Anti-MUC1 aptamers: radiolabelling with $^{99m}$Tc and biodistribution in MCF-7 tumour-bearing mice
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($^{99m}$Tc anti-MUC1 aptamers)

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Abstract

Introduction: Aptamers previously selected against the protein core (AptA) or the
tumour glycosylated (AptB) MUC1 glycoprotein have been conjugated to MAG2 and
labelled with $^{99m}$Tc, for the potential use as radiopharmaceuticals for diagnostic
imaging of breast cancer.

Methods: The conjugation was achieved in high yield using standard peptide
coupling reactions between an amino modification on the aptamer and the activated
carboxylic group on the ligands. The retention of the affinity of the MAG2 modified
AptA for the MUC1 protein core was confirmed using a FID binding assay. The
labelled aptamers were separated from free $^{99m}$Tc using microcon filter separation and monitored by HPLC at all stages, to ensure that only radiolabelled aptamers were produced. The biodistribution properties of the two aptamer-radiouclide conjugates were analysed in MCF-7 tumour bearing mice and compared.

**Results:** Efficient and convenient labelling of the two aptamers with $^{99m}$Tc was achieved as the last step of the synthesis (post-conjugation labelling). Both the aptamer-chelator conjugates had strong $^{99m}$Tc binding properties and the resulting complexes were stable *in vivo*, both in terms of nuclease degradation and leaking of the metal. The radiolabelled aptamers showed a high renal clearance and a high uptake in the intestine.

**Conclusions:** AptA and AptB have been successfully conjugated in high yield to the ligand MAG2 and labelled with $^{99m}$Tc. The radiolabelled aptamers showed different tumour uptake and clearance, but will require further development prior to diagnostic use.

*Keywords:* Aptamers; glycosylated MUC1; $^{99m}$Tc; MAG2; MCF7 tumour; FID binding assay; Biodistribution studies

1. **Introduction**

MUC1 is a large, rode-like glycoprotein consisting of an N-terminus projecting away from the cell surface as a long filament made of tandem repeats, a transmembrane region and a C-terminus cytoplasmatic tail [1, 2].
Its functions are those typically associated with mucins such as lubrication and hydration of cell surfaces as well as protection from microorganisms and degradative enzymes [2].

MUC1 is widely expressed on normal epithelial tissues but when they become malignant, its expression is increased at least ten fold, and the glycosylation and distribution of the protein at the cell surface are modified. MUC1 is highly expressed by the majority of cancers and, in particular, by primary and metastatic breast cancers [3-8].

Molecular targeted radiotherapy is an area of continuing interest that has recently seen the approval of various radiolabelled antibodies for cancer therapy, particularly in the treatment of lymphoma and leukaemia [9-11]. However, the use of monoclonal antibodies (MAbs) as such therapeutic modalities has not been without significant problems, including the time period required to produce MAbs, their immunogenicity and size. The use of humanised antibodies has overcome the immunogenicity related problems, but this can further increase the cost and time of the antibody production.

Combinatorial chemistry techniques coupled with PCR amplification allows, through exponential enrichment, the evolution of DNA based molecules (aptamers) which can bind to almost any target, including extracellular proteins, antibodies, peptides and small molecules. Research on the rapid selection of aptamers based on the SELEX methodology forms the basis for the development of high affinity and specificity molecules, which bind to surface determinants of tumour cells [12-14]. The procedure is accomplished within a short period of time, compared with several months required for the selection and purification of specific monoclonal antibodies. Aptamers offer low immunogenicity, good tumour penetration, rapid uptake and fast systemic clearance, which favour their application as effective vehicles for cytotoxic agents or
radioisotopes [15-18]. To date few examples of radiolabelling of aptamers can be
found in literature showing that aptamers can be used as targeting agents in imaging
and in radio-therapy [19-21]. With regards to the potential efficacy of aptamers as
targeted radiopharmaceuticals in cancer therapy, we have previously shown that
aptamers have improved tumour penetration, when compared with antibodies [21]. It
is speculated that radiolabelled aptamers would have great potential as imaging
agents, given that the majority of the aptamer not bound to the tumour is cleared
rapidly from the system. In comparison, intact radiolabelled antibodies remain in
circulation for a very long time, often resulting in organ toxicity. On the other hand,
for therapeutic applications, the small molecular weight, and the inherent
hydrophilicity, would result in the majority of the aptamer being washed out of the
system too rapidly. Thus, in therapeutic applications, aptamers will need to be
modified, for example PEGylated, to improve their pharmacokinetic properties [20].

The selection of high affinity and specificity DNA aptamers against the highly
immunogenic epitope of the un-glycosilated protein core of the MUC1 glycoprotein
[22] and for the malignant glycosilated form of MUC1 [23] as tumour markers on
epithelial cancer cells has been previously reported.

A previous work has studied the labelling of the MUC1 protein core targeting aptamer
(AptA) with $^{99m}$Tc. Various chelating agents have been conjugated to the aptamer and
the biodistribution data of the labelled products showed differences depending on the
size of the conjugate and on the type of ligand [21].

We report the conjugation of the two different aptamers with MAG2, their efficient
radiolabelling with $^{99m}$Tc, and subsequent biodistribution studies in MCF7 tumour
bearing mice.
2. Results and discussion

2.1. Conjugation

NHS activated S-Benzoyl-MAG2 was conjugated to the aptamers at pH=9.3. In the case of the coupling of S-Benzoyl-MAG2 to AptA the HPLC analysis of the reaction mixture showed a new peak eluting at 16.5 min while the un-conjugated AptA eluted at 15.1 min. (Fig. 1). MALDI-MS analysis of the isolated new peak confirmed it as the desired S-Benzoyl-MAG2-AptA having a MW of 8160. The conjugation yield was 70-80% in the case of AptA and >80% for AptB.

HPLC analysis of S-Benzoyl-MAG2 and AptB reaction mixture showed the product and the un-conjugated AptB eluting respectively at 16.1 min and 14.5 min. (Fig. 1) MALDI-MS analysis of the isolated new peak showed a single product having the MW of 8301. The conjugation yield also in this case was high (>84%). The conjugation yields could be determined after confirming by UV-Vis calibration lines that the S-Benzoyl-MAG2 moiety does not contribute to the absorption of the conjugates at 260nm (data not shown).

Insert Fig. 1 here

2.2. FID Binding assay

Post-selection modifications on an aptamer can alter its original structure and overall charge and therefore its binding and affinity properties for the target.

The FID assay studies the displacement of \([\text{Ru(phen)}_2\text{DPPZ}]^{2+}\) from the aptamer due to the addition of the binding peptide [24-26]. AptA and MAG2-AptA, dissolved in the same solution previously used for the selection [22], are treated with the
intercalator which displays strong photoluminescence only when in contact with the DNA. The addition of the peptide results in a decrease of fluorescence due to the displacement of the bound ruthenium complex. The percent of the decrease is directly related to the extent of binding and therefore of the affinity of the aptamer to the peptide. A plot of the change in fluorescence versus equivalents of peptide (Fig.2) shows that the displacement of the Ru complex from the aptamer by the peptide is cooperative and that the conjugation of MAG2 to the aptamer has a little effect on the affinity. AptA and MAG2-AptA show a high affinity for the MUC1 peptide and the midpoints are reached after the addition respectively of 1140.5 equivalents of peptide with a cooperativity of 3.91 and 1300.7 equivalents of peptide with a cooperativity of 4.72. It seems that the conjugation of MAG2, a small molecule, to the aptamer has some influence upon the binding affinity to the target (small increase of midpoint and cooperativity) without, on the other hand, decreasing it. The 25 bases aptamer having unspecific binding affinity used as control showed only 33.5% of intensity decrease after the addition of 3800 molar equivalents of the MUC1 peptide.

**Insert Fig. 2 here**

### 2.3. Labelling with $^{99m}$Tc

The isolated S-Benzoyl-MAG2-aptamers (0.20-0.30 nmol) were labelled with $^{99m}$Tc using tartrate as transchelator, SnCl$_2$ as reducing agent, and heating the reaction mixture at 100ºC for 10 minutes. The reaction mixture were analysed by RP-HPLC. The complexes elutes at 17.8 min ($^{99m}$Tc-MAG2-AptA) and 16.3 min ($^{99m}$Tc-MAG2-AptB). The main impurity detectable was free pertechnetate (R$_t$ = 4.7 min). Neither $^{99m}$Tc-MAG2 nor $^{99m}$Tc-tartrate, having R$_t$ of 23.7 and 2.7 min respectively, were
detected. The radiolabelled aptamers were isolated from small molecular weight impurities by ultrafiltration using microcon \textsuperscript{3kDa} MWCO filters with a recovery of $>80\%$ of the radioactivity on the cartridge. HPLC analysis of the purified labelled products shows that $>99\%$ of the activity is associated to the aptamers (Fig. 3).

Insert Fig. 3

2.4. Stability of $^{99m}$Tc-labelled aptamers

Both the used aptamers where modified on the 3’ end with an inverted thymidine to protect them from nuclease digestion [27]. Previous studies already showed the stability (non fragmentation) of the modified aptamers in serum up to 12 hours [21].

The stability of the labelled aptamers was investigated in order to study the chelation strength of the molecule and to get an indication of the stability of the complex \textit{in vivo} in relation to the possible leaking of the radionuclide.

After labelling and purification by ultrafiltration, the aqueous solutions containing the labelled aptamers were stored at room temperature and analysed by HPLC every hours up to 3 hours. Both $^{99m}$Tc-aptamers showed a $>98\%$ radiochemical purity after three hours. The only impurity detectable was free pertechnetate.

Each isolated labelled aptamer was then tested for the stability towards cysteine [28]. The percentage of dissociation is $\leq 2\%$ at molar ratios below 60:1 after 3 hours from the addition of the challenger. The instability increases to a value of 8.7\% in the case of $^{99m}$Tc-MAG2-AptA and 12\% in the case of $^{99m}$TcMAG2-AptB at molar ratio 5000:1 (Fig.4).

An activity of 55-65 MBq of the radio-labelled conjugates was added to 3 ml of human blood and the mix was incubated at 37\degree C. The radiochemical purity was assessed by analyzing samples of plasma by ITLC using butanone as eluent every
hour for 3 hours to detect a possible metal leak from the complex. Samples of blood containing the same activity of $^{99m}$TcO$_4^-$ were used as control. $^{99m}$Tc-MAG2-Apts have an R$_f$ = 0 while pertechnetate in plasma has a R$_f$ = 0.5 differently from free $^{99m}$TcO$_4^-$ in aqueous solution that has an R$_f$ = 0.9. The complexes show no leaking of the radionuclide resulting in a radiochemical purity of >97% for both $^{99m}$Tc labelled aptamers after 3 hours (Fig.5).

**Insert Fig. 4 here**

**Insert Fig. 5 here**

2.5. **Biodistribution**

Figure 6, and Tables 1 and 2 show an overview of the biodistribution of $^{99m}$Tc-MAG2-AptA and $^{99m}$Tc-MAG2-AptB in MCF7 xenografts bearing nude mice. The mean values are calculated from the three mice in each group at 3, 5, 16, and 22 hours for each radiolabelled aptamer. The data are presented as percentage of the injected $^{99m}$Tc dose per gram of tissue (%ID/g).

**Insert Table 1 here**

**Insert Table 2 here**

**Insert Fig. 6 here**

As expected, the main routes of excretion of both small aptamers was via the urinary tract and through the hepatobiliary system. Both aptamers show an excretion of more than 70% of the activity in urine and faeces after 3 hours, increasing to 99% after 22 hours. After 3h p.i., 14.3% of $^{99m}$Tc-MAG2-AptA and 25.5% of $^{99m}$Tc-MAG2-AptB was found in the small intestine. The pattern is consistent with previous studies using $^{99m}$Tc radiolabelled MAG2 conjugated oligonucleotides [29, 30]. In general the two radiolabelled aptamers showed a similar distribution throughout the test animals.
The accumulation of $^{99m}$Tc-labelled aptamers in the tumour amounted to 0.051% ID/g (for AptA) and 0.044% ID/g (for AptB) after 3 hours p.i. The uptake increased to 0.12% ID/g (AptA) and 0.14% ID/g (AptB) at 5 h. After 22 h p.i., the activity decreased to 0.008% ID/g (AptA) and 0.013% ID/g (AptB) (Fig. 7).

**Insert Fig. 7 here**

Radiolabelled AptB shows a slightly higher tumour uptake than AptA at 5 h after injection. The activity, on the other hand, was released quicker from the target in the following 11 hours, reaching a plateau at 16 h to 22 h. The decrease of tumour accumulation of radiolabelled AptA is constant in time. After 22h p.i., 0.008% ID/g and 0.013% ID/g, corresponding to 1.37% and 1.8% of the total activity in the mice treated respectively with $^{99m}$Tc-MAG2-AptA and $^{99m}$Tc-MAG2-AptB, is present in the tumour. This behavior can be explained by the fact that both aptamers have high affinity for the extracellular target MUC1 but AptB is internalized in the cancer cells quicker than AptA. This theory can be confirmed by the tumour-blood ratios of radiolabelled AptA and AptB at 3, 5, 16, and 22 h time points (Fig. 8). After 5 h not only the tumour uptake of both the radiolabelled aptamers reaches the maximum value, but also their clearance. The clearance of AptA from the tumour decreases drastically at 16 h p.i. That means that the quantity of aptamer bound to the tumour is higher than the quantity of free aptamer circulating in blood. The clearance increases again at 22 h indicating that the bound aptamer was still on the surface of the tumour cells and not internalized. The aptamer could therefore be released in circulation with the soluble portion of MUC1 before entering the cytoplasm. The tumour-blood ratio of radiolabelled AptB on the other hand is constant, showing only an increase of clearance at 5h. Since the tumour uptake of the two aptamers at the different time points is very similar, we can assume that although AptB shows a higher clearance
than AptA, especially at 16h p.i., it shows a slighter higher tumour uptake due to a quicker internalization in the cancer cells.

3. Conclusions

The two aptamers (AptA and AptB) targeting two different forms of malignant MUC1 have been successfully conjugated in high yield (70%) to the ligand MAG2. The products were isolated by HPLC and characterised. MAG2-AptA was tested for its binding affinity for the target using [Ru(phen)$_2$DPPZ]$^{2+}$ in the Fluorescent Intercalator Displacement (FID) Assay. The small MAG2 molecule showed some influence upon the binding affinity of the aptamer to the target (small increase of midpoint and cooperativity) without, on the other hand, decreasing it.

The MAG2-aptamers were successfully labelled with $^{99m}$Tc. The complexes show stability in water, in the presence of an excess of a challenging ligand, and in blood for 3 hours.

The biodistribution of the purified $^{99m}$Tc-MAG2-Apts was studied in MCF7 xenografts bearing nude mice at 3, 5, 16, and 22 hours post injection. The data showed a similar distribution of the radiolabelled aptamers throughout the test animals and a high uptake in the intestine.

The tumour uptake and clearance data showed a different behaviour between the two aptamers suggesting a quicker internalisation in the tumour cells of AptB than AptA.

The tumour/tissue ratios achieved in the present study will need to be improved before using the above described aptamers as clinical radiolabelled targeting agents. Enhancement of their short circulating biological half-life could be a way to achieve
that goal. The rapid kidney clearance of aptamers may be altered either by increasing their overall molecular weight by conjugation to PEG [20, 31] or by attaching them to the surface of carrier systems such as liposomes [32, 33].

4. Acknowledgments

This study was conducted with financial support from the Breast Cancer Campaign. We thank David Lambert and Dr Huma Kahn for assistance with the FID assay. Elaine Blackshaw and Andrew McKenzie from the Nottingham University Hospital and Clinical Science, University of Nottingham for providing MCF7 tumour xenografts and their expertise in biodistribution studies.

5. Experimental Section

5.1. Instrumentation and Materials

The two DNA aptamers (AptA and AptB) were synthesised by RNA-TEC (Leuven, Belgium). The sequence of AptA is 5’-GCA GTT GAT CCT TTG GAT ACC CTG G-3’. The sequence of AptB is 5’-GGC GTA CGG TAG GC G GGG TCA ACT G-3’. Both sequences have 5’-aminohexyl linker and 3’-inverted thymidine. S-benzoyl-MAG2 and its NHS active ester were synthesised according to the literature[29]. All chemicals were purchased from Sigma Aldrich (Gillingham, UK). MALDI analyses were performed by EPSRC National Mass Spectrometry Service Centre (Swansea University). The conjugation reactions were monitored by RP-HPLC on a Waters 2690 Alliance system using a Phenomenex Gemini 3µm C18 110A column.
(150x2.00mm, 3µm) plus Gemini C18 4x2.0mm security guard cartridge (Phenomenex). The absorbance at the wavelength of 260nm was recorded on Waters 996 Photodiode Array UV-Vis detector. The solvent gradient at 0.25ml/min was: 0’: 60%A/40%C; 20’: 40%A/20%B/40%C; 30’: 40%A/20%B/40%C, where A=water, B=50%water/50% acetonitrile, C= 87.5% 0.1M NaCl/12.5% 0.1M phosphate buffer, pH 6.6. The total and constant ionic strength of the eluent was 0.04. [Ru(phen)_2DPPZ](PF_6)_2 was synthesised according to literature [25]. The MUC1 core peptide (APDTRPAPG) was prepared using standard peptide coupling automated synthesis and was supplied by the Oligonucleotide and Peptide Synthesis Unit of the Queen’s Medical Centre, University of Nottingham. The composition of the control aptamer is 12%A, 20%C, 32%G, and 36%T. Emission measurements were carried out with FluoroMax-P (Jobin Yvon Horiba). The calculation of the midpoints and cooperativity was done using the Hill equation [34]: y = y_∞ + (y_0 - y_∞)/(1 + (x/K)^n). The technetium-labelled products were analysed by Waters LC Module1 RP-HPLC using a Phenomenex Gemini 5 µm C18 110A column (250x4.60mm, 5 µm) plus Gemini C18 4x3.0mm security guard cartridge (Phenomenex). The eluate was monitored by UV-detection at 260 nm and on-line radiochemical detection (Harwell 600) using the same eluents and gradient as before but at a flow rate of 1ml/min. The MAG2-aptamers and the radiolabelled conjugates were desalted by ultrafiltration using Microcon 3kDa MWCO cartridges (Millipore). Radiochemical purity was determined by ITLC using acetone as solvent on 1cm x 6cm strips of Whatman No 1 paper and scanned using a NaI(Tl) scintillation detector with a collimated lead slit connected to a photomultiplier base with preamplifier (ORTEC, model 276). Data were analysed using a dedicated software package provided by the radiopharmacy unit in Medical Physics (Nottingham University Hospital). 20-21 weeks old MF1
nude female mice with MCF7 xenografts in the left flanks were supplied by the Cancer Studies Unit, University of Nottingham. Radioactivity in each organ was measured in a Packard Cobra II scintillation γ-counter. The animal experiments were conducted according to ethical committee approved protocols and regulations.

5.2. Syntheses of the conjugates

5-10 µl of a 1 mM solution of the aptamers in 0.1 M Carbonate buffer pH 9.3 were added to 5-10 µl of a 20mM solution of NHS activated S-Benzoyl-MAG2 in DMF. The reaction was allowed to proceed under vortex mixing at room temperature for 2 hours.

After conjugation, the S-Benzoyl-MAG2-aptamers were purified by RP-HPLC. The isolated peaks were lyophilised and desalted using microcon cartridges (3kDa MWCO). MALDI mass spectroscopy: S-Benzoyl-MAG2-AptA:[M+H]+=8160; S-Benzoyl-MAG2-AptB: [M+H]+ = 8301.

5.3. FID Binding Assay

A 50 nM solution of either AptA, MAG2-AptA or the control aptamer in 5mM MgCl₂·6H₂O, 100 mM NaCl (pH = 7.2) was titrated with a 0.1 mM solution of [Ru(phen)₂DPPZ](PF₆)₂ in 5mM MgCl₂·6H₂O, 100 mM NaCl (pH = 7.2) until saturation. The displacement of the aptamer-bound Ru complex was studied adding aliquots of a 20 mM solution of MUC1 core peptide in 5mM MgCl₂·6H₂O, 100 mM NaCl (pH = 7.2). The wavelength of excitation was 434 nm and the emission intensity was measured in the range 450-700 nm.
5.4. Labelling with $^{99m}$Tc

The isolates, desalted, and lyophilised S-Benzoyl-MAG2-aptamers (0.50-0.80 nmol) were dissolved 20 µl of 0.1 M carbonate buffer pH 9.3. Subsequently 3 mg of potassium sodium tartrate, 3-5 µl 1mM SnCl$_2$·2H$_2$O in 0.05M HCl, and 200-250 MBq of sodium pertechnetate eluate were added. The mixture was heated at 100°C for 10 min. The solution was allowed to cool down to room temperature before desalting using microcon cartridges (3kDa MWCO). The radiochemical purity was controlled by RP-HPLC.

5.5. Stability of $^{99m}$Tc-labelled MAG2-aptamers in solution

After labelling, the reaction mixtures were desalted using microcon cartridges (3kDa MWCO). The resulting solutions were stored at room temperature and analysed by HPLC every hour up to 3 hours.

5.6. Stability of $^{99m}$Tc-labelled MAG2-aptamers to excess of cysteine

To measure the stability of the $^{99m}$Tc-MAG2-conjugates to transchelation, a 60-, 500-, and -5000 fold excess of L-cysteine was added to each solution containing the isolated labelled aptamer. The reaction solutions were kept under vortex mixing at room temperature and analysed by HPLC every hour up to 3 hours. A sample without cysteine was used as control.
5.7. Stability of $^{99m}$Tc-labelled MAG2-aptamers in blood

An activity of 55-65 MBq of the isolated radio-labelled conjugates in saline was added to 3 ml of human blood. The mix was incubated at 37ºC. Samples (0.5ml) were taken every hour for 3 hours and centrifuged. The activity in the plasma fraction was analysed by instant thin layer chromatography (ITLC), eluent=butanone. $R_f$ $^{99m}$Tc-MAG2-Apts=0.
A sample of blood (3 ml) with the same activity of $^{99m}$TcO$_4^-$ was analysed as control. $R_f$ plasma-pertechnetate= 0.5.

5.8. Biodistribution in mice

Groups of 3 mice with MCF7 xenografts were intravenously injected with 250 µl of a saline solution containing ca 5 MBq of each purified and isolated $^{99m}$Tc complexes via the tail vein. The animals were then sacrificed at 3, 5, 16, and 22 hours post injection. The organs were dissected, weighed and the activity counted.


[25] Hiort C, Lincoln P, and Norden B. DNA binding of \( \Delta \) and \( \Lambda \)-

\([\text{Ru(phen)}_2\text{DPPZ}]^{2+}\) is in contact with DNA bases when it forms a luminescent complex with single-stranded oligonucleotides. J. Inorg. Biochem. 2005;99:994-1000.


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Fig.1. HPLC chromatograms of S-Benzoyl-MAG2-AptA (above) and S-Benzoyl-MAG2-AptB (below) reaction mixtures. The peak at 2.5 min is NHS. Un-conjugated S-Benzoyl-MAG2 elutes at 22.9-23.0 min. The peaks at 15.1 min and 14.5 min are respectively un-conjugated AptA and un-conjugated AptB. S-Benzoyl-MAG2-AptA elutes at 16.5 min and S-Benzoyl-MAG2-AptB elutes at 16.1 min.

Fig.2. FID curves of MUC1 peptide against AptA, MAG2-AptA and a control aptamer.

Fig.3. HPLC radiochromatograms of purified $^{99m}$Tc-labelled AptA (above) and AptB (below).

Fig.4. Dissociation of $^{99m}$Tc from labelled MAG2-AptA and MAG2-AptB after 3 hours incubation with different molar ratios of cysteine.

Fig.5. Stability of $^{99m}$Tc-MAG2-AptA and -AptB in blood.

Table1. Biodistribution in MCF7 xenografts bearing mice of $^{99m}$Tc-MAG2-AptA 3, 5, 16, and 22 hours after injection (n=3). Values are expressed as mean % uptake of injected doses per gram of tissue ± standard deviation (%ID/g ± SD).
Table 2. Biodistribution in MCF7 xenografts bearing mice of $^{99m}$Tc-MAG2-AptB 3, 5, 16, and 22 hours after injection (n=3). Values are expressed as mean % uptake of injected doses per gram of tissue ± standard deviation (%ID/g ± SD).

Fig. 6. The biodistribution of $^{99m}$Tc-MAG2-AptA (above) and $^{99m}$Tc-MAG2-AptB (below) in MCF7 xenografts bearing mice (n=3). The uptake of the radioactive tracer (percentage of injected $^{99m}$Tc dose per gram of tissue: %ID/g) is shown in various organs and tissues at 3, 5, 16, and 22 h after injection.

Fig. 7. Tumour uptake of $^{99m}$Tc-MAG2-AptA and $^{99m}$Tc-MAG2-AptB in MCF7 xenografts bearing mice at 3, 5, 16, and 22 h p.i.

Fig. 8. Tumour-blood ratio of $^{99m}$Tc-MAG2-AptA and $^{99m}$Tc-MAG2-AptB at 3, 5, 16, and 22 h p.i.