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Nigramides A−S, Dimeric Amide Alkaloids from the Roots of *Piper nigrum*

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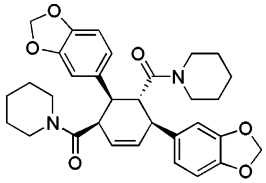
Received October 7, 2004



nigramide A

Fifteen novel dimeric amide alkaloids possessing a cyclohexene ring, nigramides A−O (**1**−**15**), as well as four novel dimeric amide alkaloids possessing a cyclobutane ring, nigramides P−S (**17**−**20**), have been isolated from the roots of *Piper nigrum*. Their structures were elucidated on the basis of their spectroscopic data. The biosynthestic hypothesis of nigramides A−O (**1**−**15**) was proposed by an intermolecular Diels−Alder reaction from the corresponding monomeric amides. On the basis of this biosynthetic hypothesis, the first study of the thermal and Lewis acid mediated Diels−Alder reactions of piperine in different organic solvents and under solventless conditions is also described.

Introduction

The genus *Piper* (Piperaceae) has over 700 species widely distributed in the tropical and subtropical regions of the world.[1] Among them, *P. nigrum* is one of the most well-known species because of its high commercial, economic, and medicinal importance. Its ripened fruit is the source of white pepper, while the unripe fruit is the source of black pepper. Biological investgations of *P. nigrum* have been mainly aimed at piperine (**22**), the main constituent of this plant, which has central nervous system depressant,[2] antiinflammatory,[3] hepatoprotective,[4] and intestinal permeability[5] properties and has been used as a bioavailability enhancer to increase the permeability of intestinal cells.[6] The fruit of *P. nigrum* has been extensively investigated for its chemical constituents and revealed the occurrence of amide alkaloids, propenylphenols, lignans, terpenes, steroids, and flavones.[7] Recently, seven novel cyclobutane-type dimeric amide alkaloids with a significant cytochrome P450 3A4 inhibitory activity have also been reported from the black and white peppers.[8] As part of our current interest in

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(1) Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597−673.

(2) (a) Woo, W. S.; Shin, K. H.; Kim, Y. S. *Soul Taehakkyo Saengyak Yonguso Opjukjip* **1979**, *18*, 12−16. (b) Lee, E. B.; Shin, K. H.; Woo, W. S. *Arch. Pharmacal Res.* **1984**, *7*, 127−132. (c) Mujumdar, A. M.; Dhuley, J. N.; Deshmukh, V. K.; Raman, P. H.; Thorat, S. L.; Naik, S. R. *Indian J. Exp. Biol.* **1990**, *28*, 486-487.

(3) (a) Mujumdar, A. M.; Dhuley, J. N.; Deshmukh, V. K.; Raman, P. H.; Naik, S. R. *Jpn. J. Med. Sci. Biol.* **1990**, *43*, 95−100. (b) Mujumdar, A. M.; Dhuley, J. N.; Deshmukh, V. K.; Naik, S. R. *Indian Drugs* **1999**, *36*, 123−126.

(4) (a) Koul, I. B.; Kapil, A. *Planta Med*. **1993**, *59*, 413−417. (b) Singh, A.; Rao, A. R. *Cancer Lett*. **1993**, *72*, 5−9.

(5) (a) Johri, R. K.; Thusu, N.; Khajuria, A.; Zutshi, U. *Biochem. Pharmacol.* **1992**, *43*, 1401−1407. (b) Gupta, S. K.; Velpandian, T.; Sengupta, S.; Mathur, P.; Sapra, P. *Phytother. Res.* **1998**, *12*, 266−269. (c) Khajuria, A.; Zutshi, U.; Bedi, K. L. *Indian J. Exp. Biol.* **1998**, *36*, 46−50.

(6) Majeed, M.; Badmaev, V.; Rajendran, R. U.S. Patent 5,536,506.

(7) (a) Siddiqui, B. S.; Begum, S.; Gulzar, T.; Farhat; Noor, F. *Phytochemistry* **1997**, *45*, 1617−1619. (b) Srinivas, P. V.; Rao, M. *Phytochemistry* **1999**, *52*, 957−958. (c) Parmar, V. S.; Jain, S. C.; Bisht, K. S.; Jain, R.; Taneja, P.; Jha, A.; Tyagi, O. D.; Prasad, A. K.; Wengel, J.; Olsen, C. E.; Boll, P. M. *Phytochemistry* **1997**, *46*, 597−673. (d) Siddiqui, B. S.; Gulzar, T.; Begum, S. *Heterocycles* **2002**, *57*, 1653−1658. (e) Siddiqui, B. S.; Gulzar, T.; Begum, S.; Rasheed, M.; Sattar, F. A.; Afshan, F. *Helv. Chim. Acta* **2003**, *86*, 2760−2767.

(8) (a) Fujiwara, Y.; Naithou, K.; Miyazaki, T.; Hashimoto, K.; Mori, K.; Yamamoto, Y. *Tetrahedron Lett.* **2001**, *42*, 2497−2499. (b) Tsukamoto, S.; Cha, B.-C.; Ohta, T. *Tetrahedron* **2002**, *58*, 1667−1671. (c) Tsukamoto, S.; Tomise, K.; Miyakawa, K.; Cha, B.-C.; Abe, T.; Hamada, T.; Hirota, H.; Ohta, T. *Bioorg. Med. Chem.* **2002**, *10*, 2981−2985.

10.1021/jo040272a CCC: $30.25
Published on Web 01/19/2005

the medicinal plants on Hainan Island, P. R. China, we investigated the chemical constituents of the roots of *P. nigrum* and reported the isolation of 39 amide alkaloids from its methanolic extract.[9] Our continuing phytochemical study of this extract led to the isolation of 21 bisalkaloids (**1**−**21**), of which **1**−**16** were determined to be the cyclohexene-type and **17**−**21** to be the cyclobutane-type dimeric amide alkaloids. Among them, **16** and **21** are known compounds.[8c,10] To best of our knowledge, except for **16**, the dimeric amide alkaloids family possessing a cyclohexene ring has not been reported to date. In this paper, we describe the isolation and structural elucidation of novel compounds **1**−**15** and **17**−**20** on the basis of their spectroscopic data.

As for these structurally related cyclohexene-type dimeric amide alkaloids **1**−**16**, we proposed a biosynthetic hypothesis for their formation from two of the same or different previously isolated monomeric amide alkaloids by an intermolecular Diels−Alder reaction. The Diels−Alder reaction is one of the most important bond-forming reactions in organic synthesis for obtaining cyclic systems and excellent stereoselectivity.[11,12] The provocative biosynthetic proposals of some secondary metabolites suggested that this valuable reaction also plays an important role in nature.[13] The structures of the cyclohexene-type nigramides inspired us to embark on investigating their biomimetic synthesis. We chose the reaction of piperine (**22**), a Diels−Alder dimerization, as proof to support the aforementioned biosynthetic hypotheses. To the best of our knowledge, the intermolecular [4 + 2] cycloaddition of **22** had not been previously reported. We now describe the study of the thermal and Lewis acid-mediated Diels−Alder reactions of **22** in different organic solvents and under solventless conditions, producing the corresponding dimeric amide alkaloids **2**, **16** and **23** (Chart 1).

Results and Discussion

Isolation. The air-dried roots of *P. nigrum* were extracted with MeOH and the extract was successively partitioned with chloroform, ethyl acetate, and *n*-BuOH. The chloroform-soluble fraction was subjected to normal-phase and reversed-phase silica gel column chromatographies followed by C_{18} HPLC to afford compounds **1**−**21**.

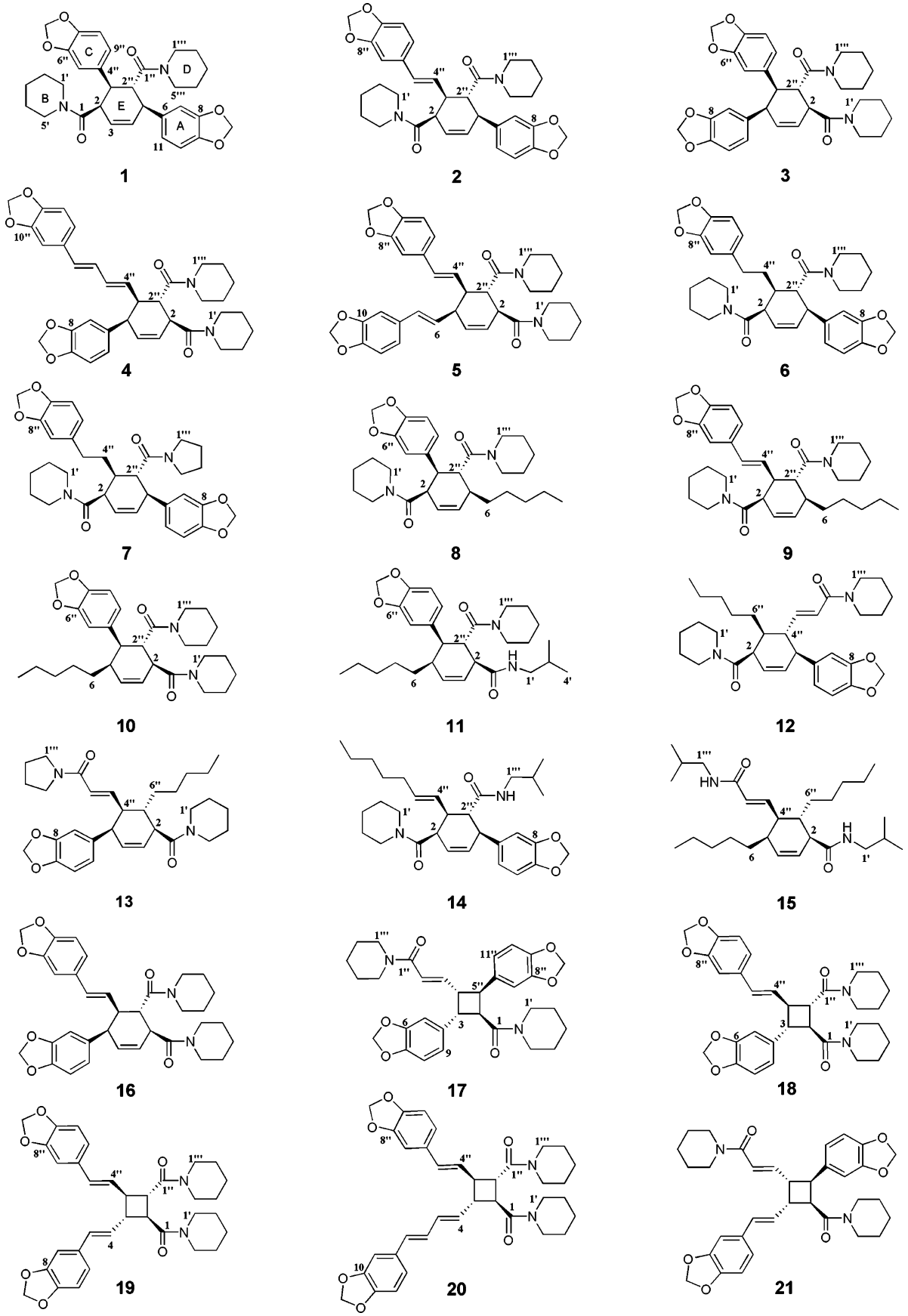
Structure Elucidation of Nigramides A−O (1−15). Nigramide A (**1**) was obtained as a colorless oil, which did not give crystals suitable for X-ray analysis. Its HRFABMS exhibited a pseudomolecular ion peak at *m/z* 545.2657 $[M + H]^+$ (calcd 545.2652), consistent with the molecular formula $C_{32}H_{36}N_2O_6$ and 16 degrees of unsaturation. Its IR absorptions implied the presence of a carbonyl (1710 cm^{-1}) functionality. The ^1H NMR spectrum of **1** displayed two sets of aromatic protons due to two 1,3,4-trisubstituted aromatic rings, A and C, at δ_H 6.98 (1H, d, J = 1.6 Hz), 6.84 (1H, dd, J = 8.1, 1.6 Hz), 6.73 (1H, d, J = 8.0 Hz), and δ_H 6.69 (1H, d, J = 1.4 Hz), 6.65 (1H, d, J = 8.0 Hz), 6.63 (1H, dd, J = 8.0, 1.4 Hz), respectively. The ^1H NMR spectrum also exhibited signals for a cis-olefin at δ_H 5.92 (1H, dt, J = 9.9, 1.8 Hz) and 5.72 (1H, ddd, J = 9.9, 5.3, 2.8 Hz), and two methylenedioxy groups at δ_H 5.90 (1H, d, J = 1.6 Hz), 5.89 (1H, d, J = 1.6 Hz), and δ_H 5.87 (1H, d, J = 1.4 Hz), 5.85 (1H, d, J = 1.4 Hz) (Table 1). Besides the above-mentioned moiety, analysis of the $^1H-^1H$ COSY spectrum also defined other spin systems: a cyclohexene ring (ring E) and two piperidine rings (rings B and D) as shown in Figure 1. The ^{13}C NMR spectrum (Table 1), in combination with analysis of the DEPT and HMQC spectra, indicated that the molecule possessed 8 sp^2 quaternary carbons, 8 sp^2 and 4 sp^3 methines, and 12 sp^3 methylenes. Among them, the two sp^2 quaternary carbons (δ_C 172.4 and 171.1) were assigned to carbonyl groups. To establish the connections between the above-described structural units, an HMBC analysis was done (Figure 1). In the HMBC spectrum, the two methylenedioxy groups were located at rings A and C based on the long-range connectivities from δ_H 5.90 and 5.89 to δ_C 147.7 (C-8) and 146.3 (C-9), and δ_H 5.87 and 5.85 to δ_C 147.4 (C-6″) and 146.3 (C-7″), respectively. The HMBC correlations for δ_H 6.98 (H-7) and 6.84 (H-11) to δ_C 47.6 (C-5), and δ_H 6.69 (H-5″) and 6.63 (H-9″) to δ_C 46.1 (C-3″) established the attachments of rings A and C at C-5 and C-3″, respectively. The locations of the two carbonyl groups at C-1 and C-1″ were based on the correlations for δ_H 3.79 (H-2) to δ_C 171.1 (C-1) and δ_H 4.28 (H-2″) to δ_C 172.4 (C-1″). The connections of two piperidine rings (B and D) were determined by the HMBC correlations for δ_H 3.14 (H-1′b) and 3.33 (H-5′a) to δ_C 171.1 (C-1) and δ_H 2.92 (H-1‴a), 3.02 (H-1‴b/H-5‴a) and 3.36 (H-5‴b) to δ_C 172.4 (C-1″). Additionally, the NOSEY correlations were found to have an effect on the location of the two piperidine rings, namely, the correlations for H-2/H-1′ and H-2″/H-1‴ were observed in the NOESY spectrum (Figure 2). Thus, the planar structure of nigramide A (**1**) was as depicted in Figure 1.

The relative configurations of the four stereogenic centers of **1** were elucidated on the basis of the $^1H-^1H$ coupling constants and NOESY correlations as shown in Figure 2 (computer-generated 3D drawing). The coupling constants of H-2″/H-3″ and H-2″/H-5 were 11.5 and 9.6 Hz, respectively, indicating the anti relations of H-2″/H-3″ and H-2″/H-5 and the axial orientations for these protons. The correlation of the 1,3-diaxial proton pair due to H-3″ and H-5 in the NOESY spectrum further supported the above result. These data indicated a β-orientation for H-2″ and an α-orientation for H-3″ and H-5. The α-orientation for H-2 was suggested by the coupling constant of H-2/H-3″ (5.7 Hz) and the absence of the NOESY correlation for H-2/H-2″. Furthermore, since the optical rotation was zero and the circular dichroism (CD) spectrum exhibited no Cotton effect, **1** was found to be a racemate. On the basis of the above spectral data, the structure of nigramide A (**1**) was unambiguously established.

Analysis of HRFABMS (*m/z* 571.2802 $[M + H]^+$, calcd for 571.2808) of nigramide B (**2**) led to the molecular formula of $C_{34}H_{38}N_2O_6$ and 17 degrees of unsaturation. The MS data showed that **2** had 26 mass units more than

(9) Wei, K.; Li, W.; Koike, K.; Pei, Y.-P.; Chen, Y.-J.; Nikaido, T. *J. Nat. Prod.* **2004**, *67*, 1005−1009.
(10) Rukachaisirikul, T.; Prabpai, S.; Champung, P.; Suksamrarn, A. *Planta Med.* **2002**, *68*, 850−853.
(11) Carruthers, W. *Cycloaddition Reactions in Organic Synthesis*; Pergamon: Oxford, 1990.
(12) (a) Ichihara, A.; Oikawa, H. *Curr. Org. Chem.* **1998**, *2*, 365−394. (b) Nicolaou, K. C.; Snyder, S. A.; Montagnon, T.; Vassilikogiannakis, G. *Angew. Chem., Int. Ed.* **2002**, *41*, 1668−1698.
(13) Stocking, E. M.; Williams, R. M. *Angew. Chem., Int. Ed.* **2003**, *42*, 3078−3115.

CHART 1



1. The ^{1}H and ^{13}C NMR spectral signals of **2** were close to those of **1** (Tables 2 and 3) except for two additional olefinic proton signals at δ_H 5.90 (1H, dd, J = 15.8, 9.8 Hz) and 6.37 (1H, d, J = 15.8 Hz), corresponding to two

TABLE 1. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for 1 (in $CDCl_3$, δ in ppm, J in Hz)

position	δ_H	δ_C	position	δ_H	δ_C
1		171.1	1″		172.4
2	3.79, m	41.8	2″	4.28, dd (11.4, 9.6)	43.4
3	5.72, ddd (9.9, 5.3, 2.8)	124.7	3″	3.50, dd (11.5, 5.7)	46.1
4	5.92, dt (9.9, 1.8)	133.6	4″		135.2
5	3.71, dq (9.6, 2.3)	47.6	5″	6.69, d (1.4)	109.1
6		137.7	6″		147.4
7	6.98, d (1.6)	109.2	7″		146.3
8		147.7	8″	6.65, d (8.0)	108.0
9		146.3	9″	6.63, dd (8.0, 1.4)	121.6
10	6.73, d (8.0)	108.1			
11	6.84, dd (8.1, 1.6)	121.6			
$-OCH_2O-$	5.89, d (1.6)	100.8	$-OCH_2O-$	5.85, d (1.4)	100.8
	5.90, d (1.6)			5.87, d (1.4)	
1′a	2.89, ddd (13.5, 6.9, 3.7)	47.1	1‴a	2.92, m	46.6
1′b	3.14, ddd (13.0, 8.2, 3.2)		1‴b	3.02, m	
2′a	0.67, m	25.9	2‴a	0.60, m	25.7
2′b	1.28, m		2‴b	1.18, m	
3′	1.43, m	24.3	3‴	1.34, m	24.4
4′a	1.34, m	25.3	4‴	1.17, m	25.5
4′b	1.46, m				
5′a	3.33, ddd (12.8, 8.4, 3.4)	43.1	5‴a	3.02, m	42.4
5′b	3.57, ddd (13.0, 6.8, 3.6)		5‴b	3.36, dt (13.3, 5.3)	

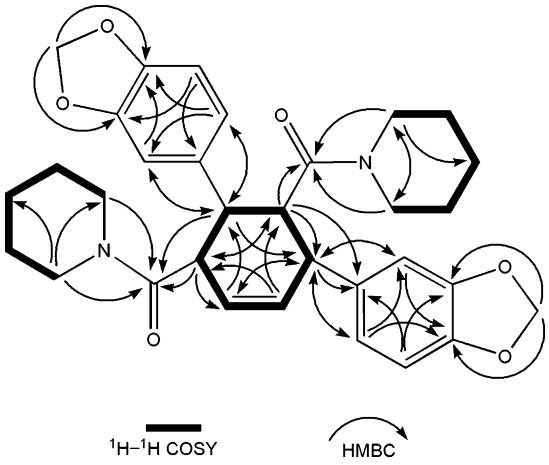


FIGURE 1. Important ^{1}H−^{1}H COSY and HMBC correlations for **1**.

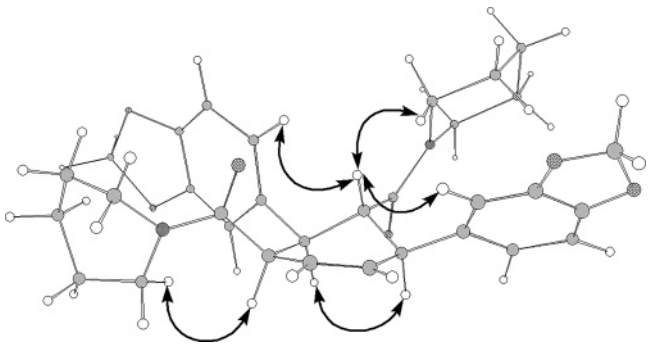


FIGURE 2. Selected NOESY correlations for **1**.

olefinic carbon signals at δ_C 127.6 and 132.0, which were assigned to a *trans*-olefin by the coupling constant of 15.8 Hz. The ^{1}H−^{1}H COSY spectrum showed cross-peaks of the olefinic proton at δ_H 5.90 (H-4″) with a methine proton at δ_H 2.94 (H-3″), suggesting the attachment of an additional double bond at C-3″. Furthermore, analyses of the 2D NMR data, including the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, resulted in the determination of the planar structure of **2** (Figure S1, Supporting Information). The relative stereochemistry of **2** was deduced from the ^{1}H−^{1}H coupling constants and NOESY spectral data. The large coupling constants of H-2″/H-3″ (10.1 Hz) and H-2″/H-5 (10.1 Hz), and the small coupling constants of H-2/H-3″ (5.5 Hz), combined with the NOESY correlations for H-2″/H-4″, H-2″/H-7, and H-3″/H-5, revealed that **2** possessed the same relative configurations at C-2, C-2″, C-3″, and C-5 as those of **1** (Figure S2, Supporting Information).

Nigramide C (**3**) had the same molecular formula, $C_{32}H_{36}N_2O_6$, as that of **1** on the basis of the HRFABMS analysis (*m/z* 545.2666 $[M + H]^+$, calcd for 545.2652). The ^{1}H and ^{13}C NMR signals of **3** (Tables 1−3) were similar to those of **1** except for the *ortho*-orientation of the two carbonyl groups in **3** instead of their *meta*-orientation in **1**, which was confirmed by the HMBC correlations for δ_H 3.89 (H-2″) to δ_C 171.2 (C-1) and 172.3 (C-1″). The observed HMBC correlations for δ_H 3.50 (H-1′), 3.57 (H-5′a), 3.61 (H-5′b) to δ_C 171.2 (C-1) and δ_H 3.29 (H-1″a), 3.50 (H-1″b), 3.07 (H-5″a), 3.37 (H-5″b) to 172.3 (C-1″) established the attachments of the two piperidine rings at C-1 and C-1″ (Figure S3, Supporting Information), supported by the NOESY correlations for H-1′/H-2 and H-1‴/H-2″ (Figure S4, Supporting Information). The analyses of the ^{1}H−^{1}H coupling constants and NOESY data allowed us to determine the relative stereochemistry of **3**. The large coupling constants for H-2″/H-2 (9.5 Hz) and H-2″/H-3″ (11.7 Hz), the small coupling constant for H-3″/H-5 (5.5 Hz), and NOESY correlations for H-2/H-3″, H-2″/H-5″, and H-2″/H-11 suggested that **3** had the same relative stereochemistry as that of **1** (Figure S4, Supporting Information). Fortunately, the X-ray analysis of a colorless prism crystal of **3** grown from a solution of methanol further confirmed the structure and relative stereochemistry (Figure 3).

The molecular formula of nigramide D (**4**) was determined as $C_{36}H_{40}N_2O_6$ by its molecular ion peak at *m/z* 597.2965 $[M + H]^+$ (calcd for 597.2965) in the HRFABMS spectrum. The ^{1}H NMR signals were similar to those of **3** (Table 2) except for four additional olefinic proton

TABLE 2. Characteristic ^{1}H NMR Spectral Data for the Cyclohexene Ring of 1−15, 23, and 24 and the Cyclobutane Ring of 18−20 (500 MHz in $CDCl_3$, δ in ppm, J in Hz)[a]

	2	3	4	5	2″(4″)[b]	3″(5″)[b]
2	3.61 m	5.72 ddd (9.8, 5.0, 2.7)	5.90 dt (10.3, 1.8)	3.78 dq (10.0, 2.3)	4.07 t (10.1)	2.94 td (10.1, 10.1, 5.5)
3	4.08 dq (9.5, 2.3)	5.72 dt (9.9, 1.8)	5.94 ddd (9.9, 5.0, 2.7)	3.48 m	3.89 dd (9.5, 11.7)	3.53 dd (11.7, 5.5)
4	4.12 dq (9.4, 2.6)	5.72 dt (9.9, 1.8)	5.84 ddd (9.8, 4.8, 2.7)	3.43 m	3.63 dd (11.7, 9.4)	2.90 ddd (11.7, 10.4, 5.8)
5	4.1 dq (9.1, 2.3)	5.64 dt (10.1, 1.6)	5.79 ddd (9.8, 5.0, 2.7)	3.07 m	3.67 dd (11.5, 9.6)	2.89 ddd (10.8, 10.8, 5.3)
6	3.59 m	5.71 ddd (9.8, 5.2, 2.7)	5.85 dt (9.8, 1.9)	3.69 dq (10.0, 2.3)	4.05 t (10.4)	2.20 tdd (10.5, 5.7, 4.5)
7	3.60 m	5.73 ddd (9.8, 5.2, 2.7)	5.85 dt (9.7, 1.8)	3.64 dq (10.0, 2.0)	3.82 t (10.1)	2.22 tt (10.0, 4.8)
8	3.69 t (5.2)	5.59 ddd (10.1, 5.1, 2.8)	5.97 dt (10.0, 1.6)	2.64 br.s	4.07 dd (11.3, 9.6)	3.34 dd (11.5, 5.8)
9	3.49 m	5.59 ddd (10.1, 5.1, 2.8)	5.91 dt (10.1, 1.8)	2.69 m	3.83 dd (11.0, 9.6)	2.75 ddd (10.6, 10.6, 5.5)
10	3.94 dq (9.4, 2.3)	5.51 dt (10.0, 1.8)	6.04 ddd (10.0, 5.2, 2.5)	2.19 m	3.86 dd (11.2, 9.4)	3.40 m
11	3.33 m	5.63 br.d (9.9)	6.05 ddd (9.8, 5.0, 2.0)	2.20 m	3.71 t (10.4)	3.36 m
12	3.62 m	5.69 ddd (9.7, 5.3, 2.8)	5.79 dt (9.4, 2.1)	3.04 dq (9.6, 2.5)	3.39 q (10.4)	1.79 tt (10.1, 4.8)
13	3.38 m	5.59 ddd (9.9, 2.0, 0.7)	5.86 ddd (9.9, 5.3, 2.5)	3.37 m	2.57 m	2.57 m
14	3.57 m	5.64 ddd (9.9, 5.0, 2.8)	5.83 dt (9.9, 1.6)	3.65 dq (9.6, 2.1)	3.07 dd (11.2, 10.1)	2.73 m
15	2.68 ddd (6.0, 3.9, 2.6)	5.66 ddd (10.1, 3.5, 2.3)	5.85 dt (10.3, 2.3)	2.18 m	2.43 dt (9.6, 5.7)	2.18 m
18	3.80 t (9.4)	3.36 t (9.4)			3.65 t (8.7)	3.15 q (8.5)
19	3.66 m	2.99 m			3.66 m	2.99 m
20	3.62 m	2.93 m			3.62 m	2.95 m
23	3.71 dq (12.1, 2.1)	5.71 dt (9.9, 1.6)	5.96 ddd (9.9, 5.3, 2.6)	3.59 t (5.3)	2.99 ddd (12.5, 9.7, 5.5)	3.44 dd (12.1, 10.1)
24		6.09 br.t (3.2)	2.42 m	3.33 ddd (9.2, 5.8, 3.5)	4.06 br.s	2.79 dt (8.5, 2.7)

[a] Complete proton signal assignments are given in the Supporting Information. [b] H-2″ and H-3″ for **1**−**11**, **14**, and **18**−**20**; H-4″ and H-5″ for **12**, **13**, and **15**.

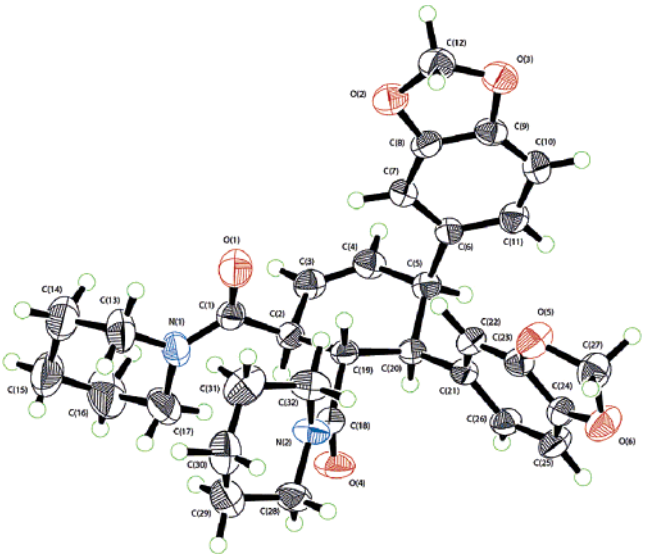


FIGURE 3. Computer-generated final X-ray model (ORTEP) of **3**.

signals at δ_H 5.05 (1H, dd, J = 14.8, 10.3 Hz), 6.13 (1H, dd, J = 14.8, 10.1 Hz), 6.57 (1H, dd, J = 15.6, 10.1 Hz) and 6.42 (1H, d, J = 15.6 Hz) (in acetone-d_6) due to two *trans*-conjugated double bonds. In the ^{1}H−^{1}H COSY spectrum, the cross-peaks of the olefinic proton at δ_H 4.95 with the methine proton at δ_H 2.90 (H-3″) indicated that the additional conjugated double bonds were attached at C-3″. Furthermore, the *ortho*-orientation of two carbonyl groups at C-1 and C-1″ were confirmed by the HMBC correlations for δ_H 4.12 (H-2) to δ_C 171.2 (C-1) and δ_H 3.63 (H-2″) to δ_C 172.3 (C-1″). The NOE for H-1‴/H-2″ observed in the 1D NOE experiment established the attachment of the two piperidine rings at C-1 and C-1″. The locations of two methylenedioxyphenyl groups at C-5 and C-7″ were elucidated by the HMBC correlations for δ_H 6.81 (H-7) and 6.81 (H-11) to δ_C 45.8 (C-5), and δ_H 6.87 (H-9″) and 6.77 (H-13″) to δ_C 131.2 (C-7″). The relative stereochemistry of **4** was also confirmed by the analysis of the coupling constants and 1D NOE spectrum. The large coupling constants of H-2″/H-2 (9.4 Hz) and H-2″/H-3″ (11.7 Hz), and the small coupling constants of H-3″/H-5 (5.8 Hz), combined with the key NOE for H-2″/H-11, suggested the β-orientation of H-2″ and the α-orientation of H-2, H-3″, and H-5. Thus, the relative stereochemistry of **4** was the same as that of **1**.

Nigramide E (**5**) possessed the same molecular formula, $C_{36}H_{40}N_2O_6$, as that of compound **4** as confirmed by HRFABMS spectrum (*m/z* 597.2958 [M + H]$^+$, calcd for 597.2964). Its ^{1}H and ^{13}C NMR spectra were very close to those of **4** (Tables 2 and 3) except for the replacement of four olefinic proton signals due to the two *trans*-conjugated double bonds in **4** by the olefinic proton signals at δ_H 6.09 (1H, dd, J = 15.8, 8.4 Hz), 6.40 (1H, d, J = 15.8 Hz), and 5.83 (1H, dd, J = 15.6, 10.1 Hz), 6.36 (1H, d, J = 15.8 Hz) ascribed to the two *trans*-nonconjugated double bonds. The locations of the two double bonds at C-5 and C-3″ were established by the cross-peaks between δ_H 3.07 (H-5) and 6.09 (H-6), and 2.89 (H-

TABLE 3. ^{13}C NMR Spectral Data for 2–15 (125 MHz in $CDCl_3$)

carbon	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	170.6	171.2	171.2	171.0	170.4	170.3	171.3	170.7	171.2	172.8	170.6	171.8	171.0	173.9
2	40.9	44.3	42.9	43.5	38.4	38.2	42.1	41.4	44.6	50.2	38.1	43.9	40.8	47.7
3	123.4	125.4	125.4	125.2	123.2	123.5	123.8	122.2	124.1	124.4	123.7	125.5	123.6	123.5
4	133.9	130.9	130.4	130.5	134.1	133.8	133.6	134.3	133.5	133.7	133.4	131.1	133.6	133.9
5	45.6	47.3	45.8	43.6	46.7	46.3	41.0	38.9	40.2	40.1	48.4	47.0	45.1	40.5
6	137.8	133.6	133.6	127.2	137.8	137.7	33.5	33.8	31.8	31.8	138.1	132.	137.8	34.0
7	108.9	111.2	111.0	132.8	109.0	108.7	25.9	25.9	27.3	27.4	109.3	111.6	108.8	26.9
8	147.6	147.1	147.4	131.9	147.5	147.4	32.4	32.3	32.1	32.1	147.3	147.4	147.6	31.9
9	146.1	146.3	146.4	105.9	146.1	146.1	22.6	22.6	22.7	22.6	145.8	146.5	146.1	22.6
10	108.1	107.6	108.0	148.0	108.1	108.0	14.1	14.1	14.0	14.0	107.7	107.9	108.1	14.0
11	121.4	123.7	123.5	147.1	121.4	121.3					122.0	123.9	121.4	
12				108.3										
13				121.0										
$-OCH_2O-$	100.7	100.8	100.9	101.1	100.7	100.7					100.7	100.8	100.7	
1′	47.4	47.4	47.1	47.1	47.1	47.1	47.1	47.3	47.2	47.1	47.2	47.0	47.6	47.1
2′	26.9	26.9	26.8	26.7	27.2	27.2	25.9	26.9	26.8	28.5	26.6	27.0	26.9	28.4
3′	24.6	24.6	24.7	24.7	24.7	24.7	24.4	24.6	24.6	20.1	24.7	24.7	24.7	20.1
4′	25.9	25.9	25.7	25.8	25.9	25.9	25.4	25.8	25.9	20.2	25.5	25.7	25.9	20.1
5′	43.2	43.5	43.3	43.4	43.0	43.0	43.1	43.1	43.4		42.9	43.4	43.2	
1″	172.4	172.3	172.3	172.3	173.2	173.5	173.6	173.6	173.0	172.9	165.3	164.4	173.5	166.3
2″	44.6	37.4	37.8	38.5	45.1	48.6	40.7	41.9	38.1	38.3	122.7	122.8	52.2	125.1
3″	44.2	47.7	45.3	45.4	39.4	39.1	46.7	45.0	46.9	46.8	146.9	147.4	43.1	144.6
4″	127.6	134.8	133.4	127.6	32.0	32.0	135.4	128.2	136.1	135.9	47.4	46.2	128.9	43.3
5″	132.0	109.5	132.4	131.7	33.6	33.6	109.4	131.8	109.3	109.3	40.6	33.0	133.9	35.7
6″	132.0	146.8	127.2	131.9	136.1	136.1	147.3	132.0	147.2	147.3	31.1	32.7	32.6	32.0
7″	105.4	145.8	131.2	105.3	108.8	108.8	146.1	105.5	145.8	145.9	27.3	24.7	28.9	26.2
8″	147.9	107.3	132.1	147.9	147.5	147.5	107.9	147.9	107.7	107.8	31.9	32.4	31.4	31.9
9″	146.9	121.8	105.4	146.9	145.6	145.6	122.0	146.8	121.5	121.4	22.7	22.6	22.6	22.6
10″	108.2		148.1	108.3	108.1	108.1		108.2			14.0	14.0	14.0	14.0
11″	120.8		147.1	120.8	121.0	121.0		120.7						
12″			108.4											
13″			121.1											
$-OCH_2O-$	101.0	100.6	101.1	101.0	100.7	100.7	100.7	101.0	100.8	100.8				
1‴	46.8	47.2	47.0	47.1	47.0	46.7	47.1	47.0	47.3	47.3	46.8	46.6	46.6	46.9
2‴	26.3	26.2	26.5	26.5	26.1	25.8	26.4	26.8	26.2	26.3	27.1	26.2	28.2	28.6
3‴	24.6	24.5	24.6	24.6	24.7	24.3	24.6	24.8	24.6	24.6	24.7	24.3	19.8	20.2
4‴	25.8	25.6	25.8	25.7	25.9	45.2	25.7	25.9	25.7	25.6	25.8	45.7	19.9	20.2
5‴	42.8	42.7	42.9	42.9	42.9		42.6	42.9	42.8	42.9	42.9			

3″) and 5.83 (H-4″) in the ^{1}H–^{1}H COSY spectrum, respectively, and further supported by the HMBC correlations for δ_H 6.36 (H-5″) to δ_C 45.4 (C-3″) and δ_H 6.40 (H-7) to δ_C 43.6 (C-5). The structure assigned to nigramide E (**5**) including the relative stereostructure, was further confirmed by the observed 2D NMR correlations, coupling constants, and the comparison of the ^{1}H NMR spectral data with those of **4**.

The molecular formula of nigramide F (**6**), $C_{34}H_{40}N_2O_6$, was established by the HRFABMS (*m/z* 573.2972 $[M + H]^+$, calcd for 573.2964), which was more than that of **2** by a 2 mass unit suggesting that one of the double bonds in **2** must be hydrogenated in **6**. This was corroborated by the ^{1}H and ^{13}C NMR spectral data of **6**, which were similar to those of **2** (Tables 2 and 3) except for the presence of two additional methylene signals at δ_H 1.60, 1.67/δ_C 32.0, and δ_H 2.40, 2.48/δ_C 33.6 in **6** instead of the olefinic signals due to the trans double bond between C-4″ and C-5″ in **2**. This was confirmed by the ^{1}H–^{1}H COSY cross-peaks between δ_H 2.20 (H-3″) and 1.60 (H-4″a), 1.67 (H-4″b), and H-4″ and 2.40 (H-5″a), 2.48 (H-5″b). Further elucidation of the 2D NMR data completely determined the planar structure of **6**. The analysis of the coupling constants and the comparison of the ^{1}H NMR spectral data with those of **2** revealed that **6** also possessed the same relative stereostructure as that of **2**.

The molecular formula of nigramide G (**7**), determined to be $C_{33}H_{38}N_2O_6$ by HRFABMS (*m/z* 559.2783 $[M + H]^+$, calcd for 559.2808), suggested the absence of a methylene when compared to that of **6**, which was confirmed by the ^{13}C NMR and DEPT spectra (Table 3). Also, the analysis of the ^{1}H–^{1}H COSY spectrum showed the presence of a pyrrolidine ring and a piperidine ring in **7** instead of the two piperidine rings of **6**. In the 1D NOE experiment, upon irradiation of the methine signal at δ_H 3.82 (H-2″), an NOE effect was observed at δ_H 2.53 and 3.40 corresponding to H-1‴a and H-1‴b. This established the location of the pyrrolidine ring at C-1″, suggesting the attachment of the piperidine ring at C-1. Furthermore, the structure of nigramide G (**7**) was determined by the interpretation of the 2D NMR spectra and the analyses of the coupling constants and proton signal patterns, indicating the same stereochemistry as that of **6**.

The molecular formula of $C_{30}H_{42}N_2O_4$ for nigramide H (**8**) was established by the HRFABMS data (*m/z* 495.3222 $[M + H]^+$, calcd for 495.3222). A comparison of the ^{1}H and ^{13}C NMR spectral data of **8** with those of **1** showed that **8** possessed the same skeleton as that of **1** (Tables 1–3) except that the signals due to a methylenedioxyphenyl group were replaced by the signals due to an *n*-amyl moiety, of which the location at C-5 was confirmed by the ^{1}H–^{1}H COSY spectral data. The HMBC correlations for δ_H 3.69 (H-2), 3.34 (H-3″) to δ_C 171.3 (C-1), and δ_H 4.07 (H-2″), 3.34 (H-3″) to δ_C 173.6 (C-1″) confirmed the *meta*-orientation of two carbonyl groups in **8** like that in **1**. Further data in support of the structure were derived from 2D NMR experiments.

The molecular formula of nigramide I (**9**) was determined to be $C_{32}H_{44}N_2O_4$ by HRFABMS (*m/z* 521.3383 $[M + H]^+$, calcd for 521.3379). The difference between **9** and **8** was analogous to the structural difference between **2** and **1** based on a comparison of their ^{1}H and ^{13}C NMR spectral data (Tables 2 and 3). Namely, **9** showed the presence of two additional olefinic proton signals due to

a trans double bond when compared to **8**. The observed 2D NMR correlations further supported the location of the additional double bond at C-3″ in **9**.

The HRFABMS (*m/z* 495.3232 [M + H]$^+$, calcd for 495.3223) of nigramide J (**10**) gave the molecular formula, $C_{30}H_{42}N_2O_4$. A comparison of the ^{1}H and ^{13}C NMR data of **10** with those of **3** (Tables 2 and 3) showed that the difference between **10** and **3** was similar to that between **8** and **1**. Namely, the NMR spectra revealed the presence of signals due to an *n*-amyl moiety instead of those due to a methylenedioxyphenyl group in **3**. The analysis of the ^{1}H−^{1}H COSY spectral data located the *n*-amyl moiety at C-5. The *ortho*-orientation of the two carbonyl groups such as that in **3** was confirmed by the HMBC correlations for δ_H 3.94 (H-2), 5.51 (H-3) to δ_C 171.2 (C-1), and δ_H 3.86 (H-2″) to δ_C 173.0 (C-1″). Further elucidation of the 2D NMR data completely determined the structure of nigramide J (**10**).

The molecular formula of nigramide K (**11**), $C_{29}H_{42}N_2O_4$, was determined by its molecular ion at *m/z* 483.3234 [M + H]$^+$ (calcd for 483.3223) in the HRFABMS spectrum. The ^{1}H NMR spectrum showed characteristic signals of an isobutylamino moiety, including a methylene protons at δ_H 2.49 and 3.16, a methine proton at δ_H 1.74, and two methyls protons at δ_H 0.89, corresponding to carbon signals at δ_C 47.1, 28.5, 20.2 and 20.1 in the ^{13}C NMR (Tables 2 and 3). The ^{1}H and ^{13}C NMR data of **11** were similar to those of **10** except for replacement of signals due to a piperidine ring in **10** by signals due to the isobutylamino group. On the basis of the NOE correlations for H-1′a/H-2, H-1′b/H-2, and H-1‴/H-2″ observed in the NOESY spectrum, the isobutylamino group and piperidine ring were located at C-1 and C-1″, respectively. Further analysis of the 2D NMR spectral data determined the structure of nigramide K (**11**).

Nigramide L (**12**) possessed the same molecular formula, $C_{32}H_{44}N_2O_4$, as that of compound **9** confirmed by the HRFABMS (*m/z* 521.3383, calcd for 521.3380 [M + H]$^+$). The analyses of the ^{1}H and ^{13}C NMR spectral data showed the occurrence of signals due to two carbonyl group, a trans double bond, a cyclohexene ring, two piperidine ring, a methylenedioxyphenyl group and an *n*-amyl unit (Tables 2 and 3). The presence of a carbonyl carbon signal at δ_C 165.3 observed in the ^{13}C NMR spectrum suggested that the functionality was conjugated to a double bond,[9] supported by the HMBC ^3J-correlation for δ_H 6.46 (H-3″) to δ_C 165.3 (C-1″). Furthermore, the ^{1}H−^{1}H COSY cross-peaks between δ_H 6.46 (H-3″) and 3.39 (H-4″), and 1.79 (H-5″) and 1.31 (H-6″a), 1.42 (H-6″b), coupled with the HMBC correlation for δ_H 5.79 (H-2″) to δ_C 47.4 (C-4″), established the attachment of the α,β-unsaturated carbonyl group and *n*-amyl group at C-4″ and C-5″, respectively. The HMBC correlation for δ_H 3.04 (H-5) to δ_C 109.3 (C-7), 122.0 (C-11) determined the location of the methylenedioxyphenyl group at C-5. Furthermore, the attachment of two piperidine rings at C-1 and C-1″ was established by the 1D NOE experiment. Upon irradiation of the methylene proton signal at δ_H 3.11 (H-1‴a), the NOE was observed at δ_H 5.79 (H-2″). The analysis of the coupling constants and the comparison of proton peak patterns of **12** with those of the above-described compounds allowed the establishment of the same relative stereochemistry like that of **9**.

The molecular formula of nigramide M (**13**) was established as $C_{31}H_{42}N_2O_4$ by the HRFABMS (*m/z* 507.3237, calcd for 507.3223 [M + H]$^+$). The analysis of the ^{1}H and ^{13}C NMR data of **13** indicated the presence of signals due to two carbonyl group, a trans double bond, a cyclohexene ring, a piperidine ring, a pyrrolidine ring, a methylenedioxyphenyl group and an *n*-amyl unit (Tables 2 and 3). Among them, the locations of the piperidine ring and pyrrolidine ring at C-1 and C-1″, respectively, were established by the NOESY correlations for H-3/H-5′b, and H-1‴a/H-2″. A further detailed analysis of the 2D NMR data including the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra determined the planar structure of **13**. The ^{1}H NMR spectrum in $CDCl_3$ was slightly complicated due to overlaps of signals from H-2/H-5 and H-4″/H-5″ for determining the relative stereochemistry of the cyclohexene ring. When acetone-d_6 was used as the solvent, the ^{1}H NMR spectrum showed a crucial signal dispersion (Table S3, Supporting Information). Thus, the relative stereochemistry was elucidated by analysis of the coupling constants of protons in the cyclohexene ring and 1D NOE experiment in acetone-d_6. The coupling constant for H-4″/H-5″ (12.2 Hz) suggested that H-4″ and H-5″ were anti relation. The relative stereochemistry was further established by the 1D NOE experiment. Upon irradiation of the methine proton signal at δ_H 2.57 (H-5″) of **13**, NOEs were observed at δ_H 6.18 (H-3″) and 6.99 (H-7). Upon irradiation of another methine proton signal at δ_H 2.65 (H-4″), an NOE was observed at δ_H 3.49 (H-2). These data allowed the establishment of the same relative stereostructure like that of the aforementioned compounds.

The HRFABMS (*m/z* 509.3397, calcd for 509.3379 [M + H]$^+$) of nigramide N (**14**) provided a molecular formula of $C_{31}H_{44}N_2O_4$. The analyses of the ^{1}H and ^{13}C NMR spectral data showed the presence of two carbonyl groups, a trans double bond, a cyclohexene ring, a piperidine ring, an isobutylamino group, a methylenedioxyphenyl group and 1-heptene unit (Tables 2 and 3). The ^{1}H−^{1}H COSY cross-peaks between δ_H 2.73 (H-3″) and 5.26 (H-4″) established the attachment of the 1-heptene unit at C-3″. The observed HMBC correlation for δ_H 6.82 (H-7) and 6.74 (H-11) to δ_C 45.1 (C-5) determined the location of the methylenedioxyphenyl group at C-5. These data suggested the *meta*-orientation of the two carbonyl groups, supported by the HMBC correlations for δ_H 3.57 (H-2) to δ_C 171.0 (C-1), and δ_H 3.07 (H-2″) to δ_C 173.5 (C-1″). In addition, the attachment of the isobutylamino group at C-1″ was established by the HMBC correlations for δ_H 2.75 (H-1‴a) and 2.89 (H-1‴b) to δ_C 173.5 (C-1″), suggesting the location of the piperidine ring at C-1. The structure assigned to nigramide N (**14**) including the relative stereostructure was further determined by the analyses of the 2D NMR data and coupling constants, and the comparison of the proton signal patterns with those of **12**.

The molecular formula of nigramide O (**15**) was determined to be $C_{28}H_{50}N_2O_2$ based on the HRFABMS (*m/z* 447.3977, calcd for 447.3951 [M + H]$^+$). Combined with the comparison of the NMR data with those of the above-described compounds, the ^{1}H and ^{13}C NMR spectra of **15** showed the presence of two carbonyl groups, a trans double bond, a cyclohexene ring, two isobutylamino groups and two *n*-amyl units (Tables 2 and 3). Further-

TABLE 4. ^{1}H NMR (500 MHz) and ^{13}C NMR (125 MHz) Spectral Data for 17 (in $CDCl_3$, δ in ppm, J in Hz)

position	δ_H	δ_C	position	δ_H	δ_C
1		169.0	1″		165.3
2	3.82, dd (10.3, 5.5)	45.8	2″	6.00, dd (15.3, 1.0)	121.8
3	4.49, dd (10.1, 5.8)	42.2	3″	6.51, dd (15.1, 8.5)	143.7
4		134.3	4″	3.62, q (8.7)	47.6
5	6.76, d (1.2)	108.5	5″	3.77, dd (10.2, 7.3)	47.4
6		147.8	6″		133.5
7		146.1	7″	6.86, d (1.8)	108.5
8	6.74, d (7.8)	108.5	8″		147.8
9	6.71, dd (8.0, 1.2)	121.1	9″		146.7
10			10″	6.73, d (7.8)	108.3
11			11″	6.76, dd (7.8, 1.8)	121.7
$-OCH_2O-$	5.92, s	100.9	$-OCH_2O-$	5.92, d (1.4)	101.0
				5.94, d (1.4)	
1′a	2.86, ddd (12.6, 7.3, 3.6)	46.0	1‴a	3.24, br.s	46.9
1′b	3.14, ddd (13.0, 8.0, 3.6)				
2′a	0.86, m	25.8	2‴	1.45, m	26.5
2′b	1.31, m				
3′	1.41, m	24.3	3‴	1.58, m	24.6
4′a	1.26, m	25.3	4‴	1.41, m	25.5
4′b	1.48, m				
5′	3.38, m	42.6	5‴	3.49, br.s	43.0

more, the detailed elucidation of the 2D NMR spectral data determined the planar structure of **15**. However, the comparison of the coupling constants of the cyclohexene ring with those of the aforementioned compounds suggested that in $CDCl_3$, **15** possessed a different conformation. Due to the overlaps of the proton signals at the positions of H-5/H-5″, the determination of the relative stereochemistry of **15** was carried out in pyridine-d_5 as the solvent, in which four methine proton signals of the cyclohexene ring showed a favorable dispersion of signals (Table S4, Supporting Information). The coupling constant of H-4″ (9.8, 9.8, 4.8 Hz) observed in the ^{1}H NMR spectrum suggested its axial orientation. The anti-axial relationships of H-2/H-5″ and H-4″/H-5″ were deduced from the coupling constant of H-2 (7.3 Hz) and NOEs for H-2/H-4″ and H-3″/H-5″ observed in the 1D NOE experiment. Furthermore, the equatorial-orientation of H-5 could have resulted from the coupling constants (4.8 Hz) of H-4″. These data revealed that in pyridine-d_5, **15** possessed the same stereochemistry as the above-described compounds. The comparison of the coupling constants of H-2 (7.3 Hz) and H-4″ (9.8, 9.8, 4.8 Hz) in pyridine-d_5 with those of H-2 (6.0, 3.9, 2.6 Hz) and H-4″ (9.6, 5.7, 5.7 Hz) in $CDCl_3$ indicated that **15** displayed different conformations in the two solvents (Figures S5 and S6, Supporting Information).

The structure of compound **16** was established by spectroscopic methods (NMR, MS, and IR). The spectral data of the isolated compound were identical with those of the reported values.[10] Thus, compound **16** was determined to be chabamide.

Structures Elucidation of Nigramides P−S (17−20). The HRFABMS (*m/z* 545.2657, calcd for 545.2652 $[M + H]^+$) of nigramide Q (**17**) gave the same molecular formula, $C_{32}H_{36}N_2O_6$, as that of **1**. The ^{1}H and ^{13}C NMR spectra of **17** showed the presence of signals due to two carbonyl groups, a trans double bond, two piperidine rings, and two methylenedioxyphenyl groups (Table 4). However, in the ^{1}H NMR spectrum, the absence of the characteristic cis-ofinic proton signals due to a cyclohexene ring suggested that **17** possessed a different structural skeleton from the above-described compounds. The analysis of the ^{1}H−^{1}H COSY spectrum revealed the presence of a cyclobutane ring (−C-2−C-3−C-4″−C-5″−). The cross-peaks between δ_H 3.62 (H-4″) and 6.51 (H-3″) observed in the ^{1}H−^{1}H COSY spectrum established the attachment of the trans double bond at C-4″. The locations of two carbonyl groups at C-1 and C-1″ were determined by the HMBC correlations for δ_H 3.82 (H-2), 4.49 (H-3) and 3.77 (H-5″) to δ_C 165.3 (C-1″), and δ_H 6.51 (H-3″) to δ_C 165.3 (C-1″). The positions of two methylenedioxyphenyl groups at C-3 and C-5″ were revealed by the HMBC correlations for δ_H 4.49 (H-3) to δ_C 108.5 (C-5) and 121.1 (C-9), and δ_H 3.77 (H-5″) to δ_C 108.5 (C-7″) and 121.7 (C-11″). Further HMBC correlations for δ_H 3.14 (H-1′b) to δ_C 169.0 (C-1) located a piperidine ring at C-1, suggesting the attachment of the other at C-1″ (Figure S7, Supporting Information). On the basis of the detailed analysis of the ^{1}H−^{1}H COSY, HMQC, and HMBC spectra, the planar structure of **17** was established. The relative stereochemistry of **17** was elucidated by the NOESY spectral data (Figure S8, Supporting Information). The NOESY correlations for H-2/H-5, H-3/H-7″, H-3/H-11″, H-2″/H-4″, H-4″/H-11″, H-3″/H-5″, and H-5″/H-5 indicated an α-orientation for H-2 and H-5″, and a β-orientation for H-3 and H-4″. Thus, the structure of nigramide P (**17**) was unambiguously established by these spectral data.

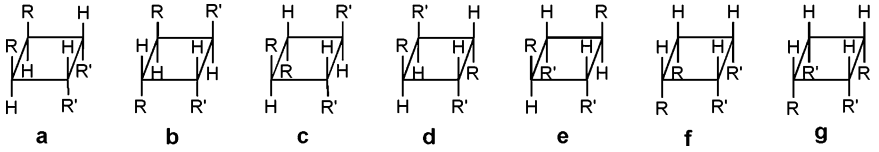
Nigramide Q (**18**) had the same molecular formula of $C_{32}H_{36}N_2O_6$ as that of **17** as established by the HR-FABMS data (*m/z* 545.2659, calcd for 545.2651 $[M + H]^+$). Coupled with the analysis of the ^{1}H−^{1}H COSY, the ^{1}H and ^{13}C NMR spectra of **18** showed the presence of the same structural units as those of **17**, namely, two carbonyl groups, a trans double bond, a cyclobutane ring, two piperidine rings, and two methylenedioxyphenyl groups (Tables 2 and 5). The differences in the chemical shifts in the ^{1}H NMR of **18** and **17** revealed a different connection between these structural units. The cross-peaks between δ_H 3.15 (H-3″) and 6.14 (H-4″) observed in the ^{1}H−^{1}H COSY spectrum determined the attachment of the trans double bond at C-3″. The locations of the two carbonyl groups and two methylenedioxyphenyl groups were established by the HMBC correlations for

TABLE 5. ^{13}C NMR Spectral Data for 18−20, 23, and 24 (125 MHz in $CDCl_3$)

carbon	18	19	20	23	24	carbon	18	19	20	23	24
1	170.6	170.5	170.4	171.3	170.5	1″	170.5	170.5	170.4	165.9	171.3
2	43.4	42.1	42.2a	47.1	131.8	2″	41.9	42.1	42.1[a]	123.8	43.4
3	47.6	46.3	46.4	126.0	129.0	3″	47.7	46.3	46.2	143.4	45.6
4	135.7	128.7	133.9	130.9	27.6	4″	128.7	128.7	128.7	46.7	125.9
5	107.7	131.1	132.1	46.4	39.8	5″	131.1	131.1	131.0	41.8	131.6
6	147.9	131.4	126.8	132.5	136.9	6″	131.4	131.4	131.5	136.6	131.6
7	146.6	105.6	131.8	111.3	108.7	7″	105.6	105.6	105.6	108.4	105.7
8	108.2	148.0	131.8	147.4	147.5	8″	148.0	148.0	148.0	147.7	147.9
9	120.7	147.2	105.4	146.5	146.0	9″	147.2	147.2	147.2	146.0	147.0
10		108.3	148.1	107.9	108.0	10″	108.2	108.3	108.3	108.1	108.2
11		120.9	147.2	124.1	121.1	11″	120.9	120.9	120.9	122.1	120.8
12			108.4								
13			121.2								
$-OCH_2O-$	101.1	101.1	101.1	100.8	101.0	$-OCH_2O-$	101.1	101.1	101.1	100.8	100.8
1′	46.6	46.7	46.7	47.1	47.3	1‴	46.7	46.7	46.7	47.0	47.3
2′	26.7	26.9	26.9	26.4	26.3	2‴	26.9	26.9	26.9	26.4	26.6
3′	24.5	24.6	24.6	24.6	24.6	3‴	24.5	24.6	24.6	24.5	24.9
4′	25.7	25.7	25.7	25.7	26.3	4‴	25.7	25.7	25.7	25.4	25.9
5′	43.3	43.3	43.3	43.3	43.2	5‴	43.2	43.3	43.3	42.7	43.2

[a] Interchangeable signals.

CHART 2



δ_H 3.65 (H-2″) and 3.80 (H-2) to δ_C 170.5 (C-1″), δ_H 3.65 (H-2″) and 3.80 (H-2) to 170.6 (C-1), δ_H 3.36 (H-3) to δ_C 107.7 (C-5) and 120.7 (C-9), and δ_H 6.32 (H-5″) to δ_C 105.6 (C-7″) and 120.9 (C-11″). In addition, the positions of the two piperidine rings at C-1 and C-1″ were deduced from the HMBC correlations for δ_H 3.22 (H-1′) to δ_C 170.6 (C-1) and δ_H 3.50 (H-1‴b) to δ_C 170.5 (C-1″). The analysis of the NOESY spectrum showed that the relative stereochemistry of **18** was different from that of **17** (Figure S9, Supporting Information). In the NOESY spectrum, correlations for H-2/H-3″, H-2/H-5, H-2″/H-4″, and H-3/H-4″ were observed, which suggested an α-orientation for H-2 and H-3″ and a *β*-orientation for H-2″ and H-3. Thus, the structure of nigramide Q (**18**) was unambiguously determined.

The molecular formula of nigramide R (**19**) was established as $C_{34}H_{38}N_2O_6$ by the HRFABMS (*m/z* 571.2802, calcd for 571.2809 $[M + H]^+$). The NMR spectra showed the presence of 19 hydrogens and 17 carbons, suggesting the symmetrical nature of **19**. The ^{1}H and ^{13}C NMR spectral data (Tables 2 and 5), coupled with the DEPT experiment, revealed the presence of all signals due to two nonconjugated carbonyl group, two trans-double bonds, two methylenedioxyphenyl groups, two piperidine rings, and four methines. By analysis of the ^{1}H−^{1}H COSY and HMBC spectra, the 1-oxopiperidine group and 2-(3,4-methylenedioxyphenyl)ethene group were connected through a C_2 unit comprised of two methines, which led to the half-unit of the molecule. A further seven types of possible symmetrical dimers **a**−**g** are depicted in Chart 2. Among them, types **f** and **g** with all the substitutents on the same side of the cyclobutane ring were eliminated due to the significant steric hindrance. Comparison of the NMR spectral data of **19** with **18** and dipiperamide C[8b] suggested that **19** contained a cyclobutane ring (−C-2−C-3−C-3″−C-2″−) with a head-to-head structure, which was supported by the ion fragments at *m/z* 250 and *m/z* 320 observed in the mass spectrum (Figure 4). Thus,

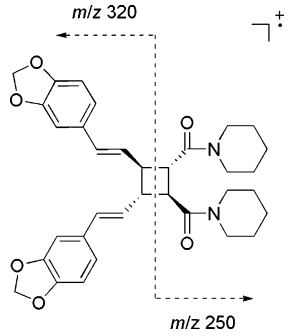
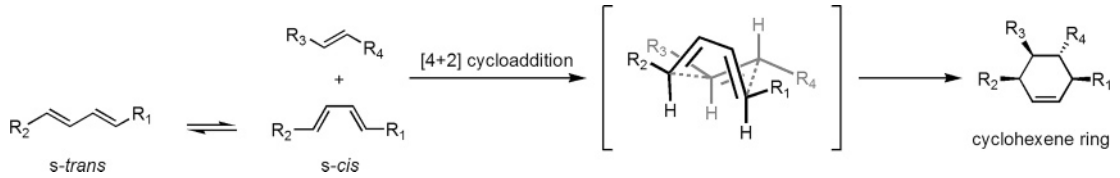


FIGURE 4. FAB-MS analysis of **19**.

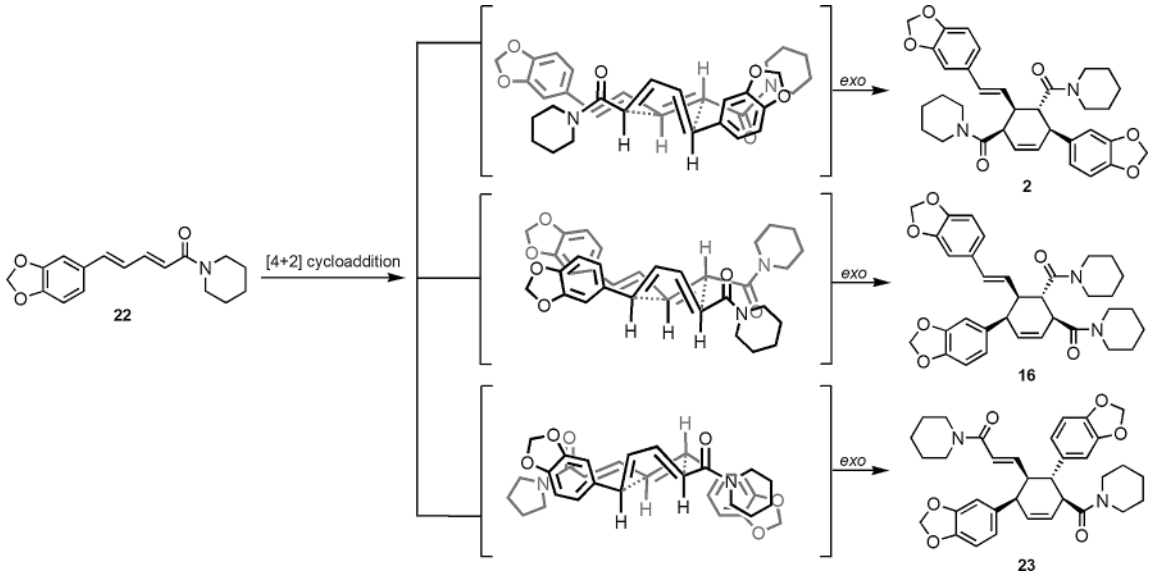
types **d** and **e** with head-to-tail structures were also eliminated. To establish the final structure of **19**, a 1D NOE experiment was undertaken. Upon irradiation of the olefinic proton signal at δ_H 6.11 (H-4 or H-4″) of **19**, NOEs were observed at δ_H 2.99 (H-3 or H-3″) and 3.66 (H-2 or H-2″) (Figure S10, Supporting Information). As far as types **a** and **b** were concerned, the structures adopting the two configurations did not show the observed NOEs. The above evidence indicated that the symmetrical structure of **19** adopted type **c** with the all-trans configuration, which was also supported by the identity of the proton peak patterns of **19** with those of theoretical spectra.[14] Thus, the structure of **19** was determined and possessed the same relative stereochemistry as that of **18**.

(14) Ulrich, H.; Rao, D. V.; Stuber, F. A.; Sayigh, A. A. R. *J. Org. Chem.* **1970**, *35*, 1121−1125.

SCHEME 1



SCHEME 2



The molecular formula of nigramide S (**20**) was established as $C_{36}H_{40}N_2O_6$ by the HRFABMS (*m/z* 597.2979, calcd for 597.2965 $[M + H]^+$). Its NMR spectral data were very similar to those of **19** except for the presence of an additional trans-olefin (Tables 2 and 5). The analysis of the ^1H NMR data showed the presence of proton signals at δ_H 5.83 (1H, dd, J = 14.8, 8.0 Hz), 6.23 (1H, dd, J = 15.1, 10.5 Hz), 6.39 (1H, d, J = 15.5 Hz) and 6.55 (1H, dd, J = 15.5, 10.3 Hz) due to two *trans*-conjugated double bonds, which were located at C-3 by cross-peaks of the olefinic proton signal at δ_H 5.83 with the methine proton signal at δ_H 2.93 observed in the $^1H-^1H$ COSY spectrum. Furthermore, the structure of **20** and the same relative stereostructure as that of **19** were established by the analysis of the 2D NMR spectra, and the comparison of the NMR data with those of **19** in the cyclobutane ring.

The structure of compound **21** was established by spectroscopic methods (NMR, MS, and IR). The spectral data of the isolated compound were identical with those of the reported values.[7c] Thus, compound **16** was identified to be dipiperamide E.

Possible Biogenesis for Cyclohexene-Type Dimeric Amide Alkaloids (1–16). Compounds (**1**–**16**) are novel cyclohexene-type dimeric amide alkaloids. By examination of these structures, we proposed a possible biosynthesis for their formation by an intermolecular Diels–Alder reaction from the same or different two monomeric amide alkaloids as postulated biosynthetic precursors, which have been reported by us.[9] Namely, one molecular monomeric amide possessing an s-*trans*-*E*,*E* system was initially isomerized to an s-*cis*-*E*,*E* system. As a diene, carried out the intermolecular [4 + 2] cycloaddition reaction with another molecular monomeric amide resulted in the formation of dimeric compounds (Scheme 1). The relative stereochemistry of the products described in the biosynthetic hypothesis was identical to that of the natural products isolated from the roots of *P. nigrum*. In addition, a possible biosynthesis for compounds (**17**–**21**) was also proposed by an intermolecular [2 + 2] cycloaddition reaction from the same or different two monomeric amide alkaloids as postulated biosynthetic precursors.[8b]

Cycloaddition Reactions of Piperine (22). To support the above-described biosynthetic hypothesis, piperine (**22**) isolated from the roots of *P. nigrum*[9] were chosen as the substrate to be used for conventional thermal and Lewis acid-mediated [4 + 2] cycloaddition reactions in different organic solvents and under solventless conditions to give the corresponding cycloadducts **2**, **16**, and **23** (Scheme 2). The structure of **23** as a new adduct was established on the basis of the 1D and 2D NMR experiments. Compound **23** had the same molecular formula, $C_{32}H_{36}N_2O_6$, as that of **2** on the basis of the HRFABMS analysis. Its ^1H and ^{13}C NMR spectral data were almost identical with those of **2** (Tables 2, 3, and 5). The presence of a carbonyl carbon signal at δ_C 165.9 observed in the ^{13}C NMR spectrum suggested that the functionality was conjugated to a double bond, as supported by the HMBC ^3J-correlation for δ_H 5.65 (H-3″) to δ_C 165.8 (C-1″). The structure assigned to **23** including the relative stereostructure was further determined by the observed 2D NMR correlations, indicating the same stereochemistry as that of **2**.

With **22** in hand, its thermal Diels–Alder dimerization reaction was performed in several solvents that were heated to their boiling points. The results of these reactions are shown in Table 6. Initially, we carried out the cycloaddition reactions in chloroform, acetone, and

TABLE 6. Diels–Alder Reactions of 22 under Thermal and Solventless Conditions

entry	solvent	conditions[a] (T (°C))	yield[b] (%)	cycloadduct (ratio%) 2	16	23
1	acetone	56	0			
2	$CHCl_3$	61	0			
3	methanol	65	0			
4	benzene	80	0			
5	dioxane	101	0			
6	toluene	110	3	11	78	11
7	xylene	135	17	11	86	3
8	DMF	153	3	22	61	17
9	4-octanol	170	47	15	77	11
10	3-octanol	177	49	14	80	8
11	2-octanol	179	26	15	77	6
12	1-octanol	194	18	22	67	8
13	neat	130	57	17	72	11

[a] All reactions were performed for 72 h. [b] Combined yields on the basis of isolated products.

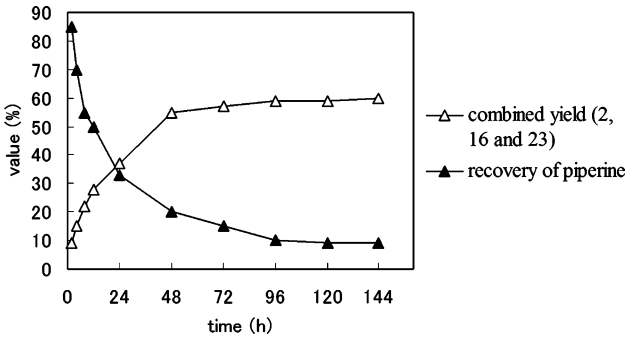


FIGURE 5. Effect of prolonging time on the Diels–Alder reaction of **22** under solventless condition.

methanol, which were used in the process of isolation and extraction of the roots of *P. nigrum*. After 12 h, the analysis of the reaction mixtures showed that no cycloadducts were formed. A prolonged reaction time (72 h) did not result in any change, which excluded the possibility of dimer formation during the process of extraction and isolation. In the cycloaddition reactions of **22**, toluene and xylene were also used, but the expected results were not obtained. In toluene, the reaction afforded an 11:78:11 mixture of **2**, **16**, and **23** in the combined isolated yield of 3%, and in xylene, the same adducts were provided in 17% yield and an 11:86:3 ratio. When the unusual solvent 3-octanol was used, the reaction was performed at 177 °C for 72 h and afforded a 14:80:6 ratio of **2**, **16**, and **23** in the combined yield of 49%. However, in DMF and 1-octanol, the combined yields of the reaction were low (3% and 18%, respectively). These results indicated that the [4 + 2] cycloaddition reactions of **22** had a dependence on the solvent. Furthermore, we also carried out the Diels–Alder reaction under solventless conditions at 130 °C (mp of piperine) for 72 h and obtained a better combined yield (57%) than those in solvents. On the basis of these results, we further investigated the reaction under solventless conditions at 130 °C for a different time. The results showed that the reaction achieved thermodynamic equilibrium after 48 h (Figure 5).

It is well-known that Diels–Alder reactions can be catalyzed with Lewis acids.[15] In our experiments, several Lewis acids were used (Table 7). During our first experiments with $ZnCl_2$, the reactions were separately performed in toluene and dioxane at 100 °C for 72 h, but no cycloadducts were formed. Furthermore, we carried out

TABLE 7. Diels–Alder Reactions of Piperine under Lewis Acid Catalyzed Conditions

entry	solvent	Lewis acid	conditions[a] (T (°C))	yield[b] (%)	cycloadduct (ratio %) 2	16	23
1	xylene	$FeSO_4{\cdot}7H_2O$	135	10	11	86	3
2	xylene	$CuSO_4{\cdot}5H_2O$	135	13	11	86	3
3	xylene	$CoCl_2{\cdot}6H_2O$	135	11	11	87	2
4	xylene	$ZnCl_2$	135	6	11	87	2
5	3-octanol	$FeSO_4{\cdot}7H_2O$	177	15	11	86	3
6	3-octanol	$CuSO_4{\cdot}5H_2O$	177	15	9	86	5
7	3-octanol	$ZnSO_4{\cdot}5H_2O$	177	22	11	86	3
8	3-octanol	$CoCl_2{\cdot}6H_2O$	177	28[c]			
9	3-octanol	$ZnCl_2$	177	9	16	78	6
10	neat	$FeSO_4{\cdot}7H_2O$	130	5	13	84	3
11	neat	$CuSO_4{\cdot}5H_2O$	130	23	14	83	3
12	neat	$ZnSO_4{\cdot}5H_2O$	130	21	15	81	4
13	neat	$CoCl_2{\cdot}6H_2O$	130	15	13	82	5
14	neat	$ZnCl_2$	130	5			

[a] All reactions were performed for 72 h. [b] Combined yields on the basis of isolated products. [c] Isolation of **2**, **16**, **23**, and **24** in a 15:41:3:41 ratio and in an overall 28% yield.

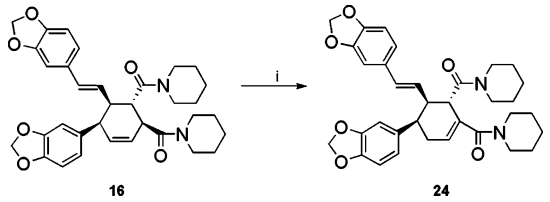
the Lewis acid-mediated Diels–Alder reactions in xylene and 3-octanol, and under solventless conditions, which produced low combined yields. A comparison of the results with those of the above-described thermal reactions showed that the presence of Lewis acids did not allow for high conversion of the substrate and low reaction temperatures and did not result in any change in the ratio of diastereomers.

The aforementioned reactions, namely, the thermal and Lewis acid-mediated [4 + 2] cycloaddition reactions in different organic solvents and under solventless conditions, all showed a complete *exo* selectivity. Additionally, the reaction yield (**2** and **16**) in the position of the α double bond of **22** as dienophile was much more than that (**23**) in the position of the γ double bond (**2** and **16/23** > 5:1), so good regioselectivity of the reaction could also be concluded. The biomimetic synthesis by means of the Diels–Alder reactions of **22** strongly supported the above-described biosynthetic hypothesis and afforded the same compounds as the natural ones (**2** and **16**) gained in the process of isolation of *P. nigrum*.

During investigating the [4 + 2] cycloaddition of **22**, an unexpected behavior was found. When in the presence of $CoCl_2{\cdot}6H_2O$ as a Lewis acid catalyst, **22** was heated to 177 °C in 3-octanol for 72 h (Table 7, entry 8), not only **2**, **16**, and **23**, but also **24** were obtained with a 15:41:3:41 ratio and a combined yield of 28%. The analyses of the HRFABMS and NMR spectral data (Tables 2 and 5) showed that **24** was also the cyclohexene-type dimer of **22**, but the double bond in the cyclohexene ring of **24** was different from that of all the above-described cyclohexene-type nigramides. The observed 2D NMR correlations supported the positions of the double bond as being located at C-2 and C-3 in **24**. As shown in Table 7, entries 5–9, a remarkable decrease in the yield of **16** in entry 8 indicated that **16** undergoes a double bond isomerization to give the isomeric product **24**. This was confirmed by the thermal transformation reaction of **16** with $CoCl_2{\cdot}6H_2O$ in 3-octanol to give **24** with 26% yield (Scheme 3).

(15) (a) Yates, P.; Eaton, P. *J. Am. Chem, Soc.* **1960**, *82*, 4436–4437. (b) Sauser, J.; Sustmann, R. *Angew. Chem.* **1980**, *92*, 773–801. (c) E.g.: Pindur, U.; Lutz, G.; Otto, C. *Chem. Rev.* **1993**, *93*, 741–761.

SCHEME 3[a]



[a] Reagents and conditions: (i) $CoCl_2 \cdot 6H_2O$, 3-octanol, 177 °C, 72 h.

Conclusion

The structures of 19 novel dimeric amide alkaloids including the relative stereostructure isolated from the roots of *P. nigrum* were determined by the analyses of the 1D and 2D NMR spectral data, and they were confirmed as racemates by the CD and $[\alpha]_D$ data. Compounds **1**−**16** are a family of dimeric amide alkaloids possessing a cyclohexene ring, which to date were not reported. By examination of the structures, a biosynthetic proposal of the cyclohexene-type nigramides was postulated to arise from an intermolecular Diels−Alder reaction of the monomeric amide alkaloids. On the basis of this proposal, a biomimetic synthetic experiment, namely, thermal and Lewis acid-mediated Diels−Alder reactions of piperine in different organic solvents and under solventless conditions was performed. The result of the reactions showed that under solventless conditions, the thermal dimerization of **22** gave a moderate yield (57%), which is the best result under all conditions, and the presence of Lewis acids was not helpful to the cycloaddition reaction. The conclusion of complete *exo* selectivity and a good regioselectivity (**2** and **16**/**23** > 5:1) were also obtained from the experimental results. In addition, under the conditions of $CoCl_2 \cdot 6H_2O$ as the Lewis acid catalyst and 3-octanol as the solvent, besides **2**, **16** and **23**, another new adduct **24** was also got through the partial isomerization of **16**. This was confirmed by the thermal transformation reaction of **16**. Furthermore, the results of the above-described biomimetic synthesis strongly supported the biosynthetic hypothesis.

Experimental Section

Plant Material. The roots of *P. nigrum* used in this study were collected on Hainan Island, People's Republic of China, in April 2001, and identified by Y.C. A voucher specimen (TH04001) was deposited in the herbarium of Toho University, Japan.

Extraction and Isolation. The dried powdered roots (7 kg) were repeatedly extracted with methanol (3 L × 4) at room temperature. The aqueous methanolic extracts were combined and evaporated under vacuum to give a residue (508 g). The residue was dispersed in H_2O (1 L) and then successively extracted with chloroform (1 L × 3), ethyl acetate (1 L × 3), and *n*-BuOH saturated with H_2O (1 L × 3). The solvents were then evaporated in vacuo. The chloroform extract (150 g) was chromatographed on a silica gel CC (2500 g) with a gradient of petroleum ether and acetone (9:1, 4:1, 2:1, and 1:1) to give four fractions A−D. Fraction B (36 g) was subjected to silica gel CC (400 g) and elution with petroleum ether and acetone (9:1, 4:1, 2:1, and 1:1) to afford four subfractions B1−B4. Fraction B1 was further chromatographed on RP-CC (MeOH−H_2O, 80:20 and 85:15 as solvent) to afford two subfractions (B1a and B1b), followed by RP-HPLC (MeOH−H_2O, 80:20) and RP-HPLC (CH_3CN−H_2O, 70:30) to yield **8** (23 mg), **9** (11 mg), **10** (22 mg), **14** (2 mg), and **15** (4 mg). Fraction C (2 g) was subjected to a silica gel CC (20 g) and elution with petroleum ether and acetone (9:1, 4:1, and 2:1). The fractions were grouped according to the TLC into three subfractions C1−C3. Fraction C1 was successively chromatographed on RP-CC (MeOH−H_2O, 85:15) and RP-HPLC (CH_3CN−H_2O, 75:25) to yield **11** (5 mg) and **12** (3 mg). Fraction C3 was further fractionated on RP-CC (MeOH−H_2O, 75:25) and RP-HPLC (CH_3CN−H_2O, 65:35) to afford **2** (26 mg) and **6** (4 mg). Fraction D (1.2 g) was subjected to a silica gel CC (12 g) and elution with petroleum ether and acetone (4:1, 2:1 and 1:1) to give three subfractions (D1, D2, and D3). Fraction D2 was subjected to RP-CC eluted with MeOH−H_2O (80:20 and 85:15) to afford two subfractions (D2a and D2b). Fraction D2a was purified by RP-HPLC (CH_3CN−H_2O, 60:40) to yield **1** (12 mg), **3** (95 mg), and **17** (6 mg). Fraction D2b was further chromatographed on RP-HPLC (MeOH−H_2O, 80:20) to give two subfractions, followed by RP-HPLC with CH_3CN−H_2O (60:40 and 70:30) to yield **13** (9 mg) and **21** (21 mg). Fraction D3 was subjected to RP-CC (MeOH−H_2O, 80:20) and RP-HPLC (MeOH−H_2O, 85:15) to afforded two subfractions (D3a and D3b). Fraction D3a was further purified on RP-HPLC (CH_3CN−H_2O, 60:40) to yield **7** (3 mg) and **18** (6 mg). Fraction D3b was chromatographed on RP-HPLC (CH_3CN−H_2O, 65:35) to yield **4** (3 mg), **5** (3 mg), **16** (188 mg), **19** (32 mg) and **20** (3 mg).

Nigramide A (1): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 1.199, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (4.50), 287 (3.83) nm; IR (KBr) ν_{max} 2924, 2856, 1626, 1481, 1240, 1128, 1035 cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 1); FABMS *m/z* 545 $[M + H]^+$; HRFABMS *m/z* 545.2657 $[M + H]^+$ (calcd for 545.2652, $C_{32}H_{36}N_2O_6$).

Nigramide B (2): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 1.512, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (4.54), 271 (4.14) nm; IR (KBr) ν_{max} 2924, 2856, 1628, 1489, 1245, 1128, 1035 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 571 $[M + H]^+$; HRFABMS *m/z* 571.2802 $[M + H]^+$ (calcd for 571.2808, $C_{34}H_{38}N_2O_6$).

Nigramide C (3): colorless oil ($CHCl_3$); colorless prism (MeOH), mp. 182−183 °C; $[\alpha]^{25}_D$ 0° (*c* 1.320, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 210 (4.44), 287 (3.85) nm; IR (KBr) ν_{max} 2922, 2855, 1629, 1457, 1240, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 545 $[M + H]^+$; HRFABMS *m/z* 545.2666 $[M + H]^+$ (calcd for 545.2652, $C_{32}H_{36}N_2O_6$).

Nigramide D (4): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.279, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.51), 285 (4.29) nm; IR (KBr) ν_{max} 2925, 2857, 1727, 1629, 1448, 1246, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 597 $[M + H]^+$; HRFABMS *m/z* 597.2965 $[M + H]^+$ (calcd for 597.2965, $C_{36}H_{40}N_2O_6$).

Nigramide E (5): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.269, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (4.63), 264 (4.31) nm; IR (KBr) ν_{max} 2926, 2857, 1628, 1498, 1448, 1249, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 597 $[M + H]^+$; HRFABMS *m/z* 597.2958 $[M + H]^+$ (calcd for 597.2964, $C_{36}H_{40}N_2O_6$).

Nigramide F (6): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.314, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.61), 287 (3.96) nm; IR (KBr) ν_{max} 2927, 2858, 1709, 1628, 1497, 1448, 1242, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 573 $[M + H]^+$; HRFABMS *m/z* 573.2972 $[M + H]^+$ (calcd for 573.2964, $C_{34}H_{40}N_2O_6$).

Nigramide G (7): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.205, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.60), 287 (3.96) nm; IR (KBr) ν_{max} 2926, 2858, 1629, 1489, 1242, 1035 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 559 $[M + H]^+$; HRFABMS *m/z* 559.2783 $[M + H]^+$ (calcd for 559.2808, $C_{33}H_{38}N_2O_6$).

Nigramide H (8): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 1.378, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.32), 286 (3.58) nm; IR (KBr) ν_{max} 2928, 2857, 1628, 1446, 1239, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 495 $[M + H]^+$;

HRFABMS *m/z* 495.3222 $[M + H]^+$ (calcd for 495.3222, $C_{30}H_{42}N_2O_4$).

Nigramide I (9): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.878, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.43), 270 (3.89) nm; IR (KBr) ν_{max} 2922, 2855, 1629, 1459, 1248 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 521 $[M + H]^+$; HRFABMS *m/z* 521.3383 $[M + H]^+$ (calcd for 521.3379, $C_{32}H_{44}N_2O_4$).

Nigramide J (10): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 1.275, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.39), 287 (3.49) nm; IR (KBr) ν_{max} 2922, 2855, 1636, 1458, 1237 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 495 $[M + H]^+$; HRFABMS *m/z* 495.3232 $[M + H]^+$ (calcd for 495.3223, $C_{30}H_{42}N_2O_4$).

Nigramide K (11): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.508, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 205 (4.26), 286 (3.58) nm; IR (KBr) ν_{max} 2927, 2859, 1617, 1551, 1450, 1238, 1036 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 483 $[M + H]^+$; HRFABMS *m/z* 483.3234 $[M + H]^+$ (calcd for 483.3223, $C_{29}H_{42}N_2O_4$).

Nigramide L (12): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.149, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (4.45) nm; IR (KBr) ν_{max} 2928, 2857, 1439, 1227, 1032 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 521 $[M + H]^+$; HRFABMS *m/z* 521.3383 $[M + H]^+$ (calcd for 521.3380, $C_{32}H_{44}N_2O_4$).

Nigramide M (13): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.810, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 208 (4.41) nm; IR (KBr) ν_{max} 2923, 2856, 1628, 1447, 1242, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 507 $[M + H]^+$; HRFABMS *m/z* 507.3237 $[M + H]^+$ (calcd for 507.3223, $C_{31}H_{42}N_2O_4$).

Nigramide N (14): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.265, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 206 (4.52) nm; IR (KBr) ν_{max} 2926, 2858, 1636, 1448, 1240, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 509 $[M + H]^+$; HRFABMS *m/z* 509.3397 $[M + H]^+$ (calcd for 507.3279, $C_{31}H_{44}N_2O_4$).

Nigramide O (15): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.257, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 216 (4.12) nm; IR (KBr) ν_{max} 3297, 2924, 2857, 1638, 1458, 1257, 1160, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 3); FABMS *m/z* 447 $[M + H]^+$; HRFABMS *m/z* 447.3977 $[M + H]^+$ (calcd for 447.3951, $C_{28}H_{50}N_2O_2$).

Nigramide P (17): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.460, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 207 (4.72), 287 (4.08) nm; IR (KBr) ν_{max} 2927, 2857, 1627, 1498, 1448, 1243, 1131, 1033 cm^{-1}; ^1H NMR and ^{13}C NMR (see Table 4); FABMS *m/z* 545 $[M + H]^+$; HRFABMS *m/z* 545.2657 $[M + H]^+$ (calcd for 545.2652, $C_{32}H_{36}N_2O_6$).

Nigramide Q (18): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.435, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 209 (4.61), 273 (4.16) nm; IR (KBr) ν_{max} 2926, 2857, 1627, 1448, 1247, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 5); FABMS *m/z* 545 $[M + H]^+$; HRFABMS *m/z* 545.2659 $[M + H]^+$ (calcd for 545.2651, $C_{32}H_{36}N_2O_6$).

Nigramide R (19): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 1.076, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 213 (4.56), 267 (4.30) nm; IR (KBr) ν_{max} 2923, 2856, 1627, 1448, 1247, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 5); FABMS *m/z* 571 $[M + H]^+$; HRFABMS *m/z* 571.2802 $[M + H]^+$ (calcd for 571.2809, $C_{34}H_{38}N_2O_6$).

Nigramide S (20): colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.178, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 211 (4.44), 274 (4.10) nm; IR (KBr) ν_{max} 2927, 2857, 1627, 1448, 1248, 1129, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 5); FABMS *m/z* 597 $[M + H]^+$; HRFABMS *m/z* 597.2979 $[M + H]^+$ (calcd for 597.2965, $C_{36}H_{40}N_2O_6$).

X-ray Crystal Data for Nigramide C (3). A colorless prism of dimensions 0.23 × 0.22 × 0.16 mm^3, obtained from MeOH, was selected for diffraction studies. Molecular formula $C_{32}H_{36}N_2O_6$; molecular mass 544.65; crystal system orthorhombic; space group *Pbca* (#61); unit cell dimensions *a* = 10.974(4) Å, *b* = 22.979(9) Å, *c* = 22.402(9) Å; *V* = 5650.1(37) $Å^3$; D_{calcd} = 1.280 g/cm^3; *F*(000) = 23420.00, *Z* = 8; μ(Mo Kα) = 0.88 cm^{-1}. Signal-crystal data were collected using a Rigaku Mercury CCD area detector with graphite-monochromated Mo Kα radiation (λ = 0.710 70 Å) at 293(2) K. Of the 45 881 reflections that were collected, 43 533 were unique (R_{int} = 0.046). The structure was solved by direct methods[16] and expanded using Fourier techniques.[17] The non-hydrogen atoms were anisotropically refined. Hydrogen atoms were refined using the riding model. The final cycle of the full-matrix least-squares refinement on *F* was based on 25 396 observed reflections [I > 3.00 σ(I)]. The final *R* and R_w factors were 0.053 and 0.066, respectively.

Representative Procedures for Thermal Diels−Alder Reactions of Piperine (22) As Presented in Table 6. Entries 10 and 13 Are Shown Below. A solution of **22** (100 mg, 0.35 mmol) in 3-octanol (2 mL) was heated at 170 °C for 72 h in an oil bath. The solution was then allowed to cool to room temperature and was subjected to silica gel CC eluting with methanol. The solvent was removed under reduced pressure, and the residue was purified by RP-CC with MeOH−H_2O (80:20) and RP-HPLC (MeOH−H_2O, 80:20) to afford the desired Diels−Alder adducts as a colorless oil **2** (6.9 mg, 6.9%), **16** (39.2 mg, 39.2%), and **23** (2.9 mg, 2.9%).

22 (100 mg, 0.35 mmol) under solventless conditions was heated at 130 °C for 72 h in an oil bath. To the mixture was added a little methanol to dissolve it. The solution was subjected to silica gel CC with MeOH, RP-CC with MeOH−H_2O (80:20), and RP-HPLC (MeOH−H_2O, 80:20) to give adducts as a colorless oil **2** (9.7 mg, 9.7%), **16** (41.0 mg, 41.0%), and **23** (6.3 mg, 6.3%).

Compound 23: colorless oil; $[\alpha]^{25}_D$ 0 (*c* 1.029, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.74), 288 (3.84) nm; IR (KBr) ν_{max} 2926, 2857, 1627, 1484, 1440, 1240, 1034 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 5); FABMS *m/z* 571 $[M + H]^+$; HRFABMS *m/z* 571.2792 $[M + H]^+$ (calcd for 571.2808, $C_{34}H_{38}N_2O_6$).

Representative Procedure for Lewis Acid-Mediated Diels−Alder Reactions of Piperine (22) As Presented in Table 7. Entry 8 Is Shown Below. A solution of **22** (100 mg, 0.35 mmol) and $CoCl_2 \cdot 6H_2O$ (4.2 mg, 0.0175 mmol) in 3-octanol (2 mL) was heated at 177 °C for 72 h in an oil bath. The mixture was then purified by silica gel CC with MeOH, RP-CC with MeOH−H_2O (80:20), and RP-HPLC (MeOH−H_2O, 80:20) to afford the adducts as a colorless oil **2** (4.2 mg, 4.2%), **16** (11.5 mg, 11.5%), **23** (1.0 mg, 1.0%), and **24** (11.5 mg, 11.5%).

Transformation of 16 into 24. To a solution of **16** (20 mg, 0.035 mmol) in 3-octanol (2 mL) was added 3.6 mg of $CoCl_2 \cdot 6H_2O$ (0.015 mmol). The reaction mixture was heated at 177 °C for 72 h in an oil bath. The solution was then subjected to silica gel CC with MeOH, RP−CC with MeOH−H_2O (80:20), and RP-HPLC (MeOH−H_2O, 80:20) to give **24** as a colorless oil (5.2 mg, 26%).

Compound 24: colorless oil; $[\alpha]^{25}_D$ 0 (*c* 0.962, $CHCl_3$); UV (MeOH) λ_{max} (log ϵ) 204 (4.64), 270 (4.05), 288 (3.88) nm; IR (KBr) ν_{max} 2926, 2857, 1628, 1485, 1439, 1246, 1036 cm^{-1}; ^1H NMR and ^{13}C NMR (see Tables 2 and 5); FABMS *m/z* 571 $[M + H]^+$; HRFABMS *m/z* 571.2797$[M + H]^+$ (calcd for 571.2808, $C_{34}H_{38}N_2O_6$).

Supporting Information Available: Experimental general procedures, complete proton signal assignments for **2**−**15**; **18**−**20**, **23**, and **24**; 1D and 2D NMR spectra for **1**−**15**, **17**−**20**, **23**, and **24**; X-ray crystallographic data of **3**. This material is available free of charge via the Internet at http://pubs.acs.org.

JO040272A

(16) Altomare, A.; Cascarano, G.; Giacovazzo, C.; Guagliardi, A.; Burla, M.; Polidori, G.; Camalli, M. *J. Appl. Crystallogr.* **1994**, *27*, 435.

(17) Beurskens, P. T.; Admiraal, G.; Beurskens, G.; Bosman, W. P.; de Gelder, R.; Israel, R.; Smiths, J. M. M. *The DIRDIF-99 Program System*; Technical Report of the Crystallography Laboratory; University of Nijmegen: The Netherlands, 1999.