First Total Synthesis of Reassigned Echinosulfonic Acid D

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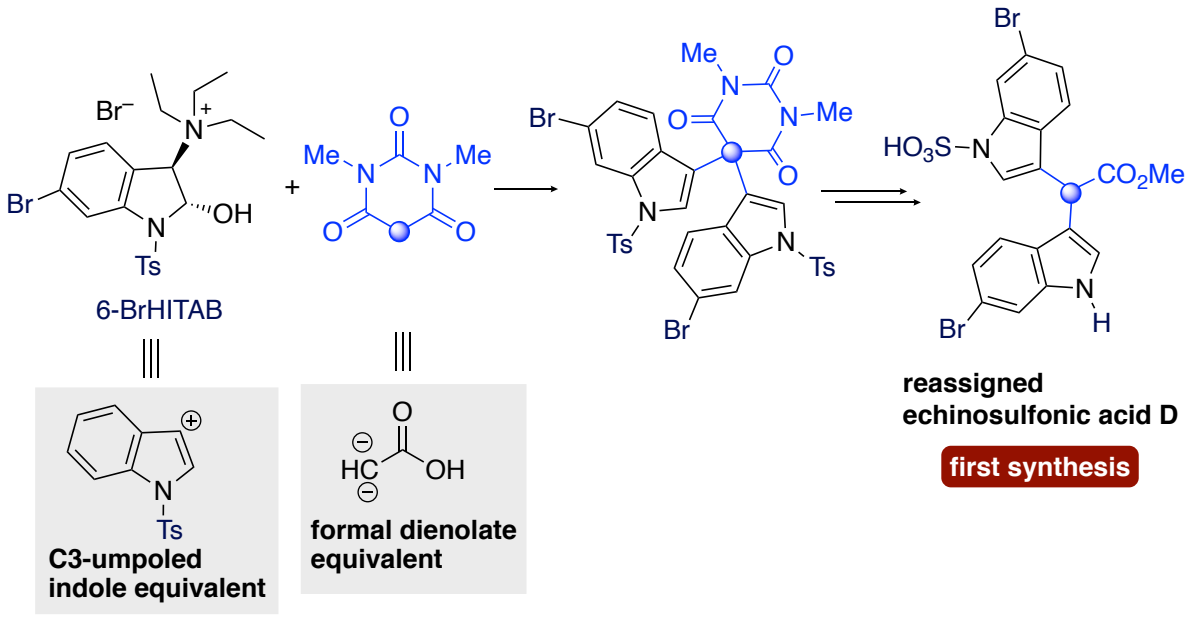


ABSTRACT. Echinosulfonic acid D, a sponge metabolite whose structure was recently reassigned, was synthesized for the first time. The key step is the double indolization of dimethylbarbituric acid using the umpolung indole reagent, followed by a hydrolysis/decarboxylation/esterification sequence.

Echinosulfonic acids A–D (**1**–**4**) and echinosulfone A (**5**) are an uncommon group of indole alkaloids that feature the unique structure of a brominated bisindole moiety with a sulfur fuctional group (Figure 1). Echinosulfonic acids A–C (**1**–**3**) and echinosulfone A (**5**) were isolated by Capon and co-workers in 1999 from an *Echinodictyum* sp. Australian marine sponge.[1] Furthermore, Sévenet et al. isolated echinosulfonic acid D (**4**) from a New-Caledonian *Psammoclema* sp. sponge in 2005.[2] However, their original structures were independently reassigned by Capon,[3] Flematti,[4] and Carroll[5] in 2020 as described in Figure 1. The structure of echinosulfone A (**5**) was revised by organic synthesis.[3,5] However, the syntheses of echinosulfonic acids A–D (**1**–**4**) have not been reported so far. In addition, echinosulfonic acid B (**2**) was found to be unstable upon isolation.[4] Thus, the related compounds may possess the same instabilities and the structures of these alkaloids may have been misassigned. Furthermore, echinosulfonic acid D (**4**) was reported without ^{13}C NMR data due to the small amount of **4** that was isolated (0.5 mg) and the inaccessibility of additional sponge material.[2] Therefore, the supply of **4** by organic synthesis and the confirmation of its structure are highly desirable.

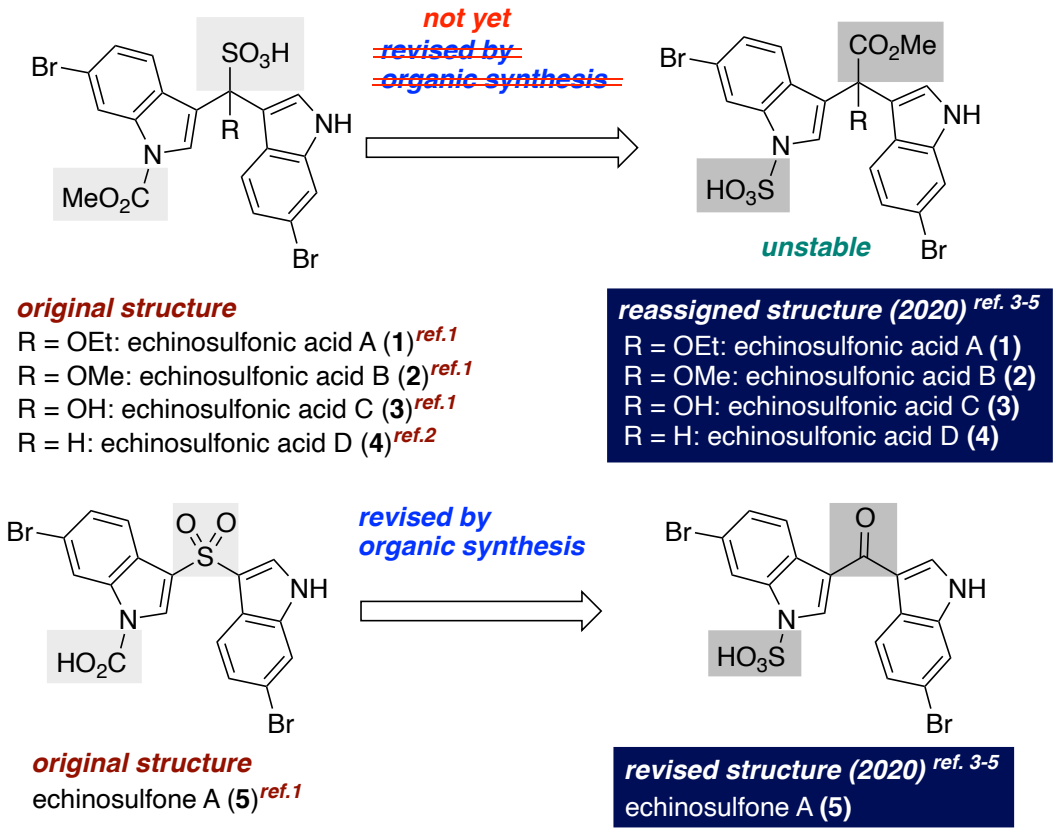


Figure 1. Structures of echinosulfonic acids A–D (**1**–**4**) and echinosulfone A (**5**).

An efficient protocol for the synthesis of bisindolylacetic acids is quite rare.[6] In 2015, Lam and co-workers reported a novel strategy for the synthesis of 5-aryl barbituric acids based on the rhodium(II)-catalyzed C–H functionalization of indoles with diazo barbituric acids.[7] However, this umpolung transformation lacks a double indolization due to the intrinsic character of the diazo compounds. Thus, it is necessary to develop a new double indolization strategy to use an active methylene compound as a dinucleophile instead of an electrophile.

We recently reported a new class of bench-stable indoline reagents that serve as an umpoled indole synthon for the formal cross-nucleophile coupling to afford 3-substituted indoles.[8,9] Using our umpoled indole reagent, α-monoindolyl carbonyl compounds were able to be synthesized from

the active methylene compounds, which are difficult to obtain by other methods.[9a,9h] Based on these results, we hypothesized a new synthetic route for bisindolylacetic acids; the desired strategy should be accomplished by a double indolization of dimethylbarbituric acid as a dinucleophile with our umpoled indole reagent as an electrophile followed by dehydration and hydrolysis of the barbituric acid moiety.

The retrosynthetic analysis of reassigned echinosulfonic acid D (**4**) is shown in Figure 2. N-Sulfonic acid **4** can be obtained by the Pedras sulfonylation[10] of bisindolyl acetic acid methyl ester **6**. The methyl ester **6** will be obtained from bisindolyl barbituric acid **7** as a masked acetic acid through hydrolysis/decarboxylation/esterification. This transformation is a new tool to use dimethylbarbituric acids as a masked dienolate of acetic acid owing to their active methylene moiety. The bisindolyl barbituric acid **7** can be prepared from dimethylbarbituric acid **8** with 6-bromo-2-hydroxyindolin-3-triethylammonium bromide (**9**, 6-BrHITAB) as an electrophile via a double indolization.

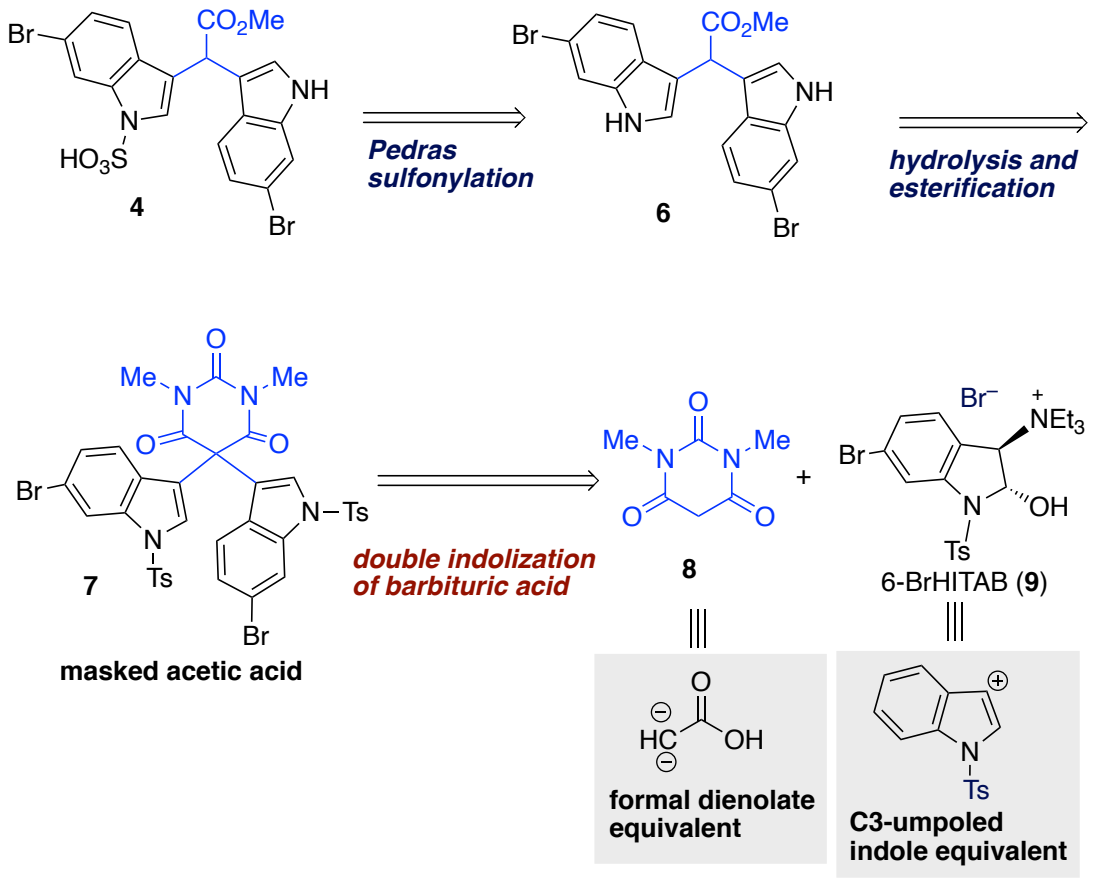
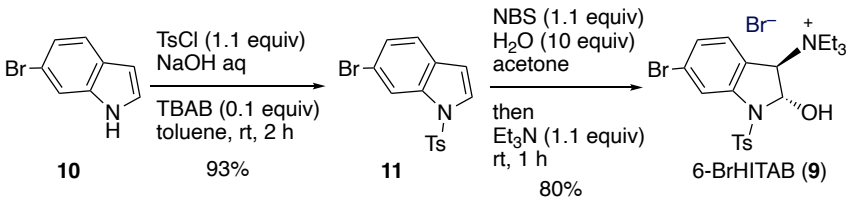


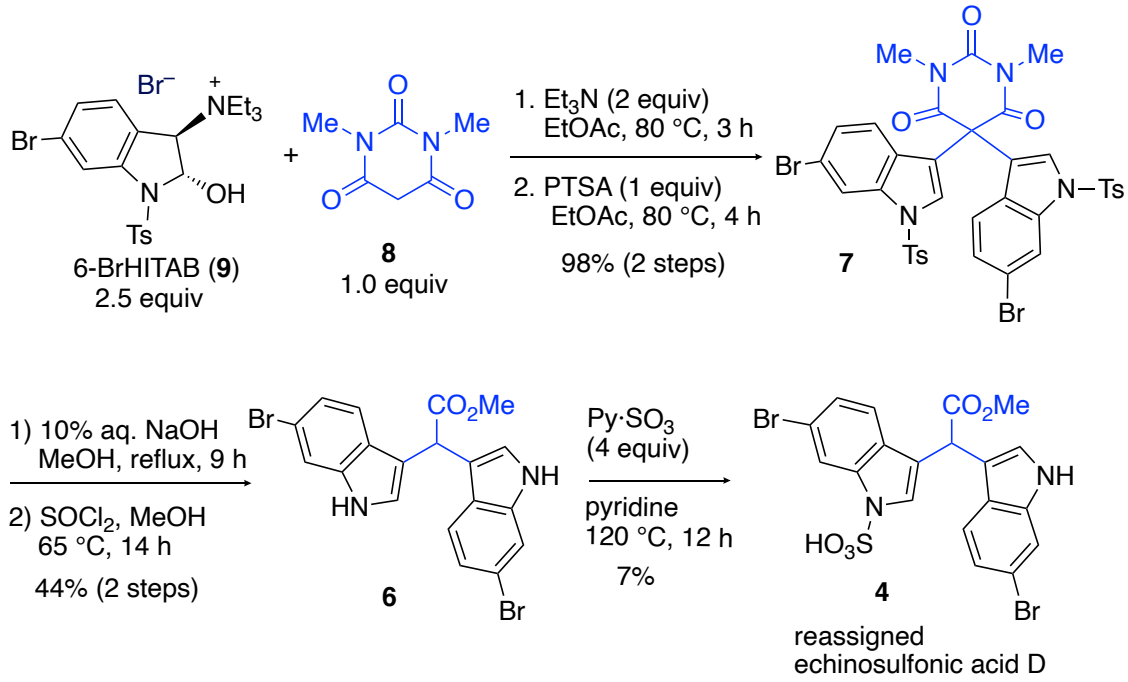
Figure 2. Our retrosynthesis of echinosulfonic acid D (**4**).

We first synthesized 6-BrHITAB (**9**) according to our previous report (Scheme 1).[9a] Treatment of commercially available 6-bromoindole (**10**) with tosyl chloride in an aqueous solution of NaOH afforded *N*-tosylindole **11**.[11] Treatment of **11** with NBS in the presence of H_2O in acetone, and then addition of Et_3N afforded **9**.

Scheme 1. Synthesis of 6-BrHITAB (**9**).



Scheme 2. Completion of the total synthesis of the reassigned structure of echinosulfonic acid D. (**9**).

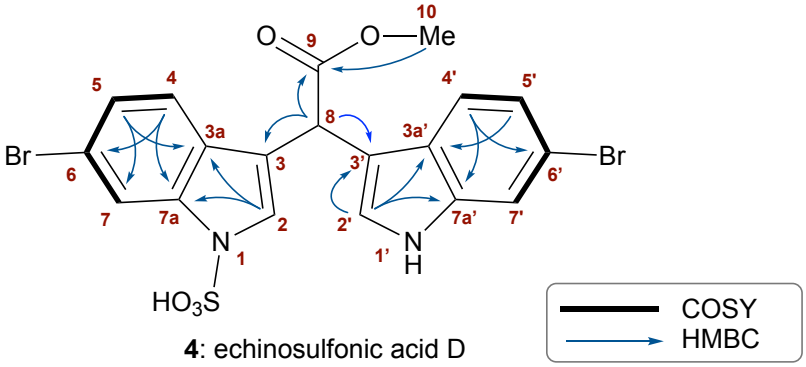


Next, we investigated the unprecedented double indolization of dimethylbarbituric acid **7** (Scheme 2).[7] Using 2.5 equivalent of **9** with **8** in the presence of Et_3N in EtOAc, followed by dehydration using PTSA, resulted in a high yield of **7**. Transformation of **7** to methyl ester **6** was accomplished by hydrolysis of the barbituric acid moieties and esterification using $SOCl_2$/MeOH. Then, a Pedras sulfonylation[10] was implemented on **6** under modified conditions (see Experimental Section). To our delight, the desired monosulfonylated product **4** was obtained in 7% yield. The

compound **4** showed rapid desulfonylation during flash column chromatography and evaporation in vacuo; therefore, rapid ^{1}H and ^{13}C NMR measurements are essential. The ^{1}H NMR, IR and HRMS data of sulfonic acid **4** were identical with the natural echinosulfonic acid D (Table 1 and Experimental Section).[2] The complete proton and carbon assignments were achieved by analysis of 2D NMR experiments (Table 2). Thus, the reassigned structure of echinosulfonic acid D was confirmed by organic synthesis. During MS analysis of synthetic **4**, fragmentations with the loss or addition of an SO_3 (–80 amu or +80 amu) were observed under ESI MS conditions in similar to the previous paper[1-5] (see Supporting Information). Echinosulfonic acids A–D are unstable and indicate reversible sulfonylation.

In conclusion, we report the first total synthesis of reassigned echinosulfonic acid D in seven steps with an overall yield of 2.2% from commercially available 6-bromoindole (longest linear sequence). Highlights of the synthesis include the double introduction of the indole moiety into dimethylbarbituric acid in a two-step protocol using HITAB and the successive hydrolysis/decarboxylation/esterification sequence. We confirmed the reassigned structure of echinosulfonic acid D[3-5] by the consistency of the spectra data for our synthetic sample with the reported literature data for natural echinosulfonic acid D.[2] We also provided ^{13}C NMR data of echinosulfonic acid D.

Table 1. Comparison of ^{1}H NMR (CD_3OD) data for synthetic **4** (600 MHz) and natural echinosulfonic acid D (300 MHz).



4: echinosulfonic acid D

Position	Synthetic **4** δ_c, type 151 MHz, CD_3OD	Synthetic **4** δ_H, mult (*J* in Hz) 600 MHz, CD_3OD	Natural **4**[2] δ_H, mult (*J* in Hz) 300 MHz, CD_3OD
2	126.3, CH	7.40, s	7.42, d (0.9)
3	113.7, C		
3a	127.2, C		
4	120.1, CH	7.33, d (8.4)	7.34, d (8.4)
5	123.4, CH	7.16, dd (8.4, 1.8)	7.17, dd (7.5, 1.7)
6	115.9, C		
7	116.3, CH	8.05, d (1.8)	8.07, d (0.7)
7a	136.1, C		
1’		–	–
2’	124.5, CH	7.12, s	7.13, d (0.7)
3’	112.0, C		
3a’	125.4, C		
4’	120.0, CH	7.41, d (7.8)	7.4, d (8.3)
5’	121.8, CH	7.07, dd (8.4, 1.8)	7.08, dd (7.5, 1.7)
6’	114.7, C		
7’	113.9, CH	7.49, d (1.8)	7.50 , d (1.7)
7a’	137.6, C		
8	40.2, CH	5.40, s	5.40, s
9	173.6, C=O		
10	51.4, CH^3	3.71, s	3.73, s

EXPERIMENTAL SECTION

General Experimental Procedures. IR spectra were measured with a Shimadzu IR Affinity-1 spectrometer. The NMR experiments were performed with a JEOL JNM-ECZ600R (^{1}H NMR: 600 MHz, ^{13}C NMR: 151 MHz) spectrometer, and chemical shifts are expressed in ppm relative to residual undeuterated solvent as an internal reference ($CDCl_3$, δ_H 7.25, δ_C 77.1; DMSO-d_6, δ_H 2.50, δ_C 39.5; CD_3OD, δ_H 3.31, δ_C 49.0). The following abbreviations were used to explain NMR peak multiplicities: s = singlet, d = doublet, t = triplet, m = multiplet, dd = doublet of doublets; coupling constants in Hz; integration. High-resolution MS spectra were recorded with a Brucker micrOTOF mass spectrometer (ESI-TOF-MS). Reactions were monitored by thin layer chromatography (TLC) carried out on a silica gel plates (60F-254) and visualized under UV illumination at 254 or 365 nm depending on the compounds monitored. Flash column chromatography was performed on silica gel (WAKO Gel 75–150 mesh, WAKO Co., Ltd.).

6-Bromo-1-tosylindole (11). To a solution of **10** (1.96 g, 10 mmol) and tetrabutylammonium bromide (TBAB) (322 mg, 1.0 mmol) in toluene (10 mL, 1 M) and 50% NaOH aq. (8 mL) was added *p*-toluenesulfonyl chloride (TsCl) (2.10 g, 11 mmol). The mixture was stirred at room temperature (rt) for 2 h. The whole was extracted with EtOAc, washed with brine. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by

recrystallization from MeOH to give **11** (3.26 g, 93% yield) as a colorless solid: IR (KBr): 3138, 3109, 1595, 1422, 1364, 1177, 665 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ: 8.16 (s, 1H), 7.75 (d, *J* = 8,4 Hz, 2H), 7.52 (d, *J* = 3.6 Hz, 1H), 7.37 (d, *J* = 8.4 Hz, 1H), 7.32 (dd, *J* = 8.4, 2.4 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 6.60 (d, *J* = 3.6 Hz, 1H), 2.34 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 145.4, 135.5, 135.1, 130.1, 129.6, 126.9, 126.7, 122.5, 118.3, 116.7, 108.8, 21.7; HRESIMS *m/z* 371.9666/373.9646 $[M+Na]^+$ (calcd for $C_{15}H_{12}{}^{79}BrNO_2SNa$, 371.9670; calcd for $C_{15}H_{12}{}^{81}BrNO_2SNa$, 373.9646).

6-Bromo-2-hydroxy-1-tosylindolin-3-triethylammonium bromide (9). To a solution of **11** (2.80 g, 8.0 mmol) and H_2O (1.44 mL, 80 mmol) in acetone (80 mL, 0.1 M) was added NBS (1.57 g, 8.8 mmol). The mixture was stirred at rt until the complete disappearance of starting material as indicated by TLC. Then, Et_3N (1.22 mL, 8.8 mmol) was added to the mixture, which was stirred 1 h further. The resulting precipitate was separated by filtration, washed with acetone, and dried *in vacuo* to give **9** (3.53 g, 80% yield) as a colorless solid: IR (KBr): 3123, 1597, 1473, 1362, 1163, 667 cm^{-1}; ^1H NMR (600 MHz, DMSO-d_6) δ: 8.02 (d, *J* = 8.4 Hz, 2H), 7.97 (d, *J* = 4.2 Hz, 1H), 7.50 (d, *J* = 8.4 Hz, 1H), 7.44 (d, *J* = 8.4 Hz, 2H), 7.42 (d, *J* = 1.8 Hz, 1H), 7.35 (dd, *J* = 8.4, 1.8 Hz, 1H), 6.38 (s, 1H), 4.83 (s, 1H), 3.45–3.30 (m, 6H), 2.35 (s, 3H), 1.02 (t, *J* = 7.2 Hz, 9H); ^{13}C NMR (151 MHz, DMSO-d_6) δ: 145.8, 144.1, 135.8, 132.4, 130.7, 128.2, 126.9, 126.0, 120.0, 116.1, 85.3, 74.8, 53.4, 21.6, 8.9; HRESIMS *m/z* 467.1003/469.0983 $[M]^+$ (calcd for $C_{21}H_{28}{}^{79}BrN_2O_3S$, 467.1004; calcd for $C_{21}H_{28}{}^{81}BrN_2O_3S$, 469.0984).

5,5-Bis(6-bromo-1-tosyl-1*H*-indol-3-yl)-1,3-dimethylpyrimidine-2,4,6(1*H*,3*H*,5*H*)-trione (7). To a solution of **9** (1.71 g, 3.13 mmol) and **8** (195 mg, 1.25 mmol) in EtOAc (25 mL, 0.05 M) was added Et_3N (0.35 mL, 2.5 mmol). The mixture was stirred at 80 °C under an argon atmosphere for 3 h. After the reaction mixture was cooled to rt, the reaction mixture was quenched with 1 M

HCl at 0 °C, then brine was added to the mixture and the whole was extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The orange residue was redissolved in EtOAc (25 mL, 0.05 M) and *p*-toluenesulfonic acid monohydrate (PTSA) (238 mg, 1.25 mmol) was added. The mixture was stirred at 80 °C under an argon atmosphere for 4 h. After the reaction mixture was cooled down to rt, brine was added to the mixture and the whole was extracted with EtOAc. The combined organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by recrystallization from EtOAc and silica gel column chromatography (EtOAc /hexane = 1/6–1/3) to give **7** (1.04 g, 98% yield) as a pale yellow solid: IR (KBr): 3152, 1692, 1422, 1377, 1175, 669 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ: 8.13 (d, *J* = 1.2 Hz, 2H), 7.69 (d, *J* = 8.4 Hz, 4H), 7.30 (d, *J* = 8.4 Hz, 4H), 7.21 (s, 2H), 7.04 (dd, *J* = 8.4, 1.8 Hz, 2H), 6.86 (d, *J* = 8.4 Hz, 2H), 3.39 (s, 6H), 2.42 (s, 6H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 166.8, 150.3, 146.1, 136.0, 134.4, 130.4, 127.2, 127.1, 127.0, 127.0, 122.3, 119.3, 117.5, 117.0, 55.8, 29.8, 21.8; HRESIMS *m/z* 872.9666/874.9642/876.9623 $[M+Na]^+$ (calcd for $C_{36}H_{28}{}^{79}Br_2N_4O_7S_2Na$, 872.9664; calcd for $C_{36}H_{28}{}^{79}Br^{81}BrN_4O_7S_2Na$, 874.9643; calcd for $C_{36}H_{28}{}^{81}Br_2N_4O_7S_2Na$, 876.9623).

Methyl 2,2-bis(6-bromo-1*H*-indol-3-yl)acetate (6). A mixture of **7** (853 mg, 1.0 mmol) in 10% NaOH aq. (5.0 mL) and MeOH (10 mL, 0.1 M) was stirred at reflux temperature under an argon atmosphere for 9 h. After the reaction mixture was cooled to rt, the reaction mixture was quenched with 1 M HCl, then brine was added to the mixture and the whole was extracted with EtOAc. The organic layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was redissolved in MeOH (10 mL, 0.1 M) and $SOCl_2$ (0.08 mL, 1.1 mmol) was added in a dropwise manner. The mixture was stirred at 65 °C under an argon atmosphere for 14 h. The reaction mixture was quenched with saturated $NaHCO_3$ aq. to pH 8 and extracted with EtOAc. The combined organic

layer was dried over Na_2SO_4 and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc /hexane = 1/10–1/3) to give **6** (201mg, 44% yield) as a pale yellow solid: IR (KBr): 3420, 1717, 1456, 1169, 667 cm^{-1}; ^1H NMR (600 MHz, $CDCl_3$) δ: 8.07 (s, 2H), 7.50 (d, J = 1.8 Hz, 2H), 7.42 (d, J = 9.0 Hz, 2H), 7.17 (dd, J = 9.0, 2.4 Hz, 2H), 7.08 (d, J = 2.4 Hz, 2H), 5.42 (s, 1H), 3.74 (s, 3H); ^{13}C NMR (151 MHz, $CDCl_3$) δ: 173.4, 137.2, 125.5, 123.9, 123.2, 120.6, 116.0, 114.3, 113.7, 52.5, 40.4; HRESIMS *m/z* 482.9319/484.9299/486.9280 $[M+Na]^+$ (calcd for $C_{19}H_{14}{}^{79}Br_2\ N_2O_2Na$, 482.9320; calcd for $C_{19}H_{14}{}^{79}Br^{81}BrN_2O_2Na$, 484.9299; calcd for $C_{19}H_{14}{}^{81}Br_2N_2O_2Na$, 486.9279).

6-Bromo-3-(1-(6-bromo-1*H*-indol-3-yl)-2-methoxy-2-oxoethyl)-1*H*-indole-1-sulfonic acid (4). To a solution of **6** (46.2 mg) in pyridine (0.2 mL, 0.5 M) was added pyridine•SO_3 (64.4 mg, 0.4 mmol) at rt. The resulting mixture was stirred at 120 °C under an argon atmosphere for 12 h. After the reaction mixture was cooled to rt, the mixture was diluted with H_2O (1 mL) and concentrated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc/acetone = 1/0–5/1) followed by evaporation of the eluent under reduced pressure at 30 °C to give **4** (3.9 mg, 7% yield) as a brown amorphous solid: IR (KBr): 3422, 2957, 2926, 1734, 1717, 1688, 1456, 1175, 667 cm^{-1}; ^1H and ^{13}C NMR data, see Table 1; HRESIMS *m/z* 538.8903/540.8888/542.8868 $[M–H]^-$ (calcd for $C_{19}H_{13}{}^{79}Br_2N_2O_5S$, 538.8912; calcd for $C_{19}H_{13}{}^{79}Br^{81}BrN_2O_5S$, 540.8892; calcd for $C_{19}H_{13}{}^{81}Br_2N_2O_5S$, 542.8871).

ASSOCIATED CONTENT

Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI:

NMR spectra of compounds (PDF).

Notes

The authors declare no competing financial interest.

ACKNOWLEDGMENT

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REFERENCES

(1) For the original isolation and assignment of echinosulfonic acids A–C, see: Ovenden, S. P.; Capon, R. J. Echinosulfonic Acids A–C and Echinosulfone A: Novel Bromoindole Sulfonic Acids and a Sulfone from a Southern Australian Marine Sponge, *Echinodictyum*. *J. Nat. Prod.* **1999**, *62*, 1246–1249.

(2) For the original isolation and assignment of echinosulfonic acid D, see: Rubnov, S.; Chevallir, C.; Thoison, O.; Debitus, C.; Laprevote, O.; Guenard, D.; Sevenet, T. Echinosulfonic acid D: an ESI MS^n exaluation of a new cytotoxic alkaloid from the New-Caledonian sponge *Psammoclemma* sp. *Nat. Prod. Res.* **2005**, *19*, 75–79.

(3) Neupane, P.; Salim, A. A.; Capon, R. J. Structure revision of the rare sponge metabolite echinosulfone A, and biosynthetically related echinosulfonic acids A–D. *Tetrahedron Lett.* **2020**, 151651.

(4) Sala, S.; Nealon, G. L.; Sobolev, A. N.; Fromont, J.; Gomez, O.; Flematti, G. R.; Structure Reassignment of Echinosulfone A and the Echinosulfonic Acids A–D Supported by Single-Crystal X-ray Diffraction and Density Functional Theory Analysis. *J. Nat. Prod*. **2020**, *83*, 105–110.

(5) Holland, D. C.; Kiefel, M. J.; Carroll, A. R. Structure Revisions of the Sponge-Derived Dibrominated Bis-indole Alkaloids, Echinosulfone A and the Echinosulfonic Acids A to D. *J. Org. Chem.* **2020**, *85*, 3490-3496.

(6) (a) Ramana, D. V.;Kumar, K. S.; Srujana, E.; Chandrasekharam, M. Copper-Catalyzed Double Friedel-Crafts Alkylation of Tetrahydroquinolines Under Aqueous Conditions: Efficient Synthesis of gem-Diarylacetic Esters. *Eur. J. Org. Chem.* **2019**, *2019*, 742–745; (b) Sakai, N.; Asano, J.; Shimano, Y.; Konakahara, T. A practical approach to non-natural or *N*-unsubstituted α-arylglycine derivatives: $Hf(OTf)_4$-doped Me_3SiCl system-catalyzed aminomethylation of electro-rich arenes with a new type *N*,*O*-acetal. *Tetrahedron* **2008**, *64*, 9208–9215; (c) Huo, C.; Wang, C.; Sun, C.; Jia, X.; Wang, X.; Chang, W.; Wu, M. Triarylaminium Salt-Initiated Aerobic Double Friedel-Crafts Reaction of Glycine Derivatives with Indoles. *Adv. Synth. Catal.* **2013**, *355*, 1911–1916; (d) Jadhav, S. D.; Bakshi, D.; Singh, A. Visible Light Mediated Organocatalytic Activation of Ethyl Bromofluoroacetate: Coupling with Indoles and Anilines, *J. Org. Chem.* **2015**, *80*, 10187–10196.

(7) For a monoindolization of barbituric acid based on rhodium(II)-catalyzed C–H functionalization, see: Best, D.; Burns, D. J.; Lam, H. W. Direct Synthesis of 5-Aryl Barbituric Acids by Rhodium(II)-Catalyzed Reactions of Arenes with Diazo Compounds. *Angew. Chem. Int. Ed.* **2015**, *54*, 7410–7413.

(8) For a review, see: Bandini, M. Electrophilicity: the "dark-side" of indole chemistry. *Org. Biomol. Chem.* **2013**, *11*, 5206–5212.

(9) For selected examples, see: (a) Abe, T.; Suzuki, T.; Anada, M.; Matsunaga, S.; Yamada, K. 2-Hydroxyindoline-3-triethylammonium Bromide: A Reagent for Formal C3-Electrophilic Reactions of Indoles. *Org. Lett.* **2017**, *19*, 4275–4278; (b) Abe, T.; Noda, K.; Sawada, D. Synthesis of α-substituted indolylacetamide using acetonitriles as acetamide enolate eauivalents through *O*-transfer reactions. *Chem. Commun*. **2021**, *57*, 7493–7496; (c) Abe, T.; Yamashiro, T.; Shimizu,

K.; Sawada, D. Indole Editing Enabled by HFIP-Mediated Ring-Switch Reactions of 3-Amino-2-Hydroxyindolines. *Chem. -Eur. J.* **2022**, *28*, e202201113.

(10) Pedras, M. S. C.; Suchy, M. Detoxification pathways of the phytoalexins brassilexin and sinalexin in Leptosphaeria maculans: isolation and synthesis of the elusive intermediate 3-formylindolyl-2-sulfonic acid. *Org. Biomol. Chem.* **2005**, *3*, 2002–2007.

(11) Hodson, H. F.; Madge, D. J.; Slawin, A. N. Z.; Widdowson, D. A.; Williams, D. J. *Tetrahedron* **1994**, *50*, 1899–1906.