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# Rapid and efficient synthesis of a novel series of substituted aminobenzosuberone derivatives as potent, selective, non-peptidic neutral aminopeptidase inhibitors

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ABSTRACT

Racemic 5-substituted 7-aminobenzocyclohepten-6-one were synthesized and evaluated for their ability to inhibit metalloaminopeptidase activities. Unexpectedly, 5-thio substituted compounds showed enhanced inhibition potency with $K_i$ values in the nanomolar range against the 'one zinc' aminopeptidases from the M1 family, while most of them were rather poor inhibitors of the 'two zincs' enzymes from the M17 family. This interesting selectivity profile may guide the design of new, specific inhibitors of target mammalian aminopeptidases with one active site zinc.



## 1. Introduction

APN/CD13 is a membrane-bound, zinc-dependent homodimeric enzyme and a member of the M1 family of aminopeptidases.[1] Like other members of this family, APN/CD13 possesses the consensus zinc binding motif (HEXXH-(X18)-E) in its extracellular domain, as well as the exopeptidase motif (GXMEN) for binding the free primary amino group of the N-terminal residue of its peptidic substrates. APN/CD13 removes the N-terminal amino acid from unsubstituted oligopeptides, amides or arylamides, with a broad substrate specificity, although a significant preference for hydrophobic residues is observed.[1] This ectoenzyme appears to be a multifunctional protein involved in the regulation of signalling peptides as well as in various cell activation and migration processes.[2] APN/CD13 is emerging as a target of significant biological and medical importance. Moreover, recent studies have indicated that APN/CD13 is an active player in angiogenesis and tumor metastasis.[3] Still, the exact role played by APN/CD13 in those pathologies, as well as the cellular pathways involved, remain to be elucidated. To this end, a small molecular weight, drug-like, selective inhibitor of APN/CD13 would be an invaluable biological tool to assess the pathophysiological roles of this multifunctional enzyme that depend solely on its catalytic activity.

Recent reviews provide an excellent overview of the currently available collection of APN/CD13 inhibitors.[4] Among the most active compounds, pseudo-peptides that mimic the transition state analogs such as α-boronic acids, sulfonamide and phosphonic surrogates of the scissile peptide bond are the most prominent. Natural products such curcumin[5] and psammaplin A[6] or synthetic flavonoid derivatives,[7] as well as compounds incorporating the hydroxamic acid moiety[8] have also been reported as potent APN/CD13 inhibitors. However, while many of these compounds display high in vitro potency, their selectivity is not always well documented. In view of the potential therapeutic relevance of some of the APN/CD13 functions, the rational design of potent and selective APN/CD13 inhibitors is of considerable interest, but remains a challenging endeavor and a daunting task.

3-Amino-2-tetralone **1** has been described as potent and selective inhibitor of the 'one zinc' APN/CD13.[9,10] Nevertheless, tetralone **1** was not stable under physiological conditions, and some more stable derivatives have been later synthesized without improvement in affinity.[11] Lately, its seven-membered homolog **2a** has been reported as more selective without loss of stability and affinity against APN (Scheme 1).[12] After having performed a SAR study around this scaffold, we were delighted to find out that the simple 4-phenyl substituted aminobenzosuberone **2′** was a nanomolar APN inhibitor ($K_i$ 7 nM).[13,14] It is noteworthy that it retained a very high selectivity towards the M1 subfamily of one-zinc aminopeptidases. However, its synthesis was problematic and gave rise to a regioisomeric mixture (in an overall yield of 2.4–6.7% from the commercially available starting materials) that required a

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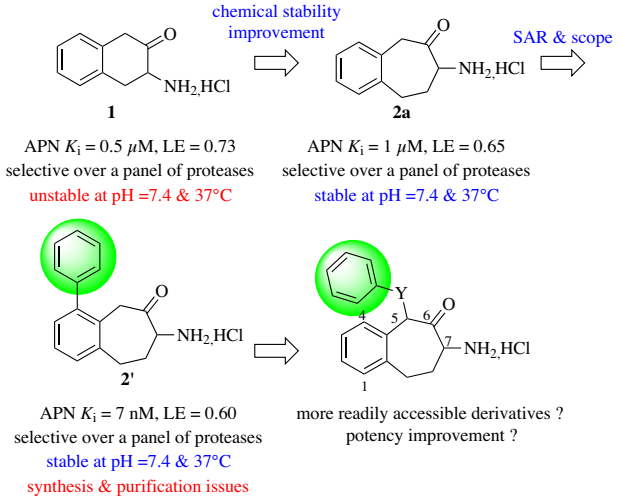
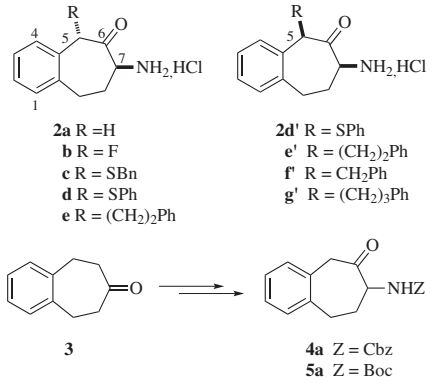



**Scheme 1.** Strategy envisioned for the development of novel APN/CD13 inhibitors. LE, ligand efficiency, LE = $-1.4\log(K_i)/N$, $N$: number of nonH-atoms.




**Scheme 2.** Chemical structures of the novel APN/CD13 inhibitors.

preparative HPLC purification to separate both regioisomers. This method was not suitable and not satisfying for a large scale synthesis needed to carry out further in vivo studies which will be reported in due course.

We thus wanted to design a straightforward synthesis allowing easy access to a large variety of derivatives retaining the phenyl moiety, a key phamacophore feature. To this end, we decided to explore the functionalization at the 5 position of the benzosuberone **2a**.

In the present work, we report the synthesis of racemic 5-substituted analogs **2b–h** of the 7-aminobenzocyclohepten-6-one **2a**, and their inhibitory activities against a panel of 'one zinc' APs. The parent compound 3-amino-2-benzosuberone **2a** and bestatin were used as reference inhibitors.

## 2. Chemistry

Since hydrated ketone is a structural mimic of the tetrahedral intermediate that forms during the hydrolysis of peptide substrates, the synthesis of 5-fluoro-5-susbtituted aminobenzosuberones should enhance considerably the inhibitory activities towards metalloproteases. Therefore, the simple fluoroketone **2b** was investigated at first. Secondly, an efficient and practical method for preparing 5-substituted analogs was sought out. Hence, we turned our attention to the ring opening of epoxides with thiols, a convenient reaction, characterized by operational simplicity, high yield and regioselectivity (addition of thiols to the benzylic carbon). Then, a series of C-substituted compounds was explored and required more complex procedures.

The preparation of the aminobenzocycloheptenone **2a** has been previously described from the known benzocycloheptanone **3**[15–17] through the N-protected derivatives **4a** or **5a**[12] (Scheme 2). Two synthetic ways have been used in order to substitute the 5-position: the *trans*-derivatives **2c–e** were prepared from the ketone **3** *via* epoxide opening, the *cis*-derivatives **2e′-g′** and the fluoro compound **2b** were directly obtained from the N-protected aminoketones **4a** or **5a**.

### 2.1. Fluoration of the N-protected aminoketones 4a or 5a

In order to mimic the tetrahedral intermediate produced during peptide hydrolysis, by all metalloenzymes, we aimed at favoring the hydrate form of ketone **2a** through incorporation of a fluorine atom. The latter was introduced at the 5-position by electrophilic reaction of Selectfluor™[18,19] with a silyl enol ether according to the literature[20,21] (Scheme 3). The silyl enol ethers **6a** and **6b** were obtained by enolization of **4a** or **5a** with LiHMDS, in the presence of HMPA as chelating agent in the case of **6b**, and quenching with $Me_3SiCl$ or better with $Me_2tBuSiCl$. The electrophilic addition of Selectfluor™ in acetonitrile at −20 °C gave different results according to the N-protection (scheme 3): the benzyloxycarbonyl derivative **6a** led to the expected fluoro compound **4b**, but the Boc-derivative **6b** gave a 1:1 mixture of **5b** and its acidic transposition product **7** (as hydrate). In the presence of aqueous $NaHCO_3$ as base at 0 °C, **6b** led to the expected fluoroketone **5b** in a 1:1 mixture with the stable silylated hemiacetal **8**, and, without separation, a subsequent treatment with fluoride anion provided **5b** as the sole product in 52% yield. Attempts to deprotect **4b** either by hydrogenolysis or with $BBr_3$ led to partial defluorination in amine **2a**, but acidic N-deprotection of **5b** gave easily the fluoroamine hydrochloride **2b** in 37% overall yield from **5a**. In $D_2O$ solution, **2b** appeared in mixture with 85% of the corresponding hydrate form **2b′**. The configuration of these compounds **2b**, **4b** and **5b** was *trans* with an axial fluorine atom.

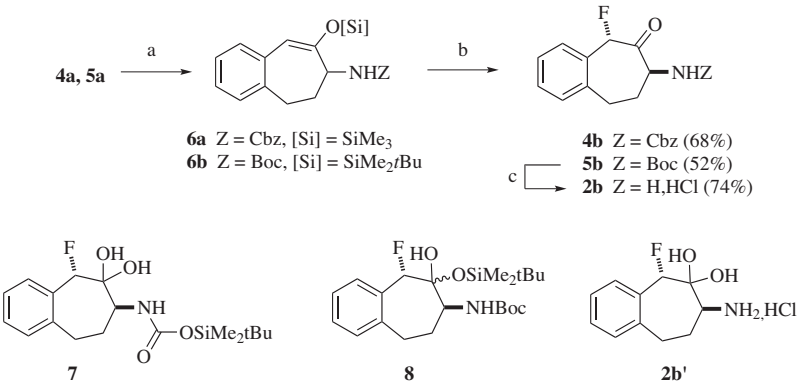
### 2.2. Thio-derivatives 2c and 2d,d′

The synthesis of the thio-derivatives **2c** and **2d,d′** was achieved via the epoxide **11b** (Scheme 4).

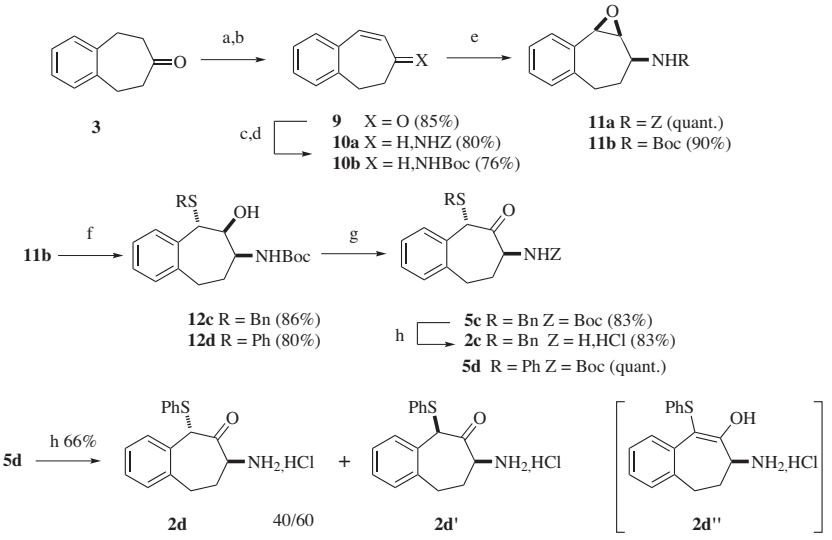
The silyl enol ether of ketone **3**, prepared by *O*-silylation with $Me_3SiOTf$ in the presence of $NEt_3$ at 85 °C, was oxidized to the enone **9** in 85% yield, with $O_2$ in DMSO and $Pd(OAc)_2$ as catalyst according to the Saegusa method.[22] The reductive amination of the ketone function according to already described procedures,[12,23] followed by N-protection with $Boc_2O$, provided the amide **10b.** This latter was epoxidized with *m*-CPBA to the *cis* epoxyamide **11b** in 65% overall yield from ketone **3**. A subsequent $SN_2$ epoxide opening reaction with benzylmercaptan or thiophenol as soft nucleophiles led stereospecifically to the thioethers **12c** and **12d** with *trans*-configuration for the 4-thioether and 5-hydroxy substituents. Then, the oxidation of the alcohol function in 6-position with DMP gave ketones **5c** and **5d** without modifications of the thioether function. Their N-deprotection with HCl led to the corresponding final amine hydrochlorides **2c** and **2d,d′** in 38% and 34% yield, respectively (overall yields calculated from ketone **3**). Unlike benzylthioether **2c**, which was isolated as a single isomer, the phenylthioether amine hydrochloride appeared in $D_2O$ solution as a 40/60 mixture of **2d**-*trans* and **2d′**-*cis*-isomers in equilibrium.

### 2.3. Aliphatic substitutions

Three methods were used to substitute the 5-position with an aliphatic chain: epoxide opening of **11a** by organocuprates,




**Scheme 3.** Synthetic scheme for the fluoro analogs. Reagents and conditions: (a) (1) LiHMDS, HMPA, in THF, 30 min, −78 °C; 2. $ClSiMe_3$ for **4a** or $ClSiMe_2tBu$ for **5a**,15 min; (b) Selectfluor in MeCN, 2 h, −20 °C for **6a**, 68% yield from **4a**; Selectfluor™ in MeCN and aqueous 1 M $NaHCO_3$, 30 min, 0 °C for **6b**, then $NBu_4F$, THF, 10 min, 52% yield from **5a**. (c) 0.6 N HCl in EtOH, 12 h.




**Scheme 4.** Synthetic scheme for the S-substituted analogs. Reagents and conditions: (a) $Me_3SiOTf$, $NEt_3$, toluene, 85 °C, 2 h. (b) $Pd(OAc)_2$, $O_2$, DMSO. (c) $Ti(OiPr)_4$, satd $NH_3$ in MeOH, 6 h then $NaBH_4$, 1 h; (d) for **10a**: CbzCl, $Na_2CO_3$, THF; for **10b**: $Boc_2O$, $Na_2CO_3$, MeOH; (e) *m*-CPBA, $CH_2Cl_2$, 0 °C, 10 h; (f) PhSH (86%) or BnSH (80%), $NEt_3$, EtOH, 1 h; (g) DMP, $CH_2Cl_2$, 2 h; (h) dry HCl 2 M in $Et_2O$, dioxane.

aldolisation–crotonisation of the amidoketone **5a** or 1,4-addition of organocuprates with the methylene ketone **4i** (Scheme 5).

The *cis N*-benzyloxycarbonylepoxide **11a** was obtained by a similar method as for the *N*-Boc derivative **11b** in 61% overall yield from ketone **3**. Its opening with phenethyl cuprate led to the alcohol **12e** which was directly oxidized with DMP to give the corresponding ketone **4e**. Its N-deprotection by hydrogenolysis in the presence of HCl provided the 5-*trans*-phenethyl amine hydrochloride **2e** in 53% yield from the epoxide **11a**.

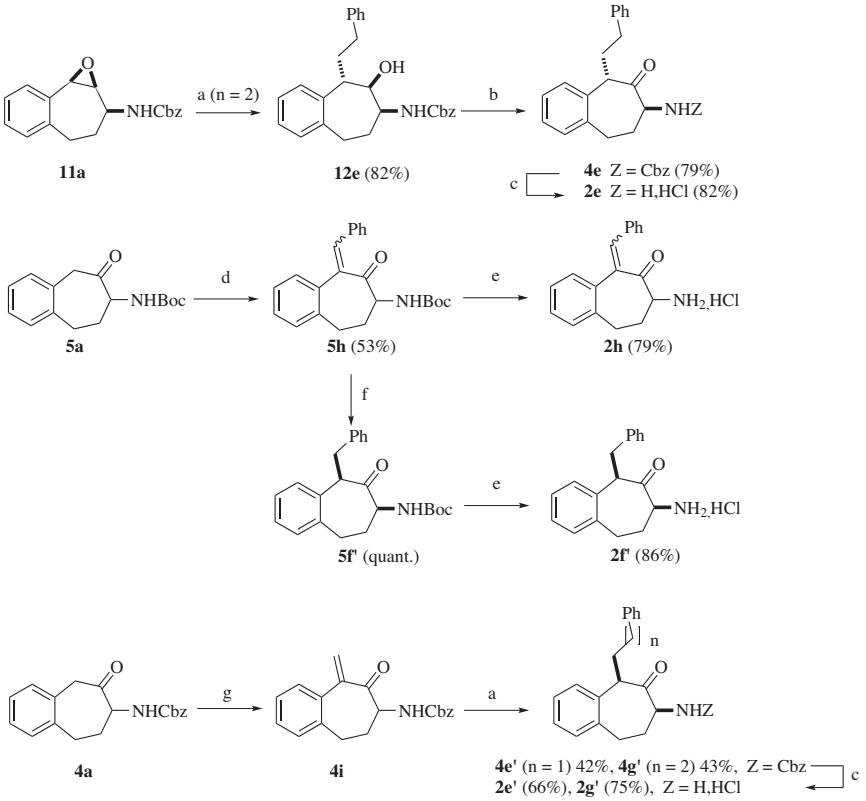
The aldolisation–crotonisation of **5a** with benzaldehyde was carried out with LiHMDS in the presence of HMPA in THF, or with DBU in toluene, to give the benzylidene derivative **5h** in 50–60% yield. Deprotection of **5h** with dry HCl in dioxane–$Et_2O$ or hydrogenolysis over Pd-C and subsequent acidic deprotection gave the amine hydrochlorides **2h** and **2f′** in 42–45% yield from **5a**.

The Mannich α-methylenation of the ketoamide **4a** was carried out with formaldehyde and pyrrolidine in acetic acid to give the reactive and unstable α-methylene ketone **4i** which was used without further purification.[24,25] Reaction of crude **4i** with the cuprates $[Ph(CH_2)_n]_2CuMgBr$ ($n$ = 1 and 2) gave the addition products **4e′** and **4g′** with moderate yield (ca. ∼40%). N-deprotection by hydrogenolysis in the presence of HCl led to the final amine hydrochlorides **2e′** and **2g′**, having *cis*-configuration, from the ketoamide **4a** in 28% and 32% yield, respectively.

## 2.4. Stereostructure and conformation of the synthesized compounds

The conformation of the cycloheptene ring has already been theoretically studied[26] and the more stable conformation was calculated to be the chair one as depicted in Figure 1 for **2a** and its derivatives. For all derivatives of 7-aminobenzocyclohepten-6-one, as amines or amides, except for the methylene derivatives **2h**, **5h** and **4i**, the amino group was in equatorial position by chelation with the ketone function. The axial Ha-8 proton was thus strongly coupled with the axial H-7 proton ($J$ = 12–14 Hz) and with the axial Ha-9 proton ($J$ = 9–13 Hz). A characteristic feature of these seven-membered rings was important axial-axial and equatorial-equatorial couplings for the H-C(8) and H-C(9) protons ($J$(8a,9a) = 9–13 Hz and $J$(8e,9e) = 6.7–9 Hz) related to a large (ca. 195°) and to a weak dihedral angle (ca. 45°) between the corresponding protons, respectively.

The fluoro, thioamines and amides **2b–d**, **4b** and **5b–d** were characterized by an important deshielding, ca. 0.5–0.7 ppm, for




**Scheme 5.** Synthetic scheme for the aliphatic-substituted analogs. Reagents and conditions: (a) $Ph(CH_2)_nMgBr$, ½CuBr·$Me_2S$, THF, −40 °C, 2 h; (b) DMP, $CH_2Cl_2$; (c) $H_2$, 5% Pd-C, dioxane, 1 N HCl, 40–50 °C, 1 d; (d) (1) LiHMDS, HMPA, −78 °C in THF; (2) benzaldehyde; (e) 1 N dry HCl/$Et_2O$–dioxane, rt, 3 d; (f) $H_2$, Pd-C, EtOH, rt, 1 h; (g) $CH_2O$, AcOH, pyrrolidine (cat), reflux, 5 h.

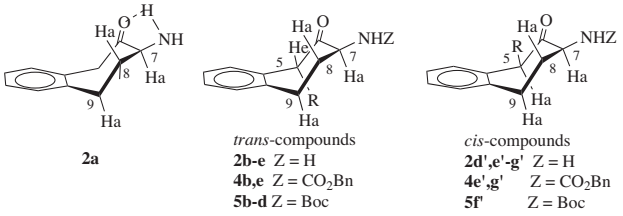



**Figure 1.** Conformation of the ketone derivatives.

the axial protons H-7 and Ha-9 by comparison with the values of the corresponding protons of the unsubstituted **2a**, **4a** and **5a**. This strong effect indicated that the electronegative substituent R was in axial position (Fig. 1), which is spatially near to Ha-7 and Ha-9. The effect of an alkyl group was weaker (ca. 0.1 ppm) for the *trans*-phenethyl compounds **2e** and **4e**.

In the *cis*-alkylated compounds **2f′**, **2g′**, **4e′** and **4g′** no important spatial effect were observed in $^1H$ NMR. Therefore NOE experiments were performed in the case of the phenethyl compound **4e′** and demonstrated the *cis*-configuration of these compounds: by irradiation of H-5 at 3.94 ppm, NOE values of 6% and 11% were observed for the axial Ha-9 at 3.05 ppm and H-7 at 4.56 ppm, respectively. These effects agreed to a spatial proximity of these protons, and consequently, to an axial Ha-5 and thus to an equatorial alkyl group in *cis*-position toward the amino group.

The observed *trans*-configuration of the thio substituents in compounds **5c** and **5d** corresponded for the compounds **11a,b** to an epoxide ring in *cis*-position of the amino group. According to the similar *J* values between H-7, H-8 and H-9 protons with those of compounds **4a** and **5a**, we can consider that the conformation of these compounds were similar. This *cis*-configuration was in agreement with the weak *J*(6,7) value between the equatorial H-6 proton and the axial H-7 and with a long-range W-coupling (*J*(6,8e) = 1.2–1.5 Hz) with the equatorial He-8 (Fig. 2).

In the case of enones **2h**, **5h** and **4i**, which possess an exocyclic double bond, a change in conformation is worth noting. The axial/equatorial nomenclature was only a convention; indeed, Ha-8 being the proton strongly coupled with H-7, and Ha-9 the proton strongly coupled with Ha-8. Then, a large coupling *J*(8e,9a) = ca. 13 Hz and a middle one *J*(8e,9e) = ca. 7 Hz were observed between the He-8 proton and both H-9 protons. These values indicate a boat conformation which would correspond to a better conjugation of the exocyclic enone moiety and to an hydrogen bond between the equatorial NH group and the CO(6) function. In these cases, the calculated dihedral angles[26] were large (148°) for (8e,9a) and near to 90° (82°) for (8e,9e), while it was near to 90° (82°) for (8a,9e).

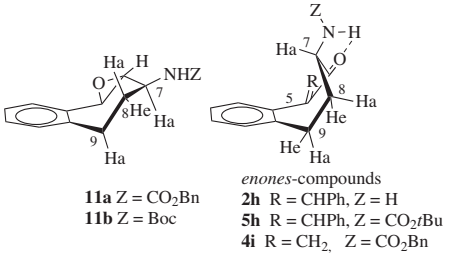



**Figure 2.** Conformation of the epoxide derivatives **11a** and **11b** and exocyclic enones compounds **2h**, **5h** and **4i**.

### 2.5. Properties of the ketoamines 2a–h

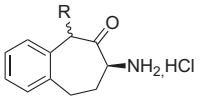
It has already been reported[12] that the ketoamine **2a** exhibited two structural properties in $D_2O$ solution: firstly, a spontaneous partial hydration of the ketone function leading to a 80/20 mixture of the ketone form and its hydrate; secondly, a very slow enolization led to a deuteration of the 5-position at higher temperature (60 °C).[12] In $CD_3OD$, the hydration occurred with concomitant formation of two isomeric hemiacetals.

In this work, all ketoamines of type **2** appeared only in preponderant ketone form (in $CD_3OD$), but two exceptions were observed:

– Firstly, the fluoro derivative **2b** appeared (in $D_2O$) as a ketone/hydrate mixture, the hydrate form being strongly preponderant (85%) and characterized by a strongly shielded H-7 at 3.9 ppm, as for the hydrate of **2a**[12], instead of 5 ppm for the ketone form.
– Secondly, in the case of the thiophenyl derivative **2d**, the enolization into **2d″** occurred spontaneously at room temperature leading, in $D_2O$, to a 60/40 mixture of **2d′**-*cis*/ **2d**-*trans* isomers in equilibrium and to the instantaneous deuteration of the H-5 position which did not appear in the $^1H$ NMR spectrum. This equilibration was observed at room temperature neither for the *trans*-thiobenzyl compound **2c**, nor for the other *trans* compounds **2e**, **4e**, **5c** and **5d**.

In the case of the *trans*-alkylated amine **2e**, an isomerization into the more stable *cis*-isomer was observed, but only in basic medium at room temperature (with $Na_2CO_3$ in $CD_3OD$ or in $D_2O$ at pH >7.4) for 24 h. and led quantitatively to the *cis*-isomer **2e′**.

**Table 1**
Inhibition of selected aminopeptidases by racemic 5-substituted 7-amino-5,7,8,9-tetrahydrobenzocyclohepten-6-one hydrochlorides




**2a-h**

| Compounds | R | $K_i$ (μM) | | |
|---|---|---|---|---|
| | | APN (EC 3.4.11.2) | LAPc (EC 3.4.11.1) | LTA4H (EC 3.3.2.6) |
| Bestatin | | 3.5[a] | 0.0005[a] | 0.5[a] |
| **1** | | 0.5[a] | 120[a] | >1000 |
| **2a** | –H | 1[a] | >1000 | >1000 |
| *trans*-**2b** | –F | 0.3[a] | >1000 | >1000 |
| *trans*-**2c** | –SBn | 0.083[a,b] | 40[a] | >100 |
| *trans*-**2d**/ *cis*-**2d′** | –SPh | 0.06[a,b] | >100 | >100 |
| *trans*-**2e** | –$(CH_2)_2$Ph | 88[a] | nd | 470[a] |
| *cis*-**2e′** | –$(CH_2)_2$Ph | 22[a] | >100 | >100 |
| *cis*-**2f′** | –$CH_2$Ph | 13[a] | >100 | >100 |
| *cis*-**2g′** | –$(CH_2)_3$Ph | 12[a] | >100 | >100 |
| **2h** | =CHPh | 80[a] | nd | 380[a] |

$K_i$ values (μM) were determined (a) from Dixon plots[27] for rapid equilibrium kinetics, (b) from Morrison plot[28] for slow binding inhibition. Inactive compounds were tested up to their solubility limit under the assay conditions. See experimental section. LAPc: cytosolic leucine aminopeptidase from bovine kidney (EC 3.4.11.1), APN: porcine kidney aminopeptidase-N (EC 3.4.11.2) and LTA4H: human leukotriene A4 hydrolase (EC 3.3.2.6). nd: not determined.

### 3. Aminopeptidases inhibition and discussion

All compounds were evaluated as racemic mixtures. All active compounds behaved as competitive inhibitors of the panel aminopeptidases. The inhibition constants ($K_i$) are reported in Table 1. The aminobenzosuberone **2a** was previously described as a selective competitive inhibitor of the 'one zinc' APN that belongs to the M1 subfamily of metalloaminopeptidases, whereas bestatin is much more potent on the 'two zincs' M17 family of enzymes.[9–11] These two compounds were used here as reference inhibitors.

These newly synthesized inhibitors maintained the selectivity profile towards mammalian APN. LTA4H was not inhibited to any significant extent and most of them were totally inactive towards LAPc, up to a concentration of 100 μM, except for compound **2c** ($K_i$ = 40 μM). However, this latter compound exhibited an interesting selectivity profile, being 500 times more active against APN ($K_i$ = 83 nM).

The most potent APN inhibitors, compounds **2c** and **2d**, behaved as reversible slow binding inhibitors; the time dependent inhibition is reported in Figure 3A. For this single-substrate reaction, the rate equation was shown to be $v = v_s + (v_o - v_s)e^{-kt}$ where v is the observed velocity at any time, $v_o$ is the initial velocity of the reaction and $v_s$ is the steady state velocity.[29] The exponential decay constant k could be determined for each individual concentration of inhibitor and the values of the association ($k_{on}$) and dissociation ($k_{off}$) constants were derived from the plot of $k$ as a function of [I] ( Fig. 3B).[28] For compound **2c**, $k_{on} = 1.2 \times 10^3$ $M^{-1}$ $s^{-1}$ and $k_{off} = 10^{-4}$ $s^{-1}$, which correspond to a $K_i$ value of 83 nM. A very close time dependent inhibition was observed with compound **2d** and similar values were calculated, with $k_{on} = 2 \times 10^3$ $M^{-1}$ $s^{-1}$, $k_{off} = 1.2 \times 10^{-4}$ $s^{-1}$ and a $K_i$ value of 60 nM. For both compounds, the observed kinetic constants are in the range of values generally encountered for time dependant slow binding inhibition.[30]

The comparison of the kinetic profiles of compound **2c** and its analog **2e** emphasized the importance of the sulfur atom in **2c** for the inhibitory potency. **2e** behaved as a reversible competitive inhibitor in rapid equilibrium and was 1000 times less potent ($K_i$ = 80 μM) than **2c**. The corresponding Dixon plot is reported in Figure 4. Similar results were obtained for compounds **2e′-g′**, which rapidly reached the steady state under our experimental conditions, with $K_i$ values reported in Table 1.

#### 3.1. Structure–activity relationships: main findings

##### 3.1.1. Fluorine substitution

Compound **2b** ($K_i$ 0.3 μM), substituted by a fluorine atom, was slightly better than the parent amino-benzosuberone **2a** ($K_i$ 1 μM) and exhibited an excellent selectivity profile. The fluorine atom might improve the affinity by favoring the hydrate form over the ketone one, and/or by undergoing multipolar C–F···H–N, C–F···C=O, and C–F···H–$C_\alpha$ interactions with the enzyme backbone.[31] The latter contributions were probably more significant, as non-fluorinated ketoamines were already hydrated in aqueous buffer.

Thus, the synthesis of 5-fluoro disubstituted analogs was not pursued due to the weak improvement in binding affinity.

##### 3.1.2. Aliphatic substitutions

In general, aliphatic substitutions at the 5-position of the aliphatic ring did not result in affinity enhancement and led to disappointing results. In particular, compounds **2e** and **2e′-g′** having the key phenyl moiety pharmacophore, connected to the aminobenzosuberone core through an aliphatic methylene chain spacer X-$(CH_2)_n$-Y, exhibited a 12- to 88-fold loss in activity. However, worth noting is an interesting trend in the activity profile related to the *cis*/*trans* conformation: *cis*-**2e′** ($K_i$ 22 μM) displayed a better inhibitory activity than its *trans*-equivalent *trans*-**2e** ($K_i$ 88 μM). Moreover, *cis*-aliphatic compounds **2e′-g′**, regardless of spacer length, were better than the *trans*-derivative **2e**, suggesting that the *cis*-conformation, with the substituent at the 5-position in

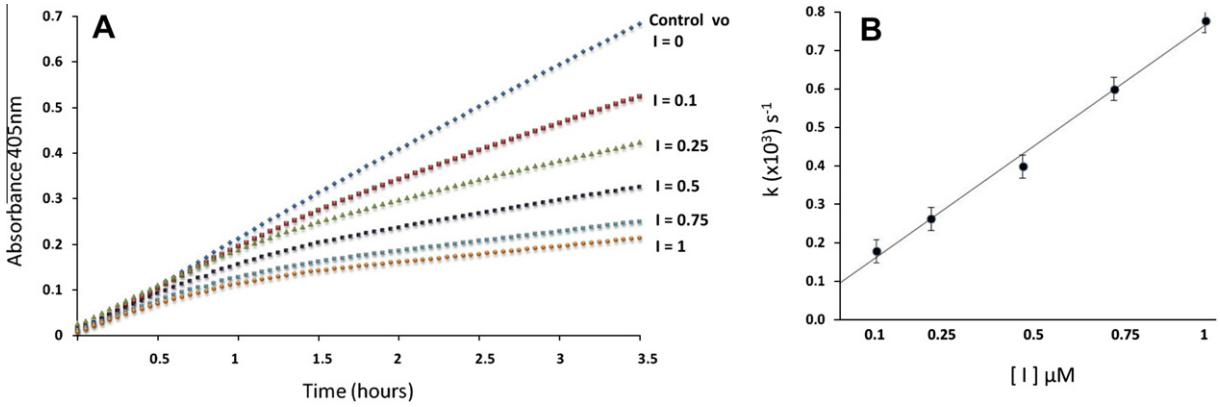



**Figure 3.** Kinetic analysis for APN inhibition by compound **2c**. (A) Progress curve in absence ($v_o$) or presence of **2c** at the reported µM concentrations. The reaction was started by the addition of enzyme at a final concentration of 3 nM with Leucine-*p*-nitroanilide as the substrate ($S = K_m = 0.2$ mM), in Tris buffer pH 7.5 at 30 °C.[13] The steady state was reached after 1.5 h ($v_s$). (B) Plot of k as a function of I ($n = 3$): $k = k_{off} + (k_{on}/(1 + S/K_m)\ I$.[28] The value of $k_{off}$ is given by the vertical intercept, while the value of $k_{on}$ can be calculated from the slope.

equatorial position, was the more favorable conformation, allowing the best fit with the enzyme active site.

### 3.1.3. Thio substitutions

Compounds **2c** and **2d**, substituted with a sulfide moiety, proved 12 and 20 times more active against APN/CD13 than the parent aminobenzosuberone **2a**, with $K_i$ values of 83 nM and 60 nM, respectively. Furthermore, these 5-axial-*S*-substituted derivatives were 1000 times more potent than their carbon analogs, which suggested a different mode of binding or new binding interactions. The comparison of the activities of **2c**/**2e** and **2d**/**2f** demonstrated that the sulfide moiety was crucial for strong affinity to APN. The strongest interaction that could possibly account for the very large increase in potency of the sulfur containing derivatives as compared to their carbon analogs would be zinc chelation. Thiols and sulfides are potent zinc-chelating group and are known to bind tightly to zinc-dependent metalloenzymes.[32]

The high inhibitory activities (in the nM range) of these two 5-axial-*S*-substituted derivatives, together with the observed slow binding kinetics, came as an intriguing result. As mentioned before, in the carbon series, the *trans*-derivatives was the less active compound. Hence, a poor fit, or even a steric clash might have been expected between the bulkier *S*-axial substituent and the protein binding site. The equilibrium (observed for the *trans*-thiophenyl **2d** and assumed for the *trans*-thiobenzyl **2c**) between the *axial* and *equatorial* S-substituent via enolization should favor the *cis*-conformation which provides a better fit to the active site and allows metal chelation by the sulfur atom. This equilibrium may account for the observed time dependent, slow binding inhibition by this APN inhibitor series. However, from a chemical standpoint, the enol intermediates **2c**″ or **2d**″ cannot be ruled out as the potential active form of these molecules.

### 3.2. Putative binding mode

To date, the three dimensional structure of a mammalian APN is still lacking. Nevertheless, it is possible to propose a model (Fig. 5) for the binding of our compounds, based on highly conserved residues in the active site of the M1 family of enzymes and the SAR discovered in the course of our studies. With the thio derivatives **2c** and **2d**, metal binding is likely to be mediated through the sulfur atom and the ketone in its hydrated form. According to the previously postulated binding mode of the aminobensosuberone **2a** ( Fig. 5A),[12] thio derivatives would have to undergo a small

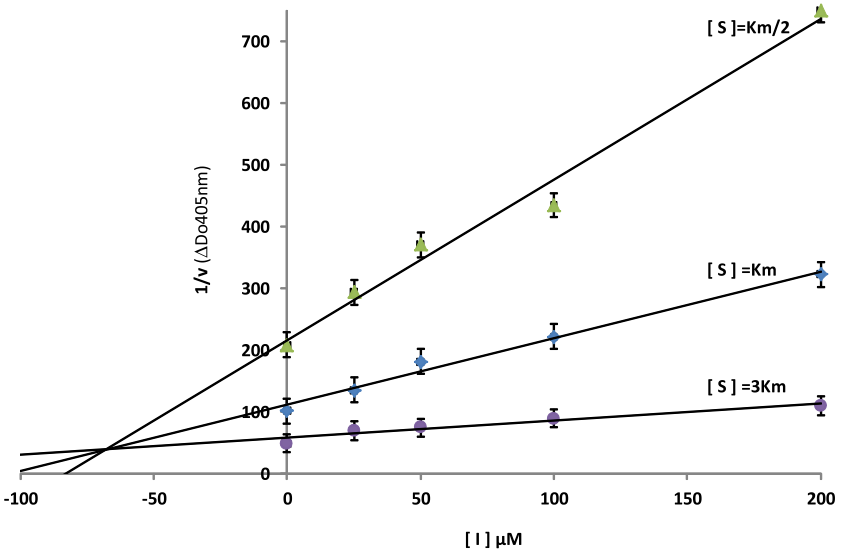



**Figure 4.** Dixon plot[27] of APN inhibition by compound **2e**. See Section 5 (d).

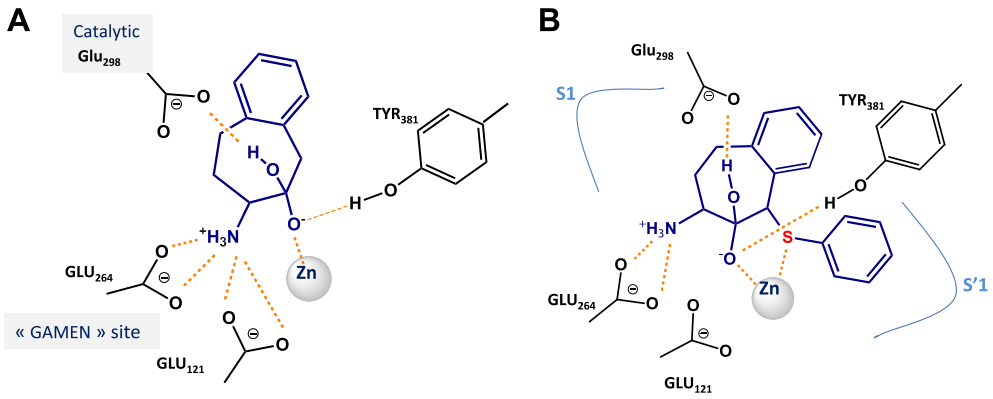



**Figure 5.** Proposed mode of binding for the hydrate form of aminobenzosuberone **2a** (A)[12] and its analog **2d** substituted with a thiophenyl moiety (B) based on zinc chelators affinity and inhibition studies. (*E. coli* numbering). The binding pose of the thiophenyl inhibitor might be slightly shifted with the sulfide moiety getting close to the zinc ion, leading to an overall rotation of the suberone, with the primary amine interacting solely with one carboxylate of the GAMEN site. The key phenyl pharmacophore would fit in the S'1 subsite. In both cases, the hydrate form of the ketone would be stabilized by Glu 298 and Tyr 381.

rotational shift of the scaffold in order to optimize zinc-chelation, interactions with the conserved essential catalytic residues and optimize contacts to the protein. This shift would govern the proper positioning of the phenyl ring in the S′1 pocket (Fig. 5B). For the carbon analogs **2e–h**, this binding would not be possible as it would induce close unfavorable contacts of the carbon linker to the zinc, thus hampering optimal binding of the phenyl ring into S′1. The primary amine, usually engaged in strong electrostatic interactions with the side-chain carboxylate of two Glu residues ('GAMEN' site), conserved in the one-zinc family of aminopeptidases and responsible for the recognition and binding of the free amino terminus of peptidic substrates,[1] may retain an interaction with at least one carboxylic function. Taken together these effects (proper binding of the phenyl ring, zinc chelation and partial interaction of the primary amine with the GAMEN site) may well account for the 1000-fold improvement in affinity observed with the thio substituted compounds. The time dependent inhibition observed with the thio derivatives may be a consequence of the *trans*/*cis* equilibrium as well as of the required rotational shift following the primary binding event.

## 4. Conclusion

In summary, we have successfully devised a straightforward synthesis allowing access to a new series of 7-aminobenzocyclohepten-6-one derivatives **2b–h**. To this end, we have explored functionalization at the 5-position of the aliphatic ring of **2a** with either a fluorine atom, or a phenyl moiety attached to the core scaffold through an aliphatic spacer or via a sulfur atom. The thio derivatives **2c** and **2d**, which show strong (60–83 nM) inhibitory activity, are the first examples of a new chemotype of APN inhibitors, derived from the aminobenzosuberone scaffold. In contrast to previously reported derivatives, which are believed to be monodentate ligands of the active site zinc ion, these new compounds are most probably bidentate zinc binders. As our understanding of APN biology and pathophysiological roles is still very limited, these new compounds have potential as chemical biology tools for shedding light into the biological functions of this enzyme, and as lead compounds for the development of therapeutic APN inhibitors.

## 5. Experimental part

### 5.1. 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 ($\nu$ in $cm^{-1}$): Nicolet 405 FT-IR. $[\alpha]_D$: Schmidt-Haensch Polartronic Universal or Perkin–Elmer 341 LC polarimeter. $^1H$ and $^{13}C$ NMR (400 MHz and 100.6 MHz resp.) spectra at 295 K: Bruker Avance 400, tetramethylsilane (TMS), or sodium ($D_4$)-trimethylsilylpropionate ($D_4$-TSP) in $D_2O$ ($^1H$ NMR), $CDCl_3$, or (in $D_2O$) dioxane [$\delta(CDCl_3) = 77.0$, in $D_2O$ $\delta$(dioxane) = 67.4 with respect to TMS] ($^{13}C$ NMR) as internal references; $\delta$ in ppm and J in Hz. High resolution MS were measured in Spectroscopy Laboratory of Strasbourg University or on a Waters Micromass Q-Tof Ultima API spectrometer in Basilea Pharmaceuticals, Basel. Microanalyses were carried out by the Service Central de Microanalyses du CNRS, F-69390 Vernaison.

### 5.2. Reagents and solvents

Dess–Martin periodinane (DMP) was obtained as a 1 M solution in $CH_2Cl_2$ from Aldrich or synthesized according to the literature.[33] Commercial products were purchased from usual suppliers. 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 $Na_2CO_3$. Dioxane, $NEt_3$ were distilled before use. Hexamethylphosphoramide (HMPA) and DMSO distilled and conserved over molecular sieves 4 Å.

The preparation of compounds **3**, **4a**, **5a** and **9** have been previously described and fully characterized in the literature.[12,13]

### 5.3. Novel chemical entities

#### 5.3.1. *trans*-7-(Benzyloxycarbonylamino)-5-fluoro-6,7,8,9-tetrahydrobenzocyclohepten-6-one (4b)

To a stirred solution of HMDS (0.3 mL, 1.42 mmol, 2.2 equiv) in dry THF (1.6 mL) at −78 °C, was successively added BuLi (1.6 M in hexane, 0.89 mL, 1.42 mmol, 2.2 equiv), and after 15 min **4a** (200 mg, 65 mmol) in dry THF (3 mL). After 45 min at −78 °C, TMSCl (0.25 mL, 1.94 mmoles, 3 equiv) was added, and the solution stirred further for 30 min. The solution was diluted with $Et_2O$, the organic phase washed with brine, dried ($MgSO_4$) and evaporated to give crude silyl enol ether **6a**.

To a solution of crude **6a** in dry $CH_3CN$ (10 mL) at −20 °C under Ar, was added Selectfluor™ (275 mg, 0.78 mmol, 1.2 equiv) and the solution stirred for 10 h at rt. $Et_2O$ was added and the organic solution washed with 2 N aqueous $NH_4Cl$, and with brine, dried ($MgSO_4$) and evaporated. Crystallization in *i*PrOH and washing with $Et_2O$ gave pure **4b** (145 mg, 68%).

Compound **4b**: colorless crystals, mp 119–121 °C (*i*PrOH). IR (KBr): 547,708, 720, 752, 757, 770, 982, 1012, 1019, 1229, 1328, 1380, 1452, 1456, 1494, 1525, 1709, 2949, 2975, 3029, 3068, 3394 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.23–7.07 (m, 9H, Har); 5.66 (d, 1H, NH); 5.48 (d, 1H, H-5); 5.22 (m, 1H, H-7); 5.02 (s, 2H, $CH_2Ph$); 3.42 (t, 1H, Ha-9); 2.68 (m, 1H, Hb-9); 2.61 (m, 1H, Ha-8); 1.48 (q, 1H, Hb-8). *J*(5,F) = 49.8, *J*(7,NH) = 7.2, *J*(7,F) = 5.4, *J*(7,8a) = 5.8, *J*(7,8b) = 11.5, *J*(8a,8b) = 12.8, *J*(8a,9b) = 7.0, *J*(8b,9a) = 13.2, *J*(9a,9b) = 14.2, *J*(9b,F) = 4.0 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 30.5 (d, *J* = 2.5 Hz, C-9); 37.2 (C-8); 58.3 (C-7); 67.1($CH_2Ph$); 98.4(d, *J* = 185.5 Hz; C(5)); 127.5 (C(3)); 128.3; 128.4; 128.7(Cp,m,o-Ph); 130.6 (C(4a)); 130.8 (d, *J* = 5.1 Hz, C(2)); 131.0 (d, *J* = 2.6 Hz, C(1)); 131.1(d, *J* = 2.6 Hz, C(4)); 136.3 (Cs-Ph); 142.0 (C(9a)); 155.4; 203.9 (d, *J* = 31.9, C(6)). Anal. calcd for $C_{19}H_{18}FNO_3$ (327.36): C, 69.71; H, 5.54; N, 4.28; F, 5.80. Found: C, 70.0; H, 5.6; N, 4.2; F, 5.5.

### 5.3.2. 5-Methylene-7-(benzyloxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-6-one (4i)

A solution of **4a** (300 mg, 0.97 mmol), formaldehyde 36% aq. (225 μL, 2.7 mmol, 2.8 equiv) and pyrrolidine (50 μL, 0.6 mmole, 0.6 equiv) in AcOH (10 mL) was heated at 110 °C for 5 h. The reaction was neutralized with aqueous 1 N NaOH, extracted with AcOEt, the organic phase washed with aqueous 1 N $NaHCO_3$, dried ($MgSO_4$) and evaporated to give crude **4i**.

Compound **4i** was used without further purification and only characterized with $^1H$ NMR.

$^1H$ NMR ($CDCl_3$, 400 MHz): 7.33–7.19 (m, 9H, Har); 6.52 (d, 1H, Ha-1′); 5.70 (d, 1H, Hb-1′); 5.67 (d, 1H, NH); 5.04 (s, 2H, O$CH_2$Ph); 4.58 (q, 1H, H-7); 2.83 (dt, 1H, Ha-9); 2.72 (dd, 1H, Hb-9); 2.61 (ddd, 1H, Ha-8); 1.76 (q, 1H, Hb-8). *J*(1′a,1′b) = 1.5; *J*(7,NH) = 7.6; *J*(7,8a) = 8.4; *J*(7,8b) = 10.0; *J*(8a,8b) = 12.2; *J*(8a,9a) = 13.4; *J*(8a,9b) = 6.8; *J*(8b,9a) = 6.8; *J*(8b,9b) = 1.4; *J*(9a,9b) = 13.8 Hz.

### 5.3.3. *trans*-7-(*tertio*Butyloxycarbonylamino)-5-fluoro-5,7,8,9-tetrahydrobenzocyclohepten-6-one (5b), and *trans*-5-fluoro-7-(dimethyl-*tertio*butyl)silyloxycarbonylaminobenzocyclo hepten-6-one, hydrate (7)

To a stirred solution of HMDS (0.34 mL, 1.6 mmol, 2.2 equiv) in dry THF (1.6 mL) at −78 °C, was successively added BuLi (1.6 M in hexane, 1.0 mL, 1.6 mmol, 2.2 equiv), and after 15 min, **5a** (200 mg, 73 mmol) in mixture of dry THF (3 mL) and HMPA (0.38 mL, 2.18 mmol, 3 equiv). After 45 min at −78 °C, *t*$BuMe_2SiCl$ (263 mg, 1.74 mmol, 2.4 equiv) was added, and the solution stirred further for 15 min at −78 °C. Aqueous 2 *M* $NH_4Cl$ (2 mL) and AcOEt were then added, the organic phase separated and washed with brine, dried ($MgSO_4$) and evaporated to give crude silyl enol ether **6b**.

To a solution of crude **6b** in dry $CH_3CN$ (8 mL) and aqueous *N* $NaHCO_3$ (2 mL) at 0 °C under Ar, was added Selectfluor™ (309 mg, 0.87 mmol, 1.2 equiv) and the solution stirred for 35 min at rt. $Et_2O$ was added and the organic solution washed with 2 N aqueous $NH_4Cl$, then with brine, dried ($MgSO_4$) and evaporated to give a mixture of **5b** and **8**. This crude mixture was stirred in THF (10 mL) with TBAF (76 mg, 0.29 mmol, 0.4 equiv) at 0 °C for 10 min, then extracted with AcOEt, the organic phase was washed with brine, dried ($MgSO_4$) and evaporated. Purification by FC (cyclohexane/ethyl acetate 8/2) gave pure **5b** (110 mg, 52%).

Without $NaHCO_3$, a mixture of **5b** (25%) and **7** (16%) was obtained. Compound **8** was neither isolated nor characterized.

Compound **5b**: colorless crystals, mp 127–129 °C (*i*PrOH). IR (KBr): 618, 760, 876, 975, 1011, 1061, 1171, 1248, 1293, 1327, 1365, 1492, 1526, 1709, 1725, 2857, 2942, 2981, 3375 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.33–7.16 (m, 4H, Har); 5.58 (d, 1H, H-5); 5.50 (d, 1H, NH); 5.27 (m, 1H, H-7); 3.50 (t, 1H, Ha-9); 2.78 (m, 1H, Hb-9); 2.67 (m, 1H, Ha-8); 1.54 (q, 1H, Hb-8); 1.46 (m, 9H, $CMe_3$). *J*(5,F) = 49.6, *J*(7,NH) = 7.0, *J*(7,F) = 5.7, *J*(7,8a) = 5.7, *J*(7,8b) = 12.0, *J*(8a,8b) = 13.0, *J*(8a,9b) = 6.7, *J*(8b,9a) = 12.6, *J*(9a,9b) = 15.0, *J*(9b,F) = 4.0 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 28.5 ($CMe_3$); 30.6 (d, *J* = 2.8 Hz, C-9); 37.3 (C-8); 58.0 (C-7); 80.1 ($CMe_3$); 98.5 (d, *J* = 188.6 Hz, C-5); 127.4 (C-3); 130.8 (d, *J* = 6.3 Hz, C-1); 130.9 (d, *J* = 3.5 Hz, C-2); 131.1 (d, *J* = 2.8 Hz, C-4); 131.1 (C(4a)); 142.1 (C-9a); 154.1 CON-7; 204.3 (d, *J* = 30.9 Hz, CO(6)). HR-MS (ESI-Q-Tof) calcd for $C_{16}H_{20}FNO_3$ $[M]^+$: 293.1427; found: 293.1405.

Compound **7**: colorless crystals, mp 114–116 °C (*i*PrOH). $^1H$ NMR ($CDCl_3$, 400 MHz): 0.10–0.14 (2 s, 6H, $SiMe_2$); 0.45 (s, 9H, Si*t*-Bu); 1.99 (m, 1H, H-8 eq); 2.16 (q, 1H, H-8ax); 2.88 (dd, 1H, H-9 eq); 2.98 (t, 1H, H-9ax); 4.50 (dt, 1H, H-7); 5.27 (s, 1H, NH); 5.43 (d, 1H, H-5); 7.22 (m, 4H, Har). *J*(5,F) = 49.4, *J*(7,F) = 3,2, *J*(7,8ax) = 12,0, *J*(7,8 eq) = 3.2, *J*(8ax,8 eq) = 12.6, *J*(8ax,9ax) = 12.3, *J*(8ax,9 eq) = 2.0, *J*(8 eq,9ax) = 3.2, *J*(8 eq,9 eq) = 5.2, *J*(9ax,9 - eq) = 15.0 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): -3.1, -3.5 ($SiMe_2$); 17.7 ($SiCMe_3$); 25.2 ($SiCMe_3$); 26.2 (C(9)); 33.9 (C(8)); 59.2 (d, *J* = 4.2 Hz,C(7)); 94.1 (d, *J* = 182.1 Hz, C(5)); 104.1 (d, *J* = 26.0 Hz, C(6)); 126.9 (d, *J* = 1.4 Hz, C(3)); 130.1 (d, *J* = 2.8 Hz, C(2)); 132.3 (d, *J* = 2.8 Hz, C(1)); 133.4 (d, *J* = 17.5 Hz, C(4a)); 133.6 (d, *J* = 5.6 Hz, C(4)); 140.1 (C(9a)); 157.7 $CO_2N$. HR-MS (ESI-Q-Tof) calcd for $C_{18}H_{26}FNO_3Si$ $[M]^+$: 351.1666, found: 351.1641.

### 5.3.4. 5-Benzylidene-7-(*tertio*butoxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-6-one (5h)

To a solution of LiHMDS (preparated from BuLi 1.6 M (0.5 mL, 0.8 mmol, 2.2 equiv) and HMDS (0.17 mL, 0.8 mmol, 2.2 equiv for 15 min) in THF (1 mL) at −78 °C, was added a solution of **5a** (100 mg, 0.36 mmol) and HMPA (0.19 mL, 1.1 mmol, 3 equiv) in THF (2 mL), then stirred 20 min at −78 °C. A solution of benzaldehyde (74 μL, 0.73 mmol, 2 equiv) in THF (2 mL) was then added and the mixture stirred 1 h at −78 °C, then 2.5 h at rt. Water (2 mL) was added and the solution extracted with AcOEt, the organic phase was washed with brine, dried ($MgSO_4$) and evaporated. Purification by FC (cyclohexane /AcOEt 8/2 to 7/3) gave pure **5h** (70 mg, 53%).

Compound **5h**: colorless crystals, mp 156–158 °C (*i*PrOH). IR (KBr): 3356, 2976, 2933, 1709, 1691, 1602, 1530, 1364, 1251, 1179, 1169, 1046, 764, 754, 691 cm−1. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.95 (s, 1H, Har); 7.29–7.09 (m, 9H, Har); 5.49 (dl, 1H, NH); 4.45 (ddd, 1H, H-7); 2.99 (ddd, 1H, Ha-9); 2.76 (ddd, 1H, Hb-9); 2.56 (dddd, 1H, Ha-8); 1.74 (m, 1H, Hb-8); 1.41 (s, 9H, $CMe_3$). *J*(7,NH) = ca. 7.6, *J*(7,8a) = 8.0, *J*(7,8b) = 10.5, *J*(8a,8b) = 12.5, *J*(8a,9a) = 13.1, *J*(8a,9b) = 7.5, *J*(8b,9a) = 7.6, *J*(8b, 9b) = 1.4, *J*(9a,9b) = 13.7 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 198.2 (CO(6)); 155.1 ($NCO_2$); 138.8 (Car); 138.6 (C(1′), 136.2, 134.9, 134.7 (3 Car); 131.2 (Co(Ph)); 130.5, 129.6, 129.4, 129.0 (4 CHar); 128.4 (Cm(Ph)); 127.2 (Car); 79.7 ($CMe_3$); 55.8 (C(7)); 34.1 (C(8)); 29.9 (C(9)); 28.5 ($CMe_3$). Anal. calcd for $C_{23}H_{25}NO_3$ (363.45): C, 76.01; H, 6.93; N, 3.85. Found: C, 75.4; H, 7.1; N, 3.9. HR-MS (ESI-Q-Tof) calcd for $C_{23}H_{25}NO_3$ $[M]^+$: 363.1834; found: 363.1846.

### 5.3.5. *N*-Benzyloxycarbonyl-6,7-dihydro-5*H*-benzocyclohepten-7-amine (10a)

To a solution of **9** (1 g, 6.33 mmol) in a 2–3 M solution of $NH_3$ in EtOH (20 mL) was added Ti(*i*PrO)$_4$ (3.8 mL, 12.7 mmol, 2 equiv) and the solution stirred for 6 h at rt. $NaBH_4$ (280 mg, 6.6 mmol, 1.1 equiv) was then added and the solution stirred for another 1 h. After evaporation of the solvent, AcOEt (20 mL) and 1 N aqueous $NH_4OH$ (20 mL) were added and the mixture stirred for 1 h. The precipitate was filtered off, washed with AcOEt/1 N aqueous $NH_4OH$ and the solutions extracted with AcOEt. The organic phases were dried ($MgSO_4$) and evaporated. The obtained amine was protected by stirring in THF (20 mL) with CbzCl (1.7 mL, 11.9 mmol, 1.8 equiv) and $Na_2CO_3$ (2.5 g, 23.9 mmol, 3.5 equiv) for 1 h. **10a**

(1.2 g, 80%) was precipitated with $H_2O$, isolated by filtration, washed with *i*$Pr_2O$ and dried.

Compound **10a**: colorless crystals, mp 135–137 °C (*i*PrOH). IR (KBr): 3320, 1685, 1534, 1304, 1248, 1048, 1019, 785, 743, 697 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.38–7.34 (m, 5H, Har); 7.23–7.14 (m, 4H, Har); 6.46 (d, 1H, H-9); 5.76 (dd, 1H, H-8); 5.12 (s, 2H, $CH_2$Ph); 4.90 (broad d, 1H, NH); 4.58 (broad s, 1H, H-7); 2.86 (m, 1H, Ha-5); 2.74 (m, 1H, Hb-5); 2.06 (m, 2H, $CH_2$(6)). *J*(NH,7) = ca. 8, *J*(7,8) = 4.0, *J*(8,9) = 12.2 Hz.

### 5.3.6. *N-tertio*butoxycarbonyl-6,7-dihydro-5*H*-benzocyclo hepten-7-amine (10b)

Same procedure as for **10a** with **9** (0.50 g, 3.15 mmol). The crude amine was protected in MeOH (6 mL) with $Na_2CO_3$ (370 mg, 3.48 mmol) and $Boc_2O$ (1.4 g, 6.33 mmol) with stirring for 2 h at rt. **10b** was isolated as above (0.70 mg, 76%).

Compound **10b**: colorless crystals, mp 146–148 °C (*i*PrOH). IR (KBr): 3361, 1682, 1517, 1245, 1168, 1156, 1005, 787, 747 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.18–7.11 (m, 4 Har); 6.44 (dd, 1H, H-9); 5.76 (dd, 1H, H-8); 4.69 (d, 1H, NH); 4.49 (broad s, 1H, H-7); 2.85 (m, 1H, Ha-5); 2.73 (m, 1H, Hb-5); 2.04 (m, 2H, H-6); 1.46 (s, 9H, $CMe_3$). *J*(7,8) = 4.0 Hz, *J*(7,9) = 1.9 Hz, *J*(8,9) = 12.3 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 155.0 ($NCO_2$); 141.8, 134.8, 131.6, 131.4, 131.0, 129.1, 127.5, 126.3 (8 Car); 79.6 (*C*$Me_3$); 51.5 (C(7)); 33.1 (C(6)); 31.5 (C(5)); 28.5 (C*Me*$_3$). Anal. calcd for $C_{16}H_{21}NO_2$ (259.34): C, 74.10; H, 8.16; N, 5.40. Found: C, 74.2; H, 8.3; N, 5.5.

### 5.3.7. *cis-N*-Benzyloxycarbonyl-5,6-epoxy-6,7,8,9-tetrahydro-5*H*-benzocycloheptен-7-amine (11a)

To a solution of **10a** (640 mg, 2.18 mmol) in $CH_2Cl_2$ (40 mL) was added *m*-CPBA (860 mg, 5.0 mmol, 2.2 equiv) and the solution stirred at 0 °C for 16 h. Aqueous solution of $Na_2S_2O_3{\cdot}5H_2O$ (2.5 g, 10 mmol) in 1 N $NaHCO_3$ (10 mL) was added and the mixture vigorously stirred for 45 min at rt, then extracted with $Et_2O$, the organic phase washed with 1 N $NaHCO_3$ then with brine, dried ($MgSO_4$) and evaporated to give **11a** (610 mg, 90%).

Compound **11a**: colorless crystals, mp 140–141 °C (EtOH). IR (KBr): 3336, 2941, 1688, 1537, 1315, 1250, 1041, 1016, 902, 754, 729, 699 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.51 (m, 1H, Har); 7,35 (m, 6H, Har); 7.24 (m, 1H, Har); 7.09 (m, 1H, Har); 5.22 (d, 1H, NH); 5.14 (s, 2H, $CH_2$Ph); 4.44 (dddd, 1H, H-7); 4.01 (d, 1H, H-5); 3.69 (m, 1H, H-6); 2.84 (ddd, 1H, Ha-9); 2.65 (dd, 1H, Hb-9); 1.98 (m, Ha-8); 1.72 (ddt, Hb-8). *J*(5,6) = 4.2, *J*(6,7) = 2.8, *J*(6,8) = 1.2, *J*(7,8a) = 4.5, *J*(7,8b) = 10.4, *J*(NH,7) = 9.2, *J*(8a,8b) = 13.6, *J*(8a,9a) = 8.8, *J*(8a,9b) = 1.3, *J*(8b,9a) = 1.7, *J*(8b,9b) = 10.6, *J*(9a,9b) = 15.6 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 155.6 ($NCO_2$); 141.5 (Cs(Bn)); 136.3 (C(9a)); 133.8 (CHar); 132.1 (C(4a)); 129.8, 128.9, 128.5, 128.1, 128.0, 126.4 (6 CHar); 66.8 ($CH_2$Ph); 61.2, 60.2 (C(5), C(6)); 52.1 (C(7)); 31.3 (C(8)); 30.6 (C(9)). Anal. calcd for $C_{19}H_{19}NO_3$ (309.36): C, 73.77; H, 6.19; N, 4.53. Found: C, 73.9; H, 6.2; N, 4.5.

### 5.3.8. *cis-N-tert* Butoxycarbonyl-5,6-epoxy-6,7,8,9-tetrahydro-5*H*-benzocycloheptен-7-amine (11b)

To a solution of **10b** (400 mg, 1.54 mmol) in $CH_2Cl_2$ (20 mL) was added *m*-CPBA (610 mg, 2.46 mmol, 1.6 equiv) and the solution stirred at 0 °C for 16 h. Same work-up as above gave **11b** (425 mg, quant.).

Compound **11b**: colorless crystals, mp 170–172 °C (*i*PrOH). IR (KBr): 3359, 2980, 2968, 2934, 1687, 1519, 1390, 1370, 1314, 1250, 1169, 1158, 1002, 753, 614 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.50 (m, 1H, Har); 7.23 (m, 2H, Har); 7.08 (m, 1H, Har); 4.98 (d, 1H, NH); 4.36 (m, 1H, H-7); 3.99 (d, 1H, H-5); 3.68 (dl, 1H, H-6); 2.84 (dd, 1H, Ha-9); 2.63 (dd, 1H, Hb-9); 1.95 (m, 1H, Ha-8); 1.69 (m, 1H, Hb-8); 1.47 (s, 9H, $CMe_3$). *J*(5,6) = 4.2, *J*(6,7) = 2.7, *J*(6,8a) = 1.3, *J*(NH,7) = 9.0, *J*(7,8a) = 4.4, *J*(7,8b) = 10.6, *J*(8a,8b) = 13.6, *J*(8a,9a) = 8.8, *J*(8a,9b) = 1.5, *J*(8b,9a) = 1.5, *J*(8b,9b) = 10.2, *J*(9a,9b) = 15.5 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 155.3 ($NCO_2$); 141.8, 132.4, (C(4a),C(9a)); 134.0, 130.0, 129.0, 126.6 (4 CHar); 79.9 (*C*$Me_3$); 61.6, 60.4 (C(5),C(6)); 51.8 (C(7)); 31.7 (C(8)); 30.8 (C(9)); 28.5 (C*Me*$_3$). Anal. calcd for $C_{16}H_{21}NO_3$ (275.34): C, 69.79; H, 7.69; N, 5.09. Found: C, 69.9; H, 7.9; N, 5.1.

## 5.4. Addition reactions—procedure A

A solution of BnMgBr (2.85 mL, 1.5 M dans $Et_2O$, 4.27 mmol) was added at −50 °C to a suspension of CuBr.$SMe_2$ (440 mg, 2.13 mmol) in anhydrous THF (20 mL) under Ar, then stirred at −50 °C for 45 min. To this suspension at −40 °C, was added dropwise a solution of crude epoxide **11a,b** or enone **4i** (1 mmol) in anhydrous THF (10 mL) and the solution further stirred for 2 h at −40 °C. After addition of 2 N aqueous $NH_4Cl$ solution, the mixture was extracted with AcOEt, the organic phases washed with brine, dried ($MgSO_4$) and evaporated.

### 5.4.1. *trans*-5-Phenylethyl-7-(benzyloxycarbonylamino)-5,7,8,9-tetrahydrobenzocycloheptен-6-one (4e)

Procedure A with $BnCH_2MgBr$ (1.7 mL, 1.3 M dans $Et_2O$, 2.13 mmol), CuBr.$SMe_2$ (220 mg, 11.1 mmol) in anhydrous THF (10 mL) and epoxyde **11a** (105 mg, 0.34 mmol) in anhydrous THF (10 mL). Purification by FC (Chx/AcOEt, 9/1 then 8/2) gave crude **12e** (115 mg, 82%). **12e** was directly oxidized according to the Procedure B with DMP (0.2 g, 0.48 mmol, 1.4 equiv) to give **4e** (90 mg, 79%).

Compound **4e**: colorless resin. IR (KBr): 3417, 3062, 3028, 2929, 2859, 1703, 1497, 1454, 1246, 749, 698 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.33–7.11 (m, 14H, Har); 5.60 (sl, 1H, NH); 5.09 (s, 2H, O$CH_2$Ph); 4.68 (ddd, 1H, H-7); 3.75 (t, 1H, H-5); 3.17 (ddd, 1H, Ha-9); 2.88 (ddd, 1H, Hb-9); 2.64 (t, 2H, H-2′); 2.56 (m, 1H, Ha-8); 2.52 (dt, 1H, Ha-1′); 2.27 (dt, 1H, Hb-1′); 1.58 (dddd, Hb-8). *J*(5,1′a) = *J*(5,1′b) = 7.5, *J*(1′a,2′) = *J*(1′b,2′) = 7.7, *J*(1′a,1′b) = 14.0, *J*(7,NH) = 6.5, *J*(7,8a) = 6.5, *J*(7,8b) = 11.0, *J*(8a,8b) = 13.4, *J*(8a,9a) = 3.6, *J*(8a,9b) = 8.2, *J*(8b,9a) = 9.5, *J*(8b,9b) = 3.7, *J*(9a,9b) = 15.2 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 207.3 (C(6)); 155.6 ($NCO_2$); 140.9, 139.8, 136.5, 135.6 (4 Car); 130.9, 129.9, 128.7, 128.6, 128.3, 128.0, 127.6, 126.4 (Car); 67.0 (O$CH_2$Ph); 59.2 (C(5)); 58.9 (C(7)); 34.5 (C(8)); 33.6 (C(2′)); 32.2 (C(1′)); 31.3 (C(9)). HR-MS (ESI-Q-Tof) calcd for $C_{27}H_{27}LiNO_3$ $[M+Li]^+$: 420.2151; found: 420.2160.

### 5.4.2. *cis*-5-Phenylethyl-7-(benzyloxycarbonylamino)-5,7,8,9-tetrahydrobenzocycloheptен-6-one (4e′)

Procedure A with BnMgBr (2.85 mL, 1.5–1.6 M dans $Et_2O$, 4.27 mmol), CuBr.$SMe_2$ (440 mg, 2.13 mmol) in anhydrous THF (20 mL), crude **4i** (ca. 0.32 g, 0.97 mmol) in anhydrous THF (10 mL). Purification by FC (Chx/AcOEt, 9/1 then 8/2) gave **4e′** (170 mg, 42%).

Compound **4e′**: colorless crystals, mp 136–138 °C (*i*PrOH). IR (KBr): 3418, 3331, 3031, 2945, 2856, 1715, 1680, 1528, 1249, 749, 696 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.36–7.11 (m, 14H, Har); 5.65 (d, 1H, NH); 5.10 (s, 2H, O$CH_2$Ph); 4.56 (m, 1H, H-7); 3.94 (t, 1H, H-5); 3.95 (dd, 1H, Ha-9); 2.84 (dd, 1H, Hb-9); 2.70–2.60 (m, 4H, Ha-1′,Ha-8, $CH_2$(2′)); 2.10 (m, 1H, Hb-1′); 1.42 (m, Hb-8). *J*(NH,7) = 7.0, *J*(7,8a) = 7.2, *J*(7,8b) = 11.0, *J*(8a,8b) = 13.0, *J*(8a,9a) = 2.8, *J*(8a,9b) = 8.6, *J*(8b,9a) = 9.6, *J*(8b,9b) = 3.2, *J*(9a,9b) = 14.6 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 206.9 (C(6)); 155.5 ($NCO_2$); 141.3, 140.5, 136.5, 135.3 (4 Car); 129.5, 128.7, 128.6, 128.3, 128.2, 127.7, 126.7, 126.3 (CHar); 67.0 (O$CH_2$Ph); 62.3 (C(7)); 52.9 (C(5)); 36.3 (C(8)); 33.7 (C(2′)); 31.0 (C(9)); 30.1 (C(1′)). Anal. calcd for $C_{27}H_{27}NO_3$ (413.51): C, 78.42; H, 6.58; N,

3.39. Found: C, 78.2; H, 6.7; N, 3.2. HR-MS (ESI-Q-Tof) calcd for $C_{27}H_{27}NNaO_3$ $[M+Na]^+$: 436.1883; found: 436.1895.

#### 5.4.3. *cis*-5-Phenylpropyl-7-(benzyloxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-6-one (4g′)

Procedure A with $BnCH_2CH_2MgBr$ (3.1 mL, 1.3 M dans $Et_2O$, 4.27 mmol), $CuBr{\cdot}SMe_2$ (440 mg, 2.13 mmol) in anhydrous THF (20 mL), crude **4i** (ca. 0.32 g, 0.97 mmol) in anhydrous THF (10 mL). Purification by FC (Cyclohexane/AcOEt, 9/1 then 8/2) gave **4g′** (182 mg, 43%).

Compound **4g′**: colorless crystals, mp 106–108 °C (*i*PrOH). IR (KBr): 3354, 3330, 3026, 2941, 2854, 1717, 1682, 1525, 1453, 1365, 1244, 751, 729, 695 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.34–7.11 (m, 14H, Har); 5.68 (d, 1H, NH); 5.08 (s, 2H, O*CH*$_2$Ph); 4.58 (m, 1H, H-7); 3.91 (t, 1H, H-5); 3.06 (dd, 1H, Ha-9); 2.84 (dd, 1H, Hb-9); 2.74–2.60 (m, 3H, Ha-8, $CH_2$(3′)); 2.36 (m, 1H, Ha-1′); 1.83 (m, 1H, Hb-1′), 1.63 (m, 2H, $CH_2$(2′)), 1.43 (m, 1H, Hb-8). *J*(1′a,1′b) = 13.0, *J*(1′,2′) = *J*(2′,3′) = 7.7, *J*(1′a,5) = 6.2, *J*(1′b,5) = 7.7, *J*(NH,7) = 7.0, *J*(7,8a) = 7.0, *J*(7,8b) = 11.2, *J*(8a,8b) = 12.6, *J*(8a,9a) = 2.5, *J*(8a,9b) = 8.4, *J*(8b,9a) = 10.0, *J*(8b,9b) = 2.8, *J*(9a,9b) = 14.8 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 207.2 (C(6)); 155.4 ($NCO_2$); 141.9, 140.2, 136.4, 135.3 (4 Car); 129.4, 128.6, 128.5, 128.4, 128.2, 128.1, 127.6, 127.5, 126.9, 125.9 (10 CHar); 66.9 (O*CH*$_2$Ph); 61.9 (C(7)); 54.0 (C(5)); 36.2, 36.1 (C(8),C(3′)); 30.8 (C(9)); 29.4 (C(2′)); 28.1 (C(1′)). Anal. calcd for $C_{28}H_{29}NO_3$ (427.53): C, 78.66; H, 6.84; N, 3.28. Found: C, 78.8; H, 6.9; N, 3.1. HR-MS (ESI-microTof) calcd for $C_{28}H_{29}NNaO_3$ $[M+Na]^+$: 450.2040; found: 450.2048.

#### 5.4.4. *r*-5-Benzylthio-*t*-7-(*tertio*butoxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-*t*-6-ol (12c)

A solution of **11c** (52 mg, 0.19 mmol), $Et_3N$ (63 μL, 0.45 mmol, 2.4 equiv) and BnSH (30 μL, 0.23 mmol, 1.2 equiv) in EtOH (1 mL) was stirred at rt for 1 h. **12c** was precipitated and washed with $H_2O$, then dried (60 mg, 80%).

*Compound **12c***: colorless resin. RMN $^1H$ ($CDCl_3$, 400 MHz): 7.32–7.15 (m, 6H, Har); 7.15 (t, *J* = 7.3 Hz, 2H, Har); 6.98 (d, *J* = 7.3 Hz, 1H, Har); 4.99 (d large, 1H, NH); 4.22 (large s, 1H, H-7); 4.09 (t large, 1H, H-6); 3.94 (d, 1H, H-5); 3.69 (d, *J* = 13.8 Hz, 1H, Ha(SBn)); 3.57 (d, *J* = 13.8 Hz, 1H, Hb(SBn); 3.38 (t l, 1H, Hax-9); 2.67 (dd, 1H, Heq-9); 2.00 (m, 1H, Heq-8); 1.46 (m, 1H, Hax-8), 1.45 (s, 9H, $CMe_3$). *J*(5,6) = 6.0, *J*(6,7) = 8.4, *J*(NH,7) = ca. 7.2, *J*(8 eq,9 eq) = 6.6, *J*(8ax,9 eq) = 1,6, *J*(8ax,9ax) = 12.2, *J*(9ax,9 eq) = 14.6 Hz. RMN $^{13}C$ ($CDCl_3$, 100 MHz): 155.0 ($NCO_2$); 142.5 (Cs(Ph)); 137.7, 134.8 (C(4a), C(9a)); 132.2, 130.9, 129.2, 128.7, 128.5, 127.3, 126.4 (7 CHar); 79.4 (*C*Me$_3$); 72.7 (C(6)); 53.0, (C(7), C(5)); 36.3 (S*CH*$_2$Ph); 31.9 (C(9)); 28.6 (C(8), C*Me*$_3$). HR-MS (ESI-microTof) calcd for $C_{23}H_{29}NO_3S$ $[M]^+$: 399.1868; found: 399.1871.

#### 5.4.5. *r*-5-Phenylthio-*t*-7-(*tertio*butoxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-*t*-6-ol (12d)

A solution of **11b** (225 mg, 0.82 mmol), $Et_3N$ (0.275 mL, 1.96 mmol) and PhSH (100 μL, 0.98 mmol) in EtOH (15 mL) was stirred at rt for 1 h. **12d** was precipitated with $H_2O$, filtrated and washed with $iPr_2O$ to give pure product (270 mg, 86%).

Compound **12d**: colorless crystals, mp 174–176 °C ($iPr_2O$). IR (KBr): 3395, 3356, 2975, 2935, 1674, 1506, 1566, 1240, 1171, 746 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.37 (m, 2H, Har); 7.25–7.14 (m, 5H, Har); 7.08 (t, *J* = 7,5 Hz, 1H, Har); 6.97 (d, *J* = 7.0 Hz, 1H, Har); 5.06 (d, 1H, NH); 4.41 (s large, 1H, H-7), 4.39 (d, 1H, H-5); 4.23 (t, 1H, H-6); 3.38 (t, 1H, Hax-9); 2.74 (dd, 1H, Heq-9); 2.07 (m, 1H, Heq-8); 1.55 (q, 1H, Hax-8); 1.46 (s, 9H, $CMe_3$). *J*(5,6) = 5.8, *J*(6,7) = 9.0, *J*(NH,7) = ca. 8.0, *J*(7,8ax) = *J*(8ax,9ax) = ca. 12.6, *J*(8ax,9ax) = 12.0, *J*(8ax,9 eq) = 1.6, *J*(8 eq,9 eq) = 6.8, *J*(9ax,9 - eq) = 14.8 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 155.1 ($NCO_2$); 142.2 (Cs(Ph)); 134.8, 134.6 (C(4a),C(9a)); 133.7, 132.3, 130.6, 129.1, 128.7, 128.1, 126.6 (7 CHar); 79.5 (*C*Me$_3$); 72.9 (C(6)); 59.6 (C(5)); 52.6 (C(7)); 32.1 (C(9)); 28.6 (C(9),C*Me*$_3$). HR-MS (ESI-microTof) calcd for $C_{22}H_{27}NO_3S$ $[M+Na]^+$: 408.1604; found: 408.1590.

### 5.5. Oxidation of alcohol—procedure B

To a solution of amido-alcohol **12** (1 mmol) in 40 mL wet $CH_2Cl_2$ (10 mL) under Ar was added Dess-Martin periodinane (DMP) ((0.6 g, 1.4 mmol, 1.4 equiv) and the mixture stirred at rt for 2 h. AcOEt (10 mL) and a solution of $Na_2S_2O_3{\cdot}5H_2O$ (4 mmol) in aqueous 1 N $NaHCO_3$ (10 mL) were added and the mixture was vigorously stirred for 1 h, then extracted with AcOEt, the organic solutions washed with aqueous 1 N $NaHCO_3$ then with brine, dried ($MgSO_4$) and evaporated.

#### 5.5.1. *trans*-5-Benzylthio-7-(*tertio*butoxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-6-one (5c)

Procedure B with **12c** (40 mg, 0.1 mmol) and DMP (47 mg, 0.11 mmol) in wet $CH_2Cl_2$ (5 mL) to give **5c** (40 mg, quant.).

Compound **5c**: colorless resin. $^1H$ NMR ($CDCl_3$, 400 MHz): 7.30–7.03 (m, 9H, Har); 5.32 (m, 2H, NH, H-C(7)); 4.40 (s, 1H, H-5); 3.68–3.70 (2 d, J = 13.7 Hz, 2H, S*CH*$_2$Ph); 3.50 (dd, 1H, Ha-9); 2.71 (ddd, 1H, Hb-9); 2.52 (m, 1H, Ha-8); 1.38 (m, 1H, Hb-8); 1.40 (s, 9H, $CMe_3$). *J*(8a,9b) = 7.8, *J*(8b,9a) = 11.2, *J*(8b,9b) = 1.8, *J*(9a,9b) = 15.0 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 204.4 (C(6)); 155.1 ($NCO_2$); 141.1 (C(9a)); 136.8 (Cs(Ph)), 132.6 (C(4a)); 131.0, 130.7, 129.3, 128.8, 128.7, 127.5, 127.4 (7 Car); 79.8 (*C*Me$_3$); 60.2 (C(5)); 57.4 (C(7)); 36.9 (*CH*$_2$Ph)); 35.3 (C(8)); 31.5 (C(9)); 28.5 (C*Me*$_3$). HR-MS (ESI-microTof) calcd for $C_{23}H_{27}NO_3S$ $[M]^+$: 397.1712; found: 397.1703.

#### 5.5.2. *trans*-5-Phenylthio-7-(*tertio*butoxycarbonylamino)-5,7,8,9-tetrahydrobenzocyclohepten-6-one (5d)

Procedure B with **12d** (230 mg, 0.60 mmol) and DMP (280 mg, 0.66 mmol) in wet $CH_2Cl_2$ (10 mL) to give **5d** (190 mg, 83%), after recristallisation in *i*PrOH.

Compound **5d**: colorless crystals, mp 121–123 °C (*i*PrOH). IR (KBr): 3428, 3369, 2975, 2927, 1693, 1493, 1484, 1366, 1169, 754, 737 $cm^{-1}$. $^1H$ NMR ($CDCl_3$, 400 MHz, 328 K): 7.38 (m, 2H, Har); 7.30–7.09 (m, 7H, Har); 5.47 (sl, 1H, H-7); 5.41 (m, 1H, H-7); 4.97 (s, 1H, H-5); 3.67 (tl, 1H, Ha-9); 2.86 (ddd, 1H, Hb-9); 2.66 (m, 1H, Ha-8); 1.53 (m, 1H, Hb-8); 1.45 (s, 9H, $CMe_3$). *J*(7,NH) = ca. 6.0, *J*(7,8a) = 6.2, *J*(7,8b) = 11.4, *J*(8a,8b) = 13.2, *J*(8a,9a) = 1.6, *J*(8a,9b) = 7.6, *J*(8b,9a) = 11.6, *J*(8b,9b) = 1.8, *J*(9a,9b) = 15.2 Hz. $^{13}C$ NMR ($CDCl_3$, 100 MHz): 204.0 (C(6)); 155.0 ($NCO_2$); 141.4, 133.4, 132.2 (3 Car); 132.4, 131.18, 131.16, 129.5, 129.2, 128.3, 127.6 (7 CHar); 79.8 (*C*Me$_3$); 65.3 (C(5)); 58.0 (C(7)); 35.6 (C(8)); 31.7 (C(9)); 28.5 (C*Me*$_3$). HR-MS (ESI-microTof) calcd for $C_{22}H_{25}NaNO_3S$ $[M+Na]^+$: 406.1453; found: 406.1420. For $C_{22}H_{25}LiNO_3S$ $[M+Li]^+$: 390.1715; found: 390.1684.

#### 5.5.3. N-deprotection

*Procedure C*: A solution of **5a–d,h** (1 mmol) in 2.2 N dry HCl in $Et_2O$ (2 mL) and dry dioxane (2 mL) was stirred under Ar at rt for 3 d. The precipitated hydrochloride was isolated by filtration or centrifugation, washed with dry $Et_2O$ and recrystallized in *i*PrOH/$Et_2O$.

*Procedure D*: A solution of N–$CO_2$Bn aminocetone **4e**,**e′**,**g′** (1 mmol) was hydrogenolysed over Pd-C 5% (20 mg) in dioxane (5–20 mL) and aqueous 1 N HCl (1.1 mL, 1.1 mmol) at 40 °C for 24 h. The catalyst was centrifuged off, the solvent evaporated. Crude **2e**,**e′**,**g′** were recrystallized in *i*PrOH/$Et_2O$.

#### 5.5.4. *trans*-7-Amino-5-fluoro-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2b)

**5b** (19 mg, 0.65 mmol) in solution in EtOH (1 mL) and aqueous 6 N HCl (0.11 mL) was stirred at rt for 12 h. After evaporation of the solvent, recrystallization in *i*PrOH/$Et_2O$ gave **2b** (11 mg, 74%).

Compounds **2b**/**2b′**: colorless crystals, mp >150 °C (dec) (*i*PrOH/$Et_2O$). IR (KBr): 408, 427, 448, 451, 620, 769, 797, 874, 955, 994, 1052, 1194, 1217, 1456, 1503, 1574, 1737, 2886, 2914, 3010, 3058, 3336, 3386, 3403 $cm^{-1}$.

#### 5.5.5. $^1H$ NMR ($D_2O$, 85/15 mixture of hydrate 2b′ and ketone 2b)

*Hydrate* ***2b′***: 7.44–7.28 (m, 4H, Har); 5.48 (d, 1H, H-5); 3.90 (dt, 1H, H-7); 3.13 (m, 1H, Ha-9); 2.86 (m, 1H, Hb-9); 2.17 (m, 1H, Ha-8); 1.77 (q, 1H, Hb-8). *J*(5,F) = 45.3, *J*(7,F) = 2.4, *J*(7,8a) = 3.6, *J*(7,8b) = 12.3, *J*(8a,8b) = 13.6, *J*(8a,9a) = 1.6, *J*(8a,9b) = 7.4, *J*(8b,9a) = 12.4, *J*(8b,9b) = 2.0, *J*(9a,9b) = 15.2, *J*(9b,F) = 2.0 Hz.

*Ketone* ***2b***: 7.44–7.28 (m, 4H, Har); 5.90 (d, 1H, H-5); 5.09 (m, 1H, H-7); 3.53 (m, 1H, Ha-9); 2.99 (m, 1H, Hb-9); 2.66 (m, 1H, Ha-8); 1.88 (q, 1H, Hb-8). *J*(5,F) = 48.6, *J*(7,F) = 4.2, *J*(7,8a) = 6.0, *J*(7,8b) = 11.8, *J*(8a,8b) = 13.2, *J*(8a,9a) = 1.8, *J*(8a,9b) = 6.4, *J*(8b,9a) = 12.2, *J*(8b,9b) = ca. 3, *J*(9a,9b) = 15.0, *J*(9b,F) = 3.0 Hz.

HR-MS (ESI-microTof) calcd for $C_{11}H_{12}FNO$ $[M]^+$: 193.0903, found: 193.0901; for the hydrate $C_{11}H_{14}FNO_2$ $[M]^+$: 211.1009, found: 211.1032.

#### 5.5.6. *trans*-7-Amino-5-benzylthio-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2c)

Procedure C from **5c** (40 mg, 0.1 mmol) was obtained the hydrochloride **2c** (20 mg, 66%).

Compound **2c**: yellowish crystals, mp 176–180 °C (*i*PrOH/$Et_2O$). IR (KBr): 3434, 2922, 2864, 1708, 1492, 1450, 767, 747, 668 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 400 MHz): 7.36–7.09 (m, 9H, Har); 5.18 (dd, 1H, H-7); 4.60 (s, 1H, H-5); 3.86 (d, 1H, Ha-C(SBn)); 3.79 (d, 1H, Hb-C(SBn)); 3.64 (dd, 1H, Ha-9); 2.97 (ddd, 1H, Hb-9); 2.53 (m, 1H, Ha-8); 1.75 (q, 1H, Hb-8). *J*(SBn) = 13.2, *J*(7,8a) = 6.0, *J*(7,8b) = 12.1, *J*(8a,8b) = 12.8, *J*(8a,9a) = 2.0, *J*(8a,9b) = 7.6, *J*(8b,9a) = 11.5, *J*(8b,9b) = 2.0, *J*(9a,9b) = 15.0 Hz. $^{13}C$ NMR ($CD_3OD$, 100 MHz): 202.0 (C(6)); 141.5 (Cs(Ph)); 137.9, 133.1 (C(4a),C(9a)); 132.1, 131.8 (C(1),C(4)); 130.3, 129.8 (CPh); 129.0, 128.7 (C(2),C(3)); 60.7 (C(5)); 57.9 (C(7)); 37.3 (C(1′)); 33.4 (C(8)); 31.6 (C(9)). HR-MS (ESI-microTof) calcd for $C_{18}H_{20}NOS$ $[M+H]^+$: 298.1260; found: 298.1241.

#### 5.5.7. *trans* and *cis* 5-Phenylthio-7-amino-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2d and 2d′)

Procedure C with **5d** (80 mg, 0.21 mmol) to give **2d**/**2d′** (55 mg, 83%).

Compounds **2d**/**2d′**: yellowish crystals, mp 148–154 °C (*i*PrOH/$Et_2O$). IR (KBr): 3420, 2925, 2867, 1719, 1490, 1481, 744, 691 $cm^{-1}$. HR-MS (ESI-microTof) calcd for $C_{17}H_{18}NOS$ $[M+H]^+$: 284.1109; found: 284.1085.

**5.5.7.1. *trans*-Isomer 2d, deuteriated at C(5).** RMN $^1H$ ($D_2O$, 400 MHz): 7.52–7.22 (m, 9H, Har); 5.29 (dd, 1H, H-7); 3.77 (tl, 1H, Ha-9); 3.08 (m, 1H, Hb-9); 2.63 (m, 1H, Ha-8); 1.87 (m, 1H, Hb-8). *J*(7,8a) = 5.8, *J*(7,8b) = 12.2, *J*(8a,8b) = 12.8, *J*(8a,9a) = 2.2, *J*(8a,9b) = 7.2, *J*(8b,9a) = 11.6, *J*(8b,9b) = 2.4, *J*(9a,9b) = 15.6 Hz.

**5.5.7.2. *cis*-Isomer 2d′, deuteriated at C(5).** RMN $^1H$ ($D_2O$, 400 MHz): 7.52–7.22 (m, 9H, Har); 4.46 (dd, 1H, H-7); 3.01 (m, 2H, $CH_2$(9)); 2.57 (m, 1H, Ha-8); 1.96 (m, 1H, Hb-8). *J*(7,8a) = 8.2, *J*(7,8b) = 10.5, *J*(8a,8b) = 13,1 Hz.

RMN $^{13}C$ ($D_2O$, 100 MHz, deuteriated at C(5)): 204.8 (C(6) **2d′**); 202.5 (C(6) **2d**); 141.0, 138.8, 134.1, 133.9, 132.5, 131.7, 131.3, 130.22, 130.20, 130.16, 129.9, 129.8, 129.7, 129.6, 129.5, 129.2, 129.1, 128.5, 128.0, 127.3 (20 Car, **2d, 2d′**); 58.1 (C(7) **2d′**); 57.7 (C(7) **2d**); 32.3 (C(8) **2d′**); 32.2 (C(8) **2d**); 30.4 (C(9) **2d**); 28.8 (C(9) **2d′**).

#### 5.5.8. *trans*-5-Phenylethyl-7-amino-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2e)

Procedure D from **4e** (85 mg, 0.21 mmol) in dioxane (1 mL) to give the hydrochloride **2e** (53 mg, 82%).

Compound **2e**: colorless crystals, mp 190–192 °C (*i*PrOH/$Et_2O$). IR (KBr): 3440, 2935, 2862, 1708, 1497, 1491, 1459, 1452, 761, 697 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 400 MHz): 7.30–7.16 (m, 9H, Har); 4.41 (dd, 1H, H-7); 3.82 (dd, 1H, H-5); 3.35 (ddd, 1H, Ha-9); 3.06 (ddd, 1H, Hb-9); 2.65 (t, 2H, $CH_2$(2′)); 2.54 (m, 1H, Ha-1′); 2.46 (m, 1H, Ha-8); 2.32 (m, Hb-1′); 1.80 (m, Hb-8). *J*(1′a,1′b) = 13.2, *J*(1′a,2′) = *J*(1′b,2′) = 7.8, *J*(1′a,5) = 6.7, *J*(1′b,5) = 8.7, *J*(7,8a) = 6.1, *J*(7,8b) = 12.4, *J*(8a,8b) = 12.8, J(8a,9a) = 4.2, *J*(8a,9b) = 7.8, *J*(8b,9a) = 9.4, *J*(8b,9b) = 4.2, *J*(9a,9b) = 15.4 Hz. $^{13}C$ NMR ($CD_3OD$, 100 MHz): 205.5 (C(6)); 142.1 (Cs(Ph)); 140.4, 136.2 (C(4a), C(9a)); 131.9, 130.8, 129.6, 129.5, 129.3, 128.8, 127.3 (9 Car); 60.0 (C(5)); 58.7 (C(7)); 34.3 (C(2′)); 32.5 (C(1′)); 32.3 (C(8)); 31.1 (C(9)). HR-MS (ESI-microTof) calcd for $C_{19}H_{22}NO$ $[M+H]^+$: 280.1696; found: 280.1664.

#### 5.5.9. *cis*-5-Phenylethyl-7-amino-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2e′)

Procedure D with **4e′** (140 mg, 0.34 mmol) in dioxane (5 mL) to give the hydrochloride **2e′** (87 mg, 81%).

Compound **2e′**: colorless crystals, mp 215–218 °C (*i*PrOH/$Et_2O$). IR (KBr): 3428, 3023, 2939, 2910, 1723, 1522, 1491, 1454, 749, 698 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 400 MHz): 7.30–7.20 (m, 9H, Har); 4.35 (dd, 1H, H-7); 4.20 (m, 1H, H-5); 3.26 (m, 1H, Ha-9); 2.98 (ddd, 1H, Hb-9); 2.70–2.60 (m, 3H, $CH_2$(2′), Ha-1′); 2.58 (m, 1H, Ha-8); 2.16 (m, 1H, Hb-1′), 1.66 (m, 1H, Hb-8). *J*(1′a,5) = *J*(1′b,5) = 6.7, *J*(7,8a) = 7.2, *J*(7,8b) = 11.7, *J*(8a,8b) = 12.7, *J*(8a,9a) = 2.4, *J*(8a,9b) = 7.9, *J*(8b,9a) = 10.7, *J*(8b,9b) = 2.6, *J*(9a,9b) = 14.8 Hz. $^{13}C$ NMR ($CD_3OD$, 100 MHz): 205.3 (C(6)); 142.9, 141.5 (C(9a), Cs(Ph)); 135.8 (C(4a)); 130.6 (CHar); 129.6, 129.4 (CHo,m(Ph)); 129.1, 129.0, 127.5, 127.2 (4 CHar); 61.6 (C(7)); 53.2 (C(5)); 34.7 (C(8)); 34.4 (C(2′)); 31.1 (C(1′)); 30.9 (C(9)). HR-MS (ESI-microTof) calcd for $C_{19}H_{22}NO$ $[M+H]^+$: 280.1696; found: 280.1693.

#### 5.5.10. *cis*-7-Amino-5-benzyl-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2f′)

**5h** (30 mg, 0083 mmol) was hydrogenolysed over Pd-C 5% (2 mg) in EtOH (5 mL) for 2 h. The catalyst was centrifugated off, the solvent evaporated. Crude **5f′** (30 mg, 0083 mmol) was deprotected according to the procedure C to give **2f′** (18 mg, 72%).

Compound **2f′**: colorless crystals, mp 220–222 °C (dec.). IR (KBr): 3425, 2923, 1722, 1496, 1490, 1453, 755, 749, 693 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 400 MHz): 7.25–7.16 (m, 9H, Har); 4.56 (dd, 1H, H-5); 4.24 (dd, 1H, H-7); 3.62 (dd, 1H, Ha-1′); 3.26 (ddd, 1H, Ha-9); 3.22 (dd, 1H, Hb-1′); 2.86 (ddd, 1H, Hb-9); 2.55 (dddd, 1H, Ha-8); 1.69 (dddd, 1H, Hb-8). *J*(1′a,1′b) = 13.7, *J*(1′a,5) = 8.5, *J*(1′b,5) = 5.6, *J*(7,8a) = 7.4, *J*(7,8b) = 11.4, *J*(8a,8b) = 12.6, *J*(8a,9a) = 2.8, *J*(8a,9b) = 8.6, *J*(8b,9a) = 9.8, *J*(8b, 9b) = 3.0, *J*(9a,9b) = 14.8 Hz. $^{13}C$ NMR ($CD_3OD$, 100 MHz): 204.9 (C(6)); 141.2, 140.2 (C(9a), Cs(Ph)); 135.7 (C(4a)); 130.5 (CHar), 130.3, 129.4 (CHo,m(Ph)), 129.2, 128.8, 128.4, 127.5 (4 CHar); 61.0 (C(7)); 55.8 (C(5)); 35.1 (C(1′)); 34.8 (C(8)); 30.7 (C(9)). HR-MS (ESI-Q-Tof) calcd for $C_{18}H_{19}NO$ $[M]^+$: 265.1467; found: 265.1481.

#### 5.5.11. *cis*-5-Phenylpropyl-7-amino-5,7,8,9-tetrahydrobenzocyclohepten-6-one, hydrochloride (2g′)

Procedure D with **4g′** (139 mg, 0.325 mmol) in dioxane (20 mL) to give the hydrochloride **2g′** (80 mg, 75%).

Compound **2g′**: colorless crystals, mp 156–158 °C (*i*PrOH/$Et_2O$). IR (KBr): 3429, 3019, 2945, 2924, 2895, 2870, 1718, 1505, 1488, 1453, 743 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 400 MHz): 7.27–7.15 (m, 9H, Har); 4.32 (dd, 1H, H-7); 4.19 (dd, 1H, H-5); 3.28 (m, 1H, Ha-9); 2.95 (ddd, 1H, Hb-9); 2.70 (m, 2H, $CH_2$(3′)); 2.55 (m, 1H, Ha-8); 2.35 (m, 1H, Ha-1′); 1.89 (m, 1H, Hb-1′); 1.67 (tt, 2H, $CH_2$(2′)); 1.63 (m, 1H, Hb-8). *J*(1a′,1b′) = 13.2, *J*(1′,2′) = 8.0, *J*(1a′,5) = 8.2, *J*(1b′,5) = 6.0, *J*(2′,3′) = 7.4, *J*(7,8a) = 7.2, *J*(7,8b) = 11.8, *J*(8a,8b) = 12.8, *J*(8a,9a) = 2.4, *J*(8a,9b) = 7.8, *J*(8b,9a) = 11.0, *J*(8b,9b) = 2.6, *J*(9a,9b) = 14.6 Hz. $^{13}C$ NMR ($CD_3OD$, 100 MHz): 205.4 (C(6)); 143.4, 141.5 ((C(9a),Cs(Ph)); 136.0 (C(4a)); 130.5 (CHar); 129.5, 129.4 (CHo,m(Ph)), 128.9, 128.8, 127.5, 126.9 (4 CHar); 61.5 (C(7)); 53.7 (C(5)); 36.9 (C(3′)); 34.7 (C(8)); 30.9 (C(9)); 30.3 (C(2′)); 28.7 (C(1')). HR-MS (ESI-microTof) calcd for $C_{20}H_{24}NO$ $[M+H]^+$: 294.1852; found: 294.1828.

#### 5.5.12. 7-Amino 5-benzylidene-5,7,8,9-tetrahydrobenzocyclohepten-6-one (2h)

Procedure C with **5h** (20 mg, 55 μmol) in HCl 2.2 *N*/$Et_2O$ (0.5 mL) and dioxane (0.5 mL) to give the hydrochloride **2h** (13 mg, 79%).

Compound **2h**: colorless resin, IR (KBr): 3407, 3019, 2984, 2943, 1702, 1591, 1510, 1482, 1188, 1096, 762, 757, 695 $cm^{-1}$. $^1H$ NMR ($CD_3OD$, 400 MHz): 8.05 (s, 1H)); 7.46–7.39 (m, 2H, Har); 7.32–7.13 (m, 7H, Har); 4.00 (dd, 1H, H-C7); 3.09 (ddd, 1H, Ha-9); 2.96 (ddd, 1H, Hb-9); 2.45 (dddd, 1H, Ha-8); 2.08 (dddd, 1H, Hb-8). *J*(7,8a) = 8.2, *J*(7,8b) = 11.0, *J*(8a,8b) = 12.4, *J*(8a,9a) = 13.0, *J*(8a,9b) = 7.4, *J*(8b,9a) = 7.4, *J*(8b, 9b) = 1.4, *J*(9a,9b) = 13.7. $^{13}C$ NMR (DMSO-$d_6$, 100 MHz): 194.2 (C(6)); 138.6, 138.2, 135.1, 134.1, 133.8, 131.0, 130.1, 130.0, 129.6, 129.2, 128.5, 128.5, 127.3 (12 Car); 54.9 (C(7)); 30.2 (C(8)); 28.3 C(9)). HR-MS (ESI-Q-Tof) calcd for $C_{18}H_{17}NO$ $[M]^+$: 263.1310; found: 263.1312.

### 5.6. Enzyme assays

#### 5.6.1. Enzyme source

Porcine kidney APN was purchased from Sigma Chemical Co. Porcine kidney LAPc was purified according to a published procedure.[34] Human recombinant LTA4H was provided by our collaborator J. Z. Haeggström.[11]

#### 5.6.2. Assay conditions[11]

(a) All enzymes: Kinetic data were collected with an HP/Agilent UV–Visible, diode array, spectrophotometer 8453 using the software 'HP chemstation' provided with the machine. Typically, spectrophotometric assays were performed with L-leucine-*p*-nitroanilide as the substrate for APN ($K_m$ = 0.2 mM), LAPc ($K_m$ = 2 mM) and Alanine-*p*-nitroanilide for LTA4H ($K_m$ = 2 mM). All kinetic studies were performed at 30 °C and the reactions were started by addition of the enzyme in 1 ml assay medium. (b) APN: 1 mUnits per assay, in 0.02 M Tris–HCl pH 7.5. (c) LAPc: 20 Units per assay in 0.1 M Tris–HCl, 0.1 mM $ZnCl_2$, 5 mM $MnCl_2$, 1 M KCl, pH 8.0 and (d) LTA4H: 5 μg per assay in10 mM Tris–HCl, 0.1 mM KCl, pH 7.5. The release of *p*-nitroanilide ($\varepsilon$ = 10,800 $M^{-1}$ $cm^{-1}$) at 405 nm was measured continuously to determine initial velocities. Assays were performed in semi microcuvettes (1 cm path).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