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# Direct Assignment of the *Threo* and *Erythro* Configurations in Polyacetylene Glycosides by $^1$H NMR Spectroscopy

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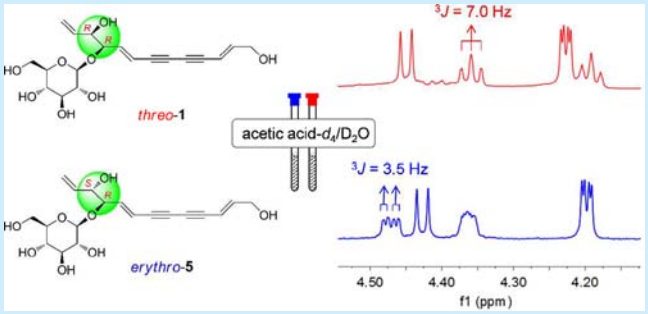
Supporting Information

**ABSTRACT:** An approach for discriminating the *threo* and *erythro* configurations of polyacetylene glycosides by $^1$H NMR spectroscopy was developed. Using acetic acid-$d_4$/$D_2O$ as the solvent, a relatively larger $^3J_{HH}$ value (7.0 Hz) for the acyclic vicinal diol group was unambiguously assigned to the *threo* configuration, whereas the smaller value (3.5 Hz) was assigned to the *erythro* configuration. This convenient method requires no hydrolysis or derivatization and is suitable for micromolar concentrations of polyacetylene glycosides. The underlying mechanism is discussed via visualized conformations.




As a class of naturally occurring open-chain compounds with two or more conjugated acetylenic and olefinic bonds, polyacetylenes are widely distributed in Araliaceae, Asteraceae, Campanulaceae, Pittosporaceae, Umbelliferae, etc.[1−5] Acyclic vicinal diol groups occur frequently in these compounds due to biological oxidation of their conjugated olefinic bonds. To date, approximately 80 such compounds have been identified[6−11] whose configurational assignments are arduous. For typical cyclic compounds with three- to six-membered rings displaying predictable conformational behavior, the relative configurations can be deduced using simple NMR parameters such as the $^3J_{HH}$ values and/or the nuclear overhauser effect (NOE) intensities.[12] However, the task of determining the relative configurations of conformationally flexible systems is significantly more challenging. In the last two decades, *J*-based configurational analysis has led to the assignment of two adjacent stereogenic carbons of an acyclic chain using a complete set of $^3J_{HH}$ and $^{2,3}J_{HC}$ values and key NOE data. However, the analytic procedure is tedious and requires a high-resolution spectrometer to obtain optimal results. Furthermore, to the best of our knowledge, the direct applicability of this method to glycosides lacks sufficient evidence and might lead to errors.[12−14] Higashibayashi and Kishi also examined 1,2-diols as one of many studies in creating their databases using trends in shift differences to determine relative stereochemistry, which needs a chiral bidentate NMR solvent.[15] Mosher's method provides another stereochemical solution but requires hydrolysis and derivatization and is thus unsuitable for minor glycosides.[16,17] Fortunately, a convenient and reliable NMR approach for discriminating the *threo* and *erythro* configurations of the acyclic vicinal diol group in polyacetylene glycosides was developed during our NMR analysis of natural compounds.

Recently, research regarding the chemical constituents of *Atractylodes lancea* led to the isolation of 18 new polyacetylene glycosides and a new polyacetylene aglycone containing an acyclic vicinal diol group, and these compounds contained linear (**1**−**14**), thiophene (**15**−**17**), and furan (**18** and **19**) skeletons. Their planar structures (Figure 1) were elucidated through spectroscopic and spectrometric analyses (UV, IR, 1D and 2D NMR, and HRESIMS). When assigning their relative configurations, we found that the $^3J_{HH}$ values of these acyclic vicinal diol groups in DMSO-$d_6$ were uninterpretable (Table 1) and that it was impossible to distinguish the *threo* and *erythro* configurations. To explore this issue, a detailed and comprehensive literature survey disclosed that the $^3J_{HH}$ values of acyclic vicinal diol groups seemingly follow an empirical rule in $CDCl_3$: a relatively larger value (more than 6.0 Hz) corresponds to a *threo* configuration, whereas a smaller value (less than 5.0 Hz) corresponds to an *erythro* configuration.[18−32] However, the poor solubility of these natural glycosides in $CDCl_3$ limited the direct application of this rule. Furthermore, preparation of the aglycones by acid hydrolysis of the minor polyacetylenes containing more than one sugar moiety was difficult due to structural instability. Thus, is there an appropriate polar solvent that can be employed to discriminate the *threo* or *erythro* configurations of these natural glycosides? Interestingly, in our previous experiments directed toward establishing the structures of dibenzoyl glycosides, acetic acid-$d_4$, which is seldom used for NMR experiments, was found to be an excellent deuterated solvent for collecting quality spectra that also display a similar spectroscopic tendency as the nonpolar $CDCl_3$ solvent.[33−35] This finding inspired us to

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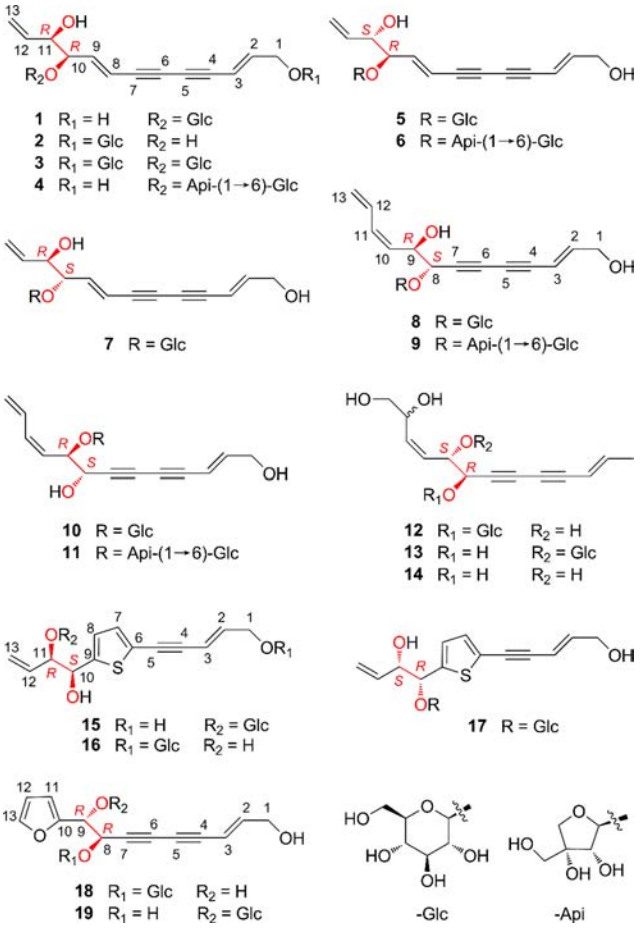


**Figure 1.** Compounds **1**–**19** isolated from *A. lancea*.

**Table 1. $^3J_{HH}$ Values (Hz) of the Acyclic Vicinal Diol Group of Compounds 1–19 in Different Solvents**

| no. | DMSO-$d_6$ | methanol-$d_4$ | $D_2O$ | acetic acid-$d_4$ solution (1:1) | assignment |
|---|---|---|---|---|---|
| **1** | 5.0 | 5.5 | 6.5 | 7.0 | *threo* |
| **2** | 5.0 | 5.5 | 6.0 | 6.5 | *threo* |
| **3** | 5.0 | 6.0 | 6.5 | 7.0 | *threo* |
| **4** | 5.0 | 5.0 | 6.0 | 6.5 | *threo* |
| **15** | 5.0 | 5.5 | 6.0 | 7.0 | *threo* |
| **16** | 5.5 | 6.0 | 6.0 | 6.5 | *threo* |
| **17** | 5.0 | 5.5 | 6.5 | 7.0 | *threo* |
| **5** | 5.0 | 3.5 | 4.0 | 3.5 | *erythro* |
| **6** | 5.0 | 4.0 | *a* | 3.5 | *erythro* |
| **7** | 5.0 | 4.0 | *a* | *a* | *erythro* |
| **8** | 3.5 | 3.5 | 4.0 | 3.5 | *erythro* |
| **9** | 3.5 | 3.5 | 4.0 | 3.5 | *erythro* |
| **10** | 5.0 | 4.0 | 4.5 | 3.5 | *erythro* |
| **11** | 5.0 | 3.5 | 4.0 | 3.5 | *erythro* |
| **12** | 5.0 | 3.5 | 3.5 | 3.5 | *erythro* |
| **13** | 6.0 | 4.0 | 4.0 | 3.5 | *erythro* |
| **14** | 5.0 | 5.0 | 4.5 | 4.5 | *erythro* |
| **18** | 4.5 | 4.5 | *a* | 4.0 | *erythro* |
| **19** | 6.5 | 5.0 | 5.0 | 4.0 | *erythro* |

[a]The signals were overlapped or broad.

thoroughly investigate the applicability of acetic acid-$d_4$ for solving the stereochemistry of polyacetylene glycosides.

Due to the poor solubility of the glycosides in acetic acid-$d_4$, a mixture of acetic acid-$d_4$ and $D_2O$ (v/v = 1:1) was first used for the $^1H$ NMR experiments. Surprisingly, the $^3J_{HH}$ values of the acyclic vicinal diol groups in all of the compounds were clearly divided into two categories: approximately 7.0 or 3.5 Hz (Table 1). To distinguish the configurational assignments of these values, several typical glycosides (**1**, **5**, **8**, **10**, **15**, and **17**) were hydrolyzed by snailase (Supporting Information, S11). The corresponding aglycones (**1a**, **5a**, **8a**, **10a**, **15a**, and **17a**) were examined by $^1H$ NMR experiments using $CDCl_3$ as the solvent. Gratifyingly, the $^3J_{HH}$ values of these aglycones (in $CDCl_3$) showed a consistent trend with those of the glycosides recorded in acetic acid-$d_4$/$D_2O$ (v/v = 1:1) (Tables 1 and 2). In

**Table 2. $^3J_{HH}$ Values (Hz) of the Acyclic Vicinal Diol Group of 1a, 5a, 8a, 10a, 14, 15a, and 17a in Different Solvents**

| no. | DMSO-$d_6$ | methanol-$d_4$ | $CDCl_3$ | acetic acid-$d_4$ solution (1:1) | assignment |
|---|---|---|---|---|---|
| **1a** | 5.0 | 6.0 | 6.5 | 6.5 | *threo* |
| **15a** | 5.0 | 6.0 | 6.5 | 6.5 | *threo* |
| **17a** | 5.0 | 6.0 | 6.5 | 6.5 | *threo* |
| **5a** | 5.0 | 5.5 | 5.0 | 5.0 | *erythro* |
| **8a** | 5.5 | 5.0 | 4.0 | 4.0 | *erythro* |
| **10a** | 5.5 | 5.0 | 4.0 | 4.0 | *erythro* |
| **14** | 5.0 | 5.0 | *a* | 4.5 | *erythro* |

[a]This compound was not soluble in $CDCl_3$.

particular, compared to **1**, **15**, and **17**, the $^3J_{HH}$ values of **5**, **8**, and **10** were significantly smaller than those of their aglycones. Therefore, the larger $^3J_{HH}$ value (7.0 Hz) of **1**–**4** and **15**–**17** could be preliminarily assigned to the *threo* configuration, whereas the smaller value (3.5 Hz) of **5**–**14**, **18**, and **19** could be assigned to the *erythro* configuration (Figure 2).

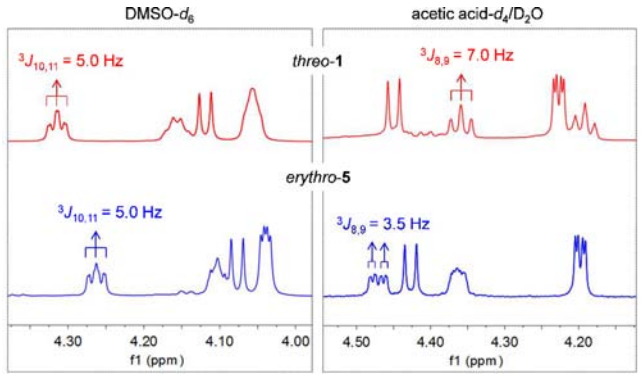


**Figure 2.** $^3J_{HH}$ value of *threo*-**1** and *erythro*-**5** in DMSO-$d_6$ and acetic acid-$d_4$/$D_2O$ (v/v = 1:1).

To further verify the above relative stereochemical assignments of the linear polyacetylenes (**1**–**14**), two acetonide derivatives, **1b** and **5b**, were prepared from the aglycones (*threo*-**1a** and *erythro*-**5a**) and 2,2-dimethoxypropane (Supporting Information). Diagnostic NOE correlations were observed for H-10 with $H_3$-15 and for H-11 with $H_3$-16 in **1b**, which indicated that H-10 and H-11 were positioned on opposite sides of the five-membered ring, whereas the correlations from $H_3$-15 to H-10 and H-11 in **5b** revealed that H-10 and H-11 were on the same side (Figure 3). This configuration was also supported by the more downfield resonances of H-10 and H-11 in **5b** compared to **1b** (Figure 3). This evidence favors the *threo*



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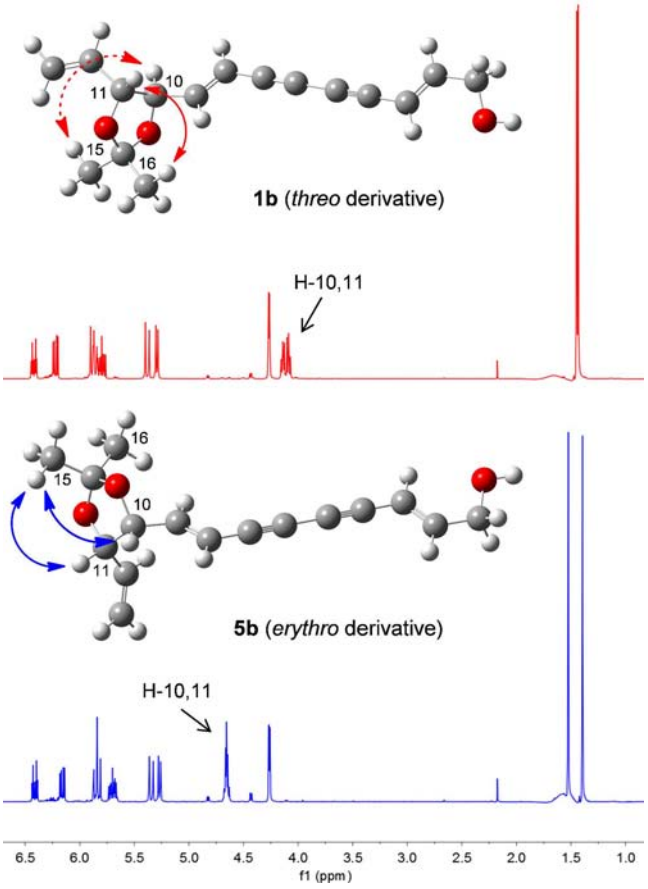


**Figure 3.** $^1$H NMR spectra and key NOE correlations (double arrows) of **1b** and **5b** in $CDCl_3$.

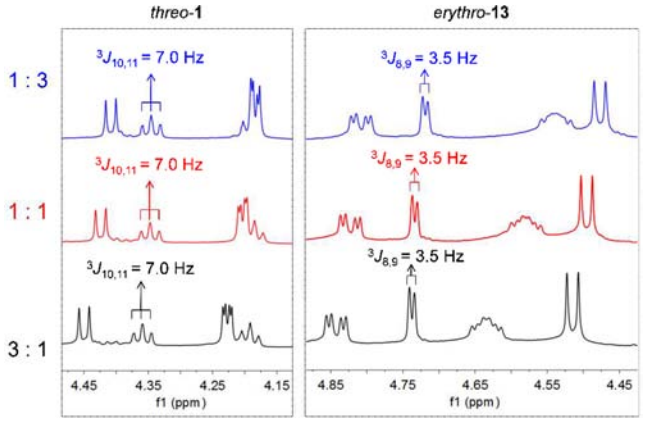


**Figure 4.** $^3J_{HH}$ values of *threo*-**1** and *erythro*-**13** in acetic acid-$d_4$/$D_2O$ with different proportions (v/v).

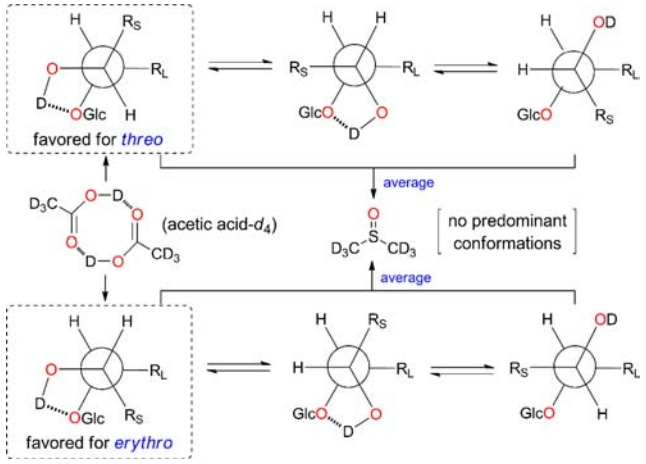


**Figure 5.** Conformational analyses of the *threo*- and *erythro*-configurations in acetic acid-$d_4$ and DMSO-$d_6$.

configuration of **1** and *erythro* configuration of **5**. For the thiophene polyacetylenes (**15**−**17**), the *threo* configuration was further confirmed by the NOE intensities of the acetonide derivative **15b**, which was produced from the typical aglycone **15a** by a similar procedure as **1b** (Supporting Information, S12). Due to the limited quantities of sample, the *erythro* configurational assignments of the furan polyacetylenes (**18** and **19**) were verified by electronic circular dichroism (ECD) calculations using an MMFF94 force field and time-dependent density functional theory at the B3LYP/6-31+G(d,p) level (Supporting Information, S13).

Other common deuterated solvents and ratios of acetic acid-$d_4$ were also investigated to further explore this phenomenon. Under the same test conditions, the $^1$H NMR spectra of **1**−**19** were recorded in methanol-$d_4$ and $D_2O$. Additionally, those of **1** and **13** were measured in a mixture of acetic acid-$d_4$ and $D_2O$ with different proportions (v/v = 1:3, 1:1, and 3:1). The differences between the $^3J_{HH}$ values for the *threo* and *erythro* configurations in methanol-$d_4$ and $D_2O$ were slightly better than in DMSO-$d_6$ but were still smaller than the differences observed in the acetic acid-$d_4$ solution (Table 1). Note that the $^3J_{HH}$ values of *threo*-**1** and *erythro*-**13** remained unchanged when the proportions of acetic acid-$d_4$ (Figure 4) were varied.

Such $^3J_{HH}$ differences in the polyacetylene glycosides can be explained by the influence of the solvent on hydrogen bonding using visualized Newman projections (Figure 5). In theory, the coupling constants between protons that are separated by three bonds are directly related to their dihedral angles through the Karplus equation,[36] which is influenced by intramolecular hydrogen bonding between the vicinal diol groups and intermolecular hydrogen bonding between the vicinal diols and deuterated solvent. More probable, an intermolecular hydrogen bond between DMSO-$d_6$ and the vicinal diol could prevent a predominant conformation. However, acetic acid-$d_4$ can interact with other acetic acid-$d_4$ molecules or with $D_2O$, which indirectly promotes the formation of intramolecular hydrogen bonds between the vicinal diol groups and also generates predominant conformations.[37−39] Thus, the staggered rotamers with an *anti* orientation in the *threo* configuration favor a larger $^3J_{HH}$ value (7.0 Hz), whereas those with a *gauche* orientation in the *erythro* configuration favor a smaller value (3.5 Hz). The reason for the smaller $^3J_{HH}$ values of the *erythro* glycosides compared to their aglycones might be the steric hindrance of the sugar moiety. Because the solvent influence on intramolecular hydrogen bonding between the vicinal diol groups is weaker in protic methanol-$d_4$ and $D_2O$ than in DMSO-$d_6$ and stronger than in acetic acid-$d_4$ and $CDCl_3$, smaller $^3J_{HH}$ differences can occur.

On the basis of the relative configurational assignments, the absolute configurations of these natural compounds were established by Snatzke's method using $Mo_2(OAc)_4$-induced electronic circular dichroism[40−43] in combination with experimental and calculated ECD spectra (Supporting Information, S29).



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In conclusion, the relative configurations of polyacetylene glycosides containing an acyclic vicinal diol group adjacent to an olefinic bond, an acetylenic bond, a thiophene ring, or a furan ring were conveniently and reliably determined by $^1$H NMR spectroscopy using acetic acid-$d_4$/$D_2O$ as the solvent. A relatively larger $^3J_{HH}$ value (7.0 Hz) was assigned to the *threo* configuration, whereas a smaller value (3.5 Hz) was assigned to the *erythro* configuration. The proportions of acetic acid-$d_4$ can be adjusted slightly based on the solubility of the samples to be tested. This convenient NMR approach, in combination with other spectral data such as ECD, facilitates determination of the absolute configurations of polyacetylene glycosides and greatly diminishes the associated labor.

## ■ ASSOCIATED CONTENT

### Supporting Information

The Supporting Information is available free of charge on the ACS Publications website at DOI: 10.1021/acs.orglett.6b03855.

Detailed experimental section; physical and chemical data; and the original UV, IR, ECD, HRESIMS, 1D and 2D NMR spectra of compounds **1**−**19** as well as the 1D NMR and HRESIMS data of compounds **1a**, **1b**, **5a**,**b**, **8a**, **10a**, **15a**,**b**, and **17a** (PDF)

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### Notes

The authors declare no competing financial interest.

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## ■ REFERENCES


(1) Hansen, L.; Boll, P. M. *Phytochemistry* **1986**, *25*, 285−293.
(2) Konovalov, D. A. *Pharm. Chem. J.* **2014**, *48*, 613−631.
(3) Ganjewala, D.; Kumar, S.; Ambika, K.; Luthra, R. *PharmacologyOnLine* **2008**, *2*, 113−131.
(4) Negri, R. *Fitoterapia* **2015**, *106*, 92−109.
(5) Dawid, C.; Dunemann, F.; Schwab, W.; Nothnagel, T.; Hofmann, T. *J. Agric. Food Chem.* **2015**, *63*, 9211−9222.
(6) Chao, C. H.; Juang, S. H.; Chan, H. H.; Shen, D. Y.; Liao, Y. R.; Shih, H. C.; Huang, C. H.; Cheng, J. C.; Chen, F. A.; Hung, H. Y.; Wu, T. S. *RSC Adv.* **2015**, *5*, 41324−41331.
(7) Jiang, Y. P.; Liu, Y. F.; Guo, Q. L.; Shi, J. G. *J. Asian Nat. Prod. Res.* **2015**, *17*, 601−614.
(8) Chan, H. H.; Sun, H. D.; Reddy, M. V. B.; Wu, T. S. *Phytochemistry* **2010**, *71*, 1360−1364.
(9) Dawid, C.; Dunemann, F.; Schwab, W.; Nothnagel, T.; Hofmann, T. *J. Agric. Food Chem.* **2015**, *63*, 9211−9222.
(10) Yuda, M.; Ohtani, K.; Mizutani, K.; Kasai, R.; Tanaka, O.; Jia, M. R.; Ling, Y. R.; Pu, X. F.; Saruwatari, Y. I. *Phytochemistry* **1990**, *29*, 1989−1993.
(11) Magalhães, A. F.; Vieira, D. M.; Magalhães, E. G.; Shepherd, G. J. *Phytochemistry* **1988**, *27*, 3827−3830.
(12) Bifulco, G.; Dambruoso, P.; Gomez-Paloma, L.; Riccio, R. *Chem. Rev.* **2007**, *107*, 3744−3779.
(13) Matsumori, N.; Kaneno, D.; Murata, M.; Nakamura, H.; Tachibana, K. *J. Org. Chem.* **1999**, *64*, 866−876.
(14) Ardá, A.; Nieto, M. I.; Blanco, M.; Jiménez, C.; Rodríguez, J. *J. Org. Chem.* **2010**, *75*, 7227−7232.
(15) Higashibayashi, S.; Kishi, Y. *Tetrahedron* **2004**, *60*, 11977−11982.
(16) Freire, F.; Seco, J. M.; Quiñoá, E.; Riguera, R. *J. Org. Chem.* **2005**, *70*, 3778−3790.
(17) Seco, J. M.; Quiñoá, E.; Riguera, R. *Chem. Rev.* **2012**, *112*, 4603−4641.
(18) Kikuchi, T.; Mori, Y.; Yokoi, T.; Nakazawa, S.; Kuroda, H.; Masada, Y.; Kitamura, K.; Kuriyama, K. *Chem. Pharm. Bull.* **1983**, *31*, 106−113.
(19) Takeshita, M.; Sato, T. *Chem. Pharm. Bull.* **1989**, *37*, 1085−1086.
(20) Ayer, W. A.; Trifonov, L. S. *J. Nat. Prod.* **1993**, *56*, 85−89.
(21) Kasahara, H.; Miyazawa, M.; Kameoka, H. *Phytochemistry* **1995**, *40*, 1515−1517.
(22) Jarvis, B. B.; Wang, S.; Ammon, H. L. *J. Nat. Prod.* **1996**, *59*, 254−261.
(23) Lehner, M. S.; Steigel, A.; Bauer, R. *Phytochemistry* **1997**, *46*, 1023−1028.
(24) Chen, X. C.; Ren, X. F.; Peng, K.; Wu, T. X.; Pan, X. F. *Chem. J. Chinese U.* **2003**, *24*, 1811−1814.
(25) Curti, C.; Zanardi, F.; Battistini, L.; Sartori, A.; Rassu, G.; Pinna, L.; Casiraghi, G. *J. Org. Chem.* **2006**, *71*, 8552−8558.
(26) Du, L.; Zhu, T. J.; Fang, Y. C.; Gu, Q. Q.; Zhu, W. M. *J. Nat. Prod.* **2008**, *71*, 1343−1351.
(27) Hanaya, T.; Baba, H.; Toyota, H.; Yamamoto, H. *Tetrahedron* **2009**, *65*, 7989−7997.
(28) Li, Y. R.; Cheng, W.; Zhu, C. G.; Yao, C. S.; Xiong, L.; Tian, Y.; Wang, S. J.; Lin, S.; Hu, J. F.; Yang, Y. C.; Guo, Y.; Yang, Y.; Li, Y.; Yuan, Y. H.; Chen, N. H.; Shi, J. G. *J. Nat. Prod.* **2011**, *74*, 1444−1452.
(29) Xia, Y.; Wang, W. *Monatsh. Chem.* **2011**, *142*, 93−96.
(30) Rong, Z. Q.; Pan, H. J.; Yan, H. L.; Zhao, Y. *Org. Lett.* **2014**, *16*, 208−211.
(31) Reddy, P. R.; Das, B. *RSC Adv.* **2014**, *4*, 7432−7434.
(32) Lu, Y. Y.; Xue, Y. B.; Liu, J. J.; Yao, G. M.; Li, D. Y.; Sun, B.; Zhang, J. W.; Liu, Y. F.; Qi, C. X.; Xiang, M.; Luo, Z. W.; Du, G.; Zhang, Y. H. *J. Nat. Prod.* **2015**, *78*, 2205−2214.
(33) Shen, Y.; Feng, Z. M.; Jiang, J. S.; Yang, Y. N.; Zhang, P. C. *J. Nat. Prod.* **2013**, *76*, 2337−2345.
(34) Ganapaty, S.; Srilakshmi, G. V. K.; Pannakal, S. T.; Rahman, H.; Laatsch, H.; Brun, R. *Phytochemistry* **2009**, *70*, 95−99.
(35) Gong, T.; Wang, D. X.; Chen, R. Y.; Liu, P.; Yu, D. Q. *Planta Med.* **2009**, *75*, 236−242.
(36) Karplus, M. *J. Chem. Phys.* **1959**, *30*, 11−15.
(37) Burwell, R. L. *Chem. Rev.* **1954**, *54*, 615−685.
(38) Fujii, Y.; Yamada, H.; Mizuta, M. *J. Phys. Chem.* **1988**, *92*, 6768−6772.
(39) Abraham, M. H.; Abraham, R. J.; Acree, W. E., Jr; Aliev, A. E.; Leo, A. J.; Whaley, W. L. *J. Org. Chem.* **2014**, *79*, 11075−11083.
(40) Snatzke, G.; Wagner, U.; Wolff, H. P. *Tetrahedron* **1981**, *37*, 349−361.
(41) Di Bari, L.; Pescitelli, G.; Pratelli, C.; Pini, D.; Salvadori, P. *J. Org. Chem.* **2001**, *66*, 4819−4825.
(42) Bari, L. D.; Pescitelli, G.; Salvadori, P. *Chem. - Eur. J.* **2004**, *10*, 1205−1214.
(43) Frelek, J.; Ruskowska, P.; Suszczynska, A.; Szewczyk, K.; Osuch, A.; Jarosz, S.; Jagodzinski, J. *Tetrahedron: Asymmetry* **2008**, *19*, 1709−1713.




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