Electronic Supporting Information

Synthesis of water-soluble polyester-based dendrimer prodrugs for exploiting therapeutic properties of two triterpenoid acids

Silvana Alfei*,†, Gaby Brice Taptue†, Silvia Catena† and Angela Bisio

Dipartimento di Farmacia, Sezione di Chimica e Tecnologie Farmaceutiche e Alimentari

Università di Genova, Viale Cembrano 4, I-16148 Genova, Italy

Table of Contents

*Corresponding Author: E-mail: alfei@difar.unige.it
Tel: (+) 39-010353-2296
Fax: (+) 39-010353-2684
ORCID: 0000-0002-4630-4371

† These authors equally contributed to this work.

Section S1 (in round parenthesis the peripheral composition in amino acids and free OH is reported)

Dendrimer G4[Arg(36)OH(12)] (1) [1]

Very hygroscopic fluffy solid, 68% isolated yield. FTIR (KBr, cm^{-1}): 3364 (NH_3^+), 1741 (C=O ester), 1653 (NH).^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.79-1.40 (m, 138H, CH_3 of dendrimer), 1.40-2.02 (m, 144H, CH_2CH_2 Arg), 3.21 (m, 72H, CH_2NH Arg), 3.34-3.62 (m, 24H, CH_2OH), 3.62-4.71 [m, 210H (CH_2O of dendrimer + $CHNH_3^+$Arg + 12OH)], 8.07 and 8.75 [br, 288H ($^{\delta}NH$ + $^{\omega'}NH_2^+$ + $^{\omega}NH_2$ + $^{\alpha}NH_3^+$ Arg)].

Dendrimer G4[ArgGly(29)OH(19)] (2)[1]

Very hygroscopic fluffy solid, 74% isolated yield. FTIR (KBr, cm^{-1}): 3412 (NH_3^+), 1739 (C=O ester), 1638 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.74-1.38 (m, 138H, CH_3 of dendrimer), 1.42-1.98 (m, 116H, CH_2CH_2 Arg), 3.21 (m, 58H, CH_2NH Arg), 3.30-3.59 (m, 38H, CH_2OH), 3.77-4.45 [m, 235H (CH_2O of dendrimer + $CHNH_3^+$ Arg + CH_2NH Gly)], 4.70 and 5.06 (br, 19H, OH), 7.56-9.51 [m, 261H (NH Gly + $^{\delta}NH$ + $^{\omega'}NH_2^+$ + $^{\omega}NH_2$ + $^{\alpha}NH_3^+$ Arg)].

Dendrimer G4[Arg(16)Lys(19)OH(13)] (3) [1]

Very hygroscopic fluffy solid, 69% isolated yield. FTIR (KBr, cm^{-1}): 3431 (NH_3^+), 1744 (C=O ester), 1628 (NH).^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90-2.07 [m, 316H (CH_3 of dendrimer + CH_2CH_2 Arg + $CH_2CH_2CH_2$ Lys)], 2.76 (m, 38H, $CH_2NH_3^+$ Lys), 3.21 (m, 32H, CH_2NH Arg), 3.54-3.82 (m, 26H, CH_2OH), 3.80-4.80 [m, 208H (CH_2O of dendrimer + $CHNH_3^+$ Lys + $CHNH_3^+$ Arg + 13OH)], 7.60-9.20 [m, 242H ($^{\delta}NH$ + $^{\omega'}NH_2^+$ + $^{\omega}NH_2$ + $^{\alpha}NH_3^+$ Arg and $^{\alpha}NH_3^+$ + $^{\varepsilon}NH_3^+$ Lys)].

Dendrimer G5[Arg(66)OH(30)] (4) [1]

Very hygroscopic fluffy solid, 46% isolated yield. FTIR (KBr, cm^{-1}): 3402 (NH_3^+), 1750 (C=O ester), 1652 (NH).^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.79-1.39 (m, 282H, CH_3 of dendrimer), 1.47-2.00 (m, 264H, CH_2CH_2 Arg), 3.21 (m, 132H, CH_2NH Arg), 3.47 (br, 60H, CH_2OH), 3.80-4.71 [m, 414H (CH_2O of dendrimer + $CHNH_3^+$ Arg + 30 OH)], 7.81 and 9.55 [m, 528H ($^{\delta}NH$ + $^{\omega'}NH_2^+$ + $^{\omega}NH_2$ + $^{\alpha}NH_3^+$ Arg)].

Dendrimer G5[ArgGly(52)OH(44)] (5)[1]

Very hygroscopic off white fluffy solid, 71% isolated yield. FTIR (KBr, cm^{-1}): 3412 (NH_3^+), 1740 (C=O ester), 1638 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.76-1.40 (m, 282H, CH_3 of dendrimer), 1.40-1.98 (m, 208H, CH_2CH_2 Arg), 3.20 (m, 104H, CH_2NH Arg), 3.33-3.61 (m, 88H, CH_2OH), 3.70-4.49 [m, 490H (CH_2O of dendrimer + $CHNH_3^+$ Arg + CH_2NH Gly + 29 OH)], 4.91 (br, 15H, OH), 7.22-9.93 [m, 468H (NH Gly + $^{\alpha}NH_3^+$ + $^{\delta}NH$ + $^{\omega'}NH_2^+$ + $^{\omega}NH_2$ Arg)].

Dendrimer G5[Arg(38)Lys(30)OH(28)] (6) [1]

Very hygroscopic pale yellow fluffy solid, 51% isolated yield. FTIR (KBr, cm^{-1}): 3411 (NH_3^+), 1743 (C=O ester), 1631 (NH). ^{1}H NMR (DMSO-d_6, 300 MHz): δ 0.90-2.05 [m, 614H (CH_3 of dendrimer + CH_2CH_2 Arg + $CH_2CH_2CH_2$ Lys)], 2.75 (m, 60H, $CH_2NH_3^+$ Lys), 3.18 (m, 76H, CH_2NH Arg), 3.39-3.55 (m, 56H, CH_2OH), 3.80-4.70 [m, 418 H (CH_2O of dendrimer + $CHNH_3^+$ Lys + $CHNH_3^+$ Arg + 28 OH)], 7.60-9.20 [m, 484H ($^{\delta}NH$ + $^{\omega'}NH_2^+$ + $^{\omega}NH_2$ + $^{\alpha}NH_3^+$ Arg and $^{\alpha}NH_3^+$ + $^{\varepsilon}NH_3^+$ Lys)].

^{1}H NMR (CD_3OD/DMSO-d_6, 300 MHz)

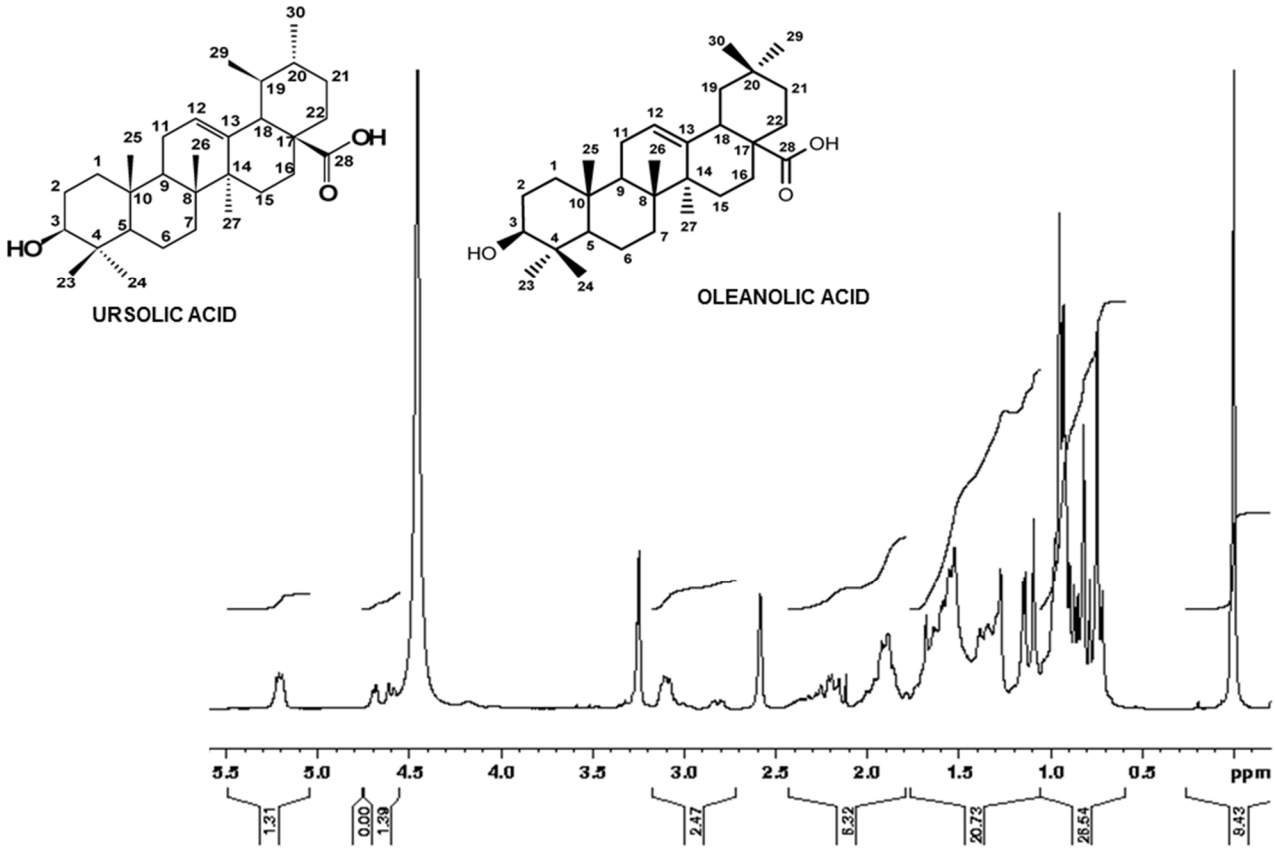


Fig. S2 ^{1}H NMR spectrum of the Ursolic and Oleanolic acids mixture extracted by Salvia Corrugata Vahl[2]

References

1 Alfei, S.; Castellaro, S. Synthesis and characterization of polyester-based dendrimers containing peripheral arginine or mixed amino acids as potential vectors for gene and drug delivery. Macromol. Res. 2017, DOI 10.1007/s13233-017-5160-3

2 Bisio, A.; Romussi, G.; Russo, E.; Cafaggi, S.; Schito, A. M.; Repetto, B.; De Tommasi, N. Antimicrobial Activity of the Ornamental Species Salvia corrugata, a Potential New Crop for Extractive Purposes. J. Agric. Food Chem. 2008, 56, 10468-10472

^{1}H NMR (CD_3OD, 300 MHz)

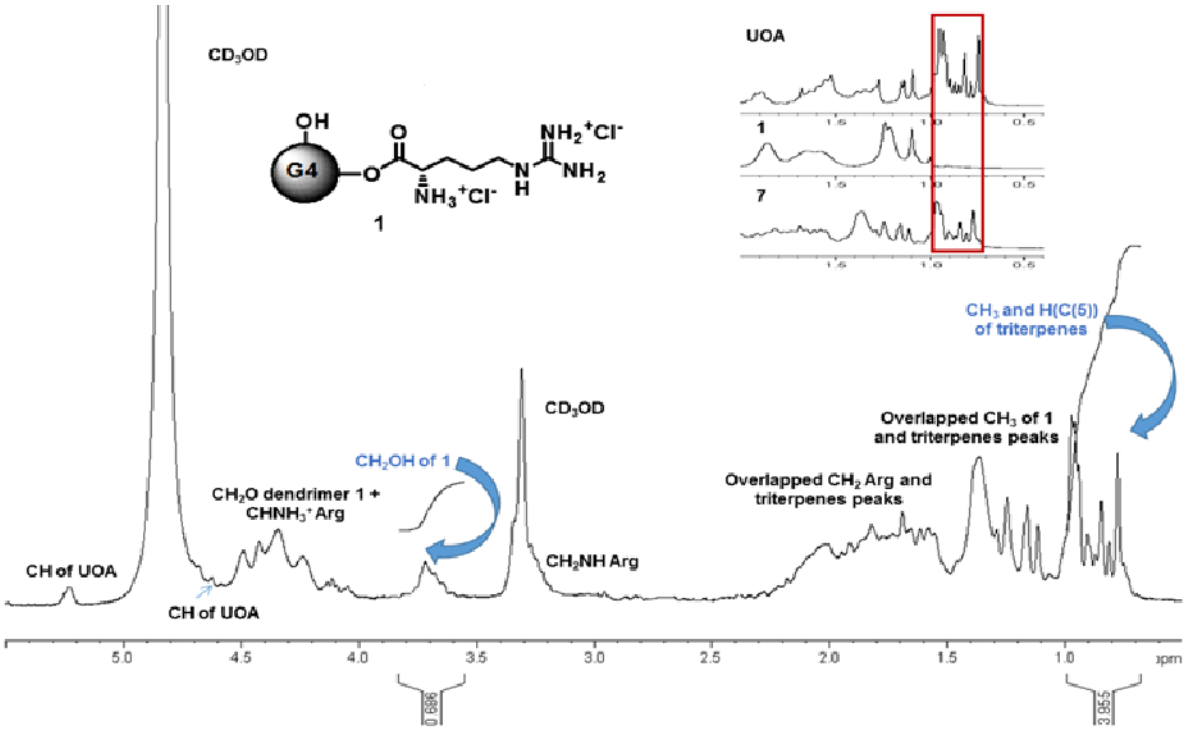


Fig. S3 ^{1}H NMR spectrum of **7** with a comparison between significant parts of spectra of UOA, **1** and **7** in expansion

^{1}H NMR (DMSO-*d*$_6$, 300 MHz)

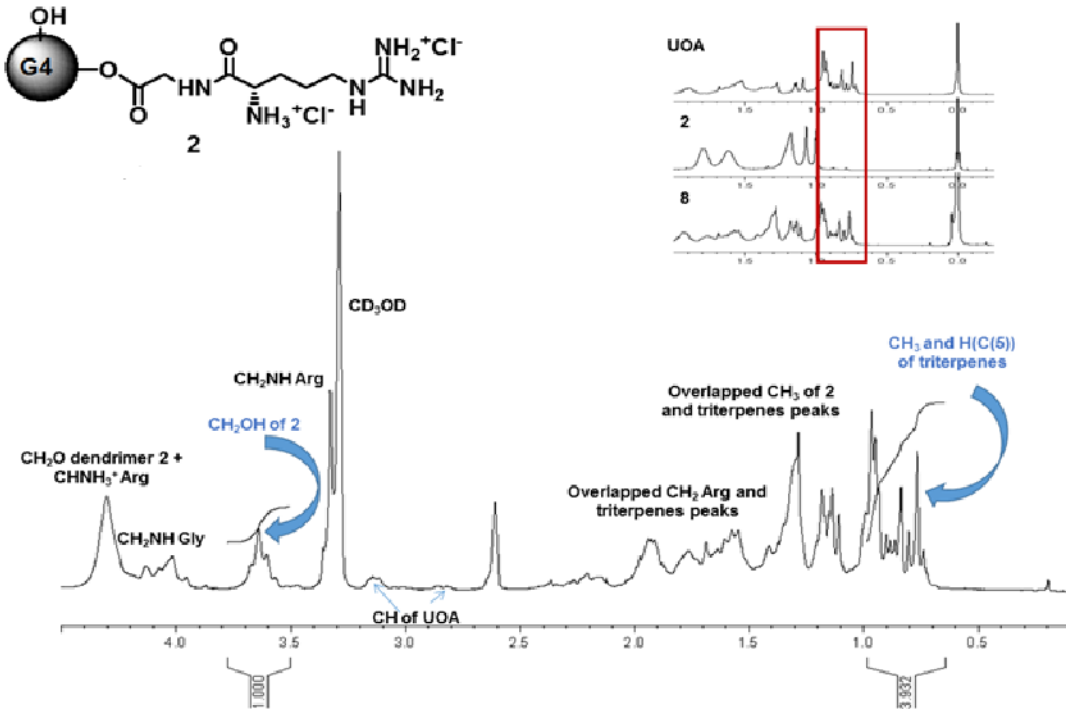


Fig. S4 ^{1}H NMR spectrum of **8** with a comparison between significant parts of spectra of UOA, **2** and **8** in expansion

^{1}H NMR (CD_3OD, 300 MHz)

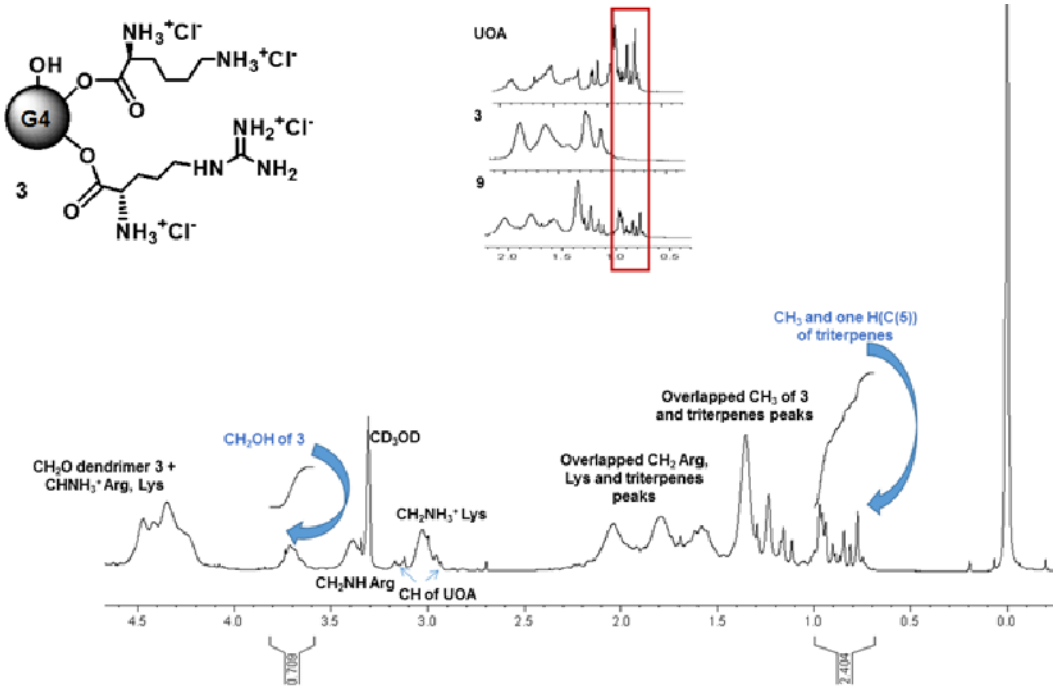


Fig. S5 ^{1}H NMR spectrum of **9** with a comparison between significant parts of spectra of UOA, **3** and **9** in expansion

^{1}H NMR (CD_3OD, 300 MHz)

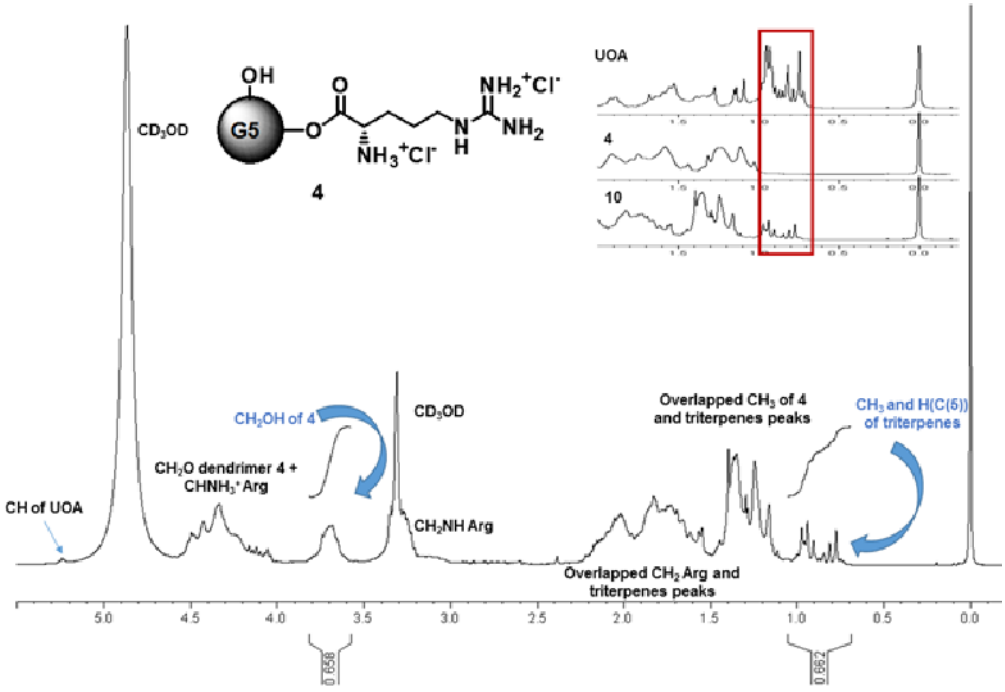


Fig. S6 ^{1}H NMR spectrum of **10** with a comparison between significant parts of spectra of UOA, **4** and **10** in expansion

^{1}H NMR (CD_3OD/DMSO-*d6*, 300 MHz)

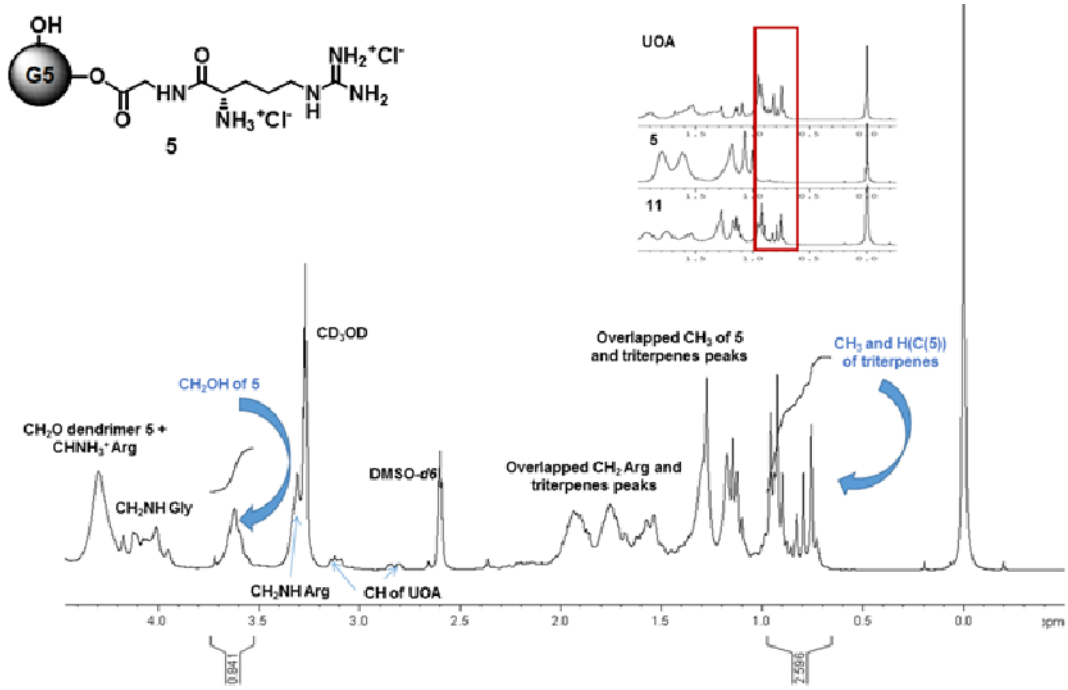


Fig. S7 ^{1}H NMR spectrum of **11** with a comparison between significant parts of spectra of UOA, **5** and **11** in expansion

^{1}H NMR (CD_3OD, 300 MHz)

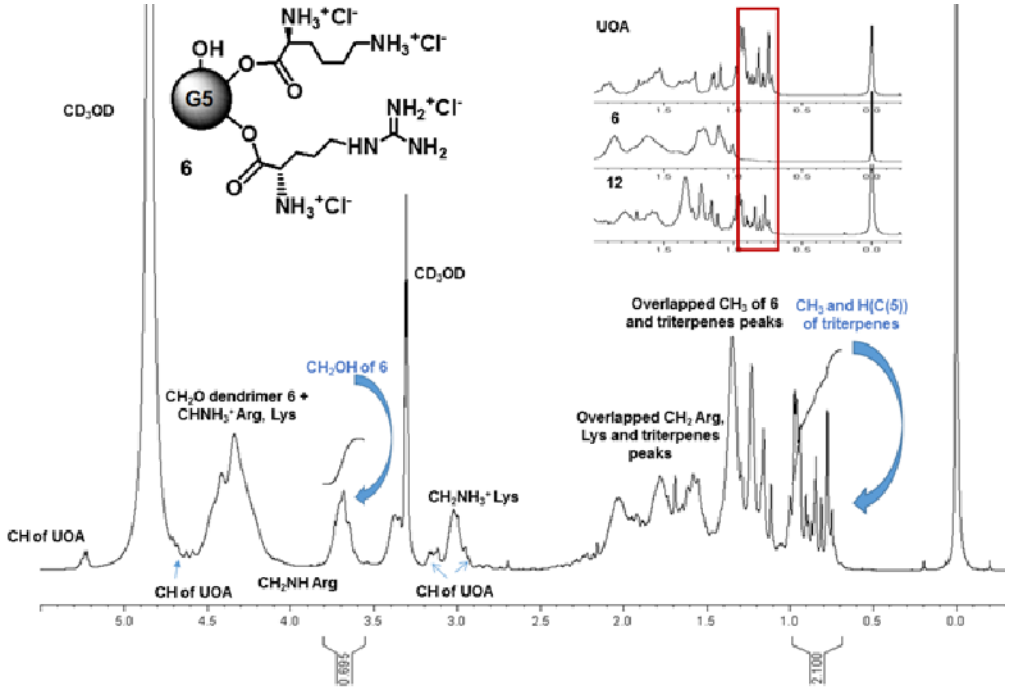


Fig. S8 ^{1}H NMR spectrum of **12** with a comparison between significant parts of spectra of UOA, **6** and **12** in expansion

^{1}H NMR (DMSO-d_6, 300 MHz)

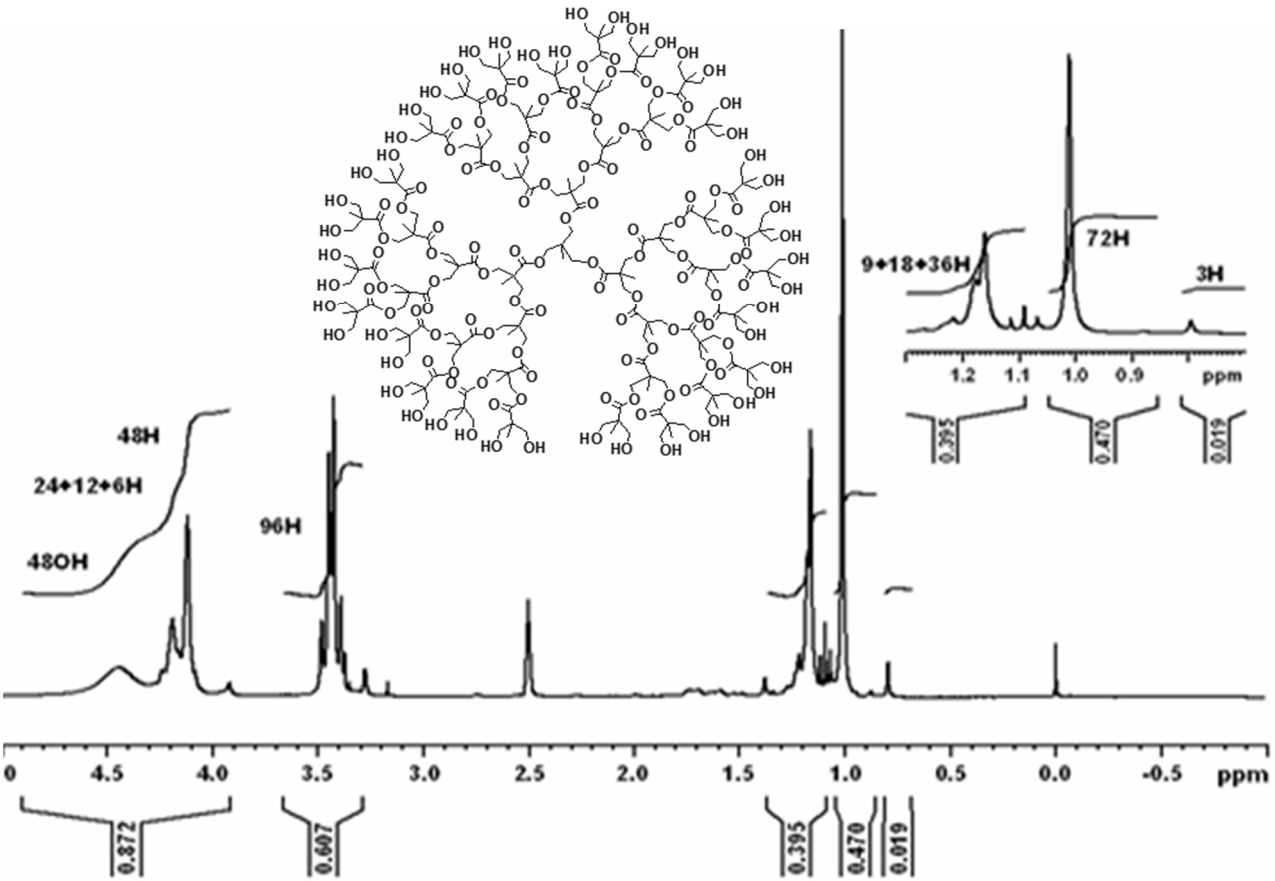


Fig. S9 ^{1}H NMR spectrum of **G4OH**

^{13}C NMR (DMSO-d_6, 75.5 MHz)

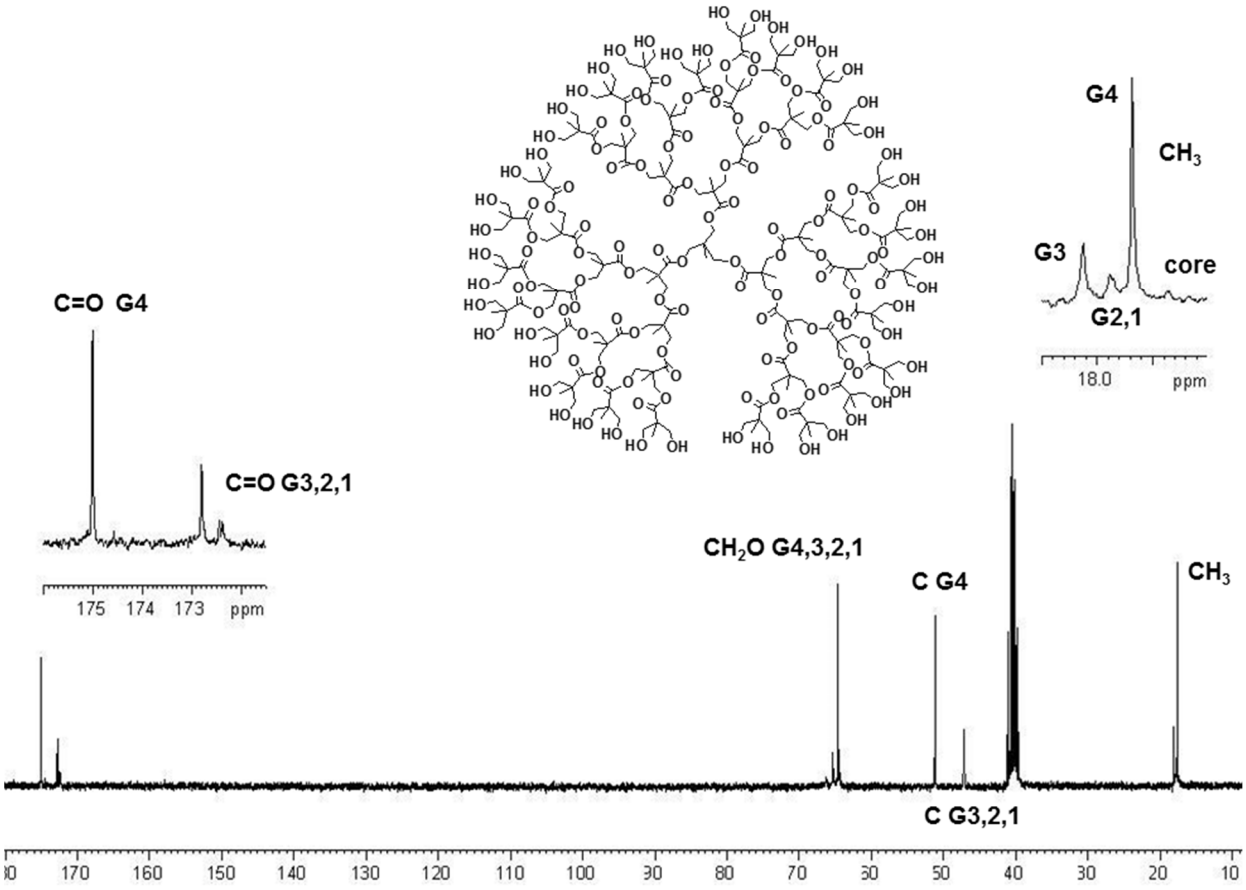


Fig. S10 ^{13}C NMR spectrum of **G4OH**

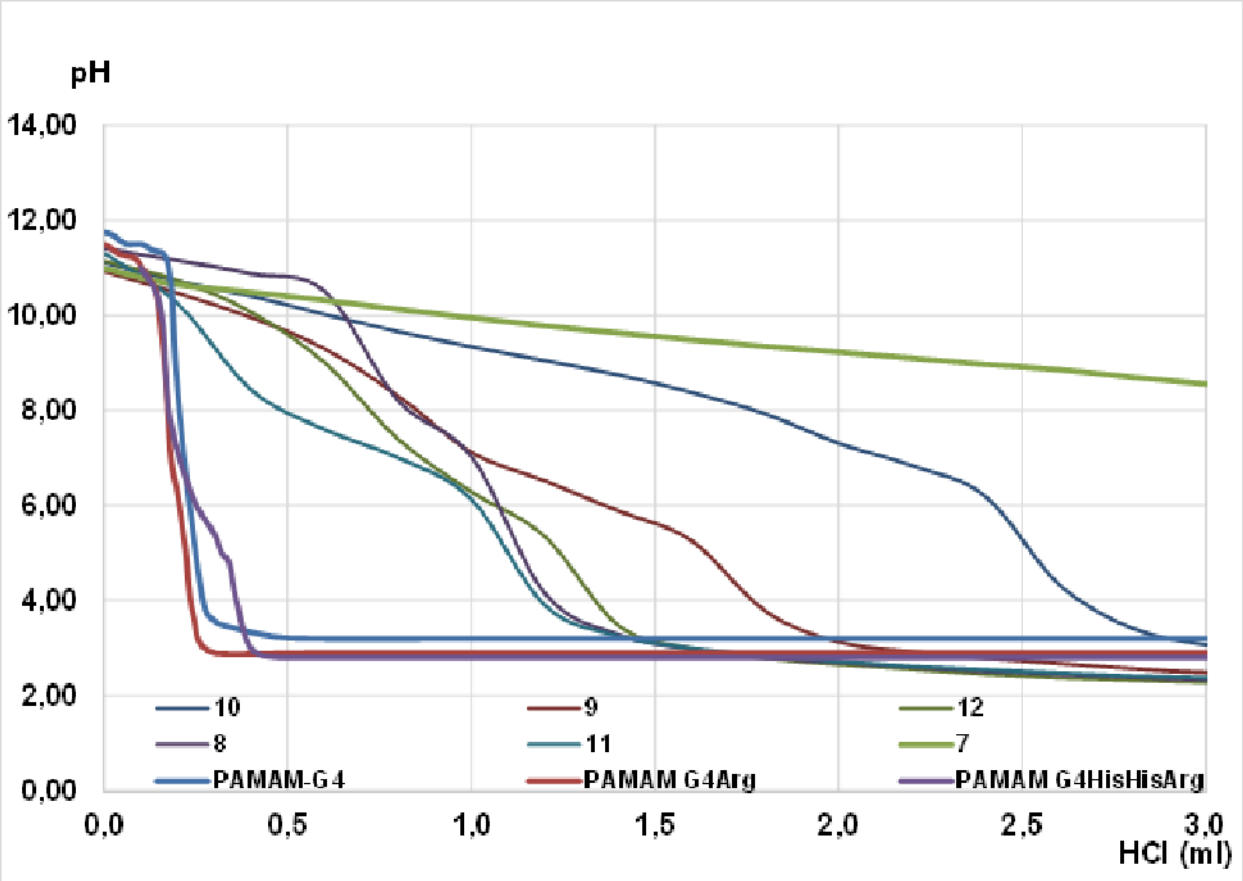


Fig. S11 Potentiometric titration curves of prepared dendriplexes and of three G4-PAMAM derivatives

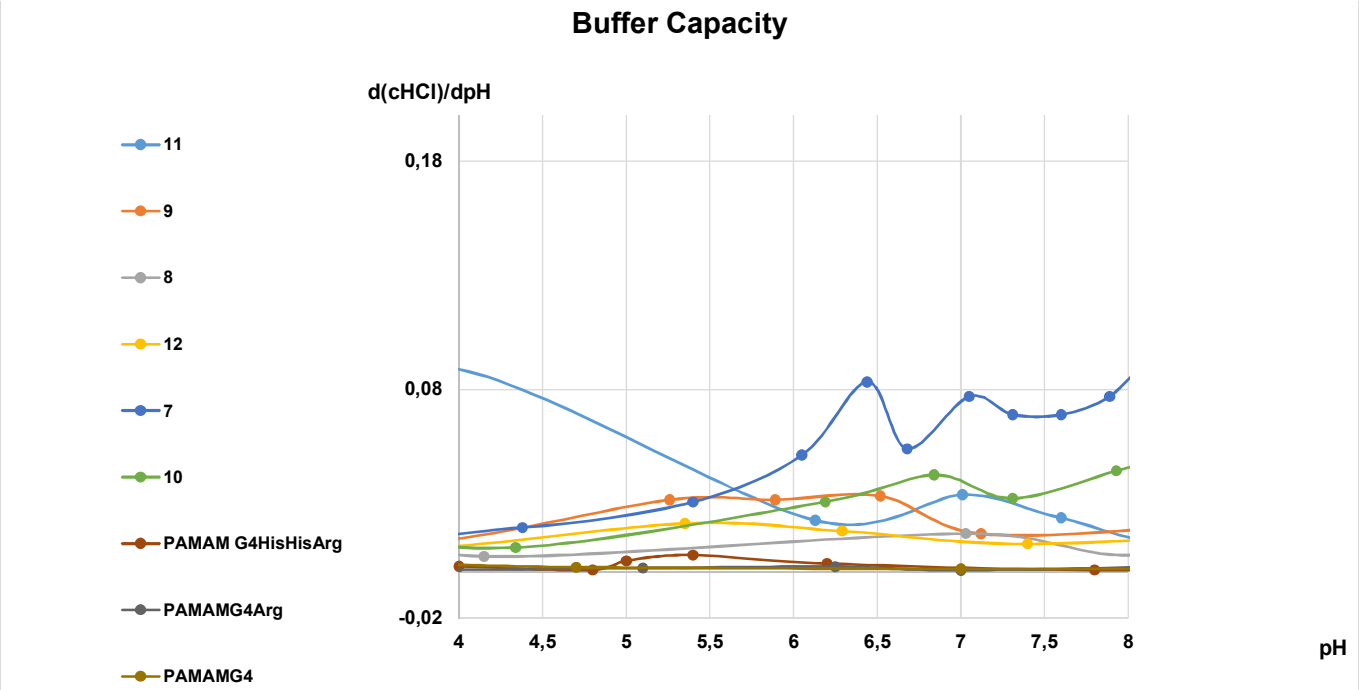
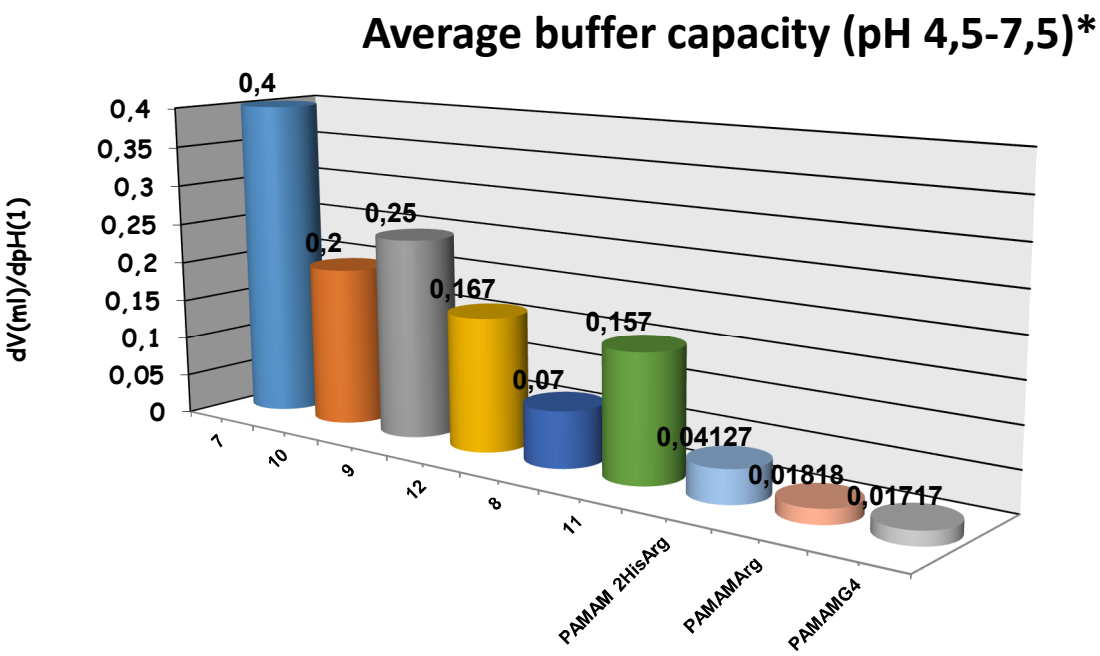


Fig. S12 Graphic of the buffer capacity (β) of prepared dendriplexes and of three G4-PAMAM derivatives



*calculated for three degrees of freedom

Fig. S13 Histogram of average buffer capacity of prepared dendriplexes and of three G4-PAMAM derivatives (pH = 4.5-7.5)

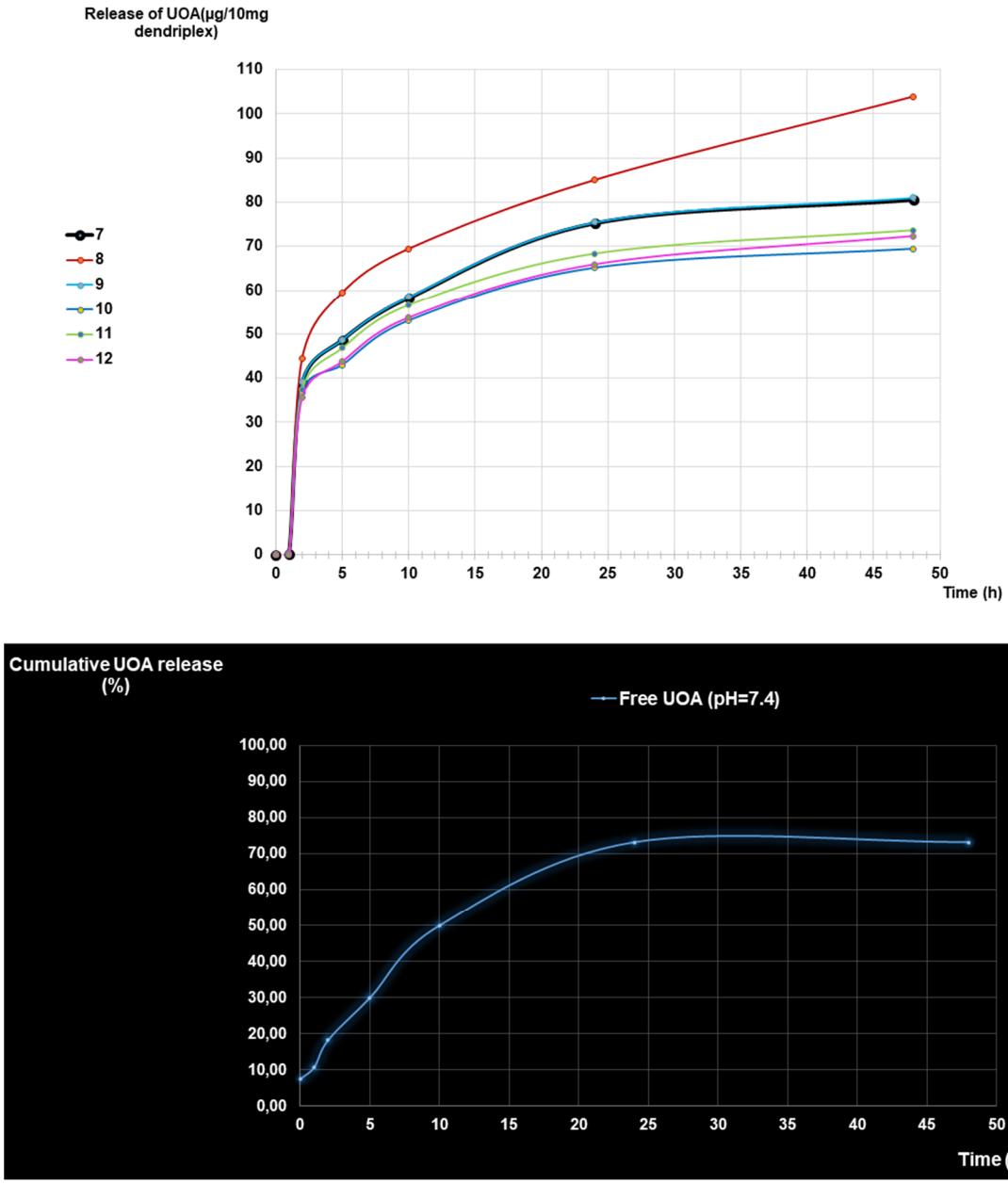


Figure S14 In time release of UOA mixture from dendriplexes **7-12** in 0.1M PBS (pH = 7.4) containing 20% erthanol (A) and cumulative release (%) of free UOA mixture (dissolved in 0.1M PBS containing 60% ethanol) from dialysis bag immersed in 0.1M PBS (pH = 7.4) containing 20% ethanol (B).