pharmaceutics

Supplementary materials: Synthesis and Properties of α-Mangostin and Vadimezan Conjugates with Glucoheptoamidated and Biotinylated 3rd Generation Poly(amidoamine) Dendrimer, and Conjugation Effect on Their Anticancer and Anti-Nematode Activities

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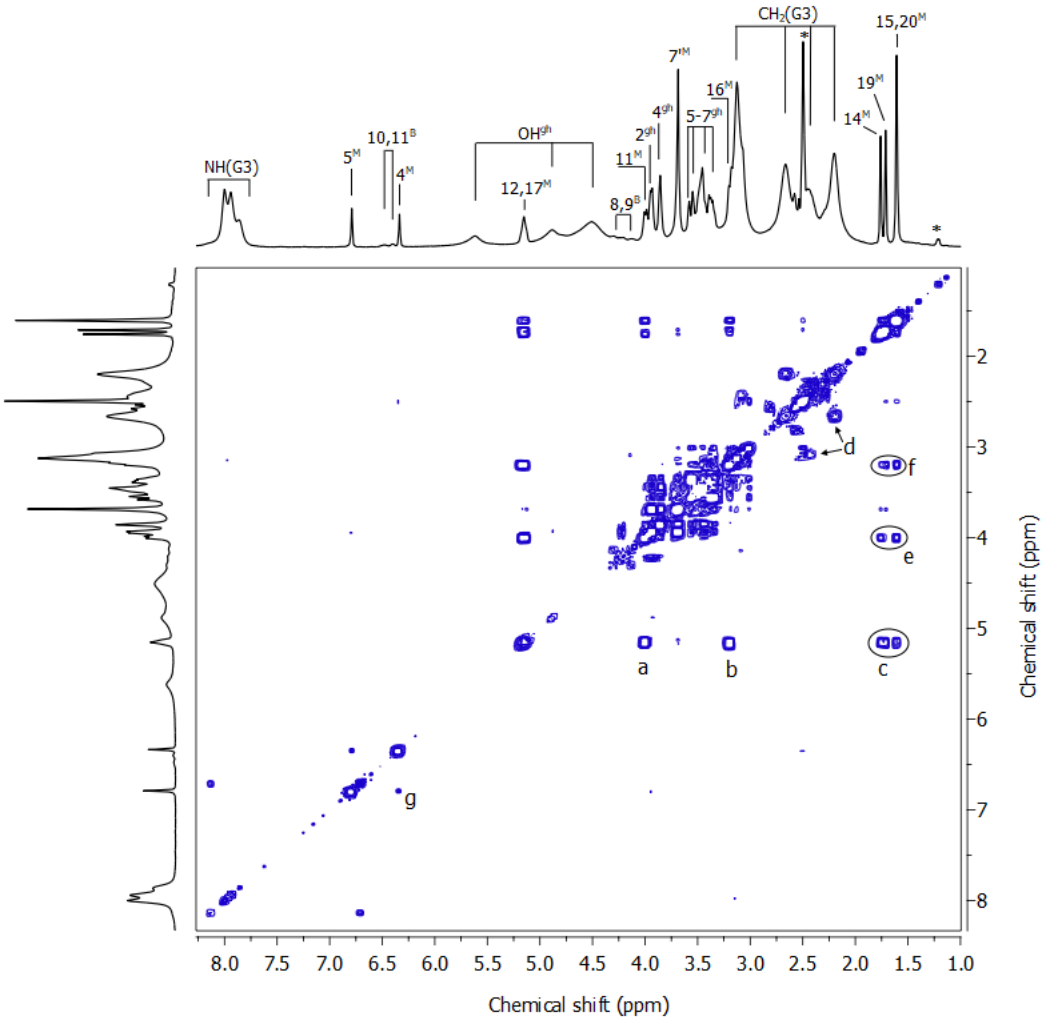

Figure S1. The ^{1}H-^{1}H COSY spectrum of G3^{gh2B5M} in DMSO-d$_6$. The relevant cross-peaks are labeled as follows: a – 11H^{M}/12H^{M}; b – 16H^{M}/17H^{M}; c – a group of scalar coupling peaks between 14H^{M}, 15H^{M}, 19H^{M}, 20H^{M} (methyl group singlets) and 12H^{M}, 17H^{M} (overlapping methylene triplets); d – a group of internal PAMAM G3 cross-peaks; e – 14H^{M},15H^{M}/11H^{M}; f – 19H^{M},20H^{M}/16H^{M}; g – 4H^{M}/5H^{M}. The chemical shifts of ^{1}H peaks are collected in Table S1.

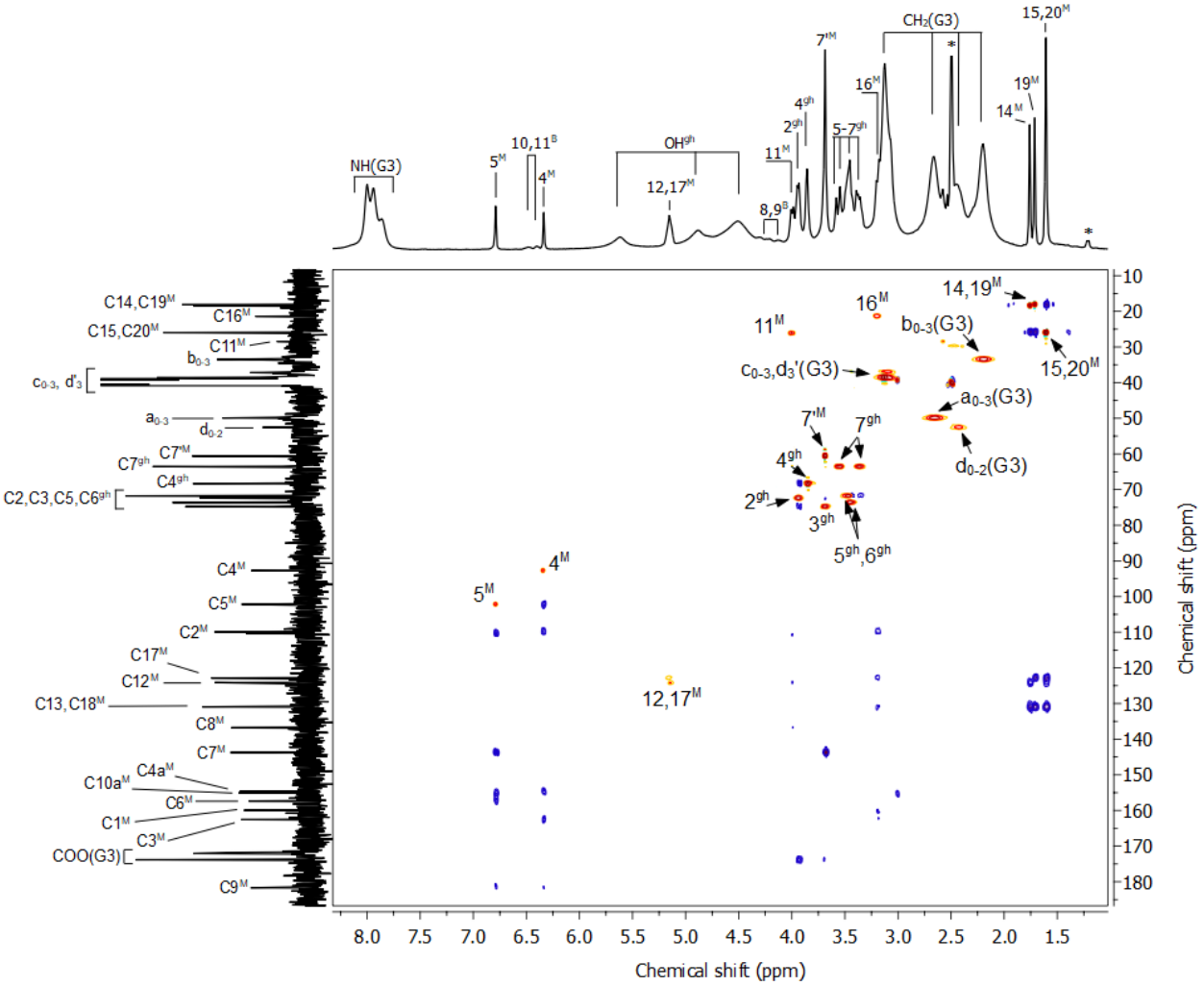

Figure S2. The HSQC/HMBC combined spectra for G3^{gh2B5M} in DMSO-d$_6$. HSQC peaks are marked yellow-red and HMBC peaks are marked blue. The one-bond ^{1}H-^{13}C peaks are labeled with locant and substituents with upper case (^{M}, ^{B}, and ^{gh}), while the peaks within PAMAM G3 arms are labeled according to numbering shown in Fig.2 in [29]. The chemical shifts of ^{1}H and ^{13}C peaks are collected in Table S1.

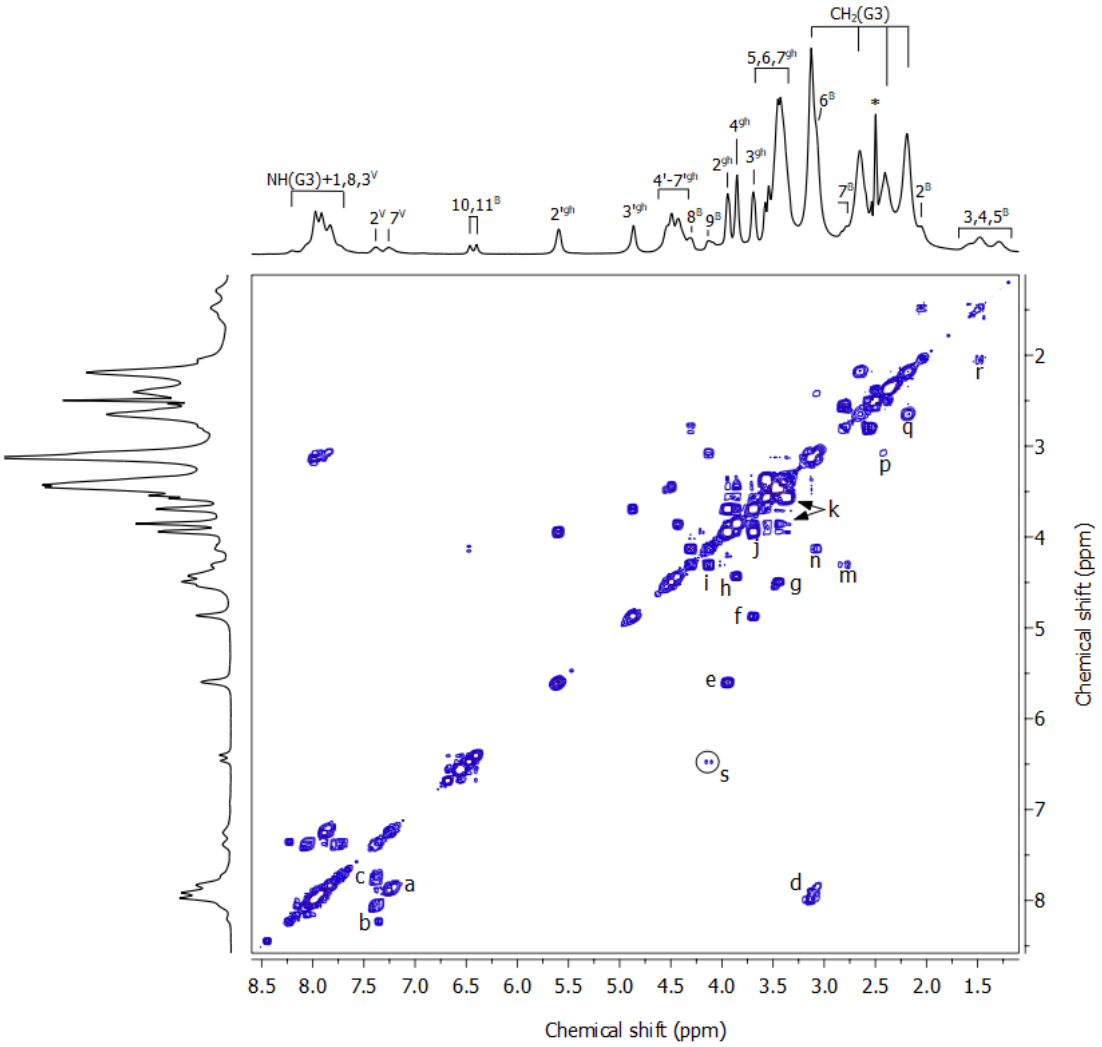

Figure S3. The ^{1}H-^{1}H COSY spectrum of **G3^{gh4B5V}** in DMSO-d$_6$. The relevant cross-peaks are labeled as follows: a – 7H^{V}/8H^{V}; b – 2H^{V}/1H^{V}; c – 2H^{V}/3H^{V}; d – c$_{0\text{-}3}$/NH (internal PAMAM G3 cross-peak; for detailed assignment see Fig. 2 in [29]); e – 2H^{gh}/2′H^{gh}; f – 3H^{gh}/3′H^{gh}; g – cross-peak between group of gh CH protons (5^{gh}, 6^{gh}, 7^{gh}) and group of OH protons (5′^{gh}, 6′^{gh}, 7′^{gh}) in gh; h – 4H^{gh}/4′H^{gh}; i – 9H^{B}/8H^{B}; j – 3H^{gh}/2H^{gh},4H^{gh}; k – 5H^{gh}/4H^{gh}; m – 7H^{B}/8H^{B}; n – 6H^{B}/9H^{B}; p – d$_{0\text{-}2}$/c$_{0\text{-}3}$ and q – b$_{0\text{-}3}$/a$_{0\text{-}3}$ (internal PAMAM G3 cross-peaks); r – 3H^{B}/2H^{B}; s – 9H^{B}/11H^{B}. The chemical shifts of ^{1}H peaks are collected in Table S1.

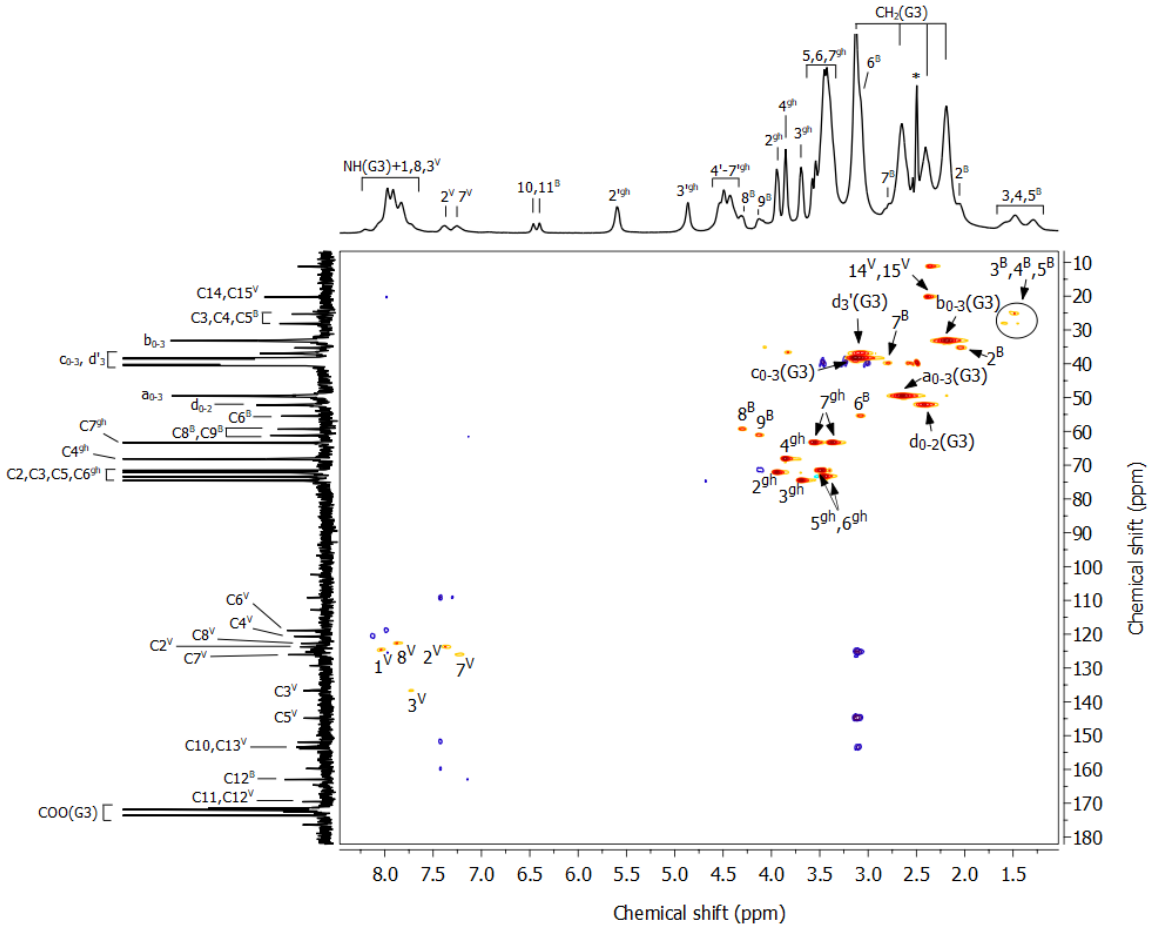

Figure S4. The HSQC/HMBC combined spectra for **G3^{gh4B5V}** in DMSO-d_6. HSQC peaks are marked yellow-red and HMBC peaks are marked blue. The one-bond ^{1}H-^{13}C peaks are labeled with locant and substituents with upper case (^{V}, ^{B}, and ^{gh}), while the peaks within PAMAM G3 arms are labeled according to numbering shown in Fig.2 in [29]. The group of HSQC peaks between 3, 4, and 5 proton nuclei of biotin and corresponding carbon nuclei are grouped within the circle on the map. The chemical shifts of ^{1}H and ^{13}C peaks are collected in Table S1.

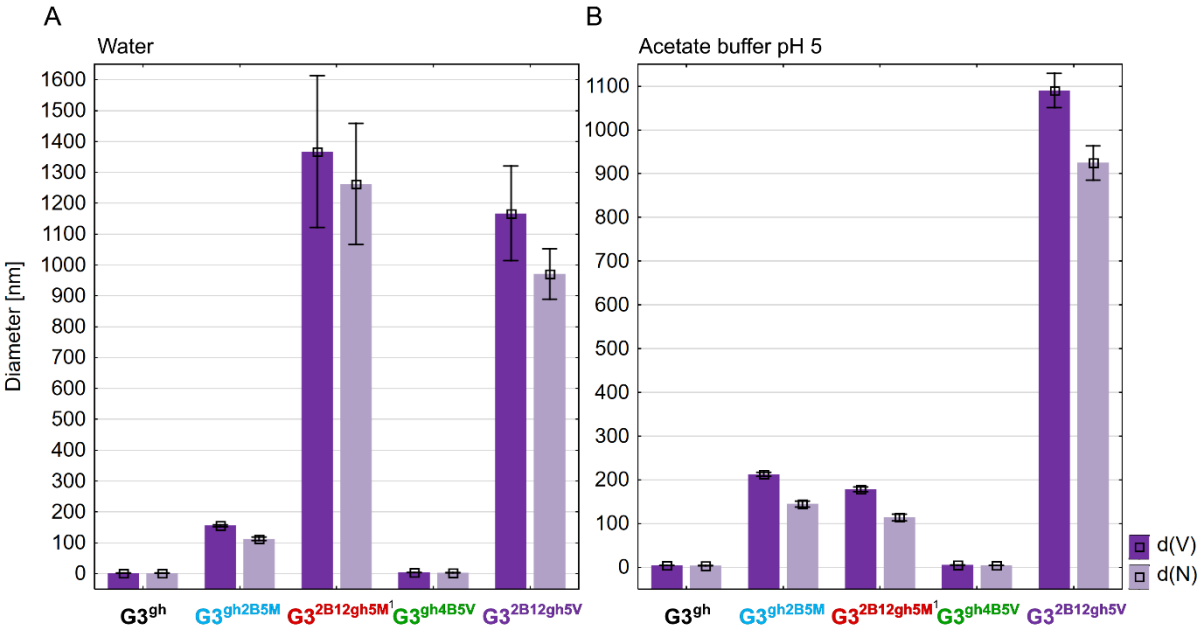

Figure S5. Diameter of conjugates averaged by volume (d(V)) and by number of molecules (d(N)) measured in water (A) and in acetate buffer pH 5 (B). Data are presented as a mean ± standard deviation. [1] Values for $G3^{2B12gh5M}$ were reproduced from [29].

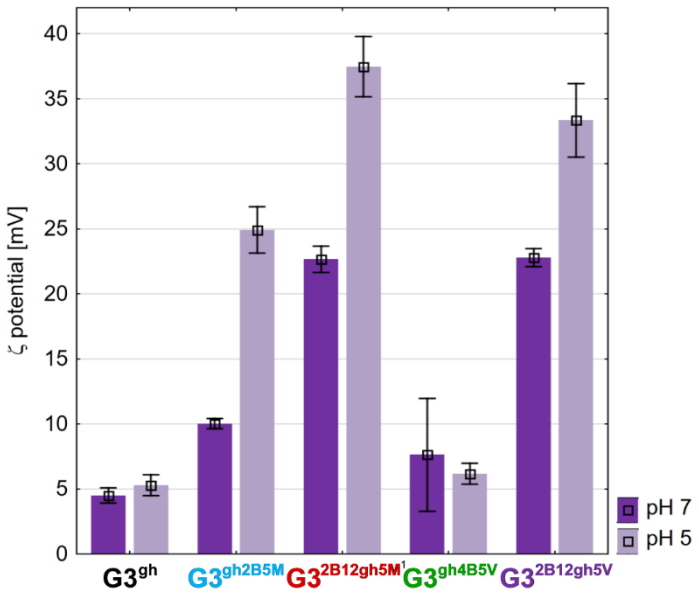

Figure S6. Zeta potential of conjugates measured in water (pH 7) and acetate buffer (pH 5). Data are presented as a mean ± standard deviation. [1] Values for $G3^{2B12gh5M}$ were taken from [29].

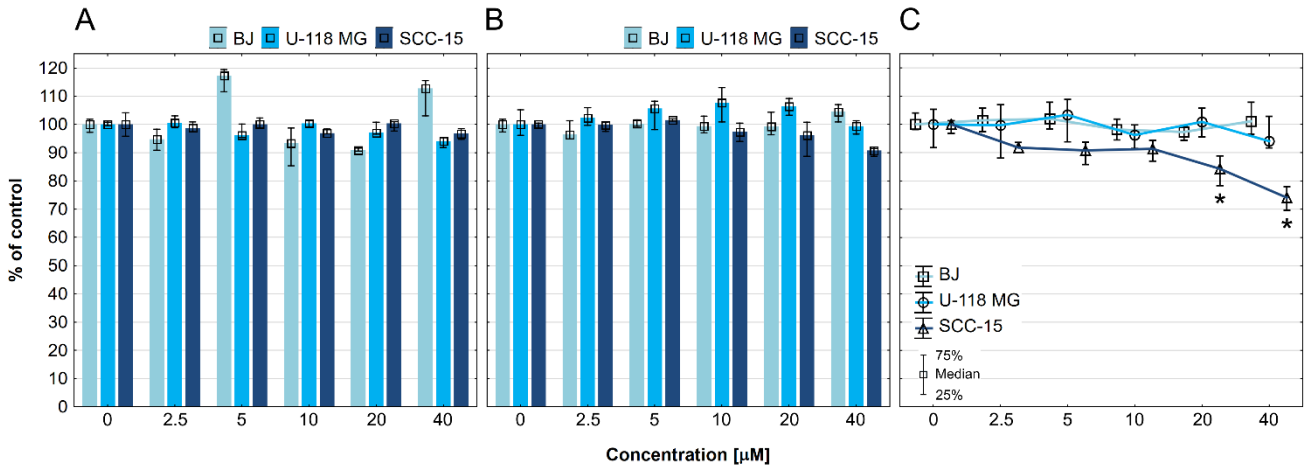

Figure S7. Cytotoxicity of **G3^{gh}** vehicle estimated with NR (**A**) and XTT (**B**) assay after 48 h incubation with normal fibroblasts (BJ), glioblastoma cells (U-118 MG) and squamous carcinoma cells (SCC-15). Anti-proliferative activity (**C**) of **G3^{gh}** against BJ, U-118 MG, and SCC-15 cells after 72 h of incubation, determined with Hoechst 33342 staining. Results are presented as medians of a percent against non-treated control (control expressed as 100%). The whiskers indicate lower (25%) and upper (75%) quartile ranges. * $p \leq 0.05$; Kruskal-Wallis test (against non-treated control).

Table S1. The ^{1}H and ^{13}C resonance assignments of the **G3^{gh4B5V}**, V, **G3^{gh2B5M}** and αM based on COSY and HSQC/HMBC spectra in DMSO-d$_6$. The superscripts: ^{B}, ^{gh}, ^{V} and ^{M} are related to Biotin, glucoheptoamide, Vadimezan and α-Mangostin, respectively. The annotation nd means not determined due to low intensity of resonances or overlapping with high intensity peaks from PAMAM.

Species	G3^{gh4B5V}		Vadimezan		G3^{gh2B5M}		α-Mangostin	
Locant	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C	^{1}H	^{13}C
b$_{0\text{-}3}$(PAMAM)	2.19	33.2			2.20	33.5		
d$_{0\text{-}2}$(PAMAM)	2.42	52.2			2.45	52.6		
a$_{0\text{-}3}$ (PAMAM)	2.65	49.5			2.66	49.9		
d$_3$'(PAMAM)	3.08	37.0			3.07	37.2		
c$_{0\text{-}3}$(PAMAM)	3.13	38.3			3.13	38.6		
-COO(G3)	-	171.5			-	171.9		
-COO(G3)	-	171.8			-	173.8		
NH(G3)	7.98				7.93			
2^{B}	2.05	35.3			nd	nd		
3^{B}, 4^{B}, 5^{B}	1.20-1.65	25.3			1.21	nd		
3^{B}, 4^{B}, 5^{B}	1.20-1.65	28.1						
3^{B}, 4^{B}, 5^{B}	1.20-1.65	28.3						
6^{B}	3.08	55.5			nd			
7^{B}	2.78	nd			nd			
8^{B}	4.31	59.4			4.30	nd		
9^{B}	4.13	61.2			4.12	nd		
10^{B}	6.40	nd			6.40	nd		
11^{B}	6.46	nd			6.48	nd		
12^{B}	-	163.0			-	nd		
1^{gh}	-	172.5			-	nd		
2^{gh}	3.94	72.2			3.94	72.4		
3^{gh}	3.69	74.6			3.69	74.8		
4^{gh}	3.85	68.1			3.86	68.3		
5^{gh} or 6^{gh}	3.46	73.4			3.45	73.7		
5^{gh} or 6^{gh}	3.48	71.6			3.48	71.8		
7^{gh}	3.60-3.30	63.3			3.60-3.30	63.5		
4'^{gh}, 5'^{gh}, 6'^{gh}, 7'^{gh}	4.54-4.43	-			5.62-4.51	-		

3′^{gh}	4.86	-				-		
2′^{gh}	5.60	-				-		
1^{V}	7.98	124.5	8.09	124.6				
2^{V}	7.38	123.7	7.41	123.8				
3^{V}	7.73	136.7	7.79	136.7				
4^{V}	-	120.6	-	120.6				
5^{V}	-	144.8	-	144.8				
6^{V}	-	118.9	-	118.9				
7^{V}	7.26	126.1	7.30	126.1				
8^{V}	7.88	122.7	7.92	122.7				
9^{V}	-	176.5	-	176.3				
10^{V}	-	153.9	-	154.0				
11^{V} or 12^{V}	-	169.6	-	169.3				
13^{V}	-	153.4	-	153.5				
14^{V}, 15^{V}	2.37	20.3	2.41	11.15 20.25				
16^{V}	nd	nd	3.98	35.7				
17^{V}	-	173.6	-	172.0				
1^{M}					-	159.9	-	160.0
1′^{M}					13.73	-	13.72	-
2^{M}					-	109.8	-	109.7
3^{M}					-	162.6	-	162.9
3′^{M}					-	-	11.01	-
4^{M}					6.34	92.7	6.34	92.6
4a^{M}					-	154.5	-	154.5
9a^{M}					-	nd	-	154.9
9^{M}					-	181.7	-	181.2
8a^{M}					-	nd	-	108.0
10a^{M}					-	155.1	-	155.8
5^{M}					6.79	102.1	6.80	101.9
6^{M}					-	157.3	-	161.6
6′^{M}					-	-	10.82	-
7^{M}					-	143.7	-	145.1
7′^{M}					3.69	60.5	3.70	60.2
8^{M}					-	136.7	-	135.6
11^{M}					3.99	26.0	4.01	26.2
12^{M}					5.15	124.1	5.16	124.8
13^{M}					-	130.8	-	130.2
14^{M}					1.76	18.6	1.77	18.4
15^{M}					1.61	26.1	1.62	26.1
16^{M}					3.19	21.4	3.21	21.6
17^{M}					5.15	122.8	5.16	123.3
18^{M}					-	130.9	-	130.6
19^{M}					1.71	18.1	1.72	18.2
20^{M}					1.61	26.0	1.62	26.0