A Radioligand for Imaging of the P2Y6 Receptor

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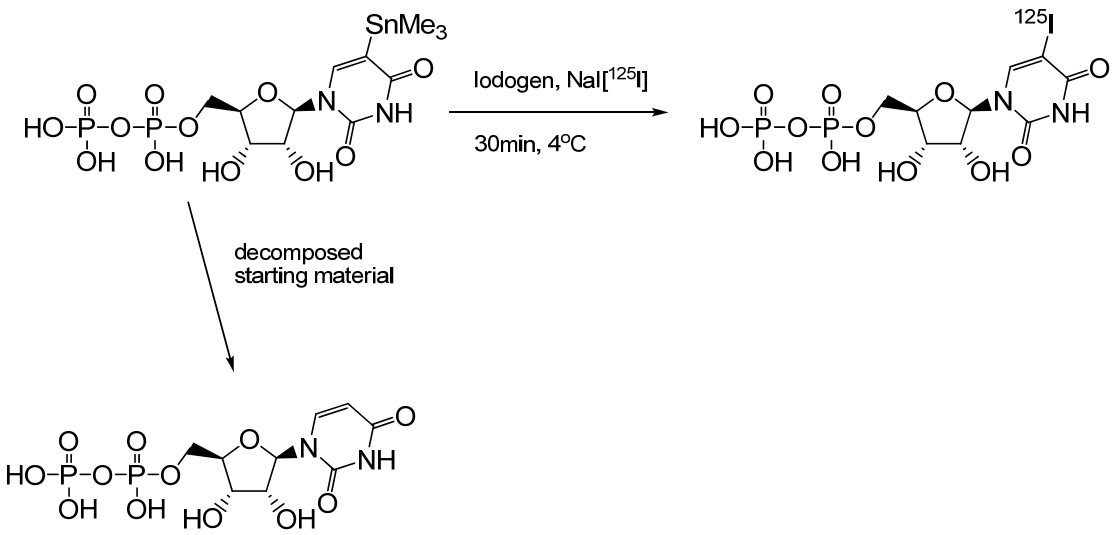
Background

P2 nucleotide receptors bind adenosine and uridine di- and triphosphates (ADP, ATP, UDP and UTP) and can be placed in two distinct groups, namely the G-protein coupled receptors (GPCRs) designated P2Y and the ligand-gated cation channels designated P2X. Both groups of receptors have multiple sub-types with the P2Y group having ay least eight (termed $P2Y_1$, $P2Y_2$, $P2Y_4$, $P2Y_6$ and $P2Y_{11}$-$P2Y_{14}$).[1] The $P2Y_6$ receptor is particularly widely distributed in body tissues and is also specifically activated by UDP (uridine-5'-diphosphate). It has multiple roles, including anti-apoptosis, while it may also enhance osteoclast survival through NF-κB activation.[2] In addition, it is suggested to have roles in the regulation of electrolyte transport in airways,[3] the production of pro-inflammatory cytokines and chemokines, and in the growth and contraction of vascular muscles.[4, 5]

Chemistry

The structure-activity relationship (SAR) of nucleotides in specifically activating the $P2Y_6$ receptor has been probed,[6] as chemists try to understand the structural basis for $P2Y_6$ ligand selectivity. The design of selective agonists and antagonists is an active area of research. Various structural modifications of UDP showed that 5-iodo-uridine-5'-diphosphate (EC_{50}=0.015μmol) has potential selectivity for the $P2Y_6$ receptor,[1] and since this analog contains a halogen it is suitable in radioiodinated form for direct labeling as a radioligand and as an agent for *in vivo* imaging. The trimethylstannyl precursor to the desired radiolabeled product was received from our collaborator at 70-80% purity and used without further purification. The 20-30% remainder of the starting material was comprised of the deactivated product shown in Scheme 1, which had no effect on the radiolabeling reaction.

Scheme 1. Radiosynthesis of 5-iodo[^{125}I]-uridine-5'-diphosphate



Experimental

All chemicals were purchased from Aldrich Chemical Company except where noted. Iodogen coated tubes were purchased from Pierce Biotechnology. Sodium [125I]iodide (17.4 Ci/mg) in NaOH (1.0x10-5M) was purchased from Perkin–Elmer Life and Analytical Science. High performance liquid chromatography was performed using a Beckman Gold HPLC system equipped with a Model 126 programmable solvent module, a Model 168 variable wavelength detector, a β–Ram Model 4 radioisotope detector, and Beckman System Gold remote interface module SS420X, using 32 Karat®software.

5-iodo[^{125}I]-uridine-5'-diphosphate (5-^{125}I-UDP). The radioiodination of the stannyled uridine diphoshate with sodium [^{125}I]iodide was accomplished in an Iodogen-coated tube as shown in **Scheme 1**. A mixture of 5-Me_3Sn-UDP (0.2 mg containing 20% uridine diphosphate) in water (20 µL), 0.1 M phosphate buffer pH 7.0 (70 µL) and sodium [^{125}I]iodide solution (10 µL, 37 MBq, 3.7 GBq/mL) was transferred into a vial coated with Iodogen. After 20 minutes in an ice bath, the labeling solution and washing solution (0.9% NaCl) (400 µL) were transferred into a new vial. The reaction mixture was loaded onto a Sep-Pak C_{18}-light cartridge previously activated by washing with 10 mL ethanol, 20 mL air and 20 mL 0.9% NaCl solution. The loaded column was washed with 0.9% NaCl solution (1 mL) and dried with 20 mL of air. 5-^{125}I-UDP was eluted with ethanol (1 mL) and the product was purified using a Beckman System Gold

HPLC equipped with a Zorbax Eclipse 5 µm XDB-C_{18} analytical column (4.6×250 mm; Agilent Technology). Solvent A was tetrabutylammonium dihydrogen phosphate (TBAP) buffer, 20 mM, and solvent B was 100% acetonitrile. A linear gradient of solvent B from 15-60% was used with a flow rate of 1mL/min. Pure 5-^{125}I-UDP was obtained in a radiochemical yield of 40 µCi (4.5%). Quality control of the final product was performed with the same HPLC system and linear gradient as described above. The 5-^{125}I-UDP eluted at a retention time of 21.1 minutes with a radiochemical purity over 98% and a specific activity of 4.12 Ci/mg.

References

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