Nontoxic Membrane-Active Antimicrobial Arylamide Oligomers**

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Scheme 1
Scheme 2
1. amino acid, POCl₃, py, R₁ OH
2. TFA
3. piperidine, DMF

Scheme 3

1. AC₂O, py, CH₂Cl₂
2. TFA

Scheme 4
Materials and Methods

2,6-Dinitro-4-t-butyl-phenyl (4-methyl)-benzenesulfonate (13). 2, 6-dinitro-4-t-butyl-phenol (80 mmol) and tosyl chloride (80 mmol) were dissolved in 300 ml CH₂Cl₂.

Diisopropylethylamine (DIEA, 80 mmol) was added to the solution. The mixture was stirred at room temperature for 2 hours. The solution was washed with 10% citric acid, saturated aqueous NaCl, and dried with MgSO₄. The solvent was removed under reduced pressure, and the product was obtained as a bright yellow solid in quantitative yield. ¹H NMR (500MHz, CDCl₃): δ = 8.12 (s, 2H), 7.80 (d, 2H), 7.40 (d, 2H), 2.51(s, 3H), 1.41 (s, 9H). ESI-MS: m/z (M+Na⁺): 417.07 (calcd), 417.2 (found).

2,6-Dinitro-4-t-butyl-1-(2-t-butoxycarbonylaminoethyl)-sulfanylbenzene (14).

Compound 1 (13 mmol), 2-Boc-aminoethanthiol (16 mmol) and DIEA(13 mmol) were dissolved in 50 ml chloroform. The solution was stirred under nitrogen for 12 hours. The solution was washed with 0.5 M NaOH, 10% citric acid, sat. Na₂CO₃ and sat. NaCl, and dried with MgSO₄. The solution volume was reduced to 15 ml by rotary evaporation.

The product crystallized as a bright yellow solid after addition of 80 ml hexane. Yield: 94%. ¹H NMR (500 MHz, CDCl₃): δ = 7.81(s, 2H), 4.87(s, 1H), 3.31(t, 2H), 3.10(t, 2H), 1.44 (s, 9H), 1.39(s, 9H). ESI-MS: m/z(M+Na⁺): 422.45 (calcd), 422.4 (found).

2,6-Diamino-4-t-butyl-1-(2-t-butoxycarbonylaminoethyl)-sulfanylbenzene(15).

Dinitro compound 2(20 mmol) and sodium acetate (200 mmol) were added to 50 ml EtOH. The mixture was heated to 78 °C, and the solid dissolved completely.

SnCl₂•2H₂O (200 mmol) was added to the solution, and the reaction mixture was stirred
at 78 °C for 35 minutes. After removal of solvent under reduced pressure, the residue was dissolved in 800 ml EtOAc, and washed with 40% KCO3. The solvent was reduced by rotary evaporation, and the product was purified by column chromatography (silica gel, CH2Cl2/MeOH 100:1 to 95:5). Yield: 93%. ¹H NMR (500 MHz, CDCl3): δ = 6.21(s, 2H), 5.41(s, 1H), 4.35(br, 4H), 3.21(t, 2H), 2.75(t, 2H), 1.35 (s, 9H), 1.24(s, 9H). ESI-MS: m/z (MH⁺): 340.51(calcd), 340.5(found).

**Synthesis of diamine 16 and 1**

Diamine 15 (5 mmol) was dissolved in 50 mL CH2Cl2 with DIEA (10 mmol). Isophthaloyl dichloride (2.3 mmol) was dissolved in 10 mL CH2Cl2/DMF (4:1) and was added dropwise to the diamine solution under argon. The addition was finished in one hour. The mixture was stirred overnight. Solvent was removed using a rotovap. The product was purified by column chromatograph (silica gel, Hexane/EtOAc 2:1 to 1:1). Yield 60%. ¹H NMR (500 MHz, CDCl3): δ = 9.69 (s, 2H), 8.57 (s, 1H), 8.19 (d, 2H), 8.18 (s, 2H), 7.70 (t, 1H), 6.61 (s, 2H), 5.22 (b, 2H), 4.47 (b, 4H), 3.25 (m, 4H), 2.83 (t, 4H), 1.34 (s, 36H). MALDI-MS: m/z (MNa⁺): 832.08 (calcd), 831.26(found).

The Boc group of 16 was removed by treatment with 50%TFA/CH2Cl2 to give compound 1. MALDI-MS: m/z (MH⁺) 608.86 (calcd), 609.26 (found).

**Dimethyl 5-(((2-((t-butyloxy)carbonyl)amino)ethoxy)benzene-1,3-dicarboxylate (17)**

To a solution of dimethyl 5-hydroxy isophthalate(5g, 23.33mmol), PPh3(6.73g, 25.66mmmol), and t-buty N-(2-hydroxyethyl)carbamate (4.05ml, 25.66mmol) in dry THF(60ml) was added diethyl azodicarboxylate(11.64ml, 25.66mmol; 40% in toluene)
dropwise at 0°C. The resulting mixture was warmed to room temperature and stirred for 24hrs. The solvent was evaporated and the residue was dissolved with EtOAc. The organic layer was washed with H₂O, saturated NaCl(aq) and dried with Na₂SO₄. The solution was filtered and concentrated. The residue was purified by column chromatography (pet. Ether/Et₂O 5:3) to give compound 17 (5.41g, 71%).

¹H NMR(500MHz, CDCl₃) : δ 1.43(s, 9H), 3.54(m, 2H), 3.92(s, 6H), 4.08(t, 2H), 5.01(s, 1H), 7.71(s, 2H), 8.26(s, 1H). Electrospray ionization-MS: m/z (MNa⁺): 376.1372 (calcd), 376.1363 (found).

5-(((2-((t-butyloxy)carbonyl)amino)ethoxy)benzene-1,3-dicarboxylic acid(18).

To a solution of compound 17 (1.66g, 4.70mmol) in MeOH/THF(10ml:10ml) was added 2N LiOH (9.28ml, 18.80ml). The resulting mixture was stirred for 24hrs. The MeOH and THF was removed under reduced pressure. The aqueous solution was diluted with H₂O (20ml) and cooled down to 0°C and acidified to pH3 with 3N HCl. The white precipitate was filtered and washed with H₂O. Compound 18 (1.46g, 96%) was obtained without further purification (TLC indicated it is pure compound).

¹H NMR(500MHz, DMSO-d₆) : δ 1.37(s, 9H), 3.32(m, 2H), 4.08(t, 2H), 6.98(t, 1H), 7.64(s, 2H), 8.07(s, 1H), 13.23(s, 2H). Electrospray ionization-MS: m/z (MNa⁺): 348.1059 (calcd), 348.1062 (found).

5-(((2-((t-butyloxy)carbonyl)amino)ethoxy)benzene-1,3-dicarboxylic acid(18).

To a solution of diacid compound 18 (1.66g, 4.70mmol) in MeOH/THF(10ml:10ml) was added 2N LiOH (9.28ml, 18.80ml). The resulting mixture was stirred for 24hrs. The MeOH and THF was removed under reduced pressure. The aqueous solution was diluted with H₂O (20ml) and cooled down to 0°C and acidified to pH3 with 3N HCl. The white precipitate was filtered and washed with H₂O. Compound 18 (1.46g, 96%) was obtained without further purification (TLC indicated it is pure compound).

¹H NMR(500MHz, DMSO-d₆) : δ 1.37(s, 9H), 3.32(m, 2H), 4.08(t, 2H), 6.98(t, 1H), 7.64(s, 2H), 8.07(s, 1H), 13.23(s, 2H). Electrospray ionization-MS: m/z (MNa⁺): 348.1059 (calcd), 348.1062 (found).

Synthesis of diamine 19 and 9.

To a solution of diacid compound 18 (200mg, 0.615mmol) in dry THF (10ml) was added pyridine (149µl, 1.845mmol) and DMF (catalytic amount). The resulting mixture was
cooled down to 0°C and then oxalyl chloride (164µl, 1.845mmol) was added dropwise. After stirring for 45min at room temperature, the solvent, excess oxalyl chloride, and pyridine were removed under vacuum to give the crude acyl chloride which was directly used in the following without further purification.

To a solution of diamine compound (630mg, 1.845mmol), Et₃N (514µl, 3.69mmol), and DMAP (7.5mg, 0.062mmol) in dry CH₂Cl₂ (10ml) was added the crude acyl chloride in CH₂Cl₂ (20ml) dropwise for 2hrs. After a total of 7hrs, the solvent was removed and then the residue was dissolved in EtOAc and washed with saturated NaHCO₃ (aq) and saturated NaCl (aq). After drying on Na₂SO₄, the organic layer was filtered and concentrated under reduced pressure. The residue was purified by column chromatography (Hexane/EtOAc 2:1) to give diamine 19 (369mg, 62%).

1H NMR (500MHz, DMSO-d₆) : δ 1.25-1.29(36H), 1.38(s, 9H), 2.63(t, 4H), 3.00(br d, 4H), 3.38(m, 4H), 4.15(t, 4H), 5.50(s, 4H), 6.70(s, 2H), 6.85(s, 2H), 7.05(s, 1H), 7.27(s, 2H), 7.70(s, 2H), 8.10(s, 1H), 9.84(s, 2H). Electrospray ionization-MS: m/z (MNa+) 990.4809 (calcd), 990.4828 (found).

The Boc group of 19 was removed by treatment with 50%TFA/CH₂Cl₂ to give compound 9. Electrospray ionization-MS: m/z (MH⁺) 667.3417 (calcd), 668.3447 (found).

**General method to synthesize amino acid appended arylamides (2-8, 11, 12).**

Boc amino acids (Boc protecting group for both α-amino and side chain amino groups) were used for the synthesis except arginine. Fmoc D-Arg (pbf) was used for synthesis of 8 and 12. Diamine 16 or 19 (2mmol) and protected amino acid (8 mmol) were dissolved...
in 30 mL anhydrous pyridine and was cooled down to –30 °C with dry ice/acetone. POCl₃ (8mmol) was added dropwise to the solution in 0.5 hour. The mixture was stirred for another 0.5 hour before the reaction was quenched with 50 mL ice water. The product was extracted with EtOAc (50 ml x 1, 30 ml x 3), washed with 10% citric acid (50 ml x 1), sat. NaHCO₃ (50 mL x 3) and sat. NaCl (50 mL x 1). The protected product was purified by column chromatograph (silica gel, CH₂Cl₂/MeOH 100:1 to 98:2). Boc and pbf groups were removed by treatment of 20 mL TFA/TIS (95:5) for one hour. Fmoc group was removed by 30 mL 20% piperidine for one hour. The product was concentrated to an oil. Water (0.1% HCl, 100 mL) was added to the oil. The aqueous solution was washed with ether (50 mL x 4) and dried on a lyophilizer. Further purification was carried out on HPLC. Yield 60%. MALDI-MS: m/z (MH⁺): 2:835.18 (calcd), 835.33(found); 3:903.21 (calcd), 903.38 (found); 4: 981.28 (calcd), 981.44 (found); 5:779.07 (calcd), 779.26 (found); 6:831.15 (calcd), 831.35 (found); 7:865.21 (calcd), 865.44 (found); 8:921.23 (calcd), 921.91 (found); 11:962.48 (calcd), 962.48 (found); 12:980.54 (calcd), 980.54 (found).

**Synthesis of 10**

To a solution of arylamide compound 19 (37mg, 0.038mmol) and DMAP(cat.) in dry CH₂Cl₂(3ml) was added pyridine(31µl, 0.38mmol). The resulting mixture was cooled down to 0°C and treated with acetic anhydride(36µl, 0.38mmol) and then warmed to room temperature. After stirring for 12hrs, the solvent was removed under reduced pressure. The residue was dissolved with EtOAc and washed with 10% citric acid(aq), saturated NaHCO₃(aq), and saturated NaCl(aq). After drying on Na₂SO₄, the organic layer was filtered and concentrated under reduced pressure. The residue was purified by
column chromatography (Hexane/EtOAc 1:1.5 to 1:2) to give diacetylated compound (31 mg, 78%).

The Boc group of diacetylated compound was removed by treatment with 50% TFA/CH₂Cl₂ to give compound 10. Electrospray ionization-MS: m/z (MH⁺): 752.3628 (calcd), 752.3661 (found).
