Supporting information

Synthesis and Anticancer Activity Novel Dimeric Azatriperoxides

Nataliya N. Makhmudiyarova*, Irina R. Ishmukhametova, Lilya U. Dzhemileva, Tatyana V. Tyumkina, Vladimir A. D'yakonov, Askhat G. Ibragimov, and Usein M. Dzhemilev

Institute of Petrochemistry and Catalysis, Russian Academy of Sciences, 141 Prospekt Oktyabrya, 450075 Ufa, Russian Federation

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A. General Information

All reactions were performed at room temperature in air in round-bottom flasks equipped with a magnetic stir bar. The NMR spectra, including two-dimensional homo- (COSY, NOESY) and and heteronuclear (HSQC, HMBC) spectra, were recorded on a Bruker Avance 500 spectrometer at 500.17 MHz for 1H and 125.78 MHz for 13C according to standard Bruker procedures. CDCl3 was used as the solvent, and tetramethylsilane, as the internal standard. The mixing time for the NOESY experiments was 0.3 sec. Mass spectra were recorded on a Bruker Autoflex III MALDI TOF/TOF instrument with α-cyano-4-hydroxycinnamic acid as a matrix. Samples were prepared by the dried droplet

method. The C, H, and N were quantified by a Carlo Erba 1108 analyzer. The oxygen content was determined on a Carlo Erba 1108 analyzer. The progress of reactions was monitored by TLC on Sorbfil (PTSKh-AF-A) plates, with a 5:1 hexane : EtOAc mixture as the eluent and visualization with I_2 vapor. For column chromatography, silica gel MACHEREY-NAGEL (0.063-0.2 mm) was used.

All calculations were carried out using a program Gaussian 09. Geometric parameter optimization, vibrational frequency analysis, and calculation of entropy and thermodynamic corrections to the total energy of the compounds were carried out on the B3LYP functional[1] using the 6-31G(d,p) basis set. No limitation was imposed on the changes in the geometric parameters of the subsystems studied. Thermodynamic parameters were determined at 298 K. The minima were confirmed through the calculation of the force constant (Hessian) matrix and the analysis of the resulting frequencies. All minima were verified to have no negative frequencies. Visualization of quantum chemical data was carried out with the programs ChemCraft.[2]

The synthesis of the heptaoxadispiroalkanes **1-3** was as reported in the literature.[3] THF was freshly distilled over $LiAlH_4$. $Sm(NO_3)_3/\gamma\text{-}Al_2O_3$ catalyst was synthesized by impregnating a porous Al_2O_3 carrier with an aqueous solution of $Sm(NO_3)_3 \times 6H_2O$ followed by a heat treatment at 400 °C for 4 hours. The content of the active component Sm^{3+} was 0.60 - 0,69 mmol/g of catalyst.

Ring transformation of heptaoxaspirocycloalkanes with alkan-α,ω-diamines catalyzed by $Sm(NO_3)_3/\gamma\text{-}Al_2O_3$. General procedure: A Schlenk vessel mounted on a magnetic stirrer was charged under argon with THF (5 mL), $Sm(NO_3)_3/\gamma\text{-}Al_2O_3$ (5 wt.%), alkan-α,ω-diamines (1 mmol), and heptaoxaspirocycloalkane (2 mmol). The reaction mixture was stirred at ~20 °C for 6 h and THF was evaporated. Then Et_2O (10 mL) was added and the mixture was washed with water (4×5 mL). The organic layer was separated and purified by column chromatography on SiO_2 using 10 : 1 PE : Et_2O as the eluent to isolate pure heterocyclic product (compounds **10-23**). The progress of reactions was monitored by TLC, with a 5 : 1 hexane : EtOAc mixture as the eluent (compounds **10-23**), visualization was performed with I_2 vapor.

1,4-Bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^8.7^5]nonadecan-16-yl)butane 10. Yellow oil, 0.378 g (63% yield), R_f 0.88 (PE/Et_2O = 10/1). ^{1}H NMR

(500.17 MHz, $CDCl_3$): δ = 1.69-1.75 (m, 24H), 1.84 and 2.09 (br.s, 4H), 2.15-2.25 (m, 8H), 2.95-3.01 and 3.51-3.53 (m, 4H), 4.82-5.37 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 22.8, 24.6, 24.3, 33.5, 32.9, 33.2, 33.7, 49.0, 84.6, 95.4, 119.3, 118.7. MALDI TOF/TOF, m/z: 603 $[M-H]^+$. Anal. calcd. For $C_{28}H_{48}N_2O_{12}$: C, 55.62; H, 8.00; N, 4.63%. Found: C, 55.57; H, 7.95; N, 4.57%.

1,5-Bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^8.7^5]nonadecan-16-yl)pentane 11. Yellow oil, 0.489 g (79% yield), R_f 0.88 (PE/Et_2O = 10/1). ^1H NMR (500.17 MHz, $CDCl_3$): δ = 1.25-1.33 (m, 4H) and 1.67-1.77 (m, 14H), 1.47-1.53 (m, 4H), 1.62-1.65 and 2.14-2.17 (m, 16H), 2.96-3.02 and 3.19-3.24 (m, 4H), 4.80-4.89 and 4.93-5.22 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 23.9, 24.4, 28.2, 32.9, 33.1, 51.5, 88.2, 90.5, 113.7. MALDI TOF/TOF, m/z: 617 $[M-H]^+$. Anal. calcd. For $C_{29}H_{50}N_2O_{12}$: C, 56.30; H, 8.15; N, 4.53%. Found: C, 56.25; H, 8.13; N, 4.49%.

1,8-Bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^8.7^5]nonadecan-16-yl)octane 12. Colorless oil, 0.455 g (69% yield), R_f 0.86 (PE/Et_2O = 10/1). ^1H NMR (500.17 MHz, $CDCl_3$): δ = 1.65-1.77 (m, 16H), 1.65-1.70 and 2.16-2.19 and 2.21-2.24 (m, 16H), 1.28-1.36 (m, 8H), 1.47-1.50 (m, 4H), 2.95-3.03 and 3.19-3.25 (m, 4H), 4.82-4.91 and 4.93-5.19 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 24.6, 24.4, 32.5, 32.9, 26.9, 28.3, 29.7, 51.6, 88.3, 119.3, 119.7. MALDI TOF/TOF, m/z: 659 $[M-H]^+$. Anal. calcd. For $C_{32}H_{56}N_2O_{12}$: C, 58.16; H, 8.54; N, 4.24%. Found: C, 58.14; H, 8.51; N, 4.21%.

1,10-Bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^8.7^5]nonadecan-16-yl)decane 13. Colorless oil, 0.495 g (72% yield), R_f 0.84 (PE/Et_2O = 10/1). ^1H NMR (500.17 MHz, $CDCl_3$): δ = 1.63-1.77 (m, 16H), 1.63-1.69 and 1.95-2.08 and 2.14-2.23 (m, 16H), 1.20-1.23 (m, 12H), 1.45-1.48 (m, 4H), 2.95-3.01 and 3.17-3.22 (m, 4H), 4.80-4.89 and 4.91-5.20 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 24.5, 24.6, 24.5, 24.4, 33.4, 33.3, 33.1, 32.9, 26.9, 28.3, 29.4, 29.5, 51.5, 88.2, 90.5, 119.6, 119.2, 122.4. MALDI TOF/TOF, m/z: 687 $[M-H]^+$. Anal.calcd. For $C_{34}H_{60}N_2O_{12}$: C, 59.28; H, 8.78; N, 4.07%. Found: C, 59.25; H, 8.76; N, 4.05%.

1,4-Bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^9.7^6]henicosan-18-yl)butane 14. Colorless oil, 0.529 g (74% yield), R_f 0.87 (PE/Et_2O = 10/1). ^1H NMR (500.17 MHz, $CDCl_3$): δ = 0.76-0.77

and 0.87-0.88 (m, 12H), 1.39-1.49 (m, 4H), 1.91-2.00 and 0.97-1.49 (m, 4H), 1.84-1.87 and 1.29-1.34 (m, 16H), 2.18- 2.24 (m, 16H), 2.83-2.88 and 3.05-3.08 (m, 4H), 4.60-4.96 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 20.9, 21.4, 25.6, 25.2, 31.0, 31.5, 30.9, 30.7, 34.6, 40.6, 51.1, 88.0, 108.2, 110.4. MALDI TOF/TOF, m/z: 715 $[M-H]^+$. Anal. calcd. For $C_{36}H_{64}N_2O_{12}$: C, 60.31; H, 9.00; N, 3.91%. Found: C, 60.29; H, 8.98; N, 3.89%.

1,5-Bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^9.7^6]henicosan-18-yl)pentane 15. Colorless oil, 0.452 g (62% yield), R_f 0.85 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 0.92-0.94 (m, 12H), 1.27-1.30 (m, 2H), 1.49-1.52 (m, 4H), 1.42-1.44 (m, 16H), 1.10-1.30 and 1.57-1.65 and 2.08-2.17 (m, 20H), 2.95-3.03 and 3.18-3.23 (m, 4H), 4.76-4.93 and 5.08-5.13 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 21.5, 24.3, 30.8, 31.0, 31.2, 31.6, 31.5, 51.4, 88.3, 90.4, 108.6, 110.7. MALDI TOF/TOF, m/z: 729 [M-H]+. Anal. calcd. For $C_{37}H_{66}N_2O_{12}$: C, 60.80; H, 9.10; N, 3.83%. Found: C, 60.75; H, 9.05; N, 3.80%.

1,7-Bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^9.7^6]henicosan-18-yl)heptane 16. Colorless oil, 0.435 g (58% yield), R_f 0.86 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 0.93-0.94 (m, 12H), 1.27-1.30 (m, 6H), 1.43-1.49 (m, 16H), 1.27-1.49 and 2.09-2.11 (m, 8H), 1.57-1.66 and 2.15-2.18 (m, 16H), 2.95-3.04 and 3.17-3.25 (m, 4H), 4.77-4.89 and 4.91-5.13 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 26.9, 28.3, 29.3, 30.6, 31.1, 31.6, 51.4, 88.3, 90.5, 108.5, 110.7. MALDI TOF/TOF, m/z: 757 [M-H]+. Anal. calcd. For $C_{39}H_{70}N_2O_{12}$: C, 61.72; H, 9.30; N, 3.69%. Found: C, 61.67; H, 9.25; N, 3.62%.

1,10-Bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^9.7^6]henicosan-18-yl)decane 17. Colorless oil, 0.552 g (69% yield), R_f 0.87 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 0.74-0.91 (m, 12H), 1.08-1.59 (m, 48H), 2.03-1.12 (m, 4H), 2.89-2.98 and 3.11-3.16 (m, 4H), 4.70-5.06 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 21.5, 26.9, 28.3, 29.4, 29.5, 29.2, 30.8, 31.0, 31.6, 31.5, 31.4, 51.4, 82.2, 90.3, 90.4, 108.3, 110.5, 110.9. MALDI TOF/TOF, m/z: 800 [M-H]+. Anal. calcd. For $C_{42}H_{76}N_2O_{12}$: C, 62.97; H, 9.56; N, 3.50%. Found: C, 62.95; H, 9.53; N, 3.48%.

1,2-Bis(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)ethane 18. Colorless oil, 0.488 g (71% yield), R_f 0.85 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 1.43-1.47 (m, 16H), 1.53-1.66 (m, 16H), 1.89-1.96 and 2.14-2.19 (m, 16H), 3.12-3.15 and 3.29-3.35 (m, 4H), 4.75-4.91 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 22.7, 29.9, 30.1, 32.3, 32.5, 50.0, 85.8, 88.1, 114.0, 115.2. MALDI TOF/TOF, m/z: 687 [M-H]+. Anal.calcd. For $C_{34}H_{60}N_2O_{12}$: C, 59.28; H, 8.78; N, 4.07%. Found: C, 59.26; H, 8.75; N, 4.04%.

1,4-Bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)butane 19. White solid, 0.497 g (70% yield), mp 99-100^{o}C, R_f 0.85 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 1.43-1.48 (m, 4H), 1.43-1.70 (m, 16H), 1.53-1.69 (m, 16H), 1.53-1.69 and 2.09-2.18 (m, 16H), 2.95-3.03 and 3.20-3.24 (m, 2H), 2.99-3.03 and 3.48-3.53 (m, 2H), 4.76-4.92 and 5.10-5.18 and 4.80-4.83 (m, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 25.8, 25.4, 22.8, 22.7, 22.9, 30.1, 30.0, 30.2, 32.8, 33.4, 51.4, 49.1, 87.9, 84.4, 112.7, 113.0, 113.9. MALDI TOF/TOF, m/z: 715 [M-H]$^+$. Anal. calcd. For $C_{36}H_{64}N_2O_{12}$: C, 60.31; H, 9.00; N, 3.91%. Found: C, 60.28; H, 8.95; N, 3.87%.

1,5-Bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)pentane 20. White oil, 0.584 g (80% yield), R_f 0.86 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 1.25-1.28 (m,2H), 1.43-1.50 (m, 4H), 1.43-1.71 (m, 16H), 1.51-1.62 (m, 16H), 1.61-1.64 and 2.13-2.18 (m, 16H), 2.96-3.01 and 3.17-3.22 (m, 4H), 4.76 and 4.84 (d, *J* = 12.0 Hz, 4H), 4.89 and 5.07 (d, *J* = 12.0 Hz, 4H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 22.7, 22.8, 24.3, 28.1, 28.2, 30.0, 30.1, 30.4, 32.4, 87.9, 90.4, 113.8. MALDI TOF/TOF, m/z: 729 [M-H]$^+$. Anal. calcd. For $C_{37}H_{66}N_2O_{12}$: C, 60.80; H, 9.10; N, 3.83%. Found: C, 60.75; H, 9.04; N, 3.79%.

1,7-Bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)heptane 21. Yellow oil, 0.570 g (75% yield), R_f 0.88 (PE/Et_2O = 10/1). ^{1}H NMR (500.17 MHz, $CDCl_3$): δ = 1.29 (m, 4H), 1.43-1.49 (m, 4H), 1.43-1.72 (m, 16H), 1.52-1.62 (m, 16H), 1.57-1.62 and 2.13-2.18 (m, 16H), 1.67-1.72 (m, 2H), 2.95-3.01 and 3.16-3.21 (m, 4H), 4.77 and 4.85 (d, *J* = 8.0 Hz, 4H), 4.90 and 5.07 (d, *J* = 12.0 Hz, 4H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 22.7, 22.8, 24.3, 26.9, 28.3, 30.1, 30.0, 30.4, 32.4, 32.8, 51.6, 88.0, 90.4, 113.8, 112.7. MALDI

TOF/TOF, m/z: 757 $[M-H]^+$. Anal. calcd. For $C_{39}H_{70}N_2O_{12}$: C, 61.72; H, 9.30; N, 3.69%. Found: C, 61.68; H, 9.25; N, 3.67%.

1,8-Bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^7]tricosan-20-yl)octane 22. Colorless oil, 0.462 g (60% yield), R_f 0.90 (PE/Et_2O = 10/1). ^1H NMR (500.17 MHz, $CDCl_3$): δ = 1.27 (m, 8H), 1.40-1.47 (m, 4H), 1.40-1.66 (m, 16H), 1.50-1.66 (m, 16H), 1.52-1.63 and 2.12-2.16 (m, 16H), 2.93-2.99 and 3.14-3.20 (m, 4H), 4.75 and 4.83 (d, *J* = 8.0 Hz, 4H), 4.88 and 5.05 (d, *J* = 12.0 Hz, 4H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 22.7, 26.9, 27.9, 28.3, 29.8, 29.9, 30.1, 32.4, 32.8, 51.6, 87.9, 90.4, 113.8, 112.7. MALDI TOF/TOF, m/z: 772 [M-H]+. Anal. calcd. For $C_{40}H_{72}N_2O_{12}$: C, 61.72; H, 9.30; N, 3.69%. Found: C, 61.66; H, 9.25; N, 3.63%.

1,10-Bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^7]tricosan-20-yl)decane 23. Colorless oil, 0.688 mg (86% yield), R_f 0.87 (PE/Et_2O = 10/1). ^1H NMR (500.17 MHz, $CDCl_3$): δ = 1.27 (m, 12H), 1.44-1.46 (m, 4H), 1.42-1.62 (m, 16H), 1.52-1.58 (m, 16H), 1.58-1.63 and 2.14-2.18 (m, 16H), 2.95-3.01 and 3.16-3.22 (m, 4H), 4.91 and 5.07 (d, *J* = 14.0 Hz, 8H), 4.85 and 4.78 (d, *J* = 12.0 Hz, 8H). ^{13}C NMR (125.78 MHz, $CDCl_3$): δ = 22.7, 22.8, 26.9, 28.4, 29.4, 29.5, 30.0, 30.1, 32.4, 32.8, 51.6, 49.5, 87.9, 90.4, 112.7, 113.8. MALDI TOF/TOF, m/z: 800 [M-H]+. Anal. calcd. For $C_{42}H_{76}N_2O_{12}$: C, 62.97; H, 9.56; N, 3.50%. Found: C, 62.93; H, 9.51; N, 3.46%.

BIOASSAY DATA

Cell culturing. Human cancer cell line HeLa was obtained from the HPA Culture Collections (UK). Cells (U937, K562, Jurkat and HEK293) were purchased from Russian Cell Culture Collection (Institute of Cytology of the Russian Academy of Sciences) and cultured according to standard protocols and sterile technique. The cell lines were shown to be free of viral contamination and mycoplasma. HEK293 cell line was cultured as monolayers and maintained in Dulbecco's modified Eagle's medium (DMEM, Gibco BRL) supplemented with 10% fetal bovine serum and 1% penicillin-streptomycin solution at 37 °C in a humidified incubator under a 5% CO2 atmosphere. Cells were maintained in RPMI 1640 (Jurkat, K562, U937) (Gibco) supplemented with 4 μM glutamine, 10% FBS (Sigma) and 100 units/ml penicillin-streptomycin (Sigma). All types of cells were grown in an atmosphere of 5% CO2 at 37 °C. The cells were subcultured at 2–3 days intervals. Adherent cells (HEK293) were suspended using

trypsin/EDTA and counted after they have reached 80% confluency. Cells were then seeded in 24 well plates at 5×10^4 cells per well and incubated overnight. Jurkat, K562, U937 cells were subcultured at 2-day intervals with a seeding density of 1×10^5 cells per 24 well plates in RPMI with 10% FBS.

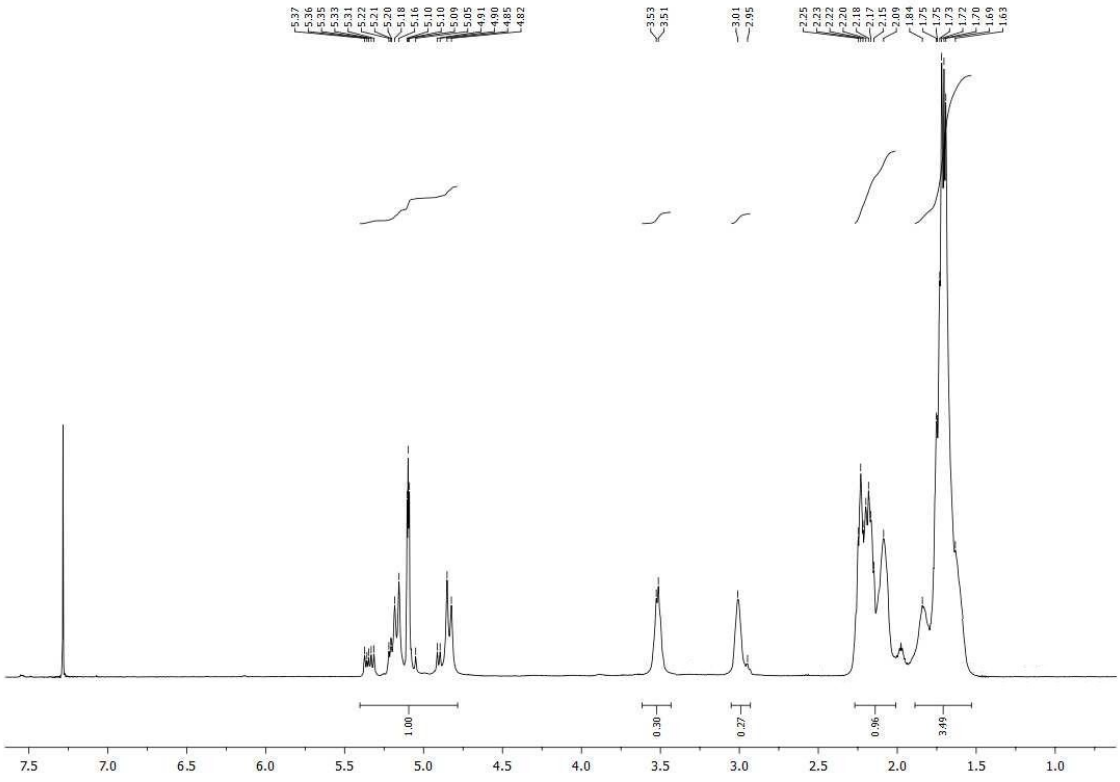
Cytotoxicity assay. Viability (Live/dead) assessment was performed by staining cells with 7-AAD (7-Aminoactinomycin D) (Biolegend). After treatment cells were harvested, washed 1-2 times with phosphate-buffered saline (PBS) and centrifuged at 400 g for 5 min. Cell pellets were resuspended in 200 µL of flow cytometry staining buffer (PBS without Ca2+ and Mg2+, 2.5% FBS) and stained with 5 µL of 7-AAD staining solution for 15 min at room temperature in the dark. Samples were acquired on NovoCyte TM 2000 Flow Cytometry System (ACEA) equipped with 488 nm argon laser. Detection of 7-AAD emission was collected through a 675/30 nm filter in the FL4 channel.

Viability and apoptosis. Apoptosis was studied using flow cytometric analysis of Annexin V and 7-aminoactinomycin D staining. After treatment cells during24 h were harvested, washed 1–2 times with phosphate-buffered saline (PBS) and centrifuged at 400 g for 5 min. Cell pellets were resuspended in 200 µL of flow cytometry staining buffer (PBS without Ca2+and Mg2+, 2.5% FBS). Then, 200 µL of Guava Nexin reagent (Millipore, Bedford, MA, USA) was added to 5×105 cells in 200 µL, and the cells were incubated with the reagent for 20 min at room temperature in the dark. At the end of incubation, the cells were analyzed on NovoCyte TM 2000 Flow Cytometry System (ACEA).

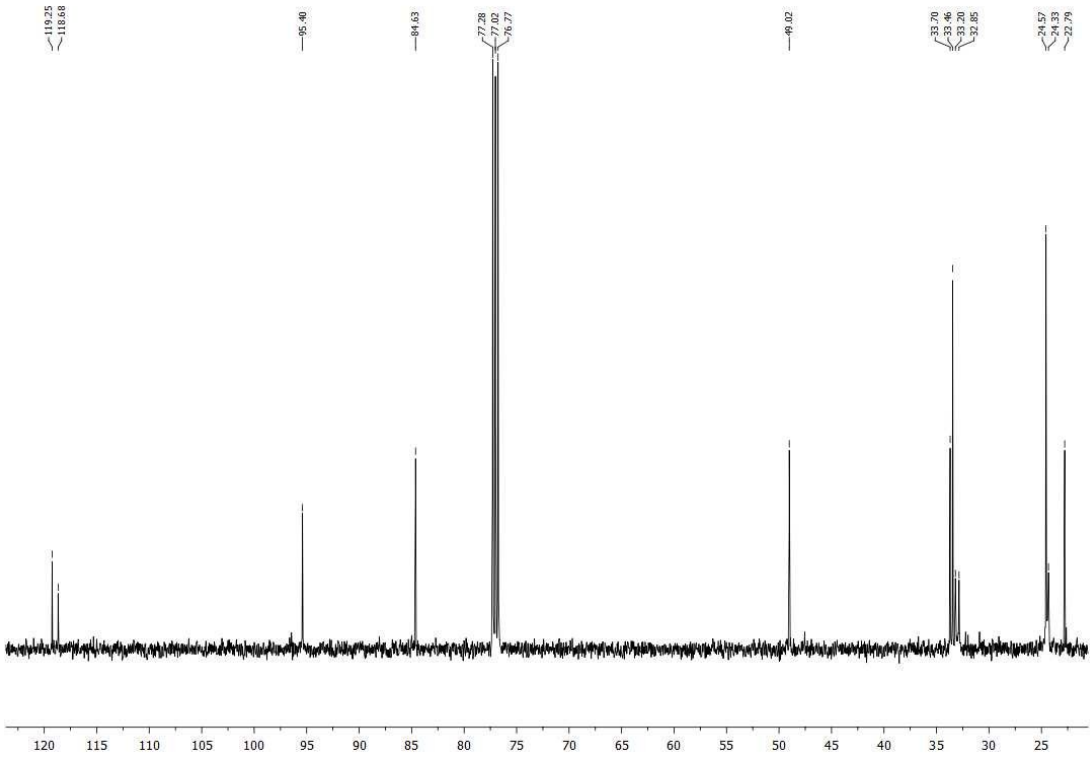
Cell cycle analysis. Cell cycle was analyzed using the method of propidium iodide staining. After treatment cells during 24 h were harvested, washed 1–2 times with phosphate-buffered saline (PBS) and centrifuged at 400 g for 5 min. Cell pellets were resuspended in 200 µL of flow cytometry staining buffer (PBS without Ca2+and Mg2+, 2.5% FBS). Then, cells were plated in 24-well round bottom plates at a density 10×105 cells per well, centrifuged at 450 g for 5 min, and fixed with ice-cold 70% ethanol for 24 h at 0°C. Cells were then washed with PBS and incubated for 30 min at room temperature with 250 µL of Guava Cell Cycle Reagent (Millipore) in the dark. Samples were analyzed on NovoCyte TM 2000 Flow Cytometry System (ACEA).

B. Copy of NMR spectra

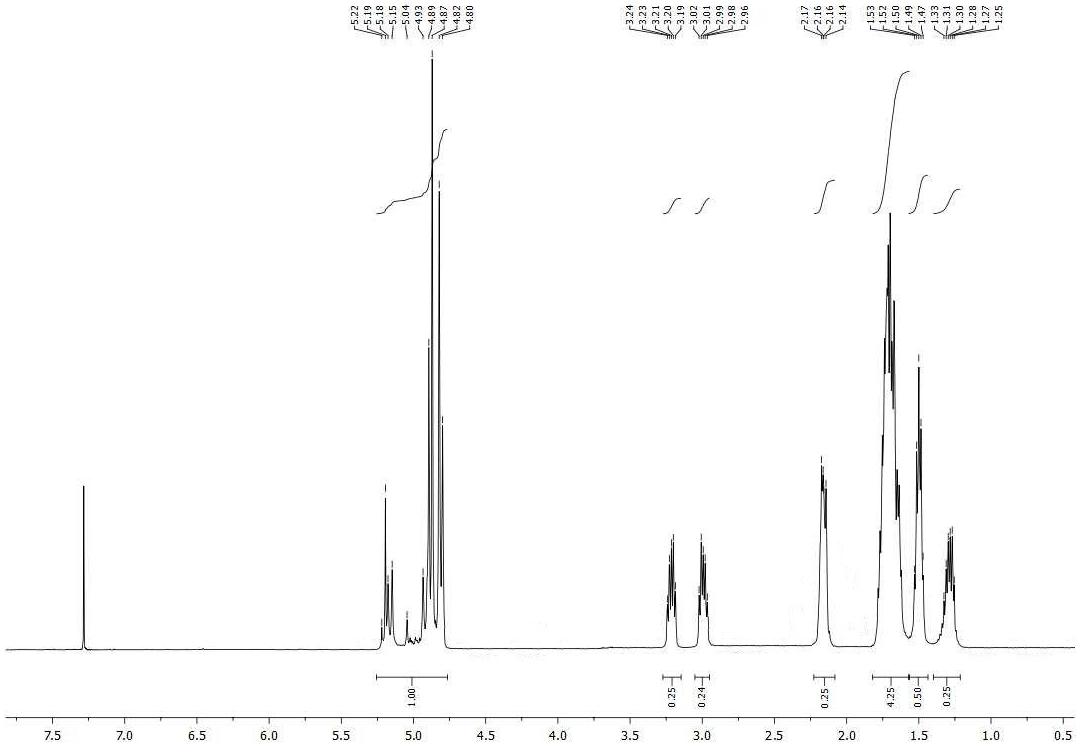
^{1}H-NMR spectrum of **1,4-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)butane (10)**



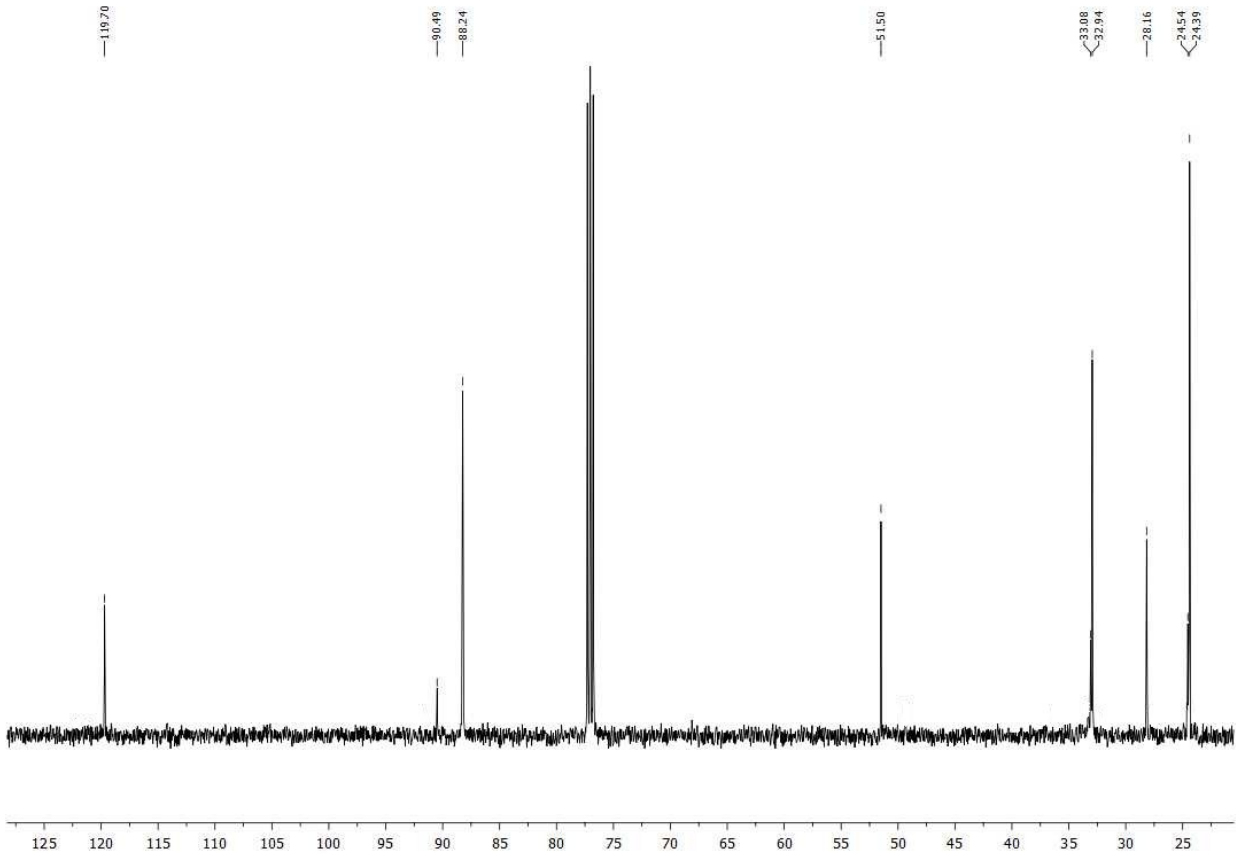
^{13}C-NMR spectrum of **1,4-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)butane (10)**



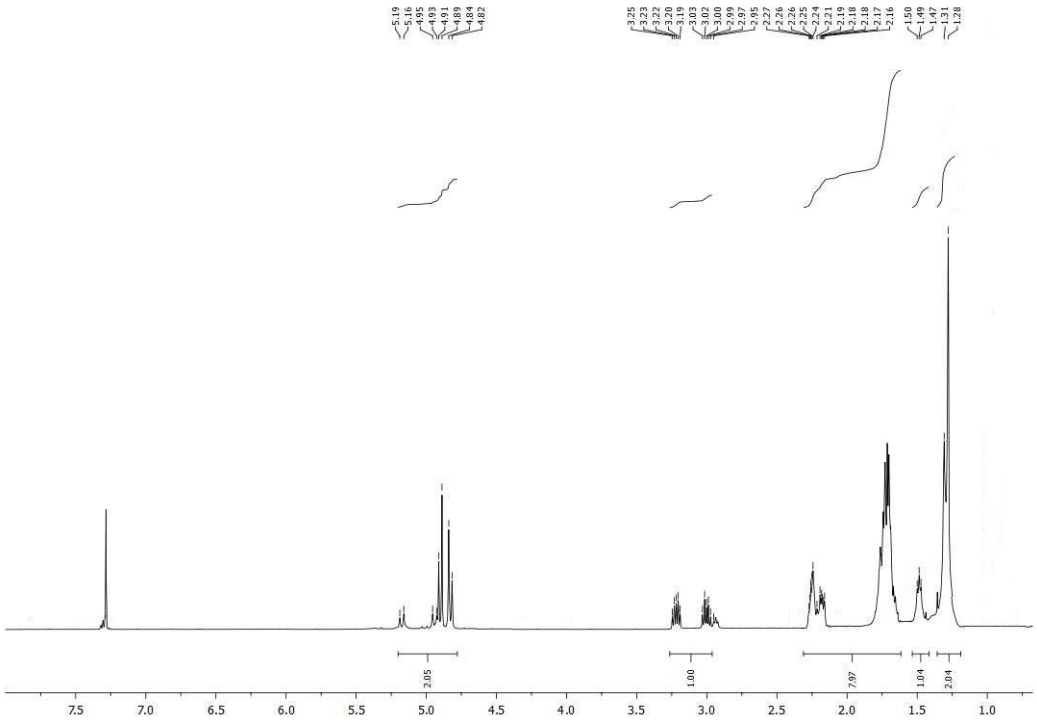
^{1}H-NMR spectrum of **1,5-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)pentane (11)**



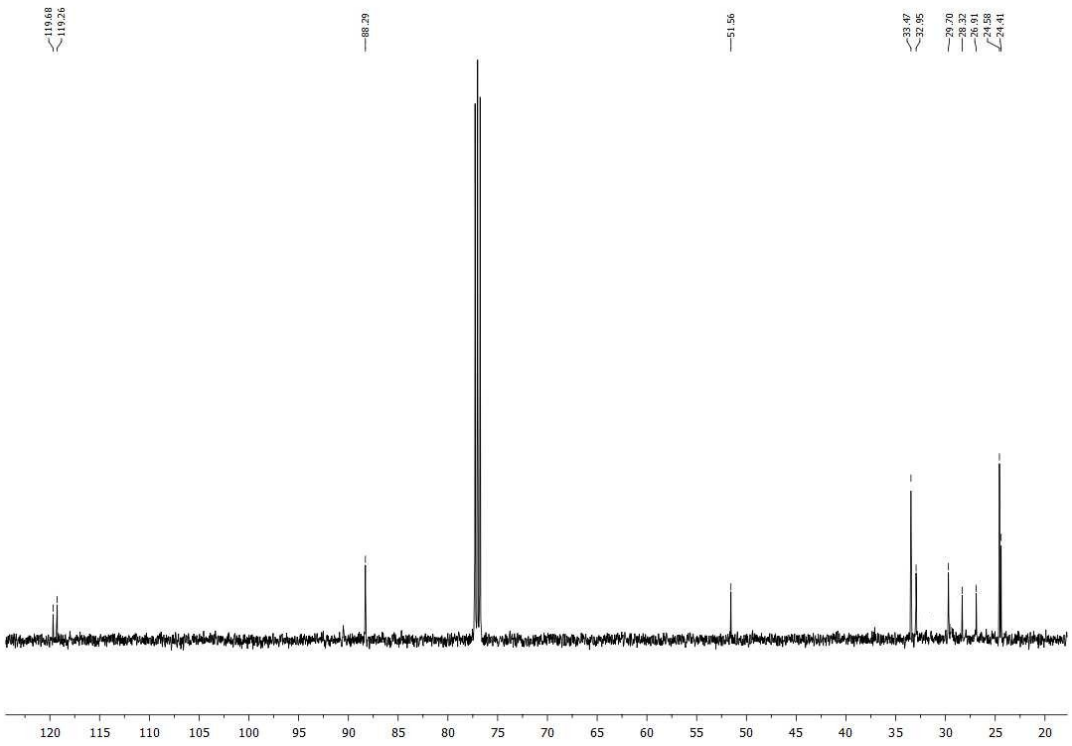
^{13}C-NMR spectrum of **1,5-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)pentane (11)**



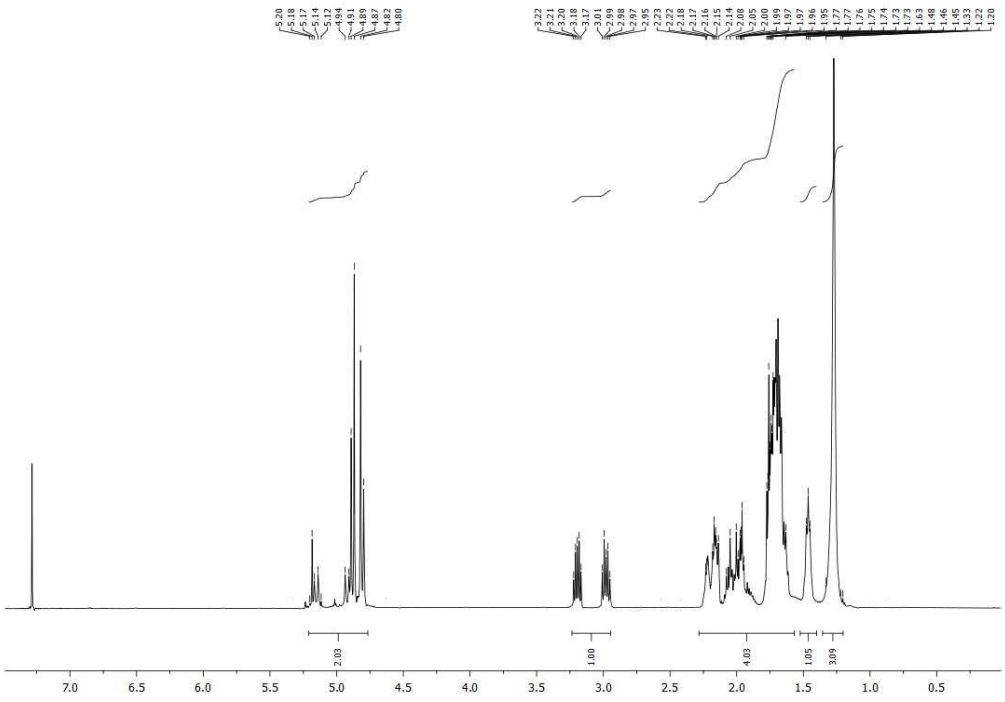
^{1}H-NMR spectrum of **1,8-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)octane (12)**



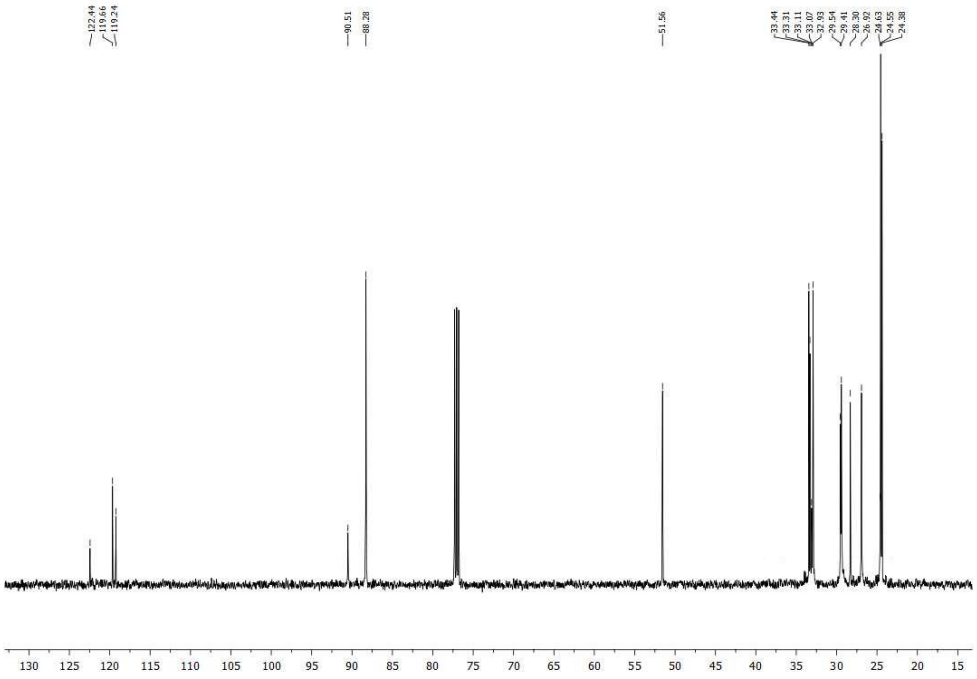
^{13}C-NMR spectrum of **1,8-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)octane (12)**



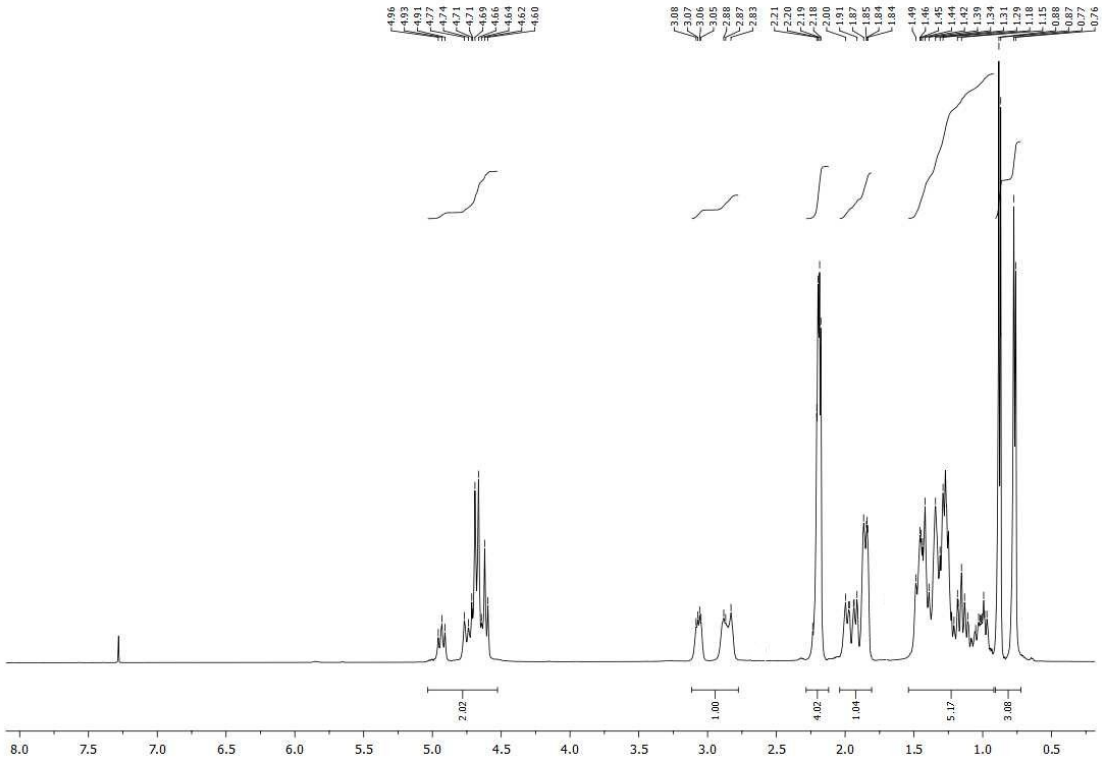
^{1}H-NMR spectrum of **1,10-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)decane (13)**



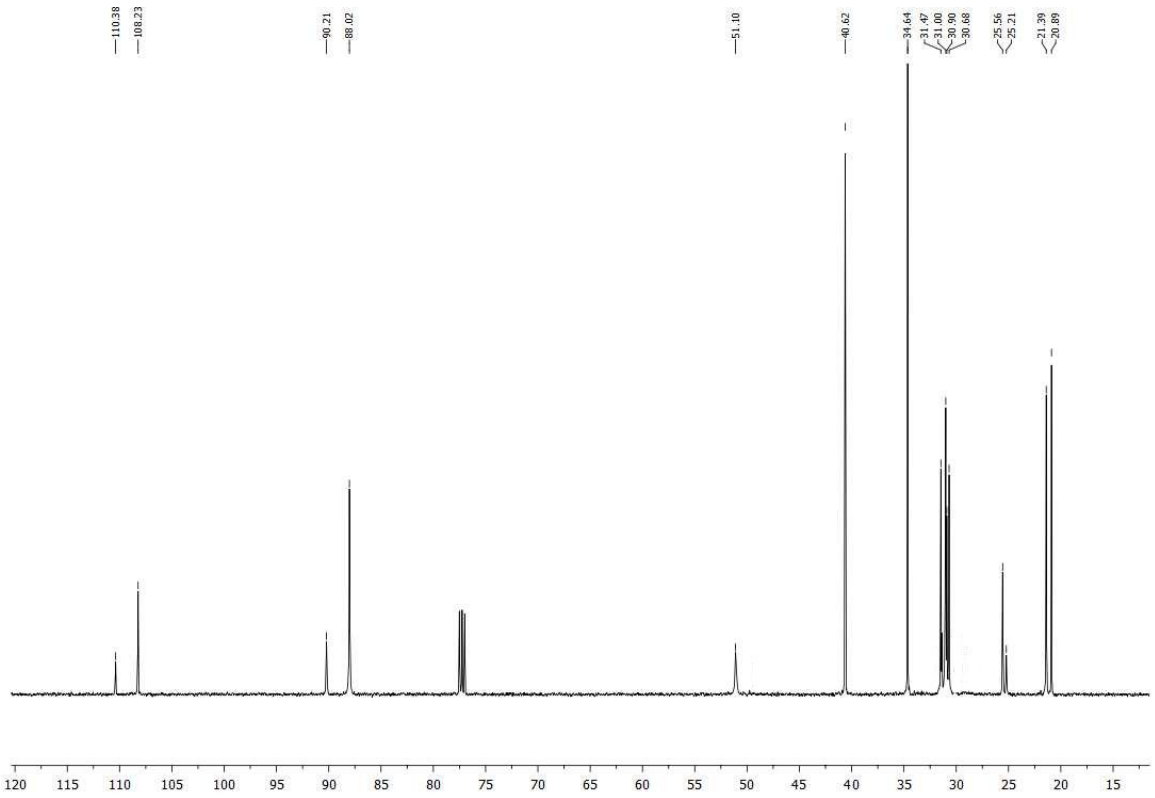
^{13}C-NMR spectrum of **1,10-bis-(6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^{8}.7^{5}]nonadecan-16-yl)decane (13)**



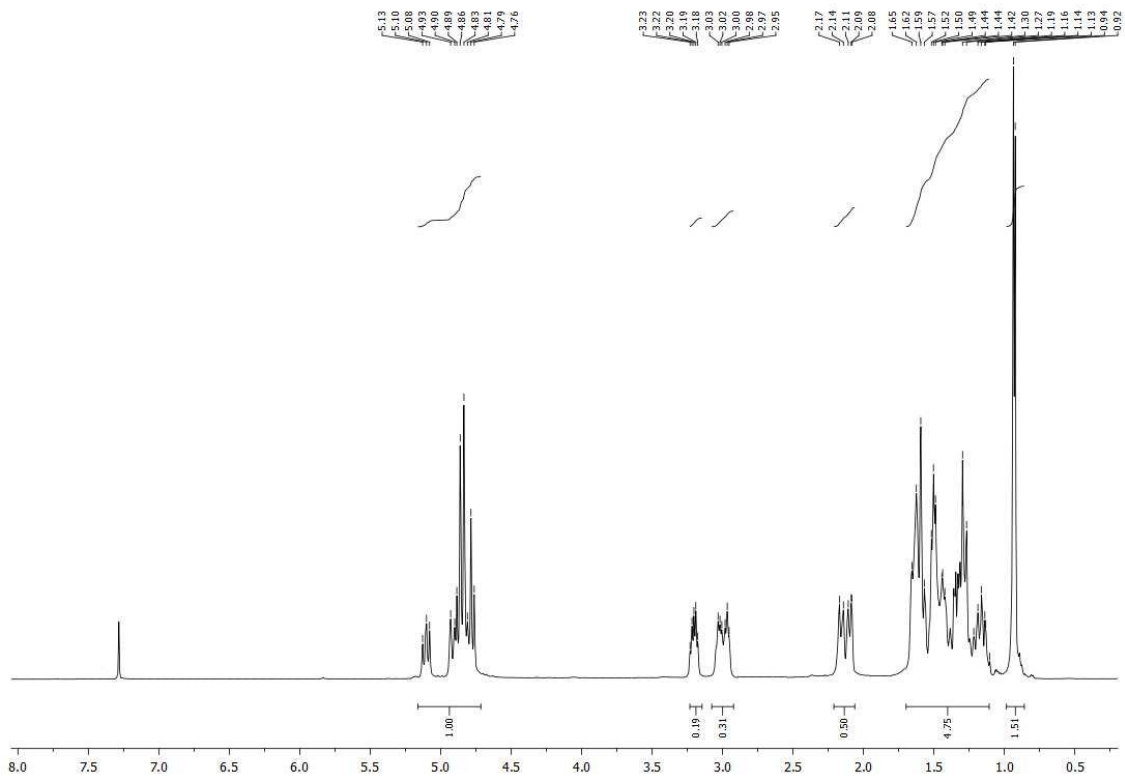
^{1}H-NMR spectrum of **1,4-bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)butane (14)**



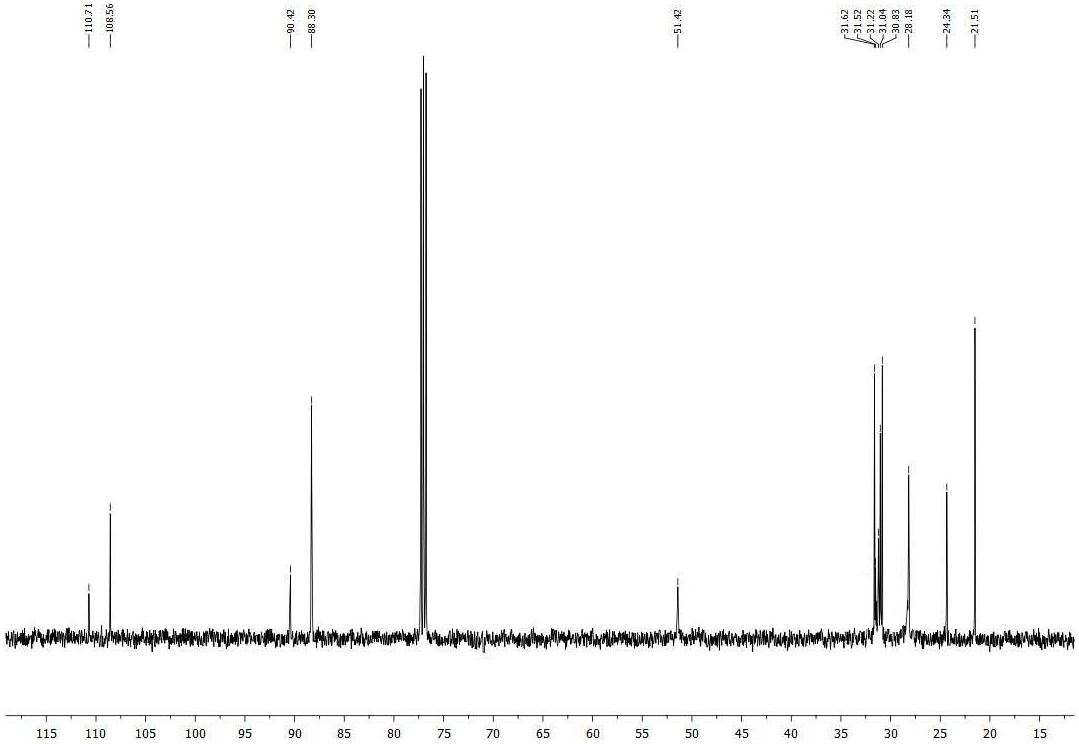
^{13}C-NMR spectrum of **1,4-bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)butane (14)**



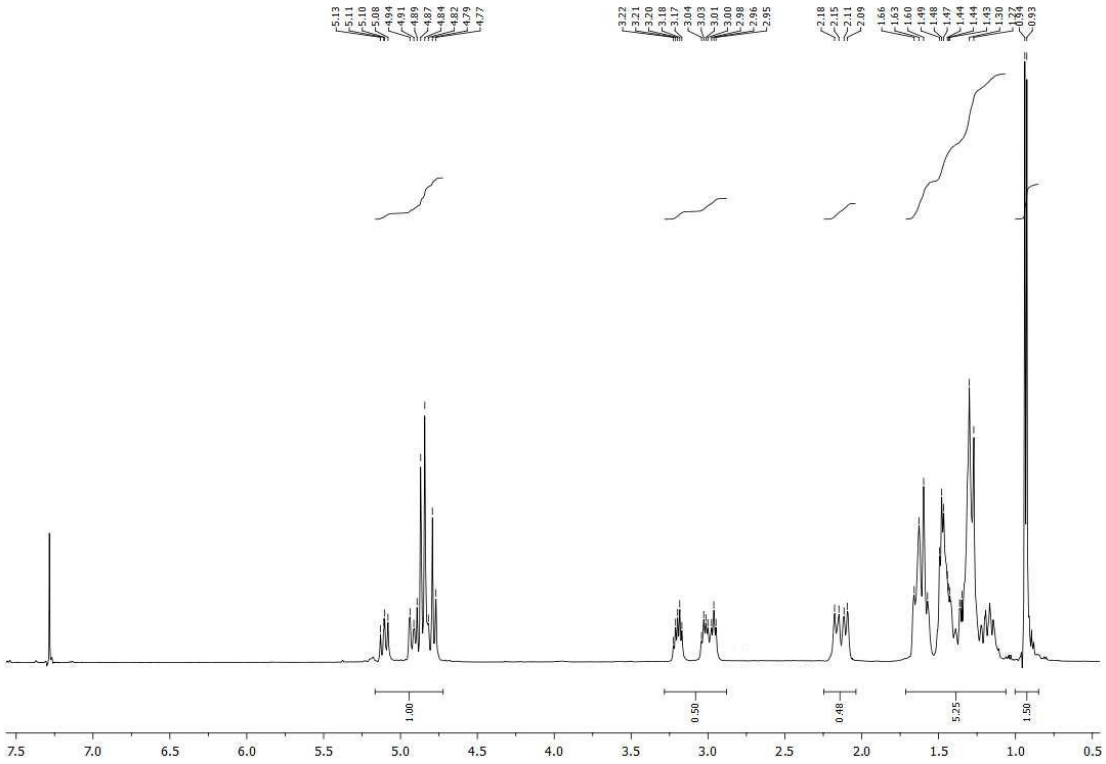
^{1}H-NMR spectrum of **1,5-bis(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)pentane (15)**



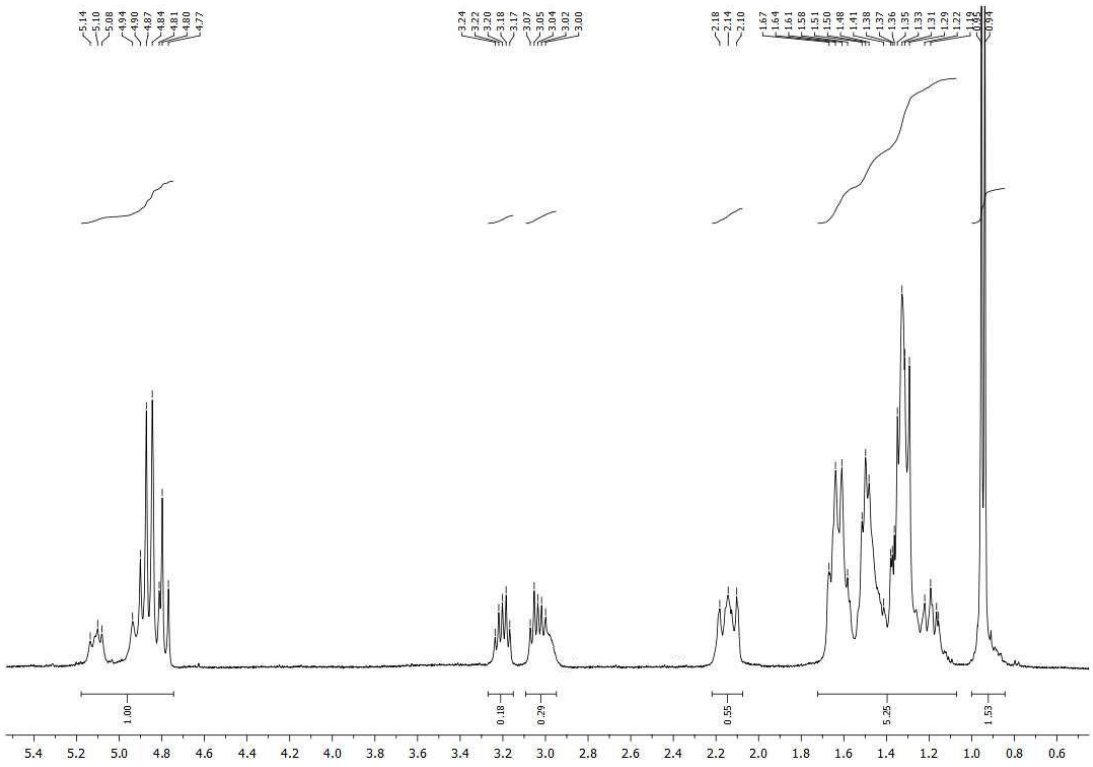
^{13}C-NMR spectrum of **1,5-bis(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)pentane (15)**



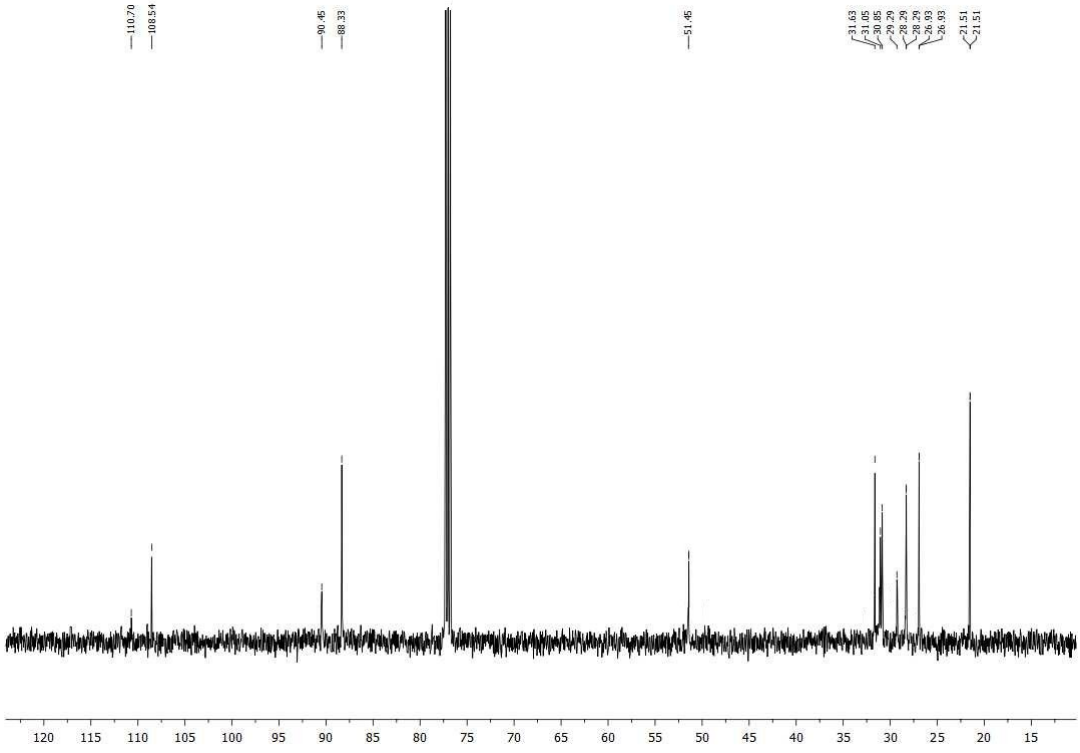
^{1}H-NMR spectrum of **1,7-bis(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)heptane (16)**



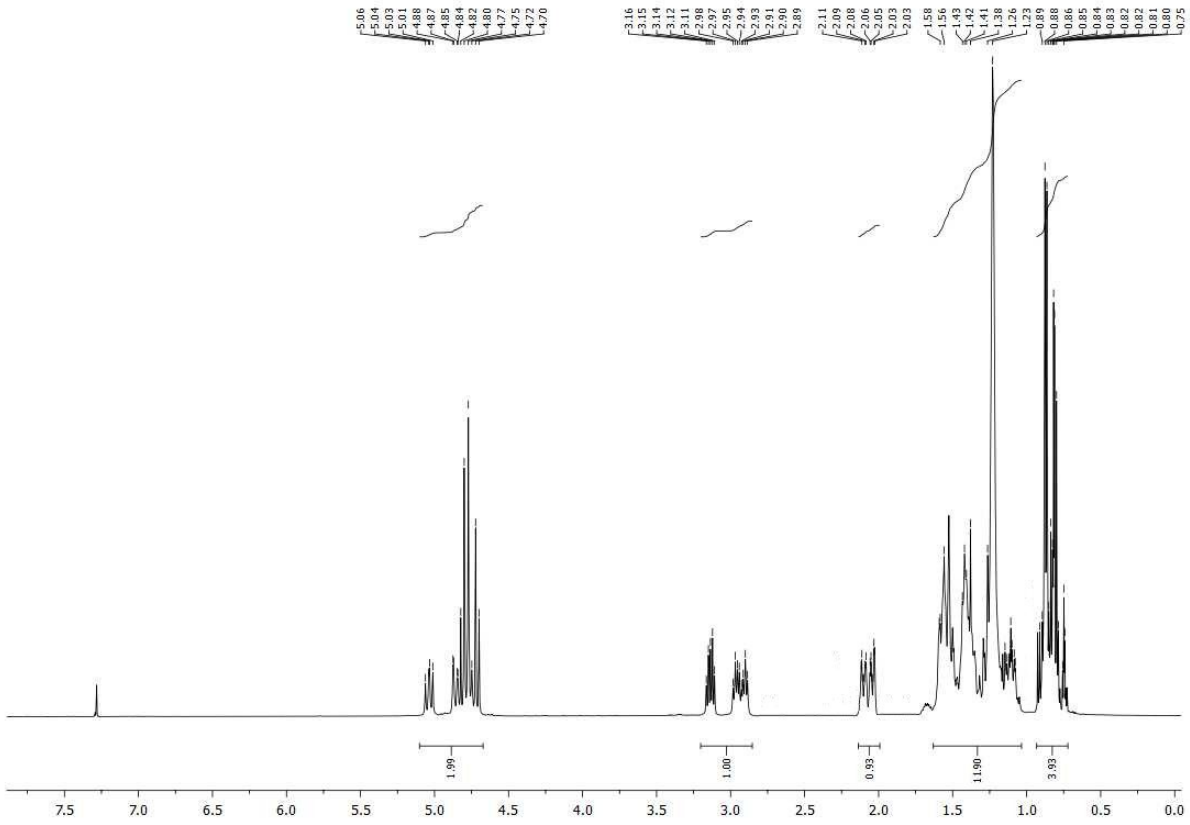
^{1}H-NMR spectrum of **1,7-bis(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)heptane (16)** at 323K.



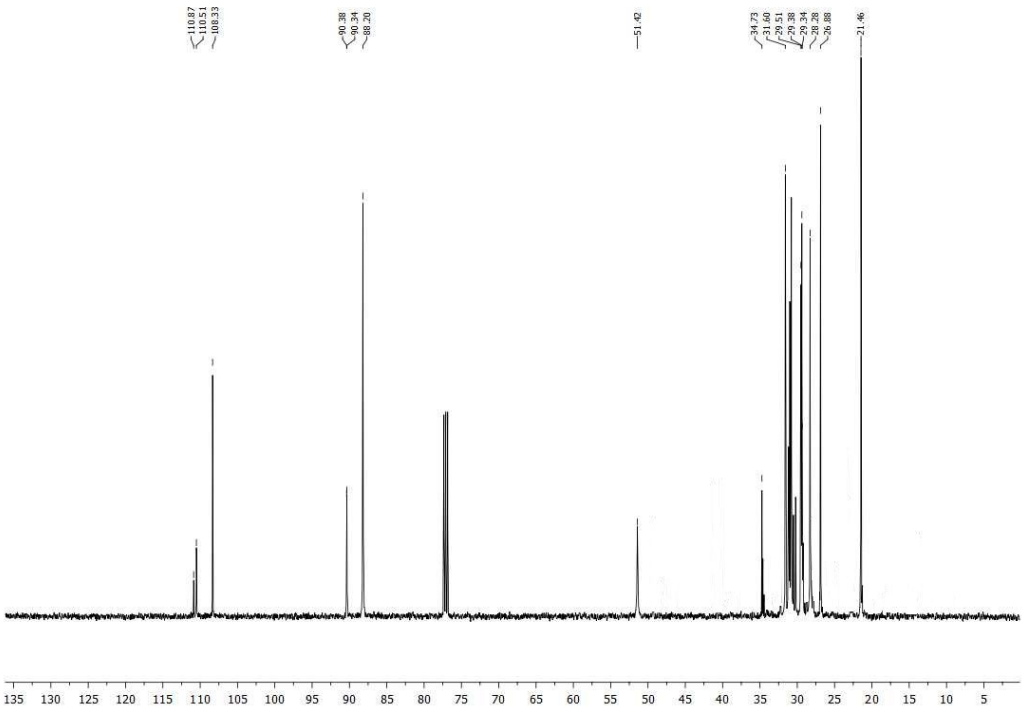
^{13}C-NMR spectrum of **1,7-bis(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)heptane (16)**



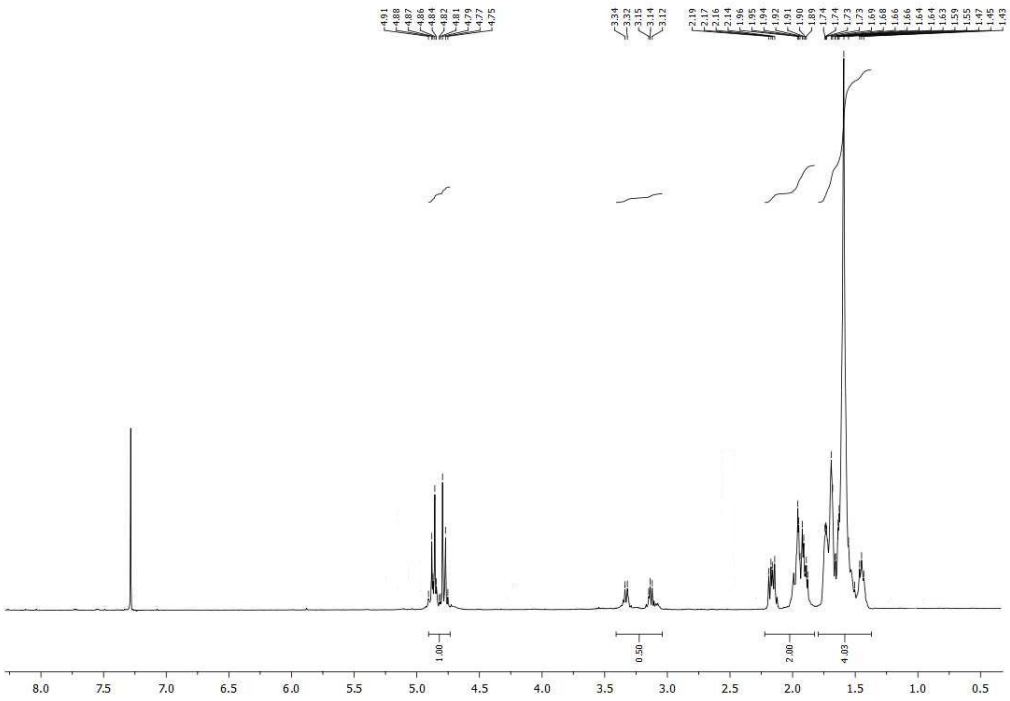
^{1}H-NMR spectrum of **1,10-bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)decane (17)**



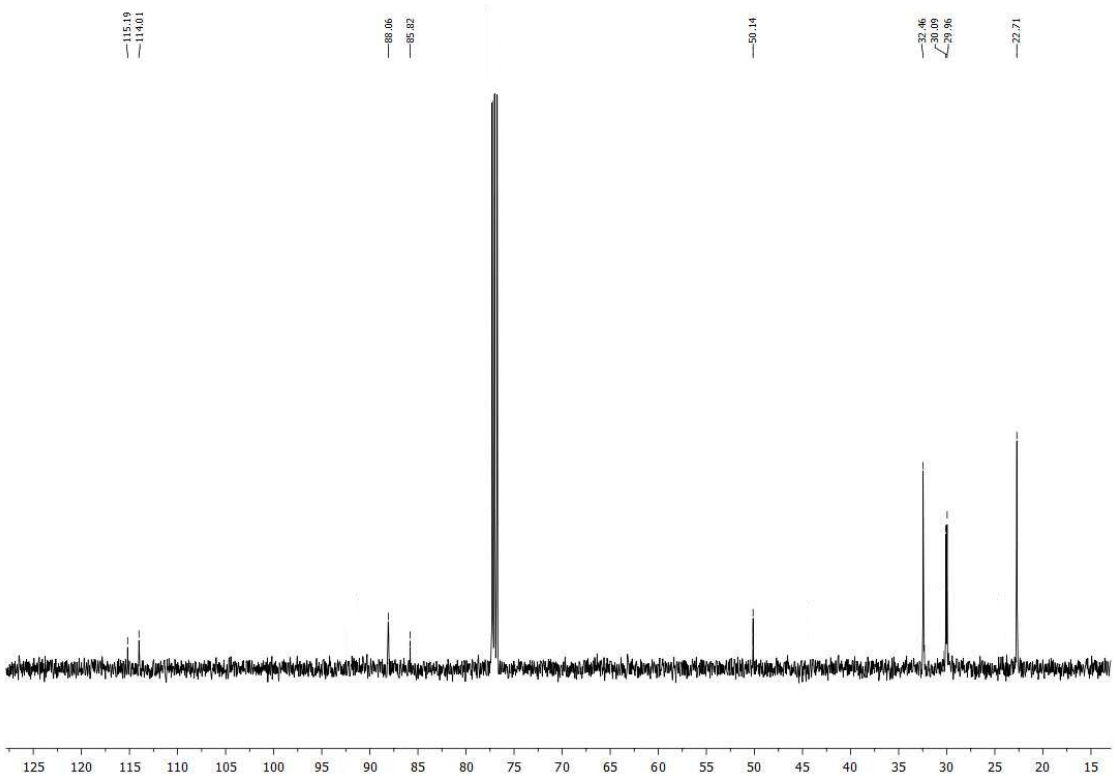
^{13}C-NMR spectrum of **1,10-bis-(3,12-dimethyl-7,8,15,16,20,21-hexaoxa-18-azadispiro[5.2.5^{9}.7^{6}]henicosan-18-yl)decane (17)**



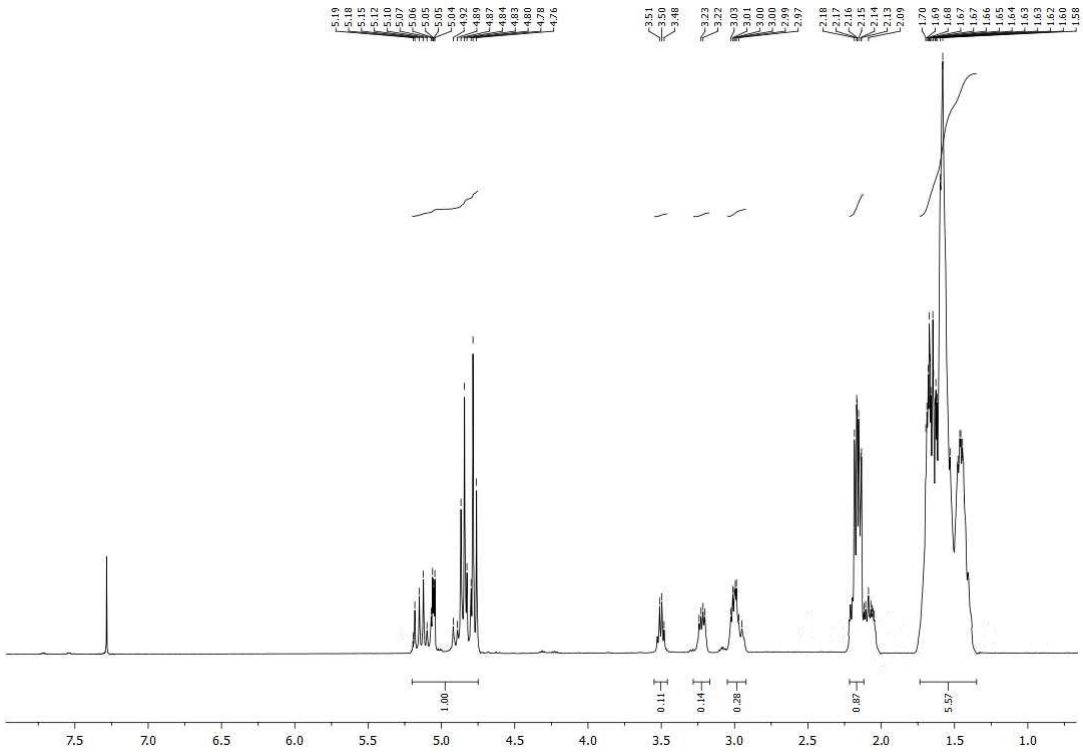
^{1}H-NMR spectrum of **1,2-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)ethane (18)**



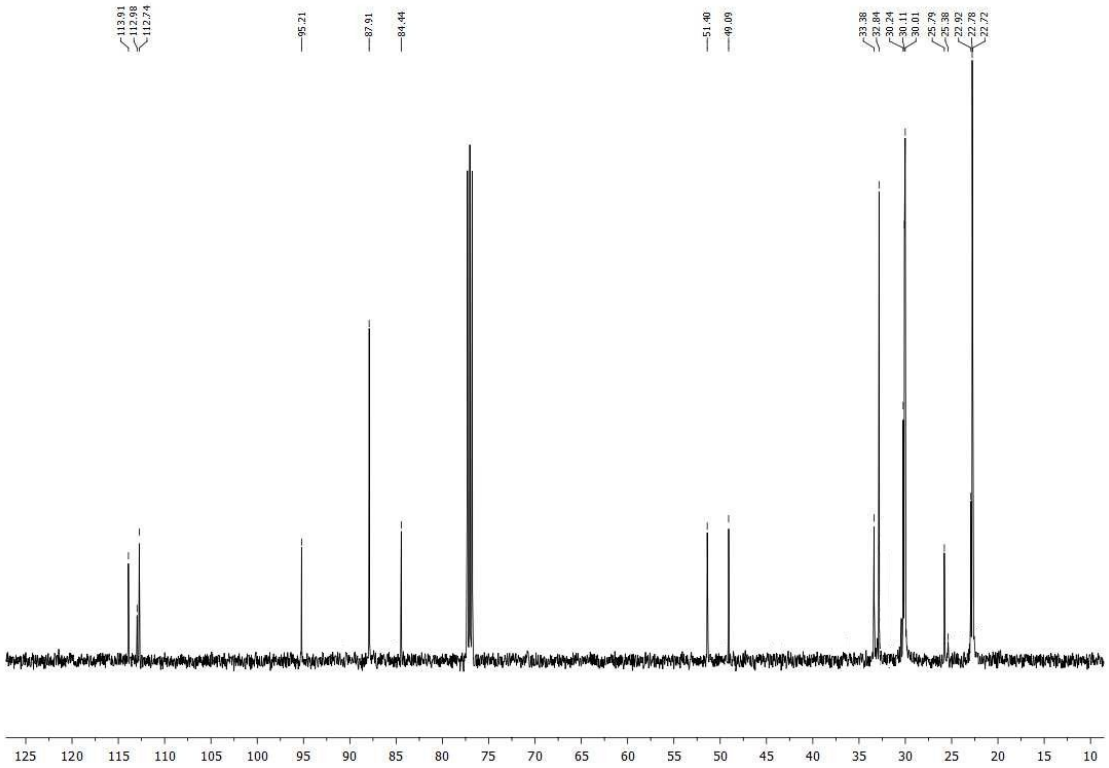
^{13}C-NMR spectrum of **1,2-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)ethane (18)**



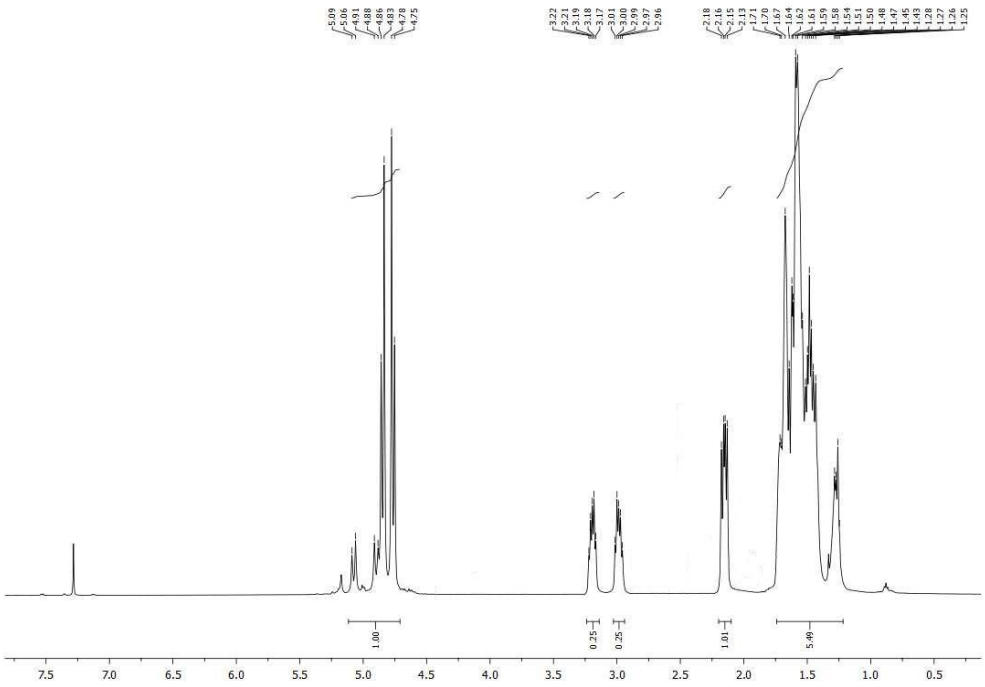
^{1}H-NMR spectrum of **1,4-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)butane (19)**



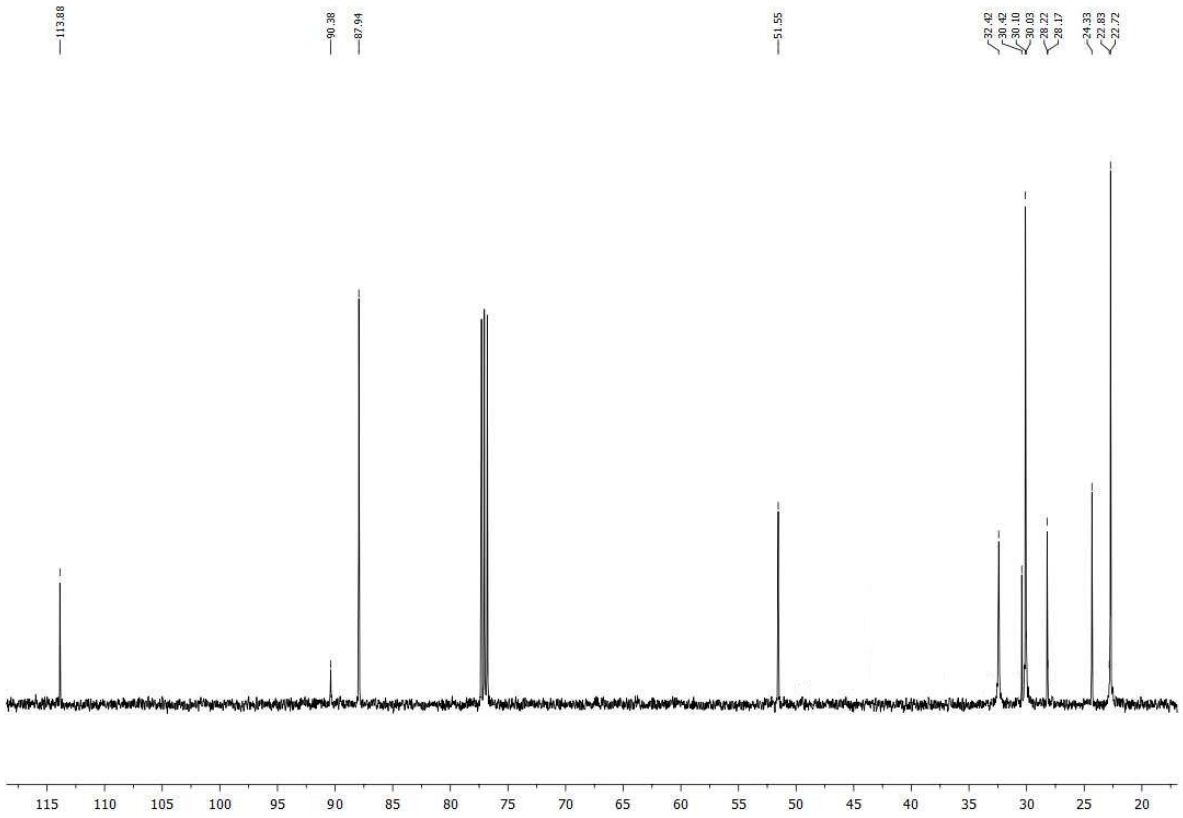
^{13}C-NMR spectrum of **1,4-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)butane (19)**



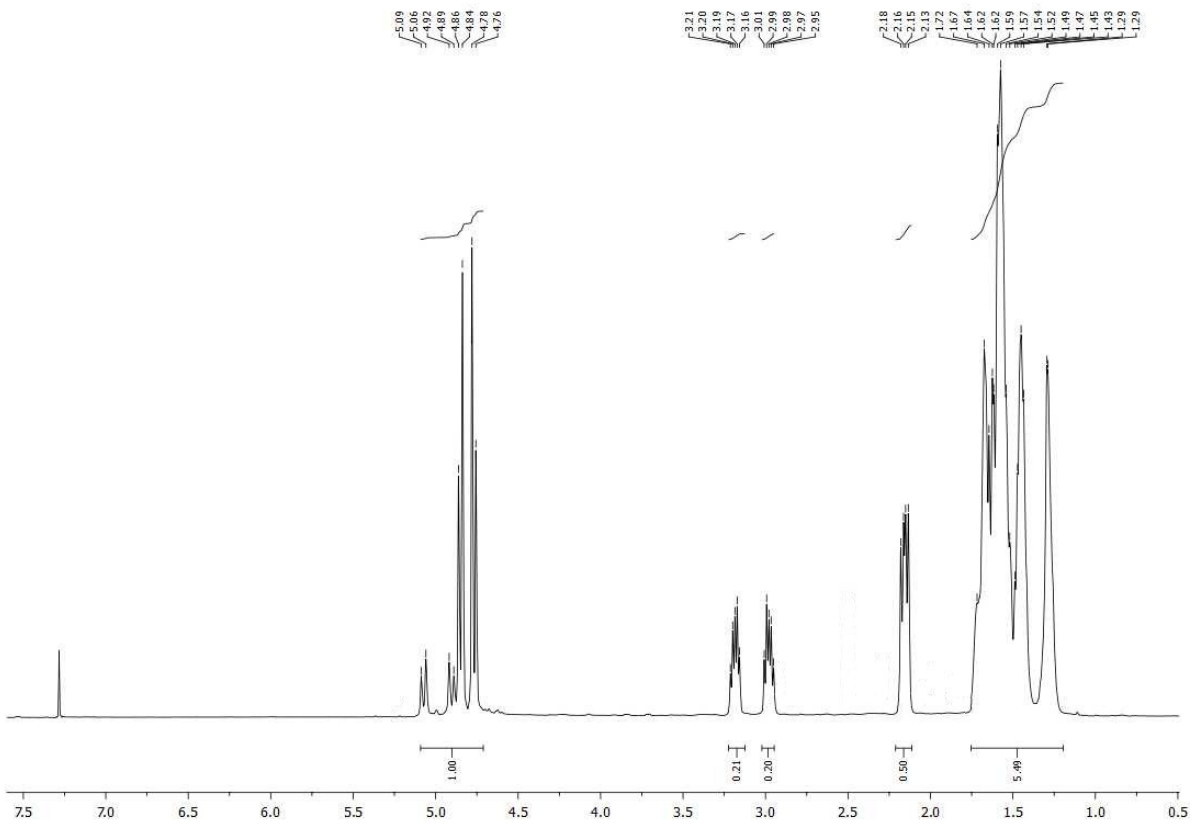
^{1}H-NMR spectrum of **1,5-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)pentane (20)**



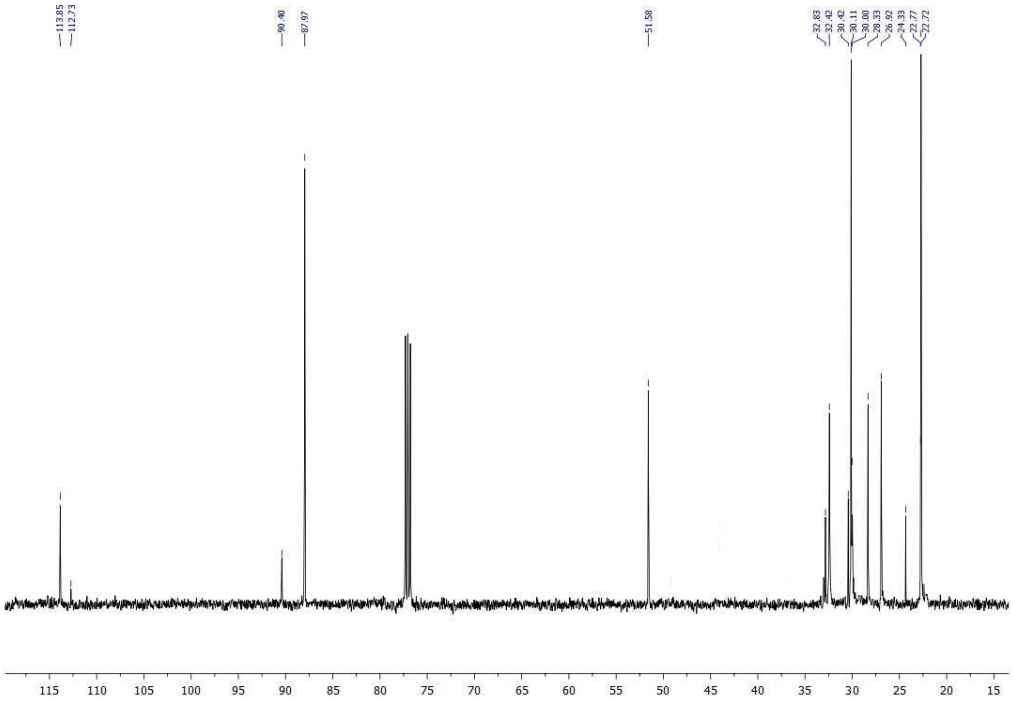
^{13}C-NMR spectrum of **1,5-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)pentane (20)**



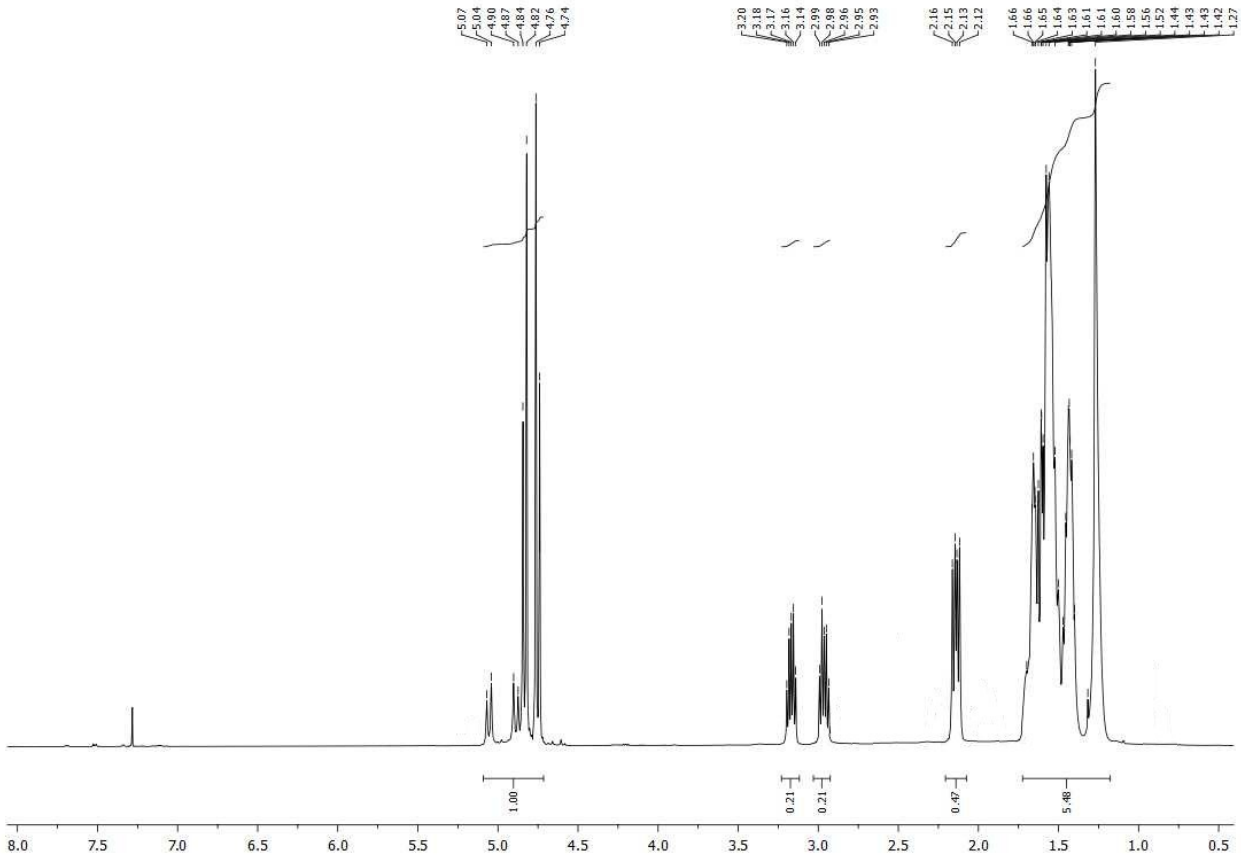
^{1}H-NMR spectrum of **1,7-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)heptane (21)**



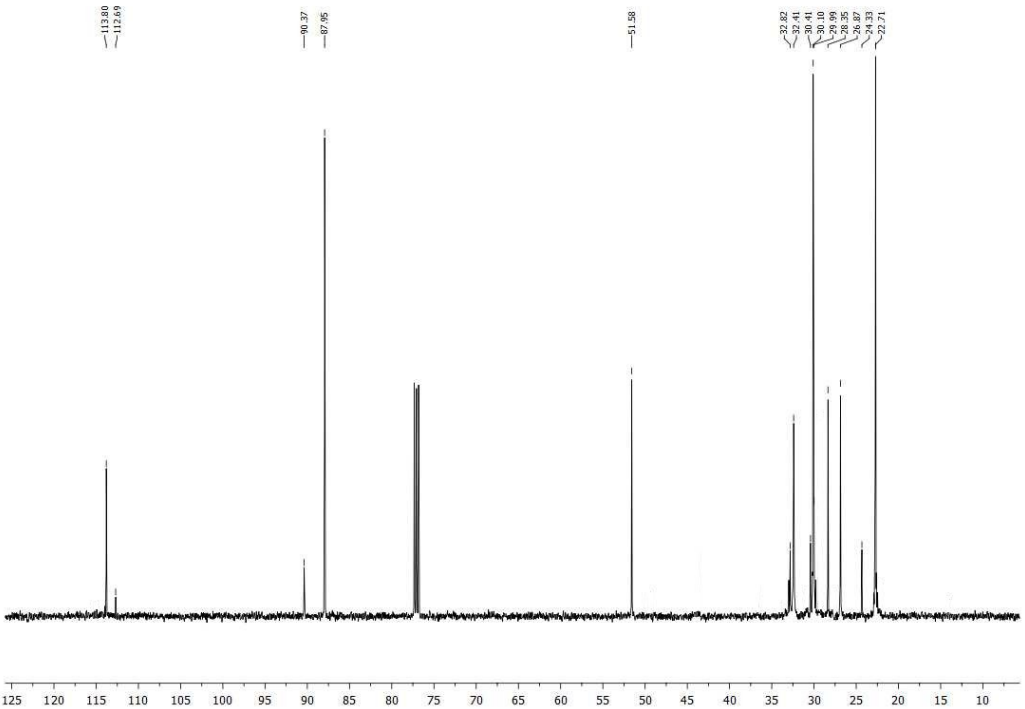
^{13}C-NMR spectrum of **1,7-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)heptane (21)**



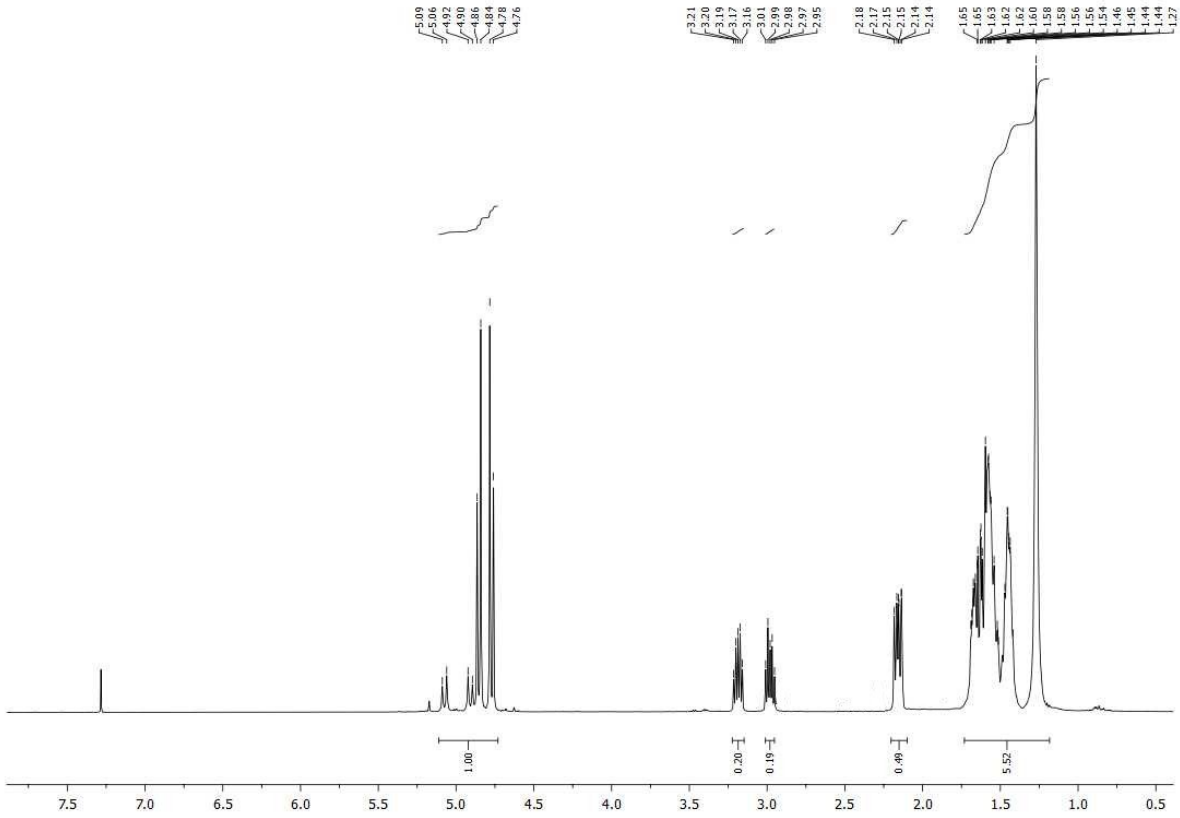
^{1}H-NMR spectrum of **1,8-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)octane (22)**



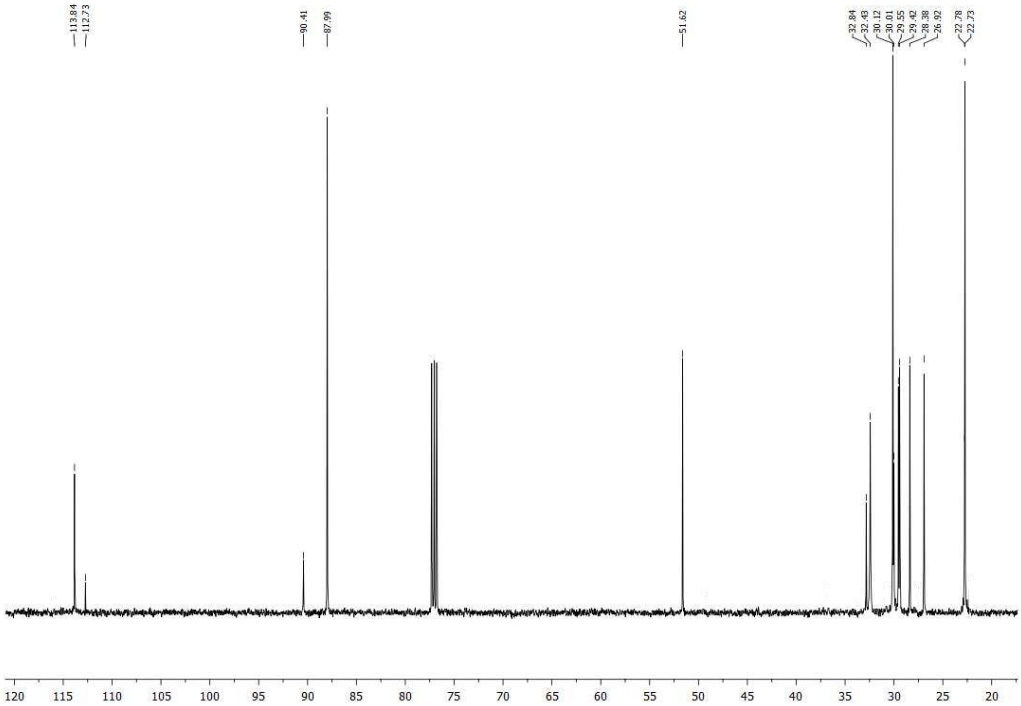
^{13}C-NMR spectrum of **1,8-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)octane (22)**



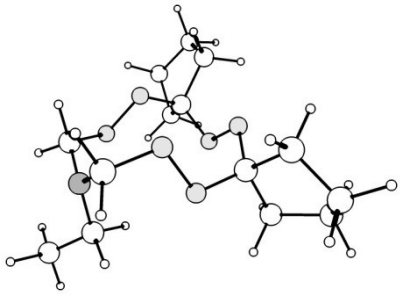
^{1}H-NMR spectrum of **1,10-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)decane (23)**



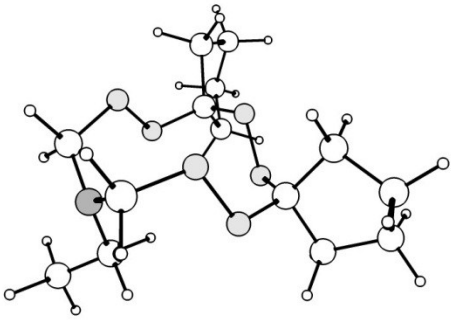
^{13}C-NMR spectrum of **1,10-bis-(8,9,17,18,22,23-hexaoxa-20-azadispiro[6.2.6^{10}.7^{7}]tricosan-20-yl)decane (23)**



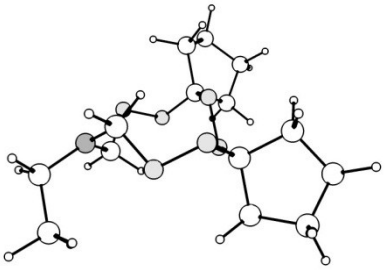
C. Figure S1. Optimized structures of primary stable conformers of *N*-ethyl-6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^8.7^5]nonadecan and their relative energies (kcal/mol).



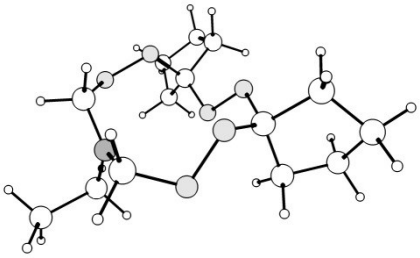
A 0.0



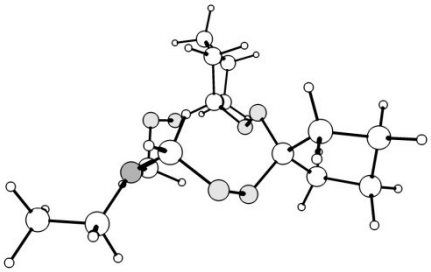
B 0.2



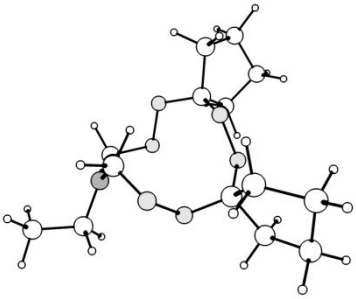
C 1.6



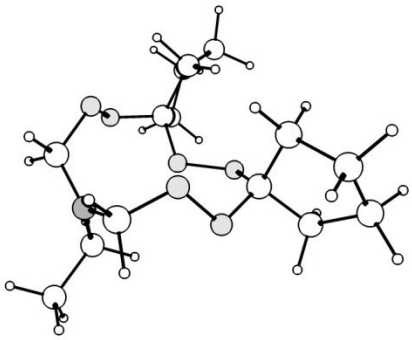
D 4.5



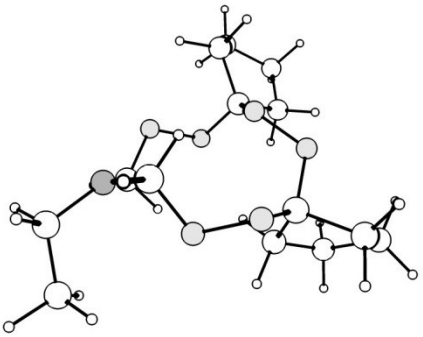
E 5.4



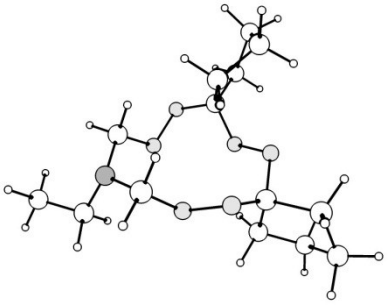
F 8.4



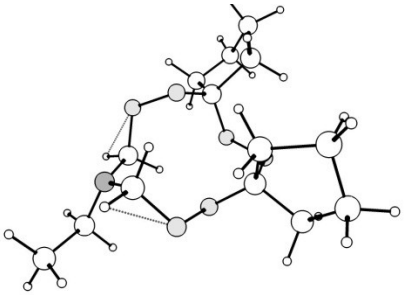
G 10.2



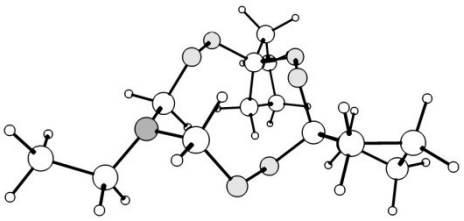
H 12.8



I 12.6



J 16.6



K 20.2

D. Table S1. ^{1}H and ^{13}C NMR shifts of ring –N-CH_2– atoms in conformers (**A**-**K**) of *N*-ethyl-6,7,13,14,18,19-hexaoxa-16-azadispiro[4.2.4^8.7^5]nonadecan.

conformer	C_2	C_2	$C_{2/5}$	C_2H_a	C_2H_b	C_5H_a	C_5H_b
A	97.6	89.7	93.7	4.7	5.1	5.3	4.9
B	98.7	89.6	94.2	4.7	5.2	5.5	4.6
C	94.8	96.4	95.6	4.8	6.2	4.9	6.2
D	93.9	96.0	95.0	4.8	5.0	5.2	4.8
E	95.4	95.9	95.7	4.2	6.4	4.9	6.2
F	92.5	95.4	94.0	4.9	6.1	5.4	4.7
G	98.9	86.1	92.5	5.1	4.9	5.2	4.9
H	94.1	93.1	93.6	4.7	5.9	4.7	6.7
I	99.4	90.8	95.1	5.2	5.3	5.5	5.2
J	103.6	88.1	95.9	4.4	6.9	4.7	5.8
K	97.3	89.3	93.3	4.2	6.2	4.9	5.7

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