Supporting Information

WELL-DEFINED STAR-SHAPED POLYGLUTAMATES WITH IMPROVED PHARMACOKINETICS PROFILE AS EXCELLENT CANDIDATES FOR BIOMEDICAL APPLICATIONS

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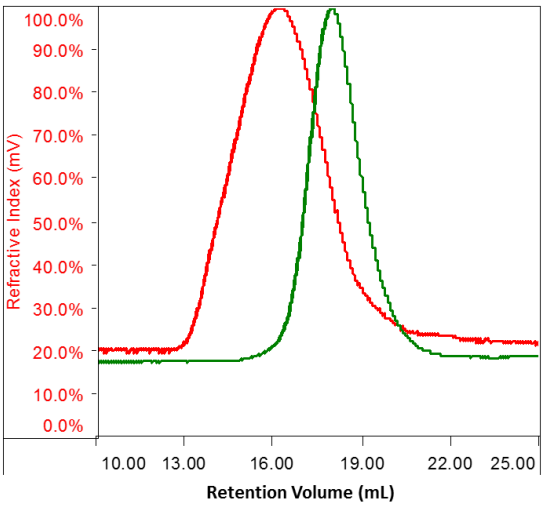
Figures



Sample	Mn (KDa)	Mw (KDa)	Rh	PDI
Star-SS initiated	43.1	52.6	5.07	1.22
Star-SS initiated with DTT	16.4	19.0	2.81	1.16

Figure S1. GPC traces in DMF/LiBr at 8 mg/mL of the cystamine initiated St-PBLG before and after treatment with DTT 1M during 72 hours.

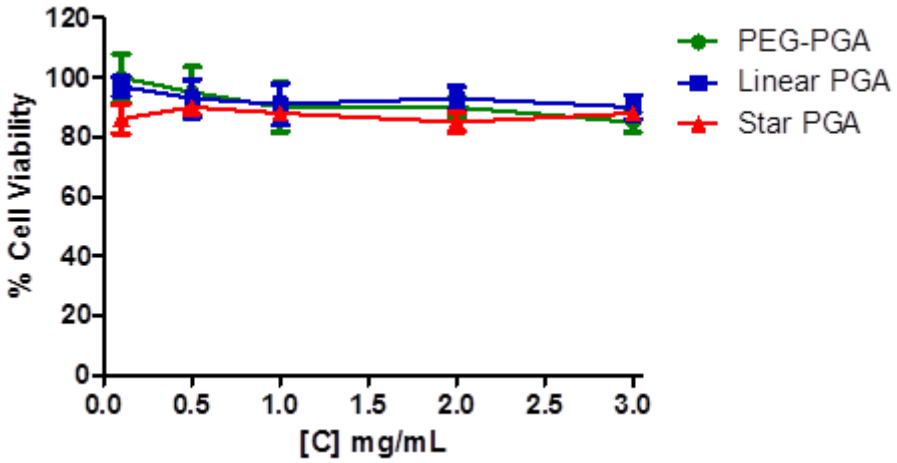


Figure S2. Toxicity assay against HUVEC cell line of different PGA based architectures measure by MTS assay at 72 hours post-treatments. DB: Di-*block* $PEG_{42}PGA_{200}$; PGA: linear PGA_{250}; STAR: St-PGA_{250}

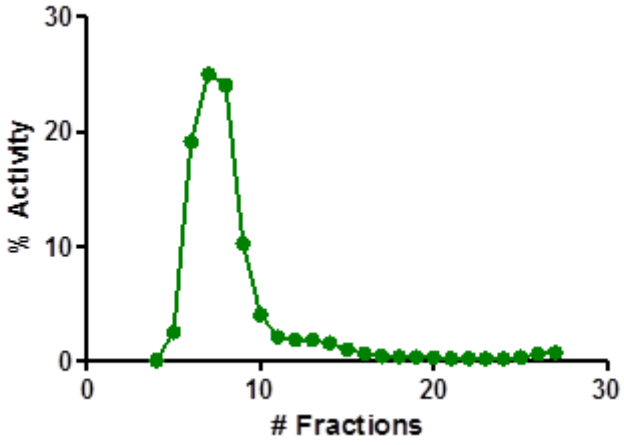
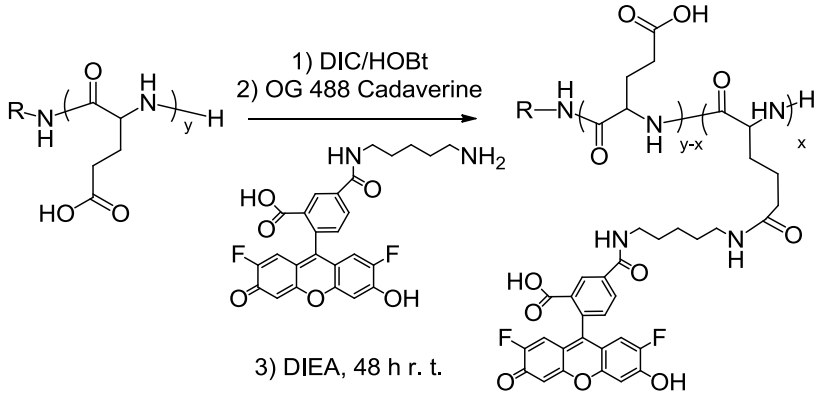
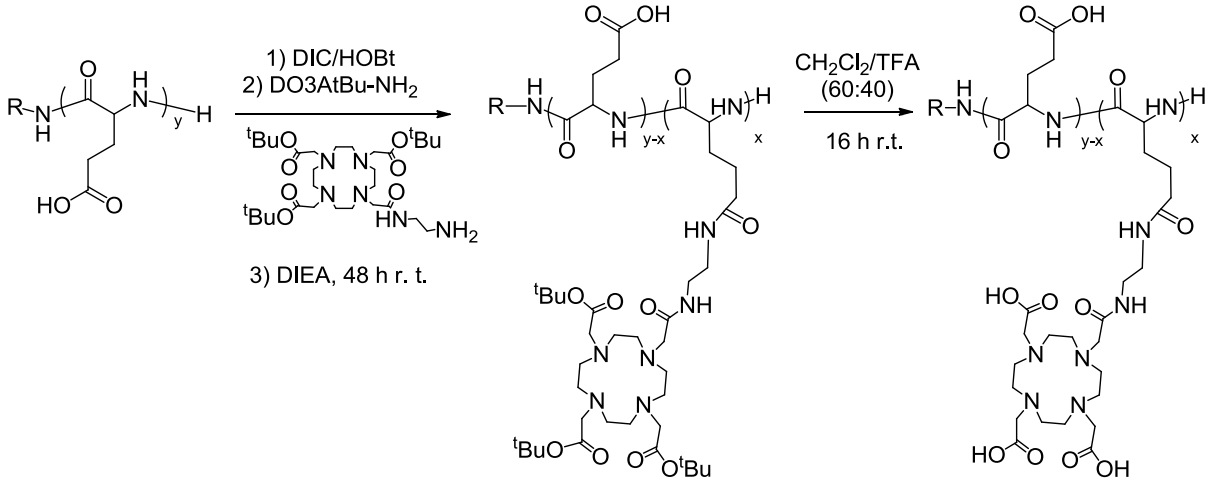


Figure S3. % Activity measured after ^{111}In labeling and purification by SEC of St-PGA-DO3A as example.



Scheme S1. Synthetic route to St-OG.



Scheme S2. Synthetic route to St-DO3A.

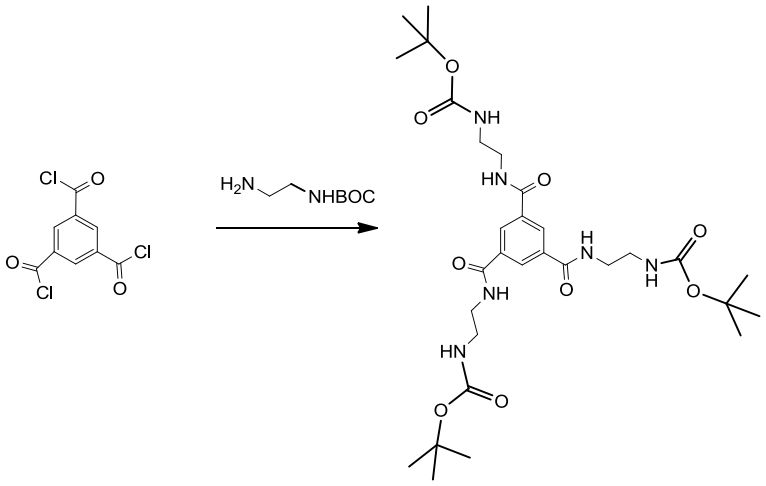
Protocols.

1.Monomer Synthesis.

As for Conejos-Sanchez et al.[1] Briefly, H-L-Glu(OBzl)-OH (17 g, 71.66 mmol, 1 equivalent (eq.)) was added to a two-neck round bottom flask fitted with a stirrer bar, reflux column, dropping funnel and an argon in and outlet. After purging with Argon, tetrahydrofuran (THF) (120 mL, anhydrous) was added and the contents were heated to 60 °C. Limonene (11.6 mL, 71.66 mmol, 1 eq.) was added to the stirring suspension before diphosgene (5.2 mL, 43 mmol, 0.6 eq.) dissolved in THF (20 mL) was added via a dropping funnel over 10 minutes (min). The reaction was stirred for 3 hours at 60 °C and later bubbled with Argon to aid the removal of remaining HCl for 2 hours. The mixture was concentrated, and precipitated into cold hexane. A white solid was isolated and recrystallized from toluene/THF induced by the addition of hexane. Yield: 70-80 %. mp: 93.4 °C. ^{1}H-NMR: (300 MHz, $CDCl_3$, δ): 2.00-2.30 (m, 2H, CH_2CH_2), 2.52-2.60 (t, *J* = 7.0 Hz, 2H, $CHCH_2$), 4.30-4.34 (t, *J* = 6.2 Hz, 1H, CH), 5.09 (s, 2H, OCH_2), 6.40 (s, 1H, NH), 7.30 (m, 5H, Ph).^{13}C-NMR: (300 MHz, $CDCl_3$, δ) 27.5, 30.6, 57.6, 67.8, 129.2, 129.4, 129.5, 135.9, 152.4, 170.2, 173.3.

2. Ethylamine initiator synthesis.

2.1. Synthesis of 1,3,5-Tri-tert-butyl ((benzenetricarbonyltris(azanediyl)) tris(ethane-2,1-diyl))tricarbamate

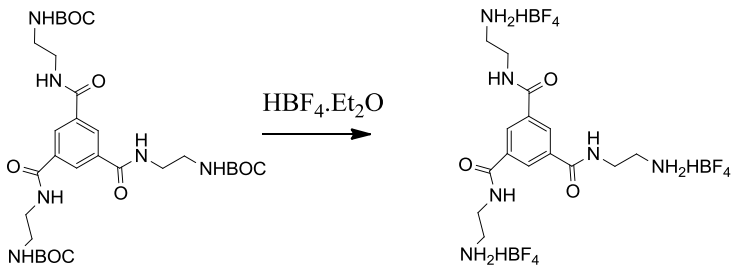


Scheme S1. Synthesis of mono-Boc-protected ethyl based initiator.

In a two-neck round bottom flask fitted with a stir bar, and a N_2 inlet and outlet, 500 mg of 1,3,5-benzenetricarbonyl thricloride (1.88 mmol, 1 eq.) was dissolved in 12 mL of dried tetrahydrofurane (THF). After that, N,N',N''-diisopropylethylendiamine (DIEA) (803.31 mg, 6.22 mmol, 3.3 eq.) was added to the reaction mixture followed by the drop wise addition of N-Boc-ethylendiamine (995.7 mg, 6.22 mmol, 3.3 eq.) over a period of 10 min. The reaction was then left to proceed for 2 hours. After that time, the solvent was completely removed under vacuum. The product was re-dissolved in chloroform and washed times with ddH_2O, and acidic water (three times each). Finally, the organic phase was isolated under vacuum and the product was recrystallized tree times from THF/Methanol/Hexane yielding a white crystalline solid. The product was then dried under high vacuum and stored at -20 °C.

Yield: 82 %. ^{1}H NMR (300 MHz, DMSO) δ 8.68-8.65 (m, 3H), 8.41 (s, 3H), 6.92-6.88(m, 3H), 3.34-3.31 (m, 6H), 3.16-3.13 (m, 6H), 1.37 (s, 27H).^{13}C NMR (75 MHz, $CDCl_3$) δ 166.80 (C=O), 156.84 (C=O), 134.58(C_{Ar} quaternary), 128.47 (CH_{Ar}), 79.57(C quaternary), 40.93 (CH_2), 40.43 (CH_2), 28.45 (CH_3).MALDI-TOF: 659.3208 [M^{+23}]

2.2 Synthesis of 1,3,5-(benzenetricarbonyltris(azanediyl))triethanamonium BF_4 salt



Scheme S2. Synthesis ethyl based initiator BF_4 salt.

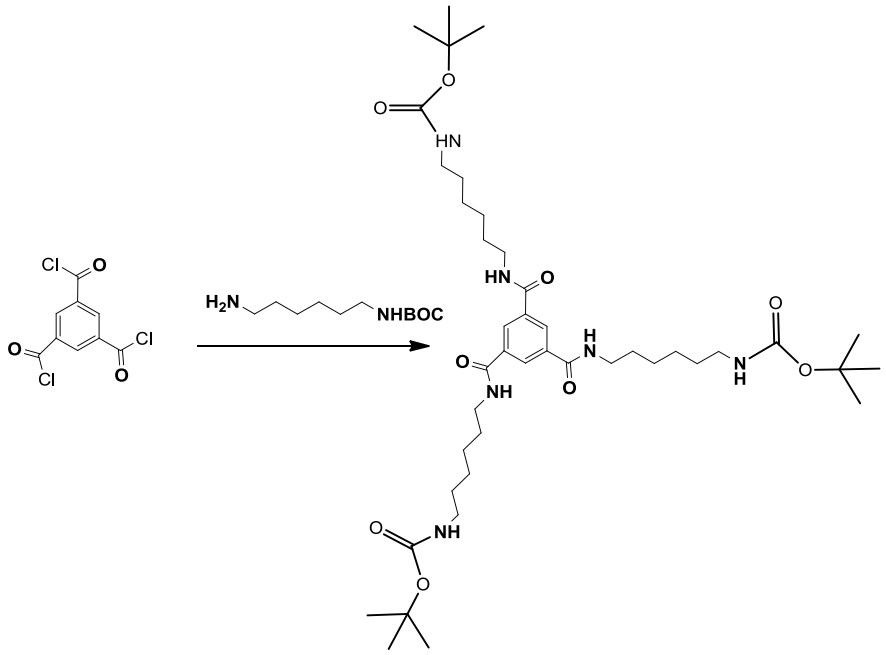
In a one-necked round bottom flask fitted with a stir bar and a stopper, 200 mg of 1,3,5-Tri-tert-butyl ((benzenetricarbonyltris(azanediyl)) tris(ethane-2,1-diyl))tricarbamate (0.33 mmol, 1 eq.) was dissolved in dichloromethane. Afterwards, 3.3 eq. of tetrafluoroboric acid diethyl ether complex, $HBF_4.Et_2O$ (139 mg, 116 µL), was added to the solution leading to the formation of a white solid salt in almost quantitative yield. The product was then filtered off and recrystallized three times from THF/methanol/hexane. The product was then dried under high vacuum and stored at -20 °C.

Yield: 98 %. ^{1}H NMR (300 MHz, D_2O) δ 8.32 (s, 3H), 3.72-3.68 (m, 6H) 3.25-3.21 (m, 6H)

^{13}C NMR (75 MHz, D_2O) δ 169.45 (C=O), 134.38 (C_{Ar} quaternary), 129.36 (C_{Ar}), 39.23(CH_2), 37.52 (CH_2). ^{19}F-NMR: -150.48 (BF_4). MALDI-TOF: 337.1709 [M^{+1}]

3.Hexylamine initiator synthesis.

3.1. Synthesis of 1,3,5-tri-tert-butyl((benzenetricarbonyltris(azanediyl)tris(hexane-1,6-diyl))tricarbamate

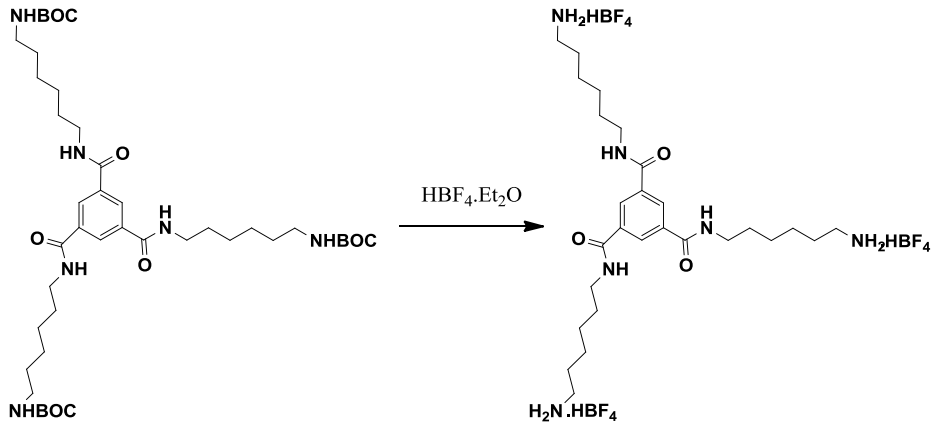


Scheme S3. Synthesis of mono-Boc-protected hexyl based initiator.

In a two-neck round bottom flask fitted with a stir bar, and a N_2 inlet and outlet, 500 mg of 1,3,5-benzenetricarbonyl thricloride (1.88 mmol, 1 eq.) was dissolved in 12 mL of dried THF. After that, DIEA (803.31 mg, 22 mmol, 3.3 eq.) was added to the reaction mixture followed by the drop wise addition of N-Boc-1,6-hexanediamine (1.34 g, 6.22 mmol, 3.3 eq.) over a period of 10 min. The reaction was then left to proceed for 2 hours. After that time, the solvent was completely removed under vacuum. The product was re-dissolved in chloroform and washed times with ddH_2O, and acidic water (three times each). Finally, the organic phase was isolated under vacuum and the product was recrystallized tree times from THF/Methanol/Hexane yielding a white crystalline solid. The product was then dried under high vacuum and stored at -20 °C.

Yield: 72 %. ^{1}H NMR (300 MHz, DMSO) δ 8.37 (s, 3H), 6.75 (m, 3H), 3.30-3.24 (m, 6H), 2.91-2.87 (m, 6H), 1.55-1.51(m, 6H), 1.37-1.29 (m, 45H). ^{13}C NMR (75 MHz, $CDCl_3$) δ 166.28 (C=O), 156.49 (C=O), 134.93 (C_{Ar} quaternary), 128.68 (C_{Ar}), 79.34 (Cquaternary), 40.33 (CH_2), 39.92 (CH_2), 29.89 (CH_2), 29.19 (CH_2), 28.42 (CH_3), 26.19 (CH_2), 25.90 (CH_2).

3.2. Synthesis of 1,3,5-(benzenetricarbonyltris(azanediyl))trishexan-1-amonium BF_4 salt



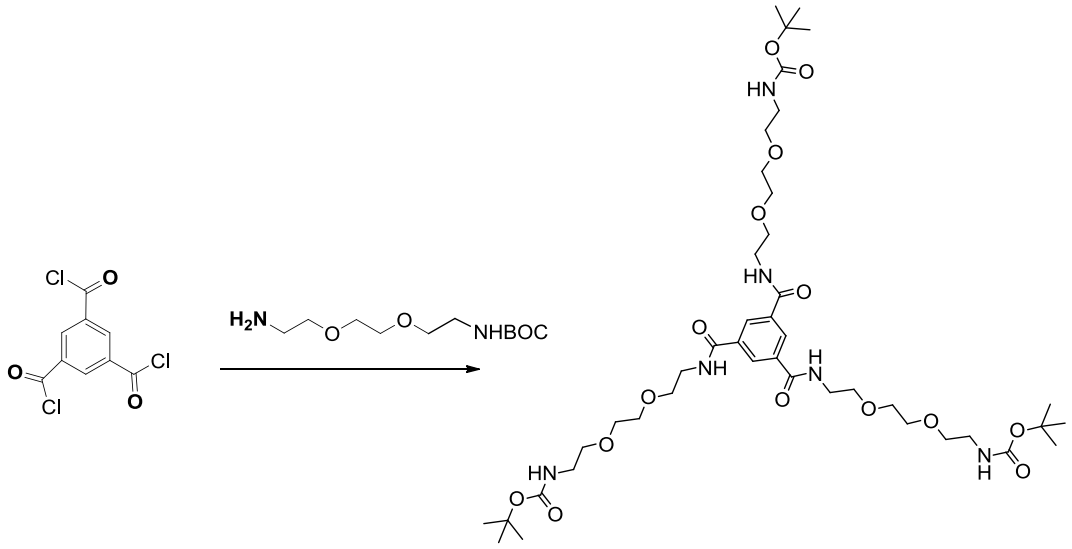
Scheme S4. Synthesis hexyl based initiator BF_4 salt.

In a one-necked round bottom flask fitted with a stir bar and a stopper, 200 mg of 1,3,5-tri-tert-butyl((benzenetricarbonyltris(azanediyl)tris(hexane-1,6-diyl))tricarbamate (1.88 mmol, 1 eq.) was dissolved in dichloromethane. Afterwards, 3.3 eq. of $HBF_4.Et_2O$ (139 mg, 116 µL), was added to the solution leading to the formation of a white solid salt in almost quantitative yield. The product was then filtered off and recrystallized three times from THF/methanol/hexane. The product was then dried under high vacuum and stored at -20 °C.

Yield: 90 %. ^{1}H NMR (300 MHz, D_2O) δ 8.12 (s, 3H), 3.37-3.32 (m, 6H), 2.96-2.91 (m, 6H), 1.61-1.57 (m, 12H), 1.39-1.34 (m, 12H).^{13}C NMR (75 MHz, D_2O) δ 168.92 (C=O), 135.00 (C_{Ar} quaternary), 128.49 (C_{Ar}), 39.97 (CH2), 39.42, (CH2)28.10 (CH2), 26.60(CH2), 5.57(CH2), 25.23(CH2). ^{19}F-NMR-150.41. MALDI-TOF: 505.3563 [M^+]

4. DOOA initiator synthesis.

4.1.-Synthesis of 1,3,5-tri-tert-butyl ((((((benzenetricarbonyltris(azanediyl)) tris(ethane-2,1-diyl))tris(oxy))tris(ethane-2,1-diyl))tris(oxy))tris(ethane-2,1-diyl))tricarbamate

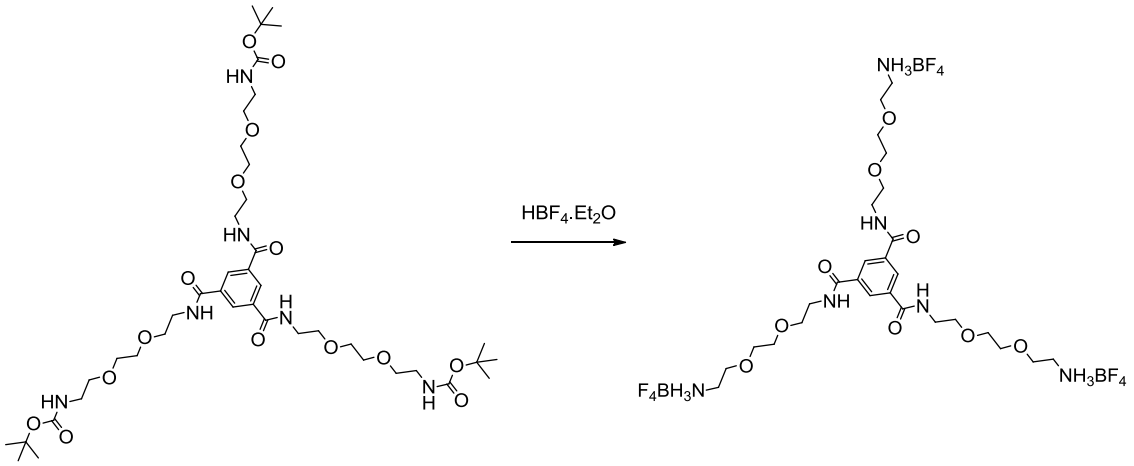


Scheme S5. Synthesis of mono-Boc-protected DOOA based initiator.

In a two-neck round bottom flask fitted with a stir bar, and a N_2 inlet and outlet, 500 mg of 1,3,5-benzenetricarbonyl thricloride (1.88 mmol, 1 eq.) was dissolved in 12 mL of dried THF. After that, DIEA (803.31 mg, 6.22 mmol, 3.3 eq.) was added to the reaction mixture followed by the drop wise addition of N-Boc-DOOA (1.54 g, 2.49 mmol, 3.3 eq.) over a period of 10 min. The reaction was then left to proceed for 2 hours. After that time, the solvent was completely removed under vacuum. The product was re-dissolved in chloroform and washed times with ddH_2O, and acidic water (three times each). Finally, the organic phase was isolated under vacuum and the product was recrystallized tree times from THF/Methanol/Hexane yielding a white crystalline solid. The product was then dried under high vacuum and stored at -20 °C.

Yield: 70 %. ^{1}H NMR (300 MHz, $CDCl_3$) δ 8.35 (s, 3H), 3.62-3.58 (m, 24H), 3.51-3.48 (m, 6H), 3.24-3.20 (m, 6H), 1.34 (s, 27H).^{13}C NMR (75 MHz, $CDCl_3$) δ 165.99 (C=O), 156.19 (C=O), 135.05 (C_{Ar} quaternary), 128.55 (C_{Ar}), 79.42 (Cquaternary), 79.32 (CH_2), 77.22 (CH_2), 70.20 (CH_2), 69.66 (CH_2), 40.51 (CH_2), 40.09 (CH_2), 28.39 (CH_3).

4.2-Synthesis of 1,3,5'-(((((benzenetricarbonyltris(azanediyl))tris(ethane-2,1-diyl))tris(oxy))tris(ethane-2,1-diyl))tris(oxy))triethanamonium BF_4 salt



Scheme S6. Synthesis DOOA based initiator BF_4 salt.

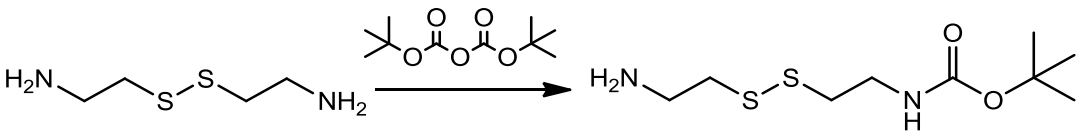
In a one-necked round bottom flask fitted with a stir bar and a stopper, 200 mg of 1,3,5-tri-tert-butyl ((((((benzenetricarbonyltris(azanediyl)) tris(ethane-2,1-diyl))tris(oxy))tris(ethane-2,1-diyl))tris(oxy))tris(ethane-2,1-diyl))tricarbamate (0.23 mmol, 1 eq.) was dissolved in dichloromethane. Afterwards, 3.3 eq. of $HBF_4.Et_2O$ (123 mg, 103 µL), was added to the solution leading to the formation of a white solid salt in almost quantitative yield. The product was then filtered off and recrystallized three times from THF/methanol/hexane. The product was then dried under high vacuum and stored at -20 °C.

Yield: 85 %. ^{1}H NMR (300 MHz, D_2O) δ 8.24 (s, 3H), 3.74-3.67 (m, 30H), 3.61-3.58 (m, 6H), 3.14-3.10 (m, 6H).^{13}C NMR (75 MHz, D_2O) δ 168.95 (C=O), 134.86 (C_{Ar} quaternary), 128.89 (C_{Ar}), 69.56 (CH_2), 69.49 (CH_2),68.78 (CH_2), 66.37 (CH_2), 39.56 (CH_2), 39.08 (CH_2).

^{19}F NMR:-150.48. MALDI-TOF: 601.3354 [M^+]

5. Cystamine initiator synthesis.

5.1. Synthesis of tert-butyl (2-((2-aminoethyl)disulfanyl)ethyl)carbamate (mono-Boc-cystamine)

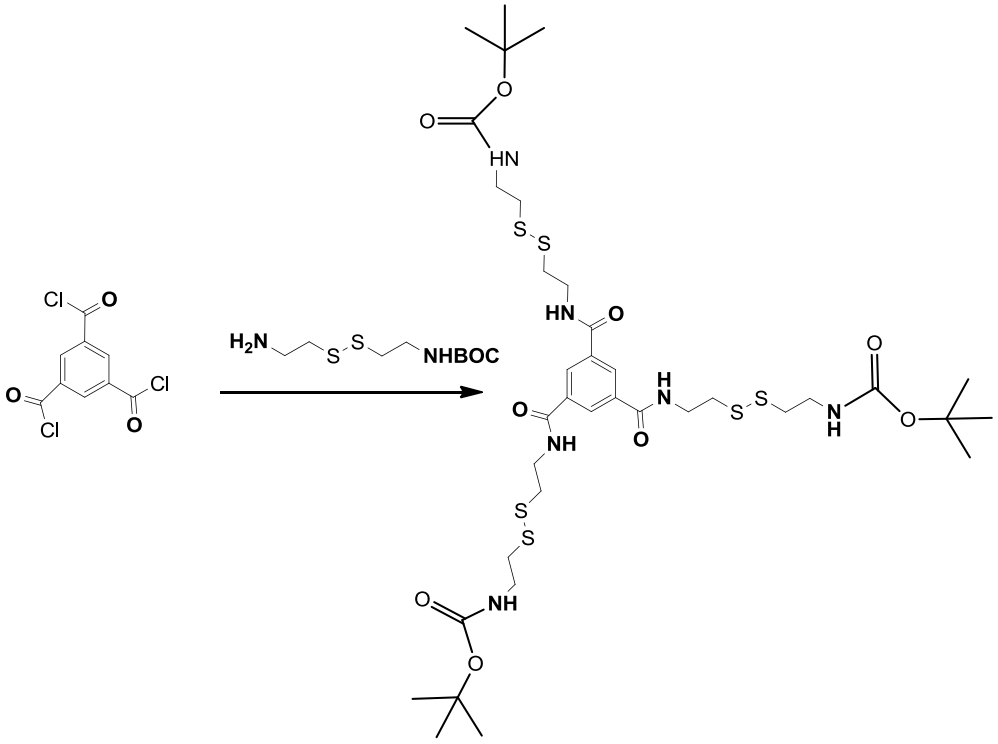


Scheme S7. Synthesis of mono-Boc-protected cystamine.

General method from Girgenti et al.[2] Briefly, a suspension of 3.37 g of cystamine dihydrochloride (15 mmol, 1 eq.) in 100 mL of anhydrous chloroform was treated with 3.04 g of triethylamine (30 mmol, 2 eq.) and stirred for twenty min at room temperature. A solution of di- 1.09 g of tert-butyl dicarbonate (5 mmol, 0.33 eq.) in another 50 mL of anhydrous chloroform was added drop wise over a period of 1.5 hours. The reaction mixture was warmed to room temperature and stirred for 16 hours more. After that time, 20 mL of ddH_2O were added and the organic layer was washed three times. A colorless oil was obtained as a pure compound after removal of the solvent under high vacuum

Yield: 50 %. ^{1}H NMR (300 MHz, DMSO) δ 6.97-6.96 (d, 1H), 3.23-3.17 (q, 2H), 2.81-2.68 (m, 6H), 1.50 (s, 1H), 1.38 (s, 9H).^{13}C NMR (75 MHz, DMSO) δ 155.94 (C=O), 78.21 (C quaternary), 42.57 (CH_2), 41.40 (CH_2), 39.90 (CH_2), 38.13 (CH_2), 28.67 (CH_3).

5.2.-Synthesis of 1,3,5-tri-tert-butyl ((((benzenetricarbonyltris(azanediyl))tris(ethane-2,1-diyl))tris(disulfanediyl))tris(ethane-2,1-diyl))tricarbamate



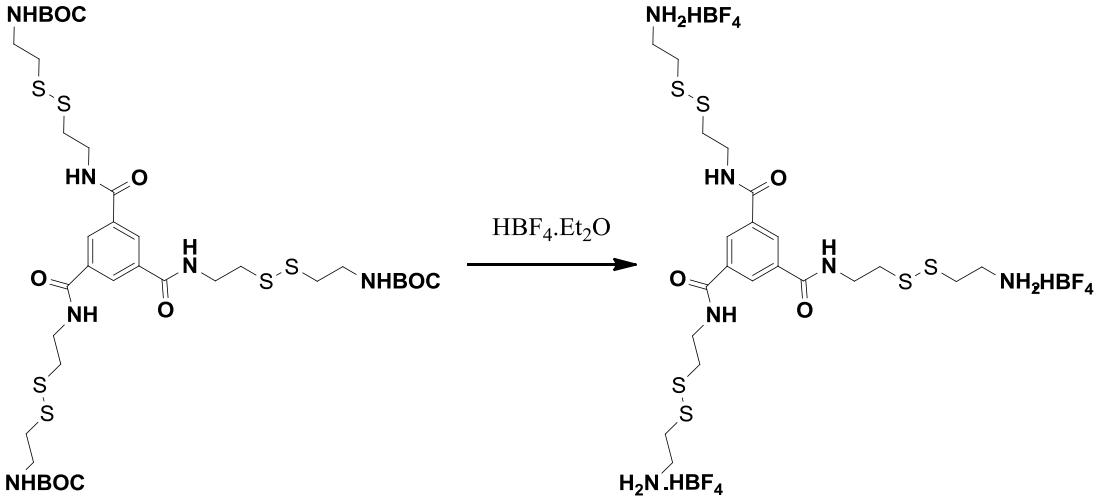
Scheme S8. Synthesis of mono-Boc-protected cystamine based initiator.

In a two-neck round bottom flask fitted with a stir bar, and a N_2 inlet and outlet, 100 mg of 1,3,5-benzenetricarbonyl trichloride (0.38 mmol, 1 eq.) was dissolved in 12 mL of dried THF. After that, DIEA (160.7 mg, 1.24 mmol, 3.3 eq.) was added to the reaction mixture followed by the drop wise addition of N-Boc-cystamine (313.7 mg, 1.24 mmol, 3.3 eq.) over a period of 10 min. The reaction was then left to proceed for 2 hours. After that time, the solvent was completely removed under vacuum. The product was re-dissolved in chloroform and washed times with ddH_2O, and acidic water (three times each). Finally, the organic phase was isolated under vacuum and the product was recrystallized tree times from THF/Methanol/Hexane yielding a white crystalline solid. The product was then dried under high vacuum and stored at -20 °C.

Yield: 60 %. ^{1}H NMR (300 MHz, DMSO-*d6*) δ 8.90 (m, 3H), 8.43 (s, 3H), 6.97 (s, 3H), 3.62-3.58 (m, 6H), 3.23-3.21 (m, 6H), 2.96-2.93 (m, 6H), 2.80-2.76 (m, 6H), 1.37(s, 27H).^{13}C NMR

(75 MHz, $CDCl_3$) δ 165.87 (C=O), 156.21 (C=O), 135.45 (C_{Ar} quaternary), 129.06 (C_{Ar}), 78.16 (Cquaternary), 39.87 (CH_2), 39.31 (CH_2), 38.07 (CH_2), 37.43 (CH_2), 28.68 (CH_3).

5.3.-Synthesis of 1,3,5-(((benzenetricarbonyltris(azanediyl))tris(ethane-2,1-diyl))tris(disulfanediyl))triethanaminium BF_4



Scheme S9. Synthesis of cystamine based initiator BF_4 salt

In a one-necked round bottom flask fitted with a stir bar and a stopper, 100 mg of 1,3,5-tri-tert-butyl ((((benzenetricarbonyltris(azanediyl))tris(ethane-2,1-diyl))tris(disulfanediyl)) tris(ethane-2,1-diyl))tricarbamate (0.11 mmol, 1 eq.) was dissolved in dichloromethane. Afterwards, 3.3 eq. of $HBF_4.Et_2O$ (58.5 mg, 49.1 µL), was added to the solution leading to the formation of a white solid salt in almost quantitative yield. The product was then filtered off and washed 3 times with ether. For further purification, the product is recrystallized from THF/methanol/hexane. The product was then dried under high vacuum and stored at -20 °C.

Yield: 80 %. ^{1}H-NMR (300 MHz, DMSO-*d6*) δ 8.43 (s, 3H), 7.81 (m, 3H), 3.63-3.58 (m, 6H), 3.23-3.21 (m, 6H), 3.17-3.13 (m, 6H), 2.96-2.94 (m, 6H).^{13}C-NMR (75 MHz, DMSO-*d6*) δ 165.47 (C=O), 134.55 (C_{Ar} quaternary), 128.06 (C_{Ar}), 39.77 (CH_2), 39.31 (CH_2), 38.28 (CH_2), 37.65 (CH_2). ^{19}F-NMR: -148.31

6. Pharmacokinetic analysis of the radioactivity experiments *in vivo*

The plasma concentration versus time data of radioactivity were analysed by a two-compartment model with bolus input and first-order elimination rate (Figure S3). The model is described by the following equation:

$$C(T) = A * \exp(-ALPHA * T) + b * \exp(-BETA * T)$$

where *D* is the dose administered by iv injection; *ALPHA* and *BETA* are constants that depend solely on K_{12}, K_{21} (transfer constants between compartments 1 and 2) and K_{10} (elimination constant). V_c is the volume of distribution in the central compartment and V_{ss} is the total volume of distribution in steady-state. Apparent terminal half-life $(t_{1/2})$ is calculated as Ln2/*BETA* and the plasma clearance (Cl) for each compound is estimated as the ratio of dose/AUC (area under the plasma concentration-time curve).

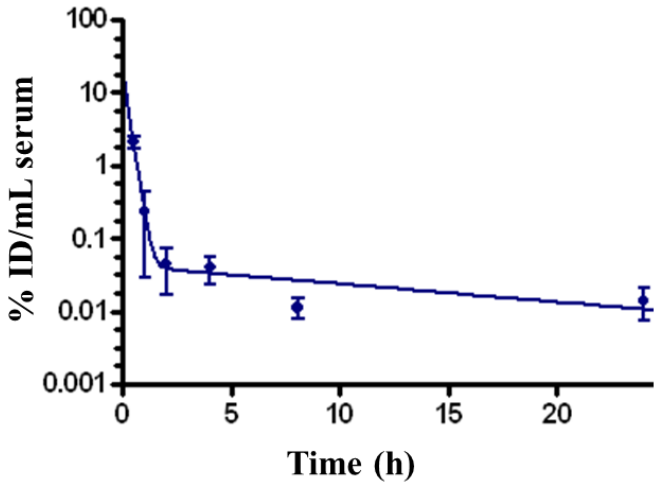


Figure S4.1 Two-compartment model fittings (PK) of % ID/mL in serum with time of St-PGA-DO3A-^{111}In.

The initial estimates of the pharmacokinetic parameters were computed by WinNonLin (ver. 5.2, Pharsight Corporation, Mountain View, CA) using curve stripping. The pharmacokinetic parameters were *A, B, ALPHA and BETA*. From these parameters, several derived pharmacokinetic parameters were computed: AUC (*A/ALPHA* + *B/BETA*), Cl (*D/AUC*), V_{ss}, C_{max} (*A+B)* and apparent terminal half-life. As plasma concentrations often span a wide range, it is useful to employ a weighting procedure for the raw data that allows one to fit low concentrations and high concentrations simultaneously. We used weighting by the deviation standard of the concentration. The Nelder-Mead simplex algorithm, which is implemented in WinNonlin, was used as fitting algorithm.

The estimated pharmacokinetic parameters were compared with the ones previously obtained for the linear construct, PGA-[^{68}Ga]-DO3A,[3] as shown in Table 1.

Obviously, in any biologic system, the loss of a radiolabelled compound is due to both the physical decay of the radionuclide, $t_{1/2}$ = 67.845 min and $t_{1/2}$ = 2.085 days for ^{68}Ga and ^{111}In respectively, and the biological elimination of the radiolabelled compound. The net or effective half-life, time required for the radioactivity in an organism to be reduced to half through the combined effects of the physical decay of the isotope and the biological elimination of the isotope, is given as follows:

$$\frac{1}{(t_{1/2})_{effective}} = \frac{1}{(t_{1/2})_{physical}} + \frac{1}{(t_{1/2})_{biological}}$$

As the effective terminal half-life was 12.05±7.96 hours and 0.59±0.29 hours for St-PGA-[^{111}In]-DO3A and PGA-[^{68}Ga]-DO3A, the biological terminal half-life was 15.87±9.47 hours and 1.23±0.40 hours for both compounds, respectively.

Table S1. Main St-PGA-[^{111}In]-DO3A and PGA-[^{68}Ga]-DO3A pharmacokinetic parameters estimated by a 2-compartment model following the equation $C(t)=Axe^{(-ALPHAxt)}+Bxe^{(-BETAxt)}$. Values represent Mean ± SD.

Parameter	St-PGA[^{111}In]-DO3A-	PGA-[^{68}Ga]-DO3A-
A (% ID/mL)	22.33±5.13	35.00±12.88
B (% ID/mL)	0.04±0.01	4.35±2.78
ALPHA (h^{-1})	4.73±0.42	7.28±2.56
BETA (h^{-1})	0.06±0.04	1.18±0.59
AUC (% ID.h/mL)	5.45±0.76	8.50±0.67
$t_{1/2}$ ALPHA (h)	0.15±0.01	0.10±0.03
$t_{1/2}$ BETA (h)	12.05±7.96	0.59±0.29
Cl (mL/h)	18.35±2.55	11.77±0.93
Vss (mL)	46.34±44.44	5.25±2.52

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