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N-[2-(maleimido)ethyl]-3-(trimethylstannyl)benzamide, a molecule for radiohalogenation of proteins and peptides

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HIGHLIGHTS

- A novel conjugation agent for radiohalogenation of proteins and peptides was synthesized.
- The characterized agent is N-[2-(maleimido)ethyl]-3-(trimethylstannyl)benzamide.
- The conjugation agent was halogenated with bromine, iodine (^{125}I) and ^{211}At.

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ABSTRACT

In this work a new coupling reagent, N-[2-(maleimido)ethyl]-3-(trimethylstannyl)benzamide, for radiohalogenation has been synthesized and characterized. The reagent is intended to either be attached to reduced disulfide bridges of proteins (making the halogenation site-specific) or to free terminal cysteine groups on peptides. The new reagent was also shown to be easily halogenated with inactive bromine and iodine as well as ^{125}I and ^{211}At, indicating potential use within targeted radiotherapy.

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1. Introduction

An efficient therapy of metastasis is one of the largest challenges within successful treatment of different types of cancers. Today chemotherapy is often employed, which is not only a very aggressive method but it is also possible that the cancer cells become resistant towards the chemicals used. This means that the patient's quality of life is affected in a negative way without resulting in therapeutic effects. Because of this, other types of treatment for disseminated cancer are desirable; one such method is targeted radiotherapy. Targeted radiotherapy often utilizes proteins such as antibodies or peptides to deliver the radioactive nuclide to the site of the tumor (Reilly, 2010). ^{131}I is one of the most commonly used nuclides for radiotherapy but due to the rather long range of the beta particles it is not the best suited nuclide for treatment of disseminated micro-tumors or single cancer cells. In such cases alpha particle emitters are promising therapeutic candidates due to the short tissue range (50–100 μm) and high LET of the alpha particle (Choppin et al., 2002). This method spares the surrounding healthy tissue from radiation damage and also increases the dose given to the tumor. One of the most intensely studied alpha emitters for this type of application is another halogen, ^{211}At (Guerard et al., 2013). Astatine has no stable isotopes (Corson et al., 1947) and despite being a halogen, astatine cannot be coupled directly to aromatic tyrosine residues on carrier proteins or peptides, like iodine, but always requires an additional coupling molecule (Guerard et al., 2013). This illustrates the difference between astatine and the other halogens, partly explained by the larger relativistic contribution in binding and hence more metallic character of the element. Due to this, alternative carrier systems such as nano-particles are also under investigation alongside more traditional methods (Kucka et al., 2006). Astatination of proteins and peptides can be performed in several

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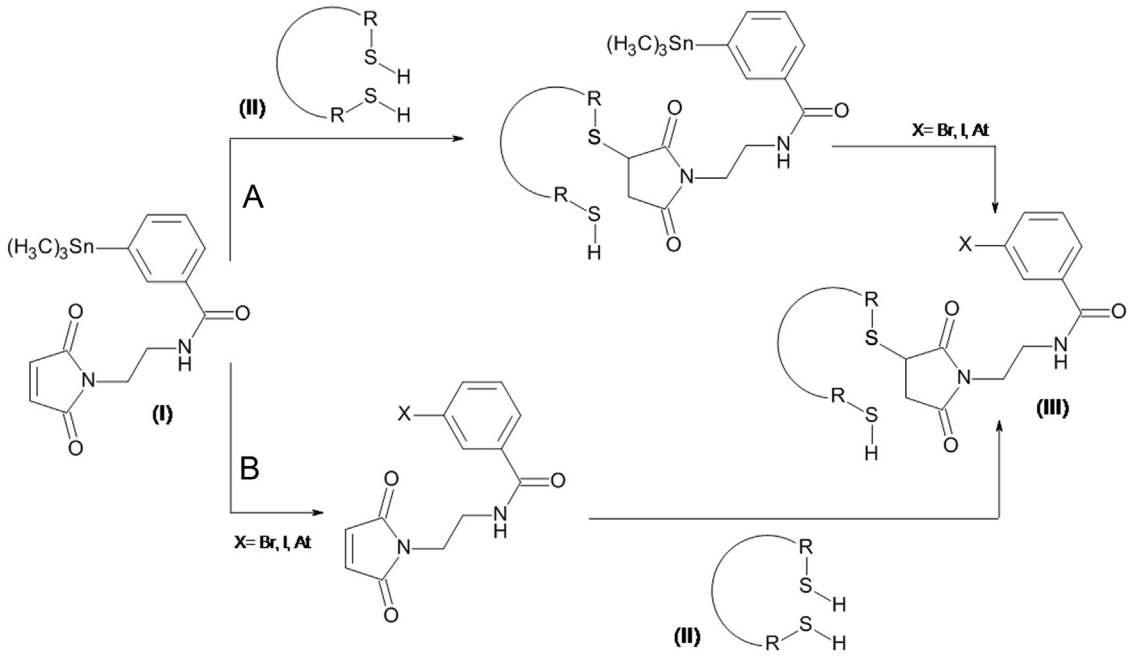

Fig. 1. Schematic picture of halogenation of proteins or peptides (II) using the new coupling reagent N-[2-(maleimido)ethyl]-3-(trimethylstannyl)benzamide (I) resulting in the final radiopharmaceutical (III). Route A: halogenation after conjugation and route B: halogenation before conjugation.

different ways (Wilbur et al., 2007; Pruszynski et al., 2006, 2008; Vaidyanathan et al., 2003). Coupling a stannylated aromatic succinimide ester, MeATE reagent, via N-acylation reactions with lysine on the biomolecule, followed or preceded by an electrophilic aromatic substitution of $Sn(CH_3)_3$ with ^{211}At is, although, the most common method (Zalutsky and Narula, 1988; Lindegren et al., 2008). However, as lysine usually is randomly distributed within proteins, such as antibodies, the astatination will, using this method, be unspecifically located in the structure. The same is true for iodination on tyrosine residues. This means that if the radionuclide and/or conjugate ends up in the antigen binding site, the immunoreactivity of the antibody might be compromised (Nikula et al., 1995). In this work, a new MeATE-type coupling reagent for radiohalogenation, N-[2-(maleimido)ethyl]-3-(trimethylstannyl) benzamide ((I), Fig. 1), has been developed. Instead of targeting lysine residues the maleimide part of the new molecule is reactive towards cystein through a thiol-ene reaction (Mark et al., 2001). In antibodies there are specifically located inter-chain disulfide bridges that can be reduced to thiol groups i.e. cystein, using mild reducing agents (Liu and May, 2012; Mark et al., 2001). As the disulfide bridges are specifically located, a halogenation using the new reagent would be site-specific instead of random. The antibody reduction required is, however, not unproblematic when using intermediate malemido-ligands for astatination as increased kidney retention previously has been reported (Wilbur et al., 2012). The new reagent can, however, also be introduced at terminal cysteine residues in solid phase peptide synthesis (Merrifield, 1963; Sosabowski and Mather, 2006), without further alterations to the peptide. In Fig. 1 there is a schematic picture that shows the possibilities for halogenation using the new coupling reagent (I). Route A is suitable for iodination of peptides without tyrosine residues or for astatination of proteins or peptides both with and without tyrosine residues. This since route A only require one radiochemical step, decreasing reaction times and hence absorbed dose in the solvent, which is beneficial when using α-emitters. For iodination of proteins, however, route B must be employed to avoid no-specific halogenation of tyrosine.

In this work the synthesis and characterization of the new molecule is described, as well as its halogenation with bromine, stable and radioactive iodine isotopes and astatine.

2. Method and materials

2.1. Synthesis and characterisation

The synthesis of N-[2-(maleimido)ethyl]-3-(trimethylstannyl) benzamide (I) was performed by contacting 0.0664 g (0.233 mmol) 3-(trimethylstannyl)benzoic acid (IV) (Toronto Research Chemicals Inc., 95%) with 0.1180 g (0.464 mmol) N-(2-aminoethyl)maleimide trifluoroacetate salt (V) (Sigma-Aldrich, ≥95% (HPLC), ≥98% (T)) in 12 ml dichloromethane with the presence of 0.3088 g (0.814 mmol) of O-(benzotriazol-1-yl)-N,N,N′,N′-tetramethyluronium hexafluorophosphate (HBTU) (Sigma-Aldrich, ≥98.0%) and 143 μl (0.813 mmol) N,N-diisopropylethylamine (DiPEA) (Sigma-Aldrich, ≥99%) in a 25 ml schlenk flask under nitrogen atmosphere at room temperature and constant stirring for 23.5 h. The product slurry was diluted with 50 ml dichloromethane and washed with 30 ml citric acid (2.2 mM) followed by 30 ml $NaHCO_3$-solution (5%) and MQ-water (18 MΩ cm) before drying on $MgSO_4$. The diluent was removed on a rotary-evaporator and the residual oil dissolved in diethyl ether and analyzed by thin layer chromatography to determine purification conditions before a second evaporation and dissolution of the oil in petroleum ether. The petroleum ether solution was purified on a silica column by elution with dichloromethane followed by 10% ethyl acetate in dichloromethane and the collected fractions identified by NMR to be the pure product was combined. Upon evaporation of the diluent 39.1 mg (0.096 mmol) of white crystalline solid product (MW = 407.06 g mol^{-1}) was formed, rendering a total synthesis yield of 41.2%.

NMR analyses of the product and starting materials were performed using a Varian Agilent 400 spectrometer with deuterated benzene (^1H NMR) or deuterated chloroform (^{13}C NMR) as diluent.

Mass determination of the product (I) was performed by SGS M-Scan Ltd., Wokingham, UK. The sample was diluted to 1 mg/ml

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