A New Group of Antibiotics, Hydroxamic Acid Antimycotic Antibiotics. IV.[1] Structures of Enactins Ia, Ib_1, Ib_2 and Va

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Structures of enactins Ia, Ib_1, Ib_2 and Va were determined by spectroscopic studies using their bis-2,4-dinitrophenyl derivatives. Both enactins and neoenactins contain L-serine to form the hydroxamic acid structure. Their physico-chemical and biological properties are closely related. The structures of neoenactin congeners have been previously reported and enactins Ia and Va are proved to be 19-hydroxyneoenactin B_2 and 14-dihydroneoenactin M_1, respectively. Both enactins Ib_1 and Ib_2 are determined to be 19-hydroxyneoenactin B_1 and considered to be diastereoisomers at the 19-position, though their steric structures at this position remain to be determined.

Keywords enactin; neoenactin; hydroxamic acid; antimycotic antibiotics; potentiator; *Streptomyces roseoviridis*

Enactins (ENs) produced by *Streptomyces roseoviridis*[1,2] and neoenactins (NEs) produced by *Streptoverticillium olivoreticuli*[3,4] are antimycotic antibiotics discovered as potentiators for polyene antifungal antibiotics (PAA) by our simple and multipurpose screening method, taking advantage of the ability of sterols to reverse the fungicidal activity of PAA.[5] Both ENs and NEs contain L-serine to form the hydroxamic acid structure (see Fig. 1) and have quite similar physico-chemical and biological properties, though NEs are more hydrophobic and more active against fungi and yeasts. Thus, we have proposed the group name "hydroxamic acid antimycotic antibiotics (HAAA)" for ENs, NEs and other related antibiotics.[6,7]

As reported in earlier, we have isolated several EN congeners called EN-Ia, -Ib, -IIc and -Va as their free bases by repetitive reverse-phase high performance liquid chromatography (HPLC) and revealed their physico-chemical and biological properties.[1]

In this paper, we will describe the separation of EN-Ib into EN-Ib_1 and -Ib_2 by HPLC as their bis-1,2-dinitrophenyl (DNP) derivatives and the structures of EN-Ia, -Ib_1, -Ib_2 and -Va elucidated by studying proton and carbon-13 nuclear magnetic resonance (^{1}H- and ^{13}C-NMR) spectra and MS data of their DNP derivatives.

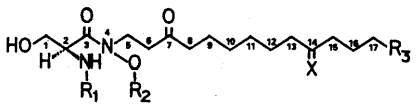
The molecular formulae of EN-Ia ($C_{20}H_{38}N_2O_6$), -Ib_1 ($C_{20}H_{38}N_2O_6$), -Ib_2 ($C_{20}H_{38}N_2O_6$) and -Va ($C_{19}H_{38}N_2O_5$) were determined by the fast atom bombardment mass spectra (FAB-MS) data and ^{1}H- and ^{13}C-NMR spectra of their DNP derivatives.

The structures of NEs were previously elucidated as

TABLE I. ^{1}H-NMR Data[a] ($CDCl_3$) of DNP Derivatives of Enactin-Ia, -Ib_1 and -Ib_2

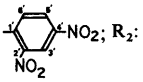
Assignment (Position)	Chemical shift (δ, ppm)				
	DNP-EN-Ia (27 °C)	DNP-NE-B_2	DNP-EN-Ib_1 (27 °C)	DNP-EN-Ib_2 (27 °C)	DNP-NE-B_1
$CHCH_3$ (20, 21)			0.88 (3H, d, J=6.9, H-21)	0.87 (3H, d, J=6.9, H-21)	0.85
			1.13 (3H, d, J=6.5, H-20)	1.12 (3H, d, J=6.2, H-20)	
CH_2CH_3 (20)					0.85
$C(CH_3)_2$ (20, 21)	1.12 (6H, s)				
$CH(CH_3)_2$ (20, 21)		0.75			
CH_2 (18, 19)		1.16 (H-18)			1.12 (H-19)
CH_2 and CH (10, 11, 17, 18, 19)	1.27 (7H, m, H-10, 11, 17, 18)	1.27 (H-10, 11, 17, 19)	1.26 (7H, m, H-10, 11, 17, 18)	1.26 (7H, m, H-10, 11, 17, 18)	1.28 (H-10, 11, 17, 18)
CH_2CH_2CO (9, 12, 16)	1.54 (6H, m)	1.54	1.54 (6H, m)	1.54 (6H, m)	1.54
CH_2CO (8, 13, 15)	2.42 (6H, m)	2.40	2.41 (6H, m, H-8, 13, 15)	2.41 (6H, m, H-8, 13, 15)	2.40
NCH_2CH_2CO (6)	2.84 (2H, t, J=5.2)	2.84	2.84 (2H, t, J=5.0)	2.84 (2H, t, J=5.5)	2.84
CHOH (19)			3.75 (1H, dq, J=4.2, 6.5)	3.63 (1H, dq, J=6.2, 6.2)	
NCH_2CH_2CO (5)	4.01 (1H, dt, J=14.5, 5.2)	4.01	4.02 (1H, dt, J=14.5, 5.0)	4.01 (1H, dt, J=15.0, 5.5)	4.02
	4.28 (1H, dt, J=14.5, 5.2)	4.28	4.27 (1H, dt, J=14.5, 5.0)	4.28 (1H, dt, J=15.0, 5.5)	4.26
$HOCH_2$ (1)	4.09 (1H, ddd, J=4.5, 4.5, 11.0)	4.09	4.10 (1H, ddd, J=5.2, 5.2, 11.5)	4.10 (1H, ddd, J=5.2, 5.2, 11.5)	4.09
	4.17 (1H, ddd, J=4.5, 4.5, 11.0)	4.18	4.17 (1H, ddd, J=5.2, 5.2, 11.5)	4.16 (1H, ddd, J=5.2, 5.2, 11.5)	4.16
NHCHCO (2)	4.86 (1H, br, m)	4.86	4.85 (1H, br, m)	4.86 (1H, br, m)	4.85
H-Ar (6′)	6.88 (1H, d, J=9.0)	6.88	6.88 (1H, d, J=9.2)	6.88 (1H, d, J=9.0)	6.85
H-Ar (6″)	7.60 (1H, d, J=9.0)	7.61	7.60 (1H, d, J=9.2)	7.61 (1H, d, J=9.0)	7.57
H-Ar (5′)	8.27 (1H, dd, J=2.7, 9.0)	8.26	8.27 (1H, dd, J=2.6, 9.2)	8.26 (1H, dd, J=2.6, 9.0)	8.25
H-Ar (5″)	8.52 (1H, dd, J=2.7, 9.0)	8.52	8.52 (1H, dd, J=2.7, 9.2)	8.52 (1h, dd, J=2.6, 9.0)	8.50
H-Ar (3′)	9.12 (1H, d, J=2.7)	9.11	9.12 (1H, d, J=2.6)	9.12 (1H, d, J=2.6)	9.12
H-Ar (3″)	8.93 (1H, d, J=2.7)	8.95	8.94 (1H, d, J=2.7)	8.95 (1H, d, J=2.6)	8.95
–CNHAr	9.17[b] (1H, d, J=8)	9.18[b]	9.17[b] (1H, d, J=8)	9.17[b] (1H, d, J=8)	9.16[b]

a) ^{1}H-NMR spectrum was recorded on a JEOL GX 400 and JEOL GX 500 spectrometer. Tetramethylsilane (TMS (0 ppm)) was used as an internal standard. Number of protons, multiplicity, coupling constants in Hz and position of protons where necessary are indicated in parenthesis. *b*) Temperature dependent.



	R_1	R_2	X	R_3
EN-Ia	H	H	=O	
EN-Ib_1	H	H	=O	
EN-Ib_2	H	H	=O	
EN-Va	H	H		
NE-A	H	H	=O	
NE-M_1	H	H	=O	
NE-M_2	H	H		
NE-B_1	H	H	=O	
NE-B_2	H	H	=O	
NE-NL_1	H	H	=O	
NE-NL_2	H	H	=O	

Fig. 1. Structures of Enactin, Neoenactin and Their DNP Derivatives[a)]

a) DNP derivatives, R_1:  R_2:

shown in Fig. 1 by studying ^{1}H- and ^{13}C-NMR spectra and MS data of their DNP derivatives.[4,8)] Thus, EN-Ia, -Ib and -Va were converted to DNP derivatives to elucidate their structures as done for NEs.

In the ^{1}H-NMR spectrum of DNP-Ib, unexplainable proton signals were observed at δ 3.63 (approximately 0.5H, dq) and δ 3.75 (approximately 0.5H, dq) and the two protons were not coupled to each other. Thus, DNP-Ib was suggested to be composed of almost equal amounts of the two closely related isomers and further separated by normal phase HPLC to the isomers designated DNP-EN-Ib_1 and -Ib_2.

^{1}H-NMR data of DNP-EN-Ia, -Ib_1 and -Ib_2 are listed in Table I together with those of DNP-NE-B_2 and -B_4[c)] and the structures of EN congeners are shown in Fig. 1 together with those of NE-congeners. EN-Ia, -Ib_1 and -Ib_2 have the same molecular formula ($C_{20}H_{38}N_2O_6$) containing one more extra oxygen atom than NE-B_1, -B_2 and -NL_2 ($C_{20}H_{38}N_2O_5$). Since EN-Ia and NE-B_2 have geminal methyl groups, EN-Ia is presumed to be a hydroxyl derivative of NE-B_2. In the ^{1}H-NMR data, the geminal methyl doublets at δ 0.75 (6H, J=6.5, H-20, -21) of DNP-NE-B_2 appear as the singlets at δ 1.12 (6H, H-20, -21) in DNP-EN-Ia by substitution of the hydroxyl group at 19-position as seen in Table I. Further, the presence of a hydroxyl group at 19-position in EN-Ia is advocated by the low field shift of methylene protons at δ 1.16 (2H, m, H-18) of DNP-NE-B_2 to δ 1.27 in DNP-EN-Ia. Other ^{1}H-NMR data of DNP-EN-Ia and DNP-NE-B_2 are substantially identical to each other from H-1 through H-17. Thus, the structure of EN-Ia is elucidated to be 19-hydroxy-NE-B_2.

NE-B_1 has the ante-iso type methyl groups and the triplet at δ 0.85 (3H, J=6.5) and the doublet at δ 0.85 (3H, J=6.5) in ^{1}H-NMR data of DNP-NE-B_1 are assigned to H-20 and -21, respectively, as seen in Table I.[4c)] EN-Ib_1 also has the ante-iso type methyl groups and is considered to be a hydroxyl derivative of NE-B_1. The triplet at δ 0.85 of DNP-NE-B_1 apppears as the doublet at δ 1.13 (3H, J=6.5)

TABLE II. ^{1}H-NMR Data[a)] ($CDCl_3$) of DNP Derivatives of Enactin-Va

Assignment (Position)	Chemical shift (δ, ppm)	
	DNP-EN-Va (50 °C)	DNP-NE-M_2
$CH_2\underline{CH}_3$ (20)		0.87
$CH(\underline{CH}_3)_2$ (19,20)	0.89 (6H, d, J=6.8)	
CH_2 and CH (10, 11, 12, 13, 15, 17, 18, 19)	1.20—1.43 (15H, m, H-10, 11, 12, 13, 15, 16, 17, 18)	1.21—1.46 (H-10, 11, 12, 13, 15, 16, 17, 18, 19)
$\underline{CH}_2CH_2CO$ (9)	1.55 (2H, m)	1.54
$CH_2\underline{CO}$ (8)	2.43 (2H, t, J=7.4)	2.42
$NCH_2\underline{CH}_2CO$ (6)	2.84 (2H, t, J=5.9)	2.83
$\underline{CH}OH$ (14)	3.59 (1H, br)	3.58
$N\underline{CH}_2CH_2CO$ (5)	4.04 (1H, dt, J=14.5, 5.9)	4.00
	4.26 (1H, dt, J=14.5, 5.9)	4.27
$HO\underline{CH}_2$ (1)	4.09 (1H, ddd, J=5.2, 5.2, 11.5)	4.07
	4.13 (1H, ddd, J=5.2, 5.2, 11.5)	4.14
$NH\underline{CH}CO$ (2)	4.87 (1H, br, m)	4.84
H-Ar (6′)	6.90 (1H, d, J=9.1)	6.87
H-Ar (6″)	7.58 (1H, d, J=9.1)	7.59
H-Ar (5′)	8.26 (1H, dd, J=2.8, 9.1)	8.26
H-Ar (5″)	8.50 (1H, dd, J=2.8, 9.1)	8.51
H-Ar (3′)	9.09 (1H, d, J=2.8)	9.11
H-Ar (3″)	8.92 (1H, d, J=2.8)	8.94
–CNHAr	9.08[b)] (1H, d, J=8)	9.15[b)]

a) ^{1}H-NMR spectrum was recorded on a JEOL GX 400 and JEOL GX 500 spectrometer. TMS (0 ppm) was used as an internal standard. Number of protons, multiplicity, coupling constants in Hz and position of protons where necessary are indicated in parenthesis. *b)* Temperature dependent.

in DNP-EN-Ib_1 by substitution of the hydroxyl group at β-position. The other methyl doublet at δ 0.85 (3H, J=6.5) in DNP-NE-B_1 is shifted to δ 0.88 (3H, d, J=6.9) in DNP-EN-Ib_1. Further, the methylene multiplet at δ 1.12 (2H, H-19) of DNP-NE-B_1 is shifted to lower field (δ 3.75, dq, J=4.2, 6.5) by the hydroxyl group at 19-position in DNP-EN-Ib_1. ^{1}H-NMR data of DNP-EN-Ib_1 from H-1 through H-17 are almost identical with those of DNP-NE-B_1. Thus, the structure of EN-$Ib_1$is elucidated to be 19-hydroxy-NE-B_1. EN-Ib_1 and -Ib_2 are closely related isomers and ^{1}H-NMR data of the two DNP derivatives are essentially the same except that the double quartet at δ 3.75 (1H, J=4.2, 6.5) in DNP-EN-Ib_1 is shifted to δ 3.63 (1H, dq, J=6.2, 6.2) in DNP-EN-Ib_2. Consequently, EN-Ib_2 is postulated to be the diastereoisomer of EN-Ib_1 at 19-position, though the steric configuration at this position remains to be solved.

EN-Va has the same molecular formula as NE-M_2 ($C_{19}H_{38}N_2O_5$). ^{1}H-NMR data of DNP-EN-Va and their assignments are listed in Table II together with those of DNP-NE-M_2. The existence of geminal methyl groups in DNP-EN-Va is shown by the methyl doublets at δ 0.89 (6H, J=6.8, H-19, -20) instead of the methyl triplet at δ 0.87 (3H, J=6.8, H-20) for DNP-NE-M_2. Further, fifteen methylene and methine protons exist as multiplets at δ 1.20—1.43 in ^{1}H-NMR data of DNP-EN-Va. On the other hand, eighteen methylene protons are observed in the same region in DNP-NE-M_2. Essentially no other difference is observed in ^{1}H-NMR data of both DNP derivatives from H-1 through H-18, as seen in Table II. Thus, the structure of EN-Va is determined to be 14-dihydro-NE-M_1 (=14-dihydrolipoxamycin).

^{13}C-NMR data of DNP-EN-Ia, -Ib_1 and -Ib_2 and their assignments are listed in Table III together with those of

TABLE III. ^{13}C-NMR Data[a] ($CDCl_3$) of DNP Derivatives of Enactin-Ia, -Ib_1 and -Ib_2

Position	Chemical shift (δ, ppm)						
	DNP-EN-Ia		DNP-NE-B_2	DNP-EN-Ib_1	DNP-EN-Ib_2		DNP-NE-B_1
	Exptl.	Calcd	Exptl.	Exptl.	Exptl.	Calcd	Exptl.
C-1	62.11		62.11	62.10	62.11		62.11
C-2	56.26		56.17	56.25	56.26		56.19
C-3	172.5		172.5	172.5	172.5		172.5
C-5	43.5		43.5	43.5	43.5		43.5
C-6	38.82		38.76	38.82	38.82		38.76
C-7	208.33		208.30	208.32	208.35		208.34
C-8	42.86[b]		42.86[b]	42.84[b]	42.84[b]		42.86[b]
C-9	23.39[c]		23.36[c]	23.36[c]	23.37[c]		23.38[c]
C-10	28.69[d]		28.72	28.67[d]	28.67[d]		28.73
C-11	28.67[d]		28.72	28.66[d]	28.65[d]		28.73
C-12	23.21[c]		23.20[c]	23.19[c]	23.20[c]		23.20[c]
C-13	42.57[b]		42.53[b]	42.60[b]	42.60[b]		42.53[b]
C-14	211.73		211.99	211.84	211.93		212.02
C-15	42.76		42.96	42.99	42.92		42.23
C-16	24.30		24.14	21.52	21.01		21.47
C-17	23.94	22.0	27.01	32.18	32.00	31.1	36.13
C-18	43.57	46.7	38.72	39.54	40.03	42.3	34.27
C-19	70.98	68.3	27.83	70.88	71.63	70.3	29.30
C-20	29.29	30.6	22.56	20.10	19.86	19.3	11.31
C-21	29.29	30.6	22.56	14.06	14.87	14.1	19.06
C-1′	146.67[e]		146.61[d]	146.66[e]	146.67[e]		146.62[d]
C-2′	131.62[e]		131.59[d]	131.61[e]	131.62[e]		131.59[d]
C-3′	124.25		124.25	124.25	124.25		124.23
C-4′	137.19[e]		137.16[d]	137.17[e]	137.18[e]		137.15[d]
C-5′	130.50		130.49	130.49	130.50		130.48
C-6′	113.99		113.93	114.27	114.00		113.94
C-1″	154.87		154.84	154.87	154.88		154.85
C-2″	137.34[e]		137.31[d]	137.17[e]	137.34[e]		137.29[d]
C-3″	122.84		122.83	122.83	122.84		122.81
C-4″	143.06[e]		143.03[d]	143.05[e]	143.06[e]		143.03[d]
C-5″	129.90		129.89	129.90	129.90		129.89
C-6″	115.43		115.37	115.42	115.43		115.40

a) ^{13}C-NMR spectra were recorded on a JEOL GX 400 and JEOL GX 500 spectrometer. TMS (0 ppm) was used as an internal standard. *b*—*e*) Values with identical superscript within a column may be interchanged.

TABLE IV. ^{13}C-NMR Data[a] ($CDCl_3$) of DNP Derivative of Enactin-Va

Position	Chemical shift (δ, ppm)		
	DNP-EN-Va		DNP-NE-M_2
	Exptl.	Calcd	Exptl.
C-1	62.31		62.11
C-2	56.28		56.16
C-3	172.5		172.5
C-5	43.76		43.5
C-6	38.80		38.78
C-7	208.15		208.37
C-8	43.01		42.96
C-9	23.47		23.36
C-10	28.97[b]		28.88[b]
C-11	29.25[b]		29.17[b]
C-12	25.34		25.63[c]
C-13	37.93[c]		37.24[d]
C-14	72.09		72.05
C-15	37.35[c]		37.59[d]
C-16	23.47	23.3	25.26[c]
C-17	39.10	39.4	29.35[b]
C-18	27.98	27.8	31.83
C-19	22.59	21.6	22.60
C-20	22.59	21.6	14.07
C-1′	146.66[d]		146.61[e]
C-2′	131.84[d]		131.60[e]
C-3′	124.18		124.25
C-4′,	137.44[d]		137.22[e]
C-5′	130.46		130.49
C-6′	113.96		113.90
C-1″	154.84		154.79
C-2″	137.44[d]		137.22[e]
C-3″	122.74		122.83
C-4″	143.22[d]		143.04[e]
C-5″	129.77		128.88
C-6″	115.41		115.33

a) ^{13}C-NMR spectra were recorded on a JEOL GX 400 and JEOL GX 500 spectrometer. TMS (0 ppm) was used as an internal standard. *b*—*e*) Values with identical superscript within a column may be interchanged.

DNP-NE-B_2 and DNP-NE-B_1.[4c] Chemical shifts from C-1 through C-16 of DNP-EN-Ia are essentially the same as those of DNP-NE-B_2, while low field shifts (α and β effects of hydroxyl group) of C-19 ($\Delta\delta_C$ 43.15 ppm), C-18 ($\Delta\delta_C$ 4.85 ppm) and C-20 ($\Delta\delta_C$ 6.73 ppm) and a high field shift (γ effect of hydroxyl group) of C-17 ($\Delta\delta_C$ 3.07 ppm) were observed on going from DNP-NE-B_2 to DNP-EN-Ia. Expected chemical shifts from C-17 through C-21 of DNP-19-hydroxy-NE-B_2 (=DNP-EN-Ia) were calculated using parameters of substituent effects[9] on ^{13}C-chemical shifts and experimental values of DNP-NE-B_2.[4c] Experimental chemical shifts from C-17 through C-21 of DNP-EN-Ia are in good agreement with those calculated for DNP-19-hydroxy-NE-B_2, as seen in Table III.

Further, ^{13}C-NMR data of DNP-EN-Ib_1 and -Ib_2 indicate that both compounds have the same structure as DNP-EN-B_1[4c] from C-1 through C-16. Experimental values from C-17 through C-21 of ^{13}C chemical shifts of DNP-EN-Ib_1 and -Ib_2 well agree with the anticipated data for DNP-19-hydroxy-NE-B_1, as seen in the same table.

^{13}C-NMR data of DNP-EN-Va and those calculated for DNP-14-dihydro-NE-M_1 are shown in Table IV in comparison with those of DNP-NE-M_2.[4c] DNP-EN-Va and DNP-NE-M_2 have the same molecular formula and DNP-EN-Va was shown to have the same structure as DNP-NE-M_2 from C-1 through C-15 by their essentially common chemical shifts. The existence of an iso type structure in DNP-EN-Va is suggested by chemical shifts at δ 22.59 (C-19, 20) and δ 27.98 (C-18), which well agree with those calculated for DNP-14-dihydro-NE-M_1 and those of DNP-NE-B_2. Thus, the EN-Va was confirmed to be 14-dihydro-NE-M_1, a positional isomer of NE-M_2.

Experimental

All chemicals and solvents used in this experiment were of reagent grade.

Isolation of Enactins Ia, Ib and Va EN-Ia, -Ib and -Va were isolated from the cultured filtrate of *Streptomyces roseoviridis*, as reported previously.[1]

Preparation of Bis-2,4-dinitrophenyl Enactins Ia, Ib and Va 0.1 ml of 2,4-dinitrofluorobenzene solution (210 mg/ml) in dry MeOH was added to 0.5 ml of dry MeOH solution of EN-Ia, -Ib or -Va (24 mg/ml). Approximately 1 ml of 1% triethylamine in dry MeOH was added dropwise to the mixture and stirred for 2 h. The reaction mixture was evaporated *in vacuo* to give yellow oil. The residual oil was purified by silica gel column chromatography using $CHCl_3$–MeOH (30:1 for DNP-EN-Ia and -Ib, 20:1 for DNP-EN-Va) as a developing solvent. The main yellow eluate was evaporated *in vacuo* to give DNP-EN-Ia, -Ib or -Va (10—12 mg) in 60% yield. DNP-EN-Va thus obtained was recrystallized from $CHCl_3$ to give yellow needles, mp 145 °C. DNP-EN-Ia and -Ib were further purified by reverse phase HPLC on an ODS column (YMC-Pack S-343 I-15, 20 × 250 mm, Yamamura Chem. Lab. Co., Ltd.) using CH_3CN–H_2O (3:2) as a mobile phase. The flow rate was maintained at 9.0 ml/min and the eluate was monitored by a ultraviolet (UV) detector at 340 nm. The retention times of DNP-EN-Ia and -Ib were 25.1 and 32.2 min, respectively. After removing the solvent of eluates, DNP-EN-Ia or -Ib was extracted with EtOAc. Each EtOAc extract was dried over Na_2SO_4 anhydride,

filtered and evaporated *in vacuo* to given yellow oil.

Separation of Bis-2,4-dinitrophenyl Enactins Ib_1 and Ib_2 DNP-EN-Ib was further separated into DNP-EN-Ib_1 and -Ib_2 by HPLC on a Radial pak nova pak silica cartridge (8 × 100 mm, Waters Assoc., U.S.A.) using *n*-hexane–tetrahydrofuran (13 : 7) as a mobile phase. The flow rate was maintained at 2.5 ml/min and the eluate was monitored by a UV detector at 340 nm. The retention times of DNP-EN-Ib_1 and -Ib_2 were 24.3 and 25.1 min, respectively. Both DNP-EN-Ib_1 and -Ib_2 were separated by repeated HPLC and recovered by evaporation of the solvent *in vacuo*.

MS Data of Enactins Ia, Ib and Va and Their DNP Derivatives MS were measured with a JEOL HX-110 mass spectrometer.

(i) EN-Ia: High resolution FAB-MS *m/z* Calcd for $C_{20}H_{39}N_2O_6^+$ $(M+H)^+$: 403.2808. Found: 403.2810.

(ii) EN-Ib: FAB-MS *m/z*: 403 $(M+H)^+$.

(iii) EN-Va: FAB-MS *m/z*: 375 $(M+H)^+$. Electron impact MS *m/z*: 271 $(M-H_2O-C_6H_{13})^+$, 253 $(M-H_2O-C_6H_{13}-H_2O)^+$.

(iv) DNP-EN-Ib_1: High resolution FAB-MS *m/z* Calcd for $C_{32}H_{43}N_6O_{14}^+$ $(M+H)^+$: 735.2837. Found: 735.2883.

(v) DNP-NE-Ib_2: High resolution FAB-MS *m/z* Calcd for $C_{32}H_{43}N_6O_{14}^+$ $(M+H)^+$: 735.2837. Found: 735.2848.

(iv) DNP-EN-Va: High resolution FAB-MS *m/z* Calcd for $C_{31}H_{43}N_6O_{13}^+$ $(M+H)^+$: 707.2888. Found: 707.2899.

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