High Efficiency Antimicrobial Thiazolium and Triazolium Side-Chain Polymethacrylates Obtained by Controlled Alkylation of the Corresponding Azole Derivatives

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Electronic Supplementary Information

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1. Determination of the Degree of Quaternization by 1H-NMR spectroscopy**:**

1.1 Determination of the Degree of Quaternization (DQth) of PMTA1 by ^{1}H-NMR spectroscopy:

We were able to determine the DQth (%) from the relationship conformed by the integrals of peaks related to thiazole/thiazolium protons as follows:

$$DQth\ (\%) = \frac{\text{Integral (Thiazolium proton } A_2)}{\text{Integral (Thiazolium proton } A_2 + \text{Thiazole proton } A_1)} \times 100$$

1.2 Determination of the Degree of Quaternization (DQth and DQtr) of PMTA4 by ^{1}H-NMR spectroscopy:

We were able to determine the DQth (%) from the relationship between the integrals of peaks associated to thiazole/thiazolium protons in every species as follows:

$$DQth\ (\%) = \frac{\text{Integral (Thiazolium } A_4 + A_3)}{\text{Integral (Thiazolium } A_4 + A_3 + \text{Thiazole } A_2 + A_1)} \times 100$$

Similarly, we were able to determine the DQtr (%) from the relationship between the integrals of peaks associated to triazole/triazolium protons in every species as follows:

$$DQtr\ (\%) = \frac{\text{Integral (Triazolium } B_4 + B_2)}{\text{Integral (Triazolium } B_4 + B_2 + \text{Triazole } B_3 + B_1)} \times 100$$

2. Kinetics and Degree of Quaternization (DQ) of PMTA1-BuI

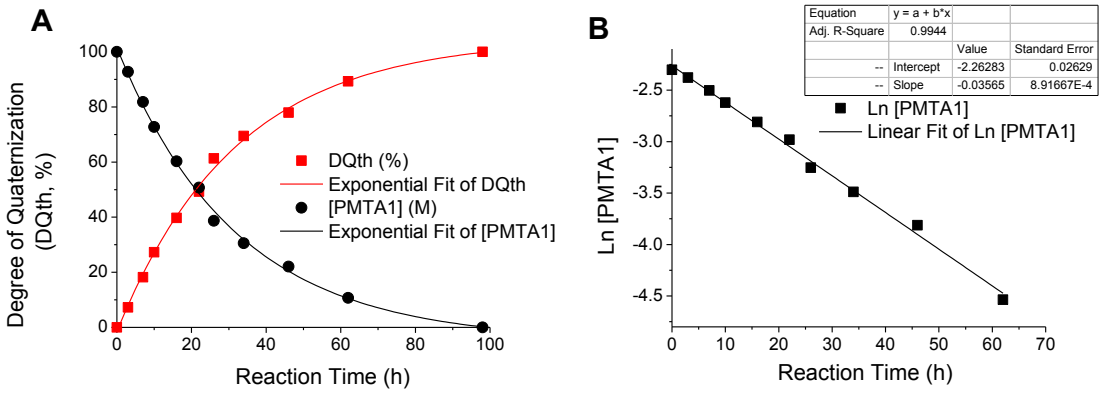


Figure S1. A) Degree of Quaternization (DQth) of **PMTA1** versus Reaction Time determined by ^{1}H-NMR spectroscopy. B) First-order Kinetics Quaternization Reaction by plotting Ln [**PMTA1**] versus Reaction Time.

The modification of PMTA1 with butyl iodide proceeds as a first-order reaction as can be seen in Figure S1B and follow the first-order rate law:

$[PMTA1] = [PMTA1]_0 e^{-kt}$

$Ln[PMTA1] = Ln[PMTA1]_0 - kt$

$k = 0.0356\ h^{-1} = 9.9 \times 10^{-6}\ s^{-1}$

3. Kinetics and Degree of Quaternization (DQ) of PMTA4-BuI

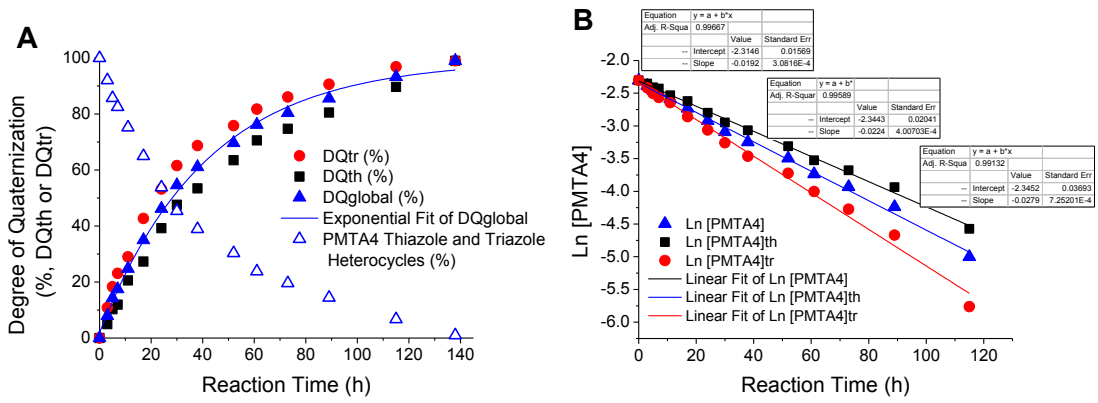


Figure S2. A) Degree of Quaternization of both heterocycles (Thiazole, DQth and Triazole, DQtr) implicit in **PMTA4** as well as the global degree, DQ_{global} versus Reaction Time determined by ^{1}H-NMR spectroscopy. B) First-order Kinetics Quaternization Reaction of **PMTA4** by plotting Ln [**PMTA4**] versus Reaction Time.

The modification of bisheterocyclic PMTA4 with butyl iodide also proceeds as a first-order reaction as can be seen in Figure S2B and follow the first-order rate law:

$[PMTA4] = [PMTA4]_0 e^{-kt}$

$Ln[PMTA4] = Ln[PMTA4]_0 - kt$

$k = 0.0224\ h^{-1} = 6.2 \times 10^{-6}\ s^{-1}$

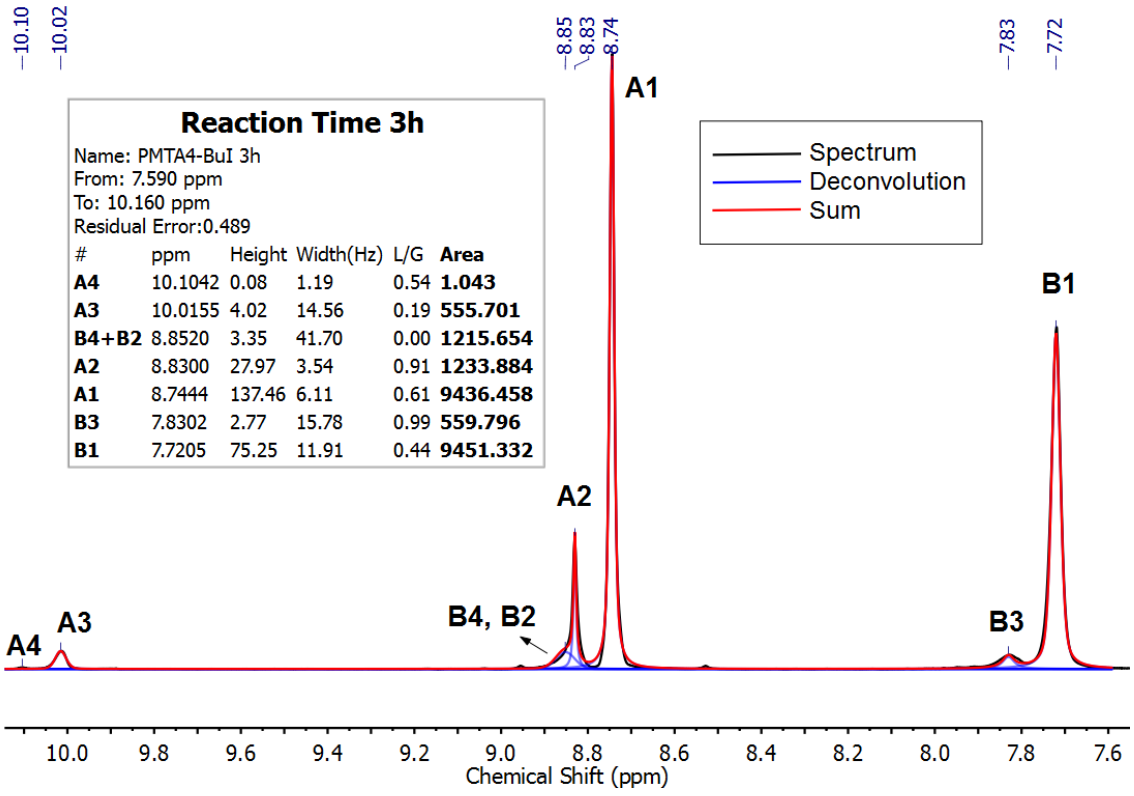
considering the amount of quaternized heterocyclic, it is possible to estimate each triazole or thiazole modification kinect constant, being $k_{th} = 0.0192\ h^{-1} = 5.3 \times 10^{-6}\ s^{-1}$ and $k_{tr} = 0.0279\ h^{-1} = 7.8 \times 10^{-6}\ s^{-1}$.

Comparing PMTA1 and PMTA4 kinetics constants of quaternization reaction, it is noticed that all of them present values of the same order ($\approx 10^{-6}\ s^{-1}$). Furthermore, PMTA4, which is

even quaternized with large excess of butyl iodide (5.0 equiv.), undergoes a quaternization kinetic slightly slower than PMTA1 maybe due to the more complex bis-heterocyclic structure as well as the repulsion of catonic species involved during the quaternization reaction.

4. Deconvolution of ^{1}H-NMR spectra

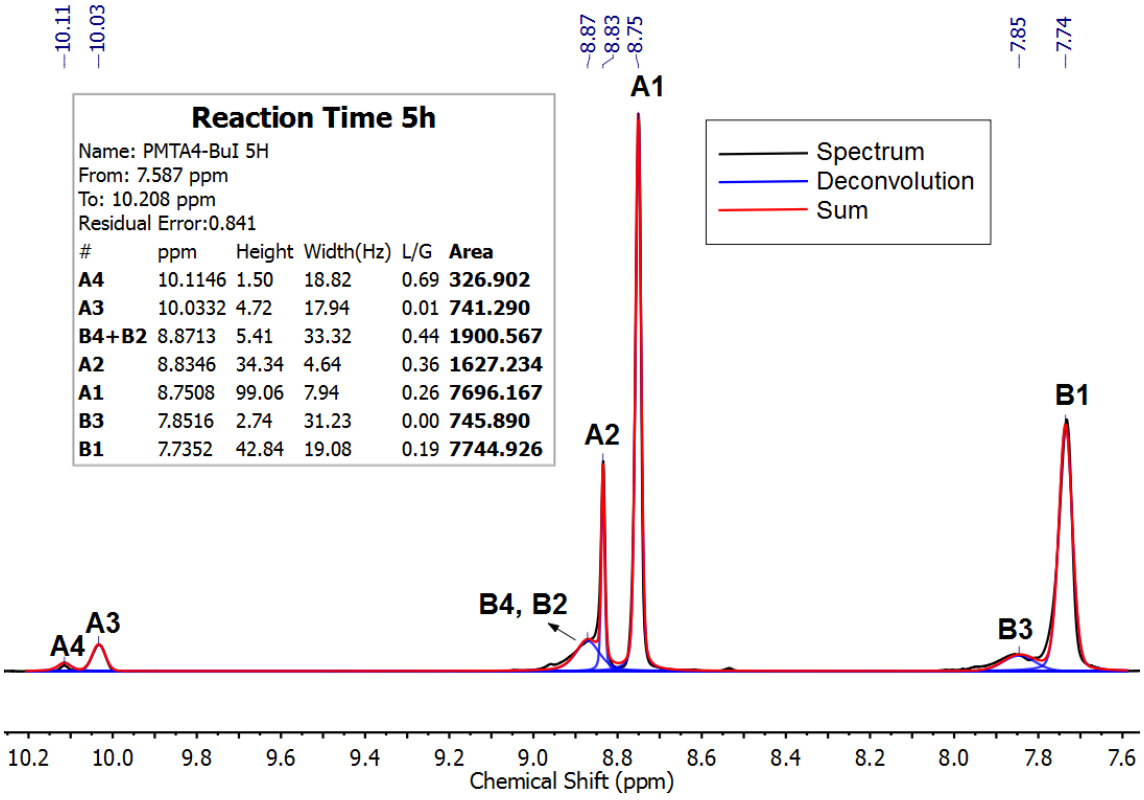
In order to analyze the percentage of chemical species of PMTA4-BuI during the quaternization reaction, deconvolution of the ^{1}H-NMR spectra and integration data were done with the support of MestReNova 8.1.1-11591 NMR analysis software as shown below.



Reaction Time 3h

Name: PMTA4-BuI 3h
From: 7.590 ppm
To: 10.160 ppm
Residual Error:0.489

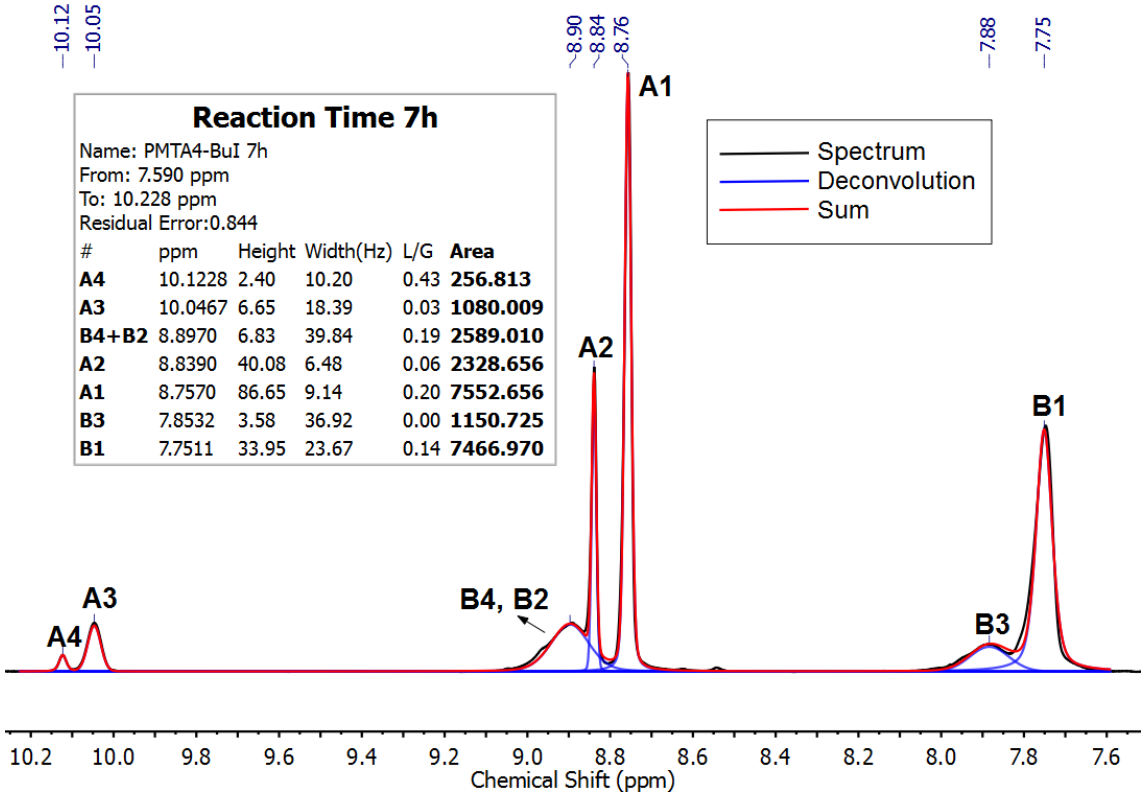
#	ppm	Height	Width(Hz)	L/G	Area
A4	10.1042	0.08	1.19	0.54	**1.043**
A3	10.0155	4.02	14.56	0.19	**555.701**
B4+B2	8.8520	3.35	41.70	0.00	**1215.654**
A2	8.8300	27.97	3.54	0.91	**1233.884**
A1	8.7444	137.46	6.11	0.61	**9436.458**
B3	7.8302	2.77	15.78	0.99	**559.796**
B1	7.7205	75.25	11.91	0.44	**9451.332**



Reaction Time 5h

Name: PMTA4-BuI 5H
From: 7.587 ppm
To: 10.208 ppm
Residual Error:0.841

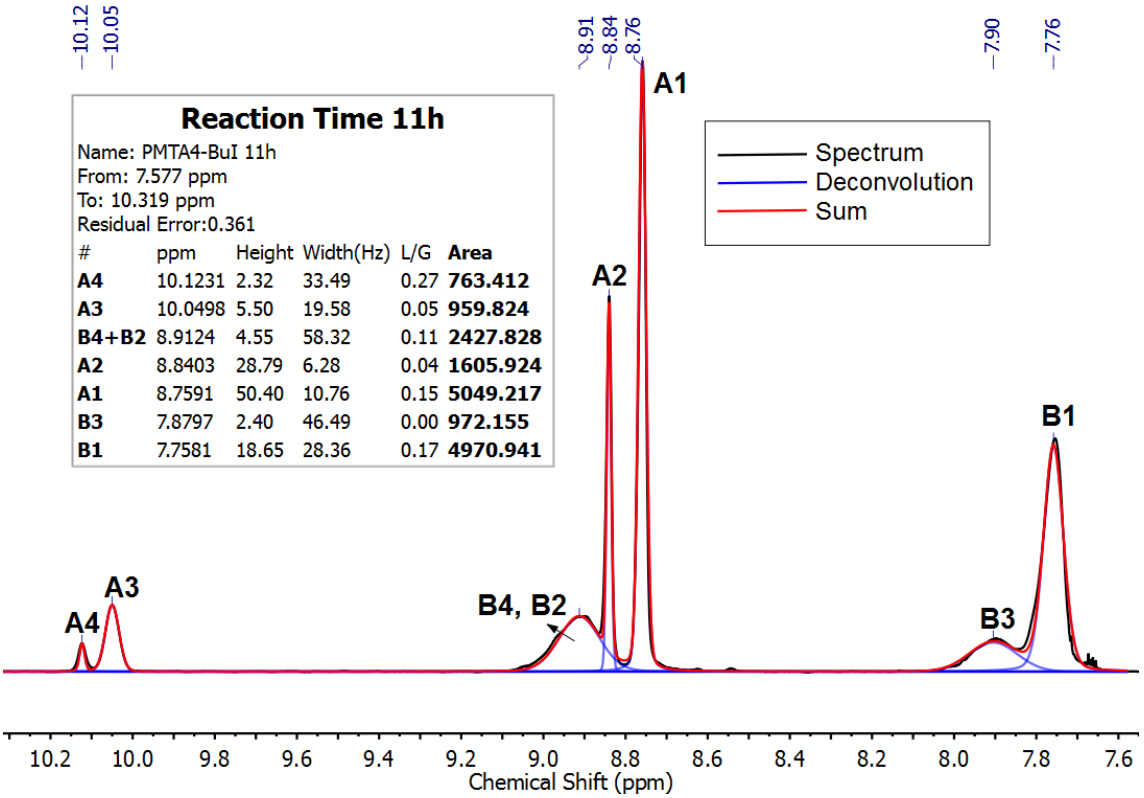
#	ppm	Height	Width(Hz)	L/G	Area
A4	10.1146	1.50	18.82	0.69	**326.902**
A3	10.0332	4.72	17.94	0.01	**741.290**
B4+B2	8.8713	5.41	33.32	0.44	**1900.567**
A2	8.8346	34.34	4.64	0.36	**1627.234**
A1	8.7508	99.06	7.94	0.26	**7696.167**
B3	7.8516	2.74	31.23	0.00	**745.890**
B1	7.7352	42.84	19.08	0.19	**7744.926**



Reaction Time 7h

Name: PMTA4-BuI 7h
From: 7.590 ppm
To: 10.228 ppm
Residual Error:0.844

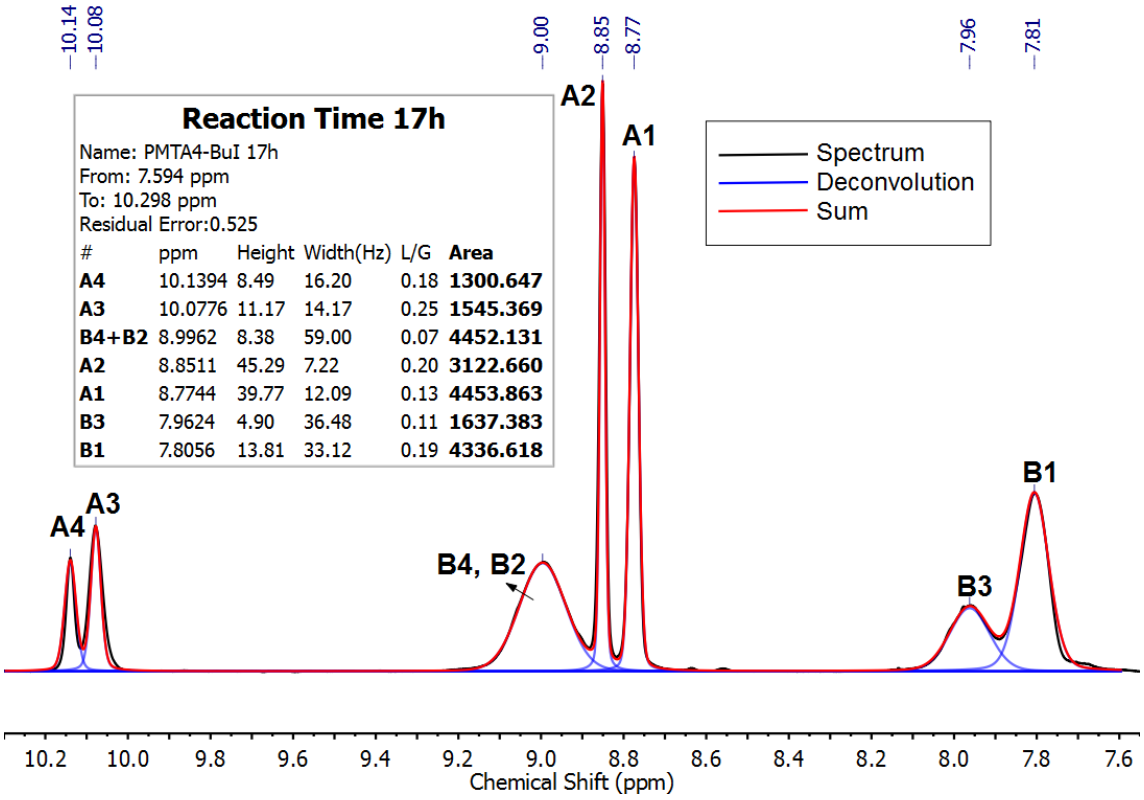
#	ppm	Height	Width(Hz)	L/G	Area
A4	10.1228	2.40	10.20	0.43	**256.813**
A3	10.0467	6.65	18.39	0.03	**1080.009**
B4+B2	8.8970	6.83	39.84	0.19	**2589.010**
A2	8.8390	40.08	6.48	0.06	**2328.656**
A1	8.7570	86.65	9.14	0.20	**7552.656**
B3	7.8532	3.58	36.92	0.00	**1150.725**
B1	7.7511	33.95	23.67	0.14	**7466.970**



Reaction Time 11h

Name: PMTA4-BuI 11h
From: 7.577 ppm
To: 10.319 ppm
Residual Error:0.361

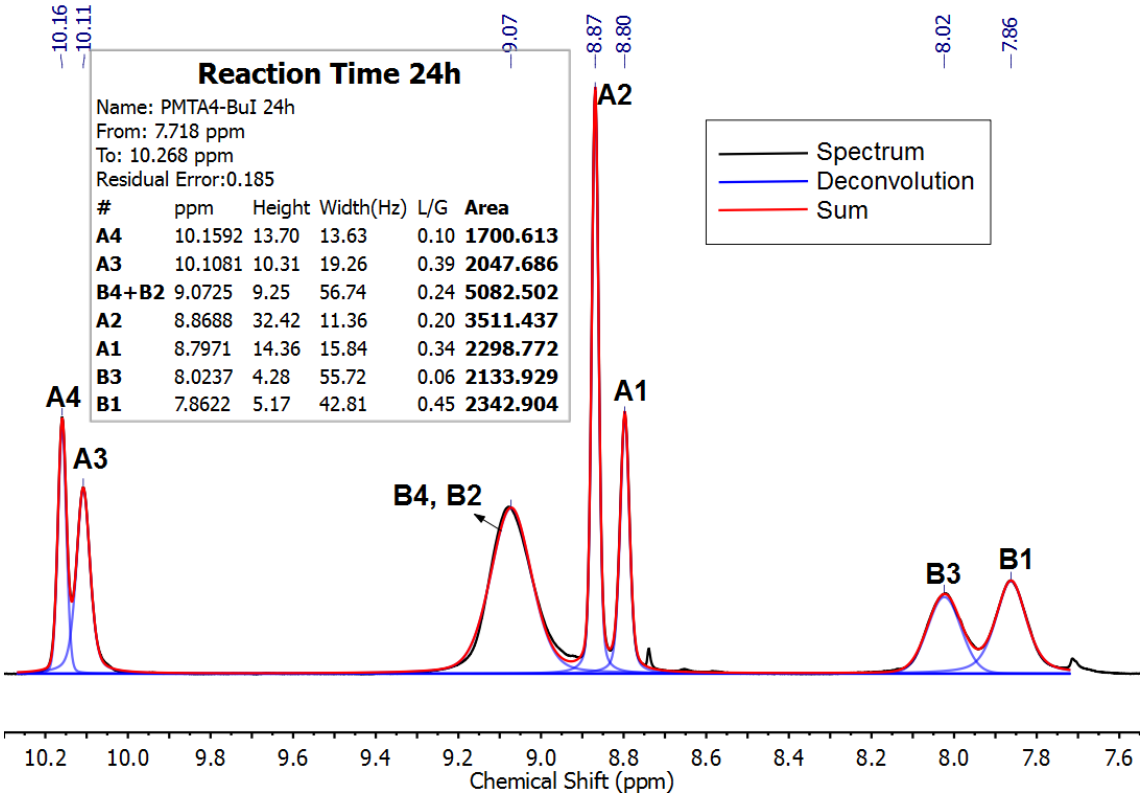
#	ppm	Height	Width(Hz)	L/G	Area
A4	10.1231	2.32	33.49	0.27	**763.412**
A3	10.0498	5.50	19.58	0.05	**959.824**
B4+B2	8.9124	4.55	58.32	0.11	**2427.828**
A2	8.8403	28.79	6.28	0.04	**1605.924**
A1	8.7591	50.40	10.76	0.15	**5049.217**
B3	7.8797	2.40	46.49	0.00	**972.155**
B1	7.7581	18.65	28.36	0.17	**4970.941**



Reaction Time 17h

Name: PMTA4-BuI 17h
From: 7.594 ppm
To: 10.298 ppm
Residual Error:0.525

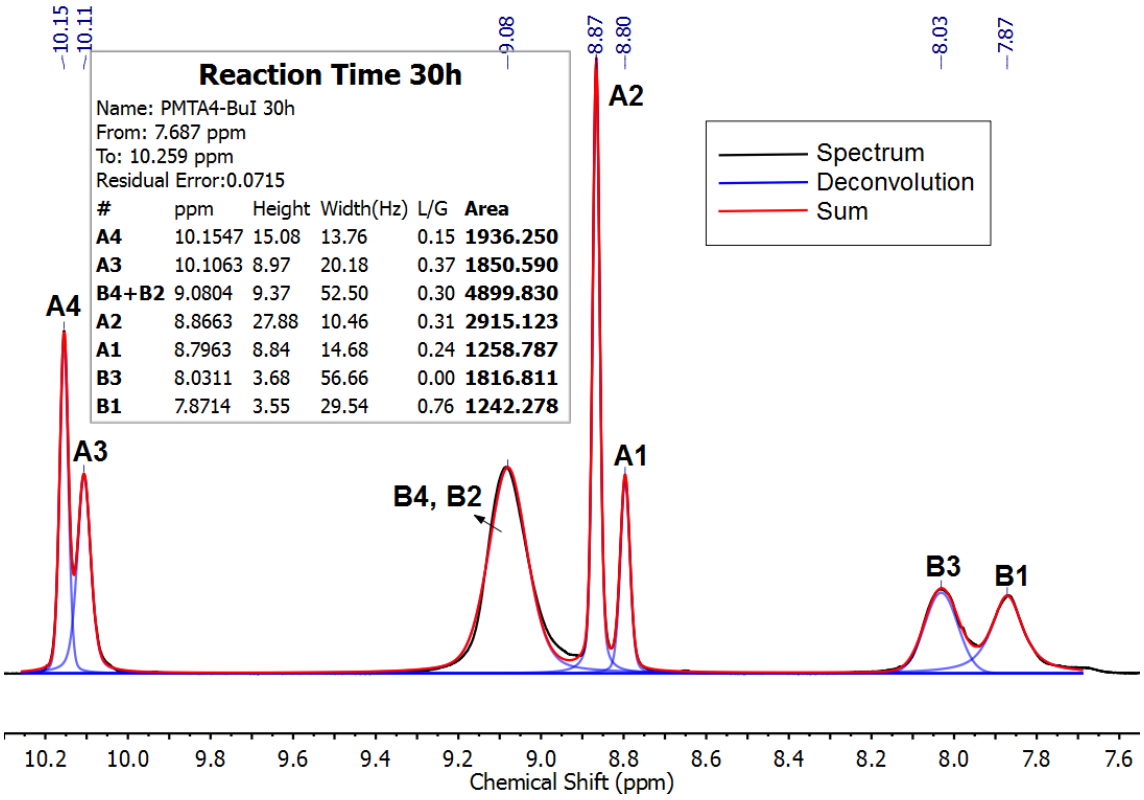
#	ppm	Height	Width(Hz)	L/G	Area
A4	10.1394	8.49	16.20	0.18	**1300.647**
A3	10.0776	11.17	14.17	0.25	**1545.369**
B4+B2	8.9962	8.38	59.00	0.07	**4452.131**
A2	8.8511	45.29	7.22	0.20	**3122.660**
A1	8.7744	39.77	12.09	0.13	**4453.863**
B3	7.9624	4.90	36.48	0.11	**1637.383**
B1	7.8056	13.81	33.12	0.19	**4336.618**

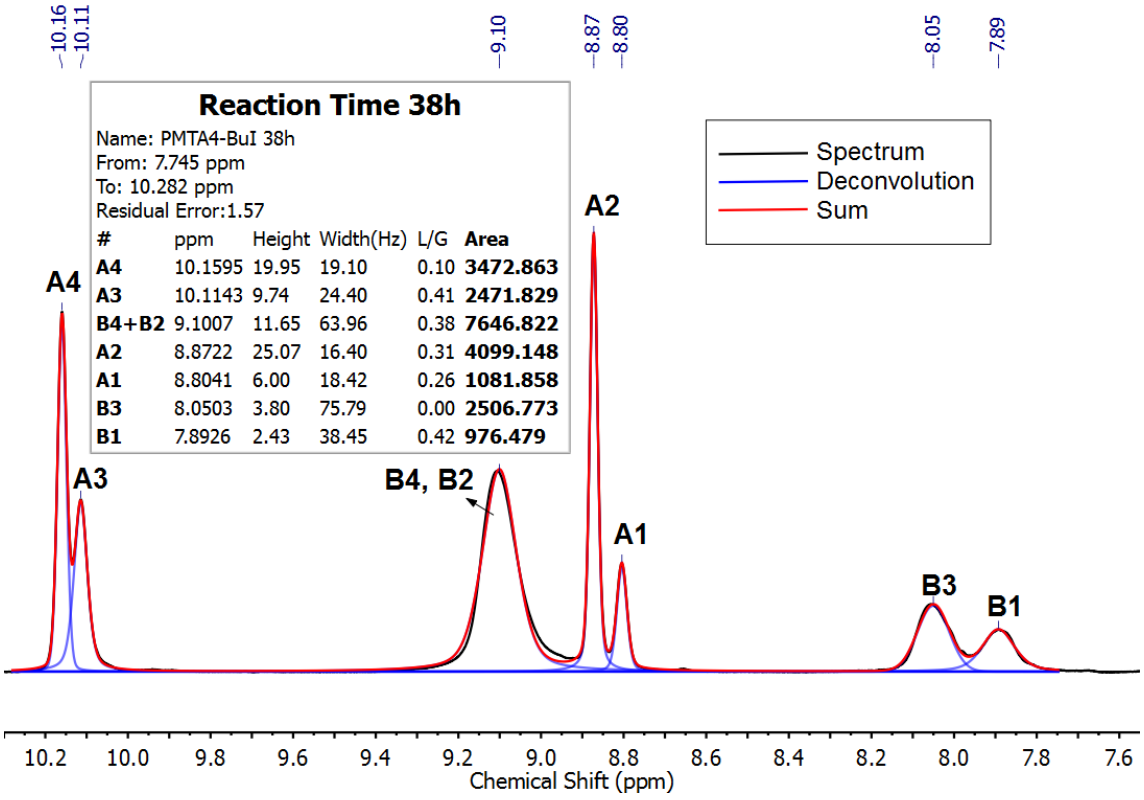


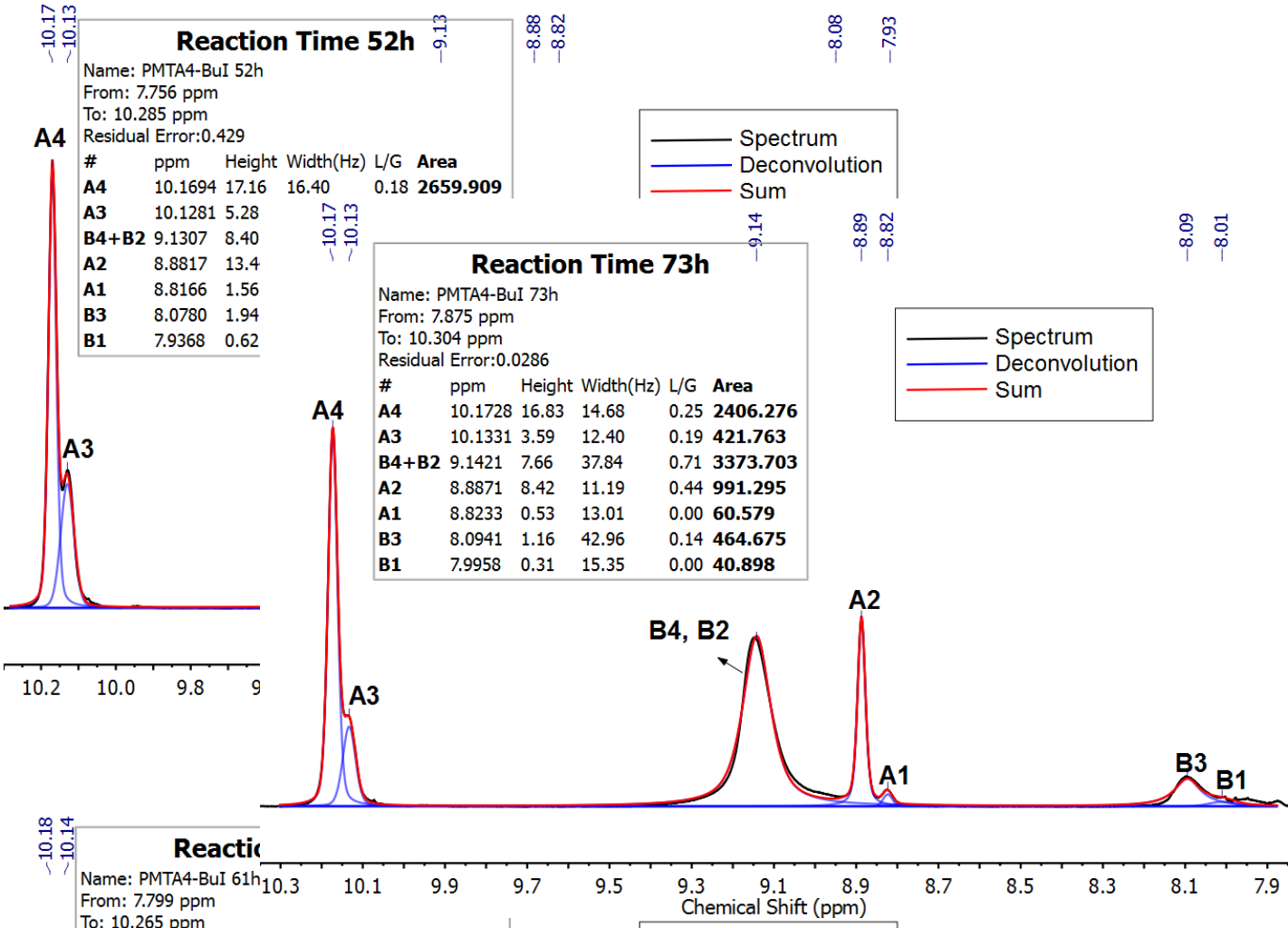
Reaction Time 24h

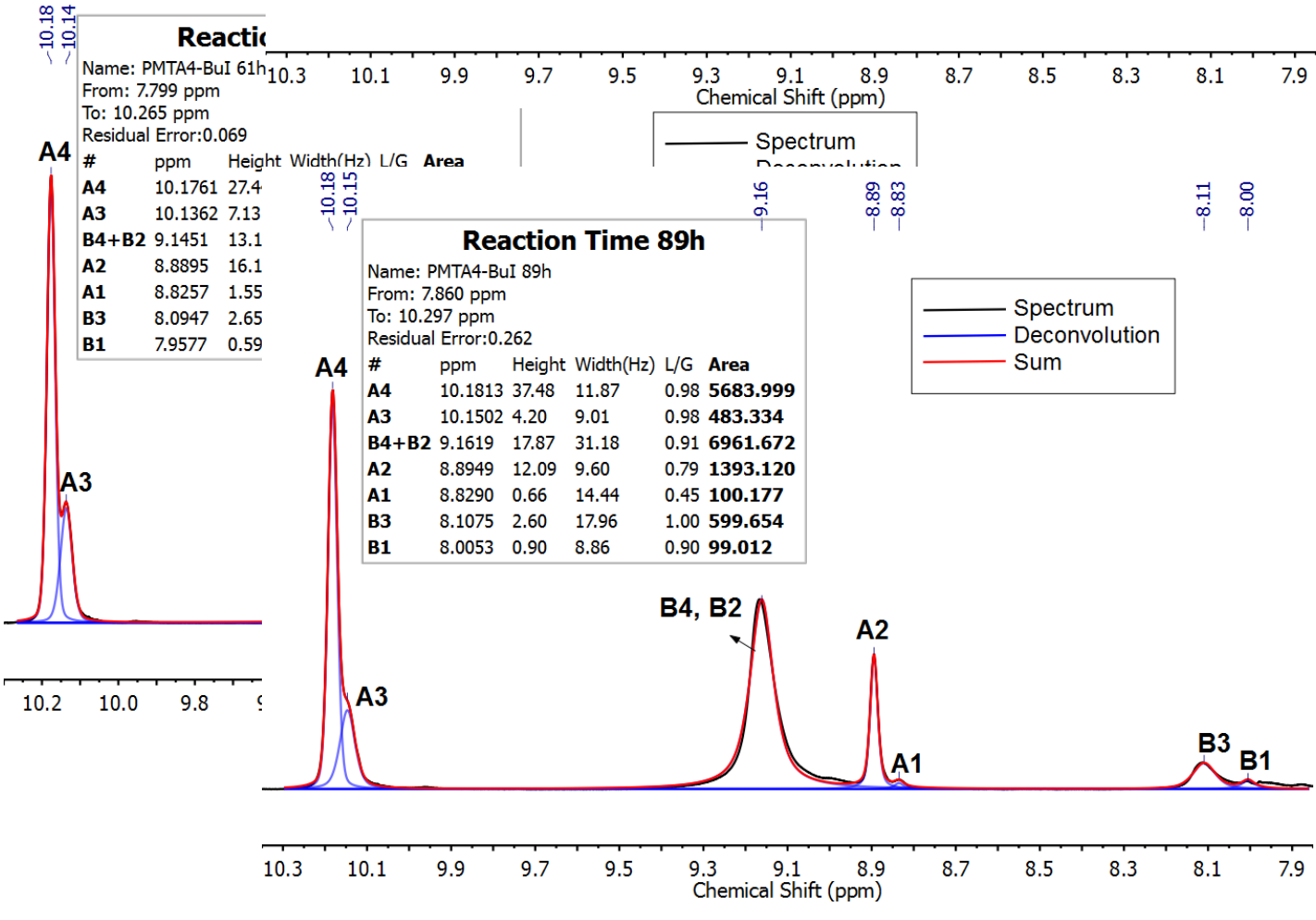
Name: PMTA4-BuI 24h
From: 7.718 ppm
To: 10.268 ppm
Residual Error:0.185

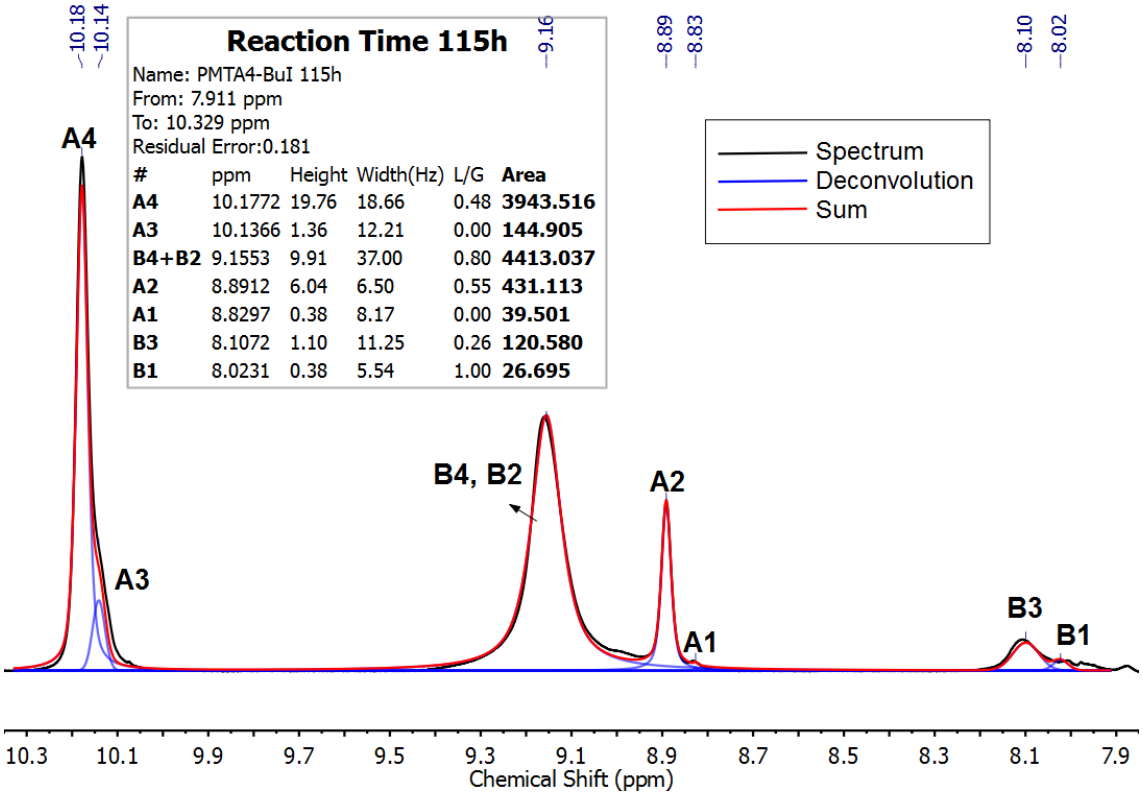
#	ppm	Height	Width(Hz)	L/G	Area
A4	10.1592	13.70	13.63	0.10	**1700.613**
A3	10.1081	10.31	19.26	0.39	**2047.686**
B4+B2	9.0725	9.25	56.74	0.24	**5082.502**
A2	8.8688	32.42	11.36	0.20	**3511.437**
A1	8.7971	14.36	15.84	0.34	**2298.772**
B3	8.0237	4.28	55.72	0.06	**2133.929**
B1	7.8622	5.17	42.81	0.45	**2342.904**


10.15
10.11
9.08
8.87
8.80
8.03
7.87
Reaction Time 30h
Name: PMTA4-BuI 30h
From: 7.687 ppm
To: 10.259 ppm
Residual Error:0.0715
ppm Height Width(Hz) L/G Area
A4 10.1547 15.08 13.76 0.15 1936.250
A3 10.1063 8.97 20.18 0.37 1850.590
B4+B2 9.0804 9.37 52.50 0.30 4899.830
A2 8.8663 27.88 10.46 0.31 2915.123
A1 8.7963 8.84 14.68 0.24 1258.787
B3 8.0311 3.68 56.66 0.00 1816.811
B1 7.8714 3.55 29.54 0.76 1242.278
Spectrum
Deconvolution
Sum
A4
A3
B4, B2
A2
A1
B3
B1
10.2 10.0 9.8 9.6 9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6
Chemical Shift (ppm)


10.16
10.11
9.10
8.87
8.80
8.05
7.89
Reaction Time 38h
Name: PMTA4-BuI 38h
From: 7.745 ppm
To: 10.282 ppm
Residual Error:1.57
ppm Height Width(Hz) L/G Area
A4 10.1595 19.95 19.10 0.10 3472.863
A3 10.1143 9.74 24.40 0.41 2471.829
B4+B2 9.1007 11.65 63.96 0.38 7646.822
A2 8.8722 25.07 16.40 0.31 4099.148
A1 8.8041 6.00 18.42 0.26 1081.858
B3 8.0503 3.80 75.79 0.00 2506.773
B1 7.8926 2.43 38.45 0.42 976.479
Spectrum
Deconvolution
Sum
A4
A3
B4, B2
A2
A1
B3
B1
10.2 10.0 9.8 9.6 9.4 9.2 9.0 8.8 8.6 8.4 8.2 8.0 7.8 7.6
Chemical Shift (ppm)


Reaction Time 52h
Name: PMTA4-BuI 52h
From: 7.756 ppm
To: 10.285 ppm
Residual Error:0.429
ppm Height Width(Hz) L/G Area
A4 10.1694 17.16 16.40 0.18 2659.909
A3 10.1281 5.28
B4+B2 9.1307 8.40
A2 8.8817 13.4
A1 8.8166 1.56
B3 8.0780 1.94
B1 7.9368 0.62
Reaction Time 73h
Name: PMTA4-BuI 73h
From: 7.875 ppm
To: 10.304 ppm
Residual Error:0.0286
ppm Height Width(Hz) L/G Area
A4 10.1728 16.83 14.68 0.25 2406.276
A3 10.1331 3.59 12.40 0.19 421.763
B4+B2 9.1421 7.66 37.84 0.71 3373.703
A2 8.8871 8.42 11.19 0.44 991.295
A1 8.8233 0.53 13.01 0.00 60.579
B3 8.0941 1.16 42.96 0.14 464.675
B1 7.9958 0.31 15.35 0.00 40.898
Spectrum
Deconvolution
Sum
A4
A3
B4, B2
A2
A1
B3
B1
Chemical Shift (ppm)


Reaction Time 89h
Name: PMTA4-BuI 89h
From: 7.860 ppm
To: 10.297 ppm
Residual Error:0.262
ppm Height Width(Hz) L/G Area
A4 10.1813 37.48 11.87 0.98 5683.999
A3 10.1502 4.20 9.01 0.98 483.334
B4+B2 9.1619 17.87 31.18 0.91 6961.672
A2 8.8949 12.09 9.60 0.79 1393.120
A1 8.8290 0.66 14.44 0.45 100.177
B3 8.1075 2.60 17.96 1.00 599.654
B1 8.0053 0.90 8.86 0.90 99.012
Spectrum
Deconvolution
Sum
A4
A3
B4, B2
A2
A1
B3
B1
Chemical Shift (ppm)



5. Integration Data and Species (%) involved During Quaternization Reaction of PMTA4

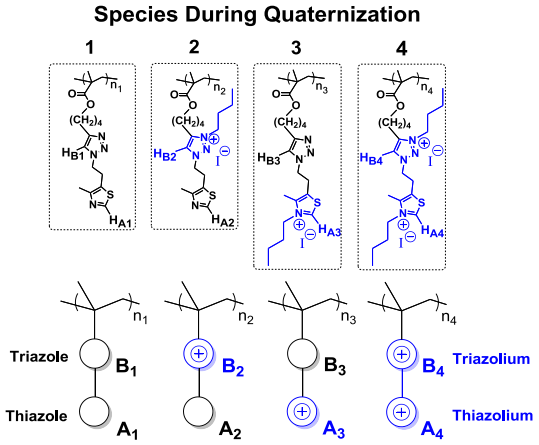


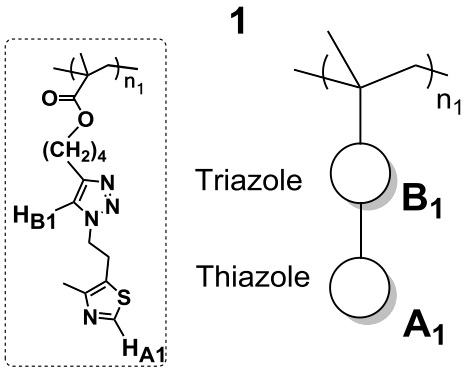
Table S1. Integration Data of Thiazole/Thiazolium protons involved in every species

Reaction Time (h)	Thiazolium A_4	Thiazolium A_3	Thiazole A_2	Thiazole A_1	Global Thiazole $A_4+A_3+A_2+A_1$	DQth (%) $(A_4+A_3)/(A_4+A_3+A_2+A_1)$
0	0	0	0	100	100	0
3	1	556	1234	9436	11227	4.96125
5	327.4	741.4	1627.3	7695.3	10391.4	10.28543
7	256.484	1080.176	2329.05	7552.05	11217.76	11.91557
11	763.965	959.659	1606.561	5049.338	8379.523	20.56948
17	1301.75	1545.75	3123.75	4454.75	10426	27.31153
24	1700	2047	3512	2298	9557	39.20686
30	1936.25	1851.25	2912.25	1258.25	7958	47.59362
38	3472	2474	4100	1082	11128	53.43278
52	2660	1394	2086	247	6387	63.47268
61	3222.193	1231.825	1818.82	93.248	6366.086	69.96478
73	2406.247	421.214	991.295	60.579	3879.335	72.8852
89	5683	483	1394	100	7660	80.49608
115	3945	145	430	40	4560	89.69298
138	100	0	0	0	100	100

Table S2. Integration Data of Triazole/Triazolium protons involved in every species

Reaction Time (h)	Triazolium B_4+B_2	Triazole B_3	Triazole B_1	Global Triazole $B_4+B_2+B_3+B_1$	DQtr (%) $(B_4+B_2)/(B_4+B_2+B_3+B_1)$
0	0	0	100	100	0
3	1216	560	9451	11227	10.83103
5	1900.945	745	7745.529	10391.474	18.29331
7	2589.24	1151.36	7477.16	11217.76	23.08161
11	2427.533	982	4970	8379.533	28.96979
17	4452	1637	4337	10426	42.70094
24	5082	2133	2342	9557	53.17568
30	4900	1816	1242	7958	61.57326
38	7646	2506	976	11128	68.70956
52	4845	1400	142	6387	75.85721
61	5160.4	1138.104	68.03	6366.534	81.05509
73	3374	465.66	40	3879.66	86.96638
89	6961	600	99	7660	90.87467
115	4413	120	27	4560	96.86128
138	100	0	0	100	99

Species 1 (%): Side chain tails with nonquaternized Triazole-Thiazole rings:



The percentage of **Species 1** can be determined following two equivalent equations due its chemical nature:

$$\text{Species 1 (\%)} = \frac{\text{Integral (Triazole } B_1\text{)}}{\text{Integral (Triazolium } B_2 + B_4 + \text{ Triazole } B_3 + B_1\text{)}} \times 100$$

$$\text{Species 1 (\%)} = \frac{\text{Integral (Thiazole } A_1\text{)}}{\text{Integral (Thiazolium } A_4 + A_3 + \text{ Thiazole } A_2 + A_1\text{)}} \times 100$$

In this case, Species 1 (%) determined by integrals of peaks related to thiazole (A_1) or triazole (B_1) protons, respectively, must be in accordance as shown in Table S3 and Figure S3A.

Table S3. Integration Data of Thiazole (A_1) and Triazole (B_1) protons involved in Species 1

Reaction Time (h)	SPECIES 1 (%): Nonquaternized Heterocycles	
	From Thiazole Peak (A_1) $A_1/(A_1+A_2+A_3+A_4)$	From Triazole Peak (B_1) $B_1/(B_1+B_2+B_3+B_4)$
0	100	100
3	84.04739	84.18099
5	74.05451	74.53735
7	67.32226	66.65466
11	60.25806	59.31118
17	42.72732	41.59793
24	24.0452	24.5056
30	15.81113	15.60694
38	9.72322	8.77067
52	3.86723	2.22327
61	1.46476	1.06856
73	1.56158	1.03102
89	1.30548	1.29243
115	0.87719	0.59211
138	0	0

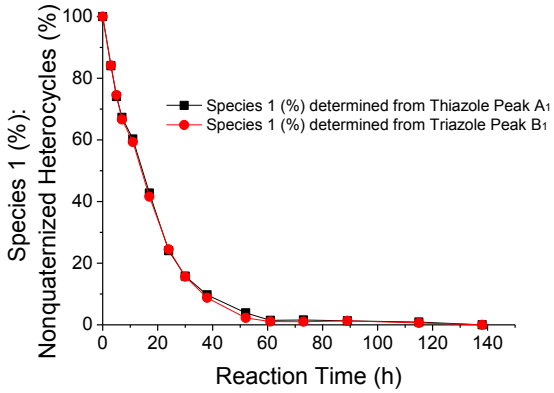
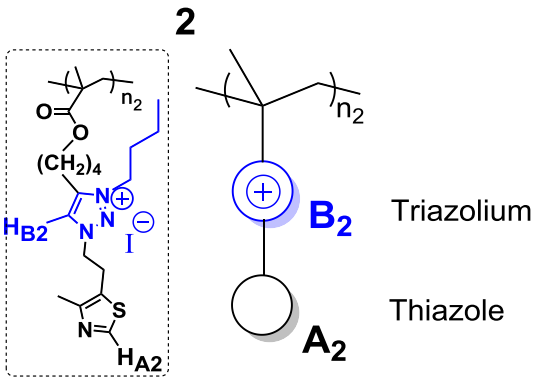


Figure S3A. Species 1 (%) determined from thiazole (A_1) or triazole (B_1) protons, respectively.

- **SPECIES 2 (%): Monocationic Side chain tails with Triazolium-Thiazole rings**



The percentage of monocationic Species 2 can be easily determined by the relationship between the integral corresponding to A_2 proton (in case of thiazole heterocycle) and the rest of thiazole protons or B_2 proton (in case of triazolium ring) and the rest of triazole protons involved applying any of the next two equations:

$$\text{Species 2 (\%)} = \frac{\text{Integral (Triazolium } B_2)}{\text{Integral (Triazolium } B_2 + B_4 + \text{ Triazole } B_3 + B_1)} \times 100$$

$$\text{Species 2 (\%)} = \frac{\text{Integral (Thiazole } A_2)}{\text{Integral (Thiazolium } A_4 + A_3 + \text{ Thiazole } A_2 + A_1)} \times 100$$

In case of triazolium heterocycle, the latter equation cannot be applied since B_2 protons are overlapped with B_4 protons during all quaternization reaction. Anyway, both equations are also equivalent.

Table S4. Integration Data of Thiazole protons involved in Species 2

Reaction Time (h)	SPECIES 2 (%): Monocationic Side Chain Tails with Triazolium-Thiazole Rings (%)
	From Thiazole Peak (A_2) $A_2/(A_1+A_2+A_3+A_4)$
0	0
3	10.99136
5	15.66007
7	20.76217
11	25.57246
17	29.96115
24	33.74793
30	36.59525
38	36.844
52	32.66009
61	28.57046
73	25.55322
89	18.19843
115	9.42982
138	0

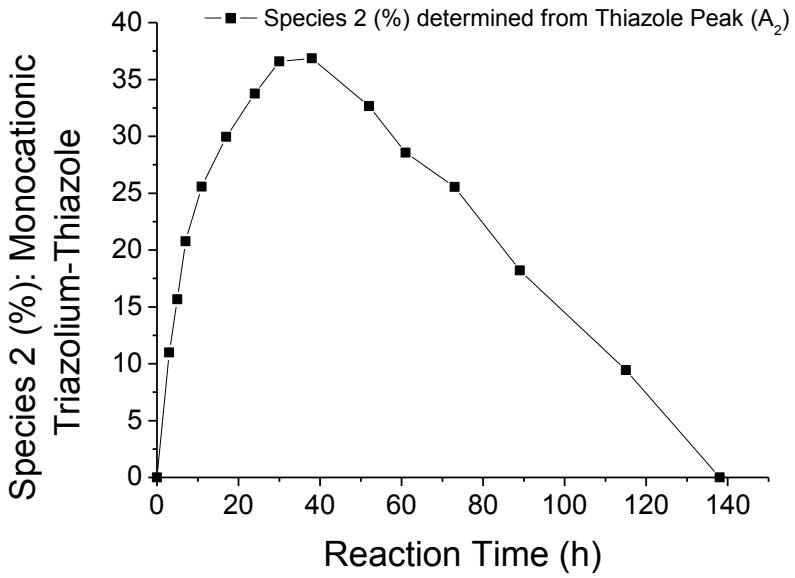
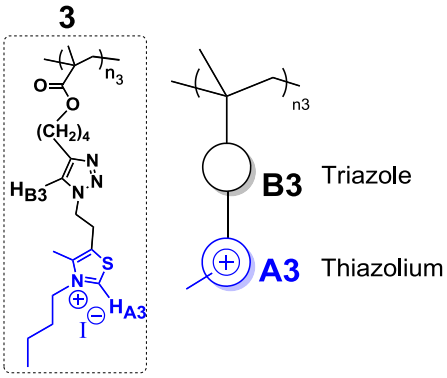


Figure S3B. Species 2 (%) determined from thiazole protons (A_2).

The representation of Species 2 (%) versus reaction time (h) gives as a result a parabolic curve (as can be seen in Figure S3B). This indicates that at the beginning of the reaction the triazole groups are logically quaternized from non-quaternized Species 1 and not from monocationic species due to the more cationic repulsion or steric hindrance evolved.

Therefore, when the quaternization of monocationic triazolium species is complete the proportion of monocationic groups decreases until the end of the reaction given rise to the formation of dicationic ones. This explains the parabolic behaviour.

SPECIES 3 (%): Monocationic side chain Thiazolium-Triazole tails:



The percentage of monocationic side-chain thiazolium and triazole groups in the N-alkylating reaction can be calculated by the relationship between the integrals corresponding to the signals A_3 (for the thiazolium group) and the rest of signals associated to this heterocycles, or alternatively, from the signal B_3 for the triazole group and the rest of signals associated to triazole. The obtained results must be similar considering to any of the heterocycles involved:

$$\text{Species 3 (\%)} = \frac{\text{Integral (Triazole } B_3)}{\text{Integral (Triazolium } B_2 + B_4 + \text{ Triazole } B_3 + B_1)} \times 100$$

$$\text{Species 3 (\%)} = \frac{\text{Integral (Thiazolium } A_3)}{\text{Integral (Thiazolium } A_4 + A_3 + \text{ Thiazole } A_2 + A_1)} \times 100$$

Table S5. Integration Data of Thiazolium and Triazole protons involved in Species 3

Reaction Time (h)	SPECIES 3 (%): Monocationic Thiazolium and Triazole Group	
	From Thiazolium Peak (A_3) $A_3/(A_1+A_2+A_3+A_4)$	From Triazole Peak (B_3) $B_3/(B_1+B_2+B_3+B_4)$
0	0	0
3	4.95235	4.98798
5	7.13475	7.16934
7	9.62916	10.26372
11	13.45243	13.71903
17	14.82592	15.70113
24	21.41886	22.31872
30	23.26275	22.8198
38	22.23221	22.51977
52	21.82558	21.91952
61	19.3498	17.87635
73	10.85789	12.0026
89	6.30548	7.8329
115	3.17982	2.63158
138	0	0

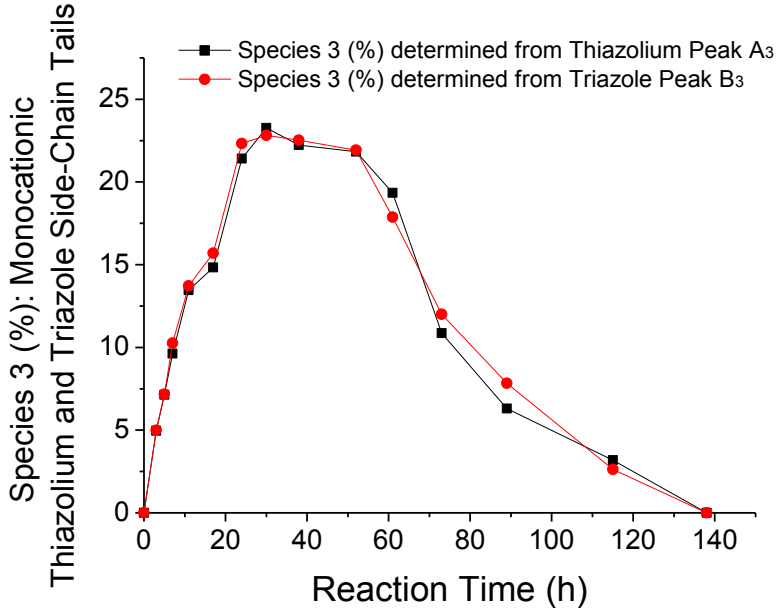
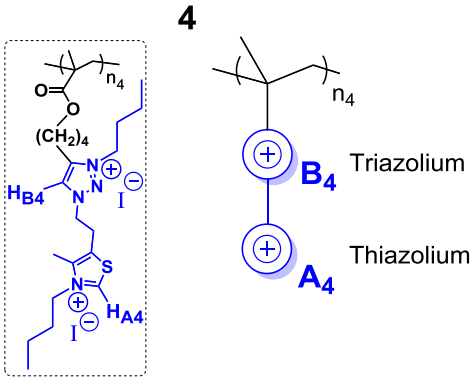


Figure S3C. Species 3 (%) determined from thiazolium (A_3) or triazole (B_3) protons, respectively.

By plotting the Species 3 (%) versus reaction time (h), also appears a parabolic behaviour during the course of the reaction (as can be seen in Figure S3C).

SPECIES 4 (%): Biscationic Side Chain Triazolium and Thiazolium Rings



The percentage of Species 4 related to biscatonic side chain quaternized heterocycles can be easily calculated by the relationship between the integral corresponding to A_4 proton in case of thiazolium heterocycle and the rest of thiazole protons applying next equations:

$$\text{Specie 4 (\%)} = \frac{\text{Integral (Triazolium } B_4)}{\text{Integral (Triazolium } B_2 + B_4 + \text{ Triazole } B_3 + B_1)} \times 100$$

$$\text{Specie 4 (\%)} = \frac{\text{Integral (Thiazolium } A_4)}{\text{Integral (Thiazolium } A_4 + A_3 + \text{ Thiazole } A_2 + A_1)} \times 100$$

In case of triazolium heterocycle, it is not possible to consider B_4 protons because they are overlapped with B_2 protons during all quaternization reaction, so the first equation cannot be applied. Nevertheless, both equations are also equivalent.

Table S6. Integration Data of Thiazolium protons involved in Species 4

Reaction Time (h)	**SPECIES 4 (%): Biscatonic Quaternized Heterocycles Side-Chain Tails**
	From Thiazolium Peak (A_4) $A_4/(A_1+A_2+A_3+A_4)$
0	0
3	0.00891
5	3.15068
7	2.28641
11	9.11705
17	12.48561
24	17.78801
30	24.33086
38	31.20058
52	41.6471
61	50.61498
73	62.02731
89	74.1906
115	86.51316
138	100

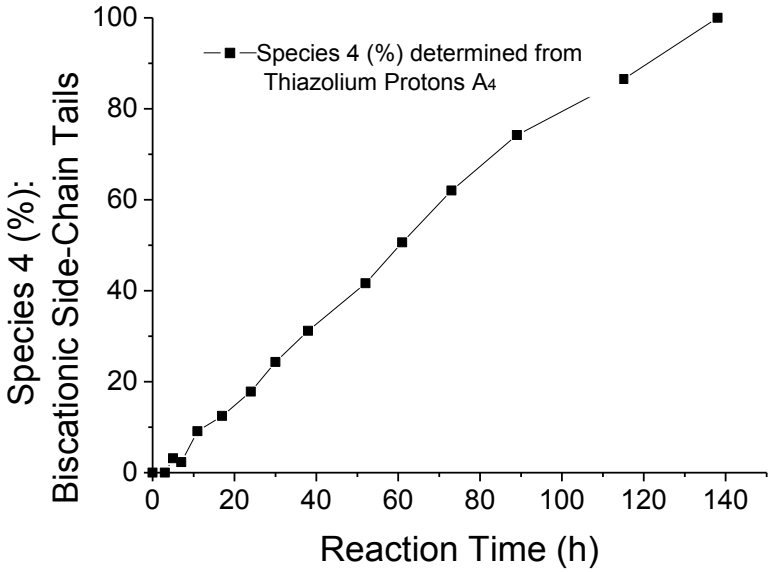


Figure S3D. Species 4 (%) determined from thiazolium protons A_4.

Finally, there are another three more calculations in order to achieve a Confirmatory Analysis of these results presented above.

1) DQth should be determined by two equivalent calculations from thiazolium peaks (A_4 + A_3) or as the sum of the Species 4 (%) (thiazolium peak A_4) and Species 3 (%) (Triazole peak B_3).

Table S7. Integration Data of Thiazolium and Triazole protons involved in the determination of DQth (%)

Reaction Time (h)	**DQth (%)** $(A_4+A_3)/(A_4+A_3+A_2+A_1)$	**SPECIES 4 (%): Biscatonic Side Chain Tails**	**SPECIES 3 (%): Monocationic Thiazolium and Triazole Groups (%)**	**DQth (%) = SPECIES 4 (%) + SPECIES 3 (%)**
		From Thiazolium Peak (A_4) $A_4/(A_1+A_2+A_3+A_4)$	**From Triazole Peak (B_3)** $B_3/(B_1+B_2+B_3+B_4)$	
0	**0**	0	0	**0**
3	**4.96125**	0.00891	4.98798	**4.99688**
5	**10.28543**	3.15068	7.16934	**10.32002**
7	**11.91557**	2.28641	10.26372	**12.55013**
11	**20.56948**	9.11705	13.71903	**20.83608**
17	**27.31153**	12.48561	15.70113	**28.18674**
24	**39.20686**	17.78801	22.31872	**40.10673**
30	**47.59362**	24.33086	22.8198	**47.15067**
38	**53.43278**	31.20058	22.51977	**53.72035**
52	**63.47268**	41.6471	21.91952	**63.56662**
61	**69.96478**	50.61498	17.87635	**68.49133**
73	**72.8852**	62.02731	12.0026	**74.0299**
89	**80.49608**	74.1906	7.8329	**82.0235**
115	**89.69298**	86.51316	2.63158	**89.14474**
138	**100**	100	0	**100**

2) In the same way, DQtr should be determined by two equivalent calculations from triazolium peaks (B_4 + B_2) or as the sum of the Species 4 (%) (thiazolium peak A_4) and Species 2 (%) (Thiazole peak A_2).

Table S8. Integration Data of Triazolium and Thiazolium and Thiazole protons involved in the determination of DQtr (%)

Reaction Time (h)	**DQtr (%)** $(B_4+B_2)/(B_4+B_2+B_3+B_1)$	**SPECIES 4 (%): Biscatonic Side Chain Tails**	**SPECIES 2 (%): Monocationic Triazolium and Thiazole Groups (%)**	**DQtr (%) = SPECIES 4 (%) + SPECIES 2 (%)**
		From Thiazolium Peak (A_4) $A_4/(A_1+A_2+A_3+A_4)$	**From Thiazole Peak (A_2)** $A_2/(A_1+A_2+A_3+A_4)$	
0	**0**	0	0	**0**
3	**10.83103**	0.00891	10.99136	**11.00027**
5	**18.29331**	3.15068	15.66007	**18.81075**
7	**23.08161**	2.28641	20.76217	**23.04858**
11	**28.96979**	9.11705	25.57246	**28.28951**
17	**42.70094**	12.48561	29.96115	**42.44677**
24	**53.17568**	17.78801	33.74793	**54.53594**
30	**61.57326**	24.33086	36.59525	**60.92611**
38	**68.70956**	31.20058	36.844	**68.04457**
52	**75.85721**	41.6471	32.66009	**74.30719**
61	**81.05509**	50.61498	28.57046	**79.18544**
73	**86.96638**	62.02731	25.55322	**87.58053**
89	**90.87467**	74.1906	18.19843	**92.38903**
115	**96.86128**	86.51316	9.42982	**95.94298**
138	**99**	100	0	**100**

3) Similarly, Species 1 (%) should be determined by three equivalent calculations from thiazole peak (A_1) or triazole one (B_1) or as the 100% of species minus the sum of the rest of calculated mono or biscationic Species (%)

Table S9. Integration Data of Thiazole and Triazole protons involved in the determination of DQth (%)

Reaction Time (h)	SPECIES 1 (%): Nonquaternized Heterocycles		SPECIES 1 (%): 100% - The Rest of Species (%)
	From Thiazole Peak (A_1) $A_1/(A_1+A_2+A_3+A_4)$	From Triazole Peak (B_1) $B_1/(B_1+B_2+B_3+B_4)$	
0	**100**	**100**	**100**
3	**84.04739**	**84.18099**	**84.01176**
5	**74.05451**	**74.53735**	**74.01991**
7	**67.32226**	**66.65466**	**66.6877**
11	**60.25806**	**59.31118**	**51.59146**
17	**42.72732**	**41.59793**	**41.8521**
24	**24.0452**	**24.5056**	**26.14534**
30	**15.81113**	**15.60694**	**16.25408**
38	**9.72322**	**8.77067**	**9.43566**
52	**3.86723**	**2.22327**	**3.77329**
61	**1.46476**	**1.06856**	**2.93821**
73	**1.56158**	**1.03102**	**0.41688**
89	**1.30548**	**1.29243**	**-0.22193***
115	**0.87719**	**0.59211**	**1.42544**
138	**0**	**0**	**0**

* In this case, the negative value is not possible but is only write it down to visualize the meaning of determination.

6. Biological Assays.

6.1 Antimicrobial Microbroth Dilution Assay

Bacterial isolates were cultured on 5% sheep blood Columbia agar for 24 h at 37 ºC, after which a microorganism suspension of 2×10^8 CFU/mL in sterile 0.9% saline was prepared to obtain a turbidity equivalent to that of the 0.5-1 McFarland opacity standard and was always used in the next step no later than 15 min. This inoculum suspension was further diluted 1:100 in Mueller-Hinton broth to yield a microorganism suspension of 2×10^6 CFU/mL. Then, each polymer was dissolved in a mixture of autoclaved water and the minimum amount of DMSO (5%, v/v) to make a stock solution of 256 µg/mL in which no precipitate appeared neither DMSO did interfere with measurements. Then, 50 µL of broth were added into all the wells of a sterile 96-well microplate (except in the first column) and 100 µL of monomer or polymer stock solution was pipetted into the first column of wells. Then, 50 µL of polymer solution was diluted by 2-fold serial dilutions in the rest of wells (except in the last column). Briefly, all wells of the broth microdilution plates were inoculated with 50 µL of each test microorganism samples for a total volume of 100 µL to yield the standard density of 5×10^5 CFU/mL and affording final monomer and polymer concentrations of 128, 64, 32, 16, 8, 4, 2, 1, 0.5, 0.25 and 0.125 µg/mL. To ensure that the solvent had no effect on bacterial growth, a negative control test was performed with test medium supplemented with DMSO at the same dilutions used in the experiment. The last column of wells with no polymer was used as positive growth control on each plate. The plates were then incubated at 37 °C in ambient air for 24 h for bacteria, respectively. Plates were read for visual turbidity, and MIC was defined as the lowest monomer or polymer concentration at which visible growth of the microorganism was inhibited. All antimicrobial tests were carried out in triplicate and were also repeated on at least two different days. MICs are reported as the most repeated value with an estimated error of +/- one order of dilution. The results are summarized in **Table S10.**

6.2 Hemolytic Assay

Human Red Blood Cells (RBC), were collected in heparinized-tubes from freshly drawn clinical samples and were centrifuged at 3100 rpm for 10 min and washed three times with cold PBS (pH 7.4) in order to remove plasma and white blood cells. The solution was suspended to 5% (v/v) in the same buffer to yield the RBC stock suspension and was always used within 24 h after collection. Each polymer was dissolved in DMSO/PBS (1:1, v/v, pH 7.4) to afford a stock solution of 20 mg/mL. Then, 50 µL of PBS were added into all the wells of a sterile 96-well microplate (except in the first column) and 100 µL of the polymer stock solution was pipetted into the first column of wells. Then, 50 µL of each polymer solution was diluted by 2-fold serial dilution in the rest of wells (except in the last column). Briefly, RBC

stock (150 µL) was then added to each well for a total volume of 200 µL to obtain the final polymer concentrations of 5000, 2500, 1250, 625, 312.5, 156, 78, 39, 19 and 9.75 µg/mL. Positive control (100% hemolysis) was monitored by adding 50 µL of Triton X-100 solution 1% in PBS (v/v) and 150 µL of RBC stock. Negative control (0% hemolysis) was performed by adding 150 µL of RBC stock in 50 µL of PBS in the last column of wells. To ensure that the solvent had no effect on hemolysis, an extra negative control test was performed with 50 µL of DMSO/PBS (1:1, v/v) and 150 µL of RBC stock. The plate was incubated at 37 °C for 1 h and centrifuged then at 3100 rpm for 10 min to settle the non-lysed cells and 100 µL of supernatant from each well was pipetted out into a new sterile 96-well microplate and the absorbance of wells was measured at 550 nm. Percentage of hemolysis was determined as follows:

Hemolysis (%) = $(A-A_0) / (A_{100}-A_0) \times 100$

where A is the absorbance of the test compound, A_0 the absorbance of the negative control (0% hemolysis) and A_{100} the absorbance of the positive control (100% hemolysis). Hemolysis (%) was plotted against polymer concentration and the concentration of polymer that causes 50% hemolysis (HC_{50}) was estimated by dose-response sigmoidal curve fitting. HC_{50} is reported as the average and standard errors from different experiments performed in triplicates. To quantify the selectivity of **PMTA1-BuI** and **PMTA4-BuI** polymers with different DQ against microorganisms over RBC, the Selectivity Index, defined as HC_{50}/MIC, was determined, which is also summarized in the Table S10. Hemolysis dose-response sigmoidal curves for each polymer are given in Figures S4A and S4B**.**

Table S10. Minimum Inhibitory Concentration (MIC), Selectivity Index (HC_{50}/MIC) and HC_{50} values with standard deviations (SD) of **PMTA1-BuI** and **PMTA4-BuI** at various **DQth.**

PMTA1-BuI DQth (%)	***P. aeruginosa* (ATCC® 27853) MIC (µg/mL)**	**Selectivity Index (HC_{50}/MIC)**	***S. aureus* (ATCC® 29213) MIC (µg/mL)**	**Selectivity Index (HC_{50}/MIC)**	**HC_{50} (µg/mL)**
10	>128	< 9	>128	< 9	1156 ± 234
20	128	12	32	49	1588 ± 183
30	32	76	16	153	2445 ± 311
40	16	194	8	389	3111 ± 260
50	8	431	4	862	3446 ± 310
60	8	528	8	528	4224 ± 203
70	8	> 625	8	> 625	> 5000
80	8	> 625	8	> 625	> 5000
90	8	> 625	8	> 625	> 5000
100	8	> 625	8	> 625	> 5000
PMTA4-BuI DQth (%)	***P. aeruginosa* (ATCC® 27853) MIC (µg/mL)**	**Selectivity Index (HC_{50}/MIC)**	***S. aureus* (ATCC® 29213) MIC (µg/mL)**	**Selectivity Index (HC_{50}/MIC)**	**HC_{50} (µg/mL)**
10	128	20	64	39	2512 ± 252
20	64	43	32	86	2764 ± 266
30	16	159	4	638	2551 ± 103
40	8	456	4	913	3651 ± 210
50	8	515	2	2058	4116 ± 302
60	8	> 625	4	> 1250	> 5000
70	8	> 625	4	> 1250	> 5000
80	8	> 625	4	> 1250	> 5000
90	8	> 625	4	> 1250	> 5000
100	4	> 1250	4	> 1250	> 5000

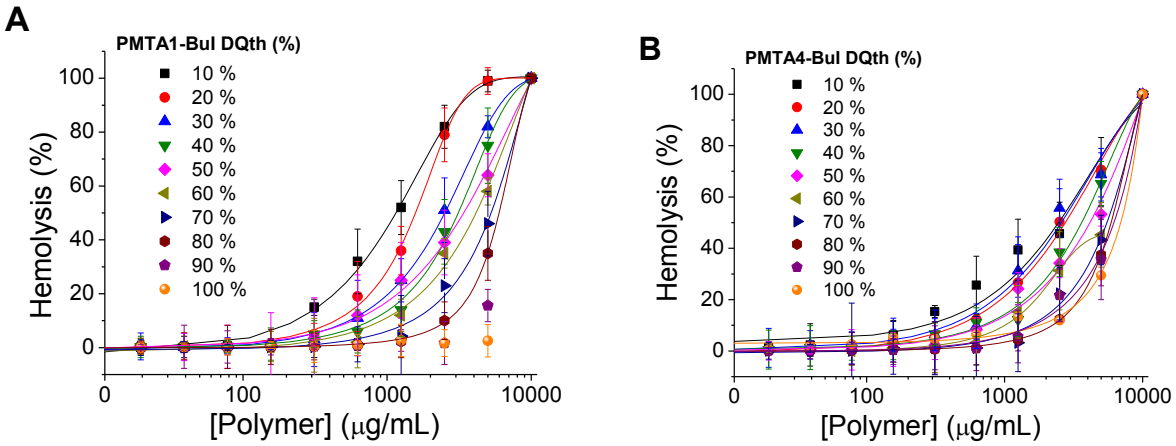


Figure S4. Dose-Response Hemolytic Curves. A) PMTA1-BuI with various DQth (%). B) PMTA4-BuI with various DQth (%).