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Feasibility of the radioastatination of a monoclonal antibody with astatine-211 purified by wet extraction

Mickaël Bourgeois,[a,b]* François Guerard,[a] Cyrille Alliot,[a] Marie Mougin-Degraef,[a] Holisoa Rajérison,[a] Patricia Remaud-Le Saëc,[a] Jean-François Gestin,[a] François Davodeau,[a] Michel Chérel,[a] Jacques Barbet,[a] and Alain Faivre-Chauvet[a,b]

Astatine-211, a most promising α-particle emitter for targeted radiotherapy, is generally obtained by high-temperature distillation. However, a liquid–liquid extraction procedure (wet extraction) has also been described. The purpose of this study was to develop and optimize the labelling of the stannylated-activated ester *N*-hydroxysuccinimidyl-*meta*-trimethylstannylbenzoate ester (MeSTB) with astatine-211 extracted in di-isopropylether (DIPE) in the presence of the oxidant *N*-chlorosuccinimide (NCS). The effect of final volume, incubation duration and NCS amounts on radiolabelling yield was studied. The best yields (85–90%) of *N*-hydroxysuccinimidyl-*meta*-[^{211}At]astatobenzoate ester (SAB) were obtained with 20 nmol of MeSTB, 100 nmol of NCS in 120 μL of DIPE after 15 min. The astatine-211-labelled-activated ester was then used to radiolabel a monoclonal antibody (mAb). The labelling yield was 20–25% and the radiochemical purity was 97–99%. These results show that mAbs may be efficiently labelled with astatine-211 obtained by wet extraction, a fully automatable technique that may prove to be a useful alternative to dry distillation for high activity labelling of radiopharmaceuticals.

Keywords: astatine-211; SAB; radioimmunotherapy; monoclonal antibody; α-particle

Introduction

Monoclonal antibodies (mAbs) directed against tumour-associated antigens armed with various radionuclides are being investigated as therapeutic agents for the treatment of malignant diseases.[1] mAbs radiolabelled with β^--emitting radionuclides such as iodine-131 or yttrium-90[2] have demonstrated their efficacy in the treatment of haematologic malignant diseases. Radioimmunotherapy (RIT) with α-particle emitters (α-RIT) is also being considered very actively.[3] The radiobiological properties of α-particles, due to a high ionization potential per unit path length, are very attractive. The high linear energy transfer of α-particles results in relatively short effective path lengths in tissues and α-particles deliver a highly localized radiation dose to malignant cells.[4] This makes them highly cytotoxic with a relative biologic effectiveness 5–20 times that of β^--particles.[5,6] Another advantage of α-particles, compared with β^--particles, is that they exhibit a low dependence on dose rate and oxygen enhancement effects.[7]

Astatine-211 is the heaviest halogen and it is one of the most promising α-emitters for medical applications. The half-life of astatine-211 is relatively long compared with that of other radionuclides available for α-RIT ($T_{1/2}$ = 7.2 h). Its potential utility for radiotherapy has been recognized 50 years ago.[8,9] The main difficulty of protein radiolabelling with astatine-211 is the imperfect knowledge of the chemistry of astatine because there is no stable isotope of this element. Moreover, proteins directly labelled with astatine-211 de-astatinate rapidly both *in vitro* and *in vivo*.[10,11]

Astatine-211 is generally purified by dry distillation.[12] However, this technique has been described as affording variable yields for the high activities required for clinical trials.[13] Thus, new approaches that could reproducibly provide high yields of astatine-211 would be interesting. An alternative to dry distillation has been described recently. In this technique, instead of distilling astatine out of the irradiated target, the layer of irradiated bismuth is dissolved in nitric acid and astatine is extracted in di-isopropylether (DIPE).[13] This technique is referred to as wet extraction. The activated ester *N*-hydroxysuccinimidyl-*meta*-trimethylstannylbenzoate ester (MeSTB) has been radiolabelled with astatine-211 obtained by wet extraction, but so far mAb radiolabelling with this method has not been reported because of hydrolysis of the *N*-hydoxysuccinimide-activated ester.[13] The aim of the present study was to

[a]*Centre de Recherche en Cancérologie de Nantes-Angers, Inserm, Institut de Biologie, Université de Nantes, U892, 9 quai Moncousu, 44093 Nantes Cedex 1, France*

[b]*Department of Nuclear Medicine, CHU de Nantes-Hôtel Dieu, Nantes, France*

**Correspondence to: Mickaël Bourgeois, Centre de Recherche en Cancérologie de Nantes-Angers, Inserm, Institut de Biologie, Université de Nantes, U892, 9 quai Moncousu, 44093 Nantes Cedex 1, France.*
E-mail: Mickael.Bourgeois@univ-nantes.fr, mickael.bourgeois@nantes.inserm.fr

investigate the feasibility of radiolabelling of an mAb with astatine-211 obtained by wet extraction. Thus, the reaction conditions were re-investigated and wet-extracted astatine-211 was coupled to the immunological vector using MeSTB and the radiochemical yield was optimized.

Results and discussion

Irradiation of a bismuth-209 target by the cyclotron of the CNRS in Orléans (CEMHTI, Orléans, France) as described in the Experimental section routinely affords 100 MBq of astatine-211 (at end of bombardment). The wet extraction procedure takes about 1.5 h because concentrated nitric acid must be evaporated to dryness and 70–90 % (decay corrected) of the activity is collected in DIPE. At the end of the procedure, 60–77 MBq of astatine is recovered in 500 μL of DIPE. Dry distillation gives similar yields in less time. Halogenated solvents (such as chloroform) are generally used to recover the astatine from a PEEK capillary loop.[12,14,15] However, methanol, which is also used to collect astatine after dry distillation, is a better solvent for SAB preparation[16] as it minimizes radiolysis. After wet extraction, astatine is obtained as a solution in DIPE containing nitric acid, in which radiolysis did not appear to be a problem.

[^{125}I]-iodine-labelled iodobenzoic acid and *N*-hydroxysuccinimidyl-*meta*-iodobenzoate ester were synthesized for reference (Figure 1). High-performance liquid chromatography (HPLC) retention times were 9.1 and 14.7 min (Figure 2) and thin-layer chromatography (TLC) R_f were 0.56 and 0.68, respectively. The amounts of both iodinated products were too small to be detected by UV light. A commercial [^{127}I]-iodobenzoic acid reference gave an HPLC retention time of 8.9 min and a TLC R_f of 0.56 detected by UV light.

[^{211}At]-astatobenzoic acid and *N*-hydroxysuccinimidyl-*meta*-[^{211}At]astatobenzoate ester gave HPLC retention times of 9.2 and 14.9 min, respectively (Figure 3). The TLC R_f were the same as that of the iodinated references. The amounts of both astatine-labelled products were too small to be detected by UV light.

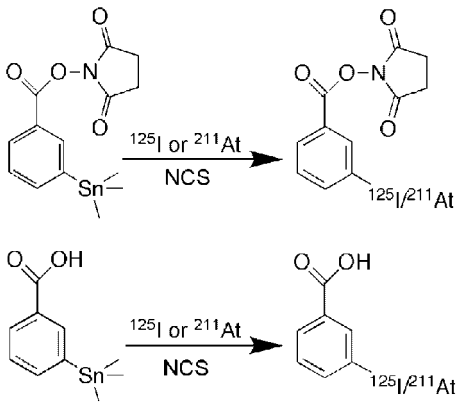

Figure 1. Halogeno-destannylation reaction of MeSTB (top) and *meta*-(trimethylstannyl)benzoic acid (bottom).

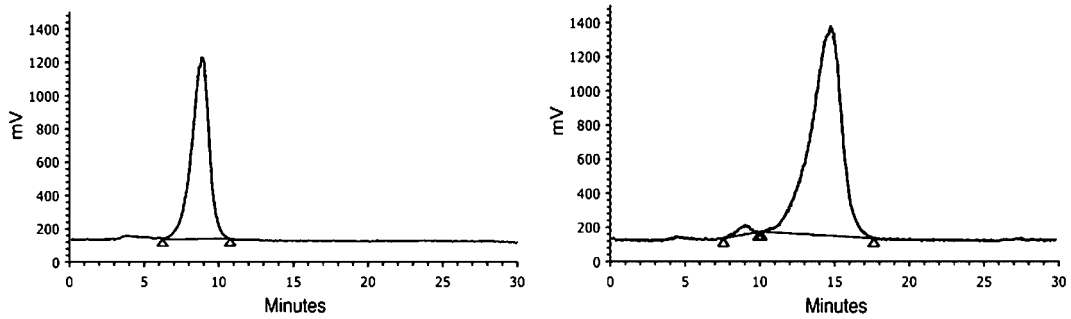

Figure 2. HPLC radiochromatogram of *meta*-iodobenzoic acid (left) and *N*-hydroxysuccinimidyl-*meta*-iodobenzoate ester (right). Reverse phase HPLC was performed using a μbondapak C_{18} analytical column under isocratic conditions (0.1% trifluoroacetic acid in water/acetonitrile, 85:15, v/v). Measured retention times were 9.1 and 14.7 min, respectively. In the radiochromatogram of *N*-hydroxysuccinimidyl-*meta*-iodobenzoate ester, a small amount of *meta*-iodobenzoic acid is observed due to the hydrolysis of activated ester.

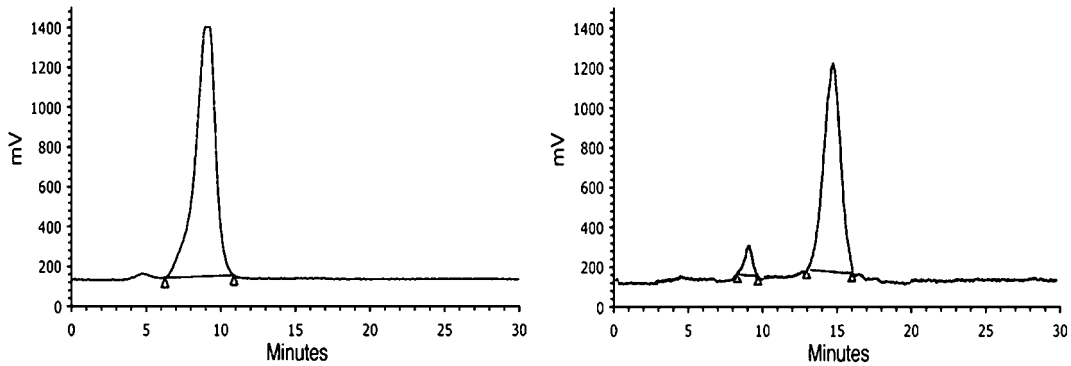

Figure 3. HPLC radiochromatogram of *meta*-astatobenzoic acid (left) and *N*-hydroxysuccinimidyl-*meta*-astatobenzoate ester (right). Reverse phase HPLC was performed under the exact same conditions as that used for the iodinated derivatives. Measured retention times were 9.2 and 14.9 min, respectively. In the radiochromatogram of *N*-hydroxysuccinimidyl-*meta*-astatobenzoate ester, a small amount of *meta*-astatobenzoic acid is observed due to the hydrolysis of activated ester.

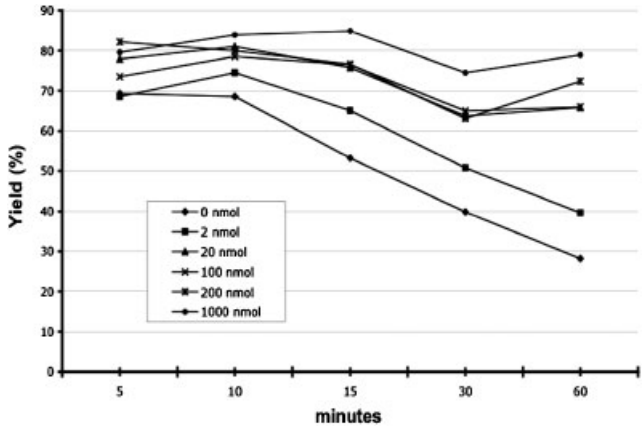

Figure 4. Kinetics of astato-destannylation of MeSTB. The reaction mixture contained 20 nmol of MeSTB and variable amounts of *N*-chlorosuccinimide (0, 2, 20, 100, 200 and 1000 nmol) in a 121 μL of final volume. Progress of the reaction was analysed at selected time intervals by TLC and the percentage of activity counted at the R_f of SAB was plotted as a function of time in the figure.

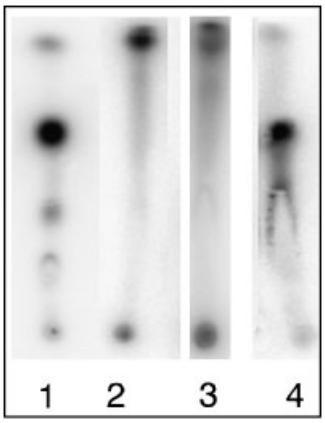

Figure 5. Radio-TLC on silica gel of astatine and SAB. TLC was performed using chloroform/ethyl acetate (8:2) as the mobile phase. The different lanes show: *N*-hydroxysuccinimidyl-*meta*-iodobenzoate ester used as a reference (1), free astatine obtained by DIPE extraction (2), free astatine obtained by DIPE extraction incubated with NCS (3) and *N*-hydroxysuccinimidyl-*meta*-astatobenzoate ester (4).

Figure 6. ITLC of astatine-labelled antibody. ITLC was performed on silica gel using 10% trichloroacetic acid in water as the mobile phase. Lane 1 shows the ITLC of crude astatine-labelled antibody and lane 2 the same preparation after size-exclusion purification. In this chromatographic condition, an IgG was retained on the baseline and free astatine was eluted to the solvent front.

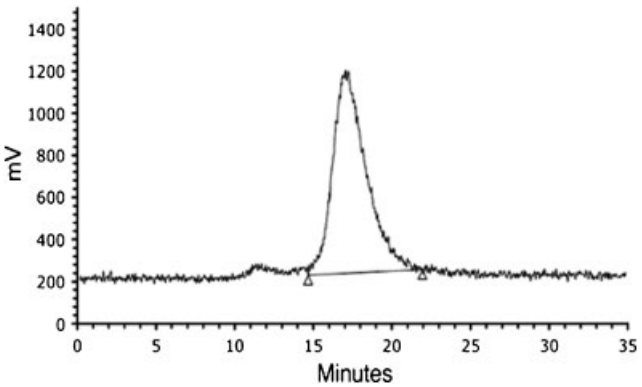

Figure 7. Size-exclusion HPLC of astatine-labelled antibody. The figure shows the radiochromatogram of astatine-labelled antibody purified by size-exclusion chromatography, with the major peak at a retention time of 17.0 min corresponding to that of the IgG.

We have analysed the effect of the quantity of oxidizing agent (*N*-chlorosuccinimide, NCS) with a constant amount of MeSTB (20 nmol). Using 100 μL of wet-extracted astatine-211, the best SAB yield was obtained with a minimum of 100 nmol of NCS and a minimum time of 5–10 min (Figure 4). It is interesting to note that in the absence of NCS or using only a small amount (2 nmol) of NCS, an astatinated species with an R_f similar to that of SAB is rapidly formed. However, the product is unstable and free astatine release is observed. Increasing the NCS amount beyond 100 nmol did not result in higher yields of SAB. The best SAB yields were obtained when the volume was kept below 120 μL. Using these parameters high repeatability was achieved with SAB yields in the 85–90% range. To keep the hydrolysis of SAB into astatobenzoic acid to a minimum (Figure 3), it appeared quite important to stop the evaporation of the reaction mixture just before complete drying.

For the radiolabelling of a mAb, we have used a volume of 400 μL of astatine solution in DIPE. The amounts of NCS and MeSTB were increased accordingly to maintain the same final concentrations as in the test experiments of SAB synthesis. Under these conditions, we obtained, in 15 min, a yield of 85–90% (Figure 5) of SAB. After the evaporation of DIPE, purification of the astatine derivative using a SepPak C_{18} cartridge followed by evaporation of the acetonitrile phase, in 30 min, 70–75% of the activity was recovered.

The conjugation of SAB to mAb was performed in 30 min with a yield of 20–25% (Figure 5). After purification on NAP-5 size-exclusion columns, the astatine-labelled IgG was collected in a volume of 0.8–1.4 mL. The radiochemical purity of this astatine-labelled mAb was 97–99%. (Figure 6) and the specific activity was 30–37 MBq/mg with a global yield of 20–25%. The fast protein liquid chromatography retention time was 17.03 min in agreement with IgG retention time on the Superdex 200 column (Figure 7). The radioastatination overall yield of the antibody was in accordance with the results obtained using dry distillation.[15,17]

Experimental

Materials

NCS was purchased from Acros Organics (Geel, Belgium). All other chemicals and solvents were obtained from Carlo Erba (Val de Reuil, France) and were used without further purification. Sodium [^{125}I]iodide was obtained from Perkin Elmer (Boston, MA, USA). MeSTB for radioiodination and radioastatination was prepared as described.[18] The mAb (MJ7/18) is a rat IgG2b mAb directed against the CD105 antigen (endoglin).[19] This mAb was produced from an hybridoma purified by our colleagues at the Centre de Recherche en Cancérologie de Nantes-Angers (Nantes, France).

HPLC was performed using a Waters HPLC system (Saint Quentin en Yvelines, France), equipped with a Waters 486 Tunable Absorbance Detector and a Packard Bioscience Flow Scintillation Analyser 150 TR (Meriden, CT, US). Data analysis was carried out using Waters Empower data acquisition and analysis software. For the iodine and astatine organic benzoate derivatives, we used a Waters μbondapak C_{18} (3.9 $\times$ 300 mm; 5 μm) analytical column. The isocratic mobile phase was composed of trifluoroacetic acid (0.1%) in water/acetonitrile (85:15, v/v). The mobile phase was filtered though a 0.45 μm Whatman (Maidstone, UK) nylon membrane filter. Outgassing was performed using a Waters inline degasser AF. Flow rate was set to 1 mL/min. The analysis time was 30 min. UV absorbance was monitored at 254 nm. For the control of the astatine-labelled antibody, we used a Superdex 200 10/300GL column (GE Healthcare Bio-science, Uppsala, Sweden). The isocratic mobile phase was 0.1 M phosphate buffer, pH 7.2, at a flow rate of 1 mL/min with an analysis time of 35 min.

Radio-TLC was carried out on precoated silica gel 60 F_{254} TLC plastic sheets (Merck, Darmstadt, Germany) with chloroform/ethyl acetate 8:2 as mobile phase. Instant TLC on silica gel (ITLC) was carried out on precoated silica gel on glass fibre sheets (Pall Life Science, New York, USA) with trichloroacetic acid in water (10%) as mobile phase. Radio-TLC plates were examined using a Typhoon 9410 Variable Mode Imager (GE Healthcare Bio-science).

Cyclotron irradiation

Astatine-211 was produced at the CNRS cyclotron of CEMHTI using the ^{209}Bi(α,2n)^{211}At reaction by bombarding a 240 μm thick natural ^{209}Bi layer on copper target with a 1.95–2.15 μA particle beam of 28 MeV α-particles. The irradiation lasted for 2 h.

Separation of ^{211}At from the bismuth target

The irradiated target was placed in a beaker and 2 mL of 65% nitric acid was added drop-wise on the irradiated target to completely dissolve the bismuth layer. After 10–15 min, the acid solution was evaporated to dryness and, after cooling, the residue was re-dissolved in 2 mL of 32% nitric acid. Astatine-211 was then extracted in DIPE (2 $\times$ 500 μL). The extracts were combined and the total harvested activity was measured in an ACAD 2000 ionization chamber (Lemer Pax, Carquefou, France).

Iodine labelling Procedure

Radioiodination of MeSTB and *meta*-trimethylstannylbenzoic acid was performed using NCS as the oxidizing agent (Figure 1). Briefly, 5 μL of saturated aqueous sodium chloride solution, 20 nmol of stannylated precursor in methanol/acetic acid 95:5, 100 nmol of NCS in methanol/acetic acid 95:5 and 2 μL (7.4 MBq) of sodium [^{125}I]iodide in 1 $\times$ 10^{-5} M NaOH were incubated for 15 min at room temperature. A 10 μL aliquot of the reaction mixture was diluted with 50 μL of methanol and 40 μL from this solution was then analysed using analytical HPLC.

Yield optimization for *N*-hydroxysuccinimidyl-*meta*-[^{211}At]astatobenzoate ester production

The following experimental plan was used to analyse the influence of kinetics, NCS concentration and volume of radiolabelling reaction. A stock solution of NCS was prepared by dissolving 66.7 mg of NCS in 50 mL of methanol/acetic acid (95:5, v/v) to yield a final concentration of 100 nmol/μL. Solutions of NCS at 20, 10, 2, 0.2 or 0 nmol/μL were prepared by dilution in methanol/acetic acid (95:5, v/v). To 100 μL of ^{211}At in DIPE were added 10.9 μL of MeSTB (20 nmol) and 10 μL of the different solutions of NCS. The yield of the reaction was determined by TLC at 5, 10, 15, 30 and 60 min.

Three concentrations of ^{211}At in DIPE were prepared: postextraction ^{211}At in DIPE (50 μL), ^{211}At (50 μL) diluted with same volume of DIPE, postextraction ^{211}At (100 μL) in DIPE diluted with three volumes of DIPE. Astatine-211 in DIPE was incubated with 10.9 μL of MeSTB (20 nmol) and 10 μL of NCS (100 nmol). The final volumes were, respectively, 71, 121 and 421 μL. The yield of the reaction was determined by TLC at 15 min.

Radiolabelling of an mAb with *N*-hydroxysuccinimidyl-*meta*-[^{211}At]astatobenzoate ester

In the first step, 400 μL of astatine-211 in DIPE was reacted with 400 nmol of NCS and 80 nmol of MeSTB in 63.6 μL of methanol/acetic acid 95:5 during 15 min at room temperature. The yield of the reaction was determined by TLC. The reaction mixture was evaporated to 50 μL under a stream of nitrogen at room temperature, loaded on a SepPak C_{18} column (Waters) and eluted with 2 mL of trifluoroacetic/water (0.1%) and second with 2 mL of acetonitrile. The acetonitrile phase was dry evaporated under a stream of nitrogen at 40°C.

The coupling reaction was performed by the addition of 200 μL of mAb (IgG MJ7/18, 0.4 mg/mL)) in carbonate buffer (300 mM, pH 8.6) to the dry residue of SAB. The yield of the radiolabelling was determined by ITLC.

Finally, the astatine-labelled antibody was purified using a NAP-5 column (Amersham Biosciences, Uppsala, Sweden) with a phosphate-buffered saline as eluent. The radiochemical purity of the antibody was determined by ITLC and Superdex G200 HPLC.

Conclusion

Wet harvesting of astatine has been discussed previously, but antibody radiolabelling with SAB obtained using this procedure has not yet been reported.[13] In this paper, we have re-evaluated the methodology and reproducibly obtained astatine-labelled mAb in a 20–25% yield based on the astatine activity involved with a typical specific activity of 26–44 MBq/mg. These results could be improved by using higher astatine activities and higher antibody concentrations. In this paper, we have studied the impact of different factors on the tin electrophilic substitution. The overall time required for the labelling and the SAB

purification remains to be further optimized. An advantage of the wet extraction procedure is that it requires no specific equipment in contrast to dry distillation[12] and that it could be easily automated. Ultimately wet extraction could be a useful alternative to dry distillation to obtain reproducible yields with high activities of astatine.

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References

[1] R. M. Sharkey, D. M. Goldenberg, *CA Cancer J. Clin.* **2006**, *56*, 226–243.
[2] S. I. Park, O. W. Press, *Curr. Opin. Hematol.* **2007**, *14*, 632–638.
[3] M. Chérel, F. Davodeau, F. Kraeber-Bodéré, J. F. Chatal, *Q. J. Nucl. Med. Mol. Imaging* **2006**, *50*, 322–329.
[4] M. R. Zalutsky, O. R. Pozzi, *Q. J. Nucl. Med. Mol. Imaging* **2004**, *48*, 289–296.
[5] T. Bäck, H. Andersson, C. R. Divgi, R. Hultborn, H. Jensen, S. Lindegren, S. Palm, L. Jacobsson, *J. Nucl. Med.* **2005**, *46*, 2061–2067.
[6] K. Vandenbulcke, P. De Vos, F. Offner, J. Philippé, C. Apostolidis, R. Molinet, T. K. Nikula, K. Bacher, V. de Gelder, A. Vral, C. Lahorte, H. Thierens, R. A. Dierckx, G. Slegers, *Eur. J. Nucl. Med. Mol. Imaging.* **2003**, *30*, 1357–1364.
[7] T. E. Wheldon, J. A. O'Donoghue, *Int. J. Radiat. Biol.* **1990**, *58*, 1–21.
[8] W. L. Hughes, D. Gitlin, *Am. Soc. Biol. Chem.* **1955**, 14, 229.
[9] P. W. Durbin, C. W. Asling, M. E. John, *Radiat. Res.* **1958**, *9*, 378–397.
[10] C. Aaij, W. R. J. M. Tschroots, L. Lindner, T. E. W. Feltkamp, *Int. J. Appl. Radiat. Isot.* **1975**, *26*, 25–30.
[11] A. T. M. Vaughan, J. H. Fremlin, *Int. J. Nucl. Med. Biol.* **1978**, *5*, 229–230.
[12] S. Lindegren, T. Back, H. Jensen, *Appl. Radiat. Isot.* **2001**, *55*, 157–160.
[13] A. T. Yordanov, O. Pozzi, S. Carlin, G. Akabani, B. Wieland, M. R. Zalutsky, *J. Radioanal. Nucl. Chem.* **2004**, *262*, 593–599.
[14] M. R. Zalutsky, X. G. Zhao, K. L. Alston, D. Bigner, *J. Nucl. Med.* **2001**, *42*, 1508–1515.
[15] A. Walte, S. S. R. Sriyapureddy, Z. Korkmaz, D. Krull, O. Bolte, M. Hofmann, G. J. Meyer, W. H. Knapp, *J. Pharm. Pharm. Sci.* **2007**, *10*, 277s–285s.
[16] O. R. Pozzi, M. R. Zalutsky, *J. Nucl. Med.* **2005**, *46*, 1393–1400.
[17] S. Palm, T. Back, I. Claesson, A. Danielson, J. Elgqvist, S. Frost, R. Hultborn, H. Jensen, S. Lindegren, L. Jacobson, *Int. J. Radiat. Oncol. Biol. Phys.* **2007**, *69*, 572–579.
[18] J. Koziorowski, C. Henssen, R. Weinreich, *Appl. Radiat. Isot.* **1998**, *49*, 955–959.
[19] A. Z. Ge, E. C. Butcher, *Gene* **1994**, *138*, 201–206.