Supporting Information

Tetrazine Ligation for the Construction of F-18 Labeled Probes

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General Considerations:

All commercially available chemical reagents were used without further purification. Chromatography was performed using Silacycle P60 silica gel. All moisture sensitive reactions were carried out in glassware that was flame-dried under vacuum and cooled under nitrogen. Solid phase extraction cartridges (silica gel, 900 mg) were purchased from Waters. Ion exchange cartridges were purchased from ABX (Germany).

Experimental Procedures:

\[
\begin{align*}
\text{Sodium hydride} (1.14 \text{ g, 26.4 mmol}) & \text{ was added to a flame dried round bottom flask. The NaH was washed with dry hexane (10 mL) and then decanted. Dry tetrahydrofuran (15 mL) was added and the mixture was allowed to stir at r.t. cis-Cyclooctene-4-ol}^1 (0.834 \text{ g, 6.61 mmol}) \text{ in tetrahydrofuran (10 mL) was added to the flask. The mixture was stirred and heated to reflux for 1 hour. \text{\(\alpha\)-Bromoacetic acid} (0.919 \text{ g, 6.61 mmol}) \text{ in tetrahydrofuran (25 mL) was added and the mixture was allowed to reflux overnight. The mixture was cooled to r.t., and then concentrated on the rotary evaporator. The residue was cooled in an ice bath and water was added followed by acidification with 3 M HCl. The aqueous layer was extracted with three portions of ether. The extracts were dried with MgSO}_4 \text{ and concentrated in vacuo to provide an oil. The title compound was used directly for the next reaction without further purification.}
\end{align*}
\]
An Erlenmeyer flask was sequentially charged with (Z)-2-(cyclooct-4-en-1-yloxy)acetic acid (1.45 g, 7.90 mmol) and diethyl ether (150 mL). Diazomethane was introduced to this flask using the apparatus developed by Lombardi. Thus, Diazald (5.07 g, 23.7 mmol) and ethanol (150 mL) were added to a stoppered flask, equipped to bubble into the aforementioned Erlenmeyer flask. The ethanol mixture was sparged with nitrogen, and a blast shield was placed in front of the two flasks. Sodium hydroxide (7.30 g, 182 mmol) in water (10 mL) was slowly added via syringe to the ethanol-containing flask. Nitrogen was bubbled through this Lombardi flask until no yellow color persisted in either flask. The reaction was purified by column chromatography using 5% ether to 30% ether in hexanes as the eluent to give 1.08 g (69%, 5.47 mmol) of the title compound as a colorless oil.
\[^1\text{H NMR}\ (400\ \text{MHz, C}_6\text{D}_6)\ \delta\ 5.61-5.45\ (m, 2H),\ 3.84\ (d, J_{AB}=16.6\ \text{Hz}, 1H),\ 3.84\ (d, J_{AB}=16.6\ \text{Hz}, 1H),\ 3.37\ (\text{app dt, J}=4.1, 9.1\ \text{Hz}, 1H),\ 3.29\ (s, 3H),\ 2.22-2.13\ (m, 1H),\ 2.05-1.78\ (m, 5H),\ 1.75-1.65\ (m, 1H),\ 1.60-1.46\ (m, 2H),\ 1.30-1.20\ (m, 1H).

\[^{13}\text{C NMR}\ (100\ \text{MHz, C}_6\text{D}_6)\ \delta\ 171.0\ (C),\ 130.4\ (\text{CH}),\ 129.5\ (\text{CH}),\ 81.5\ (\text{CH}),\ 66.1\ (\text{CH}_2),\ 51.0\ (\text{CH}_3),\ 34.3\ (\text{CH}_2),\ 33.4\ (\text{CH}_2),\ 26.0\ (\text{CH}_2),\ 25.8\ (\text{CH}_2),\ 22.8\ (\text{CH}_2).

\text{IR (liquid, CHCl}_3, \text{cm}^{-1})\ 3155,\ 3019,\ 2978,\ 2934,\ 2860,\ 1753,\ 1440,\ 1383,\ 1291,\ 1216,\ 1129,\ 900,\ 722,\ 650.

\text{HRMS-CI (NH}_3)m/z: [M+NH}_4]\ \text{calcd for C}_{11}\text{H}_{22}\text{NO}_3,\ 216.1599;\ \text{found 216.1590.}

(Z)-2-(cyclooct-4-en-1-yloxy)ethanol (6)

(Z)-methyl 2-(cyclooct-4-en-1-yloxy)acetate (2.34 g, 11.9 mmol) and anhydrous ether (150 mL) were sequentially added to a dry round bottom flask. The flask was cooled to –78 °C. DIBAL (9.03 mL, 47.6 mmol) in ether (30 mL) was added slowly via syringe to the flask. The reaction mixture was allowed to stir for another 3 hours at –78 °C, then warmed to 0 °C and stirred for a further 3 hours. The reaction was quenched at 0 °C with Na$_2$SO$_4$•10 H$_2$O. The mixture was concentrated in vacuo. The reaction was purified by column chromatography using 5% ether to 30% ether in hexanes as the eluent to give 1.55 g (78%, 9.31 mmol) of the title compound as a colorless oil.
\(^1\)H NMR (400 MHz, C\textsubscript{6}D\textsubscript{6}) \(\delta\) 5.61-5.48 (m, 2H), 3.54-3.51 (m, 2H), 3.22-3.15 (m, 3H), 2.19-2.13 (m, 1H), 2.03-1.92 (m, 3H), 1.85-1.74 (m, 3H), 1.68-1.62 (m, 1H), 1.51-1.44 (m, 2H), 1.25-1.20 (m, 1H).

\(^{13}\)C NMR (100 MHz, C\textsubscript{6}D\textsubscript{6}) \(\delta\) 130.4 (CH), 129.5 (CH), 80.9 (CH), 69.8 (CH\_2), 62.2 (CH\_2), 34.5 (CH\_2), 33.4 (CH\_2), 26.1 (CH\_2), 25.8 (CH\_2), 22.9 (CH\_2).

IR (liquid, CHCl\textsubscript{3}, cm\textsuperscript{-1}) 3456, 3011, 2975, 2936, 2861, 1650, 1447, 1392, 1252, 1100, 1049, 988, 875.

HRMS-ESI m/z: [M+Na] calcd for C\textsubscript{10}H\textsubscript{18}O\textsubscript{2}Na, 193.1204; found 193.1205.

Major diastereomer:

2-\([rel-(1R-4E-pR)]\)cyclooct-4-en-1-yloxy]ethanol (7)

Minor diastereomer:

2-\([rel-(1R-4E-pS)]\)cyclooct-4-en-1-yloxy]ethanol (7)

(Z)-2-(cyclooct-4-en-1-yloxy)ethanol (1.54 g, 9.31 mmol) and methyl benzoate (2.49 g, 18.6 mmol) were dissolved in 500 mL of 9:1 ether:hexane in a quartz flask. The photoisomerization was carried out using the flow apparatus described previously. The following minor modifications were made: a Biotage “SNAP cartridge” column (50 g, Biotage part no. FSK0-1107-0050) was used, and the FMI pump was a model QG 400.
The column was packed with 8.5 cm of silica gel, and then silver impregnated silica gel (16 g) on top. The column was flushed with 9:1 ether:hexane (250 mL). The pump was turned on at a flow rate of 100 mL/min and irradiation begun. Photoisomerization of the mixture was carried out for 6 hours. The column was flushed with 9:1 ether:hexane (250 mL) and then dried with compressed air. The silica was placed into an Erlenmeyer flask and stirred with ammonium hydroxide (200 mL) and methylene chloride (200 mL) for 5 min. The silica gel was filtered and the filtrate was placed into a separatory funnel. The organic layer was separated, and the ammonium hydroxide layer was extracted three times with methylene chloride. The organic layers were combined and twice washed with water. The organic layers were dried with MgSO₄, filtered, and purified by column chromatography with 5% ether to 30% ether in hexanes. Two diastereomers were isolated 0.572 g (3.44 mmol, 37%) of 2-[rel-(1R-4E-pR)-cyclooct-4-en-1-yloxy]ethanol and 0.275 g (1.66 mmol, 18%) of 2-[rel-(1R-4E-pS)-cyclooct-4-en-1-yloxy]ethanol as colorless oils. The major diastereomer was contaminated by 7% of the cis-isomer: peaks attributable to cis-isomer 5.61-5.48 (m), 1.51-1.44 (m). The structures were assigned on the basis of the chemical shift for the C-1 methine, as described previously.²

Spectroscopic properties of the minor diastereomer:

$^1$H NMR (400 MHz, C₆D₆) δ 5.75-5.68 (m, 1H), 5.44-5.36 (m, 1H), 3.56 (m, 2H), 3.31-3.29 (m, 1H), 3.28-3.21 (m, 1H), 3.16-3.11 (m, 1H), 2.44 (br s, 1H), 2.41-2.31 (m, 1H), 2.24-2.21 (m, 1H), 2.12-2.08 (m, 1H), 1.99-1.91 (m, 1H), 1.89-1.71 (m, 2H), 1.63-1.57 (m, 1H), 1.25-1.17 (m, 1H), 0.98-0.91 (m, 1H).

$^{13}$C NMR (100 MHz, C₆D₆) δ 136.0 (CH), 131.5 (CH), 75.2 (CH), 70.4 (CH₂), 62.2 (CH₂), 40.4 (CH₂), 34.9 (CH₂), 33.2 (CH₂), 30.2 (CH₂), 27.9 (CH)
IR (liquid, CHCl₃, cm⁻¹) 3428, 3021, 2923, 2859, 1655, 1352, 1215, 1135, 1099, 1050, 989, 907, 738

Spectroscopic properties of the major diastereomer:

¹H NMR (400 MHz, C₆D₆) δ 5.56-5.34 (m, 1H), 5.22-5.14 (m, 1H), 3.53-3.52 (m, 2H), 3.22-3.17 (m, 1H), 3.12-3.07 (m, 1H), 2.81-2.77 (m, 1H), 2.20-2.16 (m, 2H), 2.05-2.00 (m, 2H), 1.95-1.92 (m, 1H), 1.81-1.68 (m, 4H), 1.35-1.20 (m, 2H).

¹³C NMR (100 MHz, C₆D₆) δ 135.5 (CH), 132.2 (CH), 86.0 (CH), 69.6 (CH₂), 62.2 (CH₂), 41.1 (CH₂), 38.0 (CH₂), 34.8 (CH₂), 33.2 (CH₂), 31.9 (CH₂).

IR (liquid, CHCl₃, cm⁻¹) 3449, 3012, 2935, 2861, 1647, 1445, 1353, 1198, 1096, 1050, 993, 968, 797.

HRMS-CI (NH₃) m/z: [M+H] calcd for C₁₀H₁₉O₂, 171.1385; found 171.1384.

2-[rel-(1R-4E-pR)-cyclooct-4-en-1-yloxy]ethyl 4-nitrobenzenesulfonate (8)

Triethylamine (0.21 mL, 1.5 mmol) was added to a flame dried round bottom flask containing anhydrous ether (5 mL). p-Nitrosulfonyl chloride (0.073 g, 0.33 mmol) from a freshly opened bottle was added to the flask. The mixture was stirred at r.t. for 30 minutes. The mixture was cooled to 0 °C and 2-[rel-(1R-4E-pR)-cyclooct-4-en-1-yloxy]ethanol was added. The mixture was allowed to stir for 5 hours at 0 °C. The cold mixture was directly transferred to a column of silica gel. Flash chromatography using a
gradient of 5% ether/hexane to 20% ether/hexane as the eluent to afford 0.093 g (87%, 0.26 mmol) of the title compound as a white solid.

In experiments using an aged bottle of p-Nitrosulfonyl chloride, 20% of (R,Z)-2-(cyclooct-4-en-1-yloxy)ethyl 4-nitrobenzenesulfonate was formed. The E-isomer could be separated using preparative, reverse phase HPLC (C-18, 20 x 250 cm, 65% methanol/H₂O). For ¹⁸F labeling experiments 2-[rel-((1R-4E-pR)-cyclooct-4-en-1-yloxy)]ethyl 4-nitrobenzenesulfonate was purified away from the cis isomer.

¹H NMR peaks attributable to the cis isomer: 5.59-5.47 (m)

¹H NMR (400 MHz, C₆D₆) δ 7.50 (m, 4H), 5.34-5.26 (m, 1H), 5.17-5.10 (m, 1H), 3.84-3.82 (m, 2H), 3.05-3.02 (m, 1H), 2.99-2.94 (m, 1H), 2.65-2.61 (m, 1H), 2.17-2.10 (m, 2H), 2.02-1.92 (m, 1H), 1.82-1.77 (m, 1H), 1.74-1.63 (m, 2H), 1.59-1.50 (m, 2H), 1.24-1.12 (m, 2H).

¹³C NMR (100 MHz, C₆D₆) δ 142.0 (C, 2 peaks), 135.8 (CH), 131.2 (CH), 131.2 (CH), 124.1 (CH), 75.3 (CH), 70.4 (CH₂), 65.9 (CH₂), 39.8 (CH₂), 34.7 (CH₂), 33.0 (CH₂), 30.0 (CH₂), 27.6 (CH₂).

IR (liquid, CHCl₃, cm⁻¹) 3105, 3010, 2935, 1609, 1536, 1351, 1187, 1097, 932, 857, 776, 616.

HRMS-LIFDI m/z: [M+] calcd for C₁₆H₂₁NO₆S, 355.1089; found 355.1083.

rel-(1R-4E-pR)-5-(2-fluoroethoxy)cyclooct-1-ene (9)
A dry round bottom flask was sequentially charged with anhydrous acetonitrile (1 mL), 2-\[rel-(1R-4E-pR)-cyclooct-4-en-1-yloxy\]ethyl 4-nitrobenzenesulfonate (0.0037g, 0.010 mmol), and TBAF (0.17 mmol, 0.17 mL of a 1M solution in THF). The reaction was heated to 80 °C and allowed to stir for 3 hours. The mixture was cooled and then transferred directly to a column of silica gel. Flash chromatography with a gradient of pentane to 5% ether/pentane as the eluent to afford the title compound. After chromatography, most of the solvents were removed on the rotary evaporator. However, the compound was not dried in vacuo due to volatility. The yield was estimated to be 63% by adding an \(^1\)H NMR standard- mesitylene (0.015 mL, 0.010 mmol). Minor peaks attributable to the cis isomer were detected in the \(^1\)H NMR spectrum at: 5.61-5.47 (m), 1.55-1.47 (m). Minor peaks attributable to the cis isomer were detected by \(^{13}\)C NMR at: 130.4, 129.5, 80.9, 34.5, 26.0, 25.8, 22.9, 22.7. 
\(^1\)H NMR (400 MHz, C\(_6\)D\(_6\)) \(\delta\) 5.40-5.33 (m, 1H), 5.22-5.14 (m, 1H), 4.16 (dt, \(J_{HH}=48 \text{ Hz}, J_{HF}=4.3 \text{ Hz}, 2H\)), 3.26-3.21 (m, 2H), 3.19-3.13 (m, 1H), 2.85-2.74 (m, 1H), 2.21-2.14 (m, 2H), 2.09-1.99 (m, 2H), 1.83-1.67 (m, 4H), 1.36-1.21 (m, 2H).
\(^{13}\)C NMR (100 MHz, C\(_6\)D\(_6\)) \(\delta\) 135.6 (CH), 132.2 (CH), 86.2 (CH), 83.2 (d, \(J_{CF}=169 \text{ Hz}, CH_2\)), 67.5 (d, \(J_{CF}=20 \text{ Hz}, CH_2\)), 67.4 (CH\(_2\)), 41.1 (CH\(_2\)), 38.0 (CH\(_2\)), 34.8 (CH\(_2\)), 33.2 (CH\(_2\)), 31.9 (CH\(_2\)).
\(^{19}\)F NMR (376.5 MHz, C\(_6\)D\(_6\)) \(\delta\) 222.3

IR (liquid, CHCl\(_3\), cm\(^{-1}\)) 3095, 2934, 2860, 1610, 1445, 1191, 1104

HRMS-CI (NH\(_3\)) m/z: [M+H] calcd for C\(_{10}\)H\(_{18}\)OF, 173.1342; found 173.1342.
A dry round bottom flask was charged with 3,6-di(2-pyridyl)-s-tetrazine (0.005 g, 0.02 mmol) and anhydrous acetonitrile (1 mL). The mixture was allowed to stir at r.t. rel-(1R-4E-pR)-5-(2-fluoroethoxy)cyclooct-1-ene in acetonitrile (0.5 mL) was added dropwise to the flask until yellow color persisted. Flash chromatography using a gradient of 10% acetone/hexane to 60% acetone/hexane as the eluent yielded an 8:2 mixture of 7-(2-fluoroethoxy)-1,4-di(pyridin-2-yl)-4a,5,6,7,8,9,10,10a-octahydrocycloocta[d]pyridazine and 8-(2-fluoroethoxy)-1,4-di(pyridin-2-yl)-2,4a,5,6,7,8,9,10-octahydrocycloocta[d]pyridazine in 90% yield, as judged by $^1$HNMR. Rearrangement of 10 to 11 occurs at r.t., so analytical data must be collected within 10 min and without chromatographic purification, to minimize peaks from the rearrangement product.

$^1$H NMR (400 MHz, CD$_3$CN) $\delta$ 8.73-8.72 (m, 2H), 8.30-8.25 (m, 2H), 7.97-7.86 (m, 2H), 7.48-7.45 (m, 2H), 4.68 (dm, $J_{HH}$= 48 Hz, 2H), 4.08-4.03 (m, 1H), 3.98-3.93 (m, 1H), 3.84-3.77 (m, 2H), 3.73-3.70 (m, 1H), 2.18-2.08 (m, 5H), 1.85-1.81 (m, 4H), 1.71-1.62 (m, 1H)

$^{19}$F NMR (376.5 MHz, C$_6$D$_6$) $\delta$ 223.3

HRMS-CI (NH$_3$)m/z: [M+H] calcd for C$_{22}$H$_{26}$N$_4$OF, 381.2090; found 381.2080.
D_{2}O (0.1 mL) was added to 7-(2-fluoroethoxy)-1,4-di(pyridin-2-yl)-4a,5,6,7,8,9,10,10a-octahydrocycloocta[d]pyridazine in acetonitrile d-3 (1 mL). The mixture was allowed to stand at r.t. for 48 hours. Column chromatography afforded the title compound. \textsuperscript{1}H NMR analysis indicated 97\% yield of the title compound.

\textsuperscript{1}H NMR peaks attributable to aliphatic impurity: 1.30, 0.89.

\textsuperscript{1}H NMR (400 MHz, CD_{3}CN) \(\delta\) 8.99 (br s, 1H), 8.68-8.67 (m, 1H), 8.59-8.57 (m, 1H), 8.09-8.07 (m, 1H), 7.90-7.86 (m, 1H), 7.79-7.74 (m, 1H), 7.65-7.63 (m, 1H), 7.40-7.36 (m, 1H), 7.31-7.28 (m, 1H), 4.51 (dt, J_{HF}= 48 Hz, J_{HH}= 4.1 Hz 2H), 4.38-4.34 (m, 1H), 3.75-3.57 (m, 3H), 2.94-2.90 (m, 1H), 2.31-2.27 (m, 1H), 1.91-1.75 (m, 4H), 1.68-1.60 (m, 3H), 1.46-1.37 (m, 2H)

\textsuperscript{13}C NMR (100 MHz, CD_{3}CN) \(\delta\) 155.3 (C), 152.7 (C), 150.3 (CH), 149.6 (CH), 144.1 (C), 137.8 (CH), 137.1 (CH), 135.7 (C), 125.0 (CH), 124.1 (CH), 123.7 (CH), 121.3 (CH), 110.6 (C), 84.5 (d, J_{C:F}=165 Hz, CH_{2}), 80.0 (C), 68.1 (d, J_{C:F}=19 Hz, CH_{2}), 35.5 (CH), 33.4 (CH_{2}), 31.4 (CH_{2}), 27.0 (CH_{2}), 25.4 (CH_{2}), 22.0 (CH_{2})

\textsuperscript{19}F NMR (376.5 MHz, C_{6}D_{6}) \(\delta\) 223.3

IR (liquid, CHCl_{3}, cm\textsuperscript{-1}) 2934, 2861, 1708, 1599, 1571, 1462, 1361, 1225, 1117, 1047

HRMS-CI (NH_{3})m/z: [M+H] calcd for C_{22}H_{26}N_{4}OF, 381.2090; found 381.2085.
HPLC methods for analyzing radiolabeled materials and standards

The purification of the crude product was carried out on an analytical reversed-phase high performance liquid chromatography (HPLC) system equipped with a dual UV absorbance detector (Waters 2487) using a phenomenex C18 RP (150 x 4.6 mm 5 micron). The flow was 1 mL/min, with the mobile phase starting from 95% solvent A (0.1% TFA in water) and 5% solvent B (0.1% TFA in acetonitrile) (0-2 min), followed by a gradient mobile phase to 5% solvent A and 95% solvent B at 17 min, which was then kept at 95% B until 22 min. The radioactivity was detected by a model of Ludlum 2200 single-channel radiation detector. A semi-preparative C18 reverse phase column (phenomenex C18) was used in separations with a 4 mL/min flow rate under gradient conditions.

Production of no carrier added (NCA) [¹⁸F]-fluoride ([¹⁸F]-F⁻).

The radioisotope ¹⁸F (t ½ = 110 m) was prepared by the nuclear reaction ¹⁸O (p, n) to give ¹⁸F in a CTI/Siemens RDS112 11Mev cyclotron. The ¹⁸O is in the form of water with an isotopic purity of greater than 95%. The operation of the cyclotron and target functions was automatically controlled by the cyclotron computer system. The target was loaded with the required amount of [¹⁸O]-water, and bombarded for the appropriate time and beam current. The target was then unloaded to a collection vial located in the dose
calibrator; where the amount of fluoride was measured. The fluoride solution was then transferred to the chemistry operation.

**General Fluorination Method:**

The fluorination (~200 mCi) was performed on an automated synthesis module as shown in Figure 1S. As illustrated in the schematic diagram of the module, two-way valves V1-V6 were used to control the solvent and reagent containing reservoirs 1-6. Reservoirs 3-6 are connected with a nitrogen or argon gas line. Reservoir 1 is connected with reactor through several control valves. The reactor is connected with vacuum pump, gas line, and the injection port of the HPLC system. The solutions of potassium carbonate and Kryptofix K2.2.2 (or TBAB and MeCN) were loaded into Reservoirs 1 and 2, respectively. Reservoirs 3, 4, 5, and 6 were filled with precursor solution and other chemicals/solutions as needed. The target water containing $^{18}$F was passed through a preconditioned QMA cartridge where the $^{18}$F-F$^{-}$ was trapped. The $^{18}$F was released from the QMA cartridge by passing K$_2$CO$_3$ or TBAB solution from Reservoir 1 through the cartridge and allowed to enter into the reactor. Kryptofix solution or MeCN from Reservoir 2 was added into the reactor and the whole mixture was dried at 95°C in combination with nitrogen flow and vacuum. The precursor solution from Reservoir 3 was added to the dried $^{18}$F ion and heated at the desired temperature. The reaction mixture will be sampled out for analysis or loaded on HPLC for purification.
F-18 fluoride was dried as described above. Tetrazine 4 was prepared by mesylation of (MsCl, Et$_3$N, CH$_2$Cl$_2$) of 3-(4-hydroxymethyl)-phenyl-s-tetrazine, which had been prepared from 4-hydroxymethylbenzonitrile (formamidine acetate, S$_8$, hydrazine hydrate, then NaNO$_2$/HOAc).$^3$ Compound 4 was dissolved in MeCN and then allowed to react with $^{18}$F-TBAF at 85 °C for 15 min. The reaction mixture was then analyzed by HPLC. $^{18}$F-5 was eluted off at 15.6 min on HPLC, which correlates with the retention time of the
standard compound. The labeling yield was estimated to be 1% (non-decay corrected). Changing the reaction temperature to 110 °C or the solvent to DMSO/DMF did not increase the reaction yield.

**General procedure for reactions indicated in Table 1.**

Fluoride was dried as described above. Precursor 8 was dissolved in MeCN and added to the azeotropically dried fluoride from Reservoir 3. The crude mixture was heated at desired temperature and then analyzed by HPLC. The optimized labeling conditions are described in entry 3 in table 2. In the automated synthesis, the crude reaction mixture was loaded onto the semi-prep HPLC for separation. The UV-trace and Radio tracer from the purification is shown in Figure 2S. The purified sample was injected to the analytical HPLC. \(^{18}\text{F}\)-9 was eluted off at 17.4 min on HPLC, which correlates with the retention time of the standard compound as shown in Figure 3S. Under the optimized conditions, the radiochemical purity of \(^{18}\text{F}\)-9 was more than 98%.
General procedure for reactions described in Table 2.

HPLC-purified $^{18}$F-9 was mixed with 3,6-Di(2-pyridyl)-s-tetrazine (1a) under the conditions described in Table 2. Immediately after mixing, the crude reaction mixture was analyzed by HPLC. The HPLC injection was made within 10 seconds of mixing. A representative radio-trace of the crude mixture is shown in Figure 4S. $^{18}$F-10 was eluted
off at 11.8 min on HPLC, which correlates with the retention time of the standard compound as shown in Figure 4S. Small amounts of isomers $^{18}\text{F-11}$ were also observed on the HPLC radio trace. The retention time of peaks attributable to the isomers of $^{18}\text{F-11}$ were 12.7 and 13.3 min, which correlates with the retention times for the $^{19}\text{F}$ standards.

Figure 4S: (A) HPLC-UV absorbance of 10 (standard) and racemized pruduct 11. (B) Radio-trace of crude reaction mixture from Table 2 Entry 3 (Condition: 0.1mCi $^{18}\text{F-9}$, 21.2 µM tetrazine, MeCN/H$_2$O, immediate)

$^1$H NMR of (Z)-2-(cyclooct-4-en-1-yloxy)acetic acid (400 MHz, C$_6$D$_6$)
$^{13}$C NMR of (Z)-2-(cyclooct-4-en-1-yloxy)acetic acid (100 MHz, C$_6$D$_6$)
$^1$H NMR of (Z)-methy1 2-(cyclooct-4-en-1-yloxy)acetate (400 MHz, $C_6D_6$)

*: water
$^{13}$C NMR of (Z)-methyl 2-(cyclooct-4-en-1-yloxy)acetate (100 MHz, C$_6$D$_6$)
$^1$H NMR of 6 (400 MHz, C$_6$D$_6$)

*: water  
•: ethanol  
•: BHT
$^{13}$C NMR of 6 (100 MHz, C$_6$D$_6$)
$^1$H NMR of 2-[rel-(1R-4E-pR)-cyclooct-4-en-1-yloxy]ethanol (7) (400 MHz, C$_6$D$_6$) Major Diastereomer

\[
\text{H}\quad\text{H}\quad\text{O}\quad\text{C}\quad\text{H}\quad\text{H}\quad\text{O}\quad\text{C}
\]

*: water
$^{13}$C NMR of 2-[rel-(1R-4E-pR)-cyclooct-4-en-1-yl]oxy]ethanol (7) (100 MHz, C$_6$D$_6$) Major Diastereomer
$^1$H NMR of 2-[(1R)-4E-pS]-cyclooct-4-en-1-yloxy]ethanol (7) (400 MHz, $C_6D_6$) Minor Diastereomer
$^{13}$C NMR of 2-[rel-(1R-4E-pS)-cyclooct-4-en-1-yloxy]ethanol (7) (100 MHz, $C_6D_6$) Minor Diastereomer
$^1$H NMR of 8 (400 MHz, $C_6D_6$)

This sample was not HPLC purified to remove traces of cis-isomer.

*: Water

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$^{13}$C NMR of 8 (100 MHz, C$_6$D$_6$)
$^1$H NMR of 9 (400 MHz, C$_6$D$_6$)

(Prepared from nosylate which was *not* HPLC purified to remove traces of *cis*-isomer)

*: diethyl ether

*: n-hexane
$^{13}$C NMR of 9 (100 MHz, C$_6$D$_6$)

(Prepared from nosylate which was *not* HPLC purified to remove traces of cis-isomer)
$^{19}$F NMR of 9 (376.5 MHz, C$_6$D$_6$)
Conversion of 10 into 11 as a function of time (400 MHz, CD$_3$CN)

* Peaks attributable to an intermediate, presumably an aminal
** The chemical shifts of 11 differ somewhat in anhydrous CD$_3$CN
$^1$H NMR of 11 (400 MHz, CD$_3$CN)

*: water  
*: aliphatic impurity
$^{13}$C NMR of 11 (100 MHz, CD$_3$CN)

*: aliphatic impurity
$^{19}$F NMR of 11