PET AND SPECT RADIOCHEMISTRY

SELECTED EXAMPLES OF LABELLING OF MACROMOLECULES

Frédéric DOLLÉ

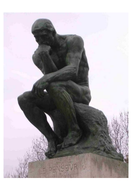
Radiochimie & Radiopharmacie
Service hospitalier Frédéric Joliot
Institut d'imagerie biomédicale - DSV/CEA
4 Place du Général Leclerc
F-91401 Orsay, France

Tél : +33 (0)1 69 86 77 04
Fax : +33 (0)1 69 86 77 49
E-mail : frederic.dolle@cea.fr

EMMI Intensive Programme
Design, synthesis and validation of imaging probes

September 19th, 2011 – 14h00-15h30
MBC, Turino, Italy

WHAT ARE PET AND SPECT ?

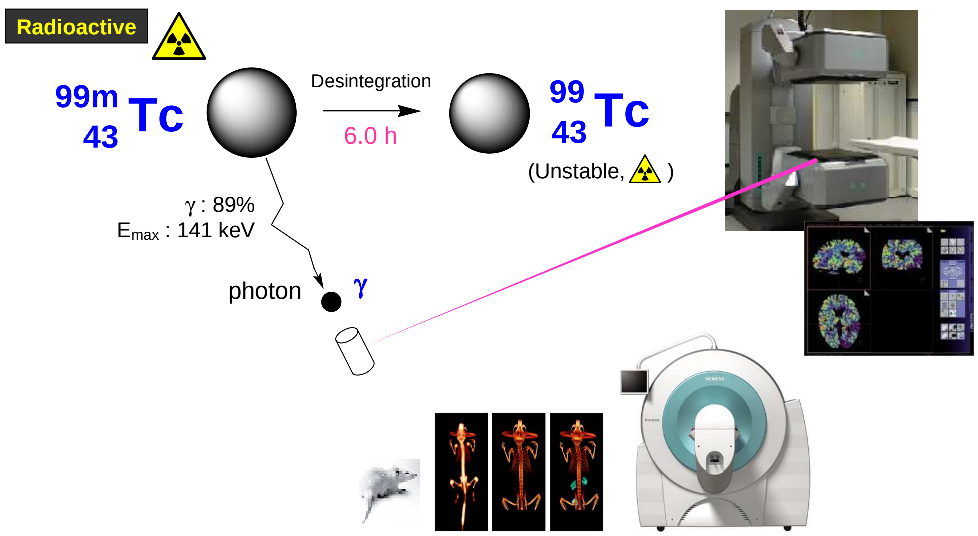


PET (Positron Emission Tomography) and SPECT (Single Photon Emission Computed Tomography) are two highly-sensitive, but relatively low-resolution functional imaging technique.

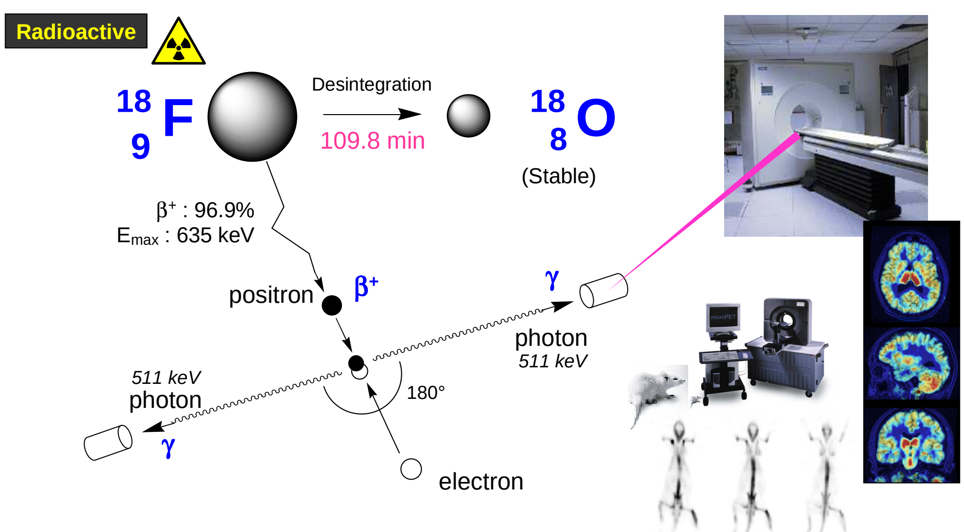
PET and SPECT permit repeated, non-invasive assessment and quantification of specific biological and pharmacological processes in living animals, humans included.

PET and SPECT are probably the most advanced technology currently available for studying *in vivo* molecular interactions, and represent the method of choice to assess *in vivo* the distribution, pharmacokinetics and -dynamics of a compound labelled with a radioactive atom.

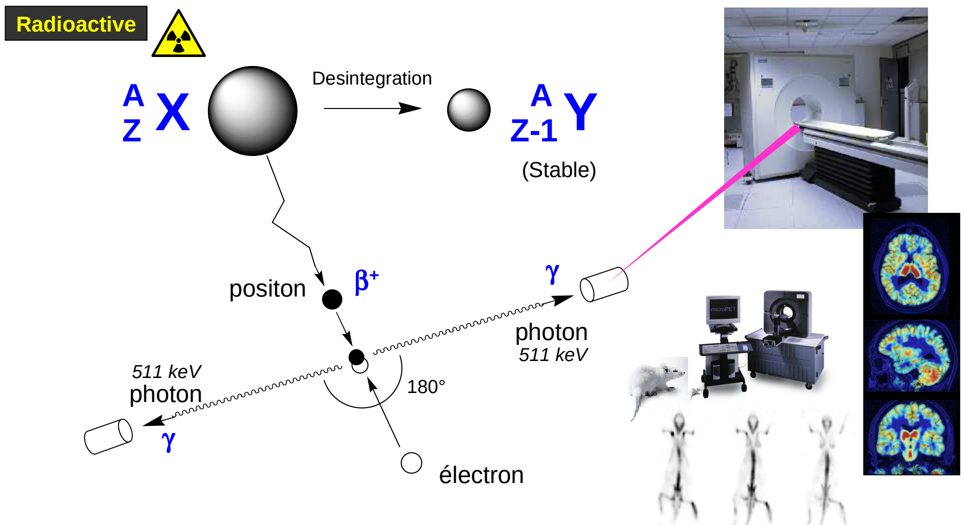
HOW DOES SPECT WORK ?



HOW DOES PET WORK ?



HOW DOES PET WORK ?



PET AND SPECT RADIOCHEMISTRY

HOW DOES PET WORK ?

SIEMENS EXACT HR+

An MRI machine
for comparaison

PET

SPECT

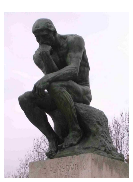
MRI

CT

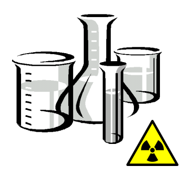
OPTICAL

PET AND SPECT RADIOCHEMISTRY

WHY RADIOCHEMISTRY ?

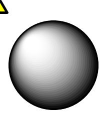


Molecular imaging with PET or SPECT requires the preparation of a positron-emitting / single-photon-emitting radio labelled probe (molecule) or radiotracer.



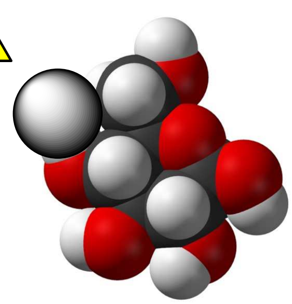
Radioactive

PET $^{18}_{9}F$

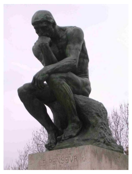


Radioactive

$^{18}_{9}F$



PET AND SPECT RADIOCHEMISTRY



PET (SPECT) RADIOCHEMISTRY …
HOW DIFFERENT IS IT FROM CHEMISTRY ?

☹ **STRESS 1 : RADIOPROTECTION**

☹ **STRESS 2 : TIME !**

☹ **STRESS 3 : AUTOMATION (PET ESPECIALLY)**

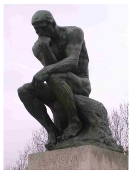
PET AND SPECT RADIOCHEMISTRY

A RADIOPHARMACEUTICAL PREPARATION IMPOSES AN EXHAUSTIVE AND ADVANCED AUTOMATION.

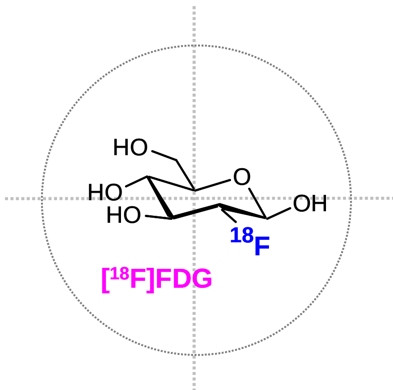
THE GAMMA RAYS FACILITATE IN THIS CASE THE PROCESS MONITORING BY EASY AND SENSITIVE RADIOACTIVITY DETECTION.

THE SUSCEPTIBILITY OF THE CHEMICAL REACTION TO AUTOMATION HAS TO BE TAKEN INTO ACCOUNT AT THE EARLY DESIGN STAGE OF THE RADIOCHEMICAL PATHWAY. CERTAIN MANIPULATIONS OF CLASSICAL CHEMISTRY, SUCH AS LIQUID-LIQUID EXTRACTION OR PRECIPITATION, CANNOT BE ENVISAGED.

AUTOMATION MAY USE BOTH ROBOTIC SYSTEMS AND MODULES ...



THE MOST COMMONLY USED PET RADIOPHARMACEUTICAL ?



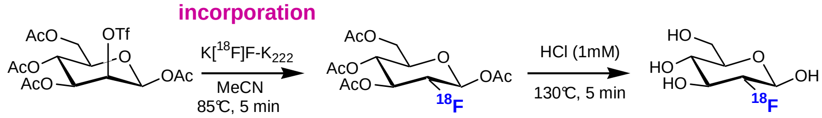
PET AND SPECT RADIOCHEMISTRY

2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG)

RADIOSYNTHESIS : 2 STEPS ONLY

a) fluorine-18 incorporation

b) deprotection

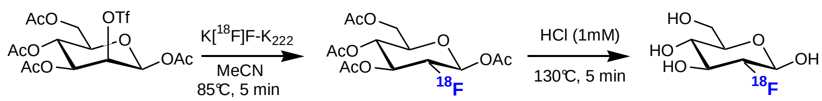


Based on Hamacher K et al. - J Nucl Med 1986, 27: 235-8.

PET AND SPECT RADIOCHEMISTRY

2-[^{18}F]Fluoro-2-deoxy-D-glucose ([^{18}F]FDG)

1. RADIOSYNTHESIS : 2 STEPS ONLY

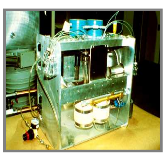


2. PURIFICATION : On CARTRIDGE (No HPLC needed)

3. AUTOMATION

PET AND SPECT RADIOCHEMISTRY

1989



CTI-CPCU^{+} *(prototype)*

cti solutions

...

1997 (2001)

TRACERlab FX_{FDG}

nuclear interface — GE Healthcare

1999 (2001)

TRACERlab MX_{FDG}

COINCIDENCE TECHNOLOGIES

2004

SYNCHROM

raytest

2005

FDG - PLUS

BIOSCAN

2005 explora

EXPLORA-FDG_4

SIEMENS medical

2005

SYNTHERA

iba Molecular

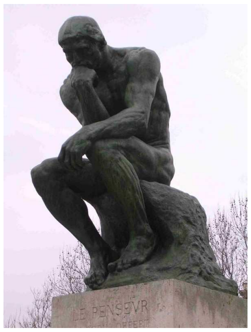
2005

FASTlab

GE Healthcare

2011 and ...

...



PET AND SPECT RADIOCHEMISTRY

SELECTED EXAMPLES OF LABELLING
OF MACROMOLECULES

WHAT IS COMMONLY TERMED « MACROMOLECULES » ?

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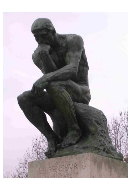
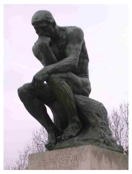


MACROMOLECULES =

High-molecular-weight bioactive chemical structures,

- Oligonucleotides
  single/double-stranded, siRNA …
- Aptamers
- Peptide Nucleic Acids,
- Peptides
- Proteins
- Antibodies (diabodies …)
- Oligosaccharides
- Nano-objects

WHAT IS COMMONLY TERMED « MACROMOLECULES » ?



MACROMOLECULES =

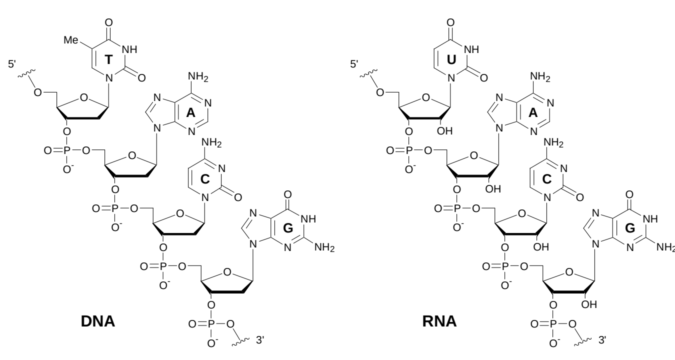
High-molecular-weight bioactive chemical structures,

- **Oligonucleotides**
  single/double-stranded, siRNA …
- **Aptamers**
- **Peptide Nucleic Acids,**
- **Peptides**
- **Proteins**
- **Antibodies (diabodies …)**
- **Oligosaccharides**
- **Nano-objects**

CHEMICAL STRUCTURE OF OLIGONUCLEOTIDES

Partial structures of DNA and RNA presenting all possible nucleobases: thymine (T), adenine (A), cytosine (C), guanine (G) and uracil (U)

C
H
O
N
P



PET AND SPECT RADIOCHEMISTRY

- ✓ WHAT IS AN OLIGONUCLEOTIDE ?

WITH WHICH RADIOISOTOPE ?

PET AND SPECT RADIOCHEMISTRY

RADIOISOTOPES: Half-life, Decay mode and Production

	Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	Production
PET	Carbon-11	20.4 min	β^+ (99.8) - EC (0.2)	β^+ 960	$^{14}N(p,\alpha)^{11}C$
PET	Gallium-68	68.3 min	β^+ (89) - EC (11)	β^+ 1900	^{68}Ge / ^{68}Ga generator (271 d)
PET	Fluorine-18	109.8 min	β^+ (97) - EC (3)	β^+ 635	$^{18}O(p,n)^{18}F$
PET	Copper-64	12.7 h	β^+ (19) - EC (41) - β^- (40)	β^+ 656 / β^- 573	$^{64}Ni(p,n)^{64}Cu$
PET	Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	$^{86}Sr(p,n)^{86}Y$
PET	Bromine-76	16.1 h	β^+ (57) - EC (43)	β^+ 3900	$^{75}As(^{3}He,2n)^{76}Br$ $^{76}Se(p,n)^{76}Br$
SPECT	Technetium-99m	6.0 h	IT (>99)	γ 140	^{99}Mo / ^{99m}Tc generator (67 h)
SPECT	Iodine-123	13.2 h	EC (100)	γ 159	$^{124}Te(p,2n)^{123}I$ $^{124}Xe(p,pn)^{123}Xe$: (β^+, EC, 2.1 h)^{123}I
SPECT	Indium-111	2.8 d	EC (100)	γ 173, 247	$^{111}Cd(p,n)^{111}In$ $^{112}Cd(p,2n)^{111}In$

RADIOISOTOPES: Half-life, Decay mode and Production

Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	CHEMISTRY
Carbon-11	20.4 min	β^+ (99.8) - EC (0.2)	β^+ 960	**NON-METALS** covalent-bond making
Fluorine-18	109.8 min	β^+ (97) - EC (3)	β^+ 635	
Iodine-123	13.2 h	EC (100)	γ 159	
Bromine-76	16.1 h	β^+ (57) - EC (43)	β^+ 3900	
Gallium-68	68.3 min	β^+ (89) - EC (11)	β^+ 1900	**METALS** prosthetic chelator
Technetium-99m	6.0 h	IT (>99)	γ 140	
Copper-64	12.7 h	β^+ (19) - EC (41) - β^- (40)	β^+ 656 / β^- 573	
Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

✓ WHAT IS AN OLIGONUCLEOTIDE ?

✓ WITH WHICH RADIOISOTOPE ?

WHICH STRATEGIE FOR LABELLING ?

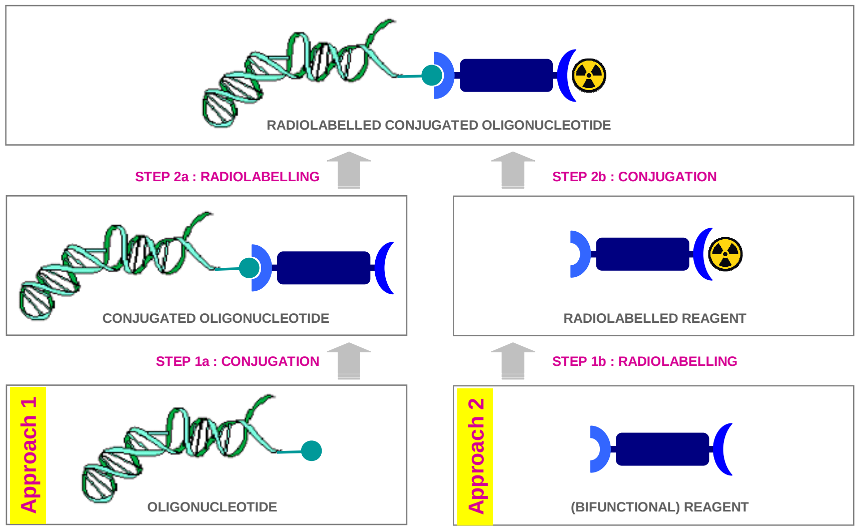
☹ **- The So-Called “True” Labelling Approach**

☹ **- Simple Addition of a Radioactive Atom**

☺ **- The Prosthetic Conjugation Approach**

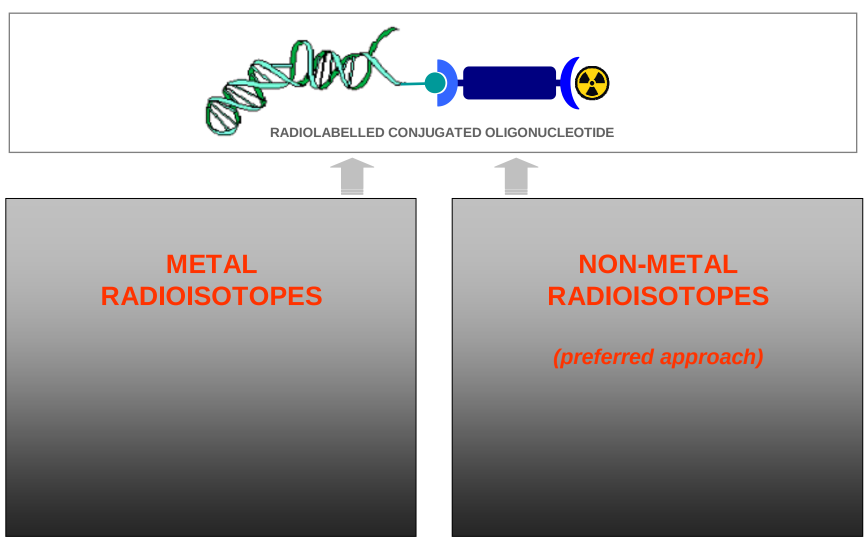
PET AND SPECT RADIOCHEMISTRY

LABELLING STRATEGIES – THE “PROSTHETIC CONJUGATION” APPROACHES



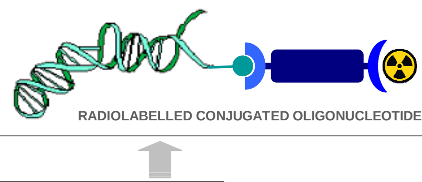
PET AND SPECT RADIOCHEMISTRY

LABELLING STRATEGIES – THE “PROSTHETIC CONJUGATION” APPROACHES



PET AND SPECT RADIOCHEMISTRY

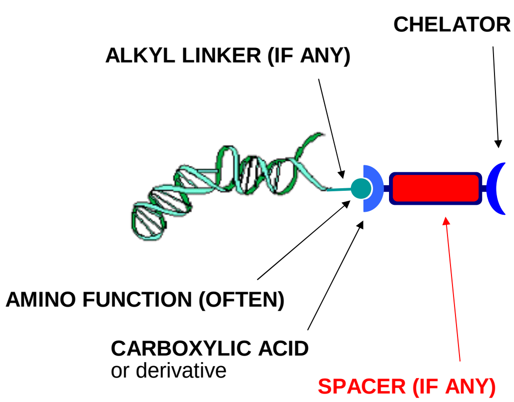
LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS

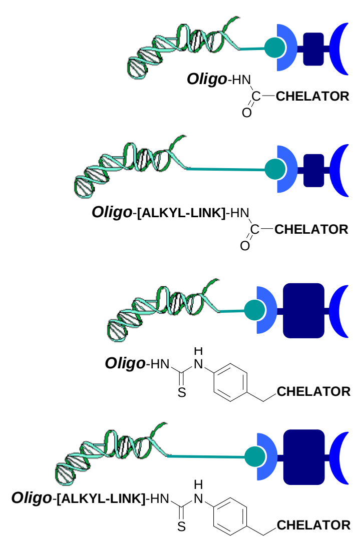


METAL RADIOISOTOPES

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS





LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS

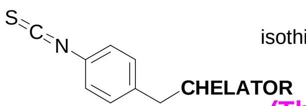
activated ester
or carboxylic acid

AMIDES

+

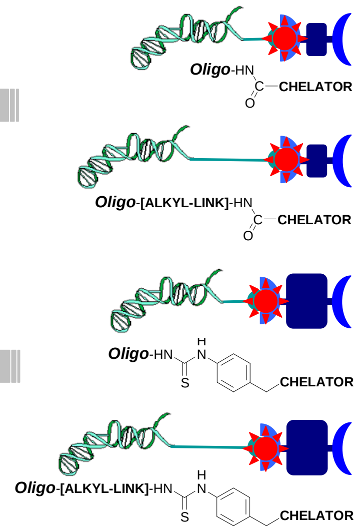
Oligo-NH_2 or ***Oligo***-**[ALKYL-LINK]**-NH_2

+

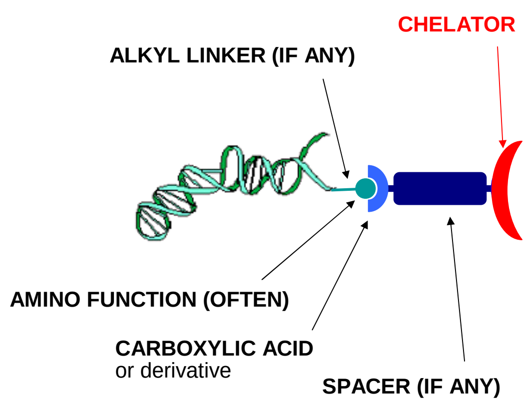


isothiocyanate

(Thio)UREAS

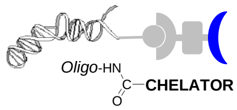


LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS



PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS
BIFUNCTIONAL CHELATING AGENTS

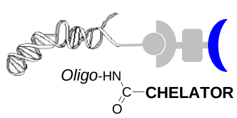


A range of chelating agents have been described, tailored to the complexation properties of each radiometal.
They all need to meet the following requirements: they should :

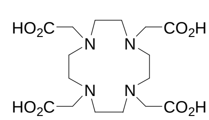
- co-ordinate the radiometal ion rapidly and quantitatively at micromolar to nanomolar concentrations,
- preferentially bind the radioisotope in the presence of contaminating metal ions,
- form discrete metal complex species to prevent lengthy purification procedures and need for excess ligand,
- co-ordinate the desired radiometal ion in the pH range of 4 to 9,
- co-ordinate the radiometal ion at mild temperatures,
- not release the radiometal ion to adventitious natural ligands in the biological fluid,
- not readily exchange with cations *in vivo*.

PET AND SPECT RADIOCHEMISTRY

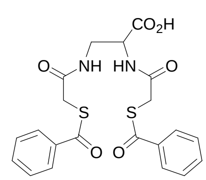
LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS
BIFUNCTIONAL CHELATING AGENTS



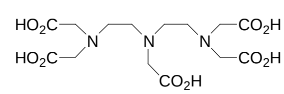
A range of chelating agents have been described, tailored to the complexation properties of each radiometal.
Below are some examples …



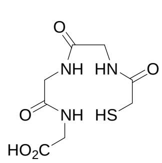
DOTA



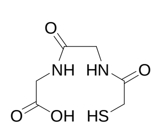
$(SBT)_2DAP$



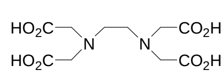
DTPA



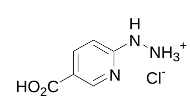
MAG3



MAG2



EDTA



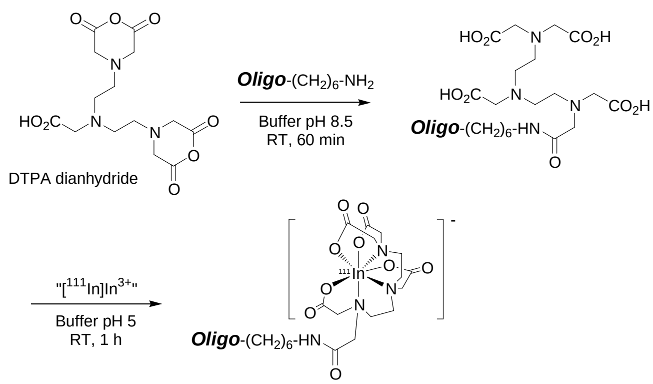
HYNIC

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS : INDIUM-111

^{111}In

DTPA activated as a *bis*-(cyclic anhydride) and conjugation with an aminohexyl-modified oligonucleotide followed by indium-111 labelling.



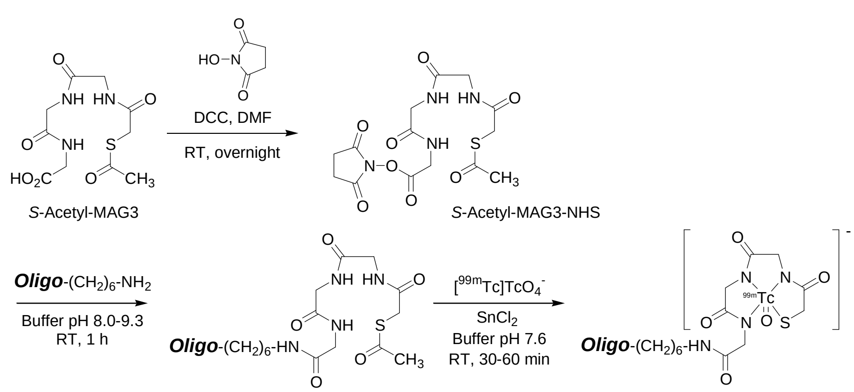
Bai J et al. - Eur J Nucl Med Mol Imag 2004, 31: 1523-1529.
Zhang YM et al. - Eur J Nucl Med 2000, 27: 1700-1707.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS : TECHNETIUM-99m

^{99m}Tc

MAG3 activation and conjugation with an aminohexyl-modified oligonucleotide followed by technetium-99m labelling.

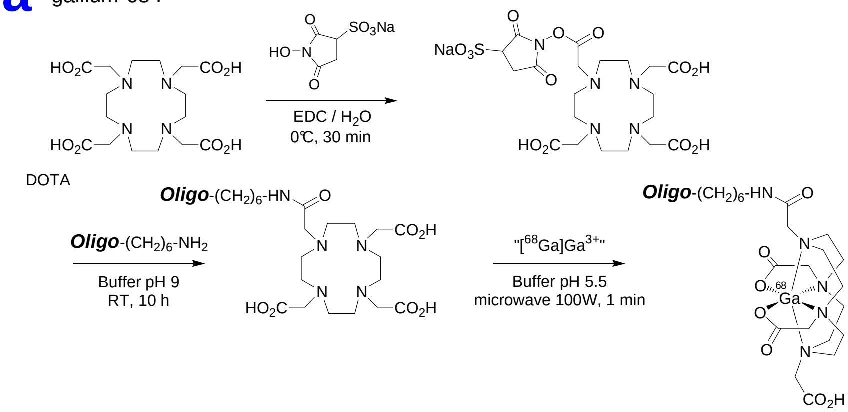


Zhang YM et al. - Eur J Nucl Med 2000, 27: 1700-1707.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH RADIOMETALS : GALLIUM-68

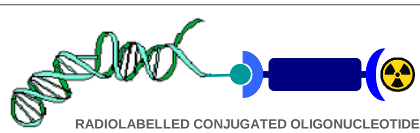
^{68}Ga DOTA activation and coupling to an oligonucleotide followed by complexation with gallium-68 .



Velikyan I et al. - J Label Compds Radiopharm 2004, 47: 79-89.
Roivainen A et al. - J Nucl Med 2004, 45: 347-355.
Lendvai G et al. - Eur J Pharmaceutical Sci 2005, 26: 26-38.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS



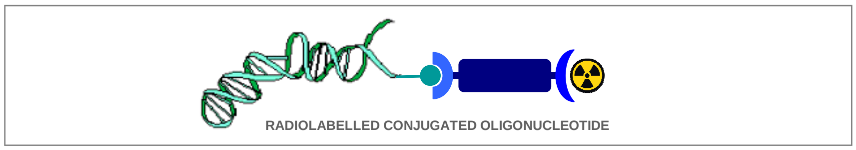
NON-METAL RADIOISOTOPES

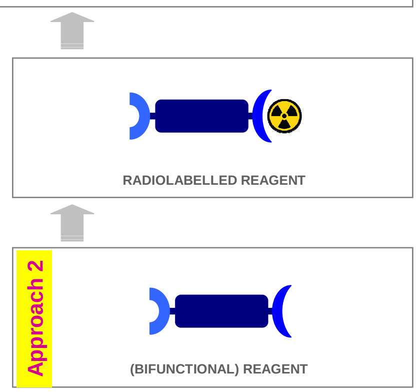
(preferred approach)

Preferred approach, except with iodine radioisotopes

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

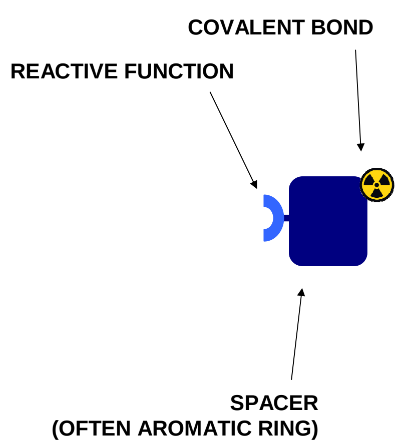




Preferred approach,
except with iodine radioisotopes

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS



PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

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Gallium-68	68.3 min	β^+ (89) - EC (11)	β^+ 1900	METALS prosthetic chelator
Technetium-99m	6.0 h	IT (>99)	γ 140	
Copper-64	12.7 h	β^+ (19) - EC (41) - β^- (40)	β^+ 656 / β^- 573	
Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : CARBON-11

Oligonucleotide labelling [^{11}C]methyl iodide.

[^{11}C]methyl iodide

[^{11}C]CO_2 —(LiAlH_4/THF, low temperature, then H_2O)→ [^{11}C]CH_3OH —(aq. HI, Δ)→ [^{11}C]CH_3I

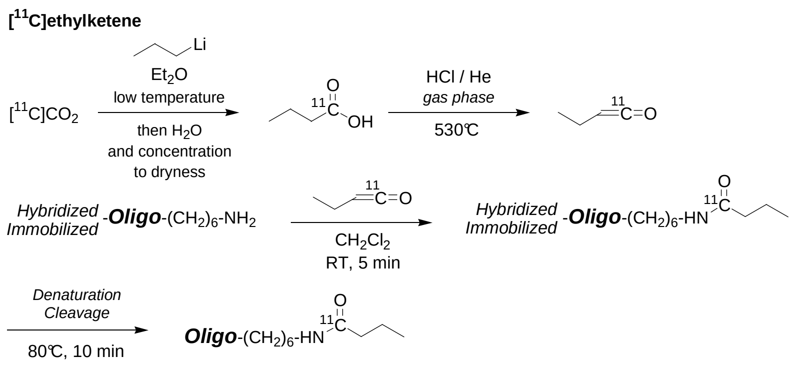
Oligo (LY2181308) —([^{11}C]CH_3I, DMF / Buffer pH 8.2, 90°C, 15 min)→ [^{11}C]CH_3-***Oligo*** ([^{11}C]CH_3-LY2181308)

Dence CS et al. - J Label Compds Radiopharm 2005, 48(Suppl 1): S161.
Dence CS et al. - J Label Compds Radiopharm 2007, 50(Suppl 1): S449.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : CARBON-11

Oligonucleotide labelling using [*carbonyl*-11C]ethylketene.



Fujii R et al. - J Label Compds Radiopharm 1991, 29: 497-505.
Kobori N et al. – Neuroreport 1999, 10: 2971-2974.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	CHEMISTRY
Carbon-11	20.4 min	β^+ (99.8) - EC (0.2)	β^+ 960	**NON-METALS** covalent-bond making
Fluorine-18	**109.8 min**	**β^+ (97) - EC (3)**	**β^+ 635**	
Iodine-123	**13.2 h**	**EC (100)**	**γ 159**	
Bromine-76	**16.1 h**	**β^+ (57) - EC (43)**	**β^+ 3900**	
Gallium-68	68.3 min	β^+ (89) - EC (11)	β^+ 1900	METALS prosthetic chelator
Technetium-99m	6.0 h	IT (>99)	γ 140	
Copper-64	12.7 h	β^+ (19) - EC (41) - β^- (40)	β^+ 656 / β^- 573	
Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	CHEMISTRY
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Fluorine-18	109.8 min	β^+ (97) - EC (3)	β^+ 635	
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Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

RADIOHALOGENS

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	CHEMISTRY
Carbon-11	20.4 min	β^+ (99.8) - EC (0.2)	β^+ 960	NON-METALS covalent-bond making
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Gallium-68	68.3 min	β^+ (89) - EC (11)	β^+ 1900	METALS prosthetic chelator
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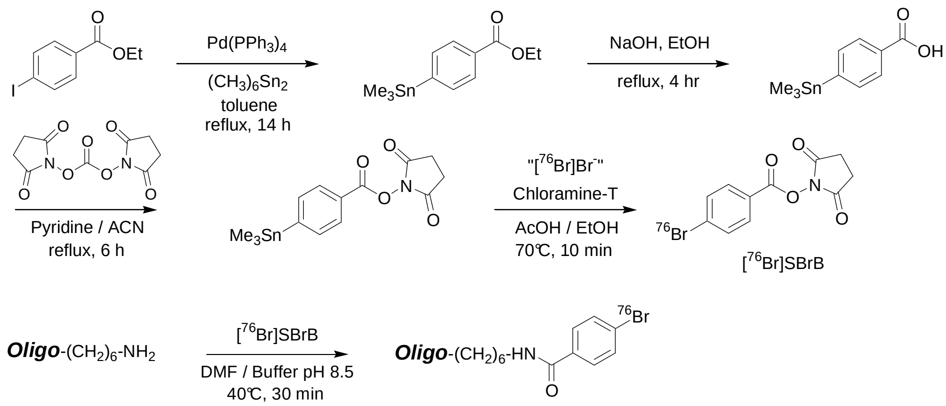
Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : BROMINE-76

Synthesis of *N*-succinimidyl 4-[^{76}Br]bromobenzoate ([^{76}Br]SBrB) and its use in oligonucleotide labelling.



Yngve U et al. - Acta Chem Scand 1999, 53: 508-512.
Wu F et al. - Eur J Pharmaceut Sci 2000, 10: 179-186.
Wu F et al. - Nucl Med Biol 2004, 31: 1073-1078.

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PET AND SPECT RADIOCHEMISTRY

SHFJ

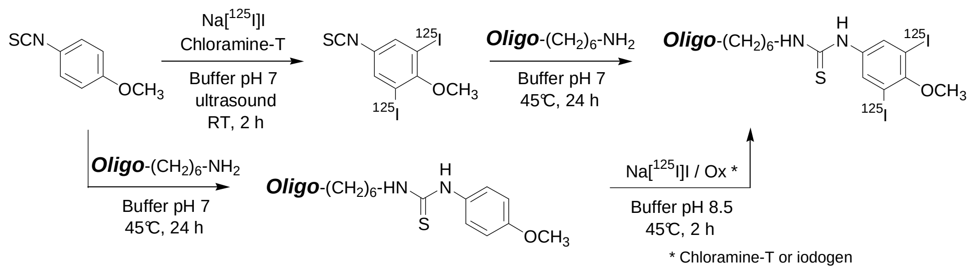
LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	CHEMISTRY
Carbon-11	20.4 min	β⁺ (99.8) - EC (0.2)	β⁺ 960	**NON-METALS** covalent-bond making
Fluorine-18	109.8 min	β⁺ (97) - EC (3)	β⁺ 635	
Iodine-123	**13.2 h**	**EC (100)**	**γ 159**	
Bromine-76	16.1 h	β⁺ (57) - EC (43)	β⁺ 3900	
Gallium-68	68.3 min	β⁺ (89) - EC (11)	β⁺ 1900	**METALS** prosthetic chelator
Technetium-99m	6.0 h	IT (>99)	γ 140	
Copper-64	12.7 h	β⁺ (19) - EC (41) - β⁻ (40)	β⁺ 656 / β⁻ 573	
Yttrium-86	14.7 h	β⁺ (34) - EC (66)	β⁺ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : IODINE-125/123

Two routes to radioiodinated oligonucleotide using *p*-methoxyphenyl isothiocyanate:
Direct labelling of an activated benzene ring.



Dewanjee MK et al. - Bioconj Chem 1991, 2: 195-200.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS

Radioisotope	Half-life	Decay mode (%)	E_β or E_γ (keV)	CHEMISTRY
Carbon-11	20.4 min	β^+ (99.8) - EC (0.2)	β^+ 960	**NON-METALS** covalent-bond making
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Gallium-68	68.3 min	β^+ (89) - EC (11)	β^+ 1900	METALS prosthetic chelator
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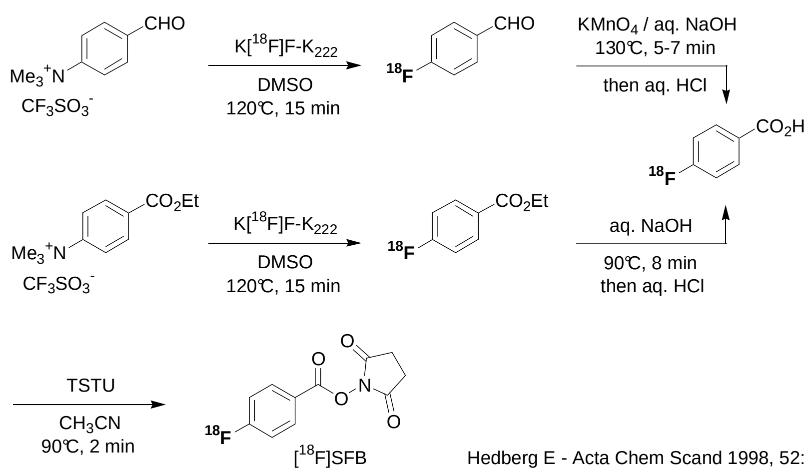
Yttrium-86	14.7 h	β^+ (34) - EC (66)	β^+ 3150	
Indium-111	2.8 d	EC (100)	γ 173, 247	

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Radiosynthesis of *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB).

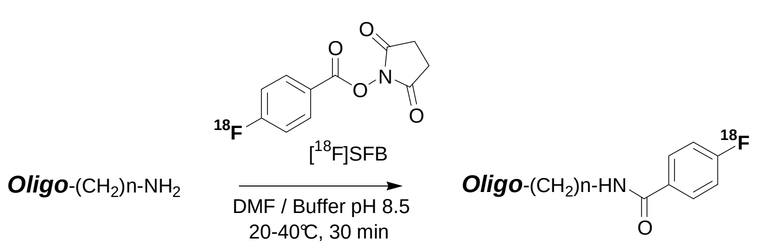


Hedberg E - Acta Chem Scand 1998, 52: 1034-9.
Li J et al. - J Label Compds Radiopharm 2006, 49: 1213-21.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Oligonucleotide conjugation with *N*-succinimidyl 4-[^{18}F]fluorobenzoate ([^{18}F]SFB).

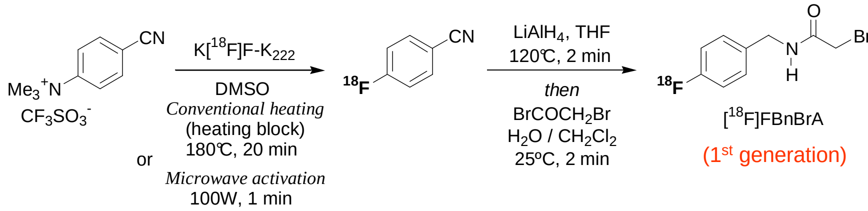


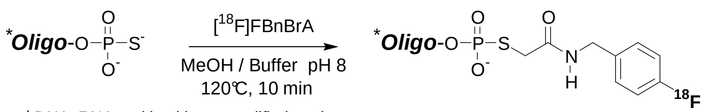
Hedberg E - Acta Chem Scand 1998, 52: 1034-9.
Li J et al. - J Label Compds Radiopharm 2006, 49: 1213-21.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Oligonucleotide conjugation with *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ([^{18}F]FBnBrA).



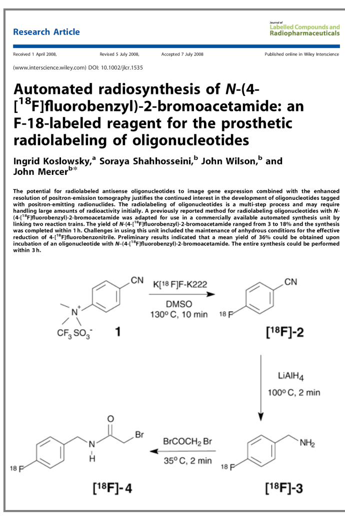


* DNA, RNA and backbone-modified analogues
Spiegelmers (L-DNA and L-RNA)

Dollé F et al. - J Label Compds Radiopharm 1997, 39: 319-30.
Kühnast B et al. - J Label Compds Radiopharm 2000, 43: 837-48.
Kühnast B et al - Bioconj Chem 2000, 11: 627-36.
Kühnast B et al - J Label Compds Radiopharm 2003, 46: 1093-103.

PET AND SPECT RADIOCHEMISTRY

MACROMOLECULE LABELING : OLIGONUCLEOTIDE and [^{18}F]FBnBrA


Research Article

Journal of Labelled Compounds and Radiopharmaceuticals

Received 1 April 2008, Revised 5 July 2008, Accepted 7 July 2008 Published online in Wiley Interscience

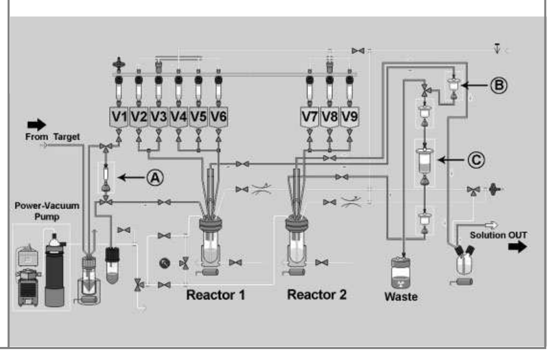
(www.interscience.wiley.com) DOI: 10.1002/jlcr.1535

Automated radiosynthesis of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide: an F-18-labeled reagent for the prosthetic radiolabeling of oligonucleotides

Ingrid Koslowsky,[a] Soraya Shahhosseini,[b] John Wilson,[b] and John Mercer[b*]

The potential for radiolabeled antisense oligonucleotides to image gene expression combined with the enhanced resolution of positron-emission tomography justifies the continued interest in the development of oligonucleotides tagged with positron-emitting radionuclides. The radiolabeling of oligonucleotides is a multi-step process and may require handling large amounts of radioactivity initially. A previously reported method for radiolabeling oligonucleotides with *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide was adapted for use in a commercially available automated synthesis unit by linking two reaction trains. The yield of *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide ranged from 3 to 18% and the synthesis was completed within 1 h. Challenges in using this unit included the maintenance of anhydrous conditions for the effective reduction of 4-[^{18}F]fluorobenzonitrile. Preliminary results indicated that a mean yield of 36% could be obtained upon incubation of an oligonucleotide with *N*-(4-[^{18}F]fluorobenzyl)-2-bromoacetamide. The entire synthesis could be performed within 3 h.

GEMS TRACERLab FX$_{FDG}$

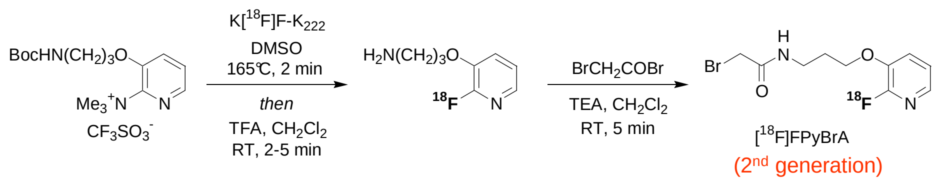


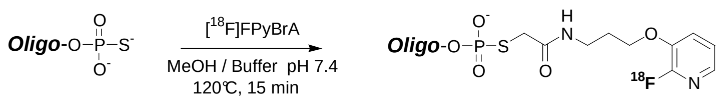
Koslowsky I et al. - J Label Compounds Radiopharm 2008, 51: 352-356.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Oligonucleotide conjugation with 2-bromo-*N*-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl] acetamide ([^{18}F]FPyBrA).



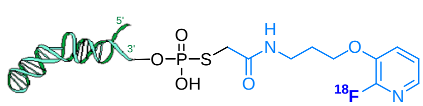


Kühnast B et al. - Bioconj Chem 2004, 15: 617-27.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

[^{18}F]Single-stranded oligonucleotides ([^{18}F]c-OLx)



[^{18}F]**c-OL1**, [^{18}F]**c-OL2a**, [^{18}F]**c-OL2b**, [^{18}F]**c-OL3**

Name	Length	Sequences	HPLC Rt (a)*	MS found (calc) (b)*	^{31}P NMR (c)*
OL1 / C-**OL1**	9 mer	A C C G A T C C G	14.0 min	2989.0 (2990.0)	δ : 14.44
OL2a / C-**OL2a**	18 mer	A G A A T A C A G G G T C C A A A T	14.1 min	5847.8 (5846.9)	δ : 14.53
OL2b / C-**OL2b**	18 mer	A G A A U A C A G G G U C C A A A U	15.4 min	6346.7 (6345.3)	δ : 13.94
OL3 / C-**OL3**	18 mer	A U U U G G A C C C U G U A U U C A	15.3 min	5989.7 (5991.0)	δ : 13.94

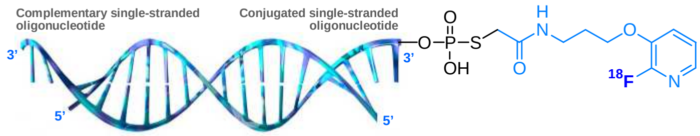
(a) HPLC column and conditions: C18 µBondapak® Waters (300 x 3.9 mm, porosity 10 µm); triethylammonium acetate, 50 mM, pH 7 (TEAA) and acetonitrile; gradient elution: linear 5 min from 95/5 to 90/10 (TEAA/acetonitrile) then linear 15 min from 90/10 to 60/40 and wash-out 10 min at 50/50, flow rate: 1.5 mL/min. (b) MALDI-TOF Spektrometer (GSG, Karlsruhe, Germany). (c) NMR Bruker AMX (300 MHz) apparatus; TMP as internal standard, spectra recorded in water at 298K.
* Analytical data (HPLC, MS, ^{31}P NMR) belong only to the conjugated oligonucleotides.

Results :
40-60 mCi of **[^{18}F]c-OLx**, ready-to-use (25-40 mCi/mL in aq. 0.9% NaCl),
starting from 1 Ci of [^{18}F]fluoride, in 140-160 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

Kühnast B et al. - Bioconj Chem 2004, 15: 617-27.

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

[^{18}F]Small Interfering RNAs ([^{18}F]c-SiRNAs)



Code	Combination of	Sequence	HPLC Rt (a)
DUPLEX-1	ON-1	5' U C G A A G U A U U C C G C G U A C G U U 3' ps	9.60 min
	ON-4	3' U U A G C U U C A U A A G G C G C A U G C 5'	
DUPLEX-2	ON-2	5' U C G A A G U A U U C C G C G U A C G U U 3' ps	10.70 min
	ON-5	3' U U A G C U U C A U A A G G C G C A U G C 5'	
DUPLEX-3	ON-1	5' U C G A A G U A U U C C G C G U A C G U U 3' ps	9.80 min
	ON-6	3' U U A G C U U C A U A A G G C G C A U G C 5'	

(a) HPLC column and conditions: C18 µBondapak® Waters (300 x 7.8 mm, porosity 10 µm); triethylammonium acetate, 50 mM, pH 7.4 (TEAA) and acetonitrile; gradient elution: linear 5 min from 95/5 to 90/10 (TEAA/acetonitrile) then linear 15 min from 90/10 to 75/25 and wash-out 5 min at 50/50, flow rate: 6.0 mL/min.

Results :
15-30 mCi of **[^{18}F]c-SiRNAs**, ready-to-use (15-30 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [^{18}F]fluoride, in 165 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

15 June 2007
Second International Conference
European Society for Molecular Imaging
June 14-15, 2007
Naples - Italy
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ORAL 27

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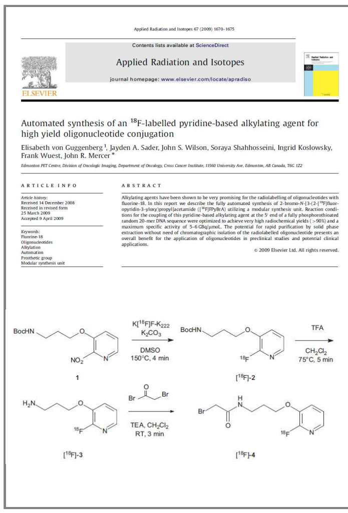
POSTER 129

Viel T et al. - J Label Compds Radiopharm 2007, 50: 1159-1168.

MACROMOLECULE LABELING : OLIGONUCLEOTIDE and [^{18}F]FBnBrA



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ELSEVIER

Automated synthesis of an ^{18}F-labelled pyridine-based alkylating agent for high yield oligonucleotide conjugation

Elisabeth von Guggenberg [1], Jayden A. Sader, John S. Wilson, Soraya Shahhosseini, Ingrid Koslowsky, Frank Wuest, John R. Mercer *

Edmonton PET Centre, Division of Oncologic Imaging, Department of Oncology, Cross Cancer Institute, 11560 University Ave, Edmonton, AB Canada, T6G 1Z2

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ABSTRACT

Alkylating agents have been shown to be very promising for the radiolabelling of oligonucleotides with fluorine-18. In this report we describe the fully automated synthesis of 2-bromo-N-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]acetamide ([^{18}F]FPyBrA) utilizing a modular synthesis unit. Reaction conditions for the coupling of this pyridine-based alkylating agent at the 5' end of a fully phosphorothioated random 20-mer DNA sequence were optimized to achieve very high radiochemical yields (>90%) and a maximum specific activity of 5–6 GBq/µmoL. The potential for rapid purification by solid phase extraction without need of chromatographic isolation of the radiolabelled oligonucleotide presents an overall benefit for the application of oligonucleotides in preclinical studies and potential clinical applications.

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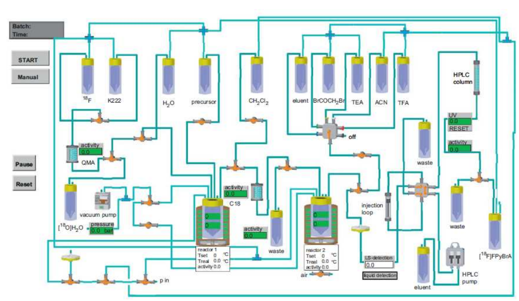
Modular Lab
Eckert & Ziegler

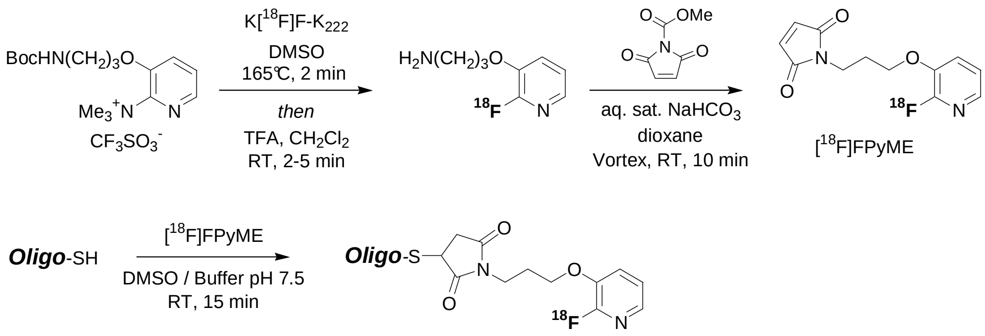


Von Guggenberg E et al. - Appl Rad Isot 2009, 67: 1670-1675.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Oligonucleotide conjugation with 1-[3-(2-[^{18}F]fluoropyridin-3-yloxy)propyl]pyrrole-2,5-dione ([^{18}F]FPyME).



15th International Symposium on Radiopharmaceutical Chemistry
Sydney 10-14 August 2003

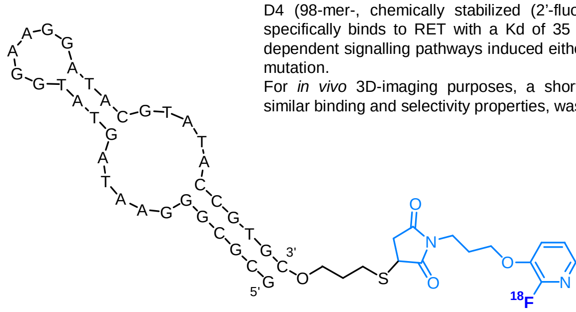
Dollé F - PCT Int. Appl., 58 pp. WO 2004002984.
Dollé F et al. - J Label Compds Radiopharm 2003, 46: S15.
de Bruin B et al. - Bioconj Chem 2005, 16: 406-20.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

[^{18}F]D4-36 Aptamer ([^{18}F]c-D4-36)

A serie of aptamers targeting the transmembrane receptor tyrosine kinase (RTK) RET (REarranged during Transfection) have been isolated from whole-living cell SELEX protocols.
D4 (98-mer-, chemically stabilized (2'-fluoropyrimidinyl modified) RNA-aptamer) specifically binds to RET with a Kd of 35 ± 3 nM and blocks RET dimerization-dependent signalling pathways induced either by GDNF or by the C634Y activating mutation.
For *in vivo* 3D-imaging purposes, a shortened version (36-mer only), showing similar binding and selectivity properties, was designed (D4-36).



Results :
24-30 mCi of [^{18}F]c-D4-36, ready-to-use (20-30 mCi/mL in aq. 0.9% NaCl), starting from 1.0-1.5 Ci of [^{18}F]fluoride, in 150-155 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

2008 World Molecular Imaging Congress
Pestourie *et al*
POSTER 1708

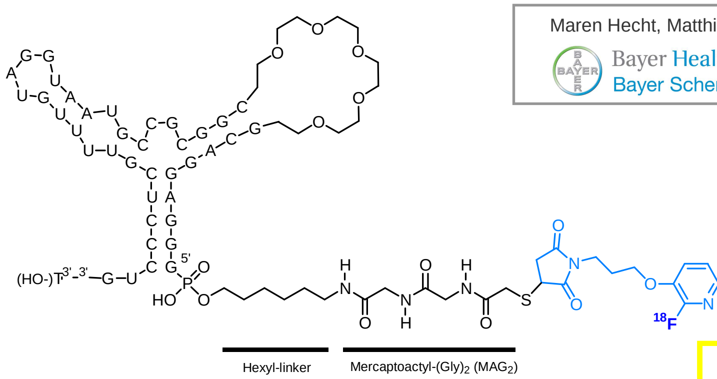
Pestourie C, Janssens I, Kühnast B, Gombert K, Thezé B, Dollé F, Tavitian B, Ducongé F - unpublished.

PET AND SPECT RADIOCHEMISTRY

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

[^{18}F]Tenascin-C Aptamer ([^{18}F]c-TTA-01)

Maren Hecht, Matthias Friebbe
Bayer HealthCare
Bayer Schering Pharma



2008 World Molecular Imaging Congress
September 10-13, 2008
Nice, France
Kuhnast *et al*
POSTER 1800

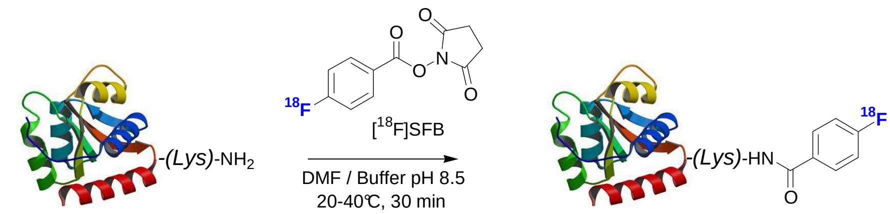
Results :
30-100 mCi of [^{18}F]c-TTA-01, ready-to-use (20-65 mCi/mL in aq. 0.9% NaCl),
starting from 1 Ci of [^{18}F]fluoride, in 140 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

Kühnast B, Boisgard R, Hinnen F, Tavitian B, Dollé F - PATENT PENDING.

PET AND SPECT RADIOCHEMISTRY

- ✓ WHAT IS AN OLIGONUCLEOTIDE ?
- ✓ WITH WHICH RADIOISOTOPE ?
- ✓ WHICH STRATEGIE FOR LABELLING ?
  - The So-Called “True” Labelling Approach
  - Simple Addition of a Radioactive Atom
  - The Prosthetic conjugation approach

WHAT ABOUT OTHER MACROMOLECULES ?



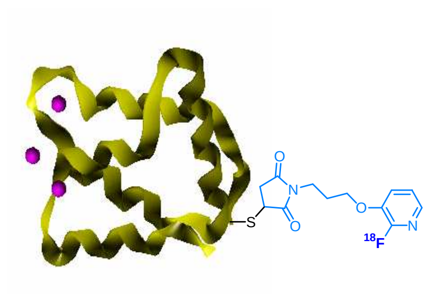
PET AND SPECT RADIOCHEMISTRY

MACROMOLECULE LABELING : PEPTIDE - PROTEIN

[^{18}F]Annexin-V fragment ([^{18}F]c-AFIM)

Alain Samson, Françoise Russo-Marie

BIONEXIS
the architect for intelligent molecules



15th International Symposium on Radiopharmaceutical Chemistry
Sydney 10-14 August 2003

Results :
25-85 mCi of **[^{18}F]c-AFIM**, ready-to-use (5-15 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [^{18}F]fluoride, in 145 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

De Bruin B et al. - Bioconj Chem 2005, 16: 406-20.

EMMI – Intensive Programme F. Dollé Service Hospitalier Frédéric Joliot – I²BM/CEA

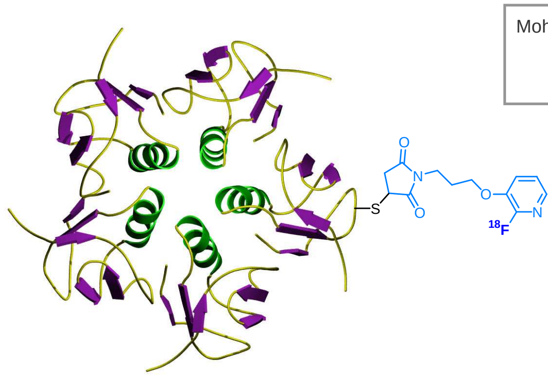
PET AND SPECT RADIOCHEMISTRY

MACROMOLECULE LABELING : PEPTIDE - PROTEIN

[^{18}F]SHIGA toxin ([^{18}F]c-STxB)

Mohamed Amessou, Ludger Johannes

institutCurie
Ensemble, prenons le cancer de vitesse.



16th International Symposium on Radiopharmaceutical Chemistry
Iowa City, Iowa
June 24-28, 2005

Results :
65-115 mCi of **[^{18}F]c-STxB**, ready-to-use (50-75 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [^{18}F]fluoride, in 145 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

De Bruin B et al. - Bioconj Chem 2005, 16: 406-20.

PET AND SPECT RADIOCHEMISTRY

- ✓ WHAT IS AN OLIGONUCLEOTIDE ?
- ✓ WITH WHICH RADIOISOTOPE ?
- ✓ WHICH STRATEGIE FOR LABELLING ?
  - - The So-Called "True" Labelling Approach
  - - Simple Addition of a Radioactive Atom
  - - The Prosthetic conjugation approach
- ✓ WHAT ABOUT OTHER MACROMOLECULES ?
- **WHAT ABOUT NANO-OBJECTS ?**

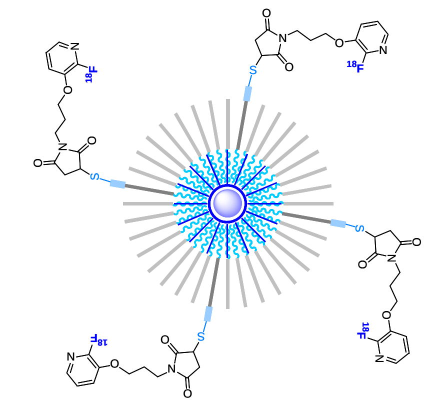
PET AND SPECT RADIOCHEMISTRY

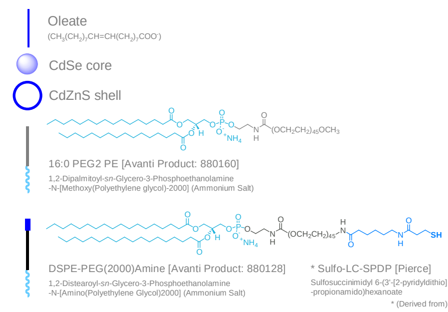
MACROMOLECULE LABELING : NANO-OBJET

[^{18}F]Quantum Dots ([^{18}F]c-QDs)

Thomas Pons, Benoit Dubertret

ESPCI





Results :
15-25 mCi of **[^{18}F]c-QD** (SRA: 8-20 Ci/µmole @ EOB), ready-to-use (10-15 mCi/mL in aq. 0.9% NaCl), starting from 1 Ci of [^{18}F]fluoride, in 145 minutes of radiosynthesis.
Automation in place on a Zymate XP (Zymark) robotic system.

Ducongé F. et al - Bioconj Chem 2008, 19: 1921-1926.

PET AND SPECT RADIOCHEMISTRY

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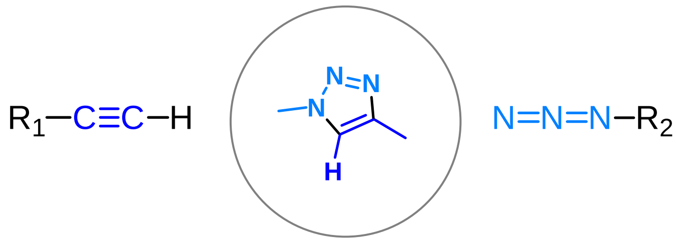
ANY NOVEL APPROCHES ?

PET AND SPECT RADIOCHEMISTRY

INTRODUCTION - 'CLICK CHEMISTRY'

Of the reactions comprising the '**Click universe**', the perfect example is the so-called **Cu(I)-catalyzed** variant of the **Huisgen 1,3-dipolar cycloaddition** of terminal alkynes to organoazides to form 1,4-disubsituted-1,2,3-triazoles ***.

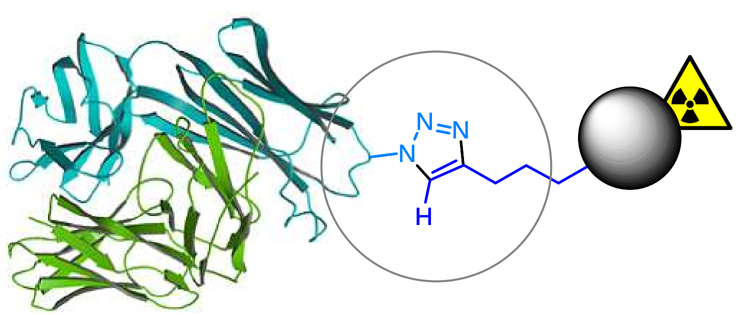


*** The triazole has also similarities to the ubiquitous amide moiety found in nature, but unlike amides, is not susceptible to cleavage and is nearly impossible to oxidize or reduce.

Hartmuth C, Kolb MG, Finn K and Barry Sharpless
Click Chemistry: Diverse Chemical Function from a Few Good Reactions
Angew Chem Int Ed 2001, 40: 2004-2021.

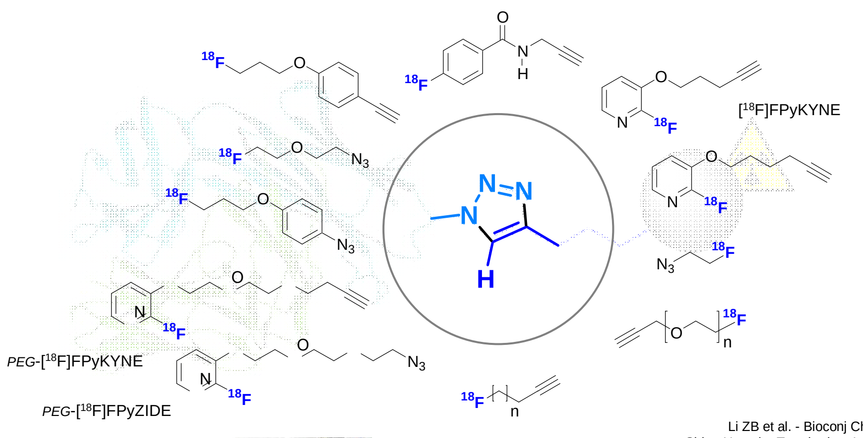
PET AND SPECT RADIOCHEMISTRY

MACROMOLECULE LABELLING – 'CLICK CHEMISTRY'



PET AND SPECT RADIOCHEMISTRY

MACROMOLECULE LABELLING – 'CLICK CHEMISTRY'



Li ZB et al. - Bioconj Chem 2007, 18: 1987-94.
Sirion U et al. - Tetrahedron Letters 2007, 48: 3953-7.
Marik J et al. - Tetrahedron Lett 2006, 47: 6681-4.
Wüst F et al. - J Label Compds Radiopharm 2007, 50(S1): S38.
Ramenda et al. - Lett Drug Design Discov 2007, 4: 279-85.
Glaser M et al. - Bioconj Chem 2007, 18: 989-93.
Inkster AHI et al. - J Label Compds Radiopharm 2008, 51: 444-52.
Kuhnast B et al. - J Label Compds Radiopharm 2008, 51: 336-42.

PET AND SPECT RADIOCHEMISTRY

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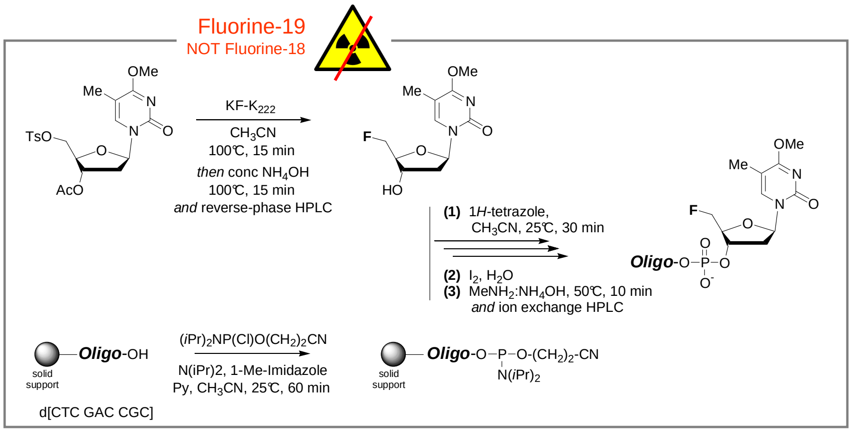
- ✓ WHAT ABOUT NANO-OBJECTS ?
- ✓ CLICK-CHEMISTRY: A NOVEL APPROACH

OTHER ALTERNATIVES ?

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Toward the fluorine-18-labelling of oligonucleotides …

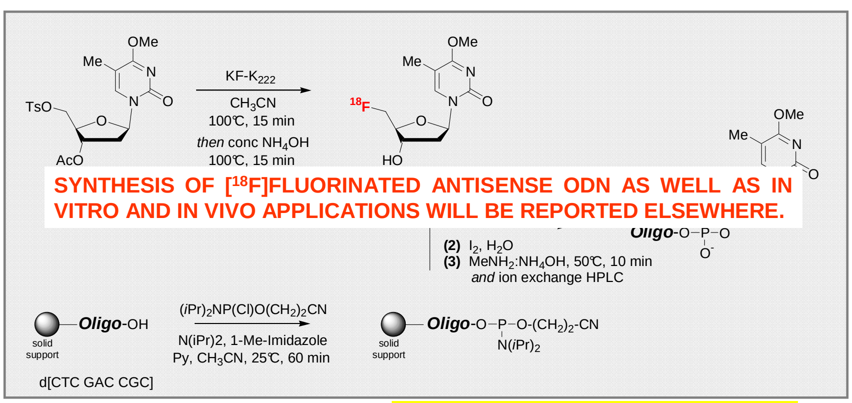


240 minutes, 2.5-5.0% yield

Pan D. E et al. - Bioorg Med Chem Letters 1998, 8: 1317-20.

LABELLING OF OLIGONUCLEOTIDES WITH NON-RADIOMETALS : FLUORINE-18

Toward the fluorine-18-labelling of oligonucleotides …



240 minutes, 2.5-5.0% yield ➡ **Radiochemical yield : 0.55-1.10% !!!**

Pan D. E et al. - Bioorg Med Chem Letters 1998, 8: 1317-20.

PET AND SPECT RADIOCHEMISTRY
SELECTED EXAMPLES OF LABELLING OF MACROMOLECULES

Fluorine-18 Chemistry for Molecular Imaging with Positron Emission Tomography,
Fluorine and Health: Molecular Imaging, Biomedical Materials and Pharmaceuticals,
Tressaud A. & Haufe G. Eds, Elsevier, 2008, 3-65.

Oligonucleotides with radioactive tags for *in vivo* imaging with SPECT and PET,
Recent Advances of Bioconjugate Chemistry in Molecular Imaging,
Xiaoyuan C. Ed., Research Signpost, 2008, chapter 1, 3-65.

***In vivo* imaging of oligonucleotidic aptamers,**
Nucleic Acid and Peptide Aptamers: Methods and Protocols,
Mayer G. (Ed.), Humana Press, 2009, volume 535, chapter 15, 241-259.

The challenge of labeling macromolecules with fluorine-18: Three decades of research,
B. Kuhnast and F. Dollé. Current Radiopharmaceuticals 2010, volume 3, issue 3, 174-201.

EMMI
EUROPEAN MASTER IN MOLECULAR IMAGING

PET AND SPECT RADIOCHEMISTRY
SELECTED EXAMPLES OF LABELLING OF MACROMOLECULES

Frédéric DOLLÉ

Radiochimie & Radiopharmacie
Service hospitalier Frédéric Joliot
Institut d'imagerie biomédicale - DSV/CEA
4 Place du Général Leclerc
F-91401 Orsay, France

Tél : +33 (0)1 69 86 77 04
Fax : +33 (0)1 69 86 77 49
E-mail : frederic.dolle@cea.fr

EMMI Intensive Programme
Design, synthesis and validation of imaging probes

September 19th, 2011 – 14h00-15h30
MBC, Turino, Italy