20], citral and citronellal derivatives [21], and polyacylated oligosaccharides [22]. Synthetic inhibitors are mainly based on heterocyclic scaffolds, such as indoles [23,24], phenylpiperidines [25], benzothiophenes [26], phenothiazines and thioxanthenes [27,28], quinolines and quinolones [29,30] and tricyclic compounds [31].

For several years, our laboratory has focused on the synthesis and physicochemical study of various heterocyclic compounds bearing boronic functions. Organoboron compounds are very well described for their key role in cross-coupling reactions [32]. Their therapeutic relevance is yet to be fully recognized but launch of Bortezomib, a boroaminoacid proteasome inhibitor in 2003 [33], and of Tavaborole, an antifungal benzoxaborole in 2014 [34] opened challenging perspectives. Biological activity of boronated compounds relies on their ability to shift from a neutral and trigonal planar sp^2 configuration to an anionic tetrahedral sp^3 one, through nucleophilic attack on the boron open shell. These compounds are thus able to form reversible covalent complexes with nucleophilic molecules such as amino acids, nucleic acids, sugars…[35] In this way, organoboron compounds have been described as insecticide [36], antifungal [37], antiparasitic [38], antiviral [39], anti-inflammatory [40] and as protease inhibitors [41]. Of particular interest are the antibacterial properties of various organoboron compounds such as diazaborines [42], benzoxaboroles [43,44], oxazaborolidines [45], borinic [46] and boronic esters [47] and boronic acids [48,49].

In our quest to explore the biological properties of a series of boronic species that were prepared in our laboratory over the past decade, we investigated their antibacterial activity. As part of this program, we previously reported the first identification of heterocyclic boronic acids as potential inhibitors of the NorA efflux pump of *Staphylococcus aureus* [50]. Starting from a chemical library of about 150 compounds, we previously identified 12 pyridine-3-boronic acids as interesting compounds, from which structure-activity relationship studies revealed that: i) the boron atom was required for biological activity as carbonylated and hydroxymethyl analogs were found to be inactive; ii) weaker activity were achieved when the boronic group was esterified; iii) best results were obtained when substituents were in *para*-position with respect to the boronic moiety; iv) activity was retained when a C-5 group was added to 6-substituted compounds or when cyclisation occurred at the C-5/C-6 positions (Figure 1) [50].

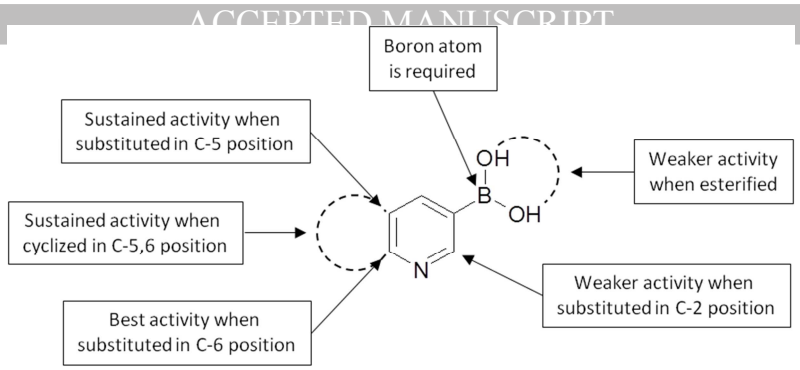


Figure 1. Previous SAR studies for boronic species of the primary screening [50].

6-benzyloxypyridine-3-boronic acid **I** (Figure 2) was identified as the most promising compound among the tested test since it was able to potentiate the activity of ciprofloxacin by a 4-fold increase at a concentration of 16µg/mL against the NorA-overexpressing SA-1199B strain. This promising result led us to deepen our study and start different chemical pharmacomodulations in order to obtain more efficient derivatives. In this paper, we describe the chemical exploration of the C-6 position of the pyridine-3-boronic scaffold by replacing the 6-benzyloxy group of **I** with various length (aryl)alkoxy chains to explore the putative hydrophobic binding site of NorA [51]. We also study the effect of the introduction of substituents on the phenyl ring of compound **I**, as well as the modification of the ether linkage between the pyridine ring and the initial 6-benzyloxy group (Figure 2).

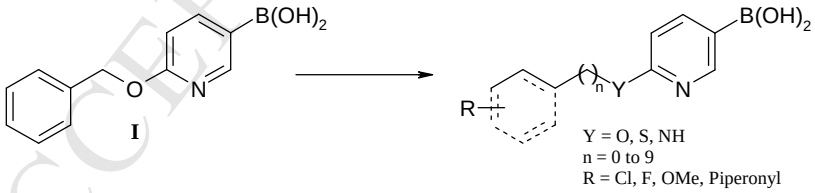


Figure 2. Considered pharmacomodulations on hit compound **I**.

RESULTS AND DISCUSSION

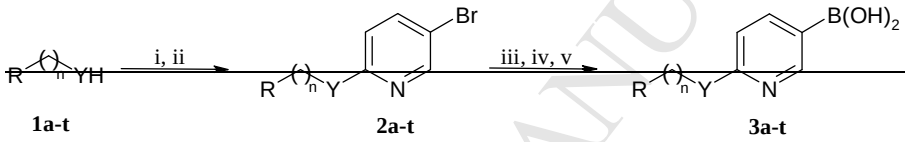
Chemistry. We first decided to explore the chemical space around the C-6 position of the pyridine-3-boronic scaffold, with the goal of obtaining more potent derivatives. 6-Substituted pyridine-3-boronic acids **3a-t** were expected to be readily available using the classical halogen-lithium

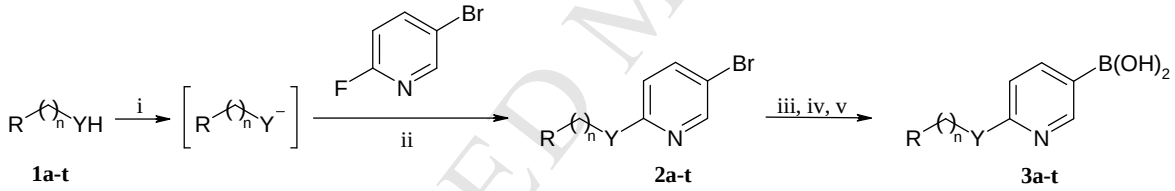
exchange methodology followed by boronation of the intermediate lithiated species. We prepared starting bromopyridines **2a-t** through aromatic nucleophilic substitution of 5-bromo-2-fluoropyridine with commercially available alcohols **1a-r**, and benzyl mercaptan **1s**. The procedure first involved deprotonation of the alcohol or the thiol in the presence of sodium hydride in anhydrous THF with subsequent addition of the dihalogenated pyridine, leading to compounds **2a-s** with high yields (76%-quant.). Attempts to synthesize 5-bromo-2-(2,4-dichlorophenoxy)pyridine **2t** with this procedure only resulted in the unmodified starting materials. **2t** was therefore prepared in good yields (81%) using a slightly modified procedure involving KOH and DMF in place of NaH and THF (Scheme 1) [52].

Bromopyridines **2a-m** and **2p-t** were then subjected to bromine-lithium exchange with *n*-BuLi in anhydrous ether at -78°C, followed by the reaction with triisopropylborate $B(O\textit{i}Pr)_3$ [53]. Specific attention must be given to the boronic acid work-up because of the concomitant presence of the boronic acid and the pyridine moieties. Thus, the desired product with protonated boronic acid and free amine is only predominant in aqueous phase in a narrow range of pH that was theoretically determined using Marvin Sketch® protonation calculator [54]. Optimized work-up involved slow addition of 4% aqueous NaOH solution to the reaction mixture allowing solubilization of the boronate sodium salt in the aqueous layer while retaining organic by-products in the ethereal phase. The resulting aqueous phase was then carefully acidified by HCl 3N until complete precipitation of the expected boronic acid. In the special case of 6-dodecanyloxypyridine-3-boronic acid **3f**, complete solubilis~~z~~ation of the boronate salt in the aqueous basic phase was prevented by the presence of the long hydrophobic dodecanyl~~e~~ chain. Optimized procedure involved direct acidification of the reaction medium with acetic acid until theoretical pH, followed by aqueous work-up and recry~~i~~stallisation of the desired product. Pyridine-3-boronic acids **3a-m** and **3p-t** were thus obtained as stable white solids in moderate to good yields, yet with unmatched purity (47-86%, Scheme 1).

Attempts to synthesize 6-(4-chlorobenzyloxy)pyridine-3-boronic acid **3n** using the above methodology only resulted in the dehalogenated 2-(4-chlorobenzyloxy)pyridine in 79% yield. Introducing the borate prior to *n*-BuLi in order to overpass the eventual instability of the lithiated intermediate prevented the dehalogenation reaction but only gave unmodified starting 2-(4-chlorobenzyloxy)-5-bromopyridine **2n**. Optimized procedure finally involved the introduction of ~~use of *in situ*~~ trimethyl borate $B(OMe)_3$ in the reaction medium prior to the ~~followed by~~ addition of *n*-BuLi in anhydrous THF at -78°C [55]. Subsequent hydrolysis led to the expected boronic acid **3n** with 60% yield. This procedure was then successfully applied to 2-(4-fluorobenzyloxy)-5-bromopyridine **2o**, leading to the corresponding boronic acid **3o** in 48% yield (Scheme 1).

Scheme 1. Synthesis of boronic acids **3a-t**.[a]





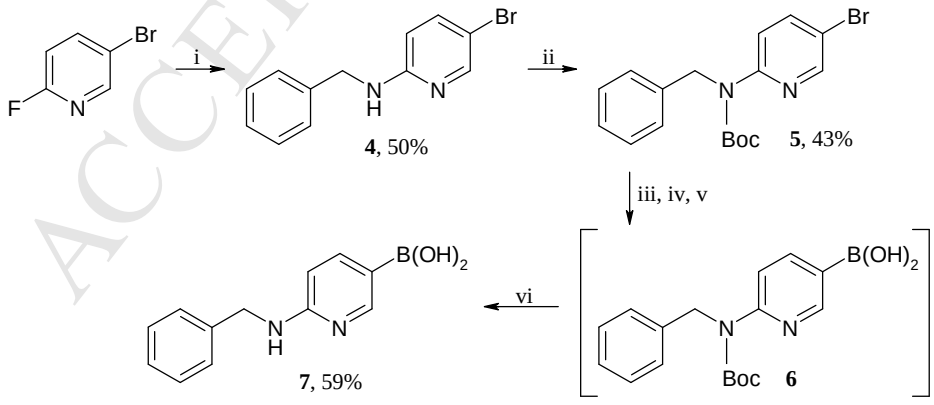
	R	n	Y
a	Me	2	O
b	Me	3	O
c	Me	4	O
d	Me	5	O
e	Me	6	O
f	Me	11	O
g	Ph	0	O
h	Ph	2	O
i	Ph	3	O
j	Ph	4	O
k	Ph	5	O
l	Ph	6	O
m	Ph	7	O
n[c]	4-Cl-Ph	1	O
o[c]	4-F-Ph	1	O
p	3,4-Cl-Ph	1	O

q	3,4,5-OMe-Ph	1	O
r	1,3-benzodioxol	1	O
s	Ph	1	S
t[b]	2,4-Cl-Ph	0	O

[a]Reagents and conditions: i) Alcohols **1a-r** or thiol **1s** 1.5 equiv., 60% NaH 2 equiv., anhyd. THF, 0°C to rt, 0.5h then reflux, 1h; ii) ~~5-bromo-2-fluoropyridine 1 equiv.,~~ reflux, 12h; iii) *n*-BuLi 1.25 equiv., anhyd. ether, -78°C, 1h; iv) B(O*i*Pr)$_3$ 1.25 equiv., -78°C to rt, 1h; v) Hydrolysis. [b]Reagents and conditions: i) Alcohol **1t** 2 equiv., KOH 2.5 equiv., anhyd. THF, reflux, 1h; ii) 5-bromo-2-fluoropyridine 1 equiv., DMF, reflux, 12h. [c]Reagents and conditions: iii) B(OMe)$_3$ 2.5 equiv., anhyd. THF, -78°C, 10min.; iv) *n*-BuLi 2.5 equiv., -78°C to rt, 1h.; v) Hydrolysis. [d]~~Isolated yields~~.

6-Benzylaminopyridine-3-boronic acid **7** was synthesized in a 4-step procedure as shown in Scheme 2. Treatment of 5-bromo-2-fluoropyridine with benzylamine and sodium hydride in DMF afforded 6-benzylamino-5-bromopyridine **4** which was then Boc-protected with di-*tert*-butyl dicarbonate to give compound **5** [56]. Bromine-lithium exchange and boronylation of **5** was performed using the *in situ* borate procedure previously described for compounds **3n-o** and the non-isolated boronic acid **6** was finally deprotected to obtain the expected boronic acid **7** in 59% yield over 2 steps.

Scheme 2. Synthesis of 6-benzylaminopyridine-3-boronic acid **7**.[a]

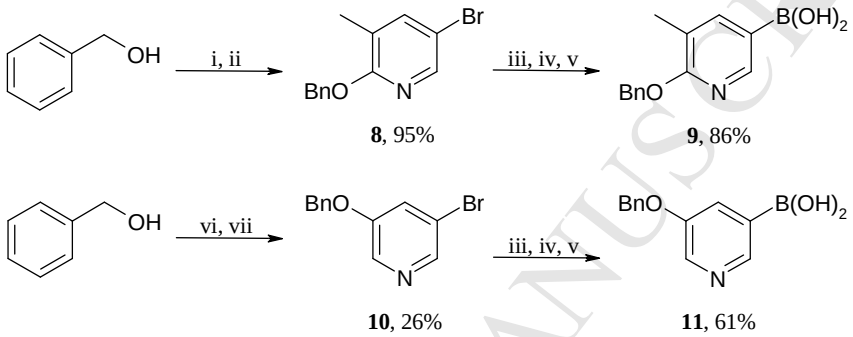


[a]Reagents and conditions : i) Benzylamine 1.2 equiv., K_2CO_3 1.5 equiv., DMF, reflux, 12h.; ii) Boc_2O 1.2 equiv., Et_3N 1.2 equiv., DMAP cat., CH_2Cl_2, reflux, 12h.; iii) B(O*i*Pr)$_3$ 1.25 equiv., THF,

-78°C, 10min.; iv) *n*-BuLi 1.25 equiv., -78°C to rt, 1h.; v) Hydrolysis; vi) EtOH/HCl 3N 1:1, reflux, 12h.

Finally, using previously described procedures, 6-benzyloxy-5-methylpyridine-3-boronic acid **9** and 5-benzyloxypyridine-3-boronic acid **11** were prepared in good yields from bromopyridines **8** and **10**. (Scheme 3). The unfavorable nucleophilic substitution at the 3-position of the pyridine ring accounts for the poor yield obtained for compound **10**.

Scheme 3. Synthesis of boronic acids **9** and **11**.[a]



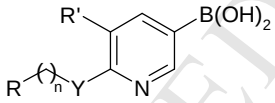
[a]Reagents and conditions : i) Benzyl alcohol 1.5 equiv., 60% NaH 2 equiv., anhyd. THF, 0°C to rt, 0.5h then reflux, 1h; ii) 2,5-dibromo-3-methylpyridine 1 equiv., reflux, 12h; iii) *n*-BuLi 1.25 equiv., anhyd. ether, -78°C, N_2, 1h; iv) B(O*i*Pr)$_3$ 1.25 equiv., -78°C to rt, 1h; v) Hydrolysis ; vi) Benzyl alcohol 0,95 equiv., 60% NaH 1.5 equiv., DMF, rt, 1h.; vii) 3,5-dibromopyridine 1.0 equiv., reflux, 48h.

Microbiological assays. In a first screening assay, the intrinsic antibacterial activity of compounds against susceptible and resistant strains of *S. aureus* was assessed and measured by their minimum inhibitory concentration (MIC). Chosen strains were the susceptible *S. aureus* ATCC25923 strain and the fluoroquinolone-resistant *S. aureus* 1199B strain that overexpresses the NorA efflux pump and is derived from a methicillin-susceptible *S. aureus* bloodstream isolate from a patient with endocarditis [57]. Ciprofloxacin has a MIC of 0.5 µg/mL against the parent susceptible *S. aureus* 1199 strain and of 8 µg/mL against the resistant strain. Compounds that were inactive alone were then investigated for their ability to restore the potency of ciprofloxacin against the resistant SA-1199B strain in order to identify potential NorA efflux pump inhibitors. Therefore, compounds

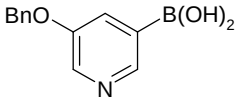
were evaluated in combination with a subinhibitory concentration of the antimicrobial agent: MIC/2, i.e. 4µg/mL or MIC/4, i.e. 2µg/mL of ciprofloxacin. Inhibitor potentiating activities are reported as minimum modulatory concentration (MMC) MMC_4 or MMC_2 values, which stand for the lowest concentration of the inhibitor required to achieve antibacterial activity in combination with corresponding subinhibitory concentrations of ciprofloxacin. Reserpine, which is a known inhibitor of the NorA efflux pump, was used as a positive control and displayed a MMC_4 of 4-8 µg/mL. Results are shown in Table 1.

Active antibacterial agents should display no or limited detrimental side effect upon the infected host cells: to evaluate this aspect the cytotoxicity of compounds was ascertained on KB cells at a concentration of 10 µM. KB are epithelial cells originated from an epidermal carcinoma of the mouth sensitive to a variety of structurally different agents and lacking the efflux pump P-gp (ABC B1). Docetaxel was used as positive control at its IC_{50} concentration (0.15 nM). The results are shown in Table 1 as percentage of cellular growth inhibition.

Table 1. Evaluation of antibacterial activity and cytotoxicity of boronic acids **3a-t**, **7**, **9** and **11**.



3a-t, **7**, **9**



11

					Activity against Se[a] and Rt[b] *S. aureus*							pKa[d]		
Id.	R	n	Y	R'	Se MIC (µg/mL)	Rt MIC (µg/mL)	Rt MMC_2 (µg/mL)	Rt MMC_4 (µg/mL)	Rt MMC_4 (µM)	Cyt.[c]	log P[d]	N	OH	OH
3a	Me	2	O	H	>128	>128	4	>128	-	0.0	1.58	1.70	8.53	10.76
3b	Me	3	O	H	>128	>128	2	64	328.17	1.0	1.98	1.70	8.53	10.76
3c	Me	4	O	H	>128	>128	2	64	306.15	16.0	2.38	1.70	8.53	10.76
3d	Me	5	O	H	N.D.	>128	2	16	71.72	46.0	2.77	1.70	8.53	10.76
3e	Me	6	O	H	>16	>16	0.5	8	33.74	73.0	3.17	1.70	8.53	10.76
3f	Me	11	O	H	>16	>16	>16	>16	-	13.0	5.15	1.70	8.53	10.76
3g	Ph	0	O	H	>128	>128	2	64	297.54	14.0	2.46	0.73	8.48	10.71
I	Ph	1	O	H	>128	>128	1	16	69.85	2.0	2.55	1.65	8.52	10.76
3h	Ph	2	O	H	>128	>128	1	16	65.82	28.0	2.80	1.69	8.53	10.76
3i	Ph	3	O	H	>128	>128	0.5	4	15.56	83.0	3.20	1.70	8.53	10.76
3j	Ph	4	O	H	64-128	32	0.25	4	14.75	13.0	3.59	1.70	8.53	10.76
3k	Ph	5	O	H	N.D.	16	2	4	14.03	60.0	3.99	1.70	8.53	10.76
3l	Ph	6	O	H	>16	>16	0.5	4	13.37	46.0	4.39	1.70	8.53	10.76

3m	Ph	7	O	H	>16	>16	0.5	4	12.77	62.0	4.78	1.70	8.53	10.76
3n	4-Cl-Ph	1	O	H	≥128	128	1	32	121.45	33.0	3.07	1.65	8.52	10.76
3o	4-F-Ph	1	O	H	>128	>128	2	32	129.54	8.0	2.69	1.65	8.52	10.76
3p	3,4-Cl-Ph	1	O	H	N.D.	16	0.125	1	3.35	44.0	3.59	1.65	8.52	10.76
3q	3,4,5-OMe-Ph	1	O	H	>128	>128	8	N.D.	-	3.0	1.79	1.65	8.52	10.76
3r	1,3-benzodioxol	1	O	H	>128	>128	64	N.D.	-	0.0	2.23	1.65	8.52	10.76
3s	Ph	1	S	H	64-128	64	0.5	16	65.28	8.0	3.35	2.26	8.42	10.63
3t	2,4-Cl-Ph	0	O	H	N.D.	128	1	16	56.36	1.0	3.49	0.69	8.48	10.71
7	Ph	1	NH	H	>128	>128	32	128	561.28	0.0	2.38	5.42	8.74	11.01
9	Ph	1	O	Me	N.D.	>128	4	16	65.82	15.0	3.02	2.19	8.55	10.79
11					>128	>128	8	>128	-	0.0	1.85	3.51	8.28	10.47
					Ampicillin									
					Ciprofloxacin	0.25	8							
					Reserpine			4-8	6.57-13.14					

[a]Se: susceptible *S. aureus* ATCC25923. [b]Rt : resistant *S. aureus* 1199B (NorA).

[c]Cytotoxicity as % of inhibition of cellular growth of KB cells in the presence of 10µM of the tested compound. [d]Calculated values [58]. N.D.: No Data.

Structural characterization of NorA, using *in silico* approaches, suggests that it is made of twelve transmembrane helices and intercalated hydrophilic loops and possesses a large hydrophobic binding site. Thus, pharmacomodulations first involved the replacement of the 6-benzyloxy group of hit compound **I** with alkoxy chains with various lengths (compounds **3a-f**) to explore the size of the putative hydrophobic pocket of NorA. We observed an overall increase of the potentiative activity in correlation with the increase of the side chain length and the increase of the logP values, which is in agreement with the binding site putative geometry. However, for compound with the longest alkyl side chain **3f**, no activity was observed. In that case, the stock solution used for the serial dilution in the MIC determination experiment was lowered to 16 µg/mL which was the highest value allowing complete solubilisation of the compound. This low solubility is incidentally correlated to a high logP calculated value, namely 5.15.

In an attempt to enhance hydrophobicity without extending the chain length of the boronic derivatives, a phenyl ring was added at the end of the side chain (compounds **3g-m**). As previously, we observed an increase in the potentiative activity with the increase of the chain length. Besides,

compounds with the phenyl ring were found to be more efficient than those with an alkyl chain. Compounds **I** and **3h-i** showed a 4-fold decrease in their MMC_4 (16 or 4 µg/mL) compared to compounds **3b-d** (64 or 16 µg/mL), whereas **3j** showed a 2-fold decrease in MMC_4 (4 µg/mL) compared to **3e** (8 µg/mL). For both alkoxy and arylalkoxy compounds **3a-m** evaluated in this study, the best activity was observed for a total-length chain of seven carbons, phenyl included (compounds **3e** and **3j**). 6-(3-Phenylpropoxy)pyridine-3-boronic acid **3i** and 6-(4-phenylbutoxy)pyridine-3-boronic acid **3j** showed the highest activities and potentiated ciprofloxacin by a 4-fold increase at MMC_4 of 4 µg/mL. No significant antibacterial activity was observed except for 6-(5-phenylpentoxy)pyridine-3-boronic acid **3k** that displayed mild activity with a MIC of 16 µg/mL. This was the first identified compound of our library with intrinsic antibacterial activity. As mentioned above for **3f**, we faced solubility problems with compounds **3e-f** and **3l-m** harboring the longest (aryl)alkoxy chains. These compounds were not soluble at the usual highest tested concentration of 128 µg/mL and were thus evaluated at most at 16 µg/mL. Therefore, possible antibacterial activity could not be evaluated above this concentration.

Then, we studied the effect of the introduction of substituents on the phenyl ring of hit compound **I** but mostly faced unsatisfactory results: the 4-chloro (**3n**) and 4-fluoro (**3o**) derivatives showed a 2-fold decrease in the potentiative activity of ciprofloxacin while the 3,4,5-trimethoxy (**3q**) and 3,4-piperonyl (**3r**) substituents led to a total loss of activity. Better results were obtained with the 3,4-dichloro derivative **3p** but its moderate intrinsic antibacterial activity (MIC=16µg/mL) invalidates its foreseeable action as an EPI. Interestingly, modification of 6-phenoxypyridine-3-boronic acid **3g** to give the triclosan-like **3t** led to a 4-fold increase in the potentiative activity of ciprofloxacin, suggesting that introduction of two chlorine atoms may be important for both antibacterial and EPI activity.

Modification of the ether linkage between the pyridine ring and the 6-benzyloxy group of **I** revealed opposite effects: conservation of a H-bond acceptor linkage with the sulfanyl compound **3s** led to similar potentiative activity (MMC_4=16 µg/mL), whereas introduction of the H-bond donor

amine linkage (**7**) was detrimental for activity with a 8-fold decrease in activity (MMC_4=128 µg/mL). Further pharmacomodulations showed that shifting the benzyloxy group from the C-6 to the C-5 position of pyridine-3-boronic acid (compound **11** vs. **I**) resulted in a total loss of activity. Finally, introduction of a methyl group in the C-5 position (compound **9**) had little influence on the activity.

Except for compound **3i**, all molecules inhibited less of 80% of the growth of KB cells and were to be considered as poorly cytotoxic. In particular, the most promising compound **3j** as a potential NorA inhibitor showed a low level of growth inhibition (13%). With an 83% inhibition of cellular growth, the other active molecule **3i** displayed a moderate level of toxicity at 10^{-5}M, but a low level of toxicity (9.0%) at 10^{-6}M. Similarly, compounds **3k** and **3p** with intrinsic antibacterial activity showed a moderate level of toxicity at 10^{-5}M, and a low level of toxicity (respectively 6.0 and 0.0%) at 10^{-6}M.

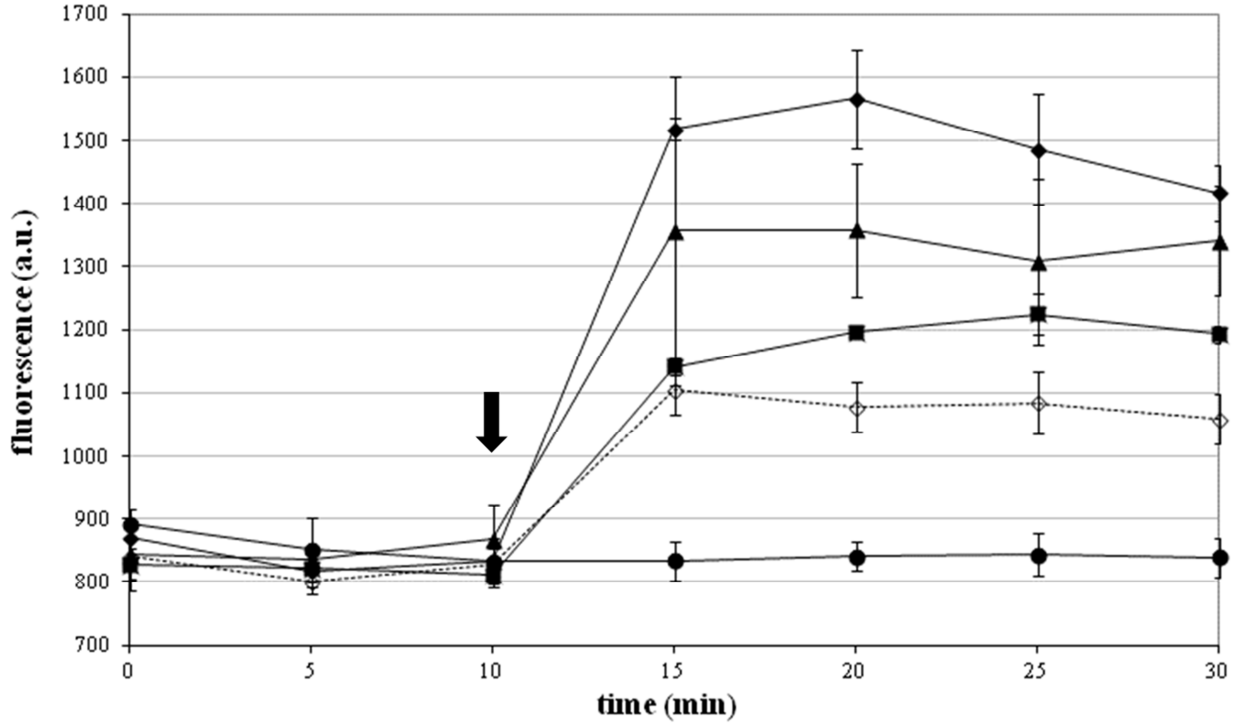
To investigate whether compounds have a specific synergistic activity with ciprofloxacin or could be used in combination with other antibacterial agents that are substrate of the NorA efflux pump, we tested their ability to potentiate the activity of norfloxacin. Norfloxacin has a MIC of 64 µg/mL against the SA 1199B strain. Fifteen compounds (**I**, **3a-e**, **3g-j**, **3n-o**, **3s-t** and **9**) were evaluated in combination with the fluoroquinolone at a subinhibitory concentration of 16 µg/mL (MIC/4). Data reported in Table 2 show that compounds displayed similar activity in the presence of ciprofloxacin or norfloxacin.

Table 2. Evaluation of antibacterial activity in combination with norfloxacin.

Antibacterial activity against SA 1199B + Nor 16 µg/mL					
Id.	MMC_4 (µg/mL)	Id.	MMC_4 (µg/mL)	Id.	MMC_4 (µg/mL)
3a	> 128	**3g**	128	**3n**	16
3b	64	**I**	32	**3o**	64
3c	64	**3h**	16	**3s**	16
3d	16	**3i**	8	**3t**	32
3e	8	**3j**	8	**9**	8

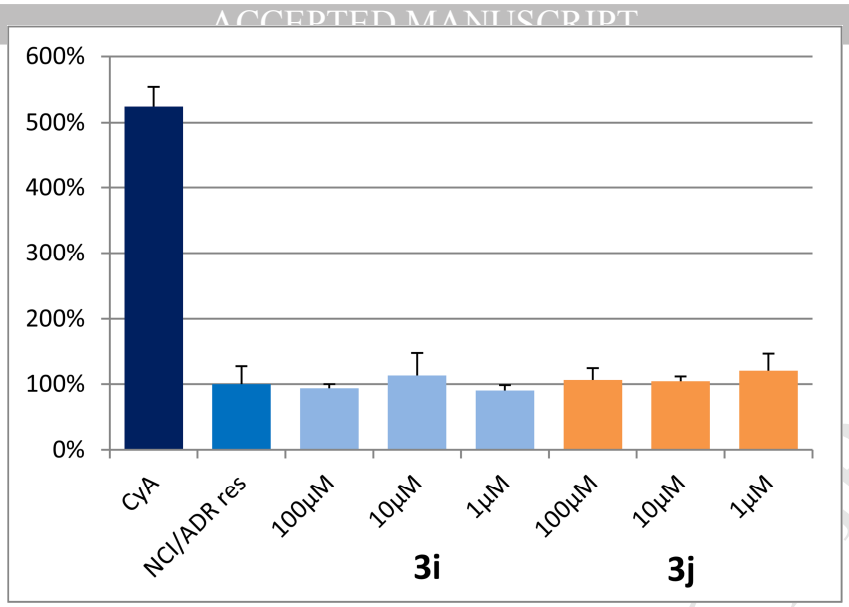
Effects on EtBr accumulation in the SA 1199B strain. As compounds **3i** and **3j**, in association with ciprofloxacin, showed a strong effect against the SA 1199B strain, we carried out their characterization as potential NorA inhibitors by measuring their effect on the accumulation of ethidium bromide (EtBr, a DNA-fluorescing agent substrate of the NorA pump). EtBr becomes fluorescent only when intercalated to bacterial DNA. Thus, when a compound is an EPI, its activity results in intracellular accumulation of EtBr and is measured by the increase in fluorescence [59]. Compounds were evaluated at a concentration of 20 µg/mL, as compared to that of the positive control reserpine. Figure 3 illustrates the effect of boronic acids **3i** and **3j** on the uptake of EtBr as a function of time. In the absence of inhibitors, the fluorescence intensity remained constant at about 800 arbitrary units (a.u.). Upon addition of reserpine, **3i** or **3j**, fluorescence reached about 1450, 1200 and 1300 a.u. respectively, after 30 minutes, resulting in a 73%, 43% and 42% increase in the EtBr uptake inside the cells. These results suggested that compounds promoted accumulation of EtBr, thus acting as inhibitors of the NorA pump, but to a lesser extent that reserpine.

Figure 3. Effect of compounds **3i** (■) and **3j** (▲) on EtBr accumulation of SA-1199B. The tested compound was added after 10 min (⬇) of bacterial incubation with EtBr. The positive control was reserpine at 5 (♢) and 20 (♦) µg/m and the negative control (●) was the bacteria in the presence of 1.6% DMSO. Vertical bars are the mean ± standard deviations for two or three independent experiments.



Effect in the human P-gp efflux pump. Although the bacterial NorA and the human P-gp efflux pumps differ in their structure and mode of action, a partial overlapping of the substrates/inhibitors had been demonstrated [60,61]. To evaluate the selectivity of the molecules **3i** and **3j** upon Nor A, we next examined their capacity to inhibit of the human P-gp efflux pump. Evaluation was carried out on the breast cancer cell line NCI/ADR-RES which overexpresses the P-gp pump. Compounds were evaluated at concentrations of 100, 10 and 1 µM for their ability to promote accumulation of Rhodamine-123, a fluorescent substrate of the pump, in NCI/ADR-RES cells. Cyclosporine A, a reference inhibitor of the P-gp pump was used as positive standard at a concentration of 50 µM. Figure 4 shows the cellular accumulation of rhodamine as a function of the concentration of P-gp potential inhibitors. The effect of the known P-gp inhibitor Cyclosporine A (CyA) was noticeable, with a 5-fold increase in fluorescence, whereas no significant increase in the uptake of Rhodamine-123 was observed upon addition of compounds **3i** and **3j**. Therefore, these compounds were not dual inhibitors for P-gp and NorA and could be used as antimicrobial agents without alteration of drug distribution and pharmacokinetics under the control of P-gp.

Figure 4. Uptake of Rhodamine-123 by the NCI/ADR-RES cell line.



CONCLUSION

In this study, we investigated the antibacterial properties of a series of boronic derivatives against susceptible and resistant *S. aureus* strains. Starting from a chemical library of about 150 compounds, we selected pyridine-3-boronic acid compounds for their ability to restore the activity of ciprofloxacin against the NorA-overexpressing SA1199B strain. Structure-activity relationships revealed the importance of the presence of an (aryl)alkoxy side chain at the C-6 position. Chemical pharmacomodulation of the hit compound **I** led to 6-(3-phenylpropoxy)pyridine-3-boronic acid **3i** and 6-(4-phenylbutoxy)pyridine-3-boronic acid **3j** that showed a 4-fold increase in activity compared to compound **I**. They emerged as potential NorA inhibitors as they potentiate the activity of ciprofloxacin and norfloxacin, have no significant intrinsic antibacterial activity, promote accumulation of EtBr, show reduced cytotoxicity and do not inhibit the mammalian P-gp efflux pump. In the course of our study, we also identified 6-(5-phenylpentoxy)pyridine-3-boronic acid **3k** and 6-(3,4-dichlorobenzyloxy)pyridine-3-boronic acid **3p** for their moderate antibacterial activity. Although the EPI activity of **3i** and **3j** appears to be lower than that of reserpine, they represent a promising chemotype for the development of new compounds with improved potency.

EXPERIMENTAL SECTION

Synthesis. All chemical products, reagents and solvents were purchased from commercial sources and used without further purification except THF, which was distilled from Na/benzophenone. Chromatography was carried out on a column using flash silica gel 60 Merck (0.063-0.200 mm) as the stationary phase. The eluting solvent indicated for each purification was determined by thin-layer chromatography (TLC) performed on 0.2 mm precoated plates of silica gel $60F_{254}$ (Merck) and spots were visualized using an ultraviolet-light lamp. Melting points were determined on a Kofler melting point apparatus. IR spectra were recorded on KBr disks using a Perkin-Elmer BX FT-IR spectrophotometer. The band positions are given in reciprocal centimeters (cm^{-1}). ^{1}H NMR (400 MHz) and ^{13}C NMR (100 MHz) spectra were recorded on a JEOL Lambda 400 spectrometer. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded on a Bruker 500 Avance III spectrometer. Chemical shifts (δ) are expressed in parts per million downfield from tetramethylsilane as an internal standard and coupling constants (J) in hertz. The abbreviations used are as follows: s, singlet; bs, broad singlet; d, doublet; dd, double doublet; t, triplet; m, multiplet. The purities of all tested compounds were analyzed by LC-MS, with the purity all being higher than 95%. Analyses were performed using a Waters alliance 2695 and MS detection was performed with a SQDetector.

General procedure A for the synthesis of compounds 2a-s. To a solution of alcohol **1a-r** or benzyl mercaptan **1s** (1.5 equiv.) in anhydrous THF (100 mL) was slowly added 60% sodium hydride (2.0 equiv.). The mixture was allowed to react at room temperature for 30 min then it was heated to reflux for 1 h. 5-bromo-2-fluoropyridine (1 equiv.) was added and the reaction was continued for 12 h. After cooling down to room temperature, the mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered, evaporated and purified by silica gel chromatography.

5-Bromo-2-propoxypyridine (2a). Starting from **1a** (3.19 mL, 42.62 mmol) using general procedure A and cyclohexane/CH_2Cl_2 98:2 as the eluent for chromatography, **2a** was obtained as a colorless oil. Yield 97%. IR (KBr): ν (cm^{-1}) 2966, 1586, 1460, 1301, 1243, 1009, 971, 824, 671. ^{1}H

NMR (400 MHz, $CDCl_3$): δ 1.01 (3H, t, J=7.1 Hz, CH_3), 1.74-1.83 (2H, m, $OCH_2CH_2CH_3$), 4.21 (2H, t, J=7.1 Hz, $OCH_2C_2H_5$), 6.65 (1H, d, J=8.8 Hz, H-3), 7.63 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.17 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 147.4, 141.0, 112.7, 111.4, 68.0, 22.2, 10.5.

5-Bromo-2-butoxypyridine (2b) [62]. Starting from **1b** (2.34 mL, 25.57 mmol) using general procedure A and cyclohexane/CH_2Cl_2 99:1 as the eluent for chromatography, **2b** was obtained as a colorless oil. Yield 93%. IR (KBr): ν (cm^{-1}) 2959, 2873, 1585, 1461, 1364, 1281, 824, 671. ^1H NMR (400 MHz, $CDCl_3$): δ 0.96 (3H, t, J=7.1 Hz, CH_3), 1.42-1.51 (2H, m, $OC_2H_4CH_2CH_3$), 1.71-1.78 (2H, m, $OCH_2CH_2C_2H_5$), 4.24 (2H, t, J=7.1 Hz, $OCH_2C_3H_7$), 6.63 (1H, d, J=8.8 Hz, H-3), 7.62 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.17 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 147.4, 141.0, 112.7, 111.3, 66.2, 31.0, 19.2, 13.8.

5-Bromo-2-pentoxypyridine (2c). Starting from **1c** (3.71 mL, 34.09 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2c** was obtained as a colorless oil. Yield 77%. IR (KBr): ν (cm^{-1}) 2956, 1586, 1463, 1358, 1243, 1090, 1003, 824, 674. ^1H NMR (400 MHz, $CDCl_3$): δ 0.92 (3H, t, J=6.8 Hz, CH_3), 1.37-1.44 (4H, m, $OC_2H_4C_2H_4CH_3$), 1.73-1.79 (2H, m, $OCH_2CH_2C_3H_7$), 4.24 (2H, t, J=6.8 Hz, $OCH_2C_4H_9$), 6.64 (1H, d, J=8.8 Hz, H-3), 7.62 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.17 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 147.4, 141.0, 112.7, 111.3, 66.5, 28.6, 28.1, 22.4, 14.0.

5-Bromo-2-hexyloxypyridine (2d). Starting from **1d** (3.21 mL, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2d** was obtained as a colorless oil. Yield 88%. IR (KBr): ν (cm^{-1}) 2956, 2858, 1586, 1461, 1281, 1003, 824, 673, 515. ^1H NMR (400 MHz, $CDCl_3$): δ 0.90 (3H, t, J=6.8 Hz, CH_3), 1.32-1.45 (6H, m, $OC_2H_4C_3H_6CH_3$), 1.71-1.79 (2H, m, $OCH_2CH_2C_4H_9$), 4.24 (2H, t, J=6.8 Hz, $OCH_2C_5H_{11}$), 6.64 (1H, d, J=8.8 Hz, H-3), 7.62 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.17 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 147.4, 141.0, 112.7, 11.3, 66.5, 31.5, 28.9, 25.7, 22.6, 14.0.

5-Bromo-2-heptyloxypyridine (2e). Starting from **1e** (3.63 mL, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2e** was obtained as a yellow oil. Yield 79%. IR (KBr): ν (cm^{-1}) 2928, 2857, 1586, 1460, 1281, 1009, 824, 673, 515. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.89 (3H, t, J=6.8 Hz, CH_3), 1.28-1.43 (8H, m, $OC_2H_4C_4H_8CH_3$), 1.72-1.79 (2H, m, $OCH_2CH_2C_5H_{11}$), 4.23 (2H, t, J=6.8 Hz, $OCH_2C_6H_{13}$), 6.64 (1H, d, J=8.8 Hz, H-3), 7.62 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.17 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 147.5, 141.0, 112.7, 111.3, 66.5, 31.8, 29.0, 28.9, 26.0, 22.6, 14.1.

5-Bromo-2-dodecanyloxypyridine (2f). Starting from **1f** (9.53 mL, 42.62 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2f** was obtained as a beige solid. Yield 91%. Mp < 50°C. IR (KBr): ν (cm^{-1}) 2917, 2851, 1587, 1459, 1279, 1024, 819, 677. ^{1}H NMR (400 MHz, $CDCl_3$): δ 0.88 (3H, t, J=6.8 Hz, CH_3), 1.26 (18H, bs, $OC_2H_4C_9H_{18}CH_3$), 1.71-1.78 (2H, m, $OCH_2CH_2C_{10}H_{21}$), 4.23 (2H, t, J=6.8 Hz, $OCH_2C_{11}H_{23}$), 6.64 (1H, d, J=8.8 Hz, H-3), 7.62 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.17 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.9, 147.5, 141.0, 112.7, 111.4, 66.5, 31.9, 29.7, 29.64, 29.6, 29.57, 29.38, 29.36, 28.9, 26.0, 22.7, 14.1.

5-Bromo-2-phenoxypyridine (2g) [63]. Starting from **1g** (3.21 g, 34.09 mmol) using general procedure A and cyclohexane/AcOEt 98:2 as the eluent for chromatography, **2g** was obtained as a colorless oil. Yield 95%. IR (KBr): ν (cm^{-1}) 3066, 1576, 1456, 1367, 1271, 1091, 758, 693. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.83 (1H, d, J=8.8 Hz, H-3), 7.12 (2H, m, H-2'), 7.22 (1H, m, H-4'), 7.41 (2H, m, H-3'), 7.75 (1H, dd, J=8.8 and 2.7 Hz, H-4), 8.22 (1H, d, J=2.7 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.5, 153.7, 148.4, 141.9, 129.7, 125.0, 121.1, 113.4, 113.0.

5-Bromo-2-(2-phenylethoxy)pyridine (2h). Starting from **1h** (3.06 mL, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2h** was obtained as a yellow oil. Yield 97%. IR (KBr): ν (cm^{-1}) 3028, 2955, 1584, 1456, 1361, 1280, 1012, 825, 699. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.07 (2H, t, J=7.1 Hz, OCH_2CH_2), 4.48 (2H, t, J=7.1 Hz, OCH_2CH_2), 6.63 (1H, d, J=8.8 Hz, H-3), 7.22-7.32 (5H, m, H-Ph), 7.62 (1H, dd, J=8.8 and 2.9 Hz,

H-4), 8.17 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.5, 147.4, 141.0, 138.3, 129.0, 128.4, 126.4, 112.8, 111.6, 66.8, 35.3.

5-Bromo-2-(3-phenylpropoxy)pyridine (2i). Starting from **1i** (5.80 mL, 42.62 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2i** was obtained as a yellow oil. Yield 93%. IR (KBr): ν (cm^{-1}) 2951, 1586, 1462, 1363, 1282, 1027, 825, 699. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.05-2.12 (2H, m, $OCH_2\mathit{CH_2}CH_2$), 2.77 (2H, t, J=6.8 Hz, $OC_2H_4\mathit{CH_2}$), 4.27 (2H, t, J=6.8 Hz, $O\mathit{CH_2}C_2H_4$), 6.65 (1H, d, J=8.8 Hz, H-3), 7.17-7.31 (5h, m, H-Ph), 7.63 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.17 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 147.4, 141.5, 141.0, 128.4, 125.9, 112.6, 111.5, 65.6, 32.2, 30.5.

5-Bromo-2-(4-phenylbutoxy)pyridine (2j). Starting from **1j** (4.05 mL, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 98:2 as the eluent for chromatography, **2j** was obtained as a white solid. Yield 85%. Mp 60-62°C. IR (KBr): ν (cm^{-1}) 2925, 1587, 1484, 1366, 1295, 1022, 830, 699. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.74-1.80 (4H, m, $OCH_2\mathit{C_2H_4}CH_2$), 2.68 (2H, t, J=6.8 Hz, $OC_3H_6\mathit{CH_2}$), 4.27 (2H, t, J=6.8 Hz, $O\mathit{CH_2}C_3H_6$), 6.63 (1H, d, J=8.8 Hz, H-3), 7.16-7.30 (5H, m, H-Ph), 7.62 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.17 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.7, 147.4, 142.2, 141.0, 128.4, 128.3, 125.7, 112.7, 111.4, 66.1, 35.5, 28.5, 27.8.

5-Bromo-2-(5-phenylpentoxy)pyridine (2k). Starting from **1k** (2.87 mL, 17.05 mmol) using general procedure A and cyclohexane/AcOEt 98:2 as the eluent for chromatography, **2k** was obtained as a colorless oil. Yield 87%. IR (KBr): ν (cm^{-1}) 2935, 1585, 1162, 1281, 1002, 825, 699. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.44-1.52 (2H, m, $OC_2H_4\mathit{CH_2}C_2H_4$), 1.65-1.73 (2H, m, $OC_3H_6\mathit{CH_2}CH_2$), 1.75-1.83 (2H, m, $OCH_2\mathit{CH_2}C_3H_6$), 2.63 (2H, t, J=7.2 Hz, $OC_4H_8\mathit{CH_2}$), 4.23 (2H, t, J=7.2 Hz, $OCH_2C_4H_8$), 6.62 (1H, d, J=8.8 Hz, H-3), 7.16-7.29 (5H, m, H-Ph), 7.61 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.16 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 147.4, 142.5, 141.0, 128.4, 128.2, 125.6, 112.7, 111.4, 66.3, 35.8, 31.2, 28.8, 25.7.

5-Bromo-2-(6-phenylhexyloxy)pyridine (2l). Starting from **1l** (1.59 mL, 8.52 mmol) using general procedure A and cyclohexane/AcOEt 98:2 as the eluent for chromatography, **2l** was obtained as a

colorless oil. Yield 91%. IR (KBr): ν (cm^{-1}) 2931, 1585, 1462, 1281, 1090, 1002, 825, 699. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37-1.50 (4H, m, $OC_2H_4C_2H_4C_2H_4$), 1.60-1.68 (2H, m, $OC_4H_8CH_2CH_2$), 1.72-1.79 (2H, m, $OCH_2CH_2C_4H_8$), 2.61 (2H, t, J=7.2 Hz, $OC_5H_{10}CH_2$), 4.23 (2H, t, J=7.2 Hz, $OCH_2C_5H_{10}$), 6.63 (1H, d, J=8.8 Hz, H-3), 7.16-7.29 (5H, m, H-Ph), 7.62 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.17 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 147.4, 142.7, 141.0, 128.4, 128.2, 125.6, 112.7, 111.4, 66.4, 35.9, 31.4, 29.0, 28.8, 25.9.

5-Bromo-2-(7-phenylheptyloxy)pyridine (2m). Starting from **1m** (1.70 mL, 8.52 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2m** was obtained as a colorless oil. Yield 76%. IR (KBr): ν (cm^{-1}) 2930, 1585, 1463, 1281, 1090, 1004, 825, 699. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.37-1.41 (6H, m, $OC_2H_4C_3H_6C_2H_4$), 1.57-1.64 (2H, m, $OC_5H_{10}CH_2CH_2$), 1.71-1.76 (2H, m, $OCH_2CH_2C_5H_{10}$), 2.60 (2H, t, J=7.2 Hz, $OC_6H_{12}CH_2$), 4.23 (2H, t, J=7.2 Hz, $OCH_2C_6H_{12}$), 6.63 (1H, d, J=8.8 Hz, H-3), 7.16-7.29 (5H, m, H-Ph), 7.62 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.17 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.8, 147.4, 142.8, 141.0, 128.4, 128.2, 125.6, 112.7, 111.4, 66.4, 35.9, 31.4, 29.2, 28.9, 26.9, 25.9.

5-Bromo-2-(4-chlorobenzyloxy)pyridine (2n). Starting from **1n** (6.08 g, 42.62 mmol) using general procedure A and cyclohexane/AcOEt 98:2 as the eluent for chromatography, **2n** was obtained as a white solid. Yield 98%. Mp 93-95°C. IR (KBr): ν (cm^{-1}) 1454, 1286, 1012, 822, 800, 642. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.31 (2H, s, OCH_2), 6.72 (1H, d, J=8.8 Hz, H-3), 7.32-7.39 (4H, m, H-2', H-3'), 7.66 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.19 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3, 147.4, 141.3, 135.4, 133.8, 129.3, 128.6, 112.9, 112.0, 67.1.

5-Bromo-2-(4-fluorobenzyloxy)pyridine (2o) [63]. Starting from **1o** (4.65 mL, 42.62 mmol) using general procedure A and cyclohexane/AcOEt 98:2 as the eluent for chromatography, **2o** was obtained as a white solid. Yield 98%. Mp 66-68°C. IR (KBr): ν (cm^{-1}) 1455, 1292, 1090, 810, 668. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.30 (2H, s, OCH_2), 6.71 (1H, d, J=8.8 Hz, H-3), 7.03-7.08 (2H, m, H-3'), 7.40-7.43 (2H, m, H-2'), 7.66 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.20 (1H, d, J=2.9 Hz, H-6).

^{13}C NMR (100 MHz, $CDCl_3$): δ 162.2, 147.4, 141.2, 132.7, 132.6, 130.0, 129.9, 115.5, 115.3, 112.9, 112.0, 67.2.

5-Bromo-2-(3,4-dichlorobenzyloxy)pyridine (2p). Starting from **1p** (4.53 g, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2p** was obtained as a white solid. Quant. yield. Mp 88-90°C. IR (KBr): ν (cm^{-1}) 1585, 1474, 1348, 1284, 1029, 802, 645. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.29 (2H, s, OCH_2), 6.73 (1H, d, J=8.8 Hz, H-3), 7.26 (1H, dd, J=8.3 and 2.0 Hz, H-6'), 7.43 (1H, d, J=8.3 Hz, H-5'), 7.53 (1H, d, J=2.0 Hz, H-2'), 7.67 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.19 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 161.8, 147.4, 141.4, 137.2, 132.5, 131.9, 130.4, 129.8, 127.1, 112.8, 112.3, 66.3.

5-Bromo-2-(3,4,5-trimethoxybenzyloxy)pyridine (2q). Starting from **1q** (2.74 mL, 17.05 mmol) using general procedure A and cyclohexane/AcOEt 8:2 as the eluent for chromatography, **2q** was obtained as a white solid. Quant. yield. Mp 76-78°C. IR (KBr): ν (cm^{-1}) 2995, 1594, 1449, 1354, 1238, 1123, 1003, 833, 706. ^{1}H NMR (400 MHz, $CDCl_3$): δ 3.86 (9H, s, OCH_3), 5.26 (2H, s, OCH_2), 6.68 (2H, s, H-2'), 6.74 (1H, d, J=8.8 Hz, H-3), 7.67 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.22 (1H, d, J=1.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3, 153.3, 147.4, 141.3, 137.7, 132.3, 112.9, 112.0, 105.3, 68.3, 60.8, 56.1.

2-(1,3-Benzodioxol-5-ylmethoxy)-5-bromopyridine (2r). Starting from **1r** (3.89 g, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 95:5 as the eluent for chromatography, **2r** was obtained as a white solid. Yield 98%. Mp 87-89°C. IR (KBr): ν (cm^{-1}) 2888, 1585, 1466, 1283, 931, 828, 807. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.23 (2H, s, OCH_2), 5.96 (2H, s, OCH_2O), 6.69 (1H, d, J=8.8 Hz, H-3), 6.80 (1H, d, J=7.8 Hz, H-2'), 6.90-6.94 (2H, m, H-5', H-6'), 7.64 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.20 (1H, d, J=1.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 162.3, 147.7, 147.41, 147.38, 141.2, 130.6, 121.9, 112.9, 111.9, 108.9, 108.2, 101.1, 67.9.

2-Benzylsulfanyl-5-bromopyridine (2s) [64]. Starting from **1s** (3.0 mL, 25.57 mmol) using general procedure A and cyclohexane/AcOEt 99:1 as the eluent for chromatography, **2s** was obtained as a beige solid. Quant. yield. Mp < 50°C. IR (KBr): ν (cm^{-1}) 1560, 1452, 1350, 1115,

821, 715, 693, 486. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.40 (2H, s, SCH_2), 7.05 (1H, d, J=7.8 Hz, H-3), 7.24-7.40 (5H, m, H-Ph), 7.57 (1H, dd, J=7.8 and 1.9 Hz, H-4), 8.50 (1H, d, J=1.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.5, 150.2, 138.5, 137.6, 128.9, 128.5, 127.2, 123.1, 116.1, 34.5.

5-Bromo-2-(2,4-dichlorophenoxy)pyridine (2t). To a solution of **1t** (3.70 g, 22.72 mmol) in anhydrous THF (100 mL) was slowly added potassium hydroxide (1.59 g, 28.41 mmol). The mixture was allowed to react at room temperature for 30 min then it was heated to reflux for 1 h. The reaction was concentrated to dryness and diluted in anhydrous DMF (100 mL). 5-bromo-2-fluoropyridine (2.0 g, 11.36 mmol) was added and the reaction was continued for 12 h. After cooling down to room temperature, the mixture was poured in water and extracted with ethyl acetate. The crude product was purified by silica gel chromatography (cyclohexane/AcOEt 98:2) affording **2t** as a white solid. Yield 81%. Mp 82-84°C. IR (KBr): ν (cm^{-1}) 1479, 1369, 1279, 1093, 804. ^{1}H NMR (400 MHz, $CDCl_3$): δ 6.93 (1H, d, J=8.8 Hz, H-3), 7.14 (1H, d, J=8.8 Hz, H-6'), 7.29 (1H, dd, J=8.8 and 2.4 Hz, H-5'), 7.48 (1H, d, J=2.4 Hz, H-3'), 7.80 (1H, dd, J=8.8 and 2.4 Hz, H-4), 8.15 (1H, d, J=2.4 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 164.5, 148.1, 148.0, 142.2, 131.2, 130.4, 128.2, 128.1, 124.7, 114.0, 112.7.

General procedure B for the synthesis of boronic acids 3a-m, **3p-t**, **9** and **11.** To a slurry of 2.5M *n*-BuLi (1.25 equiv.) in anhydrous diethyl ether (80 mL) cooled at -78°C was added a solution of 5-bromopyridine (1.0 equiv.) in anhydrous THF (10 mL). The mixture was allowed to react at this temperature for over 60 min. A solution of triisopropyl borate (1.25 equiv.) was then added and left to react for 60 min. Unless otherwise specified, treatment of the reaction proceeded as follows: the mixture was allowed to warm to room temperature and was quenched by slow addition of 4% aqueous NaOH solution (50 mL). The resulting aqueous layer was collected and acidified to adequate pH by dropwise addition of 3N HCl. The resulting precipitate was filtered on a sintered-glass filter, washed with diethyl ether and dried to give corresponding boronic acids.

6-Propoxypyridine-3-boronic acid (3a). Starting from **2a** (1.0 g, 4.63 mmol) using general procedure B (pH 5), **3a** was obtained as a white solid. Yield 77%. Mp 118-120°C. IR (KBr): ν (cm^{-}

^{1}) 3400, 2963, 1611, 1332, 1301. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.94 (3H, t, J=7.3 Hz, CH_3), 1.65-1.74 (2H, m, $OCH_2CH_2CH_3$), 4.19 (2H, t, J=7.3 Hz, $OCH_2C_2H_5$), 6.73 (1H, d, J=8.8 Hz, H-5), 7.97 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.11 (2H, s, OH), 8.48 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 109.7, 66.8, 21.9, 10.4. LC-MS (ESI): t_R = 5.34 min; $[M+H]^+$ 182,48.

6-Butoxypyridine-3-boronic acid (3b). Starting from **2b** (1.0 g, 4.35 mmol) using general procedure B (pH 5), **2b** was obtained as a white solid. Yield 79%. Mp 109-111°C. IR (KBr): ν (cm^{-1}) 3409, 2955, 1611, 1335, 1304. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.91 (3H, t, J=7.3 Hz, CH_3), 1.35-1.44 (2H, m, $OC_2H_4CH_2CH_3$), 1.63-1.70 (2H, m, $OCH_2CH_2C_2H_5$), 4.23 (2H, t, J=7.3 Hz, $OCH_2C_3H_7$), 6.71 (1H, d, J=8.8 Hz, H-5), 7.97 (1H, d, J=8.8 Hz, H-4), 8.10 (2H, s, OH), 8.48 (1H, s, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 109.7, 65.0, 30.6, 18.8, 13.7. LC-MS (ESI): t_R = 6.38 min; $[M+H]^+$ 196.50.

6-Pentoxypyridine-3-boronic acid (3c). Starting from **2c** (1.0 g, 4.10 mmol) using general procedure B (pH 5), **3c** was obtained as a white solid. Yield 86%. Mp 139-141°C. IR (KBr): ν (cm^{-1}) 3410, 2954, 1611, 1334, 1303. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (3H, t, J=6.8 Hz, CH_3), 1.33-1.37 (4H, m, $OC_2H_4C_2H_4CH_3$), 1.65-1.72 (2H, m, $OCH_2CH_2C_3H_7$), 4.23 (2H, t, J=6.8 Hz, $OCH_2C_4H_9$), 6.72 (1H, d, J=8.8 Hz, H-5), 7.97 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.10 (2H, s, OH), 8.47 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 109.7, 65.3, 28.2, 27.8, 21.9, 13.9. LC-MS (ESI): t_R = 5.71 min; $[M+H]^+$ 209.16.

6-Hexyloxypyridine-3-boronic acid (3d). Starting from **2d** (1.0 g, 3.87 mmol) using general procedure B (pH 6), **3d** was obtained as a white solid. Yield 73%. Mp 90-92°C. IR (KBr): ν (cm^{-1}) 3393, 2944, 2917, 1609, 1335, 1301. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.87 (3H, t, J=6.9 Hz, CH_3), 1.28-1.34 (6H, m, $OC_2H_4C_3H_6CH_3$), 1.65-1.72 (2H, m, $OCH_2CH_2C_4H_9$), 4.24 (2H, t, J=6.9 Hz, $OCH_2C_5H_{11}$), 6.73 (1H, d, J=8.3 Hz, H-5), 7.98 (1H, dd, J=8.3 and 2.0 Hz, H-4), 8.10 (2H, s, OH), 8.49 (1H, d, J=2.0 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 109.7, 65.3, 31.1, 28.5, 25.2, 22.1, 13.9. LC-MS (ESI): t_R = 7.20 min; $[M+H]^+$ 224.56.

6-Heptyloxypyridine-3-boronic acid (3e). Starting from **2e** (1.0 g, 3.67 mmol) using general procedure B (pH 6), **3e** was obtained as a white solid. Yield 61%. Mp 97-99°C. IR (KBr): ν (cm^{-1}) 3393, 2951, 2921, 2852, 1612, 1334, 1302. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.85 (3H, t, J=6.7 Hz, CH_3), 1.33-1.36 (8H, m, $OC_2H_4C_4H_8CH_3$), 1.64-1.71 (2H, m, $OCH_2CH_2C_5H_{11}$), 4.23 (2H, t, J=6.7 Hz, $OCH_2C_6H_{13}$), 6.72 (1H, d, J=8.3 Hz, H-5), 7.97 (1H, dd, J=8.3 and 1.9 Hz, H-4), 8.10 (2H, s, OH), 8.47 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 109.7, 65.3, 31.3, 28.53, 28.49, 25.5, 22.1, 14.0. LC-MS (ESI): t_R = 7.82 min; $[M+H]^+$ 238.57.

6-Dodecanyloxypyridine-3-boronic acid (3f) [65]. Starting from **2f** (1.0 g, 2.92 mmol), we used general procedure B and treatment proceeded as follows: after cooling down to room temperature, the mixture was acidified to pH 5 by dropwise addition of acetic acid. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. Recristallisation of the crude product from diethyl ether gave product **3f** as a white solid. Yield 58%. Mp 98-100°C. IR (KBr): ν (cm^{-1}) 3400, 2918, 2850, 1611, 1336, 1303. ^{1}H NMR (400 MHz, DMSO-d_6): δ 0.84 (3H, t, J=6.6 Hz, CH_3), 1.23 (18H, bs, $OC_2H_4C_9H_{18}CH_3$), 1.63-1.70 (2H, m, $OCH_2CH_2C_{10}H_{21}$), 4.23 (2H, t, J=6.6 Hz, $OCH_2C_{11}H_{23}$), 6.71 (1H, d, J=8.8 Hz, H-5), 7.972 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.10 (2H, s, OH), 8.47 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 109.7, 65.3, 31.3, 29.04, 29.01, 29.0 (2 C), 28.8, 28.7, 25.5, 22.1, 14.0. LC-MS (ESI): t_R = 9.62 min; $[M+H]^+$ 308.68.

6-Phenoxypyridine-3-boronic acid (3g). Starting from **2g** (1.0 g, 4.00 mmol) using general procedure B (pH 5), **3g** was obtained as a white solid. Yield 65%. Mp 147-149°C. IR (KBr): ν (cm^{-1}) 3418, 1586, 1242, 757, 690. ^{1}H NMR (400 MHz, DMSO-d_6): δ 6.95 (1H, d, J=8.1 Hz, H-5), 7.11 (2H, m, H-2'), 7.21 (1H, m, H-4'), 7.41 (2H, m, H-3'), 8.13 (1H, d, J=8.1 Hz, H-4), 8.24 (2H, s, OH), 8.45 (1H, s, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.4, 153.7, 153.5, 145.6, 129.7, 124.6, 121.3, 110.4. LC-MS (ESI): t_R = 6.48 min; $[M+H]^+$ 216.48.

6-(2-Phenylethoxy)pyridine-3-boronic acid (3h). Starting from **2h** (1.0 g, 3.60 mmol) using general procedure B (pH 5), **3h** was obtained as a white solid. Yield 79%. Mp 111-113°C. IR

(KBr): ν (cm^{-1}) 3400, 1610, 1302, 1013, 696. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.01 (2H, t, J=6.8 Hz, OCH_2CH_2), 4.46 (2H, t, J=6.8 Hz, OCH_2CH_2), 6.72 (1H, d, J=8.8 Hz, H-5), 7.18-7.30 (5H, m, H-Ph), 7.98 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.12 (2H, s, OH), 8.49 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.5, 153.3, 144.6, 138.5, 128.9, 128.3, 126.3, 109.8, 65.9, 34.7. LC-MS (ESI): t_R = 5.84 min; $[M+H]^+$ 243.13.

6-(3-Phenylpropoxy)pyridine-3-boronic acid (3i). Starting from **2i** (1.0 g, 3.42 mmol) using general procedure B (pH 5), **3i** was obtained as a white solid. Yield 76%. Mp 98-100°C. IR (KBr): ν (cm^{-1}) 3418, 1606, 1329, 1285, 1023, 697. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.97-2.04 (2H, m, $OCH_2CH_2CH_2$), 2.71 (2H, t, J=6.8 Hz, $OC_2H_4CH_2$), 4.23 (2H, t, J=6.8 Hz, $OCH_2C_2H_4$), 6.75 (1H, d, J=8.8 Hz, H-5), 7.17-7.29 (5h, m, H-Ph), 7.99 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.12 (2H, s, OH), 8.47 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.6, 153.3, 144.5, 141.4, 128.4, 128.3, 125.9, 109.8, 64.7, 31.6, 30.2. LC-MS (ESI): t_R = 6.08 min; $[M+H]^+$ 257.21.

6-(4-Phenylbutoxy)pyridine-3-boronic acid (3j). Starting from **2j** (1.0 g, 3.27 mmol) using general procedure B (pH 5), **3j** was obtained as a white solid. Yield 72%. Mp 95-97°C. IR (KBr): ν (cm^{-1}) 3401, 1607, 1334, 1300, 1017. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.66-1.71 (4H, m, $OCH_2C_2H_4CH_2$), 2.61 (2H, t, J=6.8 Hz, $OC_3H_6CH_2$), 4.26 (2H, t, J=6.8 Hz, $OCH_2C_3H_6$), 6.72 (1H, d, J=8.8 Hz, H-5), 7.14-7.280 (5H, m, H-Ph), 7.97 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.11 (2H, s, OH), 8.47 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.5, 142.0, 128.3, 128.2, 125.7, 109.7, 65.1, 34.8, 28.2, 27.5. LC-MS (ESI): t_R = 6.42 min; $[M+H]^+$ 271.20.

6-(5-Phenylpentoxy)pyridine-3-boronic acid (3k). Starting from **2k** (1.0 g, 3.12 mmol) using general procedure B (pH 5), **3k** was obtained as a white solid. Yield 69%. Mp 164-166°C. IR (KBr): ν (cm^{-1}) 3400, 2927, 1609, 1334, 1300, 1016, 737, 639. ^{1}H NMR (500 MHz, DMSO-d_6): δ 1.40-1.44 (2H, m, $OC_2H_4CH_2C_2H_4$), 1.59-1.65 (2H, m, $OC_3H_6CH_2CH_2$), 1.71-1.75 (2H, m, $OCH_2CH_2C_3H_6$), 2.59 (2H, t, J=7.1 Hz, $OC_4H_8CH_2$), 4.25 (2H, t, J=7.1 Hz, $OCH_2C_4H_8$), 6.73 (1H, d, J=8.3 Hz, H-5), 7.15-7.29 (5H, m, H-Ph), 7.99 (1H, dd, J=8.3 and 2.0 Hz, H-4), 8.12 (2H, s, OH),

8.49 (1H, d, J=2.0 Hz, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 164.6, 153.3, 144.4, 142.2, 128.3, 128.2, 125.6, 109.7, 65.2, 35.1, 30.8, 28.3, 25.2. LC-MS (ESI): t_R = 7.70 min; $[M+H]^+$ 286.61.

6-(6-Phenylhexyloxy)pyridine-3-boronic acid (3l). Starting from **2l** (1.0 g, 2.99 mmol) using general procedure B (pH 5), **3l** was obtained as a white solid. Yield 73%. Mp 86-88°C. IR (KBr): ν (cm^{-1}) 3413, 2928, 1607, 1334, 1300, 1017. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.31-1.44 (4H, m, $OC_2H_4C_2H_4C_2H_4$), 1.52-1.58 (2H, m, $OC_4H_8CH_2CH_2$), 1.60-1.69 (2H, m, $OCH_2CH_2C_4H_8$), 2.55 (2H, t, J=7.1 Hz, $OC_5H_{10}CH_2$), 4.22 (2H, t, J=7.1 Hz, $OCH_2C_5H_{10}$), 6.71 (1H, d, J=8.6 Hz, H-5), 7.12-7.26 (5H, m, H-Ph), 7.97 (1H, dd, J=8.6 and 1.9 Hz, H-4), 8.16 (2H, s, OH), 8.47 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.4, 144.5, 142.3, 128.3, 125.7, 109.8, 65.3, 65.2, 35.11, 35.10, 31.0, 28.4, 25.4. LC-MS (ESI): t_R = 8.05 min; $[M+H]^+$ 300.62.

6-(7-Phenylheptyloxy)pyridine-3-boronic acid (3m). Starting from **2m** (1.0 g, 2.87 mmol) using general procedure B (pH 5), **3m** was obtained as a white solid. Yield 64%. Mp 88-90°C. IR (KBr): ν (cm^{-1}) 3390, 2924, 1610, 1334, 1302, 1017, 745, 695, 647. ^{1}H NMR (400 MHz, DMSO-d_6): δ 1.30-1.36 (6H, m, $OC_2H_4C_3H_6C_2H_4$), 1.52-1.57 (2H, m, $OC_5H_{10}CH_2CH_2$), 1.64-1.69 (2H, m, $OCH_2CH_2C_5H_{10}$), 2.54 (2H, t, J=7.1 Hz, $OC_6H_{12}CH_2$), 4.22 (2H, t, J=7.1 Hz, $OCH_2C_6H_{12}$), 6.71 (1H, d, J=8.6 Hz, H-5), 7.12-7.26 (5H, m, H-Ph), 7.97 (1H, d, J=8.5 Hz, H-4), 8.10 (2H, s, OH), 8.48 (1H, s, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.7, 153.3, 144.49, 144.47, 142.3, 128.2, 125.6, 109.7, 65.2, 35.14, 35.1, 31.0, 28.6, 28.5, 25.5. LC-MS (ESI): t_R = 8.42 min; $[M+H]^+$ 314.64.

6-(4-Chlorobenzyloxy)pyridine-3-boronic acid (3n). To a solution of **2n** (1.0 g, 3.35 mmol) and trimethyl borate (0.93 mL, 8.37 mmol) in anhydrous THF (80 mL) cooled at -78°C was added dropwise a 2.5M solution of *n*-BuLi (3.35 mL, 8.37 mmol). The mixture was allowed to react at this temperature for over 60 min. After cooling down to room temperature, the mixture was quenched by slow addition of 4% aqueous NaOH solution (50 mL). The resulting aqueous layer was collected and acidified to pH 5 by dropwise addition of 3N HCl and then extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. Recrystallisation of the crude product from diethyl ether gave product **3n** as a white solid. Yield 60%. Mp 120-122°C.

IR (KBr): ν (cm^{-1}) 3429, 1610, 1497, 1334, 1297, 1015, 809, 639. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.35 (2H, s, OCH_2), 6.82 (1H, d, J=8.8 Hz, H-5), 7.41-7.47 (4H, m, H-2', H-3'), 8.02 (1H, dd, J=8.8 and 1.9 Hz, H-4), 8.15 (2H, s, OH), 8.50 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.1, 153.2, 144.8, 136.4, 132.3, 129.7, 128.3, 109.9, 65.9. LC-MS (ESI): t_R = 7.42 min; $[M+H]^+$ 264.50, 266.51.

6-(4-Fluorobenzyloxy)pyridine-3-boronic acid (3o). Following the procedure for **3n** synthesis, **2o** (1.0 g, 3.54 mmol) was reacted with *n*-BuLi (3.54 mL, 8.86 mmol) and trimethyl borate (0.99 mL, 8.86 mmol). The mixture was then treated as described in representative procedure (pH 5) to give compound **21o** as a white solid. Yield 48%. Mp 139-141°C. IR (KBr): ν (cm^{-1}) 3433, 1647, 1510, 1356, 1224. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.33 (2H, s, OCH_2), 6.81 (1H, d, J=7.8 Hz, H-5), 7.17-7.21 (2H, m, H-3'), 7.47-7.51 (2H, m, H-2'), 8.02 (1H, dd, J=7.8 and 1.9 Hz, H-4), 8.14 (2H, s, OH), 8.51 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.4, 160.4, 153.2, 144.7, 133.6, 130.3, 130.2, 115.3, 115.1, 109.9, 66.1. LC-MS (ESI): t_R = 5.94 min; $[M+H]^+$ 248.16.

6-(3,4-Dichlorobenzyloxy)pyridine-3-boronic acid (3p). Starting from **2p** (1.0 g, 3.00 mmol), we used general procedure B and treatment proceeded as follows: after cooling down to room temperature, the mixture was quenched by slow addition of 4% aqueous NaOH solution (50 mL). The resulting aqueous layer was collected and acidified to pH 5 by dropwise addition of 3N HCl and then extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. Recristallisation of the crude product from diethyl ether gave **3p** as a white solid. Yield 58%. Mp 131-133°C. IR (KBr): ν (cm^{-1}) 3435, 1598, 1408, 1320, 1285, 1127, 1030, 769. ^{1}H NMR (500 MHz, DMSO-d_6): δ 5.37 (2H, s, OCH_2), 6.86 (1H, d, J=8.3 Hz, H-5), 7.44 (1H, dd, J=8.3 and 1.9 Hz, H-6'), 7.64 (1H, d, J=8.3 Hz, H-5'), 7.71 (1H, d, J=1.9 Hz, H-2'), 8.04 (1H, dd, J=8.3 and 1.9 Hz, H-4), 8.17 (2H, s, OH), 8.51 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.9, 153.1, 144.9, 138.7, 131.0, 130.6, 130.2, 129.7, 128.0, 110.0, 65.3. LC-MS (ESI): t_R = 6.13 min; $[M+H]^+$ 298.46, 300.45, 302.45.

6-(3,4,5-Trimethoxybenzyloxy)pyridine-3-boronic acid (3q). Starting from **2q** (1.0 g, 2.82 mmol) using general procedure B (pH 5), **3q** was obtained as a white solid. Yield 66%. Mp 129-131°C. IR (KBr): ν (cm^{-1}) 3501, 1606, 1347, 1295, 1240, 1131, 1000, 653. ^{1}H NMR (400 MHz, DMSO-d_6): δ 3.63 (3H, s, *p*-OCH_3), 3.75 (6H, s, *m*-OCH_3), 5.26 (2H, s, OCH_2), 6.77 (2H, s, H-2'), 6.82 (1H, d, J=8.8 Hz, H-5), 8.02 (1H, dd, J=7.8 and 1.9 Hz, H-4), 8.15 (2H, s, OH), 8.52 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 153.2, 152.8, 144.7, 137.0, 132.7, 110.0, 105.5, 67.2, 60.0, 55.9. LC-MS (ESI): t_R = 5.37 min; $[M+H]^+$ 318.15.

6-(1,3-Benzodioxol-5-ylmethoxy)pyridine-3-boronic acid (3r). Starting from **2r** (1.0 g, 3.25 mmol) using general procedure B (pH 5), **3r** was obtained as a beige solid. Yield 47%. Mp 110-112°C. IR (KBr): ν (cm^{-1}) 3429, 1609, 1330, 1299, 1250, 1041. ^{1}H NMR (400 MHz, DMSO-d_6): δ 5.23 (2H, s, OCH_2), 6.00 (2H, s, OCH_2O), 6.78 (1H, d, J=8.8 Hz, H-5), 6.91 (2H, m, H-5', H-6'), 7.00 (1H, s, H-2'), 8.00 (1H, d, J=8.8 Hz, H-4), 8.14 (2H, s, OH), 8.51 (1H, s, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 164.3, 153.2, 147.2, 146.9, 144.6, 131.0, 121.9, 109.9, 108.8, 108.1, 101.0, 66.7. LC-MS (ESI): t_R = 7.07 min; $[M+H]^+$ 272.51.

6-Benzylsulfanylpyridine-3-boronic acid (3s). Starting from **2s** (1.0 g, 3.57 mmol) using general procedure B (pH 5), **3s** was obtained as a white solid. Yield 68%. Mp 149-151°C. IR (KBr): ν (cm^{-1}) 3429, 1583, 1420, 1327, 1263, 1141, 697. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.42 (2H, s, SCH_2), 7.13-7.41 (6H, m, H-Ph, H-5), 7.92 (1H, dd, J=7.8 and 1.9 Hz, H-4), 8.26 (2H, bs, OH), 8.74 (1H, s, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.9, 154.7, 141.8, 138.0, 128.9, 128.4, 127.0, 120.7, 33.0. LC-MS (ESI): t_R = 7.13 min; $[M+H]^+$ 246.50.

6-(2,4-Dichlorophenoxy)pyridine-3-boronic acid (3t). Starting from **2t** (1.0 g, 3.14 mmol), we used general procedure B and treatment proceeded as follows: after cooling down to room temperature, the mixture was quenched by slow addition of 4% aqueous NaOH solution (50 mL). The resulting aqueous layer was collected and acidified to pH 4.5 by dropwise addition of 3N HCl and then extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. Recristallisation of the crude product from diethyl ether gave **3t** as a white

solid. Yield 72%. Mp 196-198°C. IR (KBr): ν (cm^{-1}) 3435, 1599, 1471, 1420, 1340, 1248, 1097. ^{1}H NMR (500 MHz, DMSO-d_6): δ 7.10 (1H, d, J=8.3 Hz, H-5), 7.37 (1H, d, J=8.7 Hz, H-6'), 7.49 (1H, dd, J=8.7 and 2.5 Hz, H-5'), 7.77 (1H, d, J=2.5 Hz, H-3'), 8.18 (1H, dd, J=8.3 and 1.9 Hz, H-4), 8.27 (2H, s, OH), 8.41 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 163.4, 153.1, 148.3, 145.9, 129.8, 129.7, 128.5, 127.5, 125.7, 109.9. LC-MS (ESI): t_R = 7.50 min; $[M+H]^+$ 284.45, 286.45, 288.43.

2-Benzylamino-5-bromopyridine (4). To a solution of benzylamine (2.79 mL, 25.57 mmol) in anhydrous DMF (100 mL) was added K_2CO_3 (3.53 g, 25.57 mmol) and 5-bromo-2-fluoropyridine (3.0 g, 17.05 mmol). The reaction was heated to reflux for 12 h. After cooling down to room temperature, the mixture was poured in water and extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel chromatography (cyclohexane/AcOEt 9:1) affording **4** as a beige solid. Yield 50%. Mp 124-126°C. IR (KBr): ν (cm^{-1}) 3227, 1591, 1520, 1451, 1393, 810, 746, 699, 523. ^{1}H NMR (400 MHz, $CDCl_3$): δ 4.48 (2H, d, J=5.8 Hz, NH*CH*$_2$), 4.63 (1H, bs, NH), 6.28 (1H, d, J=8.8 Hz, H-3), 7.28-7.35 (5H, m, H-Ph), 7.46 (1H, dd, J=8.8 and 2.9 Hz, H-4), 8.12 (1H, d, J=2.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 157.1, 148.7, 139.8, 138.6, 128.7, 127.4, 127.3, 108.3, 107.3, 46.3.

5-Bromo-2-(*N*-*tert*-butyloxycarbonyl)benzylaminopyridine (5). To a solution of **4** (1.0 g, 3.80 mmol) in CH_2Cl_2 (50 mL) was added di-*tert*-butyl dicarbonate (1.0 g, 4.56 mmol), Et_3N (0.64 mL, 4.56 mmol) and DMAP (cat.). The reaction was heated to reflux for 12 h. After cooling down to room temperature, the mixture was concentrated to dryness, diluted with water and extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel chromatography (cyclohexane/AcOEt 95:5) affording **5** as a white solid. Yield 43%. Mp 87-89°C. IR (KBr): ν (cm^{-1}) 1694, 1468, 1393, 1165, 701. ^{1}H NMR (400 MHz, $CDCl_3$): δ 1.43 (9H, s, Boc), 5.17 (2H, s, NBoc*CH*$_2$), 7.19-7.29 (5H, m, H-Ph), 7.66-7.72 (2H, m, H-3, H-4), 8.39 (1H, d, J=2.2 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 154.0, 153.1, 148.3, 139.4, 139.1, 128.2, 127.1, 126.8, 120.4, 114.8, 81.8, 49.8, 28.1.

6-Benzylaminopyridine-3-boronic acid (7). Following the procedure for **3n** synthesis, **5** (1.0 g, 2.75 mmol) was reacted with *n*-BuLi (1.38 mL, 3.44 mmol) and triisopropyl borate (0.79 mL, 3.44 mmol) in THF (80 mL). After cooling down to room temperature, the mixture was quenched by slow addition of 4% aqueous NaOH solution (50 mL). The resulting aqueous layer was collected and acidified to pH 5 by dropwise addition of 3N HCl and then extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. The residue was then heated to reflux for 12 h in a solution of ethanol/HCl 3N 1:1 (30 mL). After cooling down to room temperature, the mixture was neutralized to pH 7 by dropwise addition of 4% aqueous NaOH solution and the aqueous phase was extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. Recristallisation of the crude product from diethyl ether gave **7** as a white solid. Yield 59%. Mp 141-143°C. IR (KBr): ν (cm^{-1}) 3414, 1602, 1317, 1255, 396. ^{1}H NMR (400 MHz, DMSO-d_6): δ 4.48 (2H, d, J=6.1 Hz, NHCH_2), 6.43 (1H, d, J=8.3 Hz, H-5), 7.18-7.30 (5H, m, H-Ph), 7.66 (1H, dd, J=8.3 and 1.9 Hz, H-4), 7.73 (2H, s, OH), 8.33 (1H, d, J=1.9 Hz, H-2). ^{13}C NMR (100 MHz, DMSO-d_6): δ 159.6, 154.4, 142.3, 140.5, 128.2, 127.2, 126.6, 107.2, 43.9. LC-MS (ESI): t_R = 4.67 min; $[M+H]^+$ 229.53.

2-Benzyloxy-5-bromo-3-methylpyridine (8). To a solution of benzyl alcohol (3.09 mL, 29.89 mmol) in anhydrous THF (100 mL) was slowly added 60% sodium hydride (1.59 g, 39.86 mmol). The mixture was allowed to react at room temperature for 30 min then it was heated to reflux for 1 h. 2,5-dibromo-3-methylpyridine (5.0 g, 19.93 mmol) was added and the reaction was continued for 12 h. After cooling down to room temperature, the mixture was poured into water and extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel chromatography (cyclohexane/AcOEt 98:2) affording **8** as a white solid. Yield 95%. Mp 54-56°C. IR (KBr): ν (cm^{-1}) 1423, 1251, 1167, 1021, 729. ^{1}H NMR (400 MHz, $CDCl_3$): δ 2.22 (3H, s, CH_3), 5.38 (2H, s, OCH_2), 7.30-7.47 (5H, m, H-Ph), 7.52 (1H, d, J=1.9 Hz, H-4), 8.05 (1H, d, J=1.9 Hz, H-6). ^{13}C NMR (100 MHz, $CDCl_3$): δ 160.7, 144.4, 140.8, 137.3, 128.4, 127.7, 127.6, 123.1, 111.6, 67.7, 15.8.

6-Benzyloxy-5-methylpyridine-3-boronic acid (9). Starting from **8** (1.0 g, 3.60 mmol) using general procedure B (pH 5), **9** was obtained as a white solid. Yield 86%. Mp 67-69°C. IR (KBr): ν (cm^{-1}) 3324, 1606, 1360, 1316, 1261, 1161, 1027, 766, 726. ^{1}H NMR (500 MHz, DMSO-d_6): δ 2.18 (3H, s, CH_3), 5.40 (2H, s, OCH_2), 7.31 (1H, t, J=7.5 Hz, H-4'), 7.39 (2H, t, J=7.5 Hz, H-3'), 7.45 (2H, d, J=7.5 Hz, H-2'), 7.86 (1H, d, J=0.8 Hz, H-4), 8.10 (2H, s, OH), 8.36 (1H, d, J=0.8 Hz, H-2). ^{13}C NMR (125 MHz, DMSO-d_6): δ 162.5, 150.4, 144.4, 137.6, 128.3, 127.5, 127.4, 118.7, 66.5, 15.5. LC-MS (ESI): t_R = 7.56 min; $[M+H]^+$ 244.54.

3-Bromo-5-benzyloxypyridine (10) [66] To a solution of benzyl alcohol (1.04g, 9.66 mmol) in anhydrous DMF (80 mL) was slowly added 60% NaH (0.58 g, 14.49 mmol). The mixture was allowed to react at room temperature for 30 min then it was heated to reflux for 1 h. 3,5-dibromopyridine (2.41 g, 10.17 mmol) was added and the reaction was continued for 12 h. After cooling down to room temperature, the mixture was poured in water and extracted with ethyl acetate. The organic phase was washed with brine, dried on $MgSO_4$, filtered and evaporated. The crude product was purified by silica gel chromatography (cyclohexane/AcOEt 95:5) affording **10** as a white solid. Yield 26%. Mp 66-68°C. ^{1}H NMR (400 MHz, $CDCl_3$): δ 5.06 (2H, s, OCH_2), 7.36-7.44 (6H, m, H-4, H-Ph), 8.30 (1H, d, J=2.3 Hz, H-6), 8.31 (1H, d, J=2.3 Hz, H-2). ^{13}C NMR (100 MHz, $CDCl_3$): δ 155.5, 143.5, 137.0, 135.7, 129.1, 128.8, 127.9, 124.7, 120.7, 71.0.

5-Benzyloxypyridine-3-boronic acid (11). Starting from **10** (1.0 g, 3.79 mmol) using general procedure B (pH 5), **11** was obtained as a white solid. Yield 61%. Mp 163-165°C. IR (KBr): ν (cm^{-1}) 3446, 1424, 1321, 1047, 723. ^{1}H NMR (500 MHz, CD_3OD+NaOD): δ 5.22 (2H, s, OCH_2), 7.32-7.47 (5H, m, H-Ph), 7.91 (1H, s, H-4), 8.19-8.22 (2H, m, H-2, H-6). ^{13}C NMR (125 MHz, CD_3OD+NaOD): δ 156.1, 147.3, 138.5, 134.8, 129.5, 129.0, 128.7, 128.4, 71.1. LC-MS (ESI): t_R = 4.38 min; $[M+H]^+$ 230.49.

Microbiological assays. *Bacterial strains. S. aureus* ATCC 25923 was purchased from the Institut Pasteur (CRBIP, Paris, France). The overproducing NorA mutant SA-1199B was generously

provided by G.W. Kaatz (University of Michigan). All strains were grown at 37°C in Mueller-Hinton broth (Bio-Rad, Mitry Mory, France) or spread on Mueller-Hinton agar plates for counting. Colony forming unit (CFU) monitoring was carried out by counting colonies present in 2 x 10 µl of a serial log dilutions of bacteria inoculum spotted on MH agar plates. Plates were examined for growth after one night at 37°C.

Media, antibiotics and culture condition. Mueller-Hinton broth (MH, Bio Rad) was used for all bacteria overnight cultures, susceptibility testing and ethidium bromide accumulation experiments. Ciprofloxacin was obtained from Sigma Aldrich (Saint Quentin Fallavier, France). Reserpine was purchased to Alfa Aesar (Schiltigheim, France). Stock solutions (ciprofloxacin and EtBr) were prepared in sterile water except for reserpine dissolved in dimethyl sulfoxide (DMSO).

MIC determination. The inhibitory potential of the boronic derivatives was tested through the determination of minimum inhibitory concentration (MIC) of these complexes against *S. aureus* ATCC25923 and *S. aureus* 1199B overexpressing the efflux pump. The bacterial inoculum was prepared from a frozen culture incubated in 5 mL of Muller-Hinton (MH) broth medium at 37°C for 18 hours. Cells were inoculated into MH broth and dispensed at 200 µL/well in 96 well microtiters plates (bacterial concentration in wells106 CFU/mL). Tested compounds, solubilised at 10 mg/mL in DMSO, were then added using a Biomek 2000 handling robot using two fold serial dilutions, the highest being 128 mg/L for fully soluble compounds. In case the tested compounds were poorly soluble in the culture medium, they were tested at the highest concentration ensuring complete solubilisation. The highest tested concentration was at least 16 mg/L. The MIC was defined as the concentration that completely inhibited cell growth after incubation at 37°C during 24 hours. Growth was assayed with a microplate reader by monitoring absorption at 620 nm. In addition, the plates were read visually. All experiments were performed in duplicate. The highest final DMSO concentration used (2.56 % v/v) induced no significant bacterial growth inhibition. MIC determination of ciprofloxacin (8 mg/L for SA-1199B) and ampicilline (0.25 mg/mL for SA-

ATCC25923) was performed as a control on microplate when necessary. The accepted variance on MIC value can be estimated to a 2-fold difference due to the microdilution method used.

Ethidium bromide accumulation assays. The accumulation of ethidium bromide by SA cells with or without inhibitors was determined by fluorimetry. SA strains were grown overnight in MH broth, diluted 20-fold in fresh, pre-warmed MH broth and were grown in a shaking water bath at 37°C under agitation (130 rpm) during 3.5 hours which corresponds to the mid-log phase of growth. 10 mL samples were aseptically withdrawn from the culture, bacteria were harvested by centrifugation (4000g, 10 min), supernatant was thrown and bacterial cells were resuspended in 20 mL fresh sterile pre-warmed MH broth containing EtBr (final concentration, 10 mg/L) and incubated again in a shaking water bath at 37°C under agitation (130 rpm) during 30 min. Aliquots (1 ml) were taken at fixed intervals (0, 5, 10, 15, 20, 25 and 30 minutes) for fluorescence measurement (λ_{ex}= 530nm, λ_{em} = 600nm). After 10 minutes, tested compounds were added (final concentration 20 mg/L) and fluorescence was measured for an additional 20 min. The fluorescence intensity difference between the compound containing assay and the fluorescence baseline before addition was indicative of the ability of the compound to inhibit the efflux of EtBr. For each experiment, it was checked that the fluorescence remained constant during the experiment in the absence of tested compound. Reserpine was used as a positive control.

Cell culture and cell proliferation assays. KB cells were provided by the NCI and grown in D-MEM supplemented with 10% FCS, penicillin-streptomycin, fungizone and glutamine at 37°C under 5% CO_2. Cells were plated in 96-well tissue culture plates in 200 µl medium and treated 24h later with 2 µl stock solution of compounds dissolved in DMSO using a Biomek 3000 (Beckman-Coulter). Controls received the same volume of DMSO (1% final volume). After 72h exposure, MTS reagent (Promega) was added and incubated for 3h at 37°C: the absorbance was monitored at

490nm and results expressed as the inhibition of cell proliferation calculated as the ratio [(1-(OD490 treated/OD490 control))×100] in triplicate experiments.

Inhibition of the P-gp efflux pump. A functional cellular assay was based on the inhibition of rhodamine 123 efflux from human cancer cells overexpressing ABC B1 (P-gp, mdr1). NCI/ADR-Res cells were seeded at 60,000 cells/well in 96w-microplates in complete RPMI medium. 24h later the medium was removed, cells were washed twice with PBS before the addition of the compound and 12.5µM rhodamine 123 dissolved in culture medium without FCS. Cells were incubated for one hour at 37°C under 5% CO_2. The medium was eliminated, cells washed three time with PBS and lysated by 100µl 20mM Tris pH 7.7, 0.2% SDS under gentle agitation. Fluorescence was monitored in a microplate reader set 507/528nm (exc/em). Cyclosporine A was used as positive control.

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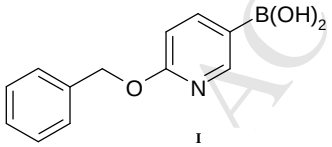
Abbreviations used: a.u., arbitrary unit; Boc, *tert*-butoxycarbonyl; *n*-BuLi, *n*-Butyllithium; EPI, Efflux Pump Inhibitor; EtBr, Ethidium Bromide; MFS, Major Facilitator Superfamily; MIC, minimun inhibitory concentration; MMC, minimum modulatory concentration; MRSA, Methicillin-resistant *Staphylococcus aureus*; P-gp, P-glycoprotein.

GRAPHICAL ABSTRACT



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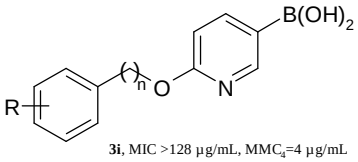
MMC_4=16 µg/mL



3i, MIC >128 µg/mL, MMC_4=4 µg/mL
3j, MIC=32µg/mL, MMC_4=4 µg/mL

ACKNOWLEDGEMENTS

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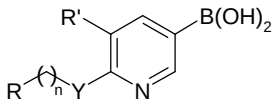
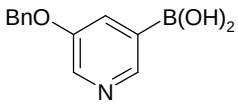
Table 1. Evaluation of antibacterial activity and cytotoxicity of boronic acids **3a-t**, **7**, **9** and **11**.



3a-t, **7**, **9**



11

					Activity against Se[a] and Rt[b] *S. aureus*							pKa[d]		
Id.	R	n	Y	R'	Se MIC (µg/mL)	Rt MIC (µg/mL)	Rt MMC_2 (µg/mL)	Rt MMC_4 (µg/mL)	Rt MMC_4 (µM)	Cyt.[c]	log P[d]	N	OH	OH
3a	Me	2	O	H	>128	>128	4	>128	-	0.0	1.58	1.70	8.53	10.76
3b	Me	3	O	H	>128	>128	2	64	328.17	1.0	1.98	1.70	8.53	10.76
3c	Me	4	O	H	>128	>128	2	64	306.15	16.0	2.38	1.70	8.53	10.76
3d	Me	5	O	H	N.D.	>128	2	16	71.72	46.0	2.77	1.70	8.53	10.76
3e	Me	6	O	H	>16	>16	0.5	8	33.74	73.0	3.17	1.70	8.53	10.76
3f	Me	11	O	H	>16	>16	>16	>16	-	13.0	5.15	1.70	8.53	10.76
3g	Ph	0	O	H	>128	>128	2	64	297.54	14.0	2.46	0.73	8.48	10.71
I	Ph	1	O	H	>128	>128	1	16	69.85	2.0	2.55	1.65	8.52	10.76
3h	Ph	2	O	H	>128	>128	1	16	65.82	28.0	2.80	1.69	8.53	10.76
3i	Ph	3	O	H	>128	>128	0.5	4	15.56	83.0	3.20	1.70	8.53	10.76
3j	Ph	4	O	H	64-128	32	0.25	4	14.75	13.0	3.59	1.70	8.53	10.76
3k	Ph	5	O	H	N.D.	16	2	4	14.03	60.0	3.99	1.70	8.53	10.76
3l	Ph	6	O	H	>16	>16	0.5	4	13.37	46.0	4.39	1.70	8.53	10.76
3m	Ph	7	O	H	>16	>16	0.5	4	12.77	62.0	4.78	1.70	8.53	10.76
3n	4-Cl-Ph	1	O	H	≥128	128	1	32	121.45	33.0	3.07	1.65	8.52	10.76
3o	4-F-Ph	1	O	H	>128	>128	2	32	129.54	8.0	2.69	1.65	8.52	10.76
3p	3,4-Cl-Ph	1	O	H	N.D.	16	0.125	1	3.35	44.0	3.59	1.65	8.52	10.76
3q	3,4,5-OMe-Ph	1	O	H	>128	>128	8	N.D.	-	3.0	1.79	1.65	8.52	10.76
3r	1,3-benzodioxol	1	O	H	>128	>128	64	N.D.	-	0.0	2.23	1.65	8.52	10.76
3s	Ph	1	S	H	64-128	64	0.5	16	65.28	8.0	3.35	2.26	8.42	10.63
3t	2,4-Cl-Ph	0	O	H	N.D.	128	1	16	56.36	1.0	3.49	0.69	8.48	10.71
7	Ph	1	NH	H	>128	>128	32	128	561.28	0.0	2.38	5.42	8.74	11.01
9	Ph	1	O	Me	N.D.	>128	4	16	65.82	15.0	3.02	2.19	8.55	10.79
11					>128	>128	8	>128	-	0.0	1.85	3.51	8.28	10.47
	Ampicillin				0.25									
	Ciprofloxacin					8								
	Reserpine							4-8	6.57-13.14					

[a]Se: susceptible *S. aureus* ATCC25923. [b]Rt : resistant *S. aureus* 1199B (NorA).

[c]Cytotoxicity as % of inhibition of cellular growth of KB cells in the presence of 10µM of the tested compound. [d]Calculated values [66]. N.D.: No Data.

Table 2. Evaluation of antibacterial activity in combination with norfloxacin.

Antibacterial activity against SA 1199B + Nor 16 µg/mL					
Id.	MMC_4 (µg/mL)	Id.	MMC_4 (µg/mL)	Id.	MMC_4 (µg/mL)
3a	> 128	**3g**	128	**3n**	16
3b	64	**I**	32	**3o**	64
3c	64	**3h**	16	**3s**	16
3d	16	**3i**	8	**3t**	32
3e	8	**3j**	8	**9**	8

Figure 1. Previous SAR studies for boronic species of the primary screening [50].

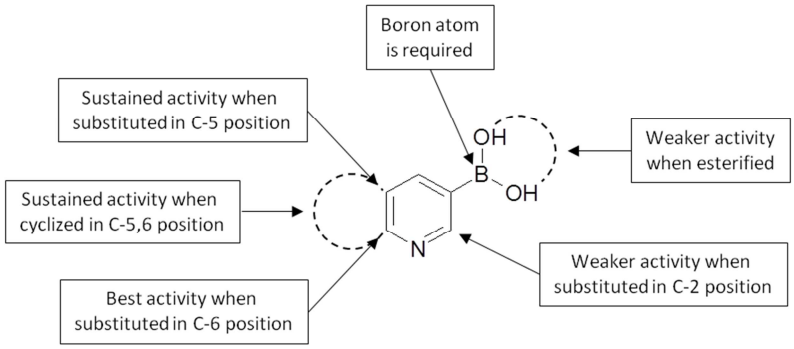


Figure 2. Considered pharmacomodulations on hit compound **I**.

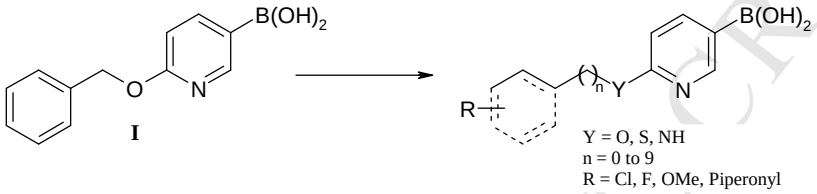


Figure 3. Effect of compounds **3i** (■) and **3j** (▲) on EtBr accumulation of SA-1199B. The tested compound was added after 10 min (⬇) of bacterial incubation with EtBr. The positive control was reserpine at 5 (◊) and 20 (♦) µg/m and the negative control (●) was the bacteria in the presence of 1.6% DMSO. Vertical bars are the mean ± standard deviations for two or three independent experiments.

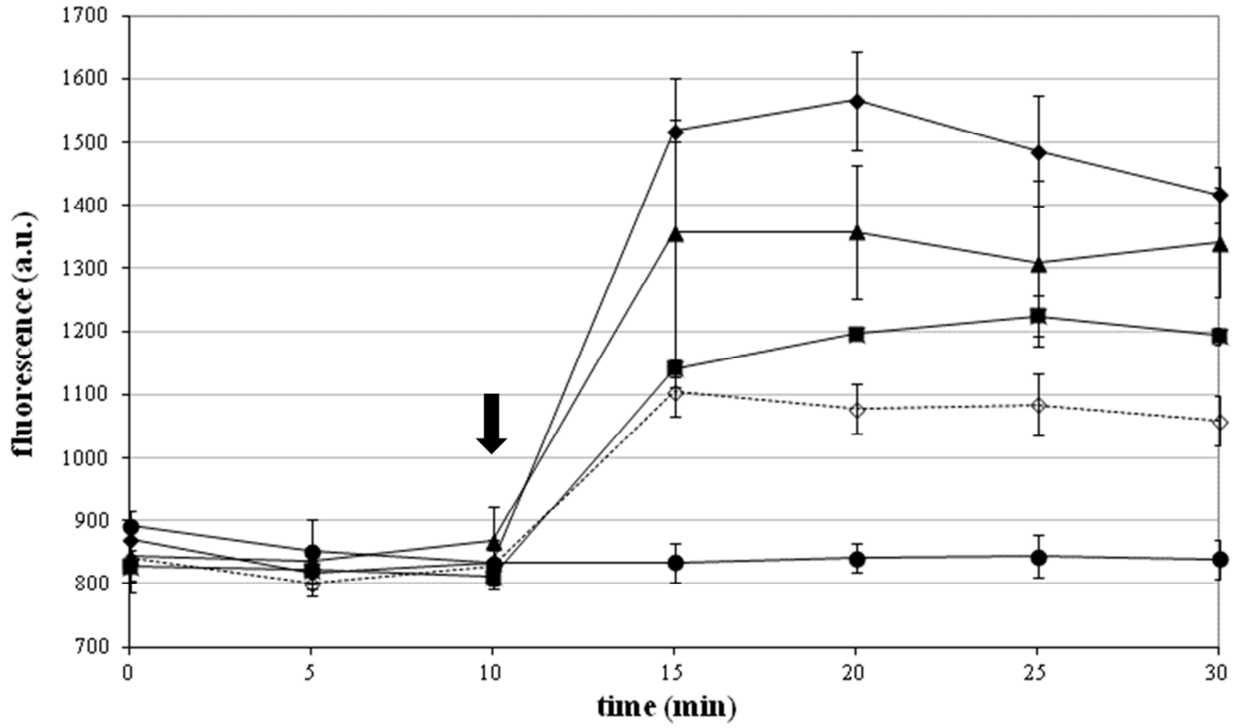
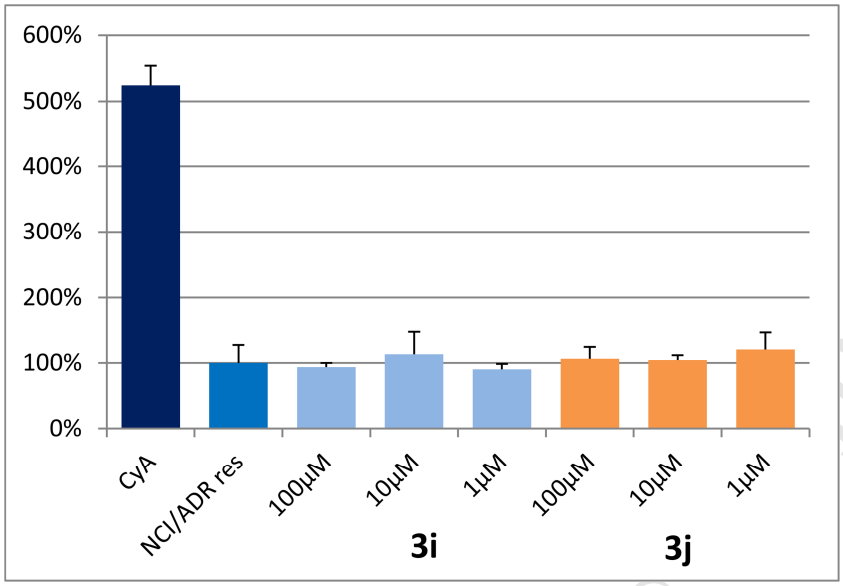
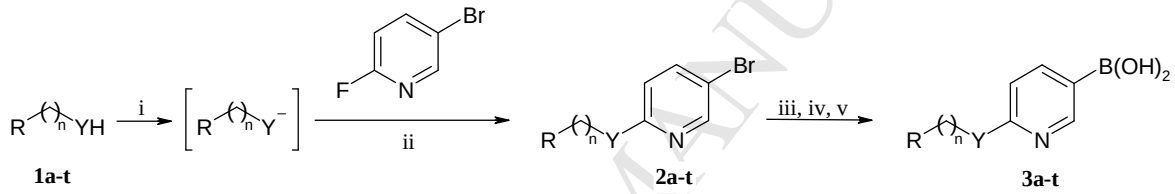


Figure 4. Uptake of Rhodamine-123 by the NCI/ADR-RES cell line.



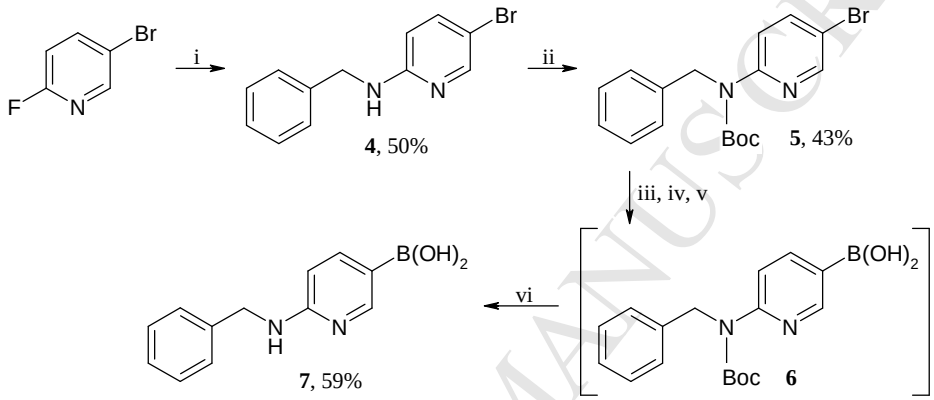
Scheme 1. Synthesis of boronic acids **3a-t**.[a]



	R	n	Y
a	Me	2	O
b	Me	3	O
c	Me	4	O
d	Me	5	O
e	Me	6	O
f	Me	11	O
g	Ph	0	O
h	Ph	2	O
i	Ph	3	O
j	Ph	4	O
k	Ph	5	O
l	Ph	6	O
m	Ph	7	O
n[c]	4-Cl-Ph	1	O
o[c]	4-F-Ph	1	O
p	3,4-Cl-Ph	1	O
q	3,4,5-OMe-Ph	1	O
r	1,3-benzodioxol	1	O
s	Ph	1	S
t[b]	2,4-Cl-Ph	0	O

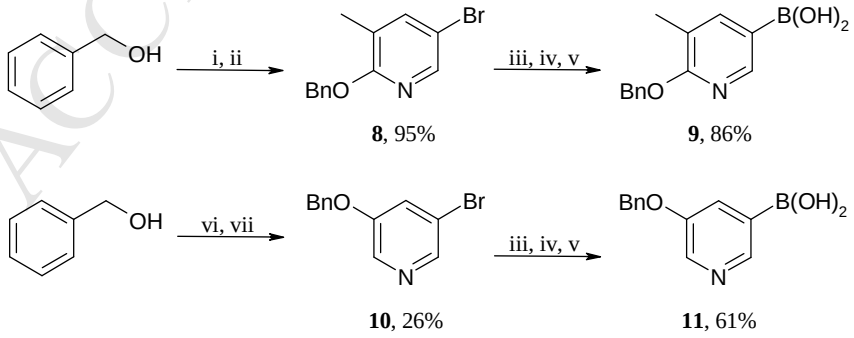
[a]Reagents and conditions: i) Alcohols **1a-r** or thiol **1s** 1.5 equiv., 60% NaH 2 equiv., anhyd. THF, 0°C to rt, 0.5h then reflux, 1h; ii) reflux, 12h; iii) *n*-BuLi 1.25 equiv., anhyd. ether, -78°C, 1h; iv) B(O*i*Pr)$_3$ 1.25 equiv., -78°C to rt, 1h; v) Hydrolysis. [b]Reagents and conditions: i) Alcohol **1t** 2 equiv., KOH 2.5 equiv., anhyd. THF, reflux, 1h; ii) 5-bromo-2-fluoropyridine 1 equiv., DMF, reflux, 12h. [c]Reagents and conditions: iii) B(OMe)$_3$ 2.5 equiv., anhyd. THF, -78°C, 10min.; iv) *n*-BuLi 2.5 equiv., -78°C to rt, 1h.; v) Hydrolysis.

Scheme 2. Synthesis of 6-benzylaminopyridine-3-boronic acid **7**.[a]



[a]Reagents and conditions : i) Benzylamine 1.2 equiv., K_2CO_3 1.5 equiv., DMF, reflux, 12h.; ii) Boc_2O 1.2 equiv., Et_3N 1.2 equiv., DMAP cat., CH_2Cl_2, reflux, 12h.; iii) B(O*i*Pr)$_3$ 1.25 equiv., THF, -78°C, 10min.; iv) *n*-BuLi 1.25 equiv., -78°C to rt, 1h.; v) Hydrolysis; vi) EtOH/HCl 3N 1:1, reflux, 12h.

Scheme 3. Synthesis of boronic acids **9** and **11**.[a]



[a]Reagents and conditions : i) Benzyl alcohol 1.5 equiv., 60% NaH 2 equiv., anhyd. THF, 0°C to rt, 0.5h then reflux, 1h; ii) 2,5-dibromo-3-methylpyridine 1 equiv., reflux, 12h; iii) *n*-BuLi 1.25 equiv., anhyd. ether, -78°C, N_2, 1h; iv) B(O*i*Pr)$_3$ 1.25 equiv., -78°C to rt, 1h; v) Hydrolysis ; vi) Benzyl

alcohol 0,95 equiv., 60% NaH 1.5 equiv., DMF, rt, 1h.; vii) 3,5-dibromopyridine 1.0 equiv., reflux, 48h.