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Synthesis and antibacterial activity of some novel chiral fluorophoric biscyclic macrocycles

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Abstract—Synthesis of chiral permanent fluorophoric biscyclic macrocycles incorporating anthraquinone and (*S*)-BINOL core is described. Interestingly, the biscyclic macrocycle **1** exhibited remarkable antibacterial activity against most of the pathogenic bacteria in the tested concentrations as compared to the other three compounds **2**, **14** and **17** as well as the test control, tetracycline. Further biscyclophanes **1** and **2** exhibited permanent fluorescence sensing property even under highly acidic conditions.

Supramolecular systems with fluorescence tag play an important role in biology.[1] Anthraquinone based fluorophoric systems find application as fluoride sensors,[2] photoactive chemosensors[3] and chemical modifications with such receptor have been also reported.[4,5] Similarly, amidoanthraquinone core units have also been used for the synthesis of cytotoxic,[6] antimicrobial[7] and human telomerase inhibiting agents.[8] Though such acid sensitive fluorescent supramolecules[9] have been reported, synthesis of permanent fluorescence sensing system[10] is of greater importance. Even though very few reports are available in the literature on pH sensitive supramolecules,[11] synthesis of fluorescent supramolecules with chiral core units[12] would be more fascinating. Synthesis of chiral cyclophanes incorporating binaphthol has been reported from our laboratory.[13] Chiral biscyclic macrocycles having anthraquinone unit are not known to the best of our knowledge. The presence of anthraquinone unit and binaphthol unit in cyclophane causes chiral as well as permanent fluorescence sensing property. The presence of anthraquinone unit would also impart antibacterial activity in the macrocyclic system. We wish to report the synthesis of permanent fluorescence sensing chiral biscyclic cyclophanes **1** and **2** having anthraquinone as well as BINOL moiety and **3** a biscyclic chiral cyclophane.

The purpose of synthesis of cyclophanes **1** and **2** is twofold. It would be of interest to examine fluorescence activity of such cyclophanes under highly acidic conditions so that they can be used as permanent fluorescence sensing tags and to investigate their antibacterial efficacy towards various bacteria such as *Escherichia coli*, *Proteus mirabilis*, *Proteus vulgaris* and *Pseudomonas aeruginosa* under different pH conditions. Thus by targeting the synthesis of cyclophanes **1** and **2** biologically active permanent fluorescence sensing supramolecules can be achieved.

The synthetic pathway leading to the synthesis of chiral biscyclophane amide **1** is outlined in Scheme 1. Reaction of ethyl *p*-toluate **4** with NBS in CCl_4 gave *p*-carbethoxybenzylbromide **5** in 82% yield. *O*-Alkylation of **5** with optically pure (*S*)-BINOL (**6**) in DMF in the presence of K_2CO_3 gave chiral diester **7** in 71% yield which was then reduced to the corresponding chiral diol **8** using $LiAlH_4$ in THF. Treatment of chiral diol **8** with PBr_3 in CH_2Cl_2 led to the chiral dibromide **9** in 72% yield. Reaction of one equivalent of chiral dibromide **9** (0.76 mmol) with one equivalent of methyl 3,5-dihydroxybenzoate (**10**) (0.76 mmol) in presence of K_2CO_3 (15.2 mmol) in acetone (250 mL) under high dilution gave the cyclophane-ester **11**, which on hydrolysis with alcoholic KOH followed by reaction with

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Keywords: Chiral fluorophoric biscyclic macrocycles; Antibacterial activity.

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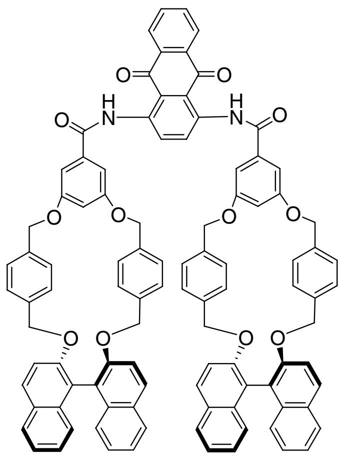

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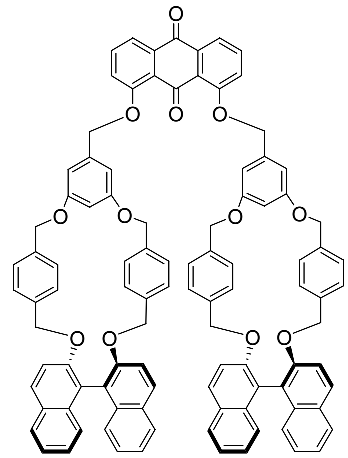

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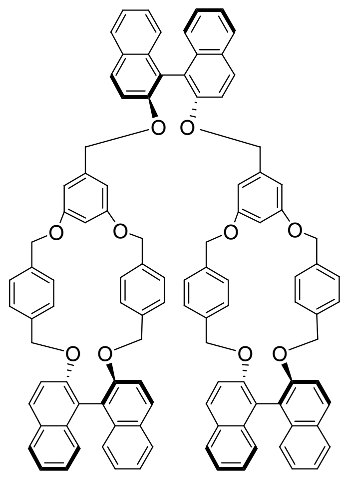

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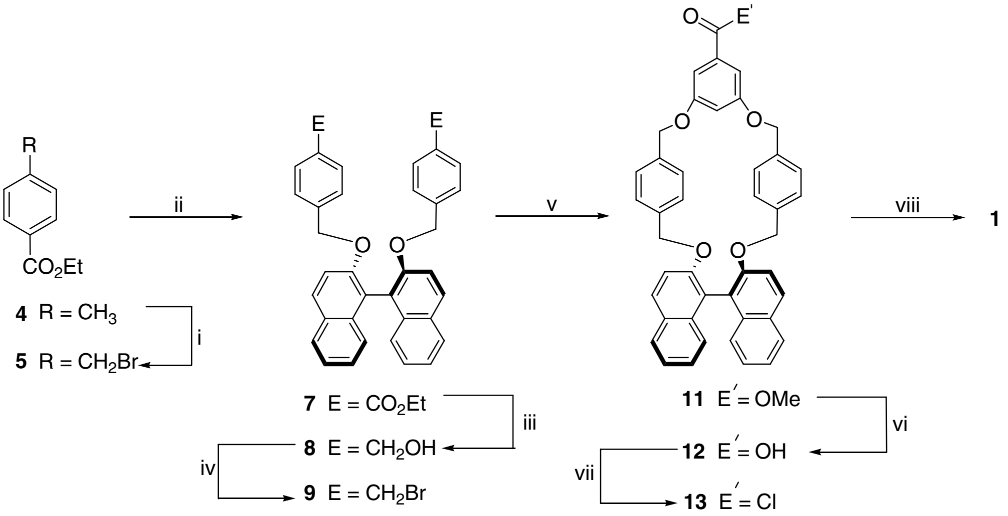
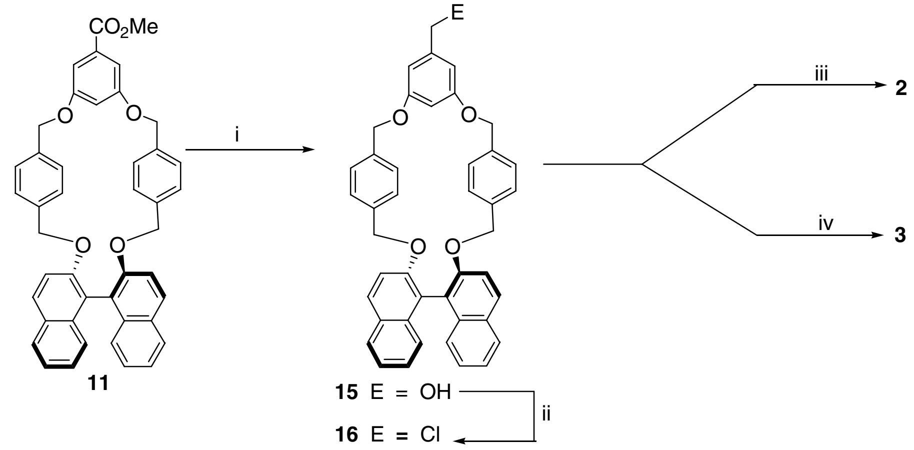
thionyl chloride gave the chiral cyclophane-acid chloride **13** in 98% yield. Synthesis of biscyclophane amide **1** was obtained by the reaction of the acid chloride **13** with 0.5 equiv 1,4-diamino-9,10-anthraquinone (**14**) in the presence of the triethyl amine in CH_2Cl_2. The chiral biscyclophane amide **1** was thus obtained in 54% yield after column chromatographic purification (Scheme 1).

The ^1H NMR spectrum of biscyclophane amide **1** showed the *O*-methylene protons attached to the BINOL unit as two doublets at δ 5.00 and 5.19, and other *O*-methylene protons as a distorted triplet at δ 5.12. The inner annular protons of the 3,5-dihydroxybenzene moiety appeared as a singlet at δ 6.44 and the outer protons as another singlet at δ 7.25 integrating for two and four protons respectively. The *p*-xylenyl protons appeared as a pair of doublets at δ 6.86 and 6.93 integrating for 16 protons in addition to the aromatic protons of the BINOL and anthraquinone unit. It is noteworthy to mention that the amide protons of **1** appeared as a singlet at δ 13.38. The ^{13}C NMR spectrum of cyclophane **1** showed the peak at δ 69.8 and 70.0 for two *O*-methylene carbons, a peak at δ 166.0 for amide carbonyl, a peak at δ 184.3 for ketocarbonyl and in addition to the other aromatic carbons. The FAB mass spectrum of **1** showed the molecular ion at *m*/*z* 1491. Thus, the structure of the cyclophane amide **1** has been completely characterized by spectral and analytical data.[14]

Permanent fluorescence sensing hyper-branched dendrimer using dihydroxy anthraquinone as a core has been explored recently from our laboratory.[10] Hence introduction of dihydroxy anthraquinone moiety in the biscyclophane would be of much biological importance. The synthetic pathway leading to biscyclophane **2** is outlined in Scheme 2. The reduction of chiral ester **11** by $LiAlH_4$ followed by reaction with thionyl chloride



Scheme 1. Reagents and conditions: (i) NBS, CCl_4, reflux, Bz_2O_2, 6 h, **5** (82%); (ii) (*S*)-BINOL, K_2CO_3, DMF, 80 °C, 48 h, **7** (71%); (iii) $LiAlH_4$, THF, reflux, 6 h, **8** (85%); (iv) PBr_3, CH_2Cl_2, 0 °C, 4 h, **9** (72%); (v) methyl 3,5-dihydroxybenzoate, K_2CO_3, acetone, rt, 3 days, **11** (35%); (vi) KOH, ethanol, 80 °C, 4 h, **12** (90%); (vii) 0.1 equiv Et_3N, $SOCl_2$, CH_2Cl_2, rt, 6 h, **13** (98%); (viii) 0.5 equiv 1,4-diamino-9,10-anthraquinone (**14**), 1 equiv Et_3N, CH_2Cl_2, rt, 8 h, **1** (54%).



Scheme 2. Reagents and conditions: (i) $LiAlH_4$, THF, 4 h, **15** (94%); (ii) $SOCl_2$, py, CH_2Cl_2, 0 °C, 3 h, **16** (30%); (iii) 0.5 equiv 1,8-dihydroxy-9,10-anthraquinone (**17**), K_2CO_3, DMF, 60 °C, 2 days, **2** (43%); (iv) 0.5 equiv (*S*)-BINOL (**6**), K_2CO_3, DMF, 60 °C, 2 days, **3** (63%).

in the presence of pyridine in CH_2Cl_2 gave the cyclophane chloride **16** in 30% yields. Reaction of 1,8-dihydroxy-9,10-anthraquinone (**17**) with 2.1 equivalent of chloride **16** in dry DMF at 70 °C in the presence of K_2CO_3 (10 equiv) as a base for 2 days afforded biscyclophane **2** in 43% yield (Scheme 2).

The ^{1}H NMR of cyclophane **2** shows two pairs of doublets at δ 4.93, 4.97, 5.03 and 5.11 and a singlet at δ 5.22 integrating to a total of 20 benzylic protons in addition to the aromatic protons. The ^{13}C NMR of cyclophane **2** showed the peak at δ 69.9 and 70.9 for two *O*-methylene carbons, a peak at δ 182.5 and 184.5 for anthraquinone carbonyl as well as for aromatic carbons. Thus, the structure of the cyclophane **2** has been thoroughly characterized by spectral and analytical data.[15]

Incorporation of (*S*)-BINOL as a core in the chiral biscyclophanes would be more promising in the recognition of large electron deficient chiral guest molecules. Further, it is of interest to study the atropisomerism of such molecules. By applying similar synthetic strategy as discussed above for cyclophane **2**, the biscyclophane **3** was prepared in 63% yield (Scheme 2). The ^{1}H NMR spectrum of biscyclophane **3** displayed *O*-methylene protons attached to the BINOL unit as multiplet at δ 4.84–4.93 integrating for 12 protons and the other *O*-methylene protons attached to the 3,5-dihydroxy benzene moiety appeared as two doublets at δ 4.98 and 5.15 integrating for four protons in addition to the protons in the aromatic region. In the ^{13}C NMR of **3**, the *O*-methylene carbons appeared at δ 69.8, 69.9 and 70.7. The FAB mass spectrum of **3** showed the molecular ion peak at *m*/*z* 1511. The structure of cyclophane **3**[16] has been completely characterized by spectral and analytical data.

Fluorescence studies. Fluorescence studies were carried out with the biscyclophanes **1** and **2**. The absorption spectrum of the biscyclophanes **1** and **2** showed λ_{max} at 552 and 382 nm. No bathochromic or hypsochromic shift could be observed on changing the solvent from $CHCl_3$ to CH_3CN. Protonation of the carbonyl chromophore in the anthraquinone moiety could lead to a change in λ_{max}. However, on adding AcOH, TFA and HCl upto 5 M for 1×10^{-5} M of the cyclophane, no shift in λ_{max} could be observed. The biscyclophanes **1** and **2** exhibited a fluorescence emission band at 632 and 400 nm. Fluorescence quenching did not occur even after adding TFA and HCl to the biscyclic cyclophanes, which shows that the biscyclophanes **1** and **2** can function as permanent fluorescence sensing material even under highly acidic conditions.

Antibacterial efficacy. Antibacterial activity studies were carried out with biscyclophanes **1** and **2** as well as parent compounds **14** and **17**. All the four compounds **1**, **2**, **14** and **17** exerted various levels of inhibitory effects against four human pathogenic bacteria (Table 1). The antibacterial activity[17] of the test compounds was dose dependent and it was remarkable at higher concentrations. Among the compounds tested, the anthraquinone compounds **14** and **17** were less effective than biscyclic macrocycles **1** and **2**. Overall analysis on the

Table 1. In vitro antibacterial activity (minimum inhibitory concentration in mM) of fluorophoric anthraquinone compounds

pH	Compound	*Escherichia coli*	*Proteus vulgaris*	*Proteus mirabilis*	*Pseudomonas aeruginosa*
7	**1**	25	15	20	25
	2	25	50	25	50
	14	75	75	100	75
	17	75	75	50	50
	Tetracycline	50	35	20	35
6	**1**	50	15	75	40
	2	60	55	20	25
	14	55	60	85	55
	17	75	65	25	50
5	**1**	25	20	45	75
	2	25	75	90	40
	14	75	40	75	100
	17	50	100	100	75
Control		NI	NI	NI	NI

NI, no inhibition.

antibacterial activity revealed that biscyclic macrocycle **1** remarkably inhibited all the pathogenic bacteria in most of the tested concentrations as compared to other three compounds and control.

In addition, the chiral biscyclic macrocycle **1** was active against the test pathogens at all three different pH values of 5–7. Further, compound **1** was also found to be superior to the commercial antibiotic, tetracycline, in controlling *E. coli*, *P. vulgaris* and *P. aeruginosa* when tested at pH 7. The minimum inhibitory concentrations[18] of compound **1** were between 15 and 25 mM as compared to 25 and 100 mM for other compounds and tetracycline. However, its effect against *P. mirabilis* was equal to that of tetracycline (Table 1).

In conclusion, the compounds **1**, **2**, **14** and **17** exhibited good antibacterial activity against all the four human pathogenic bacteria. The compound **1** may be developed as antibiotic drug as it showed superior activity against all the test pathogens than the other compounds including tetracycline. However, further studies are required to determine their potential against a wide range of human pathogens and its mode of actions. Synthesis of more permanent fluorescence sensing chiral macrocycles and their antibacterial activity as well as molecular recognition towards chiral guest molecules is on the way.

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14. Cyclophane **1**. Yield 54%; $[\alpha]_D^{30}$ −17.40, (*c* 0.01, $CHCl_3$); mp 195 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 5.00 (d, 4H, $J = 13.0$ Hz); 5.12 (t, 8H, $J = 15.3$ Hz); 5.19 (d, 4H, $J = 13.0$ Hz); 6.44 (s, 2H); 6.86 (d, 8H, $J = 8.4$ Hz); 6.93 (d, 8H, $J = 8.4$ Hz); 6.97 (d, 2H, $J = 10.0$ Hz); 7.19–7.23 (m, 8H); 7.25 (s, 4H); 7.27–7.34 (m, 4H); 7.61–7.64 (m, 2H); 7.68–7.71 (m, 2H); 7.85 (d, 4H, $J = 8.4$ Hz); 7.88 (d, 4H, $J = 9.2$ Hz); 8.25–8.28 (m, 2H); 9.06 (d, 2H, $J = 9.9$ Hz); 13.38 (s, 2H): ^{13}C NMR (125 MHz, $CDCl_3$): δ 69.8, 70.0, 104.8, 109.8, 111.2, 117.0, 120.2, 123.7, 125.4, 126.5, 126.7, 127.2, 128.1, 129.3, 129.5, 133.2, 133.5, 134.1, 134.2, 135.2, 136.0, 137.2, 148.1, 153.8, 159.5, 166.0, 184.3; *m*/*z* (FAB-MS) 1491 (M^+). Elemental Anal. Calcd for $C_{100}H_{70}N_2O_{12}$: C, 80.52; H, 4.73; N, 1.88. Found: C, 80.37; H, 4.69; N, 1.78.
15. Cyclophane **2**. Yield 43%; $[\alpha]_D^{30}$ −273.33 (*c* 0.01, $CHCl_3$); mp 205 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.93 (d, 4H, $J = 13.0$ Hz); 5.11 (d, 4H, $J = 13.0$ Hz); 4.97 (d, 4H, $J = 13.0$ Hz); 5.03 (d, 4H, $J = 13.0$ Hz); 5.22 (s, 4H); 6.17 (br s, 2H); 6.75–6.77 (m, 14H); 6.86 (d, 8H, $J = 7.7$ Hz); 7.19– 7.25 (m, 10H); 7.29–7.32 (m, 6H); 7.50 (t, 2H, $J = 8.4$ Hz); 7.83–7.85 (m, 6H); 7.88 (d, 4H, $J = 9.2$ Hz): ^{13}C NMR (125 MHz, $CDCl_3$): δ 69.9, 70.9, 101.0, 108.6, 115.3, 119.3, 119.6, 120.2, 120.7, 123.7, 124.8, 125.4, 126.6, 126.7, 128.1, 129.3, 129.4, 133.8, 134.2, 134.9, 136.3, 136.9, 139.2, 153.8, 158.3, 159.6, 182.5, 184.0; *m*/*z* (FAB-MS) 1465 (M^+). Elemental Anal. Calcd for $C_{100}H_{72}O_{12}$: C, 81.95; H, 4.95. Found: C, 81.77; H, 4.86.
16. Cyclophane **3** Yield 63%; $[\alpha]_D^{30}$ −250.94, (*c* 0.01, $CHCl_3$); mp 265 °C; ^{1}H NMR (500 MHz, $CDCl_3$): δ 4.84–4.93 (m, 12H); 4.98 (d, 4H, $J = 13.0$ Hz); 5.15 (d, 4H, $J = 13.0$ Hz); 6.06 (s, 2H); 6.20 (s, 4H); 6.73 (d, 8H, $J = 8.0$ Hz); 6.89 (d, 8H, $J = 8.0$ Hz); 7.23–7.27 (m, 12H); 7.31–7.33 (m, 10H); 7.40 (d, 2H, $J = 9.2$ Hz); 7.84–7.89 (m, 10H); 7.95 (d, 2H, $J = 9.2$ Hz): ^{13}C NMR (125 MHz, $CDCl_3$): δ 69.8, 69.9, 70.7, 100.9, 108.5, 115.2, 116.0, 120.2, 120.6, 123.7, 123.9, 125.4, 125.7, 126.5, 126.7, 128.1, 129.3, 129.5, 129.6, 134.2, 134.3, 136.4, 136.9, 140.2, 153.8, 154.2, 159.1; *m*/*z* (FAB-MS) 1511 (M^+). Elemental Anal. Calcd for $C_{106}H_{78}O_{10}$: C, 84.22; H, 5.20. Found: C, 84.01; H, 5.29.
17. Antibacterial activity. The antibacterial activity of the compounds against human pathogens was evaluated by the agar diffusion method. About 1 mL of inoculum of each test pathogen was added to the molten NA medium and poured into sterile Petri plates under aseptic conditions. After solidification, a 5-mm well was made in the center of each plate using a sterile cork borer. Each compound was dissolved in 10% DMSO to get different concentrations and filter-sterilized using 0.25 μm filter paper. Each well received 50 μL solution of each compound and the plates were incubated at room temperature. Sterile DMSO (10%) was used as control. After 48 h, the appearance of inhibition zone around the well was observed.
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