The Journal of Antibiotics (2010) 63, 157–163

www.nature.com/ja

ORIGINAL ARTICLE

Nosokomycins, new antibiotics discovered in an *in vivo*-mimic infection model using silkworm larvae. II: Structure elucidation

Ryuji Uchida[1], Masato Iwatsuki[2], Yong-Pil Kim[2,3], Satoshi Ōmura[2] and Hiroshi Tomoda[1]

The structures of nosokomycins A, B, C and D, new anti-methicillin-resistant *Staphylococcus aureus* antibiotics produced by *Streptomyces* sp. K04-0144, were elucidated by spectroscopic studies including various NMR experiments. Nosokomycins A, B, C and D are new members of the moenomycin family consisting of an oligosaccharide moiety, a 2,3-dihydroxypropionic acid and an unusual sesterterpenoid moiety. All nosokomycins lack the cyclopentenone moiety in the oligosaccharide moiety of moenomycin A.

The Journal of Antibiotics (2010) **63,** 157–163; doi:10.1038/ja.2010.10; published online 5 March 2010

Keywords: anti-methicillin-resistant *S. aureus* antibiotics; moenomycin family; nosokomycins; phosphoglycolipid; structure elucidation

INTRODUCTION

Our research group has focused on the discovery of anti-infectives from microbial metabolites.[1–3] In the course of *in vivo*-mimic screening using silkworm larvae to discovery antibiotics that are active against methicillin-resistant *Staphylococcus aureus*, nosokomycins A, B, C and D (Figure 1) were isolated as active components from the culture broth of *Streptomyces* sp. K04-0144.[4] In this study, the physico-chemical properties and structure elucidation of nosokomycins are described.

RESULTS

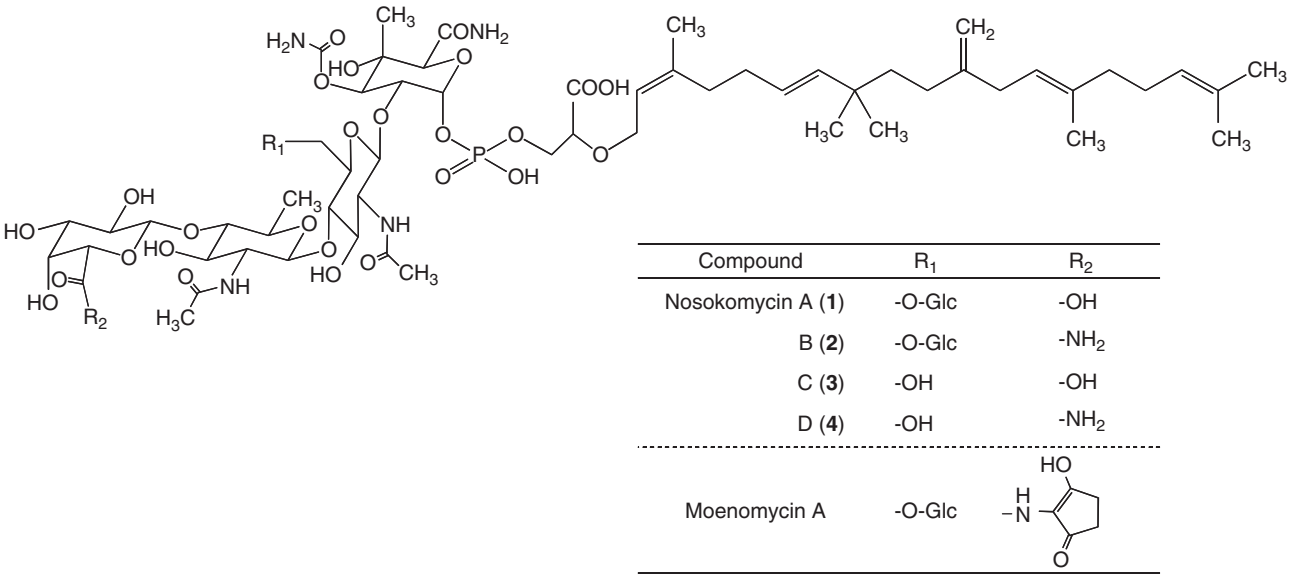
Physico-chemical properties of nosokomycins

The physico-chemical properties of nosokomycins are summarized in Table 1. The strong IR absorption at 3400 and 1720 cm^{-1} suggested the presence of a hydroxyl group and a carbonyl group. In their UV spectra, nosokomycins showed no characteristic absorption other than end absorption, and no reliable peaks related to MW were observed for this series of compounds during FAB-MS measurements. Therefore, LC-ESI-MS (magnetic sector-type) was used to elucidate the MWs of nosokomycins (Table 1). The similarities in their physico-chemical properties strongly suggested that they are structurally related.

Structure elucidation of nosokomycin B (2)

The first structure study was carried out for nosokomycin B (**2**), the major active component among them. The analysis of its ^{1}H, ^{13}C and 2D NMR spectral data indicated that **2** is a moenomycin-like phosphoglycolipid antibiotic, which consists of five substituted sugar moieties **B–F**, a 2,3-dihydroxypropionic acid (**H**) and an unusual sesterterpenoid moiety with the formula $C_{25}H_{39}$ (**I**) in **2** (Figure 2). However, the end absorption of **2** in UV spectra showed a lack of the 2-hydroxy-5-oxo-1-cyclopenten-1-ly moiety (**A**) of moenomycin A, which had the charcteristic absoption. The molecular formula of **2** was determined by HR-ESI-MS to be $C_{64}H_{104}N_5O_{32}P$ (found *m/z* 1484.6356 $(M-H)^-$, calcd 1484.6324) in conjunction with its NMR data (Table 2). The presence of a phosphorus atom in **2** was clarified by the observation of the $^{2}J_{C\text{-}P}$ coupling (4.6 and 4.4 Hz) and $^{3}J_{C\text{-}P}$ couplings (9.1 and 7.2 Hz) in the ^{13}C NMR spectra. The sesquiterpenoid moiety (**I**) was assigned as moenomycicol, the moiety of moenomycin A by COSY and HMBC analysis. The $^{3}J_{H\text{-}H}$ coupling constants ($J_{1,2}$=8.6 Hz, $J_{2,3}$=8.6 Hz, $J_{3,4}$=1.4 Hz and $J_{4,5}$=1.4 Hz) and the HMBC correlation between the proton signal of C-5 (δ_H 4.05) and the primary amide carbon signal (δ_C 173.2) proved the α-D-glucuronamate of sugar **B**. The $^{3}J_{H\text{-}H}$ coupling constants ($J_{1,2}$=8.5 Hz, $J_{2,3}$=8.6 Hz, $J_{3,4}$=8.6 Hz and $J_{4,5}$=8.6 Hz) and the HMBC correlation between the proton signal of C-2 (δ_H 3.82) and the acetyl methyl signal (δ_H 2.06) and the acetyl carbonyl carbon signal (δ_C 174.1) proved the 6-deoxy-*N*-acetyl-α-D-glucosamate of sugar **C**. The $^{3}J_{H\text{-}H}$ coupling constants ($J_{1,2}$=8.6 Hz, $J_{2,3}$=8.6 Hz, $J_{3,4}$=8.6 Hz and $J_{4,5}$=8.5 Hz) and the HMBC correlation between the proton signal of C-2 (δ_H 3.82) and the acetyl methyl signal (δ_H 2.06) proved the α-D-glucosamate of sugar **D**. The $^{3}J_{H\text{-}H}$ coupling constants ($J_{1,2}$=8.5 Hz, $J_{2,3}$=8.5 Hz, $J_{3,4}$=8.6 Hz and $J_{4,5}$=8.7 Hz) and the HMBC correlation between the proton signal of C-2 (δ_H 3.79) and acetyl methyl signal (δ_H 2.00) and

[1]Graduate School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan and [2]Kitasato Institute for Life Sciences, Kitasato University, Tokyo, Japan
[3]Current address: Faculty of Pharmacy, Iwaki Meisei University, 5-5-1 Chuodai Iino, Iwaki City, Fukushima 970-8551, Japan.
Correspondence: Professor H Tomoda, Graduate School of Pharmaceutical Sciences, Kitasato University, 5-9-1 Shirokane, Minato-ku, Tokyo 108-8641, Japan.
E-mail: tomodah@pharm.kitasato-u.ac.jp
Received 26 November 2009; revised 13 January 2010; accepted 20 January 2010; published online 5 March 2010



Compound	R_1	R_2
Nosokomycin A (**1**)	-O-Glc	-OH
B (**2**)	-O-Glc	$-NH_2$
C (**3**)	-OH	-OH
D (**4**)	-OH	$-NH_2$
Moenomycin A	-O-Glc	

Figure 1 Structures of nosokomycins A (**1**), B (**2**), C (**3**) and D (**4**).

Table 1 Physico-chemical properties of nosokomycins A (1) to D (4)

	1	**2**	**3**	**4**
Appearance	White powder	White powder	White powder	White powder
Molecular formula	$C_{64}H_{103}N_4O_{33}P$	$C_{64}H_{104}N_5O_{32}P$	$C_{58}H_{93}N_4O_{28}P$	$C_{58}H_{94}N_5O_{27}P$
MW	1487	1486	1325	1324
ESI-MS (m/z)				
Negative	1485 $[M-H]^-$	1484 $[M-H]^-$	1323 $[M-H]^-$	1322 $[M-H]^-$
HRESI-MS (m/z)				
Calcd	1485.6164	1484.6324	1323.5636	1322.5795
Found $[M-H]^-$	1485.6206	1484.6356	1323.5656	1322.5859
$[\alpha]_D^{24}$ (*c* 0.1, MeOH)	+6.7	+5.3	+4.0	+3.7
UV λ_{max}^{MeOH}, nm (ε)	End absorption	End absorption	End absorption	End absorption
IR ν_{max}^{KBr}, cm^{-1}	3299, 2960, 1722, 1673	3400, 2932, 1675, 1648	3299, 2923, 1716, 1675	3400, 2925, 1677, 1648
Solubility				
Soluble	H_2O, MeOH, DMSO	H_2O, MeOH, DMSO	H_2O, MeOH, DMSO	H_2O, MeOH, DMSO
Insoluble	Acetone, $CHCl_3$, *n*-hexane	Acetone, $CHCl_3$, *n*-hexane	Acetone, $CHCl_3$, *n*-hexane	Acetone, $CHCl_3$, *n*-hexane

the acetyl carbonyl carbon signal (δ_C 173.7) proved the *N*-acetyl-α-D-glucosaminate of sugar **E**. The $^3J_{H\text{-}H}$ coupling constants ($J_{1,\ 2}$=1.8 Hz, $J_{2,\ 3}$=10.1 Hz) and the NOE correlations between the proton signals of C-2 (δ_H 3.64) and the singlet methyl group (δ_H 1.23), and the HMBC correlations between the proton signal of C-3 (δ_H 5.10) and the ureido carbonyl carbon signal (δ_C 159.2) and between the proton signal of C-5 (δ_H 4.51) and the primary amide carbon signal (δ_C 174.7) proved the 3-*O*-carbamoyl-4-methyl-α-D-glucuronaminate of sugar **F**. The connectivity of sugar moeities was determined by the correlations between the anomeric proton signals and the corresponding carbon signals (C-4 of sugar **C** (δ_H 4.51) and C-1 of sugar **B** (δ_C 105.1), C-1 of sugar **C** (δ_H 4.57) and C-4 of sugar **E** (δ_C 82.4), C-1 of sugar **D** (δ_H 4.45) and C-6 of sugar **E** (δ_C 69.4), and C-1 of sugar **E** (δ_H 4.51) and C-2 of sugar **F** (δ_C 79.7)) through glycoside bonds in the HMBC spectra (Figure 3). The mode of linkage of all sugar moieties as the α-glycoside bond except sugar **F**, was assigned by measuring the ^1H-^1H coupling constants of anomeric protons. The connectivity between the sesterterpenoid moiety (**I**) and the 2,3-dihydroxypropionic acid (**H**) was determined by the correlations between the proton signal of C-1 of **I** (δ_H 4.22) and the carbon signal of C-2 of **H** (δ_C 81.2) observed in HMBC spectra (Figure 3). Finally, the linkage between the C-1 of the partial structure **F** and the C-3 of the partial structure **H** was elucidated by the correlation from the proton signal of C-1 of **F** (δ_H 5.95) and C-3 of **H** (δ_H 4.10) and the phosphorus signal of P of phosphate ester (δ_P-0.659) seen in ^1H-^{31}P HMBC spectra (Figure 3).

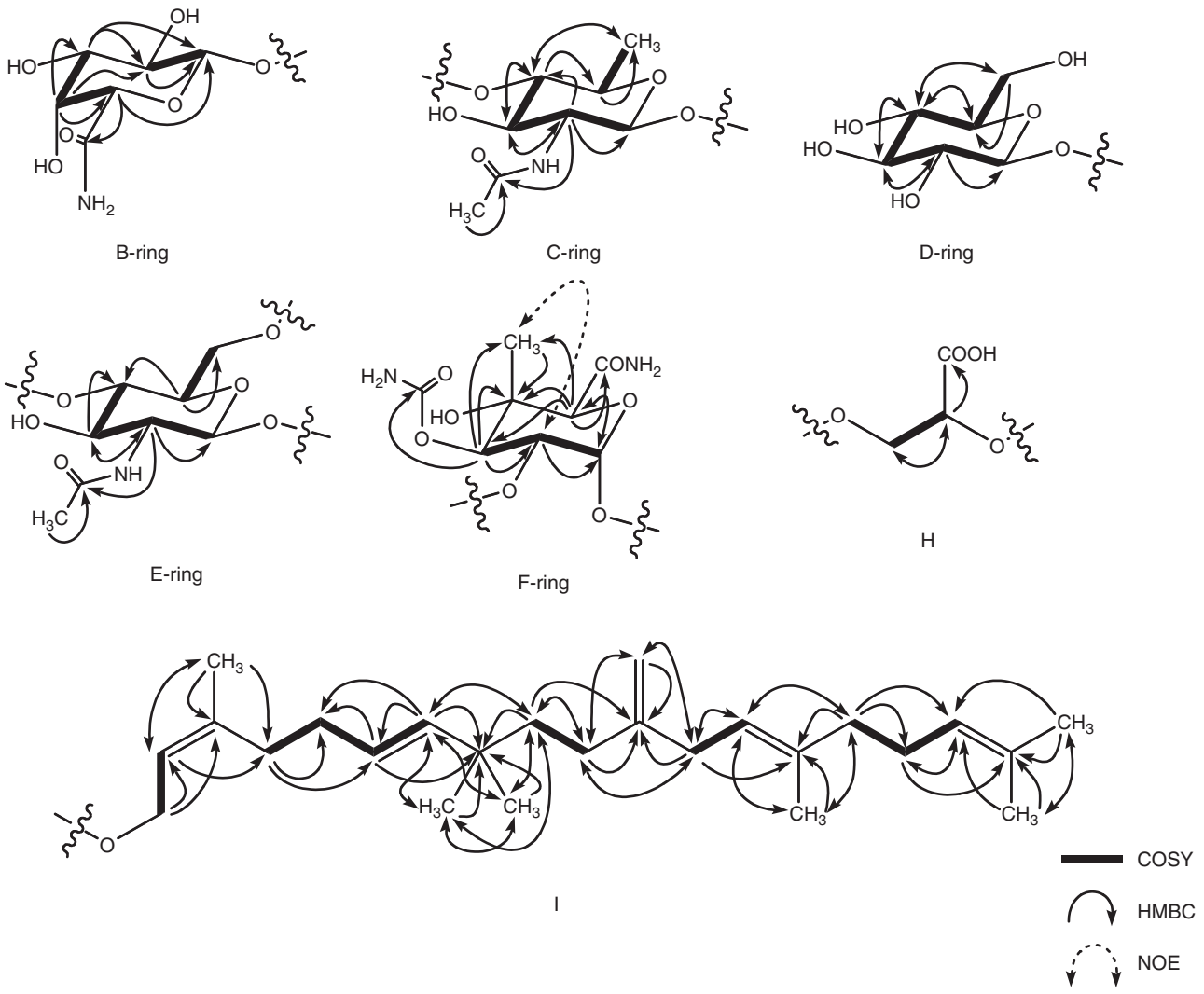


Figure 2 Partial structures of nosokomycin B (**2**).

From these results, the structure of **2** was elucidated as shown in Figure 1, which was a moenomycin A analog lacking moiety **A**. **2** was identified with the semisynthetic moenomycin derivative.[6] Very recently, a biosynthetic intermediate was found by mass and MS^2 fragmentation data.[7]

Structure elucidation of nosokomycin A (1)

The molecular formula of **1** was determined by HR-ESI-MS to be $C_{64}H_{103}N_4O_{33}P$ (found *m/z* 1485.6206 $(M\text{-}H)^-$, calcd 1485.6164) and the results indicated the replacement of NH in **2** with O in **1**. As the ^1H and ^{13}C NMR spectra of **1** (Table 2) resembled those of **2**, it was difficult to elucidate the differences in structures by only measuring the chemical shifts among candidates (the primary amide group at C-5 in sugar **B** and at C-5 in sugar **F**). To observe the correlation between the proton signal at δ_H 4.05 (C-5 of sugar **B**) and the nitrogen signal at δ_N 269.4 (N of the primary amide group of sugar **B**), ^1H-^{15}N HMBC experiments were carried out (Figure 3). However, these correlation results were not seen on **1**, indicating that the primary amino group belonging to the C-5 of sugar **B** in **2** is replaced with a hydroxyl group in **1**.

The presence of this compound in the flavomycin complex was predicted by LC-ESI-IT-MS analysis.[8] Very recently, **1** was reported to be an intermediate of moenomycin biosynthesis, the structure of which was deduced by exact mass and MS^2 fragmentation data.[7] However, we first isolated **1** as the metabolite from the culture broth of wild-type actinomycete and obtained its full spectral data.

Structure elucidation of nosokomycin C (3)

The molecular formula of **3** was determined by HR-ESI-MS to be $C_{58}H_{93}N_4O_{28}P$ (found *m/z* 1323.5656 $(M\text{-}H)^-$, calcd 1323.5636), indicating that **3** lacked $C_6H_{10}O_5$ (one sugar unit) compared with **1**. From a comparison of the ^1H and ^{13}C NMR spectra of **3** and **1** (Table 2), the signals of sugar **D** seen in **1** were absent in **3**, and the chemical shift of C-6 in sugar **E** in **3** was shifted to a higher field in the ^{13}C NMR spectra (δ_C 69.4 in **1**, δ_C 60.8 in **3**). Therefore, the structure of **3** was elucidated to be the nosokomycin A analog lacking moiety **D**, as shown in Figure 1.

Structure elucidation of nosokomycin D (4)

The molecular formula of **4** was determined by HR-ESI-MS to be $C_{58}H_{94}N_5O_{27}P$ (found *m/z* 1322.5859 $(M\text{-}H)^-$, calcd 1322.5795), which was smaller than that of **2** by $C_6H_{10}O_5$. From a comparison of the ^1H and ^{13}C NMR spectra of **4** and **2** (Table 2), the signals of

Table 2 NMR spectral data of nosokomycins A (1) to D (4)

	Nosokomycin A		*Nosokomycin B*		*Nosokomycin C*		*Nosokomycin D*		*Moenomycin A*[a]	
Position	δ_C	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C	δ_H
A-NH	—	—	—	—	—	—	—	—	—	—
A1	—	—	—	—	—	—	—	—	200.0	—
A2	—	—	—	—	—	—	—	—	111.1	—
A3	—	—	—	—	—	—	—	—	200.0	—
A4	—	—	—	—	—	—	—	—	31.7	2.33
A5	—	—	—	—	—	—	—	—	31.7	2.33
B1	105.1	4.38 d (8.6)	104.7	4.44 d (8.6)	105.20	4.37 d (7.0)	104.90	4.44 br	104.8	4.48
B2	72.2	3.55 m	72.3	3.55 ddd (8.6, 8.6)	72.10	3.56 m	72.30	3.56 m	72.4	3.58
B3	74.7	3.55 m	74.5	3.56 m	74.60	3.54 m	74.40	3.56 m	74.5	3.54
B4	71.6	4.18 m	70.7	4.16 dd (1.4, 1.4)	71.60	4.17 m	70.70	4.17 br	70.7	4.21
B5	76.4	4.09 m	76.0	4.05 d (1.4)	76.30	4.12 m	76.00	4.06 m	76.9	4.08
B5-C	173.5	—	173.7	—	173.20	—	173.70	—	170.2	—
C1	103.2	4.57 d (8.5)	103.3	4.54 d (8.5)	103.40	4.47 d (8.0)	103.50	4.46 br	103.2	4.54
C2	56.9	3.81 dd (8.6, 8.5)	56.8	3.82 dd (8.6, 8.5)	55.90	3.88 m	56.70	3.84 br	56.0	3.74
C2-C	173.8	—	174.0	—	174.10	—	173.70	—	173.9	—
C2-Ac	23.5	2.05 s	23.5	2.06 s	23.30	2.03 s	23.30	2.01 s	23.5	2.03
C3	74.0	3.60 m	74.0	3.57 m	74.10	3.58 m	73.50	3.64 m	74.2	3.61
C4	86.1	3.31 m	84.8	3.34 dd (8.6, 8.6)	86.40	3.31 m	85.10	3.34 m	84.7	3.40
C5	72.6	3.60 m	72.7	3.63 m	72.60	3.62 m	72.80	3.56 m	72.8	3.62
C5-CH_3	18.0	1.41 d (6.4)	18.0	1.42 d (6.4)	18.00	1.42 d (7.0)	17.90	1.42 d (7.0)	18.0	1.42
D1	104.3	4.45 d (8.7)	104.8	4.48 d (8.7)	—	—	—	—	104.8	4.47
D2	75.2	3.25 dd (8.7, 8.6)	75.2	3.21 dd (8.7, 8.6)	—	—	—	—	75.1	3.21
D3	78.3	3.46 dd (8.6, 8.6)	78.0	3.48 dd (8.6, 8.6)	—	—	—	—	78.1	3.47
D4	71.6	3.31 m	71.9	3.25 dd (8.6, 8.5)	—	—	—	—	71.9	3.25
D5	78.2	3.35 m	78.2	3.39 ddd (8.5, 5.7, 1.4)	—	—	—	—	78.1	3.37
D6	62.5	3.93 m	62.9	3.92 dd (11.9, 1.4)	—	—	—	—	62.8	3.90
		3.71 m		3.66 dd (11.9, 5.7)	—	—	—	—		3.66
E1	104.7	4.42 d (8.6)	104.0	4.52 d (8.6)	104.60	4.45 d (8.0)	103.90	4.54 br.d (8.0)	104.2	4.48
E2	55.7	3.90 m	56.3	3.79 dd (8.6, 8.5)	56.70	3.84 m	56.50	3.72 br.d (8.0)	57.4	3.82
E2-C	174.2	—	174.1	—	173.70	—	174.00	—	174.2	—
E2-Ac	23.3	2.04 s	23.3	2.00 s	23.10	2.02 s	23.10	2.01 s	23.3	2.00
E3	73.8	3.60 m	74.3	3.61 dd (8,6, 8.5)	73.70	3.64 m	73.90	3.64 m	74.3	3.60
E4	82.4	3.51 m	82.7	3.50 dd (8.6, 8.7)	81.50	3.56 m	81.60	3.56 m	82.5	3.47
E5	75.0	3.55 m	75.0	3.52 m	76.00	3.33 m	76.00	3.34 m	75.0	3.54
E6	69.4	4.15 m	70.1	4.11 m	60.80	3.64 m	61.00	3.64 m	70.0	4.11
		3.70 m		3.65 m		3.82 m		3.83 m		3.65

Table 2 Continued

	Nosokomycin A		*Nosokomycin B*		*Nosokomycin C*		*Nosokomycin D*		*Moenomycin A*[a]	
Position	δ_C	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C	δ_H *(J in Hz)*	δ_C	δ_H
F1	96.2	5.93 dd (3.0, 1.8)	96.0	5.94 dd (3.0, 1.8)	96.5 d (7.9)	6.04 dd (8.0, 3.2)	96.2 d (7.9)	5.99 br	95.9	5.92
F2	79.7	3.67 m	79.2	3.64 m	79.90	3.64 m	79.40	3.64 m	79.1	3.63
F3	75.4	5.06 d (10.1)	76.2	5.10 d (10.1)	75.10	5.04 d (10.0)	75.70	5.07 br.d	76.2	5.08
F3-C	159.2	—	159.3	—	159.20	—	159.20	—	159.3	—
F4	74.1	—	74.2	—	73.90	—	74.00	—	74.3	—
F4-CH_3	16.4	1.26 s	16.4	1.23 s	16.30	1.26 s	16.40	1.25 s	16.4	1.23
F5	73.8	4.47 s	73.6	4.51 s	73.80	4.44 m	73.70	4.42 br	73.7	4.50
F5-C	174.6	—	174.4	—	174.70	—	174.70	—	174.4	—
H1	68.5	4.29 m	69.3	4.30 ddd (10.8, 5.8, 2.2)	67.70	4.16 m	67.60	4.11 m	69.3	4.27
				4.12 m		4.18 m		4.21 m		4.12
H2	78.0	4.11 m	81.2	3.98 dd (5.7, 1,4)	77.90	4.08 m	78.30	4.11 m	81.1	3.96
H2-C	175.1	—	177.7	—	174.90	—	173.70	—	177.7	—
I1	66.9	4.28 m	67.0	4.22 dd (12.2, 5.7)	67.00	4.13 m	67.20	4.11 br	67.0	4.21
		4.18 m		4.08 m		4.26 m		4.21 br		4.08
I2	122.7	5.45 m	123.7	5.44 dd (5.7, 5.6)	122.80	5.39 m	122.60	5.38 br	123.6	5.43
I3	142.6	—	140.6	—	142.40	—	142.10	—	140.7	—
I4	33.5	2.17 m	33.6	2.15 m	33.40	2.14 m	33.40	2.14 m	33.6	2.12
I5	32.7	2.10 m	32.7	2.10 m	32.70	2.10 m	32.60	2.11 m	32.7	2.09
I6	126.8	5.29 ddd (15.6, 7.1, 5.6)	127.0	5.29 ddd (15.6, 7.1, 5.6)	126.80	5.29 ddd (17.0, 7.2, 6.0)	126.80	5.29 ddd (16.0, 6.0, 6.0)	127.0	5.28
I7	141.6	5.38 d (15.6)	141.4	5.37 d (15.6)	141.70	5.38 d (17.0)	141.60	5.38 d (16.0)	141.5	5.36
I8	36.5	—	36.5	—	36.50	—	36.50	—	36.5	—
I9	42.9	1.37 ddd (9.6, 4.8, 4.8)	42.9	1.37 ddd (9.6, 4.8, 4.8)	42.90	1.38 ddd (9.6, 4,8, 4,8)	42.90	1.38 ddd (9.6, 4,8, 4,8)	42.9	1.36
I10	32.4	1.91 ddd (9.6, 4,8, 4.8)	32.3	1.90 ddd (9.6, 4,8, 4.8)	32.40	1.91 ddd (9.6, 4.8, 4.8)	32.30	1.91 ddd (9.6, 4.8, 4.8)	32.3	1.89
I11	151.1	—	151.1	—	151.10	—	151.10	—	151.1	—
I12	35.9	2.70 d (7.2)	35.9	2.69 d (7.2)	36.00	2.70 d (7.0)	36.00	2.70 d (7.2)	35.9	2.68
I13	123.5	5.14 ddq (8.4, 8.4, 1.2)	123.5	5.14 ddq (8.4, 8.4, 1.2)	123.50	5.14 ddq (7.0, 7.0, 1.1)	123.50	5.14 m	123.5	5.13
I14	137.4	—	137.4	—	137.40	—	137.30	—	137.3	—
I15	40.9	2.01 m	40.9	2.02 m	40.90	2.03 m	40.90	2.04 m	40.9	2.01
I16	27.7	2.10 m	27.7	2.10 m	27.70	2.10 m	27.70	2.09 m	27.7	2.09
I17	125.4	5.10 ddq (7.0, 7.0, 1.2)	125.4	5.11 m	125.40	5.10 ddq (7.0, 7.0, 1.2)	125.40	5.11 m	125.4	5.09
I18	132.2	—	132.2	—	132.20	—	132.20	—	132.1	—
I19	26.0	1.67 s	25.9	1.67 s	26.00	1.67 s	26.00	1.67 s	25.9	1.66
I20	17.8	1.60 s	17.8	1.60 s	17.8	1.60 s	17.8	1.60 s	17.8	1.59
I21	16.1	1.62 s	16.1	1.61 s	16.1	1.61 s	16.1	1.61 s	16.1	1.60
I22	109.3	4.66 d (1.4)	109.2	4.66 d (1.4)	109.30	4.66 d (1.4)	109.30	4.66 br	109.2	4.66
		4.67 d (1.4)		4.67 d (1.4)		4.67 d (1.4)		4.68 br		
I23	27.9	0.97 s	27.9	0.97 s	27.80	0.97 s	27.90	0.97 s	27.9	0.96
I24	27.9	0.97 s	27.9	0.97 s	27.90	0.97 s	27.90	0.97 s	27.9	0.96
I25	24.0	1.78 s	24.0	1.75 s	24.00	1.77 s	23.90	1.76 s	24.0	1.74

[a]The chemical shifts of moenomycin are described in the study by Michael *et al.*[5] These data could not be compared directly.

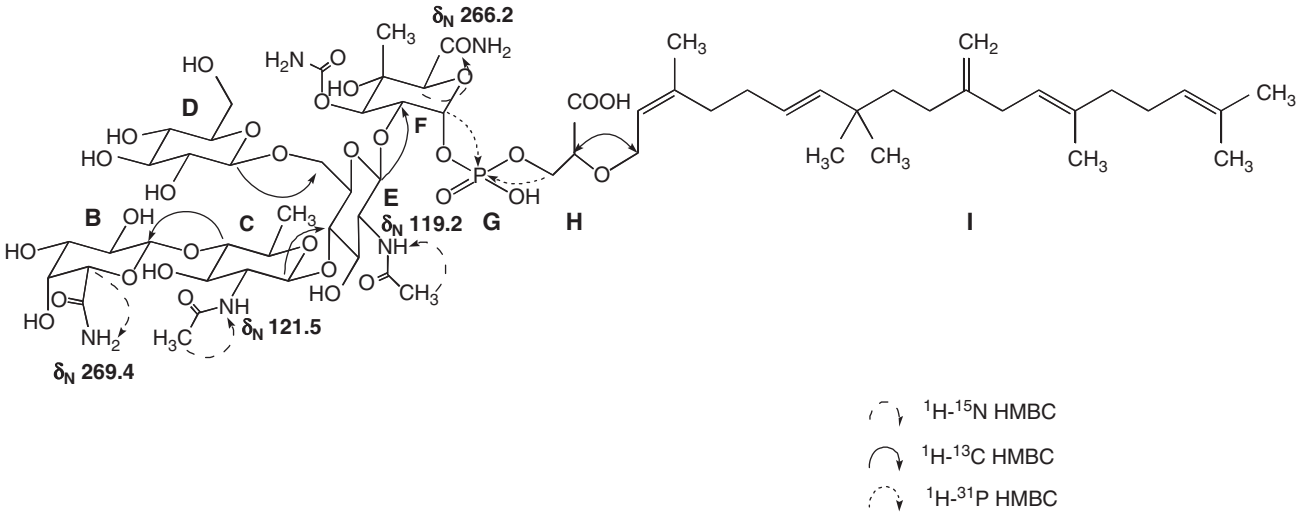


Figure 3 The linkages between the partial structures in nosokomycin B (**2**).

sugar **D** in **2** were found to be absent in **4**, and the chemical shift of C-6 seen in sugar **E** in **4** was shifted to a higher field in ^{13}C NMR spectra (δ_C 70.1 in **2**, δ_C 61.0 in **4**). Therefore, the structure of **4** was elucidated to be the nosokomycin B analog lacking moiety **D**, as shown in Figure 1.

DISCUSSION

According to the structures of the nosokomycins elucidated in this study, they belong to the moenomycin family of antibiotics. Nosokomycin B (**2**), which lacks the chromophoric cyclopentenone moiety (**A**), was identical to the semisynthetic moenomycin A derivative.[6] However, **2** was isolated as a main product from *Streptomyces* sp. K04-0144. More than 25 members of the moenomycin family have been reported from microbial metabolites.[8] However, the structures of only a few members have been fully elucidated.[5]

From the structure–activity relationship of moenomycin derivatives reported previously, it has been found that moenomycin trisaccharides containing units **C**, **E**, **F**, **G**, **H** and **I** are the smallest cores to show antibiotic activity *in vivo*,[6] whereas moenomycin disaccharides containing units **E–I** still function as transglycosylase inhibitors *in vitro*.[9] Nosokomycins are larger than the smallest cores of *in vivo*-active moenomycin derivatives and are also smaller than moenomycin A itself. Therefore, it is reasonable that nosokomycins retain activity.

Recently, Walker and coworkers[10] proposed a biosynthetic pathway for the cyclopentenone (A ring) moiety and the pentasaccharide section of moenomycin A using a whole-genome scanning approach with the producing strain *Streptomyces ghanaensis* ATCC 146723 in combination with gene knockout and complementation experiments.[10,11] They described that MoeA4 functions as an acyl-CoA ligase that cyclizes aminolevulinate to form the cyclopentenone (A ring) moiety and that MoeB4 functions as an amide synthetase that couples the **A** ring moiety to the C-6 of the **B** ring of moenomycin A. As described above, all nosokomycins lack the chromophoric cyclopentenone moiety, and no members of the moenomycin family were detected in the culture broth of the nosokomycin-producing strain, *Streptomyces* sp. K04-0144 strain. Therefore, we speculate that *moeA4* and/or *moeB4* genes do/does not work or lack(s) the nosokomycin-producing strain.

METHODS

General experiments

NMR spectra were measured on a Varian XL-400 spectrometer (Varian, Palo Alto, CA, USA) with ^1H NMR measured at 400 MHz and ^{13}C NMR measured at 100 MHz in methanol-d_4. Chemical shifts are expressed in δ values (p.p.m.) with methanol-d_4 (δ_c 49.0) used as an internal reference for ^{13}C NMR spectra and methanol-d_4 (δ_H 3.30) used an internal reference for ^1H NMR spectra. IR spectra were measured on a Horiba FT IR-710 spectrometer (Horiba, Kyoto, Japan), and UV spectra were measured on a Beckman DU-640 spectrophotometer (Beckman Coulter, Fullerton, CA, USA). Optical rotation was measured on a JASCO DIP-370 digital polarimeter (JASCO, Hachioji, Japan).

LC-ESI-MS experiments

LC-ESI-MS spectra were measured on a JEOL JMS-700 magnetic sector-type mass spectrometer (JEOL, Akishima, Japan) coupled with an Agilent 1100 G1310A liquid chromatography pump (Agilent Technologies, Santa Clara, CA, USA).[12] The LC conditions were as follows: column, PEGASIL ODS (2.0ø×50 mm, Senshu Scientific, Tokyo, Japan); mobile phase, MeOH; and flow rate, 0.2 ml min^{-1}. The ESI source was operated in negative ion mode with a ring voltage of 100 V. The standard substance YOKUDELNA (JEOL) was used for mass calibration with a scan range of *m/z* 100–2000.

^{31}P NMR and ^1H-^{31}P HMBC experiments

^{31}P NMR spectra were measured on a Mercury-300 spectrometer (Varian) at 300 MHz in methanol-d_4 at room temperature. Chemical shifts are expressed in δ values (p.p.m.), and triphenylphospine (δ_P 0.0) was used as an internal reference. ^1H-^{31}P HMBC spectra were measured under following conditions: sample 10 mg, $f_1 \times f_2$=2048×512 points, nt=16, ni=160. The pulse sequence was described in a previous study.[13]

^1H-^{15}N HMBC experiments

^{15}N NMR spectra were measured on a Varian Mercury-300 spectrometer at 300 MHz in methanol-d_4 at room temperature. Chemical shifts are expressed in δ values (p.p.m.) and benzamide (δ_N 105.4) was used as an internal reference. ^1H-^{15}N HMBC spectra were measured under the following conditions: sample 10 mg, $f_1 \times f_2$=2048×2048 points, nt=4000, ni=90. The pulse sequence was described in a previous paper.[14]

ACKNOWLEDGEMENTS

We are grateful to Ms Chigako Sakabe, Ms Akiko Nakagaw, Ms Noriko Sato and Mr Satoshi Ohte from the School of Pharmacy, Kitasato University for measuring the mass and NMR spectra and their concerted efforts in aiding this

study. This study was supported in part by a Grant-in Aid for Scientific Research 21310146 (to HT) from the Ministry of Education, Culture, Sports, Science, and Technology of Japan and by a grant from the Uehara Memorial Foundation of Japan (to HT).

1 Fukuda, T., Matsumoto, A., Takahashi, Y., Tomoda, H. & Ōmura, S. Phenatic acids A and B, new potentiators of antifungal miconazole activity produced by *Streptomyces* sp. K03-0132. *J. Antibiot.* **58,** 252–259 (2005).
2 Koyama, N. *et al.* Stemphones, novel potentiator of imipenem activity against methicillin resitant *Staphylococcus aureus*, produced by *Aspergillus* sp. FKI-2136. *J. Antibiot.* **58,** 695–703 (2005).
3 Iwatsuki, M. *et al.* Lariatins, novel anti-mycobacterial peptides with a lasso structure, produced by *Rhodococcus jostii* K01-B0171. *J. Antibiot.* **60,** 357–363 (2007).
4 Uchida, R. *et al.* Nosokomycins, new antibiotics discovered in an *in vivo*-mimic infection model using silkworm larvae. I. Fermentation, isolation and biological properties. *J. Antibiot.* **63,** 151–155 (2010).
5 Michael, K., Wolfgang, G. & Laszlo, V. Three-dimensional structure of moenomucon A, a potent inhibitor of penicillin-binding protein 1b. *Eur. J. Biochem.* **252,** 500–507 (1998).
6 Marzan, S. *et al.* Moenomycin A: reactions at the lipid part. New structure-activity relations. *Tetrahedron* **50,** 5299–5308 (1994).
7 Zehl, M., Pittenauer, E., Rizzi, A. & Allmaier, G. Characterization of moenomycin antibiotic complex by multistage MALD-IT/TOF-MS and ESI-IT-MS. *J. Am. Soc. Mass Spectrum.* **17,** 1081–1090 (2006).
8 Arai, M. *et al.* Pholipomycin, a new member of phosphoglycolipid antibiotics II. physico-chemical properties and comparison with other members of this family of antibiotics. *J. Antibiot.* **12,** 1055–1059 (1977).
9 Baizman, E. R. *et al.* Antibacterial activity of synthetic analogues based on the disaccharide structure of moenomycin, an inhibitor of bacterial transglycosylase. *Microbiology* **146,** 3129–3140 (2000).
10 Ostash, B., Saghatelian, A. & Walker, S. A streamlined metabolic pathway for the biosynthesis of moenomycin A. *Chem. Biol.* **14,** 257–267 (2007).
11 Ostash, B. *et al.* Complete characterization of the seventeen step moenomycin biosynthetic pathway. *Biochemistry* **48,** 8830–8841 (2009).
12 Sakabe, C., Iwatsuki, M., Sakayanagi, M. & Hara, R. The abstract of 'Kikibunsekigijutsukenkyuukai'. P-13-A (2005).
13 Dominika, D. *et al.* Structure of the O-specific polysaccharide of Proteus penneri 103 containing ribitol and 2-aminoethanol phosphates. *Carbohydrate Res.* **337,** 1535–1540 (2002).
14 Gary, E. M. & Chad, E. H. Long-range ^{1}H-^{15}N heteronuclear shift correlation at natural abundance. *J. Nat. Prod.* **63,** 543–585 (2000).