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Negishi cross-coupling enabled synthesis of novel NAD$^+$-dependent DNA ligase inhibitors and SAR development

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ABSTRACT

Two novel compounds, pyridopyrimidines (**1**) and naphthyridines (**2**) were identified as potent inhibitors of bacterial NAD$^+$-dependent DNA ligase (Lig) A in a fragment screening. SAR was guided by molecular modeling and X-ray crystallography. It was observed that the diaminonitrile pharmacophore made a key interaction with the ligase enzyme, specifically residues Glu114, Lys291, and Leu117. Synthetic challenges limited opportunities for diversification of the naphthyridine core, therefore most of the SAR was focused on a pyridopyrimidine scaffold. The initial diversification at R^1 improved both enzyme and cell potency. Further SAR developed at the R^2 position using the Negishi cross-coupling reaction provided several compounds, among these compounds **22g** showed good enzyme potency and cellular potency.

As bacterial pathogens are developing resistance to existing drugs from the overuse and over prescription of current therapies, the need for new and novel antibiotics has become more urgent.[1] In addition, this has been hindered through reduction of focus and resources directed towards discovery and development of new agents in the pharmaceutical industry. There are fewer options for the treatment of Gram-negative pathogens in comparison to Gram-positive pathogens and the discovery and optimization against these targets is typically more challenging due to increased difficulty in accessing the cellular targets.[2] One approach for overcoming resistance to existing antibacterial drugs is through the development of drugs that inhibit new targets. Bacterial NAD$^+$-dependent DNA ligase (LigA) was selected as a target for these reasons. LigA is essential for the growth of a broad spectrum of Gram-positive and Gram-negative pathogens,[3] and has limited homology to the human DNA ligases. DNA ligases are critical in DNA repair, replication, and recombination. Approaches to LigA inhibitors have been recently reported. Bayer reported a series of potent pyridochromanone inhibitors.[4] AstraZeneca recently reported on a series of adenosine based inhibitors[5] and napthyridines.[6] Additional reports of inhibitors from a number of classes include: aryl amino acids,[7] tetracyclic indoles,[8] glycosyl ureides and glycosylamines,[9] pyrimidopyrimidines,[10] aminoalkoxypyrimidines,[11] thienopyridines[12] and anilines.[13]

Our group recently identified two scaffolds as potent inhibitors of LigA through a fragment based screening effort pyridopyrimidines (**1**) and naphthyridines (**2**) (Fig. 1). The compounds demonstrated excellent in vitro activity and good physical properties.

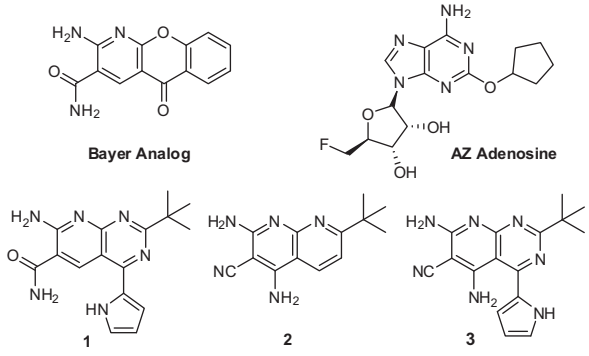

Figure 1. Reference and lead series for ligase inhibition.

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Table 1
Antibacterial activity of initial compounds

#	R^2	Core	Ligase IC_{50}[a] (μM)				MIC (μM)			Soln (μM)	AZ log D[14]
			Spn	*Sau*	*Eco*	*Hin*	*Spn* ARC 548	*Sau* ARC 516	*Hin* ARC 158		
1	2-Pyrrole	Pyridopyrimidine	0.037	0.077	0.077	0.03	50	25	12.5	130	2.5
2	H	Napthyridine	<0.02	<0.04	0.23	2.24	3.13	3.13	50	14.2	2.1
3	2-Pyrrole	Pyridopyrimidine	0.15	0.5	NT	2.09	50	50	100	50	2.5

[a] *Spn* = *Streptococcus pneumoniae*, *Sau* = *Staphylococcus aureus*, *Eco* = *Escherichia coli*, *Hin* = *Haemophilus influenzae*, NT = not tested. Pathogens; *Streptococcus pneumoniae* ARC548 (ATCC), *Staphylococcus aureus* ARC516 (CLSI QC MRSA reference strain), *Haemophilus influenzae* ARM158 (acrB gene deletion based on ARC446), *Escherichia coli* ARC524. Soln is aqueous solubility measured at pH 7.4 from dried DMSO stock as determined in a 0.1 M phosphate buffer at 20 °C after 18 h.[14]

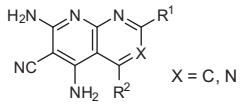


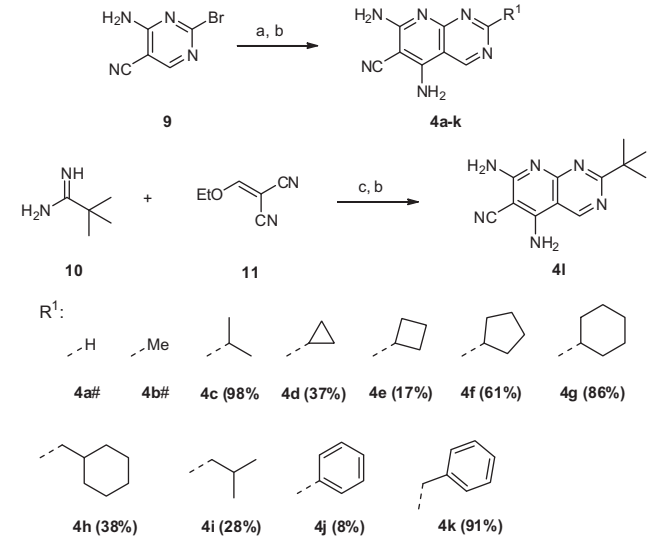
Figure 2. Generic structure of diaminonitrile scaffold.

Compound **1**, a pyridopyrimidine, demonstrated broad spectrum biochemical potency, and compound **2**, a diaminonitrile naphthyridine, demonstrated improved cellular activity (Table 1). When we combined aspects of both series in pyridopyrimidine core **3**, this resulted in a drop of both enzymatic and cellular activity.

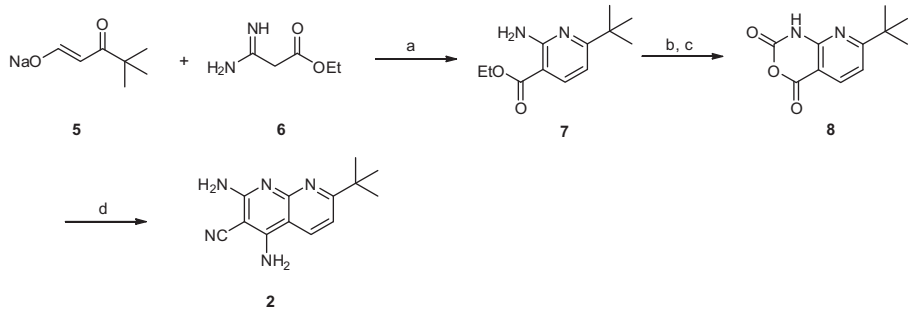
Through modeling and crystallography, it was observed the diaminonitrile pharmacophore made key interactions with the ligase enzyme and was therefore not modified, these interactions include residues Glu114, Lys291, and Leu117. For improving interaction with the enzyme, the key variables were the R^1 and R^2 substituents (Fig. 2). R^1 reaches into a hydrophobic tunnel which is conserved among ligase enzymes. R^2 affords access to the ribose pocket, a region previously shown to afford excellent biochemical potency in a series of adenosine-based inhibitors as shown in Figure 1, this series also contained a *t*-Butyl in a position analogous to R^1.[5,14]

The initial chemistry to access the diaminonitrile lead did not allow for facile diversification at R^1 and R^2. As can be seen in Scheme 1, the synthesis of diaminonitrile **2** begins by condensation of the sodium salt of (*E*)-4,4-dimethyl-3-oxopent-1-en-1-olate (**5**) and ethyl 3-amino-3-iminopropanoate (**6**) to provide pyridine **7**. Subsequent saponification of the ester, cyclization with phosgene and condensation with malononitrile, affords the target diaminonitrile **2**. In order to achieve our optimization goals, we required a synthesis that would allow for late stage diversification of the R^1 and R^2 substituents. The current naphthyridine scaffold was not amenable to this functionalization, so we modified the core to a pyridopyrimidine and developed this methodology.

We first sought to explore the SAR of the R^1 position in order to confirm that *tert*-butyl was the optimal substituent. Towards this end we developed a two-step route to final compounds which enabled a variety of alkyl based R^1 substituents to be introduced. We focused mainly on alkyl groups since this portion of the binding pocket is made up of mainly hydrophobic amino acids. As can be seen in Scheme 2, bromopyrimidine **9** undergoes Negishi cross-coupling reaction with a variety of alkylzinc bromides, yields are moderate to excellent and are unoptimized. Subsequent condensation with malononitrile afforded the desired targets. Synthesis of the *tert*-butyl derivative **4l** required an alternative synthesis since reaction of pyrimidine **9** with *tert*-butyl zinc bromide afforded only trace amounts of desired product, and instead provided *iso*-butyl derivative **4i**. Instead, we found condensation of *tert*-butyl amidine **10** and acrylonitrile **11** and subsequent cyclization provided the desired inhibitor, **4l** (R^1 = *tert*-Bu).



Scheme 2. Synthesis of R^1 substituted ligase inhibitors. Reagents and conditions: (a) R^1ZnBr, 5 mol % $Pd(PPh_3)_4$, THF, 60 °C, 18–98%; (b) $CNCH_2CN$, NaOEt, EtOH, 75 °C, 3–12%; (c) Na, EtOH, 45%. #Commercially available.



Scheme 1. Synthesis of diaminonitrile **2**. Reagents and conditions: (a) pyridine, dioxane, 100 °C, 69%; (b) NaOH, EtOH, 80 °C, 69%; (c) phosgene, Na_2CO_3, H_2O, 72%; (d) malononitrile, Et_3N, DMF, 110 °C, 40%.

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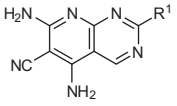
As can be seen in Table 2, compound **4a** indicates substitution at R^1 is required for activity. As the size increased (**4b**, **4c**) so did the activity, with compound **4c** showing moderate cellular activity and excellent solubility. Cyclic derivatives **4d**–**4g** show comparable biochemical activity to **4c**. Extending the alkyl substituent, as in **4h** and **4i** caused a marked decrease in biochemical activity. Aryl derivatives **4j** and **4k** were also less potent in biochemical assays when compared to the alkyl derivatives. As seen with other series,[15] *tert*-butyl derivative **4l** demonstrated the best biochemical and cellular activity as compared to the other substituents. However, when comparing the matched pair to **4l**, compound **2**, there is an overall loss in all biochemical activity with the pyridopyrimidine core in contrast to the napthyridine.

Based on this data, we planned to move forward to explore the R^2 position with a *tert*-butyl group at R^1. We chose a substrate with a chloro substituent at the R^2 position to enable access to a diverse set of compounds. Condensation of diethyl malonate and amidine **10** (Scheme 3), followed by Vilsmeier reaction, provided dichloroaldehyde **13**. Oxime formation, elimination to the nitrile, followed by mono displacement with ammonia, provided aminonitrile **14**. Due to concern about the stability of chloride **14** to the basic cyclization conditions, we performed a Stille cross-coupling at this point to provide **15**. Direct cyclization with malononitrile, analogous to the previous highlighted routes, did not afford any desired product so a two-step conversion was explored. However, after formation of amide **16**, no cyclization conditions could be found to form the desired product (see Scheme 4).

With the inability of the chloride to survive the cyclization conditions and our inability to cyclize with the R^2 substituent in place, we required a more inert cross-coupling partner. Thioethers offer a similar reactivity profile in cross-coupling reactions as aryl chlorides and should be more chemically stable.[16,17] Recent reports have demonstrated that thioethers undergo efficient transition metal catalyzed cross-coupling reactions with a variety of nucleophiles. Therefore, we sought an efficient synthesis of a thioether cross coupling partner. Beginning with dinitrile **17**, cyclization provided thiomethyl substituted pyrimidine **18**. Alkylation with cyanoacetate, followed by cyclization afforded **19**. Chlorination and two-step introduction of the amine provided the key substrate for diversification, **21**. The antibacterial activity of compound **21** (Table 3) was improved as compared to the unsubstituted derivative **4l**. The biochemical and cellular activity was comparable to the original naphthyridine **2**, indicating substitution at this position is favorable.

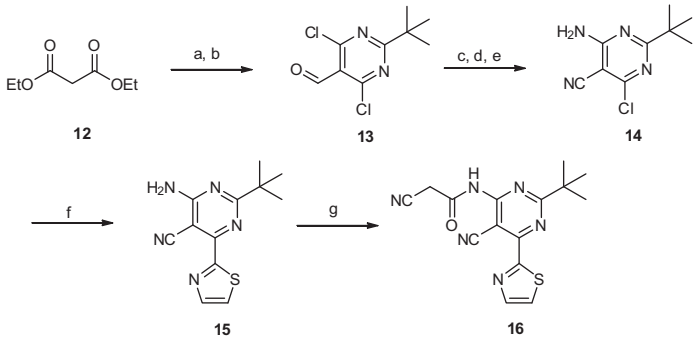
Intermediate **21** enables the exploration of a variety of cross-coupling reactions to access C-linked derivatives. Initial

Table 2
SAR of R^1 substituted pyridopyrimidines



#	R^1	Ligase IC_{50} (μM)[a]			MIC (μM)			Soln (μM)	AZ log*D*
		Spn	*Sau*	*Hin*	*Spn* ARC 548	*Sau* ARC 516	*Hin* ARC 158		
4a	H	44	13	>200	NT	NT	NT	6	0.1
4b	Me	>10	1.5	>10	NT	NT	NT	783	0.3
4c	*iso*-Pr	0.34	0.41	>10	25	>200	25	>1000	1.3
4d	Cyclopropyl	0.53	0.67	>10	100	200	200	23	1
4e	Cyclobutyl	0.46	0.69	13	50	200	>200	217	1.5
4f	Cyclopentyl	0.52	0.47	>10	50	200	200	89	2
4g	Cyclohexyl	0.59	0.81	>10	200	>200	>200	81	2.5
4h	CH_2-cyclohexyl	5	3.7	>10	NT	NT	NT	8	2.9
4i	*iso*-Bu	1.8	1.1	>10	NT	NT	NT	268	1.7
4j	Ph	0.72	0.97	>10	>200	>200	>200	NT	2.1
4k	Bn	>10	>10	>10	NT	NT	NT	13	1.7
4l	*tert*-Butyl	0.12	0.22	>200	12.5	25	200	37.2	1.9

[a] *Spn* = *Streptococcus pneumoniae*, *Sau* = *Staphylococcus aureus*, *Hin* = *Haemophilus influenzae*, NT = not tested. Pathogens; *Streptococcus pneumoniae* ARC548, *Staphylococcus aureus* ARC516 (CLSI QC MRSA reference strain), *Haemophilus influenzae* ARM158 (acrB gene deletion based on ARC446). Soln is aqueous solubility measured at pH 7.4 from dried DMSO stock as determined in a 0.1 M phosphate buffer at 20 °C after 18 h.



Scheme 3. Attempts at synthesis of R^2 substituted analogues. Reagents and conditions: (a) **10**, Na, EtOH, 90 °C, 79%; (b) $POCl_3$, DMF, 110 °C, 12 h, 48%; (c) $HONH_2$·HCl, H_2O, AcOH, 30 min, 64%; (d) $SOCl_2$, 3 h, 97%; (e) NH_3, EtOH, 75 °C, 30 min, 31%; (f) 2-tributylstannylthiazole, $Pd(PPh_3)_2Cl_2$, Ag_2O, DMF, 100 °C, 24 h, 53%; (g) 2-cyanoacetic acid, Ac_2O, 80 °C, 30 min, 39%.

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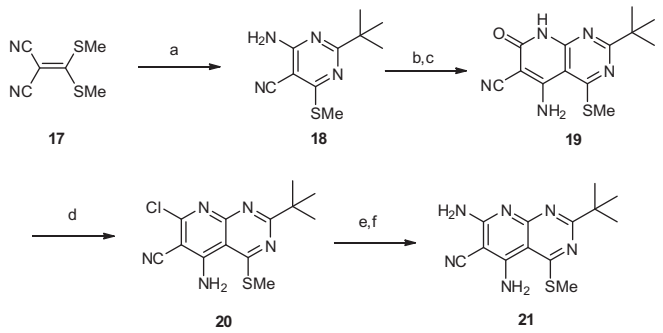


Scheme 4. Synthesis of substrate for diversification. Reagents and conditions: (a) **10**, Na, EtOH, 42%; (b) $CNCH_2CO_2H$, Ac_2O, CH_2Cl_2, 40 °C, 75%; (c) LiHMDS, THF, 70 °C, 39–93%; (d) $POCl_3$, >98%; (e) *para*-methoxybenzylamine, DMF, 50 °C, 70%; (f) TFA, 95%.

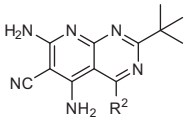
experimentation with compound **21** indicated Pd-catalyzed Cu-mediated Stille reactions were effective,[18] however the analogous Suzuki cross-couplings[19] did not afford any desired products. Early attempts at Negishi cross-couplings showed promise so we decided to pursue this further as alkyl and aryl zinc reagents are readily available[20] and we desired to avoid toxic tin reagents.[21]

Towards this end, treatment of **21** with a variety of commercially available alkylzinc bromides directly afforded C-linked analogues **22a**–**22q** (Scheme 5).[22] Based on our previous results with aryl substituted pyridopyrimidine **1** (Fig. 1) we were also interested in investigating the Pd-catalyzed cross-coupling with arylzinc bromides, especially heteroaryl zinc reagents. At the time of this work there were no reports of heteroaryl zinc bromides being utilized in a cross-coupling with thioethers as the electrophile,[23] and to date there are limited reports of cross-coupling with heteroarylzinc bromides containing multiple heteroatoms. However we were pleased to observe the desired product formation (**22g**–**22q**) when thioether **21** was treated with catalytic Pd $(PPh_3)_4$ and a heteroarylzinc bromide, yields are low to moderate and are unoptimized.

An evaluation of the SAR at the R^2-position of the pyridopyrimidine **21** was carried out next (Table 3). Substitution at R^2 with different alkyl groups (**22a**–**f**) showed moderate biochemical activity as compared to compound **2**, however the higher log*D* analogs afforded reduced MICs. Next we made a series of five membered heterocycles (**22g**–**i** and **26o**–**q**), activity in *Haemophilus influenzae*, a representative Gram-negative pathogen is generally more difficult to achieve with this substitution. The thiazole, **22g**, is the moderately potent in Gram + positive MICs, but is weaker in *Haemophilus*. Simple five membered heterocycles offer the best overall profile based on cellular potency across multiple pathogens for compounds made in this campaign. In the six membered series, pyridine **22k**–**m** at R^2 position showed only modest differences in biochemical potency compared to phenyl **22j**, however 3-pyridine **22l** showed improved Gram positive MICs. The matched pair pyrimidine **22n**, was not an improvement when compared to the other six membered aryl analogs. We next focused on further improving enzyme and cell potency of compound **22g** through the exploration of further substitution.

Though we were able to access the desired aryl substituted analogues **22g**–**22q**, the yields were often poor and attempts at introduction of N-containing heterocycles via the same methods provided the desired products in <5% yield. We hypothesized the

Table 3
SAR of R^2 substituted pyridopyrimidines (R^1 = *tert*-Butyl)



#	R^2	*Spn*	*Sau*	*Hin*	*Spn* ARC 548	*Sau* ARC 516	*Hin* ARC 158	Soln (μM)	AZ log*D*
21	-*S*-methyl	0.032	0.059	0.72	6.25	12.5	>200	14	3.4
22a	-Methyl	0.067	0.22	1.7	6.25	25	25	37	2.1
22b	-*n*-Butyl	0.043	0.11	1.1	50	>200	>200	6	3
22c	$-(CH_2)_4$-CN	0.044	0.26	0.65	12.5	50	50	16	1.9
22d	$-(CH_2)_5$-CN	0.12	0.35	1.8	50	>200	>200	6	2.2
22e	Cyclopentyl	0.051	0.12	2.1	25	>200	50	<1	3.4
22f	$-(CH_2)_5$-cyclohexyl	0.3	1.6	>200	>200	>200	>200	6	4.2
22g	-2-Thiazole	<0.02	0.057	0.49	0.78	1.56	>200	<1.5	2.7
22h	-2-Thiophene	0.044	0.16	0.52	1.56	6.25	25	56	3.1
22i	-3-Thiophene	0.31	0.74	2.19	25	50	100	40	2.8
22j	-Ph	0.15	0.47	1.8	50	>200	>200	4	2.8
22k	-2-Pyridine	0.42	1.01	6.6	50	100	>100	87	2.3
22l	-3-Pyridine	0.11	0.43	1.33	3.13	50	50	>200	1.9
22m	-4-Pyridine	0.16	0.4	2.75	50	>200	>200	44	1.9
22n	-2-Pyrimidine	0.55	1.44	13.98	100	200	>200	86	1.3
22o	-4-Carboethoxy-2-thiazole	<0.02	0.53	>10	>200	>200	>200	NT	3
22p	-5-Carboethoxy-2-thiazole	<0.39	>200	>200	>200	>200	>200	NT	3
22q	-2-Thiophene-5-carboxylate	0.034	0.55	0.62	>200	>200	>200	>1000	−0.4
26a	-2-Imidazole	0.021	0.036	0.31	3.13	6.25	>200	<1	2.2
26b	-2-(4-Methyl imidazole)	<0.02	0.068	NT	12.5	>200	>200	5	2.4
26c	-3-Pyrrazole	0.031	0.1	0.78	1.56	6.25	25	13.5	2.4
26d	-4-1,2,3 triazole	0.013	0.042	0.64	0.78	25	100	30	1.9
26e	-3-1,2,4 triazole	0.12	0.27	1.4	3.13	12.5	12.5	64.5	1.9
26f	-2-Oxazole	0.026	0.11	1.65	6.25	12.5	>200	50	2
30	-Thiazole alcohol	0.007	0.084	0.5	3.13	>200	>200	<1	2.1
32	-Thiazole methyl ether	<0.02	0.17	0.79	>200	>200	>200	<1	2.6

[a] *Spn* = *Streptococcus pneumoniae*, *Sau* = *Staphylococcus aureus*, *Hin* = *Haemophilus influenzae*, NT = not tested. Pathogens; *Streptococcus pneumoniae* ARC548, *Staphylococcus aureus* ARC516 (CLSI QC MRSA reference strain), *Haemophilus influenzae* ARM158 (acrB gene deletion based on ARC446). Soln is aqueous solubility measured at pH 7.4 from dried DMSO stock as determined in a 0.1 M phosphate buffer at 20 °C after 18 h.

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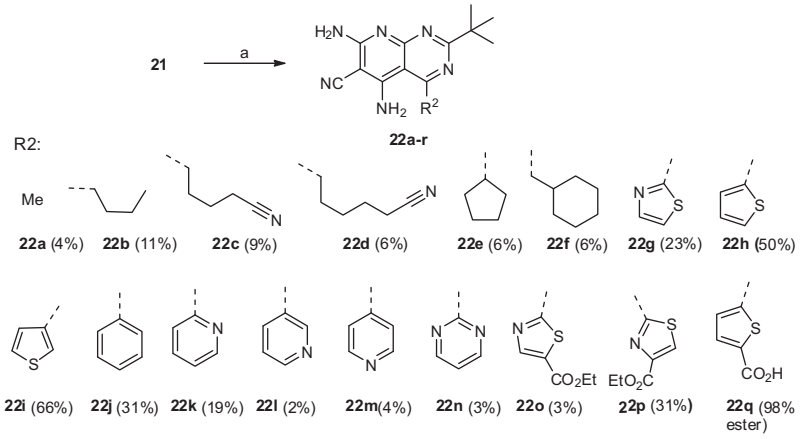
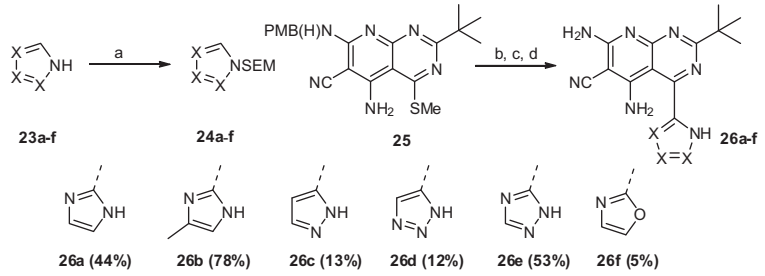


Scheme 5. Synthesis of C-linked analogues via Negishi cross-coupling. Reagents and conditions: (a) R^2ZnBr (**22a–q**), 10 mol % $Pd(PPh_3)_4$, THF, 65 °C, 3–98%.

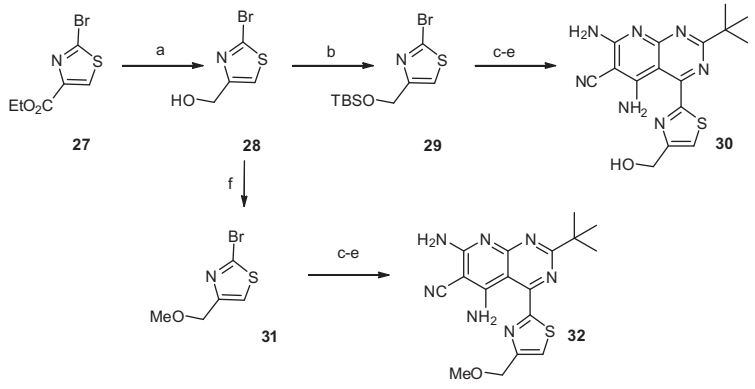


Scheme 6. Synthesis of azole substituted analogues. Reagents and conditions: (a) SEMCl, NaH, THF, rt, 16–60%; (b) **24a–f**, *n*-BuLi, $ZnBr_2$, THF, −78 °C, 1 h; $Pd(PPh_3)_4$, THF, 65 °C; (c) HCl, 65–98%; (d) TFA, 15–92%.

efficiency of the reaction suffered due to the poor solubility of the starting material. To address this, we examined the same cross coupling with PMB protected analogue **25** (see Scheme 6). To access the required arylzinc bromides we protected the desired azole (**23a–e**) with a SEM group to provide **24a–e**. Deprotonation with *n*-BuLi at −78 °C followed by treatment with dry $ZnBr_2$ formed the desired arylzinc bromide in situ. Subsequent addition of substrate **25** and Pd-catalyst afforded the desired cross coupling in good yields. Two step removal of protecting groups gave the desired final products **26a–f**.

As can be seen in Table 3, the azole analogues **26a–26f** demonstrated good biochemical potency against bacterial ligase and provided Gram-positive MICs. Methyl substituted imidazole **26b** is the only analogue that did not have any activity against *Staphylococcus aureus*. Pyrazole **26c** and triazoles **26d** and **26e** had comparable MICs to thiazole **22g** in *Streptococcus pneumoniae* and had improved solubility. However, triazoles **26d** and **26e** were less potent than **22g** against *S. aureus.*

In an attempt to improve the physical properties of the most potent analogue **22g**, we sought to introduce substitution off the thiazole. Beginning with ester **27**, since it proved to have better enzymatic activity than its isomer **22o**, $LiAlH_4$ reduction provided alcohol **28** (Scheme 7). The intermediate alcohol could be silylated or methylated to provide **29** and **31**, respectively. Treatment of bromides **29** and **31** with activated zinc dust produced the arylzinc bromides in situ. Pd-catalyzed cross coupling with methyl thioether **25** and subsequent removal of protecting groups afforded the final products **30** and **32**. Unfortunately, the introduction of the



Scheme 7. Synthesis of functionalized thiazoles. Reagents and conditions: (a) $LiAlH_4$, THF, 0 °C; (b) TBSCl, imidazole, DMF, 78%; (c) Zn dust, $Br(CH_2)_2Br$, TMSCl, THF; (d) $Pd(PPh_3)_4$, THF 65 °C, 43%; (e) TFA, 25–52%; (f) MeI, NaH, DMF, 60%.

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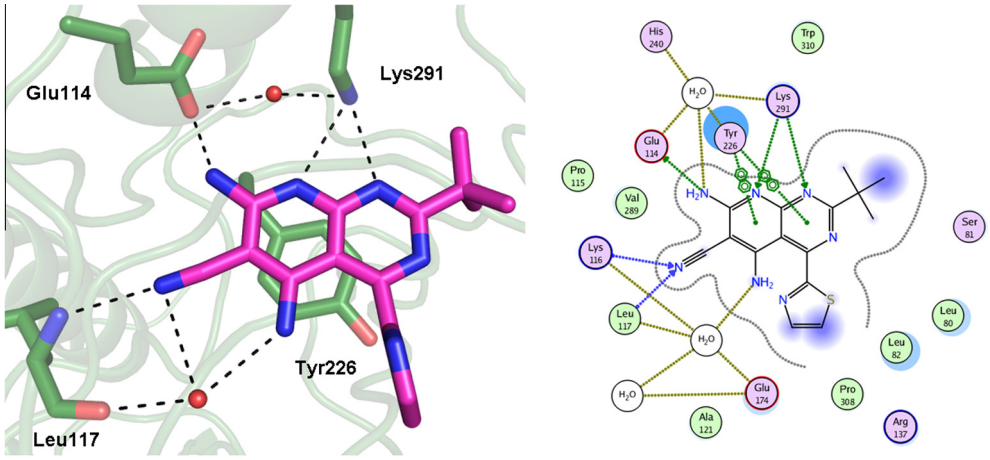

Figure 3. Structure of compound **22g** in *H. influenzae* DNA ligase (PDB Code: 4UFZ).

solubilizing groups did not increase the solubility and was detrimental to the antibacterial potency (Table 3).

Crystallographic analysis of **22g** in *H. influenzae* DNA Ligase confirmed the binding mode (Fig. 3). The inhibitor maintains multiple interactions with the active site: Leu117, Glu114, Lys291. In addition, the aromatic core π-stacks with the Tyr226 residue. The *t*-butyl in this case efficiently fills the flexible lipophilic pocket. The electron density of the thiazole was not well defined indicating it does not pick up any particular interactions. The reduction in potency of **22g** in *H. influenzae* compared to the carboxamide compound **1** may be rationalized because in compound **1**, the carboxamide is making direct H-bonds to both the backbone carbonyl and the backbone NH of Leu 117. Overall, replacing the carboxamide with the aminonitrile may reduce the overall strength of the H-bonding interactions with Leu117. In general a carboxamide NH_2 is a much stronger H-bond donor than an aromatic NH_2 moiety and the nitrile nitrogen is a weaker H-bond acceptor than an amide carbonyl.

In conclusion, we developed and utilized two Negishi cross-coupling reactions to enable evaluation of SAR in NAD^+-Dependent DNA Ligase inhibitors, our design was guided by modeling and crystallography. We utilized the earlier structures of LigA to design the novel analogs focusing on evaluation of diversity at two positions as well as incorporating a range of properties. Because of the synthetic difficulty for the diversification of R^1 and R^2 functionalization in the napthyridine core, we evaluated these positions in the pyridopyrimidine series. Initial SAR for the hydrophobic pocket was explored via Negishi cross-coupling chemistry. Evaluation of R^1 was done with R^2 = H, and it was found *tert*-butyl **4l** gave the best biochemical and cellular activity compared to other substituents, however the compound still required improvements in cellular potency. We then explored the ribose pocket, R^2. Functionalization at this position was challenging and we found Negishi cross-coupling with a variety of alkylzinc bromides readily afforded pyridopyrimidines **22a–f**. Compound **22g**, a diaminonitrile based inhibitor, was found to be more potent than compound **2**. Additional efforts are required to further improve the biochemical potency of this series including synthesis and profiling the corresponding napthyridine based aminocarboxamides.

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