Supporting Information

Long-acting, potent delivery of combination antiretroviral therapy

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Abstract: Antiretroviral therapy (ART) has revolutionized HIV treatment yet grand challenges remain: i) short blood and body residence time of the antiviral drugs, ii) relative poor anti-retroviral drug penetrance into key tissue reservoirs of viral infection, namely spleen and lymph nodes, and iii) obstacles in different pharmacokinetics of the necessary combination drugs. We present a novel drug delivery approach that simultaneously overcomes these limitations. We de-signed albumin-polymer-drug conjugates where albumin ensures long body residence time as well as lymphatic accumulation of the conjugate. The polymer enabled the delivery of combinations of drugs in precise ratios affording potency superior to the individual antiretroviral drugs and strong protection from HIV infection in primary human T cells.

MATERIALS AND METHODS

Full synthetic procedures used to obtain all the custom made monomers, polymers, and bioconjugates are presented in Supporting Information.

General procedure for polymer synthesis. HPMA, drug containing monomer (30) (31), AIBN, and 2-cyano-5-oxo-5-(2-thioxothiazolidin-3-yl)pentan-2-yl dodecyl carbonotrithioate (TA RAFT agent) agent were dissolved in DMF (0.4-0.8 mL). Four rounds of freeze-pump-thaw were performed upon which the ampule was flame sealed. The polymerization was performed at 60 °C for 17-20 h. The reaction mixture was precipitated into Et_2O:acetone 1:1 and filtered and washed yielding the pure product.

General procedure for albumin conjugation. 4 mg albumin in 200 µL PBS was added to the TA polymer (5 eq) dissolved in DMSO (0.02 mL) and diluted to 0.2 mL in PBS giving a total volume of 0.4 mL. The reaction was left for 24 h and analyzed by GPC (25 µL taken out). The reaction mixture was spin-filtered on a 30 kDa membrane and washed three times with milli Q (0.4 mL, 1100 rpm) The residue was then diluted with 525 µL milliQ (25 µL taken out for pure GPC) and aliquoted into Eppendorph tubes and lyophilized.

FcRn binding evaluation.
FcRn affinity was measured using bio-layer interferometry on a BLItz® device (ForteBio, Pall Life Sciences). Biotinylated human FcRn was immobilized on a streptavidin sensor in PBS (Sigma-Aldrich) holding 0.01% Tween 20 (PBST) (Sigma Aldrich) at pH 7.4. Increasing concentrations of albumin polymer was used for binding measurements (0.1875, 0.375, 0.750, 1.50, 3.0 µM) in association buffer (25mM sodium acetate, 25mM NaH_2PO_4, and 150mM NaCl 0,01% Tween® 20, pH 5.5). Association binding curves were performed over a period of 120 seconds, and dissociation occurred over 300 seconds. A buffer reference was subtracted for all values and curves were zero-adjusted before fitting the binding profiles to a 1:1 model using global fit in the BLItz PRO 1.2 software.

In vivo experiments.
In vivo pharmacokinetic analysis was performed with female BALB/cJRj mice (8 weeks old, Janvier-labs.com, France). Intravenous and subcutanous injections were performed in separate experimental executions on separate times. Experimental setup was however identical except the injection route. Mice were divided into groups of 12, receiving fluorescently labelled compound, dissolved in PBS. They were injected with either Cy7®-labelled polymer (4 mg/kg), Cy7®-labelled albumin-polymer conjugate (0.4 mg/kg) or vehicle (PBS), either through intravenous tain vein injection, or by injection in the subcutaneous space of the neck. Mice received injection volumes ranging from 134 µL to 170 µL depending on body weight. Blood samples from 4 mice from each group were drawn at 5, 30, 60, 120, 180, 240, 420 minutes post injection and periodically up to 1 week. Blood (~10 µL) was drawn either from retroorbital plexus, or through tail tip, into 20 µL Na-heparin coated glass capillaries (Vitrex Medical AS). Cells and plasma was separated by centrifugation for further analysis. At each blood sampling time point, mice were further anaesthazised by 3.75% isoflurane and photographed by in IVIS (in vivo imaging system, PerkinElmer, MA, USA) for a full body image of the biodistribution of the compounds. Images was taken using a 745/820 nm filterset

for Cy7 and further analyzed by using Living Image software v4.3 (PerkinElmer). Fraction of compounds in plasma samples was estimated based on fluorescence intensity. ROI (regions of interest) was manually applied to plasma and mean radiance (photons/sec/cm^2/sr) of the samples was determined. Background signal from plasma samples from animals injected with vehicle was subtracted.

Animals were terminated 1 h, 24 hand 7 days after injection. Euthanization was carried out under aneasthesia with 3.75% isofluran by perfusion of PBS through heart to displace blood from organs. Following organs were removed at necropsy for IVIS analysis of residual compound: heart, lungs, gut, spleen, kidneys, liver, bladder, thymus, and mesenteric lymph nodes. Mean radiance from ROIs of plasma, organs and full body images were compared between groups.

Ethics Statement

All animal work was performed according to national and international recognized guidelines and the animal experimental protocols approved by The Experimental Animal Inspectorate in Denmark under The Danish Veterinary and Food Administration, Ministry of Food, Agriculture and Fisheries (Id. number: 2014-15-0201-00377). All mice were housed in certified animal facilities in a 12-hours dark:light cycle, with temperature 22 $\pm$ 2°C and humidity of 55 $\pm$ 10 %. The animals had free accessibility to water and standard chow. Prior to treatment, the animals were housed 5 per cage in type III plastic cages (Techniplast, Italy) with wooden bedding (Finn Tapvei, Finland) enriched with hide shelters and gnawing softwood sticks. Experienced animal caretakers performed daily welfare monitoring and bodyweight measurement.

Cell culture

TZM-bl cells (obtained from NIH AIDS Reagent Program) were maintained in Dulbecco's Modified Essential Medium (DMEM) (Lonza, Switzerland) supplemented with 10% heat-inactivated fetal bovine serum (FBS), 50 U/mL penicillin and 50 µg/ml streptomycin (Invitrogen, Denmark), (cDMEM). Cells were grown on T75 flasks (ThermoFisher Scientific, Denmark) at 37 °C with 5% CO_2, and passaged 2-3 times each week with 1% trypsin with 0.02% EDTA in PBS pH 7.4

Viruses

HIV-1 HXB3/BaL Infectious Molecular Clone (pWT/BaL) (NIH AIDS Research and Reference Reagent Program, USA cat. no. 11414) was generated by transfection of HEK 293T cells using Lipofectamine 3000 reagents (ThermoFischer Scientific, Denmark). Briefly, HEK 293T cells were seeded at 4.5×10^4 per cm^2 on a T75 flask in cDMEM and transfected with 30 µg of HIV-1_{Bal} plasmid, 45 µL of lipofectamine and 60 µL of P3000 according to the protocol provided by the manufacturer. 24 h after transfection the cell media was renewed, and 48 h post transfection virus-containing supernatant was harvested, filtered through a 0.22 µm filter and stored at -80° C. TCID50 was determined by infecting TZM-bl cells and measuring luminescent signal. The calculations of TCID50 were done using a Reed-Muench formula. HIV-1 strain HxB2D (NIBSC Programme EVA Centre for AIDS Reagents from R. Gallo and M. Popovic, cat.no. ARP206) was generated using an identical approach as for BaL strain.

TZM-bl HIV infectivity assay

TZM-bl cells were seeded in 96-well flat-bottomed culture plates (Sarstedt, USA) at a density of 10^4 cells per well and cultured overnight. Cells were then pre-incubated with polymers at indicated concentrations for 24 h and subsequently infected with HIV-1_{Bal} strain (60 x TCID50/mL). 48 h after viral infection media was removed and cells were lysed in 100 µL 0.5% Nonidet P-40 (Struers Kebo Lab, Denmark) in PBS supplemented with 0.9 mM $CaCl_2$ and 0.5 mM $MgCl_2$ for at least 45 min in order to inactivate the virus. Luciferase activity proportional to the level of infection was measured by adding 80 µL of Britelite plus reagent (Perkin-Elmer, Denmark) per well. After mixing, 150 µL of the solution was transferred to white 96-well plates (Perkin-Elmer, Denmark). Luciferase activity was quantified by measuring luminescent signal on a FLUOstar Omega plate reader (BMG Labtech Germany).

Isolation and infection of human primary CD4+ T cells

For viral inhibition mediated by APD we used CD4+ cells harvested from healthy human donors. Briefly, whole blood was drawn from a donor and PBMCs were isolated using the Sepmate™ Ficoll (Stemcell) isolation protocol. Cells were frozen in FBS with 10% DMSO in liquid nitrogen until day of experiment. For experiment, PBMCs were thawed and quickly resuspended in cRPMI (RPMI + 10% heat-inactivated FBS + 50 U/mL penicillin and 50 µg/ml streptomycin (Invitrogen, Denmark)) +50 U/mL IL-2 and counted. CD4+ cells were subsequently isolated following Miltenyi CD4+ T cell isolation kit protocol. Briefly, target cells are selected through negative selection, where all other cells are stained by magnetically labelled antibodies, which binds to magnetic beads that retains them in a column system.

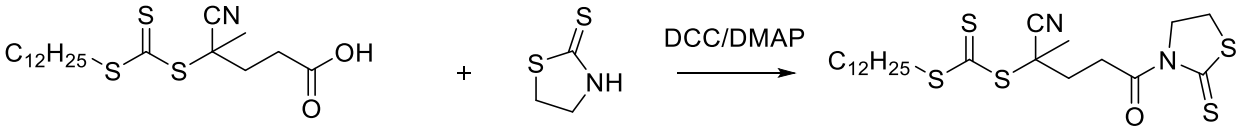
After isolation, the CD4+ cell population was stimulated in cRPMI supplemented with 1% PHA + 50 U/mL IL-2 at concentration 1 mill/mL and incubated at 37 °C with 5% CO_2. 48 h after stimulation with PHA and IL-2 cells appeared clumped which indicates that the population is activated. Cells werecounted and resuspended to 2 mill/mL in cRPMI with 50 U/mL IL-2 with either APD, the corresponding MP with AZT and 3TC, pristine AZT and 3TC, pristine albumin or nothing. Final concentration of antiviral drugs in all solutions was 1 µM AZT and 0.78 µM 3TC. Immediately following incubation with drug, cells were infected with HIV (strain: HxB2). After addition of virus, cells were incubated 1h at 37 °C with 5% CO_2, then spinoculated at 1250 g for 1 h at 30 °C, and subsequently incubated for 2 h at 37 °C with 5% CO_2. After this incubation, cells were washed 5 times with prewarmed cRPMI to remove all excess virus, and then resuspended at a concentration of 2 million cells per mL in cRPMI containing 50 U/mL IL-2 and the appropriate antiviral compounds. Cells were incubated at 37 °C with 5% CO_2, and supernatant was harvested from triplicate wells at time points 0, 24, 48, 72, 96 h after infection with HIV. Supernatants were inactivated with empigen in PBS for at least 45 minutes, and subsequent used for p24 ELISA.

p24 ELISA

Level of HIV-1 p24 in supernatants was measured by an in-house p24 ELISA, using purified sheep anti-HIV-1-p24 gag antibody (Aalto Bio) for capture of p24 in supernatants, and for detection, a biotinylated mouse anti-HIV-1-p24 gag antibody (Aalto Bio) was used.

Detailed experimental procedures.

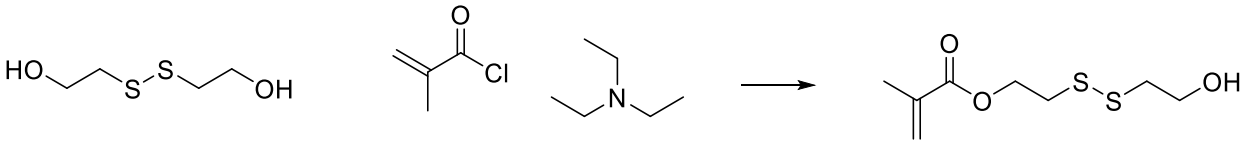
Synthesis of 2-cyano-5-oxo-5-(2-thioxothiazolidin-3-yl)pentan-2-yl dodecyl carbonotrithioate (TA RAFT agent).



The RAFT agent, 4-cyano-4-(((dodecylthio)carbonothioyl)thio)pentanoic acid, (0.217 g, 0.538 mmol, 1 eq.) was dissolved in DCM (10 mL), 2-mercaptothioazoline (0.072 g, 0.604 mmol, 1.1 eq.), DMAP (0.008 g, 0.065 mmol, 0.1 eq.) and DCC (0.128 g, 0.620 mmol, 1.2 eq.) were dissolved in DCM (5 mL) and added under N_2 atmosphere. The reaction was stirred for 5 h at room temperature. The reaction mixture was filtered and concentrated *in vacuo*. The concentrate was purified by flash column chromatography 1:3 EtOAc/Pentane yielding the pure product (174 mg, 0.344 mmol, 64 %).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 4.51 (t, J = 7.5 Hz, 2H), 3.63 – 3.37 (m, 2H), 3.25 (td, J = 7.5, 3.9 Hz, 4H), 2.48 (m, 2H), 1.82 (s, 3H), 1.63 (m, 2H), 1.39 – 1.27 (m, 2H), 1.26 – 1.14 (m, 16H,), 0.90 – 0.74 (m, 3H).
Rf (1:3 EtOAc/Pentane) 0.42

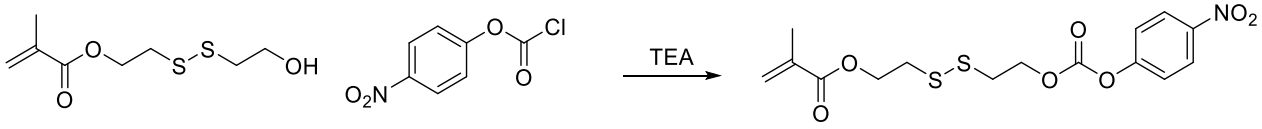
Synthesis of 2-((2-hydroxyethyl)disulfanyl)ethyl methacrylate
The synthesis was performed as described by Kock et al (30).



2-hydroxyethyldisulphide (11.7 mL, 95.7 mmol, 2 eq.) was dissolved in DCM (250 mL) and flushed with N_2. Triethylamine (13.3 mL, 95.7 mmol, 2 eq.) was added followed by methacryloyl chloride (4.7 mL, 47.8 mmol, 1 eq.) dropwise at 0 ^oC and the reaction was subsequently stirred for 1 hour. The temperature was increased to room temperature and the mixture stirred further 1.5 h. The reaction was quenched with ammonium chloride, washed with water and brine. All three aqueous phases were extracted with DCM upon washing. The combined organic phase was dried over anhydrous sodium sulphate and concentrated *in vacuo*. The crude product was purified by flash column chromatography with EtOAc/Pentane 1:4 to 4:6. The product was dried yielding the pure product as a colorless oil (8.109 g, 36.47 mmol, 76 %).

^{1}H-NMR (400 MHz, CDCl3) δ 6.14 (s, 1H), 5.60 (s, 1H), 4.42 (d, J = 6.6 Hz, 2H), 3.89 (m, 2H), 3.02 – 2.92 (m, 2H), 2.88 (m, 2H), 1.95 (s, 3H).
R_f (3:7 EtOAc/Pentane) = 0.31

Synthesis of 2-((2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)disulfanyl)ethyl methacrylate

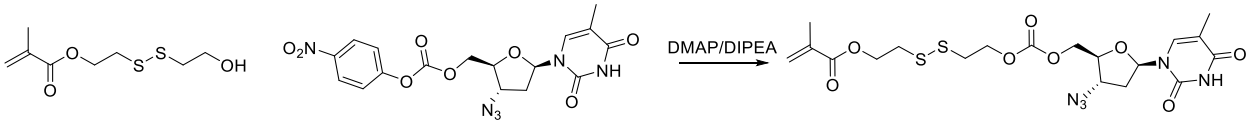


2-((2-hydroxyethyl)disulfanyl)ethyl methacrylate (1.00 g, 4.5 mmol, 1 eq.) and paranitrophenol chloroformate (0.998 g, 4.95 mmol, 1.1 eq.) were dissolved in DCM (8 mL) and cooled to 0 °C under N_2 atmosphere. Triethylamine (1.25 mL, 9.0 mmol, 2 eq.) was added dropwise at 0 °C. The reaction mixture was allowed to heat to room temperature and stirred for 1 hour. The reaction was quenched with ammonium chloride, washed with brine, dried over sodium sulphate, filtered and concentrated. The crude was purified by flash column chromatography 1:4 to 0:1 heptane/DCM yielding the pure product (1.204 g, 3.0 mmol, 67%)

^{1}H-NMR (400 MHz, $CDCl_3$) δ 8.39 – 8.21 (m, 2H), 7.50 – 7.34 (m, 2H), 6.13 (s, 1H), 5.68 – 5.56 (m, 1H), 4.55 (t, *J* = 6.5, 2H), 4.43 (t, *J* = 6.6 Hz, 2H), 3.13 – 2.93 (m, 4H), 1.94 (s, 3H).

Synthesis of 2-((2-(((((2*S*,3*S*,5*R*)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methoxy)carbonyl)oxy)ethyl)disulfanyl)ethyl methacrylate

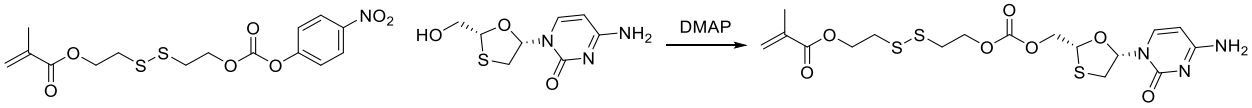
The synthesis was performed as described by Kock et al (30),



2-((2-hydroxyethyl)disulfanyl)ethyl methacrylate (1.681 g, 6.73 mmol, 3 eq.) was dissolved in DCM (200 mL). First DMAP (117 mg, 0.96 mmol, 0.4 eq.) and DIPEA (2.4 mL, 14.0 mmol, 6 eq.) dissolved in DCM (60 mL) and subsequently ((*2S,3S,5R*)-3-azido-5-(5-methyl-2,4-dioxo-3,4-dihydropyrimidin-1(2H)-yl)tetrahydrofuran-2-yl)methyl (4-nitrophenyl) carbonate (1.059 g, 2.45 mmol, 1 eq.) in DCM (60 mL). The reaction was performed at room temperature for 21.5 h. The reaction was quenched with ammonium chloride, washed with brine, washed with water, dried over Na_2SO_4 and filtered. The crude reaction mixture was purified by flash column chromatography 1:1 EtOAc/Pentane yielding the pure product (1.245 g, 2.45 mmol, 65%).

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 8.22 (s, 1H), 7.35 (q, *J* = 1.2 Hz, 1H), 6.20 (t, *J* = 6.2 Hz, 1H), 6.13 (dq, *J* = 2.0, 1.0 Hz, 1H), 5.60 (td, *J* = 1.6, 0.8 Hz, 1H), 4.59 – 4.36 (m, 6H), 4.28 (q, *J* = 5.8 Hz, 1H), 4.09 – 4.00 (m, 1H), 2.96 (m, 3H), 2.58 – 2.30 (m, 2H), 1.98 – 1.89 (m, 6H).

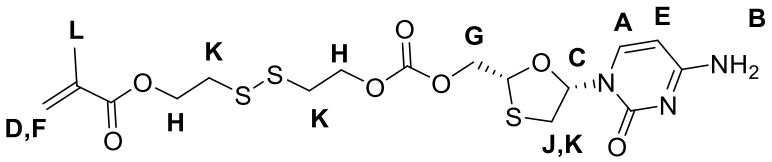
Synthesis of 2-((2-(((((2*R*,5*S*)-5-(4-amino-2-oxopyrimidin-1(2H)-yl)-1,3-oxathiolan-2-yl)methoxy)carbonyl)oxy)ethyl)disulfanyl)ethyl methacrylate



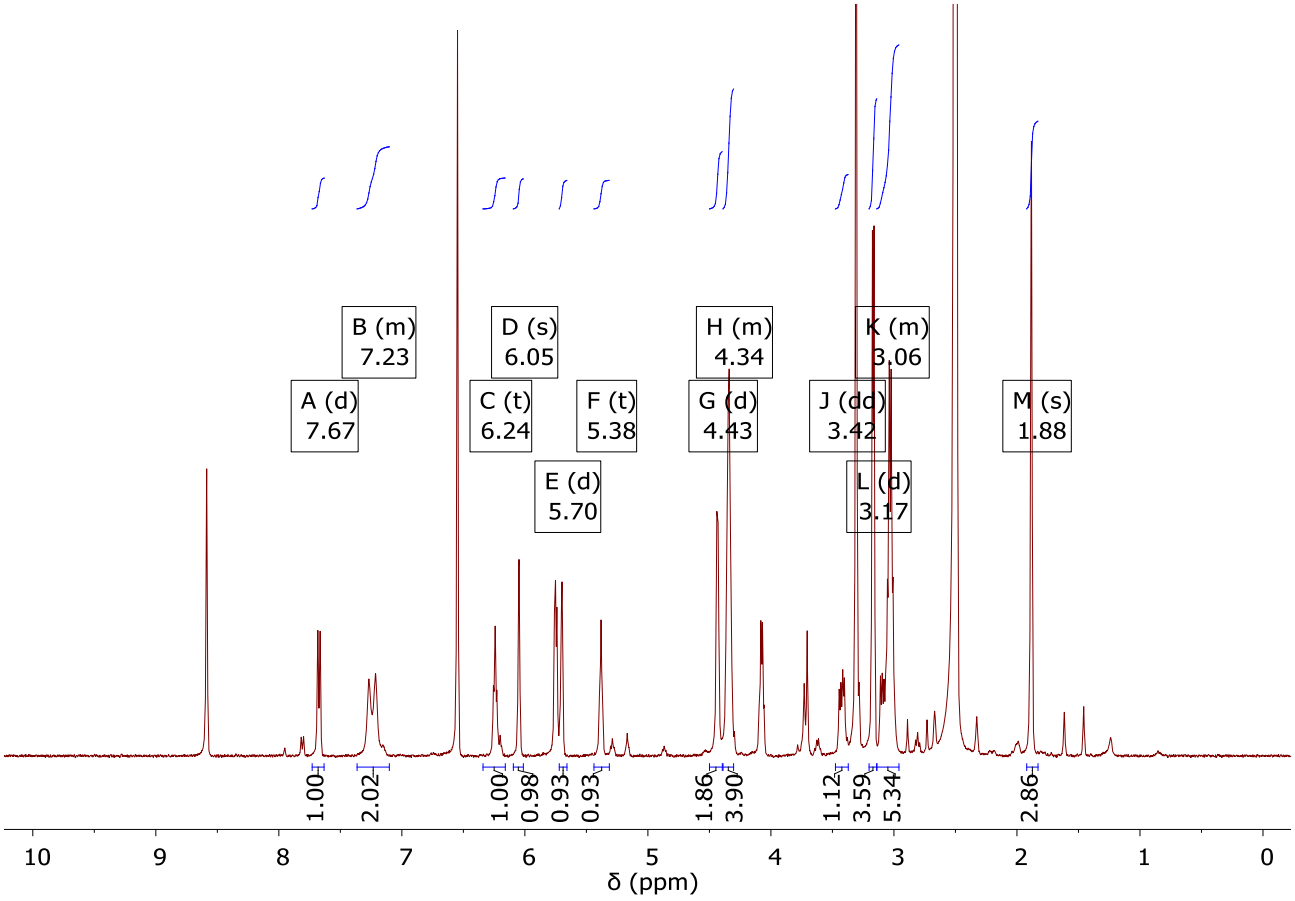
2-((2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)disulfanyl)ethyl methacrylate (0.833 g, 2.15 mmol, 1 eq.) was dissolved in THF (8 mL). DMAP (0.064 g, 0.52 mmol, 0.2 eq.) and

lamivudine (0.896 g, 3.91 mmol, 1.8 eq.) were dissolved in DMF (11 mL) and added. The reaction was stirred at room temperature for 11 h and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography 2% to 10% MeOH in DCM, yielding the product (0.845 g, 1.76 mmol, 82 %).

Rf (1:9 MeOH in DCM) 0.36

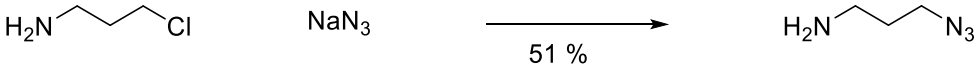


^{1}H NMR (400 MHz, DMSO-d_6) δ 7.67 (d, *J* = 7.5 Hz, 1H, A), 7.36 – 7.10 (m, 2H, B), 6.24 (t, *J* = 5.6 Hz, 1H, C), 6.05 (s, 1H, D), 5.70 (s, 1H, F), 5.38 (t, *J* = 4.5 Hz, 1H, E), 4.43 (d, *J* = 4.5 Hz, 2H, G), 4.39 – 4.30 (m, 4H,H), 3.42 (dd, *J* = 11.6, 5.4 Hz, 1H, J), 3.14 – 2.96 (m, 5H, K), 1.88 (s, 3H, L).



Synthesis of 3-azidopropan-1-amine

Synthesis of 3-azidopropan-1-amine was performed as described by Ebbesen et al (41).

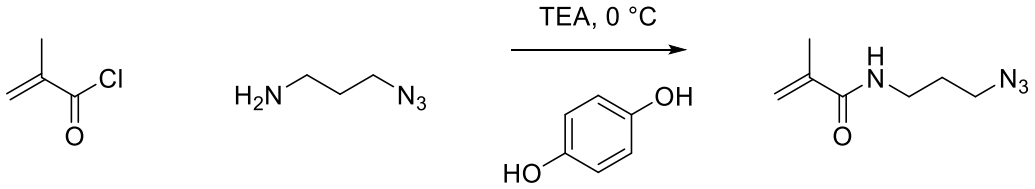


3-chloropropylamine (2.09 g, 22.35 mmol, 1 eq.) and sodium azide (4.71 g, 72.42 mmol, 3.2 eq.) were dissolved in H_2O (20 mL) and reacted at 80 °C for 17 h. The mixture concentrated *in vacuo*, then DCM (50 mL) and KOH (2.2 g) were added while stirring and keeping the temperature around 0° C. The mixture was filtered and subsequently extracted with

diethylether three times. The organic phase was dried over $MgSO_4$, filtered and concentrated yielding the pure product (0.826 g, 8.25 mmol, 51 %)

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 3.37 (t, *J* = 6.7 Hz, 2H), 2.80 (t, *J* = 6.8 Hz, 2H), 1.72 (p, *J* = 6.8 Hz, 2H), 1.20 (s, 2H).

Synthesis of *N*-(3-Azidopropyl)methacrylamide (AzMA)



3-azidopropan-1-amine (0.678 g, 6.77 mmol, 1 eq.), hydroquinone (0.09 g, 0.08 mmol, 0.01 eq.) and triethylamine (1.3 mL, 7.17 mmol, 1.1 eq.) were dissolved in DCM (6 mL) and methacryloyl chloride (0.8 mL, 8.19 mmol, 1.2 eq.) was added at 0 °C over 20 min. The reaction was then stirred for 1 hour at 0 °C followed by 14 h at room temperature. The mixture was washed twice with 1 M HCl, twice with 1 M NaOH,twice with water, dried over $MgSO_4$, filtered, and concentrated *in vacuo*. The crude mixture was purified by flash column chromatography 2% MeOH in DCM, yielding the pure product.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 6.04 (s, 1H), 5.67 (s, 1H), 5.33 (s, 1H), 3.45-3.30 (m, 4H,), 1.96 (s, 3H), 1.87-1.78 (m, 2H)

Synthesis of 2-((2-(((3-azidopropyl)carbamoyl)oxy)ethyl)disulfanyl)ethyl methacrylate

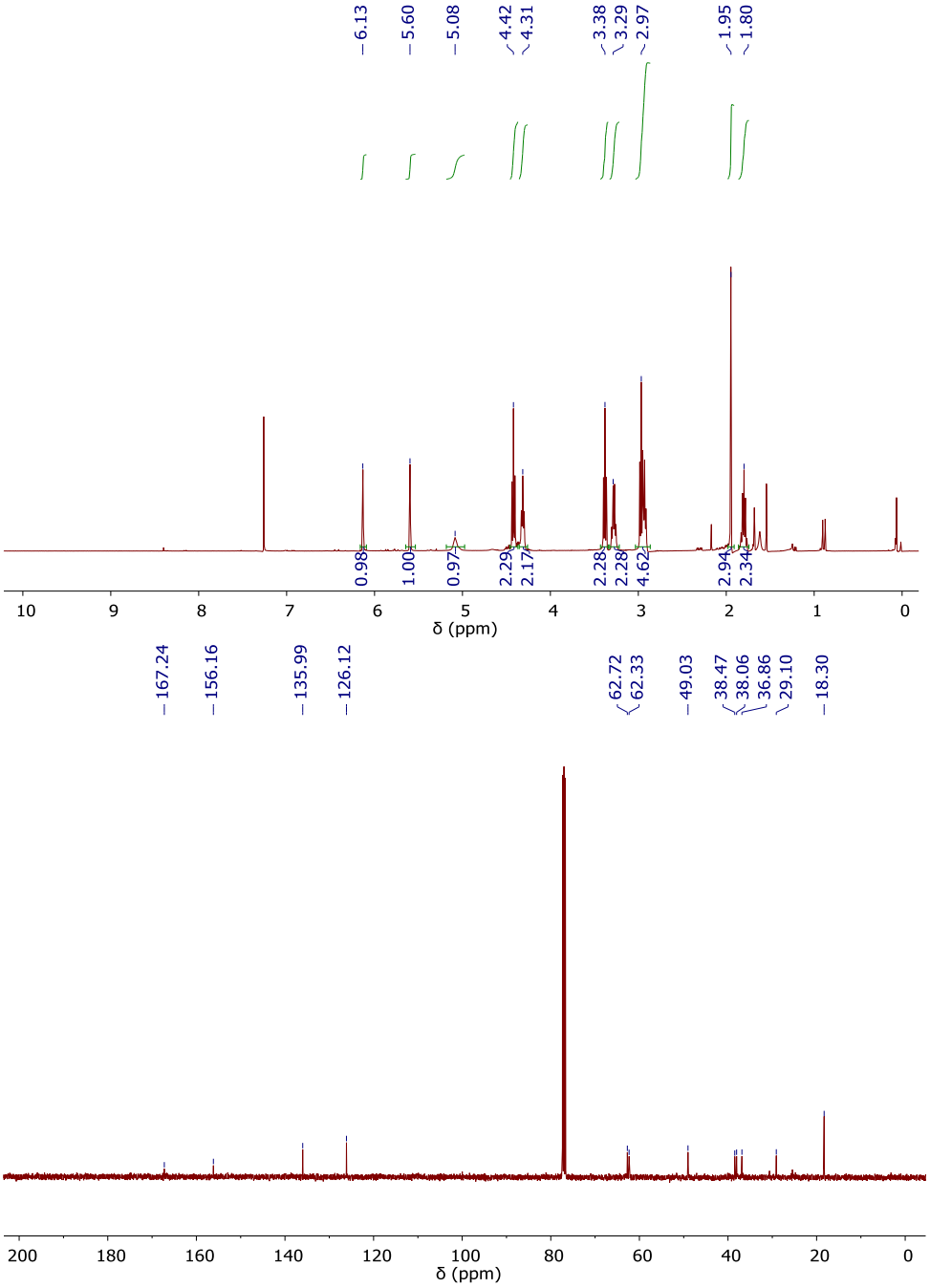
2-((2-(((4-nitrophenoxy)carbonyl)oxy)ethyl)disulfanyl)ethyl methacrylate (0.297 g, 0.80 mmol, 1 eq.) was dissolved in THF (2 mL). DMAP (0.018 g, 0.15 mmol, 0.2 eq.) and 3-azidopropan-1-amine (0.174 g, 1.74 mmol, 2.2 eq.) were dissolved in DMF (2 mL) and added dropwise at room temperature. The mixture changed color to yellow immediately and was stirred at room temperature for 16.5 h. The mixture was diluted with DCM, washed with H_2O, washed with 0.2 M $NaHCO_3$ twicce, washed with ammonium chloride twice, washed once with H_2O, dried over MgSO4, filtered, and concentrated.

^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm) 6.13 (s, 1H), 5.60 (s, 1H), 5.08 (bs, 1H), 4.42 (t, *J* = 6.8 Hz, 2H), 4.31 (t, *J* = 6.3 Hz, 2H), 3.38 (t, *J* = 6.6 Hz, 2H), 3.29 (q, *J* = 6.5 Hz, 2H), 2.97 (m, 4H), 1.95 (t, *J* = 1.3 Hz, 3H), 1.80 (m, 2H).

^{13}C-NMR (100 MHz, $CDCl_3$) δ 167.24, 156.16, 135.99, 126.12, 62.72, 62.33, 49.03, 38.47, 38.06, 36.86, 29.10, 18.30.

HRMS(ESI): *m/z* calcd. for $C_{12}H_{21}N_4O_4S_2$ [M+ H^+] = 349.0999; found 349.1002, *m/z* calcd.

for $C_{12}H_{20}N_4O_4S_2Na$ [M+Na^+]= 371,0818, found 371,0820. *m/z* calcd. for $C_{12}H_{20}N_4O_4S_2K$ [M+K^+]: 387.0558, found 387.0581



PHPMA and macromolecular prodrugs.

General procedure :
HPMA, drug containing monomer, AIBN, and TA RAFT agent were dissolved in DMF (0.4-0.8 mL). Four rounds of freeze-pump-thaw were performed upon which the ampule was flame sealed. The polymerization was performed at 60 °C for 17-20 h. The reaction mixture was precipitated into Et_2O:acetone 1:1 and filtered and washed yielding the pure product.

HPMA-co-3TC(SIL)MA:
^{1}H-NMR (400 MHz, DMSO) δ (ppm) 7.96 (s), 7.71 (s), 7.21 (s, 1H), 6.27 (s), 5.78 (s), 5.40 (s), 4.71 (s, 1H), 4.42 (m), 4.12 (s), 3.68 (s, 1H), 3.17 – 2.83 (m, 2H), 0.92 (m, 8H).

HPMA-co-AZT(SIL)MA:
^{1}H-NMR (400 MHz, DMSO) δ (ppm) δ 11.38 (s), 7.96 (s), 7.48 (s), 7.20 (s, 1H), 6.15 (s), 4.71 (s, 1H), 4.58 – 3.87 (m), 3.68 (s, 1H), 2.91 (s, 2H), 2.01 – 0.61 (m, 8H).

	n(carrier) (mmol)	n(drug monomer) (mmol)	n(TA RAFT) (mmol)	n(AIBN) (mmol)
PHPMA-3TC	1.01	0.0921	0.00803	0.00201
PHPMA-AZT $_1$	1.00	0.116	0.00887	0.000883
PHPMA-AZT $_2$	1.02	0.114	0.00559	0.00114
PHPMA-AZT $_3$	1.02	0.114	0.00559	0.00111

PHPMA-co-AZT(SIL)-co-3TC(SIL)

TA-RAFT agent, V-70, 3-TC(SIL)MA, and AZT(SIL)MA were all prepared as stock solutions in DMF. The stock solutions were added to HPMA together with additional DMF to obtain a total volume of 0.4 mL. Five rounds of freeze-pump-thaw were performed upon which the ampule was flame sealed. The polymerization was performed at 30 °C for 23 h. The reaction mixture was precipitated into DCM and filtered, yielding the pure product.
^{1}H-NMR (400 MHz, DMSO) δ (ppm) 11.28 (s) 7.71 (s), 7.27 (s, 1H), 6.21 (s), 5.77 (s), 5.41 (s), 4.71 (s, 1H), 4.42 (m), 4.12 (s), 3.68 (s, 1H), 2.98 (s, 2H), 2.19 – 0.53 (m, 8 H).

	n (HPMA)	n (AZT-MA)	n. (3TC-MA)	n(TA RAFT)	n(V-70)	Mass recovered (mg)
PHPMA-(AZT+3TC) 1	1.06	0.0749	0.0369	0.00624	0.00125	54
PHPMA-(AZT+3TC) 2	1.04	0.0367	0.0739	0.00612	0.00122	69
PHPMA-(AZT+3TC) 3	1.06	0.0562	0.0555	0.00624	0.00123	66

Synthesis of albumin conjugates

The synthesis of all albumin conjugates followed the same general procedure outlined below:

4 mg albumin in 200 µL PBS was added to the TA polymer (5 eq) dissolved in DMSO (0.02 mL) and diluted to 0.2 mL in PBS giving a total volume of 0.4 mL. The reaction was left for 24 hand analyzed by GPC (25 µL taken out). The reaction mixture was spin-filtered on a 30 kDa membrane and washed three times with milli Q (0.4 mL, 1100 rpm) The residue was then diluted with 525 µL milliQ (25 µL taken out for pure GPC) and aliquoted into Eppendorph tubes and lyophilized.

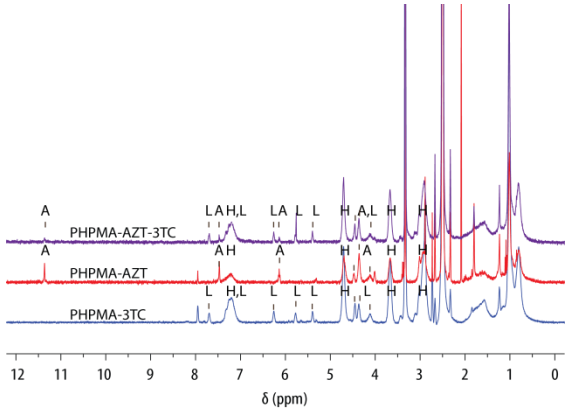


Figure S1. ^{1}H NMR spectra for MP containing AZT, 3TC, or a combination of drugs conjugated to the same carrier PHPMA polymer. The peaks are assigned with letters L=lamivudine, A=azidothymidine and H=HPMA

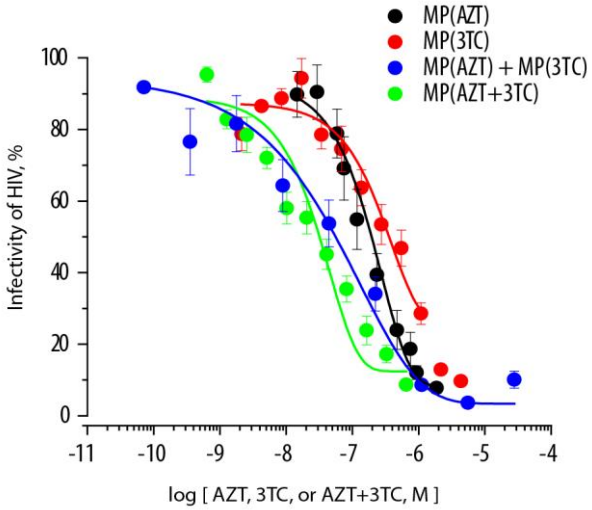


Figure S2. Dose response curves obtained for AZT, 3TC, and MP as inhibitors of infectivity of HIV, as studied in TZM-bl cells and using HIV-1_{Bal} virus strain. Results shown are average of three independent experiments ± SEM.

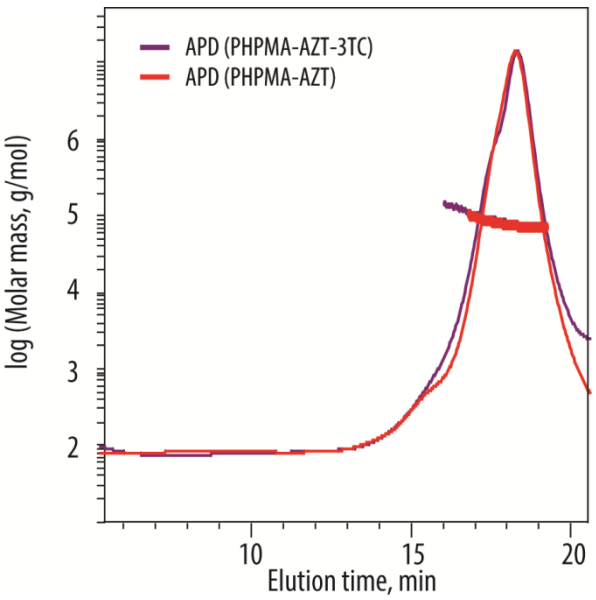


Figure S3 SEC-MALS eluegram of APD single and double drug-loaded. No aggregation is observed, illustrating the colloidal stability of the albumin-polymer drug-conjugates.

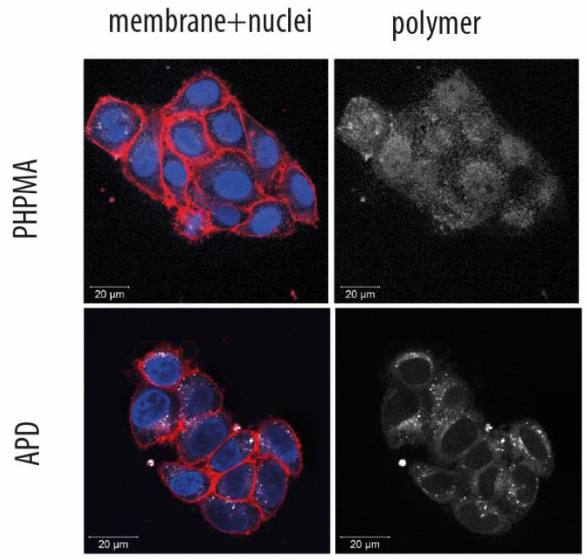


Figure S4. Confocal laser scanning microscopy imaging of TZM-bl cells upon incubation with fluorescently labelled PHPMA or albumin-PHPMA: mebrane is false-colored red, nuclei in blue, and PHPMA (as polymer and whithin APD) in white.

	Intravenous injection		Subcutaneous injection	
	Day 1	**Day 7**	**Day 1**	**Day 7**
APD	17.7 ± 0.8	19.3 ± 1.6	18.6 ± 0.9	20.9 ± 2.1
PHPMA	18.5 ± 0.8	20.2 ± 0.6	19.1 ± 1.2	20.2 ± 0.7
Control	19.2 ± 0.8	20.8 ± 0.2	18.3 ± 1.2	19.8 ± 0.7

Table S1 Mouse weight (g) ± SD. Mice were weight during the course of the experiment to assess overall health.

Table S2: Statistical evaluation of organ accumulation for APD and MP (shown in Figure 1 in the main text)

Vertical

Lymph node

	7 days	24 hours
	p value	p value
APD, S.C. vs. PHPMA, S.C.	0.0655	>0,9999
APD, S.C. vs. APD, I.V.	0.0002	0.9276
APD, S.C. vs. PHPMA, I.V.	<0,0001	0.4254
PHPMA, S.C. vs. APD, I.V.	0.0563	>0,9999
PHPMA, S.C. vs. PHPMA, I.V.	0.0108	>0,9999
APD, I.V. vs. PHPMA, I.V.	>0,9999	>0,9999

Liver

	7 days	24 hours
	p value	p value
APD, S.C. vs. PHPMA, S.C.	0.0006	0.0016
APD, S.C. vs. APD, I.V.	<0,0001	<0,0001
APD, S.C. vs. PHPMA, I.V.	<0,0001	<0,0001
PHPMA, S.C. vs. APD, I.V.	<0,0001	<0,0001
PHPMA, S.C. vs. PHPMA, I.V.	<0,0001	<0,0001
APD, I.V. vs. PHPMA, I.V.	>0,9999	>0,9999

Spleen

	7 days	24 hours
	p value	p value
APD, S.C. vs. PHPMA, S.C.	0.2033	<0,0001
APD, S.C. vs. APD, I.V.	<0,0001	<0,0001
APD, S.C. vs. PHPMA, I.V.	<0,0001	<0,0001
PHPMA, S.C. vs. APD, I.V.	0.0059	<0,0001
PHPMA, S.C. vs. PHPMA, I.V.	0.0077	<0,0001
APD, I.V. vs. PHPMA, I.V.	>0,9999	0.0614

Longitudinal

Lymph node

APD, S.C.	**p value**
1h vs. 24h	0.0263
1h vs. 7 days	0.0004
24h vs. 7 days	0.0468

PHPMA, S.C.	**p value**
1h vs. 24h	0.032
1h vs. 7 days	0.0081
24h vs. 7 days	0.7776

APD, I.V.	**p value**
1h vs. 24h	0.163
1h vs. 7 days	>0,9999
24h vs. 7 days	0.0515

PHPMA, I.V.	**p value**
1h vs. 24h	0.0961
1h vs. 7 days	0.06
24h vs. 7 days	0.0014

Liver

APD, S.C.	**p value**
1h vs. 24h	<0,0001
1h vs. 7 days	<0,0001
24h vs. 7 days	0.0097

PHPMA, S.C.	**p value**
1h vs. 24h	<0,0001
1h vs. 7 days	<0,0001
24h vs. 7 days	0.0006

APD, I.V.	**p value**
1h vs. 24h	0.5295
1h vs. 7 days	>0,9999
24h vs. 7 days	0.1589

PHPMA, I.V.	**p value**
1h vs. 24h	0.1049
1h vs. 7 days	0.0026
24h vs. 7 days	0.0001

Spleen

APD, S.C.	**p value**
1h vs. 24h	<0,0001
1h vs. 7 days	<0,0001
24h vs. 7 days	0.0005

PHPMA, S.C.	**p value**
1h vs. 24h	<0,0001
1h vs. 7 days	0.0003
24h vs. 7 days	0.0013

APD, I.V.	**p value**
1h vs. 24h	0.3421
1h vs. 7 days	0.0007
24h vs. 7 days	0.0001

PHPMA, I.V.	**p value**
1h vs. 24h	0.6831
1h vs. 7 days	0.7355
24h vs. 7 days	0.0955