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The first total synthesis and structural determination of antibiotics K1115 B_1s (alnumycins)

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ABSTRACT

K1115 B_1, isolated from the broth of *Streptomyces* species, was found to be a mixture of stereoisomers. Authors synthesized all stereoisomers of K1115 B_1 by convergent synthesis coupling a rhamnose derivative, an isobenzofuranone, and a chiral tetraol. Comparison of ^{1}H NMR spectra and optical rotations made it clear that the absolute structures of K1115 $B_{1\alpha}$ (the major isomer) and K1115 $B_{1\beta}$ (the minor isomer) were (1*R*, 17*S*)- and (1*R*, 17*R*)-configurations, respectively. The optical rotations of the stereoisomers revealed that alnumycin, reported as the identical structure with K1115 B_1, might be another mixture of stereoisomers.

Naphthopyranomycin family, involving naphthopyranomycin (**1**)[1] and K1115 B_1 (**2**),[2–4] has been studied as a unique group of bioactive natural products.[5] K1115 B_1 (**2**) (BE-41956A, alnumycin) was isolated as an antitumor antibiotic independently by Banyu group (as BE-41956A in 1997),[2] Bieber group (as alnumycin in 1998),[3] and Eisai group (as K1115 B_1 in 1998).[4] Although naphthopyranomycin analogs have been isolated widespread, their absolute structures have never been disclosed. In our laboratory, the total syntheses of bioactive pyranonaphthoquinones[6] including nanaomycins,[7] kalafungin,[7] medermycin,[8] ES-242s,[9] BE-54238B,[10] and BE-52440A[11] have been achieved. The structures and bioactivities of the naphthopyranomycin family drew our attention to determine the absolute structure. So the authors synthesized four stereoisomers of K1115 B_1 and compared the ^{1}H NMR spectra with that of the natural product isolated from the culture broth of *Streptomyces griseorubiginosus*. Consequently, we found that a red spot on TLC, of which Rf value was the same as the literature,[4] contained two compounds as a diastereomeric mixture. The mixture composed of 1.25:1 ratio of the compounds, which have been named K1115 $B_{1\alpha}$ and K1115 $B_{1\beta}$, respectively. Herein, we report the first total synthesis and the structural determination of K1115 B_1s (**2**).

Although the relative stereochemistry of the 1,3-dioxane moiety of K1115 B_1 has not been disclosed, similarity of the ^{1}H NMR data (chemical shifts & coupling constants) between K1115 B_1 and alnumycin reveals that the 1,3-dioxane moiety of K1115 B_1 should have the same relative stereochemistry of that of alnumycin (Fig. 1). Difficulty of the structural determination of K1115 B_1s is due to the distance of chiral centers between the C1 position and 1,3-dioxane. Therefore, we planed to synthesize the four diastereomers of **2** by convergent strategy coupling rhamnose derivative **3**, isobenzofuranone **4**, and diol **5** as shown in Scheme 1. The pyranonaphthoquinone skeleton would be synthesized by Michael–Dieckmann type reaction,[6–12] and the stereochemistry of the C1 carbon should be derived from C5 stereochemistry of rhamnose derivative **3**.

Synthesis of rhamnose derivative **3** and isobenzofuranone **4** is disclosed in Scheme 2. D-Rhamnal **6**[13] was oxidized to lactone **7**, which was subjected to hydrolysis with lipase[14] to afford (+)-osmundalactone (**8**)[15] in high yield. *O*-Methoxymethylation of **8** gave α,β-unsaturated lactone **3**. On the other hand, synthesis of isobenzofuranone **4** started from 2,5-dimethoxybenzaldehyde (**9**). Reduction of **9** gave alcohol **10**, which was subjected to the regioselective lithiation followed by carboxylation and the subsequent lactonization to yield **11**.[16] Regioselective de-*O*-methylation of **11** was performed by treatment with magnesium bromide and sodium iodide in refluxing acetonitrile. *O*-Benzylation of **12** afforded isobenzofuranone **4**.

Coupling of rhamnose derivative **3** and isobenzofuranone **4** was carried out by Michael–Dieckmann type cyclization (Scheme 3). Treatment of isobenzofuranone **4** with LHMDS (1.1 equiv) in THF at −78 °C for 10 min and addition of α,β-unsaturated lactone **3** to the resulting mixture (at −78 °C for 15 min) gave Michael addition adduct, to which was added another 1.1 equiv of LHMDS and the reaction mixture was stirred at room temperature for 1 h to complete Dieckmann condensation. Further addition of triethylamine

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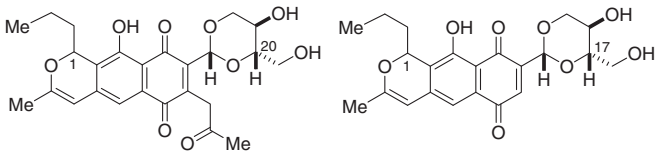
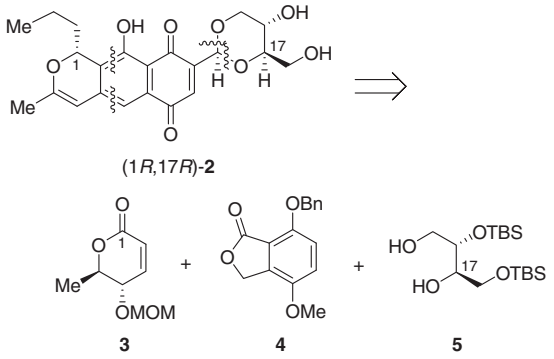


Figure 1. Structure of K1115 B_1.



Scheme 1. Synthetic plan of K1115 B_1 to determine the absolute structure.

and mesylchloride to the reaction mixture at −15 °C proceeded aromatization to give a pyranonaphthalene, which was protected as benzyl ether to afford pyranonaphthalene **13** in high yield. Reduction of lactone gave lactol **14**, of which allylation with allyltrimethylsilane proceeded smoothly in the stereoselective manner. The subsequent selective hydrogenation of *exo*-methylene gave propyl-attached pyranonaphthalene **15**, of which stereochemistry was determined by NOE between H3 and H11. Regioselective formylation of **15** accompanied with de-*O*-methoxymethylation to give alcohol **16**. The structure of **16** was determined by NOE between H7 and OMe. De-hydration with Burgess reagent afforded vinyl ether **17**. Acetalization of **17** with diol **5**[17] gave 1,3-dioxane **18** as a single isomer. The stereochemistry of the C15 position of **18** was determined by NOE (12.5% to C17). Treatment of **18** with Raney nickel in ethanol gave the labile hydroquinone monomethyl ether, which was found to be difficult to subject the direct oxidation into the corresponding quinone. After numerous experiments, we found that the unstable quinone monoacetal **20** was obtained in good yield by submitting the filtrate of the Raney nickel reduction to oxidation with [bis(trifluoroacetoxy)iodo]-benzene (PIFA). The structure of quinone monoacetal **20** was determined by NOE correlations between H4 and H5, H5 and H23, H23 and H7, as well as H7 and H15. Successive hydrolysis of quinone monoacetal **20** afforded quinone **21**. De-*O*-silylation with TBAF gave (1*R*, 17*R*)-K1115 B_1 ((1*R*, 17*R*)-**2**).

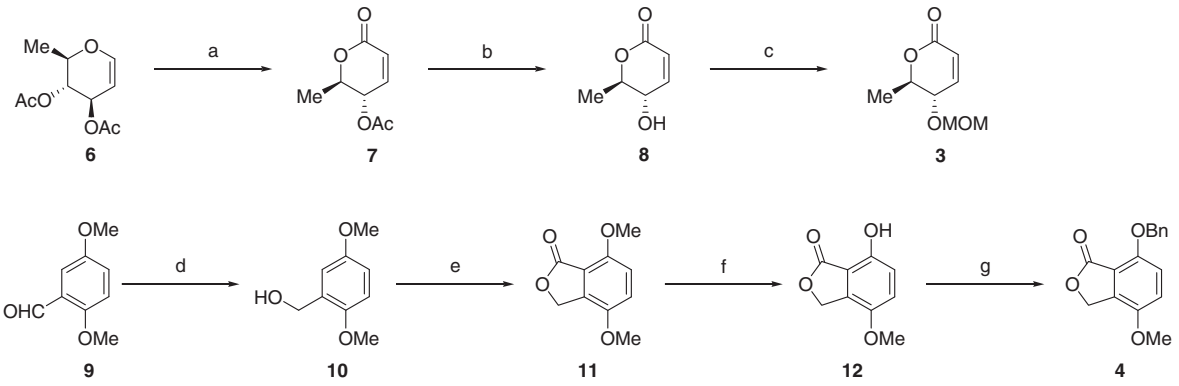
The other stereoisomers of **2** were also synthesized (Scheme 4). (1*R*, 17*S*)-K1115 B_1 ((1*R*, 17*S*)-**2**) was synthesized by coupling of aldehyde **17** and diol *ent*-**5**.[17] (1*S*, 17*R*)-K1115 B_1 ((1*S*, 17*R*)-**2**) and (1*S*, 17*S*)-K1115 B_1 ((1*S*, 17*S*)-**2**) were synthesized by coupling of aldehyde *ent*-**17** with diol **5** and *ent*-**5**, respectively.

The NMR spectra of the synthetic compounds were compared with those of the natural product, and it was found that the ^{1}H NMR spectrum of the natural product showed the mixture of diastereomers.[18]

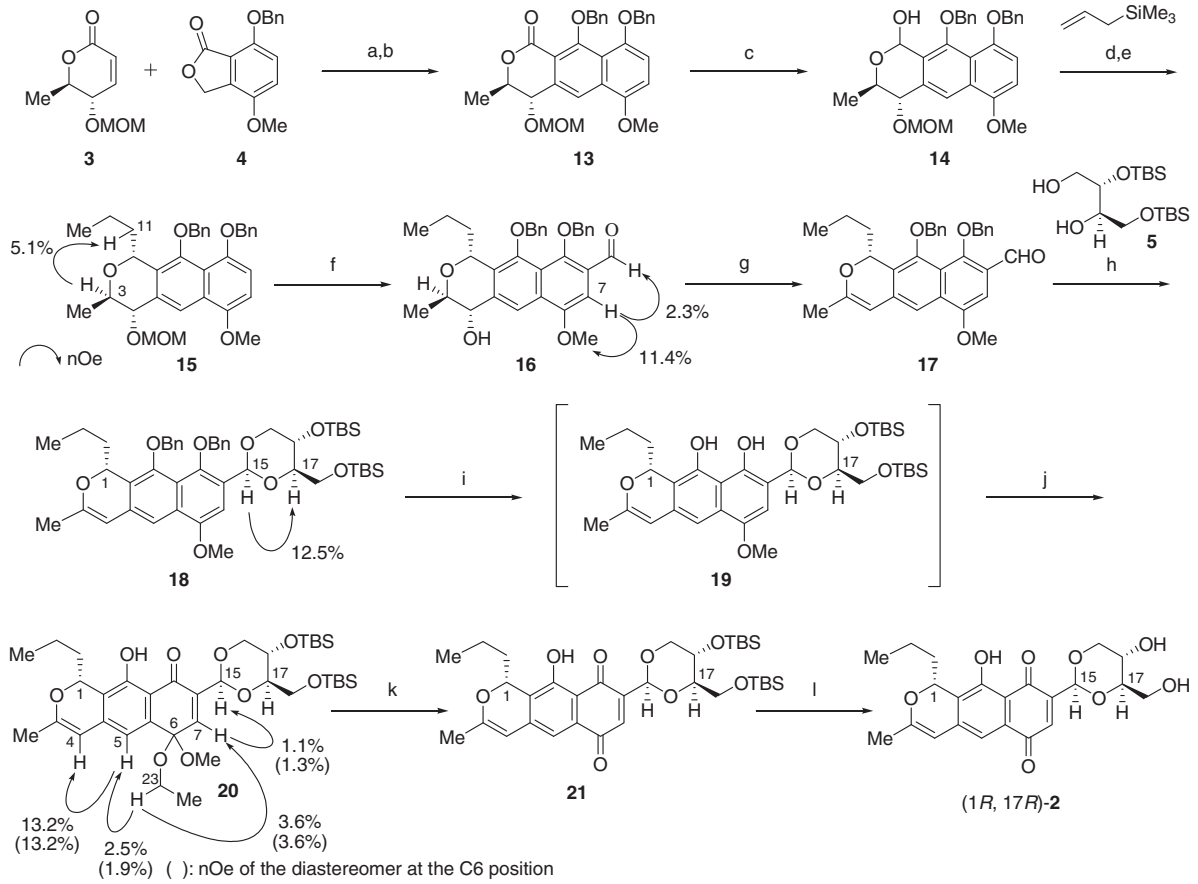
Determining the absolute structure of the natural products, we isolated the natural products from the broth of *S. griseorubiginosus* (Mer-K1115), the strain of Eisai group. Fermentation of the strain was based on the Eisai's method.[4] To the fermentation broth (2.0 L) was added ethyl acetate (2.0 L) and the mixture was stirred for 70 min at room temperature. After separation of the liquid phase from the mycelial mass by a centrifugation, the liquid phase was separated by a separatory funnel. The organic layer was dried over anhydrous sodium sulfate, filtrated, and evaporated. The residue (1.43 g) was purified by subsequent column chromatography involving SiO_2 column chromatography (hexane/2-propanol = 4:1 including 1% AcOH), LH20 chromatography (MeOH), and SiO_2 column chromatography (toluene/acetonitrile = 3:1) to give the mixture of diastereomers of **2** (27.9 mg, K1115 $B_{1\alpha}$:K1115 $B_{1\beta}$ = 1.25:1).[19]

The relative stereochemistry of the 1,3-dioxane moieties of the natural K1115 B_1s was determined by NOE (Fig. 2). Correlation between H15 and H17 as well as H15 and *axial*-H19 revealed that the relative stereochemistry of the 1,3-dioxane moieties of K1115 B_1s was identical with that of alnumycin. Comparing the ^{1}H NMR spectra of the natural K1115 B_1s and the synthetic compounds, (1*R**, 17*S**)-**2** was found to be the major isomer and (1*R**, 17*R**)-**2** to be the minor isomer (Fig. 2). Each *equatorial*-H19 of the diastereomers was unambiguously distinguished apart. H-1 and C10-OH (phenol) protons of the diastereomers were also observed individually. Additionally, the optical rotation of **2** revealed that the natural products were a mixture of (1*R*, 17*S*)- and (1*R*, 17*R*)-K1115 B_1 (**2**). Therefore, the structure of K1115 $B_{1\alpha}$ (the major product) was determined to be (1*R*, 17*S*)-configuration and K1115 $B_{1\beta}$ (the minor product) to be (1*R*, 17*R*)-configuration.[20]

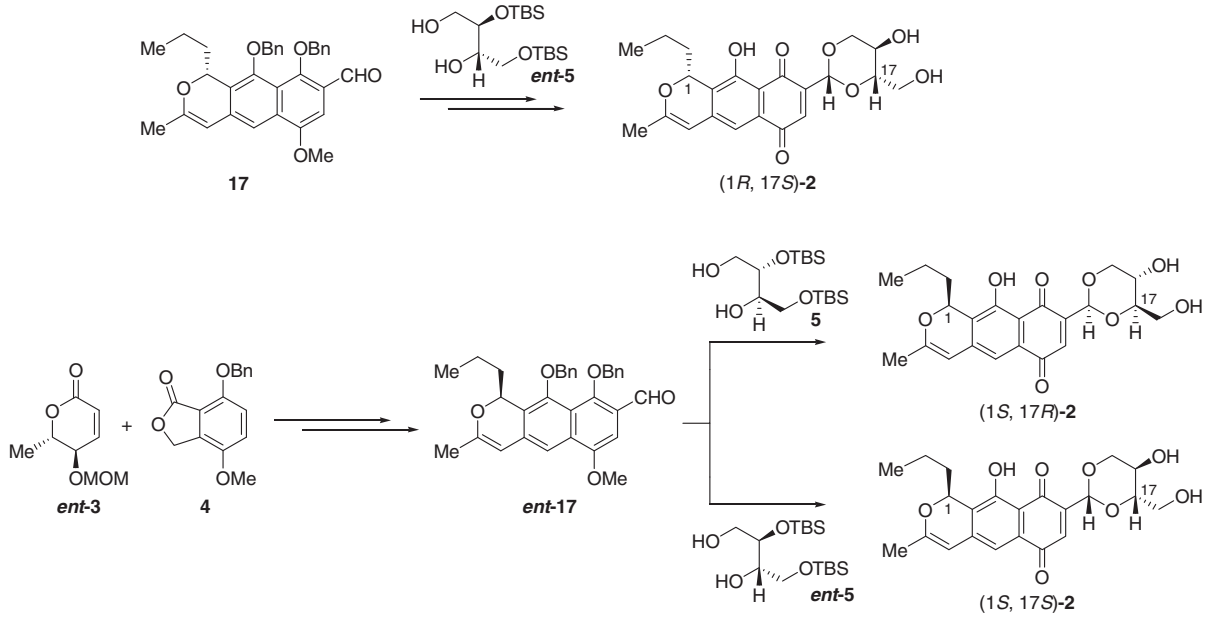
The optical rotation of each isomer of K1115 B_1s was around ±1000°,[20] while that of alnumycin was reported as $[\alpha]_D^{20}$ +170° (*c* 0.1 MeOH).[3] It indicates that alnumycin may have been a mixture of (1*R*, 17*R*)- and (1*S*, 17*R*)-**2** or (1*S*, 17*S*)- and (1*R*, 17*S*)-**2**.



Scheme 2. Reagents and conditions: (a) $BF_3{\cdot}Et_2O$, *m*CPBA, CH_2Cl_2, −20 °C, 20 min, 82%; (b) Lipase PS Amano SD, phosphate buffer (pH 8), rt, 2 d, 81%; (c) MOMCl, *i*-Pr_2NEt, $ClCH_2CH_2Cl$, 70 °C, 2 h, 88%; (d) $LiAlH_4$, THF, 0 °C, 20 min, 99%; (e) *n*-BuLi, THF, reflux, 3 h, CO_2, −78 °C to rt, 1 h, then 2 N HCl, rt, 3 h, 59%; (f) $MgBr_2$, NaI, MeCN, reflux, 6 h; (g) BnBr, K_2CO_3, 18-crown-6, DMF, rt, 1 h, 77% in two-steps.



Scheme 3. Reagents and conditions: (a) compound **4**, LHMDS, THF, −78 °C, 10 min, **3**, −78 °C, 15 min, addition of LHMDS, −78 °C to rt, 1 h, then MsCl, Et_3N, −15 °C, 5 min; (b) K_2CO_3, 18-crown-6, BnBr, acetone, reflux, 4 h, 89% in two-steps; (c) DIBAL, PhMe, −78 °C, 2 h; (d) TMSOTf, CH_2Cl_2, −78 °C, 5 min; (e) H_2, $RhCl(PPh_3)_3$, EtOH, rt, 1 h, 86% in three-steps; (f) Cl_2CHOMe, $SnCl_4$, CH_2Cl_2, −90 °C, 5 min, then HCl–MeOH, rt, 1.5 h, 53%; (g) Burgess reagent, THF, 50 °C, 1.5 h, 70%; (h) CSA, PhMe, rt, 1.5 h, 82%; (i) Raney Ni, EtOH, rt, 7 min; (j) PIFA, 0 °C, 10 min; (k) 50% TFA aq, $CHCl_3$, 0 °C, 10 min, 62% in three-steps; (l) TBAF, THF, 0 °C to rt, 20 min, 83%.



Scheme 4. Synthesis of (1*R*, 17*S*)-**2**, (1*S*, 17*R*)-**2**, and (1*S*, 17*S*)-**2**.

Alnumycin and prealnumycin, the aglycon of alnumycin, were produced by *Streptomyces* sp. CM020, which also produced K1115 A.[21] K1115 A was originally isolated with K1115 B_1s from the culture broth of *S. griseorubiginosus* (Mer-K1115).[4] Therefore, the biosynthesis of K1115 B_1s may be similar to that of alnumycin. Along the biosynthesis of alnumycin, K1115 $B_{1\alpha}$ and K1115 $B_{1\beta}$

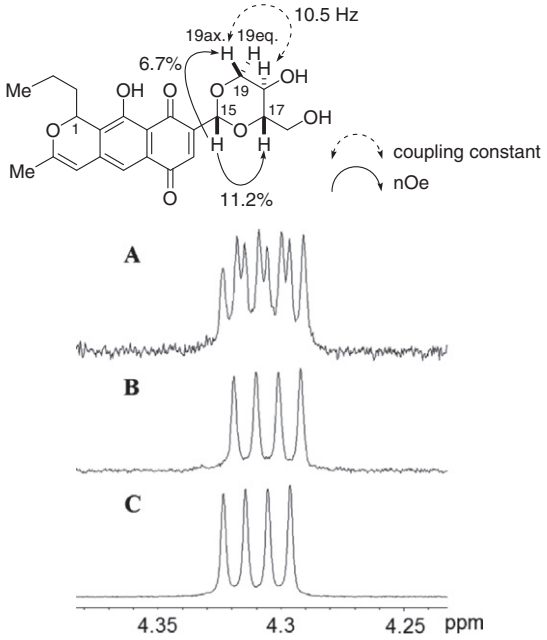


Figure 2. ^{1}H NMR spectrum of the *equatorial*-H19 of (A) natural K1115 B_1s, (B) (1*R*, 17*S*)-**2**, and (C) (1*R*, 17*R*)-**2**.

would originate in the different modes of acetalization with *meso*-erythritol and attachment of the 1,3-dioxane to prealnumycin.

We next examined the bioactivities of four compounds including (1*R*, 17*R*)-**2**, (1*R*, 17*S*)-**2**, (1*S*, 17*R*)-**2**, and (1*S*, 17*S*)-**2**.[22] Interestingly, all compounds showed almost the same antibacterial activities against Gram positive bacteria.

In conclusion, we have achieved the first total synthesis and structural determination of K1115 $B_{1\alpha}$ and K1115 $B_{1\beta}$ (**2**). Four isomers of **2** including (1*R*, 17*R*)-**2**, (1*R*, 17*S*)-**2**, (1*S*, 17*R*)-**2**, and (1*S*, 17*S*)-**2** were synthesized. By comparison of the ^{1}H NMR spectra and the optical rotation, the absolute structure of K1115 $B_{1\alpha}$ and K1115 $B_{1\beta}$ were determined to be (1*R*, 17*S*)- and (1*R*, 17*R*)-configuration, respectively. Alnumycin may have been a mixture of (1*R*, 17*R*)- and (1*S*, 17*R*)-**2** or (1*S*, 17*S*)- and (1*R*, 17*S*)-**2**.

Acknowledgments

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Supplementary data

Supplementary data (the spectrum data of compounds **3**, *ent*-**3**, **4**, **5**, *ent*-**5**, **13**, **15**, **16**, **17**, *ent*-**17**, **18**, (1*R*, 17*R*)-**2**, (1*R*, 17*S*)-**2**, (1*S*, 17*R*)-**2**, and (1*S*, 17*S*)-**2**, and ^{1}H NMR spectra (600 MHz in $CDCl_3$) of synthetic K1115 $B_{1\alpha}$, synthetic K1115 $B_{1\beta}$, and natural K1115 B_1s, as well as Table 1, which showed the antibacterial activities of four compounds ((1*R*, 17*R*)-**2**, (1*R*, 17*S*)-**2**, (1*S*, 17*R*)-**2**, and (1*S*, 17*S*)-**2**)) associated with this article can be found, in the online version, at doi:10.1016/j.tetlet.2010.12.061.

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17. For preparation of **5** and *ent*-**5**, see Supplementary data.
18. ^{1}H and ^{13}C NMR spectra of the natural product were provided by Eisai Co., Ltd.
19. The ratio of the diastereomers was determined by comparing the integration values of ^{1}H NMR at C10-O*H* (the proton of the phenol in hydrogen bonding) as well as the *equatorial*-H19.
20. Optical rotation; (1*R*, 17*R*)-**2**: $[\alpha]_D^{25}$ +1100° (*c* 0.10, MeOH), (1*R*, 17*S*)-**2**: $[\alpha]_D^{25}$ +1000° (*c* 0.14, MeOH), (1*S*, 17*R*)-**2**: $[\alpha]_D^{25}$ −900° (*c* 0.10, MeOH), (1*S*, 17*S*)-**2**: $[\alpha]_D^{25}$ −900° (*c* 0.13, MeOH). Variation in the number is due to the color of the samples. Each solution of **2** was red. The optical rotation of the natural product(s) was $[\alpha]_D^{27}$ +1000° (*c* 0.1, MeOH).
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22. See Table 1 in Supplementary data.