NEW ANTIBIOTIC NAPYRADIOMYCINS A2 AND B4 AND STEREOCHEMISTRY OF NAPYRADIOMYCINS

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Napyradiomycins A2 and B4, new members of the napyradiomycins, have been isolated from the culture broth of *Chainia rubra* MG802-AF1. The structure of napyradiomycin A2 was elucidated as 16-hydroxy-17-methylenenapyradiomycin A1 by NMR studies. The absolute structure of napyradiomycin B4 was determined as 13-hydroxy-13-methylnapyradiomycin B1 by X-ray crystallography and therefore the configuration of C(4a) in other napyradiomycins is assumed as the *R* configuration. The geometrical isomerism of napyradiomycin C1 was estimated as 12*E* and 16*E* by nuclear Overhauser effect experiments.

Isolation, physico-chemical and biological properties, and structures of napyradiomycins (NPD's) A, B1, B2, B3, C1 and C2 were reported previously[1,2] (the name of NPD-A has been changed to NPD-A1). In our successive study of NPD's, two new ones, NPD-A2 and -B4 (Fig. 1), have been isolated from the culture broth of *Chainia rubra* MG802-AF1. Isolation, physico-chemical properties and structures of NPD-A2 and -B4 are reported here, and the stereochemistry of NPD's is described.

Production and Isolation of NPD-A2 and -B4

Strain *C. rubra* MG802-AF1 was precultured on a rotary shaker at 27°C for 5 days in a 500-ml Erlenmeyer flask which contained 110 ml of medium described in the Experimental section. Three ml of this culture was inoculated into 500-ml Erlenmeyer flasks which contained 110 ml of the same medium and the organisms were cultured for 84 hours as described above.

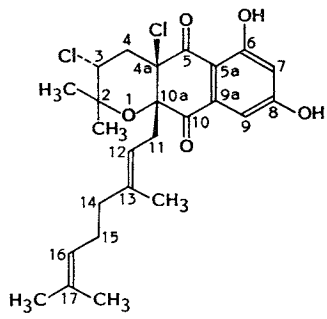
NPD-A2 and -B4 were extracted with butyl acetate from the culture filtrate (4.9 liters) and mycelia obtained by the procedure described in the Experimental section. They were purified by silica gel and Sephadex LH-20 column chromatography, and further purified by silica gel TLC. NPD-A2 (23.5 mg) and NPD-B4 (28.9 mg) were obtained as brownish yellow powder and pale yellow prismatic crystals, respectively.

Both NPD-A2 and -B4 inhibited the growth of Gram-positive bacteria in the range of 25 to 50 μg/ml.

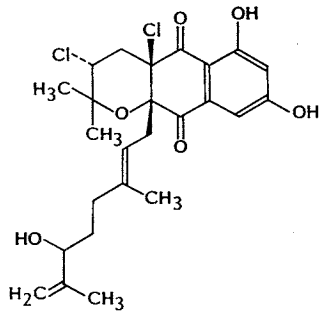
Physico-chemical Properties of NPD-A2 and -B4

NPD-A2 is soluble in methanol, ethyl acetate, chloroform and benzene, and insoluble in water and hexane. NPD-B4 is slightly soluble in chloroform and benzene, and insoluble in water and

Fig. 1. Structures of napyradiomycins.



NPD-A1



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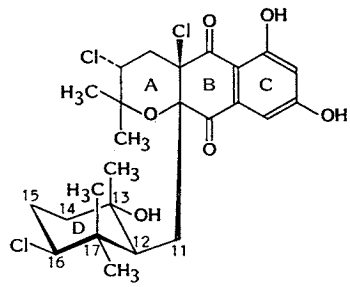
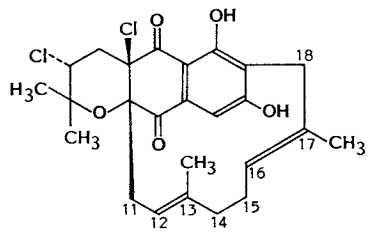


NPD-B4



NPD-C1

Table 1. Physico-chemical properties of NPD-A2 and -B4.

	NPD-A2	NPD-B4
Appearance	Brownish yellow powder	Pale yellow prisms
MP (°C)	62~67	168~171
FD-MS[a] (*m/z*)	496, 498, 500 (M)	533, 535, 537, 539 (M+H)
HR-MS[b] Found	496.1403 (M)	531.1100 (M−H)
Calcd	496.1416 ($C_{25}H_{30}O_6Cl_2$)	531.1105 ($C_{25}H_{30}O_6Cl_3$)
Molecular formula	$C_{25}H_{30}O_6Cl_2$	$C_{25}H_{31}O_6Cl_3$
Optical rotation	$[\alpha]_D^{28}$ +20° (*c* 0.3, EtOH)	$[\alpha]_D^{28}$ −81° (*c* 0.2, EtOH - benzene, 1:1)
UV λ_{max}^{MeOH} nm (log ε)	207 (4.05), 252 (4.11), 270 (sh 4.00), 295 (sh 3.84), 362 (3.77), 400 (sh 3.17)	206 (3.96), 253 (4.20), 270 (sh 4.01), 301 (3.93), 362 (3.85), 4.00 (sh 3.43)
$\lambda_{max}^{MeOH-HCl}$ nm (log ε)	207 (4.02), 251 (4.14), 270 (sh 4.01), 360 (3.76)	205 (3.91), 251 (4.26), 270 (sh 4.01), 306 (3.82), 358 (3.84)
$\lambda_{max}^{MeOH-NaOH}$ nm (log ε)	209 (4.42), 264 (3.96), 300 (4.14), 386 (3.97)	209 (4.44), 245 (sh 3.92), 264 (4.00), 297 (4.16), 384 (4.03)
IR (KBr) cm^{-1}	3400, 2980, 1700, 1640, 1620, 1380, 1260, 1080, 870, 740	3350, 2980, 1700, 1620, 1380, 1260, 1080, 1020, 880, 760

[a] Field desorption mass spectrometry.
[b] High resolution mass spectrometry.

hexane. NPD-A2 and -B4 show positive color reactions with $KMnO_4$, anisaldehyde-H_2SO_4 and Gibbs reagent. Other physico-chemical properties are shown in Table 1.

Structure of NPD-B4

The pale yellow crystals were grown in a mixed solution of chloroform and methanol as small

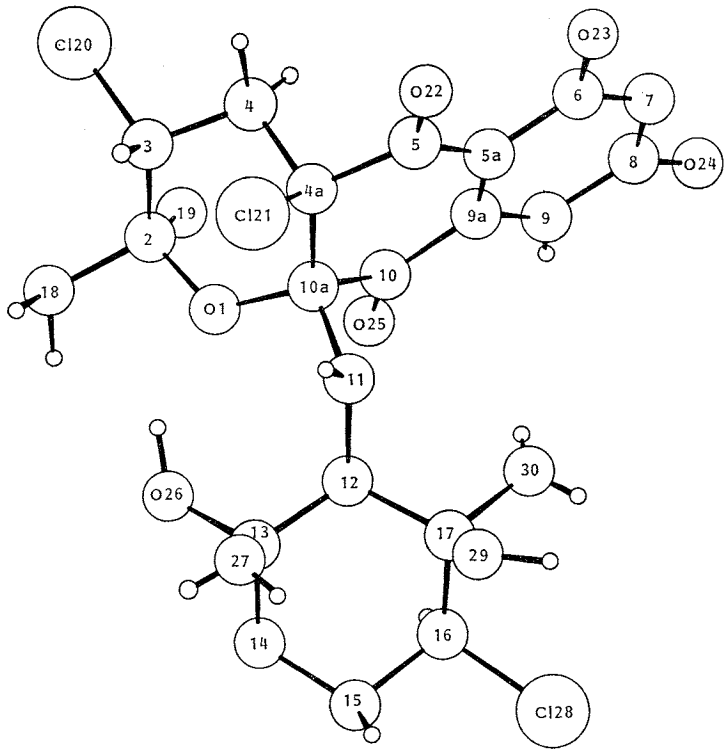
blocks of approximate dimensions of 0.1 mm. A crystal of $0.3\times0.15\times0.2$ mm in size was used for determination of cell data and the collection of intensity data on a Philips PW1100 diffractometer using CuKα radiation monochromated by a graphite plate. The crystal data are as follows: NPD-B4, $C_{25}H_{31}O_6Cl_3\cdot0.15(CH_3OH)$, FW=538.7. Monoclinic, space group $P2_1$, $Z=4$ (2 molecules per asymmetric unit), $D_{calc}=1.342$ gcm^{-3}. Cell dimensions, $a=19.156(10)$, $b=12.355(7)$, $c=11.270(7)$ Å, $\beta=91.38(5)°$, $U=2666.5$ Å^3. μ for CuKα=34.8 cm^{-1}.

The molar ratio of methanol solvate was estimated by the ratio of electron-densities of the methanol oxygen atom to those of oxygen in NPD-B4. Of the total of 5785 reflections within the 2θ range of $6°\sim156°$, 4781 (83%) could be measured as above the 2σ(I) level. Additional 782 sets of hkl and $h\bar{k}l$ reflections were measured in the 2θ range $30°\sim80°$ and $l\leq4$, in order to determine the absolute structure.

The crystal structure was solved by the direct method and subsequent use of several cycles of difference Fourier syntheses. The structural parameters were then refined by the block-diagonal-matrix least-squares calculations to give an R value of 0.085. Inclusion of dispersion correction terms for the atomic scattering factors of Cl, O and C for CuKα radiation to the structure factor calculation showed clearly the absolute structure. Of the 254 Friedel pairs giving differences between F_o (hkl) and F_o ($\bar{h}\bar{k}\bar{l}$) greater than twice their estimated standard deviations and both the ratio, $|F_o(hkl)|/|F_o(\bar{h}\bar{k}\bar{l})|$ and $|F_c(hkl)|/|F_c(\bar{h}\bar{k}\bar{l})|$ greater than 1.03 or less than 0.97, 247 pairs indicated the absolute structure as shown in Fig. 2.

Finally, a difference electron-density map was calculated which showed a diffuse and elongated

Fig. 2. Molecular structure of NPD-B4 (molecule I).



peak of about leÅ^{-3} in height along with the very low peaks assignable to hydrogen atoms. The former was taken as indicating the methanol molecule (with a multiplicity factor of 0.3) since the electron-density map could be interpreted by the two peaks separated by about 1.3 Å having the peak height ratio of 1 : 0.7 and since the crystal was grown in a mixed solution containing methanol.

The final least-squares calculations including two molecules of NPD-B4, metnanol C and O atoms with a multiplicity factor of 0.3, and 31 hydrogen atoms gave the R value of 0.062†. The molecular parameters agree well between the two molecules. As shown in Fig. 2 the molecule consists of a tricyclic chromophore (naphthopyrane) and a cyclohexyl moiety linked together by a methylene bridge. The chromophore consists of a naphthoquinone ring formed by rings B and C and a pyrane ring A, fused in the *cis* form. To relieve the repulsion between the two bulky substituent groups at the juncture (Cl and the methylene group linked to ring D), ring B is distorted in such a way that the plane of the naphthoquinone group twists at C(4a) and C(10a) in order to increase the torsion angle Cl(21)-C(4a)-C(10a)-C(11) to −46.8(0.5)° [−47.8(0.5)° in molecule II] from the exact *cis* position (0°). Rings A and D take a chair conformation; and Cl(20), Cl(28) and HO(26) are equatorial; but two methyl groups, $H_3C(27)$ and $H_3C(29)$, in D ring are oriented axially, forming a 1,3-diaxial group.

The torsion angles along the methylene bridge linking the tricyclic chromophore and the cyclohexyl group, C(4a)-C(10a)-C(11)-C(12), C(10a)-C(11)-C(12)-C(13) and C(10a)-C(11)-C(12)-C(17) are 176.5 (0.4)°, −98.3(0.5)° and 130.3(0.4)°, respectively for molecule I which can be compared with 178.5(0.3)°, −104.2(0.4)° and 126.5(0.4)° found in molecule II. Asymmetry of the latter two torsion angles may

Table 2. ^{1}H NMR chemical shifts of NPD-A1, -A2 and -B4.

Proton	Chemical shifts (δ) in ppm		
	NPD-A1[2)]	NPD-A2	NPD-B4
2-$CH_{3\ ax}$	1.18 s	1.20 s	1.33 s
2-$CH_{3\ eq}$	1.50 s	1.50 s	1.54 s
3-H	4.42 dd (4.8, 11.2)	4.45 dd (4.0, 11.8)	4.55 dd (4.0, 12.0)
4-H_{ax}	2.41 dd (11.2, 14.0)	2.41 dd (11.8, 14.0)	2.47 dd (12.0, 14.4)
4-H_{eq}	2.48 dd (4.8, 14.0)	2.52 dd (4.0, 14.0)	2.59 dd (4.0, 14.4)
6-OH	11.84 s	11.94 s	
7-H	6.73 d (2.4)	6.74 d (2.4)	6.71 d (2.4)
8-OH	3.6 br s	8.83 br s	
9-H	7.22 d (2.4)	7.28 d (2.4)	7.13 d (2.4)
11-H_A	2.70 br d (8.0)	2.82 dd (8.6, 13.8)	2.63 dd (7.6, 16.4)
11-H_B		2.47 dd (7.2, 13.8)	1.52 br d (16.4)
12-H	4.70 br t (8.0)	4.78 dd (7.2, 8.6)	1.54 br d (7.6)
13-CH_3	1.31 s	1.36 s	1.22 s
14-H_A	1.6 m	1.80 m	$_{ax}$ 1.60 ddd (4.0, 13.2, 13.6)
14-H_B			$_{eq}$ 1.86 ddd (4.0, 4.0, 13.2)
15-H_A	1.6 m	1.34 m	$_{ax}$ 1.79 dddd (4.0, 12.2, 13.6, 13.6)
15-H_B			$_{eq}$ 1.96 dddd (4.0, 4.0, 4.0, 13.6)
16-H	4.89 br s	4.01 t (6.6)	3.66 dd (4.0, 12.2)
16-OH	—	1.80 s	—
17-CH_3 or CH_2	1.52 s	4.96 s, 4.86 s	$_{ax}$ 0.70 s
17-CH_3	1.60 s	1.71 s	$_{eq}$ 0.38 s

The coupling constants (Hz) are in parentheses.

† The atomic parameters, bond lengths, and angles have been sent to the Cambridge Crystallographic Data Centre.

Table 3. ^{13}C NMR chemical shifts of NPD-A1, -A2 and -B4.

Carbon	Chemical shifts (δ) in ppm		
	NPD-A1[2,3]	NPD-A2	NPD-B4
2	78.8 s	78.9 s	(81.3)s
2-$CH_{3\ ax}$	22.3 q	22.3 q	22.7 q
2-$CH_{3\ eq}$	28.8 q	29.0 q	29.1 q
3	58.8 d	58.7 d	58.5 d
4	42.8 t	42.6 t	42.8 t
4a	79.0 s	79.2 s	(82.0)s
5	193.7 s	193.6 s	(192.7)s
5a	110.2 s	109.4 s	107.6 s
6	164.8 s	(165.1)s	(167.5)s
7	109.6 d	109.9 d	109.9 d
8	163.9 s	(164.8)s	(166.1)s
9	107.8 d	108.2 d	110.1 d
9a	135.3 s	134.9 s	134.8 s
10	196.2 s	195.6 s	(192.2)s
10a	83.6 s	84.2 s	85.0 s
11	41.3 t	40.2 t	37.8 t
12	114.9 d	116.4 d	50.5 d
13	142.8 s	141.4 s	71.2 s
13-CH_3	16.5 q	16.0 q	24.8 q
14	39.8 t	35.8 t	41.5 t
15	26.0 t	31.9 t	30.9 t
16	123.7 d	75.3 d	71.5 d
17	131.8 s	146.6 s	41.2 s
17-CH_3 or CH_2	17.5 q	111.7 t	$_{ax}$ 16.3 q
17-CH_3	25.6 q	17.7 q	$_{eq}$ 28.7 q

The chemical shifts in parentheses may be interchangeable.

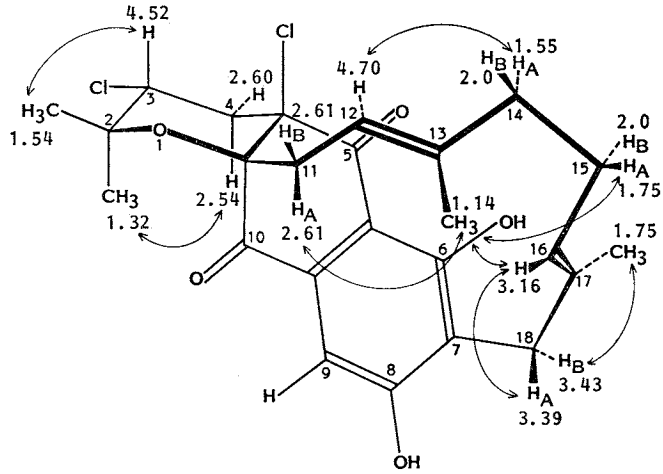
be due to the intramolecular hydrogen bond between A and D rings, O(1)···HO(26) of length 2.605(6) Å and 2.658(6) Å for molecules I and II, respectively. Agreement of the above-mentioned parameters between molecules I and II indicates the similarity of the overall conformation of the two molecules. Another intramolecular hydrogen bond is found between O(22) and HO(23), and has a length of 2.554(9) Å and 2.537(8) Å in molecules I and II, respectively.

Intermolecular hydrogen bonds are formed between the molecules of I and also between those of II. The length of the former bond is 2.658(8) Å [O(24)-H···O(26) at $x, y, -1+z$], and 2.642(7) Å [O(24)-H···O(26) of molecule II at $x, y, 1+z$] for the latter.

^1H and ^{13}C NMR spectra of NPD-B4 are shown in Tables 2 and 3. The assignment was established by the aid of ^1H-^1H shift correlation spectrum (COSY), ^1H-^{13}C COSY, nuclear Overhauser effect (NOE) experiments and the comparison with the spectra of NPD-B1[2,3]. The NOE experiments showed NOE between 9-H and equatorial 17-CH_3 protons. Therefore equatorial 17-CH_3 should be near ring C in $CDCl_3$ - MeOH-d_4 solution, thus explaining the high-field shift of the methyl protons (0.38 ppm).

In this study, the configuration of C(4a) in NPD-B4 is determined to be *R*. The configuration of 3*R* and 10a*R* is the same as in NPD-B2. There have been no studies on the stereochemistry of the chromophore of other NPD's. But the chemical shifts and the coupling constants of the chromophores obtained from NMR studies resemble each other. Consequently, the configurations of C(3), C(4a) and C(10a) in NPD's are all assumed to be *R*.

Fig. 3. NOE experiments of NPD-C1.
The values beside protons are chemical shifts (ppm) of ^1H NMR. Arrows show NOE.



Structure of NPD-A2

The UV spectrum of NPD-A2 was almost the same as those of NPD-A1. ^1H and ^{13}C NMR spectra of NPD-A2 are shown in Tables 2 and 3. The ^1H and ^{13}C spectra of the chromophore were almost coincident with those of NPD-A1[2,3]. As to the side chain, C(11), C(12), C(13), 13-CH_3, C(14) and C(15) were considered to correspond to those of NPD-A1, though the chemical shifts differed to some extent. NPD-A1 has an olefin methin C(16) (123.7 ppm), but the corresponding signal was not observed in NPD-A2. The study on ^1H-^1H COSY in NPD-A2 indicated that methylene proton 15-H (1.34 ppm) was coupled with 14-H (1.80 ppm) and 16-H (4.01 ppm). Therefore C(16) should be a methylene or methine carbon, and the signal of 75.3 ppm was attributable to C(16). Quaternary olefin (146.6 ppm), methyl (17.7 ppm) and exomethylene (111.7 ppm) carbons were assigned to C(17), C(17)-CH_3 and C(17)-CH_2, respectively. Stereochemistry of NPD-A2 at the positions of 3, 4a and 10a were estimated from that of NPD-B2 and -B4. The absolute configuration of C(16) was not determined. Consequently, the structure of NPD-A2 was determined to be (3*R*,4a*R*,10a*R*)-3,4a-dichloro-10a-(6-hydroxy-3,7-dimethylocta-2,7-dien-1-yl)-3,4,4a,10a-tetrahydro-6,8-dihydroxy-2,2-dimethyl-2*H*-naphtho[2,3-*b*]pyran-5,10-dione (Fig. 1).

Geometrical Isomerism of NPD-C1

In a previous report[2], geometrical isomerism of NPD-C1 (Fig. 1) was not described. Therefore, NOE experiments were carried out to determine this isomerism. As shown in Fig. 3, NOE between the side chain protons was observed as follows: 11-H_A···13-CH_3, 12-H···14-H_A, 13-CH_3···15-H_A, 13-CH_3···16-H, 16-H···18-H_A, 17-CH_3···18-H_B. These observations indicate that geometrical isomerism at the position of both 12 and 16 was *E*.

Experimental

NMR spectra were recorded at 400 MHz for ^1H and 100 MHz for ^{13}C on a Jeol JNM-GX400. Melting points were measured by micro melting point apparatus MP-S3 (Yanagimoto Seisakusyo Co., Japan). The mass spectra were determined by a Hitachi M-80H spectrometer. The UV and IR spectra were measured with a Hitachi 22DS and 260-10 Spectrophotometer, respectively. Optical

rotations were taken by a Perkin-Elmer 241 Polarimeter equipped with a micro-cell (light path 10 cm).

Production and Isolation of NPD-A2 and -B4

C. rubra MG802-AF1 grown on a yeast - starch agar slant was inoculated into 500-ml Erlenmeyer flasks containing 110 ml of medium [composed of Bacto-Soytone (Difco) 1.0%, galactose 2.0%, corn steep liquor 0.5%, dextrin 2.0%, $(NH_4)_2SO_4$ 0.2%, $CaCO_3$ 0.2%, silicon oil (Shin-Etsu Chemical Industry, KM-70) 0.03%, pH 7.4] and shake-cultured on a rotary shaker (180 rpm, 8 cm) at 27°C for 5 days. Then 3 ml of each culture broth was inoculated into 500-ml Erlenmeyer flasks containing 110 ml of the same medium and cultured for 84 hours as described above.

The culture broth was filtered and the filtrate (4.9 liters, pH 7.5), adjusted to pH 7.0 with 1 N HCl, was extracted with an equal volume of BuOAc. The mycelium mass was extracted with 0.8 liter of MeOH, and the extract was evaporated to dryness under reduced pressure. To this residue, 1.0 liter of BuOAc and 1.0 liter of H_2O were added and shaken at pH 7.0. BuOAc extracts of the culture filtrate and mycelia were combined and concentrated under reduced pressure to give 1.07 g of brownish oil. The residual oil was dissolved in the minimal volume of toluene and poured onto a column of silica gel (Merck, 7734, 32 g). The column was washed with 200 ml of toluene and then eluted with 700 ml of toluene - EtOAc (50 : 1) to yield the known NPD's[1]. The column was successively washed with 600 ml of toluene - EtOAc (10 : 1) and finally eluted with 500 ml of $CHCl_3$ - MeOH (40 : 1). New NPD-A2 and -B4 were detected by their fluorescence under 365 nm UV light on a silica gel TLC (Merck, 5715, developed with $CHCl_3$ - MeOH, 40 : 1). The fractions containing them were concentrated and dried to yield a brownish oil (245 mg). The oil was dissolved in MeOH and divided into two portions. Then each of them was loaded onto a Sephadex LH-20 column (Pharmacia, 1.9×42 cm, 120-ml volume), and the column was developed with MeOH. NPD-A2 and -B4-containing fractions were combined and concentrated to dryness under reduced pressure to give 101 mg of brownish yellow oil. The residual oil was applied onto 7 plates (20×20 cm) of silica gel TLC (Merck, 5715) and developed with $CHCl_3$ - MeOH (20 : 1). The zones of NPD-A2 (Rf 0.31) and NPD-B4 (Rf 0.46) were scraped off and extracted with MeOH. Each MeOH extract was concentrated under reduced pressure. Then $CHCl_3$ and H_2O were added to them and the mixture shaken at pH 3.0. Each $CHCl_3$ layer was dehydrated with anhydrous Na_2SO_4 and concentrated under reduced pressure to give brownish yellow powder of NPD-A2 (23.5 mg) and yellow powder of NPD-B4 (39.8 mg). The powder of NPD-B4 was dissolved in a small amount of the mixture of $CHCl_3$ and MeOH, and kept at room temp for 1 day to give pale yellow prismatic crystals (28.9 mg).

Measurement of NMR Spectra of NPD-A2 and -B4

NPD-A2 was dissolved in $CDCl_3$ and its chemical shifts were referred to $CDCl_3$ as 7.26 ppm and 77.0 ppm for ^1H and ^{13}C NMR, respectively. NPD-B4 was dissolved in a mixture of $CDCl_3$ - MeOH-d_4 (1 : 1), and its chemical shifts were calculated from internal reference TMS.

NOE Experiments on NPD-B4 and -C1

The sample of NPD-C1 was dissolved in $CDCl_3$, and the chemical shifts were determined relative to $CDCl_3$ (7.26 ppm). For the NOESY experiments, the pulse delay was set at 2.0s, and 0.3~0.5s was used for the mixing time. Differential NOE spectra were also measured to confirm the results of NOESY.

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