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A revised synthesis of 6-alkoxy-2-aminopurines with late-stage convergence allowing for increased molecular complexity†

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A revised synthesis of 6-alkoxy-2-aminopurines with late-stage convergence allowing for increased molecular complexity

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Abstract

6-alkoxy-2-aminopurine derivatives are potent inhibitors of Cyclin Dependent Kinases (CDKs), with some selectivity towards CDK2 and thus have potential as cancer therapeutics. Development of these inhibitors for targeting CDK2-cyclin A/E complexes has previously involved a thorough investigation of structure activity relationships of the C-2 amine moiety. However, the established synthesis of these compounds, which uses the alcohol reagent as solvent, limits the complexity of the O-6 functionality which is required for more selective targeting. Herein we report an improved and refocused synthesis of a model CDK2 inhibitor (*NU6247*), affording convenient access to inhibitors with O-6 substituents whose parent alcohol is not amenable for use as solvent. This revised synthesis allows for a meaningful exploration of the O-6 position in this class of CDK2 inhibitors.

Keywords: Cyclin-dependent kinase inhibitors, Nucleophilic aromatic substitution, Purines

Introduction

Progression through the cell cycle is driven by cyclin-dependent kinases (CDKs) that are activated by their binding partners, cyclins.[1] Increases in CDK activity, either from mutation, overexpression (either of the CDK itself or of the cyclin proteins), or changes in regulation, are strongly associated with cancer,[2, 3] making these proteins promising anti-proliferative therapeutic targets.[4-6] Many synthetic CDK inhibitors (CKIs) have been developed.[7] Although they have shown some success in pre-clinical and clinical trials, there remain many challenges in their deployment. Improved protein selectivity is still required to minimize drug toxicity and establish a clear mechanism of action.[4, 8] Guided by our computational design efforts,[9] we are synthesizing selective, competitive CDK2 inhibitors.

We have identified *NU6247* as a suitable parent scaffold for the design of a new generation of CDK2 selective CKIs (**Figure 1**).[9, 10] This compound is part of a series of 6-alkoxy-2-aminopurine derivatives, the *NU2058* series, developed by the Griffin and Golding teams at Newcastle University, to selectively inhibit CDK2-cyclin complexes.[10-12] Our molecular docking studies suggest that introducing novel functionality at the O-6 position of *NU6247*, while keeping the N-2 substituent the same, shows promise for conferring improved selectivity over CDK1. Hence, new O-6 substituents can be designed to enhance binding to the activated CDK2 complex.

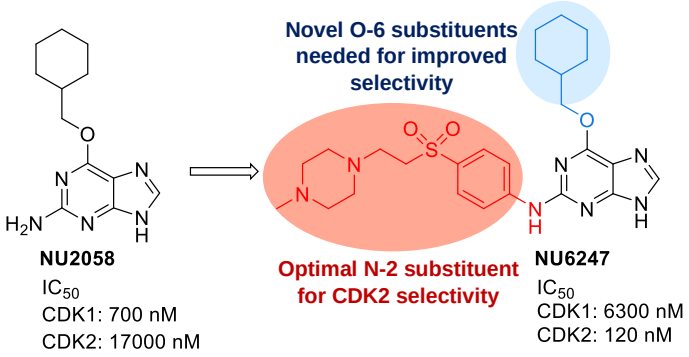
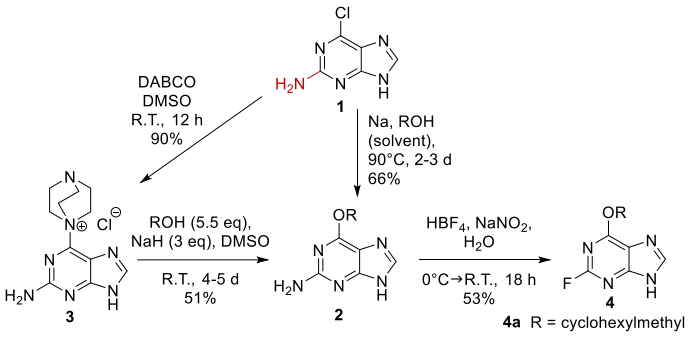


Figure 1: Structure of *NU2058* and *NU6247* and corresponding CDK1/2 half maximal inhibitory concentration (IC_{50}).[7, 9a]

The established synthesis of *NU6247* employs nucleophilic aromatic substitution (S_NAr) and subsequent side chain modifications, which involve long, linear synthetic sequences and the use of a large excess of the alcohol to introduce the O-6 substituent.[10, 13-15] Early introduction of the O-6 substituent enables simplified late-stage variation of the amine side chain. This is an excellent approach for screening chemical space at the N-2 substituent, but inefficient for generating libraries of compounds with complex O-6 substituents; this moiety needs to be carried over multiple steps, several of which are low-yielding. Finally, in the extant syntheses, this substituent is introduced with a large excess of the parent alcohol. Using difficult to synthesize alcohols, or those not amenable to use as solvent — such as those that are solids at high temperatures — under these constraints is highly impractical.

Needing more diverse O-6 substituents whose introduction is impractical under these constraints, we have developed an improved synthesis for the *NU6247* scaffold that is both more cost and time effective, with a reduced step count and higher overall yield. This synthesis not only facilitates easier access to existing CDK2 inhibitors but also makes the preparation of analogues with complex O-6 groups feasible.

Results & Discussion



Scheme 1. Synthesis of 6-alkoxy-2-fluoropurine by previous routes.[10, 13-16]

The existing synthesis of the *NU2058* series begins with a nucleophilic aromatic substitution on 2-amino-6-chloropurine **1** by an alkoxide generated from its corresponding alcohol, which also serves as reaction solvent (**Scheme 1**).[12] Despite these forcing conditions, this reaction takes several days to form appreciable amounts of product and often remains incomplete, requiring chromatography and/or distillation for product isolation. It is likely that, due to the electron donating effect of the amino group, **1** is too electron-rich to be adequately activated towards S_NAr. This justifies the large amount of alcohol, time, and heat needed to push the reaction forward. The only improvement that has previously been disclosed for this step is the substitution of the C-6 chlorine by 1,4-diazabicyclo[2.2.2]octane (DABCO) before reaction with the alcohol, generating activated intermediate **3**, which makes the 6-position more electrophilic and reduces the amount of alcohol needed to 5.5 equivalents; however, the conversion of **3** to product also requires several days and produces low yields.[15] The synthesis then proceeds with conversion of the C-2 amine group of **2** into fluoride **4** in moderate yield using Balz-Schiemann chemistry, which allows for the introduction of the unelaborated N-2 side chain through a second S_NAr reaction.

We identified two key tactical interventions that we needed to accomplish to make our library generation feasible (see SI Figure S1 for a full overview of the previous extant synthesis).[10] First, we needed to optimise the conditions for the incorporation of the O-6 substituent; second, we wished to fully elaborate the N-2 moiety prior to addition to the purine core to minimise the number of steps that the installed O-6 substituent must survive.

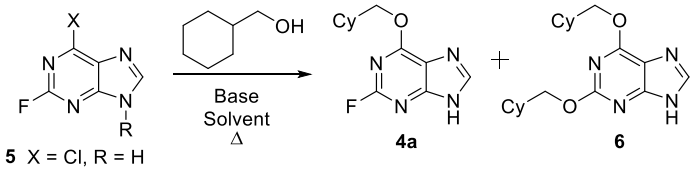
Screening for improvement of C-6 functionalization and its application to the synthesis of NU6102

S_NAr chemistry on 2,6-dihalopurines is strongly influenced by the inherent electronic bias of the heterocycle,[10] making substitution at the C-6 position much faster than at the C-2 position

regardless of the leaving group. Even if the fluorine were positioned at the C-2 position before performing C-6 substitution, the C-6 substituent would still be incorporated first. Therefore, with judicious choice of starting material we took advantage of this bias with a more electron deficient and reactive ring system, 6-chloro-2-fluoropurine (**5**).

In an initial test to assess reactivity of this substrate, previous literature conditions used with **1** were attempted, which led to rapid double substitution at both the C-6 and C-2 positions to give disubstituted purine **6** (Table 1, Entry 1). Although the desired mono-substituted product was not obtained, the enhanced S_NAr reactivity of this substrate was evident.

Table 1. S_NAr reaction screening employed to identify improved conditions for incorporation of alcohol into purine ring.



5 X = Cl, R = H
7 X = Cl, R = THP
8 X = DABCO$^+$, R = H

Cy = cyclohexyl

Entry	X	R	Alcohol Equiv.	Base Equiv.[a]	Solvent	Temperature[b,c]	Time[b,c]	Isolated Yield of 4a	Isolated Yield of 6
1	Cl	H	30	3.0[d]	-	90 °C	1.5 h	0%	69%
2	Cl	H	5.5	3.0	DMSO	R.T.	5 d	21%	0%
3	Cl	H	5.5	3.0	DMF	60 °C	24 h	39%	0%
4	Cl	H	5.5	3.0	THF	R.T.	3 d	11%	0%
5	Cl	H	5.5	5.0	THF	Reflux	4 h	60%	21%
6	Cl	H	5.5	5.0	THF	R.T.→ reflux	3.5 h	60%	13%
7	Cl	H	5.5	3.0	THF	R.T.→ reflux	3.5 h	71%	7%
8	Cl	H	3.5	3.0	THF	R.T.→ reflux	3.5 h	73%	3%
9	**Cl**	**H**	**2.5**	**3.0**	**THF**	**R.T.→ reflux**	**3 h**	**75%**	**0%**
10	Cl	H	2.0	2.5	THF	R.T.→ reflux	2 d	17 %	3 %
11	Cl	H	1.5	2.0	THF	Reflux	4 d	7%	0%
12	Cl	H	2.0	2.0[e]	THF	R.T.→ reflux	24 h	30%	0%
13	Cl	THP	2.0	2.5	THF	R.T.→ reflux	24 h	0%	0%
14	$DABCO^+$	H	2.5	3.0	DMSO	R.T.	2 d	52%	0%
15	$DABCO^+$	H	1.5	2.0	DMSO	R.T.	5 d	33%	0%

[a] NaH (60% dispersion in mineral oil) was used as the base unless otherwise indicated. [b] NaH and alcohol were mixed together at R.T. and stirred for one hour prior to adding purine substrate. Reported times and temperatures refer to the reaction after this point. [c] Reactions were run at R.T. for 2 h then heated at reflux (oil bath set to 70 °C) for 1 h. [d] Na metal was used as the base. [e] KH was used as the base with 18-crown-6 as additive.

This reaction was investigated by adjusting several parameters to identify favourable conditions able to generate good yields of the mono-substituted product (**4a**) with the minimal amount of alkoxide (**Table 1**). Cyclohexylmethoxide was generated using sodium hydride in polar aprotic solvents (DMSO, DMF, or THF). It should be noted, however, that prolonged heating of reactions containing sodium hydride and DMSO or DMF poses an explosive hazard and is far from ideal.[17,

[18] Initial attempts in these solvents at room temperature resulted in poor conversion despite long reaction times, likely due to the absence of heat, but no double substitution was observed (**Table 1, Entries 2 – 4**). Another attempt with THF was carefully heated to reflux to drive the reaction (**Table 1, Entry 5**). This modification drastically improved substrate conversion and reduced reaction time from several days to several hours, with the caveat that some disubstituted product formed. Interestingly, double substitution could be observed by TLC and NMR before complete consumption of the starting material. This was unexpected as substitution at C-2 is known to occur far slower than at C-6 due to the inherent bias of purine ring electronics. Fluoride's far higher lability than chloride's under S_NAr conditions apparently, somewhat, compensates. Considering this, we hypothesized that by heating the reaction immediately, while there is still an excess of alkoxide, we would have a greater number of alkoxide nucleophiles with sufficient energy to react at the C-2 position. Consequently, substitution, although still occurring primarily at C-6, could feasibly occur at either site, resulting in a mixture of mono- and di-substitution.

To mitigate this undesired reactivity, the reaction was initially run at room temperature for 2 h before heating to reflux; this change decreases the amount of unwanted **6** significantly (**Table 1, Entry 6**). This result suggests that it is not substitution at C-6 followed by that at C-2 that leads to product, as this pathway would not be affected by the change. Using this new thermal profile, the amount of alcohol was gradually reduced (**Table 1, Entries 7 – 10**). At 2.5 equivalents of alcohol, we obtained the highest yield of the desired product after only 3 h with no double substitution detectable. Further reduction of alcohol led to a significant loss in yield. We attempted further improvement by using a stronger base, replacing sodium hydride with potassium hydride in the presence of 18-crown-6, however the yield dropped substantially (**Table 1, Entry 11**).

Since purines exist as weakly acidic N-9/N-7 tautomers it is possible that some of the alkoxide acts as a base, potentially explaining the super-stoichiometric amount of reagent needed. To test this hypothesis, the reaction was attempted on **5**, protected at N-9 with a tetrahydropyranyl group (**7; Table 1, Entry 13**). However, no conversion was observed over 24 hours in any of our attempts at this reaction using our standard conditions; this suggests that the N−H has an essential role in the reaction.

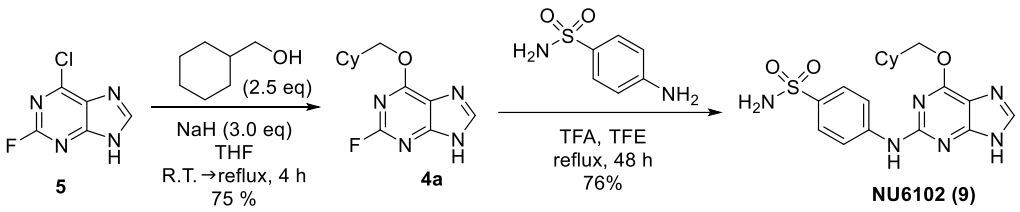
Considering the previous improvement using the 6-DABCO-2-fluoro-purine salt (**8**), we examined whether this intermediate would improve the reaction yields. Treatment of **8** with 2.5 equivalents of alcohol provided a 52% yield, comparable to substitution on **2** with 5.5 alcohol equivalents. However, it still does not provide any improvement compared to 6-chloro-2-fluoropurine **5**. Although potentially more electrophilic at the 6-position, the DABCO-purine substrate poses other synthetic hurdles as it is only soluble in DMSO, preventing reaction heating and making purification much more difficult. It also must be made which is less convenient than a readily commercially available material like **5**.

Direct substitution on **5** using 2.5 equivalents of alcohol with 3.0 equivalents of NaH in THF gives 6-cyclohexylmethoxy-2-fluoro-purine in 75% yield after 4 hours. The reaction is very clean, and the product is isolated in acceptable purity by simple neutralization, concentration, and reprecipitation out of a methanol/water solution to remove the excess alcohol.

This one step synthesis of 6-alkoxy-2-fluoro-purines is substantially more cost and time effective compared to the previously established 2-3 step method (**Scheme 1**). The amount of the nucleophilic alcohol needed is significantly less, reaction time has been decreased from several days to 4 hours, and purification is much easier as removal of large amounts of excess alcohol or unreacted starting material is not needed. This method shows a 40 to 50% increase in overall

product yield compared to previous methods. Incorporation of more complex alcohols, especially those that are not readily accessible, is now feasible, enabling exploration of chemical space at the O-6 position.

With our improved synthetic strategy on hand, accessing CDK2 inhibitors is far more convenient. To demonstrate the practicality of our C-6 substitution conditions, another member of the *NU2058* series, *NU6102* (**9**),[12] was synthesized in 2 steps (**Scheme 2**). This highlights how the C-6 substitution protocol developed in this work compliments the previously enhanced C-2 substitution conditions (TFA/TFE) for a fast and higher yielding synthesis of inhibitors (39% higher overall for *NU6102*).[14] This is especially rapid for those that incorporate commercially available alcohols and amines.



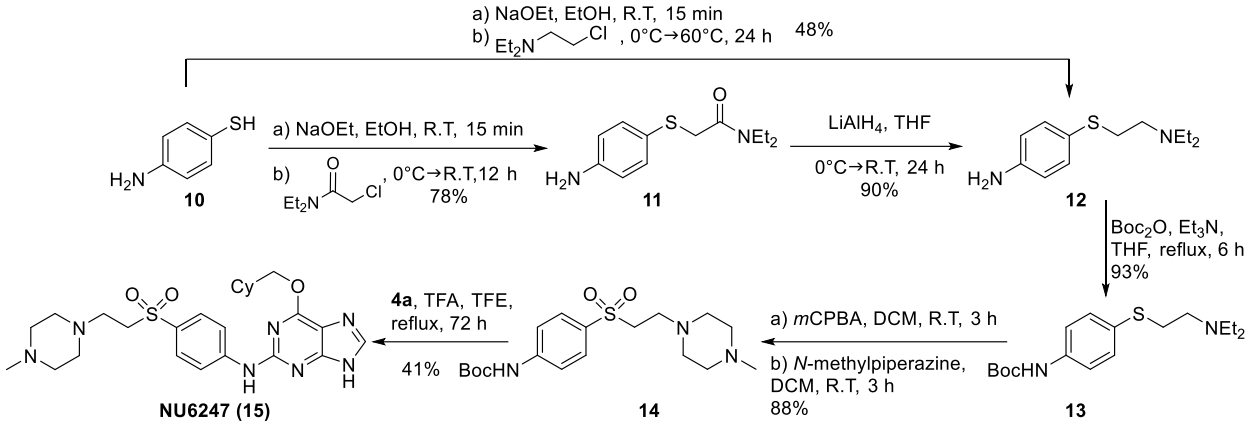
Scheme 2. Two step synthesis of *NU6102* (**9**)

Refocused synthesis and incorporation of N-2 substituent – an improved synthesis of NU6247

Another issue that impedes complex functionalization at the O-6 position of *NU6247* arises from the incorporation of the amine moiety. This has previously been done by synthesizing a precursor amine (over 3 steps), incorporating it into the C-6 substituted purine and then performing multiple side chain modification steps to obtain the fully elaborated N-2 substituent (**Scheme S1**).[10] While this approach is convenient for late stage β-aminoethylsulfone functionalization, it exposes the O-6

substituent to multiple steps (with a concomitant loss of yield), several of which involve harsh reductive and oxidative conditions incompatible with functionality more complicated than ethers.

Consequently, we needed to incorporate the fully elaborated amine into the purine scaffold to minimize step-count (**Scheme 3**). This meant redesigning the synthesis of the amine. Starting from 4-aminothiophenol (**10**), we synthesized the fully elaborated amine in 4 steps with moderate-high yields throughout. The thiophenol was first alkylated using *N*,*N*-diethylchloroacetamide to give **11**, which was then reduced to amine **12** using $LiAlH_4$. This step required the use of excess hydride as the free aniline is deprotonated by the reagent (*Caution! H_2 evolution*), fortunately this anion does not impede the reaction. An alternative strategy to synthesize **12** by alkylation of **10** using 2-chlorotriethylamine was also explored, which would have eliminated the need for reduction. However, the yield of **12** in this 1 step method was lower than for the two-step protocol—although it is more elegant. It seems that anchimeric assistance is not as beneficial as the enhanced electrophilicity of the α-halo carbonyl. The aniline was then protected with a *tert*-butyloxycarbonyl (Boc) group (**13**), and the installation of the desired β-piperazinyl functionality and concomitant oxidation of the sulfur was accomplished according to Golding and co-workers' elegant approach they had developed to access the *NU2058* series.[10] The sulfide and tertiary amine of compound **13**, when treated with *m*CPBA, oxidize to a sulfone and N-oxide respectively. An *in situ* Cope elimination generates the terminal alkene. Addition of *N*-methylpiperazine to the reaction mixture, results in conjugate addition, providing fully elaborated amine **14**.



Scheme 3. Synthesis of target amine moiety and completion of the synthesis of *NU6247* (**15**).

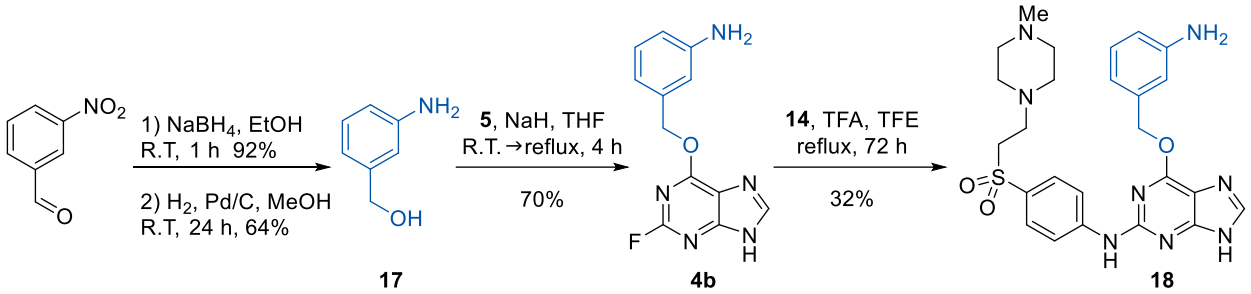
Amine **14** is then incorporated into substituted purine **4a** by S_NAr in the presence of TFA with TFE as the solvent, conditions previously shown to accelerate C-2 substitution of these compounds while simultaneously enacting Boc deprotection (**Scheme 3**).[14] This reaction is slightly lower yielding than the C-2 substitution in the literature (41% *vs* 57%); this may be ascribed to the reduced nucleophilicity of the sulfonylaniline **14** compared to the less oxidised sulfide used previously. However, the slight decrease in yield with this step is more than compensated for by the absence of subsequent steps.

Taking synthetic improvements to both C-6 and C-2 functionalization into consideration, the parent *NU6247* (**15**) scaffold was synthesized in 7 steps, cutting 3 steps out of the previously published synthesis, and more than trebling the overall yield (for comparisons, see Scheme S3). Most importantly, this approach mitigates the synthetic hurdles that have prevented broad exploration of O-6 functionality without sacrificing C-2 diversity. Modifications at C-2 can still be simply obtained either by incorporation of **12** and subsequent oxidation or derivatization of **13** with other amines before incorporation. It should be noted that the yield-limiting linear sequence

in the literature route begins with the incorporation of the alcohol. For the route developed in this work, the limiting synthetic sequence does not include C-6 substitution but instead involves the synthesis and incorporation of the amine substituent. This new synthesis allows for easy generation of libraries of compounds varying at either or both C-2 and C-6.

Accessing CDK2 inhibitors using improved synthetic methodology

To demonstrate the utility of the C-6 substitution protocol beyond the cyclohexylmethanol model, this methodology was used to prepare a novel inhibitor (**Scheme 4**). A functionalized alcohol (3-aminobenzylalcohol **17**, prepared in 2 steps from 3-nitrobenzaldehyde, see SI) was used with the C-6 substitution conditions as previously described to give **4b** in a yield similar to that using cyclohexylmethanol, the only difference being that chromatography was required to purify the product. Unsurprisingly, the specific purification procedure required after the substitution depends on the alcohol used and the properties of the product. However, the drastically reduced equivalents of alcohol left over after the reaction makes the purification much easier than when a larger excess of alcohol was used; it should also be noted that alcohol **17** is a solid over the temperature range and therefore would make for a poor solvent. N-2 Amine **14** was then incorporated into **4b** using the same methodology used to synthesize *NU6247* to give compound **18**, a simple, yet illustrative derivative that introduces hydrogen bonding, salt-bridge formation and π-stacking interactions to the O-6 position. Needless to say, the oxidation chemistry used in the traditional synthesis to access the sulfone would be complicated by the aniline on the C-6 moiety. A variety of more complex targets, with a wide variety of functional groups, are currently being prepared in our lab as part of a medicinal chemistry effort and will be disclosed in due course, but we believe that this route would be useful for others looking to investigate this or similar scaffolds.



Scheme 4. Synthesis of potential CDK2 inhibitor **18** with novel O-6 functionalization.

Conclusion

By fine-tuning the approach to C-6 S_NAr and N-2 side chain modification reactions, we have developed a synthesis for a model 6-alkoxy-2-aminopurine CDK2-cyclinA/E inhibitor (*NU6247*) that affords economical insertion of the O-6 substituent, a reduced number of steps (from 10 to 7), and over three times the overall yield from the previously reported method. This improved synthesis is not only convenient for accessing existing CDK2 inhibitors but is also being applied to synthesize inhibitors with diverse O-6 functionalization required for improving selectivity for these compounds.

Conflicts of interest

The authors declare there are no conflicts of interest.

Acknowledgements

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Author Contributions. Conceptualization, JFT and LAP; Funding acquisition JFT and LAP; Investigation LM and JJH; Methodology LM, JJH and JFT; Project administration JFT; Supervision JFT and JJH; Writing — original draft, LM; Writing — review and editing, All authors.

Experimental

General methods and materials

Reported yields in the text are the lowest from the range of replicates. Ranges of the yields from replicate experiments are noted in the experimental. Solvents were purchased from Caledon Labs (Caledon, Ontario), Sigma-Aldrich (Oakville, Ontario) or VWR Canada (Mississauga, Ontario). Other chemicals were purchased from Sigma-Aldrich, AK Scientific, Oakwood Chemicals, Alfa Aesar or Acros Chemicals and were used without further purification unless otherwise noted. 18-Crown-6 was recrystallized from acetonitrile. Anhydrous DMSO was purchased from Millipore Sigma. Anhydrous THF was obtained by distillation over benzophenone-sodium. All heated reactions were conducted using oil baths on IKA RET Basic stir plates equipped with a P1000 temperature probe. Thin layer chromatography was performed using EMD aluminum-backed silica 60 F254-coated plates and were visualized using either UV-light (254 nm), $KMnO_4$, or

ninhydrin stain. Column chromatography was carried out using standard flash technique with silica (Siliaflash-P60, 230-400 mesh Silicycle) under compressed air pressure. ^{1}H NMR spectra were obtained at 300 MHz or 500 MHz, ^{13}C NMR spectra were obtained at 75 or 125 MHz, and ^{19}F NMR spectra were obtained at 470 MHz on Bruker instruments. NMR chemical shifts (δ) are reported in ppm and are calibrated against residual solvent signals of $CHCl_3$ (δ 7.26), DMSO-d6 (δ 2.50), or methanol-d4 (δ 3.31). Spectra were processed using MNOVA 14.1.2 (Mestrelab Research S.L., Santiago de Compostela, Spain). Fourier transform was conducted using a linear phase shift group delay, an exponential-fit 1 Hz apodization, and a zero-filling linear prediction of 65536 points. An automatic phase correction was applied and manually refined to provide a flatter baseline. A baseline correction, employing a Whittaker smoothing function with a 2.60 Hz filter and a smoothing factor of 16384 was employed on the spectrum between −2.00 and 15.00 ppm. Integration was measured using the manual integration tool and referenced to a relevant aliphatic proton. Multiplet analysis was conducted using the algorithm as implemented in the software, confirmed by manual analysis. High resolution mass spectrometry (HRMS) was performed at Queen's University, Department of Chemistry, Kingston, ON, CA using an Orbitrap Velos HESI Hybrid Ion Trap-Orbitrap Mass Spectrometer (Thermo Scientific).

Specific Experimental Procedures

^{1}H, ^{13}C, and ^{19}F spectral data are provided for previously unpublished compounds, published compounds with poor or no available spectral data, and key compounds. ^{1}H spectral data are provided for all other compounds along with references to published characterization data.

6-cyclohexylmethoxy-2-fluoro-purine (4a) – CAUTION, H_2 generation

To a suspension of NaH (60% dispersion in mineral oil; 0.24 g, 6.0 mmol, 3.0 eq) in anhydrous THF (5 mL) under an inert atmosphere (N_2) was added cyclohexylmethanol (0.62 mL, 5.0 mmol, 2.5 eq) dropwise while allowing the H_2 gas generated to escape through a bubbler (**Caution!** H_2 evolution). The mixture was stirred for 1 h at room temperature, followed by dropwise addition of a solution of 6-chloro-2-fluoropurine (0.345 g, 2.0 mmol, 1.0 eq) in anhydrous THF (8 mL). The reaction was stirred at room temperature for 2 h and then heated at reflux for 1 h until TLC analysis confirmed the reaction as complete (10% MeOH/CH_2Cl_2, R_f = 0.53). Water was added (2 mL) and the mixture was neutralized with AcOH. The mixture was concentrated *in vacuo* and the resulting solid was suspended in MeOH (4 mL) and precipitated using water (30 mL). The precipitate was collected by vacuum filtration and washed with water (2 × 5 mL) to yield a white powder (0.373 g, 75%). **^{1}H NMR** (300 MHz, DMSO-d_6) δ_{ppm} 8.38 (s, 1H), 4.34-4.32 (d, J = 6.5 Hz, 2H), 1.84-1.64 (m, 6H), 1.32-1.06 (m, 5H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ_{ppm} 160.4, 157.9, 156.2, 151.4, 143.6, 72.3, 36.7, 29.0, 25.9, 25.1. **^{19}F NMR** (470 MHz, DMSO-d_6) δ_{ppm} 52.0. This reaction was repeated 5 times for this project on scales ranging from 0.3 to 2 g, with yields between 75-79%. Characterization data is consistent with previously reported values.[12] *Note: The NH signal of the purine was not observed in ^{1}H NMR spectrum. ^{13}C-^{19}F coupling in ^{13}C spectra could not be resolved due to peak broadening (see supplementary data).*

6-(3-aminobenzyloxy)-2-fluoro-9*H*-purine (4b)

To a suspension of NaH (60% dispersion in mineral oil; 0.24 g, 6.0 mmol, 3.0 eq) in anhydrous THF (5 mL) under an inert atmosphere (N_2) was added a solution of **17** (0.616 g, 5.0 mmol, 2.5 eq) in anhydrous THF (3 mL) dropwise while allowing generated H_2 gas to escape through a bubbler. The mixture was stirred for 1 h at room temperature, followed by dropwise addition of a solution of 6-chloro-2-fluoropurine (0.345 g, 2.0 mmol, 1.0 eq) in anhydrous THF (8 mL). The reaction was stirred at room temperature for 2 h and then heated at reflux for 1 h at which point TLC analysis confirmed the reaction as complete (5% MeOH/EtOAc, $R_f = 0.33$). Water was added (2 mL) and the mixture was neutralized with AcOH. The mixture was concentrated *in vacuo* and the resulting solid was purified by flash column chromatography (5% MeOH/EtOAc) by dry loading the solid using celite to yield an off-white solid (0.362 g, 70%). **^{1}H NMR** (300 MHz, DMSO-d_6) δ_{ppm} 8.33 (s, 1H), 7.06-7.00 (apparent t, J = 7.5 Hz, 1H), 6.68-6.53 (m, 3H), 5.43 (s, 2H), 5.16 (br s, 2H). **^{13}C NMR** (125 MHz, DMSO-d_6) δ_{ppm} 161.4, 157.8, 156.2, 148.8, 142.5, 136.1, 129.0, 118.7, 115.8, 114.0, 113.7, 69.3. **^{19}F NMR** (470 MHz, DMSO-d_6) δ_{ppm} 51.9. **ESI-MS** *m/z* calc'd for $C_{12}H_{11}FN_5O$ $[M+H]^+$ 260.0948, found 260.0941. This reaction was repeated 2 times for this project on a 0.6 and 1 g scale with yields of 70 and 75%, respectively. *Note: The NH signal of the purine was not observed in ^{1}H NMR spectrum. ^{13}C-^{19}F coupling in ^{13}C spectrum could not be resolved due to peak broadening (see supplementary data).*

2,6-bis(cyclohexylmethoxy)-purine (6) – CAUTION, H_2 generation

Sodium metal (0.091 g, 3.96 mmol, 3.0 eq) was added to cyclohexylmethanol (4.87 mL, 39.6 mmol, 30.0 eq) at room temperature under a Nitrogen atmosphere and the mixture was stirred at 90 °C until complete disappearance of sodium was observed (**Caution!** H_2 evolution). 6-chloro-2-fluoropurine (0.228 g, 1.32 mmol, 1.0 eq) was added and the mixture was stirred at 90°C for 1.5 h

by which time TLC analysis confirmed the reaction as complete (10% MeOH/CH_2Cl_2, R_f = 0.56). Water (2 mL) was added, and the mixture was neutralized using AcOH. The mixture was concentrated *in vacuo* and the resulting solid was washed with water, collected by vacuum filtration, and dried *in vacuo* to yield a white crystalline solid (0.317 g, 69%). **^{1}H NMR** (300 MHz, CD_3OD) δ_{ppm} 8.06 (s, 1H), 4.33-4.32 (d, *J* = 6.1 Hz, 2H), 4.15-4.14 (d, *J* = 6.1 Hz, 2H), 1.88-1.67 (m, 12H), 1.33-1.03 (m, 10H). **^{13}C NMR** (75 MHz, CD_3OD) δ_{ppm} 161.6, 160.7, 155.4, 140.6, 114.2, 72.7, 72.0, 37.4, 37.3, 29.5, 29.4, 26.2, 26.1, 25.5, 25.4. **ESI-MS** *m/z* calc'd for $C_{19}H_{29}N_4O_2$ $[M+H]^+$ 345.2291, found 345.2293. This reaction was performed once for this project.

6-chloro-2-fluoro-9-(tetrahydro-2*H*-pyran-2-yl)-9*H*-purine (7)

Under nitrogen, 6-chloro-2-fluoro-purine (2.5 g, 14.49 mmol, 1 eq) and *p*-TsOH·H_2O (0.28 g, 1.45 mmol, 0.1 eq) were dissolved in anhydrous CH_2Cl_2 (20 mL). Dihydropyran (2.00 mL, 21.73 mmol, 1.5 eq) was added dropwise at room temperature. The reaction was stirred for 12 h at room temperature at which point TLC analysis confirmed the reaction as complete (50% Hex/EtOAc, R_f = 0.45). The mixture was filtered, and the filtrate was washed with saturated Na_2CO_3 (10 mL) and brine (10 mL), dried over $MgSO_4$, and concentrated *in vacuo*. The resulting yellow oil was triturated with EtOAc (2 mL) and hexanes (10 mL) and placed in the freezer (-20 ºC) overnight to yield **3** as white crystals (2.12 g, 57%). **^{1}H NMR** (300 MHz, CD_3OD) δ_{ppm} 8.66 (s, 1H), 5.74-5.70 (dd, *J* = 10.5 Hz, 2.4 Hz, 1H), 4.14-4.08 (m, 1H), 3.83-3.74 (m, 1H), 2.29-2.05 (m, 3H), 1.88-1.62 (m, 3H). This reaction was performed once for this project. Characterization data is consistent with previously reported values.[19]

1-(2-fluoro-9H-purin-6-yl)-1,4-diazabicyclo[2.2.2]octan-1-ium chloride (8)

To a solution of 6-chloro-2-fluoro-purine (1 g, 5.78 mmol, 1.0 eq) in anhydrous THF (25 mL) was added DABCO (1.96 g, 17.39 mmol, 3.0 eq) portion wise over 1 h. The reaction mixture was stirred at room temperature for 24 h. The resulting light-yellow precipitate was collected by filtration and washed with diethyl ether (3 × 5 mL). The solid was suspended in CH_2Cl_2 (50 mL) and stirred for 1 h. After filtration and washing with CH_2Cl_2 (3 × 10 mL), **8** was obtained as a light-yellow solid which was dried *in vacuo* and used without further purification (1.51 g, 91%). **^{1}H NMR** (300 MHz, D_2O) δ_{ppm} 8.44 (s, 1H), 4.24-4.19 (t, *J* = 7.5 Hz, 6H), 3.46-3.40 (t, *J* = 7.5 Hz, 6H). **^{13}C NMR** (125 MHz, DMSO-*d*$_6$) δ_{ppm} 169.2, 157.7, 155.6, 141.2, 139.2, 54.4, 44.4. **^{19}F NMR** (470 MHz, DMSO-*d*$_6$) δ_{ppm} 49.6. **ESI-MS** *m/z* calc'd for $C_{11}H_{14}FN_6^+$ $[M]^+$ 249.1264, found 249.1267. This reaction was performed once for this project. *^{13}C-^{19}F coupling in ^{13}C spectrum could not be resolved due to peak broadening (see supplementary data).*

4-((6-(cyclohexylmethoxy)-9*H*-purin-2-yl)amino)benzenesulfonamide (NU6102) (9)

To a solution of **4a** (0.10 g, 0.4 mmol, 1.0 eq) in TFE (1 mL) was added TFA (0.15 mL, 2 mmol, 5.0 eq), followed by sulfanilamide (0.138 g, 0.8 mmol, 2.0 eq). The mixture was stirred at reflux for 48 h and then concentrated *in vacuo. T*he residue was diluted with water, washed with saturated $NaHCO_3$ (2 × 10 mL), and extracted using EtOAc (3 × 15 mL). The combined organic layers were dried over $MgSO_4$ and concentrated *in vacuo*. The resulting solid was purified by flash column chromatography using a 1-10% MeOH/EtOAc gradient (10% MeOH/EtOAc, R_f = 0.57) to yield the product as an off-white solid (0.127 g, 76%). **^{1}H NMR** (300 MHz, DMSO-*d*$_6$) δ_{ppm} 9.76 (s, 1H), 8.03 (s, 1H), 7.97-7.94 (ddd, *J* = 8.7 Hz, 2.1 Hz, 0.7 Hz, 2H), 7.72-7.68 (ddd, *J* = 8.7 Hz, 2.7 Hz, 0.7 Hz, 2H), 7.15 (s, 1H), 4.37-4.35 (d, *J* = 6.0 Hz, 2H), 1.73-1.65 (m, 6H), 1.30-1.18 (m, 5H). This reaction was performed once for this project. Characterization data is consistent with

previously reported values.[12] *Note: The NH signal of the purine was not observed in ^{1}H NMR spectrum.*

2-(4-aminophenyl)sulfanyl-*N*,*N*-diethylacetamide (11) – CAUTION, H_2 generation

Sodium metal (0.368 g, 15.98 mmol, 1.0 eq) was added to absolute EtOH (40 mL) at room temperature under an inert atmosphere (N_2) and stirred until all of the metal had reacted (**Caution!** H_2 evolution). 4--Aminothiophenol (2 g, 15.98 mmol, 1.0 eq) was added and the solution was stirred for 15 min. The solution of thiolate was then added dropwise to a solution of *N*,*N*-diethylchloroacetamide (2.20 mL, 15.98 mmol, 1.0 eq) in absolute degassed EtOH (40 mL) at 0°C under an inert atmosphere (N_2). The reaction was stirred for 12 h, water (20 mL) was added and most of the EtOH was removed under reduced pressure. The residual aqueous layer was extracted using CH_2Cl_2 (4 × 50 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in vacuo* to yield a dark yellow oil. The product was purified by flash column chromatography using (2% MeOH/EtOAc, R_f = 0.47) to yield **11** as a dark yellow solid (3.00 g, 78%). **^{1}H NMR** (300 MHz, DMSO-d_6) δ_{ppm} 7.14-7.09 (ddd, *J* = 8.7, 2.7, 2.2, 2H), 6.52-6.47 (ddd, *J* = 8.7, 2.7, 2.1, 2H), 5.27 (s, 2H), 3.55 (s, 2H), 3.28-3.18 (two overlapping q, *J* = 7.2 Hz, 4H), 1.09-1.05 (t, *J* = 7.2 Hz, 3H), 1.01-0.96 (t, *J* = 7.2 Hz, 3H). **^{13}C NMR** (75 MHz, DMSO-d_6) δ_{ppm} 167.6, 149.31, 134.7, 118.4, 114.7, 42.2, 39.8, 39.0, 14.7, 13.3. **ESI-MS** *m/z* cald for $C_{12}H_{19}N_2OS$ $[M+H]^+$ 239.1218, found 239.1220. This reaction was repeated 3 times for this project on scales ranging from 1 to 5 g with yields between 78-80%.

4-(2-(diethylamino)ethylsulfanyl)aniline (12) – CAUTION, H_2 generation

(Procedure 1)

To a suspension of $LiAlH_4$ (0.280 g, 7.4 mmol, 4.0 eq) in anhydrous THF (15 mL) was added a solution of **11** (0.43 g, 1.85 mmol, 1.0 eq) in THF (5 mL) dropwise with ice cooling (**Caution!** H_2 may be evolved). The reaction was warmed to room temperature and allowed to stir for 24 h. The reaction was quenched by sequential addition of water (0.25 mL), 15% NaOH solution (0.25 mL), and water (0.75 mL). The mixture was stirred until a white solid had formed, at which point it was filtered through celite followed by washing the celite with ether. The filtrate was dried over $MgSO_4$ and concentrated *in vacuo* to yield an oil. The oil was then taken up in CH_2Cl_2 (20 mL) and washed with water (2 × 10 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to give **12** as a yellow oil (0.367 g, 90%). **^{1}H NMR** (300 MHz, CD_3OD) δ_{ppm} 7.23-7.18 (ddd, J = 8.6 Hz, 2.9 Hz, 1.8 Hz, 2H), 6.68-6.63 (ddd, J = 8.6 Hz, 2.9 Hz, 2.0 Hz, 2H), 2.82-2.77 (t, J = 8.3 Hz, 2H), 2.66-2.61 (t, J = 8.3 Hz, 2H), 2.55-2.48 (q, J = 7.2 Hz, 4H), 0.99-0.95 (t, J = 7.2 Hz, 6H). This reaction was repeated 2 times for this project on a 0.4 and 2 g scales with yields of 90 and 94%, respectively. Characterization data is consistent with previously reported values.[20]

(Procedure 2)

Sodium metal (0.386 g, 16.78 mmol, 2.1 eq eq) was added to absolute EtOH (40 mL) at room temperature under an inert atmosphere (N_2) and stirred until all of the metal had reacted (**Caution!** H_2 evolution). 4-Aminothiophenol (1 g, 7.99 mmol, 1.0 eq) was added and the solution was stirred for 15 min. The solution of thiolate was added dropwise to a solution of 2-chloro-*N*,*N*-triethylamine hydrochloride (1.38 g, 7.99 mmol, 1.0 eq) in absolute EtOH (40 mL) at 0°C under an inert atmosphere (N_2). The reaction was stirred for 24 h at 60°C, water (10 mL) was added and EtOH was removed under reduced pressure. The aqueous layer was extracted using CH_2Cl_2 (4 × 25 mL). The combined organic layers were dried over $MgSO_4$, and the solvent was removed *in*

vacuo. The crude product was purified by flash column chromatography (30% MeOH/EtOAc, R_f = 0.33) to yield a yellow oil (0.86 g, 48%). This reaction was performed 2 times for this project on the same scale providing similar yields in both cases (48 and 49%).

tert-butyl (4-((2-(diethylamino)ethyl)thio)phenyl)carbamate (13)

To a solution of **12** (0.367 g, 1.64 mmol, 1.0 eq) in THF was added Et_3N (0.46 mL, 3.28 mmol, 2.0 eq) followed by Boc_2O (0.72 g, 3.28 mmol, 2.0 eq). The solution was stirred at reflux for 16 h after which THF was removed under reduced pressure. The residue was taken up in CH_2Cl_2 (15 mL) and washed with 1 M HCl (2 × 5 mL) and water (10 mL). The organic layer was dried over $MgSO_4$ and the solvent was removed *in vacuo* to yield a yellow oil. The product was purified by flash column chromatography (30% MeOH/EtOAc, R_f = 0.44) to give a pale-yellow oil (0.49 g, 93%). **^{1}H NMR** (300 MHz, CD_3OD) δ_{ppm} 7.40-7.32 (m, 4H), 3.02-2.96 (t, *J* = 7.8 Hz, 2H), 2.82 - 2.77 (t, *J* = 7.8 Hz, 2H), 2.73-2.66 (q, *J* = 7.2 Hz, 4H), 1.51 (s, 9H), 1.07-1.03 (t, *J* = 7.5 Hz, 6H). **^{13}C NMR** (75 MHz, CD_3OD) δ_{ppm} 155.1, 140.1, 133.2, 129.1, 120.3, 81.0, 52.9, 48.1, 31.6, 28.7, 11.0. **ESI-MS** *m/z* calc'd for $C_{17}H_{29}N_2O_2S$ $[M+H]^+$ 325.1950, found 325.1944. This reaction was carried out 2 times for this project on a 0.3 and 1.5 g scale with a yield of 93% both times.

tert-butyl (4-((2-(4-methylpiperazin-1-yl)ethyl)sulfonyl)phenyl)carbamate (14)

To a solution of **13** (0.16 g, 0.5 mmol, 1.0 eq) in CH_2Cl_2 (10 mL) was added *m*CPBA (0.41 g, 2.35 mmol, 4.7 eq) in one portion and the reaction was stirred at room temperature for 3 h. *N*-methylpiperazine (0.34 mL, 6.0 3.0 mmol, 6.0 eq) was added to the reaction mixture and the reaction was stirred for another 3 h. The reaction mixture was diluted with CH_2Cl_2 (10 mL) and washed with water (2 × 15 mL) and 5% $NaHCO_3$ (3 × 15 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo* to yield a pale-yellow oil which was triturated with hexanes to give a pale-yellow solid (0.17 g, 88%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ_{ppm} 7.82-7.79 (apparent d, *J* = 8.1 Hz, 2H), 7.56-7.53 (apparent d, *J* = 8.1 Hz, 2H), 6.81 (br s, 1H), 3.28-3.23 (t, *J* = 7.5 Hz, 2H), 2.77-2.72 (t, *J* = 7.5 Hz, 2H), 2.41 (br s, 8H), 2.24 (s, 3H), 1.53 (s, 9H). **^{13}C NMR** (75 MHz, $CDCl_3$) δ_{ppm} 152.0, 143.5, 132.9, 129.5, 117.9, 81.8, 54.8, 53.8, 52.6, 51.3, 45.9, 28.3. This reaction was repeated 2 times on a a 0.16 and 2 g scale with yields of 88 and 91%, respectively. Characterization data is consistent with previously reported values.[10]

6-(cyclohexylmethoxy)-*N*-(4-((2-(4-methylpiperazin-1-yl)ethyl)sulfonyl)phenyl)-9*H*-purin-2-amine, (NU6247, 15)

To a solution of **4a** (0.050 g, 0.2 mmol, 1.0 eq) in TFE (1 mL) was added **14** (0.154 g, 0.4 mmol, 2.0 eq), followed by TFA (0.075 mL, 1 mmol, 5.0 eq). The reaction was stirred at reflux for 72 h. The mixture is concentrated *in vacuo* and the residue was diluted with EtOAc (7 mL) and washed with saturated $NaHCO_3$ (2 × 4 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting solid was purified by flash column chromatography (10% MeOH/CH_2Cl_2, R_f = 0.19) to yield the product as an off-white solid (0.042 g, 41%). **^{1}H NMR** (300 MHz, $CDCl_3$) δ_{ppm} 7.88 (s, 1H), 7.84 (s, 1H), 7.78-7.75 (apparent d, *J* = 8.7 Hz, 2H), 7.65-7.62 (apparent d, *J* = 8.7 Hz, 2H), 4.28-4.26 (d, *J* = 6.0 Hz, 2H), 3.31-3.26 (t, *J* = 7.1 Hz, 2H), 2.76-2.72 (t, *J* = 7.1 Hz, 2H), 2.38 (br s, 8H), 2.21 (s, 3H), 1.91-1.61 (m, 6H), 1.14-0.81 (m, 5H). **^{13}C NMR** (125 MHz,

$CDCl_3$) δ_{ppm} 161.0, 154.8, 151.6, 145.5, 140.9, 130.3, 129.9, 117.7, 114.13, 72.6, 54.9, 54.0, 52.8, 51.5, 46.0, 37.4, 29.9, 26.5, 25.8. This reaction was performed once for this project. Characterization data is consistent with previously reported values.[10] *Note: The NH signal of the purine was not observed in ^{1}H NMR spectrum.*

6-((3-aminobenzyl)oxy)-*N*-(4-((2-(4-methylpiperazin-1-yl)ethyl)sulfonyl)phenyl)-9*H*-purin-2-amine (18)

To a solution of **4b** (0.050 g, 0.2 mmol, 1.0 eq) in TFE (1 mL) was added **14** (0.154 g, 0.4 mmol, 2.0 eq), followed by TFA (0.075 mL, 1.0 mmol, 5.0 eq). The reaction was stirred at reflux for 72 h. The mixture was concentrated *in vacuo* and the residue was diluted with EtOAc (7 mL) and washed with saturated $NaHCO_3$ (2 × 4 mL). The organic layer was dried over $MgSO_4$ and concentrated *in vacuo*. The resulting solid was purified by flash column chromatography (10% MeOH/CH_2Cl_2, R_f = 0.12) to yield the product as beige solid (0.033 g, 32%). **^{1}H NMR** (500 MHz, CD_3OD) δ_{ppm} 7.99 (s, 1H), 7.63-7.60 (apparent d, *J* = 8.9 Hz, 2H), 7.56-7.54 (apparent d, *J* = 8.9 Hz, 2H), 7.12-7.06 (apparent t, *J* = 8.1 Hz, 1H), 6.86-6.74 (m, 3H), 5.43 (s, 2H), 3.33-3.28 (t, *J* = 7.7 Hz, overlapping with CD_3OD peak, 2H), 2.70-2.65 (t, *J* = 7.7 Hz, 2H), 2.40 (br s, 8H), 2.23 (s, 3H). **^{13}C NMR** (125 MHz, CD_3OD) δ_{ppm} 161.4, 157.8, 156.1, 155.4, 147.9, 141.6, 135.2, 131.1, 128.7, 125.8, 121.4, 114.3, 112.5, 110.6, 110.3, 70.3, 55.5, 54.4, 53.2, 52.6, 45.9. **ESI-MS** *m/z* calc'd for $C_{25}H_{31}N_8O_3S$ $[M+H]^+$ 523.2240, found 523.2267. This reaction was performed once for this project.

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