Synthesis of surface modified TREN cored PAMAM dendrimers and their effects
on the solubility of sulfamethoxazole (SMZ) as an analogue antibiotic drug

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SUPPORTING INFORMATION

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General Experimental

IR (ATR) spectra ($4000\text{--}650\text{ cm}^{-1}$, resolution 4 cm^{-1}) were recorded on PerkinElmer Spectrum 100 FT-IR Spectrometer (Serial No:LR64912C). ^1H NMR and ^{13}C NMR spectra were recorded in CDCl_3 using Bruker Avance 500 MHz Spectrometer and TMS as an internal reference. Microwave-irradiated reactions were carried out with a CEM Focused MicrowaveTM Synthesis System, Model Discover (CEM Corporation, North Carolina, USA) with a continuous microwave power delivery system with operator selectable power output from 0 to 300W ($\pm 30\text{ W}$) programmable in 1-watt increments, infrared temperature control system programmable from 25 to $250\text{ }^\circ\text{C}$, pressure controlled and 5 to 125 mL vessel capacity was used as microwave reactor (Serial Number DU9472). Spectroscopic titrations were carried out automatically by using TitroLine® 7000 ((SI Analytics GmbH, Hattenbergstrabe, Germany)) autotitrator equipped with thermostated titration vessel under nitrogen media.

Experimental procedures for the synthesis of PAMAMs

Synthesis of PAMAMs

Microwave-assisted synthesis (MAS) of PAMAM-NH₂ and PAMAM-TRIS dendrimers with Tris(2-aminoethyl)amine core (T), from half generation PAMAM-OCH₃ dendrimers, were performed according to our previous studies (1) and (2), respectively. On the other hand, PAMAM-COOH dendrimers were prepared by the slight modification of the literature procedure (3) and our previous recent study (4). Purity of PAMAMs were characterized by ATR-FTIR, ^1H -NMR, ^{13}C -NMR, and supported by spectroscopic titrations. Synthesized PAMAMs were used in the solubilization studies of SMZ.

General procedure for the microwave-assisted synthesis PAMAM-NH₂ dendrimers

Tris(2-aminoethyl)amine (TREN) cored PAMAM-NH₂ dendrimers were synthesized according to our previous study (1), and briefly summarized hereafter. This method involves the alkylation (a) and amidation (b) steps (Scheme 1). In the alkylation steps, a methanolic solution of methyl acrylate (2.5 M eq. per terminal amine) was added over to a stirring methanolic solution of dendrimer core (C) tris(2-aminoethyl)amine (TREN) (T) or full generation PAMAM-NH₂ dendrimers (Cn.NH₂). After stirring the resulting reaction mixture for 24 h at room temperature, excess reagents and solvents were removed under vacuum at 65°C and purified by means of LPR. The resulting PAMAM-OCH₃ dendrimers (Cn.5) T1.5, T2.5, T3.5 were colorless oils and product yields were between 90-98% (Table S1)

Table S1. Preparation of TREN cored ester-terminated PAMAMs (Cn.5).

PAMAM-OCH ₃ dendrimers	R-NH ₂ g (mmol)	MA g, (mmol)	MeOH (mL)	Time (h)	Yield (%)
T1.5	8.31 (9.99)	12.9 (149)	30	24	96
T2.5	9.52 (4.32)	11.16 (129)	30	24	93
T3.5	8.63 (1.74)	9.02 (104)	30	24	90

In the amidation steps, a 1-5 mL methanolic solution of ethylenediamine (EDA) involving 10 M eq. of excess EDA per ester branched PAMAM-OCH₃ dendrimers were added to a vigorously stirring methanolic solution of half generation PAMAM-OCH₃ dendrimers (Cn.5). The final mixture was irradiated with microwave (MW) at 250 W for 80 min. Final traces of EDA were removed under vacuum below a bath temperature of 65 °C by the azeotropic mixture of n-butanol three times. The resulting product was purified by using LPR. The final products

were full generation PAMAM-NH₂ dendrimers (**Cn.NH₂**) T2.NH₂-T4.NH₂. Product yields were in the range of 90-95% (Table S2).

Table S2. Preparation of TREN cored amine-terminated PAMAMs (**Cn.NH₂**).

PAMAM-NH ₂ dendrimers	R-OCH ₃ g (mmol)	EDA g (mol)	MeOH ^a (mL)	MW (watt)	Time (min)	Yield (%)
T2.NH ₂	14.80 (7.93)	57.25 (0.95)	2.0	250	80	90
T3.NH ₂	12.6 (2.95)	42.58 (0.7)	2.0	250	80	95
T4.NH ₂	9.07 (1.06)	28.83 (0.49)	1.0	250	80	90

General procedure for the microwave-assisted synthesis of PAMAM-TRIS dendrimers

PAMAM-TRIS dendrimers were prepared according to procedure in our recent study (2), and briefly summarized hereafter. A methanolic solution of PAMAM-OCH₃ dendrimers (**C1.5-C3.5**) were added to a stirred suspension of TRIS (1.2 M equiv. per terminal ester) and anhydrous K₂CO₃ (1.5 M equiv. per terminal ester) in 10–15 mL of MeOH. The following mixture was irradiated at 200 W for 120–130 min by refluxing at a bulk temperature of 70–90 °C. The final reaction mixture was filtered to remove excess solid reagents, and the filtrate collected. Then product was purified by LPR method. The final methanolic solution of the retained product (**Cn.TRIS**) was concentrated under vacuum below a bath temperature of 65 °C, The resulting products were T2.TRIS-T4.TRIS, and yields were in the range of 92–93% (Table S3).

Table S3. Preparation of TREN cored TRIS-terminated PAMAMs (*Cn.TRIS*).

PAMAM-TRIS	R-OCH ₃	TRIS	K ₂ CO ₃	MeOH	MW	Time	Yield
dendrimers	g (mmol)	g (mmol)	g (mmol)	(mL)	(watt)	(min)	(%)
T2.TRIS	0.84 (0.45)	0.77 (6.4)	1.11 (8.04)	10.0	200	125	92
T3.TRIS	0.87 (0.2)	0.69 (5.7)	0.99 (7.17)	10.0	200	120	93
T4.TRIS	0.82 (0.09)	0.62 (5.12)	0.97 (7.02)	10.0	200	130	92

General procedure for the synthesis of PAMAM-COOH dendrimers

PAMAM-COOH dendrimers were prepared by slight modification of the literature procedure (3) and our recent study (4). A methanolic solution of ester-terminated half generation PAMAM-OCH₃ (*Cn.OCH₃*) was mixed with 1.5 M equiv. of NaOH per terminal ester. The final mixture was stirred for 24 h. Excess amount of solvent was removed under vacuum at bath temperature 65 °C. The remaining oil was dissolved in methanol and again evaporated in vacuo. Drying under vacuum resulted in a white powder product. The resulting products were T2.COOH-T4.COOH, and yields were 100% (Table S4.)

Table S4. Preparation of TREN cored carboxyl-terminated PAMAMs (*Cn.COOH*).

PAMAM COOH	R-OCH ₃	NaOH	MeOH	Time	Yield
Dendrimers	g (mmol)	g (mmol)	(mL)	(h)	(%)
T2.COOH	0.84 (0.45)	0.27 (6.95)	5.0	24	100
T3.COOH	0.86 (0.2)	0.27 (6.95)	5.0	24	100
T4.COOH	0.79 (0.08)	0.23 (5.87)	5.0	24	100

Experimental procedures for the spectroscopic titrations of PAMAM-COOH and PAMAM-TRIS dendrimers with Cu^{2+} ions

Spectroscopic titration of PAMAMs with Cu^{2+} ions

Spectroscopic titrations were conducted on the synthesized PAMAM-TRIS and PAMAM-COOH dendrimers to characterize and show the structural monodispersity or homogeneity by using a PG-70 batch type UV-VIS instrument. UV measurements were conducted within the wavelength range of 230-350 nm with a 10 mm quartz UV cells. The spectroscopic titrations experiments were carried out according to our previous work (2). In summary, Table S5 presents the concentrations of dendrimer and CuSO_4 solutions used in the spectroscopic titration.

Table S5. Concentrations used to titrate aqueous solutions of TRIS and COOH terminated PAMAMs with Cu^{2+} ions.

Type of dendrimer	Dendrimer conc. (mM)	CuSO_4 conc. (mM)	Increment of CuSO_4 (μL)
T4.TRIS	0.164	66.25	525.00
T4.COOH	0.210	66.25	748.00

Spectroscopic characterization and the determination of the number of tertiary amine groups present in PAMAM-COOH and PAMAM-TRIS dendrimers

Spectroscopic titration experiments were conducted according to our recent study (2) by simply adapting from Crooks et. al former studies (5, 6). The results of these studies showed that Cu^{2+} ions coordinate with a maximum number of four tertiary amines. In similar, our aim in this study was to show the ideal growth of the synthesized PAMAM dendrimers and monodispersity by

calculating the exact number of tertiary amines present at T4.TRIS and T4.COOH with the aid of spectroscopic titrations. After addition of Cu^{2+} ions at the specific increments (Table S5) to the PAMAM dendrimer solutions, a sudden change in the color of solutions to deep blue was observed. The intensity of this color was dependent on Cu(II)-PAMAM-COOH and Cu(II)-PAMAM-TRIS dendrimers complexes of T4.COOH and T4.TRIS characterized with a broad absorption band at λ_{max} 680 nm in UV-VIS spectrophotometer (Fig. S1 and Fig. S2). This band (copper *d-d* transition) is the indication of complexation between Cu(II), and the tertiary amine groups of dendrimers at 1:4 molar ratio and coordination (7). The maximum molar excess of Cu^{2+} that can be loaded onto PAMAM-TRIS and PAMAM-COOH dendrimers were determined from absorbance at λ_{max} 680 nm versus Cu^{2+} /PAMAM-TRIS and Cu^{2+} /PAMAM-COOH molar ratio plots (Fig. S1 and Fig. S2).

Figure S1. Absorption spectra of PAMAM-COOH dendrimers at 680 nm (a) T4.COOH (0.210 mM) solution titrated with Cu^{2+} (66.25 mM), and (b) spectroscopic titration curve of T4.COOH.

Figure S2. Absorption spectra of PAMAM-TRIS and PAMAM-COOH dendrimers at 680 nm (a) T4.TRIS (0.164 mM) solution titrated with Cu^{2+} (66.25 mM) and (b) spectroscopic titration curve of T4.TRIS.

Table S6 presents the theoretical number of tertiary amines (^3N) present in PAMAM-TRIS and PAMAM-COOH dendrimers (T4.TRIS and T4.COOH) and the experimental found numbers. The results showed that there exist a good correlation between theoretical and practical number of tertiary amine numbers in PAMAM-TRIS and PAMAM-COOH dendrimers. These results are also evidence for the purity, monodispersity therefore ideal growth of synthesized PAMAM dendrimers with no such kind of impurity like trailing generations.

Table S6. Number of tertiary amine groups on PAMAM-TRIS and PAMAM-COOH dendrimers available for binding with Cu^{2+} ions.^a

Dendrimer	^3N theoretical value	^3N practical value	% Correlation
T4.TRIS	46	43.34 ± 0.55	94.21
T4.COOH	46	43.51 ± 0.65	94.58

^aResults were calculated from potentiometric titrations for five repeated experiments, and presented as mean $\pm$ confidence intervals.

Characterization data of PAMAMs

T1.5

Yellowish gel (17.88 g, 96%). ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3311 (NH), 1731 (C=O), 1659 (HNC=O), 1543 (HNC=O). ^1H -NMR δH (500 MHz; CDCl_3) 2.38 (24H, t, $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 2.48 (24H, bm, $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 2.71 (12H, bm, $\text{CONHCH}_2\text{CH}_2\text{NR}_2$), 3.23 (12H, bm, $\text{CONHCH}_2\text{CH}_2\text{NR}_2$), 3.62 (36H, t, COOCH_3). ^{13}C -NMR δC (125 MHz; CDCl_3) 172.97 (COOCH_3), 172.05 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 51.59 (COOCH_3), 49.18 ($\text{CH}_2\text{CH}_2\text{COOCH}_3$), 32.61 ($\text{CH}_2\text{CH}_2\text{COOCH}_3$).

T2.5

Yellowish gel (17.16 g, 93%). ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3299 (NH), 1731 (C=O), 1646 (HNC=O), 1553 (HNC=O). $^1\text{H-NMR}$ δH (500 MHz; CDCl_3) 2.37 (48H, t, $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 2.48 (48H, t, $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 2.70 (24H, bm, $\text{CONHCH}_2\text{CH}_2\text{NR}_2$), 3.22 (24H, bm, $\text{CONHCH}_2\text{CH}_2\text{NR}_2$), 3.61 (72H, s, COOCH_3). $^{13}\text{C-NMR}$ δC (125 MHz; CDCl_3) 173.03 (COOCH_3), 172.04 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 51.59 (COOCH_3), 49.17 ($\text{CH}_2\text{CH}_2\text{COOCH}_3$), 32.61 ($\text{CH}_2\text{CH}_2\text{COOCH}_3$).

T3.5

Yellowish gels (14.25 g, 90%). ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3305 (NH), 1731 (C=O), 1643 (HNC=O), 1535 (HNC=O). $^1\text{H-NMR}$ δH (500 MHz; CDCl_3) 2.38 (96H, t, $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 2.48 (96H, t, $\text{CH}_2\text{CH}_2\text{COOCH}_3$), 2.70 (48H, bm, $\text{CONHCH}_2\text{CH}_2\text{NR}_2$), 3.22 (48H, bm, $\text{CONHCH}_2\text{CH}_2\text{NR}_2$), 3.61 (144H, s, COOCH_3). $^{13}\text{C-NMR}$ δC (125 MHz; CDCl_3) 173.01 (COOCH_3), 172.33 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 51.59 (COOCH_3), 49.19 ($\text{CH}_2\text{CH}_2\text{COOCH}_3$), 32.61 ($\text{CH}_2\text{CH}_2\text{COOCH}_3$).

T2.NH₂

Yellowish gel (15.72 g, 90%). ATR-IR $\nu_{\text{max}}/\text{cm}^{-1}$ 3285 (NH), 1635 (HNC=O), 1552 (HNC=O). $^1\text{H-NMR}$ δH (500 MHz; DMSO-d_6) 2.57 (24H, bm, $\text{NCH}_2\text{CH}_2\text{CONH}$), 2.68 (24H, bm, $\text{NCH}_2\text{CH}_2\text{CONH}$), 2.74 (24H, bm, $\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 3.24 (24H, bm, $\text{CONHCH}_2\text{CH}_2\text{NH}_2$). $^{13}\text{C-NMR}$ δC (125 MHz; DMSO-d_6) 171.52 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 41.69 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 41.03 ($\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 40.88 ($\text{CONHCH}_2\text{CH}_2\text{NH}_2$), 36.27 ($\text{NCH}_2\text{CH}_2\text{CONH}$).

T3.NH₂

Yellowsish gel (13.86 g, 95%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3282 (NH), 1634 (HNC=O), 1553 (HNC=O).

¹H-NMR δH (500 MHz; D₂O) 2.48 (48H, bm, NCH₂CH₂CONH), 2.59 (48H, bm, NCH₂CH₂CONH), 2.67 (48H, bm, CONHCH₂CH₂NH₂), 3.15 (48H, bm, CONHCH₂CH₂NH₂).

¹³C-NMR δC (125 MHz; D₂O) 174.88 (NCH₂CH₂CONH), 41.37 (NCH₂CH₂CONH), 41.24 (CONHCH₂CH₂NH₂), 39.66 (CONHCH₂CH₂NH₂), 35.38 (NCH₂CH₂CONH).

T4.NH₂

Yellowsish gel (9.37 g, 90%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3282 (NH), 1635 (HNC=O), 1550 (HNC=O).

¹H-NMR δH (500 MHz; D₂O) 2.46 (96H, bm, NCH₂CH₂CONH), 2.57 (96H, bm, NCH₂CH₂CONH), 2.66 (96H, bm, CONHCH₂CH₂NH₂), 3.14 (96H, bm, CONHCH₂CH₂NH₂).

¹³C-NMR δC (125 MHz; D₂O) 174.90 (NCH₂CH₂CONH), 41.43 (NCH₂CH₂CONH), 41.30 (CONHCH₂CH₂NH₂), 39.69 (CONHCH₂CH₂NH₂), 35.39 (NCH₂CH₂CONH).

T2.COOH

White solid (0,76 g, 100%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3292 (COOH), 1650 (HNC=O), 1569 (HNC=O), 1404 (O-H). ¹H NMR δH (500 MHz; D₂O) 2.31 (24H, bm, CH₂CH₂COOH), 2.63 (24H, bm, CH₂CH₂COOH). ¹³C NMR δC (125 MHz; D₂O) 181.26, 181.13 (COOH), 174.53 (NCH₂CH₂CONH), 48.58 (CH₂CH₂COOH), 34.22 (CH₂CH₂COOH).

T3.COOH

White solid (0,78 g, 100%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3292 (COOH), 1650 (HNC=O), 1569 (HNC=O), 1404 (O-H). ¹H NMR δH (500 MHz; D₂O) 2.31 (48H, bm, CH₂CH₂COOH), 2.66 (48H, bm, CH₂CH₂COOH). ¹³C NMR δC (125 MHz; D₂O) 181.46, 181.31 (COOH), 174.71 (NCH₂CH₂CONH), 48.90 (CH₂CH₂COOH), 34.13 (CH₂CH₂COOH).

T4.COOH

White solid (0.73 g, 100%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3292 (COOH), 1650 (HNC=O), 1569 (HNC=O), 1404 (O-H). ^1H NMR δH (500 MHz; D₂O) 2.28 (96H, bm, CH₂CH₂COOH), 2.62 (96H, bm, CH₂CH₂COOH). ^{13}C NMR δC (125 MHz; D₂O) 181.41, 181.22 (COOH), 174.62 (NCH₂CH₂CONH), 48.86 (CH₂CH₂COOH), 34.09 (CH₂CH₂COOH).

T2.TRIS

Opaque oil (1.21 g, 92%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3263 (COH), 1641 (HNC=O), 1562 (HNC=O), 1392 (O-H). ^1H NMR δH (400 MHz; CD₃OD) 2.54 (24H, m, CH₂CH₂CONHCR₃), 2.62 (24H, m, CH₂CH₂CONHCR₃), 3.57 (96H, s, CH₂OH). ^{13}C NMR δC (125 MHz; D₂O) 180.42, 175.43, 174.85 (NCH₂CH₂CONH), 62.48 (CONHCR₂CH₂OH), 56.81 (CONHCR₂CH₂OH), 48.69 (CH₂CH₂CONHCR₃), 33.21 (CH₂CH₂CONHCR₃).

T3.TRIS

Opaque oil (1.19 g, 93%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3254 (COH), 1636 (HNC=O), 1558 (HNC=O), 1392 (O-H). ^1H NMR δH (400 MHz; CD₃OD) 2.59 (48H, m, CH₂CH₂CONHCR₃), 2.65 (48H, m, CH₂CH₂CONHCR₃), 3.58 (192H, s, CH₂OH). ^{13}C NMR δC (125 MHz; D₂O) 180.54, 175.38, 174.81 (NCH₂CH₂CONH), 62.51 (CONHCR₂CH₂OH), 56.89 (CONHCR₂CH₂OH), 48.80 (CH₂CH₂CONHCR₃), 33.36 (CH₂CH₂CONHCR₃).

T4.TRIS

Opaque oil (1.10 g, 92%). ATR-IR $\nu_{\max}/\text{cm}^{-1}$ 3265 (COH), 1635 (HNC=O), 1553 (HNC=O), 1393 (O-H). ^1H NMR δH (500 MHz; D₂O) 2.48 (96H, m, CH₂CH₂CONHCR₃), 2.65 (96H, m, CH₂CH₂CONHCR₃), 3.58 (288H, bs, CH₂OH). ^{13}C NMR δC (125 MHz; D₂O) 180.34, 175.53,

174.83 ($\text{NCH}_2\text{CH}_2\text{CONH}$), 62.46 ($\text{CONHCR}_2\text{CH}_2\text{OH}$), 56.96 ($\text{CONHCR}_2\text{CH}_2\text{OH}$), 48.79 ($\text{CH}_2\text{CH}_2\text{CONHCR}_3$), 33.94 ($\text{CH}_2\text{CH}_2\text{CONHCR}_3$).

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