Supporting Information

The sustainable synthesis of levetiracetam by an enzymatic dynamic kinetic resolution and an *ex-cell* anodic oxidation

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1 General considerations

All reagents were used in analytical or sufficiently pure grades. Solvents were purified by standard methods.[1]

Electrochemical reactions were carried out at boron-doped diamond (BDD) anodes. The BDD electrodes were obtained in DIACHEM© quality from CONDIAS GmbH, Itzehoe, Germany. The BDD had a 15 μm diamond layer on silicon support. Stainless steel of the type EN1.4401; AISI/ASTM (V4A) was used as cathodes. Nafion™ N324 from DuPont was used as membrane. A galvanostat HMP4040 from Rhode&Schwarz was employed. NMR spectra were recorded on a Bruker Avance III HD 300 (300 MHz) equipped with 5 mm BBFO head with *z* gradient and ATM at 25 °C. Chemical shifts (δ) are reported in parts per million (ppm) relative to traces of $CHCl_3$ in $CDCl_3$ as deuterated solvent. Liquid chromatography photodiode array analysis was performed by using a DUGA-20A_3 device from Shimadzu, which was equipped with a C18 column from Knauer (Eurospher II, 100-5 C18, 150x4 mm). The column was conditioned at 25 °C and the flow rate was set to 1 mL/min. The aqueous eluent was buffered by formic acid (0.8 mL/2.5 L) and stabilised by acetone (5 vol%). Gas chromatography was performed using a GC 2010 device from Shimadzu, which was equipped with a Varian capillary column ZB-5MSi (Serial No. 334634), operating with H_2 as carrier gas. Chiral HPLC was performed with a Daicel CHIRALPAK IB-3 column. The injection volume was V = 10 μL, and the eluent was composed of 10% isopropanol and 90% hexane/ethanol. The detection followed by a photodiode array detector at λ = 210.1 nm. Infrared spectra were recorded on an ATR IR device of the type ALPHA from Bruker. Cyclic voltammetry was conducted on an AUTO LAB PGstat 204 from Metrohm AG, Herisau, Switzerland. Design-of-Experiments plans were created and analysed with the software Minitab19 from Minitab Inc.

2 Calculations of *E* factors (EF) and atom efficiencies (AE)

$$AE = \frac{M(desired\ product)}{M(all\ products)}$$

$$EF = \frac{m(raw\ materials) - m(desired\ \ product)}{m(desired\ \ product)}$$

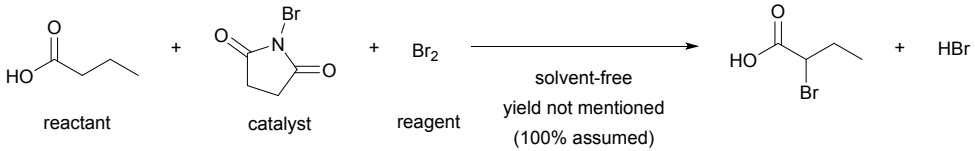
Please note that,

a) yields, solvents, catalysts and reagents for the work-up/purification are not considered in the calculations for the atom efficiency.

b) water is not considered in the calculations for the *E*-factor. The comparability of routes strongly depends on the starting material chosen for the synthesis and on the scale of the reaction. The amount of a reagent was estimated if not mentioned in the report. Flash column chromatography was generally not considered in the calculations, since no amounts of the stationary phase and the mobile phase were given in the reports. For these reasons, the calculated EF values should be considered only as a rough classification.

c) in the following tables a comma is used as the decimal separator.

2.1 α-Halogenation – nucleophilic substitution pathway[2]



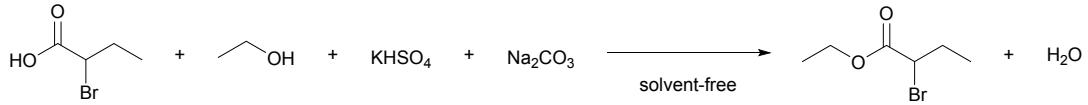
reactant | catalyst | reagent

Reactants | *Products*

Formula	$C_4H_8O_2$	$C_4H_4BrNO_2$	Br_2	Formula	$C_4H_7BrO_2$	BrH
MW	88,11	177,98	159,81	MW	167,00	80,91
Limiting?	***Yes***	No	No	Equivalents		
Equivalents	***1,00***	***0,00***	1,10	%Completion		
Sample Mass				Expected Mass	1895,47kg	918,35kg
%Weight				Expected Moles	11,35kmol	11,35kmol
Molarity				Measured Mass		
Density				Purity		
Volume				Product Mass		
Reactant Moles	11,35kmol	56,18mol	12,52kmol	Product Moles		
Reactant Mass	***1000,00kg***	***10,00kg***	***2000,00kg***	%Yield		

AE = 167.00 : (167.00 + 80.91) = 67%

EF = (1000 + 10 + 2000 - 1895.47) : 1895.47 = 0.58 [kg/kg]



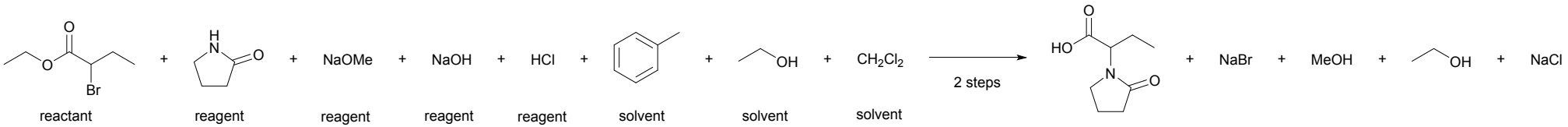
reactant | reagent | catalyst | work-up (estimated)

Reactants | *Products*

Formula	$C_4H_7BrO_2$	C_2H_6O	HKO_4S	CNa_2O_3	Formula	$C_6H_{11}BrO_2$	H_2O
MW	167,00	46,07	136,16	105,99	MW	195,06	18,02
Limiting?	***Yes***	No	No	No	Equivalents		
Equivalents	***1,00***	2,90	0,12	0,16	%Completion	***98,50%***	
Sample Mass					Expected Mass	1150,47kg	107,87kg
%Weight					Expected Moles	5,90kmol	5,99kmol
Molarity					Measured Mass		
Density					Purity		
Volume					Product Mass		
Reactant Moles	5,99kmol	17,37kmol	734,42mol	943,51mol	Product Moles		
Reactant Mass	***1000,00kg***	***800,00kg***	***100,00kg***	***100,00kg***	%Yield		

AE = 195.06 : (195.06 + 18.02) = 92%

EF = (1000 + 800 + 100 + 100 - 1150.47) : 1150.47 = 0.74 [kg/kg]

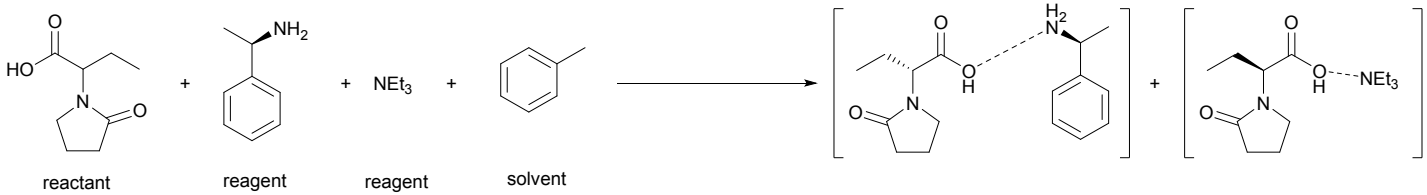


Reactants | *Products*

Formula	$C_6H_{11}BrO_2$	C_4H_7NO	CH_3NaO	HNaO	ClH	C_7H_8	C_2H_6O	CH_2Cl_2	Formula	$C_8H_{13}NO_3$	BrNa	CH_4O	C_2H_6O	ClNa
MW	195,06	85,11	54,02	40,00	36,46	92,14	46,07	84,93	MW	171,20	102,89	32,04	46,07	58,44
Limiting?	***Yes***	No	No	No	No	No	No	No	Equivalents					
Equivalents	***1,10***	1,00	1,07	***2,20***	5,00	2,71	2,19	13,33	%Completion	***88,00%***				
Sample Mass	252,00g	100,00g		103,35g		293,48g	118,35g		Expected Mass	176,94g	120,85g	37,63g	54,11g	68,64g
%Weight									Expected Moles	1,03mol	1,17mol	1,17mol	1,17mol	1,17mol
Molarity									Measured Mass					
Density					***1,19g/mL***	***867,00mg/mL***	***789,00mg/mL***	***1,33g/mL***	Purity					
Volume					***180,00mL***	***338,50mL***	***150,00mL***	***1,00kmL***	Product Mass					
Reactant Moles	1,29mol	1,18mol	1,25mol	2,58mol	5,88mol	3,19mol	2,57mol	15,66mol	Product Moles					
Reactant Mass	***252,00g***	***100,00g***	***67,70g***	103,35g	214,20g	293,48g	118,35g	1,33kg	%Yield					

AE = 171.20 : (171.20 + 102.89 + 32.04 + 46.07 + 58.44) = 42%

EF = (252 + 100 + 67.7 + 103.35 + 214.2 + 293.48 + 118.35 + 1330 - 176.94) : 176.94 = 13.0 [g/g]

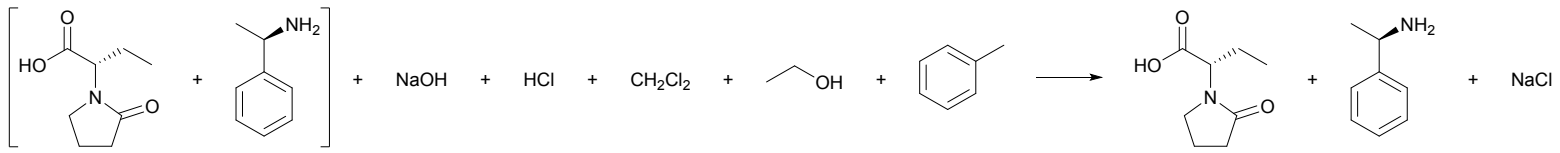


Reactants | *Products*

Formula	$C_8H_{13}NO_3$	$C_8H_{11}N$	$C_6H_{15}N$	C_7H_8	Formula	$C_{16}H_{24}N_2O_3$	$C_{14}H_{28}N_2O_3$
MW	171,20	121,18	101,19	92,14	MW	292,38	272,39
Limiting?	***Yes***	No	No	No	Equivalents		
Equivalents	1,00	***0,60***	***0,50***	12,55	%Completion	***49,10%***	
Sample Mass		31,43g	21,87g	500,00g	Expected Mass	62,05g	117,74g
%Weight					Expected Moles	212,24mmol	432,25mmol
Molarity					Measured Mass		
Density				***867,00mg/mL***	Purity		
Volume				***576,70mL***	Product Mass		
Reactant Moles	432,25mmol	259,35mmol	216,13mmol	5,43mol	Product Moles		
Reactant Mass	***74,00g***	31,43g	21,87g	500,00g	%Yield		

AE = 292.38 : (292.38 + 272.39) = 52%

EF = (74 + 31,43 + 21,87 + 500 - 62.05) : 62.05 = 9.1 [g/g]



reactant | reagent | reagent (estimated) | solvent | solvent | solvent

Reactants

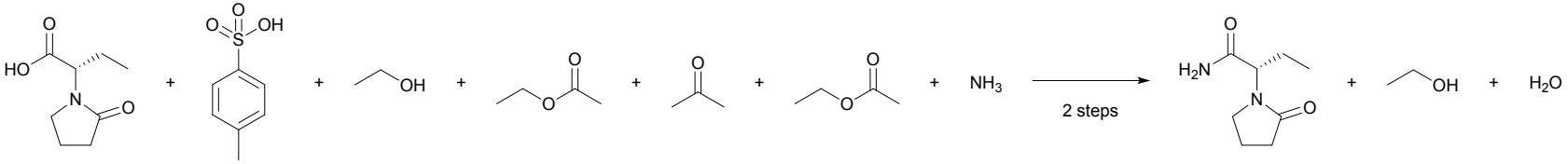
Formula	$C_{16}H_{24}N_2O_3$	HNaO	ClH	CH_2Cl_2	C_2H_6O	C_7H_8
MW	292,38	40,00	36,46	84,93	46,07	92,14
Limiting?	***Yes***	No	No	No	No	No
Equivalents	***1,00***	***4,37***	***1,00***	27,49	3,85	8,26
Sample Mass			8,77g	561,26g	42,61g	182,94g
%Weight						
Molarity						
Density				***1,33g/mL***	***789,00mg/mL***	***867,00mg/mL***
Volume				***422,00mL***	***54,00mL***	***211,00mL***
Reactant Moles	240,44mmol	1,05mol	240,44mmol	6,61mol	924,83mmol	1,99mol
Reactant Mass	***70,30g***	***42,00g***	8,77g	561,26g	42,61g	182,94g

Products

Formula	$C_8H_{13}NO_3$	$C_8H_{11}N$	ClNa
MW	171,20	121,18	58,44
Equivalents			
%Completion	***87,10%***		
Expected Mass	35,85g	29,14g	14,05g
Expected Moles	209,42mmol	240,44mmol	240,44mmol
Measured Mass			
Purity			
Product Mass			
Product Moles			
%Yield			

AE = 171.20 : (171.20 + 121.18 + 58.44) = 49%

EF = (70.3 + 42 + 8.77 + 561.26 + 42.61 + 182.94 - 35.85) : 35.85 = 24.3 [g/g]



reactant | catalyst | solvent | solvent | solvent | solvent | reagent

Reactants

Formula	$C_8H_{13}NO_3$	$C_7H_8O_3S$	C_2H_6O	$C_4H_8O_2$	C_3H_6O	$C_4H_8O_2$	H_3N
MW	171,20	172,20	46,07	88,11	58,08	88,11	17,03
Limiting?	***Yes***	No	No	No	No	No	No
Equivalents	***1,00***	***0,10***	29,32	17,53	0,66	1,50	91,47
Sample Mass		2,12g	166,48g	190,32g	4,70g	16,24g	192,01g
%Weight							
Molarity							
Density			***789,00mg/mL***	***902,00mg/mL***	***784,00mg/mL***	***902,00mg/mL***	***910,00mg/mL***
Volume			***211,00mL***	***211,00mL***	***6,00mL***	***18,00mL***	***211,00mL***
Reactant Moles	123,25mmol	12,33mmol	3,61mol	2,16mol	80,99mmol	184,28mmol	11,27mol
Reactant Mass	***21,10g***	2,12g	166,48g	190,32g	4,70g	16,24g	192,01g

Products

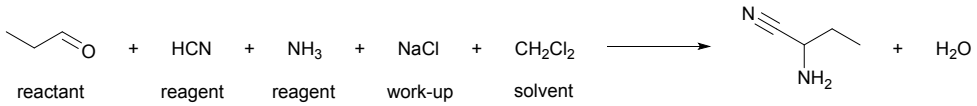
Formula	$C_8H_{14}N_2O_2$	C_2H_6O
MW	170,21	46,07
Equivalents		
%Completion	***59,00%***	
Expected Mass	12,38g	5,68g
Expected Moles	72,72mmol	123,25mmol
Measured Mass		
Purity		
Product Mass		
Product Moles		
%Yield		

AE = 170.21 : (170.21 + 46.07 + 18.02) = 73%

EF = (21.1 + 2.12 + 166.48 + 190.32 + 4.7 + 16.24 + 192.01 - 12.38) : 12.38 = 46.9 [g/g]

Π(AE) = 5%; Σ(E-factor) = 94.6, total yield = 22%

2.2 Strecker synthesis – amine alkylation pathway[3]

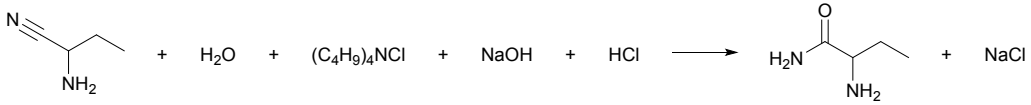


reactant reagent reagent work-up solvent

Reactants						*Products*	
Formula	**C_3H_6O**	**CHN**	**H_3N**	**ClNa**	**CH_2Cl_2**	Formula	**$C_4H_8N_2$**
MW	58,08	27,03	17,03	58,44	84,93	MW	84,12
Limiting?	***Yes***	No	No	No	No	Equivalents	
Equivalents		1,05	1,29	0,13	2,32	%Completion	***94,00%***
Sample Mass						Expected Mass	360,79g
%Weight						Expected Moles	4,29mol
Molarity						Measured Mass	
Density						Purity	
Volume						Product Mass	
Reactant Moles	4,56mol	4,81mol	5,87mol	612,60mmol	10,60mol	Product Moles	
Reactant Mass	***265,00g***	***130,00g***	***100,00g***	***35,80g***	***900,00g***	%Yield	

AE = 84.12 : (84.12 + 18.02) = 82%

EF = (265 + 130 + 100 + 35.8 + 900 - 360.79) : 360.79 = 2.97 [g/g]

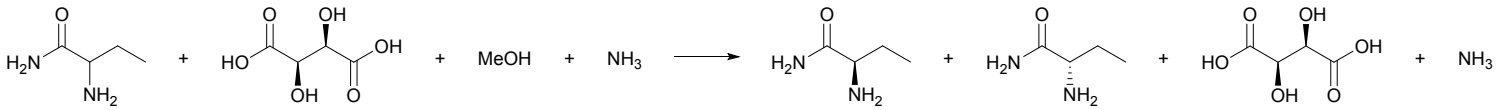


reactant reagent catalyst reagent work-up

Reactants						*Products*		
Formula	**$C_4H_8N_2$**	**H_2O**	**$C_{16}H_{36}ClN$**	**HNaO**	**ClH**	Formula	**$C_4H_{10}N_2O$**	**ClNa**
MW	84,12	18,02	277,92	40,00	36,46	MW	102,14	58,44
Limiting?	***Yes***	No	No	No	No	Equivalents		
Equivalents			0,00	0,04	0,04	%Completion	***95,00%***	
Sample Mass		77,10g				Expected Mass	415,24g	250,09g
%Weight						Expected Moles	4,07mol	4,28mol
Molarity						Measured Mass		
Density						Purity		
Volume						Product Mass		
Reactant Moles	4,28mol	4,28mol	12,95mmol	175,01mmol	164,57mmol	Product Moles		
Reactant Mass	***360,00g***	77,10g	***3,60g***	***7,00g***	***6,00g***	%Yield		

AE = 102.14 : 102.14 = 100%

EF = (360 + 77.11 + 3.6 + 7 + 6 - 415.24) : 415.24 = 0,09 [g/g]

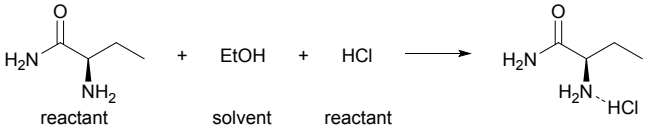


reactant | reagent | solvent | reagent

Reactants					*Products*				
Formula	**$C_4H_{10}N_2O$**	**$C_4H_6O_6$**	**CH_4O**	**H_3N**	Formula	**$C_4H_{10}N_2O$**	**$C_4H_{10}N_2O$**	**$C_4H_6O_6$**	**H_3N**
MW	102,14	150,09	32,04	17,03	MW	102,14	102,14	150,09	17,03
Limiting?	***Yes***	No	No	No	Equivalents				
Equivalents		0,38	27,82	0,75	%Completion	***46,30%***			
Sample Mass					Expected Mass	185,66g	401,00g	589,25g	66,87g
%Weight					Expected Moles	1,82mol	3,93mol	3,93mol	3,93mol
Molarity					Measured Mass				
Density					Purity				
Volume					Product Mass				
Reactant Moles	3,93mol	1,50mol	109,23mol	2,94mol	Product Moles				
Reactant Mass	***401,00g***	***225,00g***	***3,50kg***	***50,00g***	%Yield				

AE = 102.14 : (102.14 + 102.14+ 150.09 + 17.03) = 28%

E-Factor = (401 + 225 + 3500 + 50 - 185,66) : 185,66 = 21.5 [g/g]

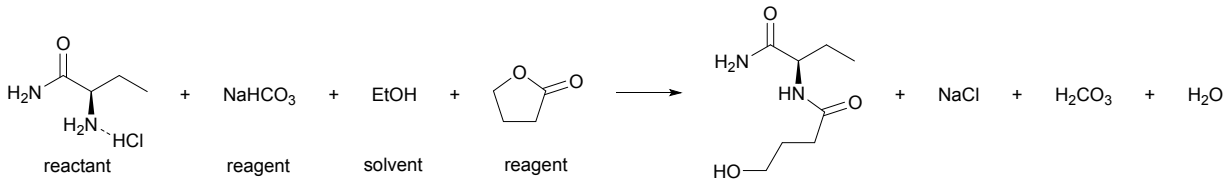


reactant | solvent | reactant

Reactants				*Products*	
Formula	**$C_4H_{10}N_2O$**	**C_2H_6O**	**ClH**	Formula	**$C_4H_{11}ClN_2O$**
MW	102,14	46,07	36,46	MW	138,60
Limiting?	***Yes***	No	No	Equivalents	
Equivalents		7,75	1,15	%Completion	***96,00%***
Sample Mass				Expected Mass	238,39g
%Weight				Expected Moles	1,72mol
Molarity				Measured Mass	
Density				Purity	
Volume				Product Mass	
Reactant Moles	1,79mol	13,89mol	2,06mol	Product Moles	
Reactant Mass	***183,00g***	***640,00g***	***75,00g***	%Yield	

AE = 138.60 : 138.60 = 100%

EF = (183 + 640 + 75) : 238.39 = 2.76 [g/g]

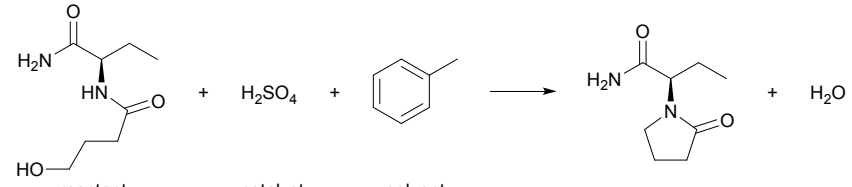


Reactants *Products*

Formula	**$C_4H_{11}ClN_2O$**	**$CHNaO_3$**	**C_2H_6O**	**$C_4H_6O_2$**	Formula	**$C_8H_{16}N_2O_3$**	**$ClNa$**	**CH_2O_3**	**H_2O**
MW	138,60	84,01	46,07	86,09	MW	188,23	58,44	62,02	18,02
Limiting?	***Yes***	No	No	No	Equivalents				
Equivalents			23,74	1,10	%Completion	***90,00%***			
Sample Mass		12,12g			Expected Mass	24,45g	8,43g	8,95g	2,60g
%Weight					Expected Moles	129,87mmol	144,31mmol	144,31mmol	144,31mmol
Molarity					Measured Mass				
Density			***789,00mg/mL***		Purity				
Volume			***200,00mL***		Product Mass				
Reactant Moles	144,31mmol	144,31mmol	3,43mol	159,14mmol	Product Moles				
Reactant Mass	***20,00g***	***12,12***g	157,80g	***13,70g***	%Yield				

AE = 188.23 : (188.23 + 58.44 + 62.02 + 18.02) = 58%

EF = (20 + 12.12 + 157.8 + 13.7 - 24.45) : 24.45 = 7.33 [g/g]



Reactants *Products*

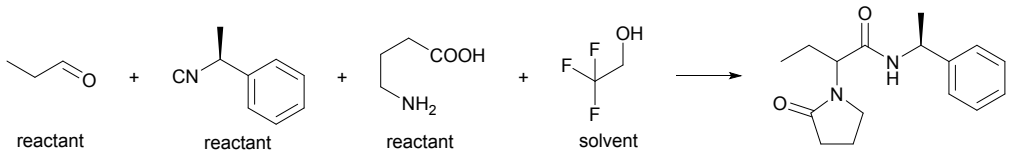
Formula	**$C_8H_{16}N_2O_3$**	**H_2O_4S**	**C_7H_8**	Formula	**$C_8H_{14}N_2O_2$**
MW	188,23	98,07	92,14	MW	170,21
Limiting?	***Yes***	No	No	Equivalents	
Equivalents		0,08	17,71	%Completion	***59,00%***
Sample Mass			173,40g	Expected Mass	10,67g
%Weight				Expected Moles	62,69mmol
Molarity				Measured Mass	
Density			***867,00mg/mL***	Purity	
Volume			***200,00mL***	Product Mass	
Reactant Moles	106,25mmol	8,16mmol	1,88mol	Product Moles	
Reactant Mass	***20,00g***	***800,00mg***	173,40g	%Yield	

AE = 170.21 : (170.21 + 18.03) = 90%

EF = (20 + 0.8 + 173.4 - 10.67) : 10.67 = 17.2 [g/g]

Π(AE) = 12%; Σ(E-factor) = 51.85, total yield: 21%

2.3 Ugi three-component reaction[4]



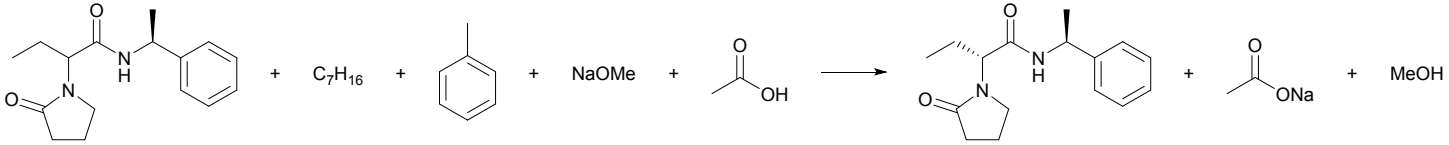
reactant reactant reactant solvent

Reactants *Products*

Formula	C_3H_6O	C_9H_9N	$C_4H_9NO_2$	$C_2H_3F_3O$	Formula	$C_{16}H_{22}N_2O_2$
MW	58,08	131,18	103,12	100,04	MW	274,36
Limiting?	***Yes***	No	No	No	Equivalents	
Equivalents	***1,00***		1,00	139,14	%Completion	***20,00%***
Sample Mass		131,00mg	103,00mg	13,90g	Expected Mass	54,80mg
%Weight					Expected Moles	199,72µmol
Molarity					Measured Mass	
Density				***1,39g/mL***	Purity	
Volume				***10,00mL***	Product Mass	
Reactant Moles	998,62µmol	998,62µmol	998,83µmol	138,94mmol	Product Moles	
Reactant Mass	***58,00mg***	131,00mg	***103,00mg***	13,90g	%Yield	

AE = 274.36 : 274.36 = 100%

EF = (0.058 + 0.131 + 0.103 + 13.9 - 0.0548) : 0.0548 = 258 [g/g]



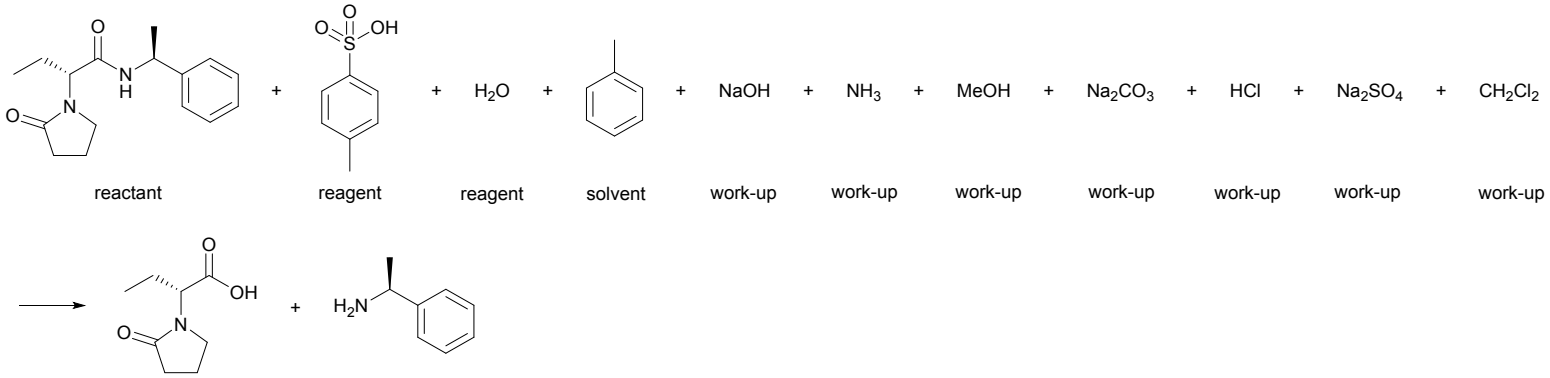
reactant solvent solvent reagent reagent

Reactants *Products*

Formula	$C_{16}H_{22}N_2O_2$	C_7H_{16}	C_7H_8	CH_3NaO	$C_2H_4O_2$	Formula	$C_{16}H_{22}N_2O_2$	$C_2H_3NaO_2$
MW	274,36	100,21	92,14	54,02	60,05	MW	274,36	82,03
Limiting?	***Yes***	No	No	No	No	Equivalents		
Equivalents		16,86	2,58	0,34	0,15	%Completion	***73,30%***	
Sample Mass		9,23g	1,30g			Expected Mass	1,10g	448,49mg
%Weight						Expected Moles	4,01mmol	5,47mmol
Molarity						Measured Mass		
Density		***684,00mg/mL***	***867,00mg/mL***			Purity		
Volume		***13,50mL***	***1,50mL***			Product Mass		
Reactant Moles	5,47mmol	92,15mmol	14,11mmol	1,85mmol	832,61µmol	Product Moles		
Reactant Mass	***1,50g***	9,23g	1,30g	***100,00mg***	***50,00mg***	%Yield		

AE = 274.36 : (274.36 + 82.03 + 32.04) = 71%

EF = (1.5 + 9.32 + 1.3 + 0.1 + 0.05 - 1.1) : 1.1 = 10.2 [g/g]

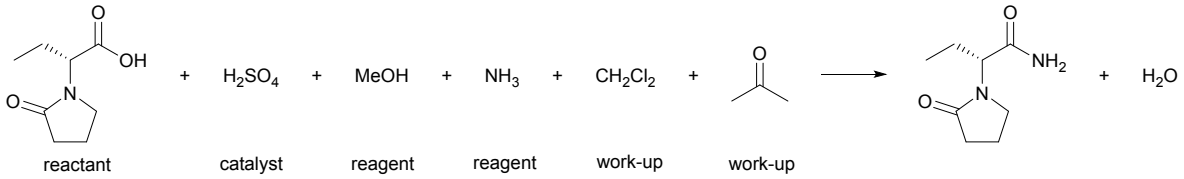


Reactants *Products*

Formula	**$C_{16}H_{22}N_2O_2$**	**$C_6H_6O_3S$**	**H_2O**	**C_7H_8**	**HNaO**	**H_3N**	**CH_4O**	**CNa_2O_3**	**ClH**	**Na_2O_4S**	**CH_2Cl_2**	Formula	**$C_8H_{13}NO_3$**	**$C_8H_{11}N$**
MW	274,36	158,17	18,02	92,14	40,00	17,03	32,04	105,99	36,46	142,04	84,93	MW	171,20	121,18
Limiting?	***Yes***	No	No	No	No	No	No	No	No	No	No	Equivalents		
Equivalents		12,66	4,01	114,88	8,23	3,84	13,56	4,12			1074,17	%Completion	***49,00%***	
Sample Mass			263,00mg	38,58g	1,20g	238,43mg	1,58g	1,59g	4,43g	517,69mg	332,50g	Expected Mass	305,75mg	441,69mg
%Weight									***3,00%***			Expected Moles	1,79mmol	3,64mmol
Molarity					***1,00M***	***7,00M***		***1,00M***				Measured Mass		
Density			***1,00g/mL***	***867,00mg/mL***			***792,00mg/mL***				***1,33g/mL***	Purity		
Volume			***263,00mmL***	***44,50mL***	***30,00mL***	***2,00mL***	***2,00mL***	***15,00mL***			***250,00mL***	Product Mass		
Reactant Moles	3,64mmol	46,15mmol	14,60mmol	418,72mmol	30,00mmol	14,00mmol	49,44mmol	15,00mmol	3,64mmol	3,64mmol	3,92mol	Product Moles		
Reactant Mass	***1,00g***	***7,30g***	263,00mg	38,58g	1,20g	238,43mg	1,58g	1,59g	132,88mg	517,69mg	332,50g	%Yield		

AE = 171.20 : (171.20 + 121.18) = 59%

EF = (1 + 7.3 + 0.263 + 38.58 + 1.2 + 0.23843 + 1.58 + 1.59 + 0.13288 + 0.51769 + 332.5 - 0.30575) : 0.30575 = 1258.89 [g/g]



reactant | catalyst | reagent | reagent | work-up | work-up

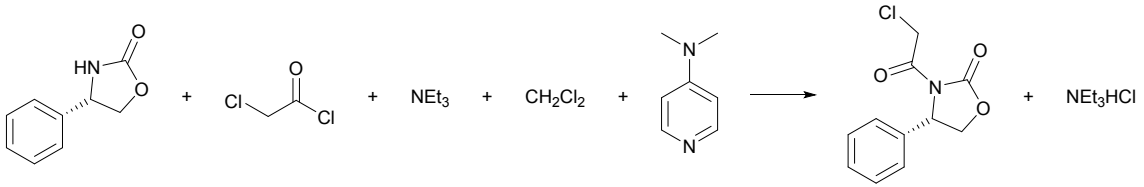
Reactants							*Products*	
Formula	**$C_8H_{13}NO_3$**	**H_2O_4S**	**CH_4O**	**H_3N**	**CH_2Cl_2**	**C_3H_6O**	Formula	**$C_8H_{14}N_2O_2$**
MW	171,20	98,07	32,04	17,03	84,93	58,08	MW	170,21
Limiting?	***Yes***	No	No	No	No	No	Equivalents	
Equivalents		0,11	21,51		5,61	12,45	%Completion	***67,80%***
Sample Mass		201,30mg	13,46g		9,31g		Expected Mass	2,25g
%Weight				***30,00%***			Expected Moles	13,24mmol
Molarity							Measured Mass	
Density		***1,83g/mL***	***792,00mg/mL***		***1,33g/mL***	***785,00mg/mL***	Purity	
Volume		***110,00mmL***	***17,00mL***	***7,50mL***	***7,00mL***	***18,00mL***	Product Mass	
Reactant Moles	19,53mmol	2,05mmol	420,20mmol	19,53mmol	109,62mmol	243,29mmol	Product Moles	
Reactant Mass	***3,34g***	201,30mg	13,46g	332,67mg	9,31g	14,13g	%Yield	

AE = 170.21 : (170.21 + 18.03) = 90%

EF = (3,34 + 0.2013 + 13.46 + 0.33267 + 9.31 + 14.13 - 2.25) : 2.25 = 17.12 [g/g]

Π(AE) = 38%; Σ(E-factor) = 1544.21, total yield: 5%

2.4 Evans' auxiliary route[5]

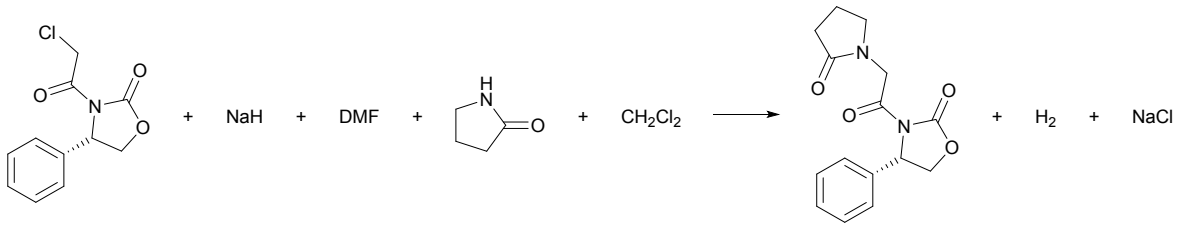


reactant reagent reagent solvent reagent

Reactants						*Products*		
Formula	**$C_9H_9NO_2$**	**$C_2H_2Cl_2O$**	**$C_6H_{15}N$**	**CH_2Cl_2**	**$C_7H_{10}N_2$**	Formula	**$C_{11}H_{10}ClNO_3$**	**$C_6H_{16}ClN$**
MW	163,18	112,94	101,19	84,93	122,17	MW	239,65	137,65
Limiting?	***Yes***	No	No	No	No	Equivalents		
Equivalents	***1,00***	***2,20***	3,98	63,89	1,27	%Completion	***70,00%***	
Sample Mass		15,23g				Expected Mass	10,28g	8,44g
%Weight						Expected Moles	42,90mmol	61,28mmol
Molarity						Measured Mass		
Density				***1,33g/mL***		Purity		
Volume				***250,00mL***		Product Mass		
Reactant Moles	***61,28mmol***	***134,82mmol***	***244,00mmol***	3,92mol	77,76mmol	Product Moles		
Reactant Mass	***10,00g***	15,23g	24,69g	332,50g	***9,50g***	%Yield		

AE = 239.65 : (239.65 + 137.65) = 64%

EF = (10 + 15.23 + 24.69 + 332.5 + 9.5 - 10.28) : 10.28 =37.12 [g/g]

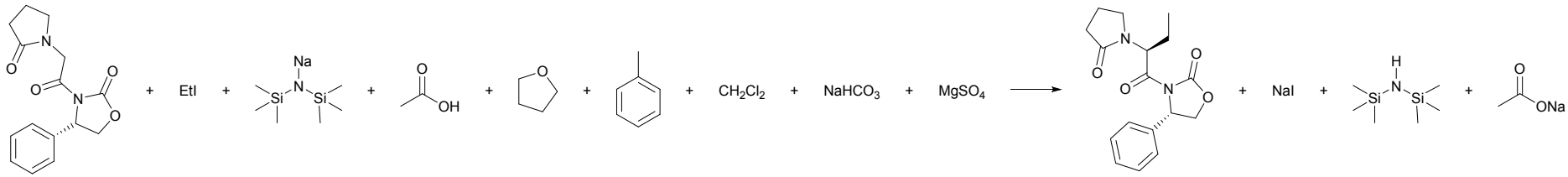


reactant reagent solvent reagent work-up

Reactants						*Products*			
Formula	**$C_{11}H_{10}ClNO_3$**	**HNa**	**C_3H_7NO**	**C_4H_7NO**	**CH_2Cl_2**	Formula	**$C_{15}H_{16}N_2O_4$**	**H_2**	**ClNa**
MW	239,65	24,00	73,09	85,11	84,93	MW	288,30	2,02	58,44
Limiting?	***Yes***	No	No	No	No	Equivalents			
Equivalents		1,29	24,76	1,19	30,02	%Completion	***90,00%***		
Sample Mass			188,80g			Expected Mass	27,07g	210,30mg	6,10g
%Weight						Expected Moles	93,88mmol	104,32mmol	104,32mmol
Molarity						Measured Mass			
Density			***944,00mg/mL***		***1,33g/mL***	Purity			
Volume			***200,00mL***		***200,00mL***	Product Mass			
Reactant Moles	104,32mmol	***135,00mmol***	2,58mol	124,55mmol	3,13mol	Product Moles			
Reactant Mass	***25,00g***	3,24g	188,80g	***10,60g***	266,00g	%Yield			

AE = 288.30 : (288.30 + 2.02 + 58.44) = 83%

EF = (25 + 3.24 + 188.8 + 10.6 + 266 - 27.07) : 27.07 = 17.24 [g/g]



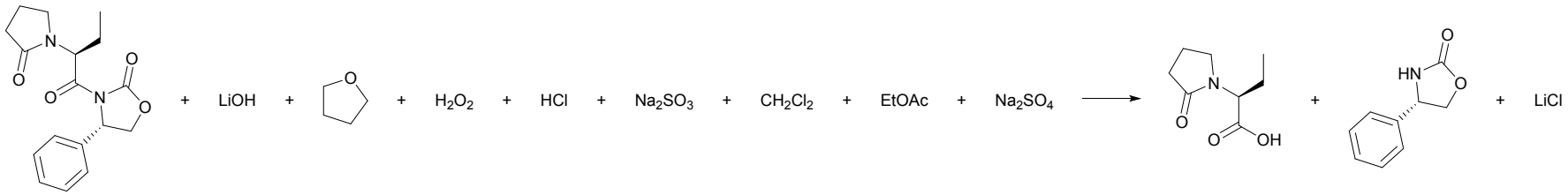
reactant | reagent | reagent | reagent | solvent | solvent | work-up | work-up | work-up

Reactants | *Products*

Formula	**$C_{15}H_{16}N_2O_4$**	**C_2H_5I**	**$C_6H_{18}NNaSi_2$**	**$C_2H_4O_2$**	**C_4H_8O**	**C_7H_8**	**CH_2Cl_2**	**$CHNaO_3$**	**MgO_4S**	Formula	**$C_{17}H_{20}N_2O_4$**	**INa**	**$C_6H_{19}NSi_2$**	**$C_2H_3NaO_2$**
MW	288,30	155,97	183,38	60,05	72,11	92,14	84,93	84,01	120,36	MW	316,36	149,89	161,40	82,03
Limiting?	***Yes***	No	No	No	No	No	No	No	No	Equivalents				
Equivalents		1,20	1,50	5,04	106,63	14,11	383,77	6,59		%Completion	***81,00%***			
Sample Mass				5,25g	133,35g	22,54g	565,25g		2,09g	Expected Mass	4,44g	2,60g	2,80g	1,42g
%Weight										Expected Moles	14,05mmol	17,34mmol	17,34mmol	17,34mmol
Molarity										Measured Mass				
Density				***1,05g/mL***	***889,00mg/mL***	***867,00mg/mL***	***1,33g/mL***			Purity				
Volume				***5,00mL***	***150,00mL***	***26,00mL***	***425,00mL***			Product Mass				
Reactant Moles	17,34mmol	20,77mmol	26,01mmol	87,42mmol	1,85mol	244,65mmol	6,66mol	114,28mmol	17,34mmol	Product Moles				
Reactant Mass	***5,00g***	***3,24g***	***4,77g***	5,25g	133,35g	22,54g	565,25g	***9,60g***	2,09g	%Yield				

AE = 316.36 : (316.36 + 149.89 + 161.40 + 82.03) = 45%

EF = (5 + 3.24 + 4.77 + 5.25 + 133.35 + 22.54 + 565.25 + 9.6 + 2.09 - 4.44) : 4.44 = 168.16 [g/g]



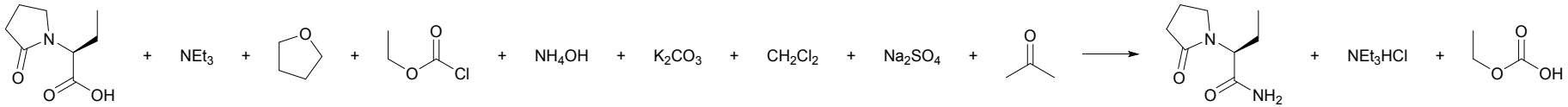
reactant | reagent | solvent | reagent | work-up | work-up | work-up | work-up | work-up

Reactants | *Products*

Formula	$C_{17}H_{20}N_2O_4$	HLiO	C_4H_8O	H_2O_2	ClH	Na_2O_3S	CH_2Cl_2	$C_4H_8O_2$	Na_2O_4S	Formula	$C_8H_{13}NO_3$	$C_9H_9NO_2$	ClLi
MW	316,36	23,95	72,11	34,01	36,46	126,04	84,93	88,11	142,04	MW	171,20	163,18	42,39
Limiting?	***Yes***	No	No	No	No	No	No	No	No	Equivalents			
Equivalents		2,99	23,40	4,68		8,28	148,63	97,16		%Completion	***85,00%***		
Sample Mass			26,67g		576,22mg		199,50g	135,30g	2,24g	Expected Mass	2,30g	2,58g	669,97mg
%Weight										Expected Moles	13,43mmol	15,80mmol	15,80mmol
Molarity										Measured Mass			
Density			***889,00mg/mL***				***1,33g/mL***	***902,00mg/mL***		Purity			
Volume			***30,00mL***				***150,00mL***	***150,00mL***		Product Mass			
Reactant Moles	15,80mmol	47,19mmol	369,87mmol	***74,00mmol***	15,80mmol	130,91mmol	2,35mol	1,54mol	15,80mmol	Product Moles			
Reactant Mass	***5,00g***	***1,13g***	***26,67g***	2,52g	576,22mg	***16,50g***	199,50g	135,30g	2,24g	%Yield			

AE = 171.20 : (171.20 + 163.18 + 42.39) = 45%

EF = (5 + 1.13 + 26.67 + 2.52 + 0.57622 + 16.5+ 199.5 + 135.3 + 2.24 - 2.3) : 2.3 = 168.32 [g/g]



reactant | reagent | solvent | reagent | work-up | work-up | work-up | work-up | work-up

Reactants | *Products*

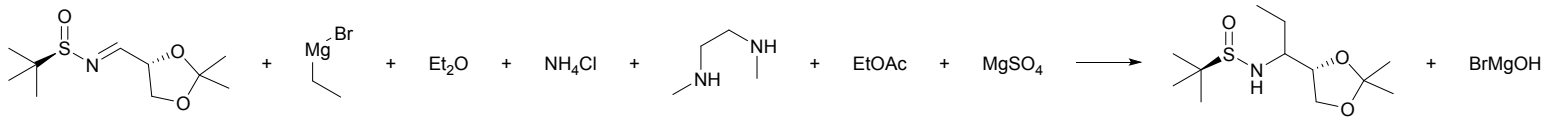
Formula	$C_8H_{13}NO_3$	$C_6H_{15}N$	C_4H_8O	$C_3H_5ClO_2$	H_5NO	CK_2O_3	CH_2Cl_2	Na_2O_4S	C_3H_6O	Formula	$C_8H_{14}N_2O_2$	$C_6H_{16}ClN$	$C_3H_6O_3$
MW	171,20	101,19	72,11	108,52	35,05	138,20	84,93	142,04	58,08	MW	170,21	137,65	90,08
Limiting?	***Yes***	No	No	No	No	No	No	No	No	Equivalents			
Equivalents		1,06	8,44	1,04	4,64	***1,03***	80,43		46,28	%Completion	***86,00%***		
Sample Mass								4,15g	78,50g	Expected Mass	4,28g	4,02g	2,63g
%Weight					***25,00%***					Expected Moles	25,12mmol	29,21mmol	29,21mmol
Molarity										Measured Mass			
Density		***726,00mg/mL***	***889,00mg/mL***	***1,14g/mL***			***1,33g/mL***		***785,00mg/mL***	Purity			
Volume		***4,30mL***	***20,00mL***	***2,90mL***	***19,00mL***		***150,00mL***		***100,00mL***	Product Mass			
Reactant Moles	29,21mmol	30,85mmol	246,58mmol	30,46mmol	135,54mmol	29,96mmol	2,35mol	29,21mmol	1,35mol	Product Moles			
Reactant Mass	***5,00g***	3,12g	17,78g	3,31g	***4,75g***	***4,14g***	199,50g	4,15g	78,50g	%Yield			

AE = 170.21 : (170.21 + 137.65 + 90.08) = 43%

EF = (5 + 3.12 + 17.78 + 3.31 + 4.75 + 4.14 + 199.5 + 4.15 + 78.5 - 4.28) : 4.28 = 73.82 [g/g]

Π(AE) = 5%; Σ(E-factor) = 464.66, total yield: 37%

2.5 Sulfinimine-auxiliary route[6]

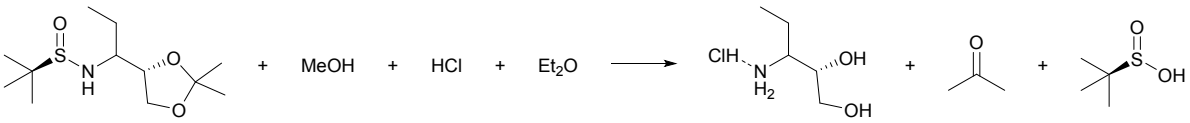


reactant reagent solvent work-up reagent work-up work-up

Reactants								*Products*		
Formula	**$C_{10}H_{19}NO_3S$**	**C_2H_5BrMg**	**$C_4H_{10}O$**	**ClH_4N**	**$C_4H_{12}N_2$**	**$C_4H_8O_2$**	**MgO_4S**	Formula	**$C_{12}H_{25}NO_3S$**	**BrHMgO**
MW	233,33	133,27	74,12	53,49	88,15	88,11	120,36	MW	263,40	121,22
Limiting?	***Yes***	No	No	No	No	No	No	Equivalents		
Equivalents		***2,00***	30,75	8,11	2,67	71,66		%Completion	***80,00%***	
Sample Mass		11,42g				270,60g	5,16g	Expected Mass	9,03g	5,20g
%Weight								Expected Moles	34,29mmol	42,86mmol
Molarity								Measured Mass		
Density			***713,00mg/mL***			***902,00mg/mL***		Purity		
Volume			***137,00mL***			***300,00mL***		Product Mass		
Reactant Moles	42,86mmol	85,72mmol	1,32mol	347,74mmol	114,57mmol	3,07mol	42,86mmol	Product Moles		
Reactant Mass	***10,00g***	11,42g	97,68g	***18,60g***	***10,10g***	270,60g	5,16g	%Yield		

AE = 263.40 : (263.40 + 121.22) = 68%

EF = (10 + 11.42 + 97.68 + 18.6 + 10.1 + 270.6 + 5.16 - 9.03) : 9.03 = 45.91 [g/g]

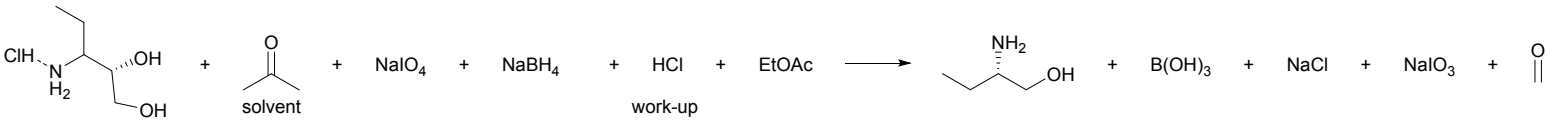


reactant | solvent | reagent | work-up

Reactants					*Products*			
Formula	**$C_{12}H_{25}NO_3S$**	**CH_4O**	**ClH**	**$C_4H_{10}O$**	Formula	**$C_5H_{14}ClNO_2$**	**C_3H_6O**	**$C_4H_{10}O_2S$**
MW	263,40	32,04	36,46	74,12	MW	155,62	58,08	122,18
Limiting?	***Yes***	No	No	No	Equivalents			
Equivalents		109,38		25,34	%Completion	***75,00%***		
Sample Mass			6,92g		Expected Mass	2,22g	1,10g	2,32g
%Weight			***10,00%***		Expected Moles	14,24mmol	18,98mmol	18,98mmol
Molarity					Measured Mass			
Density		***792,00mg/mL***		***713,00mg/mL***	Purity			
Volume		***84,00mL***	***34,00mL***	***50,00mL***	Product Mass			
Reactant Moles	18,98mmol	2,08mol	18,98mmol	480,96mmol	Product Moles			
Reactant Mass	***5,00g***	66,53g	692,08mg	35,65g	%Yield			

AE = 155.62 : (155.62 + 58.08 + 122.18) = 46%

E-Factor = (5 + 66.53 + 6.92 + 35.65 - 2.22) : 2.22 = 50.40 [g/g]

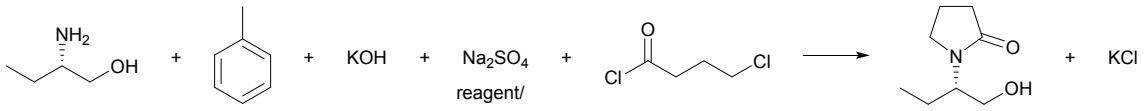


reactant | solvent (estimated) | reagent | reagent | work-up (estimated) | work-up | wrong yield reported

Reactants							*Products*					
Formula	**$C_5H_{14}ClNO_2$**	**C_3H_6O**	**$INaO_4$**	**BH_4Na**	**ClH**	**$C_4H_8O_2$**	Formula	**$C_4H_{11}NO$**	**BH_3O_3**	**ClNa**	**$INaO_3$**	**CH_2O**
MW	155,62	58,08	213,89	37,83	36,46	88,11	MW	89,14	61,83	58,44	197,89	30,03
Limiting?	***Yes***	No	No	No	No	No	Equivalents					
Equivalents		***0,05***	0,99	1,23		71,69	%Completion	***75,00%***				
Sample Mass		37,32mg			468,55mg		Expected Mass	859,18mg	794,63mg	751,05mg	2,54g	385,88mg
%Weight							Expected Moles	9,64mmol	12,85mmol	12,85mmol	12,85mmol	12,85mmol
Molarity							Measured Mass					
Density						***902,00mg/mL***	Purity					
Volume						***90,00mL***	Product Mass					
Reactant Moles	12,85mmol	642,58µmol	12,76mmol	15,86mmol	12,85mmol	921,39mmol	Product Moles					
Reactant Mass	***2,00g***	37,32mg	***2,73g***	***600,00mg***	468,55mg	81,18g	%Yield					

AE = 89.14 : (89.14 + 61.83 + 58.44 + 197.89 + 30.03) = 20%

EF = (2 + 0.03732 + 2.73 + 0.6 + 0.46855 + 81.18 - 0.85918) : 0.85918 = 100.28 [g/g]



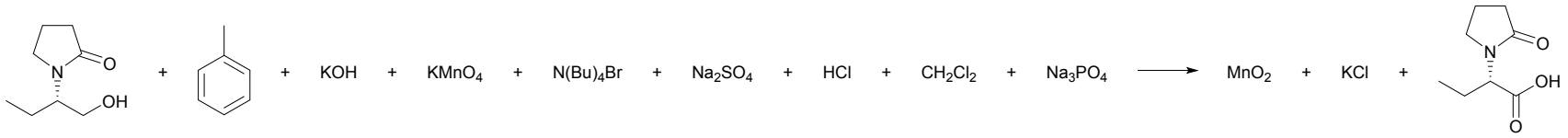
reactant + solvent + KOH + Na_2SO_4 reagent/work-up + reagent → + KCl

Reactants *Products*

Formula	**$C_4H_{11}NO$**	**C_7H_8**	**HKO**	**Na_2O_4S**	**$C_4H_6Cl_2O$**	Formula	**$C_8H_{15}NO_2$**	**ClK**
MW	89,14	92,14	56,11	142,04	140,99	MW	157,21	74,55
Limiting?	***Yes***	No	No	No	No	Equivalents		
Equivalents		6,71	2,99	1,15	1,09	%Completion	***80,00%***	
Sample Mass		69,36g				Expected Mass	14,11g	8,36g
%Weight						Expected Moles	89,75mmol	112,19mmol
Molarity						Measured Mass		
Density		***867,00mg/mL***				Purity		
Volume		***80,00mL***				Product Mass		
Reactant Moles	112,19mmol	752,76mmol	335,08mmol	129,55mmol	122,70mmol	Product Moles		
Reactant Mass	***10,00g***	69,36g	***18,80g***	***18,40g***	***17,30g***	%Yield		

AE = 157.21 : (157.21 + 2 * 74.55) = 51%

E-Factor = (10 + 69.36 + 18.8 + 18.4 + 17.3 - 14.11) : 14.11 = 8.49 [g/g]



reactant + solvent + KOH + $KMnO_4$ + $N(Bu)_4Br$ + Na_2SO_4 + HCl + CH_2Cl_2 + Na_3PO_4 → MnO_2 + KCl +

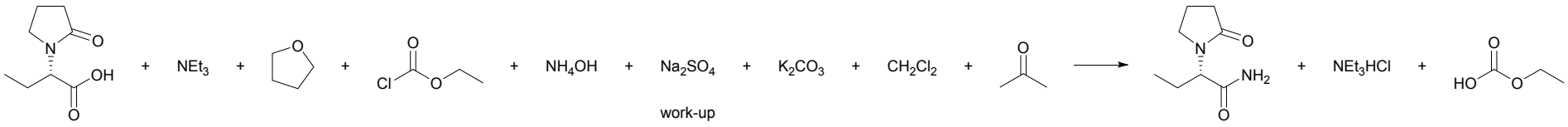
reactant solvent reagent reagent catalyst work-up work-up work-up work-up

Reactants *Products*

Formula	**$C_8H_{15}NO_2$**	**C_7H_8**	**HKO**	**$KMnO_4$**	**$C_{16}H_{36}BrN$**	**Na_2O_4S**	**ClH**	**CH_2Cl_2**	**Na_3O_4P**	Formula	**MnO_2**	**ClK**	**$C_8H_{13}NO_3$**
MW	157,21	92,14	56,11	158,03	322,38	142,04	36,46	84,93	163,94	MW	86,94	74,55	171,20
Limiting?	***Yes***	No	No	No	No	No	No	No	No	Equivalents			
Equivalents		61,56	2,80	1,49	0,10			332,37	2,40	%Completion	***100,00%***		***76,00%***
Sample Mass				1,50g		903,46mg	773,01mg			Expected Mass	552,98mg	474,19mg	827,60mg
%Weight							***30,00%***			Expected Moles	6,36mmol	6,36mmol	4,83mmol
Molarity										Measured Mass			
Density		***902,00mg/mL***						***1,33g/mL***		Purity			
Volume		***40,00mL***					***2,00mL***	***135,00mL***		Product Mass			
Reactant Moles	6,36mmol	391,57mmol	17,82mmol	9,49mmol	620,40µmol	6,36mmol	6,36mmol	2,11mol	15,25mmol	Product Moles			
Reactant Mass	***1,00g***	36,08g	***1,00g***	***1,50g***	***200,00mg***	903,46mg	231,90mg	179,55g	***2,50g***	%Yield			

AE = 171.21 : (171.21 + 74.55 + 66.94) = 55%

EF = (1 + 36.08 + 1 + 1.5 + 0.2 + 0.90346 + 0.77301 + 179.55 + 2.5 - 0.8276) : 0.8276 = 269.07 [g/g]



reactant	reagent	solvent	reagent	reagent	work-up (estimated)	work-up	work-up	work-up

Reactants *Products*

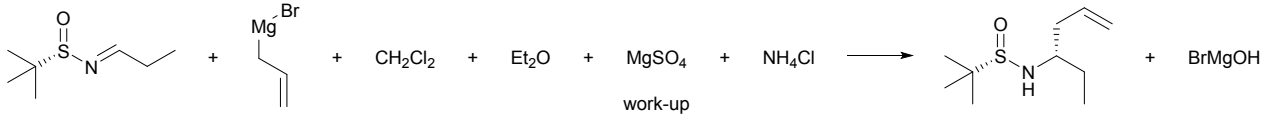
Formula	$C_8H_{13}NO_3$	$C_6H_{15}N$	C_4H_8O	$C_3H_5ClO_2$	H_5NO	Na_2O_4S	CK_2O_3	CH_2Cl_2	C_3H_6O	Formula	$C_8H_{14}N_2O_2$	$C_6H_{16}ClN$	$C_3H_6O_3$
MW	171,20	101,19	72,11	108,52	35,05	142,04	138,20	84,93	58,08	MW	170,21	137,65	90,08
Limiting?	***Yes***	No	No	No	No	No	No	No	No	Equivalents			
Equivalents		1,06	8,44	0,91	4,66			80,43	46,22	%Completion	***65,00%***		
Sample Mass		3,12g	17,78g			4,15g	4,04g	199,50g	78,40g	Expected Mass	3,23g	4,02g	2,63g
%Weight										Expected Moles	18,98mmol	29,21mmol	29,21mmol
Molarity										Measured Mass			
Density		***726,00mg/mL***	***889,00mg/mL***					***1,33g/mL***	***784,00mg/mL***	Purity			
Volume		***4,30mL***	***20,00mL***					***150,00mL***	***100,00mL***	Product Mass			
Reactant Moles	29,21mmol	30,85mmol	246,58mmol	26,72mmol	***136,00mmol***	29,21mmol	29,21mmol	2,35mol	1,35mol	Product Moles			
Reactant Mass	***5,00g***	3,12g	17,78g	***2,90g***	4,77g	4,15g	4,04g	199,50g	78,40g	%Yield			

AE = 170.21 : (170.21 + 137.65 + 90.08) = 43%

EF = (5 + 3.12 + 17.78 + 2.9 + 4.77 + 4.15 + 4.04 + 199.5 + 78.4 - 3,23) : 3,23 = 97.97 [g/g]

Π(AE) = 0.8%; Σ(E-factor) = 572.12, total yield: 18%

2.6 Ruthenium-catalysed route[7]



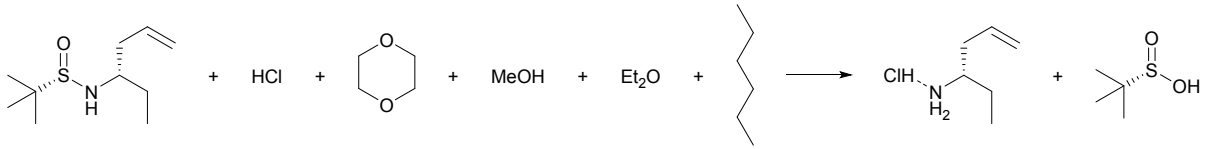
reactant | reagent | solvent | solvent | work-up | work-up

Reactants | *Products*

Formula	$C_7H_{15}NOS$	C_3H_5BrMg	CH_2Cl_2	$C_4H_{10}O$	MgO_4S	ClH_4N	Formula	$C_{10}H_{21}NOS$	BrHMgO
MW	161,26	145,28	84,93	74,12	120,36	53,49	MW	203,34	121,22
Limiting?	***Yes***	No	No	No	No	No	Equivalents		
Equivalents		2,02	1225,52	19,39		75,10	%Completion	***87,00%***	
Sample Mass			144,57g	2,00g	167,19mg		Expected Mass	245,73mg	168,37mg
%Weight							Expected Moles	1,21mmol	1,39mmol
Molarity							Measured Mass		
Density			***1,33g/mL***	***713,00mg/mL***			Purity		
Volume			***108,70mL***	***2,80mL***			Product Mass		
Reactant Moles	1,39mmol	***2,80mmol***	1,70mol	26,93mmol	1,39mmol	104,32mmol	Product Moles		
Reactant Mass	***224,00mg***	406,79mg	144,57g	2,00g	167,19mg	***5,58g***	%Yield		

AE = 203.34 : (203.34 + 121.21) = 63%

EF = (0.244 + 0.40679 + 144.57 + 2 + 0.16719 + 5.58 - 0.24573) : 0.24573 = 621.50 [g/g]



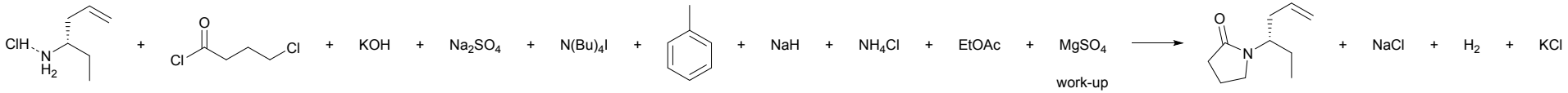
reactant | reagent | solvent | solvent | solvent | solvent

Reactants | *Products*

Formula	$C_{10}H_{21}NOS$	ClH	$C_4H_8O_2$	CH_4O	$C_4H_{10}O$	C_6H_{14}	Formula	$C_6H_{14}ClN$	$C_4H_{10}O_2S$
MW	203,34	36,46	88,11	32,04	74,12	86,18	MW	135,63	122,18
Limiting?	***Yes***	No	No	No	No	No	Equivalents		
Equivalents		***2,00***	5,70	12,57	12,54	39,63	%Completion	***89,00%***	
Sample Mass		111,88mg				5,24g	Expected Mass	185,22mg	187,47mg
%Weight							Expected Moles	1,37mmol	1,53mmol
Molarity							Measured Mass		
Density			***1,00g/mL***	***792,00mg/mL***	***713,00mg/mL***	***655,00mg/mL***	Purity		
Volume			***770,00mmL***	***780,00mmL***	***2,00mL***	***8,00mL***	Product Mass		
Reactant Moles	1,53mmol	3,07mmol	8,74mmol	19,28mmol	19,24mmol	60,80mmol	Product Moles		
Reactant Mass	***312,00mg***	111,88mg	770,00mg	617,76mg	1,43g	5,24g	%Yield		

AE = 135.63 : (135.63 + 122.18) = 53%

EF = (0.312 + 0.11188 + 0.77 + 0.61776 + 1.43 + 5.24 - 0.18522) : 0.18522 = 44.79 [g/g]



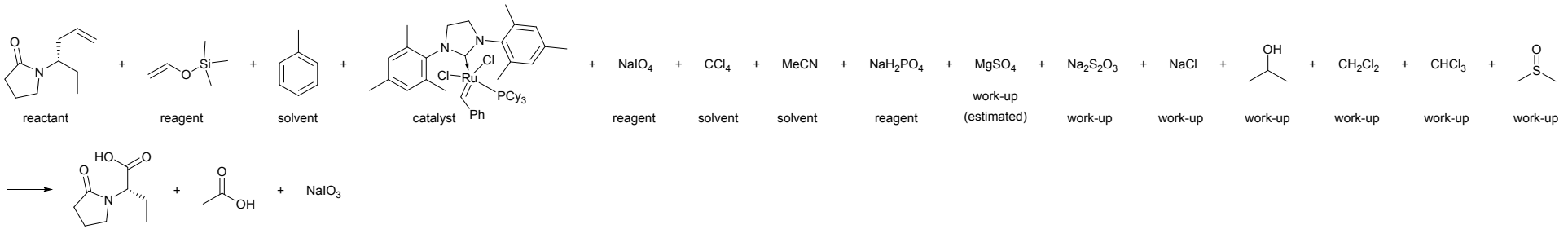
reactant + reagent + reagent + reagent + catalyst + solvent + reagent + work-up + work-up + work-up (estimated)

Reactants / *Products*

Formula	$C_6H_{14}ClN$	$C_4H_6Cl_2O$	HKO	Na_2O_4S	$C_{16}H_{36}IN$	C_7H_8	HNa	ClH_4N	$C_4H_8O_2$	MgO_4S	Formula	$C_{10}H_{17}NO$	ClNa	H_2	ClK
MW	135,63	140,99	56,11	142,04	369,38	92,14	24,00	53,49	88,11	120,36	MW	167,25	58,44	2,02	74,55
Limiting?	***Yes***	No	No	No	No	No	No	No	No	No	Equivalents				
Equivalents		***1,20***	***4,60***		0,07	127,63	3,39	282,99	1666,30		%Completion	***81,00%***			
Sample Mass		62,37mg	95,14mg	52,36mg						44,37mg	Expected Mass	49,94mg	21,54mg	743,17µg	27,48mg
%Weight											Expected Moles	298,60µmol	368,64µmol	368,64µmol	368,64µmol
Molarity											Measured Mass				
Density						***867,00mg/mL***			***902,00mg/mL***		Purity				
Volume						***5,00mL***			***60,00mL***		Product Mass				
Reactant Moles	368,64µmol	442,36µmol	1,70mmol	368,64µmol	25,99µmol	47,05mmol	1,25mmol	104,32mmol	614,26mmol	368,64µmol	Product Moles				
Reactant Mass	***50,00mg***	62,37mg	95,14mg	***52,36mg***	***9,60mg***	4,34g	***30,00mg***	***5,58g***	54,12g	44,37mg	%Yield				

AE = 167.25 : (167.25 + 58.44 + 2.02 + 74.55) = 55%

EF = (0.05 + 0.06237 + 0.09514 + 0.05236 + 0.0096 + 4.34 + 0.03 + 5.58 + 54.12 + 0.04437 - 0.04994) : 0.04994 = 1288.22 [g/g]



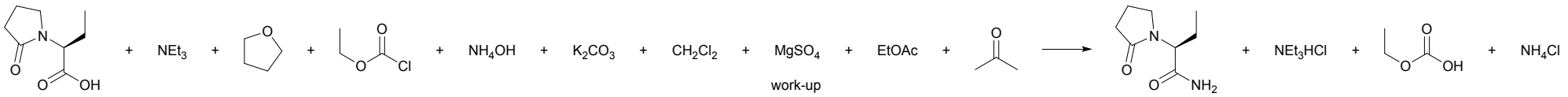
reactant + reagent + solvent + catalyst + reagent + solvent + solvent + reagent + work-up (estimated) + work-up + work-up + work-up + work-up + work-up + work-up

Reactants / *Products*

Formula	$C_{10}H_{17}NO$	$C_5H_{12}OSi$	C_7H_8	$C_{46}H_{65}Cl_2N_2PRu$	$INaO_4$	CCl_4	C_2H_3N	H_2NaO_4P	MgO_4S	$Na_2O_3S_2$	ClNa	C_3H_8O	CH_2Cl_2	$CHCl_3$	C_2H_6OS	Formula	$C_8H_{13}NO_3$	$C_2H_4O_2$	$INaO_3$
MW	167,25	116,23	92,14	848,98	213,89	153,81	41,05	119,98	120,36	158,10	58,44	60,10	84,93	119,37	78,13	MW	171,20	60,05	197,89
Limiting?	***Yes***	No	No	No	No	No	No	No	No	No	No	No	No	No	No	Equivalents			
Equivalents		10,00	486,73	0,05	4,51	37,43	69,33	213,78	14,33	38,23	29,50	488,58	67,51	2331,65		%Completion	***90,00%***		
Sample Mass			52,02g			6,68g	3,30g					34,06g	6,65g	322,84g	90,62mg	Expected Mass	178,72mg	69,66mg	229,54mg
%Weight																Expected Moles	1,04mmol	1,16mmol	1,16mmol
Molarity																Measured Mass			
Density			***867,00mg/mL***			***1,59g/mL***	***786,00mg/mL***					***786,00mg/mL***	***1,33g/mL***	***1,49g/mL***		Purity			
Volume			***60,00mL***			***4,20mL***	***4,20mL***					***43,33mL***	***5,00mL***	***216,67mL***		Product Mass			
Reactant Moles	1,16mmol	***11,60mmol***	564,57mmol	57,95µmol	5,24mmol	43,42mmol	80,41mmol	247,97mmol	16,62mmol	44,34mmol	34,22mmol	566,72mmol	78,30mmol	2,70mol	1,16mmol	Product Moles			
Reactant Mass	***194,00mg***	1,35g	52,02g	***49,20mg***	***1,12g***	6,68g	3,30g	***29,75g***	***2,00g***	***7,01g***	***2,00g***	34,06g	6,65g	322,84g	90,62mg	%Yield			

AE = 171.20 : (171.20 + 60.05 + 197.89) = 40%

EF = 0.194 + 1.35 + 0.05202 + 0.04920 + 1.12 + 6.68 + 29.75 + 2 + 7.01 + 2 + 34.06 + 6.65 + 322.84 + 0.09062 - 0.17872) : 0.17872 = 2314.61 [g/g]



reactant | reagent | solvent | reagent | reagent | work-up | work-up | work-up (estimated) | work-up | work-up

Reactants / *Products*

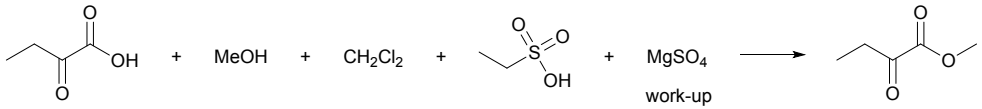
Formula	$C_8H_{13}NO_3$	$C_6H_{15}N$	C_4H_8O	$C_3H_5ClO_2$	H_5NO	CK_2O_3	CH_2Cl_2	MgO_4S	$C_4H_8O_2$	C_3H_6O	Formula	$C_8H_{14}N_2O_2$	$C_6H_{16}ClN$	$C_3H_6O_3$	ClH_4N
MW	171,20	101,19	72,11	108,52	35,05	138,20	84,93	120,36	88,11	58,08	MW	170,21	137,65	90,08	53,49
Limiting?	***Yes***	No	No	No	No	No	No	No	No	No	Equivalents				
Equivalents		***1,00***	70,36	1,11	10,00	1,03	379,13	7,18	212,44	116,71	%Completion	***45,00%***			
Sample Mass			2,93g				18,62g		10,82g		Expected Mass	44,29mg	79,60mg	52,09mg	30,93mg
%Weight											Expected Moles	260,23µmol	578,28µmol	578,28µmol	578,28µmol
Molarity											Measured Mass				
Density		***726,00mg/mL***	***889,00mg/mL***				***1,33g/mL***		***902,00mg/mL***	***784,00mg/mL***	Purity				
Volume		***88,70mmL***	***3,30mL***				***14,00mL***		***12,00mL***	***5,00mL***	Product Mass				
Reactant Moles	578,28µmol	***636,37µmol***	40,69mmol	639,97µmol	***5,78mmol***	593,32µmol	219,25mmol	4,15mmol	122,85mmol	67,49mmol	Product Moles				
Reactant Mass	***99,00mg***	***64,40mg***	2,93g	***69,45mg***	***202,57mg***	***82,00mg***	18,62g	***500,00mg***	10,82g	3,92g	%Yield				

AE = 170.21 : (170.21 + 137.65 + 90.08 + 53.49) = 38%

EF = (0.099 + 0.0644 + 2.93 + 0.06945 + 0.20257 + 0.082 + 18.62 + 0.5 + 10.82 + 3.92 - 0.04429) : 0.04429 = 841.34 [g/g]

Π(AE) = 3%; Σ(E-factor) = 5110.46, total yield: 25%

2.7 Transition metal-catalysed hydrogenation route[8]



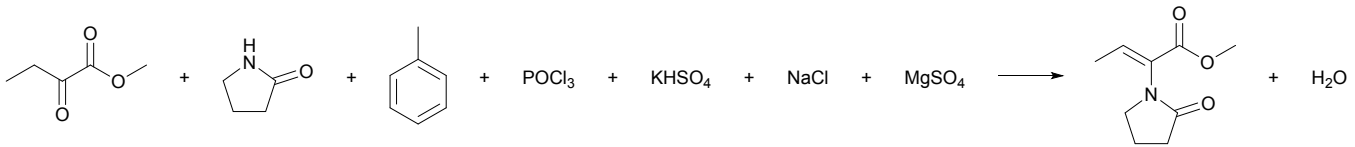
reactant | reagent | solvent | catalyst | work-up (estimated)

Reactants / *Products*

Formula	$C_4H_6O_3$	CH_4O	CH_2Cl_2	$C_2H_6O_3S$	MgO_4S	Formula	$C_5H_8O_3$
MW	102,09	32,04	84,93	110,13	120,36	MW	116,12
Limiting?	***Yes***	No	No	No	No	Equivalents	
Equivalents		3,60	9,14	***0,05***	0,30	%Completion	***48,00%***
Sample Mass		15,84g	106,40g	755,11mg		Expected Mass	7,64g
%Weight						Expected Moles	65,82mmol
Molarity						Measured Mass	
Density		***792,00mg/mL***	***1,33g/mL***			Purity	
Volume		***20,00mL***	***80,00mL***			Product Mass	
Reactant Moles	137,14mmol	494,35mmol	1,25mol	6,86mmol	41,54mmol	Product Moles	
Reactant Mass	***14,00g***	15,84g	106,40g	755,11mg	***5,00g***	%Yield	

AE = 116.12 : 116.12 = 100%

EF = (14 + 15.84 + 106.4 + 0.75511 + 5 - 7.64) : 7.64 = 17.59 [g/g]

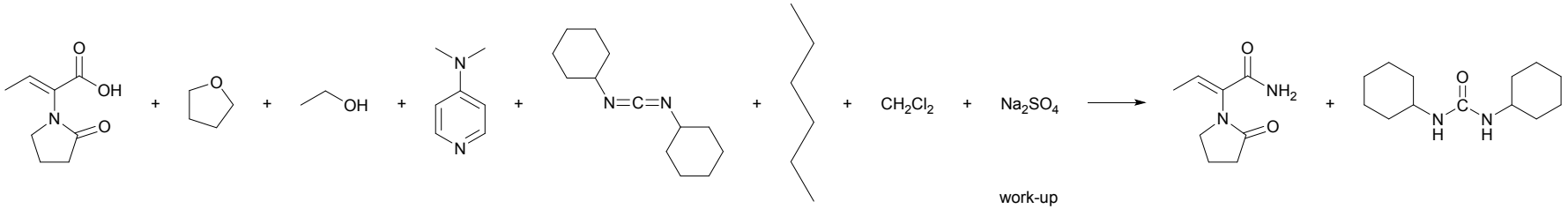


reactant | reagent | solvent | catalyst | work-up | work-up | work-up

Reactants								*Products*		
Formula	**$C_5H_8O_3$**	**C_4H_7NO**	**C_7H_8**	**Cl_3OP**	**HKO_4S**	**ClNa**	**MgO_4S**	Formula	**$C_9H_{13}NO_3$**	**H_2O**
MW	116,12	85,11	92,14	153,32	136,16	58,44	120,36	MW	183,21	18,02
Limiting?	***Yes***	No	No	No	No	No	No	Equivalents		
Equivalents		1,71	13,53	0,26	0,49	4,07		%Completion	***40,00%***	
Sample Mass		9,41g					7,77g	Expected Mass	4,73g	1,16g
%Weight					***10,00%***			Expected Moles	25,84mmol	64,59mmol
Molarity								Measured Mass		
Density		***1,12g/mL***	***867,00mg/mL***	***1,64g/mL***				Purity		
Volume		***8,40mL***	***92,90mL***	***1,60mL***	***42,90mL***			Product Mass		
Reactant Moles	64,59mmol	110,54mmol	874,14mmol	***17,11mmol***	31,51mmol	262,66mmol	64,59mmol	Product Moles		
Reactant Mass	***7,50g***	9,41g	80,54g	2,62g	***4,29g***	***15,35g***	7,77g	%Yield		

AE = 183.21 : (183.21 + 18.02) = 91%

EF = (7.5 + 9.41 + 80.54 + 2.62 + 4.29 + 15.35 + 7.77 - 4.73) : 4.73 = 25.93 [g/g]



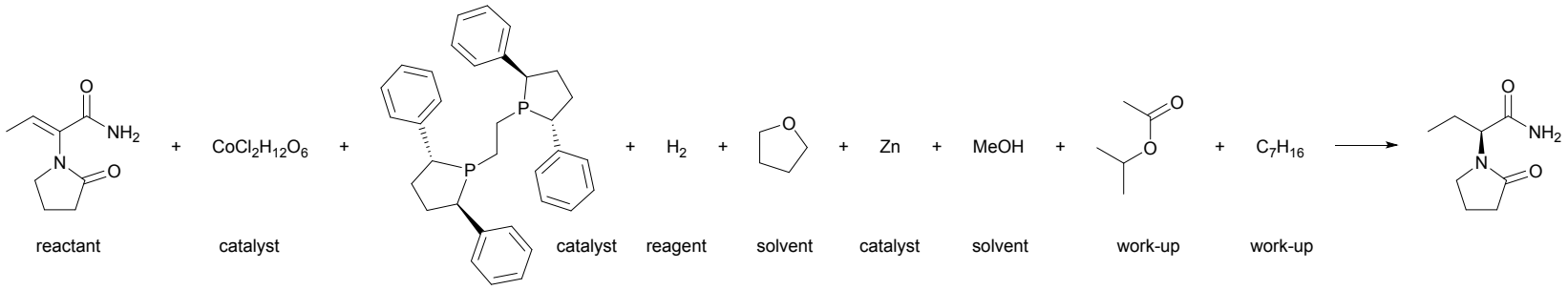
reactant | solvent | solvent | catalyst | reagent | work-up | work-up | work-up (estimated)

Reactants | *Products*

Formula	**$C_8H_{11}NO_3$**	**C_4H_8O**	**C_2H_6O**	**$C_7H_{10}N_2$**	**$C_{13}H_{22}N_2$**	**C_6H_{14}**	**CH_2Cl_2**	**Na_2O_4S**	Formula	**$C_8H_{12}N_2O_2$**	**$C_{13}H_{24}N_2O$**
MW	169,18	72,11	46,07	122,17	206,33	86,18	84,93	142,04	MW	168,20	224,35
Limiting?	***Yes***	No	No	No	No	No	No	No	Equivalents		
Equivalents		41,71	3,19	0,10	1,01	25,72	105,98		%Completion	***100,00%***	
Sample Mass			1,74g			26,20g	106,40g	1,68g	Expected Mass	1,99g	2,65g
%Weight									Expected Moles	11,82mmol	11,82mmol
Molarity									Measured Mass		
Density		***888,90mg/mL***	***789,00mg/mL***			***655,00mg/mL***	***1,33g/mL***		Purity		
Volume		***40,00mL***	***2,20mL***			***40,00mL***	***80,00mL***		Product Mass		
Reactant Moles	11,82mmol	493,10mmol	37,68mmol	1,23mmol	11,92mmol	304,02mmol	1,25mol	11,82mmol	Product Moles		
Reactant Mass	***2,00g***	35,56g	1,74g	***150,00mg***	***2,46g***	26,20g	106,40g	1,68g	%Yield		

AE = 168.20 : (168.20 + 224.35) = 43%

EF = (2 + 35.56 + 1.74 + 0.15 + 2.46 + 26.2 + 106.4 + 1.68 - 1.98) : 1.98 = 87.98 [g/g]



reactant | catalyst | catalyst | reagent | solvent | catalyst | solvent | work-up | work-up

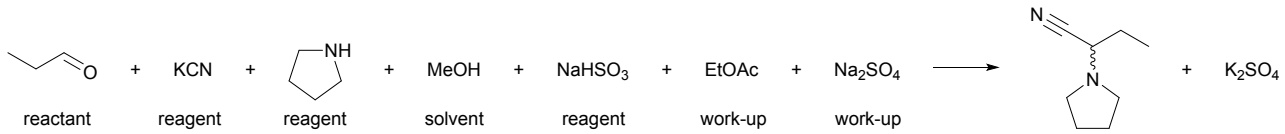
Reactants										*Products*	
Formula	**$C_8H_{12}N_2O_2$**	**$Cl_2CoH_{12}O_6$**	**$C_{34}H_{36}P_2$**	**H_2**	**C_4H_8O**	**Zn**	**CH_4O**	**$C_5H_{10}O_2$**	**C_7H_{16}**	Formula	**$C_8H_{14}N_2O_2$**
MW	168,20	237,92	506,61	2,02	72,11	65,38	32,04	102,13	100,21	MW	170,21
Limiting?	***Yes***	No	No	No	No	No	No	No	No	Equivalents	
Equivalents		0,00	0,00		0,15	0,01	40,93	17,54	1,14	%Completion	***96,00%***
Sample Mass	200,00g	170,00mg	380,00mg	2,40g		466,00mg			136,00g	Expected Mass	194,30g
%Weight										Expected Moles	1,14mol
Molarity										Measured Mass	
Density					***889,00mg/mL***		***792,00mg/mL***	***880,00mg/mL***	***680,00mg/mL***	Purity	
Volume					***14,00mL***		***1,97kmL***	***2,42kmL***	***200,00mL***	Product Mass	
Reactant Moles	1,19mol	714,52µmol	750,08µmol	1,19mol	172,60mmol	7,13mmol	48,67mol	20,85mol	1,36mol	Product Moles	
Reactant Mass	***200,00g***	***170,00mg***	***380,00mg***	2,40g	12,45g	***466,00mg***	1,56kg	2,13kg	136,00g	%Yield	

AE = 170.21 : 170.21 = 100%

EF = (200 + 0.17 + 0.38 + 2.4 + 12.45 + 0.466 + 1560 + 2130 + 136 - 194.3) : 194.3 = 19.80 [g/g]

Π(AE) = 39%; Σ(E-factor) = 151.30, total yield: 18%

2.8 Strecker synthesis – EDKR – oxidation route



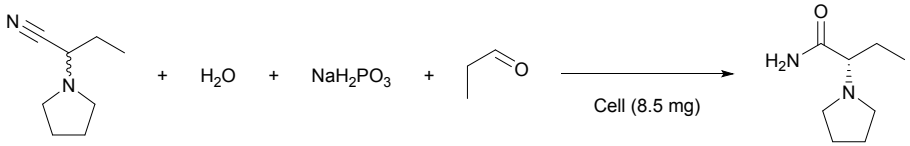
reactant reagent reagent solvent reagent work-up work-up

Reactants *Products*

Formula	**C_3H_6O**	**CKN**	**C_4H_9N**	**CH_4O**	**$HNaO_3S$**	**$C_4H_8O_2$**	**NaO_4S**	Formula	**$C_8H_{14}N_2$**	**K_2O_4S**
MW	58,08	65,12	71,12	32,04	104,05	88,11	119,05	MW	138,21	174,25
Limiting?	***Yes***	No	No	No	No	No	No	Equivalents		
Equivalents		1,82	0,91				0,14	%Completion	***86,00%***	
Sample Mass				9,91g	32,19g	27,26g		Expected Mass	36,78g	53,91g
%Weight								Expected Moles	266,08mmol	309,40mmol
Molarity								Measured Mass		
Density								Purity		
Volume								Product Mass		
Reactant Moles	309,40mmol	562,38mmol	281,20mmol	309,40mmol	309,40mmol	309,40mmol	42,00mmol	Product Moles		
Reactant Mass	***17,97g***	***36,62g***	***20,00g***	9,91g	32,19g	27,26g	***5,00g***	%Yield		

AE = 138.21 : (138.21 + 174.25) = 44%

EF = (17.97 + 36.62 + 20 + 9.91 + 32.19 + 27.26 + 5 - 36.78) : 36.78 = 3.0 [g/g]



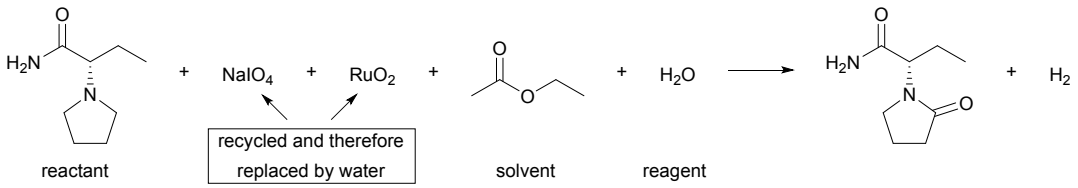
reactant reagent reagent reagent

Reactants *Products*

Formula	**$C_8H_{14}N_2$**	**H_2O**	**H_2NaO_3P**	**C_3H_6O**	Formula	**$C_8H_{16}N_2O$**
MW	138,21	18,02	103,98	58,08	MW	156,23
Limiting?	***Yes***	No	No	No	Equivalents	
Equivalents		555,09	0,50		%Completion	***67,00%***
Sample Mass	6,91mg	500,00mg	2,60mg	2,90mg	Expected Mass	5,23mg
%Weight					Expected Moles	33,50µmol
Molarity	***100,00mM***		***50,00mM***	***100,00mM***	Measured Mass	
Density		***1,00g/mL***			Purity	
Volume	***500,00mmL***	***500,00mmL***	***500,00mmL***	***500,00mmL***	Product Mass	
Reactant Moles	50,00µmol	27,75mmol	25,00µmol	50,00µmol	Product Moles	
Reactant Mass	6,91mg	500,00mg	2,60mg	2,90mg	%Yield	

AE = 156.23 : 156.23 = 100%

EF = (6.91 + 2.6 + 2.9 + 8.5 - 5.23) : 5.23 = 3,00 [mg/mg]



Reactants				*Products*	
Formula	**$C_8H_{16}N_2O$**	**$C_4H_8O_2$**	**H_2O**	Formula	**$C_8H_{14}N_2O_2$**
MW	156,23	88,11	18,02	MW	170,21
Limiting?	***Yes***	No	No	Equivalents	
Equivalents		21,27	***2,60***	%Completion	***76,00%***
Sample Mass			29,98mg	Expected Mass	82,80mg
%Weight				Expected Moles	486,47µmol
Molarity				Measured Mass	
Density		***902,00mg/mL***		Purity	
Volume		***1,33mL***		Product Mass	
Reactant Moles	640,09µmol	13,62mmol	1,66mmol	Product Moles	
Reactant Mass	***100,00mg***	1,20g	29,98mg	%Yield	

AE = 170.21 : (170.21 + 2.02) = 99%

EF = (100 + 1200 + 29.98 - 82.8) : 82.8 = 15,06 [mg/mg]

Π(AE) = 44%; Σ(E-factor) = 21.06, total yield: 44%

3 Used chemicals

(*S*)-2-Aminobutanamide hydrochloride [7682-20-4]	abcr GmbH, 98%
1,4-Dibromobutane [110-52-1]	Merck-Schuchardt
Acetonitrile [75-05-8]	Fisher Scientific, ≥99,9%
Dichloromethane [75-09-2]	Fisher Scientific, ≥99.8%
Levetiracetam [102767-28-2]	Acros Organics
Magnesium sulphate [7487-88-9]	Acros Organics, 97%
Methanol [67-56-1]	VWR Chemicals, 100.0%
Sodium bisulphate monohydrate [10034-88-5]	Merck, >99 %; Acros Organics, 99+%
Sodium thiosulphate pentahydrate [10102-17-7]	Acros Organics, 99,5%
Sodium carbonate [497-19-8]	Acros Organics, 99,5%
Sodium hydroxide [1310-73-2]	Honeywell, Pellets, ≥98%; VWR Chemicals, Pellets, 99,2%
Sodium iodate [7681-55-2]	Alfa Aesar, 99%
Sodium periodate [7790-28-5]	Fisher Scientific, 99%
Sodium sulphate [7757-82-6]	Merck
Phosphorus(V) oxide [1314-56-3]	Alfa Aesar, 98%
Ruthenium(IV) oxide hydrate [12036-10-1]	Aldrich
Nitric acid (≥65 %) [7697-37-3]	Sigma-Aldrich
Hydrochloric acid (~37 %) [7647-01-0]	Fisher Scientific
Sulphuric acid (>95 %) [7664-93-9]	Fisher Scientific
Caffeine [58-08-2]	BASF (donation)

PART I: ENZYMATIC DYNAMIC KINETIC RESOLUTION

4 Biocatalyst production

4.1 Expression in shaking flasks

Cultivation was performed typically in a total volume of 400 mL LB medium, using 1 L baffled Erlenmeyer flasks at 37 °C in LB-Amp and induction was performed at an OD_{600} of 0.8-1.0 with IPTG (0.1 mM) and $FeSO_4$ (1 mM) or $CoCl_2$ (1 mM) at 20 °C. Cultures were harvested after ~24 h by centrifugation at 5,000 *g* and 4 °C for 15 min. The supernatant was discarded and 1.2-3.0 g of pellets were stored at -20 °C or further processed.

4.2 Cell lysis by sonification

The pellets were resuspended in 25 mL of Tris-butyrate buffer (50/40 mM, pH 7.2), and lysed on ice by sonication for 6 min at 70-80% duty cycle and 7-8 output control. Cell-free extracts were obtained after centrifugation at 48,250 *g* and 4 °C for 1 h and filtered through 0.45 μm

syringe filters. Protein concentration was determined using the Pierce™ BCA Protein Assay Kit (ThermoFisher Scientific).

5 Screening for (*S*)-selective NHase

5.1 Analytical scale hydrolysis

50 μL NHase-CFE (3.7-12.7 mg/mL) was mixed with 450 μL substrate solution (11.1 mM of (*rac*)-**7** in sodium phosphate buffer, (50 mM, pH 7.2) and incubated for 16 h at 25 °C and 300 rpm in Eppendorf thermomixers. The reactions were stopped by the addition of 1 mL ethanol and vortexing. After centrifugation of precipitated protein, the supernatants were analysed by HPLC. The results are summarised in Table S1.

Table S1. Screening cell free extracts for nitrile hydratase activity towards hydrolysis of racemic **6**.

NHase	**Active site metal**	**Expr. NHase [μg/mL]**	**Hydrolysis of (*rac*)-7**	
			[%]	ee_P
*Gh*NHase (*Gordonia hydrophobica*)	Fe	340	14	78 (*S*)
PkNHase (*Pseudomonas kilonensis*)	Fe	291	8	71 (*S*)
*Pm*NHase (*Pseudomonas marginalis*)	Fe	190	3	67 (*S*)
*Re*NHase (*Rhodococcus erythropolis*)	Fe	287	18	16 (*S*)
*Ct*NHase (*Comamonas testosteroni*)	Co	340	11	89 (*S*)
*Ko*NHase (*Klebsiella oxytoca*)	Co	289	5	79 (*S*)
*Na*NHase (*Nitriliruptor alkaliphilus*)	Co	102	1	22 (*S*)

5.2 Chiral analysis of (*R*)- and (*S*)-8 by HPLC

(R)- and *(S)*-**8** were separated by a Chiralpak AD-RH (150 x 4.6 mM, 5 μm) using Na-borate buffer, (20 mM, pH 8.5), and acetonitrile in a ratio 70:30 as the mobile phase, at a flow rate of 0.5 mL/min for 15 min. The compounds were detected at 210 nm (DAD). A calibration curve of (*rac*)-**8** (external standard) was used for quantification by linear interpolation and peak areas were used to calculate the *ee*. Retention times of (*R*)- and (*S*)-**8** were 5.8 min and 6.4 min, respectively.

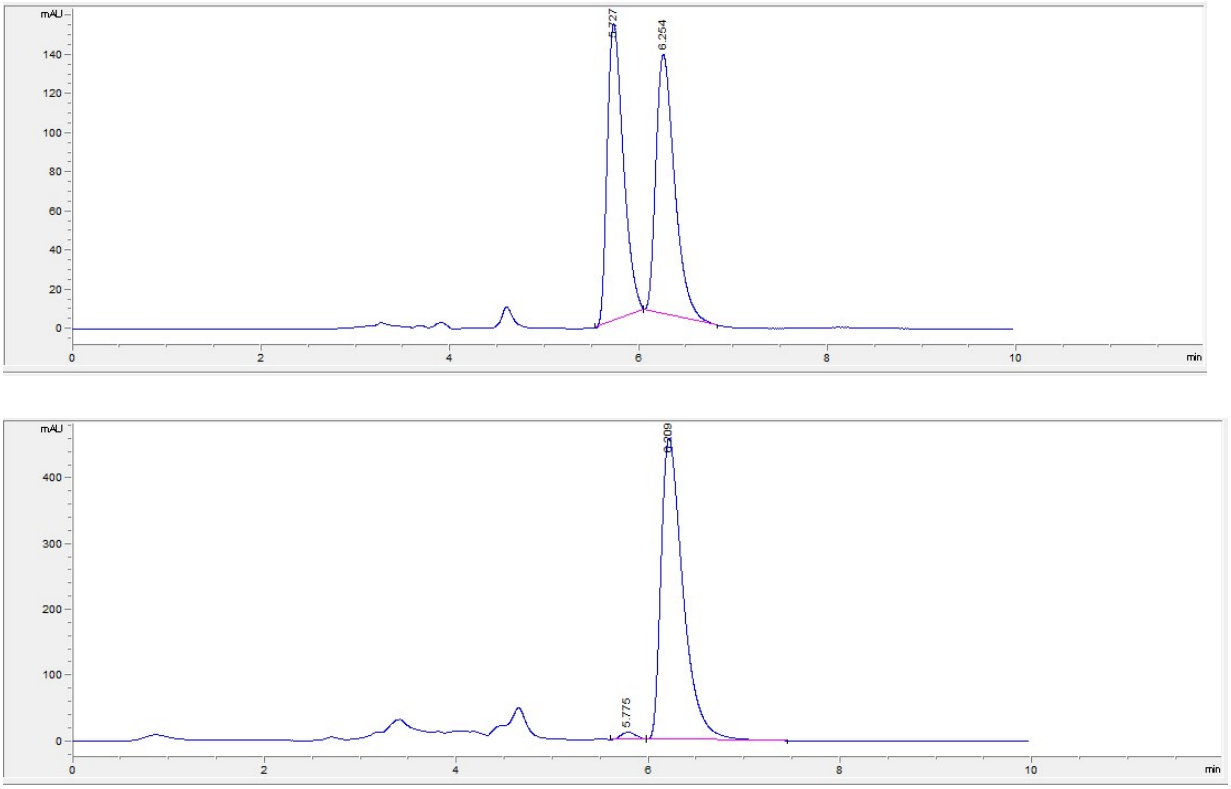


Figure S1. Top: (*rac*)-**8**. Bottom: Selected chromatogram of (*S*)-**8** from preparative scale biotransformation with *Ct*NHase variant P121T/L48R after 25 min of reaction time. *ee* = 96%

6 Characterisation of *Ct*NHase

6.1 General procedure for the spectrophotometric MAN assay (GP 1)

Physicochemical characteristics of NHases were determined by monitoring the hydrolysis of methacrylonitrile (MAN). Therefore, 10 μL NHase-CFE [diluted in Tris-butyrate buffer (50/40 mM, pH 7.2)] were mixed with 100 μL of MAN [125 mM in Tris-butyrate buffer (50/40 mM, pH 7.2)] in 96-well UV star plates. The formation of methacrylamide (MAD) was monitored at 224 nm on a Synergy Mx Plate-reader (BioTek) at 25 °C for 5 min. Appropriate blank reactions were carried out in parallel and each reaction was carried out at least in triplicate.1 Unit corresponds to the formation of 1 μmol of methacrylamide per minute.

6.2 General procedure for the biocatalytic conversions with (*rac*)-7 (GP 2)

The standard set-up of biocatalytic hydration of (*rac*)-**7** by NHases was 500 μL reaction volume comprising of either NHase-CFE or cells, the (*rac*)-**7** and buffer. Incubation was done at 25 °C in a thermomixer device. Various experiments were performed in which several parameters were investigated or additives were used. Reactions were stopped by the addition of 2 volumes of ethanol and thorough mixing for 1 min. The mixtures were left at room temperature overnight for protein precipitation before they were centrifuged for at least 20-40 min at max speed in a

table-top centrifuge. 500 μL of the supernatant were transferred into HPLC vials to be measured by chiral HPLC (5.2).

6.3 Determination of pH optimum of *Ct*NHase

For the determination of the pH optimum, the MAN assay, as according to GP 1, was used with the following buffers: citrate-phosphate buffer (100 mM, pH 5-6), sodium phosphate buffer (100 mM, pH 7-8), Tris-HCl buffer (100 mM, pH 8.5) and carbonate buffer (100 mM, pH 9-10).

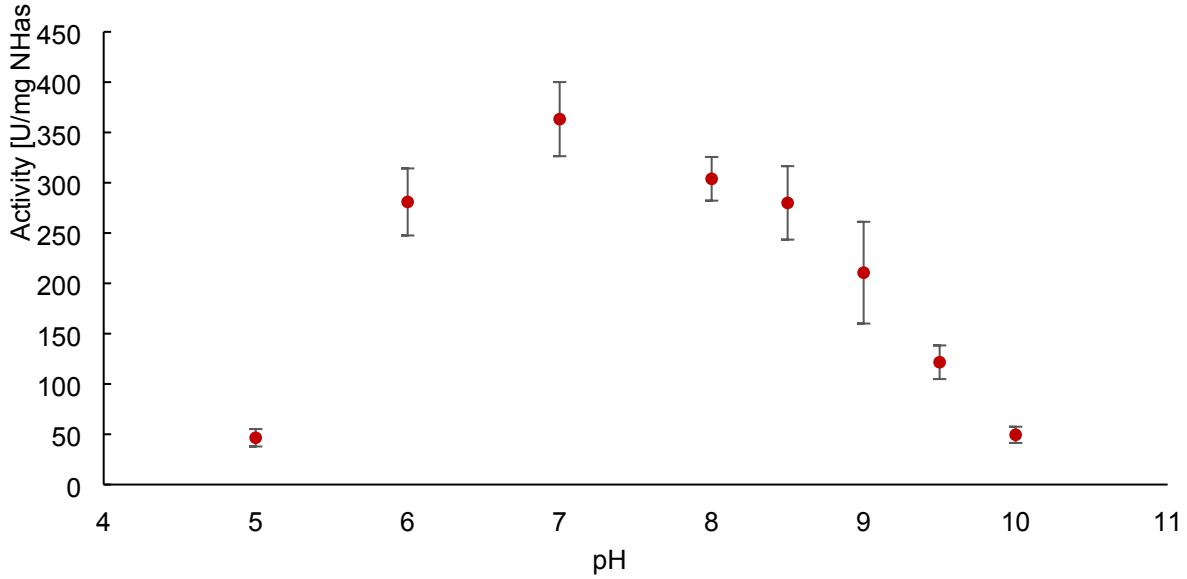


Figure S2. Activity of *Ct*NHase for the conversion of methacrylonitrile at different pH values. Reactions were performed in duplicates.

6.4 Determination of temperature optimum of *Ct*NHase

For the determination of the pH optimum, the biocatalytic reactions were carried out according to GP 2 with *Ct*NHase in the form of whole cell biocatalyst (17 mg/mL CWW) and (*rac*)-**7** [50 mM in Tris-HCl (200 mM, pH 7)]. Incubation was done at 700 rpm at the following temperatures: 25, 30, 35, 40, 45, and 50 °C. Samples were taken after 60 min to be measured by HPLC (5.2).

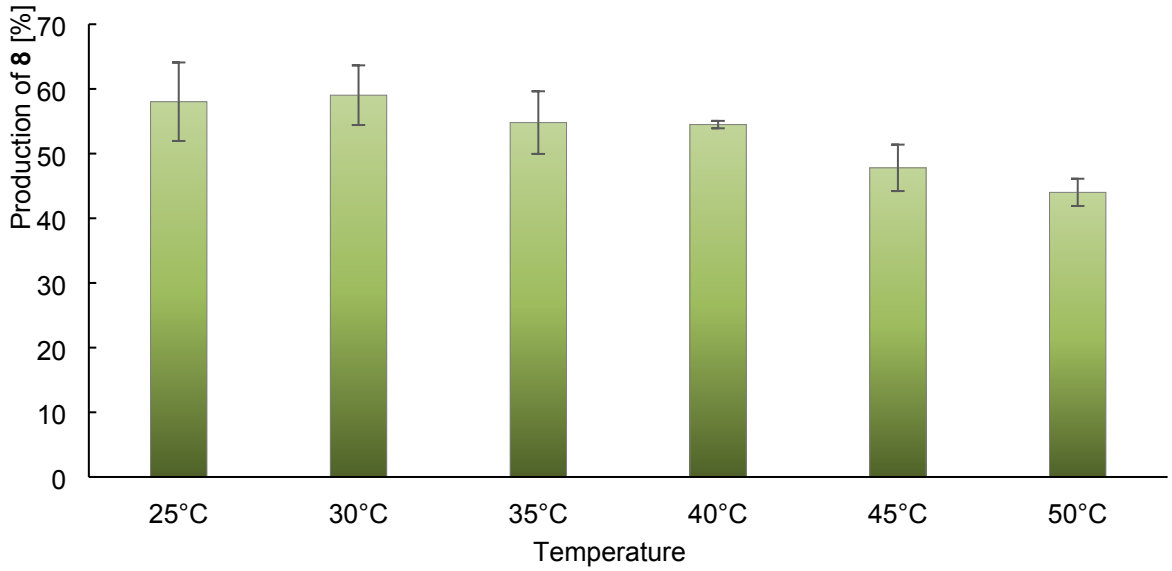


Figure S3. Activity of *Ct*NHase for hydration of (*rac*)-**7** (50 mM) by at different temperatures. Reactions were performed in triplicates.

6.5 Inhibition of *Ct*NHase by cyanide and pyrrolidine

For the determination of possible inhibition by CN^-, the MAN assay according to GP 1 was used. Prior to conversion of MAN, the CFE was incubated for 30 min at 25 °C, 300 rpm in the presence of the following concentrations of KCN: 0, 1, 5, 10, 15, 25 and 50 mM.

For the determination of possible inhibition by pyrrolidine, the MAN assay according to GP 1 was used. The buffer capacity of Tris-butyrate buffer (50/40 mM, pH 7.2)] was insufficient for high pyrrolidine concentrations and therefore, potassium phosphate buffer (1 mM, pH 7.2) was used in the presence of the following concentrations of pyrrolidine: 0, 25, 50 and 100 mM.

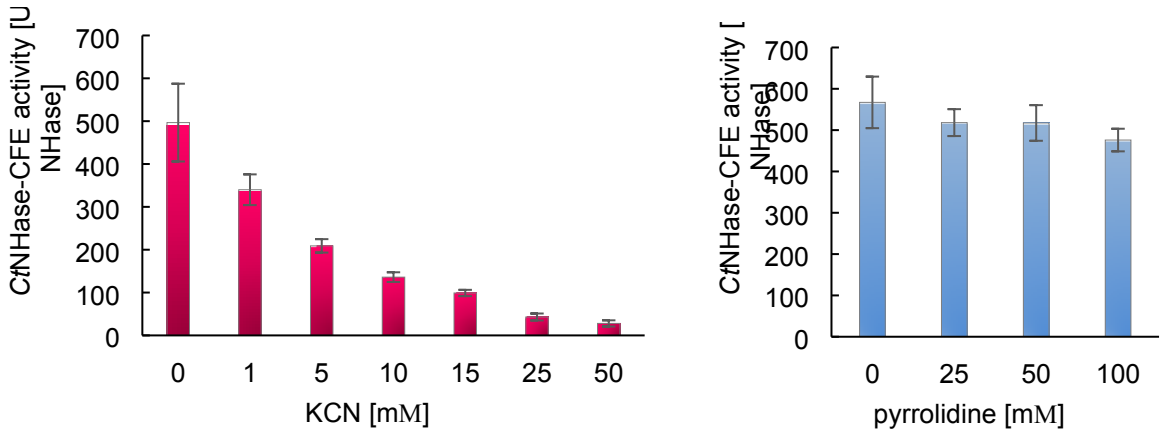


Figure S4. Left: Activity of *Ct*NHase in the presence of different concentrations of potassium cyanide. Reactions were performed in duplicates. Right: Activity of *Ct*NHase in the presence of different concentrations of pyrrolidine. Reactions were performed in six technical replicates.

6.6 Effect of propanal addition on formation of compound 8

The reaction was carried out according to GP 2. *Ct*NHase was used in the form of whole cell biocatalyst at 8.5 mg/mL cells in Tris-HCl buffer (500 mM, pH 7). The concentration of (*rac*)-**7** and propanal were 150 mM, respectively. Incubation was done at 25 °C, 700 rpm for 120 min. Reactions were measured by HPLC (5.2).

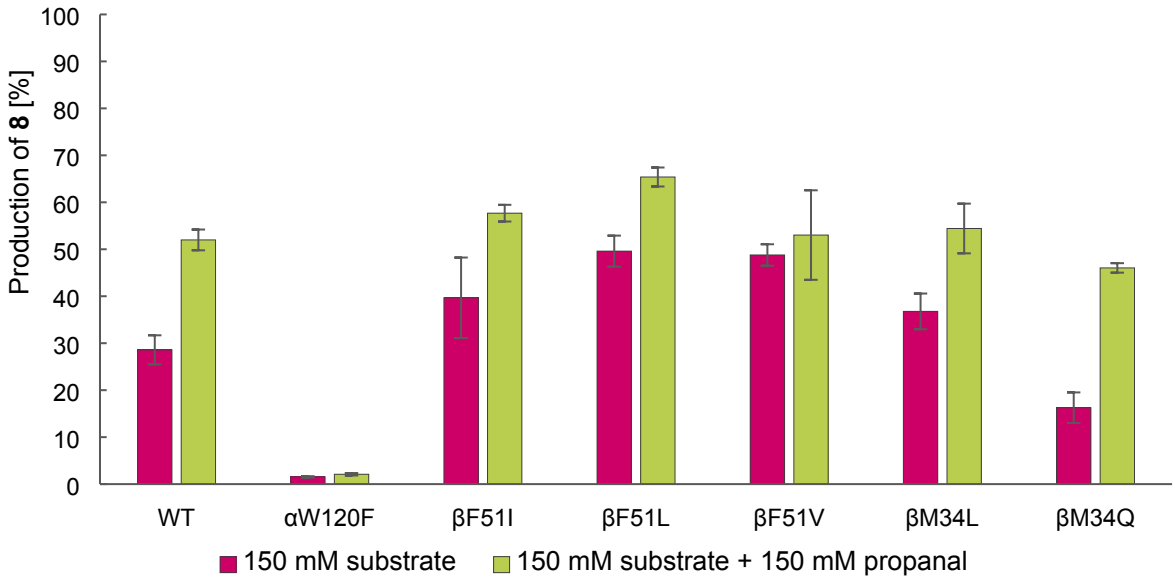


Figure S5. Formation of **8** from (*rac*)-**7** (150 mM) with and without propanal (150 mM) by wild-type *Ct*NHase in form of whole cell biocatalyst. Reactions were performed in triplicates.

6.7 Effect of pH and (*rac*)-7 concentration on formation and *ee* values of 8

The reaction was carried out according to GP 2. *Ct*NHase was used in the form of whole cell biocatalyst at 8.5 mg/mL cells in Tris-HCl buffer (200 mM, pH 7, 7.5 and 8). (*rac*)-**7** was added neat to give the following final concentrations: 50, 75, 100, 150 and 200 mM. The pH of each sample was corrected by addition of HCl (1 M) in the same volume as (*rac*)-**7**. Incubation was done at 25 °C, 700 rpm for 60 min to be measured by HPLC (5.2).

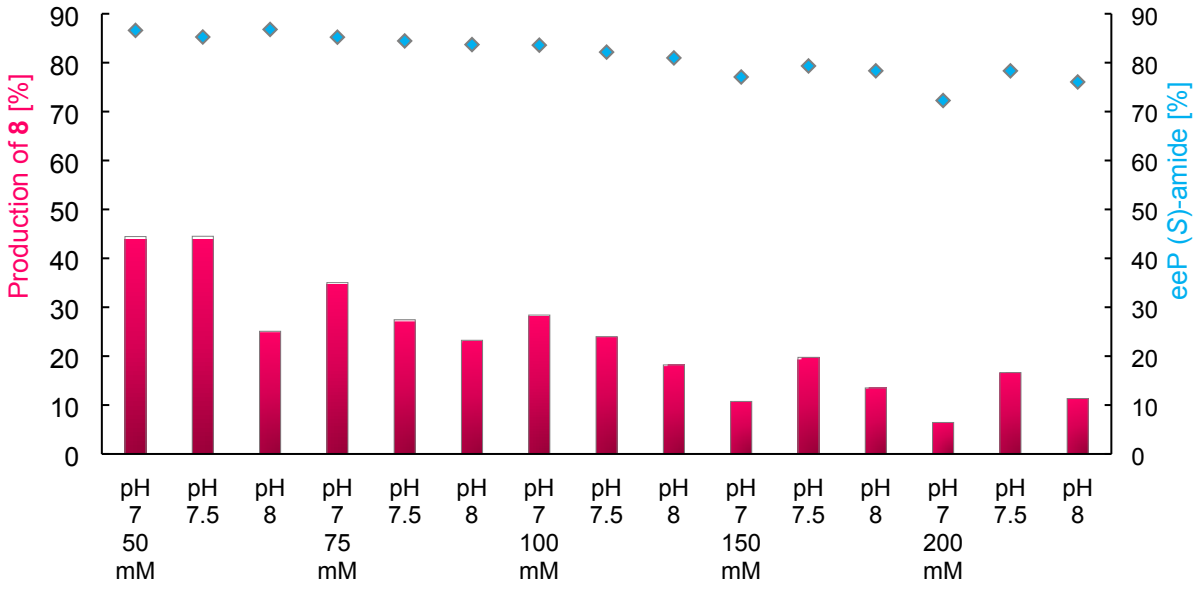


Figure S6. Formation of **8** at different concentrations of (*rac*)-**7** at different pH by *Ct*NHase.

6.8 Time-resolved hydrolysis of (*rac*)-7

The reaction was carried out according to GP 2 with *Ct*NHase in form of whole cell biocatalyst (1.7 and 8.5 mg/mL CWW, respectively), (*rac*)-**7** (50 mM in Tris-HCl buffer (200 mM, pH 7)]. Incubation was done at 25 °C, 500 rpm. Samples were taken after 0.5, 1, 2, 3, 4, 6, 8 and 25 h to be measured by HPLC (5.2).

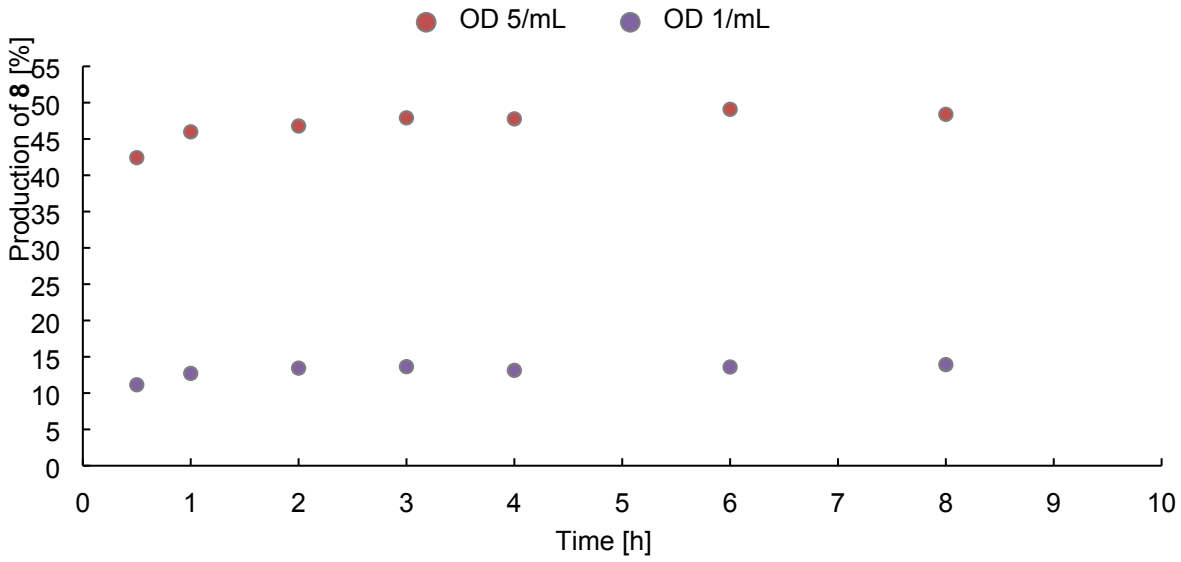


Figure S7. Time resolved view of **8** formation from (*rac*)-**7** (50 mM) by *Ct*NHase. The purple values represent production of **8** by 1.7 mg/mL cells while the red values represent production of **8** by 8.5 mg/mL cells. Amide concentrations were determined by linear interpolation of calibration curves of external standard.

6.9 Identification of side-product 2-hydroxybutaneamide

Analysis was carried out on a Shimadzu Nexera UHPLC consisting of following modules: DGU-20A5 Prominence degasser, LC-30AD pumps, SIL-30AC Nexera autosampler, CTO-20AC Prominence column oven, SPD-M20A Prominence photodiode array detector, LCMS-2020 single quadrupole mass spectrometer equipped with an ESI ionization source and a CBM-20A communications bus module. A Nucleodur C18 Gravity (EC 150/3, 3 μm, Macherey-Nagel, Germany) column was applied, using solvent A [formic acid in water (0.1%)] and solvent B (acetonitrile) at a flow rate of 0.1 mL/min for 5 min. A stepwise gradient was used: 0-0.7 min: A/B=0:100; 3.1 min: A/B=95:5; 3.1-3.7min: A/B=95:5; 3.81-5min: A/B: 0:100; Retention times of amide **8** was 2.46 min and 2-hydroxybutaneamide 1.87 min.

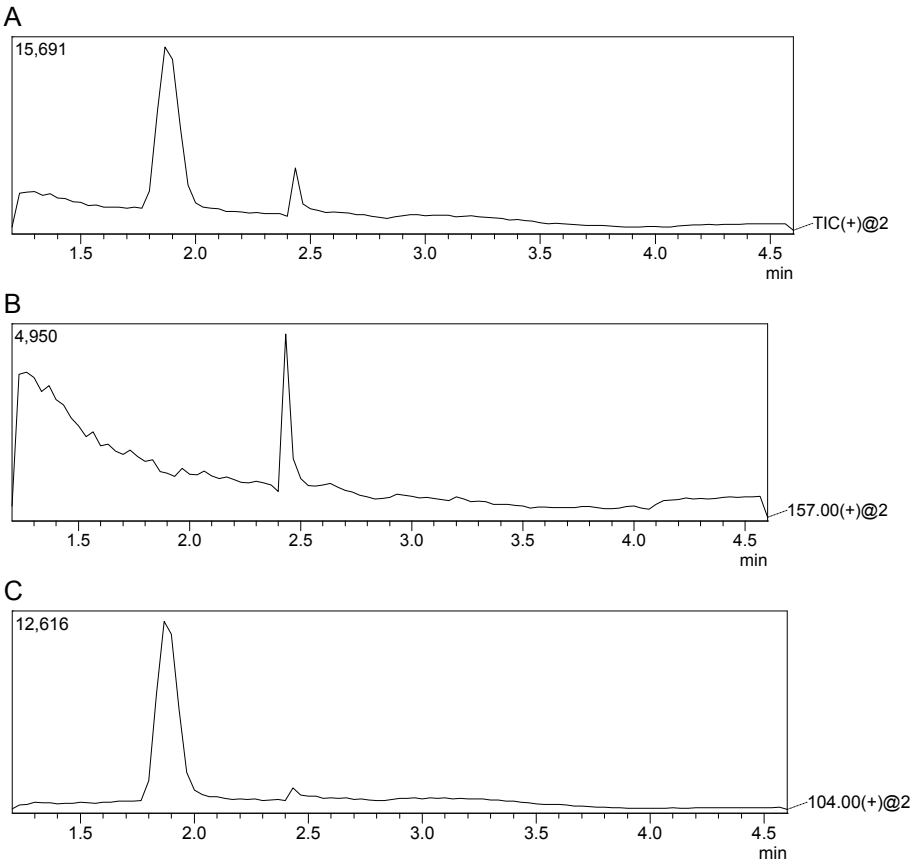


Figure S8. High performance liquid chromatography run with mass selective detection of biotransformation of (*rac*)-**7** with *Ct*NHase mutant βL48F/βG54R in form of whole cell biocatalyst. A: Total Ion Count; B: Single Ion Monitoring for M+1 of amide **8** (molecular mass: 156.23 g/mol; M+1: 157); C: Single Ion Monitoring for M+1 of 2-hydroxybutaneamide (molecular mass: 103.12 g/mol; M+1 104).

7 Homology modelling and docking

For the homology modelling the program YASARA structure[9] (version 18.2.7) was used. The hetero dimer sequence was merged into one FASTA sequence to fit to YASARA standards for hetero-dimer modelling. A free modelling was performed, restricting the best templates used

to 8 (use models with same sequence was set to 1 with maximal 5 alignments per template) and the oligomeric state was set to dimeric. The modelling speed was set to fast (no hybrid modelling was performed) and PSI-BLAST iterations were set to 4 for the initial alignment with an E-value cut-off of 0.01. Other settings were left on standard values. In the final alignment, 411 of 428 target residues (96%) are aligned (both hetero-chains combined) to residues of the template with the PDB-Code 3QYH. Among these aligned residues, the sequence identity is 95% and the sequence similarity is 96%. At this step the homology models do not include the post-translational modification of the active site cysteine residues anymore. (They are replaced by cysteine residues in the homology modelling process). Therefore, the post-translational modifications were introduced by replacing the cysteines with the sulfinoalanine present in the template with the PDB-Code 3QYH. An energy minimization step was subsequently performed using YASARA applying the AMBER03 force field[10] and the standard minimization protocol.

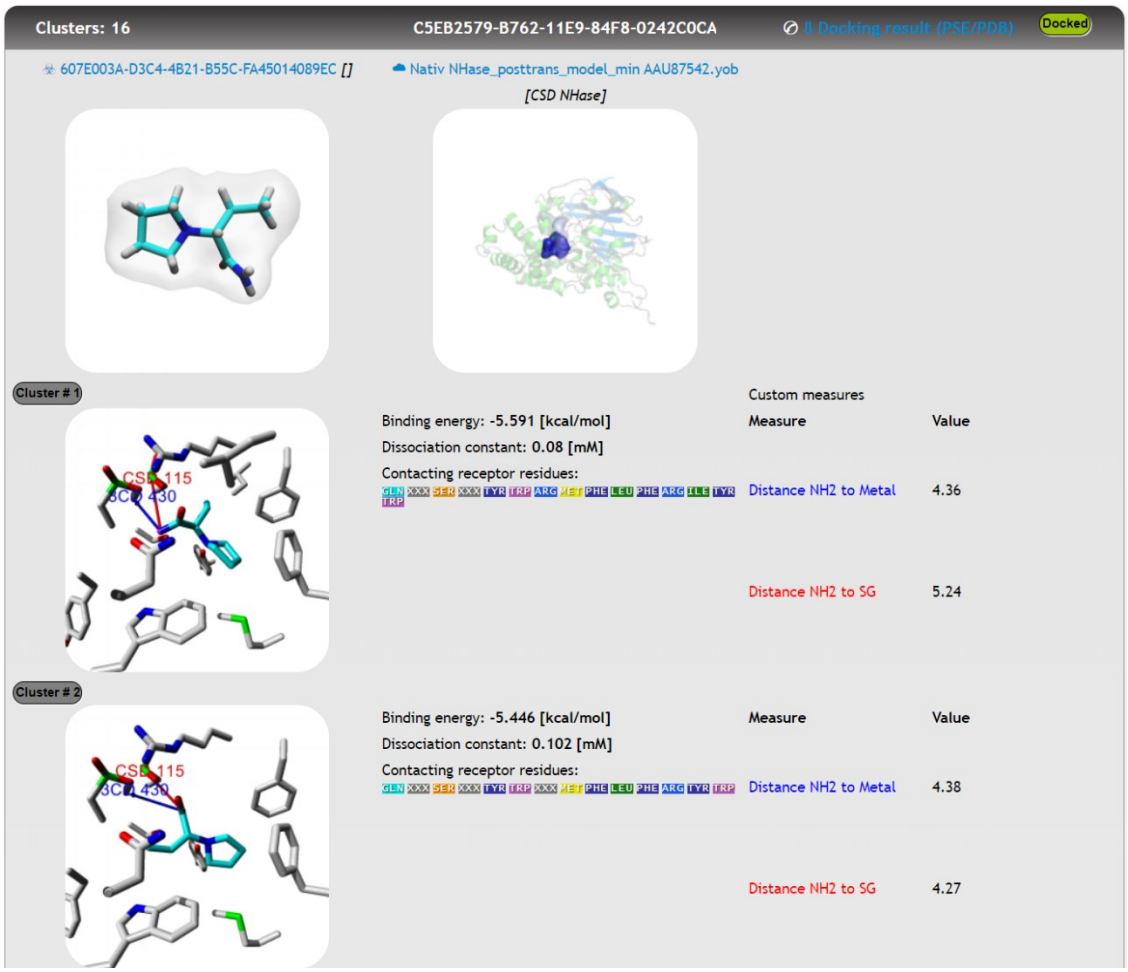


Figure S9. (*R*)**-8** Docking mode Cluster 1:4.36 [Å] distance of (*R*)**-8** NH_2 to the metal ion 3CO and -5.591 kcal/mol binding energy

The docking was performed using Autodock VINA[11] implemented within YASARA structure. The products (*R*)-**8** and (*S*)-**8** were prepared and docked into the energy minimised homology model. The docking box was set around the cavity of the proposed active site (the cavity

aligning the cobalt ion) and 25 individual VINA docking runs were performed. A clustering of the individual docking modes was performed and set to a heavy atom RMSD of 2 Å, resulting in 16 clusters for the (*R*)-**8** and 14 clusters for (*S*)-**8**, respectively. The best clusters were selected based on docking energies and compared. The best docking modes were selected by hand and based on the distance of the NH_2 group to the cobalt ion as well as the binding energy. Both docking modes (*R*) and (*S*) have a similar binding pattern and similar binding energies. Docking as described above was carried out and investigated using the Catalophore® platform.[12] Pictures were generated using the Catalophore® platform and PyMOL.[13]

Because of dockings into rigid homology model structures have to be taken with care in general, the dockings were only used as a reference for mutation hints and not for a direct comparison of binding affinities.

Residues around 4 Å of the best selected docking modes were investigated and selected for a semi-rational mutation strategy combined with random mutagenesis.

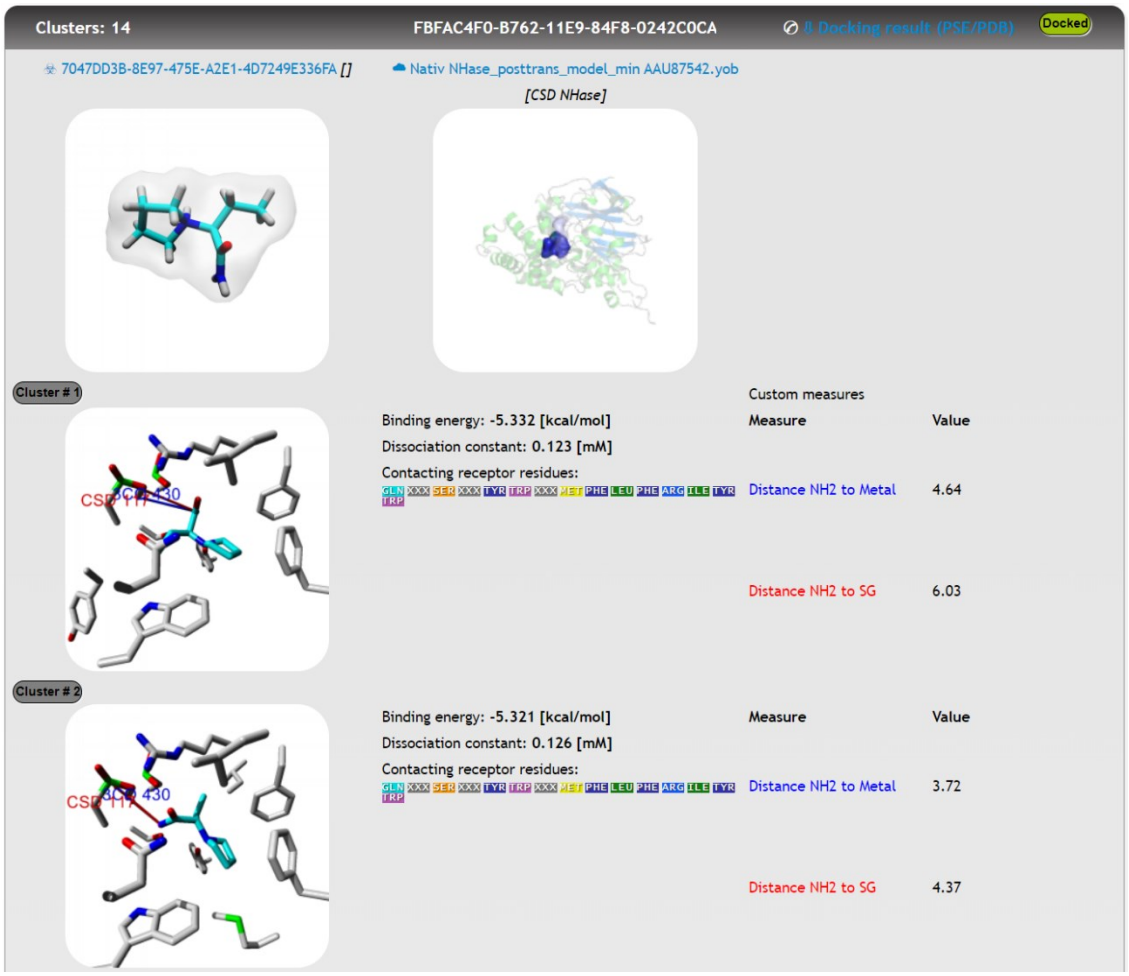


Figure S10. (*S*)**-8** docking mode cluster 2: 3.72 [Å] distance of (*S*)**-8** NH_2 to the metal ion 3CO and -5.321 kcal/mol binding energy.

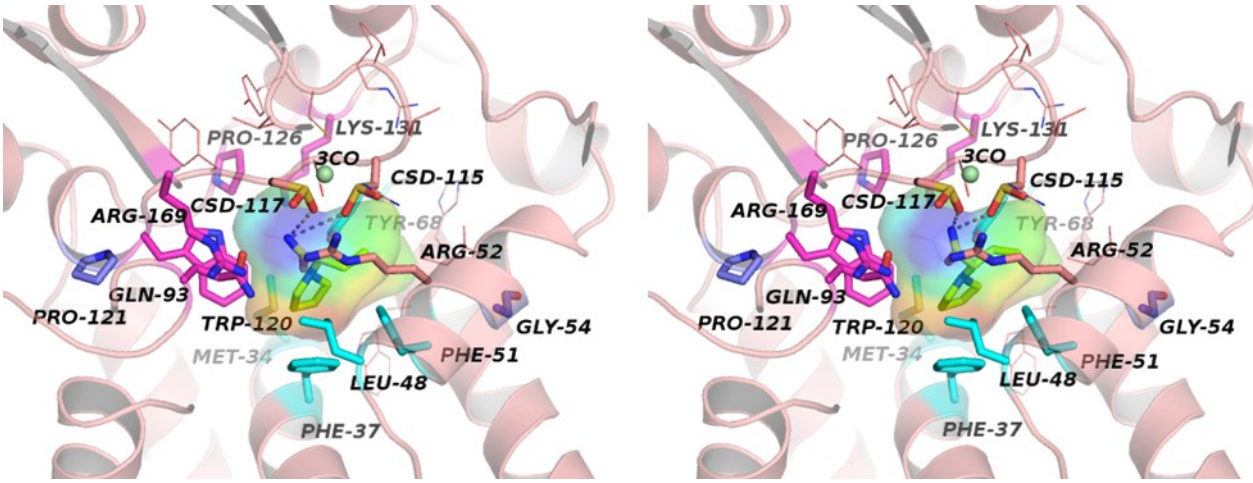


Figure S11. Docking of **8** (yellow sticks) into the homology model with altered cysteine residues (CSD). Left (*R*)-**8** (rotated) right (*S*)-**8**. The active site cavity is shown as transparent surface and coloured by hydrophobicity (blue hydrophilic to red hydrophobic). Selected residues for mutation studies are highlighted shown as stick and coloured (magenta α-subunit residues, cyan β-subunit residues). Blue highlighted residues are picked from the random mutagenesis runs. (PRO 121, GLY 54).

8 Typical methods for screening

8.1 Cultivation and screening of NHase variants in deep well plates

For the liquid screening assay in 96-well format, *E. coli* BL21 Gold (DE3) [pMS470-*Ct*NHase] cells or variants thereof were cultivated in deep well plates. Therefore, wells were filled with 750 µL LB medium containing ampicillin (100 µg/mL) and inoculated either with single colonies or from cryo-preserved cultures. The cultures were grown at 37 °C and 320 rpm overnight. The following day, 750 µL of LB-Amp media were inoculated with 10 µL of ONC and incubated at 37 °C and 320 rpm for 2 ¾ h. Cells were induced with LB-Amp medium (50 µL) containing IPTG and $CoCl_2$ to give final concentrations of 0.1 mM and 1 mM, respectively. Temperature was reduced to 20 °C. After 22 h, cells were harvested by centrifugation in a 5810R Eppendorf centrifuge for 15 min at 2970 g and pellets stored at -20 °C.

Frozen pellets were resuspended in 200 µL of Tris-HCl (200 mM, pH7), to give OD_{600} values of ~20 (corresponding to 34 mg/mL CWW). 12.5 µL of cell suspension was mixed with 37.5 µL of (*rac*)-**7** (133.33 mM; final concentration: 100 mM) and incubated at ambient temperature for 30 min at 700 rpm on a Titramax device. Afterwards, 50 µL of hydroxyl ammonium chloride (200 mM) were added as well as 50 µL of *Re*Amidase-CFE (27 mg/mL, partially purified by ammonium sulphate precipitation). After 60 min of incubation at 30 °C, 50 µL of $FeCl_3$ (0.6 M in 1 M HCl) was added, causing a yellow colouration of blank reactions whereas high nitrile hydratase activity resulted in a red colour.

8.2 Cultivation and screening of NHase variants on agar plates

For the colony-based screening assay, *E. coli* BL21 Gold (DE3) cells containing libraries of [pMS470-*Ct*NHase] were grown on LB-Amp plates for 72 h at room temperature or for 24 h at 37 °C and 20 h at room temperature before they were attached to sterilised Amersham Protran nitrocellulose membranes. The membranes were placed on LB-Amp plates containing IPTG (0.5 mM) and $CoCl_2$ (1 mM) with the colonies facing upwards. After 24-48 h of induction, colonies were used for the screening assay.

Filter paper (Whatman cellulose) was soaked with (*rac*)-**7** [100 mM in Tris-HCl (200 mM, pH 7) and the membrane with colonies was placed on top. This nitrile hydration phase was conducted for 15 min at room temperature. After that, the membrane was transferred to a new filter soaked with *Re*Amidase reaction solution, which contained 4 parts partially purified *Re*Amidase-CFE [27 mg/mL in sodium phosphate buffer (100 mM, pH 7.5)] and 1 part hydroxyl ammonium chloride [1 M in Tris-HCl (200 mM, pH 7)]. Incubation was done at 30 °C for 30 min. For the colour development phase, the membranes were transferred to fresh filter paper soaked with $FeCl_3$ (0.6 M in 1 M HCl). Colonies of active clones turned red on the yellow background.

9 Mutant libraries

Table S2. List of primers.

name	sequence
For generation of site-saturation libraries	
Ct-aQ93X_for	GGGTANNKGGCGAGGACATG
Ct-aQ93X_rev	GCCMNNTACCCCGGAGAAG
Ct-aW120X_for	TACCCANNKCCGACGCTGG
Ct-aW120X_rev	CAGCGTCGGMNNTGGGTAG
Ct-aP126X_for	TGGGCTTGNNKCCTGCCTG
Ct-aP126X_rev	GTACCAGGCAGGMNNCAAG
Ct-aK131X_for	GTACNNKGCCCCGCCCTAC
Ct-aK131X_rev	GGGCMNNGTACCAGGCAG
Ct-aR169X_for	CGAATTGNNKTACATGGTGCTG
Ct-aR169X_rev	CAGCACCATGTAMNNCAATTCG
Ct-bM34X_for	CGGTCNNKTCCCTGTTCCC
Ct-bM34X_rev	CAGGGAMNNGACCGTTTTTTC
Ct-bF37X_for	CCCTGNNKCCGGCGCTGTTC
Ct-bF37X_rev	GCCGGMNNCAGGGACATGAC
Ct-bL48X_for	CAACNNKGATGAGTTTCGACAC
Ct-bL48X_rev	GTCGAAACTCATCMNNGTTGAAG

Ct-bF51X_for	CGATGAGNNKCGACACGGC
Ct-bF51X_rev	GCCGTGTCGMNNCTCATCG
Ct-bY68X-long_for	AAGGGAACCNNKTACGAACACTGGATCCATTC
Ct-bY68X-long_rev	TGTTCGTAMNNGGTTCCCTTCAGGTAGTCG
For generation of randomly mutated CtNHase gene fragments	
Ct-alpha1_for	TGGCCAAGGCCTGGGTGGAC
Ct-alpha1_rev	GCAAGAGCACAAGGTGCAAAC
Ct-alpha2_for	CCTTGTGCTCTTGCTACCCA
Ct-alpha2_rev	TTCAGTTCCCGCGGGCCG
Ct-beta1_for	CGTCTTTCGCTACGACTGG
Ct-beta1_rev	AGGTTTCGATGGAATGGATCCA
Ct-beta2_for	GCTTCTGCCGCCCGGGAG
Ct-beta2_rev	TTTCCGTGTGCCGCGGTGTC
For generation of pMS470-CtNHase backbone strains (restriction sites in blue)	
Ct-A1bb-lig_for	AATTTCTCGAGTTTGCACCTTGTGCTCTTGCTAC
Ct-A1bb-lig_rev	AATTTCTCGAGTCCACCCAGGCCTTGGCC
Ct-A2bb-lig_for	AATTTCTCGAGGCCCGCGGGAACTGAAG
Ct-A2bb-lig_rev	AATTTCTCGAGGGTAGCAAGAGCACAAGG
Ct-B1bb-lig_for	TTTAAAGCTAGCTGGATCCATTCCATCGAAACCTTG
Ct-B1bb-lig_rev	TTTAAAGCTAGCCAGTCGTAGCGAAAGACG
Ct-B2bb-lig_for	TTTAAAGCTAGCACCGCGGCACACGGAAAGG
Ct-B2bb-lig_rev	TTTAAAGCTAGCTCCCGGGCGGCAGAAGCC
For generation of pMS470-CtNHase-β1-focused library	
Ct-beta1-focused_for	ACTTCAACYKSGATGAGVTTCGACACNDTATCGAGCGCATGAAC
Ct-beta1-focused_rev	CATGCGCTCGATAHNGTGTCGAABCTCATCSMRGTTGAAGTTGCCGTTGG
For generation of pMS470-CtNHase combination variants	
Ct-P121T_for	ATGGACGACGCTGGGCTTGC
Ct-P121T_rev	AGCGTCGTCCATGGGTAGCAAGAG
Ct-V110I_for	AACGTCATCGTTTGCACCTTGTGCTCTTG
Ct-V110I_rev	AAACGATGACGTTGTGGACGGC
Ct-L48R-G54C_for	ACTTCAACCGTGATGAGTTTCGACACTGTATCGAGCGCATGAAC
Ct-L48R-G54C_rev	CATGCGCTCGATACAGTGTCGAAACTCATCACGGTTGAAGTTGCCGTTGG
Ct-L48R-G54R_for	ACTTCAACCGTGATGAGTTTCGACACCGTATCGAGCGCATGAAC
Ct-L48R-G54R_rev	CATGCGCTCGATACGGTGTCGAAACTCATCACGGTTGAAGTTGCCGTTGG
Ct-L48R-G54V_for	ACTTCAACCGTGATGAGTTTCGACACGTTATCGAGCGCATGAAC
Ct-L48R-G54V_rev	CATGCGCTCGATAACGTGTCGAAACTCATCACGGTTGAAGTTGCCGTTGG

Ct-L48P-G54C_for	ACTTCAACCCTGATGAGTTTCGACACTGTATCGAGCGCATGAAC
Ct-L48P-G54C_rev	CATGCGCTCGATACAGTGTCGAAACTCATCAGGGTTGAAGTTGCCGTTGG
Ct-L48P-G54R_for	ACTTCAACCCTGATGAGTTTCGACACCGTATCGAGCGCATGAAC
Ct-L48P-G54R_rev	CATGCGCTCGATACGGTGTCGAAACTCATCAGGGTTGAAGTTGCCGTTGG
Ct-L48P-G54V_for	ACTTCAACCCTGATGAGTTTCGACACGTTATCGAGCGCATGAAC
Ct-L48P-G54V_rev	CATGCGCTCGATAACGTGTCGAAACTCATCAGGGTTGAAGTTGCCGTTGG
Ct-L48F-G54C_for	ACTTCAACTTCGATGAGTTTCGACACTGTATCGAGCGCATGAAC
Ct-L48F-G54C_rev	CATGCGCTCGATACAGTGTCGAAACTCATCGAAGTTGAAGTTGCCGTTGG
Ct-L48F-G54R_for	ACTTCAACTTCGATGAGTTTCGACACCGTATCGAGCGCATGAAC
Ct-L48F-G54R_rev	CATGCGCTCGATACGGTGTCGAAACTCATCGAAGTTGAAGTTGCCGTTGG
Ct-L48F-G54V_for	ACTTCAACTTCGATGAGTTTCGACACGTTATCGAGCGCATGAAC
Ct-L48F-G54V_rev	CATGCGCTCGATAACGTGTCGAAACTCATCGAAGTTGAAGTTGCCGTTGG
Ct-L48P-F51V-G54V_for	ACTTCAACCCTGATGAGGTTCGACACGTTATCGAGCGCATGAAC
Ct-L48P-F51V-G54V_rev	CATGCGCTCGATAACGTGTCGAACCTCATCAGGGTTGAAGTTGCCGTTGG
Ct-L48F_for	CAACTTTGATGAGTTTCGACAC
Ct-L48F_rev	GTCGAAACTCATCAAAGTTGAAG
Ct-F51L_for	CGATGAGCTTCGACACGGC
Ct-F51L_rev	GCCGTGTCGAAGCTCATCG

9.1 Generation and screening of site saturation libraries

Site-saturated libraries were generated by PCR reactions with degenerated oligonucleotides. The reactions contained 10 ng of template DNA (e.g. pMS470-*Ct*NHase or pMS470-*Ct*NHase-βF51L), 0.2 μM of forward and reverse primer (e.g. Ct-aQ93X_for and Ct-aQ93X_rev,Table S2), 0.2 mM of dATP, dCTP, dGTP and dTTP, 1 x Q5 Reaction buffer, 1 x Q5 High GC Enhancer and 1 U Q5 High-Fidelity DNA polymerase. The PCR program was as follows: 30 s at 98 °C, 30 cycles at 98 °C for 10 s, 60 °C for 30 s and 72 °C for 3 min and a final extension step at 72 °C for 6 min. The PCR products were digested with 10 U *Dpn*I for 2 h at 37 °C and purified (Wizard SV PCR and Clean-Up System).

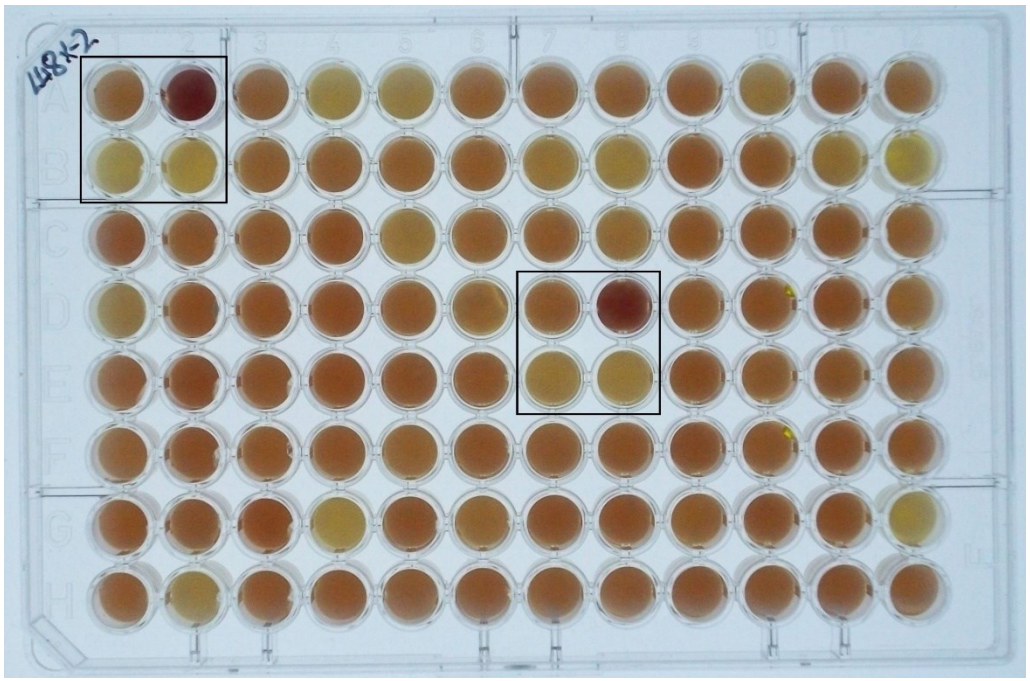


Figure S12. Typical example of screening results in deep well plates. The boxes indicate controls: *Ct*NHase (A1, D7, wild-type control), *Gh*NHase (A2, D8, positive control), empty vector (B1, E7), sterile control (B2, E8)

Table S3. Best hits of saturation library β-L48.

***Ct*NHase clone**	**formation of 8 [%]**	**ee_P for (*S*) [%]**	**amino acid at βL48**
Wild type	12.5	84.1	L
L48X-1-C4	16.5	98.7	P
L48X-1-A12	13.4	98.3	P
L48X-1-C12	12.8	99.0	P
L48X-2-E2	25.4	98.3	R
L48X-2-F9	21.6	97.9	P
L48X-2-C1	21.7	98.0	R
L48X-2-C11	19.5	98.2	P
L48X-2-D11	21.4	98.4	P
L48X-2-D2	19.7	98.5	P

Electro-competent *E. coli* Top10F' were transformed with 5 μL of digested PCR product. The number of transformants was determined to calculate the library size and mixed plasmids were isolated from overnight cultures. The plasmids of some transformants were isolated and sequenced to confirm that, next to the parent, NNK variants were also found.

The libraries were screened as described in 8.1. The plate and result of a selected library is shown in Fig. S11 and Table S3.

9.2 Generation and screening of random libraries

The Mutazyme II Kit was applied to generate randomly mutated fragments of *Ct*NHase. The 50 μL PCR reactions contained 5 ng of template DNA (pMS470-*Ct*NHase), of the forward and the reverse primer (e.g. Ct-alpha1_for and Ct-alpha1_rev, Table S2, 0.4 μM), dATP, dCTP, dGTP and dTTP (0.2 mM), $MnCl_2$ (0.5-1 mM), 1 x Mutazyme II reaction buffer and Mutazyme II DNA polymerase (2.5 U). The PCR program was as follows: 2 min at 95 °C, 30 cycles at 95 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min and a final extension step at 72 °C for 10 min. The size of the PCR products was analysed by gel electrophoresis and the products were purified (Wizard SV PCR and Clean-Up System, Promega).

50–70 ng of PCR product was cloned into pJET1.2/blunt (CloneJET PCR Cloning Kit, ThermoFisher Scientific) using the sticky end protocol. Electro-competent *E. coli* Top10F' cells were transformed with the resulting plasmids. After amplification in *E. coli* and isolation (GeneJET Miniprep Plasmid Kit, ThermoFisher Scientific), some of those plasmids were sequenced (Microysynth AG) to determine the mutation rate.

Vector backbones were generated in 50 μL PCR reactions that consisted of 1 ng of template DNA (pMS470-*Ct*NHase or pMS470-*Ct*NHase-βF51L), 0.2 μM of both primers (e.g. Ct-A1bb-lig_for and Ct-A1bb-lig_rev, Table S), dATP, dCTP, dGTP and dTTP (0.2 mM), 1 x Q5 Reaction buffer, 1 x Q5 High GC Enhancer and 1 U Q5 High-Fidelity DNA polymerase. The PCR program was as follows: 30 s at 98 °C, 30 cycles at 98 °C for 10 s, 60 °C for 30 s and 72 °C for 2 min and a final extension step at 72 °C for 2 min. The PCR products were digested with *Dpn*I (10 U) for 2 h and purified (Wizard SV PCR and Clean-Up System). The ends of 1 μg PCR product were cut with *Xho*I or *Nhe*I for 15 min at 37 °C and heat inactivated for 20 min at either 65 °C or 80 °C. The cut PCR products were purified and ligated (T4 DNA Ligase, ThermoFisher Scientific) for 10 min at 22 °C. Electro-competent *E. coli* Top10F' were transformed with the heat inactivated DNA. Circular backbone plasmids were amplified in *E. coli*, isolated and sequenced (Microsynth AG). Correct plasmids were linearised by restriction cut with either *Xho*I or *Nhe*I and gel-purified. These backbones were dephosphorylated (Shrimp alkaline phosphatase, NEB) before Gibson assembly (Gibson Assembly® HiFi 1-Step Kit, SGI-DNA).

30 ng of randomly mutated fragments were mixed with 360 ng vector backbone for Gibson assembly. Desalted reactions were applied for the transformation of electro-competent *E. coli* Top 10F'. With the number of transformants, the library size was calculated and 16-24 transformants were analysed by colony PCR for the correct insert size. In parallel, overnight cultures were inoculated with transformants and on the next day, mixed plasmids were isolated (GeneJET Miniprep Plasmid Kit, ThermoFisher Scientific).

The plasmid libraries were transformed into *E. coli* BL21 Gold (DE3) and colonies grown on LB-Amp plates. Screening was applied as described in 8.2. Clones with the strongest signal were screened in deep well plates, as described in 8.1. Clones with the strongest signal were further analysed in biocatalytic conversion reactions according to GP 1 with cell biocatalyst (CWW 8.5 mg/mL) and 100 mM substrate in Tris-HCl (200 mM) for 2 h at 25 °C and 700 rpm. Production of (*S*)-**8** was finally measured by HPLC (5.2).

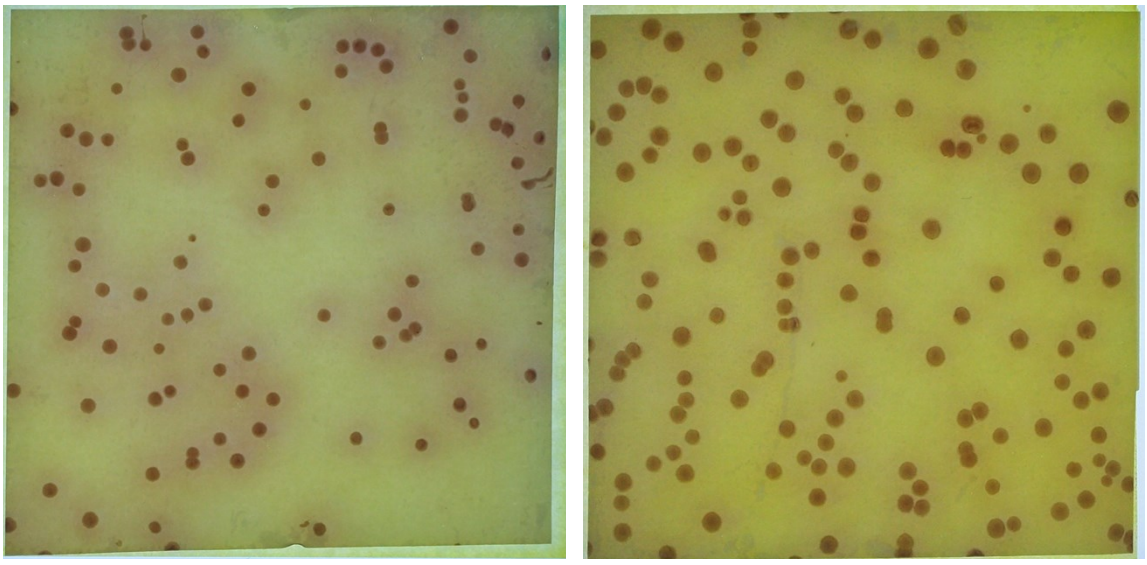


Figure S13: Typical examples of the colony-based screening assay.

9.3 Generation and screening of focused library

Beneficial amino acid exchanges of β1 region were combined (for primers see Table S2). We opted for bulky amino acids βL48. The required codon was YKS to achieve Leu (the wild type amino acid), Arg or Trp (Table S4). The YKS codon also enables cysteine or phenylalanine.

The improved variants βF51L, βF51I and βF51V were included into this library. The wild type codon was not allowed, only Ile, Leu and Val. So far, Cys, Asp and Val had been found at position βG54. We used codon NDT for this position, resulting in representatives of all chemical groups.

Table S4 Codons for focused *Ct*NHase-β1 library and their possible amino acids.

position	**βL48**	**βF51**	**βG54**
desired amino acids	L, R, W	I, L, V	variety
codon	YKS	VTT	NDT
possible amino acids	L, R, W, C, F	I, L, V	F, L, I, V, Y, H, N, D, C, R, S G

The library was constructed by overlap extension PCR with degenerated oligonucleotides (Table S2) and screened in the colony-based assay (8.2). Potential hits were re-screened using the liquid assay (8.1), the promising variants of which were applied in biocatalytic

reactions (GP 1) with cell biocatalyst (CWW 8.5 mg/mL) and substrate [100 mM in Tris-HCl (200 mM)] for 2 h at 25 °C and 700 rpm and measured by HPLC (5.2).

9.4 Combination of beneficial amino acid exchanges

After screening of more than 50,000 *Ct*NHase clones and identification of key residues for the production of enantiomerically enriched **8**, beneficial amino acid exchanges were combined.

As an example, cloning of the variants shown in Figure 2 in the main manuscript is described in the following:

- L48R: was generated in the random library (9.2), although in the presence of silent mutations.
- L48P: was generated in the site saturation library L48X (9.1).
- V110I/L48P, P121T/L48R, P121T/L48P: mutations V110I or P121T were introduced with QuikChange PCR on templates with the mutations L48R or L48P (variants above, primers in Table S2).
- L48R/G54C, L48R/G54R, L48R/G54V, L48P/G54C, L48P/G54R, L48P/G54V, L48F/G54C, L48F/G54R, L48F/G54V: mutations were introduced with QuikChange PCR on the wild type template (primers in Table S2).

The final combination variants were expressed in shaking flasks and screened for hydration of (*rac*)-**7**.

10 Typical method for preparative scale reactions

Frozen cell pellet was thawed and approximately 500 mg suspended in 50 mL of phosphate buffer (100 mM, pH 7.1). The suspension was diluted to an optical density of 5. The reaction was carried out in a Mettler Toledo T50 pH stat at 22 °C using 1 M phosphoric acid titration. The reaction was started by addition of 200 μL of (*rac*)-**7**, 100 μL of propanal and 100 μL of pyrrolidine. Nitrile hydration translates in pH increase and can be monitored on basis of acid consumption. Every 10-15 min, acid addition ceased and pulses of 100 μL of propanal and 200 μL of (*rac*)-**7** were added. To analyse reaction progress, 250 μL samples were withdrawn repeatedly and treated as according to GP 2 before analysis. Fig. 13 shows the result of a typical experiment.

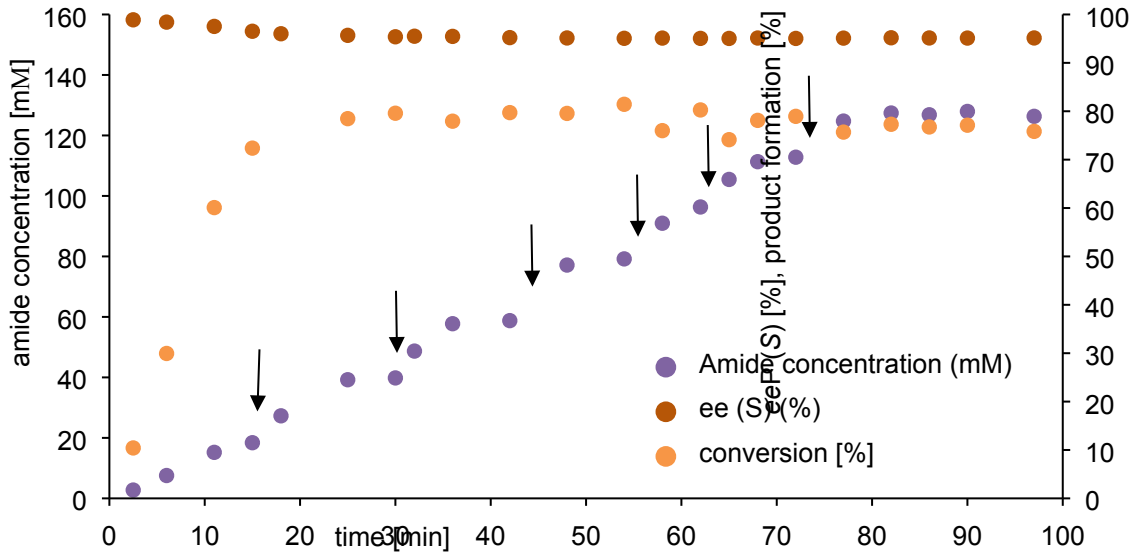
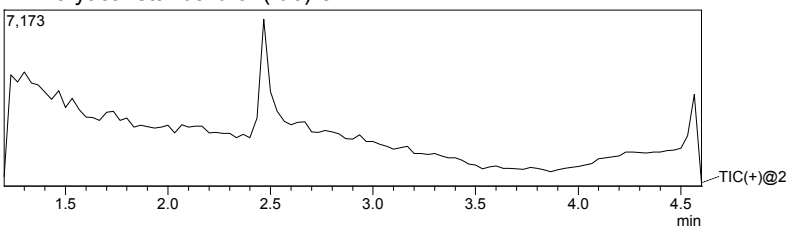
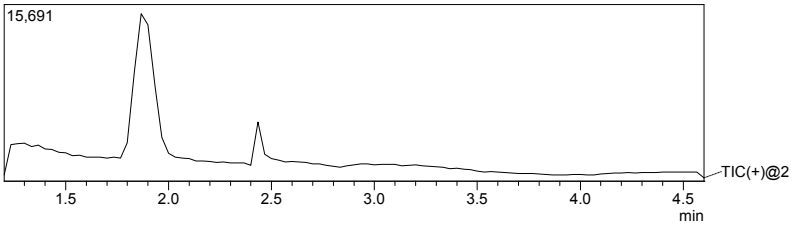


Figure S14: 50 mL scale fed batch hydration of (*rac*)-**7** by *Ct*NHase variant αP121T/βL48R in pH stat. Black arrows indicate points of substrate addition.

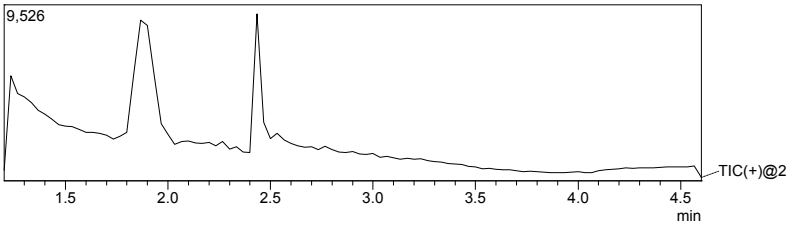
A: Analytical standard of (*rac*)-**8**



B: 50 mL fed-batch reaction with *Ct*NHase βL48F/βG54R [final analytical yield of (*S*)-**8**: 41.9%]



C: 50 mL fed-batch reaction with *Ct*NHase βL48R/βG54V [final analytical yield of (*S*)-**8**: 61.7%]



D: 50 mL fed-batch reaction with *Ct*NHase αP121T/βL48R [final analytical yield of (*S*)-**8**: 73.3%]

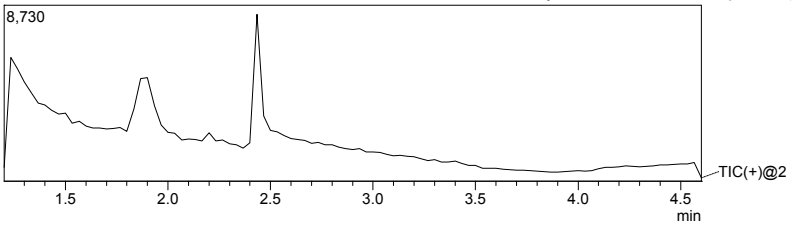


Figure S15. High performance liquid chromatography run with mass selective detection of biotransformations of (*rac*)-**7** with *Ct*NHase variants. Total Ion Count (amide **8** retention time 2.46 min; 2-hydroxybutaneamide retention time 1.87 min); For method details, see section 6.9.

PART II: PYRROLIDINE OXIDATION

11 Calibrations

11.1 Liquid chromatography photodiode array (LC-PDA)

A buffered and diluted sample solution (C = 5.341 mM) was subjected to LC-PDA analysis using an injection volume of 3 μL. The separation was carried out under isocratic conditions. I^-, IO_3^- and IO_4^- were detected by the PDA detector at 1.58 min,1.47 min and 1.92 min at a wavelength of λ = 254 nm. Yields were determined by external calibration.[14]

11.2 Gas chromatography with flame ionization detector (GC-FID)

Method: Injection volume = 1.5 μL, inlet temperature = 200 °C, initial column temperature = 50 °C (holding time = 1 min), ramping rate = 15 °C/min (gradient time = 11.5 min), final temperature = 220 °C (hold-up time = 12 min). The system was calibrated for the precursor and for levetiracetam using caffeine as internal standard (Table S5 and S6, Figure S14).

Table S5. GC-FID calibration.

Precursor (*S*)-8					
Sample	m [mg]	**n [μmol]**	R_t = 8.8 min	R_t = 12.4 min	**rel. area**
1	10	**64**	88800	165526	**0.54**
2	20	**128**	193269	167911	**1.15**
3	30	**192**	327152	172133	**1.90**
4	40	**256**	477808	199187	**2.40**
5	50	**320**	605594	192839	**3.14**
6	60	**384**	714361	176838	**4.04**
7	70	**448**	838929	186601	**4.50**
8	80	**512**	914777	183787	**4.98**
9	90	**576**	1023381	177080	**5.78**
10	100	**640**	1142542	183865	**6.21**

Table S6. GC-FID calibration.

Levetiracetam (*S*)-1					
Sample	m [mg]	**n [μmol]**	R_t = 10.4	R_t = 12.4	**rel. area**
1	21.60	**127**	368480	331274	**1.11**
2	32.40	**190**	617033	349994	**1.76**
3	43.20	**254**	742162	321520	**2.31**

4	54.00	**317**	1210293	421207	**2.87**
5	64.80	**381**	1494327	422010	**3.54**
6	75.60	**444**	1703344	412925	**4.13**
7	86.40	**508**	1663974	359865	**4.62**
8	97.20	**571**	2062582	397446	**5.19**
9	108.00	**635**	2128238	348816	**6.10**
10	118.80	**698**	1935942	303211	**6.38**

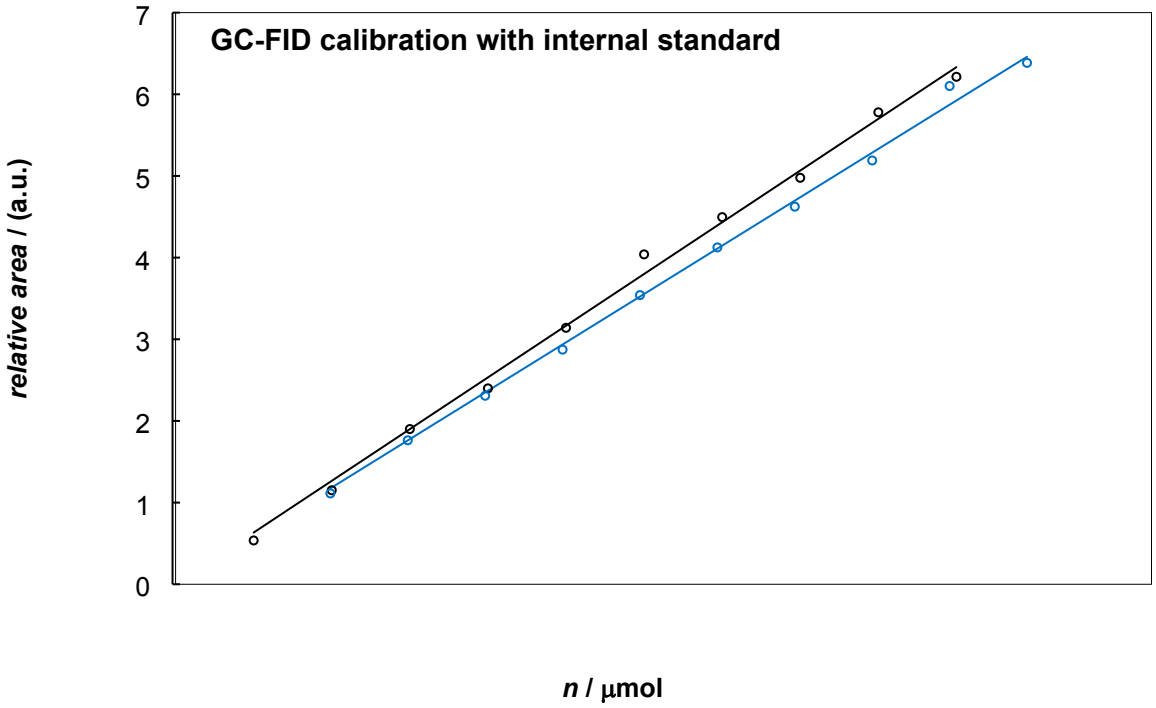
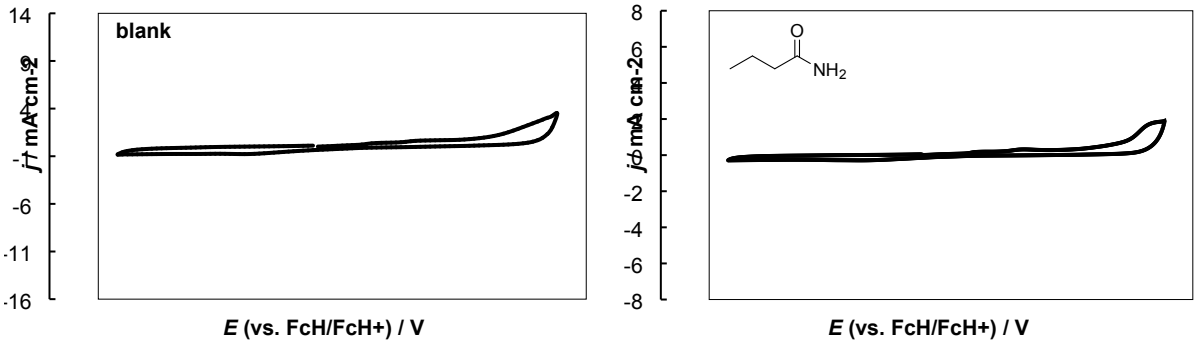
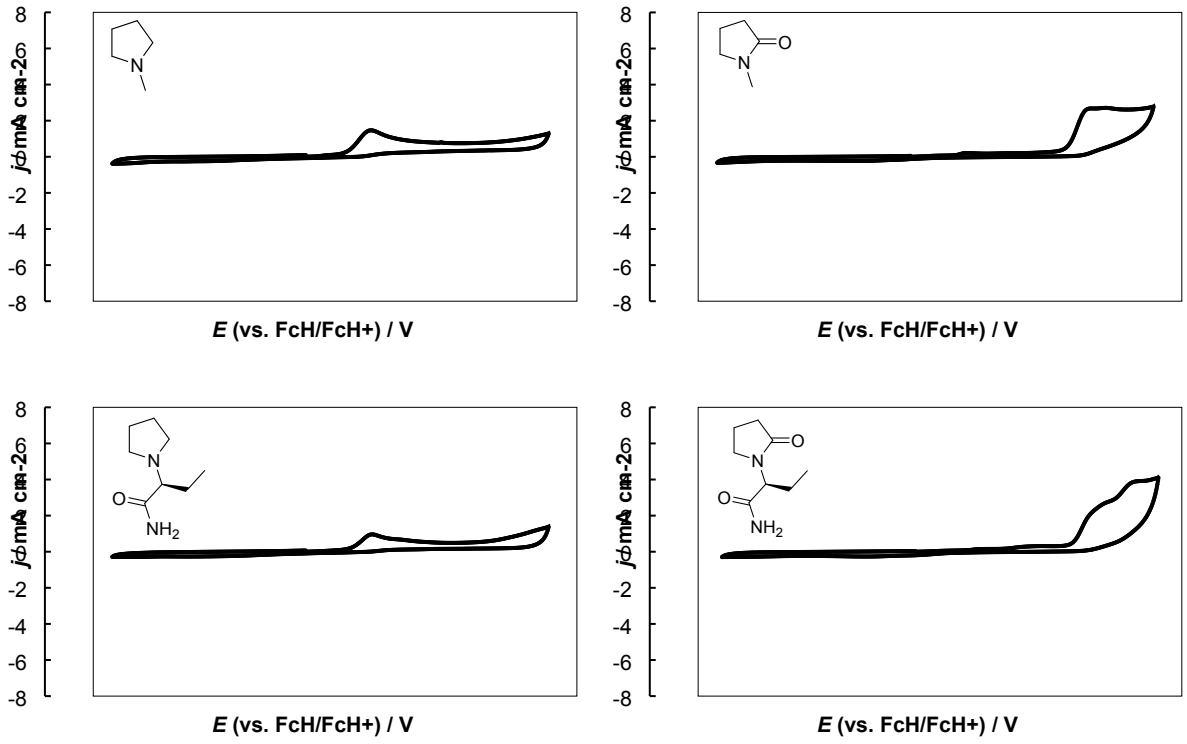


Figure S16: Calibration lines for GC-FID with caffeine as internal standard.

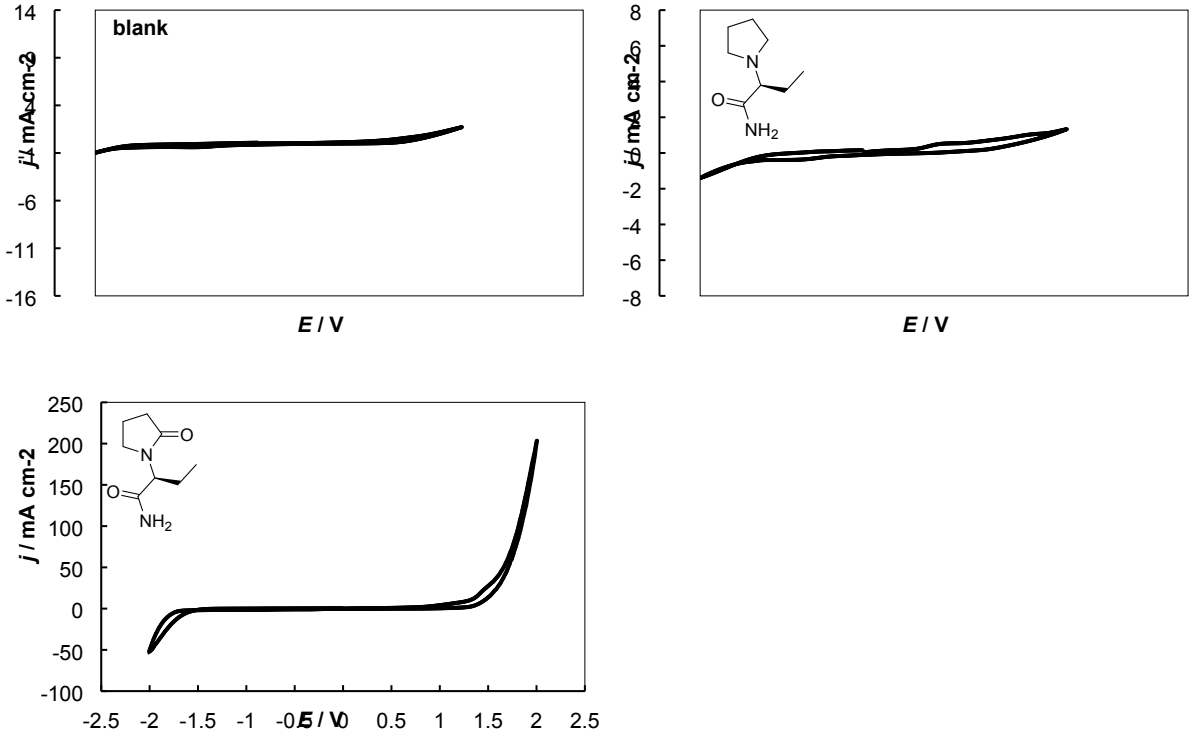
12 Cyclic voltammetry

Solvent = MeCN; glassy carbon (GC) working electrode, GC rod counter electrode, $AgNO_3$/*n*-$BuNBF_4$ reference electrode; scan rate = 0.1 V/s, scan steps = 0.01 V.

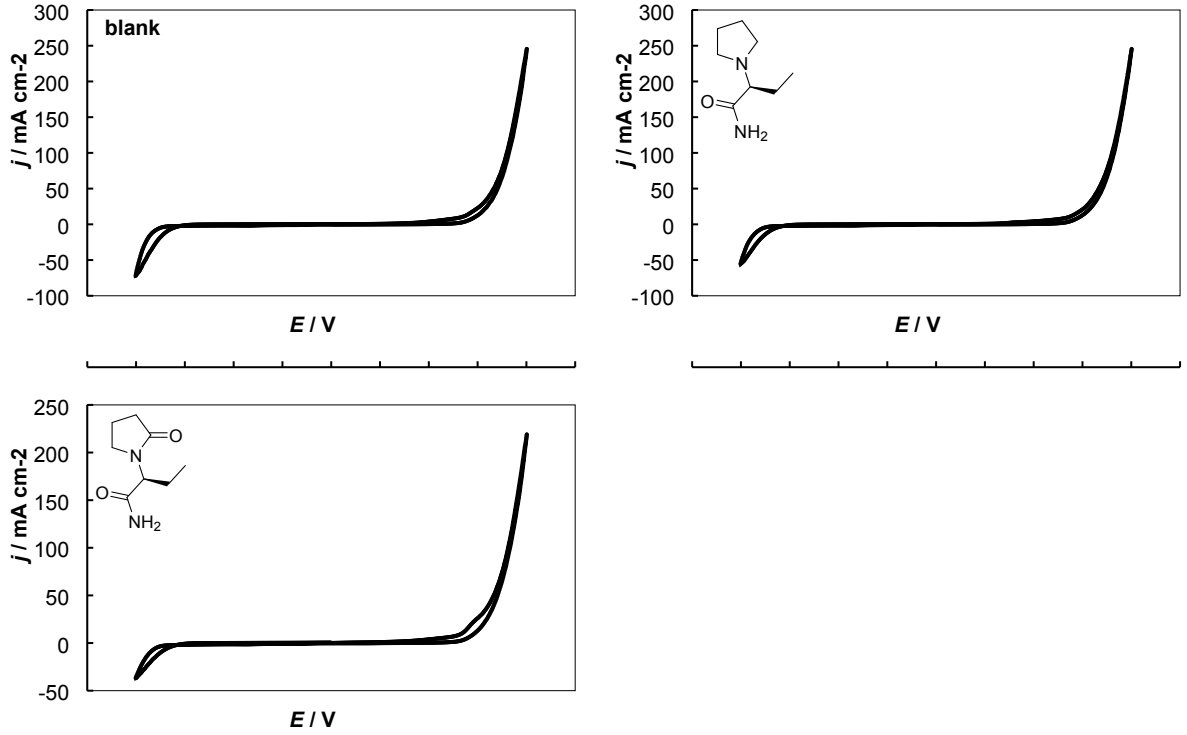




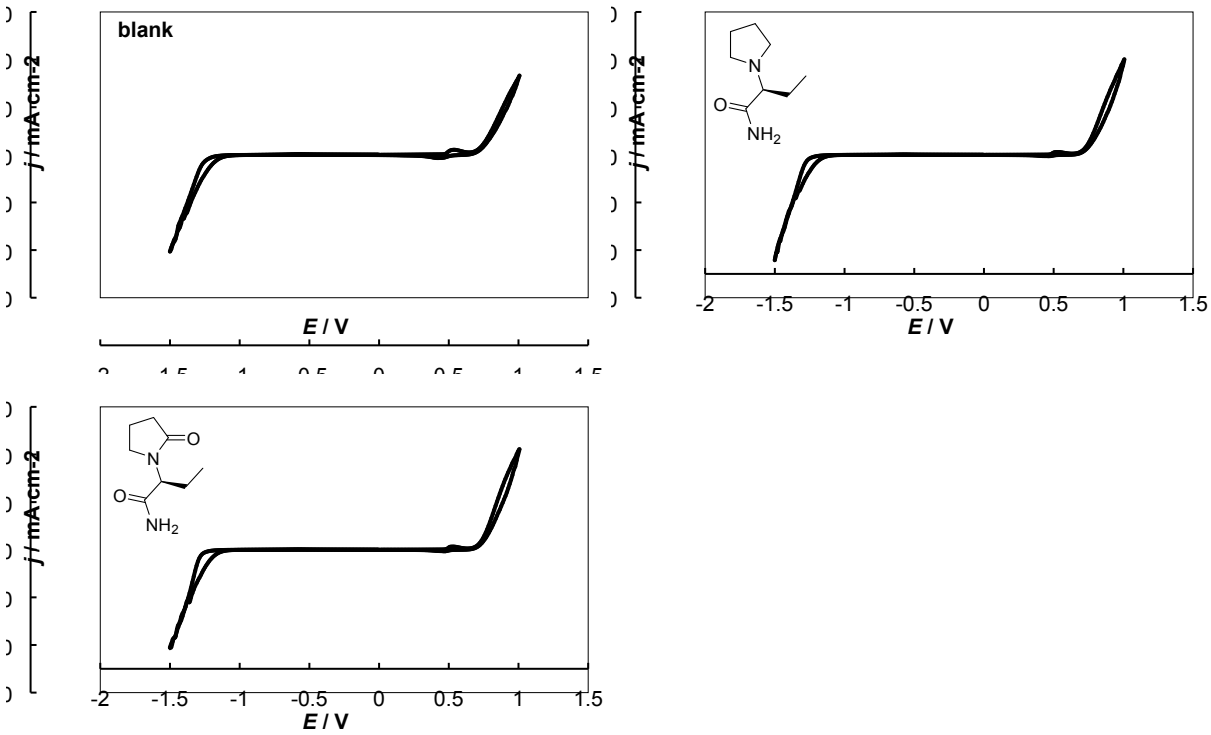
Solvent = H_2O, glassy carbon (GC) working electrode, GC rod counter electrode, Hg^0/HgO reference electrode; scan rate = 0.1 V/s, scan steps = 0.01 V. No internal reference.



Solvent = 1 M NaOH, glassy carbon (GC) working electrode, GC rod counter electrode, Hg^0/HgO reference electrode; scan rate = 0.1 V/s, scan steps = 0.01 V. No internal reference.



Solvent = 1 M NaOH, boron-doped diamond (BDD) working electrode, GC rod counter electrode, Hg^0/HgO reference electrode; scan rate = 0.1 V/s, scan steps = 0.01 V. No internal reference.



13 Representative procedures (RP)

13.1 Electrochemical synthesis of etiracetam (RP 1)

In a divided batch electrolysis-cell equipped with a Nafion membrane, a stainless-steel cathode, and a platinum anode, (*S*)-**8** (100 mg, 0.64 μmol) was electrolysed in caustic soda (0.1 M, 6 mL) using a current density of j = 2 mA/cm^2 and an applied charge of Q = 6 F. After electrolysis, the yield was determined by GC analysis using caffeine as internal standard and the crude product was purified by flash column chromatography on silica gel. The racemic etiracetam was obtained in 29% yield (17% isolated) as determined by chiral HPLC.

13.2 Ruthenium-catalysed synthesis of levetiracetam (RP 2)

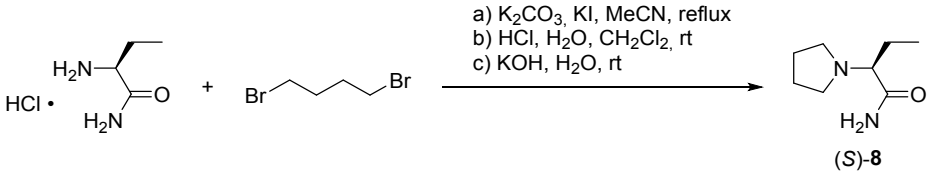
0.5-1 mol% RuO_2•xH_2O (0.5 – 1.0 mg, 3.20-6.40 µmol) and 2.60 eq. of $NaIO_4$ (356 mg, 1.66 mmol) were suspended in a suitable solvent mixture (6 mL) until the solution showed a pale-yellow colour. (*S*)-**8** (100 mg, 640 µmol) was added and the reaction was stirred at room temperature for 0.5 h. The reaction was quenched with methanol and was controlled by GC with caffeine (24.9 mg, 128 µmol, 0.20 eq.) as internal standard. Levetiracetam was obtained in 66% GC-yield. The precipitated iodate and ruthenium oxide were filtered off and the product was isolated by flash column chromatography on silica gel (12 x 2 cm, CH_2Cl_2/MeOH = 10:1). Levetiracetam was obtained in 49% isolated yield and in 99.6% *ee* as determined by chiral HPLC.

13.3 Electrochemical generation of sodium paraperiodate (RP 3)

In a divided batch electrolysis-cell equipped with a Nafion membrane, a stainless-steel cathode, and a boron-doped diamond anode, $NaIO_3$ (127 mg, 640 μmol) was electrolysed in caustic soda (0.1 M) using a charge amount of Q = 3 F, and a current density of j = 10 mA/cm^2. After complete electrolysis the yield was determined by LC-PDA.

14 Synthesis of the reference standard

14.1 (*S*)-2-(pyrrolidin-1-yl)butanamide [(*S*)-8]



(*S*)-**8**

The synthesis of *(S)*-2-(pyrrolidin-1-yl)butanamide was carried out according to Tong *et al*.[15] To a 250 mL flask was added (*S*)-2-aminobutanamide hydrochloride (20 mmol), 1,4-dibromobutane (24 mmol), K_2CO_3 (8.29 g, 60 mmol), KI (332 mg, 2 mmol), and acetonitrile (80 mL). The reaction mixture was heated to reflux. After 1 d, the reaction was quenched with concentrated HCl (50 mL), and water and dichloromethane were added for extraction. The

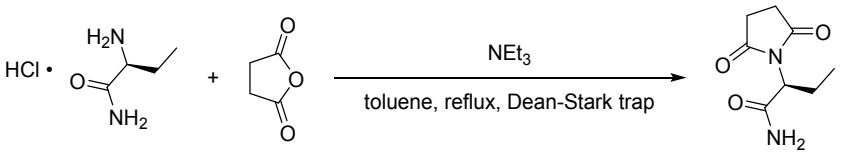
organic layer was separated and discarded. The aqueous layer was made basic with potassium hydroxide solution (~15 g in 50 mL H_2O, pH > 14) and extracted with dichloromethane (4×50 mL). The organic fractions were combined, dried over anhydrous $MgSO_4$ and concentrated under reduce pressure to give a white fluffy solid. Alternatively, the K_2CO_3 is filtered off, the solvent is removed and the solid crude product is recrystallised in acetone at 4 °C. The crystals are washed with cyclohexane and dried under vacuum. The product was yielded to 67% (2.10 g, 13.5 mmol) and in >99% *ee* as determined by chiral HPLC.

Analysis of 2-aminoacetamide hydrochloride: TLC (SiO_2, CH_2Cl_2/MeOH = 1:1, $KMnO_4$ or Bromocresol-green stain), R_f = 0.22; **^{1}H NMR** (400 MHz, Methanol-d_4) δ 3.87 (t, *J* = 6.3 Hz, 1H), 2.08 – 1.69 (m, 2H), 1.05 (t, *J* = 7.5 Hz, 3H), **^{13}C NMR** (101 MHz, Methanol-d_4) δ 172.33 (C_q), 55.27, 25.78 (CH_2), 9.36.

Analysis of (*S*)-8: TLC (SiO_2, $KMnO_4$ or Bromocresol-green stain w/o heating or ninhydrin stain with strong heating), R_f(CH_2Cl_2/MeOH = 1:1) = 0.57, R_f(CH_2Cl_2/MeOH = 10:1) = 0.22, R_f(CH_2Cl_2/MeOH = 20:1) = 0.05; **GC-FID**: R_t = 7.57 min@~150 °C; **^{1}H NMR** (400 MHz, $CDCl_3$) δ 6.65 (s, 1H), 6.00 (s, 1H), 2.69 – 2.62 (m, 1H), 2.62 – 2.50 (m, 4H), 1.89 – 1.57 (m, 6H), 0.94 (t, *J* = 7.5 Hz, 3H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 176.64 (C_q), 71.01 (CH_2), 51.93, 25.29, 23.35, 9.97 (CH_2); **LC-MS (HR ESI(+)):** calculated for $[M+H]^+$: $[C_8H_{17}N_2O]^+$ 157.1335 Da, found: 157.1338 Da. The obtained spectra are in accordance with previously reported data. High solubility in H_2O (neutral and basic), MeOH, EtOH, CH_2Cl_2, $CHCl_3$, MeCN, low solubility in THF and 1,6-Dioxane; m_p = 282 °C.

15 Synthesis of over-oxidation product

15.1 2-(2,5-dioxopyrrolidin-1-yl)butanamide



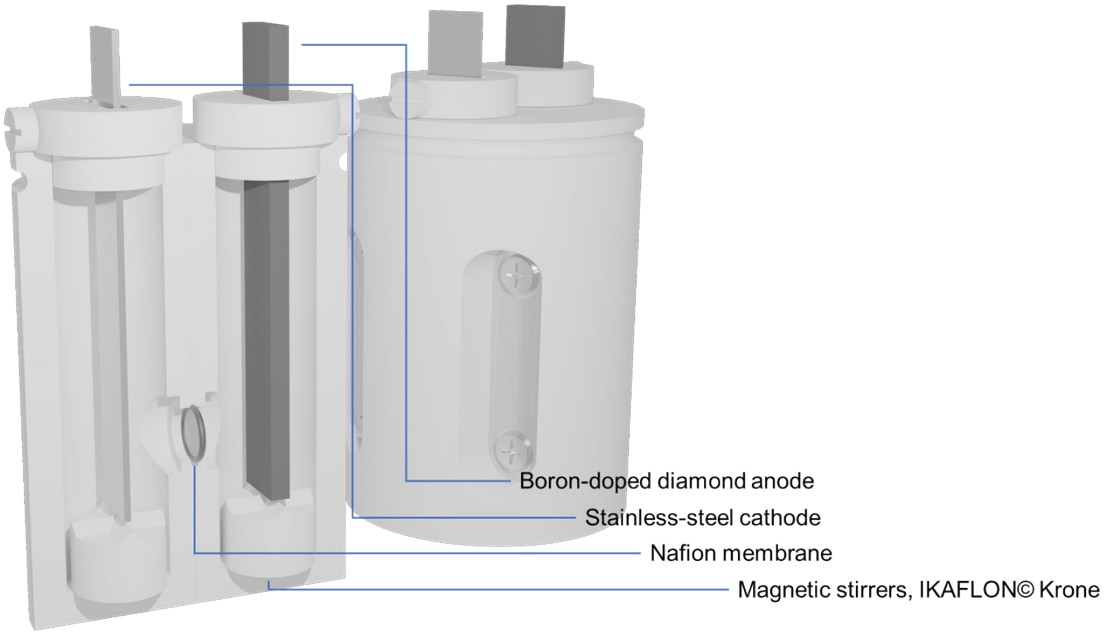
According to the procedure of Schindler and co-workers,[16] (*S*)-**8** hydrochloride (1.00 g, 7.22 mmol), 1.05 eq. of NEt_3 (1.05 mL, 7.58 mmol) and 1.05 eq. of succinic anhydride (0.75 g, 7.58 mmol) were suspended in toluene (100 mL) and refluxed overnight removing the produced water by using a Dean-Stark trap. The crude product was crystallised with acetone and was purified by flash column chromatography on silica gel (12 x 2 cm, pure CH_2Cl_2 to CH_2Cl_2/MeOH = 30:1). (*S*)-2-(2,5-dioxopyrrolidin-1-yl)butanamide was obtained as colourless solid in 42% yield (598 mg, 3.12 mmol).

^{1}H NMR (400 MHz, Chloroform-*d*) δ 6.22 (s, 1H), 5.68 (s, 1H), 4.59 (dd, *J* = 10.5, 6.0 Hz, 1H), 2.77 (s, 4H), 2.30 – 1.92 (m, 2H), 0.90 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 177.30 (C_q), 170.79 (C_q), 56.67 (CH_2), 28.22 (CH_3), 21.63 (CH), 10.99 (CH_2); **GC-FID**: R_t = 11.51 min.

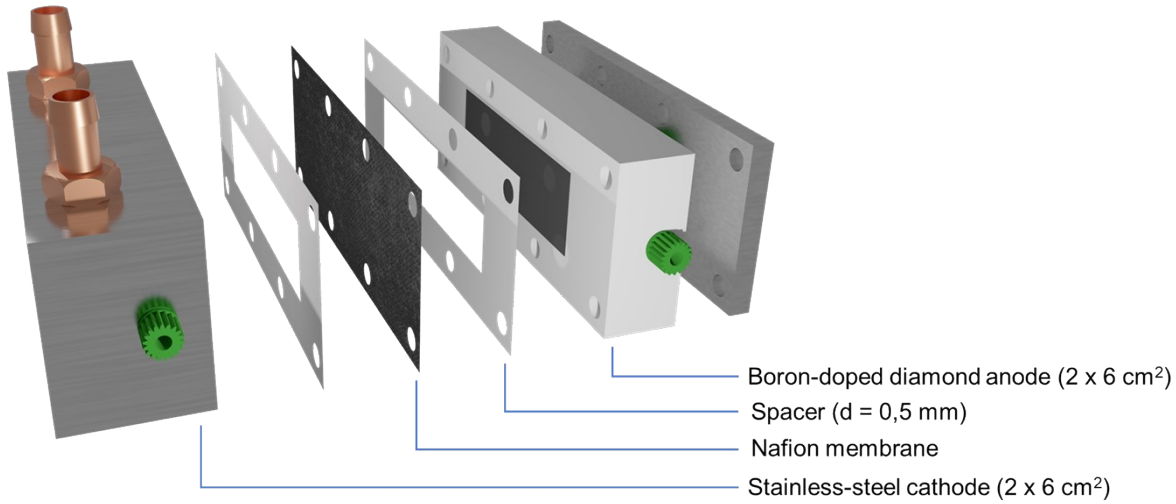
16 Electrolysis cells used

Electrolysis cells were manufactured in the work shop of the chemistry department of the Johannes Gutenberg University Mainz and are commercially available as parts of the IKA Screening System at **IKA**®-Werke GmbH&CO.KG, Staufen, Germany. The IKA company also sells the 2 x 6 cm^2-flow electrolysis cell as ElectraSyn Flow device. The stainless-steel flow electrolysis cell was purchased from CONDIAS GmbH, Itzehoe, Germany.

A:



B:



C:

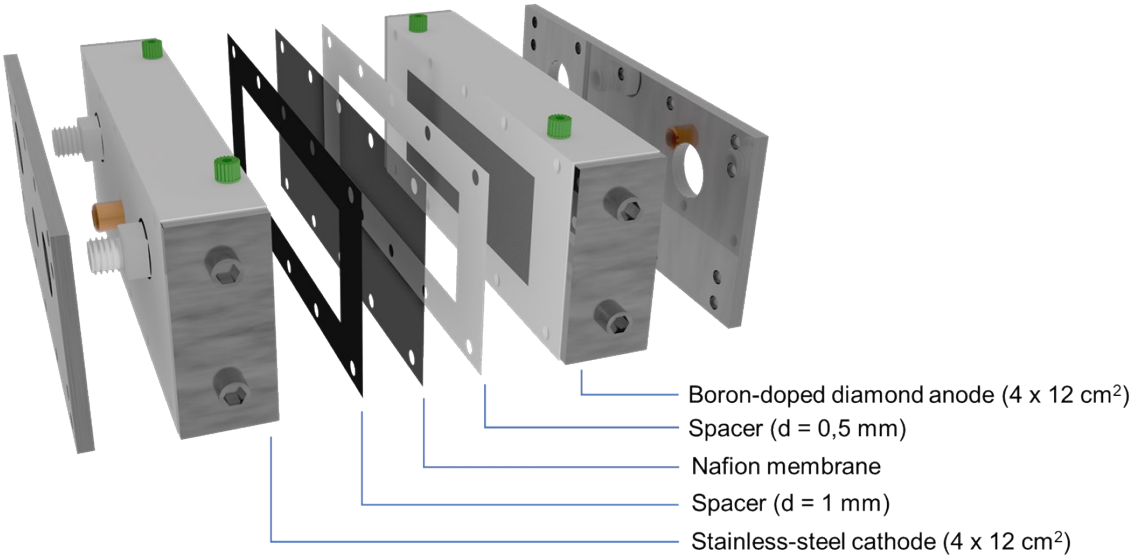
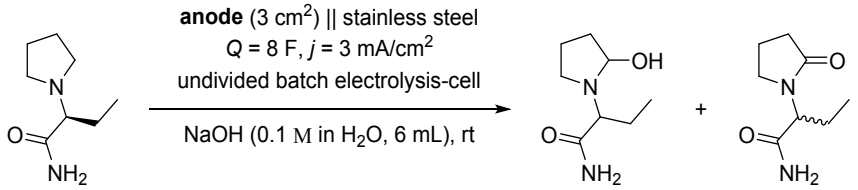


Figure S17. A: Divided batch cell with 1 x 3 cm^2 electrode surfaces. B: divided flow cell with 2 x 6 cm^2 anode surface. C: divided flow cell with a 4 x 12 cm^2 anode surface.[17] Depictions were designed with the software Blender 2.8 from Blender Foundation.

17 Experimental section for the electrochemical synthesis of etiracetam

Table S7. Anode material. According to RP 1, (*S*)-**8** was electrolysed in caustic soda.

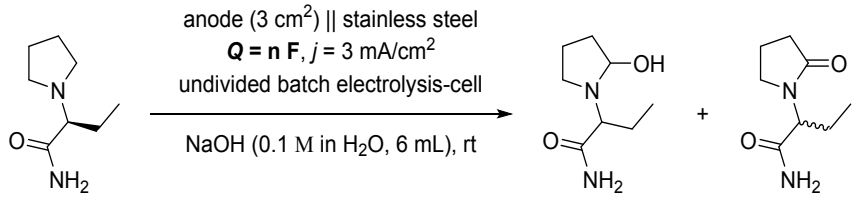


Entry	Anode	GC Yield [%]			
		8	**9**[a]	**1**	**Σ**
1	GC	0	0	6	6
2	Graphite	0	0	7	7
3	BDD	0	0	<1	<1
4	Carbon fibre	0	0	3	3
5	Ni	41	20	0	61
6	Pt	0	0	15	15
7	Pt	0	0	2	2
8	Pt	0	0	2	2
9	Cu	3	33	2	38
10	Au	0	20	6	26
11	SS	n. d.	-	-	-
12	Nb	n. d.	-	-	-

13	Mo	n. d.	-	-	-
14	W	n. d.	-	-	-
15	Pt	0	0	22	22
16	BDD	0	0	9	9

[a] relative area vs. standard.

Table S8. Applied charge. According to RP 1, (*S*)-**8** was electrolysed in caustic soda.

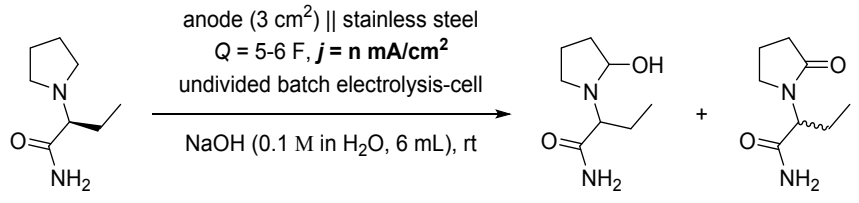


(*S*)-**8** **9** **1**

Entry	Anode	*Q* [F]	GC Yield [%]			
			8	**9**[a]	**1**	**Σ**
1	Pt	4.0	<1	32	8	41
2	Pt	5.0	0	19	15	34
3	Pt	6.0	0	0	22	22
4	Pt	7.0	0	0	18	18
5	Pt	8.0	0	0	15	15
6	BDD	4.0	4	42	4	50
7	BDD	5.0	0	0	25	25
8	BDD	6.0	0	0	21	21
9	BDD	7.0	0	0	9	9
10	BDD	8.0	0	0	9	9
11	GC	4.0	0	36	6	42
12	GC	5.0	0	12	13	25
13	GC	6.0	5	0	10	15
14	GC	7.0	0	0	9	9
15	GC	8.0	0	0	6	6

[a] relative area vs. standard.

Table S9. Current density. According to RP 1, (*S*)-**8** was electrolysed in caustic soda.



(*S*)-**8** **9** **1**

Entry	Anode	*Q* [F]	*j* [mA/cm²]	GC Yield [%]			
				8	**9**[a]	**1**	**Σ**
1	Pt	6.0 F	0.5	0	0	10	10
2	Pt	6.0 F	1	1	<1	21	23

3	Pt	6.0 F	2	0	0	28	28
4	Pt	6.0 F	3	0	0	22	22
5	Pt	6.0 F	5	0	0	26	26
6	Pt	6.0 F	8	0	<1	21	22
7	Pt	8.0 F	10	0	0	7	7
8	Pt	6.0 F	10	0	<1	20	21
9	Pt	6.0 F	12	0	0	20	20
10	Pt	6.0 F	15	0	1	18	19
11	Pt	6.0 F	18	0	2	17	19
12	Pt	8.0 F	20	<1	1	7	9
13	Pt	6.0 F	20	0	1	15	16
14	Pt	6.0 F	30	2	4	14	20
15	Pt	6.0 F	50	9	5	10	24
16	Pt	6.0 F	500	55	7	<1	63
17	BDD	5.0 F	0.5	0	0	18	18
18	BDD	5.0 F	1	4	7	22	33
19	BDD	5.0 F	2	0	<1	28	29
20	BDD	5.0 F	3	0	0	25	25
21	BDD	5.0 F	5	5	<1	22	28
22	BDD	5.0 F	10	0	2	20	22
23	BDD	5.0 F	15	<1	2	17	20
24	BDD	5.0 F	20	<1	4	17	22
25	BDD	5.0 F	30	1	5	15	21
26	Pt/Si	6.0 F	30	6	3	16	25

[a] relative area vs. standard.

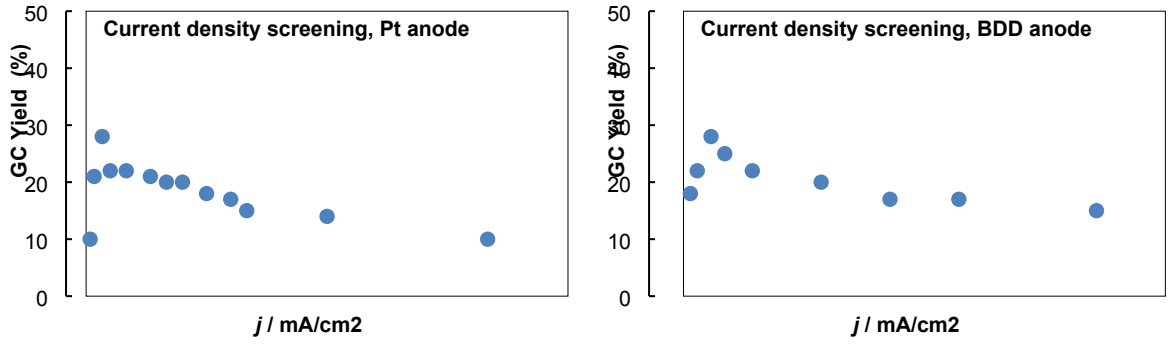
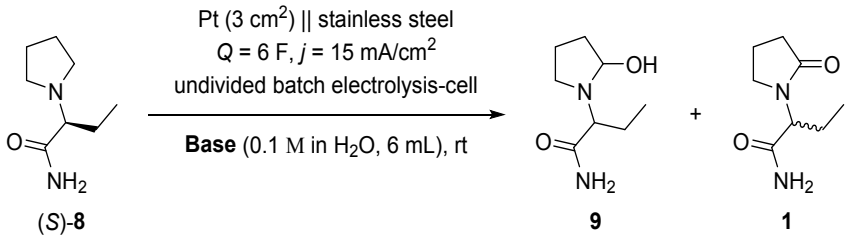


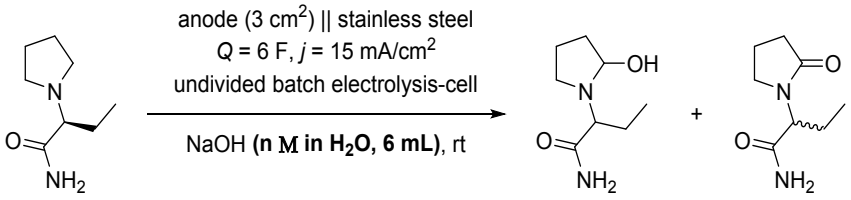
Table S10. Base. According to RP 1, (*S*)-**8** was electrolysed in alkaline solution.



Entry	Anode	Base	GC Yield [%]			
			8	9[a]	1	Σ
1	Pt	K_2CO_3	<1	<1	6	8
2	Pt	LiOH	<1	2	16	19
3	Pt	NaOH	0	1	21	22
4	Pt	KOH	7	4	10	21
5	Pt	$Ba(OH)_2$	32	15	8	55
6	Pt	CsOH	<1	<1	5	7

[a] relative area vs. standard.

Table S11. Base concentration. According to RP 1, (*S*)-**8** was electrolysed in caustic soda.

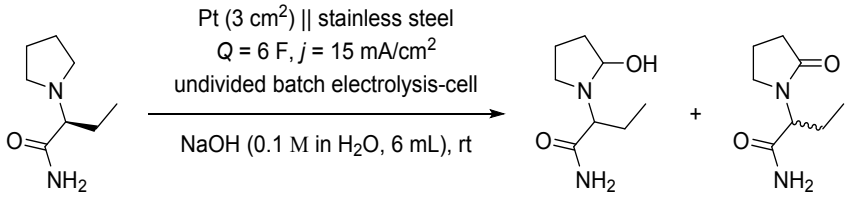


(*S*)-**8** **9** **1**

Entry	Anode	*C(NaOH)* [M]	GC Yield [%]			
			8	9[a]	1	Σ
1	Pt	0	28	3	<1	32
2	Pt	0.01	38	8	2	48
3	Pt	0.05	15	<1	5	21
4	Pt	0.07	<1	<1	13	15
5	Pt	0.09	<1	12	16	29
6	Pt	0.1	0	1	21	22
7	Pt	0.12	<1	2	17	20
8	Pt	0.15	4	4	11	19
9	Pt	0.2	<1	2	4	7
10	Pt	0.50	0	2	<1	3
11	Pt	1.00	0	3	<1	4
12	BDD	0	38	3	<1	42
13	BDD	0.05	<1	6	10	17
14	BDD	0.07	0	4	16	20
15	BDD	0.09	<1	3	17	21
16	BDD	0.1	<1	2	18	21

[a] relative area vs. standard.

Table S12. Starting material concentration. Carried out according to RP 1.

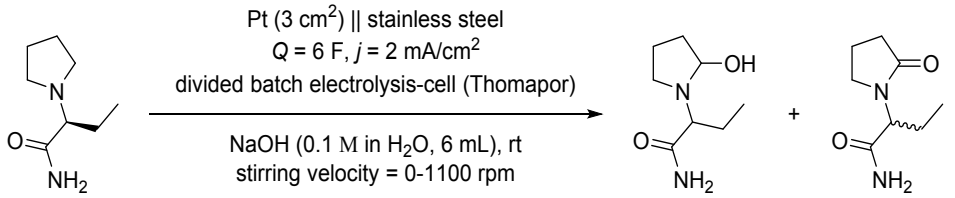


(*S*)-**8** **9** **1**

Entry	*m*(7)	GC Yield [%]			
		8	**9**[a]	**1**	**Σ**
1	5 mg	43	<1	7	51
2	20 mg	11	3	6	20
3	40 mg	1	<1	8	10
4	60 mg	<1	1	13	15
5	80 mg	0	<1	18	19
6	100 mg	0	1	21	22
7	150 mg	6	0	14	20
8	200 mg	7	0	10	17

[a] relative area vs. standard.

Table S13. Stirring velocity. According to RP 1, (*S*)-**8** was electrolysed in caustic soda.

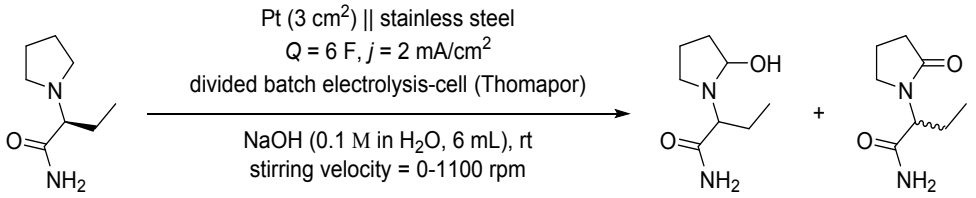


(*S*)-**8** **9** **1**

Entry	stirring velocity [rpm]	GC Yield [%]			
		8	**9**[a]	**1**	**Σ**
1	1100	0	0	27	27
2	900	<1	0	26	27
3	250	<1	0	24	25
4	100	0	0	25	25
5	0	3	5	11	19

[a] relative area vs. standard.

Table S14. Temperature. According to RP 1, (*S*)-**8** was electrolysed in caustic soda.



(*S*)-**8** **9** **1**

Entry	*T* [°C]	GC Yield [%]			
		8	**9**[a]	**1**	**Σ**
1	75-80	<1	<1	19	21
2	60	0	0	25	25
3	40	0	0	29	29
4	rt	<1	0	24	25
5	0-rt	0	0	20	20

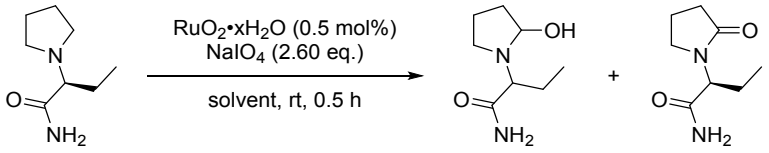
[a] relative area vs. standard.

18 Experimental section for the ruthenium catalysis

18.1 One-variable-at-a-time (OVAT) screening

Table S15. Solvent screening.

According to RP 2, RuO_2•xH_2O and $NaIO_4$ were suspended. (*S*)-**8** was added and the reaction was stirred at room temperature for 0.5 h.

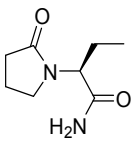


(*S*)-**8** **9** (*S*)-**1**

Entry	Solvent	Ratio [v/v]	GC Yield [%]			
			8	**9**[a]	**1**	**Σ**
1	H_2O	pure	5	9	31	45
2	H_2SO_4	0.1 M	16	0	9	25
3	NaOH	0.1 M	29	16	24	69
4	H_2O/EtOAc	4:2	3	5	32	40
5	H_2O/MeCN	4:2	1	4	57	62
6	H_2O/acetone	4:2	1	4	60	65
7	H_2O/$CHCl_3$	4:2	2	8	17	27
8	H_2O/CH_2Cl_2	4:2	3	5	21	29
9	H_2O/NMP	4:2	41	21	28	89
10	H_2O/DMSO	4:2	110	0	0	110
11	H_2O/AcOH	4:2	19	1	10	30
12	H_2O/DMF	4:2	16	18	37	72
13	Acetone	pure	132	1	0	133
14	MeCN	pure	102	0	0	102
15	H_2O/Acetone/NMP	4:2:0.5	5	14	64	83
16	0.1 M NaOH/Acetone/NMP	4:2:0.5	21	20	38	79
17	0.1 M NaOH/MeCN/NMP	4:2:0.5	16	15	42	73
18	0.1 M H_2SO_4/MeCN/NMP	4:2:0.5	3	1	27	31

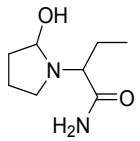
[a] relative area vs. standard

The product (*S*)-**1** and the intermediate **9** were isolated by flash column chromatography on silica gel (12 x 2 cm) using an eluent mixture of CH_2Cl_2/MeOH = 10:1; or by crystallisation in Et_2O or PE/CH_2Cl_2 at -20 °C. Levetiracetam was isolated in 49% yield and in 99.6% *ee*.



(*S*)-**1** **TLC** (SiO_2, ninhydrin stain, strong heating), R_f(CH_2Cl_2/MeOH = 10:1) = 0.56, R_f(CH_2Cl_2/MeOH = 20:1) = 0.13; **GC-FID**: R_f = 8.98 min at ~180 °C; **LC-MS (HR):** calculated for $[M+H]^+$:

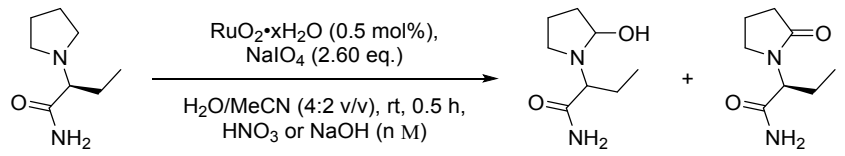
$[C_8H_{15}N_2O_2]^+$ 171.1128 Da, found: 171.1128; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 6.58 (s, 1H), 6.11 – 5.88 (m, 1H), 4.46 (dd, *J* = 9.1, 6.6 Hz, 1H), 3.45 (ddd, *J* = 9.8, 8.0, 6.3 Hz, 1H), 3.36 (ddd, *J* = 9.8, 8.0, 6.0 Hz, 1H), 2.47 – 2.29 (m, 2H), 2.10 – 1.85 (m, 3H), 1.65 (ddq, *J* = 14.6, 9.1, 7.4 Hz, 1H), 0.86 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 176.10 (C_q), 172.64 (C_q), 56.07 (*CH*), 43.89 (CH_2), 31.15 (CH_2), 21.21 (CH_2), 18.20 (CH_2), 10.59 (CH_3).



9

Characterised as a mixture of 9 and 1 (ratio = 7:1): TLC (SiO_2, ninhydrin stain, weak heating), R_f(CH_2Cl_2/MeOH = 10:1) = 0.48; **GC**: R_f = 8.20 min at ~160 °C; **LC-MS (HR ESI(+)):** calculated for $[M\text{-}OH]^+$: $[C_8H_{15}N_2O]^+$ 155.2205 Da, found: 155.1181; **^{1}H NMR** (400 MHz, Chloroform-*d*) δ 6.81 (s, 1H), 4.87 (ddd, *J* = 5.7, 3.6, 1.4 Hz, 1H), 3.17 (dt, *J* = 10.1, 5.7 Hz, 1H), 3.08 (ddd, *J* = 8.0, 4.3, 1.5 Hz, 1H), 2.71 (ddd, *J* = 10.0, 7.6, 6.3 Hz, 1H), 2.15 – 1.50 (m, 6H), 1.24 (s, 0H), 1.02 (t, *J* = 7.4 Hz, 3H); **^{13}C NMR** (101 MHz, Chloroform-*d*) δ 177.99 (Cq), 75.18 (CH), 68.93 (CH), 56.55 (CH_2), 32.87 (CH_2), 25.99 (CH_2), 23.84 (CH_2), 10.45 (CH_3).

Table S16. pH screening. According to RP 2, RuO_2•xH_2O and $NaIO_4$ were suspended. (*S*)-**8** was added and the reaction was stirred at room temperature for 0.5 h.



(*S*)-**8** **9** (*S*)-**1**

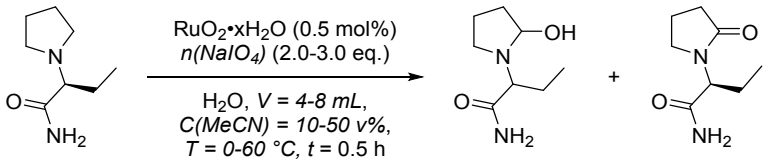
Entry	acid or base	*M* [M]	GC Yield [%]			
			8	**9**[a]	**1**	**Σ**
1	HNO_3	0.01	0	3	33	36
2	HNO_3	0.1	0	4	17	21
3	HNO_3	0.5	0	0	0	0
4	HNO_3	2.0	0	0	0	0
5	HNO_3	3.0	0	0	0	0
6	NaOH	0.01	0	5	40	45
7	NaOH	0.1	0	15	24	39
8	NaOH	0.5	51	16	0	67
9	NaOH	2.0	63	6	0	69
10	NaOH	3.0	75	4	0	79

[a] relative area vs. standard

18.2 DoE optimization

18.3 2^4 plan

Table S17. ***n(NaIO$_4$)*V*C(MeCN)*T*.** According to RP 2, RuO_2•xH_2O and $NaIO_4$ were suspended. (*S*)-**8** was added and the reaction was stirred at the given temperature for 0.5 h.



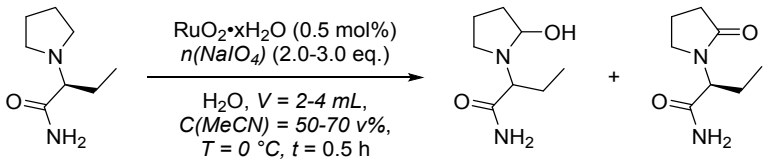
(*S*)-**8** **9** (*S*)-**1**

Entry	*n(NaIO$_4$)* [eq.]	*V* [mL]	*C(MeCN)* [v%]	*T* [°C]	GC Yield [%]			
					8	**9**[a]	**1**	**Σ**
1	3	6	10	60	2	3	22	27
2	3	4	10	60	6	4	24	34
3	2.5	4	10	0	24	8	37	69
4	3	8	30	60	11	8	16	35
5	3	6	50	0	1	2	65	68
6	2	4	50	60	13	18	26	57
7	2	4	30	0	17	7	51	75
8	2.5	8	50	60	8	17	18	43
9	3	4	50	30	0	0	56	56
10	3	8	10	0	1	4	35	40
11	2	8	50	0	26	9	37	72
12	2	8	10	30	6	19	18	43
13	2	6	10	60	4	21	9	34
14	2.5	6	30	30	1	2	48	51

[a] relative area vs. standard

18.4 DoE 2^3 plan

Table S18. ***n(NaIO$_4$)*V*C(MeCN)*.** According to RP 2, RuO_2•xH_2O and $NaIO_4$ were suspended. (*S*)-**8** was added and the reaction was stirred at the given temperature for 0.5 h.



(*S*)-**8** **9** (*S*)-**1**

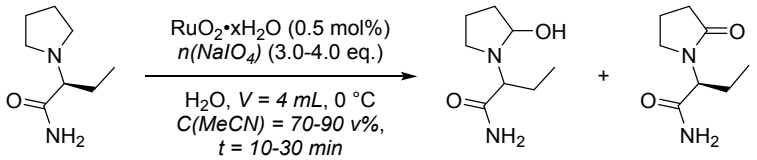
Entry	*n(NaIO$_4$)* [eq.]	*V* [mL]	*C(MeCN)* [v%]	*T* [°C]	GC Yield [%]			
					8	**9**[a]	**1**	**Σ**
1	3	4	70	0	0	1	65	66
2	3	2	70	0	0	0	66	66
3	2	4	50	-20	39	5	32	76
4	2	2	50	0	13	3	51	67
5	3	4	50	0	1	1	64	66

6	3	2	50	0	0	0	66	66
7	2	4	70	0	18	0	46	66
8	2	4	50	0	43	6	25	74
9	2	2	70	0	20	3	47	70

[a] relative area vs. standard.

18.5 DoE 2^3 plan

Table S19. ***n(NaIO$_4$)*V*C(MeCN).*** According to RP 2, RuO_2•xH_2O and $NaIO_4$ were suspended. (*S*)-**8** was added and the reaction was stirred at 0 °C for the given time.

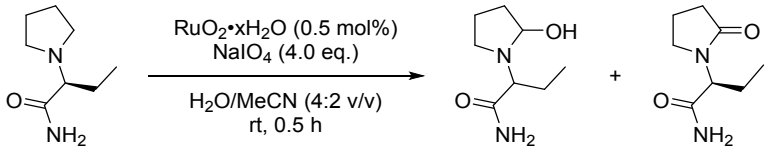


(*S*)-**8** **9** (*S*)-**1**

Entry	*n(NaIO$_4$)* [eq.]	*C(MeCN)* [v%]	*t* [min]	GC Yield [%]			
				8	**9**[a]	**1**	**Σ**
1	4	90	30	2	3	15	20
2	3	70	10	31	6	9	46
3	4	70	30	30	3	16	19
4	3	70	30	0	2	59	61
5	4	70	10	48	7	8	63
6	4	90	10	17	6	11	34
7	3	90	30	0	5	16	21
8	3	90	10	2	9	5	16
9	3.5	80	20	2	8	6	16

[a] relative area vs. standard.

18.6 Ruthenium oxide-catalysed oxidation with electrochemically produced periodate



(*S*)-**8** **9** (*S*)-**1**

According to RP 2, RuO_2•xH_2O (1 mg) and electrochemically generated $NaIO_4$ (550 mg) were suspended. (*S*)-**8** (100 mg, 640 μmol) was added and the reaction was stirred at room temperature for 0.5 h. (*S*)-**1** was obtained in 57% GC yield.

18.7 Procedure of the biphasic solvent system

(*S*)-**8** (78.1 mg, 0.5 mmol, 1.0 eq.) was dissolved in ethyl acetate (2.5 mL). An aqueous solution of RuO_2•xH_2O (366μg, 2.75 μmol, 0.55 mol%) and $NaIO_4$ (278 mg, 5 mL, 2.6 eq.) was

added. The reaction mixture was stirred at room temperature for 30 min. The mixture was concentrated under reduced pressure and the residue was purified by flash column chromatography on silica gel ($CHCl_3$/methanol = 10:1). (*S*)-**1** was isolated as a pale yellow solid in 76% yield with full retention of the stereo configuration as determined by chiral HPLC.

18.8 Scale-up experiment

(*S*)-**8** (1.00 g, 6.40 mmol, 1.0 eq.) was dissolved in ethyl acetate (25.0 mL). An aqueous solution of RuO_2•xH_2O (4.7 mg, 35.3 mmol, 0.55 mol%) and $NaIO_4$ (3.56 g, 16.6 mmol, 2.6 eq.) was added (50.0 mL). The reaction mixture was stirred at room temperature for 30 min. The crude product was extracted from the aqueous phase by a Kutscher-Steudel apparatus using ethyl acetate. The extract was dried by sodium sulphate and the solvent was removed in vacuum. The residue was purified by flash column chromatography on silica gel (cyclohexane/ethyl acetate = 3:1). (*S*)-**1** was isolated as a pale-yellow solid in 74% yield (808 mg, 4.75 mmol).

18.9 Recovery of the ruthenium and the iodate

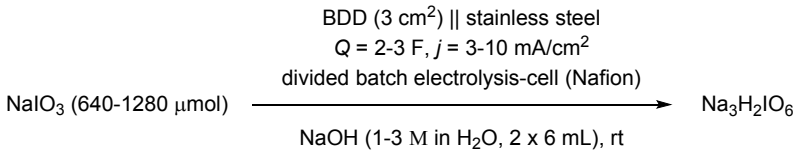
The ruthenium species was removed by filtration through a column of neutral aluminium oxide (50-200 μm, 60A, 10 cm). For the recovery of the sodium iodate, it was precipitated at 4 °C using methanol or *iso*-propanol. It was filtered off and was dried under reduced pressure. Sodium iodate was obtained in up to 96% isolated yield.

19 Experimental part for the $NaIO_3$ recycling

19.1 DoE optimisation

19.2 2^{4-1} plan with replication

Table S20. ***j*Q*C(NaOH)*C(NaIO3).*** According to RP 3, sodium iodate was electrolysed in caustic soda at a BDD anode.



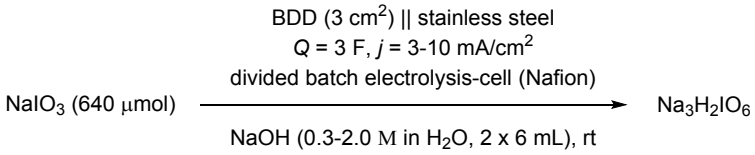
Entry	*j* [mA/cm²]	*Q* [F]	*C(NaOH)* [M]	*n(NaIO₃)* [μmol]	LC-PDA Yield [%]		
					IO_3^-	IO_4^-	Σ
1	2	3	3	640	96	4	100
2	2	2	2	640	62	38	100
3	2	1	3	1280	81	10	91
4	2	1	3	1280	77	21	98
5	2	2	3	1280	52	35	87

6	6	1.25	2	960	39	62	101
7	6	2.5	2	960	45	51	96
8	2	2	1	640	60	39	99
9	10	1.5	3	1280	102	2	104
10	10	3	3	1280	34	50	84
11	2	3	3	640	66	30	96
12	10	1	1	1280	44	62	96
13	10	2	1	1280	28	66	96
14	6	1.25	2	960	44	54	98
15	6	2.5	2	960	96	3	99
16	10	1	1	1280	98	3	101
17	10	2	1	1280	66	26	92
18	2	1.5	1	1280	10	19	29
19	2	1.5	1	1280	61	20	81
20	2	3	1	1280	37	33	70
21	10	3	1	640	93	5	98
22	10	2	3	640	30	69	99
23	2	1.5	1	1280	36	2	38
24	2	1.5	1	1280	41	53	94
25	2	3	1	1280	27	24	51
26	10	2	3	640	55	38	93
27	2	1	3	1280	22	8	30
28	2	1	3	1280	92	3	95
29	2	2	3	1280	69	13	82
30	10	3	1	640	44	34	77
31	10	3	1	640	83	8	91
32	10	1.5	3	1280	41	22	63
33	10	1.5	3	1280	51	28	79
34	10	3	3	1280	32	2	34
35	2	3	3	640	57	29	86
36	2	2	1	640	38	50	88
37	2	2	3	1280	80	4	84
38	6	2.5	2	960	69	32	101
39	2	2	1	640	77	22	99
40	10	3	3	1280	88	15	103
41	2	3	3	640	20	83	103
42	10	2	1	1280	94	3	97
43	6	2.5	2	960	92	3	95
44	10	2	1	1280	88	3	91
45	2	3	1	1280	90	6	96
46	10	3	1	640	77	7	84

47	10	2	3	640	62	24	86
48	2	3	1	1280	47	51	98
49	10	2	3	640	61	31	92
50	2	2	3	1280	89	4	93
51	10	3	1	640	36	54	90
52	10	3	3	1280	51	44	95
53	10	3	1	1280	21	86	107

19.3 DoE 2^2 plan with replication.

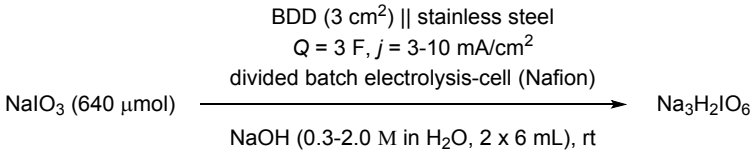
Table S21. ***j**C(NaOH).*** According to RP 3, sodium iodate was electrolysed in caustic soda at a BDD anode.



Entry	*j* [mA/cm²]	*C(NaOH)* [M]	LC-PDA Yield [%]		
			IO_3^-	IO_4^-	Σ
1	300	1.0	94	7	101
2	100	0.3	87	13	100
3	300	1.0	100	2	103
4	100	1.0	78	21	99
5	300	0.3	92	8	100
6	100	0.3	93	5	98
7	100	1.0	96	2	98
8	300	0.3	98	4	102
9	200	0.65	91	2	93
10	100	1.0	82	16	99
11	100	2.0	98	4	102
12	10	1.0	16	83	99
13	100	2.0	69	28	96
14	55	1.5	68	29	97
15	100	1.0	78	21	99
16	10	2.0	95	4	99
17	10	2.0	98	3	101
18	10	1.0	65	32	96

19.4 OVAT base-concentration screening

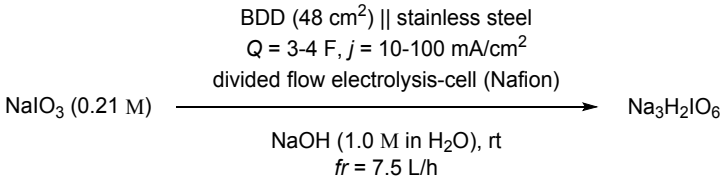
Table S22. According to RP 3, sodium iodate was electrolysed in caustic soda at a BDD anode.



Entry	*C(NaOH)* [M]	*j* [mA/cm²]	LC-PDA Yield [%]		
			IO_3^-	IO_4^-	Σ
1	0.0	3	94	2	96
2	0.1	3	74	23	97
3	0.1	10	99	7	106
4	0.3	10	103	4	106
5	0.5	10	50	25	75
6	0.8	10	30	69	99
7	0.9	10	19	80	99
8	1.0	3	11	86	97
9	1.1	10	22	75	97
10	1.2	10	97	3	100
11	1.3	10	27	70	97
12	1.4	10	97	4	101
13	1.5	10	23	77	100
14	2.0	3	73	24	97
15	3.0	3	96	4	100
16	4.0	3	12	78	90
17	5.0	3	16	85	101

19.5 Development of flow electrolysis and scale-up

Table S23. *j*Q*. Sodium iodate (0.21 M) and sodium hydroxide (1.00 M) were dissolved in water and were electrolysed in a flow electrolysis cell. The electrolysis cell was equipped with a BDD anode and a stainless-steel cathode and was divided by a Nafion membrane. The anolyte and catholyte were pumped in two independent loops in cycling-mode. Two Ritmo R033 pumps from Fink Chem+Tec GmbH (Leinfelden-Echterdingen, Germany) were used.



Entry	V [mL]	*j* [mA/cm²]	*Q* [F]	LC-PDA Yield [%]		
				IO_3^-	IO_4^-	Σ
1	50	10	3	9	83	92
2	50	10	3	20	85	105
3	50	50	3	26	73	99
4	50	50	4	16	76	92
5	50	100	3	34	63	97
6	50	100	4	24	72	96
7	50	300	3	63	37	100
8	50	300	4	44	53	97
9	500	100	3	29	70	99
10	500	100	4	14	88	102
11	500	300	3	43	56	99
12	500	300	4	32	70	102
13	1000	100	3	37	72	109
14	1000	100	4	18	78	96

* starting material did not dissolve completely before reaction, dissolved and new solid formed during the reaction.

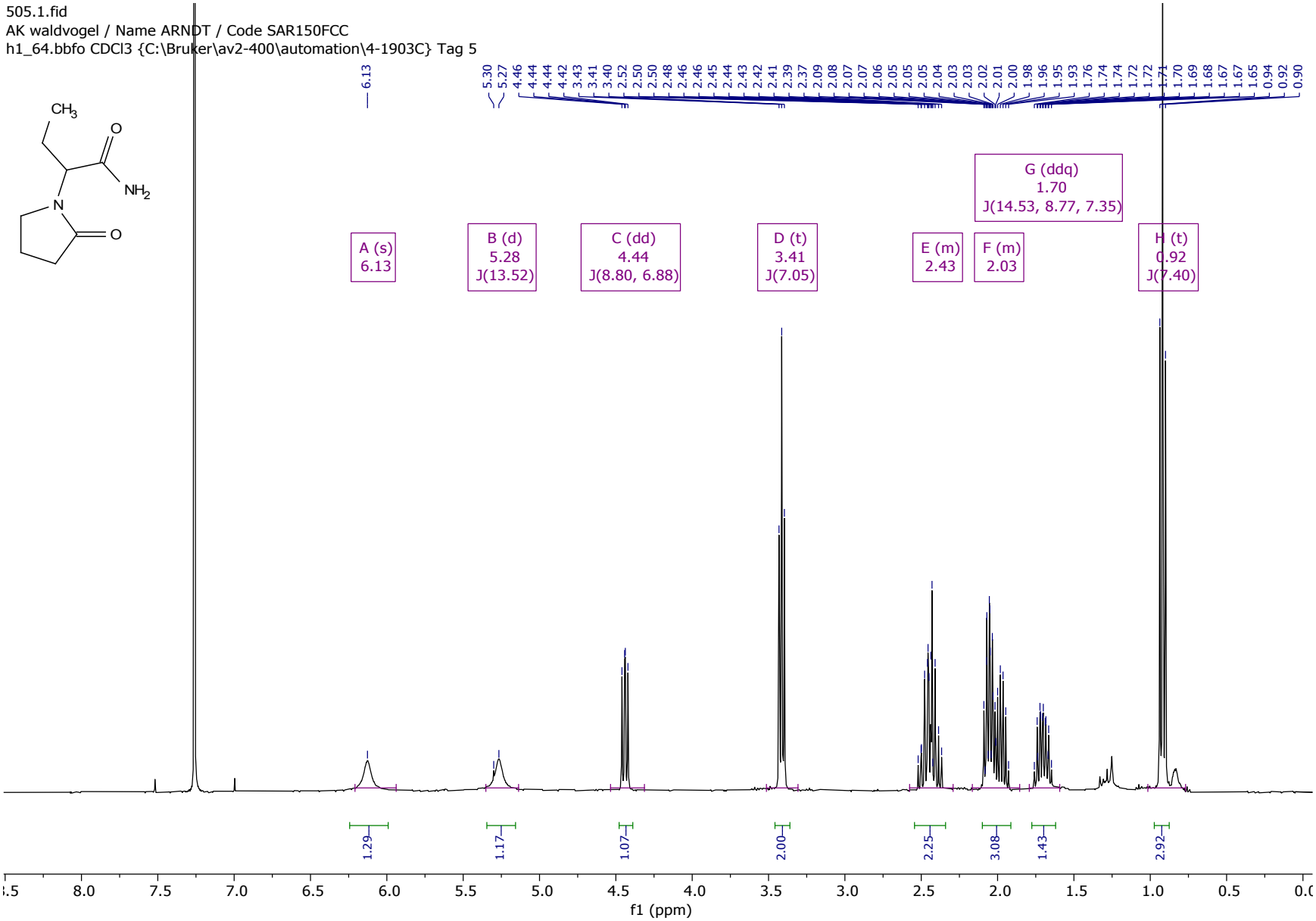
19.6 Recrystallisation of paraperiodate to metaperiodate

Sodium paraperiodate (4.00 g, 13.6 mmol), HNO_3 (2.2 mL, 65%) and water (8 mL) were refluxed at 130 °C for several minutes. Water was distilled off until crystallisation started. The mixture was cooled to 4 °C and was kept at this temperature overnight. The crystals were filtered off and were dried under vacuum. Sodium metaperiodate was obtained as colourless crystals (2.057 g, 9.62 mmol, 71%). IR data were in accordance with the Bio-Rad database.[18]

19.7 Electrolysis with recovered sodium iodate

Recovered sodium iodate (2.08 g, 10.5 mmol) and sodium hydroxide (2.00 g, 50.0 mmol) were dissolved in water (50 mL) and were electrolysed according to RP 3. A current density of j = 50 mA cm^{-2} and a charge amount of Q = 4 F (4055 C) were applied. Sodium paraperiodate was obtained in reproducible 83% yield as determined by LC-PDA.

20 NMR Spectra, GC and HPLC chromatograms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6.13
B (d)
5.28
J(13.52)
C (dd)
4.44
J(8.80, 6.88)
D (t)
3.41
J(7.05)
E (m)
2.43
F (m)
2.03
G (ddq)
1.70
J(14.53, 8.77, 7.35)
H (t)
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J(7.40)
1.29
1.17
1.07
2.00
2.25
3.08
1.43
2.92
f1 (ppm)

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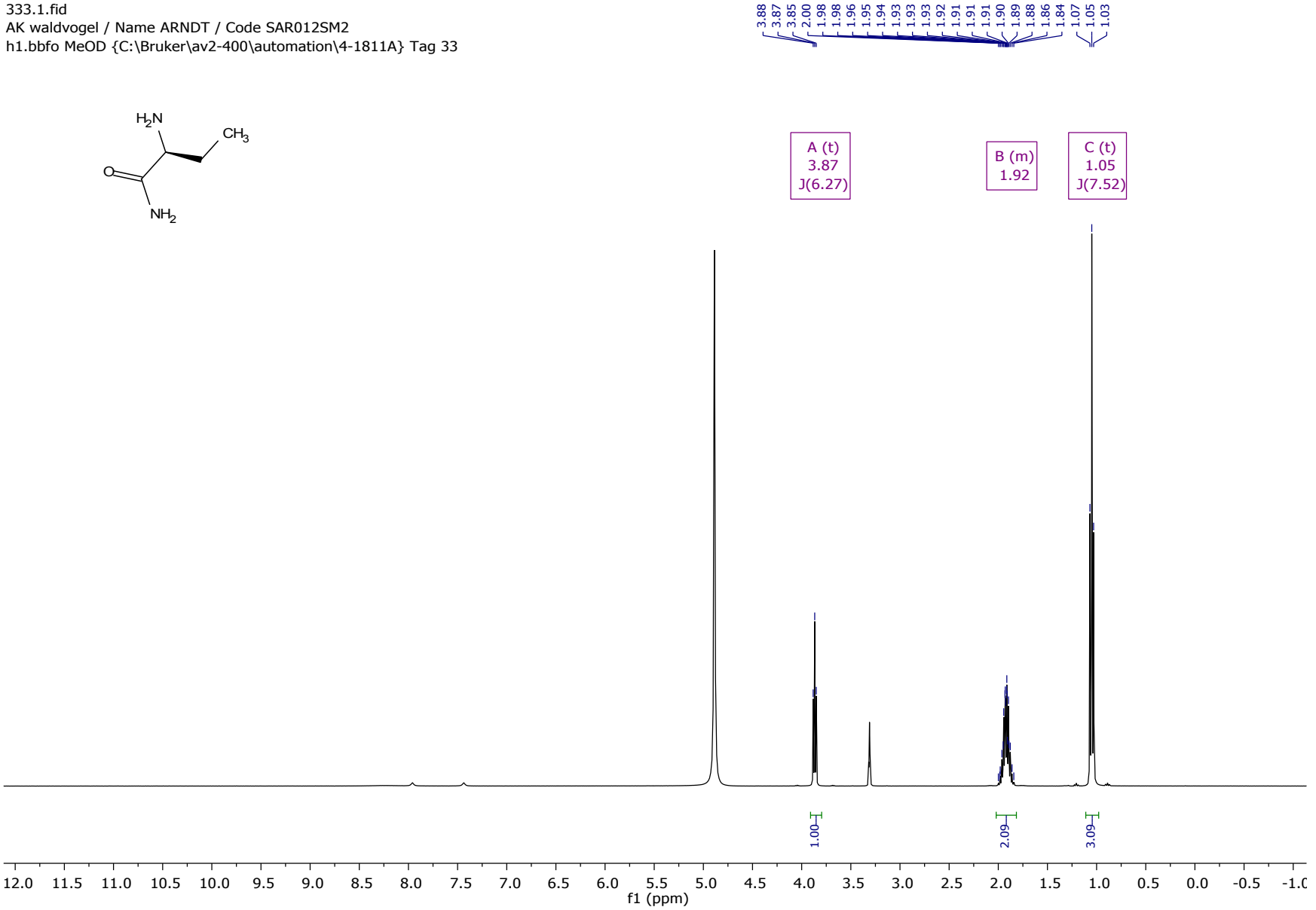

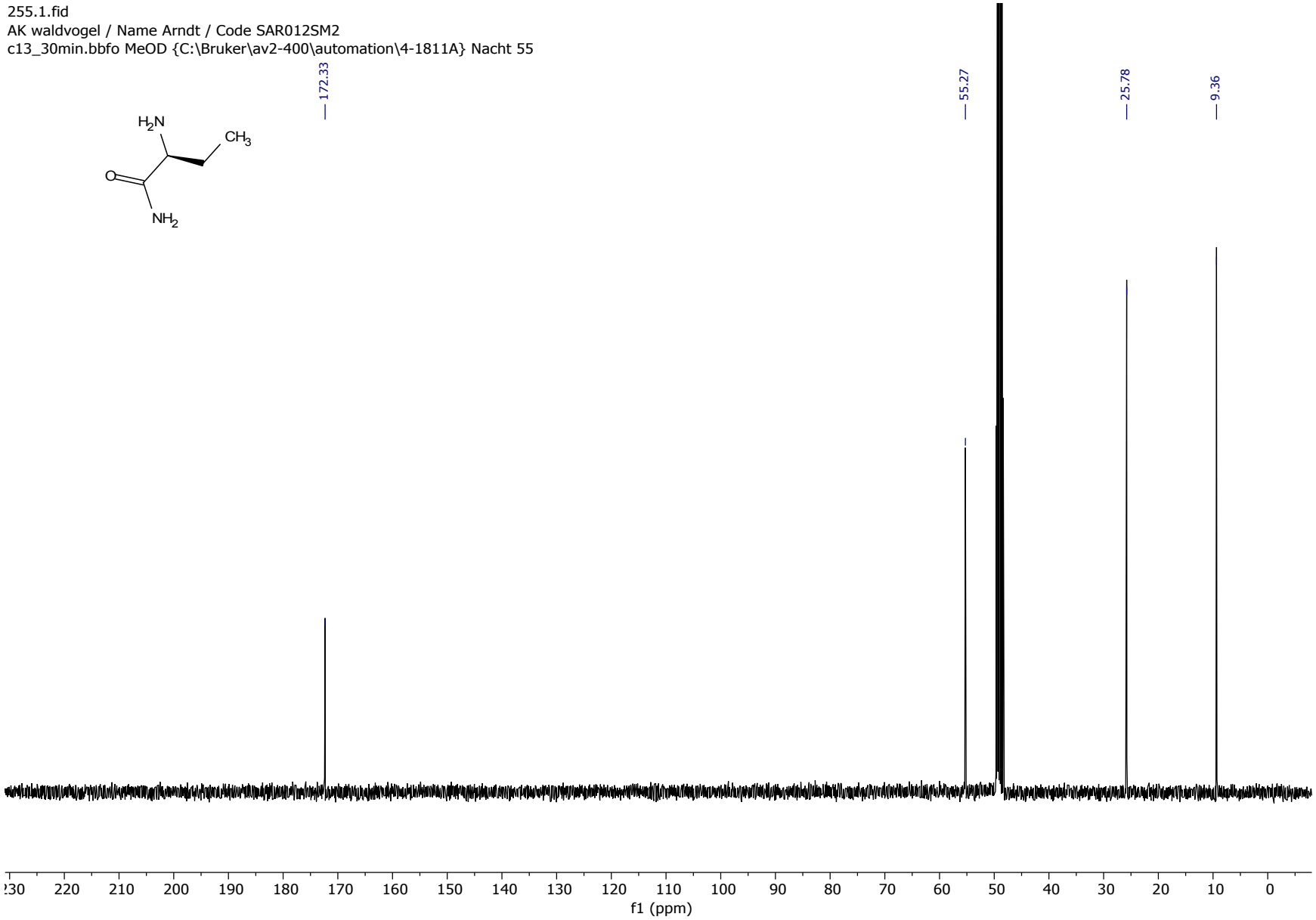
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9.36
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CH3
O
NH2
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f1 (ppm)

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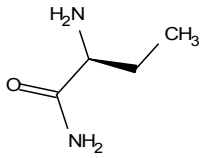

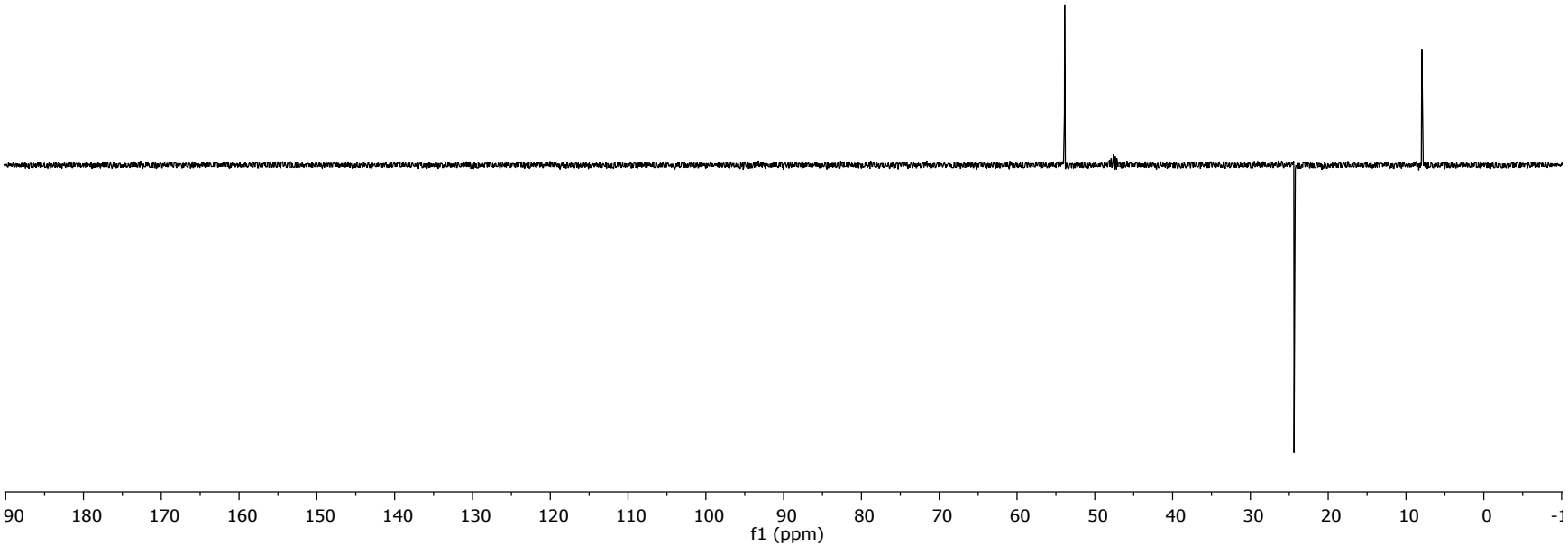

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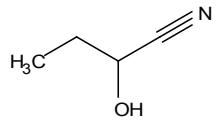

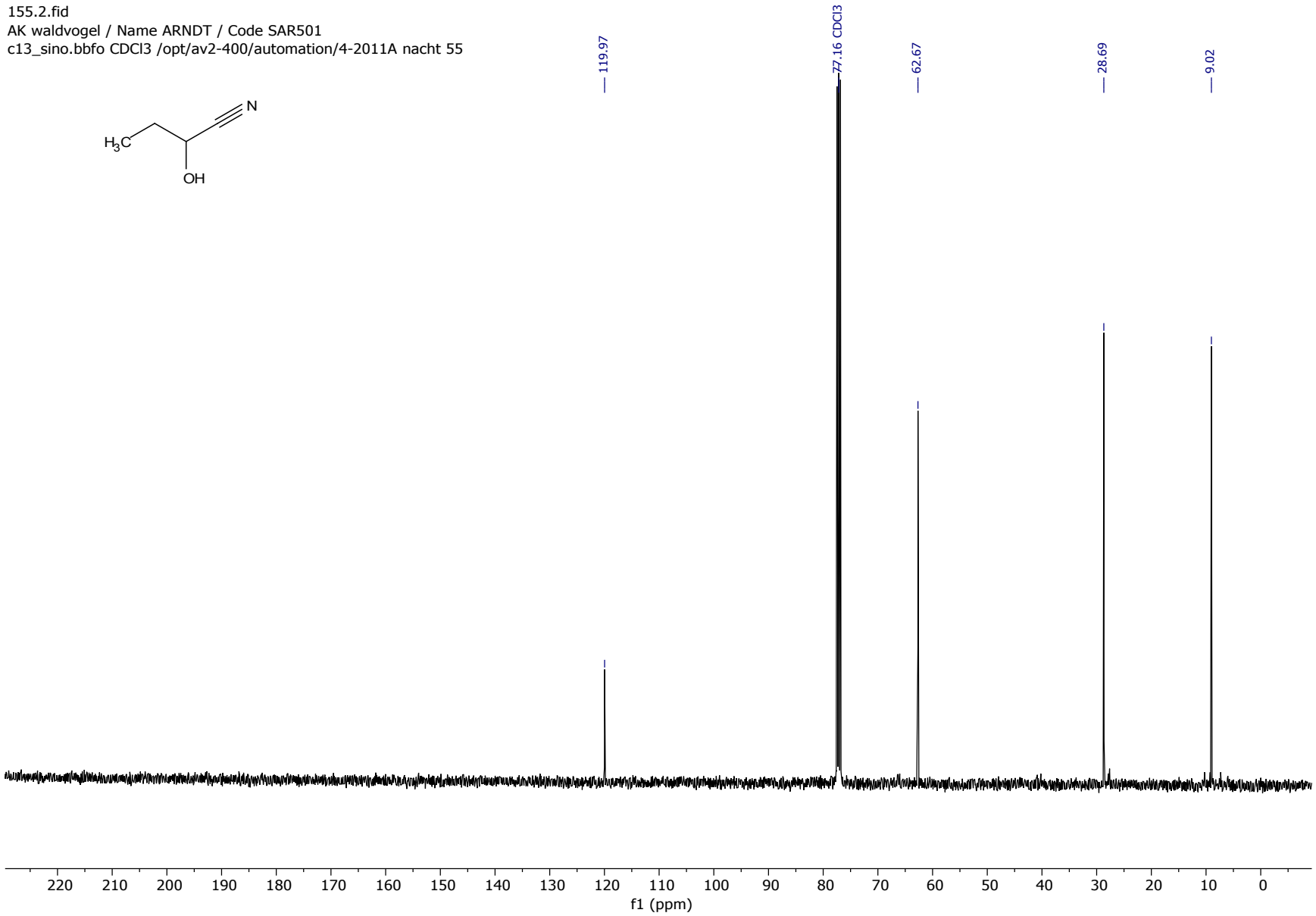
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9.02
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OH
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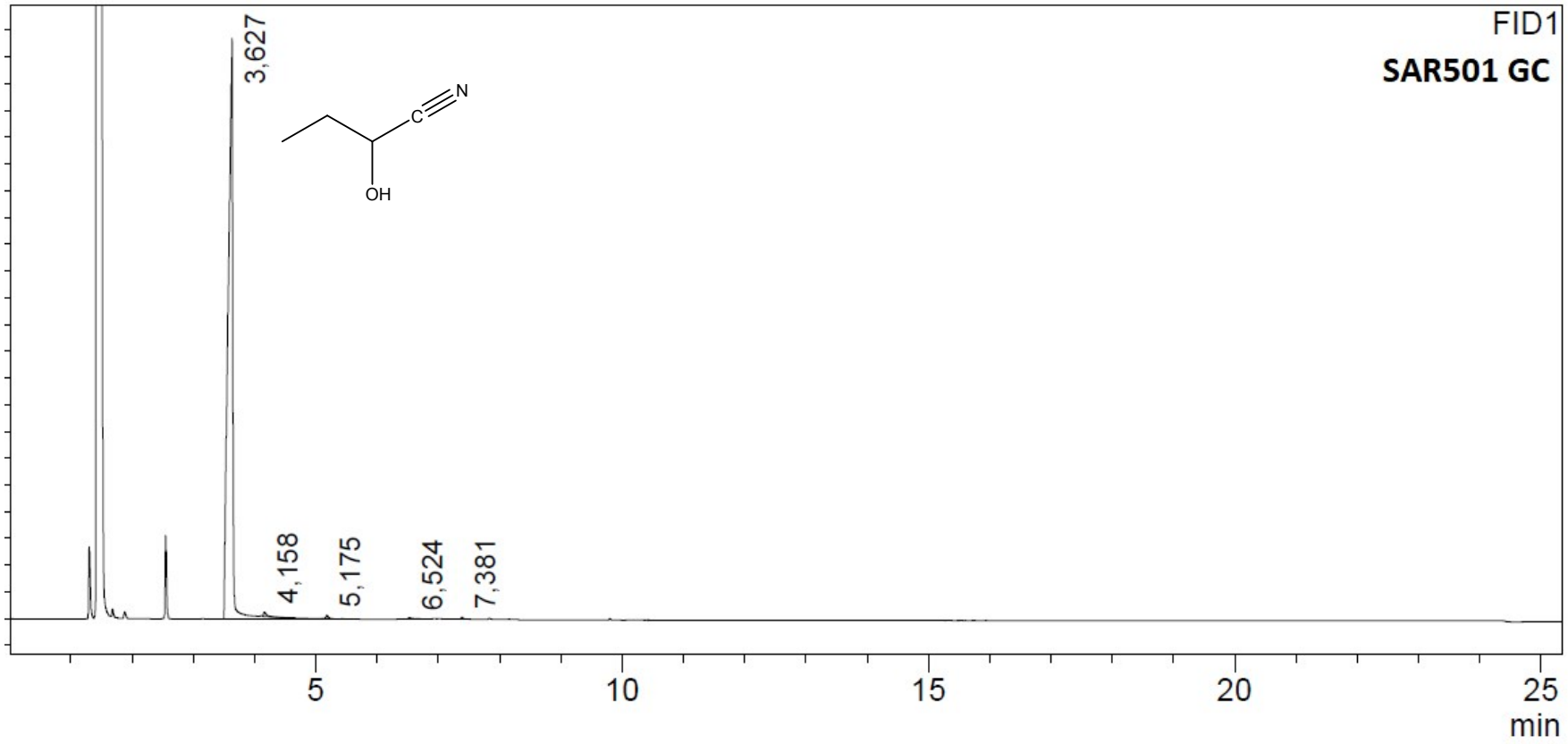
f1 (ppm)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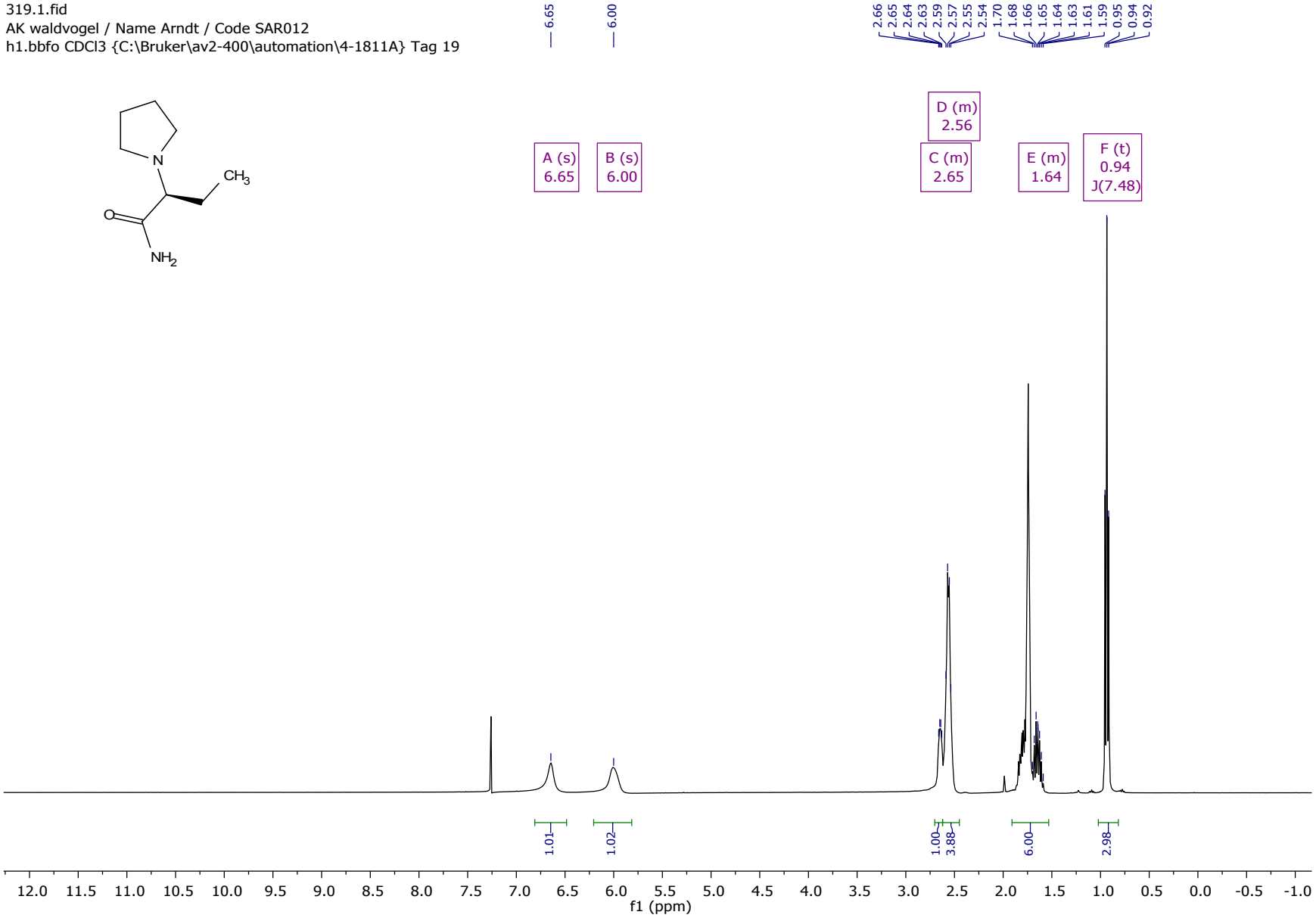
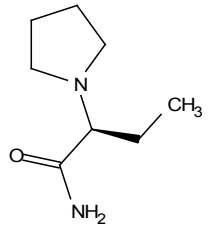
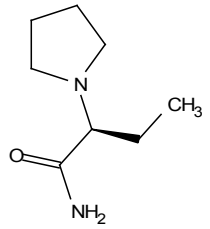
N
OH
5
10
15
20
25
min

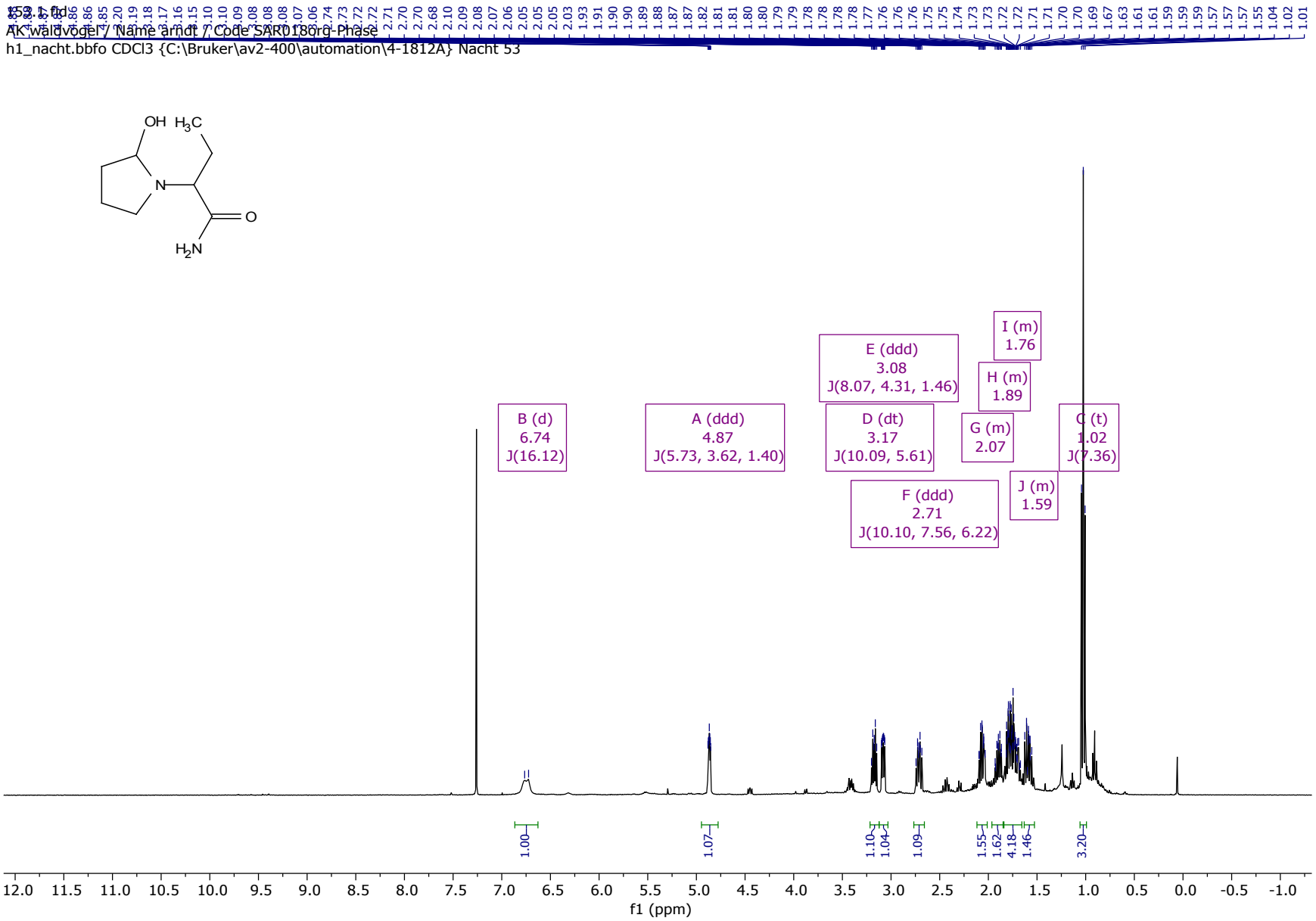

319.1.fid
AK waldvogel / Name Arndt / Code SAR012
h1.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1811A} Tag 19
6.65
6.00
2.66
2.65
2.64
2.63
2.59
2.57
2.55
2.54
1.70
1.68
1.66
1.65
1.64
1.63
1.61
1.59
0.95
0.94
0.92
A (s) 6.65
B (s) 6.00
D (m) 2.56
C (m) 2.65
E (m) 1.64
F (t) 0.94 J(7.48)
N
CH3
O
NH2
1.01
1.02
1.00
3.88
6.00
2.98
12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5 -1.0
f1 (ppm)

254.1.fid
AK waldvogel / Name Arndt / Code SAR012
c13_30min.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1811A} Nacht 54

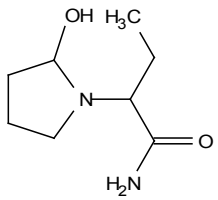


254.2.fid
AK waldvogel / Name Arndt / Code SAR012
c13_dept135.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1811A} Nacht 54

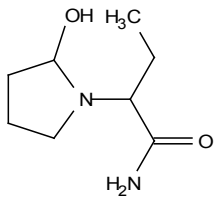


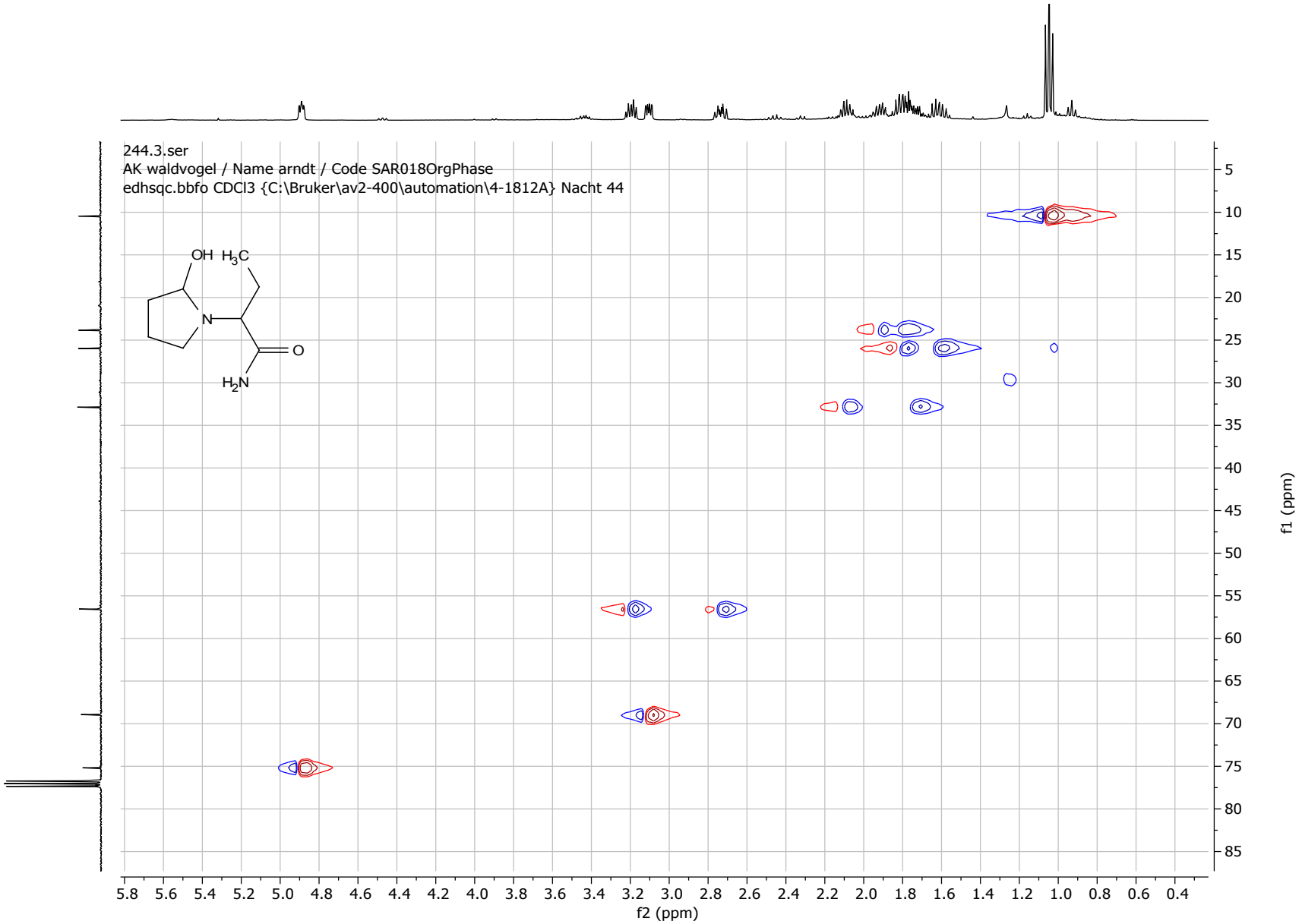

AK_waldvoger / Name arndt / Code SAR0180rg-Phase
h1_nacht.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1812A} Nacht 53
OH
H3C
N
O
H2N
B (d)
6.74
J(16.12)
A (ddd)
4.87
J(5.73, 3.62, 1.40)
E (ddd)
3.08
J(8.07, 4.31, 1.46)
D (dt)
3.17
J(10.09, 5.61)
F (ddd)
2.71
J(10.10, 7.56, 6.22)
I (m)
1.76
H (m)
1.89
G (m)
2.07
J (m)
1.59
C (t)
1.02
J(7.36)
1.00
1.07
1.10
1.04
1.09
1.55
1.62
4.18
1.46
3.20
f1 (ppm)

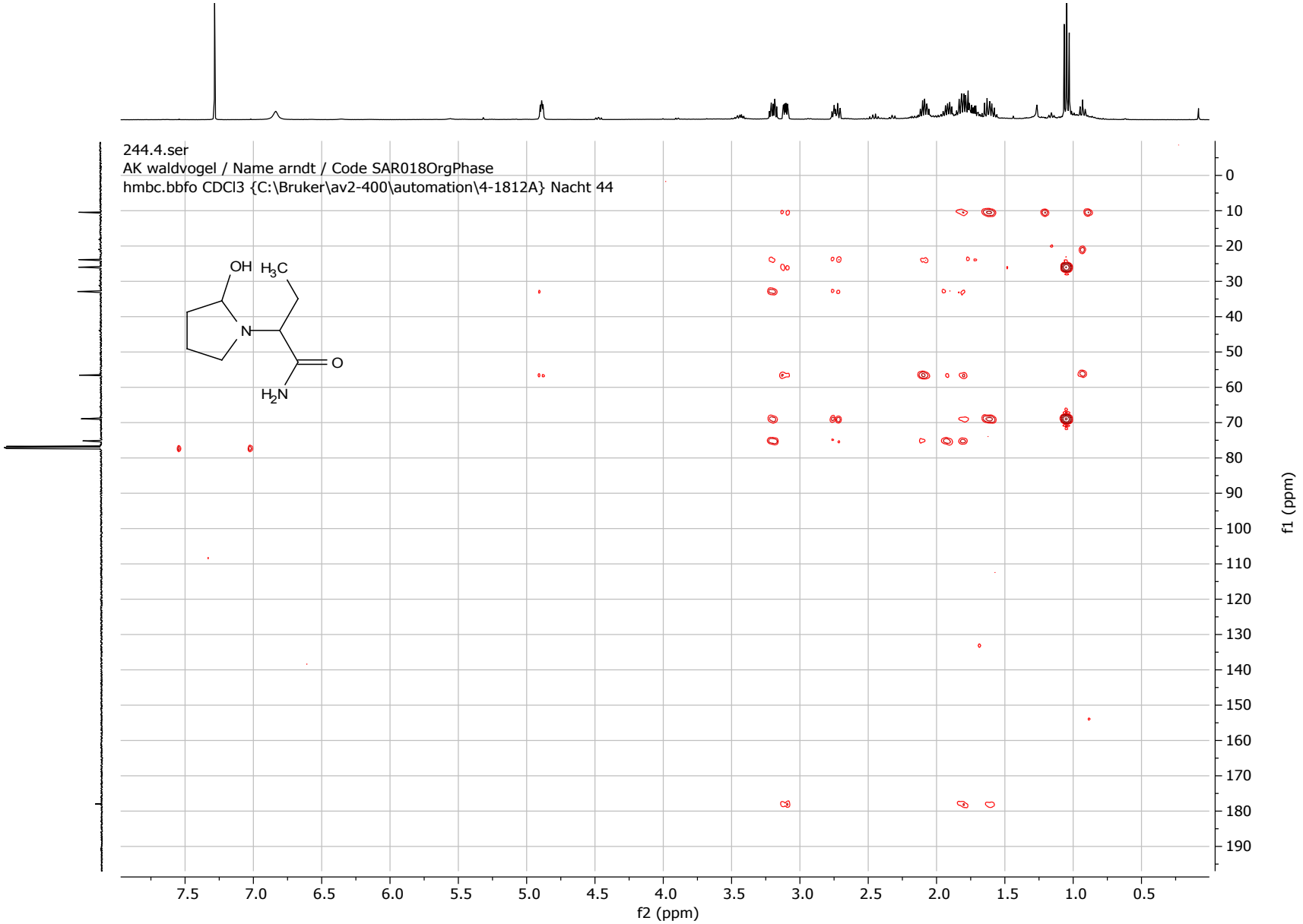
153.2.fid
AK waldvogel / Name arndt / Code SAR018org-Phase
c13_2h.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1812A} Nacht 53

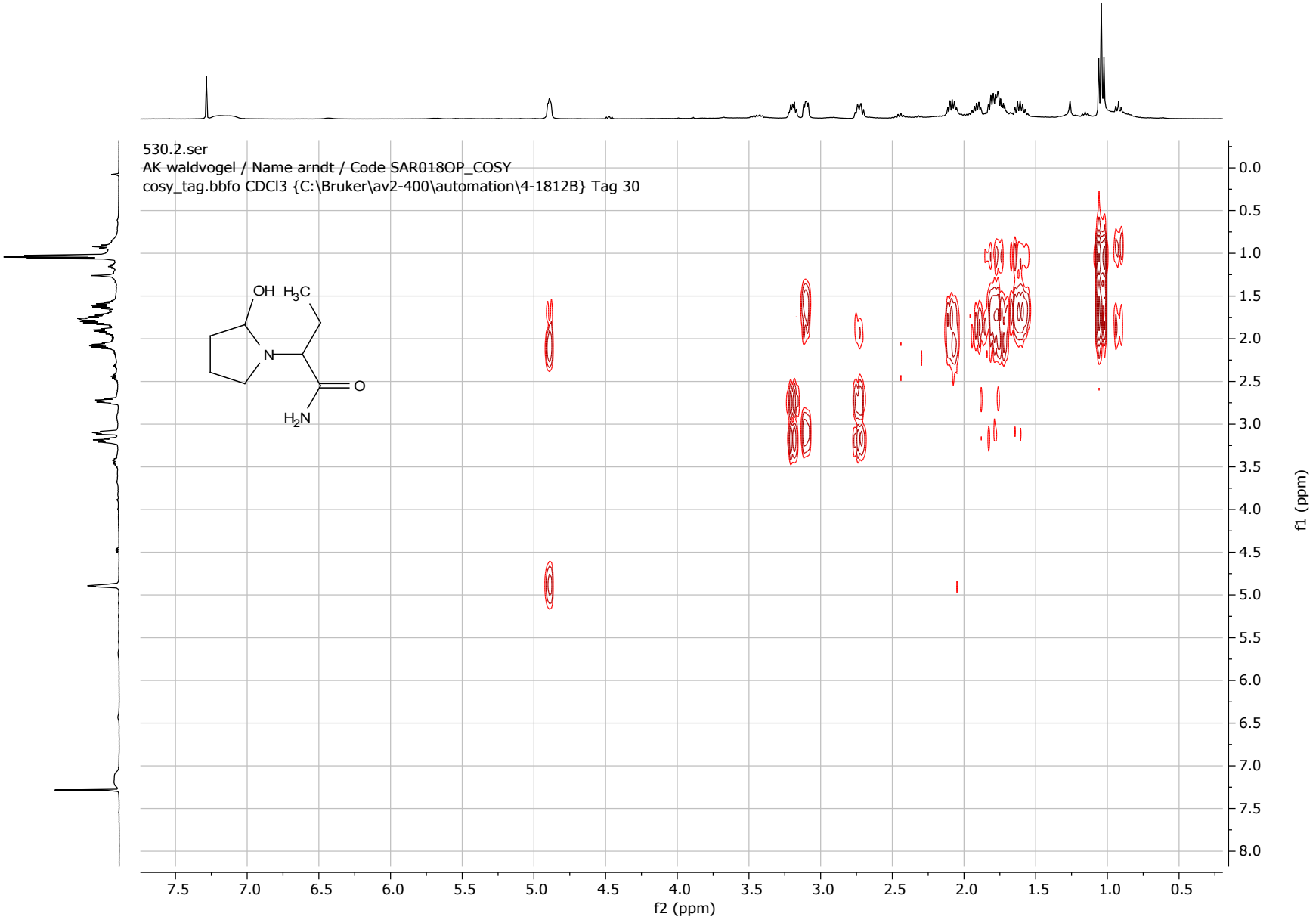


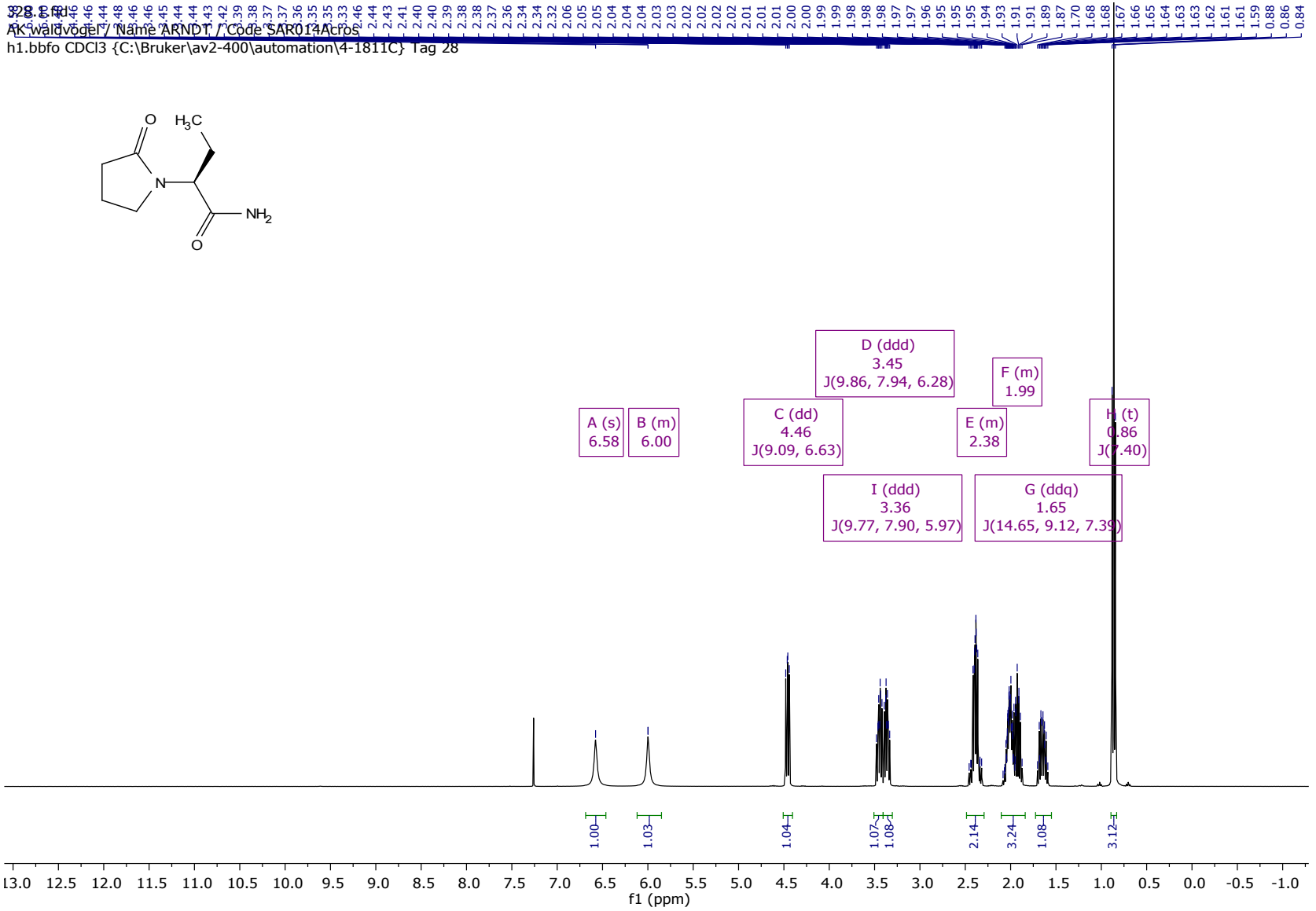
153.3.fid
AK waldvogel / Name arndt / Code SAR018org-Phase
c13_dept135.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1812A} Nacht 53



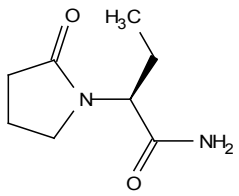

244.3.ser
AK waldvogel / Name arndt / Code SAR018OrgPhase
edhsqc.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1812A} Nacht 44
OH
H3C
N
O
H2N
f2 (ppm)
f1 (ppm)


244.4.ser
AK waldvogel / Name arndt / Code SAR018OrgPhase
hmbc.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1812A} Nacht 44
OH
H3C
N
O
H2N
f2 (ppm)
f1 (ppm)

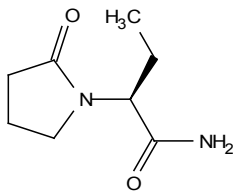

530.2.ser
AK waldvogel / Name arndt / Code SAR018OP_COSY
cosy_tag.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1812B} Tag 30
OH
H3C
N
O
H2N
7.5
7.0
6.5
6.0
5.5
5.0
4.5
4.0
3.5
3.0
2.5
2.0
1.5
1.0
0.5
f2 (ppm)
0.0
0.5
1.0
1.5
2.0
2.5
3.0
3.5
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
f1 (ppm)

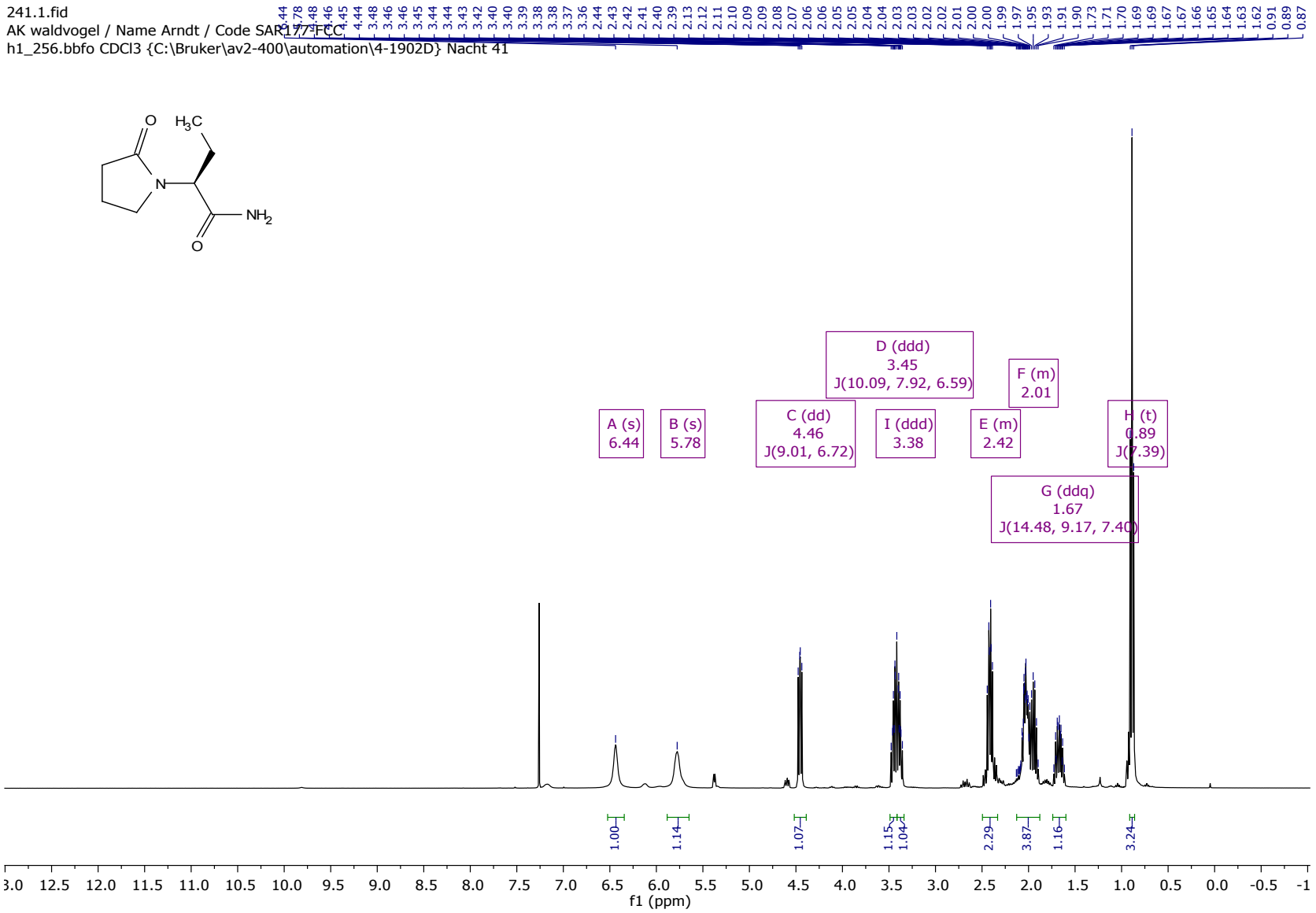

AK_waldvogel / Name ARNDT / Code SAR014Acros
h1.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1811C} Tag 28
O
H3C
N
NH2
O
A (s)
6.58
B (m)
6.00
C (dd)
4.46
J(9.09, 6.63)
D (ddd)
3.45
J(9.86, 7.94, 6.28)
E (m)
2.38
F (m)
1.99
I (ddd)
3.36
J(9.77, 7.90, 5.97)
G (ddq)
1.65
J(14.65, 9.12, 7.39)
1.00
1.03
1.04
1.07
1.08
2.14
3.24
1.08
3.12
f1 (ppm)

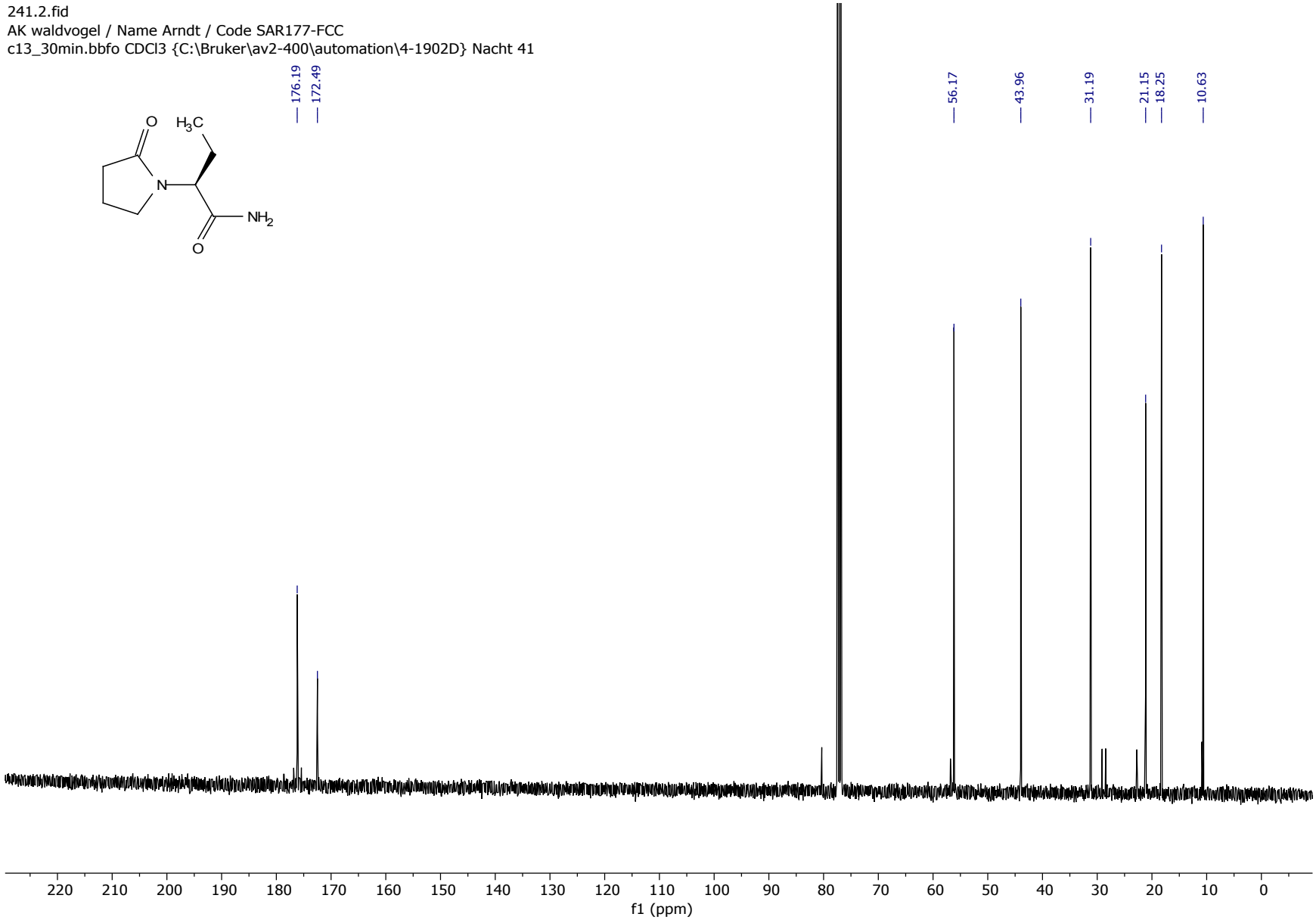
248.2.fid
AK waldvogel / Name ARNDT / Code SAR013-Acros
c13_30min.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1811C} Nacht 48



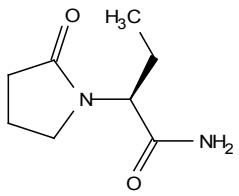
248.3.fid
AK waldvogel / Name Arndt / Code SAR013-Acros
c13_dept135.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1811C} Nacht 48

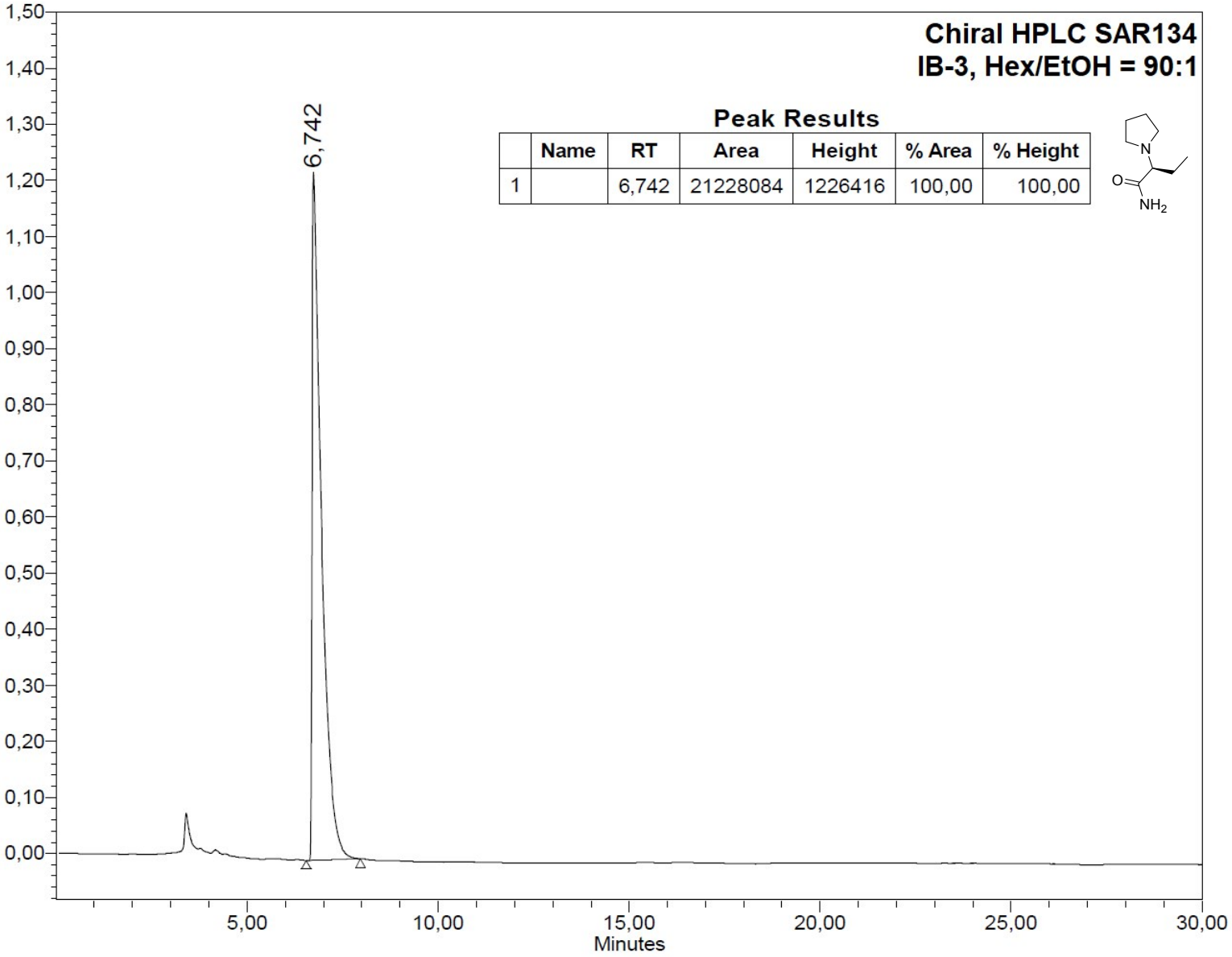


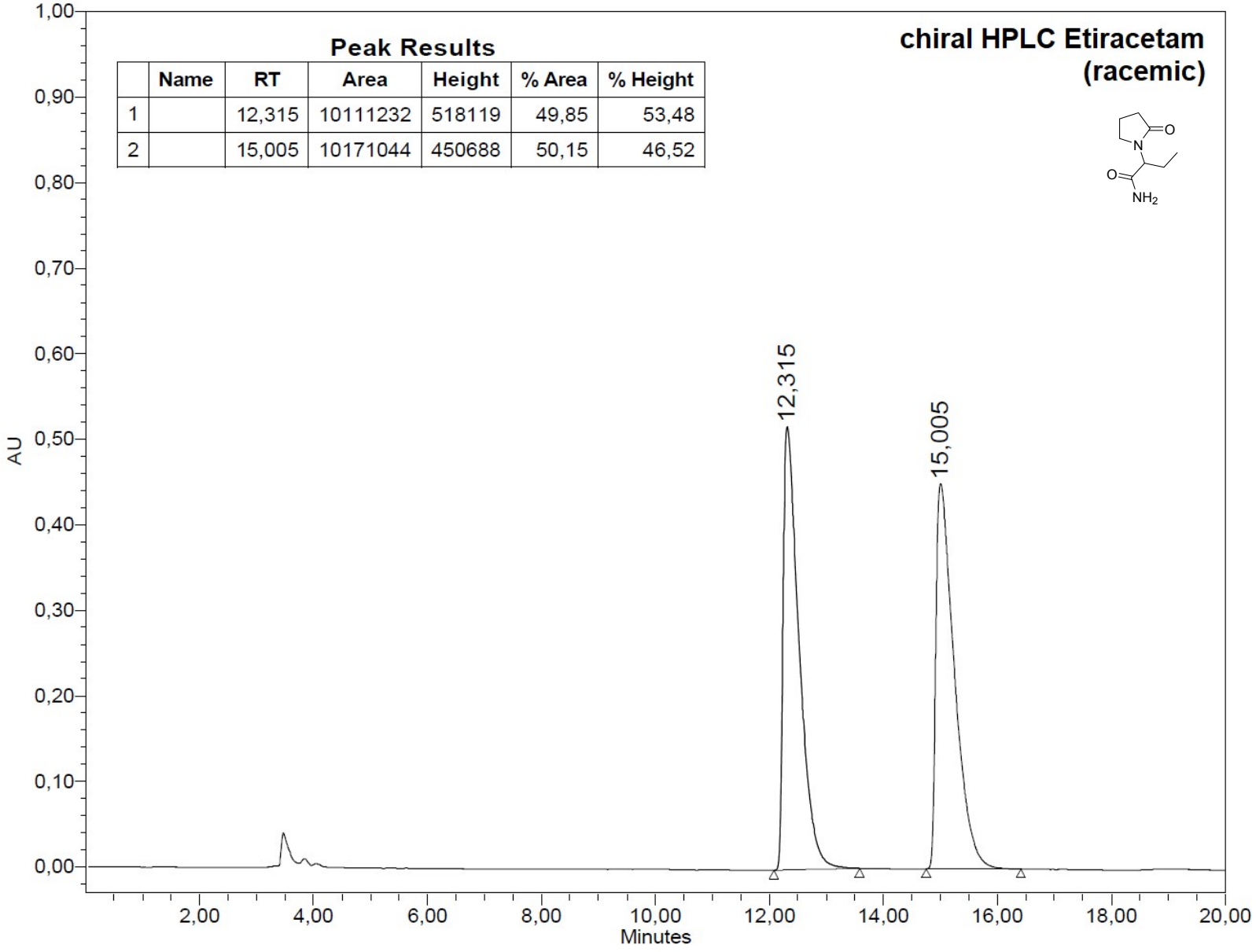

241.1.fid
AK waldvogel / Name Arndt / Code SAR177-FCC
h1_256.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1902D} Nacht 41
O
H3C
N
NH2
O
A (s)
6.44
B (s)
5.78
C (dd)
4.46
J(9.01, 6.72)
D (ddd)
3.45
J(10.09, 7.92, 6.59)
I (ddd)
3.38
E (m)
2.42
F (m)
2.01
G (ddq)
1.67
J(14.48, 9.17, 7.40)
H (t)
0.89
J(7.39)
1.00
1.14
1.07
1.15
1.04
2.29
3.87
1.16
3.24
f1 (ppm)

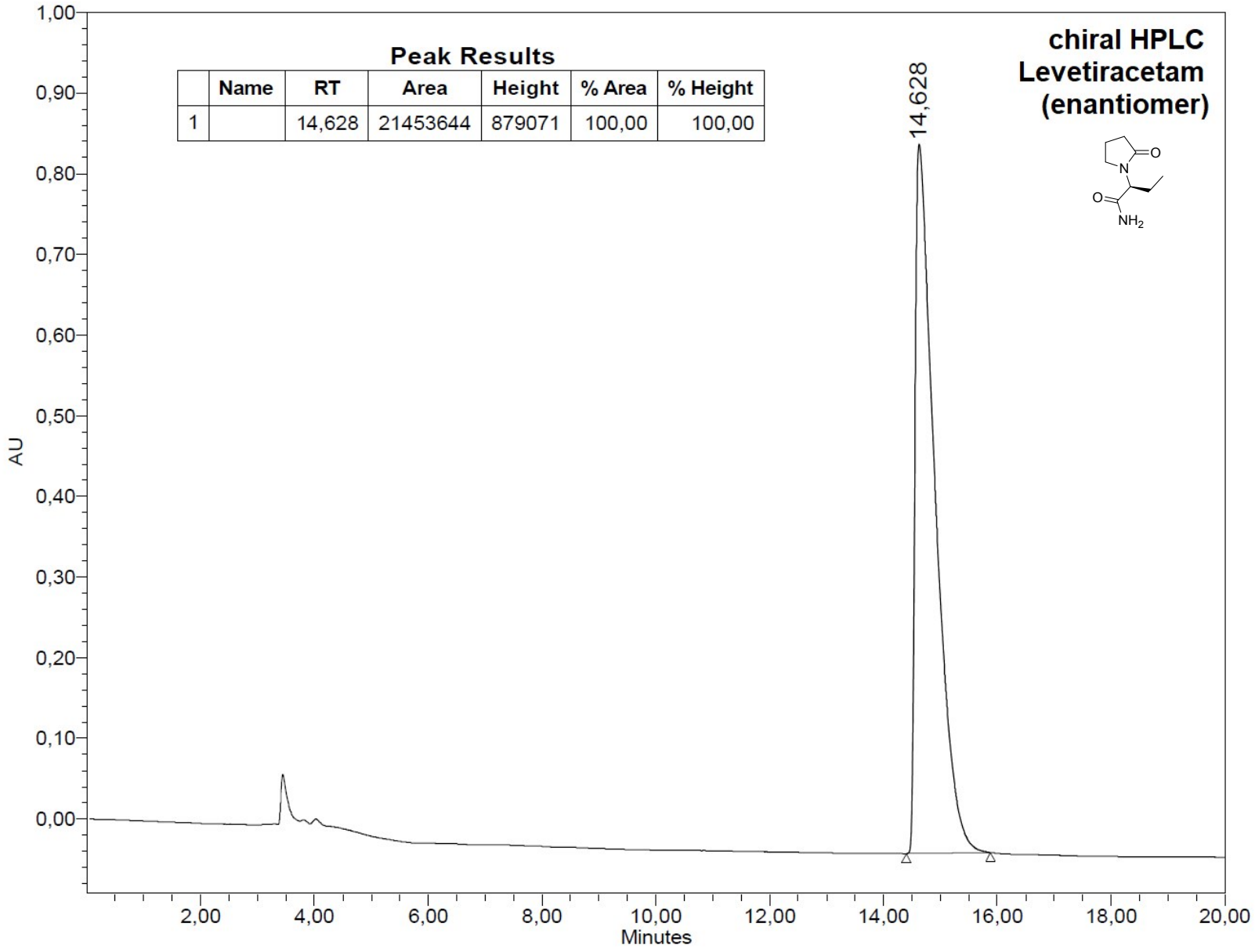

241.2.fid
AK waldvogel / Name Arndt / Code SAR177-FCC
c13_30min.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1902D} Nacht 41
176.19
172.49
56.17
43.96
31.19
21.15
18.25
10.63
O
H3C
N
NH2
O
220
210
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
40
30
20
10
0
f1 (ppm)

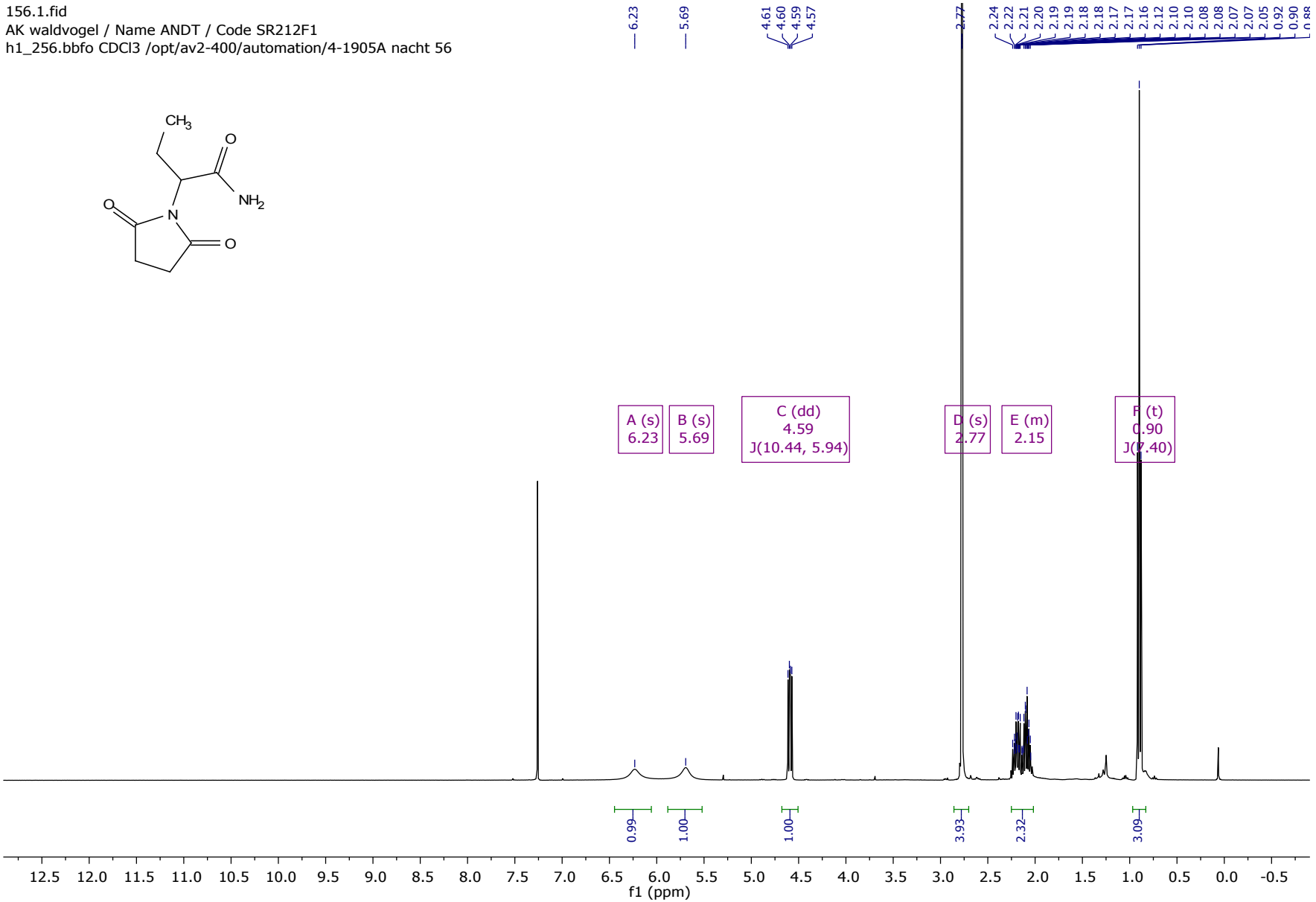
241.3.fid
AK waldvogel / Name ARNDT / Code SAR177-FCC
c13_dept135.bbfo CDCl3 {C:\Bruker\av2-400\automation\4-1902D} Nacht 41



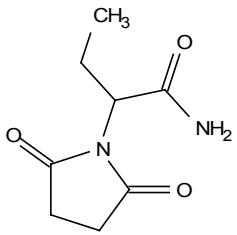

Chiral HPLC SAR134
IB-3, Hex/EtOH = 90:1
Peak Results
Name RT Area Height % Area % Height
1 6,742 21228084 1226416 100,00 100,00
6,742
1,50
1,40
1,30
1,20
1,10
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
5,00
10,00
15,00
20,00
25,00
30,00
Minutes


chiral HPLC Etiracetam
(racemic)
Peak Results
Name | RT | Area | Height | % Area | % Height
1 | | 12,315 | 10111232 | 518119 | 49,85 | 53,48
2 | | 15,005 | 10171044 | 450688 | 50,15 | 46,52
12,315
15,005
AU
Minutes
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
2,00
4,00
6,00
8,00
10,00
12,00
14,00
16,00
18,00
20,00

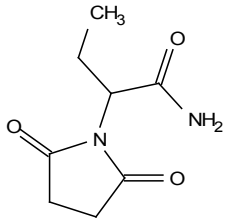

Peak Results
Name | RT | Area | Height | % Area | % Height
1 | | 14,628 | 21453644 | 879071 | 100,00 | 100,00
14,628
chiral HPLC
Levetiracetam
(enantiomer)
AU
Minutes
1,00
0,90
0,80
0,70
0,60
0,50
0,40
0,30
0,20
0,10
0,00
2,00
4,00
6,00
8,00
10,00
12,00
14,00
16,00
18,00
20,00

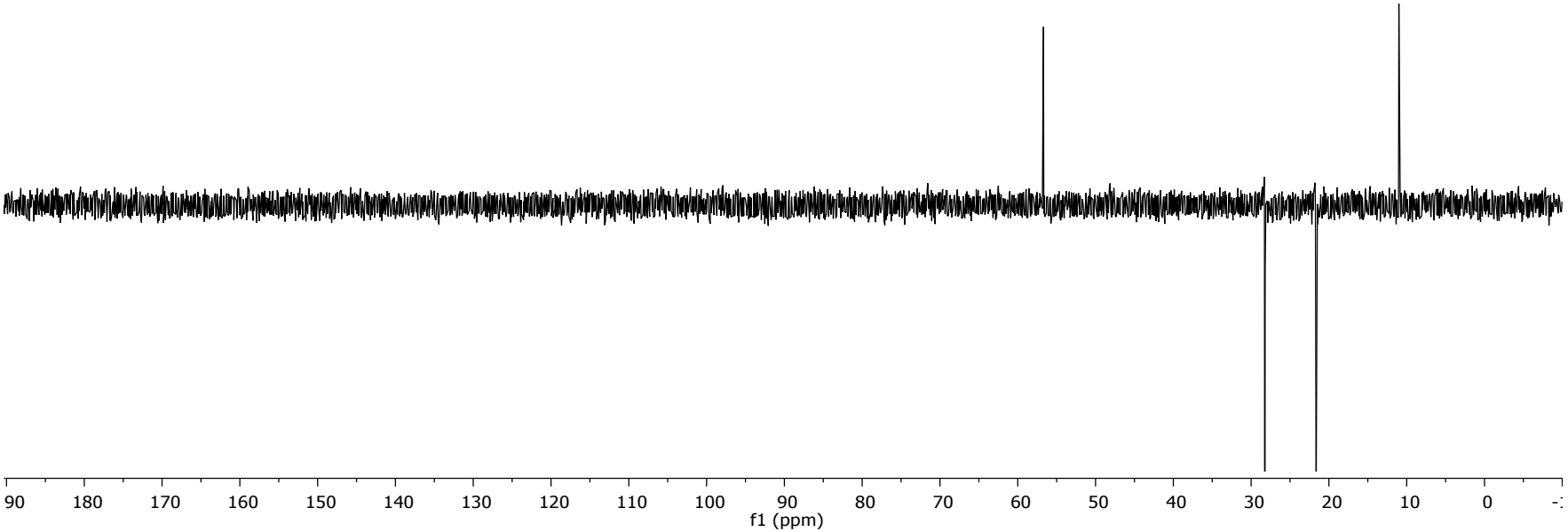

156.1.fid
AK waldvogel / Name ANDT / Code SR212F1
h1_256.bbfo CDCl3 /opt/av2-400/automation/4-1905A nacht 56
CH3
O
NH2
O
N
O
6.23
5.69
4.61
4.60
4.59
4.57
2.77
2.24
2.22
2.21
2.20
2.19
2.19
2.18
2.18
2.17
2.17
2.16
2.12
2.10
2.10
2.08
2.08
2.07
2.07
2.05
0.92
0.90
A (s)
6.23
B (s)
5.69
C (dd)
4.59
J(10.44, 5.94)
D (s)
2.77
E (m)
2.15
F (t)
0.90
J(7.40)
0.99
1.00
1.00
3.93
2.32
3.09
12.5 12.0 11.5 11.0 10.5 10.0 9.5 9.0 8.5 8.0 7.5 7.0 6.5 6.0 5.5 5.0 4.5 4.0 3.5 3.0 2.5 2.0 1.5 1.0 0.5 0.0 -0.5
f1 (ppm)

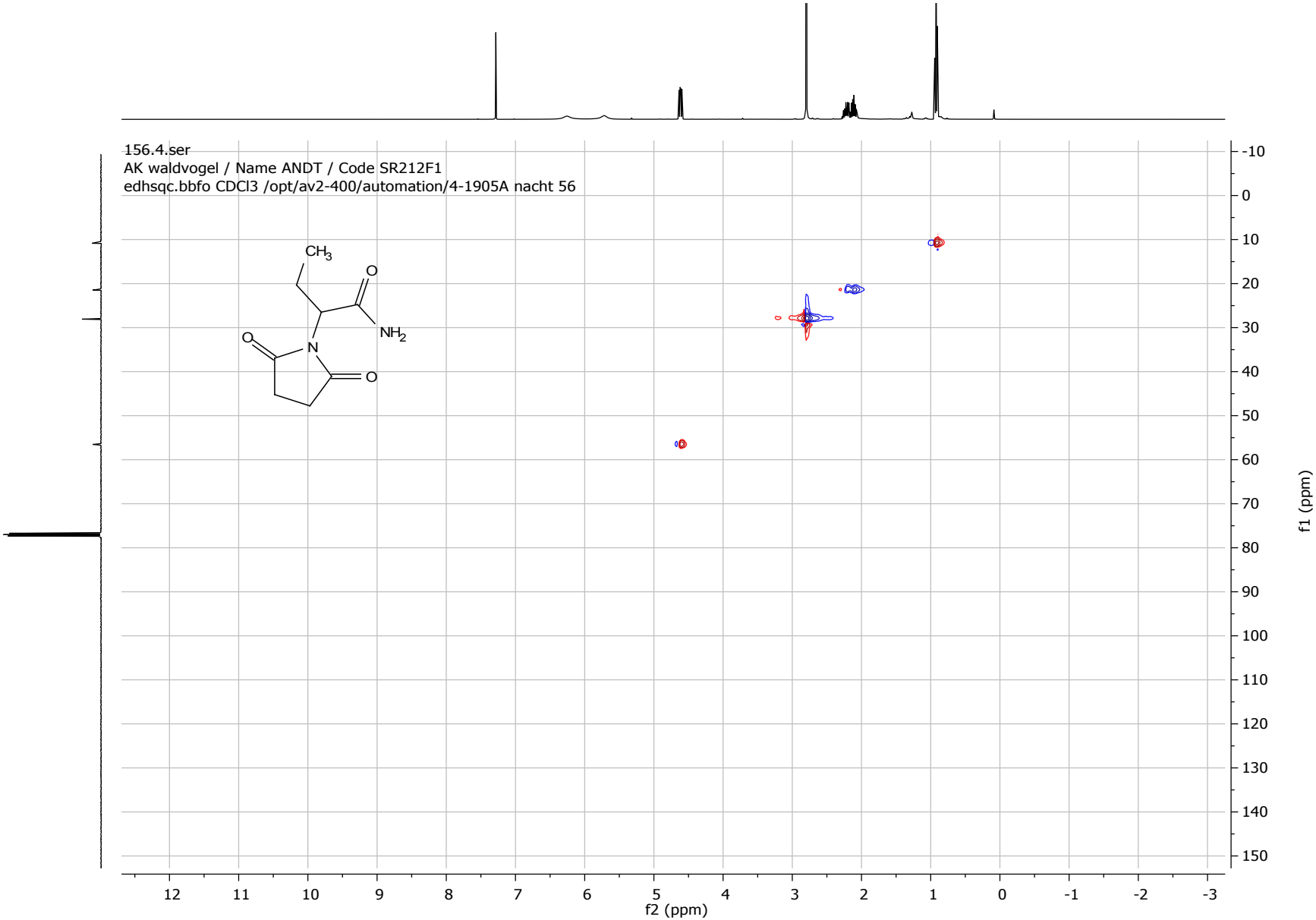
156.2.fid
AK waldvogel / Name ANDT / Code SR212F1
c13_2h.bbfo CDCl3 /opt/av2-400/automation/4-1905A nacht 56

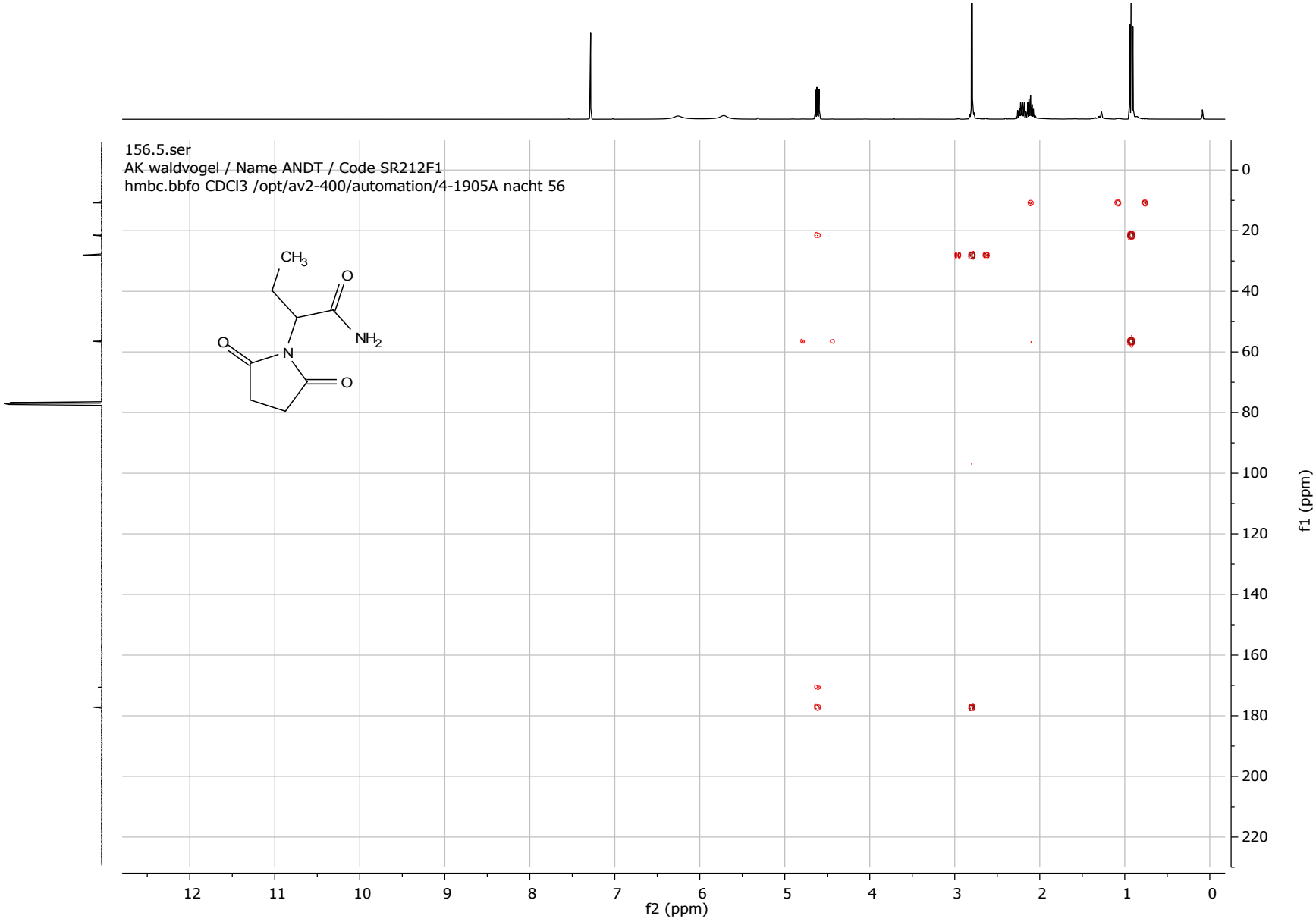


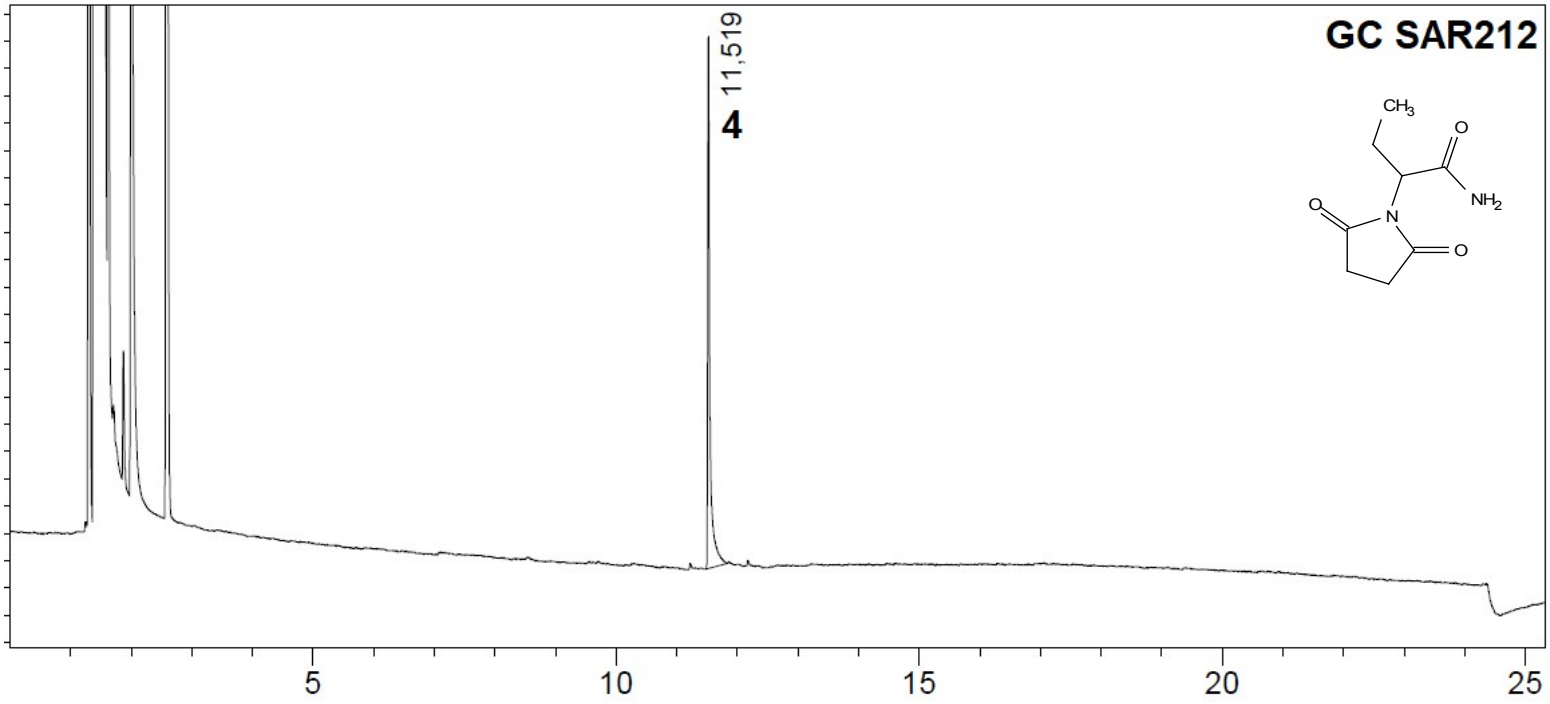
156.3.fid
AK waldvogel / Name ANDT / Code SR212F1
c13_dept135.bbfo CDCl3 /opt/av2-400/automation/4-1905A nacht 56

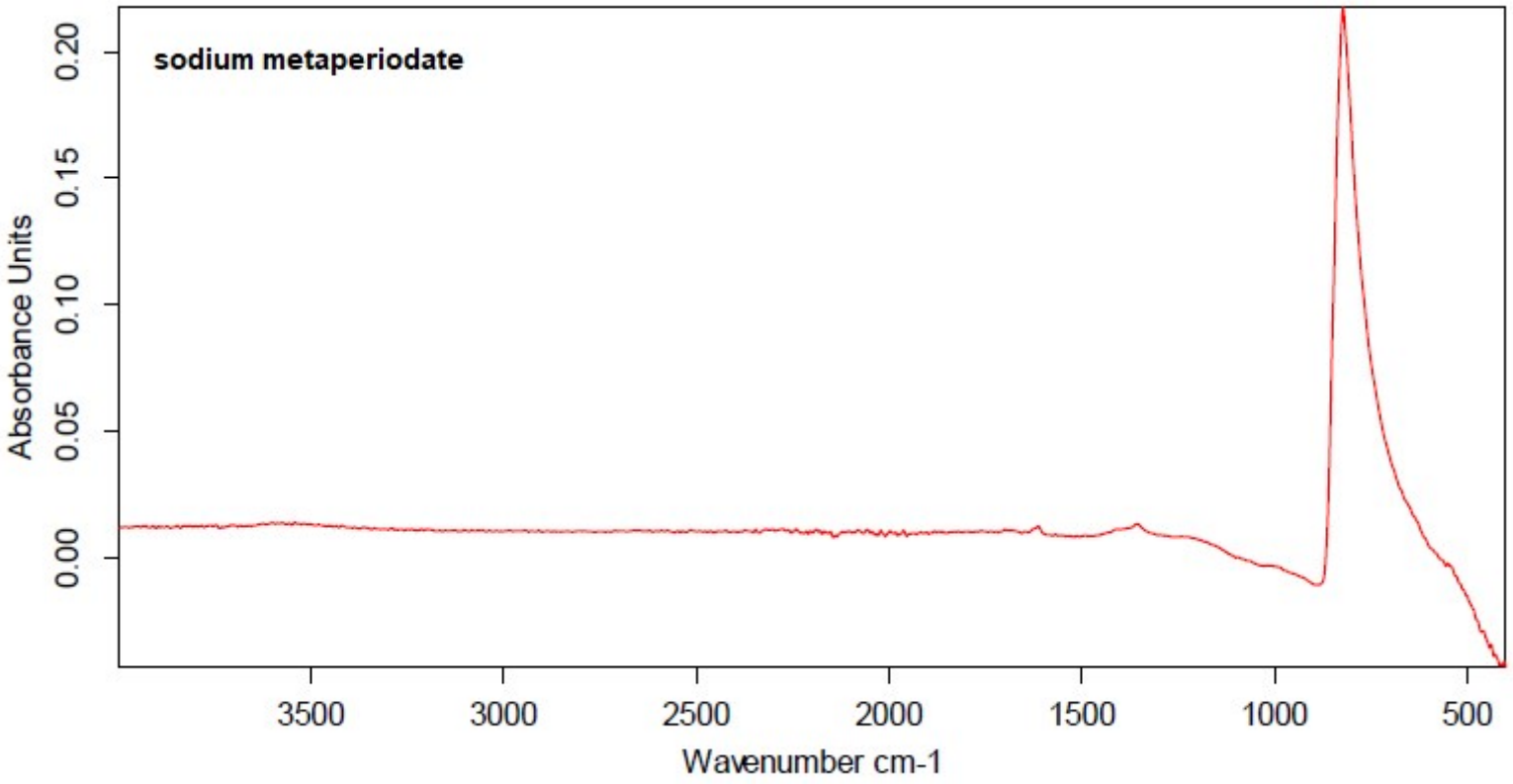





156.4.ser
AK waldvogel / Name ANDT / Code SR212F1
edhsqc.bbfo CDCl3 /opt/av2-400/automation/4-1905A nacht 56
CH3
O
NH2
O
N
O
f2 (ppm)
f1 (ppm)


156.5.ser
AK waldvogel / Name ANDT / Code SR212F1
hmbc.bbfo CDCl3 /opt/av2-400/automation/4-1905A nacht 56
CH3
O
NH2
O
N
O
f2 (ppm)
f1 (ppm)
12
11
10
9
8
7
6
5
4
3
2
1
0
0
20
40
60
80
100
120
140
160
180
200
220


GC SAR212
11,519
4
CH3
O
NH2
N
O
O
5
10
15
20
25


sodium metaperiodate
Absorbance Units
0.00
0.05
0.10
0.15
0.20
3500
3000
2500
2000
1500
1000
500
Wavenumber cm-1

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[18] Infrared spectral data were obtained from the Bio-Rad/Sadtler IR Data Collection, Bio-Rad Laboratories, Philadelphia, PA (US) and can be found under https://spectrabase.com. Spectrum ID (metaperiodate): 3ZPsHGmepSu.