

Resolution and Improved Synthesis of (\pm)-Arsenicin A: A Natural Adamantane-Type Tetraarsenical Possessing Strong Anti-Acute Promyelocytic Leukemia Cell Line Activity

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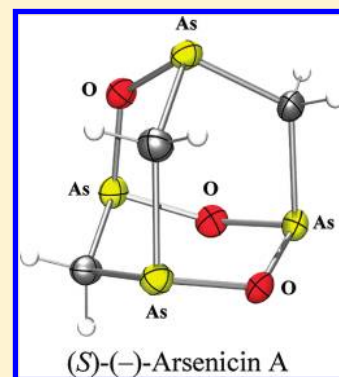
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S Supporting Information

ABSTRACT: The resolution and improved synthesis of the naturally occurring, adamantane-type, tetraarsenical (\pm)-Arsenicin A is reported. The five-step synthesis of (\pm)-Arsenicin A from methylenebis(phenylarsinic acid) affords (\pm)-Arsenicin A as air-stable, colorless crystals having an mp of 182–184 °C after column chromatography and recrystallization from benzene (overall yield: 36%). The resolution of (\pm)-Arsenicin A was achieved by preparative HPLC on a Chiralpak IA column with the use of dichloromethane as eluent to give both enantiomers in >99% enantiomeric purity (HPLC); the isolated enantiomers had $[\alpha]_{589}^{20} = -60.2$ and $+62.3$ (0.01% $\text{NEt}_3/\text{CH}_2\text{Cl}_2$). (*S*)-(-)-Arsenicin A, having an mp of 241–242 °C from dichloromethane, crystallizes in the space group $P2_12_12_1$ with one molecule having the ($S_{\text{As}_1}S_{\text{As}_2}S_{\text{As}_3}S_{\text{As}_4}$) or overall *S* configuration in the asymmetric unit. The adamantane-type structure of (\pm)-Arsenicin A is reminiscent of arsenic(III) oxide (As_4O_6), but where three of the oxygen atoms in the inorganic oxide have been replaced by methylene groups in a chiral C_2 arrangement. ((\pm)-Arsenicin A, mp 182–184 °C, crystallizes from benzene in the centrosymmetrical space group $P\bar{1}$: the unit cell of the crystal contains two independent pairs of molecules, the molecules in each pair being related by an inversion center.) The individual enantiomers of (\pm)-Arsenicin A racemize in the presence of traces of acid, and high-level ab initio calculations have been performed to examine the mechanism of the process. (\pm)-Arsenicin A exhibits a 21-fold greater inhibition of the induction of proliferation arrest and induces cell death at a 27-fold lower concentration in the acute promyelocytic leukemia cell line than the current “arsenical gold standard”, arsenic(III) oxide (Trisenox). (\pm)-Arsenicin A is also more potent than arsenic(III) oxide for the induction of proliferation arrest in two other cancers with particularly bad prognoses: pancreatic adenocarcinoma and glioblastoma.



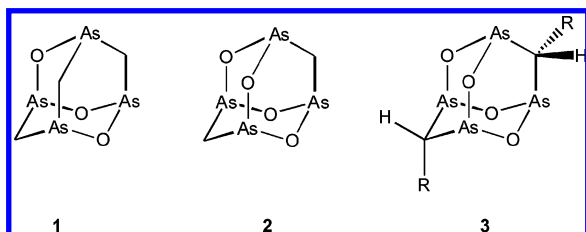
■ INTRODUCTION

The natural polyarsenical Arsenicin A was isolated in trace amounts from the New Caledonian sponge *Echinochalina bargibanti*: the chiral, adamantane-type, organometallic structure **1** was proposed for the natural polyarsenical on the basis of detailed spectroscopic measurements¹ and theoretical calculations.^{2,3} The adamantane-type structure of arsenic(III) oxide (As_4O_6) in the gas phase has long been known,⁴ and syntheses of the adamantane-type organometallics **2**⁵ and **3** ($\text{R} = \text{Me}, \text{Et}$)⁶ have been reported. Arsenic(III) oxide (Trisenox) brings about complete remission in a large proportion of patients suffering from acute promyelocytic leukemia (APL), a subtype of acute myeloid leukemia (AML).⁷ Indeed, the clinical course of APL has changed over the last few decades from one that was often fatal to one of the more treatable subtypes of acute myeloid leukemia. Arsenic(III) oxide achieves remarkable clinical responses in patients with APL by causing tumor cell

differentiation and triggering apoptosis. Arsenic(III) oxide was approved by the FDA in 2000 for use in relapsed and refractory APL. The discovery that arsenic(III) oxide induces complete remission in a high percentage of patients with APL has renewed interest in this compound for the treatment of other cancers.⁸ Arsenicin A is the first natural polyarsenical to be isolated and has a structure reminiscent of arsenic(III) oxide (As_4O_6) in the solid state, but where three of the oxygen atoms in the adamantane-type cage have been replaced by methylene groups in a contiguous, C_2 -chiral arrangement.⁹ Because of the closely related structures of Arsenicin A and arsenic(III) oxide, it was of great interest to devise a synthesis of (\pm)-Arsenicin A so that sufficient material was available for an investigation of its biological activity.

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There are known a great variety of marine arsenicals containing one arsenic atom.¹⁰ It is also known that the yeast *Scopulariopsis brevicaulis* transforms by reductive methylation, prochiral ethyl(*n*-propyl)arsinic acid into As-chiral (*S*)-ethylmethyl(*n*-propyl)arsine with 60% enantioselectivity.¹¹ It was, therefore, appropriate to investigate the resolution of (\pm)-Arsenicin A and to examine its configurational stability, bearing in mind that the biosynthesis of (\pm)-Arsenicin A, or one of its enantiomers, would require the generation of four As-chiral stereocenters of the same configuration in adjacent sites of the same adamantane-type molecule.

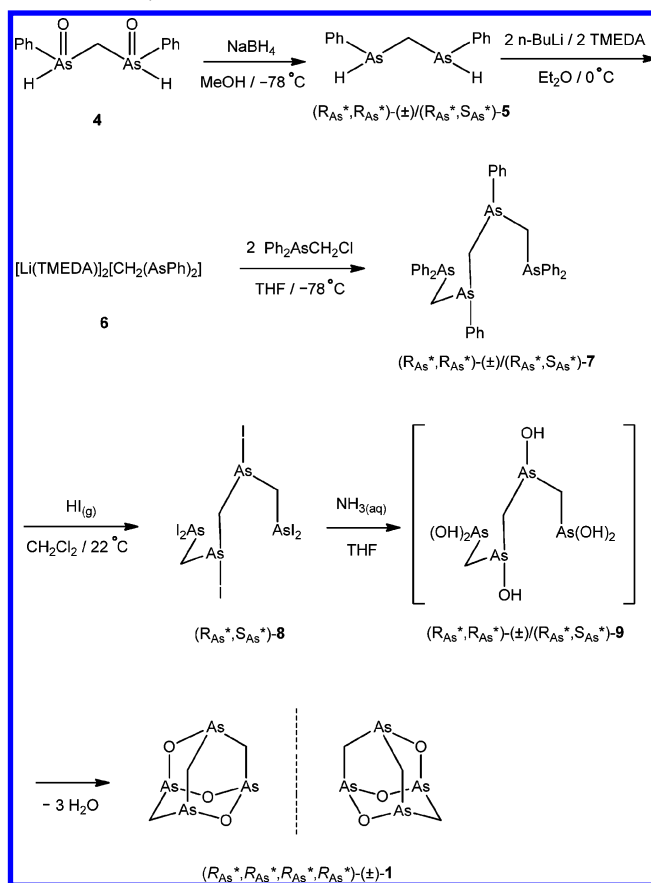


In this paper, we report the synthesis and resolution of (\pm)-Arsenicin A and the crystal structure determination of the configurationally pure enantiomer (*S*)-(-)-Arsenicin A. We also suggest a mechanism supported by high-level ab initio molecular orbital theory calculations for the observed acid-catalyzed racemization of the enantiomers of (\pm)-Arsenicin A and reveal that (\pm)-Arsenicin A is far more potent than arsenic(III) oxide against the APL cell line. A preliminary account of some of the synthetic aspects of this work and the crystal structure of (\pm)-Arsenicin A has been published.⁹

RESULTS AND DISCUSSION

Synthesis of (\pm)-Arsenicin A. The putative structure of (\pm)-Arsenicin A resembled the adamantane-type structure of As₄O₆ in which three of the oxygen atoms had been replaced by methylene groups.^{1,9} Indeed, the C₂ arrangement of the four arsenic stereocenters in the proposed structure of (\pm)-Arsenicin A suggested the five-step synthesis indicated in Scheme 1.⁹ Thus, reduction of the diarsinic acid **4** with sodium borohydride gave the bis(secondary arsine) (*R*_{As}^{*},*R*_{As}^{*})-(\pm)/(*R*_{As}^{*},*S*_{As}^{*})-methylenebis(phenylarsine), (*R*_{As}^{*},*R*_{As}^{*})-(\pm)/(*R*_{As}^{*},*S*_{As}^{*})-**5**,¹² in 73% yield after distillation. The ¹H NMR spectrum of the bis(secondary arsine) in benzene-*d*₆ was consistent with the presence of a pair of configurationally stable diastereomers chiral at arsenic, a single triplet being observed for the equivalent methylene protons of the (*R*_{As}^{*},*R*_{As}^{*})-(\pm) diastereomer and a doublet of triplets for the nonequivalent methylene protons of the (*R*_{As}^{*},*S*_{As}^{*}) diastereomer. A triplet AsH resonance was observed for each diastereomer of the bis(secondary arsine).⁹ Deprotonation of the bis(secondary arsine) with *n*-butyllithium/(*N,N,N',N'*)-tetramethyl-1,2-ethanediamine (TMEDA) in diethyl ether gave the crystalline yellow salt [Li(TMEDA)₂][CH₂(AsPh)₂] (**6**),¹⁴ which, when dissolved in THF and treated with 2 equiv of (chloromethyl)diphenylarsine, afforded the tetrakis(tertiary arsine) (*R*_{As}^{*},*R*_{As}^{*})-(\pm)/(*R*_{As}^{*},*S*_{As}^{*})-**7** as a colorless oil in 61% yield after column chromatography of the crude product on silica with dichloromethane/*n*-hexane (3/7) as eluent. The ¹H NMR spectrum of the equimolar mixture of (*R*_{As}^{*},*R*_{As}^{*})-(\pm)/(*R*_{As}^{*},*S*_{As}^{*})-**7** in chloroform-*d*₁ contains a singlet for the magnetically equivalent *inner* methylene protons of the (*R*_{As}^{*},*R*_{As}^{*})-(\pm) diastereomer of C₂ symmetry and a pair of poorly resolved singlets for the nonequivalent *inner* methylene

Scheme 1. Synthesis of (\pm)-Arsenicin A



protons of the (*R*_{As}^{*},*S*_{As}^{*}) diastereomer of C_s symmetry. The resonances for the *outer* methylene protons of the two diastereomers of the tetrakis(tertiary arsine) overlap as an apparent AB quartet.^{9,15} Exposure of the (*R*_{As}^{*},*R*_{As}^{*})-(\pm)/(*R*_{As}^{*},*S*_{As}^{*})-**7** to an excess of anhydrous hydrogen iodide in dichloromethane resulted in cleavage of all six of the arsenic–phenyl–C bonds to give the bright yellow hexaiodoarsine CH₂[AsI(CH₂AsI₂)]₂ (**8**), which crystallized in 86% yield as the (*R*_{As}^{*},*S*_{As}^{*}) diastereomer. The ¹H NMR spectrum of the hexaiodoarsine in benzene-*d*₆ exhibits two singlets in the ratio of 1/2 for the protons of the inner and outer methylene groups, respectively. This observation is consistent with the hexaiodoarsine being present in solution as a single diastereomer, or as a rapidly equilibrating mixture of the two diastereomers that furnishes the crystalline (*R*_{As}^{*},*S*_{As}^{*}) diastereomer by second-order asymmetric transformation.¹⁷ The structure of the hexaiodoarsine (*R*_{As}^{*},*S*_{As}^{*})-**8** was established by X-ray crystallography. A solution of (*R*_{As}^{*},*S*_{As}^{*})-**8** in THF is hydrolyzed instantly by the addition of an aqueous ammonia solution to give (*R*_{As}^{*},*R*_{As}^{*},*R*_{As}^{*},*R*_{As}^{*})-(\pm)-**1**, (\pm)-Arsenicin A.¹⁸ The tetraarsenic cage was isolated in 93% yield as air-stable, colorless prisms having an mp of 182–183 °C following purification of the crude product by column chromatography on silica with the use of THF/dichloromethane (1/99) as eluent and recrystallization of the residue from the evaporated eluate from benzene. The tetracyclization presumably occurs by dehydration of the diastereomers of the hexahydroxy intermediate (*R*_{As}^{*},*R*_{As}^{*})-(\pm)/(*R*_{As}^{*},*S*_{As}^{*})-**9** (Scheme 1). The overall yield of (\pm)-Arsenicin A from the bis(arsinic acid) **4** was 36%.

The 800 MHz ^1H NMR spectrum of (\pm)-Arsenicin A in chloroform- d_1 contains doublets at δ 2.42 and δ 1.38, both resonances having the same coupling constant ($^2J_{\text{HH}} = 13.8$ Hz) for the magnetically equivalent pairs of *outer* methylene protons, and a singlet at δ 2.23 for the *inner* methylene protons in the C_2 -symmetric structure.⁹ These NMR spectroscopic data agree with those reported for the polyarsenical labeled Arsenicin A isolated from the New Caledonian sea sponge *Echinochalina bargibanti*.¹

(\pm)-Arsenicin A strongly absorbs UV radiation in the absence of an obvious chromophore and contains absorption maxima at $\lambda_{\text{max}}/\text{nm}$ [CH_2Cl_2 ($\epsilon/(\text{dm}^3 \text{ mol}^{-1} \text{ cm}^{-1})$): 314 (2399), 288 (1599), 257 (3971), and 230 (11 830)].¹⁹ It was concluded from the calculations that the presence of lone pairs of electrons, especially on arsenic, and the through-space and through-bond interactions between them and the As–C and As–O framework accounted for the strong UV absorption of (\pm)-Arsenicin A.¹⁹

Resolution of (\pm)-Arsenicin A. The resolution of (\pm)-Arsenicin A was achieved by preparative HPLC on a Chiralpak IA column (5 μm , 250 mm \times 4.6 mm) with the use of dichloromethane as eluent; the separation was monitored at 254 nm. The chromatograms of the separated enantiomers of (\pm)-Arsenicin A indicated enantiomeric purities of >99% (Figure 1). The optimal conditions for the HPLC resolution of (\pm)-Arsenicin A required conditioning of the HPLC column with 0.01% triethylamine in dichloromethane, followed by the injection of the racemate in the same solvent mixture and elution with neat dichloromethane. The separated enantiomers of (\pm)-Arsenicin A had $[\alpha]_{589}^{20} = -60.2$ (c 0.073, 0.01% $\text{NEt}_3/\text{CH}_2\text{Cl}_2$) and $+62.3$ (c 0.093, 0.01% $\text{NEt}_3/\text{CH}_2\text{Cl}_2$) and melting points of 241–242 $^\circ\text{C}$ after recrystallization from dichloromethane. The individual enantiomers of (\pm)-Arsenicin A showed no significant racemization in neat dichloromethane over 4 days at room temperature, but were susceptible to racemization in the presence of traces of acid.

Crystallography. The crystal structure of (–)-Arsenicin A was determined by X-ray diffraction. The compound crystallizes from dichloromethane in the space group $P2_12_12_1$ with one molecule in the crystallographic asymmetric unit. The molecular structure of (–)-Arsenicin A is shown in Figure 2. The molecule has approximate C_2 symmetry with a 2-fold rotation axis passing through O(2) and C(3). The four stereogenic arsenic centers in the molecule have the S configuration. The As–C and As–O bond distances in the pure enantiomer agree with those for the racemate and are similar to those reported for several related oxo-arsenic compounds having the adamantane-type structure.^{5,20} (The racemate (\pm)-Arsenicin A crystallizes from benzene in the centrosymmetrical space group $P\bar{1}$: the unit cell of the crystal contains two independent pairs of molecules, the molecules in each pair being related by an inversion center.⁹) The mean bond lengths (Å) and angles ($^\circ$) in (S)-(–)-Arsenicin A are the following: As–C = 1.962(6), As–O = 1.801(4); As–C–As = 121.2(3), As–O–As = 129.6(2), C–As–C = 99.8(2), O–As–O = 102.7(18), C–As–O = 100.0(2). For the related compound 1,3,5,7-tetraarsa-2,4,6,8-tetraoxadamantane (**2**), which crystallizes in the space group $P2_1/c$, the mean interatomic distances (Å) and angles ($^\circ$) are these: As–C = 1.962(16), As–O = 1.795(18); As–C–As = 119.4(6), As–O–As = 129.0(12), O–As–O = 101.8(7), C–As–O = 99.3(10).⁵

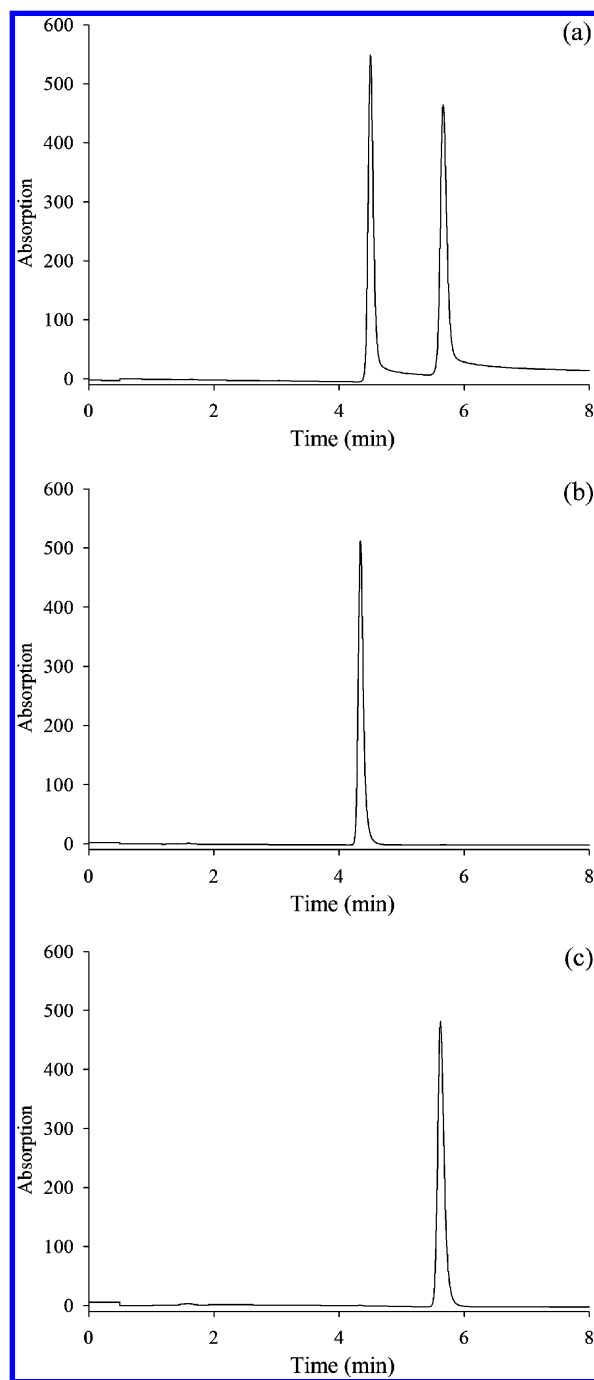


Figure 1. HPLC charts for (\pm)-, (+)-, and (–)-Arsenicin A (a–c), respectively, recorded at 254 nm during resolution on a Chiralpak IA column with the use of dichloromethane as eluent.

The hexaiodide ($R_{\text{As}}^*, S_{\text{As}}^*$)-**8** crystallizes in the space group $P2_1/c$. The structure is shown in Figure 3. The As–C and As–I distances in ($R_{\text{As}}^*, S_{\text{As}}^*$)-**8** are similar to those reported for the diiodoarsine ($R_{\text{As}}^*, R_{\text{As}}^*$)-**1,2**-phenylenebis-(iodomethylarsine).²¹

Configurational Stability at Arsenic in (\pm)-Arsenicin A: A Mechanism for the Racemization of Arsenicin A. Racemization of Arsenicin A requires ring-opening to the hexahydroxyarsenic precursor **9** (or similar), followed by ring closure to the inverted enantiomer. A possible mechanism for the observed acid-catalyzed racemization of Arsenicin A is shown in Scheme 2. There are two key steps in the proposed

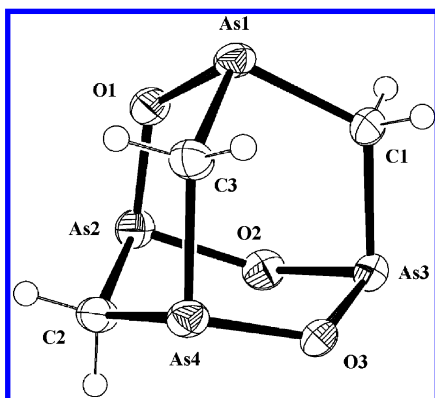


Figure 2. Structure and absolute configuration of (–)-Arsenicin A, (S)-Arsenicin A, showing 30% probability ellipsoids for non-hydrogen atoms. Selected bond distances (Å) and angles (deg): As1–C1 = 1.973(6), As1–C3 = 1.960(6), As1–O1 = 1.821(4), As2–C2 = 1.968(6), As2–O1 = 1.780(4), As2–O2 = 1.804(4), As3–C1 = 1.958(6), As3–O2 = 1.806(4), As3–O3 = 1.783(4), As4–C2 = 1.951(6), As4–C3 = 1.962(6), As4–O3 = 1.813(4); C1–As1–C2 = 100.3(2), C1–As1–O1 = 99.4(2), C3–As1–O1 = 100.1(2), C2–As2–O1 = 100.7(2), C2–As2–O2 = 98.3(2), O1–As2–O2 = 102.55(19), C1–As3–O2 = 98.9(2), C1–As3–O3 = 100.4(2), O2–As3–O3 = 102.89(18), C2–As4–C3 = 99.2(2), C2–As4–O3 = 100.7(2), C3–As4–O3 = 101.7(2), As1–O1–As2 = 130.4(2), As2–O2–As3 = 128.8(2), As3–O3–As4 = 129.6(2), As1–C1–As3 = 121.0(3), As2–C2–As4 = 120.9(3), As1–C3–As4 = 121.6(3).

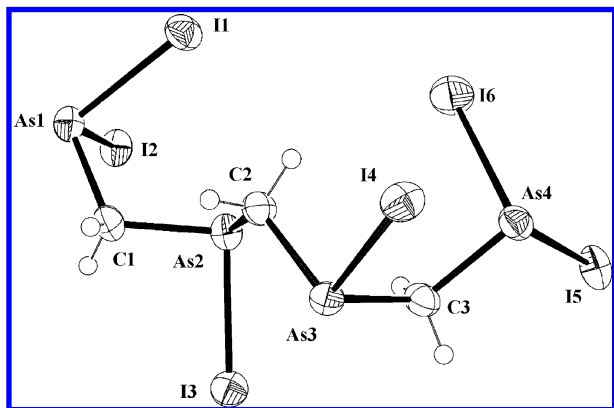
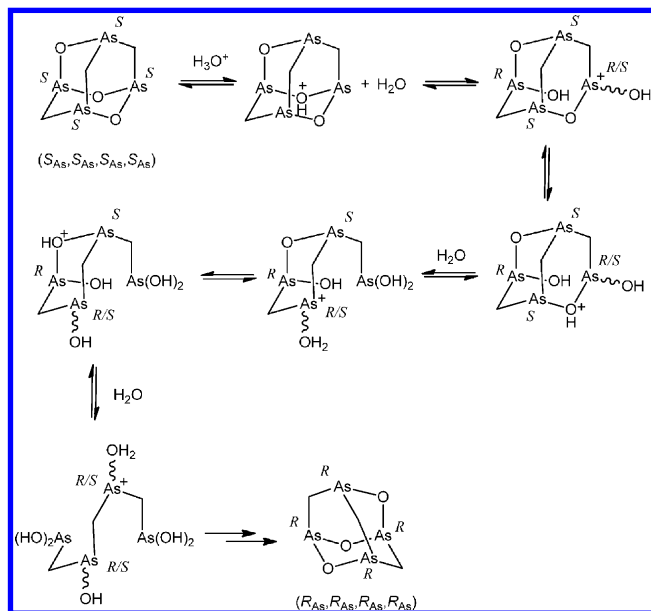


Figure 3. Structure of hexaiodoarsine (R_{As}^* , S_{As}^*)-8 showing 30% probability ellipsoids for non-hydrogen atoms. Selected bond distances (Å) and angles (deg): As3–C3 = 1.984(5), As4–C3 = 1.984(6), As1–I1 = 2.6228(8), As1–I2 = 2.5721(7), As2–I3 = 2.6312(7), As3–I4 = 2.6346(7), As4–I5 = 2.5966(6), As4–I6 = 2.5880(7); C1–As1–I1 = 96.17(17), C1–As1–I2 = 98.49(18), C1–As2–I3 = 96.96(16), C2–As2–I3 = 97.31(15), C2–As3–I4 = 97.63(16), C3–As3–I4 = 95.24(17), C3–As4–I5 = 98.61(17), C3–As4–I6 = 96.66(16), C1–As2–C2 = 100.9(2), C2–As3–C3 = 101.4(2), I1–As1–I2 = 96.76(3), I5–As4–I6 = 97.83(2), As1–C1–As2 = 116.7(3), As2–C2–As3 = 115.2(3), As3–C3–As4 = 114.1(3).

mechanism: initial protonation at bridging oxygen, followed by displacement of the arsonous acid group via hydration at arsenic and generation of a kinetically labile hydrated arsenium species. Attack by water from the opposite face of the hydrated arsenium group will result in oxygen exchange and inversion at arsenic. The proton in the hydrated arsenium ion could be transferred to an adjacent oxygen atom in the same molecule or to the oxygen of a neighboring Arsenicin A molecule, thereby generating a catalytic cycle. (This mechanism is consistent with the results of kinetic investigations on the acid-catalyzed oxygen

Scheme 2. Proposed Mechanism for Acid-Catalyzed Racemization of Arsenicin A



exchange of chiral alcohols, where racemization at carbon is considered to proceed via hydrated carbonium ions.²²)

The feasibility of the key steps of the proposed mechanism of acid-catalyzed racemization of Arsenicin A has been examined via high-level ab initio calculations on cacodyl oxide (10). The calculated Gibbs free energy profiles for the two steps are summarized in Figure 4. The calculations reveal that protonation of the bridging oxygen atom in 10 by a hydronium ion produces the protonated intermediate 11; hydration of this ion leads to the (aqua)arsenium ion intermediate 13 and dimethylarsinous acid 14 via 12. The overall process is strongly exothermic (64 kJ mol^{-1}) and barrier-free (Figure 4a). The stereochemistry around arsenic in ligand-stabilized arsenium complexes of the type 13 is based on the trigonal pyramid, where the two carbon atoms and the lone pair of electrons occupy the base and the ligand donor atom the apex.²³ Attempts to locate a barrier to the formation of the protonated intermediate 11, or to the cleavage of the original As–O bond in the nucleophilic displacement with water, by performing potential energy surface scans at the B3LYP and MP2 levels of theory in the gas phase and in dichloromethane were unsuccessful. The latter observation is consistent with ab initio calculations on ligand exchange between ligand-stabilized phosphonium complexes of the type $[L \rightarrow PH_2]^+$ (where $L = C_2H_2, NH_3, H_2O, \text{ or } H_2S$) and free ligand, which were also found to be barrier-free processes.²⁴ Subsequent attack at arsenic in (R)-13 by water from the face opposite the coordinated water will result in oxygen exchange and inversion at arsenic via 15 (Figure 4b). As found for the cleavage of the As–O bond in 12 by nucleophilic displacement with water, potential energy surface scans confirmed the absence of a barrier to the aquation of 13 to generate 15. The calculations also confirmed that protonation at the bridging oxygen in 10 is ca. 34 kJ mol^{-1} more favorable than protonation at the terminal oxygen in 14. Thus, a proton could be shuttled by repetitive intermolecular or intramolecular processes until racemization of the whole molecule was complete. We conclude from these calculations that acid catalysis provides a plausible mechanism

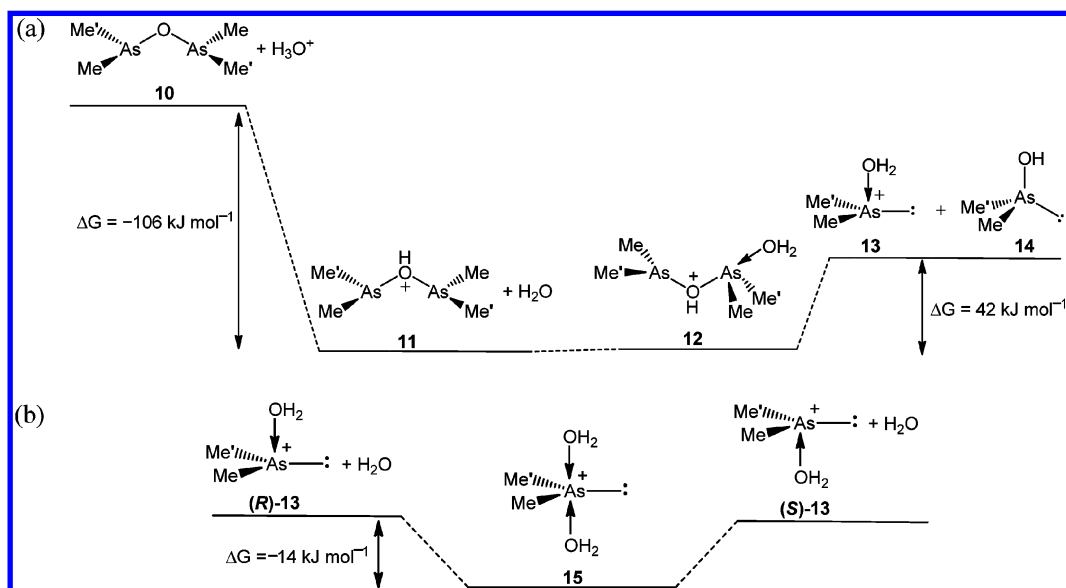


Figure 4. Solution-phase Gibbs free energy profiles for ring-opening (a) and racemization (b) of cacodyl oxide (10) obtained at the G3(MP2)-RAD level of theory in conjunction with the COSMO-RS model.

Table 1. Antiproliferative Activity of (\pm)-Arsenic A (AsA) and Arsenic(III) Oxide (As_2O_3) on Cancer Cell Lines^a

	NB4 blood		BxPC-3 pancreas		MiaPaca-2 pancreas		AsPC-1 pancreas		U87 glioblastoma	
	AsA	As_2O_3	AsA	As_2O_3	AsA	As_2O_3	AsA	As_2O_3	AsA	As_2O_3
IC ₅₀ (μM)	0.05	1.07	0.18	1.96	0.62	1.85	1.07	>25	0.20	3.35
SD	0.01	0.19	0.08	0.05	0.11	0.51	0.11		0.09	1.20
ratio	21.4		10.8		3.0		>25		16.7	

^aIC₅₀ values for proliferation inhibition are the means from at least two experiments carried out in duplicates. SD: standard deviation. Ratio = IC₅₀(As_2O_3)/IC₅₀(AsA).

for the observed racemization of the enantiomers of (\pm)-Arsenic A in dichloromethane.

Biological Activity. (\pm)-Arsenic A is more potent than arsenic(III) oxide (as Trisenox) for the induction of proliferation arrest and cell death in acute promyelocytic leukemia (APL) cells. The proliferation of NB4 cells bearing the chromosome *t*(15;17) translocation generating the PML-RAR α (promyelocytic leukemia-retinoic acid receptor α) fusion gene was determined in the presence of (\pm)-Arsenic A and arsenic(III) oxide. (\pm)-Arsenic A showed a 21-fold greater inhibition of cell proliferation than arsenic(III) oxide. The 72-h IC₅₀ values (concentration of compound inhibiting proliferation by 50%) for (\pm)-Arsenic A (AsA) and arsenic(III) oxide were 0.05 ± 0.01 and $1.07 \pm 0.19 \mu\text{M}$, respectively (Table 1). With use of flow cytometry, the IC₅₀ values for proliferation arrest and loss of viability of NB4 cells were 20 and 53 nM, respectively (Figure 5a). The corresponding IC₅₀ values for arsenic(III) oxide (As_2O_3) were 790 and 1440 nM, respectively (Figure 5b). These results show that (\pm)-Arsenic A, in addition to its antiproliferative activity, induces cell death at a 27-fold lower concentration than arsenic(III) oxide.

The antiproliferative activities of (\pm)-Arsenic A and arsenic(III) oxide were also compared on cell lines from two cancers with particularly bad prognoses: pancreatic adenocarcinoma and glioblastoma. As shown in Table 1, all three pancreatic cell lines were more sensitive to (\pm)-Arsenic A than arsenic(III) oxide; the (\pm)-Arsenic A IC₅₀ values for proliferation arrest in BxPC-3, MiaPaca-2, and AsPC-1 cells were 0.18 ± 0.08 , 0.62 ± 0.11 , and $1.07 \pm 0.11 \mu\text{M}$,

respectively; for arsenic(III) oxide, the corresponding values were 1.96 ± 0.05 , 1.85 ± 0.51 , and $>25 \mu\text{M}$, respectively. The glioblastoma cell line U87 was also clearly more sensitive to (\pm)-Arsenic A than arsenic(III) oxide. For this cell line, the (\pm)-Arsenic A and arsenic(III) oxide IC₅₀ values for proliferation arrest were 0.20 ± 0.09 and $3.35 \pm 1.20 \mu\text{M}$, respectively. Taken together, these results show that (\pm)-Arsenic A is ca. 3 to >25-fold more potent than arsenic(III) oxide for the induction of proliferation arrest in these cancer cells. With the use of 2,3-dimercapto-1-propanol (DMP),²⁵ it was determined that (\pm)-Arsenic A binds dithiols. This property is shared by arsenic(III) oxide^{8b} and the trivalent arsenicals phenylarsine oxide (PAO),²⁶ 4-(*N*-(*S*)-glutathionylacetyl)amino)phenylarsonous acid (GSAO) and its metabolites,²⁷ and 4-(*N*-(*S*)-penicillamylacetyl)amino)phenylarsonous acid (PENAO).²⁸ It, therefore, appears likely that the biological behavior of (\pm)-Arsenic A is the consequence of cross-linking of essential cellular protein cysteine thiol groups.

CONCLUSION

(\pm)-Arsenic A can be synthesized in five steps from methylenebis(phenylarsinic acid) in 36% overall yield and resolved to >99% purity by preparative HPLC on a Chiralpak IA column. (–)-Arsenic A has the overall *S* configuration. The individual enantiomers of (\pm)-Arsenic A are configurationally stable in dichloromethane, but racemize in the presence of traces of acid by a mechanism that involves ring-opening of the As–O–As bonds of the cage by protonation to form

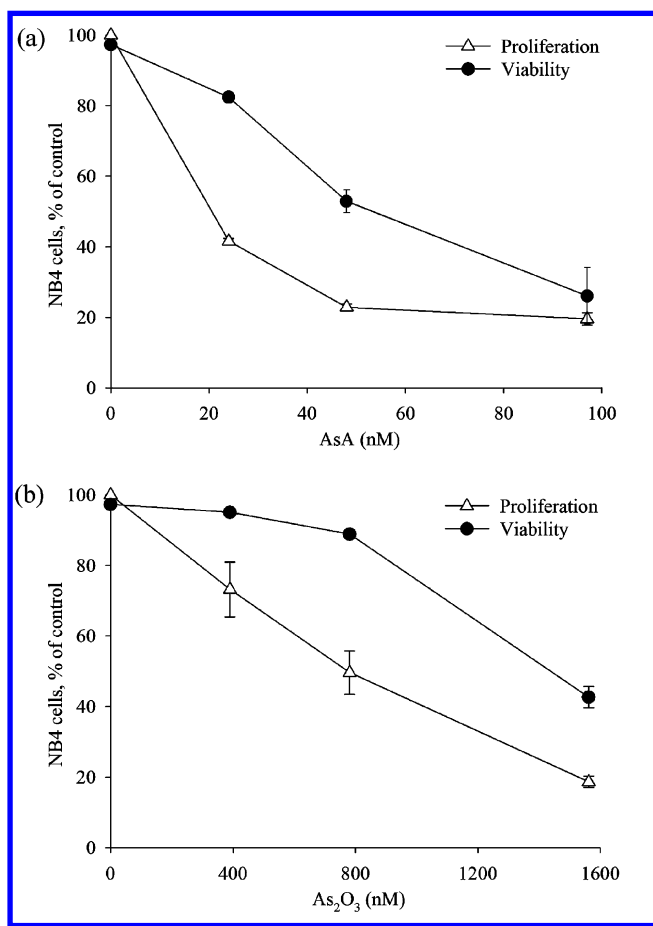


Figure 5. Proliferation arrest and cell death induction by (±)-Arsenicin A (a) and arsenic(III) oxide (b) in NB4 cells. Total numbers of cells and dead cells were counted by flow cytometry in the presence of propidium iodide. Profiles presented are representative of at least two experiments carried out in triplicate; bars: SD.

kinetically labile aquated arsenium ions; this mechanism is supported by high-level *ab initio* calculations. (±)-Arsenicin A exhibits a 21-fold greater inhibition of the induction of proliferation arrest and induces cell death at a 27-fold lower concentration in the acute promyelocytic leukemia (APL) cell line than arsenic(III) oxide. (±)-Arsenicin A IC₅₀ values for proliferation arrest and loss of viability of NB4 cells are 20 nM and 53 nM, respectively. This observation suggests that (±)-Arsenicin A may have potential as an anticancer drug. (±)-Arsenicin A is also more potent than arsenic(III) oxide for the induction of proliferation arrest in two other cancers with particularly bad prognoses: pancreatic adenocarcinoma and glioblastoma. The improved antiproliferative activity of (±)-Arsenicin A warrants further *in vitro* and *in vivo* investigations.

EXPERIMENTAL SECTION

General Comments. Operations involving air-sensitive compounds were carried out under a dry and oxygen-free nitrogen atmosphere with the use of standard Schlenk and vacuum-line techniques. Solvents were dried over appropriate drying agents and distilled before use.²⁹ Routine NMR spectra were recorded at 25 °C on a Varian Gemini 300BB spectrometer (¹H NMR at 300.066 MHz and ¹³C{¹H} at 75.428 MHz). The ¹H NMR spectrum of (±)-Arsenicin A was recorded at 800 MHz on a Bruker Advance 800 equipped TCI-cryoprobe spectrometer. EI mass spectra were

recorded on a Micromass (Waters) VG Autospec magnetic sector mass spectrometer at 800 RP, run against PFK calibrant and formulated by the MassLynx 4.0 elemental program. ESI mass spectra were recorded on a Waters LCT Premier XE instrument at 10 000 RP. The UV/vis spectrum of (±)-Arsenicin A was recorded in a 1 cm cell with the use of a SHIMADZU UV-2450 UV-Visible spectrophotometer. Optical rotations were measured on the specified solutions with a PerkinElmer model 241 spectropolarimeter. Specific rotations are within ±0.05 deg cm² g⁻¹. Elemental microanalyses were performed by the microanalytical service of the Australian National University. The resolution of the (±)-Arsenicin A was performed at The University of Queensland Analytical and Preparative Enantioselective Chromatography Facility. Methylenebis(phenylarsinic acid) (6)⁵ and (chloromethyl)diphenylarsine³⁰ were prepared by the literature methods.

(R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-Methylenebis(methylphenylarsine), (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-5. Sodium borohydride (9.6 g, 253.8 mmol) was added with vigorous stirring to a solution of methylenebis(phenylarsinic acid) (12.0 g, 31.2 mmol) in deoxygenated methanol (300 mL) at -78 °C. After 3 h, the reaction mixture was allowed to warm to room temperature and to stand overnight. Deoxygenated water (100 mL) and dichloromethane (80 mL) were then added, and the two phases separated. The aqueous phase was extracted with dichloromethane (2 × 50 mL), the combined organic fractions were dried (MgSO₄) and filtered, and the filtrate was evaporated to dryness. The resulting, air-sensitive oil was purified by distillation: bp 146–148 °C (0.07 mmHg). Yield: 7.31 g (73%). ¹H NMR (C₆D₆): δ 1.62 (dt, ²J_{HH} = 12.0 Hz, ³J_{HH} = 6.6 Hz, 1H, (R_{As}^{*}, S_{As}^{*})-AsCHH), 1.80 (t, ³J_{HH} = 6.6 Hz, 2H), (R_{As}^{*}, R_{As}^{*})-(±)-AsCH₂), 1.92 (dt, ²J_{HH} = 12.0 Hz, ³J_{HH} = 6.6 Hz, 1H), (R_{As}^{*}, S_{As}^{*})-AsCHH), 3.89 (t, ³J_{HH} = 6.9 Hz, 2H), (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-AsH), 3.92 (t, ³J_{HH} = 7.2 Hz, 2H), (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-AsH), 7.03–7.37 (m, 20H, ArH). ¹³C{¹H} NMR (75.460 MHz, C₆D₆): δ 9.3, 9.5, 128.2, 128.8, 134.4, 134.5, 137.9, 138.2. LR-EI MS (CH₂Cl₂): *m/z* 319.9 amu ([M]⁺), 152.0 ([AsPh]⁺). HR-EI MS (CH₂Cl₂): *m/z* 319.9525 amu (C₁₃H₁₄As₂ requires 319.9527 amu).

(R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-1,1,3,5,7,7-Hexaphenyl-1,3,5,7-tetraarsaheptane, (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-7. A solution of the bis(secondary arsine) (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-5 (5.83 g, 18.2 mmol) in diethyl ether (50 mL) was cooled to 0 °C, and a solution of *n*-butyllithium in *n*-hexane (25.0 mL, 1.5 M, 37.5 mmol) was slowly added. TMEDA (4.50 g, 38.7 mmol) was then added by syringe to the solution, whereupon a bright yellow, crystalline precipitate deposited. The reaction mixture was stirred for 30 min at 0 °C and then it was allowed to stand at -30 °C for a further 30 min to allow complete precipitation of the salt. The mother liquor was decanted, and the bright yellow solid was collected and washed with diethyl ether (10 mL). The air-sensitive solid [Li(TMEDA)]₂[CH₂(AsPh)₂] (6) was dried *in vacuo* for 30 min, and then it was dissolved in THF (50 mL). The deep red solution of the arsenide was cooled at -78 °C, and a solution of (chloromethyl)diphenylarsine (11.6 g, 41.5 mmol) in THF (20 mL) was slowly added. The resulting pale yellow solution was allowed to warm to room temperature, and then the reaction mixture was heated in a hot water bath for 20 min to complete the reaction. Several drops of deoxygenated water were added to the cooled reaction mixture to decompose remaining lithium arsenide, and the solvent was removed *in vacuo*. Distilled dichloromethane (80 mL) and deoxygenated water (30 mL) were added to the reaction mixture, and the organic layer was separated and the aqueous phase washed with dichloromethane (2 × 20 mL). The combined organic fractions were dried (MgSO₄) and then filtered through a Schlenk frit. The solvent was evaporated from the filtrate to give the crude product (colorless oil) that was purified by column chromatography on silica using dichloromethane/*n*-hexane (3/7) as eluent. Yield: 8.90 g (61%). Anal. Calcd for C₃₉H₃₇As₄: C, 58.23; H, 4.51. Found: C, 58.29; H, 4.87. ¹H NMR (CDCl₃): δ 2.10 (s, 2H, (R_{As}^{*}, R_{As}^{*})-(±)-CH₂AsPh), 2.17 (s, 1H, (R_{As}^{*}, S_{As}^{*})-CHHAsPh), 2.31 (s, 1H, (R_{As}^{*}, S_{As}^{*})-CHHAsPh), 2.32, 2.36 (AB q, ²J_{HH} = 11.4 Hz, 8H, (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-CH₂AsPh₂), 7.28–7.51 (m, 60H, ArH). ¹³C{¹H} NMR (CDCl₃): δ

24.7 (CH₂), 25.1 (CH₂), 25.2 (CH₂), 25.7 (CH₂), 128.4–128.7 (m, ArC), 132.6–133.1 (m, ArC), 140.7–141.3 (m, ArC). LR-EI MS (CH₂Cl₂): *m/z* 820.9 ([M + O]⁺), 804.9 ([M + H]⁺). HR-EI MS (CH₂Cl₂): *m/z* 820.9703 (C₃₉H₃₇As₄O requires 820.9708), 804.9762 (C₃₉H₃₇As₄ requires 804.9759).

(R_{As}^{*}, S_{As}^{*})-(±)-1,1,3,5,7,7-Hexaiodo-1,3,5,7-tetraarsaheptane, (R_{As}^{*}, S_{As}^{*})-8. A solution of (R_{As}^{*}, R_{As}^{*})-(±)/(R_{As}^{*}, S_{As}^{*})-7 (2.46 g, 3.06 mmol) in dichloromethane (80 mL) was treated over 12 h with a stream of freshly prepared anhydrous hydrogen iodide.³¹ The hexaiodoarsine precipitated from the reaction mixture over 15 h as yellow crystals of the (R_{As}^{*}, S_{As}^{*}) diastereomer. Yield: 2.9 g (86%). Anal. Calcd for C₃H₆As₄I₆: C, 3.66; H, 0.92. Found: C, 3.27; H, 0.55. ¹H NMR (THF-*d*₆): δ 3.55 (s, 2H, CH₂AsI), 4.00 (s, 4H, 2 CH₂AsI₂). ¹³C{¹H} NMR (THF-*d*₆): δ 26.3 (CH₂), 29.1 (CH₂). ESI MS: *m/z* 1135.28 ([M + 2O]⁺), 1149.33 ([M + 3O]⁺), 1165.30 ([M + 4O]⁺). Crystals of (R_{As}^{*}, S_{As}^{*})-8 suitable for X-ray crystallography were grown by slow evaporation of a dichloromethane solution of the crystalline product.

(R_{As}^{*}, R_{As}^{*}, R_{As}^{*}, R_{As}^{*})-(±)-1,3,5,7-Tetraarsa-2,4,6-trioxaadamantane, (R_{As}^{*}, R_{As}^{*}, R_{As}^{*}, R_{As}^{*})-(±)-1 (±)-Arsenicin A. The hexaiodoarsine (R_{As}^{*}, S_{As}^{*})-8 (0.94 g, 0.85 mmol) was dissolved in THF (25 mL), and aqueous ammonia (28% w/v, 2 mL) was added, whereupon the deep orange solution became almost colorless. The reaction mixture was stirred for 1 h at room temperature, and then the solvent was removed. Deoxygenated water (20 mL) and dichloromethane (50 mL) were added to the residue. The resulting two phases were separated, the aqueous phase was extracted with dichloromethane (2 × 20 mL), and the organic fractions were combined and evaporated to dryness. The product was isolated as air-stable, colorless prisms following column chromatography of the crude product on silica (1% THF/dichloromethane) and recrystallization of the solid from the evaporated eluate from benzene. Yield: 0.31 g (93%). mp 182–184 °C. Anal. Calcd for C₃H₆As₄O₃: C, 9.24; H, 1.55. Found: C, 9.31; H, 1.58. ¹H NMR (800.130 MHz, CDCl₃): δ 1.38 (d, ²J_{HH} = 13.8 Hz, 2H), 2.23 (s, 2H), 2.42 (d, ²J_{HH} = 13.8 Hz, 2H). ¹³C{¹H} NMR (125.697 MHz, CDCl₃): δ 17.2, δ 23.1. LR-EI MS (CH₂Cl₂): *m/z* 389.7 ([M]⁺), 359.7 ([M - CH₂O]⁺), 286.8 ([M - AsC₂H₄]⁺), 270.8 ([As₃CH₂O₂]⁺), 256.8 ([As₃O₂]⁺), 198.9 ([As₂O₃H]⁺), 181.9 ([As₂O₂]⁺), 162.9 ([CHAs₂]⁺). HR-EI MS (CH₂Cl₂): *m/z* 389.7183 (C₃H₆As₄O₃ requires 389.7181). UV/vis (0.0424 g dm⁻³ in CH₂Cl₂): λ_{max}/nm 314 (2399), 288 (1599), 257 (3971), and 230 (11830) (ε/ dm³ mol⁻¹ cm⁻¹).¹⁹

Resolution of (±)-Arsenicin A. The resolution of (±)-Arsenicin A (0.268 g) was achieved by preparative enantioselective chromatography on a Chiralpak IA column (20 mm × 250 mm) of a 0.75 mL sample (10 mg/mL) at a flow rate of 8 mL min⁻¹ with the use of dichloromethane as eluent. Detection of the bands was by UV (254 nm) spectroscopy. The resolution was performed as a service at The University of Queensland Analytical and Preparative Enantioselective Chromatography Facility. The enantiomeric purity of the (+)-enantiomer (0.131.0 g) obtained after removal of the solvent from the first fraction was >99.4%, and that of the (–)-enantiomer (0.127.6 g) from the second fraction was >99.6%. The chromatograms obtained for the racemate and the two enantiomers of (±)-Arsenicin A are shown in Figure 1. Recrystallization of (–)-Arsenicin A from dichloromethane gave crystals having an mp of 241–242 °C. Details for the chromatographic resolution are given in the Supporting Information.

X-ray Crystallography. Single-crystal X-ray diffraction data for (–)-1, (S)-Arsenicin A, and (R_{As}^{*}, S_{As}^{*})-8 were collected on a Nonius Kappa CCD diffractometer with the use of graphite-monochromated Mo Kα radiation (λ = 0.710 73 Å). The structures were solved by direct methods using SIR92³² and refined by full matrix on F² using CRYSTALS.³³ The absolute configuration of (S)-(–)-Arsenicin A was determined by refinement of the Flack parameter: final value = –0.02(3).³⁴

Compound (S)-(–)-1, (S)-Arsenicin A: C₃H₆As₄O₃, *M* = 389.77, colorless lath, 0.30 × 0.07 × 0.02 mm³, orthorhombic, space group P2₁2₁2₁. Cell parameters: *a* = 6.8594(3) Å, *b* = 9.8038(5) Å, *c* = 12.3203(6) Å, *V* = 828.52(7) Å³, *Z* = 4, *D_x* = 3.125 g cm⁻³, *F*(000) = 720. μ(Mo Ka) = 15.94 mm⁻¹, *T* = 150 K. 9310 measured reflections,

2365 independent reflections, *R* = 0.036 for 2140 observed reflections (*I* > 2σ(*I*)), *R_w*(all data) = 0.084, 111 parameters.

Compound (R_{As}^{*}, S_{As}^{*})-8: C₃H₆As₄I₆, *M* = 1103.20, yellow lath, 0.22 × 0.11 × 0.03 mm³, monoclinic, space group P2₁/c. Cell parameters: *a* = 9.3774(2) Å, *b* = 19.0852(30) Å, *c* = 10.0191(2) Å, β = 94.8205(13)°, *V* = 1786.77(6) Å³, *Z* = 4, *D_x* = 4.101 g cm⁻³, *F*(000) = 1896. μ(Mo Ka) = 17.75 mm⁻¹, *T* = 200 K. 35577 measured reflections, 4044 independent reflections, *R* = 0.032 for 3585 observed reflections (*I* > 2σ(*I*)), *R_w*(all data) = 0.083, 118 parameters.

Computational Procedures. Standard ab initio molecular orbital and density functional theory calculations were performed using Gaussian 09³⁵ and Molpro 2009.1³⁶ software; these theoretical procedures are broadly suitable for solvent-sensitive pK_a calculations.³⁷ The geometries for individual species were optimized at the B3-LYP/6-31G(d) level; scaled frequency calculations were also performed at this level, which has been shown to reproduce the geometries and frequencies obtained by high-level ab initio methods.³⁸ Gas-phase Gibbs free energies were calculated at the G3(MP2)//B3-LYP level.³⁹ For species containing one arsenic atom, the Rassolov's 6-31G⁴⁰ level was adopted rather than the default basis sets in Gaussian because it is consistent for first- and second-row atoms. Partition functions and associated thermodynamic quantities were calculated at 298.15 K using the standard textbook formulas for an ideal gas under the rigid-rotor/harmonic oscillator approximation. The gas-phase calculations were corrected for solvent effects in dichloromethane using a thermodynamic cycle in which the solvation energies were obtained using the COSMO-RS method⁴¹ at the BP/TZVP level for which it was parametrized. Free energies of each species in solution were calculated as the sum of the corresponding gas-phase free energy, the calculated free energy of solvation, and the correction term Δ*nRT* ln(*V*) to correct for differences in standard states in the gas phase (1 atm) and solution phase (1 mol L⁻¹). The COSMO-RS calculations were performed in ADF⁴² using the implementation of Pye et al.⁴³ It is not possible to perform relaxed potential energy surface scans using COSMO-RS theory,⁴¹ which were instead carried out using the SMD⁴⁴ model at the B3LYP/6-31G(d) and MP2/6-31G(d) levels of theory.

Cell Proliferation and Viability Assays. The 1 M arsenic(III) oxide solution was prepared by dissolving arsenic(III) oxide (Sigma, St. Louis, MO) in 3 M aqueous sodium hydroxide and diluting the resulting solution 10-fold with deoxygenated water, followed by adjusting the pH to 7.0 with hydrochloric acid; the neutral solution was stored at 4 °C in an airtight container. The 10 mM solution of (±)-Arsenicin A was prepared by dissolving the crystalline solid in dimethyl sulfoxide (DMSO) (Arsenic(III) oxide is insoluble in DMSO; (±)-Arsenicin A is insoluble in water at pH 7.0). BxPC-3, MiaPaca-2, and AsPC-1 cells were obtained from ATCC (Manassas, VA), NB4 cells were obtained from Shane Supple (Kanematsu Laboratories, Royal Prince Alfred Hospital, Sydney, Australia), and U87 cells were obtained from Kerrie McDonald (University of New South Wales, Adult Cancer Program, Sydney, Australia). The NB4, AsPC-1, and BxPC-3 cells were cultured in RPMI medium, and the MiaPaca-2 and U87 cells were cultured in DMEM and MEM media, respectively. The cells were supplemented with 10% fetal calf serum (FBS), 2 mM L-glutamine, and 10 units/mL penicillin and 10 μg/mL streptomycin. For MiaPaca-2 cultures, the medium was supplemented with 2.5% horse serum. All other cell culture reagents were obtained from Gibco/Invitrogen (Mulgrave, Australia). Cell culture plastic ware was purchased from Nunc (Roskilde, Denmark).

BxPC-3, MiaPaca-2, AsPC-1, NB4, and U87 cells were seeded at a density of 4 × 10³ cells per well into 96-well plates. Adherent cells were allowed to adhere overnight at 37 °C in a 5% CO₂, 95% air atmosphere. Cells were then cultured for 72 h in a medium containing or not various concentrations of (±)-Arsenicin A or arsenic(III) oxide. Viable cells were determined by incubating the samples with the tetrazolium salt MTT (Sigma, St. Louis, MO), which is metabolized by viable cells to form insoluble purple formazan crystals. DMSO was added to lyse cells, the contents of the wells homogenized, and the absorbance measured at 550 nm. The cell number in the untreated

control was normalized as 100%, and the viable cell number for all treatments was expressed as a percentage of the control.

The cytotoxic effects of (\pm)-Arsenicin A and arsenic(III) oxide were assayed by flow cytometry with propidium iodide. NB4 cells were seeded at a density of 5×10^4 cells per well into 12-well plates, allowed to grow overnight, then treated for 48 h with (\pm)-Arsenicin A or arsenic(III) oxide. The cells were pelleted, resuspended in 0.5 mL of phosphate buffered saline containing 1 $\mu\text{g/mL}$ propidium iodide (Molecular Probes, Eugene, OR), and analyzed by flow cytometry.⁴⁵ Staurosporine (6 μM) treatment for 16 h was used as a positive control for cell death induction. The number of viable cells in the untreated control was normalized to 100%, and viability was expressed as a percentage of the control.

Binding of (\pm)-Arsenicin A to 2,3-Dimercapto-1-propanol (DMP). With the use of an arsenic(III) titration technique, it was demonstrated that (\pm)-Arsenicin A reacts with 2,3-dimercapto-1-propanol (DMP).²⁵ In this method, increasing concentrations of (\pm)-Arsenicin A (dissolved in 0.14 M NaCl, 20 mM Hepes, 20 mM glycine, and 1 mM EDTA at pH 7.0) were mixed with a specific excess of DMP. After 20 min, each (\pm)-Arsenicin A solution was treated with an excess of 5,5'-dithiobis(2-nitrobenzoic acid). The excess of DMP in each of the (\pm)-Arsenicin A solutions was then determined by measuring the intensity of the absorption of the bright yellow thionitrobenzoic acid derivative of DMP at 412 nm. As the quantity of (\pm)-Arsenicin A increased, the excess of free DMP decreased, thus demonstrating the binding of (\pm)-Arsenicin A to DMP.

■ ASSOCIATED CONTENT

● Supporting Information

The report of the HPLC resolution of (\pm)-Arsenicin A (The University of Queensland Analytical and Preparative Enantioselective Chromatography Facility), B3LYP/6-31G(d) gas-phase-optimized geometries in the form of Gaussian archive entries and corresponding G3(MP2)//B3 gas-phase free energies, and COSMO-RS solvation free energies in dichloromethane (kJ mol^{-1} at 298 K). This material is available free of charge via the Internet at <http://pubs.acs.org>. Crystal data are available from the Cambridge Crystallographic Data Base as file numbers CCDC 8477337 and 847338.

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Notes

The authors declare no competing financial interest.

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