Misassigned Polyoxygenated Sterols and Reassignments of Their Structures

Yasunori Yaoita and Koichi Machida

Abstract

This review will summarize the authors’ studies on the reassignments of structures of 9 natural polyoxygenated sterols (24S)-24-ethylcholesterol-8-ene-3β,5α,6β,7α-tetrol (1), (24S)-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (2), (22E)-24-methylcholesta-8(14),22-diene-3β,5α,6β,7α-tetrol (3), (22E)-ergosta-7,22-diene-3β,7α,11α-triol (4), (22E)-ergosta-7,22-diene-3β,5α,6β,9α,14α-pentol (5), (24S)-24-ethylcholest-8(14)-ene-3β,5α,6β,7α-tetrol (6), (22E)-24-methylenecholesta-8,24(28)-diene-3β,7α,11α-triol (7), 5β,6β-epoxy-24-methylenecholesta-8,24(28)-diene-3β,7α,11α-triol (8), (22E)-24-methylenecholesta-8,24(28)-diene-3β,7α,11α-triol (9), and 5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (10). The structures of 1 to 9 have been reassigned as (24S)-5α,6α-epoxy-24-ethylcholesterol-8-ene-3β,5α,6β,7α-tetrol (11), (24S)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (12), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (13), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (14), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (15), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (16), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (17), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (18), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (19), and (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (20) (Figure 1). In this review, we summarize our studies on the reassignments of structures of 1 to 9.

Reassignments of Structures of (24S)-24-Ethylcholesterol-8-Ene-3β,5α,6β,7α-Tetrol, (24S)-24-Ethylcholesterol-8(14)-Ene-3β,5α,6β,7α-Tetrol, (22E)-24-Methylcholesta-8(14),22-Diene-3β,5α,6β,7α-Tetrol, and 3β,5α,6β,8β,14β-Pentaerythrytoidic (22E)-Ergost-22-En-7-One

First of all, we describe the structural reassignment of 4 sterols having 3β,5α,6β,7α-tetrahydroxylated and 3β,5α,6β,8β,14β-pentahydroxylated structures:

Sterols are among the most studied groups of natural products with interest commencing in the 19th century and continuing through to the present. Sterols occur in all major groups of organisms, from fungi to humans, as secondary metabolites. In a continuation of our studies on the chemical constituents from Japanese mushrooms, 28 new polyoxygenated sterols have been obtained and characterized. On the other hand, structural misassignments are prevalent in the literature. Determination of structure is a fundamental pillar of the discipline of chemistry. However, misinterpretations of spectroscopic and/or physical data have often been known to result in structural misassignments. Recently, we have reported the reassignments of structures of 5 natural polyoxygenated sterols: (24S)-24-ethylcholesterol-8-ene-3β,5α,6β,7α-tetrol (1), (24S)-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (2), (22E)-24-methylcholesta-8(14),22-diene-3β,5α,6β,7α-tetrol (3), (22E)-ergosta-7,22-diene-3β,7α,11α-triol (4), and (22E)-ergosta-7,22-diene-3β,5α,6β,9α,14α-pentol (5). The structures of 1 to 9 have been reassigned as (24S)-5α,6α-epoxy-24-ethylcholesterol-8-ene-3β,5α,6β,7α-tetrol (11), (24S)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (12), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (13), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (14), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (15), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (16), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (17), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (18), (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (19), and (22E)-5α,6α-epoxy-24-ethylcholesterol-8(14)-ene-3β,5α,6β,7α-tetrol (20) (Figure 1).

Keywords

polyoxygenated sterol, misassigned structure, structural reassignment, NMR

Received: October 27th, 2019; Accepted: January 10th, 2020.
(24S)-24-ethylcholest-8-ene-3β,5α,6β,7α-tetrol (1), (24S)-24-
eothylolest-8(14)-ene-3β,5α,6β,7α-tetrol (2), (22E)-24-
methylcholesta-8(14),22-diene-3β,5α,6β,7α-tetrol (3), and
3β,5α,6β,8β,14α-pentahydroxy-(22E)-ergost-22-en-7-one (6).

In 1995, Costantino et al.19 reported 2 new polyoxygenated
sterols 1 and 2 from the marine sponge Neofibularia nolitangere
(Figure 2). In 2006, Sun et al.20 reported a new one 3 from the
marine-derived fungus Penicillium sp. (Figure 2). Although there
is no report on the biological activities of 1 and 2, it is reported
that 3 has potent cytotoxicity against human liver cancer cell
(Hep G) with IC50 value of 10.4 µg/mL.20 The 3β,5α,6β,7α-tetrahydroxylated structures of 1 to 3 were proposed on the basis
of 1-dimensional (1D) and 2-dimensional (2D) nuclear
resonance methods.

Table 1. 1H NMR Chemical Shifts of Compounds 1, 2, 16, and 17
in CDCl3.

| No. | 1a | 2a | 16b | 17b |
|-----|----|----|-----|-----|
| 3   | 3.95 m | 3.91 m | 3.95 m | 3.91 m |
| 6   | 3.31 d (2.4)c | 3.15 d (3.5) | 3.31 d (2.5) | 3.15 d (3.5) |
| 7   | 4.22 br d (10.4) | 4.42 d (3.5) | 4.23 m (9.7 Hz) | 4.41 m (9 Hz) |
| 18  | 0.57 s | 0.85 s | 0.57 s | 0.85 s |
| 19  | 1.14 s | 0.86 s | 1.14 s | 0.86 s |
| 21  | 0.93 d (6.6) | 0.93 d (6.6) | 0.93 d (6.6) | 0.93 d (6.6) |
| 26  | 0.81 d (6.9) | 0.80 d (6.9) | 0.80 d (6.8) | 0.80 d (6.8) |
| 27  | 0.83 d (6.9) | 0.83 d (6.9) | 0.82 d (6.8) | 0.82 d (6.8) |
| 29  | 0.85 t (6.9) | 0.85 t (6.9) | 0.85 t (7.0) | 0.85 t (7.0) |

aData from Costantino et al.19
bData from Santafé et al.30
cCoupling constants (J in Hz) are given in parentheses.

dTable 2. 13C NMR Chemical Shifts of Compounds 1, 2, 16, and 17
in CDCl3.

| No. | 1a | 2a | 16b | 17b |
|-----|----|----|-----|-----|
| 1   | 30.2 | 32.2 | 30.1 | 32.1 |
| 2   | 30.9 | 31.1 | 30.8 | 31.1 |
| 3   | 68.6 | 68.7 | 68.5 | 68.6 |
| 4   | 39.2 | 72.6c | 39.1 | 39.5 |
| 5   | 65.6 | 67.8 | 65.6 | 67.7 |
| 6   | 62.6 | 61.3 | 62.5 | 61.3 |
| 7   | 67.1 | 65.1 | 67.1 | 65.1 |
| 8   | 126.9 | 125.1 | 126.8 | 125.0 |
| 9   | 134.8 | 38.7 | 134.5 | 38.6 |
| 10  | 38.0 | 35.8 | 37.9 | 35.8 |
| 11  | 23.4 | 19.1 | 23.3 | 18.9 |
| 12  | 35.8 | 36.7 | 35.7 | 36.6 |
| 13  | 42.2 | 43.1 | 42.1 | 43.1 |
| 14  | 49.5 | 152.7 | 49.5 | 152.6 |
| 15  | 23.9 | 25.0 | 23.8 | 24.9 |
| 16  | 28.8 | 26.6 | 28.7 | 26.6 |
| 17  | 53.7 | 56.7 | 53.6 | 56.6 |
| 18  | 11.1 | 17.9 | 11.0 | 17.9 |
| 19  | 22.8 | 16.6 | 22.8 | 16.5 |
| 20  | 36.6 | 35.0 | 36.6 | 34.9 |
| 21  | 18.8 | 19.0 | 18.7 | 18.9 |
| 22  | 33.8 | 33.6 | 33.7 | 33.6 |
| 23  | 26.4 | 26.2 | 26.4 | 26.2 |
| 24  | 46.0 | 46.1 | 46.0 | 46.0 |
| 25  | 28.9 | 28.9 | 28.9 | 28.9 |
| 26  | 19.0 | 19.0 | 18.9 | 19.0 |
| 27  | 19.6 | 19.6 | 19.5 | 19.5 |
| 28  | 23.0 | .d | 22.9 | 22.9 |
| 29  | 12.3 | 12.3 | 12.2 | 12.3 |

aData from Costantino et al.19
bData from Santafé et al.30
cThis chemical shift value was most likely a typographical error.
dNo data was reported in the literature.
magnetic resonance (NMR) data. During our studies on the sterol constituents from Japanese mushrooms, we reported 2 polyoxygenated sterols having the same A/B ring system as those of 1 to 3, (22E)-ergosta-8,22-diene-3β,5α,6β,7α-tetrol (10) and (22E)-ergosta-8(14),22-diene-3β,5α,6β,7α-tetrol (11), from the edible mushroom *Grifola frondosa* (Figure 2). Comparison of the NMR data of 1 and 2 for the A/B ring system with those of 10 and 11, respectively, indicated that the reported data do not fit with the proposed structures 1 and 2. Similarly, comparison of the NMR data of 3 for the A/B ring system with that of 11 indicated that the reported data also do not fit with the proposed structure 3. Consequently the structures for 1 to 3 are doubtful and reexamination of structures of 1 to 3 was necessary. Although compounds 1 and 2, and 10 and 11 differ in the structures of the side chain at C-17, we have already confirmed that the differences in the side chain at C-17 have little effect on the chemical shift values in the NMR spectrum derived from the A/B ring system of polyoxygenated sterols. In the

---

**Table 3.** 1H NMR Chemical Shifts of Compounds 3 and 13 in CDCl₃

| No. | 3ᵃ | 13ᵇ |
|-----|-----|-----|
| 3   | 3.92 m | 3.91 m |
| 6   | 3.15 d (2.5)ᶜ | 3.14 d (3.6) |
| 7   | 4.42 d (2.5) | 4.42 dd (9.6, 3.6) |
| 18  | 0.86 s | 0.86 s |
| 19  | 0.86 s | 0.86 s |
| 21  | 1.01 d (6.6) | 1.02 d (6.6) |
| 22  | 5.18 dd (15.2, 7.5) | 5.17 dd (15.3, 7.3) |
| 23  | 5.22 dd (15.2, 7.5) | 5.23 dd (15.3, 6.4) |
| 26  | 0.83 d (6.8) | 0.82 d (6.8) |
| 27  | 0.84 d (6.8) | 0.84 d (6.8) |
| 28  | 0.91 d (6.8) | 0.92 d (6.9) |

ᵃData from Sun et al. ᵇData from Ishizuka et al. ᶜCoupling constants (J in Hz) are given in parentheses. ⁴This coupling constant was derived from vicinal ¹H-¹H coupling between H-7 and OH-7.

---

**Table 4.** ¹³C NMR Chemical Shifts of Compounds 3 and 13 in CDCl₃

| No. | 3ᵃ | 13ᵇ |
|-----|-----|-----|
| 1   | 32.2 | 32.2 |
| 2   | 31.1 | 31.1 |
| 3   | 68.7 | 68.7 |
| 4   | 39.6 | 39.6 |
| 5   | 67.8 | 67.8 |
| 6   | 61.3 | 61.3 |
| 7   | 65.1 | 65.1 |
| 8   | 125.2 | 125.2 |
| 9   | 38.7 | 38.7 |
| 10  | 35.8 | 35.8 |
| 11  | 19.0 | 19.0 |
| 12  | 36.6 | 36.6 |
| 13  | 43.0 | 43.0 |
| 14  | 152.6 | 152.6 |
| 15  | 25.0 | 25.0 |
| 16  | 27.2 | 27.2 |
| 17  | 56.8 | 56.8 |
| 18  | 18.1 | 18.1 |
| 19  | 16.5 | 16.5 |
| 20  | 39.3 | 39.3 |
| 21  | 21.2 | 21.2 |
| 22  | 135.2 | 135.3 |
| 23  | 132.2 | 132.3 |
| 24  | 42.8 | 42.8 |
| 25  | 33.1 | 33.1 |
| 26  | 19.7 | 19.7 |
| 27  | 20.0 | 20.0 |
| 28  | 17.6 | 17.6 |

ᵃData from Sun et al. ᵇData from Ishizuka et al.
Table 5. $^1$H NMR Chemical Shifts of Compounds 6 and 18 in CDCl₃.

| No. | 6          | 18         |
|-----|------------|------------|
| 3   | 3.92 m     | 3.92 tt (11.4, 4.7) |
| 6   | 3.29 s     | 3.28 s     |
| 18  | 0.92 s     | 0.93 s     |
| 19  | 1.03 s     | 1.03 s     |
| 21  | 1.00 d (6.7) | 1.00 d (6.6) |
| 22  | 5.17 dd (15.3, 8.4) | 5.17 dd (15.2, 8.1) |
| 23  | 5.24 dd (15.3, 7.6) | 5.24 dd (15.3, 7.5) |
| 26  | 0.82 d (6.9) | 0.82 d (6.8) |
| 27  | 0.84 d (6.9) | 0.84 d (6.8) |
| 28  | 0.92 d (7.1) | 0.92 d (6.6) |

$^a$Data from Lee et al.$^{15}$
$^b$Data from Gao et al.$^{27}$
$^c$Coupling constants ($J$ in Hz) are given in parentheses.

$^1$H and $^{13}$C NMR data of 1 to 3, and 10 and 11 (supplemental Tables S1 and S2 for 10 and 11), there were large differences in the chemical shift values for skeletal positions C-3, C-5, C-6, C-7, and C-19. In particular, the $^{13}$C NMR chemical shift values $\delta_C$ 65.6 (C-5) and 62.6 (C-6) in 1, $\delta_C$ 67.8 (C-5) and 61.3 (C-6) in 2, and $\delta_C$ 67.8 (C-5) and 61.3 (C-6) in 3 were attributed to those of an epoxide ring,$^{22-27}$ suggesting that C-5 and C-6 of 1 to 3 were not connected to hydroxy groups. The $^{13}$C NMR chemical shift values for skeletal positions C-5 and C-6 of 1 to 3 were calculated using Kobayashi's parameters for polyhydroxysteroids.$^{28}$ Calculated values of C-5 and C-6 of 1 were $\delta_C$ 73.8 and 76.9, respectively. Those of 2 and 3 were $\delta_C$ 77.3 (C-5) and 80.4 (C-6). Kobayashi$^{28}$ concluded that deviations of 2.5 ppm between the predicted and calculated $\delta_C$ values would be sufficient for adoption, or rejection, of a particular structure. Deviations of C-5 and C-6 of 1 were 8.2 and 14.3 ppm, respectively, and those of 2 and 3 were 9.5 and 19.1 ppm. The calculated values confirmed that C-5 and C-6 of 1 to 3 were not connected to hydroxy groups. On the other hand, we reported 2 polyoxygenated sterols having an epoxide ring at C-5 and C-6, 5α,6α-epoxy-(22E)-ergosta-8,22-diene-3β,7α-diol (12) and 5α,6α-epoxy-(22E)-ergosta-8(14),22-diene-3β,7α-diol (13), from G. frondosa (Figure 2).$^{21}$ The functionality of the A/B ring

Table 6. $^{13}$C NMR Chemical Shifts of Compounds 6 and 18 in CDCl₃.

| No. | 6          | 18         |
|-----|------------|------------|
| 1   | 32.9       | 32.7       |
| 2   | 30.8       | 30.6       |
| 3   | 69.0       | 68.8       |
| 4   | 39.1       | 38.9       |
| 5   | 69.2       | 69.0       |
| 6   | 63.3       | 63.1       |
| 7   | 203.1      | 202.8      |
| 8   | 64.6       | 64.3       |
| 9   | 42.2       | 42.0       |
| 10  | 36.0       | 35.8       |
| 11  | 16.0       | 15.8       |
| 12  | 38.7       | 38.5       |
| 13  | 42.6       | 42.4       |
| 14  | 77.4       | 76.9       |
| 15  | 26.6       | 26.4       |
| 16  | 27.1       | 26.8       |
| 17  | 55.9       | 55.7       |
| 18  | 16.7       | 16.5       |
| 19  | 17.4       | 17.1       |
| 20  | 39.3       | 39.1       |
| 21  | 21.4       | 21.1       |
| 22  | 134.8      | 134.6      |
| 23  | 133.2      | 133.0      |
| 24  | 43.1       | 42.9       |
| 25  | 33.3       | 33.1       |
| 26  | 19.9       | 19.7       |
| 27  | 20.2       | 20.0       |
| 28  | 17.8       | 17.6       |

$^a$Data from Lee et al.$^{15}$
$^b$Data from Gao et al.$^{27}$
Yaoita and Machida

A system such as 12 and 13 was confirmed by the synthesis of 5α,6α-epoxycholest-8-ene-3β,7α-diol (14) from 5α,8α-epidioxy sterol utilizing microwave irradiation-induced isomerization as the key step and an x-ray diffraction experiment performed on 5α,6α-epoxycholest-8(14)-ene-3β,7α-diol-3,7-diacetate (15). The 1H and 13C NMR data of 12 and 13 for skeletal positions C-3, C-5, C-6, C-7, and C-19 were identical with those of 1 and 2, respectively. Also, the NMR data of 3 for skeletal positions C-3, C-5, C-6, C-7, and C-19 were identical with that of 13. In 2002, Santafé et al. reported 2 new sterols having the same A/B ring system as those of 12 and 13, (24S)-5α,6α-epoxy-24-ethylcholest-8-ene-3β,7α-diol (16) and (24S)-5α,6α-epoxy-24-ethylcholest-8(14)-ene-3β,7α-diol (17), from the marine sponge Polymastia tenax. The published 1H (Table 1) and 13C NMR (Table 2) data for 16 and 17 were in agreement with those of 1 and 2, respectively. Furthermore, the 1H (Table 3) and 13C NMR (Table 4) data of 13 were in agreement with those of 3. Thus, the structures of 1, 2, and 3 have been reassigned as 16, 17, and 13, respectively (Figure 3).

Table 7. 1H NMR Chemical Shifts of Compounds 4 and 12 in CDCl3.

| No. | 4a | 12b |
|-----|----|-----|
| 3   | 3.96 m | 3.95 m |
| 6   | 3.31 d (2.4)c | 3.31 d (2.6) |
| 7   | 4.22 br d (10.8) | 4.22 br d (10.4) |
| 18  | 0.59 s | 0.59 s |
| 19  | 1.14 s | 1.14 s |
| 21  | 1.02 d (6.4) | 1.02 d (6.6) |
| 22  | 5.13 dd (15.2, 5.2) | 5.14 dd (15.3, 5.8) |
| 23  | 5.22 dd (15.2, 8.0) | 5.21 dd (15.3, 6.9) |
| 26  | 0.82 d (6.8) | 0.82 d (6.8) |
| 27  | 0.84 d (6.4) | 0.84 d (6.6) |
| 28  | 0.91 d (6.8) | 0.91 d (6.8) |
| OH-7 | 1.76 d (10.8) | 1.77 d (10.4) |

aData from Su et al.31
bData from Ishizuka et al.21
cCoupling constants (J in Hz) are given in parentheses.

Table 8. 13C NMR Chemical Shifts of Compounds 4 and 12 in CDCl3.

| No. | 4a | 12b |
|-----|----|-----|
| 1   | 30.2 | 30.2 |
| 2   | 30.9 | 30.9 |
| 3   | 68.6 | 68.6 |
| 4   | 39.2 | 39.2 |
| 5   | 65.6 | 65.6 |
| 6   | 62.5 | 62.6 |
| 7   | 67.1 | 67.1 |
| 8   | 126.9 | 126.9 |
| 9   | 134.5 | 134.5 |
| 10  | 38.0 | 38.0 |
| 11  | 23.4c | 23.4 |
| 12  | 35.7 | 35.7 |
| 13  | 42.1 | 42.1 |
| 14  | 49.6 | 49.6 |
| 15  | 23.8 | 23.8 |
| 16  | 29.0 | 29.0 |
| 17  | 53.7 | 53.7 |
| 18  | 11.3 | 11.3 |
| 19  | 22.8 | 22.8 |
| 20  | 40.4 | 40.4 |
| 21  | 20.9 | 21.0 |
| 22  | 135.5 | 135.6 |
| 23  | 132.0 | 132.0 |
| 24  | 42.8 | 42.8 |
| 25  | 33.1 | 33.1 |
| 26  | 19.6 | 19.6 |
| 27  | 19.9 | 20.0 |
| 28  | 17.6 | 17.6 |

aData from Su et al.31
bData from Ishizuka et al.21
cSignals reassigned by the authors are indicated by underlining.

Figure 8. Reassignment of compound 4.

Figure 9. Structures of compounds 7 and 19.
Sterols having a $3β,5α,6β,7α$-tetrahydroxylated structure such as 10 and 11 did not show a molecular ion in the EI mass spectrum and showed the dehydration ion due to the loss of 1 molecule of $\text{H}_2\text{O}$ (supplemental material for 10 and 11). However, the dehydration ion of sterols having an above structure correspond to the molecular ion for the sterols having a $3β,7α$-dihydroxy-5α,6α-epoxy structure such as 12 and 13 (supplemental material for 12 and 13). The above-mentioned misreading of the MS data was observed in structure determination of 1 to 3. Careful interpretation of the MS data is an important point to be noticed in structure determination of polyoxygenated sterols in order to avoid deriving a wrong structure.

Furthermore, as a result of searching for literature related to the above polyoxygenated sterols, we found that the structure of $3β,5α,6β,8β,14α$-pentahydroxy-(22E)-ergost-22-en-7-one (6) from the liquid culture of the basidiomycete *Ganoderma applanatum* would need to be reassigned (Figure 4).

The molecular formula of 6 was determined to be $\text{C}_{28}\text{H}_{46}\text{O}_6$ by high-resolution (HR) fast atom bombardment mass spectrum ($m/z$ 443.3149, calcd 443.3161 for $[\text{M}+\text{H}]-2\text{H}_2\text{O}]^+$) and NMR data. Regarding compound 6, Lee et al. concluded that “The presence of five hydroxyls in the molecule was estimated from the ion peaks appearing at $m/z$ 442 [M - $2\text{H}_2\text{O}$]$^+$, 399 [(M - $2\text{H}_2\text{O}$) - $\text{C}_4\text{H}_8]^+$, 317 [(M - $2\text{H}_2\text{O}$) - side chain ($\text{C}_9\text{H}_{17}$)]$^+$, 299 [(M - $2\text{H}_2\text{O}$) - (side chain + $\text{H}_2\text{O}$)]$^+$, 281 [(M - $2\text{H}_2\text{O}$) - (side chain + $2\text{H}_2\text{O}$)]$^+$ and 263 [(M - $2\text{H}_2\text{O}$) - (side chain + $3\text{H}_2\text{O}$)]$^+$.

Figure 10. Conformations and NOEs (full-line arrows) for compounds 19 and 20.

Figure 11. Reassignment of compound 7.

Figure 12. Structures of compounds 5 and 22 to 24.
in the EI mass spectrum.7 However, as mentioned above, it is difficult to determine the number of hydroxy groups from the fragment ion peaks of the molecule such as polyhydroxy sterols in case no molecular ion was observed. On the other hand, Gao et al.27 reported a new sterol, 5α,6α;8α,14α-diepoxy-3β-hydroxy-(22E)-ergost-22-en-7-one (18), from the mangrove fungus Aspergillus awamori (Figure 4). The published 1H (Table 5) and 13C NMR (Table 6) data for 18 were in agreement with those of 6. Compound 6 did not show the molecular ion in the EI mass spectrum and showed the dehydration ion due to the loss of 2 molecules of H2O at m/z 442.15 However, the dehydration ion at m/z 442 in the EI mass spectrum of 6 corresponds to the molecular ion for 18.25 Therefore, the proposed structure of 6 has been reassigned as 18 (Figure 5).

Reassignments of Structures of 5β,6β-Epoxy-(22E)-Ergosta-8,22-Diene-3β,7β-Diol and 5β,6β-Epoxyergosta-8,24(28)-Diene-3β,7α,11α-Triol

In this section, we describe the structural reassignment of 2 polyoxygenated sterols having 3β,7β-dihydroxy-5β,6β-epoxy and 3β,7α,11α-trihydroxy-5β,6β-epoxy structures, 5β,6β-epoxy-(22E)-ergosta-8,22-diene-3β,7β-diol (4) and 5β,6β-epoxyergosta-8,24(28)-diene-3β,7α,11α-triol (7).

In 2016, Su et al.31 reported a new sterol 4 from the gorgonian Pinnigorgia sp. (Figure 6). Compound 4 was shown to significantly inhibit the accumulation of the proinflammatory inducible nitric oxide synthase and cyclooxygenase proteins in lipopolysaccharide (LPS)-stimulated RAW264.7 macrophage cells.31 The 3β,7β-dihydroxy-5β,6β-epoxy structure of 4 was proposed on the basis of 1D and 2D NMR data. As mentioned earlier, we already described the structure of 12 (Figure 6).21 The structural similarity between 4 and 12 is striking; however, the 5α,6α-epoxy-7α-hydroxy structure of 12 was substituted with a 5β,6β-epoxy-7β-hydroxy structure in 4. In contrast, a close investigation of the spectroscopic data for 4 revealed a level of equivalency with 12 that was inconsistent with this

![Figure 13. Pyridine-induced deshieldings (full-line arrows) in revised structure 25.](image)

![Figure 14. Reassignment of compound 5.](image)

![Figure 15. Structures of compounds 8 and 26.](image)

![Figure 16. NOEs (full-line arrows) for compound 8.](image)
structural divergence. Reassignment of the structure of 4 was therefore pursued.

Regarding compound 4, Su et al concluded that “comparison of these data with spectral data for a known sterol revealed that these two compounds are isomers except the configurations of 5,6-epoxy-7-hydroxy groups.” Key nuclear Overhauser effect spectroscopy (NOESY) correlations for 4 showed interactions between H-4β/H3-19 and H-4α/H-6. Also, H-7 showed a correlation with H-6. Thus, H-6 and H-7 should be located on the α-face and the oxygen of the 5,6-epoxide must be positioned on the β-face (Figure 7).

Although Su et al recorded nuclear Overhauser effect (NOE) correlation between H3-18 and H3-19, it is impossible for the structure (Figure 7). Furthermore, the NOESY correlations showed in 4 were also found to be the case for 12 (Figure 7).

The B ring of 12 adopts a boat-type conformation, as a result of the incorporation of 5α,6α-epoxide ring and double bond between C-8 and C-9 (Figure 7). When H-6 and H-7 were in pseudo-equatorial position and pseudo-axial position, respectively, of the boat-type conformation, the distances between H-4α and H-6, and H-6 and H-7 were close enough to produce the NOE correlation (Figure 7). The NOE was also observed between H3-19 and H-7. Further, the 1H (Table 7) and 13C NMR (Table 8) data of 4 were in agreement with those of 12.

From the above data, the structure of 4 has been reassigned as 12 (Figure 8).

Furthermore, as a result of searching for literature related to the above compound, we found that the structure of 5α,6β-epoxyergosta-8,24(28)-diene-3β,7α,11α-triol (7) from the marine sponge Dysidea herbacea would need to be reassigned (Figure 9).

Compound 7 was purified as the triacetate derivative 19 (Figure 9). Compound 19 was used to investigate its structure.

In the difference NOE experiment, irradiation of the signal of H-19 caused NOE enhancement in the signals of H-4β, H-7, H-11, and H3-18 (Figure 10). Furthermore, irradiation of the signal of H-6 caused NOE enhancement in the signals of H-4α and H-7 (Figure 10). Among these, the NOEs observed between H3-19 and H-7, H19 and H-11, and H19 and H3-18 are impossible for the structure 19. However, when the 5β,6β-epoxide ring of 19 was changed to the 5α,6α-epoxide ring (20), the distances between H19 and H-7, H19 and H-11, and H19 and H3-18 were close enough to produce the NOEs (Figure 10). Especially, as mentioned earlier, the NOE observed between H19 and H-7 implies that the B ring of 19 adopts a boat-type conformation, as a result of the incorporation of 5α,6α-epoxide ring and double bond between C-8 and C-9.

 Accordingly, compound 19 was revised to 20, and therefore, the structure of 7 has been reassigned as the new structure 5α,6α-epoxyergosta-8,24(28)-diene-3β,7α,11α-triol (21).

| Table 10. | 1H NMR Chemical Shifts of Compounds 8 and 26 in CDCl3. |
| --- | --- |
| No. | 8 | 26 |
| 3 | 4.08 m | 4.06 m |
| 6 | 4.84 d (5.2) | 4.84 d (5.0) |
| 7 | 5.26 d (5.3) | 5.25 d (5.0) |
| 18 | 0.59 s | 0.59 s |
| 19 | 1.06 s | 1.05 s |
| 21 | 1.02 d (6.6) | 1.02 d (6.5) |
| 22 | 5.14 dd (15.6, 8.1) | 5.16 dd (15.5, 8.0) |
| 23 | 5.20 dd (15.6, 7.5) | 5.23 dd (15.5, 7.0) |
| 26 | 0.82 d (7.2) | 0.83 d (6.5) |
| 27 | 0.83 d (7.2) | 0.85 d (6.5) |
| 28 | 0.91 d (6.8) | 0.93 d (7.0) |
| AcO-6 | 2.05 s | - |

aData from Qiao et al.17
bData from Zhang et al.38
cCoupling constants (J in Hz) are given in parentheses.
dSignals reassigned by the authors are indicated by underlining.
eNo data was reported in literature.

| Table 11. | 13C NMR Chemical Shifts of Compounds 8 and 26 in CDCl3. |
| --- | --- |
| No. | 8 | 26 |
| 1 | 32.4 | 32.4 |
| 2 | 29.7 | 30.6 |
| 3 | 67.4 | 67.3 |
| 4 | 39.2 | 39.2 |
| 5 | 75.3 | 75.3 |
| 6 | 73.4 | 73.4 |
| 7 | 114.1 | 114.1 |
| 8 | 145.7 | 145.7 |
| 9 | 43.8 | 43.4 |
| 10 | 37.3 | 37.2 |
| 11 | 22.0 | 22.0 |
| 12 | 39.2 | 39.2 |
| 13 | 43.8 | 43.8 |
| 14 | 54.9 | 54.9 |
| 15 | 22.7 | 22.8 |
| 16 | 27.8 | 27.8 |
| 17 | 55.9 | 56.0 |
| 18 | 12.3 | 12.3 |
| 19 | 18.2 | 18.2 |
| 20 | 40.4 | 40.3 |
| 21 | 21.1 | 21.1 |
| 22 | 135.4 | 135.4 |
| 23 | 132.2 | 132.2 |
| 24 | 42.8 | 42.8 |
| 25 | 33.1 | 33.1 |
| 26 | 19.6 | 19.6 |
| 27 | 19.9 | 19.9 |
| 28 | 17.6 | 17.5 |
| CH3CO-6 | 21.3 | 21.2 |
| CH2CO-6 | 170.4 | 170.5 |

aData from Qiao et al.17
bData from Zhang et al.38
cSignals reassigned by the authors are indicated by underlining.
Yaoita and Machida

The 3β,7α,11α-trihydroxy-5α,6α-epoxy moiety is unprecedented in the natural sterols previously known.

Reassignments of Structures of (22E)-Ergosta-7,22-Diene-3β,5α,6β,9α,14α-Pentol and 6β-Acetoxy-(22E)-10α-Ergosta-7,22-Diene-3β,5α-Diol

In this section, we describe the stereochemical reassignment of the hydroxy group at C-14 of (22E)-ergosta-7,22-diene-3β,5α,6β,9α,14α-pentol (5) and the methyl group at C-10 of 6β-acetoxy-(22E)-10α-ergosta-7,22-diene-3β,5α-diol (8).

Table 12. 1H NMR Chemical Shifts of Compounds 9 and 28 in CDCl₃.

| No. | 9'       | 28b   |
|-----|----------|-------|
| 3   | 4.26 m   | 4.28 tt (11.1, 5.6) |
| 6   | 5.95 d (10.0)  | 5.96 d (10.0)  |
| 7   | 5.17 d (10.0)  | 5.18 d (10.0)  |
| 18  | 0.92 s   | 0.93 s   |
| 19  | 1.10 s   | 1.12 s   |
| 21  | 1.02 d (6.5) | 1.02 d (6.7) |
| 22  | 5.19 dd (15.3, 7.7) | 5.19 dd (15.2, 7.5) |
| 23  | 5.24 dd (15.3, 7.0) | 5.26 dd (15.2, 7.6) |
| 26  | 0.80 d (7.0) | 0.82 d (6.7) |
| 27  | 0.83 d (7.0) | 0.84 d (6.7) |
| 28  | 0.89 d (6.6) | 0.92 d (6.7) |

aData from Li et al. 18
bData from Kikuchi et al. 39
Coupling constants (j in Hz) are given in parentheses.

In 2008, Zhang et al. 32 reported a new sterol 5 from the spores of the medicinal mushroom Ganoderma lucidum (Figure 12). The 3β,5α,6β,9α,14α-pentahydroxylated structure of 5 was proposed on the basis of NOESY spectrum. 32 However, the stereochemistry of the hydroxy group at C-14

Table 13. 13C NMR Chemical Shifts of Compounds 9 and 28 in CDCl₃.

| No. | 9'       | 28b   |
|-----|----------|-------|
| 1   | 25.0     | 24.9  |
| 2   | 29.7     | 29.6  |
| 3   | 66.8     | 66.7  |
| 4   | 42.1     | 42.0  |
| 5   | 73.0     | 72.9  |
| 6   | 140.8    | 140.7 |
| 7   | 124.3    | 124.2 |
| 8   | 67.5     | 67.4  |
| 9   | 75.7     | 75.6  |
| 10  | 42.3     | 42.3  |
| 11  | 28.4     | 28.3  |
| 12  | 28.3     | 28.2  |
| 13  | 42.3     | 42.2  |
| 14  | 84.2     | 84.1  |
| 15  | 25.7     | 25.6  |
| 16  | 26.7     | 26.6  |
| 17  | 52.1     | 52.0  |
| 18  | 15.5     | 15.5  |
| 19  | 21.6     | 21.5  |
| 20  | 39.5     | 39.4  |
| 21  | 21.1     | 21.0  |
| 22  | 134.6    | 134.5 |
| 23  | 132.9    | 132.8 |
| 24  | 42.8     | 42.8  |
| 25  | 33.1     | 33.0  |
| 26  | 19.7     | 19.6  |
| 27  | 20.4     | 19.9  |
| 28  | 17.6     | 17.5  |

aData from Li et al. 18
Data from Kikuchi et al. 39
Signals reassigned by the authors are indicated by underlining.
was tentatively assigned as α configuration based on the fact that the C/D ring system of ergostane-type sterols are generally trans-fused. During our studies on the sterol constituents from Japanese mushrooms, we reported a sterol having the same C/D ring system as that of 5, 3β,5α,9α,14α-tetrahydroxy-(22E)-ergosta-7,22-dien-6-one (22) from the edible mushroom *Pleurotus ostreatus* (Figure 12).33 Comparison of the 1H and 13C NMR data of 5 for the C/D ring system with those of 22 indicated that the reported NMR data do not fit well with the proposed structure 5. Although compounds 5 and 22 differ in the substituents at C-6, we have already confirmed that the differences in the substituent at C-6 have little effect on the chemical shift values in the NMR spectrum derived from the C/D ring system of polyoxygenated sterols.5 Therefore, we undertook a reexamination of the structure of 5.

In the 13C NMR data of 5 and 22 (supplemental Table S3 for 22), there were large differences in the chemical shift values for skeletal positions C-12, C-15, and C-17 (Table 9). In particular, the 13C NMR chemical shift values at C-12 and C-17 in 22 were attributed to the γ-gauche effect of the 14α-hydroxy group, as compared with 3β,5α,9α-trihydroxy-(22E)-ergosta-7,22-dien-6-one (23) (supplemental Table S3 for 23), suggesting that the difference in the C/D ring system between 5 and 22 was traced to differences in the stereochemistry of the hydroxy group at C-14. On the other hand, we reported a sterol, 6β-acetoxy-(22E)-ergosta-7,22-diene-3β,5α-diol (26), the epimer of 8 at C-10, from *Colletotrichum* sp., an endophytic fungus isolated from the island of Gomera (Figure 15). The published 1H (Table 10) and 13C NMR (Table 11) data for 26 were in agreement with those of 8. Therefore, the proposed structure of 8 has been reassigned as 26 (Figure 17).

![Figure 19. Reassignment of compound 9.](image)

Reassignment of Structure of 8α,9α-Epoxy-(22E)-Ergosta-6,22-Diene-3β,5α,14α-Triol

In this final section, we describe the structural reassignment of 8α,9α-epoxy-(22E)-ergosta-6,22-diene-3β,5α,14α-triol (9) to its regioisomer.

In 2015, Li et al.18 reported a new polyoxygenated sterol 9 from the fruiting bodies of edible mushroom *Herici um Erinaceum* (Figure 18). Compound 9 exhibited inhibitory activity against tumor necrosis factor α secretion in LPS-stimulated murine RAW264.7 macrophage cells.16 The 8α,9α-epoxy-3β,5α,14α-trihydroxylated structure of 9 was proposed on the basis of 1D and 2D NMR data.16 Regarding compound 9, Li et al.18 concluded that “Both 1H and 13C NMR spectroscopic data of 9 had some similarity to compound 27 (Figure 18), except for substituent groups at C-8, 9 and 14.” “A double bond between C-8 (δC 123.9) and C-14 (δC 149.0) was not observed, however, as it was oxidized to form the 8,9-epoxy (δC 67.5 and 75.5) and 14-OH (δC 84.2) moieties.” On the other hand, Kikuchi et al.39 reported 8α,14α-epoxy-(22E)-ergosta-6,22-diene-3β,5α,9α-triol (28), the regioisomer of 9, from the fruiting bodies of edible mushroom *Pleurotus eryngii* (Figure 18). The published 1H (Table 12) and 13C NMR (Table 13) data for 28 were in agreement with those of 9. Furthermore, Kikuchi et al.39 also reported that an x-ray diffraction analysis of 28 was conducted to confirm the position of the hydroxy and epoxy groups, and it established the relative
stereostructure of 28. From the above, the structure of 9 has been reassigned as 28 (Figure 19).

Conclusion
In this review, we summarized our studies on the reassignments of structures of 9 misassigned polyoxygenated sterols 1 to 9. The structural assignment of natural products supports research in multitude of disciplines that may lead to new therapeutic agents or new understanding of disease biology. However, structural misassignments of natural products are prevalent in the literature. Total synthesis can eventually and strongly support the real chemical structure of natural products. Only NMR assignment sometimes causes misassignment of the structures. Therefore, we want to point out the importance of careful interpretation of spectroscopic and/or physical data and eliminating preconceptions in interpretation of the data, especially when determining the structure of new compounds isolated from natural sources.

Author’s Note
This paper is dedicated to Professor Chiaki Kuroda on the occasion of his 65th birthday.

Declaration of Conflicting Interests
The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Funding
The author(s) received no financial support for the research, authorship, and/or publication of this article.

ORCID ID
Yasunori Yaoita https://orcid.org/0000-0002-0595-6701

Supplemental Material
Supplemental material for this article is available online.

References
1. Fieser LF, Fieser M. Steroids. New York: Reinhold; 1959:1-945.
2. Goad JL, Akihisa T. Analysis of Steroids. London: Blackie Academic & Professional; 1997:1-437.
3. Lednicer D. Steroid Chemistry at a Glance. West Sussex: Wiley; 2011:1-144.
4. Dewick PM. Medicinal Natural Products. West Sussex: Wiley; 2002:232-285.
5. Yaoita Y, Kikuchi M, Machida K. Terpenoids and sterols from some Japanese mushrooms. Nat Prod Commun. 2014;9(3):419-426.
6. Kutateladze AG, Kuznetsov DM, Beloglazkina AA, Holt T. Addressing the challenges of structure elucidation in natural products possessing the oxirane moiety. J Org Chem. 2018;83(15):8341-8352.
7. Kutateladze AG, Holt T, Reddy DS. Natural products containing the oxetane and related moieties present additional challenges for structure elucidation: A DU8+ computational case study. J Org Chem. 2019;84(12):7575-7586.
8. Kutateladze AG, Krenské EH, Williams CM. Reassignments and corroboration of oxo-bridged natural products directed by OSE and DU8+ NMR computation. Angew. Chem. Int. Ed. 2019;58(21):7107-7112.
9. Jensen WP, Palenik GJ, Suh IH. The history of molecular structure determination viewed through the Nobel Prizes. J Chem Educ. 2003;80(7):753-761.
10. Amagata T. Misassigned structures: Case examples from the past decade. In: Mander L, Liu H-W, eds. Comprehensive Natural Products II. London, United Kingdom: Elsevier; 2010:Volume 2. 581-621.
11. Yaoita Y, Kikuchi M, Machida K. Structure revision of two polyoxygenated sterols from the marine sponge Neofibularia multingirea. Nat Prod Commun. 2015;10(6):881-883.
12. Yaoita Y, Machida K. Structure Revision of (22E)-24-methylcholesta-8(14),22-diene-3β,5α,6β,7α-tetraol from the marine-derived fungus Penicillium sp. Nat Prod Commun. 2016;11(7):947-948.
13. Yaoita Y, Machida K. Structure revision of 5β,6β-epoxy-(22E)-ergosta-8,22-diene-3β,7β-diol from the gorgonian Pinnigorgia sp. Nat Prod Commun. 2017;12(8):1197-1198.
14. Yaoita Y, Machida K. Structure revision of (22E)-ergosta-7,22-diene-3β,5α,6β,9α,14α-pentol from the spores of the medicinal mushroom Ganoderma lucidum. Nat Prod Commun. 2016;11(2):183-184.
15. Lee SY, Kim JS, Lee S, Kang SS. Polyoxygenated ergostane-type sterols from the liquid culture of Ganoderma applanatum. Nat Prod Res. 2011;25(14):1304-1311.
16. Isaacs S, Berman R, Kashman Y, Ghebreysus T, Yosief T. New polyhydroxysterols, dysidamides, and a dideoxyhexose from the sponge Dysidea berthae. J Nat Prod. 1991;54(1):83-91.
17. Qiao M-F, Yi Y-W, Deng J. Steroids from an endophytic Eurotium rubrum strain. Chem Nat Compd. 2017;53(4):678-681.
18. Li W, Zhou W, Cha JY, et al. Sterols from Hericium erinaceum and their inhibition of TNF-α and NO production in lipopolysaccharide-induced RAW 264.7 cells. Phytochemistry. 2015;115:231-238.
19. Costantino V, Fattorusso E, Mangoni A, Pansini M. Sterols from the Caribbean sponge Neofibularia multingereae. Isolation of two novel polyhydroxysteroids. Steroids. 1995;60(11):768-772.
20. Sun Y, Tian L, Huang J, Li W, Pei Y-H. Cytotoxic sterols from marine-derived fungus Pennicillium sp. Nat Prod Res. 2006;20(4):381-384.
21. Ishizuka T, Yaoita Y, Kikuchi M. Sterol constituents from the fruit bodies of Grifola frondosa (Fr.) S. f. fr. Chem Pharm Bull. 1997;45(11):1756-1760.
22. Kobayashi M, Kanda F. Marine sterols. 18. Isolation and structure of four novel oxygenated sterols from a gorgonian coral Meldibara ocearea. J Chem Soc Perkin Trans 1. 1991;1991(5):1177-1179.
23. Greca MD, Fiorentino A, Molinaro A, Monaco P, Previtera L. Steroidal 5,6-epoxides from Arum Italicum. Nat Prod Lett. 1993;2(1):27-32.
24. Migliuolo A, Piccialli V, Sica D, Giordano F. New Δ8- and Δ24,5α,6α-epoxy-steroids from the marine sponge Spongia officinalis. Steroids. 1993;58(3):134-140.
25. Luo X, Li F, Shinde PB, et al. 26,27-cyclosterols and other polyoxygenated sterols from a marine sponge Topsentia sp. *J Nat Prod*. 2006;69(12):1760-1768.

26. Carvalho JFS, Cruz Silva MM, Moreira JN, Simões S, Sá E Melo ML. Efficient chemoenzymatic synthesis, cytotoxic evaluation, and SAR of epoxysterols. *J Med Chem*. 2009;52(13):4007-4019.

27. Gao H, Hong K, Chen G-D, et al. New oxidized sterols from *Aspergillus awamori* and the endo-boat conformation adopted by the cyclohexene oxide system. *Magn Reson Chem*. 2010;48(1):38-43.

28. Kobayashi M. Additivity relationships in the carbon-13 nuclear magnetic resonance spectra of polyhydroxy steroids. *J Chem Soc Perkin 1*. 1995;1995(1):33-40.

29. Ramesh P, Niranjan Reddy VL, Srinivasa Reddy N, Venkateswarlu Y. Synthesis of melithasterol A, a 5α,6α-epoxy-7α-hydroxy Δ8-steroid. *J Nat Prod*. 2000;63(10):1420-1421.

30. Santafé G, Paz V, Rodríguez J, Jiménez C. Novel cytotoxic oxygenated C29 sterols from the Colombian marine sponge *Polymastia tenax*. *J Nat Prod*. 2002;65(8):1161-1164.

31. Su Y-D, Cheng C-H, Wen Z-H, Wu Y-C, Sung P-J. New anti-inflammatory sterols from a gorgonian *Pinnigorgia* sp. *Bioorg Med Chem Lett*. 2016;26(13):3060-3063.

32. Zhang C-R, Yang S-P, Yue J-M. Sterols and triterpenoids from the spores of *Ganoderma lucidum*. *Nat Prod Res*. 2008;22(13):1137-1142.

33. Yaoita Y, Amemiya K, Ohnuma H, et al. Sterol constituents from five edible mushrooms. *Chem Pharm Bull*. 1993;41(1):87-89.

34. Zhang W, Draeger S, Schulz B, Krohn K. Ring B aromatic steroids from an endophytic fungus, *Colletorichum* sp. *Nat Prod Commun*. 2009;4(11):1449-1454.

35. Demarco PV, Farkas E, Doddrell D, Mylari BL, Wenkert E. Pyridine-induced solvent shifts in the nuclear magnetic resonance spectra of hydroxylic compounds. *J Am Chem Soc*. 1968;90(20):5480-5486.

36. Fujimoto Y, Yamada T, Ikekawa N. Pyridine-induced dishielding of 4-methylene protons for the determination of C-6 stereochemistry of sterols having a 5α,6-diol moiety. Revision of the C-6 stereochemistry of marine sterol isolated from a sponge, *Dysidea* sp. *Chem Pharm Bull*. 1985;33(8):3129-3133.

37. Kobayashi M, Krishna MM, Haribabu B, Anjaneyulu V. Marine sterols. XXV. Isolation of 23-demethylgorgost-7-ene-3β,5α,6β-triol and (24S)-ergostane-3β,5α,6β,7β,15β-pentol from soft corals of the Andaman and Nicobar coasts. *Chem Pharm Bull*. 1993;41(1):87-89.

38. Zhang W, Draeger S, Schulz B, Krohn K. Ring B aromatic steroids from a marine fungus, *Cyllanthus* sp. *Bioorg Med Chem*. 2011;19(22):6675-6671.

39. Nicolau KC, Snyder SA. Chasing molecules that were never there: Missassigned natural products and the role of chemical synthesis in modern structure elucidation. *Angew Chem Int Ed Engl*. 2005;44(7):1012-1044.