JOC*Article*

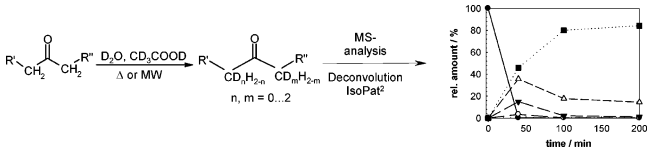
An Algorithm for the Deconvolution of Mass Spectrosopic Patterns in Isotope Labeling Studies. Evaluation for the Hydrogen−Deuterium Exchange Reaction in Ketones

Christian C. Gruber,[†] Gustav Oberdorfer,[†] Constance V. Voss,[†] Jennifer M. Kremsner,[‡] C. Oliver Kappe,[‡] and Wolfgang Kroutil*,[†]

Institute of Chemistry and Christian Doppler Laboratory for Microwave Chemistry (CDLMC), Karl-Franzens-University Graz, Heinrichstrasse 28, A-8010 Graz, Austria

wolfgang.kroutil@uni-graz.at

Received April 20, 2007



An easy to use computerized algorithm for the determination of the amount of each labeled species differing in the number of incorporated isotope labels based on mass spectroscopic data is described and evaluated. Employing this algorithm, the microwave-assisted synthesis of various α-labeled deuterium ketones via hydrogen−deuterium exchange with deuterium oxide was optimized with respect to time, temperature, and degree of labeling. For thermally stable ketones the exchange of α-protons was achieved at 180 °C within 40−200 min. Compared to reflux conditions, the microwave-assisted protocol led to a reduction of the required reaction time from 75−94 h to 40−200 min. The α-labeled deuterium ketones were reduced by biocatalytic hydrogen transfer to the corresponding enantiopure chiral alcohols and the deconvolution algorithm validated by regression analysis of a mixture of labeled and unlabeled ketones/alcohols.

Introduction

Isotope labeling, in particular ^{13}C and deuterium marking, is one of the most widespread methods for investigating molecular structures and reaction mechanisms[1] and additionally plays an important role in deducing the metabolic pathways of drugs and biologically active molecules.[2] Deuterium-labeled compounds recently also attracted attention in the high-throughput determination of enantiomeric and diastereomeric excess by mass spectrometry.[3] Not surprisingly, there is therefore an ever increasing demand for the synthesis of commercially unavailable, labeled starting materials and their accurate characterization.

Because isotope labeling generally relies on the substitution of atoms with the same chemical properties but different atomic mass, mass spectrometry is often the analytical tool of choice.[4] Thus, a significant amount of work has been carried out to date on the detection of deuterium-labeled molecules with use of mass spectrometry.[5] For our research on alcohol dehydrogenases, we required α-labeled ketones as well as *β*-labeled secondary alcohols as starting materials and envisaged applying a rapid microwave-assisted hydrogen/deuterium exchange process for their efficient synthesis.[6,7] In this context, however, we noticed that for reaction optimization a reliable characterization

* To whom the correspondence should be addressed. Phone: +43-316-380-5350. Fax: +43-316-380-9840.
[†] Institute of Chemistry.
[‡] Christian Doppler Laboratory for Microwave Chemistry.

(1) (a) Simon, H.; Palm, D. *Angew. Chem., Int. Ed.* **1966**, *5*, 920. (b) Ozaki, A. *Isotopic Studies of Heterogeneous Catalysis*; Kodansha/Academic Press: New York, 1977. (c) De Meer, K. M.; Roef, M. J. M.; Kulik, W.; Jakobs, C. *Isot. Environ. Health Stud.* **1999**, *35*, 19.

(2) (a) Guaragna, A.; De Nisco, M.; Pedatella, S.; Pinto, V.; Palumbo, G. *J. Labelled Compd. Radiopharm*. **2006**, *49*, 675. (b) Miranker, A.; Robinson, C. V.; Radford, S. E.; Alpin, R. T.; Dobson, C. M. *Science* **1993**, *262*, 896. (c) *Synthesis and Applications of Isotopically Labelled Compounds*; Pleiss, U., Voges, R., Eds.; John Wiley & Sons: Chichester, UK, 2001; Vol. 7. (d) Buncel, E. *Synthesis and Applications of Isotopically Labelled Compounds*; Kabalka, W., Ed.; Elsevier Science Ltd.: Amsterdam, The Netherlands, 1992. (e) Vederas, J. C. *Nat. Prod. Rep*. **1987**, *4*, 277. (f) Thomas, A. F. *Deuterium Labeling in Organic Chemistry*; Appleton-Century-Crofts: New York, 1971.

(3) (a) Tsukamoto, M.; Kagan, H. B. *Adv. Synth. Catal*. **2002**, *344*, 453. (b) Reetz, M. T. *Angew. Chem., Int. Ed.* **2001**, *40*, 284.

(4) Heumann, K. G. *Int. J. Mass Spectrom.* **1982**, *45*, 87.

(5) (a) Dienes, T.; Pastor, S. J.; Schurch, S.; Scott, J. R.; Yao, J.; Cui, S. L.; Wilkins, C. L. *Mass Spectrom. Rev.* **1996**, *15*, 163−211. (b) Loaiza, A.; Zaera, F. *J. Am. Soc. Mass Spectrom.* **2004**, *15*, 1366.

10.1021/jo070831o CCC: $37.00
Published on Web 06/20/2007

SCHEME 1. Hydrogen−Deuterium Exchange in the α-Positions of 3-Octanone

of the labeling degree was required to be able to properly optimize the synthesis of the desired labeled ketones. We herewith present a novel algorithm for the exact determination of the degree of labeling that is based on the evaluation of mass spectroscopic data by computerized methods.

Results and Discussion

Generation of Deuterium-Labeled Ketones. Our studies commenced with the investigation of the hydrogen−deuterium exchange reaction in simple ketones using deuterium oxide (D_2O) as the deuterium source and CD_3COOD as a catalyst. Deuterium exchange in D_2O/CD_3COOD[6] of a rather simple ketone like 3-octanone **1a** employing controlled microwave heating in sealed vessels or conventional reflux conditions proceeded via various deuterated intermediates. Considering the four acidic α-protons of **1a**, five derivatives are possible, namely d_1, d_2, d_3, d_4, and the unlabeled starting material d_0.

In order to follow the degree of deuterium labeling in 3-octanone **1a** during the reaction and to characterize the obtained products, low-resolution MS analysis may be considered as an appropriate method. However, the mixture of compounds labeled at the two different α-positions to varying degrees led to a rather complex mass spectrum (Figure 1b).

We expected that the deconvolution of such a pattern is well described in the literature. To our astonishment we found a rather unsatisfying procedure in two standard reference books.[8] Essentially, this procedure proposes to take the abundance of the lowest mass peak in the area of interest that can only be attributed to one derivative and subtract the corresponding abundance of this derivative from the abundance at higher mass and then continue with the next higher substituted derivative, and by using this iterative procedure the fraction of each derivative is obtained. It is evident that this method is rather inaccurate, since it relies completely on the accuracy of a single abundance at a single mass (the first one), which leads to a high inaccuracy. An additionally performed thorough search in the primary literature did not lead to any more sophisitcated algorithms for this specific deconvolution problem.

Analyzing the problem in more detail, we listed the abundance pattern of a hypothetical organic (carbon atom-based) starting material at the different *m*/*z* ratios of interest (Table 1, two left columns). The pattern results on the one hand from the natural distribution of ^{13}C and other atoms showing significant isotope distributions (e.g., Cl) as well as from fragmentation. For these theoretical considerations we have chosen a starting material that can be labeled only in two positions. Since we deal with low-resolution MS, the abundance pattern of the single deuterium labeled derivative d_1 has to be the same as the unlabeled one but shifted one mass unit upward. The d_2-derivative pattern is shifted two mass units to higher mass and so on. The pattern of a mixture (the analyte) results from the sum of the fraction of each derivative multiplied by the abundance at the corresponding mass. For instance, in Table 1 at *m*/*z* of M + 2 the abundance of the analyte (here z_2) is proportional to $c \cdot x(d_0) + b \cdot x(d_1) + a \cdot x(d_2)$, where $x(d_i)$ is the fraction of the corresponding derivative. Therefore, for the simple example in Table 1 five equations (for each *m*/*z* M to M + 4 one equation) are obtained.

In general, considering the number of equations that are obtained and the number of derivatives, the number of equations is higher than the number of derivatives. Taking the example of Table 1, we have three derivatives (d_0-d_2) but five equations (M until M + 4, or z_0 to z_4). Therefore we deal with an overdetermined linear equation system, which cannot be exactly solved (for real data). The equations can be written in a mathematical way, by using matrixes (eq 1), whereby **A** is the matrix (for the example in Table 1, it is the one in the box, the places without a value are set to 0), **x** is a vector containing the fraction of each derivative {for the example $[x(d_0), x(d_1), x(d_2)]^T$, T means transposed}, and **b** are the abundances of the analyte, e.g., $(z_0, z_1, z_2, z_3, z_4)^T$.

$$\mathbf{A} \cdot \mathbf{x} = \mathbf{b} \tag{1}$$

where **A** is the $m \times n$ matrix, $m \neq n$, m is the number of columns, and n is the number of rows; **x** is a vector ($1 \times m$), containing the fraction of each derivative, i.e., $[x(d_0), x(d_1), ..., x(d_m)]^T$; and **b** is a vector ($1 \times n$), representing the abundances of the analyte, i.e., $(z_0, z_1, ..., z_n)^T$.

Since we have an overdetermined system and we deal with data of measurements which have a certain error, an algorithm to minimize the error will definitely be superior to the rather cumbersome literature procedure.[8] Applying the least-squares method to the overdetermined linear equation system, the solution is found by applying the pseudoinverse matrix of **A** which is $(\mathbf{A}^T \cdot \mathbf{A})^{-1} \cdot \mathbf{A}$.[9] From a mathematical point of view, it is required that the column vectors of A have to be linear independent, which is fulfilled for our problem.

Therefore **x** of eq 1 can easily be obtained by the expression given in eq 2.

$$\mathbf{x} = (\mathbf{A}^T \cdot \mathbf{A})^{-1} \cdot \mathbf{A} \cdot \mathbf{b} \tag{2}$$

Although this expression looks rather short, the determination of **x** requires a number of mathematical operations. Standard programs like Microsoft Excel can deal with these expressions. However, to circumvent problems associated with Excel software we prepared an Excel sheet for both PC and Macintosh computer platforms.[10] Since the same question arises when dealing with ^{13}C or ^{17}O labeling, the identical sheet is also applicable for these derivatives. In the case of tritium, ^{18}O, or

(6) (a) Fodor-Csorba, K.; Galli, G.; Holly, S.; Gacs-Baitz, E. *Tetrahedron Lett.* **2002**, *43*, 3789. (b) Jones, J. R.; Lockley, W. J. S.; Lu, S. Y.; Thompson, S. P. *Tetrahedron Lett.* **2001**, *42*, 331. (c) Loupy, A.; Petit, H.; Hamelin, J.; Texier Boullet, F.; Jacquault, P.; Mathe, D. *Synthesis* **1998**, 1213. (d) Anto, S.; Getvoldsen, G. S.; Harding, J. R.; Jones, J. R.; Lu, S. Y.; Russel, J. C. *J. Chem. Soc., Perkin Trans. 2* **2000**, 2208.

(7) For a general review on controlled microwave synthesis, see: (a) Kappe, C. O. *Angew. Chem., Int. Ed.* **2004**, *43*, 6250. (b) For a review on microwave-assisted radiochemistry, see: Jones, J. R.; Lu, S.-Y. In *Microwaves in Organic Synthesis*, 2nd ed.; Loupy, A., Ed.; Wiley-VCH: Weinheim, Germany, 2006; Chapter 13, pp 435−462.

(8) (a) Hesse, M.; Meier, H.; Zeeh, B. *Spectroscopic Methods in Organic Chemistry*; Thieme: New York, 1997; pp 256−267. (b) Budzikiewicz, H. *Massenspektrometrie*; Wiley-VCH: Weinheim, Germany, 1998; pp 70−71.

(9) Poole, D. *Linear Algebra*; Thomson Brooks/Cole: New York, 2006.

(10) The Excel sheets can be downloaded free of charge from: ftp://biocatalysis.uni-graz/pub/IsoPat2/. When opening, the Excel-file macros must be activated.

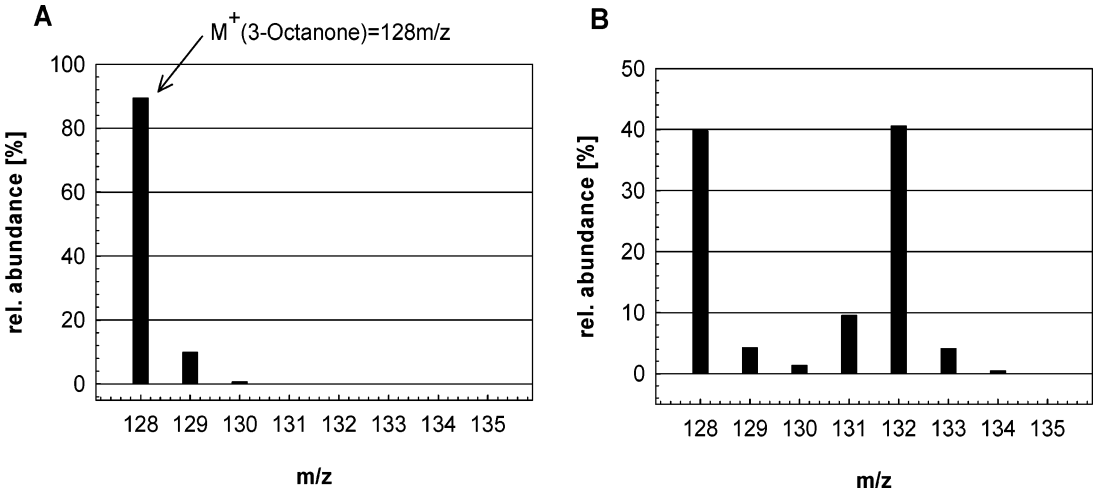


FIGURE 1. Mass spectrum of the molecule ion area of 3-octanone: (a) unlabeled 3-octanone **1a** and (b) mixtures of d_0-, d_1-, ..., d_4-3-octanone.

TABLE 1. Deconvolution Algorithm for a Compound To Be Labeled in Two Positions[a]

m/z	unlabeled d_0 (abundance)	d_1-derivative (abundance)	d_2-derivative (abundance)	analyte (abundance)
M	***a***			z_0
M + 1	***b***	***a***		z_1
M + 2	***c***	***b***	***a***	z_2
M + 3		***c***	***b***	z_3
M + 4			***c***	z_4

[a] The unlabeled compound shows abundances (*a*, *b*, *c*) at *m/z* of M, M + 1, and M + 2. The mixture of the d_i-derivatives shows abundances (z_0−z_4) at *m/z* from M to M + 4.

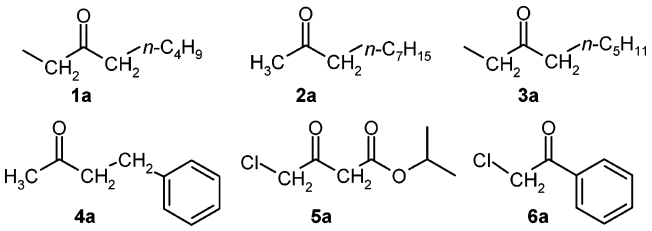


FIGURE 2. Substrates tested in hydrogen−deuterium exchange reactions.

^{14}C labeling, the matrix differs from the problem shown in Table 1 only as far as the mass of each derivative is shifted two mass units upward. The setup of such a matrix is again rather easy and was realized in an additional worksheet in the above-mentioned Excel file. For more complex analysis a Mathematica routine was developed. Further details on the Excel and Mathematica programming are provided in the Supporting Information.

Being now able to quickly analyze a mixture of derivatives varying only in their degree of isotope labeling, the transformation of ketones **1a**−**6a** (Figure 2) to their corresponding labeled derivatives was optimized by following the degree of labeling in D_2O/CD_3COOD at different reaction temperatures in a sealed vessel microwave reactor (100−180 °C) or under reflux employing traditional heating (∼100 °C).

During the deuteration reaction, samples were drawn and the complex mass patterns of the resulting mixtures were measured by MS (Figure 3a). By applying the above-described algorithm for deconvolution the time course of each derivative could be followed (Figure 3b).

Apart from 3-octanone **1a**, a variety of other ketone substrates (Figure 2) were exposed to the deuteration conditions. For example, hydrogen−deuterium exchange in ketone **2a** led to a complex mixture of all possible compounds d_0−d_5 after 40 min at 180 °C by using microwave irradiation (Figure 4, **2a**). By increasing the reaction time the amount of the fully labeled product d_5-**2a** could be increased to 80%. Compounds d_0-, d_1-, and d_2-**2a** were only visible at 40 min, afterward the amount of d_4-**2a** slowly decreased, while that of d_5-**2a** increased. The other aliphatic ketones **1a** and **3a** showed a quite similar behavior: after 200 min 85% and 82% respectively of the highest labeled derivative d_4 could be reached (Figure 4). For ketone substrates **1a**−**3a** only protons in the α-position of the carbonyl moiety were exchanged. For substrate **4a** we observed in addition to the exchange of the five α-protons an exchange of the two benzylic protons leading to a sevenfold labeled derivative d_7-**4a.** Comparing the deuterium exchange for **4a** under reflux conditions (∼100 °C) to the microwave-assisted exchange reaction at 180 °C, similarly deuterated product mixtures were obtained under reflux after 75 h while under microwave conditions only 1 h was required (Figure 4).

For more complex substrates like α-chloroketones **5a** and **6a**, optimization of the reaction parameters demonstrated that a temperature <180 °C was required to avoid the formation of side products due to the thermal instability of the educts. For substrate **5a** the main product after 180 min at 100 °C under microwave conditions did not show the maximum degree of labeling d_4. The main product was the 3-fold labeled d_3-**5a**, probably due to the lower temperature chosen as a compromise between the degree of labeling and the undesired thermal degradation of **5a**. Nevertheless, the unlabeled substrate d_0-**5a** was found in a concentration of <1%. In case of ω-chloro acetophenone (**6a**), the amount of byproducts was approximately 20% when lowering the temperature to 150 °C (40 min). Under these conditions the highest possible degree of labeling d_2 was reached with a purity of 98% (Table 2) and the corresponding product could be isolated with 73% yield. Especially for substrates with atoms possessing a significant natural isotope distribution (for example, chlorine atoms), the resulting mass patterns become rather complicated and would be tedious to solve without the program (Figure 5b). The results of the optimization of the reaction parameters are summarized in Table 2.

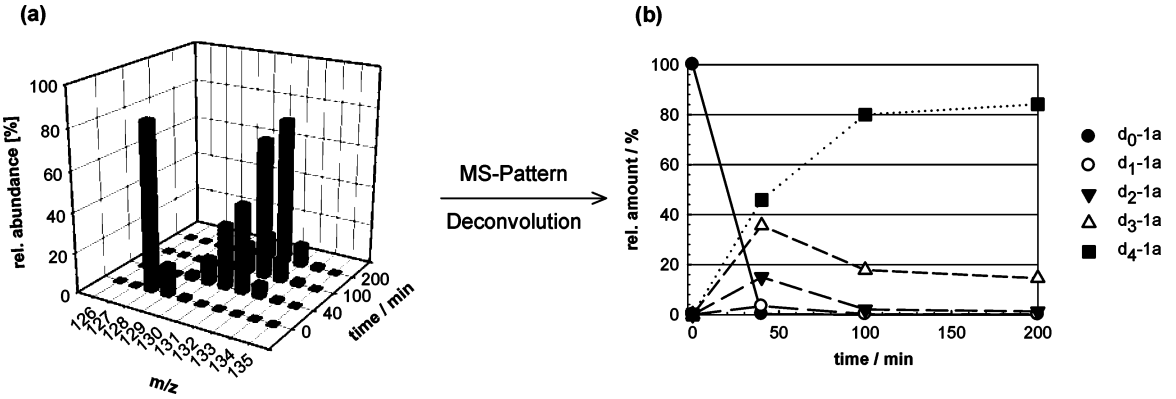


FIGURE 3. Deconvolution of the mass spectroscopic raw data (a) leading to the time course (b) of each d_0-d_4-derivative (3-octanone **1a**). Reaction conditions: 0.8 mol/L substrate, CD_3COOD, D_2O, 200 min, 180 °C, single mode microwave irradiation.

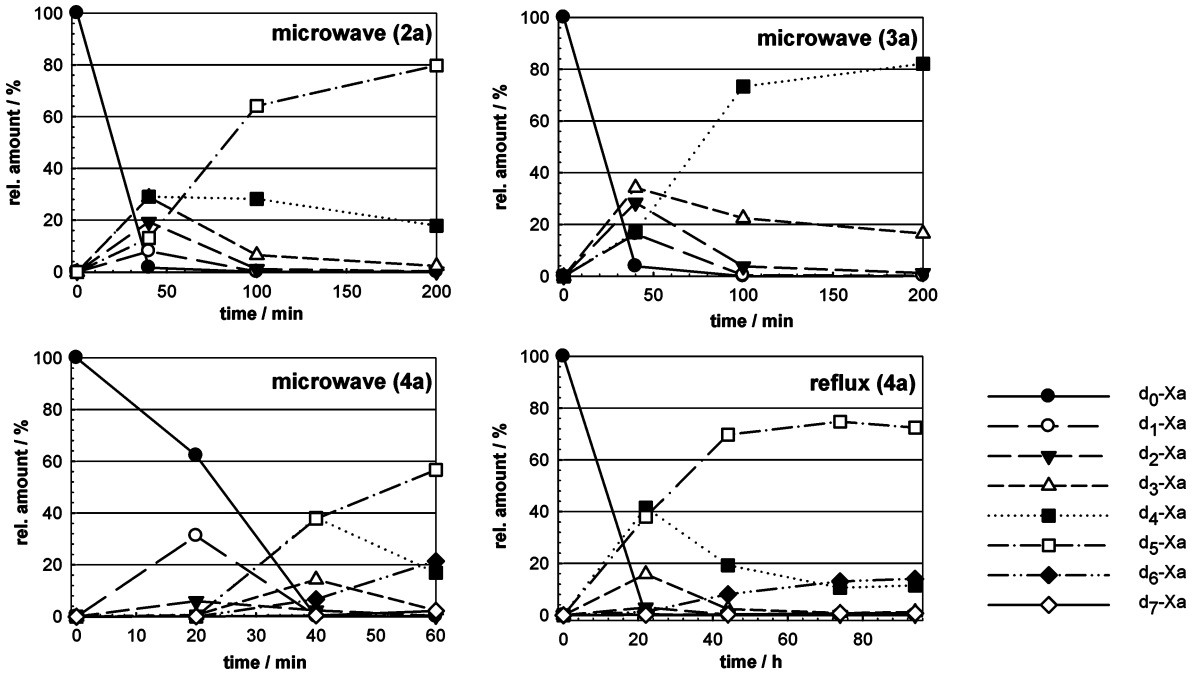


FIGURE 4. Time course of d_i-derivatives during the deuterium exchange for ketones **2a**, **3a**, and **4a** (Figure 2). Reaction conditions: 0.7−0.8 mol/L substrate, CD_3COOD, D_2O, 60 or 200 min, 180 °C, single mode microwave irradiation (for **4a** additional reflux conditions, 100 °C, 96 h).

TABLE 2. Optimized Reaction Conditions for Microwave-Assisted Hydrogen−Deuterium Exchange

		optimized reaction conditions			isolated yield		
substrate	main product	*T* (°C)[a]	time (min)	l.r. (%)	mg	%	purity[b] (%)
1a	d_4-**1a**	180, MW	200	>99.9	292	58	84
2a	d_5-**2a**	180, MW	200	>99.9	335	67	80
3a	d_4-**3a**	180, MW	200	>99.9	274	55	82
4a	d_5-**4a**	180, MW	40	>99.9	456	91	57
4a	d_5-**4a**	100, reflux	94 h	99.8	377	76	75
5a	d_3-**5a**	100, MW	180	99.7	230	46	38[c]
6a	d_2-**6a**	150, MW	40	>99.9	369	73	98

[a] MW = microwave. [b] Percentage of labeled main product to all other labeled derivatives. [c] 15% d_4-**6a**

In case of no side reactions, the conversion (the amount of converted substrate over the total starting material) is equal the sum of all labeled derivatives over the sum of labeled and unlabeled derivatives. Additionally, this parameter also gives a quantitative yield of labeled compounds or of the amount of unlabeled "impurity" and thus is a good parameter to characterize a product mixture. Due to the additional properties we named that parameter l.r. for labeled compound ratio (eq 3):

$$\text{l.r.} = \frac{\sum_{i=1}^{n} d_i}{\sum_{i=0}^{n} d_i} 100[\%] \qquad (3)$$

where l.r. is the labeled compound ratio, d_i is the concentration/amount of *i*-times labeled derivative, and *n* is the highest possible degree of labeling. For example, a l.r. of 100% corresponds to a mixture of labeled derivatives with no unlabeled substrate, while an l.r. of 0% stands for the unlabeled starting material.

Synthesis of Deuterium-Labeled Chiral Secondary Alcohols. We recently applied an alcohol dehydrogenase for deuterium transfer to obtain chiral deuterium labeled secondary

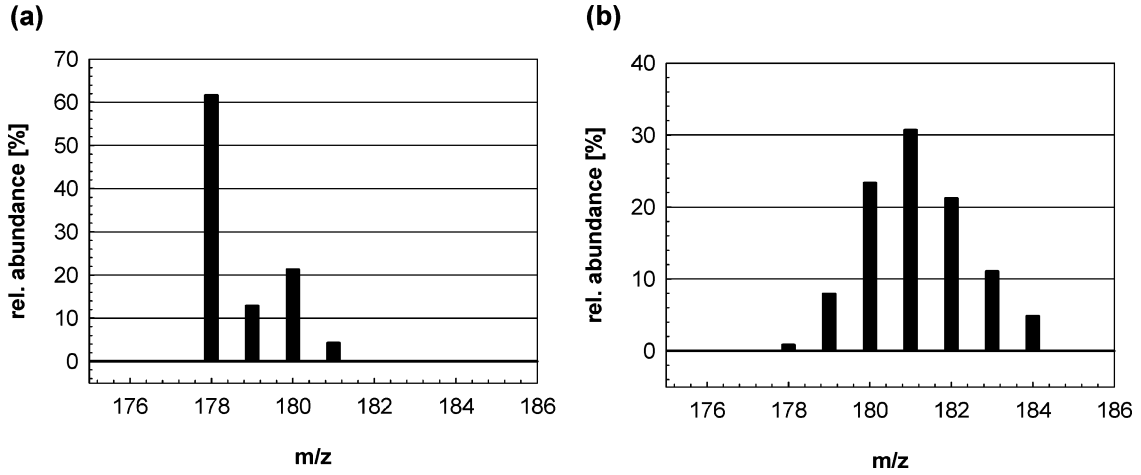


FIGURE 5. Mass pattern of (a) unlabeled compound **5a** and (b) after deuterium exchange (D_2O/CD_3COOD) after 80 min at 100 °C under microwave irradiation conditions.

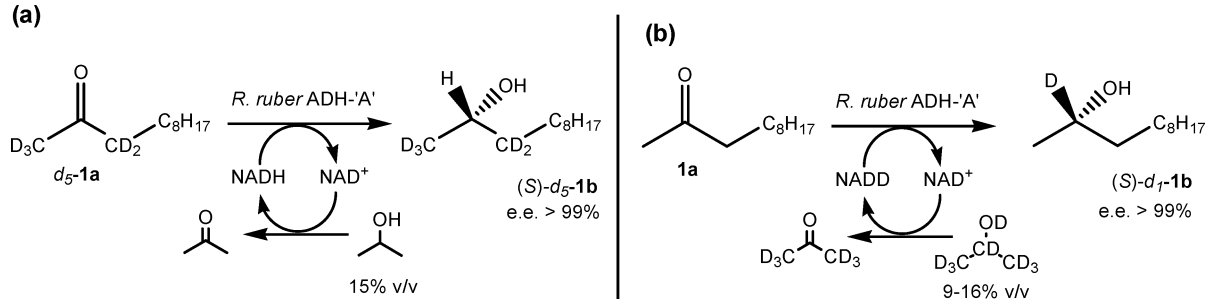


FIGURE 6. Synthesis of deuterium labeled chiral secondary alcohols in a coupled substrate approach: (a) α-Labeled starting material leading to enantiopure alcohols with a stable label and (b) unlabeled starting material, labeled hydride source for asymmetric reduction.

alcohols, for which the deuterium was located at the stereocenter. As a source of deuterium, d_8-2-propanol was applied in a coupled substrate approach (Figure 6b).[11] These labeled alcohols have the disadvantage that the label is removed during a metabolic oxidation of the alcohol. In contrast, when preparing labeled chiral alcohols from α-labeled ketones the label on the carbon adjacent to the chiral center is stable even during oxidation steps. Additionally, for monitoring these labeled compounds for kinetic and mechanistic studies during metabolism, no primary isotope effect due to cleavage of a C−D bond can cause an unwanted distortion of the observed data for oxidations. For the synthesis of such deuterium-labeled enantiopure chiral alcohols the labeled ketones d_n-**1a**−**6a** were reduced to the corresponding enantiopure labeled alcohol by a biocatalytic hydrogen transfer method (Figure 6a). By employing the alcohol dehydrogenase from *Rhodococcus ruber* ADH-"A"[10,12] and the stereocomplementary alcohol dehydrogenase from *Lactobacillus brevis* LB-ADH[13] both enantiomers were accessible in good yields and excellent enantiomeric excess (Table 3).

The obtained alcohols were analyzed by GC-MS as well as GC-FID on a chiral stationary phase. Its is worth mentioning that the labeling pattern did not change during the reduction reactions except that the mass pattern was shifted one (2-propanol) or two mass units (d_8-2-propanol) to higher mass.

TABLE 3. Biocatalytic Reduction of α-Labeled Ketones to Chiral Secondary Alcohols

			yield			
catalyst	substrate[a]	product	mg	%	ee (%)	l.r. (%)
ADH-"A"	d_4-**1a**	d_4-(*S*)-**1b**	73	71.6	>99	>99.9
	d_5-**2a**	d_5-(*S*)-**2b**	79	77.5	>99	>99.9
	d_4-**3a**	d_4-(*S*)-**3b**	61	59.8	>99	>99.9
	d_5-**4a**	d_5-(*S*)-**4b**	61	59.8	>99	>99.9
	d_3-**5a**	d_3-(*R*)-**5b**[b]	92	90.2	>99	99.7
	d_2-**6a**	d_2-(*R*)-**6b**[b]	74	72.5	>99	>99.9
LB-ADH	d_4-**1a**	d_4-(*R*)-**1b**	56	54.9	>99	>99.9
	d_5-**2a**	d_5-(*R*)-**2b**	74	72.5	>99	>99.9
	d_4-**3a**	d_4-(*R*)-**3b**	27	26.5	>99	>99.9
	d_5-**4a**	d_5-(*R*)-**4b**	98	96.1	>99	>99.9
	d_3-**5a**	d_3-(*S*)-**5b**[b]	84	82.4	>99	99.7
	d_2-**6a**	d_2-(*S*)-**6b**[b]	87	85.3	>99	>99.9

[a] 100 mg of **1a**−**6a**. [b] Switch of CIP priority.

For validation of the MS-pattern deconvolution algorithm the obtained labeled chiral alcohols as well as the labeled ketones were mixed with unlabeled compounds. The mixtures were then analyzed by MS. After deconvolution of the data the results were plotted in a diagram with the increasing mixed amount of labeled compound asthe *x*-axis and on the *y*-axis the labeled ratio. In case the algorithm performs as outlined above, a linear trend graph should result with a regression correlation coefficient (R^2) close to "1". As an example, unlabeled **1a** was admixed with the product of the microwave-assisted deuterium exchange reaction containing the d_1−d_4-derivatives of ketone **1a** (Figure

(11) Edegger, K.; Gruber, C.C.; Poessl, T. M.; Wallner, S. R.; Lavandera, I.; Faber, K.; Niehaus, F.; Eck, J.; Oehrlein, R.; Hafner, A.; Kroutil, W. *Chem. Commun.* **2006**, 2402.

(12) Stampfer, W.; Kosjek, B.; Moitzi, C.; Kroutil, W.; Faber, K. *Angew. Chem., Int. Ed.* **2002**, *41*, 1014.

(13) Wolberg, M.; Hummel, W.; Wandrey, C.; Müller, M. *Angew. Chem., Int. Ed.* **2000**, *39*, 4306.

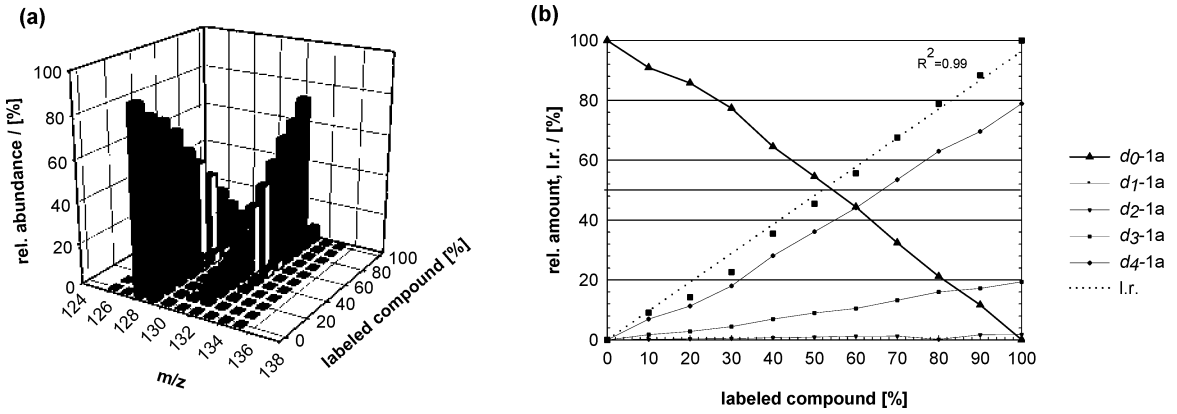


FIGURE 7. Validation of the deconvolution algorithm: (a) raw MS data morphing from unlabeled to labeled patternand (b) calibration data with linear regression.

7). Gratifyingly, after analysis as described above the R^2 coefficient was 0.99. For the other series the R^2 values were between 0.98 and 0.99. The R^2 values close to "1" therefore validate the deconvolution algorithm presented above.

Conclusion

In the context of performing isotope labeling studies by isotope exchange reactions, an error-minimizing algorithm based on the computerized analysis of readily available low-resolution mass spectroscopic patterns was developed. The algorithm allows the exact determination of the relative amounts of each labeled species differentiated in the number of incorporated deuterium, oxygen, carbon, or other isotopes. By using this methodology the preparation of α-labeled ketones employing microwave technology was optimized. The latter were then reduced in an asymmetric fashion to yield enantiopure alcohols possessing a stable label. Confirmation of the algorithm was achieved by analysis of the linear regression correlation coefficient.

Experimental Section

Typical Procedure for the Synthesis of Labeled Ketones. 3-Octanone (500 mg, 3.9 mmol) was added to D_2O (3.5 mL, 175 mmol, 99.9%) and CD_3COOD (1 mL, 16 mmol) in a 10 mL sealed microwave process vial. The exchange reaction was performed at 180 °C for 200 min in a single-mode microwave reactor. After the reactor was cooled to room temperature the product was extracted with CCl_4 (4 times), washed with a saturated $NaDCO_3$ solution (4 mL), and dried (Na_2SO_4) and the organic solvent was removed by evaporation. When required the product was purified by silica gel column chromatography. The product was analyzed by GC-MS (see the Supporting Information for more details). From the obtained MS pattern the percentage of the various labeled derivatives was analyzed with use of the above-described Excel sheet.

Typical Procedure for the Synthesis of Deuterium-Labeled *sec*-Alcohols. Lyophilized cells of *E. coli* Tuner pet22b-ADH-"A" (310 mg) were rehydrated in TRIS-HCl buffer (30 °C, 60 min, 120 rpm) in a 50 mL vessel. The reaction was started by addition of labeled 3-octanone (100 mg, 0.78 mmol) and 2-propanol (1515 μL, 15 v/v %). After 24 h at 30 °C, 120 rpm ,the reaction was stopped by extraction with ethyl acetate (3×) before the solvent was evaporated. ^{1}H NMR and MS data are provided in the Supporting Information.

Acknowledgment. This study was financed by the Austrian Science Fund (FWF Project P18537-B03). We also thank Biotage AB (Uppsala, Sweden) for use of the Emrys Synthesizer and Initiator EXP Eight microwave reactors.

Supporting Information Available: General experimental procedures, ^{1}H NMR and MS spectroscopic data, program descriptions, and source code. This material is available free of charge via the Internet at http://pubs.acs.org.

JO070831O