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Synthesis, structure, and in vitro antiproliferative activity of cyclic hypervalent organobismuth(III) chlorides and their triphenylgermylpropionate derivatives

Xiao-Wen Zhang [a,b], Jun Xia [a], Hui-Wen Yan [c], Sheng-Lian Luo [a], Shuang-Feng Yin [a,*], Chak-Tong Au [a,d,*], Wai-Yeung Wong [e,*]

[a] College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China
[b] Key Laboratory of Pollution Control and Resource Use of Hunan Province, University of South China, Hengyang 421001, PR China
[c] Cell Biology of Bioscience and Technology Academy, Central South University, Changsha 410013, PR China
[d] Department of Chemistry, Hong Kong Baptist University, Kowloon Tong, Hong Kong, PR China
[e] Department of Chemistry and Centre for Advanced Luminescence Materials, Hong Kong Baptist University, Kowloon Tong, Hong Kong, PR China

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ABSTRACT

Six compounds of cyclic hypervalent organobismuth(III) chlorides and triphenylgermylpropionates bearing a nitrogen or sulfur atom as intramolecular coordination atom have been synthesized and characterized. The results of single-crystal X-ray analysis reveal that the eight-membered tetrahydroazabismocine rings are highly flexible. The Bi–S or Bi–N bond lengths in the thiabismocine or azabismocine derivatives are dependent on how the substituted groups are acting on the Bi, S or N atom. The replacement of the chlorine atom in azabismocine and thiabismocine with the triphenylgermylpropionic group ($Ph_3GeCH_2CH_2COO$—) leads to the lengthening of Bi–N and Bi–S bond. The substituents connected with the nitrogen atom also have an effect on the Bi–N bond length of azabismocine. For example, a cyclohexyl group has electron-donating ability higher than a phenyl group; the replacement of the former by the latter would lead to the decline of Bi–N bond length and increase of C_{Ar}–Bi–C_{Ar} angle in the eight-membered ring. The in vitro antiproliferative activities of the fabricated materials were compared on gastric carcinoma cells by means of the 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) method. It was found that the compounds show antiproliferative activity on gastric carcinoma cells (MGC-803) much higher than that of cisplatin. Moreover, there is enhancement of antiproliferative activity when the chlorine atom of the bismocine compounds is replaced by the triphenylgermylpropionic group, giving a low IC_{50} value of 0.7 μM for thiabismocine triphenylgermylpropionate.

1. Introduction

Bismuth is a nontoxic and noncarcinogenic element and many of its compounds are low in toxicity and can be safely used in areas such as medicine, catalysis, and organic synthesis [1–3]. In the past decade, there has been a rapid development on bismuth chemistry, and some novel bismuth compounds have been reported [4–11]. Over 200 years, inorganic bismuth salts have been used as medicinal drugs because of their low toxicity and high effectiveness in the treatment of a variety of microbial infections [12–15]. Typical examples are the widespread use of the commercially available Pepto-Bismol (bismuth subsalicylate, BSS) and De-Nol (colloidal bismuth subcitrate, CBS) drug for gastrointestinal disorders. Since the report of Chiba that these salts are helpful in Helicobacter pylori eradication therapy [16], various bismuth compounds have been studied for antibacterial [17,18] or antitumor [14,19] purposes. For example, Murafuji et al. [17] systematically studied the antifungal activity of triarylbismuth dichlorides and halobismuthanes against *Saccharomyces cerevisiae*, and they reported that {Bi(Cl)(C_6H_4-2-$SO_2C_6H_4$-1′-)} showed the best effect on growth inhibition of the yeast. Also, Kotani et al. [18] investigated the antibacterial properties of some cyclic organobismuth compounds bearing a nitrogen or sulfur atom as an additional ring member, and found that the eight-membered bismacycles have potent antibacterial activity against gram-negative as well as gram-positive bacteria, including *methicillin-resistant S. aureus* (MRSA).

It is known that organogermanium is biologically active [20–22]. In view of the biological functions of organobismuth(V) and organogermanium compounds, Yu et al. [22] synthesized (4-BrC_6H_4)$_3$-Bi($O_2CCH_2CH_2GePh_3$)$_2$ and (4-BrC_6H_4)$_3$Bi[$O_2CCH(CH_3)CH_2GePh_3$]$_2$ and reported that the two showed good in vitro antiproliferative activity towards HCT-8, Bel-7402 and KB cells, higher than

* Corresponding authors. Address: College of Chemistry and Chemical Engineering, Hunan University, Changsha 410082, PR China (C.-T. Au). Tel./fax: +86 731 5118161.
E-mail addresses: sf_yin@hnu.cn (S.-F. Yin), pctau@hkbu.edu.hk (C.-T. Au), rwywong@hkbu.edu.hk (W.-Y. Wong).

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cisplatin which is used clinically as an antitumor drug. Although the mechanism of antifungal or antiproliferative action of the organobismuth compounds remains unclear, many researchers believe that the biological action of organobismuth compounds is related to the coordination of bismuth center [12].

In view of the "green" property of bismuth, we have made a series of studies on the chemistry of bismuth compounds [10,23,24]. We found that organobismuth compounds with cyclic framework are potentially good antiproliferative drugs. As an extension of our research, we introduced an organogermanium group to the cyclic organobismuth compounds, and examined the antiproliferative properties of the as-synthesized compounds. Overall, we synthesized six compounds of cyclic organobismuth (III) chlorides and their triphenylgermylpropionate derivatives. In this paper, we report the bonding nature and structure as well as the antiproliferative activities of these compounds. We attempted to establish a relationship between structural properties and antiproliferative activities. To the best of our knowledge, no work of this kind has been reported in the literature.

2. Results and discussion

2.1. Synthesis

Scheme 1 shows the synthesis routes for organobismuth chloride **1** and organobismuth triphenylgermylpropionate **2**. Previously, compound **1** was synthesized with the use of ^{t}BuLi [18]. In this study, we used ^{n}BuLi instead for the sake of safety. The lithiation reaction was conducted at −30 °C and the resulting dianion was treated with anhydrous bismuth chloride to produce compound **1**. The reaction of compound **1** with triphenylgermylpropionic acid in the presence of NaOH (as neutralizing agent) and THF-H_2O (as solvent) under the protection of a N_2 atmosphere yielded compound **2**. The yield of organobismuth triphenylgermylpropionate **2** (a new compound) was 90%.

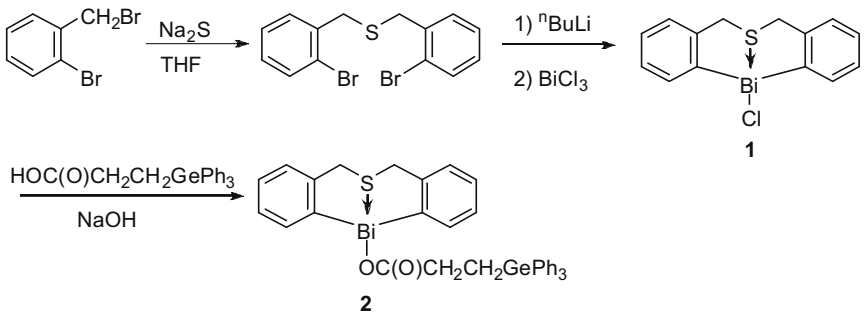
Depicted in Scheme 2 are the synthesis routes of organobismuth chlorides **4a, 4b** and organobismuth triphenylgermylpropionates **5a**, **5b**. It is noted that the synthesis and structure of 6-methyl-5,6,7,12-tetrahydrodibenz[c,f][1,5]azabismocine derivatives have been reported by Akiba and co-workers [25]. Shimada et al. also introduced a ^{t}Bu group in place of the methyl group on the N atom to increase the electron-donating ability of the N atom and to protect the N atom from being involved in undesirable side reactions [7]. In order to manipulate the electron-donating ability of the N atom, we adopted phenyl and cyclohexyl groups. Compounds **3a** and **3b** are new compounds and can be synthesized via the reaction of 1-bromo-2-(bromomethyl)benzene with RNH_2 (R = phenyl, cyclohexyl) using K_2CO_3 as neutralizing agent and DMF as solvent in refluxing conditions under N_2 atmosphere. The methodology for synthesis of compounds **4a** and **4b** was similar to that for compound **1**. The isolated yields of **4a** and **4b** are up to 87% and 92%, respectively. The reactions of compounds **4a** and **4b** with triphenylgermylpropionic acid in the presence of NaOH (as neutralizing agent) gave compounds **5a** and **5b**, respectively, in good yields (above 90%). Moreover, recrystallization of these cyclic hypervalent organobismuth compounds from CH_2Cl_2/hexane gave crystals suitable for single-crystal X-ray diffraction analysis.

2.2. Molecular structure and physiochemical property

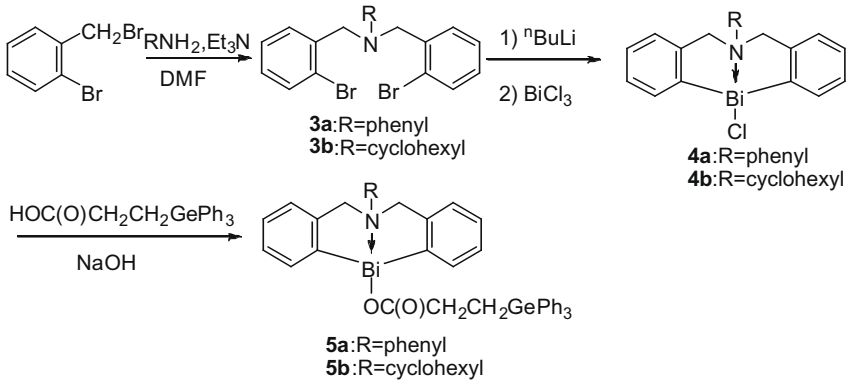
Although many bismuth compounds were found to show good biological activity, the structures of some of them are unclear or have only been characterized recently. For example, bismuth subsalicylate (BSS), colloidal bismuth citrate (CBS), and ranitidine bismuth citrate (RBC) have been used as antiulcer drugs for decades, but their structures have been discovered only recently. Considerable efforts have been made to model the structure of BSS through bismuth salicylate [Bi(Hsal)$_3$] (H_2sal = (2-HO)C_6H_4-COOH) "trapped" by chelating amines, e.g., bismuth thiosalicylate complexes [Bi(Hsal)$_3$(bpy)]$_2$·(C_7H_8)$_2$, (bpy = 2,2′-bipyridine) [26]. Recently, a new structural model of BSS with two bismuth oxosalicylate clusters, viz. [$Bi_{38}O_{44}$(Hsal)$_{26}$(Me_2CO)$_{16}$(H_2O)$_2$]·(Me_2CO)$_4$ and [Bi_9O_7(Hsal)$_{13}$(Me_2CO)$_5$]·(Me_2CO)$_{1.5}$ was reported, and the mechanism of hydrolysis and core formation proposed [15,27]. Our interest was directed towards what the intramolecular coordination between the bismuth and nitrogen (or sulfur) atom is and the effect of substitution group of the organogermanium segment.

The generation and quality of the crystals of compounds **1**, **2**, **4a**,**b**, and **5a**,**b** were verified by single-crystal X-ray diffraction. Listed in Table 1 are the crystal data, data collection and structure refinement details. The molecular structures of the six compounds are shown in Figs. 1 and 2. The values of selected bond lengths and bond angles are listed in Table 2. Despite compound **1** has been known for many years, its crystal structure has not been determined by X-ray analysis. One can see that the central bismuth-containing part of the six compounds exhibits a pseudo-trigonal bipyramidal (TBP) structure, where both the C(1) and C(14) atoms of **1** and **2**, C(1) and C(20) atoms of **4a**, **4b** and **5a**, **5b** exist in the equatorial position of the TBP structure along with a lone electron pair of bismuth, and the S(1) of **1**, **2** or N(1) of **4a**, **4b** and **5a**, **5b** are at the apical position, with C_{Ar}–Bi–C_{Ar} bond angles in the 92.9–99.5° range. These results strongly support the existence of transannular interaction between Bi and N or Bi and S atoms. For example, in compound **2**, the distance between S(1) and Bi(1) is 2.909 Å, smaller than the sum of the van der Waals radius of the sulfur (1.8 Å) and bismuth (2.4 Å) atom but longer than the covalent Bi–S bond distance (2.54 Å). In other words, there is a Bi ← S coordination bond in the thiabismocine compounds.

The Bi–S bond distance in compound **1** (2.845(4) Å) is slightly shorter than that in compound **2** (2.909(3) Å), and the Bi–C bond distance of compound **1** (ranging from 2.242(15) to 2.285(4) Å) and that of compound **2** (ranging from 2.229(12) to 2.251(12) Å)



Scheme 1. Synthesis of thiabismocine derivatives **1**, **2**.



Scheme 2. Synthesis of azabismocine derivatives **4a,b** and **5a,b**.

Table 1
Crystal data, data collection and structure refinement details for compounds **1**, **2**, **4**, and **5**.

	1	2	4a	5a	4b	5b
Chemical formula	$C_{14}H_{12}BiClS$	$C_{35}H_{31}BiGeO_2S$	$C_{20}H_{17}BiClN$	$C_{41}H_{36}BiGeNO_2$	$C_{20}H_{23}BiClN$	$C_{41}H_{42}BiGeNO_2$
Crystal habit	Colorless block	PALE yellow block	Colorless block	Colorless block	Colorless block	Colorless block
Crystal size [mm]	0.28 × 0.24 × 0.22	0.32 × 0.26 × 0.22	0.32 × 0.25 × 0.23	0.32 × 0.25 × 0.22	0.32 × 0.27 × 0.24	0.33 × 0.23 × 0.16
Crystal system	Triclinic	Monoclinic	Triclinic	Monoclinic	Monoclinic	Triclinic
Space group	P	$P2_1$	$\bar{P}$	$P2_1/c$	$P2_1/c$	P-1
a [Å]	8.9590(6)	17.8094(18)	9.2370(11)	8.9187(5)	10.2934(14)	8.9433(4)
b [Å]	9.0096(6)	9.5078(9)	9.2770(11)	35.6350(2)	16.3880(2)	19.9454(9)
c [Å]	34.2740(3)	18.0861(18)	10.8761(12)	10.7230(6)	12.0708(17)	20.0095(9)
α [°]	93.165(2)	90	78.655(2)	90	90	78.948(10)
β [°]	93.306 (10)	96.321(2)	78.668(2)	94.512 (10)	113.975(2)	88.436(10)
γ [°]	90.187 (10)	90	72.185(2)	90	90	88.354(10)
V [Å^3]	2757.6(3)	3043.9(5)	860.6 (17)	3397.4(3)	1860.5(4)	3500.7(3)
Z	8	4	2	4	4	4
D_c [g cm^{-3}]	2.2	1.74	1.99	1.674	1.863	1.636
M	456.73	797.23	515.78	856.28	521.82	862.33
F (0 0 0)	1696	1552	488	1680	1000	1704
T [°C]	20	20	20	-100	-100	-100
$2\theta_{max}$ [°]	50	56.4	50	56.6	50	50
μ(Mo Kα) [mm^{-1}]	13.106	6.86	10.397	6.09	9.62	5.91
No. of reflections measured	13143	18293	3959	20217	8331	17084
No. of unique reflections	9349	11141	2856	8204	3214	11974
No. of observed reflections (R_{int})	7386 (0.032)	9283 (0.043)	2761 (0.029)	7373 (0.032)	3025 (0.027)	10717 (0.021)
R ($I > 2\sigma(I)$)	0.057	0.051	0.034	0.039	0.029	0.026
wR^2 (F^2, all reflections)	0.164	0.141	0.093	0.109	0.08	0.07
No. of parameters	613	721	208	416	209	829
Goodness of fit on F^2	1.01	1.14	1.06	1.03	1.07	1.01

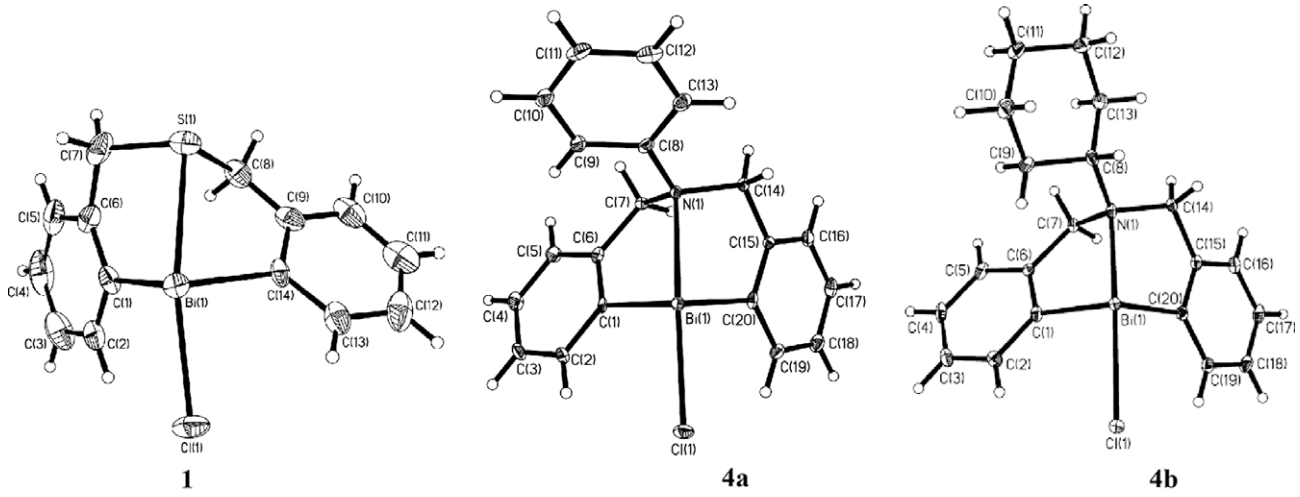


Fig. 1. Molecular structures of **1**, **4a**, and **4b**. Thermal ellipsoids are drawn at the 50% probability levels.

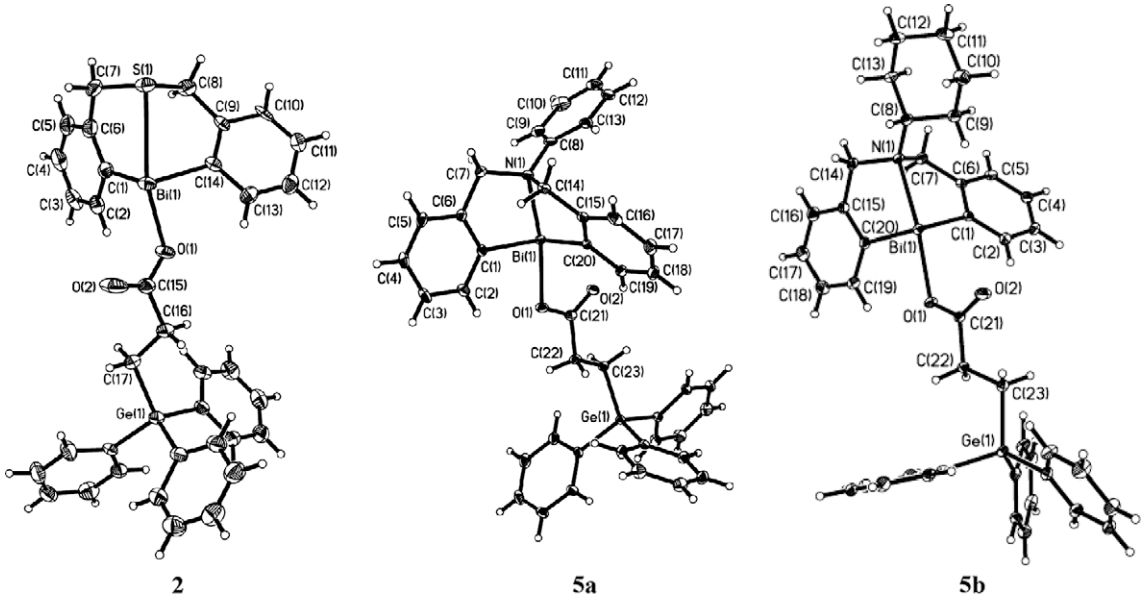
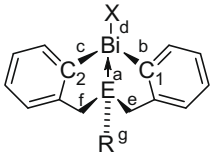


Fig. 2. Molecular structures of **2**, **5a**, and **5b**. Thermal ellipsoids are drawn at the 50% probability levels.

Table 2
Selected bond distances (Å) and bond angles (°) of compounds **1**, **2**, **4a,b** and **5a,b**.



Bond length/angle	1	2	4a	5a	4b	5b
a	2.845(4)	2.909(3)	2.607(5)	2.631(4)	2.517(4)	2.563(3)
b	2.285(14)	2.251(12)	2.259(6)	2.256(4)	2.228(6)	2.232(3)
c	2.242(15)	2.229(12)	2.238(6)	2.235(5)	2.252(5)	2.250(3)
d	2.632(4)	2.256(7)	2.597(19)	2.251(3)	2.654(13)	2.298(2)
ab	75.20(4)	74.70(3)	72.30(2)	70.32(14)	73.64(17)	73.03(11)
ac	73.30(4)	73.60(3)	73.10(2)	71.99(15)	75.23(16)	73.62(11)
ad	155.2(13)	151.0(2)	158.9(13)	148.2(13)	159.0(10)	149.3(9)
bc	98.90(5)	99.50(4)	92.90(2)	97.75(16)	96.72(19)	98.30(12)
bd	91.80(4)	91.40(4)	92.38(19)	86.35(14)	92.24(14)	83.75(11)
cd	88.40(4)	84.20(4)	93.82(17)	90.88(15)	91.49(13)	90.55(11)
ae	92.5(6)	91.9 (4)	99.4(4)	102.7(3)	103.5 (3)	102.7(19)
af	91.9(6)	92.9 (4)	106.0(4)	104.4(3)	106.6(3)	106.4(19)
ag			107.1(4)	104.1(3)	109.2(3)	111.1(2)
ef	103.9(9)	104.4(7)	111.7(5)	110.8(4)	110.6(4)	110.0(3)
Others		1.978(11)[a]		1.959(4)[b]		1.964(3)[c]

[a] The bond length of C(17)–Ge(1).
[b] The bond length of C(23)–Ge(1).
[c] The bond length of C(23)–Ge(1).

are also different. The Bi–Cl single bond of compound **1** is 2.632(4) Å, while the Bi–O single bond of compound **2** is 2.256(7) Å. In compound **2**, the bonding interactions between Bi(1) and the carbonyl oxygens of carboxylate is relatively strong. This can be attributed to the electron-donating effect of O(1) of the triphenylgermylpropionic group ($Ph_3GeCH_2CH_2COO$—). Affected by the adjacent O(1) atom, the O(1)–Bi(1)–S(1) angle of compound **2** (151.0(2)°) is smaller than Cl(1)–Bi(1)–S(1) of compound **1** (155.2(13)°). The C_{Ar}–Bi–C_{Ar} angle of compound **1** and **2** is 98.9° and 99.5°, respectively. These results suggest that the eight-membered tetrahydrothiabismocine ring forces the sulfur atom to deviate from the axial position of TBP geometry and is rather flexible in nature. Also, the Bi–S bond distance reflects the influence of the substituent that is acting on the bismuth center.

The results of X-ray diffraction analysis show that the Bi–N bond distance in compounds **4a**, **4b**, **5a** and **5b** is 2.607(5), 2.517(4), 2.631(4), and 2.563(3) Å; the Bi–Cl single bond in compounds **4a** and **5a** is 2.597(19) and 2.654(19) Å while the Bi–O bond distance in compounds **4b** and **5b** is 2.251(3) and 2.298 (2) Å, respectively. Obviously, the replacement of the Cl atom by –$OC(O)CH_2CH_2GePh_3$ lengthens the Bi–N bond, and this phenomenon can be attributed to the electron-donating effect of O(1). Com-

pared to the Cl(1)–Bi(1)–N(1) angle of compound **4a** (158.9(13)°) and compound **4b** (159.0(10)°), there is a reduction in the O(1)–Bi(1)–N(1) angle of compound **5a** (148.2(13)°) and compound **5b** (149.3(9)°). The Ge–C bonds of compounds **2**, **5a**, and **5b** are consistent with those reported in the literature [28], ranging from 1.959(4) to 1.978(11) Å. The stereochemistry of germanium is essentially tetrahedral in geometry in **5a** and **5b**.

It is known that the nature of hypervalent compounds having intramolecular Bi–N or Bi–O coordination, e.g., [2-(Me_2NCH_2)C_6H_4]Bi-[C_6H_4{$C(CF_3)_2O$}-2], [2-($MeOCMe_2$)C_6H_4]Bi-[C_6H_4{$C(CF_3)_2O$}-2] [29,30], $^tBuN(CH_2C_6H_4)_2BiX$ (X = Cl, Br, F, Ph, 3,5-$(CF_3)_2C_6H_3$–, 3,4-$(CH_2O_2)_2C_6H_3$–, 2,4,6-$(MeO)_3C_6H_2$–, CH_2=C(Me)–) and $[^tBuN(CH_2C_6H_4)_2Bi]^+$ $[B(C_6F_5)_4]^-$ [7,9,31], is highly affected by the electronic nature of the Bi atom. According to the works by Shimada et al., the Bi–N distances of 5,6,7,12-tetrahydrodibenz[c,f][1,5]azabismocines have a good linear relationship versus the Hammett's σ_m-constants of the substituents on the Bi atom [9]. All these compounds exhibit similar coordination geometries with two Bi–C bonds and the lone pair of electrons on bismuth occupying the equatorial position of TBP geometry. The equatorial position of the other compounds is occupied by two carbon atoms with the C_{Ar}–Bi–C_{Ar} angle considerably diminished (less than 120°) and with the N (O, S)–Bi–X bond angle ranging from 148.5(1) to 160.6(2) Å. The Bi–N distance (2.357(2) Å) in the cationic complex $[^tBuN(CH_2C_6H_4)_2Bi]^+[B(C_6F_5)_4]^-$ is much shorter than those of previously reported neutral 5,6,7,12-tetrahydrodibenz[c,f][1,5]azabismocines (2.559(4)–2.894(4) Å) [7,9], and those of the newly synthesized compounds **4a,b** and **5a,b** (2.517(4)–2.631(4) Å).

We found that the as-synthesized cyclic bismuth compounds are stable with high melting points. Generally speaking, ordinary organobismuth(III) compounds bearing Bi–Cl bond are moisture sensitive and readily decompose under atmospheric conditions. However, the six compounds fabricated in the present study are comparatively stable and can be stored for over one year in air without appreciable degradation. The enhanced stability of these cyclic compounds may be attributed to the efficient $6s^2$-π conjugation between the benzene and bismuth center, and to the transannular coordinative interaction between the nitrogen (or sulfur) and the bismuth center; both factors make the bismuth less susceptible to the attack of water and oxygen molecules.

2.3. Antiproliferative activity assay

The antiproliferative activity of the compounds newly fabricated by us was assessed by the MTT method as described previously by Skehan et al. [32]. We examined the viability of gastric carcinoma cells in a span of 24 h with compounds **1**, **2**, **4a,b** and **5a,b** in different concentrations. The activities of the compounds are listed in Table 3. Compared to cisplatin, the compounds are more active against the MGC-803 cell in vitro. Compound **2** shows a significant activity against the MGC-803 cell, showing inhibition ratios higher than 60%. At concentration of 1.25, 2.5, 5, 10, and 20 μg ml^{-1}, the inhibition ratio against MGC-803 cells is 62%, 65%, 77%, 82% and 86%, respectively. Moreover, the IC_{50} value for compound **2** is 0.7 μM, lower than those for other organobismuth complexes, and much lower than that for the cisplatin.

Table 3
Inhibition ratio (%) of the organobismuth complexes on MGC-803 in vitro.[a]

Compounds	Concentration (μg ml^{-1})					IC_{50} [b] (μM)
	20	10	5	2.5	1.25	
1	84	81	73	60	55	2.1
2	86	82	77	65	62	0.7
4a	76	74	71	58	50	2.0
5a	80	77	75	68	61	0.9
4b	78	72	67	60	25	5.3
5b	81	75	69	63	30	2.6
Cisplatin	63	53	40	30	10	30.5

[a] Inhibition ratio (%) = $(A_1/A_2)/A_1 \times 100\%$. A_1: mean optical densities of untreated cells; A_2: mean optical densities of drug-treated cells.
[b] The concentration of a compound needed to reduce population growth of organisms by 50% in vitro.

The results of Table 3 also illustrate how the electronic action of the substituents on Bi or N atom affects the antiproliferative activity. Due to the fact that the cyclohexyl group has an electron-donating ability stronger than the phenyl group, compounds **4a** and **5a** show better activity than compounds **4b** and **5b**, respectively. Thus, it is apparent that a proper coordination ability of Bi center is very important for good antiproliferative activity. Murafuji et al. [17] employed X-ray crystallographic method to disclose the structure-activity relationship of triarylbismuth dichlorides and halobismuthanes against the yeast *S. cerevisiae*. They found that the bismuth center of the compounds adopts a seven-coordinate geometry through intramolecular and intermolecular coordination between bismuth and oxygen atoms. The authors attributed the antifungal activity to the highly coordinated geometry which allows the bismuth center to bind tightly with biomolecules that play important roles in the growth of *S. cerevisiae*. Kotani et al. [18] deduced that the bismuth and chlorine atoms in the organobismuth compounds can undergo transannular interaction with the nitrogen or sulfur atom via a state of hypervalent transition, and attributed the bismacycle action to both the physical properties of bismuth and the chemical properties of heterocyclic structure. Comparing our work with those of Murafuji et al. [17] and Kotani et al. [18], there are apparent discrepancies. Nevertheless, it is clear that it is difficult for bacteria to develop resistance to the reported compounds. Further work is underway to disclose the antiproliferative mechanism of these complexes.

3. Conclusion

We have synthesized six cyclic hypervalent organobismuth compounds. The compounds show high degrees of stability. The results of structure determination by means of single-crystal X-ray diffraction reveal that the eight-membered tetrahydroazabismocine rings are highly flexible and the Bi–S or Bi–N bond lengths in thiabismocine or azabismocine derivatives are dependent on the influence of the substituted groups that is acting on the N and Bi atom. The replacement of Cl atom by organogermanium segment resulted in the lengthening of the Bi–S or Bi–N bonds. The six compounds show antiproliferative activities on MGC-803 better than that of cisplatin. The IC_{50} value for compound **2** is 0.7 μM. It is apparent that a proper coordination ability of Bi^{3+} and the introduction of organogermanium group is beneficial for achieving good antiproliferative activity of bismocine compound.

4. Experimental

4.1. General procedures

All manipulations of air-sensitive materials were conducted in a glovebox filled with argon or under the protection of N_2 atmosphere according to the standard Schlenk tube techniques. Tetrahydrofuran, toluene, and ether were dried and distilled from a purple solution of sodium/benzophenone ketyl. N,N-Dimethyl formamide (DMF) was dried by 4 Å molecular sieves, followed by distillation under reduced pressure. Chloroform and dichloromethane were dried with CaH_2 and distilled under nitrogen. The glassware was dried in an oven at 120 °C prior to use. The commercially available chemicals were purchased from Aldrich or Sinopharm

Chemical Reagent Co. Ltd., and were used as received without further purification unless denoted otherwise. $BiCl_3$ was purified by refluxing with $SOCl_2$, followed by sublimation under vacuum. NMR spectra were recorded on a Brucker 400M spectrometer (^1H NMR, 400 MHz; ^{13}C NMR 100 MHz); chemical shifts of ^1H (δ ppm) were reported with reference to internal tetramethylsilane standard (δ 0.0), while the chemical shifts of ^{13}C NMR were reported using $CDCl_3$ as internal standard (δ 77.0). Melting points of compounds were determined over a XT-4 micro melting point apparatus (Beijing Tech Instrument Co. LTD). The IR spectra of the compounds were recorded in the range 650∼4000 cm^{-1} on Nexus 670 FT-IR equipment (Thermo Nicolet) using powder-pressed KBr pellets. Elemental analyses were performed over a VARIO EL III instrument.

4.2. Preparation of S $(CH_2C_6H_4)_2BiCl$ (**1**)

Bis(2-bromobenzyl) sulfide (14.88 g, 40.0 mmol) was dissolved in dried ethyl ether (150 ml), and 32.6 ml (81.6 mmol, 2.5 M in hexane) of n-butyllithium was added dropwise at −30 °C. The as-obtained mixture was stirred for 3 h, which was then added a solution of $BiCl_3$ (12.87 g, 40.8 mmol) in dried ethyl ether (120 ml) at −50 °C. The resulting mixture was stirred overnight with the temperature of the mixture rose gradually to room temperature. After removal of solvent under vacuum and 3-times toluene extraction (100 ml × 3), the insoluble material was filtered out, and the resulting organic layer washed with de-ionized H_2O (300 ml × 3) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to leave oil that is yellow in color (13.86 g, 76%). The yellow oil was dissolved in CH_2Cl_2 and recrystallized from CH_2Cl_2/hexane to give compound **1** in the form of colorless crystals (8.22 g, 45%). Melting point, 179–180 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 4.26 (2H, d, J = 15.2 Hz), 4.52 (2H, d, J = 15.6 Hz), 7.37 (2H, t, J = 7.6 and 7.2 Hz), 7.49–7.56 (4H, m), and 8.80 (2H, d, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 41.05, 127.98, 130.81, 130.84 (2C), 139.12, 147.63, and 173.65. *Anal.* Calc. for $C_{14}H_{12}BiClS$ (456.74): C, 36.81; H, 2.65; Bi, 45.75; Cl, 7.76; S, 7.02. Found: C, 36.55; H, 2.75; Bi, 45.79; Cl, 7.73; S, 7.17. γ_{max}(KBr)/cm^{-1}: 3,047, 2,959, 1,574, 1,452, 1,433, 1,236, 908, 754, and 700.

4.3. Preparation of S $(CH_2C_6H_4)_2BiO_2CCH_2CH_2GePh_3$ (**2**)

The β-(triphenylgermyl)propionic acids were synthesized as described by Barton et al. [33,34] 2.0 g (19.0 mmol) GeO_2, 4.4 g (40.0 mmol) $NaH_2PO_2{\cdot}H_2O$, 25 ml HCl and 4 ml distilled water were mixed in a four-necked round bottomed flask, and the mixture was refluxed at 99–102 °C for 4 h under vigorously agitation to obtain a clear $HGeCl_3$ solution. 1.38 g (19.0 mmol) acrylic acid was added dropwise with temperature controlled in the −10 ∼ −5 °C range (using a bath of ice brine) for 2 h. The so-obtained mixture was stirred for 10 h, during which the temperature was allowed to rise gradually to room temperature. Then the mixture was subject to 3-times extraction with ethyl ether (20 ml × 3). The organic layer was separated and dried with anhydrous $MgSO_4$ for 4 h. The solvent was removed in vacuo and the resulting residue was recrystallized from CH_2Cl_2/oil ether to obtain 4.08 g (85%) of a white solid $Cl_3GeCH_2CH_2CO_2H$. The β-(triphenylgermyl)propanoic acid ($Ph_3GeCH_2CH_2CO_2H$) was prepared by reacting 3.83 g (15.0 mmol) $Cl_3GeCH_2CH_2CO_2H$ with 76.0 mmol PhMgBr in THF (51.7% yield).

A mixture of compound **1** (274.2 mg, 0.6 mmol), 237.6 mg (0.63 mmol) $Ph_3GeCH_2CH_2CO_2H$ (dissolved in 12 ml THF), and 240 mg (6.0 mmol) NaOH (dissolved in 6 ml de-ionized water) was refluxed for 10 h. Then THF was removed under reduced pressure. The residue was dissolved in 10 ml CH_2Cl_2 and washed with H_2O (10 ml × 3). The organic layer was separated and dried over $MgSO_4$. Removal of the solvent under vacuum resulted in 450.6 mg (94%) of a pale yellow solid compound **3**. Melting point, 202–204 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.04 (2H, t, J = 8.4 Hz), 2.72 (2 H, t, J = 8.4 Hz), 4.16 (2H,d, J = 14.8 Hz), 4.40(2H,d, J = 15.2 Hz), 7.25–7.56 (23 H, m), 8.18 (2H, d, J = 6.8 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.27, 31.89, 40.71, 127.69, 128.19, 128.88, 130.25, 130.98, 135.05, 136.78, 137.73, 147.48, 177.50, and 181.36. *Anal.* Calc. for $C_{35}H_{31}BiGeO_2S$ (797.30): C, 52.72; H, 3.92; Bi, 26.21; Ge, 9.11; O, 4.01; S, 4.02. Found: C, 52.63; H, 3.99; Bi, 26.16; Ge, 9.16; O, 4.09; S, 3.96. γ_{max}(KBr)/cm^{-1}: 3046, 1964, 1619, 1429, 1349, 1259, 1090, 738, and 699.

4.4. Preparation of PhN $(CH_2C_6H_4)_2BiCl$ (**4a**)

To a hexane solution of ^{n}BuLi (2.5 M, 81.6 ml, 204.0 mmol) was added an Et_2O (300 ml) solution of phenylamine **3a** (43.1 g, 100.0 mmol) at −30 °C. The mixture was gradually warmed to room temperature over 3 h. The mixture was then added to a mixture of $BiCl_3$ (31.4 g, 102.0 mmol) in Et_2O (400 ml) at −78 °C. The resulting mixture was stirred overnight, during which the temperature was allowed to rise gradually to room temperature. The solvent was removed under vacuum and the residue was subject to 3-times extraction with toluene/brine (2 M NH_4Cl) (250 ml × 3), and the insoluble material was removed by filtration. The organic layer was washed with de-ionized H_2O (450 ml × 3) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to leave an oil brownish yellow in color. The obtained residue was recrystallized from CH_2Cl_2/hexane to give compound **4a** in the form of colorless crystals (44.87 g, 87%). Melting point, 257–259 °C; ^1H NMR (400 MHz, $CDCl_3$): 4.52 (2H, d, J = 14.8 Hz), 4.76 (2H, d, J = 14.8 Hz), 6.47 (2H, d, J = 7.6 Hz), 6.65 (2H, t, J = 7.2 Hz), 7.31 (1H, t, J = 7.2 Hz), 7.44 (2H, d, J = 7.2 Hz), 7.51 (4H, t, J = 8.0 Hz), 8.59 (2H, d, J = 3.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 62.27 (2C, NCH_2), 118.06, 124.06, 127.13, 127.33, 128.79, 130.66, 137.43, 146.98, 147.47 and 172.66. *Anal.* Calc. for $C_{20}H_{17}BiClN$ (515.79): C, 46.57; H, 3.32; Bi, 40.52; Cl, 6.87; N, 2.72. Found: C, 46.49; H, 3.36; Bi, 40.48; Cl, 6.95; N, 2.62. γ_{max}(KBr)/cm^{-1}: 3,043, 2,915, 1,594, 1,491, 1,430, 1,205, 931, 754, and 698.

4.5. Preparation of $C_6H_{11}N$ $(CH_2C_6H_4)_2BiCl$ (**4b**)

Compound **4b** was prepared according to a procedure similar to that of **4a**. Amine **3b** (20.2 g, 46.0 mmol) was allowed to react with ^{n}BuLi (2.5 M, 37.7 ml, 94.0 mmol) at −30 °C, and the resulting solution was added to a mixture of $BiCl_3$ (14.8 g, 47.0 mmol) in Et_2O (200 ml) at −78 °C. The obtained mixture was gradually warmed to room temperature and stirred for 12 h. Then the solvent was removed under vacuum and the residue subject to 3-times extraction with toluene/brine (2 M NH_4Cl) (150 ml × 3), and the insoluble material was removed by filtration. The organic layer was washed with de-ionized H_2O (240 ml × 3) and dried over anhydrous $MgSO_4$. The solvent was removed under reduced pressure to leave a yellowish solid. The resulting residue was recrystallized from CH_2Cl_2/hexane to obtain compound **4b** in the form of colorless crystals (22.08 g, 92%). Melting point, 266–269 °C; ^1H NMR (400 MHz, $CDCl_3$): 1.12 (1H, td, J = 12.8 Hz), 1.23–1.42 (4H, m), 1.63 (1H, d, J = 12.8 Hz), 1.84 (2H, d, J = 12.8 Hz,), 1.99 (2H, d, J = 11.6 Hz), 2.92 (1H, td, J = 11.6 Hz), 4.15 (2H, d, J = 15.2 Hz), 4.36 (2H, d, J = 15.2 Hz), 7.31 (2H, t, J = 7.6 and 7.2 Hz), 7.39–7.48 (4H, m), and 8.62 (2H, d, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 25.48, 25.65, 30.69, 60.67, 64.81, 127.63, 128.05, 130.80, 138.15, 149.69, and 170.85. *Anal.* Calc. for $C_{20}H_{23}BiClN$ (521.84): C, 46.03; H, 4.44; Bi, 40.05; Cl, 6.79; N, 2.68. Found: C, 45.99; H, 4.48; Bi, 40.18; Cl, 6.65; N, 2.67. γ_{max}(KBr)/cm^{-1}: 3,055, 2,945, 1,579, 1,462, 1,448, 1,202, 931, 752, and 704.

4.6. Preparation of $PhN(CH_2C_6H_4)_2BiO_2CCH_2CH_2GePh_3$ (**5a**)

A mixture of compound **4a** (516 mg, 1.0 mmol), $Ph_3GeCH_2CH_2CO_2H$ (396 mg, 1.05 mmol) dissolved in 20 ml THF, and NaOH (400 mg, 10.0 mmol) dissolved in 5 ml de-ionized water was refluxed for 10 h. THF was removed under reduced pressure. The residue was dissolved in 10 ml CH_2Cl_2 and washed with H_2O (10 ml × 3). The organic layer was separated and dried over $MgSO_4$. Removal of the solvent under vacuum gave 790 mg (93%) of **5a** in the form of a white solid. Melting point, 216–217 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 2.08 (2H, t, J = 8.8 Hz), 2.71 (2H, t, J = 8.8 Hz), 4.53 (2H, d, J = 15.2 Hz), 4.80 (2H, d, J = 15.2 Hz), 7.12 (1H, t, J = 7.2 Hz), 7.18 (2H, d, J = 7.2 Hz), 7.28–7.37 (13H, m), 7.47–7.55 (10H, m), 8.13 (2H, d, J = 7.2 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.38, 31.56, 62.75, 118.75, 124.51, 127.94, 128.17, 128.24, 128.85, 129.61, 130.97, 135.03, 136.78, 137.50, 148.15, 148.44, 178.02, and 181.20. *Anal.* Calc. for $C_{41}H_{36}BiGeNO_2$ (856.35): C, 57.50; H, 4.24; Bi, 24.40; Ge, 8.48; N, 1.64; O, 3.74. Found: C, 57.43; H, 4.28; Bi, 24.28; Ge, 8.46; N, 1.68; O, 3.86. γ_{max}(KBr)/cm^{-1}: 3066, 1964, 1619, 1432, 1354, 1255, 1093, 764, and 698.

4.7. Preparation of $C_6H_{11}N(CH_2C_6H_4)_2BiO_2CCH_2CH_2GePh_3$ (**5b**)

A mixture of compound **4b** (1.04 g, 2.0 mmol), $Ph_3GeCH_2CH_2CO_2H$ (789 mg, 2.1 mmol) dissolved in 40 ml THF, and NaOH (800 mg, 20.0 mmol) dissolved in 10 ml de-ionized water was refluxed for 10 h. THF was removed under reduced pressure. The residue was dissolved in 20 ml CH_2Cl_2 and washed with H_2O (20 ml × 3). The organic layer was separated and dried over $MgSO_4$. Removal of the solvent under vacuum gave 1.62 g (94%) of **5b** in the form of white solid. Melting point, 219–223 °C; ^1H NMR (400 MHz, $CDCl_3$): δ 1.10 (1H, t, J = 12.8 Hz), 1.20∼1.39 (4H, m), 1.62 (1H, d, J = 12.8 Hz), 1.81 (2H, d, J = 12.8 Hz), 2.02 (4H, t, J = 11.6 Hz), 2.71 (2H, t, J = 8.0 Hz), 2.82 (1H, t, J = 11.2 Hz), 4.07 (2H, d, J = 15.2 Hz), 4.28 (2H, d, J = 15.2 Hz), 7.24 (2H, t, J = 14.8 Hz), 7.36 (11H, d, J = 8.0 Hz), 7.42 (2H, t, J = 7.2 Hz), 7.55 (6H, s), and 8.11 (2H, d, J = 7.6 Hz). ^{13}C NMR (100 MHz, $CDCl_3$): δ 10.39, 25.57, 25.71, 30.46, 31.74, 60.24, 64.26, 127.69, 127.78, 128.14, 128.79, 130.29, 135.05, 136.89, 137.37, 149.72, 174.59, and 181.39. *Anal.* Calc. for $C_{41}H_{42}BiGeNO_2$ (862.40): C, 57.10; H, 4.91; Bi, 24.23; Ge, 8.42; N, 1.62; O, 3.71. Found: C, 57.01; H, 4.86; Bi, 24.25; Ge, 8.39; N, 1.64; O, 3.75. γ_{max}(KBr)/cm^{-1}: 3052, 2931, 2850, 1963, 1620, 1430, 1360, 1267, 1092, 743, and 700.

4.8. Antiproliferative activity assay

Cell survival rates and IC_{50} value (the concentration of a compound needed to reduce population growth of organisms by 50% in vitro) were measured by 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl tetrazolium bromide (MTT) assay. The gastric cancer cell line MGC-803 was purchased from Xiang Ya Central Experiment Laboratory of the University of Central South China. The bismocine was dissolved in dimethyl sulfoxide (DMSO, Sigma) to 10 mg ml^{-1} as a stock solution and diluted in serum-free RPMI 1640 medium just before use. MGC-803 cells that grow exponentially were inoculated at cell concentration of 1×10^4 cells/well in a 96-well tissue culture plate. The bismocine solution was immediately added into a cell suspension after 12 h incubation and kept at different final concentrations (e.g., 0.00, 1.25, 2.50, 5.00, 10.00 and 20.00 μg ml^{-1}) with a total volume of 200 μl per well. Twenty microliter (5 mg ml^{-1}) of MTT solution was added to each well at the assigned culture time and the plate was incubated for another 4 h. Then the culture medium was poured away quickly and 100 μl DMSO was added to each wel1 and subjected to oscillation shaking. The OD value of each well was determined by ELISA Reader (SUNRISE TECAN) with 570 nm filter and the survival rate of cells calculated according to the provided formula.

Survival rate of cells = (mean optical densities of drug-treated cells/mean optical densities of untreated cells) × 100%. IC_{50} value was estimated according to the survival rate.

5. X-ray crystallography

Crystals suitable for X-ray diffraction studies were grown by slow evaporation of each of the respective solutions in CH_2Cl_2/hexane at room temperature. Geometric and intensity data were collected at 293 or 173 K using graphite-monochromated Mo Kα radiation (λ = 0.71073 Å) on a Bruker Axs SMART 1000 CCD diffractometer. The crystallinity, orientation matrix, and accurate unit-cell parameters were determined according to standard procedures. The collected frames were processed with the software SAINT [35] and an absorption correction (SADABS) [36] was applied to the collected reflections. The structure was solved by the Direct or Patterson methods (SHELXTL) [37] in conjunction with standard difference Fourier techniques and subsequently refined by full-matrix least-squares analyses on F^2. Hydrogen atoms were generated in their idealized positions and all non-hydrogen atoms were assigned with anisotropic displacement parameters.

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Appendix A. Supplementary material

CCDC 724767, 724768, 724769, 724770, 724771 and 724772 contains the supplementary crystallographic data for this paper. These data can be obtained free of charge via http://www.ccdc.cam.ac.uk/conts/retrieving.html, or from the Cambridge Crystallographic Data Centre, 12 Union Road, Cambridge CB2 1EZ, UK; fax: (+44) 1223-336-033; or e-mail: deposit@ccdc.cam.ac.uk. Supplementary data associated with this article can be found, in the online version, at doi:10.1016/j.jorganchem.2009.05.003.

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