Title
AN INTRODUCTION TO THE NATIONAL TRITIUM LABELING FACILITY

Authors
Dorsky, A.M.
Morimoto, H.
Saljoughian, M.

Publication Date
1988-06-01

LBL-25588

Lawrence Berkeley Laboratory
UNIVERSITY OF CALIFORNIA

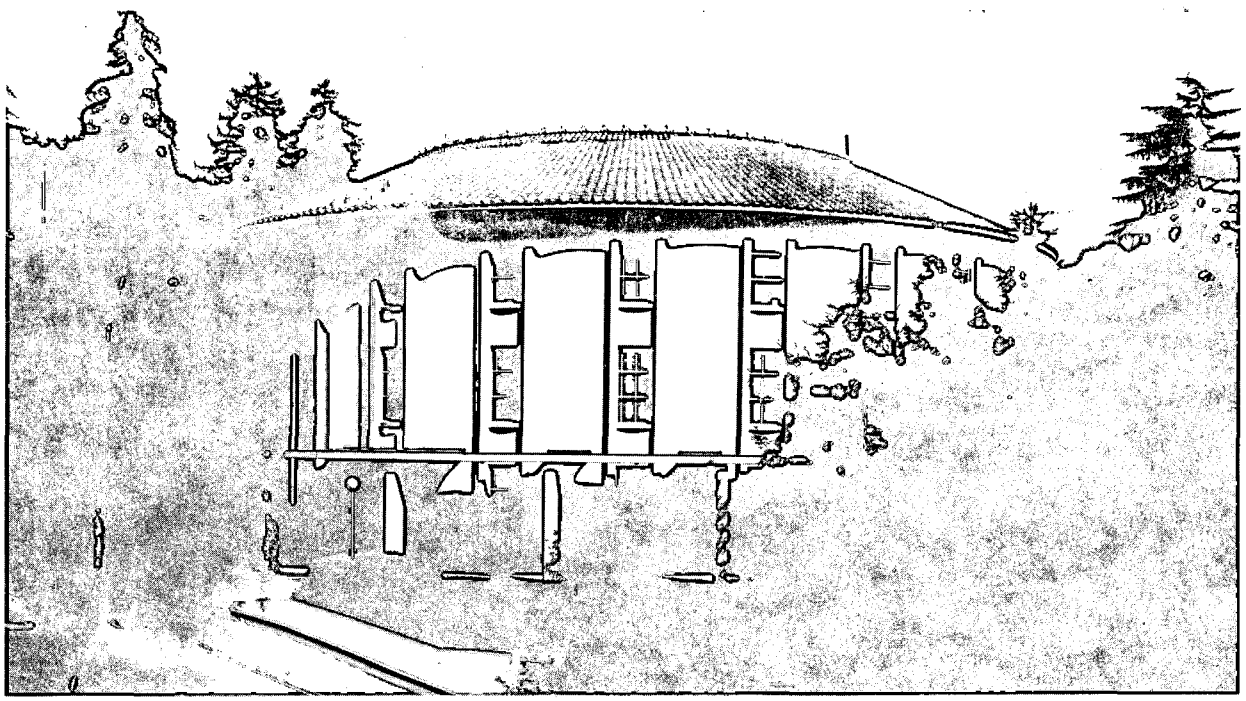
CHEMICAL BIODYNAMICS DIVISION

Presented at the Third International Symposium on Synthesis and Applications of Isotopically Labeled Compounds, Innsbruck, Austria, July 17–21, 1988, and to be published in the Proceedings

An Introduction to the National Tritium Labeling Facility

A.M. Dorsky, H. Morimoto, M. Saljoughian, and H. Rapoport

June 1988



Prepared for the U.S. Department of Energy under Contract Number DE-AC03-76SF00098.

DISCLAIMER

AN INTRODUCTION TO THE NATIONAL TRITIUM LABELING FACILITY.

A. M. Dorsky, H. Morimoto, M. Saljoughian, P. G. Williams and H. Rapoport.

National Tritium Labeling Facility, Lawrence Berkeley Laboratory, Division of Chemical Biodynamics, University of California, Berkeley, CA 94720, U.S.A.

SUMMARY

The facilities and projects of the National Tritium Labeling Facility are described.

INTRODUCTION

Tritium is the most versatile radionuclide in biological research because complex, biologically-important molecules are more readily labeled with this radioisotope than with any other. Some of the early work with non-specific labeling methods (*e.g.* Wilzbach) caused researchers great difficulty due to uncertainty in the position of the label, and because of readily exchangeable tritons in the labeled species. Emphasis was shifted to other isotopes (^{14}C, ^{35}S and ^{125}I) for metabolic fate and similar studies, particularly in the pharmaceutical industry. The development of tritium NMR spectroscopy as a non-destructive method of analysis (ref. 1) is a key factor in making tritium labeling an even more valuable and useful technique than in the past.

The National Tritium Labeling Facility was established in 1982 to function primarily as a venue where investigators from throughout the nation could carry out high level labeling and radiopurification procedures that were not possible in their own laboratories. The facility has grown to a fully equipped laboratory with a permanent staff of 4, and it routinely provides Service for approximately 20 Users per year. The established guidelines involve review of applicants and their projects, and imposition of a standard set of charges for the use of the facility in order to offset the cost of service.

OBJECTIVES AND PROJECTS

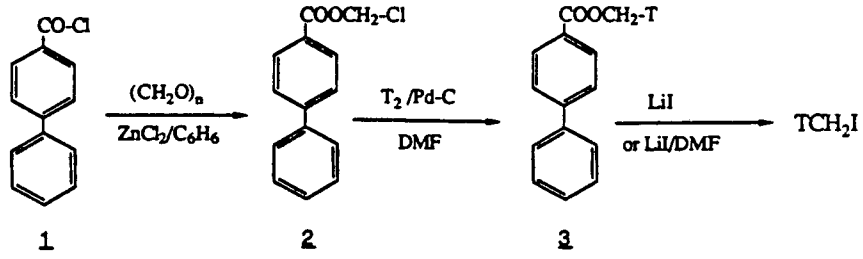
Initial core research projects were directed toward the development of a variety of novel labeling and purification methods. Other high priority work included detailed studies into the mechanisms of various tritiation methods, and the use of tritium as a probe to further the understanding of biological processes. Among the important new techniques and uses of tritium that NTLF Core Research has provided to the biomedical/biochemical community over the last several years are studies of excitation labeling (ref. 2), application of tritium NMR spectroscopy to direct monitoring of glucose metabolism (ref. 3), and new reagents for [^{3}H]-N-methylations and similar reactions (ref. 4). The objectives, areas of interest and curent projects at the NTLF are shown in Table 1.

TABLE 1 - Objectives, Areas of Interest and Projects

LONG-TERM OBJECTIVES	To serve the biomedical/biochemical community as a resource by providing unique and comprehensive facilities for high level tritiations, rigorous radiochemical purification and tritium analyses. The Service and Training functions will be carried out at approximately the same level that they have over the last three years with an anticipated 15 - 24 Users per year.		To provide leadership in the field of tritium labeling - expanding knowledge of the methods and mechanisms by which tritium is incorporated into bio-organic compounds, and the use of tritium as a tool for better understanding of biological processes.	
AREAS of INTEREST	**Analyses** Continued development of analytical techniques for the determination of site and level of tritium labeling.	**Small Molecules** Development of new methods for the synthesis and exchange labeling of tritiated small molecules, including studies of the reaction mechanisms and limitations of these procedures.	**Large Molecules** Synthesis of tritium labeled molecules of biological interest, including proteins or peptides and oligonucleotides.	**Applications** Use of tritiated substrates in the solution of problems in structural biology and general biochemistry, with particular emphasis on the application of tritium NMR techniques.
PPROPOSED PROJECTS	Mass Spectrometry and Radio-Gas Chromatography Tritium NMR of Solids Solution State Tritium NMR	Methyl Iodide Project Photoaffinity Ligand Labeling Labeling of Alanine Labeling of Primary Amines Food Mutagens Metal-Tritide Reducing Agents Excitation Labeling Thermal Activation General Labeling Studies	Synthesis of Tritiated Peptides Synthesis of Tritiated Oligonucleotides	Metabolic Studies Interaction of Maltose with MBP Tritium NMR Study of Catalytic Antibodies NMR Studies of DNA Oligomers Tritium Labeled Free Radical Traps Study of the Generation of Hydrocarbons in Mammalian Tissue

Figure 1, the synthesis of monotritiomethyliodide shown below, is an example of the type of practical and useful chemistry developed at the NTLF.

Scheme I



Scheme II

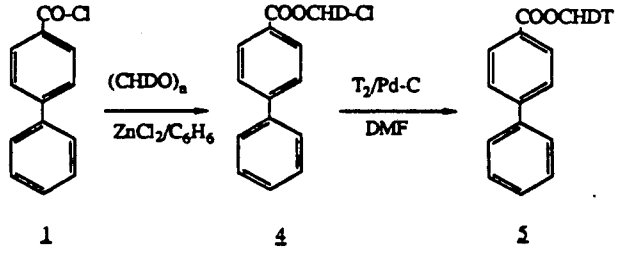


Fig. 1. SYNTHESIS OF MONOTRITIOMETHYL IODIDE

Scheme I illustrates a new and convenient preparation of high specific activity tritiomethyliodide from solid precursor 3. Scheme II illustrates the preparation of the precursor 5 for chiral methyl iodide (ref. 5).

Recently there has been increased interest within the scientific community in the use of high levels of tritium for specific labeling of molecules of biological importance (*e.g.* peptides, proteins and DNA oligomers). The highly labeled products are intended for application to the solution of particular problems in structural biology. Currently, there are very few facilities in industry or academia where this type of work can be accomplished since a combination of facilities for high level tritium labeling, synthesis with such labeled molecules, and analysis for sites and levels of tritiation is required. Although the NTLF has previously had some activity in this area, more emphasis will now be placed on work with these larger bio-molecules. The

combination of equipment already in the Facility (DNA synthesizer, NMR and tritium probe, access to a peptide synthesizer for cold work), proposed purchases over the next two years (microbore HPLC, peptide synthesizer for use with labeled compounds and a mass spectrometer), the addition of post doctoral fellows, and the momentum of several ongoing collaborative projects, will mean that the NTLF will be able to provide the leadership for this type of research.

It is clear that the availability of a facility with equipment and staff dedicated to the synthesis and use of high specific activity tritiated compounds certainly benefits the general scientific community. Hence the initial reasons for the establishment of the NTLF are satisfied by the efficient functioning of a safe, versatile, high level tritiation laboratory combined with comprehensive analytical resources. By the use of pure, well-characterized, tritiated materials in biological systems the NTLF can now make an important contribution to the understanding of fundamental biology.

REFERENCES

1. E.A. Evans, D.C. Warrell, J.A. Elvidge and J.R. Jones, Handbook of Tritium NMR Spectroscopy and Applications, John Wiley & Sons, New York (1985).

2. C.T. Peng, S.F. Ding and R.L. Hua, Preparation of [^{3}H]N,N-Dimethylaniline by Microwave Discharge Activation of Tritium Gas. Absence of Ring Saturation, J. Labelled Compd. Radiopharm., 24 (5), (1987) 505-515.

3. P.S. Carson, Application of Tritium NMR to the Study of Glycolysis in Blood, M.S. Thesis in Biophysics, University of California, Berkeley, December 1986.

4. M. Saljoughian, H. Morimoto, A.M. Dorsky and H. Rapoport, A Novel Synthesis of Monotritiomethyl Iodide: A Reagent for Tritio N-Methylation and Similar Reactions, accepted for presentation at the 1987 Pacific Conference on Chemistry and Spectroscopy, October 28-30, 1987, Irvine, CA.

5. M. Saljoughian, H. Morimoto, A. M. Dorsky, H. Andres, Y. S. Tang, A. Susán and H. Rapoport, A New and Efficient Synthesis of Monotritiomethyl Iodide, J. Labelled Compd. Radiopharm, In Press.

LAWRENCE BERKELEY LABORATORY
TECHNICAL INFORMATION DEPARTMENT
1 CYCLOTRON ROAD
BERKELEY, CALIFORNIA 94720