Results

AnxA5-DTPA was synthesised in two steps as shown in Figure 1. In the first step, cyclic DTPA anhydride 1 was reacted with N-(2-aminoethyl)maleimide trifluoroacetate 2 in half-saturated sodium bicarbonate solution at 0°C (ice/water). The pH of the resulting solution must not exceed 7.0 in order to prevent premature hydrolysis of the maleimide functionality. Due to the symmetry of cyclic DTPA anhydride 1, the di-addition product 3 may form, which is minimised by using a large excess of cyclic DTPA anhydride 1 compared to amine 2. The reaction conditions were optimised for maximising the yield of target linker 4 by modifying a variety of parameters such as concentration and amounts of

AnxA5-DTPA 6 was synthesised in two steps as shown in Figure 1. In the first step, cyclic DTPA anhydride 1 was reacted with N-(2-aminoethyl)maleimide trifluoroacetate 2 in half-saturated sodium bicarbonate solution at 0°C (ice/water). The pH of the resulting solution must not exceed 7.0 in order to prevent premature hydrolysis of the maleimide functionality. Due to the symmetry of cyclic DTPA anhydride 1, the di-addition product 3 may form, which is minimised by using a large excess of cyclic DTPA anhydride 1 compared to amine 2. The reaction conditions were optimised for maximising the yield of target linker 4 by modifying a variety of parameters such as concentration and amounts of

Figure 1. Synthesis of anxA5-DTPA 6. 1) Excess of cyclic DTPA anhydride 1 was reacted with N-(2-aminoethyl)maleimide trifluoroacetate 2 in half-saturated sodium bicarbonate solution. 2) Reaction product 4 was regiospecifically coupled with the thiol group of cys-anxA5 5. Due to complete hydrolysis of excess anhydride 1 during a 2-h reaction, the reaction solution from the first step could be used directly without purification. The conjugate 6 was purified by gel filtration.