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International Isotope Society

Abstracts of the
TWELFTH CENTRAL US MEETING
Kalamazoo, Michigan. USA.
Uses and Applications of Isotopes in CNS Research
May 20-21, 1999

Meeting Summary:

The Twelfth Meeting of the IIS Central US Chapter was held on May 20-21, 1999 at the Radisson Plaza Hotel in Kalamazoo, Michigan, USA. The meeting committee included co-host Wayne Stolle and Ashok Chaudhary, John Easter, James McGrath, Alan Walker, and Larry Wienkers (Pharmacia & Upjohn). Attendance at the meeting was excellent; 100 registrants included representatives from the major regional pharmaceutical companies, universities, vendors and contract houses, and agricultural/chemical companies. The program was divided into four sessions, following a theme of "Isotopes in CNS Research". General sessions covering "New Techniques with Isotopes", and the "Use of Isotopes in Synthesis, Metabolism, and Residue Analysis" were also included to attract as wide an audience as possible. The format chosen allowed for fourteen accepted oral presentations and one plenary lecture given by Dr. Dean F. Wong of Johns Hopkins Medical Institutions. A traditional Thursday evening reception promoted casual interaction among the registrants, discussion with the seven poster presenters, and time to browse the ten vendor exhibits.

A short chapter business meeting following lunch on Friday was brought to order by President Lennon McKendry (Dow AgroSciences). Following chapter by-laws, Bradley Keck (Procter & Gamble) will become President for the year 2000, and Kennedy O'Brien (NEN Life Science Products) was elected to replace Dr. Keck as Secretary. Surendra Gupta (American Radiolabeled Chemicals) will continue as

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chapter Treasurer through the year 2000. A number of new initiatives for this year's meeting were reviewed: preparation of an electronic mailing list for future meetings; posting of the current chapter membership on the IIS web page; the opportunity to pay meeting registration fees by personal or corporate credit card (83% of the registrants took advantage of this); and publication of the meeting abstracts in the Journal of Labelled Compounds and Radiopharmaceuticals. Next year, because of the June international IIS meeting in Dresden, Germany; the IIS Central US Meeting will be held in September 2000, in Chicago, Illinois (USA), hosted by Gary Rotert (Abbott Laboratories) and colleagues.

Contents:

Session III **Isotope Techniques in CNS Research** Chair: J. Easter

E.D. Hostetler (Washington Univ.) : "Fluorine-18 And Carbon-11 Labeled Radiopharmaceuticals For Positron Emission Tomography"
*__A.G. Chaudhary,L.C. Wienkers__ (Pharmacia & Upjohn) : "The Use of an In Vitro Inhibition Screen for the Identification of Potent CYP2D6 Inhibitors in CNS Drug Discovery"
P. Ramm (John Izzo) (Imaging Research Inc.,Brock Univ.) : "Evolution of Imaging Systems in Scintillation Detection"

Session IV **Isotopes in Synthesis, Metabolism, and Residue Analysis**
Chair: A. Walker

N. Castagnoli (Virginia Tech.) : "Single Electron Transfer: Enzyme Catalysis And Chemical Model Studies Using Deuterium Tetrahydropyridinyl Substrates"
R.E. Maleczka (Michigan State Univ.) : "Advancing Wittig Rearrangement Chemistry Via Deuterium Labeling"
J.G. Slatter (Pharmacia & Upjohn) : "Not Your Average Clinical Radiotracer Trial: Disposition Of Intravenous [^{14}C]Camptosar In Human Cancer Patients"
N.R. Pearson (Dow AgroSciences) : "The Development of a New Route to Prepare Carbon-14 Labeled XDE-350 (1), a Herbicide Used to Control Grass and Broadleaf Weeds in Corn"
C.T. Peng (NEN Life Science Products) : "Synthesis Of ^{14}C-Labeled Pesticides And Their Intermediates"

* Abstract Unavailable

Stable Isotopes: Applications in Combinatorial Chemistry

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Using Combinatorial Chemistry to create large collections of compounds for screening has become a major activity of organizations, particularly pharmaceutical companies, that are engaged in drug discovery.

Development of the solid phase chemistry often used in Combinatorial Chemistry requires following reactions of small molecules while still attached to polymer beads. Specialized NMR techniques as well as IR methods have been developed that take advantage of the properties of stable isotope labeling. Some examples from the published literature will be discussed.

Combinatorial Chemistry carried out by the "split synthesis" technique (or "one bead-one compound" method) requires an encoding method in order to identify bead-bound compounds of interest. Methods will be discussed by which stable isotope labeling has been used to encode Combinatorial libraries.

In a varient of Combinatorial Chemistry known as "SAR by NMR", 15N-labeled proteins have been used to screen compound collections for ligands that bind strongly to the protein. Some examples of drug discovery by this method will be discussed.

Uniform Tritium Labeling of Combinatorial Libraries for Affinity Selection Screening

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Charlotte Woodall□,
Yuanwei Chen□, Lin Yi□, Irini Zanze□, and Ye Yao*
*Department of Metabolism, Radiochemistry and Cellular Toxicity
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Abbott Laboratories, D46V, AP9, 100 Abbott Park Road, Abbott Park, IL, 60064-6114.

In high-throughput affinity selection screening, mixtures of compounds are passed through a column containing a stationary protein target. Compounds of high affinity remain bound to the protein target while weaker binders are washed away. This is useful for screening large mixtures of compounds such as mix and split combinatorial libraries when the small amount of individual compounds can be detected. One way to facilitate detection is to label every compound in the library.[1] Herein is described the labeling of 50x50x50 mix and split libraries (50 pools of 2500 compounds each) that were created on solid phase support beads such that the final step before cleavage from the bead was a simple reaction with a tritium-labeled reagent. The labeling chemistry was developed using single-compound models. After the labeling reaction, the products were washed with a standard battery of solvents and dried. Samples were then cleaved to determine the

[1] Bruce Beutel, Annual Reports in Medicinal Chemistry-32, 1997, Sect. VI, George L. Trainor, ed., Chapter 26: Discovery and Identification of Lead Compounds from Combinatorial Mixtures, pp.261-268.

extent and quality of reaction by scintillation counting, radio-HPLC, and mass spectroscopy. To date, two combinatorial libraries have been uniformly labeled for affinity selection screening using solid phase synthetic procedures. One was labeled by acetylation of 2° amines (10mg pools at 0.5μmol/mg) using PyBroP-activated CT_3COOH (25mCi per pool at 7.9Ci/mmol) in DCM (0.5mL) in the presence of DIEA to yield beads that were labeled at 86μCi/mg on average (4% yield, cleaved). Reactions were terminated after 2h at room temperature to avoid reactions that resulted in an unacceptable level of polar radioactive impurities. Secondly, a library of sulfonamides (10mg pools at 0.5μmol/mg) was N-methylated with CT_3I (500mCi/pool at 8Ci/mmol) overnight at room temperature in DCM (1mL) in the presence of an excess of DIEA to give beads at 1300μCi/mg on average (32% yield). Model reactions with $NaBT_3CN$ reductive methylation of amines and $NaBT_4$ ketone reduction also showed promise as means of creating libraries of compounds uniformly tritium-labeled. Thus, models of 3 different libraries containing 2° amine functionality (10mg of beads at ~0.5μmol/mg) were each treated with 37% formaldehyde (50μL) and acetic acid (15μL) in DMA (300μL). This was followed by a solution of $NaBT_3CN$ (300mCi at 13Ci/mmol) in DMA (0.5mL) and the reactions were shaken overnight to give beads labeled at 538, 834, and 1134μCi/mg (25, 38, and 52% yields, res.). Finally, two models of a ketone library (10mg each at 0.55μmol/mg) suspended in dioxane (0.4mL) each received 500mCi of $NaBT_4$ (71Ci/mmol) as a solution in 0.1M NaOH (120μL). These reactions resulted in beads labeled at 3.47 and 4.26mCi/mg (35 and 44% yields, res.) Scaffold structures cannot be revealed at this time.

ULTRASONIC $^{1}H \rightarrow ^{2}H$ ISOTOPIC EXCHANGE IN CARBOHYDRATES: STEREOSPECIFICITY AND REGIOSELECTIVITY

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In the studies of biosynthetic pathways and the cryptic stereochemistry involved in enzyme reactions, deuterated and tritiated carbohydrates play an important role; isotopic labels serve as convenient probes into the molecular organization and structural dynamics of micelles and cellular membranes. Labeled compounds also are useful for conformational analysis of oligio and polysaccharides, as well as for glycoproteins, glycolipids, and cardioglycosides, in alleviating resonance overlap in proton NMR spectroscopy; dipolar spin-coupling between ^{2}H and ^{13}C may be invaluable for unambiguous assignment of ^{13}C resonances.

We have continued our development of an ultrasonically mediated, stereospecific $^{1}H \rightarrow ^{2}H$ catalytic exchange technique using Raney nickel® catalysts in a heterogeneous reaction. This technique is facile, employs very mild reaction conditions, and is tolerant of thermally (and often chemically) sensitive moieties. Since epimerization, racemization, and degradation are absent, product isolation is generally quantitative.

The number of successfully labeled substrates, encompassing a wide variety of functionality, includes vicinal diols, hydroxy-acids, monosaccharides, polysaccharides, anhydro-sugars, deoxy-sugars, amino sugars, inositols, glycolipids, glycoconjugates, and pharmacologically important therapeutic agents.

Our research efforts have been directed towards understanding the subtleties, mechanistic implications, and potential utility of this technique for selective ^{3}H incorporation.

We have investigated in depth three approaches to regioselective control of isotopic exchange. We have found that modification of the initial or penultimate catalyst dramatically affects the initial $^{1}H \rightarrow ^{2}H$ exchange rates (*kinetic control*). Modification of the solvent system employed provides accessibility for "switching on and off" distinct stereogenic positions (*thermodynamic control*). Either derivatization of hydroxyl functionality or intramolecular conformational locking may spatially direct isotopic exchange (*steric control*); *e.g.*, a conformationally defined trisaccharide appendage inhibits reduction of the reactive enone double bond of a cardenolide aglycone. This $^{1}H \rightarrow ^{2}H$ isotopic exchange technique has shown to be reversible, which provides the unique possibility of both stereoselective and regioselective ^{3}H labeling at a singular stereogenic site by exploiting a judicious interplay of kinetic, thermodynamic, and steric effects.

The applicability and limitations of this technique, and related developmental work for catalytic tritium incorporation will be described.

The synthesis of (*1R,2R*)-(-)-1,2-diamino[1,2-^{14}C]cyclohexane

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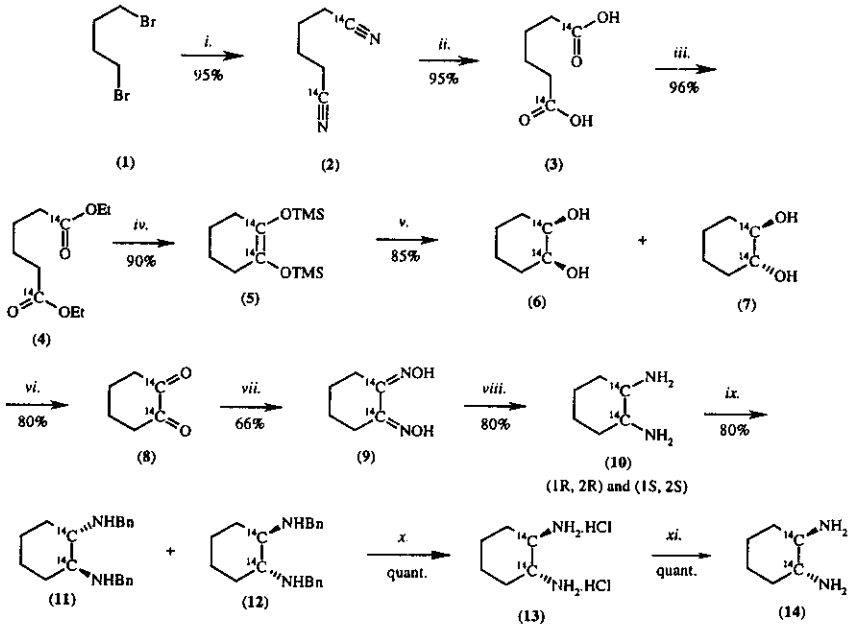
Custom Labelling and Special Synthesis Team, Amersham Pharmacia Biotech, Forest Farm Estate, Whitchurch, Cardiff CF14 7YT, Wales.

In the course of a ^{14}C labelled custom preparation we found it necessary to prepare a large amount (several hundred millicuries) of (*1R,2R*)-(-)-1,2-diamino[1,2-^{14}C]cyclohexane (**14**). This was achieved by an efficient and reproducible synthesis from potassium [^{14}C]cyanide.

Thus 1,4-dibromobutane (**1**) on treatment with potassium [^{14}C]cyanide gave [1,6-^{14}C]adiponitrile (**2**) in 95% yield. [1,6-^{14}C]Adiponitrile (**2**) was hydrolysed using concentrated potassium hydroxide solution to give [1,6-^{14}C]adipic acid (**3**), once again in 95% yield. [1,6-^{14}C]Adipic acid (**3**) was converted *via* the acid chloride to diethyl [1,6-^{14}C]adipate (**4**) in 96% yield. Diethyl [1,6-^{14}C]adipate (**4**) was cyclised to bis(trimethylsilyl)[1,2-^{14}C]cyclohexene-1,2-diether (**5**) in 90% yield using sodium and chlorotrimethylsilane as developed by Schrapler and Ruhlmann.[1] Reductive hydrolysis of the TMS diether (**5**) with sodium borohydride in aqueous ethanol gave a mixture of [1,2-^{14}C]diols (**6**) and (**7**) in 85% yield. A modified Swern oxidation of this mixture using trifluoroacetic anhydride and triethylamine in DMSO gave 1,2-[1,2-^{14}C]cyclohexanedione (**8**) in 80% yield.[2] Conversion of the [1,2-^{14}C]dione (**8**) into (+/-)-*trans*-1,2-diamino[1,2-^{14}C]cyclohexane (**10**) was achieved *via* the [1,2-^{14}C]dioxime (**9**). [1,2-^{14}C]Dioxime (**9**) was formed in 66% yield by the treatment of the [1,2-^{14}C]dione (**8**) with hydroxylamine. Reduction of [1,2-^{14}C]dioxime (**9**) using sodium in ethanol gave (+/-)-*trans*-1,2-diamino[1,2-^{14}C]cyclohexane (**10**) in 80% yield.[3] This whole sequence represents an overall conversion of potassium [^{14}C]cyanide to (+/-)-*trans*-1,2-diamino[1,2-^{14}C]cyclohexane (**10**) in 28% yield. Resolution of (+/-)-*trans*-1,2-diamino[1,2-^{14}C]cyclohexane (**10**) was achieved by formation of the dibenzyl derivatives. Thus (+/-)-*trans*-1,2-diamino[1,2-^{14}C]cyclohexane (**10**) was treated with benzaldehyde and the resulting diimines reduced *in situ* with sodium borohydride to

give N,N'-dibenzyl-1,2-diamino[1,2-^{14}C]cyclohexanes (**11**) and (**12**) in 80% yield. Compounds (**11**) and (**12**) were then separated using chiral HPLC. N,N'-Dibenzyl-1,2-diamino[1,2-^{14}C]cyclohexane (**12**) was deprotected using hydrogen and Pearlman's catalyst to give the relatively stable dihydrochloride salt (**13**) in quantitative yield. Treatment of the dihydrochloride (**13**) with 35% aqueous potassium hydroxide gave (*1R,2R*)-(-)-1,2-diamino[1,2-^{14}C]cyclohexane (**14**) quantitatively. This represents an overall yield of 80% for the resolution.



Reagents: i. K^{14}CN, EtOH, water, Δ. *ii.* KOH, water, Δ. *iii.* $(COCl)_2$, DMF, CH_2Cl_2; EtOH. *iv.* Na, TMS-Cl, toluene, Δ. v. $NaBH_4$, EtOH, water. *vi.* TFAA, TEA, DMSO, -60°C. *vii.* $NH_2OH.HCl$, KOH, water, 0°C. *viii.* Na, EtOH, 80°C. *ix.* PhCHO, EtOH; $NaBH_4$. *x.* $Pd(OH)_2/C$, H_2, MeOH, HCl, 40°C. *xi.* 35% KOH.

References

1. Schrapler U. and Ruhlmann K. - Chem. Ber. 97 : 1383 (1964)

2. Amon C.M., Banwell M.G. and Gravatt G.L. - J. Org. Chem. 52 : 4851 (1987)

3. Jaeger F.M. and Van Dijk J.A. - Proc. Akad. Sci. Amsterdam. 39 : 384 (1936).

Synthesis of Isotopically Labeled 1-[(2,4-Di-1-pyrrolidinyl-9*H*-pyrimido[4,5-b]indol-9-yl)-acetyl]pyrrolidine monohydrochloride, PNU-142731A, An Orally Active Antiasthma Agent

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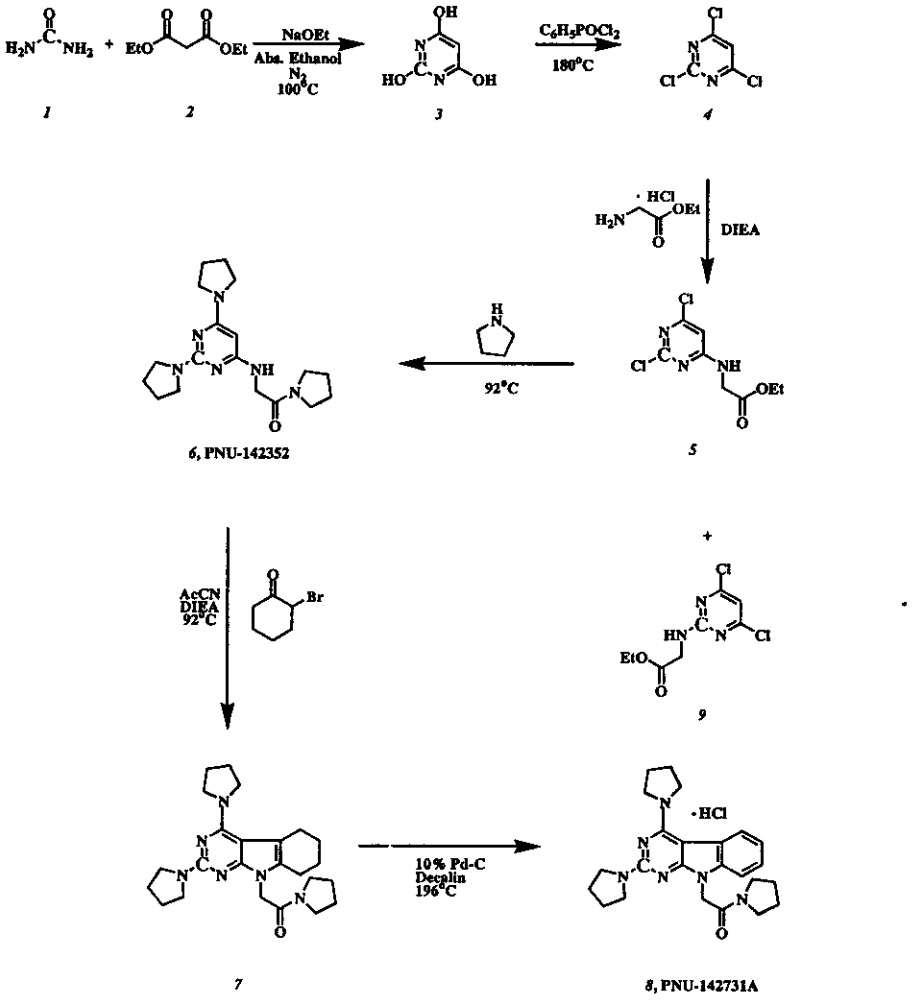
1-[(2,4-Di-1-pyrrolidinyl-9H-pyrimido[4,5-b]indol-9-yl)-acetyl]pyrrolidine monohydrochloride, PNU-142731A, is under investigation as a potential orally active antioxidant for the treatment of asthma. Stable isotope and radioisotope forms of this compound were needed for drug adsorption, distribution, excretion, and metabolism studies. The synthesis of carbon-14 PNU-142731A, labeled at the C-2 position of the pyrimidine ring was accomplished in 6 steps. [(2,6-Di-1-pyrrolidinyl-4-[2-^{14}C]pyrimidinyl)amino]acetyl pyrrolidine, [^{14}C]PNU-145325 was synthesized in 4 steps from 300 mCi (nominally 55 mCi/mmol) of carbon-14 labeled urea and unlabeled diethyl malonate. The synthesis was completed by the cyclization of [^{14}C]PNU-145325 with 2-bromocyclohexanone, followed by dehydrohalogenation to afford 31 mCi (10.3% overall yield) of [^{14}C]PNU-142731A as the monohydrochloride salt. The specific activity was 112.02 μCi/mg (51 mCi/mmol) with a radiochemical purity of 99% as determined by HPLC and TLC. In a similar manner, [^{13}C, $^{15}N_2$]urea and [1,3-^{13}C]diethyl malonate were used to prepare 379 mg of [$^{13}C_3$, $^{15}N_2$]PNU-142731A with excellent purity and 99 atom % incorporation.

Scheme 1



C= Carbon-14

Measurement of Bacterial Peptidoglycan Synthesis by Scintillation Proximity Assay

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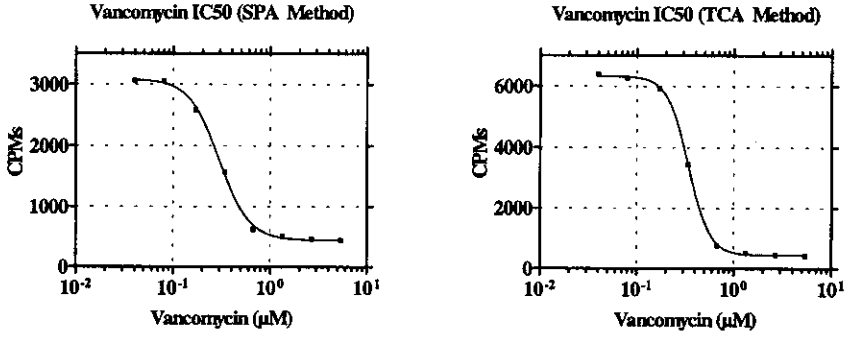
Traditional methods for monitoring macromolecular synthesis in whole bacterial cells have relied on the metabolic incorporation of soluble radiolabeled precursors into acid-precipitable that must be physically separated from unincorporated, acid-soluble radiolabel prior to quantitation by liquid scintillation counting. While TCA (trichloroacteic acid) precipitation offers significant sensitivity and specificity for monitoring metabolic pathways, the filtration requirement introduces practical barriers that severely limit the efficiency and consistency of sample analyses. We report here the development of a scintillation proximity (SPA) method that directly replaces typical TCA precipitation with the simple addition of reagents that stop metabolic incorporation of radiolabeled precursors and, provide detection via protein A-coated SPA beads in a simple, procedure.

Previously, we developed a 96-well microplate method for monitoring the synthesis of bacterial cell wall peptidoglycan. This TCA-based method measured the incorporation of [^{3}H]-glycine into glycyl containing peptidoglycan cross-bridges by intact cells of the bacterial strain *Staphylococcus aureus*. Although relatively straightforward and robust, the TCA method required multiple precipitation, filtration, washing, and filter drying steps that were both labor intensive and introduced sample-to-sample variability. We sought an improved method to facilitate more

efficient and practical signal detection without altering any of the underlying biological assay conditions. We observed that addition of protein A coated polyvinyltoluene SPA beads (Amersham, Inc.) caused the *S. aureus* cells to slowly aggregate around the beads, producing a readily detectable signal after 6 to 8 hours of incubation. This results from an association of the bacteria with the scintillation beads which measures the amount of radiolabel taken into the cell. We found that the SPA detection method yielded dose response data for control agents that correlated extremely well to parallel TCA-treated samples (see figures below).



Although count rates from the SPA data were approximately 50% lower than TCA data, repeated testing against a broad spectrum of bacterial inhibitors showed that the SPA method was generally more precise and sensitive. Moreover, IC50 endpoints derived from SPA data were the same or slightly lower than endpoints calculated from TCA data. We attribute this improved sensitivity to the physical association of beads with *S. aureus* cells, thus facilitating detection of any reduction in cell-associated radiolabel (vs TCA methods that detect only covalently incorporated radiolabel).

Application of Quantitative Whole Body Autoradiography in Candidate Drug Selection

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Quantitative whole body autoradiography is commonly used in the safety assessment of radiolabeled compounds for human clinical trials. Along with numeric data, autoradiography provides a visual snap-shot of the distribution of drug-related radioactivity in all tissues. Studies in which animals are serially sacrificed allows one to generate pharmacokinetic data as well as a time-lapse visualization of the fate of the radiolabeled drug materials.

In the push for more rapid development of drugs, opportunities arise where whole body autoradiography can be used to compare the tissue distribution of chemical analogs. These studies provide data to facilitate candidate drug selection. Three examples are presented which demonstrate the value of whole body autoradiography in comparative evaluations. The first example shows differences in accumulation of drug-related radioactivity in tissues between two analogs for treatment of metabolic diseases. The second example compares the distribution patterns of two reverse transcriptase inhibitors, atevirdine and delavirdine.
In a third example, whole body autoradiography was used to rapidly assess feto-placental transfer of two similar entities under co-development for CNS disorders.

Absorption, Distribution, Metabolism, and Excretion of OSU-6162 in the Rats.

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OSU-6162 is weak dopamine D2 receptor modulator with behavior-normalizing properties.

The absorption, distribution, metabolism, and excretion were investigated in rats following a 10 mg/kg single oral dose of [^{14}C]OSU-6162. Absorption was rapid as evidenced by tmax of less than 0.5 h. The disposition half-life of OSU-6162 was short (less than 1 h). Radioactivity was widely distributed to rat tissues, including brain. Concentrations of OSU-6162 were 2-fold higher in brain than in plasma, indicating that OSU-6162 effectively crossed the blood/brain barrier. The majority of the administered radioactivity (>90%) was excreted in urine. OSU-6162 was extensively metabolized, primarily by N-dealkylation. Systemic exposure to the N-desalkyl metabolite was 2-fold greater than systemic exposure to parent drug. The N-desalkyl metabolite accounted for 66% of the administered dose. Approximately 10% of the administered dose was excreted as unchanged parent drug in urine.

The Preparation of a Third Generation Reverse Transcriptase Inhibitor(RTI), Radioisotopically Labeled with Carbon-14 or Tritium, or Stable Isotopically Labeled with Carbon-13 and Nitrogen-15

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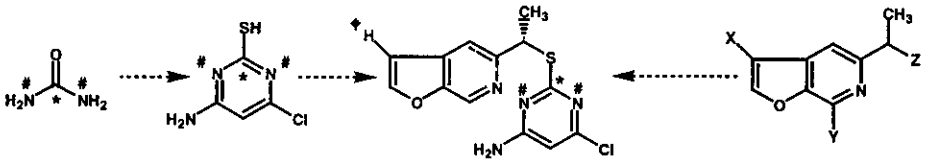
Considerable effort has been focused over the past decade on the development of HIV-1 specific non-nucleoside reverse transcriptase inhibitors (NNRTIs) due to their safety, selectivity, and antiviral profiles for the treatment of HIV-1 infection and AIDS. Such efforts at Pharmacia & Upjohn for finding new and more potent NNRTI's that target drug resistant variants of HIV-1 has led to the identification of the furo[2,3-c]pyridine pyrimidine thioether class of NNRTI's. A member of this class, ***1***, has been shown to have a potency 50-fold greater than that of delavirdine mesylate (Rescriptor®) against cultured wild type HIV-1 and is extremely potent against drug resistant viruses. As a candidate for clinical development, the preparation of radioisotope and stable isotope labeled material was requested for drug absorption, distribution, metabolism, and excretion (ADME) studies.

The initial preparation of this isotopically labeled chiral compound followed a convergent synthetic pathway in which an isotopically labeled pyrimidine moiety having suitable functionality was coupled with the furopyridine portion of the molecule. Several methods were investigated for the formation of the pyrimidine ring from either labeled urea or thiourea; both common and convenient commercial sources of the isotopic labels. The result of this effort was the preparation of 4-amino-6-chloro-2-mercaptopyrimidine, with a carbon-14 radioisotope at the 2-position, or with carbon-13 and nitrogen-15 stable isotopes at

the 2-, and 1,3-positions respectively, of the pyrimidine ring. Coupling of the carbon-14 labeled pyrimidine with 5[(1R)-1-chloroethyl]furo[2,3-c]pyridine gave 26.5 mCi (overall 18% yield, 6-steps) of ***1a***, with a specific activity of 58 mCi/mmol (93 atom %), a radiochemical purity of 99% by both HPLC and TLC, and enantiomeric purity of at least 99% by HPLC. Likewise, 298mg of ***1b***, (99 atom % ^{13}C, 98 atom % ^{15}N) having excellent chemical purity, was prepared using the same synthetic pathway.

To complete the metabolic profile of ***1***, and to provide material with high specific activity for binding studies, material with a tritium radioisotopic label in the furopyridine portion of the molecule was prepared. Exploration of selective tritium labeling by tritio-halogen exchange on a variety of halogenated precursors to ***1***, in the presence of palladium metal, eventually provided 46.1 mCi of ***1c***, having a specific activity of 12.5 Ci/mmol, radiochemical purity of 98.7% by TLC and HPLC, and enantiomeric purity of 99% by chiral phase HPLC.



1a. ★ = ^{14}C , # = ^{14}N, ✦ = ^{1}H

1b. ★ = ^{13}C , # = ^{15}N, ✦ = ^{1}H

1c. ★ = ^{12}C , # = ^{14}N, ✦ = ^{3}H

Metabolism of tirilazad mesylate in humans: Application of stable isotopic labelling and a novel bile cannulation technique in human volunteers. **Rick C. Steenwyk*, Paul G. Pearson🗁 and J. James Vrbanac***, *Drug Metabolism and Disposition Research, Pharmacia and Upjohn, Inc., Kalamazoo, MI 49001, 🗁Merck & Co., West Point PA 19486.

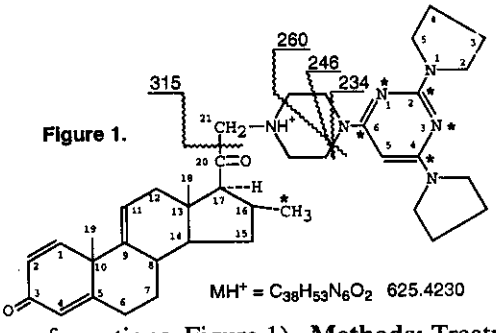
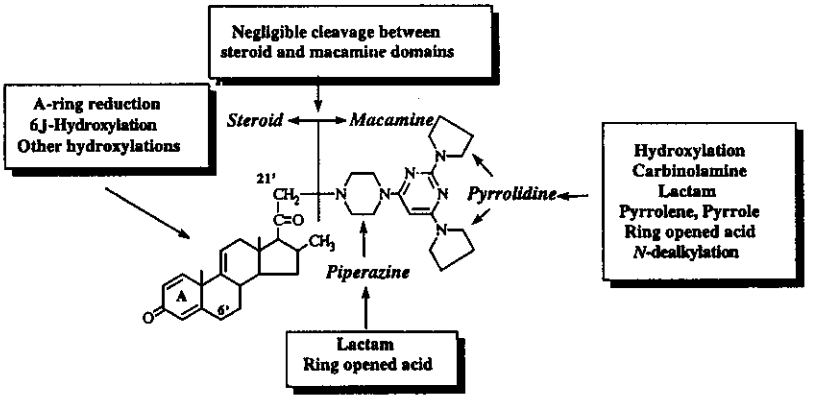


Figure 1.

The xenobiotic metabolic profile in human bile was characterized by LC-ESI-MS following IV administration of FREEDOX® (tirilazad mesylate, PNU-74006F, TM; ^{2}H, ^{13}C and ^{15}N-labeled to characterize specific metabolic transformations, Figure 1). **Methods:** Treatment A: 1.5 mg/kg [$^{13}C_3$-$^{15}N_2$]tirilazad mesylate IV Solution (1.5 mg/mL) and 1.5 mg/kg, FREEDOX® IV Solution (1.5 mg/mL) = Total dose of 3.0 mg/kg. Treatment B: 1.5 mg/kg [$^{13}H_3$]tirilazad mesylate IV Solution (1.5 mg/mL) and 1.5 mg/kg FREEDOX® IV Solution (1.5 mg/mL) = Total dose of 3.0 mg/kg. Mass Spectrometry. Online liquid chromatography-mass spectrometry (LC-MS) was performed on a TSQ 7000 triple quadrupole mass spectrometer equipped with an electrospray interface (ESI), (Finnigan MAT, San José, CA), and a Hewlett Packard HP1050 HPLC pump (Hewlett Packard, Avondale, PA). **Results:** Drug-related material was unambigously identified by the 1:1 ratio of the A ion and A+3, or A+5 ions. A total of 63 metabolites were detected in the bile sample from the subject receiving macamine-labeled tirilazad:tirilazad (1:1) and 56 metabolites were detected in the bile sample from the subject receiving steroid-labeled tirilazad:tirilazad (1:1) in

the 2-3 h collection period samples (a total of 81 different metabolites detected in both samples). The metabolic profile observed in human bile was the more complex than the bile provile observed for dog & rat. Mass spectral interpretation established complex biotransformation of the macamine domain in combination with reduction of the A-ring and one or more hydroxylations, including 6+-hydroxylation, occurring on the steroid domain.

Summary. In general, the biotransformations of tirilazad mesylate in human was characterized by; 1) insignificant, or absence of cleavage of the 21-aminosteroid resulting in free-steroid and free-macamine metabolites; 2) extensive, complex metabolism of the macamine moiety; 3) formation of steroid hydroxy metabolites including the 6B-hydroxy metabolite; and 4) reduction of the A-ring. The biotransformation of the macamine was very complex and most likely included 2′-, and 3′-hydroxylations, formation of lactams, *N*-dealkylation and subsequent oxidation to the ring opened carboxylic acid (succinimide), and *N*-dealkylation to the primary amine of the pyrrolidine moieties; and the formation of lactams and *N*-dealkylation and subsequent oxidation to the ring opened carboxylic acid of the piperazine moiety.



Synthesis of ^{14}C-Labeled S-(-)-1-Phenylethylamine and Its Application to the Synthesis of [^{14}C] CI-1021, a Potential Antiemetic Agent

Yinsheng Zhang
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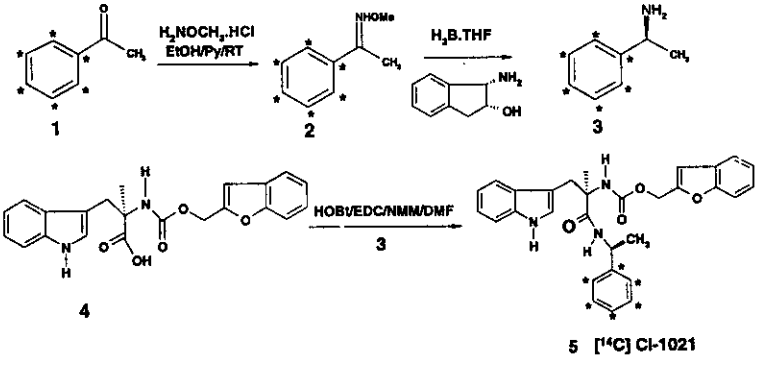
S-(-)-1-Phenylethylamine has been used in the synthesis of several drug candidates at Parke-Davis including CI-1021, an NK_1 receptor antagonist being developed as a potential antiemetic agent. CI-1021 (PD 0154075) was ^{14}C-labeled previously by using the synthetically more challenging [^{14}C] α-methyltryptophane route [I. Victor Ekhato and Yun Huang, J. Labelled Compd. Radiopharm., **1997**, *39* (12), 1020-38]. Further pharmacokinetic and metabolic studies require a second synthesis of the ^{14}C drug. Since we have seen no evidence of the *in vivo* hydrolysis of S-(-)-1-phenyl-ethylamine from the molecule, we decided to radio-label S-(-)-1-phenylethylamine because of its importance in drug design and the ease of the synthesis. [^{14}C]S-(-)-1-Phenylethylamine **3** was made in 71% yield with an ee of 96.6% from [^{14}C]acetophenone, going through a methyl oxime intermediate **2**, which underwent an enantioselective reduction with borane in the presence of a chiral ligand 1S,2R-1-amino-indan-2-ol. Coupling of [^{14}C]S-(-)-1-phenylethylamine with the chiral acid **4** provided [^{14}C] CI-1021 in 74% yield.



Characterization of *N*-Methylpiperidinyl Esters as Substrates for Cholinesterases *In Vitro* and *In Vivo*.

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A series of *N*-methylpiperidinyl esters were examined to determine the relative hydrolysis rates by both acetylcholinesterase (AChE) and the related enzyme butyrylcholinesterase (BuChE). These data were then used to identify the optimal PET radiotracers to study changes in the activity of both enzymes in Alzheimerís disease (AD). The *in vitro* hydrolysis rates for each ester, using purified enzymes, were measured in a spectrophotometric assay. Although none of the piperidinyl esters was specific for AChE, both the acetate (AMP, MPA4) and propionate (PMP) esters were hydrolyzed rapidly by AChE and slowly by BuChE. The *n*-butyrate (*n*BMP), 2-methylbutyrate (2MBMP) and *n*-valerate (*n*VMP) esters were hydrolyzed only by BuChE. The *iso*-butyrate ester (*i*BMP) was hydrolyzed slowly by both enzymes and the *iso*-valerate ester (*i*VMP) was not a substrate for either enzyme. For the five active esters, the ^{11}C-labeled analog was synthesized and *in vitro* hydrolysis rates in rodent blood samples were determined. Blood samples contain high concentrations of both AChE and BuChE; thus AMP, PMP, *n*BMP and *n*VMP were all rapidly hydrolyzed. These radiotracers were then evaluated *in vivo* in both rodents and primates. AMP and PMP have been extensively evaluated *in vivo* previously. *n*BMP exhibited high brain uptake and regional retention of radioactivity was consistent with histochemical estimates of BuChE distribution. *n*VMP exhibited lower initial uptake than *n*BMP and more rapid pharmacokinetics indicating potential difficulties with delivery dependence. Based on these results, *n*BMP seems most promising as a radiotracer for measuring BuChE activity *in vivo*. AMP or PMP, currently in clinical use, remain the best radiotracers for the *in vivo* measurement of AChE activity in patients with AD.

Fluorine-18 and Carbon-11 Labeled Radiopharmaceuticals for Positron Emission Tomography

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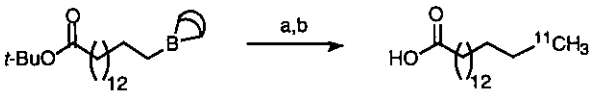
Fluorine-18 ($t_{1/2}$ = 110 min) and carbon-11 ($t_{1/2}$ = 20 min) are the most common short-lived, cyclotron-produced radioisotopes used in the design of radiopharmaceuticals for Positron Emission Tomography (PET). There are a number of methods for introducing these isotopes into simple model compounds. Recently, the range of two of these methods has been extended via the synthesis of more complex radiopharmaceuticals: [ω-^{11}C]palmitic acid, an imaging agent for studying myocardial metabolism, and 2-[^{18}F]fluoroestradiol, a potential improved diagnostic imaging agent for breast cancer.

The precursor for [ω-^{11}C]palmitic acid was synthesized in 4 steps from □-pentadecalactone. The key step in the radiosynthesis of [ω-^{11}C]palmitic acid was the incorporation of carbon-11 via a modified Suzuki coupling of [^{11}C]iodomethane with a functionalized trialkylborane (Scheme 1).[1]

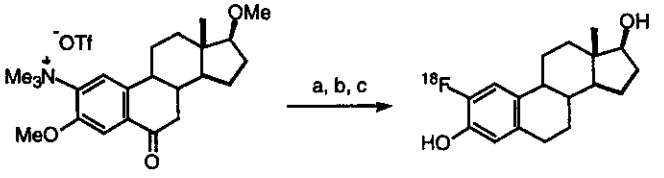
Scheme 1



(a) $Pd(PPh_3)_4$, NaOH, $^{11}CH_3I$, 90 °C, 4 min, 74%; (b) CF_3CO_2H, 90 °C, 1 min, 100%

The precursor for 2-[^{18}F]fluoroestradiol was synthesized in 8 steps from 17β-estradiol. The key step in the radiosynthesis of 2-[^{18}F]fluoroestradiol was the microwave assisted incorporation of [^{18}F]F$^-$ into a substituted phenol which has been modified to facilitate nucleophilic aromatic substitution (Scheme 2).[2]

Scheme 2



(a) [^{18}F]TBAF, microwave, 30 sec, 40- 50%; (b) $AlCl_3$, $LiAlH_4$, 5 min, 95%: (c) EtSH, $AlBr_3$, 30 min, 50%

(1) Hostetler, E.D.; Fallis, S.; McCarthy, T.J.; Welch, M.J.; Katzenellenbogen, J.A. *J. Org. Chem.*, **1998**, *63*, 1348-1351.
(2) Hostetler, E.D.; Jonson, S.D.; Welch, M.J.; Katzenellenbogen, J.A. *J. Org. Chem.*, **1999**, *64*, 178-185.

Evolution of Imaging Systems in Scintillation Detection

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Most imaging systems accept isotopic data through a conversion step in which a medium sensitive to ionizing radiation is exposed, and is then digitized using a scanning laser, CCD camera, or other detection technology. This is the case for studies in which anatomical localization of isotope is the main variable of interest. This is also the case in scintillation assays, in which alterations in molecular interactions or characteristics are studied.

High resolution localization studies continue to use emulsion coat or film as detection media. Isotope localization technologies for large format studies (such as whole body autoradiography) are evolving through a transition from film to storage phosphor detection. In recent years, storage phosphor imaging has become the standard when broad dynamic range and fast exposure are more important than high resolution and high sensitivity within a narrow dynamic range (properties of film).

Technology for the quantification of scintillation assays has remained relatively stable for some years. Typically, radioimmunoassays, receptor binding assays, and enzyme assays are detected in liquid scintillation counters. These devices, while sensitive and of high precision, are not particularly well suited to miniaturization. Therefore, there is interest in the use of imaging systems for assay detection. Imaging systems have the advantages that large numbers of wells can be quantified in a single detection procedure (favoring

miniaturization), that faint scintillation assays can be accomplished very rapidly (minutes instead of hours for a 384 well plate), and that a system that performs scintillation counting could also be adapted to luminescence and fluorescence detection.

We have developed an imaging system that performs high throughput, image-based quantification of scintillation assays. This system includes a very sensitive CCD camera and telecentric lens, that detect scintillation with better sensitivity than most scintillation counters. For example, scintillation proximity assay is detected over an entire 384 or 1536 well plate, at levels of 200 DPM with signal-to-noise of >10:1, in less than 10 minutes. The imaging system, which is developed with Amersham Pharmacia Biotech (and is sold by them under the LEADseeker label), is now being installed in high throughput screening laboratories, as a radiometric and luminometric tool. Fluorescence capabilities will be added in the near future.

Single Electron Transfer: Enzyme Catalysis And Chemical Model Studies Using Deuterium Labeled Tetrahydropyridinyl Substrates

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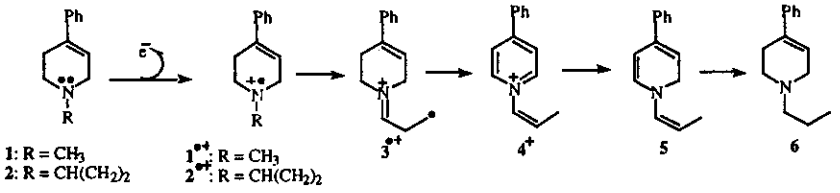
Monoamine oxidase B (MAO-B) catalyzes the allylic α-carbon oxidation of various N-substituted tetahydropyridinyl derivatives including the parkinsonian inducing neurotoxin 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine [MPTP (**1**)]. The generally accepted pathway for this type of reaction involves a single electron transfer (SET) to form an aminyl radical cation $\mathbf{1}^{\cdot+}$. The N-cyclopropylaminyl radical cation $\mathbf{2}^{\cdot+}$, derived via SET, inactivates MAO-B, presumably via the ring opened, distonic radical cation $\mathbf{3}^{\cdot+}$. We have observed normal primary (6,6-d_2 analog) and secondary (N-CH_2D) kinetic deuterium isotope effects on the MAO-B catalyzed oxidation of MPTP. We also have examined a chemical model of the SET pathway using an Fe^{+3} phenanthroline complex as the oxidizing agent and regiospecifically deuterium labeled N-cyclopropyl analogs of MPTP as substrates. The oxidation products were trapped by treatment with $NaBH_4$ in CH_3OH. The preliminary interpretation of the results from these studies led us to propose the conversion of the substrate molecule (**2**) to the corresponding N-2-propenylpyridinium species $\mathbf{4}^{+}$ which would be reduced to the N-propyltetrahydropyridine **6**, as observed experimentally. When synthetic $\mathbf{4}^{+}$ was treated with $NaBH_4$, however, the N-propenyldihydropyridinyl intermediate **5** formed in the reaction resisted further reduction. Extensive studies with $NaBD_4$ in CH_3OH and $NaBH_4$ in CH_3OD have convinced us that the reduction of $\mathbf{4}^{+}$ is influenced dramatically by the Fe^{+3} phenanthroline reagent and that the product

obtained following the SET transformation is not the N-propenylpyridinium species 4^+. The relevance of the outcomes of these model chemical studies and the kinetic deuterium isotope effect studies with respect to the mechanism by which MAO-B catalyzes the metabolic bioactivation of 1,4-disubstituted 1,2,3,6-tetrahydropyridinyl derivatives will be presented.



ADVANCING WITTIG REARRANGEMENT CHEMISTRY VIA DEUTERIUM LABELING

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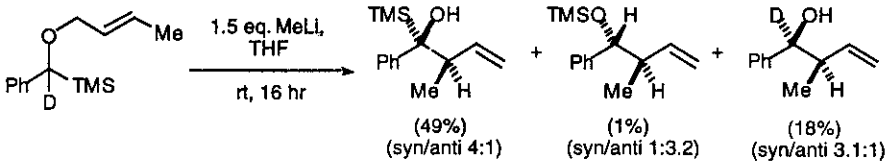
Department of Chemistry, Michigan State University, East Lansing, MI. USA

For the past twenty years Wittig rearrangements have elicited the attention of synthetic and physical organic chemists alike.[1] Reactions of this class are typically initiated by the generation of an α-metallated ether which undergoes a subsequent rearrangement, usually in the form of a [2,3], [1,2], or [1,4] shift. Formation of the α-alkoxy carbanion species is often realized by either tin-lithium exchange[2] or through direct deprotonation of an ether at the α-carbon.[1] The latter method of α-metallated ether generation is usually facilitated by the presence of anion stabilizing groups such as carbonyls, nitriles, allyl, benzyl, or propargyl moieties.

To the best of our knowledge, the deprotonation of α-alkoxysilanes has never been used to trigger a Wittig rearrangement. In contrast, Mulzer[3] has reported two cases of (trimethylsilyl)methyl allyl ethers undergoing [2,3]-Wittig rearrangements upon silicon-lithium exchange.

1. (a) Nakai, T.; Tomooka, K. *Pure Appl. Chem.* **1997**, *69*, 595-600. (b) Nakai, T.; Mikami, K. *Organic Reactions* **1994**, *46*, 106-209. (c) Marshal, J. A., in *Comprehensive Organic Synthesis*, Pattenden, G., Ed.; Pergamon: London, 1991; Vol. 3, 975-1014. (d) Brückner, R., in *Comprehensive Organic Synthesis*, Pattenden, G., Ed.; Pergamon: London, 1991; Vol. 6, 873-908.
2. Still, W. C.; Mirat, A. *J. Am. Chem. Soc.* **1978**, *100*, 1927-1928.
3. Mulzer, J.; List, B. *Tetrahedron Lett.* **1996**, *37*, 2403-2404.

Scheme I



While our initial results (represented by Scheme I) provide clear evidence of the α-alkoxysilanes undergoing deprotonation followed by subsequent Wittig rearrangement, these reactions also tend to give significant amounts of the desilylated products. Such products could be the result of a silicon-lithium exchange initiated rearrangement[3] or come from the silylated products via a Brook[4]/desilyation sequence. To better understand the origins of our reaction products and their associated stereochemistries, we employed deuterium labeled starting materials. The details of these experiments will be discussed.

4. (a) Linderman, R. J.; Ghannam, A. *J. Am. Chem. Soc.* **1990**, *112*, 2392-2398. (b) Brook, A. G.; Pascoe, J. D. *J. Am. Chem. Soc.* **1971**, *93*, 6224-6627. (c) Brook, A. G. *Acc. Chem. Res.* **1974**, *7*, 77-84. (d) Brook, A. G.; Bassendale, A. R. In *Rearrangements in Ground and Excited States*; de Mayo, P., Ed.; Academic Press; New York, 1980; Vol. 2, 149-227.

Not Your Average Clinical Radiotracer Trial: Disposition Of Intravenous [^{14}C]Camptosar In Human Cancer Patients.

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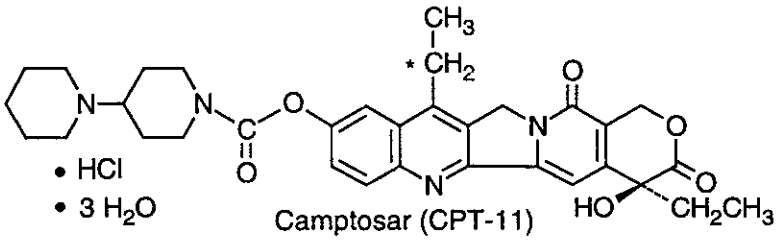
Camptosar® (irinotecan hydrochloride injection; CPT-11) is an antineoplastic agent that was recently approved for the treatment of 5-fluorouracil-refractory colorectal cancer. This presentation will focus on the solutions to some unusual challenges that were encountered during the conduct of a human radiotracer study. Radiosynthesis: The radiosynthetic sequence, starting from the natural product camptothecin, afforded only one opportunity to introduce the ^{14}C radiolabel, in the 11-ethyl substituent, by an inefficient condensation with ^{14}C propionaldehyde. Formulation Development: Camptosar was known to pi-stack when solution concentrations exceeded 2 mM and could not be frozen without precipitation. Therefore, due to concern about radiolysis and particulates in the intravenous formulation, a more dilute, freezable, unit-dose solution of neat radioactivity was necessary. Formulation efforts required facilities, training, process simulation/containment, personnel protection, cleaning and validation suitable for GMP preparation of a sterile iv formulation of a radioactive, cytotoxic agent. Patient Recruitment: Since Camptosar is cytotoxic, only cancer patients were eligible for the study. A nonradiolabeled treatment phase followed the inpatient radiolabel phase of the study. Patient recruitment was slow and the required 8 subjects were recruited and dosed individually over a 6-month period, this required a continuous state of readiness at the clinical site. All patients were ill with advanced cancer and one patient had a biliary T-tube, requiring the additional collection of bile. Dose administration: Intravenous infusion formulations required aseptic dilution of the unit dose radioactive solution with nonlabeled drug and D5W just prior to administration. Diarrhea and vomiting were both possible side effects of drug administration and either could compromise mass balance

data. Sample collection and analysis: Blood, plasma, urine, feces, and bile were collected and radioanalysis was conducted. Plasma was also assayed by fluorescence HPLC for the prodrug CPT-11, the active antineoplastic agent SN-38 as well as SN-38 glucuronide and another metabolite, APC. Methods for the near-quantitative extraction of radioactivity from biofluids were developed. Significant difficulties were overcome to recover radioactivity from bile and feces in a form suitable for HPLC analysis. Plasma and excreta were then quantitatively profiled by radiometric HPLC and unknown minor peaks were identified by HPLC-MS-MS. Brief summary of results: Results were reported in detail elsewhere (ASCO Abstract 633 1999). Briefly, a recovery of 95.8 ± 2.7% of dose (range, 92.2 to 100.3%; N=7) was obtained and parent drug and all major and most minor metabolites were quantified in plasma and excreta. Conclusion: With adequate resources, lead time, and a team-based approach, even a difficult human radiolabel clinical trial adds value by providing a quantitative understanding of the human disposition of the drug candidate. When obtained early in development, these data may prevent undue emphasis on metabolites with a low likelihood of contributing to the pharmacology, toxicology or overall clearance of the drug candidate.



Camptosar (CPT-11)

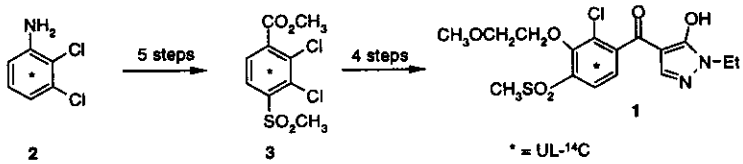
* Denotes radiolabel location

THE DEVELOPMENT OF A NEW SYNTHETIC ROUTE TO PREPARE CARBON-14 LABELED XDE-350 (1), A HERBICIDE USED TO CONTROL GRASS AND BROADLEAF WEEDS IN CORN

Norman R. Pearson

Dow AgroSciences, Specialty Synthesis, 306 Bldg., D2, 9330 Zionsville Rd., Indianapolis, IN 46268-1053.

XDE-350 (**1**), a member of the benzoylpyrazole class of herbicide chemistry, is undergoing evaluation for the control or grass and broadleaf weeds in corn. In order to obtain a registration from the EPA for this material it is necessary to conduct a broad range of environmental, biological and toxicological tests. The utilization of C-14 labeled compounds in these tests makes them easier to run and interpret. This presentation will describe the chemistry used to prepare carbon-14 labeled samples of **1**. In particular, a new synthesis of **1**-Ph-UL-^{14}C has been developed that makes the preparation of this tracer far easier than it had been by previous methods. The sequence begins with the conversion of 2,3-dichloroaniline-Ph-UL-^{14}C (**2**) in five chemical steps to methyl 2,3-dichloro-4-(methylsulfonyl)benzoate-Ph-UL-^{14}C (**3**). The reactions employed to convert **2** to **3** were thiocyanation, sulfur methylation, diazotization/iodination, sulfur oxidation and finally, low pressure carbonylation. Ester **3** was then converted to **1**-Ph-UL-^{14}C in four steps by ester hydrolysis, acid chloride formation, coupling with 1-ethyl-5-hydroxy-pyrazole and finally, alkoxylation with sodium 2-methoxyethoxide. **1**-Ph-UL-^{14}C was produced in an overall radiochemical yield of 40% for the 9 steps.



2 **3** **1**

* = UL-^{14}C

Synthesis Of ^{14}C-Labeled Pesticides And Their Intermediates

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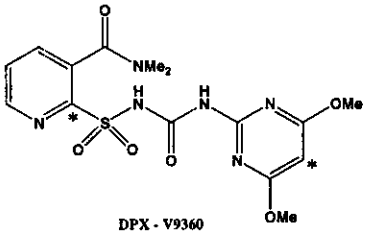
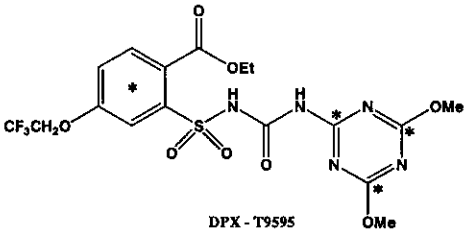
NEN Life Science Products Inc., Boston, MA 02118

The preparation of hexachlorocyclopentadiene[^{14}C(U)] an intermediate used in several insecticides will be discussed. Starting with acetylene[^{14}C], a 33% yield of the final product was obtained in four steps. The specific activity was at 140 mCi/mmol. The preparation of DPX-V9360 [Pyridine-2-^{14}C], DPX-V9360 [Pyridine-2-13C] , DPX-9360 [Pyrimidine-5-^{14}C] and DPX-V9360 [Pyrimidine-2-^{14}C] will be discussed. DPX-9360 is a corn herbicide, marketed as "ACCENT". A new synthesis of a key intermediate for DPX-V9360 [Pyridine-2-^{14}C] was developed and published in a US patent.



DPX - V9360



DPX - T9595

The preparation of DPX-T9595 [Phenyl ring-^{14}C(U)] and DPX-T9595 [Triazine-^{14}C] will be discussed. DPX-T9595 [Phenyl ring-^{14}C(U)] was synthesized from chlorobenzene [^{14}C(U)] in 11 steps with good yield. DPX-T9595 [Triazine-^{14}C] was prepared from potassium cyanide [^{14}C] in three steps with moderate yield.