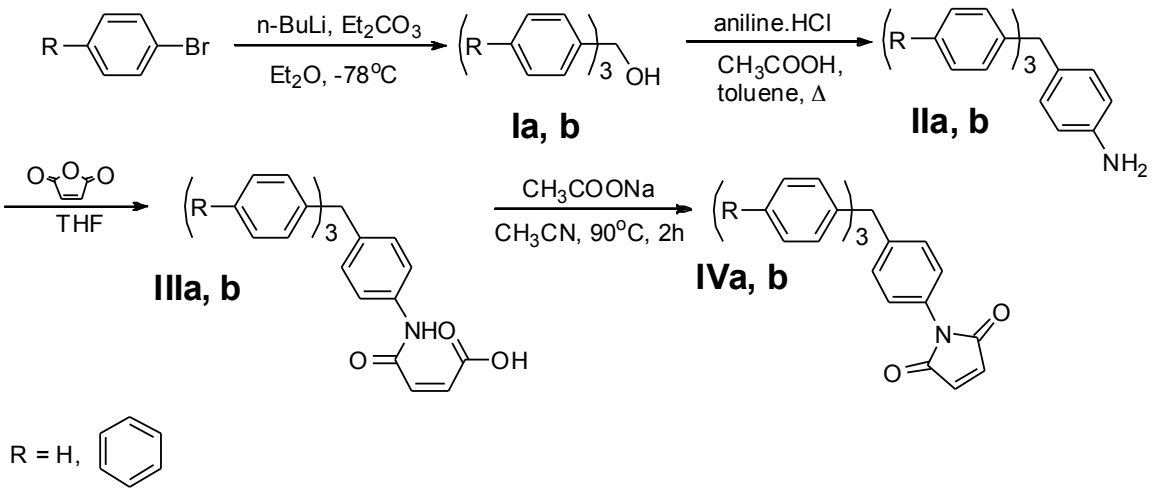
Supporting information

Materials and Methods

Tetraarylmethane Synthesis



(Z)-3-(4-trityl-phenylcarbamoyl)-acrylic acid (IIIa)

To the free amine IIa(1g, 3 mmol) in THF (6 ml) maleinanhydride (292 mg, 3 mmol) in THF (1 ml) was added and the mixture was stirred for 4 h at room temperature. The solid product was isolated by filtration, washed with THF (2 x 2 ml) and dried in vacuum to give 1.12g (86%) of a white powder.

^{1}H NMR (300 MHz, DMSO-d6) δ 6.29 (d, *J* = 12.1 Hz, 1H), 6.47 (d, *J* = 12.1 Hz, 1H), 7.07-7.23 (m, 11H), 7.27-7.33 (m, 6H), 7.54 (d, *J* = 8.4 Hz, 2H), 10.43 (s, 1H), 13.11 (bs, 1H)

^{13}C NMR (75.4 MHz, DMSO-d6) δ 64.09 (s), 118.80 (d), 125.97 (d), 127.74 (d), 130.45 (d), 130.87 (d), 131.72 (d), 136.34 (s), 141.83 (s), 146.44 (s), 163.24 (s), 166.82 (s)

MS (EI): 433 [M+]; HRMS calcd. for $C_{29}H_{23}NO_3$ 433.1678, found 433.1676

1-(4-trityl-phenyl)-pyrrole-2,5-dione (IVa)

Maleamic acid IIIa(1g, 2.3 mmol) and anhydrous sodium acetate (164 mg, 2 mmol) were suspended in acetanhydride (3 ml) and heated at 90°C. After 2 h the reaction mixture was cooled to room temperature, poured into water (15 ml) and extracted with dichloromethane (3 x 20 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ (2 x 20 ml) and water (20 ml), dried over Na_2SO_4 and the solvents were evaporated. Recrystalization from the hot toluene gave 896 mg (94%) of the product as a white solid

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.83 (s, 2H), 7.17-7.28 (m, 17H), 7.31-7.34 (m, 2H)

^{13}C NMR (100.6 MHz, $CDCl_3$) δ 64.90 (s), 124.78 (d), 126.19 (d), 127.72 (d), 129.10 (s), 131.23 (d), 131.95 (d), 134.34 (d), 146.50 (s), 146.60 (s), 169.68 (s)

MS (EI): 415 [M+]; HRMS calcd. for $C_{29}H_{21}NO_2$ 415.1572, found 415.1568

tris-biphenyl-4yl-methanol (Ib)

The 4-Bromobiphenyl (4.66 g, 20 mmol) was dissolved in diethylether (100 ml) and n-BuLi was slowly added (11.3 ml of 1.6M solution in hexanes, 18 mmol). After stirring for 1 h at room temperature diethylcarbonate (0.727 ml, 6 mmol) was added and stirring continued for 1 hour. The reaction mixture was then poured into water (100 ml), the organic layer separated and the water layer extracted with diethylether (2 x 100 ml). The combined organic extracts were dried over Na_2SO_4 and the solvents evaporated. Purification by chromatography (silicagel, hexane:toluene / 1:1, then pure toluene) yielded 2.79 g (95%) of the product as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 2.92 (s, 1H), 7.35 (t, J = 7.3 Hz, 3H), 7.42-7.45 (m, 12H), 7.57-7.62 (m, 12H)

^{13}C NMR (100.6 MHz, $CDCl_3$) δ 81.88 (s), 126.92 (d), 127.26 (d), 127.55 (d), 128.54 (d), 128.97 (d), 140.31 (s), 140.74 (s), 145.94 (s)

MS (EI): 488 [M+]; HRMS calc. for $C_{37}H_{28}O$ 488.2140, found 488.2129

4-(tris-biphenyl-4yl-methyl)-phenylamine (IIb)

Alcohol **Ib**(2.76 g, 5.65 mmol) and aniline hydrochloride (1.46 g, 11.3 mmol) were heated at reflux for 3 h in a mixture of acetic acid (10 ml) and toluene (10 ml). Then the reaction mixture was poured into water (100 ml) and extracted with chloroform (3 x 20 ml). The combined organic layers were transferred into a flask containing saturated aq. $NaHCO_3$ (100 ml) and vigorously stirred for 30 min. The organic layer was then separated and the water layer was extracted with chloroform (20 ml). The combined organic extracts were dried over Na_2SO_4 and the solvents evaporated. Purification by chromatography (silicagel, hexane:ethyl acetate / 5:1, then 3:1) yielded 2.50 g (79%) of the product as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 4.09 (s, 2H), 6.68 (d, J = 8.5 Hz, 2H), 7.12 (d, J = 8.5 Hz, 2H), 7.33 (t, J = 7.7 Hz, 3H), 7.37 (d, J = 8.4 Hz, 6H), 7.44 (t, J = 7.7 Hz, 6H), 7.53 (d, J = 8.4 Hz, 6H), 7.62 (d, J = 7.7 Hz, 6H),

^{13}C NMR (100.6 MHz, $CDCl_3$) δ 63.78 (s), 114.82 (d), 126.22 (d), 127.09 (d), 127.30 (d), 128.86 (d), 131.61 (d), 132.21 (d), 137.49 (s), 138.57 (s), 140.76 (s) 143.58 (s), 146.37 (s)

MS (EI): 563 [M+]; HRMS calc. for $C_{43}H_{33}N$ 563.2613, found 563.2619

(Z)-3-[4-(trisbiphenyl-4yl-methyl)-phenylcarbamoyl]-acrylic acid (IIIb)

To the free amine **IIb**(1,127g, 2 mmol) in $CHCl_3$ (15 ml) was added maleinanhydride (196 mg, 2 mmol) in THF (1 ml) and the mixture was stirred for 48 h at room temperature. The solid product was isolated by

filtration, washed with THF (2 x 2 ml) and dried in vacuum to give 830 mg (65%) of a white powder.

^{1}H NMR (400 MHz, DMSO-d6) δ 6.30 (d, *J* = 12.1 Hz, 1H), 6.49 (d, *J* = 12.1 Hz, 1H), 7.24 (d, *J* = 8.4 Hz, 2H), 7.32-7.37 (m, 9H), 7.45 (t, *J* = 7.7 Hz, 6H), 7.61 (d, *J* = 8.4 Hz, 2H), 7.64-7.69 (m, 12H), 10.47 (s, 1H), 13.11 (bs, 1H)

^{13}C NMR (75.4 MHz, DMSO-d6) δ 63.49 (s), 119.03 (d), 126.11 (d), 126.57 (d), 127.47 (d), 128.95 (d), 130.29 (d), 130.86 (d), 130.97 (d), 131.79 (d), 136.50 (s), 137.67 (s), 139.45 (s), 141.65 (s), 145.60 (s), 163.29 (s), 166.82 (s)

MS (EI): 661 [M+]; HRMS calc. for $C_{47}H_{35}NO_3$ 661.2617 found 661.2632

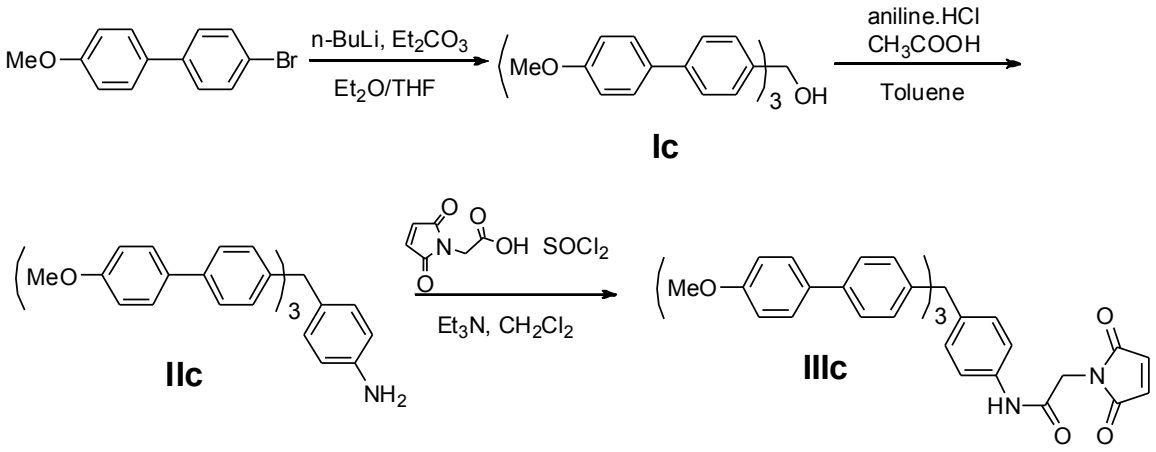
1-[4-(trisbiphenyl-4yl-methyl)-phenyl]-pyrrole-2,5-dione (IVb)

Maleamic acid **IIIb** (643 mg, 1 mmol) and anhydrous sodium acetate (40 mg, 0.5 mmol) were suspended in acetanhydride (2 ml) and heated at 90°C. After 4 h the reaction was cooled to room temperature, poured into water (15 ml) and extracted with dichloromethane (3 x 20 ml). The combined organic extracts were washed with saturated aq. $NaHCO_3$ (2 x 20 ml) and water (20 ml), dried over Na_2SO_4 and the solvents evaporated. Purification by chromatography (silicagel, hexane:chloroform / 1:1) yielded 80 mg (13%) of the product as a white solid.

^{1}H NMR (400 MHz, $CDCl_3$) δ 6.86 (s, 2H), 7.30-7.48 (m, 19H), 7.55 (d, *J* = 8.4 Hz, 6H), 7.62 (d, *J* = 8.1 Hz, 6H)

^{13}C NMR (100.6 MHz, $CDCl_3$) δ 64.28 (s), 124.95 (d), 126.44 (d), 127.11 (d), 127.36 (d), 128.86 (d), 129.24 (s), 131.58 (d), 131.91 (d), 134.34 (d), 138.90 (s), 140.61 (s), 145.53 (s), 146.48 (s), 169.68 (s)

MS (EI):643 [M+]; HRMS calc. for $C_{47}H_{33}NO_2$ 643.2511 found 643.2503



tris(4'-methoxybiphenyl-4-yl)methanol (**Ic**): Dry diethyl ether (10 ml) was added to a solution of 4-bromo-4'-methoxybiphenyl (1g, 3.8 mmol) in dry THF (10 ml), the mixture was cooled to -75 ^{o}C, and n-BuLi (2.8 ml, 4.56 mmol, 1.6 M in hexane) was added drop wise over 20 min. The mixture was stirred 30 min. Diethyl carbonate (0.11 ml, 0.95 mmol) in THF (2 ml) was added slowly and the resulting mixture was stirred at -75^{o}C for another hour. The mixture was allowed to warm to 0^{o}C (ice bath) and stirred overnight. The mixture was quenched with methanol (0.6 ml) and the solvent was removed under vacuum. The residue was extracted with ethyl acetate, washed with water, dried over $MgSO_4$ and concentrated. The product (**Ic**) (250 mg, 34%) was obtained as a white solid after purification by column chromatography on silica (hexane/EtOAc, 9:1).

^{1}H NMR ($CDCl_3$) δ 7.55 (d, *J*= 4.4 Hz, 6H), 7.54 (d, *J*= 3.6 Hz, 6H), 7.4 (d, *J*= 10.8 Hz, 6H), 6.98 (d, *J*= 12Hz, 6H), 3.85 (s, 9H).

^{13}C NMR ($CDCl_3$) δ 159.41, 145.49, 139.89, 133.35, 128.58, 128.32, 126.46, 114.45, 94.94, 55.56.

MS (EI): 578[M^+]; HRMS calcd. for $C_{40}H_{34}O_4$ 578.7023, found 578.6997

4-(tris (4'-methoxybiphenyl-4-yl) methyl) aniline (**IIc**): Acetic acid (1 ml), **Ic** (250 mg, 0.43 mmol), and aniline hydrochloride (111 mg, 0.86 mmol) were dissolved in toluene (10 ml), and the mixture was refluxed for 3 hrs. Water was added, the organic layer was separated and the water layer was extracted with $CHCl_3$ (3x). The organic layers were collected and saturated, aq$NaHCO_3$ was added and this two-layer system was stirred for 30 min. The organic layer was separated and the water layer was extracted with chloroform (2x). The chloroform solution was dried over $MgSO_4$ and the solvent evaporated under vacuum. The product (**IIc**, 50 mg, 20%) was obtained as a white solid after purified by column chromatography on silica (hexane/EtOAc, 9/1).

^{1}H NMR ($CDCl_3$): 7.54 (d, *J*= 4.4 Hz, 6H), 7.46 (d, *J*= 3.6 Hz, 6H), 7.33 (d, *J*= 10.8 Hz, 6H), 7.08 (d, *J*= 8Hz, 2H), 6.96 (d, *J*= 12Hz, 6H), 6.62 (d, *J*= 8Hz, 2H), 3.85 (s, 9H), 3.62 (s, 2H, NH_2).

^{13}C NMR ($CDCl_3$): 159.0, 146.0, 142.1, 138.0, 137.3, 133.4, 132.1, 131.7, 128.1, 125.8, 120.0, 114.2, 63.5, 54.8.

MS (EI): 652.9 [M+]; HRMS calcd. for $C_{46}H_{39}NO_3$ 653.8133 found 653.8141

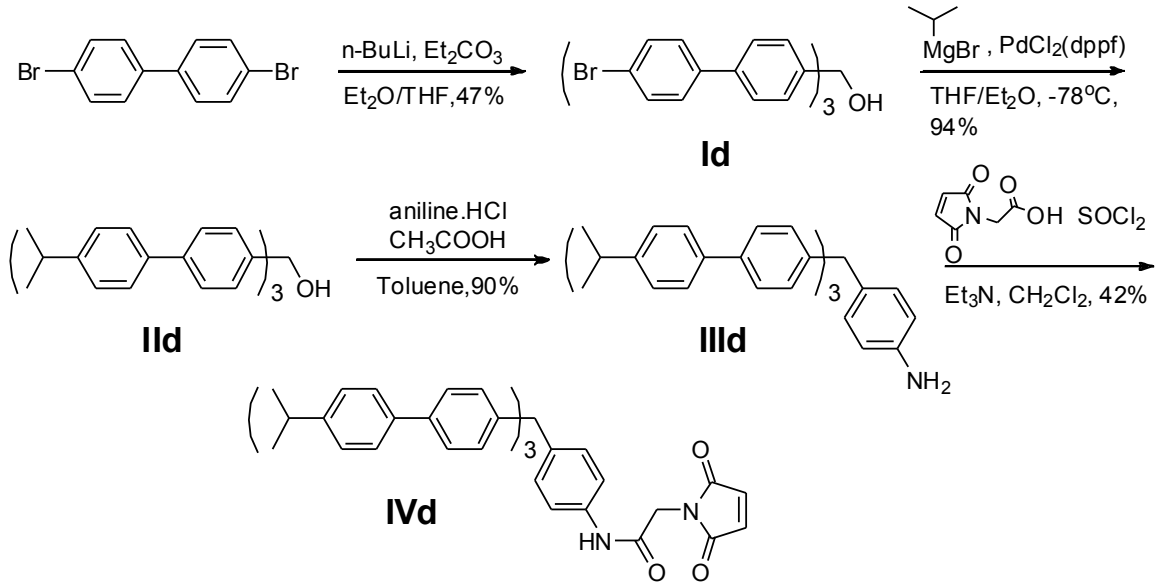
2-(2, 5-dioxo-2, 5-dihydro-1H-pyrrol-1-yl)-N-(4-(tris (4'-methoxybiphenyl-4-yl)methyl) phenyl) acetamide (**IIIc**): A mixture of (2,5-dioxo-2, 5-dihydro-pyrrol-1-yl)-acetic acid (12 mg, 0.076 mmol) and $SOCl_2$ (0.2 ml) were refluxed for 0.5 hour. The excess of $SOCl_2$ was removed by evaporation under vacuum. The residue was dissolved in toluene (2 ml) and removed under vacuum. The final product was dissolved in CH_2Cl_2 (1 ml), **IIc** (50 mg, 0.076 mmol) and Et_3N (12 µl, 0.084 mmol) dissolved in CH_2Cl_2 (1 ml). The reaction mixture was stirred overnight,

diluted with ethyl acetate and washed with aq. HCl, aq. $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$) and the solvent was removed under vacuum. The residue was purified by column chromatography on silica (hexane/ethyl acetate, 7/3) to give (**IIIc,** 30 mg, 50 %) as a white solid.

^{1}H NMR ($CDCl_3$): δ 7.58 (d, *J*= 8Hz, 2H), 7.53 (d, *J*= 4.4Hz, 6H), 7.46 (d, *J*= 3.6Hz, 6H), 7.39 (d, *J*= 8Hz, 2H), 7.31 (d, *J*= 10.8Hz, 6H), 6.96 (d, *J*= 12Hz, 6H), 6.77 (s, 2H), 4.31 (s, 2H), 3.82 (s, 9H), 1.60 (s, 1H, NH_2)

^{13}C NMR ($CDCl_3$): δ 172.3, 170.3, 159.30, 145.31, 138.41, 135.11, 133.29, 131.93, 131.59, 129.22, 128.18, 125.95, 120.11, 119.31, 114.41, 110.13, 63.36, 41.52.

HRMS calcd. for $C_{52}H_{42}N_2O_6$ 790.9045, found 790.9089



tris-(4'-bromo-biphenyl-4-yl)-methanol (Id). Diethyl ether (24 ml) was added to a solution of 4.4`-dibromo-biphenyl (3.12g, 10 mmol) in THF (24 ml), the mixture was cooled to -75 °C, and n-BuLi (7.5 ml, 12 mmol, 1.6M in hexane) was added drop wise over 20 min. The mixture was stirred for 30 min. and after 1 hour, diethyl carbonate (0.3 ml, 2.5 mmol) in THF (2 ml)

was added slowly. The mixture was allowed to warm to 0 °C (ice bath) and was stirred for 3 hrs. The mixture was quenched with methanol (2 ml) and the solvent was removed under vacuum. The residue was extracted with ethyl acetate, and the extract was washed with water, dried ($MgSO_4$) and concentrated. The residue was purified by column chromatography on silica ($CHCl_3$/hexane, 9:1). The product **Id** (991 mg, 47 %) was isolated as a white solid.

^{1}H NMR ($CDCl_3$) δ 7.59 (d, *J*= 8.5 Hz, 6H), 7.56 (d, *J*= 8.5 Hz, 6H), 7.48 (d, *J*= 8.5 Hz, 6H), 7.46 (d, *J*= 8.5 Hz, 6H), 2.88 (s, 1H).

^{13}C NMR ($CDCl_3$) δ 146.19, 139.63, 139.29, 132.88, 128.87, 128.65, 126.83, 121.96, 84.59

tris-(4'-isopropyl-biphenyl-4-yl)-methanol (IId). The Grignard reagent was prepared from 2-bromopropane (0.96 ml) and Mg (313 mg) in Et_2O (50 ml) at reflux under N_2. Alcohol **Id** (750 mg, 1.03 mmol) and $PdCl_2$ (dppf) (34 mg, 0.0414 mmol) were placed into dry flask under N_2 and Et_2O (50 ml) and THF (20 ml) were added and the reaction mixture was cooled at -78 °C. The Grignard reagent was added slowly during 15 min. The reaction mixture was stirred another 10 min. at -78 °C and subsequently at room temperature overnight. 5% aq. HCl (50 ml) was added and the reaction mixture was extracted with Et_2O (30 ml). The combined organic layers were dried ($MgSO_4$) and the solvent was evaporated under vacuum. The residue was purified by column chromatography on silica (hexane/ethyl acetate, 9:1) to give product **IId** (305 mg, 90.5 %) as white solid.

^{1}H NMR ($CDCl_3$) δ 7.59 (d, *J*= 8.5 Hz, 6H), 7.56 (d, *J*= 8.5 Hz, 6H), 7.48 (d, *J*= 8.5 Hz, 6H), 7.46 (d, *J*= 8.5 Hz, 6H), 3.0 (m, 3H), 2.01 (s, 1H), 1.36 (d, *J*= 6.8 Hz, 18H).

^{13}C NMR ($CDCl_3$) δ 148.32 145.75, 140.31, 138.37, 128.53, 127.23, 127.10, 126.95, 126.79, 82.11, 33.86, 24.24

MS (EI): 614.1 [M+]; HRMS calcd. for $C_{46}H_{46}O$ 614.8656 found 614.8649

4-[tris-(4'-isopropyl-biphenyl-4-yl)-methyl]-phenylamine (IIId). A solution of acetic acid (6 ml), **IId** (300 mg, 0.488 mmol), and aniline hydrochloride (126 mg, 0.98 mmol) in toluene (3 ml) was stirred at 100 °C for 24 hrs. The solvent was evaporated under vacuum, and then methanol (6 ml) and aq. HCl (2 M, 2 ml) were added. The resulting slurry was refluxed for 24 hrs and the solvent was evaporated under vacuum. The residue was dissolved in chloroform and washed with aq. $NaHCO_3$. The chloroform solution was dried ($MgSO_4$) and concentrated under vacuum. The residue was purified by column chromatography on silica ($CHCl_3$/hexane, 9:1) to give product **IIId** (305 mg, 90.5 %) as a pale solid. ^{1}H NMR ($CDCl_3$) δ 7.57 (d, *J*= 8.6 Hz, 6H), 7.5 (m, 12H), 7.37 (d, *J*= 8.6 Hz, 6H), 7.10 (d, *J*= 8.5 Hz, 2H), 6.67 (d, *J*= 8.6 Hz, 2H), 3.52 (s, 2H), 3.0 (m, 3H), 1.36 (d, *J*= 6.8 Hz, 18H).

^{13}C NMR ($CDCl_3$) δ 148.77, 148.07, 142.79, 139.43, 138.59, 138.43, 132.31, 131.69, 129.03, 127.11, 127.04, 114.58, 87.61, 33.86, 24.26.

HRMS calcd. for $C_{52}H_{51}N$ 689.9731 found 689.9775

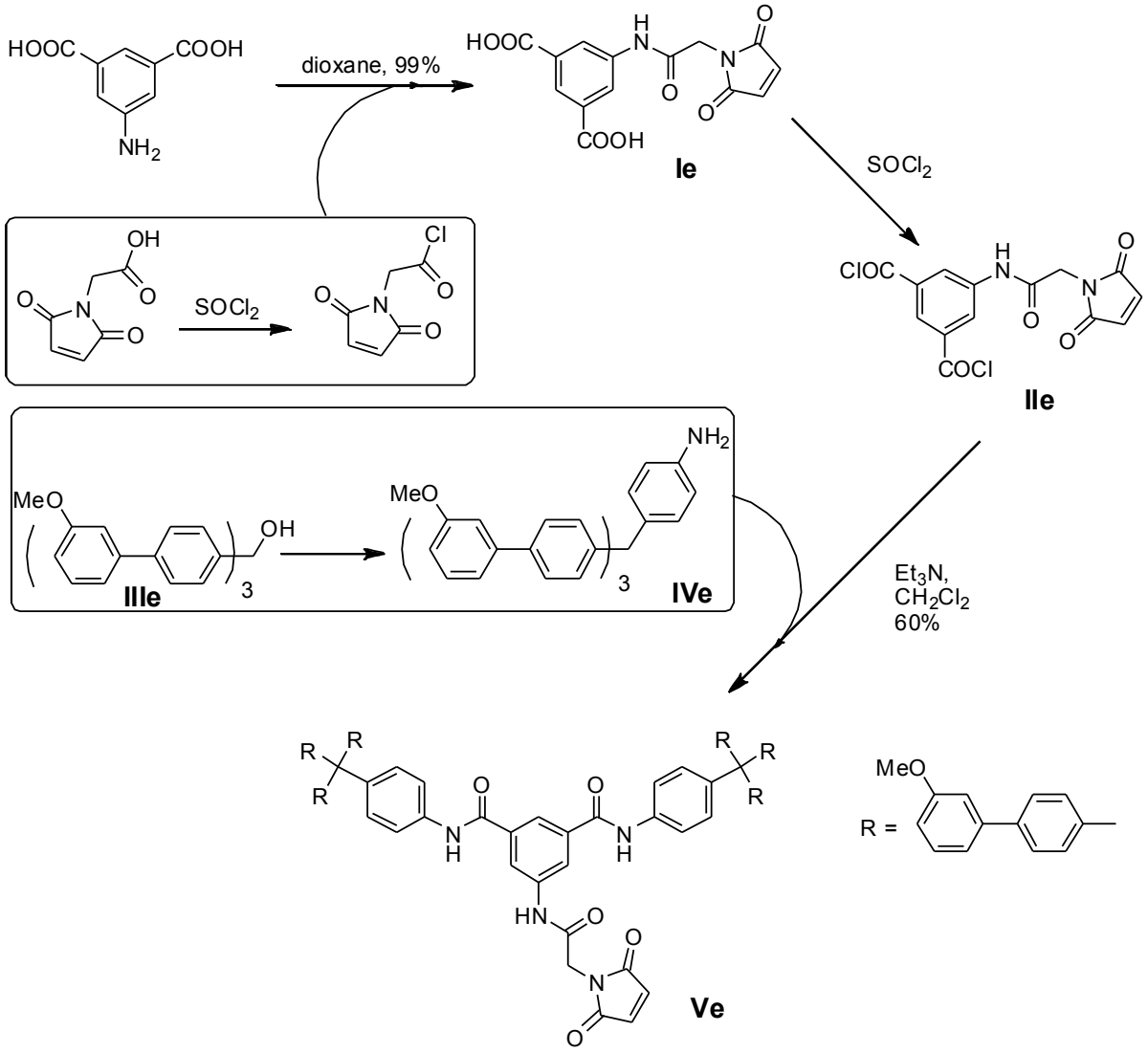
2-(2, 5-Dioxo-2, 5-dihydro-pyrrol-1-yl)-N-{4-[tris-(4'-isopropyl-biphenyl-4-yl)methyl]-phenyl}-acetamide (IVd). A mixture of (2,5-dioxo-2, 5-dihydro-pyrrol-1-yl)-acetic acid (61.7 mg, 0.39 mmol) and $SOCl_2$ (1.2

ml) was refluxed for 0.5 h. The excess of $SOCl_2$ was removed by evaporation under vacuum. The residue was then dissolved in toluene (2 ml) and evaporated again. Finally the product was dissolved in CH_2Cl_2 and **IIId** (250 mg, 0.36 mmol) and Et_3N (0.055 ml, 0.39 mmol in CH_2Cl_2 2 ml) were added. The reaction mixture was stirred overnight, diluted with ethyl acetate and washed with aq. HCl, aq. $NaHCO_3$ and water. The organic layer was dried ($MgSO_4$) and the solvent was removed under vacuum. The residue was purified by column chromatography on silica (hexane/ethyl acetate, 1:1) to give **IVd** (126 mg, 42.2 %) as a white solid.

^{1}H NMR ($CDCl_3$) δ 7.8 (s, 1H, NH), 7.5 (m, 12H), 7.4 (d, *J*= 4.2 Hz, 2H), 7.37 (m, 8H), 7.24 (d, *J*= 8.5 Hz, 2H), 6.69 (s, 2H), 4.3 (s, 2H), 3.0 (m, 3H), 1.36 (d, *J*= 6.8 Hz, 18H)

^{13}C NMR ($CDCl_3$) δ 160.3, 164.1, 148.16, 145.59, 138.83, 138.27, 134.77, 132.0, 131.6, 129.04, 127.11, 127.05, 126.27, 119.3, 64.1, 41.53, 34.02, 24.22

MS (Maldi-Tof): 849.7057 [M+Na]



5-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)isophthalic acid (Ie). A mixture of (2,5-dioxo-2, 5-dihydro-pyrrol-1-yl)-acetic acid (200 mg, 1.29 mmol) and $SOCl_2$ (4 ml) was refluxed for 1 hr. The excess of $SOCl_2$ was removed by evaporation under vacuum. The residue was then dissolved in toluene (2 ml) and the solvent was evaporated. Final product was dissolved in dioxane and added prop wise at 0 ºC to a solution of 5-aminoisophthalic (233 mg, 1.29 mmol) acid in dioxane. The reaction

mixture was stirred for 2 hrs, poured into the water, extracted with EtOAc (3 x 50 ml), combined organic layers were dried ($MgSO_4$) and solvent was evaporated under reduce pressure. Product **Ie** (350 mg, 99 %) was obtained as a yellow powder and was used without further purification in next reaction.

^{1}H NMR (DMSO)

160.13, 155.64, 146.66, 144.50, 142.41, 138.53, 136.96, 132.26, 131.66, 129.94, 126.36, 124.80, 119.73, 114.51, 112.92, 112.81, 79.17, 55.51

MS (EI): 653 [M^+], $C_{46}H_{39}NO_3$ (653.81)

5-(2-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)acetamido)-N^1,N^3-bis(4-(tris(3'-methoxybiphenyl-4-yl)methyl)phenyl)isophthalamide (Ve):

Crude diacyl dichlorode **IIe** from previous reaction was dissolved in dry CH_2Cl_2 (5 ml) and cooled at 0 ºC. Amine **IVe** (100 mg, 0.153 mmol) and Et_3N (47µl, 0.168 mmol) were dissolved in dry CH_2Cl_2 and were added dropwise into the solution of diacyl dichloride **IIe** in dry CH_2Cl_2 at 0 ºC. Reaction mixture was allowed to room temperature overnight, diluted with EtOAc and washed with diluted HCl, sat. $NaHCO_3$ and water. Organic layer was dried over Na_2SO_4 and solvents were evaporated under reduce pressure. Pure product **Ve** (71 mg, 60 %) was obtained as a brownish powder after purification by column chromatography on silica (hexane/EtOAc, 5:5).

^{1}H NMR (400 MHz, $CDCl_3$) δ 7.41 (m, 24H), 7.26 (m, 18H), 7.06 (m, 10H). 6.81 (m, 7H), 6.77 (s, 2H), 4.31 (s, 2H), 4.75 (s, 18H)

^{13}C NMR (100 MHz, $CDCl_3$) δ 170.61, 166.5, 165.3, 160.1, 146.05, 142.17, 138.68, 135.8, 131.49, 129.97, 126.57, 125.5, 119.7, 113.03, 112.74, 64.2, 55.44, 34.21

MS (EI): 1608.1 [M+18]; $C_{106}H_{84}N_4O_{11}$, 1589.82

ProOmpA Labeling. ProOmpA (3 mg/ml) in 8 M Urea and 50 mM Tris/HCl, pH 7 was treated with 1 mM TCEP for 30 mins at room temperature. For the labeling, the tetraarylmethane derivatives were dissolved in different organic solvents (TAM1: methanol; TAM2: chloroform/methanol (1:3, v/v); IsoTAM2: ethylacetate; MeOTAM2 and MeOTAM3: dimethylformamide) and then added to purified proOmpA (2 mg/ml in 8 M Urea, 50 mM Tris/HCl, pH 7) at a final concentration of: a) 4 mM TAM1; b) 4 mM TAM2; c) 4.5 mM IsoTAM2; and d) 8.3 mM MeOTAM2 or MeOTAM3. The suspensions were incubated for 2 hrs (a and b) or overnight (c and d) at room temperature under constant stirring. To increase the efficiency of the reaction, after the first hour of incubation, an aliquot of the fresh solutions of the compounds was added to the suspensions. ProOmpA was recovered from the suspension by 10 % TCA precipitation for 30 mins at 4 ^{o}C, followed by several washes of the protein pellet with ice-cold acetone. This effectively removed non-reacted compounds. Samples were dried for 10 mins at 37 ^{o}C and resuspended in 8 M Urea, 50 mM Tris/HCl, pH 7.

To verify the labeling efficiency, a second labeling step was performed with fluorescein-5-maleimide (Fmal). Isolated proOmpA conjugates were treated with 1 mM TCEP for 30 minutes at room

temperature and subsequently incubated with 2 mM Fmal for 1 hr at room temperature (1). Samples were analyzed on a 12% SDS-PAGE gel and fluorescence was detected at 520 nm and compared with a 10% standard of Fmal-labeled proOmpA using the Lumi-Imager F1^{TM} Workstation (Roche Molecular Biochemicals).

Translocation assays. *In vitro* translocation reactions (50 µl) were performed at 37 ^{o}C as previously described (2) using 20 µg/ml of SecA, 32 µg/ml of SecB, 1 µg of urea-denaturated proOmpA with or without the various labeled organic compounds or when indicated proOmpA-DHFR (3), 10 mM phosphocreatine and 50 mM creatine kinase in a buffer consisting of 50 mM Tris/HCl, pH 7, 30 mM KCl, 0.5 mM bovine serum albumin (BSA), 10 mM DTT and 5 mM $MgCl_2$. *In vitro* translocation reactions (50 µl) were performed and analysed at 37 ^{o}C as previously described. *E. coli* UH203 (*ompA*$^{-}$) or SE6004 (*prlA4*) IMVs were added to a final concentration of 0.2 mg/ml. Reactions were started by the addition of 2 mM ATP and terminated at various time intervals by chilling on ice. Samples were treated with proteinase K (1 mg/ml) for 30 min on ice, precipitated with 10 % (w/v) TCA, washed with ice cold acetone and analyzed by 12 % SDS-PAGE and immunoblotting using a polyclonal antibody against OmpA. Immunoblots

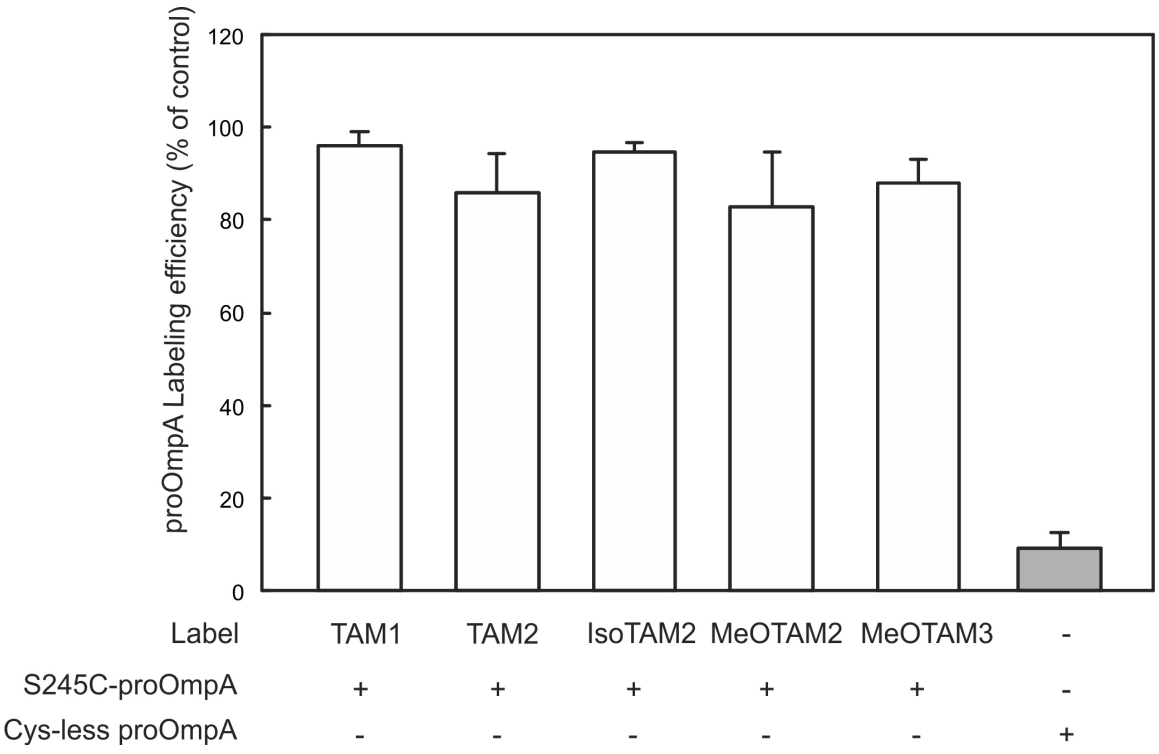
were developed using the chemiluminescent substrate disodium 4-chloro-3-(methoxyspiro {1,2-dioxetane-3,2´-(5'-chloro)tricyclo [3.3.1.1^{3,7}]decan}-1-4-yl)phenyl phosphate (CDP star, Roche Molecular Biochemicals). When indicated, the PMF was collapsed by the addition of nigericin and valinomycin at a final concentration of 2 μM.

When indicated, IMVs (from a 50 μl reaction) were recollected by centrifugation through a 0.8 M sucrose solution (15 mins; 75,000 rpm TLA110 rotor, 4 ^{o}C). The pelleted IMVs were resuspended in 50 μl of translocation buffer, and used in a second translocation reaction with Fmal-labeled proOmpA as substrate. Translocation reactions were analyzed by 12% SDS-PAGE gels and in gel fluorescence (excitation 520 nm) using the Lumi-Imager F1^{TM} Workstation (Roche Molecular Biochemicals).

References

1. de Keyzer J., van der Does C., Driessen AJM (2002) Kinetic analysis of the translocation of fluorescent precursor proteins into Escherichia coli membrane vesicles. *J. Biol. Chem.* 277**:** 46059-46065.
2. Tomkiewicz D, Nouwen N, van Leeuwen R., Tans S, Driessen AJM (2006) SecA supports a constant rate of preprotein translocation. *J. Biol. Chem.* 281**:** 15709-15713.
3. Arkowitz RA, Joly JC, Wickner W (1993) Translocation can drive the unfolding of a preprotein domain. *EMBO J.* 12**:** 243-253.

Supplementary figures



Supplemental Fig. 1. Labeling of proOmpAS245C with tetraarylmethanes derivatives. ProOmpAS245C was incubated with the indicated tetraarylmethanes derivatives. After 2 hrs at room temperature non-reacted tetraarylmethane was removed by TCA precipitation and several washes of the protein pellet with acetone where after the proOmpA tetraarylmethane conjugate was dissolved in 50 mM Tris/HCl pH 8.0 and 8 M urea. To determine the labeling efficiency a small fraction of the proOmpA tetraarylmethane conjugate was incubated with Fmal for 2 hrs at room temperature thereafter the sample was analyzed by SDS-PAGE and in gel UV fluorescence. To determine the amount of nonspecific labeling of proOmpA with Fmal, a cysteine-less variant of proOmpA was used.