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Synthesis of 3,6-diazabicyclo[3.1.1]heptanes as novel ligands for the opioid receptors

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Abstract—In an effort to improve diazabicycloalkane-based opioid receptor ligands, *N*-3(6)-arylpropenyl-*N*-6(3)-propionyl-3,6-diazabicyclo[3.1.1]heptanes (**3A**,**Ba–i**) were synthesized and their affinity and selectivity towards μ-, δ- and κ-receptors were evaluated. The results of the current study revealed a number of compounds (**3Bb**, **3Bg** and **3Bh**) having a high affinity for μ (K_i at μ-receptors ranging from 2.7 to 7.9 nM) versus δ (K_i at δ-receptors >2000 nM) and versus κ (K_i at κ-receptors >5000 nM) receptors.

Molecular modelling carried out on the pair **3Aa/3Ba** and on the **3Bh** was consistent with the hypothesis that the two series of compounds **3A** and **3B** interact with the μ-receptor in very different ways.

1. Introduction

The nucleus of 3,8-diazabicyclo[3.2.1]octane (DBO) (**1**) (Chart 1) when substituted at N_3 and N_8 by a propionyl and by an appropriate arylalkenyl group (**1A**,**B**) gave compounds provided with a central analgesic activity comparable to or higher than that of morphine.[1] Their activity was found to be related to their interaction with opioid μ-receptors with the affinity in the nanomolar range, similar to morphine but with a higher μ/δ, κ selectivity.[2] Molecular modelling studies suggest that the endoethanic bridge of **1A**,**B** plays an essential role in modulating μ-affinity by fitting lipophilic pockets of the receptor.[3]

This hypothesis was supported by the following evidences: (a) the corresponding piperazine and equatorial *cis*-2,6-dimethyl piperazine derivatives exhibited a markedly lower μ-affinity and (b) the higher homologues of **1** namely 3,9-diazabicyclo[3.3.1]nonanes (DBN) (**2**) similarly substituted on the N_3 and N_9 (**2A**,**B**) also displayed selective μ-affinity in the nanomolar range. Contrary to compounds **1**, the majority of the tested N_3-propionyl-N_9-arylalkenyl derivatives (**2B**) exhibited a selective μ-affinity significantly higher with respect to the isomeric series (**2A**). Representative DBN terms, when tested in vivo (mouse), displayed a potent analgesic effect which favourably compared with that of morphine.[4]

Quite interestingly, taking into account that the major limitation in the medical utilization of morphine and related opioids arises from two peculiar side effects closely linked to their chronic use, tolerance and dependence, we have recently observed that in the DBO class the 3-*p*-nitrocinnamyl-8-propionyl derivative (**1Ab**), provided with high affinity and selectivity towards μ opioid receptor (K_i = 25 nM) and central analgesic activity (hot plate test in mice, ED_{50} 0.44 mg/kg ip), did not induce abstinence signs connected to dependence. In addition, the isomer of **1Ab** having the N_3 and N_8 substituents reverted (**1Bb**) induced, in chronically treated mice, tolerance after 13 days as compared to 5 days for morphine.[5] The development of tolerance in a time

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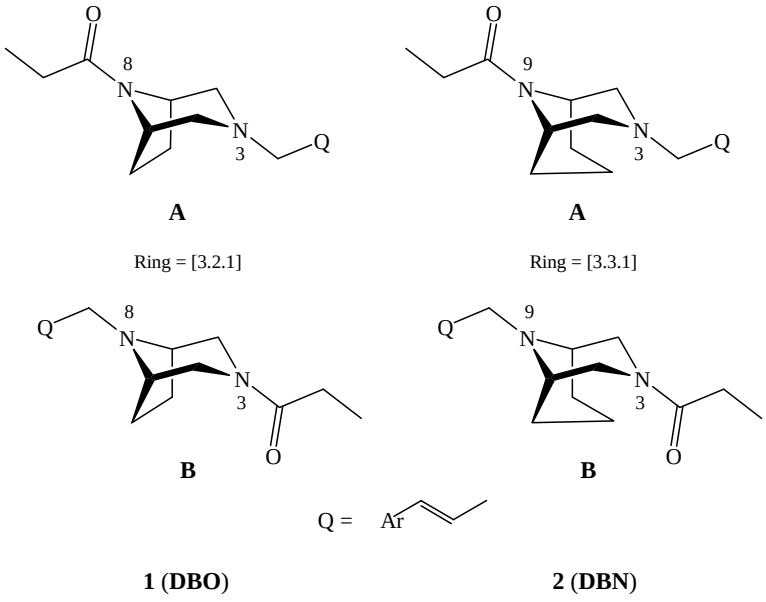
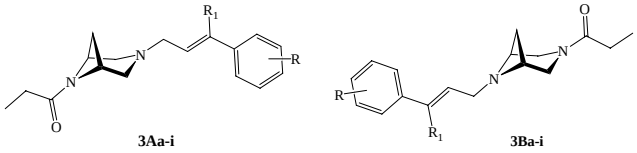


Chart 1.

significantly longer than morphine (9 days) was also observed in the representative DBN derivative *N*-9-(3,3-diphenylprop-2-enyl)-*N*-3-propionyl-diazabicyclo[3.3.1]nonane which also provided with high μ-affinity (K_i = 5 nM) and analgesic potency (ED_{50} 3.88 mg/kg ip).[4]

Our continuing interest in the bicylic systems bearing a piperazine moiety has now induced us to extend researches to the class of 3,6-diazabicyclo[3.1.1]heptanes (**3A**,**B**) (DBH) having on the piperazine moiety an endomethane bridge which determines an increased rigidity with respect to the higher homologues **1A**,**B**

Table 1. Binding affinities of **3Aa**–**i** and **3Ba**–**i** for opioid receptors K_i (nM)[a]



Compound[b]	R	R_1	μ	δ	κ	K_i ratio	
						κ/μ	δ/μ
3Aa	H	H	600 ± 75	600	>10,000	1	>16.7
3Ab	4-NO_2	H	850 ± 70	>10,000	>10,000	>11.8	>11.8
3Ac	2-Cl	H	207 ± 32	>10,000	>10,000	>48.3	>48.3
3Ad	3-Cl	H	220 ± 42	2,000	>10,000	9.1	>45.4
3Ae	4-Cl	H	452 ± 42	>10,000	>10,000	>22.1	>22.1
3Af	H	CH_3	5% inhibition at 1 μM	>10,000	>10,000	—	—
3Ag	4-Cl	CH_3	363 ± 53	>10,000	>10,000	>27.5	>27.5
3Ah	3,4-Cl_2	CH_3	223 ± 15	>10,000	>10,000	>44.8	>44.8
3Ai	H	CH_2CH_3	237 ± 25	>10,000	>10,000	>42.2	>42.2
3Ba	H	H	208 ± 8	>5,000	>5,000	>24.0	>24.0
3Bb	4-NO_2	H	5.2 ± 0.8	>10,000	>10,000	>1923.1	>1923.1
3Bc	2-Cl	H	92 ± 4	>5,000	>5,000	>54.3	>54.3
3Bd	3-Cl	H	21 ± 0.7	2060 ± 70	>5,000	98.1	>238.1
3Be	4-Cl	H	16 ± 2	4100 ± 50	>5,000	256.2	>312.5
3Bf	H	CH_3	178 ± 11	>5,000	>5,000	>28.1	>28.1
3Bg	4-Cl	CH_3	7.9 ± 0.7	2050 ± 50	>5,000	259.5	>632.9
3Bh	3,4-Cl_2	CH_3	2.7 ± 0.5	2200 ± 200	>5,000	814.8	>1851.8
3Bi	H	CH_2CH_3	384 ± 12	>5,000	>5,000	>13.0	>13.0
Morphine			2.8 ± 0.04	100.2 ± 5.14	280.8 ± 9.2	93.4	262.4

[a] The K_i values for the test ligands were determined with assays described in Section 4. Results are means ± SEM for three independent experiments assayed in triplicate.

[b] The receptor binding affinities of all compounds were carried on their hydrochlorides or fumarates.

(DBO) and **2A**,**B** (DBN) (see Table 1). The DBH nucleus is so far undescribed in the literature. In this paper, we describe the synthesis of 3-arylpropenyl-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (**3Aa–i**) and of the 6-arylpropenyl-3-propionyl-3,6-diazabicyclo[3.1.1]heptane (**3Ba–i**) isomers and their affinity towards μ-, δ-, κ-receptors.

2. Chemistry

The approach we envisaged for the synthesis of the 3,6-diazabicyclo[3.1.1]heptanes **3A**,**B** was initially based on the procedure we employed for the synthesis of the higher homologues DBO, DBN.[2,4,6]

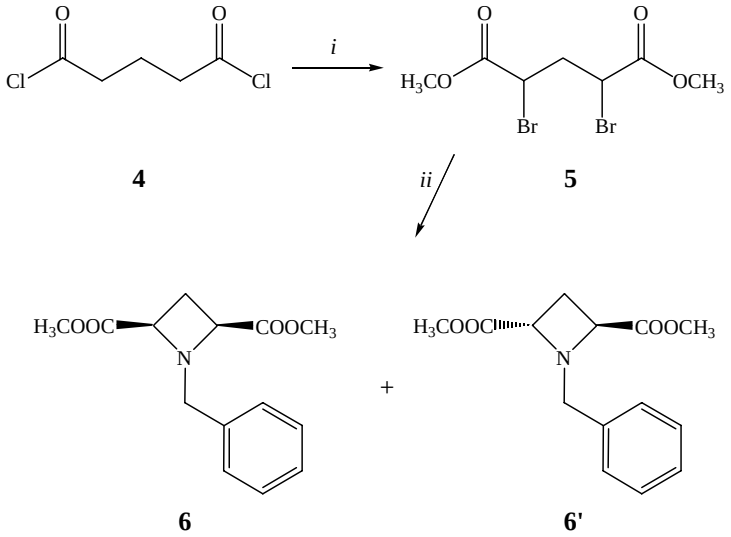
In particular, dimethyl 2,4-dibromoglutarate (**5**) (see Scheme 1), easily obtained by reacting the commercially available acid dichloride **4** with bromine and dry methanol, was condensed with 3 molar equivalent of benzylamine at 80 °C to give a 3:1 ratio of the known *cis–trans*-2,4-dicarbomethoxy-1-benzylazetidines **6** and **6′**.[7] Reaction of **6** with benzylamine in toluene under reflux for 12 h failed to give the desired imide bicyclic product **7** contrary to the trend reported in our previous papers for the synthesis of DBO and DBN.[2,4,6] This unexpected result did induce us to investigate alternative reactions starting from *cis*-1-benzylazetidine-2,4-dicarboxylic acid (**8**) or from the corresponding amide **10**, easily obtained from **6**. However, attempts to convert **8** in refluxing acetic anhydride into **9** or **10** into the imide **7** were unsuccessful (see Scheme 2).

The procedure that enabled us to obtain the desidered DBH intermediate 3,6-dibenzyl-3,6-diazabicyclo[3.1.1]-heptan-2-one (**14**) is reported in Scheme 3. Accordingly, the *cis*-diester **6** was converted with benzylamine in refluxing toluene into 55% of the monobenzylamide **11**. The structure of compound **11** was determined by analysis of its ^{1}H NMR. The spectrum showed a characteristic AB system centred at δ_H 3.75 for diastereotopic hydrogens of the methylene group attached to the amine nitrogen and two multiplets at δ_H 2.74 and δ_H 2.22 for the protons located on the C_3 carbon of the azetidine ring. The reduction of **11** with $NaBH_4$ in methanol led in almost quantitative yields to *cis*-1-benzyl-2-benzylcarbamyl-4-hydroxymethyl-azetidine (**12**) which with mesyl chloride and triethylamine at room temperature gave the mesylate **13**.

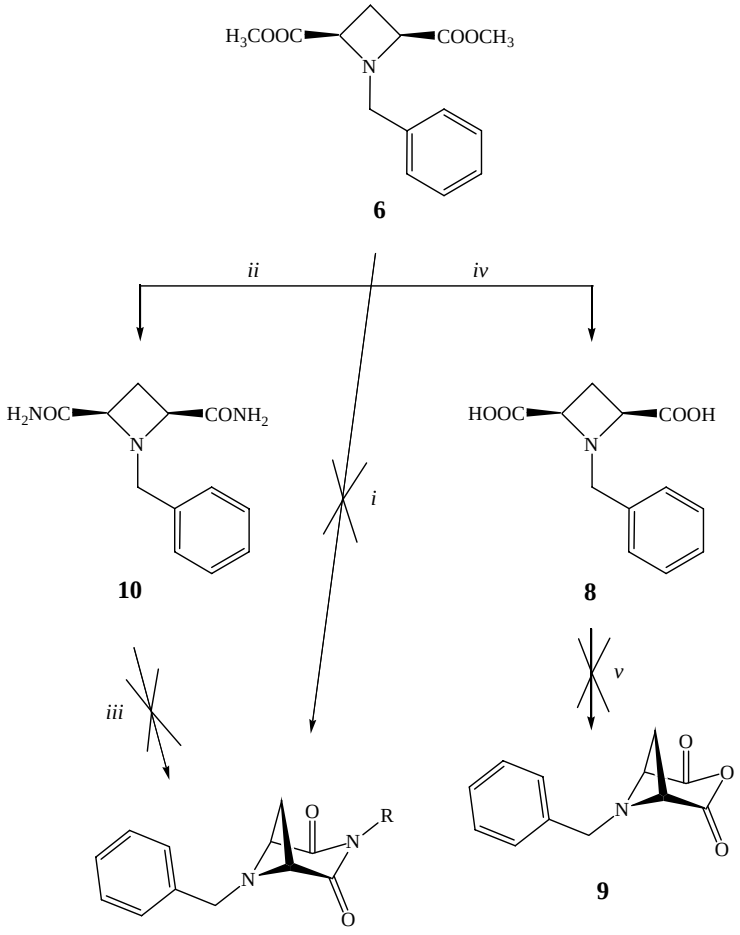
We had the chance to directly convert **13** into 84% of **14** by cyclization in refluxing toluene in the presence of sodium hydroxide, potassium carbonate and tetrabutylammonium hydrogensulfate. The structure of **14** was supported by IR, ^{1}H NMR and ^{13}C NMR (see Section 4). The availability of **14** allowed the synthesis of 6-propionyl-3,6-diazabicyclo[3.1.1]heptane (**18**) as the key intermediate of the final compounds **3A**. In particular, removal of its N_6-benzyl group by hydrogenolysis (Pd/C) led to 3-benzyl-3,6-diazabicyclo[3.1.1]heptan-2-one **15**, which, by $LiAlH_4$ reduction, was converted to 3-benzyl-3,6-diazabicyclo[3.1.1]heptane **16** (see Scheme 4).

The N_6 propionylation of **16** with propionic anhydride followed by hydrogenolysis (Pd/C) of the thus obtained 3-benzyl-6-propionyl-3,6-diazabicyclo[3.1.1]heptane **17** afforded **18** in quantitative yield. Compound **16** was also employed for obtaining N_3-propionyl-3,6-diazabicyclo[3.1.1]heptane **22**, a key intermediate for the synthesis of **3B**, inverted isomers of **3A**. As indicated in Scheme 5, the treatment of **16** with di-*tert*-butyldicarbonate in THF afforded in 86% yield 3-benzyl-N_6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane **19** which, by removal of the *N*-benzyl group by hydrogenolysis, led to N_6-Boc derivative **20**. The N_3 propionylation of **20** with propionic anhydride to give **21** followed by removal of N_6-Boc protecting group with trifluoroacetic acid in the presence of triethylsilane gave the desired 3-propionyl-3,6-diazabicyclo[3.1.1]heptane (**22**).

Being available the intermediates **18** and **22** we expected to obtain the two series of compounds **3A**



Scheme 1. Reagents and conditions: (i) (a) Br_2, *hv*, 95 °C, 4 h, (b) CH_3OH, room temperature, 14 h; (ii) $BnNH_2$, DMF, 80 °C, 4 h.



Scheme 2. Reagents and conditions: (i) $BnNH_2$, toluene, 110 °C, 12 h; (ii) NH_4OH/EtOH, room temperature, 24 h; (iii) H_2O/DMF, Δ, 2 h; (iv) $NaOH_{aq}$ 1 N, THF/MeOH, room temperature, 12 h; (v) Ac_2O, Δ, 2 h.

and **3B** by their condensation with the requisite arylalkenyl chloride **23** or by reductive amination with the aldehydes **24** and $NaCNBH_3$, similarly to the synthesis of the homologue series DBO and DBN. Unexpectedly, both **18** and the isomer **22** allowed to react with the appropriate arylalkenyl halide **23c–e** in acetonitrile at room temperature led to the formation of compounds **3B**, any detectable amount of **3A** being isolated starting from **18**. A similar behaviour was also observed by carrying out the arylalkenylation with the appropriate aldehyde **24a**,**b**,**f–i** and $NaCNBH_3$ (see Scheme 6).

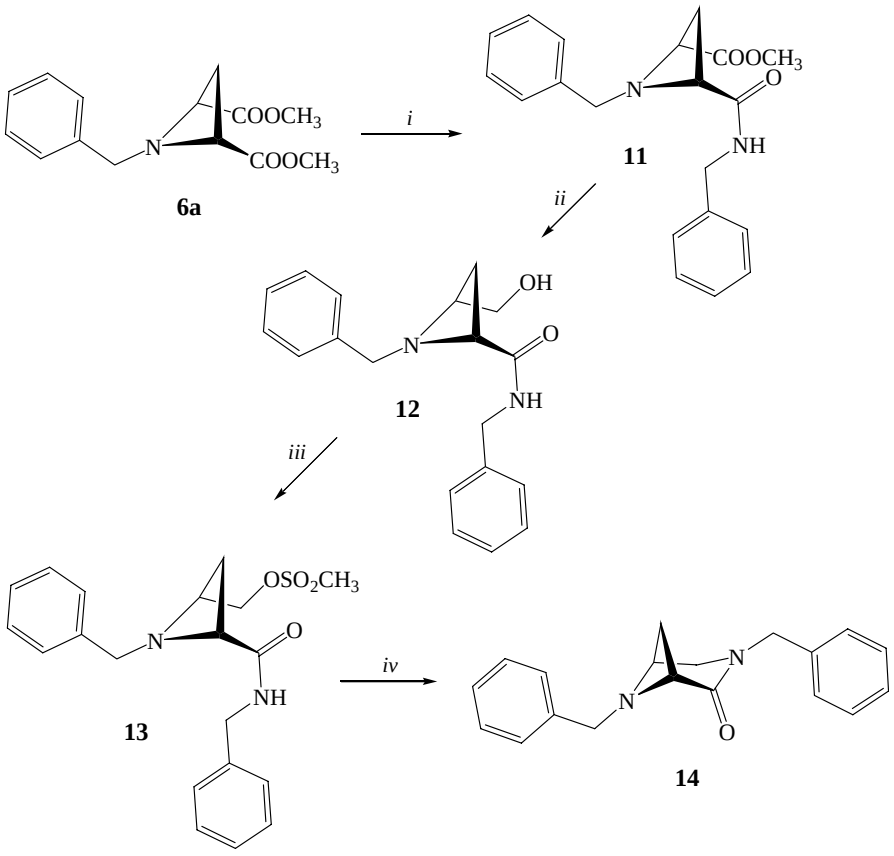
On the basis of these results it was evident that the isolation of **3B** still starting from **18** involved a $N_6 \rightarrow N_3$ propionyl migration during the reaction in acetonitrile. Interestingly, a similar rearrangement took place in the higher homologues N_8-propionyl DBO[8] and N_9-propionyl DBN[4] but under more drastic condition of heating at 120–150 °C.

Coming to the DBH derivatives two conditions are necessary for this to occur: (i) **22** should be much more stable than **18** and (ii) the kinetic barrier for the conversion should be relatively low.

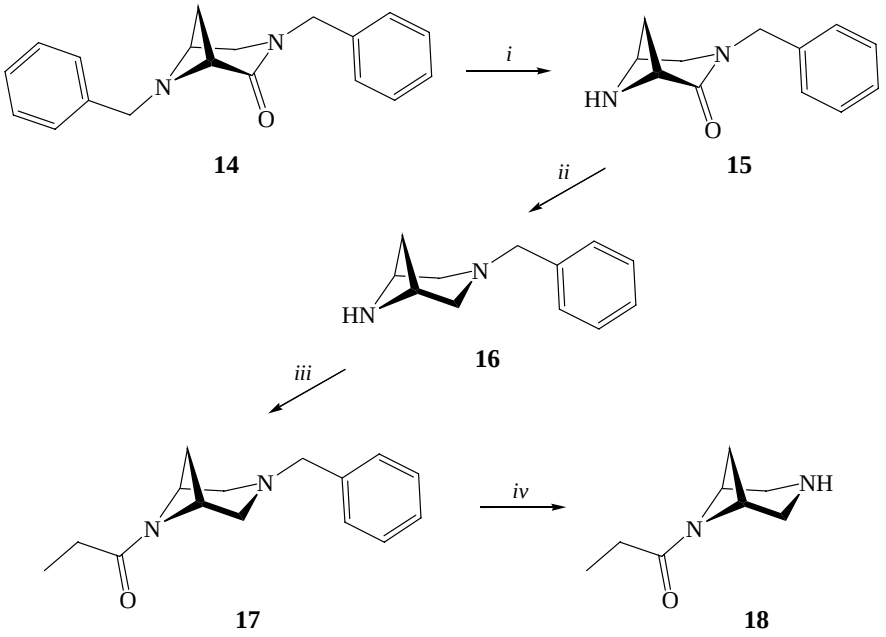
Thus, theoretical calculations were performed on the two isomeric compounds **18** and **22**. Their geometry was optimized using DFT methods at the B3LYP level with the 6-31G* basis set.[9] The DBH system could assume two different conformations corresponding to a chair or a boat geometries of the piperazine ring. Actually, in the case of **18** chair and boat geometries resulted in becoming almost equally accessible as the four conformers located (depicted in Fig. 1) were distributed in a range of less than 1 kcal/mol above the global minimum.

More interestingly, the most stable boat conformation **18c** resulted in becoming less stable than the global minimum **18a** by only 0.27 kcal/mol; an inspection of its geometry shows that its N_3 nitrogen atom points towards the acyl carbonyl group (the distance $N_3/C_{carbonyl}$ being only 2.97 Å) and all the geometry appears already distorted towards the transition state leading to migration. This justifies a very low barrier to migration.

In Figure 1 are also depicted the two allowed conformers of **22**. Their piperazine ring assumes a half-boat geometry and the carbonyl group is far away from N_6 (4.18 Å) indicating no tendency to the back migration.

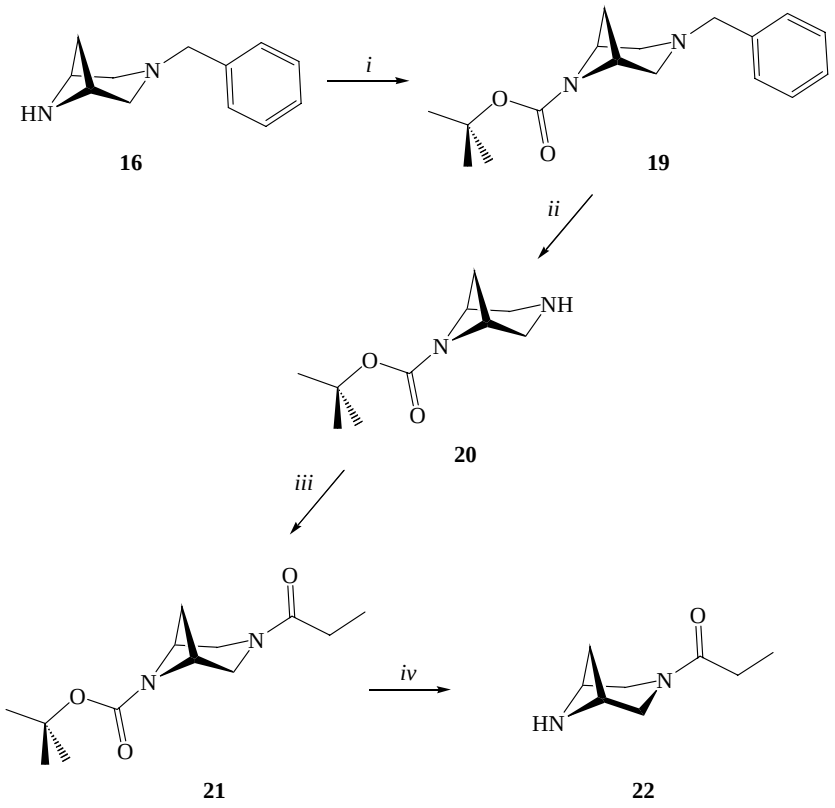


Scheme 3. Reagents and conditions: (i) $BnNH_2$, toluene, 110 °C, 60 h; (ii) $NaBH_4$, MeOH, room temperature, 14 h; (iii) CH_3SO_2Cl, Et_3N, room temperature, 2.5 h; (iv) NaOH, K_2CO_3, TBAHS, toluene, 110 °C, 4 h.



Scheme 4. Reagents and conditions: (i) H_2, Pd/C 10%, EtOH, 60 °C, 7 h; (ii) $LiAlH_4$, THF, room temperature, 14 h; (iii) $(CH_3CH_2CO)_2O$, CH_2Cl_2, Δ, 1 h; (iv) H_2, Pd/C 10%, EtOH, 60 °C, 7 h.

Moreover, **22a** is 5.34 kcal/mol more stable than **18a** ensuring the complete thermodynamic preference for **22**; this preference probably derives from the fact that the sp^2 nitrogen atom of the amide function is better accommodated in a six-membered ring as in **22** than in a four-membered ring.



Scheme 5. Reagents and conditions: (i) $(Boc)_2O$, THF, 12 h; (ii) H_2, Pd/C 10%, EtOH, room temperature, 22 h; (iii) $(CH_3CH_2CO)_2O$, CH_2Cl_2, Δ, 1 h; (iv) CF_3COOH, Et_3SiH, CH_2Cl_2, 0 °C to room temperature, 5.5 h.

The procedure which enabled us to obtain the desired **3A** started from N_6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (**20**) easily prepared from **16** and di-*t*-butyldicarbonate to give the N_3-benzyl-N_6-Boc derivative **19**, which was eventually debenzylated by hydrogenolysis. The condensation of **20** with the appropriate aldehyde **24a–i** and sodium cyanoborohydride gave the N_3-arylpropenyl-N_6-Boc DBH derivatives **25a–i** which, by cleavage of the *t*-butyloxycarbonyl substituent with trifluoroacetic acid and triethylsilane, provided the N_3-arylalkenyl DBH derivatives **26a–i**, eventually converted with propionic anhydride to the desired **3Aa–i** (see Scheme 7).

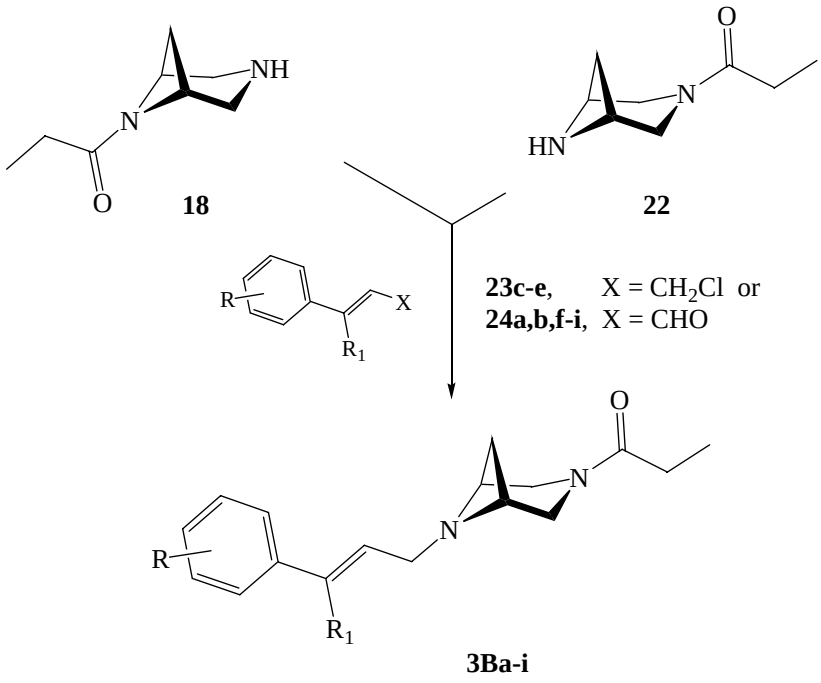
The conformational properties of compounds **3A** and **3B** were determined through the theoretical approaches described above for **18** and **22**; in these cases, optimizations were performed on the protonated form of each compound and the energy was recalculated with a continuum solvent model, C-PCM,[10] to take into account the strong influence of water on the behaviour of these compounds which bear a positive charge under physiological conditions. First, attention was focused on the bicyclic moiety by considering the simplified models **27** and **28** in which a methyl group replaces the arylpropenyl chain. Their preferred conformations are reported in Figure 2 which shows that the chair and boat conformations are accessible for the piperazine ring of **27** and the half-boat conformation is accessible for the piperazine ring of **28**.

Molecular modelling was then extended to compounds **3Aa** and **3Ba**, exploring the conformations derived from rotation of the single bonds of the phenylpropenyl group. A systematic search around the rotatable bonds showed that this group is very flexible as several conformations could be located in a range of 1 kcal/mol above the global minima. However, the geometries of the bicyclic system strictly correspond to those shown in Figure 2 for **27** and **28** as can be seen in Figure 3 where, as examples, are depicted the minimum energy conformations of **3Aa** and **3Ba** located at the B3LYP/6-31G* level.

3. Results and discussion

The new compounds **3Aa–i** and **3Ba–i** were submitted to binding studies on opioid receptors on mouse brain homogenates in the presence of [^{3}H]DAMGO for μ, [^{3}H]DADLE for δ and [^{3}H]bremazocine for κ, using morphine as the reference compound (see Table 1).

Among the compounds **3A** tested, the μ-affinity was lower by two orders of magnitude than that of morphine; on the contrary, the μ-affinity of the isomeric series **3B** was, in general, in the nanomolar range; in particular, the μ-affinity of the compounds **3Bb** (K_i 5.2 nM), **3Bg** (K_i 7.9 nM) and **3Bh** (K_i 2.7 nM) compared favourably with that of morphine (K_i 2.8 nM), while the μ/δ, κ selectivities were definitively higher than that of the reference compound (see Table 1).



3B,23,24	a	b	c	d	e	f	g	h	i
R	H	4-NO_2	2-Cl	3-Cl	4-Cl	H	4-Cl	3,4-Cl_2	H
R_1	H	H	H	H	H	CH_3	CH_3	CH_3	CH_2CH_3

Scheme 6. Reagents and conditions: **23c**–**e**, K_2CO_3, CH_3CN, room temperature, 7 h; or **24a**,**b**,**f**–**i**, $NaCNBH_3$, CH_3COOH, MeOH, room temperature, 7 h.

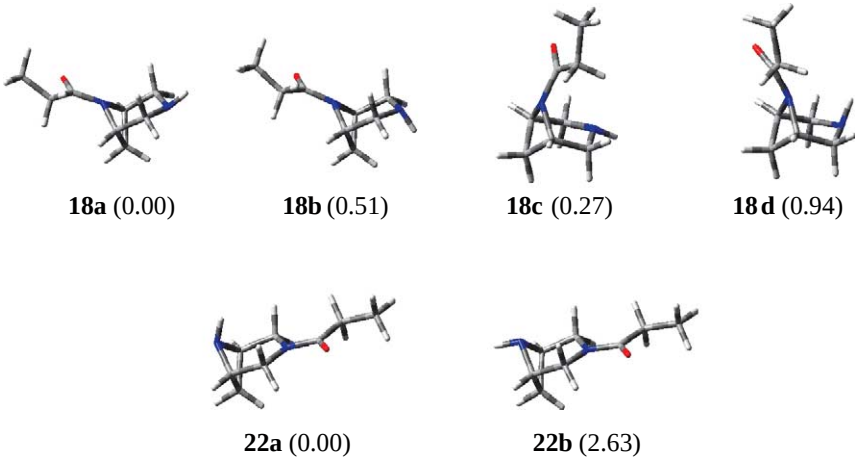
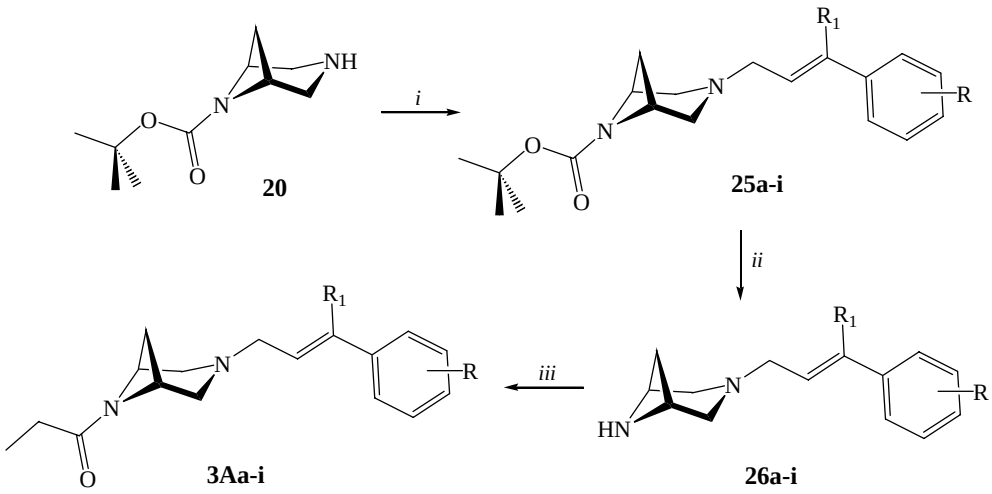


Figure 1. Three-dimensional plots of the significant conformers of compounds **18** and **22**. In parentheses are the relative energies (kcal/mol); the relative energy of **18a** with respect to **22a** is 5.34 kcal/mol.

It is interesting to note that comparison of the representative terms with the higher homologues DBO **1B** and DBN **2B** generally evidenced quite similar μ-affinity and a higher μ/δ, κ selectivity.[2,4]

The conformational preferences of **3Bh**, the compound with the best binding affinity, were determined; also in this case, the N_3 substituent is very flexible and its minimum energy conformation is also reported, as an example, in Figure 3. Its main difference from **3Ba** is the deviation from planarity of the aromatic ring with respect to the plane of the double bond due to the additional methyl group at position 3 of the propenyl chain which destabilizes the planar orientations. The conformational behaviour of compounds **3A** and **3B** here described containing the DBH moiety well compares with that of the corresponding DBO **1A** and **1B** described in previous papers.[11] In fact, the allowed



3A,25,26	a	b	c	d	e	f	g	h	i
R	H	4-NO_2	2-Cl	3-Cl	4-Cl	H	4-Cl	3,4-Cl_2	H
R_1	H	H	H	H	H	CH_3	CH_3	CH_3	CH_2CH_3

Scheme 7. Reagents and conditions: (i) **24a**–**i**, $NaCNBH_3$, CH_3COOH, CH_3CN, room temperature, 24 h; (ii) CF_3COOH, Et_3SiH, CH_2Cl_2, 0 °C to room temperature, 5.5 h; (iii) $(CH_3CH_2CO)_2O$, CH_2Cl_2, Δ, 1 h.

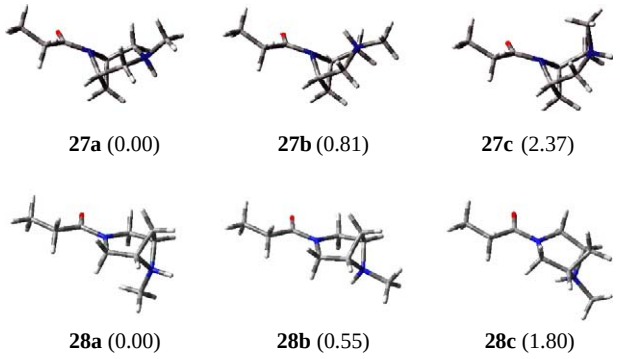


Figure 2. Three-dimensional plots of the significant conformers of compounds **27** and **28**. In parentheses are the relative energies (kcal/mol); the relative energy of **27** with respect to **28** is 7.17 kcal/mol.

conformations of the bicyclic system of **3A** and **3B** are quite similar to those of **1A** and **1B**, respectively, the main difference being the smaller size of the endomethano bridge of **3** with respect to the endoethano bridge of **1**. The affinity of the DBH compounds unsubstituted on the phenyl ring (**3Aa** and **3Ba**) is poorer than that of the corresponding **1A** and **1B** homologues, indicating that the endomethano bridge of compounds **3** induces a weaker interaction with the hydrophobic pocket. Substitution on the phenyl ring with NO_2 or Cl, in particular in the para position, improves the binding properties of the compounds of the **3B** series. The effect is particularly evident when a methyl group is present at position 3′ of the propenyl moiety thus forcing the aryl group to deviate from planarity. This observation confirms what already noticed that the phenyl ring and the double bond should not be coplanar to allow a good interaction with the μ-receptor.[12] The compounds of the **3A** series do not take advantage, neither from the substitution on the phenyl ring, nor from the methyl group on the propenyl.

All these results seem to indicate that the two series of compounds **3A** and **3B** bind the receptor in very different ways; in the case of DBOs, it has been even suggested[5] that **1A** and **1B** might activate different receptor subtypes; the results here presented for **3A** and **3B** seem to support this hypothesis suggesting that a unique model which is able to explain the affinity properties both in

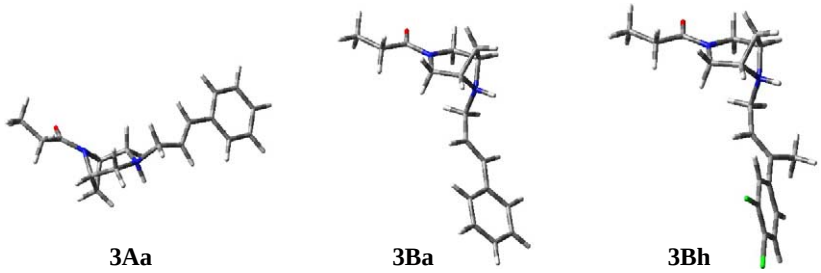


Figure 3. Three-dimensional plots of the preferred conformers of compounds **3Aa**, **3Ba** and **3Bh**.

the 'normal' (**1A** and **3A**) and in the 'reverted' (**1B** and **3B**) series cannot be used to rationalize the affinity data.

In conclusion, design and synthesis of new μ-receptor ligands were achieved by structural optimization of ancestral lead DBO. From the compound tested, terms **3Bb** and **3Bh** demonstrated best results with regard to μ-affinity and selectivity, so these compounds may be useful as probes for better studying the pharmacology of the opioid μ-receptors.

4. Experimental

4.1. General informations

Melting points were obtained on an Electrothermal IA 9100 digital melting point apparatus or on a Köfler melting point apparatus and are uncorrected. IR spectra were recorded as thin films (for oils) or nujol mulls (for solids) on NaCl plates with a Perkin-Elmer 781 IR spectrophotometer and are expressed in ν (cm^{-1}). All NMR spectra were taken on a Varian XL-200 NMR spectrometer, with ^1H and ^{13}C being observed at 200 and 50 MHz, respectively. Chemical shifts for ^1H and ^{13}C spectra were reported in δ or ppm downfield from TMS [$(CH_3)_4Si$]. Multiplicities are recorded as s (singlet), br s (broad singlet), d (doublet), dt (double triplet), t (triplet), q (quartet) and m (multiplet). Elemental analyses were performed by Laboratorio di Microanalisi, Dipartimento di Chimica, Università di Sassari, Italy, and are within ±0.4% of the calculated values. All reactions involving air or moisture-sensitive compounds were performed under argon atmosphere.

The general procedure for conversion to an HCl salt was the addition of excess ethereal HCl solution to a solution of the compound in dry ethanol or diethyl ether. The solvent was evaporated and the resulting salt was triturated with anhydrous ether and dried on vacuum.

The general procedure for conversion to a fumarate salt was the addition of a stoichiometric amount of a solution of fumaric acid in dry methanol to a solution of the compound in dry methanol. The solvent was evaporated and the resulting salt was triturated with anhydrous ether and dried on vacuum.

Unless otherwise specified, all materials, solvents, reagents and precursors **4** and **24a** were obtained from commercial suppliers.

The requisite arylalkenylchloride **23c–e** and aldehydes **24a–i** were prepared as reported in the literature.[1,13]

Flash chromatography (FC) was performed using Merck silica gel 60 (230–400 mesh ASTM). Thin-layer chromatography (TLC) was performed with Polygram® SIL N-HR/HV_{254} precoated plastic sheets (0.2 mm).

4.1.1. *cis*-1-Benzyl-azetidine-2,4-dicarboxylic acid dimethyl ester (*cis*-6) and *trans*-1-benzyl-azetidine-2,4-dicarboxylic acid dimethyl ester (*trans*-6′). Prepared according to the literature method with some modifications.[7] A solution of 2,4-dibromoglutaric acid dimethyl ester (**5**) (35.50 g, 111.64 mmol) and 3 molar equiv of benzylamine (36.60 mL, 334.92 mmol) in DMF (170 mL) was stirred at 80 °C for 4 h. The solvent was then evaporated, the residue was taken up in CH_2Cl_2, and the solution was washed with saturated aqueous $NaHCO_3$ solution. After the solution was dried over Na_2SO_4 and evaporated, the residue was purified by FC, eluting with: petroleum ether/EtOAc 8:2.

Fraction 1 contained *trans*-**6′**, yield 18%; R_f 0.43 (petroleum ether/EtOAc 8:2); bp 148 °C/0.1 mmHg; IR: 1590, 1730; ^1H NMR ($CDCl_3$) δ 2.51 (t, 2H, J = 6.6 Hz), 3.65 (s, 6H), 3.80–3.93 (m, 2H), 4.22 (t, 2H, J = 6.8 Hz), 7.20–7.35 (m, 5H).

Fraction 2 contained *cis*-**6**, yield 48%; R_f 0.26 (petroleum ether/EtOAc 8:2); bp 140 °C/0.1 mmHg; IR: 1600, 1720; ^1H NMR ($CDCl_3$) δ 2.27-2.60 (m, 2H), 3.63 (t, 2H, J = 8.0 Hz), 3.64 (s, 6H), 3.88 (s, 2H), 7.23-7.40 (m, 5H).

4.1.2. *cis*-1-Benzyl-azetidine-2,4-dicarboxylic acid (8). Prepared according to the literature method,[7] yield 84%; R_f 0.17 ($CHCl_3$/MeOH 7:3); mp 224–226 °C (lit.[7] mp 225–228); IR: 1590, 1710; ^1H NMR ($CDCl_3$) δ 1.99–2.18 (m, 1H), 2.20–2.40 (m, 1H), 3.51 (t, 2H, J = 8.4 Hz), 3.72 (s, 2H), 7.11–7.36 (m, 5H).

4.1.3. *cis*-1-Benzyl-azetidine-2,4-dicarboxylic acid diamide (10). Prepared according to the literature method with some modifications.[7] To a solution of **6** (0.50 g, 1.90 mmol) in 3 mL EtOH was added a solution of 25% NH_4OH (3 mL). The mixture was stirred at room temperature for 24 h and then concentrated to afford 0.33 g (75%) of **10** as a white solid: R_f 0.15 ($CHCl_3$/MeOH 8:2); mp 211–213 °C (lit.[7] mp 209–213); IR: 1600, 1700; ^1H NMR ($CDCl_3$) δ 1.72–1.93 (m, 1H), 2.51–2.73 (m, 1H), 3.42 (t, 2H, J = 11.6 Hz), 3.65 (s, 2H), 6.80–7.70 (m, 5H).

4.1.4. *cis*-1-Benzyl-4-benzylcarbamoyl-azetidine-2-carboxylic acid methyl ester (11). A solution of *cis*-**6** (11.01 g, 41.81 mmol) and benzylamine (4.56 mL, 41.81 mmol) in toluene (56 mL) was refluxed for 60 h. The solvent was then evaporated and the residue was purified by FC eluting with petroleum ether/EtOAc 5:5, to afford 7.77 g (55%) of **11** as a white solid: R_f 0.20 (petroleum ether/EtOAc 7:3); mp 94–96 °C (isopropyl ether); IR: 1600, 1670, 1730, 3340; ^1H NMR ($CDCl_3$) δ 2.13–2.30 (m, 1H), 2.65–2.82 (m, 1H), 3.60–3.92 (m, 4H), 3.68 (s, 3H), 4.15–4.38 (m, 2H), 7.03–7.44 (m, 10H). Anal. Calcd for $C_{20}H_{22}N_2O_3$: C, 70.90; H, 6.55; N, 8.28. Found: C, 70.80; H, 6.53; N, 8.25.

4.1.5. *cis*-1-Benzyl-4-hydroxymethyl-azetidine-2-carboxylic acid benzylamide (12). To a solution of **11** (4.00 g, 11.82 mmol) in methanol (40 mL) at 0 °C was slowly added $NaBH_4$ (1.35 g, 35.46 mmol). The reaction mixture was allowed to warm to room temperature and then stirred overnight. Water was added, the methanol was evaporated and the mixture was extracted with

CH_2Cl_2. The organic layer was washed with water and brine. After the solution was dried over Na_2SO_4 and evaporated, the pure product **12** was obtained (3.76 g, 99%) as a white solid: R_f 0.12 (petroleum ether/EtOAc 5:5); mp 92–94 °C (hexane/petroleum ether); IR: 1600, 1640, 3340, 3400; ^{1}H NMR ($CDCl_3$) δ 1.69 (br s, 1H), 1.95–2.15 (m, 1H), 2.41–2.58 (m, 1H), 3.30–3.48 (m, 3H), 3.60–3.76 (m, 3H), 4.15–4.39 (m, 2H), 7.08–7.32 (m, 11H). Anal. Calcd for $C_{19}H_{22}N_2O_2$: C, 73.52; H, 7.14; N, 9.03. Found: C, 73.50; H, 7.12; N, 9.00.

4.1.6. *cis*-1-Benzyl-4-methansulfonyloxymethyl-azetidine-2-carboxylic acid benzylamide (13). To a solution of **12** (8.77 g, 28.28 mmol) and Et_3N (11.82 mL, 84.84 mmol) in dry CH_2Cl_2 (97 mL) at 0 °C under a nitrogen atmosphere was added MsCl (2.84 mL, 36.76 mmol). The reaction mixture was stirred at the same temperature for 2.5 h, and then excess reagent was quenched with water. The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by FC eluting with petroleum ether/EtOAc 2:8, to afford 9.52 g (86%) of **13** as a light yellow oil: R_f 0.38 (petroleum ether/EtOAc 1.5:8.5); bp 155 °C/0.1 mmHg; IR: 1170, 1360, 1600, 1680, 3340; ^{1}H NMR ($CDCl_3$) δ 1.95–2.07 (m, 1H), 2.53–2.68 (m, 1H), 2.80 (s, 3H), 3.48–4.40 (m, 8H), 7.08–7.44 (m, 11H); ^{13}C NMR ($CDCl_3$) δ 24.49, 37.12, 42.35, 59.23, 60.81, 62.25, 70.16, 126.91, 127.20, 127.56, 128.30, 128.37, 129.07, 136.18, 137.99, 171.89. Anal. Calcd for $C_{20}H_{24}N_2O_4S$: C, 61.83; H, 6.23; N, 7.21; S, 8.25. Found: C, 61.68; H, 6.21; N, 7.18; S, 8.21.

4.1.7. 3,6-Dibenzyl-3,6-diazabicyclo[3.1.1]heptan-2-one (14). A mixture of **13** (4.30 g, 11.06 mmol), potassium carbonate (3.05 g, 22.12 mmol), tetrabutylammonium hydrogensulfate (0.37 g, 1.10 mmol) and finely powdered sodium hydroxide (1.54 g, 38.74 mmol) in toluene (52 mL) was refluxed for 4 h. The reaction mixture was allowed to warm to room temperature, diluted with Et_2O and washed with water. The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by FC eluting with petroleum ether/EtOAc 2.5:7.5, to afford 2.72 g (84%) of **14** as light yellow oil: R_f 0.45 (petroleum ether/EtOAc 2.5:7.5); bp 163 °C/0.1 mmHg; IR: 1600, 1670; ^{1}H NMR ($CDCl_3$) δ 1.72 (d, 1H, J = 8.4 Hz), 2.63–2.74 (m, 1H), 3.13 (d, 1H, J = 12.4 Hz), 3.29–3.45 (m, 2H), 3.55 (d, 1H, J = 13.2 Hz), 3.67–3.78 (m, 2H), 4.58 (d, 1H, J = 14.2 Hz), 4.78 (d, 1H, J = 14.4 Hz), 7.12–7.43 (m, 10H); ^{13}C NMR ($CDCl_3$) δ 29.20, 44.21, 47.88, 51.71, 57.98, 64.41, 126.94, 127.64, 128.20, 128.24, 128.53, 128.69, 136.89, 137.06, 171.30. Anal. Calcd for $C_{19}H_{20}N_2O$: C, 78.05; H, 6.89; N, 9.58. Found: C, 77.90; H, 6.87; N, 9.55.

4.1.8. 3-Benzyl-3,6-diazabicyclo[3.1.1]heptan-2-one (15). A solution of **14** (3.50 g, 11.97 mmol) in 52 mL ethanol was hydrogenated in a Parr apparatus over 1.27 g (1.20 mmol) of 10% Pd/C under a hydrogen pressure of 3 atm at 60 °C for 7 h. The mixture was filtered through Celite and the catalyst was washed with several portions of ethanol. The solution was evaporated and the residue was purified by FC eluting with $CHCl_3$/MeOH 9:1, to afford 2.23 g (92%) of **15** as a white solid: R_f 0.44 ($CHCl_3$/MeOH 9:1); mp 61–62 °C; IR: 1600, 1670, 3250; ^{1}H NMR ($CDCl_3$) δ 1.74 (d, 1H, J = 9.0 Hz), 1.94 (br s, 1H), 2.80–2.95 (m, 1H), 3.27–3.48 (m, 2H), 3.80–3.85 (m, 1H), 3.90–4.00 (m, 1H), 4.52 (d, 1H, J = 14.6 Hz), 4.72 (d, 1H, J = 14.6 Hz), 7.23–7.42 (m, 5H). Anal. Calcd for $C_{12}H_{14}N_2O$: C, 71.26; H, 6.98; N, 13.85. Found: C, 71.13; H, 6.96; N, 13.83.

4.1.9. 3-Benzyl-3,6-diazabicyclo[3.1.1]heptane (16). To a suspension of lithium aluminium hydride (1.70 g, 42.72 mmol) in dry THF (50 mL) at 0 °C under a nitrogen atmosphere was added a solution of **15** (2.16 g, 10.68 mmol) in dry THF (35 mL). The mixture was allowed to slowly reach room temperature and was then stirred overnight. The reaction was cooled to 0 °C, diethyl ether (50 mL) was added and the reaction was quenched with water (1.52 mL), 2 M aqueous NaOH (1.52 mL) and water (4.58 mL). The mixture was filtered and evaporated to give pure **16** (1.90 g, 94%) as a colourless oil: R_f 0.18 ($CHCl_3$/MeOH 9:1); bp 155 °C/0.1 mmHg; IR: 1600, 3250; ^{1}H NMR ($CDCl_3$) δ 1.80 (br s, 1H), 1.94 (d, 1H, J = 8.0 Hz), 2.40–2.53 (m, 1H), 2.65 (d, 2H, J = 11.0 Hz), 3.07 (d, 2H, J = 11.0 Hz), 3.50–3.65 (m, 2H), 3.72 (s, 2H), 7.25–7.40 (m, 5H). Anal. Calcd for $C_{12}H_{16}N_2$: C, 76.55; H, 8.57; N, 14.88. Found: C, 76.43; H, 8.55; N, 14.84.

4.1.10. 3-Benzyl-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (17). To a solution of **16** (2.00 g, 10.62 mmol) in CH_2Cl_2 (50 mL) at 0 °C was added a solution of propionic anhydride (4.94 mL, 38.55 mmol) in CH_2Cl_2 (10 mL). When addition was complete, the mixture was refluxed for 1 h. After cooling at room temperature, the mixture was made alkaline with 40% NaOH and stirred overnight. The reaction mixture was extracted with CH_2Cl_2, the organic layers were dried over Na_2SO_4, evaporated and the residue was purified by FC eluting with petroleum ether/EtOAc 2:8, to afford 2.10 g (81%) of **17** as a colourless oil: R_f 0.22 (petroleum ether/EtOAc 2:8); bp 161 °C/0.1 mmHg; IR: 1590, 1630; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.4 Hz), 2.00–2.23 (m, 2H), 2.03 (d, 1H, J = 9.5 Hz), 2.37–2.50 (m, 1H), 2.73–3.18 (m, 4H), 3.69 (s, 2H), 4.20–4.29 (m, 1H), 4.30–4.39 (m, 1H), 7.25–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 9.11, 25.54, 28.28, 52.20, 54.29, 58.50, 60.03, 61.19, 126.91, 128.10, 128.49, 138.15, 173.48. Anal. Calcd for $C_{15}H_{20}N_2O$: C, 73.74; H, 8.25; N, 11.47. Found: C, 73.45; H, 8.22; N, 11.43.

4.1.11. 6-Propionyl-3,6-diazabicyclo[3.1.1]heptane (18). A solution of **17** (1.83 g, 7.49 mmol) in 18 mL of ethanol was hydrogenated in a Parr apparatus over 0.80 g (0.75 mmol) of 10% Pd/C under a hydrogen pressure of 3 atm at 60 °C for 7 h. The mixture was filtered through Celite and the catalyst was washed with several portions of ethanol. The solution was evaporated and the residue was purified by FC eluting with $CHCl_3$/MeOH 9:1, to afford 1.09 g (95%) of **18** as a white solid: R_f 0.13 ($CHCl_3$/MeOH 8:2); mp 58–60 °C; IR: 1640; ^{1}H NMR ($CDCl_3$) δ 1.17 (t, 3H, J = 7.4 Hz), 1.74 (d, 1H, J = 10.2 Hz), 2.27 (br s, 1H), 2.40 (q, 2H, J = 7.0 Hz),

3.12–3.25 (m, 1H), 3.80–4.15 (m, 3H), 4.36 (d, 1H, $J = 12.2$ Hz), 4.45–4.60 (m, 2H); ^{13}C NMR ($CDCl_3$) δ 8.85, 26.55, 30.49, 49.10, 50.51, 55.21, 55.41, 175.27. Anal. Calcd for $C_8H_{14}N_2O$: C, 62.31; H, 9.15; N, 18.17. Found: C, 62.09; H, 9.11; N, 18.10.

4.1.12. 3-Benzyl-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]-heptane (19). To a solution of di-*tert*-butyldicarbonate (1.69 g, 7.73 mmol) in 12 mL THF at 0 °C was added a solution of **16** (0.97 g, 5.15 mmol) in 8 mL THF. The reaction mixture was allowed to reach room temperature and was then stirred overnight. The mixture was diluted with Et_2O, washed with 10% $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by FC eluting with petroleum ether/EtOAc 8:2, to afford 1.28 g (86%) of **19** as a colourless oil: R_f 0.52 (petroleum ether/EtOAc 8:2); bp 170 °C/0.1 mmHg; IR: 1600, 1670; ^{1}H NMR ($CDCl_3$) δ 1.46 (s, 9H), 1.73 (d, 1H, $J = 7.8$ Hz), 2.32–2.43 (m, 1H), 2.76 (d, 2H, $J = 10.2$ Hz), 2.90–3.25 (m, 2H), 3.69 (s, 2H), 4.00–4.10 (m, 2H), 7.25–7.35 (m, 5H). Anal. Calcd for $C_{17}H_{24}N_2O_2$: C, 70.80; H, 8.39; N, 9.71. Found: C, 70.55; H, 8.36; N, 9.67.

4.1.13. 6-*t*-Butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (20). A solution of **19** (1.49 g, 5.16 mmol) in 15 mL ethanol was hydrogenated in a Parr apparatus over 0.80 g (0.75 mmol) of 10% Pd/C under a hydrogen pressure of 3 atm at 60 °C for 7 h. The mixture was filtered through Celite and the catalyst was washed with several portions of ethanol. The solution was evaporated and the residue was purified by FC eluting with $CHCl_3$/MeOH 9:1, to afford 0.92 g (90%) of **20** as a colourless oil: R_f 0.43 ($CHCl_3$/MeOH 9:1); bp 164 °C/0.1 mmHg; IR: 1710; ^{1}H NMR ($CDCl_3$) δ 1.47 (s, 9H), 1.57 (d, 1H, $J = 8.6$ Hz), 2.20 (br s, 1H), 2.51–2.64 (m, 1H), 2.92 (d, 2H, $J = 12.6$ Hz), 3.37–3.58 (m, 2H), 4.00–4.08 (m, 2H). Anal. Calcd for $C_{10}H_{18}N_2O_2$: C, 60.58; H, 9.15; N, 14.13. Found: C, 60.38; H, 9.12; N, 14.07.

4.1.14. 3-Propionyl-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (21). To a solution of **20** (0.20 g, 1.00 mmol) in CH_2Cl_2 (6 mL) at 0 °C was added a solution of propionic anhydride (0.49 mL, 3.65 mmol) in CH_2Cl_2 (1 mL). When addition was complete, the mixture was refluxed for 1 h. After cooling at room temperature, the mixture was made alkaline with 40% NaOH and stirred overnight. The reaction mixture was extracted with CH_2Cl_2, the organic layers were dried over Na_2SO_4, evaporated and the residue was purified by FC eluting with petroleum ether/EtOAc 3:7, to afford 0.22 g (86%) of **21** as a colourless oil: R_f 0.28 (petroleum ether/EtOAc 3:7); bp 167 °C/0.1 mmHg; IR: 1650, 1700; ^{1}H NMR ($CDCl_3$) δ 1.16 (t, 3H, $J = 7.4$ Hz), 1.36 (d, 1H, $J = 8.8$ Hz), 1.42 (s, 9H), 2.32 (q, 2H, $J = 7.4$ Hz), 2.53–2.69 (m, 1H), 3.38–3.56 (m, 2H), 3.94–4.10 (m, 2H), 4.11–4.24 (m, 2H). Anal. Calcd for $C_{13}H_{22}N_2O_3$: C, 61.39; H, 8.72; N, 11.01. Found: C, 61.15; H, 8.69; N, 10.97.

4.1.15. 3-Propionyl-3,6-diazabicyclo[3.1.1]heptane (22). A solution of **21** (0.43 g, 1.69 mmol) in CH_2Cl_2 (6 mL) at 0 °C was treated with triethylsilane (0.67 mL, 4.22 mmol), followed by trifluoroacetic acid (1.69 mL, 21.97 mmol). The solution was stirred at the same temperature for 2.5 h, and then further trifluoroacetic acid (0.91 mL, 11.83 mmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 3 h, and then the solvent was evaporated. The residue was dissolved in 25 mL of saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to afford 0.20 g (77%) of **22** as a colourless oil: R_f 0.22 ($CHCl_3$/MeOH 8:2); mp 98–100 °C (as hydrochloride); IR: 1600, 1660; ^{1}H NMR ($CDCl_3$) δ 1.19 (t, 3H, $J = 7.4$ Hz), 1.47 (d, 1H, $J = 9.0$ Hz), 1.76 (br s, 1H), 2.36 (q, 2H, $J = 7.4$ Hz), 2.64–2.80 (m, 1H), 3.60–3.87 (m, 6H); ^{13}C NMR ($CDCl_3$) δ 8.83, 26.51, 30.52, 49.18, 50.57, 55.16, 55.36, 175.24. Anal. Calcd for $C_8H_{14}N_2O$: C, 62.31; H, 9.15; N, 18.17. Found: C, 62.06; H, 9.12; N, 18.09.

4.1.16. General procedures for the preparation of 3Ba–i. *Method A*. A solution of **18** or **22** (0.15 g, 0.97 mmol) and the required aldehydes **24a**,**b**,**f–i** (1.07 mmol) in MeOH (8 mL) was treated with few drops of acetic acid, followed by $NaCNBH_3$ (85 mg, 1.36 mmol). The solution was stirred at room temperature for 7 h, and then the solvent was evaporated. The residue was dissolved in 7 mL of 1 N NH_4OH solution and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by FC to afford the compounds **3Ba**,**b**,**f–i** as oils. All final compounds were converted into the HCl or $HO_2CCH{=}CHCO_2H$ salts.

Method B. A mixture of **18** or **22** (0.15 g, 0.97 mmol), the required chloride (**23c–e**) (0.97 mmol) and K_2CO_3 (0.41 g, 2.98 mmol) in CH_3CN (9 mL) was stirred at room temperature for 7 h. The inorganic salt was filtered off, the filtrate was evaporated and the oily residue was purified by FC to give the desired **3Bc–e** as oils. All final compounds were converted into the HCl salts.

4.1.16.1. 3-Propionyl-6-cinnamyl-3,6-diazabicyclo[3.1.1]-heptane (3Ba). Prepared according to the method A. Purified by FC (eluent: petroleum ether/EtOAc 4:6); yield 79%; R_f 0.39 (petroleum ether/EtOAc 4:6); mp 142–144 °C (as hydrochloride); IR: 1590, 1620, 1660; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.4$ Hz), 1.50 (d, 1H, $J = 8.8$ Hz), 2.38 (q, 2H, $J = 7.4$ Hz), 2.68–2.78 (m, 1H), 3.24 (d, 2H, $J = 6.2$ Hz), 3.38–3.89 (m, 6H), 6.19 (dt, 1H, $J = 6.2$ and 16.0 Hz), 6.56 (d, 1H, $J = 16.0$ Hz), 7.20–7.48 (m, 5 H). Anal. Calcd for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 10.36. Found: C, 75.49; H, 8.17; N, 10.33.

4.1.16.2. 3-Propionyl-6-[3′-(4-nitrophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (3Bb). Prepared according to the method A. Purified by FC (eluent: acetone/CH_2Cl_2 3:7); yield 20%; R_f 0.45 (acetone/CH_2Cl_2 3:7); mp >250 °C dec (as fumarate); IR: 1590, 1620, 1650; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.0$ Hz), 1.51–1.56 (m, 1H), 2.38 (q, 2H, $J = 7.2$ Hz), 2.60–2.66 (m, 1H), 3.28 (d, 2H, $J = 6.2$ Hz), 3.66–3.82 (m, 6H),

6.40 (dt, 1H, $J = 6.6$ and 15.8 Hz), 6.64 (d, 1H, $J = 15.8$ Hz), 7.46–8.20 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 8.91, 26.50, 29.59, 42.46, 44.08, 47.87, 57.42, 57.78, 124.01, 126.74, 129.83, 131.15, 143.28, 146.90, 174.80. Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.52; H, 6.68; N, 13.27.

4.1.16.3. 3-Propionyl-6-[3′-(2-chlorophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (3Bc). Prepared according to the method B. Purified by FC (eluent: acetone/CH_2Cl_2 6:4); yield 34%; R_f 0.20 (acetone/CH_2Cl_2 5:5); mp 137–139 °C (as hydrochloride); IR: 1600, 1640, 1660; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.8$ Hz), 1.52 (d, 1H, $J = 8.8$ Hz), 2.38 (q, 2H, $J = 7.6$ Hz), 2.59–2.78 (m, 1H), 3.28 (d, 2H, $J = 5.8$ Hz), 3.40–3.88 (m, 6H), 6.17 (dt, 1H, $J = 9.8$ and 15.8 Hz), 6.94 (d, 1H, $J = 15.6$ Hz), 7.27–7.59 (m, 4H). Anal. Calcd for $C_{17}H_{21}ClN_2O$: C, 66.99; H, 6.94; Cl, 11.63; N, 9.19. Found: C, 66.76; H, 6.91; Cl, 11.59; N, 9.15.

4.1.16.4. 3-Propionyl-6-[3′-(3-chlorophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (3Bd). Prepared according to the method B. Purified by FC (eluent: acetone/CH_2Cl_2 6:4); yield 41%; R_f 0.22 (acetone/CH_2Cl_2 6:4); mp 144–146 °C (as hydrochloride); IR: 1600, 1610, 1650; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.4$ Hz), 1.50 (d, 1H, $J = 8.8$ Hz), 2.37 (q, 2H, $J = 7.4$ Hz), 2.58–2.72 (m, 1H), 3.22 (d, 2H, $J = 6.0$ Hz), 3.40–3.84 (m, 6H), 6.10–6.30 (m, 1H), 6.50 (d, 1H, $J = 15.2$ Hz), 7.18–7.31 (m, 3H), 7.33 (s, 1H). Anal. Calcd for $C_{17}H_{21}ClN_2O$: C, 66.99; H, 6.94; Cl, 11.63; N, 9.19. Found: C, 66.75; H, 6.92; Cl, 11.61; N, 9.17.

4.1.16.5. 3-Propionyl-6-[3′-(4-chlorophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (3Be). Prepared according to the method B. Purified by FC (eluent: acetone/CH_2Cl_2 6:4); yield 35%; R_f 0.24 (acetone/CH_2Cl_2 6:4); mp 142–143 °C (as hydrochloride); IR: 1590, 1620, 1660; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.6$ Hz), 1.50 (d, 1H, $J = 8.8$ Hz), 2.38 (q, 2H, $J = 7.6$ Hz), 2.59–2.78 (m, 1H), 3.27 (d, 2H, $J = 5.8$ Hz), 3.41–3.89 (m, 6H), 6.16 (dt, 1H, $J = 6.2$ and 15.8 Hz), 6.94 (d, 1H, $J = 15.6$ Hz), 7.20–7.35 (m, 4H). Anal. Calcd for $C_{17}H_{21}ClN_2O$: C, 66.99; H, 6.94; Cl, 11.63; N, 9.19. Found: C, 66.80; H, 6.91; Cl, 11.62; N, 9.16.

4.1.16.6. 3-Propionyl-6-(3′-phenyl-but-2′-en-1′-yl)-3,6-diazabicyclo[3.1.1]heptane (3Bf). Prepared according to the method A. Purified by FC (eluent: $CHCl_3$/MeOH 9.5:0.5); yield 51%; R_f 0.39 ($CHCl_3$/MeOH 9.5:0.5); mp 123–125 °C (as hydrochloride); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.4$ Hz), 1.51 (d, 1H, $J = 8.0$ Hz), 2.05 (s, 3H), 2.37 (q, 2H, $J = 7.0$ Hz), 2.57–2.73 (m, 1H), 3.26 (d, 2H, $J = 6.0$ Hz), 3.37–3.83 (m, 6H), 5.68–5.80 (m, 1H), 7.19–7.44 (m, 5H). Anal. Calcd for $C_{18}H_{24}N_2O$: C, 76.02; H, 8.51; N, 9.85. Found: C, 75.99; H, 8.54; N, 9.88.

4.1.16.7. 3-Propionyl-6-[3′-(4-chlorophenyl)-but-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (3Bg). Prepared according to the method A. Purified by FC (eluent: acetone/CH_2Cl_2 8:2); yield 65%; R_f 0.18 (acetone/CH_2Cl_2 8:2); mp 119–121 °C (as hydrochloride); IR: 1590, 1600, 1650; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, 3H, $J = 7.4$ Hz), 1.51 (d, 1H, $J = 8.8$ Hz), 2.02 (s, 3H), 2.37 (q, 2H, $J = 7.0$ Hz), 2.58–2.76 (m, 1H), 3.24 (d, 2H, $J = 6.0$ Hz), 3.41–3.88 (m, 6H), 5.67–5.80 (m, 1H), 7.22–7.40 (m, 4H). Anal. Calcd for $C_{18}H_{23}ClN_2O$: C, 67.81; H, 7.27; Cl, 11.12; N, 8.79. Found: C, 67.55; H, 7.24; Cl, 11.08; N, 8.77.

4.1.16.8. 3-Propionyl-6-[3′-(3,4-dichlorophenyl)-but-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (3Bh). Prepared according to the method A. Purified by FC (eluent: acetone/CH_2Cl_2 8:2); yield 29%; R_f 0.31 (acetone/CH_2Cl_2 8:2); mp 122–124 °C (as hydrochloride); IR: 1590, 1610, 1630; ^{1}H NMR ($CDCl_3$) δ 1.21 (t, 3H, $J = 7.4$ Hz), 1.52 (d, 1H, $J = 9.0$ Hz), 2.02 (s, 3H), 2.37 (q, 2H, $J = 7.8$ Hz), 2.58–2.75 (m, 1H), 3.24 (d, 2H, $J = 6.4$ Hz), 3.43–3.89 (m, 6H), 5.70–5.85 (m, 1H), 7.15–7.52 (m, 3H). Anal. Calcd for $C_{18}H_{22}Cl_2N_2O$: C, 61.19; H, 6.28; Cl, 20.07; N, 7.93. Found: C, 60.94; H, 6.26; Cl, 20.05; N, 7.90.

4.1.16.9. 3-Propionyl-6-(3′-phenyl-pent-2′-en-1′-yl)-3,6-diazabicyclo[3.1.1]heptane (3Bi). Prepared according to the method A. Purified by FC (eluent: $CHCl_3$/MeOH 9.5:0.5); yield 76%; R_f 0.37 ($CHCl_3$/MeOH 9.5:0.5); mp 160–163 °C (as fumarate); IR: 1590, 1600, 1650; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, $J = 7.2$ Hz), 1.21 (t, 3H, $J = 7.6$ Hz), 1.51 (d, 1H, $J = 8.8$ Hz), 2.38 (q, 2H, $J = 7.4$ Hz), 2.51 (q, 2H, $J = 7.4$ Hz), 2.60–2.78 (m, 1H), 3.27 (d, 2H, $J = 6.2$ Hz), 3.40–3.91 (m, 6H), 5.60 (t, 1H, $J = 6.4$ Hz), 7.22–7.51 (m, 5H). Anal. Calcd for $C_{19}H_{26}N_2O$: C, 76.47; H, 8.78; N, 9.39. Found: C, 76.17; H, 8.75; N, 9.35.

4.1.17. General procedure for the preparation of 25a–i. A solution of **20** (0.50 g, 2.52 mmol) and the required aldehydes **24a–i** (2.77 mmol) in CH_3CN (20 mL) was treated with few drops of acetic acid, followed by $NaCNBH_3$ (0.36 g, 2.77 mmol). The solution was stirred at room temperature for 24 h, and then the solvent was evaporated. The residue was dissolved in 10 mL of aqueous 2 N KOH solution and extracted with diethyl ether. The organic layer was dried over Na_2SO_4, evaporated, and the residue was purified by FC to afford the compounds **25a–i** as oils.

4.1.17.1. 3-Cinnamyl-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25a). Purified by FC (eluent: petroleum ether/EtOAc 7:3); yield 41%; R_f 0.44 (petroleum ether/EtOAc 7:3); IR: 1590, 1620, 1660; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.72 (d, 1H, $J = 8.2$ Hz), 2.35–2.44 (m, 1H), 2.83 (d, 2H, $J = 10.8$ Hz), 2.97–3.30 (m, 2H), 3.33 (d, 2H, $J = 6.6$ Hz), 4.00–4.15 (m, 2H), 6.24 (dt, 1H, $J = 6.6$ and 15.8 Hz), 6.55 (d, 1H, $J = 15.8$ Hz), 7.20–7.40 (m, 5H). Anal. Calcd for $C_{19}H_{26}N_2O_2$: C, 72.58; H, 8.33; N, 8.91. Found: C, 72.33; H, 8.30; N, 8.88.

4.1.17.2. 3-[3′-(4-Nitrophenyl)-prop-2′-en-1′-yl]-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25b). Purified by FC (eluent: petroleum ether/EtOAc 5:5); yield 44%; R_f 0.32 (petroleum ether/EtOAc 5:5); IR: 1550, 1570, 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.45 (s,

9H), 1.71 (d, 1H, J = 8.0 Hz), 2.36–2.45 (m, 1H), 2.84 (d, 2H, J = 10.2 Hz), 3.00–3.36 (m, 2H), 3.38 (d, 2H, J = 6.0 Hz), 4.05–4.10 (m, 2H), 6.44 (dt, 1H, J = 6.2 and 15.8 Hz), 6.64 (d, 1H, J = 15.8 Hz), 7.47–8.20 (m, 4H). Anal. Calcd for $C_{19}H_{25}N_3O_4$: C, 63.49; H, 7.01; N, 11.69. Found: C, 63.27; H, 6.98; N, 11.28.

4.1.17.3. 3-[3′-(2-Chlorophenyl)-prop-2′-en-1′-yl]-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25c). Purified by FC (eluent: petroleum ether/EtOAc 7:3); yield 38%; R_f 0.28 (petroleum ether/EtOAc 7:3); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.73 (d, 1H, J = 8.4 Hz), 2.35–2.42 (m, 1H), 2.85 (d, 2H, J = 10.8 Hz), 2.95–3.33 (m, 2H), 3.35 (d, 2H, J = 6.1 Hz), 4.02–4.10 (m, 2H), 6.24 (dt, 1H, J = 6.6 and 15.8 Hz), 6.94 (d, 1H, J = 15.8 Hz), 7.18–7.38 (m, 4H). Anal. Calcd for $C_{19}H_{25}ClN_2O_2$: C, 65.41; H, 7.22; Cl, 10.16; N, 8.03. Found: C, 65.17; H, 7.20; Cl, 10.12; N, 8.01.

4.1.17.4. 3-[3′-(3-Chlorophenyl)-prop-2′-en-1′-yl]-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25d). Purified by FC (eluent: petroleum ether/EtOAc 6:4); yield 40%; R_f 0.32 (petroleum ether/EtOAc 6:4); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.71 (d, 1H, J = 7.4 Hz), 2.35–2.50 (m, 1H), 2.82 (d, 2H, J = 11.4 Hz), 2.95–3.25 (m, 2H), 3.33 (d, 2H, J = 6.4 Hz), 4.02–4.11 (m, 2H), 6.24 (dt, 1H, J = 6.6 and 15.8 Hz), 6.45 (d, 1H, J = 15.8 Hz), 7.16–7.34 (m, 4H). Anal. Calcd for $C_{19}H_{25}ClN_2O_2$: C, 65.41; H, 7.22; Cl, 10.16; N, 8.03. Found: C, 65.22; H, 7.20; Cl, 10.14; N, 8.00.

4.1.17.5. 3-[3′-(4-Chlorophenyl)-prop-2′-en-1′-yl]-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25e). Purified by FC (eluent: petroleum ether/EtOAc 6:4); yield 40%; R_f 0.32 (petroleum ether/EtOAc 6:4); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.44 (s, 9H), 1.74 (d, 1H, J = 7.4 Hz), 2.31–2.44 (m, 1H), 2.83 (d, 2H, J = 11.4 Hz), 2.95–3.25 (m, 2H), 3.24 (d, 2H, J = 6.4 Hz), 4.00–4.10 (m, 2H), 6.14 (dt, 1H, J = 6.6 and 15.8 Hz), 6.43 (d, 1H, J = 15.8 Hz), 7.01–7.20 (m, 4H). Anal. Calcd for $C_{19}H_{25}ClN_2O_2$: C, 65.41; H, 7.22; Cl, 10.16; N, 8.03. Found: C, 65.32; H, 7.19; Cl, 10.10; N, 8.02.

4.1.17.6. 3-(3′-Phenyl-but-2′-en-1′-yl)-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25f). Purified by FC (eluent: petroleum ether/EtOAc 6:4); yield 42%; R_f 0.27 (petroleum ether/EtOAc 6:4); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.71–1.76 (m, 1H), 2.08 (s, 3H), 2.38–2.40 (m, 1H), 2.72 (d, 2H, J = 10.6 Hz), 2.86 (d, 2H, J = 11.6 Hz), 3.36 (d, 2H, J = 7.0 Hz), 4.00–4.07 (m, 2H), 5.91 (t, 1H, J = 7.0 Hz), 7.18–7.41 (m, 5H). Anal. Calcd for $C_{20}H_{28}N_2O_2$: C, 73.14; H, 8.59; N, 8.53. Found: C, 72.88; H, 8.29; N, 8.50.

4.1.17.7. 3-[3′-(4-Chlorophenyl)-but-2′-en-1′-yl]-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25g). Purified by FC (eluent: petroleum ether/EtOAc 6:4); yield 43%; R_f 0.28 (petroleum ether/EtOAc 6:4); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.43 (s, 9H), 1.72 (d, 1H, J = 8.0 Hz), 2.05 (s, 3H), 2.35–2.40 (m, 1H), 2.71 (d, 2H, J = 10.2 Hz), 2.86 (d, 2H, J = 10.6 Hz), 3.34 (d, 2H, J = 6.6 Hz), 4.00–4.07 (m, 2H), 5.91 (t, 1H, J = 7.0 Hz), 7.17–7.40 (m, 4H). Anal. Calcd for $C_{20}H_{27}ClN_2O_2$: C, 66.19; H, 7.50; Cl, 9.77; N, 7.72. Found: C, 65.96; H, 7.47; Cl, 9.73; N, 7.69.

4.1.17.8. 3-[3′-(3,4-Dichlorophenyl)-but-2′-en-1′-yl]-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25h). Purified by FC (eluent: petroleum ether/EtOAc 7:3); yield 36%; R_f 0.21 (petroleum ether/EtOAc 7:3); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 1.45 (s, 9H), 1.68–1.75 (m, 1H), 2.03 (s, 3H), 2.34–2.41 (m, 1H), 2.85 (d, 2H, J = 10.8 Hz), 3.05 (d, 2H, J = 6.4 Hz), 3.34 (d, 2H, J = 6.4 Hz), 4.00–4.07 (m, 2H), 5.91 (t, 1H, J = 7.0 Hz), 7.23–7.45 (m, 3H). Anal. Calcd for $C_{20}H_{26}Cl_2N_2O_2$: C, 60.46; H, 6.60; Cl, 17.85; N, 7.05. Found: C, 60.25; H, 6.58; Cl, 17.79; N, 7.02.

4.1.17.9. 3-(3′-Phenyl-pent-2′-en-1′-yl)-6-*t*-butyloxycarbonyl-3,6-diazabicyclo[3.1.1]heptane (25i). Purified by FC (eluent: petroleum ether/EtOAc 6:4); yield 57%; R_f 0.27 (petroleum ether/EtOAc 6:4); IR: 1590, 1620, 1670; ^{1}H NMR ($CDCl_3$) δ 0.97 (t, 3H, J = 7.8 Hz), 1.43 (s, 9H), 1.75–1.87 (m, 1H), 2.00–2.20 (m, 3H), 2.73 (d, 2H, J = 13.4 Hz), 2.90–3.00 (m, 2H), 3.10 (d, 2H, J = 12.4 Hz), 4.06–4.08 (m, 2H), 5.91 (t, 1H, J = 7.0 Hz), 7.13–7.31 (m, 5H). Anal. Calcd for $C_{21}H_{30}N_2O_2$: C, 73.65; H, 8.83; N, 8.18. Found: C, 73.33; H, 8.79; N, 8.15.

4.1.18. General procedure for the preparation of 26a–i. A solution of **25a–i** (1.00 mmol) in CH_2Cl_2 (4 mL) at 0 °C was treated with triethylsilane (0.40 mL, 2.50 mmol), followed by trifluoroacetic acid (1.00 mL, 13.00 mmol). The solution was stirred at the same temperature for 2.5 h, and then further trifluoroacetic acid (0.54 mL, 7.00 mmol) was added. The reaction mixture was allowed to reach room temperature and stirred for 3 h. The solvent was evaporated, the residue was dissolved in 25 mL of saturated aqueous $NaHCO_3$ solution and extracted with CH_2Cl_2. The organic layer was dried over Na_2SO_4 and evaporated to afford the compounds **26a–i** as oils.

4.1.18.1. 3-Cinnamyl-3,6-diazabicyclo[3.1.1]heptane (26a). Yield quantitative; R_f 0.20 ($CHCl_3$/MeOH 9:1); IR: 1590, 1620, 3300; ^{1}H NMR ($CDCl_3$) δ 1.97 (d, 1H, J = 8.4 Hz), 2.50–2.60 (m, 1H), 2.77 (d, 2H, J = 11.2 Hz), 3.04 (br s, 1H), 3.18 (d, 2H, J = 11.2 Hz), 3.38 (d, 2H, J = 6.6 Hz), 3.69–3.75 (m, 2H), 6.29 (dt, 1H, J = 6.4 and 15.6 Hz), 6.58 (d, 1H, J = 15.6 Hz), 7.20–7.43 (m, 5H). Anal. Calcd for $C_{14}H_{18}N_2$: C, 78.46; H, 8.47; N, 13.07. Found: C, 78.19; H, 8.44; N, 13.03.

4.1.18.2. 3-[3′-(4-Nitrophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (26b). Yield 68%; R_f 0.25 ($CHCl_3$/MeOH 9:1 + gtt Et_3N); IR: 1550, 1570, 1590, 1620, 3300; ^{1}H NMR ($CDCl_3$) δ 2.04–2.05 (m, 1H), 2.17–2.18 (m, 1H), 2.85 (br s, 1H), 3.40–3.42 (m, 2H), 3.63–3.65 (m, 2H), 4.08 (d, 2H, J = 6.6 Hz), 4.25–4.30 (m, 2H), 6.45 (dt, 1H, J = 6.4 and 15.6 Hz), 6.81 (d, 1H, J = 15.6 Hz), 7.52–8.12 (m, 4H). Anal. Calcd for $C_{14}H_{17}N_3O_2$: C, 64.85; H, 6.61; N, 16.21. Found: C, 64.63; H, 6.58; N, 16.15.

4.1.18.3. 3-[3′-(2-Chlorophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (26c). Yield quantitative; R_f 0.14 ($CHCl_3$/MeOH 9:1); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 1.79–1.94 (m, 1H), 2.24 (br s, 1H), 2.59–2.67 (m, 1H), 2.75 (d, 2H, $J = 11.2$ Hz), 3.14 (d, 2H, $J = 9.6$ Hz), 3.41 (d, 2H, $J = 6.6$ Hz), 3.63–3.65 (m, 2H), 6.29 (dt, 1H, $J = 6.4$ and 15.6 Hz), 6.98 (d, 1H, $J = 15.6$ Hz), 7.12–7.37 (m, 4H). Anal. Calcd for $C_{14}H_{17}ClN_2$: C, 67.60; H, 6.89; Cl, 14.25; N, 11.26. Found: C, 67.36; H, 6.86; Cl, 14.20; N, 11.22.

4.1.18.4. 3-[3′-(3-Chlorophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (26d). Yield quantitative; R_f 0.20 ($CHCl_3$/MeOH 9:1); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 1.79–1.94 (m, 1H), 2.26–2.36 (m, 1H), 2.60–2.68 (m, 2H), 2.98 (br s, 1H), 3.22 (d, 2H, $J = 14.2$ Hz), 3.46 (d, 2H, $J = 5.8$ Hz), 4.19–4.25 (m, 2H), 6.25 (dt, 1H, $J = 6.4$ and 15.6 Hz), 6.58 (d, 1H, $J = 15.6$ Hz), 7.16–7.37 (m, 4H). Anal. Calcd for $C_{14}H_{17}ClN_2$: C, 67.60; H, 6.89; Cl, 14.25; N, 11.26. Found: C, 67.44; H, 6.85; Cl, 14.22; N, 11.24.

4.1.18.5. 3-[3′-(4-Chlorophenyl)-prop-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (26e). Yield quantitative; R_f 0.43 ($CHCl_3$/MeOH 9:1 + gtt Et_3N); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 1.65–1.76 (m, 1H), 2.35–2.41 (m, 1H), 2.58 (d, 2H, $J = 7.8$ Hz), 2.82 (d, 2H, $J = 11.4$ Hz), 3.10 (br s, 1H), 3.31 (d, 2H, $J = 5.6$ Hz), 4.00–4.10 (m, 2H), 6.25 (dt, 1H, $J = 6.4$ and 15.6 Hz), 6.51 (d, 1H, $J = 15.6$ Hz), 7.08–7.27 (m, 4H). Anal. Calcd for $C_{14}H_{17}ClN_2$: C, 67.60; H, 6.89; Cl, 14.25; N, 11.26. Found: C, 67.54; H, 6.90; Cl, 14.23; N, 11.20.

4.1.18.6. 3-(3′-Phenyl-but-2′-en-1′-yl)-3,6-diazabicyclo-[3.1.1]heptane (26f). Yield quantitative; R_f 0.37 ($CHCl_3$/MeOH 9:1); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 1.56 (br s, 1H), 1.81–1.89 (m, 1H), 2.11 (s, 3H), 2.41–2.48 (m, 1H), 2.73 (d, 2H, $J = 7.8$ Hz), 3.14 (d, 2H, $J = 10.2$ Hz), 3.40 (d, 2H, $J = 6.8$ Hz), 3.58–3.63 (m, 2H), 5.98 (t, 1H, $J = 6.4$ Hz), 7.18–7.40 (m, 5H). Anal. Calcd for $C_{15}H_{20}N_2$: C, 78.90; H, 8.83; N, 12.27. Found: C, 78.67; H, 8.80; N, 12.23.

4.1.18.7. 3-[3′-(4-Chlorophenyl)-but-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (26g). Yield quantitative; R_f 0.28 ($CHCl_3$/MeOH 9:1); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 1.84–1.86 (m, 1H), 2.07 (s, 3H), 2.37–2.40 (m, 1H), 2.73 (d, 2H, $J = 7.8$ Hz), 3.07 (br s, 1H), 3.14 (d, 2H, $J = 10.2$ Hz), 3.40 (d, 2H, $J = 6.8$ Hz), 3.63–3.66 (m, 2H), 5.96 (t, 1H, $J = 6.4$ Hz), 7.11–7.31 (m, 4H). Anal. Calcd for $C_{15}H_{19}ClN_2$: C, 68.56; H, 7.29; Cl, 13.49; N, 10.66. Found: C, 68.32; H, 7.26; Cl, 13.44; N, 10.62.

4.1.18.8. 3-[3′-(3,4-Dichlorophenyl)-but-2′-en-1′-yl]-3,6-diazabicyclo[3.1.1]heptane (26h). Yield quantitative; R_f 0.53 ($CHCl_3$/MeOH 9:1 + gtt Et_3N); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 1.85–1.90 (m, 1H), 2.06 (s, 3H), 2.54–2.65 (m, 1H), 2.76 (d, 2H, $J = 10.6$ Hz), 3.03 (br s, 1H), 3.08 (d, 2H, $J = 8.2$ Hz), 3.40 (d, 2H, $J = 6.8$ Hz), 3.60–3.69 (m, 2H), 5.98 (t, 1H, $J = 6.4$ Hz), 7.21–7.50 (m, 3H). Anal. Calcd for $C_{15}H_{18}Cl_2N_2$: C, 60.61; H, 6.10; Cl, 23.86; N, 9.43. Found: C, 60.40; H, 6.08; Cl, 23.79; N, 9.40.

4.1.18.9. 3-(3′-Phenyl-pent-2′-en-1′-yl)-3,6-diazabicyclo-[3.1.1]heptane (26i). Yield quantitative; R_f 0.53 ($CHCl_3$/MeOH 7:3 + gtt Et_3N); IR: 1590, 1620, 3300; ^1H NMR ($CDCl_3$) δ 0.97 (t, 3H, $J = 7.8$ Hz), 1.78–1.84 (m, 1H), 2.00–2.18 (m, 1H), 2.30–2.45 (q, 2H, $J = 7.4$ Hz), 2.69 (br s, 1H), 2.76–2.79 (m, 2H), 2.96 (d, 2H, $J = 5.4$ Hz), 3.13 (d, 2H, $J = 7.6$ Hz), 4.05–4.09 (m, 2H), 5.80 (t, 1H, $J = 6.4$ Hz), 7.13–7.35 (m, 5H). Anal. Calcd for $C_{16}H_{22}N_2$: C, 79.29; H, 9.15; N, 11.56. Found: C, 79.02; H, 9.12; N, 11.52.

4.1.19. General procedure for the preparation of 3Aa–i. To a solution of **26a–i** (1.60 mmol) in CH_2Cl_2 (30 mL) at 0 °C was added a solution of propionic anhydride (0.72 mL, 5.60 mmol) in CH_2Cl_2 (6 mL). When addition was complete, the mixture was refluxed for 1 h. After cooling at room temperature, the mixture was made alkaline with 40% NaOH and stirred overnight. The reaction mixture was extracted with CH_2Cl_2, the organic layers were dried over Na_2SO_4, evaporated, and the residue was purified by FC (eluent: CH_2Cl_2/acetone 7:3) to afford the compounds **3Aa–i** as oils. All final compounds were converted into the HCl or $HO_2CCH{=}CH$-CO_2H salts.

4.1.19.1. 3-Cinnamyl-6-propionyl-3,6-diazabicyclo[3.1.1]-heptane (3Aa). Yield 75%; R_f 0.32 (CH_2Cl_2/acetone 7:3); mp 191–193 °C (as hydrochloride); IR: 1590, 1620, 1690; ^1H NMR ($CDCl_3$) δ 1.14 (t, 3H, $J = 7.6$ Hz), 1.97–3.15 (m, 8H), 3.32 (d, 2H, $J = 6.6$ Hz), 4.20–4.30 (m, 1H), 4.30–4.40 (m, 1H), 6.25 (dt, 1H, $J = 6.4$ and 15.6 Hz), 6.54 (d, 1H, $J = 15.6$ Hz), 7.17–7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 9.26, 25.68, 27.87, 52.17, 54.41, 58.21, 58.53, 61.30, 126.19, 126.32, 127.39, 128.45, 132.55, 136.74, 173.26. Anal. Calcd for $C_{17}H_{22}N_2O$: C, 75.52; H, 8.20; N, 10.36. Found: C, 75.44; H, 8.16; N, 10.30.

4.1.19.2. 3-[3′-(4-Nitrophenyl)-prop-2′-en-1′-yl]-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ab). Yield 74%; R_f 0.24 (CH_2Cl_2/acetone 7:3); mp 170–172 °C (as fumarate); IR: 1590, 1620, 1650; ^1H NMR ($CDCl_3$) δ 1.15 (t, 3H, $J = 7.0$ Hz), 2.06–2.19 (m, 2H), 2.25–2.38 (m, 2H), 2.80–3.18 (m, 4H), 3.38 (d, 2H, $J = 6.0$ Hz), 4.30–4.42 (m, 2H), 6.40 (dt, 1H, $J = 6.6$ and 15.8 Hz), 6.62 (d, 1H, $J = 15.8$ Hz), 7.47–8.20 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 9.33, 25.79, 29.69, 52.20, 54.63, 58.52, 61.95, 124.00, 126.77, 128.82, 130.39, 130.89, 131.95, 173.57. Anal. Calcd for $C_{17}H_{21}N_3O_3$: C, 64.74; H, 6.71; N, 13.32. Found: C, 64.66; H, 6.69; N, 13.30.

4.1.19.3. 3-[3′-(2-Chlorophenyl)-prop-2′-en-1′-yl]-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ac). Yield 60%; R_f 0.30 (CH_2Cl_2/acetone 7:3); mp 149–151 °C (as hydrochloride); IR: 1590, 1620, 1650; ^1H NMR ($CDCl_3$) δ 1.13 (t, 3H, $J = 7.6$ Hz), 1.90–3.19 (m, 8H), 3.36 (d, 2H, $J = 7.8$ Hz), 4.20–4.40 (m, 2H), 6.21 (dt, 1H, $J = 6.6$ and 15.8 Hz), 6.92 (d, 1H, $J = 15.8$ Hz), 7.10–7.51 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 9.13, 25.57, 27.89, 51.97, 54.18, 57.89, 58.41, 61.17, 126.64, 126.68, 128.29, 128.56, 129.25, 129.42, 132.60, 134.81, 173.20. Anal. Calcd for

$C_{17}H_{21}ClN_2O$: C, 66.99; H, 6.94; Cl, 11.63; N, 9.19. Found: C, 66.75; H, 6.90; Cl, 11.60; N, 9.18.

4.1.19.4. 3-[3′-(3-Chlorophenyl)-prop-2′-en-1′-yl]-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ad). Yield 50%; R_f 0.32 (CH_2Cl_2/acetone 7:3); mp 202–204 °C (as fumarate); IR: 1595, 1644, 1644; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.6 Hz), 1.32–1.42 (m, 1H), 1.72–1.85 (m, 1H), 2.14 (q, 2H, J = 7.6 Hz), 2.46–3.10 (m, 4H), 3.32 (d, 2H, J = 6.6 Hz), 4.06–4.10 (m, 2H), 6.20 (dt, 1H, J = 6.6 and 15.8 Hz), 6.44 (d, 1H, J = 15.8 Hz), 7.11–7.28 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 9.30, 25.76, 27.96, 52.18, 54.48, 58.47, 58.53, 61.31, 124.47, 126.17, 127.37, 128.12, 128.53, 128.79, 129.73, 131.21, 173.35. Anal. Calcd for $C_{17}H_{21}ClN_2O$: C, 66.99; H, 6.94; Cl, 11.63; N, 9.19. Found: C, 66.80; H, 6.92; Cl, 11.60; N, 9.17.

4.1.19.5. 3-[3′-(4-Chlorophenyl)-prop-2′-en-1′-yl]-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ae). Yield quantitative; R_f 0.20 (CH_2Cl_2/acetone 7:3); mp 113–115 °C (as fumarate); IR: 1590, 1620, 1650; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.6 Hz), 1.32–1.42 (m, 1H), 1.63–1.65 (m, 1H), 2.06 (q, 2H, J = 7.6 Hz), 2.40–2.60 (m, 4H), 3.32 (d, 2H, J = 6.6 Hz), 4.06–4.11 (m, 2H), 6.19 (dt, 1H, J = 6.6 and 15.8 Hz), 6.43 (d, 1H, J = 15.8 Hz), 7.11–7.28 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 9.03, 25.76, 27.69, 52.18, 54.48, 58.13, 58.56, 61.34, 127.16, 127.45, 128.34, 128.66, 128.75, 128.79, 129.76, 130.87, 173.20. Anal. Calcd for $C_{17}H_{21}ClN_2O$: C, 66.99; H, 6.94; Cl, 11.63; N, 9.19. Found: C, 66.78; H, 6.92; Cl, 11.61; N, 9.16.

4.1.19.6. 3-(3′-Phenyl-but-2′-en-1′-yl)-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Af). Yield quantitative; R_f 0.26 (CH_2Cl_2/acetone 7:3); mp 245–246 °C (as fumarate); IR: 1590, 1620, 1690; ^{1}H NMR ($CDCl_3$) δ 1.14 (t, 3H, J = 7.6 Hz), 1.61–1.65 (m, 5H), 2.04–2.08 (m, 1H), 2.30–2.41 (m, 1H), 2.70–3.11 (m, 4H), 3.35 (d, 2H, J = 6.0 Hz), 4.20–4.40 (m, 2H), 6.25 (t, 1H, J = 6.4 Hz), 7.17–7.39 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 9.28, 19.13, 25.74, 27.71, 29.67, 51.99, 54.53, 58.35, 61.17, 122.85, 126.90, 127.70, 128.36, 130.89, 173.26. Anal. Calcd for $C_{18}H_{24}N_2O$: C, 76.02; H, 8.51; N, 9.85. Found: C, 75.75; H, 8.48; N, 9.81.

4.1.19.7. 3-[3′-(4-Chlorophenyl)-but-2′-en-1′-yl]-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ag). Yield 50%; R_f 0.45 (CH_2Cl_2/acetone 7:3); mp 67–68 °C (as fumarate); IR: 1590, 1620, 1645; ^{1}H NMR ($CDCl_3$) δ 1.13 (t, 3H, J = 7.6 Hz), 1.61–1.65 (m, 3H), 2.04 (s, 3H), 2.37–2.42 (m, 1H), 2.50–3.10 (m, 4H), 3.35 (d, 2H, J = 6.4 Hz), 4.20–4.25 (m, 2H), 5.88 (t, 1H, J = 6.4 Hz), 7.08–7.32 (m, 4H); ^{13}C NMR ($CDCl_3$) δ 9.21, 19.05, 25.67, 28.80, 53.98, 54.33, 58.38, 58.47, 61.15, 124.52, 126.85, 128.70, 130.78, 132.30, 136.70, 173.18. Anal. Calcd for $C_{18}H_{23}ClN_2O$: C, 67.81; H, 7.27; Cl, 11.12; N, 8.79. Found: C, 67.57; H, 7.25; Cl, 11.10; N, 8.76.

4.1.19.8. 3-[3′-(3,4-Dichlorophenyl)-but-2′-en-1′-yl]-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ah). Yield quantitative; R_f 0.23 (CH_2Cl_2/acetone 7:3); mp 85–87 °C (as fumarate); IR: 1590, 1620, 1645; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, 3H, J = 7.2 Hz), 1.74 (s, 3H), 1.98–2.10 (m, 4H), 2.37–2.45 (m, 2H), 2.60–2.75 (m, 2H), 3.04 (d, 2H, J = 7.8 Hz), 4.20–4.30 (m, 2H), 5.91 (t, 1H, J = 6.4 Hz), 7.08–7.42 (m, 3H); ^{13}C NMR ($CDCl_3$) δ 9.30, 19.15, 25.72, 28.20, 53.72, 54.20, 54.57, 58.58, 61.29, 124.97, 127.31, 127.60, 128.82, 129.93, 130.08, 130.26, 130.90, 173.28. Anal. Calcd for $C_{18}H_{22}Cl_2N_2O$: C, 61.19; H, 6.28; Cl, 20.07; N, 7.93. Found: C, 60.97; H, 6.26; Cl, 20.00; N, 7.91.

4.1.19.9. 3-(3′-Phenyl-pent-2′-en-1′-yl)-6-propionyl-3,6-diazabicyclo[3.1.1]heptane (3Ai). Yield 93%; R_f 0.48 (CH_2Cl_2/acetone 7:3); mp 132–134 °C (as fumarate); IR: 1590, 1620, 1690; ^{1}H NMR ($CDCl_3$) δ 0.98 (t, 3H, J = 7.6 Hz), 1.12 (t, 3H, J = 7.6 Hz), 1.80 (d, 1H, J = 6.8 Hz), 1.90–2.20 (m, 4H), 2.30–2.40 (m, 1H), 2.65–3.18 (m, 6H), 4.21–4.38 (m, 2H), 5.80 (t, 1H, J = 6.4 Hz), 7.11–7.35 (m, 5H); ^{13}C NMR ($CDCl_3$) δ 9.30, 10.93, 25.72, 27.23, 28.41, 51.99, 54.54, 58.49, 61.33, 123.81, 126.08, 126.47, 128.16, 138.71, 143.09, 173.04. Anal. Calcd for $C_{19}H_{26}N_2O$: C, 76.47; H, 8.78; N, 9.39. Found: C, 76.20; H, 8.74; N, 9.36.

4.2. Biology

4.2.1. General information. Male Albino CD1 mice weighing 26–30 g (Charles River, Italy) were used.

Animals were kept on a 12 h artificial light/dark cycle (lights on at 7:00 a.m.) at a constant temperature of 22 ± 2 °C and relative humidity of 60%. Food and water were available ad libitum.

All testing was performed according to the recommendations and policies of the National Institutes of Health (USA) Guidelines for the Use of Laboratory Animals.

[D-Ala^2,*N*-Me-Phe^4,Gly-ol^5]-enkephalin ([^{3}H]DAMGO), [D-Ala^2,D-Leu^5]-enkephalin ([^{3}H]DADLE) and [^{3}H]bremazocine were purchased from NEN (Life Science Products, Boston, Maryland, USA).

4.2.2. Opioid binding assay. Ligand binding assays were determined for compounds at μ-, δ- and κ-opioid receptors as described in detail elsewhere.[14] Binding affinities for μ-, δ-, and κ-receptors were determined by displacing, respectively, [^{3}H]DAMGO (1 nM), [^{3}H]DADLE (1 nM) and [^{3}H]bremazocine (1 nM) from mouse brain membrane binding sites. Brain membranes were incubated with the appropriate ^{3}H-ligand in 50 mM Tris–HCl buffer, pH 7.4, at 25 °C for 60 min in the absence or presence of 10 μM naloxone. [^{3}H]Bremazocine binding was carried out in the presence of unlabelled DAMGO (100 nM) and DADLE (100 nM) to prevent the binding at μ, δ sites. IC_{50} values were determined from log dose-displacement curves, and K_i values were calculated from the obtained IC_{50} values by means of the equation of Cheng and Prusoff,[15] using values of 1.03, 1.45 and 0.5 nM for the dissociation constants of [^{3}H]DAMGO, [^{3}H]DADLE and [^{3}H]bremazocine, respectively.

4.3. Molecular modelling

4.3.1. Computational methods. All calculations were carried out using the Gaussian 03[16] program package. The

conformational space of compounds **18**, **22**, **30**, **31**, **3Aa**, **3Ba** and **3Bh** was explored using systematic variation at each rotatable bond in order to generate all possible conformation which were optimized at the B3LYP level with the 6-31G* basis set.[9] Also the ring flexibility of the bicyclic moiety was considered by generation of geometries with all the conceivable puckerings of the rings, followed by optimization. While **18** and **22** were modelled as neutral molecules, the other compounds were modelled as cations in their protonated forms. The energies of the conformers so obtained were recalculated with the C-PCM approach[10] as implemented in Gaussian 03.

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