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Discovery of a Highly Potent, Nonabsorbable Apical Sodium-Dependent Bile Acid Transporter Inhibitor (GSK2330672) for Treatment of Type 2 Diabetes

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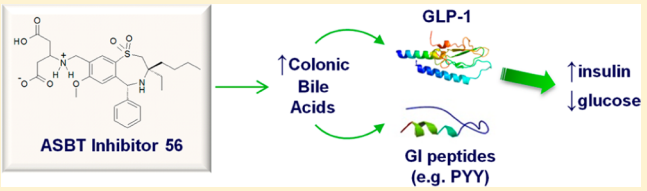
ABSTRACT: The apical sodium-dependent bile acid transporter (ASBT) transports bile salts from the lumen of the gastrointestinal (GI) tract to the liver via the portal vein. Multiple pharmaceutical companies have exploited the physiological link between ASBT and hepatic cholesterol metabolism, which led to the clinical investigation of ASBT inhibitors as lipid-lowering agents. While modest lipid effects were demonstrated, the potential utility of ASBT inhibitors for treatment of type 2 diabetes has been relatively unexplored. We initiated a lead optimization effort that focused on the identification of a potent, nonabsorbable ASBT inhibitor starting from the first-generation inhibitor 264W94 (**1**). Extensive SAR studies culminated in the discovery of GSK2330672 (**56**) as a highly potent, nonabsorbable ASBT inhibitor which lowers glucose in an animal model of type 2 diabetes and shows excellent developability properties for evaluating the potential therapeutic utility of a nonabsorbable ASBT inhibitor for treatment of patients with type 2 diabetes.



INTRODUCTION

The World Health Organization estimates that 3.4 million people died from diabetes in 2004 and projects that worldwide deaths from this debilitating disease will increase by two-thirds between 2008 and 2030. Approximately 90% of all cases of diabetes are classified as type 2 diabetes, a condition that arises when the pancreas produces insufficient insulin and/or tissues become insulin resistant, resulting in excessively high blood sugar levels which can eventually lead to severe microvascular and macrovascular complications as well as death.[1]

Current guidelines for treatment of type 2 diabetes outline lifestyle modification including weight loss and increased physical activity as an initial approach.[2] However, this approach predominantly fails, and patients will require pharmacological therapies that can include metformin, sulfonylureas, thiazolidinediones, insulin, dipeptidyl peptidase-4 (DPP-4) inhibitors, and glucagon-like-1 (GLP-1) analogues. While effective in subsets of patients with type 2 diabetes, these agents have limitations and side effects. Clearly, type 2 diabetes remains a significant unmet medical need.

The incretin peptides GLP-1 and glucose dependent insulinotropic peptide (GIP) are secreted by L and K enteroendocrine cells, respectively, from the gastrointestinal tract into the bloodstream following ingestion of nutrients. This important physiological response serves as the primary signaling mechanism between nutrients in the gastrointestinal tract and peripheral organs. Upon secretion, one of the effects of both circulating peptides is to initiate signals in beta cells of the pancreas to enhance glucose-stimulated insulin secretion, which, in turn, controls glucose concentrations in the bloodstream.[3−5]

Bile salts have been shown to enhance secretion of peptides from the gastrointestinal tract that enhance glucose-stimulated insulin secretion from the pancreatic beta cells. In particular, bile salts have been shown to promote secretion of GLP-1 from colonic L cells in a vascularly perfused rat colon model as well as GLP-1, peptide YY (PYY), and neurotensin in a vascularly perfused rat ileum model.[6,7] In humans, infusion of deoxycholate into the sigmoid colon produces a rapid and marked dose responsive increase in plasma PYY and enteroglucagon concentrations.[8] Novel mechanisms that can increase ileal and colonic bile salt concentrations would be expected to induce secretion of GLP-1 and other peptides from the distal gastrointestinal tract, providing a means to elicit an antidiabetic effect.

Bile acids are synthesized from cholesterol in the liver then, upon conjugation, released from the gallbladder into the proximal small intestine after each meal to facilitate digestion of nutrients, in particular fat, lipids, and lipid-soluble vitamins.[9−11] Conjugated bile acids then descend down the small intestine to the distal ileum, where 90% are reabsorbed into enterocytes via

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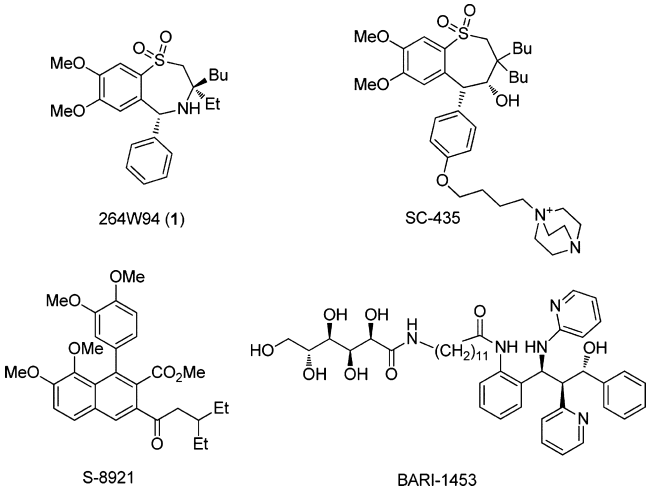
the apical sodium-dependent bile acid transporter (ASBT; also known as the ileal bile acid transporter, iBAT). Pharmacological disruption of bile acid reabsorption with an inhibitor of ASBT has been shown to increase the conversion of hepatic cholesterol to bile acids to compensate for fecal loss of bile acids.[12] While ASBT inhibitors have been explored in humans as lipid-lowering agents (Chart 1),[13] the physiological consequences of increasing the concentrations of bile salts in the colon with an ASBT inhibitor on glucose homeostasis has not been extensively explored. Data from exploratory studies in preclinical animal models of type 2 diabetes with the ASBT inhibitor 264W94 (**1**) or other inhibitors supports this strategy as a potential target for treatment of this debilitating disease.[14,15] Unfortunately, **1** is not an ideal clinical tool for testing this hypothesis in humans because the compound is extensively metabolized following oral administration, an undesirable profile which did not lead to maximal concentrations of **1** at the primary site of action in the distal ileum. Thus, we set out to identify a potent, highly soluble, nonabsorbable inhibitor of ASBT for evaluation in a preclinical animal model of type 2 diabetes and ultimately humans.

Chart 1. Chemical Structures of Select ASBT Inhibitors That Have Advanced to Clinical Development



CHEMISTRY

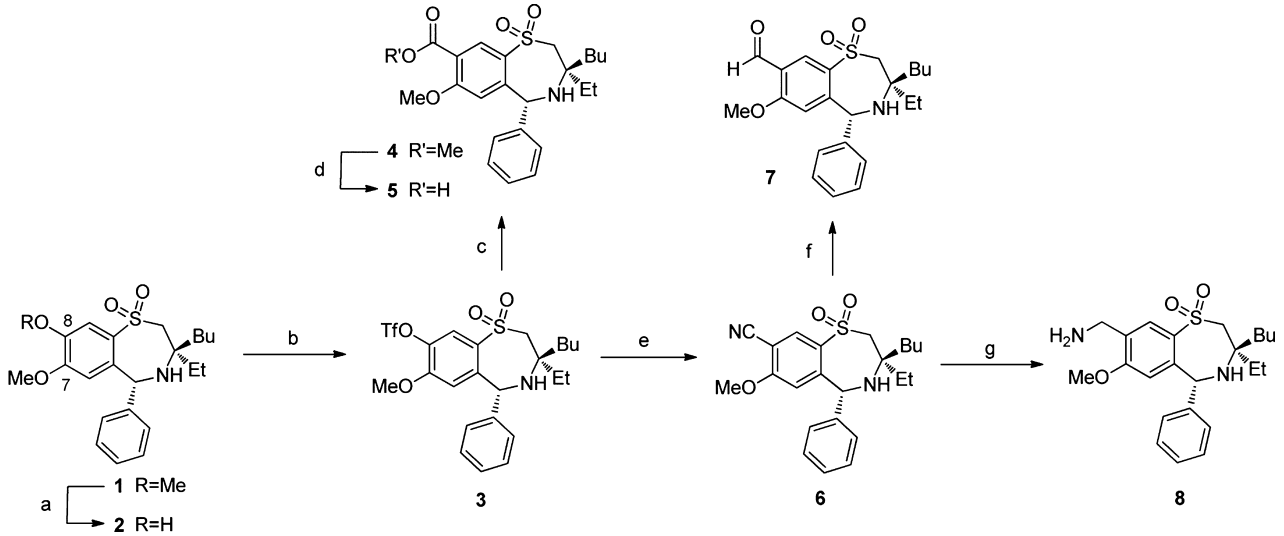
Compound **1**[16] served as the key starting material for the lead optimization program because kilogram quantities of the compound were available in our chemical stores (Scheme 1). Not surprisingly, regioselective demethylation of the C8-methoxy (see Scheme 1, compound **1** for numbering) proved to be very challenging; however, highly reproducible, scalable conditions were ultimately identified to affect clean demethylation of **1**. Pretreatment of **1** with HCl gas followed by addition of $AlCl_3$ provided a 1:1 mixture of phenol **2** and the regioisomeric C7-phenol in 96% yield. Separation of the regioisomers by chiral column chromatography provided intermediate phenol **2** in approximately 40% overall yield.

Several key synthetic intermediates were prepared from **2** (Scheme 1). Conversion of **2** to the corresponding triflate **3** was uneventful. Pd-catalyzed carbonylation in the presence of methanol provided methyl ester **4**, which was subjected to basic hydrolysis using LiOH to give the corresponding C8-carboxylic acid. Triflate **3** was also converted to C8-nitrile following subjection to $ZnCN_2$ in the presence of $Pd_2(dba)_3$.[17] DiBAL-H mediated reduction of **6** followed by hydrolysis of the intermediate imine generated aldehyde **7**. Nitrile **6** was also reduced to aminomethyl intermediate **8** upon exposure to H_2 atmosphere at 40 psi in the presence of 10% Pd/C.

A small number of C8-phenolic ethers were prepared via base-catalyzed alkylation of **2** with haloalkyl esters, haloalkyl sulfonic acids, haloalkyl phosphonates, dimethyl α-(4-chlorobenzenesulfonyl)methylphosphonate,[18] or 1,2-dibromoethane followed by bromide displacement with sulfite or trialkylphosphonate (Scheme 2). Subsequent LiOH or TMSBr-mediated deprotection of the alkyl esters or alkyl phosphonates, respectively, provided compounds **9**–**12** and **14**–**15**.

Triflate intermediate **3** proved to be a key intermediate for preparation of analogues containing alkyl and amide-linked acidic functionalities at C8 (Scheme 3). Heck reaction of **3** with

Scheme 1. Preparation of Key Intermediates[a]



[a]Reagents and conditions: (a) HCl(*g*), $AlCl_3$, DCE; (b) Tf_2O, pyridine, DCM; (c) $Pd_2(dba)_3$, dppf, CO, MeOH, 70 °C; (d) LiOH, THF, MeOH, H_2O; (e) $ZnCN_2$, $Pd_2(dba)_3$, dppf, Zn°, DMF, 80 °C; (f) DiBAL-H, toluene, DCM; (g) H_2, 10% Pd/C, EtOH, 40 psi.

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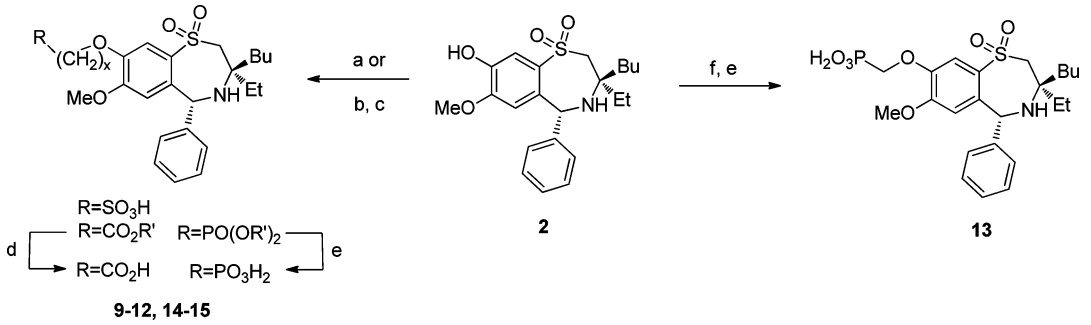
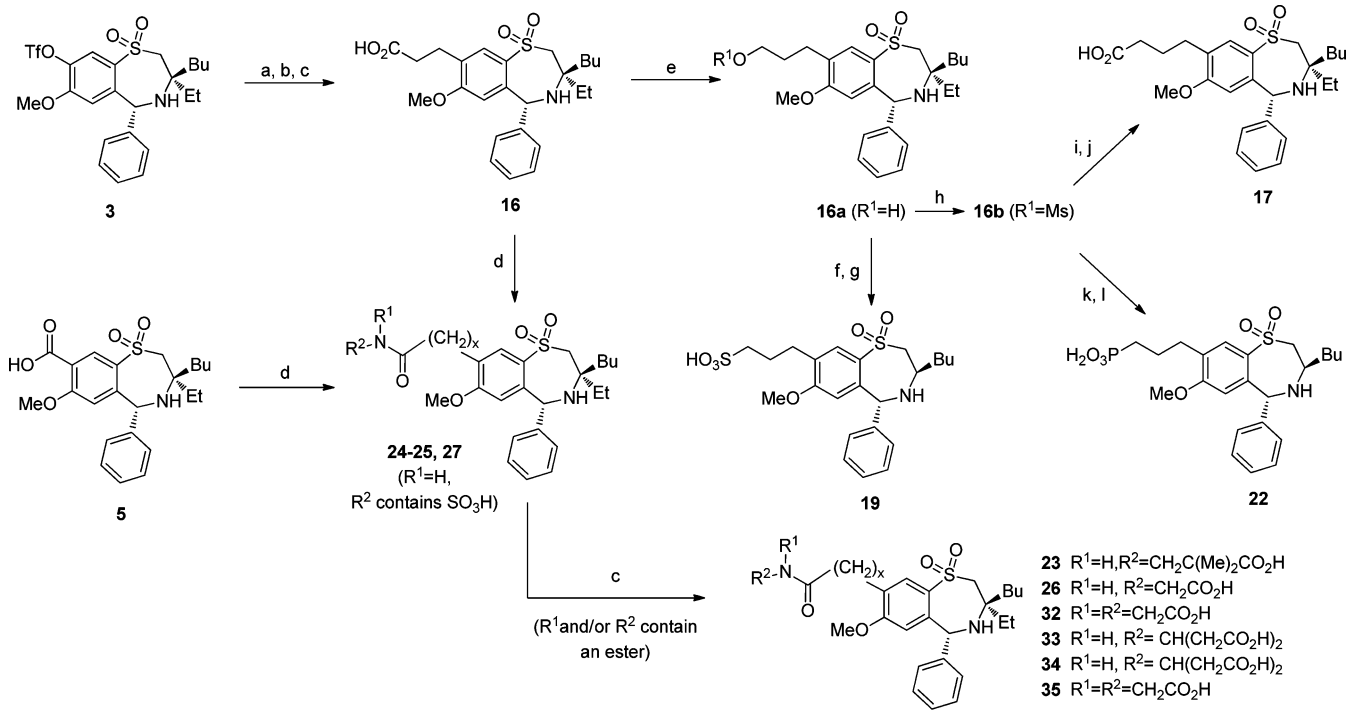
Scheme 2. General Synthetic Route to Oxygen-Linked Analogues[a]



[a]Reagents and conditions: (a) RX, K_2CO_3, DMSO, 70−100 °C; (b) 1,2-dibromoethane, 1 M KOH, Bu_4NBr, DMSO, 100 °C; (c) Na_2SO_3, EtOH, 100 °C; (d) 4 N HCl, 1,4-dioxane, DCM or LiOH, THF, MeOH, H_2O; (e) TMSBr, DCM; (f) $(EtO)_2P(O)CH_2OSO_2$-(4-Cl-Ph), NaHMDS, THF, DMSO.

Scheme 3[a]



[a]Reagents and conditions: (a) ethyl acrylate, $Pd(PPh_3)_2Cl_2$ Et_3N, DMF, 120 °C; (b) H_2, 10% Pd/C, EtOH; (c) LiOH, THF, MeOH, H_2O; (d) RNH_2, HATU, DIPEA, DCM or EDC, DMAP, DCM; (e) BH_3-THF, THF; (f) PPh_3, CBr_4, THF; (g) Na_2SO_3, EtOH, H_2O, Δ; (h) MsCl, Et_3N, DCM; (i) NaCN, DMSO, 60 °C; (j) 37% aq HCl, 100 °C; (k) $HP(O)(OEt)_2$, NaH, THF; (l) TMSBr, DCM.

ethyl acrylate in the presence of palladium catalyst at elevated temperature followed by hydrogenation of the intermediate ethyl cinnamate and saponification with LiOH provided compound **16**. Subjection of carboxylic acids **5** and **16** to substituted amines and HATU with base or EDC with DMAP led directly to amide analogues **24**−**25** and **27**. Amide analogues possessing an ester moiety were saponified with LiOH to give derivatives containing a terminal carboxylic acid (compounds **23**, **26**, and **32**−**35**). Reduction of **16** with BH_3-THF provided alcohol **16a**, which was converted to compound **19** upon bromination then reaction with Na_2SO_3. Mesylate **16b**, derived from **16a** using standard conditions, was reacted with NaCN then subjected to acidic hydrolysis to give carboxylic acid **17**. In a similar fashion, the corresponding phosphonic acid **22** was also prepared from **16b** upon conversion to a phosphonate ester followed by deprotection.

Additional amide and amine-linked analogues containing acidic functionalities were readily prepared from benzylamine intermediate **8** (Scheme 4). Compound **28** was prepared from **8** upon exposure to methyl chlorooxoacetate and Et_3N followed by saponification of the methyl ester. Acylation of **8** with haloalkyl acid chlorides in the presence of base led to intermediate **8b**, which was subsequently treated with Na_2SO_3 at elevated temperature to give sulfonic acid analogues **29**−**30**. Phosphonic acid analogue **31** was synthesized in a similar fashion using $P(OEt)_3$ as nucleophile followed by deprotection with TMSBr. Intermediate **8b** was also treated with KI and a substituted amine to give, following base-catalyzed saponification, dicarboxylic acid analogue **36**. Amine-linked analogues containing one (**47**) or two (**54**) terminal sulfonic acid moieties were generated upon heating in the presence of bromoethylsulfonic acid sodium salt, and similarly,

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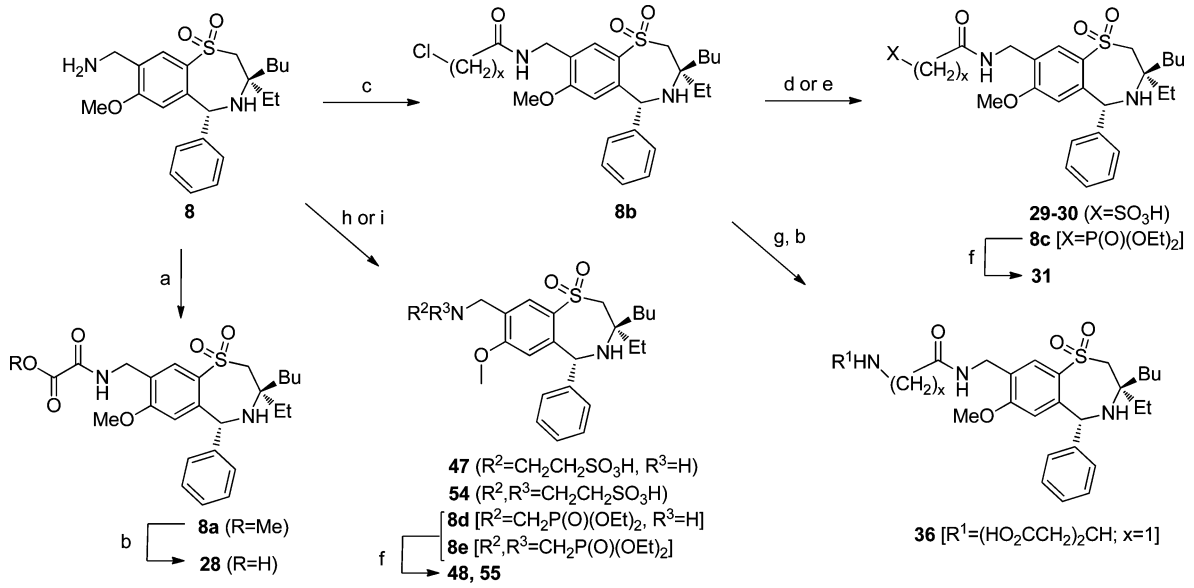
Scheme 4[a]

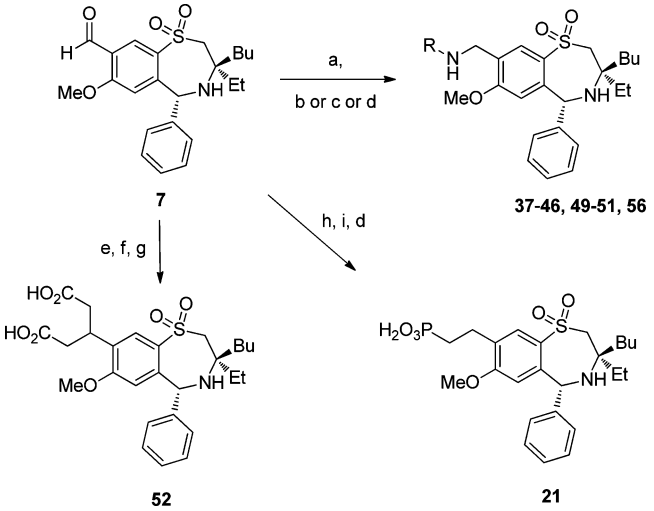


[a]Reagents and conditions: (a) ROCOCOCl, Et_3N, DCM; (b) LiOH, THF, H_2O; (c) $Cl(CH_2)_xCOCl$, pyridine, DCM; (d) Na_2SO_3, EtOH, H_2O; (e) $P(OEt)_3$, 135 °C; (f) TMSBr, DCM; (g) R^1NH_2, KI, K_2CO_3, DMF, 60 °C; (h) $Na^{+-}O_3SCH_2CH_2Br$, DMF, 70 °C; (i) paraformaldehyde, $HP(O)(OEt)_2$, THF or toluene, 70 °C.

the corresponding analogues containing one (**48**) or two (**55**) phosphonic acids were synthesized following condensation with formaldehyde in the presence of diethyl phosphate, then TMSBr-catalyzed deprotection.[19]

Reductive amination of aldehyde **7** using several substituted aminocarboxylic and aminophosphonic acid starting materials under standard reaction conditions followed by deprotection (LiOH, HCl, or TMSBr as appropriate) provided compounds **37**–**46**, **49**–**51**, and **56** in a highly efficient manner (Scheme 5). Mannich reaction of **7** with diethyl malonate in the presence of piperidine followed by base-catalyzed Michael addition of diethyl malonate then acidic hydrolysis led to the formation of compound **52**. Condensation of **7** with tetraethyl methanediylbis(phosphonate) in the presence of NaH followed by Pd^0-catalyzed olefin hydrogenation then deprotection provided compound **21**.

Scheme 5[a]



[a]Reagents and conditions: (a) RNH_2, $NaHB(OAc)_3$, AcOH, DCE; (b) LiOH, THF, MeOH, H_2O; (c) 4 N HCl, 1,4-dioxane; (d) TMSBr, DCM; (e) piperidine, diethylmalonate, toluene, 100 °C; (f) diethyl malonate, NaOEt, EtOH; (g) 37% HCl, Δ; (h) $(EtO)_2P(O)CH_2P(O)(OEt)_2$, NaH, THF; (i) H_2, 10% Pd/C, EtOH.

Two-step conversion of methyl ester intermediate **4** to bromomethyl moiety (**4a**) was achieved upon reduction with DiBAL-H to the alcohol followed by conversion to the bromide upon addition to a premixed solution of PPh_3, Br_2, and imidazole at 0 °C (Scheme 6).[20] Importantly, use of low temperature was critical for minimizing degradation of the sensitive benzyl bromide. Bromide **4a** was aminated upon heating in the presence of diethyl iminodiacetate then saponified to give compound **53**. Compounds **18** and **20** were readily synthesized from **4a** upon exposure to Na_2SO_3 and $P(OEt)_3$, respectively, with deprotection of the phosphonate ester being achieved via acidic hydrolysis.

RESULTS AND DISCUSSION

All compounds were initially evaluated in a series of assays beginning with a ^{3}H-taurocholate uptake assay in HEK293 cells expressing human ASBT. In general, compounds showing potencies comparable to or better than **1** were evaluated for cellular permeability using a MDR1-MDCK assay. Compounds with low to medium permeability (P_{app} < 15 nm/s) were subsequently evaluated in vivo in fed male SD rats for determination of maximum portal vein and systemic blood parent drug concentrations (C_{max}) following a single oral dose at 10 mg/kg. The data from profiling of potential ASBT inhibitors in these assays are summarized in Table 1. Consistent with previous data, **1** was a modestly potent ASBT inhibitor with good cellular permeability but showed unexpectedly low portal vein drug levels (C_{max} = 100 ng/mL) in vivo, which may be due to extensive intestinal and hepatic first pass metabolism (unpublished data). These results and other unpublished data led us to initiate a lead optimization effort to identify a nonabsorbable derivative of **1** with improved ASBT potency,

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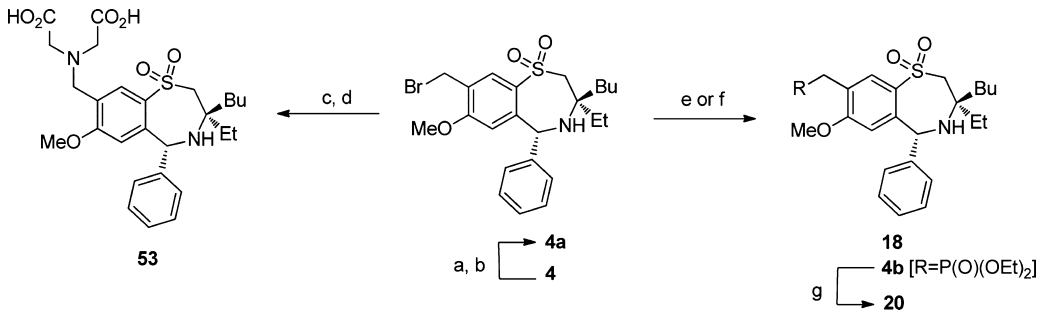
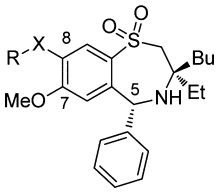
Scheme 6[a]



[a]Reagents and conditions: (a) DiBAL-H, toluene; (b) PPh_3, Br_2, imidazole, DCM, 0 °C; (c) diethyl iminodiacetate, CH_3CN, 65 °C; (d) LiOH, THF, H_2O; (e) Na_2SO_3, 1,4-dioxane, H_2O; (f) $P(OEt)_3$, toluene; (g) 6 N HCl, EtOH, Δ.

Table 1. C(8) Oxygen-Linked and Carbon-Linked ASBT Inhibitor Potency and Rat Portal Vein Drug Concentrations



compd	X	R	hASBT IC_{50} (nM)[a]	MDCK P_{app} (nm/s)	portal C_{max} (ng/mL)[b]	systemic C_{max} (ng/mL)[b]
1	O	Me	180 ± 55	107	100	19
9	O	HO_2CCH_2–	34 ± 5	10	1458	26
10	O	$HO_2C(CH_2)_3$–	13 ± 1	303	NT	–
11	O	$HO_3S(CH_2)_2$–	4 ± 3	12	220	7
12	O	$HO_3S(CH_2)_3$–	50 ± 25	8	289	1
13	O	$H_2O_3PCH_2$–	900 ± 150	NT[c]	–	–
14	O	$H_2O_3P(CH_2)CH_2$–	44 ± 28	8	82	16
15	O	$H_2O_3P(CH_2)_3$–	133 ± 60	10	260	7
16	CH_2	HO_2CCH_2–	17 ± 19	383	NT	–
17	CH_2	$HO_2C(CH_2)_2$–	2 ± 2	342	NT	–
18	CH_2	HO_3S–	4 ± 1	9	170	3
19	CH_2	$HO_3S(CH_2)_2$–	4 ± 1	7	NT	–
20	CH_2	H_2O_3P–	23 ± 1	7	14	3
21	CH_2	$H_2O_3PCH_2$–	51 ± 4	12	NT	–
22	CH_2	$H_2O_3P(CH_2)_2$–	51 ± 14	7	NT	–

[a]IC_{50} values are expressed as the mean ± standard error of ≥2 replicates. [b]dosed via oral gavage at 10 mg/kg. [c]NT or – means not tested.

decreased cellular permeability, improved metabolic stability in the gastrointestinal tract, and low (<50 ng/mL) portal vein parent drug levels when dosed orally at 10 mg/kg.

We initiated our optimization efforts by exploring the SAR of the 5-aryl and 7,8-dimethoxy moieties, however, our focus quickly shifted to modifications of the 7- and 8-methoxy substituents because highly potent compounds with low cellular permeability were rapidly identified. While a full description of the SAR of the 7 and 8-dimethoxy substituents is beyond the scope of this article, data with analogues containing structural modifications of the 8 position clearly revealed that this position was more tolerant of structural modifications and led to compounds with superior ASBT inhibitory activity. Numerous 8-substituted derivatives of **1** were synthesized containing a range of functional groups to explore whether basicity, acidity, lipophilicity, ionization state, and conformational rigidity impacted ASBT potency and cellular permeability. However, compounds with acidic moieties consistently showed potent ASBT inhibition and, in general, low to moderate cellular permeability (Table 1). Substitution of the 8-methoxy carbon with a carboxylic acid (**9**) led to a 6-fold improvement in ASBT inhibition potency and low cellular permeability. Further evaluation of **9** in a rat portal vein study revealed the compound to be highly absorbed into the portal vein as parent or the corresponding glucuronide with drug levels surpassing 1 μM. Insertion of two additional methylene groups between the acid and methoxy carbon as in **10** led to further improvement in potency, however, the additional lipophilicity led to a significant increase in cellular permeability in the MDCK assay. Analogues containing more acidic sulfonic and phosphonic acid moieties were targeted because these modifications would lead to a higher percentage of sulfonate or phosphonate anion in the intestine, which would be predicted to decrease passive cellular permeability. While the direct phosphonic acid analogue (**13**) of **9** showed significantly reduced ASBT potency, analogues with extended alkyl chains containing terminal sulfonic or phosphonic acids (compounds **11**–**12**, **14**–**15**) showed improved ASBT potency and decreased cellular permeability relative to **1** but did not show significantly reduced portal vein drug concentrations. Overall, while improved potencies were observed with several

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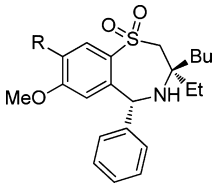
Table 2. C(8) Carbon-Linked ASBT Inhibitor Potency, Permeability, And Rat Portal Vein Drug Concentrations



compd	R	hASBT IC_{50} (nM)[a]	MDCK P_{app} (nm/s)	portal C_{max} (ng/mL)[b]	systemic C_{max} (ng/mL)[b]
23	$HO_2CC(Me)_2CH_2NHCO-$	32 ± 21	43	NT[c]	–
24	HO_3SCH_2NHCO-	11 ± 1	7	92	4
25	$HO_3S(CH_2)_2NHCO-$	2 ± 1	4	132	38
26	$HO_2CCH_2NHCO(CH_2)_2-$	276 ± 39	12	NT	–
27	$HO_3SCH_2NHCO(CH_2)_2-$	167 ± 25	7	NT	–
28	$HO_2CCONHCH_2-$	6 ± 1	NT	87	–
29	$HO_3SCH_2CONHCH_2-$	9 ± 1	19	122	26
30	$HO_3S(CH_2)_2CONHCH_2-$	128 ± 102	NT	–	–
31	$H_2O_3PCH_2CONHCH_2-$	249 ± 29	3	NT	–
32	$(HO_2CCH_2)_2NCO-$	2540	NT	–	–
33	$(HO_2CCH_2)_2CHNHCO-$	97 ± 61	NT	–	–
34	$(HO_2CCH_2)_2CHNHCO(CH_2)_2-$	>3000	NT	–	–
35	$(HO_2CCH_2)_2NCO(CH_2)_2-$	728 ± 44	NT	–	–
36	$(HO_2CCH_2)_2CHNHCH_2CONHCH_2-$	>3000	NT	–	–
37	$HO_2C(CH_2)_3NHCH_2-$	1287	NT	–	–
38	$HO_2C(CH_2)_2NHCH_2-$	367 ± 25	NT	–	–
39	$HO_2CC(Me)_2NHCH_2-$	190 ± 8	NT	–	–
40	(L)-*N*-Pro-CH_2-	>3000	NT	–	–
41	(D)-*N*-Pro-CH_2-	751	NT	–	–
42	(L)-*α*-*N*-Lys-CH_2-	1458	NT	–	–
43	(L)-*N*-Thr-CH_2-	425 ± 248	NT	–	–
44	(L)-*N*-Ser-CH_2-	59 ± 34	5	12	24
45	$HO_2CCH_2NHCH_2-$	4 ± 1	27	27	1
46	$HO_2CCH_2N(Me)CH_2-$	41 ± 33	10	224	1
47	$HO_3S(CH_2)_2NHCH_2-$	54 ± 6	10	7	0
48	$H_2O_3PCH_2NHCH_2-$	>3000	NT	–	–
49	$H_2O_3P(CH_2)_2NHCH_2-$	2698 ± 2225	NT	–	–
50	(D)-*N*-Asp-CH_2-	55 ± 11	7	11	1
51	(L)-*N*-Asp-CH_2-	47 ± 6	6	93	1
52	$(HO_2CCH_2)_2CH-$	>3000	5	333	19
53	$(HO_2CCH_2)_2NCH_2-$	245 ± 21	4	56	43
54	$(HO_3SCH_2CH_2)_2NCH_2-$	365 ± 79	8	NT	–
55	$(H_2O_3PCH_2)_2NCH_2-$	>3000	NT	–	–
56	$(HO_2CCH_2)_2CHNHCH_2-$	42 ± 3	7	4	0

[a] IC_{50} values are expressed as the mean ± standard error of ≥2 replicates. [b] Dosed via oral gavage at 10 mg/kg. [c] NT or – means not tested.

analogues, almost all of the analogues were highly absorbed from the GI tract into the portal vein.

Early in the course of our optimization effort, data emerged from exploratory intestinal stability studies with several C8 oxygen-linked compounds showing the presence of the corresponding 8-phenol (compound **2**) as a metabolite. As a result, the optimization effort shifted focus to evaluation of terminal acid analogues containing a methylene instead of the 8-oxygen. With carboxylic acid analogues, this modification led to a 2–6-fold improvement in ASBT potency (compounds **16** vs **9** and **17** vs **10**) but did not decrease cellular permeability. In contrast, two sulfonic acid analogues (**18**, **19**) were highly potent and showed low permeability, however, portal vein drug levels for **18** were higher than the targeted threshold of 50 ng/mL. Structurally similar phosphonic acid compounds (**20**, **21**, **22**) showed equivalent or better ASBT potency and cellular penetration as compared to the oxygen-containing analogues (**21** vs **13**, **22** vs **14**), and of these, compound **20** showed low oral absorption (portal C_{max} = 14 ng/mL) when tested in a rat portal vein study. Of all of the 8-oxygen and carbon analogues, compound **20** had the best overall profile and was selected for further evaluation in more advanced in vitro and in vivo assays (vide infra).

Compounds containing a lipophilic linker between the aryl ring and a terminal acid moiety were highly potent ASBT inhibitors with highly variable cellular permeability; however, portal vein drug levels for the majority of these derivatives were higher than the targeted threshold. Our strategy for maintaining potency while decreasing cellular permeability was to conserve the terminal acid group but incorporate more polar atoms within the 8-linker region. Toward this end, a limited number of compounds containing terminal carboxylic, sulfonic, and phosphonic acids with benzamide, benzylamide, and propanamide linkers were synthesized (Table 2). ASBT potency was

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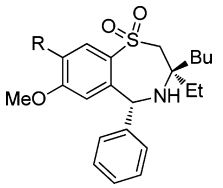
Table 3. Effects of Nonabsorbable ASBT Inhibitors in Rodent ASBT Inhibition, Intestinal Stability, and Fecal Bile Acid Excretion Assays



compd	R	mouse/rat ASBT IC_{50}[a]	rat fecal drug recovery (%)[b]	rat intestinal contents stability (h)[c]	mFBA excretion ED_{50}[d]
1	OMe	3/22	3	h[e]	0.13 (100)[f]
20	$H_2O_3PCH_2$	0.5/0.9	87	>12	0.12 (92)
44	(L)-*N*-Ser-CH_2–	NT[g]	63	8	0.012 (47)
45	$HO_2CCH_2NHCH_2$–	0.3/0.2	92	>12	0.017 (98)
47	$HO_3S(CH_2)_2NHCH_2$–	0.9/2.3	100	>12	2.7 (73)
50	(D)-*N*-Asp-CH_2–	NT	100	NT	>1 (70)
56	$(HO_2CCH_2)_2CHNHCH_2$–	2.1/1.9	98	>12	0.022 (94)

[a]IC_{50} values are in nM and are expressed as the mean of ≥2 replicates (variation <25%). [b]Percentage of parent compound in rat feces following a 10 mg/kg oral dose. [c]Data are expressed as $t_{1/2}$ of parent compound. [d]Mouse fecal bile acid excretion (mFBA) data are in mg/kg, numbers in parentheses are relative efficacy versus compound **1**. [e]The corresponding phenol metabolite (R = OH) was detected at increasing concentrations at 2, 4, and 24 h. [f]Active metabolites of compound **1** undergo enterohepatic recirculation in mice. [g]NT means not tested.

above 100 nM for compounds containing propanamide (**26**, **27**) or benylamide (**30**, **31**) linkers. In contrast, compounds with a decreased overall linker length such as benzamides (**23**–**25**) and benzylamides (**28**, **29**) were highly potent ASBT inhibitors with low to moderate cellular permeability. Unfortunately, exemplars (**24**, **25**, **28**, **29**) with good ASBT potency and low cellular permeability showed high oral absorption in the rat portal vein assay. In an attempt to decrease portal vein drug levels, a small set of amide analogues (**32**–**36**) with more polar dicarboxylic acids were evaluated, however, the majority were very weak ASBT inhibitors, leading us to abandon this approach.

The cumulative data suggested the distance between the terminal acidic functionality and the aryl ring was critical for potent inhibition of ASBT, as compounds with a total of 6 or more contiguous atoms (**26**, **27**, **34**–**36**) at the 8 position led to weak inhibition of ASBT whereas compounds with 4 or 5 (for example compounds **24**, **28**, **33**) contiguous atoms were potent ASBT inhibitors. Recognizing this general trend, our strategy shifted toward synthesis and evaluation of several substituted amino acid-derived analogues containing 4–5 contiguous atoms with an amine as one of these atoms. Our rationale for insertion of an amine was to explore whether this basic atom would impact ASBT activity and, more importantly, decrease cellular permeability and oral absorption (Table 2, **38**–**49**). β-Aminoglycine (**38**) and α-aminoisobutyryl derivatives (**39**) showed moderate to weak inhibition of ASBT while proline-derived analogues with reduced conformational flexibility (**40**, **41**) were very weakly active. Consistent with SAR from our initial exploratory studies, substitution with a basic lysine (**42**) led to very weak ASBT inhibition whereas neutral threonine (**43**) and serine (**44**) analogues were active, with the latter being a potent ASBT inhibitor with low cellular permeability and portal vein drug levels. Removal of the α-amino acid side chain to give a glycine analogue (**45**) resulted in a highly potent analogue with very low portal vein and systemic drug levels despite showing moderate permeability in the MDCK assay. Not surprisingly, we did not observe a linear correlation between the in vitro MDCK permeability and in vivo portal vein drug level assays. While the MDCK assay did effectively identify compounds with moderate to high cellular permeability, data from the in vivo assay was viewed as more physiologically relevant for making decisions about compound progression. *N*-Methylation of **45** to give compound **46** led to a potent ASBT inhibitor, however, portal vein drug levels increased substantially despite low cellular permeability in the MDCK assay. A β-aminosulfonic acid analogue (**47**) showed potent ASBT inhibitory activity as well as low cellular permeability and oral absorption, whereas structurally related α and β-aminophosphonic acid derivatives (**48**, **49**) were significantly less potent than the corresponding sulfonic acid analogue (**47**), a trend that was also observed with amide-linked analogues **29** versus **31**. Two diastereomeric aspartic acid-derived analogues (**50**, **51**) potently inhibited ASBT and showed low cellular permeability, however, only the D-diastereomer showed low oral absorption when tested in vivo. The data with diacids **50** and **51** prompted us to explore other diacid-containing analogues **52**–**56**. Not surprisingly, diacid **52** lacking an amine did not inhibit ASBT. The addition of an acetic acid moiety to glycine analogue **45** to give aminodicarboxylic acid **53** resulted in a weakly potent ASBT inhibitor. Similar data was also observed with a structurally related aminodisulfonic analogue (**54**), whereas the respective aminodiphosphonic analogue (**55**) was inactive. Speculating that the weak activities were due to the presence of a tertiary amine (see **46**), a methylene was inserted adjacent to the amine to give compound **56**, resulting in potent inhibition of ASBT and very low oral absorption in vivo in the rat. Of all the analogues in Table 2, compounds **44**, **45**, **47**, **50**, and **56**, along with compound **20** in Table 1, met our key criteria of potent ASBT inhibitory activity and low oral absorption in vivo to warrant further evaluation as potential development compounds.

Prior to evaluation in a rodent model of type 2 diabetes, the lead compounds were evaluated for mouse and rat ASBT activity, stability in the gastrointestinal tract as measured by GI-tract luminal contents and fecal parent drug recovery assays, and efficacy in a mouse fecal bile acid excretion assay (Table 3). Compound **1** more potently inhibited mouse and rat versus human ASBT but was unstable when tested in the rat fecal drug recovery and intestinal contents stability assays. Despite this instability, **1** did increase fecal bile acid excretion in mice with

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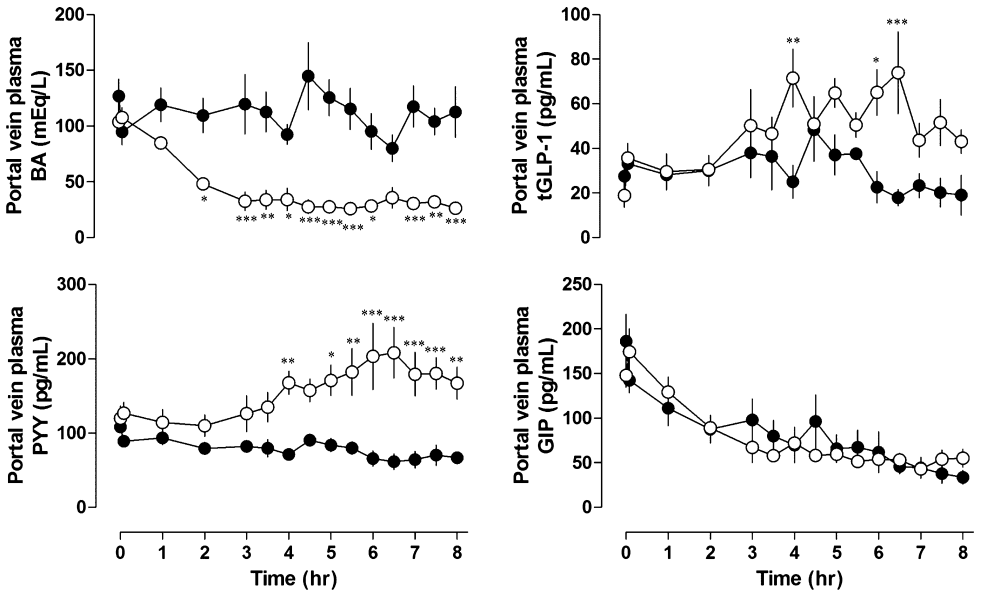

Figure 1. Effects of compound **20** on portal vein plasma bile acids (BA), total GLP-1 (tGLP-1), PYY, and GIP. Compound **20** (○, 1 mg/kg) or vehicle (●) was administered orally in portal vein cannulated male SD rats at 0800 (time 0) without fasting. Portal vein blood samples were collected at indicated time points up to 8 h. Plasma BA, tGLP-1, PYY, and GIP were measured as described in methods. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

an ED_{50} of 0.13 mg/kg, which can be attributed to extensive first pass metabolism generating active metabolites that undergo enterohepatic recirculation. Compound **20** potently inhibited mouse and rat ASBT and was very stable in the GI luminal contents and fecal parent drug recovery assays. Consistent with blocking bile acid reuptake in the ileum, **20** increased fecal bile acid excretion in mice with an EC_{50} of 0.12 mg/kg. In contrast, compound **44** was relatively unstable in the GI tract, with only 63% of parent drug being recovered in the fecal recovery assay. While potent in the mouse fecal bile acid excretion assay, this reduced intestinal stability led to an approximately 50% reduction in total amount of excreted fecal bile acids as compared to **20** and **1**. Compound **45** potently inhibited mouse and rat ASBT and showed excellent stability in the rat GI luminal contents stability and fecal parent drug recovery assays. When tested in mice, **45** potently (ED_{50} = 0.012 mg/kg) increased fecal bile acid excretion and showed equal or greater percent maximum efficacy as compared to **20** and **1**. Despite good mouse and rat ASBT potency and GI stability, compound **47** was significantly less potent in the mouse fecal bile acid excretion assay as compared to compounds **20** and **45**. While rodent ASBT activity and stability data in the GI luminal contents assay were not collected with compound **50**, significantly weaker mouse fecal bile excretion activity prompted us to abandon this compound as well as compound **47**. The dicarboxylic acid derivative **56** showed potent mouse and rat ASBT activity and was stable in the in vitro and in vivo GI stability assays. Moreover, **56** potently increased fecal bile acid excretion in mice with activity comparable to compounds **20** and **45**. Overall, these data indicated that compounds **20**, **45**, and **56** were stable in the rodent GI tract and potently induced fecal bile acid excretion in vivo in mice, leading us to select these three compounds for mechanistic and efficacy studies in vivo in lean rats and Zucker Diabetic Fatty (ZDF) rats, respectively.

Prior to in vivo efficacy studies, compound **20** was selected as an exemplar of a potent, selective, nonabsorbable ASBT inhibitor for proof of mechanism studies to test our hypothesis that inhibition of bile acid reuptake in the distal small intestine would increase portal vein concentrations of GLP-1 and PYY. Oral dosing of **20** at 10 mg/kg to portal vein cannulated lean SD rats was followed by sampling of portal vein blood for bile acids, total GLP-1, PYY, and GIP concentrations at specified time points. As can be seen in Figure 1, inhibition of ASBT led to a decrease in portal vein concentration of bile acids from 1 to 2 h postdosing relative to vehicle treated animals. Interestingly, this time frame is consistent with published transit times of small molecules through the small intestine in rats.[21] In contrast to portal vein bile acid concentrations, portal vein total GLP-1 and PYY concentrations increased with ASBT inhibitor-treated animals from 3 to 8 h postdosing whereas the concentrations of a separate upper GI-secreted peptide, GIP, did not change relative to vehicle treated animals. Agonists of the G-protein coupled receptor TGR5 have been linked to GLP-1 secretion,[22] which could be an alternative mechanism for increasing portal vein GLP-1 concentrations. However, 52 exemplars from the 1,4-benzothiazepine series did not show activity when tested in a TGR5 assay up to 10 μM, suggesting that direct TGR5 activation is not the primary mechanism of action for this chemical series of ASBT inhibitors. These data are consistent with our hypothesis that inhibition of ASBT with a non-absorbable compound could stimulate GLP-1 and PYY secretion from the distal intestines.

Encouraged by the results from the proof of mechanism studies, we next set out to compare the effects of compounds **20**, **45**, and **56** on fecal bile acid secretion and glucose homeostasis in the Zucker Diabetic Fatty (ZDF) rat, an established preclinical animal model of type 2 diabetes. Treatment of 8-week-old male ZDF rats with the three compounds at the indicated doses twice daily for 14 days and determination of fecal bile acid concentration over the last 24 h of the dosing period provided the data that is summarized in Figure 2. As expected, all three compounds stimulated bile acid excretion to a maximum of ~7−10 fold, however, in general, compounds **45** and **56** showed a higher fold increase in fecal bile acid secretion at the lowest dose (0.05 mg/kg) as

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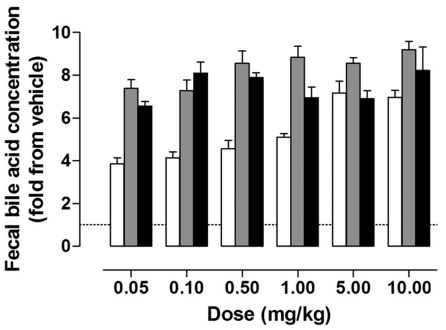

Figure 2. Effects of ASBT inhibitors at the indicated doses on fecal bile acid concentrations in ZDF rats. Male ZDF rats were treated orally twice a day with vehicle, compound **20** (open bar), compound **56** (gray bar), or compound **45** (black bar) for two weeks. Twenty-four h fecal samples were collected on day 14 and extracted for fecal bile acid (BA) measurement. Data are presented as relative fold change from vehicle groups (dashed line). All treatment groups were statistically different from vehicle groups ($p < 0.001$).

compared to **20**, a trend that was also observed at the next three higher doses (0.10, 0.50, 1.0 mg/kg). The effects of the three compounds on glucose homeostasis in ZDF rats were determined in two separate in vivo studies in which each compound was given orally at doses ranging from 0.05 to 10 mg/kg (compounds **20** and **45**) or 0.001 to 10 mg/kg (compound **56**) twice daily for 14 days. Plasma glucose, hemoglobin A1c (HbA1c), insulin, and total GLP-1 concentrations were determined at the end of the study (Figure 3). Treatment with the three nonabsorbable ASBT inhibitors led to a 1.30−1.64% reduction in HbA1c, a greater than 50% reduction in nonfasted plasma glucose to below 200 mg/dL, and statistically significant higher plasma insulin, and, importantly, greater total GLP-1 relative to vehicle treated animals. While all three compounds showed a significant improvement in glucose homeostasis, maximum efficacy was comparable with each compound at identical doses which did not permit the identification of a clearly superior compound.

The cumulative data with compounds **20**, **45**, and **56** suggested that each compound was a viable clinical candidate; however, data from preclinical development assays would be required for selection of a development candidate. The three compounds were tested in a battery of preclinical development assays including equilibrium aqueous solubility, in vitro biotransformation, general selectivity, salt selection, crystallinity, stability, hygroscopicity, and reactive metabolite assays. All three compounds showed acceptable aqueous solubility (≥200 μg/mL) and were highly selective when screened against a broad panel of unrelated targets. However, a reactive metabolite was observed with compound **20** when tested in a glutathione trapping assay and a crystalline salt derivative of **45** proved to be thermally unstable and extremely hygroscopic, properties that would slow or possibly preclude further development of these two compounds. In contrast, the zwitterionic, nonhygroscopic, crystalline salt form of **56** showed good aqueous solubility at pH 7.4 (>7 mg/mL), excellent thermal stability, and did not generate reactive or human-specific metabolite, characteristics that suggested **56** was a superior development compound versus **20** and **45**.

In preparation for safety assessment studies, compound **56** was profiled in rat, dog, and monkey PK studies to determine if low oral absorption translated across multiple preclinical species. Consistent with our previous data, oral dosing of a solution of **56** at 10 mg/kg to fed SD rats did not lead to any detectable (LLQ = 1 ng/mL) levels of parent compound in the systemic circulation. Similarly, very low systemic levels (C_{max} = 6 ng/mL) of **56** were observed following oral dosing to male beagle dogs at 10 mg/kg, and importantly, **56** could not be detected in the systemic circulation following oral dosing of cynomolgous monkeys at a very high oral dose (3 mg/kg). The

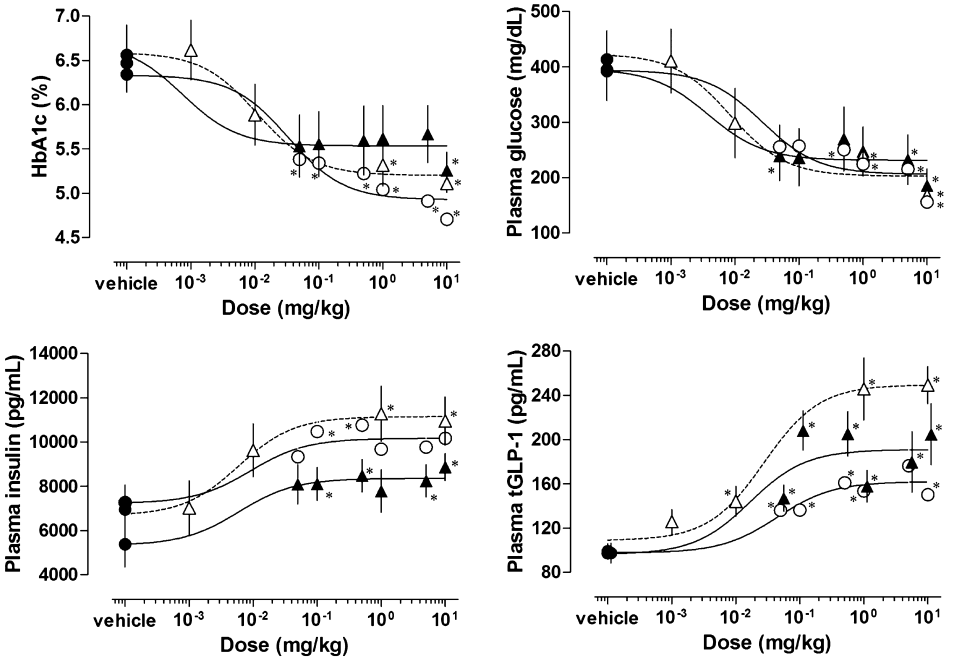

Figure 3. Effects of ASBT inhibitors on blood HbA1c, plasma glucose, insulin, and total GLP-1 in 8-week-old male ZDF rats. Rats were treated orally twice a day with compound **20** (○), **45** (▲), or **56** (△) at the indicated doses for two weeks in two separate studies. Blood samples were collected on day 14 from tail vein. Blood HbA1c, plasma glucose, insulin, and total GLP-1 were measured as described in methods. *$p < 0.05$ vs vehicle.

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data from these PK studies suggested that **56** would show very low or no oral absorption in humans at pharmacologically relevant oral doses.

CONCLUSIONS

In summary, numerous analogues were designed to probe the effects of molecular size, polarity, lipophilicity, and ionization state on ASBT inhibition, cellular permeability, and oral absorption. Compounds containing acidic moieties at C8 of the 1,1-dioxido-1,4-benzothiazepine core structure emerged as highly potent ASBT inhibitors but showed highly variable cellular permeability and oral absorption, the latter being determined by measuring portal vein parent drug concentrations after oral dosing. Three lead compounds (**20**, **45**, **56**) were identified from this optimization effort which displayed potent ASBT inhibition and very low oral absorption in rats as well as robust efficacy in an animal model of type 2 diabetes. Further characterization of the three compounds in developability assays culminated in the identification of compound **56** as a highly potent, nonabsorbable ASBT inhibitor with excellent aqueous solubility, selectivity, and developability properties for evaluation in safety studies and ultimately humans. Compound **56** will be a valuable clinical tool for exploring the therapeutic utility of a nonabsorbable ASBT inhibitor for treatment of patients with type 2 diabetes.

EXPERIMENTAL SECTION

General Protocols. All chemical reagents were purchased and used as received. ^{1}H NMR spectra were recorded on a Varian Unity-300 or Varian Unity Plus-400. Chemical shifts are expressed in parts per million (ppm, δ units). Coupling constants are in units of hertz (Hz). Splitting patterns describe apparent multiplicities and are designated as s (singlet), d (doublet), t (triplet), q (quartet), m (multiplet), or br (broad).

Unless otherwise noted, the LCMS system used to determine purity was a UPLC analysis was conducted on a Waters Acquity system with BEH C18, 2 mm × 50 mm, 1.7 μm column at 40 °C 95% H_2O, 5% MeCN to 99% MeCN in 1.1 min, holding at 100% MeCN for 40 s. Water contained 0.2% v/v formic acid. MeCN contained 0.15% v/v formic acid. The flow rate was 1 mL/min with 5 μL of solution injected. Mass spectra were recorded utilizing electrospray ionization (ESI) or atmospheric pressure chemical ionization (APCI) switching between positive and negative modes with DAD scanning from 210 to 350 nm. All compounds tested were of ≥95% purity.

Normal phase chromatography was accomplished using Teledyne Isco equipment using prepacked silica columns.

Reverse phase HPLC for purification of final compounds was accomplished on a Gilson 845Z series prep HPLC system using a C18 Sunfire 30 mm × 50 mm or 30 mm × 150 mm, 5 μm column using the gradient described with 0.05% TFA in H_2O. The flow rate was 60 or 45 mL/min and the product was collected based on UV detection at 220 or 254 nm. In some cases, basic conditions were established using the same Gilson system and a C18 XBridge 30 mm × 50 mm or 30 mm × 150 mm, 5 μm column using the gradient described with 0.2% NH_4OH in the H_2O. The flow rate was 60 or 45 mL/min and again the product was collected on UV detection at 220 or 254 nm.

HRMS. High resolution LC-MS (top) and MS-MS spectra. Spectra were acquired on a Waters qTOF Premiere mass spectrometer operating in W mode (resolving power ∼15000). A sample volume of 0.2 μL was introduced by flow injection from a Waters nanoAcquity UPLC. Mobile phase A: H_2O + 0.1% formic acid. Mobile phase B: MeOH + 0.1% formic acid. Wash: 50:50 H_2O:MeOH + 0.1% formic acid. Flow: 0.02 mL/min, 40% A, 60% B. Lock mass: 0.7 mg leucine enkephalin in 500 mL MeOH. Collision gas: argon.

(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-ol-1,1-dioxide (2). A solution of **1** (200 g, 479 mmol) (see ref 16) in DCE (1 L) was saturated with gaseous HCl then treated with $AlCl_3$ (200 g, 1.5 mol) in one portion. The reaction mixture was stirred while slowly cooling to 25 °C then stirred for 2.5 h. The reaction mixture was added to an ice−H_2O mixture with vigorous stirring. The biphasic mixture was treated with 1 N HCl (∼200 mL) then the phases were separated after stirring for 30 min. The organic phase was isolated, washed twice with dilute HCl (1.5 L H_2O/∼200 mL 1 N HCl), dried over $MgSO_4$, filtered, and concentrated to dryness to give a regioisomeric mix of 7-OH and 8-OH products (185 g, 458 mmol, 96% yield) as a white foam. ^{1}H NMR indicated a 47:53 mix of 7/8-phenols, respectively. The regioisomers were separated by chiral chromatography [stationary phase (CSP)-cellulose tris(3,5-dichlorophenylcarbamate) polymer immobilized on silica (CHIRALPAK IC), DCM and 2-propanol (98/2 v/v) as mobile phase] to give 72.5 g of **2** as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.1 Hz, 3 H), 0.79 (t, J = 7.5 Hz, 3 H), 0.95−1.28 (m, 4 H), 1.31−1.53 (m, 2 H), 1.64−1.76 (m, 1 H), 1.98−2.10 (m, 1 H), 2.43 (d, J = 9.8 Hz, 1 H), 3.06 (d, J = 14.8 Hz, 1 H), 3.42 (s, 3 H), 3.49 (d, J = 14.8 Hz, 1 H), 5.85 (d, J = 9.8 Hz, 1 H), 6.04 (s, 1 H), 7.24−7.35 (m, 1 H), 7.35−7.48 (m, 5 H), 9.72 (s, 1 H).

(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl trifluoromethanesulfonate (3). To a DCM solution of **2** (105 g, 26 mmol) and pyridine (27.3 mL, 34 mmol) cooled to 0 °C under nitrogen was added Tf_2O (57.1 mL, 34 mmol) slowly over 30 min while maintaining an internal temperature between 5 and 10 °C. Upon complete addition, the reaction was stirred until TLC and LCMS indicated complete conversion to product. H_2O (250 mL) was slowly added to the mixture, and the mixture stirred for 10 min, after which the layers separated. The aqueous layer was extracted an additional time with DCM, and the combined organics washed with 10% HCl and brine, then dried (Na_2SO_4), filtered, and concentrated to half volume. Hexanes was added until the solution became turbid, and crystallization began to occur. The solids were then filtered from the solution to give **3** (134.6 g, 95%) as a white solid, which was used without further purification. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.76−0.96 (m, 6 H), 1.03−1.40 (m, 4 H), 1.40−1.67 (m, 4 H), 1.77−1.97 (m, 1 H), 2.10−2.28 (m, 1 H), 3.07 (d, J = 14.8 Hz, 1 H), 3.48 (d, J = 14.9 Hz, 1 H), 3.62 (s, 3 H), 6.09 (s, 1 H), 6.34 (s, 1 H), 7.32−7.50 (m, 5 H), 7.96 (s, 1 H). ES-LCMS m/z 536 $(M + H)^+$.

Methyl (3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepine-8-carboxylate 1,1-Dioxide (4). To a reaction tube containing **3** (0.103 g, 0.192 mmol), $Pd_2(dba)_3$ (0.011 g, 0.012 mmol), dppf (0.013 g, 0.024 mmol), and NEt_3 (0.040 mL, 0.288 mmol) in DMF (1 mL) was bubbled in carbon monoxide gas for a period of 15 min. Anhydrous MeOH (0.039 mL, 0.962 mmol) was added, and the reaction vessel was sealed under a carbon monoxide atmosphere then heated at 70 °C overnight. The reaction was cooled to room temperature, and the contents diluted with Et_2O then poured into H_2O. The layers were separated, the aqueous extracted one additional time with Et_2O, and the combined organics washed with brine, dried (Na_2SO_4), filtered, and concentrated. The crude material was chromatographed on silica gel using hexanes/EtOAc to give **4** (0.080 g, 91%) as a a white solid. ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.75−0.96 (m, 6 H), 0.99−1.36 (m, 4 H), 1.36−1.59 (m, 5 H), 1.86 (ddd, J = 14.4, 12.0, 4.4 Hz, 1 H), 2.07−2.23 (m, 1 H), 3.02 (d, J = 14.8 Hz, 1 H), 3.45 (d, J = 14.9 Hz, 1 H), 3.57 (s, 3 H), 3.86 (s, 3 H), 6.08 (s, 1 H), 6.27 (s, 1 H), 7.29−7.48 (m, 5 H), 8.52 (s, 1 H). ES-LCMS m/z 446 $(M + H)^+$.

(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepine-8-carboxylic Acid 1,1-Dioxide (5). H_2O (0.333 mL), MeOH (0.333 mL), and THF (1 mL) were added to **4** (0.046 g, 0.103 mmol) along with LiOH (0.013 g, 0.310 mmol), and the mixture stirred for 2 h at 25 °C. The reaction mixture was concentrated to half volume, then 6 N HCl was added. The mixture was extracted with EtOAc (2×), washed with brine, dried (Na_2SO_4), filtered, and concentrated in vacuo to give **5** (0.039 g, 86%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.67−0.99 (m, 6 H), 0.99−1.64 (m, 7 H), 1.87 (t, J = 11.8 Hz, 1 H), 2.08−2.29 (m, 1 H), 3.09 (d, J = 14.8 Hz, 1 H), 3.46 (d, J = 14.9 Hz, 1 H), 3.70 (br s, 3 H),

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6.11 (br s, 1 H), 6.33 (br s, 1 H), 7.30−7.60 (m, 5 H), 8.73 (br s, 1 H). ES-LCMS m/z 432 $(M + H)^+$.

(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepine-8-carbonitrile-1,1-dioxide (6). A mixture of **3** (0.535 g, 1.0 mmol), $Pd_2(dba)_3$ (0.055 g, 0.06 mmol), dppf (0.066 g, 0.12 mmol), zinc powder (0.004 g, 0.06 mmol), and $Zn(CN)_2$ (0.117 g, 0.99 mmol) in DMF (10 mL) was stirred at 25 °C for 15 min under a stream of nitrogen. The reaction was heated to 80 °C and stirred for 8 h. After the mixture was cooled to 25 °C, DMF was removed in vacuo, Et_2O was added, and the organics were washed with 2 N NH_4OH followed by brine. The organics were dried (Na_2SO_4), filtered, and concentrated. The residue was triturated with hexanes/EtOAc, and a white solid was collected by filtration to give **6** (0.386 g, 92%). ^1H NMR (400 MHz, $CDCl_3$) δ ppm 0.73−0.97 (m, 6 H), 0.98−1.62 (m, 8 H), 1.77−1.92 (m, 1 H), 2.08−2.24 (m, 1 H), 3.01 (d, J = 14.9 Hz, 1 H), 3.45 (d, J = 14.9 Hz, 1H), 3.62 (s, 3 H), 6.08 (br s, 1 H), 6.26 (s, 1 H), 7.32−7.49 (m, 5 H), 8.28 (s, 1 H). ES-LCMS m/z 413 $(M + H)^+$.

(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepine-8-carbaldehyde 1,1-Dioxide (7). Compound **6** (7.42 g, 17.99 mmol) dissolved in DCM (150 mL) was treated with 1 M DIBAL-H in toluene (36.0 mL, 36.0 mmol) at 0 °C with stirring for 1 h after which time LCMS indicated complete conversion. The reaction mixture was poured into an ice/1 N HCl mixture and stirred vigorously for 1 h. The organic phase was isolated, dried over $MgSO_4$, filtered, and concentrated to give a yellow solid. The crude product was dissolved in 80 mL hot EtOAc to which was added hexanes (250 mL) until cloudy. The mixture was allowed to cool slowly to ambient temperature and then cooled in an ice bath. The resultant precipitate was filtered off, washed with cold 20% EtOAc/hexanes, and air-dried to give **7** (4.00 g, 53.5% yield) as a white solid. The mother liquor was concentrated to dryness, and the residue was purified on silica gel eluting with 20−40% EtOAc/hexanes to give additional product as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.74 (t, J = 7.0 Hz, 3 H), 0.81 (t, J = 7.4 Hz, 3 H), 0.96−1.29 (m, 4 H), 1.33−1.54 (m, 2 H), 1.68−1.81 (m, 1 H), 2.01−2.13 (m, 1 H), 2.80 (d, J = 9.8 Hz, 1 H), 3.13 (d, J = 15.0 Hz, 1 H), 3.58 (s, 3 H), 3.63 (d, J = 15.0 Hz, 1 H), 5.98 (d, J = 9.8 Hz, 1 H), 6.27 (s, 1 H), 7.32−7.40 (m, 1 H), 7.40−7.49 (m, 4 H), 8.23 (s, 1 H), 10.27 (s, 1 H). LC-MS (ES^+) m/z 416 [M + H].

{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}amine (8). A mixture of **6** (468 mg, 1.13 mmol) and 10% Pd/C (60.4 mg, 0.567 mmol) in EtOH (20 mL) was added 37% aqueous HCl (0.279 mL, 3.40 mmol) and subjected to H_2 atmosphere at 40 psi overnight then filtered. The residue was purified via HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) to give **8** (621 mg, 79%, TFA salt) as a white solid. ^1H NMR ($CDCl_3$) δ ppm 0.90 (t, J = 7.2 Hz, 3H), 0.97−1.15 (m, 1H), 1.14−1.38 (m, 3H), 1.44−1.75 (m, 2H), 1.80−1.99 (m, 1H), 2.14−2.38 (m, 1H), 3.29 (d, J = 15.0 Hz, 1H), 3.38−3.48 (m, 1H), 3.55 (s, 3H), 3.98- 4.12 (m, 1H), 4.12−4.27 (m, 1H), 6.17 (s, 1H), 6.27 (s, 1H), 7.31−7.57 (m, 5H), 7.98 (s, 1H), 8.07−8.46 (br s, 2H). LC-MS (ES^+) m/z 417 $(M + H)^+$.

2-(((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[*f*][1,4]thiazepin-8-yl)oxy)acetic Acid (9). To a solution of **2** (1.24 g, 3.07 mmol) in DMSO (10 mL) was added methyl bromoacetate (0.319 mL, 3.38 mmol) and potassium carbonate (0.85 g, 6.15 mmol). The reaction mixture was stirred for 2 h at 50 °C. The reaction mixture was diluted with EtOAc, washed twice with water and once with brine, dried over $MgSO_4$, filtered, and concentrated to dryness to give the intermediate ester as a clear oil. The material was dissolved in THF (30 mL) and treated with 1 M aqueous LiOH (30.7 mL, 30.7 mmol) at 25 °C for 16 h, at which time LCMS indicated complete conversion to the acid. The reaction mixture was concentrated to remove the THF, washed once with ether, and acidified with 1 N HCl with stirring and cooling in an ice bath until a heavy precipitate formed. The mixture was stirred for 30 min at ice bath temperature, filtered, and the cake washed once with dilute aqueous HCl to give **9** (1.19 g, 2.58 mmol, 84% yield) as a white solid. The mother liquor was extracted four times with ether and the organics were combined, dried over $MgSO_4$, filtered, and concentrated to give an additional batch of **9** (0.28 g, 0.61 mmol, 16% yield) as a white foam. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.95−1.28 (m, 4H), 1.31−1.53 (m, 2H), 1.65−1.78 (m, 1H), 1.97−2.09 (m, 1H), 2.55 (d, J = 9.46 Hz, 1H), 3.03 (d, J = 15.04 Hz, 1H), 3.44 (s, 3H), 0.76 (m, 6H), 3.53 (d, J = 15.04 Hz, 1H), 4.70 (s, 2H), 5.88 (d, J = 7.95 Hz, 1H), 6.10 (s, 1H), 7.27−7.44 (m, 6H), 13.12 (br s, 1H), exchangeable protons (COOH @ 13.12 and NH @ 2.55) were no longer visible by ^1H NMR upon exposure to D_2O. LC-MS (ES^-) m/z 460 [M − 1]. LC-MS (ES^+) m/z 462 [M + H]. HRMS (ES^+) calcd for $C_{24}H_{31}NO_6S$ (M + 1) m/z 462.1949, found 462.1950.

4-(((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[*f*][1,4]thiazepin-8-yl)oxy)-butanoic Acid (10). Compound **2** (50 mg, 0.124 mmol) was dissolved in DMSO (0.5 mL) and treated with 1,1-dimethylethyl 4-bromobutanoate (27.6 mg, 0.124 mmol) and K_2CO_3 (51.4 mg, 0.372 mmol) at 100 °C for 2 h, at which time LCMS indicated complete conversion to the intermediate ester. The reaction mixture was cooled to ambient temperature and then treated with excess water to precipitate the intermediate. The supernatant was decanted off, and the solid residue was washed twice with water and pumped dry to give the ester. The residue was diluted with DCM (1 mL) and treated with 4 N HCl in dioxane (2 mL, 8.00 mmol) at 25 °C for 2 h, after which time LCMS indicated complete conversion. The reaction mixture was concentrated to dryness and purified by RP-HPLC (Sunfire 30 mm × 150 mm, C18, 5 um, Waters column; 10−100% CH_3CN + 0.05% TFA in H_2O + 0.05% TFA over 10 min at 50 mL/min) to give **10** (41 mg, 0.083 mmol, 67% yield). ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.67−0.86 (m, 6H), 0.95−1.30 (m, 4H), 1.33−1.56 (m, 2H), 1.64−1.84 (m, 1H), 1.87−1.98 (m, 2H), 1.98−2.16 (m, 1H), 2.36 (t, J = 7.31 Hz, 2 H), 3.04−3.19 (m, 1H), 3.44 (br s, 3H), 3.49−3.63 (m, 1H), 3.96−4.07 (m, 2H), 5.81−6.05 (m, 1H), 6.05−6.21 (m, 1H), 7.22−7.56 (m, 6H), COOH proton not observed. LC-MS (ES^-) m/z 488 [M − 1]. LC-MS (ES^+) m/z 490 [M + H]. HRMS (ES^+) calcd for $C_{26}H_{35}NO_6S$ (M + 1) m/z 490.2263, found 490.2263.

2-(((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[*f*][1,4]thiazepin-8-yl)oxy)-ethanesulfonic Acid (11). A solution of **2** (250 mg, 0.620 mmol) and 1,2-dibromoethane (1.16 g, 6.20 mmol) in THF was treated with 1 M KOH (9.29 mL, 9.29 mmol) and tetrabutylammonium bromide (60 mg, 0.186 mmol) at 100 °C for 16 h in a sealed tube apparatus, after which time LCMS indicated complete conversion to the intermediate bromide. The reaction mixture was partitioned between EtOAc and water, and the organic phase was isolated and dried over $MgSO_4$. The mixture was filtered, and the filtrate was concentrated to dryness and pumped dry overnight to give the intermediate as an amber oil. The residue was dissolved in EtOH (6 mL) and added to a solution of sodium sulfite (78 mg, 0.620 mmol) in water (6 mL). The mixture was heated at 100 °C for 16 h with stirring, after which time LCMS indicated complete conversion to the desired sulfonic acid. The reaction mixture was concentrated to dryness and the residue was purified by RP-HPLC (30 mm × 150 mm Sunfire column at 50 mL/min; CH_3CN and H_2O + 0.05% TFA solvent system, solvent gradient consisted of 10−100 over 8 min, 100 to 100 to 10 min, sample collection at λ_{max}) to give **11** (210 mg, 0.410 mmol, 66% yield) as a white foam. ^1H NMR (DMSO-d_6) δ ppm 0.75−0.91 (m, 6H), 0.89−1.06 (m, 1H), 1.15−1.39 (m, 3H), 1.48−1.63 (m, 1H), 1.81−2.09 (m, 2H), 2.39−2.59 (m, 1H), 2.96 (td, J = 7.6, 2.0 Hz, 2H), 3.51 (s, 3H), 3.54 (d, J = 15.6 Hz, 1H), 4.01 (d, J = 15.6 Hz, 1H), 4.26 (dd, J = 8.2, 7.0 Hz, 2H), 6.37 (s, 1H), 6.22 (s, 1H), 7.45−7.66 (m, 6H), 2 exchangeable protons not observed. LCMS (ES^-) m/z 510 [M − 1]. LC-MS (ES^+) m/z 512 [M + H]. HRMS (ES^+) calcd for $C_{24}H_{33}NO_7S_2$ (M + 1) m/e 512.1777, found 512.1777.

3-(((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[*f*][1,4]thiazepin-8-yl)oxy)-propane-1-sulfonic Acid (12). Compound **2** (50 mg, 0.124 mmol) was dissolved in DMSO (0.5 mL) and treated with 3-bromo-1-propanesulfonic acid sodium salt (30.8 mg, 0.136 mmol) and K_2CO_3 (34.2 mg, 0.248 mmol) at 100 °C for 2 h, after which time LCMS indicated complete conversion. The reaction mixture was diluted with

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water, acidified with 1 N HCl, and extracted with EtOAc (3×). The organic layers were combined, dried over $MgSO_4$, filtered, and concentrated to dryness to give **12** (22 mg, 0.042 mmol, 34% yield) as a clear glass. The majority of the product, however, remained in the aqueous phase. The aqueous layer was stored overnight in the refrigerator, and the resultant precipitate was filtered off, washed with water, and air-dried to give a second batch **12** (21 mg, 0.040 mmol, 32% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6 with D_2O) δ ppm 0.70−0.86 (m, 6H), 0.88−1.03 (m, 1H), 1.13−1.34 (m, 3H), 1.48 (dd, J = 13.75, 7.09 Hz, 1H), 1.69−1.83 (m, 1H), (1.84−1.95 (m, 1H), 2.01 (qd, J = 6.88, 6.66 Hz, 2H), 2.25−2.40 (m, 1H), 2.62 (t, J = 7.41 Hz, 2H), 3.37 (d, J = 15.26 Hz, 1H), 3.46 (s, 3H), 3.68−4.23 (m, 3H), 6.09 (br s, 1H), 6.28 (s, 1H), 7.36−7.60 (m, 6H), exchangeable protons were not observed. LC-MS (ES^-) m/z 524 [M − 1]. LC-MS (ES^+) m/z 526 [M + H]. HRMS (ES^+) calcd for $C_{25}H_{35}NO_7S_2$ (M + 1) m/z 526.1934, found 526.1933.

((((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[f][1,4]thiazepin-8-yl)oxy)methyl)-phosphonic Acid (13). Step 1: A solution of **2** (100 mg, 0.248 mmol) in DMSO (3 mL) was treated with 1 M NaHMDS in THF (0.260 mL, 0.260 mmol) at ambient temperature for 20 min. [Bis(ethyloxy)phosphoryl]methyl 4-chlorobenzenesulfonate (140 mg, 0.408 mmol) was added to the reaction mixture, and the mixture was stirred at 25 °C for 16 h, after which time LCMS indicated ∼60% conversion to the desired product. The reaction mix was heated at 60 °C for 3 days, after which time LCMS indicated no discernible improvement. The reaction mixture was partitioned between EtOAc and water, and the organic phase was isolated, dried over $MgSO_4$, filtered, and concentrated to dryness. The residue was purified on silica gel eluted with 40−100% EtOAc/hexanes to give an intermediate phosphonate as a clear oil. LC-MS (ES^+) m/z 554 [M + H]. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.76 (t, J = 7.00 Hz, 3H), 0.80 (t, J = 7.42 Hz, 3H), 0.97−1.29 (m, 4H), 1.20−1.28 (m, 6H), 1.32−1.53 (m, 2H), 1.67−1.80 (m, 1H), 1.97−2.10 (m, 1H), 2.55 (d, J = 9.6 Hz, 1 H), 3.06 (d, J = 14.8 Hz, 1H), 3.45 (s, 3H), 3.56 (d, J = 15.1 Hz, 1H), 4.10 (m, 4H), 4.40−4.56 (m, 2H), 5.91 (d, J = 9.6 Hz, 1H), 6.12 (s, 1 H), 7.28−7.36 (m, 1H), 7.36−7.48 (m, 4H), 7.60 (s, 1H).

Step 2: A solution of the intermediate phosphonate (77.9 mg, 0.141 mmol) in DCM (3 mL) was treated with TMSBr (100 mg, 0.653 mmol) at 23 °C for 16 h, after which time LCMS indicated complete conversion. The reaction mixture was concentrated to dryness, chased with MeOH, and purified by RP-HPLC (30 mm × 150 mm Sunfire column at 50 mL/min. CH_3CN and H_2O + 0.05% TFA were used as solvent system. The samples were collected at λ_{max}. Gradient consisted of: 10−100 over 8 min, 100 to 100 to 10 min) to give **13** [70 mg, 0.141 mmol, 57% yield (two steps)] as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.68−0.90 (m, 6 H), 0.97−1.32 (m, 4 H), 1.34−1.62 (m, 2 H), 1.67−1.88 (m, 1 H), 1.95−2.25 (m, 1 H), 2.43−2.63 (m, 1 H), 2.97−3.24 (m, 1 H), 3.47 (s, 3 H), 3.52−3.72 (m, 1 H), 4.03−4.29 (m, 2 H), 5.80−6.02 (m, 1 H), 6.04−6.24 (m, 1 H), 7.42 (br s, 5 H), 7.60 (br s, 1 H) (acidic protons not observed). LC-MS (ES^-) m/z 496 [M − 1]. LC-MS (ES^+) m/z 498 [M + H]. HRMS (ES^+) calcd for $C_{23}H_{32}NO_7PS$ (M + 1) m/z 498.1715, found 498.1716.

(2-(((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[*f*][1,4]thiazepin-8-yl)oxy)ethyl)-phosphonic Acid (14). Step 1: To a solution of 1,2-dibromoethane (0.466 g, 2.478 mmol) and DIPEA (0.087 mL, 0.496 mmol) in THF (5 mL) was added **2** (100 mg, 0.248 mmol), and the mixture was stirred for 1 h, after which time LCMS indicated no reaction. K_2CO_3 (0.068 g, 0.496 mmol) was added to the reaction mixture, and the mixture was stirred for an additional 16 h, after which time LCMS still showed no reaction. Then 1 M KOH (3.0 mL, 3.00 mmol), tetrabutylammonium bromide (24 mg, 0.074 mmol), and additional 1,2-dibromoethane (1.07 g, 5.70 mmol) were added to the reaction mixture and stirred for 3 days, after which time LCMS indicated good conversion to the intermediate bromide. The reaction mixture was partitioned between EtOAc and water, and the organic phase was isolated, dried over $MgSO_4$, filtered, and concentrated to dryness to give the intermediate as a clear oil. The oil was dissolved in triethyl phosphite (5.26 mL, 30.1 mmol) and heated at 100 °C for 16 h with stirring, after which time LCMS indicated ∼20% conversion. The temperature was increased to 130 °C for 16 h, after which time LCMS indicated complete conversion to the desired product. The reaction mixture was concentrated to dryness, and the residue was purified on silica gel eluting with 60−100% EtOAc/hexanes to give an intermediate phosphonate (112 mg, 0.196 mmol, 79% yield) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.76 (t, J = 7.05 Hz, 3 H), 0.80 (t, J = 7.42 Hz, 3 H), 0.96−1.30 (m, 4 H), 1.23 (t, J = 6.87 Hz, 6 H), 1.32−1.53 (m, 2 H), 1.66−1.79 (m, 1 H), 1.98−2.11 (m, 1 H), 2.21−2.37 (m, 2 H), 2.51−2.56 (m, 1 H), 3.11 (d, J = 14.83 Hz, 1 H), 3.43 (s, 3 H), 3.55 (d, J = 14.83 Hz, 1 H), 3.94−4.10 (m, 4 H), 4.10−4.26 (m, 2 H), 5.90 (d, J = 9.52 Hz, 1 H), 6.11 (s, 1 H), 7.25−7.36 (m, 1 H), 7.36−7.44 (m, 4 H), 7.46 (s, 1 H).

Step 2: A solution of intermediate phosphonate (111.5 mg, 0.196 mmol) in DCM (1 mL) was treated with TMSBr (120 mg, 0.786 mmol) at 23 °C for 16 h, after which time LCMS indicated complete conversion. The reaction mixture was concentrated to dryness and purified by RP-HPLC (basic conditions) to give **14** (37 mg, 0.073 mmol, 37% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.71−0.84 (m, 6 H), 0.97−1.28 (m, 4 H), 1.32−1.53 (m, 2 H), 1.66−1.91 (m, 3 H), 1.96−2.09 (m, 1 H), 2.51−2.55 (m, 1 H), 3.11 (d, J = 14.83 Hz, 1 H), 3.42 (s, 3 H), 3.53 (d, J = 15.04 Hz, 1 H), 4.02−4.17 (m, 2 H), 5.90 (d, J = 9.67 Hz, 1 H), 6.08 (s, 1 H), 7.31 (m, J = 8.65, 4.27, 4.27, 4.08 Hz, 1 H), 7.40 (d, J = 4.51 Hz, 4 H), 7.44 (s, 1 H) (acidic protons not observed). LC-MS (ES^-) m/z 510 [M − 1]. LC-MS (ES^+) m/z 512 [M + H]. HRMS (ES+) calcd for $C_{24}H_{40}N_3O_7PS$ (M + 1) m/z 512.1872, found 512.1871.

(3-(((3*R*,5*R*)-3-Butyl-3-ethyl-7-methoxy-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydrobenzo[*f*][1,4]thiazepin-8-yl)oxy)propyl)-phosphonic Acid (15). Prepared as in compound **14**. The crude material was purified by RP-HPLC to give **15** (61 mg, 0.117 mmol, 59% yield) as a white foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.69−0.93 (m, 6 H), 0.97−1.33 (m, 4 H), 1.34−1.57 (m, 2 H), 1.58−1.71 (m, 2 H), 1.71−1.85 (m, 1 H), 1.84−1.98 (m, 2 H), 1.99−2.17 (m, 1 H), 3.00−3.26 (m, 1 H), 3.46 (s, 3 H), 3.52−3.71 (m, 1 H), 3.99−4.15 (m, 2 H), 5.70−6.05 (m, 1 H), 6.05−6.42 (m, 1 H), 7.01−7.85 (m, 6 H) (exchangeables were not observed). LC-MS (ES^-) m/z 524 [M − 1]. LC-MS (ES^+) m/z 526 [M + H]. HRMS (ES^+) calcd for $C_{25}H_{36}NO_7PS$ (M + 1) m/z 526.2028, found 526.2024.

3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]propanoic Acid (16). Step 1: In a sealed tube, a solution of **3** (3.5 g, 6.53 mmol) in DMF (30 mL) was treated with Et_3N (4.55 mL, 32.7 mmol), followed by ethyl acrylate (3.56 mL, 32.7 mmol) and bis-(triphenylphosphine)palladium(II) chloride (0.459 g, 0.653 mmol). The reaction mixture was stirred at 120 °C overnight, cooled to 25 °C, and partitioned between H_2O and EtOAc. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification on silica gel (EtOAc/hexanes = 1:6 to 2:1) to give a crude cinnamate ester (3.06 g, 94%) as a white solid. ^{1}H NMR ($CDCl_3$): δ ppm 0.81 (t, J = 7.0 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H), 1.03−1.21 (m, 2H), 1.28−1.35 (m, 3H), 1.38−1.54 (m, 3H), 1.76−1.93 (m, 1H), 2.08−2.26 (m, 1H), 3.02 (d, J = 14.9 Hz, 1H), 3.44 (d, J = 14.9 Hz, 1H), 3.55 (s, 3H), 4.18−4.30 (m, 2H), 6.06 (d, J = 6.8 Hz, 1H), 6.19 (s, 1H), 6.56 (d, J = 16.0 Hz, 1H), 7.30−7.48 (m, 5H), 7.85 (d, J = 16.2 Hz, 1H), 8.21 (s, 1H). LC-MS (ES^+) m/z 486 $(M + H)^+$.

Step 2: A mixture of cinnamate ester (0.27g, 0.556 mmol) and 10% Pd/C (0.012 g) in EtOH (10 mL) at 25 °C was hydrogenated under an atmosphere of H_2 at 1 atm overnight. The reaction mixture was filtered through diatomaceous earth and washed with EtOH. The filtrate was concentrated under reduced pressure to give an intermediate ester (182 mg, 57%) as a clear oil. ES-LCMS m/z 488 $(M + H)^+$.

Step 3: To a solution of ester (172 mg, 0.353 mmol) in a 1:1:2 mixture of THF/MeOH/H_2O (12 mL) was added lithium hydroxide (84 mg, 3.53 mmol). The reaction mixture was stirred at room temperature overnight then partially concentrated under reduced pressure to remove organic solvents. The resulting aqueous layer was

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then acidified to pH 1−2 with 1 N HCl and extracted with DCM. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give **16** (149 mg, 87%) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.81 (t, J = 7.0 Hz, 3H), 0.88 (t, J = 7.3 Hz, 3H), 0.98−1.14 (m, 1H), 1.13−1.38 (m, 5H), 1.77−1.97 (m, 1H), 2.09−2.39 (m, 1H), 2.48−2.66 (m, 2H), 2.78−2.95 (m, 2H), 3.41 (d, J = 15.0 Hz, 1H), 3.50 (s, 3H), 5.97−6.32 (m, 2H), 7.41 (d, J = 7.0 Hz, 5H), 7.85 (s, 1H). LC-MS (ES^-) m/z 458 $(M - 1)^-$. HRMS (ES^+) calcd for $C_{25}H_{33}NO_5S$ (M + 1) m/z 460.2159, found 460.2158.

3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-1-propanol (16a). To an ice-cold solution of **16** (100 mg, 0.22 mmol) in THF (5 mL) was added BH_3-THF complex (0.653 mL, 0.653 mmol). The reaction mixture was stirred at 25 °C overnight then quenched by dropwise addition of MeOH. The reaction mixture was stirred for 30 min then concentrated under reduced pressure. The residue was taken up with MeOH and evaporated under reduced pressure again. The residue was purified using silica gel (MeOH/DCM = 0:100 to 10:90) to give **16a** (96 mg, 94%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ ppm 0.80 (t, J = 7.0 Hz, 3H), 0.86 (t, J = 7.3 Hz, 3H), 0.99−1.52 (m, 14H), 1.69−1.92 (m, 3H), 2.02−2.25 (m, 1H), 2.56−2.75 (m, 2H), 3.00 (d, J = 14.8 Hz, 1H), 3.40 (d, J = 14.8 Hz, 1H), 3.48 (s, 3H), 3.58 (t, J = 6.2 Hz, 2H), 6.01 (s, 1H), 6.11 (s, 1H), 7.26−7.48 (m, 5H), 7.84 (s, 1H). LC-MS (ES^+) m/z 446 $(M + H)^+$.

4-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]butanoic Acid Trifluoroacetate Salt (17). Step 1: To an ice-cold solution of **16a** (250 mg, 0.561 mmol) in DCM (10 mL) was added Et_3N (0.235 mL, 1.683 mmol) and MsCl (0.048 mL, 0.62 mmol). The reaction mixture was stirred at 25 °C overnight and partitioned between H_2O and DCM. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give an intermediate mesylate (280 mg, 91%) as a light-yellow solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.80 (t, J = 6.8 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H), 0.97−1.34 (m, 8H), 1.35−1.61 (m, 4H), 1.73−2.27 (m, 4H), 2.46−2.78 (m, 4H), 2.85−3.07 (m, 4H), 3.31−3.43 (m, 1H), 3.47 (s, 3H), 4.04−4.26 (m, 2H), 6.01 (d, J = 8.0 Hz, 1H), 6.11 (s, 1H), 7.27−7.52 (m, 5H), 7.82 (s, 1H).

Step 2: To a solution of the above mesylate (90 mg, 0.172 mmol) in DMSO (5 mL) was added NaCN (17 mg, 0.344 mmol). The reaction mixture was stirred at 60 °C for 60 h and directly partitioned between H_2O and EtOAc. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification using silica gel (EtOAc/hexanes = 10:90 to 1:1) afforded an intermediate nitrile (76 mg, 95%) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.80 (t, J = 7.0 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H), 0.99−1.35 (m, 4H), 1.37−1.51 (m, 2H), 1.75−1.98 (m, 3H), 2.08−2.21 (m, 1H), 2.29 (t, J = 7.2 Hz, 2H), 2.64−2.80 (m, 2H), 3.01 (d, J = 14.6 Hz, 1H), 3.40 (d, J = 14.8 Hz, 1H), 6.02 (s, 1H), 6.12 (s, 1H), 7.26−7.48 (m, 5H), 7.81 (s, 1H). LC-MS (ES^+) m/z 455 $(M + H)^+$.

Step 3: A mixture of nitrile (40 mg, 0.088 mmol) and 37% HCl (4 mL) was stirred at 100 °C overnight, cooled to 25 °C, and concentrated under reduced pressure. MeOH was added to transfer the residue to a flask, and the solution was concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded trace amount of desired acid with the major product being the corresponding methyl ester of the acid, which was subsequently hydrolyzed in the presence of excess lithium hydroxide. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **17** (10 mg, 18%, TFA salt) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.86 (t, J = 7.0 Hz, 3H), 0.96 (t, J = 7.4 Hz, 4H), 1.26−1.47 (m, 3H), 1.62−2.25 (m, 5H), 2.26−2.38 (m, 1H), 2.40−2.66 (m, 2H), 2.83−2.97 (m, 1H), 3.45 (d, J = 15.4 Hz, 1H), 3.65 (d, J = 15.6 Hz, 1H), 6.32 (s, 1H), 6.59 (s, 1H), 7.41−7.65 (m, 5H), 7.87 (s, 1H). LC-MS (ES^+)m/z 474 $(M + H)^+$. HRMS (ES^+) calcd for $C_{26}H_{35}NO_5S$ (M + 1) m/z 474.2316, found 474.2314.

[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-methanesulfonic Acid Trifluoroacetate Salt (18). Step 1: To a DCM (2 mL) solution of **4** (0.275 g, 0.617 mmol) at 0 °C under nitrogen was added a 1 M solution of DIBAL-H in toluene (1.3 mL, 1.30 mmol). The reaction was warmed to 25 °C and stirred for 1.5 h then MeOH was added followed by H_2O. The reaction mixture was concentrated then redissolved in EtOAc. The layers were separated, and the aqueous layer was extracted with EtOAc, then the combined organics were washed with brine, dried (Na_2SO_4), filtered, and concentrated. Chromatography on silica using hexanes/EtOAc provided an intermediate alcohol (0.238 g, 91%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.76−0.93 (m, 6 H), 1.04−1.36 (m, 6 H), 1.36−1.59 (m, 6 H), 1.76−1.93 (m, 1 H), 2.05−2.24 (m, 2 H), 3.02 (d, J = 14.8 Hz, 1 H), 3.42 (d, J = 14.8 Hz, 1 H), 3.54 (s, 3 H), 4.65 (qd, J = 13.2, 6.3 Hz, 2 H), 6.07 (br s, 1 H), 6.17 (s, 1 H), 7.29−7.50 (m, 5 H), 8.03 (s, 2 H). ES-LCMS m/z 418 $(M + H)^+$.

Step 2: Imidazole (0.153 g, 0.563 mmol) was dissolved in DCM (2 mL), and the solution was cooled to 0 °C. Triphenylphosphine (0.295 g, 1.126 mmol) was added, followed by bromine (0.058 mL, 1.126 mmol). A solution of alcohol (0.235g, 0.563 mmol) in DCM (1 mL) was added slowly at 0 °C. The reaction was stirred at 0 °C for 2 h followed by addition of aqueous Na_2SO_3, and the resulting mixture separated. The organic layer was dried (Na_2SO_4), filtered through a silica pad, and concentrated to give a thick oil which solidified upon standing to give an intermediate bromide (0.265 g, 84%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.73−0.95 (m, 6 H), 1.02−1.36 (m, 5 H), 1.38−1.65 (m, 5 H), 1.76−1.92 (m, 1 H), 2.08−2.23 (m, 1 H), 3.02 (d, J = 14.8 Hz, 1 H), 3.43 (d, J = 14.8 Hz, 1 H), 3.57 (s, 3 H), 4.38−4.56 (m, 2 H), 6.06 (s, 1 H), 6.16 (s, 1 H), 7.28−7.45 (m, 5 H), 8.05 (s, 1 H). LC-MS m/z 480 $(M + H)^+$. LC-MS 482 $(M + H + 2)^+$.

Step 3: To a solution of bromide (0.100 g, 0.21 mmol) in 1,4-dioxane (1 mL) was added a solution of sodium sulfite (131 mg, 1.04 mmol) in H_2O (1 mL). The mixture was stirred under reflux overnight then concentrated under vacuum, and the crude reaction mixture was washed with 1 N HCl. The supernatant was decanted, leaving a white gummy solid which was triturated with DCM and hexanes to give a solid that was collected via filtration. Purification was accomplished using the Agilent prep-HPLC (C18 packing with MeCN, H_2O w/0.1% TFA as the mobile phase) to give **18** (83 mg, 66%) as a trifluoroacetate salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.62−1.13 (m, 7 H), 1.33 (br s, 3 H), 1.60 (br s, 1 H), 2.02 (br s, 2 H), 3.14−4.46 (m, 6 H + H_2O), 6.33 (br s, 2 H), 7.59 (br s, 5 H), 8.17 (br s, 1 H). LC-MS (ES^+) m/z 482 $(M + H)^+$. HRMS (ES^+) calcd for $C_{23}H_{31}NO_6S_2$ (M + 1) m/z 482.1669, found 482.1671.

3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-1-propanesulfonic Acid (19). Step 1: To a solution of compound **16a** (84.2 mg, 0.189 mmol) in THF (5 mL) was added triphenylphosphine (99 mg, 0.378 mmol) and CBr_4 (125 mg, 0.378 mmol). The reaction mixture was stirred at 25 °C overnight then partitioned between H_2O and DCM. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification using silica gel (EtOAc/hexanes = 10:90 to 50:50) afforded an intermediate bromide (78 mg, 80%) as a clear oil. ES-LCMS m/z 508 $(M + H)^+$.

Step 2: To a solution of bromide (70 mg, 0.138 mmol) in a 1:1 mixture of EtOH/H_2O (10 mL) was added sodium sulfite (868 mg, 6.88 mmol). The reaction mixture was stirred at reflux overnight, cooled to 25 °C, and partially concentrated under reduced pressure to remove the organic solvents. The aqueous layer was then acidified to pH 1 with 1 N HCl. The aqueous layer was extracted with DCM. The combined organic layers were washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification on silica gel (MeOH/DCM = 0:100 to 20:80) afforded **19** (27.2 mg, 38%) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.47−2.26 (m, 12H), 2.33−3.63 (m, 14H), 6.06 (br s, 2H), 7.16−7.60 (m, 5H), 7.78 (br s, 1H). LC-MS (ES^+) m/z 510 $(M + H)^+$. HRMS (ES^+) calcd for $C_{25}H_{35}NO_6S_2$ (M + 1) m/z 510.1988, found 510.1984.

{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-phosphonic Acid Hydrochloride Salt (20). Step 1: Prepared from

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bromomethyl intermediate (see compound **18**, step 2) (0.920 g, 1.92 mmol) upon reaction with triethyl phosphate (0.352 mL, 2.01 mmol) in toluene (10 mL) to give an intermediate phosphonate ester.

Step 2: The phosphonate ester from the previous step was dissolved in 2 mL of 6 N HCl and 1 mL EtOH then heated at reflux for 6 h. The reaction was cooled then concentrated. The residue was dissolved in EtOH then concentrated to give **20** (0.86 g, 85%) as a hydrochloride salt. ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 0.89 (t, J = 6.88 Hz, 3 H), 0.98 (t, J = 7.41 Hz, 4 H), 1.23−1.46 (m, 3 H), 1.64 (dd, J = 14.15, 7.32 Hz, 1 H), 1.97 (dd, J = 14.15, 7.12 Hz, 2 H), 2.68 (br s, 1 H), 2.99−3.12 (m, 2 H), 3.16−3.25 (m, 1 H), 3.45 (d, J = 15.51 Hz, 1 H), 3.58 (s, 3 H), 3.79 (d, J = 15.51 Hz, 1 H), 6.36 (s, 1 H), 6.46 (s, 1 H), 7.57 (s, 5 H), 8.04 (d, J = 2.34 Hz, 1 H). LC-MS (ES$^+$) m/z 482 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{23}H_{32}NO_6PS$ (M + 1) m/z 482.1766, found 482.1769.

{2-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]ethyl}-phosphonic Acid Bis-ammmonium Salt (21). Step 1: Tetraethyl methanediylbis(phosphonate) (645 mg, 2.24 mmol) dissolved in THF (7 mL) was treated with NaH (83 mg, 2.075 mmol, 60% dispersion in oil) with stirring at 25 °C for 30 min. Compound **7** (300 mg, 0.722 mmol) was added, and the mixture was stirred for 1 h at 25 °C, after which time LCMS indicated complete conversion. The mixture was quenched with H_2O and partitioned between EtOAc and brine. The organic phase was dried over $MgSO_4$, filtered, and concentrated to an oil. The residue was purified on silica gel eluting with 40−100% EtOAc/hexanes to give an intermediate phosphonate ester (342 mg, 86% yield) as a clear oil. LC-MS (ES$^+$) m/z 550 [M + H].

Step 2: The ester (240 mg, 0.44 mmol) dissolved in EtOH (20 mL) was treated with 10% Pd/C (46.5 mg, 0.44 mmol) under a balloon of H_2 at 25 °C overnight. The catalyst was filtered off, and the filtrate was concentrated to dryness to give the intermediate phosphonate as a clear oil. The residue was dissolved in DCM (10 mL) and treated with TMSBr (0.227 mL, 1.75 mmol) for 16 h at 23 °C with stirring. The reaction mixture was concentrated to dryness then purified by RP-HPLC (30 mm × 100 mm XBridge column, MeCN/H_2O containing 0.2% NH_4OH buffer, 10 to 80 to 100% over 8 min) to give **21** (88 mg, 41% yield) bis-ammonium salt as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.70−0.85 (m, 6 H), 0.97−1.30 (m, 4 H), 1.29−1.64 (m, 4 H), 1.67−1.81 (m, 1 H), 2.00−2.12 (m, 1 H), 2.58 (d, J = 9.8 Hz, 1 H), 2.63−2.79 (m, 2 H), 3.05 (d, J = 14.9 Hz, 1 H), 3.39 (s, 3 H), 3.50 (d, J = 14.9 Hz, 1 H), 5.92 (d, J = 9.6 Hz, 1 H), 6.05 (s, 1 H), 7.26−7.36 (m, 1 H), 7.36−7.46 (m, 4 H), 7.65 (s, 1 H) (phosphonic acid protons not observed). LC-MS (ES$^+$) m/z 496 [M + H]. HRMS (ES+) calcd for $C_{24}H_{34}NO_6PS$ (M + 1) m/z 496.1922, found 496.1923.

{3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]propyl}-phosphonic Acid Trifluoroacetate Salt (22). Step 1: To a solution of diethylphosphite (119 mg, 0.859 mmol) in THF (5 mL) was added NaH (27.5 mg, 0.687 mmol). The reaction mixture was stirred for 30 min, followed by dropwise addition of mesylate (90 mg, 0.172 mmol) (see compound **17**, step 1) in THF (5 mL). The reaction mixture was stirred at 25 °C overnight then concentrated under reduced pressure. The residue was partitioned between 1 N HCl and EtOAc. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification using silica gel (EtOAc/hexanes = 10:90 to 100:0) afforded an intermediate phosphonate ester (55 mg, 53%) as a white solid. ES-LCMS m/z 566 (M + H)$^+$.

Step 2: To a solution of phosphonate ester (50 mg, 0.088 mmol) in DCM (5 mL) was added TMSBr (0.115 mL, 0.884 mmol). The reaction mixture was stirred at 25 °C overnight then concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **22** (25 mg, 43%, TFA salt) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.86 (t, J = 7.0 Hz, 3H), 0.95 (t, J = 7.4 Hz, 3H), 0.99−1.13 (m, 1H), 1.20−1.45 (m, 3H), 1.48−1.72 (m, 3H), 1.75−2.10 (m, 4H), 2.51−2.66 (m, 1H), 2.73 (t, J = 7.4 Hz, 2H), 3.37 (d, J = 15.6 Hz, 1H), 3.55 (s, 3H), 3.79 (d, J = 15.6 Hz, 1H), 6.32 (s, 1H), 6.42 (s, 1H), 7.39−7.60 (m, 5H), 7.90 (s, 1H). LC-MS(ES$^+$) m/z 510 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{25}H_{36}NO_6PS$ (M + 1) m/z 510.2078, found 510.2079.

3-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]carbonyl}-amino)-2,2-dimethylpropanoic Acid (23). Step 1: To a DMF (0.5 mL) solution of **5** (0.025 g, 0.058 mmol) and ethyl 3-amino-2,2-dimethylpropanoate[23] (0.009 g, 0.064 mmol) at 0 °C was added HATU (0.024 g, 0.064 mmol) followed by DIEA (0.012 mL, 0.070 mmol). The reaction was stirred for 10 min then warmed to 25 °C. After 1 h, the reaction was concentrated to half volume, and H_2O (3 mL) was added. The precipitant was filtered and dried and then used without further purification in the next step.

Step 2: The material was dissolved in THF (0.5 mL), and H_2O (0.25 mL) was added along with excess LiOH (7 mg, 0.174 mmol). The reaction was stirred at 25 °C for 3 h then concentrated. The residue was purified by silica chromatography using DCM/MeOH to give **23** (20 mg, 63%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.71−0.96 (m, 6 H), 0.99−1.65 (m, 12 H), 1.83 (br s, 1 H), 2.18 (br s, 1 H), 3.11 (br s, 1 H), 3.31−3.70 (m, 7 H), 6.08 (br s, 1 H), 6.21 (br s, 1 H), 7.27−7.52 (m, 5 H), 8.13 (br s, 1 H), 8.71 (s, 1 H). LC-MS (ES$^+$) m/z 531 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{28}H_{38}N_2O_6S$ (M + 1) m/z 531.2530, found 531.2529.

({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]carbonyl}-amino)methanesulfonic Acid (24). Prepared in analogous fashion to compound **23** (step 1) via HATU amide-coupling using aminomethanesulfonic acid (0.013 g, 0.116 mmol) and **5** (50 mg, 0.12 mmol) to give compound **24** (45 mg, 73%). ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 0.63−0.93 (m, 6 H), 0.93−1.32 (m, 5 H), 1.45 (br s, 2 H), 1.80 (br s, 1 H), 2.14 (br s, 1 H), 3.22 (br s, 1 H), 3.43−4.72 (m, 6 H + H_2O), 6.02 (br s, 1 H), 6.29 (br s, 1 H), 7.47 (d, J = 3.62 Hz, 5 H), 8.28 (partially resolved t, J = 5.72 Hz, 1 H), 8.47 (s, 1 H). LC-MS (ES$^+$) m/z 525 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{24}H_{32}N_2O_7S_2$ (M + 1) m/z 525.1724, found 525.1729.

2-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]carbonyl}-amino)ethanesulfonic Acid Trifluoroacetate (25). Prepared in an analogous fashion to compound **23** (step 1) via HATU amide-coupling using taurine (0.014 g, 0.116 mmol) and compound **5** (50 mg, 0.116 mmol). Purification via RP-HPLC (30 mm × 150 mm H_2O Sunfire C18 column) using MeCN/H_2O 10−100% with 0.1% TFA as mobile phase over 8 min provided **25** (15 mg, 19%). ^{1}H NMR (400 MHz, MeOH-d_4) δ ppm 0.91 (t, J = 7.13 Hz, 3 H), 1.02 (partially resolved t, J = 7.48 Hz, 4 H), 1.43 (d, J = 6.55 Hz, 3 H), 1.66 (s, 1 H), 2.13 (br s, 2 H), 2.77 (br s, 1 H), 2.91−3.02 (m, 2 H), 3.55 (d, J = 15.83 Hz, 1 H), 3.72 (s, 3 H), 3.75−3.83 (m, 2 H), 4.02 (d, J = 15.83 Hz, 1 H), 6.47 (s, 1 H), 6.68 (s, 1 H), 7.48−7.72 (m, 5 H), 8.74 (s, 1 H). LC-MS (ES$^+$) m/z 539 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{25}H_{34}N_2O_7S_2$ (M + 1) m/z 539.1883, found 539.1886.

***N*-{3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-propanoyl}glycine (26).** Step 1: Prepared in an analogous fashion to compound **23** using compound **16** (50 mg, 0.109 mmol), DIPEA (0.095 mL, 0.544 mmol), HATU (124 mg, 0.326 mmol), and glycine methyl ester hydrochloride (19.39 mg, 0.218 mmol) in DCM (4 mL). Purification on silica gel (MeOH/DCM = 0:100 to 10:90) afforded an intermediate methyl ester (45 mg, 76%) as a clear oil. ES-LCMS m/z 531 (M + H)$^+$. The methyl ester was saponified in an analogous fashion to compound **23**. Step 2: Purification on silica gel (MeOH:DCM = 0:100 to 10:90) afforded **26** (21 mg, 47%) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.49−0.93 (m, 13H), 1.76−1.96 (m, 1H), 2.27−2.66 (m, 3H), 2.70−2.90 (m, 1H), 3.02 (d, J = 6.8 Hz, 1H), 3.14−3.33 (m, 1H), 3.34−3.44 (m, 1H), 3.49 (s, 3H), 3.89 (br s, 2H), 6.02−6.37 (m, 3H), 7.31−7.62 (m, 5H), 7.83 (s, 1H). LC-MS (ES$^+$) m/z 515 (M − H)$^-$. HRMS (ES$^+$) calcd for $C_{27}H_{36}N_2O_6S$ (M + 1) m/z 517.2374, found 517.2372.

({3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-propanoyl}amino)methanesulfonic Acid (27). Prepared in an analogous fashion to compound **23** (step 1) using compound **16** (50

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mg, 0.109 mmol), EDC (62.6 mg, 0.326 mmol), DMAP (39.9 mg, 0.326 mmol), and aminomethanesulfonic acid (12.1 mg, 0.109 mmol) in a 2:1 mixture of DCM/THF (6 mL). The reaction mixture was stirred at 25 °C overnight then partitioned between H_2O and DCM. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification with HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **27** (11 mg, 18%, TFA salt) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.63−0.99 (m, 6H), 1.31 (m, 3H), 1.69−1.97 (m, 2H), 2.12−2.52 (m, 2H), 2.58−2.80 (m, 2H), 2.92 (br s, 1H), 3.21−3.50 (m, 2H), 3.67 (s, 3H), 3.85 (d, J = 15.2 Hz, 2H), 6.51 (d, J = 8.1 Hz, 2H), 7.35−7.77 (m, 5H), 7.85 (s, 1H). LC-MS (ES^+) m/z 553 $(M + H)^+$. HRMS (ES^+) calcd for $C_{26}H_{36}N_2O_7S_2$ (M + 1) m/z 553.2039, found 553.2042.

({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}amino)(oxo)acetic Acid (28). Step 1: A solution of compound **8** (77 mg, 0.19 mmol) in DCM (924 μL) at 0 °C was treated with Et_3N (56.7 μL, 0.407 mmol) followed by methyl chloro(oxo)acetate (18.7 μL, 0.203 mmol). The reaction was stirred for 30 min then warmed to 25 °C and stirred for an additional 1 h. LCMS indicated 92:8/prod:SM. Added additional acid chloride (2 μL) then stirred at 25 °C for 2 h. The reaction was diluted with DCM and H_2O, the layers separated, and the aqueous layer extracted with DCM (3×). The combined organic layers were dried ($MgSO_4$), filtered, and concentrated in vacuo to give a methyl ester (93 mg, 100% yield) which was used as is in the next step. ^{1}H NMR ($CDCl_3$) δ 0.81 (t, J = 7.0 Hz, 3H), 0.88 (t, J = 7.4 Hz, 3H), 1.03−1.58 (m, 6H), 1.76−1.90 (m, 1H), 2.06−2.22 (m, 1H), 2.96−3.10 (m, 2H), 3.41 (d, J = 14.8 Hz, 1H), 3.54 (s, 3H), 3.87 (s, 3H), 4.48 (d, J = 6.0 Hz, 2H), 6.06 (s, 1H), 6.18 (s, 1H), 7.28−7.54 (m, 6H), 7.96 (s, 1H). LC-MS (ES^+) m/z 503 [M + H].

Step 2: A solution of methyl ester (93 mg, 0.185 mmol) in THF (694 μL) and H_2O (231 μL) at 0 °C was treated with 1 M LiOH (204 μL, 0.204 mmol). The reaction was stirred at 0 °C for 60 min then warmed to 25 °C and stirred for 1 h. The reaction was diluted with EtOAc and acidified with dilute HCl. The layers were separated, and the aqueous layer was extracted with EtOAc (3×). The combined organic layers were washed with brine, dried ($MgSO_4$), filtered, and concentrated in vacuo. The residue was triturated with hexanes/Et_2O then a white solid was collected by filtration. **28** (79 mg, 87% yield) was obtained as a white solid. ^{1}H NMR (DMSO-d_6) δ ppm 0.46−0.95 (m, 6H), 0.94−1.61 (m, 6H), 1.62−1.88 (m, 1H), 1.95−2.26 (m, 1H), 3.03 (br s, 1H), 3.48 (m 4H), 4.16−4.38 (m, 2H), 5.92 (br s, 1H), 6.17 (br s, 1H), 7.27−7.65 (m, 5H), 7.75 (br s, 1H), 9.37 (br s, 1H). LC-MS (ES^+) m/z 489 [M + H]. HRMS (ES^+) calcd for $C_{25}H_{32}N_2O_6S$ (M + 1) m/z 489.2062, found 489.2059.

2-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8- yl]methyl}amino)-2-oxoethanesulfonic Acid (29). Step 1: To an ice-cold solution of compound **8** (50 mg, 0.120 mmol) in DCM (6 mL) was added pyridine (0.097 mL, 1.20 mmol) and chloroacetyl chloride (0.048 mL, 0.60 mmol). The reaction mixture was stirred at 25 °C overnight then concentrated under reduced pressure to give a light-yellow oil. The intermediate chloroacetamide (55 mg, 92%) was used in the next step without further purification. ^{1}H NMR ($CDCl_3$) δ ppm 0.80 (t, J = 7.0 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H), 0.99−1.60 (m, 6H), 1.69−1.90 (m, 1H), 2.09−2.24 (m, 1H), 3.02 (s, 2H), 3.37 (s, 1H), 3.53 (s, 3H), 4.05 (d, J = 7.0 Hz, 3H), 4.44 (d, J = 6.1 Hz, 2H), 6.05 (s, 1H), 6.17 (s, 1H), 6.91−7.13 (m, 1H), 7.27−7.45 (m, 5H). LC-MS (ES^+) m/z 494 $(M + H)^+$.

Step 2: To a solution of the intermediate chloroacetamide (55 mg, 0.112 mmol) in a 1:1 mixture of EtOH/H_2O (6 mL) was added Na_2SO_3 (141 mg, 1.115 mmol). The reaction mixture was stirred at 80 °C overnight, cooled to 25 °C, acidified with AcOH to pH 3−4, and concentrated under reduced pressure. Purification using silica gel (MeOH/DCM = 0:100 to 30:70) afforded **29** (32 mg, 52%) as an off-white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.81 (t, J = 6.6 Hz, 3H), 0.89 (t, J = 7.4 Hz, 3H), 1.01−1.94 (m, 7H), 2.06−2.49 (m, 1H), 3.43−3.59 (m, 4H), 3.63−3.78 (m, 2H), 4.39 (br s, 2H), 6.06 (br s, 1H), 6.28 (br s, 1H), 7.16−7.68 (m, 5H), 7.94 (s, 1H). LC-MS (ES^+) m/z 539 $(M + H)^+$. HRMS (ES^+) calcd for $C_{25}H_{34}N_2O_7S_2$ (M + 1) m/z 539.1889, found 539.1886.

3-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}amino)-3-oxo-1-propanesulfonic Acid Ammonium Salt (30). Step 1: To an ice-cold solution of **8** (150 mg, 0.233 mmol) in DCM (10 mL) was added pyridine (0.132 mL, 1.629 mmol) and 3-chloropropanoyl chloride (0.045 mL, 0.465 mmol). The reaction mixture was stirred at 25 °C for 4 h then partitioned between H_2O and DCM. Purification via silica gel (MeOH/DCM = 0:100 to 3:97) afforded an intermediate chloropropanamide (105 mg, 89%) as a colorless oil. ES-LCMS m/z 508 $(M + H)^+$.

Step 2: To a solution of chloropropanamide (150 mg, 0.296 mmol) in a 1:1 mixture of EtOH/H_2O (10 mL) was added Na_2SO_3 (186 mg, 1.479 mmol). The reaction mixture was stirred at 60 °C overnight, cooled to 25 °C, acidified with AcOH, and concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.5% ammonium hydroxide in H_2O) afforded **30** (27 mg, 15%, ammonium salt) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.78 (t, J = 6.8 Hz, 3H), 0.86 (t, J = 7.4 Hz, 3H), 1.03−1.32 (m, 4H), 1.32−1.46 (m, 1H), 1.48−1.66 (m, 1H), 1.67−1.85 (m, 1H), 2.08−2.31 (m, 1H), 2.57−2.73 (m, 2H), 2.95−3.11 (m, 3H), 3.44 (d, J = 14.8 Hz, 1H), 3.51 (s, 3H), 4.18−4.37 (m, 2H), 6.04 (s, 1H), 6.20 (s, 1H), 7.13−7.55 (m, 5H), 7.86 (s, 1H), 8.31−8.49 (m, 1H). LC-MS (ES^+) m/z 553 $(M + H)^+$. HRMS (ES^+) calcd for $C_{26}H_{36}N_2O_7S_2$ (M + 1) m/z 553.2042, found 553.2042.

[2-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}amino)-2-oxoethyl]phosphonic Acid Trifluoroacetate Salt (31). Step 1: A mixture of intermediate chloroacetamide (see compound **29**, step 1) (82 mg, 0.166 mmol) and triethyl phosphite (2.9 mL, 16.63 mmol) was stirred at 135 °C overnight, cooled to 25 °C, and concentrated under reduced pressure. The crude diethyl phosphonate (99 mg, 98%) was used in the next step without further purification. ES-LCMS m/z 595 $(M + H)^+$.

Step 2: To a solution of intermediate diethyl phosphonate (99 mg, 0.166 mmol) in DCM (5 mL) was added TMSBr (0.065 mL, 0.499 mmol), and the reaction mixture was stirred at 25 °C overnight. Additional TMSBr (0.216 mL, 1.66 mmol) was added, and the reaction mixture was stirred overnight at 25 °C. The reaction mixture was then concentrated under reduced pressure and treated with H_2O. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **31** (69 mg, 63%, TFA salt) as a gray−white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.89 (t, J = 6.8 Hz, 3H), 0.99 (t, J = 7.4 Hz, 3H), 1.22−1.49 (m, 3H), 1.56−1.75 (m, 1H), 1.96−2.13 (m, 2H), 2.57−2.87 (m), 3H), 3.61 (s, 3H), 3.66 (br s, 1H), 3.85 (d, J = 15.4 Hz, 1H), 4.24 (d, J = 14.1 Hz, 1H), 4.43 (d, J = 14.8 Hz, 1H), 6.42 (s, 1H), 6.51 (s, 1H), 7.35−7.75 (m, 5H), 8.01 (s, 1H). LC-MS (ES^+) m/z 539 $(M + H)^+$. HRMS (ES^+) calcd for $C_{25}H_{35}N_2O_7PS$ (M + 1) m/z 539.1980, found 539.1981.

2,2′-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]carbonyl}imino)diacetic Acid Hydrochloride (32). Prepared in analogous fashion to compound **23** via HATU amide-coupling using diethyl iminodiacetate (0.022 g, 0.116 mmol) and compound **5** (0.050 g, 0.116 mmol) followed by LiOH (10 mg, 0.24 mmol) hydrolysis conditions to give **32** (45 mg, 63%). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.64−0.92 (m, 6 H), 0.95−1.63 (m, 7 H), 1.66−1.91 (m, 1 H), 2.08 (br s, 1 H), 3.01−3.53 (m, 4 H + H_2O), 3.86 (br s, 2 H), 4.15 (br s, 2 H), 5.97 (br s, 1 H), 6.15 (s, 1 H), 7.22−7.60 (m, 5 H), 7.73 (s, 1 H), 12.76 (br s, 2 H). LC-MS (ES^+) m/z 547 $(M + H)^+$. HRMS (ES^+) calcd for $C_{27}H_{34}N_2O_8S$ (M + 1) m/z 547.2115, found 547.2114.

3-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]carbonyl}amino)pentanedioic Acid (33). Prepared in analogous fashion to compound **23** via HATU amide-coupling using dimethyl 3-amino-pentanedioate (81 mg, 0.463 mmol) and compound **5** (100 mg, 0.232 mmol) followed by LiOH (10 mg, 0.24 mmol) hydrolysis in 1:1:1 mixture of MeOH/H_2O/THF (15 mL). Purification with HPLC (medium column, Gilson 845Z preparation system; Sunfire C18 5 μM,

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30 mm × 150 mm. Method, 10−100% MeCN/H_2O with 0.05% TFA over 10 min, 45 mL/min, λ = 220, 254 nm) afforded **33** as a light-yellow solid (61 mg, 38% yield) as a TFA salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.68−0.89 (m, 6H), 0.95−1.34 (m, 4H), 1.34−1.61 (m, 2H), 1.66−1.88 (m, 1H), 1.97−2.23 (m, 1H), 2.52−2.61 (m, 4H), 3.06−3.20 (m, 1H), 3.47−3.70 (m, 4H), 4.46−4.63 (m, 1H), 5.86−6.05 (m, 1H), 6.17−6.28 (m, 1H), 7.24−7.55 (m, 5H), 8.36 (s, 1H), 8.40−8.55 (m, 1H). LC-MS (ES$^+$) m/z 561 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{28}H_{36}N_2O_8S$ (M + 1) m/z 561.2273, found 561.2271.

3-({3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-propanoyl}amino)pentanedioic Acid Trifluoroacetate Salt (34). Prepared in analogous fashion to compound **23** using **16** (100 mg, 0.218 mmol), dimethyl 3-aminopentanedioate (81 mg, 0.463 mmol), DIPEA (0.190 mL, 1.088 mmol), and HATU (165 mg, 0.435 mmol) in DCM (6 mL). Saponification with LiOH (10 mg, 0.24 mmol) in 1:1:1 mixture of MeOH/H_2O/THF (15 mL). Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **34** (92 mg, 60%, TFA salt) as white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.87 (t, J = 7.0 Hz, 3H), 0.96 (t, J = 7.4 Hz, 3H), 1.33−1.39 (m, 3H), 1.49−1.67 (m, 1H), 1.86−2.13 (m, 2H), 2.37−2.46 (m, 2H), 2.48−2.58 (m, 4H), 2.60−2.75 (m, 1H), 2.78−3.03 (m, 2H), 3.46 (d, J = 15.6 Hz, 2H), 3.57 (s, 3H), 3.81 (d, J = 15.6 Hz, 1H), 4.33−4.48 (m, 1H), 6.37 (s, 1H), 6.44 (s, 1H), 7.48−7.62 (m, 5H), 7.90 (s, 1H). LC-MS (ES+) m/z 589 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{30}H_{40}N_2O_8S$ (M + 1) m/z 589.2581, found 589.2584.

2,2′-({3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]-propanoyl}imino)diacetic Acid (35). Step 1: Prepared in a similar fashion to compound **23** using compound **16** (50 mg, 0.109 mmol), diethyl 2,2′-iminodiacetate (41.2 mg, 0.218 mmol), EDC (62.6 mg, 0.326 mmol), and DMAP (39.9 mg, 0.326 mmol) in a 2:1 mixture of DCM/THF (6 mL). Purification using silica gel (EtOAc/hexanes = 10:90 to 50:50) afforded an intermediate diester (45 mg, 65%) as a clear oil. ES-LCMS m/z 631 (M + H)$^+$. Saponification of intermediate diethyl diester (45 mg, 0.071 mmol) as in compound **23**, step 2 using LiOH (85 mg, 3.55 mmol) in a 1:1:1 mixture of THF/MeOH/H_2O (9 mL). Purification with HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **35** (23 mg, 53%, TFA salt) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.55−1.45 (m, 8H), 1.69−2.09 (m, 3H), 2.31−2.48 (m, J = 14.1 Hz, 1H), 2.50−2.95 (m, 2H), 2.97−3.13 (m, 1H), 3.23−3.70 (m, 11H), 3.72−3.88 (m, J = 8.6 Hz, 2H), 3.96−4.25 (m, 2H), 6.42 (s, 1H), 6.54 (br s, 1H), 7.37−7.59 (m, 3H), 7.61−7.75 (m, 2H), 7.84 (s, 1H). LC-MS (ES$^+$) m/z 575 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{29}H_{38}N_2O_8S$ (M + 1) m/z 575.2428, found 575.2427.

3-{[2-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-amino)-2-oxoethyl]amino}pentanedioic Acid Trifluoroacetate Salt (36). Step 1: To a solution intermediate chloroacetamide (see compound **29**, step 1) (55 mg, 0.112 mmol) and diethyl 3-aminopentanedioate (45.3 mg, 0.223 mmol) in DMF (3 mL) was added K_2CO_3 (61.7 mg, 0.446 mmol) and KI (74.1 mg, 0.446 mmol). The reaction mixture was stirred at 60 °C overnight, cooled to 25 °C, and partitioned between H_2O and EtOAc. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification using silica gel (MeOH/DCM = 0:100 to10:90) afforded an intermediate diester (75 mg, 100%) as a yellow oil. ES-LCMS m/z 660 (M + H)$^+$.

Step 2: To a solution of the diester (74 mg, 0.112 mmol) in a 1:1:1 mixture of THF/MeOH/H_2O (6 mL) was added LiOH (134 mg, 5.60 mmol). The reaction mixture was stirred at 25 °C overnight, acidified with AcOH, and concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **36** (20 mg, 21%, TFA salt) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.86 (t, J = 6.8 Hz, 3H), 0.93 (t, J = 7.4 Hz, 3H), 0.97−1.13 (m, 1H), 1.24−1.45 (m, 3H), 1.49−1.72 (m, 1H), 1.77−2.11 (m, 2H), 2.45−2.72 (m, 2H), 2.85 (d, J = 5.7 Hz, 3H), 3.46 (d, J = 15.4 Hz, 1H), 3.58 (s, 3H), 3.74 (d, J = 15.4 Hz, 1H), 3.82−4.00 (m, 3H), 4.41 (d, J = 3.3 Hz, 2H), 6.30 (s, 1H), 6.45 (s, 1H), 7.34−7.66 (m, 5H), 7.95 (s, 1H). LC-MS (ES$^+$) m/z 604 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{30}H_{41}N_3O_8S$ (M + 1) m/z 604.2697, found 604.2693.

4-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-amino)butanoic Acid Trifluoroacetate Salt (37). Step 1: To a solution of **7** (200 mg, 0.481 mmol) in 1,2-dichloroethane (5 mL) was added methyl 4-aminobutanoate (111 mg, 0.722 mmol) and AcOH (0.276 mL, 4.81 mmol). The reaction mixture was stirred at 25 °C overnight, treated with $NaHB(OAc)_3$ (255 mg, 1.20 mmol), and stirred for 1 h. The reaction mixture was treated with aqueous sodium carbonate solution and extracted with DCM. The organic layer was washed with H_2O and saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification using silica gel (EtOAc/hexanes = 20:80 to 60:40) afforded an intermediate ester (210 mg, 83%) as a light-yellow oil. ES-LCMS m/z 517 (M + H)$^+$.

Step 2: To a solution of ester (210 mg, 0.406 mmol) in a 1:1:1 mixture of THF/MeOH/H_2O (3 mL) was added LiOH (10 mg, 0.406 mmol). The reaction mixture was stirred at 25 °C overnight then concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **37** (46 mg, 44%) trifluoroacetate salt as a white solid. ^{1}H NMR (DMSO-d_6) δ ppm 0.76 (t, J = 6.6 Hz, 3H), 0.81 (t, J = 7.2 Hz, 3H), 0.96−1.32 (m, 4H), 1.35−1.61 (m, 2H), 1.67−1.90 (m, 3H), 1.99−2.15 (m, 1H), 2.33 (t, J = 7.2 Hz, 2H), 2.88−3.01 (m, 2H), 3.09 (d, J = 15.0 Hz, 1H), 3.49 (s, 3H), 3.63 (d, J = 14.8 Hz, 1H), 4.16 (br s, 2H), 5.97 (s, 1H), 6.18 (s, 1H), 7.30−7.56 (m, 5H), 8.09 (s, 1H), 8.67 (br s, 2H). LC-MS (ES$^+$) m/z 503 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{38}N_2O_5S$ (M + 1) m/z 503.2584, found 503.2580.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-2-methylalanine Trifluoroacetate Salt (39).** Prepared in a similar fashion to **37** using methyl 2-amino-2-methylpropanoate. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **39** (102 mg, 66%, TFA salt) as a white solid. ^{1}H NMR (DMSO-d_6) δ ppm 0.77 (t, J = 6.8 Hz, 3H), 0.82 (t, J = 7.2 Hz, 3H), 0.98−1.31 (m, 4H), 1.35−1.49 (m, 1H), 1.70−1.86 (m, 1H), 2.00−2.23 (m, 1H), 2.46−2.55 (m, 1H), 3.13 (d, J = 14.8 Hz, 1H), 3.49 (s, 3H), 3.64 (d, J = 15.0 Hz, 1H), 4.14 (d, J = 17.6 Hz, 2H), 6.00 (s, 1H), 6.18 (s, 1H), 7.28−7.55 (m, 5H), 8.10 (s, 1H). LC-MS (ES$^+$) m/z 503 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{38}N_2O_5S$ (M + 1) m/z 503.2581, found 503.2580.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-D-aspartic Acid Trifluoroacetate Salt (50).** Prepared in a similar fashion to **37** using dimethyl D-aspartate. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **50** (11 mg, 37%, TFA salt) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.83 (t, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H), 1.04−1.41 (m, 4H), 1.43−1.57 (m, 1H), 1.58−1.78 (m, 1H), 1.80−1.96 (m, 1H), 2.92−3.14 (m, 2H), 3.58−3.67 (m, 4H), 4.13−4.24 (m, 1H), 4.31−4.45 (m, 2H), 6.18 (s, 1H), 6.42 (s, 1H), 7.33−7.54 (m, 5H), 8.10 (s, 1H). LC-MS (ES$^+$) m/z 533 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{36}N_2O_7S$ (M + 1) m/z 533.2324, found 533.2321.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-L-aspartic Acid Trifluoroacetate Salt (51).** Prepared in a similar fashion to **37** using dimethyl L-aspartate. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **51** (25 mg, 89%, TFA salt) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.81 (t, J = 6.8 Hz, 3H), 0.91 (t, J = 7.4 Hz, 3H), 1.03−1.40 (m, 4H), 1.42−1.58 (m, 1H), 1.62−1.77 (m, 1H), 1.79−1.95 (m, 1H), 2.38 (d, J = 4.1 Hz, 1H), 2.88−3.13 (m, 2H), 3.23 (d, J = 15.0 Hz, 1H), 3.55−3.66 (m, 4H), 4.19 (dd, J = 6.8, 4.3 Hz, 1H), 4.38 (d, J = 7.6 Hz, 2H), 6.17 (s, 1H), 6.40 (s, 1H), 7.32−7.56 (m, 5H), 8.10 (s, 1H). LC-MS (ES$^+$) m/z 533 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{36}N_2O_7S$ (M + 1) m/z 533.2324, found 533.2321.

3-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-amino)pentanedioic Acid (56). Prepared in a similar fashion to **37** using diethyl 3-aminopentanedioate (see below). Following saponfi-

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cation, the reaction was concentrated under reduced pressure then dissolved in H_2O and MeCN. The solution was acidified to pH 4−5 with AcOH, partially concentrated to remove MeCN under reduced pressure, and left to stand for 30 min. A white precipitate was collected by filtration then dried under reduced pressure at 50 °C overnight to give **56** as a white solid in >90% yield. ^{1}H NMR (MeOH-d_4) δ ppm 0.78 (t, J = 7.0 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H), 1.05−1.48 (m, 5H), 1.48−1.63 (m, 1H), 1.69−1.84 (m, 1H), 2.12−2.27 (m, 1H), 2.52−2.73 (m, 4H), 3.09 (d, J = 14.8 Hz, 1H), 3.47 (d, J = 14.8 Hz, 1H), 3.58 (s, 3H), 3.60−3.68 (m, 1H), 4.25 (s, 2H), 6.06 (s, 1H), 6.29 (s, 1H), 7.27−7.49 (m, 5H), 8.05 (s, 1H). ^{13}C NMR (500 MHz, DMSO-d_6) δ ppm 7.5, 13.9, 22.5, 24.5, 30.1, 32.4, 37.5, 43.2, 51.2, 54.3, 55.2, 56.8, 63.1, 110.3, 124.5, 126.9, 127.7, 128.1, 128.7, 131.8, 142.2, 147.1, 159.9, 172.7. LC-MS (ES$^+$) m/z 547 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{28}H_{39}N_2O_7S$ (M + H$^+$) m/e 547.2478, found 547.2474. $C_{28}H_{38}N_2O_7S$ requires C, 61.52%; H, 7.01%; N, 5.12%; S, 5.87%. Found: C, 61.49%; H, 6.98%; N, 5.08%; S, 5.86%.

Preparation of diethyl 3-aminopentanedioate: To a solution of β-glutamic acid (500 mg, 3.40 mmol) in EtOH (10 mL) was added thionyl chloride (0.992 mL, 13.59 mmol) dropwise. The reaction mixture was stirred at 25 °C overnight and concentrated under reduced pressure. The residue was partitioned between DCM and saturated potassium carbonate solution. The organic layer was washed with saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to give the title compound (702 mg, 97%) as a colorless oil. ^{1}H NMR ($CDCl_3$) δ ppm 1.09−1.29 (m, 6H), 2.24−2.60 (m, 4H), 3.46−3.74 (m, 1H), 4.04−4.27 (m, 4H).

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-*N*-methyl-β-alanine Trifluoroacetate Salt (38).** Step 1: To a solution of **7** (200 mg, 0.481 mmol) in 1,2-dichloroethane (10 mL) was added 1,1-dimethylethyl β-alaninate (175 mg, 0.963 mmol) and AcOH (0.276 mL, 4.81 mmol). After 1 h of stirring, $NaHB(OAc)_3$ (255 mg, 1.203 mmol) was added to the reaction mixture. The reaction mixture was stirred at 25 °C for 1 h then treated with an aqueous sodium carbonate solution. The layers were separated, and the aqueous layer was extracted with DCM. The combined organic layers were washed with H_2O and saturated brine, dried (Na_2SO_4), filtered, and concentrated under reduced pressure to afford an intermediate ester (210 mg, 80%) as an oil. ES-LCMS m/z 559 (M + H)$^+$.

Step 2: To the ester (210 mg, 0.289 mmol) was added 4 N HCl in dioxane (3.61 mL, 14.46 mmol). The reaction mixture was stirred at 25 °C overnight then concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **38** (143 mg, 82%, TFA salt) as a white solid. ^{1}H NMR ($CDCl_3$) δ ppm 0.86 (t, J = 6.8 Hz, 3H), 0.92 (t, J = 7.2 Hz, 3H), 1.00−1.17 (m, 1H), 1.18−1.46 (m, 3H), 1.51−1.68 (m, 1H), 1.68−1.82 (m, 1H), 1.89−2.10 (m, 1H), 2.35 (br s, 1H), 2.79 (br s, 2H), 3.25 (br s, 2H), 3.33−3.52 (m, 2H), 3.62 (s, 3H), 4.12−4.42 (m, 2H), 6.24 (s, 1H), 6.35 (s, 1H), 7.35−7.62 (m, 5H), 8.13 (s, 1H). LC-MS (ES$^+$) m/z 503 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{38}N_2O_5S$ (M + 1) m/z 503.2581, found 503.2580.

1-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-L-proline Ammonium Salt (40). Prepared in a similar fashion to **38** using 1,1-dimethylethyl L-prolinate HCl. Purification by RP-HPLC (30 mm × 100 mm XBridge column; MeCN + 0.2% NH_4OH and H_2O + 0.2% NH_4OH were used as the solvent system; 10−70% over 8 min, 100−100% to 10 min) to give **40** (260 mg, 84% yield) ammonium salt as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.80 (t, J = 7.4 Hz, 3 H), 0.96−1.29 (m, 4 H), 1.32−1.53 (m, 2 H), 1.64−1.94 (m, 4 H), 1.99−2.16 (m, 2 H), 2.62 (d, J = 10.0 Hz, 1 H), 2.98−3.12 (m, 2 H), 3.26−3.37 (m, 2 H), 3.44 (s, 3 H), 3.55 (d, J = 15.0 Hz, 1 H), 3.82 (d, J = 14.1 Hz, 1 H), 3.89−4.00 (m, 1 H), 5.94 (d, J = 9.2 Hz, 1 H), 6.10 (s, 1 H), 7.27−7.37 (m, 1 H), 7.42 (d, J = 4.5 Hz, 4 H), 7.99 (s, 1 H) (COOH proton not observed). LC-MS (ES$^+$) m/z 513.5 [M + H]. HRMS (ES+) calcd for $C_{28}H_{38}N_2O_5S$ (M + 1) m/z 515.2583, found 515.2583.

1-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-D-proline Ammonium salt (41). Prepared in a similar fashion to **38** using 1,1-dimethylethyl D-prolinate. Cleavage of the t-butyl ester was accomplished using TFA. Purification by RP-HPLC (30 mm × 100 mm XBridge column; MeCN + 0.1% NH_4OH and H_2O + 0.1% NH_4OH were used as the solvent system; 10−70% over 8 min) to give **41** ammonium salt (333 mg, >99% yield) as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.81 (t, J = 7.4 Hz, 3 H), 0.95−1.31 (m, 4 H), 1.34−1.56 (m, 2 H), 1.67−2.14 (m, 5 H), 2.36−2.47 (m, 1 H), 2.58−2.81 (m, 1 H), 3.06 (d, J = 15.0 Hz, 1 H), 3.14−3.27 (m, 1 H), 3.35−3.47 (m, 1 H), 3.49 (s, 3 H), 3.62 (d, J = 15.0 Hz, 1 H), 4.17−4.30 (m, 1 H), 4.36 (d, J = 13.1 Hz, 1 H), 4.50 (d, J = 13.1 Hz, 1 H), 5.96 (br s, 1 H), 6.17 (s, 1 H), 7.31−7.55 (m, 5 H), 8.09 (s, 1 H), 9.84 (br s, 1 H). LC-MS (ES$^-$) m/z 513 [M − 1]. LC-MS (ES$^+$) m/z 515 [M + H]. HRMS (ES+) calcd for $C_{28}H_{38}N_2O_5S$ (M + 1) m/z 515.2584, found 515.2580.

***N*^{2}-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-L-lysine Ammonium Salt (42).** Prepared in a similar fashion to **38** using 1,1-dimethylethyl N^6-{[(1,1-dimethylethyl)oxy]carbonyl}-L-lysinate·HCl. The crude material was purified by RP-HPLC (30 mm × 100 mm XBridge column, MeCN/H_2O containing 0.2% NH_4OH buffer, 10 to 80 to 100% over 10 min) to give **42** (297 mg, 38% yield) ammonium salt as a pink foam. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.80 (t, J = 7.4 Hz, 3 H), 0.97−1.60 (m, 12 H), 1.67−1.80 (m, 1 H), 1.99−2.14 (m, 1 H), 2.54 (d, J = 9.8 Hz, 1 H), 2.64−2.78 (m, 3 H), 3.06 (d, J = 14.9 Hz, 1 H), 3.41 (s, 3 H), 3.50 (dd, J = 15.0, 9.3 Hz, 2 H), 3.65 (d, J = 15.0 Hz, 1 H), 5.94 (d, J = 9.6 Hz, 1 H), 6.06 (s, 1 H), 7.32 (dq, J = 8.5, 4.2 Hz, 1 H), 7.41 (d, J = 4.5 Hz, 4 H), 7.99 (s, 1 H) (4 exchangeable protons not observed). LC-MS (ES$^+$) m/z 546 [M + H]. HRMS (ES+) calcd for $C_{29}H_{43}N_3O_5S$ (M + 1) m/z 546.2998, found 546.3002.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-L-threonine Ammonium Salt (43).** Prepared as in **38** using 1,1-dimethylethyl L-threoninate. The crude product mixture was purified by RP-HPLC (30 mm × 100 mm XBridge column, MeCN/H_2O containing 0.2% NH_4OH buffer, 10 to 70 to 100% over 10 min) to give **43** (188 mg, 60% yield) ammonium salt as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.80 (t, J = 7.4 Hz, 3 H), 0.97−1.28 (m, 4 H), 1.16 (d, J = 6.4 Hz, 3 H), 1.33−1.54 (m, 2 H), 1.68−1.79 (m, 1 H), 2.00−2.12 (m, 1 H), 2.59 (d, J = 9.8 Hz, 1 H), 2.96 (d, J = 4.7 Hz, 1 H), 3.06 (d, J = 14.9 Hz, 1 H), 3.44 (s, 3 H), 3.54 (d, J = 14.9 Hz, 1 H), 3.67−3.93 (m, 3 H), 5.94 (d, J = 9.4 Hz, 1 H), 6.09 (s, 1 H), 7.29−7.37 (m, 1 H), 7.37−7.46 (m, 4 H), 8.03 (s, 1 H) (3 exchangeable protons not observed). LC-MS (ES$^+$) m/z 519 [M + H]. HRMS (ES+) calcd for $C_{27}H_{38}N_2O_6S$ (M + 1) m/z 519.2532, found 519.2529.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-L-serine Bis-ammonium Salt (44).** Prepared as in **38** using 1,1-dimethylethyl L-serinate·HCl. The crude product was purified by RP-HPLC (30 mm × 100 mm XBridge column, MeCN/H_2O containing 0.2% NH_4OH buffer, 10 to 70 to 100% over 8 min) to give **44** (194 mg, 64% yield) bis-ammonium salt as a white solid. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.80 (t, J = 7.4 Hz, 3 H), 0.96−1.29 (m, 4 H), 1.32−1.54 (m, 2 H), 1.66−1.82 (m, 1 H), 1.98−2.13 (m, 1 H), 2.62 (d, J = 9.6 Hz, 1 H), 3.06 (d, J = 15.0 Hz, 1 H), 3.16 (t, J = 5.2 Hz, 1 H), 3.45 (s, 3 H), 3.56 (d, J = 14.9 Hz, 1 H), 3.65 (qd, J = 11.1, 5.2 Hz, 2 H), 3.79−3.94 (m, 2 H), 5.02 (br s, 1 H), 5.94 (d, J = 9.6 Hz, 1 H), 6.10 (s, 1 H), 7.28−7.37 (m, 1 H), 7.37−7.47 (m, 4 H), 8.02 (s, 1 H) (2 exchangables not observed). LC-MS (ES$^+$) m/z 505 [M + H]. HRMS (ES+) calcd for $C_{26}H_{36}N_2O_6S$ (M + 1) m/z 505.2372, found 505.2372.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-glycine (45).** Prepared as in **38** using 1,1-dimethylethyl glycinate. Recrystallization from CH_3CN provided **45** (5.0 g, 82%) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.78 (t, J = 6.8 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H), 1.01−1.34 (m, 4H), 1.34−1.48 (m, 1H), 1.49−1.64 (m, 1H), 1.67−1.86 (m, 1H), 2.12−2.31 (m, 1H), 3.08 (d, J = 15.0 Hz, 1H), 3.42 (s, 2H), 3.50 (d, J = 14.8 Hz, 1H), 3.58 (s, 3H), 4.22 (s,

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2H), 6.06 (s, 1H), 6.30 (s, 1H), 7.25−7.50 (m, 5H), 8.06 (s, 1H). LC-MS (ES^+) m/z 475 $(M + H)^+$. HRMS (ES^+) calcd for $C_{25}H_{34}N_2O_5S$ $(M + 1)$ m/z 475.2270, found 475.2267.

***N*-{[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-*N*-methylglycine Ammonium Salt (46).** Prepared as in **38** using 1,1-dimethylethyl *N*-methylglycinate·HCl. The residue was purified by RP-HPLC (30 mm × 100 mm XBridge column; acetonitrile + 0.2% NH_4OH and H_2O + 0.2% NH_4OH were used as the solvent system; 10% to 70% over 8 min, 100% to 100% to 10 min) to give **46** (130 mg, 44% yield) ammonium salt as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.80 (t, J = 7.4 Hz, 3 H), 0.96−1.28 (m, 4 H), 1.32−1.53 (m, 2 H), 1.68−1.80 (m, 1 H), 1.99−2.11 (m, 1 H), 2.30 (s, 3 H), 2.62 (d, J = 9.4 Hz, 1 H), 3.07 (d, J = 14.9 Hz, 1 H), 3.24 (s, 2 H), 3.42 (s, 3 H), 3.54 (d, J = 14.9 Hz, 1 H), 3.69 (s, 2 H), 5.93 (d, J = 8.2 Hz, 1 H), 6.10 (s, 1 H), 7.33 (dq, J = 8.5, 4.2 Hz, 1 H), 7.41 (d, J = 4.3 Hz, 4 H) 7.98 (s, 1 H) (COOH proton not observed). LC-MS (ES^+) m/z 489 [M + H]. HRMS (ES+) calcd for $C_{26}H_{36}N_2O_5S$ $(M + 1)$ m/z 489.2422, found 489.2423.

2-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-amino)ethanesulfonic Acid Ammonium Salt (47). To a solution of **8** (100 mg, 0.240 mmol) in DMF (4 mL) was added 2-bromoethane sulfonic acid sodium salt (25.3 mg, 0.120 mmol). The reaction mixture was stirred at 70 °C overnight, cooled to 25 °C, acidified with TFA, and concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN), followed by further purification with RP-HPLC (eluting with MeCN/H_2O with 0.5% ammonium hydroxide in H_2O) afforded **47** (28 mg, 54%, ammonium salt) as a white solid. ^1H NMR (MeOH-d_4) δ ppm ^1H NMR (MeOH-d_4) δ ppm 0.78 (t, J = 6.6 Hz, 3H), 0.87 (t, J = 7.4 Hz, 3H), 1.05−1.32 (m, 4H), 1.34−1.49 (m, 1H), 1.49−1.70 (m, 1H), 1.71−1.88 (m, J = 5.1 Hz, 1H), 2.09−2.31 (m, 1H), 2.96−3.15 (m, 3H), 3.30−3.38 (m, 2H), 3.49 (d, J = 15.0 Hz, 1H), 3.58 (s, 3H), 4.20 (s, 2H), 6.06 (s, 1H), 6.30 (s, 1H), 7.19−7.54 (m, 5H), 8.03 (s, 1H). LC-MS (ES^+) m/z 525 $(M + H)^+$. HRMS (ES^+) calcd for $C_{25}H_{36}N_2O_6S_2$ $(M + 1)$ m/z 525.2093, found 525.2093.

[({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-amino)methyl]phosphonic Acid Trifluoroacetate Salt (48). Step 1: Compound **8** (45.8 mg, 0.110 mmol) was combined with paraformaldehyde (3.5 mg, 0.11 mmol) and diethyl phosphite (0.014 mL, 0.11 mmol) in THF (4 mL) and stirred for 16 h at 75 °C, after which time all solvent was gone and LCMS indicated <20% conversion. The THF was replaced and additional diethyl phosphite (0.043 mL, 0.330 mmol) was added, and the mixture was heated an additional 24 h at 75 °C, after which time LCMS indicated ∼50% conversion. Additional diethyl phosphite (0.150 mL, 1.16 mmol) was added and heating was continued for 24 h, after which time LCMS indicated 70% completion. Additional paraformaldehyde (1.7 mg, 0.055 mmol) was added and heating was continued for 24 h, after which time LCMS indicated complete conversion of starting material to mono- and disubstituted products. The mixture was concentrated to dryness and purified on silica gel eluting with 0−20% MeOH/DCM to give an intermediate diethyl phosphonate (36 mg, 58% yield) as a clear oil. LC-MS (ES^+) m/z 567 [M + H].

Step 2: The diethyl phosphonate (36 mg, 0.064 mmol) dissolved in DCM (1 mL) was treated with TMSBr (100 mg, 0.65 mmol) at 25 °C for 18 h. The mixture was concentrated to dryness, and the crude product was purified by RP-HPLC (30 mm × 75 mm , C18, 5 um H_2O Sunfire column at 55 mL/min; MeCN + 0.05% TFA and H_2O + 0.05% TFA were used as the solvent system; 10−100 over 8 min, 100 to 100 to 10 min) to give **48** (6 mg, 18% yield) trifluoroacetate salt as a white glass. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 7.0 Hz, 3 H), 0.81 (t, J = 7.4 Hz, 3 H), 0.95−1.32 (m, 4 H), 1.33−1.56 (m, 2 H), 1.68−1.80 (m, 1 H), 1.98−2.12 (m, 1 H), 2.63−2.73 (m, 1 H), 3.00−3.13 (m, 2 H), 3.48 (s, 3 H), 3.57−3.66 (m, 2 H), 4.14−4.30 (m, 2 H), 5.86−6.02 (m, 1 H), 6.15 (s, 1 H), 7.31−7.50 (m, 5 H), 8.10 (s, 1 H) (3 exchangable protons not observed). LC-MS (ES^-) m/z 509 [M − 1]. LC-MS (ES^+) m/z 511 [M + H]. HRMS (ES+) calcd for $C_{24}H_{35}N_2O_6PS$ $(M + 1)$ m/z 511.2034, found 511.2032.

[2-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}-amino)ethyl]phosphonic Acid Trifluoroacetate Salt (49). Step 1: To a solution of **7** (50 mg, 0.120 mmol) in 1,2-dichloroethane (3 mL) was added diethyl (2-aminoethyl)phosphonate (109 mg, 0.602 mmol). The reaction mixture was stirred for 3 h then treated with $NaHB(OAc)_3$ (128 mg, 0.602 mmol). The reaction mixture was stirred at 25 °C for 2 h then treated with H_2O followed by 1 N HCl. The resulting mixture was concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded an intermediate diethyl aminophosphonate (70 mg, 71%) as a white solid. ES-LCMS m/z 581 $(M + H)^+$.

Step 2: To a solution of the diethyl aminophosphonate (70 mg, 0.121 mmol) in DCM (3 mL) was added TMSBr (0.065 mL, 0.499 mmol). The reaction mixture was stirred at 25 °C overnight. Additional TMSBr (0.16 mL, 1.21 mmol) was added. The reaction mixture was stirred overnight, concentrated under reduced pressure, and treated with H_2O. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **49** (51 mg, 55%, TFA salt) as a white solid. ^1H NMR (MeOH-d_4) δ ppm 0.81 (t, J = 7.0 Hz, 3H), 0.90 (t, J = 7.4 Hz, 3H), 1.00−1.38 (m, 4H), 1.38−1.55 (m, 1H), 1.59−1.75 (m, 1H), 1.76−2.06 (m, 3H), 2.23−2.40 (m, 1H), 3.14−3.24 (m, 3H), 3.57−3.65 (m, 4H), 4.12−4.35 (m, 2H), 6.15 (s, 1H), 6.39 (s, 1H), 7.30−7.53 (m, 5H), 8.09 (s, 1H). LC-MS (ES^+) m/z 525 $(M + H)^+$. HRMS (ES^+) calcd for $C_{25}H_{37}N_2O_6PS$ $(M + 1)$ m/z 525.2185, found 525.2188.

3-[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]pentanedioic Acid (52). Step 1: To a solution of **7** (109 mg, 0.262 mmol) in toluene (3 mL) was added diethyl malonate (0.048 mL, 0.315 mmol) and piperidine (0.013 mL, 0.131 mmol). The reaction mixture was stirred at 100 °C overnight, cooled to 25 °C, and concentrated under reduced pressure. Purification via SiO_2 chromatography (EtOAc/Hex = 10:90 to 80:20) afforded an intermediate diethyl propanedioate (146 mg, 90% pure, 90%) as a light-yellow oil. ES-LCMS m/z 558 $(M + H)^+$.

Step 2: To a solution of diethyl malonate (0.096 mL, 0.628 mmol) in EtOH (3 mL) was added sodium ethoxide (0.254 mL, 0.681 mmol). The reaction mixture was stirred at 25 °C for 20 min and treated with a solution of the intermediate diethyl malonate (146 mg, 0.262 mmol) in EtOH (2 mL). The reaction mixture was stirred at 25 °C overnight, acidified with AcOH to pH 3−4, and partially concentrated under reduced pressure to remove the organic solvents. The residue was partitioned between water and DCM. The organic layer was washed with saturated brine, dried, (Na_2SO_4), filtered, and concentrated under reduced pressure. Purification using silica gel (EtOAc/hexanes from 1:5 to 2:1) afforded an intermediate tetraethyl 1,1,3,3-propanetetracarboxylate (120 mg, 84% pure, 53%) as a colorless oil. ^1H NMR (400 MHz, $CDCl_3$) δ ppm 0.66−0.92 (m, 6H), 0.97−1.10 (m, 6H), 1.17−1.27 (m, 8H), 1.34−1.54 (m, 2H), 1.74−1.90 (m, 1H), 2.04 (d, J = 14.7 Hz, 1H), 2.07−2.23 (m, 2H), 2.90 (d, J = 14.9 Hz, 1H), 3.37 (d, J = 14.9 Hz, 1H), 3.86−4.03 (m, 4H), 4.04−4.20 (m, 6H), 4.43 (br s, 1H), 5.97 (s, 1H), 6.08 (s, 1H), 7.26−7.48 (m, 5H), 7.97 (s, 1H). LC-MS (ES^+) m/z 718 $(M + H)^+$.

Step 3: A mixture of the tetraethyl 1,1,3,3-propanetetracarboxylate (120 mg, 0.167 mmol) and 37% HCl (3 mL) was stirred at reflux for 20 h. LC-MS showed no starting material left but instead a mixture of triacid and diacid. Additional 37% HCl (3 mL) was added, and the reaction mixture was heated at reflux overnight. The reaction mixture was cooled to 25 °C then concentrated under reduced pressure. Purification with HPLC (eluting with MeCN/water with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **52** (76 mg, 95% pure, 68%) as a white solid. ^1H NMR (400 MHz, DMSO-d_6) δ ppm 0.68−0.92 (m, 6H), 0.95−2.26 (m, 7H), 2.59 (d, J = 7.2 Hz, 4H), 3.46 (s, 3H), 3.55−3.82 (m, 2H), 5.58−6.41 (m, 1H), 6.98−7.66 (m, 5H), 7.78 (br s, 1H), 12.14 (br s, 2H). LC-MS (ES^+) m/z 518 $(M + H)^+$. HRMS (ES^+) calcd for $C_{27}H_{35}NO_2S$ $(M + 1)$ m/z 518.2211, found 518.2212.

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2,2′-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}imino)diacetic Acid Hydrochloride Salt (53). Step 1: Prepared from bromomethyl intermediate (see compound **18**, step 2) (77 mg, 0.16 mmol) by heating a MeCN solution of diethyl iminodiacetic acid (33 mg, 0.18 mmol) at 65 °C for 4 h then concentrating to a thick oil. Chromatography on silica using hexanes/EtOAc provided a clear oil.

Step 2: H_2O and THF were added to the product from step 1 along with LiOH (15 mg, 0.36 mmol), and the mixture stirred for 2 h at 25 °C to hydrolyze the diacid. The reaction mixture was concentrated to half volume, and 1 N HCl added to acidify the reaction contents then the organics were extracted with DCM. The combined organics were washed with brine, dried (Na_2SO_4), filtered, and concentrated. The residue was triturated (DCM/EtOAc), filtered, and dried. The material was redissolved in DCM/EtOAc/MeOH then concentrated to a solid and kept under N_2 overnight to dry. **53** was obtained as a white hydrochloride salt (45 mg, 47% over 2 steps). ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.62−0.90 (m, 6 H), 0.98−1.56 (m, 8 H), 1.75 (br s, 1 H), 2.08 (br s, 1 H), 2.51 (br s, 14 H), 3.07 (d, J = 14.83 Hz, 1 H), 3.16−3.59 (m, 26 H), 3.81 (br s, 2 H), 5.95 (br s, 1 H), 6.09 (s, 1 H), 7.16−7.55 (m, 5 H), 8.03 (s, 1 H), 12.36 (br s, 2 H). LC-MS (ES$^+$) m/z 533 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{36}N_2O_7S$ (M + 1) m/z 533.2321, found 533.2321.

2,2′-({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}imino)diethanesulfonic Acid Trifluoroacetate Salt (54). To a solution of **8** (25 mg, 0.060 mmol) in DMF (2 mL) was added 2-bromoethane sulfonic acid sodium salt (127 mg, 0.600 mmol). The reaction mixture was stirred at 70 °C overnight, cooled to room temperature, acidified to pH 3−4 with AcOH, and concentrated under reduced pressure. Purification by RP-HPLC (eluting with MeCN/H_2O with 0.05% TFA-H_2O and 0.05% TFA-MeCN) afforded **54** (13 mg, 24%, TFA salt) as a white solid. ^{1}H NMR (MeOH-d_4) δ ppm 0.71−1.17 (m, 7H), 1.22−1.53 (m, 3H), 1.57−1.80 (m, 1H), 1.85−2.27 (m, 2H), 2.60−2.85 (m,1H), 3.41−3.83 (m, 8H), 3.95 (d, J = 15.8 Hz, 1H), 4.40 (d, J = 13.5 Hz, 1H), 4.72 (d, J = 13.3 Hz, 1H), 6.53 (s, 1H), 6.68 (s, 1H), 7.42−7.80 (m, 5H), 8.23 (s, 1H). LC-MS (ES$^+$) m/z 633 (M + H)$^+$. HRMS (ES$^+$) calcd for $C_{27}H_{40}N_2O_9S_3$ (M + 1) m/z 633.1974, found 633.1974.

[({[(3*R*,5*R*)-3-Butyl-3-ethyl-7-(methyloxy)-1,1-dioxido-5-phenyl-2,3,4,5-tetrahydro-1,4-benzothiazepin-8-yl]methyl}imino)dimethanediyl]bis(phosphonic acid) Tetraammonium Salt (55). Step 1: Compound **8** (155 mg, 0.37 mmol) was combined with TsOH (cat.) in toluene (20 mL) and concentrated to dryness to azeotrope off any residual H_2O. Paraformaldehyde (11 mg, 0.37 mmol) and toluene (5 mL) were added, and the mixture was heated at 75 °C with vigorous stirring for 30 min, after which time diethyl phosphite (0.048 mL, 0.37 mmol) was added. After 30 min, THF (20 mL) was added to the mixture, and the resultant homogeneous reaction mixture was stirred overnight at 75 °C. The mixture was concentrated to dryness and purified by RP-HPLC (30 mm × 150 mm Sunfire column under acidic conditions; MeCN + 0.05% TFA and H_2O + 0.05% TFA were used as the solvent system; 30−100% over 10 min, 100−100% to 12 min) to give an intermediate tetraethyl bis(phosphonate) (50 mg, 19% yield) as a yellow oil. LC-MS (ES$^+$) m/z 717 [M + H].

Step 2: The tetraethyl bis(phosphonate) (100 mg, 0.14 mmol) dissolved in DCM (5 mL) was treated with TMSBr (171 mg, 1.12 mmol) at 25 °C overnight. The mixture was concentrated to dryness, and the crude product was purified by RP-HPLC (30 mm × 100 mm XBridge column under basic conditions; MeCN + 0.1% NH_4OH and H_2O + 0.1% NH_4OH were used as the solvent system; 10% to 55% over 6 min, 100% to 100% to 8 min) to give **55** (45 mg, 54% yield) as an ammonium salt. ^{1}H NMR (400 MHz, DMSO-d_6) δ ppm 0.75 (t, J = 6.9 Hz, 3 H), 0.80 (t, J = 7.5 Hz, 3 H), 0.97−1.29 (m, 4 H), 1.32−1.55 (m, 2 H), 1.68−1.81 (m, 1 H), 1.98−2.14 (m, 1 H), 2.58−2.77 (m, 5 H), 3.09 (d, J = 14.8 Hz, 1 H), 3.41 (s, 3 H), 3.52 (d, J = 15.0 Hz, 1 H), 3.75−3.96 (m, 2 H), 5.94 (d, J = 8.6 Hz, 1 H), 6.08 (s, 1 H), 7.25−7.37 (m, 1 H), 7.36−7.49 (m, 4 H), 7.90 (s, 1 H) (phosphonic acid protons not observed). LC-MS (ES$^+$) m/z 605 [M + H]. HRMS (ES$^+$) calcd for $C_{25}H_{38}N_2O_9P_2S$ (M + 1) m/z 605.1852, found 605.1853.

Method for Determination of Human, Mouse, and Rat ASBT Inhibition. In preparation for measurement of bile acid uptake into cells expressing ASBT, HEK293 cells were cultured in DMEM/F12 supplemented with 10% FBS. Twenty-four h prior to running an experiment, cells were harvested when at a confluence of 80−90%. Cells were seeded in poly D-lysine coated plates at 50000 cells per well, and ASBT Bacmam virus was added such that each well contains 3.67 × 10^6 pfu (73.4 pfu/cell). Each assay plate was covered with Breathe Easy Seal and placed in an incubator for 24 h to allow expression of the transporter.

On the day of the uptake experiment, 10 mM HEPES was added to Hank's Balanced Salt Solution, and the pH was adjusted to 7.4 with TRIS (HBSSH). The assay buffer was prepared by adding 100 pM [^{3}H]-taurocholate and 10 μM cold taurocholate to room temperature HBSSH. A separate washing buffer was prepared by adding 10 μM cold taurocholate to HBSSH (∼30 mL per assay plate) and placed on ice. Using 100% DMSO, 8-point, 3-fold dilution curves for each test compound was prepared starting at 200 μM. Similarly, an 8-point dose response curve was prepared of the control compound **1** starting at 1.8 mM. Drug plates were created by adding 3 μL of each concentration to a v-bottom 96-well plate then diluted 60-fold with 177 μL of assay buffer. Plates were removed from the incubator and allowed to cool to 25 °C. Media was aspirated, and wells were washed once with 300 μL of HBSSH. Then 50 μL of each dose response curve concentration was added in triplicate by column to the assay plates, reserving column 10 for control (assay buffer + 1.67% DMSO) and columns 11 and 12 for the control compound. Plates were incubated at ambient temperature for 90 min then the plates were aspirated then washed 1× with 300 μL of wash buffer. Then 220 μL of Microscint 20 was added to each well, and the plates were sealed. The amount of [^{3}H]-taurocholate in cell lysate was quantitated using a microplate scintillation counter on the following day.

Percent inhibition of uptake was determined using the following formula at each drug concentration: 100 × (1 − ((T1 − C2)/(C1 − C2))); where T1 is average cpm for the test compound, C1 is average cpm observed in the absence of any added inhibitor, and C2 is average cpm observed in the presence of a substance known to elicit 100% inhibition of uptake (30 μM control compound). IC_{50}s can be generated using the formula, $y = (V_{max} \times x_n)/(K_n + x_n)$.

Method for Determination of MDCK Permeability. Passive permeability was measured in vitro using stably transfected human Multi-Drug Resistance 1−Madin−Darby Canine Kidney (hMDR1-MDCK, Netherlands Cancer Institute) cells incubated under conditions relevant to intestinal absorption. Briefly, hMDR1-MDCK cells were seeded at 6.6 × 10^5 cells/well onto 12-well polycarbonate Transwells filter membranes with 0.4 μm pore size (Corning (Corning, NY)) and maintained in Dulbecco's Modified Eagle's Media containing 10% fetal bovine serum (DMEM-FBS) at 37 °C in an atmosphere of 5% CO_2 and 95% relative humidity. After three days, media was removed from both the apical and basolateral chambers and replaced with transport buffer (HBSS containing 25 mM glucose and 25 mM HEPES) containing the P-gp inhibitor GF120918A at a final concentration of 2 μM. After a 30 min equilibration, the transport buffer was removed from the apical chambers and replaced with fasted-state simulated intestinal fluid (FaSSIF) containing 3 μM test compound, 2 μM GF120918A, 25 mM glucose, and 250 μM Lucifer Yellow CH. Next, the transport buffer was removed from the basolateral chambers and replaced with transport buffer containing 1% (w/v) human serum albumin and 2 μM GF120918A.

After 60 min incubation at 37 °C, samples were collected from the apical (donor) and basolateral (receiver) compartments and added to acetonitrile (1:1 and 1:2 (v/v), respectively). Receiver samples were then centrifuged and the supernatants were removed and analyzed by LC-MS/MS.

The final DMSO concentration in all dose solutions was 0.3% (v/v). Each treatment was performed in duplicate. Propranolol, a high permeability marker compound, and amprenavir, a marker compound for P-gp activity, were included in separate wells as controls for the

assay. Cell monolayer integrity was assessed by measuring Lucifer yellow transport via a fluorescence-based assay.

Rat Luminal Contents Stability Assay. A 10% (w/v) homogenate of the luminal contents from rat cecum and colon in phosphate buffered saline (PBS, pH 7.4) was prepared as follows.

Two male SD rats (Charles River Laboratories, Raleigh, NC) were fasted overnight and euthanized by CO_2 asphyxiation, followed by exsanguination. The large intestine and cecum were removed from both animals and cut lengthwise. The luminal contents were removed, pooled into a preweighed 50 mL conical tube, diluted with PBS (10 mL/g sample weight), and gently mixed by inversion. The homogenate was placed on wet ice until use.

Test compounds (10 μM final concentration) were added to a 4 mL glass screwcap vial containing 3 mL of the homogenate of the luminal contents from rat cecum and colon. Immediately after the addition of test compound, the vial was gently mixed and 3 × 100 μL aliquots were removed (t = 0) and placed into a 96-deepwell plate containing 400 μL of stopping solution (80% acetonitrile/20% methanol). Next, the glass vial was purged under a gentle stream of nitrogen gas for approximately 30 s, capped, and placed in a 37 °C shaking water bath. At t = 2, 4, and 24 h, 3 × 100 μL aliquots were removed from the vial and placed into a 96-deepwell plate containing 400 μL of stopping solution. The vial was purged under a gentle stream of nitrogen gas for approximately 30 s, capped, and placed in a 37 °C shaking water bath after each time point. Samples were covered and stored at −10 °C until LC-MS/MS analysis.

Rat Oral Absorption Assay. Male Sprague−Dawley (SD) rats (271−303 g; Charles River Laboratories, Raleigh, NC) were housed with free access to standard chow (PMI 5002 block chow) and water, unless otherwise noted. Animals for intravenous treatment groups were surgically implanted with a jugular and femoral vein cannula. Animals for oral treatment groups were surgically implanted with a jugular and portal vein cannula. Food was withheld from rats overnight prior to dosing and was returned at approximately 4 h postdose.

Oral treatment groups received test compounds formulated as a homogeneous suspension in 0.5% HPMC/0.1% Tween via oral gavage at a dose of 10 mg/kg. Blood samples were collected from both jugular and portal vein cannulae at 0.25, 0.5, 1, 2, 4, and 8 h postdose. Plasma samples were prepared and stored at −70 °C until analysis.

Rat Fecal Drug Recovery. *Fecal Recovery.* Male SD rats (Charles River Laboratories, Raleigh, NC) were administered test compounds formulated as a homogeneous suspension in 0.1% HPMC:0.5% Tween via oral gavage at a dose of 10 mg/kg. Fecal samples were collected across the following intervals: 0−6, 6−12, 12−24, 24−36, 36−48, 48−60, and 60−72 h postdose. After each collection interval, the samples were capped and stored at −70 °C until analysis. Prior to analysis, the samples were diluted with 5 volumes of 20% EtOH:80% H_2O, soaked overnight at 10 °C, and then homogenized using a Polytron hand-held homogenizer. The homogenates were extracted with 3 volumes of acetonitrile and then centrifuged for 15 min at 2304g and 4 °C. Aliquots of each acetonitrile supernatant was transferred to clean 96-well plates and diluted with an equal volume of water. Drug concentrations were quantified via LC-MS/MS.

Animals for Efficacy Studies. Male C57BL/6J mice were purchased from Jackson Laboratories (Bar Harbor, Maine) at seven weeks old and fed with normal rodent diet (Purina 5001, Harlan Teklad, Indianapolis, IN). Male Zucker Diabetic Fatty (ZDF/GmiCrl-fa/fa) mice were purchased from Charles River (Raleigh, NC) and had free access to rodent food (Purina 5008, Harlan Teklad, Indianapolis, IN). All animals were housed under controlled conditions (12/12 light−dark cycle, 24 °C and 50% relative humidity). All procedures performed were in compliance with the Animal Welfare Act and U.S. Department of Agriculture regulations and were approved by the GlaxoSmithKline Animal Care and Use Committee.

Fecal Collection in Mice. Male C57BL/6J mice were dosed with vehicle (0.5% hydroxypropyl methylcellulose (HPMC), 0.1% Tween80) or six doses (0.0001, 0.001, 0.01, 0.1, 1, and 10 mg/kg) of compounds at 0700 and 1500 for one day, and fecal samples were collected for 24 h (0700−0700). Animals were used for up to five studies with one week washout between studies.

Fecal Collection in Rat. Male ZDF rats arrived at seven weeks of age (±3 days). After a one-week acclimation period, rats were assigned to different treatment groups (n = 6−8/group) based upon baseline glucose/vehicle (0.5% hydroxypropyl methylcellulose (HPMC), 0.1% Tween80); one vehicle group for each compound) and six doses (0.05, 0.1, 0.5, 1, 5, and 10 mg/kg) of compounds **20**, **45**, and **56**. All treatments were given via oral gavage twice a day. Fecal samples were collected for 24 h on day 7 of treatment.

Metabolic Effects in ZDF Rats. Male ZDF rats arrived at seven weeks of age (±3 days). After a one-week acclimation period, rats were anesthetized with isoflurane (Abbott Laboratories, IL) and tail-vein blood samples were collected at 0900 without fasting. To ensure balanced treatment groups, ZDF rats were assigned to six treatment groups based upon baseline glucose/vehicle (0.5% hydroxypropyl methylcellulose (HPMC), 0.1%Tween80) and selected doses of compounds (0.05, 0.1, 0.5, 1, 5, and 10 mg/kg or 0.001, 0.01, 0.1, 1, and 10 mg/kg for compounds **20** and **45** or **56**, respectively). All treatments were given via oral gavage twice a day, and animals were followed for two weeks with blood samples collected from tail vein on day 14 at 0900 without fasting. Plasma samples were stored at −80 °C for further analyses.

Measurement of Clinical Chemistry Parameters. Plasma glucose and bile acids in fecal extraction were measured using the Olympus AU640 clinical chemistry analyzer (Beckman Coulter, Irving, TX). Glucose test reagents were manufactured by Beckman Coulter. Bile acids reagents were manufactured by Trinity Biotech. HbA1c was measured by the Primus Affinity Ultra2 HPLC system using Primus Affinity Assay reagents (Primus Diagnostics, Kansas City, MO). Insulin, total GLP-1 (tGLP-1), PYY, and GIP were assayed using the Meso Scale Discovery (MSD) assay kits. Total Glp-1 was assayed by the MSD Total Glp-1 assay kit and analyzed on an MSD Sector Imager 6000 (Meso Scale Discovery, Gaithersburg, MD).

Fecal Bile Acid Extraction. Fecal samples were air-dried for five days and extracted in methanol−KOH (300 mM) at 60 °C for 24 h. Fecal extract was then mixed with 150 mM Mg_2SO_4 (1:1). After centrifugation, the supernatant was saved and submitted for bile acids measurement as described above.

Data Analysis. All of the in vivo studies described above were analyzed as described in this paragraph.

Values are given as mean ± SEM. The data were analyzed in JMP 7.0.0 (SAS Institute, 174 Cary, NC) using one-way ANOVA with prespecified contrasts to compare each group to the appropriate control group (fecal BA data) or two-way ANOVA with Bonferroni post-tests. P values <0.05 were considered to indicate a significant difference between treatment groups. Dose−responses were fit to four-parameter logistic functions (GraphPad Prism v5.04, San Diego, CA).

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Notes

The authors declare no competing financial interest.

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ABBREVIATIONS USED

ASBT, apical sodium-dependent bile acid transporter; BA, bile acids; DPP-4, dipeptidyl peptidase-4; GLP-1, glucagon-like-1; GIP, glucose dependent insulinotropic peptide; HEK293, human embryonic kidney 293; iBAT, ileal bile acid transporter; HbA1c, hemoglobin A1c; hMDR1-MDCK, human multidrug

resistance 1−Madin−Darby canine kidney; PYY, peptide YY; tGLP-1, total GLP-1; ZDF, Zucker Diabetic Fatty

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