Synthesis and monoamine uptake inhibition of conformationally constrained 2β-carbomethoxy-3β-phenyl tropanes†

Patrick Johannes Riss,*[a] René Hummerich[b] and Patrick Schloss[b]

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A series of 2β-carbomethoxy-3β-phenyl tropanes with conformationally constrained nitrogen substituents were synthesized as potential selective dopamine transporter ligands. These novel compounds were examined for their monoamine uptake inhibition potency at the human dopamine transporter (hDAT), the human serotonin transporter (hSERT) and the human noradrenalin transporter (hNET), stably expressed in human embryonic kidney cells (HEK). A SAR-study was conducted to determine the contribution of extended, 4-fluorinated, conformationally constrained C_4 chains at the tropane nitrogen to human monoamine transporter affinity and selectivity.

Introduction

The dopaminergic system remains an important molecular target for basic research, drug development and diagnostic imaging.[1] This is due to its relevance to addiction, psychiatric and neurodegenerative diseases.[2] The dopamine transporter (DAT) mediated dopamine reuptake, in particular, has been of significant interest.[3] Quantification of the availability of neuronal DAT-binding sites with positron emission tomography (PET) is a sensitive measure of the function and integrity of the dopaminergic system.[4]

Two different classes of compounds display high *in vitro* affinity and selectivity to the DAT. These are benzhydryl substituted piperazine derivatives and ligands based on the methyl 3β-phenyltropane-2β-carboxylate lead (Fig. 1).[5] Radiolabelled high affinity analogues of the latter proved to be the most promising candidates for DAT imaging.[6,7]

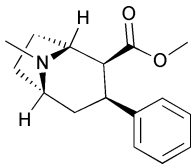


Fig. 1 2β-Carbomethoxy-3β-phenyl tropane, lead structure for DAT-selective monoamine transporter ligands.

Considerable effort has already been spent on the modification of the available lead structure.[8] However, the development of highly potent, selective DAT inhibitors was often complicated by the mixed binding profile of this class of compounds. Various highly potent cocaine-derived DAT inhibitors show a similar inhibition of norepinephrine or serotonin uptake.[8,9] In fact, the hDAT is closely related to the human serotonin transporter (hSERT; 49% amino acid homology) and the human norepinephrine transporter (hNET; 67% amino acid homology).[10]

Nevertheless, modifications of the phenyltropane carboxylate lead have already generated promising examples of hDAT or hSERT selective radio-ligands. These successful molecular modifications indicate that the selectivity for the DAT can be affected by substitutions at the ester function, at the phenyl ring as well as at the nitrogen.[8–9,11,12] With regard to a potential application as fluorinated DAT radio-ligands for PET, variation of ω-fluorohydrocarbon chains at the tropane nitrogen showed the most promising results.[7,11] A trend in selectivity as well as in affinity was found in the ω-fluoroalkyl series.[9,11] While nortropanes are more potent at the SERT, DAT affinity, SERT–DAT and NET–DAT ratios correlate with increasing chain lengths.[8,9,11,12] The 4-fluorobutyl moiety has rarely been investigated in this relationship. Instead, (*E*)-configured 3-iodoallyl substituted nortropanes showed encouraging improvements.[13a] Isosteric substitution of the (*E*)-iodoallyl moiety by an (*E*)-4-fluorobut-2-ene-1-yl residue led to the development of (*E*)-fluorobutenyl substituted 4′-halophenyl (FBCFT) and 4′-methylphenyl (LBT999, **7q**) derivatives. Most of these displayed remarkable selectivity and affinity (Fig. 2).[13,14]

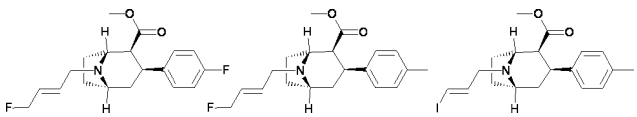


Fig. 2 FBCFT, LBT-999 and PE2I.

It has been proposed that this improvement might be particularly effected by an olefinic residue in the vicinity of the nitrogen. These findings may also indicate preferred binding of extended conformations of the *N*-C_4 substituted derivatives to the DAT. This is presumably due to their reduced flexibility upon binding to the DAT in the slow isomerisation step.[15] Although the concept of conformational restriction based monoamine transporter ligand design has been studied previously,[16] no further systematic elucidation of the contribution of conformational restriction at the *N*-substituent has been reported so far.[17]

Para-substitution at the phenyl ring is known to have a significant effect on potency and selectivity of cocaine-analogue tropanes. Bulky groups may increase SERT affinity, and even 4-iodo and 4-bromo derivatives exhibit a remarkable increase

[a]*Institute of Nuclear Chemistry, Johannes Gutenberg-University Mainz, Fritz Strassmann-Weg 2, 55128, Mainz, Germany. E-mail: riss@uni-mainz.de; Fax: +49 6131 3924692; Tel: +49 6131 3925302*
[b]*Institute of Mental Health, J 5, 68159, Mannheim, Germany*

† Electronic supplementary information (ESI) available: ^{1}H NMR and ^{13}C NMR spectra of compounds **7c** and **7j**. See DOI: 10.1039/b902863c

in SERT and NET affinity. In contrast, their DAT-inhibition-potencies and binding affinities are in a similar range as the ones of the methyl analogues.[18]

Herein, the influence of conformationally constrained, extended C_4-chains in rigid tropane-based monoamine transporter ligands to hDAT, hSERT and hNET selectivity and inhibition potency is examined. The 4-position at the phenyl ring was varied from hydrogen *via* fluoride and chloride to methyl. For comparison, the non-restricted 4-fluorobutyl derivatives have also been prepared.

Results and discussion

The synthesis of Michael-acceptor **3** was performed as published elsewhere (Scheme 1).[19a,b] Stereoselective 1,4-addition of the corresponding Normant-cuprates furnished phenyltropanes **4a–d** in high yield (88%) and high diastereomeric excess (*de* >95%).[20] Demethylation to nortropanes **5a–d** was achieved *via* a modified procedure,[19c] providing up to 97% of nortropane. More than 85% of the demethylated product was isolated after flash chromatography in a preparative scale run.

Compound **6a** (Fig. 3) was obtained from but-2-yne-1,4-diol *via* acetylation of both alcohol functions (to give compound **14**) followed by enzyme catalysed selective hydrolysis of one acetyl group using porcine pancreatic lipase (PPL, E.C. 3.1.1.1.; 96 and 97% respectively). The alcohol product **15** was mesylated in dichloromethane at 0 °C. Subsequent fluorination with CsF in 2-propanol furnished fluoride **17** in 85% yield. Fluoride **17** was deprotected in methanol containing KOH (10%) at room temperature to afford alcohol **18** in 88% yield. Appel halogenation using hexachloroethane in 1,2-dichloroethane followed by bulb-to-bulb distillation afforded 4-fluorobut-2-yne-1-yl chloride **6a** in 88% yield, resulting in an overall yield of 60% over six steps (Scheme 2).

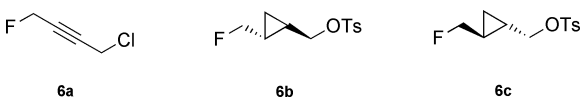
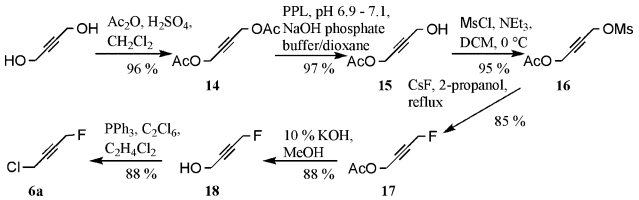


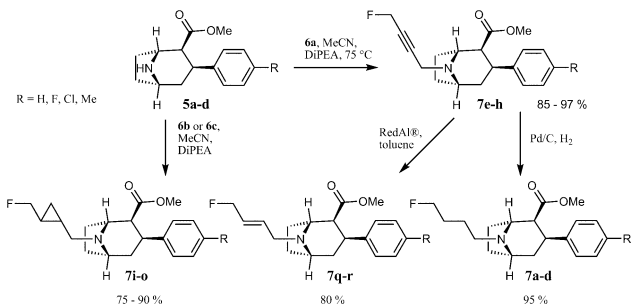
Fig. 3 Electrophilic building blocks for the introduction of terminally fluorinated C_4-residues.



Scheme 2 Synthetic route to 4-fluorobut-2-yne-1-yl chloride.

Cyclopropane **6b** and the opposite enantiomer **6c** (Fig. 3) were synthesised stereoselectively as reported recently.[21]

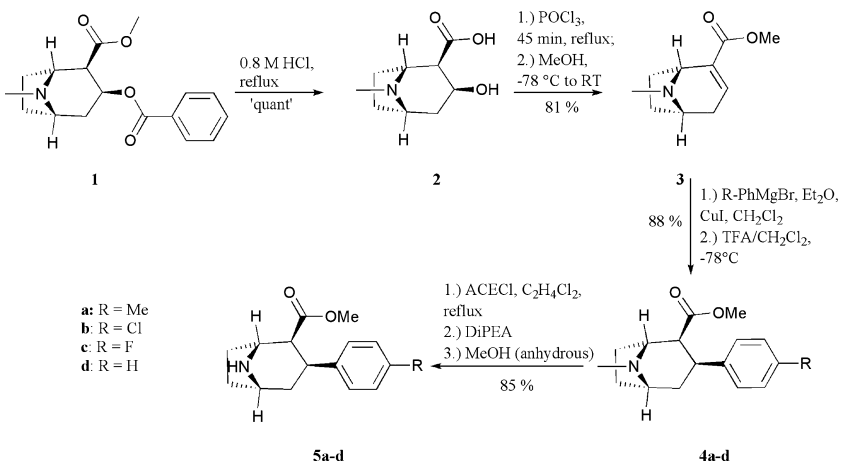
Nortropanes **5a–d** were alkylated using electrophiles **6a–c** (Scheme 3) with acetonitrile as solvent and diisopropylethylamine as base in 75–97% yield. Compounds **7a–d** were obtained directly from **7e–h** *via* catalytic hydrogenation with Pd° on activated carbon. Compounds **7q** and **7r** were obtained *via* reduction of alkynes **7e** and **7h**, respectively, with sodium (bismethoxyethoxy)aluminium hydride (RedAl®).



Scheme 3 Synthesis of compounds **7a–r**; see Table 1 for compound numbers and structures.

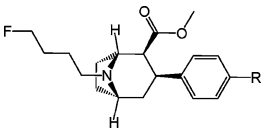
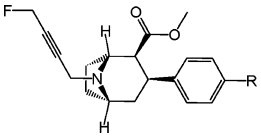
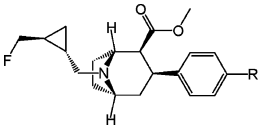
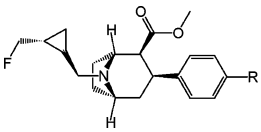
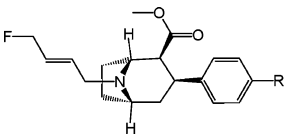
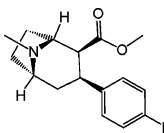
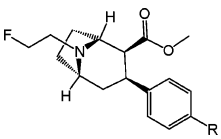
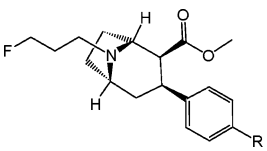
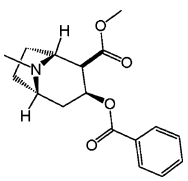
The final compounds were purified by semi-preparative HPLC and converted into water soluble hydrochlorides for cell studies. The purity of all assayed compounds exceeded 99% (by HPLC peak-area at UV_{254}).

Saturation analyses of [^{3}H]dopamine ([^{3}H]DA), [^{3}H]serotonin ([^{3}H]5HT) and [^{3}H]noradrenalin ([^{3}H]NE) uptake into HEK293 cells stably expressing the human monoamine transporters hDAT, hNET and hSERT, respectively, were performed. The resulting IC_{50} values are summarized in Table 2. A representative experiment



Scheme 1 Synthesis of nortropanes **5a–d**; see Table 1 for compound numbers and structures.

Table 1 Ligand structures of new compounds and references

Compound[a]	Structure[b]	R[c]	Name[d]
7a		Me	PRD01
7b		Cl	PRD07
7c		F	PRD11
7d		H	PRD15
7e		Me	PRD04
7f		Cl	PRD08
7g		F	PRD12
7h		H	PRD16
7i		Me	PRD05
7j		Cl	PRD09
7k		F	PRD13
7l		H	PRD17
7m		Me	PRD06
7n		Cl	PRD10
7o		F	PRD14
7p		H	PRD18
7q		Me	LBT999
7r		H	PRD19
8		F	β-CFT
9		I	β-CIT
10		Cl	FECNT
11		I	FE-β-CIT
12		I	FP-β-CIT
13			Cocaine

[a] Compound number. [b] General structure. [c] Phenyl substituent. [d] Project tag or common name of compound.

is shown in Fig. 4.[9] The selectivity was expressed as IC_{50}-ratios between the hSERT and the hDAT as well as the hNET and the hDAT, respectively.

To elucidate the effect of non-constrained C_4-chains, compounds **7a–d** were synthesised. Among these, the degree of inhibition of DA reuptake increases in the sequence H < Cl ≈ F < Me.

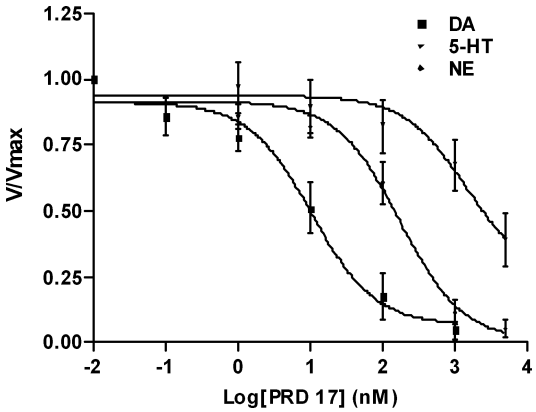


Fig. 4 Inhibition of [^{3}H]DA, [^{3}H]NE and [^{3}H]5-HT uptake into HEK293 cells stably expressing human DAT, NET or SERT, respectively. HEKhDAT, HEKhNET and HEKhSERT cells were incubated with transporter buffer containing 250 nM [^{3}H]DA (■), [^{3}H]NE (♦) and [^{3}H]5-HT (▼), respectively, and increasing concentrations of **7l**. The IC_{50} values for the inhibition of the single monoamines obtained in these representative experiments were as follows: 10.5 ± 0.2 nM for [^{3}H]DA, 172.5 ± 0.3 nM for [^{3}H]NE and 1456 ± 0.2 nM for [^{3}H]5-HT.

This effect is even more pronounced at the hSERT. Inhibition potency significantly increases with the size of the *para*-substituent: H (4 μM) < F (2.6 μM) < Cl (1.4 μM) < Me (0.8 μM). At the hNET, both electron withdrawing substituents show a similar potency, comparable to the hDAT. Potency decreases from chlorine to hydrogen (Cl ≈ F > Me > H). Both compounds with an electron donating substituent (**7a** and **7d**) show a remarkable selectivity over the hSERT (>100). They also show a reasonable selectivity over the hNET (4–5). However, hydrogen derivative **7d** is ~4-fold less potent at the hDAT (IC_{50} = 33 nM), compared to **7a** (8 nM). On the other hand, both compounds with electron withdrawing substituents show a similar potency at hDAT and hNET. As a result, **7b** and **7c** display a threefold lower selectivity over the hNET. Selectivity over the hSERT is retained (60–140).

To examine the contribution of both a linear C_4-segment as well as the necessity of an olefinic nitrogen-residue, alkynes **7e–h** were prepared. Both compounds containing an electron donating substituent **7e** and **7h** display a low nanomolar potency of 3 nM and 6 nM at the hDAT, respectively. Compared to the flexible analogues **7a** and **7d**, a 2-fold to 6-fold increase in potency is achieved. The results at the hSERT are even more significant. The introduction of a 4-fluorobut-2-yne-1-yl chain strikingly increases hSERT potency for **7e–h**. The size dependency of potency and selectivity, observed for the flexible analogues **7a–d**, is absent within the alkyne series. Interestingly, methyl derivative **7e** displays outstanding characteristics. This is consistent with the known restricted methyl analogues *e.g.* PE2I and LBT999 (**7q**). Its high potency is combined with good selectivity over the hSERT (73-fold) and the NET (10-fold). A significant increase in hDAT potency is observed for **7h**. This is accompanied by even higher increases at both the hSERT (16 fold) and the hNET (11-fold). The 4-fluorophenyl and 4-chlorophenyl derivatives **7f** and **7g** show a moderate potency (~16 nM) at the hDAT. This is comparable to their flexible analogues. Nonetheless, their hSERT-potency is partially augmented. In particular **7f** displays a 4-fold loss in

Table 2 [b] IC_{50} values and monoamine transporter selectivity of novel phenyl tropanes, as determined in human embryonic kidney cells (HEK 293) stably transfected with hDAT, hSERT and hNET-RNA

Compound	$hDAT_{IC_{50}}$ [c] ($hDAT_{K_i}$)/nM	$hSERT_{IC_{50}}$ [c] ($hSERT_{K_i}$)/nM	$hNET_{IC_{50}}$ [c] ($hNET_{K_i}$)/nM	hSERT/hDAT	hNET/hDAT
7a	7.8 ± 0.3	810 ± 5	37 ± 1	104	5
7b	22 ± 1	1400 ± 0.3	29 ± 1.2	64	1.5
7c	19 ± 1	2600 ± 0.4	31 ± 1	137	1.5
7d	33 ± 1	4000 ± 0.5	136 ± 1	121	4
7e	3.3 ± 0.5	240 ± 4	31 ± 1	74	10
7f	17 ± 1	270 ± 0.2	41 ± 1	17	2.5
7g	16 ± 1	1400 ± 0.2	21 ± 1.3	89	1.5
7h	5.8 ± 0.6	250 ± 0.1	13 ± 0.5	44	2
7i	14 ± 0.5	950 ± 3	56 ± 0.4	70	4
7j	4.3 ± 0.5	220 ± 0.4	16 ± 0.4	50	3.5
7k	31 ± 1	690 ± 5	76 ± 1	22	2.5
7l	11 ± 0.5	1400 ± 3	175 ± 1	134	17
7m	5.7 ± 0.3	290 ± 4	25 ± 0.5	51	4.5
7n	8.9 ± 0.3	160 ± 2	24 ± 1	18	3
7o	12 ± 0.6	420 ± 2	37 ± 1	34	3
7p	34 ±1	560 ± 3	84 ± 1	16	2.5
7q	26 ± 1	700 ± 2	150 ± 2	27	6
7r	53.3 ± 0.8	2650 ± 200	210 ± 0.6	50	4
8	40 ± 1	2300 ± 0.3	120 ± 2	57	3
9[a]	(6.3 ± 1.7)	(29 ± 6.4)	(33 ± 13)	4.5	5
10[a]	2.5 ± 0.2	530 ± 1.5	10.6 ± 0.5	210	4.2
11[a]	(91 ± 5)	(130 ± 31)	(130 ± 50)	1.5	1.5
12[a]	(28 ± 7)	(110 ± 64)	(70 ± 15)	4	2.5
13	(320 ± 130)	(580 ± 110)	(180 ± 25)	2	0.6

[a] Taken from ref. 9: IC_{50} values were converted to K_i values using the Cheng and Prusoff equation by the authors, $K_i = IC_{50}/(1 + [L]/K_m)$ (where $[L]$ is the concentration of [^{3}H]DA, [^{3}H]5-HT or [^{3}H]NE; K_m values from Eadie–Hofstee-plots. [b] Values are mean ± SD (nM). [c] Compounds **7a–7r** did not exhibit competitive inhibition of substrate transport.

hSERT selectivity. Also, **7f** has a 2-fold lower potency at the hNET, resulting in a higher hNET selectivity.

To investigate the effect of (*E*)-configuration, *trans*-cyclopropanes **7i–p** were examined. These derivatives facilitate the evaluation of particular effects of olefinic double bonds in the same position. Both diastereoisomeric forms lead to clearly distinguishable characteristics. Within the (*S,S*)-configurated cyclopropanes, the hDAT activity increases in the sequence F < Me < H < Cl. In contrast, the hDAT potency within the (*R,R*)-analogues increases in the sequence H < F < Cl < Me. The hSERT and the hNET potencies follow the order H < F < Me < Cl, completely independent of the absolute configuration. Compared to the 4-fluorobutyl- and 4-fluorobutynyl-residues, profound changes can be observed for the compounds containing an electron withdrawing phenyl substituent. Chloro derivatives **7j** and **7n** display a remarkably high hDAT potency of 4 and 9 nM, respectively. However, the (*S,S*)-analogue **7j** is 2.5-fold more selective to the hSERT (SERT/DAT ~ 50) and 1.3-fold more selective to the hNET (NET/DAT ~3.6). In contrast, the overall activities of fluoro-analogues **7k** and **7n** are significantly lower (31 nM and 12 nM, respectively). In this case the (*R,R*) analogue **7o** exhibits a 1.5-fold higher hSERT selectivity and a 1.25-fold higher selectivity over the hNET. Among the compounds containing an electron donating phenyl substituent, the hSERT and hNET potencies of the (*S,S*)-derivatives are slightly (at least 2-fold) lower than the corresponding (*R,R*)-analogues. Conversely, (*R,R*)-analogue **7p** shows the lowest hDAT potency (34 nM) of all the novel compounds. Methyl derivative **7i** has a lower hDAT-potency (14 nM) than its (*R,R*)-counterpart **7m** (6 nM). Both display similar selectivity over the hNET. However, the (*S,S*)-diastereoisomer **7i** is more selective over the hSERT. Finally, the introduction of the (*S,S*)-2-fluoromethylcyclopropylmethyl-residue led to the discovery of a potent hDAT inhibitor **7l** (11 nM) which provides remarkable selectivity over the hSERT (134-fold) and the hNET (17-fold). Interestingly, the correlation between activity and the characteristics of the phenyl substituent is absent between **7i–l**.

Conclusion

In summary, the overall potency within the 4-fluorobutyl series is dependent on the size of the phenyl-substituent. In comparison, the introduction of a linear alkyne strongly increases activity at all transporters. Among the cyclopropane containing ligands, both diastereoisomeric isomers lead to clearly distinguishable characteristics.

The above findings indicate a strong influence of inflexible nitrogen substituents. In particular, the extended (*S,S*)-configurated side chain. The *N*-substituent might influence the overall orientation of the molecule, which leads to a different arrangement of the phenyl substituent in the proximity of the aromatic binding site. This preset orientation might furthermore limit the beneficial effect of aromatic interactions on binding and thereby inhibition potency.

In all other cases, the overall effect of the phenyl-substituents remains similar as described for the *N*-methyl analogues. More or less the same order of affinity is found within the present study. This could give rise to the conclusion that the derivatives presented herein still bind in the same environment as the non-*N*-modified tropanes.

Most novel derivatives provide low to moderate nanomolar IC_{50} at the hDAT (**7a**,**c**,**e–j**,**l–o**) and selectivity over the hSERT (**7a–p**) and also over the hNET (**7a**,**d–f**,**h–p**). Two potent compounds, **7e** and **7l**, emerge as outstandingly selective over the hNET (10 and 17-fold, respectively), while maintaining significant selectivity over the hSERT (74 and 134-fold, respectively). In both cases, the inhibition potencies remain in a range comparable to the reference compounds (**7q**, **8–9**, **11–12**), whereas their selectivity ratios exceed the values for these reference compounds.

The only exception in terms of a superior hSERT–hDAT ratio is the clinically established imaging-agent FECNT (**10**) which, on the other hand, provides only moderate selectivity over the hNET. Furthermore, LBT-999 (**7q**) has recently been validated as an appropriate radio-ligand for PET-studies of the striatal and extra-striatal DAT. Based on these findings we conclude that two potent DAT-inhibitors (**7e** and **7l**) of improved *in vitro* selectivity have been discovered.

Compounds **7e** and **7l** are currently under investigation regarding their potential as ^{11}C and ^{18}F-labelled radio-probes for the non-invasive quantification of DAT availability in living subjects.

Experimental section

Melting points were determined on an Electrothermal® 9100 melting point apparatus and reported uncorrected. NMR-spectra were recorded with a Bruker AC 300 FT-NMR-spectrometer, *J* values are given in Hertz, chemical shifts are reported downfield from TMS ($\delta = 0$ ppm), referred to the solvent residual signal ^1H NMR ($CHCl_3$ 7.24 ppm) and ^{13}C NMR ($CDCl_3$ 77.0 ppm). Field desorption (FD) mass spectra were recorded on a Finnigan MAT90 FD spectrometer. HRMS-spectra were measured on a Micromass QTOF Ultima 3 spectrometer. IR-spectra were obtained from a Nicolet 6700 FTIR spectrometer. Optical rotations were determined using a Perkin-Elmer polarimeter 241 at 546 and 578 nm (Hg-lamp) and were extrapolated to the sodium D line. $[\alpha]_D$-values are given in 10^{-1} deg cm^2 g^{-1}. Boiling points are uncorrected. All chemicals were obtained in commercial quality from Acros Organics, Sigma Aldrich, VWR, TCI or STREM and used without further purification. Enzymes were obtained from Sigma-Aldrich. TLC was conducted on self-cut Merck silica gel 60 covered aluminium plates. Detection and staining were performed either using iodine on silica gel, potassium permanganate solution, UV fluorescence, vanillin–sulfuric acid, Seebach-reagent (phosphomolybdic acid, cerium sulfate, H_2SO_4) or Dragendorff-reagent (basic bismuth nitrate, potassium iodide and tartaric acid). Column chromatography was performed on Acros silica gel 60, 0.063–0.200 mesh, p. a. solvents for chromatography were washed with aqueous acid and base and distilled once, prior to use. Anhydrous solvents were used for reactions.

2β-Carbomethoxy-3β-phenyltropanes (4a–d)

A solution of *p*-substituted phenylmagnesium bromide (40 mmol, 40 ml) 1 M in THF was added to a suspension of CuI (7.59 g, 40 mmol) in THF (50 ml) under nitrogen and stirred at 0 °C for 30 min. The mixture was cooled to –43 °C and ecgonidine methyl ester (3.6 g, 20 mmol) in THF (6 : 4, 75 ml) was added dropwise, so that the temperature inside the flask did not exceed –40° C. Stirring was continued for 5 h after which the reaction mixture was cooled to −78 °C and TFA (4.56 g, 40 mmol) in CH_2Cl_2 was added dropwise over 30 min. The solvents were removed *in vacuo* to leave a semisolid residue that was partitioned between CH_2Cl_2 and cold 28% ammonium hydroxide solution (25 ml). The aqueous phase was extracted with CH_2Cl_2 (2 × 40 ml) followed by Et_2O (40 ml). Purification by flash column chromatography (Et_2O–hexanes, 1 : 9, 10% NEt_3) provided **4a–d** as colourless to slightly yellow solids in 88–90% yield (4.78 g, 35 mmol). NMR-spectra and FD-mass were in accordance with those published elsewhere.[19a,b]

Procedure A: general procedure for *N*-alkylation

Nortropane **5a–d** (100 mg, 0.35–0.45 mmol) was added to a stirred solution of Hünig's base (1.01 equiv.) in 10 ml of acetonitrile. Electrophile **6a–c** (1 equiv.) was added and the mixture was stirred at 70 °C for 12 h. The mixture was carefully concentrated *in vacuo* to leave a mobile residue that was chromatographed on silica gel (20 g, ether–hexanes, 1 : 9, 10% NEt_3) to afford products **7e–p** in 75–97% yield.

Procedure B: general procedure for *N*-demethylation

Tropanes **4a–d** (1.5 g, 5.4–6.1 mmol) were dissolved in dichloroethane (30 ml) and refluxed with 1-chloroethyl chloroformate (7 equiv.) for one hour. One equiv. of *N*,*N*-diisopropyl-*N*-ethylamine (700–790 mg) was added and the mixture was refluxed for one additional hour. Subsequently, the reaction mixture was concentrated *in vacuo* to leave a colourless, viscous residue that was taken up in MeOH (30 ml) with cooling and stirring. After refluxing for 2 additional hours, the resultant, pale yellow solution was concentrated, and the residue was taken up in cold ammonium hydroxide solution (28%, 15 ml) with intense cooling and extracted with Et_2O (2 × 25 ml), followed by dichloromethane (2 × 25 ml) and again Et_2O (25 ml). Combination, drying (anhydrous K_2CO_3) and concentration of the organic layers afforded crude nortropanes **5a–d**. Purification was performed on silica gel 60 (AcOEt–hexanes, 3 : 7, 10% NEt_3) to obtain products **5a–d** in 85–88% yield. NMR spectra were in accordance with those published previously.[13a,19a,b]

1,4-Diactyl but-2-yne-1,4-diol (14)

But-2-yne-1,4-diol (8.61 g, 0.1 mol) was dissolved in CH_2Cl_2 (75 ml) and cooled to 0 °C. Acetic anhydride (25 ml) was added with efficient stirring and the reaction was initiated *via* the addition of 1–2 drops of 97% sulfuric acid. The reaction mixture rapidly heated to reflux and darkened. Stirring was continued overnight at RT. The reaction mixture was washed with 1 M potassium carbonate solution (35 ml), followed by water (35 ml), dried over Na_2SO_4 and concentrated. Distillation afforded 94% (15.9 g, 0.094 mol) of a colourless liquid that crystallized upon standing: mp. 30–31 °C; $C_8H_{10}O_4$ requires C 56.5, H 5.9, found C 56.7, H 5.9%; δ_H (300 MHz, $CDCl_3$): 4.11 (s, 2H), 2.06 (s, 3 H); δ_C (100 MHz, $CDCl_3$): 170.1, 80.7, 52.0, 20.6; ν_{max}/cm^{-1} (neat): 2944, 2359, 1739, 1433, 1377, 1360, 1210, 1153, 1021, 964, 604; *m*/*z* (FD) 170.1 (100) $C_8H_{10}O_4$ requires 170.0579.

4-Acetoxy-but-2-yne-1-ol (15)

A solution of 1,4-diactyl but-2-yne-1,4-diol (5.0 g, 0.03 mol) in dioxane (10 ml) was added at once to 500 mg of porcine pancreatic

lipase in 0,1 M phosphate buffer (25 ml, pH 6,9) at 25 °C. The reaction mixture was stirred at 25 °C for 12 h while maintaining the pH stable *via* the controlled addition of 1,3 M NaOH solution. To determine the progress of the reaction, 500 µl samples were taken from the mixture, extracted with Et_2O and the organic extracts were analyzed by TLC. After 12–14 h the reaction was interrupted leaving less than 10% of 1,4-diactyl but-2-yne-1,4-diol unaffected. Chromatography on silica gel (30g/g; 40% ethyl acetate in hexanes, $R_f = 0.6$) afforded 3.31 g (0.029 mol) of **15** as a colourless liquid (88%). $C_6H_8O_3$ requires C 56.2, H 6.3, found C 56.4, H 6.4%; ν_{max}/cm^{-1} (neat): 3437, 2940, 2860, 2359, 1736, 1436, 1378, 1359, 1219, 1136, 1015, 965, 606; δ_H (300 MHz, $CDCl_3$): 4.69 (t, J = 1.8 Hz, 2 H), 4.26 (t, J = 1.8 Hz, 2 H), 2.28 (brs, 1 H, OH), 2.07 (s, 3 H); δ_C (100 MHz, $CDCl_3$): 170.5, 85.1, 79.5, 52.3, 50.8, 20.7; m/z (FD) 128.1 (100) $C_6H_8O_3$ requires 128.0473.

4-Acetoxy-but-2-yne-1-yl mesylate (16)

4-Acetoxy-but-2-yne-1-ol (1.28 g, 10 mmol) was dissolved in CH_2Cl_2 (10 ml) and triethylamine (1.02 g, 10 mmol) was added. After stirring at 0 °C for 30 min, methanesulfonyl chloride (1.146 g, 10 mmol) was added dropwise with stirring. After all the methanesulfonyl chloride had been added (~10 min), TLC indicated complete conversion of **15**. The reaction mixture was filtered and the filter cake was washed with cold CH_2Cl_2 (2 × 10 ml). The filtrate was washed with 1 M K_2CO_3 (15 ml), followed by water (15 ml). Subsequent drying and concentration *in vacuo* afforded **16** (1.95 g, 9.5 mmol; 95%) as a slightly turbid, colourless oil. $C_7H_{10}O_5S$ requires C 40.77, H 4.89, S 15.55, found C, 40.7 H, 4.95, S 15.6%. ν_{max}/cm^{-1} (neat): 3028, 2942, 2362, 1739, 1435, 1350, 1220, 1171, 1029, 936, 803, 526; δ_H (300 MHz, $CDCl_3$): 4.86 (t, J = 2 Hz, 2 H), 4.71 (t, J = 2 Hz, 2 H), 3.10 (s, 3 H), 2.07 (s, 3 H). δ_C (100 MHz, $CDCl_3$): 170.1, 83.9, 78.7, 57.3, 51.7, 39.0, 20.6; m/z (FD) 206.1 (100) $C_7H_{10}O_5S$ requires 206.0249.

4-Acetoxy-but-2-yne-1-yl fluoride (17)

Caesium fluoride (1.51 g, 10 mmol) was suspended in 2-propanol (15 ml) and heated to reflux. After the inorganic material had dissolved, 4-acetoxy-but-2-yne-1-yl mesylate (1.35 g, 6.6 mmol) was added dropwise with stirring. The reaction mixture was refluxed for a further 90 min. A waxy caesium mesylate precipitate indicated the reaction's progress. After all the mesylate had been consumed (TLC-monitoring) the reaction mixture was cooled to RT, filtered and the filter cake was washed with cold Et_2O and concentrated. The oily brown residue was chromatographed on silica gel (Et_2O–hexanes) to obtain **17** (730 mg, 85%, 5.6 mmol) as a slightly yellow liquid: $C_6H_7FO_2$ requires C 55.4, H 5.4, found: C 55.3, H 5.4%; ν_{max}/cm^{-1} (neat): 2943, 2850, 1742, 1434, 1376, 1360, 1216, 1150, 1025, 988, 969; δ_H (300 MHz, $CDCl_3$): 5.01 (t, J = 1.8 Hz, $J_{H\text{-}F}$ = 47.7 Hz, 1 H), 4.85 (t, J = 1.8 Hz, $J_{H\text{-}F}$ = 47.7 Hz, 1 H), 4.05 (t, J = 1.8 Hz, 2 H), 2.04 (s, 3 H); δ_C (100 MHz, $CDCl_3$): 170.1, 83.9, 81.3, 70.4 (d, $J_{C\text{-}F}$ = 161.5 Hz), 52.1, 20.6; m/z (FD) 130.1 (100) $C_6H_7FO_2$ requires 130.0430.

4-Fluorobut-2-yne-1-ol (18)

4-Acetoxy-but-2-yne-1-yl fluoride (1.3 g, 10 mmol) was dissolved in methanol (10 ml) containing KOH (560 mg, 10 mmol) and stirred at RT for 30 min after which all acetate **17** had been consumed. The reaction mixture was concentrated *in vacuo* to leave a waxy, solid residue that was taken up in Et_2O (20 ml). The solids were filtered off and the ethereal solution was washed with water (10 ml), followed by brine (5 ml). The organic layer was dried and concentrated to afford **18** in 88% yield: C_4H_5FO requires C 54.5, H 5.7, found C 54.1, H 5.8%; ν_{max}/cm^{-1} (neat): 3358, 2872, 2855, 1454, 1295, 1138, 1025, 982, 907, 731; δ_H (300 MHz, $CDCl_3$): 5.00 (t, J = 1.8 Hz, $J_{H\text{-}F}$ = 47.4 Hz, 1 H), 4.84 (t, J = 1.8 Hz, $J_{H\text{-}F}$ = 47.4 Hz, 1 H), 4.05 (t, J = 1.8 Hz, 2 H), 2.04 (s, 3 H); δ_C (100 MHz, $CDCl_3$): 89.0 (d, $J_{C\text{-}F}$ = 22 Hz), 85.1 (d, $J_{C\text{-}F}$ = 22 Hz), 70.6 (d, $J_{C\text{-}F}$ = 163.9 Hz), 50.4; m/z (FD) 88.1 (100) C_4H_5FO requires 88.0324.

4-Fluorobut-2-yne-1-yl chloride (6a)

4-Fluorobut-2-yne-1-ol (1.0 g, 11 mmol) was dissolved in dry $C_2H_4Cl_2$ (10 ml) containing triphenylphosphine (1.00 equiv.). The mixture was cooled to 0 °C with stirring over 30 min. Then C_2Cl_6 (2.6 g, 11 mmol) was added in portions. The mixture was stirred for one additional hour. Hexane was added until the mixture turned slightly turbid. The mixture was passed through a short silica column to remove the triphenylphosphine oxide and the solvent was evaporated. The volatile residue was purified *via* bulb to bulb distillation to obtain **6a** as a colourless, mobile oil. C_4H_4ClF requires C 45.10, H 3.78, found: C 45.3, H 3.7%; ν_{max}/cm^{-1} (neat): 2955, 1454, 1430, 1373, 1264, 1155, 987, 787, 699, 524; δ_H (300 MHz, $CDCl_3$): 4.99 (dt, J = 1.8 Hz, $J_{H\text{-}F}$ = 47.4 Hz, 2 H), 4.19 (dt, J = 1.8 Hz, J = 7.0 Hz, 1 H), 4.17 (dt, J = 1.8 Hz, J = 7.0 Hz, 1 H); δ_C (100 MHz, $CDCl_3$): 84.6, 79.8, 70.4 (d, $J_{C\text{-}F}$ = 166.2 Hz), 29.8; m/z (FD) 106.0 (100) C_4H_4ClF requires 106.0.

Methyl 8-(4-fluorobutyl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7a, PRD01)

7e (100 mg, 0.3 mmol), was dissolved in EtOH (5 ml) and Pd° (5%) on activated carbon was added (10 mg). H_2 was passed through this solution until all the olefin had been consumed (monitored by TLC). The reaction mixture was filtered through a pad of celite®, the filter cake was washed with EtOH (5 ml) followed by CH_2Cl_2 (10 ml). The organic phases were combined and concentrated *in vacuo*. The residue was purified *via* chromatography on silica gel (Et_2O–hexanes, 1 : 4, 10% NEt_3) to obtain **7a** as colourless crystals (90 mg, 89%): mp. 66–77 °C; $[\alpha]_D^{23}$ −39.2 (c 1.25 in MeOH). $C_{20}H_{28}FNO_2$ requires C 72.04, H 8.46, N 4.20, found: C 72.16, H 8.47, N, 4.22%; δ_H (300 MHz, $CDCl_3$): 7.13 (d, J = 8.5 Hz, 2 H, ArH), 7.05 (d, J = 8.5 Hz, 2 H, ArH), 4.42 (dt, J = 6 Hz, J_{HF} = 47.4 Hz, 2 H), 3.65 (brs, 1H), 3.45 (s, 3 H, OCH_3), 3.36 (brs, 1 H), 2.97 (dt, J = 4.8 Hz, J = 13.2 Hz, 1 H), 2.88 (t, J = 4 Hz, 1 H), 2.54 (J = 2.9 Hz, J = 12.5 Hz, 1 H), 2.32–2.20 (m, 2H), 2.27 (s, 3H, $ArCH_3$), 2.10–1.90 (m, 2 H), 1.82–1.53 (m, 5 H), 1.52–1.38 (m 2 H); δ_C (100 MHz, $CDCl_3$): 172.2, 138.1, 127.9, 127.3, 125.7, 84.0 (d, $J_{C\text{-}F}$ = 161.4 Hz), 62.8, 61.4, 56.8, 52.9, 50.9, 34.3, 33.9, 28.2, 27.9, 26.0, 25.9, 24.7, 24.6, 21.0; m/z (FD) 334.2 (100) $C_{20}H_{29}FNO_2$ requires 334.2; HRMS(ESI): exact mass calcd for $C_{20}H_{29}FNO_2$: 334.2182, found: 334.2180.

Methyl 8-(4-fluorobut-2-en-1-yl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1] octane-2-carboxylate (7q, LBT999)

7e (100 mg) was dissolved in dry THF (5 ml) and cooled to 0 °C. RedAl® (0.2 mL, 0,36 mmol; 70% in toluene) was added dropwise under nitrogen. The reaction mixture was stirred at RT for 1 h. The reaction was terminated by the dropwise addition of saturated ammonium chloride solution. The mixture was further stirred and allowed to warm to RT. The reaction mixture was filtered through a pad of celite® and the filter cake was washed with THF (5 ml) and acetone (2 × 5 ml). The filtrate was dried ($MgSO_4$) and concentrated *in vacuo*. The residue was purified *via* column chromatography (Et_2O–hexanes, 1 : 4, 10% NEt_3) to obtain **7q** as colourless crystals (86 mg, 85%): mp. 113.5 °C; $[\alpha]_D^{23}$ −16.9 (*c* 1.42 in MeOH). $C_{20}H_{26}FNO_2$ requires C 72.48, H 7.91, N 4.23, found: C 72.20, H 8.09, N 4.41%; δ_H (300 MHz, $CDCl_3$): 7.14 (d, $J = 8$ Hz, 2 H, ArH), 7.06 (d, $J = 8$ Hz, 2 H, ArH), 5.82–5.74 (m, 2 H), 4.89 (d, $J = 5$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 4.73 (d, $J = 3$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 3.69 (brs, 1H), 3.48 (s, 1H, OCH_3), 3.40 (brs, 1 H), 3.06–2.92 (m, 2 H), 2.91–2.81 (m, 2 H), 2.59 (dt, $J = 12.5$ Hz, $J = 2.7$ Hz, 1 H), 2.28 (s, 3 H, NCH_3), 2.13–1.92 (m, 2 H), 1.78–1.57 (m, 3 H); δ_C (100 MHz, $CDCl_3$): 172.0, 139.9, 135.2, 134.4 (d, $J_{C\text{-}F} = 12$ Hz), 128.6, 127.2, 126.2 (d, $J_{C\text{-}F} = 17$ Hz), 83.1 (d, $J_{C\text{-}F} = 161$ Hz), 62.3, 61.3, 54.9, 52.7, 50.9, 34.1, 33.8, 26.1, 25.9, 21.0; *m/z* (FD) 332.2 (100) $C_{20}H_{27}FNO_2$ requires 332.2; HRMS(ESI): exact mass calcd for $C_{20}H_{27}FNO_2$: 332.2026, found: 332.2024.

Methyl 8-(4-fluorobut-2-yn-1-yl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1] octane-2-carboxylate (7e, PRD04)

Synthesised according to procedure A, 97% yield. Yellowish crystals: mp. 73.5 °C; $[\alpha]_D^{23}$ −14.7 (*c* 1.46 in MeOH). $C_{20}H_{24}FNO_2$ requires C 72.92, H 7.34, N 4.25, found: C 72.79, H 7.03, N 4.53%. δ_H (300 MHz, $CDCl_3$): 7.14 (d, $J = 8.5$ Hz, 2 H, ArH), 7.06 (d, $J = 8.5$ Hz, 2 H, ArH), 5.02 (t, $J = 1.5$ Hz, $J_{H\text{-}F} = 47.8$ Hz, 1 H), 4.86 (t, $J = 1.5$ Hz, $J_{H\text{-}F} = 47.8$ Hz, 1 H), 3.89 (brs, 1 H), 3.51 (s, 3 H, OCH_3), 3.47 (brs, 1 H), 3.25 (ddt, $J = 1.5$ Hz, $J = 7.5$ Hz, $J = 16.5$ Hz, 1 H), 3.10 (ddt, $J = 1.5$ Hz, $J = 7.5$ Hz, $J = 16.5$ Hz, 1 H), 2.98 (dt, $J = 5$ Hz, $J = 12.9$ Hz, 1 H), 2.94–2.89 (m, 1 H), 2.62 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.27 (s, 3 H, $ArCH_3$), 2.16–1.92 (m, 2 H), 1.82–1.61 (m, 3 H); δ_C (100 MHz, $CDCl_3$): 171.7, 139.6, 135.3, 128.7, 127.2, 127.1, 87.7, 87.5, 70.8 (d, $J_{C\text{-}F} = 163.5$ Hz), 62.6, 61.2, 52.8, 52.6, 51.1, 42.9, 34.1, 33.7, 25.9, 25.7, 21.0; *m/z* (FD) 330.2 (100) $C_{20}H_{25}FNO_2$ requires 330.2; HRMS(ESI): exact mass calcd for $C_{20}H_{25}FNO_2$: 330.1869, found: 330.1885.

Methyl 8-(((1*S*,2*S*)-2-(fluoromethyl)cyclopropyl)methyl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7i, PRD05)

Synthesised according to procedure A, 87% yield. Colourless crystals: mp. 79 °C; $[\alpha]_D^{23}$ −26.0 (*c* 1.38 in MeOH). $C_{21}H_{28}FNO_2$ requires C 73.01, H 8.17, N 4.05, found C 72.9, H 8.2, N 4.0%. δ_H (300 MHz, $CDCl_3$): 7.14 (d, $J = 8$ Hz, 2 H, ArH), 7.06 (d, $J = 8$ Hz, 2 H, ArH), 4.23 (dm, $J_{H\text{-}F} = 48.5$ Hz, 2 H), 3.81 (brs, 1H), 3.48 (s, 1H, OCH_3), 3.45 (brs, 1 H), 2.98 (dt, $J = 12.5$ Hz, $J = 5$ Hz, 1 H), 2.89 (t, $J = 4$ Hz, 1 H), 2.57 (td, $J = 12.5$ Hz, $J = 2.6$ Hz, 1 H), 2.42 (dd, $J = 12.5$ Hz, $J = 5$ Hz, 1 H), 2.28 (s, 3 H, NCH_3), 2.07 (dd, $J = 12.5$ Hz, $J = 7$ Hz, 1 H), 1.98 (m, 2 H), 1.76–1.54 (m, 3 H), 1.01 (m, 1 H), 0.82 (m, 1 H), 0.52 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.2, 140.1, 135.1, 128.6, 127.2, 87.4 (d, $J_{C\text{-}F} = 161.5$ Hz), 62.9, 61.3, 56.5, 52.9, 50.9, 34.1, 33.8, 26.0, 25.9, 21.0, 16.6, 16.5, 16.2, 15.9, 9.5, 9.4; *m/z* (FD) 346.2 (100) $C_{21}H_{29}FNO_2$ requires 346.2; HRMS(ESI): exact mass calcd for $C_{21}H_{29}FNO_2$: 346.2182, found: 346.2181.

Methyl 8-(((1*R*,2*R*)-2-(fluoromethyl)cyclopropyl)methyl)-3-*p*-tolyl-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7m, PRD06)

Synthesised according to procedure A, 86% yield. Colourless crystals: mp. 77 °C; $[\alpha]_D^{23}$ −27.7 (*c* 1.21 in MeOH). $C_{21}H_{28}FNO_2$ requires C 73.01, H 8.17, N 4.05, found C 73.4, H 8.1, N 3.95%. δ_H (300 MHz, $CDCl_3$): 7.14 (d, $J = 8$ Hz, 2 H, ArH), 7.06 (d, $J = 8$ Hz, 2 H, ArH), 4.33 (m, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 4.17 (m, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 3.91 (brs, 1H), 3.48 (s, 1H, OCH_3), 3.40 (brs, 1 H), 2.98 (dt, $J = 12.9$ Hz, $J = 4.4$ Hz, 1 H), 2.91 (t, $J = 4.4$ Hz, 1 H), 2.58 (td, $J = 12.5$ Hz, $J = 2.9$ Hz, 1 H), 2.47 (dd, $J = 12.5$ Hz, $J = 3.7$ Hz, 1 H), 2.28 (s, 3 H, NCH_3), 2.1 (m, 3 H), 1.83–1.53 (m, 3 H), 1.03 (m, 1 H), 0.81 (m, 1 H), 0.45 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.1, 140.0, 135.1, 128.6, 127.2, 87.3 (d, $J_{C\text{-}F} = 161.5$ Hz), 62.0, 61.8, 56.4, 52.7, 50.9, 34.0, 33.9, 26.0, 25.8, 21.0, 18.5, 18.2, 16.6, 16.5, 7.3, 7.2; *m/z* (FD) 346.2 $C_{21}H_{29}FNO_2$ 346.2; HRMS(ESI): exact mass calcd for $C_{21}H_{29}FNO_2$: 346.2182, found: 346.2188.

Methyl 8-(4-fluorobutyl)-3-(4-chlorophenyl)-8-aza-bicyclo [3.2.1]octane-2-carboxylate (7b, PRD07)

For preparative details see **7a**, 75% yield. Colourless crystals: mp. 70 °C; $[\alpha]_D^{23}$ −44.1 (*c* 0.33 in MeOH). $C_{19}H_{25}ClFNO_2$ requires C 64.49, H 7.12, N 3.96, found C 64.6, H 6.9, N 3.9%. δ_H (300 MHz, $CDCl_3$): 7.22 (d, $J = 8.8$ Hz, 2 H, ArH), 7.15 (d, $J = 8.8$, 2 H, ArH), 4.50 (t, $J = 6.2$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 4.34 (t $J = 6.2$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 3.66 (brs, 1 H), 3.46 (s, 3H, CH_3), 3.37 (brs, 1 H), 2.95 (dt, $J = 4.8$ Hz, $J = 12.9$ Hz, 1 H), 2.86 (m, 1 H), 2.52 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.26 (m, 2 H), 2.02 (m, 2 H), 1.65 (m, 5 H), 1.46 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 171.8, 141.8, 131.4, 128.7, 128.0, 84.2 (d, $J_{C\text{-}F} = 161.5$ Hz), 62.9, 61.3, 52.9, 50.9, 38.7, 34.0, 33.8, 28.2, 27.9, 26.0, 25.9, 24.7, 24.6; *m/z* (FD) 353.2 $C_{21}H_{29}FNO_2$ requires 353.2; HRMS(ESI): exact mass calcd for $C_{19}H_{25}ClFNO_2$: 353.1558, found: 353.1560.

Methyl 8-(4-fluorobut-2-yn-1-yl)-3-(4-chlorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7f, PRD08)

Synthesised according to procedure A, 95% yield. Yellowish crystals: mp. 76 °C; $[\alpha]_D^{23}$ −13.2 (*c* 1.58 in MeOH). $C_{19}H_{21}ClFNO_2$ requires C 65.23, H 6.05, N 4.00, found C 65.5, H 6.15, N 3.8%; δ_H (300 MHz, $CDCl_3$): 7.23 (d, $J = 8.5$ Hz, 2 H, ArH), 7.16 (d, $J = 8.5$, 2 H, ArH), 5.01 (t, $J = 2.2$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H) 4.85 (t $J = 2.2$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 3.90 (brs, 1 H), 3.51 (s, 3H, OCH_3), 3.47 (brs, 1 H), 3.25 (ddt, $J = 1.5$ Hz, $J = 7.5$ Hz, $J = 16.5$ Hz, 1 H), 3.10 (ddt, $J = 1.5$ Hz, $J = 7.5$ Hz, $J = 16.5$ Hz, 1 H) 2.97 (dt, $J = 5.1$ Hz, $J = 12.5$ Hz, 1 H), 2.90 (m, 1 H), 2.59 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.05 (m, 2 H), 1.70 (m, 4 H); δ_C (100 MHz, $CDCl_3$): 171.5, 141.3, 131.6, 128.7, 128.0, 87.4, 70.8 (d, $J_{C\text{-}F} = 163.5$ Hz), 62.5, 61.0, 52.5, 51.2, 42.9, 42.8, 34.0, 33.7, 25.8, 25.6; *m/z* (FD) 350.1 (100) $C_{19}H_{22}ClFNO_2$ 350.1; HRMS-(ESI): exact mass calcd for $C_{19}H_{22}ClFNO_2$: 350.1323, found: 350.1331.

Methyl 8-(((1*S*,2*S*)-2-(fluoromethyl)cyclopropyl)methyl)-3-(4-chlorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7j, PRD09)

Synthesised according to procedure A, 85% yield. Yellowish crystals: mp. 76–77 °C; $[\alpha]_D^{23}$ −90.6 (hydrochloride) (*c* 0.96 in MeOH). δ_H (300 MHz, $CDCl_3$): 7.21 (d, $J = 8.8$ Hz, 2 H, ArH), 7.16 (d, $J = 8.5$, 2 H, ArH), 4.30 (dq, $J = 7.3$ Hz, $J = 9.56$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 1 H), 4.14 (dq, $J = 7.3$ Hz, $J = 9.56$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 1 H), 3.81 (brs, 1 H), 3.48 (s, 3 H, CH_3) 3.45 (brs, 1H), 2.95 (dt, $J = 5$ Hz, $J = 12.5$ Hz, 1 H), 2.86 (dt, $J = 4.4$ Hz, 1 H), 2.53 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.40 (dd, $J = 6$ Hz, $J = 12.5$ Hz, 1 H), 2.07 (dd, $J = 6$ Hz, $J = 12.5$ Hz, 1 H), 2.05–1.85 (m, 2 H), 1.75–1.55 (m, 3 H), 0.99 (m, 1 H), 0.80 (m, 1 H), 0.50 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 171.9, 141.7, 131.4, 129.8, 128.7, 128.0, 87.4 (d, $J_{C\text{-}F} = 161.5$ Hz), 62.9, 61.1, 56.6, 52.8, 51.0, 34.0, 33.8, 26.0, 25.9, 16.6, 16.5, 16.3, 16.0, 9.3, 9.2; *m*/*z* (FD) 366.2 (100) $C_{20}H_{26}ClFNO_2$ 366.2; HRMS-(ESI): exact mass calcd for $C_{20}H_{26}ClFNO_2$: 366.1636, found 366.1631.

Methyl 8-(((1*R*,2*R*)-2-(fluoromethyl)cyclopropyl)methyl)-3-(4-chlorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7n, PRD10)

Synthesised according to procedure A, 88% yield. Off white crystals: mp. 75 °C; $[\alpha]_D^{23}$ −25.5 (*c* 0.42 in MeOH). $C_{20}H_{25}ClFNO_2$ requires C 65.66, H 6.89, N 3.83, found C 65.95, H 7.16, N 3.81%. δ_H (300 MHz, $CDCl_3$): 7.21 (d, $J = 8.5$ Hz, 2 H, ArH), 7.16 (d, $J = 8.5$, 2 H, ArH), 4.32 (d, $J = 7.4$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 1 H), 4.17 (dq, $J = 7.4$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 1 H), 3.91 (brs, 1 H), 3.47 (s, 3 H, CH_3) 3.40 (brs, 1H), 2.96 (dt, $J = 5$ Hz, $J = 12.5$ Hz, 1 H), 2.88 (t, $J = 4.4$ Hz, 1 H), 2.55 (dt, $J = 12.5$ Hz, $J = 2.9$ Hz, 1 H), 2.38 (dd, $J = 12.5$ Hz, $J = 5$ Hz, 1 H), 2.11–1.87 (m, 3 H), 1.79–1.52 (m, 3 H), 1.01 (m, 1 H), 0.80 (m, 1 H), 0.44 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 171.9, 141.8, 131.5, 128.7, 128.0, 87.2 (d, $J_{C\text{-}F} = 160.5$ Hz), 62.0, 61.6, 56.4, 52.7, 51.0, 34.0, 33.8, 26.0, 25.8, 18.5, 18.2, 16.6, 16.5, 7.4, 7.3; *m*/*z* (FD) 366.2 (100) $C_{20}H_{26}ClFNO_2$ 366.2; HRMS-(ESI): exact mass calcd for $C_{20}H_{26}ClFNO_2$: 366.1636, found: 366.1620.

Methyl 8-(4-fluorobutyl)-3-(4-fluorophenyl)-8-aza-bicyclo [3.2.1]octane-2-carboxylate (7c, PRD11)

For experimental details see **7a**, 92% yield. **7c** was obtained as colourless oil that solidified upon standing: mp. 65 °C; $[\alpha]_D^{23}$ −90.6 (hydrochloride) (*c* 1.75 in MeOH). $C_{19}H_{25}F_2NO_2$ requires C 67.64, H 7.47, N 4.15, found C 67.5, H 7.6, N 4.05%. δ_H (300 MHz, $CDCl_3$): 7.20 (dd, $J = 5.5$ Hz, $J = 2.9$ Hz, 2 H, ArH), 6.93 (t, $J = 8.8$ Hz, 2 H, ArH), 4.49 (t, $J = 5.9$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 4.33 (t, $J = 5.9$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 3.67 (brs, 1 H), 3.46 (s, 3H, CH_3), 3.36 (brs, 1 H), 2.96 (dt, $J = 5.1$ Hz, $J = 12.5$ Hz, 1 H), 2.85 (t, $J = 3.7$ Hz, 1 H), 2.53 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.26 (m, 2 H), 2.02 (m, 2 H), 1.67 (m, 6 H), 1.47 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 171.9, 161.2 (d, $J_{C\text{-}F} = 243$ Hz), 138.8, 128.8, 128.7, 114.7, 114.4, 84.2 (d, $J_{C\text{-}F} = 162$ Hz), 62.9, 61.4, 52.946, 50.893, 34.2, 33.7, 28.2, 27.9, 26.0, 25.9, 24.7, 24.6; *m*/*z* (FD) 338.3 (100) $C_{19}H_{26}F_2NO_2$ requires 338.2; HRMS(ESI): exact mass calcd for $C_{19}H_{26}F_2NO_2$: 338.1933 found: 338.1932.

Methyl 8-(4-fluorobut-2-yn-1-yl)-3-(4-fluorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7g, PRD12)

Synthesised according to procedure A, 96% yield. Colourless crystals: mp. 72 °C; $[\alpha]_D^{23}$ −60.2 (*c* 1.17 in MeOH). $C_{19}H_{21}F_2NO_2$ requires C 68.45, H 6.35, N 4.20, found: C 68.7, H 6.6, N 4.40%; δ_H (300 MHz, $CDCl_3$): 7.20 (dd, $J = 8.1$ Hz, $J = 5.5$ Hz, 2 H, ArH), 6.93 (dd, $J = 8.8$ Hz, 2 H, ArH), 5.02 (t, $J = 2.0$ Hz, $J_{H\text{-}F} = 49.6$ Hz, 1 H), 4.85 (t, $J = 2.0$ Hz, $J_{H\text{-}F} = 49.6$ Hz, 1 H), 3.89 (brs, 1 H), 3.51 (s, 3H, CH_3), 3.48 (brs, 1 H), 3.26 (ddt, $J = 1.5$ Hz, $J = 7.4$ Hz, $J = 16.5$ Hz, 1 H), 3.11 (ddt, $J = 1.5$ Hz, $J = 7.4$ Hz, $J = 16.5$ Hz, 1 H), 2.98 (dt, $J = 5.1$ Hz, $J = 12.5$ Hz, 1 H), 2.82 (m, 1 H), 2.61 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.05 (m, 2 H), 1.70 (m, 4 H); δ_C (100 MHz, $CDCl_3$): 171.5, 161.4 (d, $J_{C\text{-}F} = 253$ Hz), 138.3, 128.9, 128.8, 114.8, 114.5, 87.5, 70.7 (d, $J_{C\text{-}F} = 163.5$ Hz), 62.5, 61.1, 52.7, 51.1, 42.9, 34.2, 33.6, 25.8, 25.7; *m*/*z* (FD) 334.2 (100) $C_{19}H_{22}F_2NO_2$ requires 334.2; HRMS(ESI): exact mass calcd for $C_{19}H_{22}F_2NO_2$: 334.1619 found: 334.1627.

Methyl 8-(((1*R*,2*R*)-2-(fluoromethyl)cyclopropyl)methyl)-3-(4-fluorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7k, PRD13)

Synthesised according to procedure A, 89% yield. Colourless to off white crystals: mp. 74 °C; $[\alpha]_D^{23}$ −22.5 (*c* 0.58 in MeOH). $C_{20}H_{25}F_2NO_2$ requires C 68.75, H 7.21, N 4.01, found: C 68.9, H 7.1, N 3.9%. δ_H (300 MHz, $CDCl_3$): 7.19 (dd, $J = 8.5$ Hz, $J = 5.5$ Hz, 2H, ArH), 6.93 (t, $J = 8.8$ Hz, 2 H), 4.30 (dq, $J = 4.4$ Hz, $J = 7.4$ Hz, $J = 9.6$ Hz, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 4.14 (dq, $J = 4.4$ Hz, $J = 7.5$ Hz, $J = 9.5$ Hz, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 3.79 (brs, 1 H), 3.49 (s, 3 H, CH_3), 3.43 (brs, 1H), 2.97 (dt, $J = 5$ Hz, $J = 12.5$ Hz, 1 H), 2.86 (dt, $J = 4.4$ Hz, 1 H), 2.53 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.41 (dd, $J = 12.8$ Hz, $J = 5.5$ Hz, 1 H), 2.17 (dd, $J = 12.1$ Hz, $J = 6.6$ Hz, 1 H), 2.03–1.85 (m, 2 H), 1.76–1.53 (m, 3 H), 1.00 (m, 1 H), 0.82 (m, 1 H), 0.50 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.0, 161.0 (d, $J_{C\text{-}F} = 248$ Hz), 138.8, 128.8, 128.6, 114.7, 114.5, 87.5 (d, $J_{C\text{-}F} = 160.5$ Hz), 86.6, 62.9, 61.2, 56.5, 53.0, 51.0, 34.2, 33.8, 26.0, 25.9, 16.6, 16.5, 16.3, 16.0, 9.4, 9.3; *m*/*z* (FD) 350.0 (100) $C_{20}H_{26}F_2NO_2$ requires 350.2; HRMS(ESI): exact mass calcd for $C_{20}H_{26}F_2NO_2$: 350.1932 found: 350.1940.

Methyl 8-(((1*S*,2*S*)-2-(fluoromethyl)cyclopropyl)methyl)-3-(4-fluorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7o, PRD14)

Synthesised according to procedure A, 83% yield. Colourless crystals: mp. 72 °C; $[\alpha]_D^{23}$ −75.9 (hydrochloride) (*c* 1.13 in MeOH). $C_{20}H_{25}F_2NO_2$ requires C 68.75, H 7.21, N 4.01, found: C 68.69, H 7.0, N 4.3%. δ_H (300 MHz, $CDCl_3$): 7.21 (dd, $J = 8.5$ Hz, $J = 5.5$ Hz, 2H, ArH), 6.93 (t, $J = 8.8$ Hz, 2 H), 4.30 (dq, $J = 7.5$ Hz, $J = 9.5$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 1 H), 4.16 (dq, $J = 7.5$ Hz, $J = 9.5$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 1 H), 3.79 (brs, 1 H), 3.49 (s, 3 H, CH_3), 3.43 (brs, 1H), 2.97 (dt, $J = 5$ Hz, $J = 12.5$ Hz, 1 H), 2.89 (dt, $J = 4.4$ Hz, $J = 1.1$ Hz, 1 H), 2.53 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.41 (dd, 1 H), 2.00 (m, 3 H), 1.63 (m, 4 H), 1.00 (m, 1 H), 0.80 (m, 1 H), 0.50 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.1, 161.2 (d, $J_{C\text{-}F} = 248$ Hz), 138.6, 128.9, 128.4, 114.7, 114.5, 87.4 (d, $J_{C\text{-}F} = 160.0$ Hz), 62.5, 61.5, 56.4, 52.9, 51.0, 34.2, 33.9, 26.0, 25.9, 18.5, 18.2, 16.6, 16.5, 7.4, 7.3; *m*/*z* (FD) 350.2 (100) $C_{20}H_{26}F_2NO_2$ requires 350.2;

HRMS(ESI): exact mass calcd for $C_{20}H_{26}F_2NO_2$: 350.1932, found: 350.1926.

Methyl 8-(4-fluorobutyl)-3-phenyl-8-aza-bicyclo[3.2.1] octane-2-carboxylate (7d, PRD15)

For experimental details see **7a**, 89% yield. Colourless oil that solidified upon standing: mp. 61–62 °C; $[\alpha]_D^{23}$ –96.5 (hydrochloride) (*c* 1.75 in MeOH). $C_{19}H_{26}FNO_2$ requires C 71.44, H 8.20, N 4.39, found: C 71.7, H 8.0, N 4.5%; δ_H (300 MHz, $CDCl_3$): 7.23 (m, 4 H, ArH), 7.14 (m, 1 H, ArH), 4.51 (t, $J = 5.9$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 4.34 (t, $J = 5.9$ Hz, $J_{H\text{-}F} = 47.4$ Hz, 1 H), 3.66 (brs, 1 H), 3.45 (s, 3H, CH_3), 3.37 (brs, 1 H), 3.01 (dt, $J = 5.2$ Hz, $J = 12.5$ Hz, 1 H), 2.92 (t, $J = 4.0$ Hz, 1 H), 2.57 (dt, $J = 2.6$ Hz, $J = 12.5$ Hz, 1 H), 2.27 (m, 2 H), 2.02 (m, 2 H), 1.69 (m, 6 H), 1.47 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.0, 143.2, 127.9, 127.3, 125.8, 84.2 (d, $J_{C\text{-}F} = 161.4$ Hz), 62.9, 61.4, 52.9, 50.9, 34.2, 34.0, 28.2, 26.0, 25.1, 24.6; *m/z* (FD) 320.2 (100) $C_{19}H_{27}FNO_2$ requires 320.2; HRMS(ESI): exact mass calcd for $C_{19}H_{27}FNO_2$: 320.2026, found: 320.2021.

Methyl 8-(4-fluorobut-2-yn-1-yl)-3-phenyl-8-aza-bicyclo [3.2.1]octane-2-carboxylate (7h, PRD16)

Synthesised according to procedure A, 96% yield. Colourless crystals, mp. 70 °C; $[\alpha]_D^{23}$ –95.7 (hydrochloride) (*c* 1.75 in MeOH). $C_{19}H_{22}FNO_2$ requires C 72.4, H 7.0, N 4.4, found C 72.8, H 6.9, N 4.2%; δ_H (300 MHz, $CDCl_3$): 7.24 (m, 4 H, ArH), 7.14 (m, 1 H, ArH), 5.02 (t, $J = 2$ Hz, $J_{H\text{-}F} = 47.8$ Hz, 1 H), 4.86 (t, $J = 2$ Hz, $J_{H\text{-}F} = 47.8$ Hz, 1 H), 3.90 (brs, 1 H), 3.50 (s, 3H, CH_3), 3.40 (brs, 1 H), 3.25 (ddt, $J = 1.5$ Hz, $J = 7.5$ Hz, $J = 16.5$ Hz, 1 H), 3.12 (ddt, $J = 1.5$ Hz, $J = 7.5$ Hz, $J = 16.5$ Hz, 1 H), 3.02 (dt, $J = 5.2$ Hz, $J = 12.9$ Hz, 1 H), 2.94 (t, $J = 3.3$ Hz, 1 H), 2.69 (dt, $J = 2.6$ Hz, $J = 12.9$ Hz, 1 H), 2.05 (m, 2 H), 1.68 (m, 4 H); δ_C (100 MHz, $CDCl_3$): 171.6, 142.7, 127.9, 127.4, 127.3, 125.9, 87.5, 70.8 (d, $J_{C\text{-}F} = 163.5$ Hz), 62.6, 61.1, 52.6, 51.1, 42.9, 42.8, 34.1, 34.0, 25.9, 25.9, 25.7; MS(FD) 316.2 (100) $C_{19}H_{23}FNO_2$ requires 316.2; HRMS(ESI): exact mass calcd for $C_{19}H_{23}FNO_2$: 316.1713, found: 316.1722.

Methyl 8-(((1*S*,2*S*)-2-(fluoromethyl)cyclopropyl)methyl)-3-phenyl-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7l, PRD17)

Synthesised according to procedure A, 84% yield. Colourless to yellowish crystals: mp. 76 °C; $[\alpha]_D^{23}$ –27.0 (*c* 1.42 in MeOH). $C_{20}H_{26}FNO_2$ requires C 72.48, H 7.91, N 4.23, found: C 72.4, H 8.0, N 4.2%; δ_H (300 MHz, $CDCl_3$): 7.25 (brs, 4H, ArH), 7.14 (m, 1H, ArH), 4.30 (dq, $J = 7$ Hz, $J = 9.6$ Hz, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 4.13 (dq, $J = 7$ Hz, $J = 9.6$ Hz, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 3.82 (brs, 1 H), 3.47 (s, 3 H, CH_3), 3.45 (brs, 1H), 3.00 (dt, $J = 5.2$ Hz, $J = 12.9$ Hz, 1 H), 2.91 (dt, $J = 4.4$ Hz, $J = 0.7$ Hz, 1 H), 2.59 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.52 (q, $J = 7$ Hz, 1 H), 2.0 (m, 3 H), 1.67 (m, 4 H), 1.02 (m, 1 H), 0.82 (m, 1 H), 0.52 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.1, 143.2, 127.9, 127.3, 125.8, 87.4 (d, $J_{C\text{-}F} = 161.5$ Hz), 63.0, 61.2, 56.5, 52.9, 50.0, 34.2, 34.0, 26.0, 25.9, 16.6, 16.5, 16.3, 16.0, 9.4, 9.3; MS (FD) 332.2 (100) $C_{20}H_{27}FNO_2$ requires 323.2; HRMS(ESI): exact mass calcd for $C_{20}H_{27}FNO_2$: 332.2026, found: 332.2034.

Methyl 8-(((1*R*,2*R*)-2-(fluoromethyl)cyclopropyl)methyl)-3-phenyl-8-aza-bicyclo[3.2.1]octane-2-carboxylate (7p, PRD18)

Synthesised according to procedure A, 84% yield. Colourless crystals: mp. 88 °C; $[\alpha]_D^{23}$ –30.0 (*c* 1.17 in MeOH). $C_{20}H_{27}FNO_2$ requires C 72.48, H 7.91, N 4.23, found: C 72.2, H 8.1, N 4.4%. δ_H (300 MHz, $CDCl_3$): 7.25 (brs, 4H, ArH), 7.14 (m, 1H, ArH), 4.33 (dq, $J = 7$ Hz, $J = 9.6$ Hz, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 4.13 (dq, $J = 7$ Hz, $J = 9.6$ Hz, $J_{H\text{-}F} = 48.9$ Hz, 1 H), 3.92 (brs, 1 H), 3.46 (s, 3 H, OCH_3), 3.42 (brs, 1H), 3.01 (dt, $J = 5$ Hz, $J = 12.9$ Hz, 1 H), 2.93 (t, $J = 4.4$ Hz, 1 H), 2.6 (dt, $J = 2.9$ Hz, $J = 12.5$ Hz, 1 H), 2.47 (dd, $J = 12.5$ Hz, $J = 4.8$ Hz, 1 H), 2.11–1.87 (m, 2 H), 1.79–1.54 (m, 3 H), 1.03 (m, 1 H), 0.82 (m, 1 H), 0.45 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 172.3, 143.1, 127.9, 127.4, 125.7, 87.3 (d, $J_{C\text{-}F} = 160.5$ Hz), 62.1, 61.8, 56.4, 52.8, 50.9, 34.3, 34.0, 26.0, 25.9, 18.5, 18.2, 16.6, 16.5, 7.4, 7.3; MS (FD) 332.2 (100) $C_{20}H_{27}FNO_2$ requires 323.2; HRMS(ESI): exact mass calcd for $C_{20}H_{27}FNO_2$: 332.2026, found: 332.2035.

Methyl 8-(4-fluorobut-2-en-1-yl)-3-phenyl-8-aza-bicyclo[3.2.1] octane-2-carboxylate (7r, PRD19)

Synthesised from **7h** according to the procedure described for **7q**. Colourless crystals (90 mg, 91%): mp. 107–109 °C; $[\alpha]_D^{23}$ –18.6 (*c* 1.42 in MeOH). $C_{19}H_{24}FNO_2$ requires C 71.90, H 7.62, N 4.41, found C 72.15, H 8.0, N 4.25%. δ_H (400 MHz, $CDCl_3$): 7.30–7.26 (m, 4H, ArH), 7.21–7.14 (m, 1H, ArH), 5.81 (brs, 2H), 4.85 (dd, $J = 2$ Hz, $J = 5$ Hz, $J_{H\text{-}F} = 48.5$ Hz, 2 H), 3.69 (brs, 1 H), 3.49 (s, 3 H, OCH_3), 3.45 (brs, 1H), 3.06 (dt, $J = 5$ Hz, $J = 12.5$ Hz, 1 H), 2.94 (t, $J = 4$ Hz, 1 H), 2.94–2.87 (m, 1 H), 2.65 (dt, $J = 3$ Hz, $J = 12.5$ Hz, 1 H), 2.17–1.97 (m, 2 H), 1.82–1.63 (m, 3 H); δ_C (100 MHz, $CDCl_3$): 171.9, 142.9, 134.3, 127.9, 127.4, 125.7, 83.2 (d, $J_{C\text{-}F} = 166.5$ Hz), 62.3, 61.4, 54.9, 52.7, 51.0, 34.2, 34.0, 26.0, 25.9; MS (FD) 318.2 (100) $C_{19}H_{25}FNO_2$ requires 318.2; HRMS(ESI): exact mass calcd for $C_{19}H_{25}FNO_2$: 318.1869, found: 318.1870.

Methyl 8-(2-fluoroethyl)-3-(4-chlorophenyl)-8-aza-bicyclo[3.2.1]octane-2-carboxylate (10, FECNT)

Synthesised according to procedure A, 83% yield. Colourless crystals: mp. °C; $[\alpha]_D^{23}$ –43.5 (*c* 1.25 in MeOH). $C_{17}H_{21}ClFNO_2$ requires C 62.67, H 6.50, N 4.30, found C 62.5, H 6.9, N 4.1%. δ_H (400 MHz, $CDCl_3$): 7.24 (d, $J = 8.6$ Hz, 2H, ArH), 7.19 (d, $J = 8.6$ Hz, 2H, ArH), 4.57–4.33 (dm, $J_{H\text{-}F} = 47.5$ Hz, 2 H), 3.79 (brs, 1 H), 3.52 (s, 3 H, OCH_3), 3.44 (brs, 1H), 2.98 (dt, $J = 6$ Hz, $J = 12.5$ Hz, 1 H), 2.91 (t, $J = 4$ Hz, 1 H), 2.66–2.53 (m, 2 H), 2.65 (dt, $J = 3$ Hz, $J = 12.5$ Hz, 1 H), 2.2–2.08 (m, 1 H), 2.06–1.96 (m, 1H), 1.77 (dt, $J = 4$ Hz, $J = 12.5$ Hz, 1 H), 1.72–1.63 (m, 2 H); δ_C (100 MHz, $CDCl_3$): 171.8, 141.6, 131.5, 128.7, 128.0, 125.7, 83.9 (d, $J_{C\text{-}F} = 166.5$ Hz), 63.5, 62.3, 53.8, 53.6, 52.6, 51.1, 34.0, 33.6, 26.2, 25.7; MS (FD) 326.1 (100) $C_{17}H_{22}ClFNO_2$ requires 326.1; HRMS(ESI): exact mass calcd for $C_{17}H_{22}ClFNO_2$: 326.1323, found: 326.1328.

Formation of hydrochloride salts for cell assay

Compounds **7a–q** were purified by HPLC (Phenomenex Luna® RP-18 10 µ semi-preparative HPLC-column (250 × 40 mm) using 10–20% 7 mM aqueous ammonia solution in MeCN. The product

fractions were collected and the eluent was evaporated *in vacuo*. The residue was taken up in MilliQ®-water and the resultant solution was lyophilised over night. The lyophilised tropanes were re-dissolved in dry ether and 2 M ethereal HCl was added dropwise (2 equiv.). The mother liquor was removed and the hygroscopic hydrochlorides were dried *in vacuo*. 1 mg of the dried hydrochloride was re-dissolved in 1 ml CH_3CN containing 20% of 0.05 M ammonium acetate buffer and analysed by HPLC. The HPLC-purity of all compounds used for biological assays exceeded 99% (by UV-area at 254 nm).

Generation of cell lines stably expressing the hSERT, hDAT and hNET

Selection of clonal lines stably expressing the human dopamine transporter, the human norepinephrine transporter and the human serotonin transporter (HEKhDAT, HEKhNET and HEKhSERT), respectively, was performed as described previously.[22]

Cell culture

HEKhSERT, HEKhNET and HEKhDAT cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum, penicillin (100 U ml^{-1}), streptomycin (100 lg ml^{-1}), and geneticin (G418, 200 lg ml^{-1}) at 37 °C in 95% humidified air with 5% CO_2. Cells were split in a defined dilution to reach 80% confluency at the beginning of the experiment. [^{3}H]5-HT, [^{3}H]NE and [^{3}H]DA transport measurement of monoamine uptake was performed as described previously and modified for IC_{50}-determination.[8a,22b] Briefly, HEKhSERT, HEKhNET and HEKhDAT cells were plated into 24-well dishes (2 cm in diameter), which had previously been treated with poly-L-lysine (0.1 mg ml^{-1}) and allowed to grow to 80% confluency. Culture medium was replaced by TB1 buffer (200 lL: containing 120 mM NaCl, 2 mM KCl, 1 mM $CaCl_2$, 1 mM $MgCl_2$ and 10 mM HEPES, pH 7.5) containing 250 nM of [^{3}H]5-HT, [^{3}H]NE or [^{3}H]DA with various concentrations (0.1–5000 nM) of each tracer ([^{3}H]DA: K_M = 1.37 µM, [^{3}H]5HT: K_M = 1.1 µM and [^{3}H]NE: K_M = 0.61 µM). After 6 min at room temperature, the medium was removed quickly and cells were washed twice with ice-cold TB1 before lysing with 10% (w/v) sodium dodecyl sulfate. Radioactivity was determined by scintillation counting. Specific uptake is determined as the difference between HEKhSERT, HEKhNET and HEKhDAT-mediated and control HEK293 uptake in parallel culture dishes. All transport measurements were analysed by non-linear regression analysis using the graphics program GraphPad Prism®.

Data analysis

All uptake data represent the means of quadruplicate determinations; each experiment was repeated at least three times. Data were analyzed by non-linear regression analysis program (GraphPad Prism®), which fitted sigmoidal uptake curves to eqn (1) and (2):

$$V = \frac{V_{max}}{\left[1 + \left(\frac{K_M}{S}\right)^{nH}\right]} \quad (1)$$

and

$$\frac{V}{V_{max}} = \frac{IC_{50}^{nH}}{\left(I^{nH} + IC_{50}^{nH}\right)} \quad (2)$$

V represents transport rate; V_{max}, maximal transport rate; S, substrate concentration; I, inhibitor concentration; IC_{50}, inhibitor concentration for half maximal transport inhibition; K_M, the Michaelis–Menten constant; and n_H, the Hill-coefficient.

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