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Laboratory note

Simple synthesis of carbon-11 labeled styryl dyes as new potential PET RNA-specific, living cell imaging probes

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Abstract

A new type of styryl dyes have been developed as RNA-specific, live cell imaging probes for fluorescent microscopy technology to study nuclear structure and function. This study was designed to develop carbon-11 labeled styryl dyes as new probes for biomedical imaging technique positron emission tomography (PET) imaging of RNA in living cells. Precursors (*E*)-2-(2-(1-(triisopropylsilyl)-1*H*-indol-3-yl)vinyl)-quinoline (**2**), (*E*)-2-(2,4,6-trimethoxystyryl)quinoline (**3**) and (*E*)-4-(2-(6-methoxyquinolin-2-yl)vinyl)-*N*,*N*-diemthylaniline (**4**), and standards styryl dyes E36 (**6**), E144 (**7**) and F22 (**9**) were synthesized in multiple steps with moderate to high chemical yields. Precursor **2** was labeled by [^{11}C]CH_3OTf, trapped on a cation-exchange CM Sep-Pak cartridge following a quick deprotecting reaction by addition of $(n\text{-Bu})_4NF$ in THF, and isolated by solid-phase extraction (SPE) purification to provide target tracer [^{11}C]E36 ([^{11}C]**6**) in 40–50% radiochemical yields, decay corrected to end of bombardment (EOB), based on [^{11}C]CO_2. The target tracers [^{11}C]E144 ([^{11}C]**7**) and [^{11}C]F22 ([^{11}C]**9**) were prepared by *N*-[^{11}C]methylation of the precursors **3** and **4**, respectively, using [^{11}C]CH_3OTf and isolated by SPE method in 50–70% radiochemical yields at EOB. The specific activity of the target tracers [^{11}C]**6**, [^{11}C]**7** and [^{11}C]**9** was in a range of 74–111 GBq/μmol at the end of synthesis (EOS).

Keywords: Positron emission tomography; Carbon-11; Styryl dyes; RNA; Live cell; Imaging

1. Introduction

There is great interest in imaging of RNA in human diseases such as various neurological and psychiatric disorders, heart and cancer diseases [1–6]. However, finding a RNA-selective probe for living cell imaging has proved to be difficult [7], and only a few RNA visualization agents are currently available. Commercial cyanine dyes are widely used for RNA imaging in living cell, but their photostability and RNA selectivity are limited, making them difficult to use for cellular time-lapse RNA imaging [8,9]. Recently, fluorescent styryl dyes E36 [(*E*)-2-(2-(1*H*-indol-3-yl)vinyl)-1-methylquinolinium iodide (**6**)], E144 [(*E*)-1-methyl-2-(2,4,6-trimethoxystyryl)quinolinium iodide (**7**)] and F22 [(*E*)-2-(4-(dimethylamino) styryl)-6-methoxy-1-methylquinolinium iodide (**9**)] have been identified as RNA-specific, live cell imaging probes for fluorescent microscopy technology to study nuclear structure and function [7,10]. These probes are more selective for RNA and more photostable than cyanine agents and are particularly advantageous for visualizing RNA sites in live cell nuclei. These RNA probes possess *N*-methyl position amenable to labeling with a positron emitting radioisotope such as carbon-11 as RNA radioligands. The same properties are often beneficial in a diagnostic radiotracer. RNA also

Abbreviations: PET, positron emission tomography; SPE, solid-phase extraction; EOB, end of bombardment; EOS, end of synthesis; rt, room temperature; HPLC, high pressure liquid chromatography; SPECT, single photon emission computed tomography; MRI, magnetic resonance imaging; TMS, tetramethylsilane; HRMS, high resolution mass spectra; TLC, thin-layer chromatography; RDS, radionuclide delivery system; INGEN, Indiana genomics initiative.

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provides an attractive target for the *in vivo* biomedical imaging technique positron emission tomography (PET) to map RNA and its related diseases. Compared to fluorescent microscopy technology, only PET has sufficient sensitivity and quantitation to measure the expression of genes *in vivo* [1]. We are interested in the development of PET RNA probes. In our previous works, we have developed radiolabeled O^6-benzylguanine derivatives for PET imaging of DNA repair protein [11–14], radiolabeled penciclovir and ganciclovir analogues as PET reporter probes for herpes simplex virus thymidine kinase (HSV-tk) gene [15–17], and radiolabeled D-luciferin derivatives as PET reporter probes for luciferase gene [18]. In this ongoing study, we have designed and synthesized new carbon-11 labeled styryl dyes, (*E*)-2-(2-(1*H*-indol-3-yl)vinyl)-1-[^{11}C]methylquinolinium triflate ([^{11}C]E36, [^{11}C]**6**), (*E*)-1-[^{11}C]methyl-2-(2,4,6-trimethoxystyryl)quinolinium triflate ([^{11}C]E144, [^{11}C]**7**), and (*E*)-2-(4-(dimethylamino)styryl)-6-methoxy-1-[^{11}C]methylquinolinium triflate ([^{11}C]F22, [^{11}C]**9**), as potential PET RNA-specific, living cell imaging probes.

2. Results and discussion

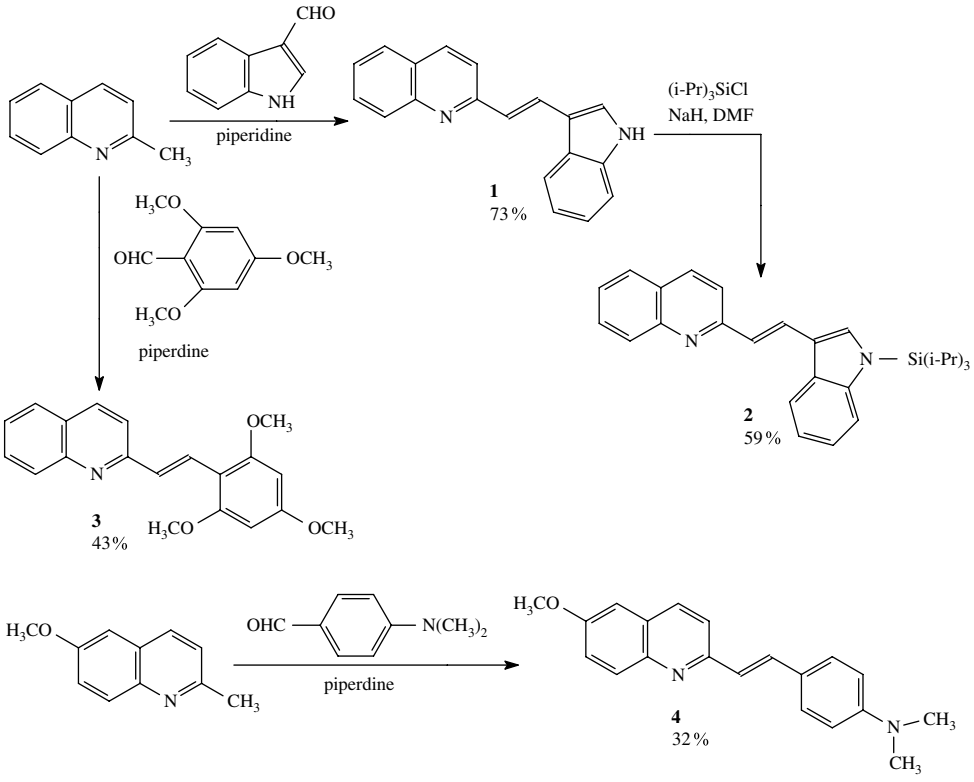
2.1. Chemistry

Synthesis of precursors (**2**–**4**) is shown in Scheme 1. As illustrated in Scheme 1, condensation of 2-methylquinoline or 6-methoxy-2-methylquinoline with aromatic aldehydes in the presence of catalytic amounts of piperidine afforded *E*-2-styrylquinolines (**1**, **3** and **4**) [19,20] in 32–73% yields. Triisopropylsilyl group for protection of indole nitrogen of compound **1** was employed since its silyl moiety could be removed easily by tetrabutylammonium fluoride [(*n*-Bu)$_4$NF] in THF at room temperature (rt) [21,22]. Thus, compound **1** was reacted with sodium hydride and triisopropylsilyl chloride in DMF to give precursor **2** in 59% yield.

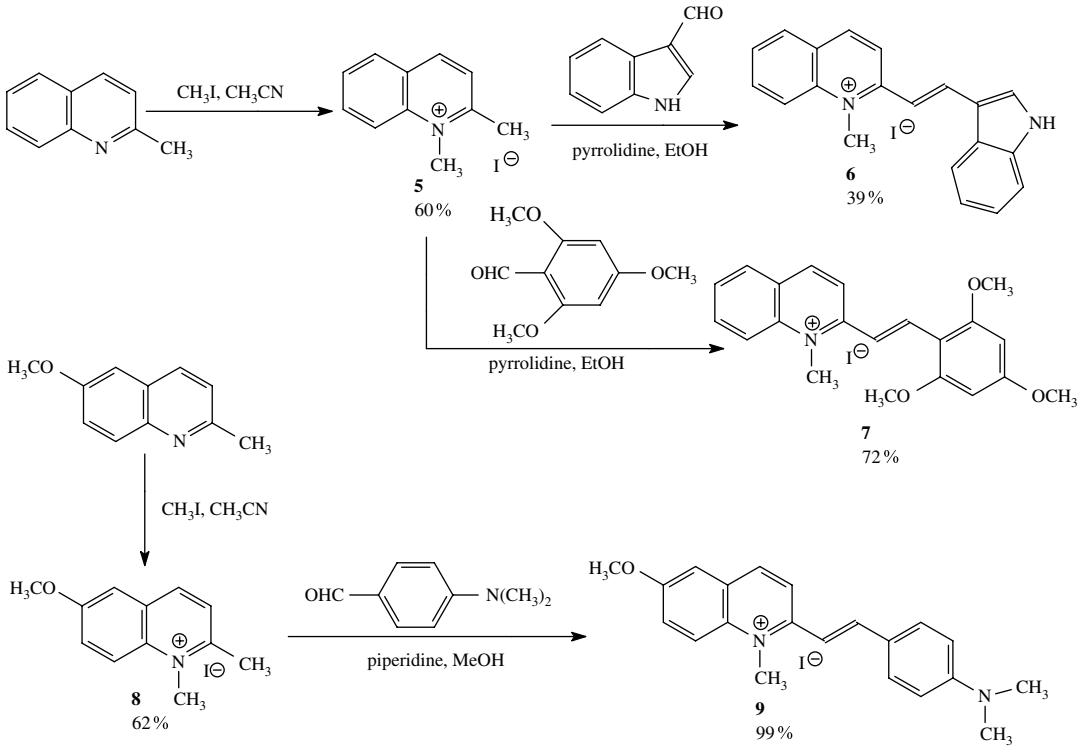
Synthesis of standard compounds E36 (**6**), E144 (**7**) and F22 (**9**) according to literature procedures with modifications [7,10] is outlined in Scheme 2. *N*-methylation of 2-methylquinoline or 6-methoxy-2-methylquinoline with iodomethane in acetonitrile provided 1,2-dimethylquinolinium iodide **5** and 6-methoxy-1,2-dimethylquinolinium iodide **8** in 60 and 62% yields, respectively. Compound **6** could be prepared by straightforward condensation of compound **5** and indole-3-aldehyde, instead of the acetylated indole-3-aldehyde [7], using catalytic amounts of pyrrolidine in EtOH in 39% yield. Compounds **7** and **9** were prepared in a manner similar to compound **6** from corresponding aromatic aldehydes by reaction with methylquinolinium iodides (**5** and **8**) with catalytic amounts of pyrrolidine or piperidine in 72 and 99% yields, respectively.

2.2. Radiochemistry

Synthesis of target probes [^{11}C]E36 ([^{11}C]**6**), [^{11}C]E144 ([^{11}C]**7**) and [^{11}C]F22 ([^{11}C]**9**) is indicated in Scheme 3. Precursor **2** was labeled by a reactive [^{11}C]methylating agent, [^{11}C]methyl triflate ([^{11}C]CH_3OTf) [23,24] prepared from

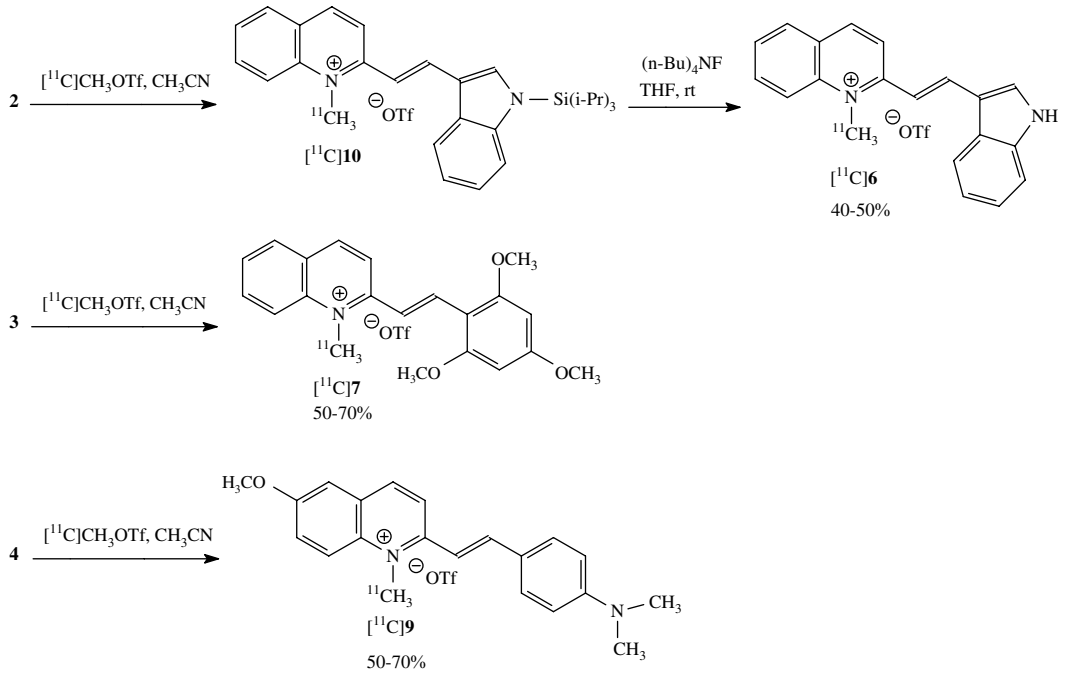


Scheme 1. Synthesis of styryl dye precursors.



Scheme 2. Synthesis of styryl dyes as standards.

[^{11}C]CO_2, in acetonitrile through the *N*-[^{11}C]methylation and trapped on a cation-exchange CM Sep-Pak cartridge to release the non-reacted indole nitrogen protected tertiary amine precursor with ethanol and to retain the pure *N*-[^{11}C]-methylated quaternary ammonium intermediate, (*E*)-2-(2-(1-triisopropylsilyl)-1*H*-indol-3-yl)vinyl-1-[^{11}C]methylquinolinium triflate ([^{11}C]**10**), on the same CM Sep-Pak. The ^{11}C-labeled intermediate underwent the deprotecting reaction [21,22] by addition of a 1.0 M solution of $(n\text{-Bu})_4$NF in THF to the same cartridge. After 2 min, non-reacted $(n\text{-Bu})_4$NF was removed from the cartridge by rinsing with ethanol. The final carbon-11 labeled product [^{11}C]E36 was then eluted from the cartridge with saline in 40–50% radiochemical yields, decay corrected to end of bombardment (EOB), based on



Scheme 3. Synthesis of carbon-11 labeled styryl dyes.

[^{11}C]CO_2. The synthesis was performed in an automated multipurpose ^{11}C-radiosynthesis module, allowing measurement of specific activity during synthesis [25–27]. The specific activity of [^{11}C]E36 was in a range of 74–111 GBq/μmol at the end of synthesis (EOS). The purification technique we used in the radiosynthesis of [^{11}C]E36 is the solid-phase extraction (SPE) method [28,29], and the key part in this technique is a CM Sep-Pak cartridge. Similarly, tertiary amine precursors **3** and **4** were labeled with [^{11}C]CH_3OTf and purified by SPE technique to provide target quaternary ammonium tracers [^{11}C]E144 and [^{11}C]F22, respectively, in 50–70% radiochemical yields, decay corrected to EOB, based on [^{11}C]CO_2. The large polarity difference between the tertiary amine precursor and the labeled quaternary ammonium product permitted the use of SPE technique for purification of labeled product from radiolabeling reaction mixture. A cation-exchange CM Sep-Pak cartridge was used in SPE purification technique. The reaction mixture was loaded onto the cartridge by gas pressure. The cartridge column was washed with ethanol and water to remove non-reacted [^{11}C]CH_3OTf, precursor and reaction solvent, and then final labeled product was eluted with saline from the CM Sep-Pak. SPE technique is fast, efficient and convenient and works very well for the quaternary ammonium tracer production. The specific activity for [^{11}C]E144 and [^{11}C]F22 was in a range of 74–111 GBq/μmol at EOS under the same targetry conditions which have been used in the radiosynthesis of [^{11}C]E36, determined by analytical high pressure liquid chromatography (HPLC) method [30].

Methods available to image gene expression in living animals are rapidly increasing. Several techniques, including radionuclide approaches PET and single photon emission computed tomography (SPECT), magnetic approach magnetic resonance imaging (MRI), and optical approaches green fluorescent protein and luciferase, are all under active investigation [1]. Fluorescent styryl molecules have been synthesized and screened for an *in vitro* RNA response and live cell nuclear imaging [7,10]. However, fluorescent microscopy technology is only available for small animals in preclinical study and has not sufficient sensitivity and quantitation to measure the expression of genes *in vivo*. PET coupled with appropriate radiotracers has the unique capability of non-invasively measuring biochemical and metabolic processes, is particularly suited for quantitatively imaging animals and humans with a relatively high sensitivity, and has become a clinically valuable and accepted diagnostic tool to image diseases. The further *in vivo* evaluation of PET imaging of transgene expression in animal models with the probes [^{11}C]E36, [^{11}C]E144 and [^{11}C]F22 is currently underway, and the results will be reported in due course.

3. Materials and methods

3.1. General

All commercial reagents and solvents from Aldrich and Sigma were used without further purification. [^{11}C]CH_3OTf was prepared according to a literature procedure [23]. Melting points were determined on a MEL-TEMP II capillary tube apparatus and were uncorrected. ^{1}H NMR spectra were recorded on a Bruker QE 300 NMR spectrometer using tetramethylsilane (TMS) as an internal standard. Chemical shift data for the proton resonances were reported in parts per million (ppm, δ scale) relative to internal standard TMS (δ 0.0), and coupling constants (J) were reported in hertz (Hz). The high resolution mass spectra (HRMS) were obtained using a Thermo MAT 95XP-Trap spectrometer. Chromatographic solvent proportions are indicated in a volume:volume ratio. Thin-layer chromatography (TLC) was run using Analtech silica gel GF uniplates (5×10 cm^2). Plates were visualized under UV light. Preparative TLC was run using Analtech silica gel UV 254 plates (20×20 cm^2). All moisture- and/or air-sensitive reactions were performed under a positive pressure of nitrogen maintained by a direct line from a nitrogen source. Analytical HPLC was performed using a Prodigy (Phenomenex) 5 μm C-18 column, 4.6×250 mm; 3:1:1 CH_3CN:MeOH:20 mM, pH 6.7 $KHPO_4^-$ (buffer solution) mobile phase; flow rate 1.5 mL/min; and UV (254 nm) and γ-ray (NaI) flow detectors. Semi-prep cation-exchange CM Sep-Pak cartridges were obtained from Waters Corporate Headquarters, Milford, MA. Sterile vented Millex-GS 0.22 μm filter unit was obtained from Millipore Corporation, Bedford, MA.

3.2. (*E*)-2-(2-(1*H*-Indol-3-yl)vinyl)quinoline (**1**)

A mixture of indole-3-aldehyde (1.0 g, 6.89 mmol), 2-methylquinoline (1.18 g, 8.25 mmol), and a catalytic amount of piperidine (80 μL) in a tube was heated at 158 °C overnight. After cooling, the resulting solid was triturated with *i*-PrOH, filtered, and recrystallized from MeOH to afford **1** (1.35 g, 73%) as a yellow solid, mp 209–210 °C (lit [19], 210–211 °C). ^{1}H NMR ($CDCl_3$): δ 8.24 (d, $J = 8.7$ Hz, 1H, Ar-H), 8.10–8.06 (m, 1H, Ar-H), 7.96 (d + d, $J = 8.6$, 16.5 Hz, 2H, Ar-H and CH=CH), 7.91 (d, $J = 8.7$ Hz, 1H, Ar-H), 7.85 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.74–7.68 (m, 1H, Ar-H), 7.64 (s, 1H, Ar-H), 7.52–7.38 (m + d, $J = 16.5$ Hz, 3H, Ar-H + CH=CH), 7.23–7.18 (m, 2H, Ar-H).

3.3. (*E*)-2-(2-(1-(Triisopropylsilyl)-1*H*-indol-3-yl)vinyl)quinoline (**2**)

Compound **1** (300 mg, 1.11 mmol) was added to a stirred suspension of NaH (32 mg, 60% dispersion in mineral oil, 1.33 mmol) in anhydrous DMF (3 mL) at 0 °C. After 20 min, at this temperature, triisopropylsilyl chloride (0.35 mL, 1.67 mmol) was added dropwise and the stirring was continued for 3 h. Water was added and the resulting mixture was extracted with $CHCl_3$. The organic layer was washed with H_2O, brine and dried over anhydrous Na_2SO_4. The solvent was evaporated and the crude product was purified by preparative TLC plate with CH_2Cl_2–MeOH (100:1) to afford **2** (280 mg, 59%) as a foam yellow solid, mp 134–135 °C. ^{1}H NMR ($CDCl_3$): δ 8.15 (d, $J = 7.7$ Hz, 2H, Ar-H), 8.04 (d, $J = 8.6$, 16.0 Hz, 1H, CH=CH), 7.78 (d, $J = 8.0$ Hz, 1H, Ar-H), 7.75–7.72 (m, 2H, Ar-H), 7.63 (s, 1H, Ar-H), 7.54

(d, $J = 7.6$ Hz, 2H, Ar-H), 7.52–7.46 (m, 1H, Ar-H), 7.31–7.22 (m + d, $J = 15.3$ Hz, 3H, Ar-H + CH=CH), 1.74 (sept, 3H, $J = 7.4$ Hz, CH), 1.17 (d, $J = 7.4$ Hz, 18H, $6 \times CH_3$). HRMS (CI) *m/z* calculated for $C_{28}H_{24}N_2Si$ ($[M]^+$), 426.2486; found, 426.2485.

3.4. (E)-2-(2,4,6-Trimethoxystyryl)quinoline (3)

A mixture of 2,4,6-trimethoxybenzaldehyde (200 mg, 1.02 mmol), 2-methylquinoline (175 mg, 1.22 mmol), and a catalytic amount of piperidine (30 μL) in a tube was heated at 150 °C overnight. After cooling, the resulting solid was triturated with *i*-PrOH, filtered, and recrystallized from MeOH to afford **3** (141 mg, 43%) as a green solid, mp 99–101 °C. ^{1}H NMR (DMSO-d_6): δ 8.25 (d, $J = 8.6$ Hz, 1H, Ar-H), 8.00 (d, $J = 16.6$ Hz, 1H, CH=CH), 7.93 (d, $J = 8.5$ Hz, 1H, Ar-H), 7.88 (d, $J = 8.9$ Hz, 1H, Ar-H), 7.72–7.65 (m, 2H, Ar-H), 7.59 (d, $J = 16.6$ Hz, 1H, CH=CH), 7.48 (t, $J = 7.4$ Hz, 1H, Ar-H), 6.31(s, 2H, Ar-H), 3.90 (s, 6H, $2 \times OCH_3$), 3.83 (s, 3H, OCH_3). HRMS (CI) *m/z* calculated for $C_{20}H_{19}NO_3$ ($[M]^+$), 321.1359; found, 321.1351.

3.5. (E)-4-(2-(6-Methoxyquinolin-2-yl)vinyl)-N, N-dimethylaniline (4)

A mixture of *p*-dimethylaminobenzaldehyde (200 mg, 1.34 mmol), 6-methoxy-2-methylquinoline (278 mg, 1.61 mmol), and a catalytic amount of piperidine (40 μL) in a tube was heated at 160 °C overnight. After cooling, the resulting solid was triturated with *i*-PrOH, filtered, and recrystallized from MeOH to afford **4** (130 mg, 32%) as a green solid, mp 198 °C (dec.). ^{1}H NMR (DMSO-d_6): δ 8.15 (d, $J = 7.3$ Hz, 1H, Ar-H), 7.83 (d, $J = 8.1$ Hz, 1H, Ar-H), 7.72 (d, $J = 6.6$ Hz, 2H, Ar-H), 7.62 (d, $J = 16.6$ Hz, 1H, CH=CH), 7.36–7.30 (m, 3H, Ar-H), 7.14 (d, $J = 16.1$ Hz, 1H, CH=CH), 6.73 (d, $J = 6.8$ Hz, 2H, Ar-H), 3.87 (s, 3H, OCH_3), 2.94 (s, 6H, $N(CH_3)_2$). HRMS (CI) *m/z* calculated for $C_{20}H_{20}N_2O$ ($[M]^+$), 304.1570; found, 304.1585.

3.6. 1,2-Dimethylquinolinium iodide (5)

To a solution of 2-methylquinoline (5.0 g, 34.92 mmol) in acetonitrile (10 mL) was added iodomethane (4.36 mL, 69.84 mmol). The solution was heated to reflux for 1 h. After cooling, the resulting precipitate was filtered, washed with cooled acetonitrile and dried to yield **5** (6.02 g, 60%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 9.11 (d, $J = 8.4$ Hz, 1H, Ar-H), 8.60 (d, $J = 8.8$ Hz, 1H, Ar-H), 8.41 (dd, $J = 2.5$, 8.1 Hz, 1H, Ar-H), 8.28–8.19 (m, 1H, Ar-H), 8.13(d, $J = 8.4$ Hz, 1H, Ar-H), 7.80 (t, $J = 7.5$ Hz, 1H, Ar-H), 4.45 (s, 3H, NCH_3), 3.09 (s, 3H, CH_3).

3.7. (E)-2-(2-(1H-Indol-3-yl)vinyl)-1-methylquinolinium iodide (E36, 6)

To a solution of compound **5** (100 mg, 0.35 mmol) and indole-3-aldehyde (153 mg, 1.05 mmol) in anhydrous EtOH (25 mL) was added pyrrolidine (12 μL). The solution was heated to reflux overnight. After cooling, the resulting precipitate was filtered, and recrystallized from anhydrous EtOH to provide **6** (56 mg, 39%) as a red solid. ^{1}H NMR (DMSO-d_6): δ 12.30 (s, 1H, NH), 8.81 (d, $J = 9.1$ Hz, 1H, Ar-H), 8.63 (d, $J = 16.3$ Hz, 1H, CH=CH), 8.59 (d, $J = 9.6$ Hz, 1H, Ar-H), 8.43 (d, $J = 8.9$, 1H, Ar-H), 8.37 (s, 1H, Ar-H), 8.26–8.18 (m, 2H, Ar-H), 8.07(t, $J = 8.0$ Hz, 1H, Ar-H), 7.83 (t, $J = 8.0$ Hz, 1H, Ar-H), 7.58–7.53 (d + m, $J = 15.8$ Hz, 2H, CH=CH + Ar-H), 7.31–7.25(m, 2H, Ar-H), 4.45 (s, 3H, NCH_3).

3.8. (E)-1-Methyl-2-(2,4,6-trimethoxystyryl) quinolinium iodide (E144, 7)

To a solution of compound **5** (200 mg, 0.70 mmol) and 2,4,6-trimethoxybenzaldehyde (147 mg, 0.75 mmol) in anhydrous EtOH (20 mL) was added pyrrolidine (30 μL). The solution was heated to reflux for 7 h. After cooling, the resulting precipitate was filtered, and recrystallized from anhydrous EtOH to provide **7** (233 mg, 72%), as a red solid. ^{1}H NMR (DMSO-d_6): δ 8.89 (d, $J = 9.0$ Hz, 1H, Ar-H), 8.47 (d, $J = 9.0$ Hz, 1H, Ar-H), 8.38 (d, $J = 9.1$ Hz, 1H, Ar-H), 8.33–8.29 (m, 1H, Ar-H), 8.17 (d, $J = 15.8$ Hz, 1H, CH=CH), 8.12–8.10 (m, 1H, Ar-H), 7.94 (d, $J = 16.4$ Hz, 1H, CH=CH), 7.87 (d, $J = 7.5$ Hz, 1H, Ar-H), 6.38 (s, 2H, Ar-H), 4.39 (s, 3H, NCH_3), 3.97 (s, 6H, $2 \times OCH_3$), 3.89 (s, 3H, OCH_3).

3.9. 6-Methoxy-1,2-dimethylquinolinium iodide (8)

To a solution of 6-methoxy-2-methylquinoline (3.0 g, 17.32 mmol) in acetonitrile (6 mL) was added iodomethane (2.70 mL, 43.30 mmol). The solution was heated under reflux for 2 h. After cooling, the resulting precipitate was filtered, washed with cooled acetonitrile and dried to yield **8** (3.36 g, 62%) as a yellow solid. ^{1}H NMR (DMSO-d_6): δ 8.94 (d, $J = 8.4$ Hz, 1H, Ar-H), 8.54–8.49 (m, 1H, Ar-H), 8.05 (d, $J = 8.8$ Hz, 1H, Ar-H), 7.88–7.81(m, 2H, Ar-H), 4.42 (s, 3H, NCH_3), 3.99 (s, 3H, OCH_3), 3.02 (s, 3H, CH_3).

3.10. (E)-2-(4-(Dimethylamino)styryl)-6-methoxy-1-methylquinolinium iodide (F22, 9)

To a solution of compound **8** (2.0 g, 6.35 mmol) and *p*-dimethylaminobenzaldehyde (1.42 g, 9.53 mmol) in anhydrous MeOH (20 mL) was added piperidine (0.2 mL). The solution was heated to reflux for 5 h. After cooling, the resulting precipitate was filtered, washed with EtOAc and dried to provide **9** (2.8 g, 99%) as a purple solid. ^{1}H NMR (DMSO-d_6): δ 8.69 (d, $J = 9.1$ Hz, 1H, Ar-H), 8.45 (d, $J = 9.3$ Hz, 1H, Ar-H), 8.36 (d, $J = 9.1$ Hz, 1H, Ar-H), 8.11 (d, $J = 15.5$ Hz, 1H, CH=CH), 7.80 (d, $J = 8.9$ Hz, 2H, Ar-H), 7.71–7.67 (m, 2H, Ar-H), 7.50 (d, $J = 15.5$ Hz, 1H, CH=CH), 6.79 (d, $J = 8.9$ Hz, 2H, Ar-H), 4.42 (s, 3H, NCH_3), 3.95 (s, 3H, OCH_3), 3.04 (s, 6H, $N(CH_3)_2$).

3.11. (E)-2-(2-(1H-Indol-3-yl)vinyl)-1-[^{11}C]methylquinolinium triflate ([^{11}C]E36, [^{11}C]**6**)

[^{11}C]CO_2 was produced by the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in small volume (9.5 cm^3) aluminum gas target (CTI) from 11 MeV proton cyclotron on research purity nitrogen (+1% O_2) in a Siemens radionuclide delivery system (Eclipse RDS-111). Precursor **2** (0.1–0.3 mg) was dissolved in CH_3CN (300 μL). The mixture was transferred to a small reaction vial. No-carrier-added (high specific activity) [^{11}C]CH_3OTf that was produced by the gas-phase production method [23] from [^{11}C]CO_2 through [^{11}C]CH_4 and [^{11}C]CH_3Br with silver triflate (AgOTf) column was passed into the reaction vial, which was cooled to ~0 °C, until radioactivity reached a maximum (~2 min), and then the reaction mixture was heated at 80 °C for 2 min. The reaction vessel was connected to a CM Sep-Pak cartridge. The labeled product mixture solution was passed onto the Sep-Pak cartridge to release the non-reacted excess precursor with ethanol and to retain the pure *N*-[^{11}C-methylated quaternary ammonium intermediate ([^{11}C]**10**), on the same CM Sep-Pak. Then the ^{11}C-labeled intermediate underwent the deprotecting reaction by addition of a 1.0 M solution of $(n\text{-Bu})_4NF$ in THF (2 mL) to the same cartridge. After 2 min, the Sep-Pak cartridge was washed with ethanol (5 mL) and water (2 mL) to remove non-reacted $(n\text{-Bu})_4NF$, and the washing solution was discarded to a waste bottle. The final product [^{11}C]**6** was eluted from the CM Sep-Pak with saline (2–4 mL) and sterile-filtered through a 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and the total volume was noted. The overall synthesis time was 20–25 min from EOB. The radiochemical yields decay corrected to EOB, from [^{11}C]CO_2, were 40–50%, the radiochemical purity was >99%, and the chemical purity of the target tracer was >95% measured by analytical HPLC. Retention times in the analytical HPLC system were: t_R **2** = 5.20 min, t_R **6** = 1.88 min, t_R [^{11}C]**6** = 1.88 min.

3.12. (E)-1-[^{11}C]Methyl-2-(2,4,6-trimethoxystyryl) quinolinium triflate ([^{11}C]E144, [^{11}C]**7**) and (E)-2-(4-(dimethylamino)styryl)-6-methoxy-1-[^{11}C] methylquinolinium triflate ([^{11}C]F22, [^{11}C]**9**)

Precursor **3** or **4** (0.1–0.3 mg) was dissolved in acetonitrile (300 μL). The mixture was placed in a sealed reaction vessel. [^{11}C]CH_3OTf was passed through the reaction solution, which was cooled at ~0 °C, until radioactivity reached a maximum (~2 min), and then the reaction mixture was heated at 80 °C for 2 min. The reaction tube was connected to a CM Sep-Pak cartridge. The labeled product mixture solution was passed onto the Sep-Pak cartridge for SPE purification by gas pressure. The reaction vessel and Sep-Pak cartridge were washed with ethanol (5 mL) and water (2 mL), and the washing solution was discarded to a waste bottle. The final product [^{11}C]**7** or [^{11}C]**9** was eluted from the CM Sep-Pak with saline (2–4 mL) and sterile-filtered through a 0.22 μm cellulose acetate membrane and collected into a sterile vial. Total radioactivity was assayed and the total volume was noted. The overall synthesis time was 15–20 min from EOB. The radiochemical yields decay corrected to EOB, from [^{11}C]CO_2, were 50–70%, the radiochemical purity was >99%, and the chemical purity of the target tracer was >95%. Retention times in the analytical HPLC system were: t_R **3** = 2.60 min, t_R **7** = 1.80 min, t_R [^{11}C]**7** = 1.80 min; and t_R **4** = 2.71 min, t_R **9** = 1.83 min, t_R [^{11}C]**9** = 1.83 min.

4. Conclusions

An efficient and convenient synthesis of new carbon-11 labeled styryl dyes has been well-developed. The synthetic methodology employed classical organic chemistry such as condensation, protecting and deprotecting, and methylation reactions to synthesize unlabeled styrylquinoline derivatives. Carbon-11 labeling at nitrogen position of the precursor through *N*-[^{11}C]methylation was incorporated efficiently using [^{11}C]CH_3OTf, a signature reaction of carbon-11 radiochemistry from our laboratory. Radiosynthesis produced new probes in amounts and purity suitable for the preclinical application in animal studies using PET. Labeled products are suitable for injection, with the higher specific radioactivities in a range of 74–111 GBq/μmol at EOS, and can be obtained within 25 min from EOB including fast and efficient SPE purification and formulation. These chemistry results combined with the reported imaging data using fluorescent microscopy technology encourage further PET imaging evaluation of carbon-11 labeled styryl dyes as new potential probes for imaging of RNA in living cells.

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References

[1] D.C. MacLaren, T. Toyokuni, S.R. Cherry, J.R. Barrio, M.E. Phelps, H.R. Herschman, S.S. Gambhir, Biol. Psychiatr. 48 (2000) 337–348.
[2] D.W. Bartlett, H. Su, I.J. Hildebrandt, W.A. Weber, M.E. Davis, Proc. Natl. Acad. Sci. U.S.A. 104 (2007) 15549–15554.
[3] X. Tian, M.R. Aruva, K. Zhang, N. Shanthly, C.A. Cardi, M.L. Thakur, E. Wickstrom, J. Nucl. Med. 48 (2007) 1699–1707.
[4] S.R. Cherry, J. Nucl. Med. 47 (2006) 1735–1745.
[5] E.F.J. de Vries, J. Vroegh, G. Dijkstra, H. Moshage, P.H. Elsinga, P.L.M. Jansen, W. Vaalburg, Nucl. Med. Biol. 31 (2004) 605–612.
[6] R.J. Perera, A. Ray, BioDrugs 21 (2007) 97–104.
[7] Q. Li, Y.-T. Chang, Nat. Protoc. 1 (2006) 2922–2932.
[8] B. Ballou, G.W. Fisher, J.S. Deng, T.R. Hakala, M. Srivastava, D.L. Farkas, Cancer Detect. Prev. 22 (1998) 251–257.
[9] A.A. Bogdanov Jr., C.P. Lin, M. Simonova, L. Matuszewski, R. Weissleder, Neoplasia 4 (2002) 228–236.

[10] Q. Li, Y. Kim, J. Namm, A. Kulkarni, G.R. Rosania, Y.-H. Ahn, Y.-T. Chang, Chem. Biol. 13 (2006) 615–623.
[11] Q.-H. Zheng, X. Liu, X. Fei, J.-Q. Wang, D.W. Ohannesian, L.C. Erickson, K.L. Stone, T.D. Martinez, K.D. Miller, G.D. Hutchins, J. Labelled Compd. Radiopharm. 45 (2002) 1239–1252.
[12] X. Liu, Q.-H. Zheng, X. Fei, J.-Q. Wang, D.W. Ohannesian, L.C. Erickson, K.L. Stone, G.D. Hutchins, Bioorg. Med. Chem. Lett. 13 (2003) 641–644.
[13] Q.-H. Zheng, X. Liu, X. Fei, J.-Q. Wang, D.W. Ohannesian, L.C. Erickson, K.L. Stone, G.D. Hutchins, Nucl. Med. Biol. 30 (2003) 405–415.
[14] J.-Q. Wang, E.L. Kreklau, B.J. Bailey, L.C. Erickson, Q.-H. Zheng, Bioorg. Med. Chem. 13 (2005) 5779–5786.
[15] J.-Q. Wang, Q.-H. Zheng, X. Fei, B.H. Mock, G.D. Hutchins, Bioorg. Med. Chem. Lett. 13 (2003) 3933–3938.
[16] Q.-H. Zheng, J.-Q. Wang, X. Fei, G.D. Hutchins, Synthesis (2003) 2785–2794.
[17] J.-Q. Wang, X. Fei, T.A. Gardner, G.D. Hutchins, Q.-H. Zheng, Bioorg. Med. Chem. 13 (2005) 549–556.
[18] J.-Q. Wang, K.E. Pollok, S. Cai, K.M. Stantz, G.D. Hutchins, Q.-H. Zheng, Bioorg. Med. Chem. Lett. 16 (2006) 331–337.
[19] M. Hranjec, M. Kralj, I. Piantanida, M. Sedic, L. Suman, K. Pavelic, G. Karminski-Zamola, J. Med. Chem. 50 (2007) 5696–5711.
[20] C.T. Bahner, H. Kinder, L. Gutman, J. Med. Chem. 8 (1965) 397–398.
[21] A. Alvarez, A. Guzman, A. Ruiz, E. Velarde, J. Org. Chem. 57 (1992) 1653–1656.
[22] B.L. Bray, P.H. Mathies, R. Naef, D.R. Solas, T.T. Tidwell, D.R. Artis, J.M. Muchowski, J. Org. Chem. 55 (1990) 6317–6328.
[23] B.H. Mock, G.K. Mulholland, M.T. Vavrek, Nucl. Med. Biol. 26 (1999) 467–471.
[24] D.M. Jewett, Int. J. Rad. Appl. Instrum. [A] 43 (1992) 1383–1385.
[25] B.H. Mock, Q.-H. Zheng, T.R. DeGrado, J. Labelled Compd. Radiopharm. 48 (2005) S225.
[26] B.H. Mock, B.E. Glick-Wilson, Q.-H. Zheng, T.R. DeGrado, J. Labelled Compd. Radiopharm. 48 (2005) S224.
[27] Q.-H. Zheng, M. Gao, B.H. Mock, S. Wang, T. Hara, R. Nazih, M.A. Miller, T.J. Receveur, J.C. Lopshire, W.J. Groh, D.P. Zipes, G.D. Hutchins, T.R. DeGrado, Bioorg. Med. Chem. Lett. 17 (2007) 2220–2224.
[28] M. Gao, M.A. Miller, T.R. DeGrado, B.H. Mock, J.C. Lopshire, J.G. Rosenberger, C. Dusa, M.K. Das, W.J. Groh, D.P. Zipes, G.D. Hutchins, Q.-H. Zheng, Bioorg. Med. Chem. 15 (2007) 1289–1297.
[29] M. Gao, M. Wang, Q.-H. Zheng, Appl. Radiat. Isot. 66 (2008) 194–202.
[30] Q.-H. Zheng, B.H. Mock, Biomed. Chromatogr. 19 (2005) 671–676.