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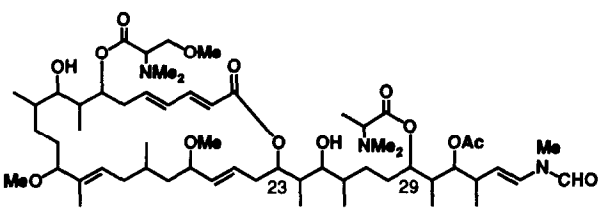
# Further Studies on Aplyronine A, an Antitumor Substance Isolated from the Sea Hare *Aplysia kurodai*

**Makoto Ojika, Hideo Kigoshi, Takeshi Ishigaki, and Kiyoyuki Yamada***

Department of Chemistry, Faculty of Science, Nagoya University, Chikusa, Nagoya 464, Japan

*Abstract*: By a series of reactions aplyronine A (**1**) an antitumor substance of marine origin was transformed into six fragments, and their structures were characterized by spectroscopic methods. Relative stereochemistry of four contiguous chiral centers (C29–C32) in **1** was established and that of three contiguous chiral centers (C23–C25) in **1** was deduced on the basis of the $^1$H NMR spectral analysis of the fragment **8** and the derived acetonide **9**, respectively.

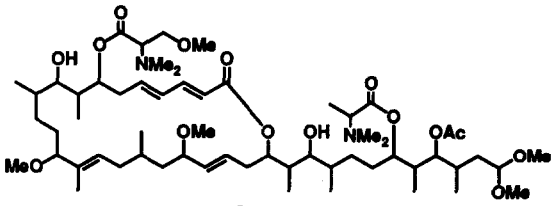
Aplyronine A (**1**) is a potent antitumor substance isolated from the sea hare *Aplysia kurodai* and its gross structure has been elucidated on the basis of the spectral data and degradation experiments.[1] To obtain further information on the chemical properties of aplyronine A (**1**), we have carried out the degradation experiments on **1**. We describe herein the preparation of the six fragments, **4**, **5**, **6a**, **6b**, **7**, and **8** from aplyronine A (**1**) together with their spectral characterization and report the stereochemical findings with respect to the fragment **8**.

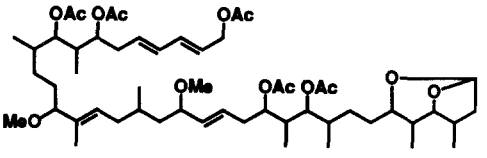


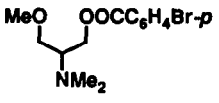

**1** aplyronine A

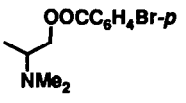
Selective hydrolysis of the *N*-methyl-*N*-vinylformamide group in **1** (0.5 M HCl, $H_2O$-dioxane, 50 °C) gave an aldehyde, which was converted ($(MeO)_3CH$, camphorsulfonic acid, MeOH, 23 °C) into acetal **2**[2] (87% from **1**). Reduction of **2** ($LiAlH_4$, ether, 23 °C) provided 2-dimethylamino-3-methoxypropanol, 2-dimethylaminopropanol, and a heptaol, treatment of which with acid ($CF_3COOH$, toluene, 23 °C) followed by acetylation ($Ac_2O$, DMAP, pyridine, 23 °C) afforded pentaacetate **3**[3] (35% from **2**). It is worthy to note that while the doubled NMR signals for some protons of aplyronine A (**1**) were observed because of the restricted rotation about the *N*-methyl-*N*-vinylformamide terminus (2:1 ratio) and the scalemic[4] property of two amino acid esters (1.1:1 and 3:1 for *N,N,O*-trimethylserine and *N,N*-dimethylalanine parts, respectively),[1] such doubled signals were no more observed in the $^1$H NMR spectrum of pentaacetate **3**. 2-Dimethylamino-3-methoxypropanol and 2-dimethylaminopropanol were converted (*p*-$BrC_6H_4COCl$, pyridine, 23 °C) into the corresponding *p*-bromobenzoates, **4**[1,5] and **5**,[1,6] respectively. Oxidative cleavage of the olefinic bonds in **3** (i. $OsO_4$, pyridine-THF, 0 °C. ii. $NaIO_4$, $H_2O$-EtOH, 23 °C) and subsequent reduction ($NaBH_3CN$, AcOH-MeOH, 23 °C) yielded a mixture of alcohols, which was converted (*p*-$BrC_6H_4NCO$, pyridine, 23 °C) into a

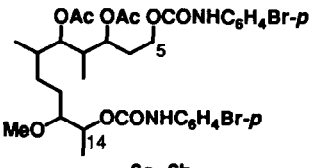
mixture of *p*-bromophenylurethanes. The mixture was separated by chromatography[7] to afford the C5–C14 fragments, **6a**[8] (19% from **3**) and **6b**[9] (26% from **3**) diastereomeric at C14, the C15–C20 fragment **7**[10] (31% from **3**) and the C21–C34 fragment **8**[11] (56% from **3**). The structures of these fragments **4**, **5**, **6a**, **6b**, **7**, and **8** were firmly established on the basis of the spectral data (cf. References and Notes).

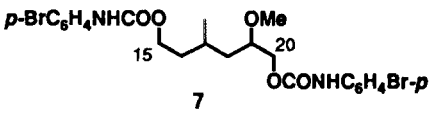


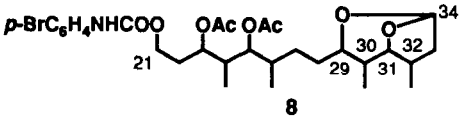



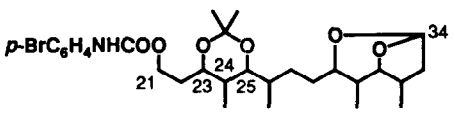



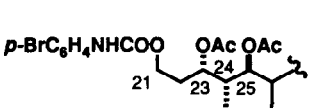



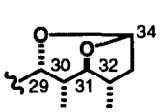

The stereochemistry of the dioxabicyclo[3.2.1]octane moiety in the fragment **8** was examined by the coupling constants and NOEs in the $^1$H NMR spectra of **8**, which are depicted in Figure 1. The relative stereochemistry of four contiguous chiral centers (C29–C32) in **8** was thus established to be *syn-anti-anti*, which is represented by the structure II.

The fragment **8** was converted by two steps (i. $K_2CO_3$, MeOH, 23 °C. ii. $Me_2C(OMe)_2$, camphorsulfonic acid, acetone, 0 °C) into acetonide **9**[12] in order to obtain the stereochemical information on the three contiguous chiral centers C23–C25. In the $^1$H NMR spectrum of **9** the coupling constants, $J_{23,24}$ and $J_{24,25}$ were shown to be 4.2 Hz and 7.3 Hz, respectively. The stereochemistry of C23–C24 and C24–C25 was deduced to be *syn* and *anti*, assuming that the acetonide ring in **9** adopted a somewhat distorted chair conformation. This assumption was supported by the computer-aided conformational analysis.[13] Thus, the relative stereochemistry of three contiguous chiral centers (C23–C25) in the fragment **8** was deduced to be represented by the structure I.

Further studies on the stereochemistry of aplyronine A (**1**) are currently in progress.



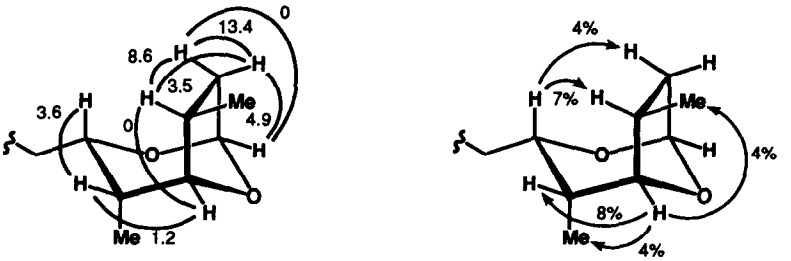
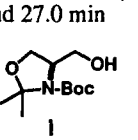



**Figure 1.** Coupling constants and NOEs in the $^1$H NMR spectra of **8**.

**Acknowledgment.** This work was supported in part by Grant-in-Aid for Scientific Research (No. 04403009 and 03640472) from the Ministry of Education, Science, and Culture, Japan, the Fujisawa Foundation, the Shorai Foundation for Science and Technology, and the Naito Foundation.

## REFERENCES AND NOTES.

1. Yamada, K.; Ojika, M.; Ishigaki, T.; Yoshida, Y.; Ekimoto, H.; Arakawa, M. *J. Am. Chem. Soc.* in press.
2. **2**: amorphous powder; $[\alpha]^{25}_D$ +44° (*c* 0.32, $CHCl_3$); UV (MeCN) $\lambda_{max}$ 261 nm ($\varepsilon$ 24100); IR ($CHCl_3$) 3680, 3600, 3490, 1730, 1695 (sh), 1645, 1620, 1245 cm$^{-1}$; FABMS *m/z* 1081 (M + H)$^+$; selected $^1$H NMR (500 MHz, acetone-$d_6$) $\delta$ 4.44 (dd, *J* = 8.1, 3.7 Hz, 1 H, H34), 3.30 (s, 3 H, OMe), 3.29 (s, 3 H, OMe).
3. **3**: oil; $[\alpha]^{24}_D$ –15° (*c* 0.16, $CHCl_3$); UV (MeCN) $\lambda_{max}$ 228 nm ($\varepsilon$ 20300); IR ($CHCl_3$) 1730, 1250 cm$^{-1}$; DCIMS *m/z* 961 (M + H)$^+$, 931, 900, 840, 717; selected $^1$H NMR (500 MHz, $CDCl_3$) $\delta$ 6.25 (dd, *J* = 15.2, 10.8 Hz, 1 H, H3), 6.06 (dd, *J* = 15.6, 10.4 Hz, 1 H, H4), 5.66 (dt, *J* = 15.2, 6.4 Hz, 1 H, H2), 5.64 (m, 1 H, H5), 5.40 (d, *J* = 4.8 Hz, 1 H, H34), 5.01 (ddd, *J* = 7.0, 7.0, 1.5 Hz, 1 H, H23), 4.88 (dd, *J* = 9.2, 2.0 Hz, 1 H, H9), 4.79 (dd, *J* = 9.6, 3.2 Hz, 1 H, H25), 4.68 (m, 1 H, H7), 4.57 (d, *J* = 6.4 Hz, 2 H, H1), 3.80 (d, *J* = 1.0 Hz, 1 H, H31), 3.73 (m, 1 H, H29), 2.07 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.02 (s, 3 H, Ac), 2.01 (s, 3 H, Ac), 1.99 (s, 3 H, Ac).
4. The term "scalemic" has been used to describe an unequal mixture of enantiomers: Heathcock, C. H.; Finkelstein, B. L.; Jarvi, E. T.; Radel, P. A.; Hadley, C. R. *J. Org. Chem.* **1988**, *53*, 1922–1942.
5. **4**: oil; IR ($CHCl_3$) 1720, 1590, 1270 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 7.89 (d, *J* = 8.6 Hz, 2 H), 7.58 (d, *J* = 8.6 Hz, 2 H), 4.49 (dd, *J* = 11.5, 5.6 Hz, 1 H), 4.39 (dd, *J* = 11.5, 5.6 Hz, 1 H), 3.59 (dd, *J* = 9.8, 5.6 Hz, 1 H), 3.49 (dd, *J* = 9.8, 5.6 Hz, 1 H), 3.37 (s, 3 H), 3.01 (m, 1 H), 2.43 (s, 6 H); EIMS *m/z* 317 (M + 2)$^+$, 315 (M$^+$), 272, 270, 185, 183; HREIMS *m/z* 317.0474 (M$^+$), calcd for $C_{13}H_{18}{}^{81}BrNO_3$ 317.0450. The ratio of enantiomers of **4** was determined by chiral HPLC analysis: column, CHIRALCEL OD (4.6 x 250 mm) (Daicel Chemical Ind., Ltd,); solvent, hexane/2-propanol 99.5:0.5; flow rate, 0.8 mL/min; detection at 254 nm. Two peaks were detected at 22.8 [(*S*)-**4**] and 27.0 min [(*R*)-**4**] in the ratio of 52:48. The authentic sample of (*R*)-**4** was prepared from alcohol **i** (Garner, P.; Park, J. M. *J. Org. Chem.* **1987**, *52*, 2361–2364) under the following reaction conditions: 1) NaH, MeI, THF, 23 °C. 2) *p*-TsOH, MeOH, 23 °C. 3) *p*-$BrC_6H_4COCl$, pyridine, 23 °C. 4) 0.6 M HCl, EtOAc-$H_2O$, 23 °C. 5) HCHO, HCOOH, $H_2O$, 70 °C. The specific rotation of (*R*)-**4**: $[\alpha]^{32}_D$ –2.12° (*c* 0.231, MeOH).



**i**

6. **5**: oil; IR ($CHCl_3$) 1715, 1590, 1270 cm$^{-1}$; $^1$H NMR (270 MHz, $CDCl_3$) $\delta$ 7.90 (d, *J* = 8.9 Hz, 2 H), 7.58 (d, *J* = 8.9 Hz, 2 H), 4.41 (dd, *J* = 11.2, 5.6 Hz, 1 H), 4.21 (dd, *J* = 11.2, 5.6 Hz, 1 H), 2.96 (m, 1 H), 2.35 (s, 6 H), 1.12 (d, *J* = 6.9 Hz, 3 H); EIMS *m/z* 287 (M + 2)$^+$, 285 (M$^+$), 272, 270, 185, 183; HREIMS m/z 285.0368 (M$^+$), calcd for $C_{12}H_{16}{}^{79}BrNO_2$ 285.0364. The ratio of enantiomers of **5** was determined by chiral HPLC analysis: column, SUMICHIRAL OA-4500 (4.0 x 250 mm) (Sumika Chemical Analysis Service, Ltd.); solvent, hexane/1,2-dichloroethane/EtOH/$CF_3COOH$ 80:15:5:0.2; flow rate, 1.0 mL/min; detection at 254 nm. Two peaks were detected at 35.0 [(*S*)-**5**] and 40.0 min [(*R*)-**5**] in the ratio of 72:28. The authentic sample of (*S*)-**5** was prepared from (*S*)-alaninol under the

following reaction conditions: 1) $(t\text{-BuOCO})_2O$, NaOH, $H_2O$-dioxane, 23 °C. 2) $p\text{-BrC}_6H_4COCl$, pyridine, 23 °C. 3) 1.5 M HCl, EtOAc-$H_2O$, 23 °C. 4) HCHO, HCOOH, $H_2O$, 80 °C. The specific rotation of (*S*)-**5**: $[\alpha]^{31}_D$ –0.33° (*c* 1.42, $CHCl_3$).

7. The mixture was separated by reversed-phase HPLC [column, Develosil ODS-10 (20 x 250 mm) (Nomura Chemical Co., Ltd.); solvent, MeOH/MeCN/$H_2O$ 57:26:17; flow rate, 8.0 mL/min; detection at 254 nm] to afford **7** (31%) and **8** (56%), and a mixture of **6a** and **6b**. The retention times (min): **6** (36.0–42.0), **7** (27.2), and **8** (25.2). The mixture of **6a** and **6b** was separated under the same conditions as described above except for the solvent (MeCN/$H_2O$ 75:25) to afford **6a** (19%) and **6b** (26%). The retention times (min): **6a** (42.8) and **6b** (46.1).

8. **6a**: oil; $[\alpha]^{17}_D$ +25° (*c* 0.082, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 244 nm (ε 44000); IR ($CHCl_3$) 3690, 3430, 3340 (br), 1730, 1595, 1520, 1250 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.9 Hz, 4 H, aromatic), 7.30 (m, 4 H, aromatic), 7.02 (br s, 1 H, NH), 6.81 (br s, 1 H, NH), 5.03 (dq, *J* = 4.0, 6.4 Hz, 1 H, H14), 4.93 (dd, *J* = 8.7, 3.0 Hz, 1 H, H9), 4.88 (ddd, *J* = 8.5, 7.5, 2.7 Hz, 1 H, H7), 4.26 (ddd, *J* = 10.8, 6.0, 5.0 Hz, 1 H, H5), 4.07 (m, 1 H, H5), 3.43 (s, 3 H, OMe), 3.14 (ddd, *J* = 7.4, 4.7, 4.0 Hz, 1 H, H13), 2.07 (m, 1 H, H8), 2.05 (s, 3 H, Ac), 2.00 (s, 3 H, Ac), 2.00 (m, 1 H, H6), 1.88 (m, 1 H, H6), 1.73 (m, 1 H, H10), 1.64 (m, 1 H, H11), 1.52 (m, 1 H, H12), 1.39 (m, 1 H, H12), 1.27 (d, *J* = 6.4 Hz, 3 H, 14-Me), 1.18 (m, 1 H, H11), 0.95 (d, *J* = 6.7 Hz, 3 H, 8-Me), 0.89 (d, *J* = 6.7 Hz, 3 H, 10-Me); HRFABMS *m/z* 757.1298 $(M + H)^+$, calcd for $C_{32}H_{43}{}^{79}Br_2N_2O_9$ 757.1335.

9. **6b**: oil; $[\alpha]^{17}_D$ +26° (*c* 0.10, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 244 nm (ε 44000); IR ($CHCl_3$) 3690, 3430, 3340 (br), 1730, 1595, 1520, 1250 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.40 (d, *J* = 8.5 Hz, 4 H, aromatic), 7.30 (m, 4 H, aromatic), 6.89 (br s, 1 H, NH), 6.83 (br s, 1 H, NH), 5.01 (dq, *J* = 3.5, 6.7 Hz, 1 H, H14), 4.93 (dd, *J* = 8.3, 3.0 Hz, 1 H, H9), 4.88 (ddd, *J* = 9.2, 8.5, 2.5 Hz, 1 H, H7), 4.23 (ddd, *J* = 10.8, 6.0, 5.0 Hz, 1 H, H5), 4.03 (m, 1 H, H5), 3.41 (s, 3 H, OMe), 3.17 (m, 1 H, H13), 2.07 (m, 1 H, H8), 2.06 (s, 3 H, Ac), 2.03 (s, 3 H, Ac), 2.00 (m, 1 H, H6), 1.88 (m, 1 H, H6), 1.73 (m, 1 H, H10), 1.63 (m, 1 H, H11), 1.61 (m, 1 H, H12), 1.40 (m, 1 H, H12), 1.26 (d, *J* = 6.7 Hz, 3 H, 14-Me), 1.13 (m, 1 H, H11), 0.95 (d, *J* = 7.0 Hz, 3 H, 8-Me), 0.89 (d, *J* = 7.0 Hz, 3 H, 10-Me); HRFABMS *m/z* 757.1361 $(M + H)^+$, calcd for $C_{32}H_{43}{}^{79}Br_2N_2O_9$ 757.1335.

10. **7**: amorphous powder; $[\alpha]^{17}_D$ –1.6° (*c* 0.13, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 244 nm (ε 42000); IR ($CHCl_3$) 3430, 1730, 1595, 1520, 1070 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.39 (d, *J* = 8.9 Hz, 2 H, aromatic), 7.38 (d, *J* = 8.9 Hz, 2 H, aromatic), 7.27 (d, *J* = 8.9 Hz, 2 H, aromatic), 7.26 (d, *J* = 8.9 Hz, 2 H, aromatic), 6.93 (br s, 1 H, NH), 6.85 (br s, 1 H, NH), 4.38 (dd, *J* = 11.8, 3.1 Hz, 1 H, H20), 4.26 (dt, *J* = 11.0, 6.5 Hz, 1 H, H15), 4.21 (dt, *J* = 11.0, 6.1 Hz, 1 H, H15), 4.10 (dd, *J* = 11.8, 5.2 Hz, 1 H, H20), 3.52 (m, 1 H, H19), 3.43 (s, 3 H, OMe), 1.78 (m, 1 H, H17), 1.70 (m, 1 H, H16), 1.63 (m, 1 H, H16), 1.55 (ddd, *J* = 14.0, 6.4, 6.4 Hz, 1 H, H18), 1.47 (ddd, *J* = 14.0, 7.0, 7.0 Hz, 1 H, H18), 1.00 (d, *J* = 6.7 Hz, 3 H, 17-Me); HRFABMS *m/z* 557.0294 $(M + H)^+$, calcd for $C_{22}H_{27}{}^{79}Br_2N_2O_5$ 557.0287.

11. **8**: oil; $[\alpha]^{15}_D$ –31.5° (*c* 0.40, $CHCl_3$); UV (MeOH) $\lambda_{max}$ 244 nm (ε 19000); IR ($CHCl_3$) 3410, 1730, 1590, 1515, 1250, 1075, 1075, 960 $cm^{-1}$; $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.9 Hz, 2 H, aromatic), 7.29 (d, *J* = 8.9 Hz, 2 H, aromatic), 6.78 (br s, 1 H, NH), 5.41 (d, *J* = 4.9 Hz, 1 H, H34), 5.10 (ddd, *J* = 7.8, 5.4, 2.3 Hz, 1 H, H23), 4.79 (dd, *J* = 9.5, 3.1 Hz, 1 H, H25), 4.21 (dt, *J* = 11.0, 6.4 Hz, 1 H, H21), 4.10 (dt, *J* = 11.0, 7.0 Hz, 1 H, H21), 3.80 (d, *J* = 1.2 Hz, 1 H, H31), 3.76 (ddd, *J* = 8.2, 4.6, 3.6 Hz, 1 H, H29), 2.29 (dd, *J* = 13.4, 8.6 Hz, 1 H, H33), 2.20 (m, 1 H, H32), 2.02 (s, 6 H, Ac), 2.00 (m, 1 H, H22), 1.99 (m, 1 H, H24), 1.88 (dddd, *J* = 14.2, 7.6, 6.4, 5.4 Hz, 1 H, H22), 1.78 (dddq, *J* = 10.0, 3.3, 3.1, 6.7 Hz, 1 H, H26), 1.52 (m, 1 H, H27), 1.48 (ddd, *J* = 13.4, 4.9, 3.5 Hz, 1 H, H33), 1.40 (m, 1 H, H28), 1.36 (m, 1 H, H30), 1.31 (m, 1 H, H28), 1.09 (d, *J* = 7.0 Hz, 3 H, 32-Me), 1.08 (d, *J* = 7.0 Hz, 3 H, 30-Me), 0.95 (d, *J* = 6.7 Hz, 3 H, 24-Me), 0.95 (m, 1 H, H27), 0.88 (d, *J* = 6.7 Hz, 3 H, 26-Me); HRFABMS *m/z* 612.2167 $(M + H)^+$, calcd for $C_{29}H_{43}{}^{79}BrNO_8$ 612.2172.

12. **9**: oil; selected $^1H$ NMR (500 MHz, $CDCl_3$) δ 7.41 (d, *J* = 8.7 Hz, 2 H, aromatic), 7.28 (d, *J* = 8.7 Hz, 2 H, aromatic), 6.57 (br s, 1 H, NH), 5.42 (d, *J* = 4.9 Hz, 1 H, H34), 4.27 (ddd, *J* = 11.0, 6.6, 4.6 Hz, 1 H, H21), 4.22 (ddd, *J* = 11.0, 8.2, 6.1 Hz, 1 H, H21), 3.91 (ddd, *J* = 10.2, 4.2, 3.5 Hz, 1 H, H23), 3.80 (d, *J* = 1.5 Hz, 1 H, H31), 3.78 (ddd, *J* = 6.4, 3.3, 3.3 Hz, 1 H, H29), 3.11 (dd, *J* = 7.3, 4.9 Hz, 1 H, H25), 2.31 (dd, *J* = 13.3, 8.3 Hz, 1 H, H33), 2.21 (m, 1 H, H32), 1.48 (ddd, *J* = 13.3, 4.9, 4.0 Hz, 1 H, H33), 1.31 (s, 3 H, acetonide Me), 1.30 (s, 3 H, acetonide Me), 1.09 (d, *J* = 6.7 Hz, 6 H, 30-Me and 32-Me), 0.96 (d, *J* = 7.0 Hz, 3 H, 26-Me), 0.85 (d, *J* = 6.7 Hz, 3 H, 24-Me); HRFABMS *m/z* 568.2272 $(M + H)^+$, calcd for $C_{28}H_{43}{}^{79}BrNO_6$ 568.2274.

13. The global minimum was calculated in a Multiconformer conformational search using MacroModel (Version 2.0). We are grateful to Professor W. Clark Still for allowing us to use the program through Prof. S. Murata (Graduate School of Human Informatics, Nagoya University) and informing us of the following literature: Mohamadi, F.; Richards, N. G. J.; Guida, W. C.; Liskamp, R.; Lipton, M.; Caufield, C.; Chang, G.; Hendrickson, T.; Still, W. C. *J. Comp. Chem.* **1990**, *11*, 440.

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