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An Improved Synthesis of (−)-5,11-Dideoxytetrodotoxin

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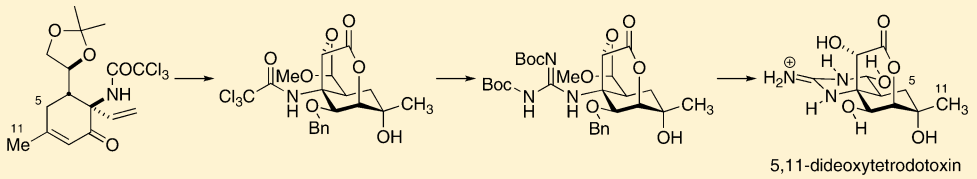
Supporting Information



ABSTRACT: We describe an improved synthesis of (−)-5,11-dideoxytetrodotoxin from an enone, which was used for synthesis of tetrodotoxin and its analogues in this laboratory. One of the major modifications was to establish a two-step guanidinylation of trichloroacetamide of a highly functionalized intermediate, which allowed us to prepare $^{15}N_2$-labeled 5,11-dideoxytetrodotoxin for biosynthetic investigations.

Tetrodotoxin[1,2] (TTX, **1**), a well-known marine natural product, was originally isolated as a toxic principle of puffer fish intoxication. The structure was determined in 1964 by three groups including Hirata-Goto, Tsuda, and Woodward to be shown in Figure 1, which consists of a densely oxygenated

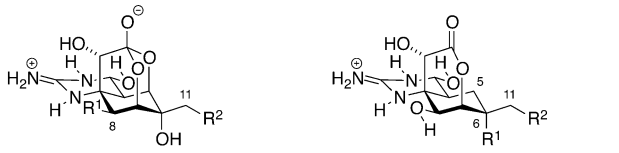


Figure 1. Structures of tetrodotoxin and its analogues.

cyclohexane possessing a cyclic guanidine with a hemiaminal, an orthoester, and eight contiguous stereogenic centers.[3−5] The same year, Mosher reported that tarichatoxin isolated from terrestrial California newts (*Taricha torosa*) was identical to tetrodotoxin.[6,7] Since then, tetrodotoxin and its analogues have been isolated from many small animals such as newt, frog, octopus, and crab.[8,9] This diversity of TTX-bearing animals suggests tetrodotoxin is not synthesized by the host animals but is derived from external sources such as their diets. Since tetrodotoxin-producing bacteria were identified from marine sources in 1986,[10,11] it is believed that tetrodotoxin in TTX-bearing animals such as puffer fish is accumulated from bacteria in the food chain. However, the biosynthetic origin of tetrodotoxin has been completely unclear. We have been interested in naturally occurring analogues of tetrodotoxin from the standpoint of biosynthetic investigations, because those analogues may be biosynthetic precursors or metabolites. One experiment designed to elucidate the tetrodotoxin biosynthesis is tracer analysis using isotope-labeled compounds of possible precursors of tetrodotoxin. We planned to prepare isotope-labeled tetrodotoxin analogues by total synthesis, because the chemical modification of tetrodotoxin is extremely difficult due to its complex structure and unique chemical properties.

The first total synthesis of racemic tetrodotoxin (**1**) was achieved by Kishi and co-workers in 1972.[12,13] We reported the stereocontrolled synthesis of (−)-5,11-dideoxytetrodotoxin (**5**), an unnatural analogue of tetodotoxin in 1999.[14,15] This is the first example of asymmetric synthesis of a tetrodotoxin analogue, which has been a basis for the subsequent synthesis of a variety of its analogues, including tetrodotoxin (**1**), in this laboratory.[16−20] In addition, we have also succeeded in the synthesis of several naturally occurring and unnatural tetrodotoxin analogues, such as 11-deoxytetrodotoxin (**2**)[21] and 8,11-dideoxytetrodotoxin (**3**)[22,23] from a common synthetic intermediate.[24] Quite recently, Yotsu-Yamashita identified 5,11-dideoxytetrodotoxin (**5**) in nature,[25] indicating that **5** might be a biosynthetic precursor of tetrodotoxin. We therefore planned to prepare stable isotope-$^{15}N_2$-labeled 5,11-dideoxytetrodotoxin (at the guanidine group) for tracer analysis. In our previous synthesis of 5,11-dideoxytetrodotoxin (**5**), however, installation of the guanidine group required a considerable number of steps[26] because deprotection of *N*-trichloroacetyl group was difficult. Since the route is not suitable for the desired $^{15}N_2$-labeled 5,11-dideoxytetrodotoxin for this purpose, we reexamined the synthesis of **5** with emphasis on improvement of the guanidinylation (vide infra).

On the basis of our previous synthesis of 5,11-dideoxytetrodotoxin (**5**), we planned to modify the synthetic route as shown in Scheme 1. An enone **7**, the same synthetic intermediate of tetrodotoxin and its analogues as used in this laboratory, would be transformed into a compound by cleavage of the 1,2-diol protected as an acetonide of **7**. Elaboration of the enone **A** followed by ozonolysis of the vinyl group would provide an aldehyde **B**. Stereocontrolled addition of acetylide

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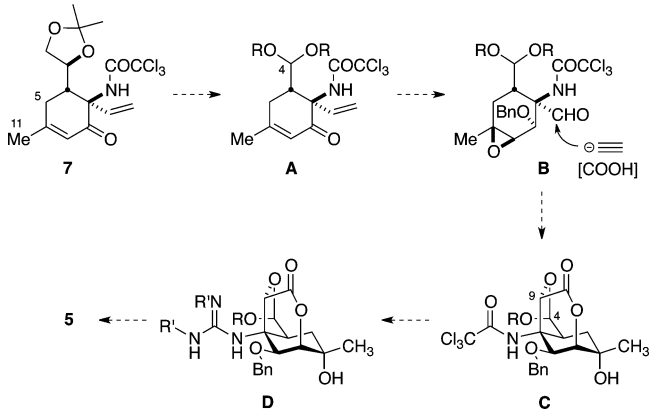
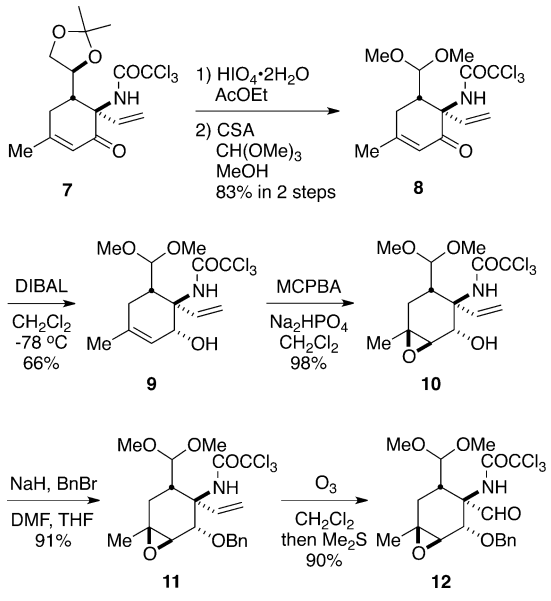
Scheme 1. Improved Synthetic Plan for (−)-5,11-Dideoxytetrodotoxin (5)



and then oxidative cleavage of the acetylenic moiety would afford lactone intermediate **C**. Guanidinylation of the trichloroacetamide, followed by global deprotection under an acidic condition, should provide 5,11-dideoxytetrodotoxin (**5**). This new route includes two major modifications from the previous one; (i) cleavage of 1,2-diol protected as acetonide to acetal at an early stage (**7** to **A**), (ii) two-step guanidinylation from trichloroacetamide (**C** to **D**). The aim of the first modification (i) is to set up the oxidation state of aldehyde at the C-4 position before hydroxylation of the cyclohexane moiety, which includes potentially cleavable 1,2-diol structure with periodic acid oxidation. With regard to the second modification (ii), we planned to utilize Cs_2CO_3-promoted deprotection of *N*-trichloroacetyl group reported by this laboratory in 2004,[27] because the condition was compatible with ester and acetal functional groups in some model compounds. When amine could be obtained under the condition, the resulting amine would be directly condensed with a derivative of methylisothiourea to give guanidine-containing intermediate **D**.

The synthesis commenced with cleavage of acetonide of enone **7** with periodic acid in AcOEt (Scheme 2). The resulting

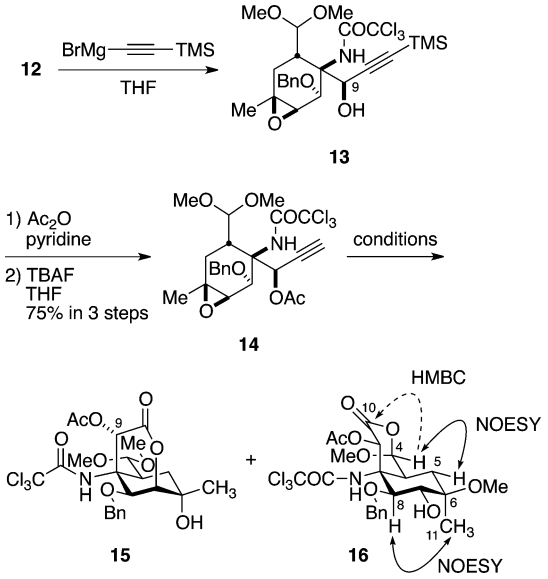
Scheme 2. Synthesis of Aldehyde 12 from Enone 7



aldehyde was protected as dimethyl acetal **8**. When the dimethyl acetal **8** was reduced under the Luche reduction used in our previous synthesis of **5**,[14,15] allylic alcohol **9** was obtained in a low yield and a considerable amount of starting material **8** was recovered, even when an excess amount of the reagents was employed. Extensive examination revealed that DIBAL in CH_2Cl_2 at −78 °C was the optimal condition for the stereoselective reduction, giving the allylic alcohol **9** in a moderate yield as a single product (dr >20:1). According to the previous synthesis of **5**, **9** was then transformed into an aldehyde **12**; epoxidation of **9** with MCPBA was followed by protection of the secondary alcohol with benzyl ether to give **11**. Subsequent ozonolysis of the vinyl group gave **12** (as intermediate **B** in Scheme 1).

With multigram quantities of **12** in hand, transformation into lactone **15** by oxidative cleavage of the acetylenic moiety was investigated (Scheme 3). Addition of magnesium trimethylsi-

Scheme 3. Synthesis of Lactone 15 by Oxidative Cleavage of the Acetylene and Lactonization



lylacetylide in THF to **12** afforded the desired **13** as a single diastereomer.[28,29] The resulting adduct was unstable, and thus was directly conducted to acetylation and then desilylation, giving propargyl acetate **14**. When **14** was treated with RuO_4 under Sharpless conditions,[30] which was employed in our first synthesis of 5,11-dideoxytetrodotoxin (**5**),[14,15] the product was not the expected **15**, but the different lactone **16**. The structure of **16** was determined by extensive NMR analysis; location of the lactone was undoubtedly determined by HMBC correlation between H-4 and C-10. The newly generated stereogenic centers of C-4 and C-6 positions were determined by the NOESY correlations. The reaction mechanism of the production of **16** is proposed in Scheme 4. The acetylenic moiety of **14** is first cleaved with RuO_4 to give carboxylic acid **17**. Under the acidic conditions, however, epoxide of the intermediate **17** opens to generate a carbocation intermediate **18**, which might be trapped by dimethyl acetal. Migration of methoxy group of the resulting **19** affords an oxocarbenium intermediate **20**, which is captured by the carboxylic acid group to give the observed lactone **16**. To avoid production of **16**, alternative reagents with mild basic conditions for cleaving the acetylenic moiety were explored. Finally, $KMnO_4$ and $NaIO_4$ in

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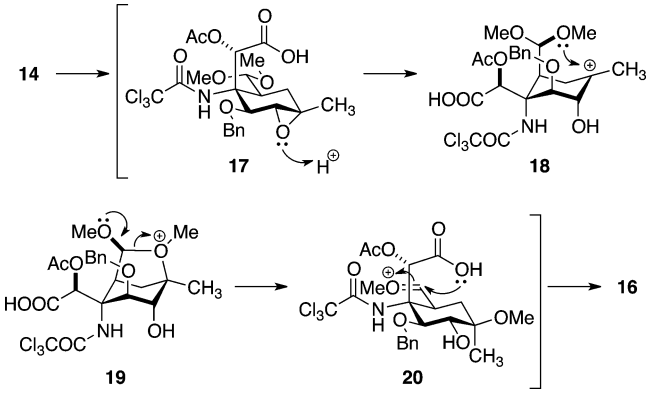
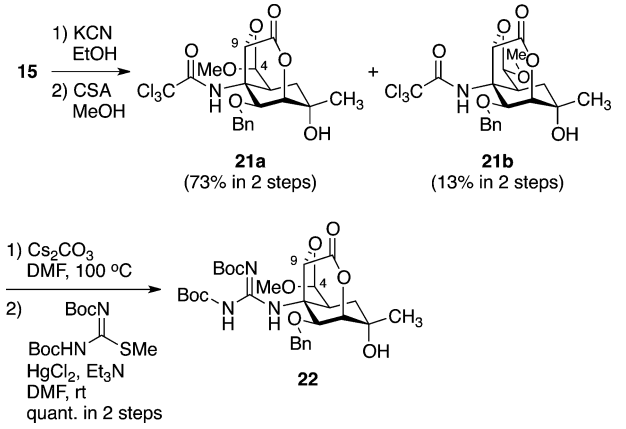
Scheme 4. Proposed Mechanism for the Formation of Lactone 16



aqueous *tert*-butanol with an excess amount of $NaHCO_3$ as a base was found to be the best condition, giving the desired lactone **15** in 88% yield without formation of **16**.[31]

Prior to guanidinylation, the hydroxy group at the C-9 position was protected as an intramolecular acetal with the aldehyde at the C-4 position in two steps (Scheme 5).

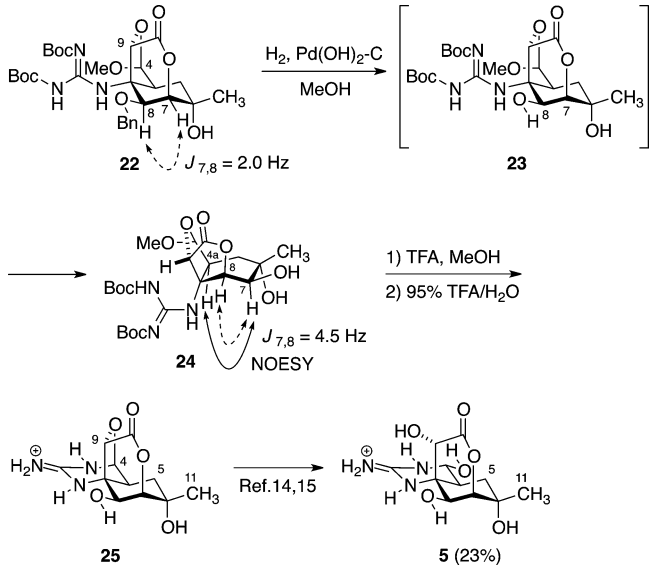
Scheme 5. Synthesis of di-Boc Guanidine 22 by a Two-step Guanidinylation



Deacetylation with KCN in EtOH followed by treatment with CSA in MeOH gave the acetal **21a** and its epimer **21b** in 73 and 13% yields, respectively.[32] Guanidinylation of the resulting acetal **21a** was next attempted. As we anticipated, *N*-trichloroacetyl group of **21a** was deprotected with Cs_2CO_3 in DMF at 100 °C to provide an amine, which was directly subjected to the guanidinylation with *N*,*N*′-bis-Boc-*S*-methylisothiourea in the presence of $HgCl_2$ to give the desired di-Boc guanidine **22** in a good overall yield. This is the first application of the deprotection condition for the highly functionalized synthetic intermediate in the synthesis of tetrodotoxin and its analogues. The successful deprotection of trichloroacetamide of **21a** demonstrates the usefulness of the deprotection condition in the synthesis of complex natural products.

To complete the synthesis of 5,11-dideoxytetrodotoxin (**5**), deprotection of the benzyl group of **22** was first attempted (Scheme 6). However, hydrogenolysis of **22** under conventional conditions with Pearlman's catalyst ($Pd(OH)_2$-C) gave an unexpected γ-lactone **24**, instead of the desired alcohol **23**. The γ-lactone structure of **24** was determined by extensive NMR analysis; the NOESY correlation observed between H-4a

Scheme 6. Synthesis of 5,11-Dideoxytetrodotoxin (5)



and H-7 indicates the boat conformation adopted in **24**, which was also supported by the observed coupling constant ($J_{7,8}$ = 4.5 Hz), whereas similar δ-lactone compounds such as **22** showed smaller coupling constant ($J_{7,8}$ = 2.0 Hz). The unexpected product **24** might be formed by intramolecular transesterification of the desired **23**. Unfortunately, retransesterification from **24** back to **23** proved to be difficult under several basic conditions. However, when Boc groups of **24** were deprotected with TFA in MeOH, and the crude products were then treated with 95% aqueous TFA, 4,9-anhydro-5,11-dideoxytetrodotoxin (**25**) was obtained.[14,15] Finally, according to the previous synthesis, **25** was hydrolyzed with 2% TFA-*d* in D_2O to afford a mixture of **25** and 5,11-dideoxytetrodotoxin (**5**), which were separated by an ion-exchange resin column (Hitachi-gel #3013-c). The ^1H and ^{13}C NMR spectra of the synthesized 4,9-anhydro-5,11-dideoxytetrodotoxin (**25**) and 5,11-dideoxytetrodotoxin (**5**) were identical to those reported by this laboratory.[14,15] It is worth noting that the successful conversion of **24** to **25** indicates the γ-lactone-type intermediate is also a possible precursor of tetrodotoxin through an intramolecular transesterification.

By utilizing the above-mentioned route, $^{15}N_2$-labeled 4,9-anhydro-5,11-dideoxytetrodotoxin (**25A**) was also synthesized from acetal **21a** as shown in Scheme 7; deprotection of the trichloroacetamide of **21a** with Cs_2CO_3 in DMF and subsequent guanidinylation with ^{15}N,$^{15}N'$-bis-Boc-*S*-methylisothiourea under the same conditions gave $^{15}N_2$-labeled di-Boc guanidine **22A**. The successive removal of protective groups was achieved by the same procedure to afford $^{15}N_2$-labeled 4,9-anhydro-5,11-dideoxytetrodotoxin (**25A**) in 50% yield.

In conclusion, we have developed an improved asymmetric synthesis of (−)-5,11-dideoxytetrodotoxin (**5**) by utilizing the two-step guanidinylation from trichloroacetamide. This new route enables us to prepare $^{15}N_2$-labeled 5,11-dideoxytetrodotoxin for biosynthetic studies of tetrodotoxin. Continuing efforts toward biosynthetic investigations of tetrodotoxin by using the synthesized $^{15}N_2$-labeled compound are in progress.

EXPERIMENTAL SECTION

2,2,2-Trichloro-*N*-((1*R*)-6-(dimethoxymethyl)-4-methyl-2-oxo-1-vinylcyclohex-3-en-1-yl)acetamide (8). To a solution of

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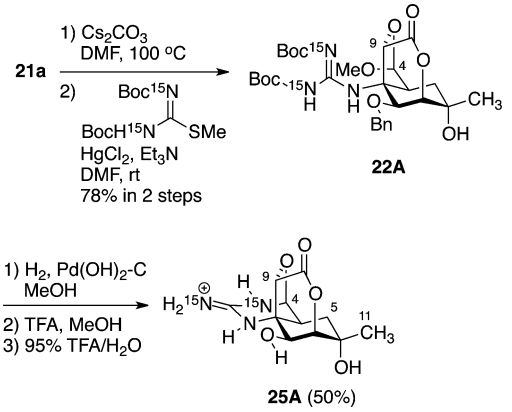
Scheme 7. Synthesis of $^{15}N_2$-labeled 4,9-Anhydro-5,11-dideoxytetrodotoxin (25A)



enone **7** (2.68 g, 6.78 mmol) in AcOEt (167 mL) was added HIO_4•$2H_2O$ (2.01 g, 8.82 mmol) at rt. After stirring for 4 h, the reaction mixture was quenched with an ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. To a solution of the residue in MeOH (100 mL) were added CSA (788 mg, 3.39 mmol) and $CH(OMe)_3$ (50 mL) at rt. After stirring for 1 h, the reaction mixture was quenched with an ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:2) to give **8** (2.07 g, 83% in 2 steps) as a colorless oil. $[\alpha]_D^{29}$ +27.0 (*c* 1.05, $CHCl_3$); IR (film) ν_{max} 2938, 1717, 1682, 1498, 1123, 1053, 852 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.98 (3H, s, CH_3 −C═CH), 2.45 (1H, dd, *J* = 20, 6.5 Hz, C−CH_AH_B-CH), 2.53 (1H, dd, *J* = 20, 11.5 Hz, C−CH_AH_B−CH), 3.18 (1H, ddd, *J* = 11.5, 6.5, 4.5 Hz, C−CH_AH_B-CH), 3.34 (3H, s, CH-$(OCH_3)_2$), 3.40 (3H, s, CH−$(OCH_3)_2$), 4.39 (1H, d, *J* = 4.5 Hz, CH−$(OCH_3)_2$), 5.27 (1H, d, *J* = 17.5 Hz, CH═CH_AH_B), 5.36 (1H, d, *J* = 11 Hz, CH═CH_AH_B), 5.97 (1H, s, CH_3−C═CH), 6.30 (1H, dd, *J* = 17.5, 11 Hz, CH═CH_AH_B), 7.23 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 24.2, 29.3, 41.9, 54.7, 55.4, 65.3, 92.7, 104.8, 118.8, 124.5, 131.4, 160.6, 161.4, 191.5; Anal. Calcd for $C_{14}H_{18}Cl_3NO_4$: C, 45.37; H, 4.89; N, 3.78. Found: C, 45.38; H, 4.88; N, 3.66.

2,2,2-Trichloro-*N*-((1*R*,2*R*)-6-(dimethoxymethyl)-2-hydroxy-4-methyl-1-vinylcyclohex-3-en-1-yl)acetamide (9). To a solution of dimethyl acetal **8** (1.11 g, 2.78 mmol) in dry CH_2Cl_2 (25 mL) was added DIBAL (1.01 M in toluene; 6.6 mL, 6.68 mmol) at −78 °C. After stirring for 30 min, the reaction mixture was quenched with acetone. The mixture was allowed to warm to rt, and a mixture of saturated aqueous potassium sodium tartrate and water was added. The mixture was vigorously stirred for 12 h, and the aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:4) to give **9** (738 mg, 66%) as a colorless oil. $[\alpha]_D^{30}$ +32 (*c* 0.97, $CHCl_3$); IR (film) ν_{max} 3853, 3307, 1397, 1085, 750 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.73 (3H, s, CH_3−C═CH), 1.77−1.90 (1H, m, C−CH_AH_B−CH), 2.05 (1H, dd, *J* = 18, 5 Hz, C−CH_AH_B−CH), 2.25 (1H, ddd, *J* = 12, 9, 5 Hz, C−CH_AH_B−CH), 3.32 (3H, s, CH-$(OCH_3)_2$), 3.45 (3H, s, CH−$(OCH_3)_2$), 4.40 (1H, d, *J* = 9 Hz, CH−$(OCH_3)_2$), 4.43 (1H, br s, CH−OH), 5.23 (1H, d, *J* = 18 Hz, CH═CH_AH_B), 5.34 (1H, br s, CH_3−C═CH), 5.40 (1H, d, *J* = 11 Hz, CH═CH_AH_B), 5.84 (1H, dd, *J* = 18, 11 Hz, CH═CH_AH_B), 5.89 (1H, br s, CH−OH), 9.84 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.4, 29.9, 42.0, 51.7, 55.0, 65.8, 73.7, 93.5, 104.7, 116.9, 123.3, 131.4, 133.0, 163.0; Anal. Calcd for $C_{14}H_{20}Cl_3NO_4$: C, 45.12; H, 5.41; N, 3.76. Found: C, 45.12; H, 5.42; N, 3.73.

2,2,2-Trichloro-*N*-((1*R*,2*S*,3*R*,6*S*)-4-(dimethoxymethyl)-2-hydroxy-6-methyl-3-vinyl-7-oxabicyclo[4.1.0]heptan-3-yl)-acetamide (10). To a solution of allylic alcohol **9** (555 mg, 1.49 mmol) in dry CH_2Cl_2 (21 mL) were added Na_2HPO_4 (1.02 g, 7.17 mmol) and MCPBA (412 mg, 2.39 mmol) at rt. After stirring for 30 h, the reaction mixture was quenched with an ice-cooled saturated aqueous Na_2SO_3. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was washed with saturated aqueous Na_2SO_3, saturated aqueous $NaHCO_3$ and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:5) to give **10** (614 mg, 98%) as a white solid. mp 81−83 °C; $[\alpha]_D^{30}$ +2.2 (*c* 0.88, $CHCl_3$); IR (film) ν_{max} 3651, 3318, 3137, 1703, 1535, 1274, 1121, 1053, 822 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.40 (3H, s, CH_3−C), 1.59 (1H, dd, *J* = 15, 13 Hz, C−CH_AH_B−CH), 2.08 (1H, dd, *J* = 15, 4.5 Hz, C−CH_AH_B−CH), 2.19 (1H, ddd, *J* = 13, 8.5, 4.5 Hz, C−CH_AH_B−CH), 3.08 (1H, br s, epoxidic), 3.27 (3H, s, CH−$(OCH_3)_2$), 3.41 (3H, s, CH-$(OCH_3)_2$), 4.04 (1H, br s, CH−OH), 4.38 (1H, d, *J* = 8.5 Hz, CH−$(OCH_3)_2$), 5.18 (1H, d, *J* = 17.5 Hz, CH═CH_AH_B), 5.43 (1H, d, *J* = 11 Hz, CH═CH_AH_B), 5.80 (1H, dd, *J* = 17.5, 11 Hz, CH═CH_AH_B), 5.81 (1H, br s, CH−OH), 9.85 (1H, br s, NH); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.9, 29.0, 36.7, 50.6, 54.8, 57.6, 61.9, 65.0, 73.3, 93.2, 103.9, 117.2, 130.8, 163.2; Anal. Calcd for $C_{14}H_{20}Cl_3NO_5$: C, 43.26; H, 5.19; N, 3.60. Found: C, 43.46; H, 5.26; N, 3.51.

***N*-((1*R*,2*S*,3*R*,6*S*)-2-(Benzyloxy)-4-(dimethoxymethyl)-6-methyl-3-vinyl-7-oxabicyclo[4.1.0]heptan-3-yl)-2,2,2-trichloroacetamide (11).** NaH (60% purity, 235 mg, 5.88 mmol) was washed with anhydrous hexane, and dry THF (10 mL) and DMF (5.0 mL) were added. To this suspension was added a solution of epoxyalcohol **10** (614 mg, 1.47 mmol) in dry THF (5.0 mL) through a cannula at rt. After stirring for 30 min, BnBr (0.4 mL, 2.94 mmol) was added to the solution. After stirring for 10 h, the reaction mixture was poured into an ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with water and brine, dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:8) to give **11** (639 mg, 91%) as a colorless oil. $[\alpha]_D^{30}$ −33.4 (*c* 1.55, $CHCl_3$); IR (film) ν_{max} 3679, 1720, 1504, 1068, 820 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, s, CH_3−C), 1.80 (1H, dd, *J* = 15, 13 Hz, C−CH_AH_B−CH), 2.17 (1H, dd, *J* = 15, 4.5 Hz, C−CH_AH_B−CH), 2.90 (1H, br dt, *J* = 13, 4 Hz, C−CH_AH_B−CH), 3.03 (1H, s, epoxidic), 3.30 (3H, s, CH−$(OCH_3)_2$), 3.32 (3H, s, CH−$(OCH_3)_2$), 4.22 (1H, d, *J* = 4 Hz, CH−$(OCH_3)_2$), 4.43 (1H, s, CH-OBn), 4.63 (1H, d, *J* = 11.5 Hz, benzylic), 4.74 (1H, d, *J* = 11.5 Hz, benzylic), 5.35 (1H, d, *J* = 17.5 Hz, CH═CH_AH_B), 5.45 (1H, d, *J* = 11.5 Hz, CH═CH_AH_B), 6.03 (1H, dd, *J* = 17.5, 11.5 Hz, CH═CH_AH_B), 6.96 (1H, br s, NH), 7.18−7.38 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.5, 27.4, 35.7, 54.3, 55.6, 58.5, 61.6, 63.1, 73.2, 76.7, 93.1, 104.5, 117.2, 127.8 × 2, 128.4, 134.3, 137.6, 160.2; Anal. Calcd for $C_{21}H_{26}Cl_3NO_5$: C, 52.68; H, 5.47; N, 2.93. Found: C, 52.68; H, 5.60; N, 2.80.

***N*-((1*R*,2*S*,3*R*,6*R*)-2-(Benzyloxy)-4-(dimethoxymethyl)-3-formyl-6-methyl-7-oxabicyclo[4.1.0]heptan-3-yl)-2,2,2-trichloroacetamide (12).** Benzyl ether **11** (498 mg, 1.04 mmol) was dissolved in CH_2Cl_2 (21 mL). Ozone gas was passed into the solution at −78 °C for 1.5 h. The remaining ozone gas was purged with nitrogen, and Me_2S (0.76 mL, 10.4 mmol) was added. The reaction mixture was allowed to warm to rt and quenched with an ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:4) to give **12** (451 mg, 90%) as a colorless oil. $[\alpha]_D^{32}$ −67.4 (*c* 1.08, $CHCl_3$); IR (film) ν_{max} 3710, 3141, 1714, 1594, 1498, 1066 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.53 (3H, s, CH_3−C), 2.31 (1H, dd, *J* = 16, 13 Hz, C−CH_AH_B−CH), 2.46 (1H, dd, *J* = 16, 5 Hz, C−CH_AH_B−CH), 3.22 (1H, s, epoxidic), 3.30−3.37 (1H, m, C−CH_AH_B−CH), 3.34 (3H, s, CH−$(OCH_3)_2$), 3.40 (3H, s, CH-$(OCH_3)_2$), 4.17 (1H, br d, *J* = 2 Hz, CH−$(OCH_3)_2$), 4.59 (1H, s, CH−OBn), 4.64 (1H, d, *J* = 11.5 Hz, benzylic), 4.73 (1H, d, *J* = 11.5 Hz, benzylic), 7.20−7.45 (5H, m, aromatic), 8.23 (1H, br s,

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NH), 9.69 (1H, s, CHO); ^{13}C NMR (100 MHz, $CDCl_3$) δ 22.2, 25.2, 36.4, 56.7, 56.9, 58.8, 60.1, 67.0, 73.7, 74.9, 92.6, 104.4, 127.8, 128.2, 128.6, 136.6, 160.1, 194.2; Anal. Calcd for $C_{20}H_{24}Cl_3NO_6$: C, 49.96; H, 5.03; N, 2.91. Found: C, 49.96; H, 5.12; N, 2.81.

(1*R*)-1-((1*R*,2*S*,3*S*,6*S*)-2-(Benzyloxy)-4-(dimethoxymethyl)-6-methyl-3-(2,2,2-trichloroacetamido)-7-oxabicyclo[4.1.0]-heptan-3-yl)prop-2-yn-1-yl acetate (14). To an ice-cooled solution of trimethylsilylacetylene (2.3 mL, 16.5 mmol) in dry THF (53 mL) was added EtMgBr (0.98 M in THF; 14 mL, 13.7 mmol) over 15 min. The solution was allowed to warm to rt and stirred for 15 min, and cooled to −20 °C. To the solution was added a solution of aldehyde **12** (660 mg, 1.37 mmol) in dry THF (5.0 mL) through a cannula. After stirring for 30 min, the reaction mixture was allowed to warm to rt and stirred for 30 min. The reaction mixture was quenched with an ice-cooled hydrochloric acid (0.12 N). The aqueous layer was extracted with AcOEt. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. To a solution of the residue in pyridine (16.7 mL) was added Ac_2O (16.7 mL) at rt. After stirring for 12 h, the reaction mixture was diluted with toluene and concentrated. To a solution of the residue in THF (25.6 mL) was added TBAF (1.0 M in THF; 1.4 mL, 1.37 mmol) at rt. After stirring for 15 min, the reaction mixture was poured into an ice-cooled mixture of saturated aqueous NH_4Cl and H_2O. The aqueous layer was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:4) to give **14** (568 mg, 75% in 3 steps) as a colorless oil. $[\alpha]_D^{29}$ −48.7 (*c* 1.11, $CHCl_3$); IR (film) ν_{max} 3545, 2929, 1721, 1523, 1372, 1223, 1053, 820 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.36 (3H, s, CH_3−C), 1.98 (1H, dd, *J* = 16, 13 Hz, C−CH_AH$_B$−CH), 2.07 (3H, s, CH_3), 2.21 (1H, dd, *J* = 16, 5 Hz, C−CH$_A$H$_B$−CH), 2.73 (1H, d, *J* = 2 Hz, CH−C≡C*H*), 3.02 (1H, dt, *J* = 13, 5 Hz, C−CH$_A$H$_B$−C*H*), 3.08 (1H, s, epoxidic), 3.30 (3H, s, CH−(OCH_3)$_2$), 3.31 (3H, s, CH−(OCH_3)$_2$), 4.53 (1H, d, *J* = 5 Hz, C*H*−(OCH$_3$)$_2$), 4.56 (1H, s, C*H*−OBn), 4.68 (1H, d, *J* = 11.5 Hz, benzylic), 4.74 (1H, d, *J* = 11.5 Hz, benzylic), 6.34 (1H, d, *J* = 2 Hz, C*H*−C≡CH), 7.25−7.40 (5H, m, aromatic), 7.74 (1H, br s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 21.1, 22.2, 27.5, 35.2, 52.9, 55.5, 58.8, 62.3, 62.9, 64.1, 73.7, 76.8, 77.7, 78.7, 93.2, 103.9, 127.8, 128.4 × 2, 137.5, 160.2, 169.1; Anal. Calcd for $C_{24}H_{28}Cl_3NO_7$: C, 52.52; H, 5.14; N, 2.55. Found: C, 52.54; H,5.17; N, 2.35.

(1*S*,4*S*,5*R*,6*S*,8*S*,9*S*)-9-(Benzyloxy)-6-(dimethoxymethyl)-8-hydroxy-8-methyl-3-oxo-5-(2,2,2-trichloroacetamido)-2-oxabicyclo[3.3.1]nonan-4-yl acetate (15). To a solution of acetate **14** (301 mg, 0.55 mmol) in *t*-BuOH (16.7 mL) and H_2O (8.0 mL) were added $NaHCO_3$ (692 mg, 8.24 mmol), $NaIO_4$ (1.41 g, 6.59 mmol) and $KMnO_4$ (86.7 mg, 0.55 mmol) at rt. After stirring for 30 min, $NaHCO_3$ (692 mg, 8.24 mmol) was added, and the mixture was quenched with a saturated aqueous Na_2SO_3. The mixture was stirred for 2 h, and the aqueous layer was extracted with CH_2Cl_2. To the aqueous layer was added a saturated aqueous potassium sodium tartrate at rt. After stirring for 12 h, the mixture was extracted with CH_2Cl_2. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 3:1) to give **15** (276 mg, 88%) as a white solid. mp 123−124 °C; $[\alpha]_D^{28}$ −35 (*c* 0.94, $CHCl_3$); IR (film) ν_{max} 3481, 2934, 1744, 1718, 1535, 1374, 1219, 1177, 1080, 821 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.38 (3H, s, HO−C−CH_3), 1.47 (1H, dd, *J* = 16, 14 Hz, CH−CH_AH$_B$−C), 1.89 (1H, br dd, *J* = 16, 4 Hz, CH−CH$_A$H$_B$−C), 2.29 (3H, s, CH_3), 3.24 (3H, s, CH−(OCH_3)$_2$), 3.26 (3H, s, CH−(OCH_3)$_2$), 3.47 (1H, ddd, *J* = 14, 8, 4.5 Hz, C*H*−CH$_A$H$_B$−C), 4.24 (1H, br s, COO−C*H*), 4.64 (1H, d, *J* = 8 Hz, C*H*−(OCH$_3$)$_2$), 4.64 (1H, d, *J* = 11 Hz, benzylic), 4.73 (1H, d, *J* = 11 Hz, benzylic), 5.36 (1H, d, *J* = 2 Hz, BnO−C*H*), 5.93 (1H, s, C*H*-COO), 7.28−7.37 (5H, m, aromatic), 7.66 (1H, br s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 20.8, 27.0, 33.6, 36.2, 50.2, 54.1, 63.0, 70.1, 71.0, 72.4, 73.2, 82.5, 92.6, 103.9, 128.0, 128.1, 128.5, 137.1, 160.6, 165.8, 171.3; Anal. Calcd for $C_{23}H_{28}Cl_3NO_9$: C, 48.56; H, 4.96; N, 2.46. Found: C, 48.55; H, 4.96; N, 2.26.

Spectroscopic data of lactone **16** were obtained from the purified compound in the separate experiment. **(1*S*,4*S*,4a*R*,5*S*,6*R*,7*R*,8a*S*)-5-(Benzyloxy)-6-hydroxy-1,7-dimethoxy-7-methyl-3-oxo-4a-(2,2,2-trichloroacetamido)octahydro-1*H*-isochromen-4-yl acetate (16)**: $[\alpha]_D^{29}$ +1.4 (*c* 0.64, $CHCl_3$); IR (film) ν_{max} 3650, 3134, 1760, 1716, 1558, 1214, 1081, 822 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.42 (3H, s, CH_3−C−OCH$_3$), 1.64 (3H, s, CH_3), 1.75 (1H, t, *J* = 14 Hz, CH−CH_AH$_B$−C), 1.98 (1H, dd, *J* = 14, 4 Hz, CH−CH$_A$H$_B$−C), 3.31 (3H, s, COO−CH−OCH_3), 3.48 (3H, s, CH$_3$−C−OCH_3), 3.74 (1H, br dd, *J* = 14, 4 Hz, C*H*−CH$_A$H$_B$−C), 3.94 (1H, d, *J* = 10 Hz, BnO−CH−C*H*−OH), 4.26 (1H, d, *J* = 10 Hz, BnO−C*H*−CH−OH), 4.48 (1H, d, *J* = 10 Hz, benzylic), 4.83 (1H, d, *J* = 10 Hz, benzylic), 5.06 (1H, d, *J* = 1 Hz, COO−C*H*−OCH$_3$), 6.08 (1H, s, C*H*−COO), 7.25−7.36 (5H, m, aromatic), 7.61 (1H, br s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 16.1, 19.8, 33.8, 34.4, 49.7, 57.3, 61.9, 64.7, 76.1, 76.3, 77.1, 79.8, 92.8, 106.8, 128.1, 128.2, 128.5, 137.7, 160.9, 165.8, 168.2; HRMS (ESI) for $C_{23}H_{28}Cl_3NO_9Na$ [M + Na]$^+$, calcd 590.0727, found: 590.0744.

***N*-((3*S*,3a*R*,4*S*,5*S*,6*S*,7a*S*)-4-(Benzyloxy)-6-hydroxy-1-methoxy-6-methyl-9-oxooctahydro-5,3-(epoxymethano)isobenzofuran-3a-yl)-2,2,2-trichloroacetamide (21).** To a solution of lactone **15** (276 mg, 0.49 mmol) in EtOH (9.5 mL) was added KCN (31.6 mg, 0.49 mmol) at rt. After stirring for 1 h, the reaction mixture was diluted with a saturated aqueous $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. To a solution of the residue in MeOH (9.5 mL) was added CSA (31.6 mg, 0.49 mmol) at rt. After stirring for 12 h, the reaction mixture was quenched with an ice-cooled saturated aqueous $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was dried over anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 1:2→1:1) to give **21a** (175 mg, 73% in 2 steps) as a colorless oil and **21b** (31.0 mg, 13% in 2 steps) as a colorless oil. **21a**: $[\alpha]_D^{30}$ −1.4 (*c* 0.97, $CHCl_3$); IR (film) ν_{max} 3490, 2932, 1747, 1714, 1525, 1189, 1025, 822 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.32 (1H, dd, *J* = 15.5, 11 Hz, CH−CH_AH$_B$−C), 1.38 (3H, s, HO−C−CH_3), 2.02 (1H, br dd, *J* = 15.5, 8 Hz, CH−CH$_A$H$_B$−C), 3.20 (1H, dd, *J* = 11, 8 Hz, C*H*−CH$_A$H$_B$−C), 3.34 (3H, s, O−CH−OCH_3), 4.43 (1H, br s, COO−C*H*), 4.57 (1H, d, *J* = 11 Hz, benzylic), 4.62 (1H, s, C*H*−COO), 4.66 (1H, d, *J* = 11 Hz, benzylic), 4.83 (1H, s, O−C*H*−OCH$_3$), 5.34 (1H, d, *J* = 2.5 Hz, BnO−C*H*), 7.03 (1H, br s, N*H*), 7.23−7.33 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 27.5, 33.6, 44.3, 55.8, 64.5, 69.3, 71.7, 72.3, 80.6 × 2, 92.6, 111.4, 127.9, 128.2, 128.5, 137.0, 160.6, 167.5; Anal. Calcd for $C_{20}H_{22}Cl_3NO_7$: C, 48.55; H, 4.48; N, 2.83. Found: C, 48.54; H, 4.66; N, 2.76. **21b**: $[\alpha]_D^{28}$ −29 (*c* 0.20, $CHCl_3$); IR (film) ν_{max} 3330, 2926, 1716, 1519, 1456, 1262, 1215, 1178, 1074, 1019, 822 cm^{-1}; ^{1}H NMR (400 MHz, $CDCl_3$) δ 1.43 (3H, s, HO−C−CH_3), 1.75−1.83 (2H, m, CH−CH_2−C), 3.38 (1H, ddd, *J* = 10, 9, 4 Hz, C*H*−CH$_2$−C), 3.50 (3H, s, O−CH−OCH_3), 4.36 (1H, s, C*H*−COO), 4.42 (1H, br d, *J* = 2.5 Hz, COO−C*H*), 4.55 (1H, d, *J* = 11.5 Hz, benzylic), 4.68 (1H, d, *J* = 11.5 Hz, benzylic), 5.12 (1H, d, *J* = 2.5 Hz, BnO−C*H*), 5.26 (1H, d, *J* = 4 Hz, O−C*H*−OCH$_3$), 6.72 (1H, br s, N*H*), 7.26−7.37 (5H, m, aromatic); ^{13}C NMR (100 MHz, $CDCl_3$) δ 28.0, 29.3, 40.6, 57.9, 65.4, 69.6, 71.6, 72.1, 77.6, 80.3, 108.6, 128.0, 128.3, 128.6, 136.7, 161.2, 167.7 (one peak was missing); HRMS (ESI) for $C_{20}H_{22}Cl_3NO_7Na$ [M + Na]$^+$, calcd 516.0360, found: 516.0354.

Carbamic Acid, *N*,*N*′-((3*S*,3a*R*,4*S*,5*S*,6*S*,7a*S*)-4-(Benzyloxy)-6-hydroxy-1-methoxy-6-methyl-9-oxooctahydro-5,3-(epoxymethano)isobenzofuran-3a-yl)bis-, C,C′-Bis(1,1-dimethylethyl) Ester (22). A mixture of acetal **21a** (61.6 mg, 0.125 mmol) and Cs_2CO_3 (81.0 mg, 0.249 mmol) in dry DMF (15.6 mL) was stirred at 100 °C for 2 h. After cooling to rt, the reaction mixture was diluted with a saturated aqueous $NaHCO_3$. The aqueous layer was extracted with AcOEt. The combined organic layer was washed with brine, dried over anhydrous Na_2SO_4, and concentrated. To a solution of the residue in DMF (15.6 mL) were added Et_3N (0.11 mL) followed by *N*,*N*′-bis-Boc-*S*-methylisothiourea (89.2 mg, 0.249 mmol) and $HgCl_2$ (67.6 mg, 0.249 mmol) at rt. After stirring for 1 h, the reaction mixuture was diluted with AcOEt and filtered through a pad of Super-Cel. The filtrate was washed with H_2O and brine, dried over

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anhydrous Na_2SO_4, and concentrated. The residue was purified by flash column chromatography (ether/hexane 3:1) to give **22** (84.2 mg, quant. in 2 steps) as a colorless oil. $[\alpha]_D^{30}$ −6.7 (*c* 0.99, $CHCl_3$); IR (film) ν_{max} 3282, 2979, 1791, 1720, 1644, 1528, 1482, 1369, 1253, 1139, 1059, 1027 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, s, HO−C−CH_3), 1.43 (9H, s, $(CH_3)_3$−C), 1.45 (1H, dd, *J* = 15, 12 Hz, CH−CH_AH_B−C), 1.52 (9H, s, $(CH_3)_3$−C), 1.98 (1H, dd, *J* = 15, 8.5 Hz, CH−CH_AH_B−C), 3.29 (3H, s, O−CH−OCH_3), 3.42 (1H, dd, *J* = 12, 8.5 Hz, C*H*−CH_AH_B−C), 4.41 (1H, br d, *J* = 2.5 Hz, COO−C*H*), 4.45 (1H, d, *J* = 12 Hz, benzylic), 4.69 (1H, s, C*H*−COO), 4.71 (1H, d, *J* = 12 Hz, benzylic), 4.81 (1H, s, O−C*H*−OCH_3), 5.29 (1H, d, *J* = 2.5 Hz, BnO−C*H*), 7.23−7.30 (5H, m, aromatic), 8.66 (1H, br s, N*H*), 11.05 (1H, br s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.7, 28.0, 28.1, 32.8, 44.8, 55.3, 62.7, 69.5, 71.5, 71.8, 79.4, 80.7, 82.1, 83.6, 111.5, 128.1, 128.2, 128.3, 137.1, 152.6, 154.1, 161.4, 168.5; HRMS (ESI) for $C_{29}H_{42}N_3O_{10}$ $[M + H]^+$, calcd 592.2870, found: 592.2887.

5,11-DideoxyTTX (5). To a solution of di-Boc guanidine **22** (84.2 mg, 142 μmol) in MeOH (3.5 mL) was added $Pd(OH)_2$-C (17.5 mg), and the reaction vessel was charged with H_2 gas. After stirring vigorously at rt for 3 h, the reaction mixture was filtered through a pad of Super-Cel, and concentrated. The residue was dissolved in Et_2O and passed through a column packed with anhydrous Na_2SO_4 and silica gel, and concentrated to give γ-lactone **24**, which was used without further purification. To a solution of γ-lactone **24** in MeOH (13 mL) was added TFA (13 mL) at rt. After stirring for 30 h, the reaction mixture was concentrated. The residue was dissolved in 95% aqueous TFA (26 mL) at rt. After stirring for 30 h, the reaction mixture was concentrated to give 4,9-anhydro-5,11-dideoxyTTX (**25**), which was used without further purification. 4,9-Anhydro-5,11-dideoxyTTX (**25**) was dissolved in TFA-*d* (2% in D_2O, 0.7 mL). After 7 days, the solution was concentrated. The residue was purified by HPLC on a Hitachi-gel #3013-c column (H^+ form, 0.4 × 15 cm, 0.1N AcOH) to give 5,11-dideoxyTTX (**5**) (6.9 mg, 23%), 4-*epi*-5,11-dideoxyTTX (**26**) (3.7 mg, 12%), and 4,9-anhydro-5,11-dideoxyTTX (**25**) (7.3 mg, 24%), respectively. Spectroscopic data of γ-lactone **24** were obtained from the purified compound in the separate experiment. **Carbamic acid, *N*,*N*′-((2a*S*,2a^1*R*,4*S*,4a*S*,6*S*,7*S*,7a*S*)-6,7-dihydroxy-4-methoxy-6-methyl-2-oxooctahydro-2*H*-1,3-dioxacyclopenta[*cd*]inden-2a^1-yl)bis-, C,C′-bis(1,1-dimethylethyl) ester (24)**: IR (film) ν_{max} 3249, 2980, 1792, 1721, 1646, 1617, 1527, 1487, 1369, 1252, 1138, 1060, 751 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, s, HO−C−CH_3), 1.45 (9H, s, $(CH_3)_3$−C), 1.50 (9H, s, $(CH_3)_3$−C), 1.71 (1H, dd, *J* = 15.0, 10.5 Hz, CH−CH_AH_B−C), 1.98 (1H, dd, *J* = 15.0, 8 Hz, CH−CH_AH_B−C), 2.36 (1H, br s, CH−O*H*), 2.74 (1H, dd, *J* = 10.5, 8 Hz, C*H*−CH_AH_B−C), 3.37 (3H, s, O−CH−OCH_3), 4.09 (1H, br s, C*H*−OH), 4.92 (1H, s, C*H*−COO), 5.04 (1H, s, O−C*H*−OCH_3), 5.10 (1H, d, *J* = 4 Hz, COO−C*H*), 8.97 (1H, br s, N*H*), 11.34 (1H, br s, N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 26.8, 28.0 × 2, 34.9, 47.8, 55.5, 65.6, 71.1, 73.4, 80.1, 81.6, 83.3, 84.0, 113.6, 152.9, 154.5, 162.0, 171.8; HRMS (ESI) for $C_{22}H_{36}N_3O_{10}$ $[M + H]^+$, calcd 502.2401, found: 502.2397. **5**: $[\alpha]_D^{27}$ −8.3 (*c* 0.23, 0.05N AcOH); ^1H NMR (400 MHz, 4% CD_3COOD/D_2O) δ 1.31 (1H, dd, *J* = 15.6, 13.2 Hz, H-5β), 1.38 (3H, s, H-11), 2.06 (1H, ddd, *J* = 15.6, 4.0, 1.2 Hz, H-5α), 2.26 (1H, ddd, *J* = 13.2, 9.6, 4.0 Hz, H-4a), 4.37 (1H, dd, *J* = 2.4, 1.2 Hz, H-7), 4.43 (1H, d, *J* = 2.4 Hz, H-8), 4.64 (1H, s, H-9), 5.18 (1H, d, *J* = 9.6 Hz, H-4); ^{13}C NMR (100 MHz, 4% CD_3COOD/D_2O) δ 27.5, 33.3, 42.6, 61.2, 71.2, 72.2, 73.9, 77.2, 87.4, 155.9, 176.6; HRMS (ESI) for $C_{11}H_{18}N_3O_6$ $[M + H]^+$, calcd 288.1196, found: 288.1172. **26**: $[\alpha]_D^{25}$ −46 (*c* 0.21, 0.05N AcOH); ^1H NMR (400 MHz, 4% CD_3COOD/D_2O) δ 1.39 (3H, s, H-11), 1.47 (1H, dd, *J* = 15.9, 13.8 Hz, H-5β), 1.81 (1H, ddd, *J* = 15.9, 4.0, 1.5 Hz, H-5α), 2.70 (1H, dt, *J* = 13.8, 4.0 Hz, H-4a), 4.37 (1H, dd, *J* = 2.2, 1.5 Hz, H-7), 4.49 (1H, d, *J* = 2.2 Hz, H-8), 4.71 (1H, s, H-9), 4.95 (1H, d, *J* = 4.0 Hz, H-4); ^{13}C NMR (100 MHz, 4% CD_3COOD/D_2O) δ 27.3, 33.1, 38.9, 60.1, 70.6, 71.9, 73.7, 74.4, 87.2, 155.6, 176.1; HRMS (ESI) for $C_{11}H_{18}N_3O_6$ $[M + H]^+$, calcd 288.1196, found: 288.1197. **25**: $[\alpha]_D^{28}$ −22 (*c* 0.35, 0.05N AcOH); ^1H NMR (400 MHz, 4% CD_3COOD/D_2O) δ 1.22 (1H, dd, *J* = 16.0, 11.6 Hz, H-5β), 1.34 (3H, s, H-11), 2.09 (1H, ddd, *J* = 16.0, 7.2, 1.2 Hz, H-5α), 2.79 (1H, dd, *J* = 11.6, 7.2 Hz, H-4a), 4.51 (1H, dd, *J* = 2.4, 1.2 Hz, H-7), 4.84 (1H, d, *J* = 2.4 Hz, H-8), 5.03 (1H, s, H-9), 5.25 (1H, s, H-4); ^{13}C NMR (100 MHz, 4% CD_3COOD/D_2O) δ 27.7, 32.1, 42.4, 62.7, 65.2, 73.9, 84.0, 86.5, 86.8, 156.5, 172.1; HRMS (ESI) for $C_{11}H_{16}N_3O_5$ $[M + H]^+$, calcd 270.1090, found: 270.1086.

Carbamic Acid, ^{15}N,^{15}N′-((3*S*,3a*R*,4*S*,5*S*,6*S*,7a*S*)-4-(Benzyloxy)-6-hydroxy-1-methoxy-6-methyl-9-oxooctahydro-5,3-(epoxymethano)isobenzofuran-3a-yl)bis-, C,C′-Bis(1,1-dimethylethyl) Ester (22A). According to the procedure for synthesis of **22**, **21a** (43.8 mg, 88.5 μmol) was converted to $^{15}N_2$-labeled di-Boc guanidine **22A** (40.7 mg, 78% in 2 steps) as a colerless oil. $[\alpha]_D^{30}$ −5.0 (*c* 0.96, $CHCl_3$); IR (film) ν_{max} 3689, 2979, 2934, 1755, 1723, 1646, 1602, 1395, 1369, 1139, 1105 cm^{-1}; ^1H NMR (400 MHz, $CDCl_3$) δ 1.35 (3H, s, HO−C−CH_3), 1.43 (9H, s, $(CH_3)_3$−C), 1.45 (1H, dd, *J* = 15, 11.5 Hz, CH−CH_AH_B−C), 1.52 (9H, s, $(CH_3)_3$−C), 1.98 (1H, dd, *J* = 15, 9 Hz, CH−CH_AH_B−C), 3.29 (3H, s, O−CH−OCH_3), 3.42 (1H, dd, *J* = 11.5, 9 Hz, C*H*−CH_AH_B−C), 4.41 (1H, br d, *J* = 2.4 Hz, COO−C*H*), 4.45 (1H, d, *J* = 12.5 Hz, benzylic), 4.69 (1H, s, C*H*−COO), 4.71 (1H, d, *J* = 12.5 Hz, benzylic), 4.81 (1H, s, O−C*H*-OCH_3), 5.28 (1H, d, *J* = 2.4 Hz, BnO−C*H*), 7.22−7.31 (5H, m, aromatic), 8.66 (1H, br s, N*H*), 11.05 (1H, br d, ^{15}N*H*); ^{13}C NMR (100 MHz, $CDCl_3$) δ 25.8, 28.0, 28.1, 32.8, 44.8 (d, *J* = 2 Hz), 55.3, 62.7, 69.4 (d, *J* = 3 Hz), 71.5, 71.8, 79.3 (d, *J* = 2 Hz), 80.7, 82.1, 83.5, 111.5, 128.1, 128.2, 128.3, 137.1, 152.7 (d, *J* = 24 Hz), 154.1 (dd, *J* = 14, 7 Hz), 161.5 (d, *J* = 12 Hz), 168.5; HRMS (ESI) for $C_{29}H_{42}N^{15}N_2O_{10}$ $[M + H]^+$, calcd 594.2811, found: 594.2808.

$^{15}N_2$-labeled 4,9-Anhydro-5,11-dideoxyTTX (25A). According to the procedure for synthesis of **25**, **22A** (40.7 mg, 68.6 μmol) was converted to $^{15}N_2$-labeled 4,9-anhydro-5,11-dideoxyTTX (**25A**) (9.3 mg, 50%). $[\alpha]_D^{29}$ −20 (*c* 0.37, 0.05N AcOH); ^1H NMR (400 MHz, 4% CD_3COOD/D_2O) δ 1.24 (1H, dd, *J* = 16.0, 11.6 Hz, H-5β), 1.36 (3H, s, H-11), 2.11 (1H, ddd, *J* = 16.0, 7.2, 1.2 Hz, H-5α), 2.81 (1H, dd, *J* = 11.6, 7.2 Hz, H-4a), 4.53 (1H, dd, *J* = 2.4, 1.2 Hz, H-7), 4.86 (1H, d, *J* = 2.4 Hz, H-8), 5.05 (1H, s, H-9), 5.27 (1H, d, *J* = 2.0 Hz, H-4); ^{13}C NMR (100 MHz, 4% CD_3COOD/D_2O) δ 28.3, 32.7, 43.0, 63.3, 65.8, 74.6, 84.6, 87.1 (d, *J* = 9 Hz), 87.4, 172.7 (one peak was missing); HRMS (ESI) for $C_{11}H_{16}N^{15}N_2O_5$ $[M + H]^+$, calcd 272.1031, found: 272.1019.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

Copies of ^1H and ^{13}C NMR spectra for all new compounds. This material is available free of charge via the Internet at http://pubs.acs.org.

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Notes

The authors declare no competing financial interest.

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