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New classes of potent and bioavailable human renin inhibitors

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ABSTRACT

New classes of de novo designed renin inhibitors are reported. Some of these compounds display excellent in vitro and in vivo activities toward human renin in a TGR model. The synthesis of these new types of mono- and bicyclic scaffolds are reported, and properties of selected compounds discussed.

The Renin–Angiotensin–Aldosterone system (RAAS) is known to be a major regulator of the cardiovascular, renal, and adrenal functions, playing a major role in water and salt homeostasis, as well as blood pressure control. From the point of view of medicinal chemist, renin proved to be a challenging target as already summarized in our previous publication[1a] and summarized recently by Tice.[1b]

As a part of our renin inhibitors program, we developed 3,9-diazabicyclononene[1a] derivatives with subnanomolar IC_{50} values for renin (in buffer), and showed in vivo efficacy after oral administration of 10 mg/kg in the double transgenic rat (TGR) model.[2] Compounds **1a** and **1b** are representatives of such bicyclic renin inhibitors[3] (Fig. 1). We could show that substituents attached at the N^3-position of this diazabicyclic scaffold had only a minor influence on binding affinity, but allowed significant modulation of other compound properties.[1a] Here, we describe bicyclic compounds (**2a–6b**, Fig. 1) with a newly designed central template exploring the role of the introduced C^2–N^3–C^4 bridge of the 3,9-diazabicyclononene moiety.

Vinyl triflates **11–14** (Scheme 1) were prepared from the corresponding known 9-methyl-3-oxa-9-aza-bicyclo[3.3.1]nonan-7-one and 9-methyl-3-thia-9-aza-bicyclo[3.3.1]-nonan-7-one,[4] and from the commercially available 9-methyl-9-aza-bicyclo[3.3.1]nonan-3-one and tropinone. These educts were first converted into their corresponding β-ketoesters (LDA,[5] then MeOCOCN, THF, −78 °C), which were deprotonated with NaH in THF, and triflated using Tf_2NPh. Negishi or Suzuki couplings yielded compounds **16** and **19–21** in 58–82% yields. Compound **15a** was prepared from the corresponding aryl bromide,[1a] with pinacolborane, $PdCl_2(PPh_3)_2$, and Et_3N in dioxane at 100 °C overnight. Removal of the *N*-methyl group with 1-chloroethyl chloroformate, followed by hydrolysis of the respective 1-chloroethyl carbamate with methanol,[6] and reaction with Boc_2O yielded the *N*-Boc-protected compounds **17** and **22–26** in 55–93% yields. Oxidation of compound **17** to the corresponding sulfoxide **18a** and sulfone **18b**, respectively, followed by Mitsunobu couplings with 2-chloro-3,6-difluorophenol, led to compounds **24** and **25**. Ester hydrolysis of **22–26**, subsequent amide couplings, and deprotections delivered compounds **2a–6a** and **2b–6b**.

In order to judge the importance of the C^2–X^3–C^4 bridge, we prepared tetrahydropyridine analogs **7a** and **7b** (Fig. 1) as reference compounds. Since the double bond of these tetrahydropyridines isomerized easily from the 3,4-position to the 4,5-position, and back (vide infra), we also prepared the monocyclic analogs **8a, 8b, 9a, 9b, 10a**, and **10b** with a fully blocked C^5-position with two methyl groups, a cyclopropyl group, or two fluorine atoms, respectively. Compound **27** (Scheme 2) was prepared from commercially available 4-oxo-piperidine-3-carboxylic acid methyl ester, which was Boc-protected, and triflated (NaH, Tf_2NPh, THF). Commercially available *N*-Boc-piperidone, was alkylated with (2-chloroethyl)dimethylsulfonium iodide[7] in *tert*-butanol using potassium *tert*-butylate as base, to give Boc-protected 5-aza-spiro[2.5]octan-8-one which was carboxymethylated (LDA, then MeOCOCN, THF, −78 °C), and triflated (NaH, Tf_2NPh, THF) to yield compound **28**. Double methylation of *N*-Boc-piperidone, with NaH and methyl iodide in THF, followed by carboxymethylation (LDA, then MeOCOCN, THF, −78 °C) and triflation (NaH, Tf_2NPh, THF) gave compound **29**. Suzuki couplings of **27–29** with **15a**

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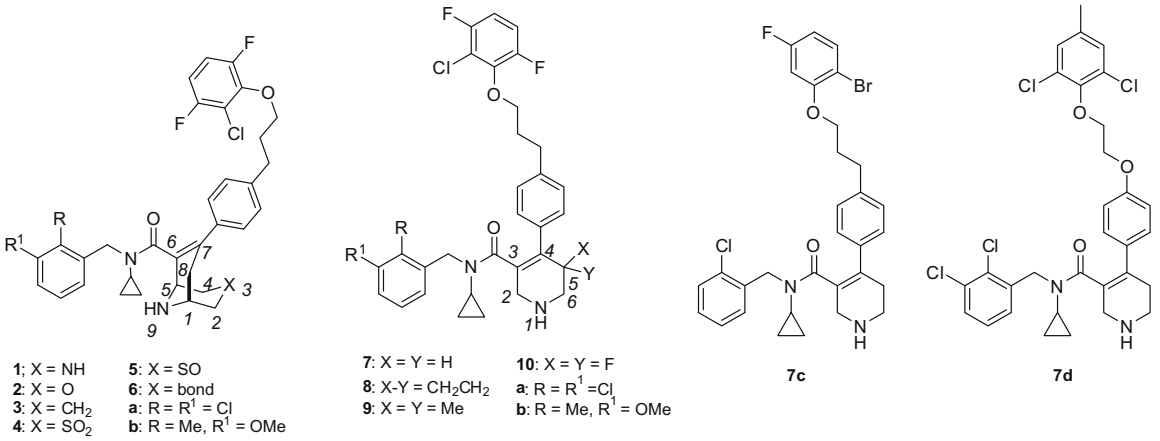
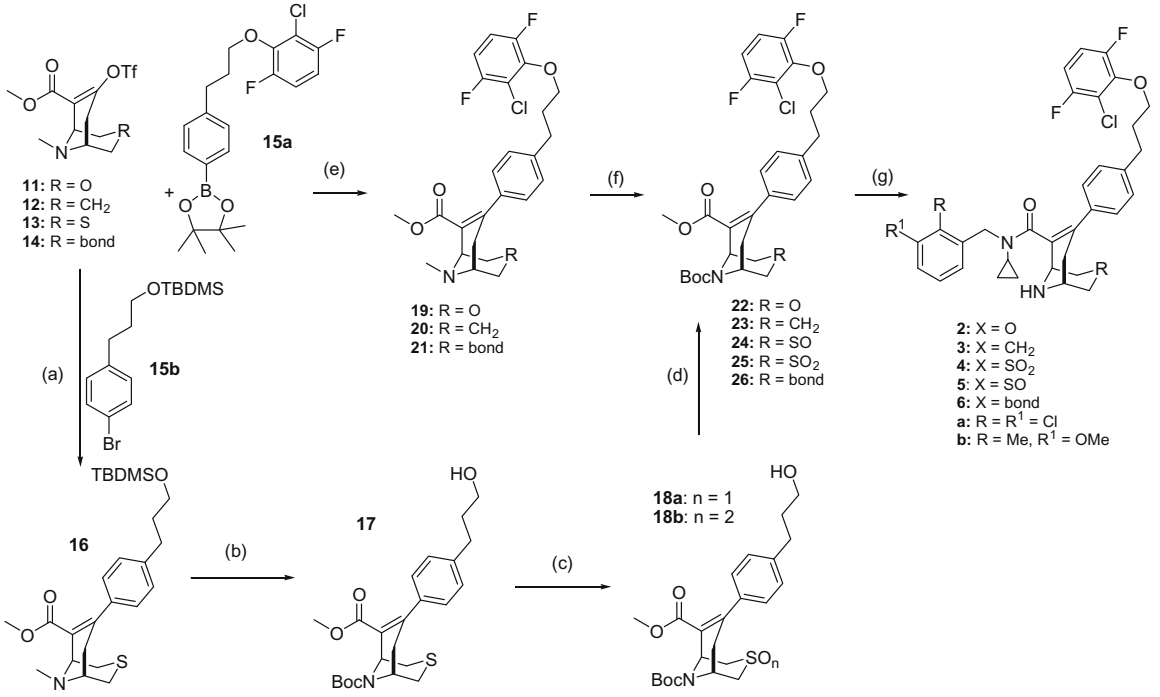
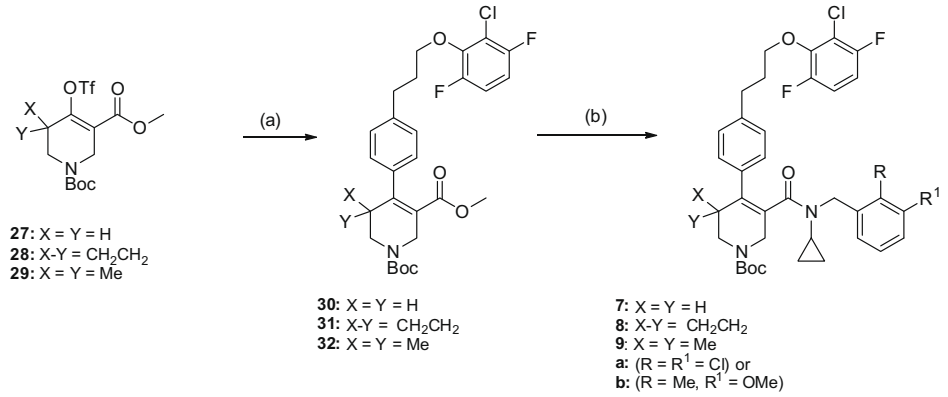


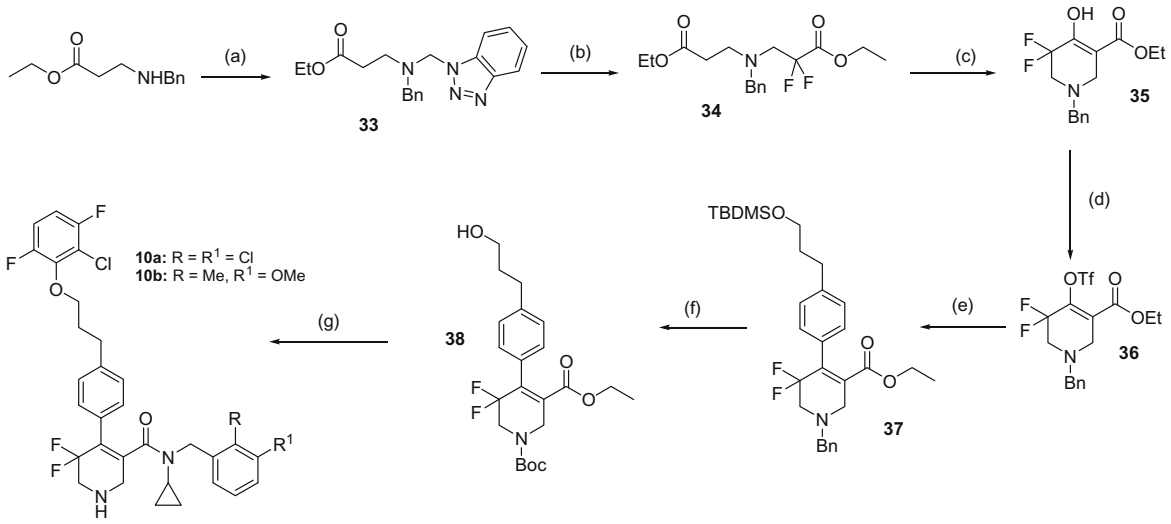
Figure 1. Described compounds with their corresponding numbering systems.



Scheme 1. Reagents and conditions: (a) (i) **15b**,[1a] *n*-BuLi, THF, −78 °C, 30 min, (ii) $ZnCl_2$, −78 °C to 0 °C, 15 min, (iii) $Pd(PPh_3)_4$, **13**, 0 °C to reflux, 1.5 h, 80%; (b) (i) $CH_3CClHOCOCl$, $ClCH_2CH_2Cl$, reflux, 4 h, (ii) MeOH, rt, 4 h, (iii) Boc_2O, DIPEA, CH_2Cl_2, 0 °C to rt, 2 h, 55%; (c) **18a**: mCPBA, CH_2Cl_2, 0 °C, 30 min, 93%. **18b**: mCPBA, CH_2Cl_2, 0 °C to rt, overnight, 92%; (d) 2-chloro-3,6-difluorophenol, ADDP, PBu_3, toluene, reflux, 1 h, 88–98%; (e) **15a**, $PdCl_2(PPh_3)_2$, Na_2CO_3, H_2O, propanol, DMF, 80 °C, 1 h, 58–82%; (f) $CH_3CClHOCOCl$, $NaHCO_3$, $ClCH_2CH_2Cl$, reflux, 2 h, (ii) MeOH, 50 °C, 1 h, (iii) Boc_2O, DIPEA, CH_2Cl_2, 0 °C to rt, 2 h, 82–93%; (g) (i) NaOH, H_2O, EtOH, 70 °C, 8 h, (ii) amine,[1a] EDC·HCl, HOBt, DMAP, DIPEA, CH_2Cl_2, rt, 4–5 days, (iii) HCl, dioxane, CH_2Cl_2, 0 °C to rt, 1–2 h, 20–67%.



Scheme 2. Reagents and conditions: (a) **15a**, $PdCl_2(PPh_3)_2$, Na_2CO_3, H_2O, propanol, DMF, 80 °C, 1 h, 48–84%; (b) (i) LiOH, H_2O, THF, 60 °C, 3 days, (ii) amine,[1a] EDC·HCl, HOBt, DMAP, DIPEA, CH_2Cl_2, rt, 3–10 days, (iii) HCl, dioxane, CH_2Cl_2, 0 °C to rt, 1–2 h, 20–38%.



Scheme 3. Reagents and conditions: (a) benzotriazole, formaldehyde, MeOH, rt, 94%; (b) BrF_2CCO_2Et, Zn, Me_3SiCl, THF, 93%; (c) LDA, −78 °C, THF, 18 h, 81%; (d) (i) NaH, THF, 0 °C, 30 min, (ii) Tf_2NPh, THF, 55 °C, 72 h, quant.; (e) (i) **15b**, *n*-BuLi, THF, −78 °C, 30 min, (ii) $ZnCl_2$, −78 °C to 0 °C, 30 min, (iii) **36**, $Pd(PPh_3)_4$, THF, 0 °C to 45 °C, 18 h, 59%; (f) H_2, Pd/C,Boc_2O, AcOH, EtOH, rt, 4 h; (g) (i) 2-chloro-3,6-difluorophenol, ADDP, PBu_3, toluene, reflux, 2 h, 90%, (ii) NaOH, EtOH, H_2O, 5 h, (iii) amine,[1a] EDC·HCl, HOBt, DMAP, DIPEA, CH_2Cl_2, rt, 3 days, (iv) HCl, dioxane, CH_2Cl_2, 0 °C to rt, 2 h, 38%.

Table 1
Measured IC_{50} values in nM

	Compound									
	1a	**2a**	**3a**	**4a**	**5a**	**6a**	**7a**	**8a**	**9a**	**10a**
IC_{50} (buffer)	0.40	0.68	1.1	6.4	NP	0.55	1.2	34	31	800
IC_{50} (plasma)	36	55	113	ND	NP	47	53	4000	2000	ND
	1b	**2b**	**3b**	**4b**	**5b**	**6b**	**7b**	**8b**	**9b**	**10b**
IC_{50} (buffer)	1.37	0.49	0.92	NP	64	0.74	2.0	210	170	330
IC_{50} (plasma)	54	42	101	NP	ND	43	87	7400	7400	ND

ND = not determined, NP = not prepared.

yielded compounds **30**–**32**, respectively. Ester hydrolysis followed by amide couplings and final removal of the Boc-groups led to the desired piperidine derivatives **7a**–**9b**.

Saponification of compound **30** led to a mixture of two isomers—the desired α,β-unsaturated carboxylic acid and the β,γ-unsaturated acid. Such a partial displacement of the double bond has actually already been observed with our diazabicyclononene derivatives described earlier.[1a] The amide coupling with this mixture delivered the corresponding amide products, which could only be separated after extensive chromatographic purification.

Compound **34** (Scheme 3) was prepared starting from the known *N*-benzyl-β-alanine ethyl ester via the benzotriazol derivative **33**, using a Reformatsky reaction.[8] A Dieckmann cyclization led to tetrahydropyridine **35**, which was converted into its corresponding vinylic triflate **36**. A Negishi coupling led to compound **37**, and the desired tetrahydropyridine **10** was obtained following standard chemistry.

The IC_{50} values of these compounds for renin were determinated as described previously[1a] and are presented in Table 1. As expected, optimal substituents from the diazabicyclononene scaffold (compounds **1a** and **1b**) led to compounds with high affinities on other scaffolds as well, as long as this scaffold fit in the active site. The IC_{50} values in buffer for compounds **1a**, **2a**, **3a**, **6a**, and **7a** on one hand, and for compounds **1b, 2b**, **3b**, **6b**, and **7b** on the other hand, were almost identical. All these scaffolds could be considered as being very effective in terms of binding. This confirmed our hypothesis that the C^2–N^3–C^4 bridge of diazabicyclononene scaffold does not influence the binding of these molecules to renin

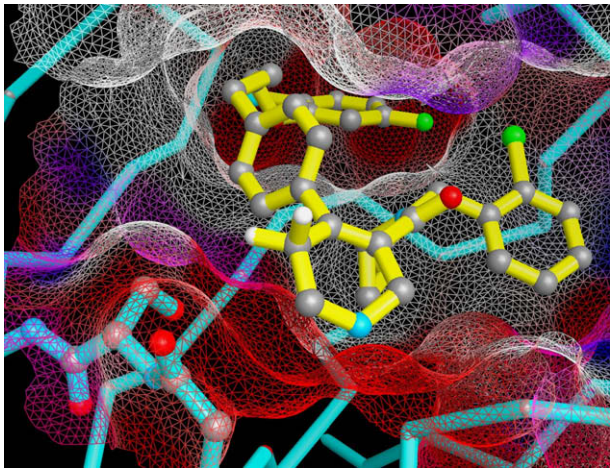


Figure 2. X-ray structure analysis of compound **7c** in renin; Gly40 and Ser41 are pictured with their carbonyl groups and side-chain. PDB Code: 3k1w.

in an essential manner. Even the absence of any bridge (compounds **7a** and **7b**) led to excellent renin inhibitors. As a matter of fact, the tetrahydropyridine moiety differs only by an endocyclic double bond from the chiral piperidine template that served as basis to the development of our renin inhibitors.[9] Thus, the tetrahydropyridine renin inhibitors of type **7** represent the first achiral, potent renin inhibitors known in the literature.

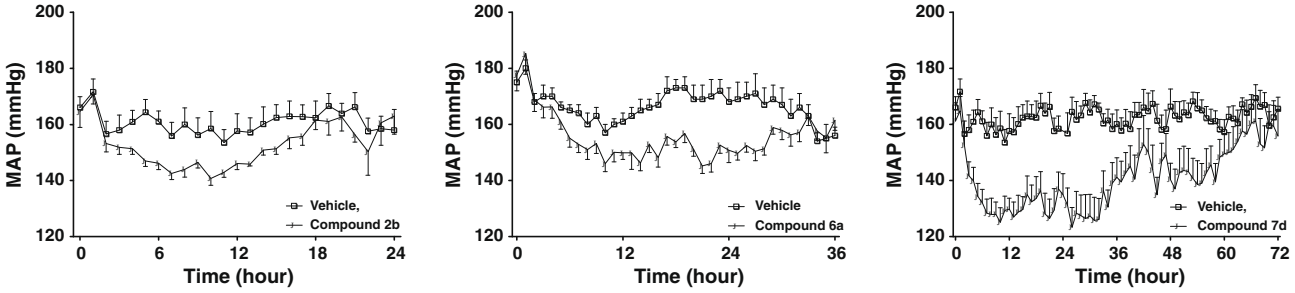


Figure 3. Oral hypotensive activity of compounds **2b** (10 mg/kg), **6a** (10 mg/kg), and **7d** (30 mg/kg) in the TGR model.

On the other hand, compounds **4a** and **5b**, as well as compounds **8a**, **8b**, **9a**, **9b**, **10a**, and **10b** turned out to be much less potent. A possible explanation for the lack of affinity of compounds of type **4**, **5**, and **10** resides in the decreased pK_a values of the secondary amines in the various templates. Diazabicyclononene compounds (e.g., **1)** displayed pK_a values around 8.2 and 3.6, while oxazobicyclononanes (e.g., **2b)** displayed a pK_a value around 6.5. The absence of an electronwithdrawing heteroatom in the bridge led to slightly enhanced pK_a values around 8.4 for the tropane scaffold (e.g., **6**), and 8.0 for a tetrahydropyridine scaffold (e.g., **7**). The pK_a value for the sulfone **4a** was measured at 3.5, while the corresponding sulfoxide **5b** displayed a pK_a value of 3.9. The two fluorine atoms in compounds **10a** and **10b** had a strong pK_a lowering effect. Since these compounds were not well soluble in aqueous medium in their neutral forms, the pK_a values had to be determined in solvent mixtures and could not be determined accurately. From these experiments, they were estimated to be between 3 and 4, resulting in a difference of 4–5 pK_a units compared with the nonfluorinated compounds. Models predict[10] a difference of 3.7 log-units. With pK_a values in this range, compounds of type **4**, **5**, and **10** are only partially protonated at the optimal pH for renin to cleave its substrate (pH 5.5–6). A protonated nitrogen of the piperidine heterocycles forms a tight salt bridge with the catalytically active aspartyl residues, and is therefore indispensable for high binding affinity.[1a,9]

Compounds of type **7**, **8**, or **9** do not comprise electronwithdrawing substituents attached to the piperidine ring and display pK_a values in the range of 8.0. Modeling studies based on X-ray structure analysis of compound **7c** (Figs. 1 and 2), led to the hypothesis that a double substitution at the 5-position of the tetrahydropyridine scaffold should not be favorable due to potential clashes of the equatorial substituent with Gly40 and/or Ser41.

The IC_{50} values of compounds **1a**–**9b** in plasma (Table 1) were higher by a factor of 50–100 independently of the scaffold. These values indicate a strong plasma protein binding for these compounds. The more polar 3-methoxy-2-methyl benzyl amides (**b**-series) tended to show slightly less shifted plasma values if compared with the corresponding, less polar, 2,3-dichlorobenzyl amides (**a**-series).

The pharmacological efficacy of selected compounds was evaluated in the TGR model[2] at 10 and 30 mg/kg (Fig. 3). Compound **2b** showed a slightly inferior profile to analogous diazabicyclononene derivatives.[1a] Obviously the exchange of the N^3-nitrogen with an oxygen exerts only minor effects on compound properties. The hydrophobic compound **6a** stands out by its exceptionally long duration of action. Tetrahydropyridine **7d** (Fig. 1; IC_{50} = 0.30 nM in buffer, 35 nM in plasma, prepared in analogy to other compounds of type **7**) also displayed a pronounced pharmacological effect at 30 mg/kg, validating this achiral series as potent renin inhibitors. Pharmacokinetic experiments (wistar rats, 10 mg/kg, po) on similar tetrahydropyridines of type 7 showed bioavailabilities varying between 10% and 70%.

Inhibition of the cytochrome P450 3A4 by these compounds emerged as a selection criteria. While compound **1a** inhibited CYP3A4 only weakly (IC_{50} >10 μM), the pure, active enantiomer **2b** proved to be a rather potent CYP3A4 inhibitor (IC_{50} = 1 μM). Compound **6a** (IC_{50} = 1.9 μM), and compound **7a** (IC_{50} = 1.3 μM) were potent CYP3A4 inhibitors as well. Clearly, the absence of a second polar group beside the charged secondary amine, leads to compounds significantly inhibiting the CYP3A4 enzyme.

In conclusion, we identified new scaffolds leading to highly potent, orally active renin inhibitors. We also identified some achiral, low-nanomolar inhibitors for human renin. Modulation of an existing bridge in these bicyclic scaffolds[1a] influences neither the binding affinity for renin nor the pharmacological efficacy, as long as the basic properties of the essential secondary amine are conserved.

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3. Compond **1a** is a pure enantiomer. All other compounds containing chiral centers are racemates.
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5. List of abbreviations: ADDP, 1,1′-(azodicarbonyl)-dipiperidine; Boc, *tert*-butoxycarbonyl; *n*-BuLi, *n*-butyl lithium; DIPEA, diisopropylethylamine; DMAP, 4-(*N*,*N*-dimethylamino)pyridine; EDC·HCl, *N*-(dimethylaminoproyl)-*N*′-ethylcarbodiimide hydrochloride; HOBt, 3-hydroxybenzotriazol; LDA, lithium diisopropilamide; mCPBA, *meta*-chloro peroxobenzoic acid; Tf, CF_3SO_2–.
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