Site-Specific Tritium Labeling at the Predefined Internal Position of the Chemically-Modified RNA

Abstract

In nucleic acid drug discovery, it is extremely important to develop a technology to understand the distribution in target organs and to trace the degradation process in the body in order to optimize the structure and improve the efficiency of the clinical trial process. Since nucleic acid drugs are essentially metabolically degraded into numerous fragments, labeling at the internal position is preferable to that at the terminus. Due to the high molar specific activity of tritium, various approaches for tritium-labeling have been studied for nucleic acid drugs. Nevertheless, a generally-applicable method for tritium labeling of the internal position of a nucleic acid has not been established. In this study, we have demonstrated a new and efficient method for site-specific tritium labeling of the cytosine base at a predefined internal position in nucleic acid drugs. This method was developed by the chemical modification of the cytosine 4-amino group with the pyridinyl vinyl keto group by the functionality-transfer reaction using the reactive oligodeoxynucleotide (ODN), followed by reduction with NaBT4. Applicability to a variety of chemical structures, such as 5-methyl cytosine, 2-Omethyl, 2-fluoro ribose derivatives, Locked/Bridged nucleic acid (LNA/BNA) derivatives, as well as phosphorothioate bonds, has been evidenced using nine oligoribonucleic acid (ORN) substrates. It has been clearly demonstrated that this method is an excellent method for tritium-labeling of nucleic acid with an average conversion efficiency of 74%, an average isolated labeling yield of 60%, and an average specific activity of 61 GBq/mmol. This method is expected to contribute to the preclinical absorption, distribution, metabolism, excretion (ADME) studies of nucleic acid drug candidates.