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ABBREVIATIONS

SAN = sino-atrial node

I_f = f-current

DD = diastolic depolarization

cAMP = cyclic adenosine monophosphate

HCN = Hyperpolarization-activated and Cyclic Nucleotide-gated

CNBD = cyclic nucleotide-binding domain

AC = adenylyl cyclase

CNS = central nervous system

DRG = dorsal root ganglion

MDR = multidrug resistance

SHR = spontaneously-hypertensive rats

HEK293 = Human Embryonic Kidney 293 cells

ER = eudismic ratio

e.e. = enantiomeric excess (%)

TOS = target-oriented synthesis

DOS = diversity-oriented synthesis

b/c/p = build/couple/pair

CMLD = chemical methodology and library development

RCM = ring-closing metathesis

OTIPS = triisopropylsiloxane

HG-II = Hoveyda-Grubbs 2nd generation catalyst

"Da chimico un giorno avevo il potere di sposar gli elementi e di farli reagire, ma mai gli uomini mi riusci' di capire affinche' si combinassero attraverso l'amore"

F. De Andre'

Design, synthesis and preliminary biological evaluation of new HCN blockers

1. INTRODUCTION

The autonomous beating of the heart is initiated by specialized pacemaker cells in the sino-atrial node (SAN), which generate spontaneous, rhythmic electrical stimuli in the absence of any external input. Different region of the brain are also able to generate rhythmic oscillations that are implicated in the modulation of vital physiological functions[1]. In fact, spontaneous action potentials in cells isolated from these natural pacemaker regions demonstrate a slow membrane depolarization phase, which is a typical characteristic in the time course of action potentials.

The so-called "pacemaker" current, with the description of the main part of conductance underlying the pacemaker activity, was first reported in the late 1970s[2]. When this current was discovered, its properties were deemed unique, in particular its characteristically slow activation in response to hyperpolarization. For this reason the current has been termed I_h (h for hyperpolarization activated), I_f (f for funny) or I_q (q for queer).

1.1 I_f and I_h

The "funny" current has unusual characteristics[3]. A first unusual feature is its voltage dependence: I_f is indeed activated by hyperpolarization with a threshold of approximately -40/-50 mV in the SAN. The fully activated current/voltage relation reverses near -10/-20 mV in physiological solutions as a consequence of the channel mixed permeability to Na^+ and K^+, which is a second unusual feature of I_f[4]. These two properties are critical with respect to the role of I_f in the generation of diastolic depolarization (DD) and hence of spontaneous activity. Thus, "f channel" is opened by hyperpolarization in the pacemaker range voltages and carries the inward current, which generates the DD, eventually leading to the threshold for Ca^{2+} channel activation and action potential firing.

Moreover, I_f is directly modulated by cAMP and regulated also by neurotransmitter receptors coupled to cyclic nucleotide second messengers. These important properties will be deeply discussed later.

A similar current to I_f was found in other tissues, as for example in neurons, where is termed I_h.

Despite these currents were known since 1970s, only in 1997 the molecular basis of $I_{f/h}$ were revealed. In fact, a "mouse brain cyclic nucleotide-gated channel" (mBCNG) was cloned[5], and it was proven that this particular channel was compatible with the pacemaker functions.

This channel belongs to a family of channels activated by hyperpolarization (HAC) and comprises four different subtypes. In 1998, further studies confirmed that pacemaking channels of mammals SAN belong to HAC channel[6] and finally, in 1999, it was proposed the nomenclature as HCN channels (Hyperpolarization-activated and Cyclic Nucleotide-gated)[7].

1.2 HCN channels

1.2.1 Structure

HCN channels form, together with cyclic nucleotide-gated (CNG) channels and Eag-like K^+ channel, the subgroup of cyclic nucleotide-regulated cation channels within the large superfamily of the poor-loop cation channels[8-10]. Their core unit consists of four subunits that are arranged around the centrally located pore (**figure 1**). In mammals, four homologous HCN channel subunits (HCN1-4) exist, which form four different homotetramers with distinct biophysical properties. There is evidence that the number of potential HCN channel types is increased *in vivo* by the formation of homotetramers[1,11]. Each HCN channel subunit consists of two major structural modules, the transmembrane core and the cytosolic C-terminal domain. The first one harbours the gating machinery and the ion conducting pore, the latter one confers modulation by cyclic nucleotides.

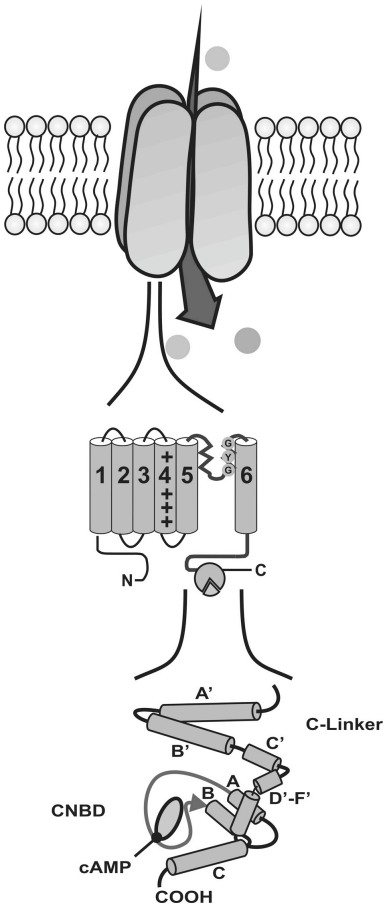


Figure 1. Structure of HCN channels.Top: HCN channels are tetramers. One monomer is composed of six transmembrane segments including the voltage sensor (S4), the selectivity filter and the pore region between S5 and S6.The C-terminal channel domain contains the cyclic nucleotide-binding domain (CNBD; middle).Bottom: the C-terminal channel domain is composed of two domains. The C-linker domain consists of six α-helical segments, designated A' to F'. The CNBD follows the C-linker domain and consists of ahelices A–C with a β-roll between the A- and B-helices (arrow).

Both modules allosterically cooperate with each other during channel activation. As for the K^+ voltage-dependent channel, the transmembrane channel core of HCN channels consists of six α-helical segments (S1–S6) and an ion conducting pore loop between S5 and S6. A highly conserved asparagine residue in the extracellular loop between S5 and the pore loop is glycosylated. It was shown that this post-translational channel

modification is crucial for normal cell surface expression[12]. The voltage sensor of HCN channels is formed by a charged S4-helix carrying nine arginine or lysine residues regularly spaced at every third position[11,13]. Positively charged S4 segments are found in all voltage-dependent members of the pore-loop cation channel superfamily[14]. Moreover, inward movement of S4 charges through the plane of the cell membrane leads to the opening of HCN channels and triggers the closure of depolarization-activated channels. The molecular determinants underlying the different polarity of the gating mechanism of HCN and depolarization-gated channels remain to be determined. However, there is initial evidence that the loop connecting the S4 with the S5 segment plays a crucial role in conferring the differential response to voltage[15-21].

A further structural analogy between the K^+ selective channel and HCN channel is the presence of a glycine-tyrosine-glycine (GYG) motif between S5 and S6 loops. This motif is particularly important in the K^+ selective channel because it forms the selectivity filter for K^+ ions. HCN channels conduct Na^+ and K^+ with permeability ratios of about 1:4, and also seem to display a small permeability for Ca^{2+}.[22,23]
Despite this preference for K^+ conductance, HCN channels carry an inward Na^+ current under physiological conditions; a possible explanation could be that in the tetrameric HCN channel complex, the GYG motif is coordinated in a less rigid fashion than in K^+ channels, allowing the entrance of cations of different size.

1.2.2 Role of cyclic nucleotides

The sensitivity of HCN channels to cAMP is mediated by the proximal portion of the cytosolic C-terminus[23]. This part of the channel contains a cyclic nucleotide-binding domain composed of about 120 amino acids (CNBD) and an 80 amino acids long C-linker region that connects the CNBD with the S6 segment of the channel core. cAMP binding to CNBD speeds up channel opening and shifts the voltage-dependence of activation to more positive voltages (**figure 2**)[23]. Thus, at a given voltage the current flowing through HCN channels is larger in the presence than in the absence of cAMP.

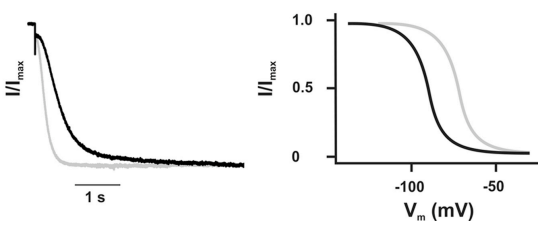


Figure 2. Left: sinoatrial I_h activates faster in the presence of cAMP (grey) than in the absence (black) of cAMP at maximal activation voltage (–140 mV). Right: activation curve of I_h in the absence (black) and the presence (grey) of cAMP. The activation curve of I_h is shifted to the right in the presence of cAMP.

The determination of the crystal structure of the C-linker-CNBD of HCN2[23] has revealed that the CNBD shows a highly conserved fold, consisting of an initial α-helix (A-helix), followed by an eight-stranded antiparallel β-roll, a short B-helix and a long C-helix (**figure 1**). The C-linker consists of six a-helices (A'–F'). Most of the subunit-subunit interactions in the tetramer are mediated by amino acid residues in the C-linker.

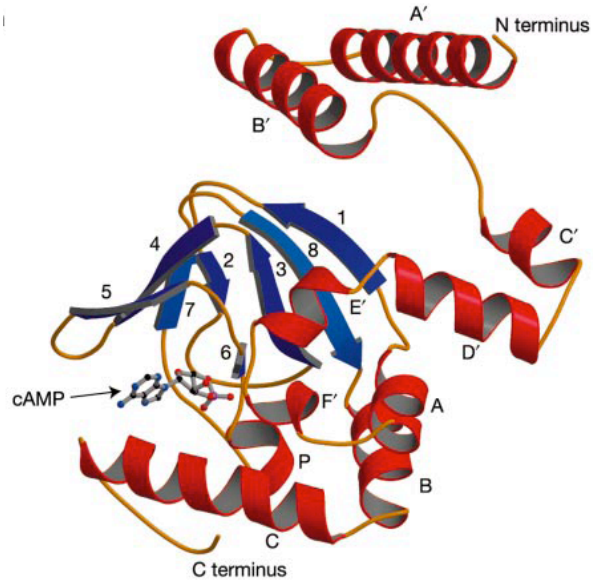


Figure 3. Representation of a single protomer of HCN2 with cAMP.[23]

The binding pocket for cAMP (**figure 3**) is formed by a number of residues at the interface between the β-roll and the C-helix: three of these residues are located in the β-roll and four in the C-helix and, among them, only one key residue contributes to the higher selectivity of the channel toward cAMP respect of cGMP. In fact, both nucleotides enhance channel opening but the interaction is more selective for cAMP[24].

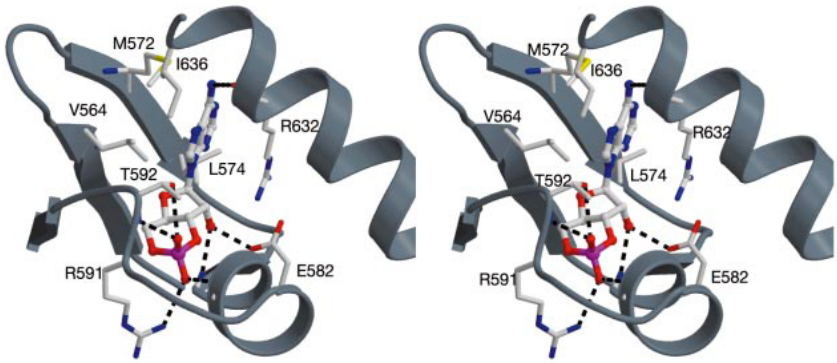


Figure 4. cAMP and cGMP bind to the same site with different stereochemistry. Stereoview of the cAMP-binding site, showing hydrogen bonds and salt bridges (brokenlines)[23].

However, it is not clear how these residues generate cAMP selectivity (**figure 4**). It was suggested that cAMP selectivity is, at least partially, not due to its preferential contacts with the protein, but rather reflects the greater hydration energy of cGMP relative to cAMP. This could result in a greater energetic cost for cGMP binding to the channel[15,23-25].

In 2003[23], a study from HCN2 crystal structure shed light on the dynamics of the allosteric process that couples cAMP binding with channel opening. According to this recent model, in the absence of cAMP the C-linker is thought to be in a "compact" conformation that produces an inhibitory effect on channel opening. In turn, cAMP binding induces a conformational change in the CNBD involving the C-helix. The conformational change in the C-helix is then coupled to the C-linker that occupies a more "loose" conformation leading to an alteration of the intersubunit interface between helices of neighboring subunits. The resulting change in the quaternary conformation removes the inhibition of the C-terminus and destabilizes the closed state, thus promoting the opening of the channel.

1.2.3 Biophysical properties of HCN channel types.

Although all the four different HCN channel types display the principal biophysical properties of native $I_{f/h}$, they differ from each other with respect to their voltage dependence, activation time constant and activation by cAMP.

- *voltage-dependent activation:* there are some differences between the HCN channel subtypes. In **table 1** are reported values for the midpoint of activation ($V_{0.5}$)[16,26,27]. These values can be altered significantly by several conditions, as reported by many studies[20].

HCN channel subtypes	midpoint of activation ($V_{0.5}$)
HCN1	-70 mV
HCN2	-95 mV
HCN3	-77 to -95 mV
HCN4	-100 mV

Table 1.

- *activation kinetics:* they also differ between the HCN channel subtypes (**figure 5**). HCN1 is the fastest channel (25-300 ms, depending on the voltage) whereas HCN4 is the slowest channel with values between a few hundred milli-seconds (at strongly hyperpolarized voltages) up to several seconds (normal resting potential). HCN2 and HCN3 activate with kinetics between the previous two[20,26-32].

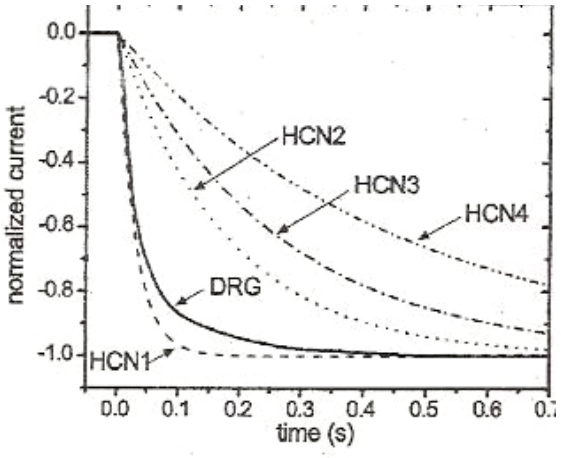


Figure 5. Different activation kinetics curves of the HCN channel subtypes respect of DRG (dorsal root ganglion)[33].

- *modulation by cAMP:* all the four HCN channel subtypes are modulated in a different way by cAMP even if they carry a highly conserved C-linker-CNBD portion. In fact, cAMP shifts the activation curves of HCN2 and HCN4 by about +10 to 25 mV and so it speeds up the opening kinetics whereas it weakly affect HCN1 and HCN3[21,23,26,30,33-37]. Interestingly, when the CNBD of HCN4 is replaced by the corresponding domain of HCN3, cAMP sensitivity is fully maintained, suggesting that the CNBD of HCN3 is mainly able to bind cAMP and to mediate cAMP-dependent gating[29]. Thus, within the HCN3 channel, the CNBD may be functionally silenced by a structural change in channel domains that relate cAMP binding with channel gating[27,38,39].

1.2.4 Dual channel activation.

The dual activation of HCN channels by voltage and by cyclic nucleotides has been described by a cyclic allosteric model[40-42]. Each of the four subunits of the tetrameric channel are independently gated by voltage. Every time a voltage sensor switches to the activated state the probability for channel opening increases. The opening/closing reactions occur allosterically and involve concerted transitions of all four subunits. This transition occurs if the channel is unliganded, partially liganded or fully liganded and is energetically stabilized by a constant amount for each cAMP bound. The model further assumes that cAMP has a higher binding affinity to open rather than to closed channels (**figure 6**).

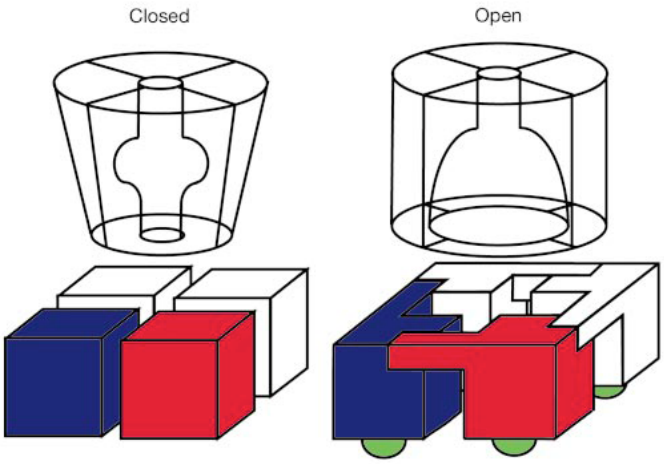


Figure 6. Cartoon of an unliganded closed channel (left) and a liganded open channel (right)[23].

1.2.5 HCN channels regulation.

HCN channels are tightly regulated by interacting proteins and by low molecular factors located in the cytosol and in the extracellular space.

- *G-Protein coupled receptors (GPCRs)*: binding with cAMP is certainly the most important factor for the HCN channel modulation and it also explains the many effects on $I_{f/h}$ mediated by GPCRs. Modulation is dependent on the type of G-protein coupled with a particular receptor (G_i, G_0, G_s), followed by activation/inhibition of adenylyl cyclase (AC) responsible of cAMP level. An example of this is given by adrenergic and cholinergic receptors: they are coupled to G_s and G_i proteins, respectively, which activate and deactivate AC, causing an increment or a decrement of $I_{f/h}$. This is the reason that explains the opposite effects on the heart of acetylcholine (inhibition) and catecholamine as adrenaline (activation) as represented in **figure 7**.

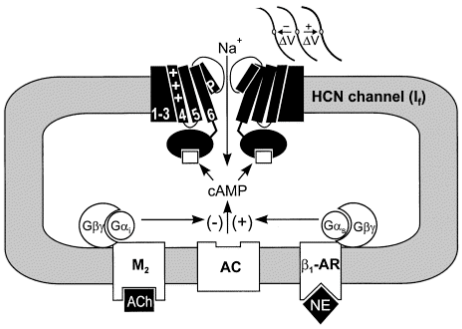


Figure 7. Regulation of HCN channel by up or down-regulation of cAMP[25].

Also morphine, an analgesic and a full agonist of opioid receptor coupled to G_i/G_0, decreases sensibly I_h by decreasing of cAMP production. On the other hand, addiction

to morphine is given by an increasing of I_h due to a rebound effect of cAMP production[43,44]. Other GPCRs can modulate $I_{f/h}$ as shown in **figure 8**.

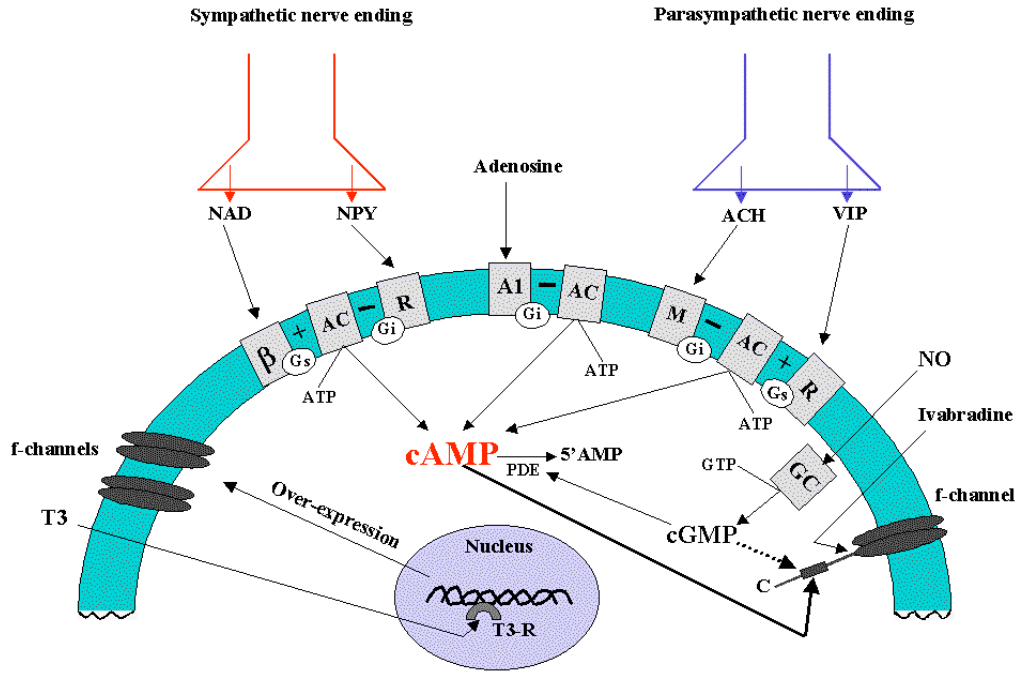


Figure 8. Schematic representation of the main signaling pathway involved in the regulation of I_f in a SAN cell. β, adrenergic receptor; A1, adenosine receptor; AC, adenylyl cyclase; ACH, acetylcholine; AMP, adenosine monophosphate; ATP, adenosine triphosphate; cAMP, cyclic adenosine monophosphate; cGMP, cyclic guanosine monophosphate; GC, guanylyl cyclase; GTP, guanosine triphosphate; M, muscarinic receptor; NAD, nicotinamide adenine dinucleotide; NO, nitric oxide; NPY, neuropeptide Y; PDE, phosphodiesterase; R, NPY or VIP receptor; T3, triiodothyronine; T3-R, triiodothyronine receptor; VIP, vasoactive intestinal peptide. Ivabradine is taken as an example for a heart rate slowing agent.

- *phosphatidylinositol-4,5-bisphosphate (PIP_2):* voltage-dependent gating of HCN channel is allosterically regulated by membrane lipids, which shift of the voltage toward positive potentials without interaction with cyclic nucleotides. Perhaps, this HCN channel activation is due to an electrostatic interaction between the negatively charged head groups of lipids and the channel protein.

- *pH:* the activity of HCN channels depends on both intracellular[45,46] and extracellular[47] concentration of protons. An increase of the intracellular protons shifts the voltage-dependence of channel activation to more negative (hyperpolarized) potentials and slow down the speed of channel opening. On the other hand, increasing intracellular pH gives the opposite result with increasing $I_{f/h}$. Intracellular pH sensitivity has been conferred to a protonable histidine residue (His321), localized at the boundary between the voltage-sensing S4-helix and the cytoplasmic S4-S5 linker[45].

- *chloride ion:* the steady-state conductance of I_h is regulated by extracellular chloride and this effect is more pronounced for HCN2 and HCN4 while being rather weak for HCN1[48]. This regulation could be relevant for heart physiology where hypochloremia could be involved in the reduction of the amplitude of sinoatrial I_h with consequent arrhythmias[11]. As for pH, also for chloride ion a single amino acid residue was identified as the molecular determinant of extracellular Cl^- sensitivity: HCN2 and HCN4 channels (high Cl^- sensitivity) carry an arginine residue (R405 in HCN2, R483 in HCN4) while HCN1 carries an alanine (A352)[48]. Chloride ion can regulate HCN channel function also from the intracellular site, although the physiological relevance of this regulation remains to be determined[48,49].

- *tyrosine phosphorylation:* tyrosine kinase c-Src constitutively binds to the C-linker-CNBD and, as a result of this phosphorylation, the activation kinetic of the channel is enhanced[50]. The residue that confers this particular modulation is located in the B'-helix of the C-linker and is conserved in all the HCN channel isoforms[11].

- *p38-MAP (mitogen-activated protein)-kinase:* it significantly shifts the voltage-dependent activation towards more positive potentials, and this regulation could be important to modulate neuronal excitability[51].

- *interacting protein:* there is a growing evidence that ion channels usually are macromolecular protein complexes that, in addition to the principal pore-forming subunits, contain auxiliary proteins that are required for the fine-tuning of electrophysiological properties. Also HCN channels are subjected to this rule (**figure 9**), as in the case of the MinK-related protein MiRP1[52,53], which reacts with different HCN channel types. In fact, MiRP1 belongs to a family of a single transmembrane-spanning proteins and is considered as an auxiliary subunit of HCN channels. In 2008[54], another transmembrane protein KCR1 was reported to interact with HCN2. Owing to its capacity to reduce the current density of native I_h, KCR1 could be an inhibitory auxiliary subunit of HCN channels and could be a regulator of cardiac automaticity. Finally, other proteins (TRIP8b, tamalin, filaminA), mainly found in neurons, are able to interact with the C-terminus of HCN channels.

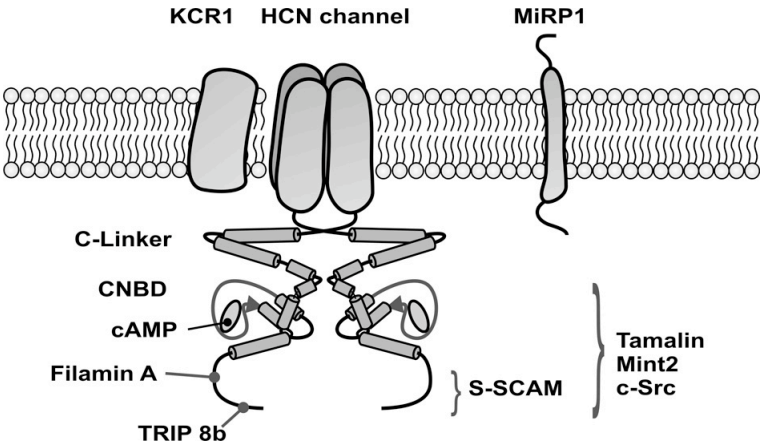


Figure 9. Regulation of HCN channels by interacting proteins. Only two C-termini of the tetrameric channel complex are shown. Proteins interacting with HCN channels are indicated. KCR1, MiRP1, Filamin A, TRIP8b, S-SCAM, Tamalin, Mint2 and c-Src interact with different channel portions as indicated.

1.3 Tissue expression and distribution of HCN channels

Quantitative rt-PCR studies showed that the different isoforms of HCN channels are mainly expressed in the nervous system and the heart (**figure 10**), but they are widely expressed also in other excitable or non-excitable tissues[55-57]. HCN channels are also located in perception and sense transmission tissues, such as retina, auditory neurons, lingual taste buds, and olfactory pathways[47,58-60].

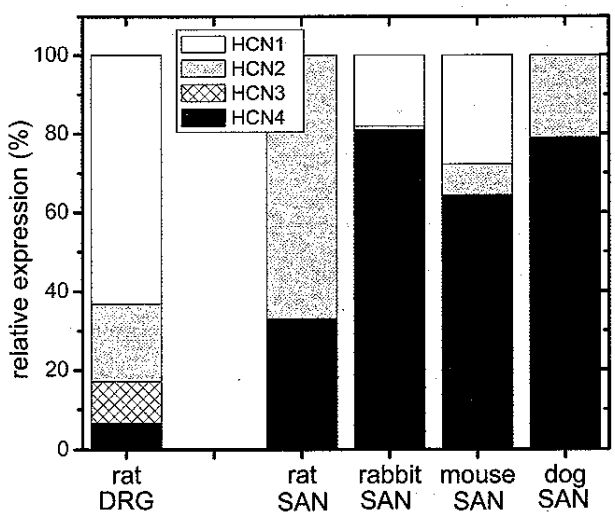


Figure 10. Relative expression of HCN subtypes in large DRG neurons versus SAN cells, as determined by rtPCR[33].

HCN1: it is expressed in many regions of the brain, in the spinal cord and in the peripheral nervous system where it is the most abundant isoform[55,61].

HCN2: it is distributed nearly ubiquitous throughout most brain regions and also in the heart[31,62].

HCN3: it is sparsely distributed at very low levels in the central nervous system, heart and in the olfactory bulbs[62].

HCN4: it is the major isoform expressed in SAN, accounting for about 80% of I_h[63,64].

It is important to underlie that expression levels of HCN channels are low in normal heart muscle compared to cells of the conduction system. However, during heart disease[65-68], upregulation of HCN channels may occur, as for example in cardiac hypertrophy and heart failure, where HCN2 and HCN4 are up-regulated in the atrial and ventricular myocardium[65-71]. Thus, I_h overexpression could well be an important trigger of arrhythmogenic activity in the hypertrophied heart[65-68,70,71].

1.4 Functional properties of HCN channels on different tissues

There are many different functional properties of $I_{f/h}$[75-77], depending on the different location of HCN channels. The functionality of different isoforms was made possible utilizing knockout phenotype technique.

1.4.1 Heart

Heart beating regulation is surely the most widely studied and important effect of $I_{f/h}$. In fact, this current generates a special kind of action potential (called "pacemaker") in SAN, which is characterized by the presence of a progressive DD in the -65 to -45 mV range. Consequently, after the repolarization phase, the DD drives the membrane potential back to the threshold of calcium channel activation, thereby maintaining firing; in this way, the repetitive rhythm of action potential is generated.

$I_{f/h}$ plays also an important role in the heart rate regulation by the autonomic nervous system. In fact, stimulation of sympathetic or parasympathetic systems can activate or inhibit respectively $I_{f/h}$ through the modulation of cAMP level.

With the exception of HCN3, all HCN isoforms are significantly expressed in the adult heart and, among them, the dominant isoform is HCN4, accounting for almost the total HCN message[78], so HCN4 has been considered to be crucial for the generation of heart beating. Concerning HCN2, it contributes for about 20% of $I_{f/h}$ expressed in SAN and may serve as a complementary channel to HCN4[74]. These data are confirmed by the

knockout phenotype technique: when a global- or heart-specific disruption of the HCN4 gene occurred, mice died *in utero* within 10 - 11.5 days, showing before day 10 an about 40% reduction of the beating frequency without any response to the β-adrenergic stimulation[72]. Surprisingly, the same experiments conducted on adult mice displayed a rather mild cardiac phenotype[73]. In conclusion, these experiments showed how this channel is absolutely crucial for autonomous heart rate regulation[72] and for maintaining a stable cardiac rhythm but it is not needed for principal pacemaking.

The analysis of human HCN4 channel reveals that patients suffering from these mutations display bradycardia. Bradycardic agents (e.g. Zatebradine, Ivabradine and Cilobradine) act blocking heart HCN channel and, as a consequence, a decrease of intensity of $I_{f/h}$ occurred, followed by reduction of firing frequency in SAN cells.

1.4.2 Brain

All four HCN channel isoforms are expressed throughout the brain[6,31,55,79,80], where they play a role in the setting of membrane potential, dendritic summation, action potential threshold and firing frequency[79]. The exact role (both excitatory and inhibitory) of HCN channels depends on where they are located and expressed.

The importance of this channel in CNS function was revealed by HCN knockout mice. Deletion of HCN1 impairs motor learning but enhances spatial learning and memory, whereas deletion of HCN2 results in absence epilepsy and tremor[74,81,82]. Moreover, expression and/or function in the CNS can vary depending on prevailing levels/patterns of normal and pathological activity; thus, HCN channel expression either increases or decreases following changes in network activities[83-86].

1.4.3 Spinal Cord

HCN1, HCN2 and HCN4 are found in the dorsal and ventral horn of the spinal cord. In 2003[87], it was reported that, when spinal intathecal is treated with ZD7288 (HCN channel blocker), no effect occurred on allodynia thresholds, suggesting that spinal I_h did not contribute substantially to the nerve injury pain state.

1.4.4 Sensory Nerves

I_h and HCN channel isoforms are highly expressed in primary afferent neurons (sensory), where they may function to control neuronal excitability and transmitter

release. Their presence was demonstrated in a variety of peripheral sensory nerve preparations, such as rat dorsal root ganglion (DRG)[87-95]. A consistent result from these studies is that I_h appears more prominent in large diameter Aβ neurons compared to small or medium diameter cells.

The subcellular localization of HCN channels depends on different factors, such as cell type, function and recent history. Using in-situ hybridization, the most abundant isoform found in DRG cells was HCN1 followed by HCN2, with the highest expression level in large diameter neurons[64]. HCN2 and HCN3 mRNAs are still undetectable.

As a complementary approach to the identification of the key isoforms responsible for I_h in sensory neurons, different attempts were made in order to correlate the properties of I_h in sensory nerves with those of heterologously expressed recombinant HCN channels. In large and medium sensory neurons, their activation rates are fast and comparable to those measured for HCN1 and/or HCN2, whereas activation appears to be slower in small cells.

Moreover, HCN isoforms also differ for the different sensitivity to cAMP, which increases HCN-based current by shifting the voltage-dependence of activation in a depolarizing direction, meaning that there is a greater open probability at physiological potential. Anyway, it is important to underlie how cAMP has only modest effects on I_h in sensory neurons[33].

All these data suggest that HCN1 channel gives a significant contribution to I_h in large diameter, mechanosensitive fibers. However, since most cells express more than one HCN isoform and because of the different biophysical properties of I_h among the four different HCN sub-types, also HCN2, HCN3 and HCN4 isoforms (either as homo- or heterotetrameric channels) may also contribute to I_h in many sensory neurons.

1.4.5 Retina

HCN channels are also expressed in the retina, where I_h plays a role in the shaping of retinal responses to light, including limitation of the retinal hyperpolarization that encodes brightness[96,97].

Apart from expected effects on heart rate, one of the most commonly described side effects of treatment with an HCN channel inhibitor in humans is visual disturbance.

These disturbances consist of photopsia (light flashes) or phosphenes (visual perceptions evoked by stimuli other than changes in light), particularly with abrupt changes in illumination[98-101].

1.5 Known HCN channels antagonists

Heart rate modulation forms part of standard angina-prevention strategies. Although β-adrenergic blocking drugs decrease heart rate and thus prevent angina, side effects that may be associated with therapy have stimulated the search for selective heart rate lowering agents, specifically targeting HCN channels.

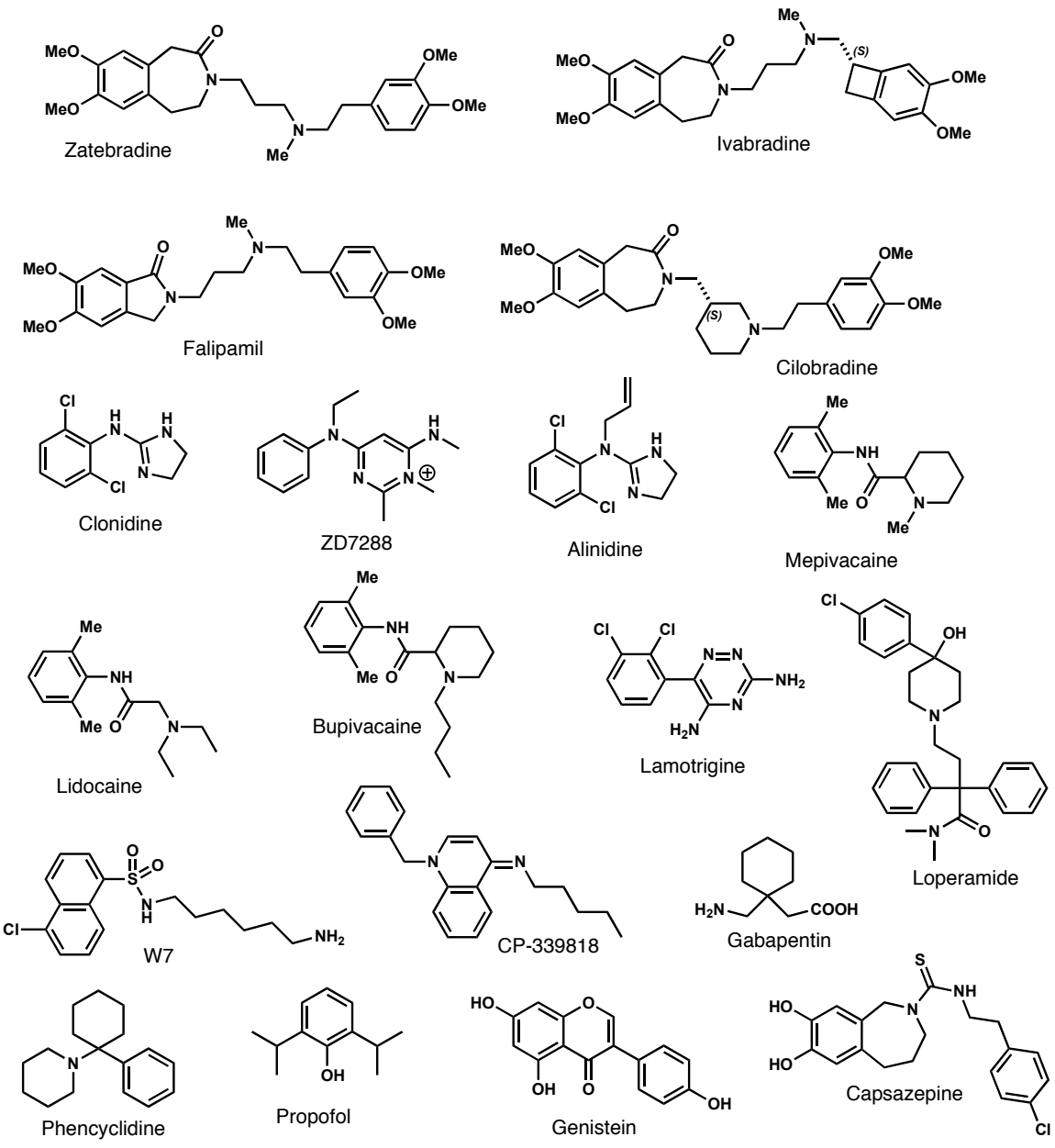


Figure 11. Structures of known HCN channel antagonists.

Nowadays, most of HCN channel blockers are specific bradycardic agents, which modulate I_f because of its relevant physiological activity in SAN cells. These drugs, however, have low selectivity among the different isoforms of HCN channels with an increase of the side effects. Achievement of selectivity is a stimulating challenge to medicinal chemists to develop new molecules that are able to discriminate for a specific isoform, HCN4 in the case of heart. A molecule that is able to selectively target isoform HCN1 would lead to block I_h in neurons and would represent a possible therapeutic option for the treatment of neuropatic pain and epilepsy[24].

The structures of the known HCN modulators are summarized in **figure 11**. They will be briefly discussed according to their mechanisms of action.

1.5.1 Pore Blockers

Monovalent cations, such as *cesium* (Cs^+), *rubidium* (Rb^+), *thallium* (Tl^+) and *ammonium* (NH_4^+), enter the permeation pathway, binding tightly enough to prevent sodium or potassium flowing[102]. Channel block and washout are nearly instantaneous, and block does not appear to be use-dependent. However a drawback of these cations is that they inhibit numerous other ion channels in a similar concentration range, which limits their use as therapeutic agents. Only Cs^+ (at 1 mM concentration) is commonly used as a diagnostic for HCN currents.

1.5.2 Inner Pore Antagonists

Most of these antagonists (*falipamil*, *zatebradine*, *ivabradine*, *cilobradine*) are analogues of the L-type calcium channel inhibitor verapamil. They all exhibit slow use-dependent block of I_f, requiring channel activation to reach the binding site. When the channel is activated the gate is opened so these molecule can enter the pore from the intacellular side[103]. Channel block occurs without shifting activation potential and can be relieved by strong hyperpolarization of the membrane[104].

Zatebradine is not a specific blocker of I_f since it has been shown to have inhibitory effects on cardiac potassium channel[105]. As a result, its application not only decreases the firing rate of autorythmic tissues but also prolongs action potentials recorded from these tissues[106-108], behaving like the III class antiarrythmic drugs[105]. Despite a significant reduction in heart rate, it did not improve exercise tolerance in angina patients[109,110]. Finally, due to the concomitant inhibition of hyperpolarization-activated

channels in neuronal tissues, Zatebradine presented undesired effects on vision, leading to its consequent withdrawal from clinical trials[109].

Falipamil, as zatebradine, was hampered by lack of selectivity. Although efficacious against angina[111], it showed anticholinergic effects[112] and a relatively short-life in human[113].

Cilobradine is structurally related to zatebradine but with less conformational freedom. Its structure bears a stereogenic center and the *(+)-S* enantiomer is a more potent HCN channel blocker than the *(-)-R* enantiomer. As Zatebradine, it reduces the maximal conductance in a use- and frequency-dependent manner[114,115]. More precisely, it induces a concentration- and voltage-dependent inhibition of I_f in Pukinje fibers and in isolated rabbit SAN cells[114,116,117], which results in a decrease in the spontaneous firing rate. In comparison to zatebradine, the inhibitory effect is faster and with higher intensity perhaps because of the higher drug-target association rates[118]. Moreover, it has not side effects on retina so it has a safer profile than zatebradine. The *(+)-S* enantiomer is patented for cardiac insufficiency whereas the other enantiomer is patented for the neuropathic pain treatment.

Ivabradine is also related to zatebradine structure. It is a new therapeutic agent (Procoralan®) recomended in the treatment of stable angina pectoris[119]. Ivabradine has been shown to induce heart rate slowing by acting specifically on I_f[120-122]. The cardiac effects are specific to the SAN with no effect on the myocardial contractility, relaxation, intra-ventricular conduction, or repolarization. The blockade of the channel is more efficient when the current is outward (i.e. during the deactivation of the pacemaker current). Ivabradine is an open channel blocker that produces a "current-dependent" channel blockade. The dependence of blockade upon current flow was limited to HCN4 and is not significant for HCN1[123]. This last property distinguishes ivabradine from the other heart-rate reducing agents that reduce I_f in a voltage-dependent manner independently of the electrochemical gradient.

ZD7288 appears to have a mechanism of blockade similar to verapamil analogues. It reduces the spontaneous beating rate without affecting the contractile force[124]. ZD7288, like alanidine (see 1.5.3 chapter) but unlike zatebradine, depresses I_f in a use- and frequency-independent way, suggesting that it interacts with its channel binding site independently from the channel gating configuration[125]. Moreover, this bradycardic

agent has also the capacity to selectively block I_h in central neurons[125-128]. Finally, it has been proposed that ZD7288, when applied externally, may behave as a 'lipophilic' quaternary cation, capable of passing into the cell interior to block pacemaker channel activity[126]; for this reason it could be used as a tool in place of Cs^+ for studying the properties and significance of the pacemaker current in controlling heart and/or neuronal function.

1.5.3 Activation Modulators

Several compounds have been identified as HCN channel antagonists, whose mechanism of action involves a shift in activation potential. It has been shown that these compounds alter the stability of the closed state relative to the open state. Generally they do not show use-dependence, but instead they demonstrate strongly voltage-dependent modulation of the current.

Alinidine is structurally related to clonidine, an adrenergic α_2-agonist. It induces bradycardia with no observable effects on action potential shape or inotropy[129]. Perhaps, due to its structure, alinidine has also an interesting analgesic effect acting on α-adrenergic receptors[129,130]. Alinidine directly inhibits HCN current by shifting the activation potential toward more negative potential, stabilizing the closed state[131]. Drawbacks of this molecule are the numerous side effects, including sedation, dry mouth, hypotension and vision[132] due to the blocking of HCN channel in retina, which would compromise its therapeutic use.

Recently[89,133], also clonidine has been shown to directly inhibit HCN channel; its affinity (1-10 nM) for adrenergic receptors is two orders of magnitude higher than the IC_{50} for HCN channels blockade.

Propofol, an intravenous general anesthetic acting *via* $GABA_A$ receptors, was shown to inhibit HCN channels in neurons at relevant concentration[134]. It slows activation and speeds deactivation of HCN gating with no apparent effect on the fully activated conductance[135,136]. It seems that it has no effect on HCN2 at concentrations at which it inhibits HCN1[137].

Loperamide is an opioid agonist that directly modulates HCN channels by slowing inactivation and shifting activation toward hypepolarized potential[138]. It is active in the micromolar range against HCN currents whereas it requires nanomolar concentration to

inhibit the opiate receptors, so it is unlikely that HCN inhibition contributes to the clinical properties of this drug.

1.5.4 Positive Allosteric Agents

Lamotrigine, an anticonvulsivant thought to operate primarily as a use-dependent antagonist of voltage-gated Na^+ and Ca^{2+} channels, was recently shown to enhance HCN currents in rat hyppocampal pyramidal cells; its mechanism is still unclear but it seems to involve a shift in activation toward more depolarized potentials.

1.5.5 Unclassified Mechanisms and other antagonists

Bupivacaine, *lidocaine* and *mepivacaine*, local anesthetics, inhibit HCN currents in DRG neurons[139]. Their potencies at HCN are similar to those at Na^+ channels, suggesting that HCN inhibition occurs at clinically relevant concentrations *in vivo*. As for the sodium channels, HCN channel blockade appears to be from the inside of the cell, so it could be interesting to see if the binding site is intracellulary located in the pore as with Na^+ channels.

Genistein, a tyrosine kinase and a DNA topoisomerase inhibitor[140], blocks f-current at concentrations similar to its potency on the enzymes[141]. This compound acts from the intracellular face of the channel and is not use-dependent. It behaves like a pore blocker, since antagonism occurs *via* reduction in open-channel conductance with no alteration of gating kinetics or voltage dependence.

W7 is a calcium-calmodulin inhibitor but it also directly blocks HCN currents. Its mechanism is unclear with both a reduction in fully-open conductance and a shift in activation toward more hyperpolarized potentials.

CP-339,818 is a voltage-gated K^+ channel inhibitor, but it blocks HCN currents with an unclear mechanism. It seems to have voltage-dependent blockade and to have a slight preference for HCN1 isoform relative to the others.

In summary, the activation of HCN1 channels, the most abundant isoform in brain and peripheral nervous system, produces I_h, which is the prominent current in many peripheral sensory nerves. Consequently, HCN1 channels may represent a valid target for the treatment of spontaneous pain and allodynia associated with nerve injury. In contrast, HCN4 channels are the predominant isoform in heart, especially in SAN cells, so they could be an ideal target in treating diseases associated with heart failures as angina and arrhythmia.

Antagonists that are selective for a specific HCN isoform are of paramount importance to eliminate, or at least minimize, adverse side effects.

2. BACKGROUND AND AIM OF THE WORK

In a series of papers produced over the past fifteen years, Prof. Gualtieri and coworkers have shown that modifications of the structure of the well-known calcium antagonist and multidrug resistance (MDR) modulator verapamil can change its cardiovascular and MDR-modulating pharmacological profile[142-148]. In particular, by suitably modifying the substituent on the nitrogen atom and the conformational freedom of the molecule, some dissociation between negative chronotropic and inotropic or vasorelaxant activity were obtained. Moreover, a clear-cut dissociation of calcium antagonism and MDR inhibitory activities was also achieved. Taking advantage of the experience acquired in that research, a similar modulation was performed on the molecule of zatebradine, which is structurally related to verapamil. As a consequence, several derivatives of zatebradine were designed where the N-methyl-homoveratryl group has been replaced by different amines or the conformational freedom of the molecule has been reduced by introducing double and triple bonds. Moreover, since it is known that the dehydro- and the desmethyl- analogues of zatebradine show potency similar to that of the parent compound[155,156], some of the previously-mentioned modifications were applied also to these series.

The aim of the work was to find substances able to reduce cardiac I_f through a direct blockade of the channel. However, before testing the substances on I_f, a screening test was necessary in order to avoid expensive and time-consuming electrophysiological experiments on inactive substances. Therefore, all the synthesized compounds were tested first for their negative chronotropic activity on guinea pig spontaneously-beating isolated atria; the absence of bradycardic activity could reasonably rule out a possible interaction with cardiac HCN channels. Then, the activity on I_f of the most interesting compounds was tested on ventricular cardiomyocytes of old spontaneously-hypertensive rats (SHR): these myocytes re-express I_f, whose biophysical and pharmacological properties closely resemble those reported for the sinoatrial node f-current[65,66]. This research, published in 2005, disclosed a structural analog of zatebradine, (**1**, **EC4**, chart 1) which was able to reduce heart rate in guinea-pig spontaneously-beating right atria and to block the I_f current with a potency comparable to the lead[144].

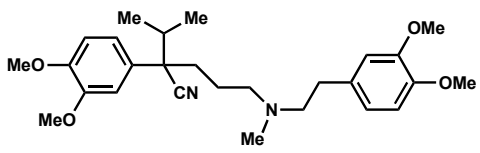
As a continuation of this research, the *cis*-isomer of **1**, **2** (**EC32**) and another reduced-flexibility analogue of zatebradine, **3** (**EC18**)[151], were prepared; they were found to possess good negative chronotropic activity in guinea-pig isolated atria[152], but their activity was not published. In fact, since the old spontaneously-hypertensive rats is an

expensive and time-consuming model, we decided to test the I_f-blocking activity on more accessible biological preparations. In 2006, HEK293 cells stably expressing HCN1, HCN2 and HCN4 isoforms were made available to us by the group of Prof. M. Biel from Munich; with this experimental model the compounds can be tested on different isoforms, and structure-activity relationships can be derived to optimize potency and selectivity.

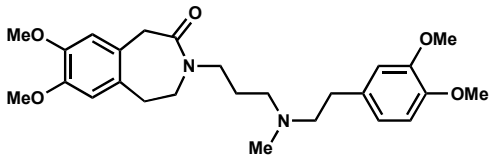
The aim of the present work is to find isoform-selective HCN blockers, to be used as pharmacological tools to study the role of these channels in physiological processes and/or in pathological conditions, or to understand the stoichometry of the channels in native tissues. Moreover, isoform-selective substances can be useful to design safe drugs for different pathologies involving HCN channels. To date, no selective substance has been reported in the literature and this may be due to several reasons. First, electrophysiological studies are needed to characterize HCN blockers, but this kind of tests require time and skill, and therefore they are not easily accessible. Second, cloned isoforms stably expressed in host cells have been available only in recent times. Third, the interest in HCN blockers has been strengthened only after the commercialization of Ivabradine (2005): in fact, the intensive efforts on the synthesis of specific bradycardic agents, produced by industries between '80s and '90s[153-157], were hampered by the disappointing result of zatebradine, the first HCN blocker to enter clinical trials[158,159]. Actually, only for a few compounds the activity on I_f has been reported, while the majority of the synthesized compounds have been characterized only for their negative chronotropic activity *in vitro* or *in vivo*: since the bradycardic effect can be due to several mechanisms, no information can be obtained on the activity of the compounds on cardiac HCN channels.

We decided to test **EC4**, **EC32** and **EC18** on the three HCN isoforms available to us, to see if the reduction of conformational flexibility could introduce some selectivity. After the promising preliminary results (see pharmacological section) we decided to continue this research in two directions: A) to optimize the synthetic pathway leading to **EC18**, trying to obtain also its *trans* isomer, in order to study the effect of geometrical and optical isomerism on the potency and selectivity of this cyclohexane derivative; B) to make some additional structural manipulations on the easily-accessible linear compounds. Therefore, a chiral center was placed on the phenethyl moiety of **EC4** and **EC32**, and/or the size of the molecule was increased by introducing on the basic nitrogen a second (7,8-dimethoxybenzazepin-3-yl)butenyl moiety or other

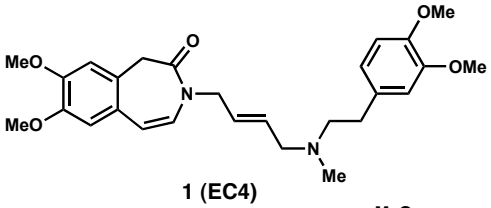
alkyl groups. During this research, the results of the enantioselectivity study prompted us also to hybridize the structure of ivabradine with that of **EC4** and **EC32**.



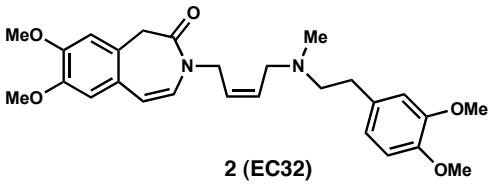
Verapamil



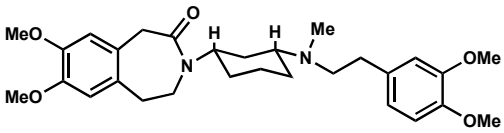
Zatebradine



1 (EC4)



2 (EC32)



***(±)–cis*-3 (EC18)**

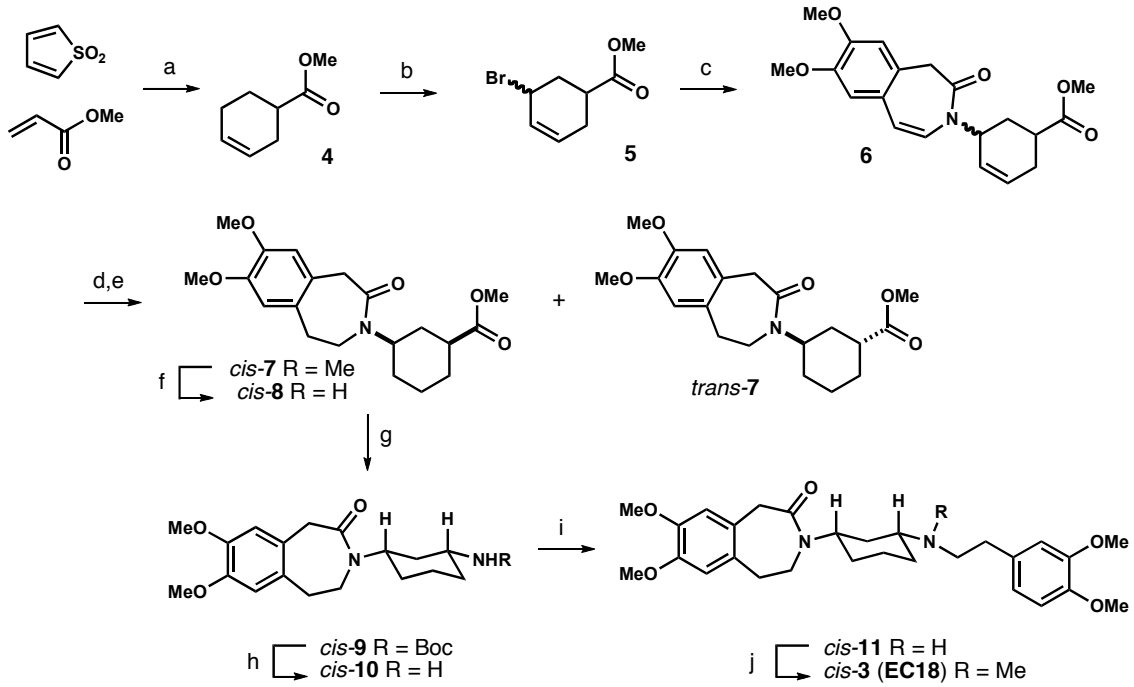
"People should continue to work hard at organic synthesis; it is hard, frustrating, but always fascinating. Synthesis can create whole new worlds"

S. Danishfesky

3. CHEMISTRY

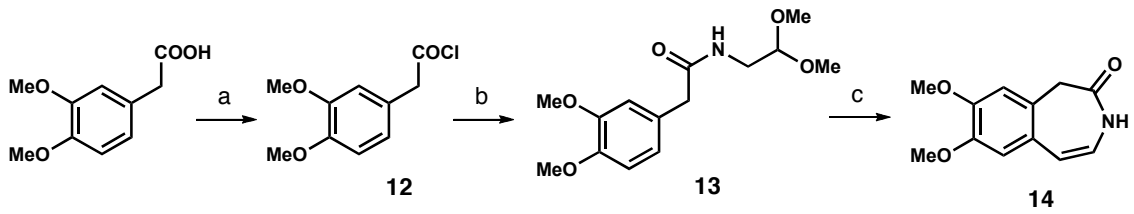
3.1 Synthesis of EC18 (*cis*-3)

The first synthesis of **3** is reported in **Scheme 1**. The cyclohexane ring was built through Diels-Alder reaction of sulpholene with methyl acrylate to give methyl 3-cyclohexene carboxylate **4**, which was brominated in the allylic position with NBS/AIBN[160] and reacted with 6,7-dimethoxy-1,3-dihydro-(2H)-3-benzazepin-2-one **14**[149] to give, after catalytic hydrogenation and chromatographic separation, the intermediate *cis*-**7**; this compound was hydrolyzed to *cis*-**8** under basic conditions and transformed into carbamate *cis*-**9** by Curtius rearrangement following a one-pot procedure reported in the literature[161]. Acid hydrolysis gave *cis*-**10**, which was treated with 3,4-dimethoxyphenylethyl bromide and methylated with formaldehyde and formic acid to give the desired compound *cis*-**3** (EC18).



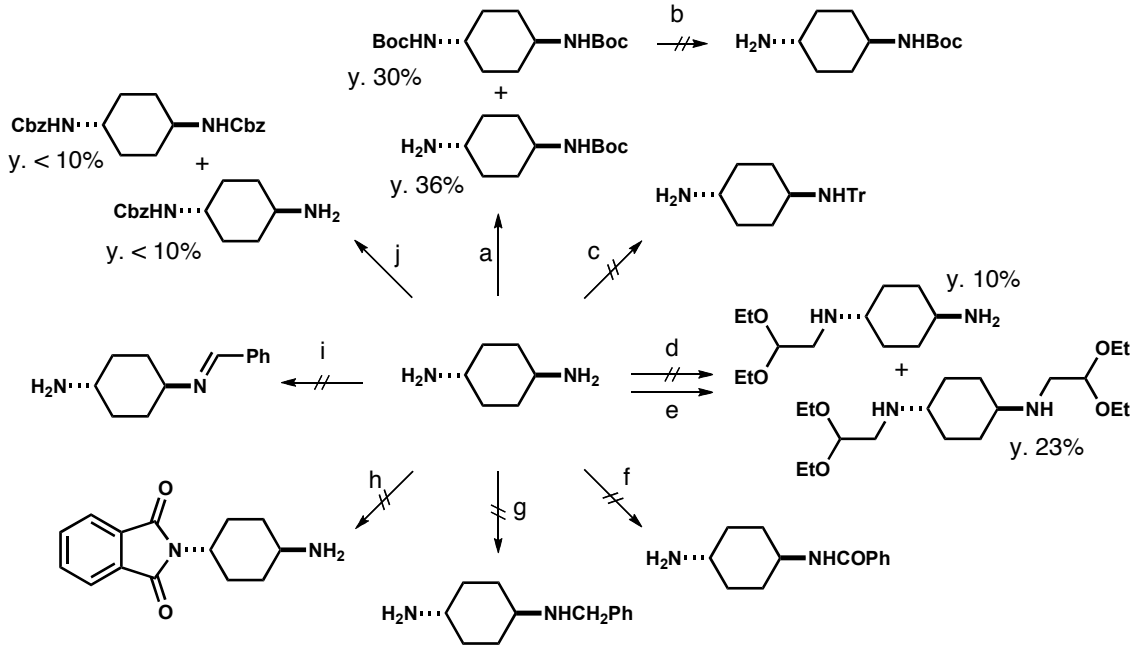
Scheme 1. a) 120 °C. b) NBS, AIBN. c) *t*-BuOK, **14**. d) H_2, Pd/C. e) chromatographic separation. f) NaOH. g) *t*-BuOH, DPPA. h) HCl. i) 3,4-dimethoxyphenethyl bromide. j) CH_2O, HCOOH.

This pathway allowed us to obtain a small amount of **EC18** for the preliminary test on spontaneously-beating guinea-pig right atria, but was not suitable for a large scale synthesis. In fact, while compound **14** could be easily prepared as reported in the literature (**Scheme 2**)[149], several steps of the reaction sequence gave very poor yields.



Scheme 2. a) $SOCl_2$. b) $NH_2CH_2CH(OCH_3)_2$, NEt_3. c) HCl, CH_3COOH.

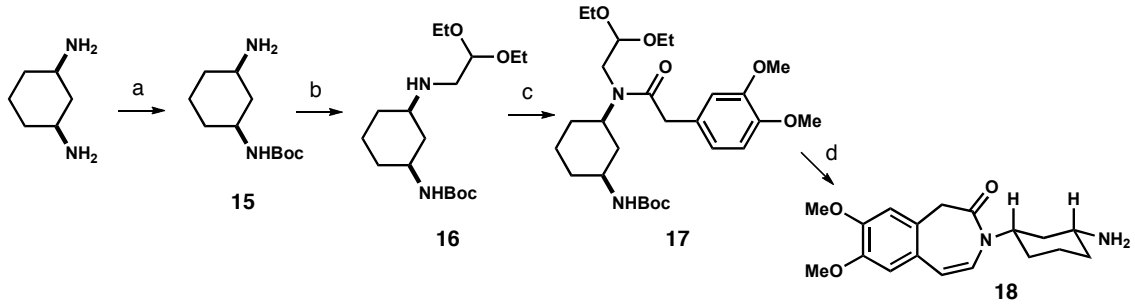
In order to obtain suitable amounts of **EC18**, a different approach was attempted, starting from *cis* 1,3-diaminocyclohexane. Since this compound is expensive, attempts to get the monoprotected diamine were carried out on the cheaper 1,4-diaminocyclohexane. Several methods are reported in the literature for the monoprotection of a diamine[162], but unfortunately, none of these was really successful (**scheme 3**): in fact, in all instances the diprotected compound was the most abundant, while the monoprotected one was obtained in poor yields, and only after chromatographic separation.



Scheme 3. a) $(Boc)_2O$ 0.5eq, $CHCl_3$ (diluted condition), 0 °C. b) AcCl, MeOH. c) Ph_3CCl, NEt_3, dryTHF. d) $BrCH_2CH(OEt)_2$, NEt_3, CH_3CN. e) $BrCH_2CH(OEt)_2$, K_2CO_3, dryDMF, Δ. f) 9-BBN in hexane, dryTHF; benzoyl chloride. g) Benzyl bromide, $CHCl_3$, Δ. h) Phthalic anhydride, $CHCl_3$, Δ. i) Benzaldheyde, MgSO4, DCM, Δ. j) Benzyl chloroformiate 0.5eq, $CHCl_3$ (diluted condition), Δ.

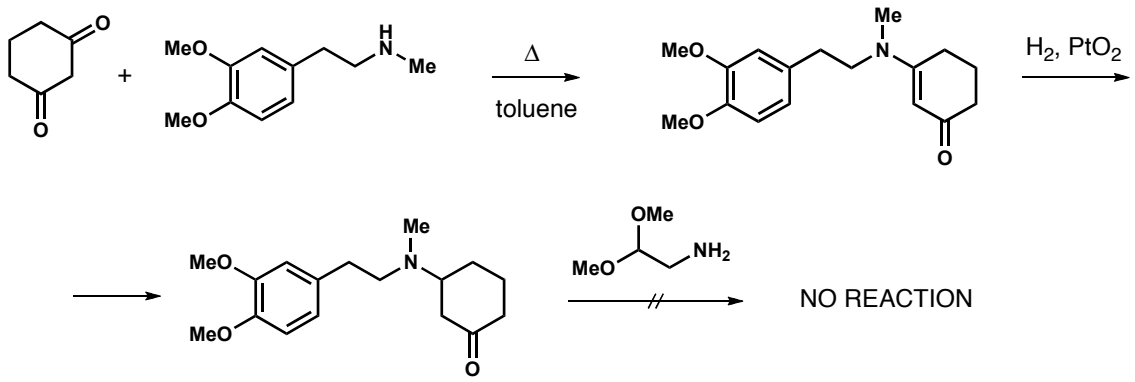
Anyway, we decided to try also on 1,3-diaminocyclohexane, using the BOC as protecting group, following the pathway reported in **Scheme 4**. Therefore, the diamine was treated with di-*t*-butyldicarbonate, obtaining the monoprotected derivative **15** in

20% yield, along with the diprotected analogue and some unreacted starting material. After chromatographic separation, the synthesis was continued by sequentially treating **15** with iodoacetaldehyde diethyl acetal[175] and then with compound **12**, obtaining **17**, which was treated with conc. HCl in AcOH to give **18**. Unfortunately, this pathway gave only a small amount of the desired compound **18**, owing to the low yields of the last reaction (10%). In order to try again the cyclization step, we attempted the synthesis of the monoprotected diamine following a different strategy.



Scheme 4. a) Di-tert-butyl dicarbonate. b) 1,1-diethoxy-2-iodoethane. c) 2-(3,4-dimethoxyphenyl)acetyl chloride, NEt_3. d) HCl, CH_3COOH.

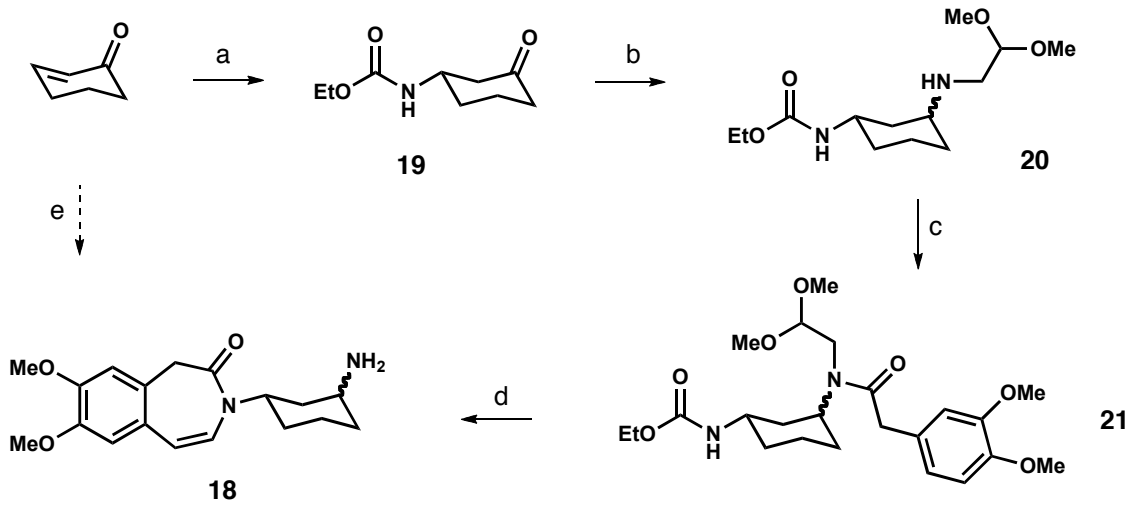
Previous work[163] had explored the use of 1,3-cyclohexanedione (**Scheme 5**): when this compound was treated with commercially-available *N*-methyl-homoveratrylamine in toluene, the enaminone was obtained, which was hydrogenated over PtO_2, but subsequent reaction with aminoacetaldehyde dimethylacetal was not successful. Therefore, we decided to explore the use of 2-cyclohexen-1-one (**Scheme 6**).



Scheme 5[163].

The *aza*-Michael addition with urethane gave compound 1**9**. The ketone was first reacted with 2,2-dimethoxyethanamine and titanium tetraisopropoxide[164], and then reduced to amine **20** by sodium cyanoborohydride. Due to the different reactivity of the two nitrogen atoms, the nucleophilic amine was treated with 2-(3,4-dimethoxyphenyl)acetyl chloride **12** to yield **21**, which underwent cyclization under

strong acidic condition. The latter one was again the limiting step of this pathway, due to the very poor yields. The cyclization and the formation of the benzazepinone moiety seemed to be crucial and difficult to obtain. For this reason, we tried a direct attachment of compound **14** to cyclohexenone under several experimental conditions[165], but without success. Therefore, we decided to switch back to the old synthesis, and optimize it.

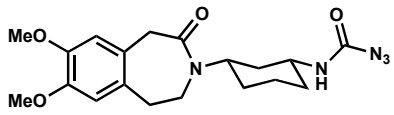


Scheme 6. a) Urethane, TMSCl, PPh_3. b) 2,2-dimethoxyethanamine, Ti(*i*OPr)$_4$, $NaCNBH_3$. c) **12**, NEt_3. d) HCl, CH_3COOH. e) **14** and DABCO, or $FeCl_3$/TMSCl, or PPh_3/TMSCl, or *t*-BuOK, or PPh_3/DEAD.

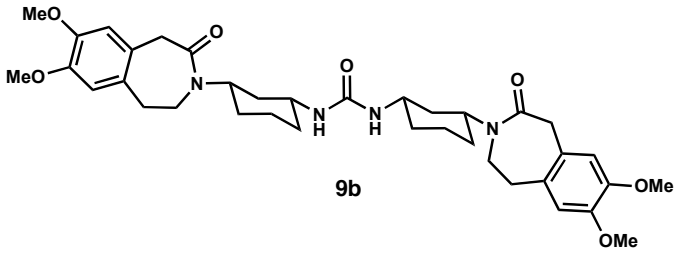
The problematic steps of the old synthesis were analyzed in details. The bromination of methyl 3-cyclohexane carboxylate (**scheme 1**; step b) gave a mixture of products; GC-MS analysis showed that at least four different products with the same desired molecular weight (219) were present. We reckoned that bromination could occur at both allylic positions (on C2 and C5) obtaining isomers with different reactivity, since the subsequent reaction with **14** gives only the desired compound **6**. A possible way to limit bromination at C2 could be the increase of steric bulkiness of the ester group; therefore, bromination was accomplished on the *t*-butyl ester but the subsequent treatment with **14** failed to give the desired compound: apparently, the large increase in steric bulkiness stopped the reactivity of the molecule.

Catalytic hydrogenation of **6** gave a *cis/trans* mixture of esters **7** in approximately 3 to 2 ratio; chromatographic separation afforded *cis*-**7** in good yields, but only a small amount of *trans*-**7**, together with mixed fractions. Basic hydrolysis of *cis*-**7** gave *cis*-**8** in quantitative yields, but previous work had shown that when the same reaction conditions were applied to *trans*-**7**, isomerization occurred, giving a *cis/trans* mixture of acids[163].

Treatment of *cis*-**7** with diphenylphosphorylazide (DPPA) and triethylamine in *t*-butanol gave *cis*-**9** in low yields: chromatographic separation of the complex reaction mixture showed the presence of at least two major by-products, which were later identified as the *cis*-carbamoyl azide **9a** and the urea **9b**. In addition, when the same reaction conditions were applied to *trans*-**8**, only a small amount of compound *cis*-**9** could be isolated from the reaction mixture[151].



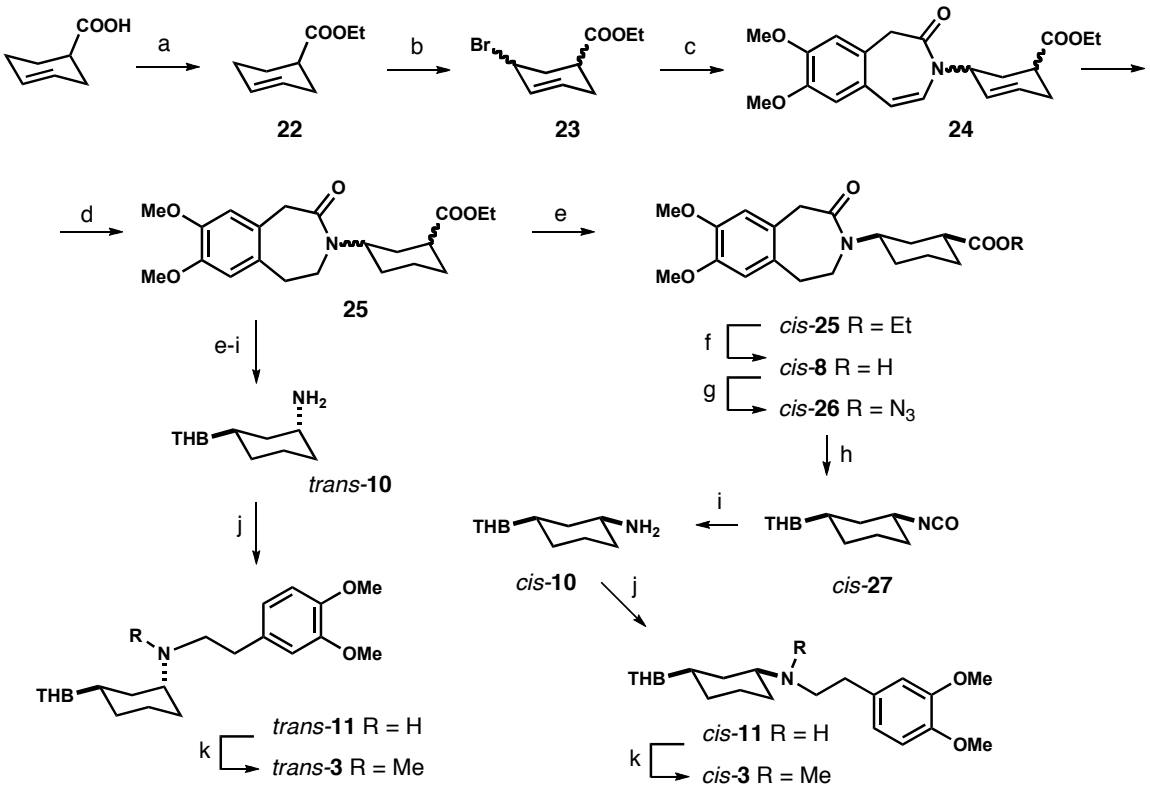
9a



9b

Therefore, we decided to repeat the reaction sequence reported in **scheme 1** on ethyl 3-cyclohexanecarboxylate, changing the conditions of the Curtius rearrangement (**scheme 7**). Compound **22** was obtained by esterification of the now commercially available 3-cyclohexene carboxylic acid, and transformed into **25** as reported for **6**. We found that a small difference in the ester group could lead us to a different ratio of diastereisomers: in fact, on the methyl ester **6**, after catalytic hydrogenation the *cis/trans* ratio of the ester mixture was approximately 3 to 2, whereas on the ethyl ester **25**, the ratio changed in 8 to 1. Therefore, the ethyl ester **25** was the ideal intermediate to get higher amount of **EC18** (*cis* isomer), while the methyl ester was a more suitable compound to obtain some amount of the trans isomer (*trans*-**3**). The *cis* ester **25** was separated from the *trans* isomer by column chromatography and the synthesis continued on the single diastereoisomer. Despite many attempts, column chromatography was not efficient to obtain substantial amount of pure *trans*-**25**; in addition, to avoid isomerization due to basic conditions, we tried to use acid hydrolysis, but the reaction was so much slower and, above all, it never got completion. For this reason the synthesis of *trans*-**3** continued on the diastereoisomeric mixture of the esters. Compound *cis*-**25** underwent basic hydrolysis; *cis*-**8** was transformed into the acylazide **26** by treatment first with $SOCl_2$ and then with NaN_3 in acetone-water, and heating in toluene afforded isocyanate **27**: these reactions were followed by IR spectroscopy and the two compounds were not fully characterized. Careful workup of **26** is needed: removal of acetone must be done at room T, in order to avoid the rearrangement of acylazide in presence of traces of water, which can lead to the formation of

carbamoylazide **9a** and urea **9b**. Finally, the amine *cis*-**10** was obtained by hydrolysis of the isocyanate under acidic condition.



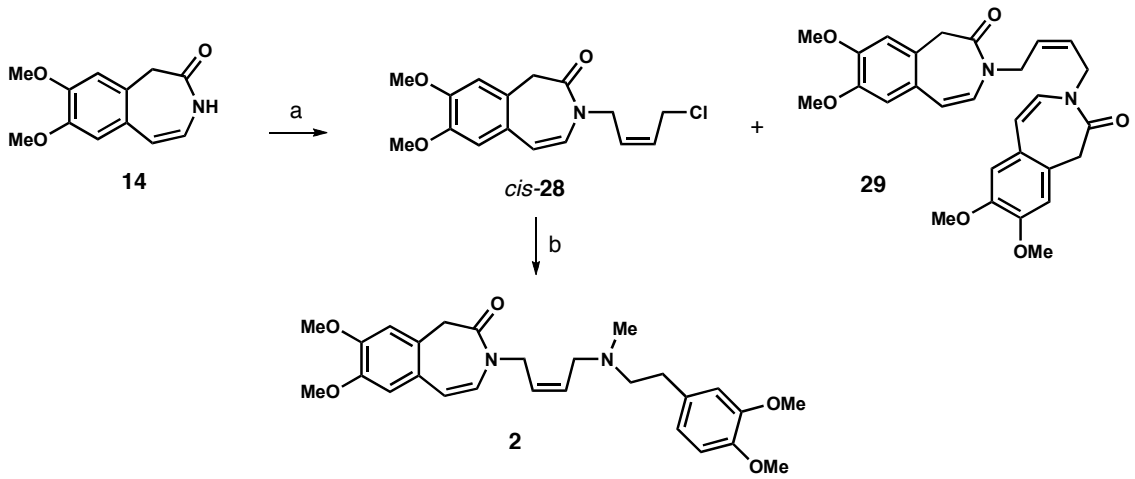
Scheme 7. a) EtOH, H_2SO_4. b) NBS, AIBN. c) **14**, *t*-BuOK, DMSO. d) H_2, Pd/C, 90psi, EtOH-EtOAc. e) Chromatographic separation. f) NaOH, MeOH. g) $SOCl_2$, NaN_3. h) Toluene, Δ. i) HCl 2N, THF. j) 3,4-dimethoxyphenethyl bromide, dryDMF, NEt_3. k) HCOOH, HCHO, MeOH. **THB** = 6,7-dimethoxy-1,3,4,5-tetrahydro-2H-3-benzazepin-2-one.

The same reaction sequence was performed on the *cis/trans* mixture of esters, and a *cis/trans* mixture of amine **10** was obtained, from which *trans*-**10** (the first eluting isomer) was isolated by column chromatography. Compounds *(±)-cis*-**11** and *(±)-trans*-**11** were prepared by treatment of the corresponding amines with 3,4-dimethoxyphenetyl bromide and NaI. Finally, methylation with formic acid and formaldehyde occurred to yield the desired compounds *(±)-cis*-**3** and *(±)-trans*-**3**. These compounds proved to be unstable if stored as free bases, and must be transformed into their stable hydrochloride salts.

Due to the promising biological activity of *cis*-**3**, we thought it important to study enantioselectivity. At the moment, the synthesis of higher amount of derivatives is ongoing; at the same time, the separation of a small amount of enantiomers is being attempted by chiral HPLC.

3.2 Synthesis of linear compounds

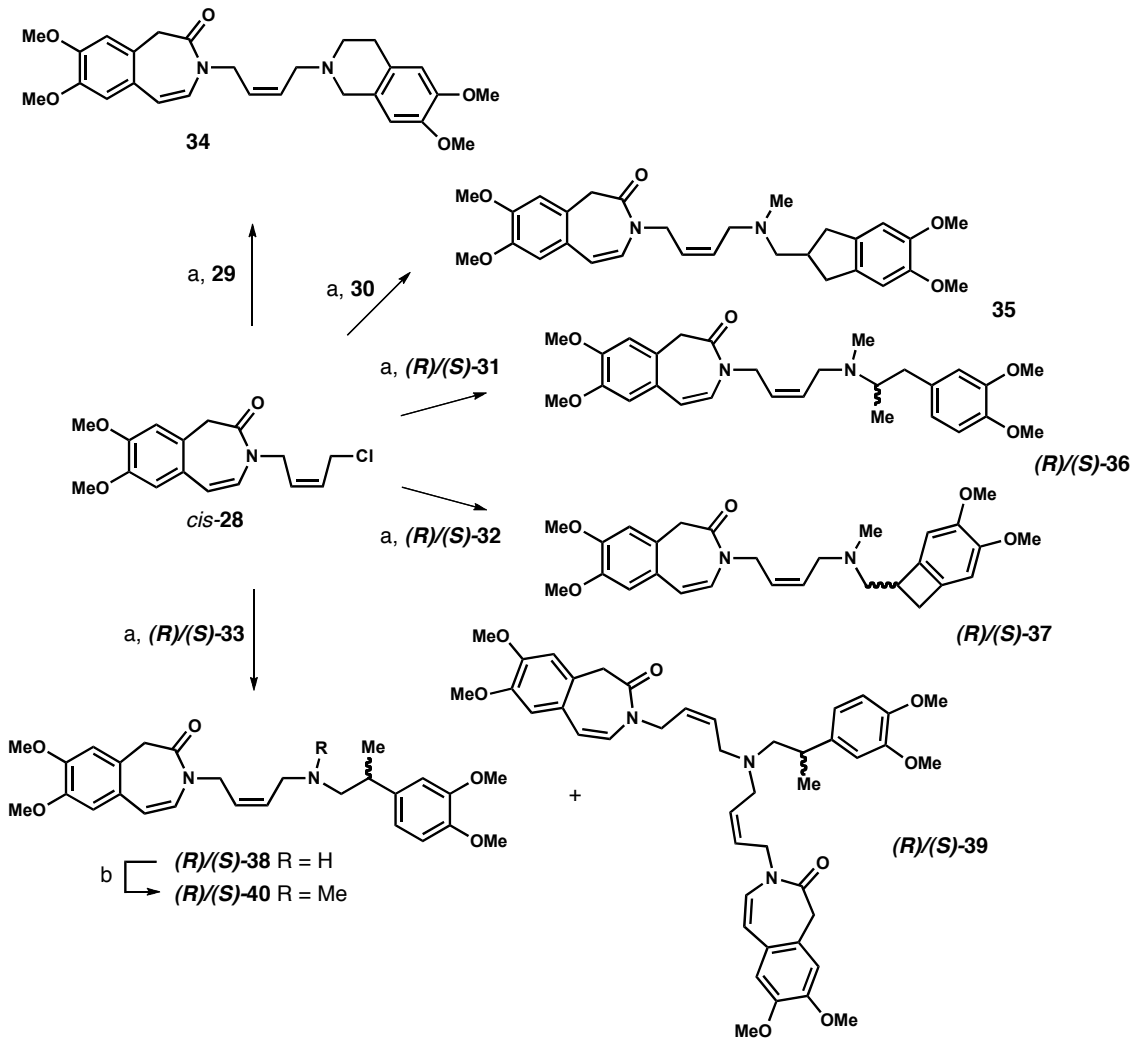
The synthesis of **2** (EC32) is reported in **scheme 8**.



Scheme 8. a) *t*-BuOK, *cis*-1,4-dichlorobutene. b) *N*-methyl-homoveratrylamine.

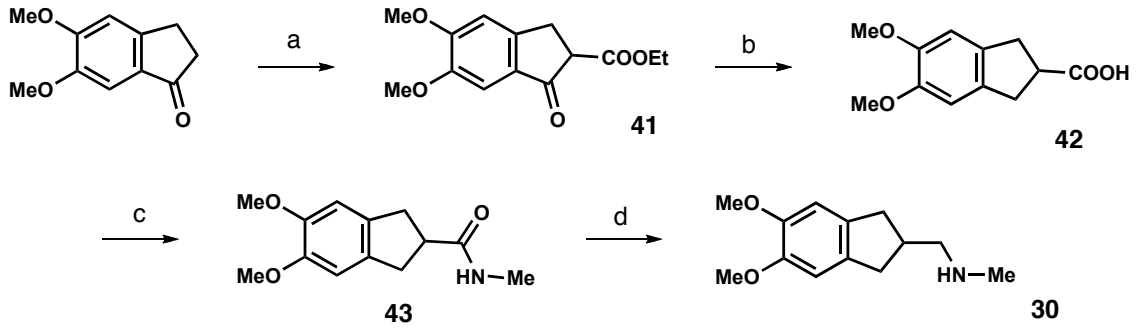
N-alkylation of **14** with commercially-available *cis* 1,4-dichlorobutene gave Z-3-(4-chlorobut-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one *cis*-**28**; the low yield of the reaction can be accounted for the competition with the double addition of **14** to the *cis* dichlorobutene, yielding 3,3'-((Z)-but-2-ene-1,4-diyl)bis(7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one) **29**. Changes in the ratio between reactants, or reverse addition of the anion to the halogen derivative did not stop the formation of the by-product. The formation of the dimeric product had not been observed when the reaction was carried out on *trans* 1,4-dichlorobutene[144]. Reaction of *cis*-**28** with commercially available *N*-methyl-homoveratrylamine gave **2** in good yields.

In a similar way (**scheme 9**), nucleophilic displacement of the chlorine of *cis*-**28** by amines **29**-**33** gave compounds **34**-**39**. Reaction of primary amine **33** with *cis*-**28** in 1:1 ratio gave a mixture of products resulting from mono- and double alkylation (compounds **38** and **39**, respectively), which were separated by column chromatography. The secondary amine **38** was transformed into **40** by methylation under standard conditions.



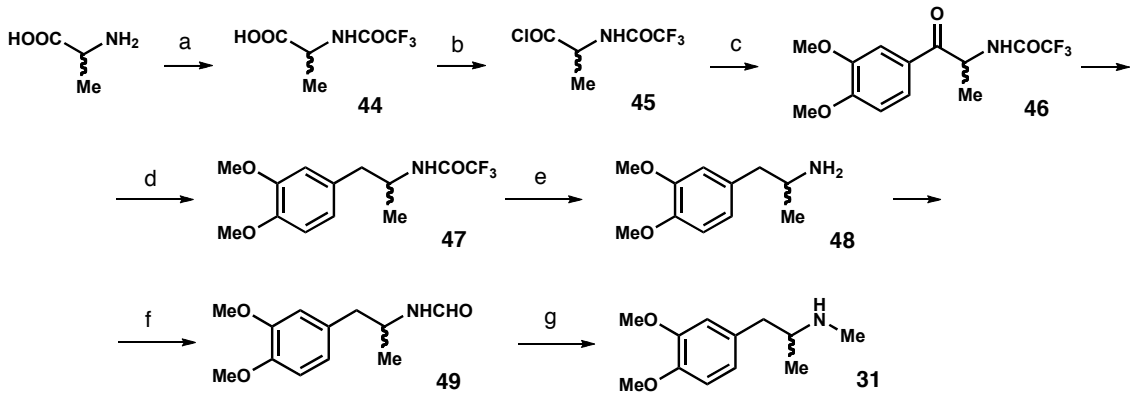
Scheme 9. a) NEt_3, MeCN, Δ. b) HCOOH, HCHO, MeOH.

Amines **30-33** were synthesized following the procedures reported in **schemes 10-13**, while **29** (6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline) was commercially available. Secondary amine **30** was synthesized as shown in **scheme 10**[166]. Commercially available 5,6-dimethoxy indan-1-one was treated with diethyl carbonate and sodium hydride to give **41**, which was reduced and hydrolyzed to acid **42**. The carboxylic group was first converted into amide (**43**) and finally reduced to the desired compound.



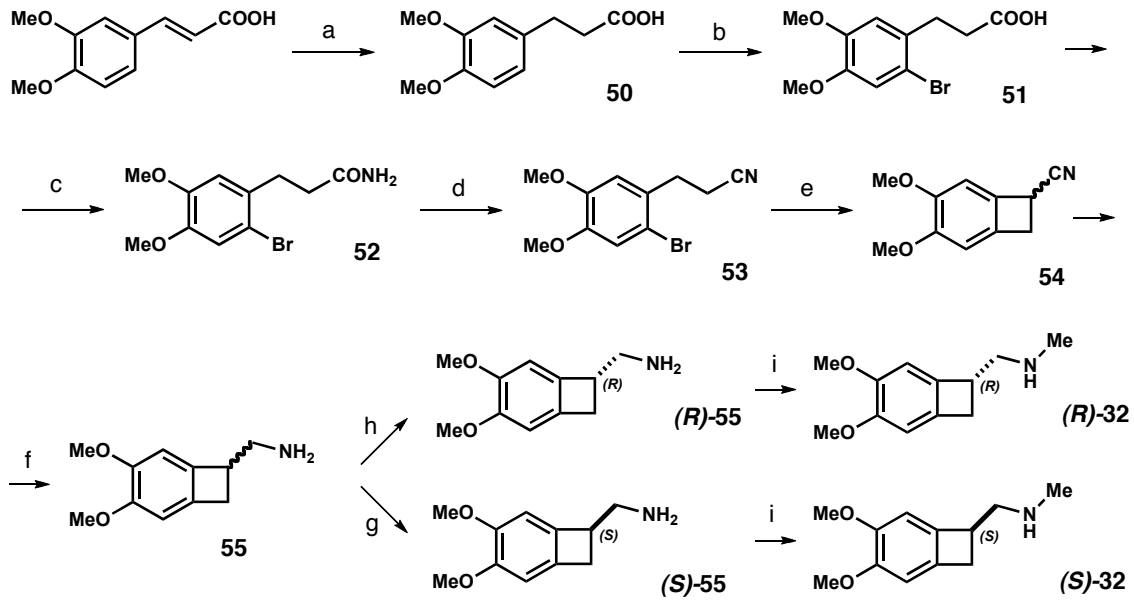
Scheme 10. a) diethyl carbonate, NaH, dryTHF. b) Zn, $HgCl_2$; NaOH 1N. c) CDI, NH_2CH_3, DCM. d) $LiAlH_4$, dryTHF.

The synthesis of secondary amines ***(R)*-31** and ***(S)*-31**, reported in **Scheme 11**, started from *D*-Alanine and *L*-Alanine, respectively. The aminoacid was N-protected as trifluoroacetate (**44**), then the acid was activated by ossalyl chloride (**45**) and treated with veratrol and $AlCl_3$, to yield compound **46**. Carbonyl reduction afforded **47**, which was hydrolyzed to **48**, transformed into the N-formyl derivative **49** and finally reduced to the desired secondary amine **31**.



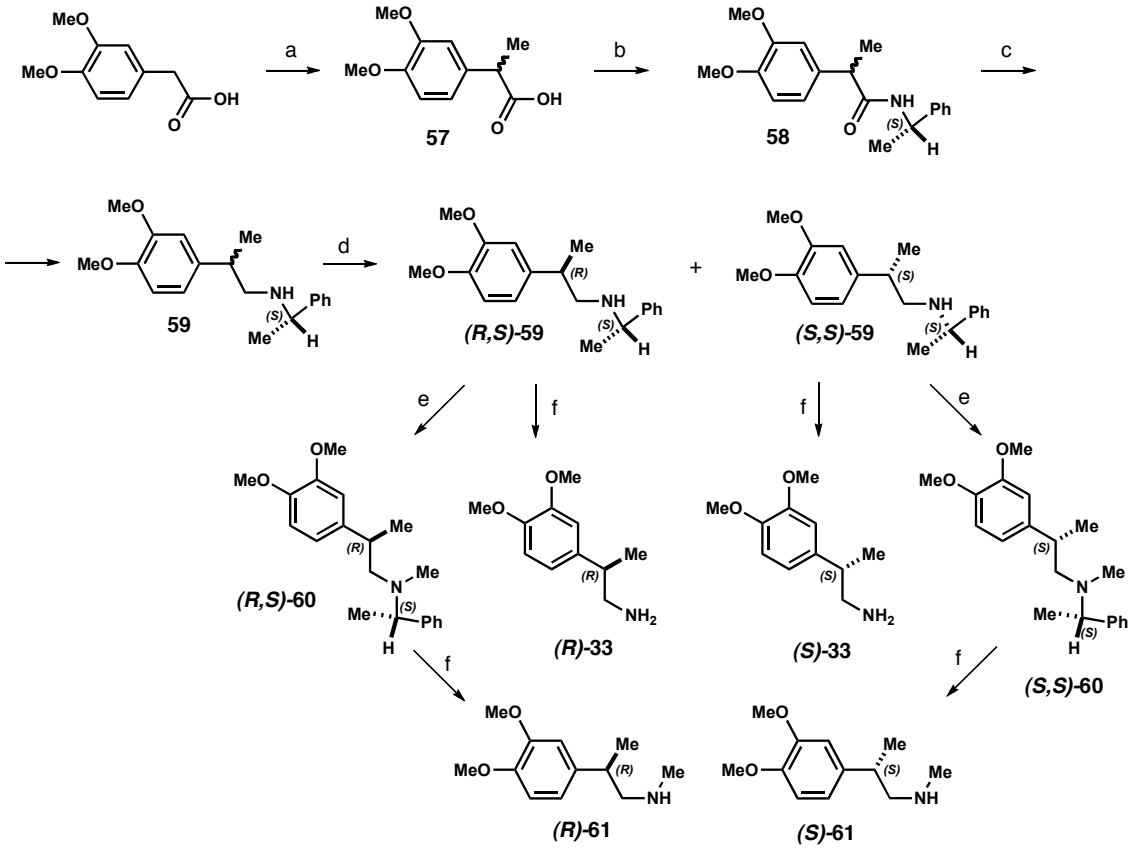
Scheme 11. a) Ethyltrifluoroacetate, NEt_3. b) Oxalyl chloride, pyridine. c) Veratrol, $AlCl_3$. d) Et_3SiH, CF_3COOH. e) K_2CO_3. f) HCOOEt. g) $LiAlH_4$.

Amines ***(R)/(S)*-32** were synthesized as reported in **scheme 12**. 3,4-Dimethoxycinnamic acid was hydrogenated to give **50**[167], which was first brominated to **51**[168] and then transformed into the amide **52**. Dehydration with phosphoryl chloride gave nitrile **53**. Formation of compound **54**[169] was the key step of the whole synthesis: treating compound **53** with sodium amide in liquid ammonia gave both deprotonation in position α to the nitrile, and dehydrobromination with formation of the arine; the reactive intermediate rapidly undergoes cyclization[176]. Reduction of **54** with borane[170] yielded the primary amine **55**. Resolution of the racemic mixture by fractional crystallization first with *N*-acetyl-L-glutamic acid[171] and then with *N*-acetyl-D-glutamic acid afforded respectively ***(S)*-55** and ***(R)*-55** as pure enantiomers, which were treated with ethyl formate and then reduced to N-methyl amines ***(S)*-32** and ***(R)*-32**.



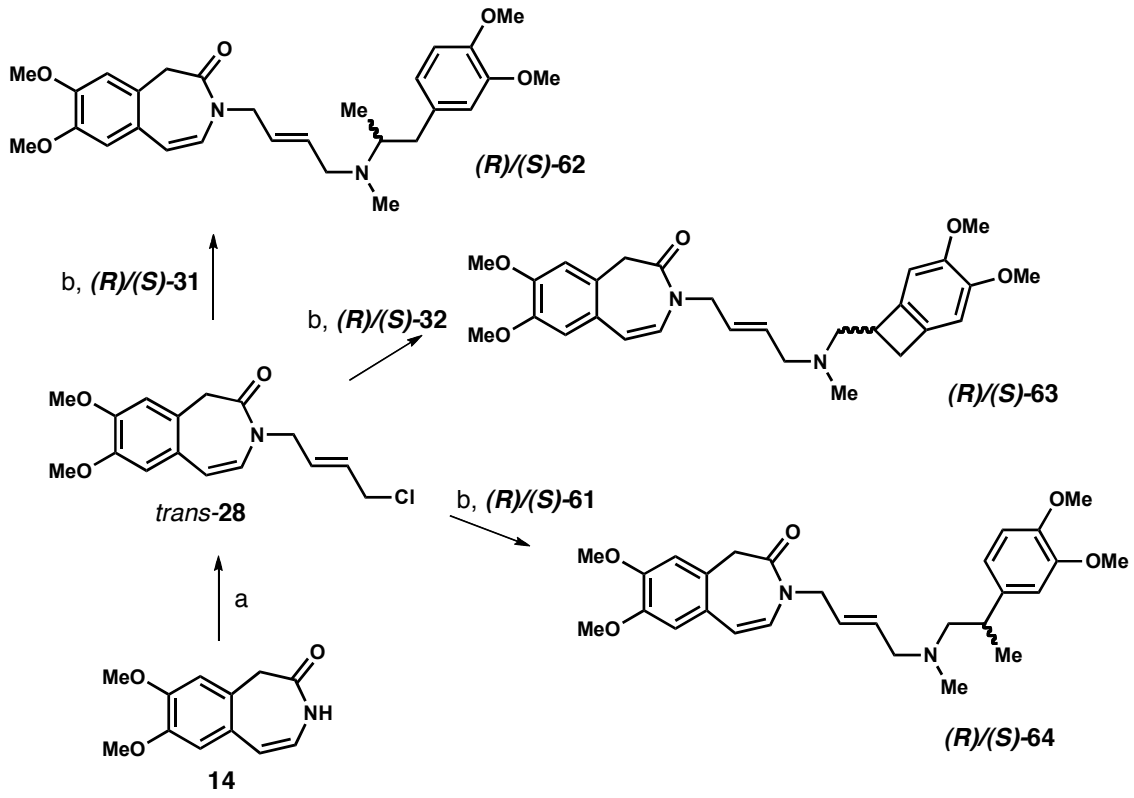
Scheme 12. a) H_2, Pd/C, 60psi, THF. b) Br_2, AcOH. c) $SOCl_2$; NH_3. d) $POCl_3$ e) liq NH_3, $NaNH_2$, -33 C. f) BH_3·THF. g) *N*-acetyl-L-glutamic acid, EtOH. h) *N*-acetyl-D-glutamic acid, EtOH. i) HCOOEt; $LiAlH_4$.

The enantiomers of amines **33** were synthesized from the commercially available 3,4-dimethoxyphenylacetic acid according to a literature procedure, with slight modifications (**scheme 13**)[172]. First, α-alkylation was performed with lithium diisopropylamide and ethyl iodide to give **57**, which was converted into the acyl chloride and then treated with *(S)*-α-methylbenzylamine to give **58**. Reduction with borane afforded the intermediate **59** as mixture of diastereoisomers that were separated by column chromatography yielding ***(R,S)*-59** and ***(S,S)*-59**. Primary amines ***(R)*-33** and ***(S)*-33** were obtained through hydrogenation in a PARR apparatus utilizing the Pearlman's catalyst in methanol. In order to obtain N-methylated secondary amines, ***(R,S)*-59** and ***(S,S)*-59** were first reacted with formic acid and formaldehyde obtaining ***(R,S)*-60** and ***(S,S)*-60**, which were hydrogenated to the desired compounds ***(R)*-61** and ***(S)*-61**[173,174].



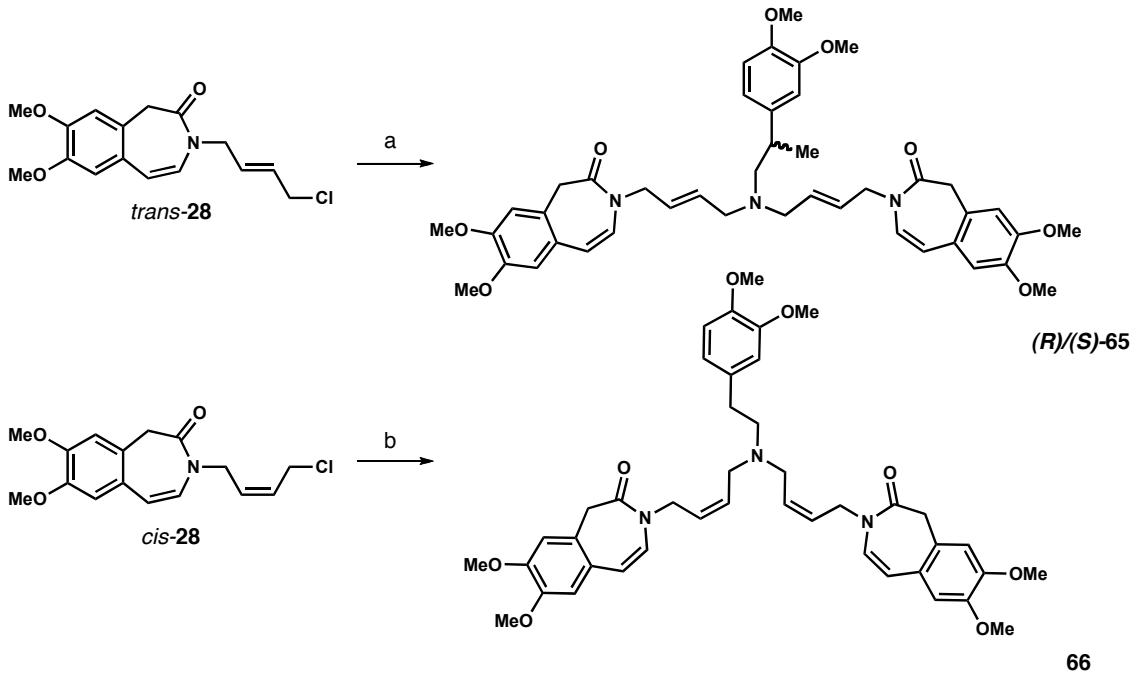
Scheme 13. a) LDA, CH_3I, dryTHF. b) $SOCl_2$, *(S)*-α-methylbenzylamine, benzene, pyr. c) 1M BH_3·THF, HCl, CH_3OH. d) Chromatographic separation of diastereisomers. e) HCOOH, HCHO. f) H_2, $Pd(OH)_2$, 60psi, CH_3OH.

In order to compare the activity of *cis* and *trans* isomers, the enantiomers of amines **31**, **32** and **61** were reacted with E-3-(4-chlorobut-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one *trans*-**28**, prepared from **14** and E-1,4-dichlorobutene as previously reported[144]. The enantiomers of compounds **62-64** were then obtained (**scheme 14**).



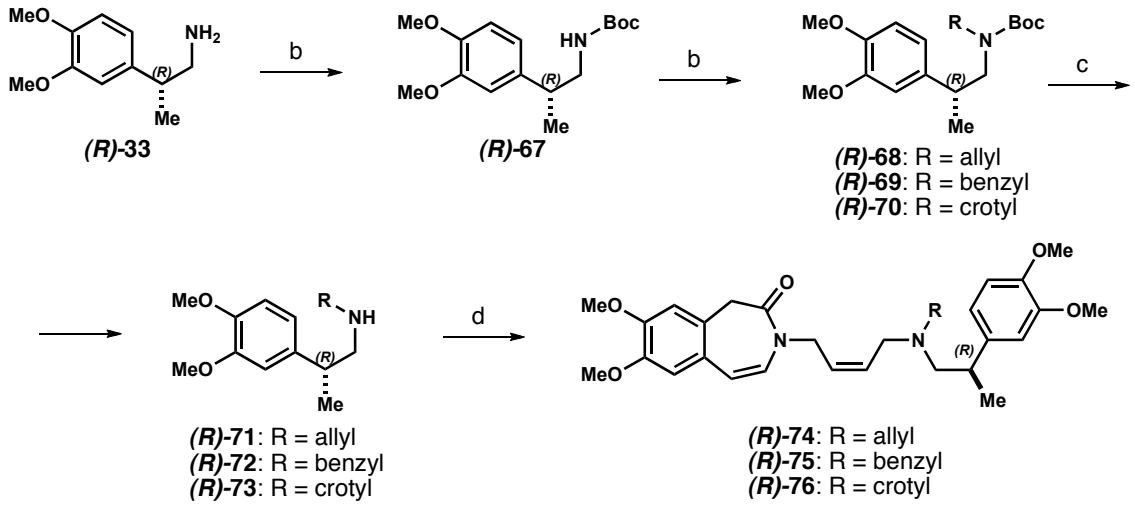
Scheme 14. a) *t*-BuOK, *trans*-1,4-dichlorobutene. b) NEt_3, MeCN, Δ.

To prepare the *trans* analogue of **39**, in order to optimize yields, amine ***(R)/(S)*-33** was reacted with *trans*-**28** in a 1:2 ratio obtaining **65** (**scheme 15**). In the same way, the achiral compound **66** was also synthesized.



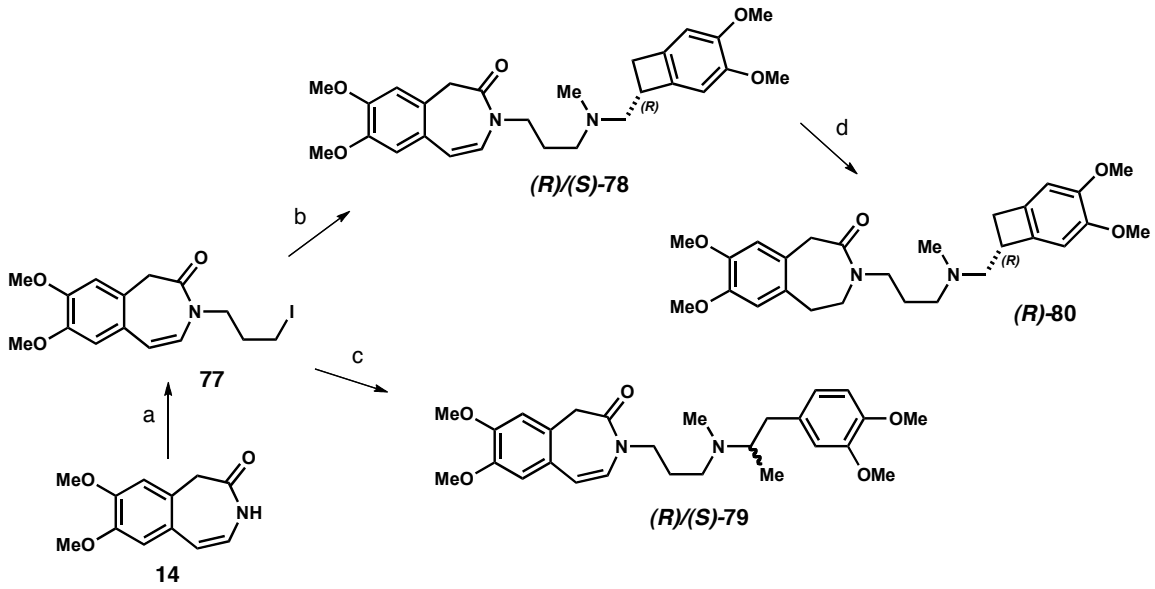
Scheme 15. a) NEt_3, ***(R)/(S)*-33**. b) NEt_3, MeCN, homoveratrylamine.

The promising activity of compound ***(R)*-39** (see results) prompted us to make some modification in its structure. In fact, this compound has a high molecular weight, and low water solubility even as hydrochloride salt. In order to reduce its size, one of the (7,8-dimethoxybenzazepin-3-yl)butenyl moiety was replaced by smaller alkyl groups bearing unsaturation (benzyl, crotyl and allyl). These modifications were made only on the *cis*-derivative and only in the R-configuration. Amines **71**-**73** were therefore prepared from ***(R)*-33**, which was treated with di-*t*-butyldicarbonate to give **67**, deprotonated with NaH and treated with allyl, benzyl and crotyl bromide to give carbamates **68**-**70**. Deprotection with trifluoroacetic acid gave amines **71**-**73** that were reacted with *cis*-**28** in 1:1 ratio, obtaining compounds **74**-**76** (**scheme 16**).



Scheme 16. a) Di-*tert*-butyl dicarbonate, dryTHF. b) NaH, allyl or benzyl or crotyl bromide, dryDMF. c) Trifluoroacetic acid, DCM. d) *cis*-**28**, NEt_3, MeCN.

Finally, in order to include Ivabradine in the study of enantioselectivity, its R-enantiomer was synthesized. At the same time, we thought it interesting to see the effect of placing a stereogenic center on the phenylethyl moiety of Zatebradine, maintaining the conformational flexibility on this part of the molecule. Therefore we synthesized the saturated derivatives reported in **scheme 17**.



Scheme 17. a) i: *t*-BuOK, $Cl(CH_2)_3Br$. ii: NaI. b) NEt_3, MeCN, ***(R)/(S)*-32**. c) NEt3, MeCN, ***(R)/(S)*-31**. d) H_2, Pd/C.

Compound **77**, prepared by reaction of **14** with 1-bromo-3-chloropropane utilizing potassium *tert*-butoxide as a base, followed by the displacement of chlorine with iodine to increase the reactivity of the leaving group[150], was reacted with ***(R)/(S)*-32** and ***(R)/(S)*-31**, obtaining compounds **78**-**79**. Compound ***(R)*-78** was finally hydrogenated to ***(R)*-80** (*R*-Ivabradine) using palladium on carbon as catalyst.

"Il prodotto e' un condensato di sudore chimico"

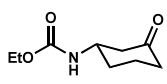
G. Appendino

4. EXPERIMENTAL SECTION

Material and Methods:

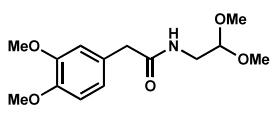
All melting points were taken on a Büchi apparatus and are uncorrected. Infrared spectra were recorded with a Perkin-Elmer Spectrum RX I FT-IR spectrophotometer in Nujol mull for solids and neat for liquids. NMR spectra were recorded on a Bruker Avance 400 spectrometer. Chromatographic separations were performed on a silica gel column by gravity chromatography (Kieselgel 40, 0.063-0.200 mm; Merck) or flash chromatography (Kieselgel 40, 0.040-0.063 mm; Merck). Yields are given after purification, unless otherwise stated. Where analyses are indicated by symbols, the analytical results are within ±0.4% of the theoretical values. We have chosen to perform and report only the combustion analyses of final compounds. The identity and purity of the intermediates was ascertained through IR, NMR and TLC chromatography. Compounds were named following IUPAC rules as applied by Beilstein-Institut AutoNom 2000 (4.01.305) or CA Index Name. When reactions were performed in anhydrous conditions, the mixtures were maintained under nitrogen.

Ethyl 3-oxocyclohexylcarbamate; **19**



In a two-necked flask, urethane (0.0062 mol; 0.56 g) and 2-cyclohexen-1-one (0.0052 mol; 0.5 mL) were added under nitrogen to a solution of PPh_3 (0.0052 mol; 0.14 g) in dichloromethane (5 mL). After 5 minutes trimethylchlorosilane (0.0057 mol; 0.73 mL) was slowly added to the solution and the resulting mixture was stirred for 24 hours at room temperature. The reaction was quenched with a saturated aqueous solution of sodium bicarbonate and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: petroleum ether-ethyl acetate, 1:1) to give **19** as a clear oil: 0.33 g; 34%; ESI-MS $[M + 1]^+$ *m/z* 186, $[M + Na]^+$ *m/z* 208; ^1H NMR ($CDCl_3$) δ 1.22-1.26 (m, 3H),1.71-1.88 (m, 3H), 1.97-2.09 (m, 1H), 2.10-2.12 (m, 1H), 2.23-2.31 (m, 2H), 2.35-2.41 (m, 1H), 2.68-2.73 (m, 1H), 3.97 (bs, 1H), 4.08-4.14 (m, 2H), 4.71 (bs, 1H).

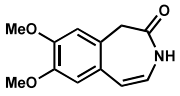
N-(2,2-dimethoxyethyl)-2-(3,4-dimethoxyphenyl)acetamide; **13**



Thionyl chloride (0.158 mol; 11 mL) was added dropwise to a suspension of 3,4-dimethoxyphenylacetic acid (0.051 mol; 10 g)

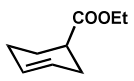
in chloroform (50 mL). The mixture was heated at 61 °C for 3 hours under nitrogen. The reaction was monitored by TLC (eluent: cyclohexane-ethyl acetate, 1:1). The solvent was removed under vacuum and the residue washed with cyclohexane (2 × 30 mL, removed under vacuum). The obtained dark red oil (**12**) was dissolved in chloroform (50 mL) yielding a solution that was added dropwise to a solution at 0 °C of amino acetaldehyde dimethyl acetal (0.05 mol; 7.5 mL) and anhydrous triethylamine (7 mL) in chloroform (50 mL). The mixture was stirred for 1 hour at room temperature and then was quenched with water and extracted with chloroform. Removal of the dried solvent (Na_2SO_4) under vacuum afforded **13** as a yellow oil: 13.95 g; 98%; ^{1}H NMR ($CDCl_3$, δ): 3.36 (s, 6H), 3.37 (t, J=5.5 Hz, 2H), 3.52 (s, 2H), 3.89 (s, 6H), 4.33 (t, J=5.5 Hz, 1H), 5.67 (bs, 1H), 6.79-6.86 (br, 3H).

7,8-Dimethoxy-1H-benzo[d]azepin-2(3H)-one; **14**



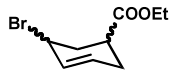
A solution of **13** (0.049 mol; 13.9 g) in glacial acetic acid (1.14 mol, 70 mL) and 37% HCl (70 mL) was stirred for 1 day at room temperature. The reaction was monitored by TLC (eluent: cyclohexane-ethyl acetate, 3:7). Ice was then added to the mixture to give a white precipitate that was filtered off under vacuum, washed with water, and dried for 2 hours at 70 °C affording **25**: 7.2 g; 66.7%; mp 240-244 °C; ^{1}H NMR (DMSO) δ 3.29 (s, 2H), 3.74 (s, 3H), 3.77 (s, 3H), 6.15-6.25 (m, 2H), 6.85 (s, 1H), 6.89 (s, 1H). IR (Nujol, cm^{-1}) ν 3174 (NH), 1606, 1631, 1664 (C=O).

Ethyl cyclohex-3-enecarboxylate; **22**



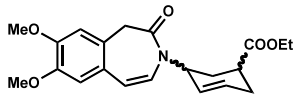
Concentrated sulfuric acid (0.35 mL) was added to a solution of 3-cyclohexene-1-carboxylic acid (0.039 mol; 5 g) in absolute ethanol (35 mL). The mixture was heated at reflux for 1 day and monitored by TLC (eluent: cyclohexane-ethyl acetate, 1:1). The solvent was removed under vacuum to give a residue that was treated with sodium bicarbonate and extracted with dichloromethane. Removal of the dried (Na_2SO_4) solvent under vacuum afforded **22** as a pale yellow oil: 5.22 g; 0.034 mol; 87%; ^{1}H NMR ($CDCl_3$) δ 1.20 (t, J=7.2 Hz, 3H), 1.57-1.67 (m, 1H), 1.92-1.97 (m, 1H), 2.02-2.06 (m, 2H), 2.18-2.20 (m, 2H), 2.45-2.52 (m, 1H), 4.09 (q, J=7.2 Hz, 2H) 5.65 (s, 2H).

Ethyl 5-bromocyclohex-3-enecarboxylate; **23**



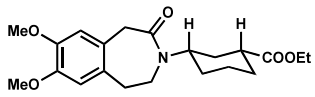
N-bromosuccinimmide (0.034 mol; 6.03 g) and a catalytic amount of AIBN were added to a solution of **22** (0.034 mol; 5.22 g) in CCl_4 (10 mL). The mixture was heated at reflux for 3 hours and monitored by TLC (eluent: cyclohexane-ethyl acetate, 9:1). During the reaction a white precipitate (succinimide) was formed that was filtered off once the reaction was finished. The filtrate was collected and the solvent was removed under vacuum to afford **23** as a *cis/trans* mixture (7.75 g; yellow oil), which was used in the next reaction without further purification.

Ethyl-5-(7,8-dimethoxy-2-oxo-1H-benzo[d]azepin-3(2H)-yl)cyclohex-3-enecarboxylate; **29**



Potassium terbutoxyde (5.15 mmol; 0.22 g) was added under nitrogen to a milky brown suspension of **14** (1.29 mmol; 0.75 g) in anhydrous DMSO. The complete formation of the anion was observed because of the formation of a dark red solution. Then, **23** (1.29 mmol; 0.30 g) was added dropwise to the solution. After stirring overnight at room temperature, DMSO was partially distillated under reduced pressure. Ice was then added to the mixture and a formation of a milky brown precipitate (**14** not reacted) was observed. The aqueous layer was extracted with chloroform (3 × 30 mL). The organic layers were collected, dried on Na_2SO_4 and evaporated under vacuum to give a residue that was purified by flash chromatography. Eluting with cyclohexane-ethyl acetate (6:4) afforded **24** (clear oil) as a mixture of *cis/trans* isomers: 0.20 g; 0.54 mmol; 42%; ^{1}H NMR ($CDCl_3$) δ 1.22 (t, J=7.20 Hz, 3H), 1.54-1.60 (m, 1H), 2.1-2.2 (m, 1H), 2.25-2.38 (m, 2H), 2.68-2.80 (m, 1H), 3.3-3.4 (m, 1H), 3.5-3.6 (m, 1H), 3.88 (s, 3H), 3.89 (s, 3H), 4.10 (q, J=7.20 Hz, 2H), 5.21-5.69 (m, 2H), 5.92-6.12 (m, 1H), 6.18-6.40 (m, 2H), 6.73 (s, 1H), 6.79 (s, 1H).

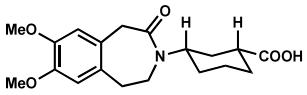
(±)-cis-Ethyl-3-(7,8-dimethoxy-2-oxo-4,5-dihydro-1H-benzo[d]azepin-3(2H)-yl)cyclohexanecarboxylate; ***(±)-cis*-25**



Pd/C (10% p/p) was added to a solution of **24** (8.63 mmol; 3.2 g) in absolute ethanol (50 mL). The mixture was placed

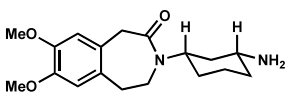
in a Parr apparatus at 90 Psi and allowed to react for 4 days at room temperature. The reaction was monitored by TLC (eluent: dichloromethane-methanol, 96:4). The mixture was filtered and the solvent removed under vacuum to give 2.42 g of a white solid that was purified by gravity column chromatography. Eluting with dichloromethane-methanol (98:2) afforded ***(±)-cis*-25** as a clear oil: 2.11 mmol; 0.79 g; 25%; ^{1}H NMR ($CDCl_3$) δ 1.24 (t, J=7.20 Hz, 3H), 1.27-1.50 (m, 3H), 1.59 (q, J=12.4 Hz, 1H), 1.77 (d, J=9.6 Hz, 1H), 1.90-1.99 (m, 3H), 2.43-2.51 (m, 1H), 3.01-3.04 (m, 2H), 3.72 (t, J=5.6 Hz, 2H), 3.84-3.86 (m, 8H), 4.11 (q, J=7.20 Hz, 2H), 4.50-4.56 (m, 1H), 6.53 (s, 1H), 6.60 (s, 1H).

(±)-cis-3-(7,8-Dimethoxy-2-oxo-4,5-dihydro-1H-benzo[d]azepin-3(2H)-yl)cyclohexanecarboxylic acid; ***(±)-cis*-8**



A solution of ***(±)-cis*-25** (2.11 mmol; 0.79 g) in NaOH 2.5M (2.53 mmol; 1.01 mL) and methanol (15 mL) was stirred for 24 hours at room temperature. The reaction was monitored by TLC (eluent: dichloromethane-methanol, 9:1). The solvent was evaporated under vacuum and the residue was taken up in water and extracted with ethyl acetate. The water layer was collected, acidified (pH 1) with 2N HCl, and extracted with dichloromethane. Removal of the dried (Na_2SO_4) solvent under vacuum gave ***(±)-cis*-8** as a gummy white solid: 0.76 g; quantitative yield; ^{1}H NMR ($CDCl_3$,) δ 1.29-1.50 (m, 3H), 1.59 (q, J=12.3 Hz, 1H), 1.77 (d, J=10 Hz, 1H), 1.92 (d, J=11.7 Hz, 1H), 2.04 (d, J=12.1 Hz, 2H), 2.53 (t, J=11.5 Hz, 1H), 3.04 (m, 2H), 3.72 (m, 2H), 3.84 (s, 2H), 3.86 (s, 6H), 4.55 (t, J=11.2 Hz, J=10.6 Hz, 1H), 6.54 (s, 1H), 6.62 (s, 1H).

(±)-cis-3-(3-Aminocyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one; ***(±)-cis*-10**



In a two-necked flask under nitrogen atmosphere, thionyl chloride (5.54 mmol; 0.4 mL) was added dropwise to a solution of ***(±)-cis*-8** (0.58 mmol; 0.2 g) in chloroform (50 mL). The mixture was heated at 61 °C for 3 hours and monitored by TLC (eluent: dichloromethane-methanol, 96:4). The solvent was removed under vacuum to give a residue that was washed with cyclohexane (2 × 30 mL) and dried under vacuum. Anhydrous acetone (10 mL) was poured into the flask followed by a saturated aqueous solution of NaN_3 (2 mL). After

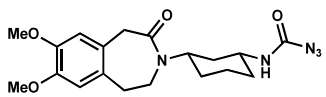
stirring for 10 minutes, a small excess of water was added to the solution. Acetone was evaporated under vacuum and the aqueous layer was extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give ***(±)-cis*-26** as a gummy yellow solid (0.18 g; 87%) that was used in the next step without further purification. The formation of acylazide was detected by IR spectroscopy (ν= 2100 cm^{-1}; CON_3).

A solution of ***(±)-cis*-26** (1.29 mmol; 0.48 g) in toluene was heated at reflux overnight to allow the formation of isocyanate. The reaction was monitored by IR spectroscopy and continued until the peak of acylazide (ν 2100 cm^{-1}) disappeared because of the formation of isocyanate ***(±)-cis*-27** (ν 2250 cm^{-1}). The toluene was removed under vacuum to give a residue that was dissolved in THF (3 mL) and 2N HCl (3 mL). The resulting solution was stirred at room temperature overnight and monitored by TLC (dichloromethane-methanol-ammonia, 90:10:0.5). Organic solvents were evaporated under vacuum and the aqueous layer was washed with diethyl ether, basified with 2.5M NaOH and extracted with dichloromethane. The organic layers were collected, dried on Na_2SO_4 and removed under vacuum. ***(±)-cis*-10** was obtained as a gummy yellow solid (0.41 g).

It is important to notice that a careful workup of **26** is required; in fact, acetone must be removed at room temperature in order to avoid the rearrangement of the acylazide in presence of traces of water, which can lead to the formation of side products such as carbamoylazide (**9a**) and urea (**9b**).

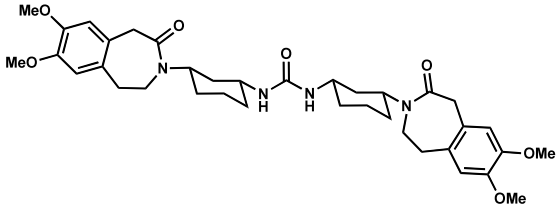
***(±)-cis*-10**: ^{1}H NMR ($CDCl_3$) δ 1.01 (qd, J=12.3 Hz, J=3.7 Hz, 1H), 1.24-1.47 (m, 3H), 1.70 (d, J=11.9 Hz, 1H), 1.80-1.91 (m, 5H), 2.86 (tt, J=11.0 Hz, J=3.8 Hz, 1H), 3.02 (dd, J=6.3 Hz, J=5.6 Hz, 2H), 3.69 (t, J=6.1 Hz, 2H), 3.81 (s, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 4.51 (tt, J=12.1 Hz, J=3.5 Hz, 1H), 6.53 (s, 1H), 6.61 (s, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 23.20, 29.66, 33.95, 35.32, 40.36, 40.83, 43.03, 49.74, 50.91, 55.93, 113.28, 114.13, 123.49, 127.22, 147.18, 147.95, 172.05.

9a: ^{1}H NMR ($CDCl_3$) δ 1.10-2.10 (m, 8H), 3.01-3.02 (m, 2H), 3.66-3.69 (m, 3H), 3.83-

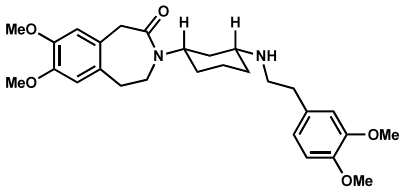


3.85 (m, 8H), 4.45-4.51 (m, 1H), 5.06 (bs, 1H), 6.52 (s, 1H), 6.60 (s, 1H); ^{13}C NMR ($CDCl_3$) δ 22.93, 29.43, 31.94, 33.79, 36.98, 40.99, 42.78, 49.68, 51.39, 55.90, 113.20, 114.07, 123.17, 126.90, 128.07, 128.48, 147.22, 147.96, 155.35, 172.15; IR (Nujol, cm^{-1}) ν 2141; LC-MS $[M + 1]^+$ *m/z* 388.

9b: ^{1}H NMR ($CDCl_3$) δ 1.20-2.17 (m, 16H), 2.99-3.01 (m, 4H), 3.50-3.51 (m, 2H), 3.66-3.70 (m, 4H), 3.77-3.80 (m, 16H), 4.37-4.40 (m, 2H), 4.71 (bs, 2H), 6.53 (s, 2H), 6.58 (s, 2H); IR (Nujol, cm^{-1}) ν 1636 (CO); LC-MS $[M + 1]^+$ *m/z* 663.

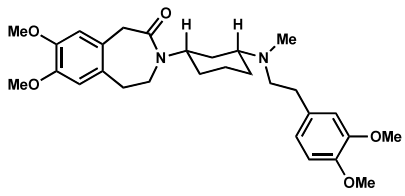


(±)-cis-3-(3-(3,4-Dimethoxyphenethylamino)cyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one; ***(±)-cis*****-11**



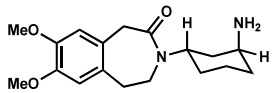
A solution of ***(±)-cis*****-10** (0.63 mmol; 0.20 g), anhydrous triethylamine (0.94 mmol; 0.13 mL) and 3,4-dimethoxyphenethyl bromide (0.94 mmol; 0.23 g) in dry DMF (2 mL) was heated at 60 °C overnight under anhydrous conditions. The reaction was monitored by TLC in dichloromethane-methanol (96:4). The mixture was cooled to room temperature and then the solvent was evaporated under vacuum to give a residue that was treated with 2N HCl (3 mL) and washed with diethyl ether (2 × 15 mL). The acidic aqueous layer was basified with a sodium carbonate saturated aqueous solution and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to afford a residue that was purified with gravity column chromatography. Eluting with dichloromethane-methanol-ammonia (90:10:0.5) gave ***(±)-cis*****-11** as a clear oil: 0.15 g; 0.31 mmol; 49%; ^{1}H NMR ($CDCl_3$) δ 1.05 (q, J=11.9 Hz, 1H), 1.32-1.46 (m, 3H), 1.72 (d, J=10.1 Hz, 1H), 1.78-2.05 (m, 4H), 2.65 (t, J=10.9 Hz, 1H), 2.77 (t, J=7 Hz, 2H), 2.92 (t, J=6.4 Hz, 2H), 3.00 (d, J=5.8 Hz, 2H), 3.67 (t, J=6.0 Hz, 2H), 3.82 (d, J=4.5 Hz, 2H), 3.83 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 4.50-4.56 (m, 1H), 6.53 (s, 1H), 6.60 (s, 1H), 6.74 (d, J=7.1 Hz, 2H), 6.81 (d, J=8.5 Hz, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 23.09, 30.07, 32.40, 33.96, 35.71, 37.54, 40.31, 43.01, 48.22, 50.79, 55.89, 55.94, 56.08, 56.66, 111.50, 112.05, 113.31, 114.13, 120.54, 123.43, 127.21, 132.15, 147.21, 147.60, 147.94, 149.04, 172.05.

(±)-cis-3-(3-((3,4-Dimethoxyphenethyl)(methyl)amino)cyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one; ***(±)-cis*****-3** (EC18)



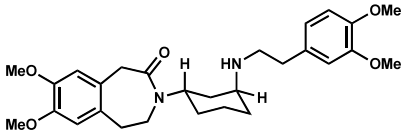
A solution of ***(±)-cis*-11** (0.31 mmol; 0.147 g), formic acid (5.12 mmol; 0.196 mL) and 37% aqueous formaldehyde (1.52 mmol; 0.115 mL) in absolute ethanol (2 mL) was heated at reflux for 15 hours. The reaction was monitored by TLC (eluent: dichloromethane-methanol, 85:15). The solvent was then removed under vacuum and the residue (yellow solid) treated with $NaHCO_3$ (saturated solution) and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and removed under vacuum to give a brown oil that was purified by gravity column chromatography. Eluting with dichloromethane-methanol-ammonia (95:5:0.5) afforded ***(±)-cis*-3** as a clear oil: 0.10 g; 0.20 mmol; 65.9%; ^{1}H NMR ($CDCl_3$) δ 1.17-1.44 (m, 4H), 1.72 (d, J=12 Hz, 1H), 1.85-1.92 (m, 3H), 2.38 (s, 3H), 2.62-2.75 (m, 5H), 3.01 (t, J=5.6 Hz, 2H), 3.69 (t, J=5.8 Hz, 2H), 3.82 (s, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 4.51 (t, J=12 Hz, 1H), 6.54 (s, 1H), 6.61 (s, 1H), 6.74-6.80 (m, 3H); ^{13}C NMR ($CDCl_3$, APT) δ 23.48, 28.39, 30.29, 32.24, 34.02, 37.82, 40.49, 43.05, 51.68, 55.89, 55.92, 55.95, 56.10, 61.44, 111.31, 112.14, 113.21, 114.05, 120.55, 123.41, 127.15, 132.98, 147.16, 147.38, 147.91, 148.86, 172.01.

(±)-trans-3-(3-Aminocyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one; ***(±)-trans*-10**



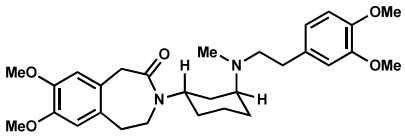
It was synthesized following the procedure described for *(±)-cis*-**10**, starting from a *cis/trans* mixture of **26** (0.80 g, 0.0021 mol). After the workup, the crude was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 90:10:0.5). ***(±)-trans*-10** was eluted first (clear yellowish oil; 50 mg) and then a mixture of the *cis/trans* amines. ^{1}H NMR ($CDCl_3$) δ 1.43-1.45 (m, 2H), 1.58-1.67 (m, 4H), 1.71-1.77 (m, 2H), 2.04 (bs, 2H), 2.96-2.99 (m, 2H), 3.41-3.42 (m, 1H), 3.63-3.66 (m, 2H), 3.78 (s, 2H), 3.80 (s, 3H), 3.81 (s, 3H), 4.79-4.82 (m, 1H), 6.50 (s, 1H), 6.58 (s, 1H). ^{13}C NMR ($CDCl_3$, APT) δ 19.40, 30.65, 31.61, 33.84, 37.41, 40.31, 42.98, 46.58, 47.12, 55.80, 55.92, 113.08, 113.95, 123.38, 127.06, 147.00, 147.76, 172.04.

(±)-trans-3-(3-(3,4-Dimethoxyphenethylamino)cyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one; ***(±)-trans*-11**



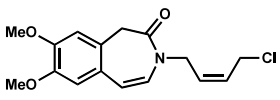
It was synthesized following the procedure described for *(±)-cis*-**11**, starting from *(±)-trans*-**10** (0.267 mmol; 85 mg), anhydrous triethylamine (0.267 mmol; 0.037 mL) and 3,4-dimethoxyphenethyl bromide (0.267 mmol; 65 mg) in dry acetonitrile. After workup, the crude was purified by flash chromatography (eluent: dichloromethane-methanol, 85:15) to give *(±)-trans*-**11** obtained as a clear oil: 25 mg; 19%; ^{1}H NMR ($CDCl_3$) δ 1.40-1.46 (m, 2H), 1.55-1.63 (m, 2H), 1.72-1.88 (m, 4H), 2.82-2.87 (m, 4H), 2.92-2.93 (m, 2H), 3.21-3.22 (m, 1H), 3.65-3.68 (m, 2H), 3.81-3.88 (m, 14H), 4.78-4.84 (m, 1H), 6.51 (s, 1H), 6.64 (s, 1H), 6.73-6.77 (m, 3H); ^{13}C NMR ($CDCl_3$, APT) δ 19.73, 28.75, 30.41, 33.86, 40.57, 43.05, 47.65, 48.59, 53.40, 55.86, 55.93, 111.26, 112.05, 113.13, 114.08, 120.52, 123.33, 127.03, 147.12, 147.42, 172.24.

(±)-trans-3-(3-((3,4-Dimethoxyphenethyl)(methyl)amino)cyclohexyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one; ***(±)-trans*-3**



It was synthesized following the procedure described for *(±)-cis*-**3**, starting from *(±)-trans*-**11** (0.052 mmol; 25 mg), formic acid (0.881 mmol; 0.033 mL) and 37% aqueous formaldehyde (0.259 mmol; 0.019 mL) in absolute ethanol (1 mL). After workup, the crude was purified by gravity column chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5) to give *(±)-trans*-**3** as a clear oil: 20 mg; 78%; ^{1}H NMR ($CDCl_3$) δ 1.38-2.00 (m, 8H), 2.35 (s, 3H), 2.62-2.63 (m, 1H), 2.70-2.71 (m, 4H), 2.99-3.02 (m, 2H), 3.66-3.69 (m, 2H), 3.81 (s, 2H), 3.84 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 4.82-4.88 (m, 1H), 6.54 (s, 1H), 6.60 (s, 1H), 6.70-6.78 (m, 3H).

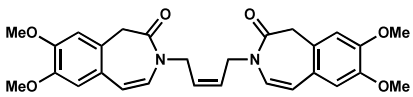
(Z)-3-(4-chlorobut-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***cis*-28** (EC30)



Potassium terbutoxyde (1.7 mmol; 0.2 g) was added to a suspension of **14** (1.1 mmol; 0.25 g) in dry DMSO (4 mL)

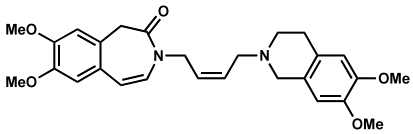
under nitrogen. The resulting solution was added dropwise to a solution of *cis*-1,4.dichlorobut-2-ene (2.3 mmol; 250 µL) in dry DMSO (4 mL), and the mixture was stirred for 1 hour at room temperature. Ice was then poured into the reaction mixture that was extracted with diethyl ether. The organic layers were collected, dried (Na_2SO_4) and removed under vacuum. The residue was purified by flash chromatography (eluent: cyclohexane-ethyle acetate, 5:5) to give ***cis*-28** as a yellow oil (30%) and the double addition's product **29** (6%).

28: ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 2H), 3.89 (s, 3H), 3.91 (s, 3H), 4.17 (d, J=7.9 Hz, 2H), 4.28 (d, J=7.1 Hz, 2H), 5.54 (dt, J=10.7 Hz, J=7.1 Hz, 1H), 5.81 (dt, J=10.6 Hz, J=7.9 Hz, 1H), 6.21 (d, J=9.1 Hz, 1H), 6.39 (d, J=9.1 Hz, 1H), 6.75 (s, 1H), 6.79 (s, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 38.64, 43.09, 43.90, 55.99, 109.54, 111.21, 117.64, 124.59, 126.26, 127.41, 128.86, 129.15, 148.05, 149.95, 167.65.



29: ^{1}H NMR ($CDCl_3$) δ 3.46 (s, 4H), 3.89 (s, 6H), 3.91 (s, 6H), 4.29 (d, J=4.4 Hz, 4H), 5.45 (t, J=4.4 Hz, 2H), 6.24 (d, J=9.2 Hz, 2H), 6.35 (d, J=9.2 Hz, 2H), 6.73 (s, 2H), 6.80 (s, 2H).

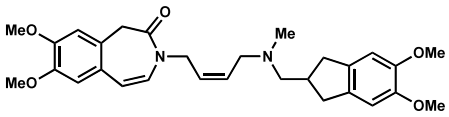
(Z)-3-(4-(6,7-Dimethoxy-3,4-dihydroisoquinolin-2(1H)-yl)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; **34**



In a two-necked flask under nitrogen, 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (commercially available; 0.65 mmol; 0.15 g) was added to a solution of *cis*-**28** (0.32 mmol; 0.1 g) in dry triethylamine (5 mL). The mixture was stirred for 5 hours at 60 °C. The solvent was removed under vacuum and the residue was taken up with water and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol, 9:1) to give **34** as a yellow oil: 80 mg; 53%; ^{1}H NMR ($CDCl_3$) δ 2.74-2.77 (m, 2H), 2.83-2.86 (m, 2H), 3.30 (d, J=6.8 Hz, 2H), 3.45 (s, 2H), 3.60 (s, 2H), 3.83-3.88 (m, 14H), 4.27 (d, J=6.8 Hz, 2H), 5.52-5.58 (m, 1H), 5.76-5.82 (m, 1H), 6.22 (d, J=9.2 Hz, 1H), 6.36 (d, J=9.2 Hz, 1H), 6.50 (s, 1H), 6.59 (s, 1H), 6.73 (s, 1H), 6.78 (s, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 28.26, 43.26, 44.75, 50.61, 54.45, 55.32, 56.00, 56.09, 109.56, 109.69,

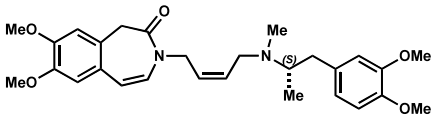
111.35, 111.47, 117.48, 124.74, 125.56, 125.73, 126.46, 127.77, 128.40, 129.63, 147.47, 147.85, 148.19, 150.08, 167.62.

(Z)-3-(4-(((5,6-Dimethoxy-2,3-dihydro-1H-inden-2-yl)methyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; **35**



In a two-necked flask under nitrogen, **30** (0.36 mmol; 80 mg) was added to a solution of *cis*-**28** (0.24 mmol; 74 mg) in chloroform (3 mL) and anhydrous triethylamine (3 mL). The mixture was stirred for 5 hours at 60 °C. The reaction was monitored by TLC (eluent: dichloromethane-methanol-ammonia, 99:1:0.5). The solvent was removed under vacuum and the residue was dissolved in water and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol, 9:1) to give **35** as a clear oil: 0.04 mmol; 18 mg; 15%; ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 2.39 (d, J=7.2 Hz, 2H), 2.60-2.73 (m, 3H), 3.00 (dd, J=7.2 Hz, J=14.4 Hz, 2H), 3.10-3.12 (m, 2H), 3.45 (s, 2H), 3.85 (s, 6H), 3.87 (s, 3H), 3.90 (s, 3H), 4.25 (d, J=6.8 Hz, 2H), 5.43-5.49 (m, 1H), 5.66-5.72 (m, 1H), 6.18 (d, J=9.2 Hz, 1H), 6.33 (d, J=8.8 Hz, 1H), 6.72-6.74 (m, 3H), 6.79 (s, 1H); ^{13}C NMR ($CDCl_3$, δ, APT): 37.57, 37.65, 42.47, 43.15, 44.41, 54.57, 55.94, 56.00, 62.84, 107.87, 109.42, 111.15, 117.21, 124.61, 126.32, 127.59, 134.37, 147.84, 147.98, 149.84, 167.47.

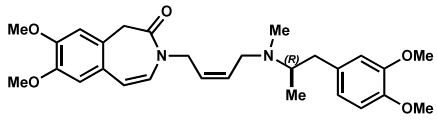
(S,Z)-3-(4-((1-(3,4-Dimethoxyphenyl)propan-2-yl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*****-36**



It was synthesized following the procedure described for ***(S)*****-62**, starting from ***(S)*****-31** (0.0005 mol; 0.10 g), *cis*-**28** (0.15 g; 0.0005 mol), dry triethylamine (0.07 mL; 0.0005 mol) and dry CH_3CN (5 mL). ***(S)*****-36** was obtained as a whitish solid: 0.13 g; 55%; mp 75 °C; $[\alpha]^{20}_D$ = +11.4° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.93 (d, J=6.8 Hz, 3H), 2.25 (s, 1H), 2.37 (dd, J=12.8 Hz, J=9.2 Hz, 1H), 2.84-2.95 (m, 2H), 3.17 (d, J=6.4 Hz, 2H), 3.45 (s, 2H), 3.85 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H),

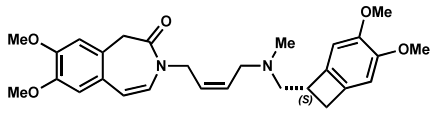
3.89 (s, 3H), 4.24 (d, J=14 Hz, 2H), 5.41-5.47 (m, 1H), 5.62-5.67 (m, 1H), 6.19 (d, J=9.0 Hz, 1H), 6.34 (d, J=9.0 Hz, 1H), 6.69-6.72 (m, 3H), 6.7-6.79 (m, 2H).

(R,Z)-3-(4-((1-(3,4-Dimethoxyphenyl)propan-2-yl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-36**



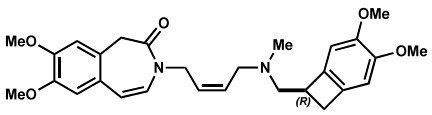
It was synthesized following the procedure described for ***(S)*****-62**, starting from ***(R)*****-31** (0.0005 mol; 0.10 g), *cis*-**28** (0.15 g; 0.0005 mol), dry triethylamine (0.07 mL; 0.0005 mol) and dry CH_3CN (7 mL). ***(R)*****-36** was obtained as a whitish solid: 52%; mp 70 °C; $[\alpha]^{20}_D$ = -11.4° (c=1; $CHCl_3$). The ^1H NMR spectrum was identical to that of ***(S)*****-35**. ^{13}C NMR ($CDCl_3$, APT) δ 13.82, 36.86, 39.23, 43.35, 44.64, 50.31, 56.02, 56.06, 56.13, 60.15, 109.78, 111.39, 111.45, 112.71, 117.33, 121.25, 124.87, 126.57, 126.97, 127.82, 132.07, 133.20, 147.46, 148.25, 148.94, 150.12, 167.65.

(S,Z)-3-(4-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl) amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*****-37**



It was synthesized following the procedure described for ***(S)*****-63**, starting from *cis*-**28** (0.10 g; 0.0005 mol), dry triethylamine (0.07 mL; 0.0005 mol) and ***(S)*****-32** (0.15 g; 0.0005 mol). ***(S)*****-37** was obtained as a pale yellow solid: 26%; mp 61-63 ^0C; $[\alpha]^{20}_D$ = +13.8° (c=1; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 2.31 (s, 3H), 2.58 (dd, J=12.8 Hz, J=8.8 Hz, 1H), 2.71-2.79 (m, 2H), 3.17 (d, J=6.4 Hz, 2H), 3.27 (dd, J=12.8 Hz, J=5.2 Hz, 1H), 3.43 (s, 2H), 3.55-3.62 (m, 1H), 3.83 (s, 6H), 3.86 (s, 3H), 3.87 (s, 3H), 4.18-4.29 (m, 2H), 5.42-5.52 (m, 1H), 5.65-5.75 (m, 1H), 6.17 (d, J=9.2 Hz, 1H), 6.32 (d, J=9.2 Hz, 1H), 6.67 (s, 1H), 6.70 (s, 1H), 6.71 (s, 1H), 6.76 (s, 1H). ^{13}C NMR ($CDCl_3$, APT) δ 35.25, 40.62, 42.49, 43.17, 44.53, 54.63, 55.98, 56.25, 56.33, 61.55, 106.78, 107.46, 109.52, 111.21, 117.32, 124.63, 126.35, 127.63, 129.21, 130.44, 134.96, 138.63, 148.05, 149.36, 149.92, 167.51.

(R,Z)-3-(4-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl) amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-37**



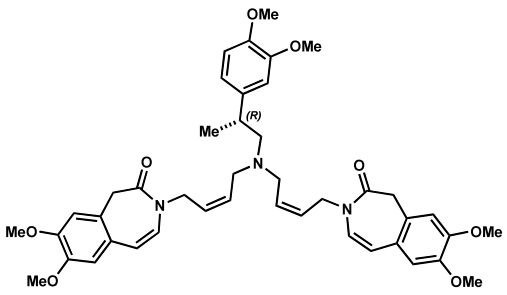
It was synthesized following the procedure described for ***(S)*****-63**, starting from *cis*-**28** (0.09 g; 0.0003 mol), dry triethylamine (0.04 mL; 0.0003 mol) and ***(R)*****-32** (0.06 g; 0.0003 mol). ***(R)*****-37** was obtained as a pale yellow solid: 31%; mp 63-66 ^{0}C; $[\alpha]^{20}_D$ = -11.3° (c=0.75; $CHCl_3$). The ^{1}H NMR spectrum was identical to that of ***(S)*****-37**.

(R,Z)-3-(4-((2-(3,4-Dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-38**

3,3'-(2Z,2'Z)-4,4'-((R)-2-(3,4-Dimethoxyphenyl)propylazanediyl)bis(but-2-ene-4,1-diyl)bis(7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one); ***(R)*****-39**

Dry triethylamine (0.11 mL; 0.001 mol) and ***(R)*****-33** (0.16 g; 0.01 mol) were added, under nitrogen, to a solution of *cis*-**28** (0.18 g; 0.001 mol) in dry CH_3CN (10 mL). The reaction mixture was stirred overnight at room temperature and monitored by TLC (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). Removal of the solvent gave a residue that was dissolved in dichloromethane and washed with 2M NaOH (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give a residue that was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). ***(R)*****-39** was eluted first (R_f=0.71; 0.095 mmol; 70 mg; 12%; yellow solid; mp 97-98 °C), followed by ***(R)*****-38** (R_f=0.42; yellow oil, 0.06 mmol; 30 mg; 8%).

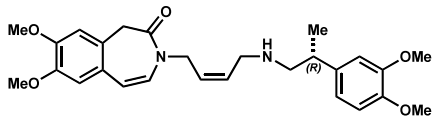
(R)**-39** (hydrochloride): $[\alpha]^{20}_D$ = -22.25° (c=0.5; CH_3OH); ^{1}H NMR ($CDCl_3$) δ 1.21 (d,



J=6.8 Hz, 3H), 2.43-2.48 (m, 1H), 2.54-2.59 (m, 1H), 2.83.2.89 (m, 1H), 3.11-3.15 (m, 4H), 3.44 (s, 4H), 3.83 (s, 3H), 3.84 (s, 3H), 3.86 (s, 6H), 3.88 (s, 6H), 4.12-4.24 (m, 4H), 5.39-5.45 (m, 2H), 5.56-5.62 (m, 2H), 6.13 (d, J=9.2 Hz, 2H), 6.31 (d, J=9.2 Hz, 2H), 6.70-6.72 (m, 4H), 6.77-6.79 (m, 3H); ^{13}C NMR ($CDCl_3$, APT) δ 20.38, 37.87, 43.14, 44.53, 51.00, 55.80, 55.82, 55.92, 61.70, 109.50, 110.58, 111.09, 111.17, 117.10, 119.00,

124.61, 126.34, 127.27, 127.63, 131.01, 138.71, 147.26, 147.99, 148.71, 149.84, 167.41.

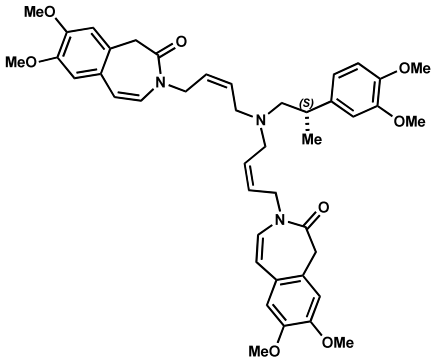
***(R)*-38** (free base): $[\alpha]^{20}_D$ = -1.70° (c=1; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.25 (d, J=6.8 Hz,



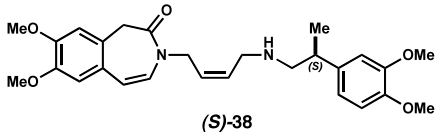
3H), 2.73-2.75 (m, 2H), 2.86-2.89 (m, 1H), 3.29 (d, J=6.8 Hz, 2H), 3.43 (s, 2H), 3.86 (s, 3H), 3.88 (s, 6H), 3.89 (s, 3H), 4.18 (d. J=6.8 Hz, 2H), 5.36-5.39 (m, 1H), 5.58-5.61 (m, 1H), 6.13 (d, J=9.2 Hz, 1H), 6.31 (d, J=9.2 Hz, 1H), 6.72-6.76 (m, 4H), 6.78-6.82 (m, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 20.29, 39.64, 43.15, 44.39, 46.11, 55.84, 55.89, 55.96, 56.73, 109.48, 110.44, 111.20, 111.36, 117.22, 118.89, 124.63, 126.26, 126.32, 127.54, 131.92, 137.72, 147.52, 148.01, 148.98, 149.89, 167.46.

(S,Z)-3-(4-((2-(3,4-Dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*-38**

3,3'-(2Z,2'Z)-4,4'-((S)-2-(3,4-dimethoxyphenyl)propylazanediyl)bis(but-2-ene-4,1-diyl)bis(7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one); ***(S)*-39**



***(S)*-39**



***(S)*-38**

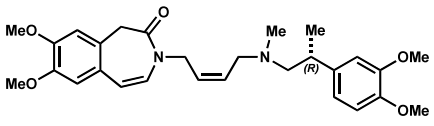
They were synthesized following the procedure described for ***(S)*-39** and ***(S)*-38**, starting from ***(S)*-33**: 0.61 mmol; 0.12 g.

***(S)*-38:** 0.13 mmol; 60 mg; yellow oil; 21%); $[\alpha]^{20}_D$ = +1.58° (c=1; $CHCl_3$.

***(S)*-39:** 0.122 mmol; 90 mg; 20%; yellow solid; mp 98-102 °C; ***(S)*-39** (hydrochloride): $[\alpha]^{20}_D$ = +25.13° (c=0.5; CH_3OH).

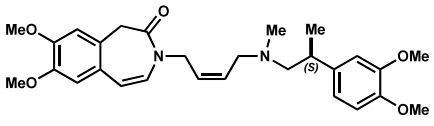
Their NMR spectra were identical to those of their enantiomers.

(R,Z)-3-(4-((2-(3,4-Dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-40**



A solution of ***(R)*****-38** (0.06 mmol; 30 mg), formic acid (17 eq), 37% aqueous formaldehyde (5 eq) in absolute ethanol (4 mL) was heated at reflux for 4 hours. The reaction was monitored by TLC in dichloromethane-methanol (96:4). The solvent was then removed by rotary evaporation and the residue was treated with $NaHCO_3$ (saturated solution) and extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and the solvent was removed under vacuum to give a residue that was purified by flash chromatography: Eluting with dichloromethane-methanol (96:4) afforded ***(R)*****-40** as a clear oil: 0.06 mmol; 28.5 mg; 92%; $[\alpha]^{20}_D$ = -8.84° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24 (d, J=7.2 Hz, 3H), 2.22 (s, 3H), 2.40-2.53 (m, 2H), 2.86-2.91 (m, 1H), 3.02-3.12 (m, 2H), 3.44 (s, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 4.15-4.26 (m, 2H), 5.30-5.47 (m, 1H), 5.60-5.66 (m, 1H), 6.15 (d, J=9.2 Hz, 1H), 6.31 (d, J=9.2 Hz, 1H), 6.72-6.80 (m, 5H); ^{13}C NMR ($CDCl_3$, APT) δ 20.41, 37.66, 42.64, 43.16, 44.48, 54.64, 55.81, 55.84, 55.95, 65.07, 109.49, 110.49, 111.19, 117.08, 118.88, 124.65, 126.37, 127.18, 127.66, 131.03, 138.79, 147.29, 148.00, 148.79, 149.86, 167.46.

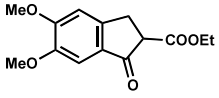
(S,Z)-3-(4-((2-(3,4-Dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*****-40**



It was synthesized following the procedure described for ***(R)*****-40**, starting from ***(S)*****-38** (0.13 mmol; 60 mg). ***(S)*****-40** was obtained as a clear oil: 21 mg; 0.04 mmol; 34%. The ^{1}H NMR spectrum was identical to that of ***(R)*****-40**.

(S)**-40** (free base): $[\alpha]^{20}_D$ = +8.00° (c=1; $CHCl_3$).

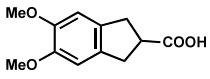
Ethyl 5,6-dimethoxy-1-oxo-2,3-dihydro-1H-indene-2-carboxylate; **41**



Diethyl carbonate (0.0052 mol; 0.63 mL) was added under anhydrous condition to a suspension of NaH (60% in hexane; 0.0104 mol; 0.42 g) in dry THF (15 mL). The mixture was stirred and heated at reflux under nitrogen. After 1 hour, 5,6-dimethoxy indan-1-one (0.0026 mol; 0.5 g) was added to the milky suspension, which turned into an orange-brown color. The mixture was heated at reflux for 3 hours and monitored by TLC (eluent: ethyl

acetate-cyclohexane, 1:1). The residue was diluted with water, neutralized with acetic acid and extracted with ethyl acetate (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give **41** as an orange oil: 0.0025 mol; 0.65 g; 95%; ^{1}H NMR ($CDCl_3$) δ 1.31 (t, J=6.8 Hz, 3H; CH_3), 3.27 (dd; J=8.0 Hz, J=17.2 Hz, 1H; H_B), 3.45 (dd, J=3.6 Hz, J=17.2 Hz, 1H; H_A), 3.71 (dd, J=3.6 Hz, J=8.0 Hz, 1H; H_C), 3.91 (s, 3H; OCH_3), 3.98 (s, 3H; OCH_3), 4.25 (q, J=7.2 Hz, 2H; OCH_2), 6.91 (s, 1H; H aromatic), 7.18 (s, 1H; H aromatic).

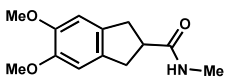
5,6-Dimethoxy-2,3-dihydro-1H-indene-2-carboxylic acid; **42**



Water (2 mL), hydrochloric acid (3.75 mL), Zn (1.125 g) and $HgCl_2$ (0.1125 g) were added to a suspension of **41** (1.89 mmol; 0.5 g) in toluene (10 mL). The resulting mixture was heated at reflux (112 °C) for 4 hours and stirred overnight at room temperature. The reaction was monitored by TLC (eluent: ethyl acetate-cyclohexane, 1:1). The two layers were separated and the organic phase was extracted with 10% NaOH (3 × 15 mL). The separator funnel was shaken vigorously for allowing the complete hydrolysis. The basic aqueous layer was acidified with 2N HCl and extracted with ethyl acetate (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and removed under vacuum to give **42**: 0.23 g; 1.04 mmol; 55%.

Ethyl 5,6-dimethoxy-indan-2-carboxylate was recovered as secondary product from the toluene's layer after evaporation of the solvent and converted into **42** after hydrolysis with absolute ethanol (10 mL) and 1N NaOH (10 mL). ^{1}H NMR ($CDCl_3$) δ 3.16-3.28 (m, 4H), 3.37-3.42 (m, 1H), 3.86 (s, 6H), 6.76 (s, 2H).

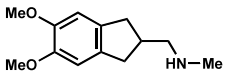
5,6-Dimethoxy-N-methyl-2,3-dihydro-1H-indene-2-carboxamide; **43**



In a round bottom flask, carbonyl diimidazole (2.39 mmol; 0.39 g) was slowly added to a solution of **42** (2.39 mmol; 0.53 g) in dichloromethane (10 mL). The mixture was stirred for 2 hours at room temperature. The reaction was monitored by TLC (eluent: ethyl acetate-cyclohexane, 1:1) and, once the starting material was disappeared, methylamine (2M solution in THF; 23.9 mmol; 11.9 mL) was added. The resulting mixture was stirred for additional 2 hours at room temperature, then it was poured into a separator funnel and

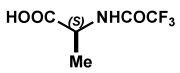
the organic layer was washed with 10% NaOH (3 × 15 mL). The organic phase was then dried (Na_2SO_4) and evaporated under vacuum to give **43** that was crystallized from absolute ethanol: 1.28 mmol; 0.30 g; 53.5%; ^{1}H NMR ($CDCl_3$) δ 2.83 (d, J=4.8 Hz, 3H), 3.05-3.19 (m, 5H), 3.84 (s, 6H), 5.73 (bs, 1H), 6.73 (s, 2H).

1-(5,6-dimethoxy-2,3-dihydro-1H-inden-2-yl)-N-methylmethanamine; **30**



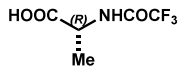
Under anhydrous condition, a solution of **43** (1.70 mmol; 0.40 g) in anhydrous THF (10 mL) was added to a suspension of $LiAlH_4$ (1.70 mmol; 0.065 g) in anhydrous THF (2 mL). The mixture was heated at reflux for 1 day under nitrogen. The reaction was monitored by TLC (ethyl acetate-cyclohexane, 1:1) until the starting material disappeared, then water (5 mL) and 10% NaOH (1 mL) were added to the mixture. The white precipitate was filtered off and the solvent was removed under vacuum to give a residue that was dissolved in ethyl acetate and washed with water (3 × 15 mL). The organic layer was dried (Na_2SO_4) and the solvent evaporated under vacuum yielding a residue that was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5) to give **30**: 1.45 mmol; 0.32 g; 85%; ^{1}H NMR ($CDCl_3$) δ 1.59 (bs, 1H), 2.47 (s, 3H), 2.59-2.60 (m, 1H), 2.64-2.68 (m, 4H), 3.00-3.03 (m, 2H), 3.84 (s, 6H), 6.73 (s, 2H).

(S)-2-(2,2,2-Trifluoroacetamido)propanoic acid; ***(S)*-44**



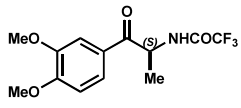
Under anhydrous condition, dry triethylamine (0.056 mol; 7.82 mL) and ethyltrifluoroacetate (0.066 mol; 8.35 mL) were added to *L*-Alanine (0.056 mol; 5.00 g) in dry methanol (30 mL). The suspension was stirred for 26 hours at room temperature until it became a clear solution. The solvent was removed under vacuum and the residue was dissolved in water, which was acidified to pH 3 with 2N HCl. The acidic aqueous layer was extracted three times with ethyl acetate. The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to afford ***(S)*-44** as a white solid: 9.42 g; 91%; mp 54 °C. The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = +11.5° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.55 (d, J=7.2Hz, 3H), 4.60-4.67 (m, 1H), 7.22 (bs, 1H), 9.19 (bs, 1H).

(R)-2-(2,2,2-Trifluoroacetamido)propanoic acid; ***(R)*-44**



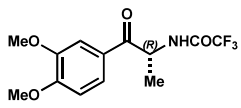
It was obtained following the procedure described for ***(S)*-44**, starting from *D*-Alanine (0.056 mol; 5.00 g), dry triethylamine (0.056 mol; 7.82 mL) and ethyltrifluoroacetate (0.066 mol; 8.35 mL), as a white solid (9.22 g; 89%; mp 55 °C). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -12.9° (c=1; $CHCl_3$). The ^{1}H NMR spectrum was identical to that of ***(S)*-44**.

(S)-N-(1-(3,4-Dimethoxyphenyl)-1-oxopropan-2-yl)-2,2,2-trifluoroacetamide; ***(S)*-46**



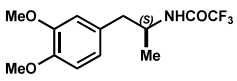
Under anhydrous condition, dry pyridine (2 drops) and oxalyl chloride (0.0172 mol; 1.46 mL) were added to a solution (0 °C) of ***(S)*-44** (0.0015 mol; 1.50 g) in dry dichloromethane (30 mL). The mixture was stirred for 1 day at room temperature, then the solvent was removed to afford crude ***(S)*-45** that was dissolved in dry dichloromethane (15 mL) under nitrogen. The flask was placed into an ice-bath and $AlCl_3$ (0.0089 mol; 1.19 g) and veratrol (0.0089 mol; 1.13 mL) were added to the reaction mixture, which was stirred for 48 hours at room temperature and then poured into a separator funnel where the organic layer was washed with 1N HCl, aqueous $NaHCO_3$ (saturated solution) and brine. The organic phase was then dried (Na_2SO_4) and evaporated under vacuum to give a residue, which was purified by flash chromatography (eluent: cyclohexane-ethyl acetate, 7:3) yielding ***(S)*-46** as a white solid: 0.686 g; 35%; mp 87 °C. The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -8.8° (c=1; $CHCl_3$). ^{1}H NMR ($CDCl_3$) δ 1.53 (d, J=6.8 Hz, 3H), 3.96 (s, 3H), 3.98 (s, 3H), 5.46-5.53 (m, 1H), 6.92 (d, J=8.6 Hz, 1H), 7.51 (d, J=1.4 Hz, 1H), 7.61 (dd, J=8.6 Hz, J=1.4 Hz, 1H).

(R)-N-(1-(3,4-Dimethoxyphenyl)-1-oxopropan-2-yl)-2,2,2-trifluoroacetamide; ***(R)*-46**



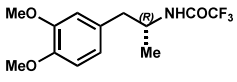
It was obtained following the procedure described for ***(S)*-46**, starting from ***(R)*-44** (0.052 mol; 9.71 g) in dichloromethane (105 ml) and utilizing $AlCl_3$ (0.058 mol; 7.7 g) and veratrol (0.058 mol; 7.36 ml). ***(R)*-46** was obtained as a white solid (29%; mp 85 °C). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = +8.8° (c=1; $CHCl_3$).The ^{1}H NMR spectrum was identical to that of ***(S)*-46**.

(S)-N-(1-(3,4-Dimethoxyphenyl)propan-2-yl)-2,2,2-trifluoroacetamide; ***(S)*****-47**



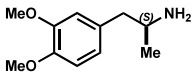
Under a nitrogen atmosphere, Et_3SiH (1.23 ml; 0.0077 mol) was added to a red solution of ***(S)*****-46** (0.0026 mol; 0.69 g) in CF_3COOH (0.010 mol; 4 mL). The resulting discolored mixture was stirred first for 2 hours at 72 °C and then for 15 hours at room temperature. The solution was basified with $NaHCO_3$ and extracted with ethyl acetate. The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give ***(S)*****-47**, which was used in the next step without further purification.

(R)-N-(1-(3,4-Dimethoxyphenyl)propan-2-yl)-2,2,2-trifluoroacetamide; ***(R)*****-47**



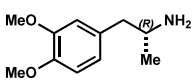
It was synthesized following the procedure described for ***(S)*****-47**, starting from ***(R)*****-46** (0.015 mol; 4.58 g), CF_3COOH (0.058 mol; 26.70 mL) and Et_3SiH (8.18 ml; 0.058 moli). Crude ***(R)*****-47** was used in the next step without further purification.

(S)-1-(3,4-Dimethoxyphenyl)propan-2-amine; ***(S)*****-48**



K_2CO_3 (2.50 g) was added to a solution of ***(S)*****-47** (0.0093 mol; 1.81 g) in methanol (49.5 mL) and water (2.5 mL). The mixture was stirred for 24 hours at 64 °C. The pricipitate was filtered off and the filtrate was diluted with water and extracted with dichloromethane (3 × 25 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated to afford a residue that was purified by flash chromatography (dichloromethane-methanol, 95:5) yielding ***(S)*****-48** as a yellow oil (0.0032 mol; 0.62 g; 34%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = +24.6° (c=1; $CHCl_3$); e.e.=91% (determined by chiral HPLC); ^1H NMR ($CDCl_3$) δ 1.14 (d, J=6.4 Hz, 3H), 2.31 (bs, 2H), 2.51 (dd, J=13.4 Hz, J=8.0 Hz, 1H), 2.67 (dd, J=13.4 Hz, J=5.6 Hz, 1H), 3.15-3.20 (m, 1H), 3.82 (s, 3H), 3.86 (s, 3H), 6.71-6.73 (m, 2H), 6.80 (d, J=8.0 Hz, 1H).

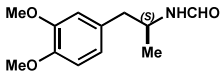
(R)-1-(3,4-Dimethoxyphenyl)propan-2-amine; ***(R)*****-48**



It was synthesized following the procedure described for ***(S)*****-48**, starting from ***(R)*****-47** (0.015 mol; 4.37 g), K_2CO_3 (0.0457 mol; 6.30 g), methanol (125 ml) and water (6 ml). ***(R)*****-48** was obtained as a yellow oil (20%). The

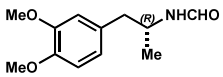
free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -29° (c=1; $CHCl_3$); e.e.=98.6% (determined by chiral HPLC). The ^{1}H NMR spectrum was identical to that of ***(S)*-48**.

(S)-N-(1-(3,4-Dimethoxyphenyl)propan-2-yl)formamide; ***(S)*-49**



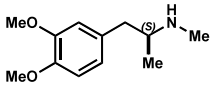
Under a nitrogen atmosphere, a solution of ***(S)*-48** (0.0029 mol; 0.59 g) in HCOOEt (0.036 mol; 2.88 mL) was stirred for 96 hours at 45 °C to give, after removal of volatiles, ***(S)*-49** that was used in the next step without any purification.

(R)-N-(1-(3,4-Dimethoxyphenyl)propan-2-yl)formamide; ***(R)*-49**



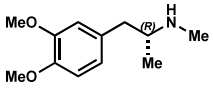
Under a nitrogen atmosphere, a solution of ***(R)*-48** (0.0031 mol; 0.60 g) in HCOOEt (0.018 mol; 1.49 mL) was stirred for 96 hours at 45 °C to give, after removal of volatiles, ***(S)*-49** that was used in the next step without any purification.

(S)-1-(3,4-Dimethoxyphenyl)-N-methylpropan-2-amine; ***(S)*-31**



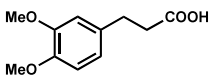
Under anhydrous condition and nitrogen, a solution of the above ***(S)*-49** in dry THF (12.5 mL) was slowly added to a suspension of $LiAlH_4$ (0.0036 mol; 0.14 g) in dry THF (25 mL) at 0 °C. The mixture was stirred at reflux for 15 hours and then the flask was placed into an ice-bath and water and 10% NaOH (1.5 mL) were slowly added to the mixture. The precipitate was filtered off and the filtrate was evaporated yielding a residue that was dissolved in ethyl acetate and washed with water. The organic layer was collected, dried (Na_2SO_4) and evaporated to afford a crude that was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5) giving ***(S)*-31** as a yellow oil (0.0006 mol; 0.13 g; 21%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = +6.6° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.07 (d, J=6.4 Hz, 3H), 1.53 (bs, 1H), 2.40 (s, 3H), 2.59 (dd, J=13.6 Hz, J=6.4 Hz, 1H), 2.64 (dd, J=13.6 Hz, J=7.0 Hz, 1H), 2.72-2.80 (m, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 6.7-6.74 (m, 1H), 6.8 (d, J=8.0 Hz, 1H).

(R)-1-(3,4-Dimethoxyphenyl)-N-methylpropan-2-amine; ***(R)*-31**



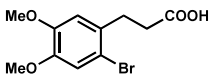
It was obtained following the procedure described for ***(S)*-31**, starting from ***(R)*-49** (0.0009 mol; 0.20 g), $LiAlH_4$ (0.0012 mol; 46 mg) and dry THF (30 ml). ***(R)*-31** was obtained as a yellow oil (45%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -8.8° (c=1; $CHCl_3$). The ^1H NMR spectrum was identical to that of ***(S)*-31**.

3-(3,4-Dimethoxyphenyl)propanoic acid; **50**



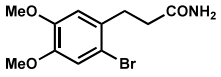
Palladium on charcoal (10% p/p) was added to a solution of 3,4-dimethoxy cinnamic acid (0.024 mol; 5 g.) in THF (50 mL). The mixture was placed in a Parr apparatus at 60 Psi and allowed to react at room temperature for 2 days. The catalyst was filtered off and the solvent was removed under vacuum, giving **50** as a white solid: 0.023 mol; 98%; mp 96-98 °C; ^1H NMR ($CDCl_3$) δ 2.68 (t, J=7.6 Hz, 2H), 2.92 (t, J=7.2 Hz, 2H), 3.87 (s, 3H), 3.88 (s, 3H), 6.75-6.77 (m, 2H), 6.80-6.82 (m, 1H).

3-(2-Bromo-4,5-dimethoxyphenyl)propanoic acid; **51**



Bromine (0.023 mol, 1.22 mL) was slowly added to a solution of **50** (0.023 mol; 4.98 g) in glacial acetic acid (25 mL) at 0 °C (ice-water bath). The mixture was stirred for 3 hours at room temperature and monitored by TLC (eluent: hexane-ethyl acetate, 1:1). The mixture was poured into ice with formation of a precipitate, which was filtered off, washed with water and dried under vacuum to give **51**: 5.94 g; 0.020 mol; 87%; mp 126-128 °C; ^1H NMR ($CDCl_3$) δ 2.69 (t, J=7.8 Hz, 2H), 3.01 (t, J=7.6 Hz, 2H), 3.86 (s, 6H), 6.79 (s, 1H), 7.01 (s, 1H).

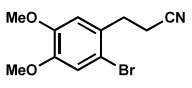
3-(2-Bromo-4,5-dimethoxyphenyl)propanamide; **52**



In a two-necked flask under nitrogen atmosphere, thionyl chloride (0.041 mol; 2.99 mL) was added dropwise to a solution of **51** (0.020 mol; 5.94 g) in chloroform (50 mL). The mixture was heated at 61 °C for 3 hours monitoring the progress of the reaction by TLC (eluent: hexane-ethyl acetate, 1:1). The solvent was removed under vacuum and the residue was washed with cyclohexane (2 × 30 mL, removed under vacuum) and dissolved in ammonia (33% in water). After

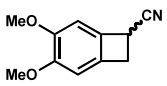
stirring overnight at room temperature, the solvent was removed under vacuum. The residue was dissolved in dichloromethane and washed twice with aqueous Na_2CO_3 solution. The organic layers were collected, dried (Na_2SO_4) and removed under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5) yielding **52** as a pale yellow gummy solid: 0.017 mol; 5 g; 84%; ^{1}H NMR ($CDCl_3$) δ 2.47 (t, J=8 Hz, 2H), 2.96 (t, J=7.2 Hz, 2H), 3.80 (s, 6H), 5.69 (bs, 1H), 6.07 (bs, 1H), 6.76 (s, 1H), 6.95 (s, 1H). ^{13}C NMR ($CDCl_3$, APT) δ 31.48, 35.88, 55.91, 56.00, 113.14, 113.66, 115.37, 131.76, 147.97, 148.25, 174.56. IR (Nujol, cm^{-1}) ν 1662 ($CONH_2$).

3-(2-Bromo-4,5-dimethoxyphenyl)propanenitrile; **53**



In a two-necked flask under nitrogen atmosphere, phosphoryl chloride (0.035 mol; 3.18 mL) was added dropwise to a solution of **52** (0.017 mol; 5 g) in chloroform (50 mL). The mixture was heated at 61 °C for 5 hours monitoring the reaction by TLC (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). The mixture was poured into ice and then extracted with chloroform (3 × 25 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane) yielding **53** as a white solid: 0.016 mol; 4.41 g; 96%; mp 77-80 °C; ^{1}H NMR ($CDCl_3$) δ 2.65 (t, J=7.2 Hz, 2H), 3.01 (t, J=7.2 Hz, 2H), 3.86 (s, 3H), 3.88 (s, 3H), 6.81 (s, 1H), 7.02 (s, 1H); IR (Nujol, cm^{-1}) ν 2245 (CN).

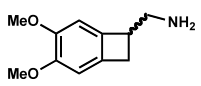
4,5-Dimethoxy-1,2-dihydrocyclobutabenzene-1-carbonitrile; ***(R)/(S)*-54**



A 100 mL three-necked flask was thoroughly dried and fitted with a large condenser, a nitrogen inlet and a thermometer. The flask was dipped into a liquid nitrogen-ethanol bath (-33 °C) and ammonia was allowed to condense (50 mL). Then commercial sodium amide (0.06 mol; 2.34 g) was rapidly added; the temperature went down so the suspension was stirred until the temperature reached –33 °C. Then **53** (0.015 mol; 4.05 g) was rapidly added to the suspension. The dark green reaction mixture was stirred for 1 hour and monitored by TLC (eluent: cyclohexane-ethyl acetate, 1:1). The mixture was then treated with solid ammonium chloride (0.06 mol; 3.21 g) allowing ammonia to evaporate. The residue was taken up

in dichloromethane (50 mL) and washed twice with 2N HCl (2 × 25 mL) and once with aqueous saturated solution of ammonium chloride (1 × 25 mL). The organic layer was collected, dried (Na_2SO_4) and evaporated under vacuum. The yellow-brown oil was purified by flash chromatography (eluent: cyclohexane-ethyl acetate, 1:1) to give ***(R)/(S)*-54** as a pale yellow solid: 0.0106 mol; 2 g; 71%; mp 85-89 °C; ^{1}H NMR ($CDCl_3$) δ 3.44 (dd, J=13.6 Hz, J=2.2 Hz, 1H), 3.57 (dd, J=13.6 Hz, J=5.2 Hz, 1H), 3.84 (s, 6H), 4.15 (dd, J=5.2 Hz, J=2.2 Hz, 1H), 6.69 (s, 1H), 6.77 (s, 1H). ^{13}C NMR ($CDCl_3$, APT) δ 28.00, 35.47, 56.16, 56.20, 106.17, 106.99, 119.84, 129.72, 134.18, 150.45, 151.39.

(4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methanamine; ***(R)/(S)*-55**



***(R)/(S)*-54** (0.0095 mol; 1.8 g) was dissolved in dry THF (50 mL) under nitrogen flow in a flame-dried flask. Then BH_3·THF (1M; 0.019 mol; 19 mL) was added at room temperature and the mixture was stirred for 6 hours at reflux. Once the mixture was cooled to room temperature, 9.5 mL of 1N methanolic HCl was carefully added, and the mixture was heated at 40 °C for 30 minutes to hydrolyze the intermediate boramine complex. The solvent was removed under vacuum and the residue was taken up in 1N NaOH (25 mL) and extracted with dichloromethane (3 × 25 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue (yellow oil) was purified with flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:10:1) to give ***(R)/(S)*-55** as a pale yellow oil: 0.0078 mol; 1.50 g; 82%; ^{1}H NMR ($CDCl_3$) δ 1.67 (bs, 2H), 2.73 (d, J=13.6 Hz, 1H), 2.98 (d, J=6.8 Hz, 2H), 3.20 (dd, J=13.6 Hz, J= 5.2 Hz, 1H), 3.43-3.51 (m, 1H), 3.84 (s, 6H), 6.70 (s, 1H), 6.73 (s, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 33.00, 45.27, 45.90, 56.19, 56.25, 106.57, 107.58, 135.02, 138.33, 149.32, 149.84.

Resolution of ***(R)/(S)*-55** *by fractional crystallization*

N-acetyl-L-glutamic acid dissolved in absolute ethanol (10 mL) was added to a solution of ***(R)/(S)*-55** in absolute ethanol (10 mL). The mixture was kept at room temperature until there was the formation of a precipitate. The solid was filtered off, dried under vacuum and then it was crystallized more times to reach constant values for melting point and [α], following the procedure shown in **scheme A**.

Number of crystallization	Volume of Ethanol used	Precipitate mass	$[\alpha]^{20}_D$ (c=1; MeOH)	Melting point (^{0}C)
1	20 mL	2.25 g	$+12.1^0$	100-105
2	20 mL	1.31 g	$+16.8^0$	130-133
3	15 mL	1.04 g	$+18.5^0$	136-137
4	10 mL	0.92 g	$+19.0^0$	136-137

Scheme A.

The solid product obtained from the last crystallization was treated with 10% NaOH and extracted with dichloromethane. Removal of the solvent afforded ***(S)*-55** as a clear oil (0.44 g): $[\alpha]^{20}_D$ = -11.7° (c=0.75; $CHCl_3$).

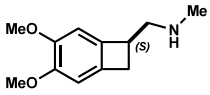
The solvents of previous crystallizations were collected and evaporated. The residue was treated with 10% NaOH and extracted with dichloromethane. Removal of the solvent afforded an unbalanced racemic mixture of ***(R)/(S)*-55**, which was dissolved in absolute ethanol (5 mL) and poured into a solution of *N*-acetyl-D-glutamic acid in absolute ethanol (5 mL). The mixture was kept at room temperature until there was the formation of a precipitate. The solid was filtered off and dried under vacuum and then it was crystallized more times to reach constant values for melting point and [α],following the procedure shown in **scheme B**.

Number of crystallization	Volume of Ethanol used	Precipitate mass	$[\alpha]^{20}_D$ (c=1; MeOH)	Melting point (^{0}C)
1	10 mL	1.70 g	-10.1^0	110-130
2	10 mL	1.00 g	-13.0^0	120-130
3	5 mL	0.819 g	-14.8^0	120-130
4	5 mL	0.702 g	-16.2^0	122-134
5	4 mL	0.600 g	-18.1^0	131-133
6	4 mL	0.515 g	-19.2^0	135-136

Scheme B.

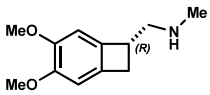
The solid product obtained from the last crystallization was treated with 10% NaOH and extracted with dichloromethane. Removal of the solvent afforded ***(R)*-55** as a clear oil: 0.25 g; $[\alpha]^{20}_D$ = +11.9° (c=0.75; $CHCl_3$).

(S)-1-(4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)-N-methylmethanamine; ***(S)*-32**



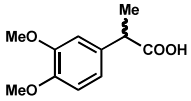
Under anhydrous condition, ethyl formiate (0.010 mol; 0.81 mL) was slowly added to ***(S)*-55** (0.002 mol; 0.32 g) and the mixture was stirred for 24 hours at room temperature. After removal of the solvent, the residue was dissolved in dry THF (4 mL) and poured into a suspension of $LiAlH_4$ (0.003 mol; 0.10 g) in dry THF under nitrogen atmosphere. The reaction mixture was stirred and heated at reflux for 4 hours. Then ice was added and the formed precipitate was filtered off and washed with THF. The filtrate was evaporated and the residue was taken up in ethyl acetate and washed with water. The organic layers were collected, dried (Na_2SO_4) and evaporated to give a residue that was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5) affording ***(S)*-32** as a red oil: 55%; $[\alpha]^{20}_D$ = -8.7° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.80 (bs, 1H), 2.48 (s, 3H), 2.75 (dd, J=13.6 Hz, J=1.8 Hz, 1H), 2.83 (dd, J=11.8 Hz, J=7.6 Hz, 1H), 2.90 (dd, J=11.8 Hz, J=6.8 Hz, 1H), 3.22 (dd, J=13.6 Hz, J=4.8 Hz, 1H), 3.53-3.59 (m, 1H), 3.83 (s, 3H), 3.84 (s, 3H), 6.67 (s, 1H), 6.70 (s, 1H).

(R)-1-(4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)-N-methylmethanamine; ***(R)*-32**



It was obtained following the procedure described for ***(S)*-32**, starting from ***(R)*-55** (0.001 mol; 0.25 g), ethyl formiate (0.008 mol; 0.63 ml) and $LiAlH_4$ (0.002 mol; 0.08 g). ***(R)*-32** was obtained as a red oil: 92%; $[\alpha]^{20}_D$ = +8.0° (c=1; $CHCl_3$). The ^{1}H NMR spectrum was identical to that of ***(S)*-32**.

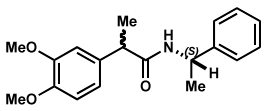
2-(3,4-Dimethoxyphenyl)propanoic acid; **57**



In a two-necked flask, butyl lithium (1.6M in hexane; 0.0204 mol; 12.74 mL) was added under nitrogen to a cooled (-50 °C, ethanol-liquid nitrogen bath) solution of diisopropylamine (0.0204 mol; 2.86 mL) in dry THF (90 mL). The mixture was then allowed to warm to -20 °C and stirred

for 20 minutes. A solution of 3,4-dimethoxyphenylacetic acid (0.0102 mol; 2 g) in anhydrous THF (10 mL) was then added and the resulting mixture was stirred at 25 °C. After 1 hour, methyl iodide (0.0102 mol; 0.63 mL) was added to the reaction mixture that was stirred overnight at room temperature and then diluted with water (100 mL). THF was removed under vacuum and the aqueous solution was washed with diethyl ether (3 × 50 mL) and acidified to pH=1 with 6N HCl and extracted with diethyl ether (3 × 50 mL). The organic layers were collected, dried (Na_2SO_4) and the solvent was removed under vacuum to give a residue that was crystallized from ethyl acetate/hexane yielding **57**: 1.40 g; 70%; mp 62-64 °C; ^{1}H NMR ($CDCl_3$) δ 1.51 (d, J=27.2 Hz, 3H), 3.70 (q, J=7.2 Hz, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 6.81-6.87 (m, 3H).

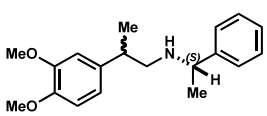
(S)-2-(3,4-Dimethoxyphenyl)-N-(1-phenylethyl)propanamide; **58**



Thionyl chloride (11.9 mmol; 0.86 mL) was added dropwise to a solution of **57** (4.76 mmol; 1 g) in chloroform (50 mL). The mixture was stirred and heated at reflux for 2 hours. The solvent was removed under vacuum to give a residue that was dissolved in benzene (10 mL) and added to a solution of *(S)*-α-methylbenzylamine (6.18 mmol; 0.80 mL) in benzene (10 mL) and pyridine (1 mL). The reaction mixture was stirred for 15 minutes at room temperature and then washed with water (25 mL), 1N HCl (25 mL) and 5% Na_2CO_3 (25 mL). The organic layers were collected, dried (Na_2SO_4) and the solvent was removed under vacuum.

The residue was purified by flash chromatography (eluent: hexane-ethyl acetate, 1:1), giving **58** as a yellow solid: 83%; 3.96 mmol; 1.24 g; mp 101-103 °C; ^{1}H NMR ($CDCl_3$) δ 1.36-1.41 (m, 3H), 1.50 (t, J=7.2 Hz, 3H), 3.47-3.56 (m, 1H), 3.79-3.89 (m, 6H), 5.10-5.14 (m, 1H), 5.54-5.56 (m, 1H), 6.72-6.87 (m, 3H), 7.11-7.23 (m, 5H).

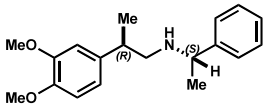
(S)-2-(3,4-Dimethoxyphenyl)-N-(1-phenylethyl)propan-1-amine; **59**



In a two-necked flask, BH_3·THF was added (1M; 6.90 mmol; 6.9 mL) to a solution of **58** (1.72 mmol; 0.54 g) in dry THF (20 mL). The reaction mixture was stirred for 24 hours at room temperature. THF was removed under vacuum affording a residue that was treated with methanol (20 mL) and concentrated HCl (1 mL). The resulting mixture was stirred at reflux for 2 hours. After cooling, the solvents were removed under vacuum to give a

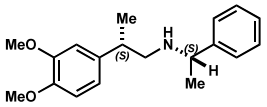
residue that was dissolved in 2N HCl and washed with diethyl ether (3 × 15 mL). The aqueous layer was basified to pH=10 with 10% NaOH and then extracted with dichloromethane (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and removed under vacuum yielding the diasteromeric amines that were purified by gravity column chromatography (eluent: toluene-ethyl acetate, 1:1) to give the two diastereoisomers.

(R)-2-(3,4-dimethoxyphenyl)-N-((S)-1-phenylethyl)propan-1-amine; ***(R,S)*-59**



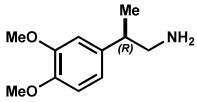
Clear oil; 0.53 mmol; 0.16 g; 31%. The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -34.9° (c=0.5; H_2O); ^{1}H NMR ($CDCl_3$) δ 1.21 (d, J=7.2 Hz, 3H), 1.28 (d, J=6.8 Hz, 3H), 2.56-2.66 (m, 2H), 2.80-2.89 (m, 1H), 3.70 (q, J=6.4 Hz, 1H), 3.87 (s, 6H), 6.73-6.84 (m, 3H), 7.22-7.33 (m, 5H).

(S)-2-(3,4-dimethoxyphenyl)-N-((S)-1-phenylethyl)propan-1-amine; ***(S,S)*-59**



Clear oil; 0.43 mmol; 0.13 g; 25%. The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -20.7° (c=0.5; H_2O).;^{1}H NMR ($CDCl_3$) δ 1.18 (d, J=7.2 Hz, 3H), 1.27 (d, J=6.8 Hz, 3H), 2.51-2.65 (m, 1H), 2.65-2.69 (m, 1H), 2.84-2.91 (m, 1H), 3,73 (q, J=6.8 Hz, 1H), 3.84 (s, 3H), 3.88 (s, 3H), 6.64-6.83 (m, 3H), 7.12-7.29 (m, 5H).

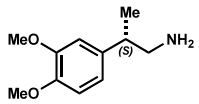
(R)-2-(3,4-dimethoxyphenyl)propan-1-amine; ***(R)*-33**



The Pearlman's catalyst (10% p/p) was added to a solution of ***(R,S)*-59** (1.13 mmol; 0.34 g) in methanol (50 mL). The mixture was placed in a Parr apparatus at 60 Psi and allowed to react for 1 day at room temperature. The reaction was monitored by TLC (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). The catalyst was filtered off and the solvent removed under vacuum. The residue was then purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 90:10:0.5) yielding the free amine ***(R)*-33** obtained as a clear oil (0.82 mmol; 0.16 g; yield: 72%) that was converted into the HCl salt: $[\alpha]^{20}_D$ = +27.56° (c=0.5; H_2O); ^{1}H NMR ($CDCl_3$) δ 1.24 (d, J=6.8 Hz, 3H), 2.66-

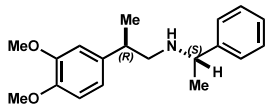
2.75 (m, 1H), 2.77-2.84 (m, 2H), 3.86 (s, 3H), 3.88 (s, 3H), 6.72-6.76 (m, 2H), 6.81-6.83 (m, 1H).

(S)-2-(3,4-Dimethoxyphenyl)propan-1-amine; ***(S)*-33**



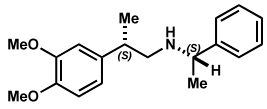
It was obtained following the procedure described for ***(R)*-33**, starting from ***(S,S)*-59** (0.73 mmol; 0.22 g). ***(S)*-33** free base was obtained as a clear oil (0.66 mmol; 0.13 g; 91%) and converted into the HCl salt: $[\alpha]^{20}_D$ = -23.3° (c=0.5; H_2O). The ^{1}H NMR spectrum was identical to that of ***(R)*-33**.

(R)-2-(3,4-Dimethoxyphenyl)-N-((S)-1-phenylethyl)propan-1-amine; ***(R,S)*-60**



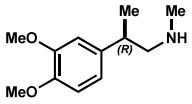
Formic acid (0.060 mol; 2.30 mL) and formaldehyde (37% in H_2O; 0.018 mol; 1.43 mL) were added to a solution of ***(R,S)*-59** (0.004 mol; 1.05 g) in absolute ethanol (20 mL). The mixture was stirred at reflux for 6 hours. The solvent was removed and the residue was dissolved in dichloromethane and washed twice with $NaHCO_3$. The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give ***(R,S)*-60** as a yellow oil (98%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -4.0° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.27 (d, J=7.2 Hz, 3H), 1.33 (d, J=6.2 Hz, 3H), 2.20 (s, 3H), 2.37 (dd, J=11.6 Hz, J=6.4 Hz, 1H), 2.43-2.48 (m, 1H), 2.77-2.94 (m, 1H), 3.60 (q, J=6.2 Hz, 1H), 3.81 (s, 3H), 3.89 (s, 3H), 6.63 (s, 1H), 6.68 (d, J=8.0 Hz, 1H), 6.78 (dd, J=8.0 Hz, J=1.6 Hz, 1H), 7.21-7.31 (m, 5H).

(S)-2-(3,4-Dimethoxyphenyl)-N-((S)-1-phenylethyl)propan-1-amine; ***(S,S)*-60**



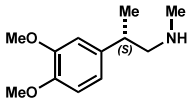
It was synthesized following the procedure described for ***(R,S)*-60**, starting from ***(S,S)*-59** (0.003 mol; 0.81 g), formic acid (0.046 mol; 1.77 mL) and formaldehyde (37% in H_2O; 0.014 mol; 1.10 mL). ***(S,S)*-60** was obtained as a yellow oil (98%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -6.3° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.19 (d, J=6.8 Hz, 3H), 1.27 (d, J=5.6 Hz, 3H), 2.19 (s, 3H); 2.36 (dd, J=11.2 Hz, J=7.6 Hz, 1H), 2.51 (dd, J=11.2 Hz, J=7.4 Hz, 1H), 2.78-2.91 (m, 1H), 3.56 (q, J=5.6 Hz, 1H), 3.84 (s, 3H); 3.87 (s, 3H); 6.62 (s, 1H), 6.69 (d, J=8.3 Hz, 1H), 6.80 (dd, J=8.3 Hz, J=1.8 Hz, 1H), 7.15-7.31 (m, 5H).

(R)-2-(3,4-Dimethoxyphenyl)-N-methylpropan-1-amine; ***(R)*****-61**



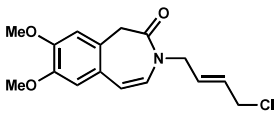
$Pd(OH_2)/C$ (0.33 g) was added to a solution of ***(R,S)*****-60** (0.004 mol; 1.13 g) in methanol (20 ml). The mixture was placed in a Parr apparatus at 65 Psi and allowed to react at room temperature for 1 day. The catalyst was filtered off and the solvent was removed to give ***(R)*****-61** as a gummy-yellow solid (80%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = +19.4° (c=1; $CHCl_3$). ^1H NMR ($CDCl_3$) δ 1.35 (d, J=6.8 Hz, 3H), 2.48 (s, 3H), 2.87 (dd, J=14.0 Hz, J=7.6 Hz, 1H), 2.93 (dd, J=14.0 Hz, J=7.2 Hz, 1H), 3.10-3.19 (m, 1H), 3.83 (s, 3H), 3.87 (s, 3H), 6.34 (bs, 1H), 6.77-6.82 (m, 3H).

(S)-2-(3,4-Dimethoxyphenyl)-N-methylpropan-1-amine; ***(S)*****-61**



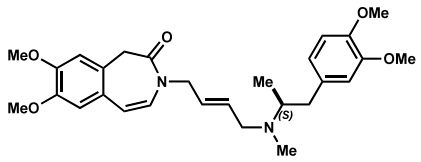
It was synthesized following the procedure described for ***(R)*****-61**, staring from ***(S,S)*****-60** (0.003 mol; 0.83 g) and $Pd(OH_2)/C$ (0.25 g). ***(S)*****-61** was obtained as a yellow oil (85%). The free base was converted into the HCl salt: $[\alpha]^{20}_D$ = -16.2° (c=1; $CHCl_3$); ^1H NMR ($CDCl_3$) δ 1.27 (d, J=6.8 Hz, 3H), 2.41 (s, 3H), 2.75 (dd, J=11.8 Hz, J=8.4 Hz, 1H), 2.79 (dd, J=11.8 Hz, J=6.4 Hz, 1H), 2.90-3.04 (m, 1H), 3.84 (s, 3H); 3.87 (s, 3H), 3.94 (bs, 1H), 6.74-6.77 (m, 2H), 6.80 (d, J=7.8 Hz, 1H).

*(E)-3-(4-Chlorobut-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; trans-***28** (EC3)



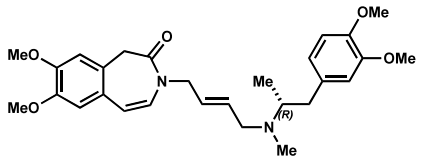
It was synthesized following the procedure described for *cis*-**28**, starting from **14** (0.018 mol; 4 g), potassium terbutoxyde (0.023 mol; 2.5 g) and *trans*-1,4-dichlorobut-2-ene (0.023 mol; 3.3 mL). **26** was obtained as a yellow solid (60%; mp 83-86 ^{0}C). ^1H NMR ($CDCl_3$) δ 3.44 (s, 2H), 3.86 (s, 3H), 3.89 (s, 3H), 3.97 (d, J=6.8 Hz, 2H), 4.17 (d, J=5.6 Hz, 2H), 5.58-5.65 (m, 1H), 5.68-5.74 (m, 1H), 6.16 (d, J=9.0 Hz, 1H), 6.37 (d, J=9.0 Hz, 1H), 6.74 (s, 1H), 6.79 (s, 1H).

(S,E)-3-(4-((1-(3,4-dimethoxyphenyl)propan-2-yl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*****-62**



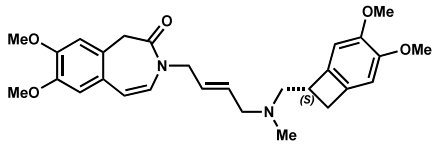
Under nitrogen atmosphere, dry triethylamine (0.07 mL; 0.0005 mol) and ***(S)*-31** (0.10 g; 0.0005 mol) in dry CH_3CN (4 mL) were added to a solution of *trans*-**28** (0.15 g; 0.0005 mol) in dry CH_3CN (3 mL). The reaction mixture was stirred for 24 hours at room temperature and monitored by TLC (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). The solvent was removed under vacuum and the residue was dissolved in ethyl acetate and washed with aqueous $NaHCO_3$ (saturated solution; 3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum to give a residue that was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). ***(S)*-62** was obtained as a whitish solid: 0.08 g; 0.0002 mol; 36%; mp 85 °C; $[\alpha]^{20}_D$ = +2.2° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.88 (d, J=6.4 Hz, 3H), 2.20 (s, 3H), 2.32 (dd, J=13.8 Hz; J=10.6 Hz, 1H), 2.80-2.89 (m, 2H), 3.00-3.10 (m, 2H), 3.44 (s, 2H), 3.84 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 3.87 (s, 3H), 4.09-4.19 (m, 2H), 5.53 (dd, J=15.6Hz; J=5.0 Hz, 1H), 5.59 (dd, J=15.6 Hz, J=5.6 Hz, 1H), 6.17 (d, J=9.2Hz, 1H), 6.31 (d, J= 9.2Hz, 1H), 6.66-6.69 (m, 3H), 6.76-6.78 (m, 2H).

(R,E)-3-(4-((1-(3,4-Dimethoxyphenyl)propan-2-yl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*-62**



It was synthesized following the procedure described for ***(S)*-62**, starting from ***(R)*-31** (0.0005 mol; 0.10 g), *trans*-**28** (0.15 g; 0.0005 mol), dry triethylamine (0.07 mL; 0.0005 mol) and dry CH_3CN (7 mL). ***(R)*-62** was obtained as a whitish solid: 41%; mp 88 °C; $[\alpha]^{20}_D$ = -3.5° (c=1; $CHCl_3$). The ^{1}H NMR spectrum was identical to that of ***(S)*-62**.

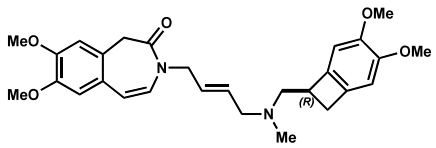
(S,E)-3-(4-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl) amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*-63**



Under nitrogen atmosphere, a solution of *trans*-**28** (0.15 g; 0.0005 mol), dry triethylamine (0.07 mL; 0.0005 mol) and ***(S)*-32** (0.10 g; 0.0005 mol) in dry CH_3CN (4 mL) was stirred for 48 hours at room temperature. The solvent was removed under vacuum and the residue was dissolved in ethyl acetate and washed with aqueous

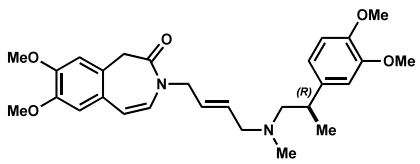
$NaHCO_3$ (saturated solution; 3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). ***(S)*-63** was obtained as a yellow solid: 40%; mp 60-62 °C; $[\alpha]^{20}_D$ = +19.6° (c=1; $CHCl_3$). e.e.=97.6% (determined by chiral HPLC); ^{1}H NMR ($CDCl_3$) δ 2.27 (s, 3H), 2.53 (dd, J=12.8 Hz, J=8.8 Hz, 1H), 2.68-2.75 (m, 2H), 3.00-3.09 (m, 2H), 3.25 (dd, J=12.8 Hz, J=5.2 Hz, 1H), 3.43 (s, 2H), 3.51-3.58 (m, 1H), 3.82 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 3.87 (s, 3H), 4.14 (d, J=5.2 Hz, 2H), 5.52-5.65 (m, 2H), 6.14 (d, J=9.2 Hz, 1H), 6.29 (d, J=9.2 Hz, 1H), 6.68 (s, 2H), 6.70 (s, 1H), 6.77 (s, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 35.39, 40.77, 42.48, 43.25, 49.97, 56.07, 56.34, 56.42, 59.67, 61.35, 106.85, 107.53, 109.55, 111.30, 117.20, 124.75, 126.49, 127.98, 128.46, 130.28, 135.06, 138.76, 148.11, 149.43, 149.96, 167.60.

(R,E)-3-(4-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl) amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*-63**



It was synthesized following the procedure described for ***(S)*-63**, starting from *trans*-**28** (0.08 g; 0.0003 mol), dry triethylamine (0.04 mL; 0.0003 mol) and ***(R)*-32** (0.06 g; 0.0003 mol). ***(R)*-63** was obtained as a pale yellow solid: 37%; mp 65-68 ^{0}C; $[\alpha]^{20}_D$ = -10.9° (c=0.34; $CHCl_3$). e.e.=87.5% (determined by chiral HPLC). NMR spectra were identical to those of ***(S)*-63**.

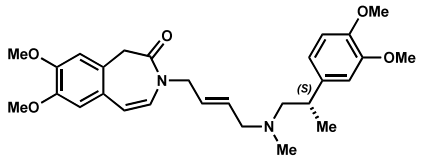
(R,E)-3-(4-((2-(3,4-Dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*-64**



It was synthesized following the procedure described for ***(S)*-63**, starting from *trans*-**28** (0.32 g; 0.001 mol), dry triethylamine (0.13 mL; 0.001 mol) and ***(R)*-61** (0.20 g; 0.001 mol). ***(R)*-64** was obtained as a pale yellow solid: 54%; mp 60-62 ^{0}C; $[\alpha]^{20}_D$ = -11.4° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.14 (d, J=6.8 Hz, 3H), 2.11 (s, 3H), 2.28-2.40 (m, 2H), 2.74-2.96 (m, 3H), 3.39 (s, 2H), 3.77 (s, 3H), 3.83 (s, 3H), 3.81 (s, 6H), 4.02-4.15 (m, 2H), 5.39-5.53 (m, 2H), 6.12

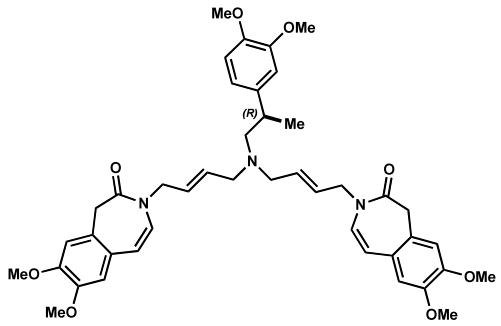
(d, J=9.2 Hz, 1H), 6.30 (d, J=9.2 Hz, 1H), 6.65-6.75 (m, 5H); ^{13}C NMR ($CDCl_3$, APT) δ 20.28, 37.43, 42.43, 43.02, 48.76, 55.76, 55.81, 56.00, 59.39, 64.43, 109.37, 110.50, 111.08, 111.12, 116.89, 118.70, 124.51, 126.29, 127.61, 127.68, 130.42, 138.59, 147.18, 147.87, 148.65, 149.71, 167.28.

(S,E)-3-(4-((2-(3,4-Dimethoxyphenyl)propyl)(methyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*****-64**



It was synthesized following the procedure described for ***(S)*****-63**, starting from *trans*-**28** (0.32 g; 0.001 mol), dry triethylamine (0.13 mL; 0.001 mol) and ***(S)*****-61** (0.20 g; 0.001 mol). ***(S)*****-64** was obtained as a pale yellow solid: 9%; mp 59-61 ^{0}C; $[\alpha]^{20}_D$ = +6.4° (c=1; $CHCl_3$). NMR spectra were identical to those of ***(R)*****-64**.

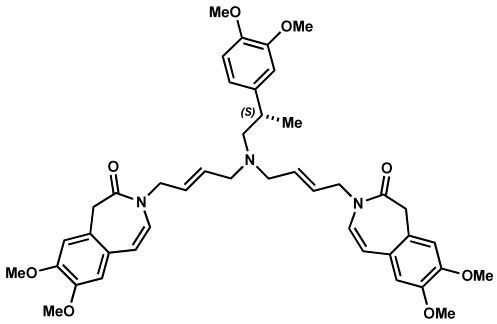
3,3'-(2E,2'E)-4,4'-((R)-2-(3,4-Dimethoxyphenyl)propylazanediyl)bis(but-2-ene-4,1-diyl)bis(7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one); ***(R)*****-65**



It was synthesized following the procedure described for ***(S)*****-63**, starting from *trans*-**28** (0.445 g; 0.00145 mol) and ***(R)*****-33** (0.141 g; 0.00072 mol). ***(R)*****-65** free base was obtained as a pale yellow solid: 7%; mp 89-93 ^{0}C; $[\alpha]^{20}_D$ = -14.7° (c=1; $CHCl_3$).

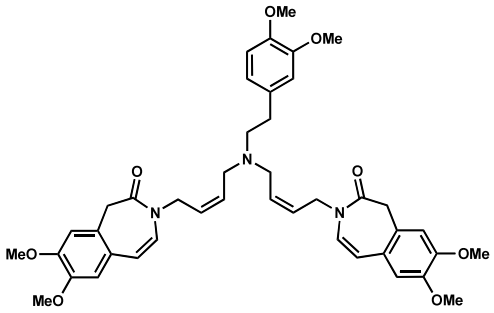
Due to the impossibility to purify the compound, the free base was converted into the HCl salt: $[\alpha]^{20}_D$ = +12.5° (c=1; CD_3OD); ^{1}H NMR ($CDCl_3$) δ 1.13 (d, J=6.0 Hz, 3H), 2.87-3.10 (m, 3H), 3.19-3.49 (m, 4H), 3.45 (s, 4H), 3.79 (s), 3.81 (s), 3.82 (s), 3.83 (s), 3.85 (s) (18H), 4.01-4.23 (m, 4H), 5.08-5.26 (m, 2H); 5.51-5.61 (m, 1H), 5.66-5.74 (m, 1H), 6.24-6.29 (m, 2H), 6.49-6.52 (m, 1H), 6.65 (d, J=8.0 Hz, 1H), 6.82-6.91 (m, 7H); ^{13}C NMR (CD_3OD, APT) δ 21.38, 36.65, 43.34, 49.96, 54.85, 56.57, 56.64, 56.78, 59.01, 111.51, 112.29, 112.64, 113.70, 119.51, 119.59, 119.83, 120.49, 126.19, 127.86, 129.20, 135.45, 138.79, 138.84, 149.81, 150.13, 151.15, 161.70, 169.48.

3,3'-(2E,2'E)-4,4'-((S)-2-(3,4-Dimethoxyphenyl)propylazanediyl)bis(but-2-ene-4,1-diyl)bis(7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one); ***(S)*-65**



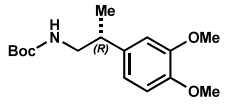
It was synthesized following the procedure described for ***(S)*-63**, starting from *trans*-**28** (0.445 g; 0.00145 mol) and ***(S)*-33** (0.141 g; 0.00072 mol). ***(S)*-65** was obtained as a pale yellow solid: 6%; mp 84-87 ^{0}C. NMR spectra were identical to those of ***(R)*-65**. Free base: $[\alpha]^{20}_D$ = +11.5° (c=1; $CHCl_3$); HCl salt $[\alpha]^{20}_D$ = -13.1° (c=1; CD_3OD).

3,3'-(2Z,2'Z)-4,4'-(3,4-Dimethoxyphenethylazanediyl)bis(but-2-ene-4,1-diyl)bis(7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one); **66**



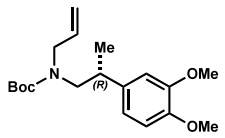
Under nitrogen atmosphere, a solution of *cis*-**28** (0.17 g; 0.0006 mol), dry triethylamine (0.04 mL; 0.0003 mol) and homoveratrylamine (0.05 g; 0.0003 mol) in dry CH_3CN (10 mL) was stirred at reflux for 16 hours. The solvent was removed under vacuum and the residue was taken up with dichloromethane and washed with 2M NaOH (3 × 15 mL). The organic layers were collected, dried (Na_2SO_4) and evaporated under vacuum. The residue was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 97:3:0.3). **66** was obtained as a pale yellow solid: mp 60-62 °C; ^{1}H NMR ($CDCl_3$) δ 2.69 (bs, 4H, CH_2CH_2); 3.22 (d, J=6.4 Hz, 4H, 2 x CH=CH*C*H_2N); 3.42 (s, 4H, 2 x CH_2CO); 3.81 (s, 3H, OCH_3); 3.83 (s, 6H, 2 x OCH_3); 3.85 (s, 6H, 2 x OCH_3); 3.87 (s, 3H, OCH_3); 4.22 (d, J=6.8 Hz, 4H, 2 x CH=CH*C*H_2NCO); 5.42-5.48 (m, 2H, 2 x NCH_2*C*H=CHCH_2NCO); 5.62-5.68 (m, 2H, 2 x NCH_2CH=*C*HCH_2NCO); 6.16 (d, J=9.2 Hz, 2H, 2 x Ar*C*H=CHNCO); 6.31 (d, J=9.2 Hz, 2H, 2 x ArCH=*C*HNCO); 6.68-6.78 (m, 7H, aromatics); ^{13}C NMR ($CDCl_3$, APT) δ 33.05, 43.08, 44.52, 50.55, 55.63, 55.79, 55.83, 55.88, 109.50, 111.15, 111.19, 112.04, 117.18, 120.46, 124.56, 126.28, 127.45, 127.55, 130.55, 132.79, 147.26, 147.97, 148.74, 149.84, 167.39.

(R)-tert-Butyl 2-(3,4-dimethoxyphenyl)propylcarbamate; ***(R)*-67**



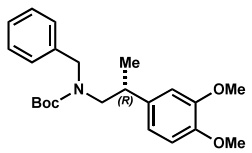
Under nitrogen atmosphere, di-*tert*-butyl-dicarbonate (0.15 g; 0.0012 mol) in dry THF (5 mL) was added dropwise to a solution (0 °C) of ***(R)*-33** (0.15 g; 0.0007 mol) in dry THF (5 mL). The mixture was stirred for 15 hours at room temperature. Removal of the solvent afforded a residue that was taken up in dichloromethane and washed with brine. The organic layers were collected, dried (Na2SO4) and evaporated to give a crude that was purified by flash chromatography (eluent: dichloromethane-methanol, 98:2). ***(R)*-67** was obtained as a pale yellow oil: 0.18 g; 0.0006 mol; 78%; $[\alpha]^{20}_D$ = +48.5° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.23 (d, J=7.2 Hz, 3H), 1.41 (s, 9H), 2.83-2.88 (m, 1H), 3.10-3.17 (m, 1H), 3.35-3.37 (m, 1H), 3.86 (s, 3H), 3.87 (s, 3H), 4.43 (bs, 1H), 6.71-6.75 (m, 2H), 6.82 (d, J=8.0 Hz, 1H).

(R)-tert-Butyl allyl(2-(3,4-dimethoxyphenyl)propyl)carbamate; ***(R)*-68**



Under anhydrous condition, sodium hydride (0.04 g; 0.0009 mol) and allyl bromide (0.08 ml; 0.0009 mol) were added to a solution (0 °C) of ***(R)*-67** (0.18 g; 0.0006 mol) in dry DMF (30 mL). After stirring for 90 minutes at 0 °C, the reaction mixture was stirred for 24 hours at room temperature. The reaction was then quenched with aqueous NH_4Cl (saturated solution) and extracted with ethyl acetate. The organic layers were collected, dried (Na_2SO_4) and evaporated to give a residue that was purified by flash chromatography (eluent: cyclohexane-ethyl acetate, 4:1). ***(R)*-68** was obtained as a clear oil: 0.082 g; 0.0002 mol; 33%; $[\alpha]^{20}_D$ = +60.8° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.31 (d, J=13.2Hz, 3H), 1.43 (s, 9H), 3.00-3.15 (m, 2H), 3.23-3.39 (m, 1H), 3.42-3.57 (m, 1H), 3.66-3.87 (m, 3H), 3.81 (s, 3H), 3.85 (s, 3H), 4.98-5.08 (m, 2H), 5.55-5.77 (m, 1H), 6.68-6.82 (m, 3H).

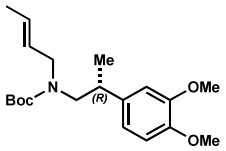
(R)-tert-Butyl benzyl(2-(3,4-dimethoxyphenyl)propyl)carbamate; ***(R)*-69**



It was synthesized following the procedure described for ***(R)*-68**, starting from ***(R)*-67** (0.22 g; 0.0007 mol), sodium hydride (0.05 g; 0.0011 mol) and benzyl bromide (0.13 ml; 0.0011 mol). It was purified by flash chromatography (eluent: cyclohexane-ethyl acetate, 99:1). ***(R)*-69** was obtained as a clear oil: 0.19 g; 0.0005 mol; 71%; $[\alpha]^{20}_D$ = +51.7° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (2 rotamers: x and y) 1.21 (d, J=6.8Hz,

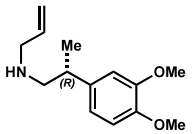
$3H_x+3H_y$), 1.40 (s, $9H_y$), 1.47 (s, $9H_x$), 3.02-3.14 (m, 3H), 3.27-3.30 (m, 1H), 3.52-3.54 (s, 1H), 3.83 (s, 3H), 3.84 (s, 3H), 3.84-3.87 (m, $1H_y$), 4.00 (d, J=15.6 Hz, $1H_x$), 4.37 (d, J=16.0 Hz, $1H_y$), 4.48 (d, J=15.6 Hz, $1H_x$), 6.62-6.80 (m, 3H), 7.08-7.34 (m, 5H).

(R)-tert-Butyl but-2-enyl(2-(3,4-dimethoxyphenyl)propyl)carbamate; ***(R)*-70** *(E+Z mixture)*



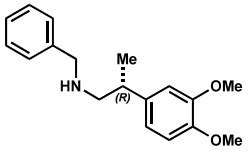
It was synthesized following the procedure described for ***(R)*-68**, starting from ***(R)*-67** (0.18 g; 0.0006 mol), sodium hydride (0.05 g; 0.0012 mol) and crotyl bromide (0.10 ml; 0.0012 mol). It was purified by flash chromatography (eluent: cyclohexane-ethyl acetate, 99:1). ***(R)*-70** (*E+Z* mixture) was obtained as a clear oil: 0.13 g; 0.0004 mol; 67%; $[\alpha]^{20}_D$ = +55.0° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.19 (d, J=6.8 Hz, 3H), 1.41 (s, 9H), 1.62 (d, J=6.0 Hz, 3H), 2.90-3.17 (m, 1H), 3.18-3.42 (m, 1H), 3.36-3.52 (m, 1H), 3.53-3.79 (m, 2H), 3.82 (s, 3H), 3.84 (s, 3H), 5.27-5.60 (m, 2H), 6.66-6.79 (m, 3H).

(R)-N-(2-(3,4-Dimethoxyphenyl)propyl)prop-2-en-1-amine; ***(R)*-71**



Trifluoroacetic acid (0.40 mL) was slowly added to a solution of ***(R)*-68** (0.08 g; 0.00024 mol) in dichloromethane (1 mL) and. The mixture was stirred for 90 minutes at room temperature. Removal of the solvent gave a residue that was taken up with aqueous $NaHCO_3$ (saturated solution) and extracted with ethyl acetate. The organic layers were collected, dried (Na_2SO_4) and evaporated to afford ***(R)*-71** as a yellow oil: 0.05 g; 0.00021 mol; 89%; $[\alpha]^{20}_D$ = +9.5° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.24 (d, J=6.8 Hz, 3H), 1.90 (bs, 1H), 2.70-2.76 (m, 2H), 2.78-2.92 (m, 1H), 3.18-3.25 (m, 2H), 3.85 (s, 3H), 3.87 (s, 3H), 5.04-5.14 (m, 2H), 5.78-5.88 (m, 1H), 6.77-6.82 (m, 3H).

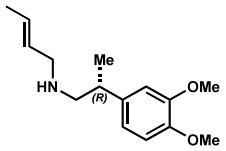
(R)-N-benzyl-2-(3,4-dimethoxyphenyl)propan-1-amine; ***(R)*-72**



It was synthesized following the procedure described for ***(R)*-71**, starting from ***(R)*-69** (0.18 g; 0.00047 mol), dichloromethane (2.3 mL) and trifluoroacetic acid (0.93 ml). ***(R)*-72** was obtained as a clear oil: 0.12 g; 0.00042 mol; 90%; $[\alpha]^{20}_D$ = +13.4° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.25 (d, J=6.8 Hz, 3H), 2.36 (bs, 1H), 2.77-2.79 (m, 2H), 2.89-2.98 (m, 1H), 3.73 (d,

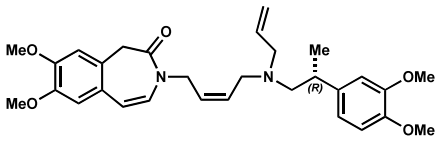
J=13.2 Hz, 1H), 3.80 (d, J=13.2 Hz, 1H), 3.85 (s, 3H), 3.86 (s, 3H), 6.72 (d, J=1.6 Hz, 1H), 6.76 (dd, J=8.0 Hz, J=1.6 Hz, 1H), 6.82 (d, J=8.0 Hz, 1H), 7.22-7.30 (m, 5H).

(R)-N-(2-(3,4-Dimethoxyphenyl)propyl)but-2-en-1-amine; ***(R)-73*** *(E+Z mixture)*



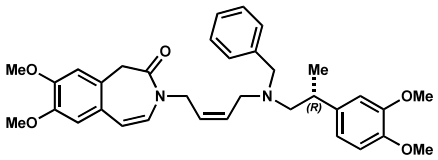
It was synthesized following the procedure described for ***(R)-71***, starting from ***(R)-70*** (0.13 g; 0.00037 mol), dichloromethane (1.8 mL) and trifluoroacetic acid (0.71 ml). ***(R)-73*** (*E*+*Z* mixture) was obtained as a clear oil: 0.09 g; 0.00036 mol; 98%; $[\alpha]^{20}_D$ = +8.0° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ratio *E/Z*=2.7/1) 1.24 (d, J= 7.2 Hz, 3H), 1.59 (d, J= 6.8 Hz, $3H_Z$), 1.64 (d, J= 6.0 Hz, $3H_E$), 2.76-2.80 (m, 2H), 2.89-2.96 (m, 1H), 3.16 (d, J= 6.4 Hz, $1H_E$), 3.27 (d, J= 6.8 Hz, $1H_Z$), 3.82 (s, 3H), 3.85 (s, 3H), 5.37-5.48 (m, 1H), 5.54-5.62 (m, 1H), 6.72-6.80 (m, 3H).

(R,Z)-3-(4-(Allyl(2-(3,4-dimethoxyphenyl)propyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)-74***



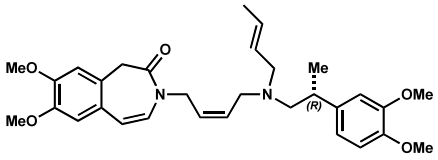
Under anhydrous condition, dry triethylamine (0.043 mL; 0.0003 mol) was added to a solution of ***(R)-71*** (0.05 g; 0.0003 mol) in dry CH_3CN (2 mL). The resulting mixture was poured into a solution of *cis*-**28** (0.08 g; 0.0003 mol) in dry CH_3CN (2 mL). The reaction mixture was stirred for 24 hours at room temperature. Removal of the solvent gave a residue that was taken up with aqueous $NaHCO_3$ (saturated solution) and extracted with dichloromethane. The organic layers were collected, dried (Na_2SO_4) and evaporated to afford a residue that was purified by flash chromatography (eluent: dichloromethane-methanol, 99:1). ***(R)-74*** was obtained as a grayish oil: 0.03 g; 0.00006 mol; 24%; $[\alpha]^{20}_D$ = +26.7° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.22 (d, J=6.8Hz, 3H), 2.46 (dd, J=12.8Hz, J=7.6Hz, 1H), 2.56 (dd, J=12.8 Hz, J=6.8 Hz, 1H), 2.82-2.90 (m, 1H), 3.02 (dd, J=14.2 Hz, J=6.6Hz, 1H), 3.08-3.19 (m, 3H), 3.43 (s, 1H), 3.84 (s, 3H), 3.86 (s, 3H), 3.87 (s, 3H), 3.88 (s, 3H), 4.11-4.23 (m, 2H), 5.10-5.16 (m, 2H), 5.37-5.43 (m, 1H), 5.63-5.71 (m, 1H), 5.75-5.85 (m, 1H), 6.12 (d, J=9.2Hz, 1H), 6.30 (d, J=9.2Hz, 1H), 6.71-6.73 (m, 2H), 6.77-6.80 (m, 3H); ^{13}C NMR ($CDCl_3$, APT) δ 20.86, 29.79, 37.17, 43.19, 44.62, 50.89, 56.02, 56.09, 56.98, 60.66, 109.74, 110.83, 111.32, 111.46, 117.62, 119.07, 124.69, 126.42, 127.66 129.02, 137.85, 147.73, 148.23, 149.08, 150.11, 167.62.

(R,Z)-3-(4-(Benzyl(2-(3,4-dimethoxyphenyl)propyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-75**



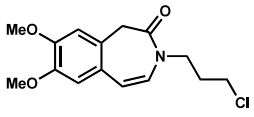
It was synthesized following the procedure described for ***(R)*****-74**, starting from ***(R)*****-72** (0.12 g; 0.0007 mol), *cis*-**28** (0.22 g; 0.0007 mol), dry triethylamine (0.10 ml; 0.0007 mol) and dry CH_3CN (10 mL). ***(R)*****-75** was obtained as a white solid: 0.13 g; 0.0003 mol; 42%; mp 105 °C (HCl salt); $[\alpha]^{20}_D$ (free base)= -12.5° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.21 (d, J=6.8 Hz, 3H), 2.45-2.50 (m, 1H), 2.56-2.59 (m, 1H), 2.88-2.90 (m, 1H), 3.11-3.12 (m, 2H), 3.42 (s, 2H), 3.55-3.58 (m, 2H), 3.81 (s, 3H), 3.86 (s, 3H), 3.88 (s, 3H), 3.89 (s, 3H), 4.00-4.18 (m, 2H), 5.39-5.42 (m, 1H), 5.61-5.63 (m, 1H), 6.05-6.07 (m, 1H), 6.27-6.29 (m, 1H), 6.64-6.79 (m, 5H), 7.20-7.28 (m, 5H); ^{13}C NMR ($CDCl_3$, APT) δ 20.28, 37.99, 43.27, 44.62, 50.84, 55.90, 56.01, 58.96, 61.91, 109.65, 110.80, 111.20, 111.35, 117.20, 119.22, 124.67, 126.49, 126.90, 127.18, 127.77, 128.20, 128.92, 131.42, 138.91, 139.75, 147.38, 148.14, 148.82, 150.01, 167.55.

3-((Z)-4-((E/Z)-But-2-enyl((R)-2-(3,4-dimethoxyphenyl)propyl)amino)but-2-enyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-76** *(E+Z mixture)*



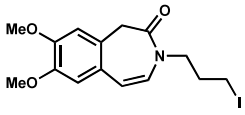
It was synthesized following the procedure described for ***(R)*****-74**, starting from ***(R)*****-73** (0.10 g; 0.0008 mol), *cis*-**28** (0.24 g; 0.0008 mol), dry triethylamine (0.10 ml; 0.0008 mol) and dry CH_3CN (10 mL). ***(R)*****-76** (*E+Z* mixture) was obtained as a white solid: 0.13 g; 0.0003 mol; 42%; mp 71 °C; $[\alpha]^{20}_D$ = +5.8° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ (ratio *E/Z*=2.7/1) 1.21 (d, J=6.8 Hz, $3H_Z$), 1.20 (d, J=6.8 Hz, $3H_E$), 1.61 (d, J=6.8 Hz, $3H_Z$), 1.67 (d, J=6.4 Hz, $3H_E$), 2.40-2.46 (m, 1H), 2.51-2.55 (m, 1H), 2.80-2.88 (m, 1H), 2.92-2.99 (m, 2H), 3.01-3.15 (m, 2H), 3.41 (s, 2H), 3.82 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 3.86 (s, 3H), 4.10-4.22 (m, 2H), 5.35-5.44 (m, 2H), 5.49-5.63 (m, 2H), 6.10-6.12 (d, J=9.2 Hz, 1H), 6.27-6.29 (d, J=9.2 Hz, 1H), 6.70-6.72 (m, 3H), 6.76-6.78 (m, 2H); ^{13}C NMR ($CDCl_3$, APT) δ 13.19, 17.87, 20.42, 21.88, 37.78, 43.18, 44.52, 50.55, 50.70, 50.94, 55.87, 55.90, 55.99, 56.37, 61.19, 61.49, 109.59, 110.70, 111.22, 111.27, 117.13, 119.05, 124.69, 126.43, 127.00, 127.17, 127.25, 127.69, 127.82, 128.79, 130.97, 131.06, 138.90, 147.33, 148.07, 148.80, 149.93, 167.52, 174.97.

3-(3-Chloropropyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; (SB4)



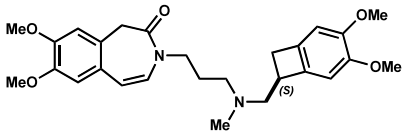
It was synthesized following the procedure described for *cis*-**28**, starting from **14** (0.019 mol; 4.1 g), potassium terbutoxyde (0.023 mol; 2.5 g) and 1-bromo-3-chloropropane (0.023 mol; 2.2 mL). SB4 was obtained as a yellow-brown solid: 55%; mp 104-106 °C; ^{1}H NMR ($CDCl_3$) δ 1.95-2.02 (m, 2H), 3.41-3.45 (m, 4H), 3.70 (t, J=6.4 Hz, 2H), 3.88 (s, 3H), 3.89 (s, 3H), 6.23 (d, J=9.2 Hz, 1H), 6.35 (d, J=9.2 Hz, 1H), 6.73 (s, 1H), 6.78 (s, 1H).

3-(3-Iodopropyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; **77**



NaI (0.41 g; 0.003 mol) was added to a solution of SB4 (0.40 g; 0.001 mol) in acetone (20 mL). The reaction mixture was stirred for 48 hours at reflux. After removal of the solvent, the residue was taken up with ethyl acetate and washed with brine. The organic layers were collected, dried (Na_2SO_4) and evaporated to afford **77** as a pale yellow solid: 76%; mp 122-124 °C); ^{1}H NMR ($CDCl_3$) δ 2.00-2.07 (m, 2H), 3.04 (t, J=6.8 Hz, 2H), 3.43 (s, 2H), 3.64 (t, J=6.4 Hz, 2H), 3.88 (s, 3H), 3.90 (s, 3H), 6.23 (d, J=9.2 Hz, 1H), 6.35 (d, J=9.2 Hz, 1H), 6.73 (s, 1H), 6.78 (s, 1H).

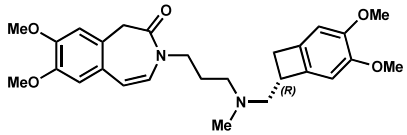
(S)-3-(3-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl)amino) propyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*-78**



It was synthesized following the procedure described for ***(S)*-79**, starting from **77** (0.001 mol; 0.39 g), Na_2CO_3 (0.16 g; 0.002 mol), ***(S)*-32** (0.21 g; 0.001 mol) dissolved in dry DMF (5 mL). ***(S)*-78** was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 97:3:0.3) and obtained as a yellow solid: 42%; mp 48-50 °C; $[\alpha]^{20}_D$ = +7.0° (c=1; $CHCl_3$); e.e.=93.8% (determined by chiral HPLC); ^{1}H NMR ($CDCl_3$) δ 1.64-1.71 (m, 2H), 2.22 (s, 3H), 2.28 (t, J=6.8 Hz, 2H), 2.45 (dd, J=12.3 Hz, J=8.2 Hz, 1H), 2.61 (dd, J=12.3 Hz, J=6.8 Hz, 1H), 2.65 (dd, J=14.4 Hz, J=1.6 Hz, 1H), 3.16 (dd, J=14.4 Hz, J=5.0 Hz, 1H), 3.40 (s, 2H), 3.42-3.48 (m, 1H), 3.50-3.68 (m, 2H), 3.81 (s, 3H), 3.82 (s, 6H), 3.85 (s, 3H), 6.18 (d, J=9.0 Hz, 1H), 6.27 (d, J=9.0 Hz, 1H), 6.66-6.67 (m, 3H), 6.75 (s, 1H); ^{13}C NMR ($CDCl_3$, APT) δ 26.24, 35.13, 40.62, 42.42, 43.32, 46.21, 54.65,

55.98, 56.28, 56.36, 61.86, 106.79, 107.44, 109.39, 111.18, 117.04, 124.78, 126.41, 128.72, 135.00, 139.02, 147.99, 149.29, 149.81, 167.63.

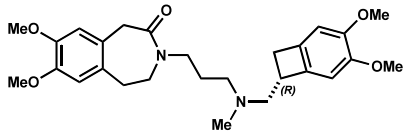
(R)-3-(3-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl)amino) propyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*****-78**



It was synthesized following the procedure described for ***(S)*****-79**, starting from **77** (0.001 mol; 0.37 g), Na_2CO_3 (0.14 g; 0.001 mol), ***(R)*****-32** (0.18 g; 0.001 mol) dissolved in dry DMF (5 mL). ***(R)*****-78** was obtained as a yellow solid: 49%; mp 55-60 °C; $[\alpha]^{20}_D$ = -6.0° (c=1; $CHCl_3$). e.e.=97.9% (determined by chiral HPLC). The NMR were identical to those of ***(S)*****-78**.

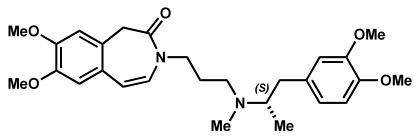
(R)-3-(3-(((4,5-Dimethoxy-1,2-dihydrocyclobutabenzen-1-yl)methyl)(methyl)amino) propyl)-7,8-dimethoxy-4,5-dihydro-1H-benzo[d]azepin-2(3H)-one;

(R)**-80 (R-Ivabradine)**



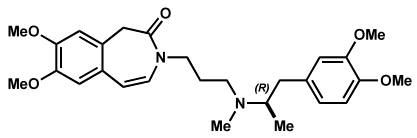
Pd/C (10% p/p; 35 mg) was added to a solution of ***(R)*****-78** (0.07 g; 0.0002 mol) in acetic acid (15 mL). The mixture was placed in a Parr apparatus at 60 Psi and allowed to react for 24 hours at room temperature. The catalyst was filtered off and the filtrate was basified with 2.5M NaOH and extracted with dichloromethane. The organic layers were collected, dried (Na_2SO_4) and removed under vacuum to afford a residue, which was purified by flash chromatography (eluent: dichloromethane-methanol-ammonia, 95:5:0.5). ***(R)*****-80** was obtained as a white solid: 24%; mp 55-58 °C). $[\alpha]^{20}_D$ = -5.0° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 1.74-1.81 (m, 2H), 2.31 (s, 3H), 2.42 (t, J=6.4 Hz, 2H), 2.45 (dd, J=12.4 Hz, J=8.4 Hz, 1H), 2.69-2.73 (m, 2H), 3.03 (t, J=6.4 Hz, 2H), 3.22 (dd, J=13.6 Hz, J=4.8 Hz, 1H), 3.40-3.54 (m, 3H), 3.70 (t, J=6.4 Hz, 2H), 3.79 (s, 2H), 3.80 (s, 3H), 3.82 (s, 3H), 3.83 (s, 3H), 3.84 (s, 3H), 6.54 (s, 1H), 6.58 (s, 1H), 6.67 (s, 1H), 6.70 (s, 1H); ^{13}C NMR ($CDCl_3$, APT, δ): 26.38, 32.46, 35.26, 40.68, 42.57, 42.78, 45.14, 46.69, 55.31, 55.97, 56.31, 56.43, 62.03, 106.87, 107.50, 113.19, 114.03, 123.55, 127.50, 135.04, 138.95, 147.21, 147.91, 149.38, 149.90, 172.15.

(S)-3-(3-((1-(3,4-Dimethoxyphenyl)propan-2-yl)(methyl)amino)propyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(S)*-79**



Under anhydrous condition, a solution of ***(S)*-31** (0.17 g; 0.0008 mol) in dry DMF (6 mL) was added to a solution of **77** (0.31 g; 0.0008 mol) and Na_2CO_3 (0.13 g; 0.0012 mol) in dry DMF (3 mL). The mixture was stirred for 5 hours at 55 °C. Removal of the solvent gave a residue that was taken up with 1N HCl and extracted with ethyl acetate. The acidic aqueous phase was then basified with aqueous Na_2CO_3 (saturated solution) and extracted with ethyl acetate. The organic layers were collected, dried (Na_2SO_4) and evaporated to afford a residue, which was purified by flash chromatography (eluent: absolute ethanol-ammonia-dichloromethane-petroleum ether, 65:8:340:60). ***(S)*-79** was obtained as a white solid: 0.13 g; 0.0003 mol; 34%; mp 81 °C). $[\alpha]^{20}_D$ = +5.0° (c=1; $CHCl_3$); ^{1}H NMR ($CDCl_3$) δ 0.82 (d, J=6.2 Hz, 3H), 1.65-1.68 (m, 2H), 2.20 (s, 3H), 2.20-2.25 (m, 1H), 2.33 (t, J=6.6 Hz, 2H), 2.74-2.80 (m, 2H), 3.39 (s, 2H), 3.52 (t, J=6.8 Hz, 2H), 3.80 (s, 3H), 3.82 (s, 3H), 3.84 (s, 3H), 3.85 (s, 3H), 6.08 (d, J=9.2 Hz, 1H), 6.28 (d, J=9.2 Hz, 1H), 6.61-6.67 (m, 3H), 6.74-6.76 (m, 2H).

(R)-3-(3-((1-(3,4-Dimethoxyphenyl)propan-2-yl)(methyl)amino)propyl)-7,8-dimethoxy-1H-benzo[d]azepin-2(3H)-one; ***(R)*-79**



It was synthesized following the procedure described for ***(S)*-79**, starting from ***(R)*-31** (0.0015 mol; 0.32 g), **77** (0.59 g; 0.0015 mol), Na_2CO_3 (0.24 g; 0.0023 mol) and dry DMF (6 mL). ***(R)*-79** was purified by flash chromatography (eluent: absolute ethanol-ammonia-dichloromethane-diethyl ether-petroleum ether, 180:9.9:360:360:900) and obtained as a white solid: 28%; mp 83 °C; $[\alpha]^{20}_D$ = -5.0° (c=1; $CHCl_3$). The ^{1}H NMR spectrum was identical to that of ***(S)*-79**. ^{13}C NMR ($CDCl_3$, APT) δ 13.27, 26.75, 36.20, 38.90, 43.08, 45.95, 50.06, 55.69, 55.74, 60.02, 109.33, 110.02, 112.42, 116.57, 120.96, 124.63, 126.29, 128.65, 133.22, 147.03, 147.83, 148.51, 149.65, 167.30**.**

"..dove la Natura finisce di produrre le sue spezie, l'uomo quivi comincia con le cose naturali, con l'aiutoro di essa Natura, a creare infinite spezie.."

Leonardo da Vinci

5. RESULTS AND CONCLUSIONS

5.1 Biological results

The compounds were tested on HEK293 cells stably expressing mouse HCN1, mouse HCN2, or human HCN4; their ability to block f-current was measured by means of patch clamp recording (whole-cell configuration). The experiments were made on the three isoforms as shown in **figure 1** for **2** (EC32) on HCN1. I_f was recorded in control conditions (A) and in the presence of a 10 mM concentration of **2** (B). The activation curve under control conditions (black) and in the presence of **2** (gray) is shown in panel C; the midpoint of activation was not modified by the drug, thus suggesting that the biophysical property of the current did not change. In panel D the f-current amplitude elicited by steps to -120mV in presence of increasing doses (1-30μM) of **2** is shown: under these conditions the I_f amplitude is progressively reduced, showing that the effect is use-dependent. This has been demonstrated for all the tested compounds.

The mean activation curves for compound **1** (EC4) and ***cis*-3** (EC18), tested at the dose of 10 mM on the three isoforms are shown in figures 2 and 3. **1** (**figure 2**) reduces I_f in the three channel isoforms; the reduction at -120mV is more pronounced on HCN1 (A, 70 %, CTR: 0.95±0.01 pS/pF, n=5; **1**: 0.29±0.1 pS/pF, n=3) and HCN4 (B, 44%, CTR: 0.89±0.03 pS/pF, n=8; **1**: 0.5±0.08 pS/pF, n=7) with respect to HCN2 (C, 34%, CTR: 0.83±0.03 pS/pF, n=8; **1**: 0.55±0.07 pS/pF, n=8). ***cis*-3** (**figure 3**) minimally reduces HCN1 I_f at -120mV (A, 7%, CTR 0.96±0.02 pS/pF, n=5; ***cis*-3**: 0.89 ±0.02 pS/pF, n=4); on HCN2 the reduction is about 30% (B), while on HCN4 is much higher (C, 70%, CTR 0.92±0.05 pS/pF, n=4; ***cis*-3**: 0.3±0.06 ps/pF, n=4).

For an easier evaluation of the potency of the compounds, the EC_{50} values for the I_f blocking activity of **1**, **2** and ***cis*-3** were determined from the dose-response curve, and compared with those of ivabradine, zatebradine and cilobradine, used as reference (**table 1**). As expected[177], ivabradine does not show selectivity for one of the three channel isoforms. Although cilobradine and zatebradine in our hands show slightly different potencies (cilobradine is more potent on HCN1, while zatebradine is equipotent on HCN2 and HCN4 but less potent on HCN1), the ratios between their EC_{50} values on the three isoforms are low (≤ 4); therefore, these compounds too cannot be considered isoform-selective.

The results reported in **table 1** show that structural modifications, such as the introduction of a double bond on the methylene chain, can change the pharmacological profile of zatebradine: in fact, **1**, showing a trans butane moiety, is more active on HCN1 while **2** (the *cis*-butene analogue) is equipotent on HCN1 and HCN4 but less active on HCN2. A more marked effect is associated to the reduction of the

conformational flexibility: in fact ***cis*-3**, in which the three-methylene linker has been incorporated into a cyclohexane ring, is able to discriminate between HCN4 and HCN1. In this respect, it could be very interesting to test its enantiomers, and also its geometrical isomer ***trans*-3**: work is underway to obtain these molecules.

Since the linear compounds are synthetically more accessible, we made some modifications on **2**. First, we investigated the effect of reducing conformational flexibility on the basic part of the molecule, synthesizing **34** (MEL41) and **35** (MEL47). We found that the incorporation of the phenylethylamino group into a tetrahydroisoquinoline ring reduced the potency, since **34** is four times less potent than **2** on HCN1 and HCN4 and equipotent on HCN2. However, the 2-aminomethyl indane moiety gives a different result, since **35** is twice less potent than **2** on HCN1 and HCN4, but four times more potent on HCN2.

As a second modification, we introduced a stereogenic center on the ethyl linker, while keeping free rotation on this part of the molecule. Therefore, the enantiomers of **40** were prepared and studied. We found that the R-enantiomer is more active than the S-form on HCN1 and HCN2, where the eudismic ratio are 3 and 11, respectively, while there is lower potency and no enantioselectivity for the interaction with HCN4. While ***(S)*-40** is not selective, the introduction of a methyl group in the R configuration on the benzylic position gives another compound [***(R)*-40**] showing a preference for HCN2, since this compound is four times and ten times more potent on this isoform than on HCN1 and HCN4, respectively.

Third, we investigated the effect of increasing the size of the molecule by introducing a second (7,8-dimethoxybenzazepin-3-yl)butenyl group on the basic nitrogen, preparing **66** and the enantiomers of **39**. ***(R)*-39** shows an unexpected pharmacological profile, with high selectivity for HCN1. As shown in **figure 4A**, this compound markedly reduces I_f on this isoform, and its activity is significantly lower on the other two isoforms. On HCN1 at -120mV I_f reduction caused by ***(R)*-39** at 10mM is about 80% (A, CTR 0.94±0.02 pS/pF, n=5; ***(R)*-39** 0.2±0.1pS/pF, n=3), being 40% on HCN2 (CTR 0.82±0.05 pS/pF, n=4; 10mM 0.6±0.1pS/pF, n=4) and only 7% on HCN4 (CTR 0.92±0.09 pS/pF, n=6; ***(R)*-39** 0.86±0.1pS/pF, n=5). **66** is much less active than **2** or ***(R)*-39** on the three isoforms, its potency being similar to that of ***(S)*-39**. The interaction of **39** with the HCN1 channel is enantioselective (ER = 26), the eutomer being once again the R enantiomer; on HCN2 the eudismic ratio is much lower (4) while the interaction with HCN4 is not enantioselective.

The results of enantioselectivity are somehow puzzling. In fact, in **40** and **39** the stereogenic center is located at the same position as in ivabradine, which has the *S* configuration: this enantiomer has been developed as drug due to its higher specificity for HCN channels with respect to its *R*-isomer [***(R)*-80**], which interacts also with K^+ channels[121,178,179]. Ivabradine and its R enantiomer show the same potency on cardiac HCN channels, but the activity of ***(R)*-80** on homomeric channels has not been reported in the literature.

These reasonings prompted us to make on zatebradine the following structural modifications:

A) The structure of **1** and **2** were hybridized with that of ivabradine, synthesizing compounds ***(S)*-63**, ***(S)*-37** and their R enantiomers ***(S)*-63** and ***(S)*-37**. Moreover, we prepared also ***(R)*-80** and its dihydrobenzazepinone analogue***(R)*-78**, and to extend the comparison, also the S-form of the latter [***(S)*-78**].

B) The chiral center was shifted from the benzylic position to the carbon atom alfa to the basic nitrogen, to give compounds ***(R)/(S)*-62**, ***(R)/(S)*-36** and ***(R)/(S)*-79**.

C) The *trans* analogues of compounds **40** and **39** (compounds **64** and **65**) were prepared, in order to study the effect of *cis/trans* isomerism.

D) Since ***(R)*-39** has not good drug-like properties (high molecular weight, low water solubility of the hydrochloride salt) one of the (7,8-dimethoxybenzazepin-3-yl)butenyl groups on the basic nitrogen has been replaced by a smaller unsaturated moiety, such as allyl, benzyl and crotyl, obtaining compounds ***(R)*-74**, ***(R)*-75** and ***(R)*-76**.

Since the determination of EC_{50} is time-consuming, in order to have a preliminary indication of the activity of the compounds, we decided to speed-up the experiments by measuring the percentage of I_f blockade by the compounds at a fixed dose (5 mM) on HCN1 and HCN4 channels, the two most interesting isoforms. By this way, we could select the most interesting substances on which a better characterization should be performed. Unfortunately, only few of the synthesized compounds have been tested so far, and for this reason we can derive only limited structure-activity relationships.

The results of the tested compounds are reported in **table 2**, in comparison with their close analogues. The *trans* derivatives are always less active than their *cis* analogues as we can see comparing ***(R)*-64** with ***(R)*-40**, ***(R)*-65** with ***(R)*-39** and ***(S)*-65** with ***(S)*-39**, and ***(S)*-63** with ***(S)*-37**. When enantiomeric couples have been tested [***(R)*-65** and ***(S)*-65**], also in the *trans* series the eutomer is the R isomer. ***(S)*-37**, the hybrid between **2** and ivabradine, shows high potency (the I_f is reduced by > 40%) but it is not selective

for HCN1 or HCN4, while ***(S)*-63**, its *trans* analogue, reduces I_f on HCN1 (19 %) but does not show activity on HCN4.

We calculated the ratio between the EC_{50} values as a measure of the selectivity of the compounds for one isoform over the others. We arbitrary select the value 5 as threshold for selectivity, considering isoform-selective a compound showing a ratio higher than 5. The selectivity ratios are reported in **figure 5**. According to this criterion, ***cis*-3**, ***(R)*-40**, ***(R)*-39** and **1** can be considered isoform-selective. ***cis*-3** is more active on HCN4, the selectivity ratios being 17 and 6 for, respectively, HCN4 *vs* HCN2 and HCN4 *vs* HCN1. ***(R)*-40** shows selectivity for HCN2 over HCN4, with a ratio of 11, but it does not discriminate between HCN1 and HCN2. **1** is more potent on HCN1 than on HCN2 (ratio = 7.5) but it does not discriminate between HCN1 and HCN4. ***(R)*-39** is the most potent and selective compounds among those tested so far, since its activity is 170-fold and 30-fold higher on HCN1 than, respectively, on HCN4 and HCN2.

Selective compounds may be useful as drugs, providing they have high specificity for HCN with respect to other ion channels: for instance, a HCN1 selective blocker may be useful for the treatment of neuropathic pain[33], a HCN4 selective inhibitor can be useful to control sinus node rhythm and ventricular arrhythmia[69], a HCN2 selective compound may be useful to study the role of I_f in pathologies where HCN2 channels are overexpressed (i.e. cardiac hypertrophy). Moreover, they may be important tools to study the stoichiometry of the channels in native tissues (as reported in the introduction, there is evidence that *in vivo* the channels may be heteromeric, but the exact composition is not known), providing that they are specific for HCN channels and do not modulate other ion currents, especially at the cardiac level. Therefore, it is important to determine the effect of our compounds on other ion currents; these experiments are carried out at present in the laboratory of Prof. Andras Varro at the University of Szeged (Hungary)

5.2 Conclusions

Our data show that, by manipulating the chemical structure of phenylalkylamines related to zatebradine, it is possible to achieve selectivity, at least on the homomeric channels. The compounds reported in this work represent analogues with different steric and conformational characteristics, and some of them show a different preference for the homomeric HCN channel isoforms. Selectivity may be related to the ability of these compounds to adopt different conformations, to the presence of specific

interactions due to the introduction of additional moieties, or to the different shape or volume of the molecules. Work is underway to derive sound structure activity relationships in this class of compounds in order to optimize their potency and selectivity.

"..quegli che pigliavano per altore altro che la Natura, maestra de' maestri, s'affaticavano invano.."

Leonardo da Vinci

6. BIOLOGICAL METHODS

These experiments were carried out at the department of Preclinical and Clinical Pharmacology by the group of Prof. Alessandro Mugelli and Prof. Elisabetta Cerbai.

Materials and Methods:

6.1 Cell culture and isolation

Human embryonic kidney cells, transfected with mHCN1, mHCN2 and hHCN4 cDNA were cultured in DMEM medium (Gibco, DMEM + GlutaMax^{TM}-I x1) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, 100 μg/ml streptomycin, 200 μg/ml G418 (Gibco) in T25 flasks and incubated at 37 °C with 5% CO_2. When confluent (3-5 days after plating), cells were detached using an enzymatic dissociation with trypsin-EDTA. Digestion was stopped by adding medium, and the sedimented cells were either re-plated or used for electrophysiological measurements.

Electrophysiological Recordings

Prior to electrophysiological recordings, HEK293 cells were incubated in normal Tyrode's solution for 2-3 hours at room temperature. Measurement of f-current was performed by patch-clamp technique in the whole-cell configuration. The experimental set-up for patch-clamp (whole-cell) recording and data acquisition was similar to that described previously. Patch-clamp pipette had a resistance of 4.5-6.5 MΩ when filled with the internal solution. The patch-clamped cell was superfused by means of a temperature-controlled microsuperfusor, allowing rapid changes of the solution bathing the cell. Temperature was maintained in the range of 36 ± 0.5 °C. Cell membrane capacitance (C_m) was measured by applying a ± 20mV pulse from holding potential of -40mV. The f-current was evoked from a holding potential of -20mV to more negative voltages in a range of -40 to -150, in 10mV increments. Cells were superfused with a modified Tyrode's solution. After recording I_f properties in control conditions, the cells were superfused with solutions containing one of the test compounds. I_f amplitude was measured as the difference between the steady-state current and the peak current, measured at the end and the beginning of the hyperpolarizing step, respectively and normalized to C_m. Dose-effect curves were fitted by using the Hill equation: $y= E_{max}[x^n/(k^n+x^n)]$ where E_{max} is the maximum effect, k corresponded to EC_{50}, concentration at which 50% E_{max} is obtained, x the drug's concentration and n the coefficient of Hill. All the results were expressed like mean ± s.e.

6.2 Solutions

The composition of solutions used was the following (in mmol): Tyrode's solution: D-(+)-glucose 10, NaCl 140, KCl 5.4, $MgCl_2$ 1.2, $CaCl_2$ 1.8, HEPES-NaOH 5.0, (pH 7.3); modified Tyrode's solution: Tyrode's solution containing 25mM KCl in order to amplify I_f. Pipette solution: K-aspartate 130; Na_2-ATP 5, $MgCl_2$ 2, $CaCl_2$ 5, EGTA 11, HEPES-KOH 10 (pH 7.2; pCa 7.0). Drug solutions were obtained from stock solutions (10^{-2}M) in water and diluted in the different modified Tyrode's solution to reach the final concentration (range 1 -30 μM).

6.3 Figures and Charts

Figure 1. Effect of **2** (EC32) on HCN1. A: Current recordings at -120mV in control condition (A) and superfusing with **2** 10μM (B). C: Activation curve of I_f current in absence (black line) and in presence of **2** 10μM (grey line). D: f-current amplitude elicited by steps to -120mV in presence of increasing doses (1-30μM) of **2**.

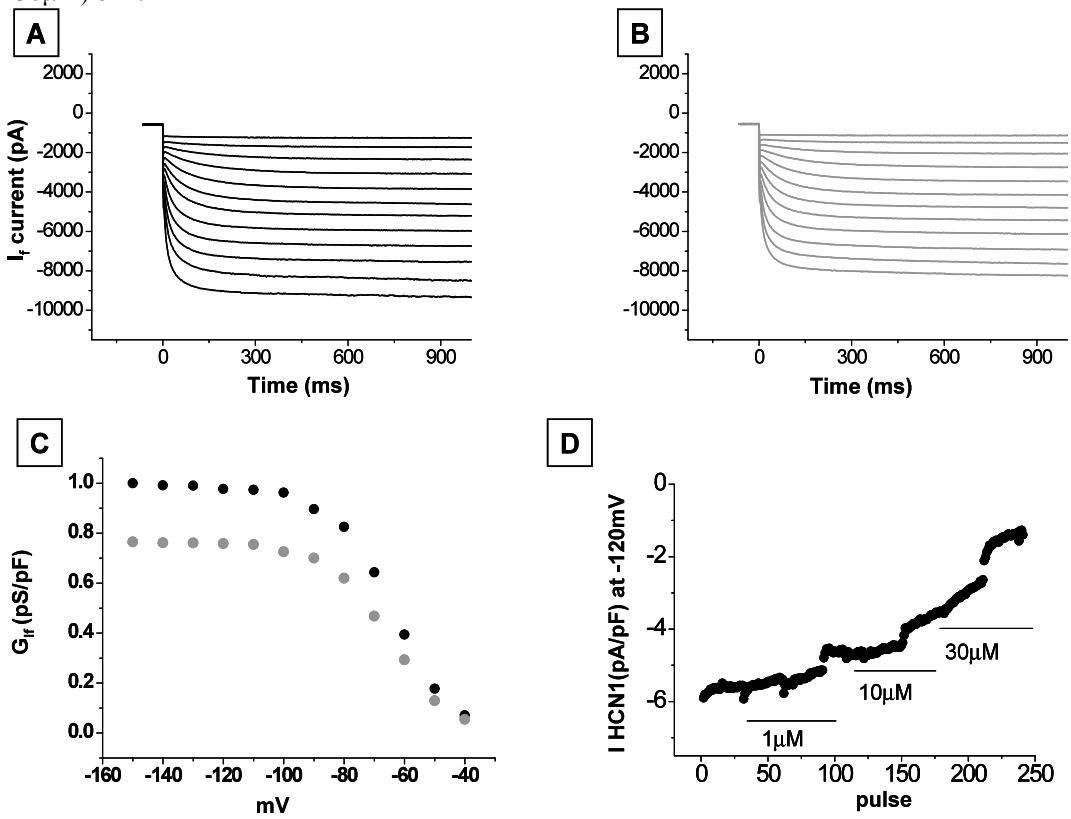


Figure 2. Effect of **1** (EC4) 10μM on HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C).

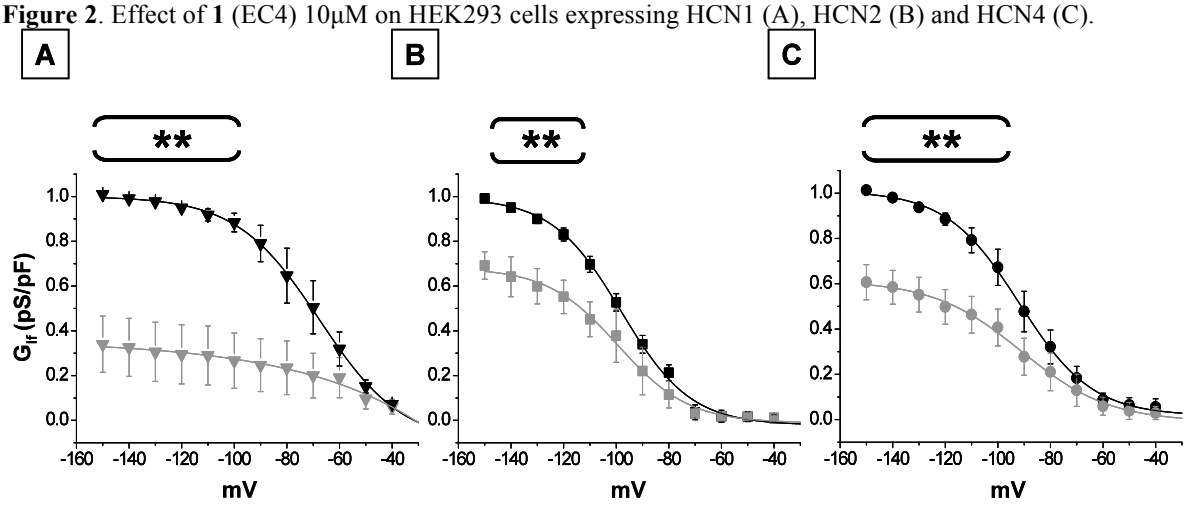


Figure 3. Effect of ***cis*-3** (EC18) 10μM on HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C).

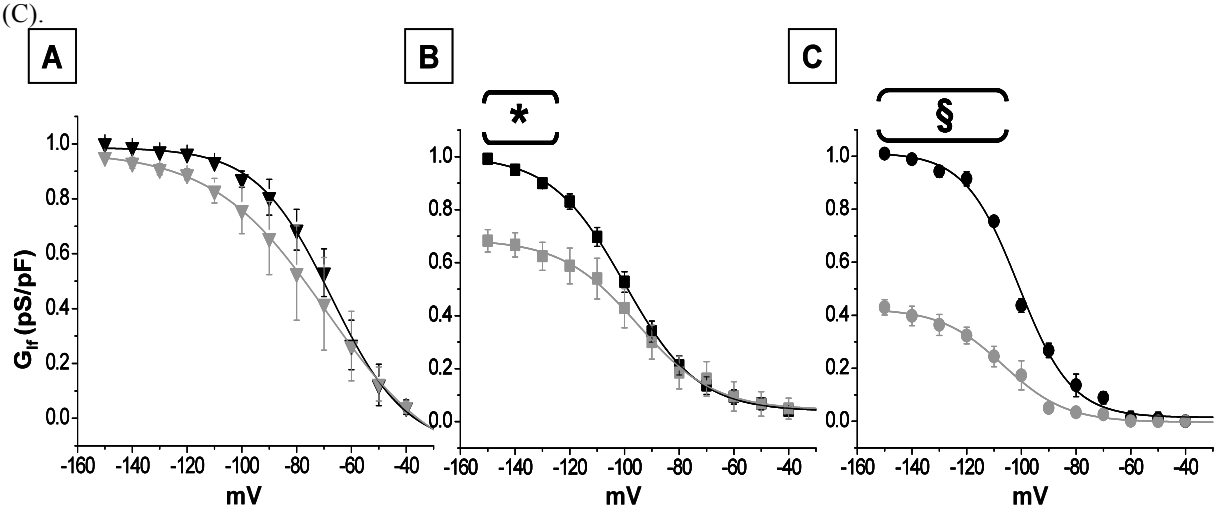


Figure 4. Effect of ***(R)*-39** (MEL57A) 10µM on HEK293 cells expressing HCN1 (A), HCN2 (B) and HCN4 (C).

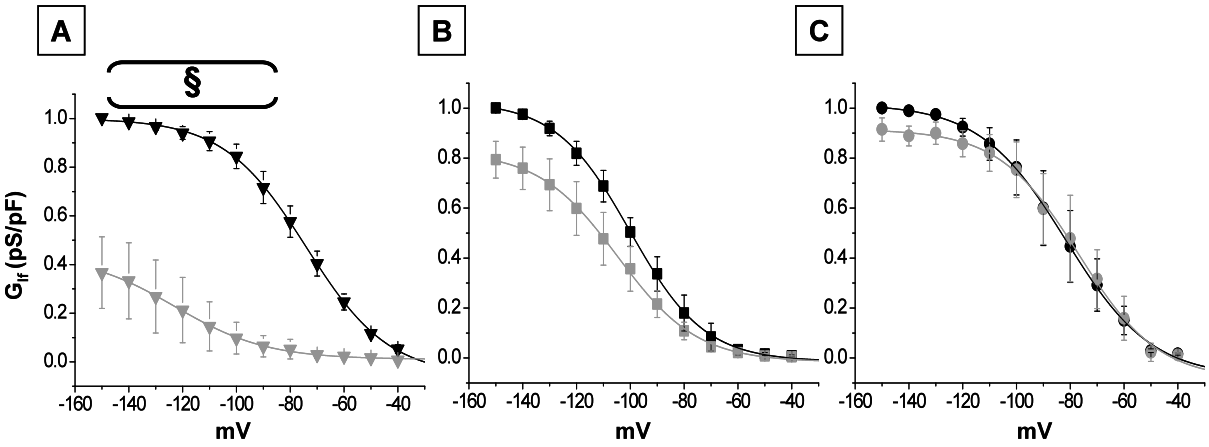


Figure 5. Selectivity ratios of selected compounds on the three channel isoforms. Blue: HCN1/HCN2. Red: HCN4/HCN1. Green: HCN4/HCN2

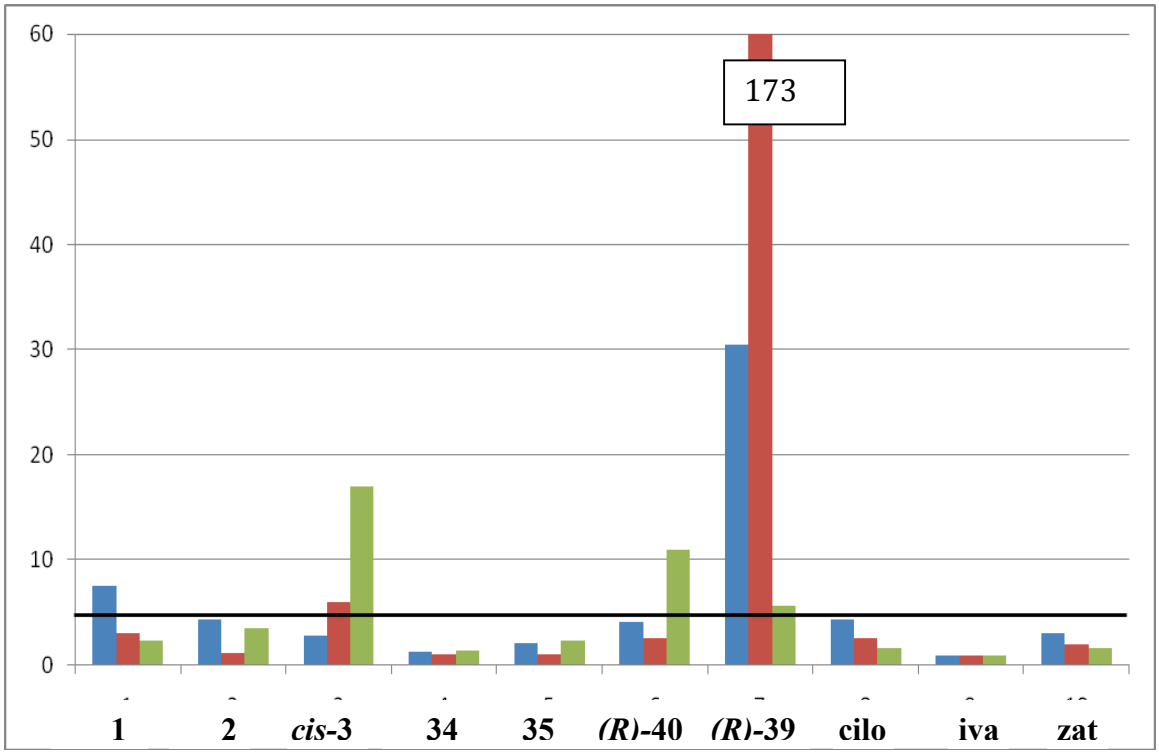


Table 1. I_f-blocking potency of selected compounds in comparison with ivabradine, cilobradine and zatebradine.

compound	EC_{50} (μM) mHCN1[a]	ER[b]	EC_{50} (μM) mHCN2[a]	ER[b]	EC_{50} (μM) hHCN4[c]	ER[c]
1 (EC4)	2.31 ± 0.37	-	17.22 ± 1.74	-	7.23 ± 2.60	-
2 (EC32)	5.60 ± 0.26	-	24.58 ± 4.89	-	6.81 ± 0.37	-
***cis*-3** (EC18)	30.82 ± 0.95	-	87.15 ± 25.61	-	5.19 ± 0.63	-
34 (MEL41)	25.5 ± 3.22	-	19.2 ± 9.20	-	27.68 ± 7.40	-
35 (MEL47)	12.34 ± 0.20	-	5.78 ± 4.30	-	13.63 ± 2.00	-
***R*40** (MEL55A)	9.41 ± 0.25	3	2.3 ± 0.60	11	24.94 ± 0.10	1
***S*40** (MEL55B)	31.27 ± 6.69		25.47 ± 3.70		31.59 ± 3.49	
66 (PK1)	106.9 ± 49.9	-	147.4 ± 0.62	-	70.67 ± 23.80	-
***R*39** (MEL57A)	0.60 ± 0.07	26	18.3 ± 0.14	4	103.78 ± 29.80	1
***S*39** (MEL57B)	17.94 ± 0.48		50.11 ± 11.9		135.73 ± 74.70	
Ivabradine	4.50 ± 0.44	-	4.52 ± 2.82	-	4.28 ± 0.40	-
Zatebradine	16.78 ± 0.9	-	5.45 ± 1.04	-	8.57 ±1.85	-
Ciloradine	4.66 ± 1.44	-	20.47 ± 4.77	-	12.01 ± 3.78	-

[a] Mouse HCN channel expressed in HEK 293 cells. [b] Eudismic Ratio: ratio between the activity of the two enantiomers. [c] Human HCN channel expressed in HEK 293 cells.

Table 2. Blockade of I_f at 5 mM, in comparison with ivabradine.

compound	mHCN1[a] (%)	hHCN4[c] (%)
1 (EC4)	64.9	43.7
2 (EC32)	47.4	41.0
***S*63** (PK8)	19.0	0
***S*37** (PK9)	45.3	42.7
***R*64** (PK10)	13.2	24.2
***S*64** (PK11)	/	/
***R*40** (MEL55A)	37.5	17.1
***S*40** (MEL55B)	9.2	10
66	18.5	24
***R*65** (PK12)	37.5	23.2
***S*65** (PK13)	3	0
***R*39** (MEL57A)	73.0	5.8
***S*39** (MEL57B)	27.8	5.3
Ivabradine	52.8	51.4

[a] Mouse HCN channel expressed in HEK 293 cells. [c] Human HCN channel expressed in HEK 293 cells.

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"Genome biology is shining a bright light on the origins of human disease."

S.L. Schreiber

Expanding the potential of enyne metathesis chemistry toward high skeletal diversity

10-months training period (Nov2008-Aug2009)

Department of Chemistry and Chemical Biology, Harvard University

Howard Hughes Medical Institute, Broad Institute

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1. INTRODUCTION

1. 1 Small molecule: the missing piece in the "central dogma"

Recently[1], the crucial role of small molecules in biological systems has been highlighted including them in the "central dogma" of biology (**Figure 1**). According to this principle the information into cells flows through macromolecules, from DNA to RNA to proteins. Nowadays Chemical Biology provides the missing piece in the central dogma, which is small molecules.

In fact they are key players in a range of topics at the heart of the life sciences such as memory, cognition, sensing, signaling and treatment of diseases. Moreover connections exist between the three family of macromolecules and small molecules in both directions.

Small molecules can bind and modulate DNA, RNA or proteins functions and these macromolecules have been used as templates for design and synthesis of small molecules.

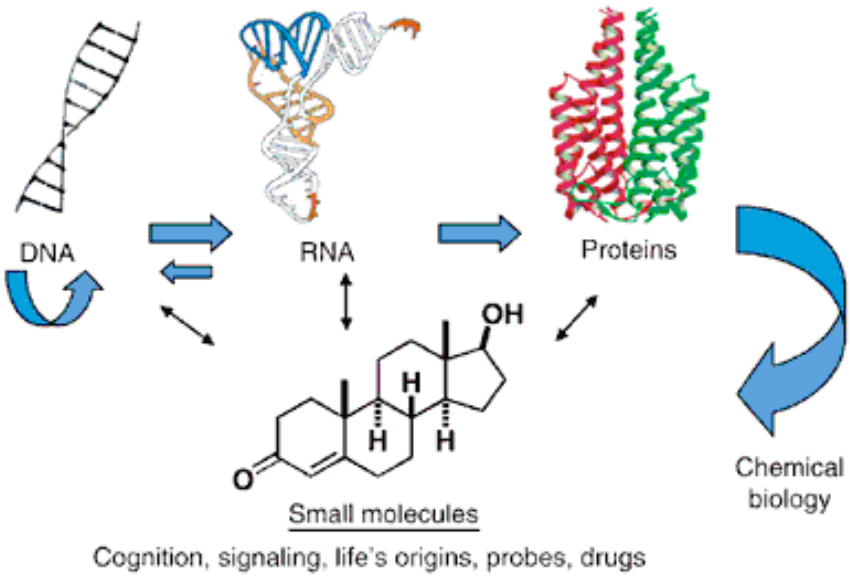


Figure 1. Central Dogma (as viewed by a chemical biologist).

1. 2 The Role of Organic Chemistry

Given the powerful role of small molecules both as probes for understanding cell states and circuits and as pharmacological agents for promoting and restoring health, the new intriguing challenge for synthetic organic chemistry[2] is to become the tool to gain

access to collections of compounds in highly efficient way. In other words, the key for synthetic organic chemistry is to set itself on a course which allows it to tackle any problem in biology, from the dissection of a pathway to the understanding of complex networks, and eventually even to global biology or molecular physiology. For organic chemistry to play an initiating role in biological investigation, it is important that the organic chemist is able to direct effectively synthetic chemistry efforts toward a set of probes of two sorts.

In the first case, a chemist might want to prepare small molecules having overall properties never seen before. In technical terms this means that such molecules address a currently poorly populated region of chemical space, which in that way can be interrogated.

In the second case, a chemist might want to prepare small molecules capable of targeting a region of chemical space known to be optimal for modulating an area of biological interest. In this case it will be possible to study and understand the relationship of chemical space to biological space.

Generally chemical or biological space is defined as *n*-dimensional space described by *n* descriptors, which can be of chemical or biological nature and are either computed or measured (**Figure 2**).

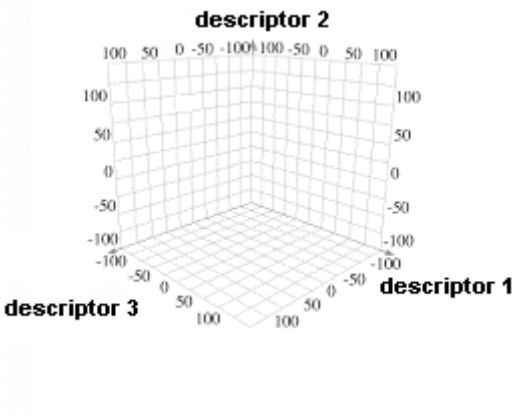


Figure 2. Chemical or biological space representation.

In order to gain access to small molecules, synthetic organic chemistry uses three main approaches:

- Target-oriented synthesis (TOS)

- Targeted Library Synthesis

- Diversity-oriented synthesis (DOS)

1.2.1 Target-Oriented Synthesis (TOS)

This approach[2-4] has a long history in organic chemistry and relies on Nature to discover small molecules endowed with interesting macromolecule-perturbing properties. Natural compounds with an already known or just hypothesized biological activity can be identified in screens of extract mixtures, isolated and then structurally characterized using a variety of spectroscopic techniques.

Once such a structure has been identified, it often becomes the target for chemical synthesis. In terms of chemical space, targeted-oriented synthesis aims at gaining access to a precise region of chemistry space, most often defined by a complex natural product known to have a useful biological function (**Figure 3**).

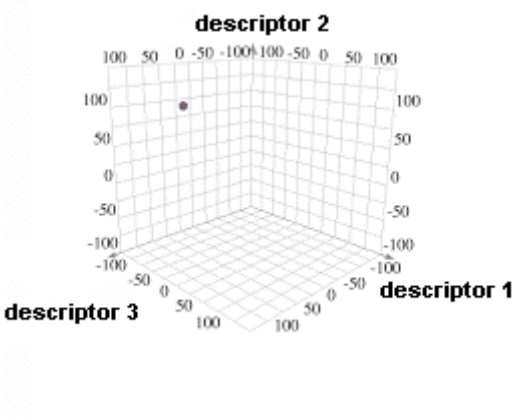


Figure 3. The aim of TOS is to synthesize a single target molecule, which addresses a precise region of chemical space (black sphere).

Target-oriented synthesis of these small molecules can be efficiently planned with *retrosynthetic analysis*[3, 4]. This strategy considers the target molecule as a reaction product for which a substrate and proper reaction conditions have to be sought in order to generate the complex target. For this purpose retrosynthetic analysis is based on the recognition of key structural elements in reaction product that code for synthetic transformations. Repetitive application of this process allows the synthetic chemist to start with a structurally complex target and find structurally simpler retrons that can be used to start the synthesis

As for chemical reactions, two classes are of considerable value and widely used in TOS; the first one is represented by the so-called “fragment coupling reactions” which join together two different building blocks and the second one corresponds to all the family of reactions that generate complexity in stereoselective way (i.e. Oxi-Cope Rearrangement and Diels-Alder Reactions).

1.2.2 Targeted Library Synthesis

The second approach[4] aims to address a dense region of chemical space in proximity to a precise region defined by a molecule known to have useful properties (**Figure 4**). The source of lead compounds can be represented by a bioactive natural product, a known drug or a rationally designed molecule.

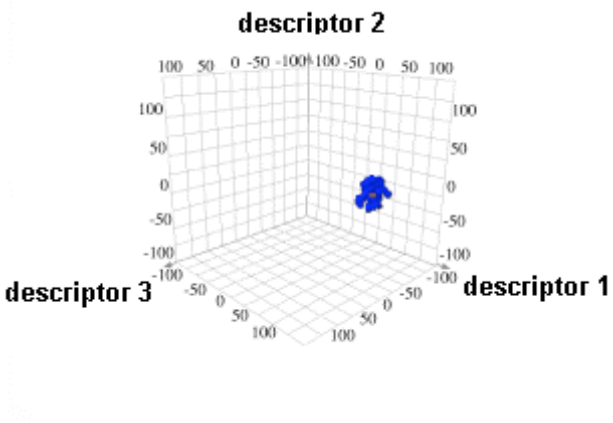


Figure 4. The goal in medicinal and combinatorial chemistry is to synthesize a collection of analogs (blue sphere) of a bioactive target. These compounds populate a dense region of chemical space.

As for planning strategy, collections of compounds analogs to an identified lead (targeted libraries) can still be effectively designed through retrosynthetic analysis.

1.2.3 Diversity-Oriented Synthesis (DOS)

The two previous approaches have led to major advances in the chemical and life sciences, but none of them is able to answer the following question: *Are the regions of chemistry space defined by natural products and known drugs, which have been so intensely scrutinized to date, the best or most fertile regions for discovering small molecules that modulate macromolecular function in useful ways?*

One of the goals of the third general synthetic chemistry-based approach, *diversity-oriented synthesis* (DOS)[2-7], is to meet this challenge.

In DOS, the synthesis effort aims at creating broad representations of compounds in chemistry space, including currently poorly populated (or even vacuous) space (**Figure 5**).

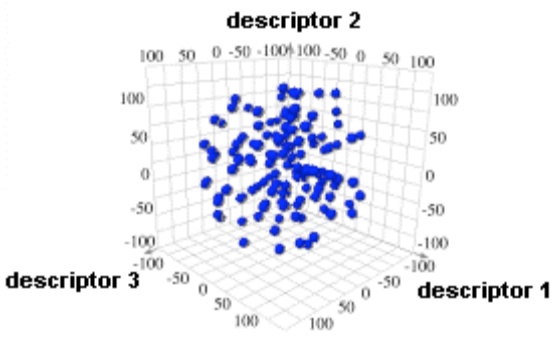


Figure 5. The aim in DOS is to populate chemistry space broadly with collections of diverse and complex small molecules having unknown properties.

The first step toward achieving this goal is to recognize that the problem of accessing broad regions of chemistry space is different from the problem of accessing precise or dense regions and because of this diversity these problems present distinct challenges and require distinct solutions.

DOS, as it has been evolving in the last years, entails the development of synthetic pathways leading to the efficient synthesis of collections of small molecules having structurally complexity, rich skeletal and stereochemical diversity and the potential to attach appendages. Complexity and diversity intuitively favors a greater variation of assay outcomes than would be obtained with a collection of more similar compounds. DOS libraries in fact are not directed toward a single biological target, but their utility is based on their ability to provide selective probes for multiple different biological targets.

In contrast to TOS, diversity-oriented synthesis is not aimed at one particular target and thus, retrosynthetic analysis cannot be applied. However the foundations of retrosynthetic analysis have been used to develop a complementary strategy to facilitate synthetic planning in DOS, named *forward-synthetic analysis*[3,4].

Beginning with a simple building block, this strategy provides a synthetic pathway leading to a large collection of structurally complex and diverse compounds.

In order to achieve compounds with these characteristics, synthetic pathways are branched and divergent and they are planned in the forward-synthetic direction, moving from simple and similar to complex and diverse.

Therefore in forward-synthetic analysis a key element is the transformation of a collection of substrates into a collection of products by performing a number of

chemical reactions in the forward-synthetic direction. To plan an efficient DOS synthesis, it is crucial to identify products-equals-substrates relationships, such that the products of one process could have some common inherent chemical reactivity that makes them all potential substrates for the subsequent reaction.

Skeletal Complexity

The structures and functions of natural products suggest that structural complexity[3,4,8] may be positively correlated with macromolecule-perturbing function and specificity of action. Besides, complexity is important since the increase of the size and number of rigidifying elements in small molecules is essential for these compounds to bind tightly to sites of protein-protein interaction, which tend to be flat in comparison to the concave topography characteristic of enzymes active sites. It is known that many biological processes are critically dependent on protein-protein interactions and many small molecules able to disrupt these interactions are complex natural products.

Therefore one of the goals of DOS is to access small molecules with complex molecular skeletons and forward synthetic analysis proceeds in the direction simple → complex.

For this purpose it is crucial to identify and to implement complexity-generating reactions that rapidly assemble complex molecular scaffolds. Moreover the identification in the forward direction of pairwise relationships, where the product of one complexity-generating reaction is the substrate for a second one, can lead to high levels of complexity in a very efficient manner.

Skeletal Diversity

Small molecule diversity[2-4] is a critical feature to gain access to broad regions of chemical space. Moreover a collection of diverse compounds is essential in chemical genetics assays where there is no one particular target in cell-based or organism-based screens and potentially any one of the entire cell's or organism's macromolecule could be an eventual target.

1.3 How a DOS library should be prepared?

The best, but most difficult, strategy is to make compounds in a way that anticipates problems at each step of the drug-discovery process in which organic synthesis is involved[9]. Usually this process requires three main steps:

1) Find a molecule that modulates a disease: synthesize thousands of structurally diverse compounds.
2) Optimize the biological properties: synthesize analogues of the compounds ideally modifying every atom of the compound.
3) Synthesize the optimal compound

Usually pharmaceutical industry has dealt with each of these steps in a serial fashion and the associated problems have been addressed independently. Each step is challenging and can create bottlenecks in the overall process. DOS aims to address, if not overcome, all of these challenges before the first compounds have even been screened[9].

For doing that, the DOS synthetic route has to follow some particular features as:

- Modularity. Many modifications can in principle be made to each skeleton simply by using different variants of the reactant at the first step. This is ideal for the optimization stage of drug discovery.
- Small number of steps.
- Purification.

The products obtained in this way differ from the compounds found in most small-molecule screening collections. Typically purchased from commercial vendors, the compounds in such collections frequently lack chirality and are structurally simple so they can bind to only a small number of biological targets. Moreover these compounds are structurally similar, where their diversity is limited to variations in appendages attached to a small number of common skeletons[9].

1.3.1 Planning DOS with the build/couple/pair strategy.

The build/couple/pair strategy[10] is aiming at flexible, modular and short constructions of transformative screening collections of small molecules, poised for optimization and modifications in follow-up studies.

This is a very useful approach to plan new DOS libraries in very few steps (ideally from 3 to 5) to rapidly achieve high stereochemical and skeletal diversity. The process is shown in **figure 6**.

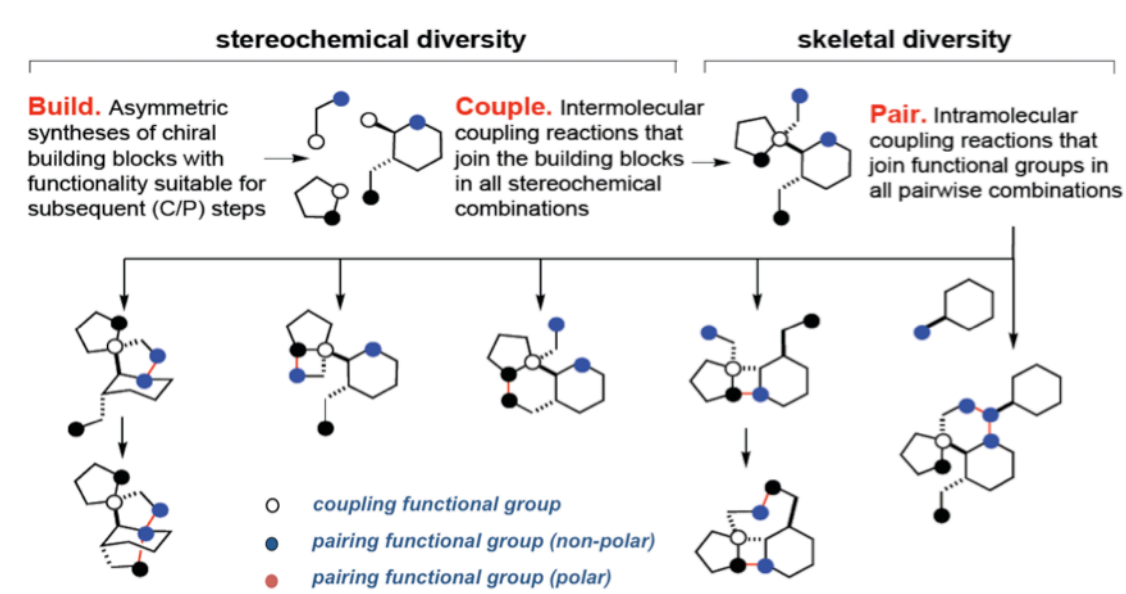


Figure 6. Generation of stereochemical and skeletal diversity with the build/couple/pair strategy.

In the *build phase*, building blocks are synthesized. Usually asymmetric synthesis of chiral building blocks containing orthogonal sets of functionality suitable for subsequent coupling and pairing steps are performed. This process when combined with the couple phase provides the basis for stereochemical diversity.

In the *couple phase*, intermolecular coupling reactions are performed to join the building block previously synthesized. To achieve the full matrix of all possible stereoisomeric products, coupling reactions are used that either generate no new stereogenic elements or that can provide every possible stereoisomeric outcome.

In the *pair phase*, intramolecular coupling reactions are performed that join strategically placed appendages in the building blocks and result in compounds with diverse skeletons. This process provides the basis for skeletal diversity that could be afforded by a chemoselective and intramolecular joining of strategically polar and non-polar functional groups.

1.4 ChemBank v. 2.0

All compounds generated by DOS chemistry are then used to probe a broad swath of biological space; in fact they are tested in a wide variety of biological assays (termed high-throughput screening). The results of these works are then reported to the international research community through a web-based database and analysis environment named ChemBank[11].

1.4.1 ChemBank's history

Since[12] 1997 the National Cancer Institute (NCI) has been interested in Chemical Genetics and some of the successes of this approach has reinforced the belief that it could be a powerful way to accelerate the discovery of potential drugs. To expand this approach the NCI created the Initiative in Chemical Genetics (ICG) and, after a national competition Harvard's Institute of Chemistry and Chemical Biology (ICCB) was chosen as the first recipient of ICG funding.

One of the goals of this collaboration is to create a proper tool to make chemical genetics approach available to the whole scientific community.

For this purpose at ICCB Schreiber and co-workers, who are making a pioneering work in this field both from a chemical and a biological point of view, have been developing a freely available collection of data about small molecules and resources for studying their properties, especially their effects on biology. This powerful tool (ChemBank), is in continuous evolution and since 2006 it is accessible online the renewed, more complete and efficient version 2.0[11].

ChemBank is a suite of informatics tools and databases aimed at promoting the development and use of Chemical Genetics by scientists worldwide. It aims at assisting chemists designing novel compounds or libraries, biologists who wish to identify small molecules that can be used to perturb a particular biological system and serves as a source of data for cheminformatic analyses. The ICCB is populating ChemBank's databases with baseline information generated by biologist's and chemist's work with the additional aim to incorporate the large body of data produced outside ICCB. In fact the growing community of academic and industrial scientists interested in data exchange is encouraged to submit information on the structures and activities of their small molecules.

Analysis and study of biological and chemical knowledge deposited in ChemBank will provide a critical link from genomic discovery to drug development.

As more biological data and small molecule collections are added to this database, more ChemBank should become increasingly powerful.

2. AIM OF THE WORK

During my 10-month training period at Harvard University, I was involved in a project called CMLD (Chemical Methodology and Library Development). The development of new methodologies useful to set up new DOS libraries was the target I was aiming at. With this idea stacked in my mind, I have tried to expand the potential of enyne ring-closing metathesis (enyne RCM)[13a-b], an extraordinary reaction in the context of DOS[14]. The remarkable functional group tolerance combined with the ability to form rings of varied sizes gives this class of reactions a degree of generality not often realized by the vast majority of reactions. Even still, the enyne metathesis products are not well poised for diverse modifications since they are simple 1,3-butadienes (see **figure 7**).

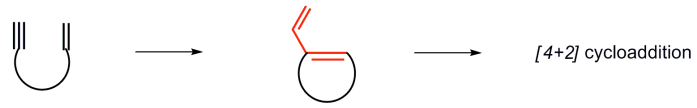


Figure 7. Enyne RCM. The limitation of this extraordinary reaction is that the product obtained is not well poised for diverse modification; in fact, it can undergo only Diels-Alder reaction.

I have therefore turned my attention to the development of metathesis-based methods that rely on alternative substrates, which can be modified post-metathesis to give diverse structures.

The idea was to modify the diene moiety into a dienophile one because of the different reactivity of the two functional groups (see **figure 8**).

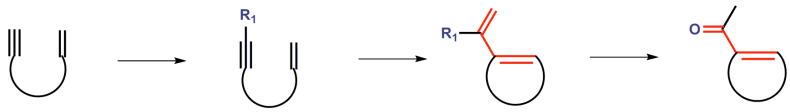


Figure 8. Post-metathesis transformation: from diene to enone for higher skeletal diversity.

Why do I have chosen the enone? The enone has unique features as for its structure. In fact, all the four carbon atoms are very different one from the other in reactivity (**figure 9**), and this peculiarity can allow us to address diverse synthesis manipulating one single atom in order to achieve high skeletal diversification.

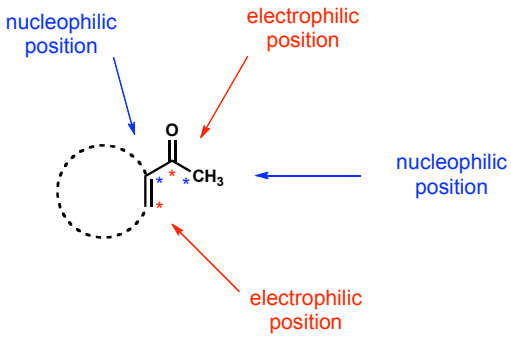


Figure 9. Reactivity of enone.

So, from one single scaffold we can obtain many different structures for higher skeletal diversity as shown in **picture 10**.

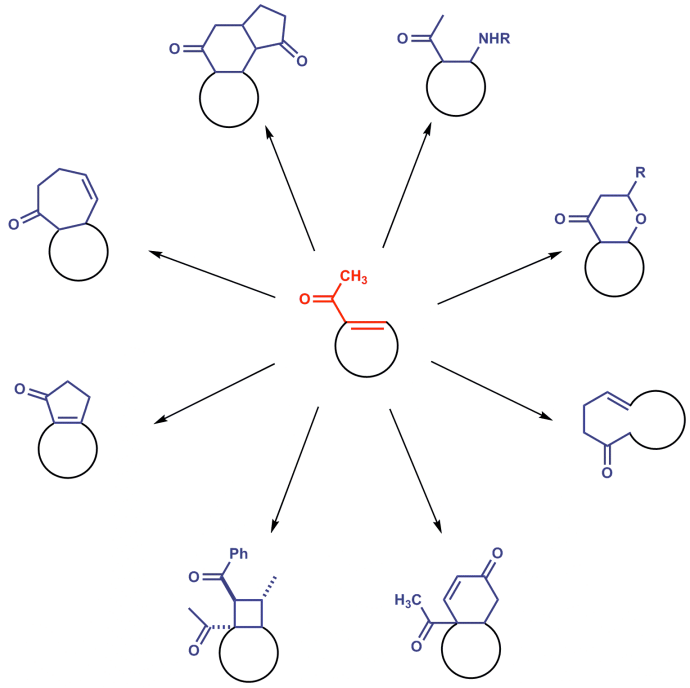


Figure 10. Planning a DOS-library: from one scaffold to many others.

Specifically, I have focused on the chemistry of siloxy and silyl alkynes (R^1 group).

3. CHEMISTRY

3.1 Siloxy alkyne's methodology

I have explored the potential of siloxy alkynes to form substituted dienes poised for protodesilylation to deliver the corresponding enone.

In 2001[15], Kozmin et al. showed the utility of triisopropylsiloxane (-OTIPS) in enyne RCM. They proved that the -OTIPS group did not interfere with the ruthenium of the Hoveyda-Grubbs 2nd generation catalyst (HG-II) during the catalytic cycle of the metathesis reaction, and it means that it is possible to get the formation of the cyclic compound in good yield.

This point is crucial, because usually the presence of the oxygen atom directly bonded on terminal position of the alkyne does interfere with ruthenium due to the formation of a stable complex. As consequence of that, the catalytic cycle of metathesis is stopped and the catalyst goes under degradation without closure of the compound.

For this reason I have decided to use the –OTIPS group as the ideal siloxane to use in the development of a new methodology for setting up a new DOS library.

The formation of the siloxy alkyne (**4**) was the crucial step of the whole synthesis due to the instability of the compound. Many different procedures[16a-d] were followed but only the procedure adapted from the Kozmin's paper[15] was the successful one.

The synthesis (**figure 11**) started with the 1,4-Michael addition of allyl amine to methyl acrilate followed by the protection of the secondary amine as tosylate (**2**)[17]. Then, dibromomethane was deprotonated by lithium tetramethylpiperidine (LiTMP) and the formed anion reacted with the ester to afford the dibromoketone (**3**)[16c,18]. The acidic proton between the two bromine atoms and the carbonyl group was deprotonated by LHMDS and then 2 equivalents of *n*-butyl lithium were added to give the formation of the ynolate that was finally quenched with triisopropylsilyl trifluoromethanesulfonate (TIPS-OTf) to yield the siloxy alkyne (**4**).

In order for the last reaction to work, the atom in β to the carbonyl has not to be an heteroatom but a methylene moiety, because the α protons have to be not acid enough to react with LHMDS and *n*-butyl lithium; in this case in fact, the reaction stops and the product goes under decomposition.

Moreover, given the instability of siloxy alkyne, I have set up a one-pot ynol formation-silylation-enyne metathesis to get the cyclic diene. Once the siloxy alkyne

was formed, the solvent was removed then the crude was immediately dissolved in benzene and HG-II (10% mol) was added to yield **5**.

Finally, protodesilylation occurred treating the compound with TBAF at 0 C giving **6**.

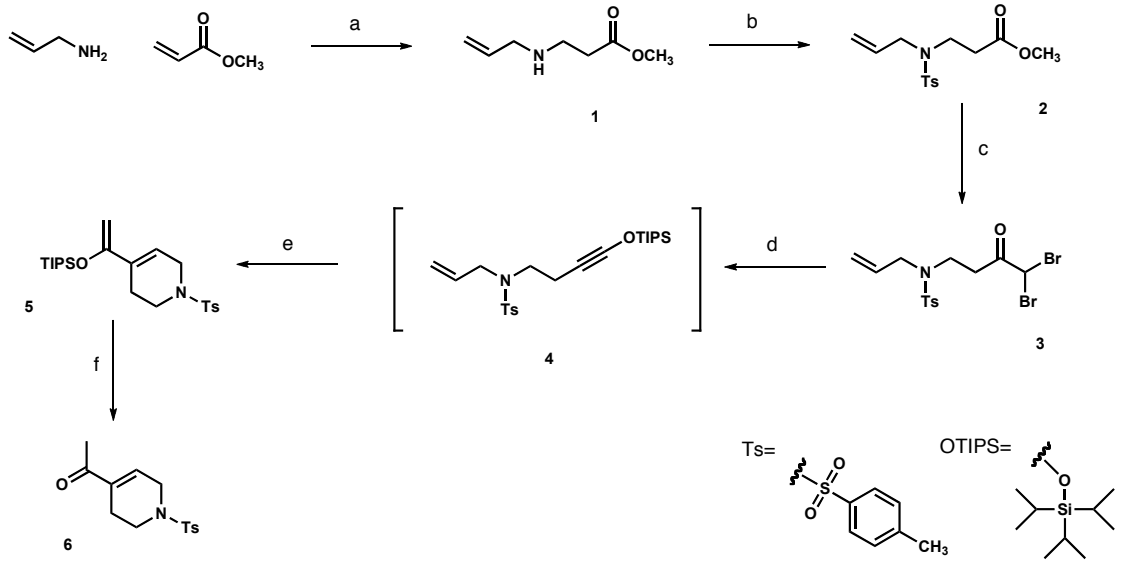


Figure 11. a) methyl acrilate 1eq, allylamine 1.03eq, MeOH 0.1M, 40 C, 4 h. b) **1** 1eq, Ts-Cl 1.2eq, NEt_3 1.2eq, DMAP 0.1eq, DCM 0.2M, rt, 14 h. c) **2** 1eq, CH_2Br_2 2.2eq, *n*-BuLi 2.2eq, 2,2,6,6-tetramethylpiperidine 2.4eq, dryTHF 0.065M, -78 C, 20 min. d) *n*-BuLi 1.05eq, HMDS 1.2eq, **3** 1eq, *n*-BuLi 2.2eq, TIPS-OTf 1.1eq, dryTHF 0.1M, -78 to 0 C, 10 min. e) **4** 1eq, HG-II 0.05eq, benzene 0.05M, 50 C, 30 min. f) **5** 1eq, TBAF 1.1eq, dryTHF 0.1M, 0 C, 10 min.

To expand the potential of the developed methodology, it is possible to form diverse heterocyclic scaffolds poised for further skeletal diversification as shown in **figure 12**, changing the heteroatom and the length of the chain. The synthesis of these scaffolds is still undergoing.

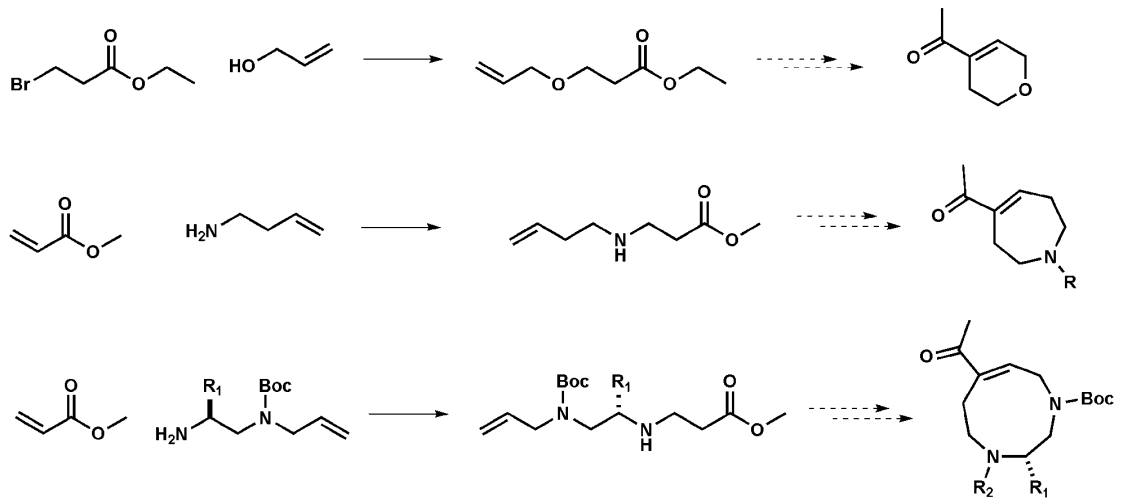


Figure 12. Different heterocyclic scaffolds poised for further skeletal diversification.

Moreover, it is also possible to change the allylamine with chiral homo allylamines in order to achieve stereochemical diversity (**figure 13**).

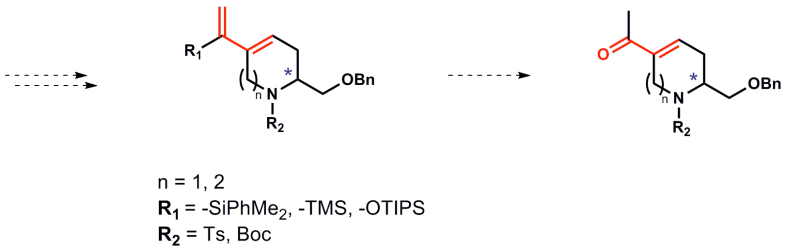
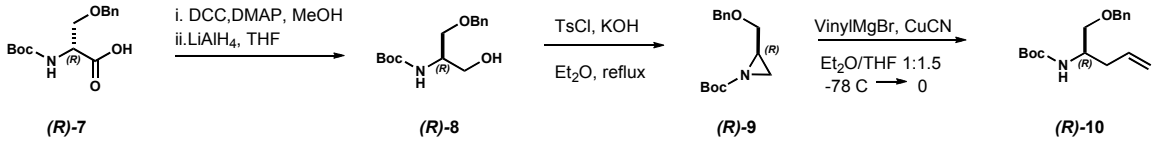


Figure 13. Introducing stereochemical diversity.

To do that, different chiral homo allyl amines with different protecting groups were synthesized.

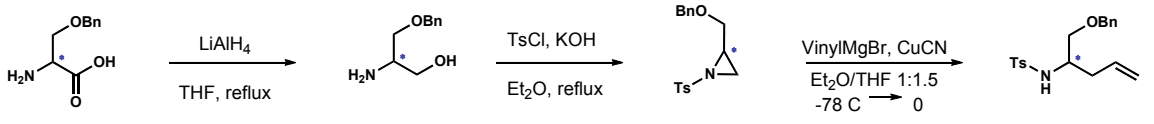
Boc-protected compound ***(R)*-10** was synthesized (**figure 14**) starting from commercially available protected Serine **7**. The carboxylic acid was first activated by DCC, and then was treated with methanol to give the ester that was finally reduced to alcohol ***(R)*-8** with lithium aluminum hydride. Treating compound ***(R)*-8** with tosyl chloride under basic condition has afforded the aziridine ***(R)*-9**, which was then opened with vinyl magnesium bromide to yield the desired compound.



***(R)*-7** ***(R)*-8** ***(R)*-9** ***(R)*-10**

Figure 14. Synthesis of Boc-protected chiral homo allylamine.

The synthesis (**figure 15**) of Ts-protected homo chiral allyl amines ***(R)*-14** and ***(S)*-14** is slightly different from the previous one. In this case, the commercially available enantiopure Serine **11** is not protected on the aminic function. First, the carboxylic acid was reduced to alcohol **12** then a one-pot procedure afforded both aziridine and tosylated amine **13**. The desired compounds were obtained opening the aziridine in presence of vinyl magnesium bromide.



***(R)*-11**
***(S)*-11**
***(R)*-12**
***(S)*-12**
***(R)*-13**
***(S)*-13**
***(R)*-14** [a]ee > 99%
***(S)*-14** [a]ee > 99%

Figure 15. Synthesis of Ts-protected chiral homo allylamine. Both ***(R)*-11** and ***(S)*-11** are commercially available.
[a] ee was determined by SFC using Chiralcel® AS-H column, mobile phase 20% MeOH/ 80% CO_2.

In conclusion, starting from an old methodology I have developed a new method to get substrates containing high skeletal diversifications. The principal features are:

- Versatility and generality of the method for ring size variation.
- Possibility to form a dense stereochemical matrix.
- Formation of diverse heterocyclic scaffolds poised for further skeletal diversification.

Unfortunately, this methodology requires some particular conditions to work, as for example the presence of an ester not directly bonded to atoms bearing acidic protons as described before, and this is a limitation for its generality in other field of organic synthesis.

To overcome to this problem, I have decided to set up a new methodology that can be more powerful and more general than the previous one. So I have focused my attention on the chemistry of silane utilizing as a key reaction the Fleming-Tamao oxidation.

3.2 Toward a new methodology: the Fleming-Tamao oxidation of Silane

The Fleming-Tamao oxidation constitutes a powerful method for the conversion of silanes into alcohols[19] (**figure 16**). In fact the silyl group masks the hydroxyl group, and this particular behavior is very useful in organic synthesis because silanes are more stable and less reactive than alcohols under different reaction conditions.

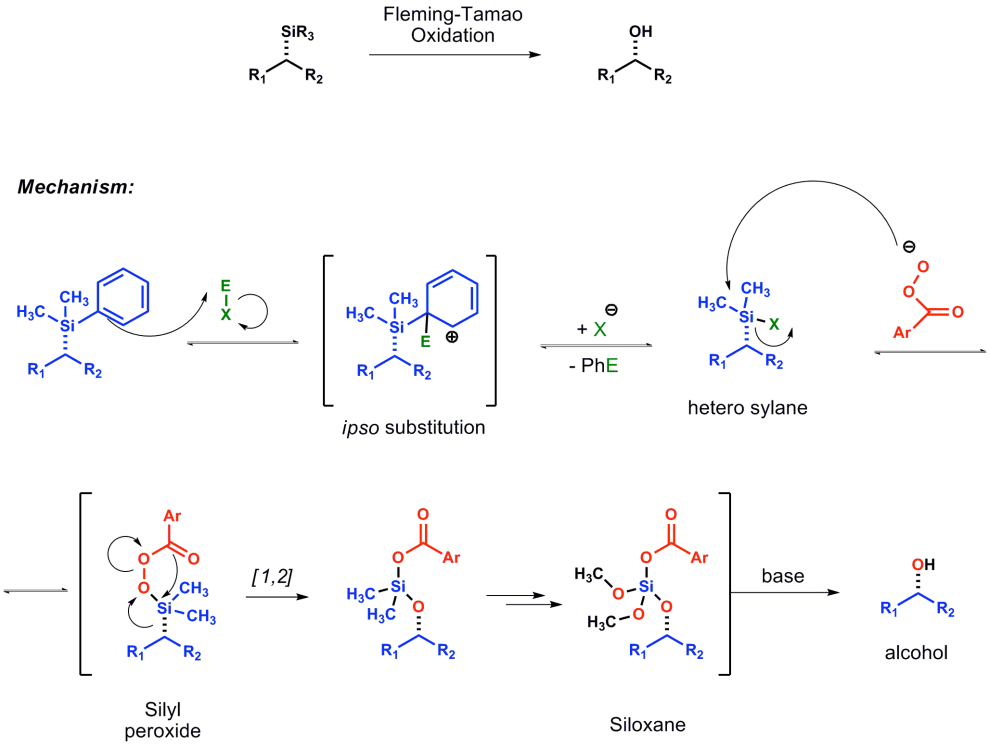


Figure 16. Fleming-Tamao oxidation: the conversion of silanes into alcohols.

Also for this new methodology, the goal was to get properly substituted compounds, which can be modified post-metathesis reaction to give the enone.

I have set up a new method that generates enone from substituted diene through enyne RCM in only three steps: silylation, metathesis reaction and Fleming-Tamao oxidation.

The first step was the silylation of the terminal alkyne of N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide[20], used as testing compound. Then, the silylated product reacted with alkene and underwent efficient enyne RCM to provide a cyclic substituted diene (**figure 17**). Many different silyl groups were screened (**table 1**) in order to find the best one over the two steps.

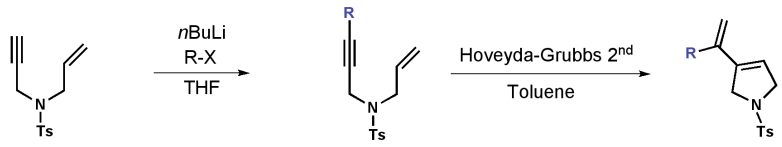


Figure 17. General scheme of silylation and enyne RCM.

In the silylation step, the terminal alkyne portion of the substrate was first deprotonated by *n*-butyl lithium to form the lithium salt that was then quenched with an excess of the silyl group. From the results summarized in **table 1**, it seemed clear that the silyl group (**R** group) required an halogen (**-X**) as leaving group to react with the substrate. In fact entries **2** and **3**, which have respectively a -methoxy or -etoxy leaving group, did not give the silylated compound.

All the other compounds (entries **1**, **4-8**) were screened in enyne RCM step, utilizing different procedure and conditions as for catalyst[21a-c] (Grubbs 1st gen., Grubbs 2nd gen., $InCl_3$ and Hoveyda-Grubbs 2nd gen.), atmosphere[22] (argon or ethylene gas), solvent (toluene, benzene and dichloromethane) and temperature (from room temperature to 50 C).

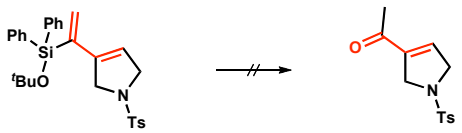
Entry	R group	-X	Silylation yield%	cpd.	Enyne RCM yield%	cpd.	Entry	R group	-X	Silylation yield%	cpd.	Enyne RCM yield%	cpd.
1	$SiClMe_2$	-Cl	80%	15	NO	-	5	$SiPh_2O^tBu$	-Cl	95%	17	60%	21
2	SiMePhOMe	-OMe	NO	-	-	-	6	$SiMe_2H$	-Cl	76%	18	NO	-
3	$SiPh_2OEt$	-OEt	NO	-	-	-	7	$SiPhMe_2$	-Cl	72%	19	91%	22
4	$SiMe_2OMe$	-Cl	32%	16	NO	-	8	TMS	-Cl	91%	20	77%	23

Table 1.

Silylated compounds bearing other leaving groups (entries **1** and **4**) or mobile proton (entry **6**) did not give the cyclic products under any condition.

The best results were obtained with entries **5**, **7** and **8** that were utilized in the next and final step.

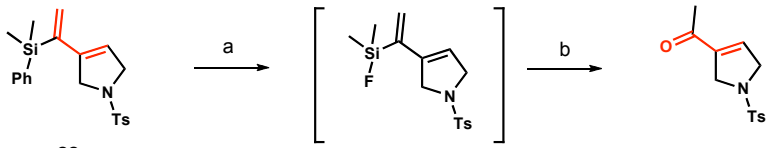
Regarding on entry **5**, compound **21** did not undergo Fleming-Tamao oxidition protocol (**scheme 18**); many procedures[23a-b,19d] and attempts were followed under different condition without any result.



21 25

Figure 18. Unsuccessful application of Fleming-Tamao oxidation protocol to diene **21**.

If the R group was the phenyldimethylsilane (entry **7**), the resulting metathesis product was subjected to a two-steps Fleming-Tamao protocol to yield the enone **25**. According to literature[23b,19d], the first step was the formation of the fluorosilane **24** (not isolated) followed by the oxidation with *m*-CPBA (**figure 19**).



22 24 25

Figure 19. Application of Fleming-Tamao oxidation to properly substituted diene.
a) $BF_3 \cdot 2AcOH$ or $HBF_4 \cdot OEt_2$, DCM. b) *m*-CPBA, KF, DMF.

When the R group was instead the trimethylsilane (entry **8**), a different procedure was pursued (**figure 20**). In fact, the TMS group cannot undergo Fleming-Tamao oxidation due to the impossibility to form the hetero silane. To get the enone **25**, an alternative route was followed: the post-metathesis product **23** treated with *m*-CPBA gave first the epoxide[24] **26**, which was then opened[25] to deliver the corresponding α,β-unsatured ketone.

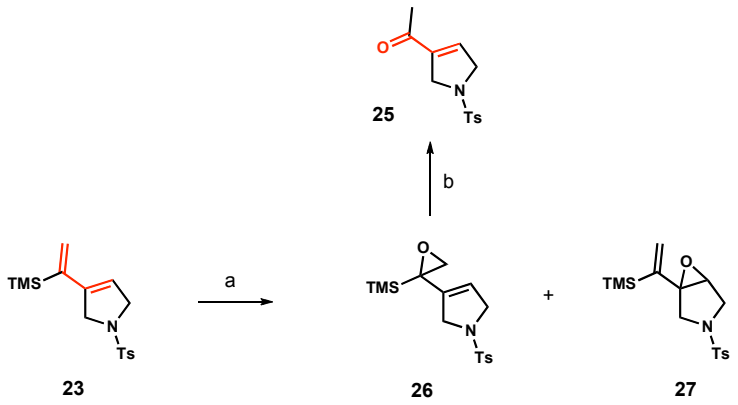


Figure 20. Alternative route to the enone. To notice that the two epoxides formed (**26**, **27**) after the first step were easily separated by column chromatography in a ratio 5 to 1.
a) *m*-CPBA, $NaHCO_3$, DCM. b) H_2SO_4/MeOH.

In conclusion, the new developed methodology is much more powerful respect of the previous one. In fact, the formation of substituted diene and its conversion into the corresponding enone is achieved in few steps with high yields. Moreover, this new method is also more general because, theoretically, it can be applied at any point of the synthesis and over different scaffolds.

The principal features are:

- Appropriate silane groups can be easily introduced in diverse molecular scaffolds bearing a terminal alkyne.
- The silyl-functionalized enyne efficiently undergoes metathesis reaction.
- The silyl-substituted diene is poised for post-metathesis modification leading to the α,β-unsatured ketone.

3.3 Conclusion

I have carried out new methodological studies on the synthesis of siloxy- and silane-substituted alkynes to expand the potential of enyne metathesis chemistry.

The developed methodologies opened new avenues to rapidly achieve skeletal diversity in a library setting from efficient synthetic manipulations of properly functionalized 1,3-dienes.

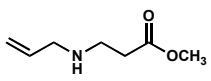
4. EXPERIMENTAL SECTION

Material and Methods:

Dry solvents were dispensed from a solvent purification system that passes solvents through packed columns (THF and CH_2Cl_2: dry neutral alumina; toluene: dry neutral alumina and Q5 reactant). Unless otherwise stated, all reagents were obtained from commercial sources and used without further purification. Infrared spectra were recorded on a Nicolet IR100 FTIR from Thermo Scientific. ^1H NMR spectra were recorded on Varian Unity/Inova I500 (500MHz) spectrometer. ^1H data are reported as follows: chemical shift in parts per million relative to residual protonated solvent ($CHCl_3$: d 7.26, C_6H_6: d 7.15), multiplicity (s, singlet; d, doublet; t, triplet; q, quartet; m, multiplet; br, broadened), coupling constant (Hz), and integration. ^{13}C magnetic resonance spectra were recorded on Varian Unity/Inova I500 and I600 (126MHz) spectrometer. ^{13}C chemical shifts are reported in parts per million relative to solvent ($CHCl_3$: d 77.0). All ^{13}C spectra were determined with broadband decoupling. Microwave heating was performed using Explorer®-48 positions, CEM. High-resolution mass spectra were obtained through the Harvard University mass spectrometry facility. All reactions were magnetically stirred and monitored by thin-layer chromatography (TLC) using E. Merck silica gel 60 F254 precoated plates (0.25 mm). Flash chromatography was performed either on EM Science silica gel 60 (230–400 mesh) or using a CombiFlash companion system (Teledyne ISCO, Inc.) with pre-packed FLASH silica gel columns (Biotage, Inc.). Optical rotations were obtained using digital polarimeter Autopol IV (Rudolph research Analytical) with a 1 mL cell and a 1 dm path length. SFC/MS chromatography was performed with a Berger analytic SFC (Waters ZQ Mass Spectrometer) using CO_2 and MeOH or MeOH/*i*-PrOH as the mobile phase and using a Chiralcel® OD-H column, or a Chiralpak® AD-H column purchased from Chiral Technology Inc. (column length: 4.6x250mm, pore size: 5um).

4.1 Siloxane's methodology

Methyl 3-(allylamino)propanoate (**1**)



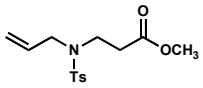
A solution of allyl amine (0.899 mL, 11.96 mmol) and methyl acrylate (1.053 mL, 11.62 mmol) in MeOH (116 mL, 0.1 M) was

stirred at 40 C for 4 hours. The reaction was controlled by TLC in dichloromethane-methanol (9-1).

The solvent was removed under reduced pressure and the crude was purified by flash chromatography. The desired product **1** was obtained (1.12 g, 7.82 mmol, 67.3 % yield) as a colorless-yellowish oil.

^{1}H NMR (CDCl3, δ): 2.50 (t, J=13 Hz, 3H), 2.86 (t, J=13 Hz, 3H), 3.23 (d, J=5.5 Hz, 3H), 3.66 (s, 3H), 5.06-5.17 (m, 2H), 5.82-5.89 (m, 1H).

Methyl 3-(N-allyl-4-methylphenylsulfonamido)propanoate (**2**)

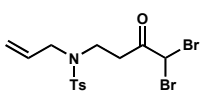


In a 100 mL pear flask, **1** (0.56 g, 3.91 mmol) was dissolved in dichloromethane (19.56 mL, 0.2 M) and triethylamine (0.651 mL, 4.69 mmol) and DMAP (0.048 g, 0.391 mmol) were added. Then, a solution of tosyl chloride (0.895 g, 4.69 mmol) in dichloromethane was added dropwise at 0 C. The reaction mixture was allowed to reach RT and stirred for additional 14 hours.

After addition of *t*-BuOMe, the organic layer was washed consecutively with 1% aqueous solution of HCl, a saturated aqueous solution of $NaHCO_3$, brine and finally was dried over Na_2SO_4. Removal of the solvent under reduced pressure followed by flash chromatography (hexane-ethyl acetate 8-2) gave the desired compound **2** (0.92 g, 3.09 mmol, 79 % yield) as a colorless oil.

^{1}H NMR (CDCl3, δ): 2.42 (s, 3H), 2.63 (t, J=15 Hz, 3H), 3.39 (t, J=15 Hz, 3H), 3.65 (s, 3H), 3.79 (d, J=6.5 Hz, 3H), 5.14-5.20 (m, 2H), 5.60-5.67 (m, 1H), 7.30 (d, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H).

N-allyl-N-(4,4-dibromo-3-oxobutyl)-4-methylbenzenesulfonamide (**3**)

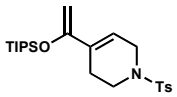


A solution of dibromomethane (0.207 mL, 2.96 mmol) in THF (10 mL) was cooled to -78 C. In a separate flask, a solution of 2,2,6,6-tetramethylpiperidine (0.548 mL, 3.23 mmol) in THF (5 mL) was cooled to 0 C and treated with *n*-butyl lithium (2.5 M in hexane, 1.184 mL, 2.96 mmol), and this yellow LiTMP solution was immediatly added dropwise via an addition funnel to the dibromomethane solution. After 5 min **2** (0.400 g, 1.345 mmol) dissolved in THF (5 mL) was added dropwise (-78 C).

After 10 minutes, the reaction was quenched by slow addition to a 1.2 M aqueous solution of HCl, extracted with ethyl acetate, washed with water, dried over Na_2SO_4, filtered and concentrated. The crude was purified by flash chromatography (hexane-ethyl acetate 8-2) to give **3** (0.35 g, 0.797 mmol, 59.2 % yield) as a colorless-yellowish solid.

^{1}H NMR (CDCl3, δ): 2.43 (s, 3H), 3.27 (t, J=7.5 Hz, 2H), 3.40 (t, J=7.5 Hz, 2H), 3.80 (d, J=6.5 Hz, 2H), 5.17-5.22 (m, 2H), 5.61-5.69 (m, 1H), 5.79 (s, 1H), 7.30 (d, J=8 Hz, 2H), 7.69 (d, J=8 Hz, 2H).

1-tosyl-4-(1-(triisopropylsilyloxy)vinyl)-1,2,3,6-tetrahydropyridine (**5**)



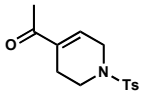
A flame-dried round bottom flask under argon atmosphere was charged with **3** (0.205 g, 0.467 mmol), THF (2.4 mL), and cooled to -78 °C. In a separate flame-dried flask, a solution of HMDS (0.117 mL, 0.560 mmol) was diluted with THF (2.3 mL), cooled to 0 °C and subsequently treated with *n*-butyl lithium (0.196 mL, 0.490 mmol). This colorless LHMDS solution was immediately added to the dibromoketone solution via canula over 5 minutes. After 5 minutes of stirring, *n*-butyl lithium (0.411 mL, 1.027 mmol) was added over a 5 minutes period to the reaction mixture. TLC showed no starting material remaining. TIPS-OTf (0.139 mL, 0.513 mmol) was then added to quench the formed ynolate. The reaction mixture was stirred for 10 minutes and brought to 0 °C, after which time TLC showed predominately one spot corresponding to the siloxyalkyne. The reaction was quenched into 20 mL hexanes *via* canula; water (0.1 mL) was added, the solution was allowed to stir for 10 minutes, and then dried with anhydrous Na_2SO_4. The solution was filtered through a glass frit (fine porosity) and concentrated. The residue was diluted with a small portion of benzene, filtered, and concentrated to afford crude siloxyalkyne (**4**) that was used in the next step without purification due to its instability both on silica gel and on solution.

4 was dissolved in benzene (9.32 mL, 0.05 M) and 10%mol (0.029 g, 0.047 mmol) of Hoveyda-Grubbs 2nd generation catalyst were added. The reaction was left stirring at 50 C for 1 hour and a variation of color from green to brown was observed. The TLC showed a new product and no starting material remaining, so the solvent was removed under vacuum and the residue was purified via flash chromatography (hexane – ethyl

acetate 10-1) to afford the desired cyclic compound **5** (0.073 g, 0.168 mmol). The yield over the two steps was 36%.

^{13}C NMR (CDCl3, δ): 12.70, 18.05, 21.51, 25.25, 42.79, 45.10, 90.13, 119.33, 127.74, 127.79, 129.62, 143.54

1-(1-tosyl-1,2,3,6-tetrahydropyridin-4-yl)ethanone (**6**)

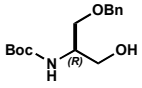


5 (0.020 g, 0.046 mmol) was dissolved in THF (0.459 mL, 0.1 M) and TBAF (0.050 mL, 0.050 mmol) was slowly added at 0 C. The reaction was left stirring at 0 C and controlled by TLC in hexane-ethyl acetate 1-1.

After 10 minutes the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with ethyl acetate. The organic layer was dried on Na_2SO_4, filtered and concentrated. The crude material was purified by prep-TLC (0.5 mm plate) to give **6** (0.007 g, 0.025 mmol, 54.6% yield).

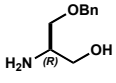
<u>Synthesis of chiral homoallyl amines:</u>

(R)-tert-butyl 1-(benzyloxy)-3-hydroxypropan-2-ylcarbamate ***(R)*****-8**



(R)**-8** was synthesized starting from commercially available (*S*)-*O*-Benzyl-*N*-BOC-serine ***(R)*****-7** by intermediate conversion to methyl ester.[26]

(R)-2-amino-3-(benzyloxy)propan-1-ol ***(R)*****-12**



(R)**-12** was synthesized starting from commercially available *O*-benzyl-L-serine ***(R)*****-11**.[27]

General procedure to synthesize N-protected-Aziridines

(R)-tert-butyl 2-(benzyloxymethyl)aziridine-1-carboxylate ***(R)*****-9**

To a solution of ***(R)*****-8** (48 mg, 0.17 mmol) and TsCl (39 mg, 0.20 mmol) in diethyl ether (3.4 mL) was added freshly powdered KOH (38 mg, 0.68 mmol) at room temperature. The stirred mixture was refluxed until TLC indicates disappearance of the reactants. The mixture was poured into a separatory funnel filled with crushed ice. The organic phase was separated, washed with brine, dried over Na_2SO_4 and the solvent was removed under reduced pressure. The crude was purified

by flash chromatography (20% of ethyl acetate in hexanes) to give the desired aziridine in 60% of yield.

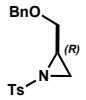
^{1}H NMR (CDCl3, δ): 1.45 (s, 9H), 2.09 (d, J=3.5 Hz., 1H), 2.29 (d, J=6 Hz., 1H), 2.65-2.67 (m, 1H), 3.57-3.59 (m, 2H), 4.61 (ddd, J=37 Hz., J=9 Hz., J=2 Hz., 2H), 7.24-7.35 (m, 5H).

^{13}C NMR (CDCl3, δ): 28.10, 29.20, 36.70, 70.10, 72.90, 81.30, 127.80, 127.90, 128.60, 138.20, 162.10

UPLC–MS: m/z 263.8 $[M + 1]^+$.

$[\alpha]^{20}_D$ = +62.85° (c=0.14; $CHCl_3$).

(R)-2-(benzyloxymethyl)-1-tosylaziridine ***(R)*****-13**



^{1}H NMR (CDCl3, δ): 2.20 (d, J=4.4 Hz., 1H), 2.41 (s, 3H), 2.67 (d, J=7.2 Hz., 1H), 3.00-3.04 (m, 1H), 3.40-3.44 (m, 1H), 3.60-3.63 (d, J=11.2 Hz., 1H), 4.43 (s, 2H), 7.20 (d, J=7 Hz., 2H), 7.27-7.32 (m, 5H), 7.85 (d, J=8.5 Hz., 2H).

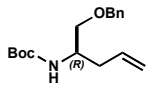
^{13}C NMR (CDCl3, δ): 21.80, 30.90, 39.20, 69.30, 73.10, 127.80, 127.90, 128.30, 128.60, 130.00, 135.10, 138.00, 144.90

UPLC–MS: m/z = 318.28 $[M + H]^+$.

$[\alpha]^{20}_D$ = +46.43° (c=0.14; $CHCl_3$).

General procedure to synthesize homo-allylamines

(R)-tert-butyl 1-(benzyloxy)pent-4-en-2-ylcarbamate ***(R)*****-10**



Vinylmagnesium bromide (17.09 mL, 17.09 mmol, solution 1M in THF) was added to a -78 °C slurry of copper(I) cyanide (0.286 g, 3.19 mmol) in diethyl ether (18.99 mL). After stirring for 20 min, a solution of ***(R)*****-9** (1.5 g, 5.70 mmol) in THF (28.5 mL) was added. After the addition, the reaction mixture was allowed slowly to warm to 0 °C for about 2 h. The mixture was quenched with a solution composed of 10% conc. NH_4OH/ 90% sat. aq. NH_4Cl solution. The mixture was diluted with Et_2O and stirred at room temperature until all solids were dissolved (ca 4h). The layers were separated and the organic layer was washed with brine, dried over Na_2SO_4, filtered, concentrated and purified by flash chromatography (15% ethyl acetate in hexanes) to give the desired ***(R)*****-10** in 60% of yield.

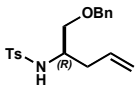
^{1}H NMR (CDCl3, δ): 1.45 (s, 9H), 2.32-2.40 (m, 2H), 3.47-3.49 (m, 2H), 3.82 (bs, 1H), 4.51 (dd, J=25 Hz., J=15 Hz., 2H), 4.82 (bs, 1H), 5.05-5.10 (m, 2H), 5.75-5.81 (m, 1H), 7.26-7.37 (m, 5H).

^{13}C NMR (CDCl3, δ): 28.60, 36.70, 50.10, 71.40, 73.40, 79.30, 117.90, 127.80, 127.90, 128.00, 128.60, 134.80, 138.40, 155.70

UPLC–MS: m/z 314.4 $[M + Na]^+$.

$[\alpha]^{20}_D$ = +12.94° (c=0.17; $CHCl_3$).

(R)-N-(1-(benzyloxy)pent-4-en-2-yl)-4-methylbenzenesulfonamide ***(R)*-14**



^{1}H NMR (CDCl3, δ): 2.29-2.30 (m, 2H), 2.42 (s , 3H), 3.40-3.43 (m, 2H), 4.39 (s, 2H), 5.00 (bs, 1H), 5.03-5.06 (m, 2H), 5.56-5.61 (m, 1H), 7.24-7.26 (m, 2H), 7.30-7.36 (m, 5H), 7.75 (d, J=8.5 Hz., 2H).

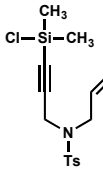
^{13}C NMR (CDCl3, δ): 21.80, 37.00, 53.20, 71.00, 73.40, 118.70, 127.40, 127.90, 128.00, 128.60, 129.80, 133.80, 138.00, 138.10, 143.50

UPLC–MS: m/z 345.9 $[M + H]^+$.

$[\alpha]^{20}_D$ = +27.37° (c=0.095; $CHCl_3$).

4.2 Silane's methodology

N-allyl-N-(3-(chlorodimethylsilyl)prop-2-ynyl)-4-methylbenzenesulfonamide (**15**)

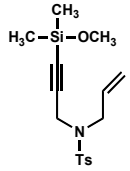


In a 25 mL dried-pear flask under Argon atmosphere, N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide (0.1 g, 0.401 mmol) was dissolved in dry THF (4.01 mL, 0.1 M) to give a colorless solution, then *n*-butyl lithium (2.5M in hexane; 0.160 mL, 0.401 mmol) was carefully added at -78C. The solution changed color and became pale yellow. After 1 hour the dichlorodimethylsilane (0.483 mL, 4.01 mmol) was added. The reaction was controlled by TLC in hexane-ethyl acetate (9-1).

After 1 hour at -78C, the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with dichloromethane (3 × 15 mL). The organic layers were washed once with water and once with brine and then collected, dried on Na_2SO_4 and evaporated under vacuum. The residue was finally purified via flash chromatography and the desired product was obtained as a clear oil in 80% yield (0.11 g, 0.322 mmol).

^{1}H NMR (CDCl3, δ): 0.00-0.14 (m, 6H), 2.42 (s, 3H), 3.81-3.82 (m, 2H), 4.10 (s, 2H), 5.21-5.27 (m, 2H), 5.67-5.75 (m, 1H), 7.28 (d, J=8.5 Hz, 2H), 7.71 (d, J=8.5 Hz, 2H).

N-allyl-N-(3-(methoxydimethylsilyl)prop-2-ynyl)-4-methylbenzenesulfonamide (**16**)

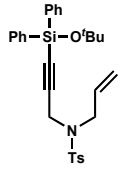


In a 50 mL dried-pear flask under Argon atmosphere, N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide (0.300 g, 1.203 mmol) was dissolved in dry THF (12.03 mL, 0.1 M) to give a colorless solution, then *n*-butyl lithium (2.5M in hexane; 0.481 mL, 1.203 mmol) was carefully added at -78C. The solution changed color and became pale yellow. After 1 hour the chloro(methoxy)dimethylsilane (0.750 mL, 6.02 mmol) was added. The reaction was controlled by TLC in hexane-ethyl acetate (9-1).

After 2 hours at -78C, the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with dichloromethane (3 × 15 mL). The organic layers were washed once with water and once with brine and then collected, dried on Na_2SO_4 and evaporated under vacuum. The residue was finally purified via flash chromatography and the desired product was obtained as a clear oil in 32% yield (0.13 g, 0.385 mmol).

^{1}H NMR (CDCl3, δ): 0.08 (s, 6H), 2.39 (s, 3H), 3.70-3.73 (m, 2H), 3.79-3.80 (m, 3H), 4.07 (s, 2H), 5.20-5.28 (m, 2H), 5.67-5.75 (m, 1H), 7.28 (d, J=8.5 Hz, 2H), 7.71 (d, J=8.5 Hz, 2H).

N-allyl-N-(3-(tert-butoxydiphenylsilyl)prop-2-ynyl)-4-methylbenzenesulfonamide (**17**)

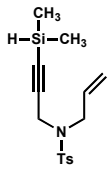


In a 50 mL dried-pear flask under Argon atmosphere, N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide (0.300 g, 1.203 mmol) was dissolved in dry THF (12.03 mL, 0.1 M) to give a colorless solution, then *n*-butyl lithium (2.5M in hexane; 0.481 mL, 1.203 mmol) was carefully added at -78C. The solution changed color and became pale yellow. After 1 hour the tert-butoxychlorodiphenylsilane (1.626 mL, 6.02 mmol) was added. The reaction was controlled by TLC in hexane-ethyl acetate (9-1).

After 2 hours at -78C, the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with dichloromethane (3 × 15 mL). The organic layers were washed once with water and once with brine and then collected, dried on Na_2SO_4 and

evaporated under vacuum. The residue was finally purified via flash chromatography and the desired product was obtained as a clear oil in 99% yield (0.60 g, 1.191 mmol).

N-allyl-N-(3-(dimethylsilyl)prop-2-ynyl)-4-methylbenzenesulfonamide (**18**)

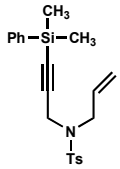


In a 250 mL dried-pear flask under Argon atmosphere, N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide (1.60 g, 6.42 mmol) was dissolved in dry THF (64.2 mL, 0.1 M) to give a colorless solution, then *n*-butyl lithium (2.5M in hexane; 2.57 mL, 6.42 mmol) was carefully added at -78C. The solution changed color and became pale yellow. After 1 hour the chlorodimethylsilane (6.97 mL, 64.2 mmol) was added. The reaction was controlled by TLC in hexane-ethyl acetate (9-1).

After 30 minutes at -78C, the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with dichloromethane (3 × 20 mL). The organic layers were washed once with water and once with brine and then collected, dried on Na_2SO_4 and evaporated under vacuum. The residue was finally purified via flash chromatography and the desired product was obtained as a clear oil in 76% yield (1.50 g, 4.88 mmol).

^{1}H NMR (CDCl3, δ): 0.05-0.06 (m, 6H), 2.42 (s, 3H), 3.81-3.82 (m, 2H), 3.87-3.90 (m, 1H), 4.10 (s, 2H), 5.23-5.30 (m, 2H), 5.71-5.78 (m, 1H), 7.29 (d, J=8.5 Hz, 2H), 7.73 (d, J=8.5 Hz, 2H).

N-allyl-N-(3-(dimethyl(phenyl)silyl)prop-2-ynyl)-4-methylbenzenesulfonamide (**19**)



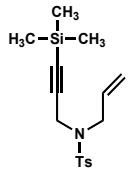
In a 100 mL dried-pear flask under Argon atmosphere, N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide (0.500 g, 2.005 mmol) was dissolved in dry THF (20.05 mL, 0.1 M) to give a colorless solution, then *n*-butyl lithium (2.5M in hexane; 0.802 mL, 2.005 mmol) was carefully added at -78C. The solution changed color and became pale yellow. After 1 hour the chlorodimethyl(phenyl)silane (1.67 mL, 10.03 mmol) was added. The reaction was controlled by TLC in hexane-ethyl acetate (9-1).

After 2 hours at -78C, the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with dichloromethane (3 × 15 mL). The organic layers were washed once with water and once with brine and then collected, dried on Na_2SO_4 and evaporated under vacuum. The residue was finally purified via flash chromatography

and the desired product was obtained as a clear oil in 81% yield (0.625 g, 1.629 mmol).

^{1}H NMR (CDCl3, δ): 0.24 (s, 6H), 2.33 (s, 3H), 3.82-3.84 (m, 2H), 4.18 (s, 2H), 5.22-5.28 (m, 2H), 5.71-5.79 (m, 1H), 7.17 (d, J=8.5 Hz, 2H), 7.33-7.45 (m, 5H), 7.72 (d, J=8.5 Hz, 2H).

N-allyl-4-methyl-N-(3-(trimethylsilyl)prop-2-ynyl)benzenesulfonamide (**20**)

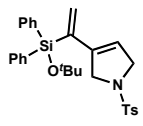


In a 100 mL dried-pear flask under Argon atmosphere, N-allyl-4-methyl-N-(prop-2-ynyl)benzenesulfonamide (0.500 g, 2.005 mmol) was dissolved in dry THF (20.05 mL, 0.1 M) to give a colorless solution, then *n*-butyl lithium (2.5M in hexane; 0.802 mL, 2.005 mmol) was carefully added at -78C. The solution changed color and became pale yellow. After 1 hour the chlorotrimethylsilane (2.54 mL, 20.05 mmol) was added. The reaction was controlled by TLC in hexane-ethyl acetate (9-1).

After 1 hour at -78C, the mixture was quenched with saturated aqueous solution of NH_4Cl and extracted with dichloromethane (3 × 15 mL). The organic layers were washed once with water and once with brine and then collected, dried on Na_2SO_4 and evaporated under vacuum. The residue was finally purified via flash chromatography and the desired product was obtained as a clear oil in 91% yield (0.585 g, 1.820 mmol).

^{1}H NMR (CDCl3, δ): 0.00 (s, 9H), 2.42 (s, 3H), 3.81-3.82 (m, 2H), 4.10 (s, 2H), 5.23-5.30 (m, 2H), 5.71-5.78 (m, 1H), 7.29 (d, J=8.0 Hz, 2H), 7.73 (d, J=8.0 Hz, 2H).

3-(1-(tert-butoxydiphenylsilyl)vinyl)-1-tosyl-2,5-dihydro-1H-pyrrole (**21**)



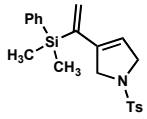
In a 15 mL dried-pear flask, **17** (0.101 g, 0.201 mmol) was dissolved in Toluene (4.01 mL, 0.05 M) to give a colorless solution. Then, under ethylene atmosphere at room temperature, Hoveyda-Grubbs 2nd generation catalyst (0.013 g, 0.020 mmol) was added. After the addition of the catalyst the solution became green and after 20 minutes it became dark green-brown.

The reaction was controlled by TLC in hexane - ethyl acetate (9-1).

The solvent was removed under vacuum and the residue was purified by flash chromatography. The NMR spectrum showed that the product isolated was **21** (0.062 g, 0.123 mmol, 61.4% yield).

^{1}H NMR (CDCl3, δ): 1.17 (s, 9H), 2.43 (s, 3H), 4.08-4.09 (m, 2H), 4.26-4.27 (m, 2H), 5.67-5.68 (m, 1H), 5.75-5.78 (m, 2H), 7.29-7.35 (m, 6H), 7.39-7.42 (m, 2H), 7.58-7.60 (m, 4H), 7.69-7.71 (m, 2H).

3-(1-(dimethyl(phenyl)silyl)vinyl)-1-tosyl-2,5-dihydro-1H-pyrrole (**22**)

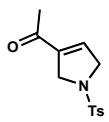


In a 250 mL dried-pear flask, **19** (0.87 g, 2.268 mmol) was dissolved in Toluene (45.4 mL, 0.05 M) to give a colorless solution. Then Hoveyda-Grubbs 2nd generation catalyst (0.071 g, 0.113 mmol) was added at room temperature under ethylene gas and the solution became green. After the addition of the catalyst the solution became green and after 20 minutes it became dark green-brown. The reaction was controlled by TLC in hexanes-ethyl acetate (4-1). After 1 hour, the solvent was removed under vacuum and the residue was purified by flash chromatography. **22** was obtained in 93% yield (0.81 g, 2.112 mmol).

^{1}H NMR (CDCl3, δ): 0.38 (s, 6H), 2.43 (s, 3H), 4.08-4.09 (m, 2H), 4.23-4.24 (m, 2H), 5.38-5.39 (m, 1H), 5.55-5.56 (m, 1H), 5.66-5.70 (m, 1H), 7.31-7.34 (m, 5H), 7.43 (d, J=8.5 Hz, 2H), 7.71 (d, J=8.5 Hz, 2H).

- Fleming-Tamao oxidation: procedure A

1-(1-tosyl-2,5-dihydro-1H-pyrrol-3-yl)ethanone (**25**)



To a solution of **22** (0.089 g, 0.232 mmol) in DCM (2.320 mL, 0.05 M) was slowly added tetrafluoroboric acid-diethylether complex (0.086 mL, 1.160 mmol) at 0 C. The reaction mixture was left stirring at RT for 1 day. The reaction was quenched with saturated aqueous solution of $NaHCO_3$ at 0 C and extracted with Et_2O (3 × 10 mL). The organic layers were collected, washed with brine, dried, filtered and concentrated to give the crude fluorosilane (**24**) that was used in the next step without any purification.

24 was dissolved in DMF (2.320 mL, 0.05 M) and then KF (0.027 g, 0.464 mmol) and *m*-CPBA (0.120 g, 0.696 mmol) were added at 0 C. The reaction was left stirring at room temperature overnight.

The reaction mixture was diluted with dichloromethane and washed with saturated aqueous solution of sodium thiosulfate, saturated aqueous solution of Na_2CO_3 and brine. The organic layer was dried on Na_2SO_4, filtered and concentrated under

vacuum. The residue was finally purified via flash chromatography yielding the desired product **25** (0.036 g, 0.136 mmol, 59% yield).

- Fleming-Tamao oxidation: procedure B

22 (0.036 g, 0.093 mmol) was dissolved in DCM (1.867 mL, 0.05 M) and then BF_3-acetic acid complex (0.185 mL, 0.467 mmol) was slowly added at 0 C. The reaction was left stirring overnight at room temperature.

The reaction was quenched with K_2CO_3 (0.064 g, 0.467 mmol) in water (saturated solution). The mixture was dried on Na_2SO_4, filtered and the solvent was evaporated under vacuum.

The crude fluorosilane **24** (0.026 g, 0.080 mmol, 86 % yield) was dissolved in DMF (3 mL, 0.05 M) and then KF (10.84 mg, 0.187 mmol) and *m*-CPBA (0.064 g, 0.280 mmol) were added at 0 C. The reaction was left stirring at room temperature overnight.

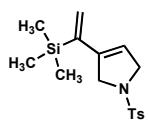
The TLC showed a new product and no starting material remaining, so the mixture was diluted with dichloromethane and washed with aqueous solution of sodium thiosulfate, aqueous solution of Na_2CO_3 and brine. The organic layer was dried on Na_2SO_4, filtered and concentrated. The residue was finally purified via flash chromatography (hexane – ethyl acetate 1-1) yielding the desired product **25** (0.014 g, 0.053 mmol, 56.5% yield).

^{1}H NMR (CDCl3, δ): 2.26 (s, 3H), 2.42 (s, 3H), 4.23-4.25 (m, 2H), 4.34-4.36 (m, 2H), 6.48-6.49 (m, 1H), 7.32 (d, J=8.5 Hz, 2H), 7.72 (d, J=8.5 Hz, 2H).

^{13}C NMR (CDCl3, δ): 21.50, 26.29, 53.28, 55.76, 127.45, 129.88, 133.61, 135.52, 140.41, 143.82, 193.66.

-Alternative route to the enone:

1-tosyl-3-(1-(trimethylsilyl)vinyl)-2,5-dihydro-1H-pyrrole (**23**)



In a 10 mL dried-pear flask, **20** (0.087 g, 0.271 mmol) was dissolved in Toluene (5.41 mL, 0.05 M) to give a yellow solution. Then, under ethylene atmosphere at room temperature, Hoveyda-Grubbs 2nd generation catalyst (0.017 g, 0.027 mmol) was added.

After the addition of the catalyst the solution became green and after 20 minutes it became dark green-brown. The reaction was controlled by TLC in hexanes-ethyl acetate (9-1). The solvent was evaporated under vacuum and the residue was purified via flash chromatography to give the desired product **23** as a white solid in 77% yield

(0.067 mg, 0.208 mmol).

^{1}H NMR (CDCl3, δ): 0.12 (s, 9H), 2.42 (s, 3H), 4.20-4.24 (m, 4H), 5.47-5.52 (m, 2H), 5.59-5.60 (m, 1H), 7.32 (d, J=8.5 Hz, 2H), 7.73 (d, J=8.5 Hz, 2H).

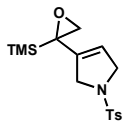
1-tosyl-3-(2-(trimethylsilyl)oxiran-2-yl)-2,5-dihydro-1H-pyrrole (**26**)

In a 25 mL pear flask, **23** (0.11 g, 0.342 mmol) and $NaHCO_3$ (0.043 g, 0.513 mmol) were dissolved in dichloromethane (6.84 mL, 0.05M) to give a colorless solution. Then *m*-CPBA (0.079 g, 0.342 mmol) dissolved in 1 mL of dichloromethane was added dropwise over a period of 2 hours at 0 C.

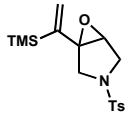
The reaction was left stirring at room temperature for one day and was controlled by TLC in dichloromethane. The TLC showed two new products so the reaction was quenched with $NaHSO_3$ at 0 C. The organic layer was separated and was washed twice with saturated aqueous solution of $NaHCO_3$ and brine. The solvent was dried over Na_2SO_4, filtered and concentrated.

The crude material was purified by column chromatography (the column was equilibrated with hexanes) and was eluted with dichloromethane. The first spot (less polar) eluted was **27** (0.013 g, 0.039 mmol, 11.3% yield) whereas the second one (more polar) was the desired product **26** (0.058 g, 0.172 mmol, 50.2% yield).

(**26**) **^{1}H NMR** (CDCl3, δ): 0.02 (s, 9H), 2.43 (s, 3H), 2.65 (d, J=5.5 Hz, 1H), 2.73 (d, J=5.5 Hz, 1H), 4.01-4.03 (m, 2H), 4.12-4.13 (m, 2H), 5.49-5.50 (m, 1H), 7.32 (d, J=8.5 Hz, 2H), 7.70 (d, J=8.5 Hz, 2H).



(**27**) **^{1}H NMR** (CDCl3, δ): 0.07 (s, 9H), 2.43 (s, 3H), 3.37 (s, 1H), 3.43 (dd, J=12 Hz, J=1 Hz, 1H), 3.53 (d, J=12.5 Hz, 1H), 3.59 (d, J=12 Hz, 1H), 3.70 (d, J=12.5 Hz, 1H), 5.56 (d, J=2.5 Hz, 1H), 5.88 (d, J=2.5 Hz, 1H), 7.31 (d, J=8.5 Hz, 2H), 7.68 (d, J=8.5 Hz, 2H).



1-(1-tosyl-2,5-dihydro-1H-pyrrol-3-yl)ethanone (**25**)

In a 15 mL pear flask, **26** (0.009 g, 0.027 mmol) was dissolved in a solution of sulfuric acid (0.2 mL) in methanol (2 ml) at 0 C. The reaction was left stirring at room temperature for 2 hours and was controlled by TLC in hexane-ethyl acetate (6-4).

The mixture was diluted with ethyl acetate and was washed twice with saturated aqueous solution of $NaHCO_3$ and brine. The organic layer was dried over Na_2SO_4, filtered and concentrated. Purification of the crude material by flash chromatography using ethyl acetate-hexane (1-1) afforded compound **25** in good yield (0.004 g, 0.015 mmol, y. 56%).

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