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The Structure−Activity Relationship of the Antimalarial Ozonide Arterolane (OZ277)

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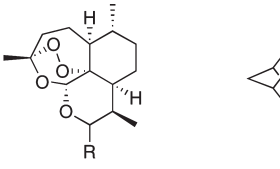
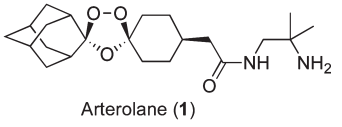
The structure and stereochemistry of the cyclohexane substituents of analogues of arterolane (OZ277) had little effect on potency against *Plasmodium falciparum* in vitro. Weak base functional groups were not required for high antimalarial potency, but they were essential for high antimalarial efficacy in *P. berghei*-infected mice. Five new ozonides with antimalarial efficacy and ADME profiles superior or equal to that of arterolane were identified.

The semisynthetic artemisinins artemether and artesunate (AS)[a] are important first-line antimalarial drugs.[1] They are employed with partner drugs in artemisinin combination treatments (ACT) to allow for treatment regimens of 3 days[2] and to protect against potential artemisinin drug resistance.[3] In efforts to improve upon the biopharmaceutical properties of the artemisinins, newer semisynthetic artemisinins such as artelinic acid and artemisone have been investigated.[4−6] In addition, work to identify synthetic peroxides[6−8] that combine the powerful antimalarial action of the artemisinins with good biopharmaceutical properties is ongoing. With this goal in mind, we identified the ozonide arterolane (OZ277, **1**), a synthetic peroxide drug development candidate[9] that is now in phase III clinical trials in the form of an arterolane maleate/piperaquine phosphate combination.[6,10]



Artemether R = −OCH$_3$
Artesunate R = ····OCO(CH$_2$)$_2$COOH



Arterolane (**1**)

The structure−activity relationship (SAR) for this class of dispiro synthetic ozonides can be summarized as follows: (1) the spiroadamantane ring system[11,12] and peroxide bond[11,13] are essential for activity; (2) more lipophilic ozonides tend to have better oral activities than their more polar counter parts,[11,12] an outcome consistent with that seen for other classes of synthetic peroxides;[7] (3) ozonides with a wide range of neutral and basic, but not acidic, functional groups have good antimalarial profiles.[12,14,15] In this paper, we describe new insights from the SAR of the ozonides related to **1** and our efforts to identify analogs of **1** with superior biopharmaceutical and antimalarial properties.

Chemistry

We identified HOBt active ester **2** as a key common intermediate suitable for the preparation of numerous analogs of **1** using parallel chemistry. HOBt active ester **2** was obtained (Scheme 1) starting with Griesbaum coozonolysis[16] of *O*-methyl-2-adamantanone oxime (**3**)[17] and methyl 4-oxocyclohexyl acetate (**4**)[18] to form ozonide ester **5** (78%), which was isolated by solvent removal followed by crystallization from 80% aq EtOH. Ester hydrolysis of **5** afforded ozonide acid **6** in 96% yield. Treatment of **6** with HOBt and EDCI in DMF afforded, after precipitation with water, HOBt active ester **2** in 95% yield. For these ozonides, X-ray crystallographic data[19,20] reveals that the peroxide bond is axial and the cyclohexane substituent is equatorial, establishing the stereochemistry as *cis*.

A wide range of ozonide amides and amino amides was obtained by stirring the corresponding amine or diamine with **2** in $CHCl_3$ or in a mixture of $CHCl_3$/EtOH at rt, typically for 1−4 h. For example, under these conditions, treatment of **2** with 1,2-diamino-2-methylpropane, guanidine, glycinamide, 1,2-diaminoethane, 1-amino-1-(aminomethyl)cyclopropane, 1,3-diaminopropane, 2,2-dimethyl-1,3-diaminopropane, *trans*-1,4-diaminocyclohexane, 1-piperazinecarboxaldehyde, piperazine, *N*-(2-hydroxyethyl)piperazine, piperazin-2-one, 4-hydroxypiperidine, isonipecotic acid, and isonipecotamide afforded **1** (84%), **17** (94%), **18** (75%), **19** (83%), **20** (60%), **21** (76%), **22** (35%), **23** (47%), **26** (58%), **28** (75%), **29** (65%), **30** (51%), **32** (37%), **34** (82%), and **35** (76%), respectively (Scheme 2). Similarly, treatment of **2** with 4-(*tert*-butoxycarbonyl)piperidine followed by deprotection with PTSA afforded **33** (31% overall) or with thiomorpholine and subsequent oxidation with *m*-CPBA afforded **25** (79% overall). Ozonides **15** (65%), **16**

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[a] Abbreviations: ACT, artemisinin combination treatment; AS, artesunate; CQ, chloroquine; DHA, dihydroartemisinin; ER, hepatic extraction ratios; MF, mefloquine; PfATP6, *Plasmodium falciparum* sarcoendoplasmic reticulum Ca^{2+} ATPase; SSV, standard suspension vehicle; TPP, triphenylphosphine.

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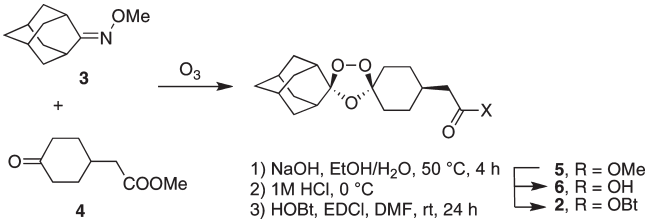
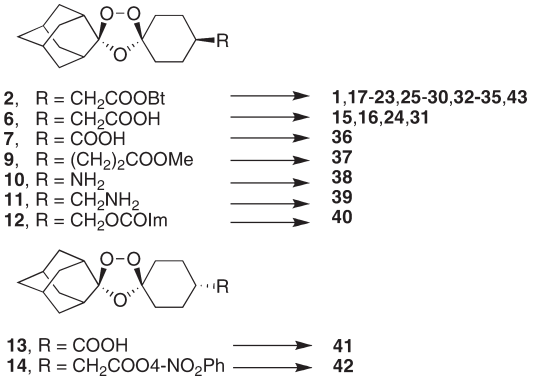
Scheme 1



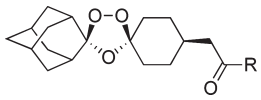
Scheme 2



(28%), **24** (74%), and **31** (62%) were obtained from the mixed anhydride of **6**, which was formed by treatment of **6** with ethyl chloroformate and Et_3N at 0 °C, followed by exposure to ammonia (7 N in MeOH), hydroxylamine, morpholine, and piperidine, respectively. Ozonide **27** (87%) was obtained by treatment of **28** with methanesulfonyl chloride/pyridine in CH_2Cl_2 at 0 °C. Ozonide tetrazole **43** was obtained by treatment of **1** with TPP, trimethylsilyl azide, and DIAD according to the method of Duncia et al.[21]

Ozonide **36** was obtained by reaction of ozonide acid **7**[20] with HOBt and EDCI in DMF followed by reaction of the thus formed HOBt active ester (95%) with 1,2-diamino-2-methylpropane in $CHCl_3$ (85%) (Scheme 2). Ozonide **37** was obtained by coozonolysis of oxime ether **3**[17] with methyl 3-(4-oxocyclohexyl)propionate (**8**)[18] to form ozonide ester **9** (59%) followed by ester hydrolysis (95%), HOSu active ester formation (100%), and reaction with 1,2-diamino-2-methylpropane (74%). Ozonide ureas **38** (31%) and **39** (25%) were obtained by treatment of the 4-nitrophenylcarbamate[22] derivatives of ozonide amines **10** and **11**[15] with 1,2-diamino-2-methylpropane. Ozonide carbamate **40** (26%) was obtained by methylation of imidazole carbamate **12**[12] with methyl trifluoromethylsulfonate followed by reaction with 1,2-diamino-2-methylpropane. *trans*-Ozonide **41** was obtained by reaction of ozonide acid **13**[23] with HOBt and EDCI in DMF followed by reaction of the thus-formed HOBt active ester (64%) with 1,2-diamino-2-methylpropane in $CHCl_3$ (82%). *trans*-Ozonide **42** was obtained starting with repeated crystallizations of **5** from the reaction mother liquor to enrich the proportion of the minor *trans*-ozonide ester isomer of **5**; using this approach, we obtained a 1:1 mixture of the *cis*- and *trans*-ozonide esters. Subsequent hydrolysis and formation of the 4-nitrophenyl ozonide esters afforded after repeated crystallizations from EtOH, the pure *trans* isomer **14** (5%). Treatment of the latter with 1,2-diamino-2-methylpropane afforded **42** (68%). As indicated in Tables 1–4, most of the weak base ozonides were isolated and tested as their tosylate salts.

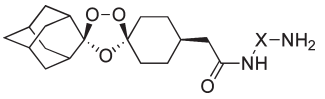
Table 1. Metabolic Stability and Activity of Hydroxamic Acid, Acylguanidine, and Neutral Amide Derivatives of Ozonide Carboxylic Acid **78** against *P. falciparum* in Vitro and *P. berghei* in Vivo (1 × 10 mg/kg po)



compd	R	IC_{50} (ng/mL),[a] K1/NF54	activity (%)[b]	ER[c]
6[d]	OH	34/45	95	0.28
15	NH_2	0.91/1.1	97	0.49
16	NHOH	1.4/2.0	97	0.88
17	NHC=NH(NH_2)	2.4/2.0	97	*e*
18	$NHCH_2CONH_2$	3.9/3.6	99.6	*e*
AS[d]		1.3/1.6	67	0.43[f]

[a] Mean from $n = 2-3$. Individual measurements differed by less than 50%. [b] Groups of five *P. berghei*-infected NMRI mice were treated orally 1 day postinfection with ozonides dissolved or suspended in SSV. Antimalarial activity was measured by percent reduction in parasitemia on day 3 postinfection. Individual measurements differed by less than 10%. [c] Predicted hepatic extraction ratios (ER) using human microsomes.[28] [d] Data from Vennerstrom et al.[9] [e] Not determined. [f] Value for dihydroartemisinin (DHA), the primary metabolite of artesunate (AS).

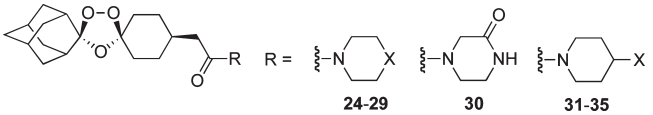
Table 2. Metabolic Stability and Activity of Ozonide Amino Amides against *P. falciparum* in Vitro and *P. berghei* in Vivo (1 × 10 mg/kg po)



compd	X	IC_{50} (ng/mL), K1/NF54	activity (%)	ER
1[a,b]	$CH_2C(CH_3)_2$	1.0/0.91	99.9	0.32
19[a]	CH_2CH_2	1.8/1.9	99.9	0.42
20[a]	$CH_2C(CH_2CH_2)$	0.40/0.52	99.9	0.50
21[a]	$CH_2CH_2CH_2$	3.5/2.3	99.9	*d*
22[c]	$CH_2C(CH_3)_2CH_2$	0.46/0.49	99.9	0.43
23[a]	*trans*-1,4-C_6H_{10}	2.8/3.0	>99.9	0.54

[a] Tosylate salt. [b] Data from Vennerstrom et al.[9] [c] Mesylate salt. [d] Not determined.

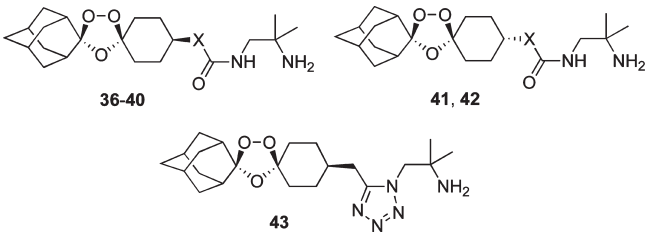
Table 3. Metabolic Stability and Activity of Ozonide Piperidine, Morpholine, Thiomorpholine, and Piperazine Amides against *P. falciparum* in Vitro and *P. berghei* in Vivo (1 × 10 mg/kg po)



compd	X	IC_{50} (ng/mL), K1/NF54	activity (%)	ER
24	O	1.7/3.0	99.8	*b*
25	SO_2	1.8/1.2	<40	*b*
26	NCOH	0.39/0.63	99.8	*b*
27	NSO_2CH_3	1.2/2.1	<40	*b*
28[a]	NH	0.21/0.24	>99.9	0.50
29	NCH_2CH_2OH	1.0/0.90	96	*b*
30		0.60/0.89	99.2	0.72
31	H	1.6/1.6	99.3	*b*
32	OH	0.49/0.76	99.7	0.75
33[a]	NH_2	0.35/0.39	>99.9	0.45
34	COOH	19/27	43	*b*
35	$CONH_2$	1.2/1.7	86	*b*

[a] Tosylate salt. [b] Not determined.

Table 4. Metabolic Stability and Activity of Homologs, Isosteres, and Stereoisomers of Ozonide Amino Amide **277** against *P. falciparum* in Vitro and *P. berghei* in Vivo (1 × 10 mg/kg po)



compd	X	IC_{50} (ng/mL), K1/NF54	activity (%)	ER
36[a]	none	0.66/0.25	96	[b]
37[a]	CH_2CH_2	0.55/0.82	>99.9	0.37
38[a]	NH	8.4/6.2	63	[b]
39[a]	CH_2NH	3.2/3.5	97	[b]
40[a]	CH_2O	0.22/0.36	>99.9	0.32
41[a]	none	1.4/0.74	84	[b]
42[a]	CH_2	1.7/1.5	96	[b]
43[a]		2.3/1.5	98	[b]

[a] Tosylate salt. [b] Not determined.

Antimalarial Activity

In vitro and in vivo antimalarial activities[9] were measured using the chloroquine-resistant K1 and chloroquine-sensitive NF54 strains of *P. falciparum* and *P. berghei*-infected mice, respectively. In vivo data were obtained using 1 × 10 and 3 × 3 mg/kg oral doses of the ozonides administered in a non-solubilizing, standard suspension vehicle (SSV) formulation comprising 0.5% w/v carboxymethyl cellulose, 0.5% v/v benzyl alcohol, 0.4% v/v Tween 80, and 0.9% w/v sodium chloride in water.

The data in Tables 1−4 reveal that with the exception of the ozonide urea **38** with IC_{50}'s between 6 and 9 ng/mL, each of the neutral and weak base ozonides had IC_{50} values in the relatively narrow range of 0.2−3.9 ng/mL, similar to that of the control artesunate. Further, there was no significant difference in in vitro potency between neutral and weak base ozonides. The weakly acidic hydroxamic acid **16** and amphoteric[24] acylguanidine **17** were similarly potent. The only compounds for which the nature of the cyclohexane substituent affected potency significantly were the acidic ozonides **6** and **34**, which, in contrast to the acidic semisynthetic artesunate, were an order of magnitude less potent against *P. falciparum* in vitro. Studies to examine the erythrocyte partitioning characteristics for a range of ozonides indicated that in contrast to the weak base compounds, **6** did not partition well into erythrocytes, a factor that could contribute in part to its poor antimalarial activity (data not shown). Whereas the structure of the neutral and weak base ozonides had little to no effect on potency against *P. falciparum* in vitro, it significantly affected potency against *P. berghei* in vivo. Thus, all six ozonides (Table 1−4) with activities >99.9% were weak bases; of these, five were primary amines and one (**28**) was a secondary amine. None of the neutral (or acidic) ozonides had an in vivo activity that exceeded 99.8%.

The data in Table 4 focus more precisely on the SAR of the amino amide substructure of **1**. Removing (**36**) or adding (**37**) a methylene between the amide functional group and the cyclohexane ring had little effect on in vitro potency but did influence in vivo efficacy; **37** was as effective whereas **36** was more than 40-fold[25] less effective than **1**. Replacing the methylene α to the carboxamide of **1** with an NH (**38**) or with a CH_2NH (**39**) decreased overall antimalarial efficacy; in contrast, replacement with a CH_2O endowed **40** with an antimalarial profile comparable to that of **1**. The relatively low in vivo activities of ureas **38** and **39** indicate that for reasonably polar ozonides such as **1** with an experimental log $D_{pH7.4}$ value of 2.9, adding H-bond donating groups decreases overall antimalarial efficacy in vivo. Tetrazole isostere **43** was as potent as **1** in vitro but was more than 20-fold less effective in vivo. The two *cis*/*trans* isomer pairs **36**/**41** and **1**/**42** exhibited little differences in in vitro potency but considerable differences in in vivo activity; for example, **1** was more than 40-fold more effective than **42**. The latter phenomenon may be explained in part by the 3.5-fold higher aqueous degradation rate[26] and 2.2-fold higher iron(II) reactivity[27] of the *trans*-**42** vs the *cis*-**1**.

Table 5. In Vivo Activity in *P. berghei* Infected Mice Following Three Consecutive Daily Oral Doses of 3 mg/kg[a]

compd	activity (%)	survival (days)	cure[b] (%)
control	0	6−7	0
1[c]	>99.9	11.4	0
23	>99.9	22.0	0
28	>99.9	14.8	0
33	>99.9	27.0	60
37	>99.9	24.4	0
40	>99.9	9.8	0
AS[c]	70	9.2	0
CQ[c]	99.7	9.4	0
MF[c]	99	15.8	0

[a] Groups of five *P. berghei*-infected NMRI mice were treated orally on days +1, +2, and + 3 postinfection with ozonides dissolved or suspended in SSV. Antimalarial activity was measured by percent reduction in parasitemia on day 4 postinfection. [b] No detectable parasites at 30 days postinfection. [c] Data from Vennerstrom et al.[9]

To assess whether the five (**23**, **28**, **33**, **37**, and **40**) most promising new ozonides (activity >99.9% at 1 × 10 mg/kg po) could cure *P. berghei*-infected mice, we administered a 3 mg/kg daily dose on days +1, +2, and +3 postinfection (Table 5). In this experiment, only **33** cured any (3/5) of the infected mice; however the other ozonides all increased survival time compared with the untreated control and were as or more effective than AS and chloroquine (CQ) in this respect. Of these, **23** and **37** were as or more effective than mefloquine (MF) at increasing survival time.

Metabolism and Pharmacokinetics

Where measured, the predicted hepatic extraction ratios (ER)[28] for nine weak base and two neutral ozonides indicated that the former were more metabolically stable than the latter. The most metabolically stable compound was carboxylic acid **6** with an ER of 0.28; in contrast, the corresponding hydroxamic acid **16** was relatively unstable (ER = 0.88).

A subset of the most promising ozonides (**28**, **33**, **37**) along with **42**, the *trans* isomer of **1**, and the neutral compound **32** (Table 5) were administered intravenously (iv) and orally (po) to rats, and the pharmacokinetic data are shown in Table 6. The data indicated that half-lives for the four active ozonides were similar, and all were longer than that for dihydroartemisinin (DHA). Ozonide **33**, which had the highest cure rate in vivo, also had the highest oral bioavailability in rats being approximately 2-fold higher than the other more active compounds tested. In comparison, **42**, the *trans* isomer of **1**, which had reduced efficacy when tested in vivo, had a very high plasma clearance, short half-life, and low oral bioavailability.

Table 6. Pharmacokinetic Parameters[a] after Intravenous and Oral Administration to Rats

	intravenous administration			oral administration
compd	half-life (min)	vol of distribution (L/kg)	plasma (blood) clearance (mL/(min·kg))	bioavailability (%)
1	76	16	141 (62)	26
28	102	25	168 (56.3)	37
32	660	36	37[d]	5
33	83	19	157 (39.1)	78
37	60	12	134[d]	32
42	37	16	298[d]	15
DHA[b,c]	26	3.0	72.0 (72)	[e]

[a] Values represent the average of two to three determinations. [b] Dihydroartemisinin (DHA), the primary metabolite of artesunate (AS). [c] Data from Dong et al.[11] [d] Not determined. [e] Not dosed PO.

Ozonide **32** had a long half-life after IV administration due to its large volume of distribution and moderate plasma clearance; however, it exhibited very low oral bioavailability in comparison to the more active ozonides, most likely as a result of its very poor aqueous solubility. Inhibition assays[29] with the 3A4, 2C9, and 2D6 CYP450 isoforms revealed that **33**, like **1**, had no inhibitory effect on enzyme activities at concentrations up to 50 μM.

Toxicology

Preliminary toxicological experiments were performed in male rats given daily oral doses of 100 and 300 mg/kg of **33** for five days. On day 5, maximum plasma levels of approximately 1000 and 3000 ng/mL were measured for **33** at the 100 and 300 mg/(kg·day) doses. Even at the high dose, this ozonide, like **1** and AS, was minimally toxic with the liver, lymphatic organs, and possibly the kidneys, as target organs. No signs of neurotoxicity were seen. Findings tended to be reversible at the end of a 1-week recovery period. The overall toxicity of **33** was quite similar to that of **1**.[9]

Summary

Data for these ozonides provide several insights for the ongoing identification of more effective[4,6−8] synthetic peroxide antimalarials. First, it is evident that in vitro potency was not a reliable predictor of in vivo activity, and determination of IC_{50}'s against *P. falciparum* was insufficient to select compounds for further metabolic and pharmacokinetic profiling; rather, data from experiments in *P. berghei* infected mice were essential for compound differentiation. Second, acidic functional groups decrease antimalarial potency, consistent with our previous observations.[12] Third, the relatively narrow IC_{50} range of the neutral and weak base ozonides indicates that the cyclohexane substituent played only a minor role in intrinsic potency against *P. falciparum*. This suggests that once an active synthetic peroxide structural framework is identified, there is considerable latitude in the range of peroxide heterocycles and functional groups that can be employed to improve both antimalarial activity and ADME properties. One such peroxide heterocycle is the 1,2,4,5-tetraoxane analog of ozonide (1,2,4-trioxolane) **24**; this 1,2,4,5-tetraoxane has excellent antimalarial properties including an IC_{50} of 2.1 ng/mL against the 3D7 strain of *P. falciparum* and an ED_{50} of 3.2 mg/(kg·day) against *P. berghei* in the 4-day Peters test.[30] Fourth, although weak base functional groups were not required for high antimalarial potency against *P. falciparum* in vitro, they were essential for high antimalarial efficacy in *P. berghei*-infected mice; this may be a function of the superior ADME properties of weak base vs neutral ozonides. Fifth, stereochemistry had little effect on the in vitro potency of antimalarial ozonides (1,2,4-trioxolanes), similar to what has previously been found[31,32] for enantiomeric synthetic 1,2,4-trioxanes. However data for **42**, the *trans* isomer of **1**, indicated that it was more rapidly degraded in aqueous solution[26] and in the presence of Fe(II).[27] The pharmacokinetic properties of **42** also indicated that the plasma clearance was approximately 2-fold higher than that for **1** and its oral bioavailability was relatively low. The preceding observations together with the twin findings that **1** alkylates heme[33] but only weakly interacts with the putative artemisinin target PfATP6[34] suggests that hemoglobin digestion[35] in the parasite food vacuole[36−38] is the parasite biochemistry[13] that accounts for the antimalarial properties of synthetic peroxides and probably semisynthetic artemisinins as well. Sixth, we identified five new ozonides (**23**, **28**, **33**, **37**, **40**) with antimalarial efficacies and ADME profiles superior or equal to that of **1**. Ongoing studies will determine the potential of these and other weak base ozonides as antimalarial drug development candidates.

Experimental Section

General. Melting points are uncorrected. Using $CDCl_3$, CD_3OD, or DMSO-d_6 as solvents, ^1H and ^{13}C NMR spectra were recorded on a 500 MHz spectrometer. All chemical shifts are reported in parts per million (ppm) and are relative to internal $(CH_3)_4Si$ (0 ppm) for ^1H and $CDCl_3$ (77.0 ppm), CD_3OD (49.0 ppm), or DMSO-d_6 (39.7 ppm) for ^{13}C NMR. Combustion and HPLC analysis confirmed that all target compounds possessed purities ≥95%.

Starting Material Synthesis. Methyl 4-oxocyclohexyl acetate[39,40] and methyl 3-(4-oxocyclohexyl)propionate[18] were obtained in a modified two-step sequence[18] by reduction (H_2, 900 psi, Rh−C, 100 °C, 5 h, isopropanol) of the corresponding phenol esters to the diastereomeric cyclohexanol esters (97−99%) followed by Jones oxidation (83−89%). In each case, some (ca. 5−8%) over-reduction occurred to form the corresponding cyclohexyl esters. 1-Amino-1-(aminomethyl)cyclopropane dihydrochloride was obtained from *N*-(diphenylmethylene)-aminoacetonitrile in a three-step sequence according to the method of Vergne et al.[41] As indicated below, the remaining starting materials were commercially available or were prepared according to known procedures.

***cis*-Adamantane-2-spiro-3′-8′-[[[(2′-amino-2′-methylpropyl)-amino]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (1).** To a solution of **2** (13.19 g, 30 mmol, 1 equiv) in $CHCl_3$ (300 mL) was added rapidly a solution of 1,2-diamino-2-methylpropane (5.29 g, 60 mmol, 2 equiv) in $CHCl_3$ (50 mL). The resulting mixture was stirred at rt for 1 h before being quenched with water (500 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 100 mL). The combined extracts were washed with water (3 × 500 mL) and brine (300 mL), dried over $MgSO_4$, and filtered. To the filtrate was added a solution of *p*-toluenesulfonic acid monohydrate

(5.71 g, 30 mmol, 1 equiv) in EtOH (30 mL). After evaporation of the solvents, the residue was treated with EtOH (100 mL), filtered, and washed with hexanes (200 mL) to afford **1** (14.25 g, 84%) as a colorless solid. Mp 160−162 °C; ^{1}H NMR (DMSO-d_6) δ 1.01−1.17 (m, 2H), 1.16 (s, 6H), 1.58−1.99 (m, 21H), 2.05 (d, J = 7.1 Hz, 2H), 2.28 (s, 3H), 3.19 (d, J = 6.3 Hz, 2H), 7.11 (d, J = 8.0 Hz, 2H), 7.48 (d, J = 8.0 Hz, 2H), 7.70 (s, 3H), 8.02 (t, J = 6.2 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 20.92, 23.47, 25.96, 26.36, 29.68, 32.71, 33.56, 34.40, 35.91, 36.23, 41.97, 46.02, 54.52, 108.52, 110.63, 125.55, 125.73, 128.20, 137.80, 145.82, 172.49. Anal. ($C_{29}H_{44}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(1′*H*-benzotriazol-1′-yloxy)-carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (2).** A solution of **6** (12.92 g, 40 mmol, 1 equiv), HOBt (6.49 g, 48 mmol, 1.2 equiv), and EDCI (9.20 g, 48 mmol, 1.2 equiv) in DMF (300 mL) under Ar was stirred at rt for 24 h. Under ice cooling, the reaction was quenched with water (150 mL). The precipitate was collected by filtration, washed with 95% EtOH (150 mL), and dried to afford **2** (16.61 g, 95%) as a colorless solid. Mp 154−156 °C; ^{1}H NMR ($CDCl_3$) δ 1.37−1.51 (m, 2H), 1.63−2.17 (m, 21H), 2.72 (d, J = 7.1 Hz, 2H), 7.39 (d, J = 78.5 Hz, 1H), 7.43 (dd, J = 8.2, 7.2 Hz, 1H), 7.56 (dd, J = 8.0, 7.4 Hz, 1H), 8.07 (d, J = 8.5 Hz, 1H).

***cis*-Adamantane-2-spiro-3′-8′-methoxycarbonylmethyl-1′,2′,4′-trioxaspiro[4.5]decane (5).** A solution of *O*-methyl 2-adamantanone oxime (**3**)[17] (11.06 g, 61.7 mmol, 1.5 equiv) and methyl 4-oxocyclohexyl acetate (**4**)[39,40] (7.0 g, 41.1 mmol, 1 equiv) in cyclohexane (200 mL) and CH_2Cl_2 (40 mL) was treated with ozone according to the method of Dong et al.[12] After removal of solvents, the crude product was purified by crystallization from 80% aq EtOH (200 mL) to afford **5** (10.83 g, 78%) as a colorless solid. Mp 96−98 °C; ^{1}H NMR ($CDCl_3$) δ 1.20−1.33 (m, 2H), 1.61−2.09 (m, 21H), 2.22 (d, J = 6.8 Hz, 2H), 3.67 (s, 3H).

***cis*-Adamantane-2-spiro-3′-8′-carboxymethyl-1′,2′,4′-trioxaspiro[4.5]decane (6).** To a solution of **5** (10.83 g, 32.19 mmol, 1 equiv) in 95% EtOH (150 mL) was added NaOH (3.86 g, 96.57 mmol, 3 equiv) solution in water (80 mL). The mixture was stirred at 50 °C for 4 h, cooled to 0 °C, and treated with 1 M HCl (129 mL, 4 equiv). The precipitate was collected by filtration, washed with 50% aq EtOH (150 mL), and dried in a vacuum oven at 40 °C to give **6** (9.952 g, 96%) as a colorless solid. Mp 146−148 °C (95% EtOH); ^{1}H NMR ($CDCl_3$) δ 1.19−1.41 (m, 2H), 1.60−2.05 (m, 21H), 2.27 (d, J = 6.8 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 26.58, 26.97, 29.91, 33.00, 33.95, 34.86, 36.49, 36.89, 40.39, 108.38, 111.40, 177.75. Anal. ($C_{18}H_{26}O_5$) C, H.

***cis*-Adamantane-2-spiro-3′-8′-(2′-amino-2′-oxoethyl)-1′,2′,4′-trioxaspiro[4.5]decane (15).** To a solution of **6** (322 mg, 1 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added triethylamine (202 mg, 2 mmol) followed by ethyl chloroformate (217 mg, 2 mmol). After 15 min, ammonia (7 N in MeOH, 3 mL) was added, and the stirring was continued for 12 h. The precipitate was filtered and dried to afford **15** (210 mg, 65%) as a colorless solid. Mp 140−142 °C; ^{1}H NMR ($CDCl_3$) δ 1.09−1.43 (m, 3H), 1.45−2.15 (m, 20H), 2.11 (d, J = 7.1 Hz, 2H), 5.48 (s, 1H), 5.66 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.47, 26.85, 29.97, 33.32, 33.95, 34.77, 36.38, 36.78, 42.55, 108.50, 111.35, 174.39. Anal. ($C_{18}H_{27}NO_4$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-(2′-hydroxyamino-2′-oxoethyl)-1′,2′,4′-trioxaspiro[4.5]decane (16).** To a solution of **6** (322 mg, 1.0 mmol) in ether (10 mL) at 0 °C were added triethylamine (202 mg, 2 mmol) and ethyl chloroformate (217 mg, 2 mmol). The mixture was stirred at 0 °C for 15 min before a freshly prepared solution of hydroxylamine was added. The latter was prepared as follows: a suspension of hydroxylamine hydrochloride (170 mg, 2.48 mmol) and sodium bicarbonate (203 mg, 2.48 mmol) in MeOH (5 mL) was stirred at rt for 15 min and then filtered. The resulting mixture was stirred at rt for 12 h, diluted with ether (10 mL), washed with water (10 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by flash chromatography (sg, 5% MeOH in CH_2Cl_2) and by subsequent recrystallization from EtOH to afford **16** (95 mg, 28%) as a colorless solid. Mp 138−140 °C (EtOH); ^{1}H NMR (DMSO-d_6) δ 0.81−1.27 (m, 3H), 1.40−2.19 (m, 22H), 8.65 (s, 1H), 10.33 (s, 1H); ^{13}C NMR (DMSO-d_6) δ 25.84, 26.25, 29.40, 32.60, 33.35, 34.26, 35.81, 36.13, 38.68, 108.33, 110.44, 167.91. Anal. ($C_{18}H_{27}NO_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-(2′-oxo-2′-guanidinoethyl)-1′,2′,4′-trioxaspiro[4.5]decane (17).** A solution of potassium *tert*-butoxide (0.56 g, 5.0 mmol) and guanidine hydrochloride (0.48 g, 5.0 mmol) in dioxane (20 mL) was heated under Ar at 50 °C for 20 min. After the mixture was cooled to rt, a solution of **2** (0.46 g, 1.05 mmol) in $CHCl_3$ (20 mL) was added dropwise. The mixture was stirred at rt for 4 h and then concentrated. After addition of water (30 mL), the resulting precipitate was collected, washed with water, and dried to give **17** (0.36 g, 94%) as a colorless solid. Mp 146−147 °C; ^{1}H NMR ($CDCl_3$) δ 1.19−1.35 (m, 2H), 1.61−2.07 (m, 21H), 2.21 (d, J = 7.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 26.49, 26.89, 30.13, 33.71, 34.16, 34.80, 36.39, 36.82, 47.35, 108.87, 111.21, 161.10. Anal. ($C_{19}H_{29}N_3O_4$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[(2′-amino-2′-oxoethyl)amino]-carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (18).** To a solution of glycinamide hydrochloride (221 mg, 2.0 mmol), triethylamine (304 mg, 3 mmol), EtOH (5 mL), and water (1 mL) in $CHCl_3$ (10 mL) was added **2** (440 mg, 1.0 mmol). The resulting mixture was stirred at rt for 17 h before being diluted with $CHCl_3$ (20 mL) and water (40 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (3 × 10 mL). The combined extracts were washed with water (2 × 20 mL) and brine (2 × 20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was crystallized from 30% aq EtOH to afford **18** (284 mg, 75%) as a colorless solid. Mp 130−132 °C; ^{1}H NMR ($CDCl_3$) δ 1.11−1.38 (m, 2H), 1.42−2.07 (m, 21H), 2.15 (d, J = 5.4 Hz, 2H), 3.95 (s, 2H), 5.84 (br s, 1H), 6.52 (br s, 1H), 6.73 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.45, 26.84, 29.94, 33.47, 33.88, 34.75, 36.34, 36.77, 42.77, 42.88, 108.50, 111.33, 171.40, 172.88. Anal. ($C_{20}H_{30}N_2O_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[(2′-aminoethyl)amino]carbonyl]-methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (19).** To a solution of **2** (440 mg, 1.0 mmol) in $CHCl_3$ (15 mL) was added rapidly a solution of ethylenediamine (601 mg, 10 mmol) in EtOH (5 mL). The resulting mixture was stirred at rt for 2 h before being quenched with water (20 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (3 × 20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in CH_2Cl_2 (20 mL) and treated with a solution of *p*-toluenesulfonic acid monohydrate (191 mg, 1.0 mmol) in EtOH (2 mL). After evaporation of the solvents, the residue was treated with ether (20 mL), filtered, and washed with ether (20 mL) to afford **19** (448 mg, 83%) as a colorless solid. Mp 140−142 °C; ^{1}H NMR ($CDCl_3$) δ 0.91−1.11 (m, 2H), 1.59−2.07 (m, 23H), 2.37 (s, 3H), 2.99−3.05 (m, 2H), 3.39−3.44 (m, 2H), 7.16 (d, J = 7.8 Hz, 2H), 7.47 (s, 1H), 7.69 (d, J = 7.8 Hz, 2H), 7.73 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 21.33, 26.48, 26.86, 29.73, 33.08, 33.84, 34.77, 36.37, 36.80, 36.98, 40.16, 42.46, 108.44, 111.15, 111.31, 125.73, 129.32, 140.71, 141.33, 173.94. Anal. ($C_{27}H_{40}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[[(1′-aminocyclopropyl)methyl]-amino]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (20).** To a solution of 1-amino-1-(aminomethyl)cyclopropane dihydrochloride[41] (0.162 g, 1.02 mmol) in EtOH (5 mL) and $CHCl_3$ (10 mL) was added triethylamine (0.5 mL) followed by a solution of **2** (0.22 g, 0.50 mmol) in $CHCl_3$ (15 mL). The resulting mixture was stirred at rt for 1 h and then quenched with water (20 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (3 × 20 mL) and brine (20 mL), dried over $MgSO_4$, and filtered. To the filtrate was added a solution of *p*-toluenesulfonic acid monohydrate (0.10 g, 0.53 mmol) in EtOH

(10 mL). After evaporation of the solvents, the residue was purified by crystallization from EtOH to afford **20** (0.17 g, 60%) as a colorless solid. Mp 175−176 °C; ^{1}H NMR (DMSO-d_6) δ 0.72−0.77 (m, 2H), 0.78−0.85 (m, 2H), 1.02−1.17 (m, 2H), 1.56−1.94 (m, 21H), 2.02 (d, J = 6.9 Hz, 2H), 2.28 (s, 3H), 3.30 (d, J = 6.9 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 8.03−8.07 (m, 4H); ^{13}C NMR (DMSO-d_6) δ 10.66, 21.22, 26.23, 26.40, 26.63, 29.90, 33.15, 33.88, 34.69, 34.84, 36.20, 36.49, 42.35, 44.95, 108.88, 111.02, 125.94, 128.64, 138.52, 145.52, 172.71. Anal. ($C_{29}H_{42}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[(3′-aminopropyl)amino]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (21).** To a solution of 1,3-propanediamine (0.50 g, 6.74 mmol) in $CHCl_3$ (10 mL) was added dropwise the solution of **2** (0.4 g, 0.91 mmol) in $CHCl_3$ (20 mL). The resulting mixture was stirred at rt for 1 h and then quenched with water (30 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in $CHCl_3$ (20 mL), and then a solution of *p*-toluenesulfonic acid monohydrate (0.18 g, 0.95 mmol) in EtOH (10 mL) was added. After evaporation of the solvent, the crude product was purified by crystallization from EtOH to afford **21** (0.38 g, 76%) as a colorless solid. Mp 154−156 °C; ^{1}H NMR (DMSO-d_6) δ 1.01−1.18 (m, 2H), 1.54−1.94 (m, 23H), 1.97 (d, J = 6.8 Hz, 2H), 2.28 (s, 3H), 2.69−2.81 (m, 2H), 3.08 (td, J = 6.8, 5.9 Hz, 2H), 7.11 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 8.3 Hz, 2H), 7.63 (s, 3H), 7.95 (t, J = 5.6 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 20.95, 25.98, 26.38, 27.70, 29.65, 32.94, 33.59, 34.42, 35.61, 35.94, 36.26, 37.05, 42.06, 108.56, 110.67, 125.67, 128.23, 137.80, 145.88, 171.82. Anal. ($C_{28}H_{42}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[(3′-amino-2′,2′-dimethylpropyl)-amino]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane Mesylate (22).** To a solution of 2,2-dimethyl-1,3-propanediamine (0.35 g, 3.43 mmol) in $CHCl_3$ (10 mL) was added dropwise a solution of **2** (0.5 g, 1.14 mmol) in $CHCl_3$ (20 mL). The resulting mixture was stirred at rt for 1 h and then quenched with water (30 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated to afford the crude product (0.41 g, 89%) as a white solid. After being washed with ether, the crude product (0.25 g) was dissolved in $CHCl_3$ (20 mL), and then a solution of methanesulfonic acid (60 mg, 0.62 mmol) in $CHCl_3$ (10 mL) was added. After evaporation of the solvent, the crude product was purified by crystallization from EtOH to afford **22** (0.20 g, 35%) as a colorless solid. Mp 162−164 °C; ^{1}H NMR (DMSO-d_6) δ 0.88 (s, 6H), 1.02−1.18 (m, 2H), 1.54−1.95 (m, 21H), 2.07 (d, J = 6.8 Hz, 2H), 2.29 (s, 3H), 2.51 (s, 2H), 2.95 (d, J = 6.4 Hz, 2H), 7.64 (s, 3H), 8.20 (t, J = 6.3 Hz, 1H); ^{13}C NMR (DMSO-d_6) δ 23.22, 25.96, 26.36, 29.62, 32.87, 33.53, 34.41, 34.83, 35.92, 36.24, 41.78, 45.53, 46.01, 108.50, 110.69, 173.23. Anal. ($C_{24}H_{42}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[(*trans*-4′-aminocyclohexyl)-amino]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (23).** To a solution of **2** (220 mg, 0.5 mmol) in $CHCl_3$ (10 mL) was added rapidly a solution of *trans*-1,4-diaminocyclohexane (343 mg, 3.0 mmol) in $CHCl_3$ (10 mL). The resulting mixture was stirred at rt for 1 h and filtered. The filtrate was diluted with water (30 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 10 mL). The combined extracts were washed with water (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, and filtered. To the filtrate was added a solution of *p*-toluenesulfonic acid monohydrate (50 mg, 0.26 mmol) in EtOH (1 mL). After evaporation of the solvents, the residue was treated with ether (20 mL), filtered, and washed with ether (20 mL) to afford **23** (139 mg, 47%) as a colorless solid. Mp 140 °C dec; ^{1}H NMR (DMSO-d_6) δ 0.97−1.43 (m, 6H), 1.54−2.02 (m, 27H), 2.29 (s, 3H), 2.84−3.06 (m, 1H), 3.38−3.52 (m, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.71 (s, 1H), 7.73 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 20.95, 25.97, 26.37, 29.26, 29.57, 30.16, 33.03, 33.58, 34.42, 35.92, 36.25, 42.10, 46.60, 48.75, 108.57, 110.63, 125.65, 128.25, 137.87, 145.76, 170.48. Anal. ($C_{31}H_{46}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[2′-(4′-morpholinyl)-2′-oxoethyl]-1′,2′,4′-trioxaspiro[4.5]decane (24).** To a solution of **6** (322 mg, 1.0 mmol) in ether (10 mL) at 0 °C were added triethylamine (202 mg, 2 mmol) and ethyl chloroformate (217 mg, 2 mmol). The mixture was stirred at 0 °C for 15 min before morpholine (175 mg, 2 mmol) was added. The resulting mixture was stirred at rt for 12 h, diluted with ether (10 mL), washed with water (10 mL), dried over $MgSO_4$, and concentrated. The crude product was purified by crystallization from EtOH to afford **24** (290 mg, 74%) as a colorless solid. Mp 118−120 °C (EtOH); ^{1} H NMR ($CDCl_3$) δ 1.16−1.35 (m, 2H), 1.60−2.16 (m, 21H), 2.21 (d, J = 6.9 Hz, 2H), 3.38−3.54 (m, 2H), 3.55−3.82 (m, 6H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.48, 26.86, 30.28, 33.26, 34.05, 34.79, 36.39, 36.79, 39.01, 41.93, 46.18, 66.66, 66.97, 108.58, 111.35, 170.67. Anal. ($C_{22}H_{33}NO_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(1′,1′-dioxido-4′-thiomorpholinyl)-carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (25). Step 1.** To a solution of **2** (0.5 g, 1.14 mmol) in $CHCl_3$ (15 mL) was added dropwise a solution of thiomorpholine (0.15 g, 1.45 mmol) in $CHCl_3$ (10 mL). The resulting mixture was stirred at rt for 1.5 h and then quenched with water (30 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, and filtered. After removal of the solvent, the crude product was purified by crystallization from ether to afford the thioether intermediate (0.45 g, 97%) as a colorless solid. Mp 126−127 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.31 (m, 2H), 1.59−2.05 (m, 21H), 2.20 (d, J = 6.8 Hz, 2H), 2.57−2.63 (m, 4H), 3.69−3.78 (m, 2H), 3.85−3.92 (m, 2H); ^{13}C NMR (125.7 MHz, $CDCl_3$) δ 26.48, 26.86, 27.46, 27.94, 30.31, 33.21, 34.05, 34.79, 36.39, 36.79, 39.36, 44.29, 48.41, 108.59, 111.35, 170.40. **Step 2**. To a solution of the above thioether intermediate (0.39 g, 0.96 mmol) in CH_2Cl_2 (10 mL) at 0 °C was added dropwise a solution of *m*-CPBA (0.52 g, 2.1 mmol) in CH_2Cl_2 (15 mL). The resulting mixture was stirred at rt for 24 h and then partitioned between CH_2Cl_2 (20 mL) and saturated aq $NaHCO_3$ (20 mL). The organic layer was washed with water (20 mL) and brine (20 mL), dried over $MgSO_4$, and filtered. After removal of the solvent, the crude product was purified by crystallization from CH_2Cl_2/EtOH to afford **25** (0.34 g, 81%) as a colorless solid. Mp 159−160 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.33 (m, 2H), 1.59−2.03 (m, 21H), 2.26 (d, J = 6.8 Hz, 2H), 3.02 (s, 4H), 3.96 (s, 2H), 4.11 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 26.46, 26.85, 30.25, 33.07, 33.96, 34.78, 36.38, 36.77, 39.04, 40.22, 43.90, 52.14, 52.28, 108.35, 111.48, 170.54. Anal. ($C_{22}H_{33}NO_6S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(4′-formyl-1′-piperazinyl)carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (26).** To a solution of **2** (440 mg, 1.0 mmol) in $CHCl_3$ (20 mL) was added a solution of 1-piperazinecarboxaldehyde (137 mg, 1.2 mmol) in $CHCl_3$ (1 mL). The resulting mixture was stirred at rt for 2 h before removal of the solvent. The residue was crystallized from 1:2 EtOH/water to afford **26** (242 mg, 58%, 2:1 mixture of rotamers) as a colorless solid. Mp 142−144 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.32 (m, 2H), 1.45−2.09 (m, 21H), 2.20−2.28 (m, 2H), 3.32−3.78 (m, 8H), 8.10 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.46, 26.84, 30.27, 33.18, 34.01, 34.77, 36.38, 36.77, 39.26, 39.99, 40.22, 41.02, 42.15, 45.15, 45.33, 45.70, 46.25, 108.49, 111.39, 160.74, 160.92, 170.55, 170.72. Anal. ($C_{23}H_{34}N_2O_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[4′-(methylsulfonyl)-1′-piperazinyl]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (27).** To a solution of the free base of **28** (0.43 g, 1.10 mmol) in CH_2Cl_2 (10 mL) at 0 °C under Ar was added pyridine (0.3 mL, 3.79 mmol),

followed by methanesulfonyl chloride (0.12 mL, 1.57 mmol). The resulting mixture was stirred at 0 °C for 1 h and then quenched with saturated aq K_2CO_3 (5 mL). After separation of the organic layer, the aqueous layer was extracted with CH_2Cl_2 (3 × 10 mL). The combined extracts were washed successively with water (10 mL), 1 M aq HCl (5 mL), and brine (10 mL), dried over $MgSO_4$, and filtered. The filtrate was concentrated to about 3 mL and diluted with ether (20 mL). The precipitate was collected and washed with hexanes (10 mL) to afford **27** (0.45 g, 87%) as a colorless solid. Mp 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.33 (m, 2H), 1.61−2.07 (m, 21H), 2.23 (d, J = 6.8 Hz, 2H), 2.80 (s, 3H), 3.16−3.28 (m, 4H), 3.55−3.63 (m, 2H), 3.70−3.79 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 26.46, 26.84, 30.26, 33.15, 34.01, 34.77, 34.85, 36.38, 36.76, 39.21, 41.14, 45.34, 45.69, 45.93, 108.48, 111.40, 170.53. Anal. ($C_{23}H_{36}N_2O_6S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[(1′-piperazinylcarbonyl)methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (28).** To a solution of **2** (13.19 g, 30 mmol, 1 equiv) in $CHCl_3$ (300 mL) was added rapidly a solution of piperazine (12.92 g, 150 mmol, 5 equiv) in $CHCl_3$ (50 mL). The resulting mixture was stirred at rt for 1.5 h before being quenched with water (500 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 100 mL). The combined extracts were washed with water (3 × 500 mL) and brine (300 mL), dried over $MgSO_4$, and filtered. To the filtrate was added a solution of *p*-toluenesulfonic acid monohydrate (5.71 g, 30 mmol, 1 equiv) in EtOH (30 mL). After evaporation of the solvents, the residue was dissolved in $CHCl_3$ (70 mL), and the product was precipitated by adding isopropanol (420 mL), filtered, and washed with 6:1 isopropanol/$CHCl_3$ (210 mL) and hexanes (300 mL). The solid was redissolved in $CHCl_3$ (60 mL), precipitated by adding hexanes (600 mL), filtered, and washed with hexanes (200 mL) to afford **28** (12.58 g, 75%) as a colorless solid. Mp 148−150 °C; ^{1}H NMR ($CDCl_3$) δ 1.03−1.29 (m, 2H), 1.49−2.05 (m, 21H), 2.15 (d, J = 6.3 Hz, 2H), 2.39 (s, 3H), 3.18 (s, 2H), 3.25 (s, 2H), 3.71 (s, 2H), 3.83 (s, 2H), 7.22 (d, J = 7.7 Hz, 2H), 7.71 (d, J = 7.4 Hz, 2H), 9.25 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 21.35, 26.47, 26.85, 30.19, 33.00, 33.96, 34.77, 36.38, 36.78, 38.19, 38.90, 42.36, 43.68, 43.78, 108.41, 111.37, 125.66, 129.22, 140.94, 141.11, 170.45. Anal. ($C_{29}H_{42}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[4′-(2′-hydroxyethyl)-1′-piperazinyl]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (29).** To a solution of *N*-(2-hydroxyethyl)piperazine (325 mg, 2.5 mmol) in $CHCl_3$ (10 mL) was added **2** (440 mg, 1.0 mmol) The resulting mixture was stirred at rt for 2 h and then quenched with water (20 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (2 × 20 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. Crystallization of the residue from EtOAc gave **29** (281 mg, 65%) as a colorless solid. Mp 143−145 °C; ^{1}H NMR ($CDCl_3$) δ 1.26−1.31 (m, 2H), 1.62−2.04 (m, 21H), 2.21 (d, J = 6.3 Hz, 2H), 2.44−2.54 (m, 4H), 2.56 (t, J = 4.4 Hz, 2H), 2.62 (brs, 1H), 3.49 (t, J = 4.0 Hz, 2H), 3.64 (t, J = 4.4 Hz, 4H); ^{13}C NMR ($CDCl_3$) δ 26.43, 26.81, 30.25, 33.29, 34.02, 34.74, 36.34, 36.74, 39.10, 41.54, 45.71, 52.62, 53.15, 57.76, 59.25, 108.56, 111.28, 170.41. Anal. ($C_{24}H_{38}N_2O_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(3′-oxo-1′-piperazinyl)carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (30).** To a solution of **2** (440 mg, 1.0 mmol) in $CHCl_3$ (20 mL) was added piperazin-2-one (120 mg, 1.2 mmol). The resulting mixture was stirred at rt for 3 h before removal of the solvent. The residue was crystallized from 1:1 EtOH/water to afford **30** (207 mg, 51%, 3:2 mixture of rotamers) as a colorless solid. Mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 1.17−1.34 (m, 2H), 1.57−2.09 (m, 21H), 2.21 (d, J = 6.8 Hz, 1.2H), 2.24 (d, J = 6.8 Hz, 0.8H), 3.39 (s, 1.2H), 3.42 (s, 0.8H), 3.67 (t, J = 5.0 Hz, 0.8H), 3.82 (t, J = 5.4 Hz, 1.2H), 4.12 (s, 1.2H), 4.25 (s, 0.8H), 6.42 (s, 0.6H), 6.58 (s, 0.4H); ^{13}C NMR ($CDCl_3$) δ 26.47, 26.85, 30.20, 30.26, 32.98, 34.00, 34.78, 36.38, 36.78, 38.31, 39.34, 39.48, 40.83, 41.34, 42.40, 46.06, 49.01, 108.46, 108.50, 111.35, 111.43, 166.60, 167.91, 170.37, 170.62. Anal. ($C_{22}H_{32}N_2O_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[(1′-piperidinylcarbonyl)methyl]-1′,2′,4′-trioxaspiro[4.5]decane (31).** To a solution of **6** (322 mg, 1.0 mmol) in CH_2Cl_2 (10 mL) at 0 °C were added triethylamine (202 mg, 2 mmol) and ethyl chloroformate (217 mg, 2 mmol). The mixture was stirred at 0 °C for 15 min before piperidine (100 mg, 1.2 mmol) was added. The resulting mixture was stirred at rt for 12 h, concentrated, and triturated with water. The crude product was purified by crystallization from EtOH to afford **31** (0.24 g, 62%) as a colorless solid. Mp 98−100 °C (EtOH); ^{1}H NMR ($CDCl_3$) δ 1.15−1.39 (m, 2H), 1.43−2.17 (m, 27H), 2.21 (d, J = 6.8 Hz, 2H), 3.30−3.49 (m, 2H), 3.50−3.65 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 24.57, 25.66, 26.49, 26.60, 26.87, 30.31, 33.43, 34.11, 34.79, 36.39, 36.81, 39.26, 42.69, 46.87, 108.72, 111.29, 170.28. Anal. ($C_{23}H_{35}NO_4$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(4′-hydroxy-1′-piperidinyl)carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (32).** To a solution of **2** (440 mg, 1.0 mmol) in $CHCl_3$ (20 mL) was added 4-hydroxypiperidine (121 mg, 1.2 mmol). The resulting mixture was stirred at rt for 1 h before being quenched with water (20 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 20 mL). The combined extracts were washed with water (3 × 30 mL) and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was crystallized from 10:1 ether/$CHCl_3$ to afford **32** (152 mg, 37%) as a colorless solid. Mp 154−156 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.35 (m, 2H), 1.41−1.59 (m, 2H), 1.60−2.09 (m, 23H), 2.23 (d, J = 7.1 Hz, 2H), 3.09−3.31 (m, 2H), 3.68−3.82 (m, 1H), 3.87−4.03 (m, 1H), 4.05−4.21 (m, 1H); ^{13}C NMR ($CDCl_3$) δ 26.49, 26.86, 30.28, 33.39, 34.01, 34.07, 34.66, 34.78, 36.39, 36.80, 38.92, 39.22, 42.97, 67.20, 108.64, 111.32, 170.39. Anal. ($C_{23}H_{35}NO_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(4′-amino-1′-piperidinyl)carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (33). Step 1.** To a solution of **2** (880 mg, 2 mmol) in $CHCl_3$ (40 mL) was added 4-(*tert*-butoxycarbonylamino)piperidine (481 mg, 2.4 mmol). The resulting mixture was stirred at rt for 1 h before solvent removal. The residue was crystallized from 50% aq EtOH (80 mL) to give the BOC ozonide (995 mg, 99%). Mp 146−148 °C; ^{1}H NMR (DMSO-d_6) δ 1.02−1.29 (m, 4H), 1.38 (s, 9H), 1.59−1.94 (m, 23H), 2.19 (d, J = 6.8 Hz, 2H), 2.63 (dd, J = 11.8, 11.8 Hz, 1H), 3.02 (dd, J = 12.2, 12.2 Hz, 1H), 3.39−3.51 (m, 1H), 3.80 (d, J = 13.7 Hz, 1H), 4.23 (d, J = 13.2 Hz, 1H), 6.84 (d, J = 7.8 Hz, 1H). **Step 2**. A mixture of the above BOC ozonide (505 mg, 1.0 mmol) and *p*-toluenesulfonic acid monohydrate (951 mg, 5.0 mmol) in EtOAc/isopropanol (9:1, 50 mL) was stirred at rt for 48 h. The precipitate was filtered, washed with EtOAc (20 mL), dissolved in 20% aq EtOH (90 mL), and basified with 14% aq KOH (10 mL). The solid was filtered, dissolved in $CHCl_3$, dried over $MgSO_4$, and concentrated. To a solution of the above free base (170 mg) in EtOAc (10 mL) was added a solution of *p*-toluenesulfonic acid monohydrate (80 mg, 0.42 mmol) in EtOAc (10 mL). The precipitate was collected by filtration, washed with EtOAc (10 mL), and dried to give **33** (180 mg, 31%) as a colorless solid. Mp 154−156 °C; ^{1}H NMR (DMSO-d_6) δ 1.05−1.18 (m, 2H), 1.20−1.29 (m, 1H), 1.30−1.42 (m, 1H), 1.59−1.97 (m, 23H), 2.22 (d, J = 5.4 Hz, 2H), 2.29 (s, 3H), 2.57 (dd, J = 11.8, 11.8 Hz, 1H), 3.02 (dd, J = 12.2, 12.2 Hz, 1H), 3.24 (br s, 1H), 3.92 (d, J = 13.7 Hz, 1H), 4.39 (d, J = 13.2 Hz, 1H), 7.12 (d, J = 7.8 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.82 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 20.94, 25.97, 26.37, 29.61, 29.77, 30.48, 32.73, 33.67, 34.41, 35.93, 36.25, 38.37, 43.32, 47.66, 47.75, 108.58, 110.62, 125.64, 128.23, 137.81, 145.84, 169.78. Anal. ($C_{30}H_{44}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(4′-carboxy-1′-piperidinyl)carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (34).** To a solution of **2** (220 mg, 0.5 mmol), water (5 mL), and EtOH (10 mL) in

$CHCl_3$ (10 mL) was added isonipecotic acid (129 mg, 1.0 mmol). The resulting mixture was stirred at rt for 16.5 h before removal of the solvents. The residue was crystallized from 1:1 EtOH/water to afford **34** (179 mg, 82%) as a colorless solid. Mp 159−161 °C; ^{1}H NMR ($CDCl_3$) δ 1.18−1.35 (m, 2H), 1.58−2.06 (m, 25H), 2.24 (d, J = 6.8 Hz, 2H), 2.60 (tt, J = 10.7, 3.9 Hz, 1H), 2.86 (t, J = 11.2 Hz, 1H), 3.14 (t, J = 11.2 Hz, 1H), 3.85 (d, J = 13.7 Hz, 1H), 4.44 (d, J = 13.6 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 26.48, 26.86, 27.72, 28.33, 30.27, 33.37, 34.06, 34.79, 36.39, 36.80, 39.23, 40.46, 40.93, 44.96, 108.62, 111.34, 170.53, 178.19. Anal. ($C_{24}H_{35}NO_6$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[4′-(aminocarbonyl)-1′-piperidinyl]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (35).** To a solution of **2** (220 mg, 0.5 mmol) in $CHCl_3$ (10 mL) and EtOH (10 mL) was added isonipecotamide (128 mg, 1.0 mmol). The resulting mixture was stirred at rt for 4 h before removal of the solvents. The residue was crystallized from 2:1 EtOH/water to afford **35** (164 mg, 76%) as a colorless solid. Mp 149−151 °C; ^{1}H NMR ($CDCl_3$) δ 1.16−1.32 (m, 2H), 1.58−2.06 (m, 25H), 2.22 (d, J = 6.8 Hz, 2H), 2.39 (tt, J = 11.2, 3.9 Hz, 1H), 2.69 (t, J = 11.5 Hz, 1H), 3.06 (t, J = 11.7 Hz, 1H), 3.92 (d, J = 13.7 Hz, 1H), 4.60 (d, J = 13.2 Hz, 1H), 5.54 (s, 2H); ^{13}C NMR ($CDCl_3$) δ 26.46, 26.84, 28.60, 29.02, 30.25, 30.30, 33.31, 34.05, 34.77, 36.37, 36.78, 39.24, 41.04, 42.37, 45.15, 108.61, 111.31, 170.39, 176.16. Anal. ($C_{24}H_{36}N_2O_5$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[(2′-amino-2′-methylpropyl)-amino]carbonyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (36). Step 1.** A mixture of **7**[12] (8.7 g, 28.3 mmol), HOBt (4.59 g, 34.0 mmol), and EDCI (6.52 g, 34.0 mmol) in DMF (70 mL) was stirred at rt overnight before addition of water (70 mL). The precipitate was collected by filtration and dried to afford the ozonide active ester (11.0 g, 91%) as a colorless solid. Mp 153−154 °C; ^{1}H NMR ($CDCl_3$) δ 1.64−2.16 (m, 20 H), 2.20−2.30 (m, 2H), 2.88−2.98 (m, 1H), 7.35 (d, J = 8.3 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 8.07 (d, J = 8.3 Hz, 1H). **Step 2**. A mixture of the above active ester (425 mg, 1 mmol) and 1,2-diamino-2-methylpropane (229 mg, 2.5 mmol) in $CHCl_3$ (10 mL) was stirred at rt for 4 h. The reaction mixture was washed with water (10 mL) and brine (10 mL) and dried over $MgSO_4$. After filtration, a solution of *p*-toluenesulfonic acid monohydrate (190 mg, 1 mmol) in MeOH (2 mL) was added. The resulting mixture was concentrated and triturated with EtOAc to afford **36** (360 mg, 65%) as a colorless solid. Mp 154−156 °C; ^{1}H NMR ($CDCl_3$) δ 1.19−1.31 (m, 2H), 1.36 (s, 6H), 1.48−2.03 (m, 21H), 2.40 (s, 3H), 3.45 (d, J = 6.3 Hz, 2H), 7.24 (d, J = 8.3 Hz, 2H), 7.33 (d, J = 6.6 Hz, 1H), 7.67 (d, J = 8.3 Hz, 2H), 7.75 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 21.33, 23.89, 26.43, 26.52, 26.85, 33.34, 34.74, 34.76, 36.36, 36.75, 43.04, 46.13, 56.44, 107.69, 111.15, 125.50, 129.39, 140.40, 141.67, 175.98. Anal. ($C_{28}H_{42}N_2O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[2′-[[(2′-amino-2′-methylpropyl)-amino]carbonyl]ethyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (37). Step 1.** A solution of **3**[17] (22.35 g, 124.7 mmol) and methyl 3-(4-oxocyclohexyl)propionate[18] (15.3 g, 83.1 mmol) in cyclohexane (400 mL) and CH_2Cl_2 (80 mL) was treated with ozone according to the method of Dong et al.[12] After removal of solvents, the crude product was purified by crystallization from 80% aq EtOH (150 mL) to afford adamantane-2-spiro-3′-8′-(2′-methoxycarbonylethyl)-1′,2′,4′-trioxaspiro[4.5]decane (17.18 g, 59%) as a colorless solid. Mp 102−104 °C; ^{1}H NMR ($CDCl_3$) δ 1.15−1.23 (m, 2H), 1.24−1.37 (m, 1H), 1.54−1.61 (m, 2H), 1.62−2.05 (m, 20H), 2.32 (t, J = 7.8 Hz, 2H), 3.66 (s, 3H); ^{13}C NMR ($CDCl_3$) *d* 26.46, 26.86, 29.72, 31.10, 31.74, 34.04, 34.75, 34.77, 35.53, 36.36, 36.78, 51.49, 108.79, 111.20, 174.17. **Step 2**. To a solution of the above ozonide ester (14.43 g, 41.2 mmol) in 95% aq EtOH (200 mL) was added NaOH (4.90 g, 123.7 mmol) solution in water (100 mL). The mixture was stirred at 50 °C for 4 h, cooled to 0 °C, and treated with 1 M HCl (162 mL). The precipitate was collected by filtration, washed with 50% aq EtOH (150 mL), and dried in a vacuum oven at 40 °C to give *cis*-adamantane-2-spiro-3′-8′-(2′-carboxyethyl)-1′,2′,4′-trioxaspiro[4.5]decane (13.2 g, 95%) as a colorless solid. Mp 144−146 °C; ^{1}H NMR ($CDCl_3$) δ 1.12−1.26 (m, 2H), 1.27−1.38 (m, 1H), 1.57 (dt, J = 7.8, 7.1 Hz, 2H), 1.62−2.04 (m, 20H), 2.36 (t, J = 7.6 Hz, 2H), 11.58 (br s, 1H); ^{13}C NMR ($CDCl_3$) δ 26.47, 26.86, 29.72, 30.80, 31.71, 34.02, 34.76, 34.78, 35.44, 36.37, 36.79, 108.76, 111.25, 180.06. **Step 3**. A solution of the above ozonide acid (0.5 g, 1.5 mmol), HOSu (0.21 g, 1.79 mmol), and EDCI (0.34 g, 1.79 mmol) in DMF (20 mL) under Ar was stirred at rt for 24 h before being quenched with water (25 mL) at 0 °C. The precipitate was filtered, washed with 95% aq EtOH (10 mL), and dried to afford the active ester (0.64 g, 100%) as a colorless solid. Mp 144−146 °C; ^{1}H NMR (500 MHz, $CDCl_3$) δ 1.16−1.29 (m, 2H), 1.35−1.44 (m, 1H), 1.63−2.02 (m, 22H), 2.62 (t, J = 7.6 Hz, 2H), 2.83 (br s, 4H). **Step 4**. To a solution of the ozonide active ester (0.5 g, 1.15 mmol) in $CHCl_3$ (25 mL) was added in one portion a solution of 1,2-diamino-2-methylpropane (0.20 g, 2.3 mmol) in $CHCl_3$ (15 mL). The reaction mixture was stirred at rt for 1 h before being quenched with water (50 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 25 mL). The combined extracts were washed with water (3 × 50 mL), dried over $MgSO_4$, and filtered. To the filtrate was added a solution of *p*-toluenesulfonic acid monohydrate (0.22 g, 1.16 mmol) in EtOH (10 mL). After evaporation of the solvents, the residue was treated with ether (15 mL). The solid was collected by filtration, washed with ether (30 mL), and dried to afford **37** (0.49 g, 74%) as a colorless solid. Mp 114−118 °C; ^{1}H NMR ($CDCl_3$) δ 0.86−1.02 (m, 3H), 1.33 (s, 6H), 1.34−1.58 (m, 6H), 1.62−2.06 (m, 18H), 2.39 (s, 3H), 3.42 (d, J = 5.9 Hz, 2H), 7.23 (d, J = 7.8 Hz, 2H), 7.64 (br s, 1H), 7.67 (d, J = 7.8 Hz, 2H), 7.78 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 21.36, 23.85, 26.45, 26.85, 29.52, 31.53, 33.33, 33.98, 34.76, 35.58, 36.36, 36.77, 46.54, 56.17, 108.61, 111.11, 125.64, 129.32, 140.03, 141.70, 175.48. Anal. ($C_{30}H_{46}N_2O_7S \cdot 1.2H_2O$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[(2′-amino-2′-methylpropyl)-amino]carbonyl]amino]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (38). Step 1.** To a solution of the free base of **10**[15] (0.70 g, 2.51 mmol) in CH_2Cl_2 (100 mL) was added pyridine (0.3 mL) followed by a solution of the 4-nitrophenyl chloroformate (0.6 g, 2.98 mmol) in CH_2Cl_2 (50 mL) at 0 °C. The resulting mixture was stirred at rt for 24 h, then diluted with CH_2Cl_2 (100 mL), washed with 1 M aq $NaHCO_3$ (2 × 100 mL), water (2 × 100 mL), and brine (100 mL), dried over $MgSO_4$, and filtered. After removal of solvent, the crude product was purified by crystallization from EtOH to afford the *cis*-adamantane-2-spiro-3′-8′-[[(4′-nitrophenoxy)carbonyl]amino]-1′,2′,4′-trioxaspiro-[4.5]decane (1.0 g, 90%). **Step 2**. To a solution of 1,2-diamino-2-methylpropane (0.40 g, 4.64 mmol) in $CHCl_3$ (10 mL) was added dropwise a solution of the above 4-nitrophenyl carbamate (0.4 g, 0.90 mmol) in $CHCl_3$ (10 mL) and MeOH (20 mL). The resulting mixture was stirred at rt for 4 h and then quenched with water (30 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 30 mL). The combined extracts were washed with 1 M aq $NaHCO_3$ (2 × 20 mL), water (3 × 20 mL), and brine (20 mL), dried over $MgSO_4$, filtered, and concentrated. The residue was dissolved in $CHCl_3$ (20 mL) and then treated with a solution of *p*-toluenesulfonic acid monohydrate (0.17 g, 0.89 mmol) in EtOH (10 mL). After evaporation of the solvent, the crude product was purified by crystallization from 1:4 EtOH/CH_2Cl_2 to afford **38** (0.16 g, 31%) as a colorless solid. Mp 172−173 °C; ^{1}H NMR (DMSO-d_6) δ 1.13 (s, 6H), 1.27−1.39 (m, 2H), 1.59−1.93 (m, 20H), 2.28 (s, 3H), 3.10 (d, J = 5.8 Hz, 2H), 3.44−3.54 (m, 1H), 6.01−6.11 (m, 2H), 7.10 (d, J = 7.8 Hz, 2H), 7.47 (d, J = 7.8 Hz, 2H), 7.65 (s, 3H); ^{13}C NMR (DMSO-d_6) δ 20.95, 23.31, 23.36, 25.95, 26.35, 30.04, 32.16, 34.41, 34.43, 35.87, 36.22, 46.37, 47.26, 54.51, 108.08, 110.87, 125.66, 128.20, 137.72, 145.97, 158.14. Anal. ($C_{28}H_{43}N_3O_7S$) C, H, N.

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

***p*-Tosylate (39).** A mixture of *cis*-adamantane-2-spiro-3′-8′-[[[(4′-nitrophenoxy)carbonyl]amino]methyl]-1′,2′,4′-trioxaspiro-[4.5]decane[23] (0.32 g, 0.7 mmol) and 1,2-diamino-2-methylpropane (154 mg, 3.5 mmol) in $CHCl_3$ (10 mL) was stirred at rt overnight. The mixture was then diluted with $CHCl_3$ (10 mL), washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The crude product was dissolved in CH_2Cl_2 (10 mL) and added to a solution of *p*-toluenesulfonic acid monohydrate (100 mg, 0.53 mmol) in MeOH (1 mL). After concentration, the solid was crystallized from 1:4 CH_2Cl_2/ether to afford **39** (100 mg, 25%) as a colorless solid. Mp 184 °C dec.; ^{1}H NMR ($CDCl_3$) δ 0.85−1.03 (m, 2H), 1.27 (s, 6H), 1.41−2.05 (m, 21H), 2.40 (s, 3H), 2.72 (d, J = 5.9 Hz, 2H), 3.26 (d, J = 7.3 Hz, 2H), 5.23−5.39 (m, 1H), 5.91−6.07 (m, 1H), 7.24 (d, J = 8.3 Hz, 2H), 7.56 (s, 3H), 7.69 (d, J = 8.3 Hz, 2H); ^{13}C NMR ($CDCl_3$) δ 21.37, 23.57, 26.48, 26.88, 27.56, 33.71, 34.79, 36.38, 36.57, 36.81, 45.34, 47.00, 56.55, 108.68, 111.16, 125.62, 129.42, 140.42, 141.69, 158.63. Anal. ($C_{29}H_{45}N_3O_7S$) C, H, N.

***cis*-Adamantane-2-spiro-3′-8′-[[[[(2′-amino-2′-methylpropyl)-amino]carbonyl]oxy]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (40).** To a solution of imidazole carbamate **12**[12] (358 mg, 1 mmol) in THF (10 mL) at 0 °C was added methyl trifluoromethanesulfonate (164 mg, 1 mmol). The mixture was stirred at 0 °C for 30 min before 1,2-diamino-2-methylpropane (440 mg, 5 mmol) was added. The mixture was stirred at 0 °C for additional 2 h and then stirred at rt for 18 h. After removal of the solvent, the residue was dissolved in CH_2Cl_2 (30 mL), washed with water and brine, dried over $MgSO_4$, filtered, and concentrated. The residue was triturated with 60% aq EtOH (20 mL) and filtered. The solid was dissolved in $CHCl_3$ (10 mL) and added to a solution of *p*-toluenesulfonic acid monohydrate (65 mg, 0.34 mmol) in MeOH (2 mL). After concentration, the crude product was triturated with ether to afford **40** (150 mg, 26%) as a colorless solid. Mp 66−70 °C; ^{1}H NMR ($CDCl_3$) δ 0.99−1.14 (m, 2H), 1.31 (s, 6H), 1.36−2.05 (m, 21H), 2.39 (s, 3H), 3.30 (d, J = 6.3 Hz, 2H), 3.71 (d, J = 6.8 Hz, 2H), 6.51 (t, J = 6.3 Hz, 1H), 7.20 (d, J = 7.8 Hz, 2H), 7.72 (d, J = 7.8 Hz, 2H), 7.79 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 21.38, 23.59, 26.48, 26.54, 26.88, 33.57, 34.80, 35.66, 36.39, 36.80, 48.40, 56.08, 68.98, 108.56, 111.26, 125.82, 129.06, 140.81, 141.02, 157.56. Anal. ($C_{29}H_{44}N_2O_8S$) C, H, N.

***trans*-Adamantane-2-spiro-3′-8′-[[(2′-amino-2′-methylpropyl)-amino]carbonyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (41). Step 1.** A mixture of **13**[23] (8.0 g, 26 mmol), HOBt (5.3 g, 39 mmol), and EDCI (6.9 g, 36.4 mmol) in DMF (70 mL) was stirred at rt overnight. The precipitate was collected by filtration and dried to afford the desired active ester (7.0 g, 64%) as a colorless solid. Mp 145−147 °C; ^{1}H NMR ($CDCl_3$) d 1.64−2.10 (m, 18H), 2.11−2.20 (m, 2H), 2.22−2.30 (m, 2H), 2.90−2.98 (m, 1H), 7.38 (d, J = 8.3 Hz, 1H), 7.43 (t, J = 7.8 Hz, 1H), 7.55 (t, J = 7.8 Hz, 1H), 8.07 (d, J = 8.3 Hz, 1H). **Step 2**. A mixture of the above active ester (425 mg, 1 mmol) and 1,2-diamino-2-methylpropane (229 mg, 2.5 mmol) in $CHCl_3$ (10 mL) was stirred at rt for 4 h. The reaction mixture was washed with water (10 mL) and brine (10 mL) and dried over $MgSO_4$. After filtration, a solution of *p*-toluenesulfonic acid monohydrate (190 mg, 1 mmol) in MeOH (2 mL) was added. The resulting mixture was concentrated and triturated with EtOAc to afford **41** (450 mg, 82%) as a colorless solid. Mp 162−165 °C; ^{1}H NMR ($CDCl_3$) δ 1.04−1.17 (m, 2H), 1.34 (s, 6H), 1.58−2.03 (m, 21H), 2.39 (s, 3H), 3.42 (d, J = 6.8 Hz, 2H), 7.23 (d, J = 7.8 Hz, 2H), 7.31 (d, J = 6.3 Hz, 1H), 7.65 (d, J = 7.8 Hz, 2H), 7.76 (s, 3H); ^{13}C NMR ($CDCl_3$) δ 21.58, 24.12, 26.64, 26.68, 27.06, 33.38, 34.95, 35.03, 36.56, 36.99, 43.04, 46.33, 56.65, 107.98, 111.94, 125.68, 129.65, 140.62, 142.11, 176.22. Anal. ($C_{28}H_{42}N_2O_7S$) C, H, N.

***trans*-Adamantane-2-spiro-3′-8′-[[[(2′-amino-2′-methylpropyl)-amino]carbonyl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane *p*-Tosylate (42). Step 1.** A 1:1 mixture of the *cis* (**5**) and *trans* ozonide esters (4.14 g, 21%) was obtained from a 59 mmol scale preparation of ozonide ester **5**, after chromatography and repeated crystallizations from EtOH to remove **5**. **Step 2**. To the above mixture of ozonide ester isomers (4.1 g, 12.20 mmol) in 95% EtOH (50 mL) was added a solution of NaOH (1.46 g, 36.60 mmol) in water (10 mL). The mixture was stirred at 50 °C for 4 h, cooled to 0 °C, and neutralized with 1.0 M aq HCl. The precipitate was collected by filtration, washed with 50% aq EtOH (50 mL), and dried in vacuo at 40 °C to give a 1:1 mixture of *cis* (**6**) and *trans* ozonide acids (3.10 g, 77%) as a colorless solid. **Step 3**. A solution of the above ozonide acid isomers (3.10 g, 9.62 mmol), HOBt (1.56 g, 11.55 mmol), and EDCI (2.28 g, 11.55 mmol) in DMF (50 mL) was stirred at 0 °C for 2 h before 4-nitrophenol (1.6 g, 11.55 mmol) was added. After being stirred for an additional 2 h at 0 °C, the reaction was stirred at rt overnight. The reaction was quenched with ice−water (50 mL) at 0 °C and extracted with EtOAc (3 × 50 mL). The organic phase was washed with water (3 × 100 mL) and brine and dried over $MgSO_4$. After removal of the solvent, the residue was purified by chromatography followed by repeated crystallizations from EtOH to give pure *trans*-4-nitrophenyl ozonide ester (0.210 g, 5%). ^{1}H NMR ($CDCl_3$) δ 1.51−2.05 (m, 23 H), 2.57 (d, J = 6.84 Hz, 2H), 7.27 (d, J = 9.3 Hz, 2H)), 8.27 (d, J = 9.3 Hz, 2H). **Step 4**. To a solution of *trans*-4-nitrophenyl ozonide ester (0.180 g, 0.42 mmol) in $CHCl_3$ (5 mL) was rapidly added a solution of 1,2-diamino-2-methylpropane (0.184 g, 2.10 mmol) in $CHCl_3$ (5 mL). The reaction mixture was stirred at rt overnight and then quenched with water (15 mL). After separation of the organic layer, the aqueous layer was extracted with $CHCl_3$ (2 × 10 mL). The combined extracts were washed with water (3 × 25 mL), dried over $MgSO_4$, and concentrated to afford **42** free base (0.130 g, 80%) as a colorless solid. ^{1}H NMR ($CDCl_3$) δ 1.11 (s, 6H), 1.20 (brs, 2H), 1.34−1.44 (m, 2H), 1.58−2.03 (m, 21H), 2.15 (d, J = 6.8 Hz, 2H), 3.14 (d, J = 5.8 Hz, 2H), 6.02 (brs, 1H); ^{13}C NMR (MHz, $CDCl_3$) δ 26.43, 26.88, 28.74, 29.79, 33.75, 33.79, 34.69, 34.90, 36.32, 36.75, 43.46, 49.74, 50.22, 108.53, 111.52, 172.13. To a solution of the free base of **42** in $CHCl_3$ (5 mL) was added a solution of *p*-toluenesulfonic acid monohydrate (0.072 g, 0.38 mmol) in EtOH (1 mL). After removal of the solvent, the residue was treated with ether (15 mL). The precipitate was filtered, washed with ether (20 mL), and dried to afford **42** (0.156 g, 85%) as a colorless solid. Mp 150−152 °C; ^{1}H NMR ($CDCl_3$) δ 1.17 (s, 6H), 1.21−1.34 (m, 2H), 1.51−1.98 (m, 21H), 2.10 (d, J = 7.3 Hz, 2H), 2.29 (s, 3H), 3.20 (d, J = 6.4 Hz, 2H), 7.12 (d, J = 7.8 Hz, 2H), 7.48 (d, J = 7.8 Hz, 2H), 7.71 (brs, 3H), 8.05 (t, J = 6.3 Hz, 1H); ^{13}C NMR ($CDCl_3$) δ 20.95, 23.48, 25.96, 26.37, 29.45, 32.85, 32.26, 34.38, 34.56, 35.93, 36.23, 41.71, 46.02, 54.53, 108.59, 110.92, 125.66, 128.24, 137.83, 145.81, 172.54.

***cis*-Adamantane-2-spiro-3′-8′-[[1′-(2′-amino-2′-methylpropyl)-1′*H*-tetrazol-5′-yl]methyl]-1′,2′,4′-trioxaspiro[4.5]decane (43).** To a mixture of the free base of **1** (0.46 g, 1.17 mmol), triphenylphosphine (0.77 g, 2.94 mmol), and trimethylsilyl azide (0.34 g, 2.95 mmol) in THF (20 mL) at 0 °C under Ar was added dropwise diisopropyl azodicarboxylate (0.71 g, 3.51 mmol). The mixture was slowly warmed to rt and stirred for 72 h. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (2 × 50 mL). The combined extracts were washed with saturated aqueous $NaHCO_3$ (2 × 50 mL) and brine (50 mL), dried over $MgSO_4$, filtered, and concentrated. To a solution of the residue $CHCl_3$ (20 mL) was added a solution of *p*-toluenesulfonic acid monohydrate (0.22 g) in EtOH (10 mL). After evaporation of the solvents, the crude product was purified by crystallization from 1:4 MeOH/CH_2Cl_2 to afford **43** (0.12 g, 17%) as a colorless solid. Mp 220 °C dec; ^{1}H NMR (CD_3OD) δ 1.28−1.38 (m, 2H), 1.38 (s, 6H), 1.65−2.09 (m, 21H), 2.37 (s, 3H), 2.86 (d, J = 6.8 Hz, 2H), 4.58 (s, 2H), 7.23 (d, J = 7.8 Hz, 2H), 7.70 (d, J = 7.8 Hz, 2H); ^{13}C NMR (CD_3OD) δ 21.30, 23.79, 27.97, 28.37, 30.04, 30.87, 34.87, 35.77, 35.78, 36.04, 37.80, 37.88, 54.02, 55.39, 109.53, 112.40, 126.97, 129.81, 141.66, 157.09.

Antimalarial Screens. In vitro and in vivo antimalarial data was obtained as previously described.[42,43]

Partition Coefficients. Log $D_{pH7.4}$ values, calculated as previously described,[9,11] denote the octanol/buffer partition coefficients at pH 7.4 that are relevant for the ionizable ozonides. Experimental log $D_{pH7.4}$ values (elog *D*) for ozonides **277** and **339** were determined using a modification of a previously reported chromatographic method.[44]

Pharmacokinetic Experiments. In vivo pharmacokinetic studies were conducted in conscious male Sprague−Dawley rats. Compounds were dosed by intravenous infusion over 5−10 min through a cannula previously implanted in the jugular vein at doses of 2.5−3.5 mg/kg. The IV formulations consisted of either DMSO (4−10% v/v)/aqueous buffer or propylene glycol (10−40% v/v)/ethanol (10−15% v/v)/water. Oral doses were administered by gavage as aqueous suspensions prepared in 0.5% w/v hydroxypropylmethyl cellulose or carboxymethyl cellulose, 0.4% Tween 80, and 0.5% benzyl alcohol. Blood samples were collected via a cannula previously implanted in the carotid artery, and plasma was immediately obtained by centrifugation after which samples were stored at −20 °C. For analysis, samples were thawed, plasma proteins were precipitated with acetonitrile, and aliquots of the supernatant were analyzed by LC/MS. Quantitation was conducted by comparison to the response obtained for calibration standards also prepared in plasma using the same methods.

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Supporting Information Available: Elemental analysis and HRMS data for **1**, **6**, and **15**−**43**. This material is available free of charge via the Internet at http://pubs.acs.org.

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