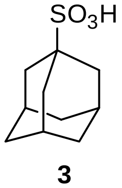
Supporting Information

Exploring the Requirements for the Hydrophobic Scaffold and Polar Amine in inhibitors of M2 from Influenza A Virus

Jun Wang, [#] Chunlong Ma, [§] Victoria Balannik, [§] Lawrence H. Pinto, [§] Robert A. Lamb, [¶, &] and William F. DeGrado [#,+,*]

Chemical Synthesis. All chemicals were purchased from commercial vendors and used without further purification unless otherwise noted. ^{1}H and ^{13}C NMR spectra were recorded on a DMX-360 NMR spectrometer. Chemical shifts are reported in parts per million referenced with respect to residual solvent ($CHCl_3$) 7.26 ppm (CH_3OH) 3.31 ppm and (DMSO-d_6) 2.50 ppm or from internal standard tetramethylsilane (TMS) 0.00 ppm. The following abbreviations were used in reporting spectra: *s*) singlet, *d*) doublet, *t*) triplet, *q*) quartet, *m*) multiplet, dd) doublet of doublets. All reactions were carried out under a N_2 atmosphere, unless otherwise stated. HPLC grade solvents were used for all the reactions. Column chromatography was performed using silica gel (230-400 mesh). Low-resolution mass spectra were obtained using an ESI technique on 3200 Q Trap LC/MS/MS system (applied biosystem). All compounds synthesized gave satisfactory MS, NMR purities. HRMS data for the synthesized compound were collected at LTQ Orbitrap XL mass spectrometer (ThermoFisher Scientific).



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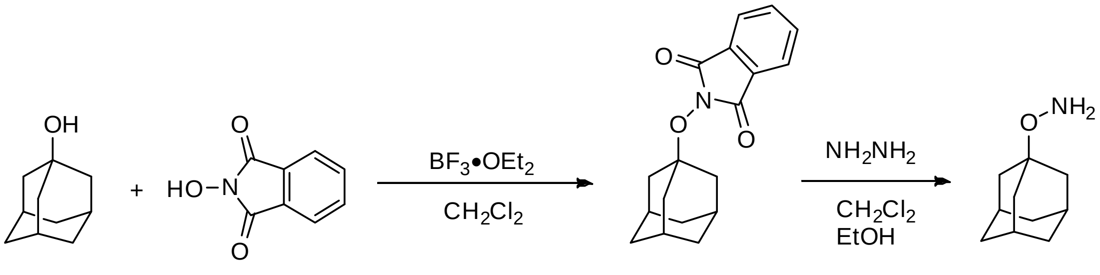
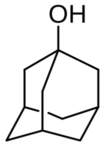


4

4 was synthesized according to the literature reported procedure.[1] (Yield: 72% over two steps). ^{1}H NMR (360 MHz, DMSO) δ 5.24 (m, 2H), 2.07 (br s, 3H), 1.65 (br s, 6H), 1.60-1.52 (m, 6H); ^{13}C NMR (90 MHz, DMSO) δ 74.13, 40.31, 36.15, 29.77; The calculated mass for $C_{10}H_{17}NO$ $(M + H)^+$ is 168.1, ESI-MS Found 168.2, HRMS Found 168.1383.



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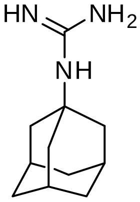
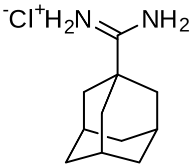


6

6 was synthesized according to the previous published procedure. [2] ^{1}H NMR (360 MHz, CD_3OD) δ 2.14-2.12 (m, 3H), 1.99-1.96 (m, 6H), 1.77-1.74 (m, 6H); ^{13}C NMR (90 MHz, CD_3OD) δ 154.26, 53.83, 42.51, 36.86, 31.03; The calculated mass for $C_{11}H_{19}N_3$ $(M + H)^+$ is 194.2, ESI-MS Found 194.7, HRMS found 194.1650.



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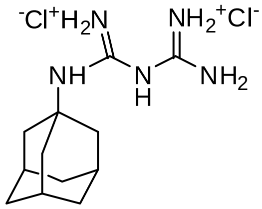

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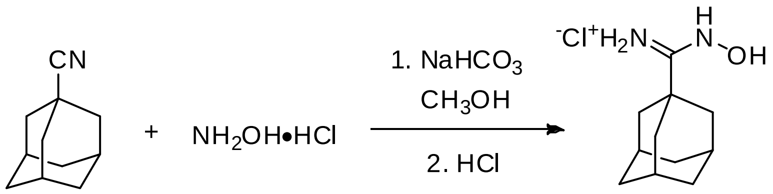
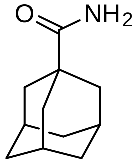


9

A suspension of 1-adamantyl carbonitrile (1.61g, 10mmol), hydroxylamine hydrochloride (1.04g, 15mmol) and $NaHCO_3$ (1.25g, 15mmol) in CH_3OH (25ml) was heated to reflux for 4hrs. After cooling down to rt, solvent was removed under reduced pressure and the crude product was extracted with ethyl acetate and H_2O. The organic layer was dried over $MgSO_4$ and filtered, 4M HCl in dioxane (3ml) was added to the filtrate and it was concentrated. The product was purified by flash column chromatography (5-15% CH_3OH/CH_2Cl_2) to give **9** as white solid (Yield 75%). ^1H NMR (360 MHz, CD_3OD) δ 2.06-2.04 (m, 4H), 2.02-2.00 (m, 3H), 1.86-1.84 (m, 2H), 1.80-1.72 (m, 6H); ^{13}C NMR (90 MHz, CD_3OD) δ 126.30, 41.09, 40.87, 37.92, 36.88, 29.88, 28.78; The calculated mass for $C_{11}H_{18}N_2O$ $(M + H)^+$ is 195.1, ESI-MS Found 195.0, HRMS found 195.1491.



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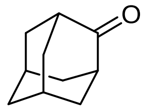

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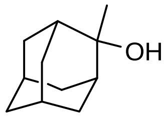

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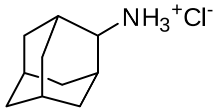

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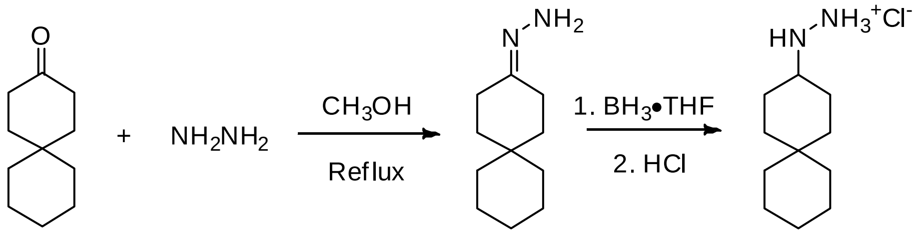
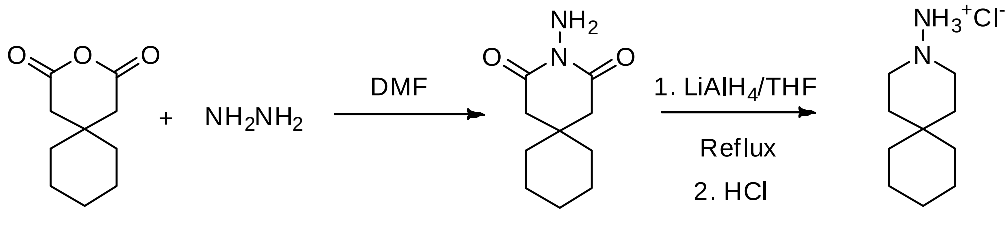


14

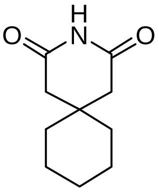
The synthesis of spiran hydrazine M2WJ124 started from spiro[5.5]undecan-3-one, which was prepared according to previous published procedure.[3] Spiro[5.5]undecan-3-one (1.66g, 10mmol) and hydrazine hydrate (0.50g, 10mmol) was refluxed in CH_3OH (20ml) for 3hrs.[4] The reaction mixture was cooled down to rt and concentrated in vacuo. The amino imine intermediate was used for the next step without purification. To a solution of the crude amino imine in THF (10ml) was added a solution of borane-THF (1M, 30ml, 30mmool) at room temperature. The reaction was stirred at room temperature for 15hrs and concentrated in vacuo. The residue was dissolved in 6M HCl (20ml) and heated to 100°C refluxing for 2hrs. After cooling to room temperature, the solution was basified to pH 12 with 6M NaOH and extracted with $CHCl_3$ three times. The combined organic layer was dried over MgSO4 and filtered. 4M HCl in dioxane (4ml) was added and the filtrate was concentrated under reduced pressure. The final product was purified by flash column chromatography (10-20% CH_3OH/CH_2Cl_2) to give **14** as white solid. (Yield: 68% over two steps). ^1H NMR (360 MHz, $CDCl_3$) δ 8.49 (br s, 4H), 3.44-3.37 (m, 1H), 2.02-2.01 (m, 2H), 1.81-1.73 (m, 4H), 1.39-1.36 (m, 8H), 1.19-1.11 (m, 2H), 1.10-1.04 (m, 2H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 61.21, 40.68, 34.37, 32.26, 32.04, 26.98, 23.12, 21.99, 21.84; The calculated mass for $C_{11}H_{22}N_2$ $(M + H)^+$ is 183.2, ESI-MS Found 183.7, HRMS found 183.1853.



17 **15**

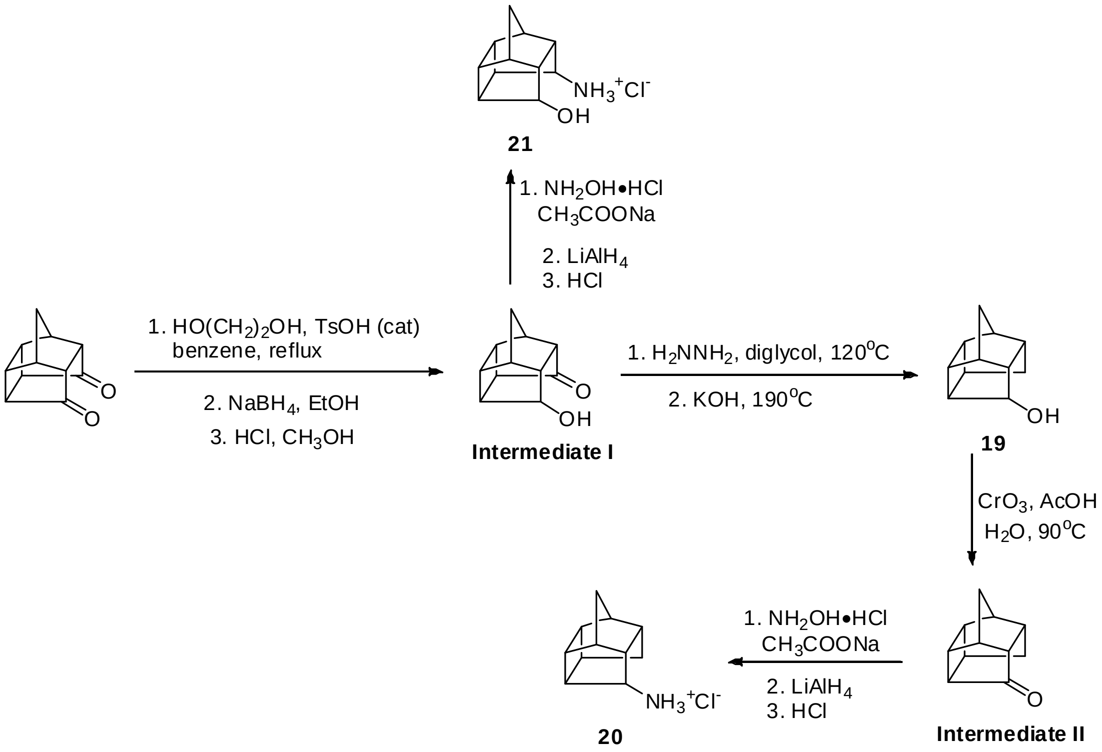
Hydrazine hydrate (0.5g, 10mmol) was added dropwise to a refluxing solution of 3-Oxaspiro[5,5]undecane-2,4-dione in DMF (20ml). After addition, the solution was slowly cooled down to room temperature. Solvent was removed under reduced pressure and the crude product was purified by flash column chromatography (2-5% CH_3OH/CH_2Cl_2) to give **17** as white solid. (Yield: 72%). ^1H NMR (360 MHz, CD_3OD) δ 2.52 (s, 2H), 2.46 (s, 2H), 1.53-1.46 (m, 10H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 176.14, 42.38, 37.12, 36.89, 27.05, 22.71; The calculated mass for $C_{10}H_{16}N_2O_2$ $(M + H)^+$ is 197.1, ESI-MS Found 197.2, HRMS found 197.1283.

15 was synthesized by $LiAlH_4$ reduction of **17**. To **17** (1.96g, 10mmol) in anhydrous THF was added $LiAlH_4$ (0.76g, 20mmol) at 0°C, the resulting slurry was heated to reflux for 4 hrs. The solution was cooled to 0°C and quenched with H_2O (0.75 mL), 15% NaOH (0.75 mL), and H_2O (2.25 mL) sequentially. The resulting slurry was filtered. The filtrate was dried over $MgSO_4$, filtered, concentrated *in vacuo*. Hydrochloride salt was made by adding 4M HCl in 1,4-dioxane to the ether solution of the above amine at 0°C, followed by centrifuge and decant the ether supernatant. The final product was purified by flash chromatography to give the hydrazine **15** as white solid. (Yield: 65%). ^1H NMR (360 MHz, $CDCl_3$) δ 2.60 (t, J = 5.52 Hz, 4H), 1.52 (t, J = 5.52 Hz, 4H), 142-1.40 (m, 6H), 1.33-1.26 (m, 4H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 56.05, 36.87, 36.44, 30.14, 26.99, 21.82; The calculated mass for $C_{10}H_{20}N_2$ $(M + H)^+$ is 169.2, ESI-MS Found 169.2, HRMS found 169.1698.



16

16 was purchased from Bosche Scientific, Cat #:P3149



Pentacyclo[$5.4.0.0^{2,6}.0^{3,10}.0^{5,9}$]undecane-8,11-dione was purchased from Sigma-Aldrich, Cat #309575, 98%

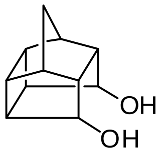
General procedure of converting ketone to amine

A solution of ketone (1mmol), NH_2OH•HCl (208.5mg, 3mmol) and CH_3COONa (328.1mg, 4mmol) in EtOH (10ml) and H_2O (1ml) was heated to reflux for 2hrs. Solvent was removed in vacuo and the residue was dissolved in CH_2Cl_2 and extracted with H_2O twice. The organic layer was dried with $MgSO_4$ and the solvent was removed in vacuo to give the oxime intermediate. Without further purification, the oxime was dissolved in anhydrous THF (10ml) and cooled to 0^oC on an ice-water bath. $LiAlH_4$ (0.4g, 10.5mmol) was added portion-wise to the stirring mixture. The solution was warmed up to ambient temperature and heated at reflux for 4hrs. The reaction was quenched by sequentially addition of 0.4 ml H_2O, 0.4 ml 15% NaOH solution and 1.2 ml H_2O. The resulting slurry was filtered and the solvent was removed in vacuo. 4M HCl in 1,4-dioxane (1ml) was added to the oily residue and the solvent was removed again in vacuo to give white solid crude product which was subsequently purified by silica gel flash column chromatography to yield the final amino hydrochloride salt.

The ketone alcohol intermediate I was synthesized according to the literature procedure without modification.[5] It was converted to the amino alcohol **21** according to the above general procedure of converting ketone to amine. (Yield: 75%). ^{1}H NMR (360 MHz, DMSO) δ 8.55 (br s, 3H), 7.09 (br s, 1H), 3.76-3.75 (m, 1H), 2.94-2.93 (m, 1H), 2.72-2.712 (m, 1H), 2.62-2.61 (m, 1H), 2.55-2.54 (m, 1H), 2.35-2.34 (m, 1H), 2.27-2.22 (m, 2H), 1.59 (d, J = 7.56 Hz, 1H), 1.59-1.45 (m, 2H), 1.00 (d, J = 7.56 Hz, 1H); ^{13}C NMR (90 MHz, DMSO) δ 69.41, 49.07, 44.23, 44.20, 42.82, 42.42, 42.25, 37.30, 35.43, 33.52, 20.31; The calculated mass for $C_{11}H_{15}NO$ $(M + H)^+$ is 178.2, ESI-MS Found 178.2, HRMS found 178.1226.

19 was synthesized from reduction of intermediate I according to the published procedure. [5] (Yield: 80%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.95-3.93 (m, 1H), 2.79-2.70 (m, 1H), 2.69-2.50 (m, 2H), 2.48-2.35 (m, 2H), 2.32-2.15 (m, 4H), 1.72-1.69 (m, 1H), 1.50 (br s, 1H), 1.19-1.12 (m, 1H), 1.11-1.06 (m, 1H). ^{13}C NMR (90 MHz, $CDCl_3$) δ 74.60, 47.25, 45.98, 43.34, 42.25, 42.23, 40.14, 39.07, 36.16, 35.36, 29.02; The calculated mass for $C_{11}H_{14}O$ $(M + H)^+$ is 163.1, ESI-MS Found 162.8, HRMS found 163.1542.

20 was synthesized from the ketone intermediate II according to the above general procedure of converting ketone to amine. (Yield: 78%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 8.23 (br s ,3H), 3.26-3.24 (m, 1H), 2.92-2.89(m, 2H), 2.68-2.58 (m, 4H), 2.32-2.30(m, 2H), 1.89-1.74 (m, 2H), 1.30-1.21 (m, 2H). ^{13}C NMR (90 MHz, $CDCl_3$) δ 51.69, 47.14, 44.83, 43.69, 42.07, 41.26, 40.57, 37.15, 35.37, 34.48, 29.58; The calculated mass for $C_{11}H_{15}N$ $(M + H)^+$ is 162.2, ESI-MS Found 162.1, HRMS found 162.1276.



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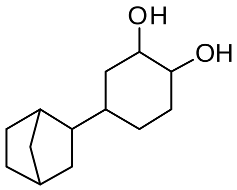

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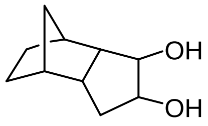

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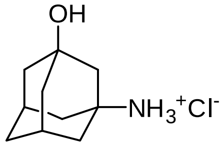

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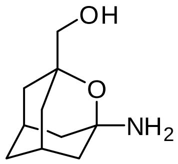

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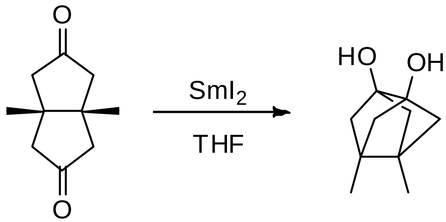
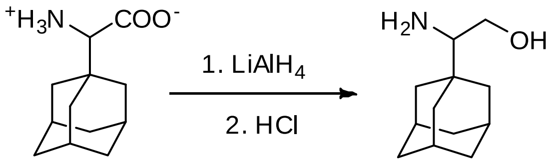


26

26 was synthesized according to the literature reported procedure using SmI_2 reduction. [6] (Yield: 32%). ^1H NMR (360 MHz, $CDCl_3$+CD_3OD) δ 1.70 (d, J = 4.68 Hz, 4H), 1.54 (d, J = 4.68 Hz, 4H), 1.04 (s, 6H). ^{13}C NMR (90 MHz, $CDCl_3$+CD_3OD) δ 78.92, 56.92, 41.85, 16.37; The calculated mass for $C_{10}H_{16}O_2$ $(M + H)^+$ is 169.1, ESI-MS Found 169.4, HRMS found 169.1672.

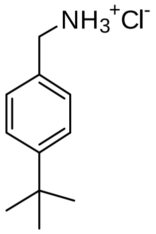
General procedure of $LiAlH_4$ mediated reduction

In anhydrous THF was added $LiAlH_4$ (X g) at 0°C, the resulting slurry was heated to reflux for 4 hrs. The solution was cooled to 0°C and quenched with H_2O (X mL), 15% NaOH (X mL), and H_2O (3X mL) sequentially. The resulting slurry was filtered. The filtrate was dried over $MgSO_4$, filtered, concentrated *in vacuo*. Hydrochloride salt was made by adding 4M HCl in 1,4-dioxane to the ether solution of the above amine at 0°C, followed by decanting the ether supernatant. The final product was purified by flash chromatography to give the ammonium hydrochloride.



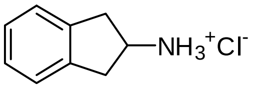
27

27 was synthesized according to the above general procedure of $LiAlH_4$ mediated reduction. The starting material 1-Adamantyl(amino)acetic acid was purchased from Matrix Scientific, Cat #: 027469. (Yield: 82%). ^1H NMR (360 MHz, CD_3OD) δ 3.88 (dd, $J = 11.52$ Hz, 3.6 Hz, 1H), 3.62-3.57 (m, 1H), 2.78 (dd, $J = 9.18$ Hz, 3.6 Hz, 1H), 2.03 (br s, 3H), 1.81-1.74 (m, 6H), 1.68-1.66 (m, 6H). ^{13}C NMR (90 MHz, CD_3OD) δ 64.01, 59.27, 39.47, 37.75, 35.16, 29.65; The calculated mass for $C_{12}H_{21}NO$ $(M + H)^+$ is 196.2, ESI-MS Found 196.2, HRMS found 196.1697.



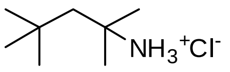
28

28 4-tert-butylbenzylamine was purchased from Sigma-Aldrich. Cat #631280 97%



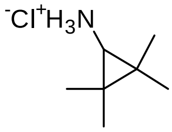
29

29 2-aminoindan was purchased from Sigma-Aldrich. Cat # 479128. 97%



30

30 tert-Octylamine was purchased from Sigma-Aldrich, Cat #: O6000 95%



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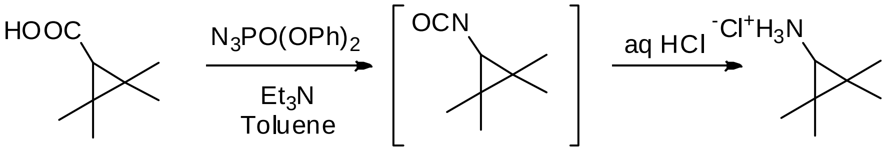
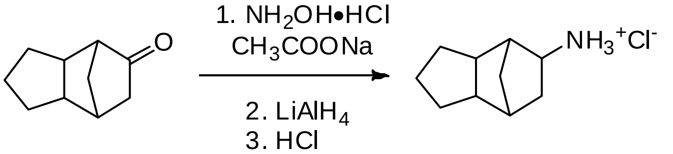
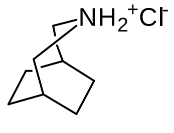


Diphenylphosphorylazide (DPPA) (1.29 ml, 6 mmol) and triethylamine (0.84ml, 6mmol) were added to a solution of 2,2,3,3-Tetramethylcyclopropanecarboxylic acid (0.71 g, 5 mmol) in 10 ml toluene at ambient temperature. The reaction was heated to reflux for 3 hours. The mixture was concentrated in vacuo. An additional 10 ml CH_2Cl_2 and 10 ml H_2O were added to the reaction mixture, the organic layer was separated and washed sequentially with H_2O, saturated $NaHCO_3$ and brine and dried over $MgSO_4$. Solvent was removed by rotary evaporation and 50ml of 6 N HCl was added. The solution was stirred at ambient temperature for 3 hours. NaOH solution was added until pH 10, the resulting solution was extracted by CH_2Cl_2 (30ml) for 3 times. 4M HCl in dioxane (3 ml) was added to the combined CH_2Cl_2 layers and the mixture was dried over $MgSO_4$. Further flash column chromatography purification gave 2,2,3,3-tetramethylcyclopropanaminium chloride as a white power (0.83g, Yield: 82%). ^{1}H NMR (360 MHz, CD_3OD) δ 3.35 (s, 1H), 1.16 (s, 12H); ^{13}C NMR (90 MHz, CD_3OD) δ 43.02, 23.24, 22.19; The calculated mass for $C_7H_{15}N$ $(M + H)^+$ is 114.1, ESI-MS Found 114.2, HRMS found 114.1274.



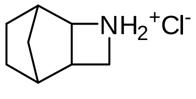
32

32 was synthesized according to the above general procedure of converting ketone to amine. (Yield: 78.2%) ^{1}H-NMR (360 MHz, CD_3OD) δ 3.49-3.44 (m, 1H), 2.25-2.24 (m, 1H), 2.11-2.05 (m, 3H), 2.00-1.93 (m, 3H), 1.72-1.70 (m, 1H), 1.60-1.57 (m, 1H), 1.29-1.20 (m, 2H), 1.04-0.93 (m, 3H); ^{13}C-NMR (90 MHz, CD_3OD) δ 50.98, 43.69, 40.69, 38.66, 33.95, 32.07, 31.71, 31.50, 26.36; The calculated mass for $C_{10}H_{17}N$ $(M + H)^+$ is 152.1, ESI-MS Found 152.5, HRMS found 152.1431.

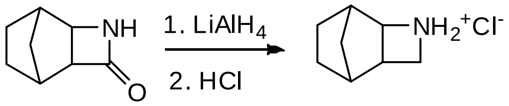


33

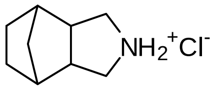
33 3-Azabicyclo[3.2.2]nonane 99% was purchased from ChemService Inc. Cat # 2040



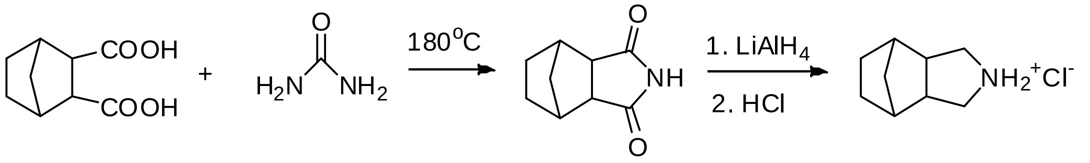
34



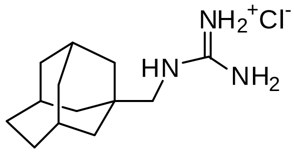
4-Oxo-3-aza-tricyclo[4.2.1.0(2.5)]nonane was purchased from Fisher Scientific Cat #26810. It was reduced to **34** with $LiAlH_4$ according to the general procedure of $LiAlH_4$ reduction described above. (Yield: 80%). ^{1}H NMR (360 MHz, CD_3OD) δ 4.40-4.36 (m, 1H), 4.19-4.17 (m, 1H), 3.85-3.82 (m, 1H), 2.78-2.10 (m, 4H), 1.70-1.64 (m, 3H), 1.19-1.16 (m, 2H); ^{13}C NMR (90 MHz, CD_3OD) δ 65.80, 48.26, 38.96, 36.97, 36.93, 30.49, 25.25, 22.63; The calculated mass for $C_8H_{13}N$ $(M + H)^+$ is 124.1, ESI-MS Found 124.2, HRMS found 124.1119.



35

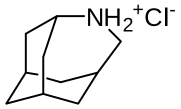


Bicyclo[2.2.1]heptane-2,3-dicarboxylic Acid was purchased from TCI America, Cat #N0753. The diacid (1.84g, 10mmol) and urea (2.40g, 40mmol) was heated to 180°C under N_2 atmosphere for 3hrs. When it was cooled down to room temperature, anhydrous THF was added, and the solution was cooled to 0°C with ice bath. $LiAlH_4$ (3.04g, 80mmol) was added portionwise to the solution, and heated to reflux for 4hrs. The solution was cooled down to 0°C with ice bath, 3ml of H_2O, 3ml 15% NaOH and 9ml of H_2O was sequentially added. The resulting slurry was filtered. 5ml of 4M HCl in 1,4-dioxane was added and the solvent was removed under reduced pressure. The crude product was purified by flash column chromatography (10% CH_3OH/CH_2Cl_2) to give **35** as white solid (1.24g, Yield: 72.0%). ^{1}H NMR (360 MHz, $CDCl_3$) δ 3.32-3.30 (m, 2H), 3.26-3.21 (m, 2H), 2.93-2.90 (m, 2H), 2.36-2.34 (m, 2H), 1.78-1.74 (m, 1H), 1.68-1.65 (m, 1H), 1.58-1.51 (m, 4H); ^{13}C NMR (90 MHz, $CDCl_3$) δ 47.11, 45.78, 44.34, 41.30, 23.23; The calculated mass for $C_9H_{15}N$ $(M + H)^+$ is 138.1, ESI-MS Found 138.2, HRMS found 138.1275.

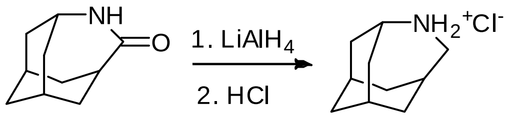


36

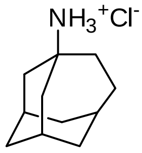
36 was purchased from Chembridge Corporation. Cat # 5875453.



37

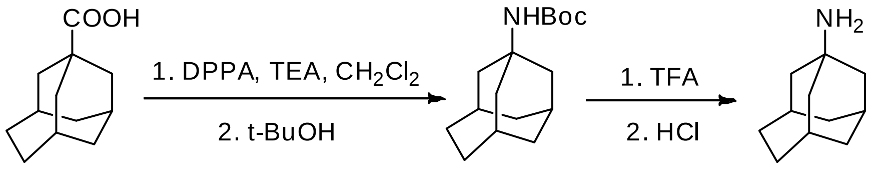


4-Azatricyclo[4.3.1.13,8]undecan-5-one was purchased from Sigma-Aldrich. Cat #403164. 98%. It was reduced to **37** according to the above general procedure of $LiAlH_4$ mediated reduction. (Yield: 82%). ^{1}H-NMR (360 MHz, CD_3OD) δ 2.80-2.77 (m, 3H), 1.84-1.82 (m, 1H), 1.61-1.52 (m, 6H), 1.36-1.31 (m, 2H), 1.17-1.36 (m, 4H); ^{13}C-NMR (90 MHz, CD_3OD) δ 53.72, 52.61, 37.17, 34.98, 34.87, 32.09, 27.27; ESI-MS: Calculated for $C_{10}H_{17}N$ $(M + H)^+$ 152.2, ESI-MS Found: 152.3, HRMS found 152.1432.



38

38 was purchased from Matrix Scientific, Cat #:038588



39

39 was synthesized according to literature reported procedure using curtius rearrangement reaction.[7, 8] The starting material was purchased from Matrix Scientific, Cat #: 037551 (Yield: 76%). ^{1}H-NMR (360 MHz, CD_3OD) δ 2.28-2.24 (m, 2H), 1.96-1.91 (m, 4H), 1.89-1.82 (m, 4H), 1.76-1.72 (m, 4H), 1.55-1.52 (m, 2H); ^{13}C-NMR (90 MHz, CD_3OD) δ 53.91, 43.16, 42.46, 40.72, 38.90, 37.36, 35.87, 33.76, 32.54, 30.98, 28.38; ESI-MS: Calculated for $C_{11}H_{19}N$ $(M + H)^+$ 166.2, ESI-MS Found: 166.3, HRMS found 166.1590.

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