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Synthesis and binding affinity at α4β2 and α7 nicotinic acetylcholine receptors of new analogs of epibatidine and epiboxidine containing the 7-azabicyclo[2.2.1]hept-2-ene ring system

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ABSTRACT

A group of novel racemic nicotinic ligands structurally related to epibatidine or epiboxidine [(±)-**10**–(±)-**17**] was synthesized through a palladium-catalyzed cross-coupling between the appropriate vinyl triflate and a range of organometallic heterocycles. The target compounds were evaluated for binding affinity at the α4β2 and α7 neuronal nicotinic receptors (nAChRs). The set of 3-pyridinyl derivatives (±)-**10**, (±)-**11** and (±)-**12** exhibited an affinity for the α4β2 nAChR subtype in the subnanomolar range (K_i values of 0.20, 0.40 and 0.50 nM, respectively) and behaved as α4β2 versus α7 subtype selective ligands. Interestingly, the epiboxidine-related dimethylammonium iodide (±)-**17**, which retained a good affinity for the α4β2 nAChR (K_i = 13.30 nM), tightly bound also to the α7 subtype (K_i = 1.60 nM), thus displaying a reversal of the affinity trend among the reference and new nicotinic ligands under investigation.

In the research field on compounds interacting with neuronal nicotinic acetylcholine receptors (nAChRs), epibatidine (−)-**1** (Fig. 1) has been one of the most investigated among the naturally occurring ligands.[1] Epibatidine, an amphibian alkaloid formerly isolated in trace amounts from the skin of the Ecuadorian frog *Epipedobates tricolor*,[2] is characterized by a very high affinity for nAChRs and behaves as a powerful antinociceptive agent.[3] This pharmacological effect, which is about 100 times more pronounced than that shown by morphine, has been primarily attributed to the activation of the α4β2 receptor population, the subtype with the highest expression levels in the mammalian central nervous system.[4] However, the lack of selectivity against the other nAChR subtypes and the marked toxicity prevented any therapeutic potential for (−)-**1**.[4] Nonetheless, epibatidine has been the subject of in depth structure–activity investigations involving changes of the different component parts of its molecular skeleton. Among the various derivatives described in the literature,[5] epiboxidine (±)-**2**[6] (Fig. 1) emerged as a high-affinity α4β2 ligand, in which the 2′-chloropyridine moiety of the model compound was replaced by the 3′-methylisoxazole ring. In particular, (±)-**2** turned out to be a potent α4β2 nicotinic receptor agonist, displaying a 10-fold reduced antinociceptive potency than epibatidine but with an improved activity/toxicity ratio.[6] Moreover, (−)-**1** and the natural α,β-unsaturated carbonyl derivative anatoxin-a (+)-**3**[7] inspired the synthesis of the hybrid 9-azabicyclo[4.2.1]non-2-ene structural analog UB-165 (±)-**4** (Fig. 1),[7,8] which behaved as a nicotinic agonist showing high affinity and selectivity for the α4β2 receptor subtype. Even the unnatural ferruginine enantiomer (−)-**5**[1,9] and its epibatidine- or epiboxidine-related analogs (±)-**6** and (±)-**7** (Fig. 1),[10] which all contain the 8-azabicylo[3.2.1]oct-2-ene moiety, were characterized as α4β2-preferring nicotinic ligands.

Recently, in describing a new synthetic approach to (±)-**2**, we re-investigated its pharmacological profile, confirming its high affinity for the α4β2 nAChRs while finding out a noteworthy affinity for the α7 subtype.[11] We prepared and tested also (±)-**8** (Fig. 1), the 'unsaturated' analog of epiboxidine, which showed a degree of α4β2 versus α7 selectivity comparable to that of the parent compound along with a reduced affinity at both receptor subtypes.[11] Conversely, the corresponding tertiary base (±)-**9** (Fig. 1) bound both α4β2 and α7 nAChRs with comparable K_i values in the submicromolar range.[11]

As a part of our ongoing interest in nicotinic agonists with enhanced subtype selectivity,[12] in this paper we synthesized and tested the racemic derivatives (±)-**10**–(±)-**17** (Fig. 1), which incorporate the 7-azabicyclo[2.2.1]hept-2-ene molecular fragment. In particular, (±)-**10** has in position 2 the 2′-chloropyridine ring of

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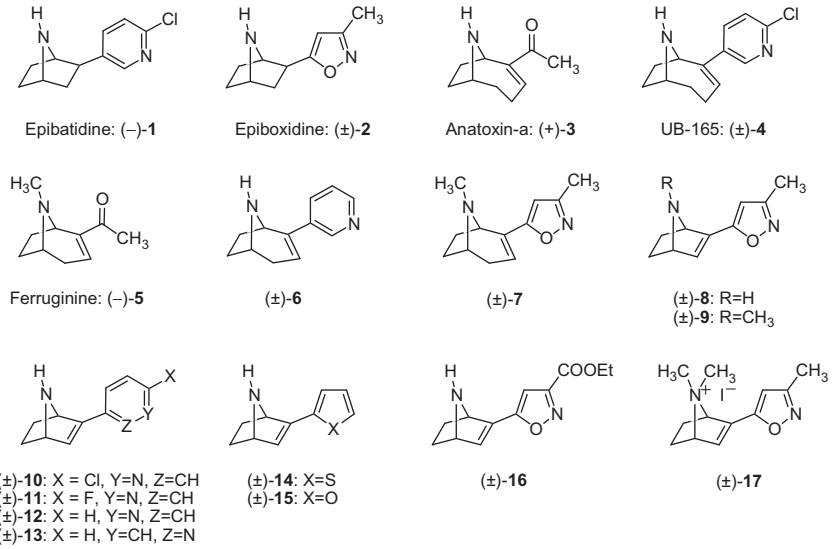
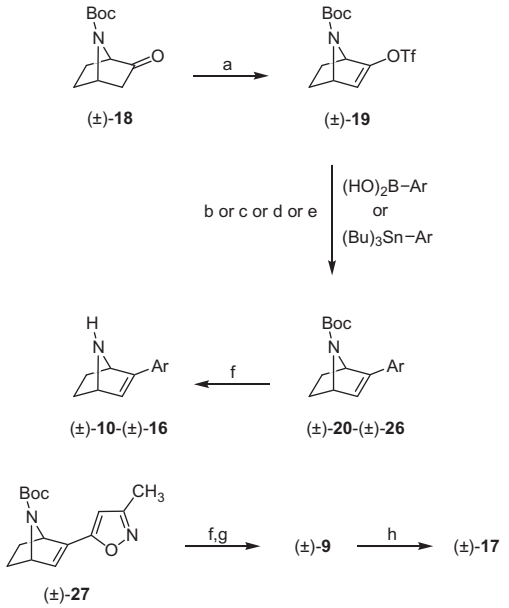


Figure 1. Structures of model nicotinic ligands (−)-**1**, (±)-**2**–(±)-**9** and derivatives (±)-**10**–(±)-**17** investigated in this study.

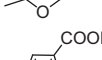


Scheme 1. Reagents and conditions: [see Table 1 for $(HO)_2B$-Ar and $(Bu)_3Sn$-Ar]: (a) $(iPr)_2NH/n$-BuLi, THF, *N*-(5-chloro-2-pyridyl)bis(trifluoromethanesulfonimide), DME, −78 °C to rt, 3 days; (b) $Pd(PPh_3)_4$, anhydrous LiCl, Na_2CO_3, DME, 85 °C, 2 h; (c) $Pd(PPh_3)_4$, anhydrous LiCl, CuI, THF, 65 °C, 10 min; (d) $Pd(PPh_3)_4$, anhydrous LiCl, THF, 80 °C, 3 h; (e) $[Pd_2(dba)_3]\cdot CHCl_3$, PPh_3, anhydrous $ZnCl_2$, THF, −78 °C to rt, 4 days; (f) 4 N HCl/dioxane, rt; (g) 37% aq. HCHO, $NaBH_3CN$, CH_3CN, rt, 1 h; (h) CH_3I, MeOH, rt, 12 h.

the two reference derivatives (−)-**1** and (±)-**4**, and (±)-**12** carries the unsubstituted pyridine ring present in (±)-**6**. The pharmacological analysis of (±)-**10**–(±)-**13**, mainly their affinity for the α4β2 subtype, would complement the data known for their structurally related analogs reported in Figure 1. To the best of our knowledge, derivative (±)-**10** has been cited in the patent literature,[13] although binding affinity data at nAChR subtypes were not determined. Among the other target derivatives, (±)-**14** and (±)-**15** have heterocyclic systems potentially able to mimic the isoxazole ring of epoxidine and its analogs (±)-**8** and (±)-**9**, whereas the substitution pattern on the isoxazole ring was changed on passing from methyl in (±)-**8** to ethoxycarbonyl in (±)-**16**. Finally, we prepared and tested the quaternary salt (±)-**17** of the tertiary base (±)-**9**, to evaluate the role of the dimethylammonium group on the molecular recognition by the two nAChR subtypes and on the α4β2/α7 selectivity.

The target compounds were synthesized exploiting the synthetic sequence recently applied by us to the preparation of derivatives (±)-**2**, (±)-**8**, and (±)-**9**, which was based on a Stille palladium-catalyzed cross-coupling reaction.[11] In the present study, we utilized a similar approach, which involved vinyl trifluoromethanesulfonate (±)-**19**[14] as the key intermediate, in turn obtained from the 7-*tert*-butoxycarbonyl-7-azabicyclo[2.2.1]heptan-2-one (±)-**18**[11,15] by a known method.[16,17] As outlined in Scheme 1 and Table 1, we carried out efficient transition metal-catalyzed cross-coupling reactions for C–C bond formation between the electrophile (±)-**19** and various organometallic reagents by using either the Suzuki–Miyaura protocol[18] with arylboronic acids or the Stille procedure[19] with

Table 1
Palladium-catalyzed cross-coupling reactions for the synthesis of intermediates (±)-**20**–(±)-**27**

Compd	Ar	Reaction conditions[a]	Yield[f] (%)
(±)-**20**		b $(HO)_2B$ Ar	50
(±)-**21**		b $(HO)_2B$ Ar	71
(±)-**22**		b $(HO)_2B$ Ar	81
(±)-**23**		c $(Bu)_3Sn$ Ar	77
(±)-**24**		d $(Bu)_3Sn$ Ar	79
(±)-**25**		d $(Bu)_3Sn$ Ar	77
(±)-**26**		e $(Bu)_3Sn$ Ar	70
(±)-**27**		e $(Bu)_3Sn$ Ar	90[g]

[a] Protocols b, c, d, and e refer to the reaction conditions reported in Scheme 1.
[f] Isolated yield, based on starting triflate (±)-**19**, after column chromatography.
[g] Ref. 11

arylstannanes. Accordingly, Suzuki-type couplings between enoltriflate (±)-**19** and the three commercially available 2-chloro-5-pyridine-, 2-fluoro-5-pyridine-, and 3-pyridine-boronic acids, under the catalysis of tetrakis(triphenylphosphine)palladium(0) in refluxing dimethoxyethane, afforded the corresponding 2-aryl-substituted adducts (±)-**20**, (±)-**21** and (±)-**22**[20] in 50%, 71% and 81% yield, respectively (Scheme 1).

The five *N*-Boc-protected 2-heteroaryl-7-azabicyclo[2.2.1]hept-2-enes (±)-**23**-(±)-**27** were prepared using Stille coupling conditions for the C–C bond formation. Treatment of enoltriflate (±)-**19** with commercial 2-(tributylstannyl)pyridine in the presence of the palladium catalyst, lithium chloride and copper(I) iodide[21] in refluxing THF led to the coupling product (±)-**23** in 77% yield (Scheme 1). With a similar experimental protocol, (±)-**24** and (±)-**25** were synthesized from (±)-**19** and 2-(tributylstannyl)thiophene or 2-(tributylstannyl)furan in 79% and 77% yield, respectively. Next, the coupling of (±)-**19** with ethyl 5-tributylstannyl-3-isoxazole carboxylate provided the desired isoxazole derivative (±)-**26** in 70% yield.[22] The starting stannyl-isoxazole[23] was in turn obtained by 1,3-dipolar cycloaddition of tributylethynyltin to ethyl 2-chloro-2-(hydroximino)-acetate[24] in a suspension of potassium carbonate in dichloromethane. Similarly, the coupling reaction of 3-methyl-5-tributylstannylisoxazole[11,23] afforded (±)-**27** in 90% yield.

Conversion of the *N*-Boc derivatives (±)-**20**–(±)-**26** into the corresponding hydrochlorides of secondary amines (±)-**10**–(±)-**16** was then achieved by treatment with a 4 N solution of HCl in dioxane (Scheme 1). Derivative (±)-**9**, prepared from (±)-**27** as previously reported,[11] was quantitatively transformed into the corresponding quaternary salt (±)-**17** upon treatment with excess methyl iodide (Scheme 1).

Compounds (±)-**10**–(±)-**17** were assayed for binding affinity at rat α4β2 and α7 nAChRs, using [^{3}H]epibatidine and [^{125}I]α-bungarotoxin as radioligands for the α4β2 and the α7 receptors, respectively, and following a reported experimental protocol.[12c] We also tested commercially available epibatidine (±)-**1** and UB-165 (±)-**4**, and epiboxidine (±)-**2**, prepared according to our synthetic procedure.[11] The K_i values, calculated from the competition curves of three separate experiments by means of the LIGAND program[25] or taken from the literature for reference compounds, have been collected in Table 2. As indicated, the novel derivatives were tested either as hydrochlorides or fumarates.[26]

The binding results evidenced that the closest congeners of epibatidine, that is, unsaturated derivatives (±)-**10**, (±)-**11**, and (±)-**12**, retained affinity for the α4β2 receptor subtype in the subnanomolar range, with K_i values equal to 0.20, 0.40, and 0.50 nM, respectively, which are about one order of magnitude higher than that determined for (±)-**1** (K_i = 0.050 nM). These data parallel previous outcomes obtained on racemic epibatidine, its 2′-fluoro analog and deschloroepibatidine.[5a] Conversely, compound (±)-**13**, in which the 3-pyridyl moiety of (±)-**12** has been replaced by the 2-pyridyl ring, showed a sharp reduction in affinity at both α4β2 (K_i = 91.5 μM) and α7 (K_i = 9.2 μM) subtype. As expected, a shortening of the average distance (4–6 Å) between the ligand cationic pharmacophoric element and the hydrogen bond acceptor moiety, here represented by the pyridine nitrogen, strongly weakened the interaction with the binding site within the receptor protein. The three analogs (±)-**10**, (±)-**11**, and (±)-**12** were also characterized by varying degrees of α4β2 versus α7 selectivity, whose ratio (2305, 565, 38, respectively) progressively decreases following the gradual enhancement of the affinity at the α7 subtype. The high value of α4β2 versus α7 selectivity of (±)-**10** (2305) should be comparable with that of UB-165 (±)-**4**, given the strict structural resemblance of the two ligands. Our affinity data provided an α4β2 versus α7 selectivity ratio of 188 for commercial (±)-**4**, resulting from K_i values of 2.80 nM at the α4β2 subtype and of 527 nM at the α7 subtype. A divergence was observed with the literature data available for (±)-**4**, particularly if the K_i values at the α7 nAChRs are taken into account (2.76 μM[8] and 12 nM,[7] Table 2), which both derive from the same rat brain tissue preparation in the presence of the same of radioligand ([^{3}H]MLA, i.e., labeled methyllycaconitine). These discrepancies strongly affect the known α4β2 versus α7 selectivity ratio of (±)-**4** (10220[8] and 300,[7] Table 2). Hence, based on our analysis, if the affinity/selectivity profile of (±)-**10** and (±)-**11** is compared with that of (±)-**4**, the two new analogs show high affinity for the α4β2 nAChRs and a noteworthy ability to discriminate the α4β2 from the α7 receptor subtype (2305 and 565, respectively, Table 2).

Compounds (±)-**14**–(±)-**16**, that is, the 2-(2-thienyl), the 2-(2-furanyl)- and the 2-(3-ethoxycarbonylisoxazol-5-yl)-substituted analogs, showed comparable affinity in the low micromolar range at the α4β2 subtype (K_i values equal to 2.3, 3.8, and 3.0 μM, respectively) and a further decline or loss in affinity at the α7 subtype. Worth noting, if the affinity data of the two isoxazole-containing

Table 2
Affinity of (±)-**1**, (±)-**2**, (±)-**4** and of target compounds (±)-**10**–(±)-**17** for native α4β2 and α7 nAChR subtypes present in rat cortical membranes, labeled by [^{3}H]epibatidine and [^{125}I]α-bungarotoxin

Entry	α4β2 [^{3}H]Epi K_i (nM)	α7[^{125}I]α-BgTx K_i (nM)	α4β2/α7 selectivity
Epibatidine (±)-**1**	0.050 (10)	0.80 (22)	16
Epiboxidine (±)-**2**	0.24 (24)	7.30 (25)	30
UB-165 (±)-**4**	2.80 (34)	527 (36)	188
UB-165 (±)-**4**	0.27[a]	2760[a]	10220
UB-165 (±)-**4**	0.040[b]	12[b]	300
(±)-**8**	50[c]	1600[c]	32
(±)-**9**	514[c]	394[c]	0.8
(±)-**10**[d]	0.20 (17)	461 (44)	2305
(±)-**11**[d]	0.40 (20)	226 (48)	565
(±)-**12**[d]	0.50 (31)	19 (27)	38
(±)-**13**[d]	91500 (41)	9200 (56)	0.1
(±)-**14**[e]	2300 (38)	7350 (58)	3.2
(±)-**15**[e]	3800 (29)	6900 (37)	1.8
(±)-**16**[e]	3000 (23)	350000 (56)	117
(±)-**17**	13.30 (28)	1.60 (29)	0.12

The K_i values were derived from three competition binding experiments. The numbers in brackets refer to the % coefficient of variation. Literature data available for (±)-**4**, (±)-**8**, and (±)-**9** have been also included.

[a] Taken from Ref. 8, [^{3}H]nicotine and [^{3}H]MLA were used as radioligands for α4β2 and α7 receptors, respectively.

[b] Taken from Ref. 7, [^{3}H]Epi and [^{3}H]MLA were used as radioligands for α4β2 and α7 receptors, respectively.

[c] Ref. 11

[d] Assayed as hydrochloride.

[e] Assayed as fumarate.

analogs (±)-**8** [K_i = 50 nM (α4β2) and 1.6 μM (α7)] and (±)-**16** [K_i = 3.0 μM (α4β2) and 350 μM (α7)] are compared, we can deduce that the significant variation in both electronic and steric properties of the heterocycle, brought about by the replacement of 3-methyl group by the ethoxycarbonyl moiety, is the reason for the remarkable drop in the ligand affinity at both studied nAChRs.

Finally, the introduction of a permanently charged nitrogen on the skeleton of the epiboxidine-related derivatives, that is, methyl iodide (±)-**17**, greatly enhanced the interaction with both receptor subtypes [K_i = 13.30 nM (α4β2) and 1.6 nM (α7)] compared with the corresponding tertiary base (±)-**9** [K_i = 0.51 μM (α4β2) and 0.39 μM (α7)]. As a consequence, iodomethylate (±)-**17** behaved as a high affinity unselective nicotinic ligand at α4β2 and α7 receptors, thus reproducing to some extent the affinity profile of epiboxidine (±)-**2** [K_i = 0.24 nM (α4β2) and 7.30 nM (α7)] (Table 2).

In summary, we applied the Suzuki–Miyaura and the Stille Pd-catalyzed coupling reaction protocols to synthesize a group of novel analogs of epibatidine and epiboxidine, in which the 7-azabicylo[2.2.1]heptane nucleus of the model compounds was replaced by the 7-azabicylo[2.2.1]hept-2-ene moiety. If the results on the closer epibatidine-related analogs (±)-**10**–(±)-**12** are considered, the loss of conformational flexibility due to the introduction of the double bond did not substantially alter the affinity for the α4β2 nAChRs. In addition, the chloro- and fluoro-substituted pyridine derivatives (±)-**10** and (±)-**11** are characterized by a meaningful degree of α4β2 versus α7 subtype selectivity. In the set of the epiboxidine-related analogs, the binding data on the permanently charged salt (±)-**17** indicated that, compared to its tertiary base (±)-**9**, the dimethylammonium moiety caused both a gain in affinity at the α4β2 receptor and a better recognition by the α7 subtype, giving rise to a ligand provided with nanomolar affinity at the two studied receptor subtypes. We are planning to further explore the pharmacological properties of selected derivatives discussed in this study through the preparation and the assessment of the affinity profile of their enantiomeric pairs.

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20. Representative procedure of a Pd-catalyzed coupling reaction under Suzuki–Miyaura conditions. 2-(Pyridin-3-yl)-7-azabicyclo[2.2.1]hept-2-ene-7-carboxylic acid *tert*-butyl ester **22**. A mixture of enoltriflate **19** (210 mg, 0.61 mmol), 3-pyridineboronic acid (113 mg, 0.92 mmol), LiCl (77.85 mg, 1.84 mmol), a saturated aqueous Na_2CO_3 solution (1.2 mL), and $Pd(PPh_3)_4$ (32.53 mg, 0.028 mmol) in DME (5 mL) was heated at reflux under nitrogen. After 2 h, the reaction mixture was cooled to room temperature, diluted with Et_2O (80 mL), filtered through a pad of Celite® into a 1:1 NH_4OH and water solution (100 mL). The water phase was extracted with Et_2O (4 × 100 mL) and the combined organic phases were dried over anhydrous sodium sulfate, filtered and concentrated under reduced pressure, to give a viscous yellow oil (190 mg). This crude material was purified by silica gel column chromatography, eluting with 10% ethyl acetate in petroleum ether to give 135 mg (81%) of the pyridinyl derivative **22** as a pale yellow oil. R_f = 0.33 (30% ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.14–1.22 (m, 2H), 1.34 (s, 9H), 1.90–2.01 (m, 2H), 4.72 (br s, 1H), 5.01 (br s, 1H), 6.47 (s, 1H), 7.18 (dd, 1H, *J* = 4.7 and 8.0 Hz), 7.59 (ddd, 1H, *J* = 1.4, 4.7 and 8.0 Hz), 8.39 (d, 1H, *J* = 4.7 Hz), 8.58 (m, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 28.47, 29.70, 61.16, 80.19, 110.01, 123.51, 132.36, 146.75, 148.78. $C_{16}H_{20}N_2O_2$ (272.34): calcd C 70.56, H 7.40, N 10.29; found C 70.67, H 7.31, N 10.13.
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22. Representative procedure of a Pd-catalyzed coupling reaction under Stille conditions. 2-(3-Ethoxycarbonylisoxazol-5-yl)-7-azabicyclo[2.2.1]hept-2-ene-7-carboxylic acid *tert*-butyl ester **26**. To a magnetically stirred solution of enoltriflate **19** (220 mg, 0.64 mmol) in anhydrous THF (6 mL) kept at −78 °C under argon were sequentially added $[Pd_2(dba)_3]\cdot CHCl_3$ (54 mg, 0.052 mmol), triphenylphosphine (13 mg, 0.013 mmol), then a solution of ethyl 5-tributylstannyl-3-isoxazole carboxylate (500 mg, 1.16 mmol) in THF (6 mL) and anhydrous $ZnCl_2$ (90 mg, 0.65 mmol). The mixture was quickly warmed at rt and stirred for about 4 days under argon until disappearance of the starting material (TLC, 20% ethyl acetate/petroleum ether). After addition of water (20 mL), the crude reaction was repeatedly extracted with ethyl acetate (4 × 30 mL). The combined organic extracts were treated with brine (2 × 20 mL), then dried over anhydrous sodium sulfate. After concentration under reduced pressure, the residue was purified by silica gel column chromatography (20% ethyl acetate/petroleum ether) to afford 150 mg (70% yield) of the expected isoxazolyl derivative **26**. Colorless prisms (from *n*-hexane), mp 78–80 °C. R_f = 0.61 (20% ethyl acetate/petroleum ether). ^{1}H NMR (300 MHz, $CDCl_3$): δ 1.15–1.33 (m, 2H), 1.42 (s, 9H), 1.42 (t, 3H, *J* = 7.0 Hz), 1.94–2.13 (m, 2H), 4.46 (q, 2H, *J* = 7.0 Hz), 4.86 (br s, 1H), 5.00 (br s, 1H), 6.65 (s, 1H), 6.82 (s, 1H). ^{13}C NMR (75 MHz, $CDCl_3$): δ 14.52, 24.70, 28.51, 60.95, 61.33, 62.55, 80.84, 101.76, 104.84, 134.53, 135.71, 154.99, 156.79, 159.86, 165.85. $C_{17}H_{22}N_2O_5$ (334.37): calcd C 61.07, H 6.63, N 8.38; found C 61.30, H 6.55, N.
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26. 2-(Pyridin-3-yl)-7-azabicyclo[2.2.1]hept-2-ene **12** dihydrochloride as an example. Pale yellow prisms (anhydrous ether washings), mp 160–165 °C, dec. ^{1}H NMR (300 MHz, CD_3OD): δ 1.60–1.76 (m, 2H), 2.29 (m, 2H), 3.30 (s, 1H), 4.96 (br s, 1H), 5.40 (d, 1H, *J* = 2.7 Hz), 7.21 (d, 1H, *J* = 2.5 Hz), 8.12 (dd, 1H, *J* = 5.8 and 8.3 Hz), 8.82 (m, 2H), 9.15 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 21.43, 22.36, 60.58, 61.85, 67.00, 128.00, 131.41, 132.42, 139.05, 141.29, 143.67. $C_{11}H_{14}Cl_2N_2$ (245.15): calcd C 53.89, H 5.76, Cl 28.92, N 11.43; found C 54.12, H 5.91, Cl 28.67, N 11.28.
2-(3-Ethoxycarbonylisoxazol-5-yl)-7-azabicyclo[2.2.1]hept-2-ene **16** fumarate as an example. Colorless prisms (from abs. ethanol/ether 1:1), mp 162–163 °C, dec. ^{1}H NMR (300 MHz, CD_3OD): δ 1.39 (t, 3H, *J* = 7.2 Hz), 1.50–1.70 (m, 2H), 2.17–2.32 (m, 2H), 4.43 (q, 2H, *J* = 7.2 Hz), 4.87 (br s, 1H), 5.14 (br s, 1H), 6.67 (s, 2H), 6.99 (d, 1H, *J* = 2.5 Hz), 7.10 (s, 1H). ^{13}C NMR (75 MHz, CD_3OD): δ 13.18, 21.45, 22.17, 60.47, 61.32, 62.22, 103.18, 131.66, 132.04, 134.94, 157.20, 159.49, 163.95, 169.96. $C_{16}H_{18}N_2O_7$ (350.32): calcd C 54.86, H 5.18, N 8.00; found C 54.71, H 5.33, N 7.82.