Supporting Information

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

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General Experimental Section

Materials: Substrates **1a**-**6a** and *rac*-**1b**-**6b** are commercially available. Alcohol dehydrogenase from *Lactobacillus brevis* (LB-ADH) was purchased from Jülich, the alcohol dehydrogenase from *Rhodococcus ruber* ADH-'A' is commercially available from Biocatalytics Inc. For the experiments described here we used the following cell-free preparation: *E. coli* Tuner^{TM} (DE3)/pET22b+-ADH-'A' was grown as previously described in LB-amp medium (250 mL) with additional Zn^{2+} (100 mg L^{-1}).[1] The medium (OD ~3) was centrifuged (Jouan KE22i, AK-500.11, 20 min, 8000 rpm, 4°C) and the debris was resuspended in buffer (50 mL, 50mM Tris-HCl pH 7.5). The cells were disrupted by ultrasonication (Branson, S250D CE, 200W, 5 mm spike, 50 mL tubes, 1 sec impulse, 2 sec pause, amplitude 50%, 16 min, 4°C) and centrifuged (Jouan KE22i, AK-100.21, 20 min, 13000 rpm, 4°C). The supernatant was transferred to an Erlenmeyer flask (250 mL) and kept at 65°C for 25 minutes. After centrifugation (Jouan KE22i, AK-100.21, 20 min, 13000 rpm, 4°C) the supernatant was used for the experiments.

Analysis: GC-MS analyses were performed on a GC system equipped with a mass selective detector and a Series injector using a (5%-phenyl)-methylpolysiloxane capillary column (5Msi, 30 m, 0.25 mm ID, 0.25 μm film). GC-FID analyses were carried out using H_2 as carrier gas (14.5 psi) with a Chirasil-DEX CB column (25 m x 0.32 mm x 0.25 μm), detector temperature 250 °C, split ratio 90:1. NMR spectra were measured at 360 MHz.

Microwave Irradiation Experiments. Microwave irradiation experiments were carried out using the Emrys^{TM} Synthesizer and Initiator eight from Biotage (Uppsala), including proprietary Workflow Manager Software (version 2.1). Experiments were carried out in sealed microwave process vials utilizing the standard absorbance level (300 W maximum power). Reaction times under microwave conditions reflect total irradiation times rather than actual reaction times at a given temperature. The temperature was measured with an IR sensor on the outside of the reaction vessel.

Analytics

The alcohols were acetylated by addition of acetic anhydride (100 μL) and DMAP (0.5 mg) within 2 hours. After work-up, the products were analyzed by GC on a chiral stationary phase.

GC-FID analysis on chiral stationary phase: Temperature program: 110/0/2.5/120/0/ 10/200/0 (temperature program (start temperature °C/ holding time [min]/ heating rate [°C min^{-1}]/ plateau temperature [°C]/ holding time [min]/ heating rate [°C min^{-1}]/ final temperature [°C]/ holding time [min]).

GC-MS analytics: Temperature program: 100/0/10/300/0, E=70eV.

Table S1. Retention times (min) of ketones and alcohols on GC-FID (chiral stationary phase).

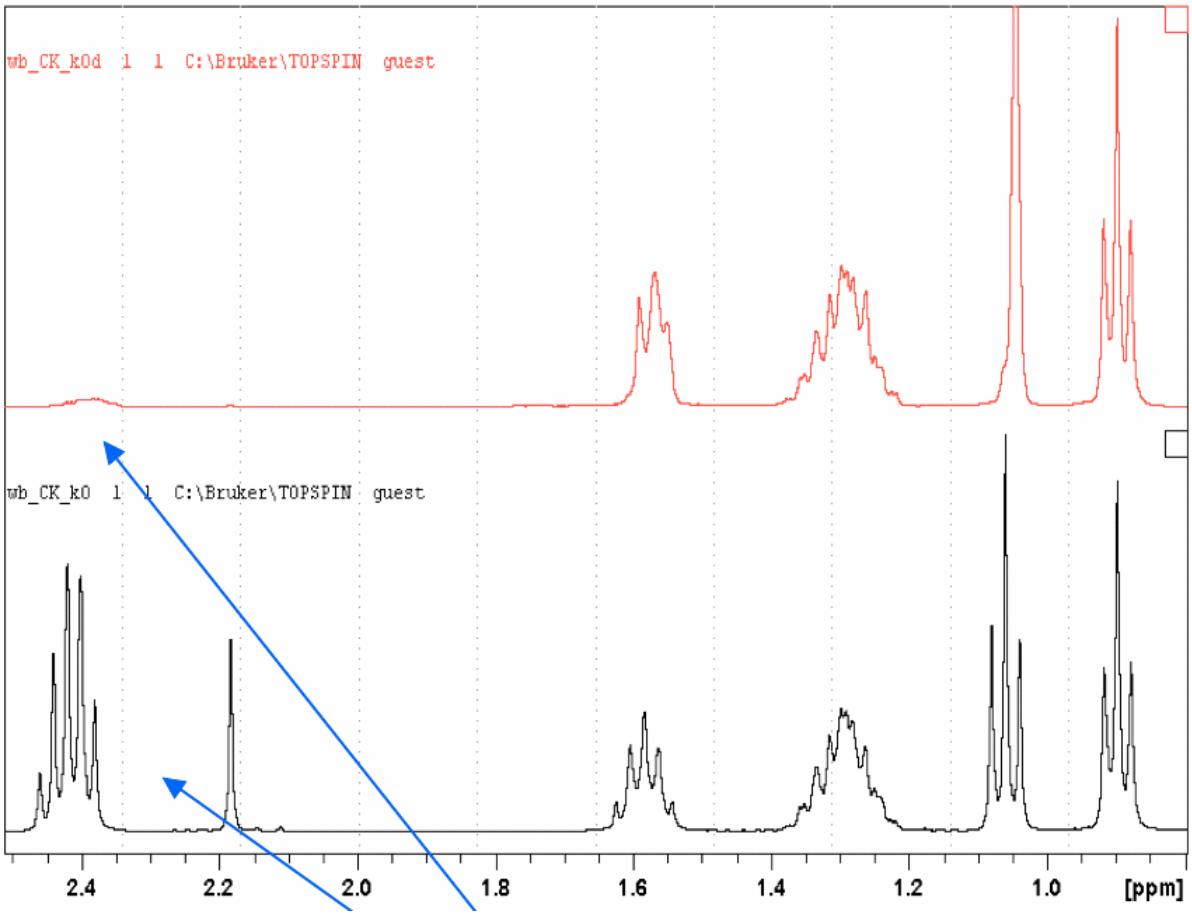
Compound	Alcohol **b**			Ketone **a**
	(*S*)	(*R*)	*rac*	
	acetylated		Not derivatized	
1	1.72	1.80	1.62	1.26
2	4.14	4.48	3.55	2.59
3	2.49	2.62	2.32	1.70
4	10.65	10.76	11.32	10.71
5	4.81	4.88	4.04	2.77
6	5.94	6.14	6.71 (*S*) 6.91 (*R*)	5.59

Table S2. Retention times (min) GC/MS

Compound	Ketone **a**	Alcohol **b**
1	4.25	4.35
2	6.00	6.10
3	5.15	5.25
4	7.90	7.99
5	5.65	8.85
6	6.61	6.50

NMR Spectra

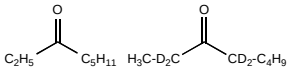
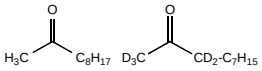
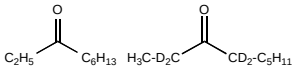
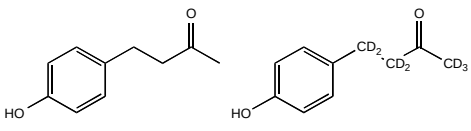
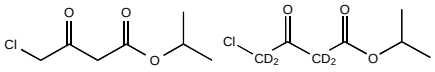
^{1}H: 360MHz, ^{13}C, 90MHz)



4H, unlabeled α-2CH_2	labeled α-2CH_2, not appearing in ^{1}H-NMR

Figure S1. Exemplary ^{1}H-NMR spectrum of labeled und unlabeled 3-octanone. The chemical shifts of all other compounds are listed in the table below.

TABLE S3. Chemical Shifts of Labeled and Unlabeled Ketones (for comparison).

Compound	**^{1}H-NMR**		**^{13}C-NMR**
	unlabeled	**labeled**	**labeled**
1a and d_4-**1a** 	0.88 (3H, t) 1.06 (3H, t) 1.29 (4H, m) 1.59 (2H, q) 2.42 (4H, m)	0.88 (3H, t) 1.04 (3H, s) 1.29 (4H, m) 1.59 (2H, m)	7.67 13.90 22.45 23.50 31.40 212.31
2a and d_5-**2a** 	0.88 (3H, t) 1.28 (10H, m) 1.59 (2H, q) 2.14 (3H, s) 2.41 (2H,t)	0.88 (3H, t) 1.27 (10H, m) 1.59 (2H, m)	14.07 22.63 23.74 29.13 29.35 31.80 210.51
3a and d_4-**3a** 	0.88 (3H, t) 1.06 (3H, t) 1.28 (6H, m) 1.59 (2H, q) 2.41 (4H, m)	0.88 (3H, t) 1.04 (3H, s) 1.28 (6H, m) 1.59 (2H, q)	7.67 14.00 22.48 23.78 28.89 31.60 212.28
4a and d_5-**4a** 	2.14 (3H, s) 2.74 (2H, t) 2.84 (2H, t) 6.74 (2H, ar) 7.01 (2H, ar)	 2.84 (2H, s) 6.74 (2H, ar) 7.01 (2H, ar)	28.80 115.32 129.36 132.60 154.12 209.69
5a and d_4-**5a** 	1.28 (6H, d) 3.63 (2H, s) 4.23 (2H, s) 5.10 (1H, m)	1.28 (6H, d) 5.10 (1H, m)	19.82 20.04 20.26 24.98

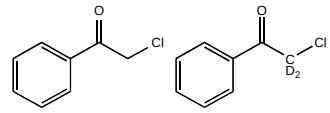
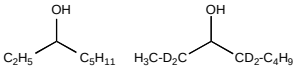
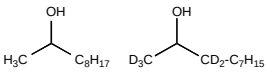
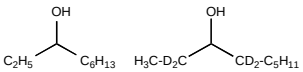
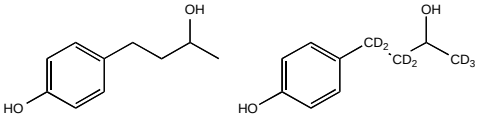
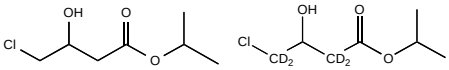
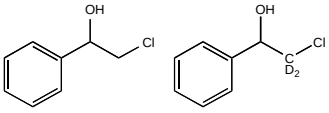
			177.23
6a and d_2-**6a** 	4.73 (2H, s) 7.51 (2H, ar) 7.64 (1H, ar) 7.98 (2H, ar)	 7.51 (2H, ar) 7.64 (1H, ar) 7.98 (2H, ar)	n.d.

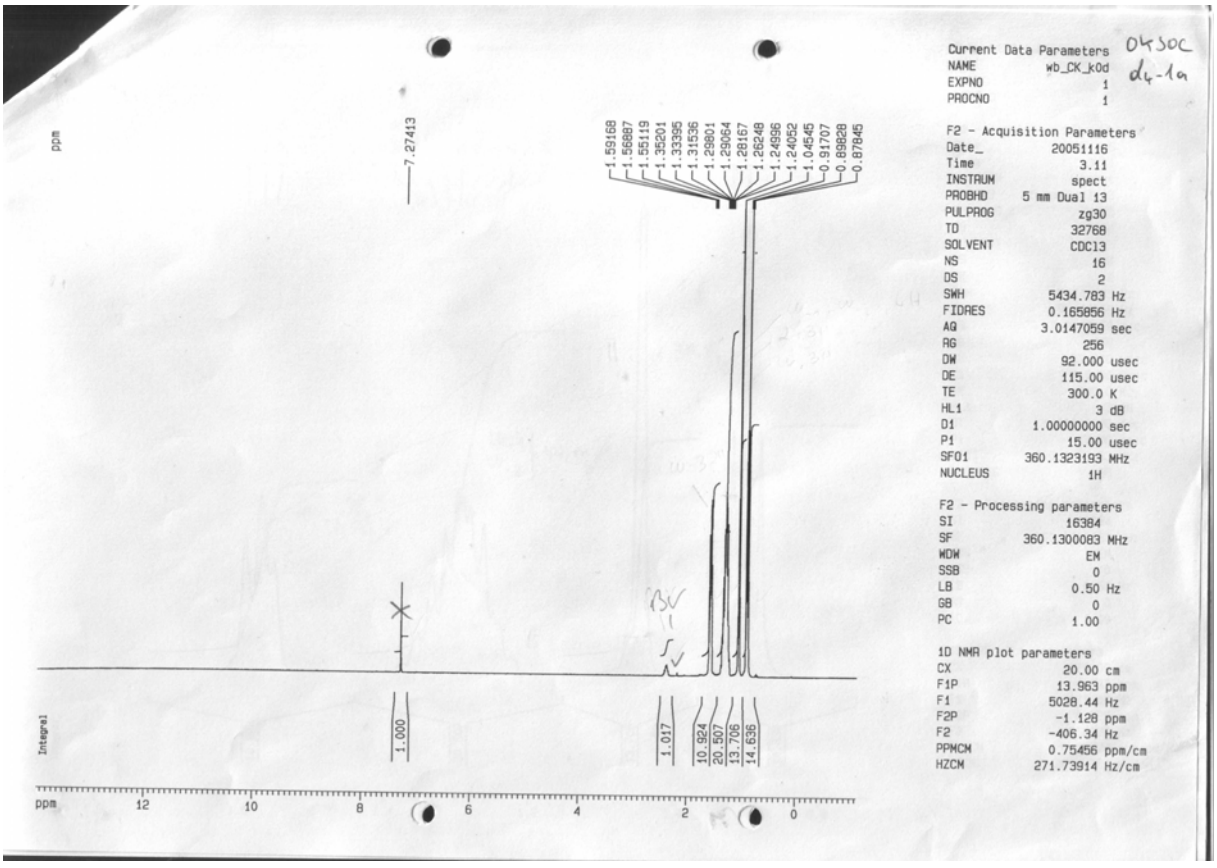
TABLE S4. Chemical Shifts of Labeled and Unlabeled Alcohols (for Comparison).

Compound	**^{1}H-NMR**		**^{13}C-NMR**
	unlabeled	**labeled**	**labeled**
1b and d_4-**1b** 	0.94 (6H, dt) 1.27 (6H, m) 1.46 (4H, m) 3.45 (1H, q)	0.94 (6H, m) 1.27 (6H, m) 3.45 (1H, s)	n.d.
2b and d_5-**2b** 	0.88 (3H, t) 1.21 (3H, d) 1.29 (12H, m) 1.44 (2H, m) 3.47 (1H, m)	 1.29 (12H, m) 1.45 (2H, m) 3.48 (1H, s)	n.d.
3b and d_4-**3b** 	0.95 (6H, m) 1.29 (8H, m) 1.46 (4H, m) 3.51 (1H, q)	0.95 (6H, m) 1.29 (8H, m) 3.51 (1H, s)	9.62 14.06 22.61 25.40 29.33 31.85 73.12
4b and d_5-**4b** 	n.d.	n.d.	n.d.

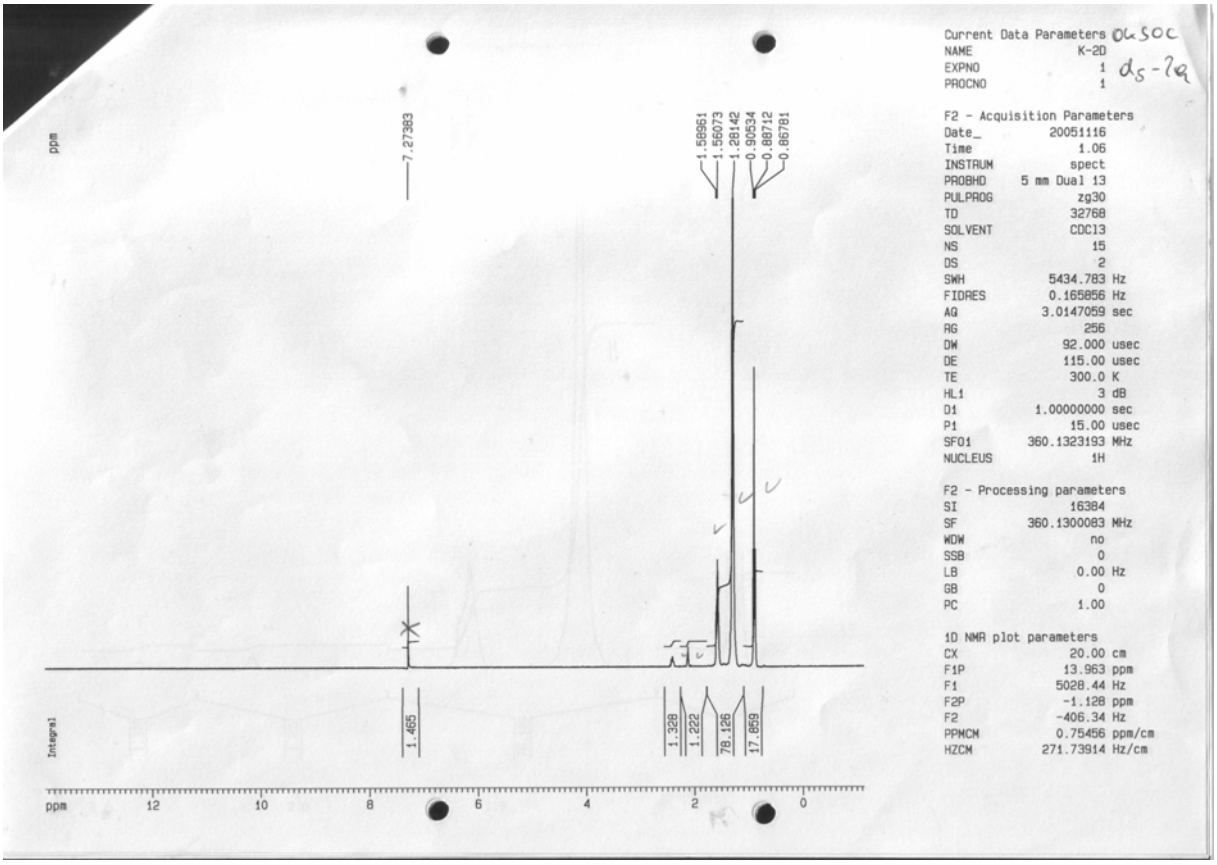
5b and d_4-**5b** 	n.d.	1.28 (6H, d) 4.23 (1H, s) 5.06 (1H, h)	21.76 38.67 67.84 68.62 171.38
6b and d_2-**6b** 	3.72 (2H, dd) 4.92 (1H, dd) 7.27-7.42 (5H, ar)	4.91 (1H, s) 7.27-7.42 (5H, ar)	73.97 126.06 128.47 128.68 139.9

Copies of the ^{1}H-NMRs

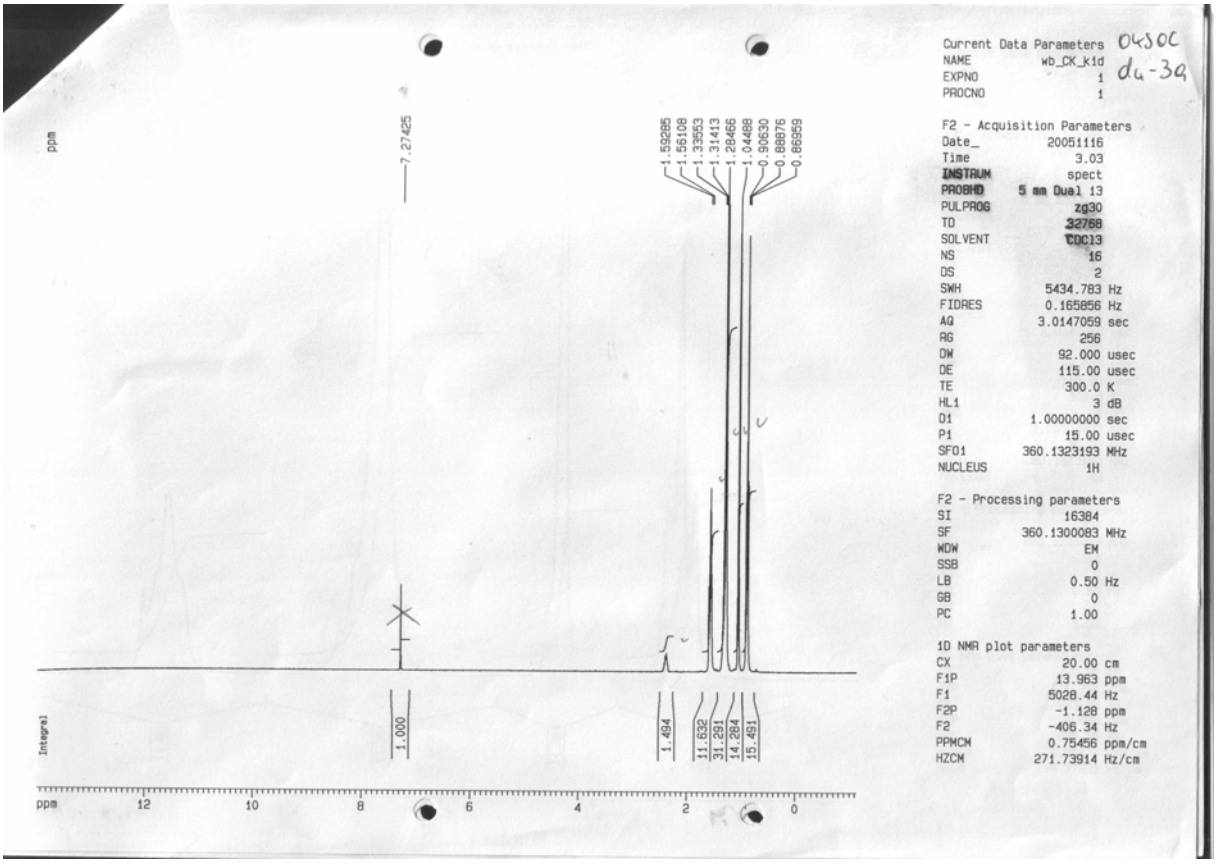
Compound d_4-**1a**



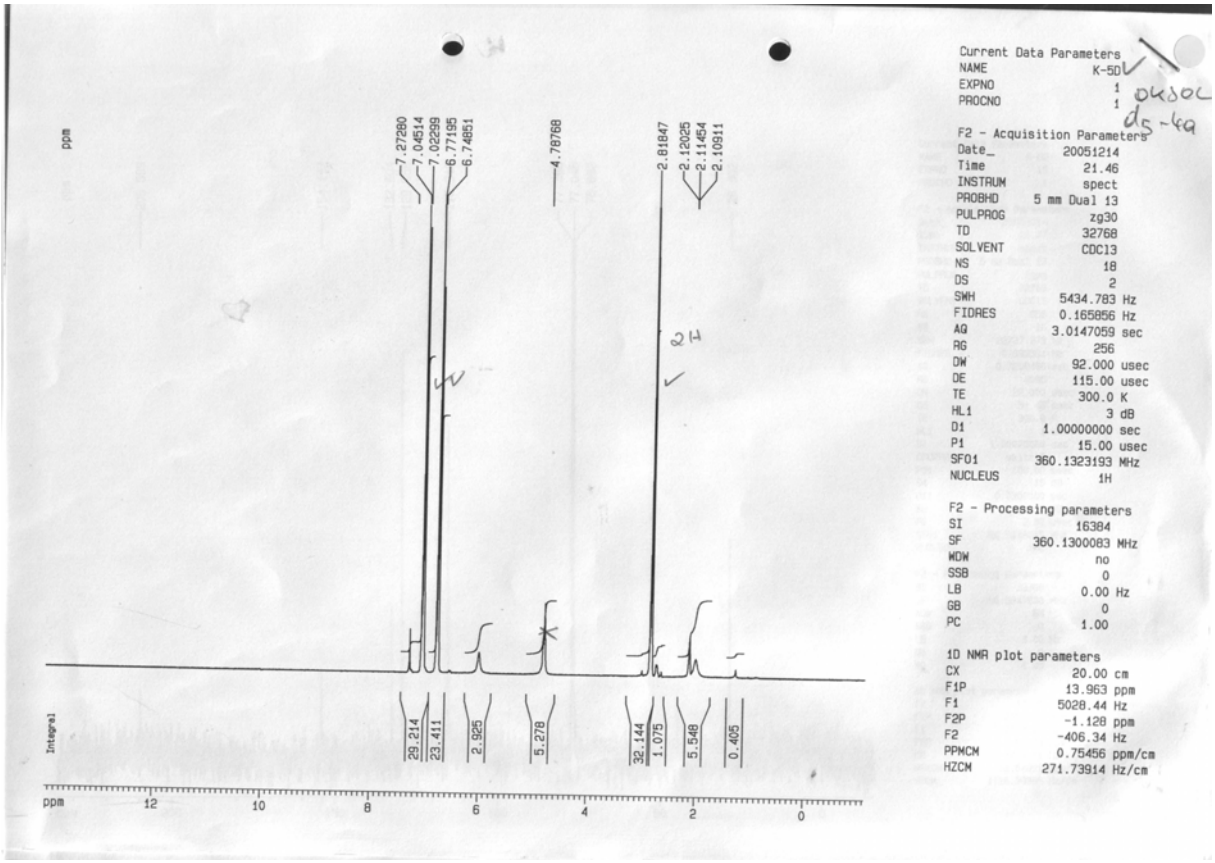
Compound d_5-**2a**



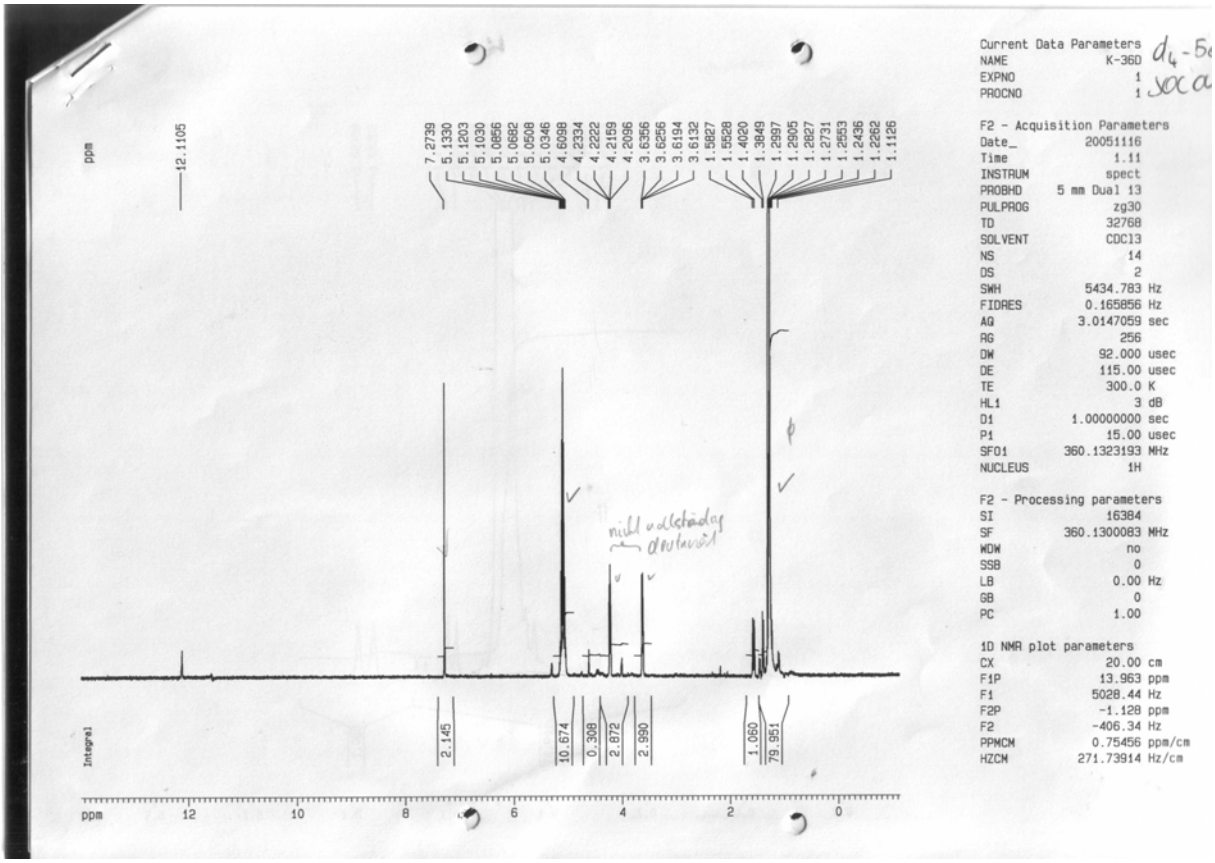
Compound d_4-**3a**



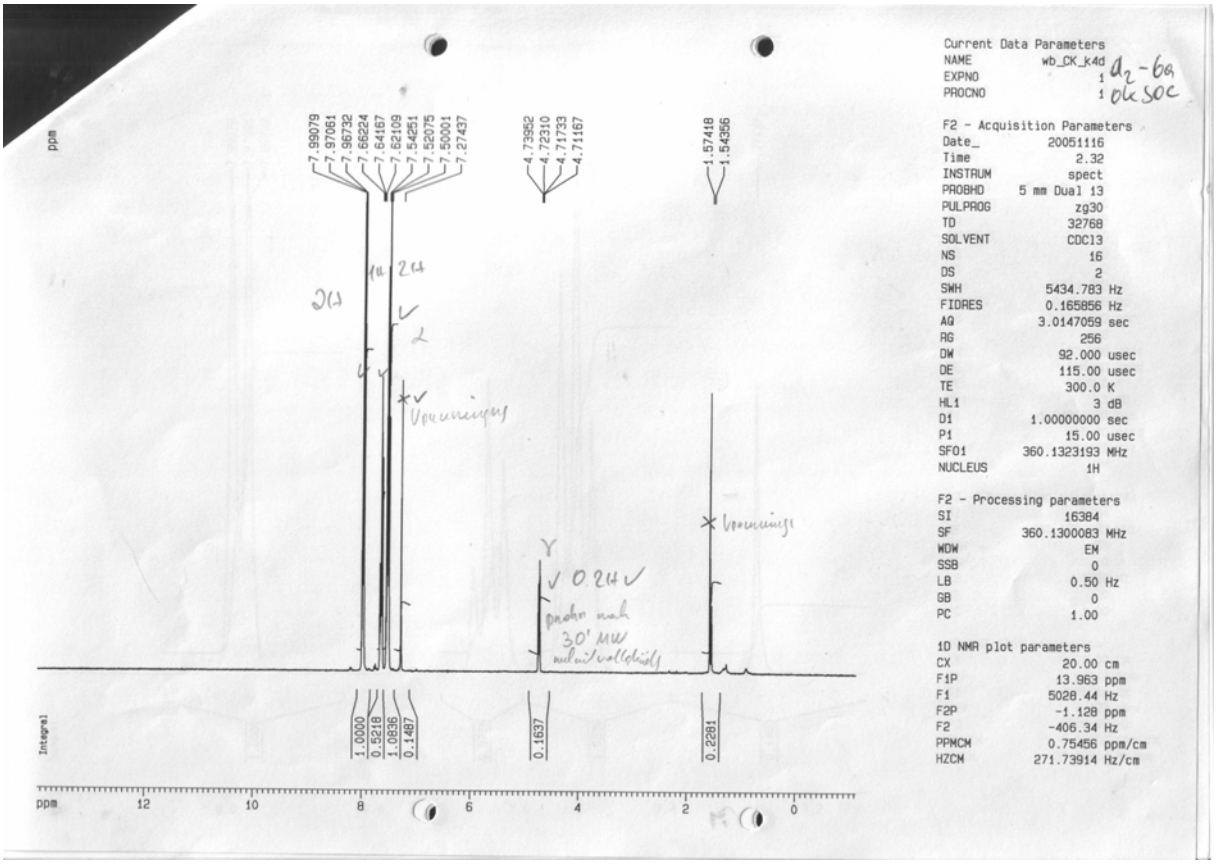
Compound d_5-**4a**



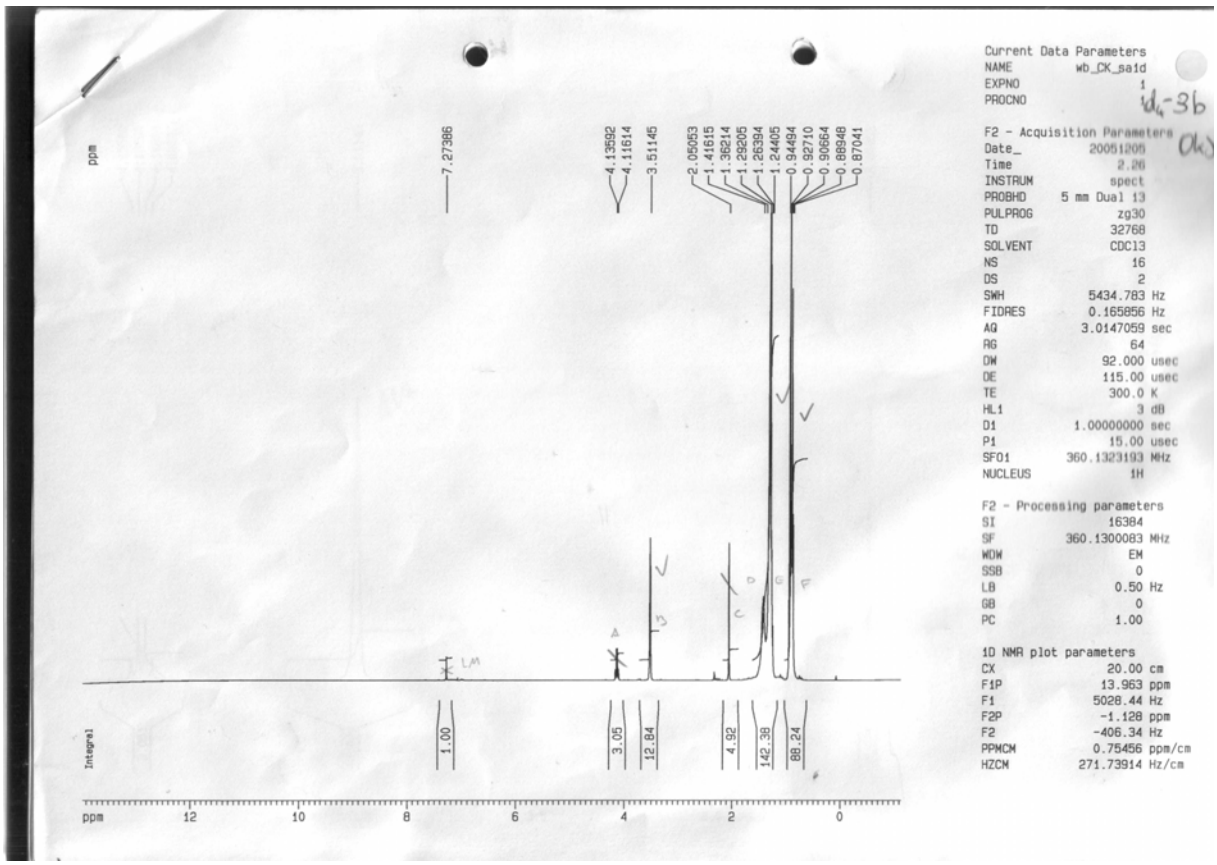
Compound d_4-**5a**



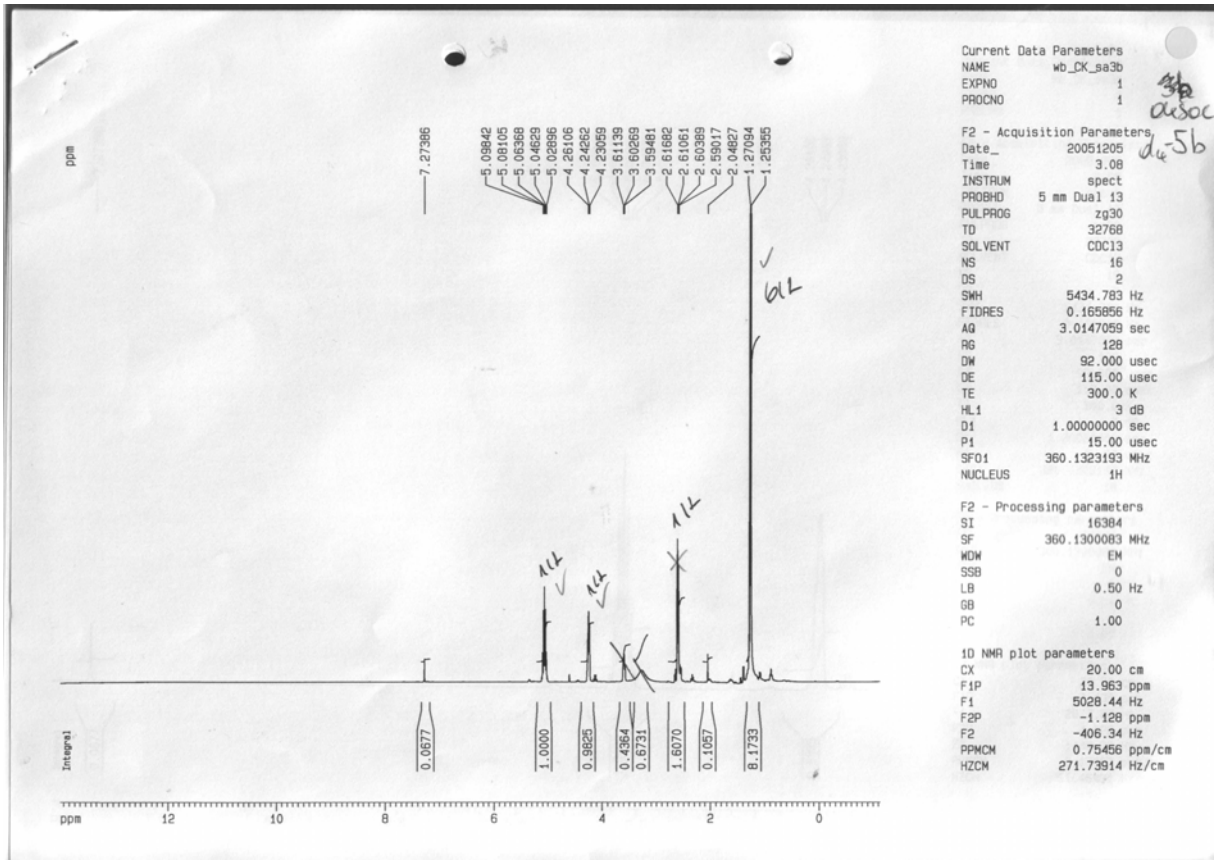
Compound d_2-**6a**



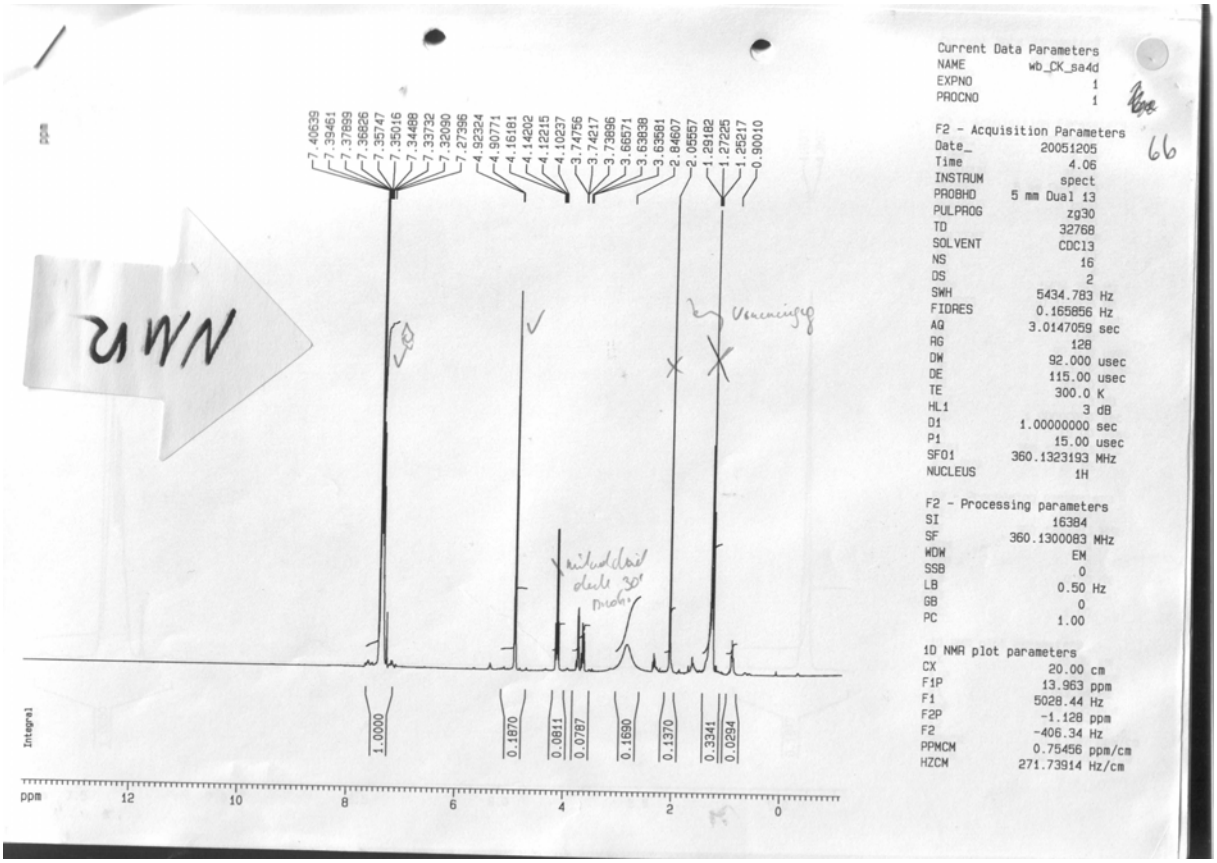
Compound d_4-**3b**



Compound d_4-**5b**



Compound d_2-**6b**

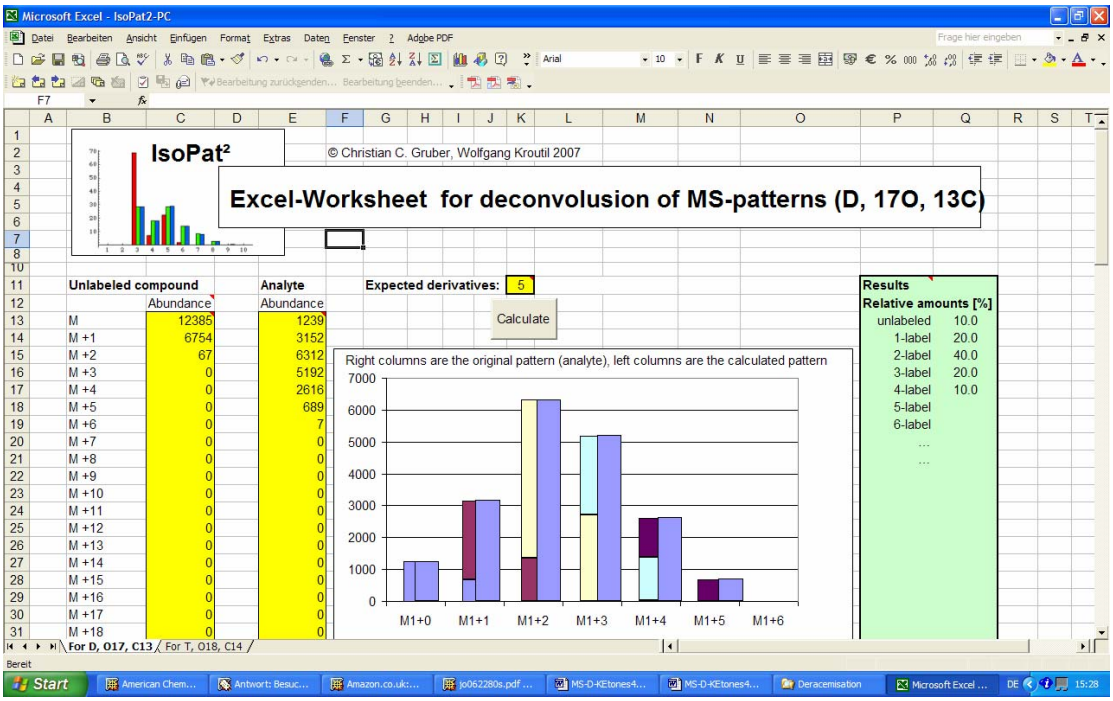


Mass Spectra

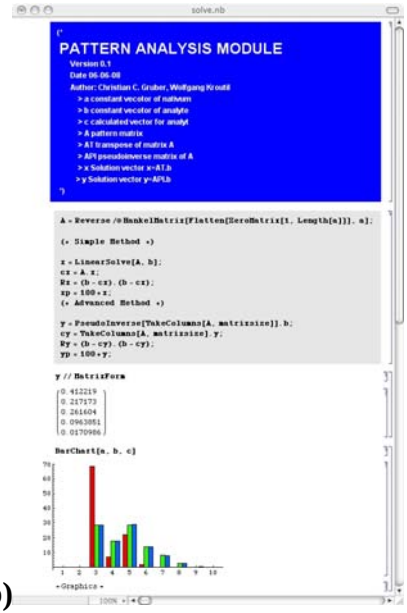
TABLE S5. Main Fragments in Mass Spectra of Labeled and Unlabeled Compounds

Compound	m/z main fragments
1a	128, 99, 85, 72, 71, 57, 43
d_4-**1a**	132, 101, 89, 76, 73, 59, 45
2a	156, 71, 58, 43
d_5-**2a**	161, 76, 63, 46
3a	142, 113, 85, 72, 57, 43
d_4-**3a**	146, 115, 87, 76, 59, 45
4a	164, 149, 121, 107, 77, 51
d_5-**4a**	169, 151, 123, 107, 77, 51
6a	154, 105, 77, 51
d_2-**6a**	156, 105, 77, 51

Program Descriptions and Source Code



(a)



(b)

Figure S2. (a) Microsoft Excel-sheet and (b) Mathematica Worksheet for deconvolusion of MS-patterns of labeled compounds (download from ftp://biocatalysis.uni-graz/pub/IsoPat2/).

Instructions. The user has to fill in the yellow areas only, which are the MS abundances of the unlabled compound, and then the abundances of the mixture. After inputting how many derivatives will be expected and pressing the button “Calculate”, the results are given on the right side of the graph. The results are given as relative amount for each labeled compound in percentage. Pressing “Calculate” is only required in case the expected number of derivatives changed or the number of filled rows of the analyte changed since the last calculation. The abundances for the input-data can be used as obtained from the measurement, no separate normalization has to be done (that is done by some additional operations implemented in the excel sheet). The graph in the middle of the figure (Figure S2A, the y-axis are arbitrary units due to normalization of the input-data sets) compares the measured MS-pattern with the derived one, therefore giving a qualitative estimation of accuracy. At each m/z (M+0, M+1…) the right column is the original abundance pattern, the left column is the calculated one. The amount of contribution of each derivative can be clearly recognized (Figure S2A).

Although the Excel file is a simple method and can be run on every PC and Macintosh platform, some restrictions had to be made: 1) the sheet is limited to 19 derivatives and 20 rows for input data of the abundances. This drawback was eliminated by an advanced implementation of the algorithm using a Mathematica based worksheet. This version can handle an unlimited number of derivatives and works with other rare isotope patterns, like higher differences of mass for each derivative (here we deal with mass differences of one like for H-D, or two like for hydrogen to tritium). Furthermore a graphical user interface for Macintosh computer running Mac OS X was developed, featuring connections to programs like Microsoft Excel for rapid further data processing. All features of the Mathematica implementation can be used within the GUI, parameters can be set and stored in a preference file. For detailed installation instructions of the GUI please read the ESI or contact the authors. The open source nature of the GUI, and in particular of the pattern analysis Mathematica module, permits to modify and extend its capabilities beyond the predefined features and easily adapt the program to new analytical problems.

The following files can be downloaded from our public ftp server under "ftp://biocatalysis.uni-graz/pub/IsoPat2/":

IsoPat2.xls	Microsoft Excel Worksheet
IsoPat2.nb	Mathematica Worksheet
IsoPat2.dmg	Graphical User Interface for Mac OS X
IsoPat2.zip	All above listed files including the source code