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Design, synthesis, and biological evaluation of conformationally constrained glycerol 3-phosphate acyltransferase inhibitors

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ABSTRACT

Glycerol 3-phosphate acyltransferase (GPAT) isozymes are central control points for fat synthesis in mammals. Development of inhibitors of these membrane-bound enzymes could lead to an effective treatment for obesity, but is thwarted by an absence of direct structural information. Based on a highly successful study involving conformationally constrained glycerol 3-phosphate analogs functioning as potent glycerol 3-phosphate dehydrogenase inhibitors, several series of cyclic bisubstrate and transition state analogs were designed, synthesized, and tested as GPAT inhibitors. The weaker in vitro inhibitory activity of these compounds compared to a previously described benzoic acid series was then examined in docking experiments with the soluble squash chloroplast GPAT crystal structure. These in silico experiments indicate that cyclopentyl and cyclohexyl scaffolds prepared in this study may be occluded from the enzyme active site by two protein loops that sterically guard the phosphate binding region. In view of these findings, future GPAT inhibitor design will be driven toward compounds based on planar frameworks able to slide between these loops and enter the active site, resulting in improved inhibitory activity.

1. Introduction

Glycerol 3-phosphate acyltransferase (GPAT) catalyzes the first committed and rate-limiting step of glycerolipid biosynthesis,[1,2] the acylation of glycerol 3-phosphate with long chain acyl-CoAs. The product of this reaction, lysophosphatidic acid (LPA), is then further acylated to phosphatidic acid, which is eventually incorporated into triacylglycerol (TAG), the primary constituent of animal fat. High bloodstream TAG levels have been most notably linked to obesity, but have been linked to other diseases such as atherosclerosis and pancreatitis as well.[3,4]

There are four known isoforms of GPAT, two located in mitochondria (mtGPAT1 and mtGPAT2), and two located in the endoplasmic reticulum (GPAT3 and GPAT4).[5,6] Of these isoforms, only mtGPAT1 appears to be selective, exhibiting a strong preference for incorporating palmitoyl-CoA (16:0) over unsaturated acyl-CoAs.[6,7] Therefore, mtGPAT1 is more heavily implicated in producing saturated phospholipids than the other three GPAT isoforms. As a result, mtGPAT1-deficient mice secrete less very low density lipoprotein (VLDL) and maintain lower hepatic TAG levels than control mice.[8] Furthermore, a significant amount of evidence indicates that a decrease in mtGPAT1 activity is coupled to an increase in β-oxidation through heightened activity of carnitine palmitoyltransferase-1 (CPT-1).[9–14] These studies indicate that inhibition of mtGPAT1 with a small molecule may be an effective treatment for obesity, diabetes, and other health problems associated with increased TAG synthesis.

Very little is known about the detailed structure of any of the four isoforms of mammalian GPAT, although a crystal structure of GPAT isolated from squash chloroplasts has been published.[15] While the latter is soluble, the mammalian enzymes are intrinsic membrane proteins of greater structural complexity and very low primary sequence similarity to the squash enzyme. Notwithstanding, all contain short motifs housing conserved residues thought to be important for substrate binding and catalysis. Examination of the squash GPAT structure has helped shed light on the mechanism of the reaction, which appears to be similar to that of a serine protease catalytic triad. In this case, however, the primary alcohol of glycerol 3-phosphate takes the place of the catalytic serine, and is deprotonated by a neighboring histidine residue prior to acylation. The putative glycerol 3-phosphate binding pocket consists of several positively charged amino acids, and could be visualized to be an advantageous binding site for an inhibitor possessing a negative charge at physiological pH. Though the location of the putative glycerol 3-phosphate binding site has been identified, little is known about the substrate's preferred bound conformation. It would be highly valuable to the development of GPAT inhibitors to learn as much as possible about the mammalian enzyme active site preference for one or a limited number of

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rotameric states of glycerol 3-phosphate. Such knowledge could be profitably used to develop a conformationally constrained and potent inhibitor that ideally is also selective for mtGPAT1 over the other isoforms.

Relatively few structural types of small molecules have been tested for GPAT inhibitory activity. At this point, the most effective compound reported to date possesses an IC_{50} value of about 25 μM in an intact mitochondrial assay.[16] That compound is based on a phenyl scaffold and utilizes a long chain alkyl sulfonamide as an alkyl acyl-CoA mimic and a carboxylate as a phosphate group mimic. A previous study on *sn*-glycerol 3-phosphate dehydrogenase, which catalyzes the oxidation of glycerol 3-phosphate to dihydroxyacetone phosphate, utilized five racemic cyclopentanetriol monophosphate isomers as stereochemically defined glycerol 3-phosphate analogs to examine the importance of specific rotameric states of the substrate on enzyme activity.[17] In this study, one of the constrained analogs uniquely functioned as a good substrate, another served as a weakly active substrate, and the other three were inhibitors of the enzyme isolated from rabbit skeletal muscle. This study indicated that the catalytically active rotameric states of glycerol 3-phosphate are those in which the C-2 and C-3 hydroxyl groups possess a *syn*- orientation, with a preference for the C-1 hydroxyl group to be *anti*- to the C-2 hydroxyl group. The fact that certain configurations of these cyclic glycerol 3-phosphate analogs were incorporated into a central metabolic mammalian pathway and thereby elicited valuable information about substrate conformational preferences is remarkable. This study suggested to us that similar glycerol 3-phosphate mimics could potentially yield analogous insights into the GPAT active site. If one or a subset of the substrate analogs functioned as significantly more effective inhibitors than others, as was observed in the glycerol 3-phosphate dehydrogenase study, the catalytically relevant substrate conformational preferences in GPAT could be determined. Therefore, we decided to synthesize analogous stereochemically-defined glycerol 3-phosphate analogs and test the compounds as GPAT inhibitors. In this case, as previously described, long chain alkyl sulfonamides were selected to serve as acyl-CoA mimics while carboxylic acids were intended to mimic the terminal phosphate of glycerol 3-phosphate.

2. Results and discussion

2.1. Synthesis of cyclopentane and cyclohexane carboxylic acids

The synthesis of the racemic cyclopentane and cyclohexane carboxylic acids is illustrated in Scheme 1. The *anti*-epoxides **1a**–**b**[18] were regio- and stereoselectively opened with sodium azide to produce azidoalcohols **2a**–**b**, which were then reduced and coupled to octanesulfonyl chloride to yield sulfonamides **3a**–**b**. The carboxylic acid functionality of products **4a**–**b** was then revealed upon hydrolysis of the esters with aqueous base. Compound **8a** was produced through a similar route, with the only exception being stereochemical inversion at C-4 of the ring, accomplished through displacement of the bromohydrin **5** with azide. The synthesis of compound **8b** was accomplished by elaboration of the known cyano-alcohol **9** to sulfonamide **10**. Hydrolysis of the nitrile under acidic conditions yielded acid **8b**. Compounds **14a**–**b** were produced in an analogous fashion to **4a**–**b**, beginning instead with *syn*-epoxides **11a**–**b**.[18]

The synthesis of cyclopentyl acetic acids **17**, **21**, and **25** is detailed in Scheme 2. Alkyl sulfonamide **16** was produced from known precursor azido-lactone **15**,[19] which was hydrolyzed to yield acid **17**. Compounds **21** and **25** were made in a similar fashion to final products **4a**–**b** and **8a**, in this case stemming from selective oxirane-opening reactions of compound **18**.[20]

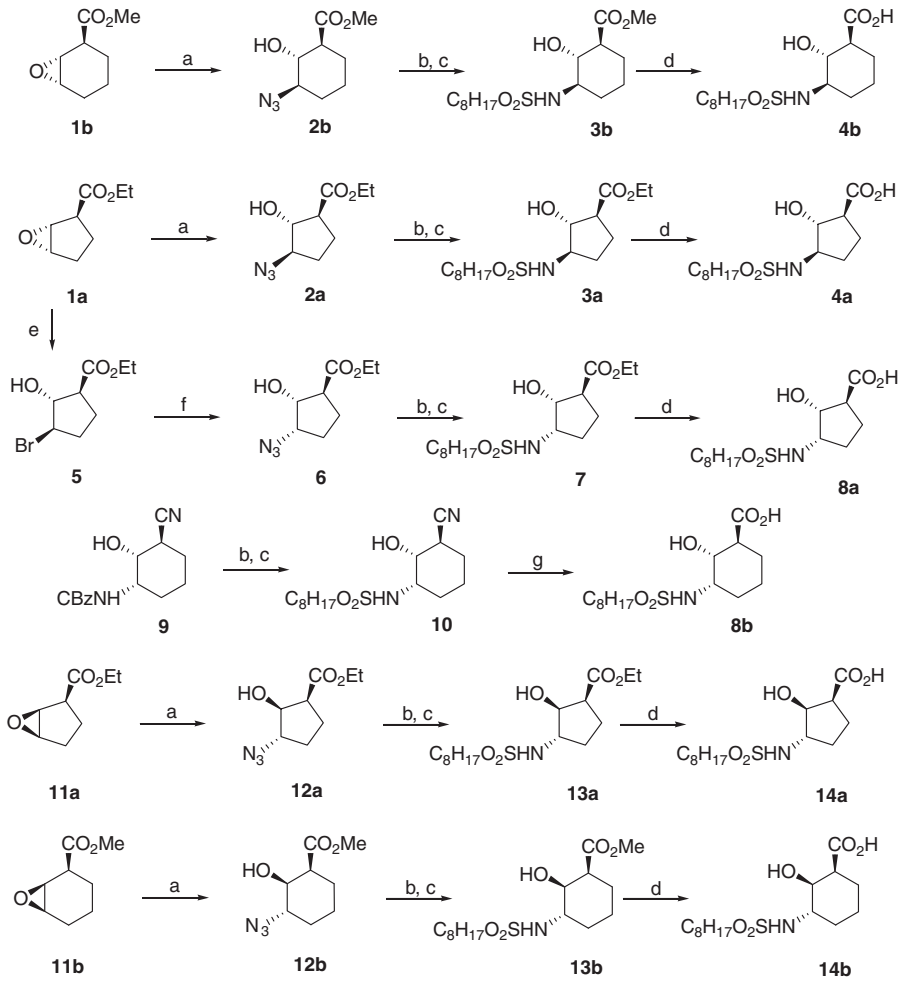
Scheme 3 depicts the synthesis of cyclohexyl acetic acids **29**, **33**, and **36**. Compounds **29** and **33** were synthesized in a similar manner as compounds **4a**–**b** and **14a**–**b**, but acid **36** required a different approach. Reaction of oxirane **26** with HBr, as in the synthesis of compound **8a** from **1a**, failed to produce the desired bromohydrin. However, compound **8b** could be converted to **36** through reduction of the acid with borane-methyl sulfide, selective tosylation of the resulting primary alcohol, displacement with cyanide, and hydrolysis of nitrile **35**.

Another proposal for a series of similar compounds stemmed from the fact that the best compounds of the earlier benzene series maintain an *ortho*- relationship between the benzoic acid and sulfonamide functional groups.[16] By analogy, if one can imagine reducing the benzene ring of these structures, then the benzoic acid and sulfonamide would be attached to vicinal carbons. In the compounds described above, however, they are not as close as possible, but are separated by an additional methylene unit. Therefore, a reasonable hypothesis was to synthesize several compounds that interchange the location of the hydroxyl group and sulfonamide groups in the acids above. The syntheses of two final products of this type are described in Scheme 4.

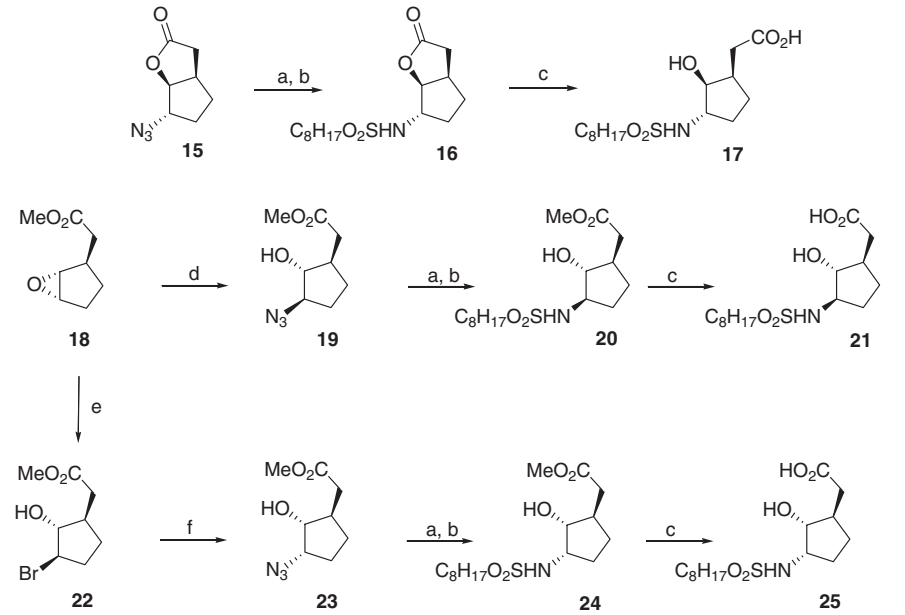
The syntheses of target acids **40** and **44**, which proceed through the same route but from different starting materials, commence with either cyclopentane **13a** or **3a**. The secondary alcohol was first mesylated in methylene chloride and the mesylate was displaced by the neighboring sulfonamide to form sulfonated aziridines **38** and **42**. The aziridines were then selectively hydrolyzed in acid to yield alcohols **39** and **43**. Finally, the esters were saponified to produce the desired cyclopentane acids **40** and **44**.

The GPAT inhibitory activity of acids **4a**–**44** in an intact mitochondrial assay is detailed in Table 1. The results are reported in terms of IC_{50} values when the raw data could be reliably fit to a dose–response curve (R^2 >0.90). Due to the poor inhibitory activity of many of the compounds tested, however, this was not always possible. For these cases, the percent of GPAT inhibition observed at an inhibitor concentration of 40 μg/mL is listed, which corresponds to 124 μM for **4a**, **8a**, **14a**, and **40**–**44**, 119 μM for **4b**, **8b**, **14b**, and **17**–**25**, and 114 μM for **29**–**36**. The most immediately striking aspect of these data is that all of these compounds are significantly weaker inhibitors than the most effective compounds based on a benzene scaffold.[16] The best inhibitor of the series described herein, cyclohexane acid **8b**, is about eight times less effective as an inhibitor than the most effective benzoic acid compound reported.[16] In three of the four series of compounds the most active inhibitor is the compound with the hydroxyl group in the *anti*- position relative to both the carboxylate and sulfonamide moieties, namely **4a**, **29**, and **21**. Comparing the series themselves, the cyclohexane carboxylic acids (**4b**, **8b**, **14b**) appear to be the most effective inhibitors, as **8b** is the best overall. The weakest inhibitor of this group, **14b**, still produces 15% GPAT inhibition at 40 μg/mL, a result comparable to the best cyclopentane acetic acid, **21**. The least effective series overall in vitro is the cyclopentane acetic acid series (**17**, **21**, **25**), which contains **14a**, the second weakest inhibitor tested. Overall, however, the cyclopentane acetic acid series was only marginally weaker than the cyclohexane acetic acid series (**29**, **33**, **36**), which also contains the least active compound (**36**). The compounds in which the positions of the sulfonamide and secondary alcohol were interchanged, **40** and **44**, unfortunately did not demonstrate any increased inhibitory activity compared to the other series of target compounds.

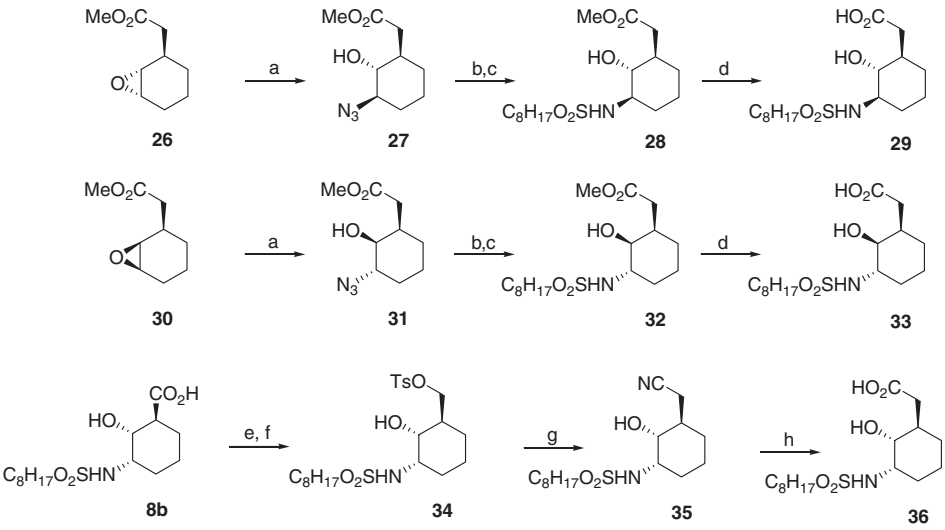
Greater potency would illuminate the precise stereochemical relationships among the carboxylate, secondary alcohol, and alkyl sulfonamide moieties that produce a pronounced impact on the efficacy of the inhibitors. However, the cyclopentane and cyclohexane scaffolds tested simply do not appear to be able to hold the two main enzyme recognition elements, the carboxylate group and the



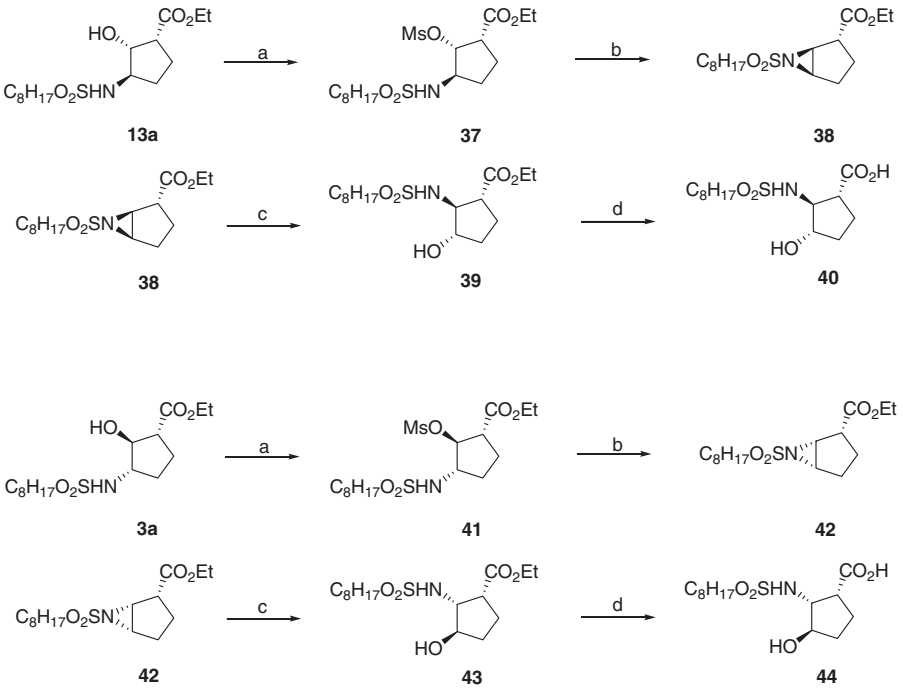
Scheme 1. Synthesis of cyclopentane and cyclohexane carboxylic acids **4a**–**b**, **8a**–**b**, and **14a**–**b**. Reagents and conditions: (a) NaN_3, NH_4Cl, MeOH, reflux; (b) H_2, Pd/C, EtOH; (c) $C_8H_{17}SO_2Cl$, Et_3N, CH_2Cl_2; (d) 1 N NaOH; (e) HBr, $CHCl_3$; (f) NaN_3, DMF, 60 °C; (g) 12 N HCl, DME, reflux.



Scheme 2. Synthesis of cyclopentyl acetic acids **17**, **21**, and **25**. Reagents and conditions: (a) H_2, Pd/C, EtOH; (b) $C_8H_{17}SO_2Cl$, Et_3N, CH_2Cl_2; (c) 1 N NaOH; (d) NaN_3, NH_4Cl, MeOH, reflux; (e) HBr, $CHCl_3$; (f) NaN_3, DMF, 60 °C.



Scheme 3. Synthesis of cyclohexyl acetic acids **29**, **33**, and **36**. Reagents and conditions: (a) NaN_3, NH_4Cl, MeOH, reflux; (b) H_2, Pd/C, EtOH; (c) $C_8H_{17}SO_2Cl$, Et_3N, CH_2Cl_2; (d) 1 N NaOH; (e) BH_3–DMS, THF; (f) TsCl, Et_3N, CH_2Cl_2; (g) KCN, DMF, 60 °C; (h) NaOH, H_2O, EtOH, reflux.



Scheme 4. Synthesis of cyclopentyl acetic acids **40** and **44**. Reagents and conditions: (a) MsCl, Et_3N, CH_2Cl_2; (b) K_2CO_3, ACN; (c) TFA, dioxane/H_2O; (d) 1 N NaOH.

long chain alkyl sulfonamide, in a relative conformation to produce favorable interactions with the phosphate and acyl-CoA GPAT binding sites. If these moieties are not appropriately positioned on the ring to interact favorably with the active site, the compound cannot adjust itself to achieve a better fit. The result is a series of weakly potent inhibitors in which the isomeric differences between the compounds do not significantly affect the inhibitory activities and a comparatively flat SAR is observed.

Another potentially important consideration for the differences in activity between the compounds in this study and the most effective inhibitors lies in the electronic nature of the sulfonamide N–H bond. In 2-(nonylsulfonamido)benzoic acid (IC_{50} = 24.7 μM),[16] the phenyl sulfonamide has a pK_a approximately 2 units lower than the alkyl sulfonamides in this study. If the sulfonamide is interacting with the catalytic histidine as was initially planned, the acidity of that N–H hydrogen should be vital for activity. It is possible that the weaker acidity of this hydrogen in all the compounds of this study reduces any such hydrogen bonding interaction, even if several of the inhibitors held the substituents in near-ideal proximity.

To attempt to determine why the cyclopentyl and cyclohexyl acids are relatively weaker GPAT inhibitors, docking simulations were performed using the CDOCKER protocol in the Accelrys Dicovery Studio (version 2.1). Each enantiomer of compound **33** was individually modeled into the presumed active site of the squash chloroplast GPAT crystal structure (PDB code: 1K30).[15] This soluble form of the enzyme catalyzes the same reaction as membrane-bound mammalian mtGPAT1, and analysis of the structure

Table 1
GPAT inhibitory activity of final products **4a**–**44**

Compound	GPAT IC_{50} value (μM)	%Inhibition at 40 μg/mL
2-(Nonylsulfonamido) benzoic acid[16]	24.7 ± 2.1	97
4a	255 ± 59	25
4b	—	17
8a	—	19
8b	164 ± 62	36
14a	324 ± 81	3
14b	—	15
17	—	8
21	—	16
25	295 ± 95	8
29	—	17
33	—	23
36	—	2
40	—	12
44	—	15

could help identify conserved residues with which potential inhibitors could form favorable interactions. The majority of the docking models inverted the hydrophobic and hydrophilic portions of the inhibitor, leaving the hydrophobic alkyl chain in the palmitoyl-CoA binding site while extending the cyclohexyl moiety into the exterior of the enzyme. In the cases where the model oriented the compound as desired, with the carboxylate extended toward the active site, however, the compound was never actually positioned inside the glycerol 3-phosphate binding site (Fig. 1a). In fact, it appears from these models that the cyclohexyl portion of **33**, for example, is sterically unable to pass between two closely positioned loops (at Gly-168 and Gly-233) that separate the putative glycerol 3-phosphate binding site from the long alkyl chain binding region. In contrast, the same experiment run with the most effective benzoic acid inhibitor described to date[16] places the benzoic acid group deep into the positively charged binding site, as the planar benzene scaffold can pass between Gly-168 and Gly-233 (Fig. 1b). The stark differences between the two docking modes produced in the models could further explain why such differences in inhibitory activity have been observed between the two structurally related classes of compounds. Although the conformationally constrained inhibitors are less effective than hoped, the in vitro results as well as the structural contrasts displayed in our docking models point successful design toward structures based on planar scaffolds that have previously demonstrated varied SAR and significant GPAT inhibitory activity.

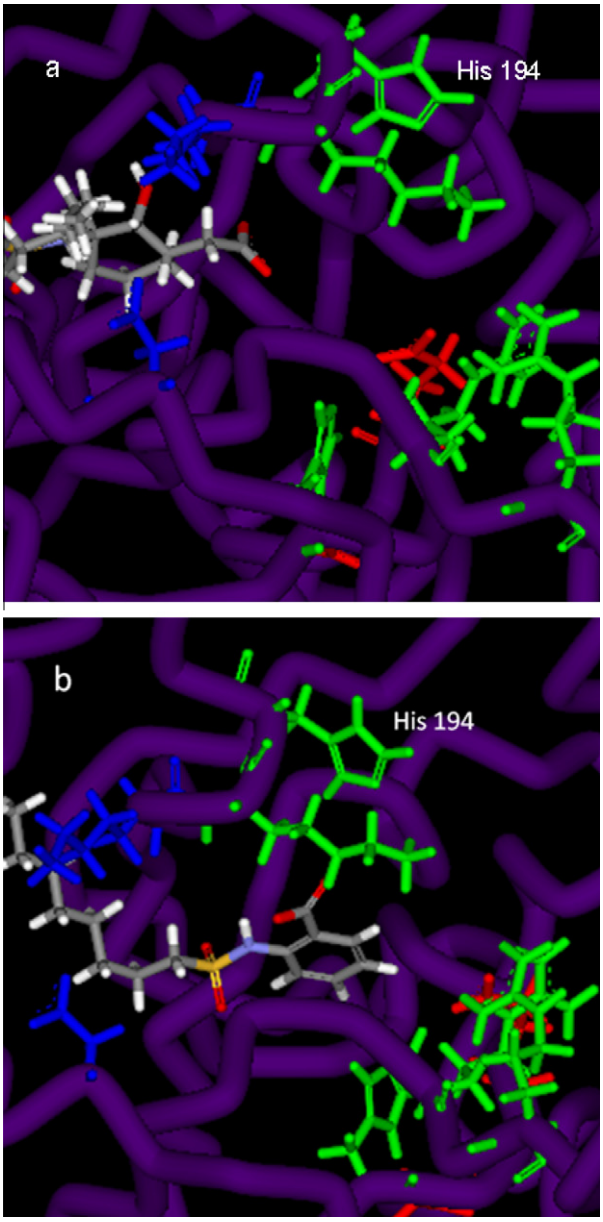


Figure 1. (a) The predominant docking model of compound **33** binding to the crystallographic structure of GPAT from squash chloroplasts (PDB code: 1K30) with the polar end of the molecule facing the active site and the alkyl chain resting in the hydroprobic binding pocket. Positively-charged active site residues and negatively-charged active site residues are shown in green and red, respectively. The salt bridge-forming residues in the acyl-CoA binding site (Asp-251 and Lys-192) are shown in blue, and the inhibitors are shown with gray carbon backbones. Note that **33** is unable to pass the two close loops guarding the enzyme active site. (b) The predominant docking model of the best GPAT inhibitor reported to date[16] in the active site of the crystallographic structure of squash GPAT (PDB code: 1K30). The model indicates that the benzoic acid portion of the molecule is able to pass between Gly-168 and Gly-233 to bind deeper into the positively charged glycerol 3-phosphate binding site.

3. Conclusions

While configurationally defined and conformationally constrained cyclopentyl analogs of glycerol 3-phosphate could function as effective substrates or inhibitors of glycerol 3-phosphate dehydrogenase, application of a similar tactic to GPAT was less successful. One obvious hazard of this experimental approach is that a subset of all the possible conformations of a flexible substrate cannot be sampled by a cyclopentyl or cyclohexyl skeleton. There are functional group orientations and intra-pharmacophore distances not represented by the several compound series prepared in this study. Despite this unavoidable limitation, the data suggest that there are several probed substrate conformations that can be eliminated. This decision is clouded, however, if the internal dimensions of the human GPAT isozymes and the squash enzyme are closely correlated. The soluble squash enzyme catalyzes the same reaction as the membrane-bound mammalian GPAT's and, although the enzymes are evolutionarily distinct, the substrate-binding and catalytic motifs are conserved. Modeling exercises using the squash GPAT crystal structure revealed a narrow passage bounded by two protein loops that occlude from the presumed phosphate-binding site all but flat inhibitor mimics of the substrate. Barring sufficient movement in the protein to overcome this barrier to binding, this feature becomes an important factor governing inhibitor design.

Finally, the alkyl sulfonamide is visualized to both mimic the tetrahedral transition state of acyl transfer from a fatty acid CoA ester to glycerol 3-phosphate and provide a hydrogen bond partner

to the catalytic histidine. Ideally, the sulfonamide hydrogen will establish a hydrogen-bonding interaction with the catalytic histidine. For this to contribute significantly to inhibitor binding affinity, distance, orientation and pK_a of this hydrogen are of central importance. The lower pK_a of an aryl sulfonamide likely produces a better match to that of this histidine residue resulting in a stronger interaction in the active site. In sum, consideration of these observations can guide further progress in the design and synthesis of effective GPAT inhibitors.

4. Experimental section

4.1. Chemistry

Commercially available reagents were used directly without purification unless otherwise stated. ^{1}H and ^{13}C NMR spectra were measured on a Bruker Avance 300 or 400 MHz NMR spectrometer. Melting points were determined on a Thomas–Hoover capillary melting point apparatus and are uncorrected. Column chromatography was carried out on Silica Gel 60 (Merck, 230–400 mesh ASTM). All solvents used for reactions were distilled prior to use (Et_2O and THF over Na/benzophenone, CH_2Cl_2 and CH_3CN over CaH). Elemental analyses were performed by Atlantic Microlab, Norcross, GA.

4.1.1. General procedure for the synthesis of epoxides 1a–b and 11a–b

The starting cyclopentene (30.7 mmol), in the case of **1a** and **11a**, prepared by the method of Bartley et al.[21] starting with the racemic alcohol[22] or the cyclohexene starting material[18] (for **1b** and **11b**) (30.7 mmol), was dissolved in CH_2Cl_2 (200 mL) and the solution was cooled to 0 °C. MCPBA (77% max, 1.35 equiv) was added, and the solution was allowed to slowly warm to room temperature. When complete by TLC (20% EtOAc in hexanes), the solution was concentrated in vacuo and the two isomers **1a** and **11a** or **1b** and **11b** were separated by column chromatography (15% EtOAc in hexanes).

4.1.2. (±) Ethyl 6-oxa-bicyclo[3.1.0]hexane-2-carboxylate (1a)

^{1}H NMR ($CDCl_3$): δ 4.15 (q, J = 7.2 Hz, 2H), 3.63 (d, J = 2.4 Hz, 1H), 3.53 (s, 1H), 3.08 (d, J = 8.4 Hz, 1H), 2.10 (m, 1H), 1.72–1.90 (m, 2H), 1.58 (m, 1H), 1.25 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$, 100 MHz): δ 172.6, 60.4, 57.5, 56.7, 44.4, 26.0, 22.6, 13.9.

4.1.3. (±) Methyl 7-oxa-bicyclo[4.1.0]heptane-2-carboxylate (1b)

Characterization data are in agreement with literature values.[18]

4.1.4. General procedure for the synthesis of *anti*-azido alcohols 2a–b, 12a–b, 19, 27, and 31

The corresponding starting epoxide (10 mmol) was dissolved in 55 mL EtOH (for **2a** and **12a**) or MeOH (for **2b** and **12b**), and NaN_3 (5 equiv) and NH_4Cl (2.25 equiv) were added. The solution was heated to reflux until all starting material had disappeared by TLC (30% EtOAc in hexanes). The mixture was poured into EtOAc (100 mL) and the organic phase was washed with H_2O (100 mL), dried over anhydrous Na_2SO_4, and concentrated in vacuo. The azido alcohol product was then purified by column chromatography (15% EtOAc in hexanes).

4.1.5. (±) Ethyl 3-azido-2-hydroxycyclopentane-carboxylate (2a)

^{1}H NMR ($CDCl_3$,): δ 4.18 (m, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.74 (q, J = 7.6 Hz, 1H), 2.78 (m, 1H), 2.72 (q, J = 8.4 Hz, 1H), 2.04 (m, 2H), 1.97 (m, 1H), 1.68 (m, 1H), 1.28 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 173.7, 79.4, 66.6, 61.0, 49.1, 26.9, 23.0, 14.1.

4.1.6. (±) Methyl 3-azido-2-hydroxycyclo-hexanecarboxylate (2b)

Characterization data are in agreement with literature values.[18]

4.1.7. General procedure for the synthesis of sulfonamides 3a–b, 7, 10, 13a–b, 16, 20, 24, 28, and 32

The corresponding starting azide (2.3 mmol) was dissolved in 35 mL anhydrous EtOH, 10% Pd/C catalyst (320 mg) was added, and the flask was flushed with argon. A balloon of H_2 was first bubbled through the solution, and the solution was then stirred under an H_2 atmosphere at 1 atm until all starting material had disappeared by TLC (30% EtOAc in hexanes). When complete, the solution was filtered through Celite to remove the catalyst, Celite was washed with EtOAc (100 mL), and the resulting solution was concentrated in vacuo and used directly in the next step without further purification.

The resulting amine (1.6 mmol) was dissolved in 8 mL CH_2Cl_2, and the solution was cooled to −78 °C. Octanesulfonyl chloride (1.1 equiv) and Et_3N (1.1 equiv) were then each added dropwise, and the solution was allowed to gradually warm to room temperature over 2 h. Reaction progress was monitored by TLC (40% EtOAc in hexanes). When complete, the reaction mixture was poured into saturated NH_4Cl (20 mL), extracted with CH_2Cl_2 (3 × 15 mL), and washed with brine (50 mL). The combined organic phases were concentrated in vacuo, and the crude product was purified by column chromatography on silica gel (30% EtOAc in hexanes).

4.1.8. (±) Ethyl 2-hydroxy-3-(octylsulfonamido)-cyclopentane-carboxylate (3a)

^{1}H NMR ($CDCl_3$): δ 4.52 (d, J = 8.4 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.61 (quintet, J = 8.0 Hz, 1H), 3.11 (dt, J = 4.4, 7.2 Hz, 2H), 2.86 (d, J = 2.8 Hz, 1H), 2.75 (q, J = 7.6 Hz, 1H), 2.19 (m, 1H), 2.05 (m, 1H), 1.96 (m, 1H), 1.84 (m, 2H), 1.63 (m, 1H), 1.44 (m, 2H), 1.28 (m, 12H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.7, 79.3, 60.9, 60.4, 53.2, 48.6, 31.5, 28.9, 28.9, 28.8, 28.1, 23.5, 23.4, 22.4, 14.0, 13.9.

4.1.9. (±) Methyl 2-hydroxy-3-(octylsulfon-amido)cyclohexane-carboxylate (3b)

^{1}H NMR ($CDCl_3$): δ 4.82 (d, J = 6.8 Hz, 1H), 3.73 (s, 3H), 3.58 (dt, J = 3.2, 10.0 Hz, 1H), 3.40 (d, J = 3.2 Hz, 1H), 3.16 (m, 1H), 3.10 (m, 2H), 2.44 (m, 1H), 2.17 (m, 1H), 2.00 (m, 1H), 1.81 (m, 3H), 1.28 (m, 13H), 0.89 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.7, 73.5, 58.0, 53.3, 52.1, 50.0, 32.8, 31.6, 29.0, 28.9, 28.2, 27.7, 23.7, 23.5, 22.5, 14.0.

4.1.10. General procedure for the synthesis of carboxylic acids 4a–b, 8a, 14a–b, 17, 21, 25, 29, 33, 36, 40, and 44

The sulfonamide ester (**3a–b**, **7**, **10**, **13a–b**, **16**, **20**, **24**,**28**, or **32**) (0.25 mmol) was placed into a dry round-bottom flask, and 2 mL 1 M NaOH was added. The solution was stirred at room temperature until the reaction was complete by TLC (40% EtOAc in hexanes). When complete, 5 mL 1 M HCl was added, and the product was extracted with Et_2O (3 × 10 mL). The combined organic phases were dried over anhydrous Na_2SO_4, concentrated in vacuo, and recrystallized (EtOAc and hexanes) to provide the corresponding carboxylic acids in high purity and yield.

4.1.11. (±) 2-Hydroxy-3-(octylsulfonamido)-cyclopentanecarboxylic acid (4a)

Mp = 129–130 °C; ^{1}H NMR (MeOD): δ 4.07 (t, J = 8.0 Hz, 1H), 3.52 (q, J = 8.4 Hz, 1H), 3.13 (m, 2H), 2.66 (q, J = 8.0 Hz, 1H), 2.08 (m, 2H), 1.80 (m, 3H), 1.62 (m, 1H), 1.44 (m, 2H), 1.33 (m, 8H), 0.92 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD): δ 178.2, 80.7, 61.8, 54.3, 50.4, 32.9, 30.3, 30.2, 30.1, 29.3, 24.8, 24.7, 23.6, 14.4. Anal. ($C_{14}H_{27}NO_5S$) C, H, N.

4.1.12. (±) 2-Hydroxy-3-(octylsulfonamido)-cyclohexanecarboxylic acid (4b)

Mp = 132–133 °C; ^{1}H NMR (MeOD): δ 3.48 (t, J = 10.0 Hz, 1H), 3.12 (m, 3H), 2.34 (m, 1H), 2.06 (m, 1H), 1.93 (m, 1H), 1.79 (m, 3H), 1.33 (m, 13H), 0.92 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD): δ 177.7, 74.7, 59.6, 54.2, 52.6, 34.8, 32.9, 30.2, 30.2, 29.7, 29.4, 25.1, 24.7, 23.6, 14.4. Anal. ($C_{15}H_{29}NO_5S$) C, H, N.

4.1.13. General procedure for the synthesis of bromo-alcohols 5 and 22

The epoxides **1** and **18** (6.5 mmol) were dissolved in 65 mL $CHCl_3$, 48% HBr (25 mL) was added, and the solution was stirred at room temperature for 30 min, or until the reaction was complete by TLC (20% EtOAc in hexanes). The solution was then diluted with $CHCl_3$ (130 mL), washed with saturated $NaHCO_3$ (300 mL), washed with H_2O (100 mL), and evaporated in vacuo. The product was then purified by column chromatography (20% EtOAc in hexanes).

4.1.14. (±) Ethyl 3-bromo-2-hydroxycyclo-pentanecarboxylate (5)

^{1}H NMR ($CDCl_3$, 400): δ 4.44 (dt, J = 3.6, 7.2 Hz, 1H), 4.19 (q, J = 7.2 Hz, 2H), 3.99 (q, J = 7.6 Hz, 1H), 2.96 (m, 1H), 2.70 (q, J = 8.0 Hz, 1H), 2.32 (m, 1H), 2.08 (m, 3H), 1.28 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 173.4, 82.1, 61.0, 53.9, 49.0, 32.5, 24.7, 14.0.

4.1.15. General procedure for the synthesis of azido compounds 6 and 23

The corresponding bromo-alcohol (2 mmol) was dissolved in 3 mL anhydrous DMF, and 2.2 equiv NaN_3 was added. The solution was stirred and heated to 60 °C until disappearance of all starting material by TLC (20% EtOAc in hexanes). The solution was then poured into 20 mL saturated NH_4Cl, extracted with Et_2O (3 × 15 mL), and washed with brine (30 mL). The combined organic phases were then concentrated in vacuo and purified by column chromatography (20% EtOAc in hexanes).

4.1.16. (±) Ethyl 3-azido-2-hydroxycyclo-pentanecarboxylate (6)

^{1}H NMR ($CDCl_3$): δ 4.31 (q, J = 6.4 Hz, 1H), 4.18 (q, J = 6.8 Hz, 2H), 3.97 (m, 1H), 2.83 (q, J = 7.6 Hz, 1H), 2.55 (m, 1H), 2.13 (m, 1H), 1.99 (m, 1H), 1.82 (m, 2H), 1.25 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.5, 76.9, 64.8, 60.8, 48.6, 27.0, 24.0, 14.0.

4.1.17. (±) Ethyl 2-hydroxy-3-(octylsulfon-amido)cyclopentanecarboxylate (7)

^{1}H NMR ($CDCl_3$): δ 5.03 (m, 1H), 4.33 (q, J = 4.8 Hz, 1H), 4.15 (q, J = 6.8 Hz, 2H), 3.73 (quintet, J = 6.8 Hz, 1H), 3.09 (m, 1H), 3.03 (t, J = 8.0 Hz, 2H), 2.81 (m, 1H), 2.10 (m, 2H), 1.78 (m, 4H), 1.26 (m, 13H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.2, 74.5, 60.9, 56.8, 53.1, 50.3, 31.6, 29.9, 29.0, 28.9, 28.2, 24.2, 23.5, 22.5, 14.1, 14.0.

4.1.18. (±) 2-Hydroxy-3-(octylsulfonamido)-cyclopentanecarboxylic acid (8a)

Mp = 88–89 °C; ^{1}H NMR (MeOD): δ 4.24 (t, J = 4.4 Hz, 1H), 3.71 (q, J = 5.6 Hz, 1H), 3.08 (t, J = 7.2 Hz, 2H), 2.78 (m, 1H), 2.12 (m, 1H), 2.02 (m, 1H), 1.77 (m, 4H), 1.35 (m, 10H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD): δ 178.1, 75.8, 58.3, 53.9, 51.5, 32.8, 30.7, 30.1, 30.1, 29.3, 25.4, 24.7, 23.6, 14.3. Anal. ($C_{14}H_{27}NO_5S$) C, H, N.

4.1.19. (±) 2-Hydroxy-3-(octylsulfonamido)-cyclohexanecarboxylic acid (8b)

Mp = 94–95 °C; ^{1}H NMR (MeOD): δ 3.97 (dd, J = 4.0, 8.0 Hz, 1H), 3.65 (m, 1H), 3.10 (m, 2H), 2.62 (m, 1H), 1.83 (m, 4H), 1.60 (m, 3H), 1.42 (m, 3H), 1.31 (m, 8H), 0.92 (t, J = 6.8 H, 3H); ^{13}C NMR (MeOD): δ 177.6, 71.2, 55.1, 53.9, 47.3, 32.9, 30.7, 30.2, 30.1, 30.1, 29.3, 24.7, 23.6, 20.7, 14.4. Anal. (($C_{15}H_{29}NO_5S$)·0.2(AcOH)) C, H, N.

4.1.20. (±) 2-Hydroxy-3-(2-oxo-2-phenylethylidene amino)-cyclohexanecarbonitrile (9)

Characterization data are in agreement with literature values.[23]

4.1.21. (±) *N*-(-3-Cyano-2-hydroxycyclohexyl)octane-1-sulfonamide (10)

^{1}H NMR ($CDCl_3$): δ 5.01 (d, J = 7.6 Hz, 1H), 4.08 (m, 1H), 3.68 (m, 1H), 3.42 (d, J = 4.4 Hz, 1H), 3.07 (m, 2H), 2.94 (m, 1H), 1.99 (m, 1H), 1.81 (m, 3H), 1.73 (m, 4H), 1.42 (m, 2H), 1.24 (m, 8H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 120.2, 69.0, 53.3, 52.6, 32.7, 31.4, 28.8, 28.7, 27.9, 27.9, 27.7, 23.3, 22.3, 19.6, 13.7.

4.1.22. (±) Ethyl 6-oxa-bicyclo[3.1.0]hexane-2-carboxylate (11a)

^{1}H NMR ($CDCl_3$): δ 4.20 (q, J = 7.2 Hz, 2H), 3.69 (m, 1H), 3.49 (m, 1H), 2.85 (t, J = 8.4 Hz, 1H), 2.12 (m, 1H), 1.82 (m, 1H), 1.69 (m, 2H), 1.28 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.9, 60.8, 57.4, 56.4, 45.3, 26.8, 21.2, 14.0.

4.1.23. (±) Methyl 7-oxa-bicyclo[4.1.0]heptane-2-carboxylate (11b)

Characterization data are in agreement with literature values.[18]

4.1.24. (±) Ethyl 3-azido-2-hydroxycyclo-pentanecarboxylate (12a)

^{1}H NMR ($CDCl_3$): δ 4.21 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.92 (m, 1H), 3.48 (m, 1H), 2.95 (dt, J = 4.8, 9.2 Hz, 1H), 2.23 (m, 1H), 2.05 (m, 2H), 1.69 (m, 1H), 1.26 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 173.8, 79.2, 66.8, 60.8, 49.1, 26.9, 23.2, 13.9.

4.1.25. (±) Methyl 3-azido-2-hydroxycyclo-hexanecarboxylate (12b)

^{1}H NMR ($CDCl_3$): δ 3.84 (m, 2H), 3.72 (s, 3H), 3.38 (s, 1H), 2.84 (m, 1H), 1.94 (m, 2H), 1.57 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 175.4, 70.0, 61.1, 51.9, 43.3, 26.0, 24.1, 19.9.

4.1.26. (±) Ethyl 2-hydroxy-3-(octylsulfon-amido)cyclopentanecarboxylate (13a)

^{1}H NMR ($CDCl_3$): δ 5.24 (d, J = 6.8 Hz, 1H), 4.16 (m, 1H), 4.14 (q, J = 7.2 Hz, 2H), 3.67 (quintet, J = 7.2 Hz, 1H), 3.53 (d, J = 4.0 Hz, 1H), 3.04 (d, J = 7.2 Hz, 2H), 2.98 (m, 1H), 2.25 (m, 1H), 1.99 (m, 2H), 1.74 (m, 2H), 1.50 (m, 1H), 1.35 (m, 2H), 1.22 (m, 11H), 0.84 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 173.8, 78.1, 60.7, 60.3, 52.8, 45.9, 31.5, 29.5, 28.9, 28.8, 28.1, 24.0, 23.4, 22.4, 14.0, 13.8.

4.1.27. (±) Methyl 2-hydroxy-3-(octylsulfon-amido)cyclohexanecarboxylate (13b)

^{1}H NMR ($CDCl_3$): δ 5.07 (d, J = 6.8 Hz, 1H), 3.70 (s, 3H), 3.62 (m, 3H), 3.04 (t, J = 8.0 Hz, 2H), 2.92 (m, 1H), 2.07 (m, 1H), 1.99 (m, 1H), 1.76 (m, 2H), 1.52 (m, 3H), 1.27 (m, 11H), 0.85 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.8, 71.8, 54.2, 53.1, 51.8, 44.0, 31.5, 28.9, 28.8, 28.8, 28.1, 25.0, 23.5, 22.4, 20.3, 13.9.

4.1.28. (±) 2-Hydroxy-3-(octylsulfonamido)-cyclopentanecarboxylic acid (14a)

Mp = 142–143 °C; ^{1}H NMR (MeOD): δ 4.21 (dd, J = 2.0, 6.0 Hz, 1H), 3.66 (m, 1H), 3.09 (dd, J = 1.6, 8.8 Hz, 2H), 3.00 (m, 1H), 2.26 (m, 1H), 2.06 (m, 1H), 1.92 (m, 1H), 1.81 (m, 2H), 1.54 (m, 1H), 1.46 (m, 2H), 1.33 (m, 8H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (MeOD) δ 176.5, 79.4, 61.9, 53.7, 48.5, 32.9, 30.6, 30.2, 30.1, 29.3, 24.8, 24.4, 23.6, 14.4. Anal. ($C_{14}H_{27}NO_5S$) C, H, N.

4.1.29. (±) 2-Hydroxy-3-(octylsulfonamido)-cyclohexanecarboxylic acid (14b)

Mp = 89–90 °C; ^{1}H NMR (MeOD): δ 3.97 (m, 1H), 3.61 (m, 1H), 3.08 (t, J = 6.8 Hz, 2H), 2.80 (m, 1H), 1.99 (m, 1H), 1.83 (m, 3H), 1.59 (m, 3H), 1.47 (m, 3H), 1.35 (m, 8H), 0.92 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD) δ 177.5, 72.0, 55.1, 53.9, 44.6, 32.8, 30.8, 30.2, 30.1, 29.3, 24.8, 23.6, 24.1, 20.7, 14.3. Anal. ($C_{15}H_{29}NO_5S$) C, H, N.

4.1.30. (±) *N*-2-Oxo-hexahydro-2H-cyclo-penta[b]furan-6-yl)-octane-1-sulfonamide (16)

^{1}H NMR ($CDCl_3$) δ 5.20 (d, J = 7.2 Hz, 1H), 4.81 (dd, J = 2.4, 7.2 Hz, 1H), 3.85 (m, 1H), 3.05 (t, J = 8.0 Hz, 2H), 2.99 (m, 1H), 2.80 (dd, J = 10.0, 18.4 Hz, 1H), 2.31 (dd, J = 2.4, 18.4 Hz, 1H), 2.14 (m, 2H), 1.75 (m, 2H), 1.67 (m, 1H), 1.52 (m, 1H), 1.41 (m, 2H), 1.37 (m, 8H), 0.87 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 176.6, 89.2, 59.8, 53.0, 36.4, 35.3, 31.6, 31.2, 30.7, 29.0, 28.9, 28.1, 23.5, 22.5, 13.9.

4.1.31. (±) 2-(2-Hydroxy-3-(octylsulfonamido)-cyclopentyl)acetic acid (17)

Mp = 73–74 °C; ^{1}H NMR (MeOD) δ 4.82 (dd, J = 2.8, 7.6 Hz, 1H), 3.82 (dt, J = 2.8, 6.4 Hz, 1H), 3.11 (t, J = 8.0 Hz, 2H), 3.01 (m, 1H), 2.86 (dd, J = 10.0, 18.4 Hz, 1H), 2.35 (dd, J = 2.8, 18.4 Hz, 1H), 2.14 (m, 1H), 2.08 (m, 1H), 1.80 (m, 2H), 1.70 (m, 1H), 1.51 (m, 3H), 1.35 (m, 8H), 0.92 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD) δ 179.4, 91.4, 61.1, 53.4, 37.9, 36.0, 32.8, 32.1, 31.6, 30.2, 30.1, 29.2, 24.7, 23.6, 14.4. Anal. ($C_{15}H_{28}NNaO_5S$)·(NaOH) C, H, N.

4.1.32. (±) Methyl 2-(3-azido-2-hydroxy-cyclopentyl)acetate (19)

Characterization data are in agreement with literature values.[24]

4.1.33. (±) Methyl 2-(2-hydroxy-3-(octylsulfon-amido)cyclo-pentyl)acetate (20)

^{1}H NMR ($CDCl_3$) δ 5.25 (d, J = 7.2 Hz, 1H), 3.80 (d, J = 2.4 Hz, 1H), 3.65 (s, 3H), 3.53 (m, 2H), 3.07 (dt, J = 4.8, 6.8 Hz, 2H), 2.55 (dd, J = 6.8, 16.0 Hz, 1H), 2.37 (dd, J = 7.2, 16.0 Hz, 1H), 2.11 (m, 2H), 1.94 (m, 1H), 1.76 (m, 2H), 1.52 (m, 1H), 1.37 (m, 2H), 1.25 (m, 9H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.9, 81.9, 60.6, 53.0, 51.6, 40.0, 37.9, 31.6, 28.9, 28.8, 28.3, 28.1, 25.6, 23.5, 22.4, 13.9.

4.1.34. (±) 2-(2-hydroxy-3-(octylsulfonamido)-cyclopentyl)-acetic acid (21)

Mp = 105–106 °C; ^{1}H NMR (MeOD) δ 3.48 (m, 2H), 3.14 (m, 2H), 2.65 (dd, J = 4.8, 15.6 Hz, 1H), 2.23 (dd, J = 9.2, 15.6 Hz, 1H), 2.08 (m, 2H), 1.96 (m, 1H), 1.80 (m, 2H), 1.57 (m, 1H), 1.42 (m, 2H), 1.34 (m, 9H), 0.93 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD): δ 176.7, 82.6, 61.5, 54.2, 42.1, 38.7, 32.9, 30.2, 30.1, 29.5, 29.3, 26.6, 24.8, 23.6, 14.4. Anal. ($C_{15}H_{29}NO_5S$) C, H, N.

4.1.35. (±) Methyl 2-(3-bromo-2-hydroxycyclo-pentyl)acetate (22)

^{1}H NMR ($CDCl_3$) δ 3.99 (q, J = 6.8 Hz, 1H), 3.89 (t, J = 7.2 Hz, 1H), 3.62 (s, 3H), 3.60 (s, 1H), 2.58 (dd, J = 7.2, 16.0 Hz, 1H), 2.40 (dd, J = 7.2, 16.0 Hz, 1H), 2.25 (m, 1H), 2.09 (m, 1H), 1.94 (m, 2H), 1.51 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.5, 84.8, 55.3, 51.5, 41.3, 38.1, 32.5, 27.8.

4.1.36. (±) Methyl 2-(3-azido-2-hydroxycyclo-pentyl)acetate (23)

^{1}H NMR ($CDCl_3$) δ 4.08 (q, J = 6.8 Hz, 1H), 3.73 (q, J = 6.0 Hz, 1H), 3.65 (s, 3H), 3.01 (d, J = 6.4 Hz, 1H), 2.51 (dd, J = 7.2, 16.0 Hz, 1H), 2.36 (dd, J = 7.2, 16.0 Hz, 1H), 2.25 (m, 1H), 2.03 (s, 1H), 1.92 (m, 1H), 1.76 (m, 1H), 1.21 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 173.7, 79.1, 64.6, 51.6, 39.9, 37.5, 26.7, 26.3.

4.1.37. (±) Methyl 2-(2-hydroxy-3-(octylsulfon-amido)cyclo-pentyl)acetate (24)

^{1}H NMR ($CDCl_3$) δ 5.18 (d, J = 7.2 Hz, 1H), 3.80 (m, 1H), 3.66 (m, 4H), 3.61 (m, 1H), 3.00 (t, J = 8.0 Hz, 2H), 2.43 (d, 2H), 2.19 (m, 1H), 2.01 (m, 2H), 1.75 (m, 3H), 1.38 (m, 2H), 1.25 (m, 8H), 1.13 (m, 1H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.9, 76.4, 56.2, 53.0, 51.7, 42.0, 37.9, 31.5, 30.4, 28.9, 28.8, 28.2, 27.0, 23.4, 22.4, 13.9.

4.1.38. (±) 2-(2-Hydroxy-3-(octylsulfonamido)-cyclopentyl)-acetic acid (25)

Mp = 98–99 °C; ^{1}H NMR (MeOD) δ 3.74 (d, J = 5.2 Hz, 1H), 3.68 (m, 1H), 3.08 (m, 2H), 2.53 (dd, J = 5.2, 9.6 Hz, 1H), 2.29 (dd, J = 5.2, 8.8 Hz, 1H), 2.23 (m, 1H), 2.03 (m, 2H), 1.80 (m, 2H), 1.71 (m, 1H), 1.46 (m, 2H), 1.34 (m, 8H), 1.24 (m, 1H), 0.91 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD) δ 176.5, 77.5, 57.6, 53.9, 43.0, 39.0, 32.9, 30.9, 30.2, 30.1, 29.3, 27.6, 24.7, 23.6, 14.4. Anal. ($C_{15}H_{29}NO_5S$) ·0.8(AcOH) C, H, N.

4.1.39. (±) Methyl 2-(3-azido-2-hydroxycyclo-hexyl)acetate (27)

From known *anti*-epoxide **26**.[25] ^{1}H NMR ($CDCl_3$): δ 3.68 (s, 3H), 3.25 (m, 1H), 3.12 (t, J = 9.6 Hz, 1H), 2.71 (dd, J = 5.2, 15.6 Hz, 1H), 2.62 (m, 1H), 2.23 (dd, J = 7.2, 15.6 Hz, 1H), 2.06 (m, 1H), 1.90 (m, 1H), 1.78 (m, 2H), 1.37 (m, 2H), 1.09 (m, 1H); ^{13}C NMR ($CDCl_3$): δ 173.6, 77.0, 66.5, 51.4, 40.1, 37.3, 30.2, 29.9, 23.2.

4.1.40. (±) Methyl 2-(2-hydroxy-3-(octylsulfon-amido)cyclo-hexyl)acetate (28)

^{1}H NMR ($CDCl_3$): δ 4.97 (d, J = 6.8 Hz, 1H), 3.66 (s, 3H), 3.24 (br s, 1H), 3.06 (m, 4H), 2.71 (dd, J = 5.2, 15.6 Hz, 1H), 2.23 (dd, J = 7.2, 15.6 Hz, 1H), 2.09 (m, 1H), 1.73 (m, 5H), 1.26 (m, 12H), 1.08 (m, 1H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 173.9, 76.6, 59.3, 53.4, 51.6, 40.9, 37.7, 33.0, 31.6, 30.5, 29.0, 28.9, 28.2, 23.8, 23.5, 22.5, 13.9.

4.1.41. (±) 2-(2-Hydroxy-3-(octylsulfonamido)-cyclohexyl)acetic acid (29)

Mp = 120–121 °C; ^{1}H NMR (MeOD): δ 3.12 (quintet, J = 5.2 Hz, 2H), 3.05 (m, 1H), 2.99 (t, J = 9.6 Hz, 1H), 2.84 (dd, J = 3.6, 15.6 Hz, 1H), 2.08 (m, 2H), 1.80 (m, 4H), 1.70 (m, 1H), 1.34 (m, 12H), 1.08 (m, 1H), 0.92 (t, J = 6.8 Hz, 3H); ^{13}C NMR (MeOD): δ 177.0, 77.3, 60.5, 54.1, 42.8, 38.5, 35.1, 32.9, 31.5, 30.2, 30.2, 29.4, 25.1, 24.7, 23.6, 14.4. Anal. ($C_{16}H_{31}NO_5S$) C, H, N.

4.1.42. (±) Methyl 2-(3-azido-2-hydroxycyclo-hexyl)acetate (31)

From known *syn*-epoxide **30**.[25] ^{1}H NMR ($CDCl_3$): δ 3.67 (s, 3H), 3.65 (m, 1H), 3.55 (m, 1H), 2.60 (dd, J = 6.4, 15.2 Hz, 1H), 2.40 (m, 2H), 2.26 (dd, J = 7.2, 15.2 Hz, 1H), 1.96 (m, 1H), 1.85 (m, 1H), 1.40–1.70 (m, 4H); ^{13}C NMR ($CDCl_3$): δ 173.8, 80.4, 61.9, 51.6, 34.9, 34.7, 25.5, 19.4, 17.9.

4.1.43. (±) Methyl 2-(2-hydroxy-3-(octylsulfon-amido)cyclo-hexyl)acetate (32)

^{1}H NMR ($CDCl_3$): δ 5.02 (d, J = 7.2 Hz, 1H), 3.65 (s, 3H), 3.60 (m, 1H), 3.28 (m, 1H), 3.09 (s, 1H), 3.03 (dt, J = 4.4, 6.4 Hz, 2H), 2.62 (dd, J = 5.6, 15.6 Hz, 1H), 2.43 (s, 1H), 2.24 (dd, J = 8.4, 15.6 Hz, 1H), 2.02 (m, 1H), 1.77 (m, 2H), 1.20–1.55 (m, 15H), 0.85 (t, J = 7.6 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 174.1, 72.7, 54.3, 53.3, 51.6, 35.3, 34.5, 33.2, 31.6, 28.9, 28.8, 28.2, 26.9, 23.5, 22.4, 19.5, 13.9.

4.1.44. (±) 2-(2-Hydroxy-3-(octylsulfonamido)-cyclohexyl)acetic acid (33)

Mp = 139–140 °C; ^{1}H NMR (MeOD): δ 3.67 (m, 1H), 3.39 (m, 1H), 3.08 (m, 2H), 2.52 (dd, J = 4.0, 14.0 Hz, 1H), 2.27 (m, 2H), 1.97 (m, 1H), 1.80 (m, 2H), 1.30–1.55 (m, 15H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (MeOD): δ 177.0, 73.0, 55.3, 54.0, 35.9, 35.7, 32.9, 30.2, 30.1, 29.3, 29.3, 27.1, 24.8, 23.6, 20.7, 14.4. Anal. (($C_{16}H_{31}NO_5S$)·0.2(C_6H_{14})) C, H, N.

4.1.45. (±) (2-Hydroxy-3-(octylsulfonamido)-cyclohexyl)methyl 4-methylbenzenesulfonate (34)

Compound **8b** (176 mg, 0.525 mmol) was dissolved in 2 mL THF, and the solution was cooled to 0 °C. 0.066 mL (1.25 equiv) BH_3–dimethyl sulfide complex was added, and the solution was allowed to slowly warm to room temperature with stirring. MeOH was added (2 mL), and the solution was evaporated in vacuo. The crude product was purified by flash chromatography (60% EtOAc in hexanes) to yield 91 mg (54%) of the diol product. ^{1}H NMR ($CDCl_3$): δ 4.76 (d, J = 4.8 Hz, 1H), 3.87 (br s, 1H), 3.69 (m, 4H),

3.08 (m, 2H), 2.60 (br s, 1H), 2.00 (m, 1H), 1.81 (m, 3H), 1.32–1.62 (m, 6H), 1.25 (m, 9H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 75.0, 67.4, 53.9, 52.7, 39.9, 31.5, 29.4, 28.9, 28.8, 28.1, 26.0, 23.3, 22.4, 18.7, 13.9. The diol (91 mg, 0.283 mmol) was dissolved in 2.4 mL CH_2Cl_2, the solution was cooled to 0 °C, then *p*-toluenesulfonyl chloride (61 mg, 1.1 equiv) was added, followed by Et_3N (0.036 mL, 1.1 equiv), and the solution was allowed to warm to room temperature slowly with stirring. After 4 h, the solution was diluted with CH_2Cl_2 (10 mL), and the organic phase was washed with saturated NH_4Cl solution (2 × 10 mL). The organic phase was dried over Na_2SO_3, and concentrated in vacuo. Flash chromatography purification (40% EtOAc in hexanes) yielded **34** (90 mg, 67%, 30% recovered starting material). ^{1}H NMR ($CDCl_3$): δ 7.76 (d, J = 8.4 Hz, 2H), 7.34 (d, J = 8.4 Hz, 2H), 4.90 (d, J = 6.4 Hz, 1H), 4.15 (dd, J = 5.2, 9.6 Hz, 1H), 4.04 (dd, J = 3.2, 9.6 Hz, 1H), 3.70 (m, 1H), 3.56 (m, 1H), 3.06 (m, 2H), 2.87 (d, J = 5.6 Hz, 1H), 2.43 (s, 3H), 1.93 (m, 1H), 1.78 (m, 4H), 1.36–1.62 (m, 4H), 1.26 (m, 10H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 144.8, 132.5, 129.8, 127.7, 71.4, 69.9, 54.0, 53.0, 38.9, 31.5, 29.7, 28.9, 28.8, 28.1, 26.1, 23.4, 22.4, 21.5, 18.8, 13.9.

4.1.46. (±) *N*-(3-(Cyanomethyl)-2-hydroxycyclohexyl)octane-1-sulfonamide (35)

Compound **34** (90 mg, 0.189 mmol) was dissolved in 3 mL anhydrous DMF, and KCN (25 mg, 2 equiv) was added. The solution was heated to 60 °C overnight, then saturated NH_4Cl solution was added (20 mL, and the product was extracted with Et_2O (3 × 10 mL). The combined organic phases were dried over Na_2SO_3, and evaporated in vacuo. Flash chromatography (40% EtOAc in hexanes) yielded 35 (65 mg, quant.). ^{1}H NMR ($CDCl_3$): δ 5.09 (d, J = 6.8 Hz, 1H), 3.77 (m, 1H), 3.45 (m, 1H), 3.10 (d, J = 6.0 Hz, 1H), 3.06 (m, 2H), 2.59 (dd, J = 4.0, 16.8 Hz, 1H), 2.48 (dd, J = 6.8, 16.8 Hz, 1H), 1.75–1.95 (m, 5H), 1.61 (m, 2H), 1.40 (m, 2H), 1.26 (m, 10H), 0.86 (t, J = 6.8 Hz, 3H); ^{13}C NMR ($CDCl_3$): δ 118.5, 72.2, 54.1, 53.3, 35.9, 31.5, 29.8, 29.0, 28.9, 28.8, 28.1, 23.4, 22.4, 20.2, 18.9, 13.9.

4.1.47. (±) 2-(2-Hydroxy-3-(octylsulfonamido)cyclohexyl)acetic acid (36)

Compound **35** (40 mg, 0.121 mmol) was dissolved in 3.75 mL EtOH, then NaOH (200 mg) in H_2O (1.25 mL) was added. The solution was heated to reflux overnight, then was cooled to room temperature. The solution was diluted with H_2O (10 mL) and washed with Et_2O (10 mL). The aqueous phase was made strongly acidic with 1 M HCl, and the product was extracted with Et_2O (3 × 10 mL). The combined organic phases were dried over Na_2SO_3 and concentrated in vacuo. Flash chromatography (7:12:1 EtOAc/hexanes/AcOH) yielded **36** (31 mg, 73%). Mp = 101–102 °C; ^{1}H NMR (MeOD): δ 3.62 (m, 1H), 3.42 (dd, J = 3.6, 8.8 Hz, 1H), 3.11 (m, 2H), 2.69 (dd, J = 4.0, 14.4 Hz, 1H), 2.03–2.23 (m, 2H), 1.75–1.90 (m, 4H), 1.40–1.59 (m, 5H), 1.31 (m, 8H), 1.19 (m, 1H), 0.92 (t, J = 7.2 Hz, 3H); ^{1}H NMR (MeOD): δ 177.1, 74.3, 55.7, 53.9, 38.1, 37.4, 32.9, 31.5, 30.2, 30.1, 30.0, 29.4, 24.7, 23.6, 20.4, 14.4. Anal. ($C_{16}H_{31}NO_5S$) C, H, N.

4.1.48. (±)-Ethyl 2-(methylsulfonyloxy)-3-(octylsulfonamido)-cyclopentane carboxylate (37)

The alcohol starting material **13a** (1.6 mmol) was dissolved in 8 mL CH_2Cl_2, and the solution was cooled to 0 °C. Methanesulfonyl chloride (1.1 equiv) and Et_3N (1.1 equiv) were then each added dropwise, and the solution was allowed to gradually warm to room temperature over 2 h. Reaction progress was monitored by TLC (40% EtOAc in hexanes). When complete, the reaction was poured into saturated NH_4Cl (20 mL), extracted with CH_2Cl_2 (3 × 15 mL), and washed with brine (50 mL). The combined organic phases were concentrated in vacuo, and the crude product was purified by column chromatography (30% EtOAc in hexanes) to yield **37**. ^{1}H NMR ($CDCl_3$) δ 5.18 (br s, 1H), 5.05 (m, 1H), 4.11 (q, J = 7.2 Hz, 2H), 3.18 (m, 1H), 3.00 (m, 5H), 2.33 (m, 1H), 2.06 (m, 1H), 1.98 (m, 2H), 1.72 (m, 2H), 1.55 (m, 1H), 1.21 (m, 13H), 0.80 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 170.9, 85.5, 61.1, 58.7, 52.8, 45.9, 38.2, 31.5, 29.5, 28.9, 28.8, 28.1, 23.4, 23.3, 22.4, 14.0, 13.8.

4.1.49. (±)-Ethyl 6-(octylsulfonyl)-6-aza-bicyclo[3.1.0]hexane-2-carboxylate (38)

400 mg (0.935 mmol) mesylate **37** was dissolved in 60 mL CH_3CN, K_2CO_3 (390 mg, 3 equiv) was added, and the solution was stirred at room temperature overnight. The mixture was filtered, the filtrate was concentrated in vacuo, and the crude product was purified by column chromatography (30% EtOAc in hexanes) to yield **38**. ^{1}H NMR ($CDCl_3$) δ 4.13 (q, J = 7.2 Hz, 2H), 3.50 (d, J = 4.8 Hz, 1H), 3.35 (m, 1H), 3.08 (m, 3H), 2.00 (m, 2H), 1.83 (m, 3H), 1.63 (m, 1H), 1.41 (m, 2H), 1.23 (m, 11H), 0.85 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 172.1, 61.0, 52.8, 45.9, 45.4, 44.6, 31.5, 28.8, 28.7, 28.1, 26.1, 24.0, 22.9, 22.4, 14.0, 13.9.

4.1.50. (±)-Ethyl 3-hydroxy-2-(octylsulfonamido)cyclopentane-carboxylate (39)

Aziridine **38** (180 mg, 0.54 mmol) was dissolved in 12 mL dioxane. H_2O (8 mL) was added, followed by 0.40 mL trifluoroacetic acid, and the solution was heated to reflux for 3 h. The solution was diluted with EtOAc, washed with saturated $NaHCO_3$ solution, concentrated in vacuo, and purified by column chromatography (30% EtOAc in hexanes) to yield **39**. ^{1}H NMR ($CDCl_3$) δ 5.32 (d, J = 7.2 Hz, 1H), 4.18 (q, J = 7.2 Hz, 2H), 4.06 (q, J = 7.2 Hz, 1H), 3.72 (m, 2H), 3.11 (t, J = 8.0 Hz, 2H), 2.68 (q, J = 8.8 Hz, 1H), 1.90–2.10 (m, 3H), 1.73 (m, 3H), 1.41 (m, 2H), 1.28 (m, 11H), 0.87 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 173.7, 77.5, 64.1, 61.1, 52.3, 47.7, 31.6, 30.3, 29.0, 28.9, 28.1, 24.1, 23.4, 22.5, 14.1, 13.9.

4.1.51. (±)-3-Hydroxy-2-(octylsulfonamido)cyclopentanecarboxylic acid (40)

Mp = 145 °C; ^{1}H NMR (MeOD) δ 3.96 (q, J = 6.4 Hz, 1H), 3.79 (m, 1H), 3.13 (t, J = 8.0 Hz, 2H), 2.69 (m, 1H), 1.97 (m, 3H), 1.79 (m, 2H), 1.68 (m, 1H), 1.44 (m, 2H), 1.33 (m, 8H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR (MeOD) δ 177.7, 78.8, 65.3, 53.9, 49.7, 32.9, 32.3, 30.2, 30.1, 29.3, 26.0, 24.8, 23.6, 14.4. Anal. ($C_{14}H_{27}NO_5S$) C, H, N.

4.1.52. (±)-Ethyl 2-(methylsulfonyloxy)-3-(octylsulfonamido)-cyclopentane carboxylate (41)

The alcohol starting material **3a** (1.6 mmol) was dissolved in 8 mL CH_2Cl_2, and the solution was cooled to 0 °C. Methanesulfonyl chloride (1.1 equiv) and Et_3N (1.1 equiv) were then each added dropwise, and the solution was allowed to gradually warm to room temperature over 2 h. Reaction progress was monitored by TLC (40% EtOAc in hexanes). When complete, the reaction was poured into saturated NH_4Cl (20 mL), extracted with CH_2Cl_2 (3 × 15 mL), and washed with brine (50 mL). The combined organic phases were concentrated in vacuo, and the crude product was purified by column chromatography (30% EtOAc in hexanes) to yield **41**. ^{1}H NMR ($CDCl_3$) δ 5.08 (m, 1H), 5.00 (s, 1H), 4.21 (q, J = 7.2 Hz, 2H), 3.92 (m, 1H), 3.13 (s, 3H), 3.08 (m, 3H), 2.29 (m, 1H), 2.17 (m, 1H), 2.00 (m, 1H), 1.82 (m, 2H), 1.74 (m, 1H), 1.44 (m, 2H), 1.24 (m, 11H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 169.9, 86.2, 61.7, 59.3, 53.7, 47.6, 38.4, 31.6, 30.4, 29.0, 28.9, 28.2, 25.1, 23.5, 22.5, 14.0, 14.0.

4.1.53. (±)-Ethyl 6-(octylsulfonyl)-6-aza-bicyclo[3.1.0]hexane-2-carboxylate (42)

400 mg (0.935 mmol) mesylate **41** was dissolved in 60 mL CH_3CN, K_2CO_3 (390 mg, 3 equiv) was added, and the solution was stirred at room temperature overnight. The mixture was filtered,

the filtrate was concentrated in vacuo, and the crude product was purified by column chromatography (30% EtOAc in hexanes) to yield **42**. ^{1}H NMR ($CDCl_3$) δ 4.16 (dq, J = 2.0, 7.2 Hz, 2H), 3.37 (m, 2H), 3.17 (m, 1H), 3.10 (m, 1H), 2.95 (t, J = 9.2 Hz, 1H), 2.13 (m, 1H), 1.99 (m, 1H), 1.84 (m, 3H), 1.65 (m, 1H), 1.40 (m, 2H), 1.25 (m, 11H), 0.84 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 171.8, 60.7, 52.3, 46.5, 44.6, 43.3, 31.5, 28.8, 28.8, 28.2, 26.9, 22.8, 22.7, 22.4, 14.1, 13.8.

4.1.54. (±)-Ethyl 3-hydroxy-2-(octylsulfonamido)cyclopentanecarboxylate (43)

Aziridine **42** (180 mg, 0.54 mmol) was dissolved in 12 mL dioxane. 8 mL H_2O was added, followed by 0.40 mL trifluoroacetic acid, and the solution was heated to reflux for 3 h. The solution was diluted with EtOAc, washed with saturated $NaHCO_3$ solution, concentrated in vacuo, and purified by column chromatography (30% EtOAc in hexanes) to yield **43**. ^{1}H NMR ($CDCl_3$) δ 5.28 (d, J = 6.8 Hz, 1H), 4.28 (m, 1H), 4.18 (q, J = 7.2 Hz, 2H), 3.66 (q, J = 7.2 Hz, 1H), 3.15 (m, 1H), 3.05 (t, J = 8.0 Hz, 2H), 2.50 (br s, 1H), 2.13 (m, 2H), 1.73–1.92 (m, 3H), 1.62 (m, 1H), 1.28 (m, 13H), 0.88 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 174.1, 76.6, 62.6, 60.9, 53.3, 45.0, 31.6, 30.1, 28.9, 28.8, 28.1, 24.6, 23.4, 22.4, 14.0, 14.0.

4.1.55. (±)-3-Hydroxy-2-(octylsulfonamido)cyclopentanecarboxylic acid (44)

Mp = 126 °C; ^{1}H NMR (MeOD) δ 4.19 (m, 1H), 3.75 (m, 1H), 3.19 (q, J = 7.6 Hz, 1H), 3.09 (t, J = 8.0 Hz, 2H), 2.14 (m, 1H), 2.00 (m, 2H), 1.80 (m, 2H), 1.59 (m, 1H), 1.45 (m, 2H), 1.31 (m, 8H), 0.92 (t, J = 7.2 Hz, 3H); ^{13}C NMR ($CDCl_3$) δ 176.8, 77.8, 64.0, 54.3, 46.9, 32.9, 31.2, 30.2, 30.1, 29.3, 24.7, 24.6, 23.6, 14.4. Anal. ($C_{14}H_{27}NO_5S$) C, H, N.

4.2. Biological testing

4.2.1. GPAT assay

The mtGPAT assay has been reported previously.[26] A mitochondrial preparation of glycerol 3-phosphate acyltransferase was added to the incubation mixture containing ^{14}C-labeled glycerol 3-phosphate, palmitoyl-CoA, and varying inhibitor concentrations to initiate the reaction. After ten min, the reaction was terminated by adding chloroform, methanol, and 1% perchloric acid. Five min later, more chloroform and perchloric acid were added, and the upper aqueous layer was removed. After washing three times with 1% perchloric acid, the organic layer was evaporated under nitrogen, and the amount of ^{14}C present was counted to determine the extent of reaction inhibition. Data points were recorded in triplicate, and IC_{50} values were calculated based on the amount of test inhibitor necessary to produce 50% of mtGPAT activity observed in the absence of inhibitor but in the presence of DMSO vehicle control.

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Supplementary data

Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.bmc.2010.06.091. These data include MOL files and InChiKeys of the most important compounds described in this article.

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