Published in final edited form as:

J Med Chem. 2010 May 27; 53(10): 4223–4233. doi:10.1021/jm100226t.

The Structure-Activity Relationship of an Ozonide Carboxylic Acid (OZ78) Against *Fasciola hepatica*

Qingjie Zhao¹, Mireille Vargas^{2,3}, Yuxiang Dong¹, Lin Zhou¹, Xiaofang Wang¹, Kamaraj Sriraghavan¹, Jennifer Keiser^{2,3}, and Jonathan L. Vennerstrom^{1,*}

¹ College of Pharmacy, University of Nebraska Medical Center, 986025 Nebraska Medical Center, Omaha, NE, USA ² Swiss Tropical and Public Health Institute, Socinstrasse 57, CH-4002 Basel, Switzerland ³ University of Basel, Petersplatz 1, CH-4003 Basel, Switzerland

Abstract

In this paper, we describe the SAR of ozonide carboxylic acid OZ78 (**1**) as the first part of our search for a trematocidal synthetic peroxide drug development candidate. We found that relatively small structural changes to **1** resulted most commonly in loss of activity against *Fasciola hepatica* in vivo. A spiroadamantane substructure and acidic functional group (or ester prodrug) were required for activity. Of twenty-six new compounds administered at single 100 mg/kg oral doses to *F. hepatica*-infected rats, eight had statistically significant worm burden reductions, seven were partially curative, and one (acyl sulfonamide **6**) was completely curative and comparable to **1** in flukicidal efficacy. This study also showed that the activity of **1** is peroxide bond-dependent suggesting that its flukicidal efficacy depends upon hemoglobin digestion in *F. hepatica*.

Some 250 million people are infected with parasitic trematode worms. Of these, the most widespread are blood flukes of the genus *Schistosoma*.¹ However, the liver flukes *Fasciola hepatica* and *F. gigantica* are also important pathogenic trematodes infecting an estimated 2.4–17 million people.² Fascioliasis is of considerable public health and great veterinary significance, and occurs worldwide, with the highest number of infected people in the Andean countries, Cuba, Western Europe, Egypt, and Iran.^{2,3} Triclabendazole, which has been routinely used since the early 1980s in veterinary medicine, is currently the sole drug used to treat human fascioliasis and is registered in only four countries.⁴ Evidence of drug-resistance to triclabendazole in veterinary medicine⁵ provides an impetus for the discovery and development of new drugs against fascioliasis. In this respect, data from a recent clinical trial in Vietnam⁶ demonstrated that artesunate, a semisynthetic artemisinin derivative, had good efficacy in the treatment of human fascioliasis, although it was less effective than triclabendazole.

tel: 402.559.5362, fax: 402.559.9543, jvenners@unmc.edu.

Supporting Information Available: Elemental analysis for **6–8**, **10**, **14**, and **16–27**. This material is available free of charge via the Internet at <http://pubs.acs.org>.

Although the semisynthetic artemisinins are best known for their powerful antimalarial properties, it is not surprising that they, as well as other peroxidic compounds, possess both antiplasmodial and trematocidal^{7–9} activities, since both plasmodia and several trematodes including *Fasciola* spp. degrade hemoglobin to generate free heme, a possible target¹⁰ for bioactive peroxides. That the artemisinins and ozonide OZ78 (1) are so much less effective against the nonhemoglobin-degrading intestinal fluke *Echinostoma caproni* than against hemoglobin-degrading flukes *F. hepatica*, *Clonorchis sinensis*, and various schistosome species^{9,11–13} supports this hypothesis.¹⁴ Thus, the artemisinins and synthetic peroxides offer excellent starting points^{15,16} for the discovery of broad-spectrum, orally-active trematocidal agents that would minimize drug-resistance and lead to superior treatment and control options. In this paper, we describe the SAR of ozonide 1 as the first part of our search for a flukicidal synthetic peroxide drug development candidate.

Chemistry

Following the method of Tsandi et al.,¹⁷ acyl sulfonamide 6 (Scheme 1) was prepared by reaction of 1 with methansulfonamide in the presence of DMAP and DCC. Hydrazide 10 was readily prepared by reaction of the corresponding methyl ester 2 with hydrazine. Ozonides 7 and 8, the glycine and taurine conjugates of 1, were synthesized by reaction of HOBt active ester 28¹⁸ with glycine ethyl ester (subsequent ester hydrolysis) and taurine, respectively.

Ozonide dicarboxylic acid 14 was synthesized in a five-step sequence (Scheme 2) starting from 29,¹⁹ the Knoevenagel condensation product of 1,4-cyclohexanedione monoethylene ketal and isopropylidene malonate (Meldrum's Acid).²⁰ Reduction (99%), deketalization (27%), and esterification (37%) afforded diester ketone 30 which underwent Griesbaum coozonolysis²¹ with oxime ether 31²² to afford diester ozonide 32 in 75% yield. The latter was hydrolyzed to afford 14 in high yield.

The synthesis of ozonide 16, the monoethyl phosphonic acid isostere of 1, began with the synthesis of ketophosphonate diester 33 (26% yield) (Scheme 3) from 4-(bromomethyl) cyclohexanone in an Arbuzov reaction following the method of Yamagishi et al.²³ 4-(Bromomethyl)cyclohexanone, in turn, was obtained by HCl deprotection of the corresponding ethylene ketal 24 in 74% yield. Ozonide diethyl phosphonate 34, obtained in good yield by Griesbaum coozonolysis²¹ between oxime ether 31²² and 33, was treated with potassium trimethylsilanolate²⁵ to afford 16. Ozonide piperidine carboxylate 17 was obtained by hydrolysis of its corresponding ester 36 in 84% yield; the latter was obtained by deprotection

of BOC ozonide **35**²⁶ with methanesulfonic acid followed by alkylation with ethyl bromoacetate in 90% overall yield. Ozonide ester **39**, the precursor of ozonide carboxylic acid **18**, the *trans* isomer of **1**, was obtained in 21% yield by Griesbaum coozonolysis²¹ of oxime ether **38** and 2-adamantanone. With this combination of reaction partners, *cis* (**2**) and *trans* (**39**) ester ozonides were produced in a ratio of 1:2.5 and **39** was purified by flash column chromatography. In contrast, **2** is the major reaction product in a Griesbaum coozonolysis of oxime ether **31** and keto ester **37**.²⁷ Ozonide carboxylic acids **19** and **20**, regioisomers of **1**, were both obtained in a straightforward two-step Griesbaum coozonolysis/ester hydrolysis sequence starting from oxime ether **31** and the corresponding keto esters **40** and **42**. Unexpectedly, both **41** and **43** were formed with high diastereoselectivity and were isolated as single isomers. Assuming the peroxide bond is axial and the alkyl ester substituent is equatorial,^{18,27–29} we assigned structures for **19** and **20** as indicated in Scheme 3, although X-ray crystallographic analysis would be required to substantiate this.

Ozonide acids **21** (2.3:1 mixture of isomers) and **22** (single isomer) (Scheme 4) were obtained by hydrolysis of ozonide esters **45** (4:1 mixture of isomers) and **47** (9:1 mixture of isomers), which, in turn, were obtained by Griesbaum coozonolysis²¹ of keto ester **37**¹⁸ and oxime ethers **44**³⁰ and **46**.²⁸ Based upon the axial peroxide bonds and equatorial cyclohexyl substituents in ozonide products from similar coozonolysis reactions,^{18,27–29} we assigned the structures for **47** and **22** as indicated in Scheme 4.

Fluorinated ozonide acids **23–25** (Scheme 5) were obtained in parallel five to six-step sequences for which the key reaction was a Griesbaum coozonolysis²¹ between fluorinated oxime ethers **51**, **57**, and **61** and keto ester **37**¹⁸ to form the corresponding ozonide esters **52**, **58**, and **62** in low to moderate yields; hydrolysis of the latter yielded **23–25** (62–99%). Ozonide esters **58** and **62** were obtained as single diastereomers and were assigned as *cis* based on the previously observed²⁷ diastereoselectivity of the Griesbaum coozonolysis reaction. Ozonide ester **52** was obtained as a 3:1 ratio of diastereomers; as depicted in Scheme 5, the major isomer was assigned as *trans*, *cis* based on the diastereoselectivity of similar coozonolysis reactions with other 5-substituted-2-adamantanones.³¹ The fluorinated oxime ethers **51**, **57**, and **61** were obtained in high overall yields by successive treatment of 5-hydroxy-2-adamantanone ethylene ketal (**48**),³² 6-hydroxy-2-adamantanone ethylene ketal (**54**), and 2,6-adamantanedione monoethylene ketal (**53**)³³ with bis(2-methoxyethyl)aminosulfur trifluoride followed by deprotection and condensation with methoxyamine.

Triethylsilylperoxyketal ester **63**, the key intermediate in the synthesis of 1,2-dioxolane **26** was obtained in a two-step sequence³⁴ from keto ester **37** (Scheme 6). Formation of the peroxycarbenium intermediate³⁵ by treatment of **63** with SnCl₄ in the presence of 2-methyleneadamantane afforded 1,2-dioxolane ester **65** (40%) that was hydrolyzed to form **26** (93%). 1,2-Dioxolane ester **65** was obtained as a single diastereomer and was assigned a *cis* configuration based on the stereochemistry observed in similar reactions.³⁶ Ketal **27**, the non-peroxidic isostere of **1**, was obtained in 79% yield as a 1.3:1 mixture of isomers by *p*-TSA catalyzed condensation¹⁴ of 2-hydroxymethyl-2-adamantanol (**66**)³⁷ and 2-(4-oxocyclohexyl) acetic acid (**67**). Ozonides **1–5**, **9**, **11–13** and **15** were obtained as previously described.^{18,27,38}

Activity against *F. hepatica*

Efficacy data for the target compounds administered at oral doses of 100 mg/kg to *F. hepatica*-infected rats³⁹ are shown in Tables 1–3. At eight weeks post-infection, rats were treated with single 50–100 mg/kg oral doses of target compounds prepared as suspensions in 7% (v/v) Tween 80 and 3% (v/v) EtOH. At day 6 post-treatment, rats were sacrificed and adult flukes were recovered from the bile ducts and livers. Target compound efficacies were

evaluated by comparing the mean total worm burdens of treated and untreated control rats. Statistical significance was calculated using the Kruskal-Wallis test.

Table 1 shows efficacy data for a range of acidic, neutral and weak base amide derivatives of **1**. Of these compounds, the only one with efficacy equal to **1** was acyl sulfonamide **6**, although methyl ester **2** and glycine conjugate **7** achieved statistically significant worm burden reductions and partial cures; a 50 mg/kg dose of ethyl ester **3** also produced partial cures. The presence of an acidic functional group was no guarantee of good activity for these ozonides. For example, hydroxamic acid **4**, amphoteric acylguanidine **5**, and taurine conjugate **8** were either completely inactive or produced marginal decreases in total worm burdens and cured no infected animals. Unlike **2** and **3**, the alkyl ester prodrugs of **1**, the primary amide (**9**), hydrazide (**10**), and piperazinamide (**11**) derivatives were inactive or only weakly active and cured no infected rats.

Table 2 shows efficacy data for compounds that probe the effect of changing the position (**12**, **13**, **18**, **19**) and stereochemistry (**18**) of the carboxylic acid functional group of **1**. The position and stereochemistry of the carboxylic acid functional group in **1** seems to be optimal since removing (**12**) or extending (**13**) the connecting alkyl link reduced efficacy, as did changing the stereochemistry from *cis* (**1**) to *trans* (**18**) and changing the position of attachment of the carboxymethyl substituent on the cyclohexyl substructure (**19**, **20**). Even though the preceding six compounds were less effective than **1**, ozonides **12**, **18** and **20** were partially curative and achieved statistically significant worm burden reductions. In addition, the effects of an additional carboxylic acid functional group (**14**), replacing the carboxylic acid with a monoethyl phosphonic acid (**16**) or carboxy oxamide (**15**), and substituting the spirocyclohexyl substructure of **1** with a spiropiperidinyl in **17** were examined. Not one of compounds **14–17** significantly reduced worm burden reductions, nor were they curative; the lack of efficacy of **17** is consistent with SAR trends of antimalarial ozonides.²⁶

Table 3 shows efficacy data for compounds that probe the effect of replacing the spiroadamantyl substructure of **1** with a spirocyclohexyl (**21**) or bicyclo[3.3.1]nonane (**22**), or of fluorine substitution (**23–25**) of the spiroadamantyl substructure of **1**. The non-existent to low efficacies for **21** and **22** is an outcome consistent with SAR trends for antimalarial ozonides.²⁸ Target ozonides **23–25** were designed to slow or block potentially inactivating CYP450 metabolism of the distal bridgehead carbon atoms of **1** based on the known inactive CYP450 metabolites³¹ of arterolane (OZ277).⁴⁰ Although **23–25** had moderate efficacies against *F. hepatica*, none was more active than **1**.

The complete loss of efficacy for **27** (Table 3), the non-peroxidic isostere of **1**, shows that the activity of **1** is peroxide bond-dependent. The lack of efficacy for the peroxidic 1,2-dioxolane **26**, consistent with our previous data³⁵ showing that 1,2-dioxolanes have very low to no antimalarial activity, provides additional mechanistic insight. 1,2-Dioxolanes react with Fe(II) primarily by two-electron vs. one-electron reduction to form inactive diol reaction products rather than carbon-centered radicals,³⁵ the latter of which are formed by β -scission reactions of the initially formed Fe(III) complexed oxy radicals. These β -scission reactions are accelerated by the adjacent oxygen atom⁴¹ present in ozonides (1,2,4-trioxolanes), but absent in 1,2-dioxolanes.

Of twenty-six new compounds tested (Tables 1–3), eight had statistically significant worm burden reductions, seven were partially curative, and one (**6**) was completely curative. Compounds that were more effective in reducing worm burdens in the *F. hepatica*-infected rats also tended to result in partial cures, although ozonide ester **3** (36% worm burden reduction at 50 mg/kg, 2/4 cures) and artesunate (30% worm burden reduction, 2/5 cures) were exceptions. Given that acyl sulfonamide **6** was the only compound with efficacy equal to that

of **1** at the 100 mg/kg dose, it was tested at the lower dose of 50 mg/kg and compared to existing data¹² for **1**. At 50 mg/kg, **1** and **6** reduced worm burdens by 53 and 17%, respectively, but only **1** cured (2/4) the infected rats. Interestingly, previous SAR^{18,26,29,42} reveals that acidic ozonides have relatively weak antimalarial activities, so that it may be possible to identify an ozonide with selectivity for inhibition of *F. hepatica*. However, **1** and **6** are clearly less effective than triclabendazole; at 10 mg/kg, the latter reduced burden reduction by 95% and cured 3/4 infected rats.¹⁶

Summary

These data indicate that relatively small structural changes to **1** led, in most cases, to substantial, if not complete, loss of activity against *F. hepatica* in vivo. A peroxide bond, spiroadamantane substructure, and acidic functional group (or prodrug) were required for activity. Although **1** seems to be an 'optimized' ozonide structure for efficacy against *F. hepatica*, its peroxide-dependent activity suggests that, like antimalarial peroxides,¹⁴ its efficacy depends upon hemoglobin digestion in *F. hepatica*. The mechanistic basis for the superior efficacy of ozonide acids is unclear, but it may derive from a favorable distribution of the unionized forms to the low pH milieu⁴³ of the trematode gut, the site of hemoglobin digestion. Indeed, in an electron microscopy study⁴⁴ of ex vivo *F. hepatica*, **1** caused extensive damage to the gut. Investigation of the flukicidal properties of other peroxy heterocycles is in progress.

Experimental Section

General

Melting points are uncorrected. ¹H and ¹³C NMR spectra were recorded on a 500 MHz spectrometer. All chemical shifts are reported in parts per million (ppm) and are relative to internal (CH₃)₄Si (0 ppm) for ¹H and CDCl₃ (77.0 ppm), CD₃OD (49.0 ppm), or DMSO-*d*₆ (39.7 ppm) for ¹³C NMR. Combustion analysis confirmed that all target compounds possessed purities ≥ 95%.

cis-Adamantane-2-spiro-3'-8'-[[[(methylsulfonyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane (**6**)

Ozonide **6** was synthesized following the method of Tsandi et al.¹⁷ To a solution of **1** (1.61 g, 5.0 mmol), methanesulfonamide (951 mg, 10.0 mmol), and DMAP (611 mg, 5.0 mmol) in CH₂Cl₂ (40 mL) was added DCC (1.14 g, 5.5 mmol). After 24 h, the reaction mixture was filtered and 1 M aq. HCl was added to the filtrate. After the aqueous layer was extracted CH₂Cl₂ (2 × 30 mL), the combined CH₂Cl₂ extracts were dried over MgSO₄, filtered, and evaporated in vacuo to produce a residue that was purified by chromatography (sg, 10:1 CH₂Cl₂:MeOH) to afford **6** (1.05 g, 72%) as a white solid. Mp 131–133 °C; ¹H NMR (CDCl₃) δ 1.26–1.30 (m, 3H), 1.68–1.96 (m, 20H), 2.22 (d, *J* = 6.4 Hz, 2H), 3.32 (s, 3H), 8.26 (s, 1H); ¹³C NMR (CDCl₃) δ 26.40, 26.79, 29.78, 32.82, 33.77, 34.74, 36.32, 36.72, 41.63, 42.88, 108.16, 111.50, 170.96. Anal. (C₁₉H₂₉NO₆S) C, H, N.

cis-Adamantane-2-spiro-3'-8'-[[[(carboxymethyl)amino]carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane (**7**)

Step 1. To a solution of *cis*-adamantane-2-spiro-3'-8'-[[[(1'-H-benzotriazol-1'-yloxy)carbonyl]methyl]-1',2',4'-trioxaspiro[4.5]decane (**28**)¹⁸ (2.00 g, 4.6 mmol) and glycine ethyl ester hydrochloride (762 mg, 5.4 mmol) in CH₂Cl₂ (60 mL) was added DIPEA (1.29 g, 10 mmol). The resulting mixture was stirred at rt for 2 h before quenching with water (30 mL). After separation of the water layer, the organic layer was washed with water (4 × 30 mL), dried over MgSO₄, filtered, and concentrated in vacuo. The residue was crystallized from 1:5 EtOH:H₂O to afford *cis*-adamantane-2-spiro-3'-8'-[[[(ethoxycarbonylmethyl)amino]

carbonyl)methyl]-1',2',4'-trioxaspiro[4.5]decane (1.50 g, 81%) as a white solid. Mp 157–159 °C; ¹H NMR (CDCl₃) δ 1.23–1.30 (m, 5H), 1.68–1.99 (m, 21H), 2.13 (d, *J* = 6.8 Hz, 2H), 4.03 (d, *J* = 4.9 Hz, 2H), 4.22 (q, *J* = 7.3 Hz, 2H), 5.97 (brs, 1H); ¹³C NMR (CDCl₃) δ 14.10, 26.42, 26.79, 29.94, 33.47, 33.92, 34.73, 36.31, 36.74, 41.29, 43.10, 61.53, 108.50, 111.30, 169.95, 172.11. **Step 2.** To a solution of *cis*-adamantane-2-spiro-3'-8'-[[[(ethoxycarbonylmethyl) amino] carbonyl)methyl]-1',2',4'-trioxaspiro[4.5]decane (1.01 g, 2.5 mmol) in EtOH (80 mL) was added a solution of NaOH (198 mg, 5.0 mmol) in water (15 mL). The mixture was stirred for 16 h at rt, evaporated to give an oil, and acidified with 1 M aq. HCl to pH = 5. After the aqueous phase was extracted with ethyl acetate (4 × 50 mL), the EtOAc extracts were combined, dried over MgSO₄, filtered, and evaporated to give an oil that was crystallized from CHCl₃ to afford **7** (752 mg, 80%) as a white solid. Mp 146–148 °C; ¹H NMR (DMSO-*d*₆) δ 1.04–1.12 (m, 2H), 1.62–1.89 (m, 21H), 2.02 (d, *J* = 6.8 Hz, 2H), 3.71 (d, *J* = 5.9 Hz, 2H), 8.15 (t, *J* = 5.9 Hz, 1H), 12.44 (brs, 1H); ¹³C NMR (DMSO-*d*₆) δ 26.00, 26.41, 29.65, 33.07, 33.64, 34.45, 35.94, 36.28, 40.72, 41.82, 108.62, 110.66, 171.60, 171.79. Anal. (C₂₀H₂₉NO₆) C, H, N.

***cis*-Adamantane-2-spiro-3'-8'-[[[(2'-sulfoethyl)amino]carbonyl)methyl]-1',2',4'-trioxaspiro[4.5]decane sodium salt (8)**

To a solution of **28**¹⁸ (1.50 g, 3.4 mmol) and taurine (387 mg, 3.1 mmol) in THF (200 mL) was added a solution of NaOH (247 mg, 6.2 mmol) in water (15 mL). After stirring for 24 h at rt, an additional portion of **28** (44 mg, 0.1 mmol) was added. After further stirring for 24 h at rt, the solvents were removed in vacuo to produce an oil that was purified by reverse phase chromatography (C18, 1:1 MeOH:H₂O) to afford **8** (1.23 g, 80%) as a white solid. Mp 154–156 °C; ¹H NMR (DMSO-*d*₆) δ 1.02–1.10 (m, 2H), 1.61–1.93 (m, 23H), 2.53 (t, *J* = 7.9 Hz, 2H), 3.28 (td, *J* = 7.9, 6.9 Hz, 2H), 7.73 (s, 1H); ¹³C NMR (DMSO-*d*₆) δ 26.02, 26.42, 29.71, 33.02, 33.66, 34.46, 35.64, 35.95, 36.30, 42.38, 50.88, 108.63, 110.63, 170.91. Anal. (C₂₀H₃₀NNaO₇S) C, H, N.

***cis*-Adamantane-2-spiro-3'-8'-(2'-oxo-2'-hydrazinoethyl)-1',2',4'-trioxaspiro[4.5]decane (10)**

To a stirred solution of **2** (0.68 g, 2 mmol) in MeOH (10 mL) and THF (5 mL) was added hydrazine monohydrate (3.0 g, 60 mmol). The resulting mixture was heated at 50–60 °C for 24 h, then cooled to rt and concentrated. The residue was dissolved in EtOAc (100 mL), washed with water (50 mL) and brine (50 mL), dried over MgSO₄, and filtered. After removal of the solvent, the crude product was purified by crystallization from 5:1 CH₂Cl₂:EtOH to afford **10** (0.56 g, 83%) as a colorless solid. Mp 124–126 °C; ¹H NMR (CDCl₃) δ 1.15–1.35 (m, 2H), 1.61–2.02 (m, 21H), 2.03 (d, *J* = 6.8 Hz, 2H), 3.55–4.09 (m, 2H), 6.76 (s, 1H); ¹³C NMR (CDCl₃) δ 26.46, 26.85, 29.99, 33.36, 33.91, 34.77, 36.37, 36.77, 41.23, 108.43, 111.40, 172.77. Anal. (C₁₈H₂₈N₂O₄) C, H, N.

***cis*-Adamantane-2-spiro-3'-8'-dicarboxymethyl-1',2',4'-trioxaspiro[4.5]decane (14)**

Step 1. A mixture of 1,4-cyclohexanedione monoethylene ketal (31.2 g, 200 mmol), isopropylidene malonate (32.4 g, 220 mmol), molecular sieves, 4 Å (9 g), and pyridine (300 mL) was stirred at rt for 2 d under Ar. The reaction suspension was filtered and the filtrate was concentrated in vacuo. The residue was dissolved in CH₂Cl₂ (500 mL), washed with 1 M HCl (2 × 150 mL) and water (3 × 150 mL). The CH₂Cl₂ layer was dried over MgSO₄ and evaporated in vacuo to give 5-(1,4-dioxaspiro[4.5]dec-8-ylidene)-2,2-dimethyl-1,3-dioxane-4,6-dione (**29**)¹⁹ (39.1 g, 69%) as a red powder. ¹H NMR (CDCl₃) δ 1.75 (s, 6H), 1.91 (t, *J* = 6.6 Hz, 4H), 3.15 (t, *J* = 6.6 Hz, 4H), 4.00 (s, 4H); ¹³C NMR (CDCl₃) δ 26.9, 30.5, 35.4, 64.6, 103.8, 106.9, 115.2, 160.8, 178.6. **Step 2.** A solution of crude **29** (39.1 g, 138.5 mmol) and 10% Pd-C (1.0 g) in CH₂Cl₂ (450 mL) was hydrogenated at 500 psi at rt for 1 d. After filtration, the filtrate was concentrated in vacuo to give crude 5-(1,4-dioxaspiro[4.5]dec-8-yl)-2,2-dimethyl-1,3-dioxane-4,6-dione (38.8 g, 99%) which was used for the next step. An analytical

sample was obtained by flash chromatography (sg, 50% ether in hexane) as a yellow powder. Mp 145–148 °C dec.; ^1H NMR (CDCl_3) δ 1.55–1.65 (m, 4H), 1.75 (s, 3H), 1.77 (s, 3H), 1.80 (s, 1H), 1.82 (s, 1H), 1.97–2.05 (m, 2H), 2.41–2.46 (m, 1H), 3.45 (d, J = 2.9 Hz, 1H), 3.95 (s, 4H); ^{13}C NMR (CDCl_3) δ 26.4, 27.4, 28.2, 34.7, 37.6, 50.2, 64.2, 64.3, 104.8, 107.8, 164.8.

Step 3. A solution of 5-(1,4-dioxaspiro[4.5]dec-8-yl)-2,2-dimethyl 1,3-dioxane-4,6-dione (38.8 g, 136.6 mmol) and pyridinium *p*-toluenesulfonate (5.0 g, 20 mmol) in acetone (400 mL) was refluxed for 20 h. The reaction solution was concentrated and then dissolved in CH_2Cl_2 (300 mL), washed with water (3×100 mL), dried over MgSO_4 , and evaporated to dryness.

Due to the poor yield of the deprotection and the difficulty of separation, the above process was repeated four times to produce crude 5-(4-oxocyclohexyl)-2,2-dimethyl-1,3-dioxane-4,6-dione (9.0 g, 27%) which was used for the next step. An analytical sample was obtained by flash chromatography (sg, 60% ether in hexane) as a white powder. Mp 152–154 °C dec.; ^1H NMR (CDCl_3) δ 1.77 (s, 3H), 1.81 (s, 3H), 1.94–1.99 (m, 2H), 2.18–2.27 (m, 2H), 2.37–2.48 (m, 4H), 2.88–2.95 (m, 1H), 3.61 (d, J = 2.9 Hz, 1H); ^{13}C NMR (CDCl_3) δ 27.0, 28.1, 28.2,

36.2, 40.7, 49.8, 105.0, 164.3 and 209.9. **Step 4.** A solution of 5-(4-oxocyclohexyl)-2,2-dimethyl-1,3-dioxane-4,6-dione (9.0 g, 37.5 mmol), MeOH (300 mL), Et₂O (300 mL) and conc. H_2SO_4 (2.0 mL, 37.6 mmol) was refluxed overnight. The reaction mixture was cooled to rt and concentrated in vacuo. The residue was dissolved in CH_2Cl_2 (150 mL) and washed with water (3×100 mL). The CH_2Cl_2 layer was dried over MgSO_4 , filtered, and evaporated to dryness. The residue was purified by flash chromatography (sg, 50% ether in hexane) to give 4-[bis(methoxycarbonyl)methyl]cyclohexanone (**30**) (3.2 g, 37%) as a white solid. Mp 59–60 °C; ^1H NMR (CDCl_3) δ 1.53–1.61 (m, 2H), 2.06–2.09 (m, 2H), 2.39–2.42 (m, 4H), 2.54–2.60 (m, 1H), 3.33 (d, J = 8.8 Hz, 1H), 3.76 (s, 6H); ^{13}C NMR (CDCl_3) δ 29.9, 35.8, 40.1,

52.4, 56.1, 168.4, 210.2. **Step 5.** A suspension of **30** (3.2 g, 14 mmol), *O*-methyl 2-adamantanone oxime (**31**)²² (5.0 g, 28 mmol), NaHCO_3 (no product was formed without the addition of solid NaHCO_3) (1.7 g, 20 mmol) in CH_2Cl_2 (80 mL) and cyclohexane (240 mL) was treated with ozone at 0 °C for 2 h. The reaction solution was concentrated and the residue was purified by flash chromatography (sg, 6% ether in hexane) to give *cis*-adamantane-2-spiro-3'-8'-[bis(methoxycarbonyl)methyl]-1',2',4'-trioxaspiro[4.5]decane (**32**) (4.2 g, 75%) as colorless crystals. Mp 94–95 °C (ethanol); ^1H NMR (CDCl_3) δ 1.29–1.37 (m, 2H), 1.68–1.99 (m, 20H), 2.09–2.16 (m, 1H), 3.19 (d, J = 8.8 Hz, 1H), 3.73 (s, 6H); ^{13}C NMR (CDCl_3) δ 26.4, 26.8, 27.7, 33.7, 34.7, 36.2, 36.3, 36.7, 52.4, 56.9, 108.0, 111.4 and 168.9. **Step 6.** To a solution of **32** (1.0 g, 2.5 mmol) in THF (50 mL) was added a solution of KOH (1.12 g, 20 mmol) in H₂O (3.0 mL). After stirring at rt for 12 h, the reaction solution was concentrated. The residue was dissolved in EtOAc (100 mL) and H₂O (50 mL) and acidified with 1 M HCl to pH = 2. The EtOAc layer was separated, washed with H₂O (3×50 mL), dried over MgSO_4 , and evaporated in vacuo to give *cis*-adamantane-2-spiro-3'-8'-(dicarboxymethyl)-1',2',4'-trioxaspiro[4.5]decane (**14**) (0.81 g, 87%) as a white powder. Mp 152–153 °C dec.; ^1H NMR ($\text{DMSO}-d_6$) δ 1.16–1.25 (m, 2H), 1.65–1.93 (m, 21H), 3.02 (d, J = 8.8 Hz, 1H), 12.7 (s, 2H); ^{13}C NMR ($\text{DMSO}-d_6$) δ 26.0, 26.5, 27.5, 33.6, 34.5, 35.3, 36.0, 36.3, 57.1, 108.4, 110.8, 170.2. Anal. ($\text{C}_{19}\text{H}_{26}\text{O}_7$) C, H.

***cis*-Adamantane-2-spiro-3'-8'-[[ethoxy]hydroxyphosphinyl]methyl]-1',2',4'-trioxaspiro[4.5]decane potassium salt (16)**

Step 1. A solution of 8-(bromomethyl)-1,4-dioxaspiro[4.5]decane²⁴ (10 g, 42.5 mmol) in EtOH (70 mL) and 6 M aq. HCl (15 mL, 90 mmol) was stirred overnight at rt. After addition of saturated aq. NaHCO_3 to bring the pH to 8, most of the solvents were removed in vacuo and water (100 mL) and ether (100 mL) were added. After separation of the ether layer, the aqueous phase was extracted with ether (3×100 mL). The combined organic phases were dried and evaporated to give an oil that was purified by sg chromatography (sg, 10% EtOAc in hexane) to afford 4-(bromomethyl)cyclohexanone (6.0 g, 74%) as a colorless oil. ^1H NMR (CDCl_3) δ 1.51–1.59 (m, 2H), 2.11–2.18 (m, 1H), 2.20–2.24 (m, 3H), 2.34–2.45 (m, 4H), 3.39 (d, J =

6.3 Hz, 2H); ^{13}C NMR (CDCl_3) δ 31.00, 37.83, 38.29, 40.02, 210.74. **Step 2.** According to the method of Yamagishi et al.,²³ a mixture of 4-(bromomethyl)cyclohexanone (3.0 g, 15.7 mmol) and triethyl phosphite (10.4 g, 62.8 mmol) was heated to 170 °C for 8 h. After cooling, saturated aq. NaHCO_3 was added to raise the pH to 8. The aq. phase was extracted with CH_2Cl_2 (3 $\times$ 100 mL). The combined organic extracts were dried with MgSO_4 , filtered, and evaporated to give an oil that was purified with chromatography (sg, 50% EtOAc and hexane to 100% EtOAc) to afford diethyl [(4-oxocyclohexyl)methyl]phosphonate (**33**) (1.0 g, 26%). ^1H NMR (CDCl_3) δ 1.33–1.36 (t, J = 7.3 Hz, 6H), 1.52–1.56 (m, 2H), 1.75–1.80 (dd, J = 18.6, 7.4 Hz, 1H), 2.23–2.26 (m, 3H), 2.37–2.40 (m, 4H), 4.08–4.16 (m, 4H); ^{13}C NMR (CDCl_3) δ 16.46 (d, J = 5.8 Hz), 31.21 (d, J = 3.8 Hz), 31.54 (d, J = 140.6 Hz), 33.79 (d, J = 11.0 Hz), 40.54, 61.51 (d, J = 6.7 Hz). **Step 3.** A solution of **31**²² (11.06 g, 61.7 mmol) and **33** (7.0 g, 41.1 mmol) in cyclohexane (200 mL) and CH_2Cl_2 (40 mL) was treated with ozone following the method of Dong et al.²⁸ After removal of solvents in vacuo, the crude product was purified by chromatography (sg, 50% EtOAc in hexane) to afford *cis*-adamantane-2-spiro-3'-8'-[(diethoxyphosphinyl)methyl]-1',2',4'-trioxaspiro[4.5]decane (**34**) (10.83 g, 78%) as an oil. ^1H NMR (CDCl_3) δ 1.29–1.34 (m, 8H), 1.65–1.99 (m, 23H), 4.04–4.13 (m, 4H); ^{13}C NMR (CDCl_3) δ 16.44 (d, J = 5.0 Hz), 26.47, 26.86, 31.15, 31.36 (d, J = 11.4 Hz), 32.10 (d, J = 133.2 Hz), 33.96, 34.77, 36.37, 36.79, 61.37 (d, J = 6.4 Hz), 108.27, 111.35. **Step 4.** According to the method of Dziemidowicz et al.,²⁵ a mixture of **34** (333 mg, 0.8 mmol) and potassium trimethylsilanolate (412 mg, 3.2 mmol) in anhydrous THF (12 mL) was stirred at 50 °C for 4 h. Removal of the solvent gave a residue that was purified by reverse phase chromatography (C18, 1:1 $\text{CH}_3\text{OH}:\text{H}_2\text{O}$) to afford **16** (264 mg, 85%) as a white solid. Mp 160–162 °C; ^1H NMR (CDCl_3) δ 1.21–1.23 (m, 5H), 1.44–1.48 (m, 2H), 1.61–1.99 (m, 21H), 3.81 (m, 2H); ^{13}C NMR (CDCl_3) δ 16.66 (d, J = 6.7 Hz), 26.45, 26.84, 31.49 (d, J = 9.6 Hz), 32.10 (d, J = 2.2 Hz), 33.00 (d, J = 130.0 Hz), 34.10, 34.73, 36.35, 36.77, 60.28 (d, J = 4.6 Hz), 108.60, 111.11. Anal. ($\text{C}_{19}\text{H}_{30}\text{KO}_6\text{P}$) C, H.

Adamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxa-8'-azaspiro[4.5]decane sodium salt (**17**)

Step 1. To a suspension of adamantane-2-spiro-3'-8'-*t*-butoxycarbonyl-1',2',4'-trioxa-8'-azaspiro[4.5]decane (**35**)²⁶ (1.462 g, 4.0 mmol) in THF (10 mL) was added dropwise a solution of methanesulfonic acid (1.54 g, 16.0 mmol) in CH_3CN (2 mL) at rt and the mixture was stirred at rt for 24 h. A solution of K_2CO_3 (2.211 g, 16.0 mmol) in H_2O (6 mL) and a solution of ethyl bromoacetate (802 mg, 4.8 mmol) in THF (12 mL) were added. After the reaction was stirred for 6 h, the solvents were removed and the residue was diluted with EtOAc (40 mL) and H_2O (30 mL). After phase separation, the aqueous phase was extracted with EtOAc (3 $\times$ 30 mL). The combined organic layers were dried over MgSO_4 , filtered, and concentrated in vacuo. The crude product was purified by sg chromatography (sg, 20% EtOAc in hexane) to afford adamantane-2-spiro-3'-8'-ethoxycarbonylmethyl-1',2',4'-trioxa-8'-azaspiro[4.5]decane (**36**) (1.26 g, 90 %) as an oil. ^1H NMR (CDCl_3) δ 1.28 (t, J = 6.8 Hz, 3H), 1.67–2.03 (m, 18H), 2.58–2.62 (m, 2H), 2.71–2.75 (m, 2H), 3.23 (s, 2H), 4.19 (q, J = 6.8 Hz, 2H); ^{13}C NMR (CDCl_3) δ 14.07, 26.26, 26.66, 34.08, 34.54, 34.60, 36.17, 36.56, 50.96, 58.94, 60.43, 106.44, 111.47, 170.21. **Step 2.** To a solution of **36** (517 mg, 1.47 mmol) in EtOH (50 mL) was added a solution of NaOH (117 mg, 2.94 mmol) in water (10 mL). After the mixture was stirred for at rt for 2 h, the resulting precipitate was collected by filtration, washed with H_2O (5 mL), and dried in vacuo at 40 °C to give **17** (305 mg, 84%) as a white solid. Mp 140–142 °C; ^1H NMR (CD_3OD) δ 1.71–2.05 (m, 18H), 2.56 (m, 2H), 2.67 (m, 2H), 2.98 (s, 2H); ^{13}C NMR (CD_3OD) δ 27.96, 28.36, 35.06, 35.75, 35.83, 37.81, 37.83, 52.38, 63.29, 108.06, 112.57, 177.62. Anal. ($\text{C}_{17}\text{H}_{24}\text{NNaO}_5$) C, H, N.

***trans*-Adamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (18)**

Step 1. To a solution of methyl 2-(4-oxocyclohexyl)acetate (**37**) (5.106 g, 30 mmol) in EtOH (100 mL) was added pyridine (3.559 g, 45 mmol) followed by methoxyamine hydrochloride (2.756 g, 33 mmol). The reaction mixture was stirred at rt for 4.5 h, concentrated in vacuo, and diluted with CH₂Cl₂ (50 mL) and water (50 mL). The organic phase was separated, and the aqueous layer was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layers were washed with 1 M HCl (40 mL), saturated aqueous NaHCO₃ (40 mL) and brine (40 mL), and dried over MgSO₄. Removal of the solvents in vacuo afforded methyl 2-[4-(methoxyimino)cyclohexyl]acetate (**38**) (5.02 g, 84%) as an oil. ¹H NMR (CDCl₃) δ 1.12–1.28 (m, 2H), 1.68–2.20 (m, 6H), 2.26 (d, *J* = 7.3 Hz, 2H), 2.37–2.41 (m, 1H), 3.16–3.21 (m, 1H), 3.68 (s, 3H), 3.81 (s, 3H); ¹³C NMR (CDCl₃) δ 23.96, 31.09, 31.35, 32.58, 33.85, 40.50, 51.47, 60.96, 158.88, 172.92. **Step 2.** A solution of 2-adamantanone (2.25 g, 14.4 mmol) and **38** (1.91 g, 9.59 mmol) in cyclohexane (50 mL) and CH₂Cl₂ (10 mL) was treated with ozone according to the method of Dong et al.²⁸ After removal of the solvents in vacuo, the crude product was purified by flash chromatography (sg, 100:1 hexane:ethyl acetate) to afford *trans*-adamantane-2-spiro-3'-8'-methoxycarbonylmethyl-1',2',4'-trioxaspiro[4.5]decane (**39**) (680 mg, 21%) as an oil. ¹H NMR (CDCl₃) δ 1.35–1.43 (m, 2H), 1.58–2.03 (m, 21H), 2.27 (d, *J* = 6.8 Hz, 2H), 3.67 (s, 3H); ¹³C NMR (125.7 MHz, CDCl₃) δ 26.38, 26.81, 29.65, 33.27, 33.61, 34.64, 34.83, 36.28, 36.70, 40.41, 51.43, 108.35, 111.51, 173.19. **Step 3.** To a solution of **39** (402 mg, 1.2 mmol) in EtOH (10 mL) was added a solution of NaOH (143 mg, 3.6 mmol) in water (3 mL). The mixture was stirred at 60 °C for 3 h and evaporated to give an oil. The residue was cooled to 0 °C and treated with 1 M HCl to pH = 3. The resulting solid was filtered, washed with water and dried in vacuo to afford **18** (346 mg, 90%) as a white solid. Mp 144–146 °C; ¹H NMR (CDCl₃) δ 1.38–1.45 (m, 2H), 1.60–2.03 (m, 21H), 2.31 (d, *J* = 6.8 Hz, 2H); ¹³C NMR (CDCl₃) δ 26.43, 26.85, 29.66, 33.10, 33.65, 34.70, 34.88, 36.32, 36.74, 40.31, 108.33, 111.65, 178.66. Anal. (C₁₈H₂₆O₅) C, H.

***cis*-adamantane-2-spiro-3'-6'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (19)**

Step 1. A solution of *O*-methyl 2-adamantanone oxime (**31**)²² (896 mg, 5.0 mmol) and ethyl 2-(2-oxocyclohexyl)acetate (**40**) (1.38 g, 7.5 mmol) in cyclohexane (30 mL) and CH₂Cl₂ (6 mL) was treated with ozone according to the method of Dong et al.²⁸ After removal of the solvents in vacuo, the crude product was purified by flash chromatography (sg, 40:1 hexane:ethyl acetate) to afford *cis*-adamantane-2-spiro-3'-6'-ethoxycarbonylmethyl-1',2',4'-trioxaspiro[4.5]decane (**41**) (380 mg, 22%) as an oil. ¹H NMR (CDCl₃) δ 1.25–1.31 (m, 5H), 1.40–2.40 (m, 23H), 2.76 (dd, *J* = 15.6, 2.4 Hz, 1H), 4.12–4.18 (m, 2H); ¹³C NMR (CDCl₃) δ 23.50, 26.18, 26.59, 27.27, 33.80, 33.94, 34.57, 35.16, 35.65, 36.10, 36.52, 36.73, 39.07, 39.26, 46.77, 60.18, 109.58, 111.26, 172.98. **Step 2.** To a solution of **41** (350 mg, 1.0 mmol) in EtOH (8 mL) was added a solution of NaOH (120 mg, 3.0 mmol) in water (3 mL). The mixture was stirred at rt for 3 h, and concentrated in vacuo to afford an oil that was cooled to 0 °C and treated with 1 M HCl to pH = 3. The resulting solid was filtered, washed with water, and dried in vacuo to afford **19** (320 mg, 93%) as a white solid. Mp 66–69 °C; ¹H NMR (CDCl₃) δ 1.29–1.39 (m, 2H), 1.44–2.37 (m, 23H), 2.84 (dd, *J* = 6.1, 3.9 Hz, 1H); ¹³C NMR (CDCl₃) δ 23.61, 24.30, 26.29, 26.72, 29.66, 33.80, 34.07, 34.72, 34.90, 35.34, 35.83, 36.63, 36.89, 39.24, 109.65, 111.62, 178.90. Anal. (C₁₈H₂₆O₅) C, H.

***trans*-adamantane-2-spiro-3'-7'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (20)**

Step 1. A solution of **31**²² (1.44 g, 8.0 mmol) and methyl 2-(3-oxocyclohexyl)acetate (**42**)⁴⁵ (1.14 g, 6.7 mmol) in cyclohexane (30 mL) and CH₂Cl₂ (10 mL) was treated with ozone according to the method of Dong et al.²⁸ After removal of the solvents in vacuo, the crude product was purified by column chromatography (sg, 50:1 hexane:ethyl acetate) to afford *trans*-adamantane-2-spiro-3'-7'-methoxycarbonylmethyl-1',2',4'-trioxaspiro[4.5]decane (**43**)

(998 mg, 45%) as an oil. ^1H NMR (CDCl_3) δ 0.93–1.00 (m, 1H), 1.40–2.09 (m, 22H), 2.20–2.29 (m, 2H), 3.66 (s, 3H); ^{13}C NMR (CDCl_3) δ 22.44, 26.30, 26.70, 30.76, 32.32, 34.02, 34.56, 34.59, 36.20, 36.58, 40.38, 40.70, 51.16, 108.44, 110.99, 172.39. **Step 2.** To a solution of **43** (627 mg, 1.8 mmol) in EtOH (16 mL) was added a solution of NaOH (224 mg, 5.6 mmol) in water (4 mL) and the mixture was stirred at 60 °C for 3 h. Removal of the solvents afforded an oil that was cooled to 0 °C and treated with 1 M HCl to pH = 3. The resulting solid was filtered, washed with water, and dried in vacuo to afford **20** (567 mg, 94%) as a white solid. Mp 66–68 °C; ^1H NMR (CDCl_3) δ 0.95–1.02 (m, 1H), 1.42–2.08 (m, 22H), 2.22–2.33 (m, 2H); ^{13}C NMR (CDCl_3) δ 22.61, 26.44, 26.83, 30.86, 32.29, 34.18, 34.76, 34.78, 36.35, 36.76, 40.53, 40.96, 108.65, 111.35, 178.46. Anal. ($\text{C}_{18}\text{H}_{26}\text{O}_5$) C, H.

3-Carboxymethyl-7,14,15-trioxadispiro[5.1.5.2]pentadecane (21)

Step 1. A solution of *O*-methyl cyclohexanone oxime (**44**)³⁰ (2.16 g, 17 mmol) and **37** (3.47 g, 20.4 mmol) in cyclohexane (120 mL) and CH_2Cl_2 (30 mL) was treated with ozone following the method of Dong et al.²⁸ After removal of solvents in vacuo, the crude product was purified by chromatography (sg, 4% EtOAc in hexane) followed by crystallization from cold MeOH to afford 3-methoxycarbonylmethyl-7,14,15-trioxadispiro[5.1.5.2]pentadecane (**45**) (2.4 g, 50%, 4:1 mixture of two isomers based on the ^1H NMR doublets at 2.22 and 2.26) as a white solid. Mp 110–112 °C; ^1H NMR (CDCl_3) δ 1.27 (m, 2H), 1.36 (m, 1H), 1.43 (m, 1H), 1.56–1.82 (m, 13H), 1.94 (d, J = 15.6 Hz, 2H), 2.22 (d, J = 7.5 Hz, 1.6H), 2.26 (d, J = 7.0 Hz, 0.4H), 3.67 (s, 3H); ^{13}C NMR (CDCl_3) δ 22.46, 22.58, 23.72, 23.77, 24.82, 29.55, 29.84, 33.08, 33.22, 33.50, 33.76, 34.54, 40.36, 40.59, 51.46, 108.32, 108.86, 109.24, 171.11, 173.21. **Step 2.** To a solution of **45** (678 mg, 2.24 mmol) in EtOH (50 mL) was added a solution of NaOH (180 mg, 4.48 mmol) in water (10 mL). The mixture was stirred at rt for 12 h, cooled to 0 °C, and treated with 1 M aq. HCl (5 mL) and H_2O (50 mL). The precipitate was collected by filtration, washed with 50% aq. EtOH (10 mL), and dried in vacuo at 40 °C to give **21** (450 mg, 74%, 2.3:1 mixture of two isomers based on the ^1H NMR doublets at 2.27 and 2.30) as a colorless solid. Mp 144–146 °C; ^1H NMR (CDCl_3) δ 1.24–1.47 (m, 4H), 1.54–1.95 (m, 15H), 2.27 (d, J = 6.8 Hz, 1.4H), 2.30 (d, J = 6.8 Hz, 0.6H); ^{13}C NMR (CDCl_3) δ 23.74, 23.79, 24.84, 24.87, 29.52, 29.80, 32.88, 33.01, 33.51, 33.76, 34.56, 40.28, 40.41, 40.51, 108.27, 108.28, 108.96, 109.33, 178.76. Anal. ($\text{C}_{14}\text{H}_{22}\text{O}_5$) C, H.

Bicyclo[3.3.1]nonane-9-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (22)

Step 1. A solution of *O*-methyl bicyclo[3.3.1]nonan-9-one oxime (**46**)²⁸ (792 mg, 4.7 mmol) and **37** (1.21 g, 7.1 mmol) in cyclohexane (30 mL) and CH_2Cl_2 (50 mL) was treated with ozone following the method of Dong et al.²⁸ After removal of the solvents, the crude product was purified by column chromatography (sg, 50:1 hexane:ethyl acetate) to afford bicyclo[3.3.1]nonane-9-spiro-3'-8'-methoxycarbonylmethyl-1',2',4'-trioxaspiro[4.5]decane (**47**) (807 mg, 53%) as a white solid. Mp 82–84 °C; ^1H NMR (CDCl_3) δ 1.22–2.06 (m, 23H), 2.22 (d, J = 7.3 Hz, 2H), 3.67 (s, 3H); ^{13}C NMR (CDCl_3) δ 20.41, 20.82, 29.33, 29.52, 29.87, 33.10, 33.94, 36.20, 40.60, 51.40, 108.27, 111.22, 173.15. **Step 2.** To a solution of **47** (785 mg, 2.4 mmol) in EtOH (20 mL) was added a solution of NaOH (290 mg, 7.3 mmol) in water (3 mL). The mixture was stirred at 60 °C for 3 h, evaporated to give an oil, cooled to 0 °C, and treated with 1 M HCl to pH = 3. The resulting solid was filtered, washed with water, and dried in vacuo to afford **22** (651 mg, 87%) as a white solid. Mp 146–148 °C; ^1H NMR (CDCl_3) δ 1.25–1.33 (m, 2H), 1.44–1.52 (m, 2H), 1.64–2.07 (m, 19H), 2.27 (d, J = 7.3 Hz, 2H); ^{13}C NMR (CDCl_3) δ 20.44, 20.84, 29.36, 29.54, 29.84, 32.91, 33.95, 36.21, 40.55, 108.24, 111.35, 178.79. Anal. ($\text{C}_{17}\text{H}_{26}\text{O}_5$) C, H.

***trans, cis*-5-Fluoroadamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5]decane (23)**

Step 1. To a stirred solution of 5-hydroxy-2-adamantanone ethylene ketal (**48**)³² (100 mg, 0.5 mmol) in CH₂Cl₂ (6 mL) at 0 °C was added bis(2-methoxyethyl)aminosulfur trifluoride (158 mg, 0.7 mmol). After 1 h of stirring, the reaction was quenched with water (2 mL) and the aq. phase was extracted with CH₂Cl₂ (2 × 15 mL). The combined organic layers were dried with MgSO₄, filtered, and concentrated in vacuo to afford a residue that was purified by crystallization from 1:1 MeOH:H₂O to afford 5-fluoro-2-adamantanone ethylene ketal (**49**) (81 mg, 80%) as a white solid. Mp 44–46 °C; ¹H NMR (CDCl₃) δ 1.55–1.57 (d, 2H), 1.73–1.75 (m, 2H), 1.89–1.92 (m, 4H), 2.01 (s, 2H), 2.18–2.22 (m, 3H), 3.92–3.98 (m, 4H); ¹³C NMR (CDCl₃) δ 29.80 (d, *J* = 10.1 Hz), 33.23 (d, *J* = 1.9 Hz), 38.76 (d, *J* = 10.6 Hz), 39.39 (d, *J* = 19.2 Hz), 42.30 (d, *J* = 16.8 Hz), 64.32, 64.48, 91.56 (d, *J* = 183.8 Hz), 109.74. **Step 2.** To a solution of **49** in 2:1 acetone:water (12 mL) was added conc. HCl (4 mL) and the reaction mixture was stirred for 1 h at rt before removal of the solvents in vacuo. CH₂Cl₂ (6 mL) and water (6 mL) were added and the two phases were separated followed by extraction of the aq. phase with CH₂Cl₂ (2 × 4 mL). The combined organic layers were dried over MgSO₄ and the solvent removed in vacuo to afford 5-fluoro-2-adamantanone (**50**) (55 mg, 86%) as a colorless solid. Mp 269–271 °C; ¹H NMR (CDCl₃) δ 1.66–2.44 (m, 11H), 2.68 (s, 2H); ¹³C NMR (125.7 MHz, CDCl₃) δ 30.43 (d, *J* = 10.1 Hz), 37.94 (d, *J* = 2.4 Hz), 41.55 (d, *J* = 17.8 Hz), 42.05 (d, *J* = 20.2 Hz), 47.05 (d, *J* = 10.1 Hz), 90.18 (d, *J* = 185.7 Hz), 214.92 (d, *J* = 1.9 Hz). **Step 3.** To a solution of **50** (800 mg, 4.8 mmol) in EtOH (40 mL) was added pyridine (564 mg, 7.1 mmol) followed by methoxylamine hydrochloride (477 mg, 5.7 mmol). The reaction mixture was stirred at rt for 2 h, concentrated in vacuo, and diluted with water (10 mL). After filtration, *O*-methyl 5-fluoro-2-adamantanone oxime (**51**) was obtained as a white solid (773 mg, 82%). Mp 70–72 °C; ¹H NMR (CDCl₃) δ 1.70–2.06 (m, 10H), 2.37 (d, *J* = 2.0 Hz, 1H), 2.78 (s, 1H), 3.69 (s, 1H), 3.82 (s, 3H); ¹³C NMR (CDCl₃) δ 30.78 (d, *J* = 10.0 Hz), 30.96 (d, *J* = 9.6 Hz), 36.08 (d, *J* = 2.4 Hz), 37.50 (d, *J* = 1.9 Hz), 37.90 (d, *J* = 10.6 Hz), 41.45 (d, *J* = 18.7 Hz), 41.74 (d, *J* = 17.3 Hz), 42.69 (d, *J* = 18.7 Hz), 61.12, 91.23 (d, *J* = 185.2 Hz), 162.81 (d, *J* = 2.4 Hz). **Step 4.** A solution of **51** (918 mg, 4.66 mmol) and **37** (1.19 g, 6.99 mmol) in cyclohexane (40 mL) and CH₂Cl₂ (20 mL) was treated with ozone according to the method of Dong et al.²⁸ After removal of solvents, the crude product was purified by crystallization from EtOH to afford *trans, cis*-5-fluoroadamantane-2-spiro-3'-8'-methoxycarbonylmethyl-1',2',4'-trioxaspiro[4.5]decane (**52**) (450 mg, 27%, 3:1 mixture of diastereomers) as a white solid. Mp 126–128 °C; ¹H NMR (CDCl₃) δ 1.23–1.28 (m, 2H), 1.55–1.95 (m, 15H), 2.18–2.23 (m, 7H), 3.67 (s, 3H); ¹³C NMR (CDCl₃) δ 29.40 (d, *J* = 10.1 Hz), 29.80, 32.99, 33.14 (d, *J* = 1.9 Hz), 33.75, 38.76 (d, *J* = 11.4 Hz), 39.20 (d, *J* = 19.7 Hz), 40.54, 41.97 (d, *J* = 17.3 Hz), 51.49, 91.20 (d, *J* = 184.3 Hz), 109.01, 109.46, 173.12. **Step 5.** To a solution of **52** (450 mg, 1.27 mmol) in EtOH (10 mL) was added a solution of NaOH (152 mg, 3.81 mmol) in water (4 mL). The mixture was stirred for 4 h at 55 °C and evaporated to give an oil. The residue was cooled to 0 °C and treated with 1 M aq. HCl (4 mL) and CH₂Cl₂ (30 mL). After separation of the organic layer, the aqueous phase was extracted with CH₂Cl₂ (2 × 20 mL). The combined organic phase was washed with saturated aqueous NaHCO₃ (2 × 20 mL) and brine (20 mL), dried over MgSO₄, filtered, and evaporated to give **23** (266 mg, 62%, 5:1 mixture of diastereomers) as a white solid. Mp 147–149 °C; ¹H NMR (CDCl₃) δ 1.24–1.32 (m, 2H), 1.55–1.93 (m, 15H), 2.19–2.28 (m, 7H); ¹³C NMR (CDCl₃) δ 29.40 (d, *J* = 9.6 Hz), 29.74, 32.78, 33.15 (d, *J* = 1.9 Hz), 33.73, 38.77 (d, *J* = 10.6 Hz), 39.21 (d, *J* = 19.7 Hz), 40.47, 41.97 (d, *J* = 17.3 Hz), 91.23 (d, *J* = 184.7 Hz), 108.93, 109.53, 178.80. Anal. (C₁₈H₂₅FO₅) C, H.

***cis*-6-Fluoroadamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5] decane (24)**

Step 1. Sodium borohydride (1.11 g, 29.2 mmol) was added portion-wise to a ice-cold solution of 2,6-adamantandione monoethylene ketal (**53**)³³ (1.74 g, 8.35 mmol) in MeOH (100 mL) at

such a rate as to keep the internal temperature below 10 °C. The reaction mixture was allowed to warm to rt and stirred for 24 h. After quenching with water (10 mL), the solvents were removed in vacuo and the residue was partitioned between CH₂Cl₂ (80 mL) and water (60 mL). After phase separation, the aq. phase was extracted with CH₂Cl₂ (3 × 40 mL). The combined organic phases were dried over MgSO₄, filtered, and concentrated in vacuo to afford 6-hydroxy-2-adamantanone monoethylene ketal (**54**) (1.6 g, 91%) as a white solid. Mp 128–130 °C; ¹H NMR (CDCl₃) δ 1.62–2.08 (m, 13H), 3.80 (s, 1H), 3.95 (s, 4H); ¹³C NMR (CDCl₃) δ 28.21, 33.29, 33.39, 35.24, 35.83, 64.19, 73.55, 110.95. **Step 2.** To a solution of **54** (808 mg, 3.8 mmol) in CH₂Cl₂ (40 mL) was added bis(2-methoxyethyl)aminosulfur trifluoride (850 mg, 3.8 mmol) and the reaction mixture was stirred at rt for 24 h before quenching with water (30 mL). The aqueous phase was extracted with CH₂Cl₂ (2 × 40 mL) and the combined organic phases were washed with saturated aqueous NaHCO₃ (2 × 30 mL) and brine (30 mL), dried over MgSO₄, and concentrated in vacuo to afford crude 6-fluoro-2-adamantanone monoethylene ketal (**55**) (540 mg, 66%) as a white solid. Mp 45–47 °C; ¹H NMR (CDCl₃) δ 1.59 (d, *J* = 12.7 Hz, 2H), 1.74 (s, 2H), 1.79 (d, *J* = 12.7 Hz, 2H), 2.01–2.11 (m, 6H), 3.94 (s, 4H), 4.59 (d, *J* = 50.8 Hz, 1H); ¹³C NMR (CDCl₃) δ 28.40 (*J* = 1.0 Hz), 31.34 (*J* = 18.2 Hz), 32.37 (*J* = 8.7 Hz), 34.92 (*J* = 1.4 Hz), 35.45, 64.11, 94.17 (*J* = 179.5 Hz), 110.29. **Step 3.** To a solution of **55** in 2:1 acetone:water (12 mL) was added conc. HCl (4 mL) and the reaction mixture was stirred at 70 °C for 1 h. After removal of the solvents in vacuo, CH₂Cl₂ (6 mL) and water (6 mL) were added. After phase separation, the aq. phase was extracted with CH₂Cl₂ (2 × 4 mL). The combined organic phases were dried over MgSO₄ and concentrated in vacuo to afford 6-fluoro-2-adamantanone (**56**) (55 mg, 86%) as a white solid. Mp 230–232 °C; ¹H NMR (CDCl₃) δ 1.81–2.49 (m, 12H), 4.85 (dt, *J* = 49.8, 3.4 Hz, 1H); ¹³C NMR (CDCl₃) δ 31.73 (*J* = 18.7 Hz), 32.68 (*J* = 1.4 Hz), 36.42 (*J* = 8.6 Hz), 44.67 (*J* = 1.9 Hz), 45.48, 92.72 (*J* = 180.9 Hz), 215.95. **Step 4.** To a solution of **56** (336 mg, 2.0 mmol) in EtOH (25 mL) was added pyridine (316 mg, 3.0 mmol) followed by methoxylamine hydrochloride (200 mg, 2.4 mmol). The reaction mixture was stirred at rt for 6 h, concentrated in vacuo, and diluted with CH₂Cl₂ (50 mL) and water (50 mL). The organic phase was separated and the aqueous layer was extracted with CH₂Cl₂ (3 × 30 mL). The combined organic phases were washed with 1 M HCl (30 mL), saturated aqueous NaHCO₃ (30 mL), and brine (30 mL) and dried over MgSO₄. Removal of the solvents in vacuo afforded *O*-methyl 6-fluoro-2-adamantanone oxime (**57**) (370 mg, 94%, 1:1 mixture of diastereomers) as a white solid. Mp 70–72 °C; ¹H NMR (CDCl₃) δ 1.61–2.49 (m, 11H), 3.41–3.44 (m, 1H), 3.81 (s, 1.5H), 3.82 (s, 1.5H), 4.71–4.82 (m, 1H); ¹³C NMR (CDCl₃) δ 31.24 (*J* = 1.0 Hz), 32.30 (*J* = 18.2 Hz), 32.36 (*J* = 18.2 Hz), 34.36, 35.00, 35.28 (*J* = 8.6 Hz), 36.53 (*J* = 8.6 Hz), 60.96, 44.67 (*J* = 1.9 Hz), 45.48, 92.72 (*J* = 180.9 Hz), 215.95. **Step 5.** A solution of **57** (370 mg, 1.9 mmol) and **37** (479 mg, 2.8 mmol) in cyclohexane (30 mL) and CH₂Cl₂ (60 mL) was treated with ozone according to the method of Dong et al.²⁸ After removal of solvents in vacuo, the crude product was purified by crystallization from cold EtOH to afford *cis*-6-fluoroadamantane-2-spiro-3'-8'-methoxycarbonylmethyl-1',2',4'-trioxaspiro[4.5]decane (**58**) (250 mg, 38%) as a white solid. Mp 114–116 °C; ¹H NMR (CDCl₃) δ 1.23–1.30 (m, 2H), 1.58–2.13 (m, 19H), 2.22 (d, *J* = 7.3 Hz, 1H), 3.67 (s, 3H), 4.59 (d, *J* = 50.8 Hz); ¹³C NMR (CDCl₃) δ 28.51 (*J* = 1.0 Hz), 28.57, 29.84 (*J* = 3.8 Hz), 31.02 (*J* = 18.2 Hz), 31.43 (*J* = 18.2 Hz), 32.38 (*J* = 4.3 Hz), 32.45 (*J* = 4.3 Hz), 33.07, 33.83 (*J* = 2.9 Hz), 34.86 (*J* = 1.4 Hz), 35.43, 40.57, 51.46, 93.90 (*J* = 179.5 Hz), 108.74, 110.32, 173.15. **Step 6.** To a solution of **58** (250 mg, 0.7 mmol) in EtOH (12 mL) was added a solution of NaOH (85 mg, 2.1 mmol) in water (3 mL). The mixture was stirred at 60 °C for 3 h and concentrated in vacuo. The residue was cooled to 0 °C and treated with 1 M aq. HCl (5 mL) and H₂O (10 mL). The precipitate was collected by filtration and dried in vacuo at 40 °C to afford **24** (237 mg, 99%) as a white solid. Mp 144–146 °C; ¹H NMR (CDCl₃) δ 1.24–1.32 (m, 2H), 1.59–2.10 (m, 19H), 2.27 (d, *J* = 6.8 Hz, 1H), 4.60 (d, *J* = 50.8 Hz); ¹³C NMR (CDCl₃) δ 28.52, 28.57, 29.78 (*J* = 3.4 Hz), 30.99 (*J* = 18.2 Hz), 31.41 (*J* = 18.2 Hz), 32.37 (*J* = 3.8 Hz), 32.44 (*J* = 3.8 Hz), 32.85, 33.80 (*J* = 2.9 Hz), 34.83, 35.40, 40.44, 93.93 (*J* = 179.0 Hz), 108.66, 110.39, 178.46. Anal. (C₁₈H₂₅FO₅) C, H.

***cis*-6,6-Difluoroadamantane-2-spiro-3'-8'-carboxymethyl-1',2',4'-trioxaspiro[4.5] decane (25)**

Step 1. To a solution of 2,6-adamantanedione monoethylene ketal (**53**)³³ (1.17 g, 5.6 mmol) in CH₂Cl₂ (85 mL) was added bis(2-methoxyethyl)aminosulfur trifluoride (2.27 g, 10.3 mmol) and the reaction mixture was stirred at rt for 24 h before quenching with water (50 mL). The aqueous phase was separated and extracted with CH₂Cl₂ (2 × 40 mL). The combined organic layers were washed with saturated aqueous NaHCO₃ (2 × 40 mL) and brine (40 mL), dried over MgSO₄, filtered, and concentrated in vacuo to give a residue that was purified by flash chromatography (sg, 40:1 hexane:ethyl acetate) to afford 6,6-difluoro-2-adamantanone monoethylene ketal (**59**) (1.10 g, 85%) as a colorless solid. Mp 76–78 °C; ¹H NMR (CDCl₃) δ 1.77 (s, 2H), 1.87–1.90 (d, *J* = 12.7 Hz, 4H), 2.00–2.02 (d, *J* = 12.2 Hz, 4H), 2.11 (s, 2H), 3.95 (s, 4H); ¹³C NMR (CDCl₃) δ 30.76 (*J* = 3.8 Hz), 34.58 (*J* = 21.6 Hz), 34.87, 64.37, 109.31, 124.43 (*J* = 246.6 Hz). **Step 2.** To a solution of **59** (1.1 g, 4.78 mmol) in 5:1 acetone:water (96 mL) was added conc. HCl (40 mL) and the reaction mixture was heated to 70 °C gradually. After the disappearance of the starting material as determined by GC-MS (0.5 h), the mixture was cooled to 0 °C and NaHCO₃ was added to adjust the pH to 7. After CH₂Cl₂ (80 mL) was added to the mixture, the organic layer was separated. The aq. phase was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic layers were dried over MgSO₄, filtered, and concentrated in vacuo to afford 6,6-difluoro-2-adamantanone (**60**) as a white solid (760 mg, 85%). Mp 252–255 °C; ¹H NMR (CDCl₃) δ 2.01 (d, *J* = 12.7 Hz, 4H), 2.29 (d, *J* = 12.7 Hz, 4H), 2.35 (s, 2H), 2.53 (s, 2H); ¹³C NMR (CDCl₃) δ 34.86 (*J* = 3.8 Hz), 35.06 (*J* = 22.1 Hz), 44.47, 123.15 (*J* = 247.6 Hz), 214.28. **Step 3.** To a solution of **60** (760 mg, 4.1 mmol) in EtOH (50 mL) was added pyridine (483 mg, 6.1 mmol) followed by methoxylamine hydrochloride (409 mg, 4.9 mmol). The reaction mixture was stirred at rt for 6 h, concentrated in vacuo, and diluted with CH₂Cl₂ (50 mL) and water (50 mL). The organic phase was separated, and the aqueous layer was extracted with CH₂Cl₂ (3 × 50 mL). The combined organic phases were washed with 1 M HCl (40 mL), saturated aqueous NaHCO₃ (40 mL) and brine (40 mL), and dried over MgSO₄. Removal of the solvent in vacuo afforded-methyl 6,6-difluoro-2-adamantanone oxime (**61**) (760 mg, 87%) as a white solid. Mp 114–116 °C; ¹H NMR (CDCl₃) δ 1.80–1.89 (m, 4H), 2.09–2.18 (m, 4H), 2.30 (s, 1H), 2.52 (s, 1H), 3.46 (s, 1H), 3.82 (s, 1H); ¹³C NMR (CDCl₃) δ 27.22, 33.59 (*J* = 3.4 Hz), 34.06, 34.87 (*J* = 3.4 Hz), 35.53 (*J* = 3.4 Hz), 61.10, 123.90 (*J* = 247.1 Hz), 162.97. **Step 4.** A solution of **61** (460 mg, 2.1 mmol) and **37** (546 mg, 3.2 mmol) in cyclohexane (30 mL) and CH₂Cl₂ (6 mL) was treated with ozone according to the method of Dong et al.²⁸ After removal of solvents in vacuo, the crude product was purified by crystallization from cold EtOH to afford *cis*-6,6-difluoroadamantane-2-spiro-3'-8'-methoxycarbonylmethyl-1',2', '-trioxaspiro[4.5]decane (**62**) (322 mg, 41%) as a white solid. mp 129–131 °C; ¹H NMR (CDCl₃) δ 1.20–1.29 (m, 2H), 1.69–2.14 (m, 19H), 2.23 (d, *J* = 6.8 Hz, 2H), 3.70 (s, 3H); ¹³C NMR (CDCl₃) δ 30.65 (*J* = 3.8 Hz), 30.68 (*J* = 3.4 Hz), 32.99, 34.11 (*J* = 21.6 Hz), 34.52 (*J* = 21.6 Hz), 34.57, 40.49, 51.47, 108.94, 109.08, 123.94 (*J* = 247.1 Hz), 173.11. **Step 5.** To a solution of **62** (322 mg, 0.87 mmol) in EtOH (8 mL) was added a solution of NaOH (104 mg, 2.6 mmol) in water (3 mL). The mixture was stirred at rt for 4 h and evaporated to give an oil. After the residue was treated with 1 M HCl to lower the pH to 3, the aqueous phase was extracted with CH₂Cl₂ (3 × 50 mL). The combined extracts were dried over MgSO₄, filtered, and evaporated to afford **25** (277 mg, 89%) as a white solid. Mp 148–150 °C; ¹H NMR (CDCl₃) δ 1.29–1.37 (m, 2H), 1.75–2.20 (m, 19H), 2.23 (d, *J* = 6.8 Hz, 2H); ¹³C NMR (125.7 MHz, CDCl₃) δ 29.72, 30.67 (*J* = 3.8 Hz), 30.70 (*J* = 3.8 Hz), 32.79, 33.73, 34.12 (*J* = 21.6 Hz), 34.54 (*J* = 21.6 Hz), 34.58, 40.53, 108.87, 109.16, 123.96 (*J* = 247.1 Hz), 179.20. Anal. (C₁₈H₂₄F₂O₅) C, H.

Adamantane-2-spiro-3'-8'-carboxymethyl-1',2'-dioxaspiro[4.5]decane (26)

Step 1. To a solution of I₂ (0.254 g, 1.0 mmol) and 50% H₂O₂ (4.5 mL, 40 mmol) in MeOH (50 mL) was added **37** (1.70 g, 10 mmol). After the mixture was stirred at rt for 24 h, it was concentrated in vacuo and the residue was partitioned between CH₂Cl₂ (30 mL) and water (30

mL). The aq. layer was extracted with CH₂Cl₂ (2 × 30 mL). The combined extracts were washed with water, brine, dried over MgSO₄, filtered, and concentrated to afford methyl 2-(4-hydroperoxy-4-methoxycyclohexyl)acetate as a 1:1 mixture of diastereomers (2.15 g, 99%) which was used immediately in the next step. ¹H NMR (CDCl₃) δ 0.92–2.46 (m, 11H), 3.30 (s, 1.5H), 3.34 (s, 1.5H), 3.70 (s, 3H), 7.42 (s, 0.5H), 7.52 (s, 0.5H). **Step 2.** To a solution of the unpurified methyl 2-(4-hydroperoxy-4-methoxycyclohexyl)acetate (2.15 g, 9.86 mmol) in DMF (100 mL) at 0 °C was added Et₃N (4.5 mL, 32 mmol) followed by Et₃SiOTf (2.54 mL, 12 mmol). The reaction mixture was stirred at rt for 24 h and then diluted with ice-cold hexane (100 mL) and ice-water (100 mL). The organic layer was separated and the aq. layer was extracted with hexane (3 × 100 mL). The organic extracts were combined, dried over MgSO₄ and concentrated to afford methyl 2-[4-methoxy-4-(triethylsilylperoxy)cyclohexyl]acetate (**63**) as a 1:1 mixture of diastereomers (3.02 g, 92%) which was used immediately in the next step. ¹H NMR (CDCl₃) δ 0.68–0.80 (m, 6H), 0.94–1.08 (m, 9H), 0.84–2.44 (m, 11H), 3.26 (s, 1.5H), 3.29 (s, 1.5H), 3.67 (s, 3H). **Step 3.** To a solution of **63** (3.02 g, 9.10 mmol) in CH₂Cl₂ (50 mL) at –78 °C was added 2-methyleneadamantane⁴⁶ (**64**) (0.67 g, 4.53 mmol) followed by 1M SnCl₄ in CH₂Cl₂ (10 mL, 10 mmol). The resulting mixture was stirred at –78 °C for 30 min and then kept at –30 °C overnight. The reaction mixture was allowed to warm to –3 °C and quenched with ice-water (50 mL). After separation of the organic layer, the aqueous layer was extracted with CH₂Cl₂ (2 × 50 mL). The combined extracts were washed with water (50 mL) and brine (50 mL), dried over MgSO₄, filtered, and concentrated in vacuo. Purification by chromatography (sg, 0 to 10 % ether in hexane) afforded adamantane-2-spiro-3'-8'-methoxycarbonylmethyl-1',2'-dioxaspiro[4.5]decane (**65**) as a colorless solid (0.60 g, 40%). Mp 119–120 °C; ¹H NMR (CDCl₃) δ 1.24–1.36 (m, 2H), 1.44–1.96 (m, 17H), 1.95–2.02 (m, 2H), 2.06–2.14 (m, 2H), 2.13 (s, 2H), 2.20 (d, *J* = 7.5 Hz, 2H), 3.66 (s, 3H); ¹³C NMR (CDCl₃) δ 26.44, 26.99, 29.07, 33.45, 34.93, 35.64, 36.24, 37.21, 41.17, 51.42, 55.47, 84.02, 88.71, 173.47. **Step 4.** To a solution of **65** (0.45 g, 1.35 mmol) in EtOH (20 mL) was added 15% aq. KOH (2 mL) and the resulting mixture was stirred at 60 °C for 20 h. The solution was concentrated to ~5 mL and the residue was diluted with water (10 mL) and acidified with acetic acid (5 mL). The precipitate was collected by filtration, washed with cold water, and dried in vacuo at 40 °C to afford **26** as a colorless solid (0.40 g, 93%). Mp 184–185 °C; ¹H NMR (CDCl₃) δ 1.27–1.40 (m, 2H), 1.42–1.96 (m, 17H), 1.95–2.04 (m, 2H), 2.06–2.14 (m, 2H), 2.13 (s, 2H), 2.24 (d, *J* = 7.5 Hz, 2H), 11.14 (brs, 1H); ¹³C NMR (CDCl₃) δ 26.44, 26.99, 29.00, 33.26, 33.45, 34.90, 35.64, 36.24, 37.20, 41.04, 55.46, 83.98, 88.75, 178.74. Anal. (C₁₉H₂₈O₄) C, H.

Adamantane-2-spiro-2'-8'-carboxymethyl-1',4'-dioxaspiro[4.5]decane (**27**)

p-Toluenesulfonic acid monohydrate (260 mg, 1.37 mmol) was added to a solution of 2-hydroxymethyl-2-adamantanol (**66**)³⁷ (1.22 g, 6.69 mmol) and 2-(4-oxocyclohexyl)acetic acid (**67**) (1.14 g, 7.29 mmol) in CH₂Cl₂ (200 mL). The reaction mixture was stirred at rt for 4 h, washed with water (100 mL) and brine (100 mL), dried over MgSO₄, filtered and concentrated in vacuo. The crude product was purified by crystallization from aq. MeOH to afford **27** as a white solid (1.69 g, 79%, 1.3:1 mixture of isomers based on the ¹H NMR singlets at 3.84 and 3.88). Mp 133–135 °C; ¹H NMR (CDCl₃) δ 1.25–1.85 (m, 21H), 2.18 (q, 2H), 2.26 (d, *J* = 6.8 Hz, 0.78H), 2.29 (d, *J* = 6.8 Hz, 1.11H), 3.84 (s, 0.87H), 3.88 (s, 1.13H); ¹³C NMR (CDCl₃) δ 26.67, 26.70, 26.79, 26.93, 30.01, 30.05, 33.08, 33.46, 33.64, 35.82, 35.91, 35.96, 36.33, 37.25, 37.34, 37.41, 40.66, 40.74, 72.14, 72.32, 84.33, 84.48, 108.35, 108.46, 178.79, 178.89. Anal. (C₁₉H₂₈O₄) C, H.

Evaluation of activity against *F. hepatica*

All animal studies³⁹ presented here were approved by regulatory authorities following Swiss National regulations. Metacercariae of *F. hepatica* were purchased from Baldwin Aquatics (Monmouth, OR, USA). Female Wistar rats (weight: ~100 g) were purchased from Harlan

(Horst, The Netherlands). Animals were kept in groups of 4 in Macrolon cages in environmentally-controlled conditions (temperature: ~25°C; humidity: ~70%; 12 h light/dark cycle) and acclimatized for 1 week. Rats were infected with ~20 metacercariae each. They had free access to water and rodent diet. At eight weeks post-infection, rats were treated with single 50–100 mg/kg oral doses of target compounds prepared as suspensions in 7% (v/v) Tween 80 and 3% (v/v) EtOH. At day 6 post-treatment, rats were sacrificed by CO₂ and adult flukes were recovered from the bile ducts and livers. Target compound efficacies were evaluated by comparing the mean total worm burdens of treated and untreated control rats. Statistical significance (at a significance level of 5%) was calculated using the Kruskal Wallis test (Statsdirect v 2.4.5; Cheshire, United Kingdom).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This investigation received financial support from NIH (R21AI076783), the Nebraska Research Initiative, the Swiss National Science Foundation (project no. PPOOA-114941), and the Medicines for Malaria Venture.

Abbreviations

AS	artesunate
DIPEA	diisopropylethylamine
DMAP	dimethylamino pyridine
DCC	dicyclohexylcarbodiimide
DMF	dimethyl formamide
EDCI	1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride
HOBt	1-hydroxybenzotriazole
<i>p</i> -TSA	<i>p</i> -toluenesulfonic acid

References and Notes

1. Fenwick A, Keiser J, Utzinger J. Epidemiology, Burden and Control of Schistosomiasis with Particular Consideration to Past and Current Treatment Trends. *Drug Fut* 2006;31:413–425.
2. Keiser J, Utzinger J. Food-Borne Trematodiasis. *Clin Microbiol Rev* 2009;22:466–483. [PubMed: 19597009]
3. Rojas L, Vazquez A, Domenech I, Robertson LJ. Fascioliasis: Can Cuba Conquer this Emerging Parasitosis? *Trends Parasitol* 2010;26:26–34. [PubMed: 19910253]
4. Keiser J, Utzinger J. Emerging Foodborne Trematodiasis. *Emerg Infect Dis* 2005;11:1507–1514. [PubMed: 16318688]
5. Keiser J, Utzinger J. Advances in the Discovery and Development of Trematocidal Drugs. *Expert Opin Drug Discov* 2007;2:S9–S23.
6. Hien TT, Truong NT, Minh NH, Dat HD, Dung NT, Hue NT, Dung TK, Tuan PQ, Campbell JI, Farrar JJ, Day JN. A Randomized Controlled Pilot Study of Artesunate versus Triclabendazole for Human Fascioliasis in Central Vietnam. *Am J Trop Med Hyg* 2008;78:388–392. [PubMed: 18337331]
7. Keiser J, Utzinger J. Artemisinins and Synthetic Trioxolanes in the Treatment of Helminth Infections. *Curr Opin Infect Dis* 2007;20:605–612. [PubMed: 17975411]
8. Keiser J, Xiao SH, Dong Y, Utzinger J, Vennertstrom JL. Clonorchicidal Properties of the Synthetic Trioxolane OZ78. *J Parasitol* 2007;93:1209–1214.

9. Xiao SH, Keiser J, Chollet J, Utzinger J, Dong Y, Vennerstrom JL, Endriss Y, Tanner M. In Vitro and In Vivo Activities of Synthetic Trioxolanes against Major Human Schistosome Species. *Antimicrob Agents Chemother* 2007;51:1440–1445. [PubMed: 17283188]
10. Robert A, Benoit-Vical F, Claparols C, Meunier B. The antimalarial drug artemisinin alkylates heme in infected mice. *Proc Natl Acad Sci USA* 2005;102:13676–13680. [PubMed: 16155128]
11. Keiser J, Brun R, Fried B, Utzinger J. Trematocidal Activity of Praziquantel and Artemisinin Derivatives: In Vitro and In Vivo Investigations on Adult *Echinostoma caproni*. *Antimicrob Agents Chemother* 2006;50:803–805. [PubMed: 16436751]
12. Keiser J, Utzinger J, Tanner M, Dong Y, Vennerstrom JL. The Synthetic Peroxide OZ78 is Effective Against *Echinostoma caproni* and *Fasciola hepatica*. *J Antimicrob Chemother* 2006;58:1193–1197. [PubMed: 17028093]
13. Keiser J, Shu-Hua X, Tanner M, Utzinger J. Artesunate and Artemether are Effective Fasciolicides in the Rat Model and In Vitro. *J Antimicrob Chemother* 2006;57:1139–1145. [PubMed: 16581915]
14. Kaiser M, Wittlin S, Nehrbass-Stuedli A, Dong Y, Wang X, Hemphill A, Matile H, Brun R, Vennerstrom JL. Peroxide Bond-Dependent Antiplasmodial Specificity of Artemisinin and OZ277 (RBx11160). *Antimicrob Agents Chemother* 2007;51:2991–2993. [PubMed: 17562801]
15. Keiser J, Utzinger J. Food-borne Trematodiasis: Current Chemotherapy and Advances with Artemisinins and Synthetic Trioxolanes. *Trends Parasitol* 2007;23:555–562. [PubMed: 17950667]
16. Keiser J, Utzinger J, Vennerstrom JL, Dong Y, Brennan G, Fairweather I. Activity of Artemether and OZ78 against Triclabendazole-resistant *Fasciola hepatica*. *Trans R Soc Trop Med Hyg* 2007;101:1219–1222. [PubMed: 17905370]
17. Tsandi E, Kokotos CG, Kousidou S, Ragoussis V, Kokotos G. Sulfonamides of Homoproline and Dipeptides as Organocatalysts for Michael and Aldol reactions. *Tetrahedron* 2009;65:1444–1449.
18. Dong Y, Wittlin S, Sriraghavan K, Chollet J, Charman SA, Charman WN, Scheurer C, Urwyler H, Santo Tomas J, Snyder C, Creek DJ, Morizzi J, Koltun M, Matile H, Wang X, Padmanilayam M, Tang Y, Dorn A, Brun R, Vennerstrom JL. The Structure-Activity Relationship of the Antimalarial Ozonide Arterolane (OZ277). *J Med Chem* 2010;53:481–491. [PubMed: 19924861]
19. Mock GA, Holmes AB, Raphael RA. Spirocyclic synthesis by [4+2]-cycloaddition: the preparation of 11,13-dioxo-1,4-dithia-12, 12,18-trimethyl-10,14,17-trioxotrispiro[4.2.0.5.4.2]eicosane. *Tetrahedron Lett* 1977;18:4539–4540.
20. The successful Knoevenagel condensation of 1,4-cyclohexanedione monoethylene ketal and dimethylmalonate with TiCl_4 and pyridine in a CCl_4/THF mixed solvent was reported by Itoh T, Wanibe T, Iwatsuki S. Synthesis and Polymerization of 7,7-Bis(alkoxycarbonyl)-1,4-benzoquinone Methides. *J Polymer Sci A: Polymer Chem* 1996;34:963–969.
21. Griesbaum K, Liu X, Kassiaris A, Scherer M. Ozonolyses of *O*-Alkylated Ketoximes in the Presence of Carbonyl Groups: A Facile Access to Ozonides. *Libigs Ann/Recueil* 1997:1381–1390.
22. Dong Y, Vennerstrom JL. Dispiro-1,2,4,5-Tetraoxanes via Ozonolysis of Cycloalkanone *O*-Methyl Oximes: A Comparison with the Peroxidation of Cycloalkanones in Acetonitrile Sulfuric Acid Media. *J Org Chem* 1998;63:8582–8585.
23. Yamagishi T, Fujii K, Shibuya S, Yokomatsu T. Asymmetric Synthesis of Phosphonic Acid Analogues for Acylcarnitine. *Tetrahedron* 2006;62:54–65.
24. Rew Y, McMin DL, Wang Z, He X, Hungate RW, Jaen JC, Sudom A, Sun D, Tu H, Ursu S, Villemure E, Walker NPC, Yan X, Ye Q, Powers JP. Discovery and optimization of piperidyl benzamide derivatives as a novel class of 11 β -HSD1 inhibitors. *Bioorg Med Chem Lett* 2009;19:1797–1801. [PubMed: 19217779]
25. Dziemidowicz J, Witt D, Sliwka-Kaszynska M, Rachon J. Potassium Trimethylsilanolate-Mediated Conversion of Dialkyl Phosphonates to Their Anhydrous Potassium Monoalkyl Phosphonates Under Mild, Non-Aqueous Conditions. *Synthesis* 2005;4:569–574.
26. Padmanilayam M, Scorneaux B, Dong Y, Chollet J, Matile H, Charman SA, Creek DJ, Charman WN, Santo Tomas J, Scheurer C, Wittlin S, Brun R, Vennerstrom JL. Antimalarial Activity of *N*-Alkylamine, Carboxamide, Sulfonamide, and Urea Derivatives of a Dispiro-1,2,4-Trioxolane Piperidine. *Bioorg Med Chem Lett* 2006;16:5542–5545. [PubMed: 16931006]

27. Tang Y, Dong Y, Karle JM, DiTusa CA, Vennerstrom JL. Synthesis of Tetrasubstituted Ozonides by the Griesbaum Cozonolysis Reaction: Diastereoselectivity and Functional Group Transformations by Post-Ozonolysis Reactions. *J Org Chem* 2004;69:6470–6473. [PubMed: 15357611]
28. Dong Y, Chollet J, Matile H, Charman SA, Chiu FCK, Charman WN, Scoreaux B, Urwyler H, Santo Tomas J, Scheurer C, Snyder C, Dorn A, Wang X, Karle JM, Tang Y, Wittlin S, Brun R, Vennerstrom JL. Spiro and Dispiro-1,2,4-Trioxolanes as Antimalarial Peroxides: Charting a Workable SAR Using Simple Prototypes. *J Med Chem* 2005;48:4953–4961. [PubMed: 16033274]
29. Tang Y, Dong Y, Wittlin S, Charman SA, Chollet J, Chiu FCK, Charman WN, Matile H, Urwyler H, Dorn A, Bajpai S, Wang X, Padmanilayam M, Karle JM, Brun R, Vennerstrom JL. Weak Base Dispiro-1,2,4-Trioxolanes: Potent Antimalarial Ozonides. *Bioorg Med Chem Lett* 2007;17:1260–1265. [PubMed: 17189686]
30. Marques CA, Selva M, Tundo P, Montanari F. Reaction of Oximes with Dimethyl Carbonate: A New Entry to 3-Methyl-4,5-disubstituted-4-oxazolin-2-ones. *J Org Chem* 1993;58:5765–5770.
31. Zhou L, Alker A, Ruf A, Wang X, Chiu FCK, Morizzi J, Charman SA, Charman WN, Scheurer C, Wittlin S, Dong Y, Hunziker D, Vennerstrom JL. Characterization of the Two Major CYP450 Metabolites of Ozonide (1,2,4-trioxolane) OZ277. *Bioorg Med Chem Lett* 2008;18:1555–1558. [PubMed: 18262417]
32. Lantvoev VI. Synthesis of 4-Substituted Adamantane-1-carboxylic acids. *Zhurnal Organicheskoi Khimii* 1976;12(11):2361–2368.
33. Ayres FD, Khan SI, Chapman OL, Kaganove SN. Improvements in the Synthesis of Adamantane-2,6-dione and Preparation of the Novel Adamantane-2,6-dione *mono*-ketal. *Tetrahedron Lett* 1994;35:7151–7154.
34. Zmitek K, Zupan M, Stavber S, Iskra J. Iodine as a Catalyst for Efficient Conversion of Ketones to *gem*-Dihydroperoxides by Aqueous Hydrogen Peroxide. *Org Lett* 2006;8:2491–2494. [PubMed: 16737296]
35. Wang X, Dong Y, Wittlin S, Creek D, Chollet J, Charman SA, Santo Tomas J, Scheurer C, Snyder C, Vennerstrom JL. Spiro and Dispiro-1,2-dioxolanes: Contribution of Iron(II)-mediated One-electron vs. Two-electron Reduction to the Activity of Antimalarial Peroxides. *J Med Chem* 2007;50:5840–5847. [PubMed: 17949067]
36. Ramirez A, Woerpel KA. Synthesis of 1,2-Dioxolanes by Annulation Reactions of Peroxycarbenium Ions with Alkenes. *Org Lett* 2005;7:4617–4620. [PubMed: 16209493]
37. Farcasiu D, Schleyer PVR, Ledlie DB. Ring Enlargements by Thallium(III) Oxidation of Double Bonds. Application to Adamantane Systems. *J Org Chem* 1973;38:3455–3459.
38. Tang Y, Wittlin S, Charman SA, Chollet J, McClennan DN, Chiu FCK, Johnson LM, Santo Tomas J, Scheurer C, Snyder C, Zhou L, Dong Y, Charman WN, Matile H, Urwyler H, Dorn A, Vennerstrom JL. The Comparative Antimalarial Properties of Weak Base and Neutral Synthetic Ozonides. *Bioorg Med Chem Lett* 2010;20:563–566. [PubMed: 19962893]
39. Keiser J. In Vitro and In Vivo Trematode Models for Chemotherapeutic Studies. *Parasitology* 2010;137:589–603. [PubMed: 19961653]
40. Vennerstrom JL, Arbe-Barnes S, Brun R, Charman SA, Chiu FCK, Chollet J, Dong Y, Dorn A, Hunziker D, Matile H, McIntosh K, Padmanilayam M, Santo Tomas J, Scheurer C, Scoreaux B, Tang Y, Urwyler H, Wittlin S, Charman WN. Identification of an Antimalarial Synthetic Trioxolane Drug Development Candidate. *Nature* 2004;430:900–904. [PubMed: 15318224]
41. Erhardt S, Macgregor SA, McCullough KJ, Savill K, Taylor BJ. Model Studies of β -Scission Ring-Opening Reactions of Cyclohexyloxy Radicals: Application to Thermal Rearrangements of Dispiro-1,2,4-trioxanes. *Org Lett* 2007;9:5569–5572. [PubMed: 18044912]
42. Dong Y, Tang T, Chollet J, Matile H, Wittlin S, Charman SA, Charman WN, Santo Tomas J, Scheurer C, Snyder C, Scoreaux B, Bajpai S, Alexander SA, Wang X, Padmanilayam M, Rao CS, Brun R, Vennerstrom JL. Effect of Functional Group Polarity on the Antimalarial Activity of Spiro and Dispiro-1,2,4-Trioxolanes. *Bioorg Med Chem* 2006;14:6368–6382. [PubMed: 16759871]
43. Lowther J, Robinson MW, Donnelly SM, Xu W, Stack CM, Matthews JM, Dalton JP. The Importance of pH in Regulating the Function of the *Fasciola hepatica* Cathepsin L1 Cysteine Protease. *PLoS Negl Trop Dis* 2009;3:e369. [PubMed: 19172172]

44. Halferty L, O'Neill JF, Brennan GP, Keiser J, Fairweather I. Electron Microscopical Study to Assess the In Vitro Effects of the Synthetic Trioxolane OZ78 Against the Liver Fluke, *Fasciola hepatica*. *Parasitol* 2009;136:1325–1337.
45. Schmoldt P, Mattay J. A Versatile Synthesis of Bicyclo[2.2.2]octan-2-one Derivatives. *Synthesis* 2003:1071–1078.
46. Hughes L, Ingold KU, Walton JC. Cycloalkylmethyl Radicals. 6. The Unexpectedly High Barrier to the Rotation of Axial CH₂ · Groups in Cyclohexylmethyl Radicals. *J Am Chem Soc* 1988;110:7494–7499.

Scheme 1a.

^aReagents and conditions: (a) MeSO₂NH₂, DMAP, DCC, CH₂Cl₂, rt, 24 h, then 1 M HCl; (b) hydrazine hydrate, MeOH/THF, 60 °C, 24 h; (c) glycine ethyl ester HCl, DIPEA, CH₂Cl₂, rt, 24 h; (d) aq. NaOH, EtOH, then 1 M HCl to pH 5; (e) taurine, THF/aq. NaOH.

Scheme 2a.

^aReagents and conditions: (a) 4 Å mol. sieves, pyridine, rt, 2d; (b) H₂, 10% Pd-C, CH₂Cl₂, rt, 24 h; (c) MeOH/Et₂O, H₂SO₄, reflux, 12 h; (d) O₃, CH₂Cl₂/cyclohexane, 0 °C, solid NaHCO₃, 2 h; (e) 1 N KOH/THF, rt, 12 h, then 1 M HCl.

Scheme 3a.

^aReagents and conditions: (a) O₃, CH₂Cl₂/cyclohexane, 0 °C; (b) Me₃SiOK, THF, 50 °C, 4 h; (c) MSA, CH₃CN, rt, 24 h; (d) BrCH₂COOEt, aq. K₂CO₃/THF, rt, 6 h; (e) aq. NaOH/EtOH, rt, 2 h; (f) MeONH₂ HCl, EtOH, pyridine, rt, 2 to 6 h; (g) 2-adamantanone, O₃, CH₂Cl₂/cyclohexane, 0 °C; (h) 1 N NaOH, aq. EtOH, 25 to 60 °C, 3 h, then 1 M HCl, 0 °C.

Scheme 4a.

^aReagents and conditions: (a) same as (a) in Scheme 3; (b) same as (h) in Scheme 3.

Scheme 5a.

^aReagents and conditions: (a) bis(2-methoxyethyl)aminosulfur trifluoride, CH₂Cl₂, 0 °C, 1 h; (b) conc. HCl, aq. acetone, rt, 1 h; (c) same as (f) in Scheme 3; (d) same as (a) in Scheme 3; (e) same as (g) in Scheme 3; (f) NaBH₄, MeOH, 0 to 10 °C to rt, 24 h; (g) bis(2-methoxyethyl)aminosulfur trifluoride, CH₂Cl₂, rt, 24 h.

Scheme 6a.

^aReagents and conditions: (a) 50% H₂O₂, I₂, MeOH, rt, 24 h; (b) Et₃SiOTf, Et₃N, DMF, 0 °C to rt, 24 h; (c) 1 N SnCl₄, CH₂Cl₂, -78 to -30 °C, 12 h; (d) 15% aq. KOH, 60 °C, 20 h; (e) *p*-TSA, CH₂Cl₂, rt, 4h.

Table 1

Worm burden reductions in adult *F. hepatica* harbored in rats following the administration of ozonides **1** to **11** at single oral doses of 100 mg/kg.

Compd	R	Worm Burden Reduction (%)	Cures
Control	-----	-----	0/12
AS ^{a,b}	-----	30	2/5
1 ^c	OH	100 ^e	10/10
2	OMe	99 ^e	2/3
3 ^d	OEt	36	2/4
4	NHOH	34	0/3
5	NHC=NH(NH ₂)	0	0/4
6	NHSO ₂ CH ₃	100 ^e	4/4
7	NHCH ₂ COOH	86 ^e	2/4
8	NH(CH ₂) ₂ SO ₃ Na	0	0/4
9	NH ₂	7	0/3
10	NHNH ₂	12	0/3
11	N(CH ₂ CH ₂) ₂ NH	0	0/4

^a AS = artesunate

^b data from Keiser et al.¹³

^c data from Keiser et al.¹²

^d tested at 50 mg/kg

^e p < 0.05 from the Kruskal-Wallis test comparing the medians of the responses between the treatment and control groups

Table 2

Worm burden reductions in adult *F. hepatica* harbored in rats following the administration of ozonides **12** to **20** at single oral doses of 100 mg/kg.

Compd	R	Worm Burden Reduction (%)	Cures
12	COOH	52 ^a	1/4
13	CH ₂ CH ₂ COOH	14	0/5
14	CH(COOH) ₂	0	0/4
15	CH ₂ NHCOCOOH	14	0/3
16	CH ₂ PO(OEt)OK	16	0/3
17	-----	0	0/3
18	-----	95 ^a	2/3
19	-----	46	0/3
20	-----	90 ^a	2/3

^a p < 0.05 from the Kruskal-Wallis test comparing the medians of the responses between the treatment and control groups

Table 3

Worm burden reductions in adult *F. hepatica* harbored in rats following the administration of ozonides **21** to **27** at single oral doses of 100 mg/kg.

<p>26, X = O, Y = CH₂ 27, X = CH₂, Y = O</p>		
Compd	Worm Burden Reduction (%)	Cures
21	0	0/3
22	51	0/3
23	80 ^a	2/3
24	76 ^b	1/3
25	80 ^a	0/3
26	7	0/3
27	0	0/3

^a p < 0.05 from the Kruskal-Wallis test comparing the medians of the responses between the treatment and control groups

^b p = 0.051