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# Synthesis of Polyamides Containing *N*-Methylpyrrole and *N*-Methylimidazole and Their Anticancer Activity

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Three hairpin polyamides were designed and synthesized by a haloform reaction and DCC/HOBt coupling reaction without amino protection and deprotection. Their anticancer activity were investigated with three kinds of cell lines-hepatic carcinoma, lung carcinoma and gastric carcinoma, and the values of $IC_{50}$ were at range of $10^{-7}$~$10^{-8}$ M.

**Key words:** Polyamides, Haloform reaction, Anticancer activity

## INTRODUCTION

Polyamides containing *N*-methylpyrrole (Py) and *N*-methylimidazole (Im) are a type of artificial DNA-binding molecules (Mrksich *et al.*, 1994; Tao *et al.*, 1999), which have been proven to permeate the cell membrane and regulate gene expression (Gottesfeld *et al.*, 1997). In recent years, polyamides became the focus of chemical, biological and medical research and inspired considerable work in molecular design, DNA-recognition (Mrksich *et al.*,1992; Geierstanger *et al.*, 1994; Kielkopf *et al.*, 1998), synthetic chemistry (Baird *et al.*, 1996; Xiao *et al.*, 2000) and gene regulation (Dickinson *et al.*, 1998; Szewczyk *et al.*, 1996). Our interest in discovery and design of new anticancer drug had led us to study on the possibility for the polyamide as the candidate of anticancer compound. Here we report the synthesis of three polyamides (Fig. 1) in solution and their anticancer activity. In the polyamides, the γ-aminobutyric acid facilitate the formation of γ-turn, the β-alanine increase polyamide-DNA binding affinity, and *N, N*-dimethylpropyldiamine increase the polarity of the polyamides (Mrksich *et al.*,1992; Xiao *et al.*, 2000).

## RESULTS AND DISCUSSION

To synthesize three polyamides [PyPyPy · PyImImDp · (**1**), $NO_2$PyPy · PyImImPy · Dp(**2**), PyPyPyPy PyImImPy · Dp (**3**); where Py = *N*-methylpyrrole, Im = *N*-methylimidazole, β = β-alanine, γ = γ-aminobutyric acid, Dp = *N*, *N*-dimethylpropyldiamine.], 4-nitro-*N*-methyl-2-trichloroace-

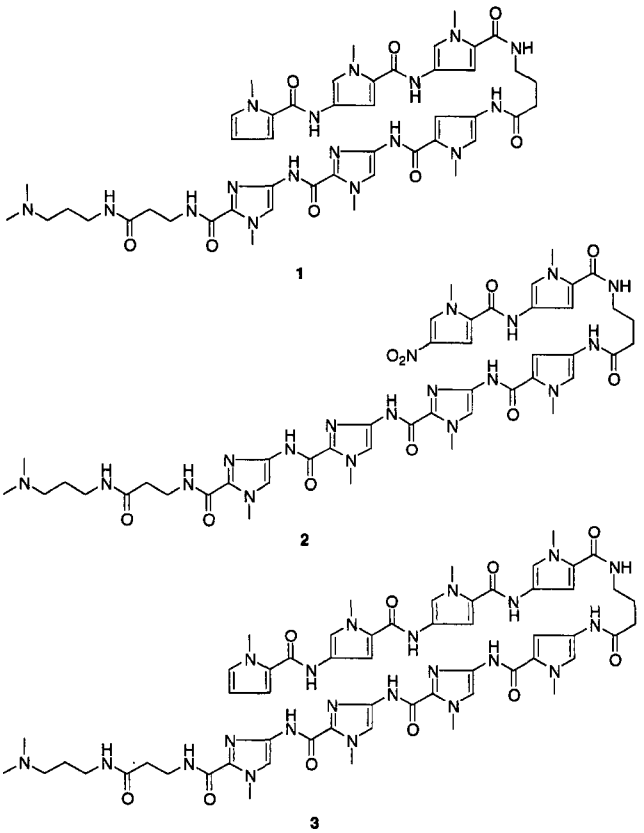

**Fig. 1.** Structures of designed and synthesized polyamides

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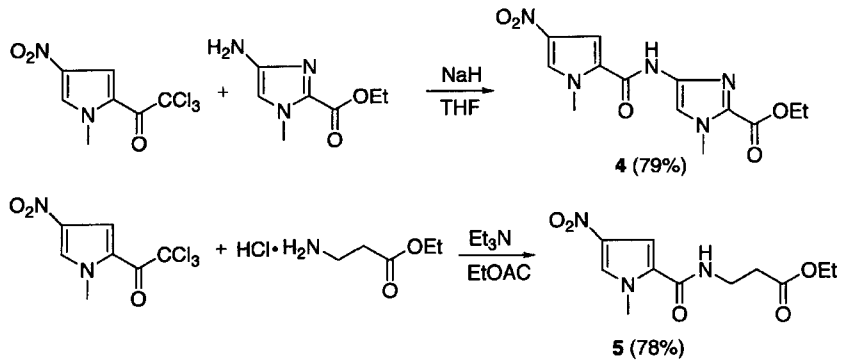
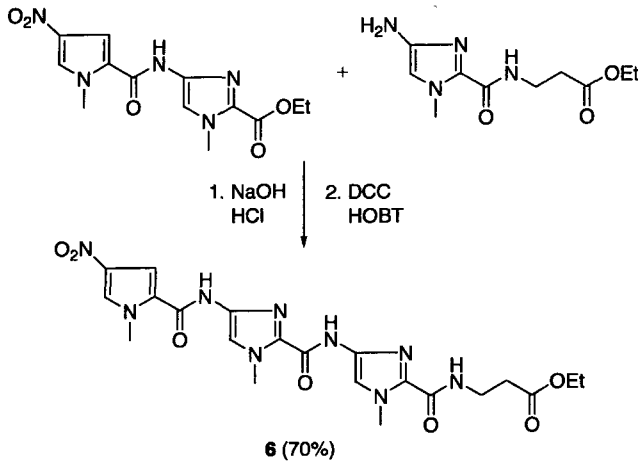



**Scheme 1.** Synthesis of building blocks by a haloform reaction.




**Scheme 2.** Synthesis of subchain by DCC/HOBT coupling reaction.

tylpyrrole, 4-nitro-*N*-methyl-2-trichloroacetylimidazole were used as key intermediates, which were easily prepared from commercially available *N*-methylpyrrole and *N*-methylimidazole through trichloroacetylization and nitration (Baird *et al.*, 1996; Xiao *et al.*, 2000).

There are two subchains in the polyamides. For example, one is PyPyPy · OEt, and another is PyImImOEt for PyPyPy · PyImIm · Dp (**1**). Being different from the former step-by-step linear synthetic strategy (Baird *et al.*, 1996), a converging synthetic strategy of the subchains condensation was employed to prepare the novel polyamides without amino protection and deprotection in a simple way.

In this research, a haloform reaction was used to synthesize the building blocks containing one or two heteocycles without column chromatography purification (Scheme 1). Then the building blocks prepared were effectively connected to synthesize the subchains by use of the DCC/HOBT coupling reaction (Xiao *et al.*, 2000) (Scheme 2).

After the hydrogenation of **6**, which was coupled with **8** to construct containing six heteocycles **9** (1.2 g, 60% yield) in one step in the presence of DCC/HOBT. After saponification with NaOH/ethanol solution and neutralization with 6N HCl of **9**, acid **10** was obtained (0.73 g, 94% yield). The final *N,N*-dimethylpropyldiamine was introduced to acid **10** to give the desired polyamide **1** (45 mg, 65% yield) by the DCC/HOBT mediated coupling reaction. The structure of the polyamide was confirmed by IR, NMR and HRMS. Polyamide **2** and **3** were prepared in the similar way.

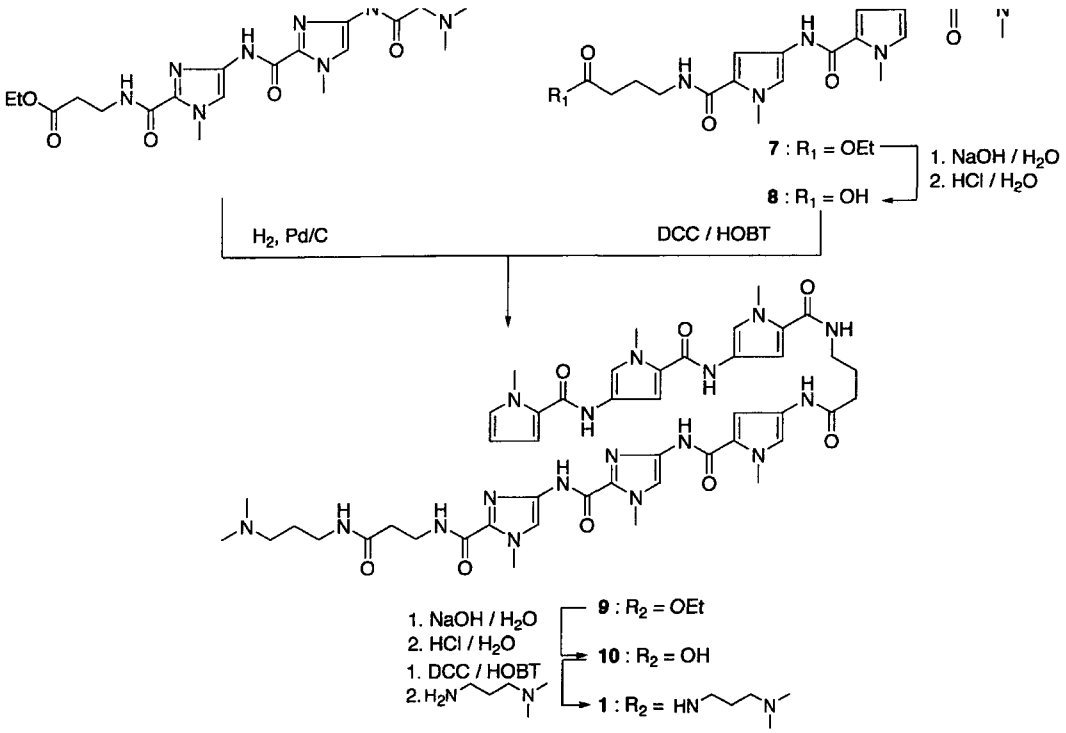
**Table I.** Cytotoxicities of designed polyamide 1, 2, 3 against tumor cell lines.

| Cancer cell type | Cell line | $IC_{50}$ [M] | | |
|---|---|---|---|---|
| | | Polyamide 1 | Polyamide 2 | Polyamide 3 |
| Hepatic carcinoma | Bel 7402 | $5.9\times10^{-8}$ | $8.5\times10^{-7}$ | $1.4\times10^{-7}$ |
| Lung carcinoma | 613 | $2.5\times10^{-8}$ | $6.3\times10^{-7}$ | $2.5\times10^{-7}$ |
| Gastric carcinoma | 803 | $1.7\times10^{-8}$ | $7.5\times10^{-7}$ | $2.2\times10^{-7}$ |

The polyamides synthesized were tested for cytotoxicity against three kinds of cell lines -hepatic carcinoma, lung carcinoma and gastric carcinoma to assess antitumor activity by the standard assay (Lee, 1991). The in vitro antitumor activities of the polyamide 1, 2, 3 against tumor cell lines are shown in Table I.

The results of this experiment revealed high inhibition potencies of the polyamides against tumor cell, and the values of $IC_{50}$ were at range of $10^{-7}$~$10^{-8}$ M. It has proven that the polyamides 1, 2, 3 possessed significant anticancer activity. The action of mode between the polyamides and DNA of tumor cell is through non-covalent interaction, such as hydrogen bond, van der waals and electrostatic (Mrksich *et al.*,1992; Kielkopf *et al.*, 1998). Binding of the polyamides in the minor groove of the tumor cell DNA inhibits the expression of the specific gene (Gottesfeld *et al.*, 1997; Dickinson et al., 1998) and then impedes the cell growth. The study of the exact mechanism of the action-mode for the polyamides against tumor cell is in the due course.

In conclusion, the polyamides containing *N*-methylpyrrole and *N*-methylimidazole are a new class of DNA-bind-




**Scheme 3.** Synthesis of PyPyPy · PyImIm · Dp (**1**)

ing molecules with potent anticancer activity and provide the new design model for anticancer drug.

## MATERIALS AND METHODS

$^1$H NMR spectra were recorded either at 400 MHz (Bruker ARX 400 NMR spectrometer) or 200 MHz (Varian HY 200 NMR spectrometer). Electron impact mass spectra (EI-MS) were recorded with an ionization voltage of 70 eV and fast-atom bombardment mass spectra (FAB-MS) were obtained using glycerol or thioglycerol as a matrix (VG-ZAB-HS mass spectrometer). MALDI-TOF-MS was obtained using a α-cyano-4-hydroxy-cinnamic acid matrix (Bruker BIFLEX-MALDI-TOF mass spectrometer), and accurate mass measurements (HRMS) were performed using an *m*-nitrobenzyl alcohol as a matrix (Bruker APEXII-FT-ICR mass spectrometer). Infrared spectra (IR) were recorded in the FT-IR mode (Bruker VECTOR22-FT-IR spectrometer).

Synthesis and characteristics of compounds are given for representative examples.

**(a) Synthesis of building block by the haloform reaction**

**$NO_2$PyImCOOEt (4)**: To a solution of $NH_2$ImCOOEt (0.57 g, 3.0 mmol) in 20 mL of THF was added *N*-methyl-4-nitro-2-trichloroacetylpyrrole (0.89 g, 3.3 mmol), followed by NaH (20 mg). The mixture was stirred for 6 h. The slight yellow precipitate was collected by filtration, washed with water and methanol, and dried to afford 0.76 g of yellow solid $NO_2$PyImCOOEt (79% yield). $^1$H NMR (DMSO-$d_6$, 200 MHz) δ: 11.18 (s, 1H), 8.19 (d, 1H, *J* = 1.4 Hz), 7.81 (d, 1H, *J* = 2.0 Hz), 7.69 (s, 1H), 4.27 (q, 2H, *J* = 7.2 Hz), 3.95 (s, 3H), 3.93 (s, 3H), 1.29 (t, 3H, *J* = 7.2 Hz); EI-MS calcd for $C_{13}H_{15}N_5O_5$ ($M^+$) *m/z* 321, found m/z 321.

**$NO_2$PyβCOOEt (5):** A synthetic procedure similar to that for **4** was followed for the preparation of **5** (6.72 g, 78% yield). $^1$H NMR ($CDCl_3$, 200 MHz) δ: 7.56 (d, 1H, *J* = 1.2 Hz), 7.08 (d, 1H, *J* = 0.8 Hz), 6.76 (b, 1H), 4.20 (q, 2H, *J* = 7.2 Hz), 3.99 (s, 3H), 3.65 (q, 2H, *J* = 6.2 Hz), 2.62 (t, 2H, *J* = 5.8 Hz), 1.29 (t, 3H, *J* = 7.2 Hz); EI-MS calcd for $C_{11}H_{15}N_3O_5$ ($M^+$) *m/z* 269, found m/z 269.

**(b) Synthesis of building block by the coupling reaction using DCC/HOBT**

**$NO_2$PyImImβ$CO_2$Et (6):** To a solution of $NO_2$PyCOOH (1.58 g, 9.31 mmol) in 6 mL of DMF was added HOBT (1.26 g, 9.32 mmol), followed by DCC (1.92 g, 9.31 mmol). The reaction solution was stirred overnight to ensure the complete formation of the active ester. DCU was removed by filtration. Separately, to a solution of $NO_2$ImImβOEt (3.05 g, 7.76 mmol) in 25 mL of DMF was added Pd/C catalyst (10%, 400 mg) and the mixture was stirred under a slight positive pressure of $H_2$ overnight. The catalyst was remov-

ed by filtration through Celite and the filtrate was directed into the active ester solution. The mixture was stirred for 20 h. The slight yellow precipitate was collected by filtration, washed with DMF, and dried to afford 2.80 g of **6** (70% yield). $^1$HNMR ($CDCl_3$, 200 MHz), δ: 9.90 (s,1H), 9.63 (s, 1H), 8.42 (t, 1H, *J* = 6.6 Hz), 8.02 (s,1H), 7.66 (s, 1H), 7.48 (s,1H), 7.41 (s, 1H), 4.04 (m, 11H), 3.74 (q · 2H, *J* = 6.0 Hz), 2.62 (2H, t, *J* = 5.8 Hz), 1.18 (t, 3H, *J* = 7.2 Hz); FAB-MS calcd for $C_{21}H_{26}N_9O_7$ (M+H) *m/z* 516, found m/z 516.

**(c) Synthesis and characteristics of PyPyPy · PyImIm · Dp (1)**

**PyPyPyγPyImImβCO$_2$Et (9):** A synthetic procedure similar to that for **6** was followed for the preparation of **9** (1.20 g, 60% yield). $^1$HNMR($CDCl_3$, 400 MHz), δ: 9.35(s, 1H), 9.24 (s, 1H), 9.06 (s, 1H), 8.69 (s, 1H), 8.14 (s, 1H), 7.99 (s, 1H), 7.50 (s, 1H), 7.35 (s, 1H), 7.30 (s, 1H), 7.24 (s, 1H), 7.16 (d, 2H, *J* = 6.8 Hz), 6.84 (d, 1H, *J* = 1.2 Hz), 6.79 (b, 1H), 6.69 (s, 1H), 6.61 (s, 1H), 6.47 (s, 1H), 6.02 (t, 1H · *J* = 3.0 Hz), 4.01 (q, 2H · *J* = 6.8 Hz), 3.91 (s, 3H), 3.89 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.80 (s, 3H), 3.71 (s, 3H), 3.58 (d, 2H, *J* = 4.0 Hz), 3.33 (b, 4H), 2.55 (t, 2H, *J* = 5.6 Hz), 2.32 (s, 2H), 1.12 (t, 3H, *J* = 7.2 Hz); FAB-MS calcd for $C_{43}H_{52}N_{15}O_9$ (M+1) *m/z*: 922, found *m/z*: 922.

**PyPyPyγPyImImβCOOH(10):** To a solution of **9** (0.80 g, 0.9 mmol) in 7 mL of ethanol was added NaOH (0.12 g in 5 mL of water). The reaction solution was stirred at room temperature overnight. After filtration, the filtrate was concentrated in vacuo to remove the ethanol solvent. The pH of the remaining aqueous solution was adjusted to about 1 by adding 6N HCl. The precipitate was collected by filtration and was washed with water and dried under IR lamp to offer 0.73 g of **10** (94% yield). $^1$HNMR(DMSO-$d_6$, 400 MHz), δ: 10.49 (s, 1H), 9.93 (s, 2H), 9.87 (s, 1H), 9.60 (s, 1H), 8.20 (t, 1H, *J* = 5.8 Hz), 8.09 (t, 1H, *J* = 5.6 Hz), 7.62 (s, 1H), 7.53 (s, 1H), 7.32 (d, 1H, *J* = 1.2 Hz), 7.24 (d, 1H, *J* = 1.4 Hz), 7.19 (d, 1H, *J* = 1.4 Hz), 7.05 (b, 1H), 6.95 (b, 2H), 6.93 (s, 1H), 6.92 (s, 1H), 6.91 (s, 1H), 6.07 (t, 1H, *J* = 2.4 Hz), 4.00 (s, 3H), 3.96 (s, 3H), 3.88 (s, 3H), 3.85(s, 6H), 3.82(s, 3H), 3.51(b, 4H), 2.55(s, 2H), 2.32 (t, 2H, *J* = 7.2 Hz), 1.89 (t, 2H, *J* = 7.2 Hz); FAB-MS calcd for $C_{41}H_{48}N_{15}O_9$ (M+H) *m/z*: 894, found *m/z*: 894.

**PyPyPy · PyImIm · Dp (1):** To a solution of **10** (63 mg, 0.07 mmol) in 1.0 mL of DMF was added HOBT (28 mg, 0.21 mmol), followed by DCC (43 mg, 0.21 mmol). The reaction solution was stirred overnight. *N,N*-dimethylpropyldiamine (10 μl) was added to the reaction solution and the stirring was continued for another 10 h. DCU was removed by filtration and the filtrate was concentrated in vacuo. Flash column chromatography of the residue afforded 45 mg of the polyamide **1** (65% yield). IR (KBr): 3283, 2926, 1652, 1533, 1466, 1409, 1253 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz ) δ: 10.46 (s, 1H), 9.89 (s, 1H), 9.87 (s, 1H), 9.82 (s, 1H), 9.43 (s, 1H), 8.25 (t, 1H, *J* = 5.6 Hz), 8.05 (t, 1H, J = 5.6 Hz), 7.99 (s, 1H), 7.80 (d, 1H, J = 8.0 Hz), 7.62 (s, 1H), 7.52 (s, 1H), 7.30 (d, 1H, *J* = 1.6 Hz), 7.23 (d, 1H, *J* = 1.6 Hz), 7.18 (d, 1H, *J* = 1.6 Hz), 7.04 (d, 1H, *J* = 1.7 Hz), 6.95 (d, 1H, *J* = 1.6 Hz), 6.92 (d, 1H, *J* = 1.6 Hz), 6.90 (d, 1H, *J* = 1.6 Hz), 6.06 (m, 1H), 4.00 (s, 3H), 3.96 (s, 3H), 3.88 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.81 (s, 3H), 3.42 (q, 2H, *J* = 6.2 Hz), 3.22 (q, 2H, *J* = 6.1 Hz), 3.09 (p, 2H, *J* = 6.2 Hz), 2.42 (m, 2H), 2.36 (t, 2H, *J* = 6.5 Hz), 2.29 (m, 2H), 2.27 (s, 6H), 1.80 (t, 2H, *J* = 7.2 Hz), 1.58 (m, 2H); HRMS calcd for $C_{46}H_{60}N_{17}O_8$ (M+1) *m/z*: 978.4811, found *m/z*: 978.4786.

**(d) Characteristics of NO$_2$PyPy · PyImImPy · Dp (2)**

A synthetic procedure similar to that for **1** was followed for the preparation of **2** (130 mg, 89% yield). IR (KBr) 3270, 2931, 1650, 1536, 1442, 1398, 1293 cm$^{-1}$;$^1$H NMR (DMSO-$d_6$, 400 MHz) δ: 10.40 (s, 1H), 10.26 (s, 1H), 10.25 (s, 1H), 9.90 (s, 1H), 8.18 (d, 1H, *J* = 1.6 Hz), 8.12 (t, 1H, *J* = 5.6 Hz), 8.04 (t, 1H, *J* = 5.6 Hz), 7.98 (s, 1H), 7.88 (t, 1H, *J* = 5.6 Hz), 7.64 (s, 1H), 7.58 (d, 1H, *J* = 2.0 Hz), 7.56 (s, 1H), 7.30 (d, 1H, *J* = 1.6 Hz), 7.23 (d, 1H, *J* = 2.0 Hz), 7.21 (d, 1H, *J* = 2.0 Hz), 6.95 (d, 1H, *J* = 1.6 Hz), 6.94 (d, 1H, *J* = 1.6 Hz), 6.89 (d, 1H, *J* = 1.6 Hz), 4.02 (s, 3H), 4.01 (s, 3H), 3.95 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.56 (q, 2H, *J* = 6.4 Hz), 3.22 (q, 2H, *J* = 6.0 Hz), 3.07 (m, 4H), 2.30 (q, 2H, *J* = 7.2 Hz), 2.20 (q, 2H, *J* = 7.2 Hz), 2.08 (s, 6H), 1.80 (pent, 2H, *J* = 7.2 Hz), 1.52 (m, 2H); MALDI-TOF-MS calcd for $C_{46}H_{58}N_{18}O_{10}Na$ (M+Na) *m/z*:1045.5, found *m/z*:1045.5.

**(e) Characteristics of PyPyPyPyγPyImImPyβDp (3)**

A synthetic procedure similar to that for **1** was followed for the preparation of **3** (90 mg, 79% yield). IR (KBr) 3750, 2934, 1648, 1535, 1467, 1255 cm$^{-1}$; $^1$H NMR (DMSO-$d_6$, 400 MHz) δ: 10.40 (s, 1H), 10.26 (s, 1H), 9.94 (s, 1H), 9.91 (d, 2H, *J* = 2.5 Hz), 9.84 (s, 1H), 9.43 (s, 1H), 8.06 (t, 1H, *J* = 5.6 Hz), 8.04 (t, 1H, *J* = 5.6 Hz), 7.90 (t, 1H, *J* = 5.4 Hz), 7.63 (s, 1H), 7.58 (s, 1H), 7.31 (d, 1H, *J* = 1.4 Hz), 7.24 (s, 2H), 7.23 (d, 1H, *J* = 1.5 Hz), 7.19 (d, 1H, *J* = 1.4 Hz), 7.07 (d, 1H, *J* = 1.7 Hz), 7.06 (d, 1H, *J* = 1.6 Hz), 6.96 (d, 1H, *J* = 1.7 Hz), 6.95 (s, 2H), 6.93 (t, 2H, *J* = 2.4 Hz), 6.06 (m, 1H), 4.02 (s, 3H), 4.01 (s, 3H), 3.89 (s, 3H), 3.86 (s, 3H), 3.85 (s, 3H), 3.84 (s, 3H), 3.82 (s, 3H), 3.81 (s, 3H), 3.37 (b, 2H), 3.23 (q, 2H, *J* = 5.6 Hz), 3.07 (m, 4H), 2.33 (t, 2H, *J* = 7.2 Hz), 2.26 (t, 2H, *J* = 7.2 Hz), 2.16 (s, 6H), 1.80 (q, 2H, *J* = 6.8 Hz), 1.5 6(q, 2H, *J* = 7.2 Hz); HRMS calcd for $C_{58}H_{72}N_{21}O_{11}$ (M+1) *m/z*: 1222.5757, found *m/z*: 1222.5783.

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