Electronic Supplementary Material (ESI) for New Journal of Chemistry. This journal is © The Royal Society of Chemistry and the Centre National de la Recherche Scientifique 2020

Structural spectroscopic study of enantiomerically pure synthetic cathinones and their major metabolites

Dita Spálovská*^a, Martin Paškan^b, Bronislav Jurásek^c, Martin Kuchař^c, Michal Kohout^b, Vladimír Setnička^a

^a Department of Analytical Chemistry, University of Chemistry and Technology, Prague 6, Czech Republic

^b Department of Organic Chemistry, University of Chemistry and Technology, Prague 6, Czech Republic

^c Forensic Laboratory of Biologically Active Substances, Department of Chemistry of Natural Compounds, University of Chemistry and Technology, Prague 6, Czech Republic

Electronic Supplementary Information

1. Synthesis and characterization

The synthesis of 4-halogen cathinones started from corresponding ketones 1b-c which underwent α -bromination by means of copper(II)bromide in ethyl acetate under reflux to afford bromo derivatives 2b-c (*).[1] Subsequent nucleophilic substitution with sodium azide in dry acetone gave rise to azides 3a-c, whose catalytic hydrogenation in hydrogen atmosphere gave rise to target 4-halogene cathinones 4a-c (norflephedrone, norclephedrone, and norbrephedrone). The target compounds were stabilized in form of hydrochlorides in order to prevent their spontaneous cyclization (*vide infra*).



Scheme S1. Synthesis of 4-halogene cathinones – the major human metabolites of the studied drugs.

* To offer an alternative route, the bromo derivative 2a was prepared by alternative procedure.

Nuclear magnetic resonance (NMR) spectra were acquired on Agilent 400-MR DDR2 spectrometer operating at 400.13 MHz for ¹H, 100.62 MHz for ¹³C and 376.50 MHz for ¹⁹F. High-resolution mass spectra (HRMS) were obtained using gas chromatography coupled to mass spectrometry Agilent 7200 Series Q-TOF GC/MS system (Agilent, Santa Clara, CA, USA) with electron ionization (+70 eV) or LC-MS LTQ-Orbitrap Velos (Thermo, Waltham, MA, USA) with positive electrospray ionization (ESI + or APCI +). Measurements performed with GC-MS/EI rendered impossible mild ionization and, thus, the localization of molecular ion. Only the main fragmentation ion (acylium cation) was observed in the spectrum (see below for compounds **2b** and **2c**).

2-Bromo-1-(4-fluorophenyl)propan-1-one (2a)

Aluminium chloride (5.30 g, 39.8 mmol) was dissolved in dry DCM (90 ml) at 0 °C to give yellow solution and, subsequently, 2-bromopropanoyl bromide (2.1 ml, 20.31 mmol) was added dropwise. The resulting mixture was stirred 30 minutes at 0 °C and then fluorobenzene (2.11 g, 22.0 mmol) was added dropwise and the reaction mixture was stirred 4 hours at 0 °C. The reaction mixture was diluted with ice cold water (90 ml) and extracted with DCM (3 × 100 mL). The organic layers were combined, washed with water (1 × 50 mL) and dried over MgSO₄. The organic solvents were concentrated under reduced pressure. Further purification was done by flash chromatography (Hexane/DCM, 65/35). 2-Bromo-1-(4-fluorophenyl)propan-1-one (4.50 g, 19.5 mmol, 96 % yield) was obtained as a colourless liquid. ¹H NMR (Chloroform-*d*) δ 8.11 – 8.00 (m, 2H), 7.21 – 7.10 (m, 2H), 5.24 (q, *J* = 6.6 Hz, 1H), 1.89 (d, *J* = 6.6 Hz, 3H). ¹³C NMR (Chloroform-*d*) δ 191.9, 166.1 (d, *J* = 256.0 Hz), 131.8 (d, *J* = 9.5 Hz), 130.5 (d, *J* = 3.0 Hz), 116.1 (d, *J* = 22.0 Hz), 41.4, 20.2. ¹⁹F NMR (282 MHz, Chloroform-*d*) δ -104.32 (tt, *J* = 8.4, 5.4 Hz). HRMS-ESI: m/z calculated for C₉H₈BrFO + H⁺ [M+H]⁺ 230.9815, found 230.9776/232.9755.



2-Bromo-1-(4-chlorophenyl)propan-1-one (2b)

A mixture of ketone **1a** (4.0 g, 23.7 mmol) and copper (II) bromide (8.0 g, 35.8 mmol) in ethyl acetate (120 ml) heated under reflux for 4 h. Then the reaction mixture was let to cool down to r.t. and then extracted with water (3 x 50 ml) and brine (50 ml). The washed organic solution was dried with anhydrous MgSO₄. The solvent was evaporated and the crude product was purified on silica to afford 4.5 g (76%) of α -bromo derivative **2a** as a white solid.¹H

NMR (Chloroform-*d*) δ 7.97 (d, J = 8.7, 2H), 7.47 (d, J = 8.4, 2H), 1.91 (d, $J = 6.6, 3H, CH_3$); 5.22 (q, J = 6.6, 1H, CH); ¹³C NMR (Chloroform-*d*) δ 192.1 (C=O), 140.2 (Ar-Cq), 132.3 (Ar-Cq), 130.3 (Ar-CH), 129.1 (Ar-CH), 41.3 (CH), 20.0 (CH₃). HRMS-EI: m/z calculated for C₉H₈BrClO + H⁺ [M+H]⁺ 246.95253, found [M–C₂H₄Br]⁺ 138,9950.



2-Bromo-1-(4-bromophenyl)propan-1-one (2c)

Compound 2c was prepared in the similar way as for **2b**.¹H NMR (Chloroform-*d*) δ 7.88 (d, *J* = 8.8, 2H), 7.62 (d, *J* = 8.8, 2H), 5.21 (q, *J* = 6.6, 1H), 1.89 (d, *J* = 6.6, 3H, CH₃). ¹³C NMR (Chloroform-*d*) δ 192.3 (C=O), 132.7 (Ar-Cq), 132.0 (Ar-Cq), 130.4 (Ar-CH), 128.9 (Ar-CH), 41.2 (CH), 20.0 (CH₃). HRMS-EI: m/z calculated for C₉H₈Br₂O + H⁺ [M+H]⁺ 290.90201, found [M-C₂H₄Br]⁺ 182.9446/184.9426.



2-Azido-1-(4-fluorophenyl)propan-1-one (3a)

To a solution of the bromide 2a (2.75 g, 11.1 mmol) in dry acetone (100 ml), sodium azide (1.25 g, 19.2 mmol) was added in one portion and the resulting suspension was stirred at room temperature under an inert argon atmosphere for 3 hours. Then, the reaction mixture was filtered, the filtration cake was washed with acetone (2×10 ml). The combined organic solution was evaporated under reduced pressure and the crude product was purified on silica (hexane/ethyl acetate, 95/5) affording **3a** (2.1 g, 90%) as yellow oil.

¹H NMR (Chloroform-*d*) δ 8.03 – 7.94 (m, 2H), 7.22 – 7.12 (m, 2H), 4.65 (q, *J* = 7.0 Hz, 1H), 1.57 (d, *J* = 7.0 Hz, 3H). ¹³C NMR (Chloroform-*d*) δ 195.18, 166.27 (d, *J* = 256.6 Hz), 131.57 (d, *J* = 9.5 Hz), 130.77 (d, *J* = 3.1 Hz), 116.26 (d, *J* = 21.8 Hz), 58.42, 16.40. ¹⁹F NMR (282 MHz, Chloroform-*d*) δ -103.78 (ddt, *J* = 11.4, 5.2, 3.2 Hz). HRMS-ESI: m/z calculated for C₉H₉FNO + H⁺ [M–N₂+H]⁺ 166.0668, found 166.0635; [M–N₂+H₃]⁺ 168.0819, found 168.0791.



Similarly, azides 3b and 3c were prepared.

2-Azido-1-(4-chlorophenyl)propan-1-one (3b)

¹H NMR (Chloroform-*d*) δ 7.89 (d, J = 8.7, 2H), 7.47 (d, J = 8.7, 2H); 4.64 (q, J = 6.7, 1H, CH), 1.57 (d, J = 6.7, 3H, CH₃). ¹³C NMR (Chloroform-*d*) δ 195.5 (C=O), 140.4 (Ar-Cq), 132.5 (Ar-Cq), 130.1 (Ar-CH), 129,2 (Ar-CH), 58.4 (CH), 16.23 (CH₃). HRMS-APCI: m/z calculated for C₉H₉ClNO + H⁺ [M–N₂+H]⁺ 182.03727, found 182.03708/184.03409.



2-Azido-1-(4-bromophenyl)propan-1-one (3c)

¹H NMR (Chloroform-*d*) δ 7.76 (d, J = 8.6, 2H), 7.58 (d, J = 8.6, 2H), 4.63 (q, J = 6.7 1H), 1.52 (d, J = 6.7, 3H). ¹³C NMR (Chloroform-*d*) δ 195.7 (C=O), 132.9 (Ar-Cq), 132.2 (Ar-Cq), 130.0 (Ar-CH), 129.2 (Ar-CH), 58.3 (CH), 16.25 (CH₃). HRMS-APCI: m/z calculated for C₉H₉BrNO + H⁺ [M–N₂+H]⁺ 225.98675, found 225.98660/227.98447.



2-Amino-1-(4-fluorophenyl)propan-1-one (4a)

To a solution of azide 3a (0.98 g, 4.67 mmol) in methanol (8 ml), palladium catalyst (98 mg, 10% Pd/C) was added. The reaction mixture was stirred at room temperature in hydrogen atmosphere for 1 h. The reaction was quenched with hydrochloric acid (1 ml; 35 ml) and evaporated to obtain white crystals of 4a (0.5 g, 49%).

¹H NMR (Methanol-*d*₄) δ 8.21 – 8.10 (m, 2H), 7.37 – 7.29 (m, 2H), 5.12 (q, *J* = 7.2 Hz, 1H), 1.57 (d, *J* = 7.2 Hz, 3H). ¹³C NMR (Methanol-*d*₄) δ 195.85, 167.82 (d, *J* = 255.3 Hz), 133.11 (d, *J* = 9.7 Hz), 130.78 (d, *J* = 2.9 Hz), 117.36 (d, *J* = 22.5 Hz), 52.81, 17.72. ¹⁹F NMR (282 MHz, Chloroform-*d*) δ -103.20 – -103.39 (m). HRMS-ESI: m/z calculated for C₉H₁₀FNO + H⁺ [M+H]⁺ 168.0819, found 168.0790.



In the same way as above, target compounds 4b and 4c were synthesized.

2-Amino-1-(4-chlorophenyl)propan-1-one (4b)

¹H NMR (Chloroform-*d*) δ 8.04 (d, J = 8.8, 2H), 7.61 (d, J = 8.8, 2H), 5.09 (q, J = 6.7, 1H), 1.55 (d, J = 6.7, 3H). ¹³C NMR (Chloroform-*d*) δ 183.6 (C=O), 143.0 (Ar-Cq), 135.1 (Ar-Cq), 130.1 (Ar-CH), 129.8 (Ar-CH), 51.4 (CH), 16.1 (CH₃). HRMS-ESI: m/z calculated for C₉H₁₀CINO + H⁺ [M+H]⁺ 184.05292, found 184.05222.





¹H NMR (Chloroform-*d*) δ 7.87 (d, J = 8.7, 2H), 7.68 (d, J = 8.7, 2H), 5.12 (q, J = 6.6, 1H), 1.47 (d, J = 6.7, 3H). ¹³C NMR (Chloroform-*d*) δ 196.6 (C=O), 133.2 (Ar-Cq), 132.0 (Ar-CH), 130.1 (Ar-CH), 128.6 (Ar-Cq), 58.3 (CH), 15.4 (CH₃). HRMS-ESI: m/z calculated for C₉H₁₀BrNO + H⁺ [M+H]⁺ 228.00240, found 228.00188/229.99960.



Dimer structures

Since we have identified dimeric structures of nor-derivatives as the major side products during chiral separation, we have synthesized a corresponding chloro-substituted dihydropyrazine derivative (Scheme S2) as an example.



Scheme S2. Structure of dihydropyrazine derivative spontaneously formed from norclephedrone free base.

3,6-Bis(4-chlorophenyl)-2,5-dihydro-2,5-dimethylpyrazine

A solution of nor-4-chlorocathinone (0.2 g, 1.09 mmol) in dry ethanol (20 ml) was heated under reflux overnight. The reaction mixture was evaporated under reduced pressure, and the crude product was purified by column chromatography (chloroform). It was obtained 0.25 g (83%) of a yellow solid. ¹H NMR (Chloroform-*d*) δ 7.88 (d, *J* = 8.8 ,2H), 7.62 (d, *J* = 8.8, 2H), 3.51 (m, 2H), 1.08 (d, *J* = 6.6, 6H). ¹³C NMR (Chloroform-*d*) δ 140.0 (C=N), 128.1 (Ar-Cq), 127.6 (Ar-Cq), 125.7 (Ar-CH), 52.3 (CH), 10.9 (CH₃).

2. Development of a chiral separation method

For convenience, the chiral separation method was developed at room temperature at a constant mobile flow rate of 1 ml·min⁻¹. For the analytical screening, the sample concentration was 1 mg mL⁻¹ and the injection volume was set to 10 μ L.

Since synthetic cathinones have been, in most cases, enantiomerically resolved on polysaccharide-based columns in normal phase mode, we have employed these conditions and screened several mobile phase compositions (Table S1). Due to the notable difference in polarity of parent drugs and their nor-derivatives (Figure S1) caused by demethylation, the composition of the screening mobile was modified accordingly (Table S2).



Fig. S1. Polarity of analytes expressed in calculated partition coefficient values.

Table S1. Screened mobile phase compositions and chromatographic parameters obtained for clephedrone and nor-clephedrone as the model analytes; conditions and results for clephedrone are colour coded (cc) in red and blue for heptane and hexane mobile phase, respectively; conditions and results for nor-clephedrone are marked in green and yellow for heptane and hexane mobile phase, respectively.

Mobile phase	c.c	tr ₁ (min)	tr ₂ (min)	k ₁	k ₂	α	R	N1	N2
HEP/IPA 95/5 + 0.1% DEA		6.44	7.04	0.82	0.99	1.21	1.11	3156	2953
HEP/IPA 90/10 + 0.1% DEA		5.73	6.16	0.64	0.76	1.19	0.91	4288	2640
HEP/IPA 85/15 + 0.1% DEA		5.44	5.80	0.55	0.65	1.19	0.70	3020	2534
HEP/IPA 95/5 + 0.1% DEA		12.56	13.65	2.55	2.86	1.12	2.10	5985	7189
HEP/IPA 90/10 + 0.1% DEA		9.58	10.14	1.74	1.90	1.09	1.26	6529	5814
HEP/IPA 85/15 + 0.1% DEA		8.47	9.16	1.41	1.60	1.14	1.56	5741	5488
HEX/IPA 95/5 + 0.1% DEA		7.21	7.95	1.03	1.24	1.20	1.55	4258	4527
HEX/IPA 90/10 + 0.1% DEA		6.32	6.83	0.79	0.93	1.18	1.06	3911	3626
HEX/IPA 85/15 + 0.1% DEA		5.34	5.72	0.53	0.63	1.18	0.78	3925	3486
HEX/IPA 95/5 + 0.1% DEA		16.59	17.36	3.67	3.88	1.06	1.43	9205	7590
HEX/IPA 90/10 + 0.1% DEA		11.96	12.94	2.38	2.65	1.12	2.04	7835	6496
HEX/IPA 85/15 + 0.1% DEA		8.88	9.49	1.52	1.70	1.11	1.27	5015	4811



Fig. S2. HPLC chromatogram of clephedrone (red) and norclephedrone (green) in heptane mobile phases: (a) 5% IPA, (b) 10% IPA, (c) 15% IPA, on the analytical column YMC ChiralArt Amylose-SA (150×4.6 mm, 5 μ m), temperature 23 °C, detection wavelength λ = 254 nm.



Fig. S3. HPLC chromatograms of clephedrone (blue) and norclephedrone (yellow) in hexane mobile phases: (a) 5% IPA, (b) 10% IPA, (c) 15% IPA, on the analytical column YMC

ChiralArt Amylose-SA (150×4.6 mm, 5 μ m), temperature 23 °C, detection wavelength λ = 254 nm.

Improved solubility of nor-derivatives in ethanol-containing mobile phases was observed. Moreover, in such mobile phases, improved chromatographic resolution of the compounds was achieved (Table 2, Figure S4). On the other hand, parent drugs were not resolved under such conditions (Figure S5).

Table S2. Optimum mobile phase compositions for nor-clephedrone - heptane-based mobile phase is marked purple, hexane mobile phase brown.

Mobile phase	c.c	tr ₁ (min)	tr ₂ (min)	k ₁	k ₂	α	R	N1	N2
HEP/EtOH 9/1 + 0.1% DEA		13.66	15.75	2.87	3.46	1.21	4.11	7892	9320
HEP/EtOH 8/2 + 0.1% DEA		8.73	9.78	1.41	1.70	1.21	2.02	4566	4722
HEX/EtOH 9/1 + 0.1% DEA		12.52	14.68	2.52	3.12	1.24	4.54	8640	9527
HEX/EtOH 8/2 + 0.1% DEA		9.01	10.22	1.56	1.90	1.22	2.63	5498	6828



Fig. S4. HPLC chromatograms of norclephedrone in (g) heptane mobile phases (purple) and (h) hexane mobile phases (orange) with different ratio of ethanol on the analytical column YMC ChiralArt Amylose-SA (150×4.6 mm, 5 μ m), temperature 23 °C, detection wavelength $\lambda = 254$ nm.



Fig. S5. HPLC chromatogram of clephedrone in heptane mobile phase (purple) and hexane mobile phase (orange) with 10 % ethanol on the analytical column YMC ChiralArt Amylose-SA (150×4.6 mm, 5 μ m), temperature 23 °C, detection wavelength λ = 254 nm.

3. Preparative enantioseparation of cathinones

Based on the results obtained in the analytical mode, the starting conditions for the preparative enantioseparation on Chiralpak IA (250×20 mm, 5 µm) were calculated as follows: flow rate 20.6 mL min⁻¹, sample concentration 21.2 mg mL⁻¹, and injection volume 0.106 mL. Due to rather low resolution values and notable peak tailing observed in the analytical mode, the flow rate was reduced to 15 mL min⁻¹ and sample concentration to 10 mg mL⁻¹ (due to solubility) for the first experiment on the preparative scale. Indeed, the resolution of enantiomers obtained in the first injection on the preparative column was not satisfactory (Figure S6).

Surprisingly, the heptane-based mobile phase, identified in the analytical mode as the less efficient, proved to be superior to the hexane-based mobile phase on the preparative scale. Therefore, in the next optimization step – reduction of the column temperature, only the heptane-based mobile phase was optimized further, eventually leading to the ideal preparative conditions used for the separation of drugs, *i.e.*, mobile phase heptane/propan-2-ol (96/4) with 0.1 % DEA, column temperature 15 °C, flow rate of 10 mL min⁻¹.



Fig. S6. Preparative chiral separation of flephedrone, using the mobile phases optimized in the analytical mode: (a) hexane/propan-2-ol (95/5) with 0.1% DEA, (b) heptane/propan-2-ol (95/5) with 0.1% DEA. Conditions: column Chiralpak IA (250×20 mm, i.d., 5 µm), column temperature 21 °C (room temperature); flow rate of 15 mL min⁻¹; and detection wavelength of 254 nm were employed. Injection volume was 0.25 mL, sample concentration 10 mg mL⁻¹.



Fig. S7. Preparative chiral separation of norclephedrone. Intervals of fraction collection are marked by coloured pillars. Conditions: column Chiralpak IA (250×20 mm, i.d., 5 µm) with the optimum mobile phase; column temperature 15 °C; flow rate of 10 mL min⁻¹; and detection wavelength of 254 nm were employed. Injection volume was 0.5 mL.



Fig. S8. Preparative chiral separation of norbrephedrone. Intervals of fraction collection are marked by coloured pillars. Conditions: column Chiralpak IA (250×20 mm, i.d., 5 µm) with the optimum mobile phase; column temperature 15 °C; flow rate of 10 mL min^[1]; and detection wavelength of 254 nm were employed. Injection volume was 0.5 mL.

4. DFT calculations

level of DFT theory	Boltzmann populations [%]			
	Ι	II		
B3LYP/6-311++G(d,p)	99.9	0.1		
B3LYP/aug-cc-pVTZ	99.9	0.1		
B3PW91/6-311++G(d,p)	99.9	0.1		
B3PW91/ aug-cc-pVDZ	99.9	0.1		
CAM-B3LYP/6-311++G(d,p)	99.8	0.2		
CAM-B3LYP/aug-cc-pVTZ	99.9	0.1		
wB97XD/6-311++G(d,p)	99.6	0.4		
wB97XD/aug-cc-pVTZ	99.9	0.1		
wB97XD/TZVP	99.9	0.1		

Table S3. Boltzmann populations of two stable conformers of (R)-flephedrone hydrochloride calculated at several levels of theory.

level of DFT theory	Boltzmann populations [%]			
	Ι	II		
B3LYP/6-311++G(d,p)	93.4	6.6		
B3LYP/aug-cc-pVTZ	94.5	5.5		
B3PW91/6-311++G(d,p)	94.8	5.2		
B3PW91/ aug-cc-pVDZ	50.1	49.9		
CAM-B3LYP/6-311++G(d,p)	93.7	6.3		
CAM-B3LYP/aug-cc-pVTZ	95.1	4.9		
wB97XD/6-311++G(d,p)	83.2	16.8		
wB97XD/aug-cc-pVTZ	91.7	8.3		
wB97XD/TZVP	86.0	14.0		

Table S4. Boltzmann populations of two stable conformers of (R)-clephedrone hydrochloride calculated at several levels of theory.

Table S5. Boltzmann populations of two stable conformers of (R)-brephedrone hydrochloride calculated at several levels of theory.

level of DFT theory	Boltzmann populations [%]			
	Ι	II		
B3LYP/6-311++G(d,p)	93.7	6.3		
B3LYP/aug-cc-pVTZ	99.9	0.1		
B3PW91/6-311++G(d,p)	90.7	9.3		
B3PW91/ aug-cc-pVDZ	99.0	11.0		
CAM-B3LYP/6-311++G(d,p)	89.8	10.2		
CAM-B3LYP/aug-cc-pVTZ	91.5	8.5		
wB97XD/6-311++G(d,p)	72.7	27.3		
wB97XD/aug-cc-pVTZ	94.4	5.6		
wB97XD/TZVP	73.3	26.7		

5. UV absorption spectroscopy

When comparing the experimental UV absorption spectra of desmethyl derivatives (Fig. S9, bottom) with the experimental spectra of flephedrone, clephedrone and brephedrone (Fig. 8, bottom, the bands were of the same wavelength or shifted by 1-2 nm.



Fig. S9. Comparison of UV absorption spectra of norflephedrone (left), norclephedrone (middle) and norbrephedrone (right) hydrochlorides: simulated spectra of individual conformers at the B3LYP/6-311++G(d,p) level (top), their population weighted spectra (middle) and experimental spectra (bottom).

6. References

1. King LC, Ostrum GK. Selective Bromination with Copper(II) Bromide1. J. Org. Chem. 29 (1964) 3459-3461.