Synthesis, absolute configuration and in vitro cytotoxicity of deschloroketamine enantiomers: Rediscovered and abused dissociative anesthetic

*Bronislav Jurásek,[1,2‡] František Králík,[3‡] Silvie Rimpelová,[4] Jan Čejka,[5] Vladimír Setnička,[3] Tomáš Ruml,[4] Martin Kuchař,[1,2] Michal Kohout[6]**

[1]Forensic Laboratory of Biologically Active Substances, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czech Republic

[2]Department of Chemistry of Natural Compounds, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czech Republic

[3]Department of Analytical Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czech Republic

[4]Department of Biochemistry and Microbiology, University of Chemistry and Technology Prague, Technická 3, 166 28, Prague 6, Czech Republic

[5]Department of solid state chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czech Republic

[6]Department of Organic Chemistry, University of Chemistry and Technology Prague, Technická 5, 166 28, Prague 6, Czech Republic

Electronic supplementary information

This document contains supplementary information on the synthesis of deschloroketamine and its enantioseparation using analytical and preparative instrumentation. Further information on spectroscopic assignment of the absolute configuration of deschloroketamine enantiomers is also included.

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1. Enantioseparation of racemic deschloroketamine

Deschloroketamine was originally proposed for clinical use in the racemic form, therefore, there is no information on its enantioseparation available in the patent literature. Recent studies focused on its characterization also neglected the fact that the substance exists in two enantiomeric forms. Thus, some estimations of the chromatographic behavior of the substance had to be performed before proceeding with the enantioseparation.

Generally, deschloroketamine is β-ketoamine, thus, it has a similar structure to cathinones - another type of new psychoactive substances. There are several reports on chiral resolution of cathinones on various types of chiral stationary phases ranging from polysaccharide over crown ethers to chiral ion exchangers.[S1-3] The highest efficiency in the enantioseparation of new chiral substances is usually achieved with polysaccharide chiral columns. Therefore, we decided to use amylose-based column, which is available in preparative size in our laboratory and optimize the chromatographic conditions for this chiral column.

Racemic DCK hydrochloride subjected to enantioseparation was well soluble only in low molecular mass alcohols. However, the enantiomer separation under such polar organic mode conditions on analytical amylose-based column (ChiralArt Amylose-SA) was not efficient. Since much higher success rate of enantioseparation on polysaccharide chiral stationary phases is generally achieved in normal mobile phase mode (mixture of alkanes with alcohols),[S4] we decided to transform DCK hydrochloride into a free base.

The samples were prepared in the following way: DCK hydrochloride (10 mg) was suspended in propan-2-ol (500 μl), diethylamine (10 μl) was added and the suspension was shaken until it cleared. Then, a mixture of heptane/propan-2-ol (9/1) was added, the achieved solution was filtered through a syringe filter (0.43 μm pores) and used for DCK resolution.

For the preparative method development, we used a screening technique proposed by the column manufacturer and started with hexane/propan-2-ol (9/1) mixture. First, mobile phase without any additives was employed. No enantiomer resolution was achieved with this first choice mobile phase. Other tested mobile phase compositions with hexane as the bulk component and propan-2-ol or ethanol as polar additives were also not successful. Thus, we changed hexane for heptane, as sometimes, there may be a slight change in enantioselectivity connected with higher density of heptane. Indeed, with a mobile phase consisting of heptane/propan-2-ol (9/1) partial resolution of DCK enantiomers was achieved. To reduce non-enantioselective interactions of the basic analyte with the acidic silica support, diethylamine (0.1% in mobile phase) was introduced. This change led to further improvement of peak resolution (Figure S1a). Reducing the amount of propan-2-ol (Figure S1b) and finally to 5%, acceptable base-line resolution was achieved. The optimum preparative method (Figure S1c) was used for the enantioseparation of DCK.

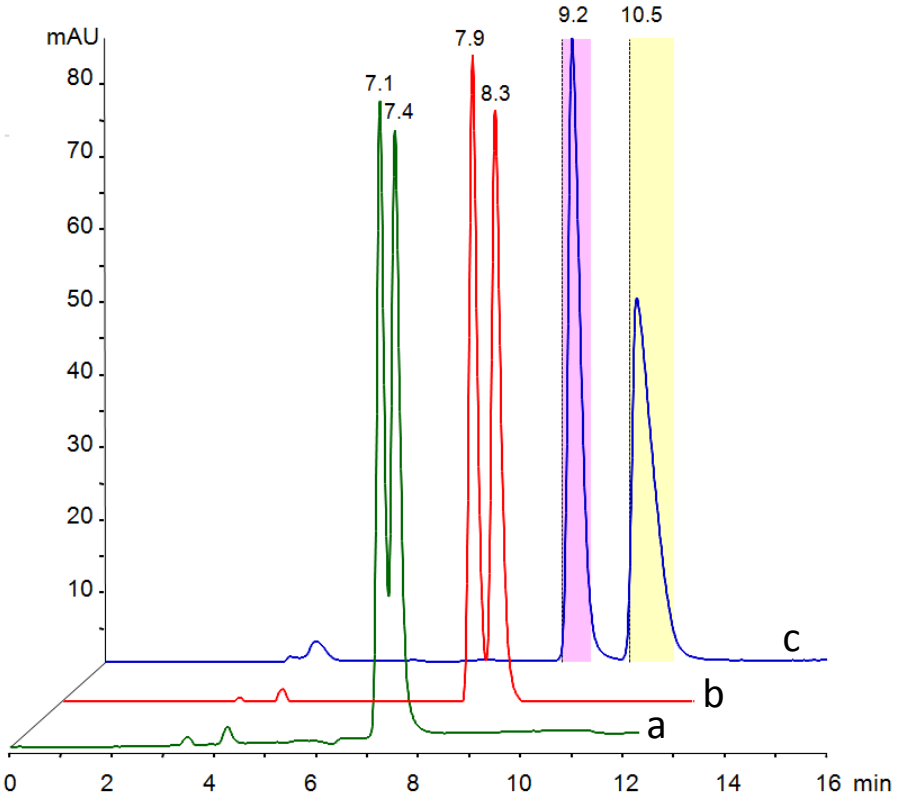


Figure S1. The optimization of enantioseparation of DCK; (a) partial resolution of enantiomers with mobile phase heptane/propan-2-ol (9/1) with 0.1% diethylamine, (b) partial resolution of enantiomers with mobile phase heptane/propan-2-ol (92.5/7.5) containing 0.1% diethylamine, (c) optimum method with the mobile phase composed of heptane/propan-2-ol (95/5), 0.1% diethylamine, flow rate 15 ml/min, injection volume 0.5 mL. Automatic peak collection at the wavelength of 254 nm based on baseline change is colored.

2. Assignment of absolute configuration

3.1 Spectroscopic assignment of absolute configuration

3.1.1 Ab initio calculations

The differences between the two major conformers of model A can be characterized by the value of the dihedral angle α (Figure S2 and Table 1). As it can be seen from the results in Table I, both levels of the theory used in calculations provided similar results.

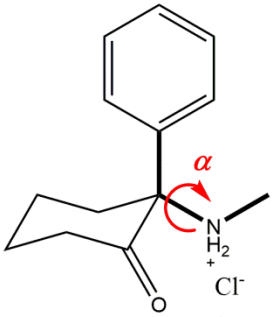


Figure S2. Definition of the dihedral angle α which describes orientation of the $-^{+}NH_2CH_3$ group.

Table 1.

The lowest-energy conformers of DCK, their relative free energies, Boltzmann populations and dihedral angle describing relative orientation of $-NH_2CH_3$ group for the model A.

Model	Conformer	CAM-B3LYP/aug-cc-pVDZ			ωB97X-D/TZVP		
		ΔG [kJ·mol^{-1}]	Population [%]	α [degrees]	ΔG [kJ·mol^{-1}]	Population [%]	α [degrees]
A	(I)	0.0	92.7	-56	0.0	90.0	-52
	(II)	6.4	7.0	47	5.5	9.8	42
	(III)	13.8	0.3	-175	15.9	0.2	-174
B	(I)	25.0	0.0	-178	27.4	0.0	-176
	(II)	28.8	0.0	-49	29.2	0.0	-48
	(III)	32.4	0.0	62	33.0	0.0	69
	(IV)	35.3	0.0	59	35.0	0.0	62

3.1.2 Infrared spectroscopy

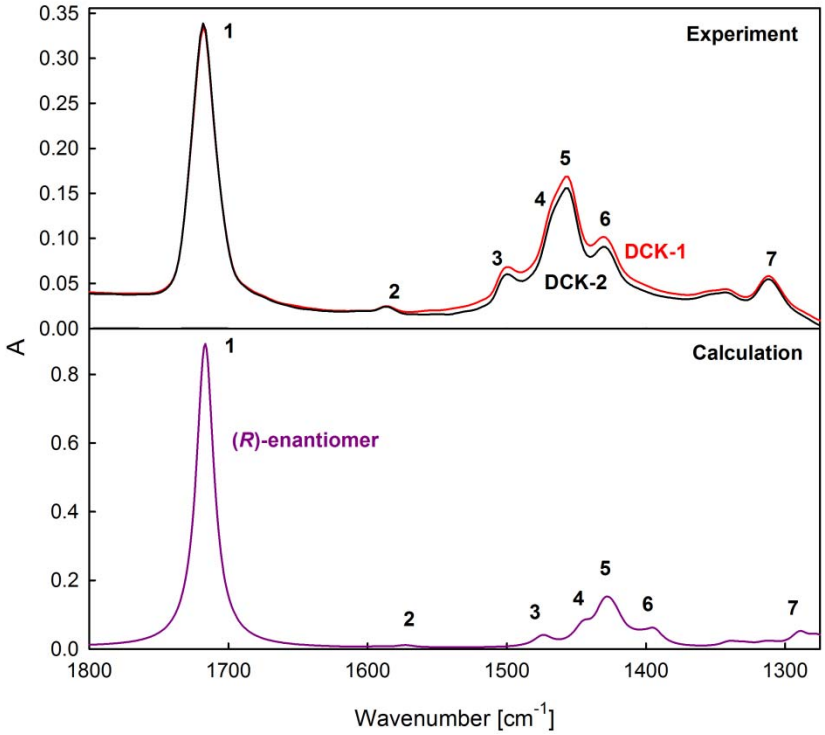


Figure S3. Experimental IR spectra of DCK-1 and DCK-2 samples (top) compared with the calculated spectra for (*R*)-DCK (bottom).

3.1.3 ECD spectroscopy

The assignment of absolute configuration obtained from experimental VCD data and *in silico* simulation of VCD spectra was further reinforced by the match between experimental and *in silico* predicted ECD spectra. In this case, also the major orbital contributions to the experimentally observed ECD bands were visualized (Figure S4).

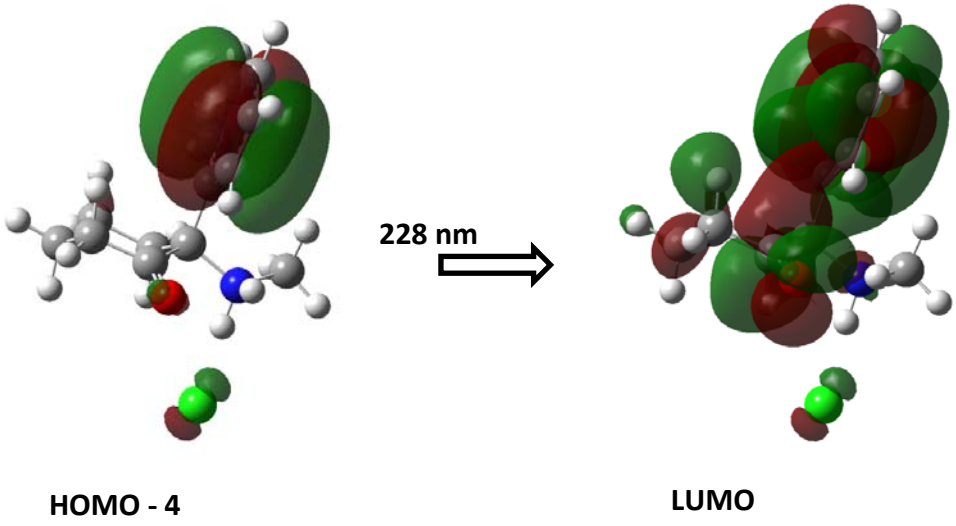
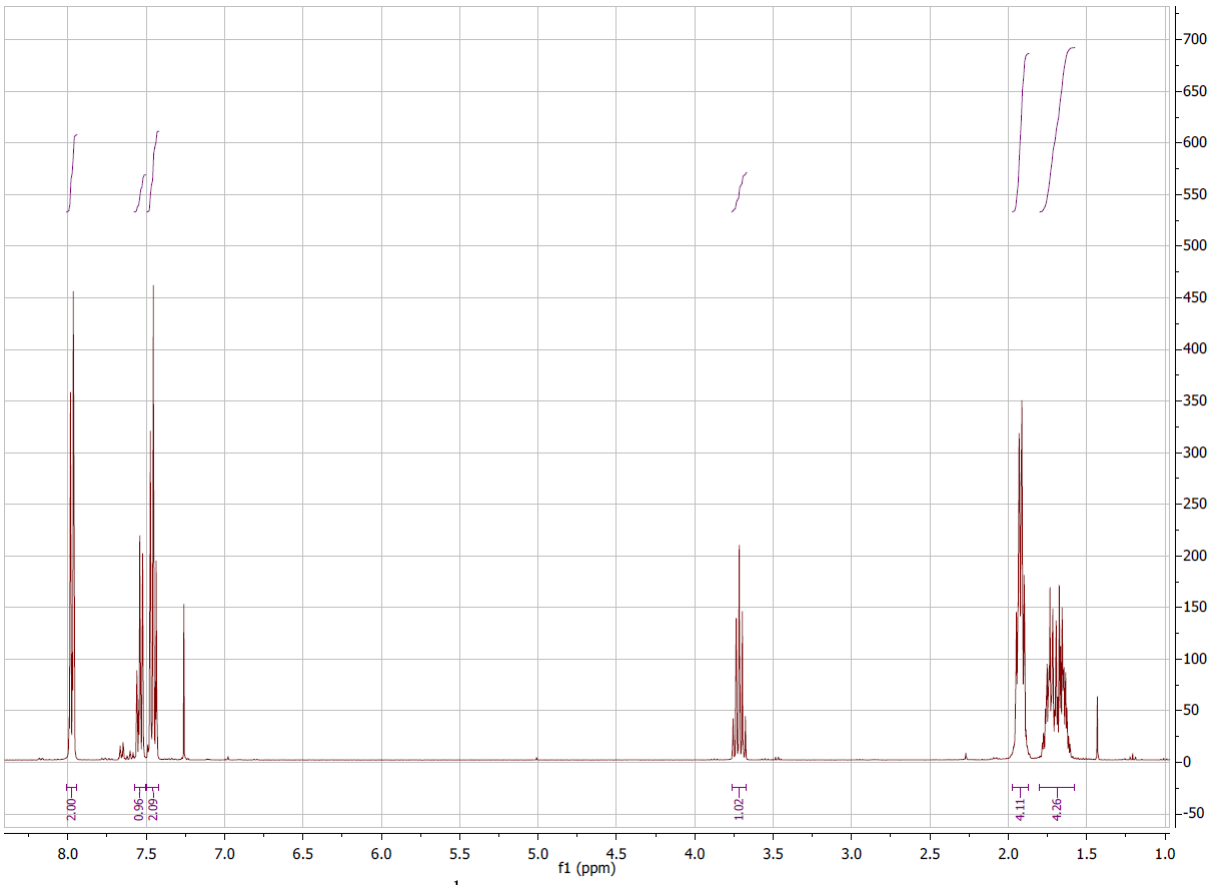


Figure S4. Major orbital contributions to experimentally observed ECD bands.

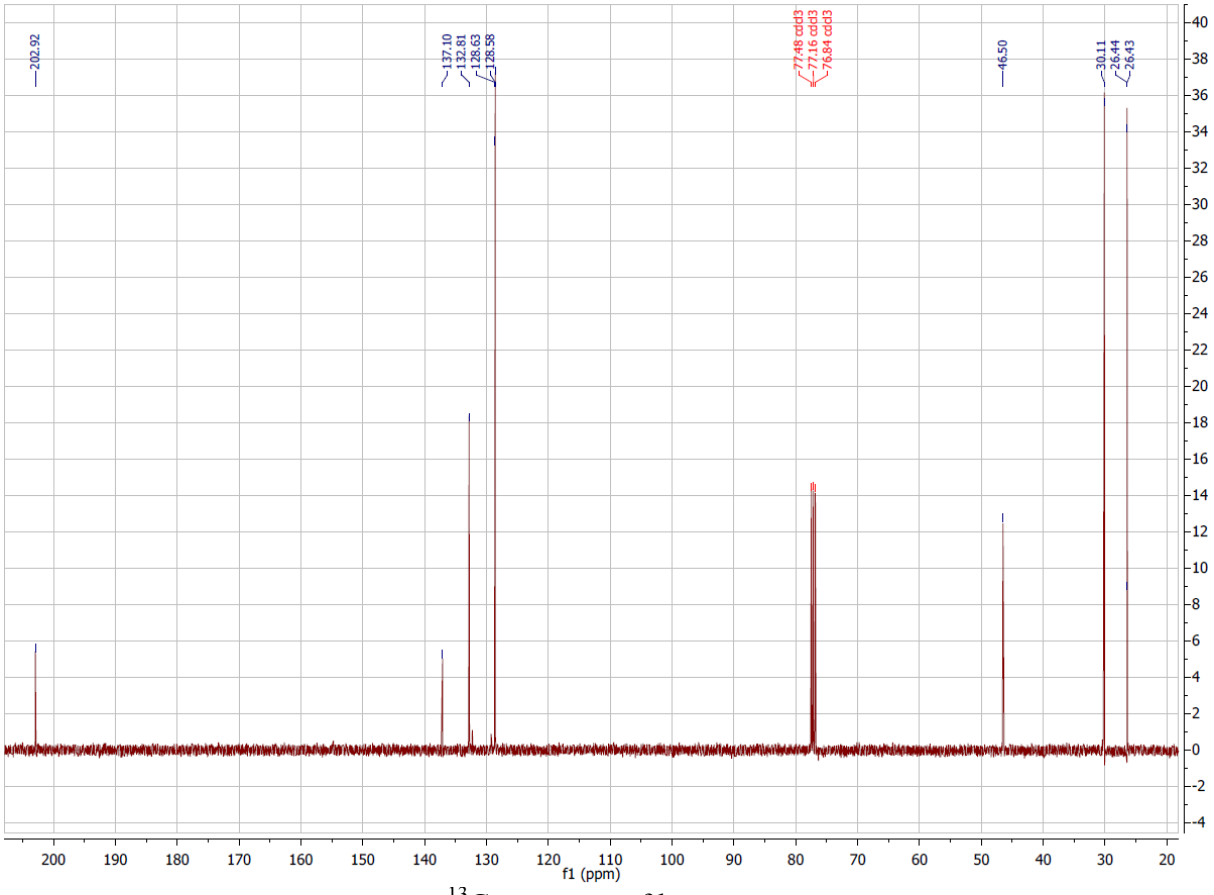
3.2 Crystallographic parameters

Table 2 Crystallographic data and structure refinement parameters for *S*-, *R*- and racemate.

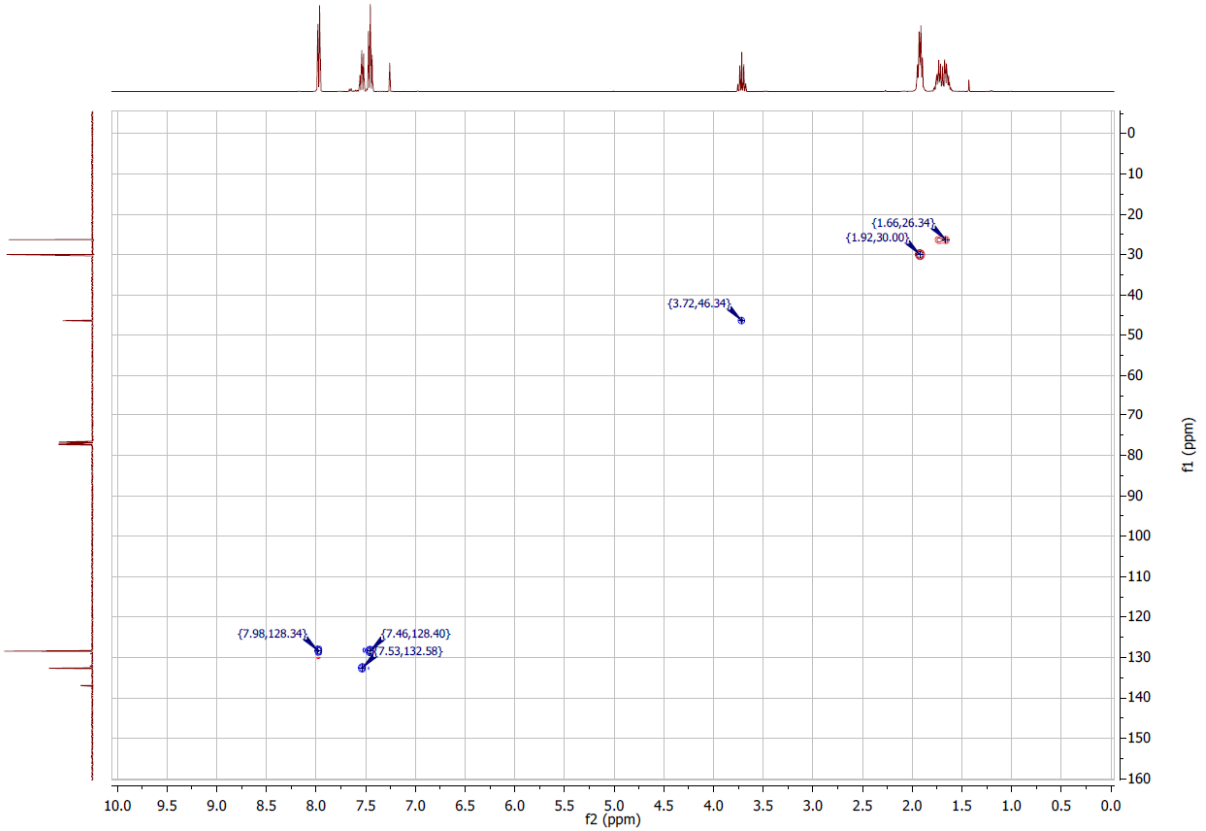
Compound	**S**	**R**	**racemate**
Chemical formula	C_{13} H_{18} Cl N O	C_{13} H_{18} Cl NO	C_{13} H_{18} Cl N O
Formula weight (g•mol^{-1})	239.74	239.74	239.74
Crystal system	monoclinic	monoclinic	monoclinic
Space group	*P* 2_1	*P* 2_1	*P* $2_1/n$
a (Å)	6.5215(3)	6.5245(3)	6.4939(3)
b (Å)	13.1197(5)	13.1240(5)	13.2876(6)
c (Å)	15.0635(6)	15.0650(6)	14.9655(7)
α (°)	90	90	90
β (°)	91.4163(8)	91.4410(10)	92.9591(12)
γ (°)	90	90	90
V (Å^3)	1288.44(5)	1289.57(5)	1289.63(6)
Temperature (K)	180	180	180
Z	4	4	4
Crystal size (mm) min/mid/max	0.374/0.425/0.467	0.368/0.443/0.536	0.185/0.198/0.481
R/*wR* (I/σ(I) > 2)	0.0251/ 0.0676	0.0261/ 0.0689	0.0378/0.0892
Goodness-of-fit	0.9997	0.9997	0.9535
R/*wR*_all refl.	0.0253/ 0.0677	0.0266/ 0.0698	0.0384/0.0895
Flack's *x*	0.095(10)	0.035(8)	-
CCDC deposition No.	1838631	1838632	1838630



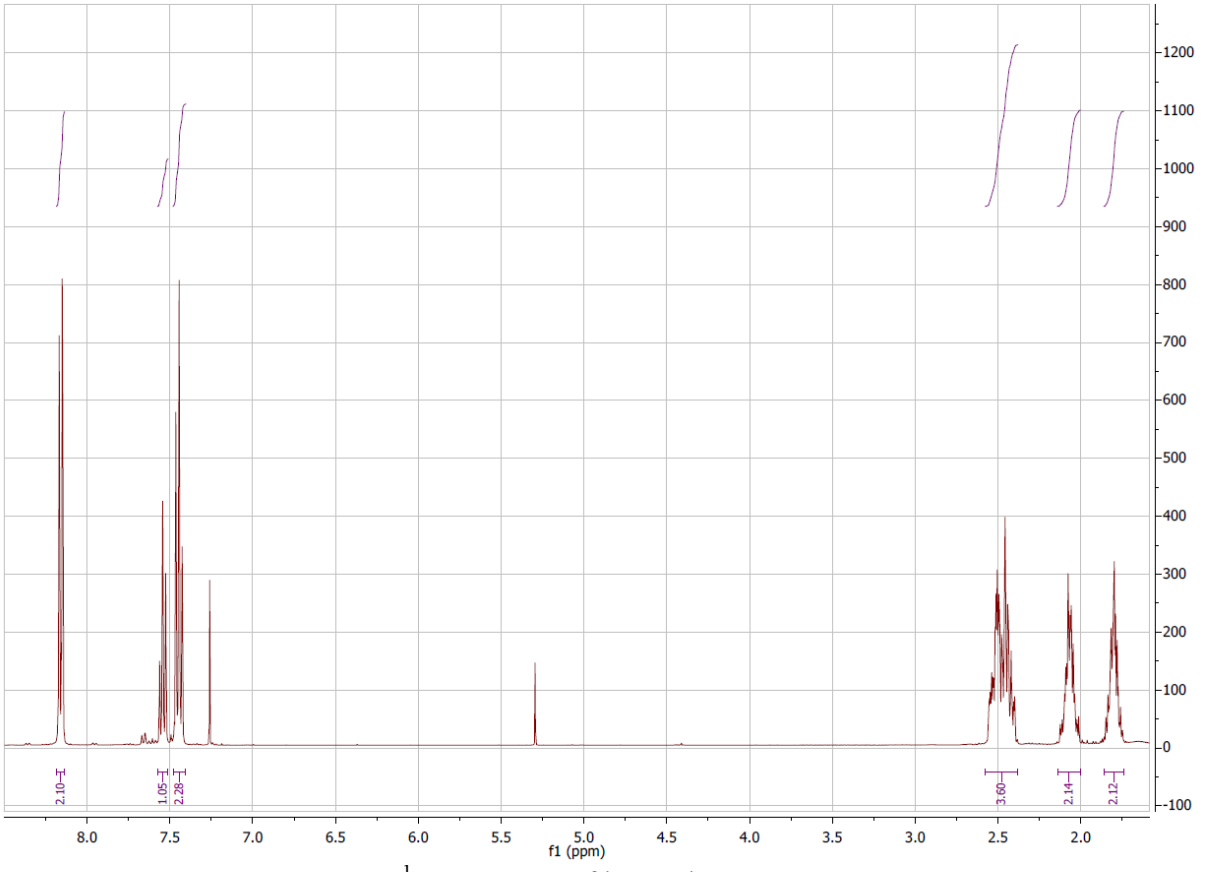
^{1}H spectrum of ketone **7**



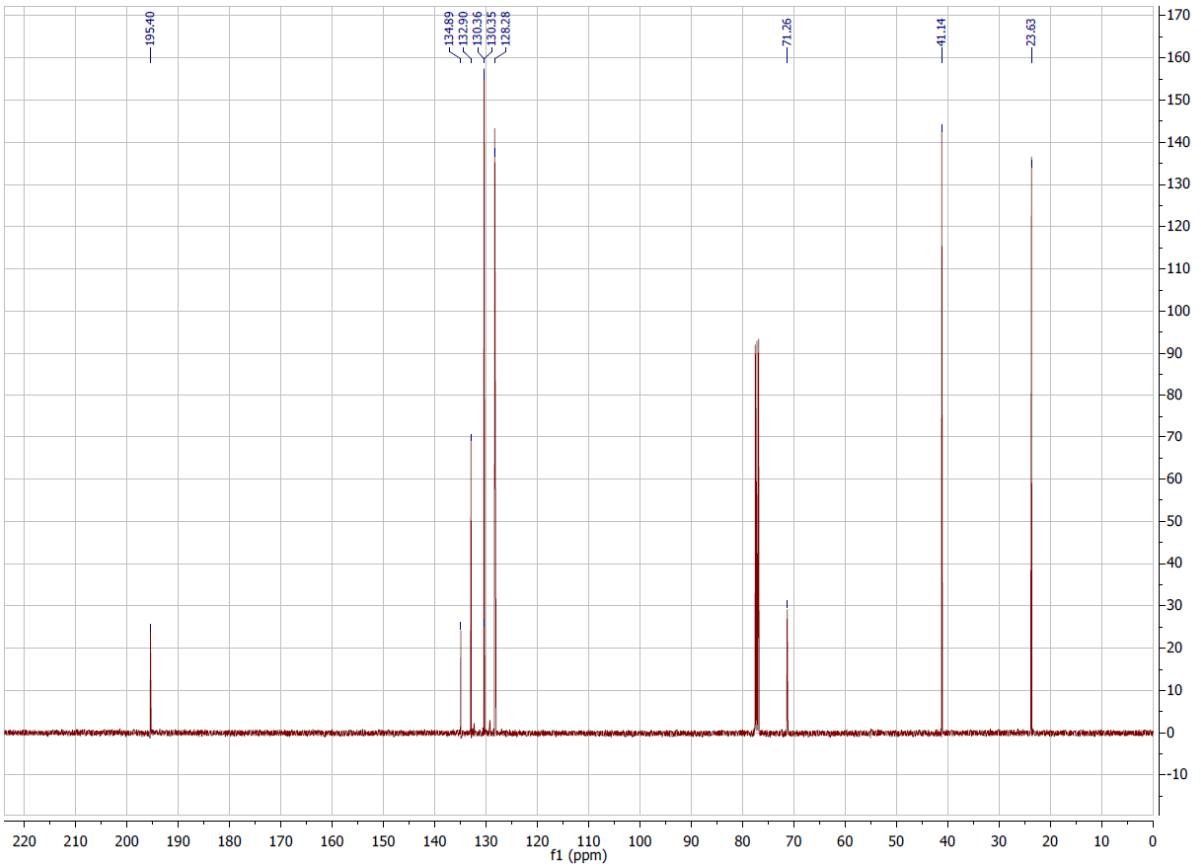
^{13}C spectrum of ketone **7**



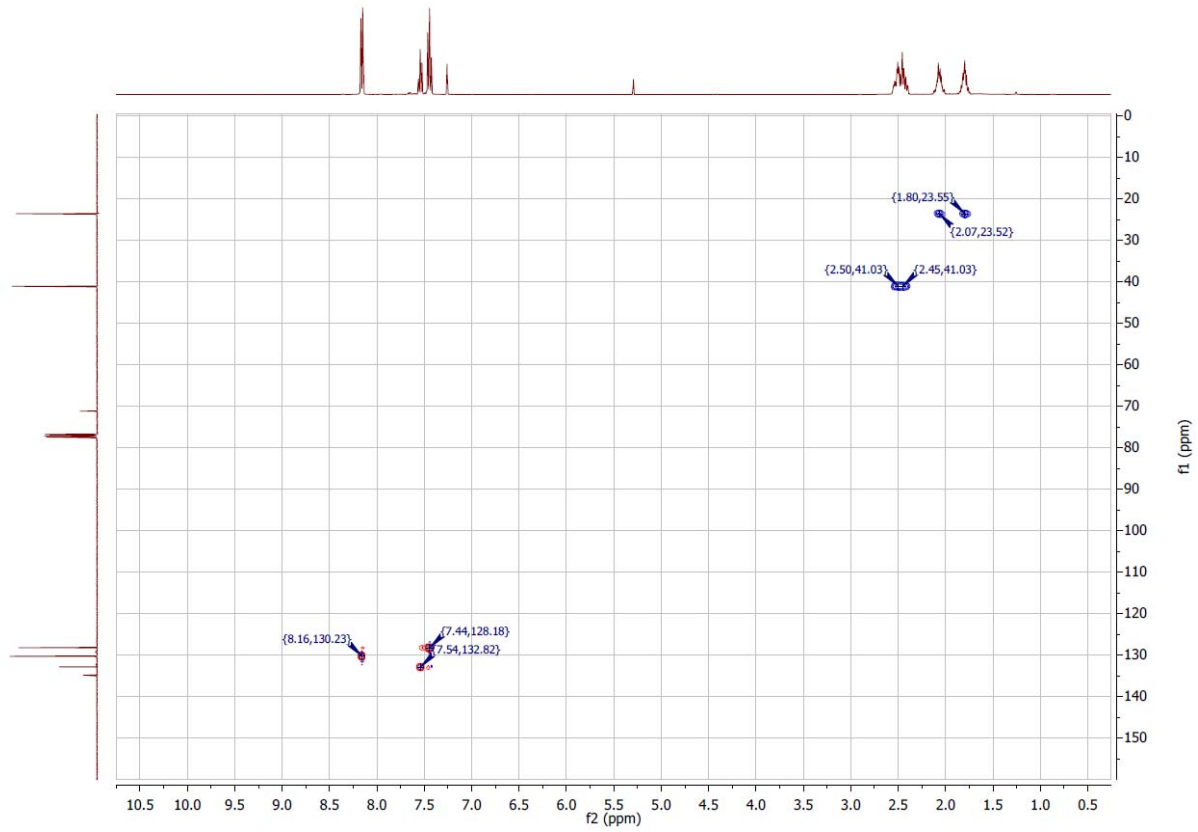
HSQC spectrum of ketone **7**



^{1}H spectrum of bromoketone **8**



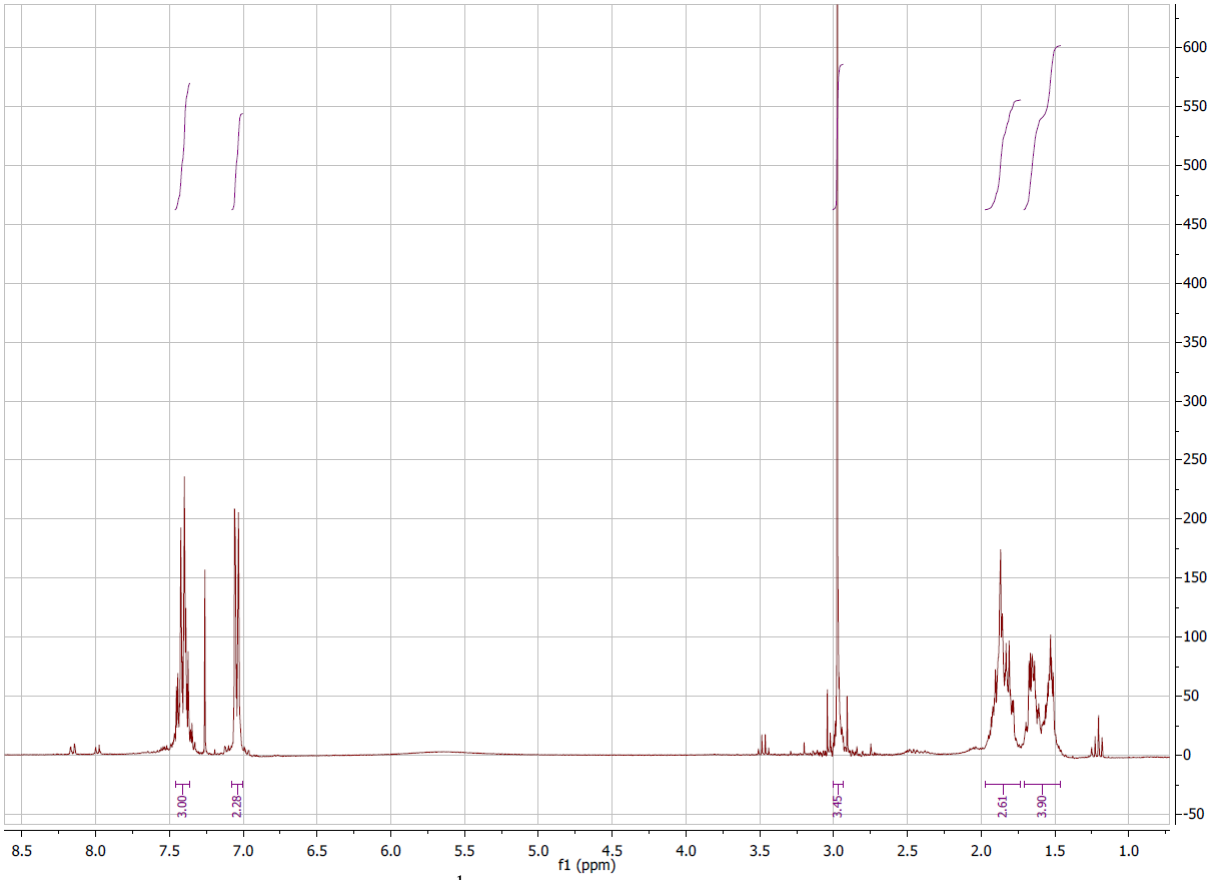
^{13}C spectrum of bromoketone **8**



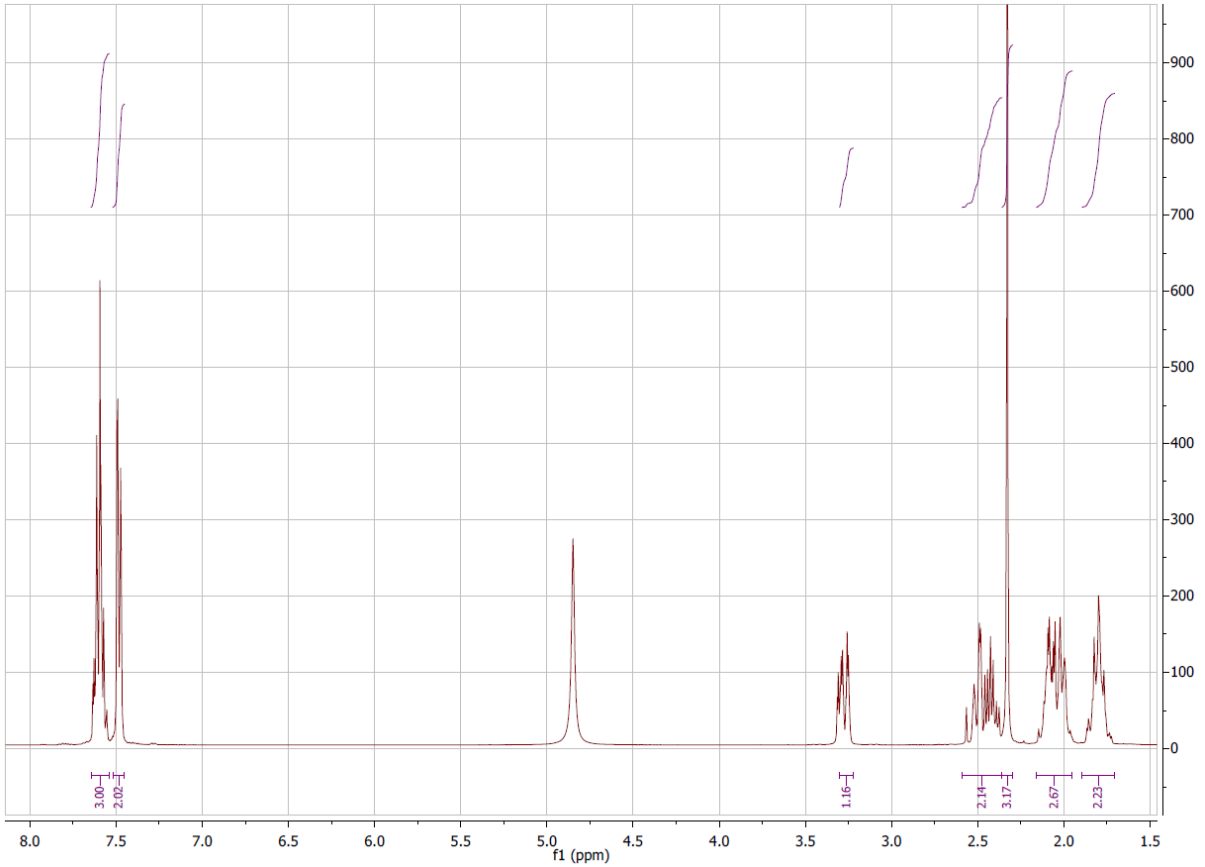
HSQC spectrum of bromoketone **8**

Since the imine **9** is highly unstable and therefore only the ^{1}H NMR spectrum was recorded (see below).

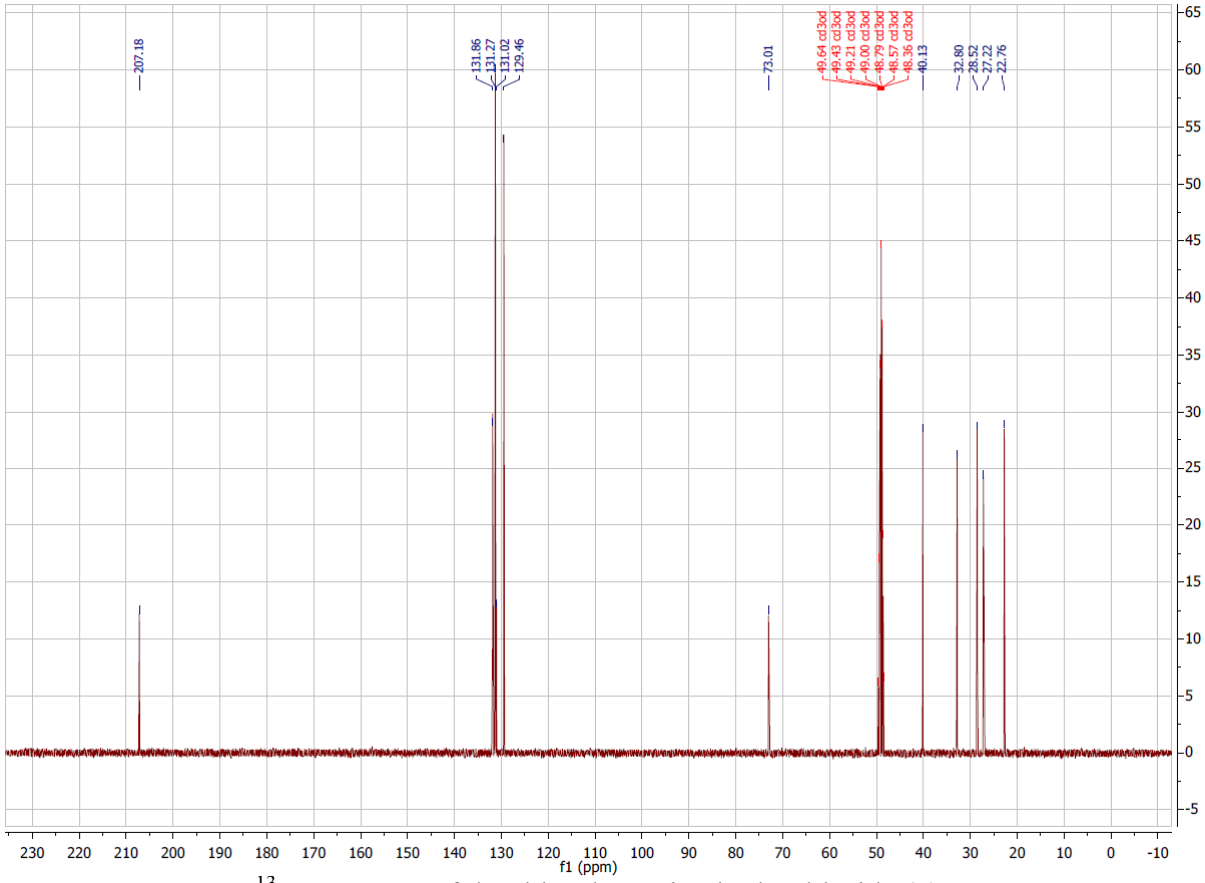
^{1}H NMR (300 MHz, Chloroform-*d*) δ 7.44 – 7.38 (m, 3H), 7.09 – 7.00 (m, 2H), 2.98 (s, 3H), 1.98 – 1.75 (m, 3H), 1.73 – 1.42 (m, 5H).



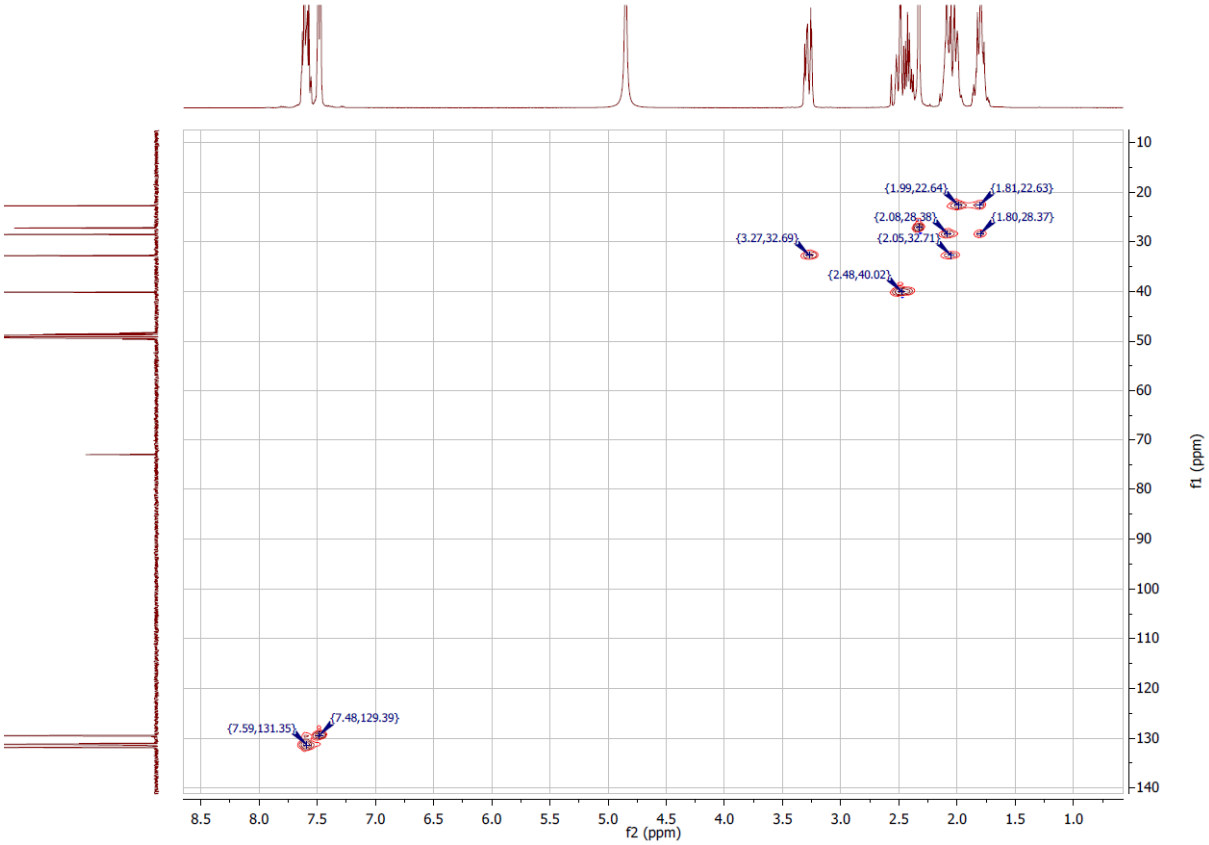
^{1}H spectrum of imine **9**



^{1}H spectrum of deschloroketamine hydrochloride (**3**)



^{13}C spectrum of deschloroketamine hydrochloride (**3**)



HMQC spectrum of deschloroketamine hydrochloride (**3**)

3. References

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S2 Mohr, S.; Taschwer, M.; Schmid M. G. *Chirality* **2012**, *24*, 486–492.

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S4 Chankvetadze, B. *J. Chromatogr. A* **2012**, *1269*, 26–51.