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First Example of Catalytic Synthesis of Cyclic S-Containing Di- and Triperoxides

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Academic Editor: Alexander O. Terent'ev

Received: 23 March 2020; Accepted: 15 April 2020; Published: 18 April 2020

Abstract: An efficient method for the synthesis of tetraoxathiaspiroalkanes, tetraoxathiocanes, and hexaoxathiadispiroalkanes was developed by reactions of pentaoxacanes, pentaoxaspiroalkanes, and heptaoxadispiroalkanes with hydrogen sulfide in the presence of a catalyst, $Sm(NO_3)_3 \cdot 6H_2O$. We found that the synthesized S-containing di- and triperoxides exhibit high cytotoxic activity against Jurkat, K562, U937, and HL60 tumor cultures, and fibroblasts.

Keywords: catalysis; lanthanide salts; hydrogen sulfide; thia-peroxides; cytotoxic activity

1. Introduction

Cyclic peroxides occur widely in nature, and they often possess desired pharmacological properties. For example, an eight-membered cyclic azaperoxide moiety is included in the biologically active alkaloid compounds fumitremorgins [1–7], namely into the fumitremorgin A *Verruculogen* produced by fungi of species *Penicillium verruculosum* [8], *Aspergillus caespitosus* [9], *A. fumigatus* [10], *A. fischeri* [11], *Penicillium piscarium* [12], *Penicillium paxilli* [13], *Penicillium estinogenum* [14], *Penicillium simplicissimum, Penicillium piceum, Penicillium nigricans, Penicillium raistricki* [15], and *Neosartorya fischeri* [16]. Fumitremorgin and related compounds are active against various cancer cells [17]. Some of these natural compounds can arrest cancer cells in their cell cycle, and some can block ABC transporters and reverse resistance in chemotherapy. Assessment of structural–functional relationships enabled prediction of biological activity in peroxide compounds due to a presence of heteroatom in the α-position with regard to the peroxide group [18–20]. Previously, we synthesized azaperoxides and demonstrated the cytotoxic activity of these compounds [21–24]. In continuation of ongoing research on the synthesis of heteroatom-containing peroxides, we attempted to synthesize S-peroxides.

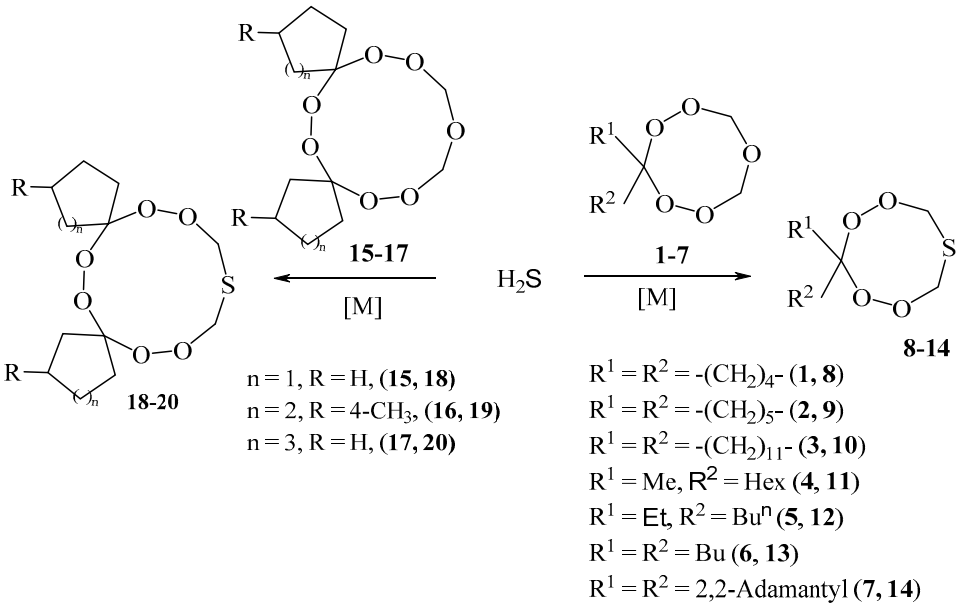
The data available on heteroatom-containing peroxides with high pharmacological activity [25–39] suggest that S-containing peroxides could be useful for the development of antimalarial and antibacterial agents. Those cyclic S-containing peroxides known from the literature are represented by thio-ozonides [40–44], obtained via photooxidation at a temperature of –78 °C. In most instances [40–44], these compounds are already unstable at 0 °C. There is no data available on stable S-containing cyclic diperoxides. This paper describes a catalytic method developed for the synthesis of cyclic thia-diperoxides with high yields and selectivity.

2. Results and Discussion

2.1. Chemistry

A classic example of the preparation of cyclic thioesters is recyclization of furan using hydrogen sulfide according to the Yuriev reaction at a temperature of 550 °C in the presence of Al_2O_3 [45]. Practically no information is available in the literature on the synthesis of cyclic thioesters at room temperature under the action of lanthanide catalysts. We developed a method for the preparation of thioperoxycarbocycles through the recyclization of pentaoxacanes and heptaoxadispiroalkanes with hydrogen sulfide under the action of lanthanide catalysts. We chose lanthanide catalysts due to their high activity in recyclization reactions involving primary amines, leading to cyclic *N*-containing di- and triperoxides [46–50].

We assumed that cyclic thia-diperoxides may be synthesized by a reaction of pentaoxacanes with hydrogen sulfide in a similar manner to what we reported previously for the synthesis of cyclic aza-diperoxides via the reaction of pentaoxacanes with primary amines [46,47,50]. Preliminary experiments demonstrated that 7,8,10,12,13-pentaoxaspiro[5.7]tridecane [51] (**1**) reacts with H_2S in the presence of the catalyst $Sm(NO_3)_3 \cdot 6H_2O$ [46–50] for 6 h at room temperature in tetrahydrofuran (THF) solvent to produce 7,8,12,13-tetraoxa-10-thiaspiro[5.7]tridecane (**8**) in 98% yield. The reaction does not proceed in the absence of a catalyst (Scheme 1).



Scheme 1. Synthesis of cyclic S-containing di- and triperoxides.

Subsequent experiments demonstrated that in certain conditions (5 mol % $Sm(NO_3)_3 \cdot 6H_2O$, 20 °C, 6 h), the yield of the target product **8** is dependent on the solvent and decreases in the following order: THF > CH_2Cl_2 > Et_2O > C_6H_{12} > EtOAc > C_2H_5OH (Table 1). To ascertain the dependency relationship between the nature of a central atom in the lanthanide catalyst and the yield of **8**, in the reaction presented here, we tested, along with the compound $Sm(NO_3)_3 \cdot 6H_2O$, a series of other lanthanide salts and complexes based on Ho, Tb, Dy, Nd, and La (Table 1). Use of the catalysts based on d- and f-elements, such as Co, Fe, Al, and Ni salts, results in decomposition of the peroxide group that enables the isolation of ketones and cyclic sulfides from the reaction mass. The reactions were conducted at ~20 °C in THF in the presence of the catalysts (5 mol %) specified earlier. Under the indicated conditions, selective formation of the 7,8,12,13-tetraoxa-10-thiaspiro[5.7]tridecane (**8**) was observed with yields of 58% to 84% (Table 1). In the determined conditions (5 mol % $Sm(NO_3)_3 \cdot 6H_2O$,

THF, 20 °C, 6 h), the reaction of cyclocondensation of pentaoxaspiroalkanes (**2,3**) with H_2S results in selective formation of tetraoxathiaspiroalkanes (**9,10**) in yields of 90% and 85%, respectively.

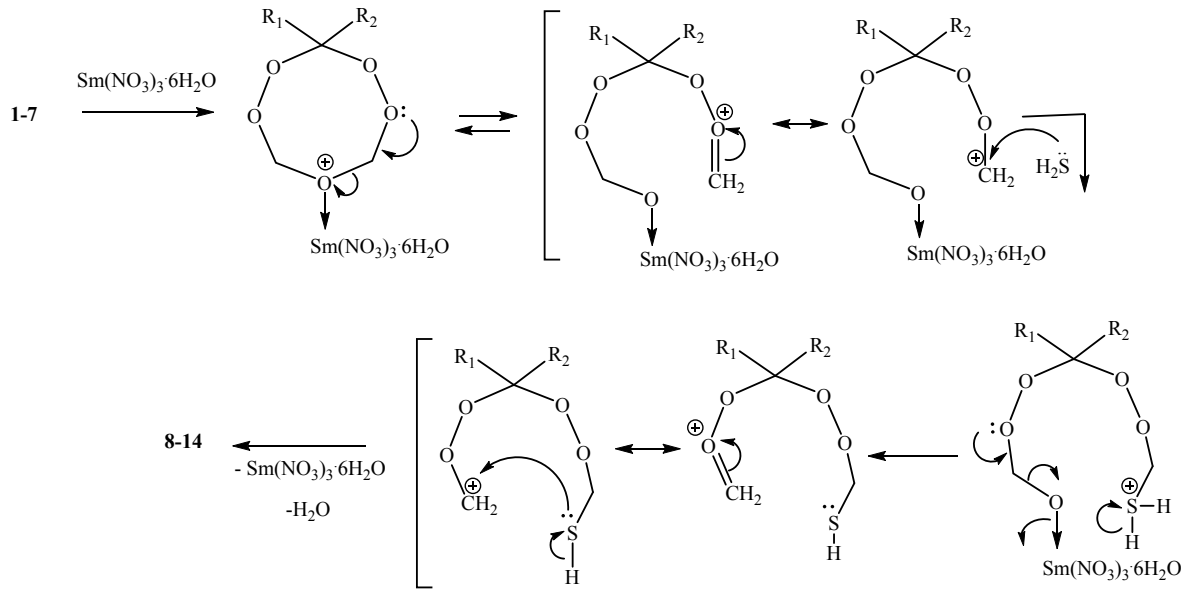
Table 1. Optimization of the reaction conditions for the synthesis 7,8,12,13-tetraoxa-10-thiaspiro[5.7] tridecane (**8**).

Entry	[M]	Solvent	Yield * of 8%
1	$Sm(NO_3)_3{\cdot}6H_2O$	THF	98
2	$Sm(NO_3)_3{\cdot}6H_2O$	CH_2Cl_2	85
3	$Sm(NO_3)_3{\cdot}6H_2O$	Et_2O	79
4	$Sm(NO_3)_3{\cdot}6H_2O$	C_6H_{12}	15
5	$Sm(NO_3)_3{\cdot}6H_2O$	EtOAc	10
6	$Sm(NO_3)_3{\cdot}6H_2O$	C_2H_5OH	7
7	$Ho(NO_3)_3$ $5H_2O$	THF	84
8	$TbCl_3$ $6H_2O$	THF	72
9	$DyCl_3$ $6H_2O$	THF	67
10	$NdCl_3$ $6H_2O$	THF	61
11	$La(NO_3)_3{\cdot}6H_2O$	THF	58

* Experimental conditions: **1**:[M] molar ratio of 1:0.05; 20 °C; 6 h; 5 mL solvent.

The reaction thus developed provides a convenient tool for preparation of various tetraoxa thiocanes. By using the described procedure, the synthesis of 3,3-disubstituted tetraoxathiocanes was implemented via the catalytic reaction of pentaoxacanes with hydrogen sulfide. In reactions of 3,3-disubstituted pentaoxacanes **4–7** with H_2S catalyzed by $Sm(NO_3)_3{\cdot}6H_2O$, 1,2,4,5,7-tetraoxathiocanes, **11–14** are selectively formed with yields of 80% to 89%.

It can be assumed [52] that formation of tetraoxathiaspiroalkanes **8–14** occurs via a pentaoxacane ring opening affected by the catalyst [53,54]. Subsequent nucleophilic addition of H_2S to the carbocation results in intramolecular cyclization, where the corresponding tetraoxathiaspiroalkanes **8–14** are obtained (Scheme 2).



Scheme 2. Formation of S-containing diperoxides (**8–14**).

To expand the scope of applicability of the method for the synthesis of cyclic thio-peroxides developed here, we produced spiro-fused hexaoxathiocanes **18–20** by reaction of heptaoxacanes **15–17** [48] with hydrogen sulfide in THF (~20 °C, 6 h), catalyzed by $Sm(NO_3)_3 \cdot 6H_2O$ (0.5 mol %). we observed that the size of the carbocycles in initial heptaoxadispiroalkanes **15–17** does not affect the yield of hexaoxathiocanes **18–20** (83–86%).

The structures of cyclic S-containing peroxides 8–14 and 18–20 were confirmed by ^1H and ^{13}C NMR spectra of the synthesized compounds. The methylene fragment signals characteristic of these –S-CH_2-O-O- systems are manifested in the regions of 4.81 to 5.31 ppm and 81.4 to 83.7 ppm in the spectra of ^1H and ^{13}C NMR, respectively. These signals reflect the process of cyclic interconversion in solution; therefore, we observed a set of signals with close chemical shifts for each of the individual compounds. The effect of the splitting of the NMR signals of the ring atoms is due to the presence of a multicomponent conformational equilibrium at room temperature, which can be assumed on the basis of published data on the identification of known heteroatom-containing peroxides, in particular azadi- and triperoxides [46–50]. The presence of one conformation was observed only in the case of 3-(adamantyl-2-yl)-1,2,4,5,7-tetraaoxatiocane (14), probably due to the rigidity of the structure of the spiroadamantane substituent.

2.2. Biological Evaluation

Cytotoxicity of azaperoxide-based compounds is well known [1–18], so we screened the representative compounds for their cytotoxicity activity against Jurkat, K562, U937, and HL60 fibroblasts cell lines. The results are summarized in Table 2.

Table 2. Cytotoxic activities in vitro of compounds **8–14** and **18–20** measured on tumor cell cultures (Jurkat, K562, U937, and HL60, fibroblasts) (μM).

Compound	Jurkat (IC_{50}, μM)	K562 (IC_{50}, μM)	HL60 (IC_{50}, μM)	U937 (IC_{50}, μM)	Fibroblasts (IC_{50}, μM)
8	5.26 ± 0.57	7.15 ± 0.64	4.59 ± 0.38	24.13 ± 1.87	118.61 ± 8.74
9	4.91 ± 0.43	6.83 ± 0.59	4.14 ± 0.34	21.17 ± 2.11	97.88 ± 6.81
10	3.52 ± 0.31	5.77 ± 0.46	2.67 ± 0.21	15.24 ± 1.26	81.42 ± 5.12
12	4.45 ± 0.49	6.29 ± 0.57	3.91 ± 0.33	19.89 ± 1.57	85.93 ± 5.47
13	10.21 ± 0.87	14.37 ± 0.96	8.56 ± 0.69	35.24 ± 2.65	142.17 ± 9.76
14	9.61 ± 0.79	11.97 ± 0.91	8.22 ± 0.74	32.81 ± 2.89	129.23 ± 8.92
18	17.11 ± 1.24	21.75 ± 1.59	14.96 ± 0.97	46.67 ± 3.76	188.36 ± 12.91
19	2.81 ± 0.37	4.37 ± 0.31	2.24 ± 0.29	11.79 ± 0.99	79.17 ± 5.41
20	23.94 ± 1.67	28.26 ± 1.48	19.61 ± 1.12	65.81 ± 4.84	195.87 ± 14.67

IC_{50}, or the concentration of half-maximal inhibition, is an indicator of the effectiveness of a ligand in inhibiting biochemical or biological interaction.

The synthesized S-containing diperoxides **8–14** and triperoxides **18–20** exhibited a cytotoxic effect against a number of suspension tumor cell lines (Jurkat, K562, U937, and HL60) in the range of 2.24 to 65.81 μM and 79.17 to 195.87 μM for normal fibroblasts. The synthesized compounds had a rather high selectivity index (SI = IC_{50} fibroblasts/IC_{50} cancer cells) for Jurkat, HL60, and K562 tumor cells, ranging from 8 to 35, whereas for the U937 culture the selectivity index ranged from 3 to 7. The highest cytotoxic activity (2.24–11.79 μM) was exhibited by triperoxide **19**, synthesized based on 4-methylcyclohexane derivative **16**, as well as a number of diperoxides **8–12**. As can be seen from Table 2, a pronounced selective effect is observed on the myelocytic (K562) and lymphocytic (Jurkat, HL60) cell lines, in comparison with the cytotoxicity of the studied compounds to a cell culture of monocytic origin (U937). The lowest cytotoxicity with respect to the studied tumor cultures was demonstrated by symmetric diperoxides with **13** dibutyl and **14** adamantane substituents.

3. Materials and Methods

3.1. Chemistry

All reactions were performed at room temperature in air in round-bottom flasks equipped with a magnetic stir bar. The NMR spectra were recorded on a Bruker Avance 500 spectrometer at 500.17 MHz for ^{1}H and 125.78 MHz for ^{13}C according to standard Bruker procedures. $CDCl_3$ was used as the solvent and tetramethylsilane as the internal standard. The mixing time for the NOESY (Nuclear Overhauser Effect SpectroscopY) experiments was 0.3 sec. Mass spectra were recorded on a Bruker Autoflex III MALDI TOF/TOF (Matrix Assisted Laser Desorption/Ionization) instrument with α-cyano-4-hydroxycinnamic acid as a matrix. Samples were prepared by the dried droplet method. C, H, and S were quantified by a Carlo Erba 1108 analyzer. The oxygen content was determined on a Carlo Erba 1108 analyzer. The progress of reactions was monitored by TLC on Sorbfil (PTSKh-AF-A) plates, with a 5:1 hexane:EtOAc mixture as the eluent and visualized with I2 vapor. For column chromatography, silica gel MACHEREY-NAGEL (0.063–0.2 mm) was used.

The synthesis of the pentaoxacanes **1–7** was as reported in the literature [51]. The synthesis of the heptaoxadispiroalkanes **15–17** was also as reported in the literature [48]. THF was freshly distilled over $LiAlH_4$. Hydrogen sulfide was obtained by the action of sodium hydrogen sulfate on hydrochloric acid.

3.1.1. Reactions of Pentaoxacanes with Hydrogen Sulfide in the Presence of a Catalyst, $Sm(NO_3)_3{\cdot}6H_2O$

General procedure: A calcined and argon-filled Schlenk vessel equipped with a magnetic stir bar was charged with THF (5 mL), $Sm(NO_3)_3{\cdot}6H_2O$ (0.5 mmol), and pentaoxacanes (10 mmol). The mixture was stirred at 20 °C for 1 h. Next, the hydrogen sulfide obtained by in situ was added while continuously bubbling for 1.5 h to the mixture, which was stirred for 5 h at 20 °C. After completion of the reaction, H_2O (5 mL) and CH_2Cl_2 (5 mL) were added. The organic layer was separated, dried (anhydrous $MgSO_4$), and concentrated to isolate products stable during storage at room temperature. Products of the reaction were purified by column chromatography on SiO_2 using 10:1 PE:Et_2O as the eluent. The progress of reactions was monitored by TLC, with a 5:1 hexane:EtOAc mixture as the eluent; visualization was performed with I_2 vapor. ^{1}H NMR and ^{13}C NMR spectra of all new compounds are in the supplementary file.

6,7,11,12-tetraoxa-9-thiaspiro[4.7]dodecane (8), colorless oil; 0.19 g (98% yield), retention factors (R_f) 0.74 (PE/Et_2O = 10/1). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.43–1.58 (m, 4H, CH_2), 1.78–1.99 (m, 4H, CH_2), 5.18–5.22 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 22.4, 24.5, 25.3, 29.7, 29.5, 33.0, 81.8, 81.9, 82.3, 110.1, 110.5. MALDI TOF/TOF, m/z: 191 $[M\text{-}H]^+$. Anal. calcd. for $C_7H_{12}O_4S$: C, 43.74; H, 6.29; S, 16.68%. Found C, 43.72; H, 6.27; S, 16.66%.

7,8,12,13-tetraoxa-10-thiaspiro[5.7]tridecane (9), colorless oil; 0.18 g (90% yield), R_f 0.76 (PE/Et_2O = 10/1). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.45–1.62 (m, 6H, CH_2), 1.74–1.90 (m, 4H, CH_2), 5.20 (s, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 22.4, 25.3, 24.9, 25.4, 29.5, 29.8, 81.8, 110.1, 110.5. MALDI TOF/TOF, m/z: 205 $[M\text{-}H]^+$. Anal. calcd. for $C_8H_{14}O_4S$: C, 46.59; H, 6.84; S, 15.54%. Found C, 46.58; H, 6.82; S, 15.52%.

1,2,6,7-tetraoxa-4-thiaspiro[7.11]nonadecane (10), colorless oil; 0.25 g (85% yield), R_f 0.78 (PE/Et_2O = 10/1). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.27–1.81 (m, 22H, CH_2), 5.17–5.20 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 19.3, 21.8, 22.2, 22.3, 22.6, 24.2, 24.6, 24.7, 25.9, 26.0, 26.1, 26.2, 26.9, 82.4, 83.6, 113.9. MALDI TOF/TOF, m/z: 289 $[M\text{-}H]^+$. Anal. calcd. for $C_{14}H_{26}O_4S$: C, 57.90; H, 9.02; S, 11.04%. Found C, 57.88; H, 9.00; S, 11.01%.

3-hxyl-3-methyl-1,2,4,5,7-tetraoxathiocane (11), colorless oil; 0.19 g (80% yield), R_f 0.73 (PE/Et_2O = 10/1). ^{1}H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.89–0.92 (m, 3H, CH_3), 1.28–1.75 (m, 13H, CH_2), 4.81–5.29 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 14.1, 18.9, 22.5, 23.9, 24.1, 29.4, 31.6, 33.9, 82.5, 83.7,

111.4. MALDI TOF/TOF, m/z: 235 $[M-H]^+$. Anal. calcd. for $C_{10}H_{20}O_4S$: C, 50.82; H, 8.53; S, 13.57%. Found C, 50.80; H, 8.51; S, 13.55%.

3-butyl-3-ethyl-1,2,4,5,7-tetraoxathiocane (12), colorless oil; 0.19 g (84% yield), R_f 0.75 (PE/Et_2O = 10/1). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.89–0.94 (m, 6H, CH_3), 1.32–1.33 (m, 4H, CH_2), 1.66–1.74 (m, 4H, CH_2), 5.00–5.26 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 7.9, 13.9, 22.4, 22.8, 25.5, 25.6, 28.5, 29.6, 81.4, 81.6, 113.7, 113.8. MALDI TOF/TOF, m/z: 221 $[M-H]^+$. Anal. calcd. for $C_9H_{18}O_4S$: C, 48.63; H, 8.16; S, 14.42%. Found C, 48.61; H, 8.14; S, 14.40%.

3,3-dibutyl-1,2,4,5,7-tetraoxathiocane (13), colorless oil; 0.22 g (87% yield), R_f 0.74 (PE/Et_2O = 10/1). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.92–0.94 (m, 6H, CH_3), 1.27–1.75 (m, 12H, CH_2), 4.97–5.31 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 7.9, 13.9, 22.8, 25.6, 25.7, 25.9, 29.1, 29.3, 29.8, 81.7, 82.4, 83.6, 113.3, 113.6. MALDI TOF/TOF, m/z: 249 $[M-H]^+$. Anal. calcd. for $C_{11}H_{22}O_4S$: C, 52.77; H, 8.86; S, 12.81%. Found C, 52.75; H, 8.85; S, 12.80%.

3-(adamantyl-2-yl)-1,2,4,5,7-tetraaoxatioocane (14), colorless oil; 0.23 g (89% yield), R_f 0.76 (PE/Et_2O = 10/1). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.67–1.71 (m, 6H, CH_2), 1.88 (s, 1H, CH), 2.01–2.03 (m, 4H, CH_2), 2.33–2.38 (m, 3H, CH, CH_2), 5.21 (d, 4H, J = 4 Hz, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 26.9, 27.0, 27.1, 31.2, 31.5, 33.7, 37.7, 37.1, 81.7, 112.1, 112.6. MALDI TOF/TOF, m/z: 257 $[M-H]^+$. Anal. calcd. for $C_{12}H_{18}O_4S$: C, 55.79; H, 7.02; S, 12.41%. Found C, 55.77; H, 7.00; S, 12.40%.

3.1.2. Reactions Heptaoxadispiroalkanes with Hydrogen Sulfide in Presence of a Catalyst, $Sm(NO_3)_3 \cdot 6H_2O$

General procedure: A calcined and argon-filled Schlenk vessel equipped with a magnetic stir bar was charged with THF (5 mL), $Sm(NO_3)_3 \cdot 6H_2O$ (0.5 mmol), and heptaoxadispiroalkanes (10 mmol). The mixture was stirred at 20 °C for 1 h. Next, the hydrogen sulfide obtained in situ was added while continuously bubbling for 1.5 h to the mixture, which was stirred for 5 h at 20 °C. After completion of the reaction, H_2O (5 mL) and CH_2Cl_2 (5 mL) were added. The organic layer was separated, dried (anhydrous $MgSO_4$), and concentrated to isolate products stable during storage at room temperature. Products of the reaction were purified by column chromatography on SiO_2 using 10:1 PE:Et_2O as the eluent. The progress of reactions was monitored by TLC, with a 5:1 hexane:EtOAc mixture as the eluent; visualization was performed with I_2 vapor.

6,7,13,14,18,19-hexaoxa-16-thiadispiro[4.2.4^8.7^5]nonadecane (15), colorless oil; 0.29 g (87% yield), R_f 0.79 (PE/Et_2O = 10/1). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.73–1.80 (m, 4H, CH_2), 1.93–2.09 (m, 4H, CH_2), 5.13–5.25 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 24.5, 24.6, 33.1, 33.3, 33.4, 33.8, 33.9, 81.9, 82.5, 120.3. MALDI TOF/TOF, m/z: 291 $[M-H]^+$. Anal. calcd. for $C_{12}H_{20}O_6S$: C, 49.30; H, 6.90; S, 10.97%. Found C, 49.28; H, 6.89; S, 10.95%.

3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-thiadispiro[5.2.5^9.7^6]henicosane (16), colorless oil; 0.29 g (83% yield), R_f 0.79 (PE/Et_2O = 10/1). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 0.93–0.94 (m, 6H, CH_3), 1.20–1.26, and 1.44–1.57 (m, 8H, CH_2), 1.60–1.64 and 2.16–2.25 (m, 8H, CH_2), 1.99–2.00 (m, 2H, CH), 5.18–5.23 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 21.3, 21.4, 22.7, 29.1, 29.2, 29.3, 29.4,29.8, 30.5, 30.6, 30.7, 31.6, 31.7, 33.1, 81.8, 81.9, 110.1, 111.1. MALDI TOF/TOF, m/z: 347 $[M-H]^+$. Anal. calcd. for $C_{16}H_{28}O_6S$: C, 55.15; H, 8.10; S, 9.20%. Found C, 55.13; H, 8.08; S, 9.17%.

8,9,17,18,22,23-hexaoxa-20-thiadispiro[6.2.6^{10}.7^7]tricosane (17), colorless oil; 0.29 g (85% yield), R_f 0.80 (PE/Et_2O = 10/1). ^1H NMR (400 MHz, $CDCl_3$, 25 °C): δ = 1.58–1.73 (m, 16H, CH_2), 1.86–2.04 (m, 8H, CH_2), 5.13–5.31 (m, 4H, CH_2). ^{13}C NMR (100 MHz, $CDCl_3$, 25 °C): δ = 22.7, 22.8, 29.8, 29.9, 30.2, 30.4, 32.4, 32.8, 32.9, 81.8, 82.5, 115.2, 116.2. MALDI TOF/TOF, m/z: 347 $[M-H]^+$. Anal. calcd. for $C_{16}H_{28}O_6S$: C, 55.15; H, 8.10; S, 9.20%. Found C, 55.14; H, 8.08; S, 9.18%.

3.2. Biology

3.2.1. Cell Culturing

Cells (Jurkat, K562, U937, HeLa, HEK293, and normal fibroblasts) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard mammalian tissue culture protocols and sterile technique. Human cell lines HEK293 and HeLa were obtained from the HPA Culture Collections (U.K.). All cell lines used in the study were tested and shown to be free of mycoplasma and viral contamination.

HEK293, HeLa cell lines, and fibroblasts were cultured as monolayers and maintained in Dulbecco's modified eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum (FBS) and 1% penicillin-streptomycin solution at 37 °C in a humidified incubator under a 5% CO_2 atmosphere.

Cells were maintained in RPMI (Roswell Park Memorial Institute medium) 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 mM glutamine, 10% FBS (Sigma), and 100 units/mL penicillin-streptomycin (Sigma). All types of cells were grown in an atmosphere of 5% CO_2 at 37 °C. The cells were subcultured at 2- to 3-day intervals. Adherent cells (HEK293, HeLa, fibroblasts) were suspended using trypsin/EDTA (Ethylenediaminetetraacetic acid) and counted after they reached 80% confluency. Cells were then seeded in 24 well plates at 5×10^4 cells per well and incubated overnight. Jurkat, K562, and U937 cells were subcultured in 2-day intervals with a seeding density of 1×10^5 cells per 24 well plates in RPMI with 10% FBS.

3.2.2. Cytotoxicity Assay

Viability (live/dead) assessment was performed by staining cells with 7-aminoactinomycin D (7-AAD) (Biolegend). After treatment, cells were harvested, washed 1 to 2 times with phosphate-buffered saline (PBS), and centrifuged at 400× *g* for 5 min. Cell pellets were resuspended in 200 µL of flow cytometry staining buffer (PBS without Ca^{2+} and Mg^{2+}, 2,5% FBS) and stained with 5 µL of 7-AAD staining solution for 15 min at room temperature in the dark. Samples were acquired on the NovoCyteTM 2000 FlowCytometry System (ACEA) equipped with a 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in the FL4 channel.

4. Conclusions

For the first time, an approach was developed that allows for the selective synthesis of new classes of stable tetraoxathiaspiroalkanes, tetraoxathiocanes, and hexaoxathiadispiroalkanes by reactions of pentaoxaspiroalkanes, pentaoxacanes, and heptaoxadispiroalkanes with hydrogen sulfide in the presence of lanthanide catalysts ($Sm(NO_3)_3 \cdot 6H_2O$, $Ho(NO_3)_3 \cdot 5H_2O$, $TbCl_3 \cdot 6H_2O$, $DyCl_3 \cdot 6H_2O$, $NdCl_3$, $La(NO_3)_3$). In addition, we found that the synthesized S-containing di- and triperoxides exhibit high cytotoxic activity against Jurkat, K562, U937, HL60 tumor cultures and fibroblasts.

Supplementary Materials: The following are available online: ^1H NMR and ^{13}C NMR spectra of all new compounds.

Author Contributions: Conceptualization, U.D. and A.G.; methodology and validation N.M. and I.I., performing the chemistry experiments; L.D. and V.D. performing the biology experiments; The manuscript was prepared through the contributions N.M., L.D., A.I., and D.A. All authors have read and agreed to the published version of the manuscript.

Funding: This work was financially supported by the Russian Science Foundation (RSF projects 18-73-00014).

Acknowledgments: The structural studies of the synthesized compounds were performed with the use of Collective Usage Centre "Agidel" at the Institute of Petrochemistry and Catalysis of RAS. The anticancer activity studies of the synthesized compounds were performed in the laboratory of molecular design and biological screening of candidate substances for the pharmaceutical industry at the Institute of Petrochemistry and Catalysis of RAS.

Conflicts of Interest: The authors declare no conflict of interest.

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Sample Availability: Samples of all compounds are available from the authors.