# Bicyclic Bioisosteres of Piperidine: Version 2.0

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***Dedicated to Ukraine***

**Introduction**. Piperidine is a popular molecule. Its ring is among the top-3 most frequently used in medicinal chemistry[1] and is found in the structure of >100 drugs. [2]

In 2010, 2-azaspiro[3.3]heptane was proposed to mimic piperidine in bioactive compounds (Figure 1a). [3] This concept became common,[4] and analogous mimetics for 2-, 3- and 4-substituted piperidines have been subsequently developed.[5,6,7] During the past decade, 2-azaspiro[3.3]heptanes appeared in at least 100 research manuscripts, 500 patents, and 7.000 newly reported compounds (Figure 1a). [8] Moreover, the numbers continue to grow (Figure 2).

In this work, we synthesed, characterized, and validated biologically *in vivo* 1-azaspiro[3.3]heptane. Our work enables the use of this molecular scaffold as a new generation of piperidine bioisosteres (Figure 1b).

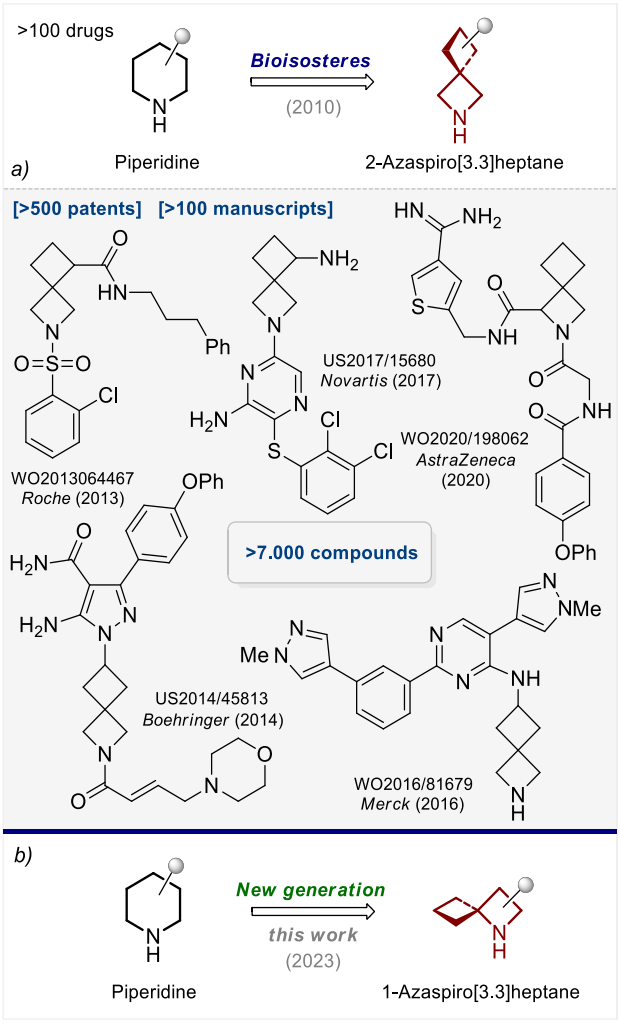



**Figure 1**. Bioisosteres of piperidine: (a) common - 2-azaspiro[3.3]heptane; (b) a new generation - 1-azaspiro[3.3]heptane (this work).

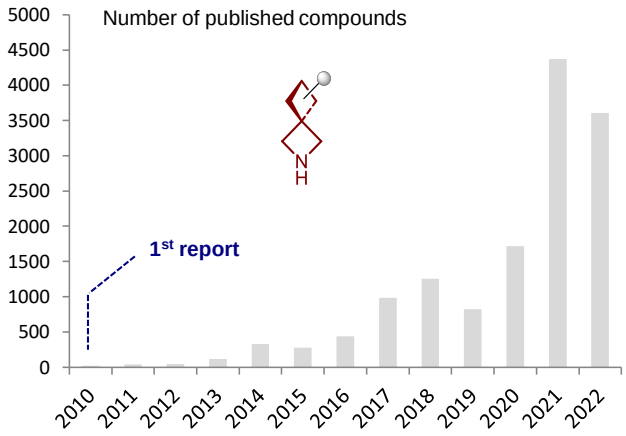


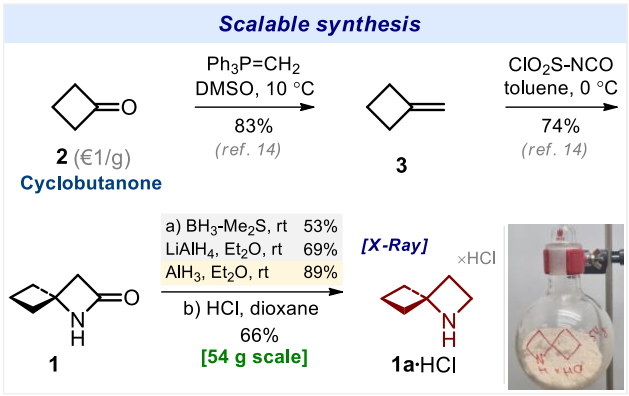

**Figure 2**. The number of 2-azaspiro[3.3]heptane published per year.

**Design**. Not surprisingly, *poly*-substituted 1-azaspiro[3.3]heptanes were known in the literature. [9,10] We needed, however, a modular approach that would produce compounds with only *one* substituent (one exit vector) at the core without additional (poly)substitution (Figure 1b). From the structural standpoint, we hoped that such compounds could mimic the *mono*-substituted piperidines.

Interestingly, even though the unsubstituted 1-azaspiro[3.3]heptane is already commercially available,[11] and its *N*-modifications can already be found in patents; [12] its preparation has not been yet reported. Only preparation of its *N*-benzyl derivative was described on a milligram scale. [13] Therefore, we needed first to develop a practical method towards 1-azaspiro[3.3]heptanes using available starting materials. From a literature survey, we noticed that β-lactam **1** (Scheme 1) was known. [14] Its reduction would directly provide the desired 1-azaspiro[3.3]heptane, however, for unclear reasons it has net been tried before. We were afraid indeed that the strained β-lactam **1** upon an attempted reduction would undergo a ring opening,[15] but nonetheless still decided to try it.

**Synthesis**. The synthesis of 1-azaspiro[3.3]heptane commenced from the commercially available cyclobutanone (**2**) (Scheme 1). Wittig reaction gave alkene **3**, which after the addition of Graff isocyanate, $ClO_2SNCO$, [16] afforded lactam **1** according to the literature procedure. [14] Its reduction was challenged next. Indeed, the reaction with $BH_3$•$Me_2S$ did produce the target amine **1a**, however, a significant ring-opening of the β-lactam ring into the corresponding amino alcohol also took place (Scheme 1). An analogous result was obtained with $BH_3$•THF. Reduction with $LiAlH_4$ worked better, but the cleavage of the β-lactam ring was still observed. [17] Finally, we could obtain the desired amine **1** in an excellent 89% yield using alane, $AlH_3$. The key reduction was attempted next on a multigram scale. After the reaction, the crude product was converted into

hydrochloride to obtain the pure amine **1a**•HCl as a white solid with a 66% isolated yield. Its structure was confirmed by X-ray analysis. [18] Despite the lower yield, this optimized protocol allowed us to synthesize 54 g of the compound in a single run.



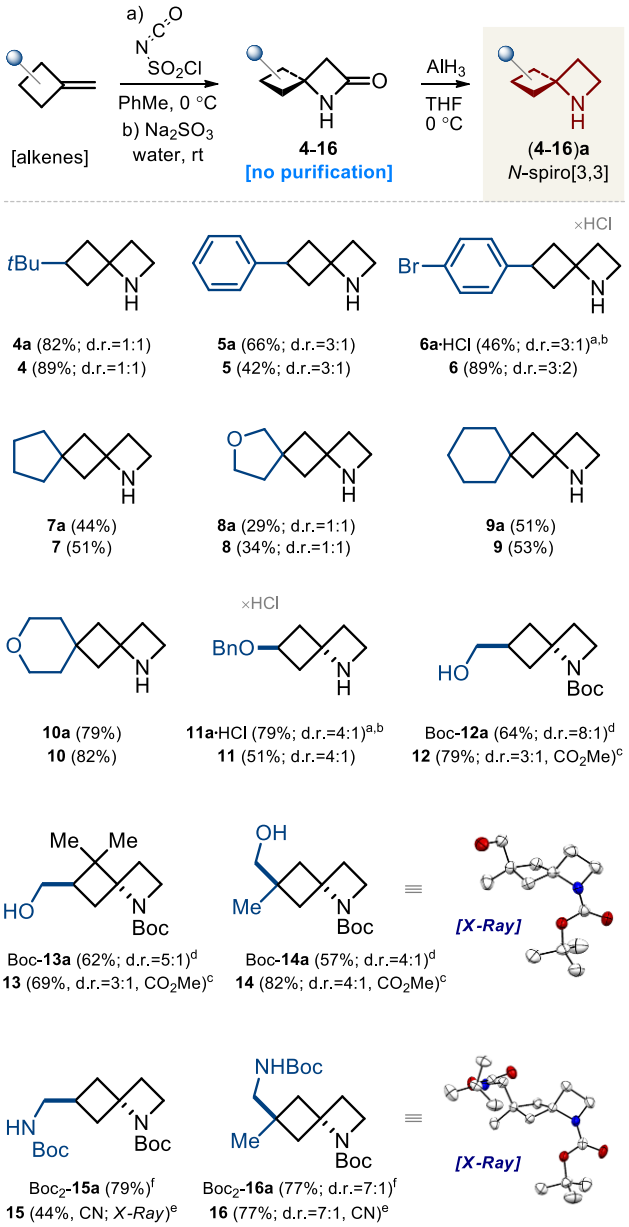

**Scheme 1**. Scalable synthesis amine **1a**·HCl.

**Scope**. Next, we studied the generality of the developed approach. Various substituted alkenes, most of which were obtained by the Wittig reaction of the corresponding cyclobutanones, were tested. Indeed, alkyl (**4**), aryl (**5**, **6**), dialkyl (**7**-**10**), and benzyloxy (**11**) alkenes were compatible with the two-step cycloaddition/reduction sequence, and β-lactams **4**-**11** were easily obtained (Scheme 2).[19]

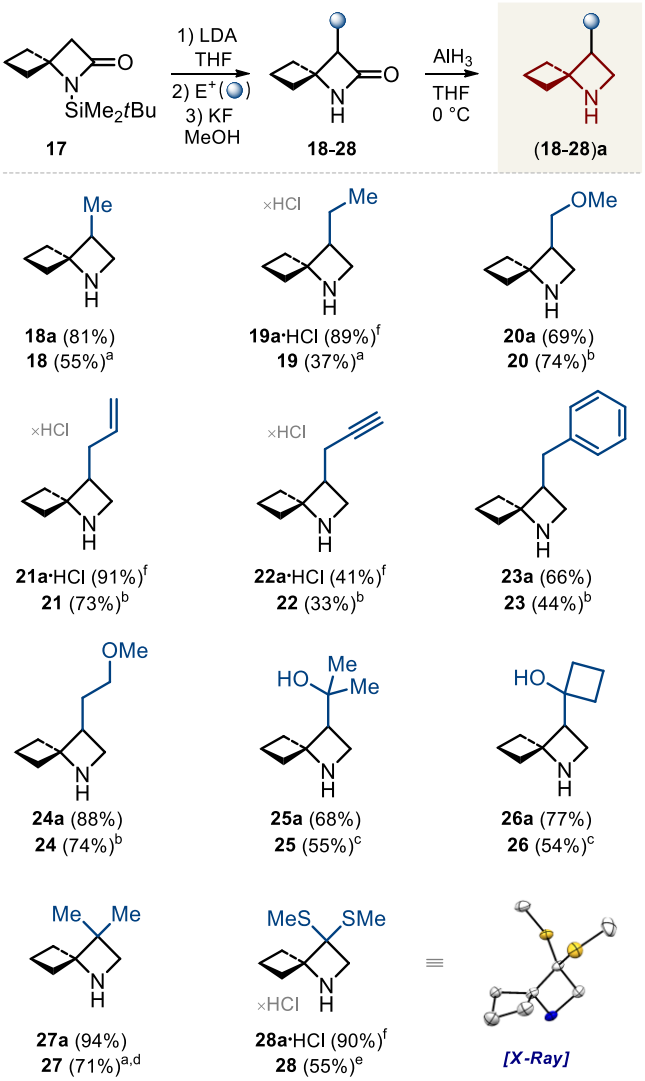
Lactams containing the $CO_2Me$-group underwent reduction into water-soluble amino alcohols **12a**-**14a** that were *N*-protected into *N*-Boc amino alcohols Boc-(**12a**-**14a**) to ease the product isolation. Similarly, lactams bearing the CN-group produced diamines **15a** and **16a** that were *N*-protected to enable the isolation of the protected species $Boc_2$-**15a** and $Boc_2$-**16a**. The structure of compounds Boc-**14a**, **15** and $Boc_2$-**16a** was confirmed by X-ray analysis (Scheme 2).[18]

Noteworthy, all products depicted in Scheme 2 were obtained in gram quantities. In most cases, β-lactams were formed with ca. 90% purity after the standard workup. On a milligram scale, we purified them to obtain analytical samples. However, on a gram scale, we directly used them in the subsequent reduction step thus ensuring a higher overall yield of the final 1-azaspiro[3.3]heptanes.

**Modifications**. The use of substituted alkenes brings diversity to 1-azaspiro[3.3]heptanes at the cyclobutane ring (Scheme 2). To bring diversity to the azetidine ring, we protected β-lactam **1** with a TBDMS group to obtain compound **17** (Scheme 3). Treatment of the latter with LDA generated carboanion that was trapped with various electrophiles - alkyl iodides, alkyl bromides, ketones, $(MeS)_2$ - to produce after *N*-deprotection the corresponding β-lactams **18**-**28**. Worth noting that an excess of electrophile led to the formation of the *bis*-functionalized products **27**, **28**. Reduction of the β-lactam ring with alane smoothly produced the desired 1-azaspiro[3.3]heptanes **18a**-**28a**. Products **19a**•HCl, **21a**•HCl, **22a**•HCl, and **28a**•HCl were isolated as crystalline hydrochloride salts. The structure of compound **28a**•HCl was confirmed by X-ray analysis.[18]




**Scheme 2**. Synthesis of cyclobutane-substituted 1-azaspiro[3.3]heptanes. Scope of the reaction. Gram scale. [a]Products **6a** and **11a** were isolated as hydrochloride salts. [b]Reduction was performed with $LiAlH_4$. [c]$CO_2Me$-group in **12**-**14** was reduced to -$CH_2OH$ in **12a**-**14a**. [d]After the reduction, the intermediate amino alcohols were treated with $Boc_2O$ (1 eq.) to obtain *N*-protected derivatives Boc-(**12a**-**14a**). [e]CN-group in **15**, **16** was reduced to -$CH_2NH_2$ in **15a**, **16a**. [f d]After the reduction, the intermediate diamines were treated with $Boc_2O$ (2 eq.) to obtain *N,N*-diprotected derivatives $Boc_2$-**15a** and $Boc_2$-**16a**. X-ray crystal structure of compounds Boc-**14a** and $Boc_2$-**16a** (carbon – white, oxygen – red, nitrogen - blue). Hydrogen and chlorine atoms are omitted for clarity. Ellipsoids are shown at a 50% probability level.




**Scheme 3** Synthesis of 3-substituted 1-azaspiro[3.3]heptanes. Scope of the reaction. Gram scale. [a]Alk-I were used as electrophiles. [b]Alk-Br were used as electrophiles. [c]Ketones were used as electrophiles. [d]MeI (2 eq.) was used. [e]$(MeS)_2$ (2 eq.) was used as an electrophile. [f]Products **19a**•HCl, **21a**•HCl, **22a**•HCl and **28a**•HCl were isolated as hydrochloride salts. X-ray crystal structure of compound **28a**•HCl (carbon – white, sulfur – yellow, nitrogen - blue). Hydrogen and chlorine atoms are omitted for clarity. Ellipsoids are shown at a 50% probability level.

Modifications of the 1-azaspiro[3.3]heptane core to obtain the medicinal chemistry-related building blocks were performed next (Scheme 4). Swern oxidation of amino alcohol Boc-**12a** gave *N*-Boc amino aldehyde **29** in 80% yield. The reaction of the latter with glyoxal/$(NH_4)_2CO_3$ gave, after *N*-deprotection, imidazole **30**•2HCl. The reaction of aldehyde **29** with hydroxylamine followed by reduction of the intermediate oxime gave *N*-Boc protected diamine **31**•HCl. Oxidation of the alcohol group in Boc-**12a** with $NaIO_4$/$RuCl_3$ (cat.) gave an interesting *N*-Boc amino acid **32** in 76% yield. Amide formation and *N*-Boc deprotection afforded azetidine **31**•HCl. The structure of products **30**•2HCl, **32,** and **34**•HCl was confirmed by X-ray analysis (Scheme 4).[18]

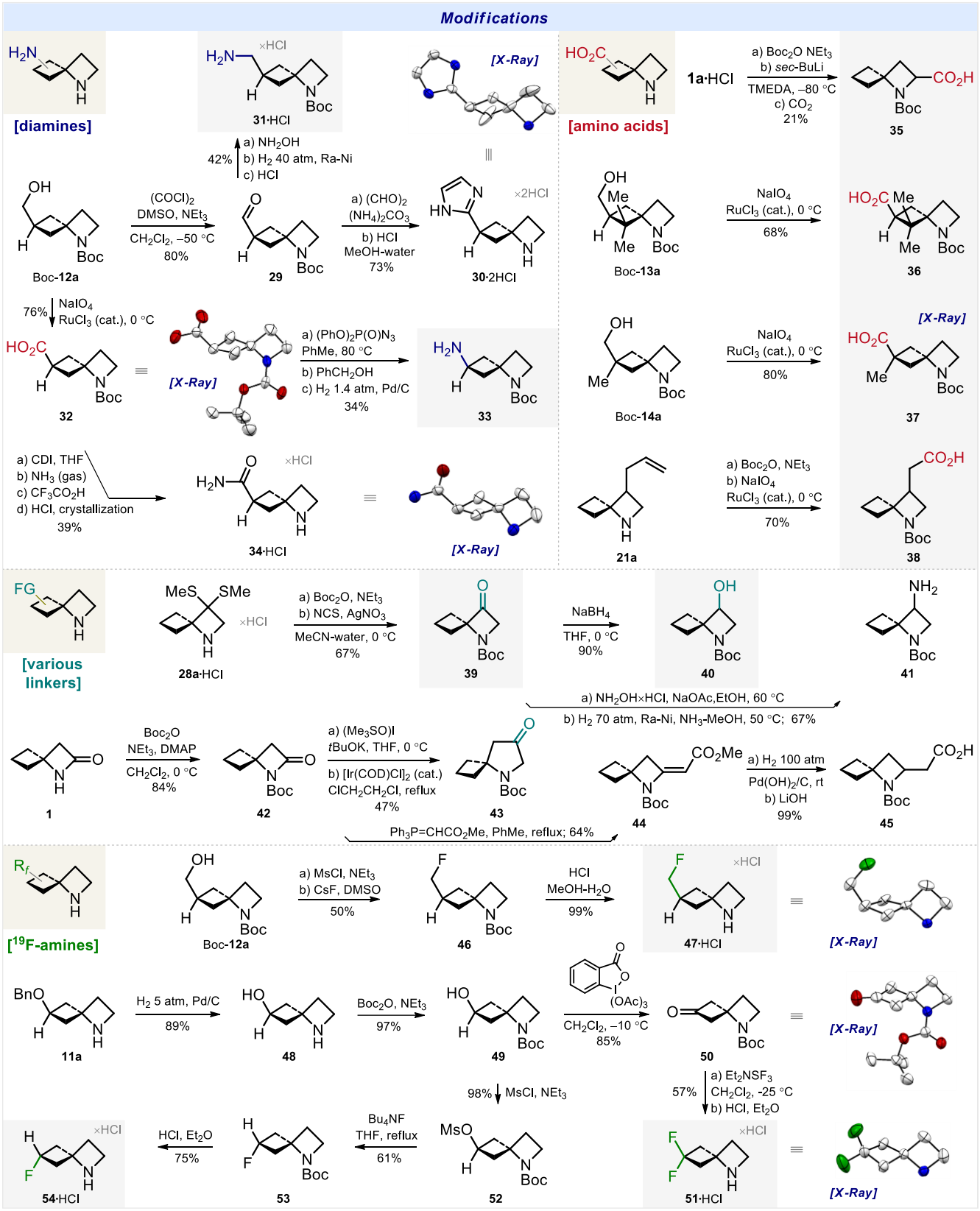
*N*-Boc protection of amine **1a**•HCl followed by *sec*-BuLi/TMEDA treatment and the addition of dry ice gave a unique amino acid **35** – an analog of α-pipecolic acid. Oxidation of the alcohol group in Boc-**13a** and Boc-**14a** with $NaIO_4$/$RuCl_3$ (cat.) gave amino acids **36** and **37**, correspondingly. *N*-Boc protection of alkene **21a** and its oxidation gave rise to another amino acid **38** in 70% yield. The structure of compound **37** was confirmed by X-ray analysis (Scheme 4).[18]

*N*-Boc protection of azetidine **28a**•HCl and the subsequent oxidative cleavage of the thioketal moiety with $Ag^+$/NCS gave amino ketone **39** in 67% yield (Scheme 4). Reduction of the carbonyl group in **39** gave amino alcohol **40** in 90% yield. In addition, the reaction of ketone **39** with hydroxylamine followed by reduction of the intermediate oxime with the Raney nickel gave *N*-Boc protected diamine **41** in 67% yield. *N*-Boc protection of β-lactam **1** with the $Boc_2O$/DMAP combination gave compound **42**. The subsequent reaction with $(Me_3SO)I$ and KO*t*Bu afforded the intermediate sulfoxonium ylide that upon treatment with $[Ir(COD)Cl]_2$ gave ketone **43** in 47% overall yield.[20] Horner–Wadsworth–Emmons reaction of the activated amide **42** gave enamide **44** in 47% yield. The standard $Pd(OH)_2$/C-catalyzed hydrogenation of the C=C bong and the subsequent saponification of the ester group provided β-amino acid **45** in almost quantitative yield – an isomer of γ-amino acid **38**.

*O*-Mesylation of amino alcohol Boc-**12a**, and the nucleophilic displacement with cesium fluoride in dimethyl sulfoxide afforded compound **46** in 50% yield. Acidic cleavage of the *N*-Boc group gave fluoro-substituted amine **47**•HCl (Scheme 4). Hydrogenative cleavage of *O*-Bn bond in amine **11a** led to the formation of amino alcohol **48** in 89% yield. *N*-Boc protection (**49**) and the Dess-Martin oxidation of the alcohol group gave ketone **50**. The reaction of the latter with $Et_2NSF_3$ and the subsequent *N*-Boc deprotection gave another fluorinated amine **51**•HCl in 57% combined yield. Finally, *O*-mesylation of alcohol **49** gave compound **52**. Its reaction with $Bu_4NF$ in THF gave compound **53** that after the acidic *N*-Boc deprotection accomplished the synthesis of fluoroamine **54**•HCl. The structure of compounds **47**•HCl, **50**, and **51**•HCl was confirmed by X-ray analysis.[18]

**Characterization**. Having synthesized various substituted 1-azaspiro[3.3]heptanes for medicinal chemistry, next we studied their experimental physicochemical properties.

*Basicity of amines*. The basicity of a nitrogen atom is a key characteristic of amines, that is often responsible for the toxicity of bioactive compounds. [21] We, therefore, measured experimental p$K_a$ values of amine hydrochlorides **1**•HCl, **55**•HCl, and **55**•HCl (Figure 3). We found that the basicity of the nitrogen atom was nearly identical in the tested substrates, with p*K*a (**55**•HCl)=11.2, p*K*a (**56**•HCl)=11.3, p*K*a (**1**•HCl)=11.4, thus highlighting a potential of 1-azaspiro[3.3]heptane to mimic piperidine in biochemical context.




**Scheme 4**. Synthesis of functionalized 1-azaspiro[3.3]heptanes for medicinal chemistry. X-ray crystal structure of compounds **30**•2HCl, **32**, **34**•HCl, **47**•HCl, **50**, **51**•HCl (carbon – white, oxygen – red, nitrogen – blue, fluorine – green). Hydrogen and chlorine atoms are omitted for clarity. Ellipsoids are shown at a 50% probability level.

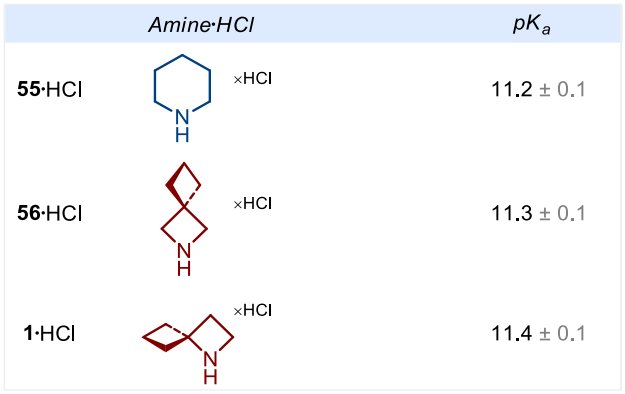



**Figure 3**. Experimental p$K_a$ values of amine hydrochlorides **1**•HCl, **55**•HCl, **56**•HCl.

*Physicochemical properties*. To better understand the impact of piperidine ring replacement with the 1-azaspiro[3.3]heptane skeleton, in the next step, we prepared three model compounds **57**-**59** – amides of piperidine (**57**), the previously used 2-azaspiro[3.3]heptane (**58**), and 1-azaspiro[3.3]heptane (**59**) (Figure 4).

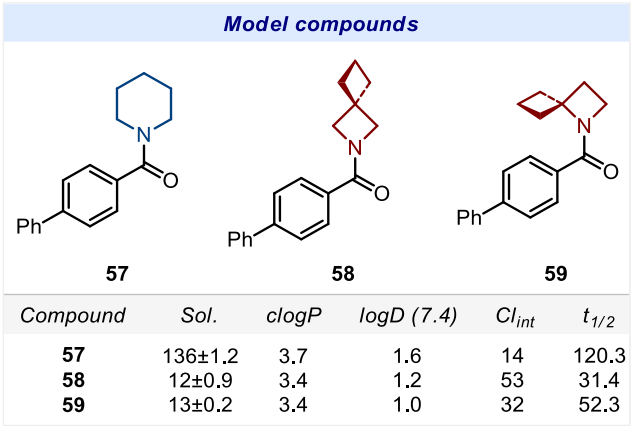
Replacement of the piperidine ring (**57**) with its both bicyclic analogs (**58**, **59**) reduced the water solubility, and this effect was almost identical: 136 μM (**57**) vs 12 μM (**58**) vs 13 μM (**59**) (Figure 4).

To estimate the influence of the replacement of the piperidine ring with the analogs on lipophilicity, we used two parameters: calculated (clogP) [22] and experimental (logD) lipophilicities. Replacement of the piperidine ring with isomeric azaspiro[3.3]heptanes led to a decrease of clogP: 3.7 (**57**) *vs* 3.4 (**58**) *vs* 3.4 (**59**) (Figure 4). A somewhat similar trend was observed with the experimental lipophilicity, logD. Replacement of piperidine with 2-azaspiro[3.3]heptane reduced the lipophilicity by 0.4 logD unit; whereby 1-azaspiro[3.3]heptane exhibited an even larger effect, logD: 1.6 (**57**) *vs* 1.2 (**58**) *vs* 1.0 (**59**).

The impact of piperidine replacement on metabolic stability was also studied. The incorporation of both azaspiro[3.3]heptanes decreased the metabolic stability in human liver microsomes: $Cl_{int}$ (mg $min^{-1}$ $\mu L^{-1}$)=14 (**57**) *vs* 53 (**58**) *vs* 32 (**59**) (Figure 4). Important to mention that the half-life time of 1-azaspiro[3.3]heptane was almost twice higher as that of 2-azaspiro[3.3]heptane: $t_{1/2}$ (min)= 31 (**58**) *vs* 52 (**59**).

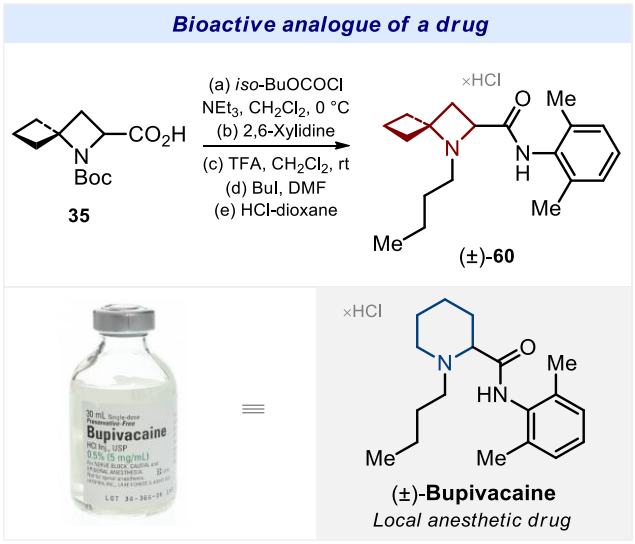
In summary, model compounds **58** and **59** had similar water solubility and lipophilicity. However, 1-azaspiro[3.3]heptane **59** was more metabolically stable than 2-azaspiro[3.3]heptane **58**.

**Incorporation into a drug**. To fully validate 1-azaspiro[3.3]heptane scaffold as a legit piperidine ring replacement option, we next aimed to incorporate this skeleton into a structure of an existing drug. We opted for the structure of the FDA-approved local anesthetic *Bupivacaine* (Scheme 5). Worth noting, *Bupivacaine* is used in practice as a racemic mixture. [23] Therefore, for primary proof of concept, we synthesized its racemic analog **60** (Scheme 5). The synthesis started from *N*-Boc amino acid **35**. Activation of the carboxyl group and the reaction with 2,6-xylidine, followed by *N*-Boc deprotection and nitrogen alkylation with BuI gave the desired compound **60**.




| Compound | Sol. | clogP | logD (7.4) | $Cl_{int}$ | $t_{1/2}$ |
|---|---|---|---|---|---|
| **57** | 136±1.2 | 3.7 | 1.6 | 14 | 120.3 |
| **58** | 12±0.9 | 3.4 | 1.2 | 53 | 31.4 |
| **59** | 13±0.2 | 3.4 | 1.0 | 32 | 52.3 |

**Figure 4**. Physico-chemical properties of model compounds **57**-**59**. Solubility (Sol.): experimental kinetic solubility in phosphate-buffered saline, pH 7.4 (μM). clogP: calculated lipophilicity. logD (7.4): experimental distribution coefficient in n-octanol/phosphate-buffered saline, pH 7.4. Reliable logD measured is obtained within a range of 1.0-4.5. $Cl_{int}$: experimental metabolic stability in human liver microsomes (μl $min^{-1}$ $mg^{-1}$). $t_{1/2}$ (min): experimental half-time of metabolic degradation.




**Scheme 5**. Synthesis of compound (±)-**60** – a spirocyclic analog of a local anesthetic drug *Bupivacaine*.

**Biological activity**. Finally, to answer the key question, of whether the 1-azaspiro[3.3]heptane core could mimic the fragment of piperidine in biologically active compounds, we experimentally measured the anesthetic activity of *Bupivacaine* and its analog **60** *in vivo*.

We studied the antinociceptive effect of *Bupivacaine* and compound **60** using the "tail flick test"[24] in Balb/cAnN male mice (for details see the Supporting Information).[25] These results are represented in Figures 5 and 6. On one hand, compound **60** was found less active compared to the original drug *Bupivacaine*. On the other hand, compound **60** demonstrated a significant level of analgesic activity compared to that of the vehicle.

This biological experiment corroborated our original hypothesis that 1-azaspiro[3.3]heptane is an actual bioisostere of the piperidine ring.

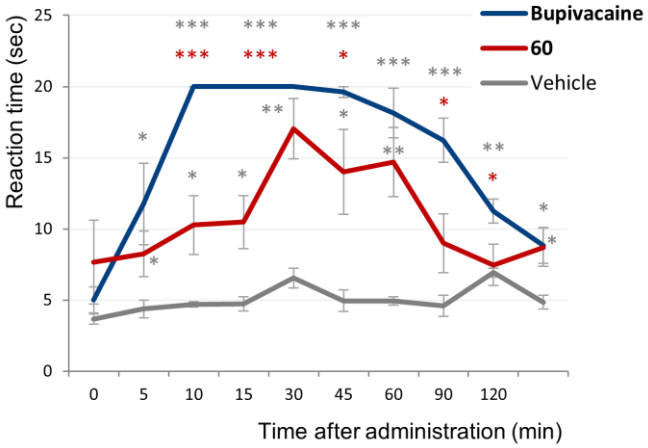



**Figure 5**. Time course of the antinociceptive effect of *Bupivacaine* and its analog **60** in tail flick test. The data were presented as mean ± SEM. * - indicates P < 0.05, ** - indicates P < 0.01, *** - P < 0.001 compared with group marked similar color.

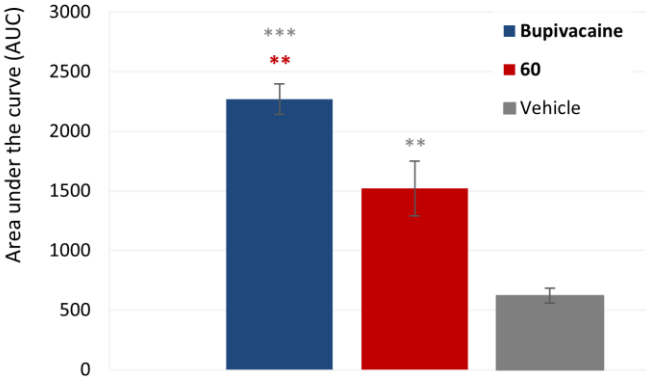



**Figure 6**. The area under the curve (AUC) of withdrawal latency of *Bupivacaine* and its analog **60** in tail flick test. The data were presented as mean ± SEM. ** - indicates P < 0.01, *** - P < 0.001 compared with group marked similar color.

**Conclusions**. Piperidine ring is found in the structure of more than one hundred drugs.[2] More than a decade ago, 2-azaspiro[3.3]heptanes were proposed to mimic the piperidine ring in bioactive compounds (Figure 1a). That finding had a beneficial effect on drug discovery: the core already appeared in more than 100 research manuscripts, 500 patents, and 7.000 bioactive compounds (Figure 1a).

Here, we have synthesized, characterized, and successfully validated biologically a new generation of piperidine bioisosteres - 1-azaspiro[3.3]heptanes (Schemes 1-4). This scaffold had similar basicity of the nitrogen atom, similar solubility, similar lipophilicity; and improved metabolic stability over the common 2-azaspiro[3.3]heptane (Figure 4). The incorporation of 1-azaspiro[3.3]heptane into the structure of the local anesthetic drug *Bupivacaine* instead of the piperidine ring was achieved. The drug analog **60** showed a significant anesthetic activity *in vivo* in mice (Figure 5, 6).

We expect that following this study, 1-azaspiro[3.3]heptanes will become common in drug discovery in the next five-ten years.

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**Keywords:** piperidine • bioisostere • 1-azaspiro[3.3]heptane • drug design • medicinal chemistry

## References


[1] (a) R. D. Taylor, M. MacCoss, A. D. G. Lawson. Rings in Drugs. *J. Med. Chem.* **2014**, *57*, 5845−5859. (b) J. Shearer, J. L. Castro, A. D. G. Lawson, M. MacCoss, R. D. Taylor. Rings in Clinical Trials and Drugs: Present and Future. *J. Med. Chem.* **2022**, *65*, 13, 8699-8712.

[2] https://go.drugbank.com (accessed on 25.03.2023). Option "Search by chemical structure." Filters used: "substructure" and "approved."

[3] J. A. Burkhard, B. Wagner, H. Fischer, F. Schuler, K. Müller, E. M. Carreira. Synthesis of Azaspirocycles and their Evaluation in Drug Discovery. *Angew. Chem. Int. Ed.* **2010**, *49*, 3524-3527.

[4] Selected examples of 2-azaspiro[3.3]heptanes in medchem campaigns: (a) K. Katayama, T. Tsunemi, K. Miyazaki, K. Uoto, R. Yoshioka, H. Terashima, M. Terakawa, K. Yamashiro, M. Haruyama, H. Maeda, T. Makino. Design, synthesis, and optimization of a series of 2-azaspiro[3.3]heptane derivatives as orally bioavailable fetal hemoglobin inducers. *Bioorg. Med. Chem. Lett.* **2020**, *30*, 127425. (b) S. L. Degorce, M. S. Bodnarchuk, J. S. Scott. Lowering Lipophilicity by Adding Carbon: AzaSpiroHeptanes, a logD Lowering Twist. *ACS Med. Chem. Lett.* **2019**, *10*, 1198–1204. (c) E. Lorthiois, E. Lorthiois, M. Gerspacher, K. S. Beyer, A. Vaupel, C. Leblanc, R. Stringer, A. Weiss, R. Wilcken, D. A. Guthy, A. Lingel, C. Bomio-Confaglia, R. Machauer, P. Rigollier, J. Ottl, D. Arz, P. Bernet, G. Desjonqueres, S. Dussauge, M. Kazic-Legueux, M.-A. Lozac'h, C. Mura, M. Sorge, M. Todorov, N. Warin, F. Zink, H. Voshol, F. J. Zecri, R. C. Sedrani, N. Ostermann, S. M. Brachmann, S. Cotesta. JDQ443, a Structurally Novel, Pyrazole-Based, Covalent Inhibitor of KRASG12C for the Treatment of Solid Tumors. *J. Med. Chem.* **2022**, *65*, 16173–16203. (d) K. Hoegenauer, J. Kallen, E. Jiménez-Núñez, R. Strang, P. Ertl, N. G. Cooke, S. Hintermann, M. Voegtle, C. Betschart, D. J. J. McKay, J. Wagner, J. Ottl, C. Beerli, A. Billich, J. Dawson, K. Kaupmann, M. Streiff, N. Gobeau, S. Harlfinger, R. Stringer, C. Guntermann. Structure-Based and Property-Driven Optimization of N-Aryl Imidazoles toward Potent and Selective Oral RORγt Inhibitors. *J. Med. Chem.* **2019**, *62*, 10816–10832. (e) Y. Wu, Q. Wang, M.-Y. Jiang, Y.-Y. Huang, Z. Zhu, C. Han, Y.-J. Tian, B. Zhang, H.-B. Luo. Discovery of Potent Phosphodiesterase-9 Inhibitors for the Treatment of Hepatic Fibrosis. *J. Med. Chem.* **2021**, *64*, 9537–9549.

[5] Mimetics for 2-substituted piperidines: (a) A. A. Kirichok, I. Shton, M. Kliachyna, I. Pishel, P. K. Mykhailiuk. 1-Substituted 2-Azaspiro[3.3]heptanes: Overlooked Motifs for Drug Discovery. *Angew. Chem. Int. Ed.* **2017**, *56*, 8865-8869. (b) L. R. Reddy, Y. Waman, P. Kallure, K. S. Nalivela, Z. Begum, T. Divya, S. Kotturi. Asymmetric synthesis of 1-substituted 2-azaspiro[3.3]heptanes: important motifs for modern drug discovery. *Chem. Commun.* **2019**, *55*, 5068-5070. (c) C. Bosset, H. Beucher, G. Bretel, E. Pasquier, L. Queguiner, C. Henry, A. Vos, J. P. Edwards, L. Meerpoel, D. Berthelot. Minisci-Photoredox-Mediated α-Heteroarylation of N-Protected Secondary Amines: Remarkable Selectivity of Azetidines. *Org. Lett.* **2018**, *20*, 6003-6006.

[6] Mimetics for 3-substituted piperidines: (a) C. Guérot, B. H. Tchitchanov, H. Knust, E. M. Carreira. Synthesis of Novel Angular Spirocyclic Azetidines. *Org. Lett.* **2011**, *13*, 780-783. (b) A. V. Chernykh, A. N. Tkachenko, I. O. Feskov, C. G. Daniliuc, N. A. Tolmachova, D. M. Volochnyuk, D. S. Radchenko. Practical Synthesis of Fluorinated Piperidine Analogues Based on the 2-Azaspiro[3.3]heptane Scaffold. *Synlett* **2016**, *27*, 1824-1827.

[7] Mimetics for 4-substituted piperidines: (a) M. J. Meyers, I. Muizebelt, J. van Wiltenburg, D. L. Brown, A. Thorarensen. Synthesis of tert-Butyl 6-Oxo-2-azaspiro[3.3]heptane-2-carboxylate. *Org. Lett.* **2009**, *11*, 3523-3525. (b) D. S. Radchenko, O. O. Grygorenko, I. V. Komarov. Synthesis of 2-azaspiro[3.3]heptane-derived amino acids: Ornitine and GABA analogues. *Amino Acids* **2009**, *39*, 515-521. (c) D. S. Radchenko, S. O. Pavlenko, O. O. Grygorenko, D. M. Volochnyuk, S. V. Shishkina, O. V. Shishkin, I. V. Komarov. Cyclobutane-Derived Diamines: Synthesis and Molecular Structure. *J. Org. Chem.* **2010**, *75*, 5941-5952.

[8] https://reaxys.com (accessed on 25.03.2023).

[9] (a) Z. Dobi, T. Holczbauer, T. Soós. Schreiner's Thiourea Promoted [2+2] Cycloaddition of Captodative Azetidinones and Nitroolefins. *Eur. J. Org. Chem.* **2017**, 1391-1395. (b) M. R. Becker, E. R. Wearing, C. S. Schindler. Synthesis of azetidines via visible-light-mediated intermolecular [2+2] photocycloadditions. *Nature Chem.* **2020**, *12*, 898–905.

[10] For higher carbocyclic analogues of 1-azaspiro[3.3]heptane, see: (a) N. E. Behnke, K. Lovato, M. Yousufuddin, L. Kürti. Titanium-Mediated Synthesis of Spirocyclic NH-Azetidines from Oxime Ethers. *Angew. Chem. Int. Ed.* **2019**, *58*, 14219-14223. (b) R. Gianatassio, D. Kadish. Direct Alkylation of 1-Azabicyclo[1.1.0]butanes. *Org. Lett.* **2019**, *21*, 2060–2063.

[11] Several chemical suppliers offer 1-azaspiro[3.3]heptane at a price 200 to 900 Euro/g.

[12] (a) J. Branalt, B. Holm, M. Johansson, O. Karlsson, D. L. Knerr, A. Nordqvist, R. J. Sheppard, M. Swanson, A. Tomberg (Astrazeneca), WO2022/130270, **2022**. (b) J. M. Baccei, Y. Bravo, A. C.-Y. Chen, R. C. Clark, B. A. Stearns, Y. P. H. Truong (Roche), WO2020/123426, **2020**. (c) D. Bauman, Z. Liu, T. Lu, B. Zhu, V. Nguyen, M. Cavitt, M. J. Hawkins (Johnson & Johnson), WO2022/136509, **2022**. (d) D. Bauman, Z. Liu, T. Lu, (Johnson & Johnson), WO2023/7009, **2023**. (e) W. Cai, J. W. J. Thuring, F. Hulpia, X. Dai, M. Li, X. Deng, C. Liang, A. T. F. Ng, Z. Sun, Z. Zhang, S. D. Demin, N. N. Dyubankova, M. D. Jouffroy, S. Lepri, N. F. J. B. Darville, V. Pande, W. B. G. Schepens, J. P. Edwards, O. A. G. Querolle (Johnson & Johnson), WO2022/253167, **2022**. (f) S. W. Reilly, L. N. Puentes, K. Wilson, C.-J. Hsieh, C.-C. Weng, M.

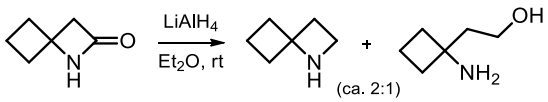
Makvandi, R. H. Mach. Examination of Diazaspiro Cores as Piperazine Bioisosteres in the Olaparib Framework Shows Reduced DNA Damage and Cytotoxicity. *J. Med. Chem.* **2018**, *61*, 5367-5379.

[13] J. A. Burkhard, C. Guerot, H. Knust, M. Rogers-Evans, E. M. Carreira. Synthesis and Structural Analysis of a New Class of Azaspiro[3.3]heptanes as Building Blocks for Medicinal Chemistry. *Org. Lett.* **2010**, *12*, 1944-1947.

[14] (a) C. Palomo, M. Oiarbide, S. Bindi. A Concise β-Lactam Route to Short Peptide Segments Containing α,α-Disubstituted α-Amino Acids. *J. Org. Chem.* **1998**, *63*, 2469-2474. (b) B. Peschke, M. Ankersen, B. S. Hansen, T. K. Hansen, N. L. Johansen, J. Lau, K. Madsen, H. Petersen, H. Thøgersen, B. Watson. Synthesis and in vitro characterization of new growth hormone secretagogues derived from ipamorelin with dipeptidomimetic *N*-terminals. *Eur. J. Med. Chem.* **1999**, *34*, 363−380.

[15] H. D. Thi, M. D'hooghe. An update on the synthesis and reactivity of spiro-fused β-lactams. *Arkivoc* **2018**, *6*, 314-347.

[16] R. Graff. Umsetzungen mit Olefinen und Aldehyden uber β-Lactame. *Liebigs Ann. Chem.* **1963**, *661*, 111-157.

[17] 


[18] Cambridge Crystallographic Data Centre (CCDC) deposition number of CIF files: 2253048 (**1a**•HCl), 2253050 (Boc-**14a**), 2253518 (**15**), 2253051 ($Boc_2$-**16a**), 2253517 (**28a**•HCl), 2253052 (**30**•2HCl), 2253053 (**32**), 2253054 (**34**•HCl), 2253055 (**37**), 2253056 (**47**•HCl), 2253057 (**50**), 2253049 (**51**•HCl).

[19] (a) S. Yu, A. Noble, R. B. Bedford, V. K. Aggarwal. Methylenespiro[2.3]hexanes via Nickel-Catalyzed Cyclopropanations with [1.1.1]Propellane. *J. Am. Chem. Soc.* **2019**, *141*, 20325−20334. (b) X. Du, D. Xu, G. Xu, C. Yu, X. Jiang. Synthesis of Imidized Cyclobutene Derivatives by Strain Release of [1.1.1]Propellane. *Org. Lett.* **2022**, *24*, 7323−7327. (c) M. L. Wrobleski, G. A. Reichard, N.-Y. Shih, D. Xiao (Schering-Plough Corp.), WO2003078376, **2003**.

[20] I. K. Mangion, I. K. Nwamba, M. Shevlin, M. A. Huffman. Iridium-Catalyzed X-H Insertions of Sulfoxonium Ylides. *Org. Lett.* **2009**, *11*, 3566-3569.

[21] F. Z. Doerwald, Lead optimizations for medicinal chemists, Wiley-VCH, Weinheim, Germany, 2012, p. 107.

[22] clogP was calculated with "Cxcalc" (ChemAxon, version 22.5.0).

[23] https://go.drugbank.com/drugs/DB00297 (accessed on 28.03.2023).

[24] O. L. Davies, J. Raventós, A. L. Walpole A method for the evaluation of analgesic activity using rats. *Br. J. Pharmacol Chemother.* **1946**, 255-264.

[25] Study design, animal selection, handling and treatment were in accordance with Bienta Animal Care and Use Guidelines, and European Union directive 2010/63/EU.

# Bicyclic Bioisosteres of Piperidine: Version 2.0

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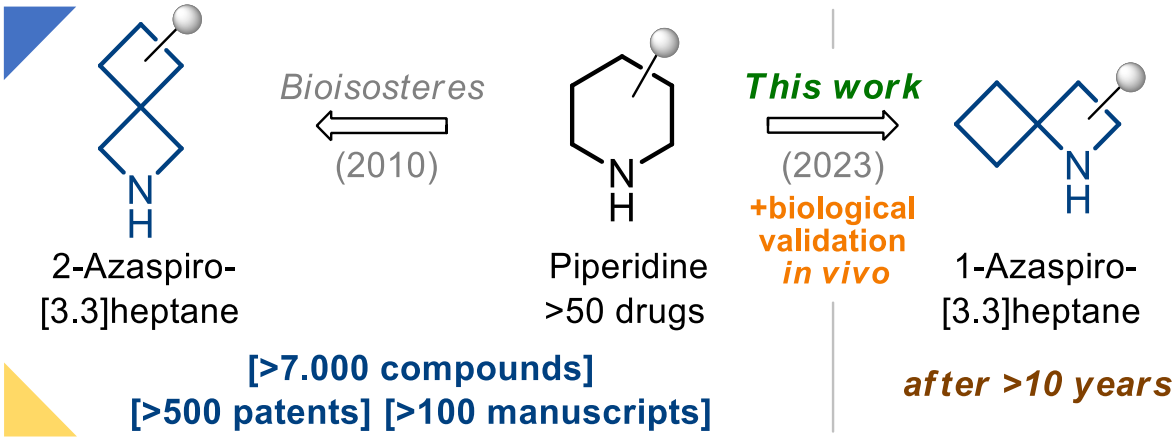


**Abstract**. 1-Azaspiro[3.3]heptanes were synthesized, characterized, and validated biologically *in vivo* as a new generation of saturated piperidine bioisosteres.