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Configurational Reassignment and Improved Preparation of the Competitive IL-6 Receptor Antagonist 20*R*,21*R*-Epoxyresibufogenin-3-formate

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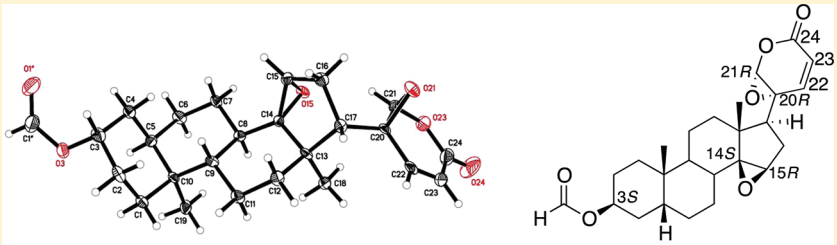
Supporting Information



20*R*,21*R*-Epoxyresibufogenin-3-formate

ABSTRACT: 20*R*,21*R*-Epoxyresibufogenin-3-formate (**1**) and 20*S*,21*S*-epoxyresibufogenin-3-formate (**2**) were synthesized from commercial resibufogenin (**3**) using known procedures. The major product (**1**) was dextrorotatory, as was the major product from the reported synthesis of epoxyresibufogenin-3-formate; however, the literature (+)-compound was assigned the 20*S*,21*S*-configuration on the basis of NMR data. We have now unequivocally determined, using single-crystal X-ray structure analyses of the major and minor products of the synthesis and of their derivatives, that the major product from the synthesis was (+)-20*R*,21*R*-epoxyresibufogenin-3-formate (**1**). Our minor synthetic product was determined to have the (−)-20*S*,21*S*-configuration (**2**). The (+)-20*R*,21*R*-compound **1** has been found to have high affinity for the IL-6 receptor and to act as an IL-6 antagonist. A greatly improved synthesis of **1** was achieved through oxidation of preformed resibufogenin-3-formate. This has enabled us to prepare, from the very expensive commercial resibufogenin, considerably larger quantities of **1**, the only known nonpeptide small-molecule IL-6 antagonist.

The only known nonpeptide small-molecule antagonist for the interleukin-6 receptor (IL-6R) that has been found to be selective and to have high affinity, an epoxyresibufogenin-3-formate, has been pharmacologically examined in a number of assay systems.[1−6] In order to obtain a relatively large quantity of the compound for our exploration of the IL-6 receptor system, we resynthesized 20*R*,21*R*- and 20*S*,21*S*-epoxyresibufogenin-3-formate (**1** and **2**, respectively, Scheme 1) using the literature[1] procedure and determined, from X-ray crystallographic analyses, that the formerly reported configurational assignments were reversed. New pharmacological assays were in agreement with our reassignment of their configuration. Future SAR studies based on these IL-6 antagonists will, of course, be dependent on their correct configuration.

Both the sympathetic and central nervous (CNS) systems play important roles in the immune response to tissue damage and infection. Involved in that response are cytokines, cell-signaling small proteins secreted by glial cells; one of them, IL-6, is an essential proinflammatory cytokine.[7,8] Many abnormal conditions and diseases are related to the overproduction and dysregulation of proinflammatory cytokines. The pathogenesis of CNS inflammation in, for example, rheumatoid arthritis[9] and osteoporosis has been linked to IL-6 dysregulation.[6] Chronic low-grade inflammation has been directly linked to an increased risk of morbidity and mortality.[10] Thus, modulation of cytokine function may be of interest for the treatment of conditions associated with a considerable number of diseases. A small-molecule nonpeptide antagonist of the IL-6-mediated response can be an invaluable research tool with potentially great therapeutic significance.

Kaneda et al.[11] found that the main constituent of a crude drug, Ch'an Su (toad cake, a folk medicine used in China and other Asian countries), was resibufogenin (**3**, Scheme 1). This medicine was prepared from skin secretions of toads of the *Bufo* genus (venenum Bufonis), and it was reported to be cardiotoxic

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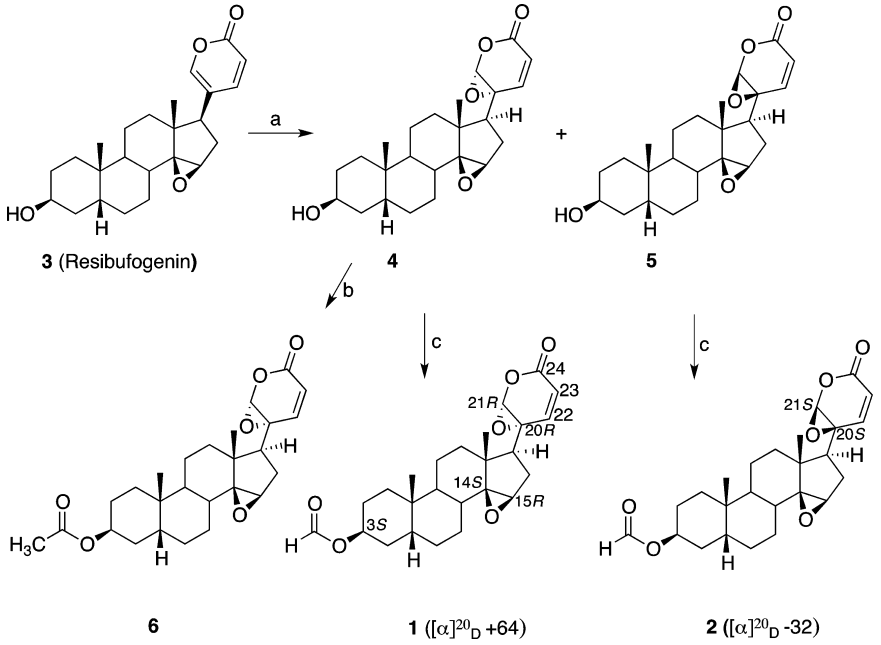
Scheme 1. Synthesis of 20*R*,21*R*-Epoxyresibufogenin-3-formate (1), 20*S*,21*S*-Epoxyresibufogenin-3-formate (2), and 20*R*,21*R*-Resibufogenin-3-acetate (6)[1a]



[a]Reagents and conditions: (a) Iron(III) acetylacetonate, H_2O_2, CH_3CN, 0 °C, 24−31%; (b) Ac_2O, pyridine, 24 h, rt, 76%; (c) HCO_2H, pyridine, rt.

with a high mortality rate when used in the United States.[12] Epoxyresibufogenin was a minor constituent of the drug. More recently, Kamano et al. also isolated the more potent bufogenin compounds 20*R*,21*R*- and 20*S*,21*S*-epoxyresibufogenin (**4** and **5**, respectively, Scheme 1) from Ch'an Su.[1] They also synthesized 20*R*,21*R*- and 20*S*,21*S*-epoxyresibufogenin-3-formate from resibufogenin (**3**), and they obtained a major (dextrorotatory) and a minor (levorotatory) product. Hyashi et al. found[4] that the dextrorotatory 20,21-epoxyresibufogenin-3-formate that they used (and that they believed to be the 20*S*,21*S*-epimeric epoxide) was an interleukin-6 receptor antagonist. Enomoto et al. also noted[2] that the 20*S*,21*S*-compound from Ch'an Su was a competitive antagonist of IL-6 and examined its SAR.

In the synthetic procedure of Kamano et al.,[1] the natural product resibufogenin (**3**) was oxidized to give a dextrorotatory major product, that was noted[1] to have the same spectral data as the natural epoxide **4**, and a minor product, the levorotatory epoxide **5** (Scheme 1). Using 2D NMR and NOESY correlations, they assigned the 20*S*-configuration to the (+)-epoxide (the major product, **4**) and the 20*R*-configuration to the (−)-epoxide (the minor product, **5**). Compounds **4** and **5** are the precursors to the pharmacologically active formates **1** and **2**, respectively (Scheme 1).

We repeated the synthesis of Kamano et al.[1] using resibufogenin (**3**, Scheme 1) that we obtained commercially from Indofine Chemical Co., Inc. (NJ, USA) and obtained a major (**4**) and minor (**5**) product. Our compound **4** had a double melting point; double and high melting point compounds in the bufalin series have been reported.[13] The published ^{1}H and ^{13}C NMR spectra[1] for the major and minor products were, in general, similar to those that we obtained. A comparison of selected chemical shifts from ^{1}H NMR and comparisons of ^{13}C NMR and IR spectra are shown in Tables 1−3. The overall yields that we obtained were consistent with the published procedure, and we initially assumed that **1**, the major diastereomeric product, had the 20*S*-configuration. Our high-resolution mass spectrometric analysis supported the preparation of **1** and **2**.

Table 1. Selected Observed and Reported ^{1}H NMR Chemical Shifts [$CDCl_3$, δ_H (*J*, Hz)] of Compounds 1 and 2

	observed	lit.[1]	observed	lit.[1]
position	**1** (300 MHz)	**1** (500 MHz)[1]	**2** (300 MHz)	**2** (500 MHz)[1]
3a	5.24	5.24 brs	5.24 s	5.23 s
15a	3.57	3.57 s	3.46 s	3.45 s
16a	2.31	2.29	2.18	2.10
18	1.03	1.03 s	1.19 s	1.19 s
19	1.01s	1.00 s	1.03 s	1.02 s
21	5.30	5.30 s	5.41 s	5.40 s
22	7.93 (10.2, 1.2)	7.93 dd (10.9, 0.5)	7.69 d (9.9)	7.69 brd (7.3)
23	6.05 (10.5)	6.04 d (10.1)	6.07 d (9.9)	6.06 d (10.1)
1′	8.07	8.07	8.08	8.07

However, our specific rotations and melting points were different from those that have been reported. The reported specific rotations for the major (dextrorotatory) and minor (levorotatory) product of Kamano et al.[1] were $[\alpha]^{20}_D$ +17.2 (*c* 0.1, $CHCl_3$) and $[\alpha]^{20}_D$ −11.5 (*c* 0.1, $CHCl_3$), respectively. The optical rotations of the major (dextrorotatory) and minor (levorotatory) product that we obtained were $[\alpha]^{20}_D$ +64 (*c* 0.1, $CHCl_3$) and $[\alpha]^{20}_D$ −32 (*c* 0.1, $CHCl_3$), respectively . Our observed melting points for the major (dextrorotatory) and minor (levorotatory) products were 159−160 °C (lit.[1] mp 180−182 °C) and 142−143 °C (lit.[1] mp 147−150 °C). Because of these differences, we decided to establish the structure of our compounds by single-crystal X-ray diffraction studies (Figure 1). Among the non-bromine-containing compounds **1**, **2**, and **6** (Figures 1 and 2) only compound **6** was characterized using likelihood methods developed by Hooft et al.,[14] as implemented in PLATON.[15] Cu Kα data were used

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Table 2. ^{13}C NMR ($CDCl_3$) Comparison of Observed and Reported Chemical Shifts of Compounds 1 and 2

	observed	lit.[1]	observed	lit.[1]
carbon	1 (125 MHz)	1 (125 MHz)[1]	2 (75 MHz)	2 (125 MHz)[1]
1	30.3	30.2	30.4	30.2
2	25.7	25.6	25.8	25.6
3	70.5	70.4	70.6	70.4
4	30.5	30.4	30.6	30.4
5	36.8	36.7	36.9	36.7
6	25.2	25.0	25.2	25.1
7	20.7	20.6	20.9, 20.8	20.7
8	33.3	33.2	33.3	33.1
9	39.5	39.4	39.7	39.5
10	35.4	35.3	35.5	35.3
11	20.8	20.6	20.9, 20.8	20.6
12	39.8	39.7	40.1	40.0
13	44.2	44.1	45.1	44.9
14	75.3	75.1	75.2	75.0
15	60.0	59.9	60.2	60.0
16	28.7	28.6	28.2	28.0
17	51.9	51.8	50.4	50.2
18	16.3	16.2	16.1	15.9
19	23.7	24.0	23.9	23.7
20	56.6	56.5	55.7	55.5
21	84.7	84.6	83.1	82.9
22	148.0	147.8	149.6	149.3
23	121.6	121.5	122.5	122.3
24	159.9	159.8	160.1	159.9
1′	160.8	160.7	160.9	160.7

Table 3. Comparison of Observed and Reported IR (cm^{-1}) Data of 4 and 5

observed		lit.[1]	observed		lit.[1]
4 (KBr)	4 (solid probe)	4 (KBr)[1]	5 (KBr)	5 (solid probe)	5 (KBr)[1]
3460		3465	3473	3475	3470
	3060	3070	3079		3070
2934	2931	2937	2936	2933	2938
2871	2864	2877	2876	2874	2877
1743	1739	1744	1744	1739	1744
1627	1626	1625	1628	1628	1632
		1533			1534
1123		1123	1123	1120	1124
1090	1088	1089	1089	1086	1093
984	983	982	985	984	980
867	867	886	876	865	875
800	796	785	790	789	782

for compounds **4** and **6** (the polymorph of **6** was collected with Mo Kα radiation). These methods indicated that the absolute stereostructure had been correctly assigned. The probability that structure **6** is inverted (as calculated using this method) is smaller than 1×10^{-26}.

When the results of the X-ray analysis contradicted a previously published result, we felt it prudent to be certain of our result and took the extra step of preparing and analyzing the heavy-atom-containing compound **8**. This allowed both confirmation of the result and a direct comparison of the methods of Hooft[14] and Flack.[16] This also seemed important, as the method of Hooft is relatively new and has not been widely applied. Compound **8** is the *p*-bromobenzoate ester of 20*R*,21*R*-epoxyresibufogenin (**4**). The crystal structure of **8** was analyzed using the method of Hooft et al.[14] and the more widely accepted method of Flack.[16] The two methods were in agreement on the absolute configuration. The Flack parameter for the bromobenzoate ester **8** was 0.007(3), and the Hooft method[14] indicated that the probability that the configuration was wrong was 0.1×10^{-115}. All of the structural features of our compounds were consistent with the literature except for the configuration of the C-20, C-21 oxirane moiety. The configuration at C-20 and C-21 was found to be opposite that of the previously reported 20*S*, 21*S*.[1]

In the ^1H NMR spectra, the (+)-diastereomer (**1**), which we determined to have the 20*R*,21*R*-configuration by X-ray analyses, showed a doublet for H-22 at δ 7.93 for the C-20,C-21 epoxide (in Kamano et al.;[1] this was associated with the compound assigned the 20*S*,21*S*-(+) configuration), and our 20*S*,21*S*-(−)-diastereomer (**2**) showed a broad singlet for H-22 at δ 7.69 for the C-20,C-21 epoxide (in Kamano et al.,[1] which was associated with the compound that they assigned the 20*R*,21*R*-(+) configuration). The optical rotations of the samples obtained subsequent to the X-ray crystallographic studies were redetermined and found to be as noted above. Since we were surprised to find that the configuration appeared to be misassigned or mislabeled in the figures or Experimental Section,[1] we converted 20*R*,21*R*-(+)-epoxyresibufogenin (**4**) to the dextrorotatory 3-acetate derivative[3] **6**, which had not formerly been examined using single-crystal X-ray diffraction studies. The results of that X-ray study (Figure 2) were in agreement with our studies of the formate compounds. We concluded that the major product of the synthesis was 20*R*,21*R*-epoxyresibufogenin (**4**) and that **1**, derived from **4**, must be (+)-20*R*,21*R*-epoxyresibufogenin-3-formate. (−)-20*S*,21*S*-Epoxyresibufogenin-3-formate (**2**) was derived from the minor product, 20*S*,21*S*-epoxyresibufogenin (**5**).

The interactions of the formates **1** and **2** with the IL-6 receptor were examined using different assays than those noted in the literature,[1,2,4] and compound **1** (Scheme 1), with the reassigned (+)-20*R*-configuration, was determined to be a pharmacologically active diastereomer; it was found to reduce pancreatitis-induced pain by peripheral mechanisms.[6] In another pharmacological assay, suppression of IL-6-induced α1-antichymotrypsin *m*RNA expression, 20*R*,21*R*-epoxyresibufogenin-3-formate (**1**) was found to specifically inhibit an IL-6-mediated signaling pathway; the 20*S*,21*S*-epoxyresibufogenin-3-formate (**2**) had a lesser effect on that pathway.[5]

We then reexamined the synthesis of (+)-20*R*,21*R*-epoxyresibufogenin-3-formate (**1**, Scheme 2) in order to obtain larger quantities for pharmacological studies. An improved path to the compound was important because the commercially available starting material, resibufogenin, was very expensive. We found that rather than an initial oxidation of resibufogenin (**3**) followed by formylation, as shown in Scheme 1, it was much better if the formylation preceded the oxidation. As shown in Scheme 2, acetic formic anhydride in pyridine at low temperatures gave the formylated product **7**[17] in 93% yield. Two methods of oxidation of the resibufogenin 3-formate (**7**)[17] were successfully implemented. In the first method a solution of dimethyldioxirane in acetone was used to oxidize the formyl compound **7** at room temperature. After 2 days, a 71% yield of the target 20*R*,21*R*-epoxyresibufogenin-3-formate (**1**) was obtained. In the second method, an acetonitrile solution of resibufogenin 3-formate was added to an aqueous disodium-EDTA solution. The cold mixture was reacted with Oxone,[21] and the reaction was complete in 1 h to give a 56% yield of **1**.

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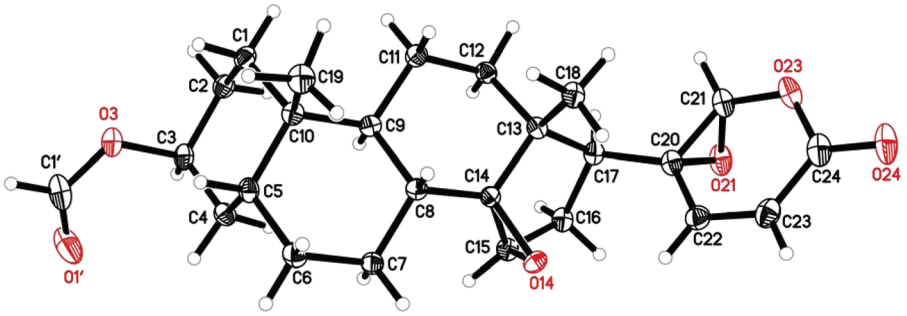
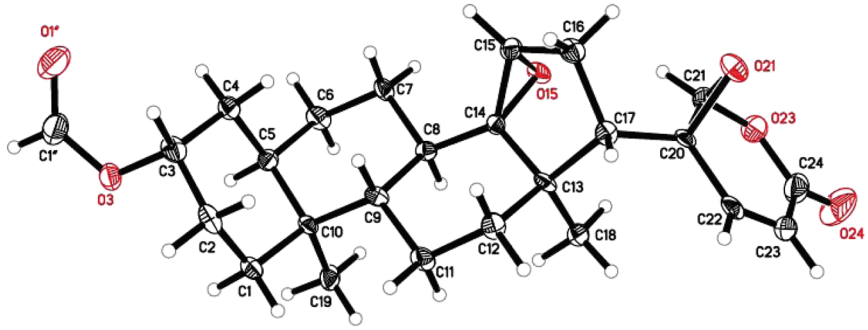


(+)-20*R*,21*R*-epoxyresibufogenin-3-formate (**1**)



(-)-20*S*,21*S*-epoxyresibufogenin-3-formate (**2**)

Figure 1. Structure of (+)-20*R*,21*R*-epoxyresibufogenin-3-formate (**1**) and (−)-20*S*,21*S*-epoxyresibufogenin-3-formate (**2**) as determined by X-ray crystallography. Displacement ellipsoids are at the 50% level.

The overall yield of **1** from **3** ranged from 65% (method 1) to 52% (method 2). Either method was a significant improvement over the procedure in Scheme 1 (ca. 20% yield over two steps), and the new synthesis was used to prepare a sufficient quantity of **1** for further pharmacological work.

EXPERIMENTAL SECTION

General Experimental Procedures. For Scheme 1: All melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. The IR data were obtained on a Perkin-Elmer Spectrum One FTIR instrument. The ^{1}H (300 MHz) and ^{13}C (75 MHz) NMR spectra were recorded at room temperature on a Varian XL-300 instrument with $CDCl_3$ as solvent, δ values in ppm (TMS as internal standard), J (Hz) assignments of ^{1}H resonance coupling. HRMS were obtained on a Waters/Micromass LCT ESI-TOF. TLC was performed on 250 mm Analtech GHLF silica gel plates using EtOAc−hexanes, 1:1, as the solvent system. Elemental analyses were performed by Atlantic Microlabs, Inc. (Norcross, GA, USA). Resibufogenin was obtained from Indofine Chemical Company, Inc. (Hillsborough, NJ, USA).

For Scheme 2 (changes from Scheme 1): ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) spectra were recorded at room temperature on a Bruker-500 instrument in $CDCl_3$ (unless otherwise noted). HRMS were obtained on a Waters/Premier LCT ESI-TOF. Flash column chromatography was performed with Bodman silica gel LC 60 A. Elemental analyses were performed by Micro-Analysis, Inc. (Wilmington, DE, USA).

Resibufogenin (3). Commercial resibufogenin was an off-white crystalline solid: mp 150−152 °C (lit.[18] mp 108−120 °C, 162−166 °C); $[\alpha]^{20}_{D}$ −6 (*c* 0.1, $CHCl_3$) (lit.[19] $[\alpha]^{16}_{D}$ −5.4 (*c* 2.3, $CHCl_3$)); ^{1}H NMR ($CDCl_3$, 500 MHz) δ 7.80 (1H, dd, J = 2.8, 12.8 Hz), 7.25 (1H, s), 6.24 (1H, d, J = 12.8 Hz), 4.14 (1H, t, J = 2.7 Hz), 3.53 (1H, s), 2.52−2.33 (2H, m), 2.18 (1H, s), 2.06−1.16 (16H, m), 0.99 (3H, s), 0.78 (3H, s); ^{13}C NMR ($CDCl_3$, 125 MHz) δ 149.5, 147.0, 122.2, 115.2, 74.7, 66.7, 59.8, 47.7, 45.2, 39.3, 39.2, 35.9, 35.4, 33.5, 33.2, 32.3, 29.5, 27.8, 25.7, 23.7, 21.0, 20.7, 16.8; HRESIMS *m*/*z* 385.2371 (calcd for $C_{24}H_{33}O_4$, 385.2379).

20*R*,21*R*-Epoxyresibufogenin (4) and 20*S*,21*S*-Epoxyresibufogenin (5). Resibufogenin (**3**) (0.31 g, 0.80 mmol) and tris(acetylacetonato)iron(III) (2.5 g, 7.1 mmol) were combined in acetonitrile (250 mL). To the solution was added 30% H_2O_2 (18 mL) dropwise at 0 °C. The mixture was filtered and poured into ice−water (3 × 150 mL). The solution was extracted with CH_2Cl_2. The combined extracts were washed with 5% aqueous sodium thiosulfate, $NaHCO_3$, and H_2O. The organic layer was dried over Na_2SO_4, and the solvent was removed under reduced pressure. The diastereomers were separated by column chromatography employing EtOAc−hexanes (1:1) as the solvent system. The major (dextrorotatory) product (**4**) was obtained in yields ranging from 24% to 31% (lit.[1] 24%), as a white crystalline solid (R_f = 0.35): mp 123−124 °C and 220−222 °C (lit.[1] mp 184−186 °C for the reported 20*S*,21*S*-epoxyresibufogenin); $[\alpha]^{20}_{D}$ +46.0 (*c* 0.1, $CHCl_3$) (lit.[1] $[\alpha]^{18}_{D}$ +18.2 (*c* 0.1, $CHCl_3$)), for the reported 20*S*,21*S*-epoxyresibufogenin)); IR (KBr and solid probe), see Table 3; ^{1}H NMR ($CDCl_3$, 300 MHz) δ 7.93 (1H, dd, J = 10.4, 1.2 Hz, H-22), 6.04 (1H, d, J = 10.2 Hz, H-23), 5.30 (1H, d, J = 1.2 Hz, H-21), 4.13 (1H, m, H-3), 3.57 (1H, s, H-15), 2.27 (2H, m, H-16), 2.18- 1.24 (19H, m), 1.02 (3H, s, H-18), 0.99 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 300 MHz) δ 160.1, 148.1, 121.6, 84.8, 75.4, 66.9, 60.1, 56.7, 51.9, 44.3, 39.9, 39.4, 36.1, 35.7, 33.4, 29.7, 28.8, 28.0, 25.9, 23.9, 20.9, 20.8, 16.4; HRESIMS *m*/*z* 401.2330 (calcd for $C_{24}H_{33}O_5$ 401.2328); anal. C 69.75, H 8.24, O 21.92%, calcd for $C_{24}H_{32}O_5 \cdot 0.7$ H_2O, C 69.78, H 8.15; O 22.07%.

The minor (levorotatory) product **5** was obtained in yields ranging from 16% to 20% (lit.[1] 13%), R_f = 0.28, as a white crystalline solid; mp 114−116 °C and 235−237 °C (lit.[1] mp 90−94 °C for the reported 20*R*,21*R*-epoxyresibufogenin); $[\alpha]^{20}_{D}$ −17.9 (*c* 0.1, $CHCl_3$) (lit.[1] $[\alpha]^{19}_{D}$ −17.0 (*c* 0.1, $CHCl_3$, for the reported 20*R*,21*R*-epoxyresibufogenin)); IR (KBr and solid probe), see Table 3; ^{1}H NMR (300 MHz) δ 7.69 (1H, d, J = 9.9 Hz, H-22), 6.07 (1H, d, J = 10.2 Hz, H-23), 5.40 (1H, s, H-21), 4.14 (1H, s, H-3), 3.45 (1H, s, H-15), 2.18−

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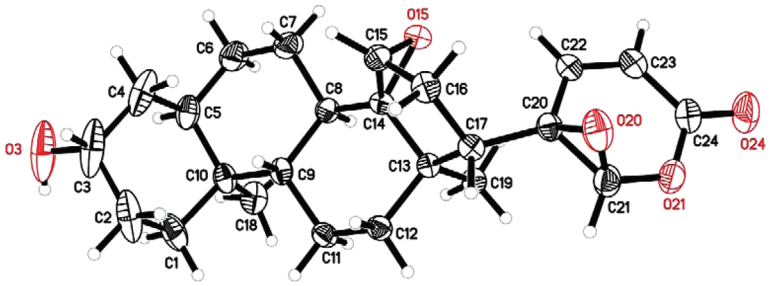
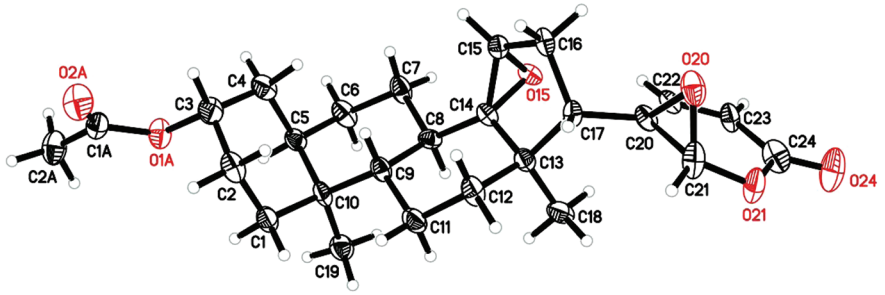
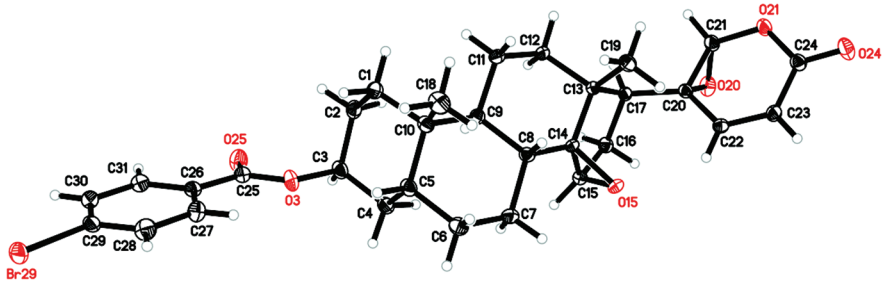


20*R*,21*R*-Epoxyresibufogenin (**4**)



(+)-20*R*,21*R*-epoxyresibufogenin 3-acetate (**6**)



(+)-20*R*,21*R*-epoxyresibufogenin 3-(4-bromobenzoate) (**8**)

Figure 2. Structure of 20*R*,21*R*-epoxyresibufogenin (**4**), (+)-20*R*,21*R*-epoxyresibufogenin 3-acetate (**6**), and (+)-20*R*,21*R*-epoxyresibufogenin 3-(4-bromobenzoate) (**8**) as determined by X-ray crystallography. Displacement ellipsoids are at the 50% level.

1.25 (19H, m), 1.19 (3H, s, H-18), 1.01 (3H, s, H-19); ^{13}C NMR ($CDCl_3$, 75 MHz) δ 160.1, 149.6, 122.4, 83.1, 75.3, 66.9, 60.1, 55.7, 50.3, 45.0, 40.1, 39.4, 36.1, 35.7, 33.4, 33.2, 29.7, 28.0, 25.9, 23.9, 20.9, 20.8, 16.1; HRESIMS *m*/*z* 401.2348 (calcd for $C_{24}H_{33}O_5$ 401.2328); anal C 69.31, H 8.16, O 21.51%, calcd for $C_{24}H_{32}O_5 \cdot 0.75\ H_2O$, C 69.62, H 8.16, O 22.21%.

20*R*,21*R*-Epoxyresibufogenin 3-acetate (6). 20*R*,21*R*-Epoxyresibufogenin (**4**) (80 mg, 0.02 mmol) and Ac_2O (2.0 mL) were combined in pyridine (4.0 mL) and stirred for 24 h at room temperature. The solution was poured into H_2O (75 mL) and extracted with CH_2Cl_2. The combined extracts were washed with H_2O (3 × 75 mL) to remove the final traces of pyridine and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield a white solid, which was recrystallized from acetone to give needles, 67 mg (0.02 mmol, 76%), mp 152−153 °C; $[\alpha]^{20}_D$ +57.3 (*c* 1.0, $CHCl_3$); ^{1}H NMR δ 7.93 (1H, dd, *J* = 10.2 Hz, 1.1 Hz, C-22), 6.05 (1H, d, *J* = 10.2 Hz, C-23), 5.30 (1H, d, *J* = 0.8 Hz, C-21), 5.09 (1H, m, C-3), 3.58 (1H, s, C-15), 2.31−1.23 (19H, m), 1.03 (3H, s, C-18), 1.00 (3H, s, C-19); ^{13}C NMR δ 170.8, 160.0, 148.1, 121.6, 84.7, 75.3, 70.4, 60.1, 56.7, 51.8, 44.2, 39.7, 39.5, 36.9, 35.4, 33.3, 31.1, 30.5, 30.4, 28.7, 25.7, 25.1, 23.8, 21.6, 20.8, 20.7, 16.4; HRESIMS *m*/*z* 443.2422 (calcd for $C_{26}H_{35}O_6$ 443.2434); anal. C 69.68, H 7.81, O, 22.51%, calcd for $C_{26}H_{34}O_6 \cdot 0.25\ H_2O$, C 69.85, H 7.78; O 22.37%.

20*R*,21*R*-Epoxyresibufogenin 3-formate (1) from 20*R*,21*R*-Epoxyresibufogenin (4). 20*R*,21*R*-Epoxyresibufogenin (**4**) (310 mg, 0.77 mmol) and HCO_2H (6.6 mL) were combined in pyridine (6.0 mL) and stirred for 24 h at room temperature. The solution was poured into H_2O (75 mL) and extracted with CH_2Cl_2. The combined extracts were washed with H_2O (3 × 75 mL) to remove the final traces of pyridine and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield a white solid, 240 mg (72%). The residue was purified by column chromatography on silica gel, eluting with EtOAc−hexanes (1:1) as solvent system to give needles. Recrystallization from acetone gave 47 mg of a crystalline solid (0.11 mmol, 14%, R_f = 0.69), mp 166−167 °C (lit.[1] mp 180−182 °C for the reported "20*S*,21*S*-epoxyresibufogenin"); $[\alpha]^{20}_D$ +64.0 (*c* 0.1, $CHCl_3$) (lit.[1] $[\alpha]^{19}_D$ +17.2 (*c* 0.1, $CHCl_3$, for the reported "20*S*,21*S*-epoxyresibufogenin"); IR (KBr) ν_{max} 3032, 2940, 2881, 1743, 1449, 1374, 1148, 1120, 1093, 984, 868, 838 cm^{-1}; ^{1}H NMR δ 8.07 (1H, s, CO_2H), 7.93 (dd, 1H, *J* = 10.3, 1.2 Hz, H-22), 6.05 (1H, d, *J* = 10.3 Hz, H-23), 5.31 (1H, s, C-21), 5.24 (1H, s, H-3), 3.57 (1H, s, H-15), 2.31−1.23 (19H, m), 1.03 (3H, s, H-18), 1.01 (3H, s, H-19); ^{13}C NMR, see Table 2;

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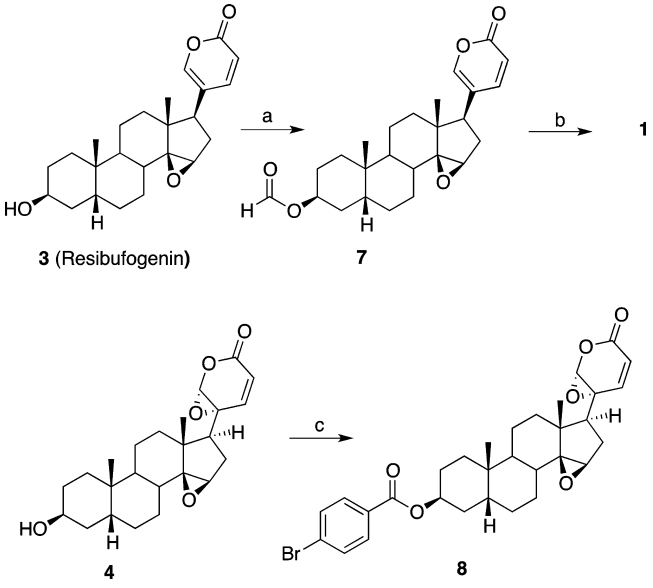
Scheme 2. Improved Synthesis of 20*R*,21*R*-Epoxyresibufogenin-3-formate (1) and Preparation of 20*R*,21*R*-Epoxyresibufogenin 3-(4-bromobenzoate) (8)[a]



[a]Reagents and conditions: (a) acetic formic anhydride, pyridine, 0−5 °C, 2 h, 93%; (b) method A: dimethyldioxirane, acetone, CH_2Cl_2, rt, 2 days, 71%, method B: Oxone,[21] $NaHCO_3$, CH_3CN, Na_2EDTA, trifluoroacetone, 5 °C, 1 h, 56%; (c) DMAP, CH_2Cl_2, *p*-bromobenzoyl chloride, rt, 14 h.

HRDEIMS *m*/*z* 428.2203 (calcd for $C_{25}H_{32}O_6$, 428.2199); anal. C 69.39, H 7.62%, calcd for $C_{25}H_{32}O_6 \cdot 0.25\ H_2O$, C 69.34, H 7.56%.

20*R*,21*R*-Epoxyresibufogenin 3-formate (1) from Resibufogenin 3-formate (7). *Method A.* To a solution of resibufogenin 3-formate (**7**, 206 mg, 0.50 mmol) in 6 mL of CH_2Cl_2 was added a solution of dimethyldioxirane[20] in acetone (4.5 mL, ~0.26 mmol) at room temperature. The progress of the reaction was followed by TLC; conversion was about 60% in 20 h. Removal of solvent gave a white, crystalline solid. The solid was dissolved in CH_2Cl_2 (50 mL) and dried with anhydrous Na_2SO_4. The drying agent was filtered and washed with CH_2Cl_2. The solvent was removed, and the residue was separated by column chromatography on silica gel, eluting with EtOAc−hexanes (1:1), to give 151 mg (0.35 mmol) of **1** as a colorless crystalline solid (conversion: 85%, yield: 71%); mp 165−167 °C; $[\alpha]^{20}_D$ +62.8 (*c* 1.0, $CHCl_3$).

Method B (ref 21). To an acetonitrile solution (5 mL) of resibufogenin 3-formate (**7**, 206 mg, 0.50 mmol) was added an aqueous disodium-EDTA solution (2.0 mL, 0.4 mM). The resulting heterogeneous solution was cooled to 5 °C, followed by addition of trifluoroacetone (1.0 mL) via a precooled syringe. To this solution was added a mixture of $NaHCO_3$ (0.65 g, 7.75 mmol) and Oxone[21] (1.53 g, 5.0 mmol), all at once. The reaction was complete in 1 h as shown by TLC. Anhydrous Na_2SO_4 was added, followed by anhydrous CH_2Cl_2 (80 mL). The mixture was filtered, and the filtrate was concentrated under reduced pressure. The residue was purified by flash column chromatography (40% EtOAc in hexanes as eluent) to provide 121 mg (0.28 mmol) of **1** as a colorless, crystalline solid (conversion: 90%, yield: 56%).

20*S*,21*S*-Epoxyresibufogenin 3-formate (2). 20*S*,21*S*-Epoxyresibufogenin (**5**) (180 mg, 0.45 mmol) and formic acid (4.4 mL) were combined in pyridine (4.0 mL) and stirred for 24 h at room temperature. The solution was poured into H_2O (75 mL) and extracted with CH_2Cl_2. The combined extracts were washed with H_2O (3 × 75 mL) to remove the final traces of pyridine and dried over Na_2SO_4. The solvent was removed under reduced pressure to yield a white solid. The residue was purified by column chromatography on silica gel, eluting with EtOAc−hexanes (1:1) as solvent system to give a white solid. Recrystallization from acetone provided 35 mg of a crystalline solid (0.08 mmol, 18%, R_f = 0.71); mp 142−143 °C (lit.[1] mp 147−150 °C); $[\alpha]^{20}_D$ −32.0 (*c* 0.1, $CHCl_3$) (lit.[1] $[\alpha]^{19}_D$ −11.5 (*c* 0.1, $CHCl_3$)); IR (KBr) ν_{max} 2937, 1748, 1707, 1448, 1376, 1193, 1152, 1119, 1094, 983, 880 cm^{-1}; HRFABMS *m*/*z* 429.2273 (calcd for $C_{25}H_{33}O_6$, 429.2277); ^1H NMR δ 8.07 (1H, s, *H*-CO_2); 7.69 (1H, d, *J* = 9.9 Hz, H-22); 6.07 (1H, d, *J* = 9.9 Hz, H-23); 5.41 (1H, s, H-21); 5.24 (1H, s, H-3); 3.46 (1H, s, H-15); 2.18−1.23 (19H, m); 1.19 (3H, s, H-18); 1.03 (3H, s, H-19); ^{13}C NMR δ 160.9, 160.1, 149.6, 122.5, 83.1, 75.2, 70.6, 60.2, 55.7, 50.4, 45.1, 40.1, 39.7, 36.9, 35.5, 33.3, 30.6, 30.4, 28.2, 25.8, 25.2, 23.9, 20.9, 20.8, 16.1; anal. C 69.87, H 7.59, O 22.35%, calcd for $C_{25}H_{32}O_6$, C 70.07, H 7.53, O, 22.40%.

Resibufogenin 3-formate (7) (ref 17). To a solution of resibufogenin (**3**, 4.23 g, 0.01 mol) in dry pyridine (100 mL) was added acetic formic anhydride[22] (30 mL). The mixture was left at 0−5 °C for 2 h, poured into ice water, and extracted with $CHCl_3$. The organic layer was washed successively with 1 N HCl, H_2O, saturated $NaHCO_3$, and H_2O, dried, and evaporated. The residue was separated by column chromatography on silica gel, eluting with EtOAc−hexanes (1:1), to give 4.20 g (0.01 mol) of **7** as colorless crystals: (93%), mp 218−220 °C; $[\alpha]^{20}_D$ −4.4 (*c* 1.04, $CHCl_3$); ^1H NMR δ8.07 (1H, s), 7.78 (1H, d, *J* = 9.5 Hz), 7.24 (1H, s), 6.25 (1H, d, *J* = 10.0 Hz), 5.25 (1H, s), 3.53 (1H, s), 2.46 (1H, d, *J* = 10.0 Hz), 2.38 (1H, dd, *J* = 15.0, 10.0 Hz), 2.05−1.82 (4H, m), 1.75−1.18 (16H, m), 1.02 (3H, s), 0.88−1.0 (1H, m), 0.78 (3H, s); ^{13}C NMR δ 162.1, 160.8, 149.7, 147.1, 122.3, 115.4, 74.7, 70.6, 60.0, 47.9, 45.4, 39.6, 39.4, 36.8, 35.4, 33.7, 32.5, 30.5, 30.3, 25.6, 25.2, 23.9, 21.2, 20.7, 17.0; HRESIMS *m*/*z* 413.2325 (calcd for $C_{25}H_{33}O_5$, 413.2328; anal. C 72.89, H 7.66%, calcd for $C_{25}H_{32}O_5$, C 72.79, H 7.82%.

20*R*,21*R*-Epoxyresibufogenin 3-(4-bromobenzoate) (8). To a solution of 20*R*,21*R*-epoxyresibufogenin (**4**, 33.0 mg, 0.08 μmol) in 1.0 mL of CH_2Cl_2 were added DMAP (25.2 mg, 0.02 mmol) and *p*-bromobenzoyl chloride (36.2 mg, 0.17 mmol). The solution changed to a suspension immediately, and the mixture was stirred overnight at room temperature. The mixture was separated by column chromatography on silica gel, eluting with EtOAc−hexanes (1:3), to afford colorless crystals, 36 mg (0.07 mmol, 81%), mp 177−178 °C and 184−186 °C; $[\alpha]^{20}_D$ +50.7 (*c* 0.4, $CHCl_3$). The ester was recrystallized from EtOAc−hexanes to obtain a crystal suitable for X-ray crystallography; ^1H NMR δ 7.95 (1H, dd, *J* = 1.0, 10.2 Hz), 7.90 (2H, d, *J* = 9.0 Hz), 7.59 (2H, d, *J* = 9.0 Hz), 6.04 (1H, d, *J* = 10.5 Hz), 5.34 (brs, 1H), 5.31 (d, 1H, *J* = 1.0 Hz), 4.13 (m, 1H), 3.59 (1H, s), 2.34−2.28 (2H, m), 2.05 (1H, s), 2.05−1.24 (16H, m), 1.05 (s, 3H), 1.04 (3H, s); ^{13}C NMR δ 165.3, 160.0, 148.0, 131.8, 131.2, 130.0, 128.0, 121.6, 84.7, 75.2, 71.4, 60.0, 56.6, 51.9, 44.2, 39.7, 39.5, 37.3, 35.5, 33.4, 33.4, 30.8, 30.7, 28.7, 25.8, 25.3, 24.0, 20.8, 16.4; HRESIMS *m*/*z* 583.1708 (calcd for $C_{31}H_{36}BrO_2$, 583.1695); anal. C 63.43, H 5.98%, calcd for $C_{31}H_{35}BrO_2$, C 63.81, H 6.05%.

X-ray Crystal Structure of Compounds 1, 2, 4, 6, and 8. Single-crystal X-ray diffraction data of compounds **1**, **2**, **4**, **6**, and **8** were collected using Mo Kα radiation (λ = 0.71073 Å) and a Bruker APEX 2 CCD area detector except where noted. The structures were solved by direct methods and refined by full-matrix least-squares on F^2 values using the programs found in the SHELXTL suite (Bruker, SHELXTL v6.10, 2000, Bruker AXS Inc., Madison, WI, USA). Parameters refined included atomic coordinates and anisotropic thermal parameters for all non-hydrogen atoms. Hydrogen atoms on carbons were included using a riding model [coordinate shifts of C applied to H atoms] with C−H distance set at 0.96 Å. Atomic coordinates for these compounds have been deposited with the Cambridge Crystallographic Data Centre (deposition numbers for compounds **1**, **2**, **4**, **6**, **6** (polymorph), and **8** are 851340, 851341, 851342, 851343, 851344, and 851345, respectively). Copies of the data can be obtained, free of charge, on application to CCDC, 12 Union Road, Cambridge, CB2 1EZ, UK [fax: +44(0)-1223-336033 or e-mail: deposit@ccdc.cam.ac.uk].

Compound 1. A 0.42 × 0.23 × 0.10 mm^3 crystal of **1** was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N, Hampton Research). The oil-coated crystal was placed

on a MicroMesh mount (MiTeGen, Ithaca, NY, USA) and transferred immediately to the cold stream (93 K) on the diffractometer. The crystal was monoclininc in space group $P2_1$ with unit cell dimensions a = 9.2499(12) Å, b = 10.3002(13) Å, c = 11.7027(15) Å, and β = 104.951(2)°. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 96.7% complete to 28.31° θ (approximately 0.75 Å) with an average redundancy of 2.79.

Compound **2**. A 0.45 × 0.44 × 0.06 mm^3 crystal of **2** was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N). The oil-coated crystal was placed on a MicroMesh mount and transferred immediately to the cold stream (93 K) on the diffractometer. The crystal was monoclininc in space group $P2_1$ with unit cell dimensions a = 8.3511(10) Å, b = 18.955(2) Å, c = 14.0356(16) Å, and β = 102.781(2)°. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 99.5% complete to 26.37° θ (approximately 0.80 Å) with an average redundancy of 3.39.

Compound **4**. A 0.632 × 0.296 × 0.275 mm^3 crystal of **4** was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N). The oil-coated crystal was placed on a MicroMesh mount and transferred immediately to the cold stream (200 K) on the diffractometer, and data were collected using Cu Kα radiation (λ = 1.54178 Å) and a Bruker Platinum-135 CCD area detector. The crystal was tetragonal in space group $P4_32_12$ with unit cell dimensions $a = b$ = 11.0131(3) Å and c = 34.7409(8) Å. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 99.7% complete to 68.45° θ (approximately 0.83 Å) with an average redundancy of 10.46. λ = 1.54178 Å.

Compound **6**. A 0.38 × 0.14 × 0.05 mm^3 crystal of **6** was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N). The oil-coated crystal was placed on a MicroMesh mount and transferred immediately to the diffractometer, and data were collected at room temperature using Cu Kα radiation (λ = 1.54178 Å) and a Bruker Platinum-135 CCD area detector. The crystal was monoclininc in space group $P2_1$ with unit cell dimensions a = 9.7084(1) Å, b = 10.6697(1) Å, c = 11.7781(2) Å, and β = 108.681(1)°. Corrections were applied for Lorentz, polarization, and absorption effects. The crystal was twinned, and data for the major component were 96.5% complete to 67.53° θ (approximately 0.83 Å) with an average redundancy of 0.97.

In an attempt to find a nontwinned crystal of **6** a polymorph was discovered. A 0.773 × 0.066 × 0.056 mm^3 crystal of **6** (orthorhombic polymorph) was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N). The oil-coated crystal was placed on a MicroMesh mount and transferred immediately to the cold stream (103 K) on the diffractometer. The crystal was orthorhombic in space group $P2_12_12_1$ with unit cell dimensions a = 8.0735(9) Å, b = 16.1265(17) Å, and c = 37.756(4) Å. Corrections were applied for Lorentz, polarization, and absorption effects. The crystal was twinned, and data for the major component were were 99.3% complete to 25.31° θ (approximately 0.83 Å) with an average redundancy of 3.93.

Compound **8**. A 0.638 × 0.498 × 0.385 mm^3 crystal of **8** was prepared for data collection by coating with high-viscosity microscope oil (Paratone-N). The oil-coated crystal was placed on a MicroMesh mount and transferred immediately to the cold stream (100 K) on the diffractometer. The crystal was monoclininc in space group $P2_1$ with unit cell dimensions a = 10.5597(4) Å, b = 10.4870(3) Å, c = 12.5566(4) Å, and β = 103.170(1)°. Corrections were applied for Lorentz, polarization, and absorption effects. Data were 95.8% complete to 25.00° θ (approximately 0.83 Å) with an average redundancy of 4.41.

■ ASSOCIATED CONTENT

Ⓢ Supporting Information

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Notes

The authors declare no competing financial interest.

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