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Synthesis of Chiral Linear and Macrocyclic Candidates: III.[1] Synthesis and Antimicrobial Activity of Linear Tetrapeptide and Macrocyclic Pentapeptide Schiff Bases[2]

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Abstract—A series of tetrapeptide Schiff bases and a macrocyclic pentapeptide were synthesized using N^{α}-dinicotinoyl-bis[L-leucyl-L-phenylalaninyl acid hydrazide] derivative and macrocyclic pentapeptide ester as starting materials and were screened for antimicrobial activity. Some of the newly synthesized compounds exhibited better antimicrobial effect compared to the reference controls. The structures of the newly synthesized compounds were confirmed by IR, NMR, and mass spectral data and elemental analyses.

Keywords: bicyclohexaazatetracarboxamides, macrocyclic pentapeptides, Schiff bases, antimicrobial activity

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Since the mid of the past century, macrocyclic peptides have become very important and represented a fascinating area of bioorganic and medicinal chemistry [3, 4]. While performing studies on new amino acids and peptides, we previously reported the synthesis of some new macrocyclic peptide candidates from pyridine dicarboxylic acids and selected amino acids and screening of their biological activity [5–11]. Synthesis and chemical modifications of existing antibacterial agents in order to generate novel macromolecules with better therapeutic properties is necessary because of the emergence of multidrug-resistant bacteria [12]. Peptides rarely function well as drugs due to their low bioavailability and rapid degradation within cells [13]. In view of these observations and in continuation of our previous works in macrocyclic and heterocyclic chemistry, we have synthesized some new macrocyclic pentapeptide Schiff bases containing a pyridine moiety and tested them for antimicrobial activity.

In this study, we report the synthesis of several tetrapeptide Schiff base and macrocyclic pentapeptide derivatives based on N^{α}-dinicotinoyl-bis[L-leucyl-L-phenylalaninyl acid hydrazide] derivative **II** and macrocyclic pentapeptide ester **III**, which were prepared from pyridine-3,5-dicarbonyl dichloride (**I**) according to the previously published procedures [1, 2] (Scheme 1).

The reaction of **II** with aromatic aldehydes in refluxing ethanol in the presence of triethylamine/diethylamine gave the corresponding N^{α}-dinicotinoyl-bis-[L-leucyl-L-phenylalaninyl *p*-substituted phenyl hydrazones] **IVa**–**IVh**. The reaction of propane-1,3-diamine with the azide prepared *in situ* from hydrazide **II** afforded chiral macrocyclic tetrapeptide derivative **V** (Scheme 2).

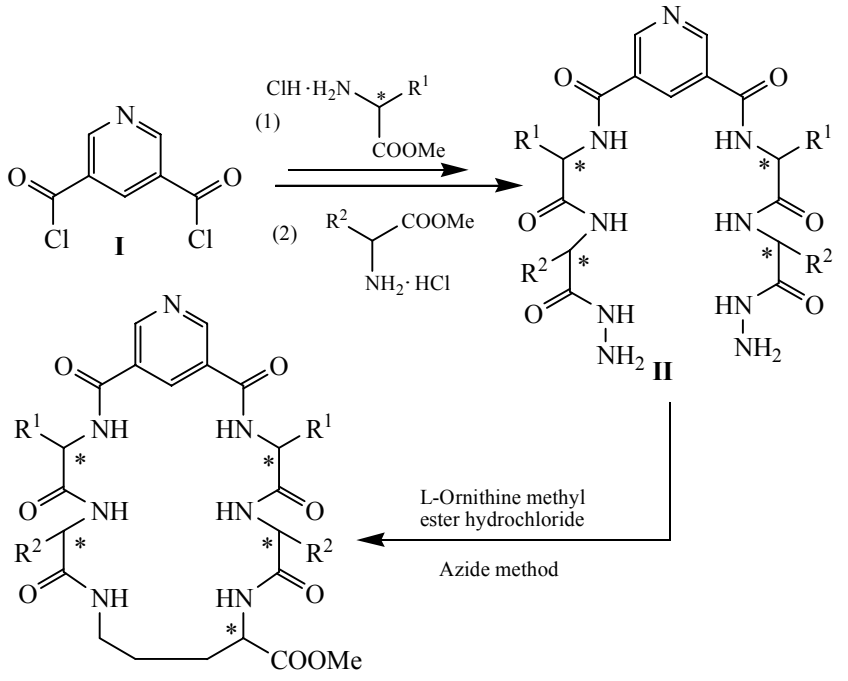
The hydrolysis of pentapeptide methyl ester **III** [1] with 1 N sodium hydroxide in methanol afforded acid **VI**. In addition, the reaction of **III** with hydrazine hydrate in methanol gave cyclic pentapeptide hydrazide derivative **VII** which was treated with aromatic aldehydes in refluxing ethanol to obtain the corresponding cyclic pentapeptide hydrazones (Schiff bases) **VIIIa**–**VIIIe** (Scheme 3).

The newly synthesized Schiff bases were screened for antimicrobial activity against Gram-positive (*Staphylococcus aureus*, *Bacillus subtilis*) and Gram-negative bacteria (*Escherichia coli*) and fungi (*Candida albicans*). The tested compounds demonstrated high

[1] For communication II, see [1].

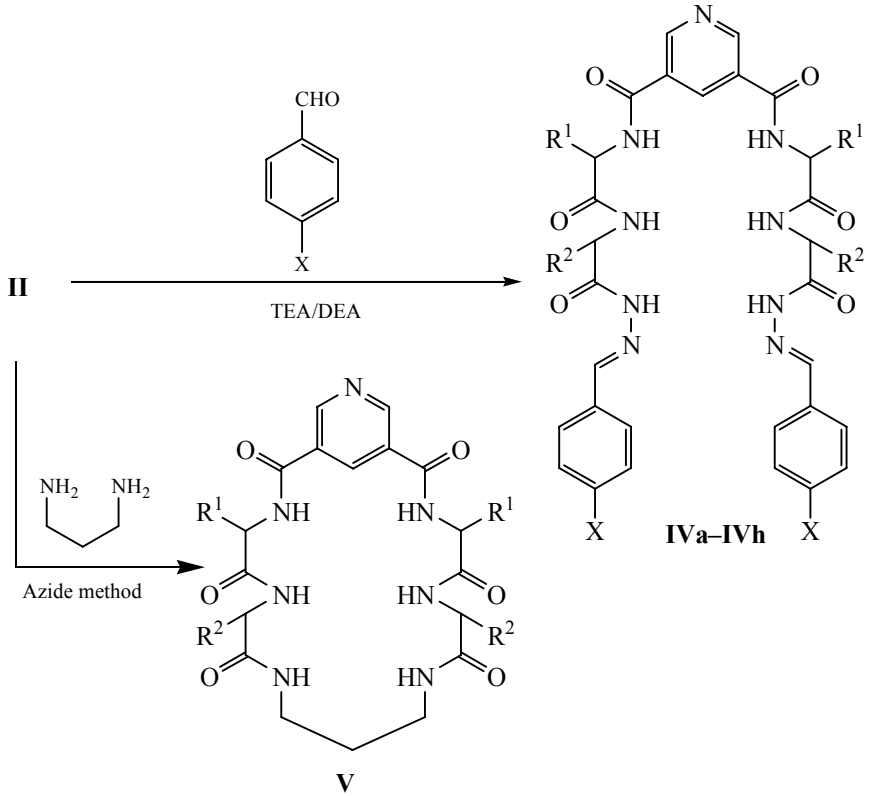
[2] The text was submitted by the authors in English.

Scheme 1.



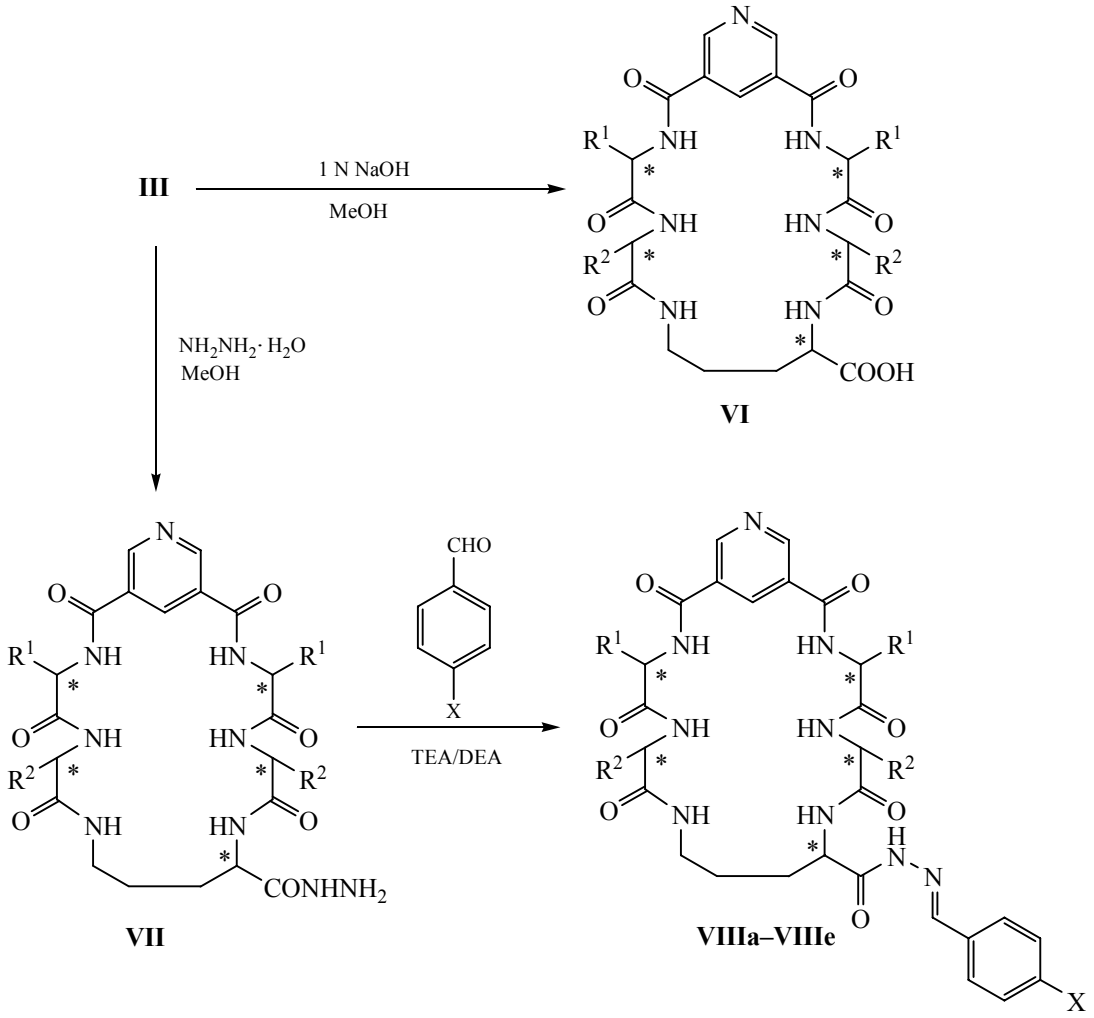
$R^1 = Me_2CHCH_2$, $R^2 = PhCH_2$.

Scheme 2.



$R^1 = Me_2CHCH_2$; $R^2 = PhCH_2$; **IVa–IVh**, X = H (**a**), Me (**b**); Me_2N (**c**), MeO (**d**), Cl (**e**), Br (**f**), F (**g**), O_2N (**h**).

Scheme 3.



$R^1 = Me_2CHCH_2$, $R^2 = PhCH_2$; **VIIIa**–**VIIIe**, X = H (**a**), Me (**b**), MeO (**c**), Cl (**d**), F (**e**).

activity at a concentration of 50 μg/mL using the bioassay technique for antibiotics [14] specified in the US Pharmacopeia (see table). Streptomycin and fusidic acid were used as antibacterial and antifungal reference drugs, respectively.

EXPERIMENTAL

The melting points were determined in open glass capillaries with an ElectroThermal digital melting point apparatus (model IA9100) and are uncorrected. The elemental microanalyses for carbon, hydrogen, and nitrogen (Microanalytical Unit, NRC) conformed to the calculated values within acceptable limits. The IR spectra (KBr) were recorded on a Nicolet Nexus 670 FTIR spectrometer with Fourier transform. The ^1H and ^{13}C NMR spectra were measured in DMSO-d_6 on a Jeol instrument at 500 MHz. The mass spectra (electron impact, 70 eV) were run on a Finnigan MAT SSQ 7000 spectrometer. Analytical thin-layer chromato-graphy (TLC) was performed on silica gel 60 F_{254} aluminum sheets (Merck). Antimicrobial assays were carried out by El-Sayed E. Mostafa, Department of Microbial Chemistry, National Research Center, Cairo, Egypt.

Tetrapeptide hydrazones IVa–IVh *(general procedure).* A mixture of 1 mmol of hydrazide **II**, 2 mmol of substituted benzaldehyde, 2 mL of triethyl-amine, and 2 mL of diethylamine in 25 mL of anhydrous ethanol was heated for 4–6 h under reflux. The mixture was then concentrated under reduced

Antimicrobial activity of compounds **II–VIII**

Compound no.	Inhibition zone, mm			
	gram-positive		gram-negative	fungi
	Staphylococcus aureus	*Bacillus subtilis*	*Escherichia coli*	*Candida albicans*
II	1.65	1.79	0.78	0.94
III	1.68	1.78	0.64	–
IVa	1.65	1.64	0.76	1.02
IVb	1.76	1.85	0.92	1.10
IVc	1.92	1.86	0.75	0.95
IVd	1.78	1.59	0.65	–
IVe	1.75	1.72	0.66	–
IVf	1.88	1.75	0.74	0.95
IVg	1.75	1.74	0.76	1.00
IVh	1.65	1.79	0.81	1.00
V	1.65	1.75	0.60	–
VI	1.98	1.82	0.65	–
VII	1.68	1.86	0.78	0.92
VIIIa	1.96	1.92	0.92	1.18
VIIIb	1.50	1.80	0.65	–
VIIIc	1.92	1.86	0.85	0.98
VIIId	1.56	1.58	0.66	–
VIIIe	1.45	1.52	0.68	–
Streptomycin	2.00	2.00	0.95	–
Fusidic acid	–	–	–	1.9

pressure, the residue was poured into ice water with stirring, and the precipitate was filtered off, washed with water, and recrystallized from appropriate solvent.

***N*,*N′*-Bis{(2*S*)-1-[(2*S*)-1-(2-benzylidenehydrazinyl)-1-oxo-3-phenylpropan-2-ylamino]-4-methyl-1-oxopentan-2-yl}pyridine-3,5-dicarboxamide (IVa).** Yield 75%, mp 210–212°C (from dioxane), $[\alpha]_D^{25}$ = –109° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3478–3340 (N–H), 3088 (C–H_{arom}), 2990 (C–H_{aliph}), 1658 (C=O), 1534 [δ(N–H)], 1253 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.85–0.99 m (12H, CH_3), 1.62–1.70 m (4H, CH_2), 2.24–2.30 m (2H, CH), 3.40 d (4H, CH_2), 4.26–4.35 m (2H, CH), 4.66–4.74 m (2H, CH), 6.95–7.62 m (22H, H_{arom}, CH=N), 8.35 s and 9.12 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.68 s, 8.80 s, and 9.25 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.65 and 19.25 (4C, CH_3), 22.45 (2C, CH), 40.12 (2C, CH_2), 42.45 (2C, CH_2), 52.42 and 53.33 (4C, CH); 125.85, 127.45, 128.32, 128.55, 129.05, 131.02, 133.24, 138.68 (24C, C_{arom}); 131.66, 140.37, 152.48 (5C, pyridine); 143.15 (2C, CH=N), 163.90 and 169.43 (4C, C=O, amide), 178.44 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 892 (I_{rel} 24%) $[M]^+$. Found, %: C 68.58; H 6.32; N 14.05. $C_{51}H_{57}N_9O_6$. Calculated, %: C 68.67; H 6.44; N 14.13.

***N*,*N′*-Bis[(2*S*)-4-methyl-1-{(2*S*)-1-[2-(4-methylbenzylidene)hydrazinyl]-1-oxo-3-phenylpropan-2-ylamino}-1-oxopentan-2-yl]pyridine-3,5-dicarboxamide (IVb).** Yield 82%, mp 227–229°C (from

AcOH/H_2O), $[\alpha]_D^{25}$ = –90° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3444–3330 (N–H), 3085 (C–H_{arom}), 2979 (C–H_{aliph}), 1654 (C=O), 1534 [δ(N–H)], 1254 (C–N). ^1H NMR spectrum, δ, ppm: 0.92–0.99 m (12H, CH_3), 1.62–1.74 m (4H, CH_2), 2.20–2.32 m (8H, CH, CH_3), 3.43 d (4H, CH_2), 4.25–4.35 m (2H, CH), 4.65–4.78 m (2H, CH), 6.96–7.55 m (20H, H_{arom}, CH=N), 8.42 s and 9.14 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.66 s, 8.84 s, and 9.25 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.68, 19.28, 24.15 (6C, CH_3); 23.42 (2C, CH), 40.16 (2C, CH_2), 42.44 (2C, CH_2), 52.46 and 53.42 (4C, CH); 125.88, 127.68, 128.56, 128.95, 129.15, 130.45, 139.42, 140.62 (24C, C_{arom}); 131.60, 140.48, 152.58 (5C, pyridine); 142.92 (2C, CH=N), 164.12 and 169.56 (4C, C=O, amide), 178.65 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 920 (I_{rel} 24%) $[M]^+$. Found, %: C 69.10; H 6.60; N 13.61. $C_{53}H_{61}N_9O_6$. Calculated, %: C 69.18; H 6.68; N 13.70.

***N*,*N*′-Bis{(2*S*)-1-[(2*S*)-1-{2-[4-(dimethylamino)-benzylidene]hydrazinyl}-1-oxo-3-phenylpropan-2-ylamino]-4-methyl-1-oxopentan-2-yl}pyridine-3,5-dicarboxamide (IVc).** Yield 72%, mp 245–247°C (from DMF/H_2O), $[\alpha]_D^{25}$ = –65° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3425–3330 (N–H), 3081 (C–H_{arom}), 2975 (C–H_{aliph}), 1653 (C=O), 1532 [δ(N–H)], 1253 (C–N). ^1H NMR spectrum, δ, ppm: 0.90–0.98 m (12H, CH_3), 1.63–1.73 m (4H, CH_2), 2.22–2.35 m (2H, CH), 2.76 s (12H, NCH_3), 3.42 d (4H, CH_2), 4.24–4.34 m (2H, CH), 4.64–4.75 m (2H, CH), 6.92–7.60 m (20H, H_{arom}, CH=N), 8.45 s and 9.10 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.68 s, 8.85 s, and 9.26 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.76 and 19.34 (4C, CH_3), 23.40 (2C, CH), 39.78 (4C, NCH_3), 40.18 (2C, CH_2), 42.45 (2C, CH_2), 52.45 and 53.45 (4C, CH); 114,32, 123.05, 125.98, 127.68, 128.56, 129.95, 139.42, 151.62 (24C, C_{arom}); 131.62, 140.47, 152.57 (5C, pyridine), 142.84 (2C, CH=N), 164.14 and 169.53 (4C, C=O, amide), 178.63 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 978 (I_{rel} 32%) $[M]^+$. Found, %: C 67.42; H 6.82; N 15.68. $C_{55}H_{67}N_{11}O_6$. Calculated, %: C 67.53; H 6.90; N 15.75.

***N*,*N*′-Bis[(2*S*)-1-{(2*S*)-1-[2-(4-methoxybenzylidene)hydrazinyl]-1-oxo-3-phenylpropan-2-ylamino}-4-methyl-1-oxopentan-2-yl]pyridine-3,5-dicarboxamide (IVd).** Yield 78%, mp 215–217°C (from AcOH/H_2O), $[\alpha]_D^{25}$ = –98° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3465–3335 (N–H), 3082 (C–H_{arom}), 2978 (C–H_{aliph}), 1656 (C=O), 1536 [δ(N–H)], 1255 (C–N). ^1H NMR spectrum, δ, ppm: 0.88–1.01 m (12H, CH_3), 1.65–1.71 m (4H, CH_2), 2.25–2.33 m (2H, CH), 3.41 d (4H, CH_2), 3.72 s (6H, OCH_3), 4.27–4.37 m (2H, CH), 4.69-4.75 m (2H, CH), 6.88–7.56 m (20H, H_{arom}, CH=N), 8.36 s and 9.11 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.69 s, 8.81 s, and 9.29 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.64, 19.28 (4C, CH_3), 23.47 (2C, CH), 40.17 (2C, CH_2), 42.47 (2C, CH_2), 52.45 and 53.39 (4C, CH), 54.98 (2C, OCH_3); 114.32, 125.65, 125.86, 127.48, 128.35, 129.05, 138.60, 162.45 (24C, C_{arom}); 131.62, 140.46, 152.55 (5C, pyridine); 142.95 (2C, CH=N), 164.02 and 169.55 (4C, C=O, amide), 178.62 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 952 (I_{rel} 16%) $[M]^+$. Found, %: C 66.80; H 6.40; N 13.20. $C_{53}H_{61}N_9O_8$. Calculated, %: C 66.86; H 6.46; N 13.24.

***N*,*N*′-Bis[(2*S*)-1-{(2*S*)-1-[2-(4-chlorobenzylidene)-hydrazinyl]-1-oxo-3-phenylpropan-2-ylamino}-4-methyl-1-oxopentan-2-yl]pyridine-3,5-dicarboxamide (IVe).** Yield 65%, mp 242–244°C (from DMF/H_2O), $[\alpha]_D^{25}$ = –96° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3488–3335 (N–H), 3092 (C–H_{arom}), 2986 (C–H_{aliph}), 1655 (C=O), 1535 [δ(N–H)], 1255 (C–N). ^1H NMR spectrum, δ, ppm: 0.86–0.98 m (12H, CH_3), 1.64–1.76 m (4H, CH_2), 2.28–2.33 m (2H, CH), 3.42 d (4H, CH_2), 4.25–4.37 m (2H, CH), 4.65–4.74 m (2H, CH), 7.05–7.58 m (20H, H_{arom}, CH=N), 8.32 s and 9.08 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.65 s, 8.85 s, and 9.28 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.68, 19.35 (4C, CH_3), 23.40 (2C, CH), 40.18 (2C, CH_2), 42.52 (2C, CH_2), 52.40 and 53.28 (4C, CH); 125.80, 127.42, 128.36, 129.05, 130.15, 131.85, 136.55, 138.65 (24C, C_{arom}); 131.42, 140.68, 152.56 (5C, pyridine); 142.85 (2C, CH=N), 163.82 and 169.56 (4C, C=O, amide), 177.98 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 961 (I_{rel} 8%) $[M]^+$. Found, %: C 63.64; H 5.70; Cl 7.30; N 13.00. $C_{51}H_{55}Cl_2N_9O_6$. Calculated, %: C 63.74; H 5.77; Cl 7.38; N 13.12.

***N*,*N*′-Bis[(2*S*)-1-{(2*S*)-1-[2-(4-bromobenzylidene)-hydrazinyl]-1-oxo-3-phenylpropan-2-ylamino}-4-methyl-1-oxopentan-2-yl]pyridine-3,5-dicarboxamide (IVf).** Yield 65%, mp 242–244°C (from DMF/H_2O), $[\alpha]_D^{25}$ = –114° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3468–3334 (N–H), 3085 (C–H_{arom}), 2990 (C–H_{aliph}), 1655 (C=O), 1535 [δ(N–H)], 1253 (C–N). ^1H NMR spectrum, δ, ppm: 0.89–0.97 m (12H, CH_3), 1.63–1.74 m (4H, CH_2), 2.25–2.36 m (2H, CH), 3.44 d (4H, CH_2), 4.23–4.35 m (2H, CH), 4.63–4.71 m (2H, CH), 7.15–7.68 m (20H, H_{arom}, CH=N), 8.38 s and 9.11 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.66 s, 8.87 s, and 9.27 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C,

ppm: 18.78 and 19.45 (4C, CH_3), 23.46 (2C, CH), 40.25 (2C, CH_2), 42.53 (2C, CH_2), 52.47 and 53.25 (4C, CH); 125.24, 125.92, 127.62, 128.44, 129.18, 131.18, 131.35, 138.65 (24C, C_{arom}); 131.34, 140.64, 152.54 (5C, pyridine); 142.76 (2C, CH=N), 163.72 and 169.52 (4C, C=O, amide), 177.78 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 928 (I_{rel} 32%) $[M]^+$. Found, %: C 58.35; H 5.28; N 12.01. $C_{51}H_{55}Br_2N_9O_6$. Calculated, %: C 58.22; H 5.20; N 11.65.

***N*,*N*′-Bis[(2*S*)-1-{(2*S*)-1-[2-(4-fluorobenzylidene)-hydrazinyl]-1-oxo-3-phenylpropan-2-ylamino}-4-methyl-1-oxopentan-2-yl]pyridine-3,5-dicarboxamide (IVg).** Yield 72%, mp 228–230°C (from DMF/H_2O), $[\alpha]_D^{25}$ = –104° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3475–3356 (N–H), 3082 (C–H_{arom}), 2992 (C–H_{aliph}), 1656 (C=O), 1537 [δ(N–H)], 1258 (C–N). ^1H NMR spectrum, δ, ppm: 0.92–0.98 m (12H, CH_3), 1.65–1.75 m (4H, CH_2), 2.26–2.35 m (2H, CH), 3.45 d (4H, CH_2), 4.24–4.32 m (2H, CH), 4.62–4.70 m (2H, CH), 6.98–7.62 m (20H, H_{arom}, CH=N), 8.36 s and 9.10 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.64 s, 8.88 s, and 9.26 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.65 and 19.36 (4C, CH_3), 23.41 (2C, CH), 40.24 (2C, CH_2), 42.50 (2C, CH_2), 52.43 and 53.29 (4C, CH); 115.45, 125.86, 127.48, 128.35, 129.12, 130.18, 138.68, 164.52 (24C, C_{arom}); 131.35, 140.62, 152.50 (5C, pyridine); 142.80 (2C, CH=N), 163.75 and 169.48 (4C, C=O, amide), 177.92 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 928 (I_{rel} 32%) $[M]^+$. Found, %: C 65.82; H 5.90; N 13.50. $C_{51}H_{55}F_2N_9O_6$. Calculated, %: C 66.00; H 5.97; N 13.58.

***N*,*N*′-Bis[(2*S*)-4-methyl-1-{(2*S*)-1-[2-(4-nitroro-benzylidene)hydrazinyl]-1-oxo-3-phenylpropan-2-ylamino}-1-oxopentan-2-yl]pyridine-3,5-dicarboxamide (IVh).** Yield 60%, mp 254–256°C (from DMF/H_2O), $[\alpha]_D^{25}$ = –86 (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3475–3322 (N–H), 3089 (C–H_{arom}), 2983 (C–H_{aliph}), 1656 (C=O), 1537 [δ(N–H)], 1253 (C–N). ^1H NMR spectrum, δ, ppm: 0.85–0.97 m (12H, CH_3), 1.63–1.72 m (4H, CH_2), 2.25–2.35 m (2H, CH), 3.45 d (4H, CH_2), 4.26–4.36 m (2H, CH), 4.65–4.73 m (2H, CH), 7.05–7.45 m (12H, H_{arom}, CH=N), 7.72–8.10 m (8H, H_{arom}), 8.39 s and 9.10 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.67 s, 8.87 s, and 9.24 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.65 and 19.38 (4C, CH_3), 23.42 (2C, CH), 40.17 (2C, CH_2), 42.54 (2C, CH_2), 52.41 and 53.27 (4C, CH); 120.85, 125.81, 127.46, 128.36, 130.05, 138.68, 139.25, 150.32 (24C, C_{arom}); 131.48, 140.62, 152.57 (5C, pyridine); 142.75 (2C, CH=N), 163.76 and 169.62 (4C, C=O, amide), 177.76 (2C, C=O, hydrazone). Mass spectrum: *m*/*z* 982 (I_{rel} 16%) $[M]^+$. Found, %: C 62.37; H 5.64; N 15.69. $C_{51}H_{55}N_{11}O_{10}$. Calculated, %: C 62.37; H 5.64; N 15.69.

(4*S*,7*S*,15*S*,18*S*)-7,15-Dibenzyl-4,18-diisobutyl-3,6,9,13,16,19,23-heptaazabicyclo[19.3.1]pentacosa-1(25),21,23-triene-2,5,8,14,17,20-hexaone (V). *Azide method*. A solution of 1 mmol of hydrazide **II** [1, 2] in a cold (–5°C) mixture of 5 N hydrochloric acid (2 mL), glacial acetic acid (3 mL), and water (10 mL) was stirred for 15 min. A solution of 0.2 g of sodium nitrite in 2 mL of water was added in one portion, and the mixture was stirred for 0.5 h. The azide formed *in situ* was extracted with cold methylene chloride (60 mL). The organic layer was washed with ice water, 3% aqueous sodium hydrogen carbonate, and water again and dried over anhydrous calcium chloride. The azide solution was added in one portion under stirring to a cold (–5°C) solution of propane-1,3-diamine in 25 mL of anhydrous methylene chloride. The mixture was stirred for 3 h at –5°C and then for 20 h at room temperature, washed with water, 0.5 N hydrochloric acid, and water again, and dried over anhydrous calcium chloride. The solvent was evaporated under reduced pressure, and the residue was purified by chromatography on silica gel using chloroform–ethanol (9 : 1, by volume) as eluent. Yield 65%, mp 196–198°C (from EtOH/Et_2O), $[\alpha]_D^{25}$ = –114 (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3435–3345 (N–H), 3080 (C–H_{arom}), 2988 (C–H_{aliph}), 1655 (C=O), 1536 [δ(N–H)], 1255 (C–N). ^1H NMR spectrum, δ, ppm: 0.90–0.96 m (12H, CH_3), 1.8 m (2H, CH_2), 1.66–1.78 m (4H, CH_2), 2.25–2.34 m (2H, CH), 3.20 t (4H, CH_2), 3.46 d (4H, CH_2), 4.27–4.35 m (2H, CH), 4.65–4.75 m (2H, CH), 6.98–7.52 m (10H, H_{arom}), 8.37 s and 9.08 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.65 s, 8.82 s, and 9.22 s (6H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 18.78 and 19.46 (4C, CH_3), 32.45 and 38.65 (3C, CH_2), 23.65 (2C, CH), 39.45 (2C, CH_2), 42.15 (2C, CH_2), 52.95 and 54.15 (4C, CH); 125.82, 127.52, 128.29, 138.75 (12C, C_{arom}); 131.48, 141.05, 152.36 (5C, pyridine); 166.75, 169.85, 173.15 (6C, C=O). Mass spectrum: *m*/*z* 725 (I_{rel} 15%) $[M]^+$. Found, %: C 66.10; H 7.00; N 13.42. $C_{40}H_{51}N_7O_6$. Calculated, %: C 66.19; H 7.08; N 13.51.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-4,19-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]hexacosa-1(26),22,24-triene-10-carboxylic acid (VI). A solution of 1 mmol of cyclic pentapeptide methyl ester **III** in 20 mL of methanol was cooled to –5°C, 25 mL of 1 N aqueous

sodium hydroxide was added, and the mixture was stirred for 6 h at –5°C and for 3 h at room temperature and concentrated under reduced pressure. The residue was acidified with 1 N hydrochloric acid to pH ~3, and the precipitate was filtered off, washed with water, dried, and crystallized from aqueous ethanol. Yield 75%, white powder, mp 210–212°C, $[\alpha]_D^{25}$ = –128° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 4474–3375 (O–H, N–H), 3086 (C–H_{arom}), 2976 (C–H_{aliph}), 1728 (C=O, acid), 1653 (C=O, amide), 1535 [δ(N–H)], 1255 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.82–0.95 m (12H, CH_3), 1.18–1.45 m (4H, CH_2), 1.58–1.72 m (4H, CH_2), 2.28–2.36 m (2H, CH), 3.05–3.24 m (2H, CH_2), 3.42 d (4H, CH_2), 3.84–4.12 m (4H, CH), 4.39–4.45 m (1H, CH), 7.04–7.54 m (10H, H_{arom}), 8.43 s and 9.12 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.76 s, 8.95 s, and 9.26 s (6H, NH, exchangeable with D_2O), 11.45 s (1H, OH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 17.55 and 18.96 (4C, CH_3); 28.32, 30.38, 38.05 (3C, CH_2); 23.82 (2C, CH), 41.98 and 41.95 (4C, CH_2), 52.25 and 52.84 (4C, CH), 58.43 (1C, C^{10}); 124.30, 128.31, 129.40, 138.75 (12C, C_{arom}); 131.45, 140.10, 152.18 (5C, pyridine); 163.80, 169.16, 170.65 (6C, C=O, amide), 174.65 (COOH). Mass spectrum: *m*/*z* 784 $[M]^+$. Found, %: C 64.30; H 6.74; N 12.45. $C_{42}H_{53}N_7O_8$. Calculated, %: C 64.35; H 6.81; N 12.51.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-4,19-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]hexacosa-1(26),22,24-triene-10-carbohydrazide (VII). Hydrazine hydrate, 0.35 mL (10 mmol) was added under stirring to a solution of 1 mmol of cyclic pentapeptide methyl ester **III** in 20 mL of methanol. The mixture was refluxed for 3 h and evaporated under reduced pressure, the residue was treated with diethyl ether, and the precipitate was filtered off and recrystallized from methanol–diethyl ether. Yield 68%, white powder, mp 235–237°C, $[\alpha]_D^{25}$ = –102° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3465–3332 (NH, NH_2), 3066 (C–H_{arom}), 2973 (C–H_{aliph}), 1656 (C=O, amide), 1528 [δ(N–H)], 1238 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.84–0.98 m (12H, CH_3), 1.24–1.46 m (4H, CH_2), 1.58–1.70 m (4H, CH_2), 2.28–2.35 m (2H, CH), 3.12–3.28 m (2H, CH_2), 3.48 d (4H, CH_2), 3.84–4.12 m (4H, CH), 4.34 br.s (2H, NH_2, exchangeable with D_2O), 4.39–4.45 m (1H, CH), 7.00–7.52 m (10H, H_{arom}), 8.38 s and 9.09 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.46 s, 8.75 s, 8.83 s, and 9.22 s (7H, NH, exchangeable with D_2O). Mass spectrum: *m*/*z* 798 $[M]^+$. Found, %: C 63.08; H 6.90; N 15.72. $C_{42}H_{55}N_9O_7$. Calculated, %: C 63.22; H 6.95; N 15.80.

Macrocyclic pentapeptide hydrazones VIIIa–VIIIe *(general procedure)*. A mixture of 1 mmol of hydrazide **VII**, 2 mmol of the corresponding substituted benzaldehyde, 2 mL of triethylamine, and 2 mL of diethylamine in 25 mL of anhydrous ethanol was heated for 4–6 h under reflux. The solvent was evaporated under reduced pressure, and the residue was crystallized by treatment with diethyl ether. The solid was collected by filtration, washed with diethyl ether, and recrystallized from appropriate solvent.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-*N*′-benzylidene-4,19-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]hexacosa-1(26),22,24-triene-10-carbohydrazide (VIIIa). Yield 74%, white powder, mp 265–267°C, $[\alpha]_D^{25}$ = –115° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3412–3342 (N–H), 3072 (C–H_{arom}), 2987 (C–H_{aliph}), 1654 (C=O), 1532 [δ (N–H)], 1236 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.87–0.99 m (12H, CH_3), 1.22–1.45 m (4H, CH_2), 1.57–1.72 m (4H, CH_2), 2.25–2.34 m (2H, CH), 3.10–3.27 m (2H, CH_2), 3.46 d (4H, CH_2), 4.05–4.10 m (4H, CH), 4.40–4.44 m (1H, CH), 7.12–7.67 m (16H, H_{arom}, CH=N), 8.35 s and 9.10 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.52 s, 8.78 s, 8.84 s, and 9.24 s (7H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 17.64 and 18.98 (4C, CH_3); 28.30, 30.34, 38.00 (3C, CH_2), 23.75 (2C, CH), 40.96 and 41.76 (4C, CH_2), 52.26 and 52.86 (4C, CH), 58.46 (1C, CH); 124.34, 128.35, 128.68, 129.12, 129.42, 131.00, 133.56, 138.71 (18C, C_{arom}); 142.78 (1C, CH=N); 131.48, 140.13, 152.19 (5C, pyridine); 163.81, 169.15, 170.62 (6C, C=O, amide); 177.75 (1C, C=O, hydrazone). Mass spectrum: *m*/*z* 886 (I_{rel} 8%) $[M]^+$. Found, %: C 66.31; H 6.60; N 14.14. $C_{49}H_{59}N_9O_7$. Calculated, %: C 66.42; H 6.71; N 14.23.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-4,19-diisobutyl-*N*′-(4-methylbenzylidene)-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]-hexacosa-1(26),22,24-triene-10-carbohydrazide (VIIIb). Yield 65%, white powder, mp 212–214°C, $[\alpha]_D^{25}$ = –68° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3430–3342 (N–H), 3080 (C–H_{arom}), 2986 (C–H_{aliph}), 1654 (C=O), 1535 [δ(N–H)], 1236 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.90–0.98 m (12H, CH_3), 1.22–1.40 m (4H, CH_2), 1.55–1.72 m (4H, CH_2), 2.20–2.34 m (5H, CH, CH_3), 3.14–3.26 m (2H, CH_2), 3.48 d (4H, CH_2), 4.04–4.12 m (4H, CH), 4.45–4.49 m (1H, CH), 7.02–7.60 m (15H, H_{arom}, CH=N), 8.48 s and 9.12 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.60 s, 8.85 s, 8.92 s, and 9.28 s (7H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 17.78, 18.97, 24.12 (5C, CH_3);

28.35, 30.38, 38.12 (3C, CH_2); 23.75 (2C, CH), 40.96 and 41.72 (4C, CH_2), 52.28 and 52.82 (4C, CH), 58.36 (1C, CH); 125.98, 127.48, 128.35, 129.05, 129.20, 130.12, 138.92, 140.45 (18C, C_{arom}), 142.74 (1C, CH=N); 131.48, 140.36, 152.23 (5C, pyridine); 163.82, 169.14, 170.62 (6C, C=O, amide); 177.60 (1C, C=O, hydrazone). Mass spectrum: *m*/*z* 900 (I_{rel} 16%) $[M]^+$. Found, %: C 66.60; H 6.75; N 13.88. $C_{50}H_{61}N_9O_7$. Calculated, %: C 66.72; H 6.83; N 14.01.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-4,19-diisobutyl-*N*′-(4-methylbenzylidene)-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]hexacosa-1(26),22,24-triene-10-carbohydrazide (VIIIc). Yield 78%, white powder, mp 254–256°C, $[\alpha]_D^{25}$ = –98° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3444–3356 (N–H), 3078 (C–H_{arom}), 2980 (C–H_{aliph}), 1655 (C=O), 1534 [δ(N–H)], 1237 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.92–0.96 m (12H, CH_3), 1.24–1.42 m (4H, CH_2), 1.54–1.70 m (4H, CH_2), 2.24–2.36 m (2H, CH), 3.12–3.25 m (2H, CH_2), 3.45 d (4H, CH_2), 3.71 s (3H, OCH_3), 4.00–4.14 m (4H, CH), 4.42–4.48 m (1H, CH), 6.97–7.64 m (15H, H_{arom}, CH=N), 8.42 s and 9.14 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.50 s, 8.82 s, 8.88 s, and 9.25 s (7H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 17.78 and 18.97 (4C, CH_3); 28.31, 30.35, 38.04 (3C, CH_2); 23.76 (2C, CH), 40.92 and 41.78 (4C, CH_2), 52.25 and 52.85 (4C, CH), 55.76 (1C, OCH_3), 58.38 (1C, CH); 114.43, 124.45, 125.43, 128.38, 129.35, 129.72, 138.76, 162,45 (18C, C_{arom}); 142.67 (1C, CH=N); 131.52, 140.22, 152.16 (5C, pyridine); 163.85, 169.18, 170.60 (6C, C=O, amide), 177.63 (1C, C=O, hydrazone). Mass spectrum: *m*/*z* 916 (I_{rel} 12%) $[M]^+$. Found, %: C 65.50; H 6.61; N 13.70. $C_{50}H_{61}N_9O_8$. Calculated, %: C 65.56; H 6.71; N 13.76.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-*N*′-(4-chlorobenzylidene)-4,19-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]hexacosa-1(26),22,24-triene-10-carbohydrazide (VIIId). Yield 72%, white powder, mp 232–234°C, $[\alpha]_D^{25}$ = –72° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3415–3325 (N–H), 3086 (C–H_{arom}), 2974 (C–H_{aliph}), 1655 (C=O), 1533 [δ(N–H)], 1233 (C–N). ^{1}H NMR spec-trum, δ, ppm: 0.86–0.95 m (12H, CH_3), 1.25–1.38 m (4H, CH_2), 1.52–1.65 m (4H, CH_2), 2.18–2.35 m (2H, CH), 3.15–3.25 m (2H, CH_2), 3.46 d (4H, CH_2), 4.00–4.15 m (4H, CH), 4.44–4.48 m (1H, CH), 7.05–7.62 m (15H, H_{arom}, CH=N), 8.42 s and 9.15 s (3H, 232-, 4-, 6-$H_{pyridine}$); 8.64 s, 8.84 s, 8.90 s, and 9.24 s (7H, NH, exchangeable with D_2O). ^{13}C NMR spectrum, δ_C, ppm: 17.86 and 18.96 (4C, CH_3); 28.36, 30.45, 38.18 (3C, CH_2); 23.78 (2C, CH), 40.93 and 41.75 (4C, CH_2), 52.34 and 52.84 (4C, CH), 58.35 (1C, CH); 125.95, 127.45, 128.32, 128.85, 130.18, 131.90, 136.34, 139.16 (18C, C_{arom}); 142.77 (1C, CH=N); 131.49, 140.39, 152.25 (5C, pyridine); 163.84, 169.18, 170.60 (6C, C=O, amide); 177.56 (1C, C=O, hydrazone). Mass spectrum: *m*/*z* 920 (I_{rel} 12%) $[M]^+$. Found, %: C 63.85; H 6.30; Cl 3.80; N 13.60. $C_{49}H_{58}ClN_9O_7$. Calculated, %: C 63.94; H 6.35; Cl 3.85; N 13.69.

(4*S*,7*S*,10*S*,16*S*,19*S*)-7,16-Dibenzyl-*N*′-(4-fluorobenzylidene)-4,19-diisobutyl-2,5,8,15,18,21-hexaoxo-3,6,9,14,17,20,24-heptaazabicyclo[20.3.1]hexacosa-1(26),22,24-triene-10-carbohydrazide (VIIIe). Yield 78%, white powder, mp 198–200°C, $[\alpha]_D^{25}$ = –104° (*c* = 0.5, DMF). IR spectrum, ν, cm^{-1}: 3433–3328 (N–H), 3080 (C–H_{arom}), 2978 (C–H_{aliph}), 1654 (C=O), 1534 [δ(N–H)], 1234 (C–N). ^{1}H NMR spectrum, δ, ppm: 0.87–0.97 m (12H, CH_3), 1.20–1.35 m (4H, CH_2), 1.50–1.64 m (4H, CH_2), 2.19–2.32 m (2H, CH), 3.19–3.28 m (2H, CH_2), 3.45 d (4H, CH_2), 3.98–4.16 m (4H, CH), 4.43–4.45 m (1H, CH), 6.96–7.56 m (15H, H_{arom}, CH=N), 8.46 s and 9.12 s (3H, 2-, 4-, 6-$H_{pyridine}$); 8.66 s, 8.88 s, 8.94 s, and 9.25 s (7H, NH, exchangeable with D_2O). ^{13}C NMR spec-trum, δ_C, ppm: 17.86 and 18.98 (4C, CH_3); 28.35, 30.48, 38.24 (3C, CH_2); 23.83 (2C, CH), 40.92 and 41.75 (4C, CH_2), 52.35 and 52.85 (4C, CH), 58.44 (1C, CH); 115.42, 125.90, 127.56, 128.42, 129.08, 130.65, 139.24, 165.02 (18C, C_{arom}); 142.68 (1C, CH=N); 131.65, 140.42, 152.28 (5C, pyridine); 163.80, 169.14, 170.62 (6C, C=O, amide); 177.64 (1C, C=O, hydrazone). Mass spectrum: *m*/*z* 904 (I_{rel} 35%) $[M]^+$. Found, %: C 65.00; H 6.40; N 13.85. $C_{49}H_{58}FN_9O_7$. Calculated, %: C 65.10; H 6.47; N 13.94.

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