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DESIGN OF DNA-CLEAVING MOLECULES WHICH INCORPORATE A SIMPLIFIED METAL-COMPLEXING MOIETY OF BLEOMYCIN AND LEXITROPSIN CARRIERS

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Abstract. The syntheses of **1a-c**, functional models for bleomycin, which are composed of a simplified metal-complexing moiety of bleomycin and poly-N-methylpyrrole peptides are described. Their functional cleavage of DNA in the presence of reductants is demonstrated.

The bleomycins(BLM) are a family of glycopeptide antitumor antibiotics isolated from the cultures of streptomyces verticillus by Umezawa and coworkers in 1966, and are clinically used in combination chemotherapy against several types of cancer.[1] Therefore they, especially bleomycin A_2 which is the main constituent of the clinically used mixture of BLM, have attracted considerable current interest both synthetically and biologically (Figure 1). The therapeutic effect of BLM is believed to arise from its ability to cause DNA

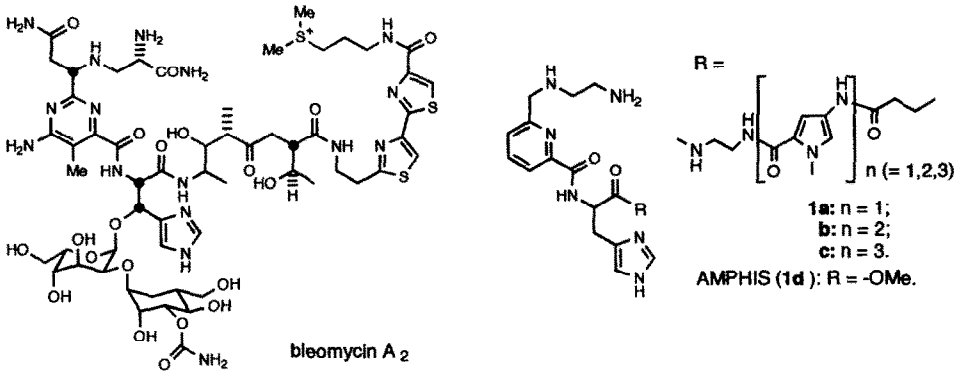
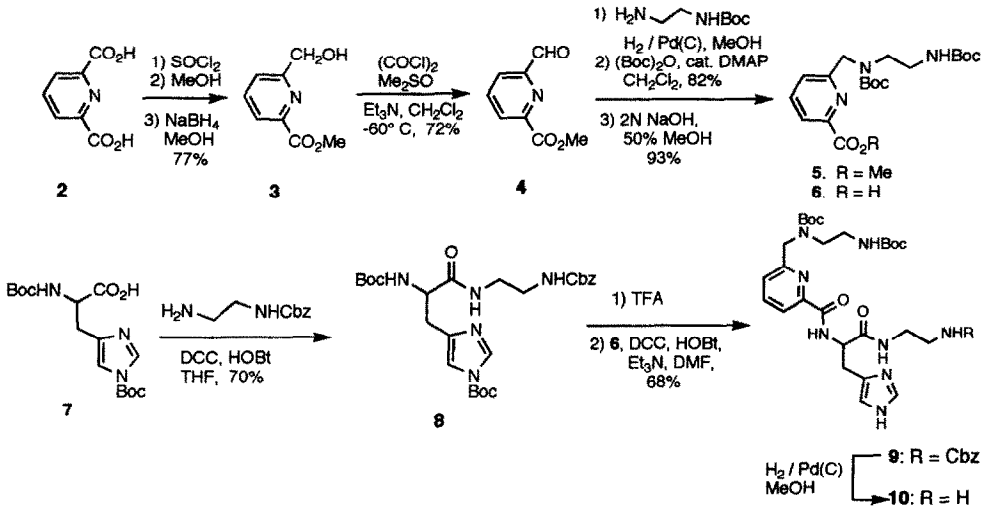


Figure 1

degradation based on a unique mechanism, in which the subunit of the amine-pyrimidine-imidazole coordinates with metal ions such as Fe^{2+} susceptible to oxygen activation; while the subunit of tetrapeptide S bithiazole and terminal sulfonium cation are responsible for DNA binding affinity.[2] Many studies have been reported on the synthesis of simplified analogues of bleomycin in the last decade.[3] Most of them focused on the simplification of the DNA active moiety, i.e metal-complexing subunit.[4] Henichart has reported the synthesis of the simplest subunit, methyl 2-(2-aminoethyl)-amino-methyl-pyridine-6-carboxyl-histidinate (AMPHIS) **1d** and its BLM analog which retains the major characteristics of the natural products.[3c,d;5] However, little work has been reported on the carrier -- DNA binding subunit.[6] Ohno et al has reported a synthetic hybrid of PYML-distamycin.[6b] We report herein another simplified synthetic functional model of bleomycin. Unlike other approaches that have been

adopted in which attempts were made to build a model by simplifying the natural bleomycin, we have constructed a effective hybrid agent by upgrading the basic structural features of the natural product. We decided to conjugate the simplest model AMPHIS with lexitropsin residues which are recognized as sequence-selective DNA binding moieties,[7] eliminating, at first, the linker and the terminal charged moiety. Furthermore, in contrast to most reported on syntheses of bleomycin analogs, we connect the metal-complexing moiety to the C-terminal of the carrier.

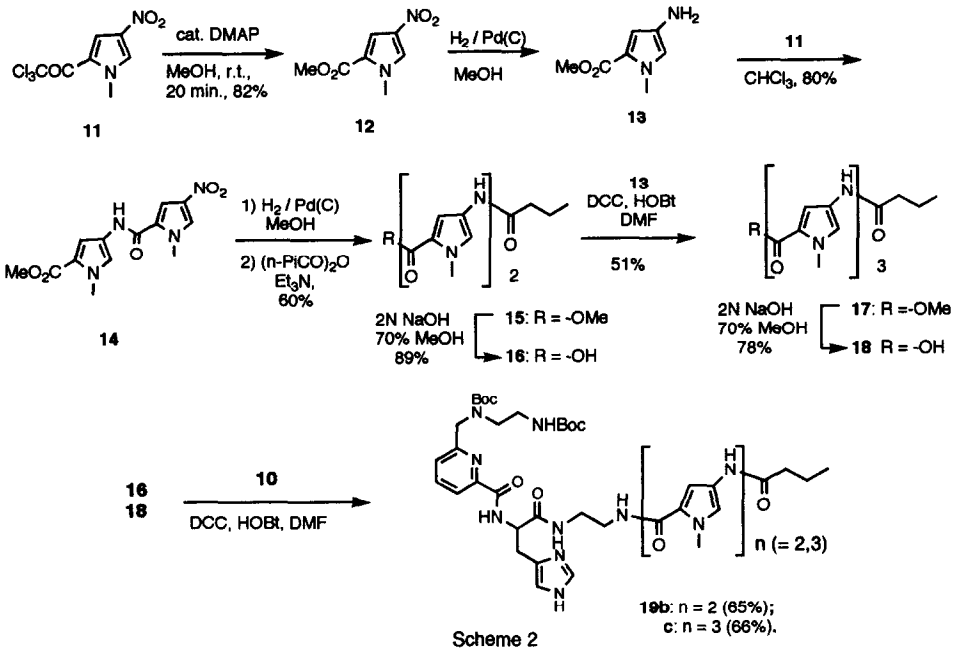
Syntheses of 1a-c. Synthesis of the protected complexing portion **10** was accomplished by the procedure Henichart et al. reported with some modifications.[5a,b] Swern oxidation of methyl 6-hydroxymethyl-2-pyridinecarboxylate **3** derived from the reduction of the corresponding dicarboxylate[8] with $NaBH_4$ in methanol afforded methyl 6-formyl-2-pyridinecarboxylate **4**[9] in a yield of 72% (Scheme 1). Catalytic hydrogenation of a solution of **4** and mono N-Boc-ethylenediamine[10] (1:1) in methanol in hydrogen atmosphere (1 atmospheric



Scheme 1

pressure) in the presence of 10% of 10% Pd(C) followed by the protection of the secondary amine group by di *t*-butyl dicarbonate in CH_2Cl_2 provided **5** in excellent yield. Hydrolysis of **5** afforded the acid **6** quantitatively. Coupling of diprotected histidine **7** with mono N-Cbz-ethylenediamine[10] in the presence of 1,3-dicyclohexylcarbodiimide(DCC), and 1-hydroxybenzotriazole(HOBt) in THF afforded amide **8** in 70%. Deprotection of **8** by trifluoroacetic acid(TFA) followed by coupling with **6** in the presence of DCC, HOBt and Et_3N in DMF provided the fully protected metal-complexing subunit **9**. Selective deprotection of **9** by catalytic hydrogenation afforded **10** quantitatively, which was used directly in the coupling with the lexitropsin carriers.

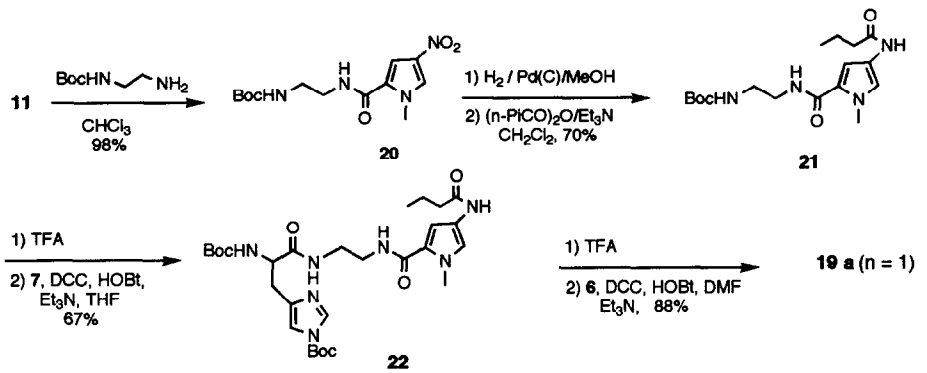
There are many possible routes to synthesize dipyrrole and tripyrrole peptides. In our synthesis, we selected 1-methyl-2-trichloroacetylpyrrole **11** as a key intermediate.[11] Solvolysis of **11** in methanol in the presence of catalytic amount of 4-dimethylaminopyridine(DMAP) provided the methyl ester **12** in 82% yield(Scheme 2).[12] Conversion of **12** to **13** followed by coupling with **11** afforded the dipeptide **14** in 80% yield.[12] Reduction of **14** by catalytic hydrogenation, acylation of the resulting amine with butyric anhydride, and hydrolysis of the ester gave rise to the netropsin moiety **16**. Coupling of **16** with **13** with DCC and HOBt in DMF provided tripeptide



Scheme 2

17 in modest yield, which was then hydrolyzed to the corresponding acid **18** in 78% yield. Coupling of the metal-complexing subunit **10** with the carriers **16** and **18** in the presence of DCC and HOBt in DMF resulted in the protected form of the hybrids **19b** and **19c** in 65% and 66% respectively.

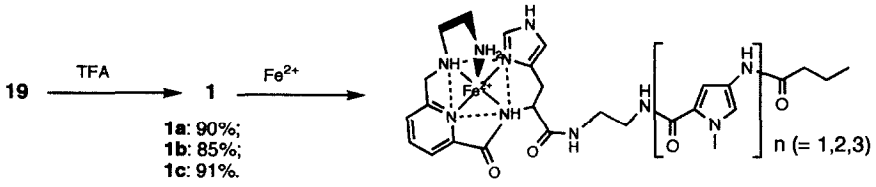
Compound **19a** was synthesized by a different strategy. Condensation of **11** with mono N-Boc-ethylenediamine afforded the amide **20** quantitatively (Scheme 3). Subsequent hydrogenation and acylation with butyric anhydride provided **21** in 70% yield. Coupling of the deprotected **21** with **7** afforded **22** in a yield of 67%. Coupling of the deprotected **22** with **6** under similar conditions afforded the protected hybrid **19a** in 88%



Scheme 3

yield.

Finally, deprotecting **19a-c** in TFA, purifying the residues on Amberlite RAD-2 resin provided the pure hybrids **1a-c** in excellent yields.[13]



Scheme 4

DNA cleavage studies. Examination of the ability of the Fe(II) complexes of **1a-c** to cleave duplex DNA was carried out through the inspection of the reaction of the hybrids in the presence of Fe(II) and thiol reductants with pBR322 supercoiled DNA by both ethidium fluorescence assay and agarose gel electrophoresis. It was observed that the ability of complexes to cleave DNA is increased with the pyrrole units in DNA binding subunit of the hybrids (Figure 2), especially under aerobic condition, consistent with the anticipated mechanism of affinity cleavage. The agarose gel electrophoresis indicates that these complexes cleave DNA very efficiently, resulting in

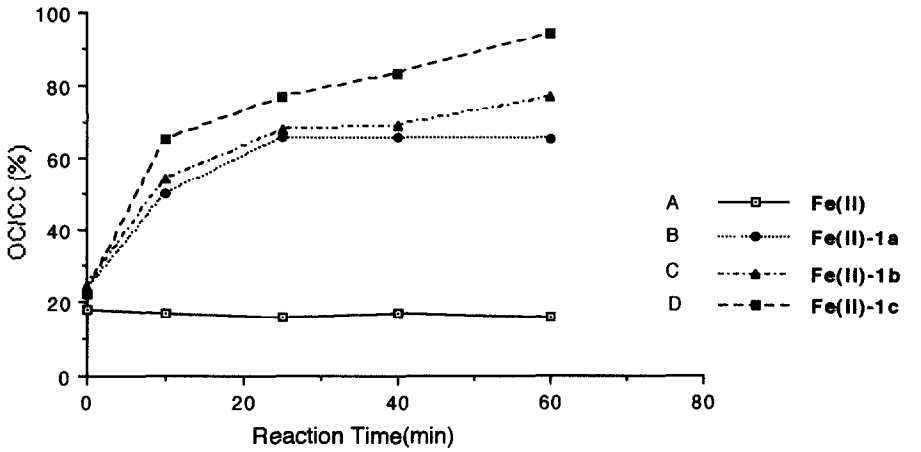


Figure 2. Plots of the percentage of the opened circular DNA(OC) , (OC /CC) measured by ethidium fluoresence assay against reaction time. The reactions were run at 24°C under aerobic condition . 70 μL of reaction mixture contained 50 μg/mL of pBR322 closed circular supercoiled DNA(CC) in 8.5 mM of Tris buffer pH 8.0, 1 mM of 1,4-dithiothreitol(DTT), and Line A: 5 μM of Fe(II); Line B, C, D: 80 μM of Fe(II)-**1a, b, c**(1:1)respectively. 10 μL of reaction mixtures were used for each point.

mainly single-strand breaks of duplex DNA (Figure 3). The detailed study of the DNA cleavage and the DNA affinity including auto-cleavage footprinting, and syntheses of upgraded functional models are currently in progress and will be reported in due course.

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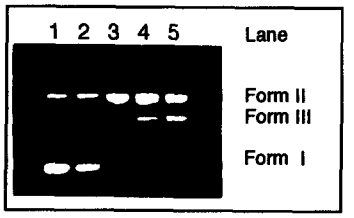

Figure 3. Supercoiled DNA cleavage by Fe(II)-1 (1:1). The reactions were run under the same conditions as shown in Figure 2. Lane 1, control DNA; Lane 2, 5 μM of Fe(II); Lane 3, 80 μM of Fe(II)-**1a** ; Lane 4, 80 μM of Fe(II)-**1b** ; Lane 5, 80 μM of Fe(II)-**1c**. Form I = closed circular DNA ; Form II = opened circular DNA; Form III = linear DNA.

References and Notes

1. a) Umezawa, H.; Maeda, K.; Takeuchi, T.; Okami, Y. J. Antibiot. Ser. A **1966**, *19*, 20. b) Blum, R. H.; Carter, S. K.; Agre, K. A. *Cancer* **1973**, *31*, 903.
2. a) Hecht, S. M. *Acc. Chem. Res.* **1986**, *19*, 383. b) Chein, M.; Grollman, A. P.; Horwitz, S. B. *Biochemistry* **1977**, *16*, 3641. c) Kross, J.; Henner, D.; Haseltine, W. A.; Rodriguez, L.; Levin, M. D.; Hecht, S. M. *Biochemistry* **1982**, *21*, 3711. d) Sugiura, Y.; Suzuki, T. *J. Biol. Chem.* **1982**, *257*, 10544. e) Kuwahara, J.; Sugiura, Y. *Proc. Natl. Acad. Sci. USA* **1988**, *85*, 2459. f) Review: Stubbe, J.; Kozarich, J. W. *Chem. Rev.* **1987**, *87*, 1107.
3. a) Otsuka, M.; Kittaka, A.; Ohno, M.; Suzuki, T.; Kuwahara, J.; Sugiura, Y.; Umezawa, H. *Tetrahydron Lett.* **1986**, *27*, 3639. b) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2821. c) Kenani, A.; Lohez, M.; Houssin, R.; Helbecque, N.; Bernier, J. L.; Lemay, P.; Henichart, J. P. *Anti-cancer Drug Design* **1987**, *2*, 47. d) Saito, I.; Morii, T.; Obayashi, T.; Sera, T.; Sugiura, H.; Matsuura, T. J. *Chem. Soc. Chem. Commun.* **1987**, 360. e) Boger, D. L.; Menezes, R. F.; Dang, Q.; Yang, W. BioMed. *Chem. Lett.* **1992**, *2*, 261. f) Owa, T.; Haupt, A.; Otsuka, M.; Kobayashi, S.; Tomioka, N.; Itai, A.; Ohno, M.; Shiraki, T.; Uesugi, M.; Sugiura, Y.; Maeda, K. *Tetrahedron* **1992**, *48*, 1193.
4. a) Otsuka, M.; Yoshida, M.; Kobayashi, S.; Ohno, M. *J. Am. Chem. Soc.* **1981**, *103*, 6986. b) Kilkuskie, R. E.; Suguna, H.; Tellin, B.; Murugesan, N.; Hecht, S. M. *J. Am. Chem. Soc.* **1985**, *107*, 260. c) Sugano, Y. Kittaka, A.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3635. d) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M.; Sugiura, Y.; Umezawa, H. *Tetrahedron Lett.* **1986**, *27*, 3631. e) Brown, S.; Masoharak, P. K. *J. Am. Chem. Soc.* **1988**, *110*, 1996. f) Kittaka, A.; Sugano, Y.; Otsuka, M.; Ohno, M. *Tetrahedron* **1988**, *44*, 2811. g) Lomis, T. J.; Siuda, J. F.; Shepherd, R. E. J. Chem. *Soc. Chem. Commun.* **1988**, 290. h) Cristini, M.; Scrimin, P.; Tonellato, U. *Tetrahedron Lett.* **1989**, *30*, 2987. i) Lomis, T. J.; Elliott, M. G.; Siddiqui, S.; Moyer, M.; Koepsel, R. R.; Shepherd, R. E. Inorg.*Chem.* **1989**, *28*, 2369. j) Lomis, T. J.; Martin, J.; McCloskey, B.; Zhang, S.; Siddiqui, S.; Shepherd, R. E. Siuda, J. F. *Inorganica Chimica Acta* **1989**, *157*, 99. k) Shepherd, R. E. *Inorganica Chimica Acta* **1990**, *171*, 139. l) Scheich, L. A.; Gosling, P.; Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *Inorg. Chem.* **1991**, *30*, 1677. m) Suga, A.; Sugiyama, T.; Otsuka, M.; Ohno, M.; Sugiura, Y,; Maeda, K. *Tetrahedron* **1991**, *47*, 1191. n) Tan, J. D.; Hudson, S. E.; Brown, S. J.; Olmstead, M. M.; Mascharak, P. K. *J. Am. Chem. Soc.* **1992**, *114*, 3841.
5. a) Henichart, J. D.; Houssin, P.; Bernier, J. L.; Catteau, J. P. *J. Chem. Soc. Chem. Commun.* **1982**, 1295. b) Henichart, J. P.; Bernier, J. L.; Houssin, R.; Lohez, M.; Kenani, A.; Catteau, J. P. *Biochem.*

Biophys. Res. Commun. **1985**, *126*, 1036. c) Shinozuka, K.; Morishita, H.; Yamazaki, T.; Sugiura, Y.; Sawai, H. *Tetrahedron Lett.* **1991**, *32*, 6869.

6. a) Hamamichi, N.; Natrajan, A.; Hecht, S. M. *J. Am. Chem. Soc.* **1992**, *114*, 6278. b) Otsuka, M.; Masuda, T.; Haupt, A.; Ohno, M.; Shiraki, T.; Sugiura, Y.; Maeda, K. *J. Am. Chem. Soc.* **1990**, *112*, 838.
7. a) Taylor, J. S.; Schultz, P. G.; Dervan, P. B. *Tetrahedron* **1984**, *40*, 457. b) Dervan, P. B. *Science* **1986**, *232*, 464. c) Review: Lown, J. W. *Anti-Cancer Drug Design* **1988**, *3*, 25; and *Antiviral Res.* **1992**, *17*, 179. d) Review: Zimmer, C.; Wahnert, U. *Prog. Biophys. Molec. Biol.* **1986**, *47*, 31.
8. Fife, T. H.; Przystas, T. J. *J. Am. Chem. Soc.* **1982**, *104*, 2251.
9. Mathes, W.; Sauermilch, W.; Klein, T. *Chem. Ber.* **1953**, *86*, 584.
10. Reaction of 3 equivalents of ethylenediamine with 1 equivalent of di *t*-butyl dicarbonate or benzyl chloroformate at room temperature in CH_2Cl_2 provided mono N-Boc- or N-Cbz-ethylenediamine in more than 70% yield.
11. a) Nishiwaki, E.; Tanaka, S.; Lee, H.; Shibuya, M. *Heterocycles* **1988**, *27*, 1954. b) Nishiwaki, E.; Nakagawas, H.; Takasaki, M.; Matsumoto, T.; Sakurai, H.; Shibuya, M. *Heterocycles* **1990**, *31*, 1763.
12. a) Bialer, M.; Yagen, B.; Mechoulan, R. Tetrahedron **1978**, *34*, 2389. b) Lown, J. W.; Krowicki, K. *J. Org. Chem.* **1985**, *50*, 3774.
13. For **1a**: ^{1}H-NMR(DMSO-d_6, 300 MHz): δ 9.73(s, 1H, -CONH-), 8.94(d,J = 8.0Hz, 1H,-CONH-), 8.29(br. 1H, -CONH-), 8.17(s, 1H), 8.11(br. 1H, -CONH-), 8.02(t, J = 8.0 Hz, 1H), 7.83(t, J = 8.0 Hz, 1H), 7.68(d, J = 8.0 Hz, 1H), 7.10(d, J = 2 Hz, 1H), 7.05(s, 1H), 6.68(d, J = 2.0 Hz, 1H), 4.72(m, 1H), 4.24 (s, 2H), 3.73(s, 3H), 3.30--2.98(m, 10H), 2.18(t, J = 7.5 Hz, 2H), 1.56(sex., J = 7.5 Hz, 2H), 0.88(t, J = 7.5 Hz, 3H). FT-IR(CH_2Cl_2 cast): 3600 - 2400(br.); 2966(w); 2940(w); 1675(s); 1596(m); 1576(m); 1528(s); 1204(s); 1183(m); 1134(m) cm^{-1}. FABHRMS m/e 567.3156(M^++1, $C_{27}H_{38}N_{10}O_4H$, requires: 567.3155); **1b**: ^{1}H-NMR(DMSO-d_6, 300MHz): δ 9.88(s, 1H, -CONH-), 9.78(s, 1H, -CONH-), 9.00(d, J = 8.5 Hz, 1H, -CONH-), 8.23(br. 1H, -CONH-), 8.16(br. 1H, -CONH-), 7.95(t, J = 7.5 Hz, 1H, -CONH-), 7.88(d, J = 7.5 Hz, 1H), 7.63(dd, J = 7.5 Hz, J = 1.0 Hz, 1H), 7.60(s, 1H), 7.18(d, J = 2.0 Hz, 1H), 7.13(d, J = 2.0 Hz, 1H), 6.85(m, 3H), 3.94(s, 2H), 3.80(s, 3H), 3.76(s, 3H), 3.28--3.10(m, 4H), 3.10--3.03(m, 2H), 2.93(t, J = 5.0 Hz, 2H), 2.79(t, J = 5.0 Hz, 2H), 2.19(t, J = 7.5 Hz, 2H), 1.57(sex., J = 7.5 Hz, 2H), 0.88(t, J = 7.5 Hz, 3H); FT-IR(CH_2Cl_2 cast): 3600-2400(br.); 2963(m); 2937(m); 1674(s); 1593(m); 1580(m); 1528(s); 1437(m); 1205(s); 1180(s); 1134(m) cm^{-1}. FABHRMS m/e: 689.3636(M^++1, $C_{33}H_{44}N_{12}O_5H$, requires: 689.3602). **1c**: ^{1}H-NMR(DMSO-d_6, 300 MHz): δ 9.90(s, 1H, -CONH-), 9.89(s, 1H, -CONH-), 9.04(d, J = 6.0 Hz, 1H, -CONH-), 8.25(br. 1H, -CONH-), 8.16(br. 1H,-CONH-), 8.00--7.86(m, 2H), 7.70--7.60(m, 3H), 7.23(d, J = 2.0 Hz, 1H), 7.19(d, J = 2.0 Hz, 1H), 7.15(d, J = 2.0 Hz, 1H), 7.03(d, J = 2.0 Hz, 1H), 6.89(s, 1H), 6.88(s, 1H), 6.87(s, 1H), 4.64(m, 1H), 4.02(s, 2H), 3.83(s, 3H), 3.82(s, 3H), 3.76(s, 3H), 3.22(m, 4H), 3.07(t, J = 6.0 Hz, 2H), 2.98(t, J = 6.0 Hz, 2H), 2.86(d, J = 6.0 Hz, 2H), 2.20(t, J = 7.5 Hz, 2H), 1.57(sex., J = 2.0 Hz, 2H), 0.88(t, J = 7.5 Hz, 3H). FT-RI(CH_2Cl_2 cast): 3600 - 2400(br.); 2961(m); 2935(m); 1677(s); 1594(m); 1580(m); 1530(s); 1436(m); 1206(s); 1183(m); 1136(m) cm^{-1}. FABHRMS m/e: 811.4101(M^++1, $C_{39}H_{50}N_{14}O_6H$, requires: 811.4116).