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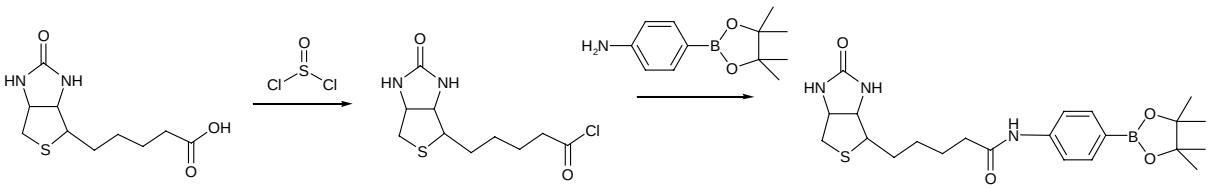
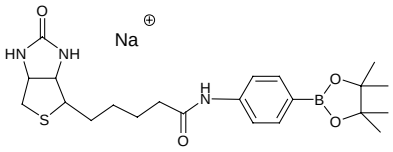
Materials: biotin, thionyl chloride, 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline, 3-aminopropyltriethoxysilane, hydrofluoric acid, potassium fluoride, and acetic anhydride were purchased from Sigma-Aldrich. Streptavidin magnetic particles were purchased from Roche Diagnostics.

Synthesis of the pinacol diester of biotinoylaminophenylboronic acid (1).



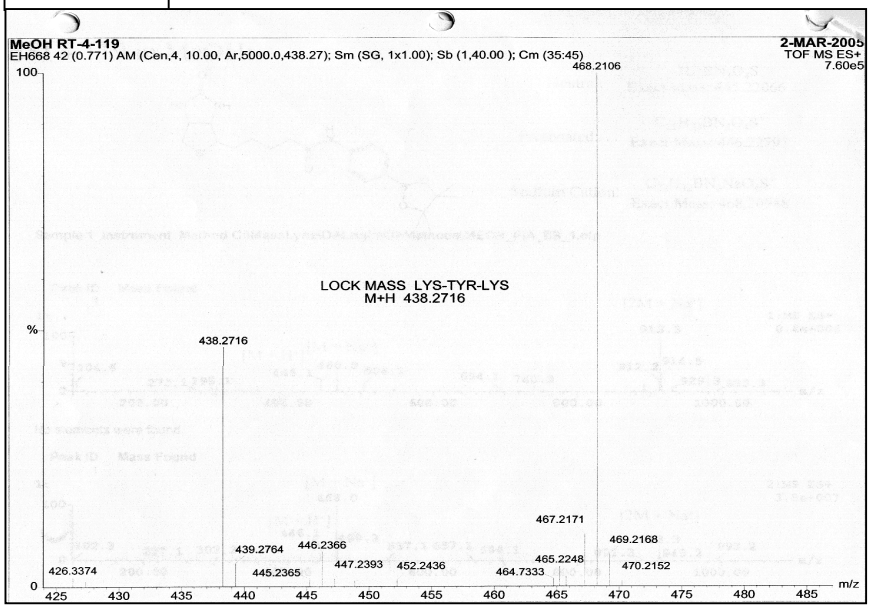
(1)

(1) A flame dried 50 ml round bottom flask was charged with a magnetic stirrer and 110 mg (0.44 mmol) of (+) biotin. A volume of 2 ml of neat excess thionyl chloride was added to the stirring solution at room temperature. The reaction was allowed to proceed for 20 min before the excess thionyl choride was removed under vacuum. The brown oil was resuspended in 25 ml of chloroform and dried down to ensure the complete removal of thionyl chloride. The resulting residue was resuspended in 2 ml of acetonitrile and a solution of 96 mg (0.44 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline in 2 ml of acetonitrile was added. The reaction was allowed to proceed for 20 min before 50 ml of diethyl ether was added, forming a precipitate that was collected by decanting. This precipitate was suspended in 10 ml of chloroform, which was washed three times with 10 ml of water, and dried over anhydrous sodium sulphate. Filtration gave **(1)** as a brown solid. ESI^+ (in MeOH): $[M+Na]^+$= 468.2106 found (468.2099 calculated). ^1H NMR (300 MHz, $CDCl_3$): δ 8.37 (1H, NH), δ 7.72 (2H, d), δ 7.68 (2H, d), δ 4.47 (1H), δ 4.30 (1H) δ 3.09 (1H), δ 2.85 (1H) δ 2.72 (1H), δ 2.36 (2H), δ 1.68 (4H), δ 1.42 (2H), δ 1.30 (12H, s). ^1H NMR (300 MHz, DMSO-d6, 50% D_2O:H_2O wash) (Shifts and integrations of exchangeable protons not listed): δ 7.50 (2H, s), δ 7.59 (2H, s), δ 4.29 (1H), δ 4.12 (1H) δ 3.11 (1H), δ 2.81 (1H) δ 2.56 (1H), δ 2.31 (2H), δ 1.70 to 1.43 (4H), δ 1.36 (2H), δ 1.26 (12H, s).

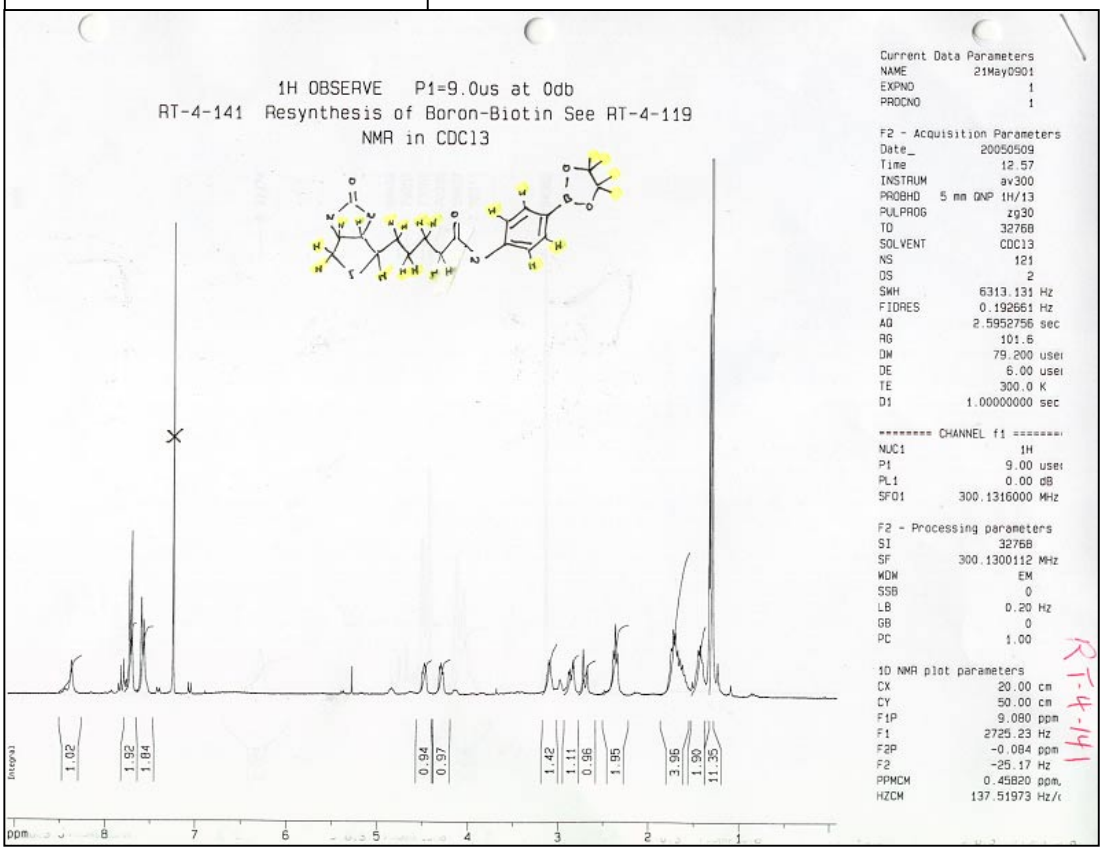


$C_{22}H_{32}BN_3NaO_4S^+$ **(1)**
Exact Mass: 468.2099
Mol. Wt.: 468.3724

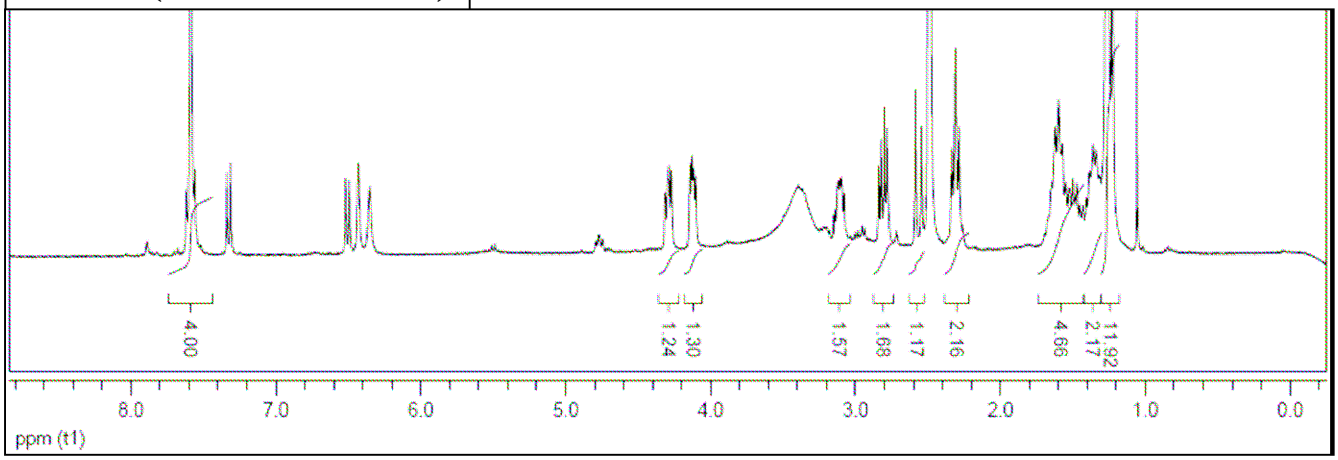
ESI MS^+



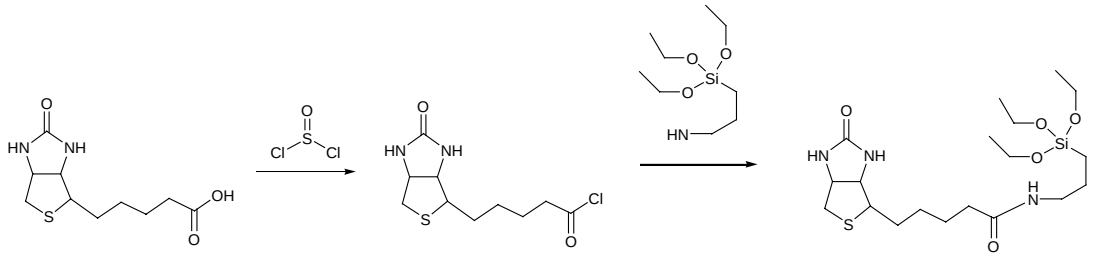
^{1}H-NMR (300 MHz, $CDCl_3$)



^{1}H-NMR (300 MHz, DMSO-d6)

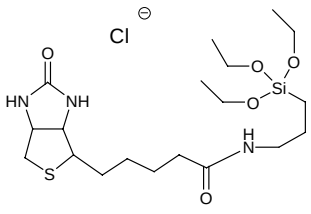


Synthesis of biotinoylaminopropyltriethoxysilane (2).



(2)

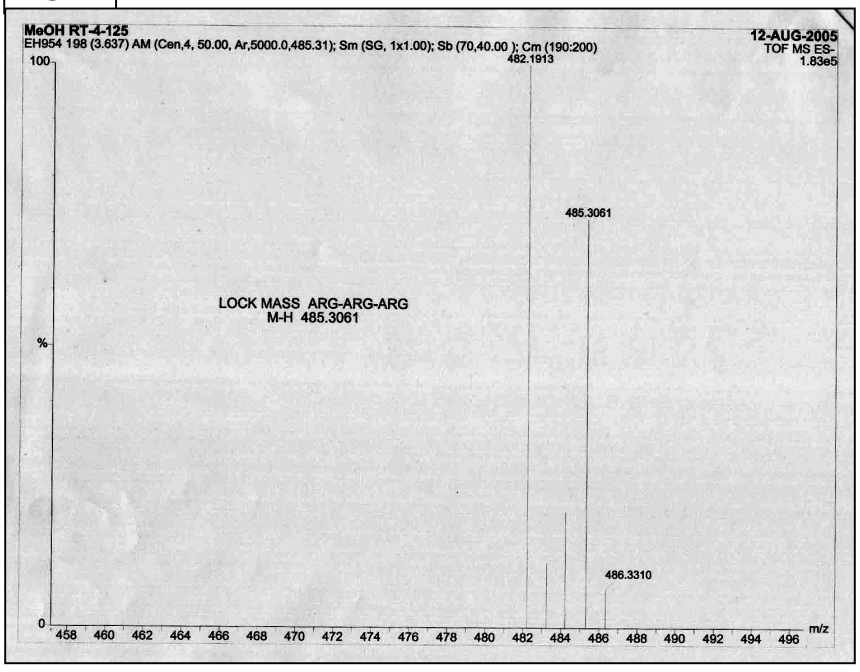
(2) A flame dried 50 ml round bottom flask was charged with a magnetic stirrer and 550 mg (2.25 mmol) of (+) biotin. A volume of 10 ml of neat thionyl chloride was added to the stirring solution and reaction was allowed to proceed for 20 min. Excess thionyl chloride was removed under vacuum. The resulting brown oil was resuspended in 25 ml of methylene chloride and dried down to quantitatively remove excess thionyl chloride. A volume of 5 ml of methylene chloride was added to the resulting oil followed by 25 ml of diethyl ether. The formation of a precipitate was observed. This solution was concentrated again to ensure the quantitative removal of thionyl chloride. To the resulting concentrated oil, 4 ml (17 mmol) of 3-aminopropyltriethoxysilane and 25 ml of methylene chloride were added. This addition guaranteed the complete solublization of the reaction mixture. Precipitation was observed within the first 10 min of the reaction. This precipitate was filtered off through a glass wool plug. The supernatant was collected, evaporated, resuspended in 5 ml of methylene chloride, and **(2)** was precipitated with 50 ml of diethyl ether. **(2)** was collected by filtration, washed with 100 ml of diethyl ether and placed under vacuum. ESI$^-$ (in MeCN): $[M+Cl]^-$ = 482.1913 found (482.1917 calculated). ^{1}H NMR (300 MHz, $CDCl_3$): δ 6.25 (1H, NH), δ 6.05 (1H, t, NH), δ 5.43 (1H, NH), δ 4.48 (1H, dd, J= 7.4 Hz, 4.9 Hz), δ 4.29 (1H, t, J= 4.8 Hz), δ 3.79 (q, 6H, J= 7.0 Hz), δ 3.20 (q, 2H, J= 6.5 Hz), δ 3.12 (q, 1H, J= 4.6 Hz), δ 2.87 (1H, dd, J= 8.0 Hz, 4.8 Hz), δ 2.71 (1H, d, J= 12.7 Hz) δ 2.17 (2H, t, J= 7.4 Hz), δ 1.65 (6H, m) δ 1.42 (2H, quintet, J= 7.5 Hz), δ 1.19 (9H t, J=7.0 Hz), δ 0.60 (2H, t, J= 8.0 Hz). ^{1}H NMR (300 MHz, DMSO): δ 7.75 (1H, NH), δ 6.42 (1H, NH), δ 6.35 (1H, NH), δ 4.28 (1H), δ 4.11 (1H), δ 3.72 (q, 6H, J= 6.9 Hz), δ 3.08 (2H), δ 2.98 (q, 1H, J= 5.9 Hz), δ 2.80 (1H), δ 2.56 (1H), δ 2.03 (2H, t, J= 7.2 Hz), δ 1.65 to 1.35 (6H, m), δ 1.29 (2H, m), δ 1.23 (9H t, J=6.9 Hz), δ 0.50 (2H, t, J= 8.9 Hz).



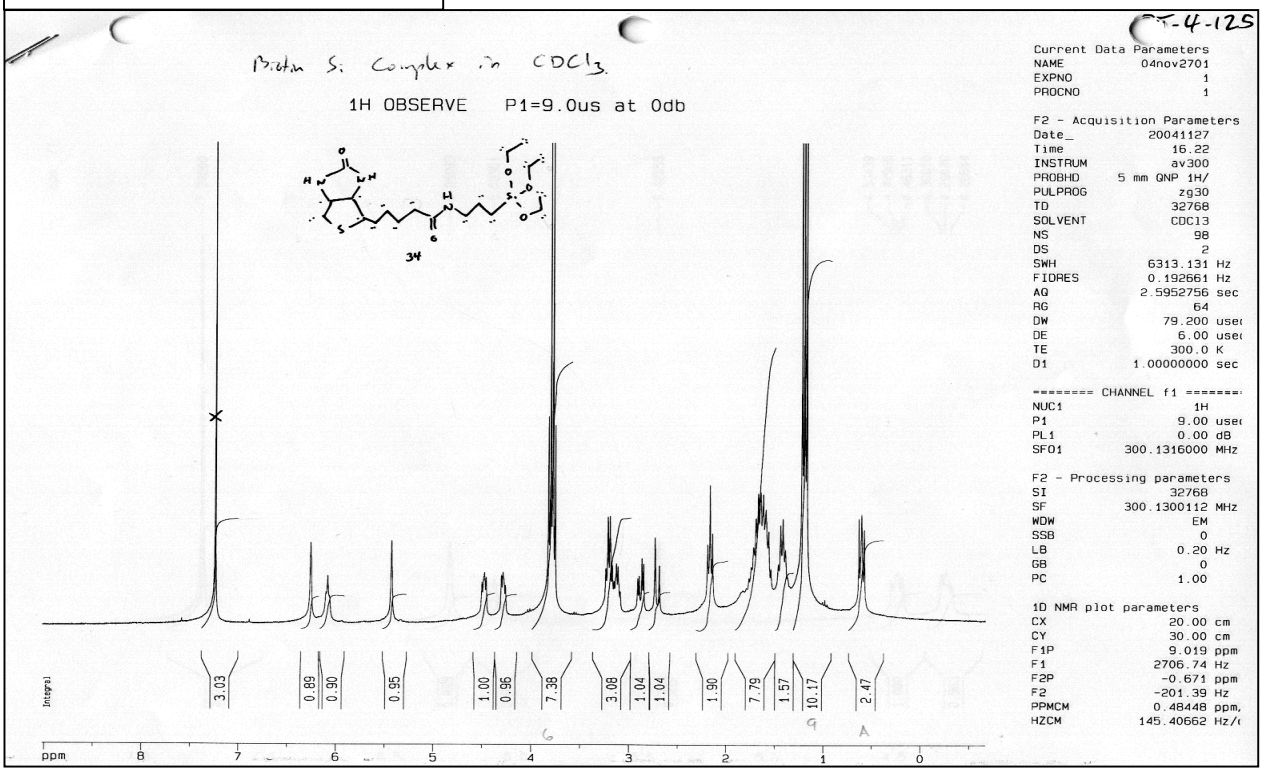
$C_{19}H_{37}ClN_3O_5SSi^-$
Exact Mass: 482.1917
Mol. Wt.: 483.1182

(2)

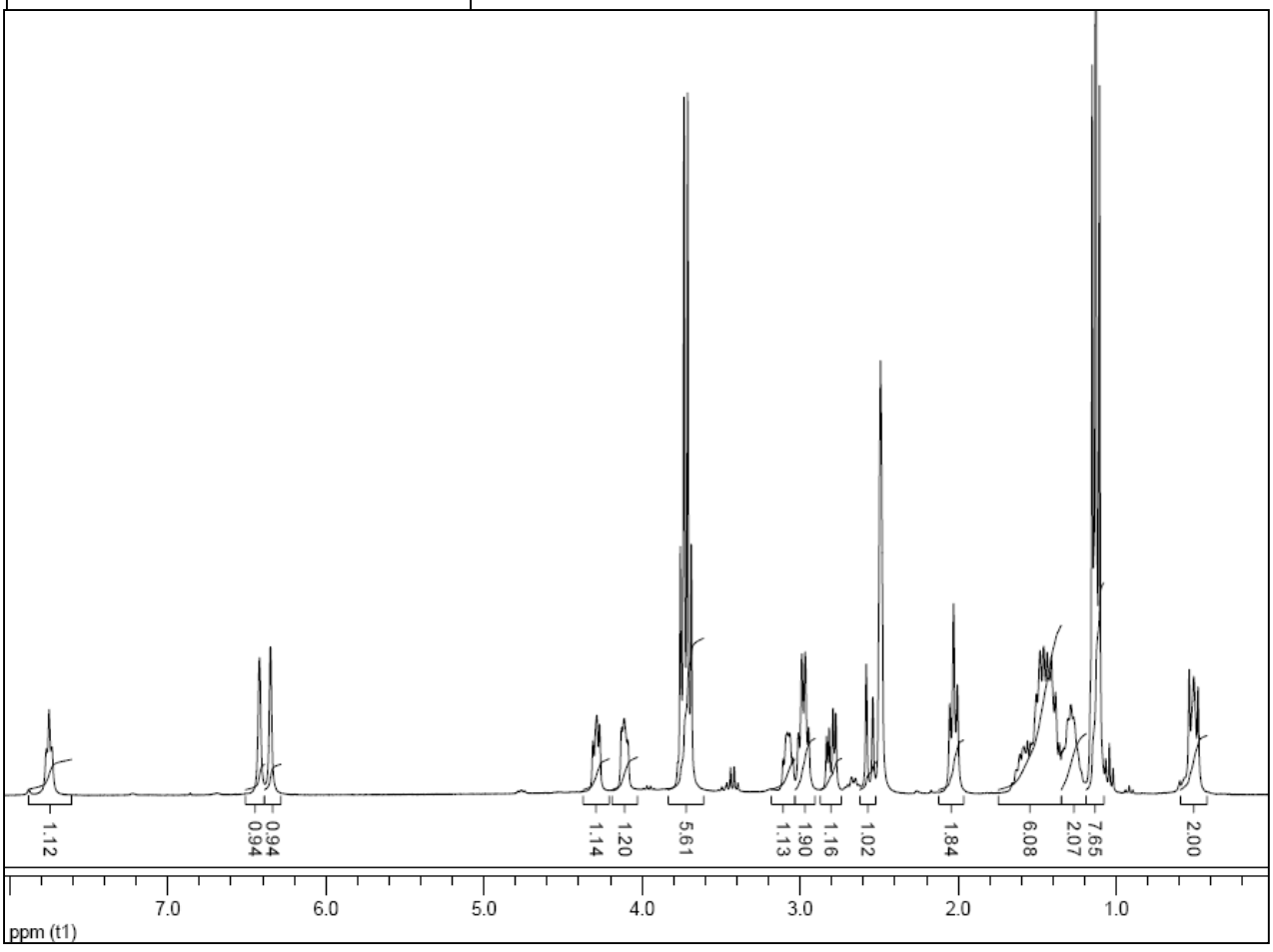
ESI



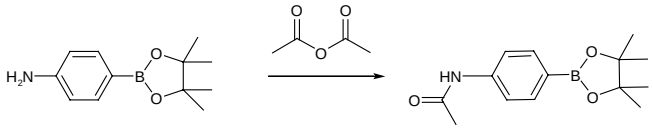
^{1}H-NMR (300 MHz, $CDCl_3$)



^{1}H-NMR (300 MHz, DMSO-d6)

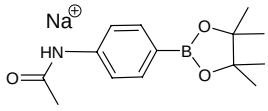


Synthesis of the pinacol diester of N-Acetylphenylboronic acid (3).



(3)

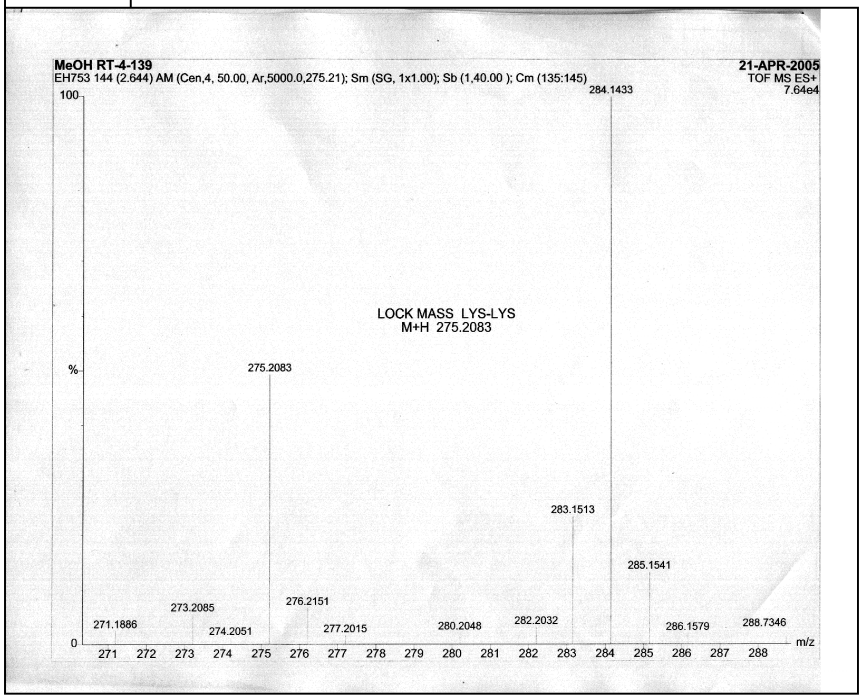
(3) A flame dried 50 ml round bottom flask was charged with a magnetic stirrer and 100 mg (0.46 mmol) of 4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)aniline. A volume of 25 ml of acetic anhydride was added to dissolve the boron compound and the reaction was allowed to proceed with stirring for 1 hour. Excess acetic anhydride and acetic acid were removed under vacuum. The resulting white solid was dissolved in 25 ml of ether, which was subsequently removed under vacuum. This process was repeated 3 times in order to facilitate the quantitative removal of any remaining acetic anhydride. **(3)** was obtained as a crystalline white solid. ESI^+ (in MeOH): $[M+Na]^+$ = 284.1433 found (284.1428 calculated), ^1H NMR (300 MHz, $CDCl_3$): δ 8.38 (1H, NH), δ 7.71 (2H, d, 8.16 Hz), δ 7.52 (2H, d, 8.14 Hz), δ 2.10 (3H, s), δ 1.29 (12H, s).



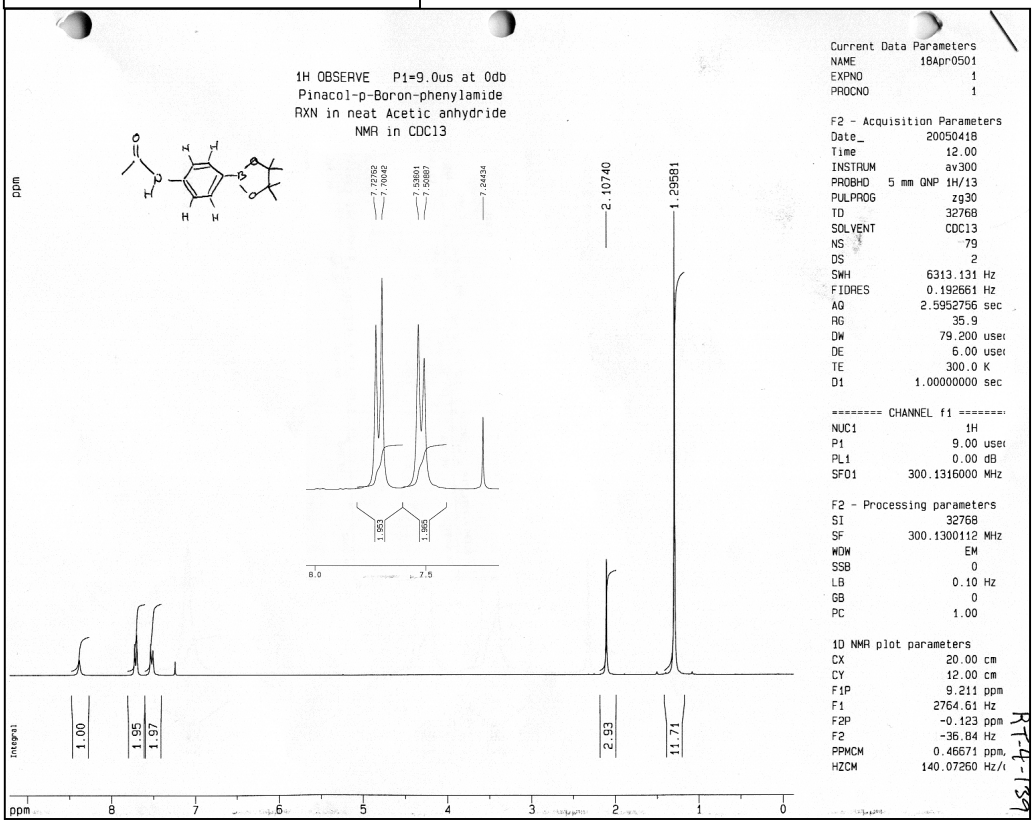
$C_{14}H_{20}BNNaO_3^+$
Exact Mass: 284.1428
Mol. Wt.: 284.1137

(3)

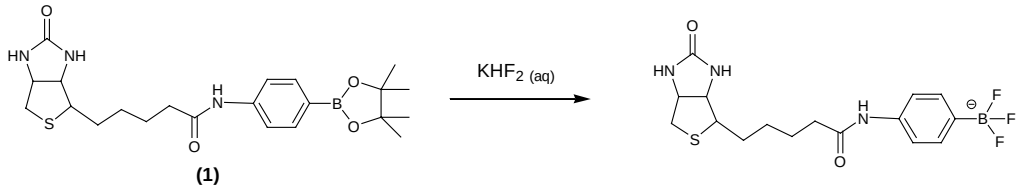
ESI



^{1}H-NMR (300 MHz, $CDCl_3$)



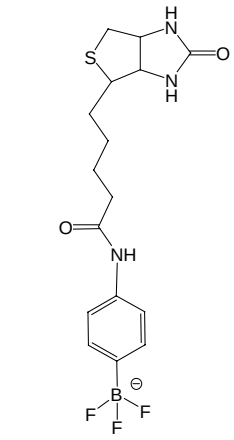
Characterization of the trifluoroborate of (1).



(1)

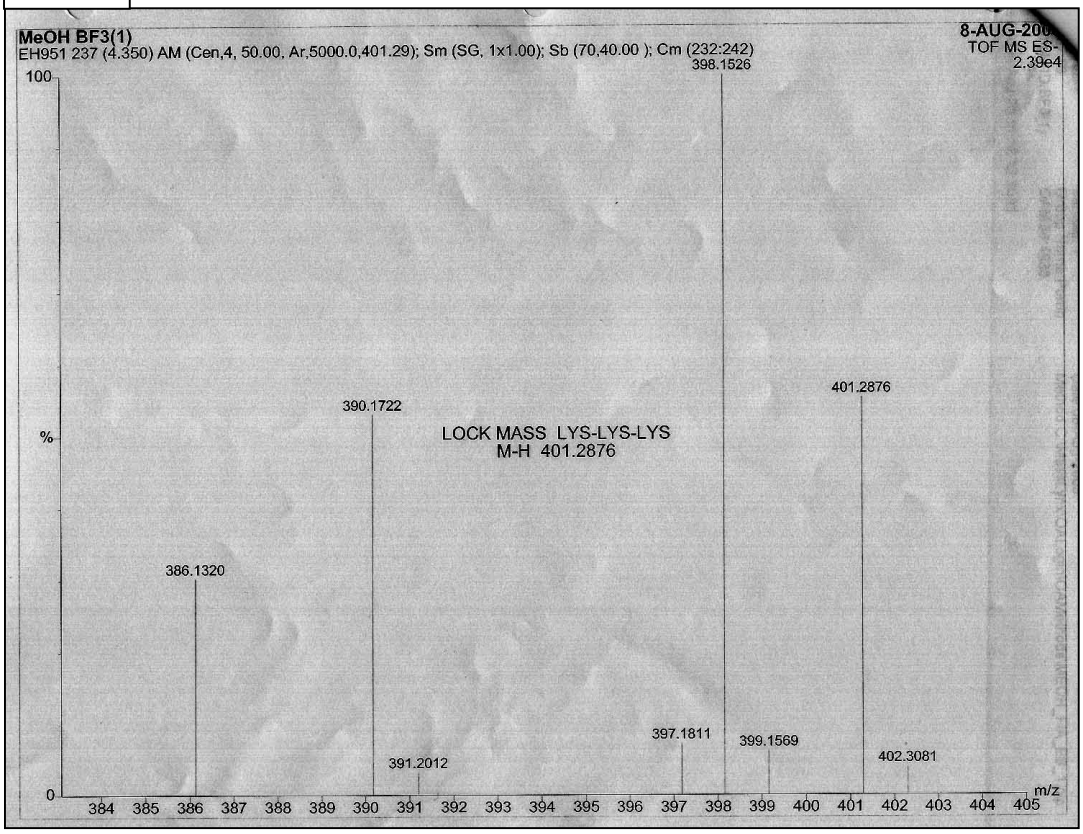
A 1.5 ml conical tube was charged with 10 µl of a 300 mM solution of **(1)** (3 µmol) in methanol and 1.12 µl of a 4M KHF_2 solution in water. A precipitate was formed in the first 5 seconds of reaction which was dissolved in a further 1.5 ml of methanol. After 5 min of reaction, excess methanol was removed under vacuum. The resulting brown powder was washed with 1 ml of acetonitrile and dried. ESI^- (in MeOH): $[M]^-$ = 386.1320 found (386.1321 calculated). ^{1}H NMR (300 MHz, DMSO-d6): δ 9.51 (1H, NH), δ 7.29-7.18 (4H), δ 6.43 (1H, NH), δ 6.34 (1H, NH), δ 4.29 (1H), δ 4.13 (1H) δ 3.11 (1H), δ 2.80 (1H), δ 2.585 - 2.544 (1H), δ 2.25 (2H), δ 1.719-1.428 (4H), δ 1.36 (2H). ^{19}F NMR (300

MHz, DMSO-d6 vs. TFA): δ -62.00 to -62.10 (br signal with some multiplet character characteristic of ^{19}F-^{11}B coupling).

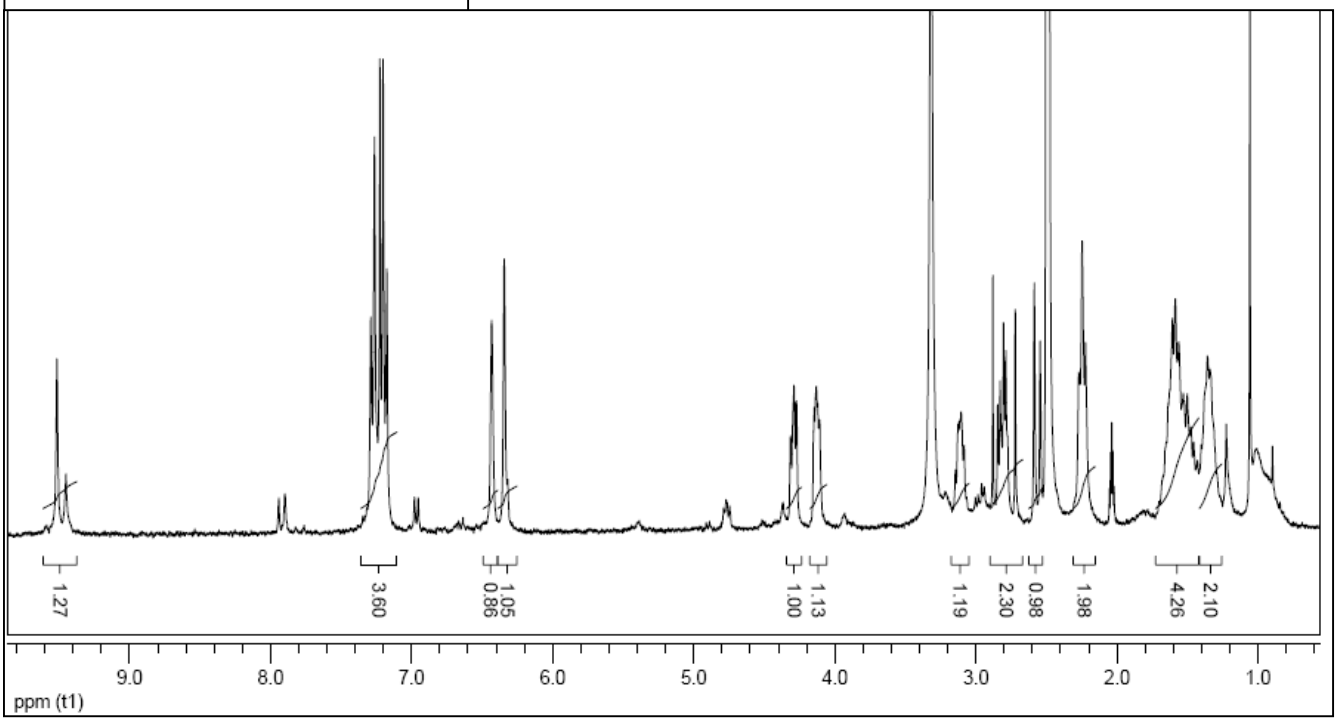


$C_{16}H_{20}BF_3N_3O_2S^-$
Exact Mass: 386.13214
Molecular Weight: 386.22011

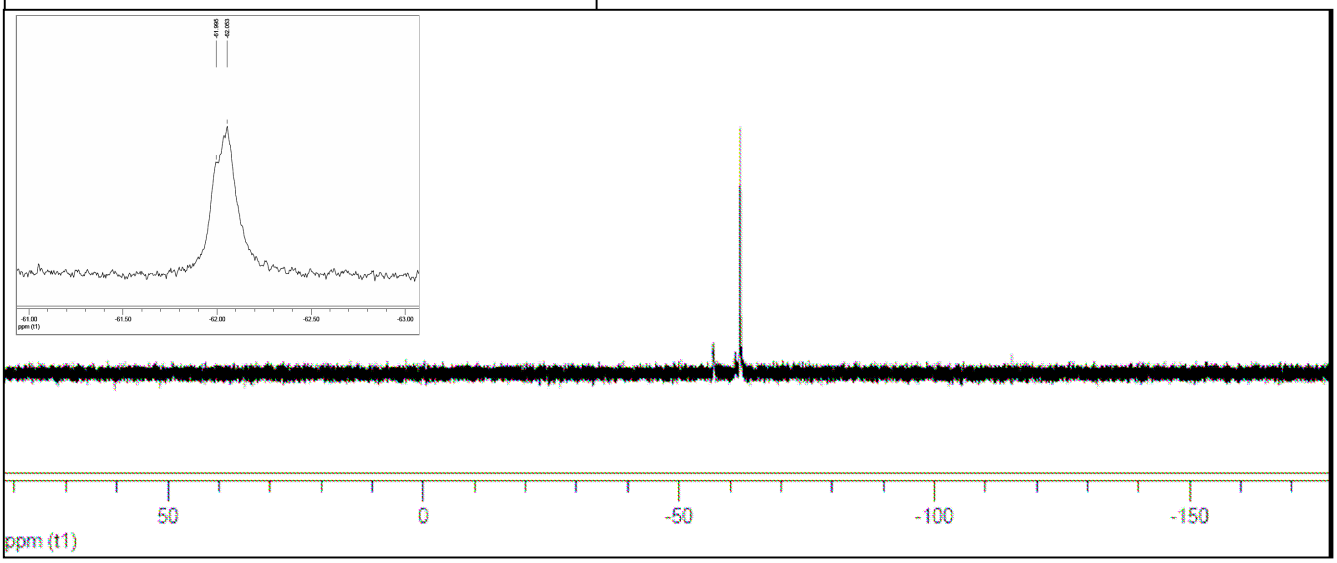
ESI



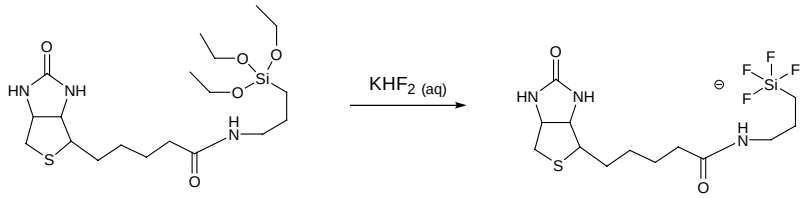
^1H-NMR (300 MHz, DMSO-d6)



^{19}F-NMR (300 MHz, DMSO-d6) ref. TFA

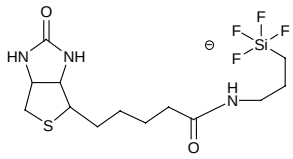


Characterization of the tetrafluorosilicate of (2).



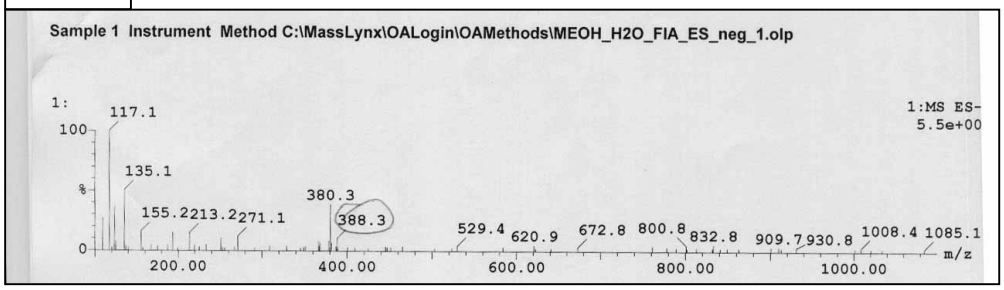
(2)

A 1.5 ml conical tube was charged with 7 mg of **(2)** (14.5 µmol) and 7.25 µl of a 4M KHF_2 solution (58.0 µmol F^-) in water. A volume of 35 µl of DMSO-d6 and 35 µl of D_2O was added to dissolve all material. After 5 min of reaction, the reaction was placed under vacuum and dried. The resulting white powder was suspended with 1 ml of DMSO and dried again in order to remove any evolved ethanol. ESI$^-$ (in MeOH): $[M]^-$ = 388.3 found (388.1 calculated). ^{1}H NMR (300 MHz, DMSO): δ 7.85 (1H, NH), δ 6.46 (1H, NH), δ 6.38 (1H, NH), δ 4.29 (1H), δ 4.10 (1H, buried in solvent peak), δ 3.08 (2H), δ 2.99 (1H), δ 2.80 (1H), δ 2.60 (1H), δ 2.06 (2H), δ 1.67 to 1.37 (6H, m), δ 1.30 (2H, m), δ 0.55 (2H). ^{19}F NMR (300 MHz, DMSO-d6 vs. TFA): δ -35.87.

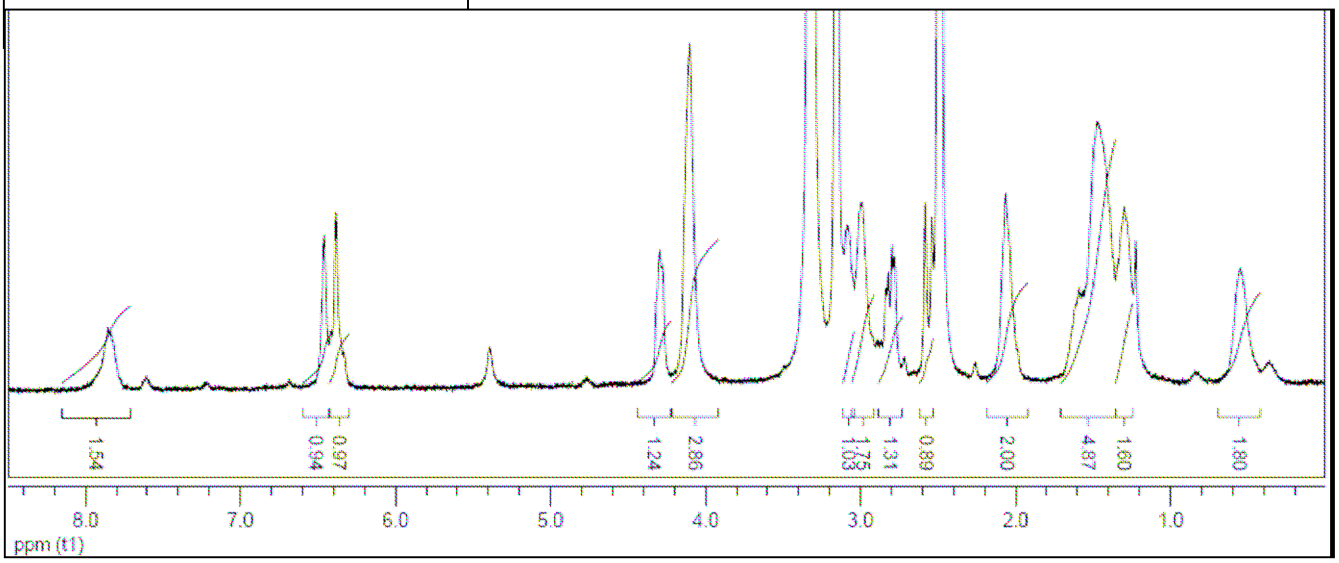


$C_{13}H_{22}F_4N_3O_2SSi^-$
Exact Mass: 388.1
Molecular Weight: 388.5

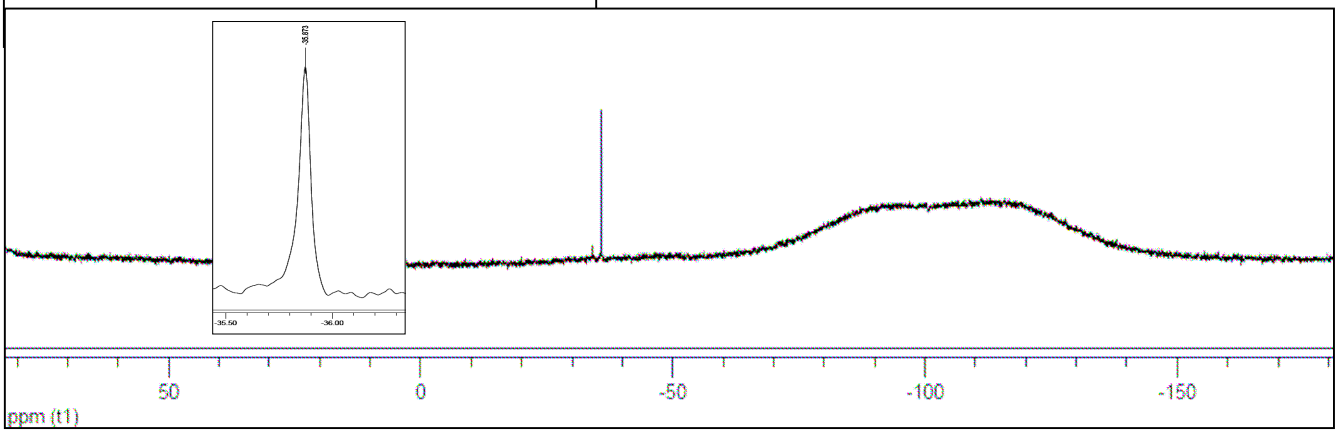
ESI



^{1}H-NMR (300 MHz, DMSO-d6)



^{19}F-NMR (300 MHz, DMSO-d6) ref. TFA



I. Quantitation of Roche Diagnostic Streptavidin Magnetic Particles (Lot no. 92544721, exp 31 Mar 2006).

Literature supplied by Roche Diagnostics that accompanied the streptavidin magnetic particles provided data on only the lower avidin binding limit (3.5 to 1.5 pmol of biotin labeled substrate per µl of particle suspension). Based on these values, fluoridation efficiencies were as high as 2000%. In order to calculate an accurate value of ^{18}F transfer onto **(1)** and **(2)**, an accurate measure of the actual number of sites per µl of particle suspension was needed. This value was established by a ^{32}P-based titration experiment that was carried out on the streptavidin magnetic particles:

^{32}P Labeling of 8-C DNA:

DNA sequence 8-C (5´-GCGTGCC(rC)GTCTGTT-biotin-3´ (where rC is a ribose C and the 3´ biotin was synthesized onto the oligo through a biotin containing controlled pore glass bead) was 5´-labeled with ^{32}P. The 5´-labeling reaction was prepared by mixing together 1 µl of 100 µM DNA sequence 8-C (100 pmol), 4 µl of NEB 10x PNK buffer (New England Biolabs), 32 µl of water, and 2 µl (20 µCi) of γ-^{32}P-rATP (Perkin-Elmer). The reaction was started upon addition of 1 µl of Polynucleotide Kinase (New England Biolabs). This reaction was allowed to proceed at 37 °C for 2 hours, after which it was placed at 95 °C for 5 min then passed over a Sephadex G-25 desalting column (Pharmacia). The desalted, labeled oligonucleotide was suspended in a final volume of 100 µl to give an approximate concentration of 1 µM (1 pmol/µl) and a specific activity of ~ 0.1 µCi/µl based on the observation that 50% of the radioactivity was recovered in the spin column eluant. The ribose linkage (which has found use in other studies in our lab) was introduced to allow cleavage of the labeled portion by RNaseA so as to demonstrate that any labeling of the beads was due to a specific oligonucleotide interaction with the avidin particles, as RNaseA treatment removed all bound radioactivity (data not shown).

Streptavidin Magnetic Particles Preparation:

A volume of 120 µl of Roche Diagnostic streptavidin magnetic particles Lot no. 92544721 contained within a 1.5 ml microcentrifuge tube was placed on a Roche Diagnostic Magnetic Particle Separator (MPS). After 2 min of magnetization, the supernatant was removed. Wash solution consisting of 300 µl of 1M NaCl, 10 mM Tris, and 1 mM EDTA for washes 1 and 2 and 300 mM Sodium Bicarbonate pH 7.5 for washes 3-5 were prepared. In each case, the tube was vortexed, and the magnetic particles were allowed to soak in the wash solution for 1 min before they were replaced on the MPS. After a total of 5 washes, the particles were suspended in 240 µl of bicarbonate wash solution. Aliquots of 30 µl were distributed to 8 1.5 ml microcentrifuge tubes. Each tube thus received a quantity of particles that corresponded to 15 µl of original suspension. To each aliquot was then added another volume of 470 µl bicarbonate buffer to mimic the binding conditions used in the fluoridation reaction.

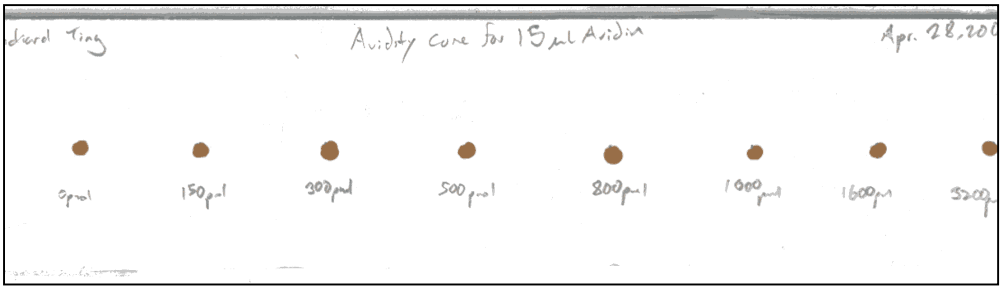
Streptavidin Magnetic Particle Titration:

To each tube, increasing quantities of biotin were added: 0, 150, 300, 500, 800, 1000, 1600, 3200 pmol (1.5 µl of a 100 µM biotin stock for the second tube, 3 µl for the third tube, ... and 32 µl for the eighth tube). Tubes were then allowed to equilibrate at room temperature for 7 hours in order to ensure the quantitative transfer of biotin onto the streptavidin magnetic particles. Following this incubation, 2 µl (2 pmol at ~0.1 µCi) of the ^{32}P labeled 8-C DNA were added. Oligonucleotide capture was allowed to proceed for 15 min, after which magnetic particles were magnetized, washed 4 times with 200 µl of

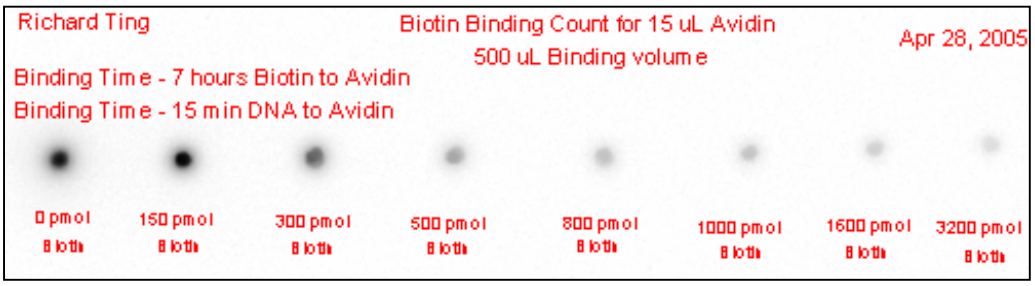
wash solution, suspended in 3 µl of water and transferred to a TLC plate. This experiment was repeated two other times.

Quantitation:

The 3 µl slurries of oligonucleotide bound magnetic particles were pipetted onto a silica plate which was affixed with cellophane tape and exposed to a Molecular Dynamics phosphor screen for 3 hours. Audioradiography of this screen was accomplished on a Molecular Dynamics Typhoon 9200 phosphoimager. Polygons were drawn around distinct bands with ImageQuant v5.2. Quantified volumes were corrected for background phosphorescence by ImageQuant v5.2 histogram peak correction prior to any mathematical treatment. The following autoradiogram and counted volume quantities were generated:



Actual Visible Light Photo of Avidin Spots on the Silica

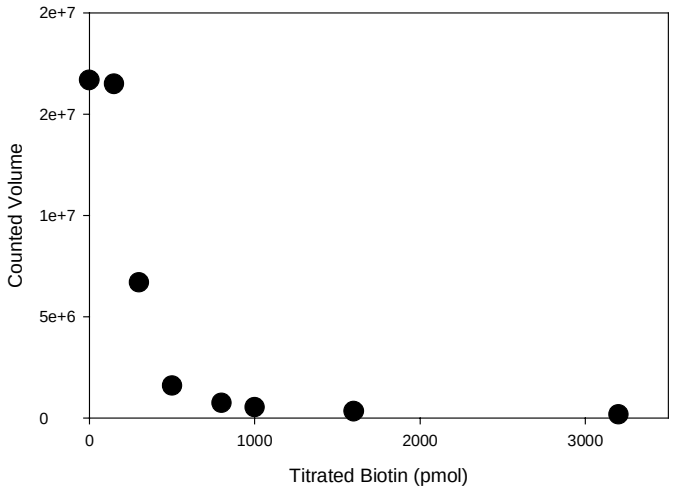


Phosphoimage of Avidin Spotted on the Silica Plate

Titration Expt Apr 28, 2005

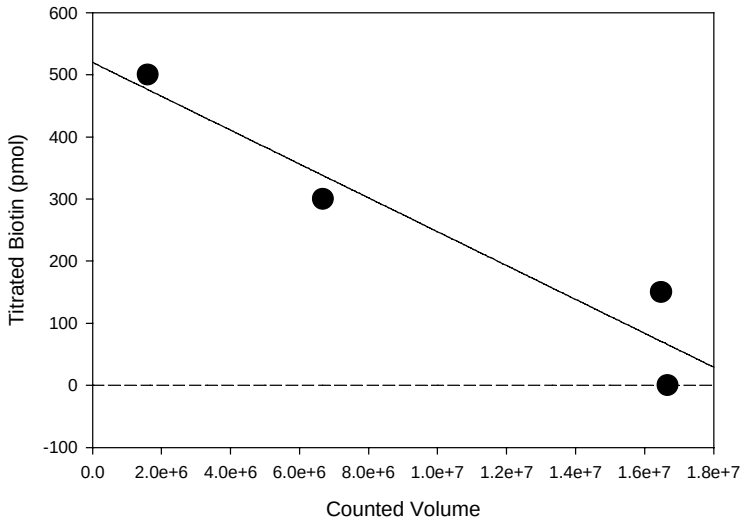
Counted Volume	Biotin (pmol)
16668347	0
16478721	150
6682183	300
1599694	500
741791	800
540483	1000
331820	1600
176002	3200

Quantitated Phophoimager Data



Titration of AMP with ^{18}F Labeled Biotin

Early data points were analyzed in a linear regression plot using the Sigma Plot 2001 v7.101 data analysis program. Titrated biotin was plot against count quantities to generate the following plot:



Quantitation of AMP Biotin Binding Sites.

The y-intercept for this plot is 520 ± 75 pmol of biotin per 15 µl of streptavidin magnetic particles or 35 ± 5 pmol of biotin per 1 µl streptavidin magnetic particles. This value is used to calculate ^{18}F transfer onto **(1)** and **(2)** in the following experiments where streptavidin magnetic particles Lot no. 92544721 were used. This titration was repeated two independent times with slight differences in sample preparation. In one case biotin was incubated with the particles in 200 µl wash buffer rather than 500 µl. In another case 5 µl of avidin particles were evaluated in a titration that used 7, 15, 30, 60, 150, and 300 pmol of biotin prior to oligo capture. Only the data from this experiment are shown.

II. Compounds (1) and (2) can be ^{18}F labeled at different pHs and solvent conditions.

Compound Preparation:
A quantity corresponding to 300 nmol (300 µl of a 1 mM solution in DMF) of compounds **(1)**, **(2)**, along with biotin as a control were dried down in 600 µl microcentrifuge tubes and resuspended in 5 µl of either methanol, DMF, DMSO, acetonitrile, or water 16 hours prior to reaction.

^{18}F Preparation:
Cyclotron bombardment of ^{18}O-water on a niobium target produced aqueous ^{18}F that was used without further purification. Samples of ^{18}F had concentration values of 25-100 mCi/ml where these values were measured after approximately 30 minutes following bombardment to allow for decay of contaminating amounts of ^{13}N. For this experiment, at 10:34 A.M. on January 14, 2005, 34 minutes post bombardment, each solution had a concentration 74 µCi/µl. This time, the start of reaction time, is referred to as the SOR.

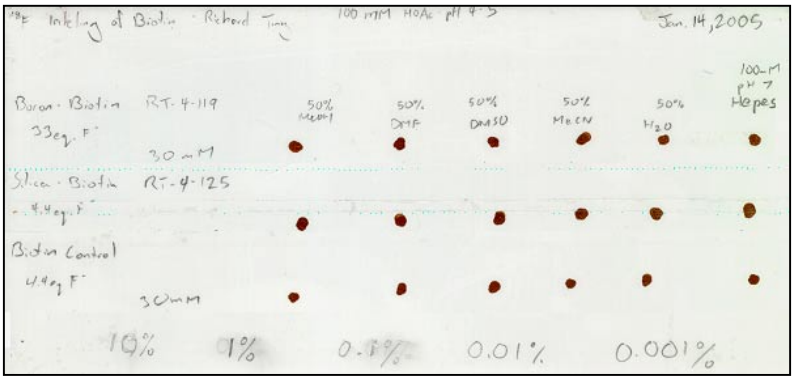
The aqueous sample of ^{18}F was used to prepare two solutions of fluoride at two different pH's. Radioactive fluoride was added in trace quantities to cold fluorine in the form of KHF_2 to give a solution that would contain either 3.3 or 4.4 equivalents of fluoride for transfer onto boron or silicon respectively. The first solution at pH 4.5, **(A1)**, consisted of 200 mM acetic acid pH 4.5 and 100 mM KHF_2. This solution was used to deliver exactly 3.3 equivalents of fluorine to reactions containing **(1)** and control biotin. A modified solution **(A2)** consisting of 200 mM acetic acid pH 4.5 and 130 mM KHF_2 was employed for the delivery of 4.4 equivalents of fluorine to reactions containing **(2)**. The second solution at pH 7.5, **(B1)**, consisted of 200 mM HEPES (N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)) pH 7.5 and 100 mM KHF_2 for boron and control reactions. **(B2)** consisted of 200 mM Hepes (N-(2-Hydroxyethyl)piperazine-N'-(2-ethanesulfonic acid)) pH 7.5 and 130 mM KHF_2 for silicon reactions. The concentration of the prepared solutions was 44 µCi/µl at the SOR.

Fluorine transfer:
Fluoridation at pH 7.5 proceeded in Hepes buffered water. Fluoridation in other solvents proceeded at pH 4.5 in acetate and KHF_2 buffered water. In each case, a volume of 5 µl of radioactive solution **(A1), (A2), (B1)** or **(B2)** was added to 5 µl of the resuspended compounds of **(1), (2),** or biotin such that 5 µl (50 %) of each reaction was water (originating from the aqueous sample of ^{18}F) and 5 µl was either methanol, DMF, DMSO, acetonitrile, or water. The final concentrations during fluoridation were: 30 mM of the potential ^{18}F acceptor (compound **(1), (2),** or biotin), 100 mM of acetic acid pH 4.5 or Hepes pH 7, and 50 mM of KHF_2 (100 mM in fluoride) for compounds **(1)** and biotin or 65 mM of KHF_2 (130 mM in fluoride) for compound **(2)**. This 10 µl reaction solution had a concentration of 220 µCi (22 µCi/µl) at the SOR. Fluoridation was allowed to proceed for 50 min at room temperature. Reactions were then quenched with 200 µl of 300 mM sodium bicarbonate pH 7.5 to give a final volume of 210 µl.

Workup:
Roche Diagnostic streptavidin magnetic particles Lot no. 92544721 were washed 3 times with wash solution as described (*vide supra* - page S11). Aliquots of 15 µl (525 pmol of predicted binding capacity) were distributed to 1.5 ml microcentrifuge tubes. An additional 300 µl of wash solution was added to each aliquot of the streptavidin magnetic particles to bring the final volume of the slurry to

315 µl. These streptavidin magnetic particles were prepared prior to the quenching of the fluorine transfer reactions.

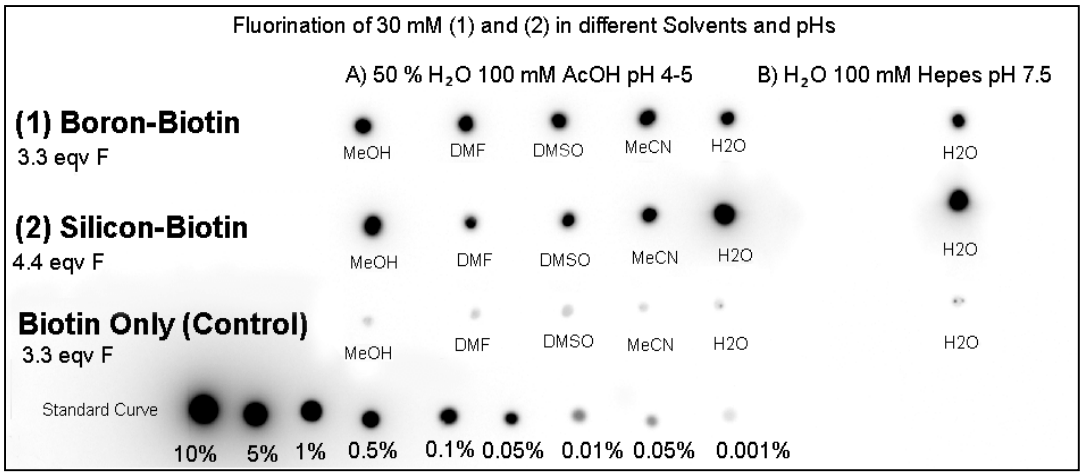
Quenched fluorine transfer reactions were added to this slurry, bringing the final volume to 525 µl. This solution was vortexed and biotin capture was allowed to proceed at room temperature for 20 min before the particles were placed on the MPS. Following magnetization, liquid was decanted away from the magnetic particles and replaced with 180 µl of wash solution (1M NaCl, 10 mM Tris, 1 mM EDTA). After the second wash, particles were transferred to a second tube and washed 3 more times with wash solution before they were suspended in 5 µl of water. The 5 µl slurries of bound magnetic particles were transferred by pipette onto a silica plate. Shown below is a visible-light photograph of the plate. The numbers 10%, 1%, 0.1%, 0.01%, and 0.001% correspond to invisible spots containing defined quantities of radioactivity used to generate the standard curve (*vide infra*)



Visible Light Photograph of Avidin Magnetic Particles Spotted on the Silica Plate

Standard Curve:
Serial dilutions, each an order of magnitude lower than the original fluoridation solution were prepared and a volume from these solutions that corresponded to 10 % (22 µCi at the SOR), 5 % (11 µCi at the SOR), 1 % (2.2 µCi at the SOR), 0.5 % (1.1 µCi at the SOR), 0.1 % (0.22 µCi at the SOR), 0.05 % (0.11 µCi at the SOR), and 0.01 % (0.022 µCi at the SOR) of the total radioactivity of the *fluorine transfer* reaction were spotted onto the same silica plate such that quantitation of fluorine transfer could be achieved. A standard curve was generated from data obtained following autoradiography. Because the $H^{18}F$ in water can be volatile, the diluent used in making up the standard solutions was 200 mM KHF_2, 300 mM $NaHCO_3$ pH 7.5.

Autoradiography:
The plate was allowed to dry for 5-10 minutes whereupon Scotch-brand tape (#3710) was applied to the silica plate to fix the particles. The plate was exposed on a Molecular Dynamics phosphor screen for either 30 minutes (short exposure) or 16 hours (long exposure). Show below is the autoradiogram following 16 hour exposure.



Autoradiogram (Phosphoimager) of Avidin Magnetic Particles Spotted on the Silica Plate, Standard curve represents the percentage of total fluoride used in the fluoridation reaction. This standard curve provided a correlation of autoradiographic density with the percentage of fluoride used.

Quantitation:

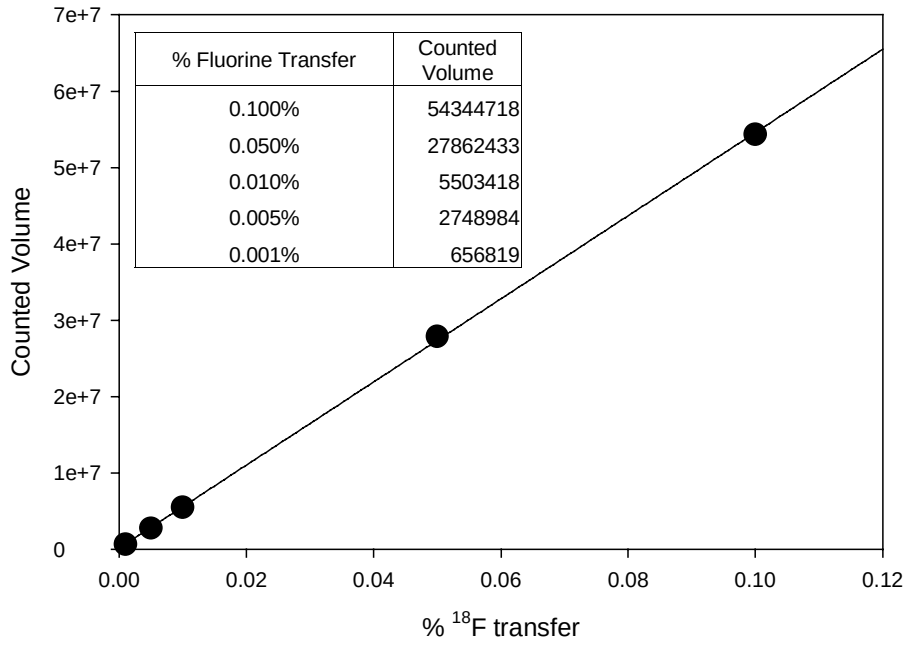
See section I (page S12-S13) Autoradiographic intensities (volumes) were quantified as described previously. Shown below is a table of raw data that represent the autoradiographic intensities of the spots on the plate.

^{18}F Experiment Jan 14, 2005

Molecule	Conditions	Counted Volume
(1)	pH 4.5, MeOH	76543535
(1)	pH 4.5, DMF	73993038
(1)	pH 4.5, DMSO	66485647
(1)	pH 4.5, MeCN	73957514
(1)	pH 4.5, H_2O	55071322
(1)	pH 7.0, H_2O	39532669
(2)	pH 4.5, MeOH	108905687
(2)	pH 4.5, DMF	19392938
(2)	pH 4.5, DMSO	36236442
(2)	pH 4.5, MeCN	66330575
(2)	pH 4.5, H_2O	151525887
(2)	pH 7.0, H_2O	140899506
biotin	pH 4.5, MeOH	717083
biotin	pH 4.5, DMF	644042
biotin	pH 4.5, DMSO	890028
biotin	pH 4.5, MeCN	434040
biotin	pH 4.5, H_2O	601636
biotin	pH 7.0, H_2O	1056945

Quantitated Phophoimager Data

Values for fluorine transfer percentages were extrapolated from a linear regression plot generated from the standard curve using the Sigma Plot 2001 v7.101 data analysis program:



Standard Curve for ^{18}F transfer

For the equation $y = mx + y_0$, $y_0 = 1.5 \cdot 10^5 \pm 2.0 \cdot 10^5$ units of volume and $m = 5.44 \cdot 10^8 \pm 4 \cdot 10^6$ volume units per percent ^{18}F. This curve remained entirely linear up to 0.1% with some deviation occurring at 0.5% due to saturation of the phosphoimager screen (not shown).

To use this equation, an assumption is made that the biotin-avidin interaction is complete within the 20 minute incubation and essentially quantitative (i.e. no avidin goes unbound under these conditions, and no biotin dissociates during washings). Based on this assumption, the 15 µl of avidin used in the assay would effectively retain 525 pmol of biotin, or 0.175 % of the 300 000 pmol biotin used (i.e. compound **(1)**, **(2)**, and biotin). This percentage (0.175 %) represents the maximum theoretical yield for ^{18}F transfer onto avidin.

For molecule (**1**) at pH 4.5 in MeOH, $7.65 \cdot 10^7$ phosphoimager volume units were experimentally measured. Using the standard curve, this value corresponds to a 0.140 % transfer [(76543535-y0)]/ *m*]:

The efficiency of fluoridation for this work is taken to be the ratio of calculated yield divided by the theoretical yield (Actual % ^{18}F transferred / Theoretical % ^{18}F transfered)*100 %). Thus for molecule (**1**) at pH 4.5 in MeOH, an efficiency of 79 % is observed (0.140 %/ 0.175 %).

The following table was generated for the rest of the data in accord with the above. Efficiency values are also calculated for the unmodified biotin control to demonstrate that the efficiency of fluoridating either biotin itself or the avidin magnetic particle is less than 1%.

^{18}F Experiment Jan 14, 2005

Molecule	Conditions	% ^{18}F transfer
(1)	pH 4.5, MeOH	79
(1)	pH 4.5, DMF	76
(1)	pH 4.5, DMSO	68
(1)	pH 4.5 MeCN	76
(1)	pH 4.5, H_2O	58
(1)	pH 7.0, H_2O	41
(2)	pH 4.5, MeOH	112
(2)	pH 4.5, DMF	20
(2)	pH 4.5, DMSO	38
(2)	pH 4.5 MeCN	68
(2)	pH 4.5, H_2O	157
(2)	pH 7.0, H_2O	146
biotin	pH 4.5, MeOH	0.5
biotin	pH 4.5, DMF	0.5
biotin	pH 4.5, DMSO	0.7
biotin	pH 4.5 MeCN	0.4
biotin	pH 4.5, H_2O	0.5
biotin	pH 7.0, H_2O	0.9

Table of Calculated Fluorine Transfer Efficiencies

III. Measurement of fluorine loss and exchange.

Compounds **(1)** and **(2)** were both fluoridated in DMF in accord with the above procedure. To further ensure the irreversibility of any fluoride loss under dilute conditions where fluoridation is not kinetically favored, samples were diluted 100-fold into a solution containing 200 mM KHF_2 at pH 7.5 such that the ratio of $^{19}F_{free}$:$^{18}F_{free}$ was greater than 400:1. The presence of excess ^{19}F would ensure that if any ^{18}F were to dissociate, its departure would be met by replacement with an atom of ^{19}F since fluoridation at 200 mM KHF_2 is both kinetically and thermodynamically favorable. This is a variation of a classic "pulse-chase" or isotope exchange reaction for estimating an exchange rate constant.

Compound Preparation:
A quantity corresponding to 3000 nmol (300 µl of a 10 mM solutions in DMF) of compounds (**1**), (**2**), and biotin was dried down in 600 µl microcentrifuge tubes and each was resuspended in 50 µl of DMF 16 hours prior to reaction to give a final concentration of 60 mM.

^{18}F Preparation:
^{18}O-water was irradiated as previously described**.** This sample of aqueous ^{18}F had a concentration of 246 µCi/µl at the SOR on February 25, 2005. The ^{18}F – water was added to acetic acid and KHF_2 such that a solution resulting in 200 mM acetic acid pH 4.5 and 100 mM KHF_2 was obtained for reactions containing **(1)** and biotin and a solution resulting in 200 mM acetic acid pH 4.5 and 130 mM KHF_2 was obtained for reactions containing **(2)**. The concentration of the prepared solutions were 134 µCi/µl at 11:23 A.M. referred to as the SOR.

Fluorine transfer:
A volume of 50 µl $KH^{18}F_2$ (134 µCi/µl) at either 100 mM or 130 mM total fluoride in acetate buffered water was added to 50 µl of the resuspended compounds **(1), (2),** or biotin in DMF. The composition of the resulting mixture was 30 mM of the potential ^{18}F acceptor (compound **(1), (2),** or biotin), 100 mM acetic acid pH 4.5, and 50 mM of KHF_2 (100 mM in fluoride) for compounds **(1)** and biotin, or 65 mM of KHF_2 (130 mM in fluoride) for compound **(2)**. Each 100 µl reaction had a total radioactivity of 6700 µCi (67 µCi/µl) at the SOR. The fluoridation reaction was started at 11:30 AM, 7 minutes following the SOR.

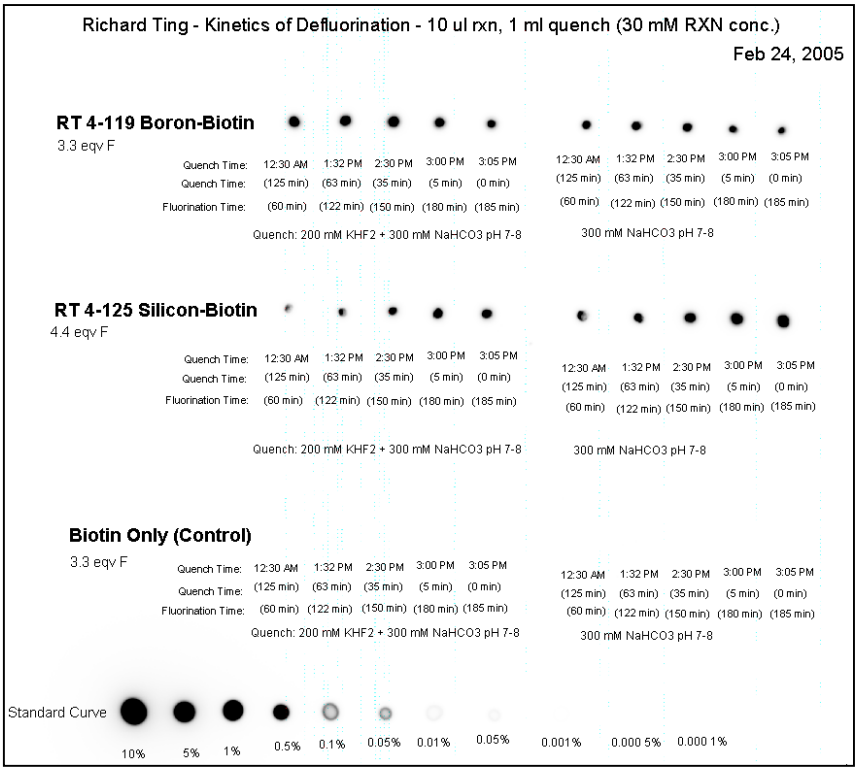
Kinetics of ^{18}F exchange:
At the following time points; 12:30 PM, 1:32 PM, 2:30 PM, 3:00 PM, or 3:05 PM, a volume of 10 µl of the reacting solutions was removed and quenched in either, **(C)**, 1 ml of a 200 mM KHF_2 & 300 mM $NaHCO_3$ pH 7.5 solution, or **(D)**, 1 ml of a 300 mM $NaHCO_3$ pH 7.5 solution in 1.5 ml microcentrifuge tubes. At 3:05 PM all quench reactions were applied to avidin particles. The periods of time **(1), (2),** or biotin remained incubating in exchange solutions **(C)** and **(D)** were 125 min, 63 min, 35 min, 5 min and 0 min respectively. Use of solution **(C)** was intended to measure the rate of ^{18}F-^{19}F exchange under physiological pH and salt concentrations (the ratio of ^{18}F-labeled **(1)**, **(2)**, or biotin : ^{19}F was 1:400 at the given conditions). Solution **(D)** was intended to measure the rate of hydrolytic decomposition (^{18}F-water exchange) under physiological pH and salt concentrations.

Workup:

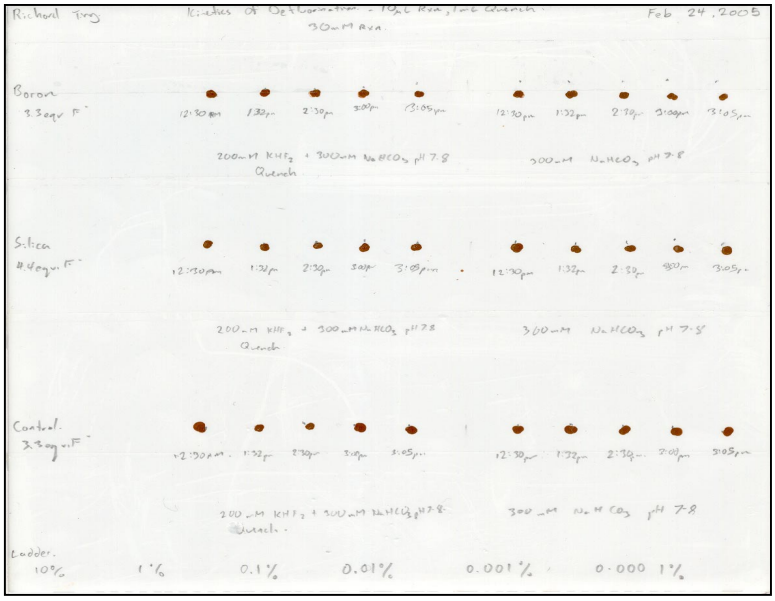
Roche Diagnostic streptavidin magnetic particles Lot no. 92544721 were washed as previously described. Aliquots of 15 µl (525 pmol of predicted binding capacity) were distributed at 3:05 P.M. to reactions that had been quenched in solutions **(C)** or **(D)** varying amounts of time, bringing the final volume of each slurry to 1025 µl. Tubes were vortexed and biotin capture was allowed to proceed at room temperature for 15 min before the reactions were placed on the MPS. Following magnetization, liquid was decanted away from the magnetic particles and replaced with 200 µl of wash solution. The particles were washed 3 more times with wash solution before the magnetic particles were finally suspended in 5 µl of water. The 5 µl slurries of bound magnetic particles were pipetted onto a silica plate. Standards were also spotted on the plate to correspond to 10 % (67 µCi at the SOR), 5 % (33.5 µCi at the SOR), 1 % (6.70 µCi at the SOR), 0.5 % (3.35 µCi at the SOR), 0.1 % (0.670 µCi at the SOR), 0.05 % (0.335 µCi at the SOR), 0.01 % (0.067 µCi at the SOR), 0.005 % (0.0335 µCi at the SOR), and 0.001 % (0.00670 µCi at the SOR), of the radioactivity of a 10 µl aliquot of the fluorine transfer reaction was spotted onto the same silica plate such that quantitation of fluorine transfer could be achieved. The silica plate was protected with Scotch tape (#3710) and exposed to a Molecular Dynamics phosphor screen for 16 hours.

Quantitation:

Radioactive avidin count volumes were quantified as described previously. Shown below is the resulting autoradiogram as well as a visible light image of the actual plate. Volume quantities were obtained and are shown in the following table.



*Autoradiogram of ^{18}F exchange for **(1)**, **(2)** and a biotin control.*



Actual Visible Light Photo of Avidin Spots on the Silica Plate

Quench solution **(C)** 200 mM KHF_2 & 300 mM $NaHCO_3$ pH 7.5

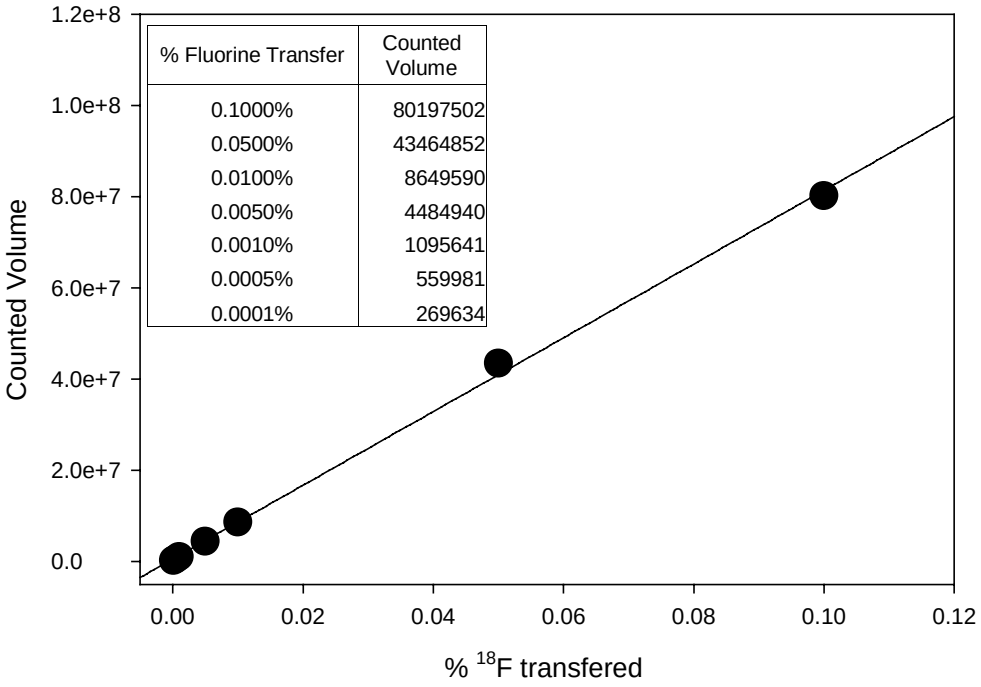
Quench solution **(D)** 300 mM $NaHCO_3$ pH 7.5

^{18}F Experiment 25 Feb 2005

Molecule	Exchange Solution	Exchange Time	Counted Volume
(1)	**(C)**	0 min	62928451
(1)	**(C)**	5 min	82993260
(1)	**(C)**	35 min	104999859
(1)	**(C)**	63 min	99132351
(1)	**(C)**	125 min	98460493
(1)	**(D)**	0 min	43657656
(1)	**(D)**	5 min	48589711
(1)	**(D)**	35 min	65576776
(1)	**(D)**	63 min	66829466
(1)	**(D)**	125 min	59967448
(2)	**(C)**	0 min	78811303
(2)	**(C)**	5 min	79563571
(2)	**(C)**	35 min	58004363
(2)	**(C)**	63 min	37850607
(2)	**(C)**	125 min	22410580
(2)	**(D)**	0 min	127085473
(2)	**(D)**	5 min	123072754
(2)	**(D)**	35 min	97929099
(2)	**(D)**	63 min	65865580
(2)	**(D)**	125 min	57410037
biotin	**(C)**	0 min	315437
biotin	**(C)**	5 min	402333
biotin	**(C)**	35 min	619352
biotin	**(C)**	63 min	743165
biotin	**(C)**	125 min	1057186
biotin	**(D)**	0 min	134113
biotin	**(D)**	5 min	172264
biotin	**(D)**	35 min	158702
biotin	**(D)**	63 min	218266
biotin	**(D)**	125 min	281478

Quantitated Phophoimage Data.

The standard curve was generated from the data in order to make it possible to correlate exposure values with the amount of fluoride incorporated. Values for the percent fluorine transfer were calculated using the standard curve shown below and the raw data values from the autoradiogram.



Standard Curve for ^{18}F transfer

For this plot $y_0 = 5.8 \cdot 10^5 \pm 5.8 \cdot 10^5$ volume units and $m = 8.08 \cdot 10^8 \pm 1.4 \cdot 10^7$ volume units per % of ^{18}F used in the fluoridation reaction with **(1)**, **(2)**, or biotin.

Based on the fact the avidin would capture 525 pmol of the 300 nmol of compound **(1)**, **(2)**, or biotin the following fluorine transfer values are presented in the following table:

Solution **(C)** 200 mM KHF_2 & 300 mM $NaHCO_3$ pH 7.5

Solution **(D)** 300 mM $NaHCO_3$ pH 7.5

^{18}F Experiment 25 Feb 2005

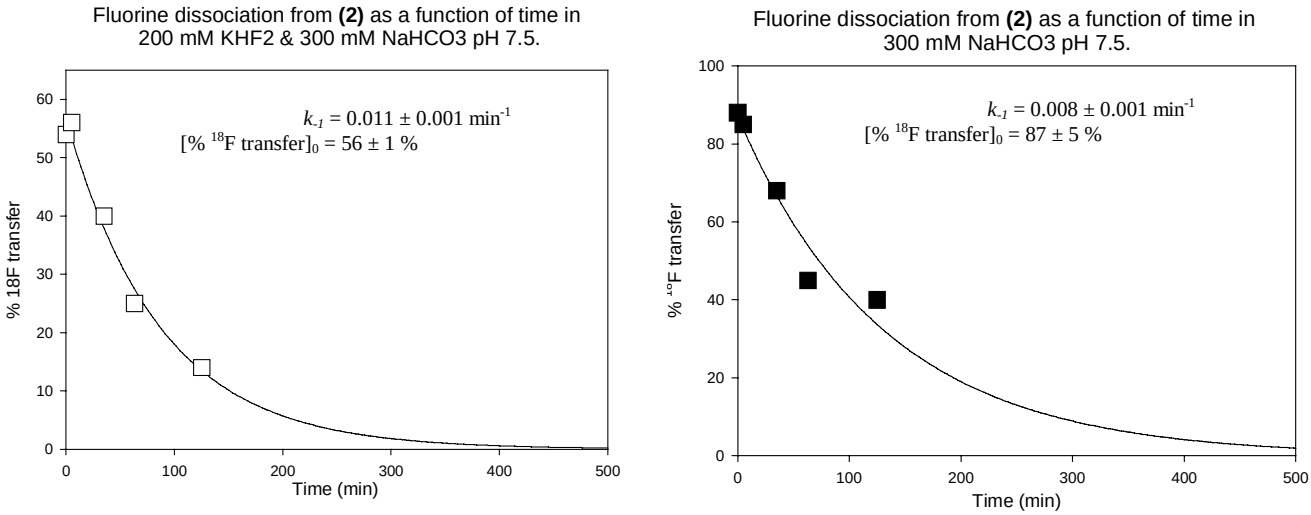
Molecule	Exchange Solution	Exchange Time	% ^{18}F transfer
(1)	**(C)**	0 min	43
(1)	**(C)**	5 min	58
(1)	**(C)**	35 min	72
(1)	**(C)**	63 min	68
(1)	**(C)**	125 min	68
(1)	**(D)**	0 min	31
(1)	**(D)**	5 min	34
(1)	**(D)**	35 min	45
(1)	**(D)**	63 min	47
(1)	**(D)**	125 min	41
(2)	**(C)**	0 min	54
(2)	**(C)**	5 min	56
(2)	**(C)**	35 min	40
(2)	**(C)**	63 min	25
(2)	**(C)**	125 min	14
(2)	**(D)**	0 min	88
(2)	**(D)**	5 min	85
(2)	**(D)**	35 min	68
(2)	**(D)**	63 min	45
(2)	**(D)**	125 min	40
biotin	**(C)**	0 min	-0.2
biotin	**(C)**	5 min	-0.2
biotin	**(C)**	35 min	0.0
biotin	**(C)**	63 min	0.2
biotin	**(C)**	125 min	0.4
biotin	**(D)**	0 min	-0.4
biotin	**(D)**	5 min	-0.4
biotin	**(D)**	35 min	-0.4
biotin	**(D)**	63 min	-0.2
biotin	**(D)**	125 min	-0.2

Table of Calculated Fluorine Transfer Efficiencies

First order rate constants, k_{-1}, for fluorine exchange were obtained by fitting % ^{18}F transfer data to the following first order single-exponential equation by the least square method using Sigmaplot 2001 v7.101:

$$[\%\ ^{18}\text{F transfer}]_t = [\%\ ^{18}\text{F transfer}]_0(\ e^{-k_{-1}t})$$

where $[\%\ ^{18}\text{F transfer}]_t$, and $[\%\ ^{18}\text{F transfer}]_0$ are percentages of ^{18}F retained by **(1)** and **(2)** at time *t* and zero respectively. The rate of fluorine exchange was too slow to be measured for fluoridated compound **(1).** Plots depicting fluorine exchange for **(2)** as a function of time, $[\%\ ^{18}\text{F transfer}]_0$ and the fluorine dissociation rate constant, k_{-1}, and are presented:



*First Order Decay Plots of ^{18}F from **(2)** under different buffer conditions.*

IV. Streptavidin magnetic particles are specific for the biotin on fluoridated compounds (1) and (2). Streptavidin magnetic particles have little affinity for fluorine labeled boron.

Compound Preparation:
A volume of 300 nmol (300 µl of a 1 mM solution in DMF) of compounds **(1), (2),** biotin, or a mixture of 300 nmol biotin and 300 nmol of **(3)** were dried down in 600 µl microcentrifuge tubes and resuspended in 5 µl of DMF 16 hours prior to reaction.

^{18}F Preparation:
^{18}O-water was irradiated as previously described. The ^{18}F containing water contained a concentration of 60.6 µCi/µl at the SOR on April 19, 2005.

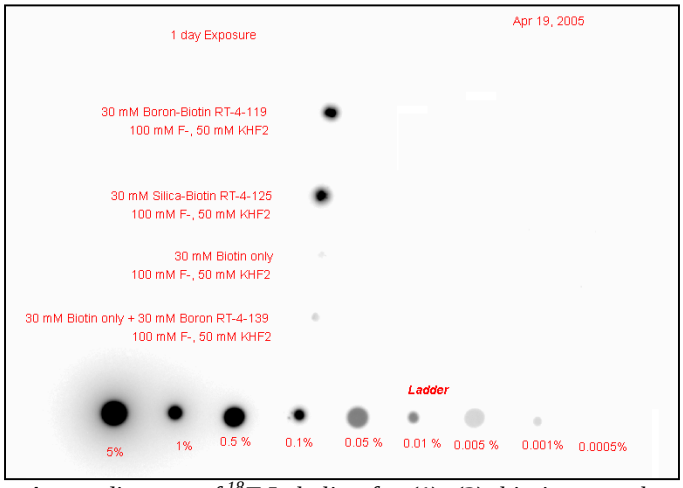
This $^{18}F^-$ water was used to generate a solution consisting of 200 mM acetic acid pH 4.5 and 100 mM KHF_2 for reactions containing **(1)**, biotin alone, and the mixture of **(3)** and biotin. A solution resulting in 200 mM acetic acid pH 4.5 and 130 mM KHF_2 was generated using the ^{18}F – water for reactions containing **(2)**. The concentration of the prepared solutions was 52.8 µCi/µl at the SOR.

Fluorine transfer:
A volume of 5 µl of the two solutions were mixed with 5 µl of the resuspended compounds of **(1), (2),** biotin, or the mixture of 300 nmol biotin and 300 nmol of **(3)**. The composition of the reacting mixture was 30 mM of the potential ^{18}F acceptor (compound **(1), (2),** biotin, or **(3)** + biotin)**,** 100 mM acetic acid pH 4.5, and 50 mM of KHF_2 (100 mM in fluoride). The 10 µl reaction solution had total radioactivity of 264 µCi (26.4 µCi/µl) at the SOR. This reaction was allowed to proceed for 50 min before 40 µl of 300 mM sodium bicarbonate pH 7.5 was added as a reaction quench to give a final volume of 50 µl.

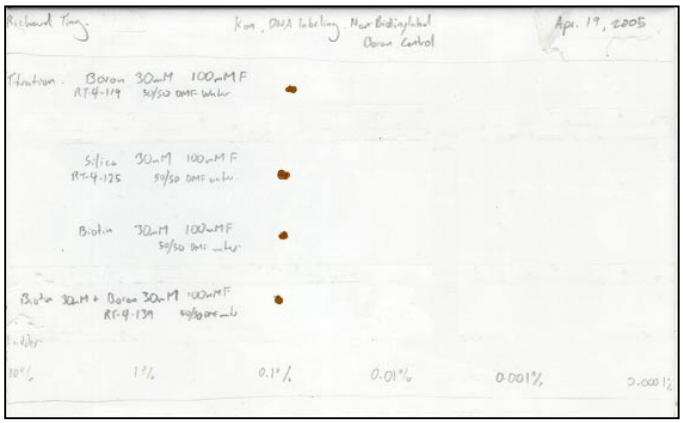
Workup:
Roche Diagnostic streptavidin magnetic particles Lot no. 92544721 were washed 3 times with wash solution as described (vide supra - page S11**).** Aliquots of 20 µl (350 pmol of predicted binding capacity) were distributed to 1.5 ml microcentrifuge tubes. An additional 500 µl of wash solution was added to each aliquot of the streptavidin magnetic particles, bringing the final volume of the slurry to 520 µl. These streptavidin magnetic particles were prepared prior to the quenching of the fluorine transfer reactions.

The 50 µl fluorine transfer reactions were added to this slurry, bringing the final volume to 570 µl. This solution was vortexed and biotin capture was allowed to proceed at room temperature for 20 min before the reactions were placed on the MPS. Following magnetization, the liquid phase was decanted away from the magnetic particles and replaced with 200 µl of wash solution. The particles were washed 3 more times using wash solution before they were suspended in 3 µl of water. The 3 µl slurries of bound magnetic particles were pipetted onto a silica plate. Serially diluted quantities of ^{18}F corresponding to 5 % (13.2 µCi at the SOR), 1 % (2.64 µCi at the SOR), 0.5 % (1.32 µCi at the SOR), 0.1 % (0.264 µCi at the SOR), 0.05 % (0.132 µCi at the SOR), 0.01 % (0.0264 µCi at the SOR, and 0.005 % (0.0132 µCi at the SOR) of the radioactivity of the fluoridation reaction was spotted onto the same silica plate such that quantitation of fluorine transfer could be achieved. The silica plate was protected with Scotch tape (#3710) and exposed to a Molecular Dynamics phosphor screen for 15 min.

Quantitation:
Radioactive avidin count volumes were quantified as described previously (see pages S12-S13). The following autoradiogram, shown above a figure of the actual plate, and volume quantities were generated:



*Autoradiogram of ^{18}F Labeling for **(1)**, **(2)**, biotin control, and **(3)** + biotin.*



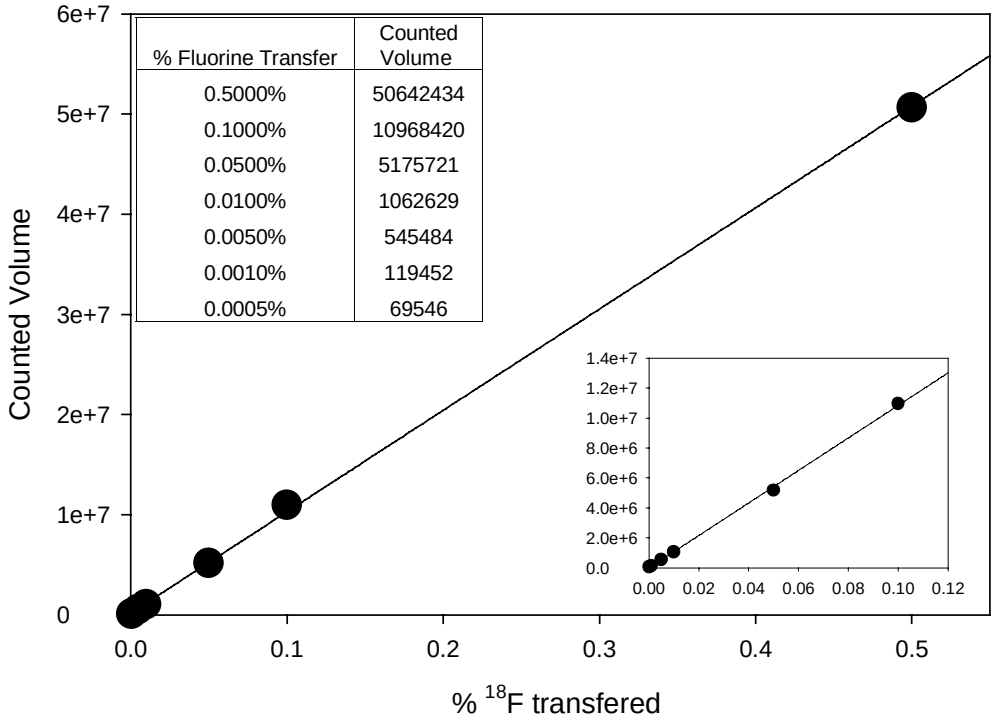
Actual Visible Light Photograph of Avidin on the Silica Plate

^{18}F Experiment Apr 19, 2005

Molecule	Conditions	Counted Volume
30 mM **(1)**	pH 4.5, DMF	16522119
30 mM **(2)**	pH 4.5, DMF	15789413
30 mM biotin	pH 4.5, DMF	58127
30 mM **(3)** + 30 mM biotin	pH 4.5, DMF	126411

Raw Phophoimage Data

Values for fluorine transfer %'s were extrapolated from a linear regression plot generated from the radioactivity ladder using the Sigma Plot 2001 v7.101 data analysis program:



Standard Curve for ^{18}F transfer

The y-intercept, y_0, for this plot is $1.6 \cdot 10^5 \pm 1.4 \cdot 10^5$ counts. The slope, *m*, is $1.01 \cdot 10^8 \pm 7.5 \cdot 10^5$ counts per % of ^{18}F transferred onto **(1)**, **(2),** the biotin control, and compound **(3)** in the presence of free biotin. Assuming that 300 nmol of ^{18}F acceptor was titrated with 350 pmol of avidin, the following fluorine transfer values were calculated:

^{18}F Experiment Apr 19, 2005

Molecule	Conditions	% ^{18}F transfer
30 mM **(1)**	pH 4.5, DMF	137
30 mM **(2)**	pH 4.5, DMF	130
30 mM biotin	pH 4.5, DMF	-0.9
30 mM **(3)** + 30 mM biotin	pH 4.5, DMF	-0.4

Table of Calculated Fluorine Transfer Efficiencies

The streptavidin magnetic particles are specific for the biotin on fluoridated compounds **(1)** and **(2)**. streptavidin magnetic particles have little affinity for fluorine labeled boron component alone.

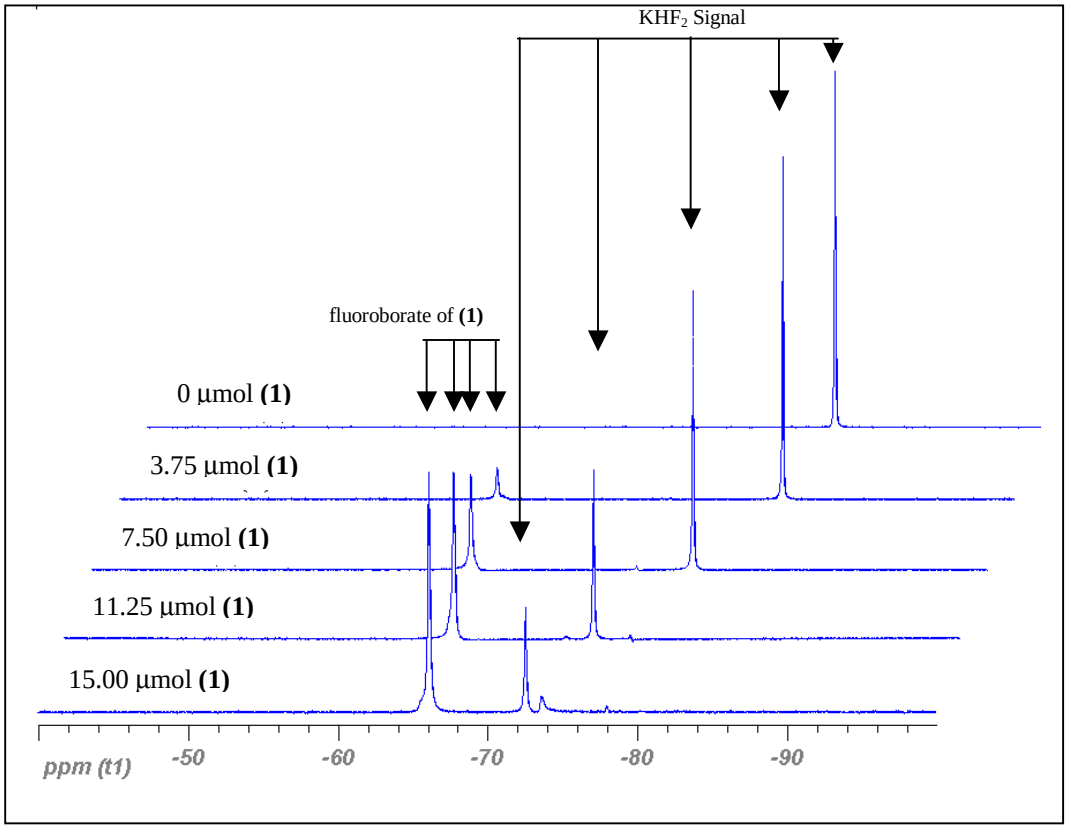
V. At 30 mM (1) and 100 mM F^-, each molecule of (1) complexes 3 F^- atoms: (1) is quantitatively converted to the trifluoroborate and no intermediates are observed.

Compound Preparation:
A mass of 0.180 g of KHF_2 (Aldrich #463671-50G, FW 78.11) was dissolved in 4.6 ml of D_2O resulting in a 500 mM KHF_2 solution (1000 mM F^-).
A mass of 0.030 g of **(1)** was dissolved in 222 µL of MeOH resulting in a 300 mM solution of **(1)**.

Fluorine Titration:
A volume of 50 µL of the 500 mM KHF_2 solution (25 µmol of KHF_2 or 50 µmol of F^-) was added to 450 µl of MeOH-d4 resulting in a 50 mM KHF_2 (100 mM F^-) solution. This mixture was placed in an NMR tube and a ^{19}F-NMR spectra (referenced to TFA at 0 ppm) was acquired.

A volume of 12.5 µl of the 300 mM solution (3.75 µmol) of **(1)** was added to the NMR tube containing 100 mM (50 µmol) of F^- and an ^{19}F-NMR spectrum was recorded. 12.5 µl aliquots of the 300 mM solution (3.75 µmol) of **(1)** was continually titrated into the NMR tube as ^{19}F-NMR spectra was recorded. The following spectra were generated:

^{19}F NMR of a 50 mM KHF_2 (25 µmol KHF_2, 100 mM F^-, or 50 µmol F^-) titration with **(1)**:



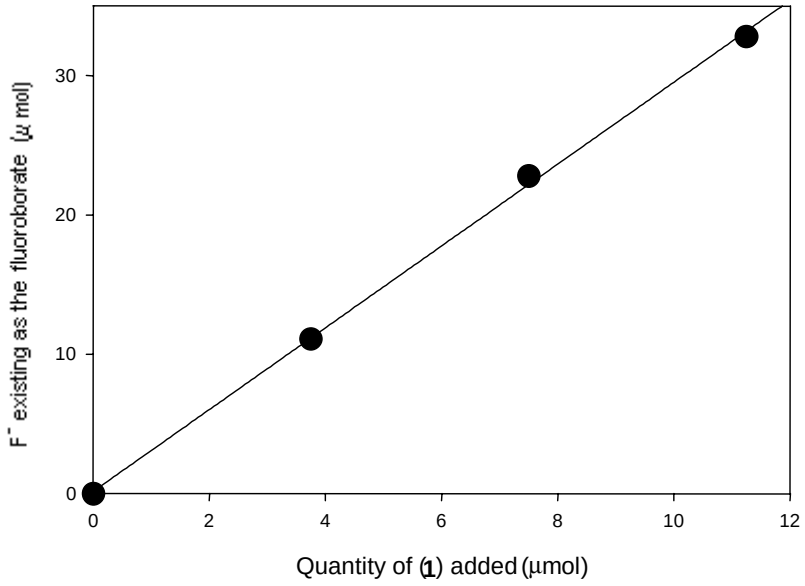
^{19}F-NMR Titration of (1) with KHF_2

he ^{19}F signals on the spectra were integrated and the following table was generated:

NMR Time	Quantity of **(1)** added (μmol)	F^- existing as KHF_2 (μmol)	F^- existing as the fluoroborate of **(1)** (μmol)
12:28 PM	0.00	50.0	0.0
1:12 PM	3.75	38.9	11.1
1:46 PM	7.50	27.2	22.8
2:54 PM	11.25	17.2	32.8
3:41 PM	15.00	15.0	35.0

Integration Data from ^{19}F-NMR

The quantity of **(1)** added was plot against the quantity of F^- existing as the fluoroborate of **(1)**:



*Plot measuring # of Fluorides incorporated per molecule **(1)***

The y-intercept for this plot is 0.2 ± 0.5 μmol. The slope for this plot is 2.94 ± 0.07 equivalents of F^- per molecule of **(1)**.

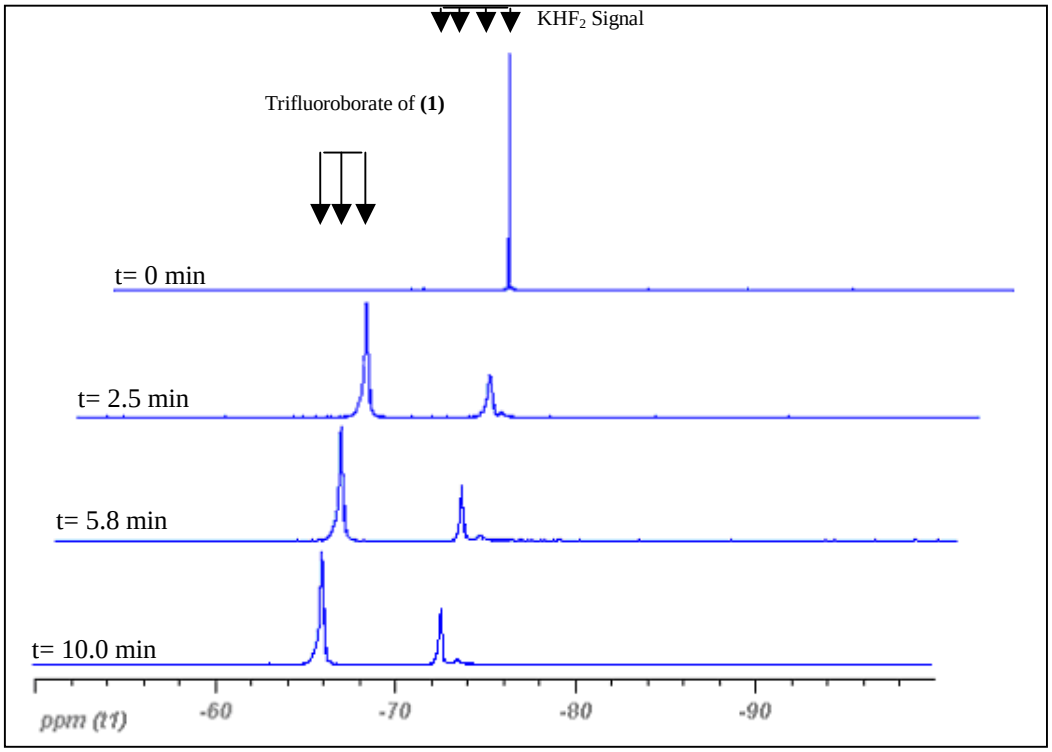
The slope of this graph indicates that approximately 3 fluoride atoms associate with each molecule of **(1)**, thus the trifluoroborate of **(1)** is formed. Because other ^{19}F signals in the NMR spectra are not detected, it appears that no bis or monofluoridated boron intermediates accumulate during the course of this reaction.

VI. At 30 mM (1) and 100 mM F^-, the rate of trifluoroborate formation is too rapid to measure as the formation of the trifluoroborate is complete in 2.5 minutes.

Compound Preparation:
Solutions from Section V. page S28 were used.
A volume of 50 µl of 500 mM KHF_2 (25 µmol of KHF_2 or 50 µmol of F^-) was added to 200 µl of MeOH-d4.
A volume of 39 µl of the 300 mM solution of **(1)** (11.3 µmol of **(1)**) was added to 200 µl of MeOH-d4.

Fluorine transfer:
The prepared solutions of **(1)** and KHF_2 were mixed in an NMR tube to give a ~500 µl solution containing 50 mM KHF_2 (100 mM or 50 µmol of F^-) and 23 mM of **(1)**. ^{19}F-NMR spectra referenced to TFA at 0 ppm were obtained at different time points. The following ^{19}F NMR spectra were obtained:

^{19}F NMR time course of a reaction with 23 mM of **(1)** and 50 mM KHF_2 (25 µmol KHF_2, 100 mM F^-, or 50 µmol F^-)



^{19}F-NMR for Fluoridation Kinetics of (1) with KHF_2

(Time points earlier than 2.5 min were difficult to obtain considering that NMR signal locking, shimming, tuning and data acquisition are not instantaneous)

From spectra integrations, the following table was generated:

time (min)	F^- (μmol) existing as KHF_2	F^- existing as the trifluoroborate of **(1)** (μmol)	Fraction of F^- existing as KHF_2	Fraction of F^- existing as the trifluoroborate of **(1)** (μmol)
0.0	**50.00**	**0.00**	**1.00**	**0.00**
2.5	16.51	33.49	0.33	0.67
5.8	16.09	33.91	0.32	0.68
10.0	15.62	34.38	0.31	0.69

^{19}F Integration Data from kinetic NMR fluorine association experiment

It was not possible to use NMR data to obtain an accurate rate constant for fluorination. At the concentrations used, the reaction was found to be complete within in 2.5 minutes, the period of time needed to perform 100 scans.

At 30 mM **(1)** and 100 mM F^-, the rate of trifluoroborate formation is > 95% complete within the first 2.5 minutes. Again, no other ^{19}F signals in the NMR spectra were detected, therefore no bis or monofluoridated boron intermediates appear to accumulate during the course of the reaction.

VII. Discussion of maximum attainable specific activity.

The reader may be concerned with two questions i) whether this method can generate specific activities that will be useful for PET imaging, and ii) whether this method will work under "no carrier added" conditions.

It is important to note that in this report we added carrier ^{19}F because the 440 μCi at 5 Ci/μmol contains only trace ^{18}F (88 pmol total fluoride). Nevertheless, if 1 Ci of "no carrier added" $^{18}F^-$ had been used, specific activities would have increased proportionately to give specific activities suitable for imaging.

Is it necessary to add carrier ^{19}F? The answer depends on two issues 1) the specific activity that can be obtained at the end of bombardment and 2) how much imaging agent is required. Routinely 1 Ci $^{18}F^-$ is prepared with variable specific activity. At very high specific activity (e.g. >100 Ci/μmol), 1 Ci would contain only 10 nmol of total fluoride. In order to label 25 nmol of triethoxysilane or 33 nmol of boronic ester (assuming this quantity is desired) one would need to add 90 nmol carrier ^{19}F to allow for stoichiometric addition of fluoride the metalloid. Addition of 90 nmol of carrier ^{19}F would essentially dilute the specific activity of the fluoride to ~10 Ci/μmol. This quantity of fluoride could be reacted with 0.25-0.33 eq. (25-30 nmol) of metalloid precursor in 5 μL that will result in "ate" salts with specific activities in the range of 30-40 Ci/μmol. The need to add carrier in this case assumes that a) the preparation of ^{18}F was at an atypically high specific activity and b) that 25 or 33 nmol of labeled imaging agent are needed. Alternatively, one could have simply reacted 2.5 nmol of triethoxysilane or 3.3 nmol of boronic ester with the 10 nmol of ^{18}F in a carrier free labeling where the specific activity is >100 Ci/μmol.

Typically, a standard "no carrier added" ^{18}F preparation has a specific radioactivity of 5 Ci / μmol due to contaminating environmental fluoride. Thus 1 Ci of ^{18}F at 5 Ci/μmol actually contains 200 nmol of total fluoride (^{18}F + ^{19}F) where approximately 0.3% of the total fluoride is ^{18}F. Under these conditions, significant quantities of ^{19}F are already present and no extra carrier is needed. Reaction of the 200 nmol of "no carrier added" ^{18}F with 50-60 nmol of metalloid (0.25 eq. Si or 0.33 eq. B) would generate "ate" salts in quantitative yield as shown here with specific activities of 16-20 / μCi.

Of note is that this approach affords an increase in the effective specific activity as each metalloid acquires 3 or 4 atoms of fluoride. Since these metalloids complex 3 or 4 atoms of ^{18}F, they differ from traditional ^{18}F-acceptors that normally accept one ^{18}F atom per acceptor. Such specific radioactivities are certainly sufficient for PET imaging. More importantly, no method to date provides for an effective *increase* in specific radioactivity over the no carrier added ^{18}F.

VIII. Stability check of fluoridated compounds in Blood and Blood Serum.

Compounds **(1)** and **(2)** were both fluoridated as before. The stabilities of the "ate" salts of (**1**) and (**2**) were examined in the presence of human blood and fetal bovine serum. It is important to note that the presence of serum or blood affected the efficiency of biotin capture by the avidin magnetic particles as well as the ability to effectively magnetize the particles for quantitative separation, particularly in blood that required a centrifugation step as well. This was attributed to the considerable viscosity of both fluids. To verify that the "ate" salts would be stable in the presence of serum/humoral proteases and the overall protein complement, ^{18}F-labeled "ate" salts were incubated either in carbonate, serum or blood for one hour. Just prior to the addition of avidin magnetic particles, an equal volume of blood, serum or carbonate was added to all samples to normalize for the viscosity of blood and serum. A detailed protocol follows.

Compound Preparation:
A quantity of 1.5 μmol (25 μl of a 60 mM solutions in DMF) of compounds (**1**), (**2**), and biotin were dried down in 1500 μl microcentrifuge tubes and each was resuspended in 25 μl of MeOH 2 hours prior to reaction to give a final concentration of 60 mM.

^{18}F Preparation:
^{18}O-water was irradiated as previously described**.** This sample of aqueous ^{18}F – water was added to acetic acid and KHF_2 such that solutions resulting in 200 mM acetic acid pH 4.5 and 130 mM KHF_2 were obtained. This ^{18}F/^{19}F preparation had a concentration of 56.4 μCi/μl at the SOR (12:57 P.M.) on August 03, 2005.

Fluorine transfer:
A volume of 25 μl $KH^{18}F_2$ (56.4 μCi/μl) at either 100 mM or 130 mM total fluoride in acetate buffered water was added was added to tubes containing 25 μl of **(1), (2),** or biotin resuspended in MeOH. The final specific activity of the prepared solutions was 28.2 μCi/μl and 0.56 mCi/μmol for boron and 0.43 mCi/μmol for silicon at 12:57 P.M.; one min following the SOR.

Evaluation of ^{18}F loss in blood serum or blood:
At 53 min following the SOR (1:51 PM), 10 μl aliquots (300 nmol of either (**1**) or (**2**)) used in each fluoridation was added to one tube of 200 μl of fetal bovine serum, one tube of 200 μl of human blood, and two tubes of 200 μl 300 mM $NaHCO_3$ pH 7.5 solution.

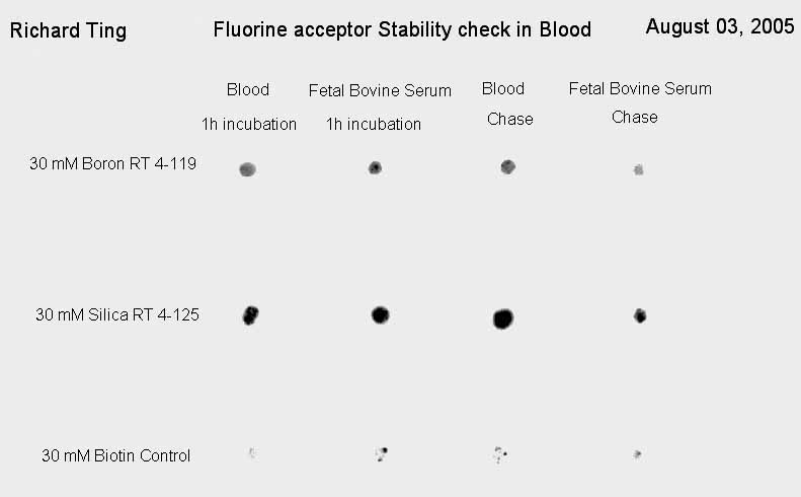
At 110 min following the SOR (2:54 P.M.), 800 μl of 300 mM $NaHCO_3$ pH 7.5 solution was added to the tubes containing human blood and fetal bovine serum and by the same token, 600 μl of 300 mM $NaHCO_3$ pH 7.5 solution and 200 μl of either human blood or fetal bovine serum was added to each of the tubes already containing 200 μl of acceptor in 200 μl 300 mM $NaHCO_3$ pH 7.5 solution. This addition was made to control the viscosity of the tubes containing serum or blood. At 110 min following the SOR, all tubes contained 1000 μl of solution, 80% of which was 300 mM $NaHCO_3$ pH 7.5, and the remaining 20 % contained either human blood or fetal bovine serum. The control tubes that received serum and blood just prior to the addition of the avidin magnetic particles are referred to as "blood chase" and "serum chase" in the table below.

Workup:

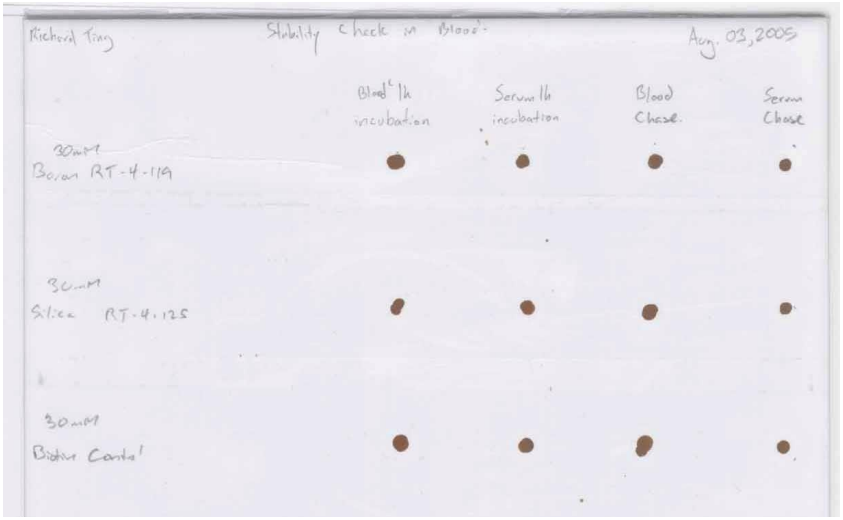
Directly following dilution (114 min following the SOR (2:58 P.M.)), aliquots of 30 µl Roche Diagnostic streptavidin magnetic particles Lot no. 92544721 washed as previously described (1050 pmol of predicted binding capacity) were distributed to the diluted reactions. Tubes were vortexed and biotin capture was allowed to proceed at room temperature for 19 min before the reactions were placed on the magnetic particle separator. Following magnetization, the supernatant was removed from the magnetic particles and replaced with 200 µl of wash solution. The particles were washed 3 more times with wash solution before the magnetic particles were finally suspended in 5 µl of water. The 5 µl slurries of bound magnetic particles were transferred onto a silica plate. The silica plate was protected with Scotch tape (#3710) and exposed to a Molecular Dynamics phosphor screen for 16 hours.

Quantitation:

Avidin plates were quantified as described previously. Shown below are the resulting autoradiograms as well as a visible light image of the actual plate.



*Autoradiogram of ^{18}F Labeled **(1)**, **(2)**, and biotin control following 1 hour (left most 6 spots) or <1 min (right most 6 spots, columns denoted "chase") of incubation of each acceptor in human blood or fetal bovine serum.*



Actual Visible Light Photograph of Avidin on the Silica Plate.

Time dependent ^{18}F dissociation was not observed qualitatively upon washing fluoridated acceptors with human blood or fetal bovine serum. This result suggests that the ^{18}F derivatives of **(1)** and **(2)** are stable under *in vivo* conditions.

Quantitated data is shown below:

Row	Molecule	Experiment	Volume above Background	Count % Remaining after 1 hour (Row A/ Row B *100 %)
A	Boron	200 ul Blood 1h incubation	411963	>100
B	Boron	200 ul Blood Chase	369979	-
A	Boron	200 ul Serum 1h incubation	408405	>100
B	Boron	200 ul Serum Chase	150059	-
A	Silica	200 ul Blood 1h incubation	1406315	33
B	Silica	200 ul Blood Chase	4239419	-
A	Silica	200 ul Serum 1h incubation	2928105	>100
B	Silica	200 ul Serum Chase	652137	-

This preliminary data suggests that suggests that the ^{18}F derivative of **(1)** is stable in human blood and fetal blood serum.

IX. Error Analysis and Attribution.

Experimentally calculated fluoridation efficiencies ranged from 20% to greater than 100% based on the biotin-avidin capture method that is used in correlation with the standard curve. The reliability of the biotin-avidin capture method here is based on the fact that the autoradiographic intensities we calculate for "ate" salts of (**1**) and (**2**) are generally greater than 40 times the background value provided by the unmodified biotin control. As such, the signal-to-noise ratio was very high. Clearly this labeling is quite selective for biotins appended with either a silicon or boron atom. More generally, we observed fluoridation efficiencies that were reasonably calculated to be in the range of 70-80%. This yield was also measured experimentally for the aryltrifluoroborate using ^{19}F-NMR and is consistent with literature reports describing syntheses of both aryltrifluoroborates and alkyltetrafluorosilicates. Despite some outlying values that are as low as 20% and higher than 100%, the range of radiochemical yields obtained here is generally consistent with those reported for purely chemical yields of similar "ate" salts. There is no reason to believe that the presence of ^{18}F should change the overall yield of "ate" production. We attribute the range of efficiencies seen in the table to several explanations:

1) Working with short lived ^{18}F required rapid manipulation. Assays generally involved as many as 30 tubes, wherein each required 5 washes and 5 magnetizations and one transfer to new tubes (40-120 minutes).

2) We noticed that magnetizations were not always consistently rapid and removal of the wash solution sometimes caused some particles to be aspirated. We paid special attention to this and did our best to ensure that the particles were properly magnetized and not aspirated.

3) When working with a total of 5-10 mCi, we were very concerned with minimizing exposure. In addition, we were considerably encumbered by lead block shielding that may have resulted in some systematic error.

4) Finally, we noticed that the slurry of avidin magnetic particles required constant agitation at the time of distribution to ensure that all tubes received the same amount of slurry. Without agitation, the magnetic particles settled to the bottom of the tube making distribution of particles somewhat problematic.

Despite these noted potential sources of error, the data here are generally internally consistent. Furthermore, the experimental yields fall within a range that is externally consistent with regards to other reports on the preparation of such "ate" salts.