Pergamon

Bioorganic & Medicinal Chemistry Letters 12 (2002) 2941–2944

BIOORGANIC & MEDICINAL CHEMISTRY LETTERS

Nodulisporic Acid B, B_1, and B_2: A Series of 1′-Deoxy-nodulisporic Acids from *Nodulisporium* sp.

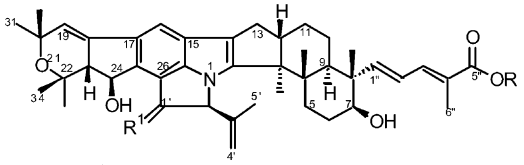
John G. Ondeyka,* Arlene M. Dahl-Roshak, Jan S. Tkacz, Deborah L. Zink, Michelle Zakson-Aiken, Wesley L. Shoop, Michael A. Goetz and Sheo B. Singh*

Merck Research Laboratories, PO Box 2000, Rahway, NJ 07065, USA

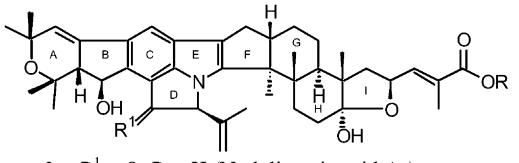
Received 13 March 2002; accepted 5 July 2002

Abstract—During the re-isolation of the lead compound nodulisporic acid A (**1a**) and targeted chemical screening for related compounds, we discovered a series of 1′-deoxy congeners named herein nodulisporic acids B (**1b**), B_1 (**2b**), and B_2 (**3b**). In comparison with nodulisporic acid A, these compounds were less active and were chemically unstable resulting into formation of Δ^{23} dehydro derivatives. Therefore, these compounds were stabilized and isolated as sodium salts and methyl ester. Nodulisporic acid B is 100-fold less active than nodulisporic acid A against fleas. The isolation, structure elucidation, and biological activities of these compounds are described.

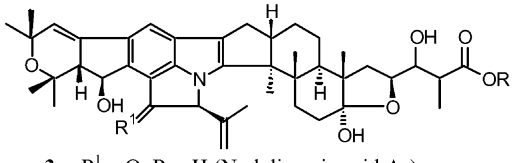
Fleas and ticks pose a significant health hazards to companion animals. Although several topical flea-killing agents are available for dogs and cats, such as, fipronil,[1] imidacloprid,[2] and selamectin,[3] none is orally active. A long-duration, systemically active, non-toxic agent that can kill fleas on dogs or cats would be highly preferred over topical agents. A few years ago we reported[4] the discovery of nodulisporic acid A (**1a**),[4a] A_1 (**2a**),[4b] and A_2 (**3a**).[4b] Nodulisporic acid A is a potent, long-lasting, nontoxic systemic orally-active agent that kills fleas on dogs[4c,d] that became a significant medicinal chemistry lead for further potency and duration-of-action improvements. The mechanism-of-action of nodulisporic acid A was elucidated.[5] It exerts its biological activity by selectively opening an insect (invertebrate)-specific, glutamate-gated chloride channel, thus paralyzing insects without affecting the mammalian host.[5] During these investigations we sought other structurally-related natural product derivatives by targeted chemical and biological screening either as minor congeners in the existing fermentation or as major products from mutants of the producing organism. These efforts led to the discovery of a series of 1′-deoxy congeners, isolated initially from the original producing fungus *Nodulisporium* sp. as very minor components, named herein nodulisporic acids B (**1b**), B_1 (**2b**), and B_2 (**3b**). Subsequently, a series of *Nodulisporium* mutants was isolated that yielded these compounds as major components of the fermentation. The isolation, structure elucidation, and activity of these compounds are described in this paper.



1a: R^1 = O, R = H (Nodulisporic acid A)
1b: R^1 = H, H, R = H (Nodulisporic acid B)
1c: R^1 = H, H, R = Na
1d: R^1 = H, H, R = CH_3
1e: R^1 = O, R = CH_3



2a: R^1 = O, R = H (Nodulisporic acid A_1)
2b: R^1 = H, H, R = H (Nodulisporic acid B_1)
2c: R^1 = H, H, R = Na



3a: R^1 = O, R = H (Nodulisporic acid A_2)
3b: R^1 = H, H, R = H (Nodulisporic acid B_2)
3c: R^1 = H, H, R = Na

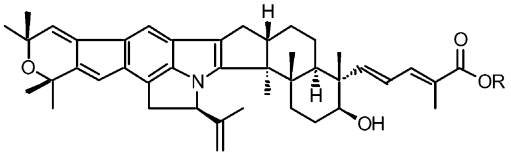
*Corresponding authors. Tel.: +1-732-594-3222; fax: +1-732-594-6880; e-mail: john_ondeyka@merck.com (J. G. Ondeyka); sheo_singh@meck.com (S. B. Singh)

0960-894X/02/$ - see front matter © 2002 Elsevier Science Ltd. All rights reserved.
PII: S0960-894X(02)00621-2

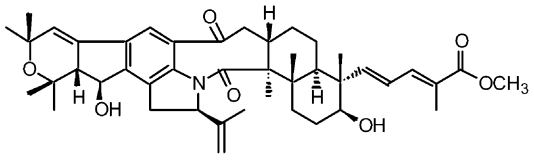
A methyl ethyl ketone extract of *Nodulisporium* sp. (ATCC 74245) was processed as described for the isolation of nodulisporic acid A.[4a] Use of this isolation procedure led to the decomposition of the new compounds to less polar products. One of these was identified as a Δ^{23} derivative **4a**, which was characterized as its methyl ester **4b** produced by reaction with diazomethane. Decomposition of the un-methylated material was accelerated upon concentration of the sample during isolation and was attributed to the presence of the free carboxyl group (methyl ester is stable). Therefore, a new isolation procedure was developed for the isolation of these compounds.

A mutant[6] of the *Nodulisporium* sp., designated ATCC74382, produced the three new compounds as major components. A 1-L portion of fermentation broth[7] from the mutant culture was diluted with an equal volume of MeOH, stirred for 1/2 h, and filtered through a filter aid. An aqueous solution of $NaHCO_3$ was added to the filtrate to adjust the pH to 9.0, and the extract was charged to a 200 cc SP207 column, which was eluted with a step gradient of 40–100% aqueous MeOH. The fractions eluting with 100% MeOH contained nodulisporic acids. These fractions were concentrated under reduced pressure and lyophilized to yield a yellow solid. A portion of the solid was dissolved in MeOH and subjected to preparative chromatography on RP-HPLC (Zorbax RX C-8) at neutral pH to afford sodium salts of nodulisporic acids B (22 mg, 55 mg/L), B_1 (7 mg, 17.5 mg/L) and B_2 (1.5 mg, 3.8 mg/L), as yellow powders.[8]

A portion of the nodulisporic acid fraction obtained from the SP207 column was dried and reacted with trimethylsilyl-diazomethane followed by silica gel chromatography to yield the methyl esters of nodulisporic acid B (**1d**)[8] and the oxidized product **5**.[9]



4a: R = H
4b: R = CH_3



5

Nodulisporic acid B (1b). HRESI-FTMS analysis of **1c** showed a molecular ion at *m/z* 666.4141 (M + H, calcd 666.4158) and produced a molecular formula $C_{45}H_{56}NO_5$ which was supported by the formula of methyl ester $C_{44}H_{58}NO_5$ (observed 679.4165) derived from the HREIMS analysis of **1d**. These formulae indicated the presence of two additional hydrogens and one less oxygen atom compared to the corresponding molecular formulae of nodulisporic acid A (**1a**) and its methyl ester (**1e**).[4a] Comparison of the ^{13}C NMR (Table 1) of **1c** and **1d** with that of nodulisporic acid A (**1a**) and **1e**[4a] indicated the absence of the C-1′ carbonyl carbon and the presence of a new methylene signal at δ 41.1 (δ 40.9 for **1d**) as only major differences. The ^1H NMR (Table 1) showed corresponding pair of doublets of doublets for methylene protons at δ 4.0 and δ 3.5, which exhibited COSY correlations to the C-2′ methine doublet at δ 5.25. The structures of the Δ^{23}-deoxy derivative of the nodulisporic acid B methyl ester was accordingly assigned as **4b**.[10] The oxidation of the indole ring has been observed[4a] earlier with nodulisporic acid A and therefore the structure of **5** was assigned with a similar comparison of spectral data.[9]

Nodulisporic acid B_1 (2b) and B_2 (3b). HRESI-FTMS analyses of **2c** and **3c** produced molecular formulae of $C_{43}H_{56}NO_6$ (observed for M + H, 682.4090) and $C_{43}H_{58}NO_7$ (observed for M + H, 700.4172), respectively. These formulae when compared to the corresponding formulae of nodulisporic acid A_1 (**2a**) and A_2 (**3a**) showed the same differences as observed between nodulisporic acids A and B. Like the ^1H and ^{13}C NMR spectra of nodulisporic acid B, the corresponding spectra of B_1 and B_2 (Table 1) showed the presence of a benzylic methylene group in ring D and the absence of the C-1′ carbonyl, thus establishing the 1′-deoxy structures for **2c** and **3c**, and corresponding acids **2b** and **3b**. Comparison of the NMR spectra[4b] of these compounds with nodulisporic acids A_1 and A_2 suggested identical stereochemistry in the ring I.

The stable salts of the nodulisporic acids B, B_1, and B_2, (**1c**, **2c**, and **3c**) along with the methyl ester **1d** and oxidized product **5** were evaluated in the flea (*Ctenocephalides felis*) membrane feeding assay[4d] and the data is summarized in Table 2. The data derived from nodulisporic A (**1a**) and its methyl ester **1e** is also presented for comparison. Nodulisporic acid B was fully effective in killing fleas at 100 ppm (LD_{90}) and was 100-fold less active than nodulisporic acid A. While the methyl ester derivative of nodulisporic acid A (**1e**) was 10-fold less active than the corresponding acid (**1a**), the activities of nodulisporic acid B (**2c**) and its methyl ester (**1d**) were similar. In fact the latter methyl ester (**1d**) may be actually slightly more potent than the corresponding acid **1c**. Nodulisporic acids B_1, B_2, and oxidized product **5** were not active at 100 ppm. This activity profile clearly demonstrated the critical importance of the 1′-ketone of β-keto-dihydropyrrole and the dienoic side chain, which parallels with the results obtained with synthetic derivatives of nodulisporic acid A.[11]

In conclusion, we report here three new nodulisporic acids belonging to the larger family of fungal indole diterpenes. These compounds have a resemblance to janthitrems (**6**),[12] lolitrol (**7**),[13] and shearinines (**8**)[14] (Fig. 1). The B-series nodulisporic acids are plausible biosynthetic precursors of the A-series nodulisporic acids. Nodulisporic acids (B, B_1, and B_2) that do not

Table 1. ^{1}H (500 MHz) and ^{13}C (125 MHz) NMR assignments[a] of nodulisporic acids B (**1b**), B_1 (**2b**), B_2 (**3b**) and methyl ester **1d** in acetone-d_6 and CD_2Cl_2

	1c (acetone-d_6)		**2c** (acetone-d_6)		**3c** (acetone-d_6)		**1d** (CD_2Cl_2)	
No.	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H	δ_C	δ_H
2	151.9		152.0		152.1		155.0	
3	56.1		55.6		55.7		55.7	
4	39.6		41.4		41.1		40.8	
5	32.8	1.8 (m), 2.0 (m)	30.0	2.0 (m, 2H)	33.1	1.8 (m), 1.95 (m)	32.2	1.75 (m), 1.92 (m)
6	27.3	1.78 (m, 2H)	30.7	1.7 (m), 1.85 (m)	30.5	1.75 (m), 1.9 (m)	25.9	1.70 (m, 2H)
7	76.9	3.48 (m)	106.4		105.9		77.0	3.40 (m)
8	47.9		48.0		49.6		47.7	
9	45.4	1.75 (m)	41.1	1.8 (m)	42	1.7 (m)	45.3	1.62 (m)
10	25.0	1.62 (m, 2H)	25.3	1.74 (m, 2H)	25.4	1.55 (m), 1.7 (m)	24.8	1.45 (m, 2H)
11	26.2	1.45 (m, 2H)	26.6	1.45 (m), 1.65 (m)	26.6	1.4 (m), 1.65 (m)	25.8	1.50 (m), 1.70 (m)
12	47.9	2.70 (m)	49.9	2.75 (m)	50.3	2.75 (m)	47.5	2.80 (m)
13	28.5	2.25 (m), 2.60 (m)	28.5	2.20 (m), 2.60 (m)	28.5	2.2 (m), 2.6 (m)	28.2	2.20 (m), 2.68 (m)
14	120.9		120.3		120.8		120.7	
15	124.1		124.0		124.1		124.0	
16	107.9	7.25 (s)	107.8	7.20 (s)	107.9	7.20 (s)	107.5	7.30 (s)
17	133.9		133.9		133.9		133.6	
18	135.6		135.3		135.3		133.9	
19	119.6	5.93 (d, $J=3.0$)	119.4	5.95 (d, $J=3.0$)	119.6	5.95 (d, $J=3.0$)	119.8	5.95 (d, $J=3.0$)
20	72.6		72.9		72.9		72.6	
22	74.1		74.0		74.4		73.9	
23	60.5	2.67 (dd, $J=3.0$, 6.0)	60.2	2.64 (dd, $J=3.0$, 6.0)	60.3	2.64 (dd, $J=3.0$, 6.0)	60.5	2.65 (dd, $J=3.0$, 6.0)
24	75.6	4.92 (d, $J=6.0$)	75.5	4.92 (d, $J=6.0$)	75.5	4.90 (d, $J=6.0$)	76.0	4.95 (d, $J=6.0$)
25	137.4		137.3		137.4		136.2	
26	118.5		118.8		118.6		118.3	
27	155.7		155.6		155.7		155.7	
28	15.3	0.96 (s)	15.3	0.90 (s)	16.8	0.85 (s)	15.0	0.94 (s)
29	19.5	1.13 (s)	17.6	1.07 (s)	17.7	1.04 (s)	19.4	1.11(s)
30	11.7	1.07 (s)	17.1	1.00 (s)	16.3	0.95 (s)	11.2	1.05 (s)
31	30.3	1.29 (s)	30.3	1.27 (s)	30.5	1.27 (s)	30.0	1.32 (s)
32	32.4	1.25 (s)	32.3	1.23 (s)	32.3	1.23 (s)	32.1	1.30 (s)
33	23.6	1.10(s)	23.5	1.07 (s)	23.5	1.07 (s)	23.2	1.10 (s)
34	30.5	1.40 (s)	30.2	1.40 (s)	30.5	1.38 (s)	30.1	1.43 (s)
1′	41.1	3.5 (d, $J=16.0$)	41.0	3.5 (d, $J=16.0$)	41.1	3.5 (d, $J=16.5$)	40.9	3.5 (d, $J=16.4$)
		4.0 (dd, $J=8.0$,16.0)		4.0 (dd, $J=8.0$,16.0)		4.0 (dd, $J=8.0$, 16.5)		4.0 (dd, $J=8.5$, 16.4)
2′	69.8	5.35 (d, $J=8.0$)	69.9	5.35 (d, $J=8.0$)	69.7	5.35 (d, $J=8.0$)	69.3	5.25 (d, $J=8.5$)
3′	147.5		147.4		147.6		146.5	
4′	112.7	4.79 (s), 4.82 (s)	112.9	4.78 (s, 2H)	112.9	4.78 (s, 2H)	112.8	4.75 (s), 4.85 (s)
5′	17.0	1.24 (s)	16.7	1.17 (s)	17.4	1.15 (s)	17.1	1.29 (s)
1″	154.8	5.96 (d, $J=15.0$)	45.3	1.7 (m), 2.3 (m)	37.9	1.8, 2.0 (m)	153.8	5.72 (d, $J=15.5$)
2″	125.7	6.38 (dd, $J=15.0$,13.5)	74.3	4.3 (t, $J=6.0$)	79.3	4.20 (m)	126.0	6.40 (dd, $J=15.5$, 11.5)
3″	139.6	7.22 (d, $J=13.5$)	140	6.56 (d, $J=6.0$)	74.7	3.90 (m)	138.7	7.22 (d, $J=11.5$)
4″	125.7		120.3		42.9	2.3 (m)	125.8	
5″	170.4		171.5		175.6		169.2	
6″	12.9	1.9 (brs)	13.9	1.78 (brs)	16.2	1.16 (brs)	12.9	1.92 (brs)

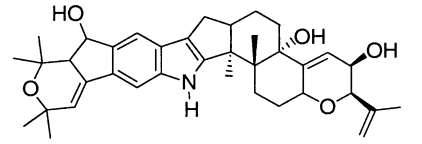
[a]Assignments were confirmed by COSY, HMQC and HMBC experiments.

Table 2. Flea killing activities of nodulisporic acids and ivermectin

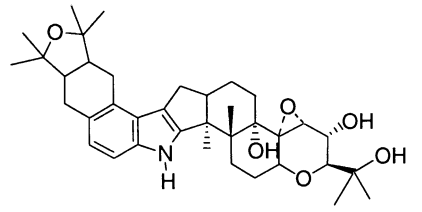
Compd	Flea assay (LD_{90}, ppm)
Nodulisporic acid A (**1a**)	1
Nodulisporic acid B (**1c**)	100
Nodulisporic acid A_1 (**2a**)	5
Nodulisporic acid B_1 (**2c**)	NA[a]
Nodulisporic acid A_2 (**3a**)	10
Nodulisporic acid B_2 (**3c**)	NA
Methyl ester of acid A (**1e**)	10
Methyl ester of acid B (**1d**)	100[b]
Compound **5**	NA
Ivermectin	10

[a]NA (not active at 100 ppm).
[b]Partial activity at 10 ppm.

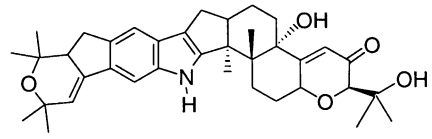
contain C-1′ ketone in the highly strained five-membered β-keto-dihydropyrrole ring have propensity to lose the C-24 hydroxy group by an internal acid-catalyzed dehydration that takes placed during drying of the free acid. The rate of the dehydration is increased at lower pH (~2.0). The sodium salt of these compounds is stable. The C-24 hydroxy group is stabilized by a six-centered H-bond in the A-series nodulisporic acids with the C-1′ keto group. The facile elimination of the C-24 hydroxy group in the B-series nodulisporic acids confirms our previous hypothesis[4a] that the C-1′ keto group plays a seminal role in the stability of this class of compounds. Since both the C-1′ keto group and the C-24 hydroxy group appear to be essential for the potency of this class of compounds, it is conceivable



Janthitrem B (**6**)



Lolitrol (**7**)



Shearinine B (**8**)

Figure 1. Structure of selected known indole diterpenes.

that they are involved in potential H-bonding in the molecular target, the glutamate gated Cl ion channels.[5]

Acknowledgements

The authors greatly thank Dr. Z. Guan for high-resolution FTMS data.

References and Notes

1. Bloomquist, J. R. *Annu. Rev. Entomol.* **1996**, *41*, 163.
2. Hopkins, T. J.; Kerwick, C.; Gyr, P.; Woodley, I. *Aus. Vet. Practioner*. **1996**, *26*, 150.
3. Banks, B. J.; Bishop, B. F.; Evans, N. A.; Gibson, S. P.; Goudie, A. C.; Gration, K. A. F.; Pacey, M. S.; Perry, D. A.; Witty, M. J. *Bioorg. Med. Chem.* **2000**, *8*, 2017.
4. (a) Ondeyka, J. G.; Helms, G.; Hensens, O. D.; Goetz, M. A.; Zink, D. L; Tsipouras, A.; Shoop, W. L.; Slayton, L.; Dombrowski, A. W.; Polishook, J. D.; Ostlind, D.; Tsou, N.; Ball, R. G.; Singh, S. B. *J. Am. Chem. Soc.* **1997**, *119*, 8809. (b) Hensens, O. D.; Ondeyka, J. G.; Dombrowski, A. W.; Zink, D. L. *Tetrahedron Lett.* **1999**, *40*, 5455. (c) Ostlind, D.; Felcetto, T.; Misura, A.; Ondeyka, J.; Smith, S.; Goetz, M.; Shoop, W.; Mickle, G. *Med. Vet. Entomol.* **1997**, *11*, 407. (d) Shoop, W. L.; Gregory, L. M.; Zakson-Aiken, M.; Michael, B. F.; Haines, H. W.; Ondeyka, J. G.; Meinke, P. T.; Schmatz, D. M. *J. Parasitol.* **2001**, *87*, 419.
5. Smith, M. M.; Warren, V. A.; Thomas, B. S.; Brochu, RM.; Ertel, E. A.; Rohrer, S.; Schaeffer, J.; Schmatz, D.; Petuch, B. R.; Tang, Y. S.; Meinke, P. T.; Kaczorowski, G. J.; Cohen, C. J. *Biochemistry* **2000**, *39*, 5543.
6. The mutants were generated by treating mycelia with *N*-methyl-*N'*-nitro-1-nitrosoguanidine and screening progeny for altered nodulisporic acid production by HPLC.
7. For seed and growth media, see for example: Byrne, K. M.; Smith, S. K.; Ondeyka, J. G. *J. Am. Chem. Soc.* **2002**, *124*, 7055.
8. **1c**: $[\alpha]_D^{23} = -40°$ (*c* 0.4, MeOH), UV (MeOH) λ_{max} 273 ($\varepsilon = 69{,}000$), 266 (64,700), 322 (8600), 335 (8000) nm, IR (ZnSe) ν_{max} 3275, 2920, 2852, 1634, 1454, 1410, 1377, 1224, 1151, 1078, 1000 cm^{-1}, **2c**: $[\alpha]_D^{23} = -36°$ (*c* 0.4, MeOH), UV (MeOH) λ_{max} 272 ($\varepsilon = 44{,}000$), 265 (43,700), 322 (6900), 335 (6700) nm, IR (ZnSe) ν_{max} 3347, 2921, 2853, 1715, 1648, 1553, 14554, 1376, 1228, 1152, 1077, 977 cm^{-1}, **3c**: $[\alpha]_D^{23} = -30°$ (*c* 0.4, MeOH), UV (MeOH) λ_{max} 272 ($\varepsilon = 49300$), 264 (47000), 322 (7200), 335 (7000) nm, IR (ZnSe) ν_{max} 3264, 2978, 2837, 1634, 1448, 1384, 1116, 1014 cm^{-1}, **1d:** $[\alpha]_D^{23} = -40°$ (*c* 0.4, MeOH), UV (MeOH) λ_{max} 272 ($\varepsilon = 85{,}100$), 265 (83,000), 322 (10,400), 335 (9500) nm, IR (ZnSe) ν_{max} 3465, 2974, 2878, 2840, 1698, 1633, 1436, 1376, 1363, 1228, 1154, 1113, 1093, 1003, 980, 736 cm^{-1}.
9. **5**: $[\alpha]_D^{23} = +410°$ (*c* 0.4, MeOH), UV (MeOH) λ_{max} 266 ($\varepsilon = 57{,}800$), 290 (32,900), 308 (26,700), 365 (2700) nm, IR (ZnSe) ν_{max} 3447, 2972, 2879, 2841, 1658, 1653, 1650, 1448, 1411, 1376, 1273, 1194, 1157, 1135, 1076, 1008, 980, 735 cm^{-1}, HRESI-FTMS *m/z* 712.4216 (M + H, calcd for $C_{44}H_{57}NO_7$: 712.4213), ^1H NMR (CD_2Cl_2, 500 MHz) δ 7.85 (1H, s, H-16), 7.15 (1H, d, $J = 11.5$ Hz, H-3″), 6.35 (1H, dd, 11.5, 15.5 Hz, H-2″), 6.1 (1H, d, 3.0 Hz, H-19), 5.76 (1H, d, 15.5 Hz, H-1″), 5.45 (1H, d, 8.5 Hz, H-2′), 4.95 (1H, 3.0, 8.5 Hz, H-24), 4.85 (1H, s, H-4′), 4.75 (1H, s, H-4′), 3.71 (3H, s, OCH3), 3.6 (1H, m, H-13), 3.3 (1H, m, H-7), 3.25 (1H, m, H-1′), 3.15 (1H, m, H-1′), 2.95 (1H, m, H-12), 2.65 (1H, m, H-23), 2.35 (1H, m, H-13), 2.1 (1H, m, H-5), 1.95 (1H, m, H-5), 1.92 (3H, s, H-6″), 1.74 (3H, s, H-5′), 1.6 (2H, m, H-11), 1.7 (2H, m, H-6), 1.5 (1H, m, H-9), 1.48 (3H, s, H-29), 1.45 (1H, m, H-10), 1.41 (3H, s, H-34), 1.32 (3H, s, H-31), 1.30 (1H, m, H-10), 1.27 (3H, s, H-32), 1.1 (3H, s, H-33), 1.07 (3H, s, H-28) and 1.05 (3H, s, H-30), ^{13}C NMR (CD_2Cl_2, 125 MHz) δ 192.0 (C-14), 175.7 (C-5″), 169.1 (C-2), 153.1 (C-1″), 146.2 (C-27), 145.6 (C-3′), 138.6 (C-3″), 135.3 (C-25), 133.8 (C-18), 132.4 (C-17), 128.1 (C-26), 128.0 (C-4″), 126.2 (C-2″), 126.1 (C-15), 124.5 (C-19), 118.6 (C-16), 109.9 (C-4′), 76.9 (C-24), 76.5 (C-7), 73.7 (C-22), 72.7 (C-20), 69.6 (C-2′), 60.3 (C-23), 55.3 (C-3), 51.9 (OCH_3), 47.3 (C-8), 45.3 (C-13), 45.0 (C-4), 44.2 (C-9), 42.9 (C-12), 31.7 (C-32), 30.8 (C-5), 30.1 (C-34), 29.9 (C-1′), 29.6 (C-31), 27.5 (C-11), 26.4 (C-6), 23.1 (C-10), 22.9 (C-33), 19.5 (C-5′), 19.3 (C-29), 15.9 (C-28), 12.9 (C-6″), 11.7 (C-30).
10. **4b**: IR (ZnSe) ν_{max} 3484, 2937, 1708, 1635, 1436, 1376, 1287, 1244, 1110, 751 cm^{-1}, HREIMS *m/z* 661.4114 (calcd for $C_{44}H_{55}NO_4$, 661.4131), ^1H NMR (CD_2Cl_2, 500 MHz) δ 7.4 (1H, s, H-16), 7.22 (1H, d, $J = 11.1$ Hz, H-3″), 6.43 (1H, dd, 1.1, 15.5 Hz, H-2″), 6.35 (1H, s, H-19), 5.92 (1H, d, 15.5 Hz, H-1″), 5.3 (1H, m, H-2′), 6.52 (1H, s, H-24), 4.85 (1H, s, H-4′), 4.75 (1H, s, H-4′), 3.75 (3H, s, OCH3), 3.6 (1H, m, H-13), 3.4 (1H, m, H-7), 4.05 (1H, dd $J = 8.2$, 16.4 Hz, H-1′), 3.52 (1H, d $J = 16.4$ Hz, H-1′), 2.7 (1H, m, H-12), 2.25 (1H, dd $J = 10.5$, 13.2 Hz, H-13), 2.6 (1H, dd $J = 6.6$, 13.2 Hz, H-13), 1.2 (2H, m, H-5), 1.95 (3H, s, H-6″), 1.29 (3H, s, H-5′), 1.6 (2H, m, H-11), 1.7 (1H, m, H-6), 1.9 (1H, m, H-6), 1.62 (1H, m, H-9), 1.68 (3H, s, H-29), 1.40 (1H, m, H-10), 1.52 (3H, s, H-34), 1.40 (3H, s, H-31), 1.40 (3H, s, H-32), 1.4 (3H, s, H-33), 1.00 (3H, s, H-28) and 1.05 (3H, s, H-30).
11. Meinke, P. T.; Ayer, M. B.; Colletti, S. L.; Li, C.; Lim, J.; Ok, D.; Salva, S.; Schmatz, D. M.; Shih, T. L.; Shoop, W. L.; Warmke, L. M; Wyvratt, M. J.; Zakson-Aiken, M.; Fisher, M. H. *Bioorg. Med. Chem. Lett.* **2000**, *10*, 2371.
12. deJesus, A. E.; Steyn, P. S.; vanHeerden, F. R.; Vleggaar, R. *J. Chem. Soc., Perkin Trans. 1* **1984**, 697.
13. Ede, R. M.; Miles, C. O.; Meagher, L. P.; Munday, S. C.; Wilkins, A. L. *J. Agric. Food Chem.* **1994**, *42*, 231.
14. Belofsky, G. N.; Gloer, J. B.; Wicklow, D. T.; Dowd, P. F. *Tetrahedron* **1995**, *51*, 3959.