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Preparation and in vivo evaluation of a novel stabilized linker for ^{211}At labeling of protein

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Abstract

Significant improvement of in vivo stability of ^{211}At-labeled radioimmunoconjugates achieved upon employment of a recently reported new linker, succinimidyl *N*-2-(4-[^{211}At]astatophenethyl)succinamate (SAPS), prompted additional studies of its chemistry. The ^{211}At radiolabeling of succinimidyl *N*-2-(4-tributylstannylphenethyl)succinamate (**1**) was noted to decline after storage at −15°C for greater than 6 months. Compound **1** was found to degrade via a ring closure reaction with the formation of *N*-2-(4-tributylstannylphenethyl)succinimide (**3**), and a modified procedure for the preparation of **1** was developed. The *N*-methyl structural analog of **1**, succinimidyl *N*-2-(4-tributylstannylphenethyl)-*N*-methyl succinamate (SPEMS), was synthesized to investigate the possibility of improving the stability of reagent–protein linkage chemistry. Radiolabeling of SPEMS with ^{211}At generates succinimidyl *N*-2-(4-[^{211}At]astatophenethyl)-*N*-methyl succinamate (Methyl-SAPS), with yields being consistent for greater than 1 year. Radiolabelings of **1** and SPEMS with ^{125}I generated succinimidyl *N*-2-(4-[^{125}I]iodophenethyl)succinamate (SIPS) and succinimidyl *N*-2-(4-[^{125}I]iodophenethyl)-*N*-methyl succinamate (Methyl-SIPS), respectively, and showed no decline in yields. Methyl-SAPS, SAPS, Methyl-SIPS and SIPS were conjugated to Herceptin for a comparative assessment in LS-174T xenograft-bearing mice. The conjugates of Herceptin with Methyl-SAPS or Methyl-SIPS demonstrated immunoreactivity equivalent to if not superior to the SAPS and SIPS paired analogs. The in vivo studies also revealed that the *N*-methyl modification resulted in a superior statinated product.

Keywords: ^{211}At; Astatine; SAPS; SPEMS; Methyl-SAPS; Radioimmunotherapy; Monoclonal antibody; α-Particle

1. Introduction

The development of therapeutic monoclonal antibodies (mAbs) remains a burgeoning area of research. This is made evident by the numerous mAbs being evaluated in clinical trials, by the two FDA-approved radiolabeled mAbs (Zevalin and Bexxar) and by continuing FDA approvals [1,2]. The aforementioned two drugs, with the abundant literature, typify the success achieved in the treatment of lymphoma and other diseases with mAbs radiolabeled with β^--emitting radionuclides [3–6]. However, further refinements are needed before ideal radioimmunotherapy (RIT) agents are translated to appropriate clinical use.

An important factor continues to be the nature of the radionuclide chosen as the cytotoxic element. Physical characteristics, half-life and associated emissions are critical factors. The pursuit and development of α-emitting radionuclides continue to be a field of intense focus stemming from their potential superiority over β^--emitters for treatment of smaller tumor burdens, disseminated disease and micrometastatic disease; the effective range of β^--emitters may not be appropriate for the treatment of "small" diseases [7–14]. The short range of the α-emission (40–100 μm) may also limit irradiation of neighboring normal

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tissue while retaining a much higher relative biological effectiveness as compared with β^--emitters [15]. Of the α-emitting radionuclides currently under investigation for use in RIT, that is, ^{212}Bi ($t_{1/2}$=60.60 min), ^{213}Bi ($t_{1/2}$=45.59 min) and ^{211}At ($t_{1/2}$=7.2 h), the last one is an attractive candidate if based only on half-life considerations [16,17]. Unfortunately, ^{211}At production and evaluation have been constrained due to requirements of cyclotron production and associated processing and isolation requirements.

A pivotal criterion in the design of a targeted radiation therapeutic agent remains the choice of the actual chemistry employed to conjugate the chosen radionuclide to the mAb [18]. The chemistry must retain the radionuclide minimizing premature release of the isotope from the mAb to achieve maximal delivery of the agent to the targeted tissue while curtailing the localization of free radionuclide to normal tissues.

Astatine has generally been regarded as a halogen, and thus, radioiodination protocols, chemistry and reagents have been adapted for ^{211}At labeling procedures. However, proteins directly labeled with ^{211}At deastatinate rapidly both in vitro and in vivo [19,20]. Methods for astatinating proteins via acylation with a variety of astato-benzoic acid or nicotinic acid derivatives prepared from their corresponding trialkylstannyl precursors have addressed this issue with varying levels of success [21–27].

This laboratory previously reported the novel linking agent, succinimidyl *N*-2-(4-tributylstannylphenethyl)succinamate (**1**) (Fig. 1), for radiolabeling proteins with ^{211}At and iodine isotopes [26]. This agent was designed to address the inadequate in vivo stability of prior ^{211}At radiolabeled conjugates. Significant improvement of in vivo stability was achieved with the succinimidyl *N*-2-(4-[^{211}At]astatophenethyl)succinamate (SAPS) conjugate formed from **1**.

However, during the course of extended studies of SAPS-labeled antibodies, a notable decline in conjugation and radiolabeling efficiency was observed. Despite the fact that no measurable changes in nuclear magnetic resonance (NMR) spectra (^{1}H, ^{13}C) of **1** were recorded after −15°C storage for 1 year, a significantly diminished yield of SAPS was observed. This decline in potency coupled with consumption of that specific lot of **1** prompted a careful resynthesis of **1**. During this process, inconsistencies in yields were observed upon preparation and isolation of **1**. Partial degradation of **1** via a spontaneous ring closure to form an imide was found to occur during the chromatographic purification of **1** during the course of resynthesis. This competing reaction pathway provided the impetus to formulate an improved preparation of **1** with a concurrent investigation into the development of analogs that might eliminate this potential result.

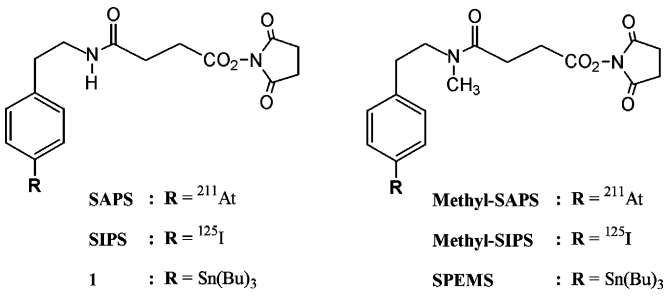

Fig. 1. Structures of SAPS precursor **1**, SIPS, SAPS, SPEMS, Methyl-SIPS and Methyl-SAPS.

A structural modification was hypothesized to obviate the inconsistencies in radiolabeling and conjugation efficiencies. The succinic half-amide was proposed to be N-methylated not only to block the ring closure pathway and concomitantly improve the long-term storage stability of the linker but also to potentially block this ring closure pathway from contributing to in vivo cleavage of the radiolabeled linker from the protein. No impact on the actual stability of the radiolabel chemistry was anticipated by this modification; however, direct comparison to the established parental compound is available. Herein, an improved procedure for the preparation of the previously reported SAPS precursor, organotin linker **1**, is presented along with the synthesis of the *N*-methyl structural analog, succinimidyl *N*-2-(4-tributylstannylphenethyl)-*N*-methyl succinamate (SPEMS), along with a long-term assessment of its radiolabeling chemistry and its in vivo evaluation for ^{211}At labeling of proteins as referenced to SAPS.

2. Methods and materials

Anhydrous solvents were purchased from Mallinckrodt (Paris, KY) and Aldrich Chemical Company (Milwaukee, WI) and used without further purification. Reagents were purchased from Aldrich Chemical Company, Sigma Chemical Company (St. Louis, MO) or as otherwise noted. Low-resolution mass spectra and high-resolution mass spectra (HRMS) were acquired on a JEOL JMS-SX 102 instrument from samples dissolved in a mixture of dithiothreitol and dithioerythritol (5:1, v/v) bombarded with 8-keV fast xenon ions. Additional HRMS were obtained using an electrospray ionization mode on a Waters LCT Premier time-of-flight mass spectrometer (ESI/TOF/MS) operated in a positive ion detection mode with a resolution of 10,000. The electrospray capillary voltage was 3 kV, and the sample cone voltage was 60 V. Desolvation temperature was 225°C, and the desolvation gas was N_2 at 300 L/h. Accurate masses were obtained using the lock spray mode with Leu-Enkephalin as the external reference compound. Proton and ^{13}C NMR spectra were measured with a Varian Mercury spectrometer, resulting to a value of 299.9 MHz for ^{1}H and 75.4 MHz for ^{13}C. Chemical shifts (δ) are expressed in ppm referenced to TMS, and values of coupling constants (J) are given in Hz. NMR peak patterns are described by the following abbreviations: br, broad; d, doublet; t, triplet; q, quartet; m, multiplet and arom, aromatic protons. Melting points were measured using a Thomas Unimelt capillary melting point apparatus (Philadelphia,