Synthesis and Characterization of New Binaphthyl-Linked Phenanthroline-, Bipyridine-, or Pyridine-Derived Ligands, and the Study of Their Cytotoxic Activity

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In the present work, we describe the synthesis and characterization of five new versatile acyclic or macrocyclic ligands containing binaphthyl-linked pyridine, bipyridine, or phenanthroline groups in their framework (see *Schemes 1–4*). The structures of the ligands were elucidated on the basis of elemental analyses, IR, ^{1}H-NMR, ^{13}C-NMR, and FAB mass spectra. The cytotoxicity of these compounds was tested *in vitro* by using the tetrazolium salt reduction (MTT) assay on A549 (human lung carcinoma epithelial like) cells. All of the tested compounds induced time- and concentration-dependent cytotoxic effect.

Introduction. – The chemistry of ligands containing the (±)-1,1′-binaphthalene-2,2′-diol (BINOL) unit in their framework is a fascinating area of research for the development of chiral ligands for transition metal catalysts [1–6] and for the development of metallosupramolecular chemistry [3]. The synthesis of chiral ligands is an important topic in modern coordination chemistry, as the coordination with metal ions can produce highly stereoselective catalysts for organic transformations [2][4]. In addition, 2,2′-bipyridine (bpy) can be also viewed as another potential promising unit. Many chiral ligands based on the bpy unit with central, axial, and planar chirality have been prepared and have been used as chiral ligands in asymmetric reactions and in the field of self-assembly reactions [7]. The dramatic progress in the synthesis and investigation of these ligands and their metal complexes has received particular attention due the variety of industrial and biochemical applications [8][9].

In the present work, we describe the synthesis and characterization of five new versatile macrocyclic or acyclic binaphthylic ligands: 1,1′-bis(bis-2,6-oxymethylenyl-pyridine)binaphthyl[1]) (**L1**), 1,1′-bis(bis-6,6′-oxymethylenyl-2,2′-bipyridine)binaphthyl[1]) (**L2**), and 1,1′-bis(bis-2,9-oxymethylenyl-1,10-phenanthroline)binaphthyl[1]) (**L3**), each of them containing two 1,1′-binaphthyl units linked through two pyridine, bipyridine, and phenanthroline units, respectively, and 1,1′-bis(6-methyl-6′-oxymethyl-enyl-2,2′-bipyridine)binaphthyl[1]) (**L4**), and 1,1′-bis(2-methyl-6-oxypyridine)binaph-

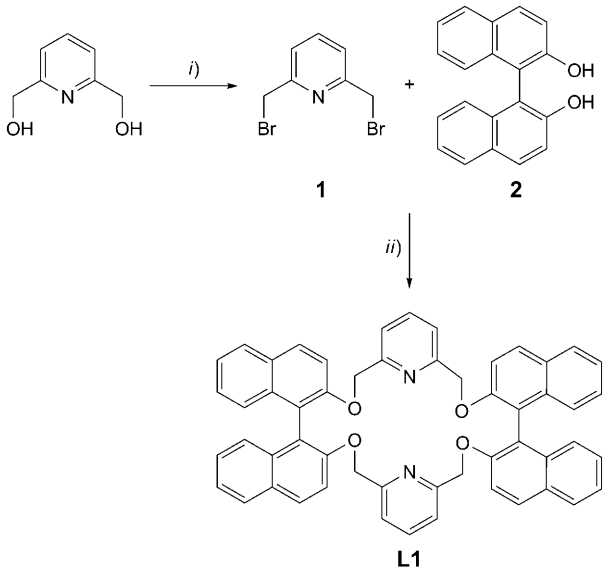
[1]) For systematic names, see *Exper. Part.*

thyl[1]) (**L5**), each of them containing two bipyridine and pyridine units, respectively, linked *via* a 1,1′-binaphthyl unit (see *Schemes 1–4* below). The structures of the compounds were elucidated on the basis of elemental analysis, IR, ^{1}H-NMR, ^{13}C-NMR, and FAB mass spectra.

Recently, we have synthesized metal complexes of a binaphthylic macrocyclic ligand and studied their genotoxic activity [10]. To the best of our knowledge, there are no other reports of the cytotoxic activity of binaphthylic ligands and their metal complexes. For this reason, the new compounds were also investigated for cytotoxic activity. The cytotoxicity was tested *in vitro* by using the tetrazolium salt reduction (MTT) assay on A549 (human lung carcinoma epithelial like) cells. All of the tested compounds showed time- and concentration-dependent cytotoxic effects.

Results and Discussion. – *Preparation of the Macrocyclic Ligand* **L1**. 2,6-Bis(bromomethyl)pyridine (**1**) and (±)-1,1′-binaphthalene-2,2′-diol (**2**, BINOL) were synthesized according to [11] and [12], respectively. The macrocyclic compound **L1** was obtained as a cream solid in a good yield using a *Williamson* ether synthesis with **1** and **2** in DMF (*Scheme 1*).

Scheme 1



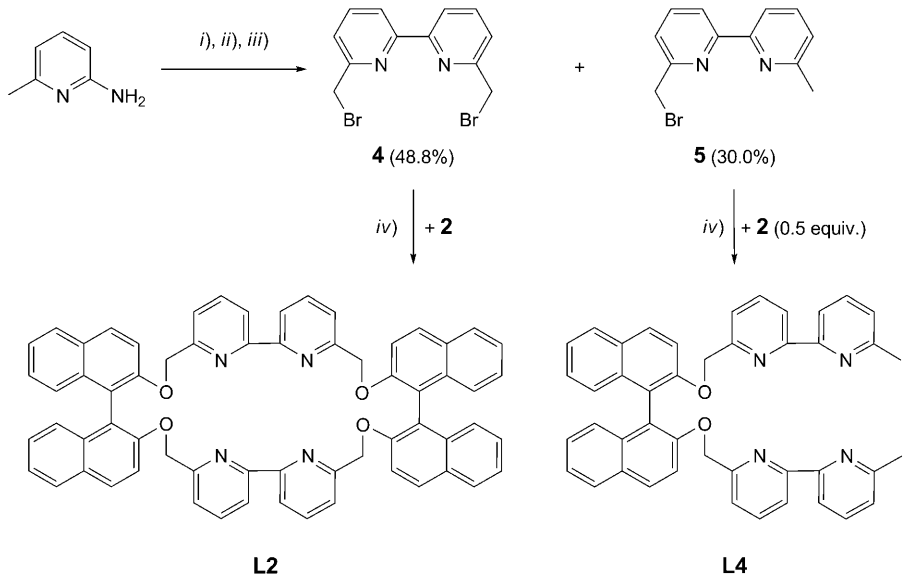
i) 47% HBr, then NaOH. *ii*) K_2CO_3, DMF.

The ^{1}H- and ^{13}C-NMR data were in agreement with the structure of ligand **L1**. The CH_2 groups appeared as an *AB* system at 5.14 and 5.51 ppm. The observation of two signals for these CH_2 groups at room temperature is in agreement with the diastereotopic nature of the geminal H-atoms of **L1**. The *AB* spin behavior of these CH_2 groups on NMR timescale evidences the non-planar arrangement of the two naphthol moieties and the chirality of the ligand [13]. Thus, macrocycle helicity is

induced by the position of the two naphthol moieties in **L1**. Elemental analysis confirmed the molecular formula of **L1**. Positive-ion FAB mass spectrometry showed a $[M+H]^+$ ion peak at 779 (40%) and a $[M+Na]^+$ ion peak at 801 (100%).

Macrocyclic Ligand **L2**. 6,6′-Dimethyl-2,2′-bipyridine was synthesized from 2-bromo-6-methylpyridine (**3**), which was prepared according to a published procedure [14], *via* an oxidative coupling reaction using a stoichiometric amount of a Ni(0) species generated *in situ*, from the reduction of $NiCl_2(PPh_3)_2$ in the presence of Zn and Et_4NI in THF. The route described is a modification of the procedure reported by *Iyoda et al.* [15]. This bipyridine compound was obtained in 87.5% yield. Bromination with *N*-bromosuccinimide in refluxing CCl_4 gave the 6,6′-bis(bromomethyl)-2,2′-bipyridine (**4**, 48.8%) and 6′-(bromomethyl)-6-methyl-2,2′-bipyridine (**5**, 30%). The macrocyclic compound **L2** was obtained as a cream solid in 55% yield using a *Williamson* ether synthesis from **2** and **4** in DMF (*Scheme 2*).

Scheme 2

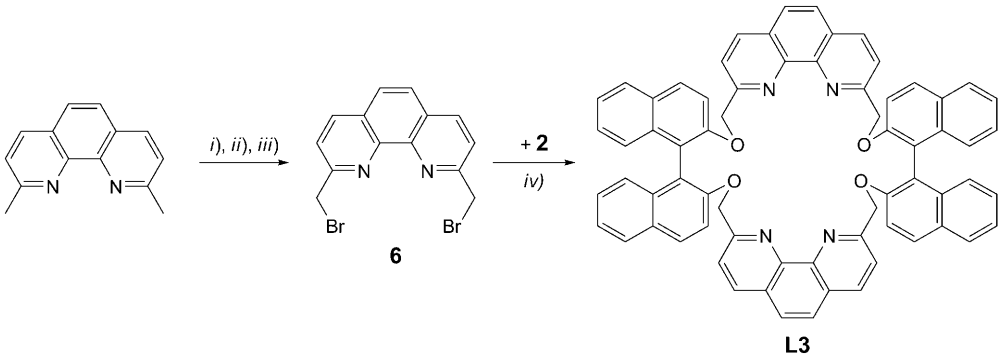


i) 47% HBr, Br_2, $NaNO_2$, then NaOH. *ii*) $NiCl_2(PPh_3)_2$, Zn, Et_4NI, THF, under Ar. *iii*) NBS, CCl_4, benzoyl peroxide. *iv*) K_2CO_3, DMF.

The ^{1}H-NMR data were in agreement with the structure of **L2**. The CH_2 groups in **L2** appeared as a broad *singlet*, and this may be attributed to rapid flipping of the helical macrocyclic ring on the NMR time scale [16]. The ^{13}C-NMR spectrum of **L2** showed 16 peaks, which was consistent with the given formula. The ^{13}C-DEPT experiment confirms the expected number of secondary, tertiary, and quaternary C-atoms. The elemental analysis was in accordance with the composition of **L2** · H_2O. Positive-ion FAB mass spectrometry gives a $[M+H]^+$ ion peak at 933 (70%) and a $[M+Na]^+$ ion peak at 955 (70%).

Macrocyclic Ligand **L3**. For the preparation of the phenanthroline unit, the starting 2,9-dimethyl-1,10-phenanthroline was oxidized with SeO_2 to the dialdehyde, which was reduced with $NaBH_4$ to give the dihydroxy compound. Then, treatment with HBr gave the desired 2,9-bis(bromomethyl)-1,10-phenanthroline (**6**). The macrocyclic compound **L3** was obtained as a cream solid in 85% yield by using a *Williamson* ether synthesis from **2** and **6** in DMF (*Scheme 3*).

Scheme 3

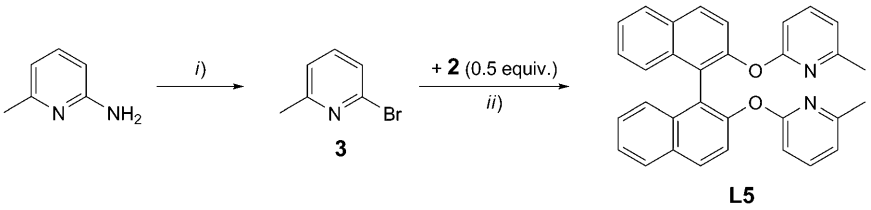


i) SeO_2, 1,4-dioxane. *ii*) $NaBH_4$. *iii*) 47% HBr. *iv*) K_2CO_3, DMF.

The ^{1}H- and ^{13}C-NMR spectra for **L3** were consistent with the proposed formula. As in the case of **L2**, the CH_2 groups were observed as a broad *singlet* at 5.03 ppm. The FAB mass spectrum of **L3** showed a ion peak at 981 ($[M+H]^+$).

Acyclic Ligands **L4** *and* **L5**. The acyclic compounds **L4** and **L5** were obtained as cream solids in good yields using a *Williamson* ether synthesis from one mol of **2** and two moles of **5** and 6-bromo-2-methylpyridine, respectively, in DMF (*Schemes 2* and *4*).

Scheme 4



i) 47% HBr, Br_2, $NaNO_2$, then NaOH. *ii*) K_2CO_3, DMF.

The ^{1}H- and ^{13}C-NMR spectra for **L4** and **L5** were consistent with the proposed structures. The Me groups were assigned to the most upfield signal in the ^{1}H-NMR spectra (δ(H) 2.63 and 2.49, resp.). The CH_2 groups for **L4** were observed as a broad *singlet* at 5.27 ppm as in **L2** and **L3**. Positive-ion FAB mass spectras of **L4** and **L5** gave ion peaks for $[M+H]^+$ at 651 and 469, respectively.

Activity. We have investigated the cytotoxic effects of the samples using the tetrazolium salt reduction (MTT; 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay on A549 cells. The MTT assay is a quick effective method for testing mitochondrial impairment and correlates well with cell proliferation. In recent years it has been frequently used as a preliminary screen for the evaluation of *in vitro* cytotoxicity of ligands. Dose-dependent cytotoxicity curves, as quantified by the MTT assay for one, two, three, and four day exposures of samples on A549 cells, are shown in the *Figure.* Our results showed that samples had a dose- and time-dependent cytotoxic activity on A549 cells.

Evaluation of the Five Samples. Ten- and twenty- μM doses of **L1**, **L2**, and **L5** caused decreases in the number of A549 cells at the first day. At the second day, same doses of three ligands caused an increase of the cell number. At the third day, 10- and 20-μM doses of **L5** and all doses of **L1** caused a decrease of the cell viability.

Cell viability was nearly the same for the 10-μM dose of **L6** and the control group of A549 cells at the first day. But cell viability was decreasing significantly at 20- and 40-μM doses of **L3** at the first day.

The 40-μM dose of **L3** was the most cytotoxic dose at the first day. Decreasing of cell viability continued during four days for all doses of **L4**. In the search for anticancer drugs, the most common screening methods employ cytotoxicity tests against panel of cancer cell lines [17]. The aim of the study was to evaluate of the cytotoxicity of five ligands. All of test compounds induced time- and concentration-dependent cytotoxic effects. The MTT assay employed in this study is as an indicator of mitochondrial function, although it is often used as an indirect indicator of cell proliferation [18].

Experimental Part

Materials and Chemicals. 2,6-Bis(bromomethyl)pyridine (**1**) [11], 2-bromo-6-methylpyridine (**3**) [14], 6,6′-dimethyl-2,2′-bipyridine [15], 6,6′-bis(bromomethyl)-2,2′-bipypridine (**4**) [15], 6′-(bromomethyl)-6-methyl-2,2′-bipyridine (**5**) [15], 2,9-bis(bromomethyl)-1,10-phenanthroline (**6**) [19], and (±)-1,1′-binaphthalene-2,2′-diol (**2**) [12] were synthesized according to published procedures. THF was distilled from Na in the presence of benzophenone immediately prior to use. Zn powder was washed successively with diluted HCl, H_2O, EtOH, acetone, and Et_2O, and dried under reduced pressure before use. Et_4NI was dried at 100° under reduced pressure. All other reagents were used as purchased from commercial suppliers without further purification. M.p.: *Gallenkamp MPD350.BM2.5* digital melting point apparatus; uncorrected. TLC on SiO_2 *60* F_{254} (*Merck*). IR: *Shimadzu 470* IR spectrophotometer. ^{1}H- and ^{13}C-NMR Spectra: *Varian Mercury Plus* instrument (300 and 75.5 MHz, resp.) in $CDCl_3$. FAB-MS (pos.): *Finnigan Mat 95* mass spectrometer with 3-(nitrophenyl)methanol as matrix. Elemental analyses: *CHNS-O Carlo Erba EA 1108* elemental analyzer.

Synthesis of the Macrocyclic Ligands **L1**–**L3**. A soln. of (±)-1,1′-binaphthalene-2,2′-diol (**2**, 0.29 g, 1 mmol) in hot anh. DMF (5 ml) was treated with K_2CO_3 (0.14 g, 1 mmol) and gently boiled. To this soln., 2,6-bis(bromomethyl)pyridine (**1**, 0.27 g, 1 mmol) for **L1** or 6,6′-bis(bromomethyl)-2,2′-bipyridine (**3**, 0.34 g, 1 mmol) for **L2**, or 2,9-bis(bromomethyl)-1,10-phenanthroline (**6**, 0.39 g, 1 mmol) for **L3** in 3 ml anh. DMF was added during 30 min. Gently reflux was maintained for 2 h and a part of the solvent (2 ml) was distilled from the mixture, which was then poured into H_2O (40 ml). Creamy solids were filtered off and washed with dilute aq. NaOH soln. and H_2O, and then dried.

1,1′-Bis(bis-2,6-oxymethylenylpyridine)binaphthyl (=*8*H,*29*H-*9,13*:*30,34-Di(azeno)tetranaphtho[2,1*-b:*1′,2′*-d:*2″,1‴*-o:*1⁗,2⁗′*-q*][1,6,14,19]tetraoxacyclohexacosine*; **L1**). Yield: 0.39 g (50%). M.p. 198–205°. IR: 3440*w*, 3056*w*, 1619*s*, 1593*s*, 1504*s*, 1459*m*, 1433*m*, 1379*w*, 1353*m*, 1331*s*, 1273*s*, 1244*w*, 1212*s*, 1148*s*, 1088*s*, 1046*w*, 1017*m*, 860*w*, 809*s*, 774*w*, 748*s*, 624*w*. ^{1}H-NMR: 5.14 (*AB*, *J* = 14.9, 4 H of

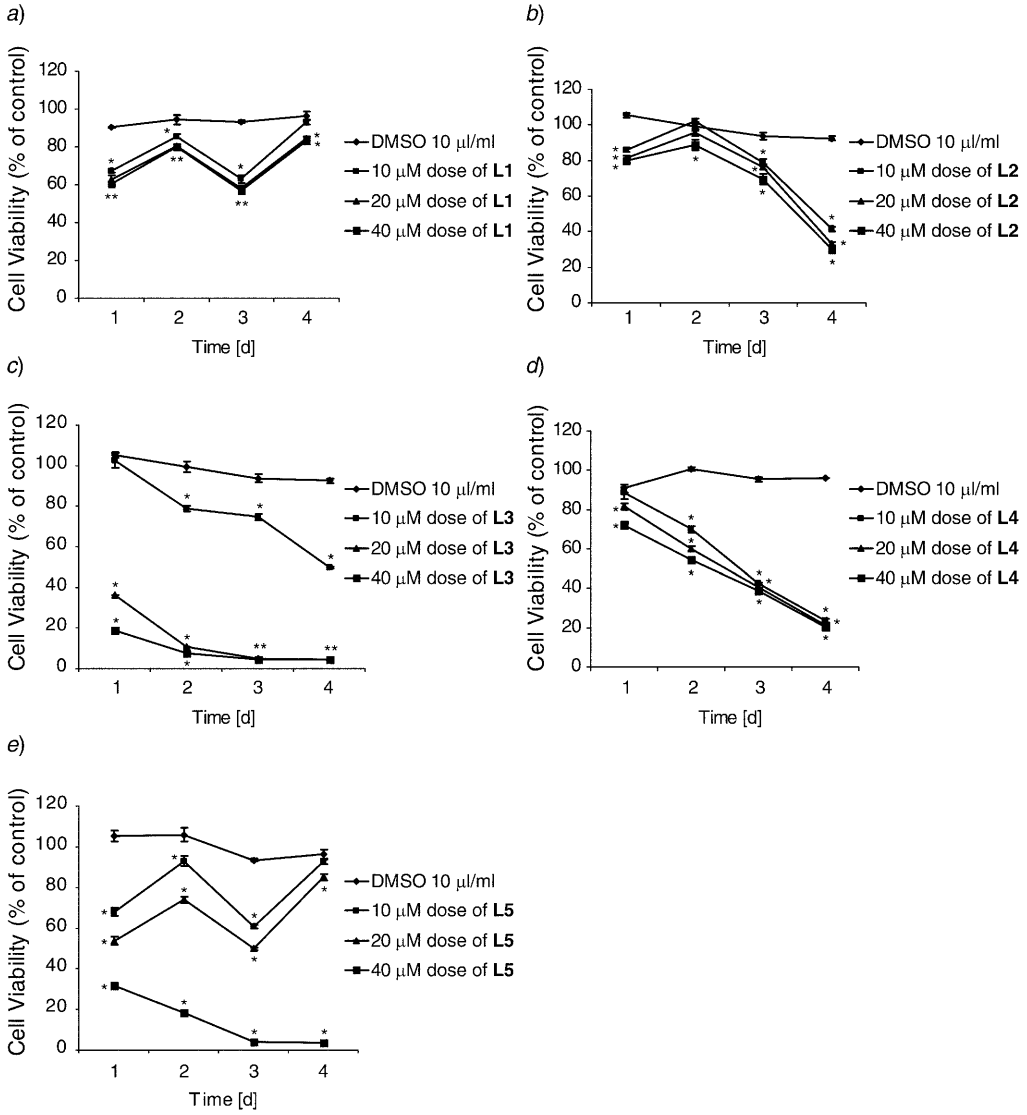


Figure. *Comparison of* a) **L1**, b) **L2**, c) **L3**, d) **L4**, *and* e) **L5** *cytotoxicity on A549 cell lines as determined in the MTT assay.* Dose-dependent cytotoxicity curves for MTT assays performed after one, two, three, and four day exposures. The results are expressed as the mean ± S.D. * Indicates significant difference from the control group by the *Tukey* test ($p < 0.05$).

4 CH_2 groups); 5.51 (*AB*, $J = 14.9$, 4 H of 4 CH_2 groups); 6.79 (*d*, $J = 7.5$, 4 H); 7.26–7.39 (*m*, 14 H); 7.49 (*d*, $J = 9.2$, 4 H); 7.80 (*t*, $J = 7.0$, 4 H); 7.87 (*d*, $J = 8.8$, 4 H). ^{13}C-NMR: 72.98 (CH_2); 118.35; 118.96; 124.39; 126.09; 126.32 (C); 126.46; 128.01; 129.43; 130.08 (C); 134.65 (C); 136.31; 155.19 (C); 156.64 (C). FAB-MS: 779 (40, $[M + H]^+$), 801 (100, $[M + Na]^+$). Anal. calc. for $C_{54}H_{38}N_2O_4$ (778.91): C 83.27, H 4.92, N 3.60; found: C 83.38, H 4.81, N 3.85.

1,1′-Bis(bis-(6,6′-oxymethylenyl-2,2′-bipyridine)binaphthyl (=*8,29,42,63-Tetraoxa-69,70,71,72-tetraazatridecacyclo[63.3.1.1*^{2,6}*.1*^{31,35}*.1*^{36,40}*.0*^{9,18}*.0*^{12,17}*.0*^{19,28}*.0*^{20,25}*.0*^{43,52}*.0*^{46,51}*.0*^{53,62}*.0*^{54,59}*]doheptaconta-1(69),2(72),3,5,9(18),10,12,14,16,19,21,23,25,27,31(71),32,34,36(70),37,39,43(52),44,46,48,50,53,55,57,59,61,65,67-dotriacontaene*; **L2**). Yield: 0.79 g (85%). M.p. 210–215°. IR: 3440*w*, 3072*m*, 1619*s*, 1571*s*, 1504*s*, 1436*s*, 1401*w*, 1350*w*, 1328*s*, 1270*s*, 1244*w*, 1212*s*, 1148*s*, 1017*s*, 908*w*, 860*w*, 803*s*, 787*s*, 745*s*, 633*m*. ^{1}H-NMR: 5.17 (*s*, 4 CH_2); 6.65–6.83 (*m*, 4 H); 7.23–7.65 (*m*, 18 H); 7.85–8.06 (*m*, 14 H). ^{13}C-NMR: 71.93 (CH_2); 115.37; 119.66; 120.38 (C); 120.89; 123.99; 125.64; 126.68; 128.14; 129.66; 134.34 (C); 137.36; 153.98 (C); 155.15 (C); 156.20 (C); 157.30 (C). FAB-MS: 933 (70, $[M+H]^+$), 955 (70, $[M+Na]^+$). Anal. calc. for $C_{64}H_{44}N_4O_4 \cdot H_2O$ (951.09): C 80.82, H 4.87, N 5.89; found: C 80.45, H 4.86, N 5.90.

1,1′-Bis(bis-(2,9-oxymethylenyl-1,10-phenanthroline)binaphthyl (=*3,24,37,58-Tetraoxa-69,72,73,76-tetraazapentadecacyclo[58.8.4.4*^{26,35}*.0*^{4,13}*.0*^{7,12}*.0*^{14,23}*.0*^{15,20}*.0*^{29,75}*.0*^{32,74}*.0*^{38,47}*.0*^{41,46}*.0*^{48,57}*.0*^{49,54}*.0*^{63,71}*.0*^{66,70}*]hexaheptaconta-1(69),4(13),5,7(12),8,10,14(23),15(20),16,18,21,26(76),27,29(75),30,32(74),33,35(73),38,40,42,44,46,48,50,52,54,56,60(72),61,63(71),64,66(70),67-tetratriacontaene*; **L3**). Yield: 0.75 g (76%). IR: 3408*m*, 3072*w*, 1619*s*, 1596*s*, 1555*w*, 1491*s*, 1388*w*, 1347*w*, 1328*s*, 1280*s*, 1238*w*, 1113*m*, 1084*m*, 947*w*, 857*w*, 838*w*, 784*s*, 726*s*, 636*m*, 604*w*. ^{1}H-NMR: 5.03 (*s*, 4 CH_2); 7.14 (*d*, J = 7.0, 4 H); 7.29–7.39 (*m*, 20 H); 7.88 (*d*, J = 7.0, 4 H); 7.96–7.99 (*m*, 8 H). ^{13}C-NMR: 71.82; 111.12; 117.99; 124.22; 124.43; 126.92; 127.67; 128.61; 129.68; 131.61; 133.64; 136.05; 140.52; 146.02; 150.90; 152.99; 162.73. FAB-MS: 981 (100, $[M+H]^+$). Anal. calc. for $C_{68}H_{44}N_4O_4$ (981.12): C 83.25, H 4.52, N 5.71; found: C 83.42, H 4.76, N 5.98.

Syntheses of Acyclic Ligands **L4** *and* **L5**. To a soln. of **2** (0.29 g, 1 mmol) in hot anh. DMF (5 ml) was added K_2CO_3 (0.14 g, 1 mmol). The soln. was gently boiled, and 6-methyl-6′-bromomethyl-2,2′-bipyridine (**5**, 0.53 g, 2 mmol) for **L4** or 2-bromo-6-methylpyridine (0.35 g, 2 mmol) for **L5** in 3 ml anh. DMF was added during 30 min. Gentle reflux was maintained for 2 h, and 2 ml of the solvent were distilled off from the mixture, which was then poured into H_2O (40 ml). Creamy solids were filtered off and washed with dilute aq. NaOH soln. and H_2O, then dried.

1,1′-Bis(6-methyl-6′-oxymethylenyl-2,2′-bipyridine)binaphthyl (=*6,6′-[1,1′-Binaphthalene-2,2′-diylbis(oxymethanediyl)]bis(6′-methyl-2,2′-bipyridine)*; **L4**). Yield: 0.52 g (80%). M.p. 90–95°. IR: 1619*w*, 1571*s*, 1504*m*, 1440*s*, 1392*w*, 1347*w*, 1328*m*, 1270*m*, 1241*m*, 1212*m*, 1148*s*, 1084*s*, 806*s*, 784*s*, 748*s*, 633*m*. ^{1}H-NMR: 2.63 (*s*, 2 Me); 5.27 (*s*, 2 CH_2); 6.74 (*d*, J = 7.3, 2 H); 7.14 (*d*, J = 7.3, 2 H); 7.34–7.39 (*m*, 8 H), 7.48 (*d*, J = 9.0, 2 H); 7.66 (*t*, J = 7.6, 2 H); 7.88 (*d*, J = 7.6, 2 H); 7.96 (*d*, J = 9.0, 2 H); 8.08 (*d*, J = 7.9, 2 H); 8.16 (*d*, J = 7.9, 2 H). ^{13}C-NMR: 24.67 (Me); 72.05 (CH_2); 115.48; 115.61; 118.12; 118.53; 119.94; 120.92; 123.48; 124.01; 125.36; 125.70 (C); 126.68 (C); 128.17; 128.31 (C); 129.694; 134.42 (C); 137.50; 154.09 (C); 155.58 (C); 157.42 (C); 157.99 (C). FAB-MS: 651 (70, $[M+H]^+$). Anal. calc. for $C_{44}H_{34}N_4O_2$ (650.78): C 81.21, H 5.27, N 8.61; found: C 80.84, H 5.03, N 8.64.

1,1′-Bis(2-methyl-6-oxypyridine)binaphthyl (=*2,2′-[1,1′-Binaphthalene-2,2′-diylbis(oxy)]bis(6-methylpyridine)*; **L5**). Yield: 0.23 g (50%). M.p. 215–223°. IR: 3328*m*, 3072*w*, 1616*s*, 1587*s*, 1555*w*, 1500*s*, 1440*s*, 1398*w*, 1334*s*, 1273*s*, 1235*m*, 1209*w*, 1171*m*, 1145*w*, 1129*w*, 1004*m*, 976*s*, 966*s*, 931*m*, 809*s*, 787*m*, 752*s*, 675*m*, 627*m*. ^{1}H-NMR: 2.49 (*s*, 2 Me); 7.10–7.13 (*m*, 4 H); 7.26–7.36 (*m*, 8 H); 7.84–7.95 (*m*, 6 H). ^{13}C-NMR: 24.32 (Me); 111.12 (C); 117.98; 122.28; 124.22; 124.42; 125.21; 127.66 (C); 128.60; 129.67; 131.59; 133.64 (C); 138.74; 141.53 (C); 152.97 (C); 160.22 (C). FAB-MS: 469 (100, $[M+H]^+$). Anal. calc. for $C_{32}H_{24}N_2O_2$ (468.55): C 82.03, H 5.16, N 5.98; found: C 81.91, H 4.97, N 5.78.

Activity. Cell Cultures. A549 (Human Lung Carcinoma Epithelial like) cell lines were obtained from the Institute for Fermentation, Osaka (IFO, Japan). A549 Cells were maintained as a monolayer in *Nutrient Mixture F-12 HAM* Medium (*Sigma*) containing 10% (*v*/*v*) heat-inactivated fetal bovine serum (*Sigma*), penicillin-streptomycin (*Sigma*), and $NaHCO_3$. The A549 cells were incubated at 37° in a humidified atmosphere of 5% (*v*/*v*) CO_2 in air. Cells were plated at 5×10^3 cells/ml into 96-well microtiter tissue culture plates (*Techno Plastic Products AG*) and then incubated for 24 h before addition of the test samples. Stock solns. of these samples were initially prepared in DMSO (*Sigma*), stored at 4° and further diluted in fresh complete medium.

In vitro *Cytotoxicity Assay.* The growth inhibitory effects of the compounds were measured using the MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) assay [20]. Cells were seeded in 96-multiwell plates at a density of 5×10^3 cells/well. After a 24-h preincubation period, the medium was changed and the cells were treated with different concentrations of the freshly prepared test compounds in complete medium. Negative control groups were untreated cells and positive control groups were the

ones treated with DMSO solely. In the positive control groups, DMSO was added to obtain the final concentration of 1% [21][22]. Just before the experiments, stock solns. were diluted with the supplemented medium to obtain final concentrations of 10, 20, and 40 μM. After 1, 2, 3, and 4 d exposure of either of the samples containing medium from each well, the medium was then replaced with 200 μl fresh medium containing 0.5 mg/ml MTT (*Sigma*) dissolved in phosphate buffer saline (PBS). The plates with added MTT soln. were then wrapped in aluminium foil and replaced in the 5% CO_2 incubator for 2 h. At the end of this period, the medium was removed and the formazan crystals formed by MTT metabolism were dissolved by addition of 200 μl DMSO to each well. Then, the plates were gently mixed on a plate shaker for *ca*. 5 min, and their absorbances were read at 570 nm with a microtiter plate reader (*Bio-Tek*, *ELX808IU*, USA). All experiments were repeated at least three times.

Statistical Data Analysis. The SPSS software has been used for the statistical analyses of assessment of the MTT assay. Data were evaluated using one-way *ANOVA* followed by the *Tukey* test. A value of $p < 0.05$ was considered significant.

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