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Chemical synthesis of the organoarsenical antibiotic arsinothricin†

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We report two routes of chemical synthesis of arsinothricin (AST), the novel organoarsenical antibiotic. One is by condensation of the 2-chloroethyl(methyl)arsinic acid with acetamidomalonate, and the second involves reduction of the N-acetyl protected derivative of hydroxarsinothricin (AST-OH) and subsequent methylation of a trivalent arsenic intermediate with methyl iodide. The enzyme AST N-acetyltransferase (ArsN1) was utilized to purify L-AST from racemic AST. This chemical synthesis provides a source of this novel antibiotic for future drug development.

Introduction

Arsenic has been utilized therapeutically since the eras of Ancient Greece and China. Numerous arsenic species present in the environment1 are used in the treatment of a number of human diseases2 and are biotransformed in microbes and animals, including humans.3 Synthesis of organoarsenic compounds is crucial to characterize arsenic species produced by arsenic biotransformations. A variety of synthetic protocols are being developed for novel organoarsonicals.4 The structures of the new aromatic arsenical metabolites discovered in chicken liver were confirmed by chemical synthesis.4

Recently a new arsenic-containing compound, arsinothricin [2-amino-4-(hydroxymethylarsinoyl)butanoic acid, AST (1); Scheme 1], was shown to be synthesized by the rice rhizosphere bacterium Burkholderia gladioli GSRB05.7 A three-gene cluster, arsQML, responsible for the AST biosynthesis has been identified recently.8 AST, a nonproteogenic amino acid analog of glutamate, is a broad-spectrum antibiotic effective against both Gram-positive and Gram-negative bacteria.9 Importantly, AST effectively inhibits growth of major pathogens such as carbapenem-resistant Enterobacter cloacae, one of the World Health Organization’s global priority pathogens. It can control growth of Mycobacterium bovis, a causative agent of both human and animal tuberculosis. The mechanism of action of AST is by inhibition of bacterial glutamine synthetase (GS), an essential enzyme for production of glutamine and for control of ammonia toxicity. AST is chemically similar to the glutamate acylphosphate intermediate in the GS catalytic cycle and binds to the active site of the enzyme, producing inhibition.

We predict that 1 and related arsenic-containing compounds may be the progenitors of a new class of antibiotics. They may prove to be more effective as drugs than chemically related phosphonates, including some of the most effective commercially available herbicides, pesticides and human drugs. While modest amounts of 1 can be generated using the source organism, drug development requires a reliable source of 1. For that reason, we recently developed a semi-synthesis of racemic 1 that involves chemical synthesis of the 2-amino-4-(dihydroxyarsonoyl)butanoic acid [hydroxarsinothricin, AST-OH].

![Scheme 1](Image)

Scheme 1 Reported synthesis of 2 (AST-OH) and its enzymatic conversion to 1 (AST).10

References

† Electronic supplementary information (ESI) available: RP-HPLC traces after derivatization of AST with Marfey’s reagent and copies of 1H, 13C NMR and HRMS spectra for all new compounds. See DOI: 10.1039/d1ra06770b
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We predict that 1 and related arsenic-containing compounds may be the progenitors of a new class of antibiotics. They may prove to be more effective as drugs than chemically related phosphonates, including some of the most effective commercially available herbicides, pesticides and human drugs. While modest amounts of 1 can be generated using the source organism, drug development requires a reliable source of 1. For that reason, we recently developed a semi-synthesis of racemic 1 that involves chemical synthesis of the 2-amino-4-(dihydroxyarsonoyl)butanoic acid [hydroxarsinothricin, AST-OH].

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OH; (2) Scheme 1] precursor; and its enzymatic methylation to AST\textsuperscript{m} using the robust thermostable enzyme CmArsM, the As(III) S-adenosylmethionine (SAM) methyltransferase from the acidothermophilic eukaryotic alga Cyanidioschyzon sp. 5508.\textsuperscript{11}

Synthesis of AST-OH from (2-chloroethyl)arsenate ester 4b that was prepared from 2-chloroethanol 3a in 5 steps that included elaboration via trivalent arsenic species has been reported (Scheme 1).\textsuperscript{12} Coupling of 4b with sodium salt of diethyl acetamidomalonate followed by deprotection and decarboxylation gave 2. We reported that condensation of 1,2-dichloroethane 3b with sodium arsenite provided (2-chloroethyl)arsenic acid 4a in a single step; which eliminates the necessity of conversion of the pentavalent (2-hydroxyethyl)arsenic acid to trivalent dichloro(2-hydroxyethyl)arsine and challenging displacement of hydroxy group with chloride. We also found that esterification of 4a to 4b was not necessary, as subjection of 4a directly to coupling with acetamidomalonate and deprotection/decarboxylation sequence provided 2 in higher yield.\textsuperscript{13} Reduction of 2 and enzymatic methylation of the resulting trivalent arsenicals by CmArsM provided 1.\textsuperscript{10} To reduce effort and complexities associated with synthesis of pure 1 from either bacterial culture medium\textsuperscript{7} or enzymatic buffer\textsuperscript{11} and to provide reliable source of larger quantities of 1 for future drug development, we report here two straightforward methods for the synthesis of racemic AST: one by condensation of the 2-chloroethyl(methyl)arsinic acid with acetamidomalonate and the second by methylation of an AST-OH derivative with methyl iodide. Enzymatic separation of racemic AST is also described.

Results and discussion

In our first route, pentavalent 2-hydroxyethyl(methyl)arsinic acid 5 was designed as crucial precursor for the synthesis of AST, which in turn could be converted to 2-chloroethyl(methyl)arsinic acid 7 via direct chlorination or a path involving trivalent arsines of type 6b. Thus, nucleophilic displacement of chloride in 2-chloroethanol 3a with sodium methyl arsonite [MeAs(O)\textsubscript{2}Na\textsubscript{3}] provided 5 in 86% yield (Scheme 2). The sodium methyl arsonite\textsuperscript{14} was prepared in 97% yield by \textit{in situ} reduction of the sodium salt of methyl arsonate [MeAs(O)\textsubscript{2}Na\textsubscript{3}] with SO\textsubscript{2} gas in the presence of HCl and catalytic amount of KI\textsuperscript{14} followed by hydrolysis of the resulting diiodo(methyl)arsine (MeAsI\textsubscript{2}) with aqueous NaOH. Reduction of 5 with SO\textsubscript{2}/HCl/KI yielded less polar trivalent chloro(2-hydroxyethyl)(methyl)arsine 6a, which appears to be susceptible to hydrolysis as it was observed for dichloro(2-hydroxyethyl)arsine.\textsuperscript{15} Treatment of crude 6a with SOCl\textsubscript{2} resulted in vigorous reaction and failed to give 6b, instead producing dichloro(2-hydroxyethyl)arsine with loss of the methyl group. Since other approaches for the synthesis of 6b by halogenation of 6a were also unsuccessful,\textsuperscript{15} we turned our attention to prepare 7 by direct chlorination of 5.

Treatment of the purified and iodide-free sodium salt of 5 with TMSCl in DMSO afforded minor quantities of 7 in addition to unchanged 5 [8 : 92 mixture based on \textsuperscript{1}H NMR and HPLC coupled with ICP-MS (inductively coupled plasma mass spectrometry)]. Subsequent reaction of this mixture with acetamidomalonate in the presence of sodium ethoxide at 70 °C yielded malonate 8. Reflux of crude 8 in 6 M HCl effected global deprotection and decarboxylation providing a mixture of products containing 5% of AST as estimated based on ICP-MS and \textsuperscript{1}H NMR. However, we were fortunate to find that treatment of sodium salt of 5 with SOCl\textsubscript{2} effected efficient chlorination to provide 7 contaminated by the acidic form of substrate 5 as 80 : 20 mixture (based on \textsuperscript{1}H NMR).\textsuperscript{16} Additional purification yielded pure 7 (4%) and a mixture of 7 and 5 (85 : 15, 69%). Treatment of the latter mixture with acetamidomalonate followed by deprotection and decarboxylation of the resulting 8 yielded AST\textsuperscript{m} (13% from 5) after purification by Dowex and Sephadex column chromatography.

Building on our enzymatic methylation of AST-OH to AST,\textsuperscript{10} we chemically methylated reduced As(III)T-OH 9 with MeI as a source of an electrophilic methyl group. Reduction of 2 with SO\textsubscript{2}/HCl/KI (rt/15 min) followed by treatment with 6 M NaOH gave the reduced arsenic salt 9 (Scheme 3). Treatment of the alkaline solution of crude 9 with excess MeI effected
methylation at arsenic atom. However, the reaction also resulted in methylation of the amino group yielding, after purification on cation exchange resin (Dowex® H⁺ form) with NH₄OH, the trimethylammonium salt 10 (70%, from 2).

Following encouraging methylation of 9 with MeI, we envisioned that reduction/methylation sequence of the AST-OH derivative bearing the protected amino group would result in an alternative straightforward synthetic route to AST. We selected N-acyl protection for the amino group in AST-OH, especially since the original synthesis of AST-OH² and our improved protocol³ required synthesis of the N-acetyl protected derivative of type 11 (ref. 10) (Scheme 4). Thus, treatment of 4a with acetamidomalonate as described³ following purification from the excess of malonate afforded 11. Reduction of pure 11 with SO₂/HCl and catalytic KI followed by pH adjustment to ~11 with 6 M NaOH gave sodium salt of the trivalent arsenic compound 12. Subsequent treatment of 12 with MeI (50 °C/4 h) resulted in the methylation at the arsenic atom, providing protected pentavalent AST derivative 8. Excess MeI and elevated temperature were critical for the optimal yield. The progress of the methylation reaction was monitored by HPLC-ICP-MS. Formation of RAs(O)Me(OMe) byproduct was not observed in the methylation reaction was monitored by HPLC-ICP-MS.

We propose that the methylation of 12 with MeI in basic solution involves Sₘ,2 attack of the nucleophilic arsenic species on the electrophilic methyl iodide with concurrent formation of the arsenic–oxygen double bond, which also oxidized trivalent arsenic to the pentavalent species 8. The reaction resembles a Michaelis–Arbuzov reaction of trivalent phosphorus esters with alkyl halides to form pentavalent phosphonate esters. Analogous conversion of trivalent to pentavalent organoarsenicals with alkyl halides has been noted.³⁸

The antibiotic properties of the chemically synthesized AST 1 (cAST, a mixture of the D/L-enantiomers) were characterized and compared with those of biogenic AST−⁷ (bAST, the l-enantiomer) and semisynthesized AST³⁹ (sAST, a mixture of the D/L-enantiomers). Approximately twice as much cAST or sAST was required to inhibit growth (Fig. 1) and GS activity (Table 1) of Escherichia coli as bAST, consistent with the l-enantiomer of b-AST as the active species.³⁹ ArsN1, the bacterial enzyme that confers AST resistance, catalyzes transfer of the acetyl group of acetyl coenzyme A (AcCoA) to the amine group of 1, generating acetyl-AST (AcAST, 13; Scheme 5). Purified PpArsN1 (ArsN1 from Pseudomonas putida KT2440) nearly completely converted bAST to an arsenic species predicted to be AcAST, while only 50% of racemic CAST or SAST were converted to the putative species and the other half was unmodified (Fig. 2), consistent with only the

Scheme 4  Synthesis of AST from N-acetyl protected AST-OH derivative 11 via reduction and methylation.

Scheme 5  Enzymatic acetylation of CAST to l-AcCAST 13 and chemical deacetylation of 13 to l-AST.

Table 1  Inhibition of E. coli glutamine synthetase by AST

| AST                  | \(K_i\) (\(\mu\)M) |
|----------------------|-------------------|
| Chemically synthesized (cAST) | 0.75 ± 0.20       |
| Semisynthetic (sAST)    | 0.65 ± 0.20       |
| Biogenic (bAST)         | 0.30 ± 0.10       |

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values are reported in parts per million (ppm) and referenced to the residual solvent peaks of CDCl$_3$ (7.26) and D$_2$O (4.79 ppm) for $^1$H NMR and the CDCl$_3$ (77.16) peaks for $^{13}$C NMR spectra, with coupling constant ($J$) values reported in Hz. HRMS were obtained in TOF (ESI) negative or positive mode. TLC was performed on Merck Kieselgel 60 F$_{254}$, and products were detected by staining with 1% ninhydrin solution. Merck Kieselgel 60 (230–400 mesh) was used for column chromatography. All reagents and solvents were purchased from commercial suppliers and used without further purification.

2-Hydroxyethyl[methyl]arsinic acid (5)

(a) Preparation of diido(methyl)arsine (MeAsI$_2$). A solution of KI (41.5 g, 0.252 mol) in H$_2$O (40 mL) was added into the solution of monosodium salt of the commercially available methylvanionate (MeAs(O)OH)ONa; 0.126 mol, 3.15 M H$_2$O, 40 mL). Conc. HCl (30 mL) was slowly added into the mixtures with continuous stirring. Then SO$_2$ gas was passed into the mixtures for 30 min. The resulting mixture was extracted with CH$_2$Cl$_2$ (3 × 70 mL) and dried over anhydrous Na$_2$SO$_4$. The volatiles were evaporated to afford MeAsI$_2$ (46 g, 97%) as orange liquid.

(b) Condensation with 2-chloroethanol. 12 M aqueous NaOH (40 mL, 19.2 g, 0.48 mol) was slowly added into MeAsI$_2$ (46 g, 0.122 mol) placed in a round bottom flask (0 °C, ice bath) over a 20 min with vigorous stirring. During the addition of NaOH, the yellow color was disappeared resulting in colorless solution. The resulting MeAs(ONA)$_2$ solution was stirred for 15 min and then 2-chloroethanol (3a; 8.2 mL, 9.8 g, 0.122 mol) was slowly added over 10 min. The mixture was allowed to warm to ambient temperature (approximately 1 h) and stirring was continued for 12 h. The mixture was acidified with 6 M HCl to pH ~4 and white precipitate was filtered out. The filtrate was evaporated at reduced pressure yielding a white solid, which was suspended in MeOH. The white precipitate was removed by vacuum filtration and the mother liquor was evaporated at reduced pressure to give 5 (19.9 g, 86%) as a white solid: $^1$H NMR $\delta$ 1.92 (s, 3H), 2.55 (t, $J = 6.4$ Hz, 2H), 3.97 (t, $J = 6.4$ Hz, 2H); $^{13}$C NMR $\delta$ 18.06, 37.30, 55.63; HRMS m/z calcd for C$_2$H$_5$AsO$_2$ [M + H]$^+$ 1669694, found 1669693.

Note: removal of volatiles from the reaction mixture prior neutralization with HCl and purification of the residue on silica column with 10–30% MeOH/CH$_2$Cl$_2$ provided iodide-free sodium salt of 5.

2-Hydroxyethyl(chloro)methylarsinic acid (6a). Conc. HCl (37%, 70 mL) was slowly added into the stirring solution of 5 (15 g, 79 mmol) in H$_2$O (40 mL) over a 10 min at rt. Next, catalytic amount of KI (200 mg, 1.2 mmol) was added and SO$_2$ gas was passed into this solution for 30 min with continuous stirring. The mixtures were extracted with CH$_2$Cl$_2$ (3 × 70 mL) and dried over anhydrous Na$_2$SO$_4$. The volatiles were evaporated under reduced pressure to give 6a (12.3 g, 92%) as yellowish oil: $^1$H NMR $\delta$ 1.72 (brs, 1H), 2.04 (s, 3H), 2.50 (t, $J = 6.8$ Hz, 2H), 4.01 (t, $J = 6.8$ Hz, 2H); $^{13}$C NMR (CDCl$_3$) $\delta$ 22.0, 35.8, 61.4. Molecular ion for 6a was not detected in HRMS experiment instead molecular ion for 5 was observed (HRMS m/z calcd for C$_2$H$_10$AsO$_3$ [M + H]$^+$ 168.9840, found 168.9840).

Note: treatment (0 °C to rt, 3 h) of the stirred solution of 6a (12 g, 70.4 mmol) in anhydrous CH$_2$Cl$_2$ (60 mL) with SOCl$_2$ (12.3

Fig. 2 Enzymatic acetylation of 1 to produce 13. Chemically synthesized d/-cAST (A), semisynthetic d/-L-cAST (B), biogenic L-BAST (C) or l-enantiomeric (D) 1 was incubated in the absence (red lines) or presence (blue lines) of PpArsN1. The arsenic species in the reaction solutions were analyzed by HPLC-ICP-MS. Abbreviations: As(ii), arsenite; As(iii), arsenite; DMAAs(v), dimethylarsinate; DMAAs(v), dimethylarsinate; AST (1), arsinothricin; AST-OH (2), hydroxysarinonic acid; As(iii), arsenite; AcAST (13). N-acytethylarsinonic acid.
mL, 20.1 g, 0.17 mol) followed by removal of volatiles under reduced pressure provided dichloro-(2-hydroxyethyl)arsine (8.7 g, 65%) as brown liquid with the 1H and 13C NMR spectra as reported18 (HRMS m/z calcd for C3H9AsClO2 [M – H] 188.8860, found 188.8862) instead of desired chloro-(2-chloroethyl)(methyl)arsine 6b.

2-Chloroethyl(methyl)arsinic acid (7)

Method A. SOCl2 (10 mL) was added to an iodide-free sodium salt of 5 (500 mg, 2.63 mmol) in a dry flask at 0 °C (ice-bath) and was stirred at rt for 15 h. The reaction was quenched by adding 20 mL water and extracted with CH2Cl2. The aqueous phase was separated and volatiles were evaporated at reduced pressure. The residue was purified by column chromatography (20 to 30% MeOH/CH2Cl2) to give mixture of 7 and 5 as a sticky solid (450 mg; 80:20). Second silica column purification afforded pure 7 (20 mg, 4%), as colorless gummy solid in addition to mixture of 7 and acidic form of 5 as a gummy solid (85:15; 400 mg, ~69% of 7) to give overall yield for the conversion of 5 to 7 of approximately 73%.

Compound 7 had: 1H NMR δ 2.08 (s, 2H), 2.94 (t, J = 6.6 Hz, 1H), 3.98 (t, J = 6.8 Hz, 1H); 13C NMR δ 17.47, 36.86, 36.97; HRMS m/z calcd for C3H9AsClO2 [M + H]+ 186.9501, found 186.9500.

Method B. Trimethylsilyl chloride (755 μL, 647 mg, 5.96 mmol) was added to a stirring solution of sodium salt of 5 (500 mg, 2.98 mmol) in DMSO (1 mL) at rt. The resulting mixture was stirred at rt for 14 h. The reaction was quenched by adding 10 mL water and extracted with EtOAc (5 × 10 mL) to remove DMSO. The aqueous phase was separated, and volatiles were evaporated at reduced pressure to give a gummy solid (~400 mg) containing 7 (8%, based on 1H NMR and HPLC-ICP-MS) and unchanged 5 (92%); HRMS m/z calcd for C3H9AsClO2 [M + H]+ 186.9501, found 186.9500 for 7 and m/z calcd for C4H10AsO3 [M + H]+ 168.9840, found 168.9840 for 5.

N-[1-Carboxy-3-(hydroxyl(methyl)arsinoyl)propan-1-yl]-N,N,N-trimethylammonium hydroxide (10)

(a) Reduction. AST-OH18 [2, 50 mg, 0.22 mmol] was dissolved in the mixture of concentrated HCl and water (1 : 1, 5 mL). Then catalytic amount of KI (2.2 mg, 0.013 mmol) was added and SO2 gas was passed into this solution for 15 min at rt. The pH was then adjusted around ~11 with 6 M NaOH [aq] solution under N2.

(b) Methylation. To the solution from step a, MeI (1.1 mL) was added and the mixture was stirred at 50 °C for 2 h. After 2 h, the pH of the reaction mixture showed around 6.5. The volatiles were evaporated under reduced pressure and the residue was suspended in methanol. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave brown solid. The residue was dissolved in 2 mL H2O and applied to a Dowex 50WX8 (H+ form) column (30 × 1 cm, 10 g) which was washed with 50 mL of H2O. The product was eluted with a solution of NH4OH (0.5 M, 50 mL). The appropriate fractions (TLC, Rf 0.70, i-ProOH/H2O/NH4OH, 5 : 2 : 3; identified by staining with 1% ninhydrin solution) from the ammonium elution (~20 mL) were evaporated under reduced pressure to afford 10 (41 mg, 70%) as an off-white solid: 1H NMR δ 1.69 (s, 3H), 1.90–2.06 (m, 2H), 2.09–2.19 (m, 1H), 2.23–2.32 (m, 1H), 3.19 (s, 9H), 3.68 (dd, J = 11.6, 3.6 Hz, 1H); 13C NMR δ 15.84, 19.61, 28.99, 51.95, 78.62, 170.65; HRMS m/z calcd for C8H19AsNNaO4 [M + H]+ 290.0344, found 290.0346.

Ethyl2-acetamido-2-ethoxycarbonyl-4-(dihydroxyarsanonyl)butanoate (11). Sodium (1.46 g, 63.6 mmol) was added into a dry flask containing 40 mL of anhydrous EtOH and the mixture was stirred at ambient temperature until the sodium dissolved. Then diethyl acetamidomalonate (10.4 g, 47.7 mmol) was added, and the resulting mixture was stirred for 1 h. During this time, the reaction was turned into milky white mixture. To the above mixture solid 4a (ref. 10) (3.0 g, 15.9 mmol) was added and the resulting mixture was stirred at 70 °C in an oil bath for 4 h. Volatiles were evaporated under reduced pressure to give crude 11 (ref. 10) as a brownish solid, which was suspended in H2O and transferred to a separatory funnel. The mixture was extracted with CH2Cl2 (6 × 50 mL) to remove excess malonate and aqueous layer was collected. The volatiles were evaporated under reduced pressure and residue was suspended in 30% MeOH in CH2Cl2. The off-white precipitate was removed by vacuum filtration. Evaporation of the volatiles at reduced pressure gave brown solid which was loaded on the silica column. The residual malonate was removed by 5–10% MeOH in CH2Cl2 until all the yellow color eluted. Then the arsenic compound was eluted by 30% MeOH. Only the colorless fractions were collected. Volatiles were evaporated under reduced pressure to afford 6.0 g white solid which on 1H NMR spectra showed approximate 10% malonate impurity. Second silica column purification gave pure 11 (5.2 g, 89%) as white solid: 1H NMR δ 1.25 (t, J = 7.2 Hz, 6H), 1.99–2.04 (m, 2H), 2.07 (s, 3H), 2.54–2.58 (m, 2H), 4.29 (q, J = 7.2 Hz, 4H); 13C NMR δ 13.73, 22.14, 26.80, 27.88, 64.77, 67.41, 168.97, 174.21; HRMS m/z calcd for C11H21AsNO8 [M + H]+ 370.0475, found 370.0475.

2-Amino-4-(hydroxymethylarsinoyl)butanoic acid (AST, 1) Procedure A (from 11)

(a) Reduction. Compound 11 (500 mg, 1.35 mmol) was dissolved in the mixture of concentrated hydrochloric acid and water (1 : 1, 14 mL). Then catalytic amount of KI (13.5 mg, 0.08 mmol) was added and SO2 gas was passed into this solution for 15 min at ambient temperature. The pH was then adjusted to around 11 with 6 M NaOH [aq] solution under N2 to give crude 12 which was directly used in next step.

(b) Methylation. To the product from step a, MeI (7 mL) was added and the mixture was stirred at 50 °C for 4 h. The reaction progress was monitored by HPLC-ICP-MSA. After 4 h, the pH of the reaction mixture showed around 6.7. The volatiles were evaporated under reduced pressure and the residue was suspended in methanol. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave brown solid crude 8.

(c) Deprotection and decarboxylation. 6 M HCl (20 mL) was added into the crude 8, and the resulting mixture was refluxed at 120 °C in an oil bath for 3 h. The mixture was neutralized with 6 M HCl around pH ~7 and white precipitate was filtered out. The volatiles were evaporated under reduced pressure and the residue was suspended in methanol. The off-white precipitate was removed by vacuum filtration. Evaporation of volatiles from the filtrate at reduced pressure gave brown solid crude 1. The residue was dissolved in 15 mL H2O and applied to a Dowex
50WX8 (H+ form) column (30 × 1 cm, 10 g) which was washed with 100 mL of H2O. The product was eluted with a solution of NH4OH (0.5 M, 100 mL). The appropriate fractions (TLC, Rf 0.70, i-PrOH/H2O/NH4OH, 5:2:3; identified by staining with 1% ninhydrin solution) from the ammonium elution (~40 mL) were evaporated under reduced pressure to afford 1 as an off-white solid with some impurities. The residue was dissolved in 5 mL H2O and was applied onto a Sephadex LH-20 (GE Healthcare) column with a mobile phase 70% (v/v) EtOH at a flow rate of 1.0 mL min−1. Arsenic species in each fraction was analyzed by HPLC-ICP-MS. Fractions containing AST with high purity (>95%) were combined and concentrated by a rotary evaporator to afford 1 (ref. 7 and 10) (200 mg, 65% from 11) as an off-white solid: 1H NMR δ 2.03 (s, 3H), 2.27–2.34 (m, 2H), 2.42–2.48 (m, 2H), 2.52–2.58 (m, 1H), 3.97 (t, J = 6.4 Hz, 1H);13C NMR δ 16.39, 23.34, 29.39, 54.39, 172.93; HRMS m/z calced for C5H13AsNO4 [M + H]+ 226.0055, found 226.0055. Note: use of pure N,N-diethyl acetamidomalonate is critical since during deprotection and decarboxylation steps these impurities are converted to glycine that is difficult to separate from the AST product during purification on Dowex.

Procedure B (from 7) (a) Condensation. Sodium (198 mg, 8.66 mmol) was added into a dry flask containing anhydrous EtOH (5 mL) and the mixture was stirred at rt until the sodium dissolved. Then diethylacetamidomalonate (1.40 g, 6.45 mmol) was added, and the resulting mixture was stirred for 30 min, followed by addition of 7 as a mixture of 7 and 5 (85:15, 400 mg from Method A) dissolved in 2 mL EtOH. The resulting mixture was stirred at 70 °C for 4 h. Volatiles were evaporated under reduced pressure, yielding crude 8.

(b) Deprotection and decarboxylation. Subjection of crude 8 to the same protocol as described for Procedure A (step c) gave 1 (72 mg, ~17% from 7) as an off-white solid: 1H NMR δ 1.97 (s, 3H), 2.11–2.29 (m, 2H), 2.33–2.41 (m, 1H), 2.43–2.51 (m, 1H), 3.83 (t, J = 6.2 Hz, 1H);13C NMR δ 16.17, 23.53, 29.26, 55.01, 173.73; HRMS m/z calced for C5H13AsNO4 [M + H]+ 226.0055, found 226.0055.

Note: treatment of crude 7 (350 mg, from Method B) with EtONa and diethylacetamidomalonate as described for Procedure B afforded 1 (3% from 5, based on 1H NMR and HPLC-ICP-MS). The presence of 1 was confirmed by HPLC-ICP-MS and HRMS: calced for C5H13AsNO4 [M – H]− 223.9909, found 223.9909.

(S)-2-Acetamido-4-(hydroxymethylarsinoyl)butanoic acid (S-AcAST, 13).

(a) Enzymatic N-acetylation of AST (1). PpArsN1, the AST-selective N-acetyltransferase from Pseudomonas putida KT2440, was purified as described previously.10 10 μM of 1 (cAST, SAST bAST, or t-AST) was incubated in a buffer consisting of 20 mM Tris–HCl (pH 7.4), 1 mM ethylenediaminetetraacetic acid, 0.2 mM acetyl coenzyme A (AcCoA) at 37 °C for 30 min, with or without 0.2 μM PpArsN1. The reaction solution was filtered using an Amicon Ultra centrifugal filter with a 3K cutoff membrane (MilliporeSigma), and arsenic species in the filtrate were analyzed by HPLC-ICP-MS.

(b) Purification of t-AcAST. A larger amount of D/t-AcAST 1 (0.9 mM, 35 mL, 7 mg) was incubated overnight with 1 mM AcCoA and 20 μM PpArsN1 in a buffer consisting of 20 mM Tris–HCl, pH 7.4 at 37 °C. The reaction solution was filtered using an Amicon Ultra centrifugal filter with a 3K cutoff membrane to remove protein. The filtrate was concentrated to 5 mL by rotary evaporation at reduced pressure and separated by Sephadex LH-20 size-exclusion chromatography to give t-AcAST 13 and D-AST with little impurities. Arsenic species in each fraction was analyzed by HPLC-ICP-MS. Fractions containing putative t-AcAST with high purity (>90%) were combined and concentrated by a rotary evaporation. The concentrated t-AcAST solution was applied again to Sephadex LH-20 size-exclusion chromatography for further purification. Fractions containing purified t-AcAST (>95%) were combined and concentrated to give 13 (3.0 mg, 36%):1H NMR δ 1.92 (s, 3H), 19.8–2.07 (m, 1H), 2.04 (s, 3H), 2.24–2.15 (m, 1H), 2.37–2.26 (m, 2H), 4.24 (dd, J = 8.2, 4.6 Hz, 1H);13C NMR δ 15.22, 21.89, 24.07, 29.42, 55.01, 173.80, 177.32; HRMS m/z calced for C7H15AsNO5 [M + H]+ 268.0161, found 268.0162.

Note: fractions containing D-AST (>95%) were combined and concentrated to give D-AST (2.1 mg, 30%); HRMS m/z calced for: C7H13AsNO4 [M + H]+ 226.0055, found 226.0059.

Derivatization of AST with Marfey’s reagent

Sample of racemic AST or t-AST (5 μmol) is dissolved in 100 μL of water. 2 mg of 1-fluoro-2,4-dinitrophenyl-5-L-alanine amide (Marfey’s reagent) is dissolved in 200 μL of acetone (1% solution). Solution of Marfey’s reagent is added to the dissolved AST sample followed by 40 μL of 1 M NaHCO3. Reaction is set up at 40 °C for 1 h after which is quenched by addition of 20 μL of 2 M HCl. Small aliquot is taken, diluted with DMSO and injected on RP HPLC (column: Vydac Denali C-18, 5 μm, 120 Å). Elution method 100% A for 5 min followed by linear gradient of 0–100% B over 40 min where A is 0.1% TFA in H2O and B is 0.1% TFA in ACN. Eluting products were detected by UV at 340 nm. Results are shown in Fig. S1 in ESI.†

Arsenic speciation by HPLC-ICP-MS

Arsenic species were determined by HPLC-ICP-MS using the HPLC retention time of known standards as described previously.18

Assay of antibiotic activity. Single colonies of E. coli W3110 were inoculated in M9 medium (47.7 mM Na2HPO4, 22 mM
KH2PO4, 8.6 mM NaCl, 18.7 mM NH4Cl, 2 mM MgSO4 and 0.1 mM CaCl2 supplemented with 0.2% glucose (w/v) and cultured in the absence or presence of the indicated concentrations of chemically synthesized AST (cAST), semisynthetic AST (sAST),7 biogenic AST (bAST),8 and L-AST for 16 h at 37 °C. The A600nm was determined to compare the antibiotic activity of each compound.

**Assay of glutamine synthetase (GS) inhibition.** Inhibition of GS activity by bAST, sAST, cAST or L-AST was analyzed by a coupled assay using GS from *E. coli* (Millipore-Sigma), as described previously9 with minor modifications. Briefly, the GS reaction was initiated by addition of L-glutamate (5, 10, 20 or 50 mM) to the reaction mixture (100 mM Tris-acetate (pH 8.6), 9 mM ATP, 1 mM phosphoenolpyruvate, 60 mM MgCl2, 19 mM KCl, 45 mM NH4Cl, 0.25 mM NADH, 13–20 units of L-lactate dehydrogenase, 8–14 units of pyruvate kinase and 1 unit of GS) and incubated at 37 °C in the absence or presence of 0.5 or 2.0 μM of bAST, sAST, cAST or L-AST and the decrease in A340 nm was monitored to quantify oxidation of NADH to NAD+ using an extinction coefficient 6230 M–1 cm–1. Activities were corrected with the values from control assays without enzyme. The inhibition constant (Ki) for each AST was determined based on the apparent Km of GS using Sigma Plot (Systat Software, Inc., Sun Jose, CA).

**Author contributions**

AHH and SHS conducted the synthetic experiments. VSN, AEG and MY conducted the HPLC-ICP-MS analysis and biological studies. AN and PC established chiral purity of r-AST, AHH, BPR, MY and SFW designed the experiments and wrote the manuscript.

**Conflicts of interest**

There are no conflicts of interest to declare.

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