JOURNAL OF LABELLED COMPOUNDS AND RADIOPHARMACEUTICALS
J Label Compd Radiopharm 2007; **50**: 1276–1286.
Published online 29 October 2007 in Wiley InterScience (www.interscience.wiley.com). DOI: 10.1002/jlcr.1453

Abstracts

14th Workshop of the Central European Division e.V. of the International Isotope Society

Bad Soden, Germany
June 21–22, 2007

Selected Abstracts

Edited by

Volker Derdau* and Jens Atzrodt*

Sanofi-Aventis Deutschland GmbH, GMPK,
Isotope Chemistry and Metabolite Synthesis,
G876 65926 Frankfurt/Main, Germany

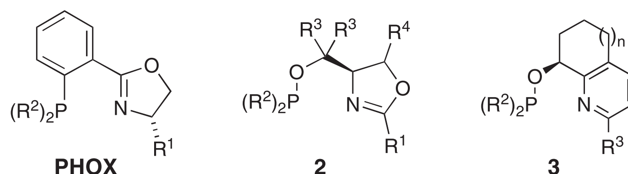
*Correspondence to: Volker Derdau and Jens Atzrodt, Sanofi-Aventis Deutschland GmbH, GMPK, Isotope Chemistry and Metabolite Synthesis, G876, 65926 Frankfurt/Main, Germany.
E-mail: Volker.Derdau@sanofi-aventis.com, Jens.Atzrodt@sanofi-aventis.com

Copyright © 2007 John Wiley & Sons, Ltd.

IRIDIUM-CATALYZED ASYMMETRIC HYDROGENATION

ANDREAS PFALTZ
Department of Chemistry, University of Basel, St Johannsring 19, CH-4056 Basel, Switzerland

For a long time, C_2-symmetric ligands were dominating in asymmetric catalysis. However, more recently it was found that in several metal-catalyzed processes non-symmetric mixed-donor bidentate P,N-ligands are superior to C_2-symmetric P,P- or N,N-ligands. Especially phosphinooxazolines (PHOX ligands) have proven to be highly versatile, very effective ligands in a number of enantioselective catalytic reactions, including hydrogenation of C = N and C = C bonds, Heck reactions, allylic substitutions and conjugate addition to enones.[1]



We have found that iridium complexes with PHOX or other chiral P,N-ligands such as **2** and **3**, which are efficient catalysts for the asymmetric hydrogenation of olefins.[2] The catalysts are readily prepared, air stable and easy to handle. In contrast to rhodium– and ruthenium–phosphine complexes, they do not require the presence of a coordinating group next to the C = C bond and, therefore, considerably expand the application range of asymmetric hydrogenation. Promising results have also been obtained with certain functionalized alkenes, furans and benzofurans. In this lecture, the special properties, the scope and recent applications of these catalysts will be discussed.

REFERENCES

1. Helmchen G, Pfaltz A. *Acc Chem Res* 2000; **33**: 336.
2. (a) Pfaltz A, Blankenstein J, Hilgraf R, Hörmann E, McIntyre E, Menges F, Schönleber M, Smidt SP, Wüstenberg B, Zimmermann N. *Adv Synth Catal* 2003; **345**: 33; (b) Bell S, Wüstenberg B, Kaiser S, Menges F, Netscher T, Pfaltz A. *Science* 2006; **311**: 642; (c) Kaiser S, Smidt SP, Pfaltz A. *Angew Chem Int Ed* 2006; **45**: 5195.

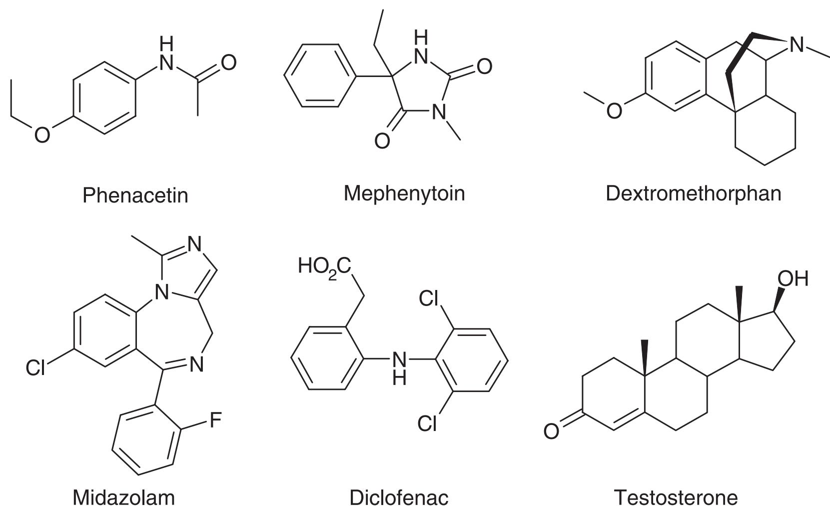
SYNTHESIS OF STABLE ISOTOPICALLY LABELLED INTERNAL STANDARDS FOR DRUG–DRUG INTERACTION (DDI) STUDIES

J. ZIMMERMANN[1*], J. ALLEN[2], J. ATZRODT[1], B. DE-BRUIN[2], V. DERDAU[1], D. GANTZ[1], J. HERBERT[3], S. RADDATZ[1] and S. ROY[2]

[1]Global Isotope Chemistry & Metabolite Synthesis Department, Sanofi-Aventis, Industriepark Höchst, 65926 Frankfurt am Main, Germany
[2]Global Isotope Chemistry & Metabolite Synthesis Department, Sanofi-Aventis, 1, Avenue Pierre Brossolette, 91385 Chilly-Mazarin, France
[3]Global Isotope Chemistry & Metabolite Synthesis Department, Sanofi-Aventis, Willowburn Ave., Alnwick, Northumberland NE66 2JH, UK

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr



The presentation summarizes the recent efforts made to synthesize these compounds. Besides the synthesis starting from commercially available labelled precursors, special emphasis is laid on CH/CD-exchange reactions in combination with biocatalytical methods for the preparation of stable isotopically labelled drug metabolites.[1–3]

REFERENCES

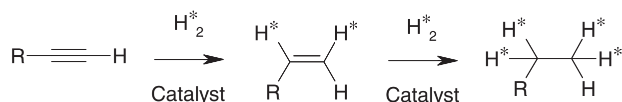
1. For a recent review of CH/CD-exchange reactions, see: (a) Junk T, Catallo WJ. *Chem Soc Rev* 1997; **26**: 401–406; (b) Atzrodt J, Derdau V, Fey T, Zimmermann J. *Angew Chem Int Ed* 2007, in print; doi: 10.1002/anie.200700039.
2. Derdau V, Atzrodt J. *Synlett* 2006; 1918–1922.
3. Webster R, Pacey M, Winchester T, Johnson P, Jezequel S. *Appl Microbiol Biotechnol* 1998; **49**: 371–376.

SOME STUDIES OF THE REDUCTION AND ISOTOPIC EXCHANGE OF UNSATURATED SYSTEMS WITH ISOTOPIC HYDROGEN OVER PT GROUP METALS

WILLIAM J. S. LOCKLEY and E. ALEXAKIS

School of Biomedical and Molecular Sciences, University of Surrey, Guildford GU2 7XH, UK

The reduction of terminal alkynes and alkenes with isotopic hydrogen gas has been studied using a number of model substrates,[1,2] over a range of heterogeneous and homogeneous catalysts (Scheme 1).



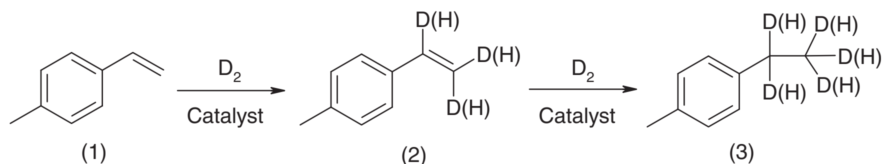
Scheme 1

The studies showed the expected overall cis-addition of isotopic hydrogen but also exemplified a known facile and general terminal alkene isotopic exchange process[3] that takes place concurrently with the reduction and which lowers the isotopic purity of the products in most cases. This process has significance both for the labelling of alkenes by exchange with hydrogen isotopes[4] and for understanding the mechanism of alkene isomerization and hydrogenation.[5] Hence it was examined in some detail.

By employing a screening approach utilizing 4-methylstyrene (1) as a model substrate, the exchange regiochemistry of a range of catalysts (comprising Pd, Ru, Rh, Ir, Pt and Mo species) was examined under conditions of deuterium deficiency (Scheme 2).

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr



Scheme 2

The extent of isotopic exchange at various olefinic positions of the recovered substrate (2) and the extent of reduction and scrambling in the 4-ethyltoluene product (3) were determined by a combination of ^{1}H- and ^{2}H-NMR and GC-MS for 23 catalysts.

The results of the screen, in conjunction with ancillary regiochemistry studies, have thrown light upon the nature of some of the key metal alkyl species[5] involved in alkene hydrogenation. They have also explained some aspects of the regiochemistry of the exchange process, and have identified catalysts that have significantly different labelling orientations to the commonly utilized palladium systems.

REFERENCES

1. Some early studies in this area were carried out as part of the ATHENA collaborative project. See: http://www.chem.gla.ac.uk/Athena [accessed 25 April 2007].
2. Alexakis E. *Ph.D. Thesis*, University of Surrey, 2005.
3. (a) Elvidge JA, Jones JR, Lenk RM, Tang YS, Evans EA, Guildford GL, Warrell DC. *J Chem Res (S)* 1982; 82; (b) Guildford GL, Evans EA, Warrell DC, Jones JR, Elvidge JA, Lenk RM, Tang YS. *Synth Appl Isotop Lab Comp.*; *Proceedings of the First International Symposium*, Duncan WP, Susan AB (eds). Elsevier: Amsterdam, 1983; **1**: 327–330; (c) Alexakis E, Jones JR, Lockley WJS. *J Label Compd Radiopharm* 2006; **49**: 408–409; (d) Alexakis E, Jones JR, Lockley WJS. *Synth Appl Isotop Lab Comp.*; *Proceedings of the 9th International Symposium*, Willis CL, Lockley WJS (eds). *J Label Compd Radiopharm* 2007; in press.
4. See for example: (a) Vader J, Sperling F, Kaspersen, Wagenaars G, Funke C. *Synth Appl Isotop Lab Comp.*; *Proceedings of the 4th International Symposium*, Buncel E, Kabalka GW (eds). Elsevier: Amsterdam, 1992; **4**: 72–73; (b) Voges R, Wartburg BR, Loosli HR. *Synth Appl Isotop Lab Comp.*; *Proceedings of the 2nd International Symposium*, Muccino RR (ed.). Elsevier: Amsterdam, 1986; **2**: 371–376; (c) Moenius Th, Voges R, Susan AB, Jones L, Tarapata R, Shapiro M, Jarema MAC. *Synth Appl Isotop Lab Comp.*; *Proceedings of the 4th International Symposium*, Buncel E, Kabalka GW (eds). Elsevier: Amsterdam, 1992; **4**: 149–154.
5. (a) Doyle AM, Shaikhutdinov ShK, Freund HJ. *J Catal* 2004; **223**: 444–453; (b) Zaera F. *Catal Lett* 2003; **91**: 1–9 and references therein.

Keywords: deuterium; isotope exchange; scrambling; terminal alkene; hydrogenation

NEW DEVELOPMENTS IN ENANTIOSELECTIVE BRØNSTED ACID CATALYSIS – CHIRAL ION PAIR CATALYSIS

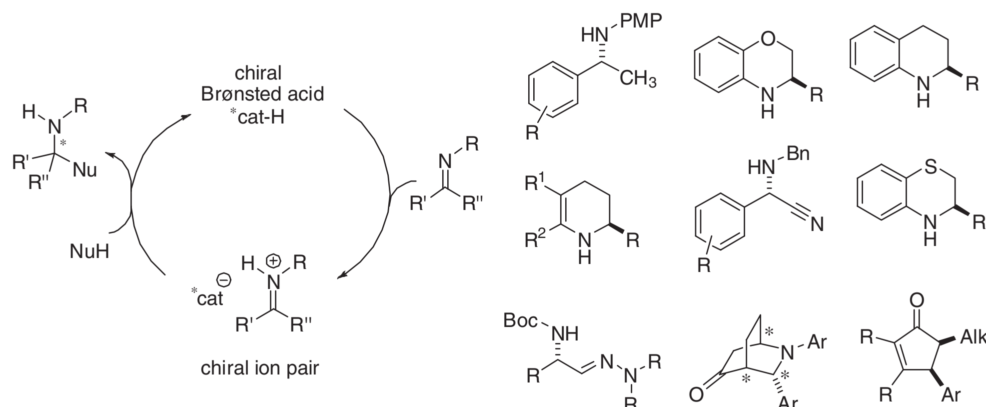
MAGNUS RUEPING

Degussa Endowed Professorship, Institute of Organic Chemistry & Chemical Biology, Johann Wolfgang Goethe-University Frankfurt am Main, Max-von-Laue-Str. 7, 60438 Frankfurt am Main, Germany

The development and application of metal-free catalysts have become an important topic in organic synthesis and catalysis. Recently, chiral Brønsted acids have been shown to be vital alternatives to metal catalysts and examples of highly enantioselective transformations have been reported. These reactions, similar to several enzymatic processes, proceed through hydrogen-bond activation or ion-pair formation.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr



In this presentation our introduction to enantioselective Brønsted acid catalysis will be discussed and new and valuable transformations based on chiral ion pair activation will be highlighted; including the development of the first enantioselective transferhydrogenation of ketoimines[1] and heteroaromatic compounds,[2] hydrocyanations,[3] new cascade reactions for the synthesis of *N*-heterocyclic compounds,[4–6] as well as the new concept of co-operative Brønsted acid catalysis.[5] Furthermore, the first enantioselective Brønsted acid catalyzed activations of carbonyl compounds will be presented.[7] Additionally, efforts to delineate the general requirements for performing asymmetric Brønsted acid catalyzed reactions and the applicability of these catalytic processes to the synthesis of natural products will be outlined.

REFERENCES

1. Rueping M, Sugiono E, Azap C, Theissmann T, Bolte M. *Org Lett* 2005; **7**: 3781.
2. Rueping M, Antonchick AP, Theissmann T. *Angew Chem Int Ed* 2006; **45**: 6751.
3. Rueping M, Sugiono E, Azap C. *Angew Chem Int Ed* 2006; **45**: 2617.
4. Rueping M, Antonchick AP, Theissmann T. *Angew Chem Int Ed* 2006; **45**: 3683.
5. Rueping M, Azap C. *Angew Chem Int Ed* 2006; **45**: 7832.
6. Rueping M, Antonchick AP. *Angew Chem Int Ed* 2007; **46**: 4562–4565.
7. Rueping M, Ieawsuwan W, Antonchick AP, Nachtsheim BJ. *Angew Chem Int Ed* 2007; **46**: 2097.

BINDING STUDIES OF [^{14}C]CAFFEINE TO ARTIFICIAL RECEPTORS AND NOVEL STRATEGIES FOR THE ANODIC PHENOL COUPLING

SIEGFRIED R. WALDVOGEL[1], MARTIN BOMKAMP[1], CARSTEN SIERING[1], ITAMAR MALKOWSKY[1], KARIN LANDROCK[2] and HOLGER STEPHAN[2]

[1]Kekulé-Institut für Organische Chemie und Biochemie, Universität Bonn, Gerhard-Domagk-Str. 1, 53121 Bonn, Germany.

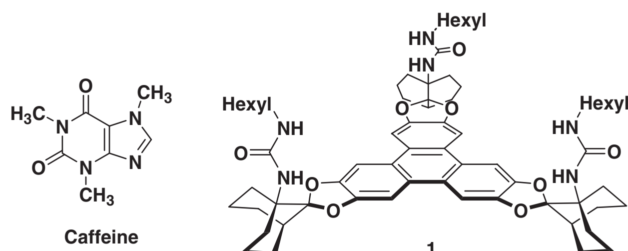
[2]Forschungszentrum Dresden-Rossendorf, Institut für Radiopharmazie, PF 510119, 01314 Dresden, Germany

Caffeine is among the most frequently consumed alkaloidal compounds and is omnipresent in many plants. The traditional significant sources of caffeine in daily life are coffee, black tea and cocoa. Actually, the alkaloid is an ingredient of Cola beverages and energy drinks. Many analgesics sold over the counter contain also caffeine. Because of the wide range of applications and the potential of new analytical tools, caffeine is currently gathering an increasing attention. Recently, we reported the synthesis of functionalized triphenylene ketals which represent the rigid C3v symmetric scaffold of our novel receptors **1**. The cleft-like structure exhibits a high affinity to caffeine and does not interfere with the alkyl groups of the oxopurine system, leading to a novel concept for the molecular recognition of caffeine and related compounds. On the basis of this concept several methods for the detection of

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

caffeine in real life samples have been established. With extraction studies using ^{14}C-labelled caffeine, the potential of this receptor systems and their binding action could be elucidated. This is a key prerequisite for the further development of caffeine detection systems on a dip-stick model. Furthermore, first experiments were done in view of the development of controlled release systems for caffeine in aqueous media.[1–9]

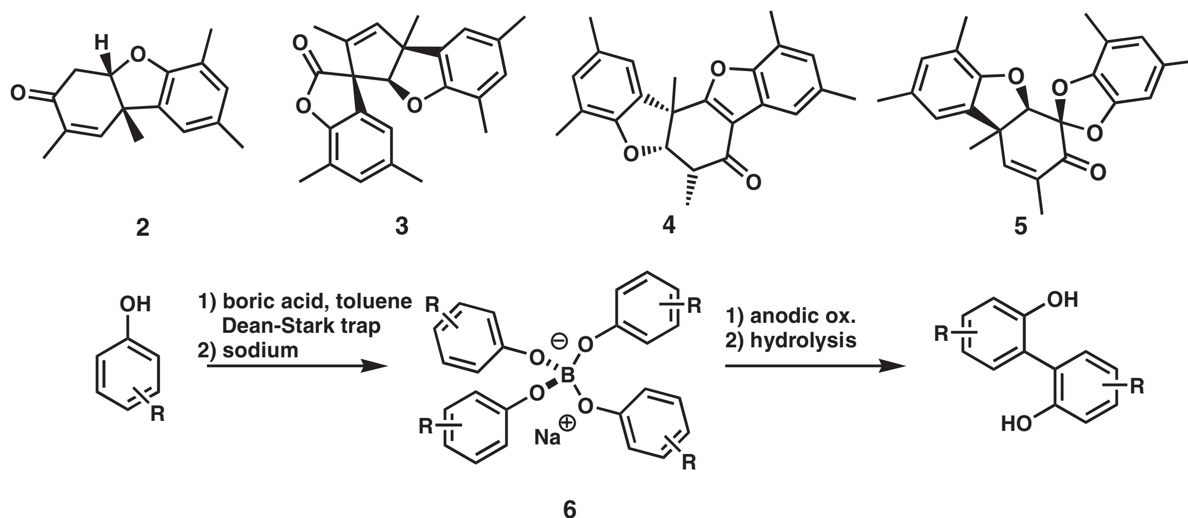


The second part of the talk will deal with the oxidative phenol coupling reaction of simple phenols exhibiting methyl substituents. Such a challenging substrate is 2,4-dimethylphenol. This particular transformation can be difficult since several by-products occur. We studied the electrochemical access to the ortho-coupled dehydrodimer.

Anodic treatment in a basic electrolyte that supports the formation of a molecular architecture called Pummerer's ketone **2** and a variety of pentacyclic structures **3–5**. Noteworthy, **3** is formed as an exclusive diastereomer. The selective ortho-coupling reaction was achieved by two ways which will be outlined in detail.

On the one hand, a borate template **6** can be used. The tetraphenoxy borates can be easily made in large scale. Owing to the ionic nature of **6** no supporting electrolyte is required. This methodology can be applied to several related phenolic substrates.

On the other hand, 2,4-dimethyl phenol can directly be converted on boron-doped diamond anodes. Surprisingly, the electrochemical transformation does not destruct or mineralize the substrate.[10–15]



REFERENCES

1. Waldvogel SR. *Angew Chem Int Ed Engl* 2003; **42**: 604.
2. Waldvogel SR, Froehlich R, Schalley CA. *Angew Chem Int Ed* 2000; **39**: 2472.
3. Schopohl MC, Siering C, Kataeva O, Waldvogel SR. *Angew Chem Int Ed Engl* 2003; **42**: 2620.
4. Siering C, Grimme S, Waldvogel SR. *Chem Eur J* 2005; **11**: 1877.
5. Schopohl MC, Faust A, Mirk D, Froehlich R, Kataeva O, Waldvogel SR. *Eur J Org Chem* 2005; 2987.
6. Siering C, Kerschbaumer H, Nieger M, Waldvogel SR. *Org Lett* 2006; **8**: 1471.
7. Siering C, Beermann B, Waldvogel SR. *Supramol Chem* 2006; **18**: 23.
8. Bomkamp M, Siering C, Landrock K, Stephan H, Froehlich R, Waldvogel SR. *Chem Eur J* 2007; **13**: 3724.
9. Bomkamp M, Artiukhov A, Kataeva O, Waldvogel SR. *Synthesis* 2007; 1107.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

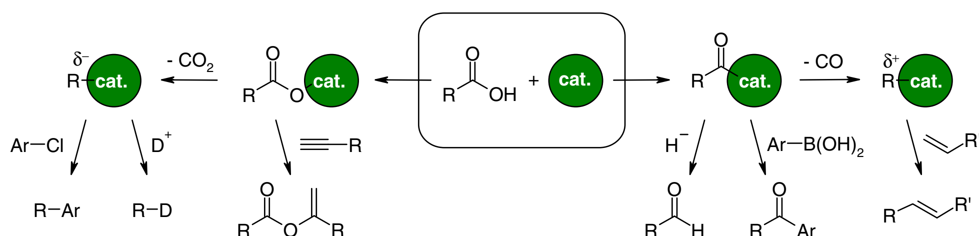
10. Malkowsky I, Rommel CE, Wedeking K, Froehlich R, Bergander K, Nieger M, Quaiser C, Griesbach U, Puetter H, Waldvogel SR. *Eur J Org Chem* 2006; 241.
11. Malkowsky IM, Froehlich R, Griesbach U, Puetter H, Waldvogel SR. *Eur J Inorg Chem* 2006; 1690.
12. Malkowsky IM, Rommel CE, Froehlich R, Griesbach U, Puetter H, Waldvogel SR. *Chem Eur J* 2006; **12**: 7482.
13. Rommel CE, Malkowsky I, Waldvogel SR, Puetter H, Griesbach U. WO 2005075709 A2.
14. Malkowsky IM, Griesbach U, Putter H, Waldvogel SR. *Eur J Org Chem* 2006; 4569.
15. Griesbach U, Puetter H, Waldvogel SR, Malkowsky I. WO 2006077204 A2.

CARBOXYLIC ACIDS AS SUBSTRATES IN CATALYSIS

L. J. GOOßEN, N. RODRÍGUEZ, B. MELZER, C. LINDER, C. OPPEL

Institut für Organische Chemie, TU Kaiserslautern, Erwin-Schrödinger-Straße, D-67663 Kaiserslautern, Germany

Carboxylic acids promise to be advantageous substrates for catalytic transformations as they are available in great structural diversity at low cost, and can be produced by environmentally benign methods. Over the last years, we have developed catalytic transformations in which different reaction modes of carboxylic acids are utilized. On the one hand, they can react as *carbon electrophiles*: after activation with coupling reagents or simple conversion to esters, they oxidatively add to transition metal catalysts giving rise to acyl-metal species, which can either be reduced to aldehydes or coupled with nucleophiles. At elevated temperatures, such acyl-metal species decarbonylate, so that carboxylic acids can also serve as synthetic equivalents for aryl or alkyl halides.



On the other hand, carboxylic acids can be efficiently decarboxylated using new copper catalysts to give aryl-metal intermediates. Usually, these *carbon nucleophiles* are protonated by the reaction medium to give the corresponding arenes – in the presence of D_2O, the deuterated arenes are produced selectively. If carboxylate salts are employed instead of the free acids, the aryl-metal species resulting from the decarboxylation can be utilized for the synthesis of biaryls in a novel cross-coupling reaction with aryl halides, thus replacing organometallic reagents.[1–4]

REFERENCES

1. (a) Gooßen LJ, Deng G, Levy LM. *Science* 2006; **313**: 662–664; (b) Gooßen LJ, Rodríguez N, Melzer B, Linder C, Deng G, Levy LM. *J Am Chem Soc* 2007; **129**: 4824–4833.
2. Gooßen LJ, Paetzold J. *Angew Chem* 2004; **116**: 1115–1118.
3. Gooßen LJ, Paetzold J. *Angew Chem* 2002; **114**: 1285–1289.
4. Gooßen LJ, Ghosh K. *Angew Chem* 2001; **113**: 3566–3568.

TETRAMIC ACIDS AND TROPANES: FROM NATURAL PRODUCT SYNTHESIS TO ASYMMETRIC CATALYSIS WITH NATURAL PRODUCTS

SABINE LASCHAT

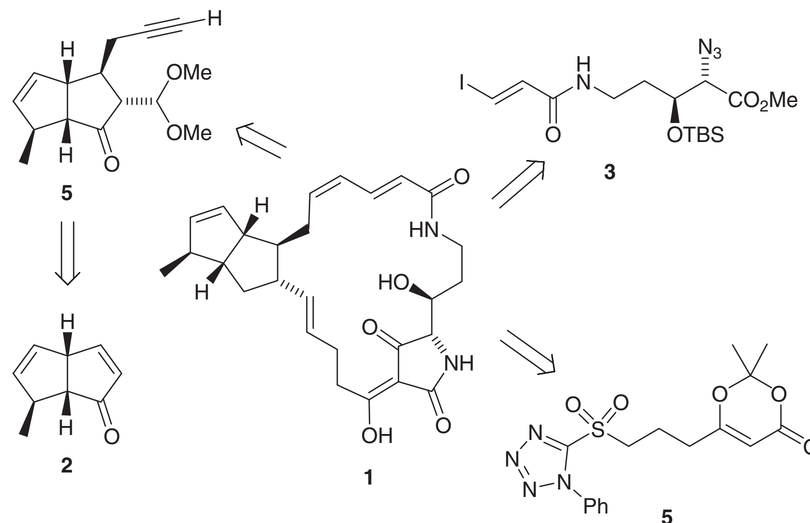
Institut für Organische Chemie, Universität Stuttgart, Pfaffenwaldring 55, D-70569 Stuttgart, Germany

Marine organisms produce a variety of secondary metabolites. The cytotoxic natural product cylindramide **1** has been isolated in 1993 by Fusetani from the marine sponge *Halichondria cylindrata*.[1] We have recently accomplished

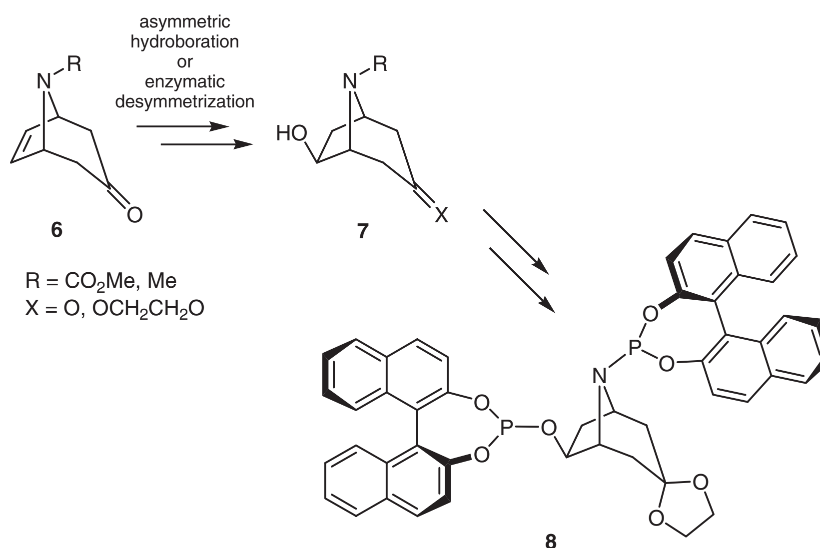
Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

the first enantioselective total synthesis of cylindramide **1**.[2] Key steps of our convergent approach are: (1) a tandem Michael addition/electrophilic trapping starting from pentalene derivative **2** to give the bicyclic ketone **5**; (2) a Sonogashira coupling of hydroxyornithine unit **3**; (3) a Julia–Kocienski olefination of tetrazolylsulfone **4**; and (5) a macrolactamization. The tetramic acid was formed by a Lacey–Dieckmann condensation. In addition to the synthetic challenges, recent results of the biological studies will be reported.



However, natural products are not only interesting because of their biological properties. Especially, the small ones are often useful chiral building blocks for ligands, which can be applied in asymmetric catalysis. We have functionalized tropane alkaloids such as tropenone **6** via asymmetric hydroboration[3] or enzymatic desymmetrization[4] to the corresponding tropinol **7**, which could be further converted to the phosphoramidite **8**. Ligand **8** and derivatives thereof were successfully employed in catalytic asymmetric hydrogenations and 1,4-additions.[5]



REFERENCES

1. Kanazawa S, Fusetani N, Matsunaga S. *Tetrahedron Lett* 1993; **34**: 1065.
2. Cramer N, Buchweitz M, Laschat S, Frey W, Baro A, Mathieu D, Richter C, Schwalbe H. *Chem Eur J* 2006; **12**: 2488.
3. Cramer N, Laschat S, Baro A, Frey W. *Synlett* 2003; 2175.
4. Cramer N, Laschat S, Baro A. *Synlett* 2003; 2178.
5. Cramer N, Laschat S, Baro A. *Organometallics* 2006; **25**: 2284.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

[^{18}F]FBAM AND [^{18}F]FBOM: NOVEL THIOL-REACTIVE PROSTHETIC GROUPS DERIVED FROM 4-[^{18}F]FLUOROBENZALDEHYDE

L. VOGLER, M. BERNDT, J. PIETZSCH AND F. WUEST

Institut für Radiopharmazie, Forschungszentrum Dresden-Rossendorf, Dresden, Germany

The incorporation of ^{18}F into peptides and proteins usually takes advantage of the prosthetic groups, also referred to as bifunctional labeling agents. This approach comprises ^{18}F incorporation into a small organic molecule capable of being linked to peptides and proteins under mild conditions.

Two new prosthetic groups derived from 4-[^{18}F]fluorobenzaldehyde for the mild and selective conjugation to thiol group-containing biomacromolecules are described: *N*-[6-(4-[^{18}F]fluorobenzylidene)aminooxyhexyl]-maleimide ([^{18}F]FBAM) and 4-[^{18}F]fluorobenz-aldehyde-*O*-(2-{2-[2-(pyrol-2,5-dion-1-yl)ethoxy]ethoxy}ethyl)oxim ([^{18}F]FBOM).

The aminooxy-functionalized labeling precursor for radiosynthesis of [^{18}F]FBAM was prepared in a convenient three-step synthesis sequence in a total yield of 59%. The corresponding labeling precursor for the radiosynthesis of [^{18}F]FBOM succeeded in a four-step reaction sequence in 14% total yield. Formation of the prosthetic groups [^{18}F]FBAM and [^{18}F]FBOM was achieved through condensation reactions with [^{18}F]fluorobenzaldehyde to form the desired oximes in radiochemical yields of 20–30% ([^{18}F]FBAM) and of 14–19% ([^{18}F]FBOM), respectively. The syntheses were carried out in a remotely controlled radiofluorination module allowing the convenient and reliable performance of the radiolabeling reactions. The radiochemical purity exceeded 95% and the specific activity ranged from 50 to 80 GBq/μmol. The total synthesis time was 70–80 min. The lipophilicity was determined to be $\log P = 2.71$ for [[^{18}F]FBAM and $\log P = 0.84$ for [^{18}F]FBOM. Both prosthetic groups could successfully be used in the radiolabeling of thiol group-containing compounds such as glutathion, low-density lipoproteins and modified neurotensin derivatives.

MONO TRITIUM-LABELLED COMPOUNDS: CALCULATION OF SPECIFIC ACTIVITY AS A FUNCTION OF TIME VERSUS RENEWED DETERMINATION – PRACTICAL OUTCOMES

COR G. M. JANSSEN*, STEVE V. H. VAN HOEGAERDEN and WILLY L. M. VERLUYTEN

Johnson & Johnson Pharmaceutical Research & Development, A Division of Janssen Pharmaceutica N.V., B-2340 Beerse, Belgium

Some 20 years ago the growing number of mono-tritiated compounds in stock in our laboratories and the emerging routine availability of computers suggested a mathematical approach for the calculation of the specific activity (s.a.) as a function of time, based on a s.a. measured in the past. Our customers would benefit from the ability to compute the s.a. at any moment and efficiency would be increased by avoiding time-consuming re-determinations.

Calculation of the specific activity is only feasible if the ratio of the various labelled forms (i.e. unlabelled versus mono-labelled versus di-labelled, etc.) is known. In our hands, most products were obtained via Br–T exchange of mono-bromo compounds, giving rise to the formation of mono-tritiated compounds. (Traditionally exchanges use long reaction times at atmospheric pressure, sometimes leading to the formation of some di-tritiated compounds. Recently, it was shown that excellent results were obtained with lower tritium pressure and much shorter reaction times. This might diminish the formation of di-tritiated compounds.[1]) Assuming labelled and unlabelled compounds to suffer equally from radiolysis, a formula was developed for the mathematical calculation of their s.a. For three compounds, the outcome of the calculation was compared with the re-determination of the specific activity via the traditional UV, weight determination and counting method and also via mass spectrometric determination, a technique made possible by the now general availability of LC-MS. For one of the compounds, the LC-MS technique also allowed for a previously unreported check on identity and quantity of the decay product (only one reference reports the reaction of a tritium-derived cation from 1,4-ditritiobenzene in gaseous methanol mixtures, stored for 3.5 months and a qualitative analysis of the formed anisole isomers[2]).

Tritium-specific activity as a function of time

The general formula for isotopic decay is expressed as follows:

$$N_t = N_0.\mathrm{e}^{c(t_0-t)}$$

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

where N_t is the number of radioactive molecules at time t, N_0 the original number of radioactive molecules, t_0 the start time of measurement, and t the present time.

For tritium, c can be derived from the half-life (expressed in days):

$$0.5 = e^{-c.t}0.5 \quad \text{or} \quad \ln 0.5 = -ct_{0.5}$$

From $t_{0.5}= 12.26 \times 365$ days follows $c= 0.0001549$/day.

The specific activity at any time is

$$\frac{\text{fraction of ra molecules}}{\text{fraction of ra molecules} + \text{original fraction of non-ra molecules}} \times \text{s.a.}_{max}$$

or

$$\left[\left(e^{c(t_0-t)} \times \frac{\text{s.a.}_{init}}{\text{s.a.}_{max}}\right) / \left\{\left(e^{c(t_0-t)} \times \frac{\text{s.a.}_{init}}{\text{s.a.}_{max}}\right) + \left(1 - \frac{\text{s.a.}_{init}}{\text{s.a.}_{max}}\right)\right\}\right] .\text{s.a.}_{max}$$

where s.a._{init} is the originally measured s.a. and s.a._{max} is the maximum s.a. for the isotope, i.e. 1080 GBq/mmol.

Dividing by $\text{s.a.}_{init}/\text{s.a.}_{max}$ leads to the final formula used for calculation:

$$\left\{(e^{c(t_0-t)}) / \left(e^{c(t_0-t)} + \frac{\text{s.a.}_{max}}{\text{s.a.}_{init}} - 1\right)\right\} .\text{s.a.}_{max}$$

Traditional measurement via UV, weight determination and counting: In the traditional determination, a short LC method is applied. Two known amounts of unlabelled reference compound are injected and the UV areas are measured. Next, the radioactive compound is injected, the area of the UV peak is determined and the complete peak is collected for counting, finally giving the radioactivity per mg compound and hence the s.a. per mmol. This determination is only possible if the compound is UV visible. Since a short chromatographic method is applied, pure reference material must be used for calibration; chemically impure radiolabelled material may also introduce errors. The estimated deviation using this technique will be approximately 3–5 %.

Measurement of the specific activity via the LC-MS technique: A single injection of the radiochemical compound on LC-MS gives a chromatogram of which the product peak is scanned to yield a mass spectrum. In this mass spectrum, the protonated peak of the unlabelled compound (protonated $(M+0)$-peak: Area 1) and the protonated peak of the radiolabelled compound (protonated $(M+2)$-peak: Area 2) can clearly be distinguished. Both areas are measured, and the $(M+2)$-peak is corrected for contributions of the unlabelled $(M+0)$-peak (from $^{13}C_2$ and ^{37}Cl).

The corrected $(M+2)$ area can be calculated as follows and equals:

$$(\text{Area } 2) - n(n-1)(1.1/98.9)^2.(\text{Area } 1) - m.(24.47/75.53).(\text{Area } 1)$$

where n and m are number of carbons and chlorines in the compound, 98.9 and 1.1: natural abundance of ^{12}C and of ^{13}C, 75.53 and 24.47: natural abundance of ^{35}Cl and of ^{37}Cl. Lesser ^{18}O (0.20%) and ^{15}N (0.37%) contributions are omitted.

The s.a. for tritium-labelled compounds follows the from:

$$\text{s.a.} = \{\text{corr. Area } 2/(\text{Area } 1 + \text{corr. Area } 2)\}.1080 \text{ GBq/mmol}$$

Some limiting factors also hold for the LC-MS method: most important, the compound must be ionizable. The equipment needs tuning to enhance mass resolution. Choice of correct exact mass may give some deviation (up to approximately 3–5 %) and the amount of material must be within the area of 'non-saturation' (linear area). Inside this area the ratio of protonated $(M+0)$- and $(M+2)$-peaks remains constant.

Practical outcomes: Results from the measurements via the mentioned techniques on three different compounds are given in Table 1.

The product formed from decay of the tritium label in ethanol-dissolved levocabastine (Scheme 1) was identified via LC-MS. If ^3H-levocabastine (^3H-**1**), levocabastine **1** and ethoxy levocabastine (EtO-**1**) suffer equally from radiolysis, then the decrease of radioactive material should equal the increase of the latter. The initial s.a. (591 GBq/mmol) gives the original ratio **1**:^3H-**1**: 0.453:0.547. LC-MS compound areas (after correction for the contribution of the unlabelled compound in the area of the labelled compound) as of today gives the areas (**1**:^3H-**1**:EtO-**1**): 436:284:232; or normalized to the original amount of unlabelled **1** : 0.453:0.295:0.241. In this the sum of ^3H-**1** and of EtO-**1** (0.536) equals the original amount of ^3H-**1** (0.547), proving the quantitative formation of only one decay product (EtO-**1**) and of no unlabelled product (Scheme 1).

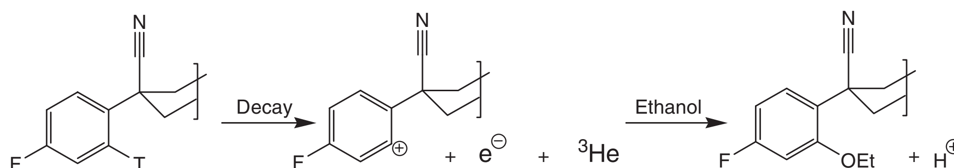
Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr

Table 1 Comparison of specific activities via the methods described

Compound	Original s.a. (GBq/mmol)	Calculated s.a. (GBq/mmol)	UV-det. s.a. (GBq/mmol)	LC-MS det. s.a. (GBq/mmol)
1	591 (May 1994)	399	396	426
2	607 (May 1999)	487	492	482
3	814 (August 1980)	438	393	430

1, Levocabastine ($C_{26}H_{29}FN_2O_2$), RA 96% pure, stored in ethanol; **2**, Loperamide ($C_{29}H_{33}ClN_2O_2$), RA 89.5% pure; **3**, Haloperidol ($C_{21}H_{23}ClFNO_2$), RA 45% pure, a 3-year-old batch (RA 95% pure) obtained via purification was used for the UV measurement.



Scheme 1

Remarks

- The s.a. of the original compounds was determined immediately after purification of the compound via the UV method.
- Possible formation of small amounts of di-tritiated compounds was never checked in the past.
- Corrections for the redetermination of the s.a. via the UV method in loperamide and haloperidol were needed, because impure material was used. The amount of decay product as determined via LC-MS and co-measured under the UV peak was taken into account.
- For repurified batches the calculation of the s.a. based on the mother batch still holds if attention is paid not to cut off part of the product peak. In the latter case isotope effects might shift the ratio labelled/unlabelled.

Conclusion

- Calculation of the s.a. as a function of time on mono-labelled compounds (here: tritium) leads to correct results, improving efficiency and providing up-to-date information to our customers.
- Determination via LC-MS is most reliable and independent on purity of sample. This determination needs less manipulation and does not depend on availability of reference compound.
- Provided purity of reference compound and radioactive sample, both techniques lead to the same results within the same error range.
- The decay product stemming from the decay-derived cation and the solvent is quantitatively formed. There is no indication for formation of unlabelled compound (i.e. no T–H replacement).

REFERENCES

1. Janssen CGM, Verluyten WLM, Vliegen M. *J label Compd Radiopharm* 2006; **49**: 219–222.
2. Speranza M. *Tetrahedron Lett* 1980; **21**: 1983–1986.

Copyright © 2007 John Wiley & Sons, Ltd.

DOI: 10.1002.jlcr