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Structure—Activity Relationship of Antischistosomal Ozonide Carboxylic Acids

Jianbo Wu,

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 986125, United States

Xiaofang Wang,

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 986125, United States

Francis C. K. Chiu,

Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia

Ćecile Häberli,

Swiss Tropical and Public Health Institute, CH-4002 Basel, Switzerland; University of Basel, CH-4003 Basel, Switzerland

David M. Shackelford,

Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia

Eileen Ryan,

Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia

Sriraghavan Kamaraj,

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 986125, United States

Vivek J. Bulbule,

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 986125, United States

Alexander I. Wallick,

Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska 68182, United States

Corresponding Author: Jonathan L. Vennerstrom Phone: 402 559 5362; jvenners@unmc.edu.

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ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge at <https://pubs.acs.org/doi/10.1021/acs.jmedchem.0c00069>.

¹H and ¹³C{¹H} NMR spectra (500 MHz) of **5–10** and **13–25** (PDF)

SMILES string computer readable identifiers for **2–25** (CSV)

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Yuxiang Dong,

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 986125, United States

Karen L. White,

Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia

Paul H. Davis,

Department of Biology, University of Nebraska at Omaha, Omaha, Nebraska 68182, United States

Susan A. Charman,

Centre for Drug Candidate Optimisation, Monash Institute of Pharmaceutical Sciences, Monash University (Parkville Campus), Parkville, Victoria 3052, Australia

Jennifer Keiser,

Swiss Tropical and Public Health Institute, CH-4002 Basel, Switzerland; University of Basel, CH-4003 Basel, Switzerland

Jonathan L. Vennerstrom

College of Pharmacy, University of Nebraska Medical Center, Omaha, Nebraska 986125, United States

Abstract

Semisynthetic artemisinins and other bioactive peroxides are best known for their powerful antimalarial activities, and they also show substantial activity against schistosomes—another hemoglobin-degrading pathogen. Building on this discovery, we now describe the initial structure—activity relationship (SAR) of antischistosomal ozonide carboxylic acids OZ418 (**2**) and OZ165 (**3**). Irrespective of lipophilicity, these ozonide weak acids have relatively low aqueous solubilities and high protein binding values. Ozonides with *para*-substituted carboxymethoxy and *N*-benzylglycine substituents had high antischistosomal efficacies. It was possible to increase solubility, decrease protein binding, and maintain the high antischistosomal activity in mice infected with juvenile and adult *Schistosoma mansoni* by incorporating a weak base functional group in these compounds. In some cases, adding polar functional groups and heteroatoms to the spiroadamantane substructure increased the solubility and metabolic stability, but in all cases decreased the antischistosomal activity.

Graphical Abstract

1,2,4,5-tetraoxane isosteres; and (3) ozonides with carboxylic acid, but not neutral or basic, functional groups show the highest antischistosomal activity. For example, **1** shows no antischistosomal activity.²⁴ In this paper, we profile ozonides **4–25** to expand the structure–activity relationship (SAR) of **2** and **3** (Figure 2).

CHEMISTRY

Ozonides **4–25** were prepared as described in Schemes 1–12. Ozonide carboxylic acids **9** and **10** were prepared in 77 and 72% yields by one-pot alkylation of ozonide phenol **26**²⁷ with the corresponding bromoalkyl esters followed by hydrolysis (Scheme 1). Ozonide acylsulfonamide **13** was prepared in 62% yield by a dicyclohexylcarbodiimide (DCC)-mediated coupling of **2** with methanesulfonamide (Scheme 2). Hydroxybenzotriazole/1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (HOBT/EDC)-mediated condensation of **2** with the ethyl ester of glycine followed by ester hydrolysis afforded ozonide carboxamide **14** in 91% yield (Scheme 2).

The reaction of ozonide acetate **27**²⁷ with N-chloroethylpiperidine **28** according to the method of Charman et al.²⁵ furnished ozonide ester **29** in 45% yield; hydrolysis of **29** gave ozonide piperidine carboxylic acid **15** as its sodium salt in 86% yield (Scheme 3). Alkylation of ozonide piperidine **30**²⁵ with ethyl bromoacetate produced ozonide ester **31** in 69% yield (Scheme 4). Hydrolysis of **31** afforded ozonide piperidine carboxylic acid **16** in 84% yield.

Ozonide esters **33** and **34** were formed in 99–100% yield by alkylation of ozonide benzyl chloride **32**²⁸ with the ethyl esters of glycine and azetidine-3-carboxylic acid, respectively (Scheme 5). Ester hydrolysis of **33** and **34** followed by treatment with methanesulfonic acid afforded the aza ozonide carboxylic acids **17** and **18** as their mesylate salts in 77 and 84% yield, respectively.

Chlorination of ozonide phenol **26**²⁷ with one and nine molar equivalents of N-chlorosuccinimide (NCS) furnished the chlorinated phenol intermediates **35** (77%) and **36** (63%), respectively (Scheme 6). Alkylation of **35** and **36** with ethyl bromoacetate afforded a quantitative yield of ozonide esters **37** and **38**; ester hydrolysis of the latter produced the target ozonide carboxylic acids **7** and **8** in 97 and 89% yield, respectively.

Ozonide salicylic acid **5** was synthesized in a multistep sequence starting from phenol ketal **39**²⁹ (Scheme 7). Formylation of **39** led to a quantitative yield of salicylaldehyde **40**, which then underwent successive silver oxide oxidation and ketal deprotection to afford keto salicylate **41** in 96% yield. Successive Fisher esterification and acetylation of **41** furnished the keto diester **42** in 99% yield. Griesbaum coozonolysis³⁰ of **42** and oxime ether **43**³¹ formed ozonide diester **44** in 70% yield. Ester hydrolysis of **44** afforded the desired ozonide salicylate **5** in quantitative yield. Ozonide dicarboxylic acid **6** was obtained in 84% yield by alkylation of **5** with ethyl bromoacetate followed by hydrolysis.

Coozonolysis³⁰ of oxime ether **47**²⁸ and keto ester **46**, formed by alkylation of keto phenol **45** with ethyl bromoacetate, furnished ozonide ester **48** in 15% yield (Scheme 8). Ester hydrolysis of **48** afforded the desired ozonide carboxylic acid **19** in 93% yield.

As shown in Scheme 9, ozonide carboxylic acids **20** and **21** were synthesized from **53**, a common ozonide keto phenol precursor. The synthesis of **53** began with the conversion of the *mono*-ketal of adamantane-2,6-dione (**49**)³² to its oxime ether **50** in high yield. This was followed by cozonolysis³⁰ of **50** and keto ester **51**²⁷ to form ozonide ester ketal **52** in 43% yield. Successive ester and ketal deprotection of **52** furnished **53** in an overall yield of 89%. Alkylation of **53** with ethyl bromoacetate to form ozonide ester **54** followed by hydrolysis afforded ozonide carboxylic acid **20** in 95% overall yield. Reduction of **53** with sodium borohydride afforded the corresponding secondary alcohol **55** in 99% yield. Alkylation of **55** with ethyl bromoacetate to form ozonide ester **56** followed by hydrolysis afforded carboxylic acid **21** in 95% overall yield.

Reductive amination of ozonide ester **54** followed by acetylation afforded ozonide ester **57** in 80% yield (Scheme 10). Ester hydrolysis of **57** furnished ozonide acetamide carboxylic acid **22** in 94% yield. Similarly, Boc protection of the crude amino ester ozonide formed by reductive amination of **54** afforded **58** in 69% yield. Ester hydrolysis of **58** followed by treatment with methanesulfonic acid afforded ozonide amino acid **23** as its mesylate salt in 98% yield.

The synthesis of ozonide **24** began with the formation of oxime ether **60** from the Boc-protected azaadamantanone **59**,³³ which then underwent cozonolysis³⁰ with keto ester **51** to form ozonide ester **61** in 36% yield (Scheme 11). Ester hydrolysis of **61** furnished ozonide phenol **62** in 98% yield, which was then alkylated with ethyl bromoacetate to form ozonide ester **63** in quantitative yield. Ester hydrolysis of **63** produced ozonide carboxylic acid **64** in 90% yield. Boc deprotection of **64** with methanesulfonic acid afforded the mesylate salt of ozonide carboxylic acid **24** in quantitative yield.

The synthesis of target ozonide **25** began with cozonolysis³⁰ of oxime ether **60** and ketone **65** to form ozonide **66** in 26% yield (Scheme 12). Alkylation of glycine ethyl ester with **66** afforded ozonide ester **67** in 97% yield. Intermediate **67** then underwent successive ester hydrolysis and Boc deprotection with methanesulfonic acid to afford the dimesylate salt of ozonide carboxylic acid **25** in 82% yield. Ozonides **2–4**, **11**, and **12** were obtained as previously described.^{34–36}

PHYSICOCHEMICAL, IN VITRO ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION (ADME), AND ANTISCHISTOSOMAL PROPERTIES

When considering the physicochemical and in vitro ADME properties of these ozonides (Tables 1–3), we note several overarching trends. First, the calculated polar surface area (PSA) values of between 68 and 117 Å² indicate that the polarity of these compounds is unlikely to be a rate-limiting factor for membrane permeability and oral bioavailability.³⁷ Second, most of the compounds have low aqueous solubilities even those with log *D*₇₄ values ≤ 3. Third, these compounds had high estimated protein binding values ranging from 94.2 to >99.5%, an unsurprising result for these ozonide weak acids.

The data in Table 1 show the SAR of the phenyl substructure of **2** and **3**. The only compounds with high aq. solubilities at pH 6.5 were salicylate **5** and dicarboxylic acid **6**; the latter was also metabolically stable. Ozonide **7**, the more lipophilic chloro analogue of **2**, was also metabolically stable. Ozonide **4**, the meta isomer of **3**, was somewhat less stable to metabolism; interestingly, addition of a phenol functional group in **5** improved the metabolic stability in mouse microsomes but substantially decreased metabolic stability in human microsomes. Of these analogues (Table 1), only **7** showed in vivo activity against chronic *S. mansoni* infections equal or superior to that of the two prototype ozonides **2** and **3**. For comparison, at this same 400 mg/kg oral dose, dihydroartemisinin⁵ and praziquantel⁴¹ reduce worm burden by 66 and 96%, respectively.

The data in Table 2 show the SAR of the carboxymethoxy substructure of **2**. Extending the length of the side chain to carboxypropoxy (**9**) or carboxybutoxy (**10**) decreased aq. solubility, increased protein binding, and decreased the antischistosomal activity compared to **2**. Compound **11**, the *gem*-dimethyl analogue of **2**, also had very high protein binding and was less active than **2**. With the exception of ethyl ester **12** and glycine conjugate **14**, the other compounds were metabolically stable; **12** was rapidly converted to **2** in both human and mouse liver microsomes, whereas **14** was converted to **2** in human but not mouse liver microsomes. Of these two compounds, only **14** showed significant antischistosomal activity. Interestingly, **14** was more and less soluble than **2** at pH 2.0 and 6.5, respectively. Acylsulfonamide **13** had the same solubility profile as **2**, but showed only a modest antischistosomal activity. The amphoteric analogs **15–18** show the effects of adding a weak base functional group to the overall profiles of these ozonides. Compound **17**, an aza isostere of **9**, had a much superior profile than the latter. For example, **17** was less lipophilic, showed lower protein binding, and was much more active than **9** against *S. mansoni* in vivo. However, for the two piperidine carboxylic acids **15** and **16**, only the former showed significant antischistosomal activity. Like **15**, azetidine carboxylic acid **18** was less lipophilic than **2**, but showed only weak antischistosomal activity.

The data in Table 3 summarize the SAR of the spiroadamantane substructure of **2**. Replacing the distal methylene carbon of **2** with a difluoromethylene (**19**) or a ketone (**20**) increased aq. solubility, decreased protein binding, and decreased both metabolic stability and antischistosomal activity. Decreasing lipophilicity by adding a secondary alcohol (**21**), acetamide (**22**), or primary amine (**23**) to the distal methylene carbon of **2** increased aq. solubility at pH 6.5 for **21** and **22**, but abolished the antischistosomal activity for all three ozonides. Interestingly, the amphoteric **23** showed the lowest protein binding (94.2%) for this series of ozonides. Compounds **24** and **25**, azaadamantane analogues of **2** and **17**, show the consequences of replacing the methylene carbon of **2** with a nitrogen atom. Unfortunately, **24** showed low aq. solubility, intermediate metabolic stability, and low antischistosomal activity. Even though **25** was metabolically stable and was the sole ozonide in this series with high aq. solubility at both pH 2.0 and 6.5, it also showed weak antischistosomal activity.

As the data in Tables 1–3 reveal, we identified **7**, **14**, **16**, and **17**—four new ozonides with high activity against adult *S. mansoni* in a mouse model. As a measure of their

antischistosomal selectivity, these ozonides were tested for cytotoxicity against four human cell lines: foreskin fibroblast (HFF), osteosarcoma (U-2 OS), kidney (HEK 293T), and hepatocyte (HC-04). None of the compounds inhibited these cell lines at concentrations of up to 50 μM (data not shown). Although our primary objective was to identify ozonides with high activity against the adult stage of *S. mansoni*, we wanted to find compounds with high activities against both adult and juvenile stages. Therefore, the most active ozonides were also tested for activity against juvenile *S. mansoni* in infected mice (Table 4). At a single 200 mg/kg dose, all of the compounds reduced worm burden by more than 80% in mice with juvenile stage *S. mansoni* infections (Table 4); of these, **2**, **14**, and **17** were the most effective with WBR values of 97–100%.

SUMMARY

In summary, these ozonide weak acids showed relatively low aqueous solubilities and high protein binding values that were independent of lipophilicity. It was possible to increase the solubility, decrease the protein binding, and maintain the high antischistosomal activity in mice infected with juvenile and adult *S. mansoni* by incorporating a weak base functional group in these compounds. Ozonides with *para*-substituted carboxymethoxy and *N*-benzylglycine substituents showed high antischistosomal activities. These are exemplified by **7**, **14**, **16**, and **17**—four new ozonides with log $D_{7.4}$ values ranging from 3.0 to 3.3, similar to that of prototype ozonide **2** (log $D_{7.4}$ of 2.9). Adding polar functional groups and heteroatoms to the spiroadamantane substructure could increase the solubility and metabolic stability, but in all cases, decreased the antischistosomal activity.

EXPERIMENTAL SECTION

General

Melting points are uncorrected. Unless otherwise noted, one-dimensional (1D) ^1H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer using CDCl_3 or $\text{DMSO}-d_6$ as solvents. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(\text{CH}_3)_4\text{Si}$ (0 ppm) for ^1H and CDCl_3 (77.2 ppm) or $\text{DMSO}-d_6$ (39.5 ppm) for ^{13}C NMR. Silica gel (sg) particle size of 32–63 μm was used for all flash column chromatography experiments. Reported reaction temperatures are those of the oil bath. Gas chromatography–mass spectrometry (GCMS) data were generated with an Agilent 6890–5973N GC-MS system using a DB-5 column and helium flow rate of 1 mL/min. Combustion analysis confirmed that all target compounds have a purity of at least 95%. X-ray crystallographic data for all ozonides with 8'-aryl substituents (like **2–25**) demonstrate that they have *cis* configurations.^{27,42}

5-(Dispiro[adamantane-2,3' -[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)-2-hydroxybenzoic acid (5**)—Step 1.** To a mixture of anhydrous magnesium chloride (12.21 g, 128.2 mmol), paraformaldehyde (5.78 g, 192.3 mmol), and triethylamine (17.9 mL, 128.2 mmol) in anhydrous THF (150 mL) at rt was added dropwise a solution of 4-(1,4-dioxaspiro[4.5]decan-8-yl)phenol (**39**)²⁹ (15.0 g, 64.1 mmol) in THF (100 mL) under Ar. The reaction mixture was then refluxed for 5 h. After completion of the reaction, an insoluble solid was filtered and washed with THF (2 \times 25 mL). After solvent removal in

vacuo, the residue was dissolved in DCM (150 mL), washed with 10% HCl (3 × 25 mL) and water (3 × 25 mL), and dried over MgSO₄. After filtration, the solvent was removed in vacuo to afford 2-hydroxy-5-(1,4-dioxaspiro[4.5]decan-8-yl)benzaldehyde (**40**) as a viscous oil (16.8 g, 100%). GCMS: *m/z*: 262 [M⁺], ¹H NMR (CDCl₃) δ 1.66–1.90 (m, 8H), 2.50–2.60 (m, 1H), 3.98 (s, 4H), 6.90–6.92 (m, 1H), 7.40–7.42 (m, 2H), 9.85 (s, 1H), 10.86 (s, 1H). ¹³C NMR (CDCl₃) δ 31.4, 34.8, 41.8, 64.1, 108.1, 117.3, 120.2, 131.1, 135.9, 137.99, 159.79, 196.5. **Step 2.** To a stirred solution of silver nitrate (7.13 g, 41.98 mmol) in water (25 mL) was added dropwise a solution of NaOH (3.35 g, 83.96 mmol) in water (25 mL) at 0 °C. After stirring for 5 min, silver oxide was obtained as a brown semisolid. To this mixture, a solution of **40** (5.5 g, 20.99 mmol) in water (25 mL) was added and the reaction mixture was stirred at 100 °C for 12 h. After filtration of the black silver suspension, the aq. solution was neutralized (pH = ~7) with 10% HCl at 0 °C to obtain a solid that was filtered, washed with water, and dried to afford 2-hydroxy-5-(1,4-dioxaspiro[4.5]decan-8-yl)benzoic acid as a colorless solid (5.6 g, 96%). To a stirred solution of hydroxy-5-(1,4-dioxaspiro[4.5]decan-8-yl)benzoic acid (5.6 g, 20.1 mmol) in THF (75 mL) was added 6 N HCl (20 mL) dropwise at rt. After completion of the reaction, the solvent was removed in vacuo and the residue was dissolved in DCM (100 mL), washed with water (3 × 25 mL), and dried over anhydrous MgSO₄. After filtration, the solvent was removed in vacuo to afford 2-hydroxy-5-(4-oxocyclohexyl)-benzoic acid (**41**) as a colorless solid (4.69 g, 100%). ¹H NMR (DMSO-*d*₆) δ 1.78–1.90 (m, 2H), 2.02–2.08 (m, 2H), 2.22–2.30 (m, 2H), 2.52–2.62 (m, 2H), 3.01–3.08 (m, 1H), 6.92 (d, *J* = 8.42 Hz, 1H), 7.47 (dd, *J* = 1.47, 8.42 Hz, 1H), 7.71 (d, *J* = 1.47 Hz, 1H), 11.2 (bs, 1H), 14.0 (bs, 1H). ¹³C NMR (DMSO-*d*₆) δ 33.6, 40.8, 40.9, 112.8, 117.4, 128.1, 134.5, 136.2, 159.9, 172.2, 210.4. **Step 3.** To a solution of **41** (6.54 g, 27.9 mmol) in MeOH (200 mL) was added acetyl chloride (2 mL) dropwise at 0 °C. Then, the reaction mixture was heated to reflux for 4 h. Solvents were then removed in vacuo, and the residue was dissolved in DCM (100 mL), washed with water (3 × 25 mL), and dried over anhydrous MgSO₄. After filtration, the solvent was removed in vacuo to afford methyl 2-hydroxy-5-(4-oxocyclohexyl)benzoate as a viscous oil (6.98 g, 100%). GCMS: *m/z*: 248 [M⁺]. Successive dropwise additions of pyridine (11.28 mL, 139.6 mmol) and acetic anhydride (13.2 mL, 139.6 mmol) were made to a solution of methyl 2-hydroxy-5-(4-oxocyclohexyl)benzoate (6.98 g, 28.2 mmol) in DCM (50 mL) at 0 °C. The reaction mixture was stirred at rt for 5 h before solvent removal in vacuo. The residue was cooled to 0 °C to obtain a solid that was filtered, washed with water (3 × 25 mL), and dried to afford methyl 2-acetoxy-5-(4-oxocyclohexyl)benzoate (**42**) as a colorless solid (8.0 g, 99%). GCMS: *m/z*: 290 [M⁺], ¹H NMR (CDCl₃) δ 1.80–1.90 (m, 2H), 2.02–2.08 (m, 2H), 2.26 (s, 3H), 2.38–2.46 (m, 4H), 2.96–3.04 (m, 1H), 3.79 (s, 3H), 6.98 (d, *J* = 8.30 Hz, 1H), 7.35 (dd, *J* = 1.95, 8.30 Hz, 1H), 7.81 (d, *J* = 1.95 Hz, 1H). **Step 4.** A solution of **42** (5.89 g, 20.29 mmol) and *O*-methyl-2-adamantanone oxime (**43**)³¹ (5.45 g, 30.44 mmol) in cyclohexane (320 mL) and DCM (80 mL) at 0 °C was treated with ozone according to the method of Dong et al.⁴² Solvents were removed in vacuo, and the residue was triturated with 95% aq. EtOH to obtain a solid that was filtered, washed with 95% aq. EtOH, and dried to afford methyl 2-acetoxy-5-(dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)benzoate (**44**) as a colorless solid (6.5 g, 70%). Mp 132–134 °C. ¹H NMR (CDCl₃) δ 1.68–2.08 (m, 22H), 2.34 (s, 3H), 2.56–2.62 (m, 1H), 3.86 (s, 3H), 7.01 (d, *J* = 8.30 Hz, 1H), 7.38 (dd, *J* = 2.44 & 8.30 Hz, 1H), 7.85 (d, *J* = 1.95 Hz, 1H). **Step 5.** To a solution of **44** (2.0 g, 4.39

mmol) in 4:4:1 H₂O/MeOH/THF (45 mL) was added solid KOH (2.46 g, 43.9 mmol) at rt. After the addition, the reaction mixture was stirred at 50 °C for 4 h. After cooling to rt, the reaction mixture was concentrated to 10 mL in vacuo, diluted with water (50 mL), and neutralized (pH = ~7) with 10% aq. HCl at 0 °C to obtain a solid that was filtered, washed with water, and dried to afford **5** (1.8 g, 100%) as a colorless solid. Mp 159–161 °C. ¹H NMR (DMSO-*d*₆) δ 1.46–1.56 (m, 2H), 1.64–1.96 (m, 20H), 2.43–2.52 (m, 1H), 6.55 (d, *J* = 8.30 Hz, 1H), 6.99 (dd, *J* = 2.44, 8.30 Hz, 1H), 7.52 (d, *J* = 1.95 Hz, 1H). ¹³C NMR (DMSO-*d*₆) δ 26.0, 26.5, 31.7, 34.4, 34.5, 36.0, 36.3, 41.1, 108.5, 110.7, 115.8, 120.1, 127.8, 129.2, 133.4, 161.2, 171.8. Anal. calcd for C₂₃H₂₇NaO₆ H₂CO₃: C, 59.50; H, 6.03. Found: C, 60.09; H, 5.77.

cis-Adamantane-2-spiro-3'-8'-[3-carboxy-4-(carboxymethoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane (6)—Step 1.

A mixture of **5** (200 mg, 0.50 mmol), ethyl bromoacetate (167 mg, 1.00 mmol), and potassium carbonate (208 mg, 1.50 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. A solid was filtered and washed with acetone, and the filtrate was concentrated in vacuo to afford cis-adamantane-2-spiro-3'-8'-[3-chloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as a colorless oil (250 mg, 87%). ¹H NMR (CDCl₃) δ 1.28 (m, 6H), 1.77–1.65 (m, 8H), 1.87–1.77 (m, 6H), 1.93 (d, *J* = 12.9 Hz, 2H), 2.07–1.97 (m, 6H), 2.54 (t, *J* = 12.3, 1H), 4.25 (m, 4H), 4.69 (s, 2H), 4.81 (s, 2H), 6.85 (d, *J* = 8.6 Hz, 1H), 7.30 (d, *J* = 8.4, 1H), 7.78 (s, 1H). ¹³C NMR (CDCl₃) δ 14.1, 26.5, 26.9, 31.4, 34.6, 34.8, 36.4, 36.8, 41.88, 61.0, 61.2, 61.3, 67.0, 108.2, 111.4, 114.9, 119.9, 130.6, 131.9, 139.5, 156.4, 164.8, 167.8, 168.6. **Step 2.** A mixture of cis-adamantane-2-spiro-3'-8'-[3-chloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (250 mg, 0.44 mmol) and 1 M aq. NaOH (2 mL) in THF (10 mL) was stirred at rt for 12 h. The solvent was removed in vacuo, and the residue was diluted with H₂O (10 mL) and acidified with AcOH (0.2 mL). The resultant precipitate was collected by filtration, washed with cold water, and dried in vacuo at 50 °C to afford **6** as a white solid (190 mg, 95%). Mp 164–165 °C. ¹H NMR (DMSO-*d*₆) δ 1.51 (q, *J* = 13.0, 2H), 1.98–1.62 (m, 20H), 2.59 (t, *J* = 12.3, 1H), 4.61 (s, 2H), 7.4 (d, *J* = 8.6 Hz, 1H), 7.28 (d, *J* = 8.2 Hz, 1H), 7.39 (s, 1H). ¹³C NMR (DMSO-*d*₆) δ 26.3, 26.7, 31.6, 34.5, 34.7, 36.2, 36.6, 40.9, 68.4, 108.5, 111.1, 116.2, 124.2, 128.9, 131.0, 139.4, 155.5, 168.3, 171.7. Anal. calcd for C₂₅H₃₀O₈ H₂O: C, 63.01; H, 6.77. Found: C, 63.38; H, 6.58. HRMS (ESI-TOF) *m/z*: [M + Na]⁺ calcd for C₂₅H₃₀O₈Na 481.1838; found 481.1838.

cis-Adamantane-2-spiro-3'-8'-[3-chloro-4-(carboxymethoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane (7)—Step 1.

A solution of *N*-chlorosuccinimide (1.25 g, 9.36 mmol) in dry DMF (10 mL) was added to a solution of **26**²⁷ (3.32 g, 9.31 mmol) in dry DMF (30 mL) and stirred under nitrogen at rt for 24 h protected from light. The mixture was poured into ice water and then extracted with EtOAc (2 × 50 mL). The combined organic layers were washed with water (100 mL) and brine (25 mL), dried with MgSO₄, and concentrated in vacuo to give a semisolid that was purified by chromatography (sg, 9:1 hexane:ethyl acetate) to afford 2-chloro-(4-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenol (**35**) (2.80 g, 77%) as a white solid. Mp 119–121 °C. ¹H NMR (CDCl₃) δ 1.62–2.01 (m, 22H), 2.44–2.50 (m, 1H), 5.41 (s, 1H), 6.92 (d, *J* = 8.0 Hz, 1H), 7.1 (dd, *J* = 8.0, 2.0 Hz, 1H), 7.15 (d, *J* = 2.0 Hz, 1H). ¹³C NMR (CDCl₃) δ 26.7, 27.1, 31.5,

31.7, 34.7, 34.8, 34.99, 35.00, 36.6, 37.0, 42.1, 108.4, 111.7, 116.2, 119.8, 126.8, 126.9, 127.3, 139.8, 149.7. **Step 2.** A mixture of **35** (210 mg, 0.54 mmol), ethyl bromoacetate (180 mg, 1.08 mmol), and potassium carbonate (224 mg, 1.62 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. The solid was filtered and washed with acetone, and the filtrate was concentrated in vacuo to afford *cis*-adamantane-2-spiro-3'-8'-[3-chloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**37**) as a colorless oil (0.257 g, 100%). ¹H NMR (CDCl₃) δ 7.22 (d, *J* = 2.1 Hz, 1H), 7.01 (dd, *J* = 8.5, 2.1 Hz, 1H), 6.77 (d, *J* = 8.5 Hz, 1H), 4.66 (s, 2H), 4.25 (q, *J* = 7.1 Hz, 2H), 2.48 (t, *J* = 12.1 Hz, 2H), 2.07–1.97 (m, 6H), 1.93 (d, *J* = 12.8 Hz, 2H), 1.87–1.59 (m, 15H), 1.28 (t, *J* = 7.2 Hz, 3H). ¹³C NMR (CDCl₃) δ 14.1, 26.5, 26.9, 31.4, 34.6, 34.8, 36.4, 36.8, 41.8, 61.4, 66.5, 108.2, 111.4, 114.2, 123.1, 125.5, 129.0, 140.8, 151.9, 168.5. **Step 3.** A mixture of **37** (110 mg, 0.23 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. The solvent was removed in vacuo, and the residue was diluted with H₂O (10 mL) and acidified with AcOH (0.2 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford **7** as a white solid (100 mg, 97%). Mp 145–146 °C. ¹H NMR (CDCl₃) δ 2.12–1.54 (m, 22H), 2.48 (t, *J* = 12.4 Hz, 1H), 4.67 (s, 2H), 6.79 (d, *J* = 8.4 Hz, 1H), 7.02 (d, *J* = 8.4 Hz, 1H), 7.23 (s, 1H), 8.07 (brs, 1H). ¹³C NMR (CDCl₃) δ 26.5, 26.9, 31.4, 34.6, 34.8, 36.4, 36.8, 41.9, 66.2, 108.2, 111.5, 114.4, 123.2, 125.9, 129.1, 141.3, 151.4, 173.2. Anal. calcd for C₂₄H₂₉ClO₆ H₂O: C, 61.73; H, 6.69. Found: C, 62.00; H, 6.40.

cis-Adamantane-2-spiro-3'-8'-[3,5-dichloro-4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (8**)—Step 1.** To a solution of **26**²⁷ (410 mg, 1.0 mmol) in dry DMF (5 mL) was added dropwise a solution of *N*-chlorosuccinimide (1.25 g, 9.36 mmol) in dry DMF (3 mL). The reaction mixture was stirred under nitrogen at rt for 24 h. The mixture was poured on ice and then extracted with EtOAc (2 × 20 mL). The combined organic extracts were washed with water (30 mL) and brine (20 mL), dried over anhydrous MgSO₄, and concentrated in vacuo. The crude product was purified by chromatography (sg, hexane/EA, 25:1) to afford *cis*-adamantane-2-spiro-3'-8'-[3,5-dichloro-4-hydroxyphenyl]-1',2',4'-trioxaspiro[4.5]decane (**36**) (270 mg, 63%) as a white solid. Mp 143–145 °C. ¹H NMR (400 MHz, CDCl₃) δ 1.61–1.87 (m, 14H), 1.90–2.08 (m, 8H), 2.45 (tt, *J* = 12.5, 3.2 Hz, 1H), 5.70 (s, 1H), 7.10 (s, 2H); ¹³C NMR (100 MHz, CDCl₃) δ 26.5, 26.9, 31.4, 34.5, 34.8, 36.4, 36.8, 41.8, 108.0, 111.6, 120.8, 126.6, 139.6, 146.0. **Step 2.** A mixture of **36** (270 mg, 0.63 mmol), ethyl bromoacetate (159 mg, 0.95 mmol), and potassium carbonate (175 mg, 1.26 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. The solid was removed by filtration and washed with acetone, and the filtrate was concentrated in vacuo to afford *cis*-adamantane-2-spiro-3'-8'-[3,5-dichloro-4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]-decane (**38**) (322 mg, 100%) as a white solid. Mp 99–100 °C. ¹H NMR (CDCl₃) δ 1.30 (t, *J* = 7.1 Hz, 3H), 1.58–1.69 (m, 6H), 1.691.84 (m, 8H), 1.87–1.93 (m, 2H), 1.94–2.05 (m, 6H), 2.46 (tt, *J* = 12.3, 3.3 Hz, 1H), 4.28 (q, *J* = 7.1 Hz, 2H), 4.56 (s, 2H), 7.11 (s, 2H); ¹³C NMR (CDCl₃) δ 14.1, 26.4, 26.8, 31.1, 34.4, 34.8, 36.4, 36.7, 41.9, 61.3, 69.3, 107.8, 111.5, 127.3, 128.8, 144.3, 148.4, 167.9. **Step 3.** A mixture of **38** (322 mg, 0.63 mmol) and 1 N aq. NaOH (2 mL) in THF (20 mL) was stirred at rt for 12 h. The removal of the solvents in vacuo gave a white residue, which was suspended in H₂O (10 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford **8** (270 mg, 89%) as a

white solid. Mp 142–143 °C. ¹H NMR (CDCl₃) δ 1.61–1.87 (m, 14H), 1.90–2.09 (m, 8H), 2.49 (tt, *J* = 12.3, 3.4 Hz, 1H), 4.64 (s, 2H), 7.15 (s, 2H); ¹³C NMR (CDCl₃) δ 26.5, 26.9, 31.2, 34.4, 34.8, 36.4, 36.8, 42.0, 68.9, 107.9, 111.6, 127.4, 128.7, 144.8, 148.0, 171.2. Anal. calcd for C₂₄H₂₈Cl₂O₆: C, 59.63; H, 5.84. Found: C, 59.89; H, 6.00.

cis-Adamantane-2-spiro-3'-8'-[4-(carboxypropoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane (9)—To a solution of **26**²⁷ (250 mg, 0.70 mmol) in dry dimethoxyethane (25 mL) were added powdered NaOH (168 mg, 4.20 mmol) and Bu₄NHSO₄ (48 mg, 0.15 mmol), and the resulting mixture was stirred at rt for 30 min before addition of ethyl 4-bromobutanoate (205 mg, 1.05 mmol). The reaction mixture was stirred at 60 °C for 12 h, then concentrated in vacuo. The residue was suspended in H₂O (20 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with H₂O, and dried in vacuo at 50 °C to afford **9** (240 mg, 77%) as a white solid. Mp 148–149 °C. ¹H NMR (CDCl₃) δ 1.87–1.57 (m, 14H), 1.94 (d, *J* = 12.8 Hz, 2H), 2.06–1.97 (m, 6H), 2.09 (p, *J* = 6.7 Hz, 2H), 2.49 (tt, *J* = 12.1, 3.3 Hz, 1H), 2.57 (t, *J* = 7.3 Hz, 2H), 3.98 (t, *J* = 6.1 Hz, 2H), 6.81 (d, *J* = 8.3 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H); ¹³C NMR (CDCl₃) δ 24.5, 26.5, 26.9, 30.6, 31.7, 34.77, 34.83, 36.4, 36.8, 42.1, 66.5, 108.5, 111.4, 114.4, 127.6, 138.5, 157.1, 179.2. Anal. calcd for C₂₆H₃₄O₆: C, 70.56; H, 7.74. Found: C, 70.70; H, 7.75.

cis-Adamantane-2-spiro-3'-8'-[4-(caroxybutoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (10)—To a solution of **26**²⁷ (250 mg, 0.70 mmol) in dry dimethoxyethane (25 mL) were added powdered NaOH (168 mg, 4.20 mmol) and Bu₄NHSO₄ (48 mg, 0.15 mmol), and the resulting mixture was stirred at rt for 30 min before the addition of methyl 5-bromopentanoate (220 mg, 1.05 mmol). The reaction mixture was stirred at 60 °C for 12 h, then concentrated in vacuo. The residue was suspended in H₂O (20 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with H₂O, and dried in vacuo at 50 °C to afford **10** (230 mg, 72%) as a white solid. Mp 144–145 °C. ¹H NMR (CDCl₃) δ 1.87–1.61 (m, 18H), 1.94 (d, *J* = 12.8 Hz, 2H), 2.06–1.97 (m, 6H), 2.43 (d, *J* = 6.9 Hz, 2H), 2.48 (tt, *J* = 15.7, 5.0 Hz, 1H), 3.93 (t, *J* = 5.3 Hz, 2H), 6.80 (d, *J* = 8.4 Hz, 2H), 7.10 (d, *J* = 8.4 Hz, 2H); ¹³C NMR (CDCl₃) δ 21.5, 26.5, 26.9, 28.7, 31.7, 33.8, 34.77, 34.83, 36.4, 36.8, 42.1, 67.3, 108.5, 111.4, 114.4, 127.6, 138.3, 157.3, 179.3. Anal. calcd for C₂₇H₃₆O₆: C, 71.03; H, 7.95. Found: C, 70.84; H, 7.78.

cis-Adamantane-2-spiro-3'-8'-[4-[2-[methylsulfonamino]-2-oxoethoxy]phenyl]-1',2',4'-trioxaspiro[4.5]decane (13)—To a mixture of **2** (150 mg, 0.36 mmol), methanesulfonamide (47 mg, 0.40 mmol), and DMAP (10 mg, 0.07 mmol) in DCM (20 mL) was added DCC (82 mg, 0.40 mmol). The resulting mixture was stirred at rt for 24 h. The solid was filtered, and the filtrate was concentrated in vacuo to produce a residue that was purified by chromatography (sg, DCM) to afford **13** (110 mg, 62%) as a white solid. Mp 148–149 °C; ¹H NMR (CDCl₃) δ 2.08–1.68 (m, 22H), 2.55 (td, *J*₁ = 12 Hz, *J*₂ = 3 Hz), 3.38 (s, 3H), 4.85 (s, 2H), 6.88 (d, *J* = 8.5 Hz, 2H), 7.21 (d, *J* = 8.5 Hz, 2H), 8.84 (br, 1H); ¹³C NMR (CDCl₃) δ 26.5, 26.9, 31.6, 34.7, 41.7, 42.1, 67.4, 108.3, 111.5, 114.7, 128.2, 140.9, 154.8, 167.7. Anal. calcd for C₂₅H₃₃NO₇S 0.25 H₂O: C, 60.53; H, 6.81; N, 2.82. Found: C, 60.87; H, 6.86; N, 2.84.

cis-Adamantane-2-spiro-3'-8'-[4-[2-[(carboxymethyl)amino]-2-oxoethoxy]phenyl]-1',2',4'-trioxaspiro[4.5]decane (14)—Step 1. To a mixture of **2** (415 mg, 1.0 mmol) and HOBt (162 mg, 1.2 mmol) in DMF (10 mL) was added EDC (230 mg, 1.2 mmol) followed by the addition of glycine ethyl ester hydrochloride (154 mg, 1.1 mmol) and DIPEA (155 mg, 1.2 mmol). The resulting mixture was stirred at rt for 12 h; diluted with EA (500 mL); washed with brine (2 × 50 mL), 1 N HCl (20 mL), 1 N NaOH (20 mL), and brine (20 mL); dried over anhydrous MgSO₄; filtered; and concentrated in vacuo to give a colorless residue that was dissolved in a mixture of ethanol (40 mL) and 2% aq. NaOH (4 mL). The resulting mixture was stirred at rt for 12 h and then concentrated in vacuo to afford a white residue that was suspended in H₂O (50 mL), acidified with AcOH to pH 3, filtered, washed with H₂O, and dried in vacuo to afford **14** (430 mg, 91%) as a white solid. Mp 147–148 °C; ¹H NMR (DMSO-*d*₆) δ 1.50–1.58 (m, 2H), 1.66–1.93 (m, 19H), 2.59 (t, *J*=15 Hz, 1H), 3.81 (d, *J*= 7.2 Hz, 2H), 4.49 (s, 2H), 6.91 (d, *J*= 11.2 Hz, 2H), 7.15 (d, *J*= 11.2 Hz, 2H), 8.35 (t, *J*= 7.2 Hz, 1H), 12.63 (bs, 1H); ¹³C NMR (DMSO-*d*₆) δ 26.3, 26.7, 31.7, 34.6, 34.7, 36.3, 36.6, 40.9, 41.2, 67.4, 108.6, 111.0, 115.2, 127.9, 139.2, 156.5, 168.7, 171.4. Anal. calcd for C₂₆H₃₃N₃O₇: C, 66.23; H, 7.05; N, 2.97. Found: C, 66.40; H, 6.96; N, 2.60.

Sodium 1-(2-(4-Dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)ethyl)piperidine-4-carboxylate (15)—Step 1. To a solution of cis-adamantane-2-spiro-3'-8'-(4'-acetoxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**27**)²⁷ (430 mg, 1.1 mmol) in dry acetonitrile (20 mL) were added powdered NaOH (259 mg, 6.5 mmol) and tetrabutylammonium hydrogensulfate (110 mg, 0.3 mmol) according to the method of Charman et al.²⁵ The mixture was stirred at rt for 0.5 h. Then, ethyl 1-(2-chloroethyl)piperidine-4-carboxylate (**28**)⁴³ (475 mg, 2.2 mmol) was added and the reaction stirred at 60 °C for 12 h. The inorganic solid was filtered off and washed with CH₂O₂. After removal of the solvents in vacuo, the residue was dissolved in EtOAc (50 mL). The organic layer was washed with water and brine and dried over MgSO₄. The removal of the solvent in vacuo afforded ethyl 1-(2-(4-dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)ethyl)piperidine-4-carboxylate (**29**) (260 mg, 45%) as a white solid. Mp 70–72 °C; ¹H NMR (CDCl₃) δ 1.25 (t, *J*= 7.3 Hz, 3H), 1.69–2.04 (m, 26H), 2.15–2.19 (m, 2H), 2.26–2.30 (m, 1H), 2.46–2.51 (m, 1H), 2.77 (t, *J*= 5.9 Hz, 3H), 2.95 (brs, 2H), 4.07 (t, *J*= 5.9 Hz, 2H), 4.13 (q, *J*= 7.3 Hz, *J*= 14.2 Hz, 2H), 6.83 (d, *J*= 8.3 Hz, 2H), 7.10 (d, *J*= 8.3 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 26.5, 26.9, 28.2, 31.6, 34.7, 34.8, 36.4, 36.8, 41.0, 42.0, 53.4, 57.4, 60.3, 66.0, 108.4, 111.3, 114.4, 127.6, 138.5, 157.1, 175.0. **Step 2.** To a solution of **29** (223 mg, 0.41 mmol) in EtOH (5 mL) was added powdered NaOH (50 mg, 1.2 mmol), and the reaction mixture was stirred for 24 h at rt. Filtration afforded **15** (190 mg, 86%) as a white solid. Mp 165–167 °C; ¹H NMR (CD₃OD) δ 1.66–2.23 (m, 29H), 2.50–2.55 (m, 1H), 2.77 (t, *J*= 5.5 Hz, 3H), 3.01 (brs, 2H), 4.09 (t, *J*= 5.5 Hz, 2H), 6.84 (d, *J*= 8.5 Hz, 2H), 7.21 (d, *J*= 8.5 Hz, 2H); ¹³C NMR (CD₃OD) δ 28.2, 28.6, 30.4, 33.0, 36.0, 38.0, 38.1, 43.4, 45.6, 55.3, 58.7, 66.7, 109.8, 112.4, 115.7, 128.8, 140.0, 158.7, 183.9. HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₃₀H₄₂N₃O₆ 512.3012; found 512.3026.

2-(4-(4-dispiro[adamantane-2,3'-[1,2,4]trioxoiane-5',1''-cyclohexan]-4''-yl)phenoxy)piperidin-1-yl)acetic acid (16)—**Step 1.** To a solution of *cis*-adamantane-2-spiro-3-8-[4-(4-piperidinyloxy)phenyl]-1,2,4-trioxaspiro[4.5]decane (**30**)²⁵ (0.30 g, 0.68 mmol) and ethyl 2-bromoacetate (0.14 g, 0.84 mmol) in THF (12 mL) was added a solution of potassium carbonate (0.09 g, 0.65 mmol) in water (1 mL). The reaction mixture was stirred at rt for 12 h. After solvent removal in vacuo, the crude product was purified by crystallization from 4:1 EtOH/H₂O to afford ethyl 2-(4-(4-(dispiro[adamantane-2,3'-[1,2,4]trioxolane-5',1''-cyclohexan]-4''-yl)phenoxy)piperidin-1-yl)acetate (**31**) as a colorless solid (0.25 g, 69%). Mp 106–107 °C; ¹H NMR (CDCl₃) δ 1.28 (t, *J* = 7.0 Hz, 3H), 1.64–2.08 (m, 26H), 2.44–2.56 (m, 3H), 2.76–2.85 (m, 2H), 3.24 (s, 2H), 4.19 (q, *J* = 7.0 Hz, 2H), 4.26–4.34 (m, 1H), 6.82 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 26.5, 26.9, 30.6, 31.6, 34.7, 34.8, 36.4, 36.8, 42.0, 50.3, 59.6, 60.6, 71.9, 108.4, 111.4, 116.0, 127.7, 138.5, 155.7, 170.5. **Step 2.** To a solution of **31** (0.53 g, 1.0 mmol) in EtOH (20 mL) was added 1 N aq. NaOH (2 mL). The resulting mixture was stirred at 50 °C for 20 h. The reaction mixture was then diluted with water (5 mL) and AcOH (5 mL). The precipitate was filtered, washed with water, and dried in vacuo at 40 °C to afford **16** (0.42 g, 84%) as a colorless solid. Mp 171–172 °C. ¹H NMR (DMSO-*d*₆) δ 1.42–1.60 (m, 2H), 1.61–2.10 (m, 24H), 2.44–2.56 (m, 1H), 2.68–2.82 (m, 2H), 2.90–3.08 (m, 2H), 3.22 (s, 2H), 4.32–4.46 (m, 1H), 6.88 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 26.3, 26.7, 30.7, 31.7, 34.6, 34.7, 36.3, 36.6, 41.2, 48.3, 55.4, 71.0, 108.6, 111.0, 116.4, 128.1, 139.2, 155.2, 167.7, HRMS (ESI-TOF) *m/z*: [M + H]⁺ calcd for C₂₉H₄₀NO₆ 498.2856; found 498.2854.

cis-Adamantane-2-spiro-3'-8'-[4-[[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (17)—**Step 1.** A mixture of *cis*-adamantane-2-spiro-3'-8'-[4-(chloromethyl)-phenyl]-1',2',4'-trioxaspiro[4.5]decane (**32**)²⁸ (0.30 g, 0.77 mmol), glycine ethyl ester hydrochloride (1.30 g, 9.32 mmol), and DIPEA (1.50 g, 11.65 mmol) in DMA (20 mL) was stirred at 50 °C for 5 h. The reaction mixture was then cooled to rt, diluted with EA (100 mL), and washed successively with H₂O (50 mL), 1 M HCl (15 mL), and brine (2 × 50 mL). The organic layer was dried over MgSO₄, filtered, concentrated, and dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-[[[(2-ethoxy-2-oxoethyl)amino]methyl]-phenyl]-1',2',4'-trioxaspiro[4.5]decane (**33**) (340 mg, 97%) as a light yellow solid. Mp 77–78 °C. ¹H NMR (CDCl₃) δ 1.27 (t, *J* = 7.0 Hz, 3H), 1.69–2.05 (m, 22H), 2.53 (t, *J* = 12.0 Hz, 1H), 1.89 (s, 2H), 3.40 (s, 2H), 4.18 (q, *J* = 7.0 Hz, 2H), 7.16 (d, *J* = 8.0 Hz, 2H), 7.24 (d, *J* = 8.0 Hz); ¹³C NMR (CDCl₃) δ 14.3, 26.5, 26.9, 31.5, 34.7, 42.6, 50.2, 53.0, 60.7, 108.4, 111.4, 126.9, 128.4, 137.4, 145.1, 172.5. **Step 2.** A mixture of **33** (340 mg, 0.75 mmol) and 1 M aq. NaOH (1 mL) in THF (20 mL) was stirred at rt for 12 h. After removal of the solvents in vacuo, the residue was suspended in H₂O (20 mL) and acidified with AcOH (0.2 mL). The resultant precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-[[[(carboxymethyl)amino]methyl]-phenyl]-1',2',4'-trioxaspiro[4.5]decane (290 mg, 90%) as a white solid. To a suspension of *cis*-adamantane-2-spiro-3'-8'-[4-[[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]-decane (230 mg, 0.54 mmol) in Et₂O (10 mL) was added methanesulfonic acid (150 mg, 1.56 mmol) in Et₂O (2 mL), and the resulting mixture was stirred at rt for 1 h.

The precipitate was then filtered, washed with Et₂O, and dried in vacuo at 50 °C to afford **17** as a white solid (240 mg, 85%). Mp 142–143 °C. ¹H NMR (DMSO-*d*₆) δ 1.55–1.93 (m, 22H), 2.30 (s, 3H), 2.65 (t, *J* = 12.0 Hz, 1H), 3.85 (s, 2H), 4.12 (s, 2H), 7.29 (d, *J* = 8.0 Hz, 2H), 7.40 (d, *J* = 8.0 Hz, 2H), 9.24 (br, 2H), 13.79 (br, 1H); ¹³C NMR (DMSO-*d*₆) δ 26.3, 26.7, 31.4, 34.5, 34.7, 36.3, 36.6, 41.7, 46.9, 50.2, 108.5, 111.1, 127.4, 129.7, 130.7, 147.5, 168.4. Anal. calcd for C₂₆H₃₇NO₈S: C, 59.64; H, 7.12; N, 2.67. Found: C, 59.26; H, 6.90; N, 2.41.

cis-Adamantane-2-spiro-3'-8'-[4-((3-(carbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (18)—Step 1. A mixture of **32** (0.22 g, 0.57 mmol), ethyl azetidine-3-carboxylate hydrochloride (1.02 g, 6.17 mmol), and DIPEA (0.99 g, 7.65 mmol) in DMA (15 mL) was stirred at 50 °C for 5 h. Then, the reaction mixture was cooled to rt, diluted with EA (100 mL), and washed successively with H₂O (50 mL), 1 M HCl (15 mL), and brine (2 × 50 mL). The organic layer was dried over MgSO₄, filtered, and concentrated. The obtained residue was dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-((3-(ethoxycarbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**34**) (270 mg, 99%) as a white solid. Mp 144–145 °C. ¹H NMR (CDCl₃) δ 1.64–1.76 (m, 8H), 1.76–1.86 (m, 6H), 1.92 (d, *J* = 12.9 Hz, 2H), 1.96–2.5 (m, 6H), 2.51 (tt, *J* = 12.0, 3.2 Hz, 1H), 3.34 (d, *J* = 3.8 Hz, 2H), 3.60 (d, *J* = 11.2 Hz, 4H), 4.14 (qd, *J* = 7.1, 1.8 Hz, 2H), 7.14 (t, *J* = 4.7 Hz, 2H), 7.19 (d, *J* = 7.6 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 26.5, 26.9, 31.4, 33.9, 34.7, 34.8, 36.4, 36.8, 42.6, 56.6, 60.9, 62.7, 108.4, 111.4, 126.9, 128.7, 134.6, 145.4, 172.9. **Step 2.** A mixture of **34** (270 mg, 0.56 mmol) and 1 M aq. NaOH (2 mL) in THF (15 mL) was stirred at rt for 12 h and then concentrated in vacuo. The residue was diluted with H₂O (20 mL) and acidified with AcOH to pH 5. The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford *cis*-adamantane-2-spiro-3'-8'-[4-((3-(carbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]-decane (250 mg, 98%) as a white solid. To a stirred solution of *cis*-adamantane-2-spiro-3'-8'-[4-((3-(carbonyl)azetidin-1-yl)methyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (250 mg, 0.55 mmol) in EA (10 mL) as added dropwise a solution of methanesulfonic acid (106 mg, 1.10 mmol) in EA (2 mL). The resulting mixture was stirred at rt for 1 h. The precipitate was filtered, washed with Et₂O, and dried in vacuo at 50 °C to afford **18** as a white solid (262 mg, 86%). Mp 151–152 °C. ¹H NMR (DMSO-*d*₆) δ 1.57 (qd, *J* = 13.0, 4.1 Hz, 2H), 1.62–1.96 (m, 20H), 2.34 (s, 3H), 2.64 (tt, *J* = 12.2, 3.5 Hz, 1H), 3.62 (q, *J* = 9.1 Hz, 1H), 4.08–4.28 (m, 4H), 4.34 (d, *J* = 17.8 Hz, 2H), 7.29 (d, *J* = 7.8 Hz, 2H), 7.39 (d, *J* = 7.9 Hz, 2H), 10.16 (s, 1H), 13.12 (brs, 1H); ¹³C NMR (DMSO-*d*₆) δ 26.3, 26.7, 31.4, 32.5, 34.5, 34.7, 36.3, 36.6, 41.7, 55.2, 55.5, 57.3, 108.5, 111.1, 127.7, 128.6, 130.6, 147.7, 171.8. Anal. calcd for C₂₈H₃₉NO₈S: C, 61.18; H, 7.15; N, 2.55. Found: C, 61.00; H, 7.30; N, 2.37.

cis-6,6-Difluoroadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane (19)—Step 1. A mixture of 4-(4-hydroxyphenyl)cyclohexanone (**45**) (5.71 g, 30 mmol), ethyl bromoacetate (6.01 g, 36 mmol), and K₂CO₃ (8.29 g, 60 mmol) in acetone (150 mL) was stirred at reflux for 12 h. The solid was removed by filtration, and the filtrate was concentrated in vacuo to afford ethyl 2-(4-(4-oxocyclohexyl)phenoxy)acetate (**46**) as a light yellow oil (8.29 g, 100%). ¹H

NMR (CDCl₃) δ 1.30 (t, J = 7.0 Hz, 3H), 1.90 (dt, J_1 = 12.5 Hz, J_2 = 6.0 Hz, 2H), 2.19–2.22 (m, 2H), 2.48–2.53 (m, 4H), 2.98 (tt, J_1 = 9.0 Hz, J_2 = 3.0 Hz, 1H), 4.27 (q, J = 7.0 Hz, 2H), 4.60 (s, 2H), 6.87 (d, J = 8.5 Hz, 2H), 7.17 (d, J = 8.5 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 34.1, 41.4, 41.9, 61.4, 65.6, 114.8, 127.7, 138.0, 156.5, 169.0, 211.1. **Step 2.** A mixture of **46** (0.51 g, 1.86 mmol) and 6,6-difluoroadamantan-2-one O-methyl oxime (**47**)²⁸ (0.40 g, 1.86 mmol) in cyclohexane (100 mL) and DCM (40 mL) was treated with ozone according to the method of Dong et al.⁴¹ After removal of the solvents in vacuo, the residue was recrystallized from MeOH to afford cis-6,6-difluoroadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**48**) (0.13 g, 15%) as a white solid. Mp 112–113 °C. ¹H NMR (CDCl₃) δ 1.28 (t, J = 7.0 Hz, 3H), 1.68 (m, 2H), 1.79–2.06 (m, 18H), 2.50 (t, J = 12.5 Hz, 1H), 4.27 (q, J = 7.0 Hz, 3H), 4.59 (s, 2H), 6.83 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 30.7 (t, ³ J = 3.5 Hz), 30.9 (t, ³ J = 3.5 Hz), 34.2 (t, ² J = 34.3 Hz), 34.4, 34.7, 42.2, 61.3, 65.6, 108.9, 109.5, 114.6, 124.0 (¹ J = 121.7 Hz), 127.8, 139.2, 156.3, 169.0. **Step 3.** A mixture of **48** (120 mg, 0.25 mmol) and 15% aq. KOH (0.5 mL) in THF (10 mL) was stirred at rt for 12 h. The removal of the solvents in vacuo gave a white residue which was suspended in H₂O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford **19** (105 mg, 93%) as a white solid. Mp 155–156 °C. ¹H NMR (CD₃OD) δ 1.65 (qd, J_1 = 13.0 Hz, J_2 = 3.0 Hz, 2H), 1.81–2.10 (m, 18H), 2.56 (t, J = 12.0 Hz, 1H), 4.48 (s, 2H), 6.86 (d, J = 8.5 Hz, 2H), 7.13 (d, J = 8.5 Hz, 2H); ¹³C NMR (CD₃OD) δ 30.36 (t, ³ J = 3.9 Hz), 30.42 (t, ³ J = 3.9 Hz), 31.4, 34.2, 34.3 (t, ² J = 21.9 Hz), 34.7 (t, ³ J = 21.9 Hz), 34.8, 41.8, 53.4, 66.0, 108.77, 108.80, 114.2, 123.7 (t, ¹ J = 150.8 Hz), 127.1, 138.6, 156.9, 173.6. Anal. calcd for C₂₄H₂₈F₂O₆: C, 63.99; H, 6.27. Found: C, 63.81; H, 6.07.

cis-6-Oxadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane (20)—Step 1. To a solution of adamantane-2,6-dione mono ethylene ketal (**49**)³² (3.90 g, 18.72 mmol) in dry EtOH (100 mL) was added pyridine (2.45 g, 30.90 mmol) followed by methoxyamine hydrochloride (2.40 g, 28.09 mmol). The reaction mixture was stirred at rt for 12 h, concentrated in vacuo, and partitioned between DCM (100 mL) and H₂O (50 mL). The organic phase was separated, and the aq. layer was extracted with DCM (3 × 30 mL). The organic layers were combined and washed with 1 M HCl (30 mL), sat. aq. NaHCO₃ (30 mL) and brine (30 mL), dried over MgSO₄, and concentrated to afford adamantane-2,6-dione mono ethylene ketal O-methyl oxime (**50**) (4.27 g, 96%) as a white solid. Mp 90–91 °C. ¹H NMR (CDCl₃) δ 1.74 (d, J = 12.5 Hz, 2H), 1.80 (d, J = 12.5 Hz, 2H), 1.91 (s, 2H), 2.47 (s, 2H), 3.41 (s, 2H), 3.82 (s, 3H), 3.99 (s, 4H); ¹³C NMR (CDCl₃): δ 27.9, 34.5, 34.7, 35.8, 36.3, 61.0, 64.4, 109.8, 165.2. **Step 2.** A mixture of **50** (4.24 g, 17.91 mmol) and 4-(4-oxocyclohexyl)phenyl acetate (**51**) (4.16 g, 17.91 mmol) in cyclohexane (450 mL) and DCM (150 mL) was treated with ozone according to the method of Dong et al.⁴² After solvent removal in vacuo, the residue was recrystallized from EtOH to afford cis-6-oxadamantane-2-spiro-3'-8'-(4-acetoxyphenyl)-1',2',4'-trioxaspiro[4.5]decane ethylene ketal (**52**) (3.35 g, 43%) as a white solid. Mp 156–157 °C. ¹H NMR (CDCl₃) δ 1.66–2.05 (m, 20H), 2.28 (s, 3H), 2.54 (t, J = 12.0 Hz, 1H), 3.94 (s, 4), 7.21 (d, J = 8.5 Hz, 2H); ¹³C NMR (CDCl₃): δ 21.1, 31.5, 31.6, 34.6, 34.8, 35.1, 35.3, 42.4, 64.3, 108.5, 109.9, 110.3, 121.4, 127.7, 143.6, 148.9, 169.7. **Step 3.** A mixture of **52** (3.35 g, 7.34 mmol), 1 M NaOH (10 mL) and THF (100 mL) was

stirred at rt overnight, then concentrated in vacuo. The residue was suspended in H₂O (30 mL) and acidified with AcOH (2 mL). The resulting precipitate was filtered, washed with water, and dried in vacuo to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane ethylene ketal (2.97 g, 98%) as a white solid. Mp 164–165 °C. ¹H NMR (500 MHz, CDCl₃) δ 1.64–2.04 (m, 20H), 2.48 (t, *J* = 12.0 Hz, 1H), 3.95 (s, 4H), 4.98 (brs, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (125 MHz, CDCl₃) δ 31.6, 34.7, 34.8, 35.1, 35.3, 42.0, 64.3, 108.6, 110.0, 110.2, 115.2, 127.8, 138.4, 153.9. **Step 4.** A suspension of *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane ethylene ketal (3.10 g, 7.48 mmol) in 1 M methanesulfonic acid in 5:1 acetone/H₂O (42 mL) was stirred at rt for 12 h. The resulting solid was filtered, washed with water, and dried in vacuo to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**53**) (2.52 g, 91%) as a white solid. Mp 187–188 °C. ¹H NMR (DMSO-*d*₆) δ 1.52 (q, *J* = 12.0 Hz, 2H), 1.75 (d, *J* = 11.5 Hz, 2H), 1.86 (d, *J* = 12.0 Hz, 2H), 1.95 (s, 4H), 2.7 (s, 4H), 2.18 (d, *J* = 12.0 Hz, 2H), 2.33 (d, *J* = 16.0 Hz, 2H), 2.51 (s, 1H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.99 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (DMSO-*d*₆) δ 31.8, 34.5, 35.50, 35.53, 35.7, 41.2, 44.5, 45.0, 109.2, 109.4, 115.5, 127.8, 136.5, 156.0, 214.6. **Step 5.** A mixture of **53** (600 mg, 1.62 mmol), ethyl bromoacetate (325 mg, 1.94 mmol), and potassium carbonate (447 mg, 3.24 mmol) in acetone (30 mL) was stirred at 55 °C for 12 h. After filtering and washing with acetone, the filtrate was concentrated in vacuo to afford *cis*-6-oxoadamantane-2-spiro-3'-8'-(4-(2-ethoxy-2-oxoethoxy)phenyl)-1',2',4'-trioxaspiro[4.5]decane (**54**) as a white solid (740 mg, 100%). Mp 138–139 °C. ¹H NMR (CDCl₃) δ 1.30 (t, *J* = 7.0 Hz, 3H), 1.68–1.75 (m, 2H), 1.84–1.90 (m, 4H), 1.92 (d, *J* = 13.0 Hz, 2H), 1.99 (d, *J* = 13.0 Hz, 2H), 2.07 (d, *J* = 13.0 Hz, 2H), 2.15 (s, 2H), 2.27 (d, *J* = 13.0 Hz, 2H), 2.35 (d, *J* = 12.5 Hz, 2H), 2.48 (d, *J* = 16.0 Hz, 2H), 2.52 (t, *J* = 12.0 Hz, 2H), 4.27 (q, *J* = 7.0 Hz, 2H), 4.60 (s, 2H), 6.84 (d, *J* = 8.5 Hz, 2H), 7.12 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 31.5, 34.6, 35.68, 35.74, 35.9, 42.0, 44.7, 45.2, 61.3, 65.6, 109.0, 109.3, 114.7, 127.7, 139.2, 156.3, 169.1, 215.9. **Step 6.** A mixture of **54** (230 mg, 0.50 mmol) and 1 M aq. NaOH (2 mL) in THF (15 mL) was stirred at rt for 12 h. Removal of the solvents in vacuo gave a white residue, which was suspended in H₂O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford **20** (205 mg, 95%) as a white solid. Mp 171–172 °C. ¹H NMR (CD₃OD) δ 1.67–1.75 (m, 2H), 1.85–1.90 (m, 4H), 1.94 (d, *J* = 13.0 Hz, 2H), 1.99 (d, *J* = 13.0 Hz, 2H), 2.7 (d, *J* = 12.5 Hz, 2H), 2.15 (s, 2H), 2.27 (d, *J* = 13.0 Hz, 2H), 2.35 (d, *J* = 12.0 Hz, 2H), 2.49 (d, *J* = 16.5 Hz, 2H), 2.53 (t, *J* = 12.5 Hz, 1H), 4.66 (s, 2H), 6.86 (d, *J* = 8.5 Hz, 2H), 7.15 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (CDCl₃) δ 31.5, 34.6, 35.7, 35.8, 35.9, 42.0, 45.1, 65.0, 109.1, 109.2, 114.7, 127.9, 139.7, 155.8, 172.8, 216.3. Anal. calcd for C₂₄H₂₈O₇: C, 67.28; H, 6.59. Found: C, 67.54; H, 6.36.

***cis*-6-Hydroxyadamantane-2-spiro-3'-8'-(4-(carboxymethoxy)phenyl)-1',2',4'-trioxaspiro[4.5]decane (**21**)**—**Step 1.** To a solution of **53** (100 mg, 0.27 mmol) in methanol (10 mL) at 0 °C was added NaBH₄ (17 mg, 0.68 mmol). The resulting mixture was stirred at 0 °C for 2 h and then quenched with H₂O (1 mL). After solvent removal in vacuo, the residue was extracted in EA (20 mL) and washed with saturated NaHCO₃ (10 mL brine (10 mL)). The organic layer was dried over MgSO₄, filtered, and concentrated to afford *cis*-6-hydroxyadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane

(55) (99 mg, 99%) as a white solid. Mp 159–160 °C. ¹H NMR (DMSO-*d*₆) δ 1.46–2.10 (m, 20H), 2.48 (t, *J* = 12.0 Hz, 1H), 5.59 (d, *J* = 2.0 Hz, 1H), 4.67 (d, *J* = 3.0 Hz, 1H), 6.66 (d, *J* = 8.5 Hz, 2H), 6.98 (d, *J* = 8.5 Hz, 2H), 9.13 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 28.5, 31.8, 32.9, 33.3, 34.61, 34.63, 35.2, 35.9, 41.2, 71.5, 108.6, 111.0, 115.5, 127.8, 136.6, 156.0.

Step 2. A mixture of **55** (90 mg, 0.24 mmol), ethyl bromoacetate (48 mg, 0.29 mmol), and potassium carbonate (67 mg, 0.48 mmol) in acetone (10 mL) was stirred at 55 °C for 12 h. The solid was filtered and washed with acetone, and the filtrate was concentrated in vacuo to afford *cis*-6-hydroxyadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**56**) as a white solid (110 mg, 100%). Mp 138–139 °C. ¹H NMR (CDCl₃) δ 1.29 (t, *J* = 7.0 Hz, 3H), 1.62–2.14 (m, 20H), 2.49 (t, *J* = 12.0 Hz, 2H), 3.81 (s, 1H), 4.26 (q, *J* = 7.0 Hz, 2H), 4.58 (s, 2H), 6.82 (d, *J* = 8.5 Hz, 2H), 7.11 (d, *J* = 8.5 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 28.2, 31.6, 32.9, 33.29, 33.32, 34.7, 35.1, 35.8, 42.0, 61.3, 65.6, 73.2, 108.6, 110.8, 114.6, 127.7, 139.4, 156.3, 169.1. **Step 3.** A mixture of **56** (190 mg, 0.41 mmol) and 1 M aq. NaOH (2 mL) in THF (15 mL) was stirred at rt for 12 h. The removal of the solvents in vacuo gave a white residue, which was suspended in H₂O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford **21** (170 mg, 95%) as a white solid. Mp 153–154 °C. ¹H NMR (DMSO-*d*₆) δ 1.46–1.92 (m, 18H), 2.02 (d, *J* = 11.5 Hz, 1H), 2.8 (d, *J* = 11.0 Hz, 1H), 2.56 (t, *J* = 12.0 Hz, 1H), 2.58 (s, 1H), 4.60 (s, 2H), 4.66 (br, 1H), 6.79 (d, *J* = 8.5 Hz, 2H), 7.10 (d, *J* = 8.5 Hz, 2H), 12.96 (br, 1H); ¹³C NMR (DMSO-*d*₆) δ 28.5, 31.8, 32.9, 33.3, 34.6, 35.2, 35.9, 41.2, 65.0, 71.5, 108.6, 111.0, 114.8, 127.89, 138.9, 156.5, 170.8. Anal. calcd for C₂₄H₃₀O₇: C, 66.96; H, 7.02. Found: C, 66.96; H, 6.89.

cis-6-Acetamidoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (22)—Step 1.

To a solution of **54** (1.02 g, 2.23 mmol) in MeOH (50 mL) were added ammonium acetate (2.27 g, 26.81 mmol) and acetic acid (0.5 mL). The resulting mixture was stirred at rt for 0.5 h before addition of sodium cyanoborohydride (0.58 g, 8.94 mmol). The reaction mixture was stirred at rt for 12 h and then quenched with 1 M aq. NaOH (10 mL). After solvent removal in vacuo, the residue was extracted with EA (100 mL) and washed with water (30 mL) and brine (30 mL). The organic layer was dried over MgSO₄, filtered, and concentrated to afford *cis*-6-aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as a gray residue (1.02 g, 100%), which was used as starting material for next step without purification. **Step 2.** *cis*-6-Aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as a gray residue (1.02 g, 100%), which was used as starting material for next step without purification. **Step 2.** *cis*-6-Aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (220 mg, 0.48 mmol) was treated with acetyl chloride (57 mg, 0.72 mmol) and pyridine (76 mg, 0.96 mmol) in DCM (10 mL) at 0 °C. After stirring at rt for 6 h, the reaction mixture was quenched with H₂O (1 mL); diluted with DCM (10 mL); washed successively with 1 M HCl (5 mL), H₂O (5 mL), and brine (5 min); and then dried over MgSO₄. After filtration and solvent removal in vacuo, the residue was purified by chromatography (sg, 10:1 DCM/MeOH) to afford *cis*-6-acetamidoadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**57**) (200 mg, 83%) as a white solid. Mp 89–90 °C. ¹H NMR (CDCl₃) δ 1.29 (t, *J* = 7.0 Hz, 2H), 1.69–2.10 (m,

23H), 2.49 (t, $J=12.0$ Hz, 1H), 3.97 (d, $J=7.0$ Hz, 1H), 4.26 (q, $J=7.0$ Hz, 2H), 4.58 (s, 2H), 5.77 (d, $J=7.0$ Hz, 1H), 6.83 (d, $J=8.5$ Hz, 2H), 7.11 (d, $J=8.5$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 14.2, 23.7, 29.1, 29.2, 30.2, 30.5, 31.5, 31.6, 34.0, 34.6, 34.7, 35.3, 35.5, 42.0, 52.4, 61.3, 65.6, 108.8, 110.5, 114.6, 127.7, 139.3, 156.3, 169.1, 169.5. **Step 3.** A mixture of **57** (170 mg, 0.34 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. After solvent removal in vacuo, the resulting residue was suspended in H_2O (10 mL) and acidified with AcOH (0.5 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford **22** (150 mg, 94%) as a white solid. Mp 163–164 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.51–2.02 (m, 26H), 2.55 (t, $J=12.0$ Hz, 1H), 3.75 (d, $J=6.0$ Hz, 1H), 4.56 (s, 2H), 6.79 (d, $J=8.5$ Hz, 2H), 7.10 (d, $J=8.5$ Hz, 2H), 7.81 (d, $J=7.0$ Hz, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 23.2, 28.8, 28.9, 30.3, 30.7, 31.7, 34.2, 34.5, 34.6, 35.4, 35.5, 41.2, 52.4, 65.4, 108.7, 110.7, 114.7, 127.8, 138.7, 156.7, 169.2, 170.9. Anal. calcd for $\text{C}_{26}\text{H}_{33}\text{NO}_7$: C, 66.23; H, 7.05; N, 2.97. Found: C, 66.44; H, 6.90; N, 2.73.

cis-6-Aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (23)—**Step 1.** *cis*-6-Aminoadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (700 mg, 1.53 mmol) was treated with $(\text{Boc})_2\text{O}$ (667 mg, 3.07 mmol) and DIPEA (793 mg, 6.13 mmol) in DCM (20 mL) at 0 °C. The reaction mixture was then stirred at rt overnight, diluted with DCM (30 mL), washed successively with 1 M HCl (10 mL), saturated NaHCO_3 (10 mL) and brine (20 min), dried over MgSO_4 , filtered, and concentrated. The residue was purified by chromatography (sg, hexane/EA 5:1) to afford *cis*-6-((tert-butoxycarbonyl)amino)adamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**58**) (590 mg, 69%) as a white solid. Mp 89–90 °C. ^1H NMR (CDCl_3) δ 1.29 (t, $J=7.0$ Hz, 3H), 1.45 (s, 9H), 1.63–2.09 (m, 20H), 2.49 (t, $J=12.0$ Hz, 1H), 3.67 (s, 1H), 4.26 (q, $J=7.0$ Hz, 2H), 4.56 (s, 2H), 4.86 (s, 1H), 6.82 (d, $J=8.5$ Hz, 2H), 7.11 (d, $J=8.5$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 14.1, 28.4, 28.9, 29.0, 30.6, 30.9, 31.49, 31.53, 34.0, 34.1, 34.6, 35.3, 35.5, 42.0, 53.5, 61.3, 65.6, 79.2, 108.6, 110.6, 114.6, 127.7, 139.3, 155.2, 156.2, 169.0. **Step 2.** A mixture of **58** (250 mg, 0.75 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. After solvent removal in vacuo, the residue was suspended in H_2O (20 mL) and acidified with AcOH (0.2 mL). The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford *cis*-6-((tert-butoxycarbonyl)amino)adamantane-2-spiro-3'-8'-[4-(carboxymethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane as a white solid, which was then treated with 1 M methanesulfonic acid in EtOAc (6 mL) at rt for 12 h. The precipitate was filtered, washed with EA, and dried in vacuo at 50 °C to afford **23** as a white solid (232 mg, 98% over two steps). Mp 182–183 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.52 (q, $J=12.5$ Hz, 2H), 1.65 (d, $J=13.5$ Hz, 1H), 1.75–2.04 (m, 17H), 2.34 (s, 3H), 2.55 (t, $J=12.0$ Hz, 1H), 3.30 (s, 1H), 4.61 (s, 2H), 6.81 (d, $J=8.5$ Hz, 2H), 7.11 (d, $J=8.5$ Hz, 2H), 7.95 (s, 3H), 12.92 (br, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 27.28, 27.33, 28.8, 29.2, 31.7, 33.57, 33.61, 34.5, 34.9, 35.1, 40.2, 41.1, 53.9, 64.9, 109.0, 110.0, 114.8, 127.9, 138.8, 156.5, 170.8. Anal. calcd for $\text{C}_{25}\text{H}_{35}\text{NO}_9\text{S}$: C, 57.13; H, 6.71; N, 2.66. Found: C, 56.95; H, 6.59; N, 2.57.

cis-6-Azaadamantane-2-spiro-3'-8'-[4-(carboxymethoxy)-phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (24)—**Step 1.** To a solution of 2-Boc-2-

azaadamantan-6-one (**59**)³³ (4.00 g, 15.91 mmol) in dry EtOH (100 mL) was added pyridine (2.08 g, 26.25 mmol) followed by methoxyamine hydrochloride (1.99 g, 23.87 mmol). The resulting mixture was stirred at rt for 12 h and then concentrated in vacuo. The residue was partitioned between DCM (100 mL) and H₂O (50 mL). The organic phase was separated, and the aqueous layer was extracted with DCM (3 × 30 mL). The combined organic layers were successively washed with 1 M HCl (30 mL), saturated NaHCO₃ (30 mL), and brine (30 mL). After drying over MgSO₄, the solvent was removed in vacuo to afford 2-Boc-2-azaadamantan-6-one *O*-methyl oxime (**60**) (4.41 g, 99%) as a white solid. Mp 90–91 °C. ¹H NMR (CDCl₃) δ 1.48 (s, 10H), 1.78 (t, *J* = 10.2 Hz, 1H), 1.86 (t, *J* = 9.6 Hz, 1H), 1.96 (t, *J* = 13.5 Hz, 1H), 2.05 (t, *J* = 13.5 Hz, 1H), 2.69 (s, 1H), 3.61 (s, 1H), 3.83 (s, 3H), 4.30 (s, 1H), 4.42 (s, 1H). ¹³C NMR (CDCl₃) δ 27.7, 28.5, 34.6, 35.7, 36.0, 37.0, 37.4, 45.6, 47.1, 61.1, 79.5, 154.2, 163.8. **Step 2.** A mixture of **60** (841 mg, 3.0 mmol) and ethyl 4-(4-oxocyclohexyl)phenyl acetate (**51**) (697 mg, 3.0 mmol) in cyclohexane (120 mL) and DCM (20 mL) was treated with ozone according to the method of Dong et al.⁴² After solvent removal in vacuo, the residue was recrystallized from MeOH to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-(4-acetoxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**61**) as a white solid (541 mg, 36%). Mp 162–163 °C. ¹H NMR (CDCl₃) δ 1.47 (s, 9H), 2.09–1.66 (m, 16H), 2.15 (s, 2H), 2.28 (s, 3H), 2.56 (t, *J* = 12.3 Hz, 1H), 4.14 (d, *J* = 11.2 Hz, 1H), 4.27 (d, *J* = 21.8 Hz, 1H), 7.00 (d, *J* = 8.5 Hz, 2H), 7.20 (d, *J* = 8.3 Hz, 2H); ¹³C NMR (CDCl₃) δ 21.1, 28.5, 31.4, 33.2, 33.5, 34.6, 35.0, 42.3, 44.4, 44.8, 45.8, 46.2, 79.3, 108.9, 110.0, 121.4, 127.7, 143.5, 148.9, 154.2, 169.6. **Step 3.** A mixture of **61** (250 mg, 0.50 mmol) and 15% aq. KOH (1 mL) in THF (10 mL) was stirred at rt for 12 h and then concentrated in vacuo. The residue was suspended in H₂O (20 mL) and acidified with AcOH to pH 4. The precipitate was filtered, washed with water, and dried in vacuo to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-(4-hydroxyphenyl)-1',2',4'-trioxaspiro[4.5]decane (**62**) (225 mg, 98%) as a white solid. Mp 176–177 °C. ¹H NMR (CDCl₃) δ 1.47 (s, 9H), 1.73–1.66 (m, 2H), 1.75–2.08 (m, 14H), 2.15 (s, 2H), 2.49 (t, *J* = 11.4 Hz, 1H), 4.15 (d, *J* = 11.5 Hz, 1H), 4.27 (d, *J* = 21.7 Hz, 1H), 5.56 (brs, 1H), 6.77 (d, *J* = 8.3 Hz, 2H), 7.06 (d, *J* = 8.2 Hz, 2H); ¹³C NMR (CDCl₃) δ 28.5, 31.6, 33.2, 33.5, 34.7, 34.9, 42.0, 44.5, 44.9, 45.9, 46.3, 79.6, 109.1, 109.9, 115.2, 127.7, 138.0, 154.2, 154.3. **Step 4.** A mixture of **62** (225 mg, 0.49 mmol), ethyl bromoacetate (100 mg, 0.60 mmol), and potassium carbonate (104 mg, 0.74 mmol) in acetone (20 mL) was stirred at 60 °C for 12 h. A solid was filtered and washed with acetone before concentrating the filtrate in vacuo to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-(2-ethoxy-2-oxoethoxy)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**63**) (266 mg, 100%) as a white solid. Mp 139–140 °C. ¹H NMR (CDCl₃) δ 1.30 (t, *J* = 7.1 Hz, 2H), 1.47 (s, 9H), 1.61–2.08 (m, 16H), 2.15 (s, 2H), 2.51 (t, *J* = 11.8 Hz, 1H), 4.14 (d, *J* = 10.9 Hz, 1H), 4.25–4.30 (m, 3H), 4.59 (s, 2H), 6.84 (d, *J* = 8.6 Hz, 2H), 7.12 (d, *J* = 8.4 Hz, 2H); ¹³C NMR (CDCl₃) δ 14.2, 28.5, 31.5, 33.2, 33.5, 34.6, 35.0, 42.0, 44.4, 44.8, 45.8, 46.2, 61.3, 65.6, 79.3, 109.0, 110.0, 114.6, 127.7, 139.3, 154.2, 156.3, 169.1. **Step 5.** A mixture of **63** (265 mg, 0.49 mmol) and 15% aq. KOH (1 mL) in THF (10 mL) was stirred at rt for 12 h. The removal of the solvent in vacuo gave a white residue, which was suspended in H₂O (10 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford *cis*-6-Boc-6-azaadamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (**64**) (232 mg, 90%) as a white solid. Mp 158–160 °C. ¹H NMR (CDCl₃) δ 1.46 (d, *J* = 1.7 Hz, 9H), 1.65 (q, *J* = 12.4 Hz, 2H), 1.73–

2.06 (m, 14H), 2.13 (s, 2H), 2.47 (t, $J = 12.2$ Hz, 1H), 4.14 (d, $J = 11.7$ Hz, 1H), 4.26 (d, $J = 28.8$ Hz, 1H), 4.48 (s, 2H), 6.78 (d, $J = 8.2$ Hz, 2H), 7.06 (d, $J = 8.2$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 28.5, 31.5, 33.2, 33.5, 34.6, 35.0, 41.9, 44.4, 44.8, 45.8, 46.2, 65.4, 79.5, 108.9, 109.9, 114.6, 127.8, 154.3, 155.9. **Step 6.** A mixture of **64** (232 mg, 0.45 mmol) and methanesulfonic acid (480 mg, 5.00 mmol) in THF (5 mL) was stirred at rt for 12 h. The precipitate was filtered and washed with Et₂O to afford **24** (230 mg, 100%) as a white solid. Mp 183–184 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.53 (q, $J = 13.3$, 2H), 1.71–2.18 (m, 16H), 2.37 (s, 3H), 2.57 (t, $J = 12.1$ Hz, 1H), 3.55 (s, 1H), 3.59 (s, 1H), 4.62 (s, 2H), 6.82 (d, $J = 8.6$ Hz, 2H), 7.12 (d, $J = 8.7$ Hz, 2H), 8.73 (brs, 2H), 12.95 (brs, 1H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 30.8, 31.7, 33.2, 34.3, 41.0, 45.5, 45.9, 65.0, 108.7, 109.7, 114.8, 127.9, 138.7, 156.6, 170.8. Anal. calcd for C₂₄H₃₁N₃O₂S: C, 56.35; H, 6.50; N, 2.74. Found: C, 56.01; H, 6.70; N, 2.59.

cis-6-Azaadamantane-2-spiro-3'-8'-[4-[[[(carboxymethyl)-amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane mesylate (25)—Step 1. A mixture of **60** (400 mg, 1.43 mmol) and 4-(4-(chloromethyl)phenyl)cyclohexan-1-one (**65**) (318 mg, 1.43 mmol) in cyclohexane (120 mL) and DCM (20 mL) was treated with ozone according to the method of Dong et al.⁴² After solvent removal in vacuo, the residue was recrystallized from MeOH to afford cis-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-(chloromethyl)phenyl]-1',2',4'-trioxaspiro[4.5]decane (**66**) as a white solid (182 mg, 26%). Mp 157–158 °C. ^1H NMR (CDCl_3) δ 1.47 (d, $J = 2.1$ Hz, 9H), 1.66–2.9 (m, 20H), 2.15 (s, 2H), 2.56 (t, $J = 11.5$ Hz, 1H), 4.15 (d, $J = 10.8$ Hz, 1H), 4.27 (d, $J = 21.9$ Hz, 1H), 4.56 (s, 2H), 7.20 (d, $J = 8.0$ Hz, 2H), 7.31 (d, $J = 7.9$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 28.5, 31.3, 33.2, 33.5, 34.9, 34.5, 42.5, 42.6, 44.3, 44.7, 45.8, 46.1, 46.2, 79.3, 108.8, 110.0, 127.1, 128.7, 135.4, 146.3, 154.2. **Step 2.** A mixture of **66** (182 mg, 0.37 mmol), glycine ethyl ester hydrochloride (622 mg, 9.32 mmol), and DIPEA (1.50, 11.65 mmol) in DMA (20 mL) was stirred at 50 °C for 5 h. Then, the reaction mixture was cooled to rt, diluted with EA (100 mL), and washed successively with H₂O (3 × 50 mL) and brine (50 mL). The organic layer was separated and dried over MgSO₄, filtered, concentrated, and dried in vacuo at 50 °C to afford cis-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-[[[(2-ethoxy-2-oxoethyl)amino]methyl]phenyl]-1,2,4-trioxaspiro[4.5]decane (**67**) (200 mg, 97%) as a white solid. ^1H NMR (CDCl_3) δ 1.27 (t, $J = 7.0$ Hz, 3H), 1.47 (d, $J = 2.2$ Hz, 9H), 1.66–2.07 (m, 16H), 2.16 (s, 2H), 2.55 (t, $J = 11.8$ Hz, 1H), 3.41 (s, 2H), 3.77 (s, 2H), 4.15 (d, $J = 10.8$ Hz, 1H), 4.19 (q, $J = 7.1$ Hz, 2H), 4.28 (d, $J = 21.2$ Hz, 1H), 7.17 (d, $J = 8.2$ Hz, 2H), 7.26 (d, $J = 8.0$ Hz, 2H); ^{13}C NMR (CDCl_3) δ 14.2, 28.5, 31.4, 33.2, 33.5, 34.6, 35.0, 42.5, 44.4, 44.8, 45.8, 46.2, 50.1, 53.0, 60.8, 79.3, 109.0, 110.0, 126.8, 128.4, 137.3, 144.9, 154.2, 172.3. **Step 3.** A mixture of **67** (200 mg, 0.36 mmol) and 1 M aq. NaOH (1 mL) in THF (10 mL) was stirred at rt for 12 h and then concentrated in vacuo. The residue was suspended in H₂O (10 mL) and acidified with AcOH to pH 3. The precipitate was filtered, washed with water, and dried in vacuo at 50 °C to afford cis-6-Boc-6-azaadamantane-2-spiro-3'-8'-[4-[[[(carboxymethyl)amino]methyl]phenyl]-1',2',4'-trioxaspiro[4.5]decane, which was treated with methanesulfonic acid (480 mg, 5 mmol) in THF (5 mL). The resulting mixture was stirred at rt for 12 h before the addition of Et₂O (10 mL). The supernatant was removed and to the oily residue was added Et₂O (20 mL). The resulting mixture was stirred at rt for 12 h. The precipitate was filtered and washed with Et₂O to afford **25** (183 mg, 82%) as a white solid. Mp 174–175 °C. ^1H NMR ($\text{DMSO}-d_6$) δ 1.58 (qd, $J = 13.1$, 3.9 Hz, 2H), 1.76–2.18

(m, 16H), 2.34 (s, 6H), 2.67 (tt, $J = 12.4, 3.2$ Hz, 1H), 3.55 (s, 1H), 3.59 (s, 1H), 3.85 (s, 2H), 4.13 (s, 2H), 7.30 (d, $J = 7.9$ Hz, 2H), 7.41 (d, $J = 7.9$ Hz, 2H), 8.73 (s, 2H), 9.26 (s, 2H), 13.77 (brs, 1H); ^{13}C NMR (DMSO- d_6) δ 30.77, 30.84, 31.4, 33.2, 34.2, 41.6, 45.5, 45.9, 46.9, 50.2, 108.7, 109.6, 127.4, 129.8, 130.7, 147.3, 168.4. Anal. calcd for $\text{C}_{26}\text{H}_{40}\text{N}_2\text{O}_{11}\text{S}$: C, 50.31; H, 6.50; N, 4.51. Found: C, 50.66; H, 6.58; N, 4.34. HRMS (ESI-ToF) m/z : $[\text{M} + \text{H}]^+$ calcd for $\text{C}_{24}\text{H}_{33}\text{N}_2\text{O}_5$ 429.2389; found 429.2397.

Polar Surface Area (PSA)

PSA values were calculated using ChemAxon Instant JChem (ver 16.4).

Partition Coefficient

Partition coefficient values ($\log D$) of the test compounds were estimated by correlation of their chromatographic retention properties against the characteristics of a series of standard compounds with known partition coefficient values using gradient HPLC (modification of a method reported by Lombardo et al.).³⁸

Protein Binding

Protein binding values of the test compounds were estimated by correlation of their chromatographic retention properties on a human albumin column against the characteristics of a series of standard compounds with known protein binding values. The method employed is a gradient HPLC-based derivation of the method developed by Valko et al.⁴⁰

Kinetic Solubility

Compounds in DMSO (10 mg/mL) were diluted into either pH 6.5 phosphate buffer or 0.01 M HCl (approx. pH 2.0) with the final DMSO concentration being 1%. After 30 min, samples were then analyzed via nephelometry to determine a solubility range.³⁹

In Vitro Metabolic Stability

As fully described in Coteron et al.,⁴⁴ metabolic stability assays were performed by incubating test compounds in liver microsomes at 37 °C and 0.4 mg/mL protein concentration. The metabolic reaction was initiated by the addition of an NADPH-regenerating system and quenched at various time points over a 60 min incubation period by the addition of acetonitrile containing diazepam as internal standard. Control samples (containing no NADPH) were included (and quenched at 2, 30, and 60 min) to monitor for potential degradation in the absence of cofactor.

Antischistosomal Screen

All animal experiments were performed in line with national guidelines (permit 2070). All animals were kept in polycarbonate cages under environmentally controlled conditions (temperature: 25 °C, humidity: 70%, 12:12 h light/dark photoperiod), had free access to tap water and rodent food, and acclimatized for 1 week prior to infection. Cercariae of *S. mansoni* were obtained from infected *Biomphalaria glabrata*. As described by Lombardo et al.,⁴⁵ 4-week-old female NMRI mice (Charles Rivers, Sulzfeld, Germany) were infected subcutaneously with approximately 100 *S. mansoni* cercariae. At 21 or 49 days after

infection, groups of four mice were treated with single oral doses of compounds in a 7% (v/v) Tween 80% and 3% (v/v) ethanol vehicle (10 mL/kg). Untreated mice ($n = 8$) served as controls. At 21 days post-treatment, the animals were killed by the CO₂ method and dissected. Worms were removed by picking, then sexed and counted.

Cytotoxicity Screen

As fully described in Sanford et al.,⁴⁶ cell lines were maintained in D10 media. Once confluent, increasing concentrations of test compound (0–100 μ M) were added and incubated for 24 h. Alamar blue (10 mM) was then added to each well and incubated for 4 h. A BioTek Synergy HT plate reader was then used to determine fluorescence.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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ABBREVIATIONS USED

CL_{int}	in vitro intrinsic clearance
DCC	dicyclohexylcarbodiimide
EDC	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide
HOBt	hydroxybenzotriazole
WBR	worm burden reduction

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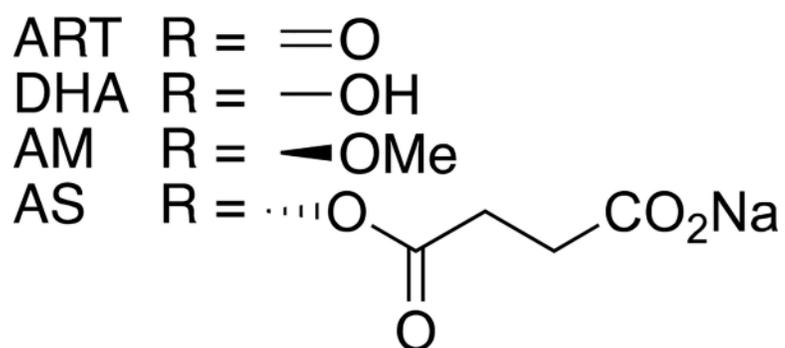
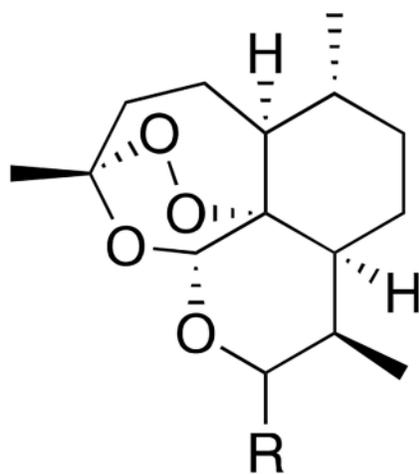


Figure 1. Artemisinin (ART) and its semisynthetic derivatives dihydroartemisinin (DHA), artemether (AM), and artesunate (AS).

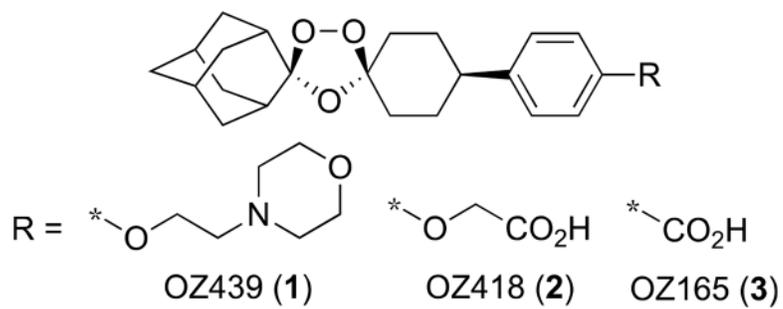
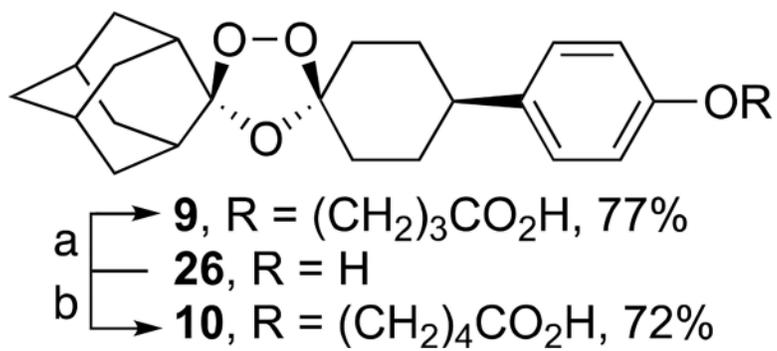
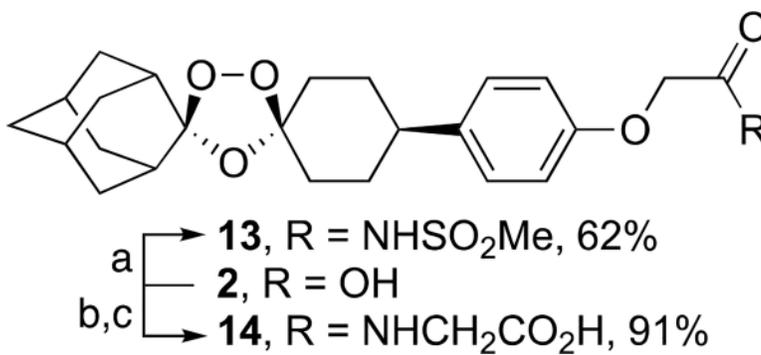


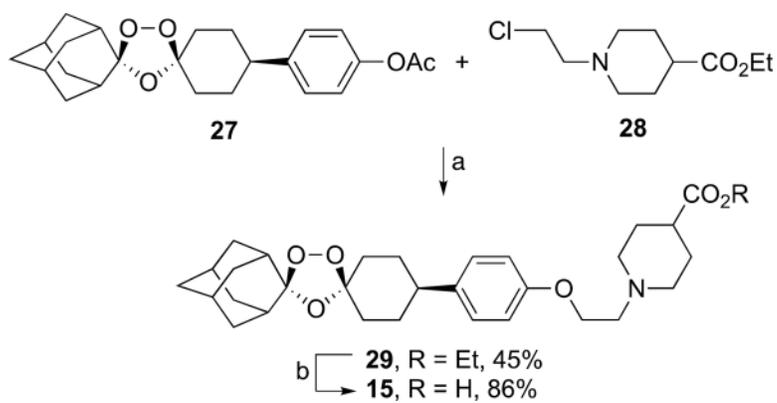
Figure 2.
Ozonides OZ439 (1), OZ418 (2), and OZ165 (3).

**Scheme 1.**

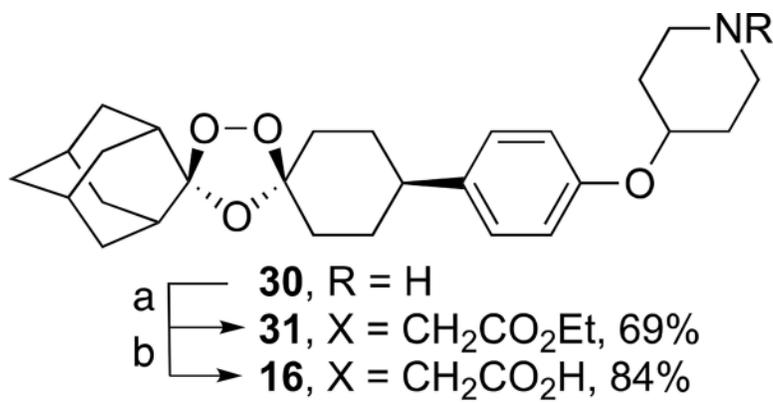
^aReagents and conditions: 4-bromobutanoate (a) or 5-bromopentanoate (b), powdered NaOH, tetrabutylammonium hydrogensulfate, dimethoxyethane, room temperature (rt) to 60 °C, 12 h, then aq. AcOH.

**Scheme 2.**

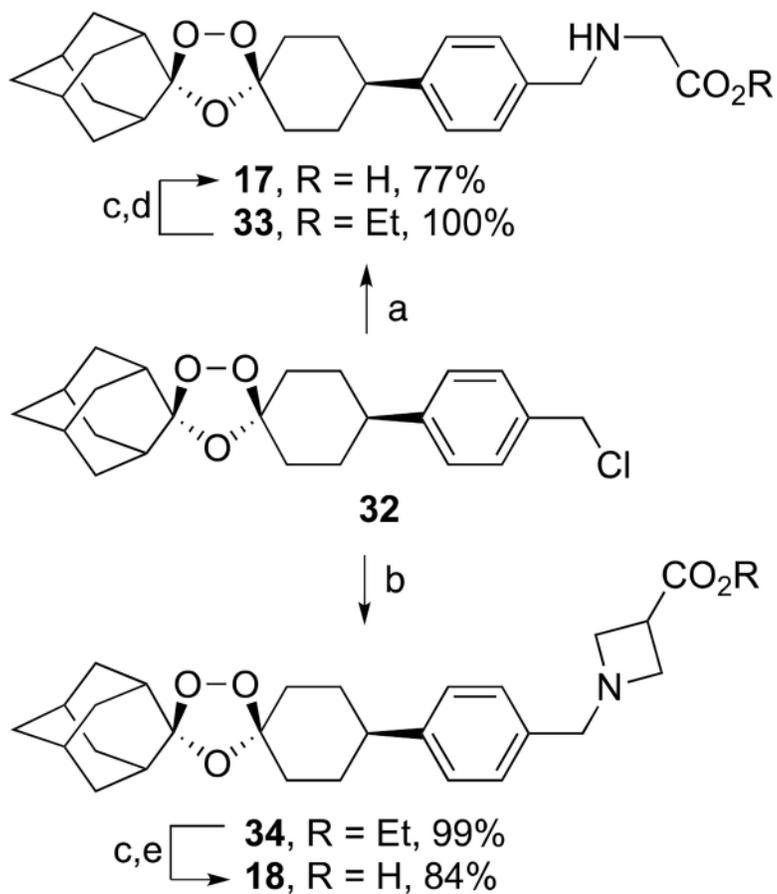
^aReagents and conditions: (a) methanesulfonamide, dicyclohexylcarbodiimide (DCC), 4-dimethylaminopyridine (DMAP), DCM, rt, 24 h; (b) glycine ethyl ester hydrochloride, HOBt, EDC, *N,N*-diisopropylethylamine (DIPEA), *N,N*-dimethylformamide (DMF), rt, 12 h; (c) 2% aq. NaOH, EtOH, rt, 12 h, then aq. AcOH.

**Scheme 3.**

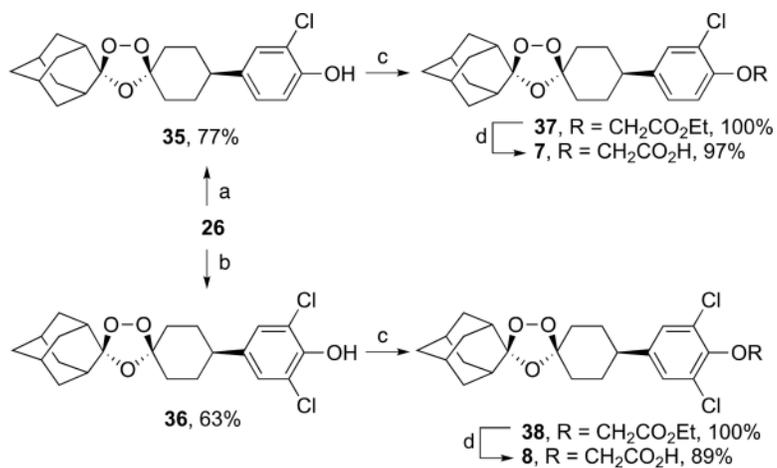
^aReagents and conditions: (a) powdered NaOH, tetrabutylammonium hydrogensulfate, CH₃CN, rt to 60 °C, 12 h; (b) powdered NaOH, EtOH, rt, 24 h.

**Scheme 4.**

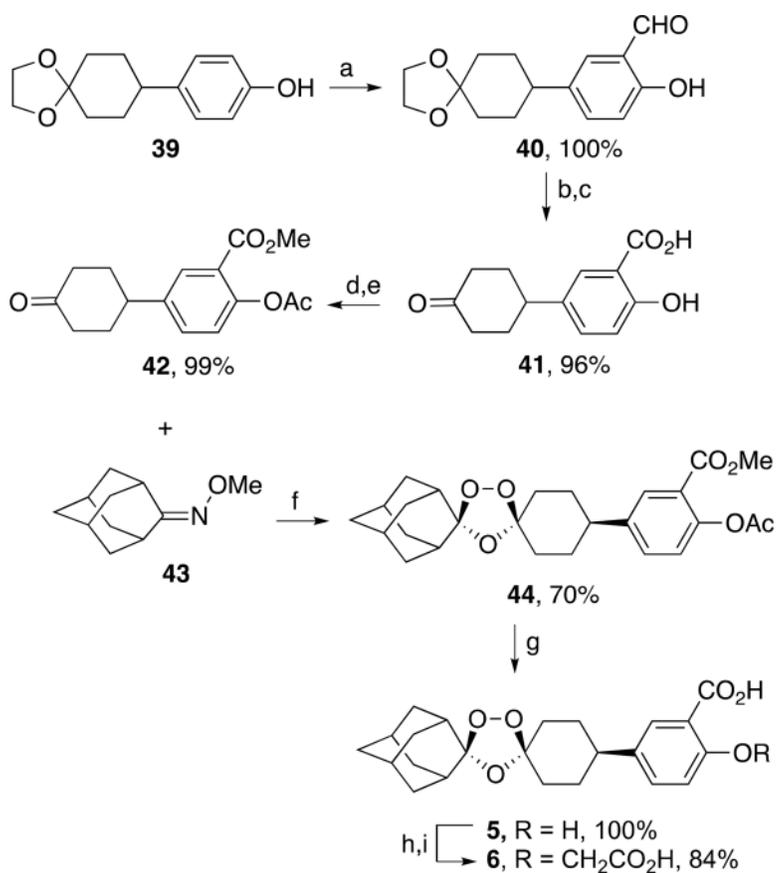
^aReagents and conditions: (a) ethyl bromoacetate, K₂CO₃, 12:1 THF/H₂O, rt, 12 h; (b) 1 M aq. NaOH, EtOH, 50 °C, 20 h, then aq. AcOH.

**Scheme 5.**

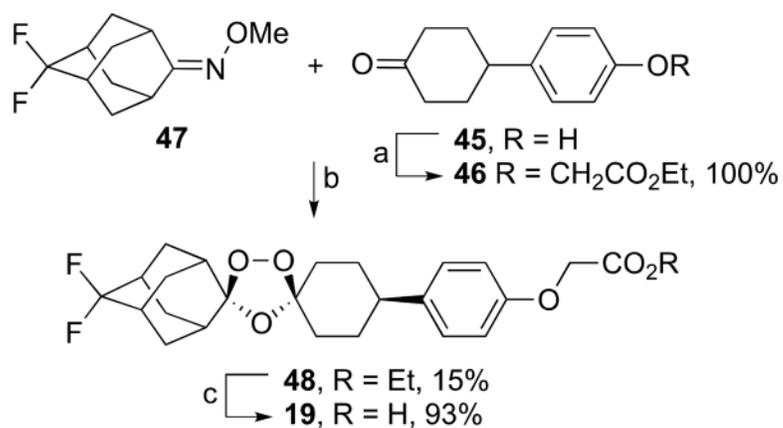
^aReagents and conditions: glycine ethyl ester hydrochloride (a) or ethyl azetidine-3-carboxylate hydrochloride (b), DIPEA, DMA, 50 °C, 5 h; (c) 1 M NaOH, aq. THF, rt, 12 h, then aq. AcOH; (d) methanesulfonic acid, Et₂O, rt, 1 h; (e) methanesulfonic acid, EA, rt, 1 h.

**Scheme 6.**

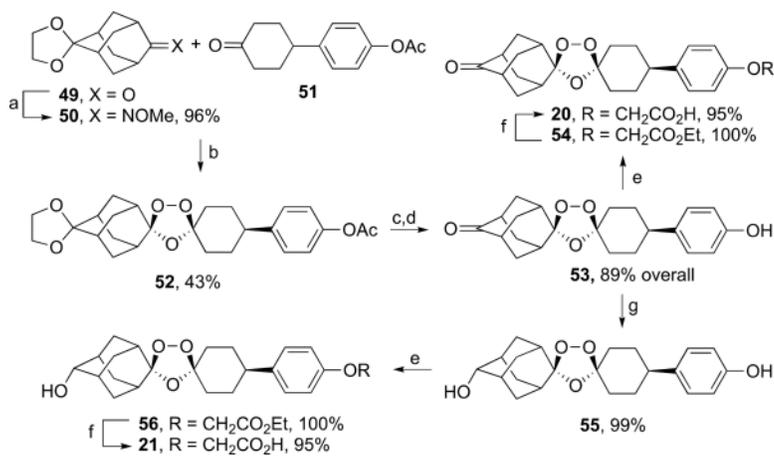
^aReagents and conditions: (a) 1 equiv NCS, DMF rt, 24 h; (b) 9 equiv NCS, DMF rt, 24 h; (c) ethyl bromoacetate, K₂CO₃, acetone, 60 °C, 12 h; (d) 1 M aq. NaOH, THF, rt, 12 h, then aq. AcOH.

**Scheme 7.**

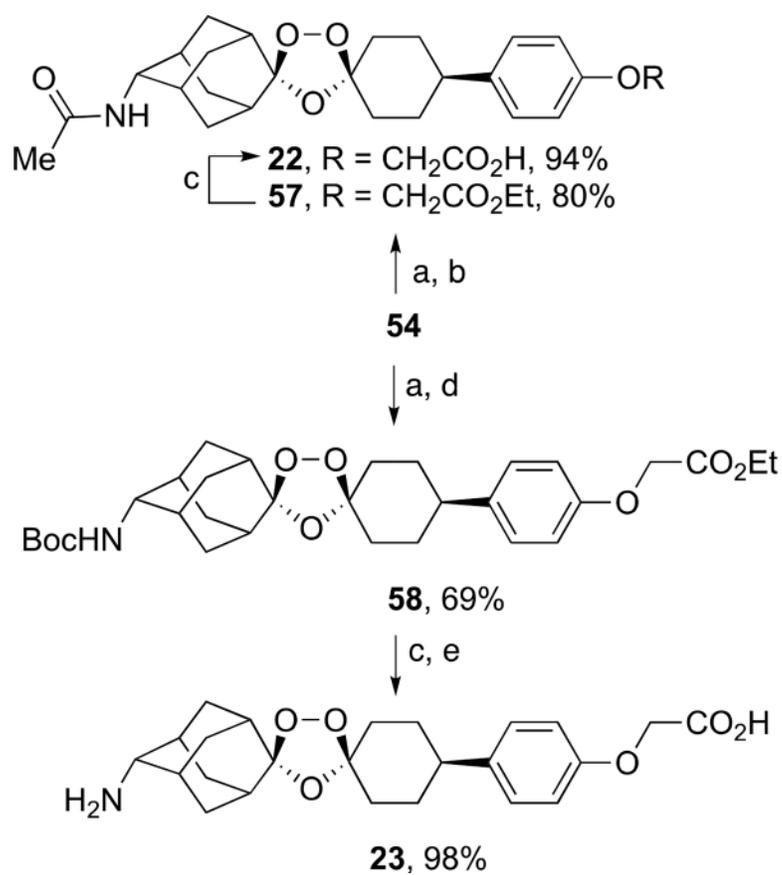
^aReagents and conditions: (a) paraformaldehyde, MgCl₂, Et₃N, THF, rt to reflux, 5 h; (b) Ag₂O, H₂O, 100 °C, 12 h; (c) 6 N HCl, THF, rt, 4 h; (d) acetyl chloride, MeOH, 0 °C to reflux, 4 h; (e) Ac₂O, pyridine, DCM, 0 °C to rt, 5 h; (f) O₃, 4:1 cyclohexane/DCM, 0 °C, 0.25 h; (g) KOH, 4:4:1 H₂O/MeOH/THF, rt to 50 °C, 4 h; (h) ethyl bromoacetate, K₂CO₃, acetone, 60 °C, 12 h; (i) 1 M aq. NaOH, 5:1 THF/H₂O, rt, 12 h, then aq. AcOH.

**Scheme 8.**

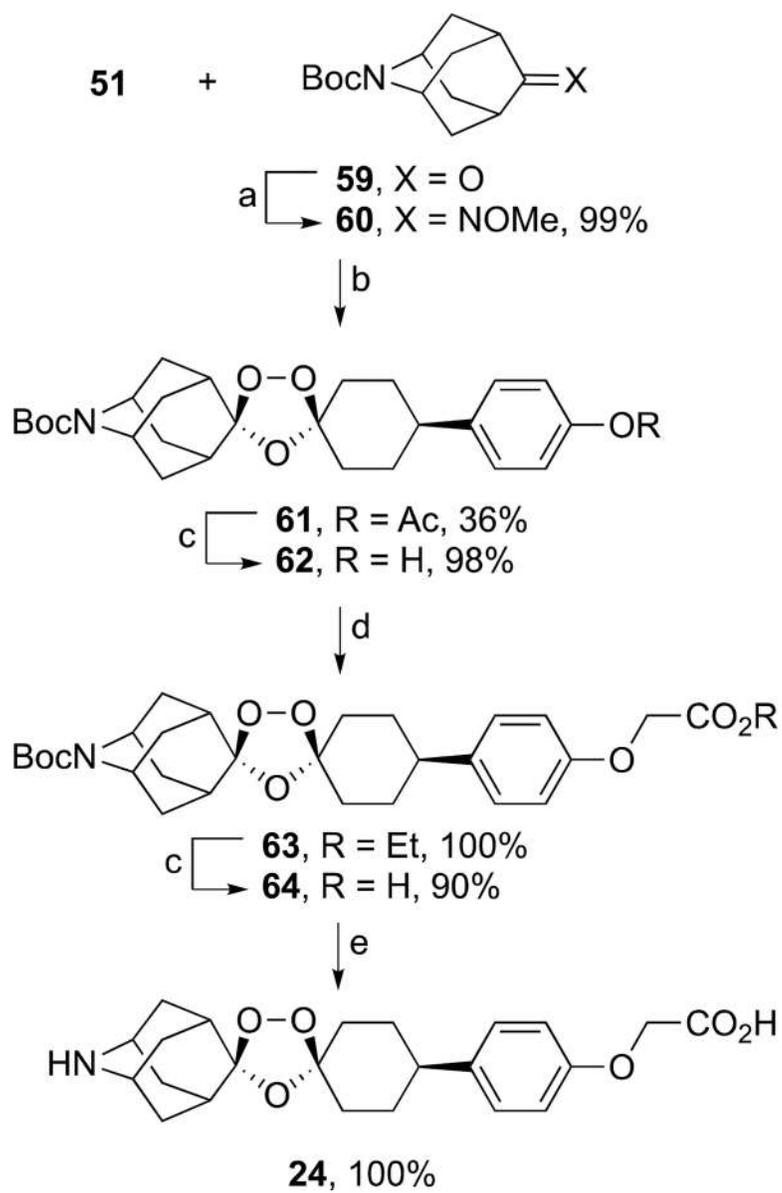
^aReagents and conditions: (a) ethyl bromoacetate, K_2CO_3 , acetone, 60°C , 12 h; (b) O_3 , 5:2 cyclohexane/DCM, 0°C , 0.25 h; (c) 15% aq. KOH, THF, rt, 12 h, then aq. AcOH.

**Scheme 9.**

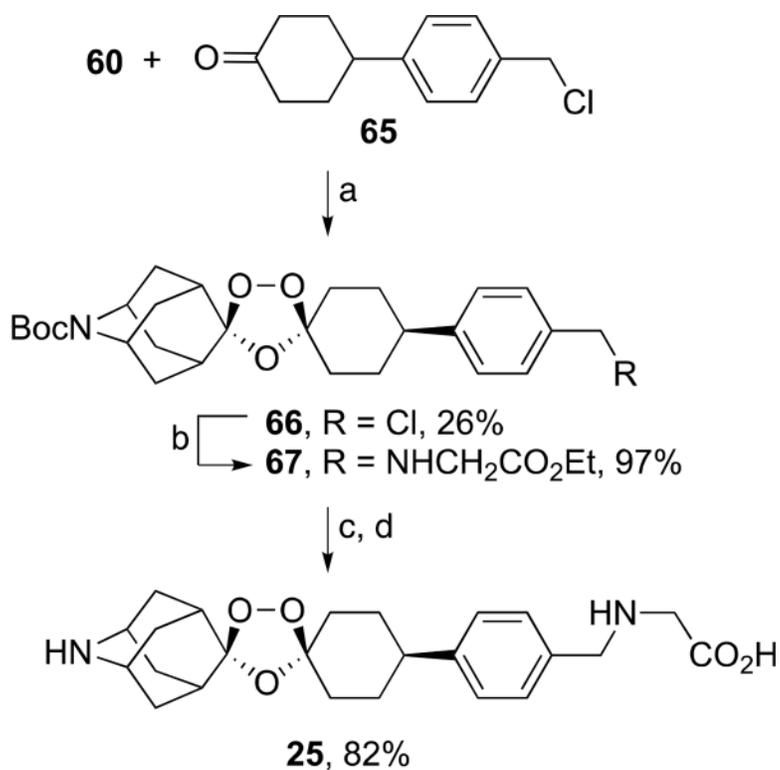
^aReagents and conditions: (a) methoxyamine HCl, pyridine, EtOH, rt, 12 h; (b) O₃, 3:1 cyclohexane/DCM, 0 °C, 0.25 h; (c) 1 M aq. NaOH, THF, rt, 12 h; (d) 1 M MsOH, 5:1 acetone/H₂O, rt, 12 h; (e) ethyl bromoacetate, K₂CO₃, acetone, 55 °C, 12 h; (f) 5:1 1 M aq. NaOH/THF, rt, 12 h, then aq. AcOH; (g) NaBH₄, MeOH, 0 °C, 2 h.

**Scheme 10.**

^aReagents and conditions: (a) NaBH₃CN, NH₄OAc, AcOH, MeOH, rt, 12 h, then 1 M aq. NaOH; (b) acetyl chloride, pyridine, DCM, rt, 6 h; (c) 1 M aq. KOH, THF, rt, 12 h, then aq. AcOH; (d) (Boc)₂O, DIPEA, DCM, 0 °C to rt, 12 h; (e) 1 M methanesulfonic acid in EtOAc, rt, 12 h.

**Scheme 11.**

^aReagents and conditions: (a) methoxyamine hydrochloride, pyridine, EtOH, rt, 12 h; (b) O₃, 10:1 cyclohexane/DCM, 0 °C, 0.25 h; (c) 15% aq. KOH, THF, rt, 12 h, then aq. AcOH; (d) ethyl bromoacetate, K₂CO₃, acetone, 60 °C, 12 h; (e) methanesulfonic acid, THF, rt, 12 h.

**Scheme 12.**

^aReagents and conditions: (a) O₃, 10:1 cyclohexane/DCM, 0 °C, 0.25 h; (b) glycine ethyl ester hydrochloride, DIPEA, DMA, 50 °C, 5 h; (c) 1 M aq. NaOH, tetrahydrofuran (THF), rt, 12 h, then aq. AcOH; (d) methanesulfonic acid, THF, rt, 12 h.

Table 1.

Physicochemical, In Vitro ADME, and Antischistosomal Data for Ozonides 2–8

compd	$\log D_{7,4}$ ^a	PSA (Å ²) ^b	sol _{2,0} /sol _{0.5} (μg/mL) ^c	h/m CL _{int} (μL/(min mg protein)) ^d	cPPB (%) ^e	<i>S. mansoni</i> WBR (%) 1 × 400 mg/kg po ^f
2	2.9	77.1	<1.6/6.3–12.5	ND ^g	ND ^h	80 ^{i,j}
3	3.0	67.8	<3.1/<1.6	ND ^g	98.8	74 ^{i,j}
4	3.0	67.8	<1.6/<1.6	12/30	98.9	63
5	3.0	88.1	<1.6/>100	108/20	99.0	36
6	1.9	117.2	<3.1/>100	<7/8	99.1	0
7	3.2	77.1	<3.1/3.1–6.3	<7/8	>99.5	84
8	3.3	77.1	<3.1/3.1–6.3	ND ^g	ND	32

^alog $D_{7,4}$ values were estimated by chromatography.³⁸^bCalculated PSA values were generated using JChem for Excel.^cKinetic solubilities at pH 6.5 phosphate buffer and 0.01 M HCl (approx. pH 2.0).³⁹^dIn vitro intrinsic clearance (CL_{int}) values in human and mouse liver microsomes.^eProtein-binding values were estimated by chromatography.⁴⁰^fGroups of five *S. mansoni*-infected NMRI mice were treated on day 49 post-infection with ozonides dissolved or suspended in 7% v/v Tween 80, 3% v/v ethanol. At 28 days post-treatment, animals were sacrificed and dissected to assess total worm burden reduction (WBR).^gAnalytical difficulties precluded measurement.^hPoor chromatography precluded determination.ⁱData from Keiser et al.²⁴^j $p < 0.02$ from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.^kND = not determined.

Table 2. Physicochemical, In Vitro ADME, and Antischistosomal Data for Ozonides 9–18

compd ^g	$g \log D_{7.4}$	PSA (\AA^2)	sol _{2,0} /sol _{6.5} ($\mu\text{g/mL}$)	h/m CL _{int} ($\mu\text{L}/(\text{min mg protein})$)	cPPB (%)	<i>S. mansoni</i> WBR (%)	$1 \times 400 \text{ mg/kg po}$
9	3.6	77.1	<3.1/<1.6	<7/8	>99.5	55	
10	4.0	77.1	<1.6/<1.6	<7/13	>99.5	51	
11	3.1	77.1	<1.6/3.1–6.3	<7/7	>99.5	48	
12	>5.3	63.2	<1.6/<0.78	ND ^a	98.9	60	
13	3.0	97.4	<1.6/6.3–12.5	<7/12	99.0	53	
14	3.0	106.2	12.5–25/<3.1	<7/CND ^{a,b}	98.5	76 ^e	
15	3.8	81.5	3.1–6.3/<1.6	<7/9	98.0	0	
16	ND ^c	81.5	ND ^d	<7/<7	ND	75 ^e	
17	3.3	84.4	<12.5/<6.3	<7/10	97.6	90 ^e	
18	ND ^c	72.3	6.3–12.5/<1.6	7/10	ND	43	

^a Apparent non-NADPH-mediated degradation.

^b Putative amide hydrolysis product **2** was detected in controls and NADPH samples.

^c Poor chromatography precluded determination.

^d Poor solubility in DMSO (<1 mg/mL) precluded determination of solubility.

^e $p < 0.02$ from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.

^f CND = could not determine.

^g ND = not determined.

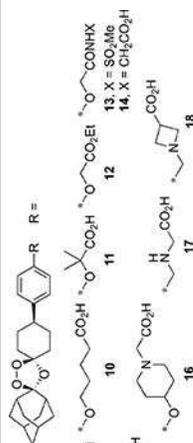
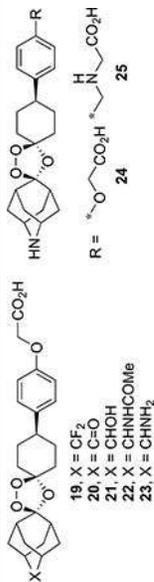


Table 3. Physicochemical, In Vitro ADME, and Antischistosomal Data for Ozonides 19–25

compd	$g \log D_{7.4}$	PSA (\AA^2)	$\text{sol}_{2.0}/\text{sol}_{6.5}$ ($\mu\text{g}/\text{mL}$)	$\text{h}/\text{m CL}_{\text{int}}$ ($\mu\text{L}/(\text{min mg})$ protein)	ePPB (%)	<i>S. mansoni</i> WBR (%)	$1 \times 400 \text{ mg}/\text{kg po}$
19	2.9	77.1	<1.6/12.5–25	23/130	99.4	57	
20	1.9	94.1	<1.6/50–100	ND ^a	97.8	24	
21	1.7	97.3	<1.6/25–50	<7/<7	98.5	14	
22	1.8	106.2	3.1–6.3/12.5–25	10/<7	98.2	0	
23	1.4	104.7	6.3–12.5/<1.6	13/8	94.2	30	
24	1.3	93.7	3.1–6.3/<1.6	35/27	ND ^b	29	
25	1.6	101.0	>100/50–100	<7/<7	ND ^b	41	



^a Apparent non-NADPH-mediated degradation.

^b Poor chromatography precluded determination.

Table 4.In Vivo Activity of Selected Ozonides against the Juvenile Stage of *S. mansoni*

compd	<i>S. mansoni</i> WBR (%) 1 × 200 mg/kg po
2	100 ^a
3	84 ^a
7	92 ^b
14	97 ^b
16	74 ^b
17	97 ^b

^aData from Keiser et al.²⁴^b $p < 0.05$ from the Kruskal–Wallis test comparing the medians of the responses between the treatment and control groups.