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Article

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Design, Synthesis and Characterization of Novel Tetrahydropyran-based Bacterial Topoisomerase Inhibitors with Potent Anti-Gram Positive Activity.

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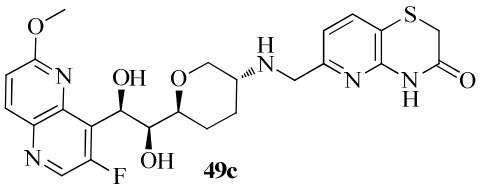
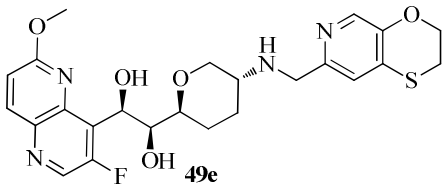
ABSTRACT

There is an urgent need for new antibacterial drugs that are effective against infections caused by multi-drug resistant pathogens. Novel non-fluoroquinolone inhibitors of bacterial type II topoisomerases (DNA gyrase and topoisomerase IV) have the potential to become such drugs as they display potent antibacterial activity and exhibit no target-mediated cross-resistance with fluoroquinolones. Bacterial topoisomerase inhibitors that are built on a tetrahydropyran ring linked to a bicyclic aromatic moiety through a *syn*-diol linker, show potent anti Gram-positive activity, covering isolates with clinically relevant resistance phenotypes. For instance, analog **49c** was found to be a dual DNA gyrase-topoisomerase IV inhibitor, with broad antibacterial activity and low propensity for spontaneous resistance development, but suffered from high hERG K^+ channel block. On the other hand, analog **49e** displayed lower hERG K^+ channel block, while retaining potent *in vitro* antibacterial activity and acceptable frequency for resistance development. Furthermore, analog **49e** showed moderate clearance in rat and promising *in vivo* efficacy against *Staphylococcus aureus* in a murine infection model.



49c



49e

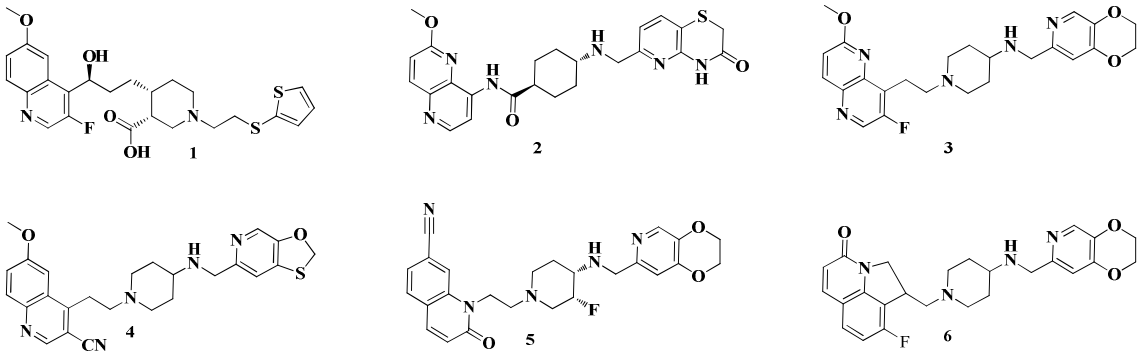
INTRODUCTION

The selective pressure exerted by the use of antibiotics has inevitably resulted in the dissemination of drug resistant bacteria, among which the ESKAPE pathogens (*Enterococcus faecium*, *Staphylococcus aureus, Klebsiella pneumoniae*, *Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* species)[1] are of utmost concern. These pathogens cause not only the major part of hospital-acquired infections (leading to a death toll of approximately 100,000/year in the US) but also constitute a financial burden to healthcare systems all over the world[2-4]. The development of novel small molecule antibacterial drugs is therefore urgently needed. At the dawn of the 21st century, it was common belief that genomic and associated new technologies would deliver a plethora of new antibacterial drug targets[5,6] and thus new chemical entities lacking cross-resistance with clinically used antibacterial drugs. Unfortunately, the efforts have not yet borne fruit and a significant research effort is still dedicated to the 'old' but clinically validated targets[7] to fill the antibiotic pipeline[8].

Bacterial type II topoisomerases (DNA gyrase and topoisomerase IV (Topo IV)) are heterotetramers of $GyrA_2GyrB_2$ and $ParC_2ParE_2$ (*E. coli*) or $GrlA_2GrlB_2$ (*S. aureus*) respectively. They have been validated as targets for antibacterial therapy ever since it was recognized that the fluoroquinolone class of drugs can inhibit the topoisomerase function. The antibacterial spectrum of activity was expanded from nalidixic acid to newest fluoroquinolones[9], but cross-resistance still emerged. Among several resistance mechanisms[10], single amino acid changes in the quinolone resistance-determining region (QRDR) of GyrA (Ser84Trp) or GrlA (Ser80Phe)[11,12] of *S. aureus* reduces dramatically fluoroquinolones affinity binding and therefore their inhibitory effect[13,14]. However, a new avenue was opened in the field of bacterial topoisomerase inhibition when researchers at GlaxoSmithKline (GSK) and Aventis independently disclosed closely related novel non-fluoroquinolone based topoisomerase inhibitors (so called NBTIs)[15,16], devoid of cross resistance with fluoroquinolones. The Aventis program, which was continued at Novexel, capitalized on the initial hit scaffold and its attractive properties, leading to **1** (NXL-101)[17] (Figure 1). Unfortunately, its development had to be discontinued after detection of unacceptable QT interval prolongation[18]. The GSK program took a different direction resulting in successive generations of NBTIs **2**[19], **3**[20] and **6**[21] and could even profit at a later

stage from the resolution of the X-ray structure of **4** (Figure 1) bound to DNA and *S. aureus* DNA gyrase[22]. The crystallographic studies provided the basis to rationalize the lack of cross resistance between NBTIs and fluoroquinolones and also led to the design of novel inhibitors (Figure 1). Recently, a publication by AstraZeneca showed that **2** and **3** block the hERG K^+ channel significantly (IC_{50}<10μM) which may lead to unacceptable cardiac safety liabilities due to QT interval prolongation. Using compound **3** as lead compound, the AstraZeneca team has successfully improved the cardiac safety profile of their newly designed NBTIs[23]. However, the resulting lead compound **5** suffered from low exposure in mice and no data concerning the stability toward resistance development were presented. Researchers at Johnson and Johnson[24] and Pfizer[25] have also reported their efforts on NBTIs.

Figure 1: Representative novel bacterial topoisomerase inhibitors (NBTIs)



Here we report the path to design and synthesize novel dual inhibitors of bacterial DNA gyrase and Topo IV from lead molecules **1** and **2**. The Structure-Activity-Relationships (SARs) of bacterial type II topoisomerase inhibitors were elaborated and used in the course of our optimization program that led to compound **49e**[26], whose characterization will be presented.

WORKING HYPOTHESIS

Biochemical evaluation of compound **1** indicated that it owns antibacterial activity against Gram-positive bacteria (including methicillin- and fluoroquinolone-resistant strains) primarily through potent *S. aureus* DNA gyrase inhibition (Table 1, supercoiling assay). By contrast, in Gram-negative pathogens, the primary target of **1** is likely Topo IV, as indicated by its better inhibitory effect on *E.*

coli Topo IV (Table 1, relaxation assay). DNA gyrase and Topo IV differ in their relative sensitivities to **1**, in opposition to the mode of action of fluoroquinolones which target preferentially Topo IV in Gram-positive and DNA gyrase in Gram-negative pathogens[27]. Compound **2** is not only more potent against Gram-positive bacteria, but also displays a broader antibacterial spectrum including *E. coli* as well as other Gram-negative pathogens (Table 2). Compound **2** is a true dual inhibitor of Gyrase-Topo IV in both *S. aureus* and *E. coli* (Table 1). A *S. aureus* single-step mutant strain, obtained from wild-type *S. aureus* grown in the presence of **1,** contained a single mutation in the *gyrA* gene corresponding to a D83N amino acid exchange. Notably, while compound **2** showed a marginal inhibitory activity against this mutated DNA gyrase, it still inhibits growth of this strain (MIC 0.25 mg/L) through Topo IV inhibition. We therefore concluded that a balanced dual inhibition profile would be highly advantageous in preventing rapid emergence of spontaneous resistance.

Table 1: Inhibitory activities of compounds **1**, **2**, **5** and **6** against bacterial topoisomerases

Compds[a]	Topo IV (IC_{50}, μM) Relaxation Inhibitory Activity		Gyrase (IC_{50}, μM) Supercoiling Inhibitory Activity			
	S. aureus wt[b]	*E. coli* wt[c]	*S. aureus* wt[b]	*S. aureus* *QR*[d]	*S. aureus* D83N[e]	*E. coli* wt[c]
1	128	2	0.125	0.125	128	32
2	0.125	0.008	0.03	0.008	8	0.03
5	8	0.125	0.03	0.03	32	8
6	2	0.5	0.125	0.03	2	0.125
CIP[f]	8	8	128	>256	>256	0.5

[a]Compounds **1**, **2**, **5** and **6** were prepared in our laboratories as reported in literature. [b]Isolated from *Staphylococcus aureus* ATCC 29213. [c]Isolated from *Escherichia coli* ATCC 25922. [d]Quinolone-resistant (QR) enzyme containing a S84L mutation isolated from *Staphylococcus aureus* A-798. [e]Enzyme containing the D83N mutation isolated from a laboratory strain resistant to **1.** [f]Ciprofloxacin, commercially available.

Table 2: Antibacterial activities of compounds **1**, **2**, **5** and **6** (MIC in mg/L)[28]

Compds	*S. aureus* wt[a]	*S. aureus* MRSA, QR[b]	*S. aureus* D83N[c]	*E. faecium* QR[d]	*S. pneumoniae.* wt[e]	*E. coli* wt[f]	*P. aeruginosa* wt[g]
1	0.125	0.25	>8	1	1	>8	>8
2	0.015	0.015	0.25	0.063	0.063	0.25	8
5	0.015	0.015	1	0.5	0.25	8	>8
6	0.015	0.015	1	0.06	0.015	1	>8
CIP[h]	0.25	>8	0.25	>8	1	0.016	0.25

[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-798. [c]Laboratory strain. [d]*Enterococcus faecium* A-949 vancomycin-resistant. [e]*Streptococcus pneumoniae* ATCC 49619. [f]*Escherichia coli* ATCC 25922. [g]*Pseudomonas aeruginosa* ATCC 27853. [h]Ciprofloxacin (fluoroquinolone).

At the start of our investigations, no crystallographic data for GyrA complexed to a NBTI was available to support rational drug design of dual GyrA/ParC inhibitors. Enzymatic data recorded for compound **2**, showed cross-resistance with **1**, suggesting that both entities share a common binding site in the proximity of Asp83. NBTIs chemical structure can be divided in three parts: a methoxy substituted bicyclic aromatic left-hand side (LHS), a mono- or bicyclic lipophilic right-hand side (RHS) and a variable linker joining the two units. Superposition of compounds **1** and **2** (Figure 2) displayed a good overlay for LHS and RHS thereby defining the length of the linker as well as directionalities of the respective exit vectors. The linker, which can itself be divided in three parts (i.e. a central ring spacer and two flanked connecting units reaching the LHS and RHS respectively), differs significantly in compounds **1** and **2**, sharing only a saturated 1,4-disubstituted six-membered ring as common motif. The amide functionality in **2** is co-planar with its LHS, leading to a straighter linker if compared with the hydroxy-propylidene (-CHOH-$(CH_2)_2$-) functionality in **1**. Both inhibitors comprise a basic nitrogen in close vicinity to the RHS.

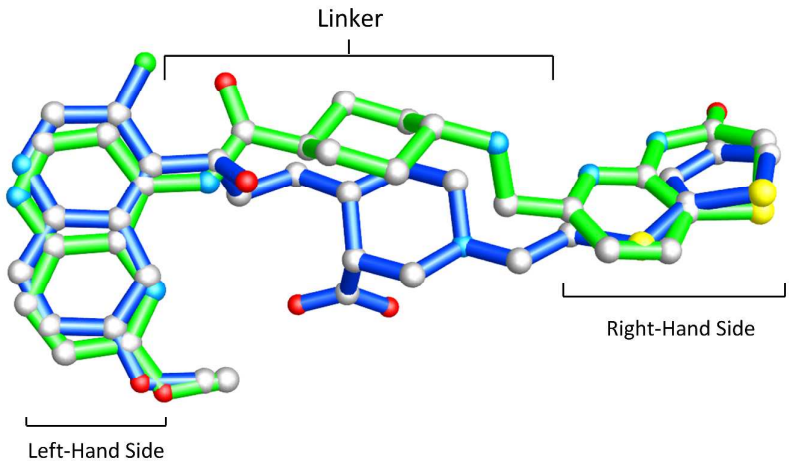


Figure 2: a. Modeled superimposition of inhibitors **1** (blue bonds) and **2** (green bonds). Red: oxygen atoms; blue: nitrogen atoms; white: carbon atoms; yellow: sulfur atoms; green: fluorine atoms.

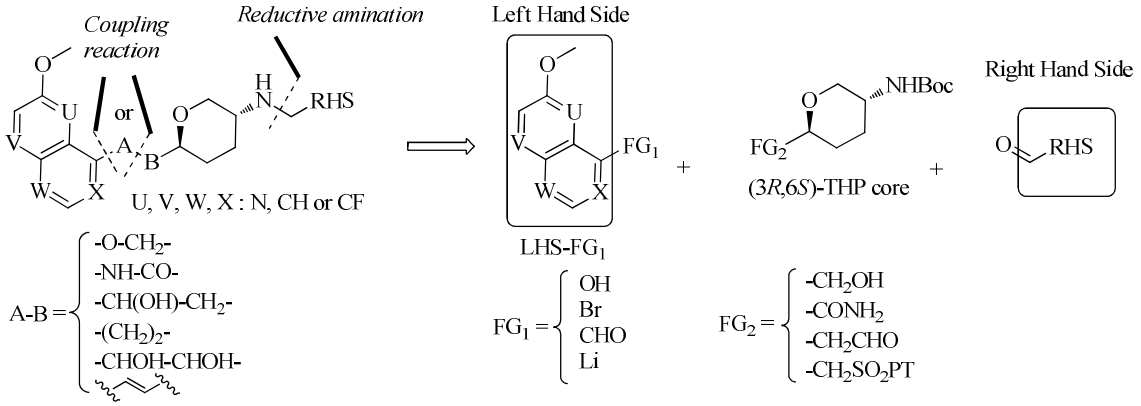
The more balanced GyrA/ParC activity of **2** led us to design NBTIs containing a *trans*-3,6-disubtituted tetrahydropyran (THP) ring and a two atom (-A-B-) unit to connect this ring to the LHS (Scheme 1). We hypothesized that such a linker should be suitable to establish geometrically restricted compounds with favorable physicochemical and pharmacological profiles. Therefore, benzylic alcohols or diols, respectively (-A-B- = -CHOH-CH_2- or -CHOH-CHOH-), were assumed to produce superior aqueous solubility if compared to amide (-A-B- = -HN-CO-) or ether (-A-B- = -O-CH_2-) units. As side products on the way to the desired diol compounds, the (*E*)-alkene (-A-B- = -CH=CH-) and its saturated analog (-A-B- = -CH_2-CH_2-) could be obtained. As we could not predict the effect of the THP-ring stereochemistry, we decided to explore both enantiomeric series. In case the –A-B- motif is a diol, we have chosen to limit our investigations to *syn*-diols for synthetic reasons. Finally, known RHS-units were connected to the THP-core through the aminomethyl side chain.

CHEMISTRY

As illustrated in Scheme 1, the exploration of the SAR of the newly designed topoisomerase inhibitors was facilitated by using a modular synthetic approach based on the following three key building blocks: i) a bicyclic aromatic LHS, ii) a central THP core, and iii) a bicyclic aromatic RHS. The

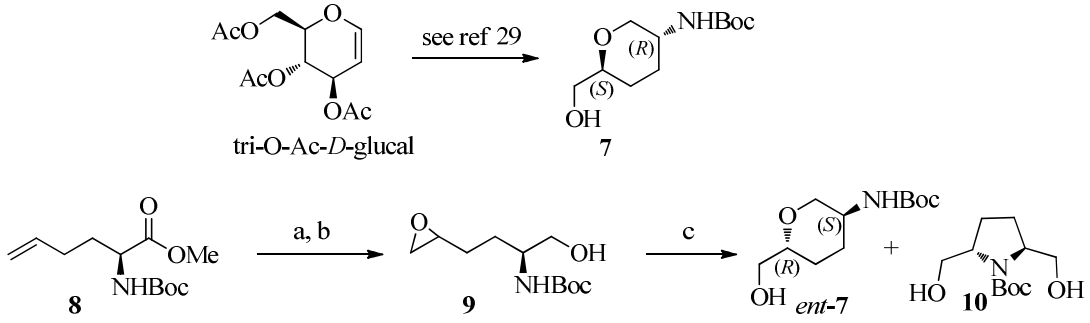
impact of the stereochemistry of the central THP core can be rapidly explored by using building blocks **7** and its enantiomer *ent*-**7**.

Scheme 1



In contrast to the well-documented synthesis of the alcohol (3*R*,6*S*)-**7** from tri-O-Ac-*D*-glucal[29], the synthetic access to the enantiomer (3*S*,6*R*)-*ent*-**7** had to be developed first. According to Scheme 2, the known ω-alkenoate **8**[30] was reduced to the corresponding alcohol with lithium borohydride in tetrahydrofuran, and a treatment with *meta*-chloroperbenzoic acid under buffered conditions yielded epoxide **9** as an equimolar mixture of diastereomers. The following acid-promoted cyclization, using (+/-)-10-camphorsulfonic acid (CSA), afforded a separable mixture of enantiopure *ent*-**7** (arising from of the specific attack of the alcohol on the (*S*)-configurated epoxide) and the pyrrolidine derivative **10** (resulting from the cyclization of –NH-*tert*-butoxycarbonyl on the (*R*)-configurated epoxide).

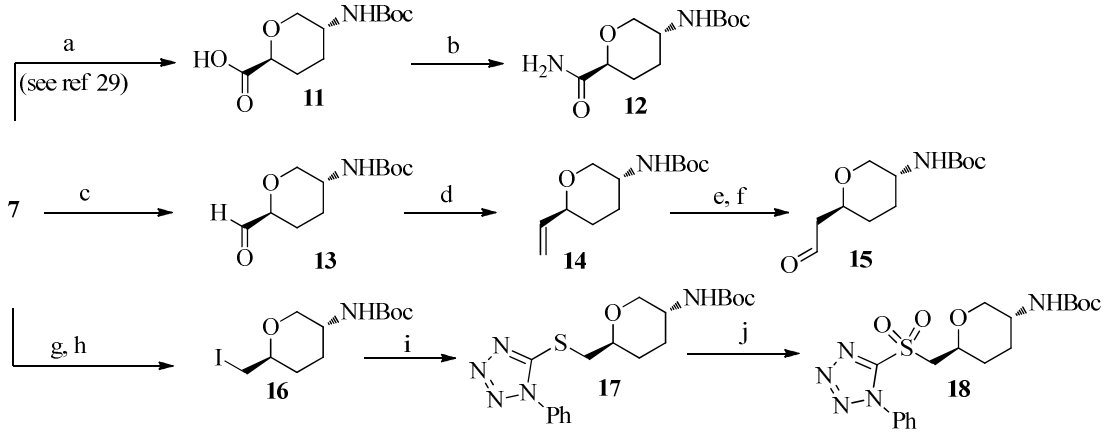
Scheme 2[a]



[a]Reagents : (a) $LiBH_4$, THF, rt; (b) *m*-CPBA, pH 8 phosphate buffer, DCE, rt; (c) CSA, DCM, rt.

According to Scheme 1, the introduction of the A-B linker required the preparation of appropriately substituted central building blocks featuring a proper reaction handle FG_2. The following modifications of alcohol (3*R*,6*S*)-**7** leading to central building blocks **12, 15** and **18** are summarized in Scheme 3. Amide **12** was obtained in two steps starting with the oxidation of alcohol **7** to acid **11** by sodium periodate in presence of a catalytic amount of ruthenium trichloride[29]. A subsequent treatment of the acid **11** with N-hydroxysuccinimide (NHS) and N,N-dicyclohexylcarbodiimide provided its activated form which upon treatment with gaseous ammonia yielded the targeted amide **12**, with no loss of stereochemical information[31] (Scheme 3).

Scheme 3[a]

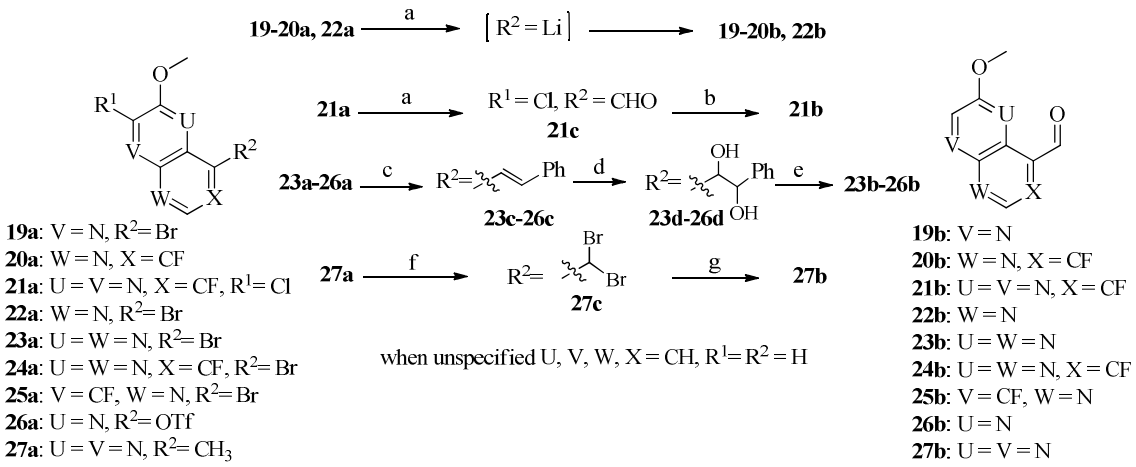


[a]Reagents : (a) $RuCl_3$, $NaIO_4$, DCM, aq CH_3CN, rt; (b) DCC, NHS, rt then gaseous NH_3; (c) py.SO_3, DMSO, DIPEA, DCM, 0 °C; (d) methyltriphenylphosphonium bromide, *t*-BuOK, THF, rt; (e) 9-BBN-H, THF, rt then aq NaOH, aq H_2O_2, 0 °C to rt; (f) Dess-Martin periodinane, DCM, 0 °C to rt; (g) tosyl chloride, Et_3N, DCM, 0 °C to rt; (h) NaI, acetone, reflux; (i) Phenyltetrazole thiol, KOH, EtOH, reflux; (j) ammonium molydbate, aq H_2O_2, EtOH, reflux.

The oxidation of alcohol **7** using pyridine-sulfur trioxide complex (py.SO_3) and dimethylsulfoxide[32] in dichloromethane afforded aldehyde **13**. The Wittig olefination[33] leading to alkene **14** was performed by the reaction of methylenetriphenylphosphorane (generated from methyltriphenylphosphonium bromide and potassium *tert*-butoxide) with aldehyde **13**. The hydroboration of alkene **14** with 9-borabicyclo[3.3.1]nonane in tetrahydrofuran led after oxidative work-up to the intermediate primary alcohol[34] that was further oxidized to aldehyde **15** using Dess-Martin periodinane[35] (Scheme 3). As observed in the case of amide **12**, it is noteworthy to state that no epimerization occurred by passing from alcohol **7** to homologated aldehyde **15**. Phenyltetrazole sulfone **18** was obtained by oxidation of sulfide **17** with hydrogen peroxide in presence of ammonium molybdate. The formation of **17** was initially achieved by direct coupling of alcohol **7** with phenyltetrazole thiol (PT-SH) under Mitsunobu-type conditions (combining triphenylphosphine (PPh_3) and diisopropyl azodicarboxylate (DIAD))[36]. However, nucleophilic substitution of iodide **16** with PT-SH in presence of potassium hydroxide in refluxing ethanol turned to be more effective. The intermediate iodide **16** was obtained through tosylation followed by displacement with sodium iodide in refluxing acetone (Scheme 3).

The bromides **19a** and **22a**-**25a** (R^2 = Br) were prepared by refluxing the corresponding hydroxyl derivatives (R^2 = OH)[37] in neat phosphorous tribromide. As illustrated in Scheme 4, the preparation of aldehydes **19b**-**22b** consisted in trapping a lithio anion that was generated either from bromides **19a** or **22a** (treatment with *n*-butyl lithium or lithium diisopropylamide at -78°C), or by fluorine-directed ortho-lithiation of compounds **20a** or **21a** with dimethylformamide. Dechlorination of aldehyde **21c** was done by hydrogenolysis using palladium on carbon and triethylamine under hydrogen atmosphere to yield aldehyde **21b**. The syntheses of aldehydes **23b**-**26b** started from bromides **23a**-**25a** (R^2 = Br) or triflate **26a** (R^2 = OTf)[38] *via* Suzuki coupling[39] with *trans*-phenylvinyl boronic acid giving rise to the respective phenyl alkenes **23c**-**26c** which were converted to the corresponding intermediate diols **23d**-**26d**. The desired aldehydes **23b**-**26b** were then subsequently formed under standard periodic cleavage conditions. On the other hand, aldehyde **27b** was obtained by hydrolysis of dibromide **27c** obtained from methyl quinoxaline **27a**[37] by treatment with an excess of N-bromosuccinimide in presence of azobisisobutyronitrile (Scheme 4).

Scheme 4[a]

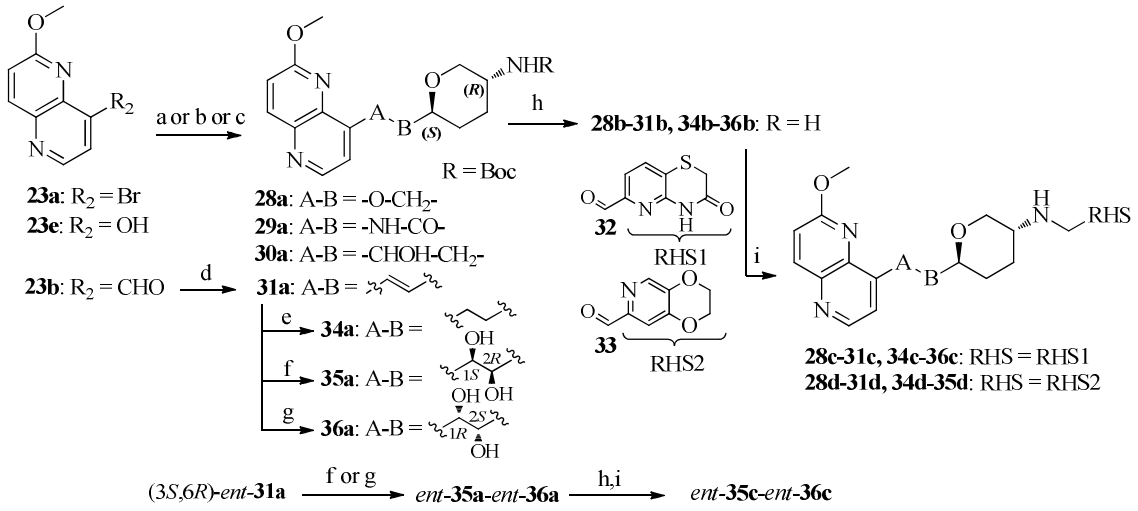


[a]Reagents: (a) *n*-butyl lithium or LDA, THF, -78 °C then DMF; (b) H_2, Pd on carbon, Et_3N, THF, rt; (c) cat $(PPh_3)_4Pd$, K_2CO_3, Ph-CH=CH-B$(OH)_2$, aq 1,4-dioxane, reflux; (d) cat OsO_4, NMO, aq DCM, rt; (e) $NaIO_4$, aq acetone, rt; (f) NBS, AIBN, CCl_4, reflux; (g) $AgNO_3$, aq CH_3CN, rt.

According to Scheme 5, the general synthetic access to final NBTI described herein involved the coupling of the central THP core to the bicyclic aromatic LHS, followed by a reductive amination using readily accessible RHS aldehydes. The ether linkage of derivative **28a** was constructed using a Mitsunobu coupling reaction (DIAD, PPh_3) between alcohol **7** and hydroxy-naphthyridine **23e**[26]. In contrast, amide **12** was coupled to bromide **23a** *via* a Buchwald-type reaction[40] to form the amide-containing derivative **29a**. Treatment of bromide **23a** with *n*-butyl lithium at -78°C generated the lithio anion that reacted with aldehyde **15** to yield benzylic alcohol **30a** as an equimolar mixture of diastereomers[41]. Julia-Kocieński coupling[42] of PT-sulfone **18** and aldehyde **23b** using potassium bis(trimethylsilyl)amide as a base provided (*E*)-alkene **31a** which was hydrogenated over palladium on carbon to afford the corresponding saturated derivative **34a**. On the other hand, Sharpless asymmetric dihydroxylation[43] of alkene **31a** gave rise to *syn*-diols with an excellent diastereoselectivity (>95%)[41]. The use of commercial AD-mix-α or AD-mix-β gave access to both diastereomeric series as demonstrated with the formation of intermediates (1*S*,2*R*)-**35a** and (1*R*,2*S*)-**36a**. The corresponding free amines **28b-31b** and **34b-36b** were obtained by treatment with trifluoroacetic acid (TFA), either neat or diluted in dichloromethane. From these amines, variable RHS moieties were introduced *via* reductive amination reaction[44] with aldehydes **32**[45] or **33**[46], giving rise to NBTIs **28c-31c, 28d-31d**,

34c-36c, **34d** and **35d**. A set of THP-based topoisomerase inhibitors belonging to the enantiomeric series could be prepared by the same synthetic pathway but using sulfone *ent*-**10** as a partner in the Julia-Kocieński coupling. Likewise, asymmetric dihydroxylation of *ent*-**31a** using either AD-mix-α or -β proceeded smoothly to afford the corresponding *syn*-diols *ent*-**35c** and *ent*-**36c** (Scheme 5).

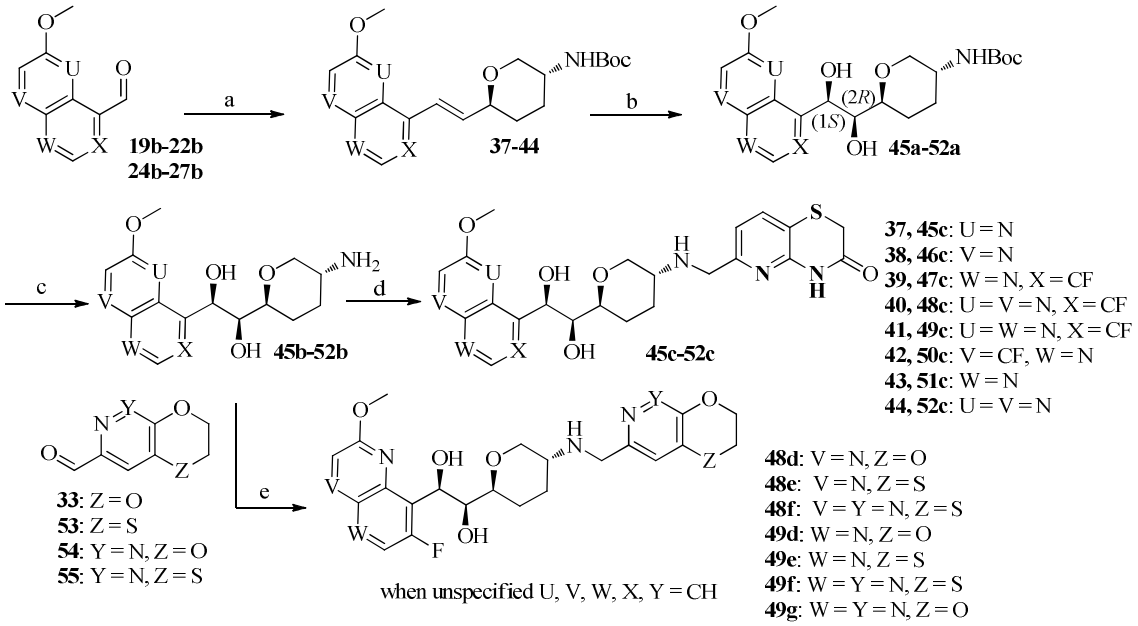
Scheme 5[a]



[a]Reagents : (a) for **28a**: **7**, **23e**, DIAD, PPh_3, THF, rt; (b) for **29a**: **12**, **23a**, cat $(dba)_3Pd_2$, BINAP, K_2CO_3, aq dioxane, reflux; (c) for **30a**: **23a**, *n*-butyl lithium, THF, -78 °C then **15**; (d) **18**, KHMDS, DME, -78 °C; (e) H_2, 10% Pd on C, MeOH, rt; (f) for **35a** and *ent*-**36a**: AD-mix-β, $CH_3SO_2NH_2$, *t*-BuOH-H_2O, 0 °C; (g) for **36a** and *ent*-**35a:** AD-mix-α, $CH_3SO_2NH_2$, *t*-BuOH-H_2O, 0 °C; (h) TFA, rt (i) **32** or **33**, 3 Å MS, DCE, MeOH, 50 °C, then $NaBH_4$, 0 °C.

The Julia-Kocieński coupling of aldehydes **19b-22b, 24b-27b** and sulfone **18** provided (*E*)-alkenes **37-44** with (*E*)/(*Z*)-selectivities bigger than 6/1[41]. The (1*R*,2*S*)-*syn*-diols **45a-52a** were prepared by using AD-mix-β as dihydroxylating reagent. After removal of the *tert*-butoxycarbonyl (Boc) group with TFA, the resulting amines **45b-52b** were transformed to NBTIs **45c-52c** using aldehyde **32** in the reductive amination reaction. Finally, the intermediate amines **48b** and **49b** were coupled under reductive amination conditions with aldehydes **33**, **53**[47], **54**[48] and **55**[49] to access NBTIs **48d-f** and **49d-g** (Scheme 6).

Scheme 6 [a]



[a]Reagents : (a) **18**, LiHMDS, THF, -78 °C (b) AD-mix-β, $CH_3SO_2NH_2$, *t*-BuOH, H_2O, 0 °C (c) TFA, rt (d) **32**, 3 Å MS, DCE, MeOH, 50 °C, then $NaBH_4$, 0 °C; (e) **48b** or **49b**, **33**, **53-55,** MeOH, 50 °C, then $NaBH_4$, 0 °C.

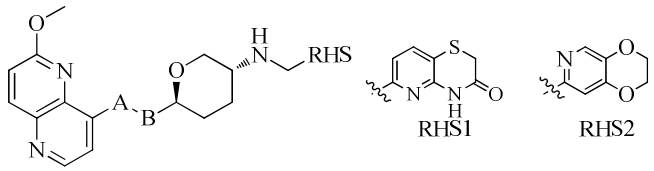
RESULTS AND DISCUSSION

The antibacterial activity, expressed as MIC (minimal inhibitory concentration, [mg/L]) of the novel chemical entities, was measured against a panel of bacterial strains including *Staphylococcus aureus* (*S. aureus*), *Streptococcus pneumoniae* (*S. pneumoniae*), *Escherichia coli* (*E. coli*) and *Pseudomonas aeruginosa* (*P. aeruginosa*) ATCC strains as well as a *S. aureus* D83N DNA gyrase mutant strain, resistant to NBTIs such as **1**. All the novel chemical entities were also evaluated for inhibition of DNA gyrase and topo IV isolated from wild type *S. aureus* and *E. coli* and for block of hERG K^+ channels.

As shown in Table 3, all molecules based on a 6-methoxy-1,5-naphthyridine scaffold showed potent binding to *S. aureus* DNA gyrase and *E. coli* Topo IV, with IC_{50} in the nanomolar range for the best compounds such as **30**, **34** and **35**. The ether linker present in **28c-d** led to lower enzyme potency, and accordingly, weaker antibacterial activities. The two amide derivatives **29c-d** displayed much weaker antibacterial activities compared to the related GSK inhibitor **2**. However, precipitation of **29c** due to low solubility prevented reliable inhibition measurements. The most potent antibacterial activities

were measured for derivatives containing an ethylene, a hydroxy- or a 1,2-dihydroxy-ethylene unit (**30c-d**, **34c-d** and **35c-d** respectively) whereas alkenes **31c,d** showed higher MICs. Compounds comprising a pyridothiazinone moiety as RHS displayed consistently superior target potency compared to molecules containing the dioxinopyridine moiety, which translated into lower MICs and a broader antibacterial spectrum, as exemplified by compounds **30c** and **30d.** While both molecules displayed good antibacterial activities on *S. aureus*, **30c** showed a 4-fold lower MIC on wild type *S. pneumoniae* (0.03 mg/L), and at least a 8-fold lowered MIC on *E. coli* (1 mg/L) when compared to **30d**. The same trend was observed for **34c** and **34d** as well as for **35c** and **35d**. The lack of antibacterial activity of compounds **28d**-**35d** against wild-type *E. coli* was not further investigated, but may result from a combination of reduced enzyme potency on target and lower cytoplasmic accumulation in *E. coli* compared to the related derivatives having a pyridothiazinone RHS (**28c**-**36c**). Moreover, the pyridothiazinone inhibitors exhibit good target potency against *S. aureus* Topo IV and *E. coli* DNA gyrase respectively, whereas inhibitors comprising a dioxinopyridine failed to do so. Similar to inhibitor **2**, the most potent dual *S. aureus* DNA gyrase-Topo IV inhibitors (**34c** and **35c**) displayed antibacterial activities against the *S. aureus* D83N mutant (MIC range 0.5-1 mg/L). In general, as shown in Table 3, a high level of hERG K^+ channel block was measured for this set of compounds with the exception of compound **35d**, suggesting that designing compounds with high antibacterial activity and low hERG K^+ channel block should be possible.

Table 3: SAR of two-atom linker A-B



Cpds	RHS	Linker	MIC (mg/L)				Gyrase SCIA (IC_{50})		Topo IV RIA (IC_{50})		hERG block[e]
			S. a.[a]	*S. a.*[R b]	*S. p.*[c]	*E. c.*[d]	*S. a.*	*E. c.*	*S. a.*	*E. c.*	
28c	1		0.5	4	0.25	4	0.125	2	8	0.125	70
28d	2		2	>8	4	>8	0.5	>8	>8	0.5	19
29c[f]	1		0.25	>8	0.5	>8	0.125	0.5	2	0.125	99
29d	2		0.125	>8	0.25	>8	0.125	>8	>8	0.125	71
30c	1		0.03	0.5	0.03	1	<0.03	0.5	>8	<0.03	99
30d	2		0.03	>8	0.125	>8	0.125	>8	>8	0.125	66
31c	1		0.125	4	1	4	0.125	0.5	2	0.03	94
31d	2		0.25	>8	1	>8	0.125	>8	>8	0.125	88
34c	1		0.03	1	0.06	1	0.03	0.12	0.5	0.03	97
34d	2		0.06	4	0.5	8	<0.03	8	>8	0.125	82
35c	1		0.03	0.5	0.06	1	<0.03	0.5	0.5	0.125	36
35d	2		0.06	4	0.5	8	0.03	8	8	0.125	27

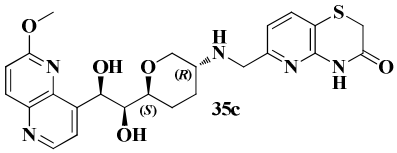
[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922, [e]% block of hERG K^+ channel measured at 10 μM. [f]Precipitation observed during assay measurements. All compounds showed MIC >8 mg/L on *Pseudomonas aeruginosa* ATCC 27853.

Lead Optimization

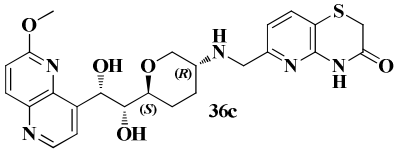
The absolute stereochemistry of the *syn*-diol linker modulated the antibacterial activity to a significant extent (Table 4). While inhibitors **35c** and *ent*-**36c** featuring a (1*S*,2*R*)-configurated dihydroxy-ethylene linker displayed low MICs against Gram-positive *S. aureus* and *S. pneumoniae* (0.03 to 0.06 mg/L) and Gram-negative *E. coli* (1-2 mg/L), the MICs recorded for their respective (1*R*,2*S*)-

configurated diastereomers **36c** and *ent*-**35c**, were more than 10-fold higher against those pathogens. These observations were in agreement with the lower DNA topoisomerases inhibitory activities recorded for **36c** and *ent*-**35c** in comparison to those of **35c** and *ent*-**36c** and **52c** respectively. Interestingly, the (3*R*,6*S*)- or (3*S*,6*R*)- configuration of the THP core (referring to the progenitor building blocks **7** and *ent*-**7**) had no influence on antibacterial potency. All diastereomers or enantiomers of **35c** that were evaluated for hERG K^+ channel block, exerted higher affinity for this channel if compared to **35c** and no clear trend could be observed with respect of the different stereochemistries.

Table 4: Influence of the stereochemistry of the 1,2-dihydroxy-ethylene linker and the THP ring



35c



36c

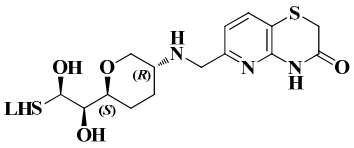
Cpds	MIC (mg/L)				Gyrase SCIA (IC_{50})		Topo IV RIA (IC_{50})		hERG block[e]
	S. a.[a]	*S. a.*[R b]	*S. p.*[c]	*E. c.*[d]	*S. a.*	*E. c.*	*S. a.*	*E. c.*	
35c	0.03	0.5	0.06	1	0.03	0.5	0.5	0.125	36
ent-**35c**	0.5	>8	0.5	4	0.125	8	>8	0.125	45
36c	1	>8	1	>8	0.5	8	>8	0.125	93
ent-**36c**	0.03	0.5	0.03	1	0.03	0.5	8	0.125	70

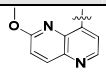
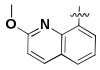
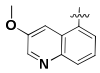
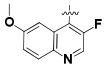
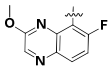
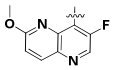
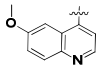
[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922, [e]% block of hERG K^+ channel measured at 10 μM. All compounds showed MIC >8 mg/L on *Pseudomonas aeruginosa* ATCC 27853.

As the stereochemistry of the central THP-core had no influence on antibacterial potency, we chose to pursue our investigations on the series stemming from the more readily available alcohol **7** and several analogs of **35c** featuring various bicyclic aromatic LHS moieties, were evaluated. As shown in Table

5, its 6-methoxyquinoline analog **51c** was less active. The nitrogen atom at the generic position noted U contributes to an enhanced antibacterial potency. This was confirmed by comparing **47c** with **49c** and **46c** with **52c**, respectively. Indeed, derivatives **45c**, **48c**, **49c** and **52c**, wherein U = N, shared a similar antibacterial profile, with good MICs against wild-type *S. aureus* and *S. pneumoniae*. As previously observed, these dual inhibitors of *S. aureus* and *E. coli* type II topoisomerases, exhibited antibacterial activity against D83N *S. aureus* mutant and wild type *E. coli*. In contrast, the nature of the groups in generic positions V, W and X (N, CH or CF) did not modulate potency to a significant extent. In general, derivatives **46c**, **47c**, **50c** and **51c**, wherein U = CH did not exhibit a dual target inhibitor profile and lacked antibacterial activity against D83N *S. aureus* mutant. Nonetheless, less block of the hERG K^+ channel was observed for derivatives with U = CH. For compounds with U = N, slight differences were observed with respect to hERG K^+ channel block, with IC_{50}s in the 10μM range in case of X = CF (**48c** and **49c**), whereas analogs for which X = CH (**35c** and **52c**) showed slightly more potent hERG K^+ channel block.

Table 5: SAR of Left Hand Sides



Cpds	LHS	MIC (mg/L)				Gyrase SCIA (IC_{50})		Topo IV RIA (IC_{50})		hERG block[e]
		S. a.[a]	*S.a.*[R b]	*S. p.*[c]	*E. c.*[d]	*S. a.*	*E. c.*	*S. a.*	*S. a.*	
35c		0.03	0.5	0.06	1	0.03	0.5	0.5	0.125	70
45c		0.03	0.25	0.06	2	0.03	0.125	0.5	0.125	72
46c		0.25	>8	0.25	8	0.125	2	0.5	0.125	17
47c		0.25	>8	0.25	4	0.125	2	8	0.125	15
48c		0.06	1	0.5	4	0.03	0.5	2	0.125	44
49c		0.03	0.25	0.06	1	0.03	0.5	0.5	0.03	54
50c		0.03	4	0.25	4	0.125	2	8	0.125	20
51c		0.25	>8	0.25	4	0.25	2	8	0.125	24
52c		0.03	0.5	0.06	2	0.03	0.5	2	0.125	55

[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922, [e]% block of hERG K^+ channel measured at 10 μM.. All compounds showed MIC >8 mg/L on *Pseudomonas aeruginosa* ATCC 27853.

From the latter observations, the influence of the RHS-moiety on hERG K^+ channel block was established based on the 3-fluoronaphthyridine and 6-fluoroquinoxaline scaffolds (Table 6). Whereas both compounds **48c** and **49c** blocked hERG K^+ channel in the low micromolar range, significantly reduced block was observed for the inhibitors **48d**, **48f**, **49d** and **49g** containing either a dioxinopyridine, a dioxinopyridazine, an oxathiinopyridine or an oxathiinopyridazine moiety. However, reduced hERG K^+ channel block tracked in parallel with lower topoisomerase inhibition.

For instance, compounds **48d**, **48f**, **49f** and **49g**, featuring a dioxinopyridine or a pyridazine-based moiety, still displayed potent inhibition of *S. aureus* DNA gyrase, but weak *S. aureus* Topo IV activity, thus resulting in limited antibacterial activity on the *S. aureus* D83N mutant strain (MIC 8mg/L or higher). The exchange of dioxino ring for a oxathiino ring resulted still a low *S. aureus* Topo IV activity but to an increased potency against *S. aureus* DNA gyrase. A good compromise between antibacterial activity and block of hERG K^+ channel could be obtained for compound **49e**.

Structural analysis of diol 49e

With the X-ray structure of compound **4** in complex with *S. aureus* DNA gyrase and a 20-bp DNA duplex in hand[22], inhibitor **49e** was docked into the active site of the protein-DNA complex in order to rationalize the proposed SAR (Figure 3). The 6-methoxy-1,5-naphthyridine moiety of **49e** sits between two stretched DNA base pairs. Saturated linkers (ethylene, hydroxyl- or 1,2-dihydroxy-ethylene) allowed an unstrained fit of compounds as observed for **4**, whereas amides, ethers and alkenes diverged from a linker path as defined by **4**, ultimately leading to a suboptimal positioning of a (charged) H-bond donor that can interact with Asp83. Furthermore, the two hydroxyl units of **49e** appear to be in a position to establish H-bonds with neighboring base pairs. The RHS of **49e** resides in a hydrophobic pocket at the interface of the two GyrA subunits. The oxathiolopyridine ring of compound **4** and similarly the hydrophobic RHS of **49e** establish van der Waals interactions with the surrounding hydrophobic residues, thereby featuring interactions between the sulfur atoms of the inhibitors and methionine side chains of GyrA.

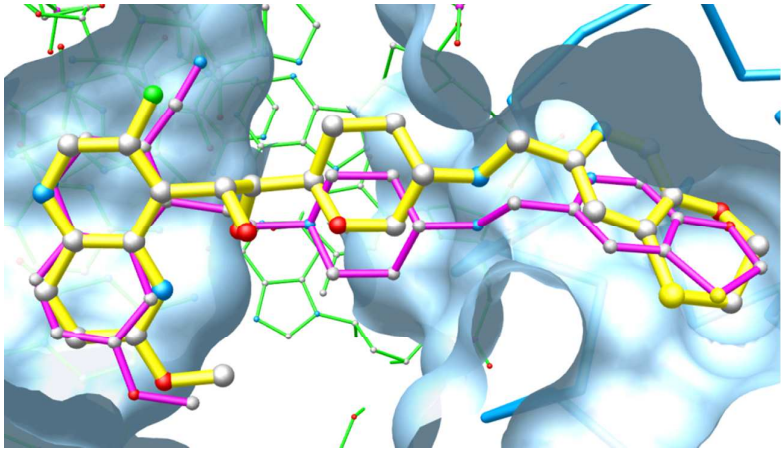
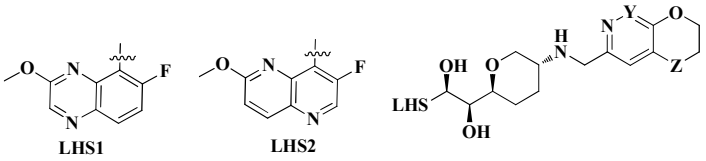


Figure 3. Docking pose of compounds **49e** (yellow) superimposed on original inhibitor **4** (pink) in GyrB27-A56 complex X-ray (PDB-code: 2XCS). DNA is shown in green, while gyrase C-alpha backbone is blue.

Table 6: Novel bacterial topoisomerase inhibitors with reduced hERG K^+ channel block



Cpds	LHS	Y	Z	MIC (mg/L)				Gyrase SCIA (IC_{50})		Topo IV RIA (IC_{50})		hERG block[e]
				S. a.[a]	*S. a.*[R b]	*S. p.*[c]	*E. c.*[d]	*S. a.*	*E. c.*	*S. a.*	*S. a.*	
48d	1	CH	O	≤0.015	8	0.125	8	0.125	8	8	0.5	11
48e	1	CH	S	≤0.015	2	0.03	8	0.03	8	8	0.125	33
48f	1	N	S	≤0.015	8	0.125	>8	0.125	8	>8	2	0
49d	2	CH	O	0.03	2	0.03	>8	0.03	8	8	0.125	10
49e	2	CH	S	≤0.015	2	≤0.015	8	0.03	8	8	0.125	19
49f	2	N	S	≤0.015	8	0.06	>8	0.125	8	8	0.5	3
49g	2	N	O	0.125	>8	0.5	>8	0.125	>8	>8	2	7

[a]*Staphylococcus aureus* ATCC 29213. [b]*Staphylococcus aureus* A-234 gyrA: D83N. [c]*Streptococcus pneumoniae* ATCC 49619. [d]*Escherichia coli* ATCC 25922. [e]% block of hERG K^+ channel measured at 10 μM. All compounds showed MIC >8 mg/L on *Pseudomonas aeruginosa* ATCC 27853.

Antibacterial profile of NBTI 49e

As shown in Table 7, compound **49e** was highly active against all *S. aureus* isolates tested including MRSA and fluoroquinolone-resistant strains (MIC_{90}, 0.03 mg/L)[50]. In addition it showed good activity against other clinically relevant Gram-positive bacteria including *S. pneumoniae* and *E. faecium* VRE with MIC_{90}s of ≤ 1 mg/L. Significant activity was also observed against *Moraxella catarrhalis* and *Haemophilus influenzae*. However, only weak or no activity was detected against other Gram-negative bacteria such as *Escherichia coli* or *Pseudomonas aeruginosa*. In summary, compound **49e** has an antibacterial spectrum suitable for the treatment of Gram-positive and respiratory tract infections.

Table 7. Antibacterial activities of **49e** against clinical isolates (in mg/L)

Organisms	Nb of strains	MIC_{50}	MIC_{90}	Range
Staphylococcus aureus	35	≤ 0.015	0.03	≤ 0.015-0.06
MRSA subset	25	≤ 0.015	0.03	≤ 0.015-0.03
Staphylococcus epidermidis	16	0.06	0.25	0.03-0.25
Enterococcus faecalis	20	0.5	0.5	0.06-1
Enterococcus faecium VRE	20	0.25	1	0.03-4
Streptococcus pneumoniae	24	0.06	0.125	0.03-0.25
Streptococcus pyogenes	14	0.03	0.06	0.015-0.125
Streptococcus agalactiae	13	0.25	0.5	0.125-0.5
Haemophilus influenzae	11	0.5	4	0.25-8
Moraxella catarrhalis	11	0.03	0.125	≤0.015-125

Spontaneous resistance frequencies of selected compounds were measured in *S. aureus*. Both **49c** and **49e** that displayed dual target activity (gyrase and Topo IV) showed 10 to 100-fold lower resistance frequencies compared to compound **1** targeting only gyrase (Table 8). This finding confirmed our working hypothesis that emergence of resistance should be slower with inhibitors acting on both targets.

Table 8. Frequencies of spontaneous resistance development in *S. aureus* ATCC 25923

Cpds	Resistance frequency	
	at 4x the MIC	at 16x the MIC
1[a]	2.7×10^{-6}	2.1×10^{-7}
49c	8.8×10^{-8}	$<2.1 \times 10^{-9}$
49e	5.0×10^{-8}	1.1×10^{-8}

[a] assayed as an equimolar mixture of diastereomers (at benzylic position).

In order to assess the pharmacokinetic properties, mouse, rat and human liver microsomal stabilities, expressed as intrinsic clearances, were determined (Table 9). With decreasing lipophilicity achieved through introduction of hydroxyl groups on the ethylene linker (drop in *c*logP of 1.2 unit), a lower metabolic turnover was observed, with similar trends for the three species. A marked enhancement in *in vitro* metabolic stability was further obtained for quinoxaline **52c**.

Table 9: Hepatic intrinsic microsomal clearance (μL/(min*mg))

Cpds[a]	mouse	Rat	Human	*c*LogP
34c	954	>1250	347	2.43
49e	291	344	30	1.20
52c	39	92	45	0.57

[a]Assayed at 1μM compound concentration.

Compounds **34c** and **52c**, both featuring a pyridothiazinone RHS, showed a high *in vivo* clearance in the rat, whereas **49e** with an oxathiinopyridine RHS, was cleared much more slowly and displayed significant bioavailability (F = 41%). Rat clearance measured for compounds **34c**, **49e** and **52c** (Table 10) only partially matched the corresponding *in vitro* microsomal stability data in line with the notion that *in vitro* CL_{int} is by and large an unbound parameter not confounded by plasma protein binding. Not surprisingly, the *in vivo* clearance of **49e** and **52c** reflects the *in vitro* metabolic stability once corrected for the fraction unbound in plasma (unbound clearance, CL_u)[51].

Table 10: Pharmacokinetic parameters in rat[a]

Cpds	CL[a,b] (mL/min//kg)	f_u[c]	CL_u[d] (mL/min/kg)	Vss[e] (L/kg)	$t_{1/2}$[f] (h)	AUC[g] (iv) (ng*h/mL)	AUC (po) (ng*h/mL)	F[h] (%)
34c	36	nd[i]	nd	3.2	1.2	463	1320	29
49e	8.7	0.017	512	1	3	959	776	41
52c	53	0.18	294	18	5.9	313	817	26

[a] Pharmacokinetic parameters following iv bolus injection of a 1 mg/kg dose (compounds **34c** and **52c**) or 0.5 mg/kg dose (compound **49e**). [b]Clearance. [c]Fraction unbound in plasma. [d]Unbound clearance. [e]Volume of distribution at steady state. [f]Half-life. [g]AUC, area under the curve. [h]Oral bioavailability F was assessed following the administration of a 10 mg/kg dose (compounds **34c** and **52c**) or 1 mg/kg dose(compound **49e**).[i]Not determined.

The anti-staphylococcal activity of compound **49e** was confirmed *in vivo* in a neutropenic murine thigh infection model[52] (MIC *S. aureus* = 0.06 mg/L). When administered subcutaneously at a dose of 40 mg/kg, **49e** mediated a net 1-log reduction of colony forming units (CFU) in the thigh measured 6 h post infection.

CONCLUSION

We have reported the design of novel bacterial dual DNA gyrase and Topo IV inhibitors comprising a tetrahydropyran core and have elaborated SAR findings that led us to identify an ethylene-diol motif as a suitable moiety connecting the central THP-ring to the LHS. Our efforts to optimize antibacterial and pharmacological profile and simultaneously decrease block of hERG K^+ channel have resulted in synthesis and characterization of a series of potent dual GyrA/ParC inhibitors which display strong antibacterial activity against clinically relevant pathogens such as Staphylococci, Enterococci, and Streptococci. Furthermore, we have demonstrated that dual inhibition of gyrase and Topo IV is required to minimize the rate of resistance development. Although a fully balanced GyrA/ParC inhibition profile could be achieved, our efforts to further advance such balanced inhibitors were thwarted by unacceptable hERG K^+ channel block. However, we have identified compound **49e** which shows an exquisite antibacterial profile, while having a low to moderate resistance frequency *in vitro*

and significantly decreased hERG K^+ channel block. The pharmacokinetic parameters of **49e** were sufficient to reach exposures needed for *in vivo* efficacy in a murine thigh infection model.

THP-based bacterial topoisomerase inhibitors show promising properties and deserve further efforts to be eventually transformed into new clinically used antibacterial agents.

EXPERIMENTAL SECTION

MIC testing. Minimal inhibitory concentrations (MICs) were determined by the broth microdilution method according to guidelines of the Clinical and Laboratory Standards Institute (CLSI)[28]. Stock solutions of compounds were prepared in DMSO and serially diluted in cation-adjusted Mueller-Hinton broth (final DMSO concentration, 2.5 % (v/v). Reference antibiotics (ciprofloxacin, linezolid) were tested in parallel and MICs against quality control strains were within the accepted CLSI ranges.

Spontaneous resistance frequencies. Large numbers of bacteria (10^9-10^{10}) were plated on Mueller-Hinton agar plates containing the antibiotic compound at 4- and 16-fold the MIC concentration. Resistance frequencies were calculated by dividing the numbers of colonies growing after 48 h of incubation at 37°C through the total numbers of viable bacteria plated (as determined by colony count on drug-free agar).

DNA gyrase and Topo IV inhibition assays. DNA gyrase supercoiling inhibition assay was performed with relaxed pBR322 (purchased from Inspiralis, UK) as a substrate. For *E. coli*, the reaction mixture (20 µL) contained 25 mM Tris HCl pH 7.5, 24 mM KCl, 4 mM $MgCl_2$, 2 mM DTT, 1 mM ATP, 1.6 mM spermidine, 6.5% glycerol, 50 µg/mL Bovine Serum Albumin (BSA) and 0.1 µg relaxed plasmid. For *S. aureus* the reaction mixture (20 µL) contained 50 mM Tris HCl pH 7.5, 20 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 3 mM ATP, 700 mM potassium glutamate, 50 µg/mL BSA and 0.1 µg relaxed plasmid. The reactions were carried out at 37 °C for 1 h. DNA Topo IV relaxation assay was performed with supercoiled pBR322 as a substrate. For *E. coli*, the reaction mixture (20 µL) contained 50 mM Tris HCl pH 7.8. 20 mM KCl, 6 mM $MgCl_2$, 10 mM DTT, 1 mM ATP, 1 mM

spermidine, 100 µg/mL BSA and 0.1 µg supercoiled pBR322. For *S. aureus*, the reaction mixture (20 µL) contained 50 mM Tris HCl pH 7.5. 20 mM KCl, 5 mM $MgCl_2$, 5 mM DTT, 1.5 mM ATP, 5 mM spermidine, 100 mM potassium glutamate, 5 µg/mL BSA and 0.1 µg supercoiled pBR322. The reaction was carried out at 37 °C for 1 h. Both supercoiling and relaxation reactions were stopped by adding a mixture of EDTA, bromophenol blue and glycerol. 10 µL of each reaction mixture were loaded on a 1% agarose gel in TAE buffer (40 mM Tris-acetate, 1 mM EDTA pH 8.0) and run at 1.0 V/cm for 14 to 16 h. Gels were stained in water with ethidium bromide and visualized under ultraviolet light. For supercoiling inhibition assays (SCIA), 50% inhibitory concentration (IC50) was determined visually as being the compound concentration at which the supercoiled band was reduced by 50%. For relaxation inhibition assays (RIA), IC_{50} was defined as the concentration of inhibitor necessary to inhibit the formation of 50% relaxed DNA from supercoiled. The IC_{50} was taken as an average of two inhibitory concentrations if inhibition did not occur exactly within a particular concentration range (for representative gels, see Supporting Information) . All gels were also subject to analysis using AlphaEase Stand Alone Software (Alpha Innotech).

hERG assays[53]**.** Compounds were evaluated for block of the hERG K channel using CHO cells stably expressing the hERG gene (bSys, Witterswil, Switzerland) and the QPatch platform (Sophion, Ballerup, Denmark). K tail currents were measured at -40 mV following a 500-ms depolarization to +20 mV from a holding voltage of -80 mV. The external solution contained 4 mM K^+, 1 mM Mg^{2+} and 1.2 mM Ca^{2+}. Compound effects were quantified 3 minutes after application to the cells.

Mice, in life procedures. All animal housing and experiments were performed in agreement with the Swiss Federal Ordinance for animal protection, the animal welfare guide lines from the Cantonal Vetenary Office Basellandschaft, and the Actelion Pharmaceuticals Ltd internal animal welfare guidelines. Eight to twelve week-old, specific pathogen-free, female NMRI mice weighing 27 to 30 g were used for all studies (Harlan, The Netherlands). Mice were rendered neutropenic (neutrophils < $100/mm^3$) by injecting cyclophosphamide (Sigma) intraperitoneally 4 days (150 mg/kg) and 1 day

(100 mg/kg) before thigh infection. Previous studies have shown that this regimen produces neutropenia in this model for 5 days. Broth cultures of freshly plated *S. aureus* (laboratory descendent of DSM 11823) were grown to logarithmic phase overnight to an absorbance of 0.3 to 0.9 at 580 nm. Thigh infections with *S. aureus* were performed by injection of 0.05 ml of inoculum (107.0-8.0 CFU/ml) intra muscularly into both thighs of mice 2 h before administration of compound **49e** (formulated in 5% mannitol in water, administered at 10 ml/kg). Post-mortem, thigh samples were homogenized (Ultra-Turrax® tube drive - IKA), serially diluted and plated (spiral platter EddyJet® (IUL)). Results were expressed as colony forming units (CFU; CFU-counter Flash & Grow® - IUL), correlating to remaining bacterial load in the thigh at the end of the study.

Pharmacokinetics in the Rat. The pharmacokinetic profile was determined in the male Wistar rat (n = 3). For this purpose, compound **34c** was formulated as an aqueous solution at pH 4.5 for intravenous administration at a dose of 1 mg/kg and as aqueous suspension pH 5.6 for oral dosing at 10 mg/kg; compound **49e** was formulated as a solution in 5% DMSO in pure water for intravenous administration at a dose of 0.5 mg/kg, and as a microsuspension in 7.5% aqueous modified gelatin for oral dosing at 1 mg/kg; compound **52c** was formulated as a solution in 10 % PG, 10% PEG400 in water at pH 7 for intravenous administration at a dose of 1 mg/kg and as a microsuspension in 0.5% in methylcellulose for oral dosing at 10 mg/kg. Blood samples were taken at regular time intervals over a period of 24 h from a preimplanted catheter, and plasma was generated by centrifugation at 4000 rpm at 4 °C. Plasma concentrations were determined using a specific and sensitive LCMS/ MS method with a limit of quantification of 1.5 ng/mL.

General Chemical Methods. Starting materials, reagents and solvents were obtained from commercial sources and used as received. All reactions were carried out with continuous stirring under atmosphere of dry nitrogen. The resonance frequency for ^{1}H (^{13}C) on a Varian Gemini 300 is 300MHz (75MHz). Chemical shifts (δ) are reported in parts per million (ppm) relative to deuterated solvent as the internal standard (δH: $CDCl_3$ 7.26ppm, DMSO-*d6* 2.50ppm), coupling constants (*J*) are in hertz

(Hz). Peak multiplicities are expressed as follows: singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), multiplet (m), broad singlet (br s). Melting points were recorded with a Büchi 520 apparatus and are uncorrected. Purification of intermediates and final products was carried out on normal phase using an ISCO CombiFlash system and prepacked SiO_2 cartridges eluted with optimized gradients of either heptane-ethyl acetate mixture or dichloromethane-methanol (doped with 1% v/v of aq NH_4OH for basic compounds). Progress of the reactions was monitored by thin-layer chromatography (TLC) analysis (Merck, 0.2 mm silica gel 60 F^{254} on glass plates) or by LC-MS (Methods and equipments are described in Supporting Information). Unless otherwise stated, all target compounds had purity > 95%, established on a Waters Atlantis T3, 5 μm, 4.6 mm × 30 mm, eluting with a gradient of 5−95% of acetonitrile in water containing 0.04% of trifluoroacetic acid; or on a Waters XBridge C18, OBD, 5 μm, 4.6 mm × 50 mm (Waters, Switzerland), eluting with a gradient of 5−95% of acetonitrile in water containing 13 mM of NH_4OH UV at 230 nm and 254nm. Purity and identity were further confirmed by NMR spectroscopy. Optical rotations were measured on a Jasco P-1030 apparatus. HPLC-HRMS Methods and equipments are described in Supporting Information. Retention times (^{t}R) are expressed in minutes. Personal protective equipment (mask and gloves) should be worn when handling aldehydes **19b-27b** (irritant). Compound **23a** is commercially available.

***tert*-butyl ((2*S*)-1-hydroxy-4-(oxiran-2-yl)butan-2-yl)carbamate (9).** To a suspension of $LiBH_4$ (1.15 g, 53 mmol) in THF (300 mL) at rt was added a solution of **8**[30] (12.9 g, 53 mmol) in THF (100 mL). The mixture was stirred at rt for 4 h, poured into water and extracted with EtOAc. The organic layer was washed with brine, dried over $MgSO_4$ and concentrated to give the alcohol (11.4 g, 99% yield). Colorless oil; ^{1}H NMR δ ($CDCl_3$): 5.75-5.65 (m, 1H), 5.00-4.90 (m, 2H), 4.5 (br, 1H, OH), 3.70-3.45 (m, 3H), 2.10-2.00 (m, 2H), 1.60-1.35 (m, 2H), 1.38 (s, 9H). The latter (11.4 g, 53 mmol) was dissolved in DCE (300 mL) and water (250 mL) and 1 M phosphate buffer pH 8 (150 mL) was added. *m*-CPBA (14.3 g) was added and the mixture vigorously stirred overnight. The two phases were separated and the aq phase was extracted once more with DCM. The combined organic layers were washed with a saturated $NaHCO_3$ solution, dried over $MgSO_4$, filtered and concentrated to

dryness. The residue was purified by chromatography (heptane-EtOAc) to give **9** (7.74 g, 63% yield, mixture of diastereomers). Colorless oil; ^{1}H NMR δ ($CDCl_3$): 4.85-4.90 (m, 1H), 3.50-3.75 (m, 3H), 2.90-3.00 (m, 1H), 2.51-2.80 (m, 1H), 2.6 (br, 1H, OH), 2.50-2.55 (m, 1H), 1.40-1.80 (m, 4H), 1.42 (s, 9H).

***tert*-butyl ((3*S*,6*R*)-6-(hydroxymethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (*ent*-7).** A solution of the epoxide **9** (2.3 g, 10 mmol) in DCM (50 mL) was treated with CSA (0.23 g, 1 mmol). The mixture was stirred at rt for 3 h, diluted with a saturated $NaHCO_3$ solution (100 mL). The two layers were separated and the organic layer was dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (EtOAc) to give *ent*-**7** (0.874 g, 37% yield). Colorless solid; m.p.= 112.9 °C; $[\alpha]_D$= + 7.1 (*c* 0.96, MeOH); ^{1}H NMR δ ($CDCl_3$): 4.28 (br, 1H), 4.10-4.20-10 (m, 1H), 3.30-3.70 (m, 5H), 3.04 (t, *J* =10.6 Hz, 1H), 2.00-2.20 (m, 2H), 1.20-1.75 (m, 11H); ^{13}C NMR δ (DMSO-*d6*) 155.6, 78.5, 78.3, 70.9, 65.0, 47.1, 29.8, 28.9 (3C), 27.8;; ESI-MS $(M+H)^+$ m/z 232.2; HR ESI-MS $(M+Na)^+$ m/z = 254.1370 (calc. for $C_{11}H_{21}NO_4Na$: 254.1368); ^{t}R = 0.84.

***tert*-butyl ((3*R*,6*S*)-6-carbamoyltetrahydro-2*H*-pyran-3-yl)carbamate (12)**. To a solution of **11** (3 g, 12.2 mmol, prepared from **7** as described[29]) in EtOAc (50 mL) was added NHS (1.5 g, 13 mmol) and DCC (2.7 g, 13.1 mmol). The reaction was stirred overnight at rt. After filtration and concentration to dryness, the residue was taken up in THF (180 mL). Gaseous NH_3 was bubbled through the solution for 10 min, and the reaction proceeded at rt for 1 h. Silica gel (20 g) was added, and the volatiles were removed *in vacuo*. The residue was purified by chromatography (DCM-MeOH 19-1) to afford **12** (1.3 g, 43%). White solid; ^{1}H NMR δ (DMSO-*d6*): 7.15 (br s, 1H), 7.07 (br s, 1H), 6.83 (d, *J* = 8.0 Hz, 1H), 3.85 (m, 1H), 3.56 (m, 1H), 3.32 (m, 1H), 3.01 (t, 10.6 Hz, 1H), 1.84-1.96 (m, 2H), 1.34-1.43 (m, 2H), 1.36 (s, 9H); ^{13}C NMR δ (DMSO-*d6*): 173.2, 155.5, 78.3, 76.9, 70.4, 46.5, 29.6, 28.7 (3C), 28.5; HR ESI-MS $(M+H)^+$ m/z = 245.1502 (calc. for $C_{11}H_{21}N_2O_4$: 245.1501); ^{t}R = 0.82.

(*tert*-butyl ((3*R*,6*S*)-6-formyltetrahydro-2*H*-pyran-3-yl)carbamate (13). To a solution of **7** (37.5 g, 162.13 mmol) in DCM (310 mL) cooled to -10°C was added DIPEA (84.75 mL, 495 mmol).

A solution of py.SO_3 complex (69.47 g, 218 mmol) in DMSO (225 mL) was slowly added. The reaction mixture was stirred for 2 h at 0 °C .The reaction mixture was partitioned between water (150 mL) and DCM (220 mL). The two layers were separated and the aq layer was extracted twice with DCM (2 x 150 mL). The combined organic layers were dried over Na_2SO_4, filtered and concentrated to dryness. The residue was filtered through silica gel (EtOAc-heptane) to afford **13** (33.58 g, 90%). White solid; m.p.= 108.9 °C; ^{1}H NMR δ ($CDCl_3$): 9.63 (br s, 1H), 4.37 (br s, 1H), 4.16 (ddd, *J* = 1.7, 4.5, 10.9 Hz, 1H), 3.72 (dd, *J* = 2.9, 10.7 Hz, 1H), 3.62 (br s, 1H), 3.14 (t, *J* = 10.4 Hz, 1H), 2.12 (m, 1H), 1.96 (m, 1H), 1.56 (m, 1H), 1.44 (s, 9H), 1.38 (overlaid m, 1H);^{13}C NMR δ ($CDCl_3$): 201.3, 155.1, 80.6, 79.7, 70.8, 45.9, 29.2, 28.4 (3C), 25.0; ESI-MS $(M+H)^+$ m/z 230.0; HR ESI-MS $(M+Na)^+$ m/z = 252.1214 (calc. for $C_{11}H_{19}NO_4Na$: 252.1211); ^{t}R = 0.78.

***tert*-butyl ((3*R*,6*S*)-6-vinyltetrahydro-2*H*-pyran-3-yl)carbamate (14).** *t*-BuOK (31.74 g, 282.89 mmol) was added in one portion to a white suspension of methyltriphenylphosphonium bromide (101.05 g, 282.89 mmol) in THF (340 mL) at rt under nitrogen. The resulting suspension was stirred for 1 h at rt and a solution of **13** (32.43 g, 141.44 mmol) in THF (85 mL) was added. The mixture was stirred 30 min. at rt. A 10% $NaHSO_4$ solution (120 mL) was added and the mixture was diluted with EtOAc (200 mL). The two layers were decanted and the aq layer was extracted once with EtOAc (250 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (heptane-EtOAc) to afford **14** (28.98 g, 90%). White solid; ^{1}H NMR δ ($CDCl_3$): 5.84 (ddd, *J* = 5.6, 10.5, 17.3 Hz, 1H), 5.24 (dt, *J* = 1.5, 17.3 Hz, 1H), 5.12 (dt, *J* = 1.5, 10.5 Hz, 1H), 4.26 (br. s, 1H), 4.10 (ddd, *J* = 2.1, 4.7, 10.8 Hz, 1H), 3.73 (m, 1H), 3.61 (m, 1H), 3.06 (t, *J* = 10.5 Hz, 1H), 2.10 (m, 1H), 1.79 (m, 1H), 1.25-1.60 (m, 2H), 1.44 (s, 9H); ESI-MS $(M+H)^+$ m/z= 228.2.

***tert*-butyl ((3*R*,6*S*)-6-(2-oxoethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (15).** To an ice-chilled solution of **14** (1.47 g, 6.46 mmol) in THF (30 mL) was added 9-BBN-H (2.37 g, 19.4 mmol). The mixture was stirred overnight at rt. After cooling to 0 °C, 3 N NaOH (25 mL) and 50% aq H_2O_2 (12 mL) were cautiously added. The resulting mixture was stirred at rt for 2 h. The two layers were decanted and the aq layer was extracted with EtOAc (2 x 200 mL). The combined organic layers were

washed with 10% aq $NaHSO_3$ solution (100 mL), water (50 mL) and dried over Na_2SO_4. After filtration and concentration to dryness, the residue was purified by chromatography (heptane-EtOAc) to afford the resulting alcohol (1.15 g, 72%). White solid; ^{1}H NMR δ ($CDCl_3$): 4.16 (br s, 1H), 4.01 (ddd, *J* = 2.2, 4.7, 10.7 Hz, 1H), 3.69 (t, *J* = 5.2 Hz, 2H), 3.52 (br s, 1H), 3.39 (m, 1H), 2.93 (t, *J* = 10.7 Hz, 1H), 2.02 (br s, 1H), 2.00 (m, 1H), 1.58-1.72 (m, 3H), 1.45 (m, 1H), 1.37 (s, 9H), 1.24 (qd, *J* = 4.1, 12.2 Hz, 1H); ESI-MS $(M+H)^+$ m/z 246.4. The latter alcohol (1.15 g, 4.68 mmol) was dissolved in DCM (20 mL), and after cooling to 0 °C, a solution of Dess-Martin periodinane (15 wt% in DCM, 18 mL) was added. After warming to rt, the reaction proceeded for 2 h. The reaction mixture was concentrated to dryness and the residue purified by chromatography (heptane-EtOAc) to afford **15** (1.06 g, 93%). White solid; ^{1}H NMR δ ($CDCl_3$): 9.79 (t, *J* = 2.3 Hz, 1H), 4.29 (br s, 1H), 4.07 (ddd, *J* = 2.1, 4.7, 10.7 Hz, 1H), 3.77 (m, 1H), 3.61 (br s, 1H), 3.04 (t, *J* = 10.7 Hz, 1H), 2.65 (ddd, *J* = 2.3, 7.6, 16.5 Hz, 1H), 2.49 (ddd, *J* = 2.3, 4.8, 16.5 Hz, 1H), 2.12 (m, 1H), 1.79 (m, 1H), 1.50 (overlaid m, 1H), 1.49 (s, 9H), 1.33 (qd, *J* = 4.1, 12.2 Hz, 1H); ^{13}C NMR δ ($CDCl_3$): 200.9, 155.1, 79.5, 72.3, 71.3, 49.2, 46.2, 30.7, 30.3, 28.3 (3C); HR ESI-MS $(M+Na)^+$ m/z = 266.1367 (calc. for $C_{12}H_{21}NO_4Na$: 266.1368); ^{t}R = 0.81.

***tert*-butyl ((3*R*,6*S*)-6-(iodomethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (16).** To an ice-chilled solution of **7** (40.9 g, 176.8 mmol)[29] in DCM (840 mL) were added successively Et_3N (59.5 mL, 423.9 mmol), DMAP (3.01 g, 24.56 mmol) and TsCl (42.4 g, 222.4 mmol). The reaction proceeded 4 h with warming to rt. A saturated $NaHCO_3$ solution (350 mL) was added. The two phases were separated and the organic layer was evaporated *in vacuo*. The residue was diluted with EtOAc (900 mL) and the organic layer was washed three times with a saturated $CuSO_4$ solution (3 x 200 mL), brine (200 mL), dried over $MgSO_4$, filtered and concentrated to dryness to afford the crude tosylate (75.83 g); ^{1}H NMR δ ($CDCl_3$): 7.78 (d, *J* = 8.7 Hz, 2H), 7.33 (d, *J* = 8.7 Hz, 2H), 4.20 (br s, 1H), 4.01 (ddd, *J* = 2.1, 4.7, 10.8 Hz, 1H), 3.96 (d, *J* = 5.7 Hz, 2H), 3.54 (br s, 1H), 3.48 (m, 1H), 2.93 (t, *J* = 10.8 Hz, 1H), 2.44 (s, 3H), 2.09 (m, 1H), 1.69 (m, 1H), 1.48-1.18 (m, 2H), 1.42 (s, 9H); ESI-MS $(M+H)^+$ m/z =386.2. The latter (75.83 g) was taken up in acetone (700 mL) and NaI (90.0 g, 600.4 mmol) was added. The reaction mixture was refluxed 24 h. The reaction mixture was cooled to rt and

diluted with water (500 mL). The volatiles were removed *in vacuo*. The solid was filtered off, washed with water, partitioned between EtOAc (700 mL) and water (300 mL). The organic layer was dried over $MgSO_4$, filtered, evaporated *in vacuo* to afford **16** (59.7 g, 99%). White solid; m.p.= 128.9 °C; ^{1}H NMR δ ($CDCl_3$): 4.20 (br. s, 1H), 4.10 (ddd, *J* = 2.1, 4.8, 10.8 Hz, 1H), 3.60 (br. s, 1H), 3.28 (m, 1H), 3.17 (d, *J* = 6.3 Hz, 2H), 3.04 (t, *J* = 10.8 Hz, 1H), 2.10 (m, 1H), 1.94 (m, 1H), 1.44-1.20 (m, 2H), 1.43 (s, 9H); ^{13}C NMR δ ($CDCl_3$): 155.3, 79.7, 76.7, 71.5, 46.4, 30.8, 30.2, 28.6 (3C), 8.7; HR ESI-MS $(M+H-C_4H_8)^+$ m/z = 285.9943 (calc. for $C_7H_{13}NO_3I$: 285.9940); ^{t}R = 1.26.

***tert*-butyl ((3*R*,6*S*)-6-(((1-phenyl-1*H*-tetrazol-5-yl)thio)methyl)tetrahydro-2*H*-pyran-3-yl)carbamate (17).** To a mixture of PT-SH (37.50 g, 210.4 mmol) in EtOH (700 mL) was added powdered KOH (14.0 g, 249.5 mmol). The mixture was refluxed for 1 h and a solution of **16** (59.40 g, 174.1 mmol) in EtOH (500 mL) was added. The reaction mixture was refluxed overnight. Water (400 mL) was added and the volatiles were removed *in vacuo*. The solid was filtered off, washed with water and dried to afford **17** (59.68 g). White solid; ^{1}H NMR δ ($CDCl_3$): 7.51-7.62 (m, 5H), 4.21 (br. s, 1H), 4.07 (ddd, *J* = 2.1, 4.6, 10.8 Hz, 1H), 3.62-3.71 (m, 2H), 3.57 (br. s, 1H), 3.34 (m, 1H), 2.99 (t, *J* = 10.8 Hz, 1H), 2.11 (m, 1H), 1.90 (m, 1H), 1.50 (m, 1H), 1.42 (s, 9H), 1.32 (qd, *J* = 3.8, 12.2 Hz, 1H); ESI-MS $(M+H)^+$ m/z = 392.5.

***tert*-butyl ((3*R*,6*S*)-6-(((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)methyl)tetrahydro-2*H*-pyran-3-yl)carbamate (18). 17** (59.68 g, 152.4 mmol) was dissolved in THF (400 mL) and EtOH (400 mL). A solution of $(NH_4)_6Mo_7O_{24}.4H_2O$ (18.9 g, 15.29 mmol) in 50% aq H_2O_2 (87 mL, 1.53 mol) was added at rt and the reaction mixture was heated to 65 °C for 3 h, cooled down to rt and diluted with water (500 mL). The volatiles were removed *in vacuo*. The residue was extracted with EtOAc (2 x 500 mL). The combined organic layers were washed with 10% aq $Na_2S_2O_3$ solution (3 x 400 mL), a saturated $NaHSO_3$ solution (3 x 400 mL), water (200 mL) and brine (200 mL), dried over $MgSO_4$, filtered and concentrated to dryness. The solid was recrystallized from an EtOAc-heptane mixture to afford **18** (63.72 g, 86%). White solid; m.p.= 122.7 °C; ^{1}H NMR δ (DMSO-*d6*): 7.64-7.74 (m, 5H), 6.72 (d, *J* = 7.9 Hz, 1H), 4.04 (d, *J* = 13.6 Hz, 1H), 3.61-3.76 (m, 2H), 3.53 (dd, *J* = 2.7, 9.6 Hz, 1H), 3.23 8br s, 1H), 2.88 (t, *J* = 10.8 Hz, 1H), 1.82 (br s, 1H), 1.65-1.72 (m, 1H), 1.30-1.41 (m, 2H), 1.36

(s, 9H); ^{13}C NMR δ ($CDCl_3$): 155.0, 154.0, 133.0, 131.5 (2C), 129.6 (3C), 125.9, 79.8, 71.4, 70.9, 60.8, 45.6, 30.0, 29.8, 28.4 (3C); HR ESI-MS $(M+H)^+$ m/z = 424.1660 (calc. for $C_{18}H_{26}N_5O_5S$: 424.1654); ^{t}R = 1.27.

***tert*-butyl ((3*S*,6*R*)-6-(((1-phenyl-1*H*-tetrazol-5-yl)sulfonyl)methyl)tetrahydro-2*H*-pyran-3-yl)carbamate (*ent*-18).** Compound *ent*-**18** (5.9 g) was obtained starting from ***ent*-7** (8.2 g, 35.5 mmol) and using the procedures reported for the preparation of **18**. Colorless solid; NMR identical to **18**; ESI-MS $(M+H)^+$ m/z = 424.4.

***tert*-butyl ((3*R*,6*S*)-6-(((6-methoxy-1,5-naphthyridin-4-yl)oxy)methyl)tetrahydro-2*H*-pyran-3-yl)carbamate (28a).** To a solution of **7** (1 g, 4.32 mmol), **23e**[26] (0.92 g, 5.2 mmol) and PPh_3 (1.71 g, 6.5 mmol) in THF (32 mL) and DMF (2.2 mL) was added drop wise DIAD (1.32 g, 6.5 mmol). The reaction mixture was stirred at rt for 4 h and concentrated to dryness. The residue was diluted with 0.2 N HCl (110 mL) and the aq. layer was washed with Et_2O. The pH was made basic with 1 N NaOH (22 mL) and the aq layer was extracted twice with EtOAc. The combined organic layers were washed with brine and dried over Na_2SO_4, filtered and concentrated. The residue was recrystallized from EtOAc to afford **28a** (0.5 g, 1.28 mmol, 30% yield). Yellowish solid; m.p.= 173 °C; ^{1}H NMR δ (DMSO-*d6*): 8.57 (d, *J* = 5.2 Hz, 1H), 8.17 (d, *J* = 9.0 Hz, 1H), 7.21 (d, *J* = 9.0 Hz, 1H), 7.18 (d, *J* = 5.2 Hz, 1H), 6.77 (m, 1H), 4.14-4.29 (m, 2H), 3.99 (s, 3H), 3.82 (m, 1H), 3.67 (m, 1H), 3.36 (m, 1H), 3.10 (t, *J* = 10.5 Hz, 1H), 1.81-1.97 (m, 2H), 1.33-1.63 (m, 2H), 1.36 (s, 9H); ^{13}C NMR ($CDCl_3$) δ: 161.5, 159.7, 155.2, 148.7, 142.7, 139.9, 134.3, 116.5, 105.5, 79.5, 75.1, 71.4, 71.3, 53.7, 46.4, 30.1, 28.4 (3C), 27.5; ESI-MS $(M+H)^+$ m/z =390.2.

General Procedure A: Boc deprotection

A solution of the Boc-protected amine (1 eq.) in TFA (2 mL/mmol) was stirred at rt for 1 h. The solvent was evaporated *in vacuo*, and the residue was partitioned between DCM-MeOH mixture (9-1, 10 mL/mmol) and saturated $NaHCO_3$ solution (5 mL/mmol). The pH was adjusted to 10 adding 32% aq NaOH. The two layers were separated and the aq layer was extracted three times with the same mixture (3 x 10mL/mmol) and the combined organic layers were dried over Na_2SO_4, filtered and

concentrated to dryness. The residue was purified by chromatography (DCM-MeOH 19-1, containing 1% aq NH_4OH) to afford the corresponding amine.

(3*R*,6*S*)-6-(((6-methoxy-1,5-naphthyridin-4-yl)oxy)methyl)tetrahydro-2*H*-pyran-3-amine (28b). The compound **28b** was prepared in 98% yield from **28a** according to the general procedure A. Yellowish oil; ^{1}H NMR δ (DMSO-*d6*): 8.57 (d, *J* = 5.3 Hz, 1 H), 8.17 (d, *J* = 9.0 Hz, 1 H), 7.22 (d, *J* = 9.0 Hz, 1 H), 7.18 (d, *J* = 9.0 Hz, 1 H), 4.15-4.29 (m, 2H), 3.99 (s, 3H), 3.85 (m, 1H), 3.70 (m, 1H), 3.04 (t, *J* = 10.4 Hz, 1H), 2.74 (m, 1H), 2.00 (m, 1H), 1.87 (m, 1H), 1.52 (m, 1H), 1.34 (m, 1H); ^{13}C NMR δ ($CDCl_3$): 161.6, 159.8, 148.7, 142.6, 139.9, 134.4, 116.6, 105.6, 75.2, 72.7, 71.5, 53.9, 47.3, 31.4, 27.3; ESI-MS $(M+H)^+$ m/z = 290.4.

General method B: Reductive amination using aldehyde 32 or 33

To a solution of amine (1eq.) in DCE (10 mL/mmol) and MeOH (4 mL/mmol) were added 3 Å molecular sieves (3 g/mmol) and **32** or **33** (1.1eq.). The reaction was heated at 50 °C overnight. After cooling to 0 °C, $NaBH_4$ (400 mol%) was added and the reaction was stirred for 2 h. The reaction mixture was filtered, and the filtrate was diluted with DCM-MeOH (9-1, 10 mL/mmol) and saturated $NaHCO_3$ solution (5 mL/mmol). The two layers were separated and the organic layer was dried over dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (DCM-MeOH containing 1% aq. NH_4OH) to afford the alkylated product.

6-((((3*R*,6*S*)-6-(((6-methoxy-1,5-naphthyridin-4-yl)oxy)methyl)tetrahydro-2H-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (28c). The compound **28c** was obtained in 55% yield from the compounds **28b** and **32** according to the general procedure B. White solid; m.p.= 202 °C (degradation); ^{1}H NMR δ (DMSO-*d6*): 10.8 (br s, 1H), 8.59 (d, *J* = 5.2 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.75 (d, *J* = 7.8 Hz, 1H), 7.24 (d, *J* = 9.0 Hz, 1H), 7.20 (d, *J* = 5.2 Hz, 1H), 7.10 (d, *J* = 7.9 Hz, 1H), 4.26 (dd, *J* = 6.0, 10.9 Hz, 1H), 4.19 (dd, *J* = 3.8, 10.9 Hz, 1H), 3.99 (s, 3H), 3.97 (partially overlaid m, 1H), 3.70-3.79 (m, 3H), 3.53 (s, 2H), 3.04 (t, *J* = 10.5 Hz, 1H), 2.14 (br s, 1H), 2.08 (m, 1H), 1.86 (m, 1H), 1.51 (m, 1H), 1.30 (m, 1H); ^{13}C NMR δ (DMSO-*d6*): 166.6, 161.2, 159.7, 158.2, 149.5, 149.2, 142.6 , 140.6, 136.4, 134.1, 117.1, 116.7, 113.2, 106.5, 75.5, 72.2, 71.7,

53.8, 53.4, 51.6, 30.5, 29.3, 27.4; HR ESI-MS $(M+H)^+$ m/z = 468.1699 (calc. for $C_{23}H_{26}N_5O_4S$: 468.1705); ^tR = 0.91.

(3*R*,6*S*)-N-((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)-6-(((6-methoxy-1,5-naphthyridin-4-yl)oxy)methyl)tetrahydro-2*H*-pyran-3-amine (28d). The compound **28d** was obtained in 51% yield from the compounds **28b** and **33** according to the general procedure B. Colorless solid; ^{1}H NMR δ (DMSO-*d6*): 8.57 (d, *J* = 5.2 Hz, 1 H), 8.17 (d, *J* = 9.0 Hz, 1 H), 7.99 (s, 1 H), 7.21 (d, *J* = 9.1 Hz, 1 H), 7.17 (d, *J* = 5.3 Hz, 1 H), 6.92 (s, 1 H), 4.30-4.34 (m, 2H), 4.24-4.28 (m, 2H), 4.13-4.24 (m, 2H), 3.97 (s, 3H), 3.94 (overlaid m, 1H), 3.72 (overlaid m, 1H), 3.67 (br s, 2H), 3.00 (t, *J* = 10.5 Hz, 1H), 2.50 (overlaid m, 1H), 1.99-2.10 (m, 2H), 1.83 (m, 1H), 1.47 (m, 1H), 1.26 (m, 1H); ^{13}C NMR δ ($CDCl_3$): 161.5, 159.8, 153.5, 150.2 , 148.8 , 142.8, 140.1, 140.0, 138.8, 134.4, 116.6, 110.6 , 105.6, 75.6, 72.7, 71.8, 65.0, 64.1, 53.7, 53. 4, 52.1, 30.7, 27.7; HR ESI-MS $(M+H)^+$ m/z = 439.1988 (calc. for $C_{23}H_{27}N_4O_5$: 439.1981)); ^tR = 0.89.

***tert*-butyl ((3*R*,6*S*)-6-((6-methoxy-1,5-naphthyridin-4-yl)carbamoyl)tetrahydro-2*H*-pyran-3-yl)carbamate (29a).** To a degassed mixture of **12** (0.8 g, 3.2 mmol), Cs_2CO_3 (1.3 g, 4 mmol), BINAP (0.145 g, 0.23 mmol) and $(dba)_3Pd_2.CHCl_3$ (0.057 g, 0.055 mmol) was added dioxane (41 mL). **23a** (0.76 g, 3.2 mmol) was added and the reaction proceeded overnight at 100 °C. After filtration, the filtrate was concentrated *in vacuo* and the residue was purified by chromatography (DCM-MeOH 19-1) to afford **29a** (1.3 g, 99% yield). Beige foam; m.p.= 131.2 °C; ^{1}H NMR δ ($CDCl_3$): 10.57 (s, 1H), 8.70 (d, *J* = 5.2 Hz, IH), 8.51 (d, *J* = 5.2 Hz, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 7.16 (d, *J* = 9.0 Hz, 1H), 4.32 (m, 2H), 4.12 (s, 3H), 4.01 (dd, *J* = 2.5, 11.4 Hz, IH), 3.72 (m, IH), 3.23 (t, *J* = 10.6 Hz, 1H), 2.39 (qd, *J* = 2.8, 10.2 Hz, 1H), 2.22 (m, 1H), 1.76 (m, 1H), 1.47 (overlaid m, 1H), 1.47 (s, 9H): ^{13}C NMR δ ($CDCl_3$): 170.1, 161.4, 155.2, 149.1, 141.0, 140.5, 139.0, 132.2, 116.7, 110.8, 79.8, 77.1, 71.3, 53.2, 38.6, 30.0, 28.4 (3C), 28.0; HR ESI-MS $(M+HH)^+$ m/z = 403.1985 (calc. for $C_{20}H_{27}N_4O_5$: 403.1981); ^tR = 0.84.

(2*S*,5*R*)-5-amino-N-(6-methoxy-1,5-naphthyridin-4-yl)tetrahydro-2*H*-pyran-2-carboxamide (29b). The compound **29b** was obtained in 80% yield from the compound **29a**

according to the general procedure A. Yellowish solid; m.p.= 205.7 °C; ^{1}H NMR δ (DMSO-*d6*): 10.52 (s, 1 H), 8.72 (d, *J* = 5.0 Hz, 1 H), 8.40 (d, *J* = 5.0 Hz, 1 H), 8.30 (d, *J* = 9.1 Hz, 1 H), 7.35 (d, *J* = 9.1 Hz, 1 H), 5.31 (br s, 2H), 4.09-4.16 (m, 2H), 4.07 (s, 3H), 3.30 (overlaid m, 1H), 2.98 (m, 1H), 2.18 (m, 1H), 2.07 (m, 1H), 1.60 (m, 1H), 1.50 (m, 1H); ^{13}C NMR δ ($CDCl_3$): 170.3, 161.5, 149.5, 141.2, 138.8, 131.8, 117.3, 110.4 , 76.6 , 72.0, 53.9, 46.8 , 38.7, 30.7, 27.9; ESI-MS $(M+H)^+$ m/z = 303.4.

(2*S*,5*R*)-N-(6-methoxy-1,5-naphthyridin-4-yl)-5-(((3-oxo-3,4-dihydro-2*H*-pyrido[3,2-*b*][1,4]thiazin-6-yl)methyl)amino)tetrahydro-2*H*-pyran-2-carboxamide (29c). The compound **29c** was obtained in 27% yield from the compounds **29b** and **32** according to the general procedure B. White solid; m.p.= 234 °C (degradation); ^{1}H NMR δ (DMSO-*d6*): 10.8 (br s, 1H), 10.4 (br s, 1H), 8.68 (d, *J* = 5.0 Hz, 1H), 8.36 (d, *J* = 5.0 Hz, 1H), 8.26 (d, *J* = 9.2 Hz, 1H), 7.73 d *J* = 7.9 Hz, 1H), 7.31 (d, *J* = 9.2 Hz, 1H), 7.08 (d, *J* = 7.9 Hz, 1H), 4.15 (m, 1H), 4.05 (overlaid m, 1H), 4.02 (s, 3H), 3.74 (br s, 2H), 3.50 (s, 2H), 3.30 (overlaid m, 1H), 2.61 (m, 1H), 2.04-2.27 (m, 3H), 1.32-1.54 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =481.1656 (calc. for $C_{23}H_{25}N_6O_4S$: 481.1658); ^{t}R = 1.18.

(2*S*,5*R*)-5-(((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)-N-(6-methoxy-1,5-naphthyridin-4-yl)tetrahydro-2*H*-pyran-2-carboxamide (29d). The compound **29d** was obtained in 55% yield from the compounds **29b** and **33** according to the general procedure B. White solid; m.p.= 68.8 °C; ^{1}H NMR δ (DMSO-*d6*): 10.51 (s, 1H), 8.70 (d, *J* = 5.0 Hz, 1H), 8.38 (d, *J* = 5.0 Hz, 1H), 8.28 (d, *J* = 9.0 Hz, 1H), 7.32 (d, *J* = 9.0 Hz, 1H), 7.25 (d, *J* = 7.9 Hz, 1H), 6.97 (d, *J* = 7.9 Hz, 1H), 4.38 (m, 2H), 4.22 (m, 2H), 4.18 (partially overlaid dd, *J* = 3.0, 10.6 Hz, 1H), 4.07 (partially overlaid m, 1H), 4.06 (s, 3H), 3.67 (dd, AB system, *J* = 14.6 Hz, 2H), 3.25 (t, *J* = 10.6 Hz, 1H), 2.61 (m, 1H), 2.15 (m, 3H), 1.56-1.36 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =452.1932 (calc. for $C_{23}H_{26}N_5O_5$: 452.1933) ; ^{t}R = 1.17.

***tert*-butyl ((3*R*,6*S*)-6-(2-hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (30a).** To a solution of **23a** (5.88 g, 24.6 mmol) in THF (75 mL), cooled to -78 °C, was added quickly *n*-butyl lithium (2.35*N* in Heptane, 10.2 mL). The mixture was stirred at -78 °C for 15 min and a solution of **15** (2.0 g, 8.22 mmol) in THF (10 mL)

was added. After 5 min, 10% aq $NaHSO_4$ solution (20 mL) and EtOAc (10 mL) were added. The two layers were decanted and the aq layer was extracted with EtOAc (2 x 100 mL). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (heptane-EtOAc) to yield **30a** (1.6 g, 48% yield, 1:1 mixture of epimers). Yellowish foam; ESI-MS $(M+H)^+$ m/z = 404.1.

2-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-1-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (30b). The compound **30b** was prepared in 37% yield from the compound **30a** according to the general procedure A. **30b** was recovered as a 1:1 mixture of epimers. Colorless foam; ESI-MS $(M+H)^+$ m/z = 304.2.

6-((((3*R*,6*S*)-6-(2-hydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2H-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (30c). The compound **30c** was obtained in 71% yield from the compounds **30b** and **32** according to the general procedure B. Yellowish foam; ^{1}H NMR δ (DMSO-*d6*) mixture of epimers: 10.86 (s, 1H), 8.77 (d, *J* = 4.6 Hz, 0.5H), 8.75 (d, *J* = 4.6 Hz, 0.5H), 8.25 (d, *J* = 9.1 Hz, 0.5H), 8.24 (d, *J* = 9.1 Hz, 0.5H), 7.75-7.71 (m, 2H), 7.25 (d, *J* = 9.1 Hz, 0.5H), 7.23 (d, *J* = 9.1 Hz, 0.5H), 7.08 (d, *J* = 7.8 Hz, 0.5H), 7.06 (d, *J* = 7.8 Hz, 0.5H), 5.74 (m, 0.5H), 5.63 (m, 0.5H), 5.39 (d, *J* = 4.9 Hz, 0.5H), 5.35 (d, *J* = 5.2 Hz, 0.5H), 4.01 (s, 1.5H), 4.00 (s, 1.5H), 4.00-3.79 (m, 1H), 3.71 (br s, 2H), 3.52 (s, 2H), 3.52 (overlaid m, 0.5H), 3.43 (m, 0.5H), 2.96 (t, *J* = 10.3 Hz, 0.5H), 2.90 (t, *J* = 10.5 Hz, 0.5H), 2.46 (m, 1H), 2.08-1.74 (m, 4H), 1.64-1.55 (m, 1H), 1.31-1.14 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =482.1859 (calc. for $C_{24}H_{28}N_5O_4S$: 482.1862); ^{t}R = 1.15.

2-((2*S*,5*R*)-5-(((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-1-(6-methoxy-1,5-naphthyridin-4-yl)ethan-1-ol (30d). The compound **30d** was obtained in 52% yield from the compounds **30b** and **33** according to the general procedure B. White foam; ^{1}H NMR δ (DMSO-*d6*) mixture of epimers : 8.74 (d, *J* = 4.6 Hz, 0.5H), 8.73 (d, *J* = 4.6 Hz, 0.5H), 8.20-8.24 (two overlaid d, *J* = 9.1 Hz, 2x0.5H), 7.98 (s, 0.5H), 7.97 (s, 0.5H), 7.70-7.73 (two overlaid d, *J* = 4.6 Hz, 2x0.5H), 7.19-7.24 (two overlaid d, *J* = 9.1 Hz, 2x0.5H), 6.90 (s, 0.5H),

6.89 (s, 0.5H), 5.73 (m, 0.5H), 5.61 (m, 0.5H), 5.35 (d, *J* = 5.0 Hz, 0.5H), 5.32 (d, *J* = 5.2 Hz, 0.5H), 4.28-4.33 (m, 2H), 4.23-4.27 (3, 2H), 3.98 (s, 3x0.5H), 3.97 (s, 3x0.5H), 3.77-3.91 (m, 1H), 3.58-3.70 (m, 2H), 3.36-3.54 (m, 1H), 2.92 (t, *J* = 10.3 Hz, 0.5H), 2.86 (t, *J* = 10.5 Hz, 0.5H), 2.37-2.50 (m, 1H), 2.11 (br s, 1H), 1.77-2.02 (m, 3H), 1.49-1.63 (m, 1H), 1.12-1.28 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =453.2135 (calc. for $C_{24}H_{29}N_4O_5$: 453.2137) ; ^{t}R = 1.17.

General Procedure C: Julia-Kocieński coupling to form (*E*)-alkenes

To a solution of **18** or *ent*-**18** (1 eq.) and the appropriate aldehyde (1 eq.) in DME (5 mL/mmol), cooled to -60 °C, was added drop wise KHMDS (0.5 M in toluene, 1.8 eq.) or LiHMDS (1 M in THF, 1.8eq.) over 30 min. The mixture was then allowed to warm up slowly to rt. After 45 min., water (10 mL/mmol) and EtOAc (10 mL/mmol) were added. The two layers were decanted and the aq. layer was extracted twice with EtOAc (2 x 10 mL/mmol). The combined organic layers were washed with brine, dried over Na_2SO_4 and concentrated to dryness. The residue was triturated in *tert*-butyl methyl ether (10 mL/mmol), filtered and dried to afford the corresponding (*E*)-alkene.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(6-methoxy-1,5-naphthyridin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (31a).** The compound **31a** was prepared in 68% yield from **23b** and **18** (KHMDS) according to the general procedure C. Off-white solid; m.p.= 180.9 °C; ^{1}H NMR δ (DMSO-*d6*): 8.72 (d, *J* = 4.7 Hz, 1H), 8.25 (d, *J* = 9.0 Hz, 1H), 7.84 (d, *J* = 4.7 Hz, 1H), 7.55 (d, *J* = 16.5 Hz, 1H), 7.28 (d, *J* = 9.0 Hz, 1H), 6.93 (dd, *J* = 5.3 Hz, 16.5 Hz, 1H), 6.85 (d, *J* = 7.7 Hz, 1H), 4.04 (s, 3H), 4.01 (partially overlaid m, 1H), 3.90 (m, 1H), 3.39 (br s, 1H), 3.10 (t, *J* = 10.6 Hz, 1H), 1.89 (m, 2H), 1.50 (m, 2H), 1.39 (s, 9H); ^{13}C NMR δ ($CDCl_3$): 161.5, 155.2, 147.6, 142.3, 140.6, 140.3, 139.3, 135.5, 124.5, 119.4, 116.5, 84.3, 79.5, 77.4, 71.1, 53.7, 46.3, 30.9, 30.3, 28.4 (3C); HR ESI-MS $(M+H)^+$ m/z = 386.2081 (calc. for $C_{21}H_{28}N_3O_4$: 386.2080); ^{t}R = 1.24.

***tert*-butyl ((3*S*,6*R*)-6-((E)-2-(6-methoxy-1,5-naphthyridin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (*ent*-31a).** Compound *ent*-**31a** was prepared in 75% yield from **23b** and *ent*-**18** using the general procedure C (KHMDS). Colorless solid; ^{1}H NMR (DMSO-*d6*) δ: 8.70 (d, , *J* =4.6 Hz, 1H), 8.23 (d, *J* =9.1 Hz, 1H), 7.82 (d, *J* = 4.6 Hz, 1H), 7.54 (d, *J* = 16.3 Hz, 1H), 7.26 (d, *J* =

9.1 Hz, 1H), 6.91 (dd, *J* = 5.3, 16.3 Hz, 1H), 6.88 (br s, 1H), 4.03 (s, 3H), 3.95-4.05 (m, 1H), 3.80-3.90 (m, 1H), 3.35-3.45 (m, 1H), 3.08 (t, *J* =10.6 Hz, 1H), 1.80-2.00 (m, 2H), 1.40-1.60 (m, 2H), 1.36 (s, 9H) ; ESI-MS $(M+H)^+$ m/z = 386.2.

(3*R*,6*S*)-6-((*E*)-2-(6-methoxy-1,5-naphthyridin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-amine (31b). The compound **31b** was prepared in 80% yield from the compound **31a** according to the general procedure A. Yellowish foam; ^{1}H NMR δ (DMSO-*d6*): 8.70 (d, *J* = 4.7 Hz, 1 H), 8.23 (d, *J* = 9.1 Hz, 1 H), 7.81 (d, *J* = 4.7 Hz, 1 H), 7.51 (dd, *J* = 0.5, 16.3 Hz, 1 H), 7.26 (d, *J* = 9.1 Hz, 1 H), 6.92 (dd, *J* = 5.3, 16.4 Hz, 1 H), 4.03 (s, 3H), 3.97 (overlaid m, 1H), 3.85 (m, 1H), 2.99 (t, *J* = 10.4 Hz, 1H), 2.62 (m, 1H), 1.92 (m, 1H), 1.84 (m, 1H), 1.21-1.51 (m, 4H); ^{13}C NMR δ ($CDCl_3$): 161.4, 147.6, 142.2, 140.7, 140.2, 139.2, 136.1, 124.2, 119.5, 116.4, 77.5, 74.9, 53.8, 47.3, 33.7, 31.3; HR ESI-MS $(M+H)^+$ m/z = 286.1554 (calc. for $C_{16}H_{20}N_3O_2$: 286.1555); ^{t}R = 0.62.

6-((((3*R*,6*S*)-6-((*E*)-2-(6-methoxy-1,5-naphthyridin-4-yl)vinyl)tetrahydro-2H-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (31c). The compound **31c** was obtained in 61% yield from the compounds **31b** and **32** according to the general procedure B. Beige foam; ^{1}H NMR δ (DMSO-*d6*): 10.84 (s, 1 H), 8.70 (d, *J* = 4.6 Hz, 1 H), 8.23 (d, *J* = 9.1 Hz, 1 H), 7.80 (d, *J* = 4.7 Hz, 1 H), 7.73 (d, *J* = 7.9 Hz, 1 H), 7.51 (d, *J* = 16.3 Hz, 1 H), 7.26 (d, *J* = 9.1 Hz, 1 H), 7.09 (d, *J* = 7.9 Hz, 1 H), 6.91 (dd, *J* = 5.2, 16.4 Hz, 1 H), 3.99-4.08 (overlaid m, 2H), 4.03 (s, 3H), 3.69-3.80 (br s, 2H), 3.51 (s, 2H), 3.10 (t, *J* = 10.5 Hz, 1 H), 2.56 (m, overlaid with DMSO, 1H), 2.02-2.16 (m, 2H), 1.87 (m, 1H), 1.28-1.50 (m, 2H);^{13}C NMR δ ($CDCl_3$): 165.7, 161.5, 157.0, 148.3, 147.6, 142.3, 140.7, 140.3, 139.3, 136.2, 135.9, 124.4, 119.5, 117.8, 116.4, 113.6, 78.0, 72.5, 53.8, 53.2, 51.6, 31.2, 31.0, 29.7; HR ESI-MS $(M+H)^+$ m/z =464.1759 (calc. for $C_{24}H_{26}N_5O_3S$: 464.1756); ^{t}R = 1.29.

(3*R*,6*S*)-N-((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)-6-((E)-2-(6-methoxy-1,5-naphthyridin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-amine (31d). The compound **31d** was obtained in 73% yield from the compounds **31b** and **33** according to the general procedure B. Colorless foam; ^{1}H NMR δ (DMSO-*d6*): 8.69 (d, *J* = 4.6 Hz, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 8.00 (s,

1H), 7.80 (d, *J* = 4.7 Hz, 1H), 7.50 (dd, *J* = 0.9, 16.6 Hz, 1H), 7.25 (d, *J* = 9.1 Hz, 1H), 6.93 (s, 1H), 6.90 (overlaid dd, *J* = 5.3, 16.6 Hz, 1H), 4.30-4.34 (m, 2H), 4.23-4.28 (m, 2H), 3.96-4.05 (overlaid m, 2H), 4.02 (s, 3H), 3.63-3.73 (m, 2H), 3.08 (t, *J* = 10.4 Hz, 1H), 2.51 (m, overlaid with DMSO, 1H), 2.11 (br s, 1H), 2.02 (m, 1H), 1.85 (m, 1H), 1.28-1.47 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =435.2031 (calc. for $C_{24}H_{27}N_4O_4$: 435.2032); ^{t}R = 1.30.

***tert*-butyl ((3*R*,6*R*)-6-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (34a).** To a solution of **31a** (0.975 g, 2.53 mmol) in MeOH (20 mL) was added 10% palladium on charcoal (0.5 g). The reaction was stirred under H_2 for 90 min. After dilution with EtOAc (200 mL), the catalyst was removed by filtration and the filtrate was concentrated to dryness to afford **34a** (0.97 g, 99% yield). Beige solid; m.p.= 152.5 °C; ^{1}H NMR δ (DMSO-*d6*): 8.64 (d, *J* = 4.5 Hz, 1H), 8.22 (d, *J* = 9.0 Hz, 1H), 7.50 (d, *J* = 4.5 Hz, 1H), 7.22 (d, *J* = 9.0 Hz, 1H), 6.69 (br d, *J* = 7,9 Hz, 1H), 4.00 (s, 3H), 3.79 (m, 1H), 3.30 (overlaid m, 1H), 3.04-3.28 (m, 3H), 2.91 (t, *J* = 10.6 Hz, 1H), 1.79-1.89 (m, 3H), 1.71 (m, 1H), 1.35 (s, 9H), 1.21-1.37 8m, 2H); ^{13}C NMR δ ($CDCl_3$): 161.3, 155.2, 148.3, 147.7, 141.5, 141.0, 140.3, 123.9, 116.2, 79.5, 77.3, 76.5, 53.6, 46.6, 35.4, 30.7, 30.6, 28.4 (3C), 27.0; HR ESI-MS $(M+H)^+$ m/z = 388.2239 (calc. for $C_{21}H_{30}N_3O_4$: 388.2236); ^{t}R = 1.23.

(3*R*,6*R*)-6-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-amine (34b). The compound **34b** was prepared in 84% yield from the compound **34a** according to the general procedure A. White solid; ^{1}H NMR δ ($CDCl_3$): 8.66 (d, *J* = 7.5 Hz, 1H), 8.20 (d, *J* = 9.0 Hz, 1H), 7.40 (d, *J* = 7.5 Hz, 1H), 7.11 (d, *J* = 9.0 Hz, 1H), 4.09 (s, 3H), 4.00 (ddd, *J* = 2.2, 4.4, 10.6 Hz, 1H), 3.34-3.17 (m, 3H), 3.02 (t, *J* = 10.6 Hz, 1H), 2.86 (m, 1H), 2.08-1.92 (m, 3H), 1.74 (m, 1H), 1.62 (br s, 2H), 1.46 (m, 1H), 1.26 (m, 1H); ESI-MS $(M+H)^+$ m/z = 288.3.

6-((((3*R*,6*R*)-6-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (34c). The compound **34c** was obtained in 27% yield from the compounds **34b** and **32** according to the general procedure B. White foam; ^{1}H NMR δ (DMSO-*d6*): 10.87 (s, 1H), 8.66 (d, *J* = 4.4 Hz, 1H), 8.23 (d, *J* = 9.0 Hz, 1H), 7.73

(d, *J* = 7.8 Hz, 1H), 7.52 (d, *J* = 4.4 Hz, 1H), 7.24 (d, *J* = 9.0 Hz, 1H), 7.08 (d, *J* = 7.8 Hz, 1H), 4.06 (s, 3H), 3.95 (ddd, *J* = 1.6, 4.4, 12.5 Hz, 1H), 3.72 (br s, 2H), 3.53 (s, 2H), 3.21-3.09 (m, 3H), 2.93 (t, *J* = 10.4 Hz, 1H), 2.48 (m, overlaid with DMSO, 1H), 2.09 (br s, 1H), 1.99 (m, 1H), 1.84 (m, 2H), 1.73 (m, 1H), 1.28-1.15 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =466.1915 (calc. for $C_{24}H_{28}N_5O_3S$: 466.1912) ; ^{t}R = 1.30.

(3*R*,6*R*)-N-((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)-6-(2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-amine (34d). The compound **34d** was obtained in 50% yield from the compounds **34b** and **33** according to the general procedure B. Colorless oil; ^{1}H NMR δ (DMSO-*d6*): 8.64 (d, *J* = 4.4 Hz, 1 H), 8.21 (d, *J* = 9.0 Hz, 1 H), 7.98 (s, 1 H), 7.49 (d, *J* = 4.5 Hz, 1 H), 7.22 (d, *J* = 9.0 Hz, 1 H), 6.90 (s, 1 H), 4.29-4.33 (m, 2H), 4.23-4.27 (m, 1H), 3.99 (s, 3H), 3.90 (m, 1H), 3.59-3.69 (m, 2H), 3.04-3.29 (m, 3H), 2.89 (t, *J* = 10.4 Hz, 1H), 2.41 (m, 1H), 2.00 (br s, 1H), 1.93 (m, 1H), 1.74-1.89 (m, 2H), 1.68 (m, 1H), 1.07-1.29 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =437.2188 (calc. for $C_{24}H_{29}N_4O_4$: 437.2189); ^{t}R = 1.31.

General Procedure D: Asymmetric dihydroxylation using AD-mix-α or-β

To a mixture of alkene (1eq) in *t*-BuOH (4 mL/mmol), EtOAc (1 mL/mmol) and water (5 mL/mmol) were added AD-mix-β or AD-mix-α (1.4 g /mmol) and $CH_3SO_2NH_2$ (1.1eq). The reaction mixture was stirred vigorously until completion. $NaHSO_3$ (1.5g/mmol) was added carefully and the mixture was stirred 30 min. EtOAc (10 mL/mmol) was added and the two layers were decanted. The aq. layer was extracted with EtOAc (2 x 10 mL/mmol). The combined extracts were washed with brine, dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatography (DCM-MeOH with an appropriate gradient).

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (35a).** The compound **35a** was prepared in 92% yield from **31a** according to the general procedure D (AD-mix-β). White solid; m.p.= 114.9 °C; ^{1}H NMR (*d6*-DMSO) δ: 8.76 (d, *J* = 4.5 Hz, 1H), 8.25 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 4.5 Hz, 1H), 7.25 (d, *J* = 9.0 Hz, 1H), 6.81 (br s, 1H), 6.75 (d, *J* = 7.8 Hz, 1H), 5.65 (br d, *J* = 6.6 Hz, 1H), 5.28 (d, *J* =

6.8 Hz, 1H), 4.41 (d, *J* = 6.4 Hz, 1H), 4.00 (s, 3H), 3.79 (m, 2H), 3.33 (m, 1H), 2.92 (t, *J* = 10.7 Hz, 1H), 1.96 (m, 2H), 1.47 (m, 2H), 1.38 (s, 9H); ^{13}C NMR δ ($CDCl_3$): 161.2, 155.1, 147.7, 146.6, 141.2, 140.5, 139.2, 122.2, 116.5, 79.6, 78.5, 75.1, 71.5, 71.3, 53.9, 46.3, 30.0, 28.3 (3C), 26.8; HR ESI-MS $(M+H)^+$ m/z = 420.2132 (calc. for $C_{21}H_{30}N_3O_6$: 420.2134); ^{t}R = 0.97.

***tert*-butyl ((3*S*,6*R*)-6-((1*R*,2*S*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (*ent*-35a).** Compound *ent*-**35a** was prepared in 88% yield from compound *ent*-**31a** using the general procedure D (AD-mix-α). Colorless solid; ^{1}H NMR was identical to **35a**. ESI-MS $(M+H)^+$ m/z = 464.1.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (35b). The compound **35b** was obtained in 80% yield from the compound **35a** according to the general procedure A. Yellowish foam; ^{1}H NMR δ ($CDCl_3$): 8.78 (d, *J* = 4.5 Hz, 1H), 8.25 (d, *J* = 9.3 Hz, 1H), 7.56 (d, *J* = 4.5 Hz, 1H), 7.14 (d, *J* = 9.0 Hz, 1H), 5.59 (d, *J* = 3.3 Hz, 1H), 4.04 (s, 3H), 3.98 (partially overlaid m, 2H), 3.38 (br d, *J* = 11.4 Hz, 1H), 3.01 (t, *J* = 10.8 Hz, 1H), 2.86 (m, 1H), 2.08 (m, 1H), 1.85 (m, 1H), 1.63 (m, 1H), 1.28 (br s, 4H), 1.26 (overlaid m, 1H); ESI-MS $(M+H)^+$ m/z = 320.2.

(1*R*,2*S*)-1-((2*R*,5*S*)-5-aminotetrahydro-2H-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (*ent*-35b). The compound *ent*-**35b** was prepared in 92% yield from the compound *ent*-**35a** according to the general procedure A. Colorless foam; ^{1}H NMR was identical to **35a**; ESI-MS $(M+H)^+$ m/z = 320.2.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (35c). The compound **35c** was obtained in 27% yield from the compounds **35b** and **32** according to the general procedure B. White solid; ^{1}H NMR δ (DMSO-*d6*): 10.84 (s, 1H), 8.73 (d, *J* = 4.6 Hz, 1H), 8.23 (d, *J* = 9.1 Hz, 1H), 7.67-7.75 (m, 2H), 7.22 (d, *J* = 9.1 Hz, 1H), 7.07 (d, *J* = 7.8 Hz, 1H), 5.63 (m, 1H), 5.23 (d *J* = 6.7 Hz, 1H), 4.33 (d, *J* = 6.2 Hz, 1H), 3.95 (s, 3H), 3.92 (overlaid m, 1H), 3.67-3.76 (m, 3H), 3.50 (s, 2H), 3.33 (overlaid m, 1H), 2.90 (t, *J* = 10.6 Hz, 1H), 2.50 (m, overlaid

with DMSO, 1H), 2.08 (m, 1H), 1.93 (m, 1H), 1.44 (m, 1H), 1.22-1.33 (m, 2H); HR ESI-MS $(M+H)^+$ m/z =498.1808 (calc. for $C_{24}H_{28}N_5O_5S$: 498.1811); ^tR = 1.01.

6-((((3*S*,6*R*)-6-((1*R*,2*S*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (*ent*-35c). The compound *ent*-**35c** was obtained in 32% yield from the compounds *ent*-**35b** and **32** according to the general procedure B. Beige foam; ^{1}H NMR was identical to **35c**. HR ESI-MS $(M+H)^+$ m/z =498:1815 (calc. for $C_{24}H_{28}N_5O_5S$: 498.1811); ^tR = 1.01.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (35d). The compound **35d** was obtained in 38% yield from the compounds **35b** and **33** according to the general procedure B. White foam; ^{1}H NMR δ (DMSO-*d6*): 8.73 (d, *J* = 4.5 Hz, 1 H), 8.23 (d, *J* = 9.1 Hz, 1 H), 7.99 (s, 1 H), 7.72 (d, *J* = 4.5 Hz, 1 H), 7.22 (d, *J* = 9.1 Hz, 1 H), 6.91 (s, 1 H), 5.64 (m, 1H), 5.21 (d, *J* = 6.7 Hz, 1H), 4.29-4.34 (m, 3H), 4.23-4.27 (m, 2H), 3.95 (s, 3H), 3.87-3.94 (m, 1H), 3.60-3.74 (m, 3H), 3.35 (m, 1H), 2.87 (t, *J* = 10.4 Hz, 1H), 2.45 (m, overlaid with DMSO, 1H), 1.99-2.11 (m, 2H), 1.91 (m, 1H), 1.46 (m, 1H), 1.22 (m, 1H); HR ESI-MS $(M+H)^+$ m/z =469.2095 (calc. for $C_{24}H_{29}N_4O_6$: 469.2087) ; ^tR = 0.97.

***tert*-butyl ((3*R*,6*S*)-6-((1*R*,2*S*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (36a).** The compound **36a** was obtained in 98% yield from **31a** using the general procedure D (AD-mix-α). White foam; ^{1}H NMR (*d6*-DMSO) δ: 8.75 (d, *J* = 4.5 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 4.5 Hz, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 6.81 (br s, 1H), 6.75 (br d, *J* = 8.3 Hz, 1H), 5.83 (d, *J* = 6.1 Hz, 1H), 5.25 (d, *J* = 6.6 Hz, 1H), 4.49 (d, *J* = 8.1 Hz, 1H), 4.00 (s, 3H), 3.76 (m, 1H), 3.67 (app t, *J* = 6.6 Hz, 1H), 3.36 (m, 1H), 2.99 (t, *J* = 10.9 Hz, 1H), 1.99-1.86 (m, 2H), 1.38 (s, 9H), 1.35-1.13 (m, 2H); ESI-MS $(M+H)^+$ m/z = 420.2.

***tert*-butyl ((3*S*,6*R*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (*ent*-36a).** Compound ***ent*-36a** was prepared in

54% yield from compound *ent*-**31a** using the general procedure D (AD-mix-β). Colorless solid; ^{1}H NMR was identical to **36a**. ESI-MS $(M+H)^+$ m/z = 464.1.

(1*R*,2*S*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (36b). The compound **36b** was obtained in 47% yield from the compound **36a** (0.47 g, 2.08 mmol) according to the general procedure A. Yellowish foam; ^{1}H NMR δ (DMSO-*d6*): 8.75 (d, *J* = 4.5 Hz, 1H), 8.24 (d, *J* = 9.0 Hz, 1H), 7.74 (d, *J* = 4.5 Hz, 1H), 7.23 (d, *J* = 9.0 Hz, 1H), 5.84 (d, *J* = 5.3 Hz, 1H), 5.21 (d, *J* = 6.5 Hz, 1H), 4.46 (d, *J* = 8.3 Hz, 1H), 3.99 (s, 3H), 3.76 (ddd, *J* = 2.5, 4.6, 8.8 Hz, 1H), 3.65 (td, *J* = 1.5, 8.0 Hz, 1H), 3.32 (m, 1H), 2.86 (t, *J* = 10.4 Hz, 1H), 2.54 (m, overlaid with DMSO, 1H), 1.93 (m, 2H), 1.55, br s, 2H), 1.2-1.09 (m, 2H); ESI-MS $(M+H)^+$ m/z = 320.2.

(1*S*,2*R*)-1-((2*R*,5*S*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (*ent*-36b). The compound ***ent*-36b** was prepared in 64% yield from the compound ***ent*-36a** according to the general procedure A. Colorless foam; ^{1}H NMR was identical to **36a**; ESI-MS $(M+H)^+$ m/z = 320.2.

6-((((3*R*,6*S*)-6-((1*R*,2*S*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (36c). The compound **36c** was obtained in 22% yield from the compounds **36b** and **32** according to the general procedure B. White solid; ^{1}H NMR δ (DMSO-*d6*): 10.84 (s, 1H), 8.71 (d, *J* = 4.6 Hz, 1H), 8.22 (d, *J* = 9.1 Hz, 1H), 7.66-7.78 (m, 2H), 7.20 (d, *J* = 9.1 Hz, 1H), 7.06 (d, *J* = 7.8 Hz, 1H), 5.79 (m, 1H), 5.19 (d *J* = 5.4 Hz, 1H), 4.44 (d, *J* = 6.2 Hz, 1H), 3.94 (s, 3H), 3.89 (overlaid m, 1H), 3.72 (br s, 2H), 3.61 (m, 1H), 3.50 (s, 2H), 3.32 (overlaid m, 1H), 2.95 (t, *J* = 10.6 Hz, 1H), 2.50 (overlaid with DMSO , m, 1H), 2.02 (m, 1H), 1.92 (m, 1H), 1.02-1.28 (m, 3H); HR ESI-MS $(M+H)^+$ m/z =498.1806 (calc. for $C_{24}H_{28}N_5O_5S$: 498.1811); ^{t}R = 1.01.

6-((((3*S*,6*R*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(6-methoxy-1,5-naphthyridin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (*ent*-36c). The compound *ent*-**36c** was prepared in 60% yield from the compounds *ent*-**36b** and

32 according to the general procedure B. Beige foam; ^{1}H NMR was identical to **36c**; HR ESI-MS $(M+H)^+$ m/z =498:1813 (calc. for $C_{24}H_{28}N_5O_5S$: 498.1811); ^{t}R = 1.01.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(2-methoxyquinolin-8-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (37).** The compound **37** was prepared in 30% yield from the compounds **18** and **26b** according to the general procedure C (KHMDS). White solid m.p.= 62.9°C; ^{1}H NMR (DMSO-*d6*) δ: 8.23 (d, *J* = 8.9 Hz, 1H), 7.90 (d, *J* = 6.5 Hz, 1H), 7.79 (d, *J* = 8.0 Hz, 1H), 7.61 (d, *J* = 16.0 Hz, 1H), 7.40 (dd, *J* = 6.5, 8.0 Hz, 1H), 7.03 (d, *J* = 8.9 Hz, 1H), 6.83 (d, *J* = 7.8 Hz, 1H), 6.58 (dd, *J* = 5.9, 16.0 Hz, 1H), 4.02 (s, 3H), 3.99 (m, 1H), 3.88 (dd, *J* = 3.4, 10.7 Hz, 1H), 3.40 (br s, 1H), 3.09 (t, *J* = 10.5 Hz, 1H), 1.88 (m, 2H), 1.51 (m, 2H); ESI-MS $(M+H)^+$ m/z = 385.3.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(3-methoxyquinolin-5-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (38).** The compound **38** was obtained in 42% yield from **19b** and **18** using the general procedure C (LiHMDS). Off-white solid; ^{1}H NMR δ (DMSO-*d6*): 8.64 (d, *J* = 2.8 Hz, 1H), 7.87 (d, *J* = 8.1 Hz, 1H), 7.81 (d, *J* = 2.8 Hz, 1H), 7.72 (d, *J* = 6.6 Hz, 1H), 7.53 (dd, *J* = 6.6, 8.1 Hz, 1H), 7.36 (d, *J* = 15.7 Hz, 1H), 6.82 (d, *J* = 8.1 Hz, 1H), 6.32 (dd, *J* = 6.2, 15.7 Hz, 1H), 4.00 (m, 1H), 3.97 (s, 3H), 3.88 (m, 1H), 3.38 (br s, 1H), 3.08 (t, *J* = 10.7 Hz, 1H), 1.88 (m, 2H), 1.51 (m, 2H), 1.38 (s, 9H); ESI-MS $(M+H)^+$ m/z = 385.0.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(3-fluoro-6-methoxyquinolin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (39).** The compound **39** was obtained in 39% yield from **20b** and **18** using the general procedure C (LiHMDS). Off-white solid; ^{1}H NMR δ ($CDCl_3$): 8.70 (d, J = 2.2 Hz, 1 H), 7.93 (d, J = 9.8 Hz, 1 H), 7.38 (d, J = 5.5Hz, 1H), 6.98 (dd, J = 1.3, 16.4 Hz, 1 H), 6.80 (d, *J* = 3.2 Hz, 1H), 6.50 (dd, J= 5.3, 16.4 Hz, 1 H), 4.05 (m, 1H), 3.97 (s, 3H), 3.89 (m, 1H), 3.38 (br s, 1H), 3.09 (t, *J* = 10.7 Hz, 1H), 1.88 (m, 2H), 1.51 (m, 2H), 1.38 (s, 9H); ESI-MS $(M+H)^+$ m/z = 403.2.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (40).** Starting from **18** (25.4 g, 60 mmol) and **21b** (12.4 g, 60 mmol), **40** (16.6 g, 52%) was prepared using the general procedure C (LiHMDS). White solid; ^{1}H NMR δ (DMSO-*d6*): 8.59 (s, 1 H), 7.93 (dd, *J* = 5.8, 9.1 Hz, 1 H), 7.54 (dd, *J* = 9.2, 11.0 Hz, 1 H), 7.23 (dd, *J*

= 0.6, 16.4 Hz, 1 H), 6.88 (dd, *J* = 5.3, 16.5 Hz, 1 H), 6.79 (d, *J* = 7.3 Hz, 1 H), 4.05 (s, 3H), 3.84-4.00 (m, 2H), 3.38 (m, 1H), 3.08 (t, *J* = 10.6 Hz, 1H), 1.81-1.94 (m, 2H), 1.40-1.53 (m, 2H), 1.36 (s, 9H); MS (ESI): m/z 404.4 $(M+H)^+$.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (41).** The compound **41** was prepared in 68% yield starting from **18** and **24b** using the general procedure C (LiHMDS). White solid; m.p.= 179.4 °C; ^{1}H NMR δ (DMSO-*d6*): 8.79 (d, *J* = 2.2 Hz, 1H), 8.26 (d, *J* = 9.1 Hz, 1H), 7.28 (d, *J* = 17 Hz, 1H), 7.24 (d, *J* = 9.1 Hz, 1H), 7.17 (dd, *J* = 4.5, 17 Hz, 1H), 6.81 (m, 1H), 4.02 (s, 3H), 4.01 (m, 1H), 3.91 (m, 1H), 3.09 (t, *J* = 10.6 Hz, 1H), 1.91 (d, *J* = 9.6 Hz, 2H), 1.35-1.58 (overlaid m, 1H), 1.38 (s, 9H); HR ESI-MS $(M+H)^+$ m/z = 404.1988 (calc. for $C_{21}H_{27}N_3O_4F$: 404.1985) ; ^{t}R = 1.44.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(8-fluoro-6-methoxyquinolin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (42).** The compound **42** was prepared in 74% yield from **18** and **25b** according to the general procedure A. Yellowish solid; ESI-MS $(M+H)^+$ m/z = 403.0.

***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(6-methoxyquinolin-4-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (43).** The compound **43** was prepared in 41% yield from **18** and **22b** using the general procedure A (KHMDS). Yellowish foam;. ^{1}H NMR ($CDCl_3$) δ: 8.69 (d, J = 4.6 Hz, 1H), 8.03 (d, J = 9.2 Hz, 1H), 7.28-7.42 (m, 3H), 7.25 (d, J = 15.8 Hz, 1H), 6.42 (dd, J = 5.2, 15.8 Hz, 1H), 4.33 (br s, 1H), 4.23 (ddd, J = 2.0, 4.7, 10.6 Hz, 1H), 4.05 (m, 1H), 3.96 (s, 3H), 3.70 (br s, 1H), 3.16 (t, J = 10.6 Hz, 1H), 2.18 (m, 1H), 1.95 (m, 1H), 1.65 (m, 1H), 1.45 (overlaid m, 1H), 1.45 (s, 9H); ESI-MS $(M+H)^+$ m/z = 385.3.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(2-methoxyquinolin-8-** ***tert*-butyl ((3*R*,6*S*)-6-((*E*)-2-(3-methoxyquinoxalin-5-yl)vinyl)tetrahydro-2*H*-pyran-3-yl)carbamate (44).** The compound **44** was prepared in 57% yield from **27b** and **18** (3.81 g, 9 mmol) using the general procedure A (LiHMDS). Beige solid; ^{1}H NMR δ (DMSO-*d6*): 8.62 (s, 1H), 7.99 (d, *J* = 7.4 Hz, 1H), 7.91 (dd, *J* = 1.2, 8.2 Hz, 1H), 7.60 (dd, *J* = 7.4, 8.2 Hz, 1H), 7.53 (d, *J* = 15.8 Hz, 1H), 6.84 (d, *J* = 8.0 Hz, 1H), 6.63 (dd, *J* = 5.7, 15.8 Hz, 1H), 4.08 (s, 3H), 3.99 (m, 1H), 3.89 (br dd, *J* = 3.0,

10.2 Hz, 1H), 3.38 (br s, 1H), 3.09 (t, *J* = 10.5 Hz, 1H), 1.91-1.86 (m, 2H), 1.53-1.45 (m, 2H), 1.39 (s, 9H) ESI-MS $(M+H)^+$ m/z = 386.2.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(2-methoxyquinolin-8-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (45a).** The compound **45a** was prepared in 92% yield from **37** following the general procedure D (AD mix β). ^{1}H NMR δ (DMSO-*d6*): 8.20 (d, *J* = 8.9Hz, 1H), 7.69-7.78 (m, 2H), 7.39 (t, *J* = 7.6 Hz, 1H), 6.98 (d, *J* = 8.9 Hz, 1H), 6.77 (br s, 1H), 6.68 (m, 1H), 5.65 (m, 1H), 5.00 (d, J = 6.7 Hz, 1H), 4.19 (d, J = 6.3 Hz, 1H), 3.95 (s, 3H), 3.78 (m, 1H), 3.67 (m, 1H), 3.22 (m, 1H), 2.79 (t, *J* = 10.8 Hz, 1H), 1.81-1.96 (m, 2H), 1.53 (m, 1H), 1.37 (s, 9H), 1.35 (overlaid m, 1H); HR ESI-MS $(M+H)^+$ m/z =419.2178 (calc. for $C_{22}H_{31}N_2O_6$: 419.2182); ^{t}R = 1.35.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(3-methoxyquinolin-5-yl)ethane-1,2-diol (46a). The compound **46a** was obtained in 86% yield from the compound **38** following the general procedure D (AD mix β). White foam; ^{1}H NMR δ ($CDCl_3$): 8.69 (d, *J* = 2.4 Hz, 1H), 8.04 (d, *J* = 8.4 Hz, 1H), 7.82 (d, *J* = 2.4 Hz, 1H), 7.72 (d, *J* = 6.3 Hz, 1H), 7.58 (dd, *J* = 6.3, 8.1 Hz, 1H), 5.44 (d, *J* = 6.9 Hz, 1H), 4.82 (br s, 2H), 4.13 (m, 2H), 3.96 (s, 3H), 3.67 (d, *J* = 6 Hz, 1H), 3.59 (br s, 1H), 2.94 (m, 1H), 2.79 (t, *J* = 10.8 Hz, 1H), 2.02 (m, 1H), 1.88 (qd, *J* = 4.2, 11.7 Hz, 1H), 1.50 (partially overlaid m, 1H), 1.43 (s, 9H), 1.15 (qd, *J* = 5.1, 12.6 Hz, 1H); ESI-MS $(M+H)^+$ m/z = 419.2.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-2-(3-fluoro-6-methoxyquinolin-4-yl)-1,2-dihydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (47a).** The compound **47a** was prepared in 82% yield from **39** following the general procedure D (AD mix β). ^{1}H NMR δ (DMSO-*d6*): 8.65 (d, *J* = 1.5 Hz, 1 H), 7.91 (d, *J* = 9.2 Hz, 1 H), 7.79 (d, *J* = 2.3 Hz, 1 H) 7.35 (m, 1 H), 6.51 (m, 1 H), 5.68 (d, *J* = 4.4 Hz, 1 H), 5.38 (m, 1 H), 5.04 (d, *J* = 6.0 Hz, 1 H), 3.85 (overlaid m, 1H), 3.85 (s, 3H), 3.66 (m, 1H), 3.25 (m, 1H), 2.62 (m, 1H), 2.43 (m, overlaid with DMSO, 1H), 1.74 (m, 1H), 1.63 (m, 1H), 1.39 (overlaid m, 1H), 1.37 (s, 9H), 1.11 (m, 1H); ESI-MS $(M+H)^+$ m/z = 437.2.2.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)-1,2-dihydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (48a).** Starting from **40** (10.6 g, 26.4 mmol), **48a** (10.26 g, 89%) was prepared following the general procedure D (AD-mix-β). White foam; ^{1}H NMR δ (DMSO-*d6*): 8.57 (s, 1 H), 7.96 (dd, *J* = 5.6, 9.1 Hz, 1 H), 7.47 (dd, *J* = 9.2, 10.3 Hz, 1 H), 6.49 (m, 1 H), 5.65 (t, *J* = 6.9 Hz, 1 H), 5.12 (d, *J* = 6.9 Hz, 1 H), 4.75 (d, *J* = 6.0 Hz, 1 H), 4.03 (s, 3H), 3.92 (m, 1H), 3.60 (m, 1H), 3.19 (br s, 1H), 2.76 (m, 1H), 2.50 (m, overlaid with DMSO, 1H), 1.71-1.84 (m, 1H), 1.43-1.68 (m, 2H), 1.33 (s, 9H), 1.07-1.22 (m, 1H); MS (ESI): m/z 438.4 $(M+H)^+$.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1,2-dihydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (49a).** The compound **49a** was prepared in 77% yield starting from **41** and applying the general procedure D (AD-mix-β). Yellowish solid; m.p.= 80.6 °C; ^{1}H NMR δ (DMSO-*d6*): 8.76 (s, 1H), 8.29 (d, *J* = 9.1 Hz, 1H), 6.81 (br s, 1H), 6.59 (d, *J* = 7.9 Hz, 1H), 5.72 (t, *J* = 6.2 Hz, 1H), 5.47 (d, *J* = 7.0 Hz, 1H), 4.88 (d, *J* = 5.9 Hz, 1H), 4.02 (s, 3H), 3.89 (m, 1H), 3.63 (m, 1H), 3.23 (m, 1H), 2.94 (m, 1H), 2.61 (t, *J* = 10.6 Hz, 1H), 1.82 (m, 1H), 1.56-1.70 (m, 2H), 1.35 (s, 9H), 1.12-1.27 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 438.2043 (calc. for $C_{21}H_{29}N_3O_6F$: 438.2040) ; ^{t}R = 1.10.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-2-(8-fluoro-6-methoxyquinolin-4-yl)-1,2-dihydroxyethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (50a).** The compound **50a** was prepared in 79% yield from the compound **42** applying the general procedure D (AD mix β). Brown oil; ESI-MS $(M+H)^+$ m/z = 437.3.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(6-methoxyquinolin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (51a).** The compound **51a** was prepared in 38% yield, starting from **43** and using the general procedure D (AD mix β). White foam; ^{1}H NMR ($CDCl_3$) δ: 8.72 (d, *J* = 4.5 Hz, 1H), 8.03 (d, *J* = 9.2 Hz, 1H), 7.57 (d, *J* = 4.5 Hz, 1H), 7.38 (dd, *J* = 2.7, 9.2 Hz, 1H), 7.32 (d, *J* = 2.7 Hz, 1H), 5.51 (d, *J* = 5.3 Hz, 1H), 4.21 (br d, partially overlaid, *J* = 6.2 Hz, 1H), 4.18 (ddd, *J* = 1.8, 4.6, 10.5 Hz, 1H), 3.94 (s, 3H), 3.69 (dd, *J* = 2.6, 5.2 Hz, 1H), 3.63 (br s, 1H), 3.15

(td, *J* = 2.2, 11.4 Hz, 1H), 2.91 (t, *J* = 10.6 Hz, 1H), 2.07 (m, 1H), 1.91 (qd, *J* = 4.0, 13.4 Hz, 1H), 1.90 (br s, 2H), 1.62 (m, 1H), 1.45 (s, 9H), 1.19 (qd, *J* = 4.2, 12.5 Hz, 1H); ESI-MS $(M+H)^+$ m/z = 419.4.

***tert*-butyl ((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(3-methoxyquinoxalin-5-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)carbamate (52a).** The compound **52a** was prepared in 86% yield starting from **44** using the general procedure D (AD-mix-β) White foam; ESI-MS $(M+H)^+$ m/z = 420.1.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(2-methoxyquinolin-8-yl)ethane-1,2-diol (45b). The compound **45b** was prepared in 85% yield from the compound **45a** using the general procedure A. Colorless solid; ESI-MS $(M+H)^+$ m/z = 319.2.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(3-methoxyquinolin-5-yl)ethane-1,2-diol (46b). The compound **46b** was prepared in 63% yield from the compound **46a** following the general procedure A. White foam; MS (ESI): m/z 319.2 $(M+H)^+$.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxyquinolin-4-yl)ethane-1,2-diol (47b). The compound **47b** was prepared in 61% yield from the compound **47a** following the general procedure A. White foam; ^{1}H NMR δ (DMSO-*d6*): 8.64 (d, *J* = 1.7 Hz, 1 H), 7.91 (d, *J* = 9.2 Hz, 1 H), 7.79 (d, *J* = 2.4 Hz, 1 H), 7.35 (dd, *J* = 2.6, 9.2 Hz, 1 H), 5.65 (d, *J* = 4.4 Hz, 1 H), 5.38 (dd, *J* = 4.5, 7.5 Hz, 1 H), 4.97 (d, *J* = 5.9 Hz, 1 H), 3.87 (s, 3 H), 3.85 (overlaid m, 1H), 3.64 (m, 1 H), 2.58 (m, 1 H), 2.36 (m, 1 H), 1.76 (m, 1 H), 1.61 (m, 1 H), 1.32 (m, 1 H), 1.21 (br s, 2 H), 0.87 (m, 1H); MS (ESI): m/z 337.1.2 $(M+H)^+$.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethane-1,2-diol (48b). Starting from **48a** (0.546 g, 1.25 mmol), **48b** (0.363 g, 86%) was prepared according to the general procedure A. White solid; m.p.= 65.6 °C; ^{1}H NMR δ (DMSO-*d6*): 8.57 (s, 1 H), 7.96 (dd, *J* = 5.6, 9.1 Hz, 1 H), 7.47 (dd, *J* = 9.1, 10.4 Hz 1 H), 5.66 (t, *J* = 6.9 Hz, 1 H), 5.10 (d, *J* = 6.8 Hz, 1 H), 4.68 (d, *J* = 6.0 Hz, 1 H), 4.04 (s, 3H), 3.92 (m, 1H), 3.55 (m, 1H), 2.74 (m, 1H), 2.29-2.43 (m, 2H), 1.78 (m, 1H), 1.58 (m, 1H), 1.41 (m, 1H), 1.18

(br s, 2H), 0.91 (m, 1H); ^{13}C NMR δ ($CDCl_3$): 160.6 (d, *J* = 251 Hz), 156.6, 139.9 (d, *J* = 9 Hz), 138.9 (d, *J* = 3 Hz), 136.1, 130.5 (d, *J* = 11 Hz), 121.1 (d, *J* = 15 Hz), 116.6 (d, *J* = 27 Hz), 76.9, 74.8, 69.5, 69.4, 54.3, 47.3, 33.4, 27.1; HR ESI-MS $(M+H)^+$ m/z = 338.1521 (calc. for $C_{16}H_{21}N_3O_4F$: 338.1516); ^{t}R = 0.57.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (49b). Starting from **49a,** the compound **49b** was prepared in 75% yield applying the general procedure A. White foam; ^{1}H NMR δ (DMSO-*d6*): 8.73 (d, *J* = 1.9 Hz, 1 H), 8.27 (d, *J* = 9.1 Hz, 1 H), 7.22 (d, *J* = 9.1 Hz, 1 H), 5.69 (t, *J* = 6.6 Hz, 1 H), 5.41 (d, *J* = 7.1 Hz, 1 H), 4.78 (d, *J* = 6.0 Hz, 1 H), 4.01 (s, 3 H), 3.87 (m, 1 H), 3.56 (m, 1H), 2.90 (m, 1H), 2.32-2.50 (m, 2H), 1.80 (m, 1H), 1.51-1.6 (m, 2H), 1.16-1.31 (br s, 2H), 0.97 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 338.1519 (calc. for $C_{16}H_{21}N_3O_4F$: 338.1516); ^{t}R = 0.57.

(1*R*,2*R*)-1-((2*S*,5*R*)-(5-amino-tetrahydro-pyran-2-yl)-2-(8-fluoro-6-methoxy-quinolin-4-yl)-ethane-1,2-diol (50b). The compound **50b** was prepared in 72% yield from intermediate **50a** and applying the general procedure A. Yellowish solid; ESI-MS $(M+H)^+$ m/z =337.2.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(6-methoxyquinolin-4-yl)ethane-1,2-diol (51b). The compound **51b** was obtained in quantitative yield from **51a** using the general procedure A. White solid; ESI-MS $(M+H)^+$ m/z = 319.1.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-aminotetrahydro-2*H*-pyran-2-yl)-2-(3-methoxyquinoxalin-5-yl)ethane-1,2-diol (52b). The compound **52b** was prepared in 76% yield, starting from **52a** and using the general procedure A. White foam; ^{1}H NMR δ (DMSO-*d6*): 8.60 (s, 1H), 7.90-7.85 (m, 2H), 7.62 (dd, *J* = 7.7, 7.8 Hz, 1H), 5.68 (br s, 1H), 5.11 (br d, *J* = 4.3 Hz, 1H), 4.27 (br s, 1H), 4.03 (s, 3H), 3.77 (ddd, *J* = 1.7, 4.3, 10.4 Hz, 1H), 3.62 (dd, *J* = 2.3, 6.1 Hz, 1H), 3.23 (ddd, *J* = 1.7, 6.4, 11.0 Hz, 1H), 2.75 (t, *J* = 10.4 Hz, 1H), 2.56 (m, 1H), 1.95-1.84 (m, 2H), 1.52 (m, 1H), 1.45 (br s, 2H), 1.13 (m, 1H); ESI-MS $(M+H)^+$ m/z = 320.2.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(2-methoxyquinolin-8-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2H-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (45c). The compound **45c** was prepared in 35% yield from the compounds **45b** and **32** using the general procedure B. Beige solid; ^{1}H NMR δ (DMSO-*d6*): 10.87 (s, 1H), 8.21 (d, *J* = 9.0 Hz, 1H), 7-79-7.72 (m, 3H), 7.41 (app t, *J* = 7.6 Hz, 1H), 7.09 (d, *J* = 7.6 Hz, 1H), 7.00 (d, *J* = 8.8 Hz, 1H), 5.68 (dd, *J* = 2.6, 6.6 Hz, 1H), 5.02 (d, *J* = 6.6 Hz, 1H), 4.19 (d, *J* = 6.2 Hz, 1H), 3.96 (s, 3H), 3.95 (overlaid m, 1H), 3.74 (dd, AB system, *J* = 2.7, 15.0 Hz, 2H), 3.67 (td, *J* = 2.7, 6.4 Hz, 1H), 3.53 (s, 2H), 3.28 (m, 1H), 2.87 (t, *J* = 10.5 Hz, 1H), 2.07 (m, 2H), 1.89 (br d, *J* = 12.2 Hz, 1H), 1.49 (m, 1H), 1.30-1.11 (m, 3H); HR ESI-MS $(M+H)^+$ m/z =497.1858 (calc. for $C_{25}H_{29}N_4O_5S$: 497.1858); ^{t}R = 1.35.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(3-methoxyquinolin-5-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (46c). The compound **46c** was prepared in 10% yield from the compounds **46b** and **32** using the general procedure B. Colorless foam; ^{1}H NMR δ (DMSO-*d6*): 10.85 (s, 1H), 8.65(d, *J* = 2.8 Hz, 1H), 7.85-7.90 (m, 2H), 7.72 (d, *J* = 7.9 Hz, 1H), 7.64 (dd, *J* = 1.4, 7.3 Hz, 1H), 7.56 (dd, *J* = 7.3, 8.4 Hz, 1H), 7.06 (d, *J* = 7.9 Hz, 1H), 5.31 (m, 2H), 4.76 (d, *J* = 6.1 Hz, 1H), 3.99 (overlaid m, 1H), 3.98 (s, 3H), 3.91 (m, 1H), 3.65-3.75 (m, 2H), 3.51 (s, 2H), 3.49 (m, 1H), 2.70 (t, *J* = 10.6 Hz, 1H), 2.50 (m, overlaid with DMSO, 1H), 2.10 (br s, 1H), 1.99 (m, 1H), 1.93 (m, 1H), 1.55 (m, 1H), 1.19 (m, 1H); HR ESI-MS $(M+H)^+$ m/z =497.1857 (calc. for $C_{25}H_{29}N_4O_5S$: 497.1858) ; ^{t}R = 0.95.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-2-(3-fluoro-6-methoxyquinolin-4-yl)-1,2-dihydroxyethyl)tetrahydro-2H-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (47c). The compound **47c** was prepared in 39% yield from the compounds **47b** and **32** using the general procedure B. Colorless foam; ^{1}H NMR δ (DMSO-*d6*): 10.78 (s, 1 H), 8.63 (d, J = 1.6 Hz, 1 H), 7.90 (d, J = 9.2 Hz, 1 H), 7.78 (d, J= 2.3Hz , 1 H), 7.67 (d, J = 7.9 Hz, 1 H), 7.34 (dd, J = 2.2, 9.1 Hz, 1 H), 7.00 (d, J = 7.9 Hz, 1 H), 5.65 (d, J = 4.5 Hz, 1 H), 5.37 (m, 1 H), 4.97 (d, J = 6.0 Hz, 1 H), 3.85 (s, 3H), 3.85-3.73 (m, 2H), 3.58-3.68 (m, 2H), 3.49 (s, 2H), 3.49 (m, 1H), 2.66 (m, 1H), 2.45 (m, overlaid with DMSO, 1H), 1.84-2.00 (m, 2H), 1.58 (m, 1H), 1.35 (m, 1H), 0.95 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 515.1765 (calc. for $C_{25}H_{28}N_4O_5FS$: 515.1764) ; ^{t}R = 1.06.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)-1,2-dihydroxyethyl) tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (48c). Starting from of **48b** (0.063 g, 0.23 mmol) and **32** (0.037 g, 0.24 mmol), **48c** (0.055 g, 57%) was prepared using general procedure B. Off-white foam; ^{1}H NMR δ (DMSO-*d6*): 10.79 (s, 1 H), 8.55 (s, 1 H), 7.95 (dd, *J* = 5.6, 9.2 Hz, 1 H), 7.68 (d, *J* = 7.7 Hz, 1 H), 7.46 (app t, *J* = 9.9 Hz, 1 H), 6.99 (d, *J* = 7.8 Hz, 1 H), 5.64 (m, 1 H), 5.10 (d, *J* = 6.8 Hz, 1 H), 4.68 (d, *J* = 5.9 Hz, 1 H), 4.01 (s, 3H), 3.91 (m, 1H), 3.70 (m, 1H), 3.61 (br s, 2H), 3.50 (s, 2H), 2.77 (m, 1H), 2.44 (m, overlaid with DMSO, 1H), 2.29 (m, 1H), 1.84-2.01 (m, 2H), 1.40-1.65 (m, 2H), 0.97 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 516.1710 (calc. for $C_{24}H_{27}N_5O_5FS$: 516.1717); ^{t}R = 1.13

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)-1,2-dihydroxyethyl) tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (49c). The compound **49c** was prepared in 32% yield from **49b** and **32** and using the general procedure B. White solid; m.p.= 66.6 °C; ^{1}H NMR δ (DMSO-*d6*): 10.83 (s, 1H), 8.75 (d, *J* = 1.5 Hz, 1H), 8.29 (d, *J* = 9.1 Hz, 1H), 7.68 (d, *J* = 7.8 Hz, 1H), 7.22 (d, *J* = 9.1 Hz, 1H), 7.02 (d, *J* = 7.8 Hz, 1H), 5.70 (br t, *J* = 7.1 Hz, 1H), 5.45 (d, *J* = 7.1 Hz, 1H), 4.83 (d, *J* = 6 Hz, 1H), 4.00 (s, 3H), 3.88 (m, 1H), 3.73 (m, 1H), 3.65 (br s, 2H), 3.53 (s, 2H), 2.90 (m, 1H), 2.58 (m, overlaid with DMSO, 1H), 2.32 (m, 1H), 2.09-1.90 (m, 2H), 1.67-1.53 (m, 2H), 1.09 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 516.1719 (calc. for $C_{24}H_{27}N_5O_5FS$: 516.1717) ; ^{t}R = 1.12.

6-((((3*R*,6*S*)-6-((1*R*,2*R*)-2-(8-fluoro-6-methoxyquinolin-4-yl)-1,2-dihydroxyethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (50c). The compound **50c** was obtained in 39% yield from **50b** and **32** and using the general procedure B. Yellowish solid; ^{1}H NMR ($CDCl_3$) δ: 10.85 (s, 1H), 8.76 (d, *J* = 4.4 Hz, 1H), 7.73 (d, *J* = 7.8 Hz, 1H), 7.64 (d, *J* = 4.5 Hz, 1H), 7.32 (dd, *J* = 2.4, 11.9 Hz, 1H), 7.28 (d, *J* = 2.6 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 1H), 5.46 (d, *J* = 5.5 Hz, 1H), 5.33 (t, *J* = 4.9 Hz, 1H), 5.17 (d, *J* = 5.8 Hz, 1H), 3.92 (overlaid m, 1H), 3.89 (s, 3H), 3.72 (d, *J* = 3.9 Hz, 2H), 3.53 (overlaid m, 1H), 3.52 (s, 2H), 2.96 (m, 1H), 2.74 (t, *J* = 10.4 Hz, 1H), 2.52 (m, overlaid with DMSO, 2H), 2.03 (m, 1H), 1.59 (m,

2H), 1.05 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 515.1769 (calc. for $C_{25}H_{28}N_4O_5FS$: 515.1764) ; ^tR = 1.06.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(6-methoxyquinolin-4-yl)ethyl)tetrahydro-2*H*-pyran-3-yl)amino)methyl)-2*H*-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (51c). The compound **51c** was prepared in 21% yield from **51b** and **32** and using the general procedure B. White solid ^{1}H NMR (DMSO) δ: 10.87 (s, 1H), 8.71 (d, *J* = 4.5 Hz, 1H), 7.93 (d, *J* = 9.1 Hz, 1H), 7.73 (d, *J* = 7.9 Hz, 1H), 7.54 (d, *J* = 4.5 Hz, 1H), 7.44 (d, *J* = 2.6 Hz, 1H), 7.40 (dd, *J* = 2.6, 9.1 Hz, 1H), 7.07 (d, *J* = 7.9 Hz, 1H), 5.41 (d, *J* = 5.2 Hz, 1H), 5.35 (t, *J* = 5.2 Hz, 1H), 4.79 (d, *J* = 6.4 Hz, 1H), 3.94 (m, 1H), 3.88 (s, 3H), 3.72 (br. d, *J* = 4.0 Hz, 1H), 3.53 (s, 2H), 2.92 (m, 1H), 2.71 (t, *J* = 10.8 Hz, 1H), 2.01 (m, 1H), 1.63 (m, 2H), 1.23 (m, 1H), 1.06 (m, 1H); HR ESI-MS $(M+H)^+$ m/z =497.1852 (calc. for $C_{25}H_{29}N_4O_5S$: 497.1858) ; ^tR = 0.80.

6-((((3*R*,6*S*)-6-((1*S*,2*R*)-1,2-dihydroxy-2-(3-methoxyquinoxalin-5-yl)ethyl)tetrahydro-2H-pyran-3-yl)amino)methyl)-2H-pyrido[3,2-*b*][1,4]thiazin-3(4*H*)-one (52c). The compound **52c** was prepared in 39% yield from **52b** and **32** according to the general procedure B. Yellowish foam ^{1}H NMR δ (DMSO-*d6*): 10.84 (s, 1H), 8.57 (s, 1H), 7.80-7.89 (m, 2H), 7.71 (d, *J* = 7.7 Hz, 1H), 7.60 (app t, *J* = 7.7 Hz, 1H), 7.06 (d, *J* = 7.9 Hz, 1H), 5.64 (m, 1H), 5.07 (d, *J* = 6.3 Hz, 1H) 4.25 (d, *J* = 6.3 Hz, 1H), 3.99 (overlaid m, 1H), 3.98 (s, 3H), 3.91 (m, 1H), 3.66-3.76 (m, 2H), 3.58 (m, 1H), 3.50 (s, 2H), 3.25 (overlaid m, 1H), 2.83 (t, *J* = 10.6 Hz, 1H), 2.50 (overlaid m, 1H), 2.10 (br s, 1H), 2.05 (m, 1H), 1.86 (m, 1H), 1.47 (m, 1H), 1.19 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 498.1814 (calc. for $C_{24}H_{28}N_5O_5S$: 498.1811); ^tR = 1.15.

General procedure E: Reductive amination reaction using aldehydes 33, 53-55.

To a solution of amine (1eq.) in MeOH (5 mL/mmol) was added the appropriate aldehyde (1eq.). The reaction mixture was stirred at rt overnight. The reaction mixture was cooled to 0 °C and $NaBH_4$ (400 mol%) was added. After stirring for 40 min, DCM-MeOH (9-1, 6 mL/mmol) and saturated $NaHCO_3$ (2 mL/mmol) were added. The two layers were decanted and the aq layer was extracted with a DCM-MeOH mixture (9-1, 4 x 5 mL/mmol). The combined organic layers were washed with brine,

dried over Na_2SO_4, filtered and concentrated to dryness. The residue was purified by chromatgraphy (DCM-MeOH containing aq NH_4OH) to afford the corresponding alkylated product.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethane-1,2-diol (48d). Starting from of **48b** (0.08 g, 0.23 mmol) and **33** (0.042 g, 0.25 mmol), **48d** (0.068 g, 59%) was prepared using the general procedure E. Off-white solid; ^{1}H NMR δ (DMSO-*d6*): 8.54 (s, 1H), 7.95 (dd, *J* = 5.5, 10.3 Hz, 1H)), 7.95 (s, 1H), 7.46 (dd, *J* = 9.3, 10.3 Hz, 1H), 6.83 (s, 1H), 5.64 (t, *J* = 6.9 Hz, 1H), 5.1 (d, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 6.0 Hz. 1H), 4.29-4.33 (m, 2H), 4.23-4.27 (m,2H), 4.02 (s, 3H), 3.90 (m, 1H), 3.69 (m, 1H), 3.50-3.60 (m, 2H), 2.76 (m, 1H), 2.42 (t, *J* = 10.6 Hz, 1H), 2.27 (m, 1H), 1.83-1.98 (m, 2H), 1.40-1.63 (m, 2H), 0.97 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 487.1996 (calc. for $C_{24}H_{28}N_4O_6F$: 487.1992); ^{t}R = 1.11.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((2,3-dihydro-[1,4]oxathiino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethane-1,2-diol (48e). Starting from of **48b** (0.099 g, 0.29 mmol) and **53** (0.054 g, 0.29 mmol), **48e** (0.079 g, 54%) was prepared using the general procedure E; Off-white foam**;** ^{1}H NMR δ (DMSO-*d6*): 8.55 (s, 1H), 7.95 (dd, *J* = 5.5, 10.3 Hz, 1H)), 7.89 (s, 1H), 7.46 (dd, *J* = 9.3, 10.3 Hz, 1H), 6.83 (s, 1H), 5.65 (t, *J* = 6.9 Hz, 1H), 5.1 (d, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 6.0 Hz. 1H), 4.32-4.37 (m, 2H), 4.01 (s, 3H), 3.90 (m, 1H), 3.69 (m, 1H), 3.49-3.60 (m, 2H), 3.20-3.24 (m, 2H), 2.76 (m, 1H), 2.42 (t, *J* = 10.6 Hz, 1H), 2.27 (m, 1H), 1.82-1.95 (m, 2H), 1.41-1.65 (m, 2H), 0.96 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 503.1767 (calc. for $C_{24}H_{28}N_4O_5FS$: 503.1764); ^{t}R = 1.22.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((6,7-dihydro-[1,4]oxathiino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(6-fluoro-3-methoxyquinoxalin-5-yl)ethane-1,2-diol (48f). Starting from of **48b** (0.070 g, 0.20 mmol) and **55** (0.038 g, 0.29 mmol), **48f** (0.070 g, 67%) was prepared using the general procedure E. Off-white; ^{1}H NMR δ (DMSO-*d6*): 8.55 (s, 1H), 7.95 (dd, *J* = 5.5, 10.3 Hz, 1H)), 7.46 (dd, *J* = 9.3, 10.3 Hz, 1H), 7.45 (s, 1H), 5.65 (t, *J* = 6.9 Hz, 1H), 5.1 (d, *J* = 6.8 Hz, 1H), 4.68 (d, *J* = 6.0 Hz. 1H), 4.53-4.58 (m, 2H), 4.01 (s, 3H), 3.90 (m,

1H), 3.65-3.74 (m, 3H), 3.24-3.30 (m, 2H), 2.75 (m, 1H), 2.41 (t, *J* = 10.6 Hz, 1H), 2.27 (m, 1H), 2.09 (m, 1H), 1.88 (m, 1H), 1.54 (m, 1H), 1.46 (m, 1H), 0.96 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 504.1713 (calc. for $C_{23}H_{27}N_5O_5FS$: 504.1717); ^{t}R = 1.01.

(1*S*,*2R*)-1-((2*S*,5*R*)-5-(((2,3-dihydro-[1,4]dioxino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (49d). Starting from of **49b** (1.5 g, 4.45 mmol) and **33** (0. 74 g, 4.47 mmol), **49d** (0.85 g, 39%) was obtained using the general procedure E. White solid; ^{1}H NMR δ (DMSO-*d6*): 8.72 (d, *J* = 1.8 Hz, 1 H), 8.26 (d, *J* = 9.1 Hz, 1 H), 7.96 (s, 1 H), 7.20 (d, *J* = 9.1 Hz, 1 H), 6.85 (s, 1 H), 5.68 (t, *J* = 6.6 Hz, 1 H), 5.41 (d, *J* = 7.1 Hz, 1 H), 4.79 (d, *J* = 6.1 Hz, 1 H), 4.30-4.34 (m, 2H), 4.21-4.26 (m, 2H), 3.98 (s, 3H), 3.85 (m, 1H), 3.70 (m, 1H), 3.52-3.62 (m, 2H), 2.94 (m, 1H), 2.54 (t, *J* = 10.6 Hz, 1H), 2.27 (m, 1H), 2.10 (br s, 1H), 1.91 (m, 1H), 1.49-1.61 (m, 2H), 1.03 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 487.1990 (calc. for $C_{24}H_{28}N_4O_6F$: 487.1993) ; ^{t}R = 1.11.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((2,3-dihydro-[1,4]oxathiino[2,3-*c*]pyridin-7-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (49e). Starting from of **49b** (1.9 g, 5.63 mmol) and **53** (0.036 g, 0.23 mmol), **49e** (1.5 g, 53%) was prepared using the general procedure E. White solid; m.p.= 133.1 °C; ^{1}H NMR δ (DMSO-*d6*): 8.72 (d, *J* = 1.8 Hz, 1 H), 8.26 (d, *J* = 9.1 Hz, 1 H), 7.89 (s, 1 H), 7.21 (d, *J* = 9.1 Hz, 1 H), 7.07 (s, 1 H), 5.68 (t, *J* = 6.6 Hz, 1 H), 5.41 (d, *J* = 7.1 Hz, 1 H), 4.78 (d, *J* = 6.1 Hz, 1 H),4.31-4.37 (m, 2H), 3.98 (s, 3H), 3.85 (m, 1H), 3.71 (m, 1H), 3.50-3.63 (m, 2H), 3.20-3.25 (m, 2H), 2.94 (m, 1H), 2.53 (t, *J* = 10.5 Hz, 1H), 2.26 (m, 1H), 1.85-1.96 (m, 2H), 1.47-1.63 (m, 2H), 1.03 (m, 1H); ^{13}C NMR δ ($CDCl_3$): 162.0, 155.0 (d, J = 270 Hz), 151.5, 147.4, 141.2 (d, J = 6 Hz), 141.1, 139.2, 138.7, 138.6 (d, J = 28 Hz), 129.2, 128.3 (d, J = 11 Hz), 119.9, 115.7 (d, J = 2Hz), 85.8, 72.6, 69.3 (2C), 64.8, 54.3, 53.0, 51.8, 30.7, 26.8, 25.7; HR ESI-MS $(M+H)^+$ m/z = 503.1765 (calc. for $C_{24}H_{28}N_4O_5FS$: 503.1764); ^{t}R = 0.81.

**(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((6,7-dihydro-[1,4]oxathiino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-

yl)ethane-1,2-diol (49f). Starting from of **49b** (0.105 g, 0.31 mmol) and **55** (0.060 g, 0.33 mmol), **46f** (0.090 g, 57%) using the general procedure E. Off-white foam; ^{1}H NMR δ (DMSO-*d6*): 8.73 (d, *J* = 1.6 Hz, 1 H), 8.26 (d, *J* = 9.0 Hz, 1 H), 7.47 (s, 1 H), 7.21 (d, *J* = 9.1 Hz, 1 H), 5.68 (t, *J* = 6.6 Hz, 1 H), 5.41 (d, *J* = 7.0 Hz, 1 H), 4.79 (d, *J* = 6.1 Hz, 1 H), 4.52-4.59 (m, 2H), 3.98 (s, 3H), 3.85 (m, 1H), 3.66-3.72 (m, 3H), 3.24-3.31 (m, 2H), 2.93 (m, 1H), 2.53 (t, *J* = 10.5 Hz, 1H), 2.26 (m, 1H), 1.85-1.96 (m, 2H), 1.46-1.62 (m, 2H), 1.02 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 504.1714 (calc. for $C_{23}H_{27}N_5O_5FS$: 504.1717); ^{t}R = 0.69.

(1*S*,2*R*)-1-((2*S*,5*R*)-5-(((6,7-dihydro-[1,4]dioxino[2,3-*c*]pyridazin-3-yl)methyl)amino)tetrahydro-2*H*-pyran-2-yl)-2-(3-fluoro-6-methoxy-1,5-naphthyridin-4-yl)ethane-1,2-diol (49g). Starting from of **49b** (0.071 g, 0.21 mmol) and **54** (0.036 g, 0.23 mmol), **49g** (0.038 g, 37%) was prepared using the general procedure E. Off-white foam; ^{1}H NMR δ (DMSO-*d6*): 8.74 (d, *J* = 1.7 Hz, 1 H), 8.27 (d, *J* = 9.1 Hz, 1 H), 7.22 (d, *J* = 9.1 Hz, 1 H), 7.11 (s, 1 H), 5.68 (t, *J* = 6.5 Hz, 1 H), 5.43 (d, *J* = 7.1 Hz, 1 H), 4.81 (d, *J* = 6.1 Hz, 1 H), 4.46-4.51 (m, 2H), 4.34-4.41 (m, 2H), 3.98 (s, 3H), 3.68-3.79 (m, 3H), 2.94 (m, 1H), 2.54 (t, *J* = 10.6 Hz, 1H), 2.26 (m, 1H), 2.16 (br s, 1H), 1.91 (m, 1H), 1.51-1.62 (m, 2H), 1.05 (m, 1H); HR ESI-MS $(M+H)^+$ m/z = 488.1948 (calc. for $C_{23}H_{27}N_5O_6F$: 488.1945); ^{t}R = 0.95.

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

NBTI, novel (non-fluoroquinolone) bacterial type II topoisomerase inhibitor; QR, quinolone-resistant; LHS, bicyclic aromatic left-hand side; RHS, aromatic right-hand side (as positioned in Figure 2); NHS, N-hydroxysuccinimide; CSA, (+/-)-10-camphorsulfonic acid; CFU, colony forming unit, RIA, Relaxation Inhibitory Activity; SCIA, Supercoling Inhibitory Activity; Et_2O, diethyl ether; EtOAc, ethyl acetate; PT-SH, phenyl tetrazole thiol; *t*-BuOH, *tert*-butanol (2-methyl-2-propanol); *t*-BuOK, potassium *tert*-butoxide.

ASSOCIATED CONTENT

*** Supporting Information**

Experimental details on synthesis and characterization of the aldehydes **19b-27b**. Representative gels for SCIA and RIA assays. Table containing the comparative data of **49e** with Ciprofloxacin and Linezolid. This material is available free of charge via the Internet at http://pubs.acs.organic

Accession Code

The RCSB PDB code for the co-crystal structure of compound **4** with *S. aureus* GyrB27-A56 used for modeling is 2XCS[22].

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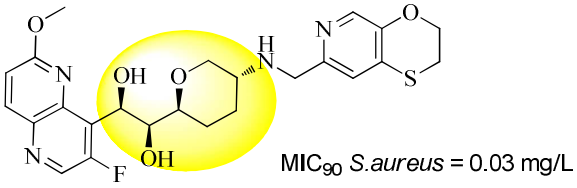
Table of Contents Graphics



MIC_{90} *S.aureus* = 0.03 mg/L