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Supplementary Information

Nano-Detoxification of Organophosphate Agents by PAMAM Derivatives

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PAMAM G5-Folate derivative (G5-FA)

Briefly, 104 mg (0.23 mmol) of FA reacted with 150 mg (0.96 mmol) of EDC·HCl and 150 of HOBt (1.11 mmol) in a mixture of 2.7 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 40 mg (1.38×10^{-3} mmol) of G5 in 3.0 mL of deionized (DI) water. The reaction mixture was vigorously stirred for 72 h. The final functionalized PAMAM was purified through dialysis (using water) membranes with cut-offs of 500 Da to take off the excess of FA. After lyophilization, the weight of G5-FA was 43 mg, affording 90% of yield.

PAMAM G5-Coumarine derivative (G5-Cou)

Briefly, 39.5 mg (0.208 mmol) of Cou reacted with 32 mg (0.206 mmol) of EDC·HCl in a mixture of 2.7 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 40 mg (1.38×10^{-3} mmol) of G5 in 3.0 mL of DI water. The reaction mixture was vigorously stirred for 72 h. The final functionalized PAMAM was purified through dialysis (using water) membranes with cut-offs of 500 Da to take off the excess of Cou. After lyophilization, the weight of G5-Cou was 55 mg affording 92% of yield.

PAMAM G4-Arginine(Tos)-OH (G4-Arg)

Briefly, 64 mg (0.15 mmol) of Boc-Arg(Tos)-OH reacted with 150 mg (0.96 mmol) of EDC·HCl and 150 mg of HOBt (1.1 mmol) in a mixture of 2.7 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 40 mg (2.81×10^{-3} mmol) of G4 in 3.0 mL of DI water. The reaction mixture was vigorously stirred for 72 h. The final functionalized PAMAM was purified through dialysis membranes with cut-offs of 500 Da to take off the excess of amino acids. Finally the product was lyophilized; the weight of Boc-G4-Arg was 70.6 mg, affording 90% of yield.

For a Boc deprotection, a solution of HCl/dioxane (4.0 mL, 4 mol L^{-1}) in a 25-mL round-bottom flask equipped with a magnetic stir-bar was cooled by an ice-water bath under nitrogen. G4-Boc-Arg(Tos)-OH was added in one portion with stirring. The ice-bath was removed and the mixture was kept stirred. After 2 h, the reaction was complete as determined by MALDI. The reaction mixture was condensed by rotary evaporation under high vacuum at room temperature. The residue was then washed with dry ethyl ether and collected by filtration,[1] the weight of G4-Arg was 65.96 mg, affording 95% yield of G4-Arg.

PAMAM G4-Lysine(Z)-OH (G4-Lys)

Briefly, 56 mg (0.147 mmol) of protected Lys reacted with 100 mg (0.64 mmol) of EDC·HCl and 100 mg of HOBt (0.74 mmol) in a mixture of 2.9 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 30 mg (2.1×10^{-3} mmol) of G4 in 3.0 mL of DI water. The reaction mixture was vigorously stirred

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for 72 h. The final functionalized PAMAM was purified through dialysis membranes with cut-offs of 500 Da to take off the excess of amino acids. After lyophilization, the weight of G4-Boc-Lys(Z)-OH was 71 mg affording 90% of yield. For a Boc deprotection, a solution of HCl/dioxane (4.0 mL, 4 mol L^{-1}) in a 25-mL round-bottom flask equipped with a magnetic stir-bar was cooled by an ice-water bath under nitrogen. G4-Boc-Lys(Z)-OH was added in one portion with stirring. The ice-bath was removed and the mixture was kept stirred. After 2 h, the reaction was complete as determined by MALDI. The reaction mixture was condensed by rotary evaporation under high vacuum at room temperature. The residue was then washed with dry ethyl ether and collected by filtration,[1] the weight of G4-Lys was 62.6 mg, affording 92% of G4-Lys.

PAMAM G4-Asn(Trt)-OH) (G4-Asn)

236.6 mg (0.49 mmol) of protected Asn reacted with 120 mg (0.77 mmol) of EDC·HCl and 120 of HOBt (0.49 mmol) in a mixture of 2.7 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 100 mg (7×10^{-3} mmol) of G4 in 5.0 mL of DI water. The reaction mixture was vigorously stirred for 72 h. The final functionalized PAMAM was purified through dialysis membranes with cut-offs of 500 Da to take off the excess of amino acids. After lyophilization, the weight of PAMAM-Boc-Asn(Trt)-OH was 242 mg affording 94% of G4-Asn. For a Boc deprotection, a solution of HCl/dioxane (7.0 mL, 4 mol L^{-1}) in a 25-mL round-bottom flask equipped with a magnetic stir-bar was cooled by an ice-water bath under nitrogen. G4-Boc-Asn (Trt)-OH was added in one portion with stirring. The ice-bath was removed and the mixture was kept stirred. After 2 h, the reaction was complete as determined by MALDI. The reaction mixture was condensed by rotary evaporation under high vacuum at room temperature. The residue was then washed with dry ethyl ether and collected by filtration,[1] the weight of G4-Lys was 234.1 mg, affording 95% of G4-Asn.

PAMAM G4-Arg(Tos)-OH/Lys(Z)-OH (PAMAM G4-Arg/Lys)

Briefly, 7.5 mg (0.018 mmol) of protected Arg reacted with 2.7 mg (0.018 mmol) of EDC·HCl and 2.3 mg of HOBt (0.018 mmol) in a mixture of 4.0 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 50 mg (3.5×10^{-3} mmol) of G4 in 3.0 mL of DI water. The reaction mixture was vigorously stirred for 72 h. The final functionalized PAMAM was purified through dialysis membranes with cut-offs of 500 Da to take off the excess of amino acids. After lyophilization, the weight of G4-Boc-Arg(Tos)-OH was 58 mg, affording 91% of yield. Then, the conjugation of G4/Boc-Arg(Tos)-OH with Boc-Lys(*Z*)-OH followed a previously described method. Briefly, 6.3 mg (0.017 mmol) of protected Lys reacted with 2.4 mg (0.017 mmol) of EDC·HCl and 2.2 mg of HOBt (0.017 mmol) in a mixture of 4.0 mL of dry DMF and 1.0 mL of dry DMSO under a nitrogen atmosphere for 1 h. The reaction mixture was slowly added dropwise to a solution of 58 mg (3.3×10^{-3} mmol) of G4-Arg in 3.0 mL of DI water. The reaction mixture was vigorously stirred for 72 h. The final functionalized PAMAM was purified through dialysis membranes with cut-offs of 500 Da to take off the excess of amino acids. After lyophilization, the weight of G4 with Boc-Arg(Tos)-OH and Boc-Lys(*Z*)-OH was 61 mg, affording 90% of yield. For a Boc deprotection, a solution of HCl/dioxane (7.0 mL, 4 mol L^{-1}) in a 25-mL round-bottom flask equipped with a magnetic stir-bar was cooled by an ice-water bath under nitrogen. G4 with Boc-Arg(Tos)-OH and Boc-Lys(*Z*)-OH was added in one portion with stirring. The ice-bath was removed and the mixture was kept stirred. After 2 h, the reaction was complete as determined by MALDI. The reaction mixture was condensed by rotary evaporation under high vacuum at room temperature. The residue was then washed with dry ethyl ether and collected by filtration,[1] the weight of G4 Arg(Tos)-OH/-Lys(Z)-OH (G4-Arg/Lys) was 51 mg, affording 88% of yield.

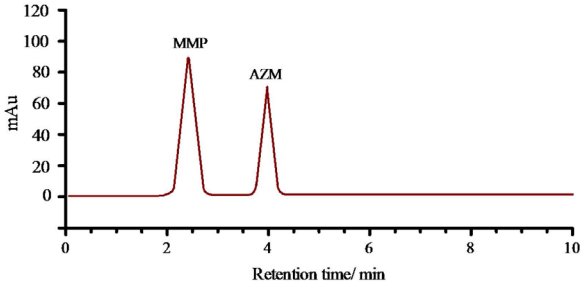

Figure S1. Chromatogram of MMP and AZM through HPLC (230 nm) by using a C-18 column.

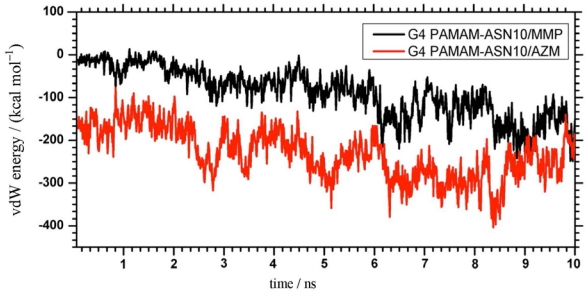

Figure S2. vdW energy calculated for PAMAM-MMP (black) and PAMAM-AZM (red) throughout the MS simulations.

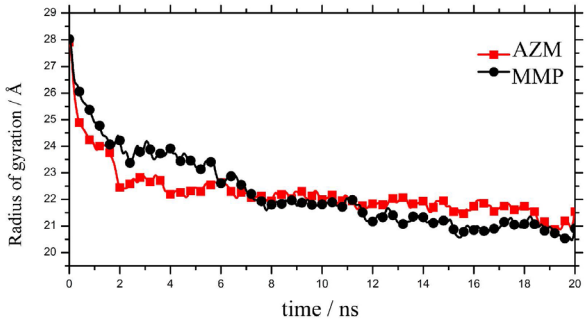

Figure S3. Radius of gyration for two systems involving G4-Asn$_{10}$ with MMP (red), as well as G4-Asn$_{10}$ with AZM (black) molecules.

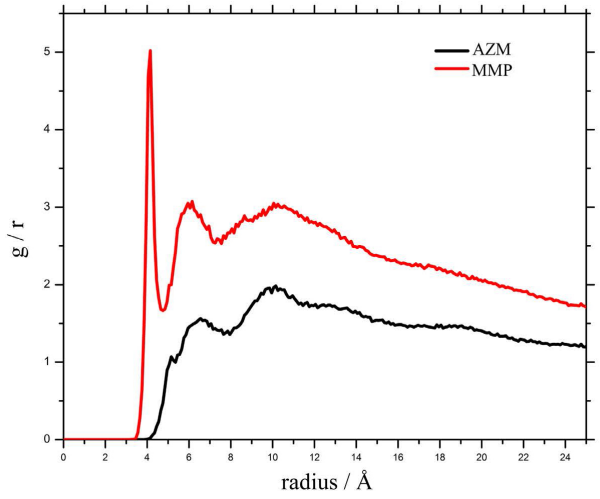

Figure S4. RDF of G4-Asn$_{10}$ with MMP (red) and AZM (black) molecules, respectively. RDF shows radius r = 4 Å as the distance from which the highest number of MMP molecules interact with functionalized PAMAM.

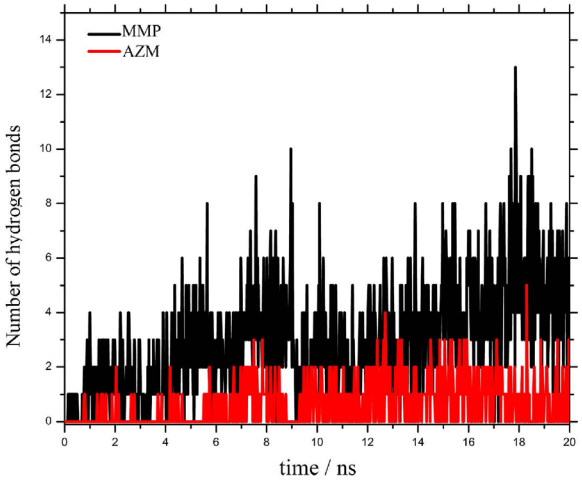

Figure S5. Number of hydrogen bonds for MMP (black) and AZM (red) molecules interacting with G4-Asn$_{10}$ as function of time. Note that after 18 ns the system reached the equilibrium showing more hydrogen bond interactions with MMP compared with AZM.

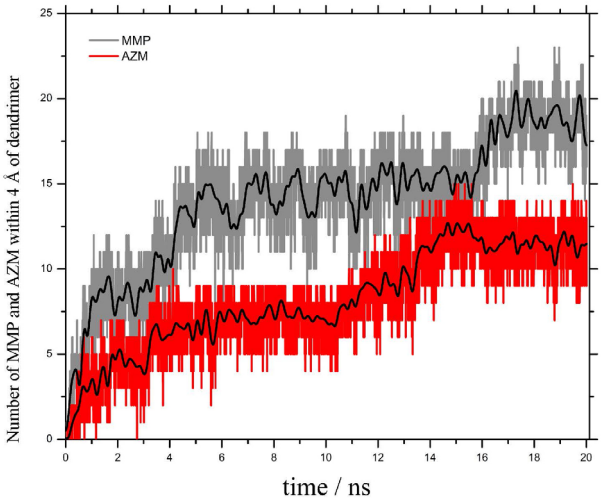

Figure S6. Number of molecules within 4 Å of G4-Asn10 for MMP (gray) and AZM (red), as function of time. The snapshots show a representation of the process of capture of MMP (a) and AZM (b). For further snapshots of the process, see the theoretical video in Supplemental Information section.

Video: supplementary data of snapshots representing the process of capture of MMP and AZM are available free of charge at http://jbcs.sbq.org.br as MOV file: PAMAMG4_ASN10.mov (9565K).

References

1. Durán-Lara, E.; Guzmán, L.; John, A.; Fuentes, E.; Alarcón, M.; Palomo, I.; Santos, L. S.; *Eur. J. Med. Chem.* **2013**, *69*, 601.