8. Experimental Part

8.1 Synthesis

12-Hydroxy-dodecanoic acid benzyl ester[38-40] (1)

A solution of 12-hydroxydodecanoic acid (10g, 26.30mmol) in DMF (120ml) was treated with potassium bicarbonate (5.10g, 50.92mmol) and benzyl bromide (9.12g, 53.24mmol) were added drop wise and stirred reaction mixture for 24h. The solvent was removed under reduced pressure. The resulting mixture was partitioned between ethyl acetate and aqueous HCl. The organic phase was separated, washed with water and dried over $MgSO_4$. Removal of the solvent in vacuum resulted in a solid compound which was recrystallized in methanol to afford white crystals of 12-hydroxy-dodecanoic acid benzyl ester **1**, yield 9.0 g (27.78 mmol, 90%).

^{1}H NMR (270 MHz, $CDCl_3$): δ 1.36 (m, 14H, 7×CH_2), 1.57 (m, 4H, 2×CH_2), 2.38 (t, 2H, CH_2COO), 3.63 (t, 2H, CH_2OH), 5.11 (s, 2H, CH_2Ph), 7.38 (s, 5H, aromatic).

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.8, 25.6, 28.9, 29.0, 29.3, 29.4, 29.5, 32.6, 34.2, 62.9, 65.9, 128.0, 128.3, 136.5, 173.9.

IR (KBr,cm^{-1}): 3444 (O-H primary), 3154 (overlap C=O stretching), 3032 (C-H stretching of phenyl), 2928 and 2855 (C-H stretching of alkane), 1724 (C=O stretching of ester), 1455 and 1466 (C-H bending of CH_2CH_3), 1182 (C-O stretching), 907 (O-H out of plane bending), 731 (methylene rock), 698.

MS m/z (EI): $C_{19}H_{30}O_3$ (Mo. Wt. 306): 306(M), 278(M-CO), 91($C_7H_7^+$)

Elemental analysis: $C_{19}H_{30}O_3$ (Mo. Wt. 306):

Calculated: C 74.50%, H 9.80%

Found : C 73.89%, H 9.37%

12-Oxo-dodecanoic acid benzyl ester[41] (2)

Pyridinium chlorochromate (3.23g, 15mmol) was suspended in methylene chloride (100ml) and the 12-hydroxy-dodecanoic acid benzyl ester **1** (3.50g, 10mmol) was rapidly added at room temperature. After 1.5 hours the oxidation was complete (followed by TLC). The black reaction mixture was diluted with 300 ml of anhydrous ether, the solvent was decanted, and the black solid was washed twice with ether. The product was isolated simply by filtration of the organic extracts through florisil and evaporation of the solvent at reduced pressure to afford 12-oxo-dodecanoic acid benzyl ester **2** in a yield of 3.15g (10.36 mmol, 91%) as an oil.

TLC (EtOAc/MeOH 1:1) R_f 0.4

$C_{19}H_{28}O_3$ (Mo. Wt. 304)

^{1}H NMR (270 MHz, $CDCl_3$): δ 1.35 (m, 12H, 6×CH_2), 1.57 (m, 4H, 2×CH_2), 2.40 (m, 4H, CH_2COO, CH_2OH), 5.10 (s, 2H, CH_2Ph), 7.40 (s, 5H, aromatic.). 9.77 (s, 1H, CHO).

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.6, 24.9, 29.0, 29.1, 29.2, 34.3, 43.7, 66.0, 128.1, 128.5, 136.0, 173.5, 202.8.

IR (KBr, cm^{-1}): 3033 (C-H stretching of phenyl), 2918 (C-H stretching of alkane chain), 2850 (C-H stretching of aldehyde), 1735 (C=O stretching of ester), 1704 (C=O stretching of Aldehyde), 1455 and 1463 (C-H bending of CH_2CH_3),1281 (C-O stretching of aldehyde), 747 (methylene rock), 697.

MS m/z (EI): $C_{19}H_{28}O_3$ (Mo. Wt. 304): 304$(M)^+$, 276(M-CO), 108 (M-PhCH_2OH), 91($C_7H_7^+$)

Teradec-2-enedioic acid 14-benzyl ester 1-tert butyl ester[42-44] **(3)**

1.32g (32.90 mmole) Sodium hydride was suspended in THF (100ml) at 0°C under the argon atmosphere. 7.38g (32.90mmole) *tert*-butyl p, p-dimethylphosphono acetate was added drop wise at this temperature; after the evolution of H_2 bubbles had ceased (0.5h), a solution of 10g (32.90mmole) 12-oxo-dodecanoic acid benzyl ester **2** in 50 ml THF was added slowly. The resulting reaction mixture was stirred for 24 hours. The solvent was removed under reduced pressure and the residue taken up with water. After extraction with four portions of ether and subsequently drying, a white solid was obtained. This was recrystallized from hexane, obtained 9.80g (24.38 mmol) teradec-2-enedioic acid 14-benzyl ester 1-tert butyl ester **3** (74%).

$C_{25}H_{38}O_4$ (Mo. Wt. 402)

^{1}H NMR (270 MHz, $CDCl_3$): δ 1.30 (m,12H,6x CH_2), 1.44 (s, 9H, $(CH_3)_3C$), 1.58 (m, 4H, 2xCH_2), 2.30 (m, 4H, 2x CH_2), 5.10 (s, 2H, CH_2Ph), 5.73 (d, 1H, J=11Hz, vinyl α-H), 6.87 (dt,1H, J_1=11, J_2= 3Hz, vinyl β-H), 7.41 (s, 5H, aromatic.).

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.9, 28.0, 28.1, 28.5, 28.7, 28.9, 29.0, 29.1, 29.3, 31.9, 34.2, 65.9, 79.8, 122.8, 128.1, 128.5, 136.0, 148.1, 166.7, 173.5

IR (KBr, cm^{-1}): 3444 (overtone of C=O stretching), 3065 (aromatic. C-H stretching), 3033 (olefinic =C-H stretching), 2926, 2854(C-H stretching of alkane), 1736 (C=O stretching of ester), 1634 (conjugated C=C stretching), 1455 and 1338 (C-H bending of CH_2CH_3), 1152 (C-O stretching of ester), 768 (C-H out of plane bending), 750 (methylene rock).

MS (FAB neg., Xe); $C_{25}H_{38}O_4$ (Mo. Wt. 402): m/z = 402$(M)^+$,

Tetradec-2-enedioic acid 14-benzyl ester[45] (4)

5g (14.45 mmol) of teradec-2-enedioic acid 14-benzyl ester 1-*tert*-butyl ester **3** in 100 ml toluene and (0.5g) *p*-toluenesulfonic (PTSA) acid was added and refluxed for 30 min. and subsequently stirred at room temperature overnight. After removing the toluene, 300ml of 5% aqueous potassium bicarbonate solution was added and stirred for 10 minutes. The white precipitate was filtered off and the filtrate was acidified to pH3 with dilute HCl. The resulting suspension was extracted with chloroform (200mL) dried with magnesium sulphate removed solvent in reduced pressure and dried in vacuum and re-crystallized with chloroform/hexane obtained white solid 3.95g (11.42mmol, 79%) of tetradec-2-enedioic acid 14-benzyl ester **4**.

$C_{21}H_{30}O_4$ (Mo. Wt. 346)

^{1}H NMR (270MHz, $CDCl_3$): δ 1.33(m,12H,6xCH_2), 1.48(m, 2H,CH_2), 1.61(m, 2H,CH_2), 2.20(m, 2H,CH_2), 2.38 (t,2H, CH_2COO), 5.10 (s, 2H, CH_2Ph), 5.73 (d, 1H, J=11Hz,vinylα-H), 6.87 (dt, 1H, J_1=11 J=3Hz, vinyl β-H), 7.41 (s, 5H, aromat.), 9.60 (m, 1H, COOH).

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.9, 24.9, 27.8, 29.0, 29.1, 29.2, 29.3, 29.4, 32.2, 34.2, 66.0, 120.5, 128.1, 128.5, 136.0, 152.2, 171.5, 173.6.

IR (KBr, cm^{-1}): 3439 (carboxylic acid free O-H stretching), 3088 (aromatic C-H stretching), 3008 (olefin =C-H stretching), 2926 and 2854 (C-H stretching), 1736 (C=O stretching of carboxylic acid), 1696 (C=O stretching of ester), 1650 (Conjugated C=C stretching), 1455 (O-H in plane bend), 1419 and 1383 (C-H bending of alkane), 1284 (C-O stretching of acid), 1168 (C-O stretching of ester), 982 (O-H out of plane bending), 750 (methylene rock).

MS (FAB, pos, Xe) m/z; $C_{21}H_{30}O_4$ (Mo. Wt. 346): 347 (M+H), 329 (M-OH), 239 (M-$C_7H_7O)^+$, 107 $(C_7H_7O)^+$ 91 $(C_7H_7)^+$.

Toluene-4-sulfonicacid 2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}ethyl ester[46] (5)

2g (10 mmole) of tri(ethylene glycol)-monomethylether was suspended in dry pyridine (10 ml) at 0 °C under the argon atmosphere. 3.9g (20 mmol) 4-methyl-benzenesulfonyl chloride was added at room temperature. The resulting mixture was stirred for 24 hours at room temperature. The reaction mixture poured into 20 ml water the resulting suspension extracted with CH_2Cl_2 , dried with magnesium sulphate, evaporation followed by flash column chromatography (ethyl acetate) afforded white solid of toluene-4-sulfonicacid 2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}ethyl ester **5**, 3.0g (88%) confirmed by ^{1}H-NMR.

TLC (EtOAc/MeOH 10:1) R_f 0.3.

^{1}H NMR (270MHz, $CDCl_3$): δ 2.4 (s, 3H); 3.24 (s, 3H, CH_3O) 3.79-3.59 (m, 14 H), 3.56 (t, 2H, CH_2O), 3.70 (t, 2H, CH_2O), 7.34 (d, 2H, aromatic), 7.73 (d, 2H, aromatic).

1-{2-[2-(2-Azido-ethoxy)-ethoxy]-ethoxy}-2-methoxy ethane (6)[47-49]

1.1g (16.5 mmole) sodium azide was added to a solution of 3.0g, (11 mmol) toluene-4-sulfonic acid 2-{2-[2-(2-methoxy-ethoxy]-ethoxy}ethyl ester **5** in dry DMF (12 ml). The mixture was heated at 90 °C for 6h and then allowed to attained at room temperature. The DMF was evaporated, and the residue was purified by flash chromatography (ethyl acetate). The obtained 1-{2-[2-(2-azido-ethoxy)-ethoxy]-ethoxy}-2-methoxy ethane **6** was light yellow oil 2.26 g (94%).

TLC (EtOAc/MeOH10:1) R_f 0.5.

^{1}H NMR (270MHz, $CDCl_3$):
δ 3.2 (t, 2H, CH_2N_3) 3.4 (t, 2H, CH_2O); 3.24 (s, 3H, CH_3O), 3.56-3.62 (m,12H, OCH_2CH_2O).

1-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy} ethylamine [$H(OCH_2CH_2)_4NH_2$] (7)[47,48]

A solution of 1-{2-[2-(2-azido-ethoxy)-ethoxy]-ethoxy}-2-methoxy ethane **6** (1.3g, 5.58 mmol) in dry THF (30 ml) was cooled to 0 °C. Triphenyl phosphine (1.8 g, 7.0 mmol) was added and the mixture was allowed to attain room temperature for 24 hours. 0.4ml water was added, and the reaction mixture was stirred for another 24 hours to hydrolyse the intermediate phosphorus adduct. The reaction mixture was diluted with water and washed with toluene. Evaporation of the aqueous layer yielded 1.1g (97%) of **7** as an yellow oil.

TLC (CH_2Cl_2/MeOH/Et_3N 3:3:1) R_f 0.5;.

^{1}H NMR (270MHz, $CDCl_3$): δ 1.54 (broad, 2H, NH_2), 2.87 (m, 2H, CH_2 NH_2); 3.24 (s, 3H, CH_3O), 3.6 (t, 2H, CH_2O), 3.56-3.63 (m,12H, OCH_2CH_2O),

^{13}C NMR (63 MHz, $CDCl_3$): δ 53.9, 73.1, 70.6, 70.9, 70.9, 70.9, 70.9, 70.6, 74.3, 43.0.

IR (KBr, cm^{-1}): 3312 (N-H stretching of primary amine), 3050 (Fermi resonance band with overtone of band at 1678 N-H bend), 2873 (aliphatic C-H stretching), 1678 (N-H bending), 1455 (C-H bending of methylene), 1106 (C-N stretching), 996-659 (N-H weg).

13-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}ethylcarbamoyl)tridec-12-enoic acid benzyl ester (8)[52-54]

0.595 g (2.89 mmol) of 1-{2-[2-(2-azido-ethoxy)-ethoxy]-ethoxy}ethylamine **7** and 1g (2.89 mmol) of tetradec-2-enedioic acid 14-benzyl ester **4** were dissolved in 200 ml of CH_2Cl_2. After cooling at 0°C for 15 min**,** 1.48g (7.22 mmol) of DCC and 1g (9 mmol) of DMAP were added. The reaction mixture was stirred at room temperature for further 24 hr. The white precipitate was filtered off and the filtrate washed successively with 0.1 M HCl, 8% $NaHCO_3$ **,** and water, dried over $MgSO_4$ The solvent was removed in vacuum and the residue purified by silica column chromatography using CH_2Cl_2: MeOH (10:1) as solvent, followed by crystallization from hexane/ethyl acetate, yielded 1.3g (84.9%) white solid of **8**.

$C_{30}H_{49}O_7N$ (Mo. Wt. 535)

^{1}H NMR (270 MHz $CDCl_3$): δ 1.34 (m, 12H, 6x CH_2), 1.49 (m, 4H, CH_2), 1.63 (m, 2H, allyl-H), 2.37 (t, 2H, CH_2COO), 3.45 (s, 3H, CH_3-O), 3.56 (m, 2H, CH_2NH), 3.56-3.7(m, 14H, CH_2O), 5.08 (s, 2H, CH_2Ph), 5.73 (d, 1H, J=11Hz, vinyl α-H), 6.30 (m,1H, NH), 6.87 (dt, 1H, J_1 = 11, J = 3Hz, vinyl β-H), 7.41 (s, 5H, aromatic)

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.8, 27.7, 28.5, 29.1, 29.2, 29.3, 29.4, 32.2, 34.2, 66.0, 62.45, 64.56, 69.67, 69.84, 70.11, 70.20, 71.60, 76.49, 77.00, 77.50, 120.4, 128.0, 128.4, 136.0, 152.2, 172.0, 173.6.

IR (KBr, cm^{-1}): 3449 (overtone of C=O stretching), 3311 (N-H stretching asymmetric amide bond), 3278 (N-H stretching symmetric amid bond), 3065 (aromatic C-H stretching), 3033 (olefin =C-H stretching), 2924 and 2851 (C-H stretching of alkane), 1736 (C=O stretching of ester), 1667 (C=O stretching, Amide), 1627 (N-H bending, Amide), 1462, 1454 and 1350 (C-H bending of CH_2CH_3), 1277 (C-O stretching of ester), 983 (C-H out of plane bend, olefin) 746 (methylene rock), 697.

MS m/z (EI): $C_{30}H_{49}O_7N$ (Mo. Wt. 535): 536(M+H), 91(M-C_7H_7)$^+$

13-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}ethylcarbamoyl)tridec-12-enoic acid (9)[55,56]

1.2 g of the 13-(2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}ethylcarbamoyl) tridec-12-enoic acid benzyl ester **8** was treated with 25 ml of 1M LiOH suspension in 10 ml THF, 5 ml methanol and 5ml water. The mixture was stirred overnight at room temperature. After removal of the solvent in vacuums, the mixture was extracted with ethyl acetate and water. The aqueous phases were combined and acidified to pH2 with dilute HCl. The suspension was extracted with chloroform, dried over magnesium sulphate and evaporated to afford 1g (93%) of **9**.

$C_{23}H_{43}NO_7$ (Mo. Wt. 445)

^{1}H NMR (270 MHz $CDCl_3$): δ 1.34 (m, 12H, 6x CH_2), 1.49 (m,4H, CH_2), 1.63 (m, 2H, allyl-H), 2.37 (t, 2H, CH_2COO), 3.45 (s, 3H, CH_3-O), 3.56 (m, 2H, CH_2NH), 3.56-3.7(m, 14H, OCH_2CH_2O), 5.89 (d, 1H, J=11Hz, vinyl α-H), 6.5 (m, 1H, NH), 6.87 (dt, 1H, J_1 = 11, J = 3Hz, vinyl β-H), 7.75(broad, 1H, COOH).

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.69, 25.66, 28.14, 28.95, 29.15, 31.91, 32.68, 33.91, 39.26, 58.90, 62.92, 69.89, 70.16, 70.44, 70.55, 71.89, 123.50, 144.86, 166.40, 177.90.

IR (KBr, cm^{-1}): 3400 (broad O-H stretching of acid), 3311 (N-H stretching asymmetric amid bond), 3278 (N-H stretching symmetric amid bond), 3064 (aromatic C-H stretching), 3009, (olefin =C-H stretching), 2914 and 2848 (C-H stretching of alkane), 1730 (C=O stretching of acid), 1699 (C=O stretching, amide), 1665 (C=C stretching), 1620 (N-H bending, amide), 1464 and 1350 (C-H bending of CH_2CH_3), 1269 (C-O stretching of amid), 985 (C-H out of plane bend, olefin), 941 (O-H out of plane bending of acid), 721 (methylene rock).

MS (EI): $C_{23}H_{43}N_1O_7$ (Mo. Wt. 445) m/z = 446(M+H), 91$(M-C_7H_7)^+$

Elemental analysis: $C_{23}H_{43}N_1O_7$ (Mo. Wt. 445)

Calculated: C 62.02%, H 9.66%, N 3.14%
Found : C 62.08%, H 9.53%, N 3.01%

13-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}-ethylcarbamoyl)-tridec-12-enoyl chloride (10)[56]

0.5g of 13-(2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ehtoxy}ethylcarbamoyl) tridec-12-enoic acid was dissolved in 50mL dichloromethane cooled to 0 ^{o}C then added equimolar amount of oxalyl chloride. Stirred reaction mixture room temperature for another 24h. After removing solvent dissolved compound in dichloromethane and used such as in next step for preparation of membrane on silica colloidal particles.

6-Oxo-hexanoic acid ethyl ester (11)[41]

Pyridinium chlorochromate (15 mmol) was suspended in methylene chloride (100ml) and the 5-hydroxy-pentanoic acid ethyl ester (purchased from Aldrich) (15 g, 9.3 mmol) was rapidly added at room temperature. After 1.5 hours the oxidation was complete (followed by TLC). The black reaction mixture was diluted with 300 ml of anhydrous ether, the solvent was decanted, and the black solid was washed twice with ether. The product was isolated simply by filtration of the organic extracts through florisil and evaporation of the solvent at reduced pressure to afford 6-oxo-hexanoic acid ethyl ester **11** in a yield of 12 g (81 %) as an oil.

$C_8H_{14}O_3$ (Mo. Wt. 158)

^{1}H NMR (270 MHz, $CDCl_3$): δ 1.12 (t, 3H), 1.35 (m, 2H, 1×CH_2), 1.57 (m, 2H, 1×CH_2), 2.40 (m, 4H, CH_2COO, CH_2OHC), 4.1, (q, 2H), 9.77 (s, 1H, CHO).

^{13}C NMR (125 MHz, $CDCl_3$): δ 14.7, 24.05, 24.27, 33.56, 33.88, 60.33, 172.0, 185.0

MS m/z (EI): $C_8H_{14}O_3$ (Mo. Wt. 158): 157.5$(M-H)^+$

Elemental analysis: $C_8H_{14}O_3$ (Mo. Wt. 158)
Calculated: C 60.00 %, H 8.86%,
Found : C 59.86 %, H 8.50%,

Oct-2-enedioic acid 1-*tert*-butyl ester 8-ethyl ester (12).[42-44]

Sodium hydride 2.77 g (11.1 mmol) was suspended in THF (100ml) at 0°C under an argon atmosphere and equimolar amount (17 g, 7.4 mmol) of *tert*-butyl p, p-dimethyl-phosphono acetate was added dropwise at this temperature; after the evolution of H_2 bubbles had ceased (0.5h), a solution of 12 g (7.4 mmol) 6-oxo-hexanoic acid ethyl ester **11** in 50 ml THF was added slowly. The resulting mixture was stirred for 24 hours. The solvent was removed under reduced pressure and the residue taken up with water. After extraction with four portions of ether and subsequently drying, a white solid was obtained. This was recrystallized from hexane to give 15 g (5.58 mmol) oct-2-enedioic acid 1-*tert*-butyl ester 8-ethyl ester **12** as a white solid (77 %).

TLC (Hexane:EtOAc/10:1) R_f 0.6.

$C_{14}H_{24}O_4$ (Mo. Wt. 256)

^{1}H NMR (270 MHz, $CDCl_3$): δ 1.12 (t, 3H), 1.35 (m, 2H, 1x CH_2), 1.44 (s, 9H, $(CH_3)_3C$), 1.58 (m, 2H, 2xCH_2), 2.30 (m, 4H, 2xCH_2), 4.1 (q, 2H), 5.73 (d, 1H, vinyl α-H), 6.87 (dt,1H, vinyl β-H).

IR (KBr, cm^{-1}): 3453 (overtone of C=O stretch), 2979, 2864 (C-H stretching of alkane chain), 1736 (C=O stretching of ethyl ester), 1715 (C=O stretching of butyl ester), 1653 (C=C stretch), 1457 and 1368 (C-H bending of CH_2CH_3), 1256 (C-O stretching of ester), 981 (C-H out of plane bending of olefinic), 762 (methylene rock).

MS m/z (EI): $C_{14}H_{24}O_4$ (Mo. wt. 256); 256.3 $(M)^+$

Oct-2-enedioic acid 8- ethyl ester (13).[45]

5.0 g (19.53 mmol) of oct-2-enedioic acid 1-*tert*-butyl ester 8-ethyl ester **12** in 100 ml toluene and (0.5g) *p*-toluenesulfonic acid (PTSA) were refluxed for 30 min. and subsequently stirred at room temperature overnight. After removing the toluene, 300ml of 5% aqueous potassium bicarbonate solution was added and stirred for 10 minutes. The white precipitate was filtered off and the filtrate was acidified to pH 3 with dil. HCl. The resulting suspension was extracted with chloroform, dried with magnesium sulphate, evaporated solvent on rotavapour and re-crystallized with chloroform/hexane 3.3 g (16.52 mmol, 84.6 %) of 8-ethyloxy-nona-2,8-dienoic acid **13** white crystals were obtained.

$C_{10}H_{16}O_4$ (Mo. Wt. 200)

^{1}H NMR (270MHz, $CDCl_3$): δ 1.12 (t, 3H), 1.33 (m, 2H, 1xCH_2), 1.48 (m, 2H, CH_2), 2.20 (m, 2H, CH_2), 2.38 (t, 2H, CH_2COO), 4.1 (q, 2H), 5.73 (d, 1H, vinyl α-H), 6.87 (dt, 1H, vinyl β-H), 8.0 (broad, 1H, COOH).

IR (KBr, cm^{-1}): 3450 (broad O-H stretching), 2982 (olefin =C-H stretching), 2933 and 2871 (C-H stretching of alkane), 1732 (C=O stretching of carboxylic acid), 1695 (C=O stretching of ester), 1652 (conjugated C=C stretching), 1465 (O-H in plane bend), 1420 and 1375 (C-H bending of alkane), 1289 (C-O stretching of acid), 1200 (C-O stretching of ester), 986(O-H out of plane bending), 740 (methylene rock).

MS m/z (EI): $C_{10}H_{16}O_4$ (Mo. Wt. 200); 200.3 $(M)^+$

Elemental analysis: $C_{10}H_{16}O_4$ (Mo. Wt. 200)
Calculated: C 60.00 %, H 8.00 %,
Found : C 59.42 %, H 7.85%,

7-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy} ethylcarbamoyl) hept-6-enoic acid ethyl ester (14).[52-54]

A 3.1 g (1.5 mmol) of 1-{2-[2-(2-azido-ethoxy)-ethoxy]-ethoxy}ethylamine **7** and 3 g (1.5 mmol) of oct-2-enedioic acid 8- ethyl ester **13** were dissolved in 50 ml of CH_2Cl_2. After cooling at 0°C for 15 min**,** 4.6 g (2.25 mmol) of DCC and catalytic amount of DMAP (2 mmol) were added. The reaction mixture was then stirred at room temperature for further 24 hr. The white precipitate was filtered off and the filtrate washed successively with 0.1 M HCl, 8% $NaHCO_3$ **,** and water, dried over $MgSO_4$ The solvent was removed in vacuum and the residue purified by silica column chromatography using CH_2Cl_2: MeOH (9:0.5) as solvent, followed by crystallization from hexane/ethyl acetate, obtained 4.9 g (83.1 %) white solid of 7-(2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy} ethylcarbamoyl) hept-6-enoic acid ethyl ester **14**.

^{1}H NMR (270 MHz $CDCl_3$): δ 1.12 (t, 3H), 1.34 (m, 2H, 1xCH_2), 1.49 (m, 2H, CH_2), 1.63 (m, 2H, allyl-H), 2.37 (t,2H, CH_2COO), 3.45 (s, 3H, CH_3O), 3.56 (m, 2H, CH_2NH), 3.56-3.7 (m, 14H, CH_2O), 4.1 (q, 2H), 5.73 (d, 1H, vinyl α-H), 6.50 (m, 1H, NH), 6.87 (dt, 1H, vinyl β-H),

MS m/z (EI): $C_{19}H_{35}O_7$ (Mo. Wt. 389); 390.2 $(M+H)^+$, 91$(C_7H_7)^+$

7-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy} ethylcarbamoyl) hept-6-enoic acid (15).[55,56]

2.5g (0.65mmol) of 7-(2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy}-ethylcarbamoyl)-hept-6-enoic acid ethyl ester **14** was treated with 25 ml of 1M LiOH suspension in 10 ml THF, 5 ml methanol and 5ml water. The mixture was stirred overnight at room temperature. After removal of the solvent in vacuum, the mixture was extracted with ethyl acetate and water. The aqueous phases were combined and acidified to pH2 with dilute HCl. The suspension was extracted with chloroform, dried with magnesium sulphate and evaporated to afford 2.25 g (96.68 %, 0.62 mmol) 7-(2-{2-[2-(2-methoxy-ethoxy)-ethoxy]-ethoxy} ethylcarbamoyl) hept-6-enoic acid **15** which was re-crystallized from CH_2Cl_2/MeOH (9:1).

^{1}H NMR (270 MHz $CDCl_3$): δ 1.34 (m, 2H, 1xCH_2), 1.49 (m, 2H, CH_2), 1.63 (m, 2H, allyl-H), 2.37 (t, 2H, CH_2COO), 3.45 (s, 3H, CH_3O), 3.56 (m, 2H, CH_2NH), 3.56-3.7 (m, 14H, OCH_2CH_2O), 5.89 (d, 1H, vinyl α-H), 6.5 (m, 1H, NH), 6.87 (dt, 1H, vinyl β-H);

^{13}C NMR (63 MHz, $CDCl_3$): δ 24.17, 27.52, 31.49, 33.68, 39.25, 60.00, 62.45, 69.90, 69.67, 70.29, 70.42, 71.81, 123.98 (C=C-CO), 143.80 (C=C-CO), 175.0 (COOH) , 177.67 (NH-C=O)

IR (KBr, cm^{-1}): 3316 (broad O-H stretching of acid), 3065 (N-H stretching of amide), 3009 (olefin =C-H stretching) 2926 and 2871(C-H stretching of alkane), 1731 (C=O stretching of acid), 1671 (C=O stretching, Amide), 1628 (N-H bending, Amide), 1449 and 1348 (C-H bending of CH_2CH_3), 1242 (C-O stretching of ester), 981 (C-H out of plane bend, olefin), 946 (O-H out of plane bending of acid), 734 (methylene rock).

MS (FAB) m/z: $C_{17}H_{31}NO_7$ (Mo. Wt. 361): 362 $(M+H)^+$

Elemental analysis: $C_{17}H_{31}N_1O_7$ (Mo. Wt. 361):

Calculated: C 56.40%, H 8.58%, N 3.87%

Found : C 56.21%, H 8.48%, N 3.78%

7-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}ethylcarbamoyl)hept-6-enoyl chloride (16) [56]

1g of 7-(2-{2-[2-(2-Methoxy-ethoxy)-ethoxy]-ethoxy}ethylcarbamoyl) hept-6-enoyl acid was dissolved in 100mL dichloromethane cooled to 0 ^{o}C then added equimolar amount of oxalyl chloride. Stirred reaction mixture room temperature for another 24h. After removing solvent dissolved compound in dichloromethane and used such as in next step for rigid membrane preparation on silica colloidal particles.

Diethyl 5-(hydroxymethyl)isophthalate (17).

Method 1:[23] To a solution of 5 g (16.7 mmol) of 1,3,5-benzenetricarboxylic acid triethylester in 250 mL of absolute THF was added 4 mL of $LiBH_4$ (2 M solution in THF, 0.5 equivalent) drop wise under a nitrogen atmosphere. The solution was refluxed for 30 min to complete the reaction. After the solution was cooled, 20 mL of water was added at 0 °C. Sulphuric acid (5%) was added drop wise until a clear solution was obtained. The organic solvent was removed by evaporation, and the aqueous residue was extracted twice with 200 mL of ether. The separated organic phase was evaporated and the residue redissolved in 50 mL of hot ethanol. Unreacted benzenetricarboxylic acid triethylester crystallized spontaneously after cooling and was removed by filtration. The ethanol solution containing the desired monoalcohol and small amounts of dialcohol and tri-alcohol was divided in the three corresponding fractions by chromatography on a silica gel column (hexane/ethyl acetate 1:1). 5-hydroxymethylisophthalic acid diethyl ester **17** 1.85 g (39%) was obtained as a white crystals.

Method 2:[57] **Diethyl 1,3,5-benzenetricarboxylate (17a).**[57] Triethyl 1,3,5-benzene tricarboxylate (28.2 g, 0.096 mol) was combined with absolute EtOH (75 mL) and THF (50 mL) in a 250 mL three necked flask. The mixture was heated to reflux thus ensuring the dissolution of all of the solids. Powdered KOH (8.7%, 5.3 g, 0.084 mol) was then added portionwise over 30 min to avoid exothermic. The solution was heated at reflux for 12 h after which a small amount of solid precipitate was produced. The reaction mixture was concentrated in *vacuo* to afford a thick slurry which was partitioned between water and CH_2Cl_2. The aqueous phase was washed with CH_2Cl_2, and then concentrated HCL (8.5 mL) was added to precipitate the product. The product was recrystallized from absolute EtOH to afford 17a as white crystals (18.9 g, 76%); mp 153-154°C.

^{1}H NMR ($CDCl_3$): δ 8.53 (d, 2H, aromatic); 8.50 (d, 2H, aromatic); 4.11 (q, 4H; J=7.11 Hz); 1.11 (t, 6H, J=7.12 Hz).

Mass (EI, 80 eV, 100ºC): [m/z] = 266 ($[M]^+$, 24 %); 221 ($[M\text{-}COOH]^+$, 100 %)

Diethyl 5-(hydroxymethyl) isophthalate (17).[57] Diethyl 1,3,5-benzenetricarboxylate **17a** (5 g, 18,7 mmol) was dissolved in THF (30 mL) in a 250 mL round bottom flask, reaction mixture cooled to 0°C in ice-bath $BH_3.(CH_3)_2S$ (2.0 M in THF, 20.4 mmol) was added over period of 3 hrs. After completion of addition the reaction mixture was heated at 60°C overnight. The reaction was neutralized by the addition of 1:2 H_2O/glacial acetic acid. The reaction mixture was then dissolved in hot ethanol and precipitated into water to retrieve a white powder. After final crystallization from ethanol yielded 17 as a white crystals 3,6 g (14,586 mmol, 78 %).

Melting point = 82°C

$C_{13}H_{16}O_5$ (Mo. Wt. 252)

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.51 (s, 1H, phenyl), 8.15 (s, 2H, 4-H, 6-H, phenyl), 4.80 (s, 2H, CH_2-benzyl), 4.33 (q, 4H, CH_2-ester, J = 7.11 Hz), 3.05 (s, 1H, OH), 1.33 (t, 6H, CH_3-ester, J = 7.13 Hz).

^{13}C NMR (63 MHz, $CDCl_3$): δ 14.13, 61.26, 66.16, 129.40, 130.49, 136.33, 141.94, 165.67

IR (KBr, cm^{-1}): 3417 (O-H stretching of primary alcohol), 3100 (C-H stretching of aromatic), 2984-2873 (C-H stretching of methylene), 1724 (C=O stretching of ester), 1445-1371 (C-H bending of methylene and methyl group), 1242 (C-O stretching of ester), 1024 (C-O stretching of primary alcohol).

MS m/z (EI): $C_{13}H_{16}O_5$ (Mo. Wt. 252); 252$(M)^+$, 207$(M-C_2H_5O)^+$

Elemental Analysis: $C_{13}H_{16}O_5$ (Mo. Wt. 252.0):
Calculated: C, 61.90%; H, 6.34%.
Found : C, 61.56%; H, 5.98%.

5-(*tert*-butyl diphenylsilanyloxymethyl) isophathalic acid diethyl ester (18).[58,59]

To a solution of diethyl 5-(hydroxymethyl)isophthalate **17** (5g, 19.82 mmol) in 40 ml CH_2Cl_2 under argon, DMAP (0.126g catalytic), Pyridine (3.2ml) cooled to 0^oC. Then TBDMS-Cl were added drop by drop (1.5 equivalent), and the mixture was stirred for 24h at room temperature. Reaction quenched with 20ml 1N HCl. Extraction of aqueous layer with 200ml CH_2Cl_2. Combined organic layer washed twice with water, dried over Na_2SO_4, filtered and removed solvent under reduced pressure. Flash chromatography using eluent chloroform/hexane (1:1) obtained as an colourless oil of **18** with 9.7g (99%) yield.

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.55 (t, 1H,); 8.25 (d, 2H, arom); 7.70-7.67 (m, 4H, arom.) ; 7.47-7.36 (m, 6H arom.); 4.80 (s, 2H, CH_2-benzyl), 4.45 (q, 4H, CH_2-ester), 1.4 (t, 6H, CH_3-ester, J = 7.0 Hz); 1.1 (s, 9H , *t*-Bu).

^{13}C-NMR: (125 Mz, $CDCl_3$): δ 165.85, 141.92, 135.51, 135.18, 134.77, 133.08, 131.18, 130.89, 129.82, 129.57, 129.21, 127.77, 61.22, 64.65, 26.84, 19.28

[3-(*tert*-Butyl diphenylsilanyloxymethyl)-5-hydroxymethyl phenyl] methanol (19).[60]

5-(*tert*-Butyl diphenylsilanyloxymethyl) isophathalic acid diethyl ester **18** (15.4 g, 3.16 mmol) was added as a solution in 50ml of dry THF to a slurry of lithium aluminium hydride (1.80g, 1.5 eq.) in 150 ml of dry THF drop wise under argon. The mixture colour turned to green and stirred at room temperature for 2-3 h. Then quenching followed by hydrolysis using 10 ml methanol and 30 ml water. The mixture then filtered, the white precipitate was washed several times with methanol and THF. The combined liquid phase evaporated to achieve yellow oil. Re-crystallized from hexane leads to a white crystals of [3-(*tert*-butyl diphenylsilanyloxymethyl)-5-hydroxymethyl phenyl] methanol **19** (11.05g, 85%).

M.P. = 93 ^{o}C

$C_{25}H_{30}O_3$ Si (Mo. Wt. 406)

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.69 (m, 4H); 7.39 (m, 6H); 7.22 (s, 1H; H-4); 7.20 (s, 2H; H-2); 4.76 (s, 2H; CH_2O); 4.61 (d, 4H; CH_2OH; J = 6Hz,); 3.34 (s, 2H; CH_2OH; J = 6Hz,); 1.11 (s, 9H; $C(CH_3)_3$)

^{13}C-NMR (125 Mz, $CDCl_3$): δ : 141.21, 141.21, 135.56, 133.36, 129.72, 127.71, 124.12, 123.92, 65.37, 65.02, 26.85, 19.28;

MS m/z (EI): 406$(M)^+$, 449$(M-C_4H_9)^+$, 151$(C_9H_{11}O_2)$.

IR (KBr, cm^{-1}): 3239 (O-H stretching of alcohol), 3070 (C-H stretching of phenyl ring), 2929 and 2856 (C-H stretching of methylene group), 1471-1309 (C-H bending of methylene), 1105 (C-O stretching of primary alcohol).

Elemental Analysis: $C_{25}H_{30}O_3$ Si (Mo. Wt. 406):
Calculated: C 73.89%; H 7.38%.
Found : C 73.50%; H 6.98%.

(3,5-bis-Bromomethylbenzyloxy)-*tert*-butyl diphenylsilane (20).[60,61]

To a solution of [3-(*tert*-butyl diphenylsilanyloxymethyl)-5-hydroxymethyl phenyl] methanol **19** (3.248 g, 8mmol) in 120 ml dry ether was cooled (ice bath) and then PBr_3 (3.03 g, 11.2 mmol) dissolved in 50 ml dry ether was slowly added. After complete addition, the ice bath was removed and then the reaction was stirred at room temperature for 4 h. Then add 50 ml water the aqueous layer was extracted with 200 ml ether and the organic layers were combined, dried over Na_2SO_4 and filtered. The solvent was evaporated in reduced pressure. The column chromatography over a silica gel eluting with hexane/ethyl acetate (9:1) to yield a white crystals (4.26 g, 78%) of (3,5-bis-bromomethyl benzyl oxy) *tert*-butyl diphenylsilane **20**.
M.P. 90 ^oC.

$C_{25}H_{28}O_1Si\ Br_2$ (Mo. Wt. 530)

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.68 (m, 4H); 7.39 (m, 6H); 7.28 (s, 1H; H-4) 7.27 (s, 2H, H-2); 4.74 (s, 2H; CH_2O); 4.44 (s, 4H, CH_2Br); 1.11 (s, 9H; $C(CH_3)_3$);

^{13}C-NMR (125 Mz, $CDCl_3$): δ 138.27, 135.56, 129.79, 128.07, 127.77, 126.73, 64.90, 32.97, 26.83, 19.27

IR (KBr, cm^{-1}): In this step all peak from primary alcohol disappear only 3070 (C-H stretching of phenyl ring), 2929 and 2856 (C-H stretching of methylene group), 1471-1309 (C-H bending of methylene) are observed.

MS m/z (EI) $C_{25}H_{28}O_1Si_1Br_2$ (Mo. Wt. 530): 530$(M)^+$, 517$(M\text{-}CH_3)^+$, 473$(M\text{-}C_4H_9)^+$, 57 $(C_4H_9)^+$.

Elemental Analysis: $C_{25}H_{28}OSiBr_2$ (Mo. Wt. 530);
Calculated: C 56.39%; H 5.26%.
Found : C 56.37%; H 5.06%.

[3-(*tert*-Butyl diphenyl silanyloxymethyl)-5-(diethoxy phosphorylmethyl)-benzyl] phosphonic acid diethyl ether (21).[65]

A mixture of (3,5-bis-bromomethyl benzyloxy) tert-butyl diphenyl silane **20** (2.70 g, 5 mmol) and triethyl phosphite (excess used as a solvent) was placed in a flask with stirring in argon atmosphere . It was immersed in an oil bath heated 120 ^{o}C 12 h. The excess triethyl phosphite was then distilled off under reduced pressure. The crude, oily products were purified over column chromatography using $CHCl_3$/MeOH (95:5). Two columns were performed to achieve the pure compound, to yield a colourless oil of [3-(*tert*-Butyl diphenylsilanyloxymethyl)-5-(diethoxyphosphorylmethyl) benzyl] phosphonic acid diethyl ether **21** (2.54 g, 3.93 mmol, 77%).

$C_{33}H_{48}O_7Si\ P_2$ (Mo. Wt. 645.8)

^{1}H NMR (250 MHz, $CDCl_3$): δ 7.68 (m, 4H); 7.39 (m, 6H); 7.10 (s, 1H; H-4) 7.00 (s, 2H, H-2); 4.74 (s, 2H; CH_2O); 3.88-4.00 (m, 8H); 3.12 (d, 4H, J = 22.0 Hz; $CH_2P(O)(OEt)_2$, J = 22.0 Hz); 1.25 (t, 12H, J = 7.1 Hz); 1.11 (s, 9H; $C(CH_3)_3$)

MS m/z (FAB neg., Xe) , $C_{33}H_{48}O_7Si\ P_2$ (Mo. Wt. 645.8): 646 $(M)^+$

[3-(Diethoxyphosphorylmethyl)-5-hydroxymethyl benzyl] phosphonic acid diethyl ether (22).[66]

To a stirred solution of [3-(*tert*-butyl diphenylsilanyloxymethyl)-5-(diethoxyphosphoryl methyl) benzyl] phosphonic acid diethyl ether **21** (6g, 9.28 mmol) in 80 ml THF was added tetrabutylammonium fluoride (14 ml, 13 mmol, 1.5 eq.) and then reaction was stirred overnight. To the solution was added glacial acetic acid (2 ml) and solvent removed in vacuum. The crude product was purified by silica gel chromatography, eluating with 1% MeOH in chloroform. The solvent was removed in vacuum to give 3.64 g (96 %) [3-(diethoxyphosphorylmethyl)-5-hydroxymethyl benzyl] phosphonic acid diethyl ether **22** as a white solid.

^{1}H NMR (250 MHz, $CDCl_3$): δ : 7.15 (s, 1H; H-4); 7.10 (s, 2H, H-2); 4.58 (s, 2H; CH_2O); 3.88-4.00 (m, 8H); 3.20 and 3.12 (dd, 4H, J = 22.10 Hz, $CH_2P(O)(OEt)_2$); 1.25 (t, 12H, J = 7.1 Hz);

^{13}C-NMR (125 Mz, $CDCl_3$): δ: 16.25, 16.34, 32.30, 34.50, 62.32, 64.45, 142.05, 131.90, 131.71, 130.02, 129.91, 127.03, 126.95,

3,5-*bis*(Diethoxyphosphorylmethyl) benzaldehyde (23).[41]

Pyridinium Chlorochromate 2.85 g (1.5 eq., 13 mmol) was suspended in 20 ml CH_2Cl_2 and 3.6 g (8.8 mmol) of [3-(diethoxyphosphorylmethyl)-5-hydroxymethyl benzyl] diethylphosphonate **22** added over it was dissolved in 10 ml CH_2Cl_2, at once upon stirring the solute becomes briefly homogeneous. After 2 h oxidation, the black reaction mixture was diluted with 200 ml ether. The solvent was decanted from black granules and washed with 200 ml ether. The organic layer was collected dried over sodium sulphate and evaporated under reduced pressure. Flash column chromatography to gave pure compound as a colourless oil yielded 2.10 g (59 %) of 3,5-bis(diethoxyphosphoryl methyl) benzaldehyde **23**.

$C_{17}H_{28}O_7P_2$ (Mo. Wt. 405.8)

^{1}H NMR (250 MHz, $CDCl_3$): δ 9.99 (s, 1H, CHO); 7.72 (m, 2H, Ar); 7.53 (broad s, 1H, Ar) 3.97-4.1 (m, 8H, 4x$CH_3\underline{CH_2}$); 3.21 (d, 4H, J = 22.0 Hz, 2xCH_2P); 1.27 (t, 12H, J = 7.0 Hz, 4x$\underline{CH_3}CH_2$).

^{13}C NMR (125 Mz, $CDCl_3$): δ 16.26, 17.01, 33.79, 32.69, 62.21, 62.32, 64.45, 191.69, 137.04, 136.74, 131.90, 131.71, 130.02, 129.91,

IR (KBr, cm^{-1}): 2817 and 2733 (aldehydic C-H stretching), 2982 - 2872 (C-H stretching of methylene group), 1699 (C=O stretching of aldehyde), 1477-1367(C-H bending of methylene), 1249 (C-O stretching of aldehyde).

MS m/z (FAB neg., Xe): $C_{17}H_{28}O_7P_2$ (Mo. Wt. 405.8); 406.9$(M-H)^+$, 391 (M-15)$^+$, 380 (M-26), 363 (M-45+H).

***meso*-5,10,15,20-Tetrakis-[3,5-bis(diethoxyphosphorylmethyl) phenyl)] porphyrin (24).**[23,24]

A 1L three neck flask fit with reflux condenser and nitrogen inlet port was filled with 600 ml of dichloromethane 3,5-bis(diethoxyphosphorylmethyl) benzaldehyde **23** (2.03 g, 5mmol) and pyrrole (0.335 g 5 mmol) were added, and then the solution was stirred at room temperature under a slow stream of nitrogen. After 15 min, BF_3-etherate (0.265ml in 10 ml of dichloromethane) was added and the reaction vessel was kept in dark. Stirring continued 14 h. Then p-chloranil (1.2 g) was added to the reaction mixture after 2 h, the colour of the mixture turned to deep violet . After standing another 30 min, the solution was concentrated and 10 g of silica was added. The slurry was dried to afford a dark powder that was poured onto the top of a chromatography column of silica. The column elute using chloroform and methanol. Performing same column three times we obtained violet crystal. The column was elute using a solvent mixture chloroform/ acetonitrile/ methanol (10:10:1). Yield 474 mg 21 % of *meso*-5,10,15,20-tetrakis[3,5-bis(diethoxypho sphorylmethyl) phenyl)] porphyrin **24**.

$C_{84}H_{118}N_4P_2O_{24}$ (Mo. Wt.1814.6)

^{1}H NMR (250 MHz, $CDCl_3$): δ 8.8 (s, 8H, pyrrole); 8.50 (broad s, 8H, arom.); 7.89 (s, 4H, arom.), 4.2-4.35 (m, 32H, CH_2CH_3); 3.58 (d, 16H, $CH_2P(O)(O\ Et)_2$, J = 22.0 Hz); 1.28 (t, 48H, CH_3CH_2, J = 7.1 Hz); -2.8 (2H, s, NH).

^{13}C-NMR (125 Mz, $CDCl_3$): δ 16.60, 33.37, 34.47, 62.19, 62.36, 62.41, 121.93, 128.11, 132.57, 134.91, 138.79, 140.16, 145.86, 190.44, 196.11

MS m/z (FAB neg., Xe) , $C_{84}H_{118}N_4P_2O_{24}$ (Mo. Wt.1814.6): 1815.6 (M+H).

***meso*-5,10,15,20-Tetrakis[3,5-bis(diphposphonoxylatophosphorylmethyl)-phenyl)] porphyrin (25).**[62]

200 mg of the *meso*-5,10,15,20-tetrakis-[3,5-bis(diethoxyphosphorylmethyl) phenyl)] porphyrin **24** was dissolved in 20 ml absolute chloroform cooled reaction mixture at –40 ^{o}C and 0.26 ml of trimethylsilyl-iodide was added drop wise by syringe. The reaction mixture kept this temperature 30 min. then left at room temperature 1 h more. 5 ml of water were added to hydrolysis, and after stirring for 5 min the solvents were removed in vacuum. 150 mg (above 95 % yield) of violet crystal obtained.

^{1}H NMR (250 MHz, DMSO d_6): δ 9.32 (broad, 8H, pyrrole); 7.97 (broad s, 8H, arom.); 7.63 (s, 4H, arom.), 3.16 (d, 16H, $CH_2P(O)(OH)_2$, J = 22.12 Hz), -2.8 (2H, s, NH).

Synthesis of *meso*-5,10,15,20-tetrakis-(3-carboxylatophenyl)porphyrin (26).[23,24]

A 1L three necked flask fit with reflux condenser and nitrogen inlet port was filled with 600 ml of dichloromethane. 3-formyl-benzoic acid ethyl ester (5mmol) and pyrrole (5 mmol) were added, and then the solution was stirred at room temperature under a slow stream of nitrogen. After 15 min, BF_3-etherate (0.265ml in 10 ml of dichloromethane) was added and the reaction vessel was kept in dark. Stirring continued 1 h. Then p-chloranil (1.2 g) was added to the reaction mixture after 2 h, the colour of the mixture turned to deep violet . After standing another 30 min, the solution was concentrated and 10 g of silica was added. The slurry was dried to afford a dark powder that was poured onto the top of a chromatography column of silica. The column elute using chloroform and methanol (10:1). Performing same column three times we obtained violet crystal. Lastly for getting pure porphyrin the column elute using ($CHCl_3$/EtOAc:10:1). Yield 21 % of porphyrin**.**

For deprotection of ester moieties from above porphyrin here we was used same method described for removal of ester moieties from compound **2** and **11** using LiOH in MeOH / THF[55,56] after acidification achieved greenish solid with 80 % yield of *meso*-5,10,15,20-tetrakis (3-carboxylatophenyl)porphyrin **26**.

Synthesis of *bis*-iminoquinone (36)

To a stirred solution of benzidine dihydrochloride (1.0 g., 3.8 mmol) in the mixture of ethanol/water (1:3, 80 mL) after 10 min, 9,10-anthraquinone-1,5-disulphonic acid disodium salt (4.8 g., 11.6 mmol) dissolved in water (20 mL) was added dropwise to the reaction mixture, which was then stirred at reflux temperature for 2 h. The completion of reaction monitored by TLC in water and after completion the solution was cooled (0 ^{o}C) for 1 h and then the radish-yellow product was collected on sintered glass, the solid material was washed three times with water, ethanol and one time with ethanol/water (1:1) mixture (10 mL each time) successively, dried solid material under *vacuo* to give pure compound **36** and was confirmed by ^{1}H NMR and mass spectrum.
TLC R_f 0.14 (in water, TLC plate saturated with aq. sodium chloride);

^{1}H NMR (DMSO d_6, 250 MHz): δ 7.35 (d, 4H, benzidine proton); 7.61 (d, 4H, benzidine proton); 8.01 (d, 4H, anthraquinone); 8.10 (d, 2H); 8.32 (d, 2H); 8.53 (s, 4H, benzyli-denimin);

MS m/z (EI): calc. $C_{40}H_{24}N_2O_{14}S_4$: 884; found: $C_{40}H_{23}N_2O_{14}S_4$: 883 (M-H, 100%);
MS m/z (FAB): calc. $C_{40}H_{20}N_2Na_4O_{14}S_4$: 971.9; found: $C_{40}H_{20}N_2Na_4O_{14}S_4$: 971.4 (100% M^+), 948.94 $[C_{40}H_{24}N_2O_{14}S_4]^-$ + $3Na^+$, and 883 $(M-H)^+$ $C_{40}H_{24}N_2O_{14}S_4$ sulphonic acid;

IR (KBr, cm^{-1}): 3112 (Overlap C=O stretching), 3052 (C-H stretching of Phenyl), 1674 (C=O stretching of ketone), 1589 (S=O stretching), 1504 (C=N stretching; Schiff's base), 1178 (C-O stretching).
The reduction potential of *bis*-aminoquinone –550 mV vs an Ag/Ag(I) electrode.[114]

Synthesis of (4-Pyridyl) viologen salts (37): The tetrapyridine with a central viologen unit was synthesized according to a literature procedure.[110]

Synthesis of Bolaamphiphile 1.5 nm (**38**)[109]**:** The applied bolaamphiphile with OEG head group was synthesized as described in reference 97.

8.2 Detailed procedures for the preparation of aminated silica particles, yoctowells and with characterization

Preparation of aminated silica particles

The silicate particles developed by van Blaaderen, are produced by hydrolysis of tetra ethoxysilane (TEOS) with aqueous ammonia in ethanol and are stabilized by subsequent treatment with (3-aminopropyl)-triethoxysilane.

Colloidal silica nanoparticles with a mean diameter of 100 nm were prepared according to the following procedure; All glass reaction vessels were cleaned extensively to ensure that no nucleation sites were present (washing procedure: filling with 3% hydrofluoric acid for an hour, rinsing with milli-Q water and finally rinsing with distilled ethanol). In a reaction vessel, which had been dried for 3 h at 120 °C, TEOS (1.5 mL) and ammonium hydroxide (3 mL, 28%) were dissolved in 50 mL of anhydrous ethanol, and the reaction mixture was slowly stirred at room temperature for 24 h in the dark. milli-Q water (400 µl) was added and the mixture was stirred for another 2 h further. Then, (3-aminopropyl) triethoxy silane (APTS) (400 µl) was added, and the mixture was stirred overnight in dark. The resulting silica particle was warmed to 80 °C and refluxed at this temperature for 10 h under an argon atmosphere. The amino-modified silica particles with a diameter of 100 nm were then used further for self-assembling work after cooling to room temperature. Unreacted (3-Aminopropyl)-triethoxysilane (APTS) was removed by repeated washing with anhydrous ethanol followed by dry DCM (each one three times) collected particles by centrifugation.

The surface of the particles could be coated through a subsequent chemical reaction with the silane coupling agent (3-aminopropyl)-triethoxysilane (APS). APS then forms a propyl amine coating on the silica particles which stabilizes them against water and organic solvents and render the surface reactive forward with acid derivatives. The qualitative reaction of APS on the silica spheres proved to be a very simple test to the presence of amino groups on the surface. If the silica particle was colored yellow in solution, the amine groups could always also be detected on dried silica. We used the

amino modified silica colloidal particles for preparation of 2 nm gaps. In such studies was required to use stable, monodisperse, unclustered colloids and also important to be able to adjust the surface properties and chemical composition of the particles.

Spherical particles with a diameter between 20 nm and 150 nm were obtained depending on concentration and hydrolysis time. The smaller 20 nm particles showed (Figure 8.1) was obtained when hydrolysis time was 2 hrs., however, a rough surface in transmission electron microscopy (TEM) which was not appropriate for the self-assembly of rigid membranes and defined nanometer gaps. The smallest uniform particles with a perfectly smooth surface had a uniform diameter of 100 ± 10 nm (Figure 8.3). The measurements were accomplished with a Philips 12 mm electron microscopy. The sample preparation took place on copper nets with 3 mm diameter and 400 meshes (type B 8010 Cu, Blazer union). These particles were stable and could be stored indefinitely as a moist powder. When resuspended in milli-Q water at pH 7-8, transmission electron microscopy (TEM) always showed observed ill-defined networks of 100-nm spheres with a smooth surface.

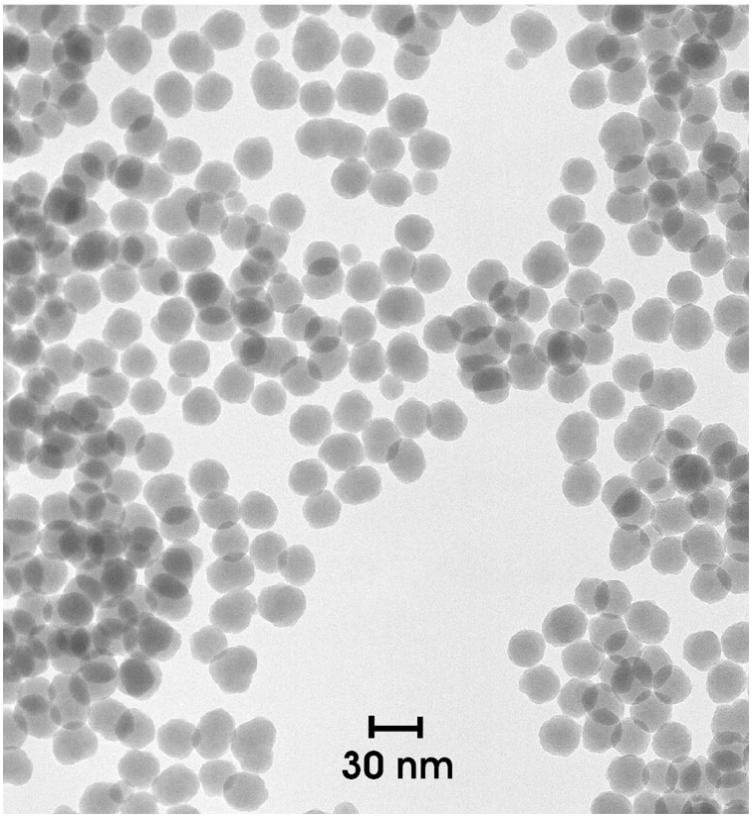


Figure 8.1 Transmission electron micrograph (TEM) of synthetic silica particles with a diameter of 20 ± 10 nm. All of these particles showed rough surface and are not spherical. Several sharp edges are usually found.

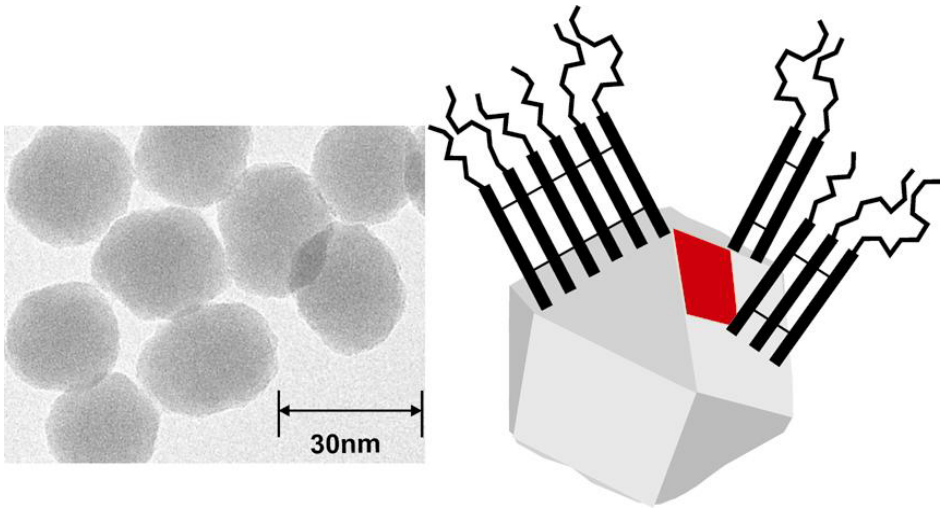


Figure 8.2 Model of a rough silica particle partially covered with a porphyrin, coated with a hybrid lipid monolayer, which cannot produce 2 nm yoctowell.

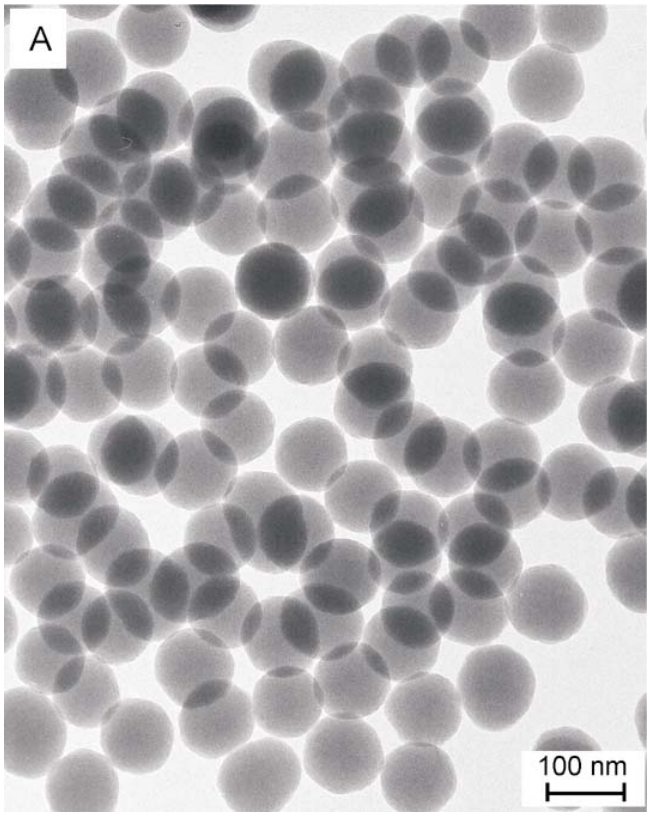
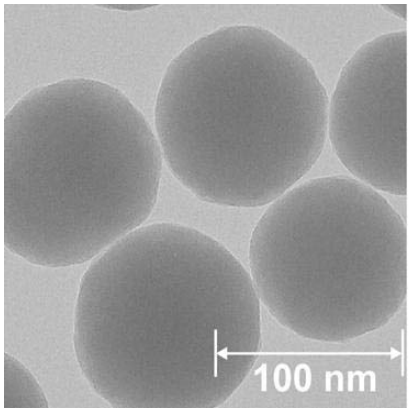


Figure 8.3 Transmission electron micrographs (TEM) of synthetic 100 nm aminated silica particles having smooth surface and moderate curvature.



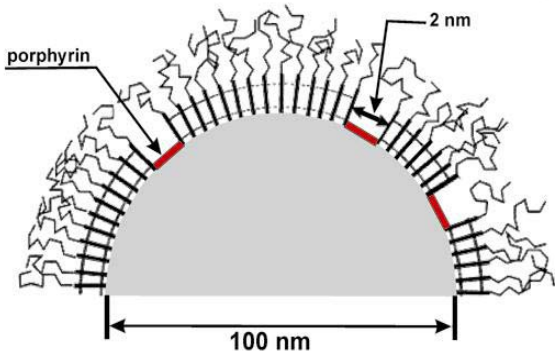


Figure 8.4 Model of a smooth silica particle coated with a closed hybrid lipid monolayer and porphyrin-based yoctowells.

The smoothness, size, and chemical self-assembly procedures were optimised in order to establish a closed monolayer with modest curvature and containing functional gaps. Furthermore, the amino silica particles are soluble in water and many solvents, and the chemistry of the amino groups is versatile enough to produce all kinds of modification on surface. Protection of the silicate surface by the thin propylamine coating as introduced by van Blaaderen solved the major problems, which were encountered with commercial CabOSil (i) no detectable roughness or irreversible aggregation occurred, (ii) organic solvents did not cause any swelling or formation of jelly like protrusions, (iii) self-assembly processes on the amino surface gave closed rigid monolayers with form-stable 2 nm gaps. After two step self assembly these particles were soluble in water as well as organic solvents (chloroform, ethanol etc.). After and before two step self assembly we observed strong fluorescence of porphyrin its fluorescence did not quench by silica particles and particles also produced extra peaks coming from scattering of light scattered in the region 510-580 nm (Figure 8.5).

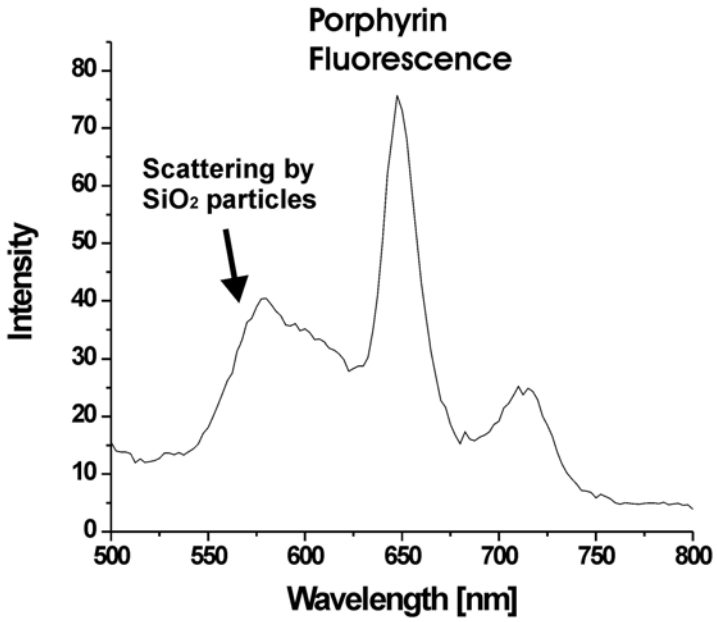


Figure 8.5 Silicate particles with bottom porphyrin **26b** within the yoctowell.

Preparation of closed monolayer (yoctowell) on silica particles

The above 100 nm aminated silica colloid (0.5 g) was washed 4 times with anhydrous ethanol and dry CH_2Cl_2 by repeated centrifugation, dispersion, and ultrasonification. Then, obtained silica particles were dissolved in 50 mL of CH_2Cl_2 containing 1 mL of dry triethylamine. With vigorous stirring, 4 mL of CH_2Cl_2 solution of meta-tetracarboxy with ethylchloroformate activated meta-tetracarboxy porphyrin **26b** (1 mg) was added dropwise. After the mixture was stirred for 2 h, 5 mL of CH_2Cl_2 solution of bola **10** or **16** or **38** (2 mg) were given. The resulting suspension was stirred in the dark overnight. The membrane coated nanoparticles were isolated by repeated centrifugation, dispersion, and ultrasonification using CH_2Cl_2 as solvent and were used for further measurements.

In the first step of self-assembly, *meso*-(tetra-*m*-benzoyl chloride) porphyrin **26a**, was applied first it did not work at all. This activated porphyrin presumably formed domains on the silicate surface rather than spots of monomeric porphyrins, because it readily formed anhydride dimers upon partial hydrolysis of the acid chlorides, which could not be totally avoided when applied. The more stable mixed anhydrides **26b** made with ethyl

chloroformate were much more reliable and give wide exact 2 nm gap. The fluorescence of the bottom porphyrin **26b** was measured and a porphyrins, which diameter is larger than the well, for example, [T2PyP] porphyrin **32** (32 Å), should not reach the bottom at all (Figure 8.6b). It proved, however, to be difficult to demonstrate this filter effect with the silicate particles. It did not work at all when the porphyrin tetracarbamoylchloride **26a** was used in the first self-assembly step porphyrin **32** (32 Å) having diameter larger than pores also enter into the gap (Figure 8.6a). This activated porphyrin **26a** presumably formed domains on the silicate surface rather than spots of monomeric porphyrins, because it readily formed anhydride dimers upon partial hydrolysis of the acid chlorides. The more stable mixed anhydrides **26b** made with ethyl chloroformate were much more reliable and give a light population of monomeric porphyrin 2 nm gaps.

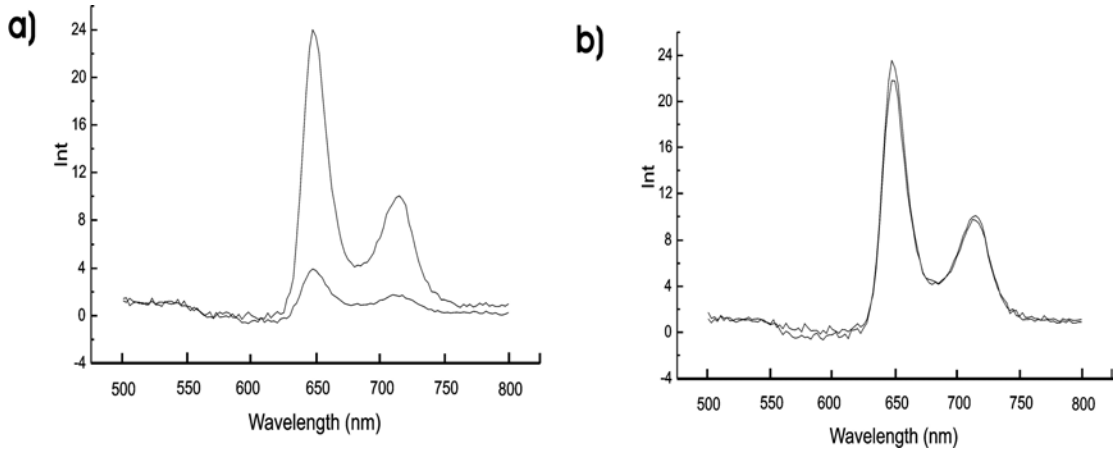


Figure 8.6 (a) Applied porphyrin **26a** formed domains and magnese porphyrin **32** enter within the yoctowell (b) applied porphyrin **26b** form 2 nm gaps porphyrin **32** doesnot enter to the gaps.

The fluorescence of bound bottom porphyrin **26b** on the particle surface was continuously checked. The results clearly indicate that no measurable domain formation of the bottom porphyrin had taken place and that the walls of the gaps are neither fluid nor contain any irregular bents. After amidation of the amino groups with meta-tetracarboxy activated with ethylchloroformate porphyrin **26b** and bolaamphiphiles **10** (10 Å) or **16** (5 Å) the solubility of the particles became pH independent. Interactions of the porphyrin on the bottom of the yoctowell with water-soluble, redox-active molecules

were now studied at pH 7-8 by fluorescence measurements. The fluorescence of the bottom porphyrin **26b** was measured and a porphyrins, which diameter is larger than the well, for example, [T2PyP] porphyrin **32** (32 Å), should not reach the bottom at all (figure 8.2b).

Transmission Electron Microscopy (TEM)

TEM samples were prepared by dropping 10 µl aliquots of colloidal solution (ethanol/H_2O 1:1) onto a carbon-coated grid. After about 1 min, the remaining solution was blotted off with a filter paper. A Philips M12 transmission electron microscope operated at 100 kV was used to obtain the images. The measurements were accomplished with a Philips's 12 cm. The sample preparation took place on copper nets with 3 mm diameter and 400 meshes (type B 8010 Cu, Blazer union)

Fluorescence Quenching Experiments

Fluorescence measurements and quenching experiments were performed on a Perkin-Elmer spectrometer (LS50B).

Silica colloid coated with perforated membranes (3 mg) was dispersed in 3 mL of water and placed in a quartz cuvette. A 30 µl aliquot of the aqueous solutions of the quenchers such as a "fitting" porphyrin: T3PyP **28** (10^{-4} M, 22 Å) or Mn(III)TPPS **27** (10^{-4} M, 22 Å), "too large" [T2PyP] porphyrin **32** (10^{-4} M, 22 Å), 9,10-anthraquinone-2,6-diamine **34** (0.1 M), or 9,10-anthraquinone-2,6-disulfonic acid sodium salt **35** (0.1 M) was added.

The fluorescence of bound bottom porphyrin **26b** on the particle surface was continuously checked. The results clearly indicate that no measurable domain formation of the bottom porphyrin had taken place and that the walls of the gaps are neither fluid nor contain any irregular bents. After amidation of the amino groups with meta-tetracarboxy activated with ethylchloroformate porphyrin **26b** and bolaamphiphiles **10** (10 Å) or **16** (5 Å) the solubility of the particles became pH independent. Interactions of the porphyrin on the bottom of the yoctowell with water-soluble, redox-active molecules were now studied at pH 7-8 by fluorescence measurements. The fluorescence of the bottom porphyrin **26b** was measured and a porphyrins, which diameter is larger than the

well, for example, [T2PyP] porphyrin **32** (32 Å), should not reach the bottom at all (page 53,54).

Functionalization of Nanogaps on Silica Particles

Amino groups were introduced on the wall of nanogaps by Michael addition of methylamine to the activated double bond of bola **10** or **16** on silica particles.

At first experiement of Michael addition of methylamine functionalised applying bolaamphiphile **10** in aqueous solution and were characterized by NMR, we observed the loss of signal trans C=C double bond. Same procedure then applied after two step self assembly on aminated silica particles. The gaps-coated 20 mg silicate particles prepared by two step self-assembly were dispersed in 10 mL of an aqueous solution of methylamine (10 mM). After the reaction mixture was stirred for 2 h, the silica particles were collected by centrifugation and washed several times using Milli-Q water. After methylammonium rim on the walls of well we observed that, fluorescence quenching of porphyrin **26b** by addition of Mn(III) TPPS **27** did not enter into the gap (Figure 6.9; page 68).

Construction of Porphyrin Heterodimer on Silica Particle

We investigate bolaamphiphilic diamide **10** and **16** with a Michael-type terminal double bond as a simple candidate for amination in aqueous medium. The ammonium groups could be used to fixate an anionic‘ top porphyrin’ relatively far away from the ‘bottom porphyrin’ and very close to the bulk water volume.

Porphyrin heterodimers separated by a defined distance were established on silica particles as follows. Silica particles (0.5 g) with amino functionalized gaps were dispersed in 5 mL of Milli-Q water, and porphyrin 27 (Mn(III) TPPS) (0.1 mg) was added at pH 8-9. The reaction mixture was stirred for 30 min and kept in the dark overnight. Silica particles with porphyrin heterodimers were obtained after washing three times with milli-Q water (see model 4.8). From UV/vis and fluorescence quenching experiment we concluded that in each yoctowell only one molecule bound to the methyl

ammonium group on the rim, it means porphyrin heterodimers were achieved, the ratio 1:1. The extinction coefficient of Mn(III)TPPS **27** λ_{max} band at 466 nm is about 99,100[116] and bottom porphyrin **26b** λ_{max} band at 419 is about 325,000[117] compare with figure 4.10 page 65 its ratio about 4:1 which means we obtained heterodimer with in the yoctowell is about 1:1 spectra (Figure 8.7).

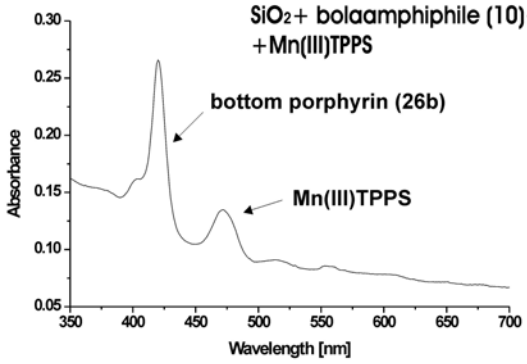


Figure 8.7 UV / VIS of porphyrin **26b** (bottom) and Mn(III) TPPS **27** within 1 nm or 0.5 nm distance (heterodimers within yoctowells).

Fluorescence decay measurements

They were carried out using the method of time-resolved single photon counting with equipment containing an SPC300 (Becker & Hickl) with a multichannel plate (Hamamatsu) and a Czerny-Turner monochromator (Oriel) on the detection pathway. For an additional exclusion of scattered light, a long-pass edge filter (530 nm) was used. As a light source, a TiSa-Laser (Mira 900, Coherent, duration 200 fs at 420 nm) was used. The sample was illuminated from below to optimize the coupling of the emitted light to the monochromator. The dimensions of the observed volume were reduced by long-distance focusing of the laser pulse into the sample and use of a 2-mm aperture directly in front of the sample. A response function of the setup shorter than 50 ps could thus be reached. The minimum time scale was also shortened by using deconvolution procedures to determine decay time values.

Blocking effect of tyrosine in yoctowell on silica particles

The particles with a coating made of *meta*-tetranhydride porphyrin **26b** and bola **10** (10 Å) were stirred overnight in a 0.05 M solution of tyrosine, centrifuged, redispersed, and centrifuged twice in distilled water, and their fluorescence was measured. It was within a possible error of ±20%, the same as that before addition of tyrosine. Addition of a large excess of quinones **34** or Mn(III)TPPS **27** did not diminish the fluorescence at all; the pore was irreversibly clogged under these conditions (see page 58 fig. 4.6)

UV/vis spectra measurements

The porphyrin on the aminated silica particles were also characterized after each self-assembly process with electronic measured with a fiber optic LAMBDA of 16 spectrometers of the company Perkin-Elmer using of quartz cuvettes.

Triplet absorption changes

The triplet absorption were measured with a conventional apparatus and signal averaging using 6-μs flashes. The oxygen was removed by bubbling with N_2. The triplet decay time did not change when the bubbling time was changed from 5 to 10 min.

The halftime of the relaxation depended on the age of the preparation. In fresh preparations, the decay of the absorption change measured under nitrogen was nearly exponential with a half time of 85 μs (overall concentration of porphyrins $C_p = 2.1 \times 10^{-6}$ M, 1% excitation). This value was obtained with fresh probes made with the *meta*-tetracarboxy acid chloride porphyrin **26a**, which is known to contain porphyrin domains, as well as with the *meta*-tetracarboxy mixed anhydride with ethylchloroformate porphyrin **26b**, which produces exclusively 2-nm-wide wells. Aged preparations (t = 2 and 22 days) showed a nonexponential decay, which was not analyzed in detail. The first halftimes were 230 μs ($C_p = 2.6 \times 10^{-6}$ M, 2.5% excitation) and 860 μs ($C_p = 2.3 \times 10^{-6}$ M, 5% excitation). *o*-Naphthoquinone sulfonate was then applied as a quencher of the triplet state. It accelerated the decay of the triplet absorption from 860 μs to 62 μs in the aged preparation and from 230 μs to 58 μs in an intermediate age. This corresponds to quenching constants of $k_q^T = (4\text{-}6) \times 10^7$ M^{-1} s^{-1} ($C_q = 3.1 \times 10^{-4}$ M). The amplitude of the

absorption decreased, indicating quenching of the singlet state also. The Stern-Volmer constant was (5-6) × 10^3 M^{-1}. Stern-Volmer traces for anthraquinone disulfonate **34**, naphthoquinone sulfonate NQS^-, and methyl viologen MV^{2+} indicate that the redox potential of the excited singlet state of **26b** is negative enough to reduce **34** and even methyl viologen. Oxidative quenching is exergonic for all three (ΔG_o= -0.29, -0.72, and -0.23 eV). The triplet state reduction power is lower by 0.47 eV and allows the reduction of NQS^- but not of **34** or MV^{2+}.

Preparation of nanoporous monolayers on Au surface

Gold electrodes [glass plate (2.5×1.5cm), with deposition of first 20 Å a layer of Cr, and then 200 nm of polycrystalline Au] were prepared as described earlier.[23,24] The electrodes were cleaned with fresh piranha solution ($3H_2SO_4$: $1H_2O_2$, Attention: piranha solution reacts violently with organic material) for 30 s, rinsed thoroughly with water and dried under a stream of N_2. These electrodes were then exposed to a 1×10^{-3}M aqueous NaOH solution (pH 12) of porphyrin **26** for 2 days. Afterwards the porphyrin-coated electrodes were rinsed with 20 mL of the solvent used for porphyrin self-assembly process, dried under a stream of N_2 and then dipped into a ethanol solution containing 1×10^{-3} M octadecanethiol. After 2 h these electrodes were again washed with ethanol and water to remove all physically adsorbed compounds and used immediately for blocking experiments.

Cyclic voltammetry (CV)

This was performed using a potentiostat PG310 (HEKA) operated with an IBM compatible PC in an one-compartment three-electrode cell. The working electrode was a circular bar gold electrode or monolayer-coated gold electrode with a surface of 0.5 cm^2. The counter electrode was a Pt wire. An aqueous SCE was chosen as reference electrode. An aqueous solution containing 0.1 M KCl and 1 mM $K_3[Fe(CN)_6]$ was used as electrolyte. Before each experiment this solution was purged with argon for 10 min at room temperature and kept under argon atmosphere during measurements. The blocking experiments were carried out as follows: the nanoporous monolayer-modified gold

electrodes were at first exposed to 0.1 M aqueous solution of the probe molecule (see Table 1). After 24 h immersion the electrodes were carefully washed with water and the blocking effect of the used probe molecules in monolayer was checked immediately using CV. The relative decrease of current: $I_{rel}=[(I_{open}-I_{blocked})/I_{open}]\times 100\%$ at potential of 0.4 V, was used as a parameter to evaluate the blocking effect of probe molecules.

FTIR Spectroscopy

FTIR measurement were carried out with Nicolet 740 FT-IR spectrometer equipped with a narrow-band, liquid nitrogen-cooled HgCdTe detector. Reflection spectra of the yoctowell, typically 1024 scans were averaged for each pair of background and sample spectra, at 2 cm^{-1} resolution with p-polarized light incident at 80^{o} C from the surface normal were collected. The whole procedure was repeated eight times, and difference spectra at the respective electrode potential were averaged to improved signal-to-noise ratio. This procedure ensures the minimization of baseline drifts and other artifacts that may appear during the long measurement time. Therefore, neither baseline correction nor any smoothing procedures needed to be carried out on the observed spectra.

FTIR spectroscopy was used to follow the detection of D_2O-Dimers with in the nanowells. The yoctowells on gold electrodes as described above were also soaked with 0.1 M D_2O-solutions of tyrosine and infrared spectra were measured in the region between 2600 and 2800 cm^{-1}. A strong and narrow D_2O-signal at 2721 cm^{-1} was found. The D_2O-monomer in Ar-matrices absorbed at 2771 cm^{-1}, the dimer at 2746 cm^{-1} and at 2725 cm^{-1} in N_2-matrices. Our spectrum thus indicates the presence of D_2O-dimers. No signal at 2623 cm^{-1} was observed, which would correspond to the 3710 cm^{-1} water monomer band.

Radioisotope Experiments

First a gold electrode was covered first with *meso*-tetra(phenyl-3,5-dicarboxy) porphyrin (~ 50 %) and then with octadecylthiol. Such a monolayer is fluid, dissolves tyrosine and partly covers the yoctowell with octadecyl-chains of neighbouring molecules. The system is thus similar to Sagiv's early monolayers containing dissolved dyes. It has, however,

been shown that such fluid gaps are blocked by tyrosine with the same efficiency observed for rigid yoctowells made of stiff diamido amphiphiles.[106] Since a closed fluid monolayer is much easier to prepare reproducibly and routinely, because eventual roughness on the gold surface are not so critical here, we applied them in a series of experiments with radioactive tyrosine. A control experiment with a rigid monolayer gave similar results for the adsorption of radioactive tyrosine.

Procedure: ^{14}C-labelled tyrosine (Amersham Pharmacia Biotech, 86 MBq mg^{-1}) with an average of 7 carbon atoms labelled per molecule was used as tracer. The solution contained 2 v% ethanol for catching radicals from radiolysis. 18.1 mg of D,L-tyrosine (ty, Fluka) was dissolved in 1 ml of this solution at pH 10.5 (NaOH) resulting in 1 mL of a 0.1 M solution. Its specific activity was measured by liquid scintillation counting[107] to be 1.85 MBq cm^{-3} for the dissolution tests (Beckman Instr. LSC 6500, counting efficiency 97.5%, Rotiszint eco). It subsequently decreased down to 0.271 MBq cm^{-3} in a 0.015 M tyrosine solution due to loss and dilution by washing and adsorption in each of the nine desorption and cv-experiments. The data in Table 1 were obtained from plates loaded with this 0.015 M solution. The conversion of CPM to molarity M was carried out as follows : activity A [Bq] = CPM/ 60 [s] x 0.975 [counts per s]. x[M] = A x 0.072 mMoles/ 1.303 x 10^6 [Bq]. The constant ratio between labelled and overall tyrosine was 1 MBq per $5.4 \cdot 10^{-5}$ moles and served as a bases for the subsequent calculations. The concentration of the labelled tyrosine was, on a molar scale, always 1000 times lower than that of the unlabelled one. Multilabelling of tyrosine did therefore did not require a correction of the concentration. The plates containing porphyrin and octadecyl thiol monolayers or thiol monolayers alone were loaded in this solution 3 days long at 23 $\pm$ 2 ^{o}C, then transferred to bidistilled water, kept there for 1 h and finally rinsed 5 times with water. In the release experiments, each of these loaded plates were kept in 3 ml water in sealed vials. After 24 h the water was exchanged and its β-activity measured by LSC using 0.5 ml aliquots. We also tried to monitor the surface activity of the plates by using a gas-flow proportional detector after each day, but the data obtained were less reliable due to low sensitivity, β-self-adsorption and an unfavourable geometry of the experimental set-up. After the cv-stirring experiments, the complete aqueous phase and

the washing solution of vessel and electrode was subjected to LSC. The given counting rates are in counts per minute (CPM) and are background-corrected. Finally, the plates were leached with aired NaCN solution until the gold coating of the electrode had completely dissolved. This leaching solution was measured immediately by LSC, as it slowly reacts with the scintillation ingredients.

The experiments were carried out together with Dr Ludwig "Institute fur Inorganic Chemi., Freie Universität, Berlin".

Preparation of silicate particles with zinc porphyrin (26c) at the bottom

It is known that zinc porphyrins form stable cation radicals, whereas free base porphyrins do not. A 100 mg of above particles containing yoctowells were dispersed in 10 mL methanol : chloroform (1:1), after dispersion 10 mg of zinc acetate was added and refluxed these particles for 4 hrs with slow stirring. After completion of conversion nanoparticles were isolated by repeated centrifugation, dispersion, and ultrasonification using methanol (three times) and chloroform (two times) to remove excess zinc acetate.

Solid State NMR measurements.

The data were obtained, using a Varian 600MHz Infinity Plus NMR spectrometer running the Spin sight software (version 4.3.2), employing 3.2mm and 4.0mm Chemagnetics HX-T3 probes. The MAS speed for ^{1}H MAS NMR measurements was set to 24 kHz, the speed for 13C VACP MAS measurements was set to 10kHz (if not otherwise noted). The measurements were made at room temperature without additional cooling. All ^{1}H spectra were referenced against TSP (Tetramethylsilyl propionic acid, Na salt), all ^{13}C NMR spectra against ^{13}C- glycine as external chemical shift standards. The repetition times of the experiments were chosen in such a way that all spectra were fully relaxed.

To suppress the considerable proton background signal of the probes, all spectra were recorded employing a rotor synchronized Hahn-Echo sequence with delay times between 800us and 3ms. The delay times were chosen as an optimized compromise between the

signal decay owing to relaxation and the resolution gain owing to longer delay times. It was shown, that by using isotopic labeling of the guest molecules of the colloids the presence of (in this case) tyrosine can be detected by ^{13}C VACP MAS NMR, despite its low concentration in the sample.

Deuteration experiments revealed the coupling of some components to nearby protons, showing the location of a water site on the coated colloids. Different charges of the colloids revealed slightly different characteristics when they were freshly prepared. These differenced however did not affect the special characteristics summarized below. Upon addition of water one very special site was detected that traps the water with a slow kinetic. One can speculate that this points to a rearrangement of the crystalline tyrosine layers within the nanowells.

The data was obtained from the group of Dr G. Buntkowsky by Thomas Emmler, "Physical Chemistry Department FU- Berlin", I thanks them for allowing me to extract few spectra from a common publication which is in preparation.

Instruments:

Nuclear resonance spectra (NMR): The spectra were taken up with the spectrometer Bruker AC 250. The values of the chemical shift δ (ppm) refer to Tetramethylsilan (TMS).

Mass spectra (ms): The measurements took place at the devices CF 5 DF or MAT 711. Used kinds of ionization were the electron collision ionization (EGG) and the atomic impact ionization (FAB) with positive and negatively charged ions.

Elementary analyses (I/O): The regulations were undertaken with burn devices of the company Perkin Perkin-Elmer working by gas chromatography.

8.3 Abbreviations used

A	Ampere
AFM	Atomic Force Microscopy
BOC	*tert*-Butyloxycyrbonyl
Bola	Bolaamphiphile
b. p.	Boiling point
CV	Cyclic Voltammetry
DCM	Dichloromethane
d	Doublet
dd	doublets doublet
D_2O	Deuterium Oxide
EI	Electron Impact
Et	Ethyl
FAB	Fast Atom Bombardement
Fs	Fluorescence spectrum
g	gram
GABA	γ-amino butyric acid
Hz	Hertz
hrs.	hours
IR	Infrared
M.P.	Melting point
m	Multiplet
MHz	Megahertz
Ms	Mass spectrum
NMR	Nuclear Magnetic Resonance
ppm	parts per million
q	Quartet
RT	Room Temperature
s	Singlet
TEM	Transmission electron microscopy
t	Triplet
T	Temperature in K
UV	Ultraviolet
vis	visible