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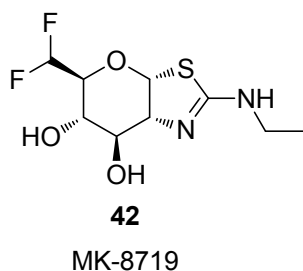
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ABSTRACT: Inhibition of O-GlcNAcase (OGA) has emerged as a promising therapeutic approach to treat tau pathology in neurodegenerative diseases such as Alzheimer's

disease and progressive supranuclear palsy. Beginning with carbohydrate-based lead molecules, we pursued an optimization strategy of reducing polar surface area to align the desired drug-like properties of potency, selectivity, high central nervous system (CNS) exposure, metabolic stability, favorable pharmacokinetics, and robust in vivo pharmacodynamic response. Herein, we describe the medicinal chemistry and pharmacological studies that led to the identification of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(ethylamino)-3a,6,7,7a-tetrahydro-5*H*-pyrano[3,2-*d*]thiazole-6,7-diol **42** (MK-8719), a highly potent and selective OGA inhibitor with excellent CNS penetration that has been advanced to first-in-human Phase I clinical trials.



INTRODUCTION

One of the defining pathological hallmarks of Alzheimer's disease (AD) is the accumulation of neurofibrillary tangles (NFTs) in the brains of patients. NFTs are composed primarily of aggregates of the microtubule-associated protein tau.¹ During the course of disease progression, aggregation of tau appears to be driven by its abnormal hyperphosphorylation leading to self-assembly into oligomers, which then form the paired helical filaments that make up NFTs. The presence of pathological hyperphosphorylated tau aggregates is a common feature of several neurodegenerative disorders that are collectively termed the tauopathies; these include progressive supranuclear palsy (PSP), corticobasal degeneration, Pick's disease, and frontotemporal dementias with parkinsonism linked to chromosome 17.² A substantial body of evidence indicates that pathological tau protein plays a central role in driving neuronal cell death in these diseases. Consistent with this hypothesis, the extent of NFTs correlate with clinical progression of AD.³ Moreover, over 44 different tau mutations have been identified that markedly increase the risk of these diseases.⁴ Emerging data supports the idea that soluble hyperphosphorylated tau species are involved in prion-like propagation of tau

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3 pathology throughout the brain in AD.^{3,5} In light of these findings, major efforts are focused
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7 on reducing pathogenic tau species as a disease-modifying therapeutic approach for AD
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10 and related tauopathies.⁶
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14 A post-translational modification (PTM) of tau that has emerged recently is O-
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17 GlcNAcylation, which involves glycosylation of serine and threonine protein residues with
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21 O-linked N-acetylglucosamine (O-GlcNAc). O-GlcNAcylation is a reversible PTM that
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24 occurs in all multicellular eukaryotes and is found on hundreds of nuclear and cytoplasmic
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27 proteins, including tau.⁷ In mammalian cells, O-GlcNAcylation is regulated by two highly-
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30 conserved enzymes: the glycosyltransferase O-GlcNAc transferase (OGT), which
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33 catalyzes the addition of O-GlcNAc to protein substrates, and the glycoside hydrolase O-
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38 GlcNAcase (OGA), which catalyzes the hydrolytic cleavage of O-GlcNAc from proteins.
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41 These modifications are illustrated by process a) in Figure 1.⁸ Because elevated O-
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60 GlcNAc levels have been found in some cases to reduce serine and threonine
phosphorylation, O-GlcNAcylation has been proposed to modulate phosphorylation either
directly, by modifying phosphorylation sites, or indirectly, through glycosylation of
residues proximal to these sites.⁷ Notably, it has been suggested that O-GlcNAcylation of

tau regulates its phosphorylation state, with increased O-GlcNAc modification correlated with lower tau phosphorylation.⁹ In addition, in vitro studies have demonstrated that increased O-GlcNAcylation of tau hinders its propensity for aggregation.^{10,11} Taken together, these data suggest that pharmacological blockade of OGA would result in an increase of O-GlcNAc modified tau and reduce the formation of pathogenic tau species and tau aggregates (Figure 1), thereby providing a therapeutic benefit in diseases associated with tau hyperphosphorylation such as AD and other tauopathies.¹² Consistent with this idea, multiple independent studies using transgenic mice expressing mutant forms of human tau have shown that administration of the small-molecule OGA inhibitor Thiamet-G (**1**)^{13,14} confers beneficial disease-modifying effects. These benefits include reduction in phosphorylated tau species and tau aggregates, reduced levels of tau in cerebrospinal fluid (CSF), decreased neuronal cell loss, and a reduction in disease-associated behavioral phenotypes.^{11,15,16,17,18} On the basis of these findings, OGA inhibition has emerged as a promising therapeutic strategy that could be used to treat tau pathology in patients. Herein we report pharmacological studies and medicinal chemistry

modification of **1** leading to identification of the first-in-class clinical OGA inhibitor, MK-8719.¹⁹

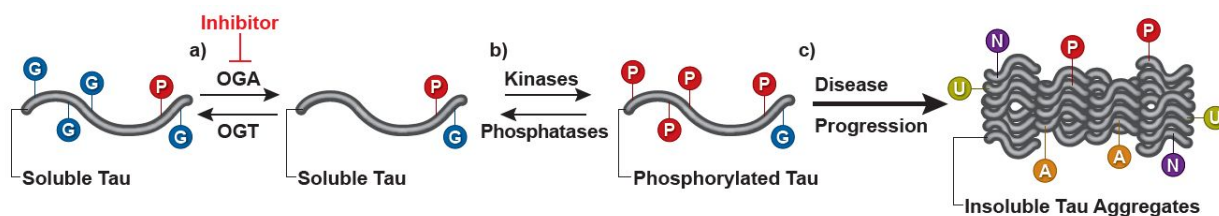


Figure 1. Hypothesis for increased O-GlcNAc modification hindering tau aggregation. a) Cellular modification of tau protein with O-GlcNAc (G) by OGT maintains the protein in a stable soluble form. Equilibrium levels of O-GlcNAc are maintained by the antagonistic actions of OGT and OGA. b) Abnormal disease-associated addition of phosphoryl residues (P) by kinases, countered by phosphatases, coupled with additional modifications including addition of nitro (N), acetyl (A) and ubiquitin (U) groups leads to oligomers and subsequently to c) insoluble tau aggregates.

OGA is a two-domain protein, with a C-terminal domain similar to the GCN5-related family of N-acetyltransferases and an N-terminal domain that is a member of Glycoside Hydrolase family 84 (GH84).^{8,20} It is the N-terminal catalytic domain of OGA that hydrolyzes the glycosidic bond of O-GlcNAc linked to proteins. As illustrated in Figure 2,

OGA uses a two-step catalytic mechanism involving participation of the substrate acetamido group, featuring an oxazoline intermediate (**A**) bound to the enzyme active site.^{21,22} During the catalytic cycle, the acetamido group acts as a nucleophile to attack the anomeric center and displace the protein hydroxyl group leading to the formation of the oxazoline intermediate **A**, which is then hydrolyzed to liberate N-acetylglucosamine. Two enzymic residues play central roles in catalysis.^{21,23,24,25} Asp174 hydrogen bonds with the acetamido group of the substrate, orienting this residue to place the carbonyl oxygen adjacent to the anomeric center and serving as a general base to enhance the nucleophilicity of this group. A second residue, Asp175, acts as a general acid, concomitantly aiding expulsion of the hydroxyl leaving group and favoring formation of **A**. NAG-thiazoline (**B**, Figure 2), which resembles the oxazoline intermediate **A**, was shown to potently inhibit human OGA ($K_i = 70$ nM) but to lack selectivity over the functionally related human lysosomal β -hexosaminidase from GH20 (hHEX $K_i = 70$ nM).²¹ Concomitant inhibition of β -hexosaminidase is not desirable, as loss-of-function mutations in these enzymes result in the lysosomal storage disorders Tay-Sachs and Sandhoff diseases. A series of NAG-thiazoline analogues with improved selectivity were identified

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3 and shown to be mimics of the OGA transition state.²⁶ Subsequently, aminothiazoline
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7 analogues were prepared that, unlike the corresponding NAG-thiazoline derivatives, are
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10 protonated at physiological pH and benefit from an ion-pair interaction of the
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13 aminothiazoline substituent with the anionic Asp174 carboxylate group.^{13,27} These
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17 inhibitors, including Thiamet-G (1, Figure 2), are stable transition state analogues for
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20 human OGA and exhibit high ligand efficiencies.²⁷ X-ray cocrystal structures of
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23 homologues of OGA²³ and the GH84 domain of human OGA in complex with 1^{28,29,30}
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27 provide support for the importance of the ionic interaction between the protonated
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30 aminothiazoline and Asp174, as well as providing a clear atomic rationale for the
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33 selectivity over lysosomal β -hexosaminidase conferred by increasing the steric bulk at the
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37 2'-position of the thiazoline moiety.^{13,21,27} Inhibitor 1 sits deeply within a pocket at one face
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41 of the $(\beta\alpha)_8$ -barrel structure where it is held within an extensive network of hydrogen
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45 bonds with the 2'-ethylamino substituent of the thiazoline tucked into a discrete pocket.
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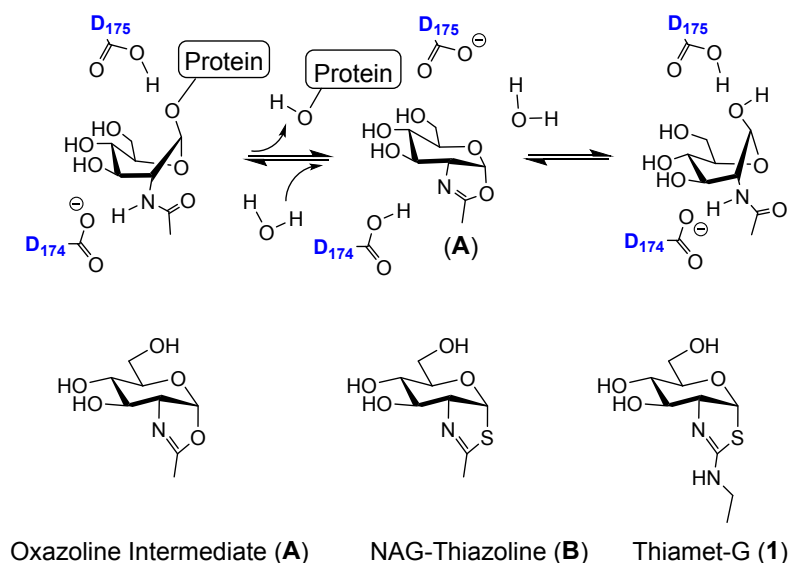


Figure 2. Mechanism-inspired basis for inhibition of OGA and its substrate-assisted catalytic mechanism. The catalytic mechanism of OGA involves two carboxyl residues within the enzyme active site (D174 and D175) that catalyze a two-step process proceeding through an oxazoline intermediate (A), which leads to cleavage of GlcNAc from modified proteins. The known inhibitors NAG-Thiazoline (B) and Thiamet-G (1) resemble the oxazoline intermediate (A) or a closely-related transition state.

RESULTS AND DISCUSSION

Several classes of carbohydrate analogues have been reported that potently inhibit human OGA in cells and tissues,^{13,21,31,32,33,34,35,36} but **1** ($K_i = 2.1 \text{ nM}$)²⁷ has emerged as

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3 a particularly effective tool compound. Within cells it has a reported EC₅₀ value of 21 nM
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7 for increasing O-GlcNAc modified protein to half-maximal level.¹³ In rodent models **1** has
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10 been shown to dramatically elevate brain protein O-GlcNAc levels^{11,13,15,16,17,18} while also
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13 being well tolerated over extended treatment periods and demonstrating beneficial effects
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17 in a range of tauopathy models.^{11,15,16,17,18,37}
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21 While **1** shows several desirable properties as a tool compound, it also possesses
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24 features that could limit its clinical utility as a therapeutic agent for the treatment of
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27 tauopathies. Initial profiling of **1** in an assay to measure its apparent permeability (P_{app})
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30 revealed a P_{app} value below the detection limit of the assay, that is, less than 1.0×10^{-6}
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33 cm/s using a monolayer of LLC-PK1 cells transfected with MDR1.³⁸ This result indicated
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37 that **1** possesses comparatively low or slow membrane penetration, which is inauspicious
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40 for achieving high brain exposure. Pharmacokinetic profiling of **1** in rat provided
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43 confirmation, showing an initial brain/plasma C_{max} ratio of ~0.1 (Figure 3) following a
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46 single oral gavage dose (po). The consistent brain concentration of **1** suggests a slow
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49 diffusion rate into and out of the CNS from systemic circulation. Additionally, **1** exhibited
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53 a high unbound fraction (f_u) in human plasma, with $f_u = 76\%$. We ascribe both features to
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the high polar surface area (PSA) of this molecule. Surveys of CNS penetrant drugs have established an optimal PSA range of 60–70 Å² for blood-brain barrier permeability, with an upper limit of 90 Å².³⁹ We examined the topological polar surface area (TPSA) of **1** and found that it was outside this range (TPSA = 105 Å²).⁴⁰ We hypothesized that we could lower the TPSA by modifying polar substituents while maintaining high intrinsic potency, which would lead to greater and more rapid biodistribution into the CNS, and thus a superior clinical candidate.

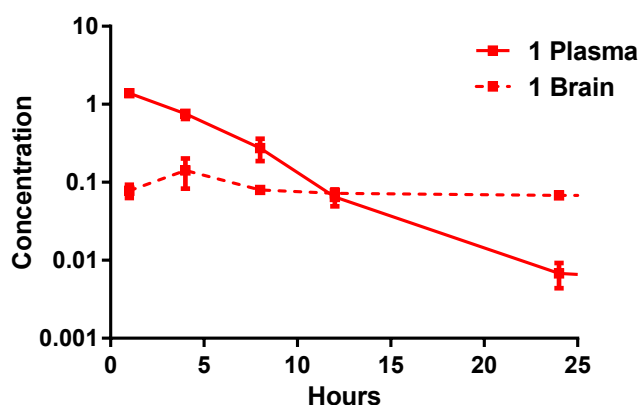


Figure 3. Rat plasma and brain pharmacokinetics for **1**, 10 mg/kg po. Brain concentration (nmol/g); plasma concentration (μM).

Our initial efforts to reduce the TPSA of **1** focused on the hydroxyl and amino groups of the molecule, and we prepared a series of analogues in which these groups were either methylated or, in the case of the OH groups, replaced by H or F (Table 1). All new compounds were tested for their inhibitory potency against recombinant hOGA as well as ancillary potency against hHEX. Cell-based activity for OGA inhibition was assessed using an ELISA-based assay with rat PC12 cells to measure EC₅₀ values for elevation of all protein O-GlcNAc levels. The MDR1-LLC-PK1 assay described above was used to measure apparent permeability (P_{app}) and Pgp efflux for selected compounds. None of the tested compounds were found to be Pgp substrates, although this transporter activity is difficult to reliably establish for compounds with $P_{app} < 7$. All compounds were assayed for activity against the cardiovascular ion channels hERG (by displacement of radiolabeled MK-0499, a known hERG blocker), Na_v1.5, and Ca_v1.2. The ion channel results are not presented in the tables, as all the compounds are devoid of significant activity against these targets (IC₅₀ > 30 μM). Similarly, all compounds were tested for inhibitory potency against the metabolic enzymes CYP3A4, CYP2D6, and CYP2C9, and were found to be inactive (IC₅₀ > 50 μM). Finally, all compounds were assayed for their

propensity to induce CYP3A4 in a PXR activation assay and are similarly inactive (EC_{50} $> 30 \mu M$).

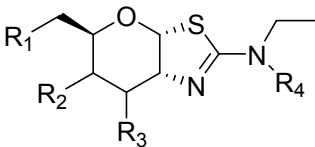


Table 1. Modification of hydroxyl or amino substituents from Thiamet-G.

Compound	Structure ^a	hOGA	rOGA	hHEX	TPSA	P_{app}
		K_i	cell EC_{50}	K_i	$(\text{\AA})^2$ ^e	(10^{-6})
		(nM) ^b	(nM) ^c	(nM) ^d		cm/s) ^f
1	Thiamet-G	0.41	13.5	$> 10,000$	105	< 1.0
2	$R_1 = OCH_3$	190	--	--	94	--
3	$R_2 = OCH_3$	$> 3,000$	--	--	91	--

4	$R_3 = \text{OCH}_3$	270	--	>	91	--
				10,000		
5	$R_4 = \text{CH}_3$	5.5	36.7	3,600	93	1.1
6	$R_1 = \text{H}$	69	--	--	84	--
7	$R_2 = \text{H}$	> 3,000	--	>	86	7.0
				10,000		
8	$R_3 = \text{H}$	44	364	>	84	2.6
				10,000		
9	$R_1 = \text{F}$	20	176	--	84	6.1
10	$R_2 = \text{F}^g$	> 3,000	--	>	84	--
				10,000		
11	$R_3 = (R)\text{-F}$	29	443	>	84	2.7
				10,000		

^aUnless otherwise noted, the substituents R_1 , = OH, R_2 = (*S*)-OH, R_3 = (*R*)-OH, and R_4 = H. ^bHuman OGA enzyme inhibition K_i values are from ≥ 2 assays with standard deviation $\leq 35\%$ of the reported mean. ^cRat cellular OGA EC_{50} values are from ≥ 2 assays with standard deviation $\leq 35\%$ of the reported mean. ^dhHEX K_i values were measured as described in the supplementary material from ≥ 2 assays with standard deviation $\leq 22\%$ of the reported mean. ^ePolar surface area (TPSA) was calculated as the sum of fragment-based contributions. ^fMDR1-LLC-PK1 cell monolayer permeability measurements are from ≥ 2 assays with a standard deviation $\leq 55\%$ of the reported mean. ^gSingle diastereomer, absolute configuration at R_2 not assigned.

The structural modifications outlined in Table 1 indicate that some alteration of **1** is tolerated to reduce the TPSA without a complete loss of OGA activity. The (6*S*)-hydroxyl substituent R_2 is critical to the OGA inhibitory potency of **1**. Any modification or removal of this substituent, as illustrated by **3**, **7**, and **10**, resulted in profound loss of activity of the corresponding compound. Modification of the (7*R*)-hydroxyl substituent R_3 was more tolerated. Methylation of this moiety (**4**) afforded a reduction in calculated TPSA but also

a significant loss of intrinsic potency. However, removal of the hydroxyl as in **8**⁴² or replacement by fluorine as in **11**⁴² afforded compounds with further reduction in calculated TPSA and superior potency as compared to the R₂ modified compounds. The decrease in TPSA correlated to measurable membrane penetration, as measured by P_{app} in LLC-PK1 cells transfected with MDR1. Modification of the (5*R*)-hydroxymethyl substituent (R₁) on **1** was more productive. Although methylation of this substituent (**2**⁴¹) resulted in a poorly active compound, removal of the hydroxyl provided **6**⁴² with improved potency relative to **2** and a further reduction in TPSA. Substitution of the hydroxyl by fluorine (**9**⁴²) afforded improved OGA inhibitory potency while maintaining the reduction in TPSA achieved by **6**, and this lower TPSA translated to significantly improved membrane permeability (P_{app}) as compared to **1**. Finally, the addition of a methyl substituent to the 2-ethylamino moiety (R₄) provided **5**¹⁴ with a moderate reduction in both TPSA and inhibitory potency as compared to **1**.

This first round of modifications suggested that further improvements in TPSA reduction could be achieved by combining the structural modifications outlined above. The (6*S*)-hydroxyl substituent R₂ was retained as critical for potency, but the combined

modifications suggested by the data in Table 1 were extended to additional substituents to further improve permeability while retaining OGA inhibitory potency. The results from these studies are provided in Table 2.

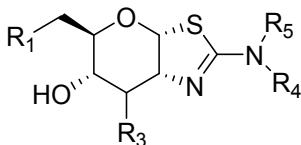
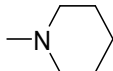
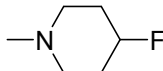
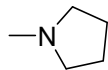


Table 2. Modification of hydroxyl or amino substituents from 1.

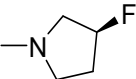
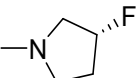

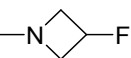
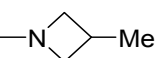

Structure ^a				hOGA	rOGA cell	hHEX A/B	TPS	<i>P</i> _{app}
				<i>K</i> _i (nM) ^b	EC ₅₀	<i>K</i> _i (nM) ^d	A	(10 ⁻⁶ cm/s) ^f
					(nM) ^c		(Å) ^{2 e}	
R ₁	R ₃	R ₄	R ₅					
1		2-F ethyl		5.6	509	> 10,000	105	2.2
2								
1		Me	allyl	2.8	152	4,500	92	0.8
3								

ACS Paragon Plus Environment

20	(<i>R</i>)-F	propyl		14	445	> 10,000	83	--
21	(<i>R</i>)-F	Me		3.0	307	8,100	84	--
22	(<i>S</i>)-F	Me		0.53	10.6	> 10,000	84	--
23	H	H		> 3,000	--	> 10,000	98	--
24	H	propyl		50	224	> 10,000	83	2.5
25	H	Me	Me	11	358	> 10,000	72	--

2	H	Me	9.4	33.1	> 10,000	85	--	
6								
2	F	(<i>R</i>)-F	Me	50	--	> 10,000	61	--
7								
2	F	(<i>S</i>)-F	Me	35	328	> 10,000	61	26
8								
2			1,800	--	> 10,000	91	--	
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3			> 3,000	--	> 10,000	92	--	
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3			13	165	> 10,000	93	--	
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3		3.3	28.0	> 10,000	96	--
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3		> 3000	--	--	95	--
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3		72	--	> 10,000	94	2.4
6						
3	F 	200	--	> 10,000	71	--
7						

^aUnless otherwise noted, the substituents R₁, = OH, R₃ = (*R*)-OH, and R₄ = H. ^bHuman

OGA enzyme inhibition K_i values are from ≥ 2 assays with standard deviation ≤ 66% of

the reported mean. ^cRat cellular OGA EC₅₀ values are from ≥ 2 assays with standard deviation \leq twofold of the reported mean. ^dhHEX K_i values were measured as described in the supplementary material from ≥ 2 assays with standard deviation $\leq 79\%$ of the reported mean. ^ePolar surface area (TPSA) was calculated as the sum of fragment-based contributions. ^fMDR1-LLC-PK1 cell monolayer permeability measurements are from ≥ 2 assays with a standard deviation $\leq 53\%$ of the reported mean.

Structural modification of the aminoethyl R₅ substituent from **1** afforded no appreciable reduction in TPSA, and diminished potency against OGA as illustrated by comparing **12**^{14,27} with **1** and **13**⁴³ with **5** (from Table 1). Like the findings from Table 1, replacement of the primary alcohol R₁ with a fluoro substituent as in **9** was more productive in reducing the TPSA, which correlated with higher membrane permeability in the *P_{app}* assay. The combination of this change with modifications to the aminothiazole substituents appear to amplify the potency impact of the amine position. The unsubstituted amine **14**⁴² lost significant potency compared to the ethylamine substituted derivative **9**, and even the corresponding allyl derivative **15**⁴² was significantly less potent than **9**. The propylamine

derivative **16**⁴² and dimethylamine derivative **17**⁴² afforded comparable potency to **9**. However, the methylamine substituent in **18**⁴² provided a significant improvement in hOGA potency, as well as in the cell-based assay using the rat enzyme, albeit with concomitant increased potency for the lysosomal hHEX enzyme.

Modification of the R₃ hydroxyl was investigated for combination SAR with other changes in the molecule. The replacement of the (*R*)-OH in R₃ with (*R*)-F afforded **19** - **21**.⁴² While the corresponding R₃-F propylamine derivative **20** displayed comparable potency to the R₁-F counterpart **16**, the methylamine derivative **21** and dimethylamino derivative **19** were less potent than their counterparts **18** and **17** respectively. The diastereomeric (*S*)-F R₃ derivative **22**⁴² was significantly more potent than the corresponding (*R*)-F diastereomer **21**, with higher selectivity against hHEX as well. Elimination of the R₃ hydroxyl group provided **23** - **26**,⁴² which were generally less potent than the corresponding R₃ fluoro derivatives. Combination of the fluoro substituents in R₁ and R₃ led to **27**⁴² and **28**⁴² with a significant reduction in TPSA. This corresponded to high membrane permeability in the *P_{app}* assay for **28**, but also to a significant loss of intrinsic potency against hOGA.

The disubstituted amine derivatives **5**, **13**, **17**, **19**, and **25** retained significant OGA potency while reducing TPSA, which led us to consider the potential of cyclic amine derivatives. The piperidine compound **29**⁴⁴ lost significant activity, whereas the pyrrolidine product **31**⁴⁴ and azetidine **34**⁴⁴ were more active. Substitution of the cyclic amine moieties with a fluoro substituent as in **30**, **32**,⁴⁴ **33**,⁴⁴ and **35**⁴⁴ resulted in a significant loss of hOGA potency relative to the corresponding non-fluorinated derivatives. We ascribe this to the inductively decreased basicity of the amino substituent, which reduces the interaction of this moiety with Asp174 in the hOGA binding pocket.²⁷

The high membrane permeability achieved with the doubly fluorinated compound **28** led us to speculate that an alternate arrangement of difluoro substituents on the pyran moiety may be optimal for both permeability and potency. To investigate this hypothesis, a variety of analogs with difluoro substituents in R₁ were synthesized, and the results are shown in Table 3.

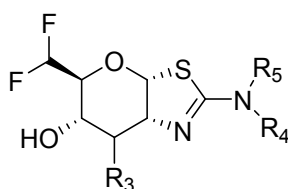


Table 3. Investigation of 5-difluoromethyl derivatives of 1.

Structure ^a				hOGA	rOGA cell	hHEX A/B	TPS	<i>P_{app}</i>
				K _i (nM) ^b	EC ₅₀	K _i (nM) ^d	A	(10 ⁻⁶ cm/s) ^f
					(nM) ^c		(Å) ^{2 e}	
	R ₃	R ₄	R ₅					
3	H	Me	Me	> 3,000	--	> 10,000	47	31
8								
3	H		Me	28	--	> 10,000	61	24
9								
4			allyl	74	--	> 10,000	82	12
0								
4			propyl	5.3	41.4	> 10,000	82	14
1								

4		ethyl	7.9	52.7	> 10,000	80	6.4
2							
4		Me	0.36	1.71	1,300	83	5.7
3							
4		H	760	2,400	> 10,000	96	9.2
4							
4	Me	Me	2.4	31.2	3,900	69	14
5							
4	(<i>R</i>)-F	Me	58	--	> 10,000	61	22
6							
4	(<i>S</i>)-F	Me	23	58.2	> 10,000	61	20
7							

4  26 -- > 10,000 71 18

8

^aUnless otherwise noted, the substituents R₃ = (*R*)-OH, and R₄ = H. ^bHuman OGA enzyme inhibition K_i values are from ≥ 2 assays with standard deviation $\leq 58\%$ of the reported mean. ^cRat cellular OGA EC₅₀ values are from ≥ 2 assays with standard deviation twofold of the reported mean. ^dhHEX K_i values were measured as described in the supplementary material from ≥ 2 assays with standard deviation \leq twofold of the reported mean. ^ePolar surface area (TPSA) was calculated as the sum of fragment-based contributions. ^fMDR1-LLC-PK1 cell monolayer permeability measurements are from ≥ 2 assays with a standard deviation $\leq 43\%$ of the reported mean.

From the difluoro-substituted compounds illustrated in Table 3, the highest reduction in TPSA was achieved with the dimethylamino derivative **38**.⁴² This TPSA reduction translated to the highest P_{app} in this lead class, albeit with complete loss of hOGA activity. The activity was restored with the removal of a single methyl from the amine substituent, affording **39**⁴² as a high permeability compound with appreciable potency.

The aminothiazoline substituents were systematically investigated with **40** - **45**,⁴² indicating that the propyl and ethyl derivatives **41** and **42** maintained a good balance of hOGA potency, P_{app} , and selectivity vs. hHEX. The potent methylamine derivative **43** was further investigated to improve selectivity against hHEX. Substitution at R₃ with a fluorine as in **46**⁴² and **47**⁴² did diminish hHEX potency, but at a significant cost to hOGA potency as well. Finally, the azetidine analog **48**⁴² was investigated, but this compound afforded significantly diminished hOGA potency as compared to the dimethylamine analog **45**.

The full collection of P_{app} data for compound in Tables 1-3 was compared to the calculated TPSA for these compounds to evaluate the design principle and strategy for optimization, and this data is provided graphically in Figure 4. As expected, there is a reasonable correlation between compounds with reduced TPSA and improved permeability as measured by P_{app} .

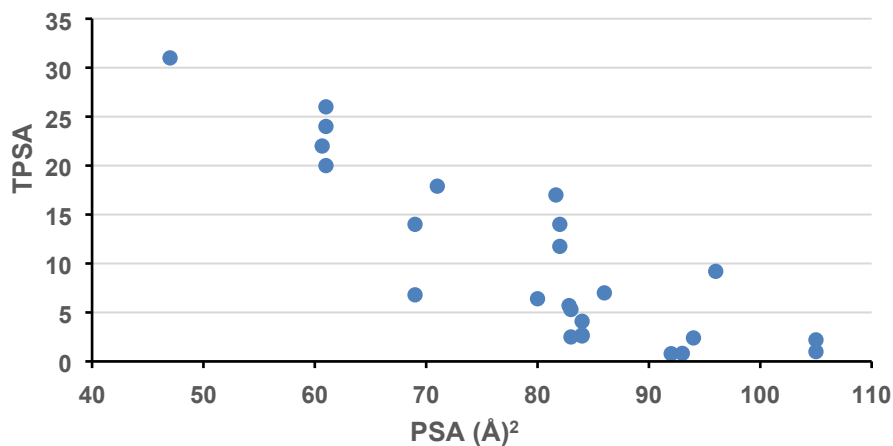


Figure 4. Correlation between *P_{app}* and calculated TPSA for OGA inhibitors in Tables 1-3.

Several compounds from each sub-series were further investigated for their pharmacokinetic properties in rats, and the results of the studies are provided in Table 4.

All compounds exhibiting hOGA $K_i \leq 35$ nM were selected for rat PK profiling unless a more potent and closely homologous compound was already included in the PK profiling set. Compounds from each sub-series in Tables 1-3 were evaluated for their free fraction in rat and human plasma, and these results are also included in Table 4. All of the compounds tested in the plasma binding assays were found to have a plasma free fraction

≥ 70%. Therefore, total PK parameters are reported in Table 4 for all compounds rather than unbound values.

Table 4. Pharmacokinetic parameters in Wister Han male rats.^a

Cpd	Plasma f_u^b	Dose	oral AUC _N ^d	iv CL	iv t _{1/2}	Vd _{ss}	F
		iv/po ^c					
#	(%, r / h)	(mg/kg)	(μM•h•kg/mg)	(mL/min/kg)	(h)	(L/kg)	(%)
1	73 / 76	2/10	0.80	25.0	1.40	1.50	30.0
5	--	2/10	0.598	66.3	1.89	5.39	62.0
9	98 / 87	2/3	1.77	37.4	2.39	3.80	102
11	--	2/10	2.16	31.9	2.40	2.00	104
12	--	2/3	0.903	33.6	1.29	1.33	48.6
13	--	2/3	0.538	45.8	4.03	16.1	108
17	>99 / >99	2/3	0.954	43.7		10.5	75.3
18	88 / 96	2/3	3.36	27.8	1.71	1.79	132
20	--	2/3	0.394	48.1	1.69	1.92	30.1

22	96 / 84	1/3	1.43	36.8	1.27	2.00	70.4
25	--	2/3	2.26	25.7	1.95	2.49	80.1
26	--	2/10	2.53	39.0	1.06	2.80	129
28	>99 / >99	1/3 ^c	2.96	14.4	1.18	0.85	60.5
34	--	2/10	1.15	53.6	1.63	2.88	96.0
39	88 / >99	2/3	1.90	30.4	1.66	2.23	74.7
41	--	2/3	0.376	72.6	3.89	5.99	44.8
42	97 / 99	2/10	1.40	36.7	1.90	3.70	80.0
43	83 / 77	2/3	2.54	27.1	1.48	1.72	93.8
45	95 / >99	2/3	0.430	70.5	3.81	10.3	49.7
47	93 / 97	2/3	4.27	11.4	4.29	2.96	90.4

^a Each experiment dosed intravenous (iv) and oral (po) are the mean values of at least two rats, with F (%) standard deviation < 30% of the mean value. All compounds were formulated in clear water or saline solution, 1.0 mg/mL for 1 mg/kg and 2.0 mg/mL for 2

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3 mg/kg iv, and 2.0 mg/mL for 10 mg/kg po or 0.6 mg/mL for 3 mg/kg po. ^b Fraction unbound
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7 (%) in plasma from rat (r) and human (h) by equilibrium dialysis at 2.5 μ M compound
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10 concentration. ^c Formulation of 1.5 mg/mL for po dose. ^d Oral area under the curve divided
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13 by the oral dose.
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18 The results in Table 4 showcase the structural features that influence the
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20 pharmacokinetic parameters of this class of compounds. As was shown in Figure 2
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22 above, **1** exhibits reasonable systemic exposure following a 10 mg/kg oral dose in rats,
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25 and the complete PK data reveals moderate clearance and distribution. The impact of the
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28 aminoalkyl substituents was first examined. Fluorination of the aminoethyl substituent as
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31 in **12**²⁷ or bis-aminoalkyl substitution as in **5** afforded modestly improved oral
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34 bioavailability as compared to **1**. More significant improvements were achieved by the
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37 azetidiny derivative **34** and allylaminomethyl derivative **13**, with the improved half-life ($t_{1/2}$)
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40 driven primarily by increases in volume of distribution ($V_{d_{ss}}$).
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49 Modification of pyranlyl substituents was also investigated. The 5-fluoromethyl
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52 derivatives **9** and **18** exhibited significantly improved oral bioavailability as compared to
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1, driven primarily by improved oral absorption in both cases. Again here, the dialkylamino derivative **17** afforded higher Vd_{ss} as compared to the alkylamine **18**. The 7-fluoro derivatives **11** and **22** provided even greater improvements in oral exposure over **1** than was afforded by the 5-fluoromethyl substituent in **9**. However, the influence of the aminoalkyl substituent in the 7-fluoro series is evident with the aminoethyl **11** providing superior oral bioavailability to the aminoethyl **22** or the aminopropyl **20**. Removal of the 7-hydroxyl substituent as in the methylamino derivative **26** and dimethylamino derivative **25** also afforded compounds with improved oral exposure and bioavailability as compared to **1**. Combining the 5-fluoromethyl and 7-fluoro substituents in **28** afforded no significant PK benefit as compared to the analogous monofluoro derivatives **18** and **21**.

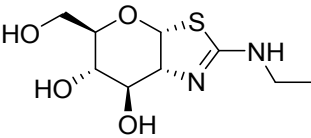
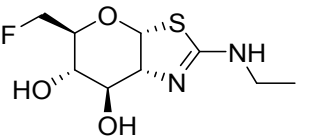
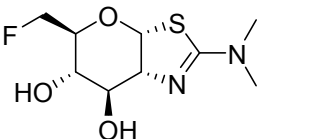
Finally, we investigated the 5-difluoromethyl derivatives for rat PK properties. The 7-deshydroxyl derivative **39** afforded slightly lower exposure than its matched pair analogous 5-hydroxyl derivative **26**. The homologous series **41**, **42**, and **43** again illustrated that the methylamino and ethylamino derivatives provide lower clearance and therefore higher oral bioavailability than the corresponding propylamino derivative. The combination of the 5-difluoromethyl and 7-fluoro substituents were investigated with **45**

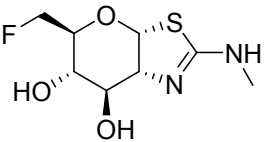
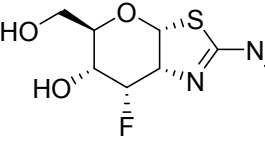
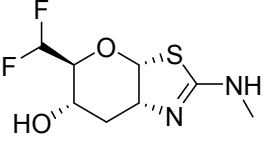
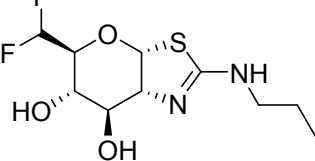
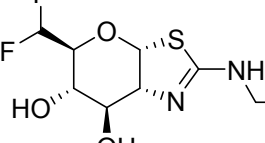
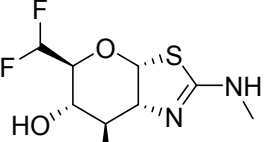
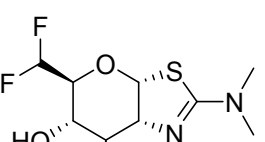
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3 and **47**, with the methylamino derivative **47** exhibiting the lowest clearance of compounds
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7 tested.
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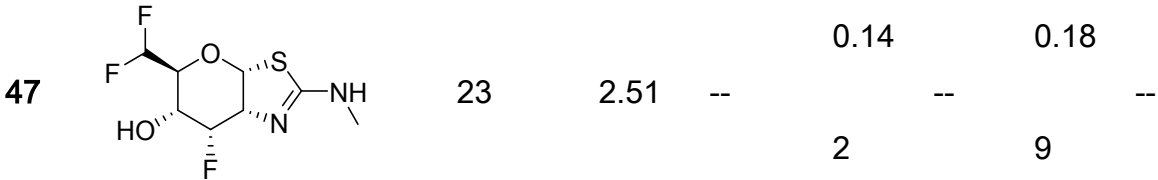
10 The bioavailability of all compounds tested was moderate to very good, and we sought
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12 to differentiate these compounds by examining their pharmacodynamic (PD) effects. We
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14 evaluated the PD effect of OGA inhibition in the CNS by measuring the time-dependent
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16 accumulation of global protein O-GlcNAcylation (referred to hereafter as O-protein) in rat
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18 brain following a single oral dose of test compound. We have previously described the
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20 development and validation of a sensitive, quantitative, high-throughput MesoScale
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22 Diagnostics (MSD) sandwich immunoassay to measure O-protein levels in homogenized
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24 brain tissue.¹⁷ Rats were sacrificed at both the 8 h and 24 h timepoints to evaluate the
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26 level of accumulated O-protein in brain homogenate compared to vehicle-dosed rats.
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28 Additionally, the concentration of inhibitor in both plasma (μM) and brain homogenate
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30 (nmol/g) was evaluated at both timepoints for comparison to the distributional delay in
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32 brain exposure observed with **1** (Figure 3). Compounds from Table 4 were selected for
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34 PD evaluation based on favorable pharmacokinetic properties, as well as potent hOGA
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36 inhibition ($K_i < 20 \text{ nM}$), moderate to potent cell-based rOGA inhibition ($\text{EC}_{50} < 200 \text{ nM}$),
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and measurable membrane permeability ($P_{app} > 2 \times 10^{-6}$ cm/s). The results are provided in Table 5. The hOGA K_i values reported in Tables 1-3 are also provided in Table 5 to clarify the analysis.

Table 5. Pharmacodynamic brain O-protein elevation in rats following 3 mg/kg oral dose of an OGA inhibitor

Compound and Structure	hOGA K_i (nM) ^a	O-Protein ^b		Brain exposure (nmol/g)		Brain / Plasma ratio ^c	
		8 h	24 h	8 h	24 h	8 h	24 h
1 	0.41	1.85	1.75	0.18 5	0.05 1	0.94	> 2.7
9 	20	1.84	1.74	0.22 8	0.04 7	1.49	≥ 18.7
17 	9.0	2.33	1.77	0.22 2	0.03 8	0.90	1.53

18		0.55	2.44	1.65	0.06	0.02	0.60	≥ 7.9
					8	5		
22		0.53	2.13	1.45	0.09	0.07	1.67	\geq
					9	4		29.5
39		28	1.80	--	0.28	--	2.22	--
					9			
41		5.3	1.81	--	0.19	--	8.81	--
					3			
42		7.9	2.06	2.17	0.21	0.08	1.84	\geq
					9	8		20.5
43		0.36	2.00	--	0.13	--	1.33	--
					3			
45		2.4	2.53	2.57	0.14	0.04	1.56	3.03
					7	9		



^aHuman OGA enzyme inhibition K_i values are from ≥ 2 assays with standard deviation $\leq 66\%$ of the reported mean. ^b Fold increase in O-protein in treated rats compared to vehicle-dosed rats. Average of 3 rats per time point, with standard deviation $\leq 34\%$ of the reported mean. ^cBrain / Plasma ratio = Brain concentration (nmol/g) / plasma concentration (μ M). The lower limit (\geq) values result from plasma exposure below the limit of detection at 24 h.

The initial inhibitor **1** afforded an 85% increase in brain O-protein as compared to vehicle-dosed rats eight hours after a single oral dose, and this result is consistent with earlier published studies.¹⁷ The O-protein levels remained elevated 75% at the 24 h time point despite approximately 3-fold decrease in brain level of **1**, which signifies a potential delayed effect in the PK / PD relationship. As illustrated in Figure 3, the low *Papp* measured for **1** does not prevent the compound from entering the brain within the time course of the experiment, and the brain exposure is sufficient to drive an appreciable and

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3 lasting PD effect. We note here that the P_{app} value represents a diffusion rate, but even
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7 the comparatively slow diffusion of **1** facilitated the accumulation of efficacious levels of
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10 inhibitor within the first eight hours of the experiment. The difference in brain / plasma
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13 ratio between 8 h and 24 h in Table 5 is also consistent with the PK experiment illustrated
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17 in Figure 3, indicating that the systemic clearance rate of the compound exceeds the
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20 diffusion rate out of the CNS. Similar pharmacodynamic results were obtained with the
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23 more lipophilic 5-fluoromethyl derivative **9**, despite the significantly decreased hOGA
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26 potency (K_i) relative to **1**. This observation is addressed below.
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31 The methylamino and dimethylamino derivatives **17**, **18**, and **22** were next examined.
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34 All three compounds provided a greater PD effect at 8 h than **1**. The elevated O-protein
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37 levels from **18** and **22** are interesting as both compounds revealed relatively low 8 h brain
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40 exposure. The higher selectivity of **22** for OGA over hHEX as compared to **18** (Table 2)
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43 identified **22** as a candidate compound for additional investigations.
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48 The 5-difluoromethyl aminomethyl derivatives **39**, **43**, **45**, and **47** also afforded
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51 consistent O-protein elevation, with **45** and **47** providing the highest O-protein levels
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54 found in these studies. The lower selectivity for **43** for OGA as compared to hHEX (Table
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2) made that compound less favorable for further study. Compound **45** was also appreciably potent against hHEX, but the significant PD effect at 24 h made a compelling case for further investigation of the compound. Compound **39** maintained high brain exposure at 8 h, and both **39** and **47** exhibit lower potency against hHEX as compared to **43** and **45** (Table 2) identifying **39** and **47** as candidates for further PD studies as well.

Finally, the homologous propylamine and ethylamine derivatives **41** and **42** were investigated. The ethylamine derivative **42** exhibited significant efficacy at both 8 h and 24 h time point, with overall good brain exposure as well. Based on this data, **42** was also selected for further studies.

The pharmacodynamic studies summarized in Table 5 illustrate that potent OGA inhibitors with biodistribution to the CNS will elevate O-protein in the brain. All of the compounds selected for these PD studies achieve appreciable brain exposure, averaging 0.175 (+/- 0.064) nmol/g concentration after 8 h. Notably, all of the selected compounds afforded comparable increases in rat brain O-protein after 8 h, averaging 2.12 (+/- 0.29) fold increase over vehicle, despite spanning a 100-fold range in intrinsic hOGA potency. This suggests that all of the compounds maintained sufficient brain OGA inhibition to

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3 produce a similar PD effect at 8 h, indicating that more detailed pharmacodynamic studies
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7 would be required to select the optimal compound.
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10 We hypothesized that the reduction of tau phosphorylation by an OGA inhibitor would
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12 require sustained preservation of tau O-GlcNAc residues. As such, we performed time-
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14 course PD experiments by measuring the elevation of brain O-protein as compared to
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20 vehicle at multiple time points over 24 hours in rat following a single oral dose of OGA
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24 inhibitor. Examples of these studies are shown in Figure 5 for compounds **1**, **42**, and **45**
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28 dosed orally at 10 mg/kg. The time of maximal O-protein elevation differs for the
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32 compounds, so the PD effect was compared by measuring the area under the curve
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35 $(AUC)_{0-24\text{ h}}$ of O-protein elevation as compared to vehicle for each compound. The relative
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39 PD effect from several compounds could then be compared by evaluating their relative
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42 AUC of O-protein elevation during the time course of the experiment.
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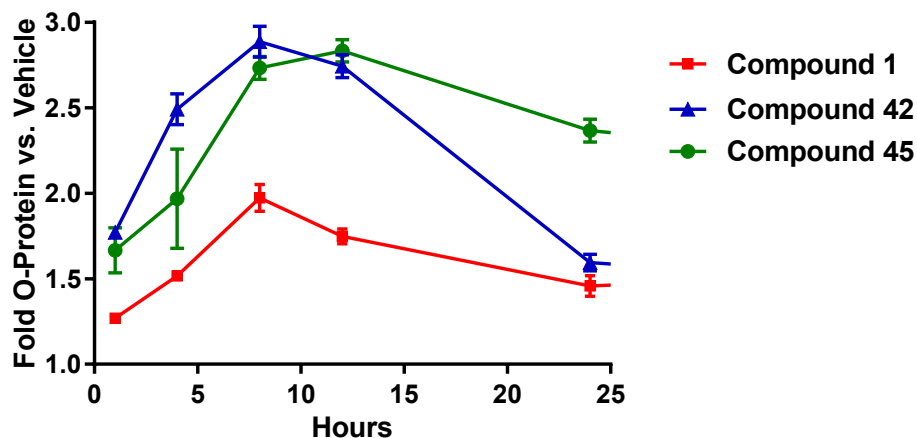


Figure 5. Elevation of brain O-protein in rat vs. vehicle following a 10 mg/kg oral dose of OGA inhibitors **1**, **42**, and **45** (n = 3 rats / time point).

These time-course PD experiments were replicated for each of the compounds of interest, and the data are provided in Table 6. The data indicate a poor correlation between the maximal O-protein elevation and total AUC O-protein elevation as compared to vehicle, with the AUC values providing a greater dynamic range for comparison. We note that the PD AUC values in Table 6 do not correlate with the intrinsic potency of the compounds. Therefore, PD reflects a more complex relationship between potency and tissue exposure to maintain a threshold inhibition for an extended time. The brain AUC₀₋ data are included in Table 6 as well. The two compounds with the highest exposure,

39 and **47**, also have the lowest intrinsic potency (Table 5), resulting in comparatively unremarkable PD AUC. These studies revealed that the ethylamine derivative **42** and analogous dimethylamine derivative **45** provided the highest sustained elevation of O-protein AUC following a single oral dose.

Table 6. Pharmacodynamic time course of OGA inhibitor O-protein elevation in rat following a single oral dose.

Compound	Dose (mg/kg)	Max O-Protein (vs. veh)	T _{max} (h)	Brain AUC ₀₋₂₄ ^a	PD AUC ^b (vs veh)
				(nM•h/g)	
1	10	1.97 (+/- 0.14)	8	1.14	14.82
22	3	2.24 (+/- 0.10)	8	1.93	18.44
39	10	2.69 (+/- 0.19)	8	53.87	19.68
42	10	2.57 (+/- 0.05)	8	9.64	31.42
45	10	2.83 (+/- 0.12)	12	4.89	34.18
47	10	2.04 (+/- 0.16)	8	35.91	11.69

^aAUC₀₋₂₄ was calculated from homogenized tissue concentration (nmol/g) at timepoints 1, 4, 8, 12, and 24 hr from an average of 3 rats. ^bPD AUC was calculated from O-protein elevation over vehicle at the same timepoints.

Considering the robust and sustained pharmacodynamic response observed for **45** in rat, we elected to characterize the metabolic profile of this compound. Metabolism of **45** was evaluated in liver microsomes from rat, dog, monkey, and human, which revealed the N-desmethyl compound **43** as the primary metabolite. We also evaluated pharmacokinetics for **45** in monkey and observed that the dominant species present in plasma following a single oral dose of **45** is its metabolite **43** (Figure 6). Based on these observations, we concluded that **45** undergoes metabolism in vivo to generate **43**, which is a more potent OGA inhibitor that exhibits reduced selectivity against hHEX. We also surmised that the pharmacodynamic response observed for **45** is likely due to the combined effects of **45** and its metabolite **43**. The additional complexity and risk associated with the presence of a circulating active metabolite led us to deprioritize **45** and related N,N-dimethyl analogues as potential development candidates.

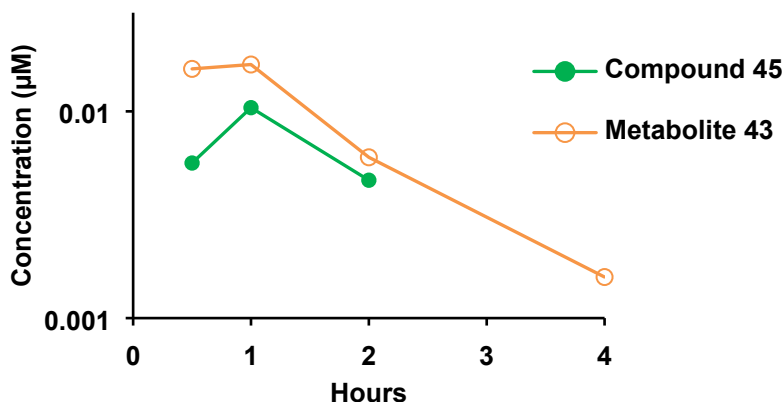


Figure 6. Monkey plasma pharmacokinetics for **45** and metabolite **43**, following 1.0 mg/kg po dose of **45**.

We repeated the PD time course experiments in rats for several compounds from Table 6 at different doses to determine whether the relative increase in O-protein was maintained across a dose range. To include the dynamic range of O-protein response, we chose compounds **1**, **42**, and **47** from the data in Table 6. These dose range studies, as well as the single dose data from Table 6, are illustrated in Figure 7. These studies show **42** affording a significantly improved PD effect at lower doses as compared to **1** or **47**.

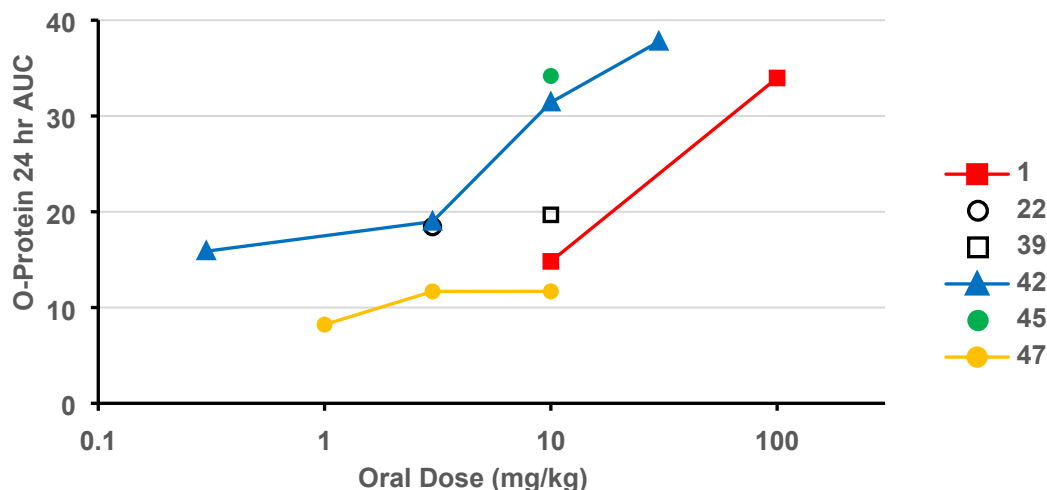


Figure 7. Fold-elevation of O-protein AUC_{0-24} over vehicle vs. dose in rats following a single oral dose at the indicated doses

Robust PD activity was observed for both **42** and **22** in the O-protein time-course experiments following a 3 mg/kg oral dose, and the brain and plasma exposure from these studies are shown in Figure 8. While both compounds afforded comparable systemic exposure, similar to the PK results in Table 4, the brain exposure differed significantly between the compounds. Indeed, **22** provided a similar elevation of brain O-protein from one fifth the relative maximal brain concentration, consistent with the 5-fold greater intrinsic potency of **22** relative to **42** in the hOGA assay (Tables 2 and 3). Although the PD potency of **22** is desirable, the low brain to plasma C_{max} ratio (≤ 0.1) of

22 illustrated in Figure 8a) introduces a development concern. A variable brain penetration profile between patients would be exacerbated by low intrinsic permeability, creating the risk of either lack of efficacy due to low brain exposure or potential toxicity with high exposure. By contrast, consistent with its higher *P_{app}*, **42** afforded a more balanced brain and plasma exposure, which is viewed as more manageable to mitigate the development risk of this novel therapy.

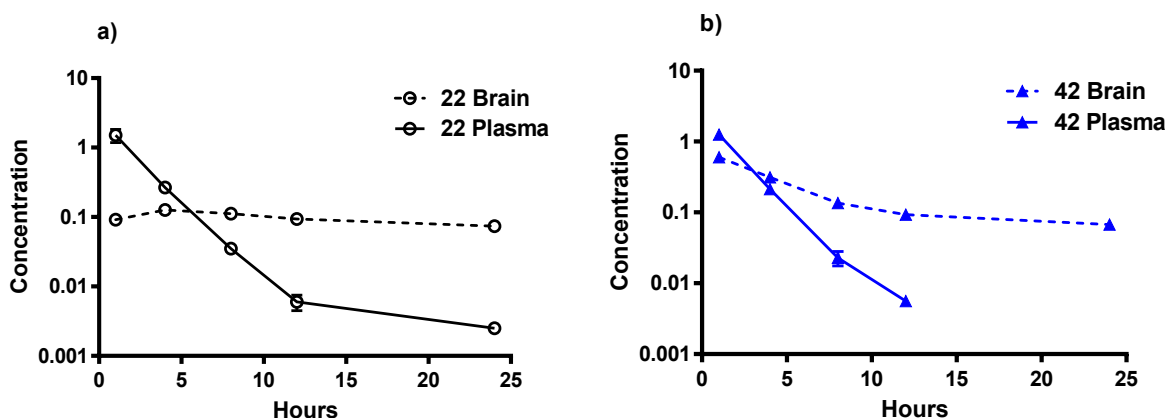


Figure 8. Rat exposure in plasma and homogenized brain from a single oral dose of a) **22** and b) **42** at 3 mg/kg from O-protein PD time course experiments. Brain concentration (nmol/g); plasma concentration (μM).

The greater efficacy of **42** as compared to **1** may be explained in part by greater brain exposure of **42** (Table 6). Figure 9 shows the plasma and brain exposure of **1** and **42**

during the rat PD experiments from Figure 5 following a 10 mg/kg oral dose. The C5-difluoromethyl derivative **42** afforded significantly higher brain exposure derived primarily from the earlier timepoints. The data correlate with success for the strategy to improve permeability in this series to drive greater efficacy.

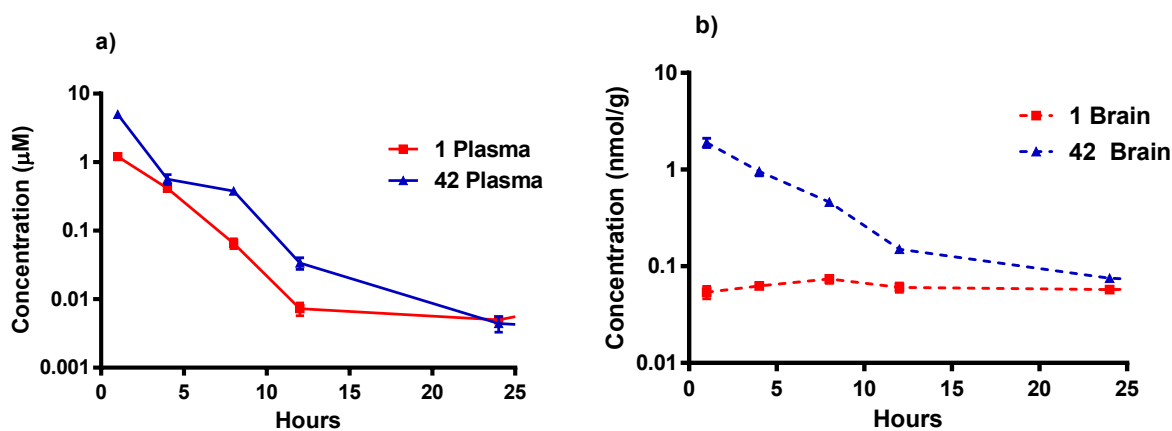


Figure 9. Exposure from a single 10 mg/kg dose in rat PD experiments in (a) plasma and (b) brain. (n=3).

The desirable properties of **42** brought our focus to this compound as a potential clinical development candidate, and it was evaluated in more advanced pharmacokinetic and safety assessment studies. The in vitro pharmacokinetic results indicate a low potential for drug-drug interactions. The compound was not an inhibitor of any of the seven major CYP isoforms ($IC_{50} > 100 \mu M$), and did not induce CYP3A4, 2B6, or 1A2 in human

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4 hepatocytes at compound concentration up to 20 μ M. The compound exhibits negligible
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7 plasma protein binding ($< 5\%$) and a blood to plasma ratio of 0.9 to 1.1 in rat, dog,
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10 monkey, and human blood components.
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14 The in vitro metabolism profile of **42** was also characterized in hepatocytes. Following
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17 120 minutes of compound incubation at 10 μ M, a significant percentage of parent
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20 compound remained when using hepatocytes from rat (96%), dog (91%), and human
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23 (90%), with significantly less parent compound using hepatocytes from monkey (58%).
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28 The primary metabolites in hepatocytes from dog and human were from direct
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31 glucuronidation of undetermined regiochemistry, whereas hepatocytes from rat and
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34 monkey afforded N-dealkylation as well.
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39 The pharmacokinetic results in beagle dog and rhesus monkey are presented in Table
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42 7, and the rat results from Table 4 are presented in Table 7 as well for comparison. The
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45 compound exhibited a large volume of distribution in all three species, and high oral
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48 bioavailability was observed in rat and dog, but this was significantly diminished in
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51 monkey. We suspect that the lower bioavailability in monkey is due to hepatic and / or
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intestinal first pass metabolism, as evidenced by the higher relative intrinsic clearance in monkey compared to rat and dog.

Table 7. Pharmacokinetic parameters of **42** in rat, dog, and monkey.

Species	Plasm	Dose	oral AUC _N ^c	iv CL	iv t _{1/2}	Vd _{ss}	F
a	a f _u ^b	iv/po					
	(%)	(mg/kg	(μM•h•kg/mg)	(mL/min/kg)	(h)	(L/kg)	(%)
)					
Rat	97	2/10	1.40 (+/-0.18)	36.2 (+/-	1.9 (+/-	3.5 (+/-	80 (+/-10)
				6.8)	0.2)	0.4)	
Dog	>99	1 / 3	14.2 (+/-1.2)	11.7 (+/-	2.3 (+/-	2.4 (+/-	90 (+/-3)
				0.6)	0.2)	0.3)	
Monkey	>99	1 / 3	0.61 (+/-0.17)	26.7 (+/-	1.4 (+/-	3.1 (+/-	9.0 (+/-
				3.9)	0.4)	0.5)	1.2)

^a Each experiment (iv and po) are the mean values of at least two animals dosed in crossover fashion, formulated in clear water or saline solution. ^b Fraction unbound (%) at

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4 1 μ M compound in plasma by equilibrium dialysis. ^c Oral area under the curve divided by
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7 the oral dose.
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11 For biodistribution studies, the radiolabeled compound was prepared as [³H]42 by
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13 introducing tritium at the (6*S*) position,⁴⁵ and the resulting compound was evaluated in
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15 bile duct cannulated rat and dog, with the results are provided in Table 8. These studies
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17 show good absorption for the compound, with most of the material excreted unchanged
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19 in the urine. Metabolite analysis revealed small amounts of N-dealkylated metabolite in
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21 rat bile, urine, and plasma, and in dog urine. The O-glucuronidated metabolites were also
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23 observed in rat bile and urine, and in dog bile, urine, and plasma.
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39 **Table 8.** Absorption and excretion of [³H]42 in bile duct cannulated rat and dog.
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Species	Dose Po	Percent of dose recovered				Total
(n)	(mg/kg)	Urine	Bile	Feces	Cage	
Rat (3)	10	75 (+/-10)	17 (+/-13)	<0.5	3	96 (+/-2)

Dog (3)	3	79 (+/-10)	8 (+/-4)	ND	1	88 (+/-6)
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The preclinical safety assessment profile of **42** was very clean and afforded high confidence in a clinical development program for this compound. This OGA inhibitor exhibited no appreciable activity (< 50% inhibition at 30 μ M) against the cardiac and hemodynamic ion channel targets $Ca_v1.2$, $Na_v1.5$, IK_r , IK_s , and no activity (< 50% inhibition at 10 μ M) against a Eurofins Panlabs panel of 118 known pharmacology targets. The compound caused no change in hemodynamic parameters in telemetered rats to a maximal oral dose of 380 mg/kg (C_{max} = 110 μ M), and no change in EKG parameters in telemetered dogs to a maximal oral dose of 300 mg/kg (C_{max} = 660 μ M). In a seven-day oral dose-limiting toxicity study in rats, no changes in physical signs or in serum chemistry were observed, and no histomorphic findings in any tissue to a maximal dose of 1,000 mg/kg/day (day 8 AUC_{0-24h} = 1340 μ M*h, C_{max} = 226 μ M). On the basis of these results, **42** was chosen as a clinical development candidate and designated MK-8719.¹⁹

We determined the X-ray crystal structure of **42** (blue) bound to hOGA,⁴⁶ which was almost superimposable with the previously determined bound structure of **1** (gray) (Figure 10).²⁹ Both inhibitors bind in the enzyme active site through a network of specific hydrogen-bonding interactions with the side chains of Asp285, Asn313, Asp174 and the peptide backbone of Gly67. Notably, the difluoromethyl group in **42** exhibits a F-H hydrogen bond with Asp285 analogous to the interaction between Asp285 and the hydroxymethyl group in **1**; this enthalpically less favorable F-H hydrogen bond may partially explain the reduced hOGA binding affinity for **42** compared to **1**. In both structures the N-ethyl group, which confers selectivity against hHEX, occupies a hydrophobic pocket sandwiched between Tyr219 and Trp278.

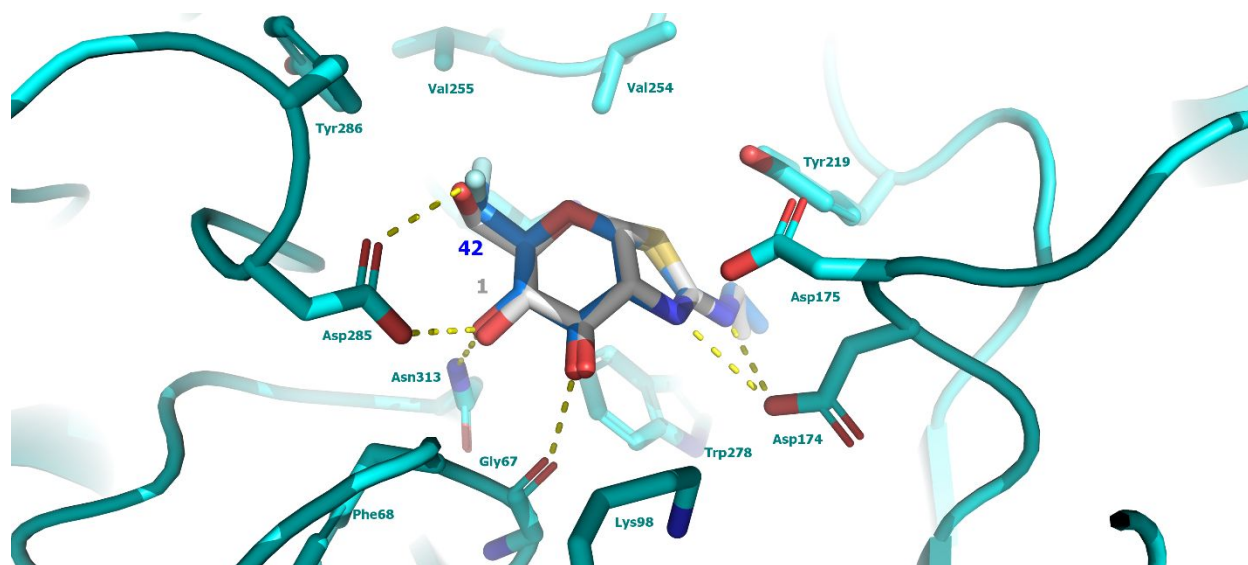


Figure 10. Overlay of structures of **1** (5UHL, grey) and **42** (6PM9, blue) bound to hOGA.

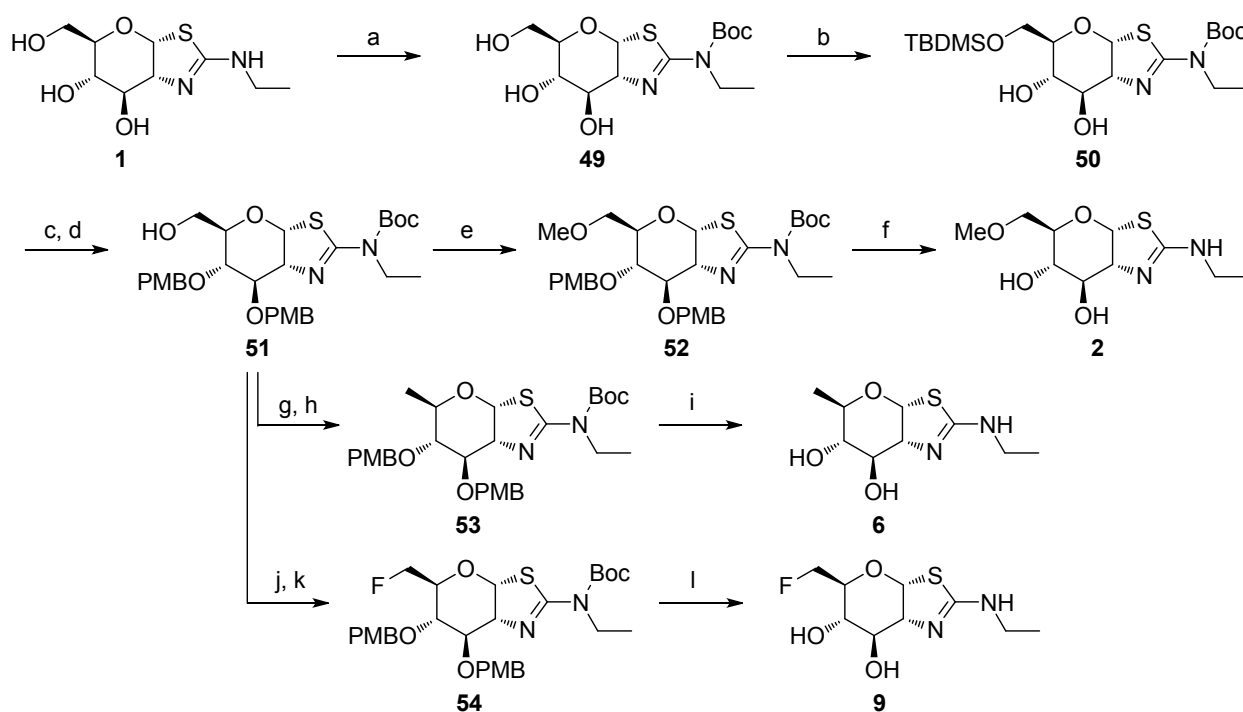
Key hydrogen-bonding interactions are highlighted.

CHEMISTRY

Analogues of **1** bearing modifications to the 5-hydroxymethyl group such as **2**, **6**, and **9** (Scheme 1) began with Boc-protection of the amino group in **1**^{13,14,27} followed by selective protection of the primary hydroxyl as the TBDMS silyl ether to provide **50**. Protection of the remaining secondary alcohols as *p*-methoxybenzyl ethers followed by TBDMS deprotection with TBAF led to the protected alcohol **51** (18% yield over 4 steps). Methylation of the free hydroxyl group in **51** with MeI in the presence of NaH followed by

global deprotection with TFA yielded in the methylated analogue **2**. Alternatively, iodination of **51** with $\text{Ph}_3\text{P}/\text{I}_2$ followed by hydrogenolysis and global deprotection provided the deoxy analogue **6**. Finally, mesylation of **51** followed by fluoride displacement with Et_4NF in CH_3CN and global deprotection led to the 5-fluoromethyl compound **9** (53% yield over 3 steps).

Scheme 1. Synthesis of Compounds **2**, **6**, and **9**^a

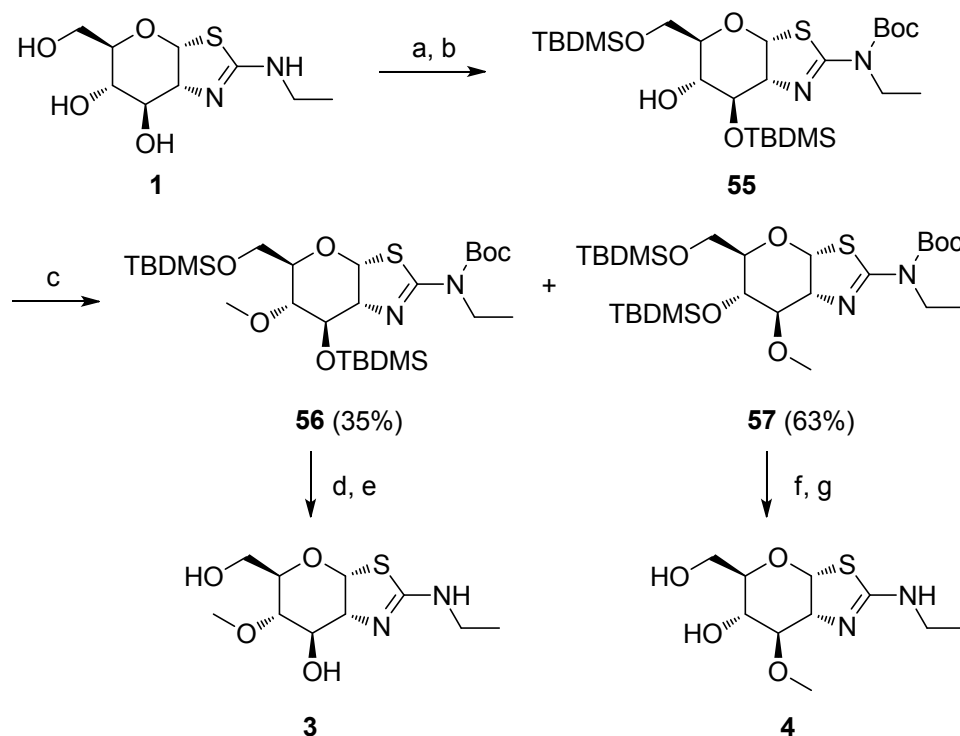


^aReagents and conditions: (a) Boc_2O , iPr_2NEt , DMF/MeOH , rt, 64%; (b) TBDMSCl , imidazole, DMF , rt, 76%; (c) PMBCl , NaH , Bu_4NI , DMF , rt, 0 °C to rt, 66%; (d) TBAF , THF ,

rt, 56%; (e) MeI, NaH, THF, 0 °C to rt, 99%; (f) TFA, CH₂Cl₂, rt, 60%; (g) Ph₃P, I₂, pyridine, toluene, rt, 92%; (h) Pd/C, H₂ (50 psi), EtOH, rt, 78%; (i) TFA, CH₂Cl₂, rt, 69%; (j) MsCl, pyridine, CH₂Cl₂, rt, 99%; (k) Et₄NF, CH₃CN, reflux, 73%; (l) TFA, CH₂Cl₂, rt, 73%.

Preparation of the O-methylated analogues **3** and **4** proceeded via a similar sequence (Scheme 2). Boc-protection of **1** followed by treatment with TBDMSCl under forcing conditions in the presence of DMAP afforded the bis-silyl ether **55** (33% yield over 2 steps). Attempted methylation of the free hydroxy group in **55** using MeI and NaH led to partial migration of the 7-OTBDMS group, with the regioisomeric methylated products **56** and **57** being isolated in a roughly 1:2 ratio. Global deprotection of both compounds using TBAF followed by HCl/MeOH provided **3** and **4**, respectively.

Scheme 2. Synthesis of Compounds **3** and **4**^a

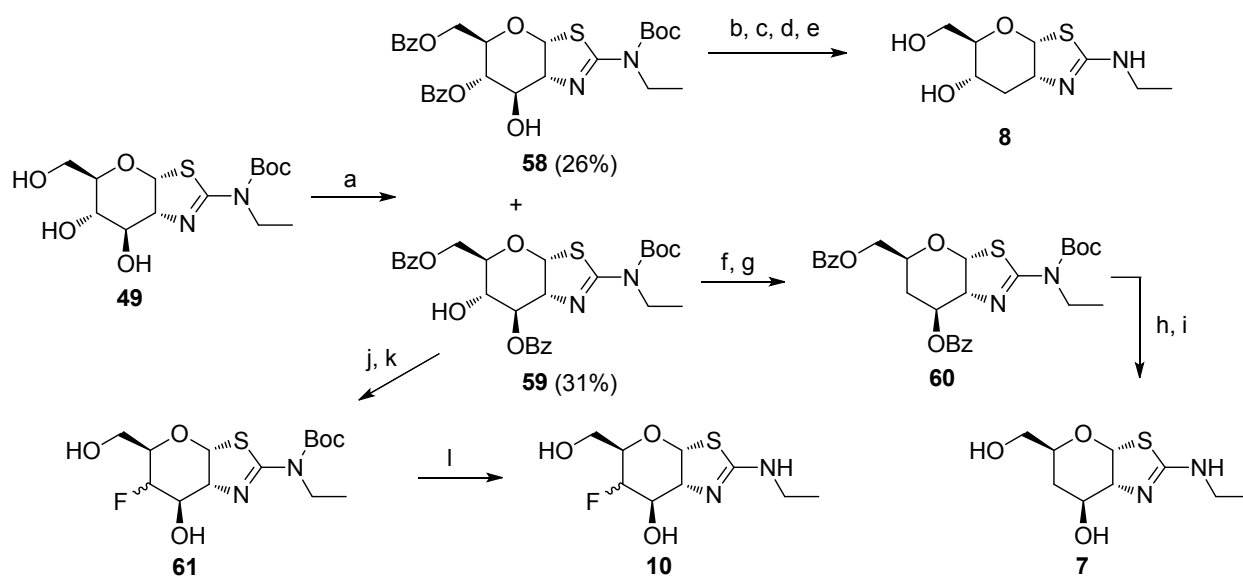


^aReagents and conditions: (a) Boc_2O , DMAP, DMF, rt; (b) TBDMSCl, imidazole, DMF, 0 °C to rt, 33% (2 steps); (c) NaH, MeI, DMF, rt; (d) TBAF, THF, 0 °C to rt; (e) HCl, MeOH, rt, 60% (2 steps); (f) TBAF, THF, 0 °C to rt; (g) HCl, MeOH, rt, 55% (2 steps).

Analogues of **1** with modifications to the 6-hydroxy group such as **7**, **8**, and **10** (Scheme 3) began with treatment of the Boc-protected triol **49** with 2.0 equivalents of benzoyl chloride in the presence of DMAP and Hünig's base to furnish a mixture of protected products, from which the dibenzoylated regioisomers **58** and **59** could be isolated in 26% and 31% yield, respectively. Deoxygenation of **59** was effected through radical reduction

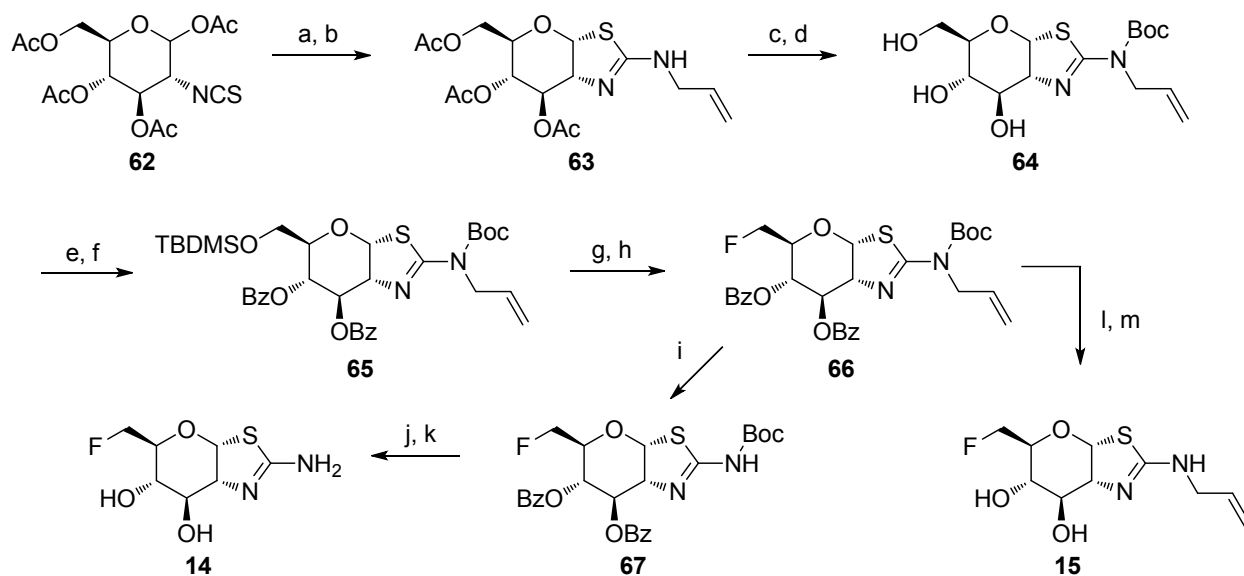
of the thio-CDI adduct using tributyltin hydride, providing **60** in 37% overall yield. Removal of the benzoyl groups with K_2CO_3 /MeOH followed by TFA deprotection led to the 6-deoxy compound **7**. Alternatively, fluorination of **59** with DAST followed by K_2CO_3 /MeOH deprotection led to a single diastereomer of **61** in very low yield (9% over 2 steps). HCl deprotection of this material resulted in the 6-fluoro analogue **10**, although it was not possible to assign the 6-F stereochemistry based on 1H NMR analysis. Dexoygenation of **58** was accomplished using conditions analogous to those used for **59**, which led to the 7-deoxy compound **8**.

Scheme 3. Synthesis of Compounds **7**, **8**, and **10**^a



^aReagents and conditions: (a) BzCl (2.0 equiv), DMAP, *i*Pr₂NEt, CH₂Cl₂, 0 °C; (b) Thio-CDI, toluene, 95 °C, 83%; (c) Bu₃SnH, ABCN, toluene, 90 °C, 51%; (d) K₂CO₃, MeOH, rt; (e) HCl, MeOH, rt, 73% (2 steps); (f) Thio-CDI, toluene, 95 °C, 92%; (g) Bu₃SnH, ABCN, toluene, 90 °C, 44%; (h) K₂CO₃, MeOH, rt, 84%; (i) HCl, MeOH, rt, 86%; (j) DAST, CH₂Cl₂, 0 °C to rt; (k) K₂CO₃, MeOH, rt, 9% (2 steps); (l) HCl, MeOH, rt, 86%.

Access to analogues of **1** bearing a 5-fluoromethyl group with modifications to the thiazoline N-ethyl moiety was achieved as indicated in Scheme 4. Treatment of the isothiocyanate **62**⁴⁷ with allylamine followed by TFA-mediated cyclization provided **63**. Sequential Boc-protection of the amino group, acetate deprotection, TBDMS-protection of the primary alcohol, and benzoyl-protection of the secondary alcohols led to the orthogonally protected material **65**. Silyl deprotection with TBAF followed by fluorination with DAST installed the fluoromethyl group in **66**, which could be globally deprotected with K₂CO₃/MeOH followed by HCl/MeOH to furnish **15**. Alternatively, Pd(0)-mediated removal of the allyl group in **66** followed by global deprotection under analogous conditions led to **14**.

Scheme 4. Synthesis of Compounds 14 and 15^a

^aReagents and conditions: (a) allylamine, CH₂Cl₂, rt; (b) TFA, CH₂Cl₂, rt, 84% (2 steps);

(c) K₂CO₃, MeOH, rt; (d) Boc₂O, Et₃N, MeOH, rt, 90% (2 steps); (e) TBDMSCl, DMAP,

Et₃N, CH₂Cl₂, 40 °C, 87%; (f) BzCl, DMAP, CH₂Cl₂, 0 °C to rt, 78%; (g) AcCl, MeOH, rt,

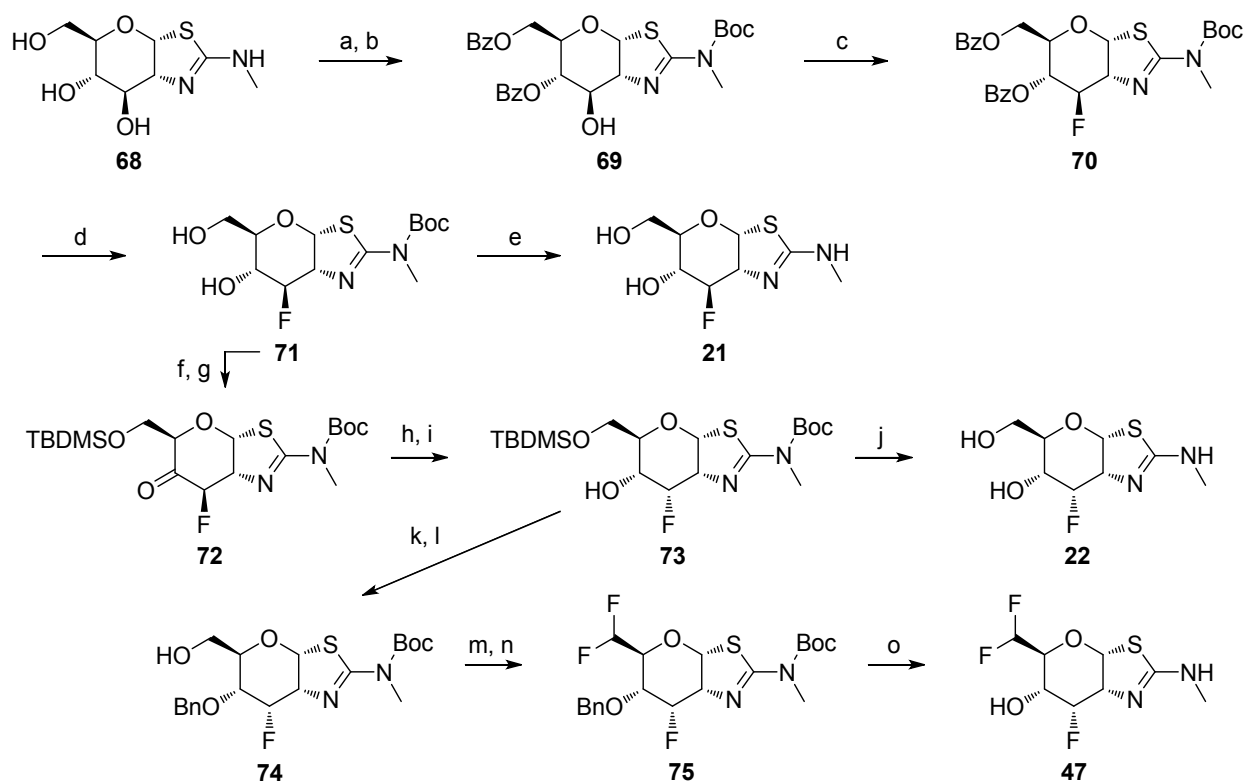
29%; (h) DAST, CH₂Cl₂, -78 °C to rt, 32%; (i) Pd(PPh₃)₄, dioxane, HCO₂H, Et₃N, 60 °C,

71%; (j) K₂CO₃, MeOH, rt, 85%; (k) HCl, MeOH, rt, 20%; (l) K₂CO₃, MeOH, rt, 86%; (m)

HCl, MeOH, rt, 58%.

Generation of analogues bearing a fluoro substituent at the 7-position, such as **21**, **22**, and **47**, required a different approach (Scheme 5). Boc-protection of **68**^{14,27} followed by

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3 treatment with 2.0 equiv of benzoyl chloride led to secondary alcohol **69** (21% yield over
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7 2 steps). Surprisingly, DAST treatment of this material proceeded with retention of
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10 configuration to produce the (7*R*)-F compound **70**, the stereochemistry of which was
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13 confirmed by ¹H NMR analysis of derivatives and X-ray crystallography studies (see
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16 Supporting Information). This result suggests an S_N1-type mechanism with the
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19 involvement of a carbocation intermediate, potentially with anchimeric stabilization by the
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22 flanking benzoyl group. Sequential deprotection of **70** with K₂CO₃/MeOH followed by
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25 HCl/MeOH led to **21**. To access the (7*S*)-F diastereomers, the primary alcohol in **71** was
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28 selectively protected as the TBDMS ether and the secondary alcohol was then oxidized
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31 to the ketone using Dess-Martin periodinane (DMP), providing **72**. Epimerization of the 7-
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34 F center was effected using NaH/MeOH, and subsequent stereoselective ketone
35
36
37 reduction with NaBH₄ gave **73** in high yield, with the desired (6*R*,7*S*) stereochemistry.
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41 Global deprotection of **73** with HCl/MeOH provided **22**. Introduction of a 5-difluoromethyl
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44 group was accomplished by benzylation of **73** followed by TBAF deprotection to afford
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47 alcohol **74**, which was then oxidized to the aldehyde and treated with DAST, furnishing
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56 **75**. Deprotection of this material with BCl₃ gave **47**.
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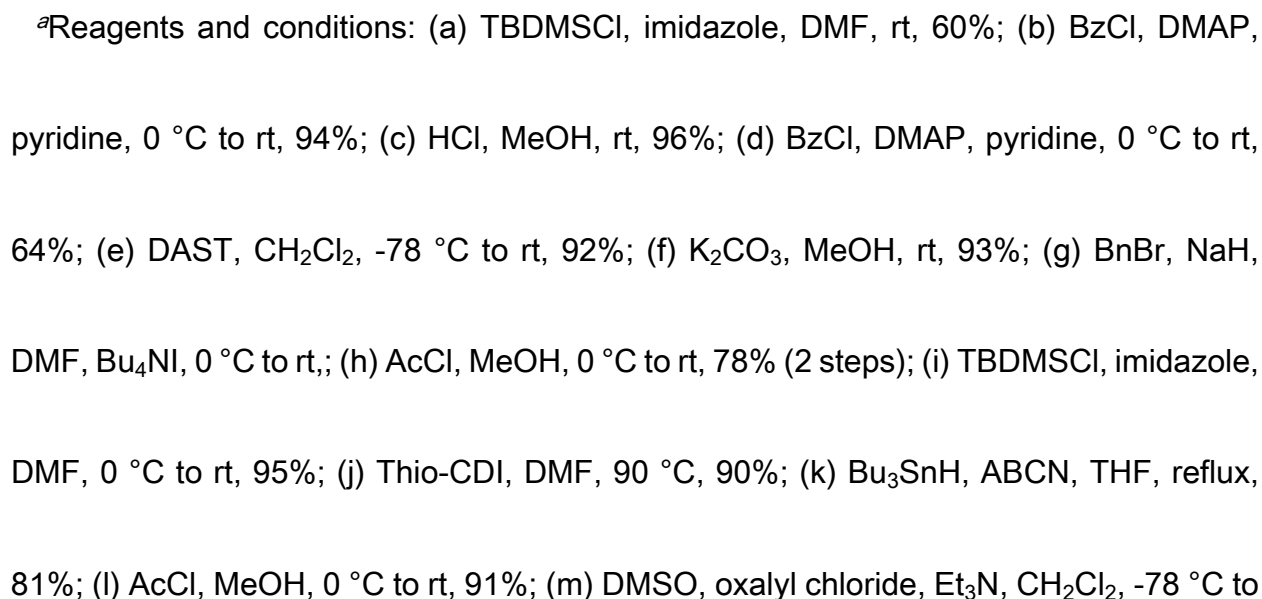
Scheme 5. Synthesis of Compounds 21, 22, and 47^a

^aReagents and conditions: (a) Boc_2O , $i\text{Pr}_2\text{NEt}$, DMF/MeOH, rt, 96%; (b) BzCl (2.0 equiv), DMAP, $i\text{Pr}_2\text{NEt}$, CH_2Cl_2 , 0 °C, 22%; (c) DAST, CH_2Cl_2 , 0 °C, 77%; (d) K_2CO_3 , MeOH, rt, 86%; (e) HCl, MeOH, rt, 95%; (f) TBDMSCl, imidazole, DMF, rt, 96%; (g) DMP, CH_2Cl_2 , rt, 97%; (h) NaH, MeOH, 0 °C; (i) NaBH_4 , MeOH, 0 °C, 76% (2 steps); (j) HCl, MeOH, rt, 89%; (k) BnBr , NaH, DMF, Bu_4NI , 0 °C to rt, 92%; (l) TBAF, THF, rt, 95%; (m)

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3 DMP, CH₂Cl₂, 0 °C to rt; (n) DAST, CH₂Cl₂, rt, 60% (2 steps); (o) BCl₃,
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7 pentamethylbenzene, CH₂Cl₂, rt, 72%.
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11 Modifications to the core scaffold of **1** involving deoxygenation at the 7-position
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13 combined with fluorination of the 5-hydroxymethyl group were generated as outlined in
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15 Scheme 6. Bis-TBDMS-protection of **76**^{14,27} at the 5- and 7-positions provided **77**, which
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18 could be benzylated at the 6-position using BnBr/NaH and then desilylated using mild
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21 acidic conditions in MeOH to furnish **79**. Selective protection of the primary alcohol in **79**
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25 using TBDMSCl at 0 °C led to **80**, which was deoxygenated at the 7-position via radical
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28 reduction of the thio-CDI adduct as described above; subsequent silyl deprotection with
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31 AcCl in MeOH provided **81**. Swern oxidation of **81** gave the corresponding aldehyde,
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34 which was then treated with bis(2-methoxyethyl)aminosulfur trifluoride to afford the 5-
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37 difluoromethyl compound **82**. Deprotection of this material using BCl₃ provided **38**.
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42 Intermediate **77** could also be converted to the 5,6-dibenzoyl-protected compound **78** via
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45 benzoyl protection of the secondary alcohol, acid-mediated silyl deprotection, and
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48 selective benzoyl protection of the primary alcohol. Fluorination of the 7-hydroxy group in
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Scheme 6. Synthesis of Compounds 19 and 38^a

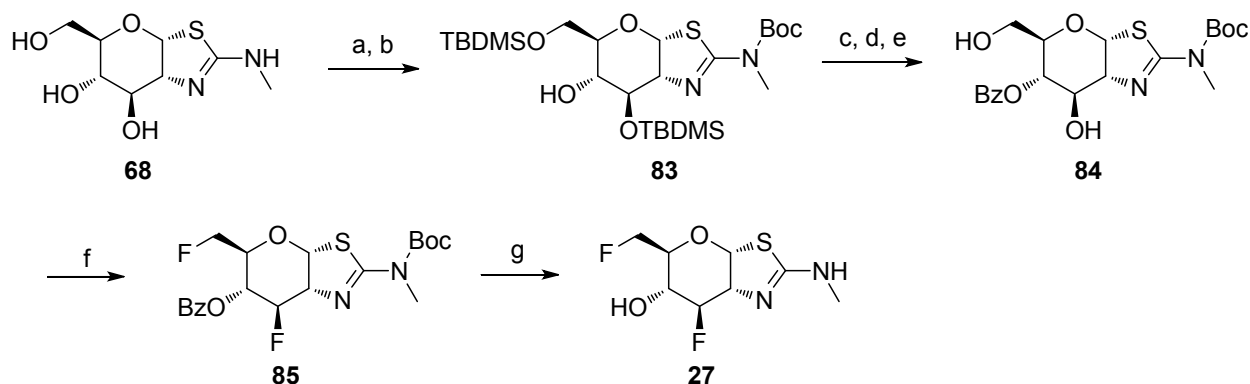


-30 °C; (n) bis(2-methoxyethyl)aminosulfur trifluoride, CH₂Cl₂, 0 °C to rt, 23% (2 steps);

(o) BCl₃, CH₂Cl₂, -78 °C to rt, 53%.

The 5,7-difluoro analogue **27** was accessed using a similar sequence of transformations (Scheme 7). Boc-protection of **68**^{14,27} followed by bis-TBDMS protection afforded **83**. The secondary alcohol in **83** was benzoyl-protected, then the silyl and Boc protecting groups were removed using HCl/MeOH; re-protection of the resulting material with Boc₂O led to **84**. DAST treatment of **84** provided the difluoro compound **85**, which was globally deprotected using MeMgCl to give **27**.

Scheme 7. Synthesis of Compound 27^a

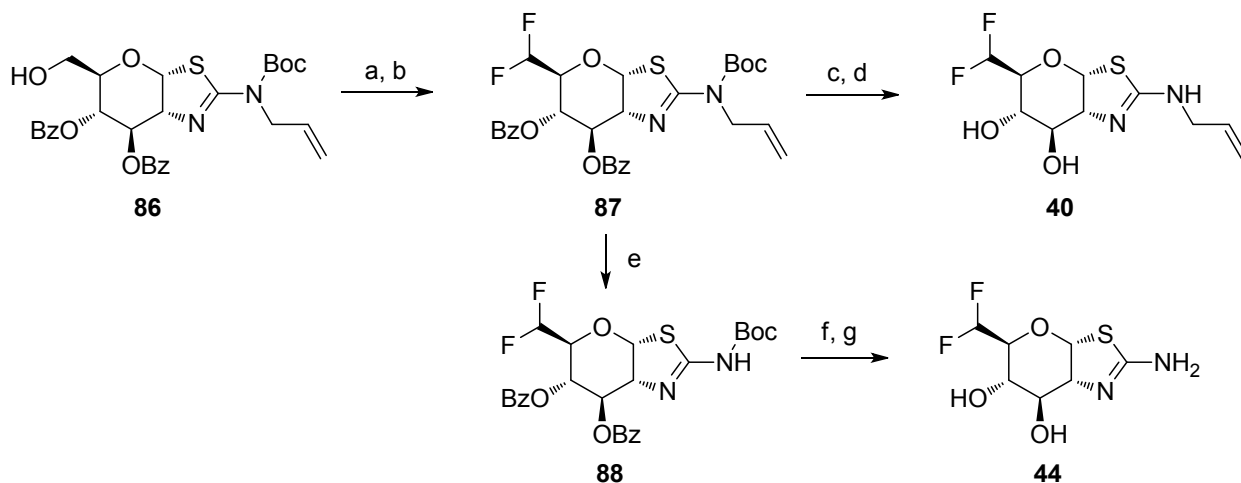


^aReagents and conditions: (a) Boc₂O, iPr₂NEt, DMF, rt; (b) TBDMSCl, imidazole, DMF, rt, 71% (2 steps); (c) BzCl, DMAP, pyridine, 0 °C to rt; (d) HCl, MeOH, rt; (e) Boc₂O,

$i\text{Pr}_2\text{NEt}$, CH_2Cl_2 , rt, 77% (3 steps); (f) DAST, CH_2Cl_2 , -78°C to rt 73%; (g) MeMgCl , THF, rt, 38%.

Introduction of the 5-difluoromethyl group in compounds **40** and **44** (Scheme 8) proceeded via intermediate **86** (prepared from **65** as indicated in Scheme 4). Oxidation of the primary hydroxy group in **86** with DMP provided the corresponding aldehyde, which was converted to the difluoro compound **87** using DAST. Global deprotection of this material using $\text{K}_2\text{CO}_3/\text{MeOH}$ followed by TFA afforded **40**. Alternatively, allyl-deprotection of **87** could be effected using $\text{Pd}(0)$ -mediated hydrogenolysis to give **88**, which was Boc-deprotected with TFA to give **44**.

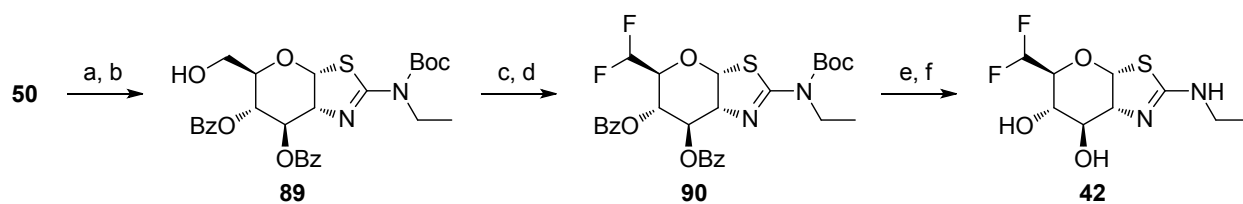
Scheme 8. Synthesis of Compounds **40** and **44**^a



^aReagents and conditions: (a) DMP, CH₂Cl₂, rt; (b) DAST, CH₂Cl₂, -78 °C to rt, 43% (2 steps); (c) K₂CO₃, MeOH, rt, 76%; (d) TFA, CH₂Cl₂, rt, 15%; (e) Pd(PPh₃)₄, dioxane, HCO₂H, Et₃N, 60 °C, 72%; (f) K₂CO₃, MeOH, rt, 81%; (g) TFA, CH₂Cl₂, rt, 27%.

Scheme 9 describes the synthesis of MK-8719 (**42**). Dibenzoylation of **50** (Scheme 1), followed by TBAF deprotection of the TBDMS group provided the primary alcohol **89**. DMP oxidation to the aldehyde followed by DAST treatment afforded the difluoro compound **90** in 40% yield over 2 steps. Removal of the benzoyl groups with K₂CO₃/MeOH followed by TFA deprotection of the Boc group led to **42**.

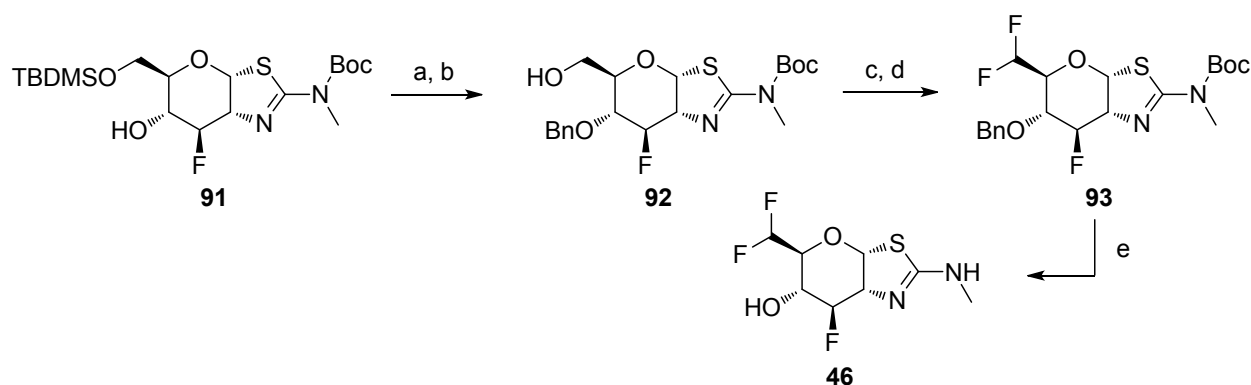
Scheme 9. Synthesis of MK-8719 (**42**)^a



^aReagents and conditions: (a) BzCl (8.0 equiv), DMAP, pyridine, 0 °C, 71%; (b) AcCl (0.2 equiv), MeOH, rt, 90%; (c) DMP, pyridine, CH₂Cl₂, 0 °C to rt; (d) DAST, CH₂Cl₂, -78 °C to rt, 40% (2 steps); (e) K₂CO₃, MeOH, rt, 92%; (f) TFA, CH₂Cl₂, 0 °C to rt, 95%.

Synthesis of the trifluoro analogue **46** (Scheme 10) proceeded via intermediate **91** (prepared from **71** as indicated in Scheme 5). Benzyl-protection of the secondary alcohol in **91** using BnBr/NaH followed by TBDMS deprotection using TBAF provided **92**. DMP oxidation of **92** to the aldehyde followed by DAST treatment gave **93**, which was globally deprotected using BCl₃ to afford **46**.

Scheme 10. Synthesis of Compound 46^a

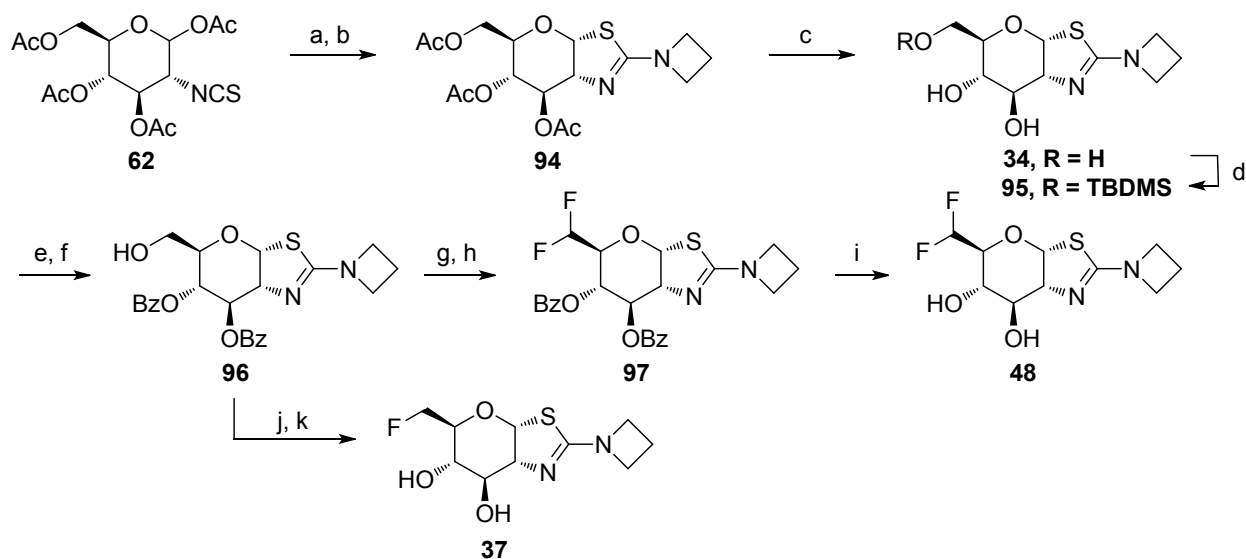


^aReagents and conditions: (a) BnBr, NaH, DMF, Bu₄NI, 0 °C to rt, 96%; (b) TBAF, THF, 0 °C to rt, 100%; (c) DMP, CH₂Cl₂, rt, 68%; (d) DAST, CH₂Cl₂, -78 °C to rt, 76%; (e) BCl₃, CH₂Cl₂, pentamethylbenzene, -78 °C to rt, 76%.

Analogues of **1** bearing cyclic amino substituents at the 2'-position such as **34** and **48** were generated as shown in Scheme 11. Treatment of the isothiocyanate **62**⁴⁷ with

azetidine/HCl provided the corresponding thiourea, which was cyclized using TFA to furnish **94**. Deprotection with K_2CO_3 /MeOH gave **34**, which could be selectively protected as the silyl ether **95**. Benzoylation of both secondary alcohols followed by TBAF deprotection gave primary alcohol **96**, which was oxidized to the aldehyde and treated with DAST to provide **97**. Deprotection with K_2CO_3 /MeOH led to **48**. Alternatively, direct treatment of **96** with DAST followed by benzoyl deprotection using K_2CO_3 /MeOH provided **37**.

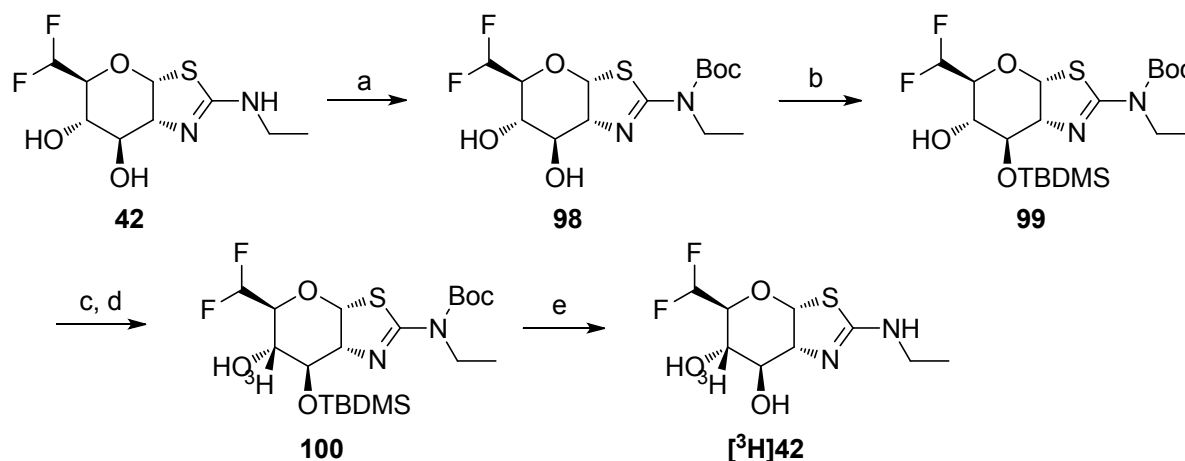
Scheme 11. Synthesis of Compounds **34**, **37**, and **48**^a



^aReagents and conditions: (a) azetidine hydrochloride, Et₃N, CH₂Cl₂, rt; (b) TFA, CH₂Cl₂, rt, 75% (2 steps); (c) K₂CO₃, MeOH, rt, 87% (compound **34**); (d) TBDMSCl, Et₃N, DMAP, CH₂Cl₂, rt, 65%; (e) BzCl, NaH, DMF, 15 °C, 30%; (f) AcCl, MeOH, rt, 40%; (g) DMP, CH₂Cl₂, 0 °C to rt; (h) DAST, CH₂Cl₂, -78 °C to rt, 46% (2 steps); (i) K₂CO₃, MeOH, rt, 22%; (j) DAST, CH₂Cl₂, -78 °C to rt; (k) K₂CO₃, MeOH, rt, 17% (2 steps).

The tritiated analogue of MK-8719, [³H]**42**, was prepared as indicated in Scheme 12. Boc-protection of **42** furnished **98**, which could be selectively protected at the 7-position as the silyl ether with TBDMSCl/imidazole to give **99**. DMP oxidation of the secondary alcohol in **99** afforded the corresponding ketone, which was reduced using sodium borotritide to provide a 2:1 mixture of the 6*R*:6*S* diastereomeric alcohols; HPLC purification of this mixture provided **100** as a single diastereomer. Boc-deprotection of **100** using HCl/DMF provided [³H]**42**.

Scheme 12. Synthesis of Compound [³H]**42**^a



^aReagents and conditions: (a) Boc₂O, MeOH, rt, 51%; (b) TBDMSCl, imidazole, DMF, rt, 57%; (c) DMP, CH₂Cl₂, 5 °C, 84%; (d) Na³H₄B, MeOH, 0 °C; (e) HCl, DMF, rt.

CONCLUSIONS

In the work described here, we have defined the key elements of the pharmacophore in **1** that are required for potent and selective inhibition of OGA. We applied this information to design analogues that possess improved brain penetration based on a strategy of lowering the calculated TPSA to increase membrane permeability as measured by *Papp*. This strategy entailed sequential removal or modification of hydrogen-bond donors and acceptors in **1** while maintaining potency and selectivity.

These efforts led to the identification of a series of highly potent and selective OGA inhibitors that exhibit good pharmacokinetic properties and excellent brain penetration *in vivo*. Characterization of these compounds in a pharmacodynamic assay to measure continued elevation of brain O-protein in rat over 24 h (PD AUC) allowed us to rank compounds in terms of their ability to elicit a robust and sustained pharmacodynamic response. Taken together, this work led us to identify **42** as the compound that integrates the desired properties of excellent intrinsic potency and selectivity, improved *P_{app}*, high CNS exposure, good pharmacokinetics, favorable metabolic stability, and robust pharmacodynamic response. In particular, when compared to **1** across a range of doses, **42** required a roughly 10-fold lower dose to achieve the same rat brain PD AUC response. On the basis of these properties, **42** was designated as MK-8719 and was selected as a development candidate that has been advanced to first-in-human Phase I clinical trials.¹⁹ Further details of these trials will be reported in due course.

EXPERIMENTAL SECTION

Synthetic Materials and Methods. Reagents and solvents were obtained from commercial sources and used without further purification. All final compounds reported are at least 95% pure judged by HPLC UV detection (254 nm), and LC-MS. Flash chromatography was performed on a Teledyne Isco CombiFlash instrument using pre-packed RediSepRf Gold silica gel columns. LC-MS was measured using HP1100 and Micromass ZQ instruments. Preparative HPLC purification were performed on Gilson 500 instrument with C8 or C18 reverse phase preparative HPLC column eluting with MeCN/water with modifiers of either TFA or NH₄OH, except where otherwise indicated. High resolution mass spectrometry was performed on a Waters Xevo G2 Qtof spectrometer: ESI+; direct infusion; sheath gas flow rate:5; capillary temperature: 120 °C. ¹H and ¹³C NMR spectra (300, 400, and 500 MHz) were collected on Bruker spectrometers at ambient temperature. Chemical shifts are reported in ppm relative to the residual solvent peak in the indicated solvent, and for ¹H NMR spectra, multiplicities, coupling constants in hertz, and numbers of protons are indicated parenthetically. All in vivo experimental protocols described in this study were approved by the Institutional Animal Care and Use Committee of Merck & Co., Inc., Kenilworth, NJ, USA, and

conducted in accordance with the Guide for Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, 1996). All efforts were made to minimize animal suffering, to reduce the number of animals used and to use alternatives to in vivo methods where possible.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-(methoxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (2). Step 1: Preparation of *tert*-butyl ((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (**49**). To a suspension of **1**¹³ (35.0 g, 141 mmol) in DMF (300 mL) cooled to 15 °C was added DIPEA (6.0 mL), Boc₂O (61.5 g, 282 mmol), and MeOH (6.0 mL). The mixture was stirred at room temperature for 16 h then MeOH (50 mL) was added. The reaction mixture was concentrated in vacuo at 35 °C. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:1, then MeOH/DCM 1:5), followed by recrystallization from EtOAc/hexanes, to afford **49** (31.5 g, 64% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 6.12 (d, *J* = 6.8 Hz, 1H), 4.23–4.22 (m, 1H), 4.17–4.14 (m, 1H), 3.91–3.86 (m, 2H), 3.81–3.77 (m, 3H), 3.59–3.55 (m, 1H), 3.17–3.16 (m, 1H, OH), 1.53 (s, 9H), 1.16 (t, *J* = 7.0 Hz, 3H).

Step 2: Preparation of *tert*-butyl ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-6,7-dihydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (50). To a solution of **49** (5.0 g, 14.4 mmol) in DMF (25 mL) was added imidazole (1.57 g, 23.1 mmol) and TBDMSCI (2.82 g, 18.7 mmol). The reaction mixture was stirred at room temperature for 30 h then diluted with EtOAc (100 mL). The organic layer was washed with saturated NH₄Cl, brine, dried over anhydrous Na₂SO₄, and concentrated. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:1) affording **50** (5.08 g, 76% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 6.12 (d, *J* = 6.7 Hz, 1H), 4.25 (t, *J* = 6.2 Hz, 1H), 4.16 (t, *J* = 6.4 Hz, 1H), 4.10–4.04 (m, 2H), 3.91–3.85 (m, 3H), 3.65–3.62 (m, 1H), 1.55 (s, 9H), 1.26 (t, *J* = 7.0 Hz, 3H), 0.89 (s, 9H), 0.08 (s, 6H).

Step 3: Preparation of *tert*-butyl ethyl((3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-(hydroxymethyl)-6,7-bis((4-methoxybenzyl)oxy)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)carbamate (51). To a solution of **50** (1.15 g, 2.5 mmol) and tetrabutyl ammonium iodide (0.092 g, 0.25 mmol) in DMF (25 mL) at 0 °C was added NaH (60%, 0.3 g, 7.5 mmol) followed by *p*-methoxybenzyl chloride (1.02 mL, 7.5 mmol). The reaction mixture was stirred at room

temperature for 6 h, then diluted with Et₂O (50 mL), and quenched with water (5 mL). The ether layer was washed with saturated NH₄Cl, brine, dried over anhydrous Na₂SO₄, and concentrated in vacuo. A crude mixture of two products, *tert*-butyl ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6-((*tert*-butyldimethylsilyl)oxy)-7-((4-methoxybenzyl)oxy)-5-((4-methoxyphenoxy)methyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate and *tert*-butyl ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-6,7-bis((4-methoxybenzyl)oxy)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (1.6 g, 66% yield) was isolated in a 1:2 ratio, respectively, as indicated by ¹H NMR. To a solution of this mixture (1.58 g, 2.26 mmol) in THF (15 mL) at 0 °C was added a 1M TBAF solution in THF (4.0 mL, 4 mmol) and the mixture was stirred at room temperature for 2 h. The reaction was diluted with EtOAc (50 mL) and the organic layer was washed with saturated NH₄Cl, dried over anhydrous Na₂SO₄, and concentrated. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:2), affording **51** (0.75 g, 56% yield) as a gummy solid. ¹H NMR (400 MHz, CDCl₃): δ 7.30 (d, *J* = 8.6, 2H), 7.15 (d, *J* = 8.6, 2H), 6.88–6.80 (m, 4H), 6.01 (d, *J* = 6.9 Hz, 1H), 4.64 (d, *J* = 11.8 Hz, 1H), 4.59 (d, *J* = 11.8 Hz, 1H), 4.48–4.45 (m, 1H),

4.37–4.34 (ddd, J = 6.8, 3.6, 1.28 Hz, 1H), 4.27 (d, J = 11.2, 1H), 4.24–4.23 (dd, J = 1.8, 1.76 Hz, 1H), 3.87–3.80 (m, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.70–3.65 (m, 1H), 3.59–3.56 (dt, J = 9.0, 1.44 Hz, 1H), 3.55–3.49 (m, 1H), 3.41–3.37 (ddd, J = 8.5, 5.3, 2.8 Hz, 1H), 1.78 (t, J = 6.4 Hz, 1H), 1.50 (s, 9H), 1.18 (t, J = 6.9 Hz, 3H).

Step 4: Preparation of *tert*-butyl ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-bis((4-methoxybenzyl)oxy)-5-(methoxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (52). To a solution of **51** (0.16 g, 0.28 mmol) in dry THF (5 mL) at 0 °C was added NaH (60%, 13.5 mg, 0.33 mmol) in small portions. After stirring at 0 °C for 20 min, methyl iodide (0.052 mL, 0.84 mmol) was added and the mixture was stirred at room temperature for 2.5 h. MeOH (2 mL) was added and the reaction mixture was concentrated in vacuo. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes, 3:7), affording **52** (0.175 g, 99% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.34 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 6.90–6.84 (m, 4H), 6.09 (d, J = 7.0 Hz, 1H), 4.69 (d, J = 11.7 Hz, 1H), 4.63 (d, J = 11.7 Hz, 1H), 4.54 (d, J = 11 Hz, 1H), 4.39–4.37 (m, 1H), 4.31–4.25 (m, 2H), 3.89–3.87 (m, 2H), 3.807 (s, 3H), 3.803 (s, 3H), 3.60 (m, 1H), 3.55–3.51 (m, 1H), 3.46–3.44 (m, 2H), 3.32 (s, 3H), 1.53 (s, 9H), 1.13 (t, J = 6.9 Hz, 3H).

Step 5: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-(methoxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (2). The ether **52** (0.120 g, 0.2 mmol) was dissolved in 10% TFA/DCM solution (10 mL) and stirred at room temperature for 2.5 h. The mixture was concentrated and co-evaporated with Et₂O (20 mL) in vacuo. To the residue was added 2M NH₃/MeOH solution (5 mL) and the mixture was concentrated in vacuo. The residue was purified by flash chromatography (SiO₂; 5% MeOH in DCM then 94:4:2 DCM-MeOH-NH₄OH (28% aqueous)) to give **2** (31 mg, 60% yield) as a white solid. ¹H NMR (400 MHz, MeOH-*d*₄) δ 6.42 (d, *J* = 5.9 Hz, 1H), 4.10 (t, *J* = 6.36 Hz, 1H), 3.89 (t, *J* = 6.08 Hz, 1H), 3.73 (ddd, *J* = 8.6, 6.0, 2.0 Hz, 1H), 3.66 (dd, *J* = 8.9, 2.08 Hz, 1H), 3.61–3.56 (dd, *J* = 6.04, 4.8 Hz, 1H), 3.51–3.47 (dd, *J* = 5.9, 3.3 Hz, 1H), 3.37 (s, 3H), 3.35–3.33 (m, 2H), 1.22 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, MeOH-*d*₄) δ 164.03, 89.03, 81.35, 76.00, 71.19, 71.11, 64.05, 59.74, 41.79, 14.99. ESI MS *m/z* 263 [M + H]⁺.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-(hydroxymethyl)-6-methoxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (3). **Step 1: Preparation of *tert*-butyl ((3a*R*,5*R*,6*R*,7*R*,7a*R*)-7-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-**

1
2
3 butyldimethylsilyl)oxy)methyl)-6-hydroxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazol-
4
5
6
7 2-yl)(ethyl)carbamate (**55**). A mixture of **1** (4.96 g, 20.0 mmol), DMAP (0.050 g, 0.41
8
9
10 mmol) and Boc₂O (9.82 g, 45.0 mmol) in DMF (20 mL) was stirred at room temperature
11
12
13 overnight. The mixture was cooled to 0 °C, then imidazole (5.45 g, 80.0 mmol) and
14
15
16 TBDMSCl (3.77 g, 25.0 mmol) were added. After stirring at room temperature overnight
17
18
19 the reaction was quenched by adding saturated aqueous NH₄Cl solution (100 mL). The
20
21
22 mixture was then extracted with DCM (2 × 100 mL), and the combined extract was
23
24
25 washed with brine (100 mL) and dried over anhydrous Na₂SO₄. After filtration the solvent
26
27
28 was evaporated under reduced pressure, and the residue was purified on silica gel by
29
30
31 flash column chromatography (EtOAc/hexanes, 1:4 to 1:2), affording **55** as a white solid
32
33
34 (3.80 g, 33% yield). ¹H NMR (400 MHz, CDCl₃) δ 5.88 (d, *J* = 6.0 Hz, 1H), 4.24 (br s, 1H),
35
36
37 4.09–3.86 (m, 3H), 3.84–3.64 (m, 3H), 3.38–3.35 (m, 1H), 1.47 (s, 9H), 1.14 (t, *J* = 7.2
38
39
40 Hz, 3H), 0.85 (s, 9H), 0.82 (s, 9H), 0.096 (s, 3H), 0.087 (s, 3H), 0.000 (s, 6H).
41
42
43
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49 **Step 2: Preparation of *tert*-butyl ((3a*R*,5*R*,6*R*,7*R*,7a*R*)-7-((*tert*-butyldimethylsilyl)oxy)-5-**
50
51
52 **(((*tert*-butyldimethylsilyl)oxy)methyl)-6-methoxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-**
53
54
55 **d]thiazol-2-yl)(ethyl)carbamate (**56**) and *tert*-butyl ((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-((*tert*-**
56
57
58
59
60

butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-methoxy-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-d]thiazol-2-yl)(ethyl)carbamate (**57**). To a solution of **55** (0.400 g, 0.693 mmol) in anhydrous THF (5 mL) was added NaH (60% in mineral oil, 0.032 g, 0.80 mmol) at room temperature, and then MeI (0.28 g, 2.0 mmol) was added immediately after. After stirring at room temperature for 1 h the reaction was quenched by adding saturated aqueous NH₄Cl solution (10 mL). The mixture was extracted with EtOAc (2 × 30 mL), and the combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:10), affording **56** as clear oil (0.14 g, 35% yield); ¹H NMR (400 MHz, CDCl₃) δ 5.90 (broad, 1H), 4.10 (br s, 2H), 3.95–3.80 (m, 2H), 3.72–3.63 (m, 2H), 3.42 (s, 3H), 3.34–3.20 (m, 2H), 1.47 (s, 9H), 1.16 (br s, 3H). 0.84 (s, 9H), 0.82 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), 0.00 (s, 6H). Also isolated was **57** (0.26 g, 63% yield) as a clear oil; ¹H NMR (400 MHz, CDCl₃) δ 5.88 (d, *J* = 6.8 Hz, 1H), 4.24 (br s, 1H), 3.88–3.84 (m, 2H), 3.76–3.72 (m, 3H), 3.65–3.60 (m, 1H), 3.48 (s, 3H), 3.31–3.27 (m, 1H), 1.50 (s, 9H), 1.16 (t, *J* = 7.2 Hz, 3H), 0.85 (s, 9H), 0.84 (s, 9H), 0.069 (s, 3H), 0.045 (s, 3H), 0.020 (s, 6H).

Step 3: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-(hydroxymethyl)-6-methoxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (3). To a solution of **56** (0.140 g, 0.236 mmol) in anhydrous THF (5 mL) at 0 °C was added TBAF (1.0 M in THF, 1.0 mL, 1.0 mmol), and the mixture was stirred at room temperature for 1 h. Brine (30 mL) was added, the mixture was extracted with EtOAc (2 × 30 mL) and the combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 5:1) affording a white foam. The foam was dissolved in MeOH (3 mL), into which HCl (g) was bubbled for 30 sec. The reaction was then stirred at room temperature for 3 h. The solvent was removed, and the residue was neutralized with 1.0 M NH₃ in MeOH and subsequently purified on silica gel by flash column chromatography (1.0 M NH₃ in MeOH/DCM, 1:8), affording **3** as an off-white solid (0.038 g, 60% yield over 2 steps). ¹H NMR (400 MHz, CD₃OD) δ 6.25–6.23 (m, 1H), 4.26–4.22 (m, 2H), 3.69 (dd, *J* = 2.3, 11.9 Hz, 1H), 3.58 (dd, *J* = 5.9, 11.9 Hz, 1H), 3.49–3.47 (m, 1H), 3.45 (s, 3H), 3.31–3.15 (m, 3H), 1.14 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 162.97, 89.69, 81.59, 76.15, 73.99, 70.75, 63.61, 58.28, 39.78, 14.83. ESI MS *m/z* 263.1 [M + H]⁺.

(3*aR*,5*R*,6*S*,7*R*,7*aR*)-2-(ethylamino)-5-(hydroxymethyl)-7-methoxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (4). Compound **4** was prepared from **57** (0.120 g, 0.203 mmol) following the procedure used to prepare **3**. The crude material was purified on silica gel by flash column chromatography (1.0 M NH₃ in MeOH/DCM, 1:8), affording **4** as an off-white solid (0.029 g, 55% yield over 2 steps). ¹H NMR (400 MHz, CD₃OD) δ 6.27 (d, *J* = 6.0 Hz, 1H), 4.22 (t, *J* = 5.8 Hz, 1H), 3.81–3.78 (m, 1H), 3.67–3.64 (m, 1H), 3.63–3.58 (m, 2H), 3.55 (s, 3H), 3.37–3.27 (m, 3H), 1.14 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 90.24, 84.96, 76.07, 69.40, 63.22, 59.31, 49.87, 39.93, 14.73. ESI MS *m/z* 263.1 [M + H]⁺.

(3*aR*,5*R*,6*S*,7*R*,7*aR*)-2-(ethyl(methyl)amino)-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diol (5). Step 1: Preparation of (2*S*,3*R*,4*R*,5*S*,6*R*)-6-(acetoxymethyl)-3-(3-ethyl-3-methylthioureido)-tetrahydro-2*H*-pyran-2,4,5-triyl triacetate (**Exp #5a**). To a stirred solution of **62** (1.10 g, 2.8 mmol) in DCM (10 mL) was added neat ethyl(methyl)amine (310 μL, 3.6 mmol) dropwise. The mixture was stirred at room temperature for 1 h. Solvents were removed by concentration. The residue was purified by flash chromatography on silica gel (hexane/EtOAc 1:1) to give (2*S*,3*R*,4*R*,5*S*,6*R*)-6-

(acetoxymethyl)-3-(3-ethyl-3-methylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate as a white foam (1.09 g, 86% yield). ¹H NMR (500 MHz, CDCl₃) δ 1.15 (t, 3H, *J* = 7.0 Hz), 2.05 (s, 3H), 2.06 (s, 3H), 2.11 (s, 3H), 2.13 (s, 3H), 3.08 (s, 3H), 3.74–3.81 (m, 3H), 4.16 (dd, 1H, *J* = 2.0, 12.5 Hz), 4.27 (dd, 1H, *J* = 4.5, 12.5 Hz), 5.14 (t, 1H, *J* = 10.0 Hz), 5.26 (t, 1H, *J* = 10.0 Hz), 5.34–5.40 (m, 2H), 5.78 (d, 1H, *J* = 8.0 Hz).

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(acetoxymethyl)-2-(ethyl(methyl)amino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-*d*]thiazole-6,7-diyl diacetate (Exp #5b). The thiourea (2*S*,3*R*,4*R*,5*S*,6*R*)-6-(acetoxymethyl)-3-(3-ethyl-3-methylthioureido)-tetrahydro-2H-pyran-2,4,5-triyl triacetate (155 mg, 0.35 mmol) was dissolved in DCM (1.5 mL) and TFA (20 μL, 2.63 mmol) was added. Then the resulting mixture was stirred at room temperature for 16 h. The solution was diluted with saturated aqueous NaHCO₃ (20 mL), then the resulting mixture was extracted with DCM (3 × 10 mL) and the combined organic extracts were dried (Na₂SO₄) and concentrated to give (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(acetoxymethyl)-2-(ethyl(methyl)amino)-5,6,7,7a-tetrahydro-3aH-pyrano[3,2-*d*]thiazole-6,7-diyl diacetate as a pale yellow foam (134 mg, 100% yield). This material was used the next reaction without further purification. ¹H NMR (500 MHz, CDCl₃) δ 1.16 (t, 3H, *J* =

7.0 Hz), 2.06 (s, 3H), 2.08 (s, 3H), 2.10 (s, 3H), 2.98 (s, 3H), 3.24–3.31 (m, 1H), 3.39–3.45 (m, 1H), 3.83–3.86 (m, 1H), 4.14 (d, 2H, J = 4.5 Hz), 4.34 (dd, 1H, J = 4.5, 6.5 Hz), 4.93 (dd, 1H, J = 3.0, 10.0 Hz), 5.40 (dd, 1H, J = 3.0, 4.5 Hz), 6.21 (d, 1H, J = 6.5 Hz).

Step 3: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(ethyl(methyl)amino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (5). The triacetate (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(acetoxymethyl)-2-(ethyl(methyl)amino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl diacetate (134 mg, 0.35 mmol) was dissolved in MeOH (2.0 mL) and then K₂CO₃ (72 mg, 0.52 mmol) was added. The resulting mixture was stirred at room temperature for 2 h. The mixture was diluted with DCM (9 mL) and then poured onto the top of a basic Al₂O₃ (1 g) column. The column was eluted with 10–25% MeOH in DCM to give **5** (57.4 mg, 57% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 1.07 (t, 3H, J = 7.0 Hz), 2.88 (s, 3H), 3.21–3.28 (m, 2H), 3.58–3.60 (m, 2H), 3.67–3.73 (m, 2H), 3.79 (dd, 1H, J = 3.5, 7.0 Hz), 3.88 (t, 1H, J = 6.5 Hz), 4.07 (t, 1H, J = 6.5 Hz), 4.60 (br s, 3H), 6.28 (d, 1H, J = 6.5 Hz); ¹³C NMR (125 MHz, CDCl₃) δ 11.88, 35.70, 46.93, 60.82, 68.02, 73.03, 73.76, 74.02, 90.19, 162.40. HRMS (ESI+) m/z [M + H]⁺ calculated for C₁₀H₁₈N₂O₄S: 263.1065; found: 263.1064. Elemental analysis

calculated for C₁₀H₁₈N₂O₄S: C, 45.79; H, 6.92; N, 10.68; Found: C, 46.01; H, 7.18; N, 10.46.

(3*aR*,5*R*,6*S*,7*R*,7*aR*)-2-(ethylamino)-5-methyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diol (6). Step 1: Preparation of *tert*-butyl ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-6,7-bis((4-methoxybenzyl)oxy)-5-methyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (53). To a solution of triphenyl phosphine (0.179 g, 0.68 mmol) in dry toluene (5 mL) was added iodine (0.145 g, 0.57 mmol), pyridine (0.1 mL, 1.2 mmol), and a solution of **51** (0.210 g, 0.357 mmol) pre-dissolved in toluene (3 mL). The reaction mixture was stirred at 65 °C for 3.5 h. Insoluble yellowish material was filtered off and the filtrate was diluted with EtOAc (50 mL) and then washed with 1N HCl (20 mL), saturated NaHCO₃ (20 mL), and brine. The EtOAc layer was separated, dried over Na₂SO₄, and concentrated in vacuo to give a residue which was purified by flash chromatography (SiO₂; EtOAc/hexanes, 3:7), affording *tert*-butyl ethyl((3*aR*,5*S*,6*S*,7*R*,7*aR*)-5-(iodomethyl)-6,7-bis((4-methoxybenzyl)oxy)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)carbamate (0.23 g, 92% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.33 (d, *J* = 8.4, 2H), 7.18 (d, *J* = 8.4, 2H), 6.89–6.82 (m, 4H), 6.07 (d, *J* = 7 Hz, 1H),

4.67 (d, $J = 11.6$ Hz, 1H), 4.61 (d, $J = 11.6$ Hz, 1H), 4.54–4.51 (m, 1H), 4.36–4.31 (m, 2H), 4.19 (t, $J = 2.64$ Hz, 1H), 3.87 (d, $J = 6.9$ Hz, 1H), 3.83 (d, $J = 6.9$ Hz, 1H), 3.77 (s, 6H), 3.50–3.48 (dd, $J = 6.8, 1.3$ Hz, 1H), 3.37–3.34 (m, 1H), 3.23–3.20 (m, 2H), 1.51 (s, 9H), 1.10 (t, $J = 6.9$ Hz, 3H). To a solution of this material (0.120 g, 0.172 mmol) in EtOH (4 mL) and Et₃N (0.1 mL) was added 10% Pd/C (0.060 g). The mixture was hydrogenated at 50 psi and at room temperature for 24 h. The catalyst was removed by filtration and solvent was removed in vacuo. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 3:7) to give **53** (0.077 g, 78% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.31 (d, $J = 6.8$, 2H), 7.17 (d, $J = 6.8$, 2H), 6.87–6.81 (m, 4H), 6.07 (d, $J = 5.6$ Hz, 1H), 4.67 (d, $J = 9.4$ Hz, 1H), 4.58 (d, $J = 9.4$ Hz, 1H), 4.50 (d, $J = 8.9$ Hz, 1H), 4.34 (m, 1H), 4.27 (d, $J = 8.9$ Hz, 1H), 4.23–4.17 (m, 1H), 3.87–3.84 (m, 2H), 3.78 (s, 3H), 3.77 (s, 3H), 3.44–3.39 (m, 1H), 3.30–3.28 (m, 1H), 1.50 (s, 9H), 1.18 (d, $J = 4.9$ Hz, 3H), 1.10 (t, $J = 5.5$ Hz, 3H).

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-methyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (6**).** A solution of **53** (0.075 g, 0.131 mmol) dissolved in 30% TFA/DCM (10 mL) was stirred at room temperature for 2.5 h. The

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4 mixture was concentrated in vacuo and co-evaporated with Et₂O (20 mL). To the residue
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6
7 was added 2M NH₃/MeOH solution (3 mL) and the mixture was concentrated in vacuo.
8
9
10 The residue was purified by flash chromatography (SiO₂; 5% MeOH in DCM then 94:4:2
11
12 DCM-MeOH-NH₄OH (28% aqueous)) to give **6** (0.021 g, 69% yield) as a white solid. ¹H
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14 NMR (400 MHz, MeOH-*d*₄) δ 6.60 (d, *J* = 6.5 Hz, 1H), 4.08 (t, *J* = 7.04 Hz, 1H), 3.77 (t, *J*
15
16 = 7.2 Hz, 1H), 3.75–3.72 (m, 1H), 3.44–3.38 (ddd, *J* = 14.6, 7.28, 2.08 Hz, 2H), 3.25–3.20
17
18 (dd, *J* = 7.7, 1.12 Hz, 1H), 1.33 (d, *J* = 6.2 Hz, 3H), 1.28 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100
19
20 MHz, MeOH-*d*₄) δ 160.40, 90.08, 75.69, 75.66, 74.21, 66.27, 55.65, 18.91, 14.51. ESI
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22 MS *m/z* 233 [M + H]⁺.
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35 **(3a*R*,5*S*,7*S*,7a*R*)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-**
36
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38 **pyrano[3,2-*d*]thiazol-7-ol (7).** Step 1: Preparation of **((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-**
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40
41 **(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-7-hydroxy-5,6,7,7a-tetrahydro-3a*H*-**
42
43 **pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (58)** and **((3a*R*,5*R*,6*S*,7*R*,7a*R*)-7-**
44
45 **(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-6-hydroxy-5,6,7,7a-tetrahydro-3a*H*-**
46
47 **pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (59).** To a solution of **49** (1.64 g, 4.73 mmol),
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DIPEA (1.34 g, 10.4 mmol) and DMAP (0.010 g, 0.082 mmol) in DCM (50 mL) at 0 °C

was slowly added BzCl (1.33 g, 9.50 mmol). The mixture was stirred at room temperature overnight, then saturated aqueous NH₄Cl (50 mL) was added and the organic layer was collected. The aqueous layer was further extracted with DCM (2 × 40 mL) and the combined organic extract was dried over anhydrous Na₂SO₄, concentrated in vacuo, and the residue was separated by flash chromatography (SiO₂; EtOAc/hexanes 1:4 to 1:2) affording **59** (0.81 g, 31% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.98 (dd, *J* = 1.2, 8.2 Hz, 2H), 7.88 (dd, *J* = 1.2, 8.2 Hz, 2H), 7.53–7.49 (m, 1H), 7.41–7.32 (m, 3H), 7.20–7.15 (m, 2H), 6.07 (d, *J* = 6.8 Hz, 1H), 5.72–5.70 (m, 1H), 4.51–4.49 (m, 1H), 4.46–4.41 (m, 2H), 4.00–3.84 (m, 3H), 3.70–3.68 (m, 1H), 1.49 (s, 9H), 1.13 (t, *J* = 7.2 Hz, 3H). Also isolated was **58** as a white solid (0.67 g, 26% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.08 (d, *J* = 8.1 Hz, 4H), 7.57–7.35 (m, 6H), 6.19 (d, *J* = 7.1 Hz, 1H), 5.21 (dd, *J* = 2.8, 9.2 Hz, 1H), 4.56–4.51 (m, 2H), 4.47–4.42 (m, 2H), 4.14–4.10 (m, 1H), 3.99–3.92 (m, 2H), 1.55 (s, 9H), 1.19 (t, *J* = 7.2 Hz, 3H).

Step 2: Preparation of ((3a*R*,5*S*,7*S*,7a*R*)-7-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (60**).** A mixture of **59** (0.570 g, 1.03 mmol) and 1,1'-thiocarbonyl diimidazole

(thio-CDI, 90% tech, 0.40 g, 2.0 mmol) in anhydrous toluene (15 mL) was stirred at 95 °C for 4 h. After cooling to room temperature, the solvent was removed in vacuo and the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 2:5) affording (3*aR*,5*R*,6*S*,7*R*,7*aR*)-6-((1*H*-imidazole-1-carbonothioyl)oxy)-5-((benzoyloxy)methyl)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-7-yl benzoate (0.63 g, 92% yield) as a yellow solid. ¹H NMR (500 MHz, CDCl₃) showed the existence of rotational isomers in CDCl₃ at room temperature. A mixture of this material (0.63 g, 0.95 mmol), tributyltin hydride (0.61 g, 2.1 mmol) and 1,1'-azobis(cyclohexane-carbonitrile) (0.015 g, 0.061 mmol) in anhydrous toluene (15 mL) was stirred at 90 °C for 3 h. After cooling to room temperature, the solvent was removed in vacuo and the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:4) affording **60** (0.23 g, 44% yield) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 8.10–8.08 (m, 2H), 7.99–7.96 (m, 2H), 7.60–7.56 (m, 1H), 7.51–7.48 (m, 1H) 7.46–7.42 (m, 2H), 7.31–7.27 (m, 2H), 6.23 (d, *J* = 6.8 Hz, 1H), 5.91–5.88 (m, 1H), 4.41–4.38 (m, 1H), 4.38–4.35 (m, 2H), 4.22–4.17 (m, 1H), 4.00–3.94 (m, 2H), 2.30–2.26 (m, 1H), 2.01–1.97 (m, 1H), 1.55 (s, 9H), 1.19 (t, *J* = 7.2 Hz, 3H).

Step 3: Preparation of (3a*R*,5*S*,7*S*,7a*R*)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (7). A mixture of **60** (0.20 g, 0.37 mmol) and K₂CO₃ (0.030 g, 0.22 mmol) in anhydrous MeOH (3 mL) was stirred at room temperature for 2 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO₂; MeOH/DCM 1:20) affording *tert*-butyl ethyl((3a*R*,5*S*,7*S*,7a*R*)-7-hydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)carbamate (0.11 g, 84% yield) as a clear oil. ¹H NMR (500 MHz, CDCl₃) δ 6.19 (d, *J* = 7.1 Hz, 1H), 4.36 (q, *J* = 5.3 Hz, 1H), 4.21 (dd, *J* = 5.0, 7.5 Hz, 1H), 3.98–3.93 (m, 3H), 3.67 (dd, *J* = 3.0, 11.9 Hz, 1H), 3.56 (dd, *J* = 6.3, 11.9 Hz, 1H), 2.03 (td, *J* = 5.5, 14.1 Hz, 1H), 1.68–1.62 (m, 1H), 1.54 (s, 9H), 1.19 (t, *J* = 7.2 Hz, 3H). To a solution of this material (0.10 g, 0.31 mmol) in MeOH (5 mL) was bubbled HCl (g) for 30 sec. The solution was then stirred at room temperature for 3 h. The solvent was removed in vacuo and the residue was neutralized with 1.0 M NH₃ in MeOH. The mixture was concentrated in vacuo and the residue was purified by flash chromatography (SiO₂; 1.0 M NH₃ in MeOH/DCM 1:8), affording **7** (0.062 g, 86% yield) as a white foam. ¹H NMR (500 MHz, MeOH-*d*₄) δ 6.31 (d, *J* = 6.3 Hz, 1H), 4.19 (q, *J* = 5.7 Hz, 1H), 3.98 (t, *J* = 5.9 Hz, 1H), 3.93–3.88 (m, 1H),

3.53–3.49 (m, 2H), 3.29–3.22 (m, 2H), 2.03–1.98 (m, 1H), 1.54–1.48 (m, 1H), 1.16 (t, J = 7.2 Hz, 3H). ESI MS m/z 233.1 $[M + H]^+$.

(3a*R*,5*R*,6*S*,7a*R*)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (8). Step 1: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-7-((1*H*-imidazole-1-carbonothioyl)oxy)-5-((benzoyloxy)methyl)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-yl benzoate (**Exp #8a**). A mixture of **58** (0.346 g, 0.623 mmol) and thio-CDI (90% tech, 0.20 g, 1.0 mmol) in anhydrous toluene (10 mL) was stirred at 95 °C for 4 h. After cooling the solvent was removed under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 2:3), affording (3a*R*,5*R*,6*S*,7*R*,7a*R*)-7-((1*H*-imidazole-1-carbonothioyl)oxy)-5-((benzoyloxy)methyl)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-yl benzoate as a yellow solid (0.343 g, 83% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.76 (s, 1H), 8.08–7.94 (m, 4H), 7.70 (s, 1H), 7.57–7.37 (m, 6H), 7.18 (s, 1H), 6.36 (dd, J = 1.9, 3.7 Hz, 1H), 6.17 (d, J = 7.1 Hz, 1H), 5.54 (td, J = 1.2, 9.2 Hz, 1H), 4.70–4.67 (m, 1H),

4.60 (dd, J = 3.2, 12.1 Hz, 1H), 4.42 (dd, J = 5.1, 12.2 Hz, 1H), 4.11–4.08 (m, 1H), 4.05–3.97 (m, 2H), 1.56 (s, 9H), 1.22 (t, J = 7.2 Hz, 3H).

Step 2: Preparation of ((3*aR*,5*R*,6*S*,7*aR*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (Exp #8b). A mixture of the above material (0.343 g, 0.515 mmol), Bu_3SnH (0.291 g, 1.00 mmol) and 1,1'-azobis(cyclohexanecarbonitrile) (ABCN) (8.0 mg, 0.033 mmol) in anhydrous toluene (10 mL) was stirred at 90 °C for 3 h. After cooling the solvent was removed under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:4), affording ((3*aR*,5*R*,6*S*,7*aR*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate as a white solid (0.141 g, 51% yield). ^1H NMR (400 MHz, CDCl_3) δ 8.03–7.94 (m, 4H), 7.57–7.52 (m, 2H), 7.44–7.35 (m, 4H), 6.07 (d, J = 7.2 Hz, 1H), 5.44–5.40 (m, 1H), 4.52–4.41 (m, 3H), 4.06–3.96 (m, 3H), 2.70–2.64 (m, 1H), 2.47–2.40 (m, 1H), 1.56 (s, 9H), 1.18 (t, J = 7.2 Hz, 3H).

Step 3: Preparation of (3*aR*,5*R*,6*S*,7*aR*)-2-(ethylamino)-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (8). A mixture of the above material (0.140 g,

0.260 mmol) and K_2CO_3 (0.030 g, 0.22 mmol) in anhydrous MeOH (6 mL) was stirred at room temperature for 3 h. The solvent was removed under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc), affording a white solid. The solid was dissolved in MeOH (3 mL), into which HCl (g) was bubbled for 30 sec. The solution was then stirred at room temperature for 3 h. The solvent was removed, and the residue was neutralized with 1.0 M NH_3 in MeOH and subsequently purified on silica gel by flash column chromatography (1.0 M NH_3 in MeOH/DCM, 1:8), affording **8** as an off-white solid (0.044 g, 73% yield). 1H NMR (500 MHz, CD_3OD) δ 6.20 (d, J = 7.1 Hz, 1H), 4.28–4.25 (m, 1H), 3.75 (dd, J = 2.6, 14.4 Hz, 1H), 3.73–3.69 (m, 1H), 3.61 (dd, J = 6.4, 14.4 Hz, 1H), 3.54–3.51 (m, 1H), 3.30–3.22 (m, 2H), 2.16–2.11 (m, 1H), 2.06–2.00 (m, 1H), 1.18 (t, J = 7.2 Hz, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 163.24, 91.35, 77.13, 69.15, 65.79, 63.36, 39.60, 34.75, 14.87. ESI MS m/z 233 $[M + H]^+$.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (9). Step 1: Preparation of *tert*-butyl ethyl((3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(fluoromethyl)-6,7-bis((4-methoxybenzyl)oxy)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)carbamate (54). Methanesulfonyl chloride

(0.038 mL, 0.495 mmol) was added in portions to a stirred solution of **51** (0.190 g, 0.323 mmol) in dry pyridine (5 mL) at -20 °C. After 3 h, the mixture was diluted with DCM (20 mL) and the DCM extract was washed with saturated NaHCO₃, brine, dried over Na₂SO₄, and concentrated in vacuo. Residual pyridine was removed by co-evaporation with hexanes in vacuo. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 3:7) to give ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-6,7-bis((4-methoxybenzyl)oxy)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methyl methanesulfonate (0.215 g, 99% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 7.32 (d, *J* = 8.5 Hz, 2H), 7.17 (d, *J* = 8.5 Hz, 2H), 6.91–6.83 (m, 4H), 6.01 (d, *J* = 6.88 Hz, 1H), 4.66 (d, *J* = 11.6 Hz, 1H), 4.60 (d, *J* = 11.6 Hz, 1H), 4.52 (d, *J* = 11.1 Hz, 1H), 4.42–4.36 (m, 2H), 4.27–4.23 (m, 3H), 3.96–3.84 (m, 2H), 3.8 (s, 3H), 3.79 (s, 3H), 3.55 (m, 2H), 2.98 (s, 3H), 1.52 (s, 9H), 1.20 (t, *J* = 6.9 Hz, 3H). To a solution of this material (0.520 g, 0.78 mmol) in CH₃CN (6 mL) was added a solution of tetraethylammonium fluoride (0.640 g, 4.28 mmol) in CH₃CN (4 mL) and the mixture was heated to reflux for 2.5 h. After concentration in vacuo, the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:1) to provide **54** (0.335 g, 73% yield) as a foamy solid. ¹H NMR (400

MHz, CDCl₃) δ 7.35 (d, J = 8.5 Hz, 2H), 7.20 (d, J = 8.5 Hz, 2H), 6.93–6.86 (m, 4H), 6.08 (d, J = 6.88 Hz, 1H), 4.72 (d, J = 11.7 Hz, 1H), 4.65 (d, J = 11.7 Hz, 1H), 4.56 (d, J = 11.6 Hz, 1H), 4.52–4.51 (m, 1H), 4.41–4.38 (m, 2H), 4.33 (d, J = 11.6 Hz, 1H), 4.26 (t, J = 2.88 Hz, 1H), 3.93–3.87 (m, 2H), 3.83 (s, 3H), 3.82 (s, 3H), 3.69–3.66 (m, 1H), 3.61–3.50 (m, 1H), 1.55 (s, 9H), 1.15 (t, J = 6.9 Hz, 3H).

Step 2: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(ethylamino)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (9). Fluoride **54** (0.115 g, 0.195 mmol) dissolved in 30% TFA/DCM at 0 °C was stirred for 3 h at room temperature. TFA/DCM was then removed in vacuo and the residue was suspended in 2M NH₃/MeOH (3 mL). The mixture was concentrated in vacuo and the crude residue was purified by flash chromatography (SiO₂; 10% MeOH in DCM) to give **9** (0.035 g, 73% yield) as a light yellowish solid. ¹H NMR (400 MHz, CD₃OD) 6.49 (d, J = 6.48 Hz, 1H), 4.66–4.52 (dt, J = 47.6, 10.5, 4.3 Hz, 2H), 4.12 (t, J = 6.6 Hz, 1H), 3.90 (t, J = 6.9 Hz, 1H), 3.80–3.73 (m, 2H), 3.55 (dd, J = 6.3, 3.2 Hz, 1H), 3.4–3.3 (m, 2H), 1.23 (t, J = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 163.78, 89.63, 83.90 (d, $J_{C6,F}$ 171.7 Hz, C-6), 76.83 (d, $J_{C5,F}$ 17.8 Hz,

C-5), 75.43, 69.51 (d, $J_{C4,F}$ 7.0 Hz, C-4), 67.71, 42.13, 14.74. HRMS (ESI+) m/z [M + H]⁺ calculated for C₉H₁₅FN₂O₃S: 251.0865; found: 251.0870.

(3a*R*,5*R*,7*R*,7a*R*)-2-(ethylamino)-6-fluoro-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (10). Step 1: Preparation of *tert*-butyl ethyl((3a*R*,5*R*,7*R*,7a*R*)-6-fluoro-7-hydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)carbamate (61). To a solution **59** (0.63 g, 0.11 mmol) in anhydrous DCM (8 mL) at -20 °C was added DAST (1.0 g, 6.3 mmol). The mixture was warmed to room temperature and stirred overnight. The reaction was then cooled at -40 °C and diluted with DCM (20 mL), then quenched by dropwise addition of saturated aqueous NaHCO₃. The organic layer was separated and the aqueous was extracted with DCM (2 × 30 mL). The combined organic extract was dried over anhydrous Na₂SO₄, concentrated in vacuo, and the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:5 to 2:7) affording impure ((3a*R*,5*R*,7*R*,7a*R*)-7-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-6-fluoro-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (0.20 g) as a pale yellow oil. This impure material was dissolved in anhydrous MeOH (6 mL) and K₂CO₃ (0.030 g, 0.22 mmol) was added, then the mixture

was stirred at room temperature for 2 h. The solvent was removed in vacuo, and the residue was purified by flash chromatography (SiO₂; MeOH/DCM 1:25) affording **61** (0.034 g, 9% yield over two steps) as a white solid. ¹H NMR (500 MHz, CDCl₃) δ 6.11 (d, *J* = 6.5 Hz, 1H), 4.63 (ddd, *J* = 3.1, 8.2, 49.1 Hz), 4.46 (td, *J* = 3.7, 18.8 Hz, 1H), 4.34 (dd, *J* = 4.4, 7.1 Hz, 1H), 3.92–3.84 (m, 3H), 3.73 (dd, *J* = 4.4, 12.2 Hz, 1H), 3.68–3.62 (m, 1H), 1.53 (s, 9H), 1.15 (t, *J* = 7.0 Hz, 3H).

Step 2: Preparation of (3a*R*,5*R*,7*R*,7a*R*)-2-(ethylamino)-6-fluoro-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (10). To a solution of **61** (0.034 g, 0.097 mmol) in MeOH (3 mL) was bubbled HCl (g) for 30 sec. The solution was then stirred at room temperature for 2 h. The solvent was removed in vacuo and the residue was neutralized with 1.0 M NH₃ in MeOH. The mixture was concentrated in vacuo and the residue was purified by flash chromatography (SiO₂; 1.0 M NH₃ in MeOH/DCM 1:8) affording **10** (0.021 g, 86% yield) as a white foam. ¹H NMR (500 MHz, MeOH-*d*₄) δ 6.27–6.24 (m, 1H), 4.51–4.36 (m, 1H), 4.31–4.22 (m, 2H), 3.74–3.60 (m, 3H), 3.30–3.19 (m, 2H), 1.16 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, MeOH-*d*₄) δ 163.18, 92.16 (d, *J* = 178.8

Hz), 89.99, 76.03 (d, $J = 2.7$ Hz), 73.04 (d, $J = 27.7$ Hz), 71.95 (d, $J = 22.5$ Hz), 63.13, 39.80, 14.93. ESI MS m/z 251.1 $[M + H]^+$.

(3a*R*,5*R*,6*R*,7*R*,7a*R*)-2-(ethylamino)-7-fluoro-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (11). To a solution of **58** (0.49 g, 0.88 mmol) in anhydrous DCM (3 mL), at -40 °C under N₂, was added DAST (1.0 g, 6.2 mmol). After addition the mixture was stirred at room temperature overnight. After the reaction mixture was again cooled at -40 °C, it was diluted with DCM (20 mL), and then quenched by adding saturated aqueous NaHCO₃ dropwise. The organic layer was collected, and the aqueous was extracted with DCM (2 × 15 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:5 to 2:7), affording ((3a*R*,5*R*,6*R*,7*R*,7a*R*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-7-fluoro-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate as a white solid (0.14 g, impure, ~28% yield). Removal of the benzoate and Boc protective groups on this material was accomplished by treatment with solid K₂CO₃ in MeOH and saturated aqueous HCl in MeOH respectively, via procedures

analogous to those described for preparation of **8**. After purification on silica gel by flash column chromatography (1.0 M NH₃ in MeOH/DCM, 1:7), **11** was obtained as an off-white solid (0.051 g, 23% yield over 2 steps). ¹H NMR (400 MHz, CD₃OD) δ 6.33 (d, *J* = 6.6 Hz, 1H), 4.77 (td, *J* = 5.0, 48.2 Hz, 1H), 4.34–4.27 (m, 1H), 3.81 (dd, *J* = 2.0, 12.0 Hz, 1H), 3.77–3.72 (m, 1H), 3.68 (dd, *J* = 5.7, 12.0 Hz, 1H), 3.63–3.59 (m, 1H), 3.31–3.23 (m, 2H), 1.18 (t, *J* = 7.2 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 163.40, 96.25 (d, *J* = 177.7 Hz), 90.55 (d, *J* = 3.9 Hz), 75.26 (d, *J* = 4.6 Hz), 73.58 (d, *J* = 24.5 Hz), 68.91 (d, *J* = 22.8 Hz), 62.85, 39.54, 14.82. ESI MS *m/z* 251 [M + H]⁺. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₉H₁₆FN₂O₃S: 251.0866; found: 251.0870.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(allyl(methyl)amino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (13). To a solution of **68** (500 mg, 2.14 mmol) in DMF (50 mL) was added 3-bromoprop-1-ene (1.28 g, 10.67 mmol) and K₂CO₃ (295 mg, 2.14 mmol). The resulting solution was stirred overnight at room temperature. The resulting mixture was concentrated under vacuum to afford a crude product (450 mg), which was purified by Prep-HPLC with the following conditions (Agilent 1200 prep HPLC; Column: Sun Fire Prep C18, 19*50mm 5um; mobile phase, water with 0.03% NH₄OH and CH₃CN

(10% CH₃CN up to 25% in 10 min; Detector: UV: 220 nm) to give **13** (116 mg, 22% yield) as a white solid. ¹H NMR (300 MHz, D₂O), δ: 6.20–6.22 (d, *J* = 6.0 Hz, 1H), 5.70–5.80 (m, 1H), 5.05–5.15 (m, 2H), 4.07–4.10 (t, *J* = 6.0 Hz, 1H), 3.91–3.94 (t, *J* = 4.8 Hz, 1H), 3.84–3.86 (m, 2H), 3.71–3.74 (t, *J* = 5.7 Hz, 1H), 3.53–3.70 (m, 2H), 3.44–3.50 (t, *J* = 4.8 Hz, 1H), 2.90 (s, 3H); ¹³C NMR (125 MHz, CD₃OD) δ 36.22, 54.82, 61.86, 69.76, 74.33, 74.76, 75.13, 90.49, 116.25, 132.71, 163.91. ESI MS *m/z* 275.0 [M + H]⁺. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₁₁H₁₈N₂O₄S: 275.1065; found: 275.1065.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-amino-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (14). Step 1: Preparation of **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(acetoxymethyl)-2-(allylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl diacetate (63).** A mixture of allylamine (9.2 g, 0.16 mol) and **62** (60 g, 0.154 mol) in DCM (300 mL) was stirred for 1 h at room temperature, followed by addition of TFA (88 g, 0.77 mol). The resulting solution was stirred overnight at room temperature and was quenched with aqueous NaHCO₃ to pH at 8, and extracted with DCM (3 × 300 mL). The combined organic layer was dried over anhydrous Na₂SO₄, and condensed to give a residue, which was purified by a silica gel column with 1%~2% MeOH in DCM to give **63** as a yellow

liquid (58 g, 84% yield). ^1H NMR (300 MHz, CDCl_3) δ 6.33 (d, J = 6.6 Hz, 1H), 5.84–5.96 (m, 1H), 5.32–5.44 (m, 4H), 4.98–5.04 (m, 1H), 4.37 (t, J = 5.7 Hz, 1H), 4.28–4.22 (m, 2H), 3.92–3.95 (m, 3H), 2.14 (s, 3H), 2.12 (s, 3H), 2.11 (s, 3H). ESI MS m/z 387.0 $[\text{M} + \text{H}]^+$.

Step 2: Preparation of *tert*-butyl allyl((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)carbamate (64). A solution of **63** (50 g, 0.13 mol) in MeOH (300 mL) was treated with K_2CO_3 (3.6 g, 0.26 mol) overnight at room temperature, and followed by addition of Boc_2O (56 g, 0.26 mol) and Et_3N (19.6 g, 0.19 mol). After an additional 2 h, the resulting solution was concentrated to give a residue, which was purified by a silica gel column, eluted with 5% MeOH in DCM to give **64** as an oil (42 g, 90% yield in two steps). ^1H NMR (300 MHz, CDCl_3) δ 6.14 (d, J = 6.9 Hz, 1H), 5.84–5.96 (m, 1H), 5.22–5.44 (m, 4H), 4.37–4.38 (m, 1H), 4.22–4.28 (m, 2H), 3.92–3.95 (m, 3H), 1.54 (s, 9H). ESI MS m/z 361.0 $[\text{M} + \text{H}]^+$.

Step 3: Preparation of (3*aR*,5*R*,6*S*,7*R*,7*aR*)-2-(allyl(*tert*-butoxycarbonyl)amino)-5-((*tert*-butyl-dimethylsilyloxy)methyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (65). A mixture of TBDMSCl (26 g, 0.17 mmol), DMAP (1.4 g, 0.01 mol), Et_3N

(23.5 g, 0.23 mmol) and **64** (42 g, 0.12 mol) in DCM (300 mL) was stirred overnight at 40 °C. The reaction mixture was quenched by saturated aqueous NaHCO₃ (20 mL), and was condensed to give a residue, which was purified by a silica gel column, eluted with 1%~2% MeOH in DCM to give *tert*-butyl allyl((3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-((*tert*-butyldimethylsilyloxy)methyl)-6,7-dihydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)carbamate as a light yellow syrup (48 g, 87% yield). ¹H NMR (300 MHz, CDCl₃) δ 6.16 (d, *J* = 6.6 Hz, 1H), 5.84–5.93 (m, 1H), 5.22–5.32 (m, 4H), 4.65–4.67 (m, 1H), 4.26–4.31 (m, 1H), 4.20–4.22 (m, 1H), 3.84–3.88 (m, 3H), 1.54 (s, 9H), 0.93 (s, 9H), 0.09 (s, 6H). ESI MS *m/z* 474.9 [M + H]⁺. A mixture of this material (12.5 g, 26.3 mmol), BzCl (11.1 g, 79 mmol), DMAP (322 mg, 2.63 mmol), and pyridine (20.8 g, 263 mmol) in DCM (150 mL) at 0 °C was stirred overnight at room temperature. The reaction mixture was quenched with saturated aqueous NaHCO₃ (200 mL), extracted with DCM (3 × 100 mL), washed with brine (3 × 50 mL), dried over anhydrous MgSO₄, and condensed to give a residue, which was purified by a silica gel column with 10% EtOAc in petroleum ether to afford **65** as a white solid (16.5 g, 78% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.05–8.10 (m, 4H), 7.56–7.61 (m, 2H), 7.42–7.47 (m, 4H), 6.27 (d, *J* = 7.2 Hz, 1H), 5.99–6.02 (m,

2H), 5.86–5.92 (m, 1H), 5.43–5.46 (m, 1H), 5.10–5.18 (m, 2H), 4.81–4.89 (m, 1H), 4.56–4.62 (m, 2H), 3.76–3.87 (m, 3H), 1.56 (s, 9H), 0.88 (s, 9H), 0.04 (s, 6H). ESI MS m/z 683.1 $[M + H]^+$.

Step 4: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(allyl(*tert*-butoxycarbonyl)amino)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (66). A solution of **65** (7.0 g, 10.3 mmol) in MeOH (50 mL) was treated with AcCl (0.5 mL, 0.07 mmol) for 3 h at room temperature. The reaction mixture was quenched with saturated aqueous NaHCO₃ (10 mL) and water (200 mL), extracted with DCM (3 × 50 mL), dried over anhydrous MgSO₄, and concentrated under vacuum to give a residue, which was purified by a silica gel column with 10% EtOAc in petroleum ether to give (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(allyl(*tert*-butoxycarbonyl)amino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate as a white syrup (1.7 g, 29% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.06–8.09 (m, 4H), 7.60–7.63 (m, 2H), 7.43–7.58 (m, 4H), 6.26 (d, *J* = 6.9 Hz, 1H), 6.06–6.27 (m, 2H), 5.37–5.41 (d, *J* = 8.4 Hz, 1H), 5.11–5.28 (m, 2H), 4.82–4.84 (m, 1H), 4.56–4.62 (m, 2H), 3.71–3.86 (m, 3H), 1.56 (s, 9H). ESI MS m/z 569.0 $[M + H]^+$. A solution of this material (1.7 g, 3.0 mmol) in DCM (40 mL) was

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3 treated with DAST (2.5 g, 15.4 mmol) for 30 min at -78 °C. After stirring overnight at room
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7 temperature, the reaction was quenched with saturated aqueous NaHCO₃ (30 mL). The
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10 organic layer was separated, and the aqueous layer was extracted with DCM (3 × 20 mL).
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13 The combined organic layer was washed with brine (20 mL), dried over anhydrous
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16 Na₂SO₄ and concentrated under vacuum. The residue was purified by a silica gel column
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19 with 5%–10% EtOAc in petroleum ether to give **66** as a white solid (580 mg, 32% yield).
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22 ¹H NMR (300 MHz, CDCl₃) δ 8.09 (d, *J* = 7.5 Hz, 4H), 7.60 (t, *J* = 7.8 Hz, 2H), 7.45 (t, *J*
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24 = 7.8 Hz, 4H), 6.27 (d, *J* = 6.3 Hz, 1H), 6.08 (s, 1H), 5.86–5.92 (m, 1H), 5.40 (d, *J* = 9.6
25
26 Hz, 1H), 5.12 (d, *J* = 9.3 Hz, 2H), 4.81–4.89 (dd, *J* = 10.0, 2.8 Hz, 1H), 4.63–4.69 (m, 2H),
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28 4.50–4.54 (m, 2H), 3.82–3.98 (m, 1H), 1.63 (s, 9H). ESI MS *m/z* 571.0 [M + H]⁺.
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38 **Step 5: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(*tert*-butoxycarbonylamino)-5-**
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41 **(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (**67**).**
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45 To a solution of **66** (500 mg, 0.88 mmol) in 1,4-dioxane (30 mL) was added Pd(PPh₃)₄
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48 (200 mg, 0.17 mmol), HCO₂H (84 mg, 1.76 mmol) and Et₃N (177 mg, 1.76 mmol) at room
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51 temperature under N₂ atmosphere. After 20 min at 60 °C, additional HCO₂H (404 mg, 8.8
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54 mmol) was added into the reaction mixture. The reaction was stirred for additional 2 h at
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60 °C, and then quenched by H₂O (40 mL), neutralized by NaHCO₃, extracted with DCM (3 × 50 mL). The combined organic layer was concentrated and purified by a silica gel column, eluted with 10% EtOAc in petroleum ether to give **67** as a white syrup (330 mg, 71% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.30 (d, *J* = 7.2 Hz, 2H), 8.06 (d, *J* = 7.2 Hz, 2H), 7.46–7.66 (m, 5H), 6.46 (d, *J* = 7.5 Hz, 1H), 5.85 (s, 1H), 5.53 (d, *J* = 6.6 Hz, 1H), 4.71–4.79 (m, 2H), 4.62 (t, *J* = 2.1 Hz, 1H), 3.95–4.03 (m, 1H), 1.57 (s, 9H). ESI MS *m/z* 513.0 [M + H]⁺.

Step 6: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-amino-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (14). A solution of **67** (330 mg, 0.62 mmol) in MeOH (10 mL) was treated with K₂CO₃ (24 mg, 0.17 mmol) for 2 h at room temperature, then neutralized by AcOH. Volatiles were removed to give a residue, which was purified by a silica gel column, eluted with 2%–5% MeOH in DCM to afford *tert*-butyl (3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(fluoromethyl)-6,7-dihydroxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-ylcarbamate as white solid (170 mg, 85% yield). ¹H NMR (300 MHz, CDCl₃) δ 6.22 (d, *J* = 6.6 Hz, 1H), 4.67–4.79 (m, 1H), 4.57–4.66 (m, 2H), 3.84–3.96 (m, 2H), 3.70 (t, *J* = 8.4 Hz, 1H), 1.52 (s, 9H). ESI MS *m/z* 323.0 [M + H]⁺. A solution of this

material (110 mg, 0.34 mmol) in MeOH (5 mL) was bubbled with dry HCl gas until saturated at room temperature. After an additional 3 h, volatiles were removed to give a residue, which was neutralized with concentrated NH₄OH and purified by a silica gel column, eluted with 10%–20% MeOH in DCM to give **14** as a white solid (15 mg, 20% yield). ¹H NMR (300 MHz, D₂O) δ 6.31 (d, *J* = 6.3 Hz, 1H), 4.63 (d, *J* = 3.3 Hz, 1H), 4.47 (d, *J* = 3.6 Hz, 1H), 4.07 (t, *J* = 5.7 Hz, 1H), 3.93 (t, *J* = 4.8 Hz, 1H), 3.66–3.80 (m, 2H), 3.51–3.58 (m, 1H). ESI MS *m/z* 222.9 [M + H]⁺.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(allylamino)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (15). A solution of **66** (540 mg, 0.94 mmol) in MeOH (10 mL) was treated with K₂CO₃ (26 mg, 0.18 mmol) for 2 h at room temperature, then neutralized with AcOH. Volatiles were removed to give a residue, which was purified by a silica gel column, eluted with 5% MeOH in DCM to give *tert*-butyl allyl((3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(fluoromethyl)-6,7-dihydroxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)carbamate as a white solid (310 mg, 86% yield). ¹H NMR (300 MHz, CDCl₃) δ 6.10 (d, *J* = 6.9 Hz, 1H), 5.85–5.96 (m, 1H), 5.11–5.18 (m, 2H), 4.61 (d, *J* = 2.4 Hz, 1H), 4.43–4.58 (m, 3H), 4.13–4.16 (m, 1H), 4.04–4.07 (m, 1H), 3.53–3.63 (m, 2H), 1.53 (s, 9H). ESI MS

m/z 363.0 $[M + H]^+$. A solution of this material (100 mg, 0.27 mmol) in MeOH (6 mL) was bubbled with dry HCl gas at room temperature until saturated. After an additional 4 h, volatiles were removed to give a residue, which was re-dissolved into MeOH (5 mL) and neutralized with concentrated NH_4OH , concentrated and purified by a silica gel column, eluted with 5%–10% MeOH in DCM to give **15** as a white solid (42.7 mg, 58% yield). 1H NMR (300 MHz, D_2O) δ 6.29 (d, J = 6.3 Hz, 1H), 5.85–5.96 (m, 1H), 5.19–5.26 (m, 1H), 5.09–5.14 (m, 1H), 4.63 (d, J = 3.3 Hz, 1H), 4.47 (d, J = 3.6 Hz, 1H), 4.07–4.11 (m, 1H), 3.95–3.99 (m, 1H), 3.86–3.89 (m, 2H), 3.68–3.79 (m, 1H), 3.51–3.56 (m, 1H). ESI MS m/z 263.0 $[M + H]^+$.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(fluoromethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (16). Compound **16** was prepared from **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol** following the procedure used to prepare **9**. 1H NMR (400 MHz, CD_3OD) 6.44 (d, J = 6.44 Hz, 1H), 4.67–4.49 (dt, J = 47.5, 10.4, 4.2 Hz, 2H), 4.11 (t, J = 6.4 Hz, 1H), 3.91 (t, J = 6.1 Hz, 1H), 3.82–3.72 (m, 1H), 3.52–3.55 (dd, J = 6.0, 3.4 Hz, 1H), 3.27–3.21 (m, 2H), 1.66–1.57 (m, 2H), 0.95 (t, J = 7.4 Hz, 3H). ^{13}C NMR (100

MHz, CD₃OD) δ 167.82, 90.26, 84.10 (d, $J_{C6,F}$ 171.5 Hz, C-6), 76.34 (d, $J_{C5,F}$ 17.7 Hz, C-5), 75.80, 75.79, 70.05 (d, $J_{C4,F}$ 6.9 Hz, C-4), 55.67, 23.98, 12.38. ESI MS m/z 264.4 [M + H]⁺.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (17). Step 1: Preparation of **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (Exp #17a).** To a solution of **76**²⁷ (0.944 g, 3.8 mmol) in DMF (5 mL) was added imidazole (0.8 g, 12 mmol) and TBDMSCI (0.75 g, 4.97 mmol). The reaction mixture was stirred at room temperature for 18 h and concentrated using high vacuum. The crude residue was purified on silica gel by flash column chromatography (DCM/MeOH, 95:5), affording **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol** as a white solid (0.76 g, 55% yield). ¹H NMR (400 MHz, CD₃OD) δ 6.31 (d, J = 6.4 Hz, 1H), 4.06 (t, J = 6.1 Hz, 1H), 3.92–3.88 (m, 2H), 3.81–3.76 (dd, J = 11.4, 5.7 Hz, 1H), 3.62–3.58 (m, 1H), 3.52–3.48 (dd, J = 9.3, 5.5 Hz, 1H), 3.02 (s, 6H), 0.93 (s, 9H), 0.10 (s, 6H).

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-6,7-bis(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-N,N-dimethyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazol-2-amine methanol (Exp #17b). To a stirred solution of the above material (1.19 g, 3.3 mmol) in DMF (18 mL) at 0 °C was added NaH (60%, 0.5g, 13 mmol) in small portions. After 20 min, BnBr (1.17 mL, 9.9 mmol) was added and the reaction mixture was then stirred at room temperature overnight. The reaction was diluted with DCM (100 mL) and the DCM extract was washed with saturated NaHCO₃, brine, dried over anhydrous Na₂SO₄ and concentrated. The crude product was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:1), affording (3a*R*,5*R*,6*S*,7*R*,7a*R*)-6,7-bis(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-N,N-dimethyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazol-2-amine methanol as a gummy solid (0.805 g, 45% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.31–7.16 (m, 10H), 6.19 (d, *J* = 6.7 Hz, 1H), 4.72 (d, *J* = 12 Hz, 1H), 4.60 (d, *J* = 12 Hz, 1H), 4.57 (d, *J* = 11.4 Hz, 1H), 4.37–4.32 (m, 2H), 4.06–4.00 (m, 1H), 3.69–3.62 (m, 2H), 3.60–3.52 (m, 2H), 2.89 (s, 6H), 0.80 (s, 9H), 0.04 (s, 6H).

Step 3: Preparation of ((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6,7-bis(benzyloxy)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazol-5-yl)methanol (Exp #17c). To a solution of

the above material (0.80 g, 1.48 mmol) in THF (6 mL) was added 1M TBAF solution (2.25 mL, 2.25 mmol) at 0 °C and the mixture was then stirred at room temperature overnight. After diluting with EtOAc (50 mL), the organic layer was washed with saturated aqueous NH₄Cl, dried over anhydrous Na₂SO₄ and concentrated. The crude residue was purified on silica gel by flash column chromatography (100% EtOAc), affording ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-bis(benzyloxy)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methanol (0.57g, 90% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃), δ 7.38–7.28 (m, 10H), 6.29 (d, *J* = 6.9 Hz, 1H), 4.79 (d, *J* = 12.1 Hz, 1H), 4.69 (d, *J* = 12.0 Hz, 1H), 4.65 (d, *J* = 11.7 Hz, 1H), 4.55–4.53 (m, 1H), 4.43 (d, *J* = 11.5 Hz, 1H), 4.24 (m, 1H), 3.77 (d, *J* = 12.1 Hz, 1H), 3.69–3.59 (m, 3H), 3.00 (s, 6H).

Step 4: Preparation of ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-bis(benzyloxy)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methyl methanesulfonate (Exp #17d).

Methanesulfonyl chloride (0.180 mL, 2.32 mmol) was added in portions to a stirred solution of the above material (0.673 g, 1.57 mmol) in dry pyridine (8 mL) at -20 °C. After 3h, reaction mixture was diluted with DCM (20 mL) and DCM extract was washed with saturated NaHCO₃ (30 mL), brine, dried over Na₂SO₄ and concentrated. Residual

pyridine was removed by co-evaporation with hexanes. The crude product was purified by silica gel column chromatography, eluted with 100% EtOAc to give ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-bis(benzyloxy)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methyl methanesulfonate (0.610 g, 76% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) 7.32–7.18 (m, 10H), 6.15 (d, *J* = 6.48 Hz, 1H), 4.68 (d, *J* = 12 Hz, 1H), 4.57 (d, *J* = 12 Hz, 1H), 4.53 (d, *J* = 11.5 Hz, 1H), 4.48–4.45 (dd, *J* = 3.8, 2.0 Hz, 1H), 4.29 (d, *J* = 11.4 Hz, 1H), 4.24–4.16 (m, 3H), 3.73–3.69 (m, 1H), 3.53–3.50 (dt, *J* = 8.9, 1.7 Hz, 1H), 2.91 (s, 6H), 2.90 (s, 3H).

Step 5: Preparation of (3*aR*,5*S*,6*S*,7*R*,7*aR*)-6,7-bis(benzyloxy)-5-(fluoromethyl)-*N,N*-dimethyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-amine (Exp #17e). To a solution of the above material (0.7 g, 1.38 mmol) in CH₃CN (15 mL) was added a solution of TBAF (1.13 g, 7.59 mmol) in CH₃CN (5 mL) and the mixture was heated to reflux for 2.5 h. After evaporation of the solvent, the residue was purified by silica gel column chromatography (7:3 EtOAc/hexanes) to provide (3*aR*,5*S*,6*S*,7*R*,7*aR*)-6,7-bis(benzyloxy)-5-(fluoromethyl)-*N,N*-dimethyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-amine (0.567 g, 95% yield) as a foamy solid. ¹H NMR (400 MHz, CDCl₃) 7.33–7.18 (m, 10H),

6.20 (d, J = 6.5 Hz, 1H), 4.72 (d, J = 12 Hz, 1H), 4.60 (d, J = 12.6 Hz, 1H), 4.57 (d, J = 11.9 Hz, 1H), 4.46–4.41 (m, 2H), 4.34–4.30 (m, 2H), 4.14 (t, J = 3 Hz, 1H), 3.74–3.64 (m, 1H), 3.59–3.55 (m, 1H), 2.91 (s, 6H).

Step 6: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (17). To a solution of the above material (0.56 g, 1.3 mmol) in dry DCM (8 mL) at -78 °C was added a solution of BCl₃ (1.0 M in DCM, 6.5 mL, 6.5 mmol) dropwise. The mixture was stirred at -78 °C to 0 °C for 3 h. The reaction was then quenched by adding a solution of 1:1 DCM-MeOH (5 mL) at -78 °C. The mixture was slowly warmed to room temperature. Solvents were evaporated under reduced pressure. 1.3M NH₃/MeOH solution (10 mL) was added to the residue and evaporated. This was repeated one more time. The residue was purified by silica gel column chromatography, eluted with 90:10 DCM-1.3M NH₃-MeOH solution to give **17** as a white solid (0.25 g, 77% yield). ¹H NMR (400 MHz, CD₃OD) 6.31 (d, J = 6.44 Hz, 1H), 4.63–4.50 (dd, J = 44.4, 3.4 Hz, 2H), 4.09 (t, J = 6.0 Hz, 1H), 3.92 (t, J = 6.9 Hz, 1H), 3.79–3.69 (m, 1H), 3.55–3.51 (dd, J = 5.4, 4.04 Hz, 1H), 3.01 (s, 6H). ¹³C NMR (100 MHz, CD₃OD) δ 166.34, 92.58, 84.50 (d, $J_{\text{C6,F}}$ 171.2 Hz, C-6), 77.09, 76.56, 76.55, 75.72 (d,

$J_{C5,F}$ 17.7 Hz, C-5), 70.73 (d, $J_{C4,F}$ 7.12 Hz, C-4), 40.97. HRMS (ESI+) m/z [M + H]⁺ calculated for C₉H₁₅FN₂O₃S: 251.0865; found: 251.0869.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(fluoromethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (18). Compound **18** was prepared from **68** following the procedure used to prepare **9**. ¹H NMR (400 MHz, CD₃OD) 6.52 (d, J = 6.48 Hz, 1H), 4.66–4.52 (dt, J = 47.6, 10.4, 4.2 Hz, 2H), 4.15 (t, J = 6.5 Hz, 1H), 3.93 (t, J = 6.9 Hz, 1H), 3.87–3.76 (m, 1H), 3.59–3.55 (dd, J = 6.4, 3.12 Hz, 1H), 2.99 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 169.32, 90.48, 84.10 (d, $J_{C6,F}$ 171.5 Hz, C-6), 76.45 (d, $J_{C5,F}$ 17.8 Hz, C-5), 69.86 (d, $J_{C4,F}$ 6.6 Hz, C-4), 55.67, 46.07, 32.11. HRMS (ESI+) m/z [M + H]⁺ calculated for C₈H₁₃FN₂O₃S: 237.0709; found: 237.0719.

(3a*R*,5*R*,6*R*,7*R*,7a*R*)-2-(dimethylamino)-7-fluoro-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (19). Step 1: Preparation of **(3a*R*,5*R*,6*R*,7*R*,7a*R*)-7-(((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (77).** To a solution of **76**²⁷ (5.20 g, 21.0 mmol) and imidazole (12.0 g, 176 mmol) in DMF (60 mL) was added TBDMSCl (15.0 g, 99.5 mmol). The mixture was stirred

at room temperature for 24 h, and then diluted with brine (200 mL). The mixture was extracted with Et₂O (3 × 100 mL). The combined extract was washed with brine (100 mL) and water (100 mL), and then dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure. The residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:4 to 1:1), affording **77** as a white solid (5.95 g, 60% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.15 (d, *J* = 5.8 Hz, 1H), 4.34–4.33 (m, 1H), 4.20 (t, *J* = 5.0 Hz, 1H), 3.80–3.72 (m, 2H), 3.48 (s, br., 2H), 3.01 (s, 6H), 0.90 (s, 9H), 0.89 (s, 9H), 0.13 (s, 3H), 0.12 (s, 3H), 0.07 (s, 6H).

Step 2: Preparation of ((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-(benzoyloxy)-2-(dimethylamino)-7-hydroxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (78). To a solution of **77** (5.95 g, 12.5 mmol) and DMAP (0.12 g, 0.98 mmol) in pyridine (50 mL), at 0 °C, was added BzCl (3.00 g, 21.3 mmol) slowly. After addition the mixture was stirred at room temperature for 24 h. Saturated aqueous NaHCO₃ solution (100 mL) was added, and the mixture extracted with EtOAc (3 × 100 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography

(EtOAc/hexanes, 1:9 to 1:3), affording (3a*R*,5*R*,6*R*,7*R*,7a*R*)-7-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-yl benzoate as a clear oil (6.85 g, 94% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.05–8.02 (m, 2H), 7.56–7.52 (m, 1H), 7.43–7.39 (m, 2H), 6.26 (d, *J* = 6.4 Hz, 1H), 5.06–5.03 (m, 1H), 4.40 (dd, *J* = 2.2, 3.8 Hz, 1H), 3.81–3.78 (m, 1H), 3.71–3.70 (m, 2H), 3.03 (s, 6H), 0.88 (s, 9H), 0.85 (s, 9H), 0.17 (s, 3H), 0.13 (s, 3H), 0.02 (s, 3H), 0.00 (3H). To a solution of this material (9.30 g, 16.8 mmol) in dry MeOH (100 mL), was bubbled HCl for 2 min, and the mixture was then stirred at room temperature for 2 h. After concentrated under reduced pressure the mixture was treated with saturated aqueous NaHCO₃ solution (150 mL), and then extracted with EtOAc (6 × 80 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, affording (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-7-hydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-yl benzoate as a white crystalline solid (5.40 g, 96% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.03–8.01 (m, 2H), 7.58–7.53 (m, 1H), 7.44–7.39 (m, 2H), 6.37 (d, *J* = 4.5 Hz, 1H), 5.12–5.09 (m, 1H), 4.41–4.37 (m, 2H), 3.94–3.85 (m, 1H), 3.78–3.74 (m, 1H), 3.67

(dd, J = 4.9, 12.5 Hz, 1H), 3.00 (s, 6H). To a solution of this material (5.35 g, 15.2 mmol) and DMAP (0.050 g, 0.41 mmol) in pyridine (50 mL), at 0 °C, was added BzCl (2.22 g, 15.8 mmol) slowly. After addition the mixture was stirred at room temperature for 4 h. Saturated aqueous NaHCO₃ solution (100 mL) was added, and the mixture was extracted with EtOAc (2 × 50 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:1 to 10:1), affording **78** as a white solid (4.45 g, 64% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.04–8.00 (m, 4H), 7.58–7.51 (m, 2H), 7.44–7.37 (m, 4H), 6.38 (d, J = 6.6 Hz, 1H), 5.23–5.20 (m, 1H), 4.55 (dd, J = 3.2, 12.0 Hz, 1H) 4.48–4.40 (m, 3H), 4.27–4.22 (m, 1H), 3.00 (s, 6H).

Step 3: Preparation of (3a*R*,5*R*,6*R*,7*R*,7a*R*)-2-(dimethylamino)-7-fluoro-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (19). To a solution of **78** (0.410 g, 0.898 mmol) in anhydrous DCM (6 mL), at -78 °C under N₂, was added DAST (0.87 g, 5.4 mmol). After addition the mixture was stirred at room temperature for 16 h. The reaction mixture was then cooled at -78 °C, diluted with DCM (20 mL), and quenched by adding saturated aqueous NaHCO₃ (20 mL). The organic layer was collected, and the

aqueous was extracted with DCM (2 × 30 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:3 to 1:2), affording ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-6-(benzoyloxy)-2-(dimethylamino)-7-fluoro-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate) as a white solid (0.380 g, 92% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.03–8.00 (m, 4H), 7.58–7.55 (m, 1H), 7.54–7.51 (m, 1H), 7.44–7.41 (m, 2H), 7.39–7.36 (m, 2H), 6.35 (d, *J* = 5.4 Hz, 1H), 5.47 (dd, *J* = 7.3, 16.3 Hz, 1H), 5.23 (ddd, *J* = 1.1, 2.8, 35.2 Hz, 1H), 4.70–4.66 (m, 1H), 4.52 (dd, *J* = 2.8, 9.6 Hz), 4.40 (dd, *J* = 4.7, 9.6 Hz, 1H), 4.14–4.10 (m, 1H), 3.05 (s, 6H). A mixture of this material (0.375 g, 0.818 mmol) and K₂CO₃ (0.113 g, 0.818 mmol) in anhydrous MeOH (8 mL) was stirred at room temperature for 2 h. The reaction mixture was neutralized with dry ice, and the solvent was then removed under reduced pressure. The residue was purified on silica gel by flash column chromatography (1.0 M NH₃ in MeOH/DCM, 1:12), affording **19** as a white solid (0.190 g, 93% yield). ¹H NMR (400 MHz, CD₃OD) δ 6.35 (d, *J* = 6.7 Hz, 1H), 4.74 (dt, *J* = 48.1, 5.0 Hz, 1H), 4.31 (dt, *J* = 13.8, 6.0 Hz, 1H), 3.79 (dd, *J* = 2.0, 12.0 Hz, 1H), 3.76–3.69 (m,

1H), 3.66 (dd, J = 5.8, 12.0 Hz), 3.61–3.57 (m, 1H), 3.01 (s, 6H); ^{13}C NMR (100 MHz, CD_3OD) δ 166.25, 96.36 (d, J = 176.8 Hz), 91.60 (d, J = 3.9 Hz), 75.45 (d, J = 4.6 Hz), 73.99 (d, J = 24.7 Hz), 68.98 (d, J = 22.9 Hz), 62.91, 40.33. ESI MS m/z 251.1 $[\text{M} + \text{H}]^+$.

(3a*R*,5*R*,6*R*,7*R*,7a*R*)-7-fluoro-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (20). Compound 20 was prepared from (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol^{14,27} following the procedure used to prepare 11. ^1H NMR (400 MHz, CD_3OD) δ 6.31 (d, J = 6.4 Hz, 1H), 4.74 (dt, J = 48.2, 4.9 Hz, 1H), 4.31–4.24 (m, 1H), 3.81–3.58 (m, 4H), 3.24–3.15 (m, 2H), 1.60–1.53 (m, 2H), 0.94 (t, J = 7.4 Hz, 3H); ^{13}C NMR (100 MHz, CD_3OD) δ 163.93, 96.41 (d, J = 177.7 Hz), 90.64 (d, J = 4.0 Hz), 75.38 (d, J = 4.5 Hz), 73.63 (d, J = 24.4 Hz), 69.03 (d, J = 22.8 Hz), 62.96, 46.72, 23.66, 11.53. ESI MS m/z 265.2 $[\text{M} + \text{H}]^+$. HRMS (ESI+) m/z $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{10}\text{H}_{18}\text{FN}_2\text{O}_3\text{S}$: 265.1022; found: 265.1026.

(3a*R*,5*R*,6*R*,7*R*,7a*R*)-7-fluoro-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (21). Step 1: Preparation of ((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(methyl)amino)-7-hydroxy-5,6,7,7a-tetrahydro-

3aH-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (69). To a suspension of **68**²⁷ (8.50 g, 37.0 mmol) in DMF (60 mL) was added DIPEA (2.0 mL), Boc₂O (23.0 g, 105 mmol), and MeOH (2.0 mL). The mixture was stirred at room temperature for 3 h, then MeOH (50 mL) was added. The mixture was concentrated in vacuo at 35 °C. The residue was purified by flash chromatography (SiO₂; MeOH/DCM 1:8), followed by re-crystallization from EtOAc/hexanes, to afford *tert*-butyl ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (11.8 g, 96% yield) as a white solid. ¹H NMR (400 MHz, CDCl₃) δ 6.14 (d, *J* = 6.9 Hz, 1H), 4.20 (d, *J* = 6.4 Hz, 1H), 4.11 (d, *J* = 5.6 Hz, 1H), 3.85–3.70 (m, 2H), 3.63–3.55 (m, 1H), 3.31 (s, 3H), 1.53 (s, 9H). To a solution of this material (11.7 g, 35.1 mmol), DIPEA (10.3 g, 80.0 mmol), and DMAP (0.040 g, 0.33 mmol) in DCM (180 mL) at 0 °C was slowly added BzCl (10.1 g, 72.0 mmol). The mixture was stirred at room temperature for 5 h. Saturated aqueous NH₄Cl solution (100 mL) was added and the organic layer was collected. The aqueous layer was extracted with DCM (3 × 50 mL). The combined organic extract was dried over anhydrous Na₂SO₄, concentrated in vacuo, and the residue was separated by flash chromatography (SiO₂; EtOAc/hexanes 1:4 to 1:1), affording **69** (4.20

g, 22% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 8.01–7.99 (m, 4H), 7.60–7.55 (m, 1H), 7.54–7.50 (m, 1H), 7.45–7.41 (m, 2H), 7.37–7.35 (m, 2H), 6.21 (d, J = 7.1 Hz, 1H), 5.23–5.20 (m, 1H), 4.55–4.51 (m, 2H), 4.48–4.42 (m, 2H), 4.15–4.07 (m, 2H), 3.36 (s, 3H), 1.56 (s, 9H).

Step 2: Preparation of ((3a*R*,5*R*,6*R*,7*R*,7a*R*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(methyl)amino)-7-fluoro-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (70). To a solution of **69** (7.91 g, 14.6 mmol) in anhydrous DCM (100 mL) at -78 °C was added DAST (11.8 g, 73.0 mmol). The mixture was stirred at room temperature for 72 h. The reaction was then cooled to -78 °C, diluted with DCM (100 mL), and quenched with saturated aqueous NaHCO_3 (150 mL). The organic layer was collected, and the aqueous was extracted with DCM (2 × 100 mL). The combined organic extract was dried over anhydrous Na_2SO_4 , concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2 ; EtOAc/hexanes 1:10 to 1:4), affording **70** (6.10 g, 77% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 8.01–7.98 (m, 4H), 7.60–7.56 (m, 1H), 7.56–7.52 (m, 1H), 7.45–7.41 (m, 2H), 7.38–7.35 (m, 2H), 6.19 (d, J = 7.2 Hz,

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4 1H), 5.52–5.46 (m, 1H), 5.40–5.28 (m, 1H), 4.61–4.56 (m, 1H), 4.52 (dd, J = 3.6, 12.0 Hz,
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7 1H), 4.43 (dd, J = 5.7, 12.0 Hz, 1H), 4.03–3.99 (m, 1H), 3.36 (s, 3H), 1.56 (s, 9H).
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11 **Step 3: Preparation of *tert*-butyl ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-7-fluoro-6-hydroxy-5-**
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13 **(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate**
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15 **(71).** A mixture of **70** (6.10 g, 11.2 mmol) and K₂CO₃ (1.00 g, 7.25 mmol) in anhydrous
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17 MeOH (50 mL) was stirred at room temperature for 3 h. Dry ice was added, and the
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19 solvent was removed in vacuo. The residue was purified by flash chromatography (SiO₂;
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21 EtOAc/hexanes 1:1 to 10:1), affording **71** (3.25 g, 86% yield) as a white solid. ¹H NMR
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23 (400 MHz, CDCl₃) δ 6.06 (d, J = 6.8 Hz, 1H), 5.15 (ddd, J = 2.4, 4.4, 45.7 Hz, 1H), 4.46–
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25 4.41 (m, 1H), 3.96–3.89 (m, 1H), 3.83 (dd, J = 3.2, 11.8 Hz, 1H), 3.73 (dd, J = 5.4, 11.8
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27 Hz, 1H), 3.46–3.42 (m, 1H), 3.32 (s, 3H), 1.54 (s, 9H).
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42 **Step 4: Preparation of (3*aR*,5*R*,6*R*,7*R*,7*aR*)-7-fluoro-5-(hydroxymethyl)-2-**
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44 **(methylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (21).** To a solution of **71**
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46 (0.490 g, 1.46 mmol) in dry MeOH (20 mL) was bubbled HCl (g) for 30 sec. The solution
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48 was then stirred at room temperature for 3 h. The solvent was removed in vacuo and the
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50 residue was neutralized with 1.0 M NH₃ in MeOH. The mixture was concentrated in vacuo
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and the residue was purified by flash chromatography (SiO₂; 1.0 M NH₃ in MeOH/DCM 1:8), affording **21** (0.328 g, 95% yield) as a white foam. ¹H NMR (500 MHz, CD₃OD) δ 6.33 (d, *J* = 6.5 Hz, 1H), 4.75 (td, *J* = 5.1, 48.2 Hz, 1H), 4.29 (td, *J* = 6.0, 13.9 Hz, 1H), 3.81–3.57 (m, 4H), 2.84 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 164.65, 96.42 (d, *J* = 178.9 Hz), 91.01 (d, *J* = 4.0 Hz), 75.37 (d, *J* = 4.7 Hz), 73.69 (d, *J* = 24.8 Hz), 69.02 (d, *J* = 22.9 Hz), 62.92, 30.54. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₈H₁₃FN₂O₃S: 237.0709; found: 237.0702.

(3*aR*,5*R*,6*R*,7*S*,7*aR*)-7-fluoro-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (**22**). Step 1: Preparation of *tert*-butyl ((3*aR*,5*R*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-fluoro-6-oxo-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (**72**). To a solution of **71** (18.7 g, 55.5 mmol) and imidazole (11.4 g, 167 mmol) in anhydrous DMF (150 mL) at 0 °C was added TBDMSCl (9.19 g, 61.0 mmol). The mixture was stirred at room temperature for 16 h, then diluted with Et₂O (300 mL) and washed with brine (2 × 300 mL). The organic layer was collected, and the aqueous was extracted with Et₂O (2 × 150 mL). The combined organic extract was washed with brine (100 mL), dried over anhydrous Na₂SO₄, concentrated in vacuo,

and the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:10 to 1:3), affording *tert*-butyl ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-fluoro-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (24.1 g, 96% yield) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 6.06 (d, *J* = 6.8 Hz, 1H), 5.19–5.02 (m, 1H), 4.43–4.38 (m, 1H), 3.98–3.93 (m, 1H), 3.85 (dd, *J* = 5.0, 10.6 Hz, 1H), 3.73 (dd, *J* = 5.2, 10.6 Hz, 1H), 3.45–3.43 (m, 1H), 3.34 (s, 3H), 1.54 (s, 9H), 0.89 (s, 9H), 0.08 (s, 6H). To a solution of this material (9.28 g, 20.6 mmol) in DCM (150 mL) was added DMP (13.1 g, 30.9 mmol). After stirring at room temperature for 1 h the reaction was diluted with Et₂O (400 mL). The resulting suspension was filtered through a Celite plug and the filtrate was concentrated in vacuo. The residue was loaded onto a layered NaHCO₃/silica gel plug, and eluted with EtOAc/hexanes 1:4, affording **72** (8.96 g, 97% yield) as a white crystalline solid. ¹H NMR (400 MHz, CDCl₃) δ 6.29 (d, *J* = 7.0 Hz, 1H), 5.09 (dd, *J* = 4.7, 48.4 Hz, 1H), 4.75–4.69 (m, 1H), 4.12–4.05 (m, 2H), 3.96–3.93 (m, 1H), 3.28 (s, 3H), 1.54 (s, 9H), 0.86 (s, 9H), 0.056 (s, 3H), 0.050 (s, 3H).

Step 2: Preparation of *tert*-butyl ((3*aR*,5*R*,6*R*,7*S*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-fluoro-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-

d[thiazol-2-yl](methyl)carbamate (**73**). To a solution of **72** (8.96 g, 20.0 mmol) in dry MeOH (250 mL) was added NaH (60% in mineral oil, 0.158 g, 3.95 mmol), and the mixture was stirred at room temperature for 15 min. The mixture was cooled to 0 °C and NaBH₄ (1.32 g, 34.9 mmol) was added. After stirring at 0 °C for 20 min, a chip of dry ice was added and the solvent was evaporated in vacuo. The residue was dissolved in DCM (100 mL) and washed with saturated aqueous NH₄Cl (100 mL). The organic layer was collected, and the aqueous was extracted with DCM (2 × 50 mL). The combined organic extract was dried over anhydrous Na₂SO₄, concentrated in vacuo, and the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:10 to 2:5), affording **73** (6.84 g, 76% yield) as a white foam. ¹H NMR (400 MHz, CDCl₃) δ 6.06 (d, *J* = 6.7 Hz, 1H), 5.01 (td, *J* = 4.3, 46.8 Hz, 1H), 4.49–4.44 (m, 1H), 4.17–4.13 (m, 1H), 3.80–3.79 (m, 2H), 3.66–3.63 (m, 1H), 3.38 (s, 3H), 2.72 (s, br., 1H, (OH)), 1.54 (s, 9H), 0.89 (s, 9H), 0.062 (s, 3H), 0.057 (s, 3H).

Step 3: Preparation of (3a*R*,5*R*,6*R*,7*S*,7a*R*)-7-fluoro-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (22**).** To a solution of **73** (0.200 g, 0.444 mmol) in dry MeOH (6 mL) was bubbled HCl gas for 30 sec. The mixture

was stirred at room temperature for 5 h. After the solvent was evaporated in vacuo, the residue was neutralized with 1.0 M NH_3 in MeOH and purified by flash chromatography (SiO_2 ; 1.0 M NH_3 in MeOH/DCM 1:8) followed by recrystallization from MeOH/ Et_2O , affording **22** (0.093 g, 89% yield) as a white solid. ^1H NMR (400 MHz, CD_3OD) δ 6.62 (d, J = 6.5 Hz, 1H), 4.89 (td, J = 3.5 Hz, 51.0 Hz, 1H), 4.54 (ddd, J = 3.5, 6.5, 20.6, 1H), 3.98–3.94 (m, 1H), 3.90–3.84 (m, 1H), 3.83–3.81 (m, 1H), 3.75 (dd, J = 5.4, 12.2 Hz, 1H), 2.99 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.62, 60.03, 60.78, 64.06, 72.28, 86.11, 88.82 (d, J = 184 Hz), 157.27. HRMS (ESI+) m/z $[\text{M} + \text{H}]^+$ calculated for $\text{C}_8\text{H}_{13}\text{FN}_2\text{O}_3\text{S}$: 237.0709; found: 237.0718.

(3a*R*,5*R*,6*S*,7a*R*)-2-amino-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazol-6-ol (23). Compound **23** was prepared from (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-amino-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazole-6,7-diol following the procedure used to prepare **8**. ^1H NMR (400 MHz, CD_3OD) δ 6.44 (d, J = 6.9 Hz, 1H), 4.56–4.52 (m, 1H), 3.90–3.85 (m, 1H), 3.77 (dd, J = 2.5, 12.1 Hz, 1H), 3.63 (dd, J = 6.4, 12.1 Hz, 1H), 3.54–3.50 (m, 1H), 2.18–2.10 (m, 2H); ^{13}C NMR (100 MHz, CD_3OD) δ 172.80, 87.98, 77.53, 63.91, 63.25, 59.96, 32.33. ESI MS m/z 205 $[\text{M} + \text{H}]^+$.

(3a*R*,5*R*,6*S*,7a*R*)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-

pyrano[3,2-*d*]thiazol-6-ol (24). Compound **24** was prepared from (3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol following the procedure used to prepare **8**. ¹H NMR (400 MHz, CD₃OD) δ 6.18 (d, *J* = 6.3 Hz, 1H), 4.26–4.22 (m, 1H), 3.74 (dd, *J* = 2.1, 11.8 Hz, 1H), 3.71–3.66 (m, 1H), 3.59 (dd, *J* = 6.3, 11.8 Hz, 1H), 3.54–3.49 (m, 1H), 3.21–3.12 (m, 2H), 2.14–2.08 (m, 1H), 2.04–1.99 (m, 1H), 1.59–1.53 (m, 2H), 0.93 (t, *J* = 7.3 Hz, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 163.32, 91.40, 77.17, 69.28, 65.94, 63.36, 46.81, 34.81, 23.67, 11.72. ESI MS *m/z* 269 [M + Na]⁺.

(3a*R*,5*R*,6*S*,7a*R*)-5-(hydroxymethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-

pyrano[3,2-*d*]thiazol-6-ol (25). A suspension of **76**²⁷ (0.800 g, 3.23 mmol) and anhydrous ZnCl₂ (1.00 g, 7.35 mmol) in benzaldehyde (5 mL) was stirred at room temperature overnight. DCM (50 mL) and saturated aqueous NaHCO₃ (50 mL) was added, and mixture was stirred for 20 min. The solid was filtered off, and the organic layer was collected from the filtrate. The aqueous layer was extracted with DCM (2 × 50 mL), and the combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was

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4 evaporated under reduced pressure, and the residue was purified on silica gel by flash
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7 column chromatography (1.0 M NH_3 in MeOH/DCM, 3:100), affording
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11 *((3aR,4aR,8aS,9R,9aR)-2-(dimethylamino)-7-phenyl-3a,4a,5,8a,9,9a-hexahydro-*
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14 *[1,3]dioxino[4',5':5,6]pyrano[3,2-d]thiazol-9-ol* as a pale yellow solid (0.76 g, 70% yield).
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17 ^1H NMR (400 MHz, CDCl_3) δ 7.51–7.48 (m, 2H), 7.40–7.34 (m, 3H), 6.39 (d, J = 6.6 Hz,
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20 1H), 5.58 (s, 1H), 4.31 (dd, J = 5.1, 10.4 Hz, 1H), 4.10–4.03 (m, 2H), 3.86 (dd, J = 8.2,
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23 9.5 Hz, 1H), 3.76 (t, J = 10.4 Hz, 1H), 3.59 (t, J = 9.5 Hz, 1H), 3.00 (s, 6H). A mixture of
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26 this material (0.67 g, 2.0 mmol) and thio-CDI (90% tech, 1.6 g, 8.0 mmol) in anhydrous
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29 DMF (20 mL) was stirred at 95 °C overnight. After cooling the solvent was removed under
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32 reduced pressure, and the residue was purified on silica gel by flash column
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35 chromatography (MeOH/DCM, 1:100 to 5:100), affording a mixture containing O-
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38 *((3aR,4aR,8aS,9R,9aR)-2-(dimethylamino)-7-phenyl-3a,4a,5,8a,9,9a-hexahydro-*
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41 *[1,3]dioxino[4',5':5,6]pyrano[3,2-d]thiazol-9-yl) 1*H*-imidazole-1-carbothioate*. This mixture
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48 was dissolved in anhydrous THF (30 mL), and Bu_3SnH (1.0 g, 3.4 mmol) and 1,1'-
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51 azobis(cyclohexanecarbonitrile) (ABCN) (0.060 mg, 0.24 mmol) was added, and the
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56 mixture was stirred at reflux overnight. The solvent was then removed under reduced
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pressure, and the residue was purified on silica gel by flash column chromatography (1.0 M NH₃ in MeOH:DCM, 3:100), affording an pale yellow solid containing (3a*R*,4a*R*,8a*S*,9a*R*)-*N,N*-dimethyl-7-phenyl-3a,4a,5,8a,9,9a-hexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-2-amine (0.17 g, impure). The solid was treated with 2% HCl in MeOH (10 mL) at room temperature for 2 h, and then the solvent was removed under reduced pressure. The residue was neutralized with 1.0 M NH₃ in MeOH, and subsequently purified on silica gel by flash column chromatography (1.0 M NH₃ in MeOH:DCM, 1:10), affording **25** as an off-white solid (0.050 g, 11% overall yield). ¹H NMR (400 MHz, CD₃OD) δ 6.23 (d, *J* = 6.4 Hz, 1H), 4.28–4.24 (m, 1H), 3.74 (dd, *J* = 2.5, 12.0 Hz, 1H), 3.70–3.65 (m, 1H), 3.59 (dd, *J* = 6.2, 12.0 Hz, 1H), 3.53–3.48 (m, 1H), 2.99 (s, 6H), 2.14–2.08 (m, 1H), 2.02–1.95 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 165.60, 92.43, 77.19, 69.67, 65.79, 63.27, 40.15, 34.93. ESI MS *m/z* 255 [M + Na]⁺. HRMS (ESI⁺) *m/z* [M + H]⁺ calculated for C₉H₁₇N₂O₃S: 233.0960; found: 233.0963.

(3a*R*,5*R*,6*S*,7a*R*)-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (26). Step 1: Preparation of benzyl ((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-

yl)(methyl)carbamate (Exp #26a). To a suspension of **68** (1.50 g, 6.40 mmol) and NaHCO₃ (1.01 g, 12.0 mmol) in THF/water (40 mL, 1 :3) was added benzyl chloroformate (1.70 g, 10.0 mmol). The mixture was stirred at room temperature for 4 days. The organic solvent was removed under reduced pressure to result in a precipitate. The precipitated solid was collected by filtration, washed with Et₂O and dried under vacuum, affording benzyl ((3*aR*,5*R*,6*S*,7*R*,7*aR*)-6,7-dihydroxy-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate as a white solid (1.72 g, 73% yield). ¹H NMR (400 MHz, CD₃OD) δ 7.44–7.32 (m, 5H), 6.13 (d, *J* = 6.9 Hz, 1H), 5.27–5.20 (m, 2H), 4.14 (t, *J* = 6.1 Hz, 1H), 4.02 (t, *J* = 4.9 Hz, 1H), 3.74 (dd, *J* = 2.4, 12.0 Hz, 1H), 3.63 (dd, *J* = 6.1, 12.0 Hz, 1H), 3.53 (dd, *J* = 4.5, 9.2 Hz, 1H), 3.47–3.43 (m, 1H), 3.39 (s, 3H).

Step 2: Preparation of benzyl ((3*aR*,4*aR*,8*aS*,9*R*,9*aR*)-9-hydroxy-7-phenyl-3*a*,4*a*,5,8*a*,9,9*a*-hexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #26b). A mixture of the above material (1.50 g, 4.08 mmol), benzaldehyde dimethyl acetal (3 mL) and *p*-toluenesulfonic acid monohydrate (0.058 g, 0.30 mmol) in DMF (10 mL) was stirred at 50 °C under a slight vacuum overnight. After cooling the solution was neutralized with solid NaHCO₃, and then concentrated under

reduced pressure. The residue was purified on silica gel by flash column chromatography (EtOAc/DCM/hexanes, 2:4:1), affording benzyl ((3*aR*,4*aR*,8*aS*,9*R*,9*aR*)-9-hydroxy-7-phenyl-3*a*,4*a*,5,8*a*,9,9*a*-hexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate as a white solid (0.91 g, 53% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.49–7.47 (m, 2H), 7.40–7.35 (m, 8H), 6.28 (d, *J* = 7.5 Hz, 1H), 5.78 (s, 1H), 5.29–5.21 (m, 2H), 4.34 (dd, *J* = 5.1, 10.4 Hz, 1H), 4.15–4.06 (m, 2H), 3.89–3.84 (m, 1H), 3.76 (t, *J* = 10.4 Hz, 1H), 3.58 (t, *J* = 9.4, 1H), 3.38 (s, 3H), 2.62 (d, *J* = 2.4 Hz, 1H).

Step 3: Preparation of O-((3*aR*,4*aR*,8*aS*,9*R*,9*aR*)-2-(((benzyloxy)carbonyl)(methyl)amino)-7-phenyl-3*a*,4*a*,5,8*a*,9,9*a*-hexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-9-yl) 1*H*-imidazole-1-carbothioate (Exp #26c).

A mixture of the above material (0.700 g, 1.54 mmol) and thio-CDI (90% tech, 0.80 g, 4.04 mmol) in anhydrous DMF (20 mL) was stirred at 90 °C overnight. After cooling the solvent was removed under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/DCM, 2:1), affording O-((3*aR*,4*aR*,8*aS*,9*R*,9*aR*)-2-(((benzyloxy)carbonyl)(methyl)amino)-7-phenyl-3*a*,4*a*,5,8*a*,9,9*a*-hexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-9-yl) 1*H*-imidazole-

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4 1-carbothioate as a yellow solid (0.71 g, 81% yield). ^1H NMR (400 MHz, CDCl_3) δ 8.36 (s,
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7 1H), 7.66 (s, 1H), 7.42–7.37 (m, 7H), 7.35–7.32 (m, 3H), 7.03 (s, 1H), 6.28 (d, J = 7.2 Hz,
8
9
10 1H), 6.20 (dd, J = 7.1, 8.6 Hz, 1H), 5.56 (s, 1H), 5.26 (s, 2H), 4.43 (t, J = 7.1 Hz, 1H), 4.39
11
12
13 (dd, J = 5.1, 10.4 Hz, 1H), 4.19–4.13 (m, 1H), 3.93 (t, J = 9.0 Hz, 1H), 3.79 (t, J = 10.4
14
15
16 Hz, 1H), 3.32 (s, 3H).

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21 **Step 4: Preparation of benzyl methyl((3a*R*,4a*R*,8a*S*,9a*R*)-7-phenyl-3a,4a,5,8a,9,9a-**
22
23
24 **hexahydro-[1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-2-yl)carbamate (Exp #26d).** A
25
26
27 mixture of the above material (0.560 g, 1.00 mmol), Bu_3SnH (0.87 g, 3.0 mmol) and ABCN
28
29
30 (24 mg, 0.10 mmol) in anhydrous THF (20 mL) was stirred at reflux for 3 h. After cooling
31
32
33
34 the solvent was removed under reduced pressure, and the residue was purified on silica
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36
37
38 gel by flash column chromatography (EtOAc/hexanes/DCM, 1:3:1), affording benzyl
39
40
41
42 methyl((3a*R*,4a*R*,8a*S*,9a*R*)-7-phenyl-3a,4a,5,8a,9,9a-hexahydro-
43
44
45 [1,3]dioxino[4',5':5,6]pyrano[3,2-*d*]thiazol-2-yl)carbamate as a white solid (0.32 g, 73%
46
47
48
49 yield). ^1H NMR (400 MHz, CDCl_3) δ 7.49–7.47 (m, 2H), 7.39–7.33 (m, 8H), 6.21 (d, J =
50
51
52 6.5 Hz, 1H), 5.56 (s, 1H), 5.29–5.20 (m, 2H), 4.52–4.25 (m, 2H), 4.08–4.02 (m, 1H), 3.72
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(t, J = 10.3 Hz, 1H), 3.61–3.54 (m, 1H), 3.33 (s, 3H), 2.41–2.35 (m, 1H), 1.76–1.68 (m, 1H).

Step 5: Preparation of (3a*R*,5*R*,6*S*,7a*R*)-5-(hydroxymethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (26). To a solution of the above material (0.20 g, 0.45 mmol) in anhydrous DCM (10 mL), cooled at -78 °C under N₂, was added boron trichloride in DCM (1.0 M, 2.0 mL, 2.0 mmol). The mixture was stirred for 3 h while the reaction temperature was gradually brought to ~ 0 °C. The mixture was then cooled at -78 °C again, and MeOH (5 mL) was added carefully. After stirring at room temperature for 30 min the mixture was concentrated under reduced pressure. The residue was neutralized with 1.0 M NH₃ in MeOH, and subsequently purified on silica gel by flash column chromatography (1.0 M NH₃ in MeOH:DCM, 1:7), affording **26** as an off-white solid (0.070 g, 72% yield). ¹H NMR (400 MHz, CD₃OD) δ 6.20 (d, J = 6.4 Hz, 1H), 4.27–4.23 (m, 1H), 3.74 (dd, J = 2.5, 12.0 Hz, 1H), 3.71–3.67 (m, 1H), 3.63 (dd, J = 6.2, 12.0 Hz, 1H), 3.54–3.49 (m, 1H), 2.83 (s, 3H), 2.16–2.10 (m, 1H), 2.04–1.97 (m, 1H); ¹³C NMR (100 MHz, CD₃OD) δ 163.99, 91.81, 77.11, 69.31, 65.82, 63.32, 34.93, 30.49. ESI MS

m/z 219 $[M + H]^+$. HRMS (ESI+) m/z $[M + H]^+$ calculated for $C_8H_{15}N_2O_3S$: 219.0803; found: 219.0806.

(3*aR*,5*S*,6*R*,7*R*,7*aR*)-7-fluoro-5-(fluoromethyl)-2-(methylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (27). Step 1: Preparation of *tert*-butyl ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-7-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (83). To a suspension of 68 (80.00 g, 341.6 mmol) in DMF (500 mL) was added DIPEA (8 mL), MeOH (8 mL) and Boc_2O (100.0 g, 458.7 mmol) in sequence. The suspension was stirred at room temperature for 6 h and became a clear solution. After the volume of the solution was reduced under reduced pressure at room temperature by around 100 mL to remove MeOH and *tert*-butanol, the solution was cooled with an ice cooling bath, and imidazole (92.9 g, 1.36 mol), and TBDMSCl (155 g, 1.03 mol) was added in sequence. After stirring at room temperature overnight the mixture was diluted with brine (1.5 L). Extraction was performed with Et_2O three times (500 mL and 2 × 300 mL). The combined ether extract was washed with brine (1 L) and dried over anhydrous Na_2SO_4 . After filtration the solvent was evaporated under reduced pressure,

and the residue of the two batches was combined and purified on silica gel by flash column chromatography (EtOAc/hexanes, 2:11) to afford **83** as a white crystalline solid (136 g, 71% yield). ¹H NMR (500 MHz, CDCl₃) δ 6.15 (d, *J* = 6.2 Hz, 1H), 4.29 (s, br. 1H), 4.06–4.05 (m, 1H), 3.81–3.75 (m, 2H), 3.71–3.68 (m, 1H), 3.47–3.43 (m, 1H), 3.36 (s, 3H), 1.54 (s, 9H), 0.915 (s, 9H), 0.893 (s, 9H), 0.161 (s, 3H), 0.149 (s, 3H), 0.068 (s, 6H).

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-((*tert*-butoxycarbonyl)(methyl)amino)-7-hydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-yl benzoate (84**).** At 0 °C, to a solution of **83** (94.30 g, 167.5 mmol) and DMAP (0.50 g, 4.1 mmol) in pyridine (350 mL) was added BzCl (28.3 g, 201 mmol). The mixture was stirred at room temperature overnight, another portion of BzCl (6.90 g, 49.1 mmol) was added and the mixture was stirred at room temperature for another 3 h. MeOH (20 mL) was added and the mixture was stirred for another 30 min. The mixture was diluted with brine (1 L) and extracted with mixed EtOAc/hexanes three times (1:4; 600 mL, 200 mL and 100 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the combined residue of all three batches was treated with 1.5 M HCl in MeOH (1.0 L) at room temperature for 16 h. The solvent was

then removed at room temperature under reduced pressure to afford a white solid. To the white solid in DCM (1.6 h) was added DIPEA (100 mL) and Boc₂O (109 g, 500 mmol) in sequence. The mixture was then stirred at room temperature for 4 days. The reaction mixture was washed with saturated aqueous NH₄Cl (1 L) and brine (1 L) and dried over anhydrous Na₂SO₄. The combined aqueous washing was basified to pH = ~9 with saturated aqueous Na₂CO₃ solution and extracted with DCM (5 × 150 mL). The combined DCM extracts were dried over anhydrous Na₂SO₄. After filtration the solution was treated with Boc₂O (30 g) for 5 h. After washed with saturated aqueous NH₄Cl (1 L) and brine (1 L) and dried over anhydrous Na₂SO₄. All DCM extracts were combined. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:4 to 3:2) to afford **84** as a white solid (56.5 g, 77% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.00–7.98 (m, 2H), 7.59–7.54 (m, 1H), 7.44–7.40 (m, 2H), 6.20 (d, *J* = 7.0 Hz, 1H), 5.15–5.12 (m, 1H), 4.55–4.50 (m, 1H), 4.41–4.39 (m, 1H), 3.80–3.76 (m, 1H), 3.70–3.66 (m, 1H), 3.49 (s, br., 1H), 3.34 (s, 3H), 1.56 (s, 9H).

Step 3: Preparation of (3a*R*,5*S*,6*R*,7*R*,7a*R*)-2-(*tert*-butoxycarbonyl(methyl)amino)-7-fluoro-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-yl benzoate (85**).**

To a solution of **84** (500 mg, 1.1 mmol) in DCM (15 mL) was added DAST (1.1 g, 6.8 mmol) at -78 °C under nitrogen atmosphere. After stirring overnight at room temperature, the reaction mixture was quenched with saturated aqueous NaHCO₃ (30 mL) solution, extracted with DCM (3 × 15 mL), dried over anhydrous Na₂SO₄, and condensed to give a residue, which was purified by a silica gel column, eluted with 10%–30% EtOAc in petroleum ether to afford **85** (370 mg, 73% yield) as light yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 8.02–8.01 (m, 2H), 7.64–7.62 (m, 1H), 7.59–7.44 (m, 2H), 6.19 (d, *J* = 7.2 Hz, 1H), 5.44–5.26 (m, 2H), 4.62–4.60 (m, 2H), 4.58–4.46 (m, 1H), 3.90–3.80 (m, 1H), 3.39 (s, 3H), 1.58 (s, 9H). ESI MS *m/z* 443.0 [M + H]⁺.

Step 4: Preparation of (3a*R*,5*S*,6*R*,7*R*,7a*R*)-7-fluoro-5-(fluoromethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (27**).** A solution of **85** (170 mg, 0.4 mmol) in THF (10 mL) was treated with MeMgCl (3 mmol, 1 mL, 3 M in THF) for 1 h at room temperature. The reaction mixture was quenched with saturated aqueous NH₄Cl (30 mL) solution, extracted with EtOAc (3 × 20 mL), washed with brine (10 mL), dried over

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4 anhydrous Na₂SO₄, and condensed to give a residue, which was purified by a silica gel
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7 column, eluted with 2%–5% MeOH in DCM to afford **27** as a white solid (35 mg, 38%
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10 yield). ¹H NMR (300 MHz, CDCl₃) δ 6.27 (d, *J* = 6.3 Hz, 1H), 5.14 (td, *J* = 45.6 Hz, 2.1 Hz,
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12
13 1H), 4.70–4.44 (m, 3H), 3.90–3.79 (m, 1H), 3.73–3.61 (m, 1H), 2.95 (s, 3H). ESI MS *m/z*
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17 239.0 [M + H]⁺.
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21 **(3a*R*,5*S*,6*R*,7*S*,7a*R*)-7-fluoro-5-(fluoromethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-**
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24 **3a*H*-pyrano[3,2-*d*]thiazol-6-ol (28).** To a stirred solution of **74** (330 mg, 0.77 mmol) in
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27 DCM (20 mL) at -78 °C was added DAST (625 mg, 3.88 mmol) dropwise over 10 min.
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29
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31 The resulting solution was stirred for 20 min at -78 °C and then for an additional 4 h at
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34 room temperature. The reaction was cooled to 0 °C and quenched by the addition of 30
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36
37 mL saturated aqueous NaHCO₃ solution. The resulting mixture was extracted with DCM
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41 (3 × 30 mL) and the combined organic layers were dried over anhydrous Na₂SO₄ and
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43
44 concentrated under vacuum. The residue was purified by a silica gel column, eluted with
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47 EtOAc/petroleum ether (1:50–1:10), to afford *tert*-butyl ((3a*R*,5*S*,6*R*,7*S*,7a*R*)-6-
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50 (benzyloxy)-7-fluoro-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-
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60 yl)(methyl)carbamate as a yellow oil (280 mg, 84% yield). ¹H NMR (300 MHz, CDCl₃) δ

7.36–7.26 (m, 5H), 6.23 (d, J = 7.5 Hz, 1H), 5.35–5.18 (m, 1H), 4.82 (d, J = 11.4 Hz, 1H), 4.70–4.59 (m, 1H), 4.57 (d, J = 11.4 Hz, 1H), 4.54–4.47 (m, 2H), 4.16–4.11 (m, 1H), 3.89–3.80 (m, 1H), 3.47 (s, 3H), 1.55 (s, 9H). ESI MS m/z 451.1 $[M + Na]^+$. To a stirred solution of this material (200 mg, 0.47 mmol) in DCM (20 mL) at -78 °C was added BCl_3 (273 mg, 2.33 mmol) dropwise over 15 min. The resulting solution was stirred for 5 h at room temperature. The reaction was then cooled to -78 °C and quenched by the addition of 20 mL of MeOH. The crude product was purified by preparative HPLC to afford **28** (53 mg, 47% yield) as a white solid. 1H NMR (300 MHz, $CDCl_3$) δ 6.31 (d, J = 3.0 Hz, 1H), 5.01–4.83 (m, 1H), 4.67–4.60 (m, 2H), 4.49 (m, 1H), 4.14–4.08 (m, 1H), 3.94–3.81 (m, 1H), 3.00 (s, 3H); ^{13}C NMR (125 MHz, CD_3OD) δ 28.85, 64.41, 68.91, 69.95, 70.07, 82.07 (d, J = 171 Hz), 89.72 (d, J = 176 Hz), 164.55. HRMS (ESI+) m/z $[M + H]^+$ calculated for $C_8H_{12}F_2N_2O_2S$: 239.0666; found: 239.0661.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(piperidin-1-yl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (29). Compound **29** was prepared from **62** and piperidine following the procedure used to prepare **34**. 1H NMR (300 MHz, $CDCl_3$) δ 1.44–1.50 (6H,

m), 3.27–3.44 (7H, m), 3.55–3.58 (1H, J = 10.2 Hz, d), 3.73–3.76 (1H, m), 3.94–3.98 (1H, m), 6.14–6.16 (1H, J = 6.6 Hz, d). ESI MS m/z 288.9 $[M + H]^+$.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(4-fluoropiperidin-1-yl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (30). Compound **30** was prepared from **62** and 4-fluoropiperidine hydrochloride following the procedure used to prepare **34**. ^1H NMR (300 MHz, D_2O) δ 6.24–6.22 (d, J = 6.3 Hz, 1H), 5.18–4.94 (m, 0.5 H), 4.90–4.78 (m, 0.5 H), 4.14–4.10 (t, J = 6.0 Hz, 1H), 3.95–3.92 (t, J = 5.1 Hz, 1H), 3.78–3.71 (m, 1H), 3.63–3.46 (m, 5H), 3.40–3.32 (m, 2H), 1.97–1.74 (m, 4H). ESI MS m/z 307.0 $[M + H]^+$.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(pyrrolidin-1-yl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (31). Compound **31** was prepared from **62** and pyrrolidine following the procedure used to prepare **34**. ^1H NMR (300 MHz, CDCl_3) δ 1.78–1.83 (4H, m), 3.27–3.32 (5H, m), 3.36–3.46 (2H, m), 3.56–3.59 (1H, J = 10.4 Hz, d), 3.74–3.78 (1H, m), 3.92–3.96 (1H, m), 6.17–6.19 (1H, J = 6.3 Hz, d). ESI MS m/z 275.0 $[M + H]^+$.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-((*S*)-3-fluoropyrrolidin-1-yl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (32). Compound **32** was prepared from **62** and (*S*)-3-fluoropyrrolidine following the procedure used to prepare **34**. ^1H NMR (300

MHz, D₂O) δ 6.28–6.26 (d, J = 6.6 Hz, 1H), 5.40 (s, 0.5 H), 5.22 (s, 0.5 H), 4.14–4.10 (t, J = 6.0 Hz, 1 H), 3.97–3.93 (t, J = 5.1 Hz, 1 H), 3.78–3.43 (m, 8 H), 2.29–1.95 (m, 2H).

ESI MS m/z 293.0 [M + H]⁺.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-((*R*)-3-fluoropyrrolidin-1-yl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (33). Compound **33** was prepared from **62** and (*R*)-3-fluoropyrrolidine following the procedure used to prepare **34**. ¹H NMR (300 MHz, D₂O) δ 6.28–6.26 (d, J = 6.3 Hz, 1H), 5.40 (s, 0.5 H), 5.20 (s, 0.5 H), 4.14–4.10 (t, J = 6.0 Hz, 1 H), 3.97–3.94 (t, J = 5.1 Hz, 1 H), 3.78–3.39 (m, 7 H), 2.22–1.96 (m, 2 H).

ESI MS m/z 293.0 [M + H]⁺.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (34). Step 1: Preparation of **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(acetoxymethyl)-2-(azetidin-1-yl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl diacetate (94).** A mixture of Et₃N (18.7 g, 185 mmol), azetidine hydrochloride (12 g, 129 mmol) and **(2*S*,3*R*,4*R*,5*S*,6*R*)-6-(acetoxymethyl)-3-isothiocyanatotetrahydro-2*H*-pyran-2,4,5-triyl triacetate⁴⁷ (62)** (48 g, 123 mmol) in DCM (500 mL) was stirred for 2 h at room temperature, followed by addition of TFA (56 g, 493 mmol). The resulting solution was

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4 stirred overnight at room temperature, neutralized by NaHCO_3 , extracted with DCM (3 ×
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7 100 mL), dried over anhydrous Na_2SO_4 , and concentrated in vacuo to give a residue,
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10 which was purified by flash chromatography (SiO_2 ; 30% EtOAc in petroleum ether) to
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13 afford **94** (36 g, 75% yield) as a light yellow syrup. ^1H NMR (300 MHz, CDCl_3) δ 6.29 (d,
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15 J = 6.6 Hz, 1H), 5.44–5.47 (m, 1H), 4.95–4.99 (m, 1H), 4.36 (t, J = 5.4 Hz, 1H), 4.05–4.18
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17 (m, 6H), 3.86–3.92 (m, 1H), 2.34–2.44 (m, 2H), 2.07–2.14 (m, 9H). ESI MS m/z 386.9 [M
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20 + H] $^+$.
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28 **Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(hydroxymethyl)-**
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31 **5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (**34**).** A solution of **94** (36 g, 93
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34 mmol) in MeOH (200 mL) was treated with K_2CO_3 (5.14 g, 37 mmol) for 4 h at room
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37 temperature. The resulting solution was filtered through a silica gel plug and solvent was
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39
40 removed in vacuo to afford **34** (21 g, 87% yield) as a light-yellow syrup. ^1H NMR (300
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43 MHz, $\text{DMSO}-d_6$) δ 6.27 (d, J = 6.3 Hz, 1H), 3.94 (t, J = 6.3 Hz, 1H), 3.85 (t, J = 7.5 Hz,
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46 4H), 3.70 (t, J = 4.8 Hz, 1H), 3.55–3.59 (m, 1H), 3.33–3.42 (m, 3H), 2.21–2.51 (m, 2H);
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52 ^{13}C NMR (125 MHz, CD_3OD) δ 16.22, 51.72, 61.82, 69.74; 74.37, 75.04, 75.11, 91.31,
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164.22. HRMS (ESI+) m/z $[M + H]^+$ calculated for $C_{10}H_{16}N_2O_4S$: 261.0909; found: 261.0916.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(3-fluoroazetidin-1-yl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (35). Compound **35** was prepared from **62** and 3-fluoroazetidine hydrochloride following the procedure used to prepare **34**. 1H NMR (300 MHz, D_2O) δ 6.32–6.29 (d, J = 6.6 Hz, 1H), 5.45–5.43 (m, 0.5H), 5.26–5.23 (m, 0.5H), 4.31–4.20 (m, 2H), 4.19–4.16 (m, 2H), 4.14–4.00 (m, 1H), 3.99–3.92 (m, 1H), 3.76–3.60 (m, 1H), 3.57–3.46 (m, 3H). ESI MS m/z 279.0 $[M + H]^+$.

(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(3-methylazetidin-1-yl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (36). Compound **36** was prepared from **62** and 3-methylazetidine hydrochloride following the procedure used to prepare **34**. 1H NMR (300 MHz, D_2O) δ 6.26–6.28 (d, J = 6.6 Hz, 1H), 4.02–4.13 (m, 3H), 3.90–3.94 (t, J = 5.1 Hz, 1H), 3.72–3.79 (m, 1H), 3.46–3.63 (m, 5H), 2.69–2.76 (m, 1H), 1.13–1.16 (d, J = 6.9 Hz, 3H). ESI MS m/z 275.0 $[M + H]^+$.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (37). Step 1: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-

(azetidine-1-yl)-5-((*tert*-butyldimethylsilyloxy)methyl)-5,6,7,7a-tetrahydro-3a*H*-

pyrano[3,2-*d*]thiazole-6,7-diol (**95**). To a solution of **34** (34 g, 131 mmol), Et₃N (20.2 g, 0.2 mol) and DMAP (0.5 g, 4 mmol) in DCM (200 mL) was added TBDMSCI (21.6 g, 143 mmol). After stirring overnight at 15 °C, the mixture was quenched by addition of saturated aqueous NaHCO₃ (200 mL) then extracted with DCM (3 × 100 mL). The combined organic extract was dried over anhydrous Na₂SO₄ and concentrated in vacuo to give a residue, which was purified by flash chromatography (SiO₂; 2% MeOH in DCM) to afford **95** (32 g, 65% yield) as a solid. ¹H NMR (300 MHz, CDCl₃) δ 6.38 (d, *J* = 6.3 Hz, 1H), 4.24 (t, *J* = 6.3 Hz, 1H), 4.04–4.09 (m, 5H), 3.85 (d, *J* = 3.6 Hz, 2H), 3.70–3.78 (m, 1H), 3.66–3.69 (m, 1H), 2.33–2.43 (m, 2H), 0.91 (s, 9H), 0.09 (s, 6H). ESI MS *m/z* 375.0 [M + H]⁺.

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (96**).** To a solution of **95** (3.0 g, 8.0 mmol) in DMF (40 mL) was slowly added NaH (1.5 g, 37.50 mmol). The mixture was stirred at room temperature for 30 min, then BzCl (3.36 g, 24 mmol) was added. The resulting solution was stirred overnight at 15 °C, quenched with water/ice (100 mL), and extracted with DCM (3 × 50 mL). The combined organic extract was

washed with brine (3 × 20 mL), dried over anhydrous Na₂SO₄, and concentrated in vacuo to give a residue which was purified by flash chromatography (SiO₂; 10% EtOAc in petroleum ether) to afford (3*aR*,5*R*,6*S*,7*R*,7*aR*)-2-(azetidin-1-yl)-5-((*tert*-butyldimethylsilyloxy)methyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (1.38 g, 30% yield) as a light yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 8.05–8.09 (m, 4H), 7.54–7.59 (m, 2H), 7.28–7.39 (m, 4H), 6.43 (d, *J* = 6.3 Hz, 1H), 5.84–5.96 (m, 1H), 5.37 (dd, *J* = 2.1, 1.5 Hz, 1H), 4.59–4.61 (m, 1H), 4.04–4.09 (m, 5H), 3.78–3.93 (m, 2H), 2.38–2.43 (m, 2H), 0.91 (s, 9H), 0.09 (s, 6H); ESI MS *m/z* 583.0 [M + H]⁺. A solution of this material (1.3 g, 2.2 mmol) in MeOH (10 mL) was stirred with AcCl (1 mL, 0.45 mmol) overnight at 15 °C. The reaction was quenched with saturated aqueous NaHCO₃ (20 mL) and extracted with DCM (3 × 40 mL). The combined organic extract was dried over anhydrous Na₂SO₄ and concentrated in vacuo to give a residue, which was purified by flash chromatography (SiO₂; 20% EtOAc in petroleum ether) to afford (**96**) (0.42 g, 40% yield) as a solid. ¹H NMR (300 MHz, CDCl₃) δ 8.04–8.11 (m, 4H), 7.55–7.62 (m, 2H), 7.42–7.48 (m, 4H), 6.48 (d, *J* = 6.6 Hz, 1H), 5.92 (t, *J* = 3.6 Hz, 1H), 5.35 (dd, *J*

= 8.0, 3.6 Hz, 1H), 4.63 (t, J = 5.4 Hz, 1H), 4.11–4.18 (m, 4H), 3.92–3.96 (m, 1H), 3.80–3.84 (m, 1H), 3.69–3.68 (m, 1H), 2.38–2.48 (m, 2H). ESI MS m/z 469.0 $[M + H]^+$.

Step 3: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(fluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (37**).** A solution of **96** (100 mg, 0.2 mmol) in DCM (10 mL) was treated with DAST (137 mg, 0.8 mmol) for 30 min at -78 °C. After stirring overnight at room temperature, the reaction was quenched with saturated aqueous NaHCO₃ (10 mL), extracted with DCM (3 × 10 mL), washed with brine (10 mL), dried over anhydrous Na₂SO₄ and concentrated under vacuum to give the crude product as a white syrup (80 mg), which was dissolved into MeOH (5 mL) and treated with K₂CO₃ (10 mg, 0.07 mmol) for 3 h at room temperature. The reaction mixture was neutralized by AcOH and concentrated. The residue was purified by a silica gel column, eluted with 5%–10% MeOH in DCM to give **37** as white solid (10 mg, 17% yield, two steps). ¹H NMR (300 MHz, D₂O) δ 6.30 (d, J = 6.3 Hz, 1H), 4.49–4.69 (dd, J = 57, 3.3 Hz, 1H), 4.11–4.15 (m, 1H), 3.93–4.01 (m, 5H), 3.70–3.82 (m, 1H), 3.59–3.69 (m, 1H), 2.24–2.34 (m, 2H). ESI MS m/z 263.0 $[M + H]^+$.

(3a*R*,5*S*,6*S*,7a*R*)-5-(difluoromethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (38). Step 1: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-(benzyloxy)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (79). To a solution of 77 (4.26 g, 8.95 mmol) in anhydrous DMF (30 mL) was added BnBr (1.68 g, 9.84 mmol) and tetrabutylammonium iodide (0.330 g, 0.895 mmol). At 0 °C NaH (60%, 0.430 g, 10.744 mmol) was added in portions and then the reaction was stirred at this temperature for 2 h. The mixture was diluted with water (200 mL), extracted with Et₂O (2 × 100 mL). The combined extract was washed with brine (100 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure to give a mixture containing ~80% (3a*R*,5*R*,6*R*,7*R*,7a*R*)-6-(benzyloxy)-7-((*tert*-butyldimethylsilyl)oxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-*N,N*-dimethyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-amine (5.06 g, 100% yield) which was inseparable on silica gel column. This mixture was directly submitted to the next reaction. To a solution of this material (9.89 g, 17.5 mmol) in MeOH (100 mL) at 0 °C was added AcCl (6.21 mL, 87.4 mmol). The mixture was stirred at room temperature for 16 h. Solvent was evaporated under reduced pressure. The residue was purified on silica gel column, eluted

with 2%–5% 2M NH₃ MeOH solution in DCM to give **79** (4.58 g, 78% yield). ESI MS *m/z* 339.1 [M + H]⁺.

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-ol (80**).** To a solution of **79** (4.58 g, 13.6 mmol) and imidazole (2.76 g, 40.7 mmol) in anhydrous DMF (30 mL) at 0 °C was added TBDMSCI (2.45 g, 16.3 mmol). The mixture was stirred at room temperature for 21 h. The reaction was diluted with water (200 mL), extracted with Et₂O (2 × 100 mL). The combined extracts were washed with brine (100 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure. The residue was purified on silica gel column, eluted with 1%–3% 2 M NH₃ MeOH solution in DCM to give **80** (5.80 g, 95% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.27–7.37 (m, 5H), 6.37 (d, *J* = 6.6 Hz, 1H), 4.88 (d, *J* = 11.4 Hz, 1H), 4.63 (d, *J* = 11.4 Hz, 1H), 4.22 (t, *J* = 6.4 Hz, 1H), 4.09–4.13 (m, 1H), 3.80–3.82 (m, 2H), 3.74–3.78 (m, 1H), 3.64 (dd, *J* = 8.7 Hz, 6.4 Hz, 1H), 2.98 (s, 6H), 0.897 (s, 9H), 0.90 (s, 9H), 0.052 (s, 3H), 0.056 (s, 3H). ESI MS *m/z* 453.2 [M + H]⁺.

Step 3: Preparation of ((3a*R*,5*R*,6*S*,7a*R*)-6-(benzyloxy)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methanol (81). A mixture of **80** (5.80 g, 12.8 mmol) and thio-CDI (90% tech, 4.57 g, 23.1 mmol) in anhydrous DMF (30 mL) was stirred at 90 °C for 2.5 h. After cooling, the mixture was diluted with water (200 mL), extracted with Et₂O (2 × 100 mL). The combined extracts were washed with brine (100 mL) and dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure. The residue was purified on silica gel column, eluted with 30%–100% EtOAc in hexanes to give O-((3a*R*,5*R*,6*S*,7*R*,7a*R*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-7-yl) 1*H*-imidazole-1-carbothioate (6.51 g, 90% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.30 (s, 1H), 7.59 (s, 1H), 7.23–7.34 (m, 5H), 7.03 (s, 1H), 6.36 (br s, 1H), 6.31 (d, *J* = 6.7 Hz, 1H), 4.91 (d, *J* = 11.6 Hz, 1H), 4.62 (m, 1H), 4.58 (d, *J* = 11.6 Hz, 1H), 3.85 (d, *J* = 8.7 Hz, 1H), 3.64–3.74 (m, 3H), 3.03 (s, 6H), 0.82 (s, 9H), 0.02 (s, 3H), 0.00 (s, 3H). ESI MS *m/z* 563.2 [M + H]⁺. A mixture of this material (6.51 g, 11.6 mmol), tributyltin hydride (7.47 g, 25.7 mmol) and ABCN (0.313 g, 1.28 mmol) in anhydrous THF (100 mL) was heated at reflux for 17 h. After cooling the solvent was evaporated under reduced pressure. The residue was

purified by silica gel column chromatography, eluted with 50%–80% EtOAc in hexanes to give (3a*R*,5*R*,6*S*,7a*R*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-*N,N*-dimethyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-amine as a white solid (4.07 g, 81% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.24–7.37 (m, 5H), 6.25 (d, *J* = 6.6 Hz, 1H), 4.71 (d, *J* = 11.6 Hz, 1H), 4.42 (d, *J* = 11.6 Hz, 1H), 4.34–4.39 (m, 1H), 3.65–3.80 (m, 4H), 2.99 (s, 6H), 2.25 (br s, 1H), 2.10–2.16 (m, 1H), 0.89 (s, 9H), 0.05 (s, 6H). ESI MS *m/z* 437.2 [M + H]⁺. To a solution of this material (4.07 g, 9.33 mmol) in MeOH (50 mL) at 0 °C was added AcCl (1.33 mL, 18.7 mmol). The mixture was stirred at room temperature for 16 h. Solvent was evaporated under reduced pressure. The residue was purified on silica gel column, eluted with 3%–5% 2 M NH₃ MeOH solution in DCM to give **81** (2.78 g, 91% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.25–7.36 (m, 5H), 6.22 (d, *J* = 6.6 Hz, 1H), 4.70 (d, *J* = 11.6 Hz, 1H), 4.42 (d, *J* = 11.6 Hz, 1H), 4.32–4.37 (m, 1H), 3.71–3.79 (m, 2H), 3.60–3.67 (m, 2H), 2.97 (s, 6H), 2.19–2.25 (m, 1H), 2.09–2.15 (m, 1H), 1.86 (br s, 1H). ESI MS *m/z* 323.1 [M + H]⁺.

Step 4: Preparation of (3a*R*,5*S*,6*S*,7a*R*)-6-(benzyloxy)-5-(difluoromethyl)-*N,N*-dimethyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-amine (82**).** To a solution of

DMSO (0.875 g, 11.2 mmol) in anhydrous DCM (15 mL) at -78 °C under N₂ was added oxalyl chloride (1.316 g, 10.36 mmol) dropwise. The mixture was stirred at ~ -30 °C for 30 min and cooled to -78 °C again. A solution of **81** (1.39 g, 4.32 mmol) in anhydrous DCM (15 mL) was added dropwise. After stirring at ~ -30 °C for 2 h the reaction mixture was cooled back to -78 °C, and Et₃N (1.74 g, 17.3 mmol) was added. The mixture was stirred at ~ -30 °C for another 30 min, and then quenched with water (50 mL). The organic layer was collected, and the aqueous was extracted with DCM (2 × 20 mL). The combined extracts were dried over anhydrous Na₂SO₄. The solvent was evaporated under reduced pressure to give the crude (3a*R*,5*S*,6*S*,7a*R*)-6-(benzyloxy)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-5-carbaldehyde (1.24 g) as a yellow foam. ESI MS *m/z* 353.1 [M + Na]⁺. To a solution of this material (200 mg) in anhydrous DCM (5 mL) at 0 °C was added bis(2-methoxyethyl)aminosulfur trifluoride (0.553 g, 2.50 mmol). The mixture was stirred at room temperature for 27 h. The reaction was quenched with saturated aqueous NaHCO₃ (10 mL), and then extracted with EtOAc (2 × 10 mL). The combined extract was dried over anhydrous Na₂SO₄. The solvents were evaporated under reduced pressure, and the residue was purified by silica gel column

chromatography (EtOAc/hexanes, 2:1 to 5:1) to afford **82** as a pale-yellow foam (0.050 g, 23% yield, 2 steps). ESI MS m/z 343.1 $[M + H]^+$.

Step 9: Preparation of (3a*R*,5*S*,6*S*,7a*R*)-5-(difluoromethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (38**).** To a solution of **82** (0.049 g, 0.14 mmol) in DCM (2 mL) at -78 °C was added a solution of BCl₃ in DCM (1.0 M, 0.19 mL, 0.19 mmol). The mixture was slowly warmed up to room temperature and stirred for 18 h. The reaction was cooled to -78 °C again and a 1:1 mixture of MeOH-DCM (2 mL) was added dropwise to quench the reaction. Solvents were evaporated, and the residue was treated with MeOH for three more times. The crude product was purified by silica gel column chromatography, eluted with 1%–2% 2 M NH₃ MeOH solution in DCM to give **38** (0.0193 g, 53% yield) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 6.22 (d, J = 6.5 Hz, 1H), 5.93 (td, J = 54.4 Hz, 2.6 Hz, 1H), 4.36–4.40 (m, 1H), 3.99–4.03 (m, 1H), 3.59–3.68 (m, 1H), 3.03 (s, 6H), 2.12–2.16 (m, 2H); ¹³C NMR (100 MHz, CD₃OD) δ 165.06, 115.86 (t, J = 192.8 Hz), 91.48, 74.91 (t, J = 17.1 Hz), 70.02, 64.40 (t, J = 2.8 Hz), 40.31, 34.09. ESI MS m/z 253.1 $[M + H]^+$.

(3*aR*,5*S*,6*S*,7*aR*)-5-(difluoromethyl)-2-(methylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (39). Step 1: Preparation of (3*aR*,5*R*,6*S*,7*R*,7*aR*)-7-((1*H*-imidazole-1-carbonothioyl)oxy)-5-((benzoyloxy)methyl)-2-((*tert*-butoxycarbonyl)(methyl)amino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-yl benzoate (Exp #39a). A mixture of 69 (5.00 g, 9.24 mmol) and thio-CDI (90% tech, 3.40 g, 19.1 mmol) in anhydrous DMF (30 mL) was stirred at 95 °C for 4 h. After cooling the solvent was removed under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:10 to 2:3), affording (3*aR*,5*R*,6*S*,7*R*,7*aR*)-7-((1*H*-imidazole-1-carbonothioyl)oxy)-5-((benzoyloxy)methyl)-2-((*tert*-butoxycarbonyl)(methyl)amino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-yl benzoate as a pale yellow solid (5.60 g, 93% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.76 (s, 1H), 8.03–8.01 (m, 2H), 7.97–7.95 (m, 2H), 7.64–7.60 (m, 1H), 7.54–7.50 (m, 1H), 7.45 (t, *J* = 7.7 Hz, 2H), 7.34 (t, *J* = 7.7 Hz, 2H), 7.02 (s, 1H), 6.38–6.37 (m, 1H), 6.15 (d, *J* = 7.1 Hz, 1H), 5.56 (td, *J* = 1.2, 9.2 Hz, 1H), 4.70–4.67 (m, 1H), 4.58 (dd, *J* = 3.2, 12.1 Hz, 1H), 4.42 (dd, *J* = 5.1, 12.1 Hz, 1H), 4.08–4.03 (m, 1H), 3.43 (s, 3H), 1.56 (s, 9H).

Step 2: Preparation of ((3a*R*,5*R*,6*S*,7a*R*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(methyl)amino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate (Exp #39b). A mixture of the above material (5.60 g, 8.59 mmol), Bu₃SnH (5.84 g, 17.0 mmol) and ABCN (0.15 g, 0.60 mmol) in mixed anhydrous toluene/THF (50/50 mL) was stirred at 90 °C for 16 h. After cooling the solvent was removed under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:10 to 1:2), affording ((3a*R*,5*R*,6*S*,7a*R*)-6-(benzoyloxy)-2-((*tert*-butoxycarbonyl)(methyl)amino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-5-yl)methyl benzoate as a white solid (3.20 g, 70% yield). ¹H NMR (400 MHz, CDCl₃) δ 8.03–7.98 (m, 4H), 7.58–7.49 (m, 2H), 7.44–7.40 (m, 4H), 6.08 (d, *J* = 7.3 Hz, 1H), 5.44–5.40 (m, 1H), 4.49–4.40 (m, 3H), 4.07–4.03 (m, 1H), 3.35 (s, 3H), 2.64–2.59 (m, 1H), 2.44–2.37 (m, 1H), 1.56 (s, 9H).

Step 3: Preparation of *tert*-butyl ((3a*R*,5*R*,6*S*,7a*R*)-6-hydroxy-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #39c). Following the procedure described for preparation of **47**, the above material (3.20 g, 6.10 mmol) was benzoyl-deprotected using K₂CO₃/MeOH. After purification on silica gel by

flash column chromatography (MeOH/DCM, 1:50 to 1:20), *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-6-hydroxy-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate was obtained as a white solid (1.82 g, 94% yield). ¹H NMR (400 MHz, CDCl₃) δ 5.91 (d, *J* = 6.9 Hz, 1H), 4.36–4.32 (m, 1H), 3.89–3.85 (m, 1H), 3.81–3.75 (m, 1H), 3.65–3.59 (m, 1H), 3.38–3.34 (m, 1H), 3.33 (s, 3H), 2.48–2.43 (m, 1H), 2.32 (d, *J* = 10.7 Hz, 1H), 2.17–2.11 (m, 1H), 1.84 (t, *J* = 6.3 Hz, 1H), 1.54 (s, 9H).

Step 4: Preparation of *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #39d). Following the procedure described for preparation of **47**, the above material (1.82 g, 5.74 mmol) was mono-TBDMS protected. After purification on silica gel by flash column chromatography (EtOAc/hexanes, 1:10 to 1:2), *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate was obtained as a colorless sticky oil (2.30 g, 93% yield). ¹H NMR (400 MHz, CDCl₃) δ 5.92 (d, *J* = 6.8 Hz, 1H), 4.31–4.28 (m, 1H), 3.92–3.90 (m, 1H), 3.73 (d, *J* = 4.6 Hz, 2H), 3.35–3.31 (m, 1H), 3.33 (s, 3H), 2.41 (d,

$J = 9.4$ Hz, 1H), 2.41–2.36 (m, 1H), 2.18–2.12 (m, 1H), 1.54 (s, 9H), 0.89 (s, 9H), 0.06 (s, 6H).

Step 5: Preparation of *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #39e). Following the procedure described for preparation of **46**, the above material (2.78 g, 6.45 mmol) was benzyl protected using BnBr. After purification on silica gel by flash column chromatography (EtOAc/hexanes, 1:10 to 1:4), *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate was obtained as a colorless sticky oil (2.7 g, 80% yield). ^1H NMR (400 MHz, CDCl_3) δ 7.36–7.27 (m, 5H), 6.02 (d, $J = 7.1$ Hz, 1H), 4.67 (d, $J = 11.6$ Hz, 1H), 4.40 (d, $J = 11.6$ Hz, 1H), 4.34–4.30 (m, 1H), 3.83–3.78 (m, 1H), 3.77–3.69 (m, 2H), 3.53–3.50 (m, 1H), 3.29 (s, 3H), 2.44–2.39 (m, 1H), 2.14–2.08 (m, 1H), 1.52 (s, 9H), 0.88 (s, 9H), 0.04 (s, 6H).

Step 6: Preparation of *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-6-(benzyloxy)-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #39f). Following the procedure described for preparation of **46**, the above material (2.70 g, 5.30

mmol) was silyl-protected using TBAF. After purification on silica gel by flash column chromatography (EtOAc/hexanes, 1:5 to 1:1), *tert*-butyl ((3*aR*,5*R*,6*S*,7*aR*)-6-(benzyloxy)-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate was obtained as a colorless sticky foam (2.0 g, 93% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.37–7.27 (m, 5H), 6.01 (d, *J* = 7.2 Hz, 1H), 4.69 (d, *J* = 11.6 Hz, 1H), 4.40 (d, *J* = 11.6 Hz, 1H), 4.36–4.34 (m, 1H), 3.77–3.72 (m, 2H), 3.62–3.54 (m, 2H), 3.30 (s, 3H), 2.53–2.48 (m, 1H), 2.09–2.02 (m, 1H), 1.71 (t, *J* = 6.3 Hz, 1H), 1.53 (s, 9H).

Step 7: Preparation of *tert*-butyl ((3*aR*,5*S*,6*S*,7*aR*)-6-(benzyloxy)-5-formyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #39g). Following the procedure described for preparation of **46**, the above material (0.663 g, 1.62 mmol) was oxidized to the aldehyde using DMP. After purification on silica gel by flash column chromatography (EtOAc/hexanes, 1:10 to 2:3), *tert*-butyl ((3*aR*,5*S*,6*S*,7*aR*)-6-(benzyloxy)-5-formyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate was obtained as a white foam (0.57 g, 86% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.63 (s, 1H), 7.36–7.27 (m, 5H), 6.04 (d, *J* = 4.3 Hz, 1H), 4.69 (d, *J* = 9.2

Hz, 1H), 4.50 (d, J = 9.2 Hz, 1H), 4.43–4.39 (m, 1H), 4.07 (d, J = 6.4 Hz), 4.02–3.99 (m, 1H), 3.29 (s, 3H), 2.64–2.59 (m, 1H), 2.10–2.03 (m, 1H), 1.53 (s, 9H).

Step 8: Preparation of *tert*-butyl ((3*aR*,5*S*,6*S*,7*aR*)-6-(benzyloxy)-5-(difluoromethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (Exp #39h).

Following the procedure described for preparation of **46**, the above material (0.550 g, 1.35 mmol) was treated with DAST. After purification on silica gel by flash column chromatography (EtOAc/hexanes, 1:10 to 1:3), *tert*-butyl ((3*aR*,5*S*,6*S*,7*aR*)-6-(benzyloxy)-5-(difluoromethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate was obtained as a pale yellow sticky oil (0.48 g, 83% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.34–7.27 (m, 5H), 6.04 (d, J = 7.4 Hz, 1H), 5.79 (dt, J = 2.2, 54.7 Hz, 1H), 4.67 (d, J = 11.4 Hz, 1H), 4.43 (d, J = 11.4 Hz, 1H), 4.43–3.40 (m, 1H), 4.01–3.97 (m, 1H), 3.82–3.73 (m, 1H), 3.27 (s, 3H), 2.59–2.54 (m, 1H), 2.10–2.05 (m, 1H), 1.53 (s, 9H).

Step 9: Preparation of (3*aR*,5*S*,6*S*,7*aR*)-5-(difluoromethyl)-2-(methylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (39). Following the procedure described for preparation of **46**, the above material (0.480 g, 1.12 mmol) was deprotected using BCl₃.

After purification on silica gel by flash column chromatography (1.0 M NH₃ in MeOH/DCM, 1: 12), **39** was obtained as a pale-yellow sticky solid (0.24 g, 87% yield). ¹H NMR (400 MHz, CD₃OD) δ 6.17 (d, *J* = 6.4 Hz, 1H), 5.91 (dt, *J* = 2.6, 54.4 Hz, 1H), 4.38–4.34 (m, 1H), 4.03–3.98 (m, 1H), 3.64–3.61 (m, 1H), 2.84 (s, 3H), 2.16–2.13 (m, 2H); ¹³C NMR (100 MHz, CD₃OD) δ 163.35, 116.00 (t, *J* = 241.0 Hz), 91.12, 74.97 (t, *J* = 42.7 Hz), 70.00, 64.54 (t, *J* = 3.6 Hz), 34.19, 30.79. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₈H₁₂F₂N₂O₂S: 239.0666; found: 239.0669.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(allylamino)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (40). Step 1: Preparation of **(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(allyl(*tert*-butoxycarbonyl)amino)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (87).** A solution of **86** (1.5 g, 2.6 mmol) in DCM (30 mL) was treated with DMP (1.7 g, 4.0 mmol) for 2 h at room temperature. The resulting solution was quenched with saturated aqueous NaHCO₃ (10 mL) and saturated aqueous Na₂S₂O₃ (10 mL), extracted with DCM (3 × 50 mL), dried over Na₂SO₄, and concentrated under vacuum to give a residue, which was purified by a short silica gel column with 30% EtOAc in petroleum to afford the crude aldehyde (1.3 g), which was dissolved into DCM (20 mL)

and treated with DAST (1.5 g, 9.3 mmol) at -78 °C. After stirring overnight at room temperature, the resulting solution was quenched with saturated aqueous NaHCO₃ (50 mL), extracted with DCM (3 × 50 mL), dried over Na₂SO₄, and concentrated under vacuum to give a residue, which was purified by a silica gel column with 10% EtOAc in petroleum to give **87** as a white solid (650 mg, 43% yield). ¹H NMR (300 MHz, CDCl₃), δ 8.03–8.13 (m, 4H), 7.56–7.61 (m, 2H), 7.48–7.51 (m, 4H), 6.11–6.14 (d, *J* = 8.7 Hz, 1H), 5.95–6.09 (td, *J* = 54.3 Hz, 2.8 Hz, 1H), 5.19–5.25 (m, 2H), 4.44–4.45 (m, 2H), 4.31–4.36 (m, 2H), 4.10–4.18 (m, 2H), 1.53 (s, 9H). ESI MS *m/z* 589.0 [M + H]⁺.

Step 2: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(allylamino)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (40**).** A solution of **87** (200 mg, 0.34 mmol) in MeOH (15 mL) was treated with K₂CO₃ (10 mg, 0.07 mmol) for 3 h at room temperature. The reaction mixture was neutralized by acetate acid, and was condensed to give a residue, which was purified by a silica gel column, eluted with 1%~2% MeOH in DCM to give *tert*-butyl allyl((3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-6,7-dihydroxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)carbamate as a yellow oil (150 mg, 76% yield). ¹H NMR (300 MHz, CDCl₃) δ 6.13 (d, *J* = 8.7 Hz, 1H), 5.95–6.09 (m, 1H), 5.19–5.25 (m,

2H), 4.44–4.45 (m, 2H), 4.31–4.36 (m, 2H), 4.10–4.18 (m, 2H), 1.53 (s, 9H). ESI MS m/z 381.0 $[M + H]^+$. A solution of this material (150 mg, 0.39 mmol) in DCM (18 mL) was treated with TFA (1.8 mL) overnight at room temperature. The reaction mixture was condensed to give a residue, which was neutralized by NH_4OH (0.5 mL, 25%~28%, w/v) for purification by Prep-HPLC under the following conditions [Agilent 1200 prep HPLC; Column: Sun Fire Prep C18, 19*50mm 5 μ m; mobile phase: Water with 0.03% NH_4OH and CH_3CN (10% CH_3CN up to 45% in 10 min; Detector: UV 220 nm)] to give **40** as a white solid (16.6 mg, 15% yield). 1H NMR (300 MHz, D_2O) δ 6.20 (d, J = 6.3 Hz, 1H), 6.14–6.16 (m, 1H), 5.74–5.78 (t, J = 2.7 Hz, 1H), 5.04–5.10 (m, 2H), 4.20–4.24 (t, J = 6.0 Hz, 1H), 4.04–4.07 (t, J = 4.2 Hz, 1H), 3.66–3.86 (m, 4H). ESI MS m/z 281.0 $[M + H]^+$.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (41). Compound **41** was prepared from **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-5-(hydroxymethyl)-2-(propylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol** following the procedure used to prepare **42**. 1H NMR (400 MHz, CD_3OD) δ 6.44 (d, J = 6.5 Hz, 1H), 6.01 (td, J = 54.2, 1.8 Hz, 1H), 4.23 (t, J = 5.9 Hz, 1H), 4.03 (t, J = 4.6 Hz, 1H), 3.79–3.72 (m, 2H), 3.32–3.23 (m, 2H), 1.69–1.60 (m,

2H), 0.98 (t, J = 7.3 Hz, 3H). ^{13}C NMR (100 MHz, CD_3OD) δ 167.42, 116.25 (t, $J_{\text{C6,F}}$ 241.4 Hz, C-6), 89.12, 75.41 (t, J = 21.4 Hz), 74.78, 69.99 (t, J = 3.2 Hz, C-4), 55.64, 48.47, 23.98, 12.36. HRMS (ESI+) m/z $[\text{M} + \text{H}]^+$ calculated for $\text{C}_{10}\text{H}_{16}\text{F}_2\text{N}_2\text{O}_3\text{S}$: 283.0928; found: 283.0934.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(ethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (42). Step 1: Preparation of **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (89).** To a solution of **50** (1.0 g, 2.2 mmol) in pyridine (20 mL) at 0 °C was added DMAP (0.024 g, 0.20 mmol) followed by slow addition of BzCl (2.0 mL, 17.6 mmol). The mixture was warmed to room temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL) then washed with saturated NaHCO_3 solution and brine. The organic extract was dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residual pyridine was co-evaporated with hexanes in vacuo and the residue was separated by flash chromatography (SiO_2 ; EtOAc/hexanes 1:5) to give **(3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl**

dibenzoate (1.05 g, 71% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 8.08 (m, 2H), 7.99 (m, 2H), 7.61–7.52 (m, 2H), 7.47–7.38 (m, 4H), 6.12 (d, J = 5.6 Hz, 1H), 5.89 (dd, J = 1.5, 1.4 Hz, 1H), 5.39 (m, 1H), 4.46 (ddd, J = 5.5, 2.9, 0.96 Hz, 1H), 3.96 (m, 2H), 3.80 (m, 1H), 3.75–3.70 (m, 2H), 1.54 (s, 9H), 1.18 (t, J = 5.5 Hz, 3H), 0.83 (s, 9H), 0.01 (s, 3H), 0.03 (s, 3H). To a solution of this material (3.2 g, 4.8 mmol) in dry MeOH (30 mL) at 0 °C was added AcCl (0.07 mL, 1.0 mmol). The mixture was stirred at 0 °C for 30 min and then at room temperature overnight. The mixture was diluted with DCM (30 mL) and neutralized with 10% aq. NaHCO_3 solution. The DCM layer was washed with brine, dried over anhydrous Na_2SO_4 and concentrated in vacuo. The residue was purified by flash chromatography (SiO_2 ; EtOAc/hexanes 3:7) to provide **89** (2.4 g, 90% yield) as a foamy solid. ^1H NMR (400 MHz, CDCl_3) δ 8.06 (m, 2H), 8.01 (m, 2H), 7.58–7.53 (m, 2H), 7.47–7.39 (m, 4H), 6.14 (d, J = 7.0 Hz, 1H), 5.93 (dd, J = 1.9, 1.8 Hz, 1H), 5.34 (m, 1H), 4.49 (ddd, J = 6.8, 3.6, 0.96 Hz, 1H), 4.03–3.92 (m, 2H), 3.80–3.65 (m, 3H), 1.53 (s, 9H), 1.19 (t, J = 6.9 Hz, 3H).

Step 2: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (90).

To a solution of **89** (1.0 g, 1.8 mmol) in dry DCM (25 mL) at 0 °C was added dry pyridine (0.30 mL, 3.7 mmol), followed by DMP (1.14 g, 2.69 mmol). The reaction was stirred at 0 °C for 10 min and at room temperature for 1.5 h. The mixture was diluted with 1M Na₂S₂O₃/saturated NaHCO₃ (30 mL, 1:1) and stirred for 10 min. The DCM layer was separated, dried over anhydrous Na₂SO₄ and concentrated in vacuo to yield crude (3*aR*,5*S*,6*S*,7*R*,7*aR*)-2-((*tert*-butoxycarbonyl)(ethyl)amino)-5-formyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate as a foamy solid (1.0 g crude) which was carried forward without further purification. ¹H NMR (400 MHz, CDCl₃) δ 9.71 (s, 1H), 8.04 (m, 4H), 7.58 (m, 2H), 7.47–7.39 (m, 4H), 6.14 (d, *J* = 6.24 Hz, 1H), 6.01 (dd, *J* = 1.8, 1.3 Hz, 1H), 5.53–5.51 (m, 1H), 4.38 (ddd, *J* = 6.2, 3.2, 1.2 Hz, 1H), 4.27 (d, *J* = 7.3 Hz, 1H), 4.0–3.9 (m, 2H), 1.53 (s, 9H), 1.15 (t, *J* = 6.8 Hz, 3H). To a stirred solution of this aldehyde (1.0 g, crude) in DCM (30 mL) at -78 °C was added DAST (1 mL, 7.7 mmol) dropwise. The cooling bath was removed, and the mixture was stirred at room temperature overnight. The reaction was diluted with saturated NaHCO₃ solution (15 mL) and the DCM layer was separated, dried over anhydrous Na₂SO₄, and concentrated in vacuo. The residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:4) to provide **90**

(0.413 g, 40% yield) as a foamy solid. ^1H NMR (400 MHz, CDCl_3) δ 8.08 (m, 2H), 8.0 (m, 2H), 7.60–7.53 (m, 2H), 7.46–7.39 (m, 4H), 6.13 (d, J = 7.0 Hz, 1H), 5.94 (m, 1H), 5.93 (td, J = 54.08, 2.9 Hz, 1H), 5.59–5.56 (m, 1H), 4.55–4.52 (ddd, J = 7.0, 3.4, 1.3 Hz, 1H), 3.98–3.86 (m, 3H), 1.55 (s, 9H), 1.17 (t, J = 6.9 Hz, 3H).

Step 3: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(ethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (42). To a stirred solution of 90 (0.41 g, 0.71 mmol) in dry MeOH (20 mL) at 0 °C was added K_2CO_3 (0.050 g, 0.36 mmol). The reaction mixture was warmed to room temperature and stirred 1.5 h. AcOH (0.5 mL) was added and the mixture was concentrated in vacuo. The residue was purified by flash chromatography (SiO_2 ; EtOAc/hexanes 1:1) to provide *tert*-butyl ((3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-6,7-dihydroxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (0.24 g, 92% yield) as a foamy solid. ^1H NMR (400 MHz, CD_3OD) δ 6.03 (d, J = 6.6 Hz, 1H), 5.93 (td, J = 54.5, 2.6 Hz, 1H), 4.19 (m, 1H), 4.11 (t, J = 4.3 Hz, 1H), 3.96–3.87 (m, 2H), 3.82 (dd, J = 4.7, 4.4 Hz, 1H), 3.56–3.47 (m, 1H), 1.53 (s, 9H), 1.16 (t, J = 6.9 Hz, 3H). This material (0.24 g, 0.65 mmol) was dissolved in 30% TFA/DCM (10 mL) at 0 °C and stirred at 0 °C for 1 h then slowly warmed to room temperature over

1 h. The mixture was concentrated in vacuo then neutralized with 2M NH₃/MeOH (5 mL) solution. The mixture was concentrated in vacuo and the residue was purified by flash chromatography (SiO₂; DCM/MeOH 95:5) to provide **42** (0.166 g, 95% yield) as a white solid. $[\alpha]_D^{20}$ -34.3 (*c* 1.00, MeOH); ¹H NMR (400 MHz, CD₃OD) δ 6.41 (d, *J* = 6.4 Hz, 1H), 6.00 (td, *J* = 54.4, 1.9 Hz, 1H), 4.22 (t, *J* = 5.9 Hz, 1H), 4.01 (t, *J* = 4.6 Hz, 1H), 3.76–3.70 (m, 2H), 3.37–3.33 (m, 2H), 1.21 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 167.35, 116.25 (t, *J*_{C6,F} 242.0 Hz, C-6), 89.10, 75.42 (t, *J* = 21.0 Hz), 74.74, 71.24, 69.96 (t, *J* = 4.0 Hz, C-4), 41.61, 15.09. mp 141.8 °C; HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₉H₁₄F₂N₂O₃S: 269.0771; found: 269.0756.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (43). Compound **43** was prepared from **68** following the procedure used to prepare **42**. ¹H NMR (400 MHz, CD₃OD) δ 6.45 (d, *J* = 6.4 Hz, 1H), 6.01 (td, *J* = 54.2, 2.2 Hz, 1H), 4.23 (t, *J* = 6.0 Hz, 1H), 4.00 (t, *J* = 4.8 Hz, 1H), 3.82–3.73 (m, 2H), 2.95 (s, 3H). ¹³C NMR (100 MHz, CD₃OD) δ 168.82, 116.17 (t, *J*_{C6,F} 241.5 Hz, C-6), 89.26, 75.42 (t, *J* = 21.2 Hz), 74.75, 69.87 (t, *J* = 3.3 Hz, C-4), 55.64, 32.20. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₈H₁₂F₂N₂O₃S: 255.0615; found: 255.0621.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-amino-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-
d]thiazole-6,7-diol (**44**). Step 1: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(*tert*-
butoxycarbonylamino)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-d]thiazole-
6,7-diyl dibenzoate (**88**). To a solution of **87** (900 mg, 1.53 mmol) in 1,4-dioxane (30 mL)
was added Pd(PPh₃)₄ (347 mg, 0.30 mmol), HCO₂H (384 mg, 8.35 mmol) and Et₃N (846
mg, 8.38 mmol) under N₂ atmosphere at 10 °C. After 20 min at 60 °C, additional HCO₂H
(1.4 g, 30.3 mmol) was added. After stirring overnight at 60 °C, the reaction mixture was
quenched with saturated aqueous NaHCO₃, extracted with DCM (3 × 30 mL), dried by
anhydrous MgSO₄, and concentrated under reduced pressure to afford a residue, which
was purified by a silica gel column, eluted with 10% EtOAc in hexane to give **88** as a
white syrup (600 mg, 72% yield). ¹H NMR (300 MHz, CDCl₃) δ 8.07–8.14 (m, 4H), 7.60–
7.65 (m, 2H), 7.51–7.59 (m, 4H), 6.34 (d, *J* = 8.7 Hz, 1H), 5.82–6.20 (td, *J* = 54.0 Hz, 2.7
Hz, 1H), 5.83–5.85 (m, 1H), 5.61–5.64 (m, 1H), 4.59–4.63 (m, 1H), 4.05–4.18 (m, 1H),
1.53 (s, 9H). ESI MS *m/z* 549.0 [M + H]⁺.

Step 2: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-amino-5-(difluoromethyl)-5,6,7,7a-
tetrahydro-3a*H*-pyrano[3,2-d]thiazole-6,7-diol (**44**). A solution of **88** (600 mg, 1.1 mmol)

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4 in MeOH (50 mL) was treated with K₂CO₃ (45 mg, 0.33 mmol) for 3 h at room temperature.
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7 The reaction mixture was neutralized by addition of AcOH, and was condensed to give a
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10 residue, which was purified by a silica gel column eluted with 1% ~ 5% MeOH in DCM to
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13 give *tert*-butyl (3*aR*,5*S*,6*S*,7*R*,7*aR*)-5-(difluoromethyl)-6,7-dihydroxy-5,6,7,7*a*-tetrahydro-
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15
16 3*aH*-pyrano[3,2-*d*]thiazol-2-ylcarbamate as a solid (300 mg, 81% yield). ¹H NMR (300
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19 MHz, CDCl₃) δ 6.17 (d, *J* = 6.6 Hz, 1H), 5.85–6.21 (td, *J* = 51.6 Hz, 2.7 Hz, 1H), 3.84–
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21
22 4.16 (m, 4H), 1.56 (s, 9H). ESI MS *m/z* 341.0 [M + H]⁺. A solution of this material (135
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25 mg, 0.4 mmol) in DCM (10 mL) was treated with TFA (1mL) overnight at room
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28 temperature. Removal of solvents gave a residue, which was dissolved into MeOH (5 mL)
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31 and neutralized by concentrated NH₄OH (0.5 mL, 25% ~ 28%, w/v). Concentration and
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33
34 purification by Prep-HPLC under the following conditions [(Agilent 1200): Column, X-
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37 Bridge C18; mobile phase, 50mmol/L NH₄HCO₃ in water with 0.05% NH₄OH and CH₃CN
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40 (CH₃CN 5% up to 20% in 10min); Detector, 220nm UV] afforded **44** as a white solid (26.7
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43 mg, 27% yield). ¹H NMR (CD₃OD, 300 MHz) δ 6.28 (d, *J* = 6.3 Hz, 1H), 5.50–6.14 (td, *J*
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46 = 54.3 Hz, 2.1 Hz, 1H), 4.16–4.20 (m, 1H), 4.02–4.05 (t, *J* = 4.5 Hz, 1H), 3.74–3.80 (m,
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49 1H), 3.63–3.71 (m, 1H). ESI MS *m/z* 240.9 [M + H]⁺.
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(3*aR*,5*S*,6*S*,7*R*,7*aR*)-5-(difluoromethyl)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diol (45). Step 1: Preparation of (3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (Exp #45a). To a solution of (3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diol (0.84 g, 2.31 mmol) in pyridine (10 mL), at 0 °C was added DMAP (0.028 g, 0.23 mmol) followed by BzCl (1.6 mL, 13.8 mmol) slowly. The mixture was warmed to room temperature and stirred overnight. The reaction was diluted with EtOAc (50 mL), washed with saturated NaHCO₃ solution and brine. The organic extract was dried over anhydrous Na₂SO₄ and concentrated under reduced pressure. The residual pyridine was co-evaporated with hexanes and the crude residue was separated on silica gel by flash column chromatography (EtOAc/hexanes, 2:3) to give (3*aR*,5*R*,6*S*,7*R*,7*aR*)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-2-(dimethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (0.9 g, 68% yield) as a foamy white solid. ¹H NMR (400 MHz, CDCl₃) δ 8.04–8.01 (m, 4H), 7.53–7.47 (m, 2H), 7.40–7.32 (m, 4H), 6.36 (d, *J* = 5.2 Hz, 1H), 5.81 (t, *J* = 2.4 Hz, 1H), 5.35–5.33 (dd, *J* = 7.1, 0.9 Hz, 1H), 4.59 (t, *J*

= 4.2 Hz, 1H), 3.92–3.89 (m, 1H), 3.80–3.73 (m, 2H), 3.05 (s, 6H), 0.83 (s, 9H), 0.01 (s, 6H).

Step 2: Preparation of (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano-[3,2-*d*]thiazole-6,7-diyl dibenzoate (Exp #45b). To a solution of the above material (0.88 g, 1.54 mmol) in dry MeOH (6 mL) was added AcCl (0.054 mL, 0.77 mmol) at 0 °C. The reaction mixture was stirred at this temperature for 30 min and then at room temperature overnight. The reaction mixture was diluted with DCM (30 mL) and neutralized with 10% aq. NaHCO₃ solution. DCM layer was further washed with brine, dried over anhydrous Na₂SO₄ and concentrated. The crude residue was purified by silica gel column chromatography (EtOAc/hexanes, 1:1) to provide (3a*R*,5*R*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano-[3,2-*d*]thiazole-6,7-diyl dibenzoate (0.57 g, 81% yield) as a foamy solid. ¹H NMR (400 MHz, CDCl₃) δ 8.03–8.01 (m, 4H), 7.55–7.49 (m, 2H), 7.41–7.37 (m, 4H), 6.36 (d, *J* = 6.5 Hz, 1H), 5.84–5.83 (dd, *J* = 4.3, 3.0 Hz, 1H), 5.32–5.28 (m, 1H), 4.57 (t, *J* = 5.2 Hz, 1H), 3.92–3.88 (m, 1H), 3.78–3.75 (dd, *J* = 12.4, 2.5 Hz, 1H), 3.70–3.65 (dd, *J* = 12.4, 5.4 Hz, 1H), 3.03 (s, 6H).

Step 3: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-5-formyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (Exp #45c). To a solution of the above material (0.57 g, 1.25 mmol) in dry DCM (10 mL) at 0 °C was added DMP (0.8 g, 1.88 mmol). The reaction was stirred at 0 °C for 10 min and at room temperature for next 1.5 h when the starting material was completely consumed. The reaction mixture was diluted 1:1 1M Na₂S₂O₃: Saturated NaHCO₃ (30 mL) and stirred for 10 min. DCM layer was separated, dried over anhydrous Na₂SO₄ and concentrated to yield crude foamy solid containing (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(dimethylamino)-5-formyl-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (0.56 g crude). The crude product was carried forward for the next reaction without any further purification.

Step 4: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (Exp #45d). The above material (0.56 g, crude) was taken in DCM (10 mL) and cooled to -78 °C. DAST (0.72 mL, 5.5 mmol) was added dropwise while stirring at -78 °C. After the addition, cooling bath was removed, and reaction mixture stirred at room temperature overnight. The reaction was diluted with saturated NaHCO₃ solution (15 mL). DCM layer was

separated, dried over anhydrous Na_2SO_4 and concentrated. The crude residue was purified by silica gel column chromatography (EtOAc/hexanes, 1:4) to provide (3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (0.330 g, 55% yield over 2 steps) as a foamy solid. ^1H NMR (400 MHz, CDCl_3) δ 8.06–8.01 (m, 4H), 7.57–7.53 (m, 2H), 7.44–7.39 (m, 4H), 6.38 (d, J = 5.3 Hz, 1H), 5.99–5.77 (m, 1H), 5.85 (m, 1H), 5.54 (d, J = 6.8 Hz, 1H), 4.66–4.64 (m, 1H), 4.06–4.01 (m, 1H), 3.06 (s, 6H).

Step 5: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(dimethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (45**).** To a stirred solution of the above material (0.33 g, 0.69 mmol) in dry MeOH (8 mL) was added K_2CO_3 (0.073 g, 0.53 mmol) at 0 °C. The reaction mixture was warmed to room temperature and stirred 1.5 h. AcOH (0.5 mL) was added to the reaction mixture and contents were concentrated. The AcOH salt thus obtained was treated with 2M NH_3/MeOH solution (8 mL) and again concentrated. The residue was purified by silica gel column chromatography (DCM/MeOH, 9:1) to provide **45** as a foamy solid (0.1 g, 54% yield). ^1H NMR (400 MHz, CD_3OD) δ 6.31 (d, J = 6.4 Hz, 1H), 5.98 (td, J = 54.1, 2.0 Hz, 1H), 4.20 (t, J = 5.6 Hz,

1H), 4.04 (t, J = 4.7 Hz, 1H), 3.78–3.66 (m, 2H), 3.03 (s, 6H). ^{13}C NMR (100 MHz, CD_3OD) δ 166.20, 116.56 (t, $J_{\text{C6,F}}$ 241.2 Hz, C-6), 91.41, 77.21, 75.57, 75.04 (t, J = 21.0 Hz), 70.68 (t, J = 3.3 Hz, C-4), 41.17. HRMS (ESI+) m/z $[\text{M} + \text{H}]^+$ calculated for $\text{C}_9\text{H}_{14}\text{F}_2\text{N}_2\text{O}_3\text{S}$: 269.0771; found: 269.0774.

(3*aR*,5*S*,6*R*,7*R*,7*aR*)-5-(difluoromethyl)-7-fluoro-2-(methylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-6-ol (46). Step 1: Preparation of *tert*-butyl ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-6-(benzyloxy)-7-fluoro-5-(hydroxymethyl)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (92). At 0 °C, to a solution of **91** (1.06 g, 2.35 mmol) and Bu_4NI (0.087 g, 0.24 mmol) in anhydrous DMF (15 mL) was added NaH (60% in mineral oil, 0.118 g, 2.94 mmol). After addition of NaH, to the reaction mixture was added BnBr (0.703 g, 4.11 mmol). The mixture was stirred at room temperature for 16 h and diluted with Et_2O (60 mL) and saturated NH_4Cl (50 mL). The organic layer was collected, and the aqueous was extracted with Et_2O (2 \times 30 mL). The combined extract was washed with brine (40 mL) and dried over anhydrous Na_2SO_4 . After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc /hexanes, 1:10 to 1:4),

affording *tert*-butyl ((3*aR*,5*R*,6*R*,7*R*,7*aR*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-fluoro-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate as a sticky oil (1.22 g, 96% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.37–7.27 (m, 5H), 6.10 (d, *J* = 7.0 Hz, 1H), 5.30–5.16 (m, 1H), 4.80 (d, *J* = 11.0 Hz, 1H), 4.55 (d, *J* = 11.0 Hz, 1H), 4.48–4.42 (m, 1H), 3.88–3.80 (m, 1H), 3.78–3.69 (m, 2H), 3.46–3.44 (m, 1H), 3.31 (s, 3H), 1.53 (s, 9H), 0.89 (s, 9H), 0.04 (s, 6H). At 0 °C, to a solution of this material (1.22 g, 2.25 mmol) in THF (15 mL) was added TBAF (1.0 M in THF, 5.0 mL, 5.0 mmol). After addition the reaction mixture was stirred at room temperature for 2 h and diluted with EtOAc (20 mL) and brine (50 mL). The organic layer was collected, and the aqueous was extracted with EtOAc (2 × 50 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:5 to 1:2), affording **92** as a white solid (0.96 g, 100% yield). ¹H NMR (400 MHz, CDCl₃) δ 7.37–7.29 (m, 5H), 6.09 (d, *J* = 6.7 Hz, 1H), 5.32 (ddd, *J* = 1.8, 3.6, 45.4 Hz, 1H), 4.80 (d, *J* = 11.6 Hz, 1H), 4.55 (d, *J* = 11.6 Hz, 1H), 4.53–4.48 (m, 1H), 3.81–3.72 (m, 2H), 3.61–3.55 (m, 1H), 3.49–3.45 (m, 1H), 3.31 (s, 3H), 1.53 (s, 9H).

Step 2: Preparation of *tert*-butyl ((3*aR*,5*S*,6*R*,7*R*,7*aR*)-6-(benzyloxy)-5-(difluoromethyl)-7-fluoro-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (**93**). To a solution of **92** (1.50 g, 3.52 mmol) in DCM (40 mL) was added DMP (2.20 g, 5.20 mmol). After stirring at room temperature for 1 h the reaction mixture was diluted with Et₂O (20 mL), and then concentrated to dryness. Saturated aqueous NaHCO₃ (30 mL) with Na₂S₂O₃ (2 g) was added, and the mixture was extracted with EtOAc (2 × 50 mL). The combined extract was dried over anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure, and the residue was purified on silica gel by flash column chromatography (EtOAc/hexanes, 1:5 to 1:2), affording *tert*-butyl ((3*aR*,5*S*,6*R*,7*R*,7*aR*)-6-(benzyloxy)-7-fluoro-5-formyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate as a white solid (1.02 g, 68% yield). ¹H NMR (400 MHz, CDCl₃) δ 9.60 (s, 1H), 7.35–7.29 (m, 5H), 6.12 (d, *J* = 7.0 Hz, 1H), 5.39–5.27 (m, 1H), 4.78 (d, *J* = 11.4 Hz, 1H), 4.66 (d, *J* = 11.4 Hz, 1H), 4.57–4.51 (m, 1H), 4.00–3.95 (m, 2H), 3.31 (s, 3H), 1.53 (s, 9H). To a solution of this material (0.156 g, 0.367 mmol) in anhydrous DCM (6 mL) at -78 °C under N₂, was added DAST (0.354 g, 2.20 mmol). After addition the mixture was stirred at room temperature for 16 h. The

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4 reaction mixture was then cooled at -78 °C, diluted with DCM (10 mL), and then quenched
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7 with saturated aqueous NaHCO₃ (10 mL). The organic layer was collected, and the
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10 aqueous was extracted with DCM (2 × 15 mL). The combined extract was dried over
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13 anhydrous Na₂SO₄. After filtration the solvent was evaporated under reduced pressure,
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16 and the residue was purified on silica gel by flash column chromatography
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19 (EtOAc/hexanes, 1:10 to 1:4), affording **93** as a pale-yellow oil (0.125 g, 76% yield). ¹H
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21 NMR (400 MHz, CDCl₃) δ 7.38–7.29 (m, 5H), 6.11 (d, *J* = 7.2 Hz, 1H), 5.79 (dt, *J* = 2.4,
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24 54.4 Hz, 1H), 5.37–5.25 (m, 1H), 4.78 (d, *J* = 11.3 Hz, 1H), 4.59 (d, *J* = 11.3 Hz, 1H),
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27 4.58–4.53 (m, 1H), 4.00–3.92 (m, 1H), 3.72–3.63 (m, 1H), 3.29 (s, 3H), 1.53 (s, 9H).
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35 **Step 3: Preparation of (3a*R*,5*S*,6*R*,7*R*,7a*R*)-5-(difluoromethyl)-7-fluoro-2-**
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38 **(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (**46**).** To a solution of **93**
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41 (0.240 g, 0.537 mmol) and pentamethylbenzene (0.26 g, 1.7 mmol) in anhydrous DCM
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44 (10 mL) at -78 °C under N₂, was added BCl₃ (1.0 M in DCM, 1.6 mL, 1.6 mmol). The
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47 mixture was stirred at room temperature for ~3 h while the temperature of the cooling trap
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50 reached at 0 °C. The reaction mixture was cooled at -78 °C, quenched with mixed
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53 MeOH/DCM, and then concentrated to dryness. The residue was purified on silica gel by
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flash column chromatography (1.0 M NH₃ in MeOH/DCM, 1: 15), affording **46** as an off-white solid (0.104 g, 76% yield). ¹H NMR (400 MHz, CD₃OD) δ 6.30 (d, *J* = 6.6 Hz, 1H), 5.96 (dt, *J* = 2.4, 54.1 Hz, 1H), 4.89 (td, *J* = 3.9, 46.5 Hz, 1H), 4.48–4.42 (m, 1H), 4.02–3.94 (m, 1H), 3.72–3.63 (m, 1H), 2.85 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 164.08 (d, *J* = 2.3 Hz), 115.63 (dt, *J* = 7.9, 241.6 Hz), 94.73 (d, *J* = 177.6 Hz), 89.90 (d, *J* = 2.0 Hz), 74.04 (d, *J* = 26.1 Hz), 72.92 (dt, *J* = 3.9, 21.3 Hz), 67.87 (td, *J* = 3.8, 25.0 Hz), 30.74. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₈H₁₁F₃N₂O₂S: 257.0571; found: 257.0580.

(3a*R*,5*S*,6*R*,7*S*,7a*R*)-5-(difluoromethyl)-7-fluoro-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (47). Step 1: Preparation of *tert*-butyl ((3a*R*,5*R*,6*R*,7*S*,7a*R*)-6-(benzyloxy)-7-fluoro-5-(hydroxymethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (74). To a solution of **73** (1.30 g, 2.89 mmol) and Bu₄NI (0.107 g, 0.290 mmol) in anhydrous DMF (12 mL) at 0 °C was slowly added NaH (60% in mineral oil, 0.145 g, 3.63 mmol) followed by dropwise addition of BnBr (0.989 g, 5.78 mmol). After stirring at 0 °C for 30 min then at room temperature overnight, the mixture was diluted with Et₂O (100 mL). The mixture was washed with saturated aqueous NH₄Cl (2 × 50 mL). The aqueous was extracted with Et₂O (2 × 40 mL).

The combined organic extract was washed with brine (50 mL), dried over anhydrous Na_2SO_4 , concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2 ; EtOAc/hexanes 1:20 to 1:4), affording *tert*-butyl ((3*aR*,5*R*,6*R*,7*S*,7*aR*)-6-(benzyloxy)-5-(((*tert*-butyldimethylsilyl)oxy)methyl)-7-fluoro-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (1.44 g, 92% yield) as a sticky oil. ^1H NMR (400 MHz, CDCl_3) δ 7.36–7.27 (m, 5H), 6.21 (d, J = 7.2 Hz, 1H), 5.30–5.16 (m, 1H), 4.80 (d, J = 11.4 Hz, 1H), 4.56 (d, J = 11.4 Hz, 1H), 4.50–4.42 (m, 1H), 3.95–3.78 (m, 4H), 3.44 (s, 3H), 1.54 (s, 9H), 0.89 (s, 9H), 0.049 (s, 6H). To a solution of this material (1.44 g, 2.66 mmol) in THF (25 mL) at 0 °C was added TBAF (1.0 M in THF, 3.5 mL, 3.5 mmol). The reaction was stirred at room temperature for 2 h and diluted with brine (50 mL). The mixture was extracted with EtOAc (2 \times 40 mL). The combined organic extract was dried over anhydrous Na_2SO_4 , concentrated in vacuo, and the residue was purified by flash chromatography (SiO_2 ; EtOAc/hexanes 1:2 to 1:1), affording **74** (1.08 g, 95% yield) as a white solid. ^1H NMR (400 MHz, CDCl_3) δ 7.37–7.27 (m, 5H), 6.18 (d, J = 7.4 Hz, 1H), 5.17–5.04 (m, 1H), 4.84 (d, J = 11.6 Hz, 1H), 4.55 (d, J = 11.6 Hz, 1H), 4.50–4.43 (m,

1H), 3.95–3.91 (m, 1H), 3.88–3.82 (m, 1H), 3.79–3.75 (m, 1H), 3.71–3.67 (m, 1H), 3.37 (s, 3H), 1.53 (s, 9H).

Step 2: Preparation of *tert*-butyl ((3*aR*,5*S*,6*R*,7*S*,7*aR*)-6-(benzyloxy)-5-(difluoromethyl)-7-fluoro-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate (75). To a solution of **74** (2.57 g, 6.03 mmol) in DCM (60 mL) at 0 °C was added DMP (3.82 g, 9.00 mmol). After stirring at room temperature for 1 h the mixture was diluted with Et₂O (100 mL). The resulting suspension was filtered through a Celite cake and the filtrate was concentrated in vacuo. The residue was extracted with EtOAc (3 × 50 mL), and the solid was filtered off. The combined organic extract was washed with mixed saturated aqueous NaHCO₃ (30 mL) and Na₂S₂O₃ (5 mL), dried over anhydrous MgSO₄, and concentrated in vacuo to give the crude aldehyde *tert*-butyl ((3*aR*,5*S*,6*R*,7*S*,7*aR*)-6-(benzyloxy)-7-fluoro-5-formyl-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(methyl)carbamate.

This material was used without further purification. ¹H NMR (400 MHz, CDCl₃) δ 9.65 (s, 1H), 7.39–7.29 (m, 5H), 6.04 (d, *J* = 7.0 Hz, 1H), 5.08 (td, *J* = 4.2, 46.7 Hz, 1H), 4.84 (d, *J* = 11.4 Hz, 1H), 4.64 (d, *J* = 11.4 Hz, 1H), 4.55–4.49 (m, 1H), 4.31 (d, *J* = 7.5 Hz, 1H), 4.19–4.15 (m, 1H), 3.30 (s, 3H), 1.52 (s, 9H). To a solution of this material (0.19 g, ~80%

pure, ~0.36 mmol) in anhydrous DCM (6 mL) at -78 °C was added DAST (0.37 g, 2.3 mmol). The reaction was stirred at room temperature for 24 h. The mixture was then cooled to -78 °C and quenched with saturated aqueous NaHCO₃ (10 mL). The organic layer was collected and the aqueous was extracted with DCM (2 × 10 mL). The combined organic extract was dried over anhydrous Na₂SO₄, concentrated in vacuo, and the residue was purified by flash chromatography (SiO₂; EtOAc/hexanes 1:20 to 1:4), affording **75** (0.097 g, 60% yield) as a white foam. ¹H NMR (500 MHz, CDCl₃) δ 7.37–7.28 (m, 5H), 6.12 (d, *J* = 6.9 Hz, 1H), 5.85 (dt, *J* = 1.6, 54.5 Hz, 1H), 5.11 (td, *J* = 4.0, 47.3 Hz, 1H), 4.83 (d, *J* = 11.2 Hz, 1H), 4.57 (d, *J* = 11.2 Hz, 1H), 4.55–4.50 (m, 1H), 4.12–4.04 (m, 2H), 3.28 (s, 3H), 1.52 (s, 9H).

Step 3: Preparation of (3a*R*,5*S*,6*R*,7*S*,7a*R*)-5-(difluoromethyl)-7-fluoro-2-(methylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-6-ol (47). To a solution of **75** (0.097 g, 0.22 mmol) and pentamethylbenzene (0.050 g, 0.34 mmol) in anhydrous DCM (3 mL) at -78 °C was added BCl₃ (1.0 M in DCM, 1.0 mL, 1.0 mmol). The mixture was allowed to warm to room temperature and stirred for 5 h. The reaction was then cooled to -78 °C, quenched with mixed MeOH/DCM, and concentrated in vacuo. The residue

was purified by flash chromatography (SiO₂; 1.0 M NH₃ in MeOH/DCM 1:12) affording **47** (0.041 g, 72% yield) as a white solid. ¹H NMR (400 MHz, CD₃OD) δ 6.37 (d, *J* = 6.6 Hz, 1H), 6.04 (dt, *J* = 1.5, 54.0 Hz, 1H), 4.86 (td, *J* = 3.3, 50.9 Hz, 1H), 4.39 (ddd, *J* = 3.8, 6.6, 20.9 Hz, 1H), 4.05–3.95 (m, 2H), 2.86 (s, 3H); ¹³C NMR (100 MHz, CD₃OD) δ 165.00, 115.89 (t, *J* = 242.6 Hz), 90.65 (d, *J* = 185.7 Hz), 90.25 (d, *J* = 3.5 Hz), 72.22–71.71 (m), 71.71 (d, *J* = 16.1 Hz), 66.22–65.97 (m), 30.48. HRMS (ESI+) *m/z* [M + H]⁺ calculated for C₈H₁₁F₃N₂O₂S: 257.0571; found: 257.0569.

(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (48). Step 1: Preparation of **(3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diyl dibenzoate (97).** To a solution of **96** (400 mg, 0.85 mmol) in DCM (20 mL) at 0 °C was added DMP (600 mg, 1.41 mmol) and the reaction was stirred 10 min at 0 °C then warmed to room temperature and stirred for 2 h. The resulting solution was quenched with saturated aqueous NaHCO₃ (10 mL) and saturated aqueous Na₂S₂O₃ (10 mL) and extracted with DCM (3 × 20 mL). The combined organic extract was dried over anhydrous Na₂SO₄ and concentrated in vacuo to afford a residue, which was dissolved in DCM (20

mL) and treated with DAST (5 mL, 4.48 mmol) at -78 °C. The resulting solution was stirred overnight at 15 °C and was quenched with saturated aqueous NaHCO₃ (10 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (2 × 20 mL). The combined organic layers were dried over anhydrous Na₂SO₄ and concentrated in vacuo. The residue was purified by flash chromatography (SiO₂; 20% EtOAc in petroleum ether) to afford **97** (240 mg, 46% yield) as light yellow oil. ¹H NMR (300 MHz, CDCl₃) δ 8.03–8.13 (m, 4H), 7.56–7.63 (m, 2H), 7.42–7.49 (m, 4H), 6.48 (d, *J* = 6.6 Hz, 1H), 5.94–5.96 (m, 2H), 5.58 (d, *J* = 8.7 Hz, 1H), 4.73 (s, 1H), 4.07–4.17 (m, 4H), 2.43–2.51 (m, 2H). ESI MS *m/z* 488.9 [M + H]⁺.

Step 2: Preparation of (3a*R*,5*S*,6*S*,7*R*,7a*R*)-2-(azetidin-1-yl)-5-(difluoromethyl)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (48**).** A solution of **97** (110 mg, 0.23 mmol) in MeOH (10 mL) at room temperature was treated with K₂CO₃ (20 mg, 0.14 mmol) for 2 h, and then neutralized by addition of AcOH. Concentration in vacuo gave a residue, which was purified by flash chromatography (SiO₂; 10% MeOH in DCM) to afford **48** (24 mg, 22% yield) as a white solid. ¹H NMR (300 MHz, D₂O) δ 6.29 (d, *J* = 6.3 Hz,

1H), 5.82–6.00 (td, $J = 54.0$ Hz, 1.8 Hz, 1H), 4.24 (t, $J = 5.7$ Hz, 1H), 3.97–4.04 (m, 5H), 3.82–3.87 (m, 1H), 3.69–3.80 (m, 1H), 2.29 (m, 2H). ESI MS m/z 280.9 $[M + H]^+$.

(3a*R*,5*S*,(³H)6*S*,7*R*,7a*R*)-5-(difluoromethyl)-2-(ethylamino)-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazole-6,7-diol (³H)42). Step 1: Preparation of *tert*-butyl ((3a*R*,5*S*,6*S*,7*R*,7a*R*)-5-(difluoromethyl)-6,7-dihydroxy-5,6,7,7a-tetrahydro-3a*H*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (**98**). To a solution of **42** (5.00 g, 18.6 mmol) in MeOH (100 mL) under a nitrogen atmosphere was added di-*tert*-butyl dicarbonate (6.10 g, 28.0 mmol). The resulting solution was stirred for 16 h at room temperature. The solvent was concentrated under vacuum and purified by reverse-phase flash chromatography (CombiFlash-1): Column, C18 silica gel; mobile phase, acetonitrile/(5%NH₄HCO₃) water=30/70 increasing to acetonitrile/(5%NH₄HCO₃) water=60/40 within 30 min; Detector, UV 210 nm. This resulted in **98** (3.60 g, 9.53 mmol, 51.1 % yield) as an off-white solid. ¹H NMR (400 MHz, CD₃OD) δ 6.08 (d, $J = 8.0$ Hz, 1H), 5.96 (td, $J = 52.0, 2.0$ Hz, 1H), 4.22 (t, $J = 6.0$ Hz, 1H), 4.14 (t, $J = 6.0$ Hz, 1H), 4.00–3.92 (m, 2H), 3.85 (dd, $J = 4.0$ Hz, $J = 12.0$ Hz, 1H), 3.59–3.50 (m, 1H), 1.56 (s, 9H), 1.20 (t, $J = 8.0$ Hz, 3H). ESI MS m/z 368.1 $[M + H]^+$.

Step 2: Preparation of *tert*-butyl ((3*aR*,5*S*,6*R*,7*R*,7*aR*)-7-((*tert*-butyldimethylsilyl)oxy)-5-(difluoromethyl)-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazol-2-yl)(ethyl)carbamate (99). To a solution of **98** (3.2 g, 8.69 mmol) in DMF (128 ml), under nitrogen was added 1*H*-imidazole (1.774 g, 26.1 mmol) and TBDMSCl (3.93 g, 26.1 mmol) at room temperature. Then the mixture was stirred 8 h at 40 °C, then quenched by the addition of 300 ml water/ice and extracted with DCM. The organic layers were combined and washed with sodium bicarbonate and brine, dried over anhydrous sodium sulfate and concentrated under vacuum. The crude product was purified by Prep-SFC with the following conditions (Prep SFC350-2): Column: Phenomenex Lux 5u Cellulose-45*25cm,5um; mobile phase, CO₂:80%, MeOH:20%; detector, UV 220 nm. This resulted in **99** (2.4 g, 4.92 mmol, 56.7 % yield) as a clear oil. ¹H NMR (300 MHz, CD₃Cl₃) δ 5.96 (td, *J* = 55.2 Hz, *J* = 3.9 Hz, 1H), 5.84 (d, *J* = 5.4 Hz, 1H), 4.41 (s, 1H), 4.05 (t, *J* = 4.8 Hz, 1H), 3.99–3.94 (m, 1H), 3.91–3.84 (m, 2H), 3.71–3.67 (m, 1H), 2.47 (s, 1H), 1.54 (s, 9H), 1.18 (t, *J* = 6.9 Hz, 3H), 0.915 (s, 9H), 0.16 (s, 6H). ESI MS *m/z* 483.3 [M + H]⁺.

Step 3: Preparation of *tert*-butyl ((3*aR*,5*S*,([³H])6*R*,7*R*,7*aR*)-7-((*tert*-butyldimethylsilyl)oxy)-5-(difluoromethyl)-6-hydroxy-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-

d[thiazol-2-yl)(ethyl)carbamate (**100**). To a solution of **99** (440 mg, 0.91 mmol) in DCM (20 mL) was added DMP (587 mg, 1.38 mmol). The resulting solution was stirred for 1 h at 5 °C, then quenched by the addition of 20 mL of saturated aqueous NaHCO₃/NaS₂O₃. The resulting solution was extracted with DCM and the organic layers combined and concentrated under vacuum. The residue was applied onto a silica gel column with EtOAc/petroleum ether (1:40). This resulted in 370 mg (84%) of *tert*-butyl *N*-[(3*aR*,5*S*,7*R*,7*aR*)-7-[(*tert*-butyldimethylsilyl)oxy]-5-(difluoromethyl)-6-oxo-3*aH*,5*H*,6*H*,7*H*,7*aH*-pyrano[3,2-*d*][1,3]thiazol-2-yl]-*N*-ethylcarbamate as a white syrup. To a 1 dram vial of this material (24.5 mg, 0.051 mmol) in 200 μL MeOH at 0 °C was added the contents of a 25 mg ampoule containing sodium borotritide (0.05 mmol) (ARC, 1 Ci, 80 Ci/mmol) followed by a 200 μL MeOH wash of the vial. After 2 h, the reaction was transferred with DCM to a 20 mL scintillation vial containing H₂O. The aqueous layer was extracted with DCM. The combined organic extracts were passed through a plug of Na₂SO₄ and concentrated to give a film that was taken up in 10 mL DCM and counted at 204.75 mCi. The batch was analyzed by RP-HPLC (Gemini C18 column 3×150mm, flow rate: 0.5 mL/min; 0.1% aqueous HCO₂H / CH₃CN) revealing a 2:1 mixture of the desired

6*R*:6*S* diastereomers. Purification by RP-HPLC (Gemini C18 column, 10x250mm, flow rate 5 mL/min, 80/20 0.05M pH 9 TEAA / CH₃CN) provided fractions that were concentrated to a thin film that was taken up in 10 mL EtOH and counted at 84.70 mCi. The batch was analyzed by RP-HPLC to be 98.7% of the 6*R* diastereomer of **100**, which was used in the next step without further characterization.

Step 4: Preparation of (3*aR*,5*S*,([³H])6*S*,7*R*,7*aR*)-5-(difluoromethyl)-2-(ethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diol ([³H]42**).** To a solution of **100** (24 mg, 0.062 mmol) (mass is approximate, 84.7 mCi) in DMF (0.25 mL) was added 6 N aqueous HCl (0.25 mL, 3.04 mmol). After 4 h, the reaction was neutralized by the addition of satd. aqueous NaHCO₃ (70 drops) followed by an equal volume of CH₃CN. Purification by RP-HPLC (Chirobiotic T column, 4.6x250 mm, flow rate 4.0 mL/min, 3:1 CH₃CN / 10 mM NH₄OAc) provided 4.24 mCi of (3*aR*,5*S*,([³H])6*S*,7*R*,7*aR*)-5-(difluoromethyl)-2-(ethylamino)-5,6,7,7*a*-tetrahydro-3*aH*-pyrano[3,2-*d*]thiazole-6,7-diol ([³H]**42**), with identical HPLC retention time to unlabeled **42**. ESI MS *m/z* 271.0 [M + H]⁺.

ASSOCIATED CONTENT

Supporting Information.

This material is available free of charge via the internet at <http://pubs.acs.org>.

Assay conditions for determination of K_i values for inhibition of β -hexosaminidase activity; ELISA assay for determination of EC_{50} values for cell-based inhibition of O-GlcNAcase activity; stereochemistry determination for **21** and **22**; X-ray crystallography for **42** bound to hOGA (PDF). Molecular formula strings (CSV).

Accession Codes.

PDB code for **42** bound to hOGA is 6PM9.

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Notes

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ABBREVIATIONS USED

ABCN, 1,1'-Azobis(cyclohexanecarbonitrile); AD, Alzheimer's disease; Boc₂O, di-*tert*-butyl decarbonate; CDI, carbonyldiimidazole; CNS, central nervous system; CSF, cerebrospinal fluid; DAST, diethylaminosulfur trifluoride; DCM, dichloromethane; DIPEA, *N,N*-diisopropylethylamine; DMAP, *N,N*-dimethylaminopyridine; DMF, *N,N*-

dimethylformamide; DMP, Dess-Martin periodinane; ELISA, enzyme-linked immunosorbent assay; EtOAc, ethyl acetate; f_u , unbound fraction; GH84, Glycoside Hydrolase family 84; hHEX A/B, human lysosomal β -hexosaminidases A and B; MDR1, multidrug resistance protein 1; NFTs, neurofibrillary tangles; O-GlcNAc, O-linked N-acetylglucosamine; OGA, O-GlcNAcase; OGT, O-GlcNAc transferase; P_{app} , apparent permeability; PO, per os (by mouth); TPSA, topological polar surface area; PSP, progressive supranuclear palsy; TBAF, tetrabutylammonium fluoride; TBDMSCl, *tert*-butyldimethylsilyl chloride; TFA, trifluoroacetic acid; THF, tetrahydrofuran.

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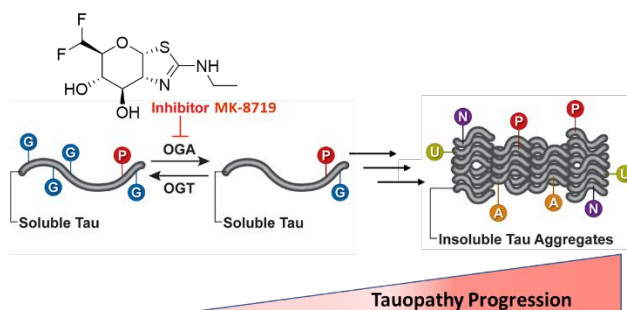
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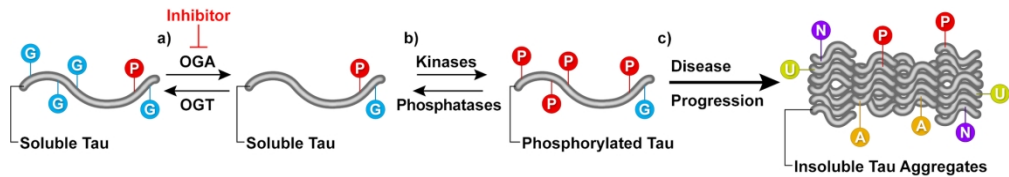


Figure 1. Hypothesis for increased O-GlcNAc modification hindering tau aggregation. a) Cellular modification of tau protein with O-GlcNAc (G) by OGT maintains the protein in a stable soluble form. Equilibrium levels of O-GlcNAc are maintained by the antagonistic actions of OGT and OGA. b) Abnormal disease-associated addition of phosphoryl residues (P) by kinases, countered by phosphatases, coupled with additional modifications including addition of nitro (N), acetyl (A) and ubiquitin (U) groups leads to oligomers and subsequently to c) insoluble tau aggregates.

176x30mm (300 x 300 DPI)