Princeton New Jersey, USA
June 7th – 11th 2015

Organized in collaboration with the IAEA

Symposium final Programme

And

Collection of Abstracts
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Welcome

On behalf of the Organising Committee and the International Isotope Society, we have the pleasure of welcoming you to IIS2015, the Twelfth International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds at Princeton University in the historic town of Princeton NJ.

The scientific sessions in the symposium are very similar to the previous meetings organised by the International Isotope Society. We have continued the theme begun at the Heidelberg meeting by placing a larger emphasis on the applications side of isotopically labelled compounds and especially the areas of drug metabolism, pharmacokinetics, metabolites, AMS and WBA studies. There will be a series of plenary lectures, invited and contributed papers, and posters. There will be a strong emphasis on recent trends and innovation. One of the main objectives of the symposium is to bring together leading scientists in the field of synthesis and applications of labelled compounds and provide them with a forum where they can discuss and exchange ideas. There will also be a commercial exhibition which will provide scientists with an overview of new technologies and services that will be available to them. An opportunity will be made available to participants to meet the exhibitors at the Poster Session and exhibitors’ evening on Monday.

This should be a wonderful week for science but also for the delights that Princeton can offer.

We welcome you all to Princeton.

Organizers IIS2015

Dr. David Hesk  Dr. Carolee Lavey  Dr. Eric Soli
Welcome

As the 2015 President of the International Isotope Society, it gives me great pleasure to welcome you to the 12th International Symposium on the Synthesis and Applications of Isotopes and Isotopically Labelled Compounds being held at Princeton University, June 7-11, 2015.

The 12th International Symposium continues in the trend of the previous 11 Symposia with an exciting mix of plenary lectures from some of the world’s leaders in science, invited lectures, contributed oral and poster presentations and exhibitors of the latest products for use with isotopes. As in the past, special lectures will be given by this year’s Melvin Calvin Award winner and the Wiley student researchers, as well as the presentation of the IIS Award to be given at the Wednesday evening symposium banquet.

I want to take this opportunity to especially thank the organizers of the 12th Symposium, David Hesk, Carolee Lavey and Eric Soli. They have worked diligently over the past three years to make this symposium a success and deserve our appreciation for their extra efforts. I also want to thank the sponsors and the exhibitors for their financial support of this symposium. The extra funds have a direct impact on the quality and scope of this symposium and we are fortunate to receive this support.

While in the Princeton area, I hope that you will take time to explore the area including the Princeton University Art Museum home to over 6000 Asian pieces of art, the Princeton Battlefield, local nightlife, restaurants and maybe even North America’s oldest bike shop where Professor Albert Einstein bought his bicycle. With Princeton’s proximity to New York City and Philadelphia, I hope you have the opportunity to visit these great cities as well during your stay in the area.

Best wishes for an enjoyable and productive symposium!

Best regards,

Brad D. Maxwell
2015 International Isotope Society President
Conference Committees

Organizers
Dr. David Hesk
Dr. Carolee Lavey
Dr. Eric Soli
Dr. Jens Atzrodt (Past organizer)
Dr. Volker Derdau (Past organizer)

Scientific Advisory Board
Dr. Brad Maxwell
Dr. Thomas Hartung
Dr. Eric Solon
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Dr. Chad Elmore

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Dr. Richard Burrell
Dr. Brad Keck
Dr. Brad Maxwell
Dr. Alain Schweitzer
Dr. Rhys Salter
Dr. Mark Seymour
Dr. Martin Sandvoss
Dr. Sam Bonacorsi
Dr. Jon Bloom
Dr. Ronghui Lin

Dr. Matt Donahue
Dr. Thomas Moenius
Dr. Joel Krauser
Dr. Eric Solon
Dr. Mihaela Plesescu
Prof. Dan Murnick
Dr. David Schenk
Dr. Thomas Hartung
Dr. Bruce McKillican
Dr. Chad Elmore
Dr. Dieter Muri
We would like to thank our Sponsors for their generous support:

Quotient BioResearch

Curachem

IAEA International Atomic Energy Agency
Atoms for Peace

Roche

Sanofi

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Vitrax

Janssen

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ISOTEC® Stable Isotopes
List of Exhibitors

Almac Sciences Inc
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Berthold
Cambridge Isotope Labs
CDN Isotopes
Combiphos Catalysts Inc
Curachem Inc
Hartmann Analytic
Isosciences LLC
Lablogic
Selcia
Quotient Bioresearch
PerkinElmer
RC TRITEC
Raytest
XenoTech
Floor Plans

McDonnell Hall
Floor plans

Frick Chemistry Building
Campus Map
## Final Scientific Program

### IIS 2015 Time Schedule

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<th>Monday</th>
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<td>June 7th</td>
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<td>7.00 am</td>
<td>Open</td>
<td>Breakfast</td>
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<td>Frick Atrium</td>
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<td>8.00 am</td>
<td>Welcome and Opening McDonnell A02</td>
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<td>Meet at Nassau Inn</td>
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<td>Session 10</td>
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<td>Symposium Banquet and IIS Award</td>
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Sunday June 7th, 2015

11:00 – 12:00 p.m. BoT Meeting (Nassau Inn)

12:00 – 1:00 p.m. BoT/ BoR Lunch (Nassau Inn)

1:00 – 3:00 p.m. BoT/ BoR Meeting (Nassau Inn)

4:00 – 8:00 p.m. Reception desk (Frick Atrium)

6:00 – 8:00 p.m. Welcome reception Sponsored by QUOTIENT BIORESEARCH (Frick Atrium)

Monday, June 8th, 2015

7:00 – 8:30 a.m. Reception desk (Frick Atrium)

7:00 – 8:30 a.m. Breakfast Sponsored by CURACHEM (Frick Atrium)

8:30 – 9:00 a.m. Welcome and Opening (McDonnell A02)

Organizer IIS2015: Dr. David Hesk, Dr. Carolee Lavey, Dr. Eric Soli
President Dr. Brad Maxwell

Chairman Dr. David Hesk (Merck & Co, USA)

9:00 – 10:00 a.m. Plenary Lecture 1 (McDonnell A02)

Prof. Angela Creager (Princeton University, USA)

PL-1 ‘Traces of the Manhattan Project: Radioisotopes in Science and Medicine’

10:00 – 10:30 a.m. Coffee break (Frick Atrium)

Session 1: Synthesis of Compounds Labelled with long lived Isotopes (including biochemical and microbiological approaches) (McDonnell A02)

Chairman Dr. Jens Atzrodt (Sanofi, Germany)

10:30 – 10:50 a.m. Dr. Ian T. Huscroft (Selcia) (McDonnell A02)

O-1 ‘Total radiosynthesis of [14C]Homoharringtonine’
O-2
‘14-step synthesis of an enantiomerically pure $^{14}$C-labelled morpholine derivative as a development candidate for the treatment of psychiatric disorders’

O-3
‘[14C]Phosgene—a building block in C-14 labelling chemistry’

O-4
‘Synthesis of isotope labelled SGLT2 inhibitor Canaglifozin (JNJ-28431754) and its metabolites’

O-11
‘Molecular isotope engineering (MIE): Industrial manufacture of Naproxen of predetermined isotopic composition’

O-12
‘Radioactive contamination limits, equivalent doses, and ’should you care’

O-13
‘Impacts on occupational radiation safety, public awareness and pharmaceutical research of ICRP 103 implementation’

Melvin Calvin Award
‘Iridium catalyst systems of enhanced utility in hydrogen isotope exchange’

Session 1: Synthesis of Compounds Labelled with long lived Isotopes (including biochemical and microbiological approaches)
Chairman
Dr. Jens Atzrodt (Sanofi, Germany)

2:20 – 2:50 p.m.
Dr. Sébastien Roy (Sanofi)
赵：‘Synthesis of a radiolabelled antibody drug conjugate using biosynthesized $^{14}$C labelled Tamaymycin’

2:50 – 3:10 p.m.  Dr. William Watters (Almac)  Page 49

赵-6 ‘Application of biocatalysis in isotope chemistry’

3.10 – 3:40 p.m.  Coffee Break  Sponsored by Roche

3:40 – 4:00 p.m.  Mr. Nelo Rivera (Merck & Co)  Page 50

赵-7 ‘Enzymatic approaches towards the synthesis of isotopically labelled 9-(2-hydroxypropyl) adenine’

4:00 – 4:20 p.m.  Dr. Neil Geach (Selcia)  Page 51

赵-8 $^{14}$C-labelling of the acaricide Hexythiazox

4:20 – 4:40 p.m.  Dr. Grégory Piéters (CEA-Saclay)  Page 52

赵-9 ‘Regioselective and enantioselective C-H deuteration using Ru nanocatalysts: application to the labelling of nitrogen and sulphur containing biomolecules’

4:40 – 5:00 p.m.  Dr. Qingfeng Pan (University of Notre Dame)  Page 53

赵-10 ‘Synthesis of selectively $^{13}$C-labelled high mannose N-Glycanes’

6:00 – 8:00 p.m.  Poster Session and Exhibition  (Frick Atrium)

Chairman  Dr. Dieter Muri (Roche, Switzerland),  Pages 108-134

A buffet dinner and drinks will be served

Tuesday, June 9th, 2015

7:00 – 8:00 a.m.  Reception desk  (Frick Atrium)

7:00 – 8:00 a.m.  Breakfast sponsored by Vitrax  (Frick Atrium)

Chairman  Dr. Thomas Hartung (Roche, Switzerland)

8:00 – 9:00 a.m.  Plenary Lecture (2)  Prof. V. Gouverneur  (University of Oxford, UK)  Page 28

(University of Oxford, UK)

PL-2 ‘Expanding the range of $^{18}$F-tags for PET Applications’
IIS2015, Princeton, June 7-11, 2015

**Chairman**  
*Dr. Brad Maxwell (BMS, USA)*

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<th>Time</th>
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<tr>
<td>9:00 – 10:00 a.m.</td>
<td><strong>Plenary Lecture 3</strong> Sponsored by Prof. Jin-Quan Yu (Scripps Institute, USA)</td>
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<td>PL-3 ‘Ligand-Accelerated C-H Activation Reactions: Near and Far’</td>
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<tr>
<td>10:00 – 10:30 a.m.</td>
<td><strong>Coffee break</strong></td>
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**Session 3: Preclinical and Clinical ADME Studies supported by Labelled Compounds**  
*(McDonnell A02)*

**Chairman**  
*Dr. Brad Maxwell (BMS, USA)*

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<th>Time</th>
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<tr>
<td>10:30 – 11:00 a.m.</td>
<td>Dr. Lisa Christopher (BMS)</td>
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<td>O-14 ‘Applications for radiolabeled and stable labeled compounds in preclinical and clinical ADME studies’</td>
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<td>11:00 – 11:20 a.m.</td>
<td>Dr. Ian Shaw (Quotient Clinical)</td>
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<td>O-15 ‘What data can a 14C clinical study deliver? A decade of innovative, integrated 14C study designs to understand drug behaviour in human subjects’</td>
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<td>11:20 – 11:40 a.m.</td>
<td>Dr. Yuexian Li (Takeda)</td>
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<td>O-16 ‘Strategies for the conduct of human ADME studies of oncology compounds’</td>
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<td>11:40 – 12:00 p.m.</td>
<td>Prof. Tomáš Elbert (Institute of Org. and Biochem. of ASCR)</td>
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<td>O-17 ‘Synthesis of derivatives of 6-amino-uracil labeled with 14C for testing of their metabolic stability’</td>
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<td>12:00 – 1:20 p.m.</td>
<td><strong>Lunch</strong></td>
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**Session 5: New Insights into metabolite identification, applications and underlying strategies**  
*(McDonnell A01)*

**Chairmen**  
*Dr. Mihaela Plesescu (Takeda, USA), Dr. Rhys Salter (Janssen, USA)*

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<tr>
<td>10:30 – 11:00 a.m.</td>
<td>Dr. Liam Evans (Hypha Discovery Ltd)</td>
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<td>O-18 ‘Biocatalytic alternatives to chemical synthesis of drug metabolites: options, advantages and limitations’</td>
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<td>11:00 – 11:20 a.m.</td>
<td>Dr. Gregory Walker (Pfizer)</td>
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Page 15
11:20 – 11:40 a.m.  Dr. Yong Gong (Janssen)  Page 63
O-19  ‘Structural characterization and NMR quantitation of drug metabolites in discovery and development’

11:40 – 12:00 p.m.  Dr. Neil Geach (Selcia)  Page 64
O-20  ‘Design, generation and utilization of radio and stable isotope co-labelled standards for metabolite quantitation’

12:30 – 1:20 p.m.  Lunch

Chairman  Dr. Eric Solon (QPS, USA)

1:20 – 2:20 p.m.  Plenary Lecture 4
(McDonnell A02)  Dr. Jack Hoppin  (inviCRO, USA)  Page 30
PL-4  ‘Translational nuclear imaging in drug discovery and development’

Session 4: Whole Body Autotradiography (WBA)-New Methods, Applications and Trends
(McDonnell A02)

Chairman  Dr. Eric Solon (QPS, USA)

2:20 – 2:50 p.m.  Dr. Claude Rouleau (University of Quebec)  Page 65
O-22  ‘Dual-radionuclide imaging in WBARG by differential absorption of charged particles’

2:50 – 3:10 p.m.  Dr. Marissa Vavrek (Merck & Co)  Page 66
O-23  ‘Imaging tools for putting the “D” in “ADME”’

3:10 – 3:40 p.m.  Coffee Break  Sponsored by  Page 67
(Frick Atrium)

3:40 – 4:00 p.m.  Dr. Jonathan Stauber (Imabiotech Corp)  Page 67
O-24  ‘Mass spectrometry imaging as a tool for ADME and PK studies’

4:00 – 4:20 p.m.  Dr. Thomas Bäck (University of Gothenburg)  Page 68
O-25  ‘Alpha camera imaging for evaluation of small scale activity and dose distributions in targeted alpha therapy’

4:20 – 4:40 p.m.  Dr. XiaoXian Chan (Novartis)  Page 69
O-26 ‘QWBA’ PBPK modeling and liquid surface sampling micro-liquid chromatography mass spectrometry to impact drug development’

4:40 – 5.00 p.m. Dr. Natasha Penner (Biogen Idec) Page 70

O-27 ‘Beyond QWBA: combining multiple imaging modalities in a single study’

Session 6: Accelerator Mass Spectrometry (AMS) - a versatile Tool in Drug Development (McDonnell A01)

Chairmen Prof. Daniel Murnick (Rutgers University, USA), Dr. Mark Seymour (Eckert and Ziegler Vitalea Science, UK)

2:20 – 2:50 p.m. Dr. Mark Seymour (Eckert and Ziegler) Page 71

O-28 ‘Adding value through AMS-enabled FIH studies’

2:50 – 3:10 p.m. Dr. Ian Shaw (Quotient Clinical) Page 72

O-29 ‘Clinical studies utilizing accelerator mass spectrometry: opportunities and challenges of 14C radiolabelled micro tracer study delivery’

3:10 – 3:40 p.m. Coffee Break Sponsored by

(Frick Atrium)

3:40 – 4:00 p.m. Dr. Ad Roffel (PRA Health Sciences) Page 73

O-30 ‘An evaluation of human ADME and mass balance studies using regular or low doses of radiocarbon’

4:00 – 4:20 p.m. Dr. Adedayo Adedoyin (Cubist Pharmaceuticals) Page 74

O-31 ‘Characterization of the absorption and disposition of Bevenopran using micro-tracer techniques’

4:20 – 4:40 p.m. Dr. Stephen English (Xceleron Inc.) Page 75

O-32 ‘Surveying the boundaries of accelerator mass spectrometry: the widening scope of an evolving technology’

4:40 – 5:00 p.m. Prof. Paul T. Henderson (UC Davis) Page 76

O-33 ‘Quantitation of DNA adducts formed by deoxynucleoside analogues and alkylating agents by accelerator mass spectrometry’

5:00 – 7:00 p.m. Workshop hosted by XenoTech

(McDonnell A01) ‘Biomimetic oxidation: A chemical approach to metabolite generation’
Wednesday, June 10th, 2015

7:00 – 8:00 a.m. Reception Desk
(Frick Atrium)

7:00 – 8:00 a.m. Breakfast
(Frick Atrium)

Chairmen
Dr. Ken Lawrie (GSK, UK), Dr. Carolee Lavey (USA)

Journal of Labelled Compounds Young Chemist Award
(sponsored by Wiley)

8:00 – 8:20 a.m.
Dr. Marc Reid (University of Edinburgh, UK)  Page 39
‘Design and application of Iridium catalysts for C-H activation towards hydrogen isotope exchange processes.’

8:20 – 8:40 a.m.
Mr. Renyuan Yu (Princeton University, USA)  Page 40
‘Iron catalysed hydrogen isotope exchange in drug molecules.’

8:40 – 9:00 a.m.
Mr. Thomas Andersen (Aarhus University, Denmark) Page 41
‘Efficient 11C-carbonylation of isolated aryl palladium complexes for PET: application to challenging radiopharmaceutical synthesis’

9:00- 9:20 a.m.
Miss Philippa Owens (University of Strathclyde, UK) Page 42
‘Iridium catalysed hydrogen isotope exchange for the regioselective deuteration of N-heterocycles’

Chairman
Dr. Jon Bloom (Quotient Biosciences)

9:30 – 10:30 a.m. Plenary Lecture 5
(McDonnell A02)
Professor Mohammad Movassaghi (MIT, USA) Page 31
PL-5 ‘Complex alkaloid total synthesis’

10:30 – 11:00 a.m. Coffee break
(Frick Atrium)

Session 7: Analytical challenges and formulation issues with labelled compounds
(McDonnell A01)

Chairmen
Dr. Martin Sandvoss (Sanofi, Germany), Dr. David Schenk (Merck, USA)

11:00 – 11:20 a.m.
Dr. Tony Pereira (Merck and Co) Page 77
O-34 ‘Determination of tritium atom location and distribution using tandem mass spectrometry’
11:20 – 11:40 a.m.  Dr. Kiernan Crowley (Quotient Clinical)  Page 78
O-35  ‘Addressing the challenges of working with 14C radiolabelled drug substance and drug product in clinical development through a translational pharmaceutics platform’

11:40 – 12:00 p.m.  Dr. Chad Elmore (Astra-Zeneca)  Page 79
O-36  ‘A collaborative study comparing the use of LC/MS to LC/UV for the determination of specific activity’

12:00 – 12:30 p.m.  Dr. Markus Walles (Novartis), Dr. Greg Warner (Perkin Elmer)  Page 80
O-37  ‘Viewlux microplate imager for metabolite profiling: applications in drug development’

12:30 – 1:40 p.m.  Lunch  Sponsored by
(Frick Atrium)

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**Session 9: Non Pharmaceutical applications of labelled compounds**

(McDonnell A02)

**Chairman**  Dr. Jon Bloom (Quotient Biosciences, UK)

11:00 – 11:20 a.m.  Prof. Roman Zubarev (Karolinska Institutet)  Page 81
O-38  ‘Escherichia coli growth rate measurements verify the isotopic resonance hypothesis’

11:20 – 11:35 a.m.  Prof. M.A. Samad Khan (Bangladesh University)  Page 82
O-39  ‘Reproductive performance of crossbred dairy cows under organized farm conditions in Bangladesh: An isotopic radioimmunoassay study’

11:35 – 11:50 a.m.  Dr. Laszlo Orha (Izotop)  Page 83
O-40  ‘Challenges in the reductions of the [ring-U-14C] labelled fluoro-nitro-benzene derivatives’

11:50 – 12:10 p.m.  Prof. Marianna Kanska (University of Warsaw)  Page 84
O-41  ‘Kinetic and solvent isotopic effects in biotransformations of aromatic amino acids and their derivatives’

12:10 – 12:30 p.m.  Dr. Del Koch (ABC Labs)  Page 85
O-42  ‘The use of stable isotope labelled analogues both as internal standards and as surrogates for the analysis of multiple pyrethroid insecticides in complex environmental samples’

12:30 – 1:40 p.m.  Lunch  Sponsored by
(Frick Atrium)
IIS2015, Princeton, June 7-11, 2015

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| 1:40 – 2:40 p.m. | **Plenary Lecture 6**  
*Prof. Mathew Thakur* (Thomas Jefferson University, USA)  
PL-6  
‘Novel emerging technologies in PET imaging of breast and prostate cancer’ |

**Session 8: Synthesis and applications of labelled compounds with short lived isotopes**  
*(McDonnell A02)*

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| 2:40 – 3:10 p.m. | Dr. Volker Derdau (Sanofi) | O-43  
‘Synthesis and characterization of novel fluorophore or isotopically labelled probes for beta cell imaging’ |
| 3:10 – 3:30 p.m. | Dr. Andrew Hoover (Harvard University) | O-44  
‘Automated synthesis of $[^{18}F]5$-fluorouracil from $[^{18}F]$fluoride for human PET imaging’ |
| 3:30 – 4:00 p.m. | Coffee break              | **Sponsored by**  
SIGMA-ALDRICH®  
ISOTEC® Stable Isotopes |
| 4:00 – 4:20 p.m. | Dr. David Donnelly (Bristol Myers Squibb) | O-45  
‘Design, synthesis of development of lysophosphatidic acid receptor 1 (LPA1) PET radioligands for lung receptor occupancy imaging’ |
| 4:20 – 4:40 p.m. | Dr. Gilles Tamagnan (MNI imaging) | O-46  
‘The development of a PET tracer from bench to large clinical studies’ |
| 4:40 – 5:00 p.m. | Dr. Luca Gobbi (F. Hoffmann-La Roche) | O-47  
‘RO6807936 as a novel radiotracer for in vitro and in vivo visualization and quantification of BACE1 in the rodent and baboon brain’ |

**Dinner – Program**  
5:45 – 7:00 p.m.  
Princeton University Campus walking Tour- meet at Nassau Inn  
7:00 p.m.  
Conference Banquet at Nassau Inn  
IIS Award ceremony

Chairman  
**Dr. Eric Soli** (AbbVie)
Thursday, June 11th, 2015

7:00 – 8:00 a.m.  Reception Desk
(Frick Atrium)

7:00 – 8:00 a.m.  Breakfast
(Frick Atrium)

Chairman  Dr. Dieter Muri (Roche)

8:00 – 9:00 a.m.  Plenary Lecture 7
(McDonnell A02) Prof. Tobias Ritter  (Harvard University, USA)  Page 33
PL-7  ‘Late stage fluorination for PET Imaging’

Chairman  Dr. Brad Maxwell (BMS)

9:00 – 10:00 a.m.  Plenary Lecture 8
(McDonnell A02) Prof. Abigail Doyle  (Princeton University, USA)  Page 34
PL-8  ‘New reagents and strategies for catalytic nucleophilic (radio)fluorination’

10:00 – 10:30 a.m.  Coffee break
(Frick Atrium)

Session 1: Synthesis of Compounds Labelled with long lived isotopes
(McDonnell A02)

Chairmen  Dr. Richard Burrell  (BMS, USA), Dr. Thomas Moenius  (Novartis, Switzerland)

10:30 – 11:00 a.m.  Dr. Sumei Ren (Merck & Co)  Page 95
O-52  ‘Synthesis of labelled compounds through manipulation of active pharmaceutical ingredients (API’s) and late stage intermediates- case studies’

11:00 – 11:20 a.m.  Dr. Ronghui Lin (Janssen)  Page 96
O-53  ‘Synthesis of metabolites of C-FMS Kinaseinhibitor JNJ-40346527 and the isotope labeled compounds’

11:20 – 11:40 a.m.  Dr. Richard C. Burrell (BMS)  Page 97
O-54  ‘Synthesis of stable isotope labelled Epothiolone D using a degradation-reconstruction approach’

11:40 – 12:00 p.m.  Dr. Sean P. Bew  (University of East Anglia)  Page 98
O-55  ‘Protocol for incorporating multiple stable isotopes into high value heterocycles’

12:00 – 1:20 p.m.  Lunch
(Frick Atrium)
**Chairman**  
Dr. Chad Elmore (Astra Zeneca, Sweden)

1:20 – 2.20 p.m.  
**Plenary Lecture 9**  
(McDonnell A02)  
Dr. Jeff Bode (ETH Zürich)  
Page 35

**PL-9**  
‘New chemoselective ligation reactions for protein synthesis and bioconjugation’

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**Session 1: Synthesis of Compounds Labelled with long lived isotopes**  
(McDonnell A02)

**Chairmen**  
Dr. Richard Burrell (BMS, USA), Dr. Thomas Moenius (Novartis, Switzerland)

2:20 – 2:50 p.m.  
Dr. Alban Allentoff (BMS)  
Page 99

O-56  
‘Synthesis of stable labelled signature peptides for use as internal standards in protein quantification’

2:50 – 3:10 p.m.  
Dr. Peter Johnson (Dow Agrosciences)  
Page 100

O-57  
‘Synthesis of isotopically labelled 6-arylpicolinate herbicides’

3:10 – 3:40 p.m.  
**Coffee break**  
(Frick Atrium)

3:40 – 4:00 p.m.  
Mr. Richard Mudd (University of Strathclyde)  
Page 101

O-58  
‘Investigating selective hydrogen isotope exchange on unsaturated moieties with active Iridium complexes’

4:00 – 4:20 p.m.  
Dr. Yui. S. Tang (Perkin Elmer)  
Page 102

O-59  
‘Tritium labelling of small molecules: Crotonic acid, Isocrotonic acid, Cystamine, Cysteamine, acetylcholine and butyrlcholine’

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**Session 3: Preclinical and Clinical ADME Studies supported by Labelled Compounds**  
(McDonnell A01)

**Chairman**  
Dr. Brad Maxwell (BMS, USA)

10:30 – 11:00 a.m.  
Dr. Wayland Rushing (ABC Labs)  
Page 91

O-48  
‘cGMP radiosynthesis for early phase clinical trials: a unique challenge’

11:00 – 11:20 a.m.  
Dr. Davide Audisio (CEA-Saclay)  
Page 92

O-49  
‘Combining efficient $^{14}$C radiolabelling and radioimaging techniques of manufactured nanoparticles for toxicological studies’

11:20 – 11:40 a.m.  
Prof. Gheorghe Mateescu (Case Western Reserve University)  
Page 93

O-50  
‘A new biomarker of mitochondrial function obtained in vivo by dynamic deuterium magnetic resonance following administration of deuterated glucose’
## Session 10: New Trends and Technologies in Isotope Science

### (McDonnell A01)

**Chairmen**

Dr. Chad Elmore (Astra Zeneca, Sweden), Dr. Ronghui Lin (Janssen, USA)

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<td>‘Deuteration-unexpected effects on the structures and properties in soft materials’</td>
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<td>3:40 – 4:00 p.m.</td>
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<td>‘Benchtop laser based radiocarbon analyzer’</td>
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### 4:20 pm – 4:40 p.m.

**Closing Remarks**

Organizer IIS2015 Dr. David Hesk, Dr. Carolee Lavey, Dr. Eric Soli
Session Index

Session 1: J. Atzrodt (Sanofi)  
R. Burrell (BMS), T. Moenius (Novartis)  
Synthesis of Compounds Labelled with Long-lived Isotopes (including biochemical and microbiological approaches)

Session 2: B. Keck (NorthStar Medical Radioisotopes)  
Handling of Isotopes, safety, political and social dimensions

Session 3: B. Maxwell (BMS) and J. Krauser (Novartis)  
Preclinical and clinical ADMe studies supported by labelled compounds

Session 4: E. Solon (QPS)  
Whole body autoradiography (WBA)- New methods, applications and trends

Session 5: M. Plesescu (Takeda) and R. Salter (Janssen)  
New insights into metabolite identification, applications and trends

Session 6: D. Murnick (Rutgers) and M. Seymour (Eckert and Ziegler)  
Accelerator mass spectrometry (AMS)- a versatile tool in drug development

Session 7: D. Schenk (Merck) and M. Sandvoss (Sanofi)  
Analytical challenges and formulation issues with labelled compounds

Session 8: T. Hartung (Roche) and S. Bonacorsi (BMS)  
Synthesis and applications of labelled compounds with short lived isotopes

Session 9: J. Bloom (Quotient) and B. McKillican (Syngenta)  
Non-Pharmaceutical applications of labelled compounds

Session 10: C. Elmore (Astra-Zeneca) and R. Lin (Janssen)  
New trends and technologies in isotope science

Poster Session: D. Muri (Roche)
Scientific Abstracts
“Traces of the Manhattan Project: Radioisotopes in Science and Medicine”

Angela Creager

Philip and Beulah Rollins Professor of History, Princeton University, Princeton NJ.

After detonating the first nuclear weapons in Japan, to devastating effects, the U.S. government turned swiftly to promoting the peaceable dividends of atomic energy. The first such benefit took the form of radioactive isotopes, produced in a former Manhattan Project reactor and distributed to civilian purchasers beginning in 1946. The consequences of this new supply of radioisotopes for science and medicine were profound and extensive, as illustrated by developments in biochemistry, nuclear medicine, and ecology. In each of these areas, one can see how government policy and infrastructure integral to the Cold War shaped scientific opportunities and knowledge. Routine practices of radiolabeling and radiotracing remained in place long after the positive political valence of radioisotopes dimmed in the 1960s and 1970s, in the wake of the debates over radioactive contamination of the environment from atomic weapons tests and nuclear waste. The talk will conclude with a consideration of how radioisotope usage served to prompt the postwar federal regulation of scientific research, which expanded considerably in the last decades of the twentieth century.
Expanding the Range of $^{18}$F-Tags for PET Applications

Véronique Gouverneur

Chemistry Research Laboratory, University of Oxford, 12 Mansfield Road
OX1 3TA Oxford (UK)
Email: veronique.gouverneur@chem.ox.ac.uk

The success of PET and renewed interest in $[^{18}\text{F}]$radiochemistry led to creative methods to incorporate $^{18}\text{F}$ into molecules of increasing complexity. Despite these advances, clinically useful radiotracers lie within a narrow accessible space with $[^{18}\text{F}]$fluoroalkanes and $[^{18}\text{F}]$fluoroarenes at the forefront. Many potentially high value PET $^{18}\text{F}$-labeled tracers and drugs lie outside this radiochemical space, and the ability to test tracers not amenable to traditional or newly developed $^{18}\text{F}$-labeling intervention would be a major boost for PET imaging. A more diverse range of $^{18}\text{F}$-tags could immediately serve medicinal chemists by informing the selection of lead compounds much earlier in the drug discovery pipeline. This lecture will present our general approach to radiochemistry and the recent contribution we have made to this field of research with the labeling of a range of $^{18}\text{F}$-tags for PET.
Ligand-Accelerated C-H Activation Reactions: Near and Far

Jin-Quan Yu

Department of Chemistry, The Scripps Research Institute, 10550 N. Torrey Pines Road, BCC-372, La Jolla, California, 92037-1000, U.S.A.

Email: Yu200@scripps.edu

Website: http://www.scripps.edu/chem/yu/

Two different classes of novel ligands are developed to drastically accelerate Pd-catalyzed C–H activation reactions. These ligands enable the activation of C-H bonds that are near or far from a functional group. Enantioselective C–H activation reactions are also made possible by using chiral version of these ligands.
Translational nuclear imaging offers a broad array of assays to drug discovery and development researchers. These assays range from micro-autoradiography to whole-body tomography and present various strengths and limitations. This talk will focus on the assessment of these techniques and technologies from the perspective of sensitivity, spatial and temporal resolution, quantification as well as cost. Results for pre-clinical and clinical case studies will be presented for multiple disease areas with a focus on the impact of isotope selection on image study design and imaging informatics.
PL5

Complex Alkaloid Total Synthesis

Mohammad Movassaghi

Massachusetts Institute of Technology,

Several representative enantioselective alkaloid total syntheses and related methodologies will be discussed. Of particular interest to these programs is the development of unifying strategies guided by biogenetic considerations for each alkaloid family of interest. These syntheses feature new stereo- and chemoselective reactions that enable maximum use of the inherent chemistry of intricate intermediates. Examples detailing strategic C-H functionalization and its application in complex settings will be discussed.
NOVEL EMERGING TECHNOLOGIES IN PET IMAGING OF BREAST AND PROSTATE CANCER

Mathew L. Thakur
Professor of Radiology and Radiation Oncology
Thomas Jefferson University

For more than five decades, certain radioisotopes have continued to contribute extensively to the management of human health and diseases. Although great strides have been made with the use of radioisotopes in oncology, there remains an unmet need in early and accurate diagnosis of breast and prostate cancer, for which histology is considered as a gold standard. In order to obtain a tissue specimen for histology, a biopsy must be performed. Data are abundant that indicate more than two third of breast and prostate biopsies find benign pathology at the expense of severe patient anxiety and morbidity, and billions of healthcare dollars.

In this presentation, I shall outline an emerging technology, which targets a genomic biomarker with a specific biomolecule that uses a positron emitting ($t_{1/2} = 12.8$ hrs.) radionuclide, $^{64}$Cu, as a tracer. $^{64}$Cu is produced by the $^{64}$Ni (p,n) $^{64}$Cu reaction using a medical cyclotron and then chelated to the biomolecule that has a high affinity to the genomic biomarker, namely VPAC1. This biomarker expresses in high density on breast and prostate cancer cells at the onset of oncogenesis, but not on the normal cells. Translated from basic investigation and preclinical evaluation, to PET image breast and prostate cancer bearing patients, the $^{64}$Cu-labeled biomolecule promises to minimize the number of millions of unnecessary breast and prostate biopsies performed each year, and to reduce the healthcare cost.

Supported by NIH CA 157372 and NIH CA 109231
Late-Stage Fluorination for PET Imaging

Tobias Ritter
Harvard University, 12 Oxford Street, Cambridge, MA 02138, USA; ritter@chemistry.harvard.edu

The unnatural isotope fluorine–18 (\(^{18}\)F) is used as a positron emitter in molecular imaging. Currently, many potentially useful \(^{18}\)F-labeled probe molecules are inaccessible for imaging, because no fluorination chemistry is available to make them. Syntheses must be rapid on account of the 110-minute half-life of \(^{18}\)F and benefit from using \([^{18}\text{F}]\)fluoride due to practical access and suitable isotope enrichment. But \([^{18}\text{F}]\)fluoride chemistry has been limited to nucleophilic fluorination reactions. I will describe the development of a palladium-based electrophilic fluorination reagent derived from fluoride and its application to the synthesis of aromatic \(^{18}\)F-labeled molecules via late-stage fluorination. In addition, I will discuss new reaction chemistry for introduction of fluorine into functionalized molecules. Late-stage fluorination enables the synthesis of conventionally unavailable positron emission tomography (PET) tracers for anticipated applications in pharmaceutical development as well as pre-clinical and clinical PET imaging.

\[ \text{Me} \quad \text{2OTf} \quad \begin{array}{c} 2^{\ominus} \quad \text{18-cr-6, KHCO}_3 \quad \text{acetone} \quad 23^\circ C, 10 \text{ min} \end{array} \]

*Science* 2011, 334, 639.
New reagents and strategies for catalytic nucleophilic (radio)fluorination

Abigail G. Doyle

Chemistry Department, Princeton University, Princeton NJ, USA

An expansive array of medicines, agrochemicals, and materials contain fluorine due to the unique chemical properties that the element confers on organic molecules. One of the chief obstacles to the discovery and production of these compounds is the availability of synthetic methods for carbon–fluorine (C–F) bond formation. The most abundant and inexpensive fluorine sources, nucleophilic fluoride salts, typically suffer from low solubility, high hygroscopicity, and strong Brønsted basicity, rendering them recalcitrant reagents for chemical synthesis. Nevertheless, our laboratory has recently identified strategies that achieve mild and efficient nucleophilic fluorination using transition metal catalysis. This lecture will describe some of our recent progress in methodology development. Mechanistic studies will be discussed that contribute a better understanding of the properties and reactivity of transition metal fluorides. Furthermore, this lecture will cover application of our methods to the preparation of small-molecule tracers containing the radionuclide $^{18}$F for positron emission tomography (PET).
NEW CHEMOSELECTIVE LIGATION REACTIONS FOR PROTEIN SYNTHESIS AND BIOCONJUGATION

Jeffrey W. Bode

1Laboratorium für Organische Chemie, ETH Zürich, Zürich,CH-8093 Switzerland
2Institute for Transformative bio-Molecules (ITbM), Nagoya University, Nagoya, JAPAN
bode @org.chem.ethz.ch

Chemical ligation reactions enable the rapid ligation of molecules in an aqueous environment, regardless of the number or nature of unprotected functional groups present in the substrates. Such reactions are critical to the fields of synthetic protein chemistry, bioconjugation, and labeling. In order to expand the scope of chemical protein synthesis and to improve the ease with which proteins can be chemically synthesized, our group has sought to identify new ligation reactions that give native peptide bonds under mild, chemoselective conditions. This work has led to the discovery of the -ketoacid–hydroxylamine amide-forming (KAHA) and potassium acyltrifluoroborate (KAT) ligations, which operate in the presence of unprotected functional groups, require no reagents or catalysts, and proceed under aqueous conditions.

This talk will describe the development of the KAHA ligation, methods to prepare peptide segments containing the key functional groups, and the application of these to the chemical synthesis of proteins. Finally, we will discuss solutions to the still unsolved problem of ligating large, unprotected molecules at submillimolar concentrations with equimolar stoichiometry by developing new ligation reactions with faster rates and perfect chemoselectivity.

References
Melvin Calvin Award
In order to more effectively alleviate the appreciable issue of drug candidate attrition within medicinal chemistry research, isotopic labelling with heavy hydrogen (and other) isotopes is widely used as a means to monitor the fate of a potential drug molecule. In recent years, organoiridium complexes have garnered considerable attention within the field of transition metal-catalysed hydrogen-isotope exchange.\(^1\) Key to their applicability in this area is the ability of such complexes to selectively target unactivated C-H bonds, whilst simultaneously allowing convenient isotope incorporation with the use of practically convenient deuterium or tritium gas (e.g. $^1\rightarrow^2$, Scheme 1). Until recently, the standard in this field has been Crabtree’s catalyst $^3$.\(^2\) However, super-stoichiometric amounts of this species are frequently required together with long reaction times, and often only low levels of labelling are delivered.\(^3\) More recently, a series of detailed studies within our laboratory revealed complexes of type 4, possessing sterically encumbered and electronically tuned ligand combinations, to be superior in both activity and efficiency.\(^4\) Furthermore, the site of labelling is predictable and reproducible. As shown in Scheme 2, at 5 mol% complex loading, high deuterium incorporation is achieved into substrate 5, whereas previously, stoichiometric amounts of Crabtree’s catalyst were required to induce a similar level of labelling into this substrate.\(^5\)

Within this presentation, our continuing efforts to develop even more effective catalyst systems will be described. In particular, the use of both theoretical and experimental techniques relating ligand properties to catalyst activity will be outlined, with this growing knowledge base having now facilitated the development of a portfolio of catalysts of types 6 and 7 with well-defined and further enhanced levels of reactivity. Application of these complexes to the isotopic labelling of a widening range of substrates will be discussed, highlighting the selectivity and low catalyst loading of these developed processes, in addition to the application of heterocyclic architectures and additional functional groups which have previously proven recalcitrant towards iridium-catalysed deuterium labelling.\(^5\) Applicability of our developed catalysts will also be demonstrated in selective and efficient deuteration and tritiation of established drug molecules.

References
Wiley Young Scientist Awards
On the Design and Application of Iridium Catalysts in Hydrogen Isotope Exchange Processes

Marc Reid,1 William J. Kerr,*2 Tell Tuttle*2

1School of Chemistry, University of Edinburgh, West Mains Road, EH9 3FJ.
2Dept. of Pure & Applied Chemistry, University of Strathclyde, Cathedral Street, G1 1XL.

The synthesis of isotopically-labelled molecules via iridium-catalysed hydrogen isotope exchange (HIE) has a sustained importance in the study of reaction mechanisms and metabolic processes. Over recent years, research at the University of Strathclyde has centred on the development of iridium-based HIE catalysts able to deliver the desired chemical tag under extremely mild and industrially applicable reaction conditions.[1–4]

In a broader programme of work, we have combined experimental knowledge with computational modelling techniques in order to describe ligand properties of our iridium catalysts on a quantifiable and predictive footing. In this JLCR Award overview, we explain how novel catalyst mapping techniques have guided the development of mild and highly selective methods for labelling aldehydes, primary sulfonamides, and N-H-tetrazoles.

References
IRON-CATALYZED HYDROGEN ISOTOPE EXCHANGE IN DRUG MOLECULES

Renyuan Pony Yu,† David Hesk,‡ Paul J. Chirik†

†Department of Chemistry, Princeton University, Princeton, New Jersey 08544, United States
‡Merck Research Laboratories, Rahway, New Jersey 07065, United States

Research abstract:

Hydrogen isotope exchange catalyzed by soluble metal compounds is one of the most widely used and attractive methods for the radiolabelling of drug and drug-like molecules. For over 2 decades, Iridium compounds have been the primary catalyst technology and typically promote ortho-directed activation of aryl C-H bonds. The discovery of new transition metal catalysts that rely on earth abundant rather than scarce elements is attractive and remains underdeveloped. In addition to potential cost and environmental benefits, catalysts based on earth abundant elements may enable new reactivity not previously observed with existing catalysts. Our laboratory has discovered a family of bis(imidazolylidine)pyridine iron dinitrogen compounds that are active pre-catalysts for the hydrogenation of hindered and unactivated alkenes under mild reaction conditions. During our mechanistic investigation on the fundamental reactivity of the iron catalyst towards H₂, it was discovered that substantial amount of hydrogen was incorporated into the benzene-d₆ solvent, with concomitant formation of HD and D₂, revealing the catalysts’ potential for hydrogen isotope exchange reactions. Initial catalytic studies were conducted on exchange reactions of simple aromatic substrates using D₂. In contrast to the iridium compounds the iron catalysts effectively deuterate unhindered (meta, para positions) C-H bonds of arenes as well as aromatic heterocycles without the need for directing groups. Notably, the site of C-H activation is predictable and governed by steric accessibility and C-H bond acidity and isotopic exchange is optimal at subatmospheric pressures of D₂ gas. The iron catalysts are active in polar aprotic solvents such as DMF, DMA and NMP, an advantageous feature over most conventional Ir-based systems. In full collaboration with Merck, catalytic deuterium exchange reactions on several drug-like molecules were investigated. Moderate to high levels of deuterium incorporation were observed in a variety of structurally divergent substrates such as Loratadine, Papaverine and Suvorexant. Deuterium exchange occurs expectedly at unhindered aromatic positions of these substrates. Tritium labeling of these substrates were subsequently performed at Merck with modest to high levels (16 – 57 Ci/mmol) of specific activity and with retained regioselectivity. Current studies are directed towards improving catalyst stability for easier handling as well as enhanced functional group tolerance.
Efficient $^{11}$C-Carbonylation of Isolated Aryl Palladium Complexes for PET: Application to Challenging Radiopharmaceutical Synthesis

In this work, we investigated the implementation of palladium-aryl complexes as stoichiometric reagents in carbonylation reactions with $^{11}$CO to produce structurally challenging, pharmaceutically relevant compounds. By this method, the first $^{11}$C-carbonyl labeling of an approved Positron Emission Tomography tracer (PET tracer) was conducted providing $[^{11}$C$]$raclopride, an antagonist for the dopamine D2/D3 receptor, with an excellent radiochemical purity (RCP) and in a synthetically good yield.$^1$ Further investigations into the use of palladium-aryl complexes as stoichiometric reagents in $^{11}$C-labeling were conducted, and provided the $^{11}$C isotopically labeled anti-cancer agent $[^{11}$C$]$olaparib, and the brain-penetrant human neuropeptide Y Y2 receptor antagonist $[^{11}$C$]$JNJ-31020028 in an equally successful manner. The suitability of the carbonyl labeled $[^{11}$C$]$-carbonyl$[^{11}$C$]$raclopride as a PET tracer is under evaluation by microPET imaging on living rats and the results are compared to images obtained by use of the commonly applied $[^{11}$C$]$-methyl$[^{11}$C$]$raclopride [figure 1].

Figure 1: microPET image of $[^{11}$C$]$carbonyl$[^{11}$C$]$raclopride distribution in the brain of living rat

References
IRIDIUM-CATALYSED HYDROGEN ISOPOE EXCHANGE FOR THE REGIOSELECTIVE DEUTERATION OF N-HETEROCYCLES
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Isotopic labelling is an essential tool within the pharmaceutical industry for monitoring the metabolism of potential drug candidates; additionally, demand for deuterated compounds for use in mechanistic studies or as drug targets in their own right is ever-increasing. As such, the development of novel, mild, and efficient labelling techniques, which avoid the need for expensive deuterated starting materials, has been the focus of significant attention within the research community. Previous work within our laboratory has involved the development of a novel range of HIE catalysts of the type 1, bearing a combination of bulky phosphine and N-heterocyclic carbene ligands.

N-Heterocycles are ubiquitous in the pharmaceutical industry and represent an important and challenging class of labelling substrates. Recently, complexes 1 have been employed in the successful deuteration of a large range of indole and pyrrole derivatives. This practically convenient methodology allows for the use of common protecting groups to direct deuteration; these can then be removed under mild conditions with retention of the isotopic label.

The regioselectivity of deuteration in substrates such as 2, containing competing labelling sites, has been investigated. Complementary DFT studies have been used to rationalise the remarkable preference for deuteration at the C2 position, and have demonstrated that regioselectivity is determined by the kinetic barrier to C–H activation.

Through careful choice of N-protecting group, selective C3 labelling of N-alkyl indoles has also been achieved. Mechanistic studies support our proposal that this process proceeds via nucleophilic attack on deuteride intermediate 3, the acidity of which can be through careful selection of the ligand set. This process offers the opportunity to label nucleophilic compounds containing acid-sensitive functional groups.

The development and understanding of these two novel and notably mild HIE procedures offers the research community expedient access to new labelled compounds, previously unattainable under such mild conditions.

Abstracts for the parallel sessions
TOTAL RADIOSYNTHESIS OF $^{14}$C]HOMOHARRINGTONINE

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Homoharringtonine (HHT) (omacetaxine mepesuccinate) was approved for pharmaceutical use in
the United States in October 2012. Commercially marketed as Synribo®, the drug is indicated for
the treatment of adult patients with chronic myeloid leukaemia, a blood and bone marrow disease.
HHT was originally isolated from the leaves and stems of Cephalotaxus harringtonia, commonly
known as the Japanese Plum Yew. HHT is one of many constituents of the crude alkaloid extract.
The antileukemic property of HHT has stimulated numerous studies towards synthetic HHT. To
support the clinical development of HHT, a $^{14}$C radiolabeled synthesis was necessary with the
label in the cephalotaxine substructure. We wish to report the first total radiosynthesis of
$^{14}$C]homoharringtonine. ($^{14}$C]HHT). Optimising the chemistries developed by Royer1, Robin2 and
Kuehne3, we have prepared 15 mCi of $^{14}$C]HHT in enantiopure form. Starting from
$^{14}$C]cephalotaxine, two GMP radiosyntheses of $^{14}$C]HHT have been conducted providing material
for use in a clinical mass balance/metabolism study.

References

2934.
53, 3439-3450.
14-STEP SYNTHESIS OF AN ENANTIOMERICALLY PURE $^{14}$C-LABELLED MORPHOLINE DERIVATIVE AS DEVELOPMENT CANDIDATE FOR THE TREATMENT OF PSYCHIATRIC DISORDERS

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Trace amines, e.g. phenyl ethylamine, $p$-tyramine and tryptamine are amino acid derived metabolites present in low concentrations in the mammalian CNS. They are structurally related to the classical biogenic amines such as dopamine and serotonin and have been shown to be endogenous agonists for the trace amine-associated receptors (TAAR). Trace amines are rapidly catabolized by monoamine oxidase (MAO). Their dysregulation is linked to highly prevalent psychiatric disorders such as depression, schizophrenia, bipolar disorder.\cite{1}

In order to perform \textit{in vitro} and \textit{in vivo} drug metabolite profiling studies of the potential clinical candidate \textit{1} the synthesis of the $^{14}$C-labelled drug was requested by DMPK scientists. Taking into account the additional requirement to introduce $^{14}$C into a metabolically stable position of the molecule $^{14}$C-labelling of the phenyl ring or the morpholine moiety was considered as only viable labelling strategy.

![Scheme 1: Retrosynthetic analysis of the potential clinical candidate 1.](image)

Retrosynthetic analysis of \textit{1} resulted in the selection of \textit{p}-nitroacetophenone (\textit{2}) as key intermediate (\textit{Scheme 1}). Due to limited own internal capacity it was decided to source this material in $^{14}$C-labelled form as starting material for the radiosynthesis from an established supplier. However, due to unexpected synthesis problems the supplier failed almost completely with the consequence that an in-house preparation of \textit{2} had to be performed on very short notice. After evaluating and optimizing different synthesis routes 4-iodonitrobenzene (\textit{3}) was chosen as unlabelled starting material. Incorporation of $^{14}$C into the acetyl moiety allowed a safe and efficient synthesis of $^{14}$C-labelled $p$-nitroacetophenone in 5 steps and 38% yield. Further transformation of $^{14}$C-labelled \textit{2} to the target compound \textit{1} was achieved in a 9-step synthesis and an overall yield of 28%. $^{14}$C-labelled \textit{1} was isolated in $>99\%$ radiochemical purity and a specific activity of 47 mCi/mmol.

Reference:
\cite{1}: Neuropsychopharmacology, \textbf{2012}, \textit{37}, 2580–2592.
Phosgene – A Building Block in C-14 Labeling Chemistry
Ulrike Glaenzel, Bettina Rudolph, Andrea Romeo and Thomas Moenius
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\[ ^{14}\text{C}]\text{Phosgene}, \ [^{14}\text{C}]\text{Carbonyldi(imidazole (CDI) and } [^{14}\text{C}]\text{Carbonyldi(1,2,4-triazole) (CDT) are well established building blocks allowing late-stage introduction of the label in urea- or carbamate-type molecules. Since the resulting position of label might be subject of metabolism-induced hydrolysis resulting in a loss of the label, this synthetically straightforward approach is often avoided in the tracer synthesis. This communication suggests an } \text{in vitro}\text{ experiment providing evidence for critical cases, in which in vivo hydrolysis might be expected.} \]
SYNTHESIS OF ISOTOPE-LABELED SGLT2 INHIBITOR CANAGLIFLOZIN (JNJ-28431754) AND ITS METABOLITES


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Canagliflozin (Invokana®, JNJ-28431754) is the first approved SGLT2 (subtype 2 sodium-glucose transport protein) inhibitor for the treatment of type 2 diabetes in the United States. Herein we report the synthesis of the isotope-labeled JNJ-28431754 and the drug metabolites. The stable isotope-labeled [13C6] JNJ-28431754 was synthesized via four steps starting from [13C6]-labeled glucose. [14C]-Labeled JNJ-28431754 was synthesized by incorporation of [14C] into the benzylic position between the thiophene and benzene ring of the compound. [3H]-Labeled JNJ-28431754 was synthesized through tritium-de-bromination on the thiophene ring. On the other hand, glucuronide M5 and M7 were identified as the two major metabolites among others.[1] The two glucuronides were isolated from human urine and in turn their structures were determined. Biosynthesis of the glucuronides was first explored by screening the incubation of the drug with various microsomes from different species in the presence of cofactors UDP-glucuronic acid and UDP-N-acetylglucosamine. A feasible and cost-effective biosynthesis was developed to generate the major M7 glucuronide in gram scale in a 31% yield. The in vitro biosynthesis of the other glucuronide M5 however gave less than 1% with this protocol. Thus an alternative chemical synthesis for M5 glucuronide was investigated. A multi-step synthesis involving selective protection of 4'- and 6'-hydroxy groups as benzaldehyde acetal, followed by coupling with protected bromo glucuronate, and deprotection to produce M5 in low yield (2.9%) was first developed. This chemical synthesis was still problematic for large scale production and the deprotection of the benzaldehyde acetal often led to break down of the glucuronide due to limited stability. To improve the synthesis we protected 4'- and 6'-hydroxyl of parent drug with sterically bulky DTBS (di-t-butylsilylene) which was much easier to deprotect under mild conditions, and increased selectivity to generate more M5 at the 2'-hydroxyl group since the DTBS increases steric hindrance at the nearby 3'-hydroxyl group. As a result, this process improved the coupling DTBS-protected parent drug molecule with the protected bromo glucuronate and subsequent deprotection, generating M5 glucuronide in 7-10% yield in two pots. Detailed synthesis of the isotope-labeled compounds and metabolites will be presented.

Canagliflozin and isoetine-labeled compounds

M5 / M7 glucuronides

References
SYNTHESIS OF A RADIO-LABELLED ANTIBODY DRUG CONJUGATE USING BIOSYNTHESIZED $^{14}$C LABELLED TOMAYMYCIN

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Antibody Drug Conjugates (ADCs) are one of the most promising technologies to treat cancer as they combine the specificity of an antibody with the high potency of a cytotoxic molecule. Tomaymycin, isolated from *Streptomyces anchromogenes* var. *tomaymyceticus*, is part of the family of DNA-interactive antitumor antibiotics. In common with other natural antitumor antibiotics such as sibiromycin, neothramycin and anthramycin, it possesses the pyrrolo[2,1-c]benzodiazepine (PBD) skeleton. The multistep chemical synthesis of PBD containing compounds is complicated because of the reactivity of the imine bond. Therefore, we turned to biosynthesis to obtain $^{14}$C radiolabelled tomaymycin for ADME studies. Following Hurley’s work, the $^{14}$C radiolabel was incorporated efficiently in one step from radiolabelled tyrosine using the strain *Streptomyces sp FH6421*. This process has been further optimized by using anthranilic acid as radiolabelled precursor, and this radiolabelled tomaymycin has been coupled to an antibody to support PK and metabolism studies.

APPLICATIONS OF BIOCATALYSIS IN ISOTOPE CHEMISTRY

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The application of biocatalysis in the pharmaceutical industry continues to grow, a move that has been facilitated by the increasing commercial “off-the-shelf” availability of enzymes for reaction screening and scale-up. Recent advances in DNA technology have enabled tailoring of enzyme properties as well as access to increased biocatalyst variety at ever decreasing cost and these enzymes are now being used in many disciplines across the pharmaceutical sector.

Almac’s isotope chemistry group routinely use enzyme platforms consisting of an extensive range of enzyme types including hydrolases, carbonyl reductases, transaminases, P450 monooxygenases, nitrile hydratases and nitrilases to introduce and manipulate isotopically labelled intermediates and API’s.

![Figure 1: chemical transformations using selectAZyme™ platform](image)

The high yields and purities typically obtained by enzymatic transformations makes them ideal for use in isotope chemistry where raw material and radioactive waste disposal costs are so high.

This presentation, through actual case studies, will illustrate screening and application of enzymes for use in isotopic labelling of APIs. The presentation will highlight the use of novel biocatalytic processes for carbonyl-reduction, transamination, hydrolase selective hydrolysis and biooxidation chemistries involving isotopic labelling.

William Watters is the isotope chemistry manager at Almac, a technical expert in isotope chemistry and organic synthesis with >20 years of extensive academic and industry experience.
Enzymatic Approach Towards the Synthesis of Deuterium Labeled Tenofovir and its Diphosphate Metabolite

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Department of Process Chemistry, Merck Research Laboratories, Rahway, NJ, 07065

Tenofovir, which is marketed as its prodrug tenofovir disoproxil fumarate, is a nucleoside analogue reverse transcriptase inhibitor (nRTI) used in the treatment of HIV/AIDS and hepatitis B infections. In order to support ongoing studies, we were asked to synthesize deuterium labeled Tenofovir and its diphosphate metabolite. The current literature procedure to prepare d6-Tenofovir was deemed to be inefficient; thus, a new synthetic approach was needed. Various routes to the target compound will be discussed; including the development of a short and highly enantioselective, highly efficient deuterium transfer enzymatic approach.
Hexythiazox is a generic non-systemic acaricide with contact and stomach action. It is generally used for the control of eggs and larvae of mites on fruit, citrus, vegetables, vines and cotton. The regulatory requirements for environmental fate studies on pesticides means that multiple rings must be separately labelled and studied, as must other relevant parts of the molecule or significant side chains. Our client required two of the rings in hexythiazox to be labelled with carbon-14. This presentation demonstrates that the reported routes required development to be applicable for the radiosynthesis of [thiazolidinyl-5-14C]hexythiazox (1) and [cyclohexyl-U-14C]hexythiazox (2).

The route followed to prepare [thiazolidinyl-5-14C]hexythiazox (1) includes the preparation of the p-chloronorephedrine intermediate (4) from the keto oxime intermediate (3). The reported conditions involve a stepwise reduction of an oxime using H₂ and a palladium catalyst followed by treatment with sodium borohydride. This method proved unsuccessful in our hands so a one pot method was developed using lithium aluminium hydride. This and other modifications to the reported literature routes will be discussed.

A short cut to the key unlabelled intermediate (5) in the radiosynthesis of [cyclohexyl-U-14C]hexythiazox (2) was also found. The degradation of unlabelled hexythiazox produced (5) in one step rather than a multi-step synthesis from commercial intermediates.

References
2. a) EU regulation 283/2013; b) EPA Guideline OPPTS 835; c) OECD Test Guidelines, OECD 501 and 503.
Regioselective and stereospecific C-H Deuteration using Ru Nanocatalysts: Application to the Labeling of Nitrogen- and Sulfur-containing Biomolecules

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b) LPCNO-IRSAMC, Université de Toulouse, INSA, UPS, France

Deuterated molecules have found numerous applications in chemistry, biology and material science. Indeed, these molecules are essential for mechanistic studies and for the resolution of structure by NMR. The development of metabolomics has also increased the demand for isotopically labelled compounds. In particular, their use as internal standards is essential for the quantitative LC-MS/MS analysis of new drug candidates and metabolites in biological fluids. Moreover, it has been demonstrated that the selective incorporation of deuterium into drugs can significantly increase their biological half-life and hence improve their therapeutic profiles. As a consequence, development of efficient, atom economical and selective labelling methodologies through catalytic C-H deuteration is of great interest.

Scheme 1: Scope and selectivity of the C-H deuteration

In this context, we have recently discovered an efficient C-H deuteration process allowing the labelling of nitrogen- and sulfur-containing biomolecules using Ru nanoparticles as catalyst and D₂ as the isotopic source (scheme 1).¹ Using a general and simple protocol which involves mild reaction conditions and simple filtration of the labelled product, the deuteration of more than 40 compounds has been achieved in good yields with high chemo- and regioselectivities. The applicability of this procedure has been demonstrated by the labelling of an additional 20 biologically active compounds (scheme 2). The level of isotopic enrichment reached by this method is suitable for metabolomic studies in most cases. In addition this approach is perfectly adapted to tritium labelling because it uses gas as an isotopic source. Remarkably, the C-H deuteration on an asymmetric sp³ carbon (chiral amines, amino acids, peptides) occurred with a total stereoretention.³ Experimental results and DFT calculations suggest that the reaction implies a four-membered dimetallacycle as key intermediate. In this communication, a particular emphasis will be made on the mechanism of this rare case of enantiospecific C-H activation process.

² Manuscript in preparation
³ Manuscript submitted
SYNTHESIS OF SELECTIVELY $^{13}$C-LABELED HIGH-MANNOSE N-GLYCANS

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Cell-surface oligosaccharides appended to proteins and lipids are key binding epitopes in many critical biological processes, including bacterial infection, cell development and the immune response. Understanding these processes at the molecular level will help sort out structure-function relationships of these biomolecules \textit{in vivo}. Determining the conformations and dynamics of glycans in solution remain challenging tasks in structural glycobiology. Recent developments in the use of NMR scalar couplings ($J$-couplings) as parameters in assess carbohydrate conformation, underpinned by density functional theory (DFT) calculations and a new mathematical algorithm (\textsc{MA'AT}), have the potential to overcome this impediment. This approach requires the use of redundant $^{13}$C-$^{13}$C and $^{13}$C-$^1$H $J$-coupling constants sensitive to the individual molecular torsion angles comprising glycosidic linkages. Both types of $J$-couplings can be conveniently measured in compounds labeled with $^{13}$C at specific sites. Synthetic methodologies to prepare high-mannose N-glycan nested fragments have been developed recently during a Phase I SBIR project funded by NIH/NCI. Synthetic methods developed in this work can be applied to the synthesis of isotopically enriched high-mannose N-glycans with minimal modification. To demonstrate the latter application, a tetrasaccharide and a hexasaccharide have been constructed using site specifically $^{13}$C-labeled D-mannoses. NMR Studies of these strategically labeled samples yield redundant $J$-couplings across each of their constituent $O$-glycosidic linkages, data that are used by \textsc{MA'AT} to experimentally determine the conformational properties of these glycans. This work also provides important new quantitative information on the effects of context on preferred linkage conformation.
Molecular Isotopic Engineering (MIE): Industrial Manufacture of Naproxen of Predetermined Stable-Isotopic Compositions for Reasons of Identity and Security

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¹Molecular Isotope Technologies LLC, ²Canaan Partners, ³Dilworth IP, LLC, and ⁴Mezes Consulting

Molecular Isotope Technologies LLC has developed four patented or patent-pending generations of stable-isotopic methods and technologies: (i) product characterization (for both small molecules and biologics), (ii) process characterization (notably, process patent protection), (iii) in-process (continuous) analysis, and now (iv) molecular isotopic engineering. Early work in cooperation with the US FDA on the product characterization of naproxen revealed manufacturer-level isotopic provenance of this small analgesic molecule (Wokovich et al., 2004) which was referred to as “The Manufacturer’s Fingerprint.” This isotopic provenance represented the convergence of the effects of the stable-isotopic compositions of starting materials and isotopic effects of the synthetic process. Rather than merely accepting the random effects of variable sourcing and synthetic process on the stable-isotopic compositions of products, we take a proactive approach to purposefully determine the stable-isotopic composition of bio/pharmaceutical products. The main rationale for MIE is to predetermine the isotopic ranges of products for reasons of product identification and of product security, and also for intellectual property considerations. As an example of MIE, we analyzed the products of the isotopic-synthetic reactions for the last two steps of naproxen synthesis:

\[
\text{2-Bromo-6-methoxynapthalene} + \text{Bromopropionate} \rightarrow \pm \text{Naproxen} \rightarrow (S)\text{-Naproxen}
\]

Pre-selection of the stable-isotopic compositions of the starting material, 2-Bromo-6-methoxynaphthalene (e.g., \(\delta^{13}\text{C} \sim -12\%\), -22\%, -32\% vs VPDB) yields the product of discrete stable-isotopic ranges (\(X \pm x\), \(Y \pm y\), \(Z \pm z\)% vs VPDB). The resulting MIE naproxen is very different from a naproxen molecule that has merely been substituted at a single position with a different isotope. Our directed isotopic synthesis is just one example of MIE to predetermine the discrete isotopic ranges of bio/pharmaceutical products. In principle, the MIE approach should be readily adapted to existing bio/pharmaceutical manufacturing units. The main difference in the manufacturing process would be the use of starting materials or synthetic intermediates of pre-measured stable-isotopic compositions. The manufacturing apparatus would remain unchanged. This approach could have broad application in securing drug identity/provenance from manufacturing plant to consumer.

Relevant Intellectual Property


Other patent applications pending.
Radioactive Contamination Limits, Equivalent Doses, and “Should You Care”.
Vincent Williams
Merck and Co, 126 E. Lincoln Avenue, Rahway NJ 07065, USA

What radioactive contamination levels are you expected to stay below at your facility? Where do those limits come from? If you ingest the equivalent amounts of radioactivity, how much radiation exposure would you receive? What amount of contamination can you ingest in a year and stay under the federal exposure limits? How do those doses compare to exposures that we receive from other naturally occurring or man-made radioactive sources? This talk will answer those questions, will walk the listener through a historical look at sources and limits, and will help answer the question “should you care”? 
Impacts on Occupational Radiation Protection, Public Perception and Pharmaceutical Research of ICRP 103 Implementation

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The latest recommendations on standards for radiation protection, known as “ICRP 103,” are on their way to implementation in many countries, including the United States. These recommendations contain changes to the practice of occupational health physics, protection of research subjects and how the public should be protected. In addition, there are new recommended approaches, changes in annual dose limits, an emphasis on SI units and a more integrated approach to radiation protection.

As the new recommendations are adopted, there will be many evolutionary changes including the use of effective dose with tissue weighting factors as a prevalent concept, an increased emphasis on SI units and changes in approach to occupational radiation protection. These will include a scheme of justification for any exposure, recommendations for many types of exposure, and a recommendation for formal dose constraints for various situations – these will be discussed in the context of radiochemistry efforts. A change in the annual limit for workers and changes in recommendations for public dose will also influence the perception of safety.

Of particular interest to pharmaceutical research will be the proposed dose limits for human subjects, and the means of internal dose assessment. These will be contrasted with the current 21.CFR.361 approach. The ICRP recommended approach will be discussed in detail and compared to the current US regulatory approach.

In the US, the adoption process is just beginning, and the effort toward these new standards with greater international harmonization is likely to become the new standard for pharmaceutical and occupational radiation protection.
Isotope-labeled compounds are utilized in a variety of preclinical and clinical studies to facilitate collection of ADME data. Routinely, stable-labeled compounds are used as internal standards for bioanalytical LC-MS/MS assays and small molecule drug candidates labeled with conventional (microcurie) amounts of $^3$H or $^{14}$C are administered to animals and humans for determining mass balance or tissue distribution. With accelerator mass spectrometry (AMS) available for detection, microtracer doses of $^{14}$C-labeled drugs (nanocurie amounts) can be administered in human ADME/mass balance studies, in situations where standard doses of radioactivity are not feasible. $^{14}$C-Microtracers have also been exploited to obtain absolute bioavailability data using an innovative study design. In this approach, an intravenous microdose of $^{14}$C-labeled drug is administered concurrently with a therapeutic oral dose of unlabeled compound. The plasma drug concentrations originating from each route of administration are measured by AMS and LC-MS/MS, respectively; the study design can also be adapted for use with stable-labeled compounds, allowing all analyses to be conducted with traditional LC-MS/MS. Radiolabeled versions of therapeutic proteins have also been synthesized to study drug disposition, tissue distribution, or to confirm delivery of drug to the target tissue/site of action. Several examples will be provided to illustrate recent applications of radio- and stable-isotope-labeled compounds for ADME-related studies.
WHAT DATA CAN A $^{14}$C CLINICAL STUDY DELIVER? A DECADE OF INNOVATIVE, INTEGRATED $^{14}$C STUDY DESIGNS TO UNDERSTAND DRUG BEHAVIOUR IN HUMAN SUBJECTS

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Introduction:
Utilizing $^{14}$C radiolabelled drug to obtain definitive mass balance data and to characterize metabolites for regulatory submissions is long established as a necessary part of the drug development program.
In addition, human $^{14}$C studies can be used to elucidate greater information about drug behavior and help build a complete understanding of the pharmacokinetics and metabolism of drugs.

Presentation:
This presentation will demonstrate through published examples the robust understanding of drug metabolism that can be achieved for regulatory submissions from human ADME studies including mass balance, routes and rates of elimination and metabolite characterization. If required, more detailed insights such as fraction absorbed, absolute bioavailability and a further understanding of metabolic disposition can be achieved from alternate study designs.

Data collated from $^{14}$C radiolabelled studies conducted by Quotient Clinical in the last 10 years will be presented to provide a visual portrayal of the complete picture of drug absorption and distribution through the use of a ‘Human Metabolism Dashboard’. Examples from conventional human mass balance and metabolism (ADME) studies, oral / intravenous crossover $^{14}$C studies, intravenous microtracer (ivMT) studies and integrated ivMT/ADME studies will be provided.

Conclusion:
Human studies involving the dosing of $^{14}$C radiolabelled drug product have the capability to deliver a reliable and thorough understanding of drug absorption and disposition. Reviewing examples from the unique experience of performing studies across a wide range of molecules and for clients from across the globe allows a comprehensive knowledge base to share with those involved in $^{14}$C enabled drug development.
STRATEGIES FOR THE CONDUCT OF HUMAN ADME STUDIES OF ONCOLOGY COMPOUNDS

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Human ADME (absorption, distribution, metabolism, and excretion) studies are an important part of the drug development process. These studies are normally performed by using a radioactive tracer (C-14 or H-3) blended with a therapeutic dose of non-radioactive drug in about four to six subjects. The radiolabeled compound is utilized to evaluate the recovery and track the metabolic fate and physiological disposition of the drug. More challenges are faced regarding subject and site selection, choice and supply of C-14 labeled drug product when performing these studies with oncology compounds. If the compound is suitable to study in healthy volunteers, the strategy employed will be similar to what is normally conducted with non oncology compounds. But if the compound is not suitable to study in healthy volunteers (e.g. a cytotoxic drug), different strategies including recruitment of patients and availability of pure radioactive drug product whenever a patient becomes available for the study must be employed. These studies generally extend over a period of six months to a year. Two approaches (Micro tracer – AMS (Accelerator Mass Spectrometry) and the traditional method) used for these studies will be described. Detailed information will be presented by using three examples (Compounds A, B, and C, micro tracer – AMS method in healthy volunteers, micro tracer – AMS method in patients, and traditional method in patients).
SYNTHESIS OF DERIVATIVES OF 6-AMINO-URACIL LABELED WITH \(^{14}\text{C}\) FOR TESTING OF THEIR METABOLIC STABILITY

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We recently discovered interesting biological activities of 6-amino-5-nitroso-uracil (1), 5-acetyl-6-amino-1,3-dimethyl-uracil (2) and some related compounds. The key question for these compounds was their metabolic stability. To prove that both compounds 1 and 2 do enter the human cells and that their metabolic stability is high enough to support the selection for \textit{in vivo} studies the radioactively labeled compounds were required. Because of the lack of hydrogen atoms in stable positions in both compounds the isotope of choice for labeling was \(^{14}\text{C}\).\textit{[cyano-\(^{14}\text{C}\)]}Cyanoacetic acid was chosen as a common precursor for labeling of 1 and 2 at C-6. The syntheses on gram scale of 1 and 2 were based on condensation of ethyl ester of cyanoacetic acid with with urea or dimethyl urea catalyzed by sodium methoxide in methanol. However, after scaling down to tens of miligrams the yields were not very promising for "hot" experiments. Optimization of reaction conditions led to use of methyl \textit{[cyano-\(^{14}\text{C}\)]}cyanoacetate for the synthesis of 6-amino-5-nitroso-[6-\(^{14}\text{C}\)]uracil (1) and to utilization of free \textit{[cyano-\(^{14}\text{C}\)]}cyanoacetic acid for the synthesis of 5-acetyl-6-amino-[6-\(^{14}\text{C}\)]uracil (2) as depicted in the scheme bellow. Results of metabolic studies will be presented.
Metabolite identification is a key element in early drug discovery and development. Often LC-MS approaches enable rapid and information-rich structural elucidation by MS ion trapping experiments and high resolution MS. Quite often however, alternative approaches are needed where insufficient or ambiguous ion fragmentation occurs and thus rapid access to standards are required for $^1$HNMR confirmation. In the discovery setting, the often time-intensive exploratory syntheses required to produce phase I and II metabolites can fall short of delivering within the expected timeframes.

Here we describe the production of drug and agrochemical metabolites using whole-cell microbial biotransformation as an effective complementary approach to chemical synthesis, providing solutions in cases where the latter proves challenging. Incubation of parent compounds with a specifically-selected panel of wild-type bacteria and fungi often results in conversion to human and environmental fate metabolites, including aliphatic/aromatic hydroxyls and glucuronides, as well as new derivatives (microbe-specific compounds). The advantage of using microbes over mammalian cells is primarily the feasibility of scaling to produce high milligram, gram or even kilogram quantities of metabolites at a reasonable cost.

This talk will summarize the production process, types of metabolites that can be produced, including reference to isotopically-labelled metabolites, and the overall pros and cons of the use of this technique.
O19

Structural Characterization and NMR Quantitation of Drug Metabolites in Discovery and Development

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In early discovery through development there are situations' where having a qualified metabolite standard is advantageous; monitoring reactive metabolites, assessing pharmacological activity of metabolites or quantification of metabolites in a clinical setting. In many cases, for a variety of reasons, timely chemical synthesis of these metabolites can be difficult. Alternatively, biological generation and isolation of metabolites in the low to mid nanomole range is feasible. However, without accurate quantitative assessments of the concentration of these isolates, their utility is limited. Definitive structural characterization of drug metabolites has long been the domain of NMR. Additionally, there is a significant history of NMR as a quantitative technique. Only recently have these two concepts been merged to provide structural and quantitative information on biologically generated isolates. We have optimized both isolation and NMR techniques to develop a process that can generate, characterize and quantitate metabolite standards in the low nanomole range. These isolates can then be used as standards in a variety of pharmacological and metabolic assays. Several practical examples of this process will be presented.
DESIGN, GENERATION AND UTILIZATION OF RADIO AND STABLE ISOTOPE CO-LABELED STANDARDS FOR METABOLITE QUANTITATION

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Abstract

In the development of more efficient and accurate analytical methods to support measurement of analytes in biological matrices, especially in the absence of authentic metabolite standards, radio isotope and stable isotope co-labeling concept was introduced into standards to facilitate metabolite quantitation and prevent cross isotopic distribution interference with real analyte samples. The selected acetaminophen was simultaneously labeled with both $^{14}$C and $^{15}$C isotopes, and transformed in vitro to corresponding radio and stable isotope co-labeled glucuronide and sulfate metabolites. The co-labeled standards were used directly in the quantitative determination of rat plasma concentration of acetaminophen metabolites by LC-MS and radiometric detection (LC-MS-RAD). The results were validated by an established LC-MS/MS method using authentic metabolite standards. The variations between two methods were within 20% range. The radio and stable Isotope co-labeled standards (RADSTIL) and LC-MS-RAD protocol developed here should enable timely and accurate metabolite quantitation especially in the early preclinical/clinical studies where metabolite standards or even identifications are not available.
Selcia was approached by a customer who required storage stability/mass balance on their Natural Product Pharmaceutical for a Registration Dossier to be submitted for FDA approval of the compound. As the compound was a mixture of components with one key active component the customer was concerned that 40% of the mass balance was unaccountable for after 6 months stressed (40°C) storage. The target active component has no natural isotope ratio and the majority of the anticipated degradation products were already present in the extract. Therefore Selcia proposed a $^{14}$C Mass balance study to quantitatively follow the key active component and its breakdown on storage. Additional $^{13}$C labelling would aid elucidation of the degradants.

The presentation will take the form of a case study on how a mixture of $^{14}$C, $^{13}$C and unlabelled active compound, was used to generate an artificial isotope ratio for structural elucidation of the breakdown products resulting in a novel test system for following the natural product storage stability. The $^{14}$C label component helps to target MS peaks of interest and provides mass balance details. The $^{13}$C label provides a ‘handle’ for the structural elucidation.
DUAL-RADIONUCLIDE IMAGING IN WBARG BY DIFFERENTIAL ABSORPTION OF CHARGED PARTICLES.

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WBARG is usually limited to single-radionuclide distribution studies, as phosphor screens used for imaging react with all charged particles, e.g., beta particles ($\beta^-$), internal conversion electrons ($\epsilon_{IC}$), and cosmic muons. However, if two given radionuclides emit charged particles at different kinetic energy levels, it should be possible to selectively stop those of the lowest energy with a suitable absorbing material. The average and maximum energies of $\beta^-$ emitted by $^{14}$C ($t_{1/2} = 5730$ y) are 49.5 keV and 156.5 keV, respectively (100% emission probability). $^{113}$Sn ($t_{1/2} = 115.09$ d) decays by electron capture to $^{113m}$In ($t_{1/2} = 99.5$ min), which in turn decays by isomeric transition to stable $^{113}$In, emitting in the process monoenergetic $\epsilon_{IC}$ at 363.8, 387.5, and 391.6 keV (emission probability 28.8, 5.6, and 1.1 %, respectively). In a WBARG study of $^{14}$C-benzo-a-pyrene and $^{113}$Sn-tributyltin chloride tissue distribution in mummichog ($Fundulus heteroclitus$), a double exposure method based on differential absorption of charged particles by Al foil was tested to visualize the distribution of both radiolabels in the same tissue section. This exposure method allowed a clear differentiation of $^{14}$C and $^{113}$Sn distribution, qualitatively and quantitatively. It could be applied to any pair of charged-particle emitters, as long as the difference in kinetic energy is sufficient to allow for a significant absorption of the less energetic one. Further improvements will also be discussed.
O23

**Imaging Tools for Putting the “D” in “ADME”**

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Quantitative whole body autoradiography (QWBA) using radiolabeled compounds is the gold standard for preclinical tissue distribution studies, providing accurate and reliable quantitation of total drug related material (DRM) in tissues. The inherent non-selectivity of QWBA and inability to resolve cellular disposition provide opportunities for innovative pairings with other methodologies. Major innovations have been made in mass spectrometric imaging and surface sampling techniques for molecular identification of DRM in tissue samples. In addition, higher resolution methodologies including microautoradiography (MARG), transmission electron microscopy, and nano-secondary ion mass spectrometry (nanoSIMS) present unique opportunities for studying various therapeutic agents at the cellular and subcellular level using either radiolabeled or isotopically labeled material. Examples highlighting pairing of more traditional QWBA methodologies with mass spectrometry, MARG, and nanoSIMS will be highlighted.
Drug efficacy and toxicity are the main causes of drug failures in clinical studies. Preclinical technologies have to be used for a better risk assessment to minimize potential failures in clinical studies. Autoradiography and Mass Spectrometry are the “Gold Standard” techniques to assess ADME (Absorption, Distribution, Metabolism and Elimination) and pharmacokinetics studies. More recently, Mass Spectrometry Imaging (MSI) becomes an established tool to answer the same questions with the advantages of these two techniques, specificity and Image resolution. MSI technique provides molecular distributions and concentrations of many molecular species (drug, metabolites, lipids and peptides) and supports ADME and pharmacokinetic investigations.

ImaBiotech developed Multimaging Platform, a combination of imaging techniques (Mass Spectrometry Imaging (MSI), Histology and Immuno-Histology) to obtain at the same time the localization and quantification of drug/metabolites with biomarkers of efficacy and toxicity. During the presentation, we would present the technology, advantages, limitations and the benefits of using it in different therapeutic areas.
Alpha Camera imaging for evaluation of small-scale activity- and dose distributions in Targeted Alpha Therapy

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The implementation of targeted alpha therapy (TAT) requires tissue specific small scale dosimetry by which tumor efficacy and normal tissue toxicity can be evaluated in relation to an absorbed dose to relevant biological volumes. We developed a novel quantitative bioimaging technique, the Alpha Camera, as a tool for image based small scale dosimetry of alpha particles. Here we present an overview of the technique and results from our Alpha Camera imaging studies.

The Alpha Camera is an ex vivo scintillator-based autoradiography imaging technique for quantification of alpha emitters in cryosections. The spatial resolution is better than 35 microns, the uniformity better than 2% and the image pixel intensity is proportional to radioactivity. The technique has been used preclinically for estimating activity and resulting dose distribution estimations on macro- and micro-tumors. Activity distribution within normal tissues following TAT was also analyzed, including kidneys, liver, spleen, stomach, femur (bone marrow) and whole-body sections. Finally, intratumoral activity distributions after direct alpha-radioimmunotherapy (alpha-RIT) and pre-targeting (alpha-PRIT) was compared.

Presented here is an overview from alpha-camera studies in settings of TAT, including results from a method for image-based small-scale 3D-dosimetry using voxel dose-point kernels (VDKs). The 3D-dosimetry was conducted in an ovarian cancer mouse model. At-211-MX35-F(Ab')_2 was i.v.-injected into mice and intratumoral (OVCAR-3) activities were quantified by α-camera imaging of cryosections. For macro-tumors, a non-uniform activity distribution of the bioconjugate was found from 10 mpi to 6 hpi, with areas 3-fold higher than the mean interspersed among lower-activity areas (0.5-fold). At 21 hpi the distribution was more uniform. Quantified activity was used for dose distribution calculations using voxel dose kernel (VDK) dosimetry (serial sectioning). Images were acquired for series of e.g. 13 consecutive tumor sections that were 12 μm-thick. Images were registered and stacked to a 3D-matrix of 12x12x12 μm voxels. VDKs were generated by an in-house Monte Carlo code. Using Matlab, the activity images were convoluted with the VDKs to calculate absorbed dose rate in each voxel. The center section in a series was used for further evaluation. Tumor cells located close to stroma received 4.2 Gy/MBq, while cells in the core of 'lobular clusters' received 2.8 Gy/MBq. For micro-tumors, we quantified the activity uptake versus time of i.p.-injected At-211-MX35-F(Ab')_2 in tumor-clusters. For clusters >100 microns, we observed two different activity distribution patterns; clusters with no stroma had a high and uniform uptake while clusters with fibroblasts had a radial distribution with high activity on the edge, but lower in the core. Estimated mean absorbed dose to tumor cells in the lobular-type ranged from 4–8 Gy, while the uniform-type from 10–25 Gy. In contrast to conventional radioimmunotherapy (RIT), a so-called pretargeted RIT showed therapeutically favorable intratumoral distributions on macro-tumors already 30 mpi.

The activity distribution in the kidneys after TAT was markedly non-uniform with clear visualization of the different renal compartments. The ‘cortex-to-whole-kidney-ratio’ varied with time and bioconjugate size. At 2 hpi, the i.v.-injected At-211-MX35-F(Ab')_2 showed a marked retention in the renal cortex, corresponding to a ratio of 1.38 ± 0.03, while an At-211-labeled IgG had a ratio of 0.77 ± 0.04. Imaging of whole-body sections 30 min after i.v.-injection of free At-211 revealed an elevated uptake in cartilage in the spine. A non-uniform activity distribution was found in spleen for both RIT and PRIT with At-211, with highly elevated uptake in the marginal zone of the white pulp.

Combining the high-resolution of autoradiography with direct quantitative optical ex-vivo imaging, the Alpha Camera system can assess tissue distribution and pharmacokinetics of drug molecules labeled with alpha emitters. Such data is also needed to improve alpha particle dosimetry, using experimental data, why this technique can support the development of TAT in important aspects, where data today is lacking.
QWBA, PBPK MODELING AND LIQUID SURFACE SAMPLING MICRO-LIQUID CHROMATOGRAPHY MASS SPECTROMETRY TO IMPACT DRUG DEVELOPMENT

Case examples:
1. Physiologically based pharmacokinetic modeling.
2. Recent advances and applications of Surface Sampling micro- Liquid Chromatography Mass Spectrometry (SSμLC-MS).

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Quantitative whole-body autoradiography (QWBA) is a powerful tool for investigating the tissue pharmacokinetics, route of elimination, and distribution of drug-related radioactivity in the preclinical setting. Moreover, QWBA data are crucial in support of dosimetry calculations used to guide clinical ADME studies in humans. The tissue distribution data obtained by QWBA are of particular significance in that they enable the calculation of tissue to plasma partition coefficients (Kp) used to predict human pharmacokinetic profiles by physiologically based pharmacokinetic (PBPK) modeling approaches. Case examples discussed include: 1) PBPK models using Kp estimates based on measured QWBA tissue distribution profiles which describe observed rat and monkey i.v. PK profiles; 2) human PBPK models based upon preclinical tissue distribution data which describe observed human PO data.

A limitation of QWBA is its inability to distinguish parent-compound related radioactivity versus that related to metabolites. This is of particular concern for extensively metabolized drugs and/or those producing biologically active metabolites. An attractive analytical technique for addressing this problem is mass spectrometry (MS). The power of MS lies in its selectivity based on a molecules mass to charge ratio (m/z). Thus, one or more species of interest can be distinguished simultaneously within the same study sample. Matrix Assisted Laser Desorption Ionization Mass Spectrometry (MALDI-MS) has been applied this problem. However, this ionization technique is not well suited to the quantitative analysis of small molecules or labile phase two metabolites thereof. More recently, Surface Sampling micro-Liquid Chromatography Mass Spectrometry (SSμLC-MS) has been used to interrogate whole body tissue sections. The ionization mode (ESI) makes this technique particularly well suited to the detection of small molecules, phase I, and II metabolites. Advances in SSμLC-MS instrumentation and case examples of its application to the evaluation of drug distribution in QWBA tissue sections will be discussed.
Tissue distribution of drugs is one of the major drivers of success of a drug candidate, as molecular targets are often located outside of the vascular space. Both pharmacodynamic response and toxicity are usually associated with respective tissue concentration. Therefore, it is important to comprehensively characterize distribution of drugs, biologics or small molecules, in tissues of interest. There are many examples when necessary answers can be obtained only by combining multiple analytical and imaging modalities such as in-vivo imaging, QWBA, 3D-QWBA, mass spec imaging, fluorescent imaging, and tissue homogenate analysis. This presentation will cover a few examples when multiple imaging techniques were combined for study of drug biodistribution.
ADDING VALUE THROUGH AMS-ENABLED FIH STUDIES
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Accelerator mass spectrometry (AMS) has been used in the biomedical field for two decades. Initially, the sensitivity of the technique in the analysis of total $^{14}$C was exploited as an alternative to conventional decay counting techniques, for example in metabolism studies where the dose of radioactivity that could be administered was restricted. AMS continues to be used in this way and the ability to obtain fully quantitative results with doses in the nCi range has facilitated developments such as the conduct of AME studies in vulnerable populations including neonates and children, late-stage cancer patients and the renally impaired.

However, perhaps the most significant development has been the use of intravenous $^{14}$C-microtracer doses concomitantly administered with non-radiolabelled extravascular doses to determine the fundamental pharmacokinetics and absolute bioavailability of compounds. This study design relies on the fact that circulating compound arising from the different dose routes can be distinguished by means of the radioactive tag. The utility of the approach has been underpinned by the development of robust, specific, accurate and precise assay methodologies using LC+AMS: liquid chromatography with fractionation and off-line quantification by AMS. Validation strategies that meet regulatory bioanalytical method validation guidelines have been developed to enable such assays to be deployed with confidence alongside the conventional LC-MS/MS assays used to quantify concentrations arising from the non-radiolabelled extravascular dose, whilst the inherent sensitivity allows the microtracer dose to be kept low enough to ensure that the observed PK is driven only by the extravascular component. The methodology is becoming ‘routine’, and is accepted (and even recommended) by regulatory agencies.

This presentation will discuss the use of $^{14}$C-microtracers in the context of early Phase 1 studies, including First In Human. By incorporating both a concomitant intravenous and an extravascular microtracer, in separate cohorts, a significant amount of additional data can be generated for a relatively small addition to the total cost of the clinical trial. Inclusion of the microtracers does not affect fulfilment of the original study objectives (typically measurement of plasma PK for the therapeutic dose), however the extra information generated (including clearance, volume of distribution, absolute bioavailability, routes and rates of excretion and biotransformation pathways) can save time and money and/or avoid unnecessary expenditure later in development by providing reliable human in vivo data on which decisions can be based. The talk will include case studies, exemplifying the information only available through the use of $^{14}$C-microtracers and the practicalities of implementing AMS-enabled Phase 1 studies.
CLINICAL STUDIES UTILIZING ACCELERATOR MASS SPECTROMETRY:
OPPORTUNITIES AND CHALLENGES OF $^{14}$C RADIOLABELLED MICRO TRACER STUDY DELIVERY

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Introduction
Accelerator mass spectrometry (AMS) can be a versatile, complementary tool in drug development enabling the conduct of alternative clinical study designs to understand human drug metabolism and pharmacokinetics (DMPK) through the uniquely sensitive analysis of biological samples. Successful utilization of such enabling instrumentation requires a concomitant understanding of the requirements for the preparation of AMS assay methods, their validation and subsequent application to sample analysis.

Presentation
This presentation will draw upon Quotient Clinical’s experience of the design and conduct of over 50 AMS-enabled clinical studies to present a comprehensive picture of the knowledge that can be made available to the pharmaceutical scientist, clinical pharmacologist and DMPK scientist from AMS derived sample analysis in early clinical development. Case studies will be presented to demonstrate how insights can be gained into:

- the performance of drug product formulations
- intravenous pharmacokinetics and absolute bioavailability
- drug metabolism

The practicalities of performing AMS enabled clinical studies, particularly the importance of assay establishment and qualification, will also be examined which will enable an assessment of the advantages and challenges presented by the technology, in comparison to other approaches available to the development team.

Summary
Quotient Clinical has been an innovator in the design and implementation of AMS enabled clinical studies over the last 10 years and has worked during that period with all of the established commercial providers of AMS services to the pharmaceutical industry. As such Quotient Clinical is in a unique position to provide insights into the types of study being carried out, the deliverables from these studies to the project team and the overall advantages and challenges of implementing AMS in the drug development program.
AN EVALUATION OF HUMAN ADME AND MASS BALANCE STUDIES USING REGULAR OR LOW DOSES OF RADIOCARBON - A.F. Roffel, S.P. van Marle, J.J. van Lier, E.-J. van Hoogdalem, PRA Health Sciences, Zuidlaren, The Netherlands

At PRA, we have been involved in the conduct of $^{14}$C and $^3$H human ADME and mass balance studies and later also $^{14}$C microdose studies (for candidate selection, or the assessment of absolute bioavailability using i.v. microdoses) since more than 20 years. Especially with the validation of Accelerator Mass Spectrometry as a bioanalytical technique, we have seen increased interest in human ADME studies that use low doses (up to 0.1 MBq) as opposed to regular doses (1.85-3.7 MBq) of radiocarbon ($^{14}$C). As advantages, low dose human ADME studies may be conducted without dosimetry calculations, and hence without animal $^{14}$C studies, and will lead to lower human radiation exposure by default, which is especially useful for long half-life drugs. In the current evaluation, we sought to compare the outcomes of low dose versus regular dose human ADME studies in healthy volunteers. A total of 40 oral human ADME studies were taken into account, as conducted in our units over a 5-yr period, among which 12 low dose studies. The average total recovery of $^{14}$C in excreta in these studies was 92±7% (range 72-103%). For regular dose studies, the average total recovery was 93±5% (range 83-103%), and 21 of 28 studies (75%) showed recoveries above 90%. For low dose studies, average total recovery was 89±9% (range 72-98%), and 6 of 12 studies (50%) showed recoveries above 90%. Recovery of total $^{14}$C in excreta had plateaued completely or almost completely in all studies, both regular and low dose, when collection was terminated, except in one low dose study (75% recovery by 11 days of sampling). Metabolite profiling was an objective in almost all studies, and was successful in all cases reported to PRA (13 regular dose studies, 4 low dose studies). There was no obvious relationship between the total recoveries of $^{14}$C in excreta in the low dose studies and the proportion of $^{14}$C excreted in feces, or between the total recoveries and the plasma elimination half-lives for parent or total $^{14}$C in these studies. Similarly, there was no obvious relationship between the total recoveries of $^{14}$C in excreta and the proportion of $^{14}$C excreted in feces or the $^{14}$C plasma elimination half-lives in the regular dose studies. By contrast, there was some indication that longer parent plasma elimination half-lives were associated with lower total recoveries of $^{14}$C in excreta in the regular dose studies. Low dose studies were more often conducted on drugs that had plasma elimination half-lives above 100 h (5 out of 12 studies) than regular dose studies (1 out of 26 studies; data for 2 studies not reported). In conclusion: (i) total recovery of $^{14}$C on average was slightly lower in low dose studies, and more often below 90%, but was generally complete, and metabolite profiling was successful in all regular dose as well as low dose studies reported to PRA; (ii) lower recovery of $^{14}$C in excreta was apparently associated with long half-life drugs, but not with the use of low versus regular doses of $^{14}$C. Overall, we conclude that low dose human ADME studies provide adequate data to support drug development, and that long half-life drugs continue to come with challenges for the proper design of human ADME studies.
CHARACTERIZATION OF THE ABSORPTION AND DISPOSITION OF BEVENOPRAN USING MICRO-TRACER TECHNIQUES

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Bevenopran, a mu opioid receptor antagonist, is under development for the treatment of opioid induced constipation (OIC), a major adverse effect of chronic opioid treatment, in patients on long term opioid treatment for chronic non-cancer pain. As part of its ongoing development, the absorption and disposition of bevenopran was further characterized in male healthy volunteers using a micro-tracer approach.

Six healthy male volunteers were administered a single oral dose of 0.5 mg of cold bevenopran followed 1 hour later by a 14C-labeled IV tracer dose (5 μg, 1 μCi) of bevenopran. Samples of blood, urine, feces and bile were collected over 72 hours and analyzed by LC-MS/MS and AMS to quantify the parent compound and total radioactivity. Data were analyzed by non-compartmental methods to determine PK parameters.

Both IV and oral pharmacokinetics of bevenopran PK were characterized. After IV administration of the tracer dose, the profiles of total radioactivity and bevenopran were consistent but levels of total radioactivity were higher than parent bevenopran. Bevenopran exhibited good absorption after the oral dose and the disposition was consistent with the IV profile. Majority of the administered dose was excreted in urine with some fecal contribution. Analysis of bile samples showed the presence of radioactive traces after the IV dose.

The absorption and disposition of bevenopran has been further characterized, including after the administration of a tracer IV dose. It has an absolute bioavailability of about 70% and majority of administered dose is eliminated with urinary excretion as the major route of elimination. Detection of radioactivity in bile suggests some biliary excretion may be involved though minimal. The application of AMS technology was a significant factor that made this study feasible.
SURVEYING THE BOUNDARIES OF ACCELERATOR MASS SPECTROMETRY: THE WIDENING SCOPE OF AN EVOLVING TECHNOLOGY

Mr. Stephen L. English, Dr. Marie Croft, Mr. Todd Pankratz, Dr. Michael Butler
Xceleron Inc.

Accelerator mass spectrometry (AMS) has been used for quantitative analysis of analytes labeled with $^{14}$C in a range of biological matrices since the early 1990s. Applications of the technique have progressed from total $^{14}$C analysis in mass balance studies (i.e. an ultra-sensitive replacement for scintillation counting) to specific analyte quantitation in microdose and microtracer studies, using LC fractionation with off-line analysis of the fractions (LC+AMS), i.e. bioanalysis. In recent years, however, the full robustness of AMS as an analytical technique is becoming more realized through its use in complimenting a variety of study designs and clinical approaches. This robustness also manifests from a cost-effectiveness point of view with time-reduction during the drug development pipeline previously unseen in contemporary analytical platforms.

Using AMS to support a patient study, for example, allows for the sensitivity demands in capturing drugs slowly excreted as a result of patients with impaired biological metabolic states. In these situations, the AMS’s ability to measure a variety of matrices, typical and unusual (dialysate and dialysate cartridges in support of a renal/hepatically impaired patient study, for instance), are critical to ensure once difficult to achieve clinical goals are met. A growing body of work with these studies has also highlighted the AMS’s role in metabolite profiling in situations where the matrix to be profiled provides difficult challenges such as very dilute concentrations, non-specific binding, poor extraction efficiencies, and ionization interference. The sensitivity of the AMS and the analytical expertise gained by working with these compounds and matrices have allowed for solutions to these increasingly common problems. Recently, profiles have been obtained with no more than 0.04 dpm on HPLC column in support of low concentration plasma pools. It is important to note that these advances are not dependent on the structure of the compound or the type. The unique intermediary graphitization process allows for an unparalleled variety of compound compatibility: NCEs, biologics, ADCs, etc.

While current and upcoming studies are evolving what the AMS is conventionally used to support, Xceleron is looking at the ability of the AMS to measure $^{14}$C and $^{13}$C as the next innovative step in the technology’s future. Research is currently being undertaken to fully understand the differences in measuring $^{13}$C from $^{14}$C: what precision is the AMS capable of when analyzing $^{13}$C; what sensitivity can be achieved in light of the difference of the natural abundance of the two isotopes, ($^{13}$C is 1.09% of all naturally occurring carbon isotopes versus $^{14}$C which is $1 \times 10^{-10}$% of all naturally occurring carbon isotopes); what is the impact of the weight of carbon graphitized to $^{13}$C analysis and AMS current measurements. Through exploring this application of the technology, a study design using dual-labeled molecules could be conceived which holds the potential for financial and timeline benefits from a drug development perspective.
QUANTITATION OF DNA ADDUCTS FORMED BY DEOXYNUCLEOSIDE ANALOGUES AND ALYLATING AGENTS BY ACCELERATOR MASS SPECTROMETRY

Paul T. Henderson, University of California Davis Department of Internal Medicine, Division of Hematology and Oncology and the UC Davis Comprehensive Cancer Center, Sacramento, CA

The advent of companion diagnostics has revolutionized targeted cancer therapies. Unfortunately, no such tests exist for more commonly used cytotoxic chemotherapy agents such as the platinum-based drugs, anthracyclines and nucleoside antimetabolites, all of which bind to or incorporate into DNA to form toxic drug-DNA complexes called adducts. Our hypothesis is that a threshold level of drug-DNA adducts is required for cancer cell killing, and that dosing cells with sub therapeutic concentrations of radiocarbon-labeled drugs, called microdoses, can enable prediction of which cancers will respond to a given therapy. We also hypothesize that pharmacokinetic analysis of patients given microdoses of some these drugs intravenously will enable dose optimization in order to maximize treatment efficacy and minimize toxicity during chemotherapy. This talk provides an overview of a decade of research that illustrates the utility of accelerator mass spectrometry (AMS) for “bench to bedside” applications of drug-DNA adducts as biomarkers. Data will be presented for cisplatin, carboplatin, oxaliplatin, doxorubicin, gemcitabine and cytarabine. All of these compounds have been tested as potential diagnostic reagents in cell culture and mice bearing patient-derived tumor xenografts. There are currently two clinical trials in progress for precision medicine applications. One trial involves dosing bladder or lung cancer patients with a microdose of $^{14}$C carboplatin followed by blood sampling and tumor biopsy over 24 hours post dose. Within twelve weeks, patients begin platinum-based chemotherapy. Progress towards the endpoint of positively correlating $^{14}$C carboplatin-DNA adducts with response will be reported. A colorectal microdosing trial is focused on correlating microdose-induced oxaliplatin-DNA adducts with response, and on determining the feasibility of using the microdose plasma area under the curve (AUC) to optimize the subsequent therapeutic dose to a target AUC. The talk will finish with a discussion of more recent technical developments, collectively called liquid sample AMS, that may make analysis faster, cheaper and more amenable to quantification of multiple $^{14}$C-labeled analytes from the same sample.
DETERMINATION OF TRITIUM ATOM LOCATION AND DISTRIBUTION USING TANDEM MASS SPECTROMETRY
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When radiolabeled compounds are used for metabolism studies, it is important to know the location of the radiolabeled atom. The presence of the radiolabeling in a location lost due to metabolism, either due to direct loss of the tritium atom or from the loss of a radiolabeled moiety, results in the inability to detect the remaining portion of the compound and any subsequent metabolites produced from that portion of the compound.

While carbon-14 radiosynthesis results in a known location of radiolabeling, the same is not necessarily true for most tritium reactions, even those involving multiple bond reductions and dehalogenation. In nearly all such tritium reactions - and to a much greater degree for any tritium exchange reactions - there are often found to be some unexpected locations of tritium.

Radiosyntheses are conducted on a small mass scale (~1 mg) with the limiting reagent being the radiochemical compound. This results in purified batch sizes of approximately 10 µg. Depending on the reaction and purification yields and the amount of compound needed by the end user, this amount of radioactivity available for analysis may or may not be enough to determine tritium atom location and distribution by the traditionally used 3H NMR analysis. Depending on spectrometer specifics, approximately one milliCurie of radioactivity is needed to achieve the desired NMR signal-to-noise level of approximately 100:1 on an uncoupled tritium resonance in one hour.

Compared to NMR spectroscopy, mass spectrometry provides very high mass sensitivity. Therefore, it is expected that a mass spectrometry based method could determine the tritium location and distribution of a mass limited sample which would otherwise prove difficult to analyze by 3H NMR spectroscopy due to the latter technique’s inherent relatively poor sensitivity. Presented here will be a comparison of these two techniques in measuring tritium location and distribution.
ADDRESSING THE CHALLENGES OF WORKING WITH $^{14}$C RADIOLABELLED DRUG SUBSTANCE AND DRUG PRODUCT IN CLINICAL DEVELOPMENT THROUGH A TRANSLATIONAL PHARMACEUTICS PLATFORM

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Introduction
During the course of product development, a project team will have limited need to work with the radiolabelled form of a drug candidate either as drug substance/active pharmaceutical ingredient (API) or as investigational drug product (IMP). Given this infrequent use of $^{14}$C-API and $^{14}$C-IMP within the development lifecycle, these studies can present atypical challenges. As $^{14}$C-API is an expensive and inherently unstable commodity, it is important to optimize use to avoid waste, minimise study timelines and assure quality of what is typically a ‘single-use’ $^{14}$C-IMP.

Presentation
This presentation will describe an integrated process for $^{14}$C-API radiosynthesis, $^{14}$C-IMP manufacture and clinical dosing. This approach minimizes stability data requirements and facilitates the rapid use of the manufactured material in clinical development. A typical clinical program will be described with particular focus on how radiochemistry activities dovetail with the associated pharmaceutical development program to enable a suitable ‘single use’ $^{14}$C-IMP to be developed and manufactured to meet all GMP requirements for immediate clinical dosing. The presentation will also address the requirements for understanding the provenance of all starting material and intermediates to facilitate use of the $^{14}$C API in drug product manufacture together with the ultimate manufacture, QC testing, QP release and clinical dosing of the drug product.

Conclusion
An integrated approach to $^{14}$C-API synthesis and $^{14}$C-IMP development/manufacture ensures optimal material use and limits extended stability testing requirements. $^{14}$C-IMP quality is assured and the proximity of the site of IMP manufacture to the clinical site allows for the rapid dosing of study subjects and hence optimization of the overall program timeline.
A Collaborative Study Comparing the Use of LCMS to LC/UV for the Determination of Specific Activity

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Labeled compounds containing tritium and carbon-14 are widely used across drug development. When determining the suitability of a radiolabeled compound for a biological assay many parameters must be taken into account including the purity (chemical and radiochemical), labeling pattern and specific activity (SA). SA is used either directly to target a tracer mass concentration in the assay or is used in the calculation and carrier dilution step when making the stock solution for the assay. Significant error in SA will lead to the assay being run outside of the target concentration range and so an accurate (within 10\%) method of determining SA is required. There are a handful of techniques to measure specific activity including: gravimetric, UV mass concentration determination with radioactive solution count, LC/MS and GC/MS measurement of isotopic distribution and NMR analysis. Each method has advantages and disadvantages.

Gravimetric SA determination is most appropriate when the solid will be directly used in the biological study as it accounts for all impurities including solvents and salts, but is typically limited to very low SA materials such as Carbon-14 or tritium tracers intended for ADME studies. Since most of the compounds handled in our two groups are solutions, gravimetric analysis is not generally appropriate. Typical alternative techniques use the ratio of isotope peaks in a mass spectrum or for lower specific activity compounds the mass concentration of the radiolabeled compound, from its UV response and a standard curve, is used in conjunction with the radioactive concentration to calculate specific activity. The later UV based technique is expected to be optimal for tracer solutions that display an appropriately intense UV signal while the MS technique is expected to be better for moderately labeled compounds (>5 to <95\%) with few heteroatoms complicating the MS spectrum (S, Cl, Br, etc).

We selected several C-14 and H-3 labeled compounds and compared the results from the MS and UV response methods. In a few examples, carrier dilutions were performed to generate a series of tracers across a range of SA values thereby allowing for a comparison of each method at different SA values. We included heteroatoms containing tracers to determine how the presence of naturally isotopically distributed elements (S, Cl, Br) affected the measurement accuracy of each method. Additionally, a comparison of the LC/MS SA results using different calculation methods is provided. The intent was that a general understanding of which technique would be most appropriate in each situation would be developed in the process of this investigation.
Viewlux Microplate Imager for Metabolite Profiling: Applications in Drug Development

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Generation of early information on metabolic pathways, metabolite structures and their systemic exposure is a highly time consuming activity during the drug development process. Since these data have become of higher interest for the health authorities, efforts have been made to provide results as early as possible. ViewLux UltraHTS Microplate Imager is an instrument originally designed for high throughput in biological assays requiring luminescence, absorbance or fluorescence detections. In this work, we evaluate the capability of the new generation of the instrument for both $^{14}$C and $^3$H detection. We discuss data processing of the Viewlux, especially the background subtraction in comparison to conventional TopCount® instruments, as this has an impact on the limit of detection for samples containing low amounts of radioactivity like samples originating from ADME studies. We demonstrate that the limit of detection can be lowered by prolonging the exposure time for $^3$H labeled compounds up to 2 h. We validated the ViewLux for our metabolite profiling applications (in vitro and in vivo ADME samples) in early drug development using UPLC followed by fraction collection in 384 well plates and demonstrated for our applications that limits of detection of 2.2 and 24 dpm/well for $^{14}$C and $^3$H, respectively could be reached and that the throughput could be increased by at least two fold compared to conventional Topcount detection. We also demonstrate in this work how endogenous interferences resulting in false positive peaks in samples containing low amounts of radioactivity can be overcome by using a customized light filter.

We also discuss in this work how the Viewlux can shorten analysis times for ADME studies when it is combined with UPLC and automated sample handling devices like the PlateCrane and how the data flow can be optimized.
Escherichia coli growth rate measurements verify the Isotopic Resonance Hypothesis

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Stable isotopes affect living organisms. The Isotopic Resonance (IsoRes) hypothesis [1] postulates that at certain “resonance” abundances of the stable isotopes of the elements C, H, N and O, the rates of chemical or biochemical reactions of some compound classes accelerate. The suggested IsoRes mechanism relates to the overall reduction of the system’s complexity, which boosts its kinetics. One isotopic resonance is observed near the terrestrial isotopic compositions (Figure, left). It acts on polypeptides following the rule $C - (N+H)/2 = 0$, which encompasses most “ancient” amino acid residues in polypeptides.

The IsoRes hypothesis has first been tested on literature data [2]. Then we tested this hypothesis experimentally by investigating the growth behavior of Escherichia coli (E. coli) through altering the isotopic composition of the growth media [3]. While high contents of heavy stable isotopes, such as $^2H$ or $^{15}N$, decreased the growth rate of E. coli and extended the lag time (an expected result), significantly accelerated growth and/or shorter lag time were observed at the predicted “resonance” isotopic compositions of $^{13}C$, $^2H$, $^{15}N$ and $^{18}O$ (Figure). In all cases, the level of enrichment was low to moderate (<12%). The bulk of observations can only be explained by the IsoRes hypothesis, the combined p-value for which is <<10^-15 [3].

The terrestrial isotopic resonance becomes perfect when the deuterium concentration nearly doubles from 150 ppm to 250-300 ppm, and thus the IsoRes hypothesis suggests that, at these concentrations, the rate of growth should further increase. There is abundant experimental evidence gathered since 1930s confirming that this is the case. The reverse is also true – according to the IsoRes hypothesis, deuterium depletion should lead to reduction in growth, particularly of fast-growing cells, such as cancer cells.

There are numerous implications of the IsoRes phenomenon. One of them is that early life on Earth has probably benefited from the presence of the terrestrial resonance. On Mars, the terrestrial $Z=0$ resonance is absent, but there are multiple, although smaller, other resonances.

Figure. Left – the line represents the terrestrial isotopic resonance [1]. E. coli growth increases at 3.4% of $^{15}N$ (center) and at 0.35% of $^{13}C$ (right), as well as some other resonance compositions predicted by the hypothesis [3].

References

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REPRODUCTIVE PERFORMANCE OF CROSSBRED DAIRY COWS UNDER ORGANIZED FARM CONDITION IN BANGLADESH: AN ISOTOPIC RADIOMICNOASSAY STUDY

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Introduction
Poor reproductive performance due to inadequate nutrition of crossbred cows in Bangladesh is a major problem for developing dairy industry. Low quality rice straw based diets have a prolonged anestrous period after calving and it is recognized as a major constraint for maintaining 12 months calving interval. The experiment was conducted to know the effect of optimum level of urea molasses block (UMB) supplement under organized farm condition on reproductive efficiency of cows by using Isotopes.

Description of the Work
The experiment was conducted at Bangladesh Agricultural University Dairy Farm, Mymensingh, Bangladesh. Thirty two cows were divided into 4 groups, each group consisting of 8 cows. Cows were grouped randomly on the basis of body weight and initial (2nd week) milk production. One group was not supplemented with UMB and designated as control (T0) and the remaining three groups of cows were supplemented with UMB at three different levels containing 300, 500 and 700 g/d/cow which were designated as T1, T2 and T3 respectively. UMB was supplied as supplemented with straw based diets in all treatments groups. Animals were upgraded popularly called Holstein crossbred (local zebu x Holstein), >2nd lactation, reared in Bangladesh Agricultural University Dairy Farm. Average body weight of cows was 256 kg and initial milk production was 5.20 kg/d.

The basal diet composed of adlibitum rice straw, 3.0 kg Para (Brachiara mutica) + German (Echinocloa crusgalli) grass and 2.50 kg concentrate mixture/cow/day. Attempts were made to keep all animals under same condition. Ten ml of milk was collected directly from udder of the individual cows during milking at an interval of 10 days beginning from 10 days after calving up to 120 days postpartum. Sodium azide tablets were used as preservatives @ 8mg/10 ml milk. The milk samples were then kept in deep freeze at -20°C until analysis. Uterine involution and ovarian activity was monitored 30 days postpartum and thereafter. Food and Agriculture Organization of the United Nations / International Atomic Energy Agency (FAO/IAEA) Progesterone (P4) Radioimmunoassay (RIA) kits were used for P4 measurement by using 125I as a tracer. The P4 concentration in the skimmed milk was determined by using a solid phase RIA technique. The laboratory of IAEA, Vienna, Austria, supplied the RIA kits for P4 measurement. P4 profile was used to calculate the intervals from calving to resumption of ovarian activities.
CHALLENGES IN THE REDUCTIONS OF THE [RING-U-$^{14}$C]-LABELLED FLUORONITROBENZENE DERIVATIVES

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The key-intermediate of a new type of pesticides (1) is the methyl-3-amino-2-fluorobenzoate (2). The preparation of the labelled compound was carried out after inactive method development. An optimized way was used in the radiosynthesis. At a step an unexpected product was formed by a side-reaction, therefore a new synthetic method had to be elaborated, which was successful. From the so synthetised compound (2) we could reach the target compound in few steps.

The preparation of the key-intermediate will be presented.

The same type of unexpected reaction occurred in the synthesis of the intermediate of Florasulam (3) which will also be presented.
KINETIC AND SOLVENT ISOTOPIC EFFECTS IN BIOTRANSFORMATIONS OF AROMATIC AMINO ACIDS AND THEIR DERIVATIVES

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Aromatic amino acids as L-phenylalanine, L-Phe, L-Tyrosine, L-Tyr, L-tryptophane, L-Trp, and their derivatives as 2,4-dihydroxyphenylacetaldehyde, DOPAL, and dihydroxyphenylethanol, DOPET, play an essential role in many metabolic processes in humans. The incorrect or slow metabolic conversions of these compounds lead to metabolic illnesses, and in some cases to neurodegenerative diseases. For this reason, the study the mechanisms of biotransformation of these compounds draws attention of biochemists, pharmacist and medical researchers.

For many years, our research group has investigated the mechanism of the above transformations using the kinetic (KIE) and solvent (SIE) isotope effect methods. Here we present an overview of results and numerical values of KIE’s and SIE’s obtained during the studies of mechanism decomposition of L-Phe, L-Tyr, L-Trp, and their derivatives, and also DOPAL catalyzed by the enzymes of the groups of lyases and oxyreductases. The kinetic and solvent isotope effects have been determined using non-competitive spectrophotometric and competitive (combined with internal radioactivity standard) radiometric methods.

The objects of the research were the biotransformations of following compounds:

1. L-Phe. Elimination of ammonia catalyzed by the enzyme Phenylalanine lyase (PAL, EC 4.1.3.5). KIE’s values were determined for deuterium and tritium in the (3R)- and (3S)-positions, and for carbon-14 at the 2-position of side chain of L-Phe.
2. L-Tyr. Kinetic isotope effect for C-14 in the 2-position of L-Tyr was determined in the decarboxylation reaction catalyzed by the tyrosine decarboxylase. (EC 4.1.1.25).
3. 5’-chloro-L-Trp. The KIE’s for deuterium and tritium in the 2-position of 5’-chloro-L-Trp were obtained in the decarboxylation reaction catalyzed by tryptophan indole-lyase (TPase, EC 4.1.99.1).

The reactions catalyzed by dehydrogenases.

1. 2’-chloro-L-Phe. KIE for deuterium in the (3S)-position was determined for deamination of 2’-chloro-L-Phe leading to 2’-phenylpyruvic acid catalyzed by enzyme L-phenylalanine dehydrogenase (EC 1.4.1.20).
2. DOPAL. Isotope effects were determined for conversion of DOPAL into DOPET, catalyzed by adehyde dehydrogenase (ALDH, EC 1.2.1.3)) using NADPH as cofactor. In this reaction deuterium KIE,s for the (4R)- and (4S)-positions of NADPH were determined Additionally, the deuterium SIE’s were determined for halogen derivatives of L-Phe and L-Trp.
THE USE OF STABLE ISOTOPE LABELLED ANALOGUES BOTH AS INTERNAL STANDARDS AND AS SURROGATES FOR THE ANALYSIS OF MULTIPLE PYRETHROID INSECTICIDES IN COMPLEX ENVIRONMENTAL SAMPLES
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The method of choice for multi-analyte pyrethroid analysis methods is gas chromatography with negative chemical ionization with mass spectral detection (NCI-GC-MS). Because of the inherent susceptibility of trace-level GC methods to matrix effects and changes in detector response over time, the Pyrethroid Working Group has invested in the synthesis of eight pyrethroid D6 stable isotope analogues as a corrective measure. The use of these materials as internal standards, introduced into sample extracts at the final reconstitution step (with sample concentrations being determined based upon the native analyte/d6 internal standard ratios), has proven to be a valuable tool for normalization of any fluctuations in the NCI-GC-MS response. This has been shown to be particularly useful in the analysis of complex matrices such as influent/effluent waters and biosolids from publicly owned treatment works (POTWs) for multiple pyrethroids. In addition to the use of eight D6 stable isotope analogues as internal standards for the corresponding representative pyrethroids, the use of two of these stable isotope standards in an additional capacity, as surrogates, will be presented. Due to significant variability in the composition of biosolids from different POTWs, adding known amounts of two d6 compounds as surrogates (which reflect the behavior of the eight targeted analytes) has been investigated. By adding the two d6 surrogates to each sample (prior to extraction), and then measuring recoveries, acceptable method performance (including the extraction and clean-up steps) has been demonstrated. Details of a validated biosolids method will be reported, with an emphasis on method performance and surrogate stable isotope analogue recovery data.
O43

Synthesis and characterization of novel fluorophore or isotopically labelled probes for beta cell imaging#

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Today, hundreds of million people suffer from diabetes, and the World Health Organization expects that deaths caused by diabetes will double between 2005 and 2030. Yet beside the importance to understand the disease, the underlying precise molecular and cellular mechanisms causing the decrease in mass and function of the insulin-producing β-cells remain to be elucidated. Imaging of β-cells would be a key to improved understanding of the pathophysiology of the disease; it would allow monitoring of therapeutic efficacy and durability (including islet transplantation) and thereby would facilitate the discovery of new drugs. So far, there is no small molecule based tool available to visualize and reliably quantify β-cells in a non-invasive manner.

Here we describe the first small molecule probe targeting the free fatty acid receptor 1 (FFAR1) which is highly expressed on the membrane of β-cells. We designed the probes starting from a selective and potent FFAR1 agonist.

*In vitro* characterization by microscopy experiments showed effective binding of the probe to FFAR1 overexpressing HEK cells (Figure 1a). In both cases the control experiments, employing excess of unlabeled agonist, indicate that the binding is specific (Figure 1b). Furthermore F-18 labelled probes have been synthesized. Details on synthesis and application of the imaging probes are reported.

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Automated Synthesis of $^{18}$F-5-Fluorouracil from $^{18}$F-Fluoride for Human PET Imaging

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$^{18}$F-5-Fluorouracil was synthesized from $^{18}$F-fluoride in an automated cGMP process that produces doses suitable for human PET imaging. To enable this process, two new technologies were developed. First, a novel transmetallation reaction was developed for the preparation of new nickel(II) aryl precursors for oxidative fluorination. Second, a new microfluidic device was developed for highly efficient fluoride concentration that proceeds without needing to evaporate water. This work illustrates that a modern transition metal-mediated fluorination reaction can enable clinical research imaging.
Design, Synthesis and Development of Lysophosphatidic Acid Receptor 1 (LPA1) PET Radioligands for Lung Receptor Occupancy Imaging

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Lysophosphatidic acid (LPA) is a bioactive phospholipid that regulates numerous aspects of cellular function and has been recognized as a novel mediator of wound healing and tissue fibrosis. LPA mediates its biological effects through the LPA receptors, of which at least six isoforms have currently been identified. Recently, LPA1 has been linked to the pathogenesis of lung fibrosis and antagonism of LPA1 is currently being explored as a target for the treatment of idiopathic pulmonary fibrosis (IPF). It has been hypothesized that blocking LPA1 will reduce lung injury by mediating fibroblast recruitment and vascular leakage. The development of a specific PET radioligand having high affinity for LPA1 may provide a method to evaluate both target engagement and drug exposure, receptor occupancy relationships of LPA1 antagonists in the human lung. Both $[^{18}F]$-BMT-083133 and $[^{11}C]$-BMT-136088 were designed to be LPA1 PET radioligands, and have low nanomolar affinity for the LPA1 receptor. These molecules are functional in ex-vivo autoradiography studies and in-vivo imaging of LPA1 expression in lung tissue. Here, we describe the discovery and design of these potent LPA1 PET ligands and their preclinical validation using disease state induced models.
The Development of a PET Tracer from bench to large clinical studies

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RO6807936 as a Novel Radiotracer for In Vitro and In Vivo Visualization and Quantification of BACE1 in the Rodent and Baboon Brain

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Objectives: Beta-amyloid is the main constituent of amyloid plaques in Alzheimer’s disease (AD). The beta-site amyloid precursor protein (APP) cleaving enzyme (BACE1) catalyzes the first step in the generation of soluble beta-amyloid from APP. As such, BACE1 is an intensely investigated target for the therapeutic inhibition of beta-amyloid production in AD. In the absence of a suitable BACE1 radioligand, little is known about the distribution pattern and density of the BACE1 protein in the brain. Our aim was to develop a specific BACE1 radioligand for visualization of BACE1 in the rodent and monkey brain.

Methods: RO6807936 was selected among the BACE1 inhibitors from an in-house drug program on account of its favorable physicochemical and pharmacokinetic profile with PET tracer like properties. After radiolabeling with tritium, ligand affinity and specificity were assessed in vitro and ex vivo in rodent brain tissue by radioligand binding assays and autoradiography. The compound was subsequently labelled with carbon-11 (Fig. 1; for carbon-11 methylation of terminal alkynes see T. Hosoya, et al. (2004), Organ. Biomol. Chem., 2, 24-27) and tested in non-human primates under baseline and blocking conditions.

Results: [3H]RO6807936 was obtained with a specific activity (SA) of 980 GBq/mmol and a radiochemical purity (RCP) > 99%. [11C]RO6807936 was produced in 33 min and 12% decay-corrected yield with SA = 218 GBq/μmol and a RCP = 100%. Saturation analysis of [3H]RO6807936 revealed high-affinity binding (K_D = 2.9 nM) and a low binding site density (B_max = 4.3 nM) in native rat brain membranes. The specificity of [3H]RO6807936 for BACE1 was demonstrated by absence of binding to BACE1 knockout mouse brain sections. A comparable BACE1 expression pattern and binding level was found in rodent, cynomolgus, baboon and human brain in vitro. [3H]- and [11C]RO6807936 displayed acceptable uptake in the rat or baboon brain, respectively, and an in vivo binding pattern comparable with that observed in vitro. Pretreatment of the animals with a structurally different BACE1 inhibitor led to a significant blockade of the signal.

Conclusions: RO6807936 allows for the specific visualization of the BACE1 protein in the rodent and monkey brain. This novel radioligand displays a consistent in vivo binding pattern across several species and is of interest for further profiling as PET radiotracer for target engagement studies of BACE1 drug candidates.

Figure 1: Radiosynthesis of [11C]RO6807936.
CGMP RADIOSYNTHESIS FOR EARLY PHASE CLINICAL TRIALS: A UNIQUE CHALLENGE

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Abstract
Radiolabeled drugs are used in Human ADME and bio-availability studies. While the regulatory requirements for traditional “cold” clinical trial materials are well understood, the regulatory requirements for radiolabeled are much less understood by industry. The synthesis of radiolabeled compounds sometimes requires development of new synthetic pathways which can be significantly different from the traditional synthetic pathway. The radiolabeled drug can have significantly different stability and impurity profiles from the non-labeled drug and thus require special considerations. These special considerations may pose challenges in terms of ensuring CGMP compliance and safety for the patient during the clinical trial.

This presentation discusses the challenges associated with radiolabeled synthesis coupled with maintaining CGMP compliance for the synthesis and analytical portions of the program.
Combining efficient $^{14}$C-radiolabeling and radioimaging techniques of manufactured nanoparticles for toxicological studies.

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Carbon nanotubes (CNTs) are very promising for a variety of industrial and biochemical applications due to their physicochemical properties. As a result, many different types of CNTs have been produced on an industrial scale over the last 20 years, raising the issue of their safety. Radiolabeling of CNTs with a long life radioactive nucleus, such as carbon-14, offers a unique tool to assess their bio-persistence over long periods in animals and plants. Our laboratory has recently developed two straightforward and efficient $^{14}$C-radiolabeling techniques. Using an innovative $^{12}$C/$^{14}$C isotope exchange strategy and a catalytic chemical vapor deposition (CCVD) method, utilizing $^{14}$C-labeled benzene as carbon source, CNTs were successfully synthesized with specific activities ranging from 0.45 to 10 MBq/mg.

After pharyngeal aspiration to mice, a small amount of MWCNT dose was observed to translocate to distant organs (spleen and bone marrow), showing the ability of MWCNTs to cross the air-blood barrier. Over 12 months, MWCNTs increasingly accumulate in these organs, indicating a bio-persistence of these nanoparticles. Such preliminary results may raise safety concerns for humans in working places.

A New Biomarker of Mitochondrial Function Obtained in vivo by Dynamic Deuterium Magnetic Resonance Following Administration of Deuteriated Glucose

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We report the discovery of a new biomarker of mitochondrial function, namely the crossing point of the glucose consumption and nascent mitochondrial water (oxygen consumption) curves simultaneously recorded, in vivo, by DDMR (Dynamic Deuterium Magnetic Resonance). In order to demonstrate that it is possible to measure differences in mitochondrial function, we performed DDMR on a mouse administered D-Glucose-6,6-d2 (G66d2) by intraperitoneal (ip) infusion, and on another mouse, administered the same amount of G66d2 via intravenous (iv) infusion. The crossing point of the glucose and oxygen consumption curves was approximately 6.5 times faster for iv infusion (at ~ 6 minutes), when compared with ip infusion (at ~ 39 minutes). All animal procedures were according to the Institutional Animal Care and Use Committee regulations. It is reasonable to assume that this new biomarker will be an excellent indicator of normal or pathological mitochondrial function, i.e., the state of health of an organism. The method can be totally noninvasive (a water solution of deuteriated glucose can be taken orally, as in the Glucose Tolerance Test); it can probe mitochondrial function in any organ or tissue; with straightforward modification, it may be implemented in commercial scanners; it is less expensive, less elaborate, and more informative than PET (Positron Emission Tomography) or 17O-MR; also, it may have therapeutic value (kinetic isotope effect), particularly in cancer treatment. Validation of the method will be made by comparing results with those obtained by PET and functional MRI.
THE SYNTHESIS AND ANALYSIS OF $[^{14}\text{C}(U)]$BMS-770767 and $[^{13}\text{C}_6]$BMS-770767 FOR USE IN DISCOVERY BIOTRANSFORMATION, HUMAN ADME AND BIOANALYTICAL STUDIES

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BMS-770767 is potent inhibitor of the 11β-hydroxysteroid dehydrogenase type 1 (11β-HSD1) enzyme which is a possible new therapy for type 2 diabetes and other disorders. As such, it was necessary to synthesize $[^{14}\text{C}(U)]$BMS-770767 for use in discovery biotransformation studies and later for the human ADME clinical study. It was also necessary to prepare $[^{13}\text{C}_6]$BMS-770767 for use as a LC/MS internal standard for bioanalytical studies. This presentation will involve a complete discussion of the syntheses and analyses of $[^{14}\text{C}(U)]$BMS-770767 and $[^{13}\text{C}_6]$BMS-770767.
Synthesis of Isotopically Labeled Compounds through Manipulation of Active Pharmaceutical Ingredients (APIs) and Late Stage Intermediates - Case Studies

**Sumei Ren**: David Hesk; Cheng Tang; Eric Soli; Yong Liu; Huifang Yao; Ryan Cohen; and Roy Helmy

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The synthesis of natural products, natural product analogs, or pharmaceuticals with high structural complexity with $^{14}$C or stable isotopes is often very challenging. They frequently suffer from long synthetic sequences, low overall yield, unavailability of unlabeled advanced intermediates and the lack of readily accessible labeled starting materials. In some cases it may even not be viable to introduce $^{14}$C or stable isotopes via a direct synthetic approach. One possible solution for this type of problems is the degradation of the parent APIs or advanced intermediates followed by reformation of the APIs or advanced intermediates incorporating $^{14}$C or stable isotopes. We will demonstrate the principles by a number of case studies:

- the decarboxylation of cholic acid to label the cholic acid with carbon-14
- the hydrolysis of O-methyloxime of Moxidectin to Keto-Nemadectin followed by introduction of a labeled methyl group to form labeled Moxidectin
- the demethylation of methylethers or N-methyl amines followed by re-methylation with labeled methyl groups
- The hydrolysis of the hydantoin moiety of SCH 900567 and then reconstruction of the hydantoin ring using potassium $^{14}$C-cyanate.

Some of the degradations are very challenging. We will share how these challenges were overcome as well as the limitations of these types of manipulations.
SYNTHESIS OF THE METABOLITES OF C-FMS KINASE INHIBITOR JNJ-40346527 AND THE ISOTOPE-LABELED COMPOUNDS

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FMS is a class III receptor tyrosine kinase and is the exclusive receptor for colony stimulating factor-1 (CFS-1). Binding of CFS-1 to FMS induces FMS dimerization and auto-phosphorylation of the intracellular FMS kinase domain. Small molecule inhibitors of the kinase catalytic site of FMS will block CFS-1 induced cellular responses. Thus, FMS inhibitors will be potentially useful in treating diseases where osteoclasts and macrophages are pathogenic, such as rheumatoid arthritis and metastatic cancer to the bone. JNJ-40346527 was discovered as orally active FMS kinase inhibitor for the potential treatment of rheumatoid arthritis.[1] Oxidative metabolites M2 and M7 were identified as two major metabolites among others.

Herein we report the synthesis of the two metabolites for the evaluation of their biological activity and the synthesis of the stable isotope labeled compounds as internal standard for bioanalysis. Enzymatic screening for biosynthesis of M2 and M7 metabolites with Codex® MicroCyp® kit was attempted and only trace of M7 in only one condition was found. Biomimetic oxidation by the catalysis of Mn and Fe metalloporphyrins for the generation of M2 and M7 was attempted; but was unsuccessful. Nonetheless, chemical synthesis of a 13-step sequence provided M7 and M2 metabolites successfully. Chemical synthesis with total 17 steps provided STIL [M+D4]M7 and STIL [M+D4]M2. Stable isotope labeled JNJ-40346527 was similarly synthesized. Both metabolites in racemic form and in enantiomeric pure form were found to be pharmaceutically active and were as potent as the parent drug.[2] Details will be presented.

References
SYNTHESIS OF STABLE ISOTOPE LABELED EPOTHILONE D USING A DEGRADATION - RECONSTRUCTION APPROACH

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The stabilization of microtubules using epothilones represents a novel mechanism of action to treat Alzheimer’s disease. Epothilone D is one such microtubule stabilizing drug that has been investigated by Bristol-Myers Squibb. An important step in the development process was the synthesis of a stable isotope labeled analog for use in bioanalytical assays to accurately quantify the concentration of the drug in biological samples.

A novel synthetic route to stable isotope labeled epothilone D will be presented. The synthetic route was based on a strategy to degrade epothilone B (2) and then use that key intermediate to reconstruct stable isotope labeled epothilone D. Epothilone B (2) was treated with potassium osmate and sodium periodate to cleanly remove the thiazole moiety. The epoxide in the macrocyclic ring of that intermediate was efficiently removed by treatment with tungsten hexachloride and n-butyllithium to give the corresponding olefin. Bis triethylysilyl protection produced ketone 3. Ketone 3 was coupled to a stable isotope labeled thiazole using a Wittig reaction as the key step to provide labeled epothilone D ([13C5, 15N]-1) after deprotection. The synthesis was completed in nine total steps, only six of which involved isotopically labeled reagents. A total of 168 mg of stable isotope labeled epothilone D ([13C5, 15N]-1) were prepared in an 8% overall yield from 13C2, 15N-labeled thioacetamide and 13C3-labeled ethyl bromopyruvate.
Protocols For Incorporating Multiple Stable Isotopes Into High Value Heterocycles
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The copper-catalysed azide-alkyne cycloaddition (CuAAC) reaction has attracted an enormous amount of interest. This can be attributed, in part, to its exceptional and unparalleled 100% successful ‘reactants to product’ track record. This extraordinary ability to transform ‘any’ azide and ‘any’ alkyne to the corresponding 1,2,3-triazole in, more often than not, excellent yield, 100% regioselectivity, 100% chemoselectivity and 100% atom-efficiency has placed it at the pinnacle of the scientific communities ‘most reliable chemistry’. Thus we anticipated the introduction of stable isotopes into 1,2,3-triazoles via CuAAC to be straightforward and efficient. In contrast to their non-isotope enhanced isotopologs we were surprised that this is not the case. Preliminary results will be discussed on our attempts thus far to include stable isotopes into the CuAAC reaction.

The first organocatalytic procedure for the efficient incorporation of multiple stable isotopes (2H, 13C and 15N) into a heterocycle will be discussed: the synthesis of optically active, stable isotope labelled aziridines.1 With equal ease our protocol allows the incorporation of either one or two deuterium nuclei or the inclusion of multiple and different isotope combinations generating structure and function diverse aziridines. Our procedure is efficient, high yielding, allows the ‘dial up and lock in’ of different stable isotope nuclei, and affords aziridines in excellent regio-, diastereo- and stereoselectivities. Exemplifying the utility of our protocol their subsequent transformation into non-natural, isotope labelled α-amino or β-substituted-α-amino acids further substantiates the value of our protocol which employs readily available starting materials and affords highly desirable, optically active α-amino acids or β-substituted-α-amino acids with >95% 2H, 13C, 15N and 18O incorporation.

References

SYNTHESIS OF STABLE ISOTOPE-LABELED SIGNATURE PEPTIDES FOR USE AS INTERNAL STANDARDS IN PROTEIN QUANTITATION BY LC-MS/MS

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In the development of therapeutic proteins, a key requirement is establishing a sensitive bioanalytical assay allowing quantitation of the protein in biological matrices for pharmacokinetic and toxicokinetic evaluations. Liquid chromatography coupled to tandem mass spectometry (LC-MS/MS) is a versatile method for protein quantitation due to its high specificity, reproducibility, and wide dynamic range. For the LC-MS/MS quantitation of larger proteins (>10,000 amu), an initial digestion is needed to afford a mixture of peptide fragments (see figure below) for analysis by LC-MS/MS. Surrogate peptides with suitable LC-MS/MS sensitivity and low interference from endogenous proteins serve as a signature peptide of the target protein. In order to develop a rugged and reproducible assay, the digest is spiked with an isotopically-labeled analogue of the signature peptide as an internal standard in the quantitation. By using selected reaction monitoring (SRM), the response ratio of the signature peptide to its internal standard is used for the quantitation because variations in signature peptide response due to variations in extraction recovery or matrix effects can be compensated by the internal standard.

Synthesis of the stable-labeled signature peptide for these analyses usually requires the use of solid phase peptide synthesis (SPPS) on an automated synthesizer. However, the efficient preparation of useful amounts of stable-labeled peptides can be problematic due to excessive hydrophobicity or steric hindrance of protected amino acid side chains. These issues can often be overcome by utilizing SPPS with microwave or heating, and pseudoproline dipeptide intermediates during the coupling steps. This presentation will include a brief background on LC-MS/MS protein bioanalytics and then detail our recent syntheses of labeled signature peptides. Examples will include the synthesis of a labeled signature peptide capable of quantifying immunoglobulin G (IgG) and Fc-fusion protein drug candidates¹, and the preparation of labeled signature peptides possessing unsymmetrical disulfide bridges.

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Synthesis of Isotopically Labeled 6-Arylpicolinate Herbicides

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Picolinic acid based herbicides have been used for decades for weed control. These picolinate herbicides act via the auxinic mode of action and have been primarily used to control broadleaf weeds in various crops. Recently, a new structural class of synthetic auxin herbicides, the 6-arylpicolinates, was introduced by Dow AgroSciences. This novel auxin herbicide class includes Arylex™ active and Rinskor™ active. To support method development, metabolism, and environmental fate studies, there was a need to prepare isotopically labeled standards of both Arylex and Rinskor. The synthesis of stable isotopes and C-14 labeled radiotracers of these two novel herbicides will be discussed.
INVESTIGATING SELECTIVE HYDROGEN ISOTOPE EXCHANGE ON UNSATURATED MOIETIES WITH ACTIVE IRIDIUM COMPLEXES

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There exist a wide range of reported iridium catalysts which can facilitate hydrogen isotope exchange (HIE) upon aromatic compounds. Within this array, several catalysts stand out for their ability to mediate the HIE under mild conditions, with high levels of isotope incorporation. One key drawback in the labeling protocol with each catalyst is the necessity of an aromatic group to accept the isotopic label, limiting the utility to compounds containing functionalised aromatic skeletons.

Our first effort to overcome this limitation was to apply aromatic unsaturated compounds, with skeletons related to aromatic moieties previously labelled. However, each currently capable of HIE under mild conditions is also capable of olefin hydrogenation, typically under similar reaction conditions. Pleasingly, we found that utilising our developed, commercially available, bulky NHC/phosphine complex (I), under optimised conditions, excellent isotope incorporation is delivered and the alkene reduction is negligible. Furthermore, isotope incorporation is observed exclusively $\beta$ to the donor group. We have proposed a mechanism for this mild and novel process, supported by experimental findings, which includes oxidative addition into the $\beta$-C-H bond, similar to aromatic HIE.

Further experimental studies regarding the selectivity of $\beta$-HIE substrates, show the preference in single donor group substrates to be dependent upon the activation energy (Eyring analysis). In contrast, for substrates with two separate donor groups, the binding energy of each group is selectivity-determining (Lineweaver-Burk analysis).

In conclusion, by coupling the detailed experimental work described above with theoretical calculations (DFT), we have established enhanced levels of understanding for a novel HIE process that is now primed for application within the labelling community.

TRITIUM LABELLING OF SMALL MOLECULES, CROTONIC ACID, ISO-CROTONIC ACID, CYSTAMINE, CYSTEAMINE, ACETYLCHOLINE, AND BUTYRYLCHOLINE.

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Attempts to prepare high specific activity tritiation of some small molecules are to be presented. Solid state exchange tritiation of crotonic acid and iso-crotonic acid were achieved by using different exchange heterogeneous catalysts and homogeneous Crabtree’s catalysts. Alternatively, poison-catalysed alkyne reduction approach yields iso-[^3H]crotonic acid, which followed by acidic conversion to give pure isomeric forms of[^3H]crotonic acid. These[^3H]crotonic acids were used as anchors for the synthesis of larger molecule[^1H]crotonyl Co-enzyme A. Similar exchange reactions for the preparation of small molecules [G-[^3H]cystamine and [G-[^3H]cysteamine will also be high-lighted. Related synthesis of acetyl[^3H]acidic anhydride and [2,3,4-[^3H]butentanoic acid for the uses of making acetyl[^1H]choline and butyryl [2,3,4-[^3H] choline respectively will be reviewed.

\[
\begin{align*}
\text{Crotonic acid [2,3-[^3H]} & \quad \text{Iso-Crotonic acid [2,3-[^3H]} \\
\text{H}_2\text{N} & \quad \text{H}_2\text{N} \\
\text{SS-S} & \quad \text{S-S} \\
\text{NH}_2 & \quad \text{SH} \\
\text{Acetyl[^3H] choline} & \quad \text{Butyryl[2,3,4-[^3H] choline}
\end{align*}
\]
USE OF STABLE ISOTOPE-LABELED TRACER TECHNIQUES FOR METABOLIC DISEASE RESEARCH IN DRUG DISCOVERY

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In 21st century, one of the major challenges in drug discovery is to translate pre-clinical animal experimental results to clinical pharmacology assessments. Biomarker strategy and implementation become critical in translational medicine. Stable isotope tracer technology provides us a unique opportunity to apply biomarkers of target engagement, mechanism of action, as well as efficacy and safety, which will facilitate go/no-go decision making, increase the success of clinical studies, and thus significantly improve the process of clinical development.

In recent years, we have developed several platforms of using stable isotope-labeled glucose or lipid tracers to track glucose and lipid metabolism in animal studies. These tracer-related methods have been used to support multiple drug discovery projects for metabolic diseases. These applications include: (1) $[{}^{13}C_6]glucose$ and $[6-{}^{13}C-6,6-D_2]glucose$ tracers for determination of glucose intestinal absorption and endogenous glucose production; (2) $[{}^{13}C]oleic$ acid and $[{}^{13}C_3,D_5]glycerol$ for differentiation of diacylglycerol O-acyltransferase-1-mediated or diacylglycerol O-acyltransferase-2-mediated triglyceride synthesis and fatty acid oxidation; and (3) $[{}^{13}C]tryglycerides$ tracer for monoacylglycerol acyltransferase 2 mediated lipid intestinal absorption.

The combination of these isotope-labeled tracer strategies with LC/MS analysis offers simplicity, speed, specificity, sensitivity, and feasibility in metabolic disease research using animal models with diabetes and obesity, and thus provide novel opportunities to implement target engagement and efficacy assessment in drug discovery and development process.

References:


Deuteration---Unexpected effects on the structures and properties in soft materials

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Abstract: Hydrogen is the most abundant element in the universe. It has two stable isotopes, protium and deuterium, though deuterium is quite rare with a natural abundance of only 0.016%. Selective incorporation of deuterium in place of protium in soft materials (organic compounds, polymeric materials) for the most part does not alter the physical and chemical properties of the compound, yet provides rich structural information due to the difference in mass and neutron scattering length densities (SLD). Thus selective deuteration can provide a powerful approach to structural elucidation and chemical dynamics in soft materials by way of neutron scattering analyses. However, deuteration can sometimes introduce subtle changes in the structures and properties of soft materials. In this presentation, we will discuss some examples of how selective deuteration can be used to elucidate the structures and dynamics of soft matters unambiguously. Meanwhile, we will also discuss the impact of deuteration on certain fundamental properties, such as optoelectronic properties, thermal properties, biocompatibility, and viscosities of some target molecules in details.

Acknowledgement: This work was performed at the Center for Nanophase Materials Sciences at Oak Ridge National Laboratory, supported by the Office of Basic Energy Sciences, U.S. Department of Energy.
In 2008 we reported a sensitive laser-based analytical technique, intracavity optogalvanic spectroscopy (ICOGS) that might meet the requirements for $^{14}$C quantitation at zeptomole $^{14}$C levels in a laboratory-based instrument. The basic laser technology upon which ICOGS is based was first demonstrated for stable carbon isotope analysis of $^{13}$C in 1994 and a clinically approved instrument was subsequently developed for G-I disease breath testing. Now with National Science Foundation Innovation Corps (I-Corps) and Instrument Development support we are working to bring the considerably more sophisticated radiocarbon technology to the laboratory. We have conducted interviews with potential users in the drug development and related communities in order to determine their needs and desired instrument specifications. Industry experience in the past decade using Accelerator Mass Spectrometry (AMS), the only competing method available for direct radiocarbon counting, has been invaluable. The anticipated device will be similar in concept to a high performance scintillation system where the laser is analogous to the scintillation detector and a gas discharge is analogous to the scintillation cocktail. Sensitivity however will be hundreds of times greater than that of a radiation detection system.

Samples will be introduced as carbon dioxide from oxidation of biological samples. Only small amounts of total carbon (sub mg to ng) are required for analysis. The instrument layout anticipates ancillary sample handling options such as an interface for various forms of sample introduction including multi-well plates for pipetted liquids or collected chromatography column fractions and continuous flow from oxidation of gas or liquid chromatography carriers. The instrument will be automated, with a versatile digital interface for simple operation. Precision and accuracy in the few percent or better range are projected over several orders of magnitude $^{14}$C content. Performance metrics will depend upon sample size, radiocarbon content and measurement time.

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'Deuterium-enabled chiral switching' (DECS) and its application to the stabilization and differentiation of the enantiomers of thalidomide analogs

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‘Chiral switching’, the development of the single preferred enantiomer from a racemic mixture, emerged in the 1990s as a successful strategy to improve the therapeutic properties of marketed drugs (pharmacokinetics, pharmacodynamics, off-target side-effects). Representative chiral switches include: Prilosec® to Nexium®, Celexa® to Lexapro®, and Savella® to Fetzima®.

However, the enantiomers of certain compounds are chemically unstable and rapidly interconvert in vitro and in vivo. Thus today, numerous drugs, such as Actos®, Aricept®, Contrave®, Effient®, and Revlimid®, are still developed and marketed with racemic active ingredients. Using DECS, replacement of the exchangeable hydrogen at the chiral center with deuterium, we have shown that we can stabilize enantiomers for several classes of compounds including thalidomide analogs, therefore enabling their development as improved therapeutics.

We will present results obtained with the deuterated enantiomers of CC-122, a racemic drug currently in human clinical trials for hematological cancers and solid tumors. We will show that the in vitro antiinflammatory and in vivo antitumorigenic activity, in a NCI-H929 mouse xenograft model of multiple myeloma, of CC-122 is due almost exclusively to the stabilized (-)-enantiomer of CC-122 (DRX-254, believed to be the (S)-enantiomer) while, by contrast, the dramatically less potent stabilized (+)-enantiomer (DRX-255) has a negligible effect on tumor growth and may even exhibit a potential tumor growth promoting effect in the mouse model where the stabilized enantiomers were tested.

These results show that DECS can stabilize chiral compounds against epimerization and clearly differentiate the beneficial and potential detrimental effects of the racemic mixture while enabling adherence to stated regulatory guidance for the development of single enantiomers.

Figure 1: Structure of CC-122 (X = hydrogen or deuterium)
Poster abstracts
A glimpse of the deliveries and efficiency of the AstraZeneca Isotope Chemistry Group and the reactions used for isotopic label incorporation.

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The desire and need to constantly improve a group’s delivery to the business has always been important, but in the current environment of budgetary pressure, globalization, and rapidly advancing science, it is even more critical. As a first step in the improvement process, AstraZeneca Isotope Chemistry collected metrics on much of the work that is done in the group so that the areas for improvement could be understood and acted upon. The information collected includes the speed of deliveries by isotope and type of work, the reagents and catalysts used for isotope incorporating steps, and comparisons for internal and external deliveries. The group has acted upon some of the results of these measurements to improve the delivery time of internal tritiation and external stable isotope syntheses. This data presents a good snap shot into the functioning of the AstraZeneca Isotope Chemistry group.

Table 1. Deliveries grouped by activity

Table 2. Delivery time grouped by activity
The development of an tritium labeled tool compound for use in *in vivo* receptor occupancy studies

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Opioid receptors play an important role in the central nervous system; in particular in their ability to mediate analgesia. Three distinct receptor subtypes have been identified, and while all show a high sequence homology and all mediate analgesia, they do so in qualitatively distinct manners. Agonists of the delta opioid receptor have been the target of drug discovery efforts because they are expected to mediate pain similar to the mu receptors (morphine receptors) while avoiding some of the typical side effects associated with the mu receptor such as sedation, addiction, and respiratory depression. Recent evidence also links agonism of the delta opioid receptor to antidepressant and anxiolytic effects, and this has been validated in preclinical models. To better understand our drug candidates, a radioligand tool was desired. Many different compounds were tritiated and tested *en route* to compound 1 which was an effective radioligand for use *in vivo* in rats. The compound showed good brain uptake, good selectivity for the target regions in the brain, and was blocked by the selective delta opioid receptor antagonist, naltrindole.
Current FDA thinking on the drug development process emphasizes the early identification of drug metabolites which are either unique to man or occur at disproportionately higher levels in humans. To accomplish this task efficiently, Carbon-14 labelled drug substances play a crucial role and are produced in accordance with ICH Q7 Good Manufacturing Practice Guidance (GMP) for active pharmaceutical ingredients (Section 19), August 2001. At PerkinElmer we have been engaged in the GMP synthesis of Carbon-14 labelled compounds for several decades. During this time our GMP program has evolved in response to emerging changes for both synthetic and analytical aspects of GMP. This poster will review our program of Carbon-14 GMP manufacturing from the standpoint of chemistry, documentation, equipment, facilities, quality review and training.
SPECIFIC ACTIVITY DETERMINATION BY MASS SPECTROMETRY-LIQUID CHROMATOGRAPHY: HOW APPROPRIATE IS THE USE OF THE ISOTOPIC DISTRIBUTION PATTERN OF THE UNLABELED COMPOUND?

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Specific activity (SA) along with radiochemical purity and radioactive solution concentration are the commonly determined parameters for radiolabeled compounds. Mass spectrometry liquid chromatography (LC/MS) is the technique often employed in the determination of tracer specific activity since it is an efficient and easy to perform process. This process is more straightforward than measurement of SA from the radioactive concentration, determined by liquid scintillation counting, and the mass concentration, determined by UV-HPLC. In the latter method, a series of accurately known-concentration standards are needed to generate a UV standard curve. This requires that the purity of the unlabeled reference standard is known. In contrast, MS-based SA measurement does not require an unlabeled compound of known purity.

All MS-based SA measurements require an appropriate natural abundance isotopic distribution pattern. This pattern is used to correct the MS of the labeled compound for the known, though generally small, contributions from heavy isotopes of the constituent elements which are present at their natural abundances. This natural abundance correction is applied across the observed mass spectrum of the tracer to remove the summed contributions of natural abundance heavy isotopes (e.g. each C atom contributes ca. 1.1% of intensity to the next highest peak due to the $^{13}$C isotope present). After correction the ion intensities due to the introduced radioactive isotopes remain and are used to calculate the SA. Normally, either the observed mass distribution of the unlabeled compound or a theoretically generated distribution pattern is used for the above correction process.

Here we examine the validity and degree of problems associated with the use of the isotopic distribution of the unlabeled compound as the correction pattern during MS-based SA measurements. Examples using the analysis of highly isotopically enriched compounds will be presented.
DEUTERIUM LABELLING OF ALKYL NITROAROMATICS AT THE BENZYLC POSITIONS BY BASE-CATALYSED EXCHANGE WITH DEUTERIUM OXIDE.

Stephen Maddocks and William J. S. Lockley
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Nitrated alkylaromatics are an important class of compounds that are utilised as intermediates in high-volume industrial processes. This poster describes an approach to the regiospecific labelling of these systems by exploiting the differences in acidity between their aromatic and benzylic protons. In alkyl nitroaromatics the benzylic protons are rendered particularly acidic by virtue of the electron withdrawing nitro-substituents. Hence the compounds are amenable to labelling provided that the appropriate benzylic anion can be generated in the presence of a suitable deuterium donor. We have investigated this labelling approach using deuterium oxide as the isotope donor in the presence of various bases and aprotic cosolvents.

In the case of dinitro-alkylaromatics, a carefully selected tertiary amine base proved ideal, yielding, in the absence of oxygen, an effective labelling reaction at room temperature. E.g. 2,4-dinitrotoluene (pKa 15.3) exchanges easily, regiospecifically and with high atom% abundance under catalysis by triethylamine (pKa 10.8) without any of the expected complications due to the formation of the Meisenheimer complex.

Although the reaction of dinitro-systems is particularly facile, mono-nitro compounds with the alkyl substituent ortho or para to the nitro group can also be smoothly labelled using this approach provided that a stronger base, a longer reaction time and a higher temperature are applied. In this case a typical procedure utilises DBN base at 95°C degrees for 18h.

Overall, we have examined the relative labelling propensities of a range of alkyl mono-nitroaromatics and two alkyl dinitro-aromatics under various conditions. Our results show clear effects of the substitution pattern and of the stereochemical orientation of the nitro and alkyl substituents upon the extent and ease of labelling. The poster will discuss these effects in addition to providing efficient protocols for the labelling of both mono- and dinitro-systems.
DEUTERIUM-LABELLING OF BASE-SENSITIVE ALKYNES IN THE TERMINAL POSITION USING DEUTERIUM OXIDE: CATALYSIS BY SILVER-ION.

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As part of our continuing studies of the isotopic exchange and reduction of alkenes over platinum group metals we frequently required a source of terminally-labelled alkynes. Exchange-labelling was often employed for this purpose.

\[
\begin{align*}
R\cdots\text{H} & \overset{\text{Deuterium oxide}}{\xrightarrow{\text{Cosolvent}} } R\cdots\text{D} \\
\text{NaOD or Na}_2\text{CO}_3
\end{align*}
\]

Since the methine proton of terminal alkynes is acidic (pKa ~26-29) isotopic exchange with a suitable isotope donor in the presence of a strong base was an option and proved quite suitable for simple alkynes. Unfortunately this approach is incompatible with systems prone to hydrolysis or elimination, or where other sites are vulnerable to base-catalysed labelling. This restricts its use with acetylenic esters, ketones, phenols, sulfoxides, tosylates, etc.

The complexation of alkynes with metals results in a significant change in terminal proton acidity leading to enhancements in isotopic exchange rates of more than $10^5$. We have studied this exchange with a range of alkynes and potential metal catalysts. Of these, Ag$^+$-catalysis proved particularly effective and was completely regiospecific. Although this catalysis was reported previously,\(^1\)\(^2\) the methodology described was quite unsuitable for $^2$H or $^3$H-labelling purposes. Hence new methodology for Ag$^+$-catalysed labelling using isotopic water as the donor was developed.

Our studies have led to a reliable protocol for labelling alkynes in general and base-sensitive alkynes in particular. It is simple, high-yielding, rapid and takes place at ambient temperature. Typical exchanges are shown below for a simple alkyne and for two alkyne esters (high recoveries and a higher percentage labelling could be achieved by employing more D$_2$O).

LABELLING OF BENZYLICS VIA PALLADIUM-CATALYSED EXCHANGE USING A DEUTERIUM GAS OR SODIUM BORODEUTERIDE DONOR.
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As part of our continuing studies of regiospecific deuterium labelling of substituted aromatics we have re-examined the well-known Evans benzylic tritiation procedure. In this approach the benzylic methylene protons of alkylaromatics are labelled by exchange using an isotopic hydrogen donor and a palladium catalyst.

Our D₂ gas screening of a wide range of potential catalysts showed that regiospecific labelling was limited to palladium derivatives. The most effective catalyst found was Pearlman’s catalyst (palladium hydroxide on carbon). Labelling of the simple symmetric substrate 1,2-diphenylethane using this catalyst with deuterium gas gave the isotopic distribution expected for a random 4-site exchange process (Figure below at ca. 50% deuteration, expt. vs theory).

In the course of our screens we investigated a range of palladium salts and complexes, and found that palladium metal prepared from palladium trifluoroacetate, was particularly interesting. Changing the reductant from D₂ gas to NaBD₄ (at 1 mol/mol of Pd) in D₂O produced a catalytic system with stoichiometric as well as catalytic activity. Although both the NaBD₄ and the D₂O donated isotope to the substrate, the borodeuteride was by far the more effective source. Even in H₂O a substantial amount (ca. 40%) of the borodeuteride deuterium content was transferred to the substrate above, auguring well for the use of this catalytic system for tritium-labelling using sodium borotritide as donor.

As expected, the reaction rate may be enhanced by the addition of a carrier agent (C, Al₂O₃) to increase Pd dispersion, and by the application of higher temperatures.

Typical benzylic substrates were labelled as shown below. Also, as expected for a palladium catalyst, the methylxanthines caffeine and theophylline could also be labelled.
The Synthesis of $[^{14}C]$Trifluoroacetic acid derivative

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While substances with low boiling points are readily synthesized, a true difficulty lies in the work-up, purification and identification process of these materials. These chemicals are in gaseous state in room temperature, making their collection very challenging. Furthermore, when the chemical is inactive to the UV light, it poses additional obstacles for identification.

$[^{14}C]$Trifluoroacetic acid, $[^{14}C]$N-Methyl-2,2,2-trifluoroacetamide, $[^{14}C]$1,1,1-Trifluoroacetone, $[^{14}C]$1,1,1-Trifluoro-2-propyl alcohol; these 4 substances have very low boiling points between 21~150 °C and are inactive to the UV light. In particular, $[^{14}C]$Trifluoroacetic acid is not detectable to H-NMR, making its identification almost impossible. However, this study successfully synthesized, purified and identified the 4 materials mentioned above. Experiments were conducted in vacuum using Manifold and by employing RI-monitor HPLC, H-NMR and LC/MS and their purity levels were correctly identified.

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| CF₃CO₂H | F₃C\text{\text{-\text{NH}} } | F₃C\text{\text{-\text{O}} } | F₃C\text{\text{-\text{OH}} } | \text{[^{14}C]} |
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References

SYNTHESIS OF ISOTOPE-LABELED PLK1 INHIBITOR: \(^{2}\text{H}\) AND \(^{14}\text{C}\)-MLN0905

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Polo-like kinase 1 (PLK1) is a serine/threonine protein kinase that plays a key role in cell-cycle control. PLK1 is overexpressed in numerous cancers including diffuse large B-cell lymphoma (DLBCL).\(^1\) MLN0905 is an experimental, potent and selective inhibitor of PLK1. The stable isotope labeled MLN0905 was required for bioanalytical studies. \(^{2}\text{H}\)\text{4-MLN0905} (1A) was synthesized in 5 steps by gradual treatment of 2 with deuterium gas, followed by coupling with the previously published intermediates 4 and 5.\(^2\)

For \textit{in vitro} and \textit{in vivo} studies, we prepared \(^{14}\text{C}\)-MLN0905 (1B) in a 4-step synthetic route. Commercially available \(^{14}\text{C}\)-cyanamide (6) was treated with hydrogen chloride in ether to generate the labeled chloroformamidine hydrochloride 7.\(^3\) The radiosynthesis was then completed in a similar manner as described above.

References
Isotopic Resonance Hypothesis: Experimental Verification by *Escherichia coli* Growth Measurements

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Isotopic composition of reactants affects the rates of chemical and biochemical reactions. As a rule, enrichment of heavy stable isotopes leads to progressively slower reactions. But the recent isotopic resonance hypothesis suggests that the dependence of the reaction rate upon the enrichment degree is not monotonous. Instead, at some “resonance” isotopic compositions, the kinetics increases, while at “off-resonance” compositions the same reactions progress slower1–3. To test the predictions of this hypothesis for the elements C, H, N and O, we designed a precise (standard error ±0.05%) experiment that measures the parameters of bacterial growth in minimal media with varying isotopic composition. A number of predicted resonance conditions were tested, with significant enhancements in kinetics discovered at these conditions. The combined statistics extremely strongly supports the validity of the isotopic resonance phenomenon (p<<10^{-15}). This phenomenon has numerous implications for the origin of life studies and astrobiology, and possible applications in agriculture, biotechnology, medicine, chemistry and other areas.

Synthesis of Isotopically Labeled Dabigatran and Dabigatran Etexilate

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Abstract:

Dabigatran is a reversible, competitive, direct thrombin inhibitor (factor IIa). It is used to prevent strokes in patients with atrial fibrillation (AFib) and the formation of blood clots in the veins (deep venous thrombosis) in adults who have had an operation to replace a hip or a knee. Dabigatran is a very polar compound and not suitable for oral administration to patients. Thus the double prodrug dabigatran etexilate (Pradaxa) is used instead for oral intake. Dabigatran etexilate was the first FDA-approved alternative to warfarin. It is converted to dabigatran, the active drug, by the ubiquitous esterases in the human body. Herein, the synthesis of dabigatran and dabigatran etexilate labeled with carbon13, carbon14 and tritium is described.
**A Novel Five-Lipoxygenase Activity Protein Inhibitor Labeled with Carbon-14 and Deuterium**

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**Abstract:**

2-[4-(3-\{(1R)-1-[4-(2-Aminopyrimidin-5-yl)phenyl]-1-cyclopropylethyl}-1,2,4-oxadiazol-5-yl)-1H-pyrazol-1-yl]-N,N-dimethylacetamide (1) is a novel and selective five-lipoxygenase activity protein (FLAP) inhibitor with excellent pharmacokinetics properties. The availability of a key chiral intermediate from in house synthesis, allowed the synthesis of [14C]-\(1\) in just six radiochemical steps and in 47% overall radiochemical yield with a specific activity of 51 mCi/mmol using carbon-14 zinc cyanide. 2-Chloro-N,N-dimethyl-\(^2\)H\(_6\)-acetamide was prepared and condensed with a penultimate intermediate to give \([^2\)H\(_6\)](1) in very high yield and in more than 99% isotopic enrichment.
A new methodology of tritium labelling through SP3 CH bond activation

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A new methodology of organoiridium catalyst (Crabtree’s catalyst) mediated isotopic hydrogen exchange was developed at BMS for tritium labelling. Isotopic exchange occurred at a sp3 carbon center without the participation of an aromatic ring structure and the chiral structure of the carbon center was retained during the exchange.
THE TRITIUM LABELING OF OLIGONUCLEOTIDES AND A BIODISTRIBUTION STUDY IN MOUSE

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Stephen Harris, Claire Henson, Andrew McEwen, Kathryn Sweeney, Stuart Wood: Quotient Bioresearch (Rushden) Ltd, Pegasus Way, Crown Business Park, Rushden, Northamptonshire, NN10 6ER, UK.

Interest in the development of oligonucleotides drugs has increased in recent years. This has resulted in an increased demand for radiolabelled oligonucleotides. This poster outlines some of the ways that Quotient chemists have labelled oligonucleotides with tritium. A number of methods including bromine-tritium exchange, tritiated water exchange, radio-tag with $[^3]$H]-N-succinimidyl propionamide ($[^3]$HNSP) and enzymatic labelling are discussed. Tritiated oligonucleotides can be used in biodistribution studies and an example is given in the poster.
The compound MMV390048, an aminopyridine derivative from Medicine for Malaria venture and H-3D center (UCT), exhibited promising in vitro activities against different *P. falciparum* K1 and NF54, 3D7 and Dd2. Preliminary in vivo studies on this aminopyridine derivative showed that it has a potential to be used as a single oral dose for malaria treatment. Moreover, it has shown that it has the potential to block transmission of the malaria parasite. The purpose of this work was to radiolabel MMV390048 with the carbon-14 isotope and to perform the tissue distribution studies of this compound in rats with particular emphasis to the distribution to the skin and eyes. The key steps to the synthesis of MMV390048 are highlighted in Scheme 1. The synthesis of 1 started from bromothiophenol (2), which was converted to the sulfone 4. Finally, the carbon-14 labeled 1 was assembled through the Suzuki-Miyaura reaction of 4 with the boronate ester 5 in good yields.

**Scheme 1.** Carbon-14 radiosynthesis of MMV390048.

The radiolabel 1 was tested in vivo in rats (Long_Evans and Sprague-Dawley rats) in order to determine its tissue distribution profile. Tissue distribution results demonstrated high local exposure in the GI and excretory organs but low exposure of all other tissues. There were minor differences in tissue exposure between pigmented and non pigmented rats but no significant differences in melanin containing tissues. Therefore, the recorded data suggests that radiotracer 1 can proceed to clinical evaluation.

**References**

Synthesis of C-14 Labeled (S)-Ketamine and Three Ketamine Metabolites

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Abstract
Ketamine is a well-known general anaesthetic that inhibits cerebral NMDA receptors. A nasal spray formulation of (S)-ketamine, the S enantiomer of ketamine or esketamine, is under development for the management of treatment resistant depression. The synthesis of C-14 labeled ketamine was achieved in five steps from cyanation of 1-chloro-2-iodobenzene with $[^{14}C]$CuCN and subsequent conversion in 24% overall radio chemical yield. The chiral resolution with L-(+)-tartaric acid, followed by salt exchange, resulted in $[^{14}C]$(S)-ketamine hydrochloride in 25% radio chemical yield and 99% ee. Three hydroxylated ketamine metabolites were also targeted in the study. Efficient syntheses of (Z)-6-hydroxy ketamine, (Z)-6-hydroxy-(S)-norketamine and its reduction product, dihydro-6-hydroxy-(S)-norketamine were developed. Amino groups in ketamine and (S)-norketamine precursors were protected as trifluoroacetamide during the transformations. Deprotection conditions in the final steps were critical to attenuate a major competing side reaction; elimination and formation of unsaturated ketone. Relative configuration / chirality of the newly introduced chiral centers in the metabolites were determined by NMR analysis.
ENZYMATIC SYNTHESIS OF $^2$H- AND $^3$H-LABELLED HALOGEN DERIVATIVES OF AROMATIC AMINO ACIDS AND BIOGENIC AMINES

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Aromatic amino acids and bioorganic amines play a very important role in many physiological processes. L-tryptophan, L-tyrosine and tyramine are intermediates in the biosynthesis of neurotransmitters, vitamins and hormones. The disturbance in their production and metabolism leads to many pathologies such as allergy, Alzheimer’s and Parkinson’s, and many other neurodegenerative diseases.

Halogen derivatives of amino acids and bioamines are often used in medical diagnostics. The mechanisms of their actions is not fully understood since the presence of halogen in their structure causes a change in the catalytic activity of enzymes involved in their degradation. For this reason we have developed the synthesis of halogen-labelled amino acid derivatives and aromatic biogenic amines to investigate mechanisms of their biodegradation using kinetic and solvent isotope effect methods.

We have elaborated and obtained of several isotopomers and isotopologues of halogen derivatives of L-phenylalanine, L-Phe, L-tryptophan, L-Trp, and tyramine, TA, selectively labelled with deuterium and tritium in the side chain. Using as a label source the deuteriated and tritiated water, the following chemical species were synthesized:

Isotopologues of L-phenylalanine, i.e.
2-chloro-[(3S)-$^2$H]-L-Phe, 2-chloro-[(3S)-$^3$H]-L-Phe, and 2-chloro-[(3S)-$^2$H/$^3$H]-L-Phe;

Isotopomers of L-tryptophan, i.e.
5’-chloro-[2-$^2$H]-L-Trp, 5’-chloro-[2-$^3$H]-L-Trp, and 5’- chloro-[2-$^2$H/$^3$H]-L-Trp;

Isotopologues of tyramine, i.e.
3’-fluoro-[(1R)-$^2$H]-TA, 3’-fluoro-[(1R)-$^3$H]-TA, 3’-fluoro-[(1S)-$^2$H]-TA, and 3’-fluoro-[(1S)-$^2$H/$^3$H]-TA;
3’-chloro-[(1R)-$^2$H]-TA.
3’-iodo-[(1R)-$^2$H]-TA and 3’-iodo-[(1R)-$^3$H]-TA.

The isotopologues of L-Phe were synthesized by addition of ammonia to the cinnamic acid derivative n the presence of enzyme Phenylalanine Lyase, PAL, (EC 4.3.5.1), carried out in deuteriated and tritiated incubation media. The isotopomers of L-Trp were obtained by condensing 5-chloroindole and S-methyl-S-cysteine, catalyzed by the enzyme Tryptophanase, TPase, EC 4.1.99.1. Generally, the labeled isotopologues of tyramine, TA, were obtained by enzymatic decarboxylation of theirs amino acids precursors prepared earlier using enzymatic methods.
CARBON-14 LABELLING OF ANTIBODY DRUG CONJUGATES

Mike Cannarsa, Sean L. Kitson, Tom S. Moody, David J. Speed, William H. Watters, Department of Biocatalysis and Isotope Chemistry, Almac Sciences, 20 Seagoe Industrial Estate, Craigavon, BT63 5QD, Northern Ireland, UK.

The radiolabelling group at Almac have synthesised a number of Antibody Drug Conjugates (ADCs) labelled with carbon-14. These radiolabelled materials are required for key ADME studies to identify the major catabolites formed in circulation. The position of the radiolabel is typically either on the linker unit or drug component. This poster provides an overview of the synthesis of radiolabelled ADCs and the major steps involved in their preparation and purification.

Figure 2: carbon-14 labelled ADC generic structure
GREATER ENDOGENOUS AND EXOGENOUS DHA AND DPAN-6 IN FEMALE RAT PLASMA WERE INDEPENDENT OF THE STATUS OF DIETARY LINOLENIC ACID

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Human studies and some animal work have shown a gender difference in PUFA compositions and metabolism. More arachidonic acid (ARA) and docosahexaenoic acid (DHA) was accumulated or converted in female subjects than in male. This study explored gender differences in fatty acid composition and PUFA metabolism in rats consumed diets containing 10 wt% fat with 15% linoleate and either 3% (LA+ALA diet) or 0.05% linoenate (LA diet). Stable isotope tracer technique coupled with gas chromatography/mass spectrometry negative chemical ionization assay was employed. Adult rats were orally administered one single dose of a mixture of deuterium labeled linoleic (d5-LA) and α-linolenic acids (d5-ALA). Caudal venous blood was drawn at 0, 2, 4, 8, 12, 24, 48, 96 and 168 h afterwards. The deuterated precursors and their metabolites in plasma total lipids were quantitated by GC/MS negative chemical ionization and the endogenous fatty acids by GC/FID analysis. On a per dosage basis, female rats accumulated more deuterated PUFA in plasma than in male rats, including deuterated n-6 docosapentaenoic acid (d5-DPAn-6), d5-ALA, d5-20:5n-3, and d5-DHA. For the endogenous PUFA, greater concentrations of DHA and DPAn-6 were observed in female rats compared to the male. On the other hand, a changed, lower concentration of arachidonic acid was observed only in female rats fed LA+ALA diet. In summary, female rats accumulated a higher concentration of DPAn-6 and DHA in the plasma than the male rats and this is reflected in the in vivo fatty acid metabolism of deuterated precursors in both dietary LA+ALA or LA diets. This study indicated that the dietary 18:3n-3 showed no significant effect on the accumulation of DHA and DPAn-6. However, in comparison to the male rats, the female rats presented a greater sensitivity and accommodation to deficiency of the dietary 18:3n-3.
A NEW METHOD FOR SYNTHESIZING ISOTOPE LABELED CARNITINE
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Carnitine deficiency is a related mitochondrial and not a rare disease. It may occur when carnitine is inadequate intake, reduced in synthesis by body, or blocked in absorption again by kidney. Carnitine deficiency can be cured after diagnosed, and can be diagnosed by isotope tracing technology together with LC-MS/MS. So, it is useful to establish a convenient route to synthesize isotope labeled carnitines.

We reported here a new method for synthesizing isotope labeled carnitine. As shown in the scheme below, the isotope labeling is happened only in the last step, so the expensive isotope starting material is highly utilized. It is pretty convenient in operations, and economic in cost. The isotope labeled carnitine can be used as an important starting compound to synthesize many isotope labeled carnitine derivatives.

Commercially available carnitine 1 was used as starting material to form (S)-4-(dimethylamino)-3-hydroxy-N-(2-hydroxyethyl) butanamide 2 by refluxing with 2-amaioethanol, and (S)-4-(dimethylamino)-3-hydroxybutanoic acid hydrochloride 3 was obtained for further reflux with 6N hydrochloric acid, with 78% yield for the two steps. The isotope labeled carnitine 4 was synthesized from 3 with isotope labeled iodo methane in DMF or DMSO or acetonitrile with 79% yield.
POTENTIOMETRIC STUDIES OF $^{117m}$Sn-POLYHEDP AND $^{177}$Lu-POLYHEDP: IN ATTEMPTS TO DEVELOP EFFECTIVE RADIOPHARMACEUTICALS FOR BONE METASTASES.

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Secondary cancer tumour formation, often called metastases, remains one of the great scientific challenges in public health. Therapeutic radiopharmaceuticals for bone pain palliation play an important role in providing quality of life for cancer patients with metastatic bone cancer. They comprise of a bone seeking bisphosphonate ligand and a radionuclide. The structural variations of the bisphosphonate affects to a great extend the effectiveness of the radiopharmaceutical with the greatest shortfall being bioavailability. A polymer of the ligand HEDP, viz. poly-HEDP was successfully synthesized from its free acid form in relatively low yields. This study seeks to understand the in vivo chemistry of the $[^{117m}$Sn]$\text{Sn(II)}$, $[^{117m}$Sn]$\text{Sn(IV)}$, and $[^{177}$Lu]$\text{Lu(III)}$ complexed with poly-HEDP in blood plasma. The formation constants of Sn(II), Sn(IV), and Lu(III) were measured by glass electrode potentiometry at 25 $^\circ$C and $I = 150$ mM. This made possible the construction of a blood plasma model of poly-HEDP, determined with the aid of thermodynamic blood plasma modeling simulations. The Sn(IV)-poly-HEDP complex was shown to be unstable, with a 100 % dissociation, while Sn(II)-poly-HEDP showed much improved stability with 10 % of the metal ion remaining bound to the ligand. However, 97% of the Lu(III) remained complexed to the ligand with less competition from the physiological ligands in vivo.
Palladium-catalyzed Br/D exchange of arenes: Selective deuterium incorporation with versatile functional group tolerance and high efficiency
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Abstract:
Selectively-deuterated compounds have been increasingly attracting attention and interest in areas such as metabolic or pharmacokinetic probes both in vitro and in vivo; drug discovery and development; and exploration of advanced functional materials. Deuteration is also a powerful tool for the study of structure and dynamics in soft matters through neutron scattering. The synthesis of selectively deuterated compounds often requires multiple steps, sometimes under harsh conditions, such as strong acid or base, and high temperature and/or high pressure. Accordingly, functional group tolerance has been a major challenge. In this presentation, we report a facile method for introducing one or more deuterium atoms onto an aromatic nucleus via Br/D exchange. Our method provides introduction of deuterium at selected positions in high atom percentage deuterium incorporation with excellent tolerance to a wide range of functional groups. We believe this approach will facilitate the synthesis of more complicated deuterium-labeled compounds through further modification of the functional groups.

FG including ester, ketone, nitrile, nitro, amine, aldehyde, alkene, boronic acid, chloride or sulfonamide

Yield: 65%-95%
deuteration incorporation:>98%

Acknowledgements: This research was conducted at the Center for Nanophase Materials Sciences, which is a DOE Office of Science User Facility.
COMPARISON of CARBON-13 and TRITIUM NMR BASED SPECIFIC ACTIVITY MEASUREMENT to UV with RADIOACTIVE CONCENTRATION and to MASS SPECTROMETRY APPROACHES

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Labeled compounds containing tritium and carbon-14 are used across the stages of drug development to identify biological targets, understand preclinical species and human metabolism (PK/PD), and to determine environmental impact. Specific activity is measured for each tracer batch since this value is required by researchers to determine what level of carrier dilution is needed to achieve the targeted mass concentration of the assay.

There are several different methods to determine specific activity including UV mass concentration with radioactive solution concentration, mass spectrometry (MS) determined isotopic distribution, NMR analysis, and gravimetric radioactive content. Each assay method has advantages and disadvantages. In some cases a given approach may fail due to the chemical properties of the compound such as when a compound does not ionize under standard MS conditions. In the case of UV-based specific activity an unlabeled standard for each compound must be available at a known purity to establish the required UV standard curve.

A comparison of these different methods as applied to a series of compounds will be presented. Cases including different specific activities for the same compound will be included. Additionally, further investigations into the use of NMR analysis as applied to specific activity measurement will be described.
IRON-CATALYZED HYDROGEN ISOTOPE EXCHANGE IN DRUG MOLECULES

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Poster abstract:
Bis(imidazolylidine)pyridine iron dinitrogen compounds, (iPrCNC)Fe(N₂)₂ and (iPrH₄-CNC)Fe(N₂)₂ have been synthesized and fully characterized as Fe(0) compounds where the supporting pincer acts a classical π-acceptor. Because of the catalytic alkene hydrogenation activity of (iPrCNC)Fe(N₂)₂, the reactivity of these compounds with H₂ and D₂ was explored. These investigations revealed facile isotopic exchange between the free gas and arene and heteroarene C-H bonds under mild conditions in solution with highly predictable site selectivity. In full collaboration with Merck, the application of these catalysts to the radiolabeling of various pharmaceutically relevant compounds has been explored. Unprecedentedly high levels of tritium incorporation were observed in several cases with selectivity distinct from known precious metal catalyst technology. The mechanism of action, functional group compatibility and potential utility in the ADME sequence will be presented.
SYNTHESIS OF DEUTERIUM LABELED $\beta$-AMINOBUTYRIC ACID AND USE AS INTERNAL STANDARD IN UPLC-MS/MS ANALYSIS OF PLANT EXTRACTS

Damien Thevenet$^{1,2}$, Victoria Pastor$^1$, Armelle Vallat$^3$, Prof. Reinhard Neier$^2$, Gaetan Glauser$^3$, Prof. Brigitte Mauch-Mani$^{1,*}$

1. Laboratory of Molecular and Cell Biology, University of Neuchâtel, Switzerland
2. Laboratory of Organic Chemistry, University of Neuchâtel, Switzerland
3. Neuchâtel Platform of Analytical Chemistry, University of Neuchâtel, Switzerland

The basal immune system of a plant can be primed for increased defence, resulting in an augmented stress resistance. Among the synthetic chemical priming agents, $\beta$-aminobutyric acid (BABA) has a special place due to its very broad range of action.[1,2] This non-proteinogenic amino acid has a stereospecific inducing capacity since only the $R$ isomer is active in plants.[3] Recently, the presence in Arabidopsis of a specific BABA receptor protein, IBI1, has been reported.[4]

$[^2H_3]$- and $[^2H_6]-(\pm)$-3-aminobutyric acid (BABA-d$_3$ and -d$_6$) were synthesized de novo starting from the commercially available $[^2H_3]$- and $[^2H_4]-(\pm)$-alanine, respectively. The synthetic approach is based on the Arndt-Eistert homologation methodology.[5,6] In an effort to monitor the fate of BABA applied externally to plants and to determine the resulting concentrations in the plant tissues, we developed a selective and sensitive UPLC-MS/MS method based on stable isotope quantification. Moreover, our protocol unequivocally separate BABA from its two isomers $\alpha$- and $\gamma$-aminobutyric acid (AABA and GABA).

Application of phosphor imaging to study the plant uptake and distribution of agrochemicals

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Abstract
The uptake, movement and systemicity of an agrochemical are essential to understand its intrinsic attributes and relation to properties such as soil mobility, plant mobility and residual characteristics. In previous studies, phosphor imaging has been utilized to investigate the plant uptake of agrochemicals by soil application as well as redistribution and translocation by foliar application. Recently, there has been an increasing need to understand the uptake, movement, and distribution of agrochemicals from treated seed through germination to early development which helps explain the intrinsic activity of seed treatment technology against early season root and foliar pests. This presentation highlights an analytical methodology and technique to investigate active movement from treated seed using radiolabeled material.
THE SYNTHESSES OF ISOTOPICALLY LABELED CB-1 ANTAGONISTS FOR THE TREATMENT OF OBESITY

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BMS-725519, BMS-811064 and BMS-812204 are potent and selective central cannabinoid receptor antagonists (CB-1) that were being investigated for the treatment of human obesity. To further understand their biotransformation profiles, radiolabeled and stable-labeled analogs were required. This poster will discuss the utility of [14C]1,1-carbonyldiimidazole (CDI) as a radiolabeled reagent for the labeled carbonyl syntheses of [14C]BMS-725519, [14C]BMS-811064 and [14C]BMS-812204. The syntheses of stable-labeled [13C6]BMS-725519 and [13CD2,13CD3]BMS-812204 involving the incorporation of [13C6]4-chloroacetophenone and [13CD3,13CD2]iodoethane, respectively, will also be discussed.
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35 Spring St., 609-921-0404

Despaña (Spanish)
235A Nassau St., 609-921-2992

Efes Mediterranean Grill
235B Nassau St., 609-683-1220

elements (interpretive American cuisine)
163 Bayard Lane (Route 206), 609-924-0078

Fruity Yogurt & Cafe
166 Nassau St., 609-921-8787

George's Roasters & Ribs (takeout)
244 Nassau St., 609-252-0419

Halo Pub (ice cream, coffee)
9 Hulfish St., 609-921-1710
Hoagie Haven (takeout)
242 Nassau St., 609-921-7723

Hunan Chinese Restaurant
157 Witherspoon St., 609-921-6950

Infini-T Café and Spice Souk (tea room)
4 Hulfish St., 609-454-3959

Jammin Crepes’
20 Nassau Street, (609) 924-5387

La Mezzaluna (Italian)
25 Witherspoon St., 609-688-8515

Mamoun’s (falafel)
20 Witherspoon Street 609-454-5936

Masala Grill (Indian)
19 Chambers St., 609-921-0500

Massimo’s (casual Italian)
110 Nassau St., 609-924-0777

Mediterra (Mediterranean)
29 Hulfish St., 609-252-9680

Méhé (Indian)
164 Nassau St., 609-279-9191

Mistral (bistro)
66 Witherspoon St., 609-688-8808

MoC MoC Sushi (traditional Japanese)
14 South Tulane St., 609-688-8788

Naked Pizza (takeout)
180 Nassau St., 609-924-4700

Nassau Sushi (Japanese and Korean)
179 Nassau St., 609-497-3275

Olives (gourmet deli)
22 Witherspoon St., 609-921-1569

Palace of India
235A Nassau St., 609-688-8923

Panera Bread Bakery and Café
136 Nassau St., 609-683-5222

The Peacock Inn Restaurant (contemporary American cuisine)
20 Bayard Lane (Route 206), 609-681-1707
PJ's Pancake House (pancakes, eggs, sandwiches, burgers)
154 Nassau St., 609-924-1353

Princeton Pi (Iano's)
86 Nassau St., 609-924-5515

Princeton Soup and Sandwich Company (soups, salads, paninis)
30 Palmer Square East, 609-497-0008

Qdoba Mexican Grill
140 Nassau St., 609-921-2031

Red Onion (deli)
20 Nassau St., 609-924-6667

Rojo’s Roastery
33 Palmer Square West

Sakura Express (Japanese)
43 Witherspoon St., 609-430-1180

Say Cheez
183 Nassau St (609) 924-2454

Sketch-Yogurt Without Limits (frozen yogurt)
84 Nassau St., 609-454-3057

Slice Between (pizza)
242 Nassau St., 609-683-8900

Small World Coffee
14 Witherspoon St., 609-924-4377; 254 Nassau St.

Starbucks Coffee Company
100 Nassau St., 609-279-9204

Subway
18 Witherspoon St., 609-924-5063

Tandoori Bite (Indian)
36 Witherspoon St., 609-385-0169

Taste of Mexico
180 Nassau St., 609-924-0500

Teresa's Pizzetta and Caffé
21 Palmer Square East, 609-921-1974

Thai Village
235 Nassau St., 609-683-3896

Tiger Noodles (Chinese)
260 Nassau St., 609-252-0663
Tomo Sushi  
236 Nassau St., 609-924-8478

Tortuga's Mexican Village  
41 Leigh Ave., 609-924-5143

Triumph Brewing Co.  
138 Nassau St., 609-924-7855

J. B. Winberie  
1 Palmer Square East, 609-921-0700

The Witherspoon Grill (steakhouse)  
57 Witherspoon St., 609-924-6011

Yankee Doodle Tap Room  
The Nassau Inn, 10 Palmer Square, 609-921-7500

Zorba's Brother Restaurant (casual bistro-style Greek diner)  
80 Nassau St., 609-279-0999
Notes