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Synthesis and study of anti-inflammatory activity of some novel cyclophane amides

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Abstract—Macrocyclic di- and tetra-amides with thia- and oxylinkages were synthesized and screened for in vitro anti-inflammatory activity. Cyclophane diamide **15** showed a dose-dependent activity, while the other cyclophane amides **16**–**20** exhibited mild activity.

Synthesis of new supramolecules architecturally novel and of potential importance in the context of designing simple models for studying biomolecular interactions stimulates the imaginative skill of synthetic chemists. The basic crown ether has been modified by substituting the oxygen donor atom by sulfur and/or nitrogen atom and introducing functional groups, viz., amide, ester in the ring to use them as models of protein–metal binding sites in biological systems,[1] synthetic ionophores, therapeutic reagents in chelate therapy, cyclic antibiotics,[2] and to study host–guest interactions.[3] Cyclic amides play important role in various biological systems.[4] Cystine based cyclic peptide has the unique ability of forming double-helical structure.[5] Cyclic peptides with open pores are useful as transport vehicles for biologically important ions or neutral molecules.[6] The self-assembly of acyclic peptides and their ability to form β-sheet structures have been demonstrated.[7,8] Adamantane-based supramolecular systems also form double-helical cyclic structures.[8,9] Macrocyclic hexa-amides, which can effectively bind peptides, have been reported.[10] Supramolecular amides have been also used as molecular receptors and in molecular recognition of biologically interacting substrates[11] including anti-HIV active macrocyclic amides.[12] Copper complexes of macrocyclic compounds exhibit increased antibiotic and antifungal activity than the uncomplexed macrocyclic compounds.[13] Thus macrocyclic compounds containing amide linkages are found to be biologically active. Hence we are interested in the synthesis and study of anti-inflammatory activity of cyclophane amides.

Diamines **1**–**6** were synthesized and used for the preparation of cyclophane amides.

Diamine **1** and **2**[14] were prepared by the reaction of their corresponding dihalides **7** and **8** with 2-aminothiophenol in methanolic KOH at room temperature. Dichloride **8**[15] was obtained by the reaction of *o*-phenylenediamine with 2 equiv of chloroacetyl chloride in the presence of triethylamine in methylene chloride. Diamine **3** was prepared by the reaction of the corresponding dibromide[16] **9** with 2-aminothiophenol in toluene and aq KOH in the presence of tetrabutylammonium bromide at reflux. Nitro compounds **10** and **11** were obtained by the alkylation of 4-nitrobenzyl bromide and 1-(4-nitrophenoxy)-2-bromo ethane with 2-aminothiophenol, respectively. Reduction of the nitro compounds **10** and **11** with iron and dil HCl gave the diamine **4**[17] and **5** in 62% and 70% yields, respectively (Scheme 1).

In order to synthesize the cyclic tetra-amide with oxy linkages, diamine **6** was obtained in 65% yield by the reaction of diacid chloride **12** with 2 equiv of *N*-phenylethylenediamine (Scheme 2).

Diacid chloride **14**[18] was obtained by the reaction of the corresponding diacid **13** with excess thionyl chloride in

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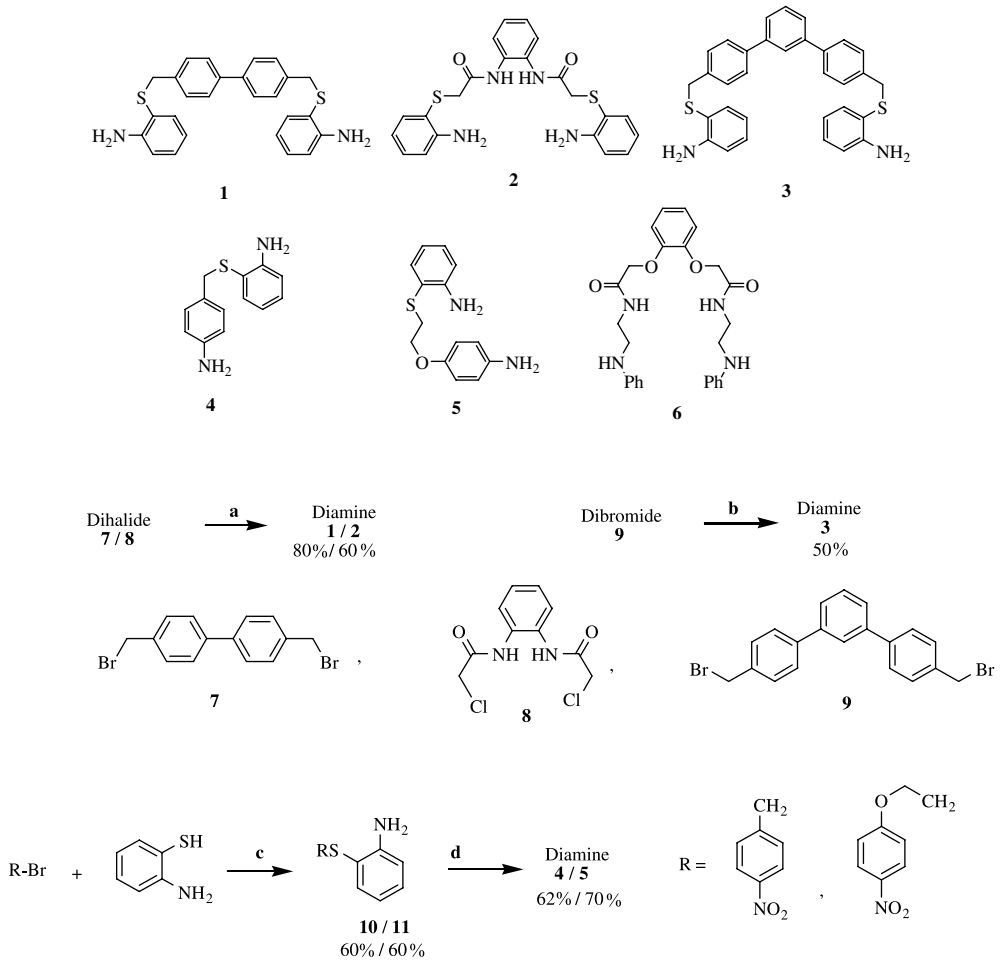
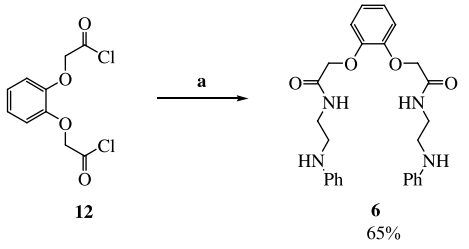


Scheme 1. Reagents and conditions: (a) 2-aminothiophenol (2 equiv), KOH, methanol, room temperature; (b) 2-aminothiophenol (2 equiv), aq KOH, toluene, TBAB, reflux, 4 h; (c) KOH, methanol, room temperature; (d) activated iron powder, dil HCl, reflux, 3 h.



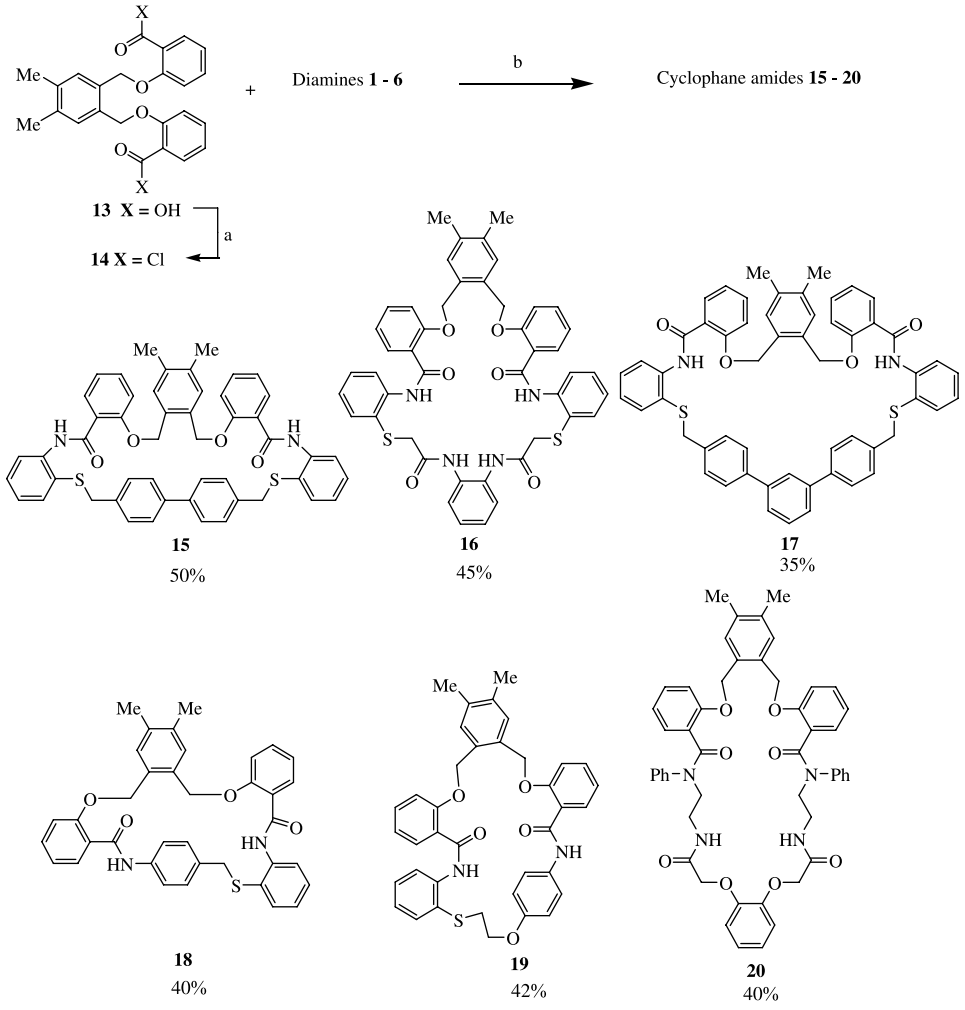
Scheme 2. Reagents and conditions: (a) *N*-phenylethylenediamine (2 equiv), TEA, CH_2Cl_2, room temperature, 1 h.

the presence of triethyl amine in methylene chloride. Thus diacid chloride **14** was reacted with diamines **1**–**6** to give cyclophane amides **15**–**20** in 50%, 45%, 35%, 40%, 42%, and 40% yields, respectively (Scheme 3).

In ^{1}H NMR spectrum cyclophane amide **15**[19] displayed the aromatic methyl, SCH_2, and OCH_2 protons as singlets at δ 2.16, δ 3.95, and δ 4.80, respectively, in addition to aromatic protons. The protons attached to the amide nitrogen appeared at δ 9.68 as a broad singlet. In ^{13}C NMR spectrum aromatic methyl, SCH_2, OCH_2, and carbonyl carbons appeared at δ 19.4, 41.9, 67.9, and 164.0 in addition to the aromatic carbons. The molecular ion appeared at *m*/*z* 798 in the FAB mass spectrum for cyclophane amide **15**, which further confirms the proposed structure. Cyclophane amides **16**[20] and **17**[21] were also completely characterized by spectral and analytical data.

Cyclophane amide **18**[22] in ^{1}H NMR spectrum displayed the aromatic methyl protons as two singlets at δ 2.25 and δ 2.27, SCH_2 protons as singlet at δ 3.65, OCH_2 protons as two singlets at δ 5.08 and δ 5.17, and the amide protons as a broad singlet at δ 9.69 in addition to the aromatic protons. In ^{13}C NMR spectrum cyclophane amide **18** displayed two aromatic methyl carbons at δ 19.6 and δ 19.8, SCH_2 carbon at δ 43.3, OCH_2 carbons at δ 69.5 and δ 70.8, and carbonyl carbons at δ 162.8 and δ 165.7 in addition to the aromatic carbons. In the mass spectrum the molecular ion appeared at *m*/*z* 600.

Cyclophane amide **19**[23] in ^{1}H NMR spectrum displayed the aromatic methyl protons as two singlets at δ 2.25 and δ 2.33, SCH_2CH_2O protons as two triplet at δ 2.97 and δ 3.77 with $J = 4.8$ Hz, OCH_2 protons as two singlet at δ 5.01 and δ 5.06 in addition to the aromatic protons, and the amide protons as a broad singlet at δ 9.13. In ^{13}C NMR spectrum cyclophane amide **19**



Scheme 3. Reagents and conditions: (a) $SOCl_2$, TEA, CH_2Cl_2, reflux, 3 h; (b) TEA, $CHCl_3$, room temperature, 6 h.

displayed two aromatic methyl carbons at δ 19.2 and δ 19.8, SCH_2CH_2O carbons at δ 37.7 and δ 64.9, OCH_2 carbons at δ 66.5 and δ 70.9, and carbonyl carbons at δ 162.5 and δ 165.0 in addition to the aromatic carbons. In the FAB mass spectrum, the molecular ion appeared at *m*/*z* 630. Similarly cyclophane amide **20**[24] was also completely characterized by spectral and analytical data.

Anti-inflammatory activity. Cyclophane amides **15**–**20** were screened for in vitro anti-inflammatory activity. The HRBC membrane stabilization[25] has been used as a method to study the anti-inflammatory activity using prednisolone as standard drug.

Blood was collected from healthy volunteers and the collected blood was mixed with equal volume of sterilized Alsever's solution (2% dextrose, 0.8% sodium citrate, 0.05% citric acid, and 0.42% sodium chloride). The blood was centrifuged at 1500 rpm and the packed cells were washed with isotonic NaCl (0.85%, pH 7.2) and a 10% v/v suspension of the packed cells was made with isotonic NaCl. The assay mixture contained the drug (concentration as mentioned in Table 1), 1 ml phosphate buffer (0.15 M, pH 7.4), 2 ml of hypotonic NaCl (0.36%), and 0.5 ml HRBC suspension. Prednisolone 100 μg was used as the reference drug. Instead of hypotonic NaCl, 2 ml of distilled water was used in the control. All the assay mixtures were incubated at 37 °C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer (systronics model) at 500 nm. The percentage hemolysis was calculated by assuming the hemolysis produced in the presence of distilled water as 100%. The percentage of HRBC membrane stabilization or protection was calculated using the formula:

Table 1. Effect of cyclophane amides **15**–**20** on HRBC membrane stabilization

Compound	Activity (% prevention of lysis)			
	10 μg/ml	50 μg/ml	100 μg/ml	200 μg/ml
15	65.17 ± 0.95	82.43 ± 0.48	89.67 ± 0.68	91.00 ± 0.58
16	40.53 ± 0.75	42.60 ± 0.95	48.72 ± 0.31	49.65 ± 0.49
17	48.27 ± 0.61	49.37 ± 0.54	51.42 ± 0.59	54.14 ± 0.38
18	50.31 ± 0.73	52.27 ± 0.39	56.04 ± 0.18	60.86 ± 0.46
19	44.28 ± 0.11	45.63 ± 0.64	50.25 ± 0.63	51.27 ± 0.63
20	38.84 ± 0.44	41.72 ± 0.61	45.25 ± 0.86	47.97 ± 0.09
Prednisolone	—	59.34 ± 0.44	86.37 ± 0.49	—

$$\text{Activity (\% prevention of lysis)} = 100 - \frac{\text{OD of drug treated sample}}{\text{OD of control}} \times 100$$

The lysosomal enzymes released during inflammation produce a variety of disorders. This extracellular activity of these enzymes is related to acute or chronic inflammation. Since the HRBC membranes are similar to lysosomal membrane components, the prevention of hypotonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drug.

The degree of anti-inflammatory activity in cyclophane amide **15** at 50 μg/ml was found to be 82.43%, whereas that of the reference drug prednisolone, at the same concentration, was 59.34%. The activity of cyclophane amide **15** was found to be more than that of prednisolone which clearly shows that cyclophane amide **15** is superior to the reference drug prednisolone. Though the degree of anti-inflammatory activity in cyclophane amide **18** at 50 μg/ml was 52.27% which is some what nearer to the reference drug prednisolone, at 100 μg/ml it was only 56.04% which indicates that cyclophane amide **18** is inferior to prednisolone. Cyclophane amides **16**, **17**, **19**, and **20** did not show much activity. The results are summarized in Table 1.

Cyclophane amides **15**–**20** were synthesized by the acylation of the corresponding diamines with acid chloride **14**. Cyclophane amides **15**–**20** showed a dose-dependent effect in HRBC membrane stabilization. Anti-inflammatory activity was more in cyclophane amide **15** than the cyclophane amides **16**–**20**.

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References and notes

1. Hosseini, M. W.; Lehn, J. M.; Duff, S. R.; Gu, K.; Mertes, M. P. *J. Org. Chem.* **1987**, *52*, 1662.
2. Dobler, M.. In *Comprehensive Supramolecular Chemistry*; Gokul, G. W., Ed.; Pergamon: New York, 1996; Vol. 1, p 267.
3. Bradshaw, J. S.; Izatt, R. M.; Bordunov, A. V.; Zhu, C. Y.; Hathway, J. K.. In *Comprehensive Supramolecular Chemistry*; Gokel, G. W., Ed.; Pergamon: New York, 1996; Vol. 1, p 35.
4. Sprengard, U.; Schudok, M.; Schmidt, W.; Kretzschmar, G.; Kunz, H. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 321.
5. Karle, I. L.; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1996**, *118*, 10916.
6. Kataoka, H.; Katagi, T. *Tetrahedron* **1987**, *43*, 4519.
7. Karle, I. L.; Ranganathan, D.; Kurur, S. *J. Am. Chem. Soc.* **1999**, *121*, 7156.
8. Karle, I. L.; Ranganathan, D.; Haridas, V. *J. Am. Chem. Soc.* **1998**, *120*, 6903.
9. Ranganathan, D.; Haridas, V.; Nagaraj, R.; Karle, I. L. *J. Org. Chem.* **2000**, *65*, 4415.
10. Eun, J. J.; Soo, Y. S. *Bull. Korean Chem. Soc.* **2002**, *23*, 1483.
11. Cheng, S.-K.; Van Ergen, D.; Fan, E.; Hamilton, A. D. *J. Am. Chem. Soc.* **1991**, *113*, 7640.
12. (a) Jhaumeer-Laulloo, B. S.; Witvrouw, M. *Indian J. Chem.* **2000**, *B*, 842; (b) Jhaumeer-Laulloo, B. S. *Asian J. Chem.* **2000**, *12*, 775.
13. Sulekh, C.; Sangeetika, T.; Shalini, T. *Transition Met. Chem.* **2004**, *29*, 925.
14. Cheng, C.-C.; Rokito, S. E.; Burrows, C. J. *Angew. Chem., Int. Ed. Engl.* **1993**, *32*, 277.
15. Halit, K.; Guelsev, D.; Yasar, G.; Uemmuehan, O.; Riza, A. *Transition Met. Chem.* **2003**, *28*, 51.
16. Du, C.-J. F.; Hart, H.; Ng, K. K. D. *J. Org. Chem.* **1986**, *51*, 3162.
17. Harada, T.; Takayama, M.; Oohashi, H.; Koike, W.; Yazawa, C. Jpn. Kokai Tokkyo Koho 80 33,348; *Chem. Abstr.* **1980**, *93*, p96593q).
18. Rajakumar, P.; Rasheed, A. M. A. *Tetrahedron* **2005**, *61*, 5351.
19. A solution of the diacid chloride **14** (0.5 mmol) in dry chloroform (100 mL) and a solution of the diamine **1** (0.5 mmol) and triethylamine (1.1 mmol) in dry chloroform (100 mL) were added simultaneously dropwise to chloroform (500 mL) with vigorous stirring during 6 h. After the addition was complete, the reaction mixture was stirred for another 6 h. The solvent was removed at reduced pressure and the residue obtained was then dissolved in chloroform (300 mL), washed with water (2× 100 mL) to remove triethylamine hydrochloride and then dried over magnesium sulphate. Removal of the chloroform gave the cyclophane **15** as a crude material, which was purified by column chromatography (SiO_2) using hexane/chloroform (1:1) as eluting agent to give pure cyclophane amide **15** as a violet crystalline solid. Yield: 50%; R_f 0.66 (toluene/ethyl acetate, 9:1); mp: 234–236 °C; IR (KBr, cm^{-1}) 3379, 1662, 1508; ^1H NMR (400 MHz, $CDCl_3$) δ 2.16 (s, 6H), 3.95 (s, 4H), 4.80 (s, 4H), 6.75–8.58 (m, 26H), 9.68 (s, 2H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 19.4, 41.9, 67.9, 113.7, 121.1, 121.2, 123.9, 123.9, 124.4, 126.6, 128.2, 128.5, 129.8, 130.4, 130.5, 135.6, 136.1, 137.3, 138.7, 140.7, 156.3, 164.0; FAB Mass spectrum: *m/z* 798 (M^+); Elemental analysis calcd for $C_{50}H_{42}N_2O_4S_2$: C, 75.18; H, 5.26; N, 3.50. Found: C, 75.35; H, 5.32; N, 3.61.
20. Cyclophane amide **16**. White solid. Eluent for column chromatography: chloroform to chloroform/methanol (99:1); yield: 45%; R_f 0.54 (chloroform/methanol, 9:1); mp: 280–283 °C; IR (KBr, cm^{-1}) 3280, 1672, 1581, 1517; ^1H NMR (400 MHz, DMSO-d_6) δ 2.08 (s, 6H), 3.38 (s, 4H), 5.62 (s, 4H), 7.10–8.29 (m, 22H), 9.47(s, 2H), 10.52 (s, 2H); ^{13}C NMR (100.4 MHz, DMSO-d_6) δ 19.0, 40.1, 68.2, 113.5, 120.9, 122.2, 122.6, 124.8, 125.5, 127.9, 130.3, 131.2, 131.6, 132.0, 133.0, 135.9, 138.0, 155.7, 163.4, 166.7; FAB Mass spectrum: *m/z* 808 (M^+); Elemental analysis calcd for $C_{46}H_{40}N_4O_6S_2$: C, 68.31; H, 4.95; N, 6.93. Found: C, 68.42; H, 5.12; N, 6.84.
21. Cyclophane amide **17**. Beige crystalline solid. Eluent for column chromatography: hexane to hexane/chloroform (1:1); yield: 35%; R_f 0.45 (toluene/ethyl acetate, 9:1); mp: 150–154 °C; IR (KBr, cm^{-1}) 3280, 1662, 1581, 1517; ^1H NMR (400 MHz, $CDCl_3$) δ 1.94 (s, 6H), 4.03 (s, 4H), 5.05 (s, 4H), 6.75–8.43 (m, 30H), 9.73 (s, 2H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 19.3, 41.5, 68.1, 114.3, 121.4, 121.9, 123.9, 124.5, 125.4, 126.8, 127.4, 128.7, 128.8, 128.9, 129.3, 130.4, 130.8, 132.7, 134.6, 136.5, 137.2, 140.0, 140.2, 140.9, 156.4, 163.9; FAB Mass spectrum: *m/z* 874 (M^+); Elemen-

tal analysis calcd for $C_{56}H_{46}N_2O_4S_2$: C, 76.88; H, 5.30; N, 3.20. Found: C, 76.79; H, 5.41; N, 3.28.

22. Cyclophane amide **18**. Beige crystalline solid. Eluent for column chromatography: hexane to hexane/chloroform (1:1); yield: 40%; R_f 0.55 (toluene/ethyl acetate, 9:1); mp: 206–208 °C; IR (KBr, cm^{-1}) 3340, 1677, 1591; ^1H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 2.27 (s, 3H), 3.65 (s, 2H), 5.08 (s, 2H), 5.17 (s, 2H), 6.54–8.50 (m, 18H), 9.69 (br s, 2H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 19.6, 19.8, 43.3, 69.5, 70.8, 113.0, 116.3, 121.0, 122.1, 122.2, 122.4, 124.2, 125.2, 127.7, 128.0, 128.5, 128.9, 130.5, 131.4, 131.7, 132.2, 133.1, 133.6, 134.4, 135.5, 136.7, 137.5, 137.5, 138.9, 141.6, 156.0, 156.8, 162.8, 165.7; Mass spectrum: *m/z* 600 (M^+); Elemental analysis calcd for $C_{37}H_{32}N_2O_4S$: C, 74.00; H, 5.33; N, 4.66. Found: C, 73.85; H, 5.49; N, 4.78.
23. Cyclophane amide **19**. Yellow crystalline solid. Eluent for column chromatography: hexane to hexane/chloroform (1:1); yield: 42%; R_f 0.60 (toluene/ethyl acetate, 9:1); mp: 202–204 °C; IR (KBr, cm^{-1}) 3355, 1674, 1595; ^1H NMR (400 MHz, $CDCl_3$) δ 2.25 (s, 3H), 2.33 (s, 3H), 2.97 (t, 2H, J = 4.8 Hz), 3.77 (t, 2H, J = 4.8 Hz), 5.01 (s, 2H), 5.06 (s, 2H), 6.17–8.97 (m, 18H), 9.13 (br s, 2H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 19.2, 19.8, 37.7, 64.9, 66.5, 70.9, 113.4, 113.8, 120.1, 120.4, 120.5, 120.6, 122.4, 122.7, 123.1, 124.5, 125.7, 127.5, 127.7, 128.2, 129.0, 130.0, 131.4, 132.0, 132.1, 132.2, 132.9, 133.0, 134.0, 135.5, 136.1, 138.5, 141.6, 154.0, 156.1, 162.5, 165.0; FAB Mass spectrum: *m/z* 630 (M^+); Elemental analysis calcd for $C_{38}H_{34}N_2O_5S$: C, 72.38; H, 5.39; N, 4.44. Found: C, 72.55; H, 5.59; N, 4.53.
24. Cyclophane amide **20**. White crystalline solid. Eluent for column chromatography: chloroform/methanol (99:1); yield: 40%; R_f 0.65 (chloroform/methanol, 9:1); mp: 160–162 °C; IR (KBr, cm^{-1}) 3234, 1672, 1664, 1590; ^1H NMR (400 MHz, $CDCl_3$) δ 2.19 (s, 6H), 3.07–3.95 (m, 8H), 4.47 (s, 4H), 5.11 (s, 4H), 6.58–7.66 (m, 24H), 8.00 (br s, 2H); ^{13}C NMR (100.4 MHz, $CDCl_3$) δ 19.3, 38.5, 47.6, 65.4, 67.6, 112.5, 114.2, 120.8, 121.8, 127.1, 127.3, 127.7, 128.3, 128.5, 128.7, 130.2, 130.8, 132.2, 132.5, 136.6, 141.6, 147.1, 154.3, 169.0, 170.1; FAB Mass spectrum: *m/z* 832 (M^+); Elemental analysis calcd for $C_{50}H_{48}N_4O_8$: C, 72.10; H, 5.81; N, 6.73. Found: C, 72.28; H, 5.74; N, 6.66.
25. Gandhidasan, R.; Thamaraichelvan, A.; Baburaj, S. *Fitoterapia* **1991**, *62*, 81.