Supplementary Information

Histamine H_3 receptor ligands with a 3-cyclobutoxy motif: a novel and versatile constraint of the classical 3-propoxy linker

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Gradient programs (ZQ Waters)

Solvent A: Water

Solvent B: Acetonitrile

Solvent C: Water/Acetonitrile/TFA : 49.75/49.75/0.5, v/v/v, pH~2.

Solvent D: Ammonium Formate ($HCOONH_4$) in water (630 mg/L) + 500 µL/L NH_4OH (30%), pH~9.5.

Acidic gradient program

Time (min)	A (%)	B (%)	C (%)	D (%)	Flow (mL/min)
0	85	5	10	0	1.8
1	85	5	10	0	1.8
7	5	85	10	0	1.8
9	5	85	10	0	2.3
12	5	85	10	0	2.3
12.2	85	5	10	0	1.8
15	85	5	10	0	1.8

Table S1. Elution conditions for the acidic gradient program.

Basic gradient program

Time (min)	A (%)	B (%)	C (%)	D (%)	Flow (mL/min)
0	85	5	0	10	1.8
1	85	5	0	10	1.8
7	5	85	0	10	1.8
9	5	85	0	10	2.3
12	5	85	0	10	2.3
12.2	85	5	0	10	1.8
15.5	85	5	0	10	1.8

Table S2. Elution conditions for the basic gradient program.

Gradient program (LCT Waters)

Solvent A: Ammonium Formate ($HCOONH_4$) in water (630 mg/L) + 500 µL/L NH_4OH

Solvent B: Acetonitrile

Basic gradient program

Time (min)	A (%)	B (%)	Flow (mL/min)
0	99	1	0.4
0.8	99	1	0.4
5.3	5	95	0.4
5.35	5	95	0.5
7.3	5	95	0.5
7.35	99	1	0.4
9	99	1	0.4

Table S3. Elution conditions for the basic gradient program.

Compound purities

Cmpd	LC-PURITY
5 [1]	B: 96.34% A: 96.86%
6 [2]	B: > 99% A: > 99%
22	B: 95.70% A: 96.35%
24	B: >99% A: >99%
25	B: >99% A: >99%
29	B: 98.58% A: 98.13%
31	B: 96.90% A: 96.45%
32 [1]	B: 95% A: 97%

Table S4. Compound purities, determined as described in the Synthetic Procedures section and in Tables S1 and S2. B: Basic gradient. A: Acidic gradient.

Pharmacological procedures

Membrane preparation. Confluent CHO cells are detached by 10 min incubation at 37°C in PBS/EDTA 0.02 %. The cell suspension is centrifuged at 1,500 x *g* for 10 min at 4°C. The pellet is homogenized in a 15 mM Tris-HCI buffer (pH 7.5) containing 2 mM $MgCl_2$, 0.3 mM EDTA and 1 mM EGTA (buffer A). The crude homogenate is frozen in liquid nitrogen and thawed. DNAse (1 µL/mL) is then added and the homogenate is further incubated for 10 min at 25°C before being centrifuged at 40,000 x *g* for 25 min at 4°C. The pellet is resuspended in buffer A and washed once more under the same conditions. The final membrane pellet is resuspended, at a protein concentration of 1-3 mg/mL, in a 7.5 mM Tris-HCI buffer (pH 7.5) enriched with 12.5 mM $MgCl_2$, 0.3 mM EDTA, 1 mM EGTA and 250 mM sucrose and stored in liquid nitrogen until used.

[^{3}H]-Nα-methylhistamine binding assay. Affinity of compounds for human histamine H_3 receptors was measured by competition with [^{3}H]-Nα-methylhistamine. Compounds were dissolved in 100 % DMSO to give a 1 mM stock solution. Compounds were diluted 100-fold in the total assay volume and final DMSO concentration in the assay did not exceed 1 %. Briefly, membranes (20-40 µg proteins) expressing human H_3 histamine receptors are incubated at 25°C in 0.5 mL of a 50 mM Tris-HCI buffer (pH 7.4) containing 2 mM $MgCl_2$, 0.2 nM [^{3}H]-Nα-methylhistamine and increasing concentrations of ligands. The non-specific binding (NSB) is defined as the residual binding observed in the presence of 10 µM thioperamide or histamine. Membrane-bound and free radioligand are separated by rapid filtration through glass fiber filters presoaked in 0.1 % PEl. Samples and filters are rinsed by at least 6 mL of ice-cold 50 mM Tris-HCI buffer (pH 7.4). The entire filtration procedure does not exceed 10 seconds per sample. Radioactivity trapped onto the filters is counted by liquid scintillation in a β-counter.

[^{35}S]-GTPγS binding assay. Inhibition (inverse agonist) of [^{35}S]-GTPγS binding to membrane expressing human H_3 histamine receptors was measured as described by Lorenzen et al.[3] with a few modifications. Compounds were dissolved in 100 % DMSO to give a 1 mM stock solution. Compounds were diluted 100-fold in the total assay volume and final DMSO concentration in the assay did not exceed 1 %. Briefly, membranes (10-20 μg proteins) expressing human H_3 histamine receptors were incubated at 25°C in 0.2 mL of a 50 mM Tris-HCI buffer (pH 7.4) containing 3 mM $MgCl_2$, 50 mM NaCl, 1 μM GDP, 2 μg saponin and increasing concentrations of ligands. After 15 min preincubation, 0.2 nM of [^{35}S]-GTPγS were added to the samples. The non specific binding (NSB) was defined as the residual binding observed in the presence of 100 μM Gpp(NH)p. Membrane-bound and free radioligand were separated by rapid filtration through glass fiber filters. Samples and filters were rinsed by at least 6 mL of ice-cold 50 mM Tris-HCl buffer (pH 7.4). The entire filtration procedure did not exceed 10 seconds per sample. Radioactivity trapped onto the filters was counted by liquid scintillation in a β-counter.

Computational studies

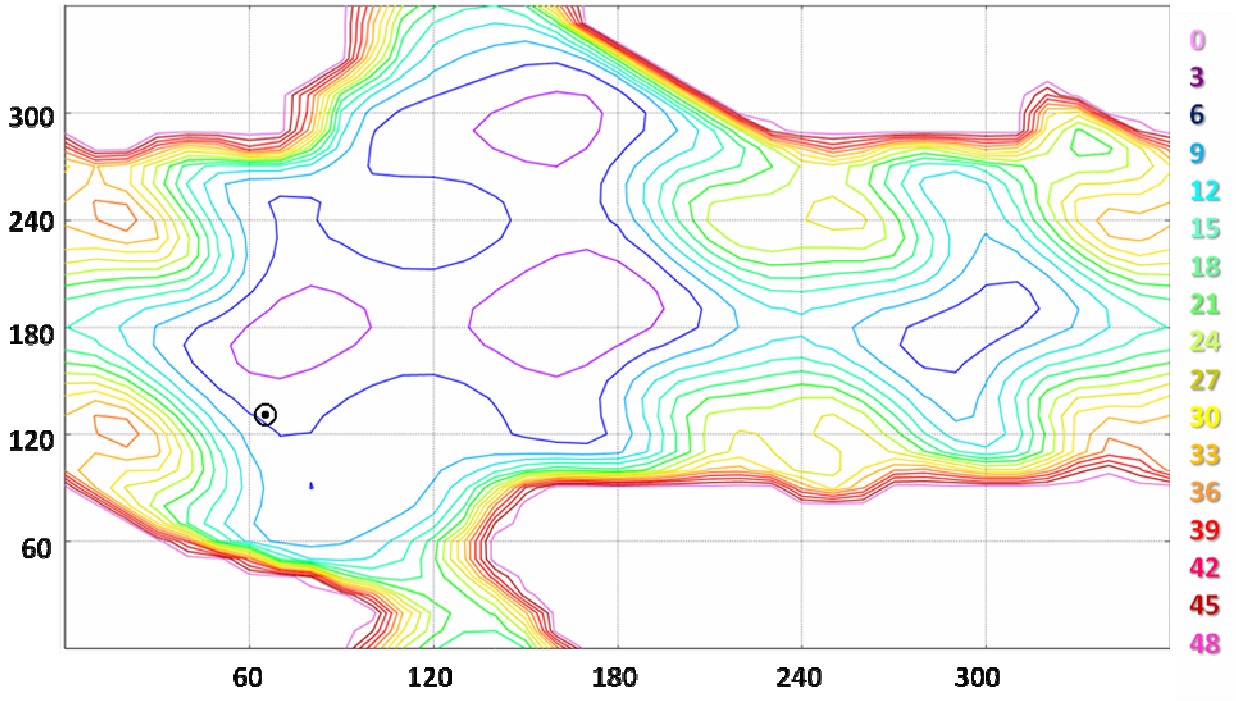
General

Computational chemistry work was performed on an Intel Core 2 Quad CPU 2.4 GHz, with 4 GB Memory (RAM) running Molecular Operating Environment (MOE, version 2009.10, Chemical Computing Group Inc., Canada).[4] All structures were drawn with the builder module. The conformational analysis and energy minimization were performed using stochastic conformation search with a rms gradient of 0.001 and iteration limit of 10000 using the MMFF94 force field.[5, 6] The failure limit was set to the high value of 250 to ensure thorough sampling of conformational space.

Conformational analyses

In an effort to understand the stringent spatial effects of the 3-cyclobutoxy linker, computational studies involving conformational analyses and energy minimizations were undertaken (see also Plot S1). These studies revealed 405 conformers within 7 Kcal/mol of the global minimum (627 conformers within 14 Kcal/mol) of the flexible analogue **32**, whereas only 35 conformers (68 within 14 Kcal /mol) were found for *cis*-cyclobutoxy compound **25** and 68 conformers within 7 Kcal/mol of the global minimum (133 conformers within 14 Kcal/mol) for the *trans*-cyclobutoxy compound **24**. It is evident that the configurational entropy penalty upon protein binding is smaller for the rigidified cyclobutoxy-compounds compared to the flexible analogue **32**.[7]

Dihedral contour plot



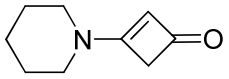
Plot S1. A dihedral contour plot displaying the potential energy of the propyl-spacer of **32** against rotational increments (given in degrees). Energy values are displayed using the colour coding given in the right side panel. The marked area indicates the corresponding dihedral angles of the *trans*-cyclobutoxy compound **24**.

Synthetic procedures

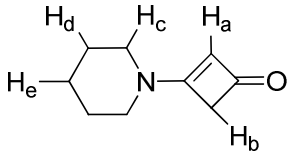
General synthetic procedures.

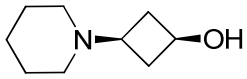
Cyclobutanedione dicyclohexylammonium salt (**16**) was obtained from Lonza. Other reagents were obtained from commercial suppliers and used without further purification. Solvents used were either AR or HPLC grade. Dry THF and CH_2Cl_2 were freshly distilled from lithium aluminium hydride and calcium hydride, respectively. Thin-layer chromatography was carried out on Merck Kieselgel 60 F254 on aluminium sheets and flash chromatography was performed using J. T. Baker Kieselgel 60 under pressure. The ^{1}H-, ^{13}C- and 2D-spectra were recorded on a Bruker 200, 250 or 400 MHz spectrometer. J-values are given in Hz. Systematic names for molecules according to IUPAC rules were generated using the Beilstein AutoNom program. Compounds were dried for extended periods of time under high vacuum. Purities were assayed using LC-MS with a ZQ Waters single quadrupole mass spectrometer equipped with an ESI source and an HPLC Waters 2795 quaternary pump with diode array detector (210 to 400 nm). Data are acquired in a full MS scan from m/z 50 to 750 in positive mode with an acidic elution and both in positive and negative modes with a basic elution. The reverse phase separation is carried out at 45 °C on a Sunfire MS C18 column (5µm, 150 x 4.6 mm) for acidic elution and on a XBridge MS C18 column (5µm, 150 x 4.6 mm) for basic elution. Tables S1 and S2 contain a detailed summary of the gradient programs used for the purity measurements. Compound purities were calculated as the percentage peak area of the analyzed compound by UV detection (all wavelengths between 210 and 400 nm). Purities of all final compounds are listed in Table S4. HRMS spectra were recorded using LC-MS with a LCT Waters time of flight mass spectrometer equipped with an ESI source and an Waters Acquity UPLC with diode array detector (210 to 400 nm). Data are acquired in a full MS scan from m/z 50 to 750 in positive mode with a basic elution. The reverse phase separation is

carried out at 55 °C on an Acquity UPLC BEH C18 column (1.7µm, 2.1 x 100 mm). Table S3 contains a detailed summary of the gradient program used.

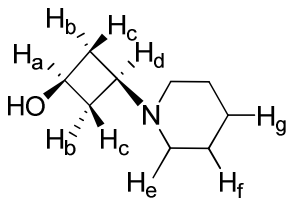


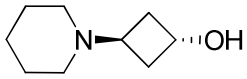
3-(Piperidin-1-yl)cyclobut-2-enone (17). Trifluoroacetic acid (64 mL, 0.86 mol) is added over 10 min to a stirred suspension of cyclobutanedione dicyclohexylammonium salt **16** (200 g, 0.75 mol) in dioxane (1 L). After 4 h of stirring at r.t., the resulting suspension is filtered and washed with dioxane (300 mL). The filtrate is then stirred at r.t. and treated dropwise with piperidine (96 mL, 0.975 mol) while maintaining the temperature below 30°C throughout the addition (20 min) with a water bath. The mixture is stirred for 18 h at r.t.. The dioxane is then removed under reduced pressure and the resulting oil is taken up in CH_2Cl_2 (400 mL). The organic layer is washed with a 1 N aq. HCl-solution (400 mL), water (400 mL), satd. aq. $NaHCO_3$-soln. (400 mL) and brine (400 mL). The organic layer is dried over $MgSO_4$ and concentrated to yield 90.7 g of a red solid. This is purified by chromatography over silicagel (eluent: CH_2Cl_2/MeOH/ammonia 98:1.8:0.2) to afford the title compound (74.8 g, 66 %) as an oil which slowly crystallizes to white crystals. δ_H (400 MHz; $CDCl_3$; Me_4Si) 4.47 (1 H, s, H_a), 3.22 (4 H, m, H_c), 2.95 (2 H, s, H_b), 1.53 (6 H, m, H_d+H_e). *m/z* (ESI) 152.3 ($M + H^+$, $C_9H_{14}NO$ requires 152.1).



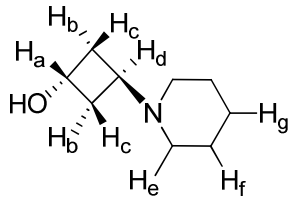


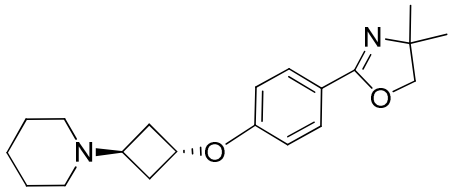
***Cis*-3-(piperidin-1-yl)cyclobutanol (18)**. A solution of **17** (10 g, 66.1 mmol) in ethanol (200 mL) is treated with portions of sodium borohydride (8.76 g, 231 mmol). At the end of the addition, the mixture is stirred at 50°C for 12 h, cooled down to 20°C and treated with acetone (20 mL). The solvents are removed under reduced pressure to leave a yellow oil which is diluted with EtOAc (200 mL). This organic layer is washed with satd. aq. $NaHCO_3$-soln. (100 mL), water (100 mL) and brine (100 mL). The organic layer is subsequently concentrated under reduced pressure. The residual crude oil (also containing ca. 5 % of *trans*-product **20**) is purified by chromatography over silicagel (eluent: CH_2Cl_2/MeOH/ammonia 95:4.5:0.5) to afford the title compound as a white solid (8.0 g, 78 %). δ_H (400 MHz; CD_3OD; Me_4Si) δ 3.91 (1H, m, H_a), 2.44 (2 H, m, H_b), 2.31 (4 H, m, H_e), 2.23 (1 H, m, H_d), 1.76 (2 H, m, H_c), 1.59 (4H, m, H_f), 1.46 (2 H, m, H_g). δ_C (100 MHz; $CDCl_3$; Me_4Si) 50.2, 51.7, 50.8, 38.1, 25.1, 24.0. *m/z* (ESI) 156.2 ($M + H^+$, $C_9H_{18}NO$ requires 156.1). The spectra-section of the Supplementary Information contains 2D-NMR data.



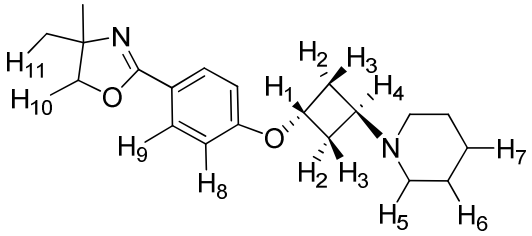


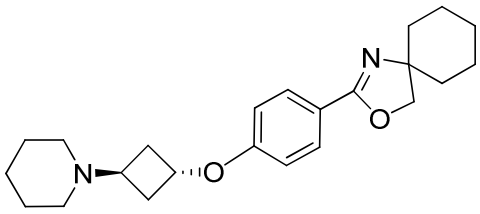
***Trans*-3-(piperidin-1-yl)cyclobutanol (20).** Benzoic acid (244 mg, 2.0 mmol), alcohol **18** (200 mg, 1.29 mmol) and triphenylphosphine (524 mg, 2.0 mmol) were dissolved in dry THF (10 mL). The mixture was cooled in an icebath and diisopropylazodicarboxylate (DIAD, 0.396 mL, 2.0 mmol) was added dropwise. After 10 min in the icebath, the mixture was stirred at r.t. overnight. TLC showed full consumption of alcohol **18**. The mixture was concentrated *in vacuo*. The residue was applied to a silica column and elution was performed with EtOAc. The relevant fractions were collected and concentrated. Trituration with ether (2x) and then hexane (1x) was conducted. This yielded intermediate **19** as a white solid (220 mg), which contained 15 % triphenylphosphine oxide. This solid was dissolved in THF/H_2O (4/1, 10 mL). LiOH.H_2O (185 mg, 5.55 mmol) was added and the mixture was stirred at reflux for 5 h and then at r.t. overnight. Aq. 2 M HCl was added to neutralization, after which THF was evaporated. More 2 M HCl was added to pH = 1. The precipitated benzoic acid and triphenylphosphine oxide (remaining from the first step) were extracted with EtOAc (3x). The aqueous layer was saturated with Na_2CO_3 and extraction was performed with EtOAc (3x). The organic layers were combined, dried (Na_2SO_4), filtered and concentrated. This yielded the title compound as a yellow oil (120 mg, 60 % from **18**). δ_H (200 MHz; $CDCl_3$; Me_4Si) 4.38 (1H, m, H_a), 3.31 (1H, br s, OH), 2.92 (1H, m, H_d), 2.23-2.16 (6H, m, H_e+H_c), 2.06-1.93 (2H, m, H_b), 1.59-1.51 (4H, m, H_f), 1.48-1.39 (2H, m, H_g). δ_C (50 MHz; $CDCl_3$; Me_4Si) 63.9, 57.1, 51.6, 36.0, 24.9, 24.0. *m/z* (ESI) 156.2 ($M + H^+$, $C_9H_{18}NO$ requires 156.1). The spectra-section of the Supplementary Information contains 2D-NMR data.





***Trans*-4,4-dimethyl-2-(4-(3-(piperidin-1-yl)cyclobutoxy)phenyl)-4,5-dihydrooxazole (22).** Phenol **21** (130 mg, 0.68 mmol)[1], alcohol **18** (80 mg, 0.52 mmol) and triphenylphosphine (PPh_3, 170 mg, 0.65 mmol) were dissolved in dry THF (8 mL). The mixture was cooled in an icebath and diisopropylazodicarboxylate (DIAD, 0.130 mL, 0.66 mmol) was added dropwise. After 10 min in the icebath, the mixture was stirred at r.t. overnight. TLC showed some remaining starting material **18**. More DIAD (0.05 mL) and PPh_3 (50 mg) were added. After 3 h at r.t., the mixture was concentrated *in vacuo*. The residue was applied to a silica column and elution was performed with EtOAc until all triphenylphosphine oxide had eluted. Subsequently, the product was eluted using 5 % Et_3N in EtOAc. The title compound was obtained as an orange solid (45 mg, 27 %). δ_H (250 MHz; $CDCl_3$; Me_4Si) 7.77 (2H, d, *J* 8.9, H_9), 6.69 (2H, d, *J* 8.9, H_8), 4.71 (1H, m, H_1), 3.99 (2H, s, H_{10}), 2.91 (1H, m, H_4), 2.40 – 2.21 (8H, m, H_2 + H_3 + H_5), 1.58-1.50 (4H, m, H_6), 1.50-1.28 (2H, m, H_7), 1.29 (6H, s, H_{11}). δ_C (62.5 MHz; $CDCl_3$; Me_4Si) 161.7, 159.8, 131.3, 120.2, 114.4, 78.7, 69.3, 67.2, 57.1, 50.7, 33.6, 28.3, 25.3, 24.1. *m/z* (ESI) 329.2214 ($M+H^+$, $C_{20}H_{29}N_2O_2$ requires 329.2229). The spectra-section of the Supplementary Information contains 2D-NMR data.





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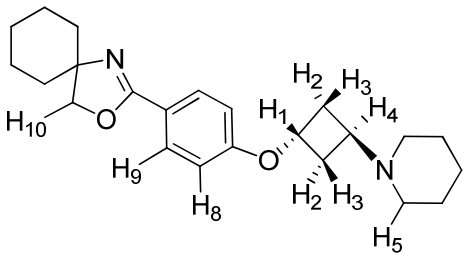
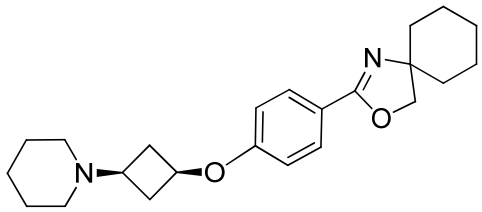
***Trans*-2-(4-(3-(piperidin-1-yl)cyclobutoxy)phenyl)-3-oxa-1-azaspiro[4.5]dec-1-ene (24)**. Phenol **23** (270 mg, 1.17 mmol),[1] alcohol **18** (120 mg, 0.77 mmol) and triphenylphosphine (300 mg, 1.15 mmol) were dissolved in dry THF (6 mL). The mixture was cooled in an icebath and diisopropylazadicarboxylate (DIAD, 0.230 mL, 1.17 mmol) was added dropwise. After 10 min in the icebath, the mixture was stirred at r.t. overnight. TLC showed full consumption of **18**. The mixture was concentrated *in vacuo*. The residue was applied to a silica column and elution was performed with EtOAc. The title compound was collected as a yellow solid (65 mg, 23 %). δ_H (250 MHz; $CDCl_3$; Me_4Si) 7.76 (2H, d, *J* 8.8, H_9), 6.68 (2H, d, *J* 8.8, H_8), 4.69 (1H, m, H_1), 4.03 (2H, s, H_{10}), 2.91 (1H, m, H_4), 2.38-2.30 (2H, m, H_3), 2.30-2.18 (6H, m, H_2+H_5), 1.77-1.64 (4H, m, CH_2), 1.64-1.49 (6H, m, CH_2), 1.49-1.37 (2H, m, CH_2), 1.37-1.19 (4H, m, CH_2). δ_C (62.5 MHz; $CDCl_3$; Me_4Si) 161.4, 159.8, 129.7, 120.4, 114.4, 77.5, 70.9, 69.3, 57.1, 50.9, 37.4, 33.6, 25.3, 25.0, 24.1, 23.0. *m/z* (ESI) 369.2555 ($M+H^+$, $C_{23}H_{33}N_2O_2$ requires 369.2542). The spectra-section of the Supplementary Information contains 2D-NMR data.





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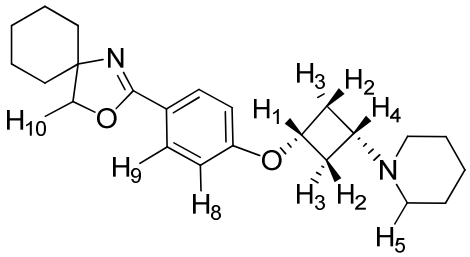
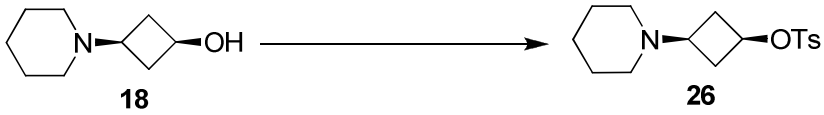
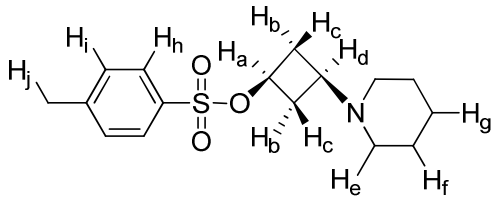
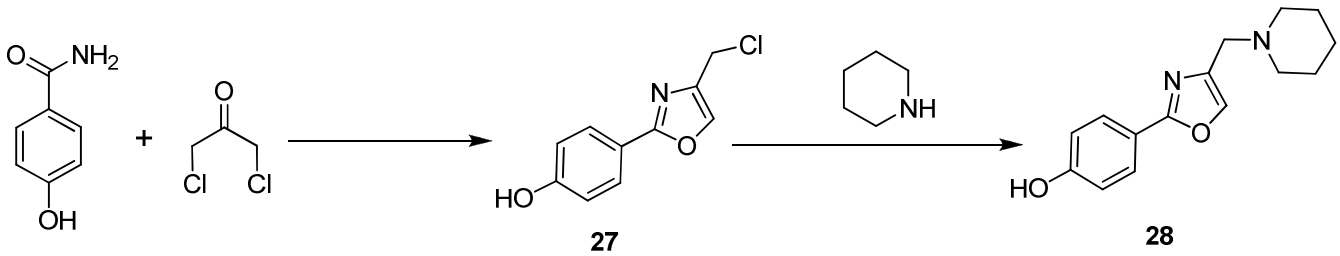
***Cis*-2-(4-(3-(piperidin-1-yl)cyclobutoxy)phenyl)-3-oxa-1-azaspiro[4.5]dec-1-ene (25).** This reaction was carried out as described for **24**, using phenol **23** (270 mg, 1.17 mmol),[1] alcohol **20** (120 mg, 0.77 mmol), triphenylphosphine (300 mg, 1.15 mmol) and diisopropylazodicarboxylate (0.230 mL, 1.17 mmol). Two subsequent purifications by column chromatography (EtOAc, then 25 % Et_3N/hexane) afforded the title compound as white crystals (50 mg, 18 %). δ_H (250 MHz; $CDCl_3$; Me_4Si) 7.75 (2H, d, *J* 8.9, H_9), 6.71 (2H, d, *J* 8.9, H_8), 4.37 (1H, m, H_1), 4.03 (2H, s, H_{10}), 2.66-2.54 (2H, m, H_2), 2.38 (1H, m, H_4), 2.30-2.16 (4H, m, H_5), 2.07-1.90 (2H, m, H_3), 1.73-1.62 (4H, m, CH_2), 1.62-1.48 (6H, m, CH_2), 1.48-1.35 (2H, m, CH_2), 1.35-1.18 (4H, m, CH_2). δ_C (62.5 MHz; $CDCl_3$; Me_4Si) 161.4, 159.6, 129.7, 120.4, 114.4, 77.5, 70.9, 65.2, 52.3, 50.7, 37.4, 35.3, 25.3, 25.0, 24.1, 23.0. *m/z* (ESI) 369.2 ($M + H^+$, $C_{23}H_{33}N_2O_2$ requires 369.2). The spectra-section of the Supplementary Information contains 2D-NMR data.





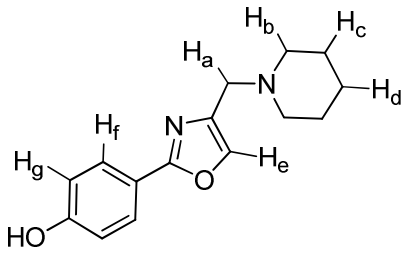
***Cis*-3-(piperidin-1-yl)cyclobutyl 4-methylbenzenesulfonate (26)**. A solution of alcohol **18** (1.0 g, 6.44 mmol) and *N*-methylimidazole (1.03 mL, 12.88 mmol) in CH_2Cl_2 (10 mL) is treated with *para*-toluenesulfonyl chloride (2.1 g, 10.95 mmol). The mixture is stirred at 20 °C for 48 h, washed with satd. aq. $NaHCO_3$-soln (10 mL), dried over magnesium sulfate, filtered and concentrated to afford 1.8 g of a red oil. This oil is purified by chromatography over silicagel (CH_2Cl_2/MeOH/ammonia 99:0.9:0.1) to yield the title compound as an orange solid (1.1 g, 55 %). δ_H (400 MHz; $CDCl_3$; Me_4Si) 7.77 (2 H, d, *J* 8.3, H_h), 7.32 (2 H, d, *J* 8.3, H_i), 4.58 (1 H, m, H_a), 2.44 (3 H, s, H_j), 2.40 (2 H, m, H_b), 2.27-2.14 (5 H, m, H_e + H_d), 2.01 (2 H, m, H_c), 1.53 (4 H, m, H_f), 1.41 (2 H, br m, H_g). *m/z* (ESI) 310 ($M + H^+$, $C_{16}H_{24}NO_3S$ requires 310.1).

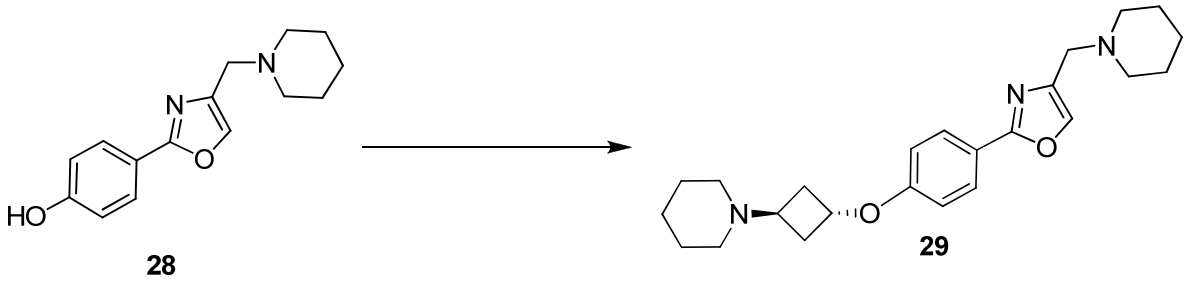




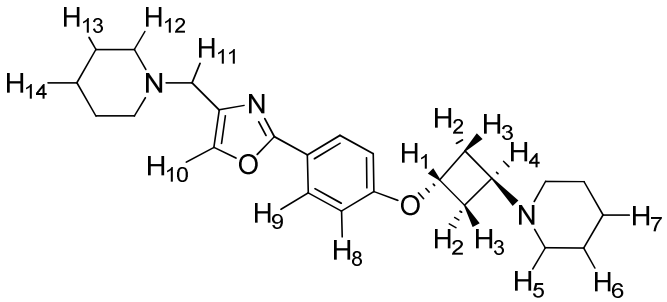
27 **28**

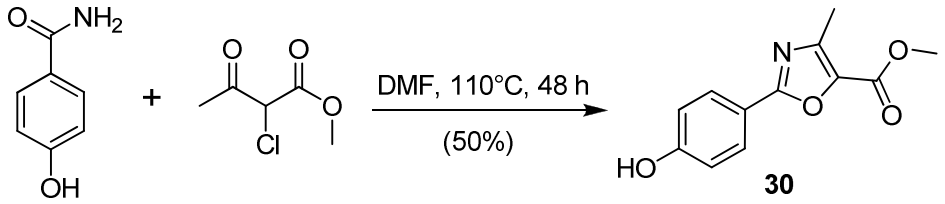
4-(4-(Piperidin-1-ylmethyl)oxazol-2-yl)phenol (28). A solution of 4-hydroxy-benzamide (0.4 g, 2.92 mmol) in propionitrile (15 mL) is treated with 1,3-dichloroacetone (0.37 g, 2.92 mmol) and the mixture is heated at 110°C for 20 h. The mixture is then concentrated under reduced pressure and the residue is mixed with EtOAc. The resulting solution is washed twice with satd. $NaHCO_3$-soln and dried over $MgSO_4$. Concentration affords crude **27** as a white solid (580 mg, 95 %), which is used directly in the next reaction. A total of crude **27** (1.56 g, 7.44 mmol) is dissolved in MeCN (50 mL). The solution is treated with piperidine (0.81 mL, 8.2 mmol), sodium iodide (0.22 g, 1.49 g) and potassium carbonate (2 g, 14.88 mmol). The suspension is stirred at reflux for 16 h. The mixture is then concentrated *in vacuo* and the residue is mixed with EtOAc and satd. $NaHCO_3$-soln. The organic layer is washed with an additional volume of satd. $NaHCO_3$-soln, dried over $MgSO_4$, filtered and concentrated *in vacuo* to yield the title product as a white solid (790 mg, 41%). δ_H (400 MHz; d_6-DMSO; Me_4Si) 7.90 (1 H, s, H_e), 7.78 (2 H, d, *J* 8.6, H_f), 6.87 (2 H, d, *J* 8.6, H_g), 3.36 (2 H, s, H_a), 2.39 (4 H, m, H_b), 1.45-1.53 (4 H, m, H_c), 1.34-1.40 (2 H, m, H_d).





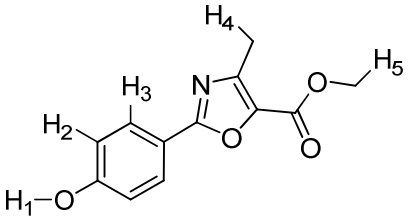
***Trans*-2-(4-(3-(piperidin-1-yl)cyclobutoxy)phenyl)-4-(piperidin-1-ylmethyl)oxazole (29).** A solution of phenol **28** (0.5 g, 1.94 mmol) in dry DMF (18 mL) is treated with sodium hydride (60% in mineral oil, 1.27 g, 2.9 mmol) at r.t. When gas evolution has subsided, tosylate **26** (0.6 g, 1.94 mmol) is added and the mixture is stirred at 85°C for 48 h. The mixture is then poured onto satd. aq. $NaHCO_3$-soln (10 mL) and extracted with ether (4 x 20 mL). The organic layers are combined, dried over $MgSO_4$, filtered and concentrated under reduced pressure. The oily residue is purified by column chromatography (CH_2Cl_2/MeOH/NH_3 95:4.5:0.5) to yield the title compound as a white solid (72 mg, 9 %). m.p.=120-121°C. δ_H (400 MHz; $CDCl_3$; Me_4Si) 7.95 (2 H, d, *J* 8.6, H_9), 7.51 (1 H, s, H_{10}), 6.82 (2 H, d, *J* 8.6, H_8), 4.79 (1 H, m, H_1), 3.48 (2 H, s, H_{11}), 2.95-3.05 (1 H, m, H_4), 2.22-2.56 (12 H, m, H_2 + H_3 + H_{12} + H_5), 1.55-1.66 (8 H, m, H_6 + H_{13}), 1.39-1.51 (4 H, m, H_7+H_{14}). *m/z* (ESI) 396.2646 ($M+H^+$, $C_{24}H_{34}N_3O_2$ requires 396.2651).

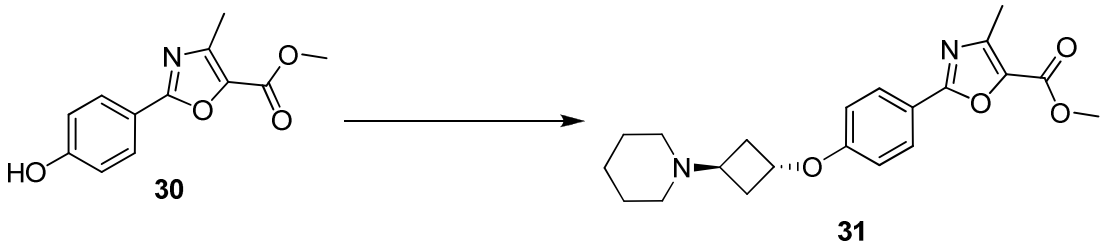




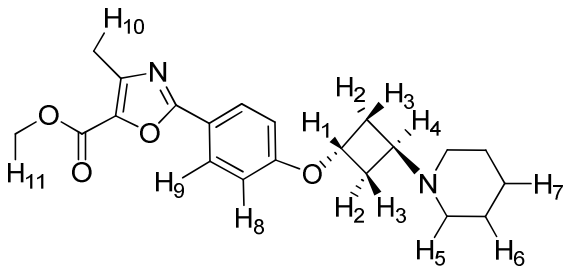
30

Methyl 2-(4-hydroxyphenyl)-4-methyloxazole-5-carboxylate (30). Prepared from 4-hydroxybenzamide and methyl 2-chloro-3-oxobutanoate in 50 % yield according to a procedure reported by us.[2] δ_H (400 MHz; d_6-DMSO; Me_4Si) 10.30 (1 H, m, H_1), 7.86 (2 H, d, J 8.6, H_3), 6.92 (2 H, d, J 8.8, H_2), 3.85 (3H, s, H_5), 2.43 (3 H, s, H_4).





Methyl *trans*-4-methyl-2-(4-(3-(piperidin-1-yl)cyclobutoxy)phenyl)oxazole-5-carboxylate (31). A solution of tosylate **26** (1 g, 3.23 mmol), phenol **30** (1.01 g, 4.35 mmol), potassium *tert*-butylate (0.98 g, 8.7 mmol) and tetrabutylammonium bromide (467 mg, 1.45 mmol) is stirred at 70°C in THF (60 mL) for 12 h. DMF (25 mL) is then added and the mixture is stirred for an additional 48 h at 100°C. The mixture is cooled and then poured into water. The aqueous phase is extracted twice with EtOAc. The combined organic layers are dried over $MgSO_4$ and concentrated *in vacuo*. The residual oil is purified by column chromatography eluting with a gradient of MeOH in CH_2Cl_2. This affords the title compound as a brown solid (346 mg, 32%). δ_H (400 MHz; $CDCl_3$; Me_4Si) 8.03 (2 H, d, *J* 8.8, H_9), 6.84 (2 H, br d, *J* 8.8, H_8), 4.80 (1 H, m, H_1), 3.93 (3 H, s, H_{11}), 3.00 (1 H, m, H_4), 2.52 (3 H, s, H_{10}), 2.42 (2 H, m, H_3), 2.31 (6 H, m, H_2+H_5), 1.61 (4 H, m, H_6), 1.47 (2 H, m, H_7). *m/z* (ESI) 371.1975 ($M+H^+$, $C_{21}H_{27}N_2O_4$ requires 371.1971).



NMR Spectra

Figure S1. ^{1}H-NMR spectrum of compound **18** in CD_3OD (400 MHz).

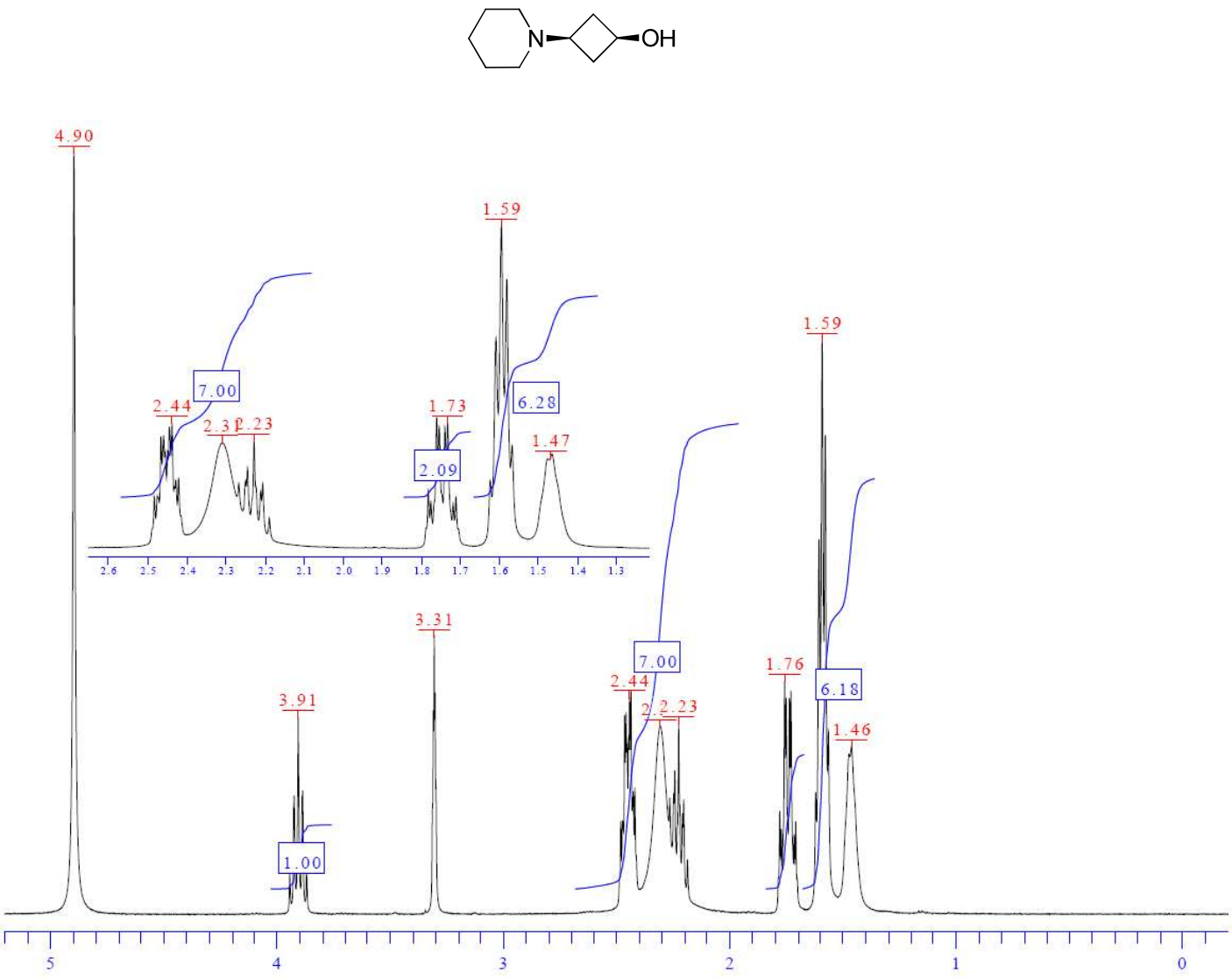
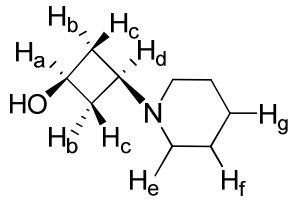


Figure S2. HSQC spectrum of compound **18** in $CDCl_3$.



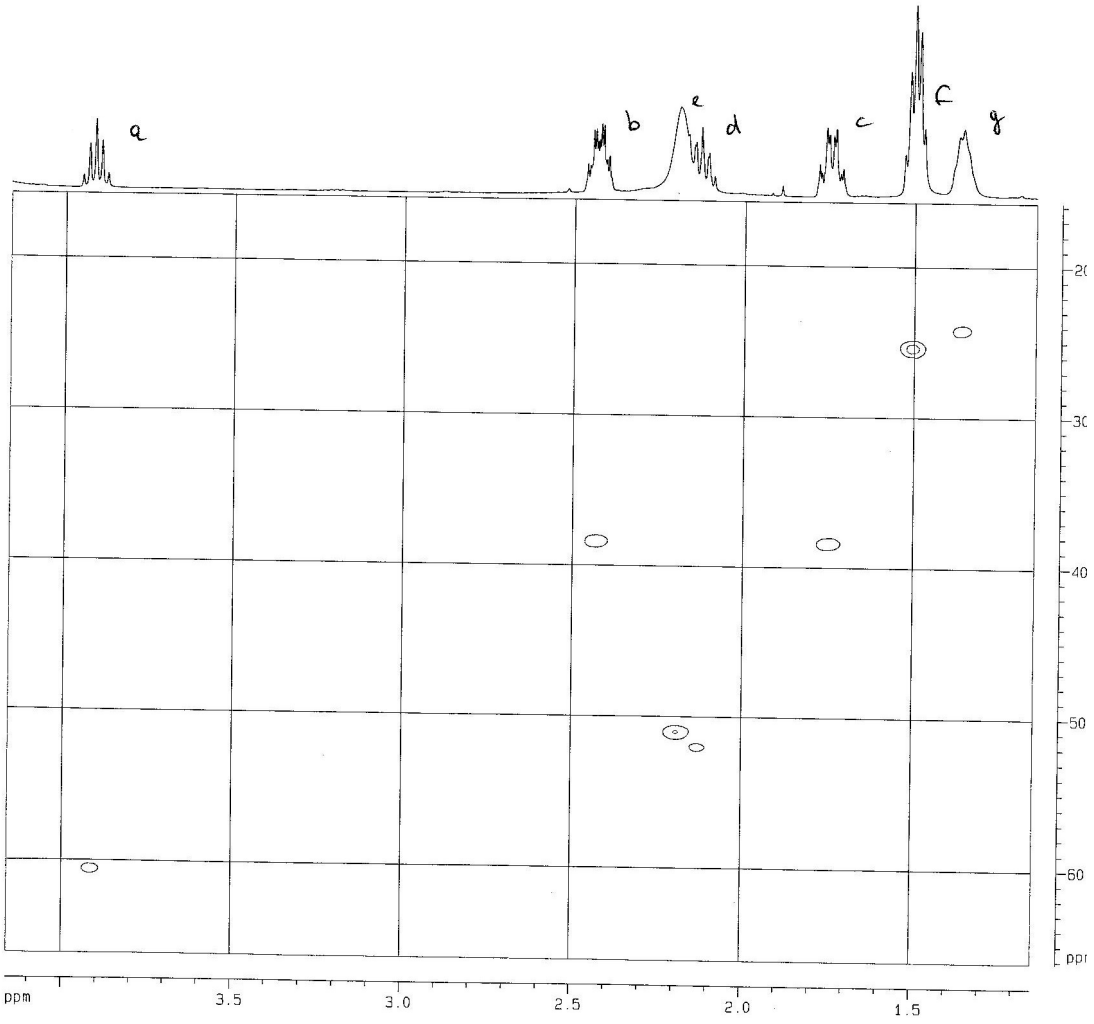


Figure S3. COSY spectrum of compound **18** in $CDCl_3$.

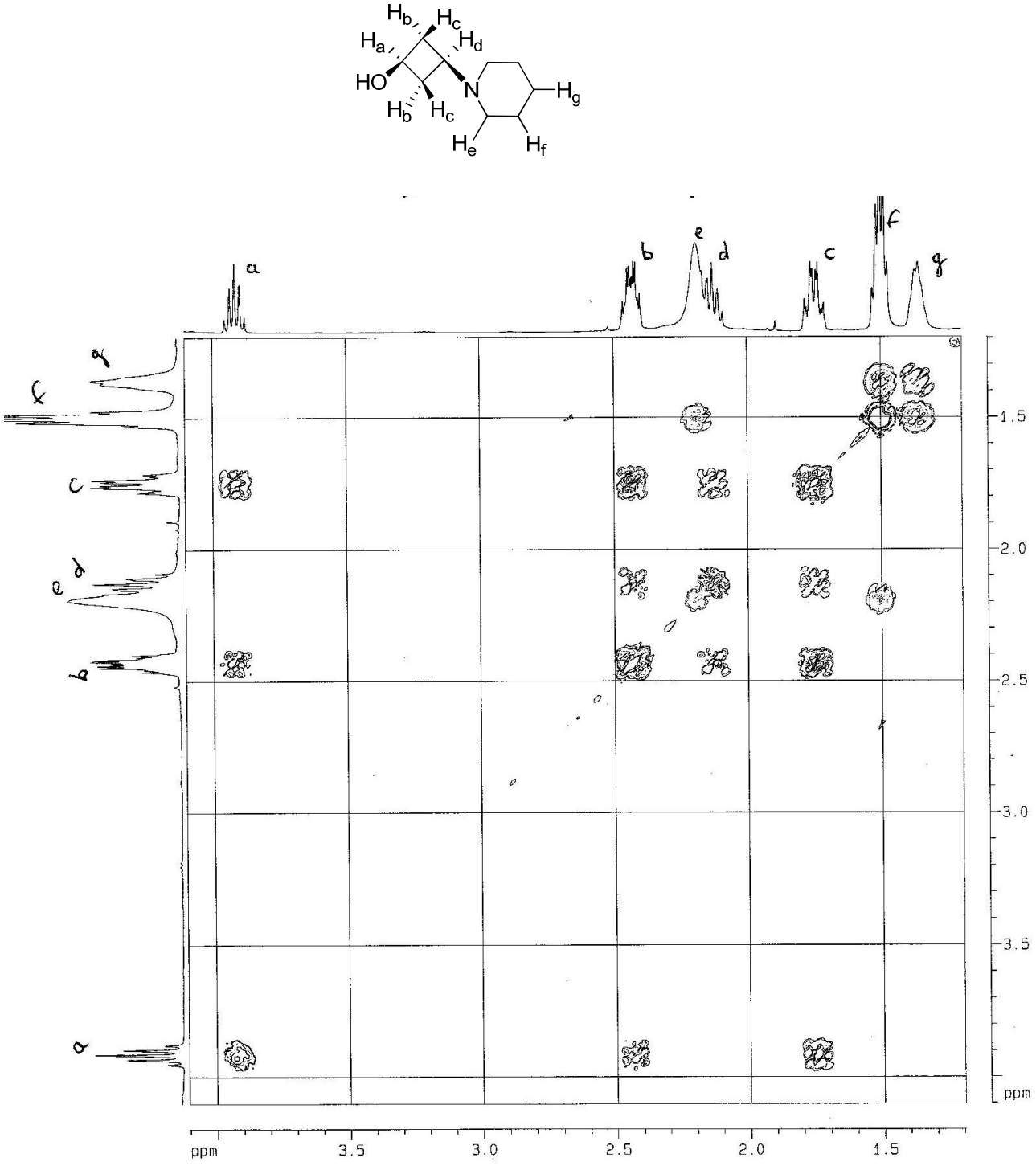


Figure S4. NOESY spectrum of compound **18** in $CDCl_3$.

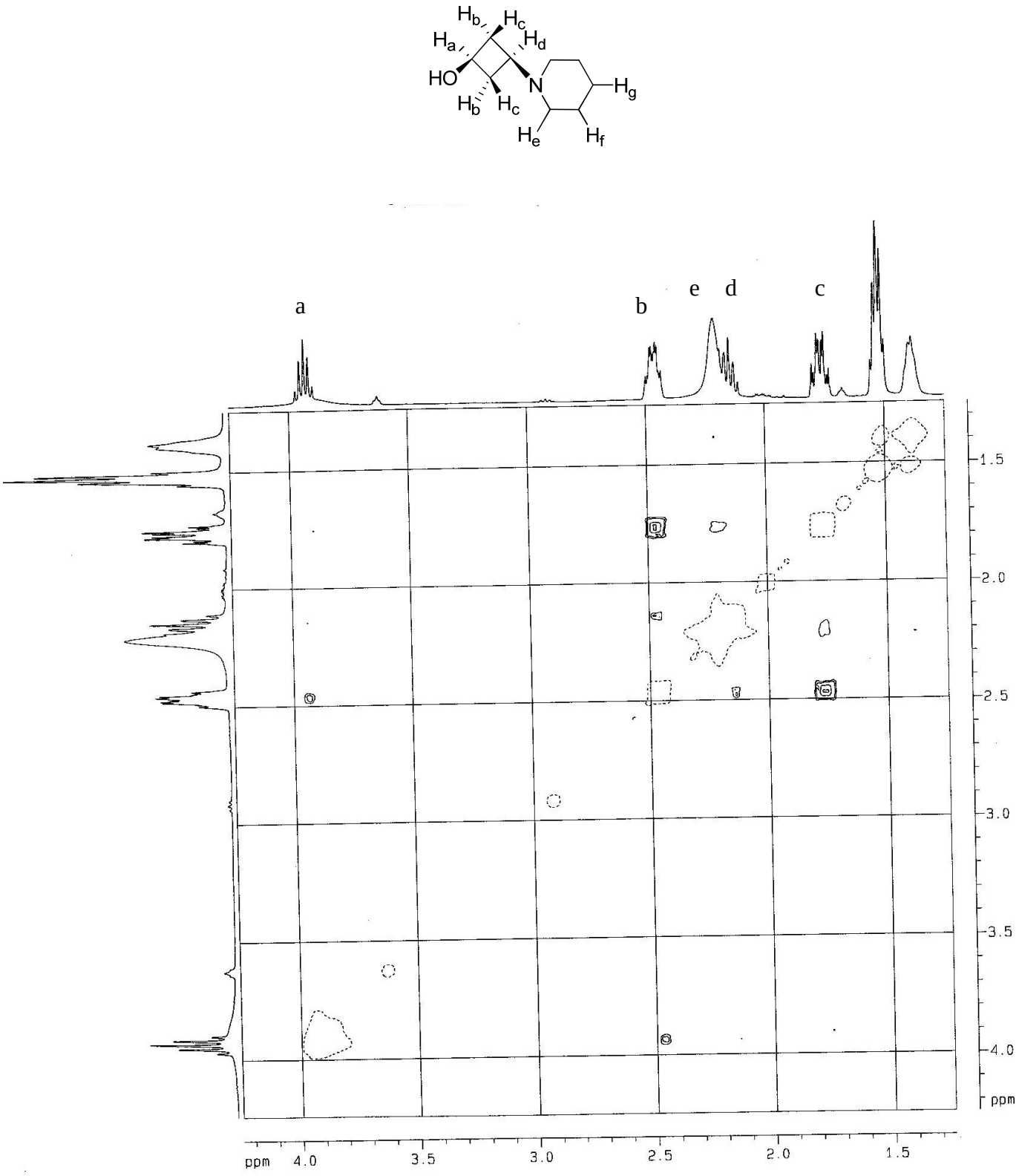


Figure S5. NOESY spectrum of compound **20** in $CDCl_3$.

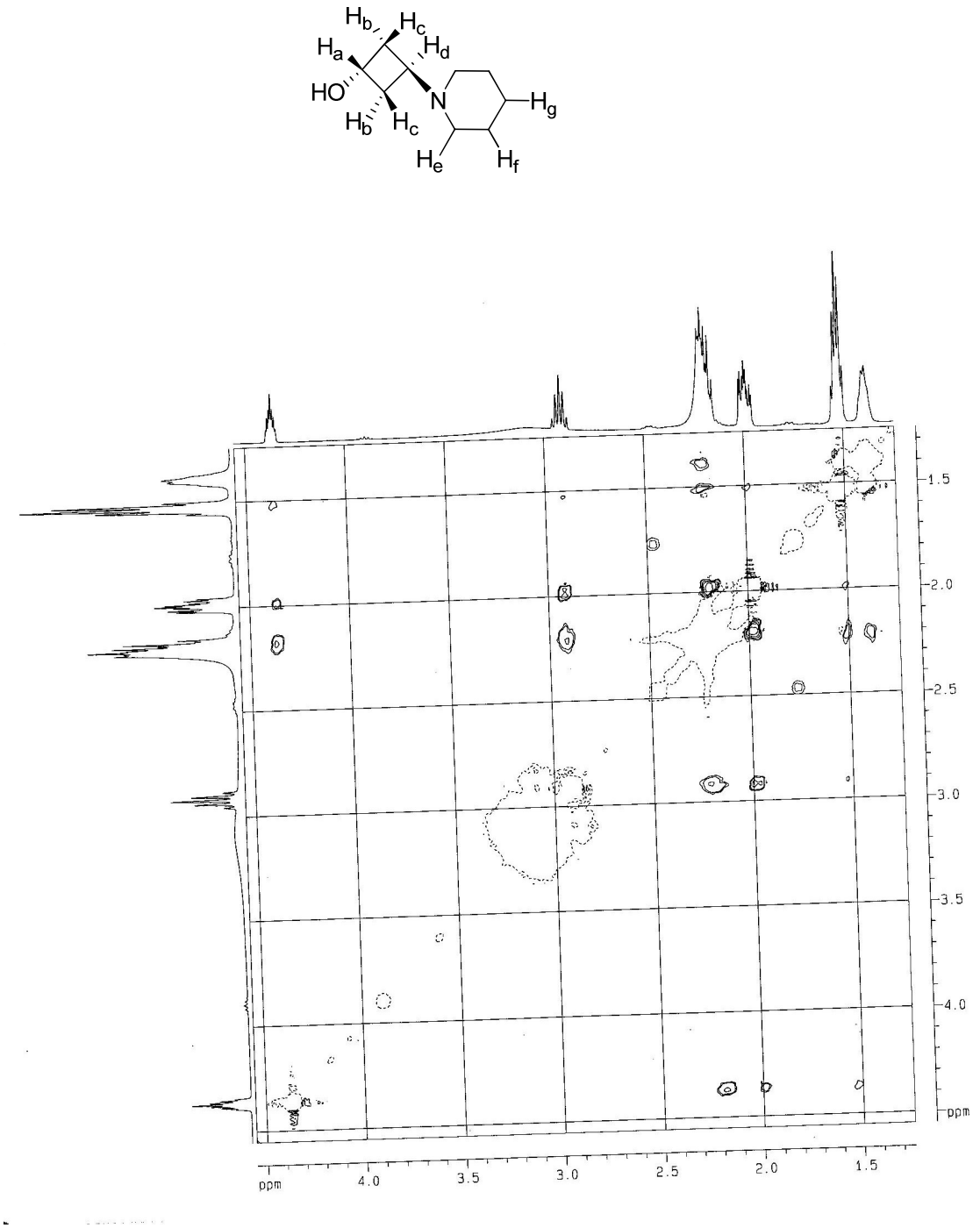


Figure S6. NOESY spectrum of compound **22** in $CDCl_3$.

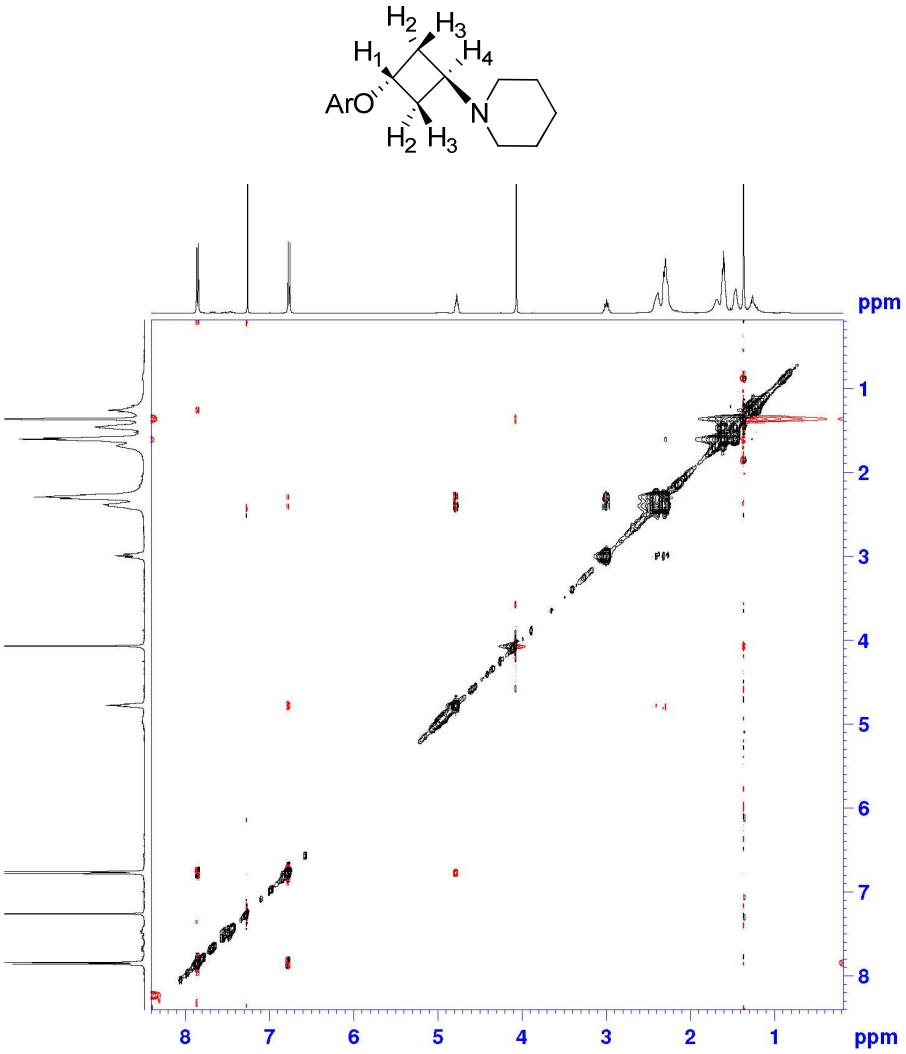


Figure S7. NOESY spectrum of compound **24** in $CDCl_3$.

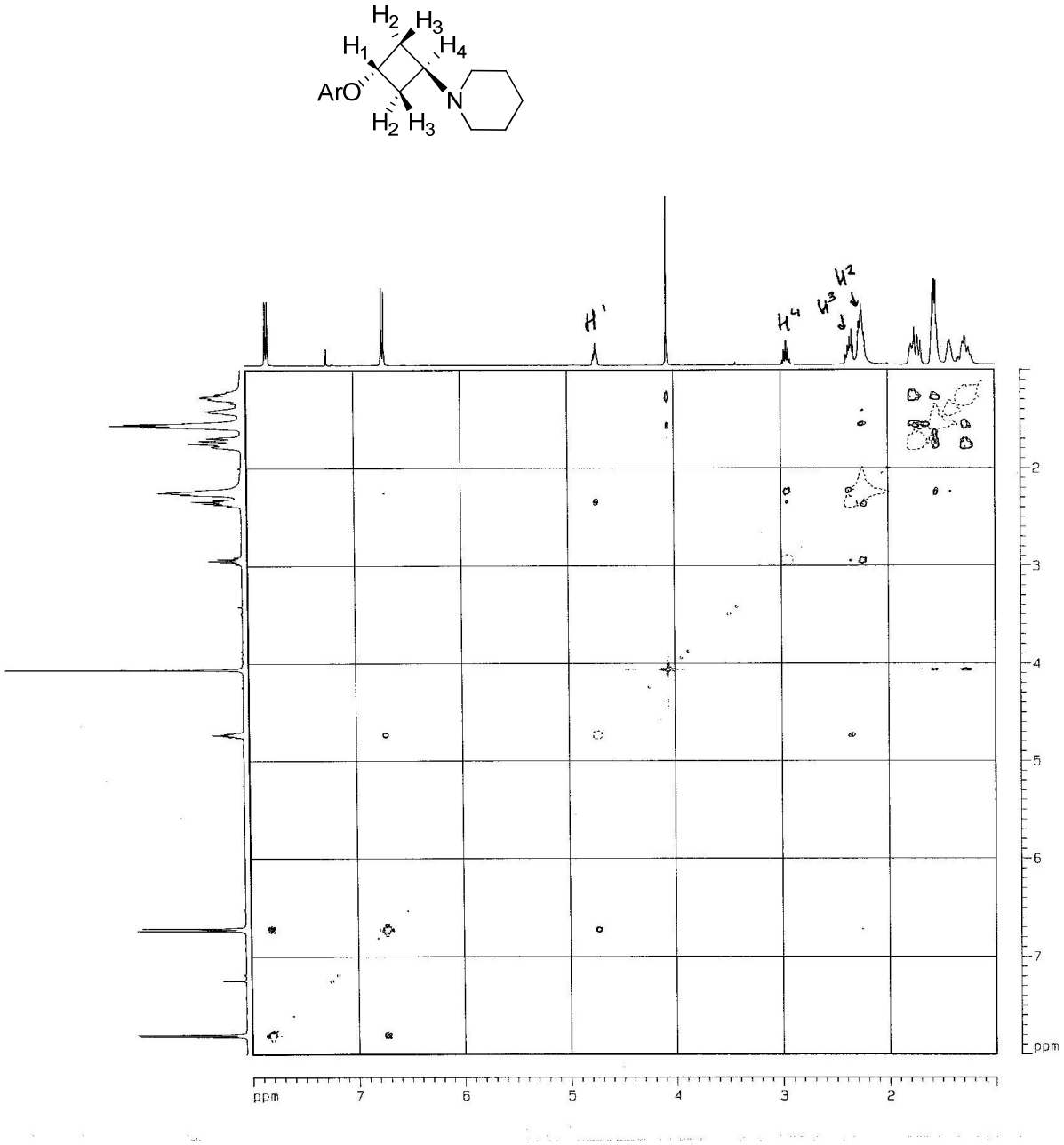
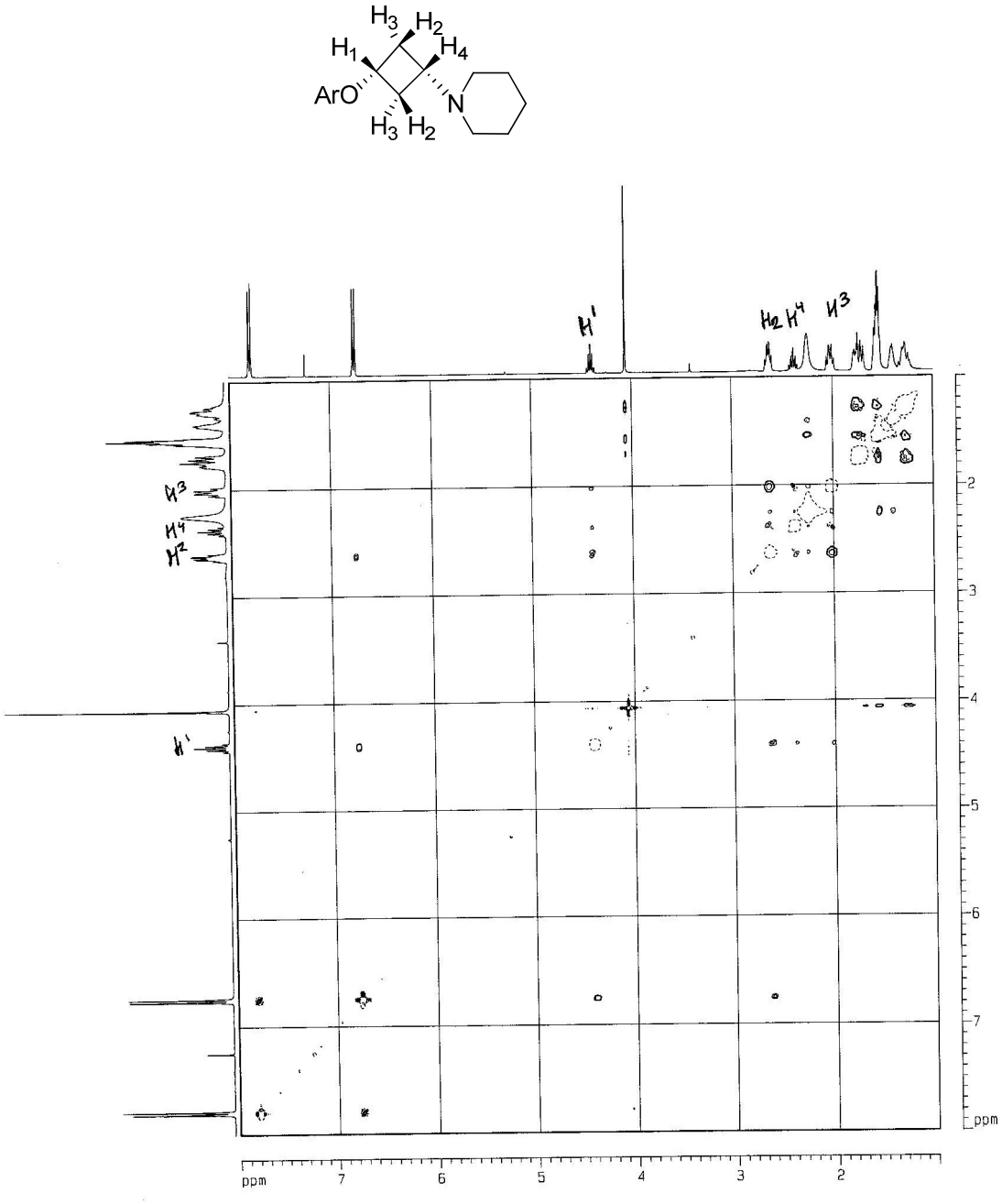


Figure S8. NOESY spectrum of compound **25** in $CDCl_3$.



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