PRODUCTION OF $^{203}Pb$ BY PROTON IRRADIATION OF Bi.

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High liver uptake and slow body clearance presently limit the usefulness of $^{111}In$-labeled antibodies for tumor imaging. Lead-203 has similar radionuclidic properties ($t_{1/2}$ = 52h, E$\gamma$=72.9, 279.2 KeV, 47%, 80.8%) and shows improved biodistribution when conjugated to monoclonal antibodies using cyclohexyl EDTA (1). Commercially available $^{203}Pb$, a by-product in $^{201}Tl$ production, has an unacceptably low specific activity for antibody labeling. Therefore we have investigated the production of $^{203}Pb$ by proton irradiation of Bi metal at the Brookhaven Linac Isotope Producer (BLIP). This method takes advantage of direct production of $^{203}Pb$ as well as indirect production from the beta decay of $^{203}Po$ and $^{203}Bi$.

Calculations using the semi-empirical spallation systematic method (2,3) predict a maximum cross section at approximately 90 MeV. Thus test targets of Bi metal (0.10 cm thick x 2.5 x 2.5 cm) were irradiated with ~90 MeV protons for periods of 1-3 h. The radionuclides found in the target solution are shown in Table 1. Preliminary work on isolating Pb investigated solvent extraction with diethyl dithiocarbamate and dithizone into $CCl_4$ from aqueous solution of proper acid strength. Due to the tendency of bulk Bi to precipitate and form emulsions, the quantitative and selective separation of lead was not achieved.

The observation that Bi precipitated as the oxynitrate without precipitating Pb became the basis for a different approach for Pb isolation. The Bi targets were dissolved in 8 M $HNO_3$, the solutions evaporated to a syrupy consistency, and $BiONO_3$ precipitated by the addition of hot water. After two precipitations at least 98% of the Bi was separated. The filtrate containing Pb was evaporated to near dryness and the residue dissolved in 8M HCl containing a few drops of 30% $H_2O_2$. Residual amounts of Tl isotopes were extracted with isopropyl ether. After heating to drive off trace ether, the acid phase was passed through a column of Bio-Rad AG1x8 anion exchange resin. Lead was then eluted with 8M HCl. Bi was retained on the column.

The average $^{203}Pb$ chemical recovery was 89%. Traces of stable Pb and Bi in the final product from one target were measured by atomic absorption spectroscopy. The values were 19$\mu$g Pb and 22$\mu$g Bi. The Bi separation factor was $5x10^{-6}$. Because of grow-in from $^{203}Po$ and $^{203}Bi$ decay, $^{203}Pb$ activity reaches a maximum approximately 30 h after end of bombardment (EOB). To maximize yield and minimize impurities, nuclear excitation function measurements from 60-190 MeV, using the stacked foil method, were performed. The cumulative excitation function of $^{203}Pb$ is given in Fig. 1. Calculated thick target yield from 91-69 MeV is 5.4 mCi/$\mu$Ah. Thus $^{203}Pb$ can be produced in large quantity from a thick target. The major impurities were $^{200}Pb$ and $^{201}Pb$, 38% and 15% of $^{203}Pb$ at EOP (end of processing, ~54h after EOB).

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Table 1. Product and Daughter Nuclides Produced in Proton Irradiated Bismuth.

| Product nuclides (half-life) | Daughter nuclides (with decay chain) |
|---|---|
| Po-202 (45 min) | Bi-202 → Pb-202g → Tl-202 → Hg-202 (*S) |
| Po-203 (35 min) | Bi-203 → Pb-203 → Tl-203 (S) |
| Po-204 (3.5 h) | Bi-204 → Pb-204 (S) |
| Po-205 (1.8 h) | Bi-205 → Pb-205($1.5 \times 10^7$y) → Tl-205 (S) |
| Po-206 (8.8 h) | Bi-206 → Pb-206 (S) |
| Po-207 (5.8 h) | Bi-207(32y) → Pb-207 (S) |
| Bi-200 (36 min) | Pb-200 → Tl-200 → Hg-200 (S) |
| Bi-201 (1.8 h) | Pb-201 → Tl-201 → Hg-201 (S) |
| Bi-202 (1.7 h) | Pb-202g($5.3 \times 10^4$y) → Tl-202 → Hg-202 (S) |
| Bi-203 (11.8 h) | Pb-203 → Tl-203 (S) |
| Bi-204 (11.2 h) | Pb-204 (S) |
| Bi-205 (15.3 d) | Pb-205 → Tl-205 (S) |
| Bi-206 (6.2 d) | Pb-206 (S) |
| Pb-200 (21.5 h) | Tl-200 → Hg-200 (S) |
| Pb-201 (9.3 h) | Tl-201 → Hg-201 (S) |
| Pb-202m (3.5 h) | Pb-202g → Tl-202 → Hg-202 (S) |
| Pb-203 (51.9 h) | Tl-203 (S) |
| Pb-204m (67 min) | Pb-204 (S) |
| Tl-200 (26.1 h) | Hg-200 (S) |
| Tl-201 (73.1 h) | Hg-201 (S) |
| Tl-202 (12.2 d) | Hg-202 (S) |

*S= stable isotope

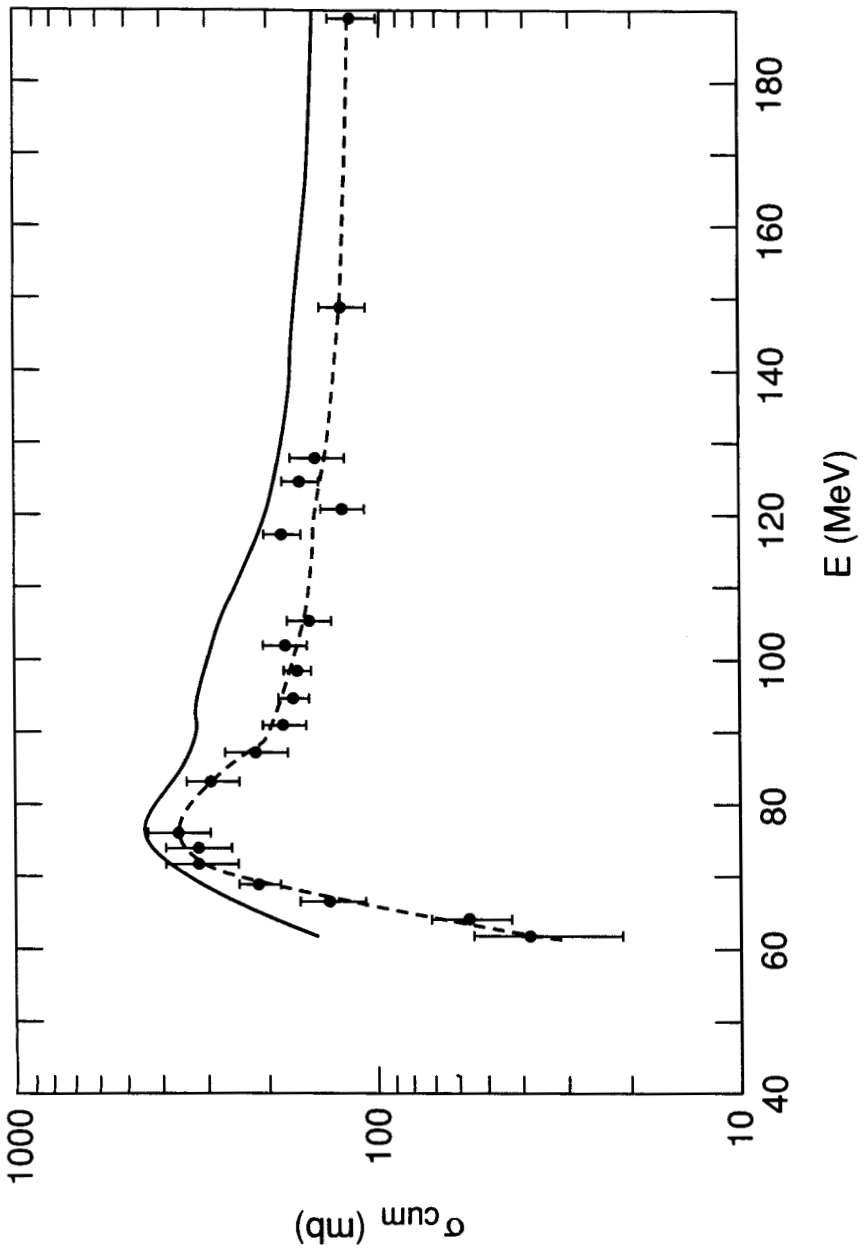


Fig. 1. Cumulative excitation functions ( $\sigma_{cum}$ ) of Pb-203 at 30 hrs (time of maximum grow-in) from EOB. The solid line represents predicted values using the code SPALL (3), employing the semi-empirical formulae (2). The broken line is drawn through our experimental points.

PRODUCTION OF A BROAD RANGE OF RADIONUCLIDES WITH AN 11 MeV PROTON CYCLOTRON. RJ Nickles, Medical Physics Dept, University of Wisconsin, Madison, WI 53706.

**Introduction.**

Most PET radiochemistry development has focussed on *de novo* synthesis of tracers labeled with $^{11}C$, $^{13}N$, $^{15}O$ and $^{18}F$, readily produced by a small cyclotron at Curie levels. Fixed-energy, proton-only cyclotrons have recently found favor because of their simplicity and compact shielding, essential in a hospital setting. There is, however, a growing need for a wider range of radionuclide precursors in the medical sciences. Research in nutrition, trace metal metabolism and monoclonal antibodies call for a more general class of labels, often with longer half-lives for protracted studies. This work was initiated to determine the production potential of an 11-MeV proton cyclotron (CTI RDS) , leading to these "other" radionuclides.

The Coulomb barrier $V_c$ (MeV) $\approx Z/A^{1/3}$ begins to seriously throttle the 11 MeV proton entrance channel as the target Z approaches the rare earths. Similarly, the Coulomb barrier favors K-capture over positron decay in nuclei beyond zirconium. These considerations suggest a systematic measurement of radionuclide yields from 11 MeV proton irradiation of thick targets from boron to lanthanum ($5 < Z < 57$). Our experience with evaporation codes (1) such as ALICE have shown that such calculations are useful for predicting the importance of competing reactions and the energy dependence of the cross sections, but they generally have a large uncertainty in absolute yields. For this reason, a methodical sequence of experimental measurements was undertaken.

**Measurements.**

In most cases, thick targets of naturally abundant target materials were irradiated with a known beam current for a known time, followed by time-resolved, high resolution gamma spectroscopy. Calibration of detector efficiency ($50 < E < 1800$ keV) made use of NBS-traceable standards at a standard geometry, relying on the literature (2) values for the gamma branching ratios. Decay corrections were then applied for finite irradiation, delay and counting times. The results are compiled as the activity (mCi) that could be expected:

- at the end of a saturated bombardment by one μA of 11 MeV protons
- on a thick, elemental target
- of 100% isotopic enrichment, to reveal the underlying systematics of reaction yields.

Most of the targets were in the form of the elemental material, isotopically enriched in the cases of $^{10}B$, $^{13}C$, $^{15}N$ and $^{18}O$. In some cases, compounds ($H_2O$, LiF, SiC, $Li_2S$, CaO LiI and La-Al) were more advantageous. The target material is pressed against a water-cooled aluminum cold-finger to remove the beam heat. Nonetheless, the beam current was held below 1 μA in most cases, where the target (S, Zn, Se, Cd, Sb, Te, I) or reaction products (C, O, P, Zn, As, Br, Cd, Te, I, Xe) were volatile. Needless to say, the practical difficulties of targetry lie in the extrapolation of these low current yield experiments to high power irradiations required in a production setting.

**Results.**

The results of the 106 reactions leading to 100 product (eg. $^{13}N$) nuclides ranging from $^{10}C$ to $^{139}Ce$ are listed below, as well as the physical decay half life, the abundance of the target isotope occurring in nature (eg. $^{13}C$), the Q-value (MeV), the "signature" energy of the major gamma peak (keV), and the activity (+/- 20%) that would result from a saturated irradiation at 1 μA on a 100% enriched target. The ninety six (p,n) reactions are listed first, followed by eight (p,α), one (p,αn) and one (p,p') reaction.

The 11 MeV proton cyclotron was chosen as the most effective means of providing conventional PET precursors at Wisconsin. The windfall of high (p,n) and (p,α) yields through the transition metals has further demonstrated the versatility of this machine.

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| PROD | $t_{1/2}$ | % | Q | $E_\gamma$ | Act |
|---|---|---|---|---|---|
| $^{10}C$ | 19 s | 20% | -4.4 | 717 | 8 |
| $^{11}C$ | 20 m | 80% | -2.8 | 511 | 93 |
| $^{13}N$ | 10 m | 1% | -3.0 | 511 | 120 |
| $^{14}O$ | 71 s | 99% | -5.9 | 2314 | 2 |
| $^{15}O$ | 2 m | 0.3% | -3.5 | 511 | 70 |
| $^{18}F$ | 2 h | 0.2% | -2.4 | 511 | 120 |
| $^{30}P$ | 2.5 m | 3% | -5.0 | 511 | 116 |
| $^{34m}Cl$ | 32 m | 4% | -6.4 | 2127 | 12 |
| $^{38}K$ | 7.7 m | .06% | -6.7 | 2167 | 5 |
| $^{43}Sc$ | 3.9 h | 0.1% | -3.0 | 373 | 11 |
| $^{44m}Sc$ | 2.4 d | 2% | -4.7 | 271 | 1.7 |
| $^{44}Sc$ | 3.9 h | 2% | -4.4 | 1157 | 2.9 |
| $^{48}Sc$ | 43 h | 0.2% | -0.5 | 984 | 40 |
| $^{45}Ti$ | 3 h | 100% | -2.8 | 511 | 47 |
| $^{47}V$ | 33 m | 8% | -3.8 | 511 | 100 |
| $^{48}V$ | 16 d | 74% | -4.8 | 1312 | 108 |
| $^{51}Cr$ | 28 d | 99% | -1.5 | 320 | 140 |
| $^{52m}Mn$ | 21 m | 84% | -5.9 | 1434 | 67 |
| $^{52}Mn$ | 5.7 d | 84% | -5.5 | 744 | 15 |
| $^{54}Mn$ | 312 d | 2.4% | -2.2 | 835 | 131 |
| $^{54m}Co$ | 1 m | 2.4% | -9.2 | 511 | 2.9 |
| $^{56}Co$ | 79 d | 92% | -5.3 | 847 | 77 |
| $^{57}Co$ | 271 d | 2% | -1.6 | 122 | 129 |
| $^{58}Co$ | 71 d | 0.3% | -3.1 | 811 | 150 |
| $^{60}Cu$ | 23 m | 26% | -6.9 | 1332 | 40 |
| $^{61}Cu$ | 3.4 h | 1% | -3.0 | 283 | 76 |
| $^{62}Cu$ | 9.8 m | 3.6% | -4.7 | 511 | 130 |
| $^{64}Cu$ | 13 h | 0.9% | -2.5 | 511 | 73 |
| $^{63}Zn$ | 38 m | 69% | -4.1 | 669 | 116 |
| $^{65}Zn$ | 244 d | 31% | -2.1 | 1115 | 228 |
| $^{64}Ga$ | 2.6 m | 49% | -7.9 | 992 | 10 |
| $^{66}Ga$ | 9.4 h | 28% | -6.0 | 1039 | 105 |
| $^{67}Ga$ | 78 h | 4% | -1.8 | 93 | 51 |
| $^{68}Ga$ | 68 m | 19% | -3.7 | 1077 | 177 |
| $^{69}Ge$ | 38 h | 60% | -3.0 | 574 | 103 |
| $^{70}As$ | 53 m | 20% | -7.0 | 1040 | 45 |
| $^{72}As$ | 26 h | 27% | -5.1 | 834 | 102 |
| $^{73}As$ | 80 d | 8% | -1.1 | 53 | 60 |
| $^{74}As$ | 18 d | 36% | -3.3 | 596 | 176 |
| $^{76}As$ | 26 h | 8% | -1.7 | 560 | 41 |
| $^{76}Br$ | 16 h | 9% | -5.4 | 560 | 37 |
| $^{77}Br$ | 57 h | 8% | -2.1 | 239 | 30 |
| $^{80m}Br$ | 4.4 h | 50% | -2.6 | 616 | 17 |
| $^{82}Br$ | 35 h | 9% | -0.9 | 776 | 44 |
| $^{82m}Rb$ | 6.3 h | 11% | -5.2 | 776 | 15 |
| $^{83}Rb$ | 83 d | 12% | -1.6 | 520 | 23 |
| $^{84m}Rb$ | 20 m | 57% | -3.9 | 248 | 8 |
| $^{84}Rb$ | 33 d | 57% | -3.5 | 881 | 38 |
| $^{86}Rb$ | 19 d | 17% | -1.3 | 1076 | 65 |
| $^{84}Y$ | 38 m | 0.6% | -7.1 | 793 | 31 |
| $^{86}Y$ | 15 h | 10% | -6.1 | 1076 | 70 |
| $^{87m}Y$ | 13 h | 7% | -2.9 | 381 | 83 |
| $^{87}Y$ | 80 h | 7% | -2.5 | 485 | 157 |
| $^{88}Y$ | 108 d | 83% | -4.4 | 898 | 96 |

| PROD | $t_{1/2}$ | % | Q | $E_\gamma$ | Act |
|---|---|---|---|---|---|
| $^{89}Zr$ | 78 h | 100% | -3.6 | 909 | 100 |
| $^{90}Nb$ | 15 h | 51% | -6.9 | 2319 | 44 |
| $^{92m}Nb$ | 10 d | 17% | -2.9 | 934 | 88 |
| $^{96}Nb$ | 23 h | 3% | -0.6 | 778 | 66 |
| $^{93m}Mo$ | 7 h | 100% | -3.6 | 685 | 1.7 |
| $^{92}Tc$ | 4 m | 15% | -8.8 | 1510 | 17 |
| $^{94}Tc$ | 5 h | 9% | -5.0 | 871 | 3.2 |
| $^{95m}Tc$ | 61 d | 16% | -2.5 | 204 | 21 |
| $^{96}Tc$ | 4 d | 17% | -3.7 | 778 | 95 |
| $^{96}Rh$ | 10 m | 5% | -7.2 | 833 | 18 |
| $^{98}Rh$ | 9 min | 2% | -5.0 | 652 | 56 |
| $^{99m}Rh$ | 5 h | 13% | -2.9 | 1261 | 40 |
| $^{99}Rh$ | 15 d | 13% | -2.9 | 528 | 18 |
| $^{100}Rh$ | 20 h | 13% | -4.4 | 540 | 63 |
| $^{101m}Rh$ | 4 d | 17% | -1.5 | 307 | 50 |
| $^{102m}Rh$ | 206 d | 32% | -3.2 | 478 | 66 |
| $^{107}Cd$ | 6 h | 52% | -2.2 | 824 | 66 |
| $^{109}Cd$ | 453 d | 48% | -1.0 | 88 | 48 |
| $^{110}In$ | 69 m | 12% | -4.7 | 658 | 179 |
| $^{111}In$ | 2.8 d | 13% | -1.9 | 245 | 54 |
| $^{112m}In$ | 21 m | 24% | -3.4 | 155 | 139 |
| $^{113m}In$ | 1.6 h | 12% | -0.9 | 392 | 143 |
| $^{114m}In$ | 49 d | 29% | -2.4 | 725 | 5.5 |
| $^{113m}Sn$ | 21 m | 4% | -2.0 | 79 | 4.3 |
| $^{116m}Sb$ | 60 m | 15% | -5.8 | 1294 | 51 |
| $^{116}Sb$ | 15 m | 15% | -5.3 | 1294 | 4.3 |
| $^{117}Sb$ | 3 h | 8% | -2.6 | 159 | 39 |
| $^{118m}Sb$ | 5 h | 24% | -4.7 | 253 | 048 |
| $^{120}Sb$ | 6 d | 32% | -3.5 | 1172 | 2.2 |
| $^{122}Sb$ | 3 d | 5% | -2.4 | 564 | 20 |
| $^{124}Sb$ | 60 d | 5% | -1.4 | 603 | 46 |
| $^{121m}Te$ | 154 d | 57% | -2.4 | 212 | 16 |
| $^{121}Te$ | 16 d | 57% | -2.1 | 573 | 21 |
| $^{123m}Te$ | 120 d | 43% | -1.1 | 159 | 17 |
| $^{120}I$ | 1.3 h | .09% | -6.2 | 560 | 19 |
| $^{123}I$ | 13 h | 0.9% | -2.1 | 159 | 34 |
| $^{124}I$ | 4 d | 4% | -4.0 | 603 | 3.2 |
| $^{126}I$ | 13 d | 19% | -2.9 | 389 | 9 |
| $^{130}I$ | 12 h | 35% | -1.2 | 536 | 2 |
| $^{127m}Xe$ | 69 s | 100% | -1.6 | 125 | 7 |
| $^{127}Xe$ | 36 d | 100% | -1.3 | 202 | 8 |
| $^{139}Ce$ | 137 d | 100% | -1.0 | 166 | 8 |
| $^{11}C$ | 20 m | 99% | -2.9 | 511 | 80 |
| $^{13}N$ | 10 m | 99% | -5.2 | 511 | 7 |
| $^{17}F$ | 66 s | 90% | -4.1 | 511 | 7 |
| $^{51}Mn$ | 46 m | 6% | -3.1 | 511 | 4.6 |
| $^{55}Co$ | 17 h | 68% | -1.3 | 931 | 1.3 |
| $^{57}Co$ | 271 d | 26% | -0.3 | 122 | 5.8 |
| $^{61}Cu$ | 3.4 h | 49% | +0.8 | 283 | 5.8 |
| $^{75}Kr$ | 95 m | 0.3% | -0.1 | 285 | .064 |
| $^{15}O$ | 2 m | 100% | -7.5 | 511 | 1.1 |
| $^{115m}In$ | 4 h | 95% | -0.3 | 336 | 0.18 |

## WATER-TARGET-CHEMISTRY OF NITROGEN-13 RECOILS REVISITED.

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We have studied the chemical effects of the $^{16}O(p,\alpha)^{13}N$-reaction as a function of dose, pH and additives with the goal to distinguish between primary hot atom and secondary radiolytic processes and to direct the complex mechanism into a dominant reaction channel for one of the major products, i.e. $^{13}NO_3^-$, $^{13}NO_2^-$, $^{13}NH_4^+$ and $^{13}NH_2OH$, respectively.

Dose effects observed from 1 to 5000 μAs show the trend observed in previous studies [1-3], i.e. an increase of higher oxidation states ($^{13}NO_3^-$) at the expense of the lower oxidation states ($^{13}NO_2^-$, $^{13}NH_4^+$). The highest $^{13}NO_3^-$ yield in pure water is 85 ± 5% in the high dose region. Dose rate effects can only be observed in the low dose region (< 10 μAs), indicating mainly $^{13}NO_2^-$ oxidation to $^{13}NO_3^-$. Only at the highest doses gaseous products such as $^{13}NO$ and $^{13}NN$ are observed in significant amounts.

The radiochemical yields of $^{13}NO_3^-$, $^{13}NO_2^-$ and $^{13}NH_4^+$ as a function of pH (1.5 to 12.8 adjusted with $H_2SO_4$ and KOH, respectively) are shown in Fig. 1 for the low dose region (60 μAs). Depending on the pH $^{13}NO_2^-$, $^{13}NO_3^-$ and $^{13}NH_4^+$ can be obtained in radiochemical yields

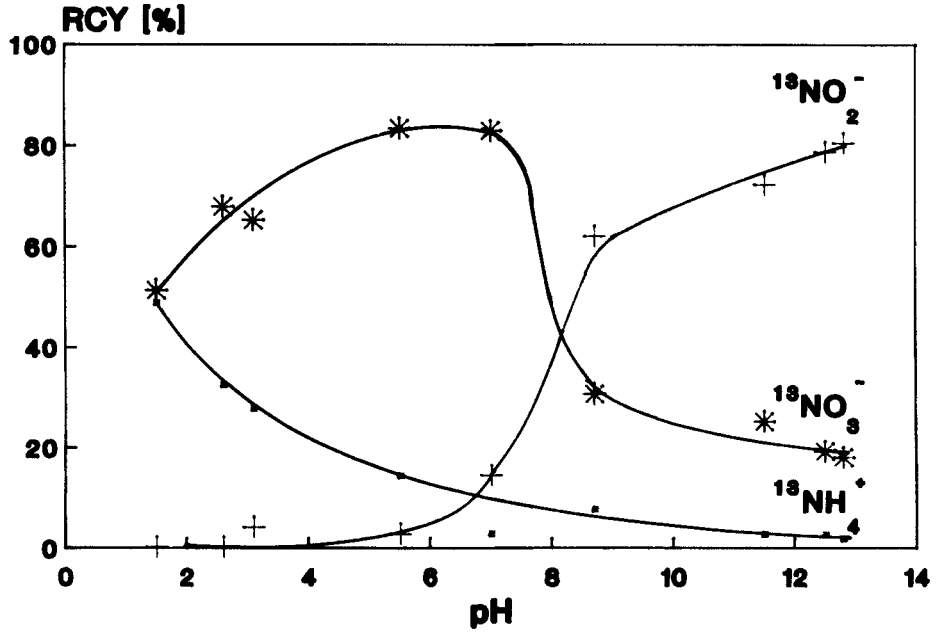


Fig. 1: pH-dependence of the radiochemical yields of $^{13}N$-products (proton dose 60 μAs)

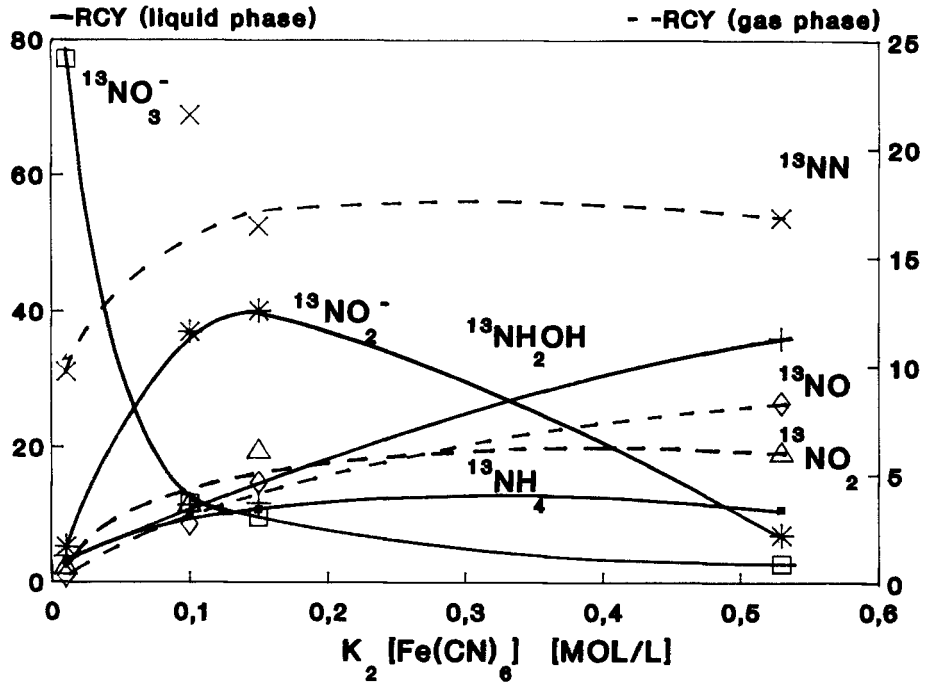


Fig. 2: Effect of OH-radical scavenger $K_2[Fe(CN)_6]$ concentration on the radiochemical product yields of $^{13}N$-products (proton dose 60 $\mu$As)

of 50 to 80%. The influence of the concentration of the OH-radical scavenger $K_2[Fe(CN)_6]$ on the product yields is shown in Fig. 2. Increasing scavenger concentration prevents the radiolytic oxidation of $^{13}NH$-species and leads to increasing yields of $^{13}NH_2OH$ and $^{13}NH_4^+$.

The changes observed can be described by reactions of radicals formed in the water radiolysis with primary recoil species, in particular $^{13}NH$, $^{13}NH_2$ and $^{13}NH_3$.

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## AN AEROSOL-SPRAY APPROACH TO TARGETRY - THEORETICAL CONSIDERATIONS

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The endothermic nature of the evaporative process suggests the introduction of target material in the form of an aerosol. Anticipated benefits include reduction of spatial inhomogeneities within the target, improved cooling of the entrance foil and irradiation at greater beam intensities than are presently feasible.

Most radionuclides used in PET are produced by bombardment of gases or liquids. In both cases, the considerable thermal energy deposited by the incident charged particle beam causes production uncertaintities. Liquids must be irradiated under considerable pressure to reduce cavitation; for gases, even high pressures do not eliminate the well-known spatial inhomogenieties and consequent reductions of target nuclei in the beam strike.

A twenty-minute irradiation at 20 microamperes with 15 Mev. protons deposits nearly 90,000 calories in a target, and about a fifth as much in the entrance foil. A single water droplet of diameter 100 microns and initial temperature 20° C,introduces into the target chamber $3 \times 10^{-4}$ calories of heat-absorption capacity, assuming a final gaseous state at 100° C. This droplet will absorb thermal energy from incident protons at approximately $3.6 \times 10^{-4}$ calories/second, with the 20 microampere beam spread over a circle of 1 cm. diameter. Thus a typical droplet would endure nearly one second in the chamber environment before vaporizing completely. This is sufficient time to allow a variety of injection geometries. By spraying the aerosol directly onto the inner face of the entrance foil, its evaporative cooling will augment that of the helium spray on the outer surface. 10,000 microdrops per second of average diameter 100 microns would carry off approximately 20% of all the beam-deposited thermal energy of the entrance foil assuming total evaporation of each droplet at the foil surface.

With external condensation and recirculation of the target gas, it should be possible to carry out irradiation at lower pressures than are presently feasible, with amounts of target aerosol on the order of 30 ml, typical in the production of $^{13}N$. Given a multi-aperture nozzle design that conforms to the natural convection currents of the chamber, target uniformity should be greatly improved.

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**A NEW SIMPLE AND ATTRACTIVE METHOD OF [$^{11}$C]HALOGENOMETHANES PRODUCTION ($Br^{11}CH_3$, $I^{11}CH_3$).**

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[$^{11}$C]Methyl iodide is a very often employed precursor for labelling molecules. Automation of the classical method used ($^{11}CO_2 \rightarrow {}^{11}CH_3OH \rightarrow I^{11}CH_3$) for the [$^{11}$C]methyl iodide preparation presents a certain complexity. Furthermore, it would not resolve the problems encountered in the preparation of the reagents and of the synthesis apparatus. Our new method avoid the use of liquid reagents (LAH/THF, HI) and renders it more simple. According to the reported method used in the production of $^{11}CHCl_3$ and $^{11}CCl_4$ (ref. 1), we studied the iodination and bromination of methane (bromomethane being also a good methylating agent). Methane iodination or bromination by radicalar reaction are not favorised (ref. 2). Effectively the enthalpy of the first reaction of the propagation step is positive in both cases :

$$X^{\cdot} + CH_4 \longrightarrow HX + CH_3^{\cdot}$$

$\Delta H$ = 138 and 71 Kj/mol for X=I and Br respectively.

But the excess of halogen compared to $^{11}CH_4$ favorises the halogenomethane formation. HI or HBr are produced in too low quantity to inhibit the reaction. The halogenation is produced by sending methane, with a nitrogen flow (15 ml/min), on a catalyst (CuI, $CuBr_2$...), previously adsorbed on pumice stone. The reaction was carried out at 600°C. Excess of halogen is trapped on soda lime at the exit of the oven. Different parameters have been studied such as the oven temperature and the contact time of methane with the catalyst, which depends of the flow rate of the carrier gas and the quantity of catalyst. The reaction is initiated with a low quantity of chlorine mixed with methane. The yield (based on $CH_4$) of halogenomethane produced is 15-20 % for $BrCH_3$ (about 10 % of $Br_2CH_2$ are also found) and 10-15 % for $ICH_3$. The products are analysed by HPLC : C18 column, water/methanol (65/35 for $BrCH_3$ and 50/50 for $ICH_3$). The HPLC retention time of $BrCH_3$, $Br_2CH_2$ and $ICH_3$ are respectively : 9.8, 15.7 and 15.1 min. Using the same method for [$^{11}$C]halagenomethanes production, 7.4 - 11.1 GBq (200-300 mCi) of $Br^{11}CH_3$ or 4.85 - 7.40 GBq (150 - 200 mCi) of $I^{11}CH_3$ were obtained from 55.5 - 74.0 GBq (1.5 - 2 Ci) of $^{11}CH_4$ with a specific radioactivity of 59 - 96 GBq/µmol (1.6 - 2.6 Ci/µmol), 10 min after EOB. $BrCH_3$ prepared by this method has been used to methylate nor RO 15 1788 (benzodiazepine ligand) with a 45 % yield (based on $BrCH_3$), according to the reported method with $I^{11}CH_3$ (ref. 3). Studies are under progress to improve the yield of methane halogenation.

This simple method, easily automated could be used routinely for labelling molecules.

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## SYNTHESIS OF ELECTROPHILIC $^{11}$C-SYNTHONS: $^{11}$C-CYANOGEN BROMIDE AND SOME $^{11}$C-CYANO REAGENTS

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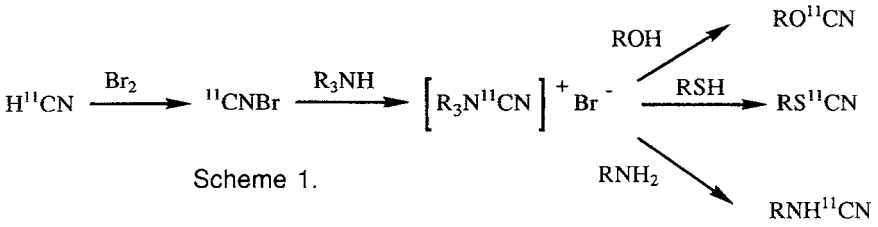
A fast, simple and reliable route has been developed to $^{11}$C-cyanogen bromide as well as to stabilized forms of this cyanation reagent. Cyanogen bromide is an electrophilic one-carbon synthon, which can be regarded as an umpoled cyanide ion.

Cyanogen bromide (CNBr) itself is a poor cyanation agent, and is rapidly hydrolysed in aqeous solution. Therefore, the CNBr is allowed to react with a tertiary amine, such as triethylamine (TEA), or 4-dimethylaminopyridine (DMAP), or a phenol to give N-cyano-ammonium ions and aromatic cyanates respectively (1).

$^{11}$C-Cyanogen bromide was prepared from H$^{11}$CN (2) according to Scheme 1. The cyanogen bromide was transferred from the reaction vessel in a stream of nitrogen or helium to the reaction vessel containing the appropriate tertiary amine base or phenol.

The characteristics of these reagents make them very versatile for the unspecific $^{11}$C-labelling of macromolecules. Preliminary studies suggest that such labelling is feasible, and work is now in progress to develop suitable labelling conditions for proteins, oligonucleotides and polysaccharides.




Scheme 1.

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## NEW SYNTHESIS METHOD OF [C-11]PROPYL KETENE USING HCL/HE GAS MIXTURE AND THE REACTIONS ON VARIOUS ALCOHOLS

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We have been reported a new type of [C-11] labeled precursor. We found out and report a new [C-11]propyl ketene synthesis technique using gas mixture of 0.1% HCl/He and He for dilution instead of the phosphoric acid. We evaluated the validity and usefulness of this technique and the [C-11]propyl ketene reactions on various alcohols.

We synthesized [C-11]propyl ketene by using the method of Y. Imahori et al.(1) with some modifications. After the trapping of $[C\text{-}11]CO_2$ in a cooled reaction vessel with $LN_2$, the propyl lithium and $H_2O$ was added, and we obtained the lithium salt of [C-11]butyric acid by evaporation of solvent. The quartz glass column(ID: 7mm, length: 240mm) containing glass beads of 1mm in diameter was heated up at 530°C beforehand, and the reaction vessel was connected to it. The gas mixture of 0.1% HCl/He and He for dilution was through the P2O5 column for exclution of the moisture from gas mixture, and swept into the reaction vessel. The vapor of the [C-11]butyric acid was extructed from the lithium salt, and carried to the quartz glass column by sweep gas. The [C-11]butyric acid was decomposed by pyrolysis at 530°C to yield [C-11]propyl ketene. The total flow rate of the gas mixture was 70ml/min.(flow rate of 0.1% HCl/He was 5ml/ min., and He for dilution was 65ml/min.).

$$^{11}CO \xrightarrow{C_3H_7Li} C_3H_7{}^{11}COOLi \xrightarrow[H^+]{} C_3H_7{}^{11}COOH \xrightarrow[530°C]{} C_3H_6={}^{11}C=O$$

The esterfication by [C-11]propyl ketene was analyzed by using PMTE(Phorbol 20-Methoxytrityl ether) as a standard. And we selected β-phenetyl alcohol as the primary alcohol, 1-phenyl 2-propanol as the secondary alcohol and 1,1-dimethyl 2-phenethyl alcohol as the tertialy alcohol to evaluate the [C-11]propyl ketene reactivity on those alcohols.

The total yield was about 22% and synthesis time was 25min. As the result of the evaluation of the reactivity of [C-11]propyl ketene to alcohols, the reactivity of the primary alcohol suposed 1, the relative reactivity of the secondary alcohol and the tertiary alcohol were 0.4 and 0.1 respectively.

Ketene is high reactive compound reported by Hurd C.D.(2). We selected this high reactive compound for [C-11] labeling agent. In synthesis method using phosphoric acid we could not succeed to get the good reproducebility of the yield of [C-11]butyric acid, and the yield of [C-11]propyl ketene depended on the condition of packing of the quartz glass wool. Those problem were resolved by new method using gas mixture and glass beads. As the effect of HCl, the vapor of [C-11]butyric acid were increased, and the synthesis steps were simplified, and as the effect of the glass beads, we could get the good reproducebility of [C-11]propyl ketene yield. The concentration of HCl are very important to vaporize [C-11]butyric acid. In case of low concentration, the vaporization of [C-11]butyric acid was poor, and in case of high concentration, a precursor, which is

sensitive to acid, may be decomposed by surplus HCl. The optimal concentration of this system was 0.007%. This method can produced another type of reaction, for example N-butyryl formation can be seen in the reaction of [C-11]THPO(4,5,6,7,Tetrahydro isoxazoro [4,5-C]pyridine-3-ol) by using [C-11]propyl ketene(yield : 42%, purity: 98%). This N-butyryl compound is useable for GABA up take mechanism. Thus this labeling method makes it possible to produce many types of bioactive compounds such as alkaloid, stealoid, amide and carbohydrate.

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Table 1 Radiochemical yields on each synthesis steps

| step | $C_3H_7Li + {}^{11}CO_2$ ↓ $H_2O$ | dry up | pyrolytic decomposition | dry up | final |
|---|---|---|---|---|---|
| Yield (%) | 100 | 96 | 32 | 28 | 22 |

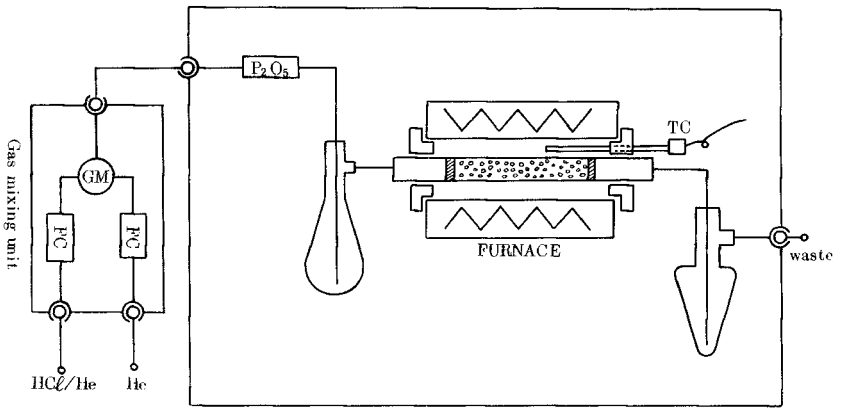


Figure 1. Diagram of the pyrolytic decomposition system

—◎—: connecter, FC: flow controller, GM: gas mixer
⁞⁞⁞: glass beads ▨: quartz glass wool, TC: thermocouple

## PREPARATION OF N.C.A. [$^{11}$C] ACRYLIC DERIVATIVES FOR [$^{11}$C] ALLYLATION OF AMINES.

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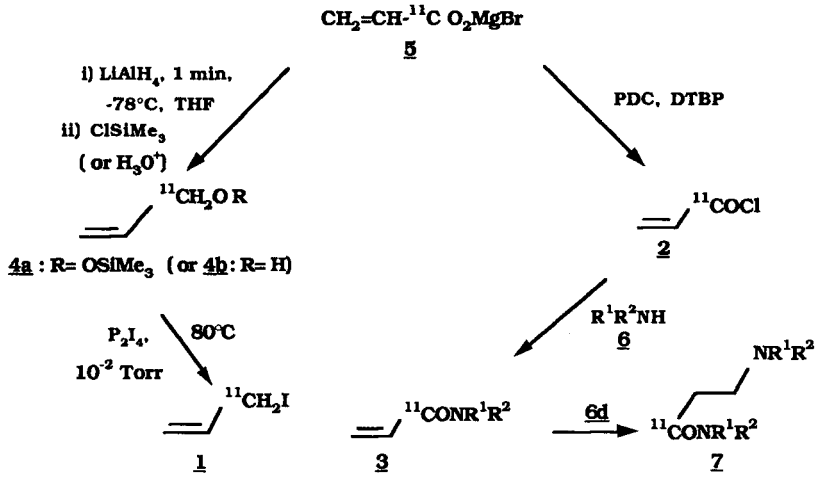
In the search for [$^{11}$C] labelled precursors, [$^{11}$C] labelled alkyl iodides and acid chlorides have proved to be useful because of their easy incorporation into many [$^{11}$C] radiopharmaceuticals (1). So far, only [$^{11}$C] alkyl and aryl iodides (2) and [$^{11}$C] saturated acid chlorides (3) have been prepared. Unsaturated analogous compounds such as 3-iodo [3-$^{11}$C]-1-propene ([$^{11}$C] allyl iodide ) **1** or [1-$^{11}$C] 2-propenoyl chloride **2** ([1-$^{11}$C] acryloyl chloride ) are potential precursors for the synthesis of [N-$^{11}$C] allyl amines such as [$^{11}$C] naloxone (4) required for PET studies of opioid receptors in the living human brain.

Due to the presence of a reactive double bond, conditions similar to those used for the synthesis of saturated iodides or acid chlorides are not *a priori* applicable (5). In this paper we report the preparation of [$^{11}$C] vinylic amides **3** *via* the acid chloride **2** and of [$^{11}$C] allyl iodide **1** *via* [$^{11}$C] allyl alcohol **4b** without protecting the double bond of [$^{11}$C] acrylic acid.




Scheme 1 :Preparation of [$^{11}$C] acrylic acid derivatives; **a** :$R^1$= Pr, $R^2$= H; **b**: $R^1$= Ph, $R^2$= H; **c**: $R^1$, $R^2$= $(CH_2)_5$,; **d** :$R^1R^2$ = tetrahydroisoquinoline (TIQ); PDC = phtaloyl dichloride; DTBP = di-*tert*-butylpyridine.

### *1-[$^{11}$C] acryloyl chloride , [$^{11}$C] vinylic amides :*

The key step in the preparation of [$^{11}$C] vinylic amides is the carboxylation of the vinylmagnesium bromide. When [$^{11}$C]$CO_2$ is trapped at room temperature for 3 min, yields up to 50% of [$^{11}$C] acrylic acid can be obtained after acid hydrolysis and HPLC isolation [see table 1 for the conditions

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and (6) for a comparison ]. Direct treatment of its magnesium salt **5** according to a previously described procedure (3) followed by immediate reaction of the distilled acid chloride **2** with the amine **6** has given the [$^{11}$C] amides **3** in 10-18% yield. In the case of the more nucleophilic tetrahydroisoquinoline **6d**, a strict control of the reaction temperature has been necessary to avoid the formation of the Michael adduct **7d**.

Table 1 :

| Compounds | | RCY(a) (%) | Synthesis time (b) | Chromato. conditions | RT (min) or Rf |
|---|---|---|---|---|---|
| $H_2C$=CH-$^{11}$COOH | | 49 | 19 | (c) $H_2O$ 0.025M $NaH_2PO_4$ | 9 |
| $H_2C$=CH-$^{11}$CONHPr | **3a** | 10-13 | 23 | (c) $H_2O/CH_3CN$,30/70 | 6.5 |
| $H_2C$=CH-$^{11}$CONHPh | **3b** | 11-18 | 25 | (c)$CH_3CN/H_2O$, 30/70 | 9.5 |
| $H_2C$=CH-$^{11}$CON$(CH_2)_5$ | **3c** | 10 | 20 | (d) Pentane/AcOEt, 50/50 | 0.23 |
| $H_2C$=CH-$^{11}$CONTIQ | **3d** | (f) | 20 | (d) $HCCl_3$/MeOH, 90/10 | 0.85 |
| $H_2C$=CH-$^{11}CH_2OH$ | **4b** | 23-28 | 25 | (e) | 12 |
| $H_2C$=CH-$^{11}CH_2I$ | **1** | 6 | 32 | (c) $H_2O$/MeOH, 50/50 | 11 |

(a) RCY : radiochemical yield after HPLC and corrected to E.O.B. starting from 200-250 MBq (b) in min after HPLC, except for **3c** and **3d** (c) µ Bondapack C18, λ : 254 nm, flow rate 2 mL.min$^{-1}$ (d) radio TLC (e) CPV : column : carbowax 20M, 4m, 70°C (f) crude : reaction at 0°C, 8 min : 100% of **3d** ; after heating 30 s at 80°C: 60% of **7d** .

*[$^{11}$C] allyl alcohol, [$^{11}$C]allyl iodide :*

[$^{11}$C] allyl alcohol **4b** can also be prepared from [$^{11}$C] acrylic acid magnesium salt **5** if the reduction with lithium aluminium hydride is achieved at low temperature (- 78°C) for 1 min . These conditions minimize the formation of [$^{11}$C] propanol ( < 30% ). [$^{11}$C] Methanol from unreacted $^{11}CO_2$ is also always present in the mixture (< 20 %). No improvement of the selectivities and yields were obtained by using more selective reagents (DIBAL, RedAL, $AlH_3$) . The transformation of the alcohol **4b** into the iodide **1** has been investigated . The best results (6% isolated after HPLC) have been obtained by treating the crude [$^{11}$C] alcohol salts by chlorotrimethylsilane and distilling under $10^{-2}$ Torr the volatiles ( **4a** was not isolated) over solid $P_2I_4$, heated at 80°C (7). Work is in progress both to improve the yields and to use **1** in [$^{11}$C] allylation reactions.


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# $^{11}$C-LABELLED MALONIC ESTERS AS MULTI-FUNCTIONAL PRECURSORS IN RAPID LABELLING SYNTHESES

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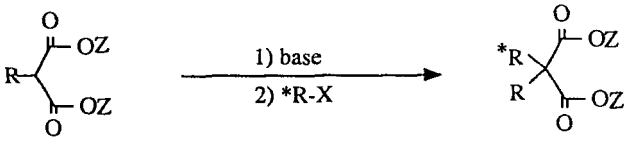
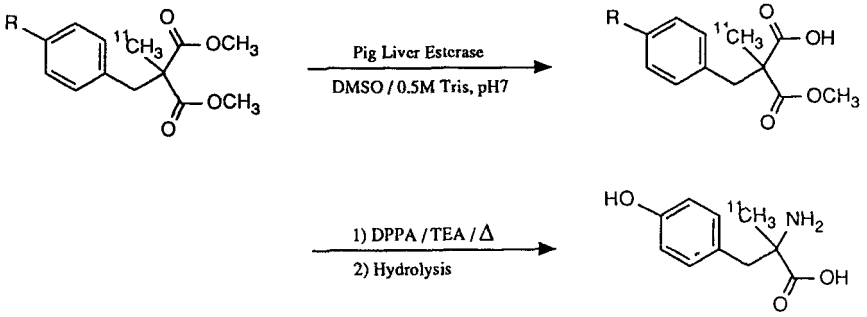
With the aim of developing multi-functional precursors labelled with short-lived positron emitting radionuclides, the possibility of using $^{11}$C-labelled malonic ester derivatives in radiolabelling syntheses is being investigated (Scheme 1).




Scheme 1. General scheme for the radio-labelling of malonic ester derivatives.
*R- = $^{11}CH_3$-, R$^{11}CH_2$-, $CH_3{}^{11}CO$- Z/Z = Me/Me, Et/Et, >C$(CH_3)_2$
R- = H, $CH_3$-, R-Benzyl

In combination with an array of chemical transformations developed for use in rapid labelling syntheses, a diversity of biologically interesting molecules have now been synthesised from labelled malonic ester substrates (1). Presented below are a few examples illustrating the flexability of this approach.

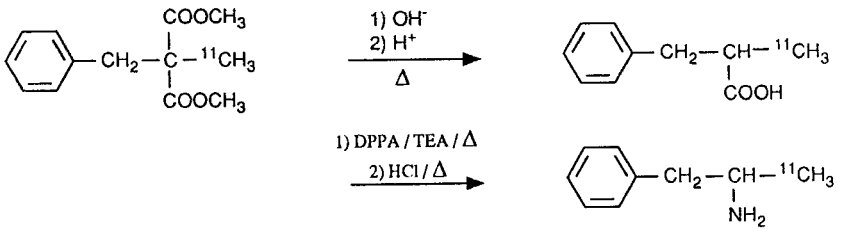
***α-[Methyl-$^{11}$C]methyl amino acids (Scheme 2.)***




Scheme 2. The synthesis of α-[methyl-$^{11}$C]methyl tyrosine.

α-Methyl tyrosine and α-methyl phenylalanine have been labelled with $^{11}$C in the α-methyl moiety starting from 2-benzyl-2-[$^{11}$C]methyl substituted malonic esters(1). A selective enzymatic hydrolysis of one of the ester groups using pig liver esterase, followed by a Curtius rearrangement using diphenyl diphosphoryl azide (DPPA)(2) produced the desired amino acids after hydrolysis. The total synthesis time was 45-50 min (counted from start of [$^{11}$C]methyl iodide synthesis) with a 14-20% decay-corrected radiochemical yield. The α-methyl phenylalanine was found to be racemic whereas the α-methyl tyrosine was obtained in 62 % e.e. in the L-form.

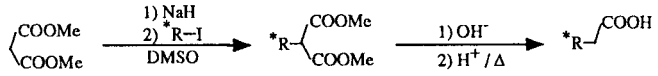
***[Methyl-$^{11}$C]Amphetamine (Scheme 3.)***




Scheme 3. The synthesis of [methyl-$^{11}$C]amphetamine.

2-Benzyl dimethylmalonate was alkylated in the 2-position with $^{11}$C-methyl iodide. After decarboxylation, the remaining carboxylic acid function was converted to the amine via a Curtius rearrangement using DPPA (2). After acidic hydrolysis and preparative LC purification, amphetamine was obtained in a 22 % decay-corrected radiochemical yield in a total synthesis time of 40 min (counted from the start of [$^{11}$C]methyl iodide synthesis).

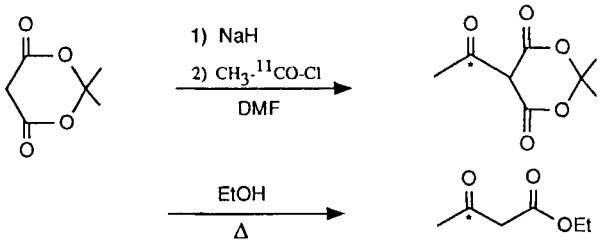
***[3-$^{11}$C]Carboxylic acids (Scheme 4.)***




Scheme 4. The synthesis of straight chain [3-$^{11}$C]carboxylic acids.

The synthesis of straight-chain [3-$^{11}$C]-carboxylic acids has been performed by the alkylation of malonic esters with various $^{11}$C-labelled alkyl halides followed by a decarboxylation. This method complements a previously reported method to synthesise 2-[methyl-$^{11}$C]methyl carboxylic acids (3).

***β-[3-$^{11}$C]Keto esters (Scheme 5.)***




Scheme 5. Meldrums acid in the synthesis of ethyl [3-$^{11}$C]acetoacetate.

An alternative source of the ⊖CH-COOR synthon is Meldrums Acid. This can be used in electrophilic substitution reactions (4), and has now been acylated with $^{11}$C-acetyl chloride. In this case, ethanolysis produces ethyl [3-$^{11}$C]acetoacetate. Optimisation of reaction conditions is now in progress.

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## A NEW APPROACH TO THE SYNTHESIS OF NO-CARRIER-ADDED FLUORINE-18 LABELED FLUOROCATECHOLS

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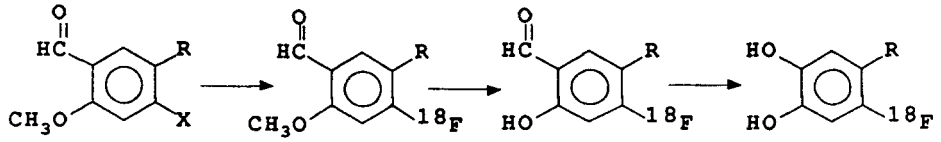
The fluorine-18 labeling of catechols is of considerable interest for the preparation of new radiopharmaceuticals to study the dopaminergic and adrenergic neurotransmitter systems via PET. Electrophilic fluorination methods are quite suitable for synthesis of 6-[$^{18}$F]fluoroDOPA, but such reactions provide final aryl[$^{18}$F]fluorides of low specific activity which are not acceptable with such proposed agents as fluorodopamine or fluoro-norepinephrine. Several investigators have reported multistep syntheses of fluorinated catechols, including 6-[$^{18}$F]fluoroDOPA and [$^{18}$F]fluorodopamine, using NCA [$^{18}$F]fluoride and nucleophilic aromatic substitution in the initial synthetic steps (1,2). Although successful, these syntheses are multistep, and in some instances (e.g., fluoroDOPA, fluoronorepinephrine) require the later synthesis of chiral center.We have begun evaluation of a new synthetic approach to fluorocatechols which we hope will obviate some of these problems. Overall, the concept is the use of a salicylaldehyde as a "synthon" for a catechol, as shown below. The aldehyde substituent allows for nucleophilic substitution of an appropriate leaving group (nitro, trimethylammonium) by [$^{18}$F]fluoride ion. Salicylaldehydes can be converted to catechols by peroxide oxidation (3); this oxidation reaction has been previously used in the synthesis of L-DOPA (4), and the feasilbility of application to syntheses with short-lived radionuclides was shown by the rapid conversion of salicyladehyde to catechol using basic hydrogen peroxide (30% $H_2O_2$) according to modifications of these literature procedures.




As a test synthesis, we have used this sequence to prepare 4-[$^{18}$F]fluorocatechol. 2-Methoxy-4-nitrobenzaldehyde was prepared in six steps from 2-amino-4-nitrotoluene (conversion of aniline to phenol, methylation, benzylic bromination, acetate-for-bromo displacement, hydrolysis of benzylic acetate, and benzylic oxidation). Reaction with NCA [$^{18}$F]fluoride ion gave the corresponding 2-methoxy-4-[$^{18}$F]fluorobenzaldehyde in 45% yield. Cleavage of the anisole was done with $BI_3$ (10 min, 25 °C) to yield the free phenol in 20-40% yield, which was then oxidized (12.5 N NaOH, 30% $H_2O_2$, 25 °C, 10 min) to the desired 4-[$^{18}$F]fluorocatechol in 45% yield (all radiochemical yields decay corrected)

This synthetic approach holds considerable promise as a rapid and simple method for fluorocatechol synthesis. Appropriate choices in protecting groups may allow the entire sequence to be done in a single pot, and might allow [$^{18}$F]fluorination reactions to proceed without racemization of a chiral center (4).

Acknowledgements. This work was supported in part by Department of Energy grant DE-AC02-76EV02031 and National Institutes of Health grant NS15655.

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## [$^{18}$F]-SUBSTITUTED AROMATIC ALDEHYDES, KEY-INTERMEDIATES FOR NCA RADIOSYNTHESES

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The ongoing interest in highly biologically specific fluorine-18 labeled traceurs for PET requires the development of new labeled secondary [$^{18}$F]-intermediates, in order to enlarge the range of radiopharmaceuticals which can be prepared in practical radiochemical yields for routine medical use.

The few primary precursors currently available belong either to the electrophilic class $F_2$, $XeF_2$, $CH_3COOF$ (requiring the addition of carrier), or to the nucleophilic species $F^-$ which remains the only way for NCA synthesis. Secondary nucleophilic agents such as [$^{18}$F]-fluoro alkyl mesylate, tosylate, triflate or halogeno derivatives have been proposed as an alternative method via fluoroalkylation at the no-carrier-added level (1, 2). Nucleophilic aromatic substitution by $^{18}F^-$ is now a general reliable route when the phenyl ring carries a leaving group (nitro) activated by an electron withdrawing functional group (cyano, ketone, nitro,..) (3). This is now one of the main methods for preparing a wide variety of NCA [$^{18}$F]-labeled fluoroaryl compounds.

The aim of the present work was to develop an additional fluorination pathway starting from the NCA [$^{18}$F]-fluoride and giving access to a family of general purpose secondary fluorinating agents: the substituted [$^{18}$F]-fluoro aromatic aldehydes. These key-precursors are obtained directly from the corresponding $NO_2$ or $N^+(Me)_3$ analogs with radiochemical yields depending on the electronic structure of the aromatic aldehydes as shown in Table 1.

Scheme 1 depicts some chemical reactions which have either been successfully applied (4, 5, 6) or show promise when starting from the selected substituted [$^{18}$F]-fluoroaromatic aldehydes. These fast reactions afford several new active intermediates such as substituted [$^{18}$F]fluorobenzoyl chlorides and also [$^{18}$F]fluorobenzyl bromides. The latter derivatives can be quantitatively prepared by means of a reducing $NaBH_4$ column followed by bromination (6).

Scheme 2 shows the main possible reactions starting from substituted [$^{18}$F]fluorobenzyl bromide some of which are key-intermediate successfully applied to the syntheses of NCA [$^{18}$F]fluorobenzylamines and also to the asymmetric synthesis of [$^{18}$F]-fluoroaromatic amino acids.

It is anticipated that derivatives of [$^{18}$F]fluorobenzaldehyde an [$^{18}$F]fluorobenzyl bromide will find widespread application in the synthesis of [$^{18}$F]-labeled radiopharmaceuticals.

**Table I. Radiochemical yields of various $^{18}$F-labeled aldehydes (% EOB)**

| | | | |
|---|---|---|---|
| 2-$^{18}$F-fluorobenzaldehyde | 70 | 4-$^{18}$F-fluoro-3-anisaldehyde | 60 |
| 3-$^{18}$F-fluorobenzaldehyde | 0 | 3-$^{18}$F-fluoro-4-anisaldehyde | 0 |
| 4-$^{18}$F-fluorobenzaldehyde | 65-70 | 6-$^{18}$F-fluoroveratraldehyde | 50 |
| 2-$^{18}$F-fluoro-4-anisaldehyde | 75-80 | 6-$^{18}$F-fluoropiperonal | 55 |
| 3-$^{18}$F-fluoro-4-anisaldehyde | 0 | | |

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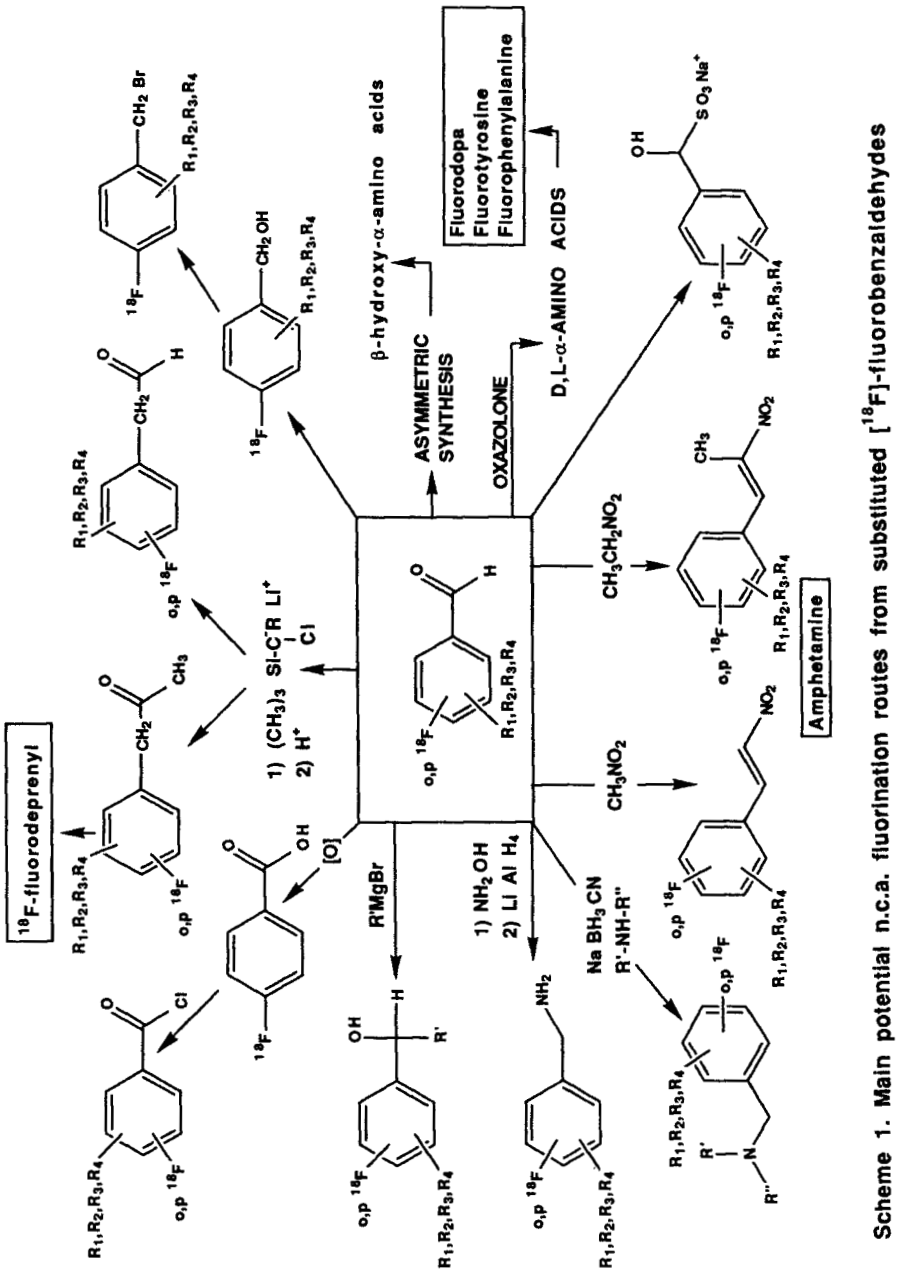
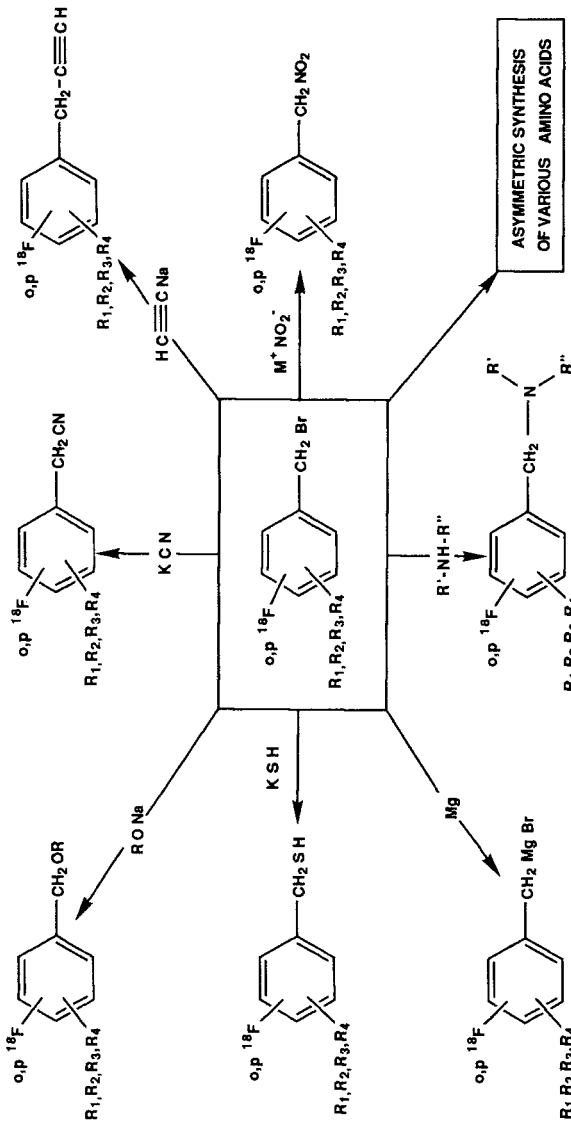



**Scheme 1. Main potential n.c.a. fluorination routes from substituted [$^{18}$F]-fluorobenzaldehydes**




**Scheme 2. Main potential n.c.a. fluorination routes from substituted [$^{18}$F]-fluorobenzylbromides**

SYNTHESES OF o- AND p-[$^{18}$F]FLUOROBENZYLBROMIDE AND THEIR APPLICATION TO PREPARATIOIN OF F-18 LABELED NEUROLEPTICS.

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Since neucleophilic substitution of activated nitro group with no-carrier-added (NCA) [$^{18}$F]fluoride was reported for the preparation of [$^{18}$F]arylfluoride (1), this method became the representative route for radiopharmaceuticals containing NCA fluorine-18 on an aromatic ring system. But, a precursor used for the reaction was restricted to substrates having strong activating groups on o- or p- (rarely m-) position to a leaving group. Consequently, several reactive fluorine-18 labeled building blocks were prepared from [$^{18}$F]fluorobenzonitrile, [$^{18}$F]fluoronitrobenzene etc. and were applied to preparations of radiopharmaceuticals. We considered [$^{18}$F]fluorobenzylbromide should be a synthon of extensive use. We present here the preparation of o- and p-[$^{18}$F]fluorobenzylbromide, and their application to the synteses of [$^{18}$F]fluorinated analogs of YM-09151-2, a potent neuroleptic, via N-[$^{18}$F]fluorobenzylation.

The synthetic procedure for [$^{18}$F]fluorobenzylbomide is indicated in Figure 1. Ortho- and p-[$^{18}$F]fluorobenzaldehyde were prepared from fluorination of respective nitrobenzaldehydes according to the reported method (2). The aldehydes were quantitatively converted to corresponding benzylalcohols with $NaBH_3CN$ in acidic conditions (pH 4). These two reaction steps could be carried out in one-pot. Rather low extractability was found for the benzylalcohols, and no significant difference was observed between o- and p-fluoro conjeners in their yields and extractabilities (Table 1).

Bromine substituion of hydroxyl group of [$^{18}$F]fluorobenzylalcohol was accomplished along with the introduction of gaseous HBr into benzene solution of the alcohol. Yields were indicated in Table 2. p-[$^{18}$F]Fluorobenzylalcohol was observed to be more reactive. The results of iodination of the same alcohols were also tabulated. The labeled synthons, o- and p-[$^{18}$F]fluorobenzylbromide were obtained in three steps of good yields. The incorporation of fluorobenzyl group into desbenzyl derivative of YM-09151-2 were carried out in three solvents in Table 3. Although p-[$^{18}$F]fluoro analog (2) of YM-09151-2 was obtained in a good yield under a fairly mild reaction condition, the yield of o-[$^{18}$F]fluoro analog (1) was lower.

The in vitro $D_2$-dopamine receptor binding of non-radioactive compounds of 1, 2, sulpiride, and YM-09151-2 were tested with [$^3$H]YM-09151-2 and rat striatal membrane preparation according to the method of Niznik et al. (4). Another fluorinated analog, 3, having fluoropropyl moiety instead of methylamino group on YM-09151-2 (3) was also tested. The low affinity of 3 was consistent with our previous in vivo observation (3). While in vitro affinity of 1 and 2 were less potent compared to YM-09151-

2, the in vivo striatal distribution of 1 in rat brain suggested that 1 was equal or more potent than carbon-11 labeled YM-09151-2.

In conclusion, syntheses of o- and p-[$^{18}$F]fluorobenzylbromide were accomplished in three steps. Preparatin of a neuroleptics having fluorine on an aromatic ring were attempted via N-fluorobenzylation using the obtained labeled synthons. The product, 1, was suggested to be potent radiopharmaceutical for the measurement of $D_2$-dopamine receptors with PET.

Authors would like to thank Yamanouchi Pharmaceutical Co.,Ltd., (Tokyo, Japan) for supplying us desbenzyl derivative of YM-09151-2 and [$^3$H]YM-09151-2. The members of CYRIC, Tohoku University are also ackowledged for their cooperations.

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Figure 1.

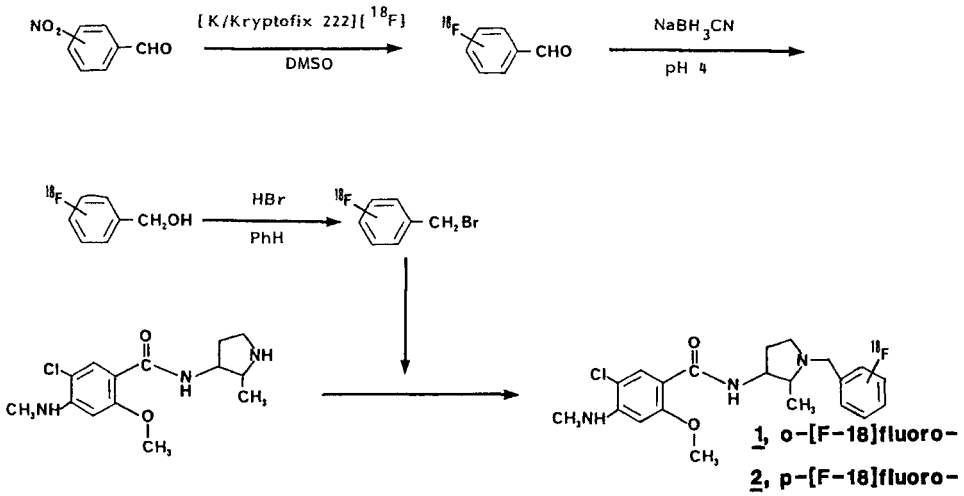


Table 1. Yields of [$^{18}$F]Fluorobenzyl Alcohols

| Product | Yield | Extractability |
|---|---|---|
| o-Fluorobenzyl Alcohol | 57 % (47 - 68) | 55 % (40 - 70) |
| p-Fluorobenzyl Alcohol | 59 % (50 - 76) | 62 % (43 - 84) |

Data represents a mean and a range in the parenthesis. Yield was obtained from HPLC analysis and extractability was calculated as follows; (extracted $^{18}$F / total $^{18}$F used in the synthesis) / yield obtained with HPLC.

Table 2. Yields of Benzylhalides from Benzylalcohols.

| Product | Temperature | Yield |
|---|---|---|
| o-[$^{18}$F]Fluorobenzyl Bromide | R.T. | 53 % |
| | 60°C | 90 % |
| p-[$^{18}$F]Fluorobenzyl Bromide | R.T. | 95 - 100 % |
| o-[$^{18}$F]Fluorobenzyl Iodide | 60°C | 15 % |
| | 120°C | 22 % |
| p-[$^{18}$F]Fluorobenzyl Iodide | R.T. | 70 % |
| | 60°C | 89 % |

For bromination, gaseous HBr was introduced to a benzene solution of benzylalcohol for 2 min. The solution was stirred for 20 min at indicated temperatures. For iodine substituion, HI/benzene solution prepared beforehand was added to alcohol. Yield was obtained by radio-TLC analysis developed in 30% $CH_3COOC_2H_5$/n-$C_6H_{14}$.

Table 3. Yields of 1 and 2 from [$^{18}$F]Fluorobenzyl Bromides.

| Product | Solvent | Base | Temperature | Yield |
|---|---|---|---|---|
| 1 | $CH_3CN$ | $NaHCO_3$ | 40°C | 0 % |
| | DMF | NaH | 40°C | Trace |
| | DMSO | NaH | 40°C | 14 % |
| | DMSO | TBA-OH* | 60°C | 36 % |
| 2 | $CH_3CN$ | $NaHCO_3$ | R.T. | 28 % |
| | $CH_3CN$ | $NaHCO_3$ | 40 C | 88 % |

Reaction time was 20 min. Yiled of the product was obtained by radio-TLC analysis developed in 5% MeOH/$CHCl_3$. *TBA-OH = tetrabutylammonium hydroxide.

Table 4. Inhibition of [$^3$H]YM-09151-2 Binding to Rat Striatal Membrane preparation by various drugs.

| Drug | $IC_{50}$ | Relative Affinity | Hill Coefficient |
|---|---|---|---|
| YM-09151-2 | $4.68 \times 10^{-9}$ | 1000 | 1.00 |
| Sulpiride | $8.13 \times 10^{-7}$ | 5.76 | 1.36 |
| 1 | $2.88 \times 10^{-8}$ | 163 | 1.41 |
| 2 | $2.14 \times 10^{-8}$ | 218 | 0.96 |
| 3 | $9.18 \times 10^{-8}$ | 51.0 | 1.07 |

$IC_{50}$ and Hill coefficient was obtained from linear regression of a Hill-plot. Relative affinity was calculated as follows; 1000 x ($IC_{50}$ of YM-09151-2)/($IC_{50}$ of drug).

## [$^{18}$F]FLUORINATION OF HETEROCYCLIC RINGS: SYNTHESES OF [$^{18}$F]FLUOROPYRIDAZINES BY NUCLEOPHILIC SUBSTITUTION

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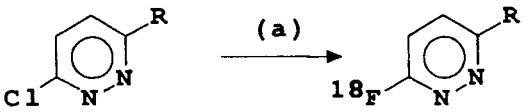
Nucleophilic aromatic substitution is now a well established method for the no-carrier-added [$^{18}$F]fluorination of aromatic rings. Most efforts have been expended in the fluorination of substituted phenyl rings, although successful nucleophilic substitutions with [$^{18}$F]fluoride ion have been described with pyridine (1), pyrimidine (2), and thiophene rings (3). We have a continuing interest in fluorine-18 labeled heterocyclic rings as a means for decreasing the lipophilicity of radiotracers through the substitution of a heterocyclic ring for a phenyl ring. In general, heterocyclic 5 and 6 membered aromatic rings maintain the steric bulk and planarity similar to a phenyl ring, but are considered more polar. Such substitutions might afford less lipophilic radiotracers with minimum alterations of drug behavior: we have recently described the substitution of a thiophene for a phenyl ring in one class of drugs, dopamine reuptake inhibitors, with apparent success (3). In many cases, heterocyclic ring substituted analogs are prepared in the original structure-activity studies on a class of drugs. Pyridazines (1,2-diazabenzenes) are a previously unexplored heterocyclic ring for nucleophilic substitution by [$^{18}$F]fluoride ion. We have therefore examined the reaction of high-specific acitivity, no-carrier-added [$^{18}$F]fluoride ion with various chloro substituted pyridazines (Table). Reactions were conducted as per the usual methods for phenyl ring substitutions. The [$^{18}$F]fluoropyridazines were isolated by liquid-liquid extraction, analyzed by TLC, and compared to authentic samples of the fluoro-pyridazines prepared by reaction of the chloro componds with excess cold fluoride ion. Radiochemical yields of [$^{18}$F]fluoropyridazines ranged from 11 to 64% (decay-corrected) with synthesis times of 25 to 40 min. Neither the reactions conditions nor the yields have been optimized. In all cases single radiolabeled products were obtained, with the remainder of the radioactivity associated with polar, water-extracted materials (assumed to be [$^{18}$F]fluoride ion).




1 2

Table

| | R | % Yield | TLC $R_f$ ($CH_2Cl_2$/silica) |
|---|---|---|---|
| (2a) | -methyl | 24 | 0.51 |
| (2b) | -benzoyl | 64 | 0.2 |
| (2c) | -4-fluorobenzoyl | 11 | 0.35 |
| (2d) | -(2-thienyl)carbonyl | 46 | 0.2 |

(a) Conditions: 1 mg substrate (1), $^{18}F^-/K^+$/Kryptofix 222, 100 µl DMSO, 155 °C, 25-40 min

As expected, the pyridazine analogs are more polar than the corresponding phenyl derivative ($R_f$ 4-fluorobenzophenone = 0.46). In vivo stability of the carbon-fluorine bond of fluoropyridazines was observed in preliminary rat biodistribution studies of compound **2b**. [$^{18}$F]Fluoropyridazines would thus appear a reasonable alternative to [$^{18}$F]fluorophenyl groups, and applications to radiopharmaceutical design are underway.

Acknowledgements. This work was supported in part by DOE grant DE-AC02-76EV02031 and National Institutes of Health grant T32-CA09015 (to A.E.M.).

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A TWO-STEP PROCEDURE FOR LABELING UNACTIVATED ALKYLBENZENES WITH NCA [$^{18}$F]FLUORIDE.

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The aromatic $^{18}$F-for-$NO_2$ displacement reaction has been used widely for the preparation of [$^{18}$F]-labeled aromatic compounds with high specific activity (1,2). The limitation of this reaction is that it precedes well only with activated nitroarenes. A two-step procedure has now been developed for labeling simple alkylbenzenes with no-carrier-added [$^{18}$F]fluoride.

The new procedure (Scheme 1) involves (A) the aromatic $^{18}$F-for-$NO_2$ reaction of 4-nitrophenones, e.g. 1a to 2a, and (B) the reduction of 4-[$^{18}$F]fluorophenones with triethyl silane (TES) and trifluoroacetic acid (TFA) to 4-[$^{18}$F]fluoro-1-alkylbenzenes, 3, 4, and 5.

The TES-TFA reduction procedure has been used successfully in the reduction of various phenones and substituted benzaldehydes to the corresponding alkylbenzenes at room temperature (3). For some phenones, e.g. bromoacetophenone, the reduction can take days. We have found these phenones could be easily reduced in <15 min by simply raising the reaction temperature to 95°C. After the reduction TFA was removed by aqueous base wash, and the reduced alkylbenzenes were dissolved in pentane and purified by column chromatography (silica gel) eluted with pentane. Using this procedure, a variety of phenones and secondary benzylalcohol could be easily reduced to the corresponding alkylbenzenes in excellent yields (Table 1). Under similar conditions, 4-fluorobenzaldehyde was reduced to a mixture of the corresponding benzyl alcohol, benzyl trifluoroacetate, and toluene; benzyl alcohol was converted to benzyl trifluoroacetate exclusively; and no reaction was observed with alkylalcohol (Entry 7, Table 1).

Using this 2-step procedure a pentane solution of 4-[$^{18}$F]fluoro-1-ethylbenzene, 3, could be obtained with a radiochemical yield of > 30% (EOS) in less than 40 min. Similarly, 2-bromo-4'-[$^{18}$F]fluoroacetophenone, prepared by brominating 2a with bromine (4), and 4-chloro-4'-[$^{18}$F]fluorobutyrophenone, prepared by HCl hydrolysis of 2b (5), was efficiently converted to the corresponding halides, 4 and 5.

Preliminary results indicated the reaction between piperidine and halide 4 in DMF at 110°C for 30 min only yielded a small amount of the alkylated amines. Efforts to find optimized reaction conditions are in progress..

This new procedure not only provides a new method for labeling simple alkylbenzenes with $^{18}$F, but also produces valuable alkylating agents like 4 and 5 which provides new methods for labeling biologically important amines, e.g. fentanyl.

This work was supported by NIH grants HL 13851 and NS 06833.

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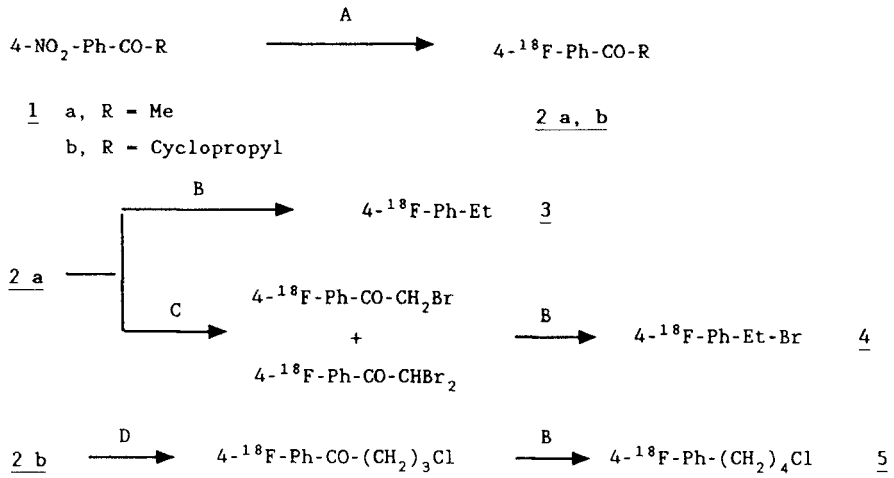
Table 1. Reduction of phenones, benzaldehydes, and benzyl alcohols with TES and TFA*.

| Entry | Starting material | Product (yields, %) |
|---|---|---|
| 1 | 4-F-Ph-CO-Me | 4-F-Ph-Et ($70^{A}$) |
| 2 | 4-F-Ph-CO-$CH_2$Br | 4-F-Ph-$(CH_2)_2$Br ($75^{A}$) |
| 3 | 4-F-Ph-CO-$(CH_2)_3$Cl | 4-F-Ph-$(CH_2)_4$Cl ($>90^{B}$) |
| 4 | Ph-CH(OH)-Ph | Ph-$CH_2$-Ph ($>90^{B}$) |
| 5 | Ph-CHO | $PhCH_2OH$ ($48^{B}$)<br>$PhCH_2OCOCF_3$ ($36^{B}$)<br>Ph-Me ($<16^{B}$) |
| 6 | Ph-$CH_2OH$ | Ph-$CH_2OCOCF_3$ ($90^{B}$) |
| 7 | $ClCH_2CH(OH)CH_2Cl$ | no reaction |

* Reaction conditions: 0.1 g of starting material, 0.2 ml of TES, and 2 ml of TFA were heated in an 95°C oil bath for 15 min.
A Isolated yield.
B Yields based on NMR.

SCHEME 1




Reaction conditions:
(A)$K^{18}F$/Kryptofix 222/DMSO; 5 min in a 500 Watts microwave oven (2a, 40-50%; 2b, 70%) (B) $Et_3SiH$ (50 µl), $CF_3CO_2H$ (0.5 ml), 95°C, 15 min (>95%). (C) $Br_2$/HOAc/HCl (0.2 M, 0.1 ml), EtOAc/$CHCl_3$ (1/1, 3 ml), 95°C, 7 min (>90% monobromo-, and <5% dibromo-acetophenone). (D) Concentrated HCl/MeOH (1/1, 2 ml), 110°C, 5 min (40-50% from 1b).

## N.C.A. [$^{73}$Se] LABELED COMPOUNDS: BASIC STUDIES ON A SELENATION STRATEGY VIA TRIPHENYLPHOSPHINESELENIDE USING N.C.A. SELENIUM-75

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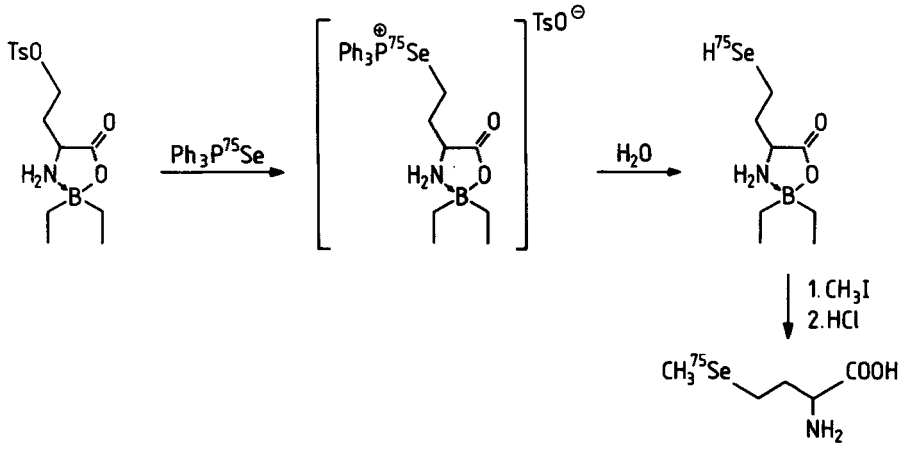
Due to its chemical properties, selenium is a suitable substitute for sulfur in thioamino acids, proteins and sulfur containing pharmaceuticals. The half-life of the positron emitter Se-73 (7.1 h) offers the possibility to label and study antibodies or other compounds with relatively slow pharmacokinetics using PET.

A new route to seleno-analogues of thioamino acids was demonstrated with selenium-75 ($T_{\frac{1}{2}}$ = 120 d). This radionuclide was generated via the $^{75}As(p,n)^{75}Se$ reaction. N.c.a. Se-75 was isolated from the arsenic target by solubilization with alkaline $H_2O_2$ and subsequent reduction with $SO_2$ and extraction with benzene [1]. In this benzene solution the elemental [$^{75}$Se]selenium was reacted with triphenylphosphine at 80°C for 30 min. The intermediate reagent [$^{75}$Se]triphenylphosphineselenide ([$^{75}$Se]$Ph_3PSe$) was prepared in near quantitative radiochemical yield of >90%. Alkylation of [$^{75}$Se]$Ph_3PSe$ and subsequent hydrolysis of the Se-alkylated phosphonium salt yields n.c.a. selenols. In a second step, the selenol can be alkylated with formation of symmetrical or unsymmetrical [$^{75}$Se]selenoethers, respectively. An example of this selenoether formation is shown in the scheme. Alkylation of [$^{75}$Se]$Ph_3PSe$ with tosyloxyethylboroxazolidone, a derivative of L-homoserine, leads to the corresponding phosphonium salt. By acidic hydrolysis the corresponding selenol is formed which is methylated with $CH_3I$ to the protected n.c.a. L-[$^{75}$Se]methionine. Finally, the boroxazolidone group is cleaved via mild acid catalyzed hydrolysis affording L-[$^{75}$Se]methionine.

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# THE USE OF ORGANOMETALLIC COMPOUNDS IN SYNTHESIS OF $^{11}$C-LABELLED AROMATICS

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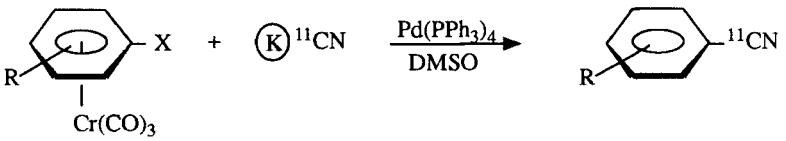
The use of organotransition metal compounds, both as stoichiometric reagents and as catalysts, has found increasing applications in organic synthesis. Various methods for carbon-carbon bond formation utilizing palladium catalyzed reactions have been developed. The cyanation of aryl halides in the presence of a catalytic amount of palladium(0) to obtain the corresponding aryl nitriles is one example.[1]

Arenetricarbonyl chromium complexes form an important class of organometallic compounds. The tricarbonylchromium moiety is strongly electron withdrawing and has an umpolung effect on the arene, resulting in enhanced reactivity towards nucleophiles and enhanced acidity of the arene hydrogens.[2] Coordination to chromium gives approximately the same activating effect as an aromatic nitro substituent, the advantage being the facility of attachment and removal of the chromium unit from aromatic systems.

It is well known that these complexes readily undergo nucleophilic displacement reactions on the aromatic ring. It has also recently been shown that $^{11}$C-labelled arylnitriles can be synthesized by displacement of fluorine with $^{11}$C-cyanide on fluoroaryl-chromiumtricarbonyl complexes.[3] We now wish to report the $^{11}$C-labelling of several aromatic model compounds by a similar nucleophilic substitution on arenetricarbonyl chromium complexes, using $^{11}$C-cyanide as nucleophile in combination with tetrakis(triphenylphosphine)-palladium(0) as catalyst, *Scheme 1.*




(1)

It is interesting to note that displacement of both fluorine, chlorine, bromine and iodine could be achieved, and that the result varied when either the arene chromium complex, the palladium catalyst or both were used in combination, see table 1. In reactions where fluorine or hydrogen acted as leaving group, no improvement was obtained by using the tetrakis(triphenylphosphine)palladium(0). When chlorine or bromine were substituted, the reactions performed in presence of the catalyst were the most succesful. In nucleophilic substitution of iodine, complexation to the chromium unit was not necessary since the aryl iodide easily reacted to aryl nitrile when the palladium catalyst was used.

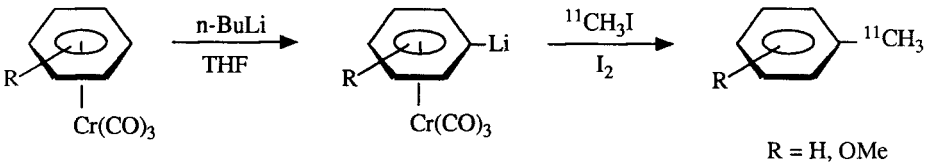
In a typical experiment, H$^{11}$CN was introduced to a solution of KOH (5-10 µmol) and 2.2.2.Kryptofix (12 µmol) in DMSO (1 ml). The K$^{11}$CN solution obtained was transferred to the palladium catalyst, immediately followed by addition of the arene chromium complex or aryl halide. The mixture was heated to 135 °C for 5-10 minutes. After cooling to room temperature the nitrile was purified by passing through a Sep-Pak® C-18 column. The product was collected and the radiochemical yields determined, see table 1. Radiochemical purity, determined by HPLC, was usually 95-100 %. Decomplexation steps to remove the chromium unit were found not to be necessary, since this occured during the reaction under the conditions used. The results are summarized in table 1.

Table 1. Labelling aryl halides and arene tricarbonyl chromium complexes with [$^{11}$C]cyanide.

| Substrate | Pd-catalyst | Reaction time(min) | Product | Radiochemical yields (%) a | b |
|---|---|---|---|---|---|
| fluorobenzene | yes | 5 | benzonitrile | 0 | -- |
| p-fluorotoluene | yes | 5 | p-tolunitrile | 0 | -- |
| chlorobenzene | yes | 5 | benzonitrile | 0 | -- |
| p-chlorotoluene | yes | 5 | p-tolunitrile | 12 | -- |
| p-bromotoluene | yes | 5 | p-tolunitrile | 90 | -- |
| iodobenzene | yes | 5 | benzonitrile | 100 | -- |
| fluorobenzene-$Cr(CO)_3$ | yes/no | 5/10 | benzonitrile | 0/74 | 62 |
| p-fluorotoluene-$Cr(CO)_3$ | yes/no | 5/10 | p-tolunitrile | 0/69 | 49 |
| m-fluoroanisole-$Cr(CO)_3$ | yes/no | 5/10 | m-cyanoanisole | 0/46 | -- |
| chlorobenzene-$Cr(CO)_3$ | yes/no | 5/10 | benzonitrile | 95/30 | 65 |
| p-chlorotoluene-$Cr(CO)_3$ | yes/no | 5/10 | p-tolunitrile | 95/50 | 74 |
| p-bromotoluene-$Cr(CO)_3$ | yes/no | 5/10 | p-tolunitrile | 89/0 | 80 |
| anisole-$Cr(CO)_3$ | yes/no | 5/10 | cyanoanisole[c] | 80/80 | -- |
| veratrole-$Cr(CO)_3$ | yes/no | 5/10 | cyanoveratrole[c] | --/80 | -- |

a) Of crude product.
b) Decay corrected yield of purified product, based on [$^{11}$C]cyanide trapped.
c) Position of cyano group not verified.

The tricarbonyl chromium moiety in arene complexes also has a strongly activating effect in reactions with n-butyllithium. This reaction is suggested to proceed via proton abstraction to yield an aryllithium tricarbonyl chromium intermediate.[4] The metalation step may be followed by reaction with carbon electrophiles to produce new alkylated complexes. In this paper the $^{11}$C-labelling of aromatic compounds is presented, using $^{11}$C-methyl iodide as electrophile in reactions with metalated arene tricarbonyl chromium complexes, *Scheme 2*.




(2)

R = H, OMe

The reaction conditions have not been optimized, but the preliminary results indicate that this method will be useful for labelling aromatic compounds.

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# SYNTHESIS OF $^{11}$C-LABELLED HALONITRILES AND EXAMPLES OF THEIR USE IN ALKYLATION REACTIONS

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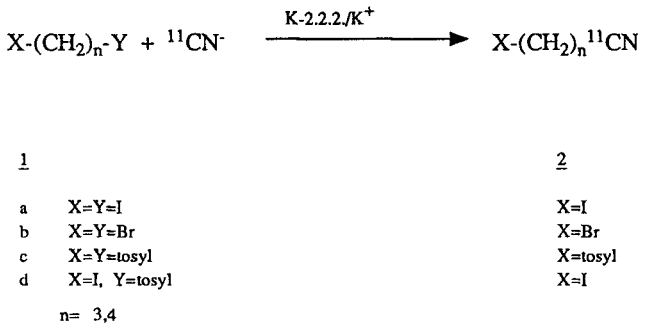
In the field of labelling synthesis with short-lived positron emitting radionuclides, access to a variety of reactive precursors is one important factor influencing the development of new compounds of potential interest for biomedical investigations using PET.
The introduction of a functional group in the labelled precursor enhances the diversity of molecular structures possible to achieve in rapid labelling synthesis.[1,2] In Scheme 1, the synthesis of $^{11}$C-labelled halonitriles are presented.




Scheme 1

Substitution reactions between K$^{11}$CN and the corresponding dibromo-, diiodo-, ditosyl-, or mixed iodotosyl compounds using kryptofix-2.2.2. as anion activating agent have been performed in different solvents. The corresponding labelled halonitriles (n=3,4) were obtained in 80-95% radiochemical yields. Interestingly, in the synthesis of bromo-, iodo- or 3-tosylpropylnitrile, the same unexpected product was obtained in 85-95% radiochemical yield, which did not correspond to the expected halonitrile. The possibility of an elimination reaction taking place to produce [$^{11}$C]acrylonitrile could be ruled out by comparing the HPLC retention times of the unknown compound with that of acrylonitrile. Work is in progress to identify the product.

As earlier presented, 4-iodobutyro[CN-$^{11}$C]nitrile has been used in an alkylation reaction on an achiral glycine derivative to produce DL-[6-$^{11}$C]lysine.[3] Work on the asymmetric synthesis of lysine using the same labelled precursor is now in progress. Other examples of alkylation reactions with these versatile difunctional $^{11}$C-labelled precursors will be presented.

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## SYNTHESIS OF [CN-$^{11}$C]ACRYLONITRILE

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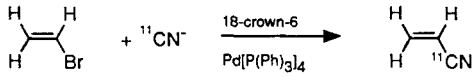
The reaction of nucleophiles with acrylonitrile is a well-known method for the introduction of a cyanoethyl group into a molecule.(1) The possibility of converting the cyano group to other functional groups is another important feature of acrylonitrile that contributes to its versatility in organic reactions. The broad spectrum of Michael addition reactions possible to perform with acrylonitrile, render labelling of acrylonitrile with $^{11}$C especially interesting as a means for increasing the available arsenal of labelled reactive precursors. Accordingly, we looked for a convenient procedure for the synthesis of [CN-$^{11}$C]acrylonitrile. In Scheme 1 the synthesis [$^{11}$C]acrylonitrile is shown.




Scheme 1

The substitution reaction between K$^{11}$CN/18-crown-6 and vinylbromide catalysed by tetrakis(triphenylphosphine)palladium (Pd$^0$-complex) has been performed in acetonitrile (MeCN), Benzene (Bz), 1,2-dichlorobenzene (*o*-DCB), tetrahydrofuran (THF) and dimethylsulfoxide (DMSO). The highest radiochemical yields were obtained in MeCN (75-85%) and *o*-DCB (50-75%), whereas the radiochemical yield in the other solvents were not higher than 50%. Rather high concentrations of vinylbromide were needed to achieve reproducible results. The oxygen sensitivity of the catalyst as well as the possibility of acrylonitrile to polymerise, are the most important factors influencing the radiochemical yield. The major by-products are probably polymers, but the formation of a complex between cyanide and palladium of a higher oxidation state can not be ruled out. No reaction took place in the absence of the palladium catalyst or anion-activating agents such as crown-ethers or cryptands.(2)

The labelled acrylonitrile has succesfully been used in Michael type addition reactions on malonic ester derivatives. Work is in progress to extend the palladium catalysed [$^{11}$C]cyanide substitution reaction to produce substituted acrylonitriles.

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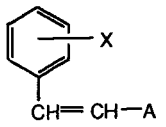
NUCLEOPHILIC AROMATIC SUBSTITUTION IN THE CINNAMALDEHYDE RING SYSTEM AS A ROUTE TO NO-CARRIER-ADDED ARYL [$^{18}$F]FLUORIDES. Y.-S. Ding, J. S. Fowler and A. P. Wolf, Brookhaven National Laboratory, Upton, New York

Within the last decade, the nucleophilic aromatic substitution reaction has been introduced and widely applied to the synthesis of NCA aryl [$^{18}$F]fluorides.[1,2] Although it is known that the minimal structural requirements for this reaction are the presence of an electron withdrawing, activating substituent as well as a leaving group on the aromatic ring, there is only limited information on the effect of other substituents on the yield of the displacement reaction with [$^{18}$F]fluoride. In the interests of maximizing the synthetic potential of this general reaction, we are investigating the effects of different substituents on the aromatic ring on the yield of the fluoride displacement reaction. For example, we have recently demonstrated that the nucleophilic aromatic substitution reaction can be effectively carried out in the presence of sterically constrained electron-donating substituents thereby making it possible to synthesize NCA fluorine-18 labeled molecules containing the catechol moiety.[3]

In this report, we have investigated the possibility of activating the aromatic ring toward nucleophilic substitution through a conjugated electron-withdrawing group.

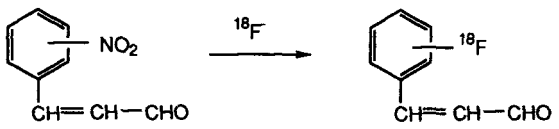



A =electron withdrawing, activating group

X = leaving group

Since the formyl group is often used as an electron withdrawing, activating substituent, we examined the reaction of [$^{18}$F]fluoride with 2 and 4-nitrocinnamaldehyde where the activating formyl group is **conjugated** to aromatic ring. This would represent a route to the [$^{18}$F]fluorophenylpropenyl substituents through appropriate synthetic manipulations of the resulting [$^{18}$F]fluorocinnamaldehydes. We have found that both 2 and 4-nitrocinnamaldehyde (**1a** and **1b**) undergo nucleophilic aromatic substitution (K[$^{18}$F]/kryptofix, DMSO, 120 °C, 10 minutes) to give 2-[$^{18}$F]fluoro or 4-[$^{18}$F]fluorocinnamaldehyde (**2a** or **2b**) in a radiochemical yield of 30-45% (**Scheme I**).

**Scheme I**

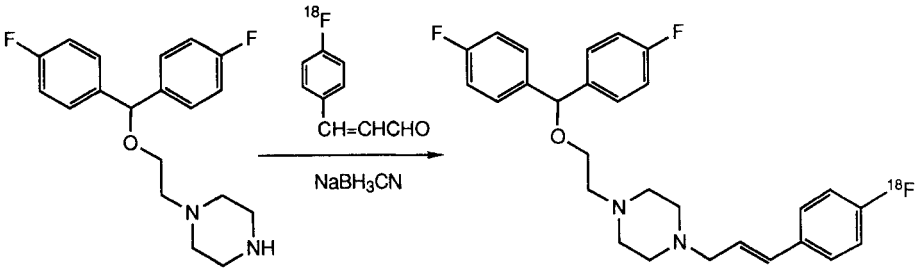



**1a** 2-$NO_2$ **2a** 2-$^{18}$F

**1b** 4-$NO_2$ **2b** 4-$^{18}$F

To demonstrate the utility of the [$^{18}$F]fluorocinnamaldehyde moiety in radiotracer synthesis, we have used it in the synthesis of [$^{18}$F]GBR 12937 (**4**), a highly specific presynaptic dopamine reuptake antagonist which has an IC50 value of 1.2 nM.[4] Reductive amination of 4-[$^{18}$F]fluorocinnamaldehyde with 1-[2-bis-(4-fluorophenyl)methoxy)ethyl]piperazine (**3**), gives [$^{18}$F]GBR 12937 in two steps (**Scheme II**).

**Scheme II**




**3** **4**

In summary, it has been demonstrated that the nucleophilic aromatic substitution reaction can be carried out on molecules in which the electron withdrawing activating substituent is conjugated to the aromatic ring. Thus the entry into other no-carrier-added fluorine-18 labeled molecules bearing the [$^{18}$F]fluorophenylpropenyl (and potentially [$^{18}$F]fluorophenylpropyl) substituent is possible. This increases the potential for preparing structurally diverse NCA fluorine-18 labeled radiotracers for PET studies and increases their potential availability from regional cyclotron centers.

This research was carried out at Brookhaven National Laboratory under contract DE-AC02-76CH00016 with the U. S. Department of Energy and supported by its Office of Health and Environmental Research and also supported by the National Institutes of Health Grant NS-15380. We also thank Novo Nordisk for an authentic sample of GBR 12937.

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## SYNTHESES OF [ω-$^{11}$C]-LABELLED FATTY ACIDS USING ALKYL HALIDES AND GRIGNARD REAGENTS.

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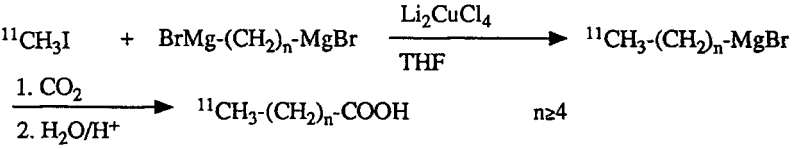
The limited availability of precursors, is a main obstacle in designing syntheses of $^{11}$C-labelled compounds. This, however, can be overcome by the development of new reactions, suitable for rapid labelling syntheses. The use of $Li_2CuCl_4$ to facilitate carbon-carbon coupling between an alkyl halide and a Grignard reagent (1) opens up new interesting pathways.The coupling method is a fast, clean and widely applicable reaction, and in this paper, its use in the syntheses of some [ω-$^{11}$C]-labelled fatty acids is reported. The syntheses are performed as shown in scheme 1.




Scheme 1.

Fatty acids labelled with $^{18}$F and $^{11}$C are ideal for studies of the metabolism of fatty acids (2,3). However, [1-$^{11}$C]-Fatty acids lose the label at an early stage in the metabolic degradation (4) and the biological properties of fluorofatty acids are not identical with natural ones(5). [ω-$^{11}$C]-Fatty acids are thus a useful complement. Access to long chain [1-$^{11}$C]-labelled alkyl halides, opens up the possibility to synthesize [m-$^{11}$C]-labelled fatty acids, were m≥6. Another interesting aspect is the possibility of obtaining deuterated fatty acids, $^{11}CD_3$-$(CD_2)_n$-COOH, which could be useful in studies of kinetic isotope effects *in vivo* using PET.

The syntheses were carried out as follows: $^{11}$C-methyl iodide was trapped in a solution of α,ω-di(bromo magnesium) alkane in tetrahydrofuran. $Li_2CuCL_4$ was added and the mixture was allowed to react for 3 min at 0 °C. A stream of $CO_2$ was introduced into the reaction mixture. The vial was heated to 80 °C until the THF had evaporated, the residue hydrolysed with HCl and the reaction mixture purified by semi-preparative LC. Confirmation of the products identity was performed by LC-analysis of the p-bromo fenacyl derivative (6).
[6-$^{11}$C]-hexanoic acid, [8-$^{11}$C]-octanoic acid and [10-$^{11}$C]-decanoic acid were synthesized with similar results: decay corrected radiochemical yields 20-30%, synthesis times 30-35 min.,

radiochemical purities >99%. However [6-$^{11}$C]-hexanoic acid, is the shortest fatty acid possible to be synthesized by this method.

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## SYNTHESIS OF FLUORINE-18 LABELED 1,1-DIFLUORO-2,2-DICHLOROETHYL ARYL ETHERS BY ISOTOPIC EXCHANGE

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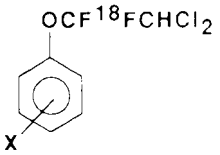
Isotopic exchange reactions involving fluorine-18 produce carrier-added radiolabeled compounds, and this method has been applied to the synthesis of alkyl and aryl fluorides, and trifluoromethyl compounds. As part of an effort to examine the reactivities of 1,1-difluoro-2,2-dichloroethyl aryl ethers, we report here the unexpected facile 18F-for-19F isotopic exchange in these compounds.

Several 1,1-difluoro-2,2-dichloroethyl aryl ethers (**1** - **4**, Table) with para- or meta- ring substituents were prepared by the condensation of the corresponding sodium phenolate with 1,1-difluoro-2,2-dichloroethylene under phase transfer conditions (1). No-carrier-added [18F]fluoride, as the K+/Kryptofix 222 salt, was prepared by standard methods. Isotopic exchange reactions were conducted in sealed vessels under a variety of conditions (see Table). Reaction workup consisted of partitioning between water and diethyl ether, and the organic layer then analyzed by TLC (silica gel) or HPLC (C18 reverse phase, $CH_3CN$/aq. $NH_4OAc$). In all cases a single radioactive product was observed. Yields in the Table are for isolated products, decay-corrected. Specific activities ranged from 0.15 to 150 Ci/mmol; neither the yields or specific activities have been optimized. The reaction products have been assigned as arising from isotopic exchange based on the following conclusions. First, ring substitution did not occur,due to the isolation of a product from the unsubstituted parent compound (**1**), as well as no formation of the 4-[18F]fluoro derivative (**2**) from substitution of the nitro analog (**4**). (A corollary of this is that the ether substituent is not activating, and perhaps deactivating, of the ring towards nucleophilic substitutions). Substitution of the fluorines is supported by the chromatographic data (products identical to starting material) and the chemical literature, where reactions of 1,1-difluoro-2,2-dichloroethyl ethers with nucleophiles (bromide, ethoxide) yields products arising from fluoride, but not chloride, displacement (2,3).

TABLE

| | X | conc (mM) | solvent (100 µl) | T °C | time (min) | yield (%) |
|---|---|---|---|---|---|---|
| 1 | H | 7 | DMSO | 155 | 30 | 45 |
| 2 | 4-F | 7 | DMSO | 155 | 30 | 25 |
| 3 | 3-$NO_2$ | 0.066 | DMSO | 155 | 30 | 31 |
| | | 6.6 | $CH_3CN$ | 100 | 30 | 70 |
| | | 66 | 20% $H_2O$ in DMSO | 155 | 75 | 47 |
| 4 | 4-$NO_2$ | 6.6 | DMSO | 155 | 15 | 34 |
| | | 66 | DMSO | 155 | 30 | 55 |




Applications of these compounds in radiopharmaceutical chemistry are currently being investigated. The biological stability of these ethers has been examined by the injection of compound **3** into rats and examination of bone levels of fluorine-18; radioactivity in bone was low at 1 h (0.13% ID/g) and decreased with time (.096% ID/g at 4 h). Thus, these compounds appear biologically stable with respect to loss of [18F]fluoride ion.

Acknowledgements. This work was supported in part by DOE grant DE-AC02-76ER02031 and National Institutes of Health grant T32-CA09015 (to R.S.).

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The Use of [F-18]4-Fluorobenzyl Iodide As A Prosthetic Group For Introducing F-18 Into Pharmacologically Active Molecules: N-Alkylation of Amines and Amides.

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Fluorine-18 ($^{18}F$) is a positron-emitting radioisotope that is gaining an increasing recognition since its relatively long half-life (110 min) places less time restraints on both radiopharmaceutical production and image acquisition. Unfortunately, the difficulties associated with incorporating high specific activity $^{18}F$ into pharmacologically active molecules has limited the rate of development of $^{18}F$-labeled radiotracers. This work describes the use of [$^{18}F$]4-fluorobenzyl iodide ([$^{18}F$]FBI) as a prosthetic group for introducing $^{18}F$ via N-alkylation of both amines and amides. Previous studies with [$^{18}F$]N-(3-fluoropropyl)spiperone and [$^{18}F$]N-(2-fluoroethyl)spiperone have shown these fluoroalkyl analogs to undergo metabolic transformation to form either $^{18}F$-fluoride (fluoropropyl) or a metabolite capable of crossing the blood-brain-barrier (fluoroethyl) (1). N-dealkylation of the N-(4-fluorobenzyl) group results in the formation of [$^{18}F$]4-fluorobenzoic acid, which should undergo elimination either directly or as an amino acid or glucuronide conjugate. Furthermore, the $^{18}F$ radiolabel is attached to an $sp^2$ (aromatic) carbon and is expected to be more stable with respect to carbon-fluorine bond cleavage.

[$^{18}F$]FBI was prepared in a three step sequence that began with the nucleophilic displacement of 4-nitrobenzaldehyde with [$^{18}F$]CsF in aqueous DMSO (150°C/40 min) to give [$^{18}F$]4-fluorobenzaldehyde, **1** (40-60%). Reduction of **1** with LAH in THF (RT/3 min) followed by treatment of the crude alcohol with 47% HI (80°C/3 min) gave [$^{18}F$]FBI in moderate yield (50-80% from **1**). Model studies were conducted in order to determine the reaction conditions required to alkylate nitrogen-containing substrates with varying degrees of nucleophilicity (Table). N-alkylation of secondary amines generally took place under relatively mild conditions (entries 1-3) whereas the less-reactive aniline derivative (entry 4) required an elevated temperature. N-alkylation of amides (entries 5 and 6) required the use of tetraethylammonium hydroxide as a base catalyst; [$^{18}F$]FBI appears to be sensitive toward hydrolysis since [$^{18}F$]4-fluorobenzyl alcohol was observed as a major by-product in entries 5 and 6.

**TABLE.** Model Alkylation Study Using [$^{18}F$]FBI.

| Entry | Substrate | Conditions | yield[a,b] |
|---|---|---|---|
| 1 | 1-phenyl-1,3,8-triaza-spiro[4.5]decan-4-one (**2**) | 2 mg/600 µL $CH_3CN$/DMF (5:1)/RT/5 min | >90% |
| 2 | m-trifluoromethyl-phenylpiperazine | 0.5 mg/300 µL $CH_3CN$/ RT/ 5 min | >99% |
| 3 | N-(piperidin-4-yl)-4-amino-2-methoxybenzamide | 0.5 mg/200µL $CH_3CN$/DMF (3:1)/RT/15 min | >90% |
| 4 | N-methylaniline | 1 mg/200 µL $CH_3CN$/DMF (1:1)/sealed vial/100°C | >99% |
| 5 | 8-N-t-butyloxycarbonyl-**2** | 2 mg/300 µL $CH_3CN$ 10 µL TEOH/RT/5 min[c] | 53-79% |
| 6 | spiperone | 2 mg/300 µL $CH_3CN$ 10 µL TEOH/RT/5 min[c] | 25-66% |

[a]Radiochemical yield as determined by normal phase HPLC; [b]$n \geq 3$; [c]TBOH gave consistently lower yields.

In vitro binding studies have shown N-(4-fluorobenzyl)spiperone (product of entry 6) to possess both a high affinity for the dopamine D2 receptor (Ki vs [$^{3}$H]spiperone at D2 sites = 0.04 nM) and low affinity for the serotonin 5-HT2 receptor (Ki vs [$^{125}$I]I-LSD at 5-HT2 sites = 25 nM). This data supports the use of $^{18}$F-FBI as a means of incorporating $^{18}$F into a pharmacologically active molecule possessing an N-atom with a certain degree of bulk-tolerance.

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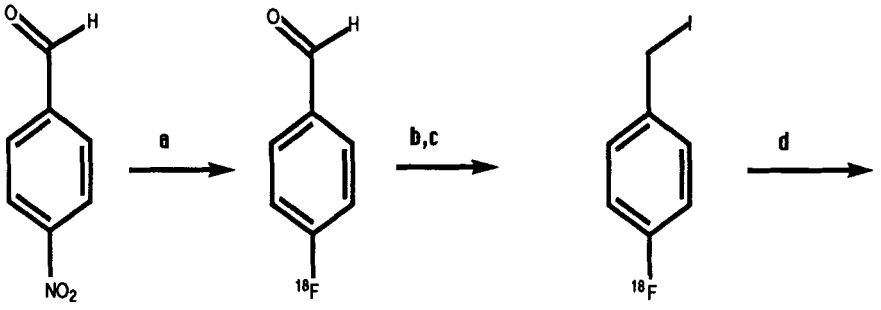
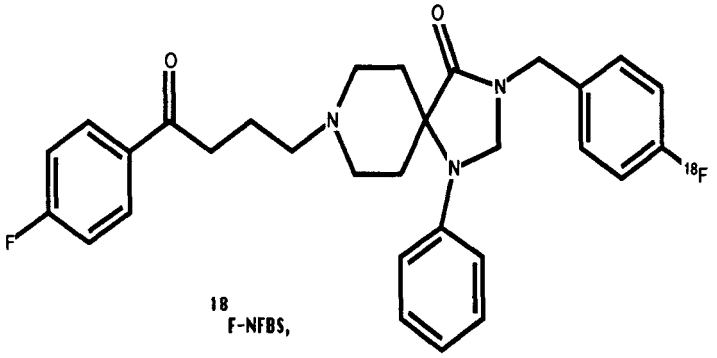
## Synthesis of [$^{18}$F]FBI and Alkylation of Spiperone[a]




1 ["F]FBI




$^{18}$F-NFBS,

[a]Reagents: a: Cs$^{18}$F/DMSO; b: $LiAlH_4$/THF; c: 47% HI; d: TEOH/$CH_3CN$/Spiperone

**In Vitro Binding Data**
**Ki Values [nM][a]**

| | $D_2$ [$^{3}$H]spiperone | 5-$HT_2$ [$^{125}$]I-LSD | 5-$HT_2$:D2 Ratio |
|---|---|---|---|
| spiperone | 0.07 | 0.40 | 5.70 |
| NMSP | 0.12 | 0.64 | 5.30 |
| [$^{19}$F]-NFBS | 0.04 | 24.7 | 617.5[b] |

[a]n = 4; [b]indicates [$^{18}$F]-NFBS will exhibit a >600-fold higher selectivity for the $D_2$ receptor vs the 5-$HT_2$ receptor as compared with 5.30 for NMSP.

## SYSTEMATIC STUDIES FOR THE OPTIMIZATION OF [$^{11}$C]-HCN PRODUCTION

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The parameters for routine production of [$^{11}$C]-HCN via the $^{14}N(p,\alpha)^{11}C$ nuclear reaction in a Nitrogen/Hydrogen gas-target have been investigated and optimized. Factors like beam current dependent in-target Ammonia formation and product composition in relation to impurities have been evaluated.

$^{11}$C was produced with our MC 35 Cyclotron by the $^{14}N(p,\alpha)^{11}C$ reaction, bombarding a 95%$N_2$/5% $H_2$ gas-target with 16 MeV protons. Beam current and irradiation times were varied from 5-40 μA and 5-30 min respectively. Two targets with different dimensions have been used.

One target was of rectangular shape with ca. 1500 ml STP inventory. It was operated at 6 bar, yielding a gas inventory of ca 10,000 ml STP. During the course of the investigations a cylindrical small Nickel target was designed with 1.6 cm diameter and a length of 16 cm (ca 65 ml STP), which was operated at 20 bar, yielding a gas inventory of 1300 ml STP.

The target-gas flow was varied from 100 - 600 ml/min, the actual Platinum catalyst temperature was measured inside the conversion tube under flow conditions and was varied from 600 to 1100 $^{o}$C.

Gas samples were collected before and after passage of the platinum contact and assayed for radioactivity and chemical composition, using ionization chamber type isotope calibrators, NaI scintillation wellcounters and radio-gas-chromatography. Inactive Ammonia was assayed by titration. The Ammonia concentration was varied by mixing additional ammonia to the target gas before entering the PT-catalyst from 0.1 - 5 vol%. The [$^{11}$C]-HCN was trapped in 5 ml 0.1n NaOH and identified by precipitation as [$^{11}$C]-AgCN and redissolution as [$^{11}$C]-$Ag(CN_2)^-$ under carrier addition. It was found that with the small target in-target Ammonia formation is sufficient for catalytic conversion of radiolytically formed [$^{11}$C]-$CH_4$ to [$^{11}$C]-HCN.

Oxygen impurities in the target gas in combination with insufficient ammonia concentrations lead to a nearly quantitative catalytic conversion of [$^{11}$C]-$CH_4$ to [$^{11}$C]-CO.

At low $NH_3$ concentrations the methane was oxidized to Carbonmonoxide nearly quantitatively. In order to account for the large amounts of [$^{11}$C]-CO in the system effluent, the

Oxygen source was searched for. The Oxygen concentration in the target gas mixture (5% $H_2$ , 95% $N_2$ (grade 99.9995) was verified to be smaller than 1 ppm. The use of oxygen absorbers like Oxysorb® did not reduce the [$^{11}$C]-CO yields. Different $NH_3$ gas flasks were analyzed and somewhere found to contain relative large amounts of Oxygen up to 1%. The $NH_3$ could not be purified from oxygen by Oxysorb® because of a reaction of the absorber with $NH_3$ . Only a special research grade $NH_3$ (also available as semiconductor production grade) met specifications of less than 100 ppm $O_2$.

It was noted that low flow rates did not increase the yield, as would have been predicted from the prolonged catalyst contact time. However, it was found, that the Ammonia decomposes with increased contact time and with increasing temperature. Therefore a higher ammonia concentration seems to be favourable for low flow rates.

The specific activity of [$^{11}$C]-HCN was determined by Merk-Aquaquant® Cyanide Test (0.03-0.7 ppm).

Absolute yields of 48.5 GBq [$^{11}$C]-HCN with a specific activity of 70 GBq/µMol are produced in 30 min / 40 µA routine production runs.

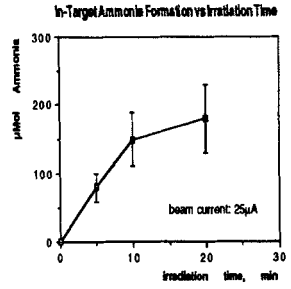


Fig. 1 In-target ammonia formation vs irradiation time

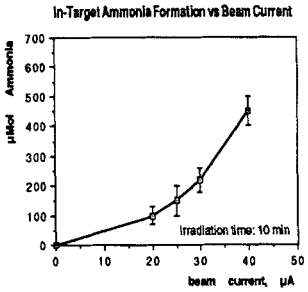


Fig. 2 In-target ammonia formation vs beam current

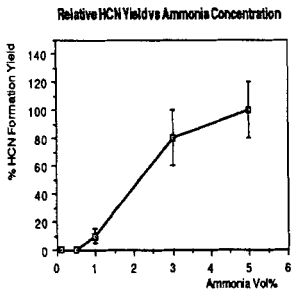


Fig. 3 Relative HCN yield vs ammonia concentration

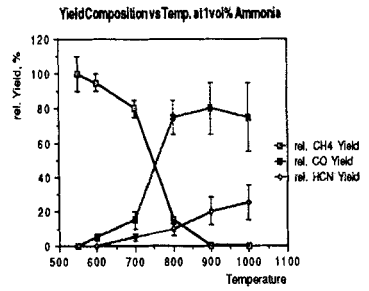


Fig 4. Yield composition vs catalyst oven temperature at 1vol% $NH_3$

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OPTIMIZATION OF CARBON-11 CARBON DIOXIDE TRAPPING FROM NITROGEN GAS STREAMS.

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An inexpensive, efficient, rapid and compact trapping and storage system has been developed for trapping high specific activity $^{11}C$-$CO_2$ from high flow nitrogen gas streams. Carbon-11 is routinely produced by the batch irradiation of nitrogen (UHP) via the $^{14}N(p,alpha)^{11}C$ reaction. The 1-2 ppm trace amounts of oxygen in the target gas are radiolytically reacted to produce $^{11}C$-$CO_2$, an important precursor for the synthesis of many $^{11}C$-radiopharmaceuticals. Traditionally the trapping of $^{11}C$-$CO_2$ has been accomplished using 3-5 m coiled lengths of 1/8" OD copper tubing at liquid nitrogen temperatures. The large size of this system places demands on the limited Hot Cell/ Fume Hood space available at most laboratories and makes the radiation shielding of the trap difficult. The requirement for heating the trap to 100° C to efficiently release the $^{11}C$-$CO_2$ increases the complexity and may add additional manipulations to the chemistry procedure.

Trapping efficiencies at gas flow rates ranging from 400 to 1000 sccm have been measured using three trapping systems. All three trapping systems are fabricated entirely from 316 stainless steel components. This permits easy and convenient maintenance and cleaning of systems used for high specific activity chemical synthesis. Recovery of $CO_2$ from these traps does not require warming to temperatures greater than 25° C thereby simplifying the processing.

Lengths of 1.6 m, 2.6 m, and 4.8 m 1/8" OD tubing coiled in 3, 5, and 9 53 cm loops were half submerged in liquid nitrogen. $^{11}C$-$CO_2$ trapping efficiencies (corrected to EOB) were 77.8%, 86.9% and 90.8% respectively. In an attempt to increase the active trapping surface area and reduce the overall trap size a 7.6 cm long by 1.27 cm OD cartridge was packed with .24 cm stainless steel shot. Trapping efficiencies for the shot system were 99.0%. A reduction in size was further achieved by packing a 5.0 cm long by 1.9 cm OD cartridge with 8 .55 cm OD by .175 cm thick 20 u pore sintered stainless steel frits. No restriction to gas flow through the system was observed at flow rates up to 1000 sccm. The measured trapping efficiency for the sintered stainless steel trap was 95.4 %. Using all three trapping methods the $^{11}C$-$CO_2$ activity was available for chemistry procedure 8-15 minutes post-EOB. A further reduction to a 5 minute post-EOB delivery time is very possible.

This work is supported by NIH grant # 14867.

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## COMPUTER-ASSISTED PREPARATION AND USER PROGRAMMABLE UTILIZATION OF [$^{11}$C]$CH_3I$

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A remotly operated free programmable instrument for the conversion of [$^{11}$C]$CO_2$ to [$^{11}$C]$CH_3I$ has been developed. Various labelling procedures incorporating the [$^{11}$C]$CH_3$- residue into a target molecule are easily programmed by the user and stored as method files in the controller. Signal output has been provided for starting a subsequent HPLC-method for purification and quantitation of the final product.

The apparatus is based on an improved procedure of our previously described synthesis of [$^{11}$C]$CH_3I$ using $P_2I_4$ for the conversion of methanol to methyl iodide (1). The complete system accommodates the most common labelling reactions using [$^{11}$C]$CH_3I$, such as the synthesis of the >N-[$^{11}$C]$CH_3$ structure units, and the formation of [$^{11}$C]$CH_3$–C- bonds starting from carbanions. Typical procedures were run for those molecules that offered importance in clinical oncology, e.g. α-[$^{11}$C]methyl alanine, α-methyl-N-[$^{11}$C]methyl alanine or $^{11}$C-labelled methionine.

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## AN AUTOMATED LABELLED COMPOUNDS PRODUCTION SYSTEM FOR POSITRON EMISSION TOMOGRAPHY.

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The growing use of cyclotron produced radiopharmaceuticals in hospitals, particularly those used in Positron Emission Tomography, has increased the demand for radiopharmaceutical agents labeled in a short time. Nowadays user's requirements are high and reproducible labelling reactions yields, low operation costs and flexibility.

A complete automated system, controlling the production from target filling or pressurizing to radiopharmaceutical packaging has been developed. The control system is based on an industrial programmable logic controller (SIEMENS 135 U), and enables different syntheses to be processed at the same time. The same system is used for PET production with two of IBA's cyclotrons, CYCLONE 30 and CYCLONE 10/5. CYCLONE 30 is a 15 to 30 MeV protons machine with dual beam extraction and up to ten target ports. CYCLONE 10/5 is a 10 MeV protons, 5 MeV deuterons machine with dual extraction beam, up to eigth target ports and self-shielded in option.

Targetry.
A complete set of targets produces the four classical PET isotopes in the appropriate chemical forms. All targets, wich can be installed on CYCLONE 30 as well as on CYCLONE 10/5, have been designed to be compatible with the same window system, and are thus fully interchangeable and adaptable to user needs. Experiments at 10 MeV have been performed since september 1989. Production yields are in agreement with those published and obtained on similar machines. For instance at 10 MeV,
(i). 700 mCi of $^{18}F$ - $F^-$ are currently produced (300 µl $^{18}O$ water, $^{18}O(p,n)$, silver body target, 25 µm Havar foil, 25 µA, 1 hr irradiation).
(ii). 100 mCi of $^{13}N$ - Ammonia, wich are more than sufficient for myocardium PET studies, are directly produced in a hydrogen pressurized water target (1) (1.5 ml water, $^{16}O(p, \alpha)$, 25 µm Havar foil, 25 µA, 20' irradiation).

Chemistry.
A complete set of chemistry modules achieve then the different syntheses and produce the radiopharmaceuticals. The only operator interaction necessary with the modules consists in the replacement of the vials (max. 6) from the previous synthesis by the vials containing the fresh sterile solutions. This user-friendly design does not require highly skilled specialists for the routine production of radiopharmaceuticals in a normal hospital environment. Some modules (e.g. the FDG module) can be operated manually or be used for other syntheses (e.g. FESP) or for new developments. Almost every module has a special wash-up program, which rinces and dries the tubing and valves, to recondition the module for the next production cycle.

Our center daily produces : $^{15}O$ - $H_2O$, $^{15}O$ - $O_2$, $^{11}C$ - Acetate, $^{13}N$ - Ammonia, $^{11}C$ - HCN (Thymidine) (2) and $^{18}F$ - 2-fluoro-2-deoxy-d-glucose (3), each of these syntheses being performed automatically by a dedicated module. We are also able to supply automatically $^{11}C$ - $CH_3I$, $^{15}O$ - CO, $^{15}O$ - $CO_2$, $^{11}C$ - CO, $^{11}C$ - $CO_2$ and in the near future F-Dopa.

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A ROBOT-ASSISTED SYNTHESIS SYSTEM APPLIED TO $^{11}$C-ALKYLATIONS

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A robot-based system for the production of positron-emitting radiopharmaceuticals has been developed. The system consists of a 7-axis SCARA robot (from Universal Machine Intelligence), supporting equipment, a synthesis module and a PC-AT personal computer as a system controller. The synthesis module may be dedicated to one or several of the same or different types of syntheses The equipment is, for convenience, located on a removable tray, so that it may easily be withdrawn from the hot cell and serviced or replaced with a new one. The supporting equipment, common for many syntheses, does not necessarily have to be removable and may be installed separately in the hot cell.

The Multifunction Editor (MFE) is a development as well as a production tool for building and interpreting control sequences for the system. The program recognizes commands to move the robot, to control valves and to control the computer screen. There are also general commands like wait for a specified time or wait for specified event to occur. The event wait commands are usually related to some sensor input, such as wait for a radiation detector to reach a maximum or until it exceeds a certain value.

When programming the system the robot is guided through the motions required for the synthesis. Manual control of the robot can be accomplished in different ways: by moving the mouse, from the keyboard or from a remote control box. Storing representative robot positions in a command sequence makes it possible to recall and repeat the motion.

With the program in the editing mode it is possible to run command sequences forward or backward, multiple or single steps one at a time, in order to facilitate debugging the control process. The editor commands include insert, replace and delete of commands in the sequence.

The screen control is used to display dynamic flowchart diagrams in the production mode. Thus. the picture on the screen is modified and up-dated to indicate significant changes in the state of the system.

The user interface of the Multifunction Editor has been based on mouse activated pulldown windows to provide a user-friendly mode of operation (Figure 1). Help information is also available in most situations.

The reproducibility of the robot arm has been found to be sufficient for its use in $^{11}$C-radiochemistry. The placement of the apparatus in a hot cell (dimensions: height= 1.3 m, width= 1.1 m, depth= 1.1 m) is shown in Figure 2. The first system tested here , the production of and alkylations with $^{11}$C-methyl iodide, is easily extendable to other alkyl halides using the same synthesis module. Work is continuing on the development of synthesis modules for other types of $^{11}$C- and $^{18}$F-radiochemistry.

Figure 1:

PC DISPLAY OF MFE FUNCTIONS

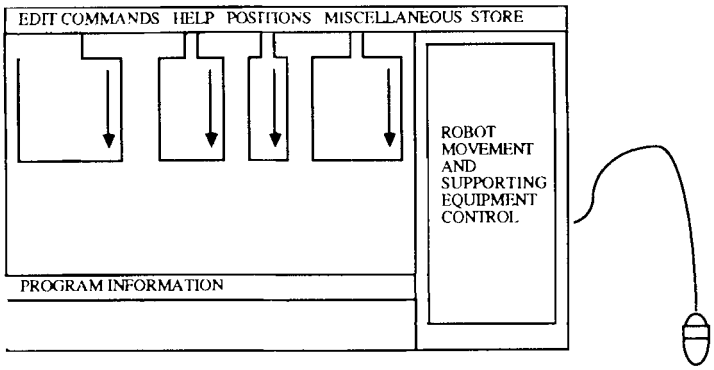
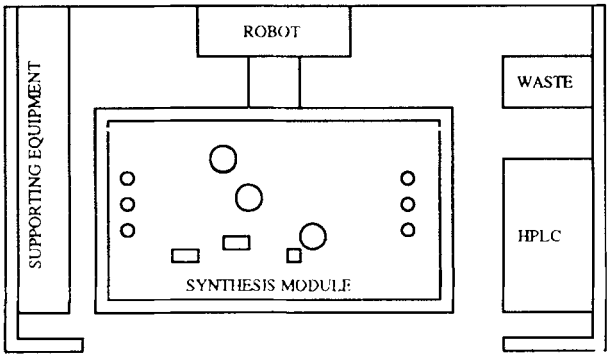



Figure 2:

ARRANGEMENT OF ROBOT-ASSISTED SYNTHESIS SYSTEM IN A HOT CELL



AN OPERATING ENVIRONMENT WITH MULTITASKING CAPABILITIES FOR RADIOCHEMISTRY AUTOSYNTHESIZERS. Anthony L. Feliu, Dept of Neurology, Memorial Sloan-Kettering Cancer Center, New York, NY. Present address: 231 River Avenue, Patchogue, NY 11772.

The proliferation of positron emission tomography centers during recent years has stimulated intensive efforts to develop reliable and efficient devices for automating radiopharmaceutical syntheses. Notwithstanding, most routine production is still performed with remotely- or manually-operated apparatus.

In order to expedite the design and operation of fully automated synthesizers, a graphical process control scheme featuring a simple method for the end-user to reconfigure the software was recently suggested (1). The flexibility and user-friendliness of this scheme were demonstrated with an application package to operate a commercially-available autosynthesizer (2). This methodology is now extended to include multitasking capabilities.

Given the variety of radiotracers of current clinical interest, a staff of only one or two may be called upon to oversee multiple syntheses. In principle, these syntheses could be controlled individually by low-end PCs. However, tending several terminals would require more operator effort than if all synthetic operations were consolidated under a single console. To evaluate the multitasking concept, the demonstration program AUTOMATE (3) was created to simulate independent operation (cf 1) of four autosynthesizers (figure 1). With AUTOMATE, a chemist could easily supervise one routine synthesis while "warming up" another synthesizer or conducting a research experiment. The graphical approach provides the simplicity of manual control, instant status information, and a choice between "hands-on" or unattended operations. The potential utility of AUTOMATE in the laboratory is discussed in figure 2.

The AUTOMATE multitasking system for radiochemistry autosynthesizers is fully compatible with standard 80x86-based PCs running under MS-DOS. It includes algorithms for isolating multiple synthetic tasks from one another, implementing foreground/background processing, maintaining system integrity after hardware and software errors, and providing "virtual" video consoles for each synthesizer. Key to the AUTOMATE multitasking scheme is a task management module which receives all user input and which coordinates execution of the various syntheses. The supporting system architecture is outlined in figure 3.

The multitasking and graphical display concepts described for radiochemistry autosynthesizers may also have benefits in designing user-friendly programs for cyclotron/targetry control, QA/QC functions, and laboratory information management. This possibility is under investigation as an application package for Microsoft Windows. Given this graphics-oriented operating system, the autosynthesizer control program can offer chemists Macintosh-HyperCard style capabilities for customizing the user interface. A brief progress report will be presented.

A sophisticated process-control interface can render mysterious "black boxes" in a chemist-familiar object-oriented way, lending convenience and reliability to both routine and research applications. The techniques developed for AUTOMATE demonstrate that a computer-controlled synthesis system is available to any radiochemistry laboratory given modest investment of programming effort.

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3. Program AUTOMATE was written in Microsoft QuickBASIC. As a fully-functional simulation, AUTOMATE lacks only program code necessary to communicate with a hardware I/O card. A color graphics adapter is required to support text-mode video paging. Color output is used to reduce screen clutter.

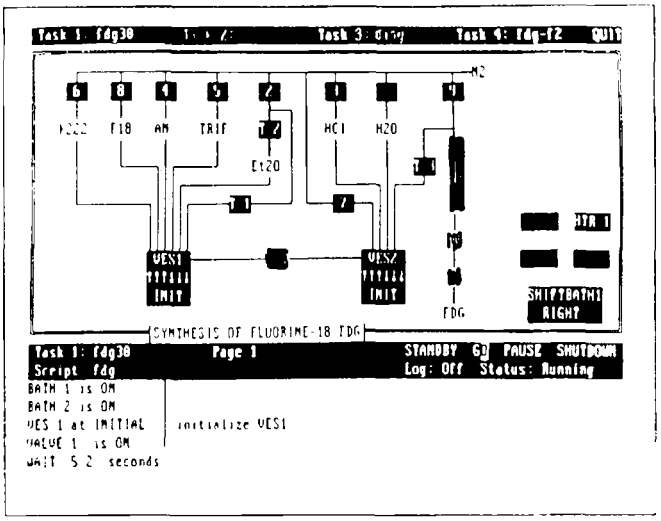


**Figure 1:** The AUTOMATE user interface is organized into four subunits.

At top, the TASK BAR continuously displays the names of the configurations corresponding to each of four independent synthesizers. Color codes signal idle, running, and error conditions thereby providing continuous status information on background tasks.

Each synthesizer has a FLOWCHART window, CONTROL PANEL and MESSAGE window. User-created flowcharts and configuration files are loaded/unloaded by clicking "Task" on the control panel. Other mouse-activated functions on the control panel direct totally automated synthetic runs.

Devices are represented on screen as rectangular icons and may be actuated manually by clicking the mouse. This arrangement obviates the need for confusing switch panels common to traditional synthesizers.

Text-mode display screens for each synthesizer are maintained in separate pages of video RAM, giving the appearance of multiple dedicated PCs. In this figure, synthesis 1 is currently visible, but any other may be called into the foreground (by clicking its name on the task bar) without disturbing automated runs.

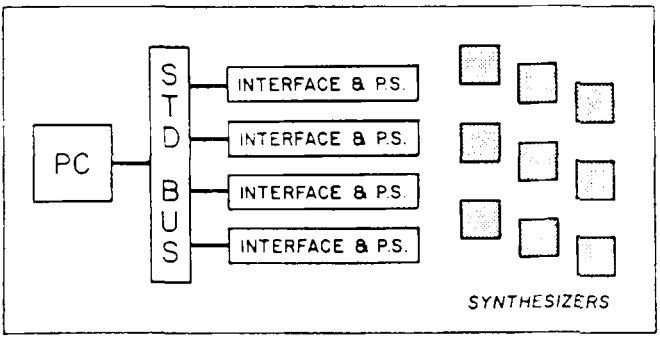


**Figure 2:** A radiochemistry laboratory using AUTOMATE might, for example, have one PC connected via a "bus" to four interface/power supply modules. Autosynthesizers would be constructed from standardized components such that any autosynthesizer could be "plugged in" to any available interface module. After plugging in a synthesizer, configuration files (created by the chemist and saved on disk) could be loaded into AUTOMATE thereby creating an optimized user interface for that application. The proposed interchangeability concept would reduce capital costs compared to the one-PC-per-synthesizer approach. Moreover, any combination of radiosyntheses, even duplicates, could be performed depending on which autosynthesizers were on hand.

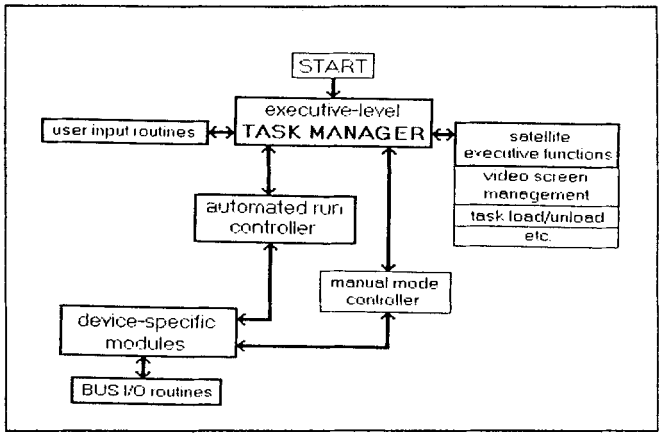


**Figure 3:** Flowchart summarizing calling relationships between various routines in AUTOMATE.

The START module initializes the mouse, video card, and key variables. The executive-level TASK MANAGER is a program loop which processes user input, manages screen I/O, and generally coordinates processing. The task manager passes execution to the four independent synthesizers in modified "round robin" fashion, using a priority scheme coordinated by task- and device-status variables. DEVICE-SPECIFIC MODULES are responsible for hardware control.

Processes relinquish control to the task manager immediately after each operation, thereby guaranteeing both foreground and background processes execution time without employing interrupts.

COMPUTER-CONTROLLED SYNTHESIS OF OXYGEN-15 BUTANOL AND WATER: AUTOMATED PRODUCTION AND DISPENSING SYSTEMS

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Oxygen-15 labeled water is the most widely used tracer for measuring regional cerebral blood flow (rCBF) using positron emission tomographic (PET) techniques. Studies comparing the extraction of a series of carbon-11 labeled alcohols and oxygen-15 labeled water by the brain during a single capillary transit show that only approximately 93% of an injected bolus of water is extracted into the brain when compared to the alcohols isopropanol and butanol which exhibit 99% and 98% extraction in the brain respectively (1-3). Additional studies comparing the behavior of oxygen-15 labeled water and carbon-11 labeled butanol demonstrated that measurements with oxygen-15 labeled water overestimated and underestimated rCBF in regions of low and high blood flow respectively, whereas with carbon-11 butanol brain permeability was shown to be unchanged over a range of blood flows (3). Therefore, carbon-11 was proposed to be the "gold standard" for measuring rCBF. Since clinical protocols for evaluating rCBF often require several successive administrations of the rCBF tracer within short time intervals and because of radiation dose considerations, syntheses employing organoborane chemistry were developed for the preparation of oxygen-15 labeled butanol (4-7) for clinical use.

We wish to report the development of computer controlled units for the automated continuous production and dispensing of oxygen-15 labeled butanol and water. The systems use a 8085 microprocessor system developed at UTMCK and a RTX286 operating system provided with the RDS 112 11 MeV negative ion cyclotron (Cyclotron Systems, Inc.). Oxygen-15 labeled oxygen gas was produced in a continuous mode in a 10 mL volume target pressurized to 200 psi followed by proton bombardment of a mixture of 99% $^{15}N_2$ (99.999% enriched) and 1% $O_2$. A menu oriented command file for oxygen-15 is set up which automatically loads and irradiates the target, produces an O-15 bolus of approximately 80% of the target contents for delivery to either the O-15 butanol (figure 1) or the O-15 water production system, then reloads the target and continues O-15 oxygen production. As many as 40 boluses may be produced with this command file.

The O-15 butanol system is shown in figure 1. It is comprised of two linked motor driven Hamilton 6-way distribution valves, five alumina Sep Paks preloaded with 0.2 mmole neat tributylborane, a series of reagent reservoirs, three 3-way solenoid valves, two C-18 purification sep paks and a filter sterilization unit. Solution addition and transfer are achieved through narrow teflon tubing by the application of nitrogen pressure.

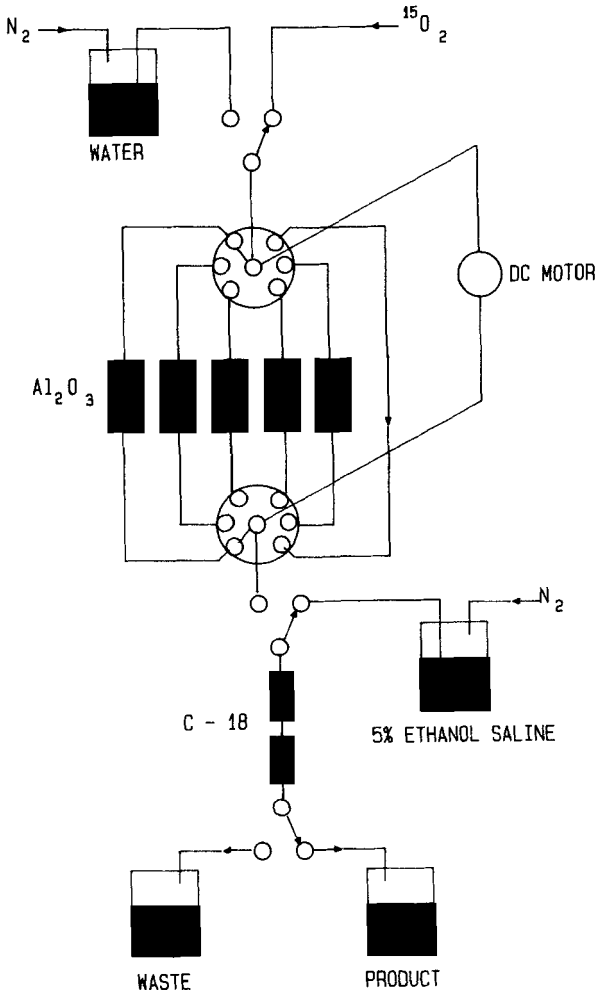
The O-15 water system consists of a precision two unit rotameter to monitor and mix hydrogen and air, a temperature controller, a catalytic converter comprised of a heated stainless steel HPLC column containing palladium-coated alumina pellets and a filter sterilization unit.

The O-15 oxygen is delivered in a transit time of 20 seconds to the modular systems which

are housed in a hot cell located 20 feet from the scanner. The products are assayed in a dose calibrator located in the hot cell and the dose is drawn into a syringe in the hot cell for safe and rapid delivery to the patient. Our experience using these systems will be presented.

This research was funded by NIH under contract GM-39081.

Figure 1. The O-15 Butanol System



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Simultaneous Production of Several PET Radiopharmaceuticals From a Single Laboratory Robot.

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The use of an automated device for the routine production of positron-emitting radiopharmaceuticals for PET is an ideal method of obtaining reproducible yields and significantly reducing radiation dose to personnel. Numerous devices have been described for the synthesis of a wide variety of PET radiopharmaceuticals with the synthesis of 2-[$^{18}$F]fluoro-2-deoxy-D-glucose (FDG) being the most automated. Both electrophilic and nucleophilic reactions have been utilized in the design of automated systems used to prepare FDG.[1,2]

In all of these devices, specific features have been incorporated to enhance the system's function and to make it more "user friendly". Some of the automated systems are capable of implementing a cleanup procedure after each synthesis in order to set itself up for another run.[3] Others, through the use of modular components, can be reconfigured to synthesize a different compound after the appropriate reagents and software have been installed.[4] In all of these cases, however, the automated device is programmed to synthesize only one compound at a time. This feature, while adequate for facilities with a single PET camera, can create scheduling problems for institutions with several PET scanners that require different complex radiopharmaceuticals at the same time. Additional automated devices could be built to make more compounds available, but budget constraints and limited laboratory space can restrict the number of such devices that could be implemented.

In recent years, our research has been to investigate the use of robotics for the automated synthesis of complex radiopharmaceuticals for PET studies. From the synthesis of the complex radiopharmaceutical 16$\alpha$-[$^{18}$F]fluoroestradiol-17$\beta$[5] to the synthesis of several different compounds using the same apparatus,[6] we have shown the versatility and flexibility that a laboratory robot has towards the automation of these types of compounds. In the past, however, the robot was programmed to prepare only one compound at a time, a task which was then suitable to the demands of our PET facility. With more compounds now being requested in addition to an expanding PET facility, scheduling conflicts arise when more than one automated synthesis needs to be performed at the same time.

To expand upon the robot's capabilities, we have investigated methods that would allow the robot to prepare more than one PET radiopharmaceutical simultaneously. Through the modification of existing software plus the addition of a Zymate® Concurrent EasyLab Module, we now report the ability of a single laboratory robot to prepare the following two compounds simultaneously: 16$\alpha$-[$^{18}$F]fluoroestradiol-17$\beta$ and 2-[$^{18}$F]fluoro-2-deoxy-D-glucose. As indicated in Scheme 1, the merging of the separate synthesis programs into one larger one was readily achieved by comparing the similarities and differences between the two programs and adjusting the final program accordingly. For example, since each

of the separate syntheses start with a similar resolubilization routine, the combined program also begins with the same procedure using twice the usual activity. During the heating steps of FDG, the robot performs steps related to the synthesis of the fluoroestrogen. Likewise, while the robot is collecting HPLC fractions during the fluoroestrogen synthesis, the robot performs the final purification step for FDG. Overall, the combined program takes approximately 20 minutes longer to execute than the synthesis of either of the two individual programs.

In conclusion, a laboratory robot in this application is extremely versatile, and its ability to perform more than one synthesis simultaneously demonstrates its power as a multifunctional device for the automated syntheses of radiopharmaceuticals for PET imaging.

This work was supported by DOE grant DE-FG02-84ER60218 and NIH grant HL 13851.

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EASYLAB PROGRAM: FLUOROESTRADIOL.SYNTHESIS

INITIALIZE
ADD.TARGET.WATER
RESOLUBILIZE.F-18
ADD.SUBSTRATE
ADD.REDUCING.AGENT
QUENCH.AND.EXTRACT
INJECT.ONTO.HPLC
HAND.REST

EASYLAB PROGRAM: FLUORODEOXYGLUCOSE.SYNTHESIS

INITIALIZE
ADD.TARGET.WATER
RESOLUBILIZE.F-18
ADD.SUBSTRATE.AND.HEAT
DILUTE.AND.EXTRACT
HYDROLYZE.PRODUCT
PURIFY.FINAL.PRODUCT
HAND.REST

EASYLAB PROGRAM: COMBINED.FES.AND.FDG.SYNTHESIS

INITIALIZE
ADD.TARGET.WATER
RESOLUBILIZE.F-18
SPLIT.F-18.BETWEEN.FES.AND.FDG
MEANWHILE.HEAT.FDG.INTERMEDIATE
ADD.REDUCING.AGENT.TO.FES
QUENCH.AND.EXTRACT.FES
DILUTE.AND.EXTRACT.FDG
MEANWHILE.HYDROLYZE.FDG
INJECT.FES.ONTO.HPLC
MEANWHILE.COLLECT.FES.FRACTIONS
PURIFY.FINAL.FDG.PRODUCT
HAND.REST

Scheme 1. Individual robot synthesis programs for 16$\alpha$-[$^{18}$F]fluoroestradiol-17$\beta$ and 2-[$^{18}$F]fluoro-2-deoxy-D-glucose and the combined program for their simultaneous production.

DISPOSABLE ALUMINA COLUMNS FOR THE PURIFICATION OF N-[$^{11}$C]METHYL AMIDES AND AMINES.

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For the routine production of the N-[$^{11}$C]methyl amides Ro151788, Ro54864, and flunitrazepam, small disposable neutral alumina columns are used rather than HPLC. As a rule alumina selectively adsorbs secondary amides relative to their N-methylated analogs. Thus the radiolabeled product is eluted first, uncontaminated by the relatively large amount of precursor. Compared to silica gel, small dry-packed alumina columns (3.2 mm x 180 mm bed) accept high loads, and solvent flow is uniform and reproducible. The low pressures and small solvent volumes permit miniature peristaltic pumps to be used for solvent delivery. Volatile solvent systems can be selected so that evaporation of the solvent from the product is accomplished simultaneously with elution simply by warming under a flow of $N_2$. For routine production a large number of identical columns can be dry-packed and sterilized by heating in an oven. The packings are confined by plugs of glass or polypropylene fiber, and connections are by way of teflon plugs that are simply pushed into the ends of the columns. The alumina is prepared by discarding the <120 mesh fines from reagent grade neutral alumina, washing with ethanol and drying at 150°.

The selectivity between secondary amides and their N-methyl analogs is greatest on basic alumina and least on acidic alumina. Neutral alumina is adequate for the above separations. Substantial amounts of water in the alumina decrease the selectivity, but levels on the order of 1% do not. As a rule, mixtures of small amounts of ethanol with a less polar solvent such as pentane give good selectivity. (Pentane 98%: EtOH 2% for Ro54864 and flunitrazepam.) For Ro151788 only $CHCl_3$ or $CHCl_3/CH_2Cl_2$ give adequate selectivity. In this case the separation depends critically on the amount of EtOH (<1%) present in the $CHCl_3$ as a preservative. Alumina shows a similar strong selective adsorption of secondary amines relative to their N-methylated analogs (eg., nor tropanyl benzylate vs tropanyl benzilate; $CHCl_3$), and should thus be applicable as well to this major group of PET radiopharmaceuticals.

This work was supported by NINCDS Grant PO1 NS 15655 and by DOE Grant DE-FG02-87ER60561.

OBSERVATIONS CONCERNING EXPERIMENTAL PARAMETERS IN MICROWAVE-INDUCED REACTIONS AND THEIR APPLICATIONS TO PET RADIOCHEMISTRY

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Microwave treatment has been shown to, in some cases, drastically accelerate the rates of organic reactions (1). Microwaves have been previously used in the production of positron-emitting labelling precursors (2) and in the production of $^{11}C$- and $^{18}F$-labelled radiopharmaceuticals (3, 4, 5), resulting in shorter reaction times and higher yields of the radiolabelled product. The equipment used was a readily available household microwave oven.

The microwave field can be described as a periodically reversed electromagnetic field. Polar molecules follow the direction of the field and are thereby set into periodic motion, resulting in heat. The effect on the molecule is thus proportional to the strength of the electromagnetic field. To investigate this phenomenon we have utilized a microwave cavity with variable geometry rather than a conventional household microwave oven.

At one given geometry the time and microwave intensity required for boiling common organic solvents were: EtOH (3 s, 40 W), acetone and dichloromethane (0.5 min, 100 W), water (1 min, 100 W), acetonitrile, DMF and DMSO (1.5 min, 100 W). Factors contributing to these results are naturally the boiling point, polarity, specific heat, and latent heats of vaporization of the molecule. However, it should be noted that the observed polarity of a molecule is a function of the applied microwave frequency. Most commercially available household microwave oven magnetrons have a set frequency of 2450 MHz as was the case in this experiment. This frequency agrees with a resonance frequency in the microwave spectrum of water. For optimal microwave absorption in the reaction solvent, a source of variable frequency may give even better results.

The effect of low intensity microwave treatment was further investigated in the reaction of $^{18}F$ with 4-nitro-4'-fluorobenzophenone (Scheme 1) and compared with thermal heating (Table 1). Not only the total incorporation but also the product distributions were affected by the geometries and intensities. In addition to the polarity of the reaction solvent, the distance between the counterions in the Kryptofix-potassium carbonate-fluoride complex should also vary with the rapidly varying electromagnetic field, thereby affecting the availability of the ionic reagent for reaction

**Scheme 1:**

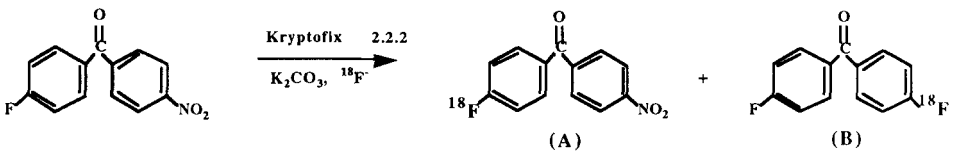

**Table 1:**

**Microwave**

| Geom. | Intens. | Time | Incorp. | % A | %B | Unident. |
|---|---|---|---|---|---|---|
| (I) | 200 | 5 | 38 | 65 | 20 | 15 |
| | 100 | 5 | 20 | 70 | 15 | 15 |
| | 200 | 8 | 71 | 60 | 30 | 10 |
| | 100 | 8 | 23 | 70 | 15 | 15 |
| (II) | 35 | 2.5 | 21 | 5 | 30 | 65 |
| (III) | 35 | 2.5 | 32 | 5 | 30 | 65 |
| (IV) | 35 | 2.5 | 54 | 5 | 35 | 60 |
| | 35 | 1 | 55 | 20 | 50 | 30 |
| | 45 | 1 | 41 | 5 | 35 | 60 |

**Thermal**

| | | | | | | |
|---|---|---|---|---|---|---|
| (I) | R.T. | 25 | 9 | 90 | 5 | 5 |
| | 80°C | 25 | 58 | 75 | 20 | 5 |
| | 100°C | 25 | 67 | 60 | 35 | 5 |

Reactions of short-lived radioisotopes in the presence of microwave fields can be optimized to very short times, thereby increasing the total radiochemical yield. Since this microwave device can be used to optimize the experimental parameters and is small enough to fit inside a conventional radiochemical set-up in a hot cell, investigations with it are being continued.

*We would like to thank Prof. G. Edvinsson and L. Klynning, Univ. of Stockholm, for the generous loan of the microwave equipment.*

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## THE USE OF SONICATION IN REACTIONS INVOLVING SHORT LIVED RADIOISOTOPES

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Most of Radioisotopes used in PET, have short half life ($^{11}C$, $^{18}F$). It would be of great interest to find a way to reduce reaction times and to increase yields of reactions. In a first place, the use of microwaves have been tried, but there were not usable in every case (1). Consequently, we tried ultrasonic irradiation (2). We report here our results.
The affects of ultrasound on chemical reactivity, could not only be attributed to mechanical results. When a medium is subjected to ultrasonic irradiation, microbubbles appear, it is probably in the process of cavitational collapse we could find the explanation.
Indeed the microbubble contains vapour from the solvent and from any volatile reagents, when it collapses the vapours of solvent and reagents are subjected to enormous increases in both temperature and pressure which could induce fragmentation to generate reactive species as radicals or carbenes. In an other hand the solvent structure could be disrupted, either by the shock wave produced by the bubble collapse or by the propagation wave itself, which could influence reactivity by altering solvation of reactive species present. We used an ultrasonic BRANSON 450 W, with horn and tip except for the synthesis of nitromethane for which we used a cup adapted to the horn.

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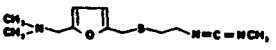
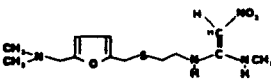
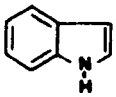
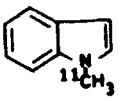
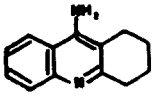
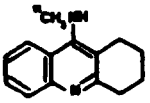
**SUMMARY OF RESULTS:**

| COLD REAGENT | [$^{11}$C]REAGENT | PRODUCT | REACTION CONDITIONS | RESULTS |
|---|---|---|---|---|
| $NaNO_2$ | $[^{11}C]CH_3I$ | $[^{11}C]CH_3NO_2$ | DMF<br>100°C<br>3 min | 35 % |
| | | | DMF<br>RT<br>(((-3 min | 80 % |
|  | $[^{11}C]CH_3NO_2$ |  <br>RANITIDINE | THF-10% HMPT<br>KOH, K222<br>60°C<br>15 min | 0 % |
| | | | THF-10% HMPT<br>KOH, K222<br>RT<br>(((-15 min | 15 % |
|  | $[^{11}C]CH_3I$ |  | Toluene<br>KOH-K222<br>80°C-10 min<br>Stirring | 14 % |
| | | | Toluene<br>KOH-K222<br>RT<br>(((-10 min | 20 % |
|  <br>THA | $[^{11}C]CH_3I$ |  <br>$^{11}CH_3$THA | Toluene<br>KOH-K222<br>80°C-10 min | 50 % |
| | | | Toluene<br>KOH-K222<br>RT<br>(((-10 min | 75 % |

THF : tetrahydrofurane. HMPT : hexamethylphosphorous triamide. DMF : dimethylformamide. K222 : kryptofix 222. ((( : ultrasound. THA : tetrahydroaminoacridine.

# OPTIMIZATION STUDIES OF AUTOMATED 2-[$^{18}$F]-FDG SYNTHESIS

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At the Department of Radiochemistry of the University of Helsinki, synthesis of 2-[$^{18}$F]-Fluoro-2-Deoxy-D-Glucose (2-FDG) is controlled automatically using a personal computer as a control unit. The system, which was developed in our laboratory/1/, was primarily designed to control what has become the most efficient method for the synthesis of 2-FDG.

$^{18}$F-fluoride for the synthesis of 2-FDG is produced at the University of Helsinki via the $^{18}$O(p,n)$^{18}$F reaction using an effective small volume water target in a tandem van de Graaff accelerator/2/. After the irradiation, the aqueous $^{18}$F$^-$ is transferred from the target to a glass reaction vial with a semiautomatic system, whereafter the procedure described for the synthesis continues automatically through remote control as reported earlier/1/.

The method is based on the aminopolyether (Kryptofix-222) supported nucleophilic $^{18}$F-fluorination of 1,3,4,6-tetra-O-asetyl-2-O-trifluoro-methanesulfonyl-ß-D-mannopyranose (triflate) described by Hamacher and co-workers/3,4/ with some minor modifications added in the light of our own experience/5/. To synthesize 2-FDG in a good and reproducible yield using automated equipment requires optimization of the main parameters. In our experience a mole ratio of at least 2:1 (triflate:$K_2CO_3$) and ~3.5 ml of substrate solvent (anhydrous acetonitrile) are needed to obtain a crude synthetic product at > 50% (EOB) yield. A bath temperature of 130-135 °C is required to remove the acetyl groups completely. The yield was raised with two Sep-Pak cartridges in series in the first purification step after the labelling reaction.

Final purification on the ion retardation resin and aluminum is presently carried out manually. The radiochemical purity of 2-FDG has varied from 97 to 99%, depending on the column length (resin bed) and the flow rate of the eluent. The radiochemical purity has been checked by the autoradiographic TLC method ($CH_3CN$:$H_2O$/95:5). In addition some TLC plates are counted manually with a $\gamma$-counter to verify the results of the autoradiographic method.

The presence of the toxic chemical impurity Kryptofix-222 was detected using the TLC method described by Chaly et al./6/ with minor modifications introduced by us. Washing the Sep-Pak:s (first purification step in the synthesis) with 10 ml 0.1M HCl was found to eliminate K-222 totally, as suggested by Moerlain et al./7/.

The yield of 2-FDG from the $^{18}$F-fluoride ion is 43% (±7,n=40) (Table 1) corrected to EOB after a synthesis time of about 110 min (this includes the time required for chromatographic purification). According to approved tests, the product is sterile, apyrogenic, and non-toxic.

We have now achieved reproducible production of 2-FDG on an experimental scale (3-50 mCi, n.c.a). Some improvements are still needed to speed up the synthesis and improve the yield. In the future, we shall use ultrasonic bath to promote labelling of triflate. In addition, automatization of the chromatographic purification process is in progress.

Table 1. ACTIVITY DISTRIBUTION DURING THE SYNTHESIS OF 2-FDG CORRECTED TO EOB

| 2-FDG yield | Sep-Pak cartridges | Remaining activity in the equipment | Unlabelled $^{18}F^-$ | Ion retardation resin |
|---|---|---|---|---|
| 43%(±7,n=40) | 4%(±2,n=40) | 25%(±7,n=40) | 19%(±6,n=40) | 9%(±3,n=40) |
| 54%(±5,n=15) | 4%(±1,n=15) | 21%(±6,n=15) | 14%(±7,n=15) | 8%(±3,n=15)* |
| 51%(±9,n=15) | 4%(±1,n=15) | 20%(±4,n=15) | 19%(±7,n=15) | 6%(±2,n=15)** |

* fifteen best ones
** mean value of last fifteen successive syntheses

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**ION CHROMATOGRAPHIC ANALYSIS OF $^{18}$FDG PRODUCED BY [$^{18}$F]FLUORIDE DISPLACEMENT: PRODUCTION OF 2-CHLORO-2-DEOXY-D-GLUCOSE AS AN IMPURITY IN THE PRESENCE OF CHLORIDE ION.** D.L.Alexoff, R.Casati, J.Fowler, A.P.Wolf, C.Shea, D. Schlyer and C.-Y. Shiue, Chemistry Department, Brookhaven National Laboratory, Upton, N.Y., 11973.

2-Deoxy-2-[$^{18}$F]fluoro-D-glucose ($^{18}$FDG) has recently been included in the United States Pharmacopeia as the first positron-emitting compound so designated.[1] This is expected to expedite its availability for clinical and basic research. The availability of an efficient synthetic route, the reaction of [$^{18}$F]fluoride with 1,3,4,6-tetra-O-acetyl-2-O-trifluoromethanesulfonyl-D-mannopyranose described first by Hamacher and co-workers, [2] has facilitated the production of the large quantities of $^{18}$FDG needed to meet this demand. The well known advantages of this method include high yield, epimerically pure compound, minimal operational complexity (hence, easy to mechanize or automate), and high specific activity of the final product. Although this method has been used by many groups for several years, little data has been published on the chemical composition of $^{18}$FDG preparations, including measurements of FDG mass. A specific activity of 900mCi/micromole has been recently reported; [3] however, most of the literature addressing the chemical composition of $^{18}$FDG preparations has focussed on detecting and minimizing the mass of 4,7,13,16,24-hexaoxa-1,10-diazabicyclo(8,8,8)-hexacosane (Kryptofix 2.2.2.).4,5

The recent introduction of convenient high sensitivity analytical methods for carbohydrate analysis [6] prompted us to examine the chemical composition of our $^{18}$FDG preparations, paying particular attention to measuring FDG mass and identifying chemical impurities.

Carbohydrate analysis on $^{18}$FDG preparations was first made using a Dionex BioLC (Sunnyvale, CA) high pressure ion chromatograph with pulse amperometric detection (HPIC/PAD) on a CarboPaq PA1 column using 75 mM NaOH at 1 ml/min with a post-column addition flow of 0.5 M NaOH at 0.7 ml/min.[7] The primary advantages of this analytical method are its high sensitivity (10-100 pmol) for many carbohydrates in complex matrices, and its ability to separate structurally similar carbohydrates. For example, this system gave baseline resolution of FDG and 2-deoxy-2-fluoromannose. The first analyses of our $^{18}$FDG using this chromatographic system showed FDG mass concentrations of up to 180 micrograms/mL for a 6 mL preparation. This value is much greater than the 18 micrograms total mass expected for a 100 mCi batch of no carrier added (NCA) $^{18}$FDG having a specific activity of 1 Ci/micromole. Carrier fluoride concentrations in all reagents were measured by ion chromatography on the Dionex BioLC using conductivity detection. [8] These values could not account for the large FDG mass since fluoride levels were consistent with expected NCA levels. This suggested the presence of an unidentified compound with an identical retention time as FDG in this particular chromatographic system. This was confirmed by capillary gas chromatography (GC) of trimethylsilyl (TMS) and O-methyloxime TMS ether [9] derivatives of these preparations which resolved FDG from the unknown compound. The retention time of the derivatized unknown was between the retention times of derivatized authentic samples of FDG and 2-bromo-2-deoxy-D-glucose.

These and other results suggested that the unknown compound was 2-chloro-2-deoxyglucose (CIDG) produced by a chloride ion displacement reaction with mannose triflate as is shown in Figure 1. Chloride ion can arise from a number of sources including the eluate of [$^{18}$F]fluoride from the resin used to recover enriched water [10] and HCl used in the hydrolysis step. An authentic sample of CIDG was synthesized by chloride displacement on mannose triflate under similar conditions used for the $^{18}$FDG synthesis and characterized by mass spectroscopy, proton NMR, and elemental analysis.

A separation method on the Carbopaq PA1 anion exchange column was developed to give a near baseline separation of CIDG and FDG. The eluent of this system was 220 mM NaOH at a flow rate of 0.2 mL/min with no post-column addition. The system was calibrated with authentic FDG and CIDG. The limit of detection for this method was about 20 picomoles of FDG for a 100 microliter injection of standard solution (PAD (gold) settings E1=.05V; E2=-.65V; E3=.95V). A shortcoming of this method is the introduction of periodic, baseline noise caused by flow fluctuations due to the low backpressure

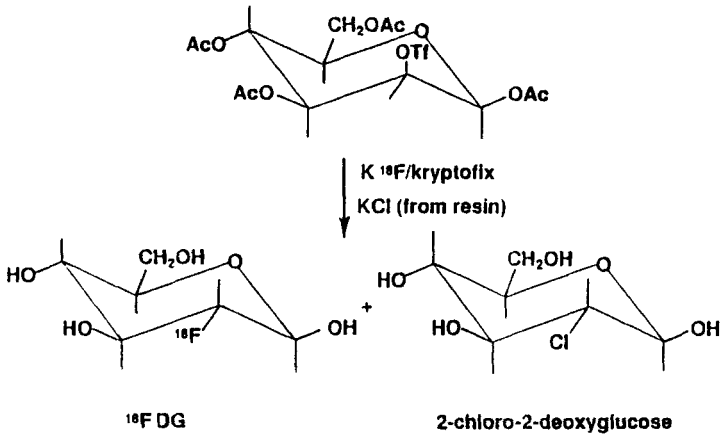


Fig. 1 Formation of 2-Cl-2-deoxy-D-glucose impurity from chloride ion in the synthesis of $^{18}$FDG by [$^{18}$F]fluoride displacement.

on the PAD cell. Ultimately, this systematic noise, combined with tailing of large glucose peaks, limits the quantitation of FDG in the presence of high glucose concentrations to ca. 0.5 microg/mL for 100 microliter injection (approximately 55 pmoles). Glucose and mannose were also assayed with this method. Typical chromatograms appear in Figure 2.

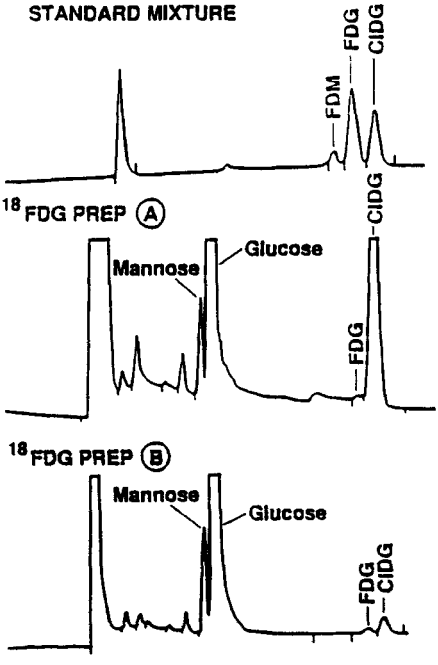


Fig. 2 Ion chromatograms of $^{18}$FDG prepared by [$^{18}$F]fluoride displacement. Preparation A used Ag1x10 hydroxide form resin and 30 mg triflate. Preparation B with Ag1x10 carbonate form resin and 10 mg triflate.

The retention time of an authentic sample of CIDG on HPIC matched that of the unknown component in the $^{18}$FDG preparations and co-injection of authentic CIDG and $^{18}$FDG samples showed identical retention times of the unknown component and CIDG on the HPIC. In addition, an authentic sample of CIDG was derivatized using the same procedures as before and analysed by capillary GC. The retention times of the TMS derivatives of the authentic CIDG matched those of the unknown component in $^{18}$FDG. Mass spectral analysis of tetrafluorocetylated (TFA) CIDG also matched that of the TFA derivatives of the unknown, exhibiting the natural Cl isotopic ratio at masses (241,243) and (174,176).

Several $^{18}$FDG samples from other institutions were analysed by HPIC/PAD. Mass concentrations of FDG, CIDG, glucose, and mannose were obtained from the HPIC/PAD chromatograms. Although the same general procedure for the displacement reaction is used at each of these institutions, the procedures differed with respect to the amounts of starting triflate and whether the enriched water was recovered using an anion exchange resin. Significant amounts (500-1000 micrograms) of CIDG were found in $^{18}$FDG preparations wherever ion exchange resins or other solid supports containing chloride ion are used. The eluent from the standard recovery resin (Ag1x10, hyroxide form) is known to have concentrations of chloride as high as 400 ppm.[11] However, commercially available AG1x10, carbonate form resin contained significantly less chloride (10-50 ppm) and also was suitable for enriched water recovery. CIDG levels decreased by a factor of 5 by using this resin. The ability of chloride to react with mannose triflate at a sufficient rate to result in the introduction of CIDG as an impurity during [$^{18}$F]fluoride displacement was confirmed by the production of increased amounts of CIDG in syntheses which were spiked with 400 ppm KCl. It was also shown that direct hydrolysis of mannose triflate with 1N HCl gave significant amounts of CIDG (ca. 190 micrograms for 30 mg of mannose triflate). The amount of CIDG can be minimized by minimizing starting materials. For example, by using the carbonate resin to recover enriched water and 10 mg of triflate, 35 micrograms of CIDG is produced along with 5 micrograms of FDG. Glucose levels are roughly proportional to the amount of triflate used, as expected. Glycerol (arising from the sterilizing filter, Gelman, Acrodisc, 0.22 micrometer) was also identified as another major chemical compound present in the FDG preparations and was often the largest peak in the ion chromatogram. When no resin is used in the preparation, only trace amounts of CIDG (range of 0-40 micrograms) are detected with the mass of FDG being in the range of 1 to 37 micrograms.[12]

Acknowledgment: This work was supported by DOE (OHER) and by NIH (NS 15380). The authors are grateful to Mark Andrews for helpful discussions and for performing the capillary gas chromatographic analyses and to Dionex Corporation for initial analysis of FDG samples.

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## High-Sensitivity Determination of Carrier Carbon Oxides in $[^{11}C]O_2$ Production using Modified Hydrogen Flame Ionization Detection

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Upton, NY 11973

Recent interests in receptor imaging have stressed the need for pharmacologically active compounds that are labeled with carbon-11 at high specific activity. $[^{11}C]H_3I$ is perhaps the most versatile and by far the most widely used precursor for labelling such compounds with carbon-11 through N-$[^{11}C]$-methylation. We have demonstrated that with careful handling of reagents for the reduction of $[^{11}C]O_2$ to $[^{11}C]H_3OH$, and for the subsequent iodination of $[^{11}C]H_3OH$ to $[^{11}C]H_3I$, as little as 3 nmol of carrier carbon is introduced at this stage of synthesis with the exclusion of the $[^{11}C]O_2$ target system contributions. However, in the routine preparation of compounds such as $[^{11}C]$-cocaine and $[^{11}C]$-cogentin through N-$[^{11}C]$-methylation using $[^{11}C]H_3I$, total masses as high as 400 nmol are sometimes obtained. This suggests that the $[^{11}C]O_2$ target system contributes a substantial amount of carrier carbon to the final product mass.

This conclusion prompted our recent re-evaluation of target materials used and of procedures followed for the routine production of $[^{11}C]O_2$, with the focus on identifying and minimizing all sources of carrier carbon both from within the high radiation field of the cyclotron target as well as from the post-irradiation manipulation of the target gas.

Unfortunately, no practical analytical method exists for the routine determination of trace carrier carbon oxide levels within the $[^{11}C]O_2$ target system. In the past, researchers have had to rely on gas chromatography (GC) coupled with thermal conductivity detection (TCD). While TCD is a nondestructive universal detector, it possesses only a modest sensitivity for carbon oxides with a detection limit of about $10^{-8}$ g/s (1) or about 10 ppm at normal GC flowrates. Because of this, it was necessary to process large quantities of target gas through cryogenically cooled traps in order to concentrate the carrier carbon to levels that could be detected. Unfortunately, this manner of processing has several drawbacks. Firstly, noncondensible sources of carrier carbon such as CO and $CH_4$ are not measureable. Secondly, any malfunction during the manipulation of target gas by this process can introduce large quantities of $CO_2$ from air (present at 300 ppm (2)). Thirdly, it is generally not possible to repeat an analysis of a single processed batch of gas for replication of data. In view of these shortcomings, we did not find the present method attractive for routine target gas analysis.

A more sensitive, but less universal method of detection relies on hydrogen flame ionization (FID). The sensitivity FID offers is several orders of magnitude higher than TCD. A detection limit of approximately $10^{-12}$ g/s (1) or 1 ppb at normal GC flowrates is routinely achieved. Unfortunately, standard FID is insensitive to $CO_2$.

Recently, we modified a commercial FID (Figure 1) by incorporating a miniature nickel catalyst methanizer into its jet and operating the detector at 400°C (3-5). The catalyst reduces CO and $CO_2$ to $CH_4$ (a detectable species) prior to their entering the flame region of the detector. The catalyst operates efficiently using the same hydrogen supply as that needed to sustain the flame.

This modified detector offers several advantages over the old method of analysis. Firstly, it can detect 10 ppb of $CO_2$ within a 4 mL volume sample of target gas at STP. Thus numerous analyses can be carried out on a rather small amount of collected sample, without the need for preconcentration of sample. Direct analysis of CO and $CH_4$ is also possible because of this at about the same levels of sensitivity. Secondly, the detector responds linearly to $CO_2$ with over three orders of magnitude change in concentration. This makes it attractive for trace as well as high-level measurements. Finally, the catalyst exhibits remarkable stability in its day-to-day usage with minimal maintainance.

The sensitivity of this modified detector rivals those achieved by many optical techniques used in trace gas analysis that are based on atomic and molecular absorption or fluorescence, with the advantages that it is less costly and far simpler to set-up and maintain in a radiopharmaceutical laboratory.

Recently, we used this technique to measure $CO_2$ and total carbon concentrations within the UHP $N_2$ gas (99.999% purity, Matheson Gas Co.) used for producing $[^{11}C]O_2$. $CO_2$ concentrations as low as 2 pmol/cm$^3$ or 0.05 ppm were found. Total carbon concentrations were determined as $CO_2$ after combustion over CuO at 850°C and were found to be 24 pmol/cm$^3$ or about 0.6 ppm. These levels were the same regardless of whether samples were taken at the supply or the target. The above concentrations suggest that at most 50 nmol of carrier carbon should be introduced in our production of $[^{11}C]O_2$ using the present target (212 cm$^3$ volume pressurized to 10 atmospheres). Typically, a 15 minute irradiation using 16 MeV $H^+$ at a 25 μA beam intensity produces 1.4 Ci of $[^{11}C]O_2$ at EOB. Thus a practical specific activity for $[^{11}C]O_2$ approaching 28 Ci/μmol should be obtainable, assumming there are no additional carrier carbon sources within our system other than the gas contribution. However, present conditions typically yield $[^{11}C]O_2$ at a specific activity of only 3 Ci/μmol at EOB. All attempts to maximize this value through the elimination of organic material sources used to seal the window and gas fittings to the aluminum target body have failed to yield an improvement. Experiments where the target window was changed from Havaar (0.2% carbon) to aluminum also showed little effect. We can only hypothesize that the aluminum body of the target is the source of our high carrier carbon levels. Investigations are under way to determine whether this is due to impurities entrained within the aluminum alloy itself, or due to some phenomenon related to the exposure surface.

**ACKNOWLEDGEMENT**: This research was supported by the U.S. Department of Energy and by National Institutes of Health (NS-15380).

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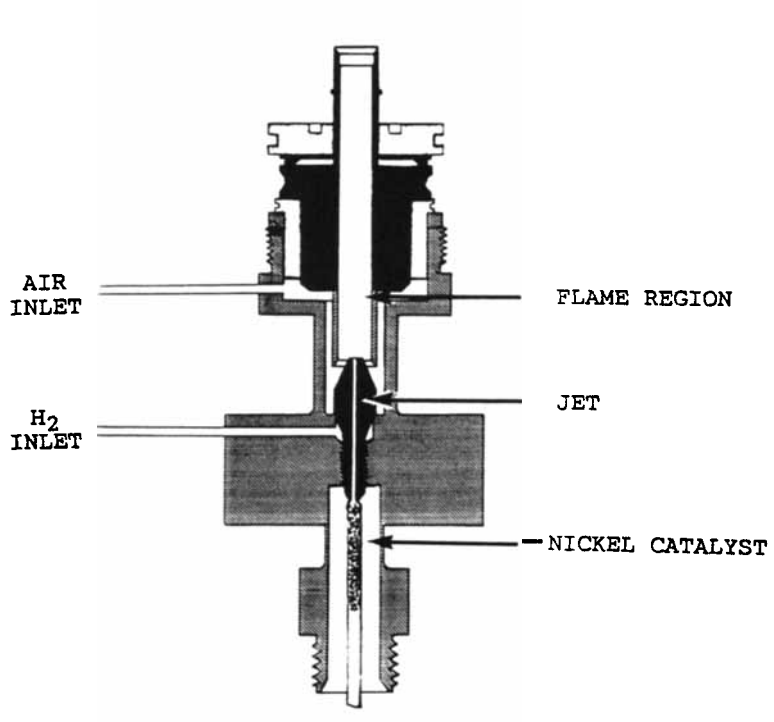


Figure 1. Flame ionization detector from a Hewlett-Packard model 5890 gas chromatograph.

## A MICROPROCESSOR-CONTROLLED RADIOCHEMISTRY SYSTEM FOR THE AUTOMATED PREPARATION OF CARBON-11 AMINO ACIDS

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Carbon-11 amino acids which are rapidly transported into malignant cells and show high uptake, slow washout, and rapid clearance from non-specific tissues are excellent agents for the diagnosis and management of treatment of cancer using positron emission tomography (PET). Only a few remotely operated semi-automated systems have been reported for the routine production of 1-C-11 amino acids (1-3). The short half-life of carbon-11 and curie quantities of carbon-11 labeled precursors require that each stage of the chemical process must be performed in a minimum number of high yield reaction steps and in a remote manner respectively. The optimum production of amino acids in a clinical facility for routine diagnosis is most efficiently accomplished by similar synthetic processes differing only by parameters such as reaction times, temperatures, and chromatographic systems. Yields from organic synthetic reactions required for amino acid synthesis can be optimized most efficiently by employing automated systems. We report the development of a microprocessor reaction system for the automated production of amino acids employing the Bucherer-Strecker amino acid synthesis (2,4).

The automated amino acid synthesis unit consists of 6 major subunits (figure 1): (1) a series of reagent vials and reservoirs (2) a stainless steel reaction vessel (3) two air actuated teflon rotary valves (4) a programmable temperature controller (5) a programmable microprocessing unit and (6) an ion retardation and strong cation exchange column and filter sterilization unit. The stainless steel vessel is heated by a nichrome ribbon seated in a copper jacket. The rate and duration of heating and reaction temperatures are regulated by the temperature controller. The reaction vessel is cooled by the circulation of nitrogen gas within the jacket. Reagent addition, solution transfers, and column purification is achieved through small bore teflon tubing by the application of gas pressure. The entire radiosynthetic process is performed by a dedicated 8085 microprocessor control unit which consists of a main control processor, keyboard and LCD display. The amino acids are synthesized using a set of subprograms that are displayed on the LCD and which direct a series of major tasks. The microprocessor control unit is designed to be programmed to prepare a variety of amino acids.

As an example of its capability, the unit has been programmed (table 1) to synthesize 1-C-11 aminocyclobutane carboxylic acid (ACBC) via a Bucherer-Strecker synthesis from cyclobutanone and C-11 potassium cyanide.

Acknowledgement: We wish to thank N.I.H. under contract GM39801 for support of this research.

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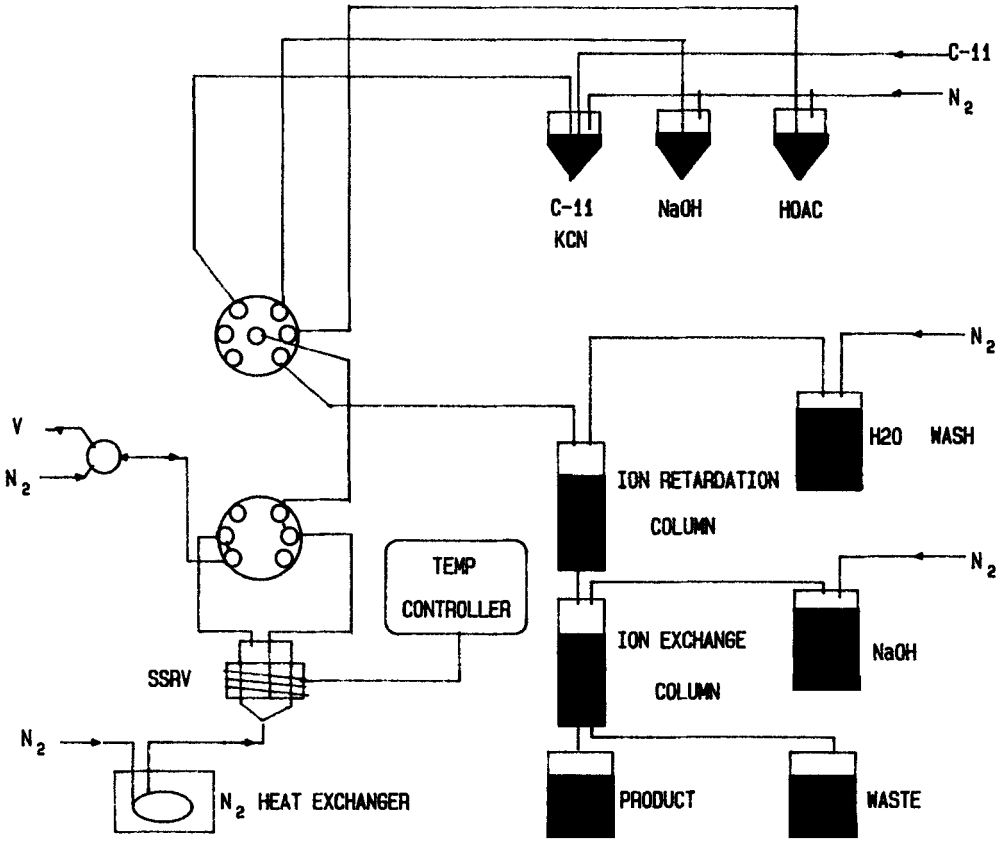


FIGURE 1. AMINO ACID SYNTHESIS UNIT

TABLE 1. 1-C-11 ACBC Synthesis Program

| COMMAND FILE/DESCRIPTION | TASK DESCRIPTION/TIME REQUIRED |
|---|---|
| **TRANSFER POSITION:** A PATH IN THE VALVES IS OPENED FOR LIQUID TRANSFERS. | VALVES ARE POSITIONED. **0 MIN.** |
| **A TO VESSEL:** C-11 CN IS TRANSFERRED TO STAINLESS REACTION VESSEL (SSRV). | VIAL CONTAINING C-11 CN/NaOH IS PRESSURIZED TO ADD C-11 CN TO SSRV. **0.25 MIN.** |
| **HEAT POSITION:** SSRV VALVES ARE CLOSED. | VALVES IN SSRV ARE CLOSED. **0 MIN.** |
| **HEAT VESSEL 1:** SSRV IS HEATED TO FORM HYDANTOIN. | SSRV SOLUTION IS HEATED FROM 25° C TO 135° C. **3 MIN.** |
| **COOL VESSEL 1:** SSRV IS COOLED BY A FLOW OF NITROGEN (-60° C). | SSRV SOLUTION TEMPERATURE IS COOLED TO 60° C. **1.5 MIN.** |
| **TRANSFER POSITION:**<br>**B TO VESSEL:** 6.25 N NaOH IS TRANSFERRED TO SSRV. | VIAL CONTAINING 1 mL 6.25 N NaOH IS PRESSURIZED TO ADD TO SSRV. **0.25 MIN.** |
| **HEAT POSITION:**<br>**HEAT VESSEL 2:** SSRV IS HEATED TO FORM AMINO ACID. | SSRV SOLUTION IS HEATED FROM 60° C TO 180° C. **10 MIN.** |
| **COOL VESSEL 2:**<br>**TRANSFER POSITION:**<br>**C TO VESSEL:** SSRV IS COOLED AND 50% HOAc IS TRANSFERRED TO SSRV. | SSRV SOLUTION IS COOLED TO 40° C AND 0.5 mL OF 50% HOAc IS ADDED TO SSRV. **2.25 MIN.** |
| **TRANSFER POSITION:**<br>**VESSEL TO COLUMN:** REACTION MIXTURE IS TRANSFERRED TO ION RETARDATION COLUMN. | REACTION MIXTURE IS TRANSFERRED ONTO ION RETARDATION RESIN. **0.5 MIN.** |
| **RINSE COLUMN 1:** AMINO ACID TRANSFERRED TO CATION COLUMN. | WATER RESERVOIR VIAL IS PRESSURIZED TO RINSE 15 mL OF $H_2O$ THROUGH RESIN COLUMNS TO WASTE TO ELUTE AMINO ACID ONTO CATION COLUMN. **3 MIN.** |
| **BASE 1 RINSE:** 0.3 N NaOH IS TRANSFERRED THROUGH CATION COLUMN. | NaOH RESERVOIR VIAL 1 IS PRESSURIZED TO RINSE 10 mL OF 0.3 M NaOH THROUGH CATION COLUMN TO WASTE. **2 MIN.** |
| **BASE 2 RINSE:** 0.3 N NaOH IS TRANSFERRED THROUGH CATION COLUMN. | NaOH RESERVOIR VIAL 2 IS PRESSURIZED TO RINSE 20 mL OF 0.3 M NaOH THROUGH CATION COLUMN TO ELUTE AND FILTER AMINO ACID INTO STERILE PRODUCT VIAL. **3 MIN.** |

RADIOLABELLED MONODISPERSE POLYSTYRENE PARTICLES

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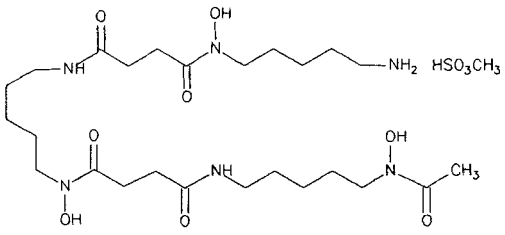
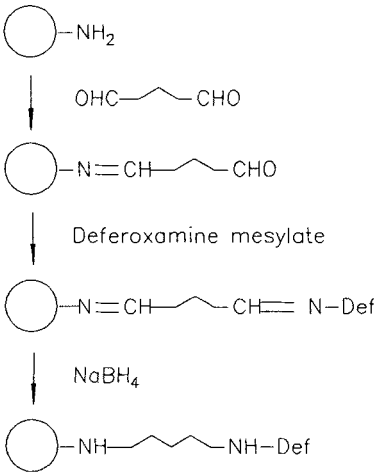
Radiolabelled aerosols play an important role in diagnostic imaging, patient management and therapy, the study of lung physiology and help us to develop an understanding of occupational exposures and inhalation toxicology. The issues of particle deposition and subsequent clearance from the lung are central to all of these activities.

We have developed a simple method of producing radioaerosols based on the principal of labelling functionalized polystyrene molecules. These particles are available as highly monodisperse suspensions in a wide range of sizes with several different functional groups. Our studies dealt mostly with amino functionalized particles and are directed toward the attachment of a chelating agent to the particles. The particles used (Polysciences) are not cross-linked and as a result are extremely sensitive to heat and organic solvents thus limiting the chemistry to that which can be performed in aqueous solutions at room temperature.




Deferoxamine Mesylate

The best results were obtained using a three-step synthesis in which Deferoxamine mesylate was used as a chelating agent and glutaraldehyde was used as the coupling agent. This route requires a $NaBH_4$ reduction of the Schiff bases formed in the reaction.




The labelled particles have been subjected to a variety of leaching tests and in all cases the loss of label was negligible. The particles have been used in a human study in which the deposition and subsequent clearance of 1μm and 3μm particles labelled with $^{111}In$ and $^{99m}Tc$ respectively was followed in a dual isotope imaging experiment.

INVESTIGATION OF [$^{18}$F]FLUORIDE EXCHANGE SITE ACCESSIBILITY IN RESINS USED FOR DIRECT NUCLEOPHILIC RADIOFLUORINATION

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While direct nucleophilic [$^{18}$F]fluoride substitution on anion exchange resins based on 2% crosslinked polystyrene gels offers a substantial simplification in the synthesis of [$^{18}$F]FDG, exchange of the radioactivity from the resin to the triflate precursor (in MeCN) is incomplete. A possible explanation appeared to lie in the swelling behavior of the resins (MeCN < $H_2O$ < acetone, EtOH, $MeCl_2$, THF, DMSO, toluene, DMF.) [$^{18}$F]Fluoride trapped by exchange from [$^{18}$O]water might become inaccessible by occlusion of some sites as a result of shrinkage of the resin in MeCN. As a model system, tetrabutyl ammonium iodide and tetrabutylammonium toluene sulfonate dissolved in a variety of solvents were used as probes of resin site accessiblity by simple ion exchange at room temperature:

$$Resin^+F^- + (Bu)_4N^+I^- \rightleftharpoons Resin^+I^- + (Bu)_4N^+F^-$$

$$Resin^+F^- + (Bu)_4N^+Toluenesulfonate^- \rightleftharpoons Resin^+Tosyl^- + (Bu)_4N^+F^-$$

The ease of ion exchange was in the order: EtOH, $H_2O$, DMSO, DMF > $MeCl_2$ > MeCN > THF, acetone, toluene. A significant fraction of the [$^{18}$F]fluoride became inaccessible to rapid exchange after brief exposure to MeCN at 90°. A modified procedure (EtOH followed by toluene at room temperature) is recommended for drying the resin after the [$^{18}$F]fluoride has been trapped. The results above suggested that solvent mixtures as opposed to pure solvents might be best able to swell the resin and to support ion exchange or nucleophilic substitution at the same time. Adding 10% EtOH to toluene markedly enhanced the rate of fluoride exchange between the resin and tetrabutyl ammonium toluenesulfonate. In a mixture of toluene and MeCN (50:50) incorporation of [$^{18}$F]fluoride into tetraacetyl mannose triflate was 70%. In toluene alone, incorporation was 50%.

This work was supported by NINCDS Grant PO1 NS 15655 and by DOE Grant DE-FG02-87ER60561.

## COLORIMETRIC MICROASSAY OF $CH_3I$ FOR THE RAPID OPTIMIZATION OF [$^{11}$C]METHYLATION REACTIONS

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A sensitive microcolorimetric assay for methyl iodide was used for development and efficient optimization of reaction systems for [$^{11}$C]methylation with [$^{11}$C]methyl iodide. The assay measured unlabeled $CH_3I$ at the 1 - 10 μg levels in order to duplicate exactly the conditions for radiolabeling with [$^{11}$C]$CH_3I$. After a given step in the radiomethylation reaction, unreacted methyl iodide was purged from the reaction mixture and trapped in 1 ml of a 1% solution of 4-(4-nitrobenzyl)pyridine (NBP) in acetone at -20°. The sample vial was sealed and heated 20 min at 80°. After cooling, 40 μl of triethylamine was added, and the resulting intense magenta chromophore was measured at 565 nm.

As a general approach, the assay was first used to adjust the volume, cooling temperature and composition of the reaction solvent to allow complete trapping of methyl iodide in a minimal reaction volume. Methyl iodide (10 μg) was passed into the reaction system in a stream of $N_2$ (40 ml/min for 3 min), and any effluent $CH_3I$ was collected in acetone/NBP solution and assayed as above. Next the effects of time, temperature and concentrations of substrate and base on $CH_3I$ consumption were determined. In these experiments, 10 μg $CH_3I$ (in $N_2$ or acetone) was added to the reaction system in the absence of $N_2$ flow. The reaction mixture was sealed and heated, then cooled, and the residual $CH_3I$ was transferred to acetone/NBP by warming and purging with a slow stream of $N_2$.

The high volatility of $CH_3I$ and the insensitivity of the assay to traces of solvents commonly used in [$^{11}$C]radiomethylation permitted this approach to be followed generally when developing a new reaction system. The intense chromophore allowed semiquantitative determinations of $CH_3I$ to be made visually. The simplicity of the assay allowed several variables to be studied quickly. For the captive solvent methylation of desmethyl Ro151788, conditions were thus quickly determined which allowed efficient trapping and reaction of [$^{11}$C]$CH_3I$ in only 40 μl of solvent (DMSO:tributyl phosphate, 1:1) adsorbed on 40 mg KOH/alumina. Greater than 90% of the 10 μg $CH_3I$ was consumed in the presence of 1 mg of the desmethyl substrate, but <20% was consumed in the absence of the substrate. Thus, specific vs nonspecific reactions of $CH_3I$ could be differentiated in this way and confirmed by subsequent HPLC analysis.

This work was supported by NINCDS Grant PO1 NS 15655 and by DOE Grant DE-FG02-87ER60561.

## A NEW METHOD FOR SPECIFIC ACTIVITY MEASUREMENT OF [$^{13}$N]$NH_3$ PRODUCED BY PROTON IRRADIATION OF WATER OR DEUTERON IRRADIATION OF METHANE.

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The specific activity of [$^{13}$N]$NH_3$, produced by proton bombardment of water, and by deuteron bombardment of methane, has been determined by measuring the change of electrical conductivity of a standard $NH_3$ absorber solution using a continuous flow instrument equipped with a differential conductivity meter. The sensitivity limit of the experimental method was 6 ppb for dissolved $NH_3$. Samples were taken from routinely used production systems without special precautions against the contamination of the target materials by enviromental nitrogen sources. Thus, we focussed on the avarage amount of ammonia which was carried into a routine preparation of a $^{13}$N-labelled compound (e.g. into the enzymatic preparation of $^{13}$N-labelled glutamate). Values of [$^{13}$N]$NH_3$, obtained from a standard water target were compared with those obtained from the deuteron irradiation of methane.

## APPLICATION OF ION CHROMATOGRAPHY TO THE ANALYSIS OF $^{18}$F-LABELLED DEOXYALDOHEXOSES. AN IMPROVED SYSTEM FOR MONITORING THE CHEMICAL PURITY OF 2-DEOXY-2[$^{18}$F]FLUORO-D-GLUCOSE AND 2-DEOXY-2[$^{18}$F]FLUORO-D-GALACTOSE

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The interaction of the weakly acidic monosaccharides with a 9% cross linked polystyrenesulfonate in the $H^+$ form, resulted in an unexpected and excellent selctivity for epimeric aldohexoses, deoxyaldohexoses and deoxyfluoroaldohexoses. This led to a new application of high performance liquid ion chromatography, in which the separation is dominated solely by the electrostatic attraction between carbohydrate oxygen atoms and $H^+$ of the stationary phase. The mobil phase was deionized water. Tedious column operation conditioning, nor metal ion contamination of the eluate, interfered with the application. Column heating was not required, and high flow rates were possible, thus allowing fast and acceptable separations of the $^{18}$F-labelled monosaccharide analogues.

Analysis and preparative separation of 2-deoxy-2-fluoro-D-glucose, 2-deoxy-2-fluoro-D-mannose and 2-deoxy-2-fluoro-D-galactose will be discussed. A summary of applications of the method for the separation of [$^{14}$C]- and [$^{18}$F]-labelled tracers of the deoxyaldohexose family will be included. A standard procedure will be presented for monitoring the chemical purity of 2-deoxy-2-[$^{18}$F]fluoro-D-glucose and 2-deoxy-2-[$^{18}$F]fluoro-D-galactose. For example, optimization of separation mode and detection method have allowed us to detect as little as $5x10^{-10}$ g of 2-deoxy-2-fluoro-D-galactose in $H_2O$.

**ETAC REAGENTS: A NEW CLASS OF SULFHYDRYL SITE-SPECIFIC RADIOLABELING PROBES FOR ANTIBODIES**

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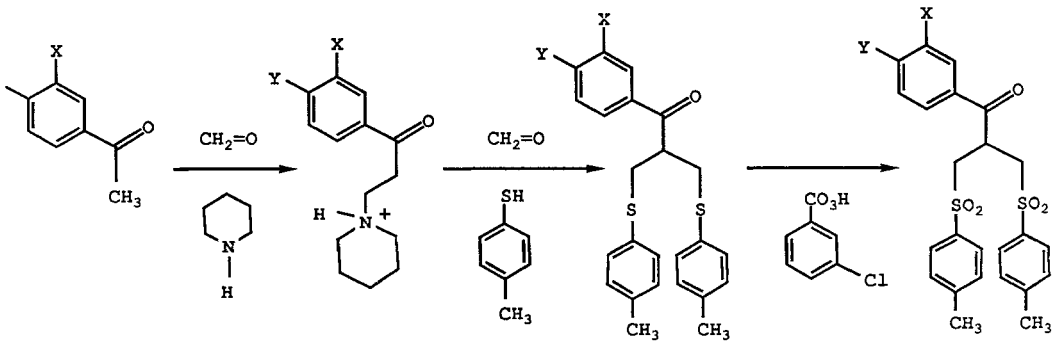
A new class of bis-alkylating Michael reagents, equilibrium transfer crosslink reagents[1], "ETAC", which combine the techniques of cross-linking with tethering have been synthesized. Following a succession of Michael and retro-Michael additions and elimination of the arylsulfone groups (Scheme I, ETAC **1-2**), reduced heavy-heavy and heavy-light disulfide links of an anti-ovarian IgG2a monoclonal antibody, 5G6.4, were site-specifically reannealed via a 3-carbon bridge having a tether branch containing a designated label.



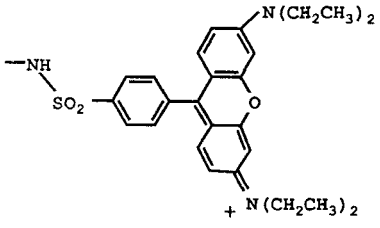

**Scheme I**

The synthetic protocol for parent ETACs **1a-2b**[2] is outlined in Scheme I. The nitro and carboxylic substituents of ETACs **1a-2a** could readily be functionalized to yield useful protein fluorescent and radiolabeling probes.

Hydrogenation of ETAC **1a** produces an amine residue which reacts with lissamine rhodamine B sulfonyl chloride to give the rhodamine adduct **1b**[3]. ETAC **2a** undergoes carbodiimide coupling with 5-(((N-biotinoyl)amino)hexanoyl)amino)-pentylamine and L-tyrosine methyl ester to yield the long-arm biotin probe **2b** and the thiol reactive Bolton-Hunter reagent analogue, ETAC **2c** respectively. ETAC **2c** was radioiodinated using Na[$^{125}$I]/ ICl in acetic acid.

1a, Y = H, X = $NO_2$

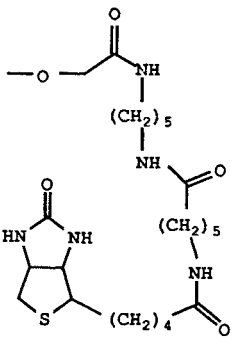
1b, Y = H, X =



rhodamine

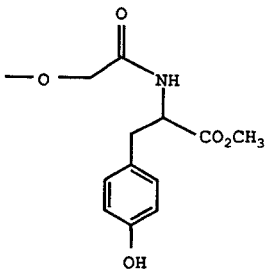
2a, X = H, Y = $-O-CH_2-CO_2H$

2b, X = H, Y =



biotin

2c, X = H, Y =



tyrosine

Electrophoresis and laser densitometry of the cross-link products of reduced 5G6.4 + ETACs **1-2** allowed direct quantification of interchain heavy-heavy and heavy-light ETAC crosslinks (≈1-2 crosslinks/ IgG2a). Retention of immunoreactivity in both ETAC **1b**, **2b** and **2c**-modified 5G6.4 was shown by flow cytometry measurements and direct cell binding assays using 77 IP3 human ovarian carcinoma cells. Direct cell binding assays of ETAC **2b**-biotinylated 5G6.4 complexed with [$^{125}$I]streptavidin and I-125 labeled ETAC **2c**-crosslinked 5G6.4 exhibited equal or better (50-80%) binding than iodogen labeled 5G6.4 (40-60%). Likewise, addition of a large excess of unlabeled 5G6.4 to target cell-bound [$^{125}$I]streptavidin-ETAC **2b**-5G6.4 showed no

detectable dissociation of radiolabel over a 3 day period. These studies illustrate the potential of utilizing ETAC compounds for site-specific radiolabeling of monoclonal antibodies.

Research was funded by the N.I.H. (R.O.I. CA 41531-02 and P.O.I. CA 42768-01A1) and NSF (CHE 8421137A02).

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SYNTHESIS OF NEW CDTA-LINKER-ANTIBODY SYSTEMS FOR IMPROVED IN-VIVO DISTRIBUTION OF In-111.

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Immunoconjugates containing an internal cleavable linking group between the antibody (MAb) and chelated indium have demonstrated faster background clearance and modified liver retention[1,2]. In one study[1], a linking group containing the diester, ethylene glycol bis(succinimidyl succinate), was added sequentially to DTPA-p-(aminoethyl)anilide and the MAb; characterization was based on the extent of hydrolysis. Such indirect techniques may not be applicable to all linkers. In another study[2], various linking groups were attached to p-aminobenzyl EDTA and its derivatives, and isolated prior to immunoconjugation. Since the starting materials in this study were the products of multi-step syntheses, the scale of each subsequent reaction was small and precluded extensive purification and characterization.

In the present study, symmetrical linking groups have been added to one of the COOH groups of trans-1,2-diaminocyclohexane N,N,N',N'-tetraacetic acid (CDTA) by two routes that utilize the CDTA monoanhydride (CDTAMA) as the starting material. Indium-111 labeled CDTA-immunoconjugates prepared from (CDTAMA) have shown similar tumor uptake and decreased kidney retention compared to DTPADA[3]. The ease of synthesis of the starting material in these routes, CDTAMA (from acetic anhydride/pyridine dehydration of commercial CDTA; CDTAMA selectively precipitates from the reation media, yield >90%) allows subsequent syntheses to be on a scale sufficient for isolation and characterization of all products.

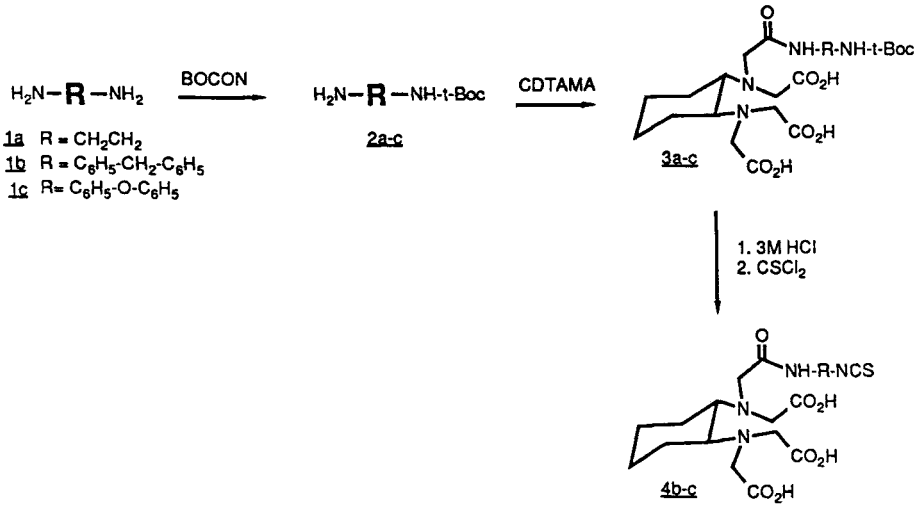
In the first route (Scheme 1), the symmetrical amines (ethylenediamine, 1a; 4,4'-methylenedianiline, 1b; and 4-aminophenyl ether, 1c) were converted to mono t-Boc derivatives 2a-c by the reaction of excess 1a-c with BOCON followed by purification by flash chromatography on silica gel. This was done to prevent CDTA from attaching to both ends of the linker. The reaction of 2a-c with CDTAMA in DMSO gave 3a-c. Hydrolysis of t-Boc followed by treatment with thiophosgene gave 4b and 4c but not 4a. Functionalization of the terminal amine of 3a may be achievable by formation of a bromoacetamide. In the second route (Scheme 2), CDTAMA is reacted with excess ethylenediamine in DMSO to introduce a primary amine into CDTA. Concentration of the reaction under vacuum removed excess ethylenediamine. Recrystallization of the residue from acetonitrile (ACN) gives pure 5. Ethylene glycol bis(hydrogensuccinate) was prepared from ethyl glycol and succinic anhydride[4], and converted to 6 using dicyclohexylcarbodiimide (DCC) and N-hydroxysuccinimide (NHS) in ACN. Compound 7 was similarly prepared from 1,10-decanedicarboxylic acid in DMF. Compound 5 was added in small portions over 12h to excess 6 and 7 respectively. Concentration of the reaction, followed by addition of excess $CH_2Cl_2$ to the residue precipitated 8 and 9 respectively. Concentration of the filtrate followed by recrystallization recovered unreacted 6 and 7. The CDTA-linkers have been characterized by NMR, conjugated to MAbs, labeled with $^{111}In$, and are currently under investigation in mice. Chemical reactivity of the CDTA linkers remains intact upon storage with desiccant.

In conclusion, the examples above demonstrate the general utility of these two routes in preparing CDTA-linkers. Additional symmetrical acids and amines containing potentially cleavable internal functional groups are either commercially available or can be readily synthesized. For these reasons and

due to the ease of synthesis of CDTAMA, this approach is attractive for the continued investigation of the ability of linking groups to modify the biodistribution of indium immunoconjugates.

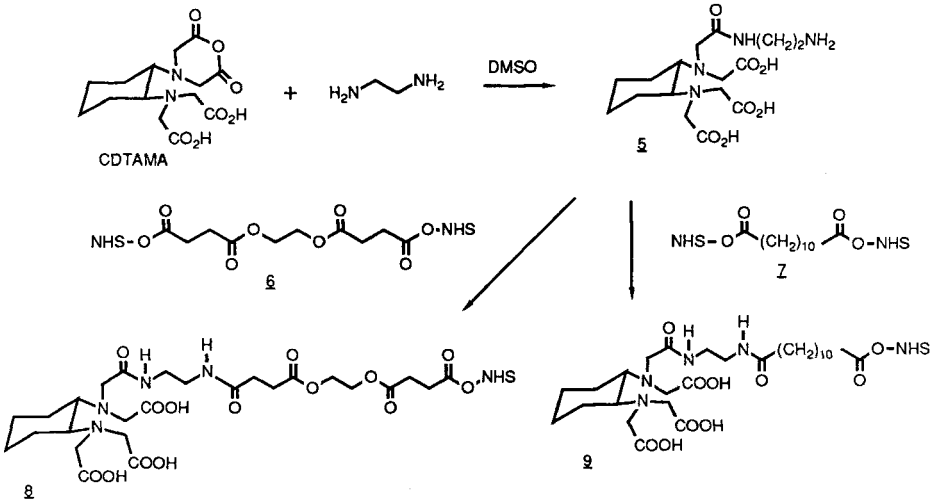
## Scheme 1

Synthesis of CDTA-Linkers from Symmetrical Amines and Cyclohexyl EDTA Monoanhydride




## Scheme 2

Synthesis of CDTA-Linkers from Symmetrical Acids and Cyclohexyl EDTA Monoanhydride

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NEW ACTIVE $N_2S_2$-ESTERS AND THE LABELING OF PROTEINS WITH Tc-99m

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Compared to DTPA-type ligands $N_2S_2$-chelates have definite advantages since they form uniform and more stable $^{99m}$Tc complexes. Appealed by these characteristics we chose this type of ligand for conjugation with monoclonal antibodies, although we were aware that by using the active ester route the $N_2S_2$-ligand might react with itself.

7-(4'-bromobutyl)-3,3,11,11-tetramethyl-1,2-dithia-5,9-diazacycloundecane (8), recently developed as a general purpose precursor for the synthesis of new $N_2S_2$-ligands, was used as the starting material. The formation of compound (8) is outlined in scheme I[1].

In order to bind the ligand to proteins by amide formation a carboxyl group was introduced into compound (8). Therefore, the bromide (8) was reacted with the sodium salt of p-hydroxybenzoic acid ethyl ester. As depicted in scheme II compound (9) was formed, because traces of $H_2O$ enabled ester hydrolysis. The reductive disulfide-bond cleavage was achieved with dithiotreitol (DTT). Compound (10) was isolated as the $BF_4$-salt. The reaction of compound (10) with dicyclohexyl carbodiimid (DCC) and the leaving groups tetraflorophenol (TFP) and thiophenol (PhSH) yielded the active esters (11a,b). The active esters (11a,b) were isolated by reverse-phase chromatography. The identity of compounds (11a,b) was proved by FAB-mass spectrometry and NMR. In protonated form the active esters were stable. Conjugation with IgG or other proteins was performed in aqueous solution at pH 8-8.5.

The conjugation yield for active ester (11a) was 65-75% estimated by chromatographic methods. Active ester (11b) was less reactive. Separation of the IgG-$N_2S_2$-ligand conjugates was performed by size-exclusion chromatography. Reconcentration to 10 mg protein/ml buffer and complexation of $^{99m}$Tc by the tin-reduction method afforded >95% protein-bound activity.

We are currently testing this labeling method with a rat sarcoma affine monoclonal antibody (A2.6). Animal experiments including the investigations of tumor affinity, organ distribution studies and activity kinetics are in progress.

Acknowledgement: A postdoctoral grant for one of us (M. M.) was kindly provided by Mallinckrodt Diagnostica B.V. (Netherlands).

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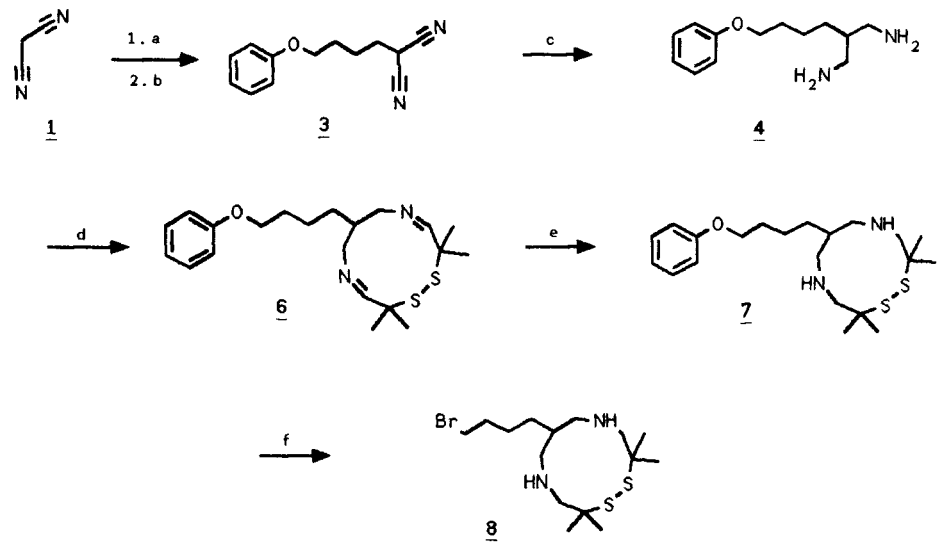
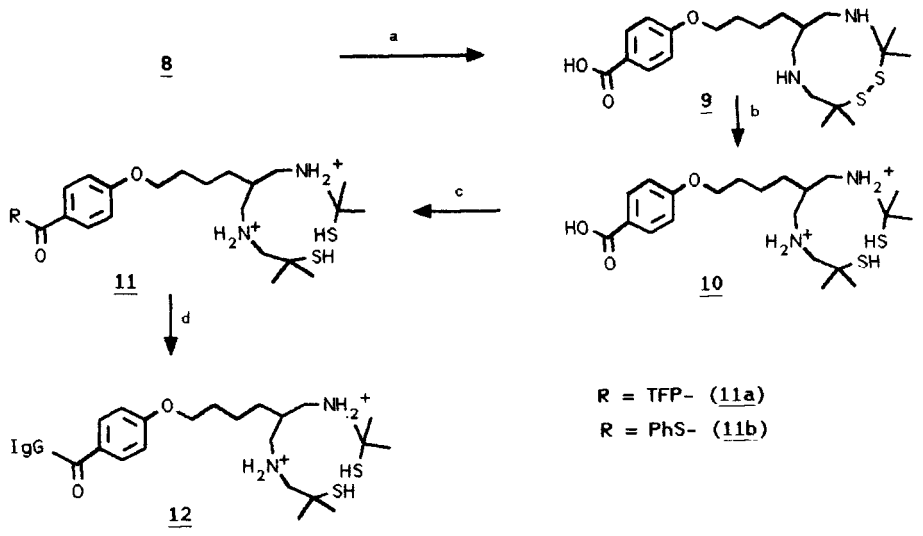
S C H E M E I




a: NaH,DMF; b: $PhO(CH_2)_4Br$ (2); c: $H_2$,Pd/C; d: $(-S-CMe_2-CHO)_2$ (5); e: $NaBH_4$ ; f: HBr,AcOH,95°C

S C H E M E I I




a: $NaOPhCO_2Et$; b: 1.DTT,2.$HBF_4$; c: DCC,TFP or PhSH; d: IgG,pH 8-8.5

ISOTHIOCYANATO BATOs: NEW $^{99m}$Tc REAGENTS FOR PROTEIN LABELING

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Introduction of an isothiocyanate (NCS) functionality into the R group of Technetium BATOs [$TcCl(dioxime)_3BR$] [BATO = Boronic Acid Adducts of Technetium DiOximes (1)] has allowed us to use BATOs for protein labeling. We have developed a novel class of NCS-containing boronic acids that can bind to both a technetium-dioxime complex and a protein to form a stable covalent bond between the two. The synthesis of two NCS-boronic acids, 3-isothiocyanatophenylboronic acid [$(OH)_2$BPITC], and 3-isothiocyanato-5-carboxyphenylboronic acid [$(OH)_2$B-NCS-COOH] are shown in Fig. 1. Fig. 2 shows the structure of two of the Tc-BATO-NCS complexes; $TcCl(DMG)_3$BPITC (Tc-PITC) and $TcCl(DMG)_3$B-NCS-COOH (Tc-NCS-COOH). (DMG=dimethylglyoxime, PITC=phenylisothiocyanate).

The Tc-BATO-NCS labeling reagents can be prepared by either of two methods. In the first method, $TcO_4^-$, a dioxime ligand, an NCS boronic acid and $SnCl_2$ are heated at pH 2-3 for 5 min. The desired complex $TcCl(dioxime)_3BR$ (R=PITC, NCS-COOH) forms in low yield (20-30%), due to side reactions between the NCS-boronic acid and other reagents in the reaction. However, Tc-BATO-NCS compounds can be formed in up to 90% yield by reaction of an NCS-boronic acid with the Tc(III) complex (2), $TcCl(dioxime)_3$. The latter compounds are made in good yield from freeze-dried kits. The Tc-BATO-NCS complexes can be prepared from $^{99}$Tc and have been characterized by elemental analysis, NMR, UV-Visible and IR spectroscopy, FAB Mass Spectrometry and X-Ray crystallography (Table 1).

When the Tc-BATO-NCS complexes are incubated with proteins, or other amine-containing compounds at pH 8-9.5, the NCS group on the Tc complex reacts with primary amines to form a thiourea bond (Fig. 3). Labeling yields are affected by pH, protein concentration and temperature. We have labeled several compounds with these reagents, including glycine, poly-lysine, IgG, and the monoclonal antibodies B72.3 and NP-4. Labeling was done at 37$^0$C, using a protein concentration of 10 mg/mL in pH 9.5 sodium phosphate buffer and a 2 hour reaction time. Labeled protein was HPLC purified via a size exclusion TSK column, using an ISRP (3) guard column to remove unreacted labeling reagent.

Analysis of the BATO-labeled antibodies B72.3 and NP-4 by gel filtration and by gel electrophoresis (SDS-PAGE) shows that no covalent aggregation has taken place. Under non-reducing SDS-PAGE conditions, at least 90% of the radioactivity was found to remain associated with the protein. This suggests that the observed labeling was through covalent bond formation. Under reducing conditions, a significant amount of delabeling occurred, presumably by reaction of the technetium with 2-mercaptoethanol. Affinity chromatography of BATO-labeled B72.3 and NP-4 showed that the labeled antibodies retain their immunoreactivity. The binding observed was always equal to, or greater than that observed with $^{125}$I labeled controls.

Biodistribution studies in GW 39 tumor-bearing nude mice were carried out with Tc-NCS-COOH labeled B72.3 (Table 2), NP-4 and NP-4 F(ab )$_2$ fragments. Significant localization of activity in tumors was seen. It appears from these results that reaction of antibodies with Tc-BATO-NCS complexes yields a stable $^{99m}$technetium-labeled protein that retains specificity for its antigenic target both *in vitro* and *in vivo*.

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Fig.1. Synthesis of Isothiocyanatophenyl Boronic Acids.

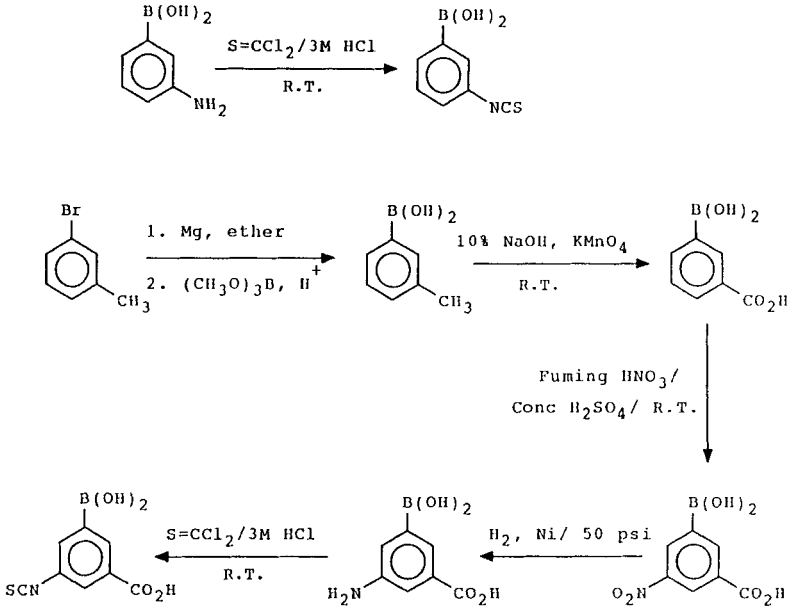
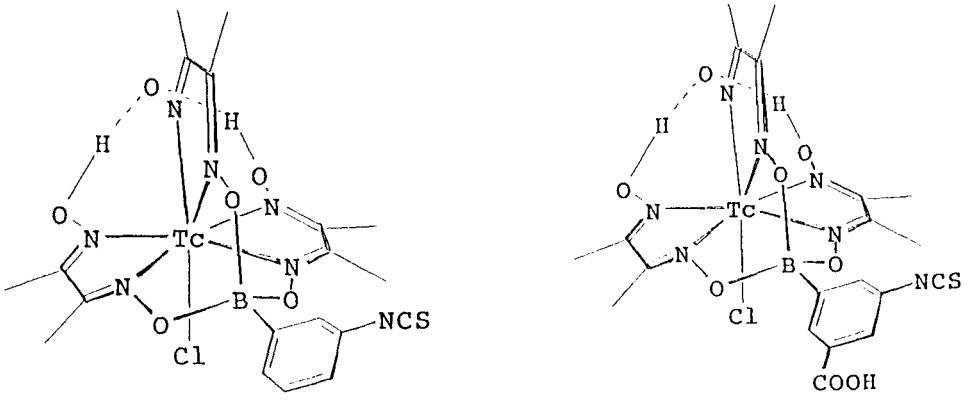



Fig. 2. Structure of Tc-BATO-NCS Complexes.




(1) $TcCl(DMG)_3BPITC$ [Tc-PITC] (2) $TcCl(DMG)_3B$(NCS-COOH) [Tc-NCS-COOH]

Fig. 3. Preparation of BATO labeled Proteins. (R=H, COOH)

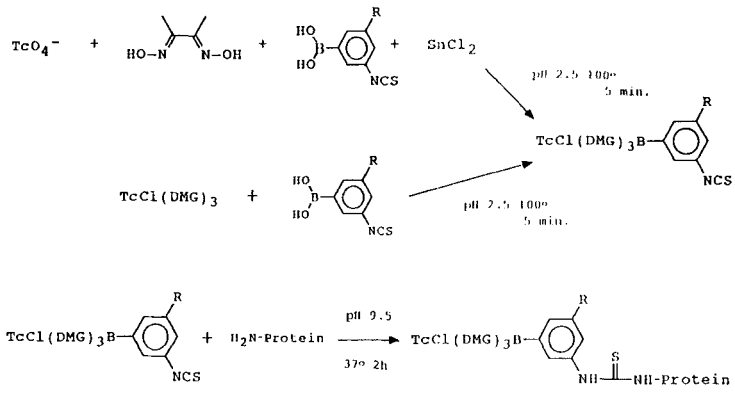



Table 1. Characterization of Tc-PITC (1) and Tc-NCS-COOH (2)

| Complex | Elemental Formula | Anal. | (Calc.) | νNCS (IR) |
|---|---|---|---|---|
| (1) | $C_{19}H_{24}N_7BClO_6STc$ | C 36.36 | (36.59) | 2056, 2120 $cm^{-1}$ |
| | | H 3.80 | (3.89) | |
| | | N 15.88 | (15.72) | |
| (2) | $C_{20}H_{24}N_7BClO_8STc$ | C 34.76 | (34.57) | 2100 $cm^{-1}$ |
| | (1.5 $H_2O$) | H 3.90 | (3.92) | |
| | | N 13.90 | (14.11) | |

Table 2. Biodistribution of $^{99m}$Tc-NCS-COOH Labeled B72.3 in Normal and Tumor-Bearing Mice at 24 hours post injection. (%I.D./gm, n=5). Comparison to $^{131}$I-B72.3.

*GW-39 tumor-bearing nude mice.

| ORGAN | NORMAL MICE | TUMOR BEARING* MICE | |
|---|---|---|---|
| | $^{99m}$Tc-B72.3 | $^{99m}$Tc-B72.3 | $^{131}$I-B72.3 |
| BLOOD | 13.37 (1.44) | 15.84 (2.26) | 18.98 (2.71) |
| LIVER | 5.63 (1.14) | 6.13 (1.23) | 5.50 (1.13) |
| INTESTINE | 1.79 (0.57) | 3.31 (0.30) | 1.45 (0.18) |
| STOMACH | N.D. | 1.60 (0.34) | 4.47 (1.12) |
| KIDNEYS | 4.48 (0.83) | 5.17 (0.87) | 6.26 (1.03) |
| URINE | N.D. | 6.50 (3.64) | 4.28 (1.70) |
| THYROID | N.D. | 4.79 (2.04) | 45.95 (14.7) |
| SPLEEN | 4.83 (2.17) | 3.82 (1.10) | 5.11 (1.51) |
| MUSCLE | 1.92 (0.80) | 1.46 (0.32) | 2.16 (0.32) |
| WASHED BONE | N.D. | 0.71 (0.29) | 1.24 (0.38) |
| TUMOR | --- | 10.47 (3.79) | 18.20 (8.36) |

**N-Succinimidyl-3-Iodo-($^{125}$I)-Benzoate. A new compound for protein iodination.**

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One of the major problems in the use of radioiodinated proteins for diagnosis and treatment of tumors, is their rapid in vivo deiodination and uptake of iodine into the thyroid. Regardless of the iodination method used (Chloramine-T, Iodogen or Bolton-Hunter), all involve substitution of the radioactive iodine ortho to the hydroxyl group on an aromatic ring. It is assumed that deiodination occurs due to the structural similarity between these iodopheyl groups and thyroid hormones. N-Succinimidyl-3-iodo-($^{125}$I)-benzoate (NSIB $^{125}$I), a compound which does not involve ortho substitution was proposed by others (1). Herein we describe two different approaches for preparing the above compound.

Procedure I: This procedure involves radioiodination of anthranilic acid, its deamination and convertion into m-iodo-($^{125}$I)-benzoic acid and then estrification of the latter with N-hydroxy succinimide (NHS) in the presence of dicyclohexylcarbodiimide (DCC), (Figure 1 A). This procedure is somewhat cumbersome as all steps are carried out on a labeled material.

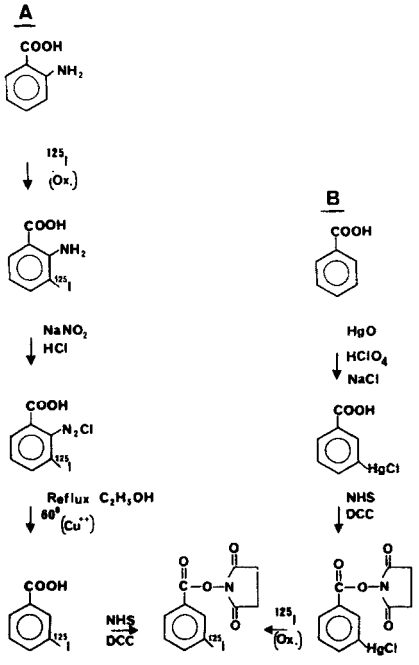


**Figure 1. Synthesis of NSIB-$^{125}$I.**

Procedure II: This approach, which overcomes the latter disadvantages, involves the synthesis of chloromercuric benzoic acid, its esterification with NHS/DCC and exchanging the mercuric moiety with radioactive iodine in the presence of an oxidant, (Figure 1 B) The desired compound (prepared by both ways) was purified on silica TLC plates extracted with tetrahydrofuran (THF) and has been attached to human serum albumin (hSA) in the presence of borate buffer 0.01 M pH=8.5 (Figure 2). About 25-50% of the radioactivity was bound to the protein. The radioiodinated protein was purified on Sephadex G-25

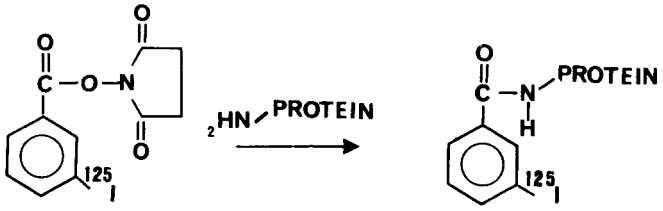


**Figure 2. NSIB-$^{125}$I iodinated protein.**

colounm resulting in 90% radiochemical purity. For comparison hSA was also radioiodinated using Chloramine-T (Ch-T). Both preparations were analysed for free iodide on paper chromatography using methanol as the mobile phase. In contrast to the Ch-T iodinated hSA which was deiodinated at a rate of 12.5% after 21 days, the hSA-NSIB-$^{125}$I did not undergo any detectable deiodination upon storage during the same period. It is believed that N-succinimidyl-3 Iodo($^{125}$I)-Benzoate provides new protein iodination sites which are more stable in vitro and in vivo.

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THE USE OF PENTAFLUOROPHENYL DERIVATIVES FOR THE $^{18}F$ LABELING OF PROTEINS.

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Specific labeling of amino acid residues in antibody proteins and peptides with positron emitting radionuclides of the halogen series ($^{18}F$, $^{76}Br$, and $^{124}I$) should increase the potential of labeled monoclonal antibodies.

We have investigated a number of pentaflourophenyl (PFP) derivatives as candidates for use as reagents in the synthesis of $^{18}F$ labeled proteins, and we have found two to be useful in the labeling of HSA , quickly and in relatively high yield.

Pentaflourophenyl derivatives have been shown to be susceptible to nucleophilic attack, primarily at the position para to the non-fluorine group (1); the rate is dependent on that group (proportional to electron withdrawing strength). We have found efficient $^{18}F$ for F exchange in PFP derivatives bearing a wide variety of electron withdrawing groups (Table 1).

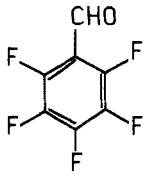
| Group | Reaction Time (min) | Decay Corrected Yield (%) | EOB Yield (%) |
|---|---|---|---|
| CHO | 40 | 50 | 39 |
| $NO_2$ | 45 | 71 | 54 |
| CN | 83 | 43 | 25 |
| $COCH_3$ | 59 | 76 | 52 |
| $CONH_2$ | 45 | 51 | 38 |
| COOEt | 47 | 73 | 54 |
| COOPh | 23 | 70 | 60 |
| COO-p-CN-phenyl | 27 | 30 | 25 |
| COO-TFP | 16 | 32 | 29 |
| $SO_2N(CH_2)OH$ | 45 | 39 | 29 |
| $SO_3N(Me)(Ph)$ | 43 | 36 | 27 |

TFP = tetrafluorophenyl

Ligands bearing aldehyde functionality may be attached to proteins by reductive alkylation in the presence of $NaBH_4$ or $NaBH_3CN$ (2). We have had some success labeling HSA with $^{18}F$-bearing pentafluorobenzaldehyde (1). Incorporation of $^{18}F$ into the aldehyde, using the tetrabutylammonium (TBA) salt in DMSO, proceeds in 60% decay-corrected yield within 30 minutes. Treatment of HSA with the aldehyde with $NaBH_4$, using water/DMSO as solvent, occurs in 15% yield (decay corrected). The total time for

the synthesis is approximately 1.5 h. Reductive alkylation using $NaBH_3CN$ gave incorporation of $^{18}F$ into HSA, but biodistribution data has suggested in vivo hydrolysis of the intermediate Schiff base and re-release of the labeled ligand. This indicates that reduction of imine to amine by $NaBH_3CN$, under these conditions, is too slow for introduction of the label.

As activated esters are useful for the covalent attachment of ligands to amino-bearing molecules, we are interested in pentafluorophenyl esters that would survive exchange conditions ($F^-$, heat, polar solvents) and react quickly with proteins (considering the short half life of $^{18}F$). A series of alkyl and phenyl esters proved to be unreactive. N-OH-succinic and pentafluorophenyl ester decomposed under exchange conditions. However, 2,3,5,6-tetrafluorophenyl-pentafluorobenzoate (2) readily incorporated $^{18}F$ and reacted quickly with HSA, to give an absolute yield of 15% labeled HSA, in 72 mins., based on starting tetrabutylammonium fluoride (TBAF).

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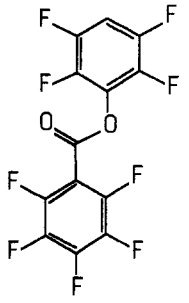


2

In an analogous method, 2,3,5,6-tetrafluorophenyl-pentafluorobenzoate (2) was radiofluorinated and used to acylate HSA.

Biodistribution studies in rats (six rats at each time point) injected with a cocktail of $^{18}F$ and $^{125}I$ labeled HSA, using the aldehyde method, showed similar distribution at 5 and 60 min. Washout from the blood was minimal. At 60 min blood activity, in % dose per gram, was 5.04+/-.05 and 6.07+/-.06 for the $^{18}F$ and $^{125}I$ labeled HSA respectively. Bone uptake was minimal.

This work was supported by DOE grant DE-FGO2-86ER60460.

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RADIOIODINATION AND ASTATINATION OF MONOCLONAL ANTIBODIES USING HETEROCYCLIC ACYLATION AGENTS.

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Direct radioiodination of proteins occurs primarily via electrophilic substitution of tyrosine residues. It is generally assumed that the dehalogenation of monoclonal antibodies (MAbs) which occurs *in vivo* reflects the recognition of these iodination sites by the deiodinases normally involved in the metabolism of thyroid hormones such as iodotyrosine and thyronine. Previously, we reported that labeling MAbs using N-succinimidyl-3-(tri-*n*-butylstannyl)benzoate (ATE), a method not involving iodination ortho to a hydroxyl group on an aromatic ring, decreased the thyroid uptake of radioiodine (1) and increased tumor retention of radioactivity (2). Since the recognition of iodinated heterocycles by endogenous deiodinases might be less likely than aryl iodides, we have explored the potential utility of labeling MAbs using N-succinimidyl-5-(tri-*n*-butylstannyl)pyridine-3-carboxylate (SPC) and N-succinimidyl-5-(tri-*n*-butylstannyl)furan-2-carboxylate (SFC).

The syntheses of SPC and SFC were performed by reacting 5-bromonicotinic acid and 5-bromo-2-furancarboxylic acid, respectively, with n-BuLi and tri-*n*-butylstannyl chloride. Subsequent reaction with dicyclohexyl carbodiimide and N-hydroxysuccinimide gave SPC and SFC whose structures were confirmed by $^1$H-NMR. Radioiodinations were performed in dichloromethane-acetic acid using t-butylhydroperoxide as the oxidant. Unlike ATE which could be labeled with I-131 in nearly quantitative yield at room temperature, labeling of SPC required heating to 60°C and proceeded in 40-60% yield. In contrast, iodination of SFC in 70-80% yield could be performed at 25°C. Coupling of the labeled esters to 200-250 $\mu$g of 81C6, a MAb reactive with gliomas, proceeded in 38% and 53% yield for SPC and SFC, respectively.

The tumor specificity of 81C6 radioiodinated using SPC, SFC and ATE were compared *in vitro* and *in vivo*. Specific binding (% bound to D-54 MG human glioma homogenate minus binding to rat liver) was 87% for SPC, 83% for SFC and 86% for ATE. Paired-label experiments were performed in athymic mice with D-54 MG tumors receiving 81C6 labeled with I-125 using ATE and labeled with I-131 using either SPC or SFC. With SPC, no differences in thyroid uptake were seen (3 d, SPC, 0.15±0.08%; ATE, 0.15±0.09%; 4 d, SPC, 0.11±0.02%; ATE, 0.12±0.03%) but tumor uptake was higher for MAb labeled using ATE (3 d, SPC, 15±2%/g; ATE, 17±3%/g; 4 d, SPC, 18±6%/g; ATE, 22±7%/g; mean SPC/ATE uptake ratio, 0.84±0.01 at 3 and 4 d). However, SPC/ATE uptake ratios in most normal tissues were 0.75-0.80, resulting in slightly higher tumor:normal tissue ratios for 81C6 labeled using SPC. At 3 d, thyroid uptake of SFC (1.3±0.4%) was higher than ATE (0.11±0.05%), tumor uptake was lower (SFC, 22±10%/g; ATE, 26±11%/g; SFC/ATE 0.84±0.03) and tumor:normal tissue ratios were slightly lower.

Both SPC and SFC could be used to label 81C6 IgG with 7.2 h At-211 in yields similar to those for I-131. In a preliminary study with MAb labeled with At-211 using SFC, at 16 h, tumor uptake was 9.5±1.0%/g, tumor/liver was 3 and tumor/blood was 2.

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Table 1 Tumor to Tissue Ratios 4 Days after Injection of Radioiodinated 81C6 IgG in Athymic Mice with D-54 MG Xenografts

| | Experiment 1 | | Experiment 2 | |
|---|---|---|---|---|
| Tissue | I-125 ATE | I-131 SPC | I-125 ATE | I-131 SFC |
| liver | 26 | 27 | 22 | 20 |
| spleen | 28 | 30 | 30 | 30 |
| lungs | 16 | 17 | 17 | 17 |
| heart | 28 | 31 | 29 | 27 |
| kidneys | 23 | 25 | 23 | 22 |
| stomach | 97 | 103 | 130 | 67 |
| sm. intestine | 64 | 58 | 71 | 77 |
| lg. intestine | 103 | 81 | 66 | 92 |
| muscle | 60 | 66 | 62 | 58 |
| blood | 7 | 7 | 8 | 7 |
| brain | 225 | 242 | 237 | 219 |

AROMATIC ACYLATION REAGENTS FOR USE IN THE RADIOIODINATION OF PROTEINS.

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The Bolton-Hunter reagent (BH) (1), N-succinimidyl 3-(4-hydroxy-3-[$^{125}$I]iodophenyl)propionate, is used frequently for *in vitro* applications of labeled proteins. We have developed a conceptually similar reagent, N-succinimidyl-3-(tri-*n*-butylstannyl)benzoate (ATE) (2), which should be more useful for *in vivo* applications since a) ATE lacks the two-carbon spacer between the aromatic ring and the active ester, which should increase conjugation yields by decreasing hydrolysis of the active ester and b) the iodophenyl group created by the electrophilic iododestannylation of ATE does not contain a hydroxyl group, decreasing the structural similarity to thyroid hormones which undergo rapid dehalogenation. Although the thyroid uptake of radioiodine for monoclonal antibodies (MAbs) labeled using ATE has been shown to be more than 20-fold less than for MAbs labeled directly using Iodogen (3), the *in vivo* behavior of MAbs labeled via BH has not been well documented, particularly in direct comparison to those labeled using ATE.

Conjugation efficiencies for BH and N-succinimidyl-3-[$^{131}$I]iodobenzoate, synthesized from ATE as described (3), were compared using goat IgG in pH 8.5 borate and a 20 min reaction at 4$^{o}$C. Yields for both were dependent on protein concentration (Table 1), and at $\geq$150 $\mu$g/75$\mu$L, yields using ATE were about twice those obtained using BH. Using 150 $\mu$g of goat IgG and a pH range of 8.5-10, optimal yields were obtained with both reagents at a pH of 9.5 and again, the coupling efficiency for ATE was at least twice that of BH (Table 2).

A paired-label study was performed in normal mice injected with MAb 81C6 $IgG_{2b}$ labeled with $^{125}$I and $^{131}$I using BH and ATE, respectively. Thyroid uptake of $^{125}$I was 2 to 2.5-fold higher than $^{131}$I (Table 3), suggesting more rapid dehalogenation of MAbs labeled using BH. No other significant differences in tissue distribution of the two nuclides were observed. Thyroid uptake at 3 d also was compared in mice receiving 81C6 IgG labeled with $^{125}$I using BH and $^{131}$I using Iodogen. Despite the fact that both methods involve iodination ortho to a hydroxyl group, thyroid accumulation of $^{131}$I (6.8$\pm$2.8%) was considerably higher than $^{125}$I (0.44$\pm$0.05%).

To further investigate the potential role of aromatic substituent on thyroid uptake and to determine whether ortho substitution of electron donating groups would increase C-I bond strength *in vivo*, N-succinimidyl-2,4-dimethoxy-3-(tri-*n*-butylstannyl)benzoate (DMATE) was synthesized as described (4). Iodination of DMATE using t-butylhydroperoxide proceeded in 75-80% yield after a 10 min reaction and after HPLC purification of the labeled ester, about 60% of the activity could be coupled to MAb. Paired-label studies 1-4 d after MAb injection indicated that thyroid uptake for DMATE (0.63-1.27%) was higher than for ATE (0.42-0.53%). Taken together, these results suggest that the presence of a hydroxyl group ortho to the site of iodination on a MAb is not the sole factor determining thyroid uptake and presumably, dehalogenation.

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Table 1 Coupling Efficiency as a Function of Protein Concentration

| μg Goat IgG per 75μL | % Yield | |
|---|---|---|
| | Bolton Hunter | ATE |
| 75 | 24.3±4.2 | 30.3±5.4 |
| 150 | 22.2±3.1 | 52.0±2.5 |
| 225 | 31.0±2.4 | 58.9±1.5 |
| 300 | 28.1±1.6 | 61.3±18.0 |
| 750 | 34.5±0.7 | 63.4±10.0 |

Table 2 Coupling Efficiency as a Function of pH

| pH | % Yield | |
|---|---|---|
| | Bolton Hunter | ATE |
| 8.5 | 22.2±3.1 | 52.0±2.5 |
| 9.0 | 31.2±2.1 | 63.9±4.5 |
| 9.5 | 38.3±0.4 | 82.0±4.9 |
| 10.0 | 34.4±3.6 | 63.8±3.3 |

Table 3 Thyroid Uptake of Radioiodinated 81C6 IgG

| Days | % Injected Dose | | Significance |
|---|---|---|---|
| | Bolton-Hunter | ATE | |
| 3 | 0.37±0.04 | 0.18±0.04 | P<0.001 |
| 4 | 0.45±0.05 | 0.22±0.03 | P<0.001 |
| 5 | 0.39±0.05 | 0.15±0.05 | P<0.001 |
| 6 | 0.33±0.06 | 0.13±0.02 | P<0.01 |
| 7 | 0.26±0.06 | 0.11±0.02 | P<0.01 |

## $^{199}$Au PRODUCTION FOR USE AS A RADIOLABEL OF GOLD CLUSTER IMMUNOCONJUGATES

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$^{199}$Au is an attractive radionuclide for radioimmunotherapy due to its 3.14 day half-life, intermediate energy $\beta^-$ emission ($\beta^-_{max}$ 295 keV), and imageable gamma ray (158 keV, 37%). A method has been developed to covalently link stable clusters of gold atoms to monoclonal antibodies(1), offering the possibility of delivering a greater amount of radiolabel per antibody than previously attainable. Initial gold radiolabeled cluster studies made use of $^{198}$Au/$^{199}$Au produced at the High Flux Beam Reactor (HFBR) at BNL by the $^{197}$Au (n,$\gamma$) route (~80 mCi/mg) which was incorporated into an eleven gold atom cluster. Fab', F(ab')$_2$ and intact IgG of the anticolon ca MAb 17-1A were radiolabeled with gold clusters and the in-vivo distribution was evaluated in mouse tumor xenografts. This data compared favorably to $^{111}$In-DTPA-Ab control data except for lower bone and liver retention for the gold labeled antibodies(2).

The purpose of the research reported here was to develop production methods for high specific activity $^{199}$Au. Since a significant advantage of radiolabeling with clusters is the increased number of radiolabels per antibody, a large portion of the 11 gold atoms in the cluster must be radioactive $^{199}$Au. We have investigated an alternative production route to higher specific activity gold, $^{198}$Pt(n,$\gamma$)$^{199}$Pt $\xrightarrow{\beta^-}$$^{199}$Au. Enriched $^{198}$Pt targets would be irradiated to produce short-lived $^{199}$Pt ($t_{1/2}$ = 30.8 min) which decays by $\beta^-$emission to $^{199}$Au. Calculations show that bombarding 100 mg of $^{198}$Pt for seven days in the HFBR at a flux of 8.2x10$^{14}$ n/cm$^2$-sec would yield 1.5 Ci of $^{199}$Au with a theoretical specific activity of 209 Ci/mg. Carrier-free radiochemical separations of $^{199}$Au from platinum targets have also been studied using targets of 20-60 mg of natural platinum metal (7.2% $^{198}$Pt), typically irradiated for 3 hours at the Brookhaven Medical Research Reactor (BMRR) at a flux of 4.5x10$^{13}$ n/cm$^2$-sec. The principal radionuclides produced are shown in Table I.

The results for three Au/Pt radiochemical separation procedures adapted from the literature are reported here. One scheme is based on the selective extraction of bromoauric acid, $HAuBr_4$, into isopropyl ether(3) with results reported in Table II. Table III shows the results of a method devised from anion exchange in mixed solvent media, acetone/HCl(4), and acetone/$HNO_3$(5). In addition, an extraction chromatography study employing tri-n-butyl phosphate(6) (TBP) is summarized in Table IV. The extraction chromatography with TBP has been shown to yield the highest separation factor and is the fastest and most reliable procedure for remote operation.

In conclusion, these studies have shown that $^{199}$Au can be produced with high specific activity and high radionuclidic purity for antibody labeling with gold clusters.

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Table I. Principal radionuclides formed from a 3 hr neutron irradiation of $^{nat}Pt$ at the BMRR at BNL.

| Nuclide | $t_{1/2}$ | $E_\gamma$ | Theoretical Ativity per mg of $^{nat}Pt$ ($\mu$Ci)* |
|---|---|---|---|
| $^{199}Au$ | 3.14 D | 158.5, 208.2 | 30.7 |
| $^{191}Pt$ | 2.96 D | 538.9 | 1.8 |
| $^{195m}Pt$ | 4.02 D | 98.9, 129.7 | 2.6 |
| $^{197}Pt$ | 18.3 H | 77.3 | 65.0 |

* Platinum targets were dissolved in aqua regia, evaporated to dryness, and taken up with 6N HCl. Actual activities averaged 90-97% of the theoretical calculations.

Table II. Carrier-free separation of $^{199}Au$ from platinum targets employing bromoauric acid extraction into ether.

| | |
|---|---|
| Separation Factor*: | $2 \times 10^{-3}$ |
| Separation Time: | 30 min. |
| $^{199}Au$ Recovery: | 95% |

* Separation factor was determined for the following scheme: The target solutions were made 2.9N in HBr and extracted twice with equal volumes of isopropyl ether. The combined organic extracts were washed with 4N HBr, evaporated to dryness, and brought up to volume. Platinum contamination was determined from the absorbance (403 m$\mu$) of the Pt-$SnCl_2$ complex, and gold recovery from radioactivity assay.

Table III. Carrier-free separation of $^{199}Au$ from platinum targets using anion exchange in mixed solvent media.

| Eluant: | 10% HCl/90% acetone | 5% $HNO_3$/5% $H_2O$/90% acetone |
|---|---|---|
| Separation Factor*: | $3 \times 10^{-4}$ | $3 \times 10^{-4}$ |
| Separation Time: | 2 hr | 2 hr |
| $^{199}Au$ Recovery: | 60-80% | 80-92% |

* Based on the following procedure: The target solution was made 0.1 N HCl and loaded on an anion exchange column, 6.5 cm x 0.7 (ID) cm, Dowex 1-X8 100-200 mesh, $Cl^-$ form. Gold was selectively eluted with 100 mL of mixed organic solvent and gold recovery was determined from radioactivity assay. Platinum contamination was calculated from absorbance measurements (403 mμ) of the Pt-$SnCl_2$ complex.

Table IV. Carrier-free separation of $^{199}Au$ from platinum targets using extraction chromatography with TBP/$HNO_3$.

| | |
|---|---|
| Separation Factor*: | $> 10^{-5}$ |
| Separation Time: | 1 hr |
| $^{199}Au$ Recovery: | 95% |

* Based on the following scheme: The target solution in aqua regia was introduced into a 2.54 cm x 0.4 cm (ID) column containing TBP adsorbed on Silica Gel, 100-200 mesh. Platinum was selectively eluted with 10 mL of 3.5 N $HNO_3$ and gold was subsequently eluted with 2 mL of 14 N $HNO_3$. Platinum was not detected by either radioactivity assay or absorbance measurements (403 mμ) of the Pt-$SnCl_2$ complex. Gold recovery was determined from radioactivity assay.

ASSESSMENT OF DRY DISTILLATION METHODS FOR IMPROVING PROTEIN LABELING YIELDS WITH ASTATINE-211.

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Labeling of proteins with astatine has been carried out by a number of research groups. Previous protein labelings have been conducted by isolation of the astatine-211 prior to reacting it with a suitable compound that is subsequently reacted with the protein. Indeed, labelings involving the reaction of astatine-211 with *para*-diazobenzoic acid were quite lengthy and required extensive manipulation of the solutions containing astatine and astatinated compounds (1,2). More recent protein labeling has utilized arylstannane intermediates (3,4) which contain protein reactive functional groups, simplifying the labeling process. Unfortunately, most protein labeling methods still involve three primary steps: isolation of the astatine from the bismuth target material; reaction with an aryltin intermediate; and conjugation with the protein. Low recoveries of astatine-211, and the safety of personnel manipulating solutions of astatine led to studies of astatine labeling in which astatine was distilled from the target directly into a solution containing the compound to be labeled.

We have investigated two methods of dry distillation of astatine-211 for labeling of a protein reactive compound, N-succinimidyl *para*-tri-*n*-butyl-stannylbenzoate (PSB). In the investigations, astatine-211 has been distilled at temperatures ranging from 250-750°C from an irradiated bismuth target into a MeOH solution of acetic acid, N-chlorosuccinimide, and PSB. However, it was found that the distillations gave the best recoveries when the oven temperature range was between 600-750°C. In one series of astatine labeling experiments, the distilled astatine was bubbled through the reaction solution (at room temperature) using an argon carrier gas. This method worked very well for several experiments providing total recoveries of astatine-211 of greater than 75%, then dramatic decreases in the recovered yields (to 25-35%) were noted. While not fully understood, the decrease may be attributable to a change (increase) in the bismuth thickness of the target. To improve recoveries, the dry distillations were conducted under a vacuum (-20 mmHg) and the distilled astatine was condensed (-78°C) onto the reaction solution. After distillation, the vessel containing the reaction solution and trapped At-211 was warmed to room temperature for 10 minutes. Once the At-211 had reacted with PSB to form the astatinated benzoate, sodium metabisulfite solution was introduced and the MeOH solvent was either removed by a stream of argon gas or under vacuum. Protein labeling was afforded by simply introducing the antibody in a basic buffer solution into the reaction vessel via a syringe. Once this was accomplished, the crude reaction mixture was placed onto a G-25 (PD-10) size exclusion column. In 8 vacuum distillations conducted with oven temperatures above 600°C, 60-82% of the astatine-211 was distilled into the reaction vessel, with yields of 75-82% being obtained when the distillations were conducted at 700-730°C.

With these investigations, we have developed a method of labeling protein with astatine-211 that can be accomplished in 60-75 min. with overall labeling yields of protein to approx. 45% (decay corrected), and that can be conducted with minimal risk to the labeling personnel.

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**Figure I:** Schematic of Vacuum Distillation Apparatus.

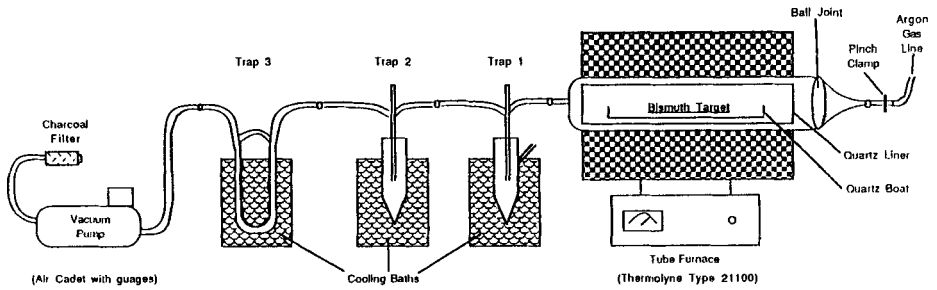


**Table I:** Radioactivity Distribution in Vacuum Distillation Setup*

| Distillation # | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Oven Temp. | 250-430 | 400-650 | 600 660 | 625-680 | 710-720 | 665-700 | 600-650 | 700-715 | 710-730 | 700-730 |
| Charcoal Trap | <1% | - | <1% | <1% | <1% | <1% | <1% | 1% | 1% | 3% |
| Distilling Tube | <1% | - | 2% | <1% | 1% | <1% | 1% | <1% | 1% | 5% |
| Target and Boat | 68% | - | 24% | 18% | 11% | 30% | 21% | 15% | 17% | 13% |
| Reaction Vessel** (bottom)*** | 25% | 50% | 70% | 66% | 79% (60%) | 60% | 74% (61%) | 82% (81%) | 75% (72%) | 77% (44%) |
| Trap #2 (-78°C) | <1% | - | 4% | 13% | 6% | 10% | 2% | <1% | <1% | <1% |
| Trap #3 (-78°C) | n a | n a | n a | 4% | 2% | <1% | <1% | <1% | <1% | <1% |

*Measured by counting radioactivity in 1 inch segments of apparatus (shielded by 2 in. lead bricks)

**Reaction Vessel same as Trap 1

***(bottom) refers to amount of activity found in bottom portion of trap #1 when it was removed for counting. Trap was cooled to -78°C in all but distillation #10, which was cooled to 0°C.

## NOVEL ALKYLAMINE LINKER TECHNOLOGY FOR CONJUGATION OF NEW HEXADENTATE RADIOMETALLIC NUCLIDES CHELATING AGENTS TO ANTIBODIES.

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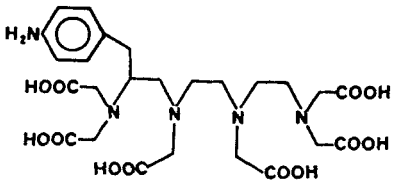
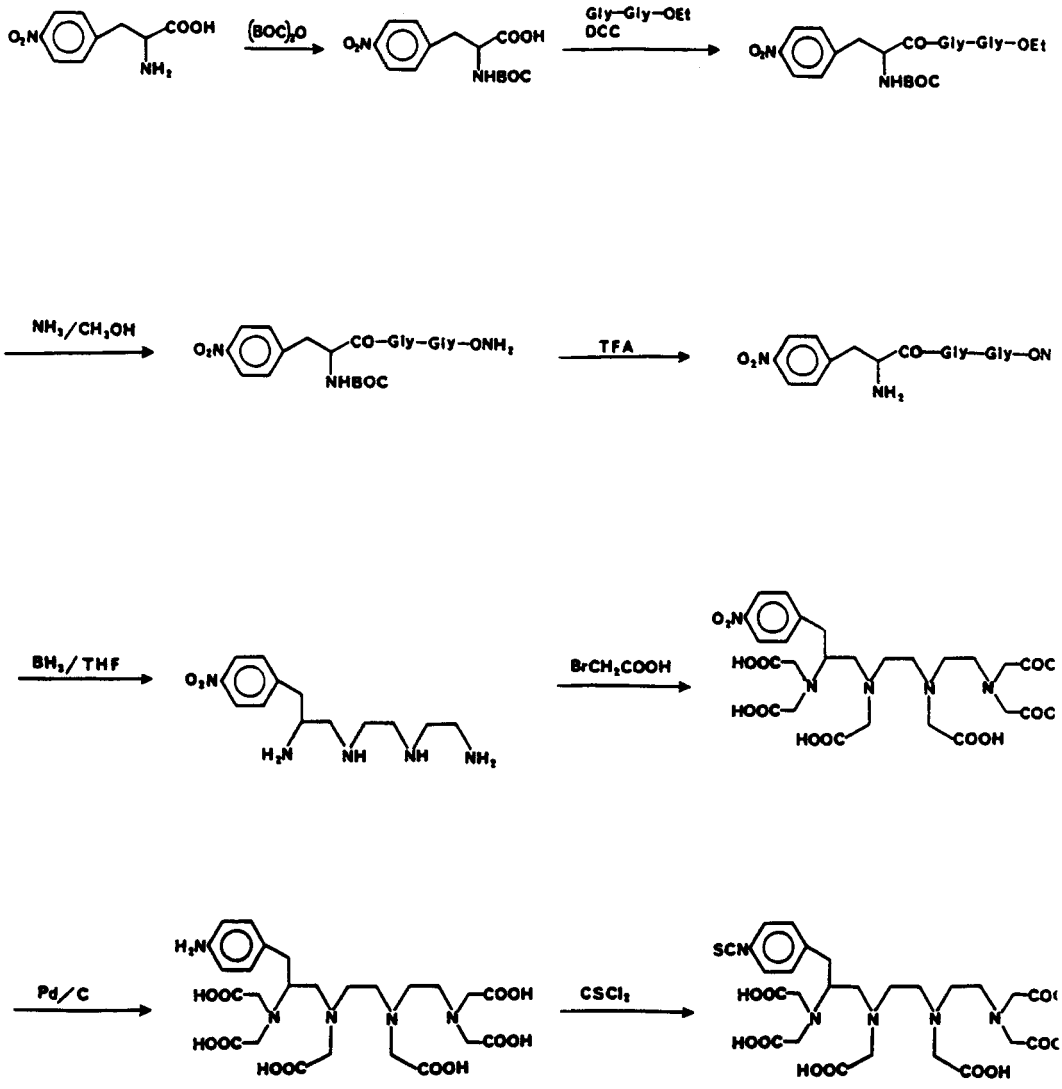
Monoclonal antibodies labeled with radiometallic nuclides are being utilized increasingly for tumor detection and radioimmunochemotherapy in place of earlier more invasive clinical methods. However, the dissociation of radioactivity from the antibody chelate nuclide complex in vivo and loss of the immunospecificity of antibody as a consequence of conjugation procedures are the two major difficulties encountered in realizing full potential of the monoclonal antibodies (1). In an attempt to overcome these difficulties, we have synthesized a hexadentate chelating agent Triethylenetetraaminehexaacetic acid (TTHA) derivative (2) and conjugated this reagent to proteins through alkylamine linkage making use of the latent cross-linking potential of α-hydroxyaldehyde, specifically glycolaldehyde.

The functional part in this new chelating agent is six carboxyl and four nitrogen atoms to increase the strength of interactions of metal nuclides and thus decrease the propensity of transchelation in vivo. The synthesis of the reagent involves the reduction of 4-nitro derivative of tripeptide, phenylalanylglycylglycine amide, to the corresponding amine followed by carboxymethylation of the amines. The Pd/C reduction of $NO_2$ group give the reagent containing a free amino group. This amino group has been designed into this chelating agent to facilitate the conjugation of this hexadentate moiety to the antibody.




For conjugation, the chelating agent was converted in situ to 2 oxoethyl amino benzyl-TTHA by incubating the reagent with glycolaldehyde (Figure 1). The Schiff base adduct of the amino group of benzyl-TTHA and glycolaldehyde undergo an intramolecular rearrangement known as Amadori rearrangement to generate the 2 oxoethyl derivative of the reagent. This aldoamine adduct prepared in situ was reacted with human serum albumin (HSA) in the presence of sodium cyanoborohydride (reductive alkylation). For comparison, isothiocyante derivative of Bz-TTHA was also conjugated to HSA which resulted in the thiocarbamoyl linkages between protein and chelating agent. After inserting $^{111}In$ into both of these preparations, the $^{111}In$ that might have non-specifically bound to HSA was removed by the addition of an excess of DTPA into samples. Subsequent size exclusion HPLC separated the protein with bound $^{111}In$ from free DTPA and DTPA bound $^{111}In$.

Preparation of HSA with the hexadentate chelating agent conjugated with alkylamine as well as thiocarbamoyl linkage have been compared for their tissue distribution after bonded with $^{111}In$ as discussed. Both preparations gave similar tissue distribution in CD-1 mice. Alkylamine linked preparation showed greater stability in vivo as compared to thiocarbamoyl




Scheme 1. Synthesis of Amino Benzyl Triethylenetetraaminehexaacetic Acid

linked preparation. The activity in the blood for alkylamine linked protein after 1 hr post injection in mice was 66% of the total injected dose as compared to 57% activity with the thiocarbamoyl linked preparation. There was no retention of activity in the liver in either case indicating that there was not transchelation of $^{111}In$ in the liver.

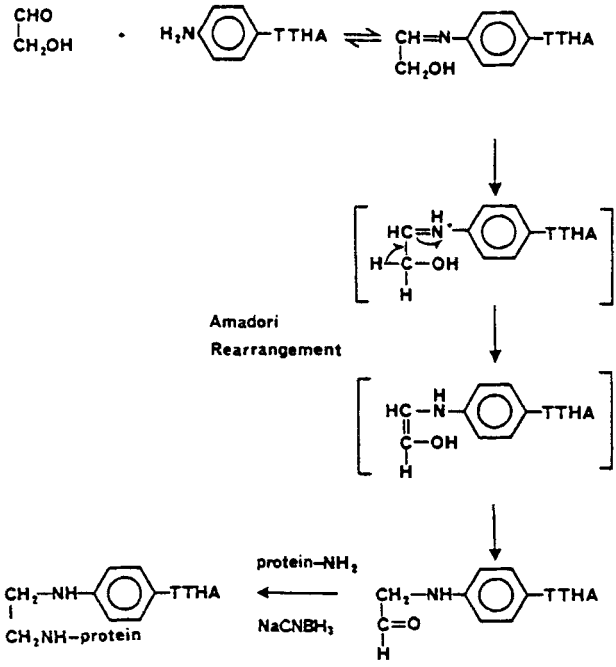



Figure 1. Covalent attachment of $NH_2$-Bz-TTHA to protein using crosslinking potential of glycolaldehyde.

The alkylamine linker technology discussed here is in principle adoptable to other chelating agents currently being used and should prove advantageous to their contemporary isopeptide and thiocarbamoyl linker technology. It is anticipated that further studies with glycolaldehyde and other $\alpha$-hydroxyaldehydes would lead to the development of an ideal, simpler linker technology and the systematic evaluation of other analogs to TTHA would provide better procedure for radioimaging of tumors with desired characteristics of high target to background ratio.

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QUANTIFICATION OF ANTIBODY (MAB) SULFHYDRYLS FOLLOWING CONTROLLED REDUCTION OF DISULFIDES; INFLUENCE ON MAB PROPERTIES.

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Reduction of MAb disulfide groups to sulfhydryls has been shown to be an efficient means of labeling MAb with Tc-99m (1-5). We have evaluated five reducing agents namely $SnCl_2$, 2-mercaptoethanol (2 ME), dithiothreitol (DTT), dithioerythritol (DTE), and ascorbic acid (AA). AA among them has given us the best results and is considered particularly suitable for a kit type of labeling procedure. Since MAb helical structure is important for the preservation of immunoreactivity and specificity of the protein (6), it is important to ascertain that such a reduction is limited to only a few disulfide groups. In order to quantify the number of disulfide groups reduced, a spectrophotometric method was developed that formed a ninhydrin complex with cysteine residues on MAb treated with the reducing agents. The complex is formed within 10 minutes in acidic media, has a maximum absorbance at 520 nm and a molar extinsion coefficient of 30250, allowing one to determine as low as 0.04 ug cysteine/ml solution. Using the quantity of cysteine produced and the following equation, the percentage of disulfides reduced per molecule of MAb was calculated.

$$\frac{A \times B \times 100}{C \times D \times E}$$ where,

A = the quantity of cysteine estimated, in ug,
B = the molecular wt. of the protein
C = the quantity of MAb, in ug, used for reduction
D = the number of theoretically possible molecules of cysteine per MAb molecule and
E = molecular wt. of cysteine

There are approx. 175 disulfide groups in an IgM molecule, 35 in IgG and 25 in $F(ab')_2$, giving approx. $1.2 \times 10^{14}$ disulfide groups/ug IgM, $1.4 \times 10^{14}$ for IgG and $1.4 \times 10^{14}$ for $F(ab')^2$. Twenty mCi Tc-99m, needed for most diagnostic procedures, equals $2.3 \times 10^{15}$ atoms. Assuming uniform reduction of each antibody molecule, 100 ug of MAb can label 20 mCi Tc-99m with <1% of its disulfide groups reduced. The actual percent of disulfide groups reduced using suboptimal, optimal and ultraoptimal quantities of the reducing agents were calculated as above and given in Table-1. These indicate that at the optimal amount of reducing agent no greater than 2.7+0.2% of the disulfide groups are reduced. These results support the observations that no immunologic properties of MAb had altered, no fragmentation had taken place, and concur with Ruddikoff and Pumphrey (7) who have suggested that the loss of a few disulfide bridges may not alter the function of an antibody.

The cysteine ninhydrin complex provides a simple, sensitive, and reliable means of determining the number of disulfide groups reduced thereby controlling the MAb reduction process that is important in the preservation of MAb properties.

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Table-1

REDUCING AGENTS IN MOLAR RATIOS TO IgG AND ESTIMATED % OF AVAILABLE DISULFIDE GROUPS REDUCED

| Reducing agent to protein molar ratio | | ug Cysteine detected | % of available disulfide groups |
|---|---|---|---|
| DTE | (1000:1) | 0.073 ± 0.01 | 0.54 ± 0.08 |
| DTE | (3000:1) | 0.135 ± 0.02 | 0.99 ± 0.15 |
| DTE | (5000:1) | 0.250 ± 0.01 | 1.85 ± 0.07 |
| DTT | (1000:1) | 0.073 ± 0.01 | 0.54 ± 0.08 |
| DTT | (3000:1) | 0.136 ± 0.02 | 0.99 ± 0.15 |
| DTT | (5000:1) | 0.26 ± 0 | 1.9 ± 0 |
| 2-ME | (1000:1) | Not detectable | -- |
| 2-ME | (3000:1) | 0.135 ± 0.02 | 0.99 ± 0.15 |
| 2-ME | (5000:1) | 0.2 ± 0 | 1.5 ± 0 |
| $SnCl_2$ | (0500:1) | Not detectable | -- |
| $SnCl_2$ | (1500:1) | 0.125 ± 0.04 | 0.92 ± 0.25 |
| $SnCl_2$ | (2500:1) | 0.250 ± 0.04 | 1.85 ± 0.35 |
| AA | (3500:1) | 0.370 ± 0.03 | 2.70 ± 0.21 |
| AA | (10,500:1) | 0.770 ± 0.01 | 5.70 ± 0.14 |
| AA | (17,500:1) | 1.070 ± 0.04 | 7.90 ± 0.28 |

KINETICS OF THE ISOTOPIC EXCHANGE BETWEEN COPPER(II) AND COPPER(II) 1,4,7-TRIAZACYLCLONONANE-N,N',N'' TRIACETATE.

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Radiation Oncology Branch, National Cancer Institute, National Institutes of Health, Bethesda, MD 20892

The radionuclides copper-64 and copper-67 have desirable properties as radiopharmaceuticals. Copper-64 [ 12.7 h, $B^+$ 17.9 %] is suitable for PET scanning, whereas copper-67 [2.58 d, $B^-$ 100%, 93.3(16.1%) KeV, 184.6 (48.7%) KeV] may be useful for radioimmunotherapy. In order to provide useful systems for the delivery of these radionuclides in vivo it is necessary to prepare coordination complexes of copper which are inert. Our preliminary results demonstrated that the macrocyclic NOTA complex of copper is inert towards isotopic exchange with Cu-67. For comparison, the CuNOTA exchange rate is ca. 100 times less than for CuDOTA or CuTETA at pH 4.6 (Table 1).

The present study examined in detail the kinetics of the exchange between Cu(II) and CuNOTA in aqueous media. The observed rate parameters for Cu(II) exchange were determined as a function of Cu(II) and CuNOTA concentrations, acidity, and temperature. These results and a possible exchange mechanism will be presented.

Table 1. Kinetic data for the isotopic exchange reactions between copper(II) and copper(II)(L), where L = NOTA, DOTA, and TETA[a]

| L | $k(hr^{-1})$[b] | half-life (hr) |
|---|---|---|
| NOTA | $5.8(0.6) \times 10^{-4}$ | 516 (46) |
| TETA | $3.5(0.2) \times 10^{-2}$ | 8.5(0.5) |
| DOTA | $1.6(0.2) \times 10^{-1}$ | 1.8(0.2) |

a) At 25.0 °C in 0.1M acetate buffer, pH 4.6, I = 0.1 M $NaClO_4$. [CuL] = $2.00 \times 10^{-5}$ M and [Cu] = $1.5 \times 10^{-5}$ M. Uncertainties are at the $2\sigma$ level are listed in parentheses.

b) Assuming the exchange rate $R_{ex} = k[CuL]$.

SYNTHESIS AND EVALUATION OF RADIOIODINATED TETRAFLUOROPHENYL m-IODOBENZOATE AND TETRAFLUOROPHENYL-5-IODOPENTENOATES AS CONJUGATING AGENTS FOR PROTEINS AND ANTIBODIES.

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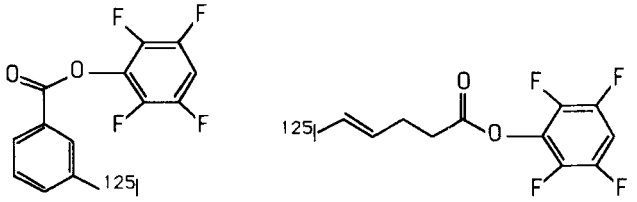
The potential of radio-immunoscintigraphy for the diagnosis of specific pathology is great. However, there are few specific agents capable of providing useful diagnostic information. This is due to the inherent difficulties associated with the introduction of the radiolabel onto the antibody. The use of labeled conjugating agents has been described by Fritzberg, et al (1).

Previous workers have prepared two radioconjugating agents, isomeric iodobenzoates (2,3) and the iodopentenoate (4). As part of our research program to apply organotin chemistry to the development of new radiochemicals, we have developed an alternative method for the preparation of the benzoate derivative, and new derivatives of the iodopentenoate compound.




Overall yields of the tri-n-butylstannylative tetrafluorophenyl esters were good, and conversion to the corresponding iodo- and radioiodo derivatives has proceeded well under standard labeling conditions.

The labeled products were conjugated to antibodies under basic conditions, and the tissue distribution determined.

This work was supported by DOE grant DE-FGO2-86ER60460.

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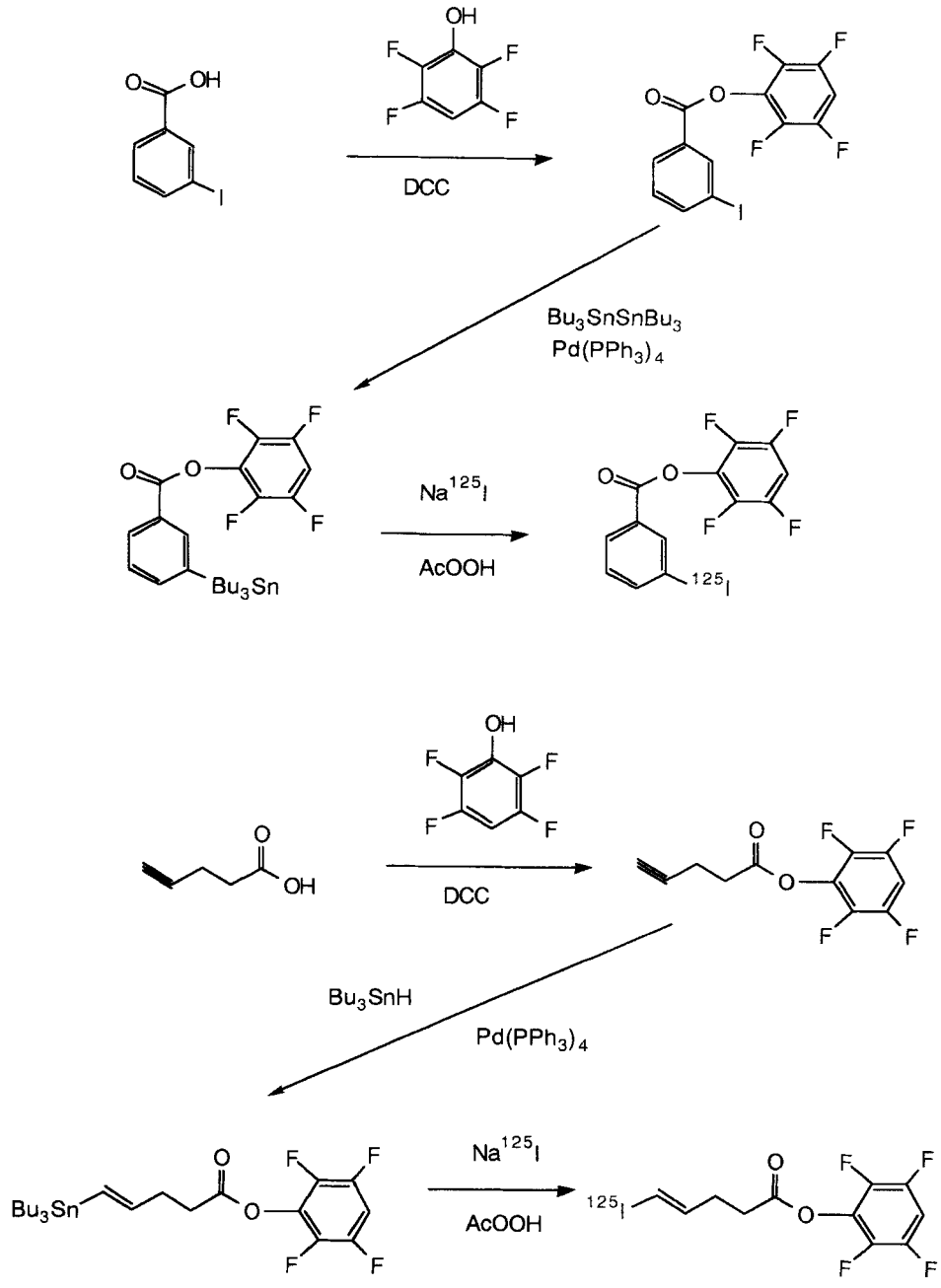
OH
F
F
F
F
DCC
O
OH
I
O
O
F
F
F
F
I
$Bu_3SnSnBu_3$
$Pd(PPh_3)_4$
$Bu_3Sn$
$Na^{125}I$
AcOOH
$^{125}I$

ESTIMATION OF CHARGE AND STABILITY OF In-111 BIFUNCTIONAL DIAMIDE DITHIOL COMPLEXES.

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The Purpose of this research was to estimate the charge and the stability of In-111 diamide dithiol complexes in a process of screening bifunctional chelating agents for In-111 labeling of antibody.

2,4- and 3,4-Bis(benzoylthioacetamido)-pentanoates were sythesized according to the method of Fritzberg, et al (1). 2,4-Bis(benzoylthioacetamido)-pentanoate (0.04 umol) was hyrdolysed in 100 ul of 1 N NaOH to the corresponding bis(thioacetamido)-pentanoic acid (N2S2). The N2S2 solution was neutralized with 100 ul of 1 N HCL, dried with a stream of nitrogen gas and reacted with 100 ul (1 mCi) of In-111 in a buffer mixture containing 0.1 M Na acetate and 0.01 M Na citrate at pH 5.0 at 80 $^{o}$C for 2 hr. The labeling yield was quantitative as assessed by silica gel TLC (Macherey-Nagel, Germany) developed with a solvent mixture containing 2:2:1 10 % ammonium formate in water:methanol:0.2 M citric acid. The $R_f$ value of In-111 N2S2 and free In-111 ion is 0.56 and 0.86, respectively in this TLC system.

In-111 N2S2 was subjected to cellulose acetate electrophoresis using In-111 DTPA as a standard. The electrophoresis in 0.06 M Na barbital at pH 8.8 showed In-111 N2S2 moving toward anode (+) with a mobility slightly higher than that of In-111 DTPA. In-111 N2S2 also moved toward anode but with a slower mobility in 0.06 M Na acetate buffer at pH 3.5 as compared to that in the barbital buffer. These results indicate that In-111 N2S2 has a negative charge equal to or higher than that (-2) of In-111 DTPA in the basic buffer. The fact that In-111 N2S2 moves toward anode at pH 3.5, where the pentanoic acid exists mainly as a neutral carboxy compound, indicates that the core of the In-111 N2S2 complex has a negative charge. For stability test, 100 ul of In-111 N2S2 was added to 1 ml of serum and incubated in a incubator at 37 $^{o}$C. The serum stability test indicates that In-111 dissociated gradually from N2S2 so that the percent In-111 N2S2 was 85, 79 and 54 at 14, 24 and 48 hr, respectively.

The polar nature of the In-111 N2S2 makes it easy to handle in an aqueous medium where antibody conjugation reaction has to be performed but the lower stability of the In-111 N2S2 suggests that N2S2 bifunctional chelators are only suitable for labeling antibody fragment such as Fab which has a short physiological half life.

This research was supported in part by CA 28462 and CA 48276

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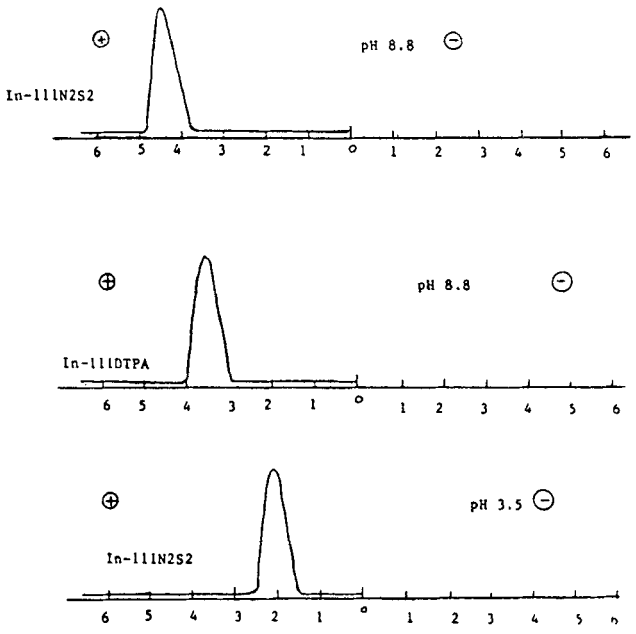
Electrophoresis Profile of In-111 Complexes




The electrophoresis was run at 2.5 mA/strip in 0.06 M barbital buffer at pH 8.8 and at 3 mA/strip in 0.06 M acetate buffer at pH 3.5 for 45 min.

## HIGH SPECIFIC ACTIVITY LABELING OF MONOCLONAL ANTIBODIES POLYLYSINE-DTTA AS A MULTIFUNCTIONAL CHELATING AGENT

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The uptake of tumor specific monoclonal antibodies (MAb) in target tissue is very low (0.01% of the injected dose / gram tissue). Thus there is an obvious need to increase the number of relevant substituents (radioactive label, paramagnetic atom, chemotherapeutic agent, toxin) i.e. to "label" these MAb's to a high specific activity (SA), if MAb's are to be used as specific therapeuticals or in magnetic resonance immunoimaging.

If simply an increased number of substituents is introduced per MAb, this hampers the immunological activity at higher label / MAb ratio's (often already at ratio's not much > 1). (1,2). Thus an approach in which a multifunctional ligand is coupled to the MAb with one of its functions, whereas the other functional groups are used to attach the label seems a reasonable approach (3,4,5).

We have prepared a diethylene triamine tetraacetic acid - polylysine multifunctional ligand (PoLys-(DTTA)m, II) by the reaction of cyclic DTPA-dianhydride (cDTPAA) with polylysine (PoLys-(NH2)n, I), which was coupled to an anti-CEA MAb with succinimidyl-S-acylthioacetate (SATA) (6). The MAb was activated with N-succinimidyl maleimido benzoate (SMB).

Scheme:

| | | | | |
|---|---|---|---|---|
| PoLys-(NH2)n (I) + cDTPAA | ⟶ | H2N-PoLys-(DTTA)m (II) | (1) |
| II + SATA | ⟶ | PoLys-DTTA-NH-CO-CH2-S-CO-CH3 (III) | (2) |
| III + NH2OH | ⟶ | PoLys-DTTA-NH-CO-CH2-SH (IV) | (3) |
| MAb.NH2 + SMB | ⟶ | MAb-NH-MB (V) | (4) |
| IV + V | ⟶ | PoLys-DTTA-MAb (VI) | (5) |

1. 1 mg of I was reacted with cDTPAA (cDTPAA / I = 178 / 1) in 80 µl DMSO . 20 ul water was added and the excess anhydride functions hydrolyzed.
To part of the reaction solution an excess of Gd, labeled with 153-Gd was added and analyzed by Sephadex G-25 chromatography. Thus 30-40 DTTA are introduced per polylysine. If a trace of Gd was used, an apparently higher number of Gd-DTTA-units was found in the polylysine. Obviously DTTA sites of different reactivity exist in II.
2. SATA was dissolved in DMF, added to II (SATA / II = 16 / 1).and the product purified over Sephadex G-25.
3 Activation of III was accomplished by hydroxyl amine. The number of activated SATA molecules per polylysine was determined with Ellman's reagent (7). 2-4 SATA molecules were thus introduced.
3b. IV was saturated with an excess of Gd-EDTA. The timing of this step is not critical: it can also be done after reaction 2 or 5. However, if done after 5, care must be taken not to damage the MAb.
4. MAb was activated towards the -SH group of IV by coupling with a maleimide group using SMB precipitated on the wall of the reactionvessel. The yield is virtually quantitative and thus this reaction can be used to control the number of polylysine substituents.

5. The preactivated polylysine-DTTA IV and MAb V give the multifunctional MAb VI. Purification and concentration is accomplished by ultrafiltration over 10 kD cut off filters. Analysis by gel permeation hplc gives the yield of the coupling reaction as about 80% of V at a twofold excess of IV, which means about 0.75 polylysine unit per MAb (30-40 DTTA functions per MAb involved). However the precise circumstances to get an optimal product have to be studied further. They will be different for various proteins.

6 Immuno integrity control. Various amounts of the multifunctional MAb VI were incubated over night with Sepharose bound antigen (CEA) and the precipitate and solution separated. The percentage VI found in the precipitate was > 70%.

By means of the reaction sequence shown it is possible to introduce 30-40 DTPA molecules (and as many metal atoms) into 7.5 kD polylysine. This multifunctional ligand can then be coupled to protein (MAb) molecules.

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## THE RADIOSYNTHESIS OF [$^{18}$F]PK 14105 AS AN ALTERNATIVE RADIOLIGAND FOR PERIPHERAL BENZODIAZEPINE BINDING SITES

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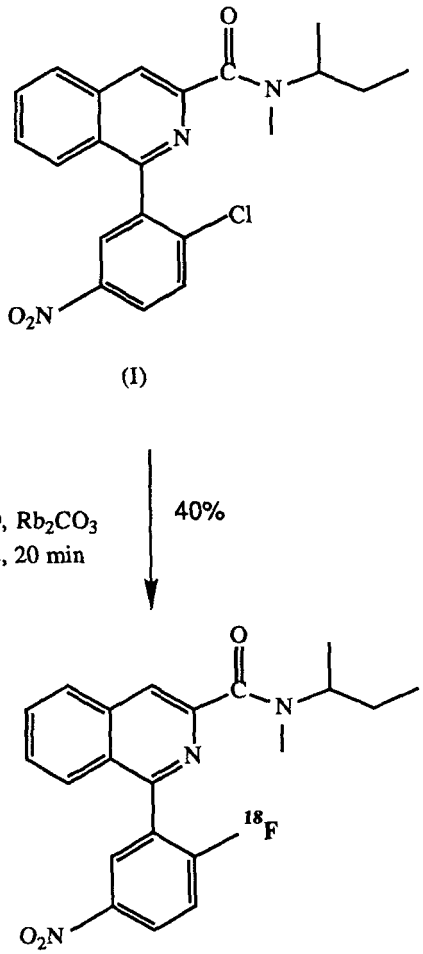
With the development[1] of carbon-11 ($t_{1/2}$ = 20.3 min ) labelled PK 11195 it became possible to study peripheral type benzodiazepine binding sites (PBBS) in living man with PET. Like PK 11195, the structurally related compound PK 14105 [*N*-methyl-*N*-(1-methyl-propyl)-1(2-fluoro-5-nitrophenyl)isoquinoline-3-carboxamide] has high affinity and selectivity for PBBS.[2] The *para* relationship of the fluoro group to the nitro group in PK 14105 suggested the possibility to label PK 14105 with fluorine-18 ($t_{1/2}$ = 109.6 min, $\beta$+ =96.9%), by aromatic nucleophilic substitution in a halo analogue with cyclotron-produced nca [$^{18}$F]fluoride, perhaps to provide a usefully longer-lived radioligand for PET studies of PBBS.

The chloro analogue (I) PK 14105 was available to us initially as a gift and subsequently by synthesis from 1-(2-chlorophenyl)isoquinoline-3-carboxylic acid and *N*-methyl-1-methyl-propylamine. Various conditions were explored for the reaction of this analogue with nca [$^{18}$F]fluoride, produced by the $^{18}$O(p,n)$^{18}$F reaction on $^{18}$O-enriched (20 atom %) water (subsequently removed azeotropically with acetonitrile in the presence of base). Optimal reaction conditions for the [$^{18}$F]fluoride were found to be 140 $^{o}$C for 20 min, using 4 mg of analogue (I), DMSO (1.5 mL) as solvent, rubidium carbonate (5 mg) as base and a platinum reaction vessel. These conditions gave *ca* 40 % radiochemical yield (decay-corrected) of crude [$^{18}$F]PK 14105 (II) (Scheme 1). Work up of this product on a C18 Sep-Pak followed by HPLC (with one recycle) on a silica gel column (30 cm X 0.7 cm i.d.) eluted at 6 mL/min with pentane/$CHCl_3$/$Et_3N$ (92.5/7.5/0.1 v/v) gave radiochemically and chemically pure [$^{18}$F]PK 14105, as assessed by analytical TLC, HPLC and mass spectrometry. Product can be formulated for *i.v.* injection into rats by removal of solvent, dissolution in absolute ethanol (0.2 mL) plus normal saline for injection (1.8 mL) The radiosynthesis requires 210 min from EOB, giving a radiochemical yield of *ca* 15%, decay-corrected.

Nca [$^{18}$F]PK 14105 (II) has been found to bind avidly to sites associated with kainic acid-induced unilateral lesions of rat striata. Such binding can be blocked by pre-dosing the rat with PK 11195, so providing evidence for specific binding to PBBS. These results suggest that [$^{18}$F]PK 14105 (II) has potential for PET studies of PBBS in man.

Acknowledgement The authors are grateful to Dr C. Gueremy (Rhone Poulenc) for the gift of PK 14105 and related compounds.




Scheme 1. Radiosynthesis of [$^{18}$F]PK 14105

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2.Doble A. *et al.*, *Mol. Pharmacol.*, (1986) **31**, 42.

The Routine Synthesis of Carbon-11 Alprazolam Utilizing Carbon-11 Acetyl Chloride as a Synthetic Intermediate.

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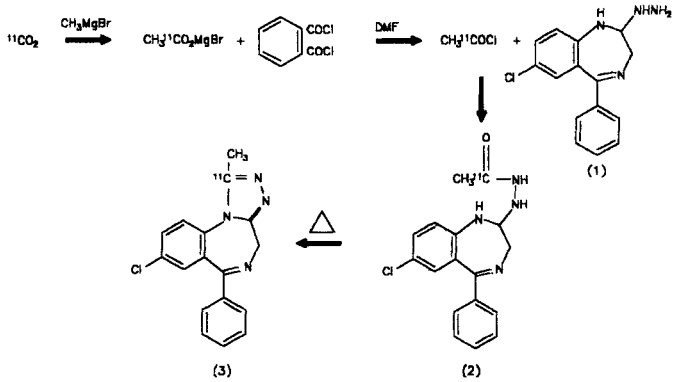
*University of Texas Health Science Center at Houston, +Division of Medicinal Chemistry and Pharmacognosy, University of Kentucky and ǂClinical Pharmacokinetics, The UpJohn Company, Kalamazoo MI.

We have recently developed a synthesis of carbon-11 alprazolam, a benzodiazepine agonist, which is potentially useful both for studying the pharmacokinetics and receptor binding of the drug and for studying the benzodiazepine receptor itself using PET. The synthesis is based upon the reaction of carbon-11 acetyl chloride with 7-chloro-5-phenyl-[3H]-1,4-benzodiazepine-2-yl hydrazine followed by ring closure at 200°C to the endocyclic carbon-11 product alprazolam (Scheme 1). The synthesis involves four reactions and two purifications and takes about forty minutes to perform. In order to produce this compound on a routine basis for PET studies it is necessary to build a remote synthesis system and thus avoid overexposure to the operators. We have built the system shown in Figure 1 and optimized the various parameters involved in the synthesis of the acetic acid derivative with respect to temperature of the reaction and flow rate of the gas Table 1. Formation and distillation of acetyl chloride, with respect to the amount of reagent, temperature of reaction and distillation has been optimized as shown in Table 2 and minimisation of the codistillation of the reagents has been achieved. Reaction of the acetyl chloride with the hydrazine has been shown to proceed to completion. Many of the earlier reaction steps have been studied using aniline instead of the precious hydrazine, analyzing the product acetanilide. Purification of the intermediate carbon-11 hydrazone on Sep-Pak cartridges has been evaluated with respect to type and quantity of rinses, Table 3, and the final purification of the product is currently being evaluated. The alprazolam can be sublimed and if this can be performed successfully then it will remove the need for a final time consuming HPLC purification. The design of a remote sublimation apparatus is being studied, with that shown in Figure 2 being the most promising design to date. The specific activity of the final product from this system varies between 900 and 2,200 Curies per millimole and the parameters necessary to stabilize this figure will be discussed.




Scheme 1

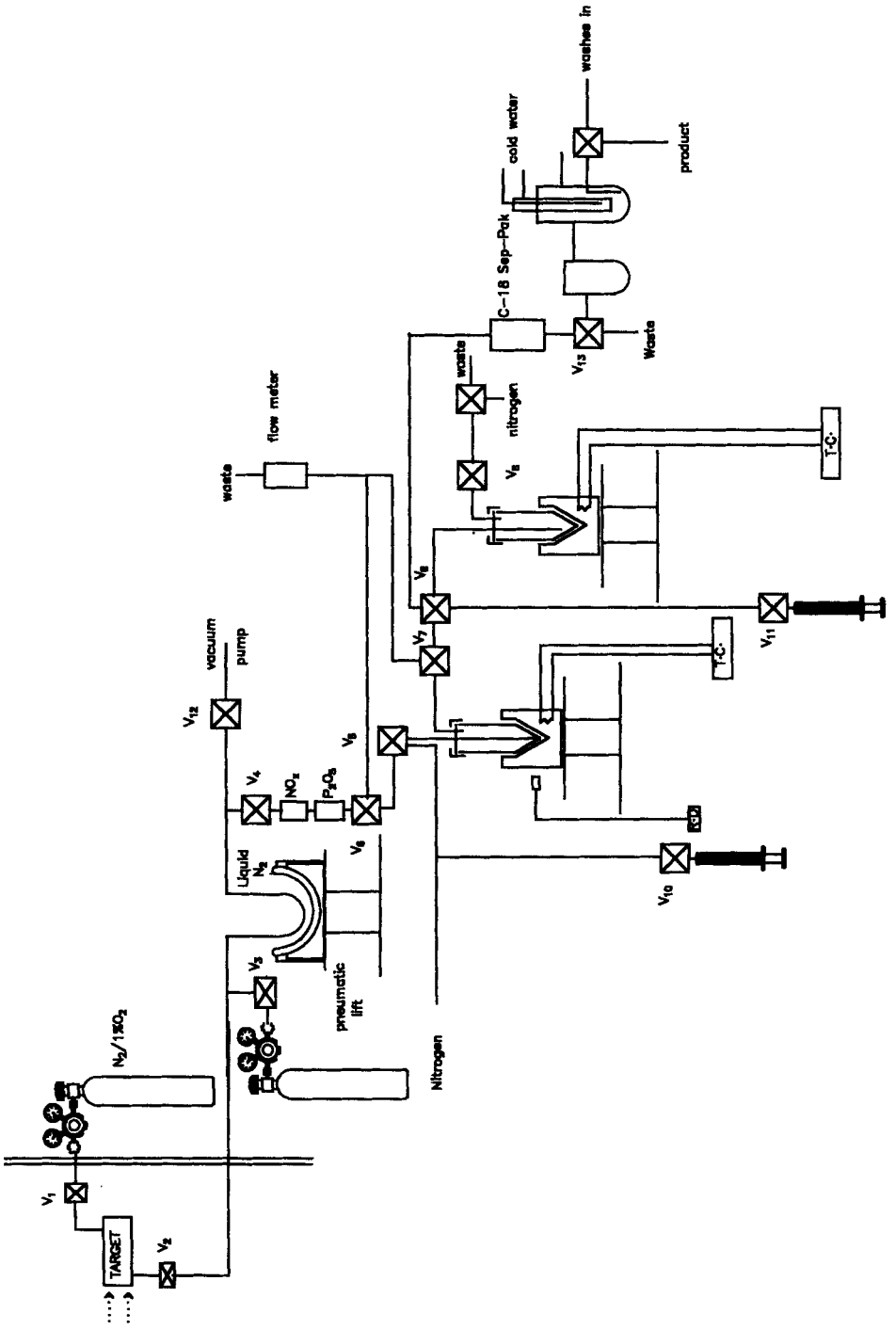

washes in
cold water
product
C-18 Sep-Pak
waste
$V_{13}$
waste
nitrogen
flow meter
waste
$V_8$
T-C
$V_6$
$V_7$
$V_{11}$
vacuum pump
$V_{12}$
$V_5$
$V_4$
$NO_x$
$P_2O_5$
T-C
RDI
Liquid $N_2$
$V_9$
$V_{10}$
$V_3$
pneumatic lift
$N_2/1\%O_2$
Nitrogen
$V_1$
TARGET
$V_2$

Table 1.Trapping Efficiency of Acetyl Chloride Using Aniline for a Beam Current of Five Microampheres for Five Minutes.a

| RUN # | 11CO2 (mCi) | TR#1 (mCi) | TR#2 (mCi) | NAOH (mCi) | Ves A (mCi) | Accout (%) | Sparge Rate | Distill Time(min) | Temp. ( C) |
|---|---|---|---|---|---|---|---|---|---|
| 9/7R3 | 56.0 | 26.6 | 0.3 | 3.7 | 9.6 | 97 | 50 | 5 | 5 |
| 9/7R5 | 67.0 | 33.6 | 0.4 | 8.4 | 9.6 | 99 | 50 | 5 | 5 |
| 9/7R6 | 58.0 | 28.8 | 0.4 | 3.4 | 5.0 | 90 | 50 | 5 | 5 |
| 9/5R2 | 57.1 | 8.9 | --- | 32.8 | 1.2 | 100 | 70 | 5 | 5 |
| 9/5R3 | 57.5 | 7.0 | --- | 36.3 | 1.4 | 103 | 70 | 5 | 5 |
| 9/6R3 | 57.0 | 19.4 | 0.8 | 16.8 | 2.4 | 95 | 70 | 5 | 5 |
| 9/8R3 | 55.2 | 31.9 | --- | 4.3 | 7.2 | 104 | 50 | 5 | 5 |
| 9/12R2 | 60.6 | 35.0 | --- | 7.1 | 3.8 | 101 | 50 | 5 | 20 |
| 11/7R2 | --- | 19.1 | --- | 2.9 | 1.0 | --- | 50 | 5 | 20 |
| 11/7R3 | --- | 28.4 | --- | 4.7 | 1.7 | --- | 50 | 5 | 20 |
| 11/8R4 | --- | 34.5 | --- | 1.4 | 1.2 | --- | 50 | 5 | 20 |

a.The trapping efficiency did not improve significantly for a concentration of aniline above the 15 umol used in traps #1 and #2.A similar trapping efficiency was found for aqueous NaOH over the same concentration range.Moisture and O2 were excluded in all runs by the use of high purity gases, and traps were utilized for the removal of nitrogen oxides formed during the bombardment.The accountability was based on the 25% expected decay of carbon-11 determined at the beginning of the run.

Table 2. Evaluation of Several Reaction Steps in the Carbon-11 Alprazolam Synthesis Using a Beam Current of Five Microampheres for Five Minutes.a

| | ACETYLATION | | SEP PAK PURIFICATION | | SUBLIMATION | | |
|---|---|---|---|---|---|---|---|
| RUN# | Hydrazone (mCi) | Loss (mCi) | Loss (mCi) | Hydrazone (mCi) | Sublimed APZ(mCi) | Loss (mCi) | Yield (%) |
| | | | | | Double-Chamber Sublimator | | |
| No NaHCO3 Wash | 27.9 | 3.3 | 2.2 | --- | 0.64 | 1.2 | 14 |
| Std Sep Pak Wash | 29.7 | 3.8 | 3.7 | --- | 0.88 | 1.5 | 13 |
| Std Sep Pak Wash | --- | 2.8 | 4.7 | --- | 0.50 | 1.1 | 13 |
| | | | | | Pear-Shaped Flask and Condenser | | |
| Std Sep Pak Wash | --- | 2.1 | 4.5 | 6.8 | 1.4 | 3.1 | 36 |
| Std Sep Pak Wash | --- | 1.8 | 0.9 | 13.5 | 1.4 | 3.6 | 38 |
| No Aqueous Wash | --- | 2.2 | 0.9 | 7.6 | 0.06 | 0.7 | 6 |
| No Citric Acid | --- | 0.4 | 0.8 | --- | 0.3 | 0.7 | 8 |
| Std Size Sep Pak | --- | --- | --- | 5.5 | 1.1 | 2.2 | 26 |
| Not Activated | --- | 2.0 | 9.4 | 0.8 | --- | --- | -- |
| Sep Pak H20 Only | 7.6 | 1.1 | 1.0 | --- | 0.2 | 3.6 | 29 |
| | | | | | Cold-Finger Sublimator | | |
| Std Sep Pak Wash | 9.7 | --- | 1.3 | --- | 2.0 | 1.9 | 30 |
| Std Sep Pak Wash | 10.5 | --- | 0.2 | --- | 0.0 | 5.9 | 45 |
| Std Sep Pak Wash | 10.4 | --- | 0.3 | --- | 1.1 | 3.2 | 33 |
| Std Sep Pak Wash | 16.7 | --- | 1.1 | 6.4 | 0.08 | 2.5 | 20 |
| Std Sep Pak Wash | --- | --- | --- | 8.1 | 0.9 | 3.1 | 30 |

a.All runs utilized 300 umol CH3MgBr, 1035 umol of phthalolyl dichloride and DMF,and 1.08 umol of hydrazine. The trapping eficiency in the acetylation of the hydrazine was comparable to that found for aniline, giving about 10-25 mCi of hydrazone.The standard sep pak wash included 4cc of H20,2% citric acid, 4% NaHCO3,H20,and hexane.The sublimation is presently being optimized as the yield penalty is still too large.

Table 3. Evaluation of Waters C18 Silica Sep Pak Plus Cartridges Using a Mixture of Possible Contaminants.a

| | | Contaminants (umol) | | | Area Count (X1000) | | | | Reduction |
|---|---|---|---|---|---|---|---|---|---|
| RUN# | Activated | HYD | 2,6L | PDC | 2,6L | HYD | PDC | TOTAL | (%) |
| Std Size | yes | 1.06 | 294 | 1030 | 2229 | 1226 | 688 | 4143 | ---- |
| Std Size | no | 1.06 | 294 | 1030 | 122 | 323 | 264 | 710 | 82 |
| 10 mm | yes | 0.53 | 147 | 1030 | 250 | 79 | 44 | 302 | 93 |
| 15 mm | yes | 1.06 | 294 | 1030 | n.d. | 85 | 364 | 449 | 89 |
| 15 mm | no | 0.53 | 147 | ---- | n.d. | 60 | n.d. | 60 | 99 |
| 15 mm | no | 0.53 | 147 | 1030 | n.d. | 20 | n.d. | 20 | 99 |
| 15 mm | no | 0.53 | --- | 1030 | n.d. | 20 | 9 | 29 | 99 |
| 15 mm | no | 0.53 | 147 | 1030 | n.d. | 13 | 4 | 17 | 99 |

a.The activation of the C18 sep pak was accomplished with absolute ethanol and then an aqueous wash. Without any activation, the radioactive product is lost to waste.The standard sep pak wash was used in this series of runs.