

Spectral Assignments and Reference Data

Complete assignments of ^1H and ^{13}C NMR data for rings A,B-*seco* limonoids from the seed of *Aphanamixis polystachya*

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Seven rings A,B-*seco* limonoids 1–7 were isolated from the EtOH extract of the seed of *Aphanamixis polystachya*. Their structures were identified as rohituka-7 (1), dregeana-1 (2), rohituka-15 (3), Tr-B (4), rohituka-3 (5), rohituka-5 (6), and rohituka-14 (7) by MS and NMR spectroscopy. The complete assignment of proton and carbon signals was achieved by 1D and 2D NMR experiments including DEPT, HSQC, ^1H – ^1H COSY, HMBC, and NOESY. Copyright © 2006 John Wiley & Sons, Ltd.

KEYWORDS: ^1H NMR; ^{13}C NMR; 2D NMR; Meliaceae; *Aphanamixis polystachya*; limonoid; dregeana-1; rohituka-3; 5; 7; 14; 15; Tr-B

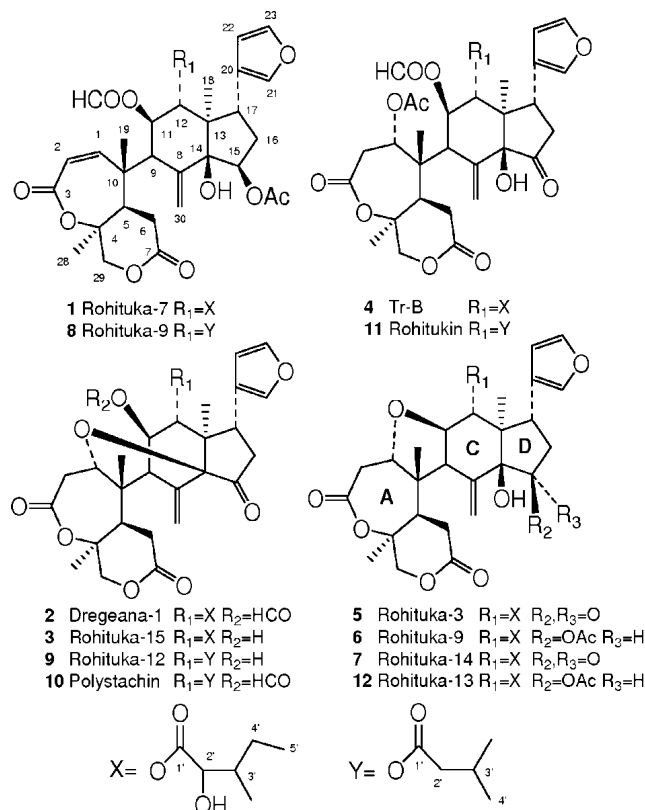


Figure 1. Structures of limonoids 1–12 from *Aphanamixis polystachya*.

INTRODUCTION

The tropical plant *Aphanamixis polystachya* J. N. Parker (synonyms: *Amoora rohituka* Weight and Arn.; *Aphanamixis rohituka* (Roxb.) Pierre) (Meliaceae) is a large evergreen and useful timber tree. It has been extensively investigated since the 1960s because of the anticancer,^{1,2} antimicrobial and antifungal,³ anti-inflammatory,⁴ and insecticidal potential,⁵ and hepatoprotective properties⁶ of the plant extracts. Earlier studies on this plant have disclosed the presence of alkaloids,⁷ fatty acids,⁸ flavonoids,^{9–12} lignans,¹³ steroids,^{14,15} diterpenoids,¹⁶ sesquiterpenoids,¹⁷ triterpenoids,^{1,18–20} and a series of complex tetranortriterpenoids (limonoids) (including dihydroamoorinin²¹ and amoorinin,²² and its glucosides,⁹ aphanamixinin,¹⁸ kihadalactone and polystachin,²³ rohituka 1–9,^{24–26} rohituka 12–14,²³ and rohitukin²⁷). Our early work on investigating the chemical constituents of the plant seed resulted in the isolation of a known dregeana-1 (2) and a novel limonoid rohituka-15 (3).²⁸ Further investigation led to the isolation and identification of five other limonoids: rohituka-7 (1), Tr-B (4), rohituka-3 (5), -5 (6), and -14 (7). Their chemical structures (Fig. 1) were characterized by MS and extensive NMR experiments including techniques like DEPT, HSQC, HMBC, ^1H – ^1H COSY, and NOESY. Although these limonoids have been previously reported in the literature, their NMR data are either incomplete or confusing. For example, rohituka-5 (6)²⁴ only had partial ^1H NMR data reported, and Tr-B (4) was presented with different stereochemistries in previous articles.^{29,30} The NMR profile of rohituka-3 (5)³⁰ is not like that of rohituka-14 (7),²³ though there is only a minor difference between 5 and 7 in the substituted ester variation on their C-12 α group. Therefore, there is a necessity to report the revised NMR data of these rings A,B-*seco* limonoids on the basis of modern 2D NMR measurements.

RESULTS AND DISCUSSION

Compound 1 had ^1H and ^{13}C NMR spectral characteristics consistent with those reported for rohituka-7, previously isolated from the same species²⁴ and *Trichilia hispida*.³¹ Although the ^1H NMR and ^{13}C NMR spectra of rohituka-7 were not completely assigned, its stereochemistry was determined by X-ray diffraction analysis.²⁶ Here, we supplement an unambiguous assignment of its ^1H and ^{13}C NMR spectral data that were obtained through the HSQC, ^1H – ^1H COSY, HMBC, and NOESY experiments. These data are listed in Tables 1 and 2.

The ^1H and ^{13}C NMR data of compound 2 are in good agreement with those of limonoid dregeana-1 found in *Trichilia dregeana*.³² A 1,14-ether linkage is substantiated by the high-frequency chemical shift value of C-14 (δ 87.3) and detailed comparison with those of polystachin (10).²³ It was also confirmed by an HMBC correlation between H-1 (δ 3.80) and C-14. The presence of a 1 α ,14 β -oxide bridge in both polystachin (10)²³ and dregeana-1 (2) was established by a NOESY correlation between H-1 and the C-19 methyl group. 2D NMR of 2 enabled the full assignment of ^1H and ^{13}C NMR resonances to be completed (Tables 1 and 2).

The ^1H and ^{13}C NMR data of rohituka-15 (3)²⁸ (Tables 1 and 2) were, in part, identical to those observed for rohituka-12 (9)²³ and 2, suggesting a close structural similarity between 3, 2, and 9 (Fig. 1). ^1H – ^1H COSY showed the existence of a fragment $\text{CH}_3\text{CH}_2\text{CH}(\text{CH}_3)\text{CHOH}$ - (this moiety is also confirmed to be present in ^1H – ^1H COSY spectrum of 2), but the substituted group $\text{CH}_3\text{CH}(\text{CH}_3)\text{CH}_2\text{COO}$ - on C-12 is lacking in 9. The structure of rohituka-15 was deduced as 3 by detailed comparison between 3, 2, 9, and 10 and HMBC correlations between H-1 (δ 3.68) and C-14 (δ 87.3), and H-12 (δ 5.84) and C-1' (δ 174.9).

The ^1H and ^{13}C NMR data of compound 4 are very similar to those of rohitukin (11)^{24,33} and 2'-hydroxyrohitukin.³⁴ The only difference between 4 and 11 is similar to the difference between 3 and 9. ^1H – ^1H COSY and HMBC spectra in 4 exhibited the presence of a 2-hydroxy-3-methylpentanoate group, which was replaced by 3-methylbutanoate moiety in 11. The ester carbon (δ 174.9, C-1') in 4

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Table 1. The ^1H NMR chemical shifts of limonoids **1–7** in CDCl_3 , coupling constants J (^1H , ^1H) (Hz) in parentheses

Position	1	2	3	4	5	6	7
1	7.56 (1H, d, 12.0)	3.80 (1H, t, 9.1)	3.68 (1H, d, 10.7, 7.2)	5.56 (1H, dd, 7.0, 6.0)	3.99 (1H, t, 3.0)	4.06 (1H, t, 3.0)	4.02 (1H, t, 3.5)
2	6.02 (1H, d, 12.0)	3.17, 2.99 (each 1H, m)	3.12, 2.88 (each 1H, m)	2.65, 2.02 (each 1H, m)	3.07, 2.78 (each 1H, m)	3.20, 2.78 (each 1H, m)	3.12, 2.74 (each 1H, m)
5	2.14 (1H, t, 2.0)	2.88 (1H, m)	3.14 (1H, m)	2.48 (1H, t, 2.0)	2.34 (1H, t, 2.0)	2.35 (1H, t, 2.0)	2.32 (1H, m)
6	2.61, 2.37 (each 1H, m)	2.55, 2.23 (each 1H, m)	3.12, 2.67 (each 1H, m)	2.63, 2.50 (each 1H, m)	2.80, 2.72 (each 1H, m)	2.72 (2H, m)	2.80 (2H, m)
9	3.03 (1H, d, 9.0)	2.75 (1H, d, 9.0)	2.69 (1H, d, 6.2)	3.23 (1H, d, 9.0)	3.24 (1H, d, 10.0)	3.20 (1H, d, 9.0)	3.21 (1H, d, 10.0)
11	5.18 (1H, t, 9.0)	5.33 (1H, t, 9.0)	4.01 (1H, dd, 6.2, 9.8)	5.28 (1H, t, 9.0)	4.16 (1H, t, 10.0)	4.11 (1H, t, 9.0)	4.16 (1H, dd, 10.0, 9.0)
12	6.15 (1H, d, 9.0)	6.10 (1H, d, 9.0)	5.84 (1H, d, 9.8)	6.16 (1H, d, 9.0)	5.87 (1H, d, 10.0)	5.84 (1H, d, 9.0)	5.78 (1H, d, 9.0)
15	5.56 (1H, m)	–	–	–	–	5.49 (1H, dd, 5.5, 8.5)	–
16	2.14, 2.01 (each 1H, m)	2.85, 2.36 (each 1H, m)	2.82, 2.33 (each 1H, m)	2.75, 2.35 (each 1H, m)	2.85, 2.36 (each 1H, m)	2.40, 1.98 (each 1H, m)	2.82, 2.34 (each 1H, m)
17	3.89 (1H, d, 9.5)	3.88 (1H, t, 9.0)	3.85 (1H, dd, 10.1, 9.3)	3.92 (1H, t, 9.5)	3.83 (1H, t, 9.0)	3.79 (1H, t, 9.5)	3.84 (1H, t, 9.0)
18	0.97 (3H, s)	0.90 (3H, s)	0.80 (3H, s)	0.97 (3H, s)	0.86 (3H, s)	0.86 (3H, s)	0.84 (3H, s)
19	1.08 (3H, s)	1.02 (3H, s)	1.08 (3H, s)	1.62 (3H, s)	1.17 (3H, s)	1.17 (3H, s)	1.17 (3H, s)
21	7.16 (1H, s)	7.21 (1H, s)	7.22 (1H, s)	7.21 (1H, s)	7.21 (1H, s)	7.15 (1H, s)	7.22 (1H, s)
22	6.26 (1H, s)	6.20 (1H, s)	6.19 (1H, s)	6.26 (1H, s)	6.22 (1H, s)	6.23 (1H, s)	6.24 (1H, s)
23	7.34 (1H, s)	7.36 (1H, s)	7.35 (1H, s)	7.38 (1H, s)	7.37 (1H, s)	7.33 (1H, s)	7.35 (1H, s)
28	1.65 (3H, s)	1.99 (3H, s)	1.83 (3H, s)	1.81 (3H, s)	1.68 (3H, s)	1.68 (3H, s)	1.68 (3H, s)
29	4.20 (1H, d, 11.0)	4.18 (1H, d, 11.5)	4.22 (1H, d, 11.7)	4.20 (1H, d, 11.0)	4.26 (1H, d, 11.0)	4.24 (1H, d, 11.0)	4.25 (1H, d, 11.0)
30	4.04 (1H, d, 11.0)	4.08 (1H, d, 11.5)	4.00 (1H, d, 11.7)	4.06 (1H, d, 11.0)	4.01 (1H, d, 11.0)	4.00 (1H, d, 11.0)	4.00 (1H, d, 11.0)
30	5.20, 5.17 (each 1H, s)	5.49 (2H, s)	5.36, 5.33 (each 1H, s)	5.89, 5.50 (each 1H, s)	6.25, 5.28 (each 1H, s)	5.18, 5.10 (each 1H, s)	6.23, 5.26 (each 1H, s)
2'	3.10 (1H, d, 3.0)	3.30 (1H, d, 3.5)	3.53 (1H, d, 4.1)	3.20 (1H, d, 3.0)	3.44 (1H, d, 3.5)	3.47 (1H, d, 3.0)	1.89, 1.86 (each 1H, d, 6.5)
3'	1.47 (1H, m)	1.50 (1H, m)	1.60 (1H, m)	1.47 (1H, m)	1.77 (1H, m)	1.58 (1H, m)	1.90 (1H, m)
4'	1.20, 0.93 (each 1H, m)	1.13, 0.95 (each 1H, m)	1.20, 0.92 (each 1H, m)	1.09, 0.91 (each 1H, m)	1.18, 0.95 (each 1H, m)	1.25, 0.92 (each 1H, m)	0.91 (3H, d, 6.5)
5'	0.76 (3H, t, 7.5)	0.77 (3H, t, 7.5)	0.78 (3H, t, 7.3)	0.76 (3H, t, 7.5)	0.82 (3H, t, 7.5)	0.81 (3H, t, 7.5)	–
3'-Me	0.83 (3H, d, 6.5)	0.83 (3H, d, 6.5)	0.82 (3H, d, 5.2)	0.83 (3H, d, 6.5)	0.89 (3H, d, 6.5)	0.89 (3H, d, 6.5)	0.87 (3H, d, 5.8)
HCOO	7.91 (1H, s)	8.09 (1H, s)	–	7.81 (1H, s)	–	–	–
CH ₃ COO	2.01 (3H, s)	–	–	2.00 (3H, s)	–	2.15 (3H, s)	–

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Table 2. The ^{13}C NMR chemical shifts (ppm) of limonoids **1–7** in CDCl_3

Position	1	2	3	4	5	6	7
1	153.2	74.2	74.2	74.0	78.4	78.3	78.3
2	120.0	38.4	38.6	38.0	37.0	37.0	37.1
3	167.0	167.5	168.2	167.8	167.8	167.6	167.7
4	79.2	78.6	79.4	79.3	79.6	79.5	79.6
5	50.7	40.7	41.0	40.0	43.7	43.7	43.7
6	29.9	32.8	32.5	35.0	31.2	31.3	31.2
7	172.4	172.3	174.0	173.5	171.2	169.7	171.5
8	140.5	134.2	135.8	138.2	138.9	142.7	139.0
9	51.6	55.3	56.4	51.3	54.7	53.9	54.6
10	43.5	50.0	50.7	49.6	51.7	51.8	51.7
11	72.4	72.1	74.4	78.1	79.1	79.1	79.5
12	76.1	74.4	79.2	71.2	75.3	75.6	73.3
13	50.9	49.2	48.8	47.8	48.0	49.4	48.2
14	84.4	87.3	87.3	80.0	79.4	84.1	79.6
15	71.1	205.0	205.6	205.6	209.2	74.1	209.3
16	36.5	41.1	41.1	42.2	41.9	37.0	42.0
17	39.4	36.8	37.1	37.9	34.8	38.8	34.8
18	13.3	12.3	12.5	12.9	12.9	13.2	12.9
19	24.0	22.2	22.6	22.6	16.6	16.1	16.6
20	123.5	121.8	122.2	122.2	121.0	123.8	121.0
21	140.5	140.6	140.5	140.5	140.4	140.1	140.5
22	110.5	110.3	110.4	110.3	110.5	110.7	110.5
23	143.0	143.3	143.5	143.3	143.0	142.8	142.7
28	26.8	29.1	28.1	28.5	26.7	26.6	26.7
29	74.6	74.3	74.1	74.2	72.7	72.7	72.7
30	119.3	119.3	117.8	122.2	122.7	117.3	122.8
1'	174.8	174.9	175.3	174.9	175.2	175.1	172.7
2'	74.8	75.1	75.1	74.6	74.3	71.1	43.2
3'	37.6	38.1	38.6	38.6	39.2	37.0	25.2
4'	22.9	23.1	24.0	22.7	24.0	24.0	22.4
5'	11.3	11.4	11.7	11.4	11.8	11.8	–
3'-Me	15.3	15.1	15.0	15.1	14.9	14.9	22.5
HCOO	159.9	160.3	–	160.3	–	–	–
CH_3CO	169.7	–	–	169.9	–	171.4	–
CCH_3CO	20.6	–	–	21.0	–	21.0	–

showed an HMBC correlation with H-12 (δ 6.16). The data suggested that compound **4** was Tr-B, which was previously reported from *Trichilia roka*²⁹ and *Trichilia emetica*.³⁰ In the latter reference, the configuration at C-1 and C-11 are wrongly drawn. The correct stereochemistry is given in Ref. 29. A complete analysis of the proton and carbon NMR data of **4** (Tables 1 and 2) was undertaken and the assignments of formate, acetyl, and methylene groups (C-2, C-6 and C-17) and the quaternary carbons (C-10 and C-14) of **4** in Ref. 30 were revised.

Compound **5** and rohituka-15 (**3**) have the same molecular formula $\text{C}_{32}\text{H}_{40}\text{O}_{11}$, similar ^1H and ^{13}C NMR spectra, same multiplicities of total 32 carbons determined by DEPT, and same functional groups in IR absorptions. This implied that these two compounds might be isomers. Moreover, the NMR data of **5** is very similar to those of rohituka-14 (**7**), suggesting that **5** was identical to rohituka-3. However, there is a slight difference between our ^{13}C NMR data of compound **5** and that of rohituka-3 isolated from *T. emetica*.³⁰ Such discrepancies prompted us to undertake a complete NMR analysis by extensive 2D NMR measurements, including HMBC correlations, between H-1 and C-11, and H-11 and C-1, which confirmed that compound **5** was rohituka-3. All

the assignments for rohituka-3 (**5**) are listed in Tables 1 and 2. Meanwhile, our result demonstrated that the previously reported compound³⁰ was not rohituka-3 but more likely to be **3**. For example, the reported chemical shift values for C-14 and C-15 (δ 87.3, 205.5) of that compound are the same as those in **2** (87.3, 205.6), **3** (87.3, 205.0), **9**²³ (87.4, 205.3), and **10**²³ (87.4, 205.9), but different from those in **5** (79.4, 209.2) and **7** (79.6, 209.3).

The ^1H NMR data of compound **6** showed similarities to rohituka-3 (**5**) and rohituka-13 (**12**).²³ Detailed comparison of ^1H NMR data for **6** with that of limonoids **1**, **5**, **7**, rohituka-9 (**8**),²⁴ and **12** suggested that **6** was identical to rohituka-5.²⁴ The absence of ^{13}C NMR data for rohituka-5 prompted us to undertake an unambiguous assignment of its NMR data, which was achieved by the application of 2D NMR techniques and are listed in Tables 1 and 2.

The NMR data of compound **7** is in agreement with that of rohituka-14 reported in the literature.²³ Since the assignments of ester carbons (C-1', C-3) and quaternary carbons (C-10, C-14) of rohituka-14 reported in the literature²³ need to be revised, the complete NMR data assignments of rohituka-14 (**7**) are also listed in Tables 1 and 2 for comparison with rings A,B-*seco* limonoids.

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EXPERIMENTAL

General experimental procedures

IR: Bio-Rab FTS-135; EIMS: VG Autospec 3000; $[\alpha]_D$: Horiba-300.

Extraction and isolation

The seeds of *A. polystachya* were collected at Xishuangbanna, Yunnan Province, P. R. China, in October 2000 and identified by Prof. Guoda Tao, Xishuangbanna Botany Garden, Chinese Academy of Sciences. Air-dried seeds (2.5 kg) were ground and extracted by 8 l hexane two times. Then the defatted seeds were extracted with 8 l of 95% EtOH three times at room temperature. After removal of the solvent by evaporation, the combined residue (200 g) was suspended in 300 ml water, followed by partition with 300 ml chloroform three times. The combined CHCl_3 extract (36 g) was subjected to column chromatography and eluted by hexane–acetone mixture containing increasing amounts of acetone to give seven fractions. Repeated CC for these fractions on silica gel, Sephadex LH-20, and Reverse-phase C18 column afforded 1 (23 mg), 2 (8 mg), 3 (22 mg), 4 (14 mg), 5 (17 mg), 6 (10 mg), and 7 (19 mg).

Rohituka-7 (1): amorphous, $\text{C}_{35}\text{H}_{44}\text{O}_{13}$; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3503, 2968, 2880, 1732, 1639, 1503, 874; EIMS m/z (%): 672 $[\text{M}]^+$ (10), 626 (11), 481 (7), 452 (13), 434 (17), 418 (10), 312 (10), 226 (40), 209 (44), 195 (24), 177 (27), 135 (26), 121 (45), 95 (47), 76 (67), 57 (100).

Dregeana-1 (2): amorphous, $\text{C}_{33}\text{H}_{40}\text{O}_{12}$; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3490, 2966, 1752, 1461, 874; EIMS m/z (%): 628 $[\text{M}]^+$ (44), 357 (5), 341 (20), 263 (19), 243 (12), 226 (21), 197 (12), 185 (15), 167 (12), 149 (37), 121 (42), 94 (53), 76 (47), 57 (100).

Rohituka-15 (3): amorphous, $\text{C}_{32}\text{H}_{40}\text{O}_{11}$; mp, 122–124 °C, $[\alpha]_D^{27}$ –105.7 (CHCl_3 , c 0.29); IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3484, 3400, 2966, 2927, 1750, 1462, 1380, 874; EIMS m/z (%): 600 $[\text{M}]^+$ (24), 488 (17), 468 (9), 452 (9), 429 (30), 410 (9), 381 (7), 357 (7), 341 (63), 315 (33), 297 (15), 273 (7), 260 (18), 243 (28), 227 (30), 213 (16), 185 (14), 167 (22), 149 (50), 121 (70), 109 (40), 94 (58), 81 (60), 76 (67), 57 (100).

Tr-B (4): amorphous, $\text{C}_{35}\text{H}_{44}\text{O}_{14}$; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3500, 2968, 2880, 1725, 1629, 1502, 1460, 1379, 874; EIMS m/z (%): 688 $[\text{M}]^+$ (3), 628 (11), 582 (15), 497 (11), 468 (13), 450 (17), 434 (15), 400 (15), 368 (10), 341 (7), 312 (10), 226 (40), 209 (44), 195 (24), 177 (27), 135 (26), 121 (45), 95 (47), 76 (67), 57 (100).

Rohituka-3 (5): amorphous, $\text{C}_{32}\text{H}_{40}\text{O}_{11}$; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3515, 2970, 1746, 1462, 1378, 874; EIMS m/z (%): 600 $[\text{M}]^+$ (24), 468 (25), 452 (21), 358 (13), 263 (5), 234 (30), 201 (8), 187 (14), 175 (16), 149 (25), 137 (55), 121 (55), 95 (50), 76 (65), 57 (100).

Rohituka-5 (6): amorphous, $\text{C}_{34}\text{H}_{44}\text{O}_{12}$; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3507, 2964, 2880, 1746, 1464, 1374, 874; EIMS m/z (%): 644 $[\text{M}]^+$ (24), 598 (27), 566 (10), 512 (8), 468 (16), 452 (20), 436 (24), 399 (9), 346 (16), 323 (13), 297 (9), 255 (10), 242 (17), 226 (30), 209 (37), 185 (14), 167 (16), 137 (40), 121 (45), 95 (47), 85 (80), 57 (100).

Rohituka-14 (7): amorphous, $\text{C}_{31}\text{H}_{38}\text{O}_{10}$; IR (KBr) $\nu_{\text{max}}(\text{cm}^{-1})$: 3432, 2962, 2875, 1746, 1635, 1503, 1465, 1371, 874; EIMS m/z (%): 570 $[\text{M}]^+$ (24), 468 (100), 452 (22), 358 (13), 346 (37), 234 (61), 221 (19), 199 (10), 187 (14), 175 (16), 149 (25), 137 (87), 121 (54), 109 (20), 95 (65), 85 (100), 57 (88).

NMR spectroscopy

NMR spectra were acquired using a Bruker AV-500 spectrometer operating at 500 and 125 MHz for ^1H and ^{13}C , respectively, and equipped with an inverse-detection 5 mm probe (TBI probe, ^1H 90° pulse width = 6.1 μs , ^{13}C 90° pulse width = 12.3 μs).

Spectra were recorded at 25 °C using tetramethylsilane as internal standard. Approximately 7–16 mg of the samples was dissolved in 0.5 ml CDCl_3 solvent. 1D NMR spectra were obtained using 64 K data points and spectral widths of 10 000 Hz and 27 500 Hz for ^1H and ^{13}C , respectively. For the processing, 32 K data points were used with an exponential function for all 1D NMR spectra.

Standard pulse sequences were used for 2D NMR spectra. Spectral widths of 5 000 Hz and 25 000 Hz were used for ^1H and ^{13}C , respectively. Relaxation delays of 2.0 s were used for all 2D

NMR experiments. The 2D NMR spectra used 1024 \times 512 (^1H – ^1H COSY), 2048 \times 512 (HSQC), and 4096 \times 512 (HMBC) data point matrices, which were zero-filled to 1024 \times 1024, 2048 \times 1024, and 4096 \times 1024, respectively. Nonshifted q-sine-bell window functions were used along with the F_1 and F_2 axes for all the 2D NMR spectra. The HMBC experiments used an 80-ms delay time to obtain ^1H and ^{13}C long-range correlation. Z-PFGs were used to obtain HSQC and HMBC spectra. Data processing was carried out with Bruker XWINNMR 3.50 programs.

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