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Amino-benzosuberone: A novel warhead for selective inhibition of human aminopeptidase-N/CD13

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ABSTRACT

This paper describes the design and synthesis of compounds belonging to a novel class of highly selective mammalian CD13 inhibitors. Racemic homologues of 3-amino-2-tetralone **1** were synthesised and evaluated for their ability to selectively inhibit the membrane-bound, zinc-dependent aminopeptidase-N/CD13 (EC 3.4.11.2). Some of these novel non-peptidic compounds are potent, competitive inhibitors of the mammalian enzyme, with K_i values in the low micromolar range in spite of their minimal size (MW <200 Da). Moreover, they show an interesting selectivity profile against representative members of the aminopeptidase family, that is leucine aminopeptidase (EC 3.4.11.1), *Aeromonas proteolytica* aminopeptidase (EC 3.4.11.10) and the aminopeptidase activity of leukotriene A4 hydrolase (EC 3.3.2.6). The amino-benzosuberone derivative **4** is the most promising compound in terms of potency, stability and selectivity. A hypothetical binding mode of **4** to the catalytic zinc and several conserved active site residues is proposed, based on the observed structure–activity relationships, structural insights from aminopeptidase-N homologues of known three-dimensional structure.

1. Introduction

Aminopeptidases constitute a large family of proteolytic enzymes that affect the maturation, degradation and regulation of proteins and oligopeptides. Aminopeptidase-N (CD13/APN) is a multifunctional ectoenzyme widely expressed in many cells and tissues, suggesting a variety of biological functions depending on its location.[1] Among its well-established roles, CD13/APN modulates the activity of numerous peptides that participate in important biological processes. For example, it regulates the metabolism of leu- and met-enkephalins,[2] angiotensins,[3] kinins[4,5] and a number of cytokines or chemokines.[6,7] Additionally, CD13/APN is involved in signal transduction and cell motility, proliferation and differentiation.[8–12] Worth of note, APN expression is deregulated in inflammatory diseases and in cancer.[9–11] In view of the potential therapeutic relevance of some of the CD13/APN functions, the rational design of potent and selective CD13/APN inhibitors is of considerable interest but remains a challenging endeavor.

CD13/APN belongs to the M1 family of zinc metallopeptidases with one essential catalytic zinc ion. As an enzyme, APN removes the aminoterminal amino acid from unsubstituted oligopeptides. As a membrane-bound protein, CD13 is well-known as a viral receptor, and virus binding does not appear to be affected by active site directed APN inhibitors. Nevertheless, there seems to be an interplay between the enzymatic activity-dependent and -independent functions of CD13/APN.[1] Unfortunately, the exact relationship between the enzymatic and non enzymatic roles of CD13 was so far obscured basically by the fact that, although many inhibitors of aminopeptidases are available most of them are poorly selective. Recent outstanding reviews summarized the main achievements in this field.[13] Among the most active compounds one can bring up Gallic acid derivatives and pseudo peptides that used the concept of transition state analogues, such as α-boronic acids, sulfonamide and phosphonic surrogates of the scissile peptide bond. Natural products such curcumin[14] and psammaplin A[15] or synthetic flavonoid[16] derivatives were also worthy of note, as well as highly potent inhibitors related in structure to hydroxamic acids derivatives.[17] However, the selectivity of this large diversity of APN inhibitors towards other metallo-aminopeptidases has not always been assessed or proved to be modest.

The rational design of truly selective APN/CD13 inhibitors is far from obvious owing to the very high structural similarity of the many zinc-dependent aminopeptidases. The problem of achieving high selectivity is further compounded by the relatively broad substrate specificity displayed by these enzymes.

We have already shown that 3-amino-2-tetralone **1** is an interesting specific inhibitor of mammalian APN with a significant selectivity towards the M1 subfamily of one-zinc aminopeptidases,

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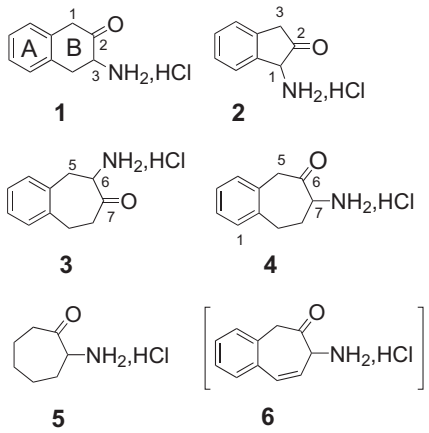
as opposed to the M17 subfamily that require co-catalytic metal ions for activity.[18,19] Nevertheless, we have found that the stability of this compound in aqueous solution at physiological pH is limited, with an approximate half life of 2 h. We thus wondered whether one could improve stability and potency with either more rigid or more flexible analogues. We have shown in our previous work,[19] that the free amine in position 3 as well as a carbonyl group in position 2 are essential features for the inhibitory activity, presumably as surrogates for the free amino terminus and the carbonyl group of the scissile bond, respectively, of peptide substrates.

In the present work, we have studied the influence of the size of cycle B of **1** on the stability, the inhibitory potency and the selectivity against a panel of representative aminopeptidases.[20] A comprehensive list of aminobenzocyclanones synthesised during the course of this work is reported in Scheme 1, including the monocyclic aminocycloheptanone **5**. All compounds were evaluated against human, porcine and murine aminopeptidase-N/CD13 (EC 3.4.11.2), bovine lens leucine aminopeptidase (EC 3.4.11.1), *Aeromonas proteolytica* aminopeptidase (EC 3.4.11.10) and the aminopeptidase activity of human leukotriene A4 hydrolase (EC 3.3.2.6). The amino-benzocycloheptenone (amino-benzosuberone) derivative **4** turned out to be the most promising analogue with much improved stability and selectivity.

The three-dimensional structure of CD13/APN has not been elucidated yet. However, in spite of a relatively low overall amino acid sequence identity (between 20% and 30%), the mammalian enzyme is expected to share substantial structural homology with the known *Escherichia coli*[21] or *Neisseria meningitidis*[22] APNs, as well as with the human leukotriene A4 hydrolase,[23] most particularly within the active site region. In support of this view, all these enzymes are inhibited to the same extent by the most widely used, non-specific inhibitor and natural compound bestatin.[13] Furthermore, these APs use a set of conserved residues for binding the free N-terminal amino group of their substrate and the zinc ion essential for catalysis. This structural homology combined with the shared catalytic mechanism, allows us to propose for the amino-benzosuberone **4** a plausible binding mode to the catalytic zinc of the mammalian APN.

2. Chemistry

The synthesis of the 3-aminotetralone **1** was already reported.[18,19] In contrast, 1-aminoindan-2-one **2** and the aminobenzosuberone derivatives **3**–**6** were prepared according to different routes.



Scheme 1.

2.1. Amino-indanone 2

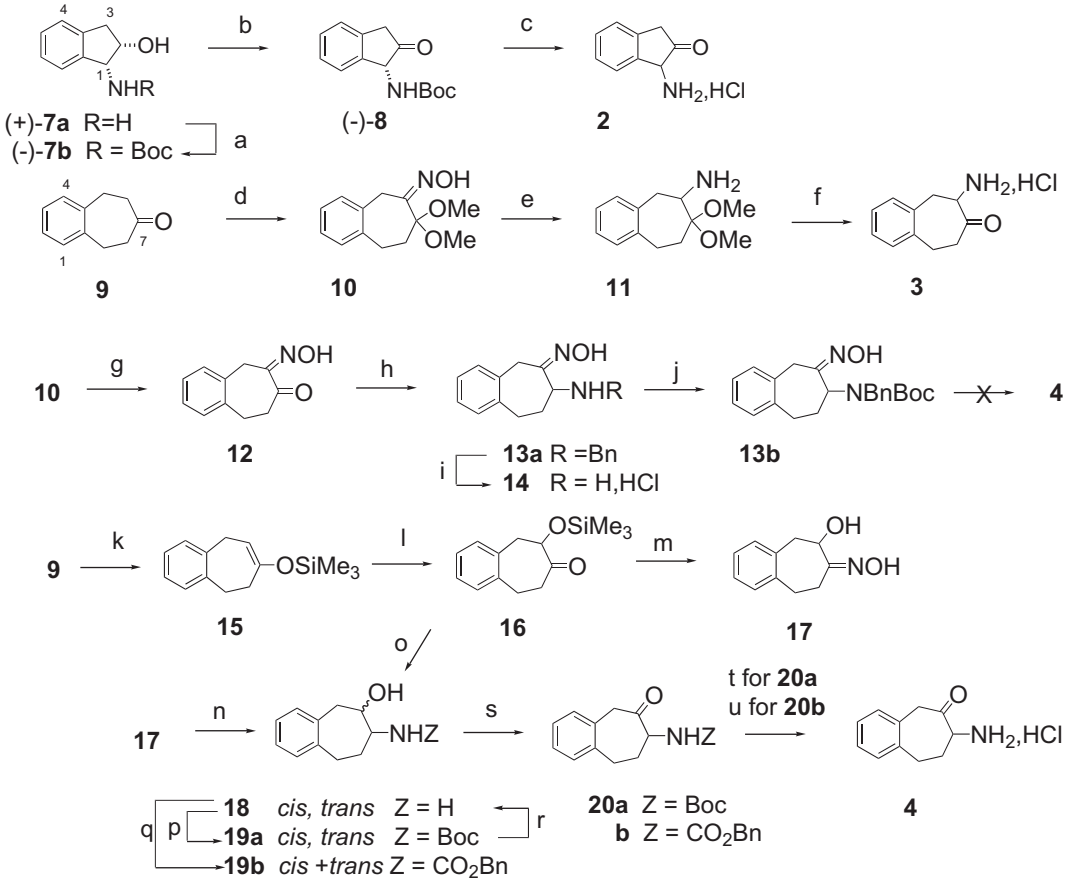
1-Aminoindan-2-one **2** was synthesised from the commercially available chiral *cis* amino-alcool (+)-**7a** in a three steps procedure (Scheme 2). Selective N-protection was first achieved using *tert*-butoxycarbonyl anhydride in methanol as for its known enantiomer.[24] The obtained amido-alcohol (−)-**7b** was then smoothly oxidized with Dess–Martin periodinane (DMP)[25] into the N-protected amino-indanone (−)-**8** in good yield. The compound slowly racemises in solution (see below). Acidic deprotection with dry HCl in ether provided the racemic aminoketone **2** as its crystalline hydrochloride salt. This sequence afforded the desired five-membered aminoketone **2** in 47% yield from (+)-**7a**.

2.2. Benzosuberones 3 and 4

The simplest member in this series was obtained from the known tetrahydrobenzocycloheptenone **9**[26–28] through α-nitrosation and reduction. Nitrosation adjacent to the keto group of **9** with *tert*-butyl nitrite in methanol[29] gave directly the ketal-oxime **10**. Reduction of the oxime function with Raney-nickel in the presence of aqueous ammonia gave the corresponding amino-ketal **11** in high yield. Hydrolysis of this crude amine with aqueous hydrochloric acid regenerated the keto group, yielding the amino-ketone **3** as its hydrochloride salt. This three steps sequence provided the desired 6-aminobenzosuberone **3** with a good overall yield (45%) from the benzosuberone **9** (Scheme 2).

The ketal-oxime **10** could be a convenient entry for the preparation of the isomeric 7-aminobenzosuberone **4** and its oxime **14**, through reductive amination and eventually oxime cleavage. Aqueous hydrolysis of the acetal group according to[29] gave the known keto-oxime **12**. Reductive amination of the ketone by imination with benzylamine in pyridine followed by reduction with sodium borohydride led to the N-benzylated amino-oxime **13a** in high yield. N-deprotection by hydrogenolysis over Pd–C in the presence of hydrochloric acid led to the amino-oxime as its hydrochloride salt **14** in high yield. However, all attempts to approach the target ketone **4** by hydrolyse or oxidise the oxime group, either from the N-diprotected derivative **13b** or from the monoprotected **13a**, yielded only unsatisfactory results.

Another route was thus planed from benzosuberone **9** through α-hydroxylation and amination (Scheme 2). α-Hydroxylation of a ketone could be achieved by epoxidation of the corresponding silyl enol ether. Thus, **9** was converted to its silyl enol ether **15** using trimethylsilyl chloride or triflate under basic conditions.[30] Oxidation with *m*-CPBA[31] gave the α-siloxyketone **16** in quantitative yield. Sensitive silylated compounds were not purified and the conversion of carbonyl to amino group was achieved by reduction of its oxime or by reductive amination. The first route started from the ketoxime **17**, easily obtained from the siloxyketone **16** under conventional conditions. Reduction over Raney-nickel provided the aminoalcohol **18**, which was directly N-protected as *tert*-butyloxycarbonyl or benzyloxycarbonyl derivatives, **19a** or **19b**, respectively. Both compounds were obtained as *cis*–*trans* mixtures in ca 35% overall yield from **9**. On the other hand, reductive amination of the siloxyketone **16** was readily performed upon treatment with tetra*iso*propyl titanate and dry ammonia in methanol, with a subsequent sodium borohydride reduction.[32] This reaction directly gave the amino-alcohol **18** in reasonable yields. N-protection gave the carbamates **19a,b**, again as *cis*–*trans* mixtures (ca 52% from **9**). The *cis* and *trans* isomers of the Boc-amide **19a** can be separated by chromatography. Their acidic deprotection was a good way to obtain separately both isomers, *cis*-**18**·HCl and *trans*-**18**·HCl, of the amino-alcohol. The oxidation of the alcohol function in **19a**,**b** with Dess–Martin periodinane[25] afforded the N-protected aminoketones **20a**,**b**. Deprotection of **20a** with dry hydrochloric acid in



Scheme 2. Reagents and conditions: (a) Boc_2O, MeOH, 5.5 h, rt, quant.; (b) DMP, CH_2Cl_2, 7 h, rt, 69%; (c) dry HCl 2.2 M in Et_2O, dioxane, rt, 72 h, 69%; (d) *t*BuONO, HCl, MeOH, 0 °C, 66%; (e) H_2/Ra-Ni, concd NH_4OH, EtOH, rt, 95%; (f) 1 N aq HCl, MeOH, rt, 90%; (g) aq HCl 6 N, Et_2O, 15 min, 0 °C, 72%; (h) $BnNH_2$, pyridine, 6 h, rt, then $NaBH_4$, MeOH, 1 h, rt, 93%; (i) H_2/Pd–C, EtOH, aq HCl 1 N, rt, 75%; (j) $ClCO_2Bn$, aq Na_2CO_3, THF, 24 h, rt, 64%; (k) Me_3SiCl, DBU, CH_2Cl_2, quant.; or Me_3SiOTf, NEt_3, toluene, 85 °C, 2 h, quant.; (l) *m*-CPBA, CH_2Cl_2, 0 °C, 2 h, quant.; (m) $NH_2OH{\cdot}HCl$, pyridine, rt, 3 h, 64%; (n) H_2/Raney-Ni, EtOH, concd NH_4OH, rt, 2.5 h; (o) $Ti(OiPr)_4$, 2–3 M NH_3 in MeOH, 6 h, then $NaBH_4$, 2 h; (p) Boc_2O, Na_2CO_3, MeOH, 2 h, rt, 56% yield from **17**, or Boc_2O, MeOH, rt, 53% yield from **16**; (q) $ClCO_2Bn$, Et_3N or Na_2CO_3, THF, rt, 52–54% yield from **16** or **17**; (r) dry HCl 2 M in Et_2O, dioxane, rt, 48 h, 85% for *cis*-isomer, quant. for *trans*-isomer (as HCl salt); (s) DMP, CH_2Cl_2, 77% and 92% yield, respectively, from **19a** or **19b**; (t) dry HCl 2.2 M in Et_2O, dioxane, rt, 72 h, 85%; (u) H_2/Pd–C, aq HCl, EtOH, rt, 60%.

ether or of **20b** by hydrogenolysis in ethanolic hydrochloric acid provided the desired aminoketone **4** as its hydrochloride salt with good overall yield of 25% or 40%, respectively, from the ketone **9**.

2.3. Monocyclic amino-suberone 5

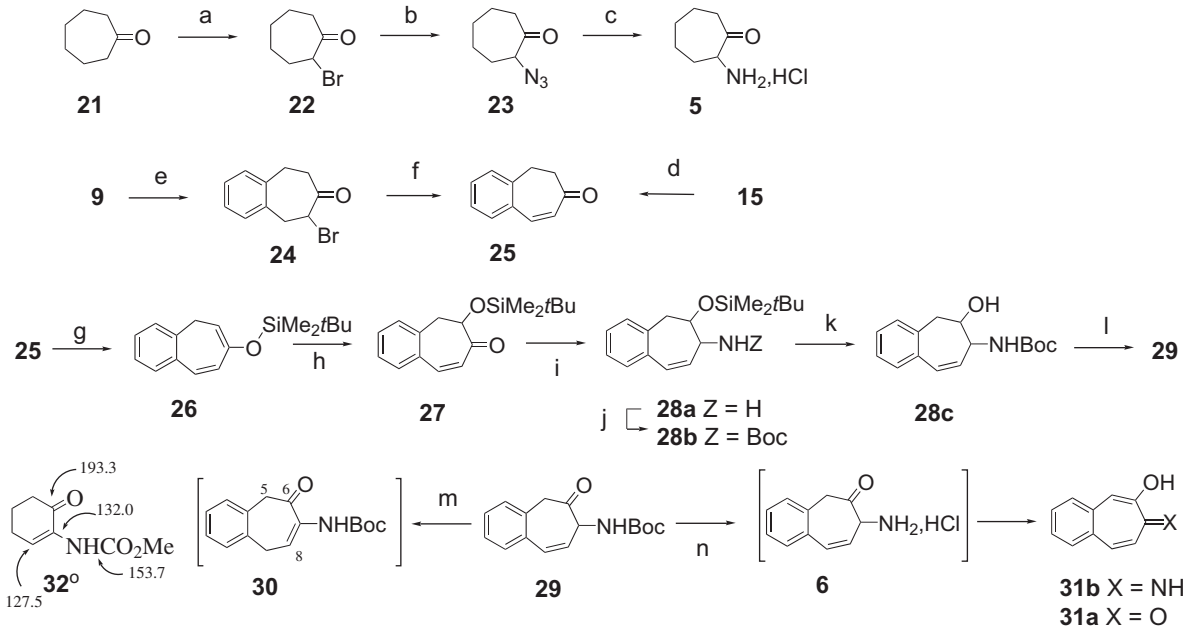
The known azido-compound **23**[33] was prepared by α-functionalisation of the suberone **21**, successively by oxidation with NBS into the α-bromoketone **22**[34] according to[35] and, directly without purification, by nucleophilic substitution with sodium azide[36] in DMF (Scheme 3). The reduction of the azide function of **23** by hydrogenolysis over Pd/C in the presence of hydrochloric acid provided the amino-suberone **5** as hydrochloride with a 50% overall yield from the ketone **21**.

2.4. Unsaturated benzocycloheptanone 6

The synthesis of the unsaturated analogue **6** of the aminobenzosuberone **4** was attempted from the ketone **9** through a similar route using the known enone **25**[37] as intermediate (Scheme 3). Enones could be obtained from the corresponding ketones either through palladium catalysed dehydrosilylation of the corresponding silyl enol ether or more classically through α-bromination and elimination. From the silyl enol ether **15** (cf above), the Pd-catalysed enone formation was first explored. In the presence of *para*-benzoquinone[38] or oxygen,[39] palladium acetate catalysed dehydrosilylation, nevertheless the enone yields proved non-reproducible (45–85%). Thus, the α-bromoketone **24** was quantitatively prepared either from the silyl enol ether **15** with NBS,[40] or directly from the ketone **9** with NBS in the presence of trimethylsilyl triflate.[41] It was submitted to DBU and HBr elimination gave the desired enone **25** in 80% overall yield from **9** in a one pot procedure.

To introduce the amine and ketone groups, we relied on the same α-hydroxylation, reductive amination, oxidation sequence successfully applied to the synthesis of **4**. The silyloxyenone **27** was obtained from the enone **25** upon silylation into the dienol ether **26** and *m*-CPBA oxidation, as before. However, stability problems occurred with the trimethylsilyl ether and the *tert*-butyldimethylsilyl enol ether was preferred. The yield was nevertheless lower than before in this case (70% vs quant.). In contrast, reductive amination proved very efficient and provided the expected siloxyamine **28a** which was directly N-protected into the O,N-diprotected aminoalcohol **28b** as a *cis–trans* 80:20 mixture. Desilylation under conventional conditions led to **28c**, which was further oxidised with Dess–Martin periodinane. This sequence provided the N-protected unsaturated aminoketone **29** in 34% overall yield from the enone **25**.

Unfortunately, this N-protected aminoketone **29** proved very sensitive both to basic or acid conditions (Scheme 3). Minute amounts of triethylamine in a $CDCl_3$ solution of **29** promoted



Scheme 3. Reagents and conditions: (a) NBS, cat. *p*-TsOH, rt, 2.5 h, quant.; (b) NaN_3, DMF, rt, 30 min, quant.; (c) H_2-Pd/C, rt, 16 h, 50% overall yield from **21**; (d) $Pd(OAc)_2$, benzoquinone, MeCN, 3 d, rt, 45–75% or $Pd(OAc)_2$, O_2, DMSO, 16 h, rt, 45–85%; (e) cat. Me_3SiOTf, NBS, MeCN, rt, quant.; (f) DBU, MeCN, rt, 80% over two steps; (g) $Me_2tBuSiCl$, DBU, CH_2Cl_2, 3 h, rt or $Me_2tBuSiOTf$, NEt_3, rt, 3 h; (h) *m*-CPBA, CH_2Cl_2, 0 °C, 16 h, 75–77% over two steps; (i) $Ti(OiPr)_4$, NH_3 in MeOH, 16 h, then $NaBH_4$, 2 h, rt, quant.; (j) Boc_2O, Na_2CO_3, MeOH, 16 h, rt, quant.; (k) Bu_4NF, THF, rt, quant.; (l) DMP, CH_2Cl_2, rt, 70–84%; (m) NEt_3, $CDCl_3$, rt; (n) aq 2 N HCl; (o) ^{13}C NMR values.

immediately a double bond migration, isomerising **29** into the α-amino-enone **30**. Its planar structure was clearly supported by NMR data, with, for example, a singlet for the 5-methylene adjacent to the keto group, a triplet for the vinylic proton H-8 coupled with a doublet for the 9-methylene. The ^{13}C NMR values for C(6), C(7) and C(8) (at δ 190, 133 and 126 ppm) were consistent with the corresponding data for the known cyclohexane analogue **32**[42] (Scheme 3).

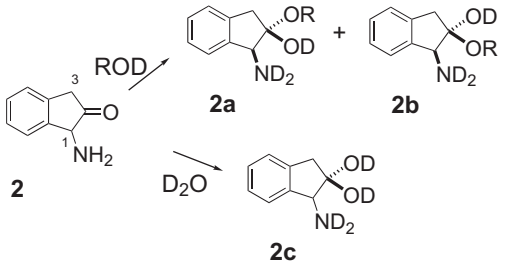
Attempts to obtain the aminoketone **6** by deprotection of **29** in aqueous 2 N hydrochloric acid gave an aromatised product, assumed to be the benzotropolone imine **31b**. This product proved relatively unstable and had only been characterised in solution by NMR (in H_2O to avoid deuteration problems). In comparison to the NMR data of the known benzotropolone **31a**, which was synthesised according to,[43] the data of **31b** were very similar, with slightly different δ-values and identical coupling values.

3. Reactivity of the carbonyl function in deuterated solvents

The reactivity of the carbonyl function of the tetralone **1**, amino-indanone **2**, and benzosuberone **4** derivatives has been studied in hydroxylated deuterated solvents by addition reaction, isotopic exchange and self condensation.

3.1. Addition reactions

The indanone derivative **2** slowly evolved in deuterated water or methanol. It was converted to the corresponding hydrate and hemiacetals (Scheme 4). In CD_3OD ^1H NMR spectra, the singlet at 5.06 ppm corresponding to the proton H-1 in α-position of the amino and the carbonyl groups decreased to the benefit of new peaks at 4.53 and 4.39 ppm. Similarly, the AB system of the CH_2(3) group was replaced by new sets of shielded signals. These shifts clearly indicated the disappearance of the carbonyl unit and of its anisotropy. These new signal sets corresponded to the isomeric hemiketals **2a** and **2b**. At equilibrium, the mixture contained the three components in a 24:33:43 ratio, the minor compound being the ketone. Interestingly, the hemiacetals were already detectable after 10 min, revealing the sensitivity of the carbonyl group to nucleophilic addition. In deuterated water, the hydrate **2c** was formed immediately and at equilibrium, a 60:40 mixture was observed with the hydrate form being the minor one.



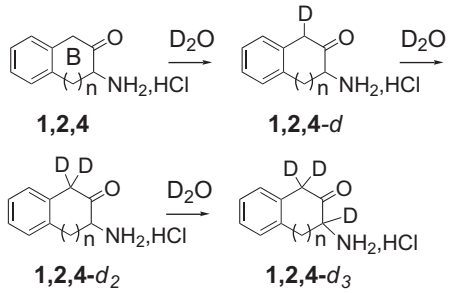
Scheme 4. Addition reactions to the carbonyl function of amino indanone **2**, in deuterated solvents (CD_3OD and D_2O).

For analogs **1** and **3** with extended six- and seven-membered rings B, respectively, similar addition reactions were observed. Nevertheless as expected, the equilibrium was clearly in favor of the ketone form, with a ketone/hydrate ratio of 80:20, due to a less constrained (rigid) cycle.

3.2. Keto-enol tautomerisation

This family of compounds was also slowly deuterated at adjacent positions to the carbonyl group (Scheme 5). Enolisation was obviously responsible for this process. Monitoring the evolution of these compounds **1**, **2** and **4** as hydrochloride in D_2O at pH 6 by ^1H NMR allowed the estimation of enolisation rates through deuteration.

Not so surprisingly, the deuteration rate proved different depending on the proton position around the carbonyl function and on the size of the saturated B cycle, it followed the general rules for enolisation (Table 1).



Scheme 5. Proton exchanges at adjacent positions to the carbonyl group.

Another result illustrating our comments, dealt with the synthetic pathway used to produce the amino-indanone **2**. The synthesis of this compound was carried out from the commercially available chiral aminoalcool **7a** (Scheme 2). The indanone ketoamide intermediate **8**, was still chiral, enolisation was then directly observed in chloroform solution and led to a spontaneous racemisation of position 1 bearing the amino group. As expected this racemisation was slow, but significant and measured by the decrease of the $[\alpha]_D$ value. The half-time of this reaction was circa 4 days at 20 °C.

For the β-tetralone derivative **1**, as expected, deuteriation of H-3, position noted γ, to give **1**-d_3 is much slower since we do not form any conjugated system with the aromatic ring A. For compounds **4**, in water at pH 6 the stability is even better since we had to increase the temperature up to 60 °C to observe any proton exchange either for H-1 in α-position or H-7 in γ-position. Not so surprisingly, under physiological conditions at pH 7.5, we have shown that deuteriation is much faster and occurs even at 20 °C.

3.3. Dimerisation at mM concentration

These stability experiments were only performed in the case of the benzosuberone **4**. We found that, in aqueous solution at pH 7.5, it precipitated with a half-time of ca 1 h at a concentration of 2.5 mM. The ^{1}H NMR spectrum in $CDCl_3$ of this precipitate was similar to the starting isomer **4** but with a clear loss of the coupling *J*(7,NH). It was in agreement with the dimeric structure **33a** (Scheme 6). The axial position of the tertiary protons H-6a and H-14a, characterised by a large coupling with one of the neighbouring protons, corresponded rather to a *trans*-arrangement in which the dihydropyridazine ring was in a more stable half-chair conformation. A spontaneous aromatisation transformed **33a** to the symmetrical pyrazine **34a** (one night at room temperature in solution). The ^{13}C NMR showed two quaternary signals at ca 149 ppm, which corresponded to the mentioned structure, by comparison with the tetramethylpyrazine **35** (δ ca 151 ppm[44]).

The dimerisation of α-amino-ketones is an already known, common reaction.[45] It was formerly described for the colchicine analogues[46] to lead to the hexamethoxy-derivative **33b**, or more probably the pyrimidine **34b**. The first step, the imine formation, is probably a reversible reaction. Worth of note, at the low concentrations used for the inhibition studies (μM range), this dimerisation did not occur to a significant extent, since, as we will see below, the enzyme was inhibited by the amino-benzosuberone **4** for at least 10 h without any changes in inhibitory potency.

4. Conformation analysis of the benzocycloheptane and amino-ketones derivatives

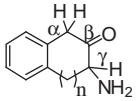
4.1. Benzocycloheptane derivatives

The conformations of the seven-membered ring was already theoretically studied[47–49] and the more stable one was determined to be the chair conformation (see Fig. 1), with protons in axial or equatorial positions. A characteristic feature is that the dihedral angle between both equatorial H-8 and H-9 protons is nearly 15° and between both axial ones nearly 195°.[49] Consequently, the vicinal coupling constant values between both equatorial protons, in one hand, and between both axial protons, on the other hand, are important: *J*(Heq-8,Heq-9) = 7–9 Hz, and *J*(Hax-8,Hax-9) = 9–12 Hz. An example was set with the aminoalcohol **18** and its N-acylated derivative **19a**. The NMR spectra of the *trans* isomers *trans*-**18** and *trans*-**19a** were fully resolved and the vicinal H–H couplings *J*(5a,6), *J*(6,7), *J*(7,8b) and *J*(8b,9a) were large (9.7–12.2 Hz) corresponding to a *trans*-diaxial relation between axial protons as depicted in Figure 1 for the aminoalcohol *trans*-**18**. The value of the *trans* equatorial-equatorial coupling was likely large [*J*(8a,9b) = 7.2 Hz]. For the *cis* isomers, their exact conformation was not studied, but the *J*(6,7) axial-equatorial value was smaller (ca 3 Hz).

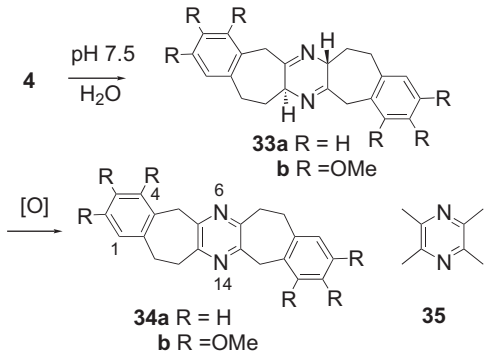
4.2. Silyloxy-ketone 16 and bromo-ketone 24

For these 6-substituted compounds, the coupling values between H-6 and methylene H-5 protons gave the conformational position of these substituents (Fig. 1).

Table 1
Hydration ratio and deuteration rates for amino-benzocyclanones (as hydrochlorides) at pH 6

Ring B	Compound	Ketone/hydrate ratio	Protons exchanged		Deuteration half-time		
			α	γ	20 °C	60 °C	
5	**2**	60/40	Ha-3		14 d		
			Hb-3		14 d		
				H-1	7 h		
6	**1**	80/20	Hb-1		3 h		
			Ha-1		6 h		
				H-3	25 d	52 h	**1** *n* = 1
							2 *n* = 0
							4 *n* = 2
7	**4**	80/20	Ha-5			31 h	
			Hb-5			44 h	
				H-7		12 d	
7	**4**[a] (pH 7.5)	nd[b]	Ha-5		3 h		
				H-7	>10 h[c]		

[a] For compound **4** the deuteration rate was also determined at pH 7.5 in Hepes or Tris buffers.
[b] nd: not determined.
[c] Deuteration of H-7 for compound **4** is slower than the observed dimerisation (see next section). 10 h represents the duration of the experiment.



Scheme 6.

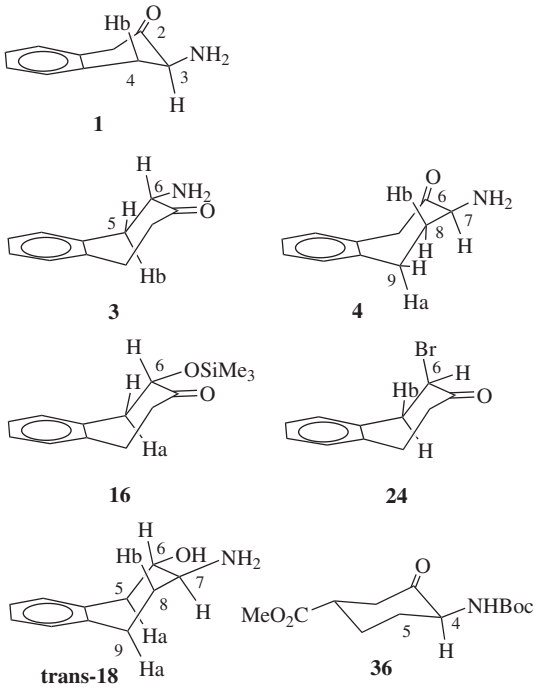


Figure 1.

For the bromo-compound **24**, the equatorial–equatorial relation of H-6 and Hb-5 (J(5b,6) = 7.8 Hz) corresponded to the axial position of the bromine atom. This was supported by the strong deshielding of 0.5 ppm of the spatially near axial Ha-8, which was in *trans*-diaxial relation with Hb-9 (J(8a,9b) = 11.0 Hz).

For the silyloxy-compound **16**, by contrast, a *trans*-diaxial relation of H-6 and Ha-5 (J(5a,6) = 10.2 Hz) corresponded to the equatorial position of the silyloxy substituent.

4.3. Conformation of the amino-ketones

The conformation of amino-ketones was determined by NMR in the case of the aminotetralone **1** and aminobenzocycloheptenone **4** in D_2O (Fig. 1) by a careful analysis of the proton–proton couplings in ^1H NMR spectra. In the first case, a clear *trans*-diaxial coupling (J(3,4b) = 13.2 Hz) for the H-3 proton in the α-position of the amino group of **1** corresponded to a strictly equatorial position of this amino group in a chair conformation, the coupling value with the equatorial H-4 proton J(3,4a) being relatively large (6.4 Hz). Similar coupling values (J(4,5*trans*) = 11.8 Hz, J(4,5*cis*) = 5.6 Hz) were found for the amido-cyclohexanone **36**[50] whose chair conformation with equatorial substituents was ascertained by X-ray structure determination.

In the case of the benzocycloheptenes **3** and **4**, the large value J(5b,6) = 10.0 Hz for **3** showed a clear *trans–trans* diaxial relationship between these two protons. For **4** the large value of the proton–proton couplings between H-7, H-8 and H-9 protons (J(7,8b) = 12.0 Hz, J(8b,9a) = 9.5 Hz) showed clearly a *trans–trans* diaxial relation between H-7 and Hb-8 and between Hb-8 and Ha-9. Thereof, the amino group of the amino-benzocycloheptenones **3** and **4** was in equatorial position. In all the compounds, the conformation was also directed by an hydrogen bond between amine and ketone groups.

In the case of the hydrate forms of **1** and **4**, the situation was not so simple. For the amino-tetralone **1**, the values of the corresponding couplings J(3,4a) = 5.8 Hz, J(3,4b) = 8.2 Hz indicated here an equilibrium between both the possible chair conformations. By contrast, the similar values of the couplings between H-7 and CH_2(8) with those of the ketone form were in agreement with the same conformation of the hydrate form for the amino-benzocycloheptenone **4** and its ketone form.

5. Aminopeptidase inhibition and discussion

All compounds were evaluated as racemic mixtures. All active compounds behaved as competitive inhibitors of the panel aminopeptidases. The inhibition parameters (K_i or IC_{50}) are reported in Table 2.

The amino-tetralone **1** was previously described as a selective competitive inhibitor of the 'one zinc' subfamily of M1 metalloaminopeptidases whereas bestatin is much more potent on the 'two zincs' M17 family of enzymes.[18,19] These two compounds were here used as reference inhibitors. Our strategy for the design of novel amino-tetralone analogues focused on investigating more constrained as well as more flexible derivatives, while also probing the position and nature of the functional groups.

5.1. Structural requirement

5.1.1. Modification of the tetralone scaffold

The five-membered ring amino-indanone **2** (K_i 20 μM), the most conformationally-constrained analogue in our series of compounds, is 40 times less active against APN than the corresponding amino-tetralone **1** (K_i 0.5 μM).[18] Nevertheless **2** remained a highly selective inhibitor of APN. Indeed we did not observe any inhibition of the mammalian 'two zincs' enzyme LAPc with up to 1 mM of compound **2**. LTA4H, an enzyme with aminopeptidase activity structurally related to APN, is not even inhibited by amino-tetralone **1**, suggesting that zinc-chelation is more difficult to achieve for LTA4H than for APN, a more typical aminopeptidase, presumably as a result of steric hindrance. In contrast, we observed weak inhibition of the 'two-zincs' bacterial enzyme APaero (K_i 150 μM).

The seven-membered aminobenzocycloheptenone **4**, with a higher conformational flexibility, appeared to be the most interesting compound in our series. It was almost equipotent to the parent compound **1** (K_i 1 μM) on APN. Moreover we observed a remarkable selectivity against our panel of aminopeptidases, since none of them was inhibited in presence of up to 1 mM concentration of **4**.

5.1.2. Key roles of the carbonyl and primary amine groups

Structural requirements for APN inhibition were previously determined in the tetralone series.[19] It was shown that the primary amine and the carbonyl group were essential features for activity. Here again, we confirm that these functional groups are key

Table 2
Inhibition data of aminopeptidase activity by the synthesised amino-ketones **1-5**, oxime **14** and amino-alcohols *cis*-**18** and *trans*-**18**[a]

Compound	APN (EC 3.4.11.2)	LAPc (EC 3.4.11.1)	LTA4H (EC 3.3.2.6)	APaero (EC 3.4.11.10)
Bestatin	3.5	0.0005	0.5	0.0016
1	0.5[b]	120	>1000	130
2	20[b]	>1000	>1000	150
3	>1000	>1000	>1000	>1000
4	1[b]	>1000	>1000	900
5	310	>100	nd	350
14	110	>1000	>1000	15
trans-**18**·HCl	300	nd	nd	nd
cis-**18**·HCl[c]	60[b]	>1000	nd	>1000

All substances were evaluated as racemic mixtures. IC_{50} (μM) values were determined from Dixon plots at a substrate concentration set to the K_m value for the corresponding enzyme (see Section 7). Inactive compounds were tested up to their solubility limit under the assay conditions that is, 1 mM. K_i values (μM) were determined from Dixon plots. LAPc: cytosolic leucine aminopeptidase from bovine lens (EC 3.4.11.1), APN: human aminopeptidase-N (EC 3.4.11.2); APaero: *Aeromonas proteolytica* aminopeptidase (EC 3.4.11.10) and LTA_4H: human leukotriene A_4 hydrolase (EC 3.3.2.6).

[a] IC_{50} (μM), nd: not determinated.

[b] K_i (μM).

[c] Containing 10% of *trans*-isomer.

pharmacophores, and, in addition, show that their positioning is also crucial. The three derivatives **1**, **2** and **4** bearing the ketone and the primary amine in positions β and γ, respectively, of the benzo group, are specific inhibitors of APN with potencies in the low micromolar range, irrespective of the size of the B cycle. In sharp contrast, when the positions of these functional groups were swapped, like in compound **3** of the seven-membered ring series, the inhibitory potency was totally lost. Our data also further emphasise the requirement for a ketone functional group. When this latter was replaced by an oxime moiety or an alcohol function, a dramatic drop in potency was observed (compound **14** (K_i 110 μM), compound *cis*-**18** (K_i 60 μM) or *trans*-**18** (K_i 300 μM) versus compound **4** (K_i 1 μM)). This result suggests that the ketone moiety plays an important role, most likely in coordinating the active site zinc, and that this role is only poorly fulfilled by the oxime or alcohol functions. As discussed before, compound **4** is readily hydrated in solution and, therefore, the higher potency afforded by the ketone functionality could originate from the binding of the hydrated form. This latter has the potential to be a close mimic of the transition state formed during the nucleophilic attack of the scissile bond carbonyl by the zinc-activated water/hydroxide ion.

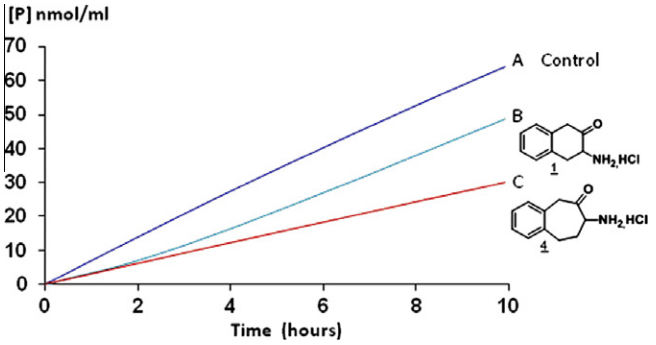


Figure 2. Enzyme assays were run at 25 °C in 20 mM Tris–HCl buffer pH 7.5, with leucine-para-nitroanilide (0.2 mM) as substrate, in a total reaction volume of 1 ml. The formation of para-nitroaniline was followed at 405 nm (ϵ = 10,800 mol^{-1} L cm^{-1}). The reaction was started by addition of 3 milliunits of porcine kidney AP-N. Under these experimental conditions linear kinetics were observed for at least 10 h. Dotted line A corresponds to control (no inhibitor; V_o = 6.8 nmol h^{-1}). In experiments B and C 2 μM of **1** and 2 μM of **4**, respectively, were used. In experiment B, 60% of inhibition (V_i = 3.1 nmol h^{-1}) was observed during the first hour. After 8 h, the enzyme activity was back to control (V_i = V_o = 6.5 nmol h^{-1}). In contrast, 60% inhibition (V_i = 3.1 nmol h^{-1}) was observed throughout experiment C.

5.1.3. Monocyclic amino-suberone 5

The loss of the aromatic A cycle led to a dramatic decrease in the inhibitory potency (Table 1). This effect confirmed the importance of this aromatic part in the recognition of the inhibitors by the enzyme.

5.2. Stablity of the amino-benzosuberone 4 under enzyme assay conditions

We showed here above that the amino-benzocycloheptenone **4** was less prone to proton exchange in position α to the carbonyl group, in comparison to the five- and six-membered ring derivatives **1** and **2**. We have further investigated the stability of this scaffold under our enzyme assay conditions. We observed that inhibition was maintained during at least 10 h while the inhibition by the amino-tetralone derivative **1** levelled off after about 2 h, as already reported[19] (Fig. 2). This result was in agreement with the stability observed through previously deuteration experiments. We have already noted that the dimerisation of **4** clearly did not occur at the micromolar concentrations used in the enzyme assay.

5.3. Potential binding mode

To date, no three dimensional structure is available for a mammalian APN. Nevertheless the crystal structures of several members of the M1 family of peptidases have already been solved.[21–23,51–54] All these enzymes are dependent on a single zinc ion for their activity and remove the amino terminal residue of polypeptides. The structural data show that their active site is related to the metallo-endoproteinase thermolysin,[54] including in particular the consensus zinc binding motif (HEXXH-(X18)-E), but with an additional recognition motif, the exopeptidase motif (GXMEN), for binding the free primary amino group of the N-terminal residue of their peptidic substrates. An example is given in Figure 3 with a partial sequence alignment of the human (P15144) and *E. coli* (P04835) APNs.

Although the one-zinc aminopeptidases of known three-dimensional structure share little overall amino acid sequence similarity, they display remarkable structural conservation, including a common fold and a highly conserved active site. Interestingly, the three-dimensional fold of aminopeptidases with a binuclear metal centre, such as LAPc, is totally different[56,57] (pdb entries 1BPN, 2EWB). This structural segregation rules out the possibility of a common ancestor for the super-family of zinc-dependent aminopeptidases.

The structural homology of the mammalian APN to the microbial enzymes of known 3D structure, inferred from the presence of characteristic sequence motifs, is confirmed by HHPRED[58] analysis, one of the most powerful structure prediction algorithm

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349 FNAGAMENWGLVTYRENSLLFDPLSSSSSNKERVVTVIAHELAHQWFGNLVTIEWWNDLW
258 FNMGAMENKGLNIFNSKYVLARTDTATDKDYLDIERVIGHEYFHNWTGNRVTCRDWFQLS
    ** ***** **        *                ** **  * * ** **   *  *

409 LNEGFASYVEYLGADYAEPTWNLKDLMVLNDVYRVMAVDALASSHPLSTPASEINTPAQI
318 LKEGLTVFRD----QEFSSDLGSRAVNRINNVRTMRGLQFAEDASPMAHPIRP-DMVIEM
    * **                        * *            *   *
```

Figure 3. Partial sequence alignment of the human (P15144, black) and the *E. coli* (P04835, blue) APNs around the active site region. The alignment was produced with the program LALIGN.[55]

currently available. The remote homology to thermolysin from *Bacillus thermoproteolyticus* around the active site (amino acids 310–530) is detected by HHPRED, and, more interestingly, structural homology spanning over 800 amino acids is detected at the 100% confidence level with four PDB entries (release January 2010): 1Z5H (Tricorn interacting factor F3 from *Thermoplasma acidophilum*), 3EBH (*Plasmodium falciparum* M1 neutral aminopeptidase), 3B34 (*E. coli* aminopeptidase-N), 2gtq (*Neisseria meningitidis* aminopeptidase-N) (Fig. 4). The structural homology to human LTA4 (3B7S, amino acids 70–770) is also detected at the highest confidence level, underscoring the relevance of this enzyme for the assessment of the selectivity of our novel inhibitors.

Based on the predicted structural similarity, we have derived a working model of the binding of the amino-benzosuberone **4**, in its hydrated and non-hydrated forms, to the human APN active site zinc. To this end, we used the X-ray structure of the *E. coli* APN, ePepN,[21] in complex with bestatin, and focused on the conserved residues involved in substrate recognition, zinc binding and catalysis. Although we do not know which enantiomer is the active species, we assumed that the amino group was in equatorial position, in order to best match the binding mode observed with bestatin. We then superimposed the primary amino groups of **4** and bestatin, and either the ketone or the *cis*-hydroxyl group of the hydrate form of **4** with the hydroxyl functionality of bestatin (Fig. 5). Both proposed binding modes account for the observation that the positions of the primary amine and carbonyl pharmacophores are crucial for inhibitory activity and cannot be interchanged. The primary amine of **4** is engaged in strong electrostatic interactions with the side-chain carboxylate of Glu121 and Glu264 (ePepN numbering scheme), fully conserved in the one-zinc family of aminopeptidases and responsible for the recognition and binding of the free amino terminus of peptidic substrates. In the case of the non-hydrated form of the ketone, the ketone oxygen binds to the zinc ion in a way similar to that proposed for the scissile amide carbonyl of a peptide substrate. In the case of the hydrated form, both hydroxyl groups contribute binding interactions. The *cis*-hydroxyl one is part of the zinc coordination sphere, and can be viewed as a mimic of the nucleophilic zinc-bound water involved in catalysis. The *trans*-hydroxyl group occupies the position of the substrate amide carbonyl oxygen following the formation of the tetrahedral transition state. This hydroxyl makes a hydrogen-bonded interaction to the side-chain of Glu298 (ePepN numbering scheme). Indeed, we observed a reduced potency of the *trans*-isomer versus the *cis*-isomer, and a synergistic effect with the hydrated ketone.

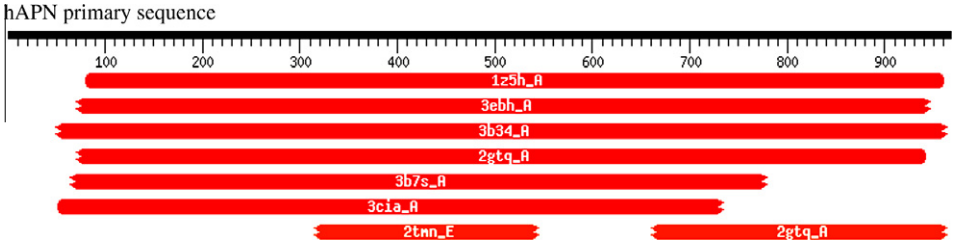

Figure 4. The human APN sequence (966 residues) was used as template (black bar). Reported here are only the top scoring hits identified by HHPRED in the January 8, 2010 release of the PDB (probability of structural homology: red, 100%; orange, 94–95%). PDB entries: 1Z5H (Tricorn interacting factor F3 from *Thermoplasma acidophilum*), 3EBH (*Plasmodium falciparum* M1 neutral aminopeptidase), 3B34 (*E. coli* aminopeptidase-N), 2GTQ (*Neisseria meningitidis* aminopeptidase-N), 3B7S (Human leukotriene A4 hydrolase), 3CIA (Cold-active aminopeptidase from *Colwellia psychrerythraea*), 2TMN (*Bacillus thermoproteolyticus* thermolysin).

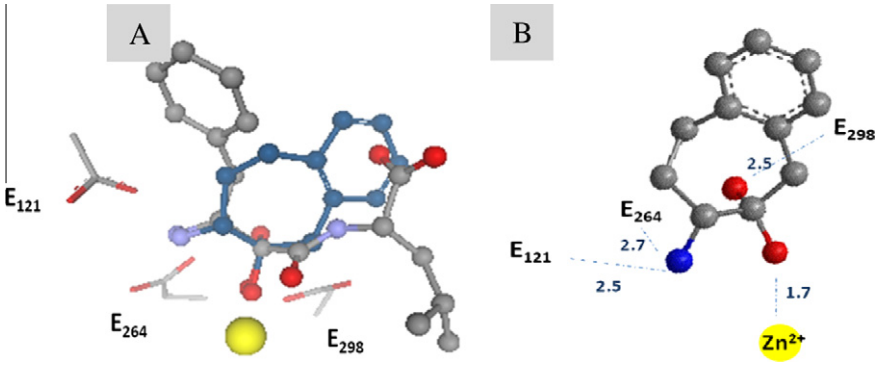

Figure 5. Schematic model of the binding interactions of amino-benzosuberone **4** to the catalytic zinc. Note: one enantiomer of **4** was arbitrarily chosen. (A) overlay with bestatin bound to the *E. coli* APN (pdb entry 2HPT, Web LaB viewer Pro, Accelrys). The primary amino groups of both inhibitors and the carbonyl and hydroxyl groups of **4** and bestatin, respectively, were used as anchoring points. (B) Schematic model showing the key interactions of the hydrated form of **4**, which mimics the transition state intermediate formed during peptide bond hydrolysis.

The seven-membered ring of compound **4** adopts chair conformation[49] but the twisted conformation is of similar energy (within 1 kcal/mol). While the relative conformational versatility of this scaffold may contribute to a more optimal fit of the inhibitor to the enzyme active site and is therefore of advantage, it is difficult to predict which is the biologically active absolute configuration and associated conformation of this new class of APN inhibitors. Furthermore, the observation that the phenylalanine side-chain of bestatin has the configuration of α-D-amino acid, while peptide substrates have the L-configuration, indicates that amino-peptidases do not strongly discriminate diastereoisomers at the C-α position of the amino terminal residue. Therefore, the development of a more refined model for the binding of **4** to the APN active site must await the provision of detailed structural information on a mammalian APN.

6. Conclusion

Mammalian APN appears to be involved in numerous and complex biological functions, but it has not been possible up to now to fully clarify which of its functions depend on its enzymatic activity and which do not. The development of highly specific and potent human APN inhibitors would greatly help shed light on the exact involvement of APN in a number of physiological and pathological processes, with potential pharmaceutical applications, notably in oncology and other, angiogenesis-dependent pathologies.[9–12] The main interest of 3-amino-2-tetralone was its high selectivity for the 'one zinc enzymes' and particularly APN whereas bestatin remained the best inhibitors to date for the 'two zincs' aminopeptidase sub-family.[18,19] We successfully identified in this work the amino-benzosuberone scaffold as an amino-tetralone analogue exhibiting greatly improved stability as well as promising potency and a remarkable selectivity profile. This compound is readily hydrated in aqueous solution. The observed SAR strongly suggests that the active species is the hydrated form, which shares many structural features with the transition state formed during the hydrolysis of a peptidic substrate. In view of its minimal size, this compound displays an excellent ligand efficiency (0.63 according to the definition by Hopkins et al.[59]) and has therefore strong potential for further potency and selectivity improvements through continued chemical elaboration and derivatisation. The working model presented here provides a basic understanding of how this class of compound may bind to the catalytic zinc of APN, and may thus provide guidance for further medicinal chemistry efforts.

7. Experimental part

General. Flash chromatography (FC): silica gel (*Merck 60*, 230–400 mesh). TLC: Al-roll silica gel (*Merck 60*, F_{254}). Mp: *Kofler* hot bench, corrected. IR spectra (ν in cm^{-1}): *Nicolet 405 FT-IR*. $[\alpha]_D$: Perkin–Elmer *341 LC* polarimeter. ^1H and ^{13}C NMR (400 and 100.6 MHz resp.) spectra: *Bruker Avance 400* at 295 K unless otherwise noted, tetramethylsilane (TMS), or natrium (D_4)-trimethylsilyl-propionate (D_4-TSP) in D_2O (^1H NMR) and $CDCl_3$, or MeOD-D_4 [$\delta(CDCl_3) = 77.0$, $\delta(CD_3OD) = 35.0$ with respect to TMS] (^{13}C NMR) as internal standard; δ in ppm and J in Hz. ^1H NMR spectra in 2 N HCl in H_2O were recorded with external deuteriated lock and irradiation of the H_2O peak, acetone at δ 2.22 ppm as internal standard. High resolution MS were measured on a *Bruker MicrOTof* spectrometer in Institut de Chimie, UMR 7177 CNRS, ULP, Strasbourg, or Agilent Technologies 6510 (QTof) spectrometer in FRE 3252 CNRS, UHA, Mulhouse, France, or in Basilea Pharmaceutical Ltd, CH-Basel. Microanalyses were carried out by the *Service Central de Microanalyses du CNRS*, F–69390 Vernaison, France.

Reagents and solvents. Raney-nickel, aqueous suspension and 5% Pd/C were obtained from Fluka, other reactants were purchased from usual furnisher. Dess–Martin periodinane (DMP) was prepared according to[25] or obtained in CH_2Cl_2 solution, and titrated by oxidation of benzyl alcohol with NMR monitoring. *tert*-Butyl nitrite was prepared according to lit.[60] Usual solvents were freshly distilled, dry EtOH and MeOH distilled over Mg/MgI_2, dry THF over Na and benzophenone, dry Et_2O was distilled and stored over Na, CH_2Cl_2 was distilled over P_2O_5 and kept over Na_2CO_3. NEt_3 and $SiMe_3Cl$ were distilled before use.

7.1. Indanone series

7.1.1. (1*R*,2*S*)-1-(*tertio*-Butyloxycarbonylamino)indan-2-ol ((−)-7b)

To a solution of (+)-**7a** (400 mg, 2.68 mmol) in MeOH (10 mL) was added Boc_2O (662 mg, 3.0 mmol, 1.1 equiv). After 3–5 h stirring at room temperature, the solvent was evaporated and the residue (0.9 g) recrystallised in cyclohexane to give pure (−)-**7b** (634 mg, 98%) as colorless crystals, mp = 77–79 °C [lit.[24] for the (1*S*,2*R*)-enantiomer, mp 76.1–76.8 °C], $[\alpha]_D^{20} = -13$ (*c* 1, $CHCl_3$). ^1H and ^{13}C NMR ($CDCl_3$) data were identical with those reported in the lit.[24]

7.1.2. (1*R*)-(*tertio*-Butyloxycarbonylamino)-indan-2-one ((−)-8)

To a solution of (−)-**7b** (320 mg, 1.34 mmol) in CH_2Cl_2 (7 mL) at 0 °C was added DMP (0.46 M in CH_2Cl_2, 4.2 mL, 1.93 mmol, 1.4 equiv). After 2 h stirring at 0 °C, AcOEt (20 ml) and an aqueous solution of 0.5 N sodium thiosulfate in 1 N $NaHCO_3$ (20 mL) were added to the reaction mixture. After 1 h stirring at rt, the organic layer was successively washed with aqueous 1 N $NaHCO_3$ (10 mL) and with brine (10 mL), dried ($MgSO_4$) and evaporated. Purification of the residue by flash chromatography (Cyclohexane/AcOEt 8:2) gave (−)-**8** (227 mg, 69%) as a colorless resin, $[\alpha]_D^{20} = -15$ (*c* 1.1, $CHCl_3$). IR (KBr): 3372, 2974, 1768, 1698, 1508, 1366, 1322, 1253, 1169, 1152, 1049, 1027, 904, 757, 747, 738 cm^{-1}. ^1H NMR ($CDCl_3$): 1.48 (s, 9H, *t*Bu); 3.54 and 3.66 (2 d, 2H, $J = 21.6$ Hz, H-3); 5.08 (br d, 1H, $J = 8.0$ Hz, H-1); 5.22 (br d, 1H, $J = 8.0$ Hz, NH); 7.32 (m, 3Har); 7.42 (m, 1Har). ^{13}C NMR ($CDCl_3$): 28.4 (C*Me*$_3$); 41.6 (C(3)); 59.8 (C(1)); 80.6 (*C*Me$_3$); 124.7, 125.1, 128.1, 128.7 (4Car); 136.0, 139.2 (C(3a),C(7a)); 156.1 (NCO_2); 212.4 (C(2)). Anal. Calcd for $C_{14}H_{17}NO_3$ (247.30): C, 68.00; H, 6.93; N, 5.66. Found: C, 67.8; H, 7.1; N, 5.5.

7.1.3. 1-Aminoindan-2-one, hydrochloride (2)

A solution of **8** (158 mg, 0.64 mmol) in a mixture of 2.2 N HCl in dry Et_2O (1.2 mL) and dry dioxane (1.2 mL) was stirred for 3 days at rt. The precipitate was isolated by filtration or centrifugation, washed with dry Et_2O, and recrystallised in *i*PrOH/Et_2O to give **2** (80 mg, 69%) as ochre crystals, mp 198–201 °C. IR (KBr): 3421, 2975–2863, 1774, 1759, 1599, 1498, 1478, 1085, 743 cm^{-1}. ^1H NMR (D_2O, 60:40 mixture of ketone and hydrate). Ketone: 3.92 and 3.70 (2 d, 2H, $J = 22.9$ Hz, H-3); 5.18 (s, 1H, H-1); 7.30–7.60 (m, 4Har). Hydrate: 3.29 and 3.38 (2 d, 2H, $J = 16.9$ Hz, H-3); 4.52 (s, 1H, H-1); 7.30–7.60 (m, 4Har); ^1H NMR (CD_3OD, 27:35:38 mixture of ketone and 2 hemiacetals). *Ketone*: 3.87 and 3.60 (2 d, 2H, $J = 22.2$ Hz, H-3); 5.06 (s, 1H, H-1); 7.3–7.6 (m, 4Har). *Hemiacetal 1*: 3.17 and 3.38 (2 d, 2H, $J = 16.1$ Hz, H-3); 4.39 (s, 1H, H-1); 7.3–7.6 (m, 4Har). *Hemiacetal 2*: 3.31 (s, 2H, H-3); 4.54 (s, 1H, H-1); 7.3–7.6 (m, 4Har). ^{13}C NMR (CD_3OD), *ketone*: 42.3 (C(3)); 58.1 (C(1)); 131.3, 129.3, 128.6, 125.7 (4CHar)); 139.0, 135.4 (C(3a),C(7a)); 209.3 (CO(6). *2Hemiacetals*: 43.3, 41.6 (2 C(3)); 63.6, 61.5, (2 C(1)); 106.0, 105.4 (2 C(2)); 131.28, 130.9, 128.5, 126.9, 126.8, 126.7, 126.6, 126.1, (8CHar)); 142.8, 142.1, 137.3, 137.1 (4Car). HR-MS (TOF ESI), calcd for $C_9H_{10}ON$ $[M+H]^+$: 148.0762. Found: 148.0764.

7.2. Tetralone series

7.2.1. 3-Amino-3,4-dihydro-1*H*-naphtalen-2-one, hydrochloride (1)

Preparation of **1** was achieved from 1,4-dihydronaphtalene according to the literature.[18b,19] Colorless crystals, mp 180–200 °C (dec.). ^{1}H NMR (D_2O, 80:20 mixture of ketone and hydrate). *Ketone*: 3.30 (dd, 1H, *J* = 13.2, 15.0 Hz, Hb-4); 3.47 (dd, 1H, *J* = 6.4, 15.0 Hz, Ha-4); 3.89 and 3.98 (2 d, 2H, *J* = 21.9 Hz, H-1); 4.40 (dd, 1H, *J* = 6.4, 13.2 Hz, H-3); 7.18–7.26 (m, 2Har); 7.33 (m, 3Har); *Hydrate*: 3.06 (dd, 1H, *J* = 8.2, 16.8 Hz, Hb-4); 3.35 (dd, 1H, *J* = 5.8, 16.8 Hz, Ha-4); 3.19 and 3.23 (2 d, 2H, *J* = 18.4 Hz, H-1); 3.67 (dd, 1H, *J* = 5.8, 8.2 Hz, H-3); 7.18–7.26 (m, 2Har); 7.33 (m, 2Har). ^{1}H NMR (CD_3OD): 3.22 (t, Hb-4); 3.40 (dd, Ha-4); 3.81 (d, 1H, Hb-1); 3.94 (d, 1H, Ha-1); 4.28 (dd, 1H, H-3); 7.23 (m, 1Har); 7.28 (m, 3Har); *J*(1a,1b) = 21.4, *J*(3,4a) = 6.4, *J*(3,4b) = 13.1, *J*(4a,4b) = 14.9 Hz.

7.3. Benzosuberone series

7.3.1. 5,6,8,9-Tetrahydrobenzocycloheptene-7-one (9)

Preparation of **9** was achieved from α,α'-dibromoxylene according to[26–28] overall yield 81%. Colorless crystals, mp 40 °C (pentane) (lit.[27] 41–42 °C, lit.[26] 43–43.8 °C). IR ($CHCl_3$): 2950, 1690, 1450, 1090 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.62 (m, 4H, CH_2(6), CH_2(8)); 2.91 (m, 4H, CH_2(5), CH_2(9)); 7.23 (s, 4Har). ^{13}C NMR ($CDCl_3$): 30.5 (C(5),C(9)); 44.6 (C(6),C(8)); 127.1 (C(2), C(3)); 129.1 (C(1),C(4)); 140.5 (C(4a),C(9a)); 211.3 (C(7)).

7.3.2. 8,9-Dihydro-6-hydroxyimino-5*H*-benzocycloheptene-7-one, 7-dimethylacetal (10)

Preparation according to[29]: To a stirred solution of **9** (1.0 g 6.24 mmol) in 2 M HCl in MeOH (13 ml) was added dropwise at 0 °C in ice bath *t*BuONO (1.1 m, 9.3 mmol, 1.5 equiv). After 1 hour, the solution was poured in aqueous N $NaHCO_3$ (30 ml) and ice. The precipitate was isolated by filtration, washed with water and dried on P_2O_5 under vacuum or washed with *i*PrOH, to give **10** (0.90 g, 61%). A second crop (70 mg, 5%) was obtained by extraction with AcOEt of the aqueous phase. Colorless crystals, mp 194–198 °C then 202–4 °C (MeOH) (lit.[29] 195–7 °C). IR (KBr): 3213, 2952, 2829, 1737, 1698, 1489, 1455, 1423, 1235, 1155, 1135, 1099, 1090, 1058, 1041, 989, 951, 938, 925, 888, 781, 753, 728 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.07 (m, 2H, CH_2(8)); 2.91 (m, 2H, CH_2(9)); 3.30 (s, 6H, 2 OMe); 3.73 (s, 2H, CH_2(5)); 7.10–7.13 (m, 3Har); 7.32 (d, 1Har); 7.46 (br s, NOH). ^{13}C NMR ($CDCl_3$): 29.3, 29.6 (C(5),C(9)); 36.5 (C(8)); 49.0 (2 OMe); 101.7 (C(7)); 126.6 (C(2),C(3)); 128.9, 129.8 (C(1),C(4)); 135.2 (C(4a)); 141.0 (C(9a)); 155.4 (C(6)). Anal. Calcd for $C_{13}H_{17}NO_3$ (235.29): C, 66.36; H, 7.28; N, 5.95. Found: C, 66.3; H, 7.5; N, 5.9.

7.3.3. 6-Amino-5,6,8,9-tetrahydrobenzocycloheptene-7-one, 7-dimethylacetal (11) and 6-amino-5,6,8,9-tetrahydrobenzocycloheptene-7-one, hydrochloride (3)

Acetal **10** (40 mg, 0.17 mmol) was hydrogenolysed in EtOH (1.7 ml) with concentrated aqueous NH_4OH (0.12 ml, 1.7 mmol) over wet Raney-Nickel (400 mg) for 6 h at rt. The catalyst was discarded by filtration on celite or by centrifugation and the solution evaporated to give **11** (36 mg, 95%). Crude amine **11** was hydrolysed by stirring in aqueous N HCl (1.8 ml) and EtOH (0.7 ml) for 6 h. Evaporation of the solvents and washing with dry Et_2O gave crystalline **3** (30 mg, 90%).

Compound **11**, colorless resin. ^{1}H NMR ($CDCl_3$): 1.54 (dd, 1H, Hb-8); 2.05 (dd, 1H, Ha-8); 2.55 (dd, 1H, Hb-9); 2.81 (dd, 1H, Ha-9); 2.87 (dd, 1H, Hb-5); 3.23 (s, 3H, OMe); 3.28 (d, 1H, Ha-5); 3.34 (d, 1H, H-6); 3.30 (s, 3H, OMe); 7.10–7.18 (m, 4Har); *J*(5a,5b) = 14.4, *J*(5b,6) = 6.8, *J*(8a,8b) = 14.8, *J*(8a,9b) = 7.2, *J*(8b,9a) = 13.0, *J*(9a,9b) = 14.2 Hz. ^{13}C NMR ($CDCl_3$): 28.6, 29.0 (C(5),C(9)); 35.0 (C(8)); 47.3, 48.0 (2 OMe); 50.1 (C(5)); 104.0 (C(7)); 126.3, 127.0, 128.4, 131.5 (4CHar); 136.2, 142.1 (2Car). HR-MS (ESI$^+$) Calcd for $C_{12}H_{16}NO$ [M−OMe]$^+$: 190.1226; found: 190.1227. Calcd for $C_{13}H_{20}NO_2$ [M+H]$^+$: 222.1489; found: 222.1484.

Compound **3**: colorless crystals, mp 210–220 °C (dec). IR (KBr): 3367, 2970, 2719, 2624, 1720, 1600, 1486, 1448, 1154, 1055, 772, 761, 754, 718, 476 cm^{-1}. ^{1}H NMR (D_2O): 2.69 (dt, 1H, *J* = 3.3, 12.4 Hz, Hb-8); 2.90 (m, 2H, Ha-8, Hb-9); 3.08 (ddd, 1H, *J* = 3.3, 7.0, 15.4 Hz, Ha-9); 3.21 (m, 2H, CH_2(5)); 4.24 (dd, 1H, *J* = 4.5, 9.8 Hz, H-6); 7.28 (m, 1Har); 7.37 (m, 3Har). ^{13}C NMR (D_2O): 29.7 (C(9)); 35.0 (C5)); 42.8 (C(8)); 59.6 (C(6)); 128.3, 129.1, 130.0, 130.7 (4CHar); 135.3, 141.3 (2Car); 207.8 (CO(7)). HR-MS (TOF ESI) calcd for [M+H]$^+$ ($C_{11}H_{14}NO$): 176.1075; found: 176.1072.

7.3.4. 6-Hydroxyimino-8,9-dihydro-5*H*-benzocycloheptene-7-one (12)

A biphasic solution of **10** (0.30 g, 1.27 mmol) in Et_2O (5 ml) and aqueous 6 N HCl (5 ml) was stirred at 0 °C for 15 min and poured in aqueous N $NaHCO_3$ (30 ml) and ice. The mixture was extracted with Et_2O, the organic phase washed with aq N $NaHCO_3$, dried (SO_4Mg) and evaporated. Pure **12** (173 mg, 72%) was obtained by flash chromatography (Cyclohexane/AcOEt 8:2), yellow crystals, mp 153–154 °C (*i*PrOH) [lit.[29] 151–153 °C (benzene)]. IR (KBr): 3282, 2954, 1683, 1583, 1460, 1427, 1411, 1395, 1266, 1095, 979, 956, 938, 917, 889, 865, 770, 713 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.87 (m, 2H, CH_2(8)); 3.10 (m, 2H, CH_2(9)); 4.03 (s, 2H, CH_2(5)); 7.23 (m, 4Har); 8.50 (br s, NOH). ^{13}C NMR ($CDCl_3$): 28.6 (C(5)); 31.0 (C(9)); 43.7 (C(8)); 127.5, 128.0, 128.6, 130.2 (C(1), C(2),C(3), C(4)); 133.2 (C(4a)); 139.9 (C(9a)); 156.8 (C(6)); 199.0 (C(7)).

7.3.5. 7-Benzylamino-5,7,8,9-tetrahydrobenzocyclohepten-6-one, oxime (13a)

To a solution of **12** (2.32 g, 12.3 mmol) in pyridine (7 ml) at 0 °C was added benzylamine (1.35 ml, 12.4 mmol, 1 equiv) and the red solution stirred at rt for 6 h. Anhydrous MeOH (7 ml) was then added, then $NaBH_4$ (0.55 g, 14.5 mmol, 1.2 equiv) and the mixture was vigorously stirred for 1 h. It was then poured in aqueous N $NaHCO_3$ (45 ml) filtered and the precipitate washed with water and dried under vacuum, to give **13a** (3.18 g, 93%), cream crystals, mp 144–146 °C (EtOH). IR (KBr): 3145, 3027, 2842, 1640, 1490, 1475, 1455, 1374, 1116, 965, 870, 852, 763, 747, 724, 699 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.85 (dddd, Hb-8); 2.07 (dddd, Ha-8); 2.65 (ddd, Hb-9); 3.14 (ddd, Ha-9); 3.50 (dd, H-7); 3.67 (d, *J* = 12.9 Hz, CHa(Bn)); 3.72 (d, Hb-5); 3.81 (d, *J* = 12.9 Hz, CHb(Bn)); 3.95 (d, Ha-5); 7.14 (m, 3Har); 7.32 (m, 6Har); *J*(5a,5b) = 14.3, *J*(7,8a) = 4.8, *J*(7,8b) = 6.4, *J*(8a,8b) = 13.6, *J*(8a,9a) = 10.8, *J*(8b,9a) = 2.6, *J*(8a,9b) = 2.9, *J*(8b,9b) = 7.2, *J*(9a,9b) = 14.4 Hz. ^{13}C NMR ($CDCl_3$): 30.1, 30.9 (C(5), C(9)); 35.2 (C(8)); 51.7 (NCH_2); 60.1 (C(7)); 126.6, 126.8, 126.9 (Car-p,C(2),C(3)); 128.2, 128.3 (Car-o, Car-m); 129.0, 129.7 (C(1),C(4)); 133.2 (C(4a)); 140.2, 141.3 (C(9a), Car-s); 159.1 (C(6)). Anal. Calcd for $C_{18}H_{20}N_2O$ (280.37): C, 77.11; H, 7.19; N, 9.99. Found: C, 77.3; H, 7.4; N, 10.0.

7.3.6. 7-Amino-5,7,8,9-tetrahydrobenzocyclohepten-6-one, 6-oxime hydrochloride (14)

Compound **13a** (100 mg, 0.36 mmol) was hydrogenolysed at rt in EtOH (3 mL) and 1 N aqueous HCl (0.36 ml) over 5% Pd/C (7 mg) for 13 h. The catalyst was discarded by centrifugation and the solvent evaporated. The crude product was recrystallised in *i*PrOH/Et_2O to give pure **14** (60 mg, 75%). Colorless crystals, mp 270–280 °C (*i*PrOH/Et_2O). IR (KBr): 3297, 3016, 2946, 2451, 1581, 1554, 1491, 1448, 1427, 1046, 977, 936, 925, 763, 755, 720, 450 cm^{-1}. ^{1}H NMR (CD_3OD): 1.66 (dddd, 1H, Hb-8); 2.41 (dddd, 1H, Ha-8); 2.93 (ddd, 1H, Hb-9; 3.04 (ddd, 1H, Ha-9); 3.42 (d, 1H,

Hb-5); 4.01 (dd, 1H, H-7); 4.23 (d, 1H, Ha-5); 7.18 (m, 3Har); 7.26 (m, 1Har); J(5a,5b) = 15.2, J(7,8a) = 5.4, J(7,8b) = 11.6, J(8a,8b) = 12.6, J(8a,9a) = 3.4, J(8a,9b) = 8.6, J(8b,9a) = 9.0, J(8b,9b) = 3.4, J(9a,9b) = 14.6 Hz. ^{13}C NMR ((CD$_3$OD): 31.3, 32.7 (C(5),C(9)); 35.8 (C(8)); 55.2 (C(7)); 128.0, 128.2 (C(2),C(3)); 130.0, 130.8 (C(1),C(4)); 136.1 (C(4a)); 141.5 (C(9a)); 155.6 (C(6)). HR-MS (FAB$^+$) calcd for [M+H]$^+$ ($C_{11}H_{15}N_2O$): 191.1184. Found 191.1186.

7.3.7. 7-[Benzyl(benzyloxycarbonyl)amino]-8,9-dihydro-5*H*-benzocyclohepten-6-one, 6-oxime (13b)

To a biphasic solution of **13a** (0.25 g, 0.90 mmol) in THF (1.4 ml) and H_2O (0.6 ml), was added Na_2CO_3 (0.38 g, 3.6 mmol, 4 equiv) and at 0 °C dropwise in two times $ClCO_2Bn$ (126 μl, 0.89 mmol, 1 equiv, and after 4 h, 63 μl, 0.45 mmol, 0.5 equiv). The reaction was stirred for 24 h, then diluted with H_2O and extracted with AcOEt. After evaporation of the solvent, purification by FC (Cyclohexane/AcOEt 9:1) gave pure **13b** (237 mg, 64%), colorless resin. IR ($CHCl_3$): 3583, 3327, 3014, 2952, 1694, 1497, 1453, 1416, 1364, 1263, 1120, 963, 909 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.79 (m, 1H, Hb-8); 2.02 (m, 1H, Ha-8); 2.77 (ddd, 1H, J(9a,9b) = 14.1 Hz, J(8,9b) = 3.5, 9.0 Hz, Hb-9); 2.85 (m, 1H, Ha-9); 3.32, 4.21 (2 d, 2H, J = 15.1 Hz, NCH_2Ph); 4.21, 4.92, (2 d, 2H, J = 17.1 Hz, CH_2(5)); ca 5.0 (br s, 1H, H-7); 5.08, 5.19 (2 d, 2H, J = 12.6 Hz, OCH_2Ph); 7.09 (m, 1Har); 7.15–7.28 (m, 13Har). ^{13}C NMR ($CDCl_3$): 31.5, 32.2, 32.6 (C(5),C(8),C(9)); 49.2 (N*C*H_2Ph); 60.2 (C(7)); 67.4 (O*C*H_2Ph); 126.3, 126.5, 127.1, 127.2, 126.7, 127.4 (Cp(NBn), Cp(OBn), C(2),C(3), Co(OBn), Co(NBn)); 128.3 (Cm(NBn), Cm(OBn); 128.8, 129.8 (C(1), C(4)); 134.6 (C(4a)); 136.5 (Cs(NBn)); 139.8, 140.1 (C(9a), Cs(OBn)); 156.8, 156.9 (C(6), NCO_2). Anal. Calcd for $C_{26}H_{26}N_2O_3$ (414.51): C, 75.34; H, 6.32; N, 6.76. Found: C, 74.9; H, 6.6; N, 6.5.

7.3.8. 7-(Trimethylsilyloxy)-6,9-dihydro-5*H*-benzocycloheptene (15) and 6-(trimethylsilyloxy)-5,6,8,9-tetrahydro-benzocyclohepten-7-one (16)

Silyl ether **15** *from ClSiMe$_3$ and DBU*: To a stirred solution of **9** (0.40 g, 2.50 mmol) in dry CH_2Cl_2 (2.5 mL) were successively added under Ar at rt DBU (0.94 mL, 6.24 mmol, 2.5 equiv) and $SiMe_3Cl$ (0.64 mL, 5.0 mmol, 2 equiv). After 1–3 h stirring, H_2O (10 mL) was added and the mixture extracted with cyclohexane (100 mL). The organic phase was dried (Na_2SO_4) and evaporated to give crude **15** as a brownish resin (0.60 g, quant.).

Silyl ether **15** *from SiMe$_3$OTf and NEt$_3$*: To a solution of **9** (3.98 g, 24.8 mmol) and Et_3N (4.8 ml, 34.8 mmol, 1.4 equiv) in dry toluene (40 mL), was added dropwise $SiMe_3OTf$ (5.4 ml, 29.8 mmol, 1.2 equiv) at rt under Ar. The mixture was stirred at 85 °C for 2 h, then cooled at 0 °C, diluted with cyclohexane (100 ml), washed successively with 2 M aq NH_4Cl and brine and dried ($MgSO_4$). Evaporation of the solvent gave the crude **15** (ca 6 g, quant.).

Silyloxyketone **16**: Crude **15** (ca 6 g, 24.8 mmol) was dissolved in CH_2Cl_2 (40 ml) at 0 °C, *m*-CPBA (5.13 g, 29.7 mmol, 1.2 equiv) was then added portionwise (or in CH_2Cl_2 solution) and the solution stirred at 0 °C for 2 h. The solid was filtered off and the solvent evaporated. Cyclohexane (100 mL) was added, the insoluble filtrated off, and the filtrate evaporated to give crude **16** (ca 4.8 g, quant.) which was used without further purification.

Compound **15**, R_f = 0.56 (AcOEt/cyclohexane 5:5), only characterised by NMR. ^{1}H NMR ($CDCl_3$): 0.12 (s, 9H, $(CH_3)_3Si$)); 2.31 (m, 2H, CH_2(6)); 2.92 (m, 2H, CH_2(5)); 3.29 (dt, 2H, CH_2(9)); 5.08 (tt, 1H, H-8); 7.06–7.26 (m, 4Har); J(6,8) = 1.4, J(6,9) = 2.1, J(8,9) = 6.2 Hz.

Compound **16**, only characterised by NMR. ^{1}H NMR ($CDCl_3$): 0.15 (s, 9H, $SiMe_3$); 2.43 (m, 1H, Hb-8); 2.84 (m, 3H, Ha-8, CH_2(9)); 2.95 (dd, 1H, Hb-5); 3.11 (dd, 1H, Ha-5); 4.26 (ddd, 1H, H-6); 7.21–7.28 (4Har); J(5a,5b) = 14.3, J(5a,6) = 10.2, J(5b,6) = 3.0, ^4J(6,8b) = 1.0 Hz. ^{13}C RMN ($CDCl_3$): 0.22 ($SiMe_3$); 31.1 (C(9)); 41.4, 42.2 (C(5), C(8)); 77.9 C(6); 127.4, 127.6 (C(2), C(3)) 129.1, 130.8 (C(1), C(4)); 136.2, 140.9 (C(4a), C(9a)); 209.5 (CO(7)).

7.3.9. 6-Hydroxy-5,6,8,9- tetrahydro-benzocyclohepten-7-one, oxime (17)

A suspension of crude **16** (from **9** (3.98 g, 24.8 mmol)) and $NH_2OH{\cdot}HCl$ (2.09 g, 30 mmol, 1.2 equiv) in pyridine (40 ml) was stirred at rt under Ar for 3.5 h. The solvent was evaporated, the residue dissolved in AcOEt and the organic solution washed with 2 M aqueous NH_4Cl and with brine, dried ($MgSO_4$) and evaporated to give crystallised **17** (3.04 g, 64%) after washing with toluene. Crystallised major oxime: Colorless crystals, mp 122–124 °C (PhMe). IR (KBr): 706, 753, 836, 915, 949, 1009, 1045, 1058, 1455, 1492, 1654, 2919, 3256, 3489 cm^{-1}. ^{1}H NMR ($CDCl_3$): 2.31 (m, 1H, Hb-8); 2.83 (m, 2H, CH_2(9)); 3.06 (dd, 1H, J = 9.6, 14.2 Hz, Ha-5); 3.16 (dd, 1H, J = 2.8, 14.2 Hz, Hb-5); 3.22 (m, Ha-9); 4.32 (dd, 1H, J = 2.8, 9.6 Hz, H-6)); 7.20 (m, 3Har); 7.26 (m, 1Har). ^{13}C NMR (1:1, CD_3OD/$CDCl_3$): 23.2 (C(9)); 31.7 (C(5)); 41.8 (C(8)); 70.8 (C(6)); 126.7, 127.1 (C(2), C(3)); 128.9, 130.9 (C(1), C(4)); 135.7 (C(4a)); 141.4 (C(9a)); 161.5, (C(7)). Anal. Calcd for $C_{11}H_{13}NO_2$ (191.23): C, 69.08; H, 6.85; N, 7.32. Found: C, 69.2; H, 7.0; N, 7.2.

Minor oxime, colorless resin, ^{1}H NMR ($CDCl_3$): 2.52 (m, 1H, Hb-8); 2.82 (m, 2H, CH_2(9)); 3.02 (m, 1H, Ha-8); 3.08 (dd, 1H, Hb-5); 3.13 (dd, 1H, Ha-5); 4.37 (dd, 1H, H-6); 7.16–7.25 (m, 4Har); J(5a,6) = 3.0, J(5b,6) = 5.3, J(5a,5b) = 14.1 Hz.

7.3.10. 7-(*tertio*-Butyloxycarbonylamino)-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-6-ol (19a)

a. *From oxime* **17**. A solution of **17** (0.80 g, 4.18 mmol) in EtOH (20 ml) and aqueous concd NH_4OH (3 ml) was hydrogenolysed over wet Raney-Ni (2 g) at rt for 15 h. The catalyst was discarded by centrifugation or filtration on celite and the solvent evaporated to give **18**. A solution of crude **18** (4.18 mmol) in MeOH (20 ml) and $(Boc)_2O$ (1.36 g, 7.84 mmol, 1.5 equiv) was stirred under Ar for 15 h at rt, then evaporated. The obtained crystals were washed with iPr$_2$O to give **19a** (0.65 g, 56%) as 1:1 *cis*–*trans* mixture.

b. *From silyloxyketone* **16**. A solution of **16** (from 1.07 g of **8**, 6.68 mmol) and Ti(O*i*Pr)$_4$ (4.0 mL, 13.3 mmol, 2.0 equiv) in a 2–3 M solution of dry NH_3 in EtOH (20 mL) was stirred at rt for 6 h. $NaBH_4$ (380 mg, 10 mmol, 1.5 equiv) was then added and the solution was stirred for further 2 h. The mixture was evaporated, the residue dissolved in AcOEt and stirred with 1 *N* aqueous NH_4OH (10 ml). The precipitate was filtrated off, washed with AcOEt/1 *N* aqueous NH_4OH and the filtrate extracted with AcOEt. The organic phases were then dried ($MgSO_4$) and evaporated to give crude **18**. A solution of crude **18** (6.68 mmol), Na_2CO_3 (780 mg, 7.34 mmol, 1.1 equiv) and Boc_2O (3.2 g, 14.6 mmol, 2.2 equiv) in MeOH (10 mL) was stirred at rt for 2 h under Ar. Precipitation with H_2O (20 ml), filtration, washing with H_2O and iPr$_2$O and drying led to **19a** (985 mg, 53%) as crystalline mixture of 50:50 *cis* and *trans* isomers which can be separated by flash chromatography (AcOEt/cyclohexane 1:1).

trans-**19a**: colorless crystals, mp 174–175 °C (*i*PrOH). IR (KBr): 756, 882, 1003, 1034, 1172, 1246, 1321, 1370, 1453, 1526, 1683, 2933, 2979, 3370 cm^{-1}. ^{1}H NMR ($CDCl_3$): 1.36 (m, 1H, Hb-8); 1.47 (s, 9H, CMe_3); 2.19 (dddd, 1H, Ha-8); 2.73 (ddd, 1H, Hb-9); 2.83 (ddd, 1H, Ha-9); 2.99 (dd, 1H, Hb-5); 3.02 (dd, 1H, Ha-5); 3.36 (ddd, 1H, H-6); 3.70 (br ddt, 1H, H-7); 4.54 (br s, 1H, NH-7); 7.05–7.20 (m, 4Har); J(5a,5b) = 14.0, J(5a,6) = 8.8, J(5b,6) = 3.6, J(6,7) = 9.2, J(7,8a) = 4.1, J (7,8b) = 11.5, J(NH,7) = ca 9; J(8a,8b) = 13.6, J(8a,9a) = 1.8, J(8a,9b) = 7.5, J(8b,9a) = 11.2, J(8b,9b) = 2.0, J(9a,9b) = 14.8 Hz. ^{13}C NMR ($CDCl_3$): 28.3 (C*Me*$_3$); 31.8 (C(9)); 32.7 (C(8)); 41.6 (C(5)); 60.0 (C(7)); 74.4 (C(6)); 80.2 (*C*Me$_3$); 126.7, 127.0, 128.6, 130.2 (4CHar); 136.5, 142.0 (C(4a), C(9a)); 156.8 (CO).

cis-**19a**: colorless crystals, mp 180–181 °C (*i*PrOH). IR (KBr): 751, 867, 957, 1013, 1045, 1081, 1166, 1250, 1372, 1393, 1453, 1528, 1664, 2936, 2982, 3005, 3377, 3472 cm^{-1}. ^1H NMR ($CDCl_3$): 1.46 (s, 10H, Hb-8, CMe_3); 2.00 (dq, 1H, *J* = 13.2, 4.4, Ha-8); 2.75 (m, 2H, $CH_2(9)$); 3.05 (dd, 1H, Hb-5); 3.08 (dd, 1H, Ha-5); 3.82 (dddd, 1H, H-7); 4.11 (br dddd, 1H, H-6); 5.06 br d, 1H, NH-7); 7.11–7.20 (m, 4Har); *J*(5a,5b) = 14.6, *J*(5a,6) = 1.6, *J*(5b,6) = 7.2, *J*(6,7) = 2.2, *J*(6,OH) = 7.8, *J*(7,NH) = 9.6, *J*(7,8a) = 4.4, *J*(7,8b) = 11.2 Hz. ^{13}C NMR ($CDCl_3$): 28.4 (CMe_3); 28.8 (C(8)); 32.3 (C(9)); 39,2 (C(5)); 56.9 (C(7)); 69,3 (C(6)); 79,4 (CMe_3); 126.7, 127.6, 128.9, 131.8 (4Car); 133.8, 142.6 (C(4a), C(9a)); 155.3 (CO). Anal. Calcd for $C_{16}H_{23}NO_3$ (277.37) for the *cis*–*trans* mixture: C, 69.29; H, 8.36; N, 5.05. Found: C, 69.3; H, 8.4; N, 4.9.

7.3.11. *cis* and *trans*-7-amino-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-6-ol (*cis*-18 and *trans*-18)

trans-**18**: to a solution of *trans*-**19a** (8 mg, 0.035 mmol) in dioxane (0.3 ml) was added 5 N HCl in dry Et_2O (0.2 ml, 1 mmol). After 2 d at rt, the precipitate of *trans*-**18** (6 mg, 97%) was isolated by centrifugation, washed with dry Et_2O (0.5 ml) and dried as colorless crystals, mp >250 °C. IR (KBr): 751, 762, 1041, 1092, 1449, 1494, 1586, 1613, 2927, 3440 cm^{-1}. ^1H NMR (CD_3OD): 1.46 (dddd, 1H, Hb-8); 2.26 (dddd, 1H, Ha-8); 2.82 (ddd, 1H, Hb-9); 2.90 (ddd, 1H, Ha-9); 2.93 (dd, 1H, Hb-5); 3.11 (dd, 1H, Ha-5); 3.24 (ddd, 1H, H-7); 3.38 (ddd, 1H, H-6); 7.13–7.23 (m, 4Har); *J*(5a,5b) = 14.0, *J*(5a,6) = 10.5, *J*(5b,6) = 2.1, *J*(6,7) = 9.7, *J*(7,8a) = 4.0, *J* (7,8b) = 12.2, *J*(8a,8b) = 13.4, *J*(8a,9a) = 1.6, *J*(8a,9b) = 7.2, *J*(8b,9a) = 11.6, *J*(8b,9b) = 1.9, *J*(9a,9b) = 14.8 Hz. ^{13}C NMR (CD_3OD): 31.5 (C(8)); 32.0 (C(9)); 43.3 (C(5)); 61.2 (C(7)); 72.3 (C(6)); 128.2, 128.4, 130.0, 131.0 (4Car); 137.4, 142.6 (C(4a), C(9a)). HR-MS calcd for $C_{11}H_{16}N$ $[M+H]^+$: 178.1232; found: 178.1226.

cis-**18**: same procedure with *cis*-**19a** (22 mg, 0.08 mmol, containing 10% of *trans*-isomer) in dioxane (0.6 ml) and 5 N HCl in dry Et_2O (0.4 ml, 2 mmol) to give *cis*-**18** (14 mg, 82%, containing 10% of *trans*-isomer) as colorless crystals, mp >250 °C. IR (KBr): 689, 747, 757, 979, 1029, 1185, 1240, 1454, 1495, 1511, 2920, 3020, 3596 cm^{-1}. ^1H NMR (CD_3OD): 1.83 (br dt, 1H, *J*(7,8b) = 11.2, *J*(8a,8b) = 14 Hz, Hb-8); 1.93 (m, 1H, Ha-8), 2.78–2.92 (m, 2H, $CH_2(9)$); 3.08 br m, 2H, $CH_2(5)$); 3.48 (ddd, 1H, *J*(6,7) = 2.8, *J*(7,8a) = 4.0, *J* (7,8b) = 11.2 Hz, H-7); 4.16 (br s, 1H, H-6); 7.13–7.23 (m, 4Har). ^{13}C NMR (CD_3OD): 27.4 (C(8)); 32.0 (C(9)); 39,8 (C(5)); 58.4 (C(7)); 67.4 (C(6)); 127.7, 128.1, 129.7, 132.4 (4Car); 136.7, 142.4 (C(4a), C(9a)). HR-MS calcd for $C_{11}H_{16}N$ $[M+H]^+$: 178.1232; found: 178.1226.

7.3.12. 7-(Benzyloxycarbonylamino)-6,7,8,9-tetrahydro-5*H*-benzocyclohepten-6-ol (19b)

a. *From oxime* **17**. Compound **19b** was obtained with the same procedure as above from **17** (2.47 g, 12.9 mmol) and Raney-Ni (wet 6.2 g) in EtOH (25 ml) and concd NH_4OH (7.5 ml). Crude **18** was N-protected in THF (40 ml) with stirring under Ar at 0 °C with NEt_3 (5 ml) and $ClCO_2Bn$ (2.6 ml, 18.1 mmol, 1.4 equiv). The solution was stirred at rt for 10 h, then hydrolysed with 2 M aqueous NH_4Cl (20 mL), extracted with AcOEt (100 mL) and the organic solution was washed with brine (20 mL), dried ($MgSO_4$) and evaporated. The obtained crystals were washed with iPr_2O to give pure **19b** (2.17 g, 54%).

b. *From silyloxyketone* **16**. Compound **19b** was obtained with the same procedure as above from **16** (3.0 g, 18.7 mmol). A solution of crude **18** in THF (40 ml) was stirred with Na_2CO_3 (5.6 g, 52.4 mmol, 2.8 equiv) and $ClCO_2Bn$ (2.6 ml, 18.1 mmol, 1.4 equiv) at rt for 16 h. Work up as for a) gave pure **19b** (3.0 g, 52%). Colorless crystals, 70:30 isomeric *cis*/*trans* mixture, mp 148–150 °C (toluene). IR (KBr): 697, 751, 1017, 1039, 1085, 1130, 1249, 1315, 1453, 1534, 1673, 1685, 2942, 3318, 3401 cm^{-1}. ^1H NMR ($CDCl_3$, 70:30 *cis* and *trans* isomers): 1.3–1.5 (m, 1H *cis* + *trans*); 2.04 (m, 1H, Ha-8 *cis*); 2.23 (m, 1H, Ha-8 *trans*); 2.7–2.9 (m, 2H *cis* + *trans*); 3.0–3.2 (m, 2H *cis* + *trans*); 3.41 (t, *J* = 9 Hz, 1H, H-6 *trans*); 3.79 (q, *J* = 8 Hz, 1H, H-7 *trans*); 3.88 (s, 1H, H-7 *cis*); 4.13 (s, 1H, H-6 *cis*); 4.77 (s, 1H, NH-7 *trans*); 5.11 (m, 2H, CH_2(Bn) *cis* + *trans*); 5.39 (d, *J* = 8 Hz, 1H, NH-7 *cis*); 7.1–7.4 (m, 9Har *cis* + *trans*). ^{13}C NMR ($CDCl_3$): 28.8 (C(8) *cis*); 31.8 (C(9) *trans*); 32.3 (C(9) *cis*); 32.8 (C(8) *trans*); 39.2 (C(5) *cis*); 41.6 (C(5) *trans*); 57.6 (C(7) *cis*); 60.6 (C(7) *trans*); 66.8, 67.3 (CH_2Ph *cis* + *trans*); 69.3 (C(6) *cis*); 74.2 (C(6) *trans*); 126.6, 126.89, 126.91, 127.2, 127.9, 128.26, 128.37, 128.41, 128.66, 128.72, 128.75, 129.1 (12CHar); 130.4 (CHar *trans*); 132.0 (CHar *cis*); 133.9 (C(4a) *cis*); 136.3, 136.4, 136.6 (Cs(Ph) *cis* + *trans*, C(4a) *trans*); 142.2 (C(9a) *trans*); 142.7 (C(9a) *cis*); 155.8 (NCO *cis*); 157.1 (NCO *trans*). Anal. Calcd for $C_{19}H_{21}NO_3$ (311.37): C, 73.29; H, 6.80; N, 4.50. Found: C, 73.5; H, 6.9; N, 4.4.

7.3.13. 7-(*tertio*-Butyloxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-6-one (20a)

To a solution of **19a** (1.0 g, 3.6 mmol) in CH_2Cl_2 (20 ml) was added at rt DMP (2.3 g, 5 mmol, 1.5 equiv) portionwise or dissolved in CH_2Cl_2. The mixture was stirred for 3 h at rt, and reduced by stirring with a solution of sodium thiosulfate (4 g, 7 equiv) in aqueous N $NaHCO_3$ (20 mL) for 1 h. The mixture was extracted with AcOEt (100 mL) and the organic phase washed with aqueous N $NaHCO_3$ (20 mL) and brine (20 mL), dried ($MgSO_4$) and evaporated. Purification by flash chromatography (AcOEt/Cyclohexane 1:9) and washing with Et_2O gave pure **20a** (765 mg, 77%), colorless crystals, mp 150–152 °C (*i*PrOH). IR (KBr): 750, 759, 1007, 1050, 1156, 1169, 1250, 1296, 1365, 1451, 1491, 1522, 1688, 1723, 2933, 2976, 3004, 3380 cm^{-1}. ^1H NMR ($CDCl_3$): 1.43 (s, 9H, CMe_3); 1.46 (m, 1H, Hb-8); 2.63 (dddd, 1H, Ha-8); 2.89 (ddd, 1H, Hb-9); 3.03 (ddd, 1H, Ha-9); 3.60 (d, 1H, Hb-5); 3.95 (d, 1H, Ha-5); 4.55 (dt, 1H, H-7); 5.43 (d, 1H, NH); 7.18 (m, 4Har); *J*(5a,5b) = 14.6, *J*(7,NH) = 7.0, *J*(7,8a) = 7.0, *J*(7,8b) = 11.3, *J*(8a,8b) = 12.8, *J*(8a,9a) = 3.4, *J*(8a,9b) = 9.0, *J*(8b,9a) = 8.7, *J*(8b,9b) = 3.4, *J*(9a,9b) = 14.6 Hz. ^{13}C NMR ($CDCl_3$): 28.4 (CMe_3); 31.1 (C(9)); 34.8 (C(8)); 48.2 (C(5)); 60.9 (C(7)); 79.8 (CMe_3); 127.6, 128.0 (C(2), C(3)); 129.3, 129.8 (C(1), C(4)); 132.3 (C(4a)); 140.3 (C(9a)); 155.1 (NCO); 204.9 (C(6)). Anal. Calcd for $C_{16}H_{21}NO_3$ (275.34): C, 69.79; H, 7.69; N, 5.09. Found: C, 69.7; H, 7.8; N, 4.9.

7.3.14. 7-(Benzyloxycarbonylamino)-6,7,8,9-tetrahydrobenzocyclohepten-6-one (20b)

Same procedure as for **20a** with **19b** (2.71 g, 8.7 mmol), DMP (5.2 g, 12 mmol, 1.4 equiv) in CH_2Cl_2 (40 ml) to obtain **20b** (2.48 g, 92%) as colorless crystals, mp 119–121 °C (*i*PrOH). IR (KBr): 697, 737, 751, 1015, 1238, 1247, 1531, 1685, 1718, 2938, 3338 cm^{-1}. ^1H NMR ($CDCl_3$): 1.50 (dddd, 1H, Hb-8); 2.68 (m, 1H, Ha-8); 2.90 (ddd, 1H, Hb-9); 3.06 (ddd, 1H, Ha-9); 3.60 (d, 1H, Hb-5); 3.97 (d, 1H, Ha-5); 4.61 (dt, 1H, H-7); 5.09 (s, 2H, CH_2Ph); 5.71 (d, 1H, NH); 7.20 (m, 4Har); 7.34 (m, 5Har); *J*(5a,5b) = 14.2, *J*(7,NH) = 7.5, *J*(7,8a) = 6.5, *J*(7,8b) = 11.2, *J*(8a,8b) = 12.7, *J*(8a,9a) = 3.2, *J*(8a,9b) = 9.0, *J*(8b,9a) = 9.0, *J*(8b,9b) = 3.2, *J*(9a,9b) = 14.6 Hz. ^{13}C NMR ($CDCl_3$): 31.0 (C(9)); 34.7 (C(8)); 48.1 (C(5)); 61.4 (C(7)); 66.9 (CH_2Ph); 127.6, 128.1, 128.2, 128.3; 128.6 (5Car); 129. 4, 129.8 (C(1), C(4)); 132.1 (C(4a)); 136.4 (Car-s); 140.3 (C(9a)); 155.5 (NCO_2); 204.3 (CO(6)). Anal. Calcd for $C_{19}H_{19}NO_3$ (309.37): C, 73.77; H, 6.19; N, 4.53. Found: C, 73.5; H, 6.2; N, 4.4.

7.3.15. 7-Amino-5,7,8,9-tetrahydro-benzocyclohepten-6-one, hydrochloride (4)

From **20a**. A solution of **20a** (765 mg, 2.78 mmol) was stirred in dioxane (5 mL) and HCl 2.2 N in Et_2O (5 mL) at rt under Ar for 72 h. The precipitate was isolated by filtration and recrystallised in *i*PrOH/Et_2O to give pure **4** (500 mg, 85%).

From **20b**. A solution of **20b** (200 mg, 0.65 mmol) in EtOH (10 ml) and aqueous 1 N HCl (0,8 ml) was hydrogenolysed over 5% Pd/C (20 mg) at rt for 1 h. The catalyst was discarded by centrifugation or filtration on celite, the solution evaporated and the product recrystallised in *i*PrOH/Et_2O to give pure **4** (120 mg, 60%). Colorless crystals, mp 230–240 °C (dec.) (*i*PrOH/Et_2O). IR (KBr): 448, 762, 1042, 1050, 1146, 1226, 1278, 1446, 1454, 1489, 1597, 1723, 2625, 2904, 2970, 3425 cm^{-1}. ^1H NMR (D_2O) 80:20 ketone–hydrate mixture). *Ketone*: 1.69 (q, 1H, Hb-8); 2.61 (m, 1H, Ha-8); 3.06 (ddd, 1H, Hb-9); 3.21 (ddd, 1H, Ha-9); 3.72 (d, 1H, Hb-5); 4.23 (d, 1H, Ha-5); 4.50 (dd, 1H, H-7); 7.24–7.32 (m, 4Har); *J*(5a,5b)) = 14.4, *J*(7,8a) = 7.0, *J*(7,8b) = 12.0, *J*(8a,8b) = 13.0, *J*(8a,9a) = 3.2, *J*(8a,9b) = 8.5, *J*(8b,9a) = 9.5, *J*(8b,9b) = 3.2, *J*(9a,9b) = 15.0 Hz. *Hydrate*: 1.46 (m, 1H, Hb-8); 2.28 (m, 1H, Ha-8); 2.88 (m, 2H, CH_2(9)); 3.10, 3.40 (2d, 2H, *J* = 14.5 Hz, CH_2(5)); 3.54 (dd, 1H, *J* = 4.2, 12.7 Hz, H-7); 7.32 (s, 4Har). ^1H NMR (CD_3OD): 1.68 (dddd, 1H, Hb-8); 2.53 (m, 1H, Ha-8); 3.03 (ddd, 1H, Hb-9); 3.25 (ddd, 1H, Ha-9); 3.62 (d, 1H, Hb-5); 4.21(d, 1H, Ha-5); 4.38 (dd, 1H, H-7); 7.19–7.26 (m, 4Har); *J*(5a,5b) = 13.8, *J*(7,8a) = 6.8, *J*(7,8b) = 12.1, *J*(8a,8b) = 12.8, *J*(8a,9a) = 2.8, *J*(8a9b) = 8.2, *J*(8b9a) = 10.0, *J*(8b,9b) = 3.0, *J*(9a,9b) = 14.8 Hz. ^{13}C NMR (CD_3OD), *ketone*: 31.0 (C(9)); 33.3 (C(8)); 48.0 (C(5)); 61.2 (C(7)); 128.9, 129.2 (C(2), C(3)); 130.4, 130.7 (C(1), C(4)); 133.0 (C(4a)); 141.3 (C(9a)); 202.4 (CO(6)). HR-MS (FAB^+), calcd for $C_{10}H_{14}NO$ $[M+H]^+$: 176.1076; found: 176.1067.

7.4. Heptanone series

7.4.1. 2-Bromocycloheptanone (22)

Compound **21** (0.5 mL, 4.5 mmol) was added dropwise to a stirred mixture of NBS (0.8 g, 4.5 mmol, 1 equiv) and *p*-toluenesulfonic acid (77 mg, 0.45 mmol). The mixture became warm and liquid. After 2 h 30 stirring, water (20 mL) was added and the solution was extracted with CH_2Cl_2 (3 × 20 mL), the organic phases washed with H_2O, dried over $MgSO_4$ and evaporated to give **22** as colorless oil (0.9 g, quant.). For analytical purpose, a sample was purified by chromatography (cyclohexane/AcOEt 70:30). IR (KBr): 1453, 1711, 2857, 2933 cm^{-1}. ^1H NMR ($CDCl_3$): 1.38 (m, 1H, Hb-4); 1.55–1.59 (m, 1H, Hb-5, Hb-6); 1.77 (m, 1H, Ha-6); 1.78–2.04 (m, 1H, Ha-4, Ha-5); 2.02 (dtd, 1H, *J* = 1.8, 9.6, 14.4 Hz, Hb-3); 2.37 (dddd, 1H, *J* = 1.8, 5.0, 8.8, 14.4 Hz, Ha-3); 2.50 (ddd, 1H, *J* = 2.8, 7.6, 13.2 Hz, Hb-7); 2.86 (ddd, 1H, *J* = 3.4, 10.4, 13.2 Hz, Ha-7); 4.38 (dd, 1H, *J* = 5.0, 9.6 Hz, H-2). ^{13}C NMR ($CDCl_3$) values are identical as in lit.[34,61] IR and ^1H NMR values are in accordance with the lit.[34,35]

7.4.2. 2-Azidocycloheptanone (23)

A solution of **22** (614 mg, 3.2 mmol) in DMF (20 mL) was stirred with NaN_3 (312 mg, 4.8 mmol, 1.5 equiv) for 30 min. Brine (10 mL) was added and the solution extracted with AcOEt (3 × 20 ml). The reunified organic phases were dried ($MgSO_4$) and evaporated to give **23** as colorless oil (490 mg, quant.). For analytical purpose, a sample was purified by chromatography (cyclohexane/AcOEt 70:30). IR (KBr): 1143, 1264, 1455, 1712, 2106, 2862, 2937 cm^{-1}. ^1H NMR ($CDCl_3$): 1.45 (m, 1H); 1.62–1.82 (m, 6H); 2.02 (m, 1H, Ha-3); 2.53 (ddd, 1H, *J* = 4.2, 10.0, 16.0 Hz, Hb-7); 2.61 (ddd, 1H, *J* = 4.2, 6.8, 16.0 Hz, Ha-7); 4.08 (dd, 1H, *J* = 3.6, 9.2 Hz, H-2). ^{13}C NMR ($CDCl_3$): 23.5, 26.5, 28.8 (C(5), C(4), C(6)); 30.7 (C(3)); 41.3 (C(7)); 67.7 (C(2)); 208.6 (C(1)). IR and ^1H NMR values are in accordance with the lit.[33]

7.4.3. 2-aminocycloheptanone (5)

Compound **23** (480 mg, 3.13 mmol) was hydrogenolysed on 5% Pd/C (63 mg) in EtOH (116 mL) and 1 N aqueous HCl (3.1 mL, 3.1 mmol) for 16 h. The catalyst was centrifuged off and the solvent evaporated. The resulted solid was washed with hot AcOEt to give **5** (241 mg, 50%) as colorless solid, mp 208–211 °C. IR (KBr): 938, 1143, 1176, 1226, 1475, 1710, 2936 cm^{-1}. ^1H NMR (D_2O): 1.38 (m, 1H); 1.66–1.97 (m, 6H); 2.12 (m, 1H); 2.56 (ddd, 1H, *J* = 4.0, 11.0, 18.0 Hz, Hb-7); 2.77 (ddd, 1H, *J* = 3.6, 6.0, 18.0 Hz, Ha-7); 4.41 (dd, *J* = 3.2, 10.0 Hz, H-2). ^{13}C NMR (D_2O): 22.9, 27.3, 28.8, 29.6 (C(5), C(4), C(6), (C(3)); 41.4 (C(7)); 60.1 (C(2)); 211.5 (C(1)). HR-MS (QTof), calcd for $C_7H_{14}NO$ $[M+H]^+$: 128.1075; found: 128.1070.

7.5. Insaturated benzosuberone series

7.5.1. 6-Bromo-5,6,8,9-tetrahydrobenzocyclohepten-7-one (24) and 5,6-dihydrobenzocyclohepten-7-one (25)

1. *Oxidation of* ***15*** *with p-benzoquinone*: To a solution of **15** (0.725 g, 3.12 mmol) in CH_3CN (10 mL) were added $Pd(OAc)_2$ (0.35 g, 1.56 mmol, 0.5 equiv) and *p*-benzoquinone (0.17 g, 1.56 mmol, 0.5 equiv). The reaction mixture was stirred at room temperature for 3 days, then filtrated through a pad of celite which was washed with CH_2Cl_2 (3 × 20 ml). The organic layer was washed with H_2O (10 mL), dried over Na_2SO_4 and evaporated. The residue was purified by flash chromatography (AcOEt/cyclohexane, 1:9) to afford **25** (0.367 g, 75%) as a colorless oil. The yields varied between 45% and 75%.

2. *Oxidation of* ***15*** *with* O_2: A solution of **15** (4.5 g, 19 mmol) and $Pd(OAc)_2$ (0.43 g, 1.9 mmol, 0.1 equiv) in DMSO (25 mL) was stirred under an oxygen atmosphere. After 16 h stirring at rt, water (10 mL) was added and the mixture extracted with Et_2O (3 × 20 mL). The organic layer was dried ($MgSO_4$), and evaporated. The residue was purified by distillation to afford **25** (2.59 g, 85%, $Bp_{0.5\ Torr}$ 85–90 °C, the yields varied between 50% and 85%).

3. *Directly from ketone* **9** *and bromoketone* **24**: To a solution of **9** (0.5 g, 3.12 mmol) in MeCN (12 mL) were successively added $SiMe_3OTf$ (0.045 mL, 0.25 mmol, 0.08 equiv) and NBS (0.61 g, 3.5 mmol, 1.1 equiv). The solution was stirred at rt for 4.5 h (TLC monitoring. Treatment of the solution allowed isolating the rather unstable **24**). DBU (1.03 mL, 7.0 mmol, 2.2 equiv) was added dropwise to the solution of crude **24** and the solution was stirred for further 30 minutes, then diluted with Et_2O or AcOEt (100 mL) and washed with brine (2 × 20 mL). The organic layer was dried over $MgSO_4$ and evaporated. Purification by flash chromatography (AcOEt/cyclohexane 2:8) gave **25** (0.42 g, 80%) as a brownish oil, and by further elution, the benzocycloheptene-3-one (40–70 mg, ca 10%, resulting from the elimination of a 6,8-dibromo-compound).

Compound **24**: colorless resin; R_f = 0.64 (AcOEt/cyclohexane 5:5). IR (KBr): 752, 773, 1090, 1188, 1456, 1493, 1703, 2854, 3024 cm^{-1}; ^1H NMR ($CDCl_3$): 2.63 (dddd, 1H, *J* = 1.6, 3.0, 7.8, 13.5 Hz, Hb-8); 2.87 (ddd, 1H, *J* = 3.0, 11.0, 14.8 Hz, Hb-9); 2.97 (ddd, 1H, *J* = 3.6, 7.8, 14.8 Hz, Ha-9); 3.17 (ddd, 1H, *J* = 3.6, 11.0, 13.5 Hz, Ha-8); 3.25 (dd, 1H, *J* = 7.8, 15.0 Hz, Hb-5); 3.44 (dd, *J* = 2.8, 15.0 Hz, Ha-5); 4.57 (ddd, *J* = 1.6, 2.8, 7.8 Hz, H-6); 7.20 (m, 4Har). ^{13}C NMR ($CDCl_3$): 30.4 (C(9)); 39.1 (C(5)); 39.5 (C(8)); 53.5 (C(6)); 127.2, 128.5, 132.1, 135.8 (4Car); 136.4, 140.0 (C(4a)), C(9a)); 203.8 (C(7)). Too unstable for MS measurement.

Compound **25**: colorless oil, R_f = 0.57 (AcOEt/cyclohexane 5:5). IR (KBr): 3022, 2953, 1662, 1612, 1275, 1202, 1101, 815, 751, 543, 443 cm^{-1}. ^1H NMR ($CDCl_3$): 2.76 (m, 2H, H-6); 3.01 (m, 2H, H-5); 6.18 (d, 1H, *J* = 12.6 Hz, H-8); 7.13 (d, 1H, *J* = 12.6 Hz, H-9); 7.22–7.37 (m, 4Har). ^{13}C NMR ($CDCl_3$): 29.3 (C(5)); 42.2 (C(6)); 132.6, 130.1, 129.1, 129.0, 127.1 (4CHar, C(8)); 134.2 (C(9a)); 141.7 (C(4a)); 142.8 (C(9)); 201.3 (CO(7)). ^1H NMR data are in agreement with those of the lit.[37] HR-MS (ESI^+) calcd for $C_{11}H_{10}NaO$ $[M+Na]^+$: 181.0629; found: 181.0668.

7.5.2. 7-*tert*-Butyldimethylsilyloxy-5*H*-benzocycloheptene (26) and 6-(*tert*-butyl-dimethylsilyl)oxy-5,6-dihydrobenzocyclohepten-7-one (27)

1. *With $Me_2tBuSiOTf$-NEt_3.* To a stirred solution of **25** (3.13 g, 19.8 mmol) in dry CH_2Cl_2 (55 mL) were added under Ar at rt Me_2tBuSiOTf (10.0 mL, 44 mmol, 2.2 equiv) and NEt_3 (6.68 g, 48 mmol, 2.4 equiv). The solution was stirred for another 3 h, then H_2O (50 mL) was added and the mixture extracted with cyclohexane (3×100 mL). The combined organic phases were dried ($MgSO_4$) and evaporated to give crude dienol ether **26** (5 g, 92%). For analytical purpose, a sample was purified by chromatography (eluent: AcOEt/cyclohexane 9:1). To the solution of the crude **26** (5 g, 18.3 mmol) in CH_2Cl_2 (53 mL) was added portionwise *m*-CPBA (3.8 g, 22 mmol, 1.2 equiv) at 0 °C under Ar and the mixture stirred for 16 h. The reaction mixture was diluted with AcOEt (200 mL), washed successively with aqueous Na_2SO_3 solution, aqueous 1 M $NaHCO_3$ (50 mL) and with H_2O (2×50 mL), dried ($MgSO_4$) and evaporated. Purification by flash chromatography (AcOEt/*n*-heptane, 1:9 or cyclohexane/CH_2Cl_2 7:3) gave **27** (3.82 g, 77%).

2. *With $Me_2tBuSiCl$-DBU.* To a stirred solution of **25** (0.1 g 0.62 mmol) in dry CH_2Cl_2 (1 mL) at rt were added DBU (0.22 mL, 1.58 mmol, 2.5 equiv) and $ClSiMe_2t$Bu (0.19 g, 1.26 mmol, 2 equiv). After 3 h stirring, same work-up as above gave **26** (0.18 g, quant.). The oxidation in CH_2Cl_2 (2 mL) with *m*-CPBA (0.13 g, 0.76 mmol, 1.2 equiv, then 0.05 g, 0.5 equiv) at 0 °C gave **27** (136 mg, 75%) after same work-up as above. Compound **26** yellowish oil, R_f (AcOEt/cyclohexane 5:5) = 0.75. IR (KBr): 796, 839, 896, 1169, 1231, 1256, 1636, 1741, 2853, 2929, 2956 cm^{-1}.^{1}H NMR ($CDCl_3$, 400 MHz): 0.07 (s, 6H, $SiMe_2$); 0.89 (s, 9H, CMe_3); 2.94 (d, 2H, *J* = 7.2 Hz, H-5); 5.06 (dt, 2H, *J* = 1.7, 7.2 Hz, H-6); 6.25 (dd, 1H, *J* = 1.7, 11.9 Hz, H-8); 6.99 (d, *J* = 11.9 Hz, H-9); 7.14 (d, 1Har, *J* = 7.5 Hz); 7.20 (ddd, 1Har, *J* = 1.5, 7.1, 7.8 Hz); 7.28 (m, 2Har). ^{13}C NMR ($CDCl_3$): −4.5 ($SiMe_2$); 18.0 (Si*C*Me_3); 25.6 (C*Me*$_3$); 31.2 (C(5)); 106.7 (C(6)); 125.5, 127.2, 128.0, 128.5 (4CHar); 128.7 (C(8)); 133.3 (C(9)); 135.5, 138.5 (C(4a), C(9a)); 149.5 (C(7)). HR-MS (ESI$^+$) calcd for $C_{13}H_{17}OSi$ [M−(C_4H_8)+H]$^+$: 217.1043; found: 217.1043; calcd for $C_{17}H_{25}OSi$ [M+H]$^+$: 273.1669; found: 273.1665.

Compound **27**: colorless resin, R_f = 0.77 (AcOEt/cyclohexane 5:5). IR (KBr): 837, 1119, 1254, 1679, 1725, 2855, 2929, 2954 cm^{-1}. ^{1}H NMR ($CDCl_3$, 400 MHz): 0.02, 0.14 (2 s, 6H, $SiMe_2$); 0.87 (s, 9H, C*Me*$_3$); 2.98 (dd, 1H, *J* = 1.6, 15.0 Hz, Hb-5); 3.39 (dd, 1H, *J* = 12.0, 15.0 Hz, Ha-5); 4.29 (dd, 1H, *J* = 1.6, 12.0 Hz, H-6); 6.09 (d, 1H, *J* = 12.8 Hz, H-8); 7.09 (d, 1H, *J* = 12.8 Hz, H-9); 7.33 (m, 4Har). ^{13}C NMR ($CDCl_3$, 100 MHz): −5.5, −4.5 ($SiMe_2$); 18.5 (Si*C*Me_3); 25.8 (Si*CMe*$_3$); 40.0 (C(5)); 75.9 (C(6)); 125.9 (C(8)); 127.4, 130.2, 130.4, 132.7 (4CHar); 134.1 (C(9a)); 137.5 (C(4a)); 142.5 (C(9)); 199.1 (CO(7)). HR-MS (ESI$^+$): calcd for $C_{17}H_{24}O_2Si$ [M+H]$^+$: 289.1618; found: 289.1602; calcd for [M+Na]$^+$: 311.1438; found: 311.1433.

7.5.3. 6-(*tert*-Butyldimethylsilyl)oxy-6,7-dihydro-5*H*-benzocyclohepten-7-amine (28a), *tert*-butyl 6-(*tert*-butyldimethylsilyl)oxy-6,7-dihydro-5*H*-benzocyclohepten-7-carbamate (28b)

To a solution of **27** (1.80 g, 6.64 mmol) in 2–3 M NH_3 in dry EtOH (40 mL) was added Ti(O*i*Pr)$_4$ (3.9 mL, 13.2 mmol, 2 equiv). After 16 h stirring at rt, $NaBH_4$ (0.3 g, 9 mmol, 1.5 equiv) was added and the mixture stirred for 2 h further. After evaporation of the solvent, the residue was dissolved in AcOEt (50 mL) and stirred with aqueous 1 N NH_4OH (10 mL) for 16 h. The precipitate was filtered off and washed with AcOEt/1 N aqueous NH_4OH (5×20 mL). The organic layer was separated, washed with aqueous N NH_4OH (20 mL), dried over $MgSO_4$ and evaporated to give the crude amine **28a** (as a 80:20 diastereoisomeric mixture). To a solution of the crude **28a** (6.64 mmol) in MeOH (20 mL) were added at rt $NaHCO_3$ (0.462 g, 5.49 mmol, 1 equiv) and Boc_2O (1.8 g, 8.24 mmol, 1.5 equiv). After 16 h stirring, the reaction mixture was diluted with ether (50 mL), washed with brine (10 mL), dried over $MgSO_4$ and evaporated. Purification by flash chromatography (AcOEt/cyclohexane 1:9) gave pure **28b** (1.48 g, 74%) as a 80:20 diastereoisomeric mixture.

Compound **28a**, only characterised by NMR. ^{1}H NMR ($CDCl_3$, 300 MHz), major diastereoisomer: 0.06, 0.08 (2s, 6H, $SiMe_2$); 0.88 (s, 9H, CMe_3); 2.67 (ddd, 1H, *J* = 1.5, 2.0, 14.3 Hz, Hb-5); 3.04 (dd, 1H, *J* = 10.0, 14.3 Hz, Ha-5); 3.49 (tdd, 1H, *J* = 1.5, 4.2, 4.8, H-7); 3.99 (ddd, 1H, *J* = 2.0, 4.2, 10.0 Hz, H-6); 5.42 (br s, 2H, NH_2); 5.81 (dd, 1H, *J* = 4.8, 12.2 Hz, H-8); 6.44 (dd, 1H, *J* = 1.5, 12.2, H-9); 7.17 (m, 4Har); minor diastereoisomer, partial data: 0.15, 0.08 (2s, 6H, $SiMe_2$); 0.92 (s, 9H, CMe_3); 2.99 (dd, 1H, *J* = 7.2, 10.4 Hz, Ha-5); 5.78 (dd, 1H, *J* = 4.0, 10.0 Hz, H-8).

Compound **28b:** yellowish resin. IR (KBr): 1074, 1169, 1254, 1494, 1717, 2857, 2930, 2955, 2978, 3454 cm^{-1}. ^{1}H NMR ($CDCl_3$, 300 MHz) major diastereoisomer: 0.05, 0.08 (2s, 6H, $SiMe_2$); 0.82 (s, 9H, Si*CMe*$_3$); 1.43 (s, 9H, C*Me*$_3$); 2.76 (dd, 1H, *J* = 9.0, 13.5 Hz, Hb-5); 2.93 (dd, 1H, *J* = 4.0, 13.5 Hz, Ha-5); 4.23 (br dddd, 1H, *J* = 2.2, 4.2, 4.5, 9.0H-7); 4.37 (dddd, 1H, *J* = 1.0, 4.0, 4.5, 9.0, H-6); 5.00 (br d, 1H, *J* = 9.0 Hz, NH); 5.73 (ddd, 1H, *J* = 1.0, 4.2, 11.5 Hz, H-8); 6.55 (dd, 1H, *J* = 2.2, 11.5 Hz, H-9); 7.13 (m, 4Har); minor diastereoisomer, partial data: 0.07 (s, 6H, $SiMe_2$); 0.83 (s, 9H, Si*CMe*$_3$); 1.43 (s, 9H, CMe_3); 2.85 (dd, 1H, *J* = 2.0, 14.6 Hz, Hb-5); 2.98 (dd, 1H, *J* = 6.8, 14.6 Hz, Ha-5); 4.04 (m, 2H, H-6 and H-7); 5.78 (m, 1H, H-8); 6.45 (dd, 1H, *J* = 2.0, 12.2 Hz, H-9). ^{13}C NMR ($CDCl_3$, 100 MHz): Major diastereoisomer: −4.5, −5.0 ($SiMe_2$); 18.0 (Si*C*Me_3); 25.7 (Si*CMe*$_3$); 28.4 (O*CMe*$_3$); 41.1 (C(5)); 54.1 (C(7)); 76.6 (C(6)); 79.3 (O*C*Me_3); 126.5, 127.2, 129.4, 130.0 (4CHar); 130.3 C(9)); 131.5 (C(8)); 136.2, 136.8 (C(4a), C(9a)); 155.2 (NCO). HR-MS (ESI$^+$): calcd for $C_{22}H_{35}NNaO_3Si$ [M+Na]$^+$: 412.2284; found: 412.2271.

7.5.4. 7-(*tert*-Butyloxycarbonyl)amino-6,7-dihydro-5*H*-benzocyclohepten-6-ol (28c), 7-(*tert*-butyloxycarbonyl)amino-5,7-dihydro-benzocyclohepten-6-one (29)

To a solution of **28b** (414 mg, 1.06 mmol) in Et_2O (5 mL) was added $Bu_4NF{\cdot}3H_2O$ (0.5 g, 1.58 mmol, 1.5 equiv). After 16 h stirring at rt under Argon, the solution was diluted with Et_2O (50 mL), washed successively with a 20% aqueous NH_4Cl solution (2×10 mL) and then with brine (10 mL), dried over Na_2SO_4 and evaporated. **28c** (207 mg, 70%) was crystallised in *i*Pr_2O as a 80:20 diastereoisomeric mixture.

To a solution of **28c** (43 mg, 0.16 mmol) in dry CH_2Cl_2 (10 mL) was added DMP (0.121 g, 0.29 mmol, 1.5 equiv). After 1 h stirring at rt, AcOEt (25 mL) and H_2O (10 mL) were then added to the reaction mixture that was stirred for 2 h further. The organic layer was separated, washed successively with aqueous 1 N $NaHCO_3$ (10 mL), aqueous Na_2SO_3 (10 mL), and brine (10 mL), dried over Na_2SO_4 and concentrated to give **29** (42 mg, quant.)

Compound **28c**: Major diastereoisomer, isolated by crystallisation in *i*Pr_2O. Colorless crystals, mp 121–123 °C (*i*Pr_2O). IR (KBr): 1164, 1325, 1389, 1529, 1667, 2875, 2954, 2975, 2988, 3360, 3501 cm^{-1}.^{1}H NMR ($CDCl_3$, 300 MHz): 1.45 (s, 9H, C*Me*$_3$); 1.85 (s, 1H, OH); 2.95 (dd, 1H, Hb-5); 3.06 (dd, 1H, Ha-5); 4.26 (ddd, 1H, H-6); 4.42 (br dddd, 1H, H-7); 5.18 (br d, 1H, NH); 5.70 (dd, 1H, H-8); 6.51 (dd, 1H, H-9); 7.19 (m, 4Har); *J*(5a,5b) = 14.4; *J*(5a,6) = 2.8 Hz; *J*(5b,6) = 9.2; *J*(6,7) = 4.2; *J*(7,8) = 4.4; *J*(7,9) = 2.0; *J*(7,NH) = 8.4; *J*(8,9) = 12.0 Hz. ^{13}C NMR ($CDCl_3$, 100 MHz): 28.4 (C*Me*$_3$); 40.0 (C(5)); 55.0 (C(7)); 73.1 (C(6)); 80.0 (*C*Me_3); 127.0, 128.0, 130.8, 130.9 (4 CH-ar); 128.9 (C(8)); 130.9 (C(9)); 135.0, 135.4 (C-(4a), C-(9a)); 156.1 (NCO). HR-MS (ESI$^+$) calcd for $C_{16}H_{21}NO_3$ [M+Na]$^+$: 298.1414; found: 298.1410.

Minor diastereoisomer, only characterised by ^{1}H NMR ($CDCl_3$, 300 MHz), partial data: 1.45 (s, 9H, C*Me*$_3$); 2.92 (dd, 1H, *J* = 2.5, 14.4 Hz, Hb-5); 3.05 (dd, 1H, *J* = 7.6, 14.4 Hz, Ha-5); 4.01 (dt, 1H,

J = 2.5, 7.6 Hz, H-6); 4.42 (m, 1H, *J* = 2.2, 4.4, 7.6, H-7); 5.68 (dd, 1H, *J* = 4.4, 12.0 Hz, H-8); 6.50 (dd, 1H, *J* = 2.2, 12.0 Hz, H-9).

Compound **29**: colorless crystals, mp 155–157 °C. IR (KBr): 3355, 2969, 2931, 1732, 1688, 1521, 1390, 1152, 1051, 1023 cm^{-1}. ^1H NMR ($CDCl_3$, 300 MHz): 1.43 (s, 9H, CMe_3); 3.65 (d, 1H, *J* = 17.7 Hz, Hb-5); 3.76 (d, 1H, *J* = 17.7 Hz, Ha-5); 4.66 (br dd, 1H, *J* = 2.5, 4.3 Hz, H-7); 5.72 (br s, 1H, NH); 5.82 (dd, 1H, *J* = 4.3, 10.6 Hz, H-8); 6.87 (dd, 1H, *J* = 2.5, 10.6 Hz, H-9); 7.25 (m, 4Har). ^{13}C NMR ($CDCl_3$, 75 MHz): 28.9 (O*CMe*$_3$); 47.7 (C(5)); 60.8 (C(7)); 80.2 (O*C*Me$_3$); 127.2, 128.1, 129.2, 130.1, 130.4, 130.7 (4CHar, C(8), C(9)); 132.4, 136.6 (C(4a), C(9a)); 155.8 (NCO); 207.6 (C(6)). HR-MS (ESI^+) calcd for $C_{16}H_{19}NNaO_3$ $[M+Na]^+$: 296.1263; found: 296.1268.

7.6. Transformations of 29

7.6.1. 7-*tert*-Butoxycarbonylamino-5,9-dihydrobenzocyclohepten-6-one (30)

To a solution of **29** (2 mg) in $CDCl_3$ (0.7 ml), was added NEt_3 (5 μl). The isomerisation was achieved after 2 h at rt (NMR monitoring). After addition of some silicagel and centrifugation, a solution of **30** in $CDCl_3$ was obtained. ^1H NMR ($CDCl_3$): 1.44 (s, 9H, CMe_3); 3.74 (d, 2H, *J* = 7.0 Hz, CH_2(9)); 3.98 (s, 2H, CH_2(5)); 7.19 (m, 4Har); 7.74 (t, 1H, *J* = 7.0 Hz, H-8). ^{13}C NMR ($CDCl_3$ 75 MHz): 28.3 (*CMe*$_3$); 33.7 (C(9)); 50.0 (C(5)); 80.3 (*C*Me$_3$); 126.1 (C(8)); 127.2, 127.3, 127.9, 129.2 (4CHar); 132.0, 134.0 (C(4a), C(7)); 140.0 (C(9a)); 153.1 ($NHCO_2$); 190.4 (CO(6)). HR-MS (ESI^+): calcd for $C_{16}H_{18}NO_3$ $[M-H]^+$ (aromatisation): 272.1287; found: 272.1276.

7.6.2. 6-Hydroxybenzocyclohepten-7-imine (31a)

A solution of **29** (2 mg) in aqueous 2 N HCl in H_2O (0.7 mL) was studied in ^1H NMR. The signals of **31a** were only observed, ^1H NMR (2 N HCl in H_2O): 7.50 (d, 1H, H-8); 7.81 (dd, 1H, H-2); 7.89 (dd, 1H, H-3);7.99 (s, 1H, H-5) 7.99 (d, 1H, H-4); 8.06 (d, 1H, H-1); 8.32 (d, 1H, H-9). *J*(1,2) = 7.9, *J*(2,3) = 7.1, *J*(3,4) = 7.4, *J*(8,9) = 12.3 Hz.

7.6.3. 6-Methoxybenzocyclohepten-7-one and 6-hydroxybenzocyclohepten-7-one (benzotropolone 31b)

The methoxybenzotropolone was prepared according to[43]: brownish crystals, mp 85–87 °C (lit.[42] 89–90 °C), yield 87% after purification by flash chromatography (AcOEt/cyclohexane (1:9). ^1H NMR ($CDCl_3$): 3.99 (s, 3H, OMe); 7.01 (d, 1H, *J* = 12.8 Hz, H-8); 7.43 (s, 1H, H-5); 7.61, 7.70 (2ddd, 2H, *J* = 7.9 Hz, 8.3, 1.3 Hz, H-2, H-3); 7.85 (d, 1H, *J* = 12.8 Hz, H-9); 7.87, 7.94 (2 d, 2H, *J* = 7.9 Hz, H-1, H-4).

Benzotropolone **31a** was prepared according to[43] by hydrolysis of the methoxy-benzotropolone: yellow needles, mp 158–160 °C (EtOH) (lit.[43] 159.5–160.5 °C (EtOH)), yield 58% after recrystallisation in EtOH. IR (KBr): 1223, 1249, 1303, 1352, 1426, 1459, 1535, 1570, 1614, 1625 cm^{-1}. ^1H NMR ($CDCl_3$): 7.19 (d, 1H, H-8); 7.55 (ddd, 1H, H-2); 7.59 (s, 1H, H-5); 7.65 (ddd, 1H, H-3); 7.74 (dm, 1H, H-4); 7.79 (dm, 1H, H-1); 7.84 (d, 1H, H-9); *J*(1,2) = 8.0, *J*(1,3) = 1.5, *J*(2,3) = 7.3, *J*(2,4) = 1.5, *J*(3,4) = 7.8, *J*(8,9) = 12.5 Hz. ^1H NMR (D_2O): 7.22 (d, 1H, H-8); 7.66 (dd, 1H, H-2); 7.77 (dd, 1H, H-3); 7.76 (s, 1H, H-5); 7.90 (d, 1H, H-4); 7.97 (d, 1H, H-1); 8.09 (d, 1H, H-9); *J*(1,2) = 8.0, *J*(2,3) = 7.3, *J*(3,4) = 7.9, *J*(8,9) = 12.5 Hz. Data in 2 N HCl in D_2O: 7.25 (d, 1H, H-8); 7.71 (t, 1H, H-2); 7.80 (t, 1H, H-3); 7.84 (s, 1H, H-5); 7.94 (d, 1H, H-4); 8.01 (d, 1H, H-1); 8.15 (d, 1H, H-9); same *J* values as in D_2O. ^{13}C NMR ($CDCl_3$): 118.6 (C(5)); 127.9 (C(2)); 128.3(C(8)); 131.1 (C(3)); 132.3 (C(4)); 132.9 (C(4a)); 134.3 (C(1)); 135.7 (C(9a)); 144.1 (C(9)); 154.8 (C(6)); 180.8 (CO(7).

7.6.4. Dimerisation of 4: 5,6a,7,8,13,14a,15,16-octahydro-dibenzo[*d*,*d*′]pyrazino[2,3-*a*;5,6-*a*′]dicycloheptene (33a) and 5,7,8,13,15,16-hexahydro-dibenzo[*d*,*d*′]pyrazino[2,3-*a*;5,6-*a*′]dicycloheptene (34a)

Compound **4** (5 mg, 0.025 mmol) was stirred in 20 mM Hepes buffer (2 ml) at pH 7.5 at rt. A precipitate appeared immediately and was isolated after 16 h by centrifugation, washing with H_2O and drying under vacuum to give a 75:25 mixture of **33a** and **34a** (ca 3 mg, 60%). In $CDCl_3$ solution, this mixture was quantitatively transformed into **34a** at rt after 16 h and isolated after evaporation of the solvent and washing with Et_2O (0.5 mL) to give **34a** (3 mg, quant.).

Compound **33a**, only characterised by ^1H NMR ($CDCl_3$): 1.42 (ddt, 2H, Hb-7, Hb-15); 2.62 (dddd, 2H, Ha-7, Ha-15); 2.93 (ddd, 2H, Hb-8, Hb-16); 3.07 (ddd, 2H, Ha-8, Ha-16); 3.45 (d, 2H, Hb-5, Hb-13); 3.73 (d, 2H, Ha-5, Ha-13); 4.01 (m, 2H, H-6a, H-14a); 7.10–7.25 (m, 4Har). *J*(5a,5b) = 13.2, *J*(6a,7a) = 5.0, *J*(6a,7b) = 11.0, *J*(7a,7b) = 13.8, *J*(7a,8a) = 2.1, *J*(7a,8b) = 7.7, *J*(7b,8a) = 11.0, *J*(7b,8b) = 2.2, *J*(8a,8b) = 13.8 Hz.

Compound **34a**: yellowish crystals, mp 234–236 °C. IR (KBr): 3023, 2926, 2901, 1488, 1456, 1436, 1428, 1394, 755, 721 cm^{-1}. ^1H NMR ($CDCl_3$): 3.20 (m, 4H); 3.24 (m, 4H); 4.25 (s, 4H, CH_2(5), CH_2(13)); 7.10–7.25 (m, 8Har). ^{13}C NMR ($CDCl_3$): 30.6, 35.4 (C(7),C(8), C(15),C(16)); 41.8 C(5),C(13)); 126.7, 127.4, 128.5, 128.8 (8CHar); 137.7 (C(4a),C(12a)); 140.0 (C(8a),C(16a)); 149.3, 149.7 (C(5a),C(6a), C(13a),C(14a)). HR-MS (FAB^+) calcd for $C_{10}H_{14}NO$ $[M+H]^+$: 313.1699; found 313.1705.

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References and notes

1. Mina-Osorio, P. *Trends Mol. Med.* **2008**, *14*, 361.
2. Xu, Y.; Wellner, D.; Scheinberg, D. A. *Biochem. Biophys. Res. Commun.* **1995**, *208*, 664.
3. Danziger, R. S. *Heart Fail. Rev.* **2008**, *13*, 293.
4. Fortin, J.-Ph.; Gobeil, F.; Adam, A.; Regoli, D.; Marceau, F. *J. Pharmacol. Exp. Ther.* **2005**, *314*, 1169.
5. Bawolak, M. T.; Fortin, J.-P.; Vogel, L. K.; Adam, A.; Marceau, F. *Eur. J. Pharmacol.* **2006**, *551*, 108.
6. Proost, P.; Mortier, A.; Loos, T.; Vandercappellen, J.; Gouwy, M.; Ronsse, I.; Schutyser, E.; Put, W.; Parmentier, M.; Struyf, S.; Van Damme, J. *Blood* **2007**, *110*, 37.
7. Hoffmann, T.; Faust, J.; Neubert, K.; Ansorge, S. *FEBS Lett.* **1993**, *336*, 61.
8. Bauvois, B. *Oncogene* **2004**, *23*, 317.
9. Carl-McGrath, S.; Lendeckel, U.; Ebert, M.; Röcken, C. *Histol. Histopathol.* **2006**, *21*, 1339.
10. Fujii, H.; Nakajima, M.; Saiki, I.; Yoneda, J.; Azuma, I.; Tsuruo, T. *Clin. Exp. Metastasis* **1995**, *13*, 337.
11. Kehlen, A.; Lendeckel, U.; Dralle, H.; Langner, J.; Hoang-Vu, C. *Cancer Res.* **2003**, *63*, 8500.
12. Petrovic, N.; Schacke, W.; Gahagan, J. R.; O'Conor, C. A.; Winnicka, B.; Conway, R. E.; Mina-Osorio, P.; Shapiro, L. H. *Blood* **2007**, *110*, 142.
13. (a) Bauvois, B.; Dauzonne, D. *Med. Chem. Rev.* **2006**, *26*, 88; (b) Mucha, A.; Drąg, M.; Dalton, J. P.; Kafarski, P. *Biochimie* **2010**, *92*, 1504.
14. Shim, J. S.; Kim, J. H.; Cho, H. Y.; Yum, Y. N.; Kim, S. H.; Park, H. J.; Shim, B. S.; Choi, S. H.; Kwon, H. J. *Chem. Biol.* **2003**, *10*, 695.
15. Kim, D.; Lee, I. S.; Jung, J. H.; Lee, C. O.; Choi, S. U. *Anticancer Res.* **1999**, *19*, 4085.
16. Bauvois, B.; Puiffe, M. L.; Bongui, J. B.; Paillat, S.; Monneret, C.; Dauzonne, D. *J. Med. Chem.* **2003**, *46*, 3900.

17. Grzywa, R.; Oleksyszyn, J.; Salvesen, G. S.; Drąg, M. *Bioorg. Med. Chem.* **2010**, *20*, 2497.
18. (a) Schalk, C.; d'Orchymont, H.; Jauch, M.-F.; Tarnus, C. *Arch. Biochem. Biophys.* **1994**, *311*, 42; (b) d'Orchymont, H.; Tarnus, C. Eur. Patent 0378456 A1, 1990.
19. Albrecht, S.; Defoin, A.; Salomon, E.; Tarnus, C.; Wetterholm, A.; Haeggström, J. Z. *Bioorg. Med. Chem.* **2006**, *14*, 7241.
20. Barret, A. J.; Rawling, N. D.; Woessner, J. F., 2nd ed. In *Handbook of Proteolytic Enzymes*; Elsevier Academic Press: Oxford, 2004; Vol. 2,
21. Addlagatta, A.; Gay, L.; Matthews, B. W. *Biochemistry* **2008**, *47*, 5303.
22. Nocek, B.; Mulligan, R.; Bargassa, M.; Collart, F.; Joachimiak, A. *Proteins* **2008**, *70*, 273.
23. Tholander, F.; Muroya, A.; Roques, B. P.; Fournié-Zaluski, M. C.; Thunnissen, M. M.; Haeggström, J. Z. *Chem. Biol.* **2008**, *15*, 920.
24. Kajiro, H.; Mitamura, S.; Mori, A.; Hiyama, T. *Bull. Chem. Soc. Jpn.* **1999**, *72*, 1553.
25. (a) Boeckman, R. K., Jr.; Shao, P.; Mullins, J. J. *Org. Synth.* **2000**, *77*, 141; (b) Dess, D. B.; Martin, J. C. *J. Org. Chem.* **1983**, *48*, 4155. www.SyntheticPages.org/pages/51.
26. Allinger, N. L.; Szkrybalo, W. *J. Org. Chem.* **1962**, *27*, 722.
27. Russell, G. A.; Blankespoor, R. L.; Trahanovsky, K. D.; Chung, C. S. C.; Whittle, Ph. R.; Mattox, J.; Myers, C. L.; Penny, R.; Ku, Th.; Kosugi, Y.; Givens, R. S. *J. Am. Chem. Soc.* **1975**, *97*, 1906.
28. Mataka, S.; Takahashi, K.; Mimura, T.; Hirota, T.; Takuma, K.; Kobayashi, H.; Tashiro, M. *J. Org. Chem.* **1987**, *52*, 2653.
29. Bianchi, M.; Butti, A.; Pfeiffer, U.; Rossi, S.; Barzaghi, F.; Marcaria, V.; Nencioni, A. *Farmaco, Ed. Sci.* **1986**, *41*, 229.
30. (a) Simchen, G.; Kober, W. *Synthesis* **1976**, 259; (b) Taniguchi, Y.; Inanaga, J.; Yamaguchi, M. *Bull. Chem. Soc. Jpn.* **1981**, *154*, 3229.
31. (a) Hassner, A.; Reuss, R. H.; Pinnick, H. W. *J. Org. Chem.* **1975**, *40*, 3427; (b) Rubottom, G. M.; Vasquez, M. A.; Pelegrina, D. R. *Tetrahedron Lett.* **1974**, 4319.
32. Miriyala, B.; Bhattacharyya, S.; Williamson, J. *Tetrahedron* **2004**, *60*, 1463.
33. Ehrenfreud, J.; Zbiral, E. *Liebigs Ann. Chem.* **1973**, 290.
34. Horiuchi, C. A.; Kiji, S. *Bull. Chem. Soc. Jpn.* **1997**, *70*, 421.
35. Pravst, I.; Zupan, M.; Stavber, S. *Tetrahedron* **2008**, *64*, 5191.
36. Effenberger, Fr.; Beisswenger, Th.; Az, R. *Chem. Ber.* **1985**, *118*, 4869.
37. Hart, H.; Dunkelblum, E. *J. Org. Chem.* **1979**, *44*, 4752.
38. Ito, Y.; Hirao, T.; Saegusa, T. *J. Org. Chem.* **1978**, *43*, 1011.
39. Larock, R. C.; Hightower, T. B.; Kraus, G. A.; Hahn, P.; Zheng, D. *Tetrahedron Lett.* **1995**, *36*, 2423.
40. (a) Blanco, L.; Amice, P.; Conia, J. M. *Synthesis* **1976**, 194; (b) Reuss, R. H.; Hassner, A. *J. Org. Chem.* **1974**, *39*, 1785.
41. Guha, S. K.; Wu, B.; Kim, B. S.; Koo, S.; Bait, W. *Tetrahedron Lett.* **2006**, *47*, 291.
42. Magnus, Ph.; Barth, L. *Tetrahedron* **1995**, *51*, 11075.
43. Tarbell, D. S.; Bill, J. C. *J. Am. Chem. Soc.* **1952**, *74*, 1234.
44. Chen, W.-X.; Zhang, J.-H.; Hu, M.-Y.; Wang, X.-Ch. *Synthesis* **1990**, 701.
45. Tonsiengsom, F.; Miyake, F. Y.; Yakushijin, K.; Horne, D. A. *Synthesis* **2006**, 49.
46. Buchanan, G. L.; Sutherland, J. K. *J. Chem. Soc.* **1957**, 2334.
47. Buchanan, G. L.; McCrae, J. M. *Tetrahedron* **1967**, *23*, 279.
48. (a) Kabuss, S.; Friebolin, H.; Schmid, H. G. *Tetrahedron Lett.* **1965**, 469; (b) Kabuss, S.; Schmid, H. G.; Friebolin, H.; Faisst, W. *Org. Magn. Reson.* **1969**, *1*, 451; (c) Kabuss, S.; Schmid, H. G.; Friebolin, H.; Faisst, W. *Org. Magn. Reson.* **1970**, *2*, 19.
49. Leong, M.; Mastryukov, V.; Boggs, J. *J. Mol. Struct.* **1998**, *445*, 149. From the given dihedral angles, it is possible to calculate the approximate dihedral angle between the different protons: (7,8a) 185°; (7,8e) 65°; (8a,9a) 195°; (8a,9e) 75°; (8e,9e) 15°.
50. Krajewski, K.; Zbigniew, C.; Siemon, I. Z. *Tetrahedron: Asymmetry* **2001**, *12*, 455.
51. Kyrieleis, O. J.; Goettig, P.; Kiefersauer, R.; Huber, R.; Brandstetter, H. *J. Mol. Biol.* **2005**, *349*, 787.
52. McGowan, S.; Porter, C. J.; Lowther, J.; Stack, C. M.; Golding, S. J.; Skinner-Adams, T. S.; Trenholme, K. R.; Teuscher, F.; Donnelly, S. M.; Grembecka, J.; Mucha, A.; Kafarski, P.; Degori, R.; Buckle, A. M.; Gardiner, D. L.; Whisstock, J. C.; Dalton, J. P. *Proc. Natl. Acad. Sci. U.S.A.* **2009**, *106*, 2537.
53. Bauvois, C.; Jacquamet, L.; Huston, A. L.; Borel, F.; Feller, G.; Ferrer, J. L. *J. Biol. Chem.* **2008**, *283*, 23315.
54. Tronrud, D. E.; Monzingo, A. F.; Matthews, B. W. *Eur. J. Biochem.* **1986**, *157*, 261.
55. Huang, X.; Miller, W. *Adv. Appl. Math.* **1991**, *12*, 373. Programm LALIGN, http://www.expasy.ch/tools/sim-prot.html.
56. Kim, H.; Lipscomb, W. N. *Proc. Natl. Acad. Sci. U.S.A.* **1993**, *90*, 5006.
57. Cappiello, M.; Alterio, V.; Amodeo, P.; Del Corso, A.; Scaloni, A.; Pedone, C.; Moschini, R.; De Donatis, G. M.; De Simone, G.; Mura, U. *Biochemistry* **2006**, *45*, 3226.
58. Söding, J. *Bioinformatics* **2005**, *21*; 951. http://toolkit.tuebingen.mpg.de/hhpred, HHPRED.
59. Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discovery Today* **2004**, *9*, 430.
60. Noyes, W. A. *Org. Synth.* **1943**, *coll. vol. 2*, 108.
61. Beak, P.; Berger, K. R. *J. Am. Chem. Soc.* **1980**, *102*, 3848.