Chemicals

Anhydrous forms of dichloromethane (DCM), dimethylformamide (DMF), dimethylsulfoxide (DMSO), acetonitrile (ACN), diethylether and trifluoroacetic acid (TFA) were purchased from Acros Organics (Geel, Belgium). HGly-2-chlorotrityl resin (1.1mmol/g), Boc-glycine, Fmoc-glycine, Fmoc-alanine, triphenylisopropylsilane, *N*,*N*-diisopropylethylamine (DIPEA), piperidine, picrylsulfonic acid aqueous solution (1 M), sodium iodide and ethanol were obtained from Fluka (Buchs, Switzerland). The L-aminoacids Fmoc(tBu)-serine, Fmoc(Pbf)-arginine, as well as O-(7-azabenzotriazol-1-yl)-*N*,*N*,*N*,*N*-tetramethyluronium hexafluorophosphate (HATU) were purchased from Genscript (Piscataway, USA). Poly-L-lysine HBr (PL; 18 kDa) were provided by Sigma-Aldrich (Buchs, Switzerland). Pheophorbide *a* (Pb*a*) was purchased from Frontier Scientific (Carnforth, UK). mPEG-SPA (20 kDa) was purchased from Nektar (San Carlos, USA). $mPEO_8$-NHS, were provided by ThermoFisher Scientific (Erembodegem, Belgium).

Synthesis of prodrug

uPA-PPP was synthesized in three steps. The L-configured peptide GSGRSAG containing the reported urokinase minimal substrate was synthesized using standard Fmoc chemistry. Subsequently, NHS-activated pheophorbide a (Pb*a*) was coupled to the N-terminus of the peptide and the corresponding Pb*a*-peptide conjugate was purified by preparative RP-HPLC (Waters Delta 600 HPLC) on a C8, Nucleosil 300-10 column (Macherey–Nagel) using a 0.01%TFA/water/acetonitrile gradient and molecular mass was analyzed by ESI-MS, with a Finnigan MAT SSQ 7000 (Thermo Electron Co. Waltham, MA).

Pb*a*-peptide was then loaded on PL (25 units per 100 free epsilon-NH_2 groups of the PL). For this propose, Pb*a*-peptide (3.06 mg, 3.1×10^{-6}mol), PL 18 kDa (2.00 mg,

0.11×10^{-6}mol, 1.1×10^{-5}mol of $-NH_2$ functions), and HATU (1.36 mg, 4.03×10^{-6}mol, 1.3 equivalents based on Pb*a*-peptides to be activated) were dissolved in DMSO (0.65 mL) and, DIPEA (3.7 mg, 3.3×10^{-5}mol, 3 equivalents of free $-NH_2$ functions of PL) was added to the stirred solution. The reaction was carried out in the dark under argon for 4 hours at room temperature. Complete loading of the Pb*a*-peptide on PL was confirmed by analytical RP-HPLC.

The polymeric carrier was further modified by the covalent coupling with high molecular weight *methoxypoly*(ethylene glycol) (mPEG) chains and secondly, by capping the remaining epsilon-lysine residues with methyl octa-ethylene oxide ($mPEO_8$). For this propose, mPEG-SPA 20 kDa (1.91/3.83 mg, 9.56/19.1 $\times 10^{-8}$mol 1.1 equivalents based on the number of $-NH_2$ of PL in 0.2 mL DMSO) was added to the PL-Pb*a*-peptide solution under stirring and at 19 C°. The reaction was kept in the dark and left to proceed overnight at room temperature. Then, $mPEO_8$-NHS (3.61mg, 7.01 x $\times 10^{-6}$mol; 0.1 mL in DMSO) was added. The reaction was kept in the dark and left to proceed overnight at room temperature. The crude product was purified by size exclusion chromatography using a sephacryl™ S-100 (Amersham Biosciences, Otelfingen, Switzerland) column and a mixture of acetonitrile/water/TFA (30:70:0.0025) as eluent. The fractions containing the product were pooled, lyophilized and stored light-protected at − 20 °C until use.