IOCB SERVICE DAYS 200

Research-Service Groups / Service Groups / Core Facilities

Synthesis of Radiolabeled Compounds

Aleš Marek



Our team



Aleš Marek





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Radio -labeling, -safety, -metry, -waste management IOCB Service Team Synthetic Radiochemistry 5 Synthetic Radiochemists

> Group location: Building A 3rd floor Office A.3.82



IOCB Service Days February 28, 2023

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Overview of services

What to consider when using radioactivity at IOCB...?



Sbírka zákonů č. **263** / 2016

263 ZÁKON ze dne 14. července 2016 atomový zákon



O1. Consultations & Paperwork Radiosafety f2f training (1-2 hours) Personal Protective Equipment Lab monitoring Dosimetry in compliance with Czech law contact Radiation Safety Officer (A. Marek)

O2. Radiolabeling Synthesis Choice of radionuclide Beta or gamma emitters? (pros and cons)

Custom synthesis + cryogenic storage (slide 4-9)





O3. Radiometry Wide portfolio of radiometric instruments available (slide 11-12)

04. Radioactive waste management Collecting, sorting and disposal in compliance with Czech law - significant paperwork -







02. Radiolabeling Synthesis

SMALL tracers -> ³H, {¹⁴C}

- State-of-the-art instrumentation for synthetic work; deuterium & tritium manifold placed in glovebox, analytical/semiprep-radio-HPLC, ³H NMR, GC-MS, LC-MS, radio-TLC
- Full characterization, Highest Quality, Cryogenic
 Storage, Stability follow up
- Delivery time: 2 weeks to months after request

Synthesis always **starts from T₂ gas,** the cleanest reducing agent - used for hydrogenation, HIE, reductive dehalogenations, and for synthesis of tritiated reagents e.g., metallic tritides, [³H]-alkylating agents etc.

Scale: few mg

LARGE molecules – peptides/proteins -> 125I

- Commercially unavailable radiotracers or expensive ones
- **Fast** (one day synthesis-purification)
- Easy to detect (No Liquid Scintillation Counting)
- Very high Specific Activity (~2100 Ci/mmol)
- No Radioactive Waste disposal eventually
- Reasonable cost of nuclide ([125-I]Nal)
- Late-stage-labeling of tyrosine, histidine moiety, S_EAr
 Scale: 5-40 ug



glove box with tritiation manifold for handling xCi-scale of carrier-free tritium gas (He-free).

Cost of label used in synthesis

³H \$\$ {¹⁴C} >\$\$\$\$\$ ¹²⁵| \$

<u>YOUR cost = 0.00 \$</u>



02. Radiolabeling Synthesis – ³H methods



Examples of labeled material synthesized recently

method of labeling, customer, results



[³H]WQE-199 - HIE -SA = 20.2 Ci/mmol (0.7 T) non-specific

Z. Janeba, IOCB Galia Maik-Rachline, The Weizmann Institute of Science, Rehovot



[³H]NAAG - building block hydrogenation -SA = 49.8 Ci/mmol (1.7 T)

> P. Šácha, IOCB Barbara Slusher, JHU

Kriegelstein et al., J Label Compd Radiopharm. 2022



[³H]Estradiol, [³H]Estrone - reductive dehalogenation -SA = 55.0 Ci/mmol (1.9 T)

H. Mertlíková Kaiserová, IOCB P. Šácha, IOCB



[³H]T0901307 - HIE -SA = 10.8 Ci/mmol (0.4 T) non-specific

H. Mertlíková Kaiserová, IOCB

Yongsong et al., J Label Compd Radiopharm. 2022



[³H]JHIII - reduction with generated borotritide -SA = 25.7 Ci/mmol (0.9 T)

> R. Hanus, IOCB Marek Jindra, Institute of Entomology, Biology Centre CAS

Miláček et al., Insect Biochem. Mol. Biol.. 2021



[³H]Naloxone - propargyl hydrogenation -SA = 56.8 Ci/mmol (2 T)

J. Konvalinka, IOCB Jiří Novotný, Department of Physiology, CU

Melkes et al., Biol. Pharm. Bull. 2020



[¹³C₆]lbrutinib - multistep synthesis cold isotope labeling with ¹³C

M. Hroch, CU, Faculty of Medicine in Hradec Králové

Kriegelstein et al., J Label Compd Radiopharm. 2021 Mžik et al., J. Pharm. Biomed. Anal., 2021



[³H]Yohimbine - reductive dehalogenation -SA = 61.8 Ci/mmol (2.1 T)

H. Mertlíková Kaiserová, IOCB

Examples of labeled material synthesized recently

method of labeling, customer, results



[³H]Deltrophin, [³H]DAMGO - reductive dehalogenation -SA = 22.0, 33.7 Ci/mmol (0.8, 1.2 T)

E. Kudová, IOCB Jan Jakubík, Inst. of Physiology CAS





[³H]Org 24598 - alkylation with generated CH_2TI -SA = 26.2 Ci/mmol (0.9 T) [³H]DS₂OMe - C-F activation -SA = 27.0 Ci/mmol (0.9 T)



[³H]Prazosin - reductive dehalogenation -SA = 13.0 Ci/mmol (0.4 T)

H. Mertlíková Kaiserová, IOCB

 NH_2



[³H]AN099 - reductive dehalogenation -SA = 26.1 Ci/mmol (0.9 T)

P. Majer, IOCB Marek Jindra, Institute of Entomology, Biology Centre CAS

[8-³H]ATP

- reductive dehalogenation -SA = 8.3 Ci/mmol (0.3 T)



[³H]PD1468 (A, B, C, parent) - reductive dehalogenation -SA = 37.7-49.1 Ci/mmol (1.3-1.7 T) G. Birkuš, IOCB



[³H]2,3-cGAMP - reductive dehalogenation -SA = 5.5-16.5 Ci/mmol (0.2-0.6 T)

Petrine Wellendorph, Bente Frølund, University of Copenhagen

G. Birkuš, IOCB

G. Birkuš, IOCB

Polidarová et al., J. Med. Chem. 2021

³H-radioligand application example

binding studies, team of Prof. P. Wellendorph, University of Copenhagen

Radioligand binding studies of ³H-HOCPCA

- Homogenate binding
 - · For ligand profiling and drug discovery efforts
- Autoradiography in vitro
 - For expression pattern studies across species, ontogenesis
- Autoradiography ex vivo For in vivo target engagement
- Uptake by MCT1
- A Autoradiography to mouse brain



B Autoradiography to pig brain C





[³H]-HOCPCA S.A. 28.9 Ci/mmol

D

P<0.0001

P=0.0081

-ortet -

2.0-

1.5

1.0-

0

0.0

Cerebellum

Normalized binding

(fmol/mg TE)



Autoradiography to mice brain



Klein et al., Neurochem Int. 2016 Griem-Krey et al., J Label Compd Radiopharm. 2020

Panel A: *In vitro a*utoradiographic determination of K_d and B_{max} values of GHB high-affinity site distribution by homogenous displacemement of [³H]HOCPCA in mouse brain tissue, illustrating the high level of specific binding in frontal cortex. **Panel B:** [³H]HOCPCA *in vitro* autoradiography to pig brain tissue.

Panel C: *Ex vivo* binding of [³H]HOCPCA. Mice (adult male C57/BL6) were injected with 5-MBq [³H]HOCPCA and brains collected 30 min postinjection. Significant specific binding levels were observed in the hippocampus and cortex compared with the cerebellum. **Panel D:** Binding curve, homogenate binding (1 = HOCPCA, GHB = γ -hydroxybutyrate)

02. Radiolabeling Synthesis – ¹²⁵I method



¹²⁵I-peptide – radioligand application example

data provided by the team of Dr. Jiráček



Domain 11 of IGF-2R

03. Radiometry direct measurement

self-operated after training located: A.3.76., A.3.75.

contact: B.B. (line 269)





Radio-TLC scanner RITAβ- radioactivity detector for Thin-Layer-ChromatographyGamma emitters as well (Compton electrons detection)

2 TLC plates of 200 x 200 mm each can be placed at the same time



WIZARD² 24/0 Gamma Counter high-throughput detection of γ-ionization (¹²⁵I, ¹³¹I, ^{99m}Tc, etc..) BRUKER Avance II[™] 300 MHz 5mm broad-band probe & tritium probe (³H NMR)

03. Radiometry using scintillation

self-service after training located: A.3.76.

contact: A.M. (line 395)



MicroBeta² Microplate Counter (96, 384-well plates)







Cell Harvester FilterMate-96 for MicroBeta² Microplate Counter

- cell proliferation through filter-based ³H-labeled thymidine uptake
- receptor-ligand binding assay, ⁵¹Cr release assays for cell cytotoxicity assays, nucleic acid degradation, cell wash station for adherent cell assays, etc.



Liquid scintillation analyzer Tri-Carb 2900TR 5- or 20-mL scintillation vials



Analytical and semi-preparative Waters Alliance HPLC system combined with a radioactivity-HPLC flow detector Ramona



04. Radioactive Waste Management

details of proper handling provided at face-to-face safety training (A.M.)

in charge: B.B, G.N. (line 269)



Inventory of solid state long-lived RAW

	Contractory of Contractory	0 Hmotnost [kg]	Počet	Aktwita (HBo)			
DATUM	JHENO		pytiú	3-H	14-C	Uznaceni, poznamky	
15.06.2018	Brož	1,1	1	444,0			
06.09.2818	Birkuš	1,0	1	1,9		Plastové nádobí (čivý pevný pytel)	
09.11.2018	Fraž	0,5	1	37,0		Rukavce, papirové utěrky a drobný odpad, vzniklý přimo ve skladu (čirý pytel)	
09.11.2018	Brož	0,7	1	444,0		Rukavice, śpičky, stříkačky, valky, papitové utěrky z [3H] dgestoře (3 čné pytiky v černém py	
19.11.2018	Brož	0,4	1	260,0		Rukavice, vata, papírové utěrky z (3H) digestole (1 čirý pytik)	
18.01.2019	Braž	0,5	1	370,0		Rukavice, vista, papirové utěrky, vlaky z [3H] digestoře (1 črý pytik)	
01.03.2019	Tipušťová	1.2	1	135130050	5,6		
01.03.2019	Tibuttions	5,0	1	3,7	100000000000000000000000000000000000000		
09.04.2019	Pokomá	0,1	1	37,0			
05.06.2019	Ero2	1,2	3	370,0		Rukavice, śpičky, stříkačky, vialky, papírové utěrky z [3H] digestoře (3 čné pytiky ve větším py	
30.07.2019	Birkus	1,0	1	3,7			
31,07,2019	Brož	5,9	1	111,0		Adsorpční pliny nasáknuté a) elejem z pumpy, b) zbytky v záchytných nádobách; modný pyte	
15.11.2019	Brož	0,5	2	311,0			
29.11.2019	Eraž	5,6	1	5 550,0		Spalitelný odpad po generálním úklidu v gloveboxu (zejména fitrační papíty a rukovice)	
20.12.2019	Braž	0,6	1	740,0		Spalitelný odpad po generálním úklidu v digestoli (zejména fitrační papity a rukavice)	
20.12.2019	Braf	1.5	1	74,0		Rukavce, papírové utěrky, adsorpční pliny a drobný odpad, vzniký přimo ve skladu	
24.04.2020	(Součet)	26,6	19	8 557,3	5,6	Připraveno k likvidaci (231.3 mCl)	
Sud 115 L "Sudička"				21. 9. 202	0 Předán	so k likvidaci UJV Řež, protokol č.;	
DATUM	JHÉNO	Hmotnost	Počet	Aktivita	[MBq]	Označení, poznámky	
	and the second	(-0)	P400	34	140	And the second se	
						Table: the fourier the constant	

DATUM	JHENO	[kg]	pythi	34	14-C	Oznacen, poznalsky	
18.01.2019	Braž	0,6	1	1 850,0		Jehly, skip (glovebox, 3H-digesto?)	
01.03.2019	Tibultiová	6,7	2	9,3		Převážně špíčky a valky.	
30.07.2019	Birkuš	1.5	2	7,4		Spalitelný odpad (mix), kovový stojánek na zkumavky. 2 kádinky	
30.07.2819	Brož	0,5	1	222,0		Spalitelný odpad (plasty po výměně olege, velké rukavice, mis z digestoře), 1 modný p	
24.04.2628	Eral	0,8	2	740,0		Rukavice, špičky, střkačky, viaky, papirové utěrky z [3H] digestoře po dvou tritiacich	
30.04.2020	Birkuš	0,3	1	3,7		Pevný mix	
04.05.2020	terkuš	4,0	2	3,7		Peuný mix (utěrky, špičky, stříčka, pláště, tác, ksták)	
07.05.2020	Erož	0,7	1	7 400,0		Pevný mix z 3H digestoře a gloveboxu	
05.08.2020	Erož	0,3	1	370,0		Rukavice, špičky, stříkačky, vlaky, papírové utěrky z [3H] digestoře	
02.09.2020	Tlouistiová	0,7	1		0.19		
02.09.2020	Tibuitiová	2,3	2	18,5		Převážně špičky a vláky, utěrky a rukavce	
21.09.2620	Brož	1,2	1	7,4		Drobný odpad vzniklý přímo ve skladu	
21.09.2020	Brož	0,4	1	1 \$50,0		Pevný mix z 34-digestoře a gloveboxu	
21.09.2020	[Součet]	53,0	40	12 482,0	0,2	Přípraveno k likvídaci (337,4 mCi)	

DATUM	JHÉNO	Hmotnost [kg]	Počet pytiú	Aktwita (MBq) 3-H 14-C		Označení, poznámky	
22.09.2020	Brož	0.7	1	3 700.0		Adscrpční materiál z úkldu po likvidaci RaO	
22.09.2628	Brož	0,8	1	3 700,0		3 kanystry po vysokoaktivním 3H-odpadu (>200 GBq)	
09.11.2020	Eraž	0,6	2	\$\$\$5,0		Rukavice, špičky, stříkačky, viaky, papítové utěrky z [3H] digestoře	
22.12.2020	Braž	4.0	1	7 400,0		Rukavce, špičky, stříkačky, viaky, adsorpční meterál po vánočním úklidu (glovebox, digesteře	
04.02.2021	Poldarová	2,0	1	8,9		Pévný me v Biohazard pyth	
12.02.2021	Ero2	1,2	1	14 800,0		Pevný mix z [3H] digestoře po třech tritacích (S pytiků, cca 80 mC každý)	
05.05.2021	Brož	0,8	1	11 100,0		Pevný mix z [3H] digestoře a gloveboxu	
24.05.2021	Braž	0,5	1	1 850.0		Pevný mix z [3H] digestoře a glovebonu po dvou tritacích	
27.07.2021	Ero2	0,7	1	555,0		Pevný mir z [3H] digestoře	
13.08.2021	Brož	1,2	1	151,0		4 kanystry po 3H-odpadu (>2 GBq) + 2 kanystry po vylékání lahvíček	
19.08.2521	Braž	1.5	1	37,0		Drobný odpad vzniklý přimo ve skladu	
03.11.2021	Brož	1,2	1	1 850,0		Rukavce, špičky, stříkačky, valky, adsorpční materál po 3 tritacich (glovebox, digestoře)	
22.04.2822	Hacháček	0,3	1	7,4		Rukavce, valky, pasteurky	
25.04.2022	Tibultiová	0,55	1	0	0,56	Rukavce, špičky, valky	
14.06.2022	Braž	0,5	1	1 850,0		Rukavice, špičky, stříkačky, viaky, adsorpční materiál	



...the end of each radioligand story at IOCB.

collecting-sorting-storage-disintegration-compressing-disposal



https://intraweb.uochb.cas.cz/radiochemie-609.html?lang=en



the IOCB radioactive waste buffer stock, B.01.01



Inventory of short-lived RAW; disintegration time calculations for its disposal in compliance with bylaw 422/2016 Sb.



Inventory of short-lived RAW in buffer stock per year (solid state)



How to reach the facility on www

https://intraweb.uochb.cas.cz/radiochemie-609.html?lang=en ÚOCHB # 9 CS Vyhledat **IOCB PRAGUE** Noticeboard Events Management Administration IR & Payrolls ITS Contacts Misc Documents Signpost Science / Úvod / Science / Core Facilities / Radiochemistry Laboratoř syntézy radioaktivně **Core** Facilities Analytical značených sloučenin Laboratories Biochemical Laboratory of the Synthesis Pharmacology of Radiolabeled Compounds MS NMR Microscopy The Laboratory of Synthesis of Radiolabeled Compounds, formerly named Central Laboratory of Radiochemistr Radioisotopes or Laboratory of Radioisotopes, has been part of the IOCB since its foundation in early fifties of Documents the 20th century. The laboratory was recently renovated offering state-of-the-art instrumentation for carrying Instrumenta out safe synthesis and analyses of radioactively labeled compounds. Virology **High Performance** Custom synthesis of radioactive molecular tracers ³H (²H), ¹⁴C (¹³C), ¹²⁵I Computing Provision of radiometric services Grants Radioactive waste management Collecting, sorting and disposal of radioactive waste 1/2 (B.01.01, close to chemical warehouse) Library Radioactive waste cover letter 😤 ORCID Inventory of ionizing sources at IOCB For Ph.D. Students... Collecting of the Cover sheets of radionuclide sources Postdoc Projects Radiation safety training – initial, annual Lectures Nomination Supervision of radiation safety rules within the Institute Mentoring **ELIXIR CZ - Resources** Contacts Special Bretislav BROŽ, 2:269 Authorizations [radioactive waste, lab manager] **GMO Documents CAS** Code of Ethics Jana HOJCSKOVÁ, 2:269 Michal KRIEGELSTEIN, 2:395 Ales MAREK, 2: 395 [consultation, radiation safety training, radiation safety officer at IOCB, dosimetry] Gabriela NOVÁKOVÁ, 2: 269

[list of radionuclides, radioactive waste]

Documents Instrumentation

Q. Search Just environg	Radiochemistry			
CALORIMETRY	K tack to categories			
CAPILLARY ELECTROPHORESIS	Tri-Carb 2900TR Liquid scintillation analyzer Tri-Carb 2900TR (Perkin Elmer) is a computer-	-		
CYTOMETRY	controlled benchtop liquid scintillation analyzer for detecting small			
ELECTROCHEMISTRY	Tritiation Manifold System	14		
ELEMENTAL ANALYSIS	Tritiation Manifold System (RC Tritec) is based on U-Bed Technology to provide fresh 346 free Tritium for tritiation by tripply beating the UT-bed	C C C C		
EPR SPECTROSCOPY	provide resile. The rest instant for protection of surface result] and of 2-dec.			
OC (GAS CHROMATOGRAPHS)	WIZARD2 2470 Gamma Counter			
GC-MS SYSTEMS	2470 WIZARD ² Automatic Gemma Counter (Perkin Elmer) is a high- throughout instrument with 5 independent well-type detectors measuring			
HPLC (LIQUID CHROMATOGRAPHS)				
LARGE MS INSTRUMENTS	Radio-TLC scanner	-		
LC-MS SYSTEMS	Radio-TLC scanner RITA (RAYTEST, Germany) with software GINA STAR TLC enables automatic quantitative measurement of radioactivity			
MICROSCOPY				
NMR SPECTROSCOPY	Mobile Contamination Monitor CoMO 170	10 m		
OTHER INSTRUMENTS	CoMo 170 (Graetz, Germany) is a portable contamination monitor for α,β and γ surface contamination. The detected events are counted over time	- And		
PCR DETECTION				
PROTEINE & PEPTIDE ANALYSIS & SYNTHESIS	Radio HPLC-LS System An analytical and semi-proparative Waters Alliance HPLC system			
RADIOCHEMISTRY	equipped with the ecoso Separation module, 2985 Yok detrictor and			
SPECIAL OPTICAL INSTRUMENTS	Radio LC-MS-LS System	c mill.		
THERMOPHORESIS	Radio HPLC (Waters Alliance) is an analytical and semi-preparative HPLC system equipped with the e2695 Separation module, 2995 PDA detector			
UV-VIS, IR, RAMAN & CD/VCD				
X-RAY DIFFRACTION	Radio HPLC System Assembly consist of Waters Delta 600 solvents dolivery system, Waters 2470 dual wavelength UV dotector, RAMONA radioactivity detector from			
	Agilent 5975B Inert MSD	ant, Caller		

https://www.uochb.cz/en/instrumentation/c-30/radiochemistry

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UOCHB I

Research Instrumentation

Instrumentation

Agilent 5975B Inert MSD is a quadrupole mass spectrometer coupled to 6890N gas chromatograph. Mass range is up to 1050 u, GC is equipped



MicroBeta2 Microplate Counter





cs Q

Services of Synthetic Radiochemistry

What to consider when using radioactivity at IOCB...?

X

Sbírka zákonů č. 263 / 2016

263 ZÁKON ze dne 14. července 2016 atomový zákon



01. Consultations & Paperwork Radiosafety f2f training (1-2 hours) Personal Protective Equipment Lab monitoring

Dosimetry

contact Radiation Safety Officer

(A. Marek)

02. Radiolabeling Synthesis

Choice of radionuclide

Beta or gamma emitters?

(pros and cons)

Custom synthesis + cryogenic storage

(slide 4-9)

Always happy to help! Questions?

03. Radiometry Wide portfolio of radiometric instruments available (slide 11-12) 04. Radioactive waste management Collecting, sorting and disposal in compliance with Czech law - significant paperwork -

(slide 13)



SBÍRKA ZÁKONŮ

