# Functionalized Derivatives of 2-azaspiro[3.3]heptane-1-carboxylic Acid and 7-oxa-2-azaspiro[3.5]nonane-1-carboxylic Acid for Drug Design

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2-azaspiro[3.3]heptane-1-carboxylic acid and 7-oxa-2-azaspiro[3.5]nonane-1-carboxylic acid, which had been reported as bioisoster of well-known pipecolic acid, were subjected to chemical transformations, resulting in a number of functionalized derivatives. The obtained molecules contained diversified functional groups, allowing their incorporation in bioactive compounds in versatile modes. Described synthetic approaches afforded multigram-scaled synthesis of the desired compounds with good yields, thus being applicable in drug design.

## Introduction

The concept of "Escape from Flatland" has changed modern trends in drug design dramatically [1]. Its influence has raised a significant interest in creating novel 3D-shaped $Fsp^3$-rich chemical structures [2, 3]. Bioisosteric replacement is among the most efficient strategies for the improvement of pharmacological properties of bioactive molecules, as it allows retaining or enhancing target activity profile [4]. Spirocyclic bioisosters are of particular attention due to their improved metabolic stability towards oxidative enzymes, which is considered a weak spot for piperidine-containing structures.

The fragment of 2-azaspiro[3.3]heptane gained significant attention in the past decades, since it had been reported to mimic piperidine in bioactive compounds back in 2010 [5] (**Figure 1**). This concept is now regarded as common [6].

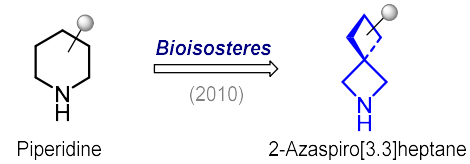



**Figure 1.** 2-azaspiro[3.3]heptane as bioisoster of piperidine.

In our recent work we have for the first time reported the synthesis of 2-azaspiro[3.3]heptane-1-carboxylic acid and validated its incorporation to drug [7]. Replacing piperidine fragment in local anesthetic drug *Bupivacaine* with the spirocyclic amino acid resulted in an enhanced activity of the analog with longer duration of action (**Figure 2**, top).

Expanding the scope of the developed strategy towards multifunctional analogs of 2-substituted piperidines led us to a number of promising spirocyclic compounds, including 7-oxa-2-azaspiro[3.5]nonane-1-carboxylic acid. Incorporating the latter into *Bupivacaine* structure resulted not only in the comparable activity and ADME properties of the analog, but also in its 5-times lower toxicity over the original compound and increased water solubility [8] (**Figure 2**, bottom).

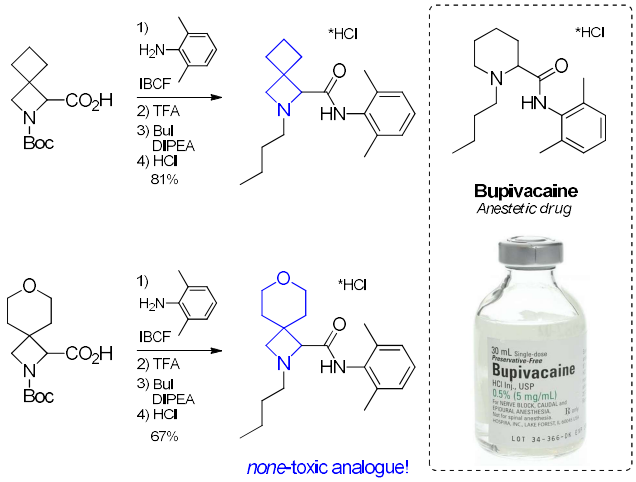



**Figure 2.** Spirocyclic analogues of the anesthetic drug *Bupivacaine.*

Thus, incorporating 3D-shaped $Fsp^3$-rich piperidine bioisosters into *Bupivacaine* molecule not only enhances lipophilicity and water solubility, but also influences the duration and potency of its anesthetic action. The keystone of controlled molecule physical and chemical properties modification are substituents. Manageable functionalization is supposed to facilitate lipophilicity, water solubility and metabolic stability fine-tuning in bioactive molecules.

Herein we report the synthesis of functionalized derivatives of 2-azaspiro[3.3]heptane-1-carboxylic acid and 7-oxa-2-azaspiro[3.5]nonane-1-carboxylic acid, which may be diversely exploited in drug discovery.

## Experimental part

*Material and methods*

Starting materials and reactants were of commercial grade and were used without additional purification. Reaction solvents were anhydrous. NMR spectra were recorded at 400 and 500 MHz $^1$H frequencies. NMR spectra were calibrated using conventional deuterium lock referencing at the machine, and were not referenced thereafter. Mass spectra were recorded on an LCMS instrument with chemical ionization (CI) or a GCMS instrument with electron impact ionization (EI).

*Synthesis* (**Scheme 1**)

*tert-butyl-1-(hydroxymethyl)-2-azaspiro-[3.3]heptane-2-carboxylate* ***(3a)*** *–* ***procedure 1****.* 19.201 g (0.506 mol, 1 equiv.) of $LiAlH_4$ was dissolved in 3 L of absolute THF. The reaction mixture was cooled down to -40°C under constant argon flow. To this mixture, a solution of 2-tert-butyl 1-methyl 2-azaspiro[3.3]heptane-1,2-dicarboxylate (2) (129.171 g, 0.506 mol, 1 equiv.) in 350 mL of absolute THF was added dropwise over a period of 15 minutes. The mixture was allowed to warm up to 0°C and stirred at this temperature for 1 hour. It was then

cooled to -20°C, and the excess of $LiAlH_4$ was carefully quenched with 20% aqueous sodium bislulfate solution (100 mL), changing its color from grey to yellowish. After that, the reaction mixture was treated with 1 L of 20% aqueous sodium bislulfate solution, the organic phase was separated, dried over anhydrous $Na_2SO_4$ and evaporated to yield **3a** as yellow oil (97 g, 0.427 mol, 84.3% yield). $^1$H NMR (400 MHz, C*D*Cl$_3$) δ 4.15 (dd, J = 8.0, 3.2 Hz, 1H), 4.01 – 3.64 (m, 4H), 2.30 – 1.99 (m, 3H), 2.02 – 1.67 (m, 3H), 1.46 (s, 9H). MS (APCI) m/z [M-(*t*-Bu)+H] calculated for $C_8H_{14}NO_3$: 172.2; found: 172.0.

*tert-butyl-1-(hydroxymethyl)-7-oxa-2-azaspiro[3.5]nonane-2-carboxylate* ***(3b)*** was obtained using **procedure 1** as yellowish oil (76 g, 91.1% yield). $^1$H NMR (400 MHz, C*D*Cl$_3$) δ 4.19 (br. s, 1H), 3.94 – 3.72 (m, 3H), 3.70 (d, *J* = 3.5 Hz, 2H), 3.39 (ddd, *J* = 14.5, 8.9, 3.6 Hz, 2H), 1.85 (td, *J* = 12.2, 4.2 Hz, 1H), 1.79 – 1.60 (m, 3H), 1.48 (S, 9H). MS (APEI) m/z [M-(*t*-Bu)] calculated for $C_9H_{15}NO_4$: 201.22; found: 201.0.

*(2-methyl-2-azaspiro[3.3]heptan-1-yl)-methanol* (4a) – procedure ***2***. $LiAlH_4$ (40.851 g, 1.076 mol, 3.8 equiv.) was added in several portions to a reactor containing anhydrous tetrahydrofuran (2 L) under argon, and the mixture was cooled down to 0 °C. Trimethylsilyl chloride (118.48 g, 1.091 mol, 3.85 equiv.) was added dropwise at the rate, which allowed keeping the temperature below 15 °C. The resulting mixture was stirred at that temperature for 2 hours to allow complete transformation to alane. The mixture was cooled down to 0 °C. A solution of 2-tert-butyl 1-methyl 2-azaspiro[3.3]heptane-1,2-dicarboxylate **(2)** (72.320 g, 0,283 mol, 1 equiv.) in anhydrous tetrahydrofuran (100 mL) was added dropwise, while keeping the temperature below 10 °C. This reaction mixture was stirred at room temperature for 36 hours. It was then cooled to 10 °C and carefully quenched by dropwise addition of 40% aqueous sodium hydroxide solution (50 mL) and water (100 mL). The inorganic solids were filtered out and discarded. The filtrate was concentrated under reduced pressure, and the residue was purified by vacuum distillation (0.3 Torr, 53°C) to yield **4a** as colorless liquid (27 g, 0.191 mol, 67.5% yield). $^1$H NMR (500 MHz, C*D*Cl$_3$) δ 3.71 – 3.59 (m, 2H), 3.56 (dd, *J* = 7.1, 1.0 Hz, 1H), 3.11 (s, 1H), 2.92 – 2.80 (m, 2H), 2.37 (dt, *J* = 11.5, 8.4 Hz, 1H), 2.29 (s, 3H), 2.02 – 1.86 (m, 3H), 1.82 (dq, *J* = 11.1, 8.2 Hz, 1H), 1.77 – 1.64 (m, 1H). MS (APEI) m/z [M] calculated for $C_8H_{15}NO$: 141.21; found: 141.0.

*(2-methyl-7-oxa-2-azaspiro[3.5]nonan-1-yl)methanol* ***(4b)*** was prepared using **procedure 2**. The product was purified by vacuum distillation to yield **4b** as colorless liquid which solidifies upon standing (31 g, 0.181 mol, 55.6% yield). M.p. 65 °C. $^1$H NMR (400 MHz, C*D*Cl$_3$) δ 3.85 (dt, *J* = 11.7, 3.9 Hz, 1H), 3.75 (dt, *J* = 11.5, 4.0 Hz, 1H), 3.68 (d, *J* = 4.9 Hz, 2H), 3.54 – 3.41 (m, 2H), 3.36 (td, *J* = 11.4, 2.6 Hz, 1H), 3.21 – 3.00 (m, 1H), 2.84 (t, *J* = 4.9 Hz, 1H), 2.72 (d, *J* = 7.1 Hz, 1H), 2.40 (s, 3H), 1.87 (dd, *J*

= 7.0, 4.0 Hz, 2H), 1.77 (ddd, *J* = 13.0, 11.1, 4.5 Hz, 1H), 1.54 (dt, *J* = 13.2, 3.0 Hz, 1H). MS (APCI) m/z [M+H] calculated for $C_9H_{18}NO_2$: 171.2; found: 171.2.

*tert-butyl-1-formyl-2-azaspiro[3.3]-heptane-2-carboxylate* ***(5a) – procedure 3***. **3a** (50.45 g, 0.222 mol, 1 equiv.) was dissolved in 1.5 L of dry DCM, and the solution was cooled to 0°C. Dess-Martin periodinane (103.55 g, 0.244 mol, 1.1 equiv.) was added to this solution in several portions, and the resulting slurry was stirred at the ambient temperature for 2 hours. After this period, the reaction mixture was poured into 2 L of saturated aqueous sodium bicarbonate solution at vigorous stirring. After 15 minutes of stirring, the organic phase was separated, dried over anhydrous $Na_2SO_4$ and evaporated. The residue was purified by flash column chromatography (MTBE/hexanes 1:5) to yield **5a** as colorless oil (21 g, 0.280 mol, 42% yield). $^1$H NMR (400 MHz, C*D*Cl$_3$) δ 9.71 (s, 1H), 4.32 (s, 1H), 4.00 (s, 2H), 2.31 (q, *J* = 9.4 Hz, 1H), 2.26 – 2.10 (m, 2H), 2.02 – 1.73 (m, 3H), 1.44 (s, 9H). MS (APEI) m/z [M-(*t*-Bu)] calculated for $C_8H_{11}NO_3$: 169.18; found: 169.1.

*tert-butyl-1-formyl-7-oxa-2-azaspiro-[3.5]nonane-2-carboxylate* ***(5b)*** was prepared using **procedure 3.** The product was purified by vacuum distillation (0.1 Torr, 121 °C) to yield **5b** as yellow oil, which solidifies upon standing (41 g, 56.4%). M.p. 44 °C. $^1$H NMR (400 MHz, C*D*Cl$_3$) δ 9.80 (s, 1H), 4.19 (s, 1H), 3.81 (q, *J* = 8.1 Hz, 3H), 3.77 – 3.68 (m, 1H), 3.60 – 3.49 (m, 1H), 3.49 – 3.39 (m, 1H), 1.96 (ddd, *J* = 13.6, 9.4, 4.0 Hz, 1H), 1.81 (ddd, *J* = 13.5, 9.3, 4.0 Hz, 2H), 1.64 (d, *J* = 13.7 Hz, 1H), 1.45 (s, 9H). MS (APEI) m/z [M-(*t*-Bu)] calculated for $C_9H_{13}NO_4$: 199.21; found: 198.97.

*tert-butyl-1-ethynyl-2-azaspiro[3.3]-heptane-2-carboxylate* ***(6a)*** – ***procedure 4***. Potassium carbonate (62.45 g, 0.452 mol, 2 equiv.) was suspended in 1 L of dry methanol. 50.9 g of **5a** was added to the reaction mixture in one portion. The mixture was cooled down to 10°C, and dimethyl-1-diazo-2-oxopropylphosphonate (65.11 g, 0.339 mol, 1.5 equiv.) was added dropwise, and the mixture was stirred at the ambient temperature for 3 hours. It was then diluted with water (1.5 L), and extracted with MTBE (2 x 1 L). The combined organic extracts were additionally washed with water (2 x 1 L), dried over anhydrous $Na_2SO_4$ and evaporated. The residue was purified by vacuum distillation (0.3 Torr, 86°C) to give **6a** as yellow oil (39 g, 0.176 mol, 78% yield). $^1$H NMR (500 MHz, C*D*Cl$_3$) δ 4.60 – 4.52 (s, 1H), 3.82 (q, *J* = 8.4 Hz, 2H), 2.60 (s, 1H), 2.53 (d, *J* = 12.2 Hz, 1H), 2.14 (ddt, *J* = 10.0, 8.2, 1.9 Hz, 2H), 2.09 – 1.95 (m, 1H), 1.88 – 1.76 (m, 2H), 1.44 (s, 9H). MS (APEI) m/z [M-(*t*-Bu)] calculated for $C_9H_{11}NO_2$: 165.19; found: 165.0.

*tert-butyl-1-ethynyl-7-oxa-2-azaspiro-[3.5]nonane-2-carboxylate* ***(6b)*** was prepared using **procedure 4**. The product was purified by flash column chromatography (MTBE/Hexane 1:20) to afford **6b** as colorless liquid (24 g, 95.6

mmol, 61.1% yield). $^1$H NMR (400 MHz, C*D*Cl$_3$) δ 4.41 (s, 1H), 3.83 (dt, *J* = 10.5, 4.2 Hz, 1H), 3.77 – 3.64 (m, 2H), 3.64 – 3.48 (m, 3H), 2.56 (s, 1H), 2.00 (ddd, *J* = 12.6, 8.0, 3.6 Hz, 1H), 1.88 – 1.69 (m, 3H), 1.47 (d, *J* = 2.1 Hz, 9H). MS (APEI) m/z [M-(*t*-Bu)] calculated for $C_{10}H_{13}NO_3$: 195.22; found: 195.07.

*1-ethynyl-2-azaspiro[3.3]heptan-2-ium trifluoroacetate* ***(7a·TFA)*** – ***procedure 5***. **6a** (10 g, 45 mmol, 1 equiv.) was dissolved in 250 mL of dry DCM. Trifluoroacetic acid (20.61 g, 181 mmol, 4 equiv.) was added to this solution in one portion, and the mixture was stirred at the ambient temperature overnight. The volatiles were then evaporated, and the residue was vacuum-dried to yield **7a·TFA** as orange viscous oil (10.58 g, 45 mmol, quantitative yield). $^1$H NMR (500 MHz, DMSO-$d_6$) δ 9.40 (br. d, 2H), 5.05 (s, 1H), 3.91 (d, *J* = 10.4 Hz, 1H), 3.74 (d, *J* = 10.4 Hz, 1H), 2.41 (ddd, *J* = 12.0, 9.3, 6.7 Hz, 1H), 2.13 (t, *J* = 8.2 Hz, 3H), 1.82 – 1.64 (m, 2H). MS (APCI) m/z [M+H] calculated for $C_8H_{11}N$: 122.18; found: 122.2.

*1-ethynyl-7-oxa-2-azaspiro[3.5]nonan-2-ium trifluoroacetate* ***(7b·TFA)*** was obtained using **procedure 5** as brown oil (7.21 g, 27.2 mmol, quantitative yield). $^1$H NMR (400 MHz, DMSO-$d_6$) δ 9.54 (br. d, 2H), 4.19 (s, 1H), 3.89 – 3.64 (m, 3H), 3.64 – 3.53 (m, 1H), 3.47 (ddd, *J* = 12.0, 8.2, 3.9 Hz, 1H), 3.37 (ddd, *J* = 12.0, 8.8, 3.2 Hz, 1H), 2.54 (s, 1H), 1.90 – 1.64 (m, 4H). MS (APCI) m/z [M+H] calculated for $C_9H_{13}NO$: 152.21; found: 152.2.

**Results and discussion**

The synthesis of functionalized derivatives of both acids **1a** and **1b** started with converting the latter ones to subsequent methyl esters **2a** and **2b** using well known procedure, involving reaction of the acid with methyl iodide in the presence of DIPEA [9]. The reaction proceeded in mild conditions with excellent yields. The obtained esters could be reduced with lithium aluminum hydride to afford the corresponding BOC-protected amino esters **3a** and **3b**. At the same time, reduction in harder conditions by using alane resulted in N-Me-amino alcohols **4a** and **4b**. Compounds **3a** and **3b** possessed appropriate purity directly after the standard work-up, and were used in the next steps with no further purification, while compounds **4a** and **4b** were additionally purified by vacuum distillation.

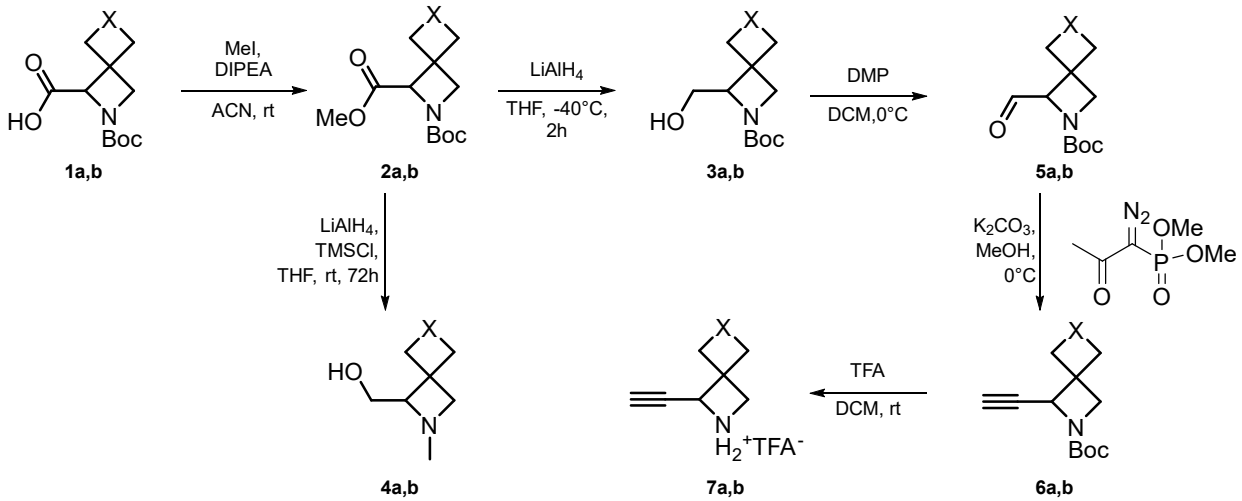
As far as building blocks containing aldehyde functional group are highly appreciated in organic synthesis due to a large scope of possible modifications of the latter moiety [10], we next attempted to convert **3a** and **3b** to corresponding aldehydes. Indeed, reaction with Dess-Martin periodinane [11] efficiently converted the corresponding BOC-protected alcohols to **5a** and **5b**. Purification of compound **5a** was performed by means of flash column chromatography, while compound **5b** required vacuum distillation to achieve the desired purity.

It is widely reported that aldehydes are facile synthons for the preparation of alkynes

using the Seyferth–Gilbert homologation [12]. Considering the growing demand of modern organic synthesis in functionalized alkynes [13], we next studied the possibility to subject **4a** and **4b** to this transformation. Both **5a** and **5b** readily afforded the corresponding alkynes **6a** and **6b** after the reaction with Bestmann-Ohira reagent [14] in the presence of potassium carbonate. Purification of the title compounds included vacuum distillation for **6a** and flash column chromatography for **6b**. Deprotection of both **6a** and **6b** proceeded easily by treatment of the latter with trifluoroacetic acid in DCM. Deprotected

**Table 1.** Summary of the synthetic results

| Compound | Yield, % | Scale, g | Purification |
|---|---|---|---|
| **3a** | 84.3 | 97 | no |
| **3b** | 91.1 | 76 | no |
| **4a** | 67.5 | 27 | distillation |
| **4b** | 55.6 | 31 | distillation |
| **5a** | 42.0 | 21 | FC |
| **5b** | 56.4 | 41 | distillation |
| **6a** | 78.0 | 39 | distillation |
| **6b** | 61.1 | 24 | FC |
| **7a** | 100 | 10.58 | no |
| **7b** | 100 | 7.21 | no |




**Scheme 1.** Synthesis of 2-azaspiro[3.3]heptane-1-carboxylic acid and 7-oxa-2-azaspiro[3.5]nonane-1-carboxylic acid derivatives; X = -$CH_2$- for **a** and –$CH_2OCH_2$- for **b**.

piperidine.alkynes **7a** and **7b** were produced with appropriate purity as trifluoroacetates by simple evaporation of the reaction mixture resulting in a quantitative yield.

It is noteworthy, that all the described transformations were scaled up to produce multigram quantities of the title compounds. Purification procedures were applied to achieve ≥95% purity of the desired products, unless they possessed the denoted purity directly after isolation from the reaction mixture. The results are summarized in **Table 1**.

## Conclusions

Herein we have synthesized and characterized ten novel functionalized derivatives of 2-azaspiro[3.3]heptane-1-carboxylic acid and 7-oxa-2-azaspiro[3.5]nonane-1-carboxylic acid. Considering the reported ability of the mentioned acids to mimic pipecolic acid fragment in bioactive molecules, the described compounds reveal significant prospects of being exploited in drug development. All the products were obtained in multigram quantities with decent yields. The synthetic approaches reported are facile and scalable, making it possible to apply them to a larger scope of spirocyclic blocks.

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