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**Efficiency and sustainability in cyclizine synthesis:
a comparative analysis of reductive amination methodologies**

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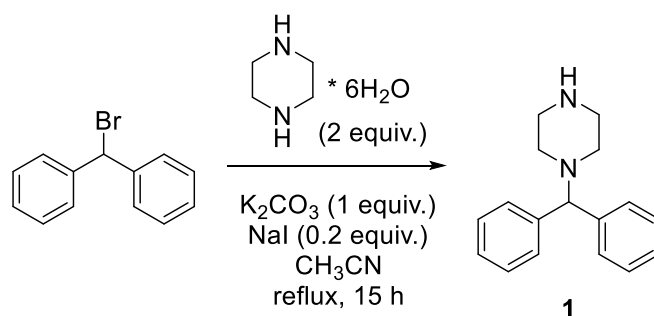
General information

Unless otherwise stated, all reagents were purchased from commercial suppliers and used without further purification. ^1H and ^{13}C spectra were recorded in CDCl_3 on Bruker Avance 300, Bruker Avance 400 spectrometers. Chemical shifts δ are reported in ppm relative to the solvent resonance signal as an internal standard. The following abbreviations were used to designate chemical shift multiplicities: s = singlet, d = doublet, t = triplet, q = quartet, sept = septet, m = multiplet, br = broad; coupling constants are given in Hertz (Hz).

For thin layer chromatographic (TLC) analyses, Merck precoated TLC plates (silica gel 60 F254) were used. Silica gel 0.060 – 0.200 mm (Acros) was used for column chromatography. Analytical gas chromatography (GC) was performed using a Chromatec Crystal 5000.2NP gas chromatograph fitted with a flame ionization detector (He was used as the carrier gas) and a MS detector. Chromatec CR-5MS (30 meters) capillary columns were used. GC settings for the yield determination using FID detector and CR5MS column: the injector temperature was 250 °C, split ratio of 10:1 at the moment of injection, the FID temperature was 250 °C. Column compartment temperature program: 100°C for 2 min, 100 °C $\rightarrow$ 250 °C at 19 °C/min, 290 °C for 1 min. Flow rate 1.5 mL/min, column CR-5MS. Retention time for cyclizine **2** is 11.2 min, for *N*-benzhydrylpiperazine **1** – 11.4 min. Retention times (Rt) and integrated ratios were obtained using Chromatec Analytic Software.

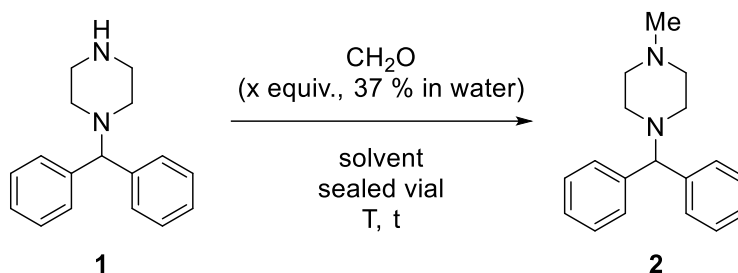
1. Experimental section

1.1 Synthesis of *N*-benzhydrylpiperazine **1**^{S1}



Solid potassium carbonate (1.863 g, 13.5 mmol) and catalytic sodium iodide (405 mg, 2.7 mmol) were added to a solution of piperazine hexahydrate (5.238 g, 27.0 mmol) in acetonitrile (45 mL) and stirred with heating for 5 minutes. Bromodiphenylmethane (3.334 g, 13.5 mmol) was added, and the reaction mixture was heated at reflux for 15 hours. After completion of the reaction (TLC), the reaction was quenched with water (30 mL), extracted with ethyl acetate (2×50 mL), dried over anhydrous sodium sulfate and evaporated to afford crude yellow oil solidifying upon cooling. The crude product was purified by flash chromatography (ethyl acetate : triethylamine = 100:1 to ethyl acetate : methanol = 15:1) to afford 1.683 g (49 %) of *N*-benzhydrylpiperazine **1** as white solid. ^1H NMR (300 MHz, CDCl_3) δ 7.47 – 7.37 (m, 4H), 7.33 – 7.22 (m, 4H), 7.22 – 7.12 (m, 2H), 4.22 (s, 1H), 2.89 (t, $J = 4.8$ Hz, 4H), 2.36 (t, $J = 4.8$ Hz, 4H), 1.89 (s, 1H). ^{13}C NMR (101 MHz, CDCl_3) δ 142.8, 128.6, 128.1, 127.0, 76.8, 53.4, 46.4. The NMR spectra are in agreement with the literature.^{S2}

1.2 Optimization studies



General procedure: to a suspension of *N*-benzhydrylpiperazine **1** (20-50 mg, 0.08-0.20 mmol) in the corresponding solvent (0.55 M) was added formalin (1.112 g/ml, 37% in water). The 10 ml test tube with a cap was sealed (described below) and placed into preheated oil bath for $\frac{3}{4}$ of its length. After heating and stirring for the corresponding time, the reaction mixture was cooled and diluted with DCM. Organic layer was dried with anhydrous Na_2SO_4 . The reaction mixture was analyzed using GC.

As shown in the photo (Figure S1), PTFE tape was used for better tube sealing to prevent solvent vapors from leaking. The cap has a silicon pad.



Figure S1
Reaction vessel
for the
optimization
studies

Table S1 Solvent screening

Entry	Solvent	Yield, %
1 ^a	CH_3CN	19
2 ^a	EtOH	11
3 ^a	<i>i</i> PrOH	12
4 ^a	<i>t</i> BuOH	25
5 ^a	Sulfolane	20
6 ^a	Ethyl acetate	19
7 ^a	Neat	20
8 ^a	Butyl acetate	10
9 ^a	H_2O	23
10 ^b	CH_3CN	60
11 ^b	<i>t</i> BuOH	83
12 ^b	<i>n</i> BuOH	63
13 ^b	Acetone	29

^a – 8 equiv. of formalin, 90 °C, 1 h; ^b – 6 equiv. of formalin, 130 °C, 4 h.

Table S2 Temperature and formaldehyde equiv. screening

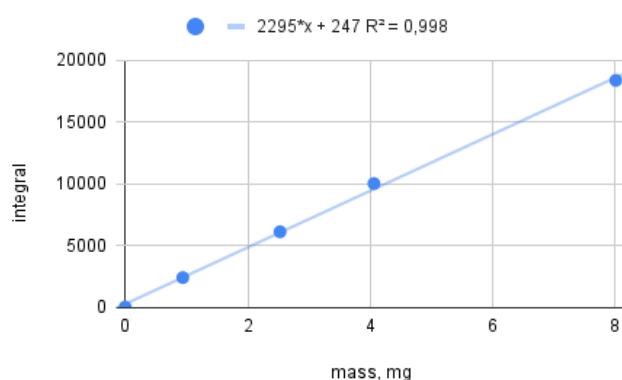
Entry ^a	Formalin equiv.	Yield, %				
		90 °C	110 °C	130 °C	130 °C (paraform) ^b	150 °C
1	6	19	43	60	19	53
2	8	19	53	71	33	55
3	10	23	61	89	37	78

^a – *N*-benzhydrylpiperazine (**1**) (20-25 mg, 0.08-0.10 mmol), CH₃CN (0.55 M), formalin (0.48-1.00 mmol, 35-73 µL 1.112 g/ml, 37% in water), T °C, 1 h; ^b – paraform (0.48-1.00 mmol, 14.4-30.0 mg) was used instead of formalin.

Table S3 Concentration of *N*-benzhydrylpiperazine (**1**) in *t*-butanol influence

Entry ^a	Concentration, M	Yield, %
1	0.55	73
2 ^b	1.1	83
3 ^c	2.2	99

^a – *N*-benzhydrylpiperazine **1** (50.4 mg, 0.2 mmol), *t*BuOH (364 µL), formalin (6 equiv., 37% in water), 130 °C, 24 h; ^b – *t*BuOH (182 µL); ^c – **1** (100.8 mg, 0.4 mmol), *t*BuOH (182 µL).

**Table S4** GC analyses data

Mass, mg	Integral ^a
0,94	2389
2,53	6103
4,06	10014
8,01	18384

^a average of 2 GC analyses.

Figure S2 Calibration curve for the optimization studies

Below there is a demonstration of the retention times order for both substances in their GC-MS spectra.

GC settings for the mass determination using MS detector and CR5MS column: the injector temperature was 250 °C. Column compartment temperature program: 60°C for 2 min, 60°C → 250 °C at 30 °C/min, 250 °C for 12 min. Flow rate 1.0 mL/min, column CR-5MS. Retention time for cyclizine **2** is 8.96 min, for *N*-benzhydrylpiperazine **1** – 9.07 min. Retention times (Rt) were obtained using Chromatec Analytic Software.

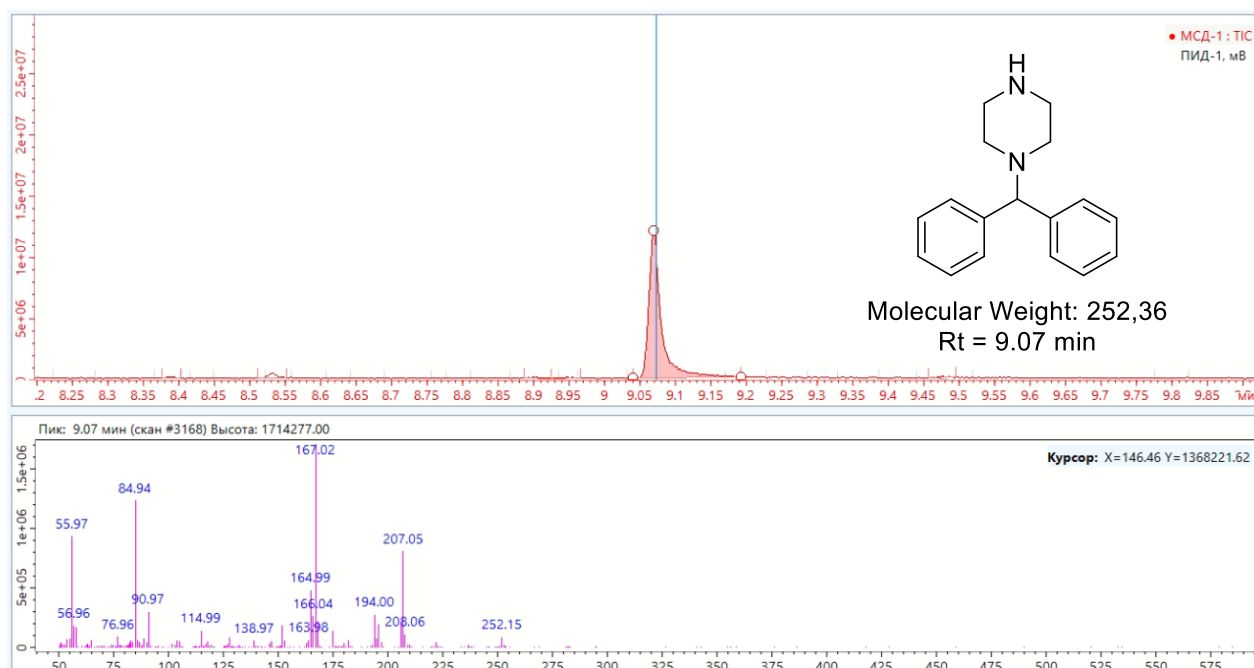


Figure S3 Retention time for *N*-benzhydrylpiperazine (**1**)

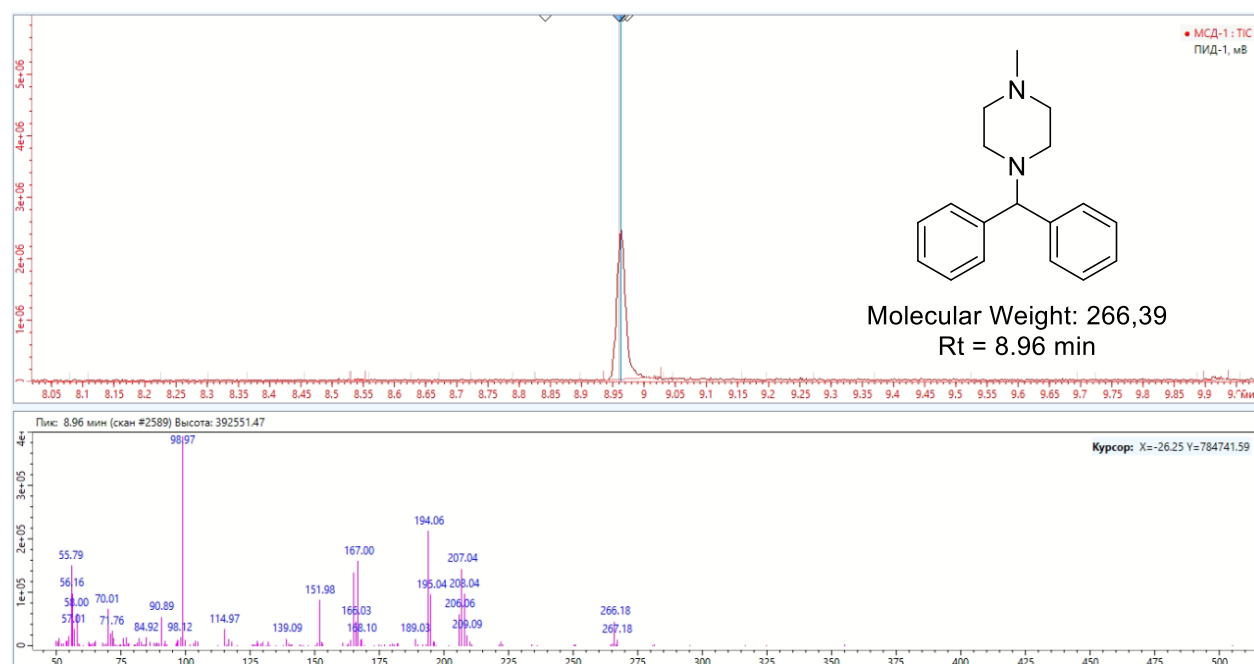


Figure S4 Retention time for cyclizine **2**

1.3 Cyclizine **2** synthesis using the optimized conditions

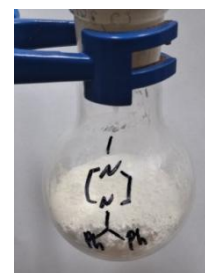
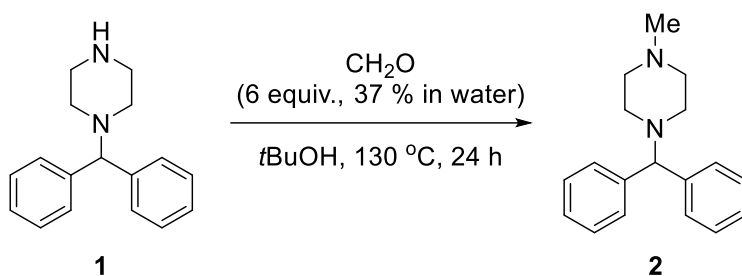


Figure S5
Scaled up
experiment result

To a suspension of *N*-benzhydrylpiperazine (**1**) (12.60 g, 50 mmol) in tert-butanol (22.7 ml, 2.2 M) in 250 ml Schlenk tube was added formalin (6 equiv., 21.9 mL, 1.112 g/mL, 37% in water). The tube was sealed and placed into preheated to $130\text{ }^\circ\text{C}$ oil bath. After 24 h the reaction mixture was cooled to room temperature and evaporated on a rotary evaporator to give crude yellowish solid. Then it was dissolved as a cloudy solution in ethyl acetate (150 ml) and washed with water ($2 \times 20\text{ ml}$). The combined water aqueous layer was washed with ethyl acetate (150 ml). The combined organic layer was dried over anhydrous sodium sulfate. The drying agent was filtered off, and the filtrate was evaporated to give yellowish solid. The crude was purified through crystallization (4 times) from acetonitrile-water to afford cyclizine **2** (12.51 g, 47 mmol, 94 %) as white to yellowish-white solid (m.p. $104\text{--}107\text{ }^\circ\text{C}$)^{S3}. ^1H NMR (300 MHz, CDCl_3) δ 7.50 – 7.40 (m, 4H), 7.35 – 7.24 (m, 4H), 7.24 – 7.15 (m, 2H), 4.24 (s, 1H), 3.00–1.90 (m appears as br s, 8H), 2.31 (s, 3H). ^{13}C NMR (101 MHz, CDCl_3) δ 143.0, 128.6, 128.0, 127.0, 76.4, 55.5, 52.1, 46.1. The NMR spectra are in agreement with the literature.^{S4}

1.4 Cyclizine **2** syntheses via reductive amination reactions variations ^{S5}

1.4.1 NaBH_4

A 10 mL round bottom flask was charged with formalin (17.5 μL , 0.24 mmol), *N*-benzhydrylpiperazine (50.4 mg, 0.20 mmol) and methanol (2 mL). The mixture was refluxed for 2 h. NaBH_4 (15.1 mg, 0.4 mmol) was added to reaction mixture. The suspension was stirred at room temperature overnight. Then reaction mixture was refluxed for additional 2 h. After reaction completion, it was diluted with water (5 mL), and the product extracted with ethyl acetate ($2 \times 15\text{ mL}$). Organic layer was washed with brine 2 times, dried over anhydrous sodium sulfate, and solvent was evaporated. GC yield 99 %.

1.4.2 $\text{NaBH}(\text{OAc})_3$

A 10 mL round bottom flask with input for Ar was charged with formalin (14.6 μL , 0.20 mmol), *N*-benzhydrylpiperazine (50.4 mg, 0.20 mmol), glacial acetic acid (12 μL , 0.20 mmol) and 1,2-dichloroethane (0.7 mL). The mixture was stirred at room temperature under Ar atmosphere for 15 min. $\text{NaBH}(\text{OAc})_3$ (59.4 mg, 0.28 mmol) was added to reaction mixture. The suspension was stirred at room temperature under Ar atmosphere for 18 h. Then the reaction mixture was quenched with aqueous saturated NaHCO_3 and extracted three times with 10 mL of ethyl acetate. The organic layer was dried over anhydrous sodium sulfate, filtered and evaporated. GC yield 10 %.

1.4.3 NaBH_3CN

A penicillin vial was charged with *N*-benzhydrylpiperazine (48 mg, 0.19 mmol), glacial acetic acid (11 μL , 0.19 mmol) and methanol (2 mL). After that, formalin (13.9 μL , 0.19 mmol) and solution of NaBH_3CN (24 mg, 0.38 mmol) in methanol (1 mL) were added. The reaction mixture was stirred at room temperature overnight. Then the solution was evaporated and the residue was taken up with water (10 mL) and extracted with dichloromethane ($2 \times 15\text{ mL}$). The organic layers were combined, dried over anhydrous sodium sulfate, filtered and evaporated. GC yield 99 %.

1.4.4 H₂/Pd/C

1% of Pd 10% Pd/C (wet support, water content is 56%) (4.8 mg, 2 μmol, 1 mol%), *N*-benzhydrylpiperazine (50.4 mg, 0.20 mmol), formalin (14.6 μL, 0.20 mmol), methanol (0.2 ml) and stirring bar were charged into a 10 ml Schlenk flask. The flask was charged with 1 atm H₂ (balloon) at room temperature. After 15 h of stirring, the reaction mixture was diluted with DCM (3 ml), centrifuged, and the clear solution was decanted. The Pd/C was once more suspended in DCM (3 ml) and methanol (2 ml), centrifuged, both organic phases were combined and analyzed using GC. GC yield 99 %. The catalyst was dried on rotary evaporator (20 mm Hg, 40 °C, 30 min) and put into the next cycle.

2. Green metrics evaluation (RME, RME generalized, E factor)

$$\text{RME} = \frac{\text{Mass of product} * 100 \%}{\text{Total mass of reactants}}$$

$$\text{Generalized RME} = \frac{\text{Mass of product} * 100 \%}{\text{Total mass of reactants and solvents}}$$

$$\text{E factor} = \frac{\text{Total mass of waste}}{\text{Mass of final product}} \text{ (kg/kg)}$$

RME is the ratio of the product mass and the reagents masses. Higher RME value means that a method deals with less waste and is considered synthetically reasonable. Generalized RME takes into account solvents. E factor is the ratio of the total amount of waste and the mass of final product. We estimated these metrics only for the reaction part. Work-up procedures were considered sharing somewhat equal amounts of solvents, thus not included into the calculations. The metrics are attributed to the GC yields. The mass of the catalyst for Pd/C was not included into E factor calculations due to its recycling.

Table S5 Green metrics values

Green metric	NaBH ₄	NaBH ₃ CN	NaBH(OAc) ₃	H ₂ /Pd/C	HCHO
RME, %	62.0	50.5	3.8	73.7	35.7
Generalized RME, %	3.2	2.0	0.5	22.9	24.1
E factor (kg/kg)	30.7	48.5	190.1	3.3	3.2

3. Cost evaluation

The total cost (\$/g, Table S6 and Table S8) is calculated for the sum of formalin and reducing agent or catalyst (in case of H₂/Pd/C) mass amount put into the reaction to form 1 mol (266 g) of cyclizine with 100 % yield. The cost of the Pd/C catalyst is divided by 4 due to 3 recycles.

Table S6 Reagents cost^a

Reagent	Pack size	Pack cost, \$	Cost per gram, \$/g
Formaldehyde solution about 37% (1040039025) ^{S6}	25 L (27.250 kg)	1760.00	0.0646
Acetic acid, glacial, ReagentPlus®, ≥99% (A6283) ^{S7}	18 L (18.720 kg)	563.00	0.0301
Sodium borohydride, ReagentPlus®, 99% (213462) ^{S8}	0.500 kg	446.00	0.8920
Sodium cyanoborohydride for synthesis (8.18053) ^{S9}	0.500 kg	1930.00	3.8600
Sodium triacetoxyborohydride for synthesis (8.43705) ^{S10}	0.100 kg	482.00	4.8200
Palladium on carbon, 10 wt. % loading (dry basis), matrix carbon powder, wet support (520829) ^{S11}	0.250 kg	6140.00	24.5600

^a – all pricing data are taken from Sigma-Aldrich catalog. The links are in the References section.

Table S7 Cost values

Method	Reagent	Reaction loading, mol	Reaction loading, g	Reagent cost, \$/g	Total cost, \$/g
NaBH ₄	NaBH ₄	2	75.6	0.8920	73.7
	HCHO	1.2	97.3	0.0646	
NaBH ₃ CN	NaBH ₃ CN	2	125.6	3.8600	491.9
	AcOH	1	60.0	0.0301	
	HCHO	1	81.1	0.0646	
NaBH(OAc) ₃	NaBH(OAc) ₃	1.4	296.8	4.8200	1437.6
	AcOH	1	60.0	0.0301	
	HCHO	1	81.1	0.0646	
H ₂ /Pd/C	Pd/C	0.01	24.2	6.1400	153.8
	HCHO	1	81.1	0.0646	
HCHO	HCHO	6	486.5	0.0646	31.4

Table S8 Reagents cost^a

Reagent	Pack size	Pack cost, \$	Cost per gram, \$/g
Formaldehyde solution, 37 wt. % in H ₂ O, contains 10-15% methanol as stabilizer (F809702) ^{S12}	25 L (27.250 kg)	234.38	0.0086
Acetic acid 99-100%, for synthesis (A992640) ^{S13}	5 L (5.245 kg)	203.13	0.0387
Sodium borohydride (no available pricing data)	—	—	—
Sodium cyanoborohydride for synthesis (S817682) ^{S14}	2.500 kg	1059.38	0.4238
STAB,97% (S817709) ^{S15}	10.000 kg	485.78	0.0486
Palladium on carbon, moistened with water, 10% Pd basis (based on dry substance) (768449) ^{S16}	0.025 kg	604.22	24.1688

^a – all pricing data are taken from Macklin catalog. The links are in the References section.

Table S9 Cost values

Method	Reagent	Reaction loading, mol	Reaction loading, g	Reagent cost, \$/g	Total cost, \$/g
NaBH ₃ CN	NaBH ₃ CN	2	125.6	0.4238	56.2
	AcOH	1	60.0	0.0387	
	HCHO	1	81.1	0.0086	
NaBH(OAc) ₃	NaBH(OAc) ₃	1.4	296.8	0.0486	17.4
	AcOH	1	60.0	0.0387	
	HCHO	1	81.1	0.0086	
H ₂ /Pd/C	Pd/C	0.01	24.2	6.0422	146.9
	HCHO	1	81.1	0.0086	
HCHO	HCHO	6	486.5	0.0086	4.2

4. NMR spectra

1. ^1H and ^{13}C NMR spectra of N-benzhydrylpiperazine (**1**)

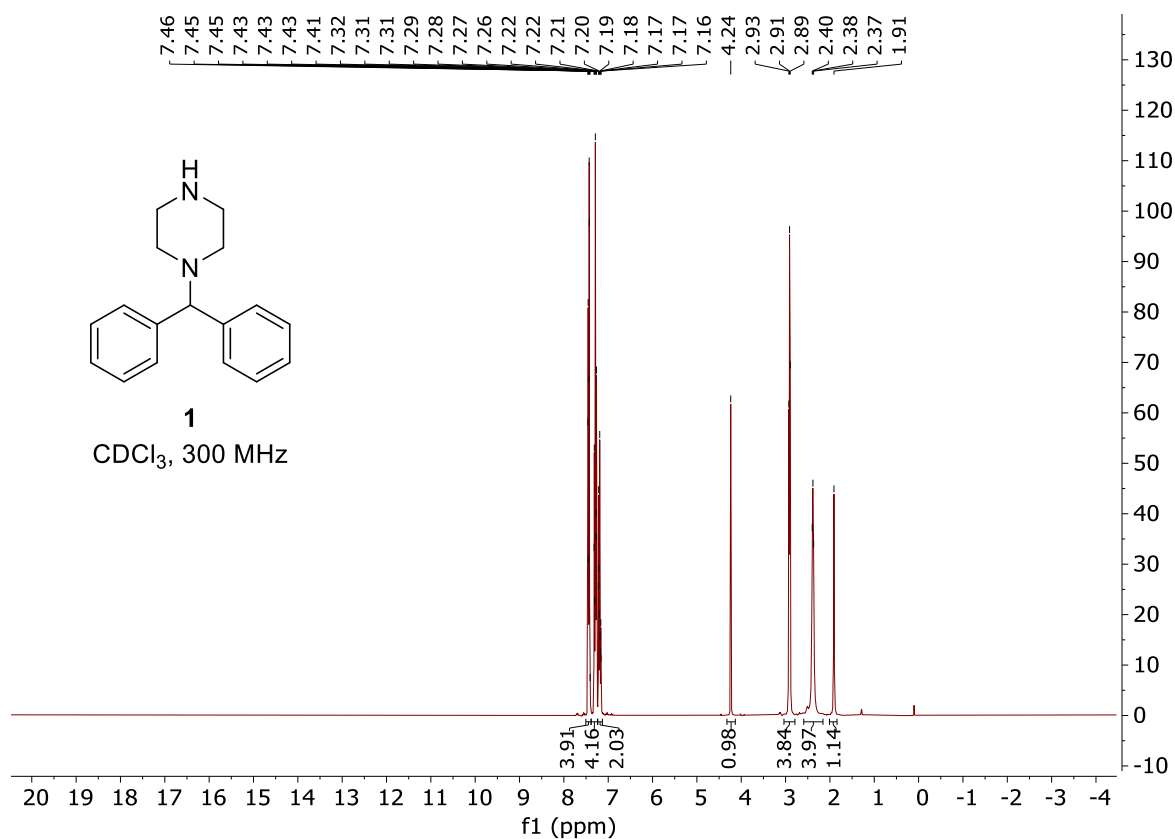


Figure S6 ^1H NMR spectrum of N-benzhydrylpiperazine (**1**)

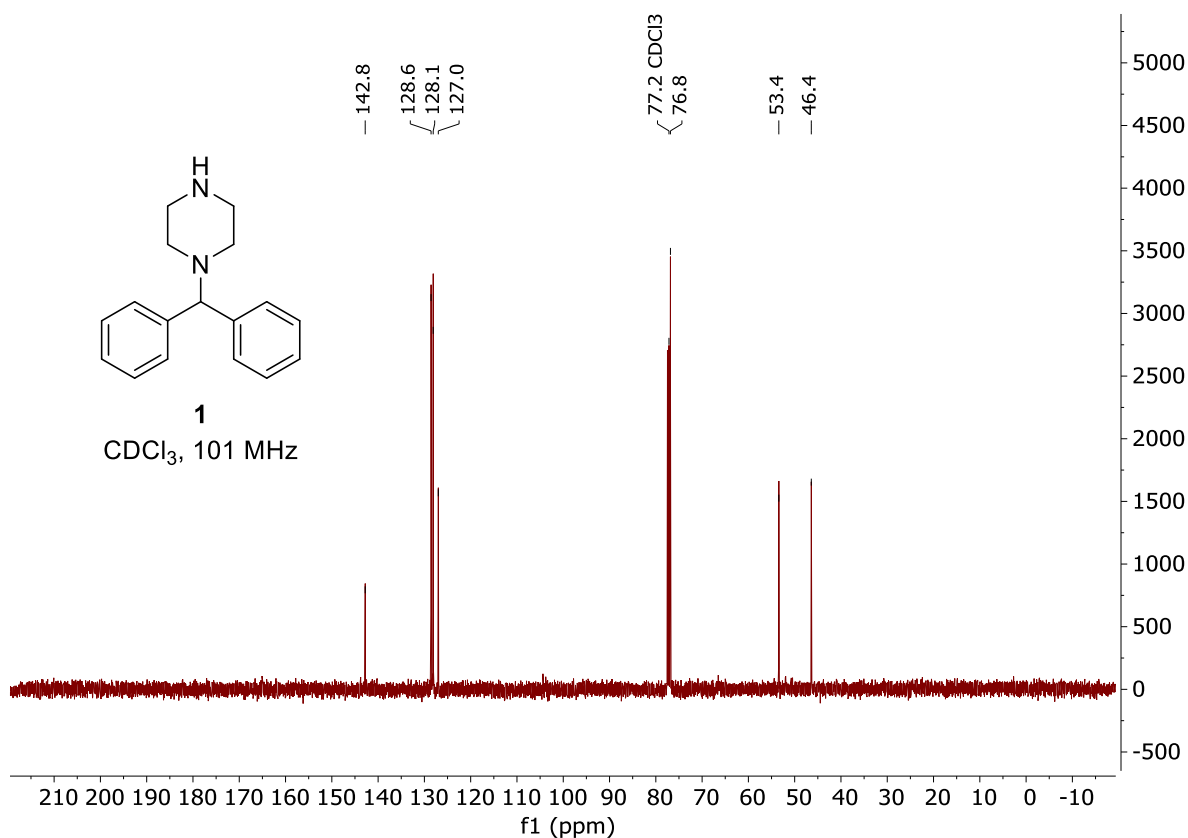


Figure S7 ^{13}C NMR spectrum of N-benzhydrylpiperazine (**1**)

2. ^1H and ^{13}C NMR spectra of cyclizine (2)

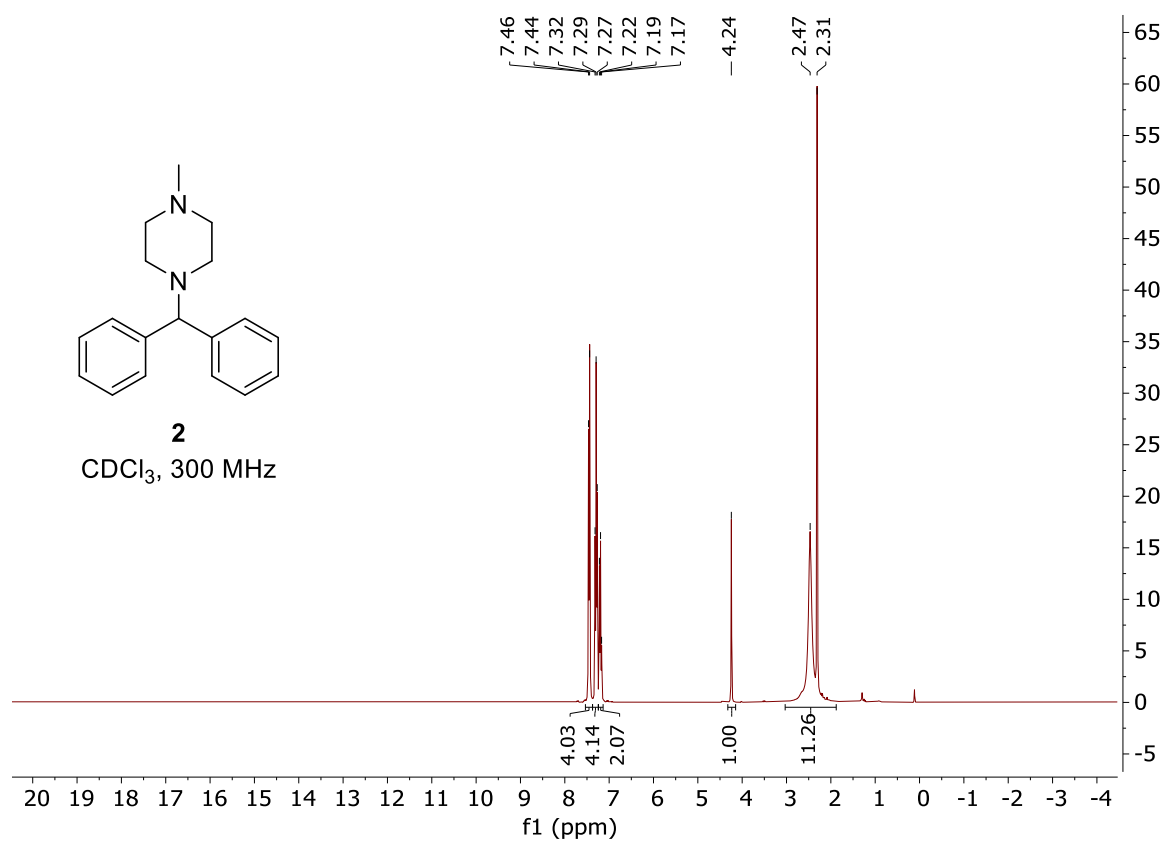


Figure S8 ^1H NMR spectrum of cyclizine (2)

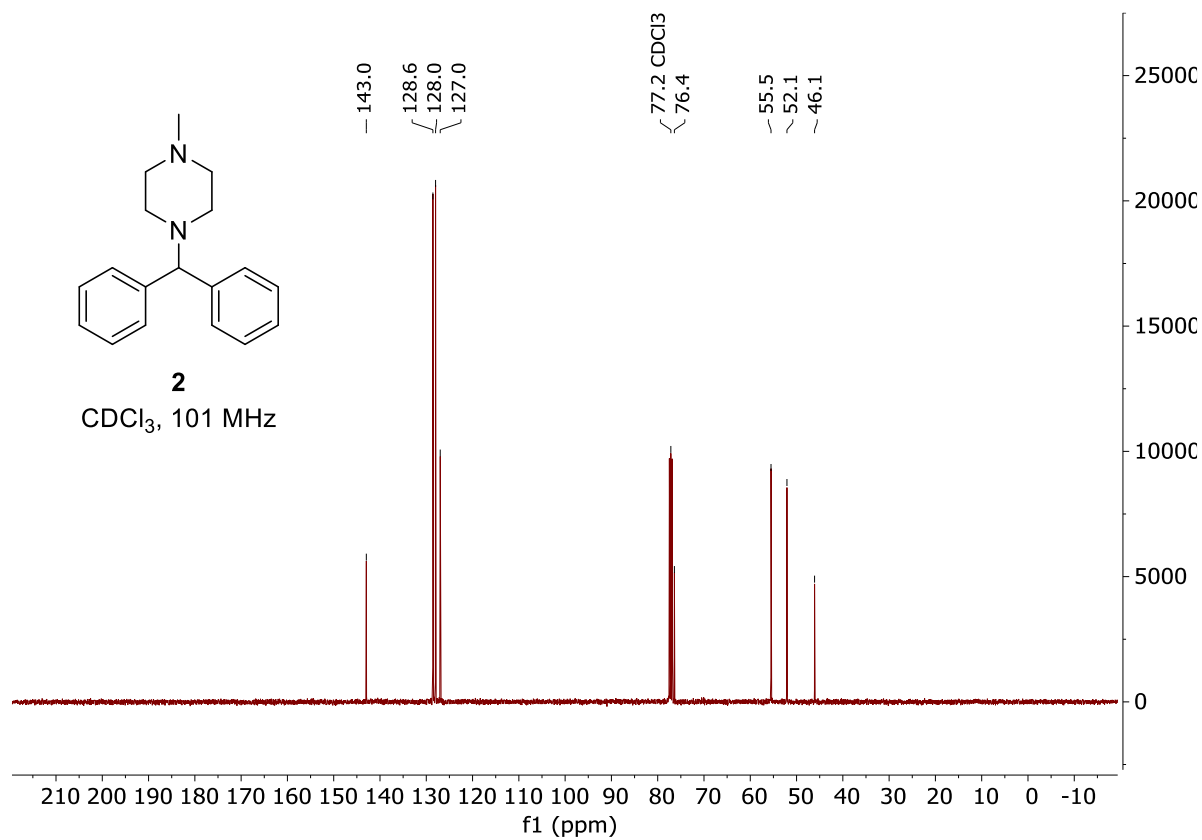


Figure S9 ^{13}C NMR spectrum of cyclizine (2)

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