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SYNTHESIS OF NEW CONFORMATIONALLY
CONSTRAINED
 β -SULFANYL- α - AMINO ACIDS AND THEIR
EXPLOITATION IN THE
SYNTHESIS OF PEPTIDOMIMETICS

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1 Introduction

1.1 From protein to stable peptidomimetics

“Protein structure defines protein function” this is the basic assumption in the field of peptidomimetics proven by studies resulting from the most disparate scientific areas like pharmacology, molecular biology, physical chemistry of macromolecules, computational chemistry and synthetic organic chemistry. Tons of scientific literature in the last 50 years have described the enormous interest in the study of the structure of certain receptors, proteins, peptides in their active conformation. The use of X-ray crystallography has allowed the undoubted clarification, when is possible to obtain a single crystal, of conformations and structures of protein receptors, proteins, and in some cases also of interaction complex between inactive molecule and its protein target in the solid state. The advances of two-dimensional NMR spectroscopy techniques, in particular the NOESY experiments, the CD spectroscopy, and the improvement in the protocol of Molecular modelling have provided a model more and more accurate to observe how these peptides and proteins are structured in solution and some pioneering studies permitted to observe interaction complex in solution. In nature and in the biological systems the function of a protein depends from its structure and finds the way to express itself by the aforementioned protein-protein interactions (PPI). This interaction is present at every level of cellular function and has a key importance in the processes needed for the life. Such interactions are regulated by a variety of different forces: hydrophobic, ionic, hydrogen bonding interactions and van der Waals forces. Such forces take place between the entire contact surfaces of the proteins. To mimic the entire exposed solvent surface of the proteins, in order to develop synthetically feasible peptide mimetics, appears to be unattainable also from the molecular design point of view. The identification of key points of interaction called hot-spots, where the free energy of protein-protein interactions has minimum value, has allowed an easier development of mimetics of such important interaction. The so-called hot-spots tend to be located in those part of the protein highly structured as helical type, turns type or sheet type. The reason of this tendency seems to lie in the ability of those molecular fragments to place in a stable manner, with the right orientation in the three dimensional space, the side chains of key amino acids involved in the PPI. For this reason the identification of synthetic approaches able to produce the stabilization of secondary structure in small peptide mimics of the protein natural fragment or even non peptide structure opens the possibility to mimic a PPI (protein-protein interaction).

The study and synthesis of systems capable to bind and interact with natural target in the same way as the natural peptide sequence from which it derived, have acquired more and more importance in the development of new therapeutic agents, pharmacological tools and of pharmacophore model and biomimetic of macromolecular systems. Such new pharmaceutical entities aim not only to mimic natural peptides but also to enhance potency and selectivity and to overcome all the problems deriving from the use of natural peptides as drugs like low proteolytic stability, low bioavailability and so on.

1.2 Stabilization of helical secondary structure in small peptide fragment.

Small peptide sequences are characterized by high conformation instability and a large number of disordered structures. In order to design small peptidomimetics, different approaches were developed to stabilize secondary structure in such small peptides. β -sheet and α -helix are the most common secondary structures present in natural proteins. Helical structures, in particular, are highly represented on the surface of proteins and play an important role in membrane proteins. In fact trans-membrane domain generally is made by multiple helical fragments, which have the ability to regulate ion channels and transduce chemical signals from out to inside the cell. This process is generally the consequence of a change of conformation following the process of PPI with the chemical entity responsible for the signal. The dysregulation of PPI due to a structural modification usually lead to a disease. For these reasons helical conformation has become one of the most important target in peptidomimetic field of study and different approaches have been developed in order to stabilize such conformation in small peptide sequence or to mimic them with non peptide sequences.¹

1.2.1 Conformational considerations

The secondary structure of a protein fragment directly derives from its primary structure, the sequence of connected amino acids, and the network of intramolecular hydrogen bonds that this primary sequence is able to form ($i, i+4$: α -helix, $i, i+3$: 3_{10} -helix etc). But we cannot forget that the stabilization of the fragment structure usually is conferred by the entire protein structure through a large number of interactions of hydrophobic nature, ionic nature, van der Waals interactions, π interactions and in some cases covalent bonds like S-S bonds.² This is true also in small natural peptides: it is known that in the peptabiotics class, which includes the cephaibol family, a random coil portion seems to have a fundamental role in stabilization and determination of screw sense of a helical folded part.^{3 4} In most cases natural helical protein fragments out from the protein, show high conformational flexibility and present an elevated structural disorder. Such behaviour is connected to the features of natural amino acids that allowed energetically favourable multiple conformations deriving from the permitted free rotation around the backbone bond. Natural peptides are

¹ Special Issue: Protein Design (Guest Ed.: W. F. DeGrado), Chem. Rev. **2001**, 101.

² R. M. Freidinger, Trends Pharmacol. Sci. **1989**, 10, 270–274.

³ G. Bunk-czi, M. Schiell, L. V_rtesy, G. M. Sheldrick, J. Pept. Sci. **2003**, 9, 745.

⁴ U. Orzel, M. De Poli, M. De Zotti, J. Clayden The N-Terminal Nonapeptide of Cephaibols A and C: A Naturally Occurring Example of Mismatched Helical Screw-Sense Control, Chem. Eur. J. **2013**, 19, 16357 – 16365

formed by L α -amino acids and for this reason we could apply the description of torsional angles of one amino acid inserted in the protein to the entire backbone.

The torsional angles of one amino acid inserted in a peptide chain are three: ϕ , ψ and ω (Figure 1). ϕ is the torsional angle define by the two plans $C(O)$ -N and $C\alpha$ -C(O) with rotation around the bond N- $C\alpha$; ψ by the plans N- $C\alpha$ and $C(O)$ -N with rotation of the bond $C\alpha$ -C(O); and ω between the plans $C\alpha$ -C(O) and N- $C\alpha$ that is generally fixed at 180° because the partial double bond character of the amide bond $C(O)$ -N doesn't permit free rotation and generally lies in a less steric hindrance trans conformation. The well know work of Ramachandran et al. has produced the Ramachandran plot that is the graphic representation of the torsion angles ϕ and ψ values allowed for the natural amino acids.

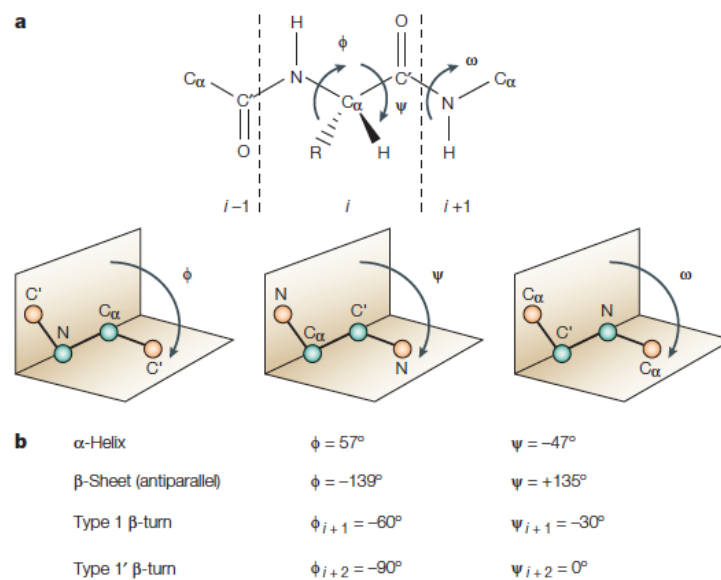


Figure 1.1 Torsional dihedral angles in a natural peptide chain

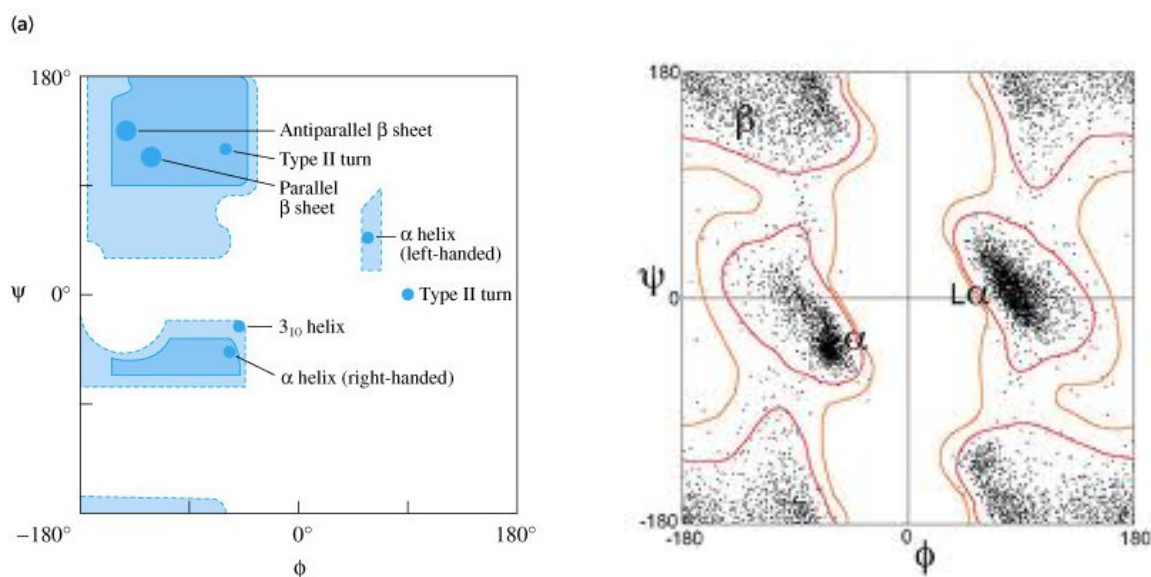


Figure 1.2 Ramachandran plot

The allowed value cover one third part of the plot and the natural L-amino acids are represented in the left part of the plot. The energetically preferred backbone conformations for a peptide are in a Ramachandran plot β sheet, α helix, extended or β turns.⁵ Despite Ramachandran plot, prediction of structure in natural peptides remains extremely difficult due to the dynamic interconversion of natural peptides in different conformations. In fact in a natural peptide of 20 amino acids the possible conformations are around 10^{20} .⁶ Only few examples of conformationally stable peptides are present in nature and reported in literature and most of them in solution are represented by dynamically interconversion of structures.

Natural peptides also possess side chains and we previously underlined the important role of side chains in the PPI for the mediation of the biological function. In the most known protein receptors, the pharmacophore structure is defined by the location in the 3D space and the nature of the side chain that could interact with charged moieties, aromatic portions or hydrophobic chains. So we could identified in the χ torsional angle and other important angle in a peptide structure outside from the backbone that are crucial in order to found a correlation between the structure and activity. The torsional angle of the side chain χ could be defined by the two plan N-C α and C β -C γ and is free to rotate around the bond C β -C α . Considering Newman projection we could identified three low energy possible conformation without a great different in energy that oriented the side chain in completely different portion of the space in respect of the backbone structure. (Figure 3)

⁵ G. N. Ramachandran, C. Ramakrishnan, Biophys. J. 1965,5, 909–933; b

⁶ Venkatraman, S. C. Shankaramma, P. Balaram, Chem. Rev. 2001, 101, 3131–3152

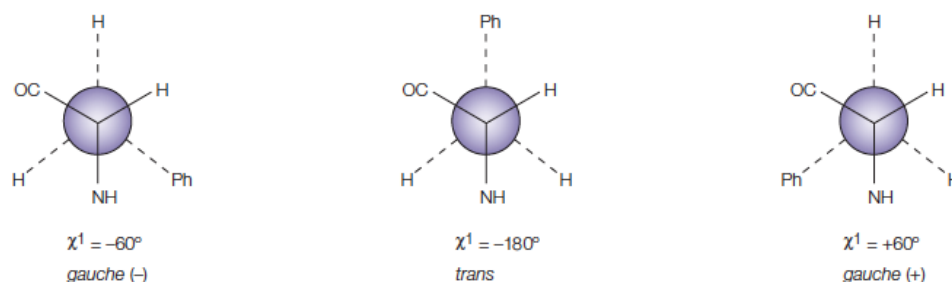


Figure 1.3 χ Dihedral angle of side chain in Newman Projection.

The nature and the bulkiness of the substituent at the α carbon of the amino acid are crucial to stabilize an helical structure instead of sheet, in the discrimination of the helical sense or the type of the helical structure and inversely the secondary structure are vital to place side chain in a correct position.⁷ Ionic and hydrophobic interaction, H-bond between side chains or very bulky group can modulate the energy barrier between the Newman projection and deeply stabilising one instead of the other and so generate different type of turn and consequently secondary structure.⁸ (Figure 4)

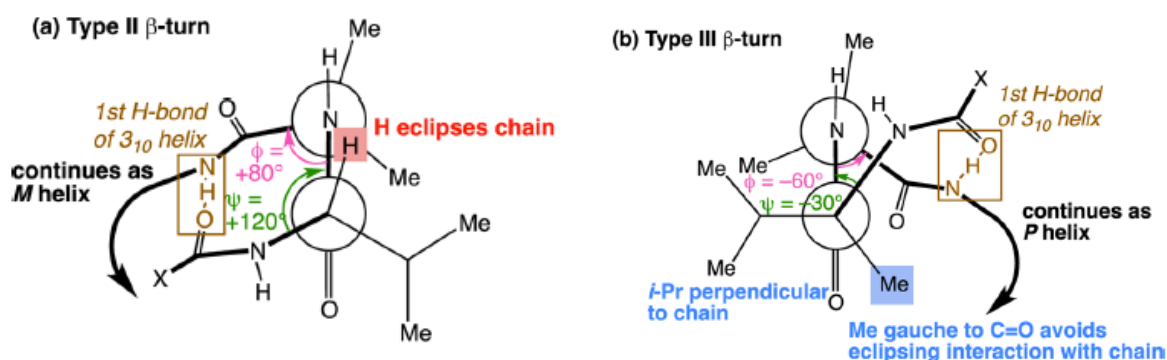


Figure 1.4 Newman projection: steric and electronic influence of side chain on the stabilization and selection of secondary structure

1.2.2 Approaches for structural stabilization of helical conformation.

Different approaches were developed in the last decades starting from various point of view and different solution have arisen exploiting broad field of organic chemistry.⁹ All of them finally follows the aim to

⁷ J. Gante, Angew. Chem. Int. Ed. Engl. 1994, 33, 1699–1720

⁸ M. De Poli, M. De Zotti, J. Raftery, J. A. A. G. A. Morris, J. Clayden J. Org. Chem. 2013, 78, 2248–2255

⁹ D. J. Hill, M. J. Mio, R. B. Prince, T. S. Hughes, J. S. Moore, Chem. Rev. 2001, 101, 3893–4012.

obtain more active, selective and stable compounds through a stabilization of well defined secondary structures. The spring that has pushed the rapid development of such solutions was the extremely high demanding and promising field of peptidomimetics as useful drug candidates or pharmacological tools. Some pioneering works nowadays start to see in the field of peptides, in particularly of helical structured peptides, a source of inspiration and application in biopolymer world as tools for electrical conduction¹⁰ or as mimetic of conduction of receptor signals¹¹.

1.2.2.1 Disulfide bridge

The disulfide bridge is the global restriction strategy that takes inspiration from the nature to stabilize α helix. Natural large protein presents multiple disulphide bridge that stabilize secondary and in some cases tertiary and quaternary structure. Placed at the correct distance, the covalent bound between two residues of cysteine or unnatural cysteine mimics, could form a cyclic peptide that tends to stabilise secondary helix structure. An example reported in literature shows the development of a cyclic enkephaline analogue active against δ -opiate receptor, using a disulphide bridge between two unnatural cysteine amino acids.¹² (Figure 1.5) The limit of such methodology is the possible reduction of the disulfide bridge that leads to destabilization of the helix. More stable different bridges between side chains have been developed to overcome this limitation.

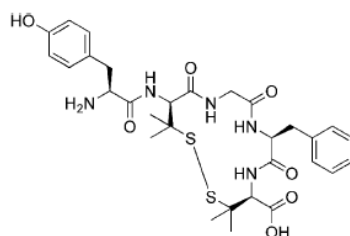


Figure 1.5 Enkephaline analogue

¹⁰ E. Gatto, A. Porchetta., F. Formaggio, C. Toniolo, B. Kaptein, Q. B. Broxterman, M. Venanzi. Conformational Effects on the Electron-Transfer Efficiency in Peptide Foldamers Based on α,α -Disubstituted Glycyl Residues *CHEMISTRY & BIODIVERSITY* – Vol. 5 (2008) 1263

¹¹ J. Sol., S. P. Fletcher, A. Castellanos, J. Clayden. Nanometer-Range Communication of Stereochemical Information by Reversible Switching of Molecular Helicity, *Angew. Chem. Int. Ed.* 2010, 49, 6836 –6839

¹² Korol'kov, V. I.; Vlasov, G. P.; Korol'kova, S. V.; Veselkina, O. S.; Shpakov, A. O.; Denisov, I. G.; Illarionova, N. G.; Petrov, A. N.; Shevchuk, M. K; Enkephalin cyclic analogs with a disulfide bridge between the second and fifth positions *Bioorganicheskaya khimiya* (1989), 15(3), 313-24

1.2.2.2 Lactam Bridge

The formation of inter side-chain lactam cyclization is another common way to stabilize secondary structure, the strong covalent lactam bond could produce an elevated steric constrain that generally improves the bioavailability of the peptide precursor and decreases the enzymatic metabolism with a consequent increase of activity. Parathyroid hormone hPTH is one example of extreme stabilization with lactam bridge placed i, i+4 amino acid residues of α -helix structure.¹³ In any case, also lactam bridges are involved in enzymatic hydrolysis process. An alternative strategy to completely overcome this problem consists in the formation of new C-C bond that became a real possibility with the advent of olefin metathesis reaction.

1.2.2.3 Ring closing metathesis

The olefin metathesis is a fundamental chemical reaction in the selective formation of C-C double bond through two other olefins or alkyne moiety. The first use of Ru complexes as catalyst for olefin metathesis was performed by Grubbs et al, and after that, different generation of catalysts have seen the light with different type of selectivity. RCM metathesis is being widely used to introduce conformational constraints into small peptides, through the generation of cyclic structures from appropriate linear precursors. The hydrocarbon bridges, less is prone to metabolic degradation than lactam and disulfide bridge gave peptide in a stable conformation with favourable pharmacokinetic proprieties. In peptide science it is used mainly to replace disulphide bridges, lactones bridges and thioether, that are the more common and chemically simple way to stabilize peptide secondary structure. For stabilize short peptides in a helical conformation with RCM different approaches were developed.

Stappled approach: It was developed manly by Groubs, Verdini, Debnath, Jacobsen and Toniolo and gave important results in construct small active peptides in highly stable helical conformation. The helix stabilization is achieved placing the linked side chains on the same side of the helix in a position i-i+4 (or i-i+7) for the α -helix and i-i+3 fo the 3_{10} -helix.^{14 15 16 17 18} The best results were obtain with the use of α,α -

¹³ S. M. Condon, I. Morize, S. Darnbrough, C. J. Burns, B. E. Miller, J. Uhl, K. Burke, N. Jariwala, K. Locke, P. H. Krolikowski, N. V. Kumar, R. F. Labaudiniere, J. Am. Chem. Soc. 2000,122, 3007–3014.

¹⁴ Blackwell HE, Grubbs RH. Highly efficient synthesis of covalently cross-linked peptide helices byring-closing metathesis. Angew Chem Int Edit 1998;37:3281–3284.

¹⁵ Blackwell HE, Sadowsky JD, Howard RJ, Sampson JN, Chao JA, Steinmetz WE, O'Leary DJ, Grubbs RH. Ring-closing metathesis of olefinic peptides: Design, synthesis, and structural characterization of macrocyclic helical peptides. J Org Chem 2001;66:5291–5302

¹⁶ Schafmeister CE, Po J, Verdine GL. An all-hydrocarbon cross-linking system for enhancing the helicity and metabolic stability of peptides. J Am Chem Soc 2000;122:5891–5892

¹⁷ Zhang H, Zhao Q, Bhattacharya S, Waheed AA, Tong X, Hong A, Heck S, Curreli F, Goger M, Cowburn D, Freed EO, Debnath AK. A cell-penetrating helical peptide as a potential HIV-1 inhibitor. J Mol Biol 2008;378:565–580.

disubstituted amino acids linked by a side chain made by 11 carbon atoms with *cis* geometry at the double bond placed between i-i+7 residues with *R* and *S* configuration, or a chain made by 7 carbon atoms with *cis* geometry at the double bond placed between i-i+4 residues with *S* and *S* configuration.^{3 19} With this strategy apoptosis promoting derivatives BCL-2 20 and resensitizer of p53-hDM2 for apoptosis process and many other active derivatives were prepared that present high metabolic resistance and are more active than the unconstrained corresponding derivatives.²¹

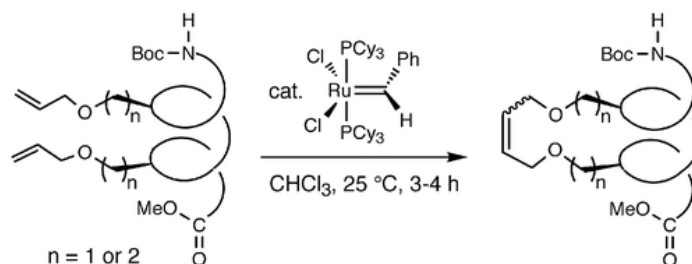


Figure 1.6 RMC in peptide helical stabilization. Stapled approach

Hydrogen Bond surrogate strategy (HBS) It was developed by Arora et al.^{22 23 24 25} and consists in a replacement of the hydrogen bond between CO_i in terminal position and the NH_{i+4} with a 4 carbon chain presented a double bond in *trans* conformation that from one side is connected directly to the amide and in terminal portion of the peptide replace the CONH groups of the last peptide. The strategy is based on helix-coil transition theory and overcomes the problem of high energetical barrier of small peptides to engage in α -helix structure. With the preformed 13 member rings α -helix turn is extremely stabilized and initiates the helical formation on the entire peptide. The advantage of this approach is that the link is inside the helix and

¹⁸ A. Boal, I. Guryanov, A. Moretto, M. Crisma, E.L. Lanni, C. Toniolo, R. H. Grubbs, D.J.O'Leary. Facile and *E*-Selective Intramolecular Ring-Closing Metathesis Reactions in 3₁₀-Helical Peptides: A 3D Structural Study. *J. Am. Chem. Soc.*, 2007, 129 (22), pp 6986–6987

¹⁹ Kutchukian PS, Yang JS, Verdine GL, Shakhnovich EI. All-atom model for stabilization of α -helical structure in peptides by hydrocarbon staples. *J Am Chem Soc* 2009;131:4622–4627

²⁰ Walensky LD, Pitter K, Morash J, Oh KJ, Barbuto S, Fisher J, Smith E, Verdine GL, Korsmeyer SJ. A stapled BID BH3 helix directly binds and activates BAX. *Mol Cell* 2006;24:199–210

²¹ Bernal F, Tyler AF, Korsmeyer SJ, Walensky LD, Verdine GL. Reactivation of the p53 tumor suppressor pathway by a stapled p53 peptide. *J Am Chem Soc* 2007;129:2456–2457.

²² Chapman RN, Dimartino G, Arora PS. A highly stable short α -helix constrained by a main-chain hydrogen-bond surrogate. *J Am Chem Soc* 2004;126:12252–12253

²³ Dimartino G, Wang D, Chapman RN, Arora PS. Solid-phase synthesis of hydrogen-bond surrogate-derived α -helices. *Org Lett* 2005;7:2389–2392

²⁴ Wang D, Chen K, Kulp JL, Arora PS. Evaluation of biologically relevant short α -helices stabilized by a main-chain hydrogen-bond surrogate. *J Am Chem Soc* 2006;128:9248–9256

²⁵ Wang D, Chen K, Dimartino G, Arora PS. Nucleation and stability of hydrogen-bond surrogate based α -helices. *Org Biomol Chem* 2006;4:4074–4081

it didn't interfere with solvent exposed surface and consequently with PPI. It is noteworthy that such methodology allowed also the stabilization of rare π -helix motif²⁶. (Figure 6)

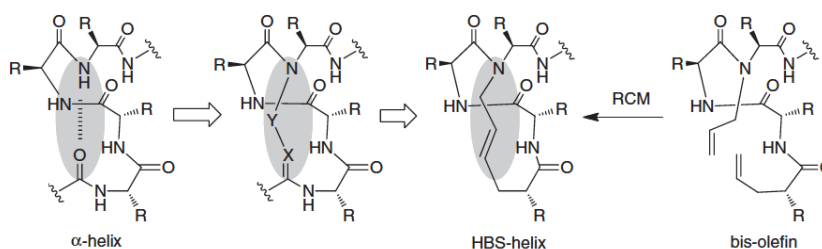


Figure 1.7 RMC in peptide helical stabilization. HBS approach

1.2.2.4 CuAAC Huisgen cycloaddition

CuAAC Huisgen cycloaddition took in this last 10 years a privileged position in organic chemistry thanks to the development of the concept of click chemistry by K. Barry Sharpless.²⁷ The high tolerance of different functional groups and the elevated yield, mild conditions and different medium where the reaction works properly permits the application of this types of reaction in different fields. In fact the field of peptides is only one of the large number of applications of this reaction. In peptide science the click reaction was widely use to bioconjugation chemistry,²⁸ dimerization and construction of unnatural amino acids and stabilization of secondary structure.²⁹ The first example of 3_{10} helix stabilisation by click chemistry is reported by Jacobsen. The side chains with azide and alkyne moieties were placed on amino acids $i, i+3$ in a small Aib rich peptides and, after cycloaddition reaction, an extreme stable 3_{10} helix was fully characterized proving the advantage of this methodology in secondary structure stabilization .³⁰

²⁶ Chapman R, Kulp JL, Patgiri A, Kallenbach NR, Bracken C, Arora PS. Trapping a folding intermediate of the α -helix: Stabilization of the π -helix. *Biochemistry* 2008;47:4189–4195

²⁷ Kolb, H.C.; Finn, M.G.; Sharpless, K.B. Click chemistry: diverse chemical function from a few good reactions. *Angew. Chem. Int. Ed.* **2001**, *40*, 2004

²⁸ Jewett, J.C.; Bertozzi, C.R. Cu-free click cycloaddition reactions in chemical biology. *Chem. Soc. Rev.* **2010**, *39*, 1272.

²⁹ Holub, J.M.; Kirshenbaum, K. Tricks with clicks: modification of peptidomimetic oligomers via copper-catalyzed azide-alkyne [3 + 2] cycloaddition. *Chem. Soc. Rev.* 2010, *39*, 1325.

³⁰ Jacobsen, H. Maekawa, N.-H. Ge, C. H Gorbitz, P. Ottersen, M. Amiry-Moghaddam, J. Klaveness Stapling of a 3_{10} -Helix with Click Chemistry. *J. Org. Chem.* 2011, *76*, 1228–1238

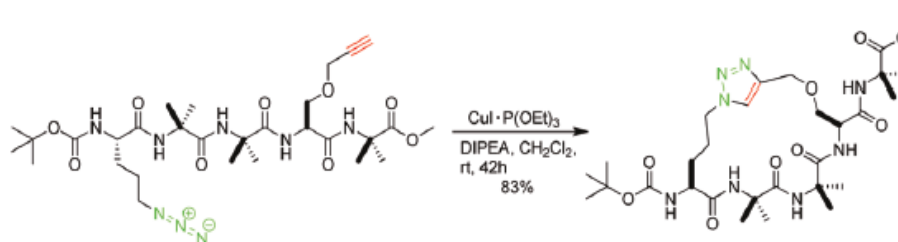


Figure 1.8 3_{10} helix stabilization by CuAAC

1.2.2.5 Peptoids

The peptoid derivatives of a specific peptide can be defined as the poly-N-substituted glycine, where the substitution on the nitrogen atom are represented by the side chain of the original peptide.^{31 32} The backbone of peptoids doesn't contain chiral centre and the hydrogen bond strictly important in the formation of the network of hydrogen bonds, that usually stabilized the secondary structure, is replaced by an alkyl chain in order to obtain an helical structure. The substituent placed on the amide has to be chiral and generally induce α - Helix of higher order secondary structure³³. This secondary structure are usually not denatured by solvent and temperature because independent from the hydrogen bond. Peptoids recently found application as surfactants and allowed the development of potent antimicrobial agent.³⁴ One of the great advantage of peptoids as new drug entities, is the higher resistance to the enzymatic proteolysis due to the completely masked amide bond. It seems that the strong activity of peptoids as their propriety as surfactant is related more to the high hydrophobicity and helical structure itself that the nature and space disposition of the side chains.¹⁶

³¹ Zuckermann, R. N., Kerr, J. M., Kent, S. B. H. & Moos, W. H. Efficient method for the preparation of peptoids [oligo(N-substituted glycines)] by submonomer solid-phase synthesis. *J. Am. Chem. Soc.* 1992, 114, 10 646–10 647

³² Stigers, K. D., Soth, M. I. & Nowick, J. S. Designed molecules that fold to mimic proteinsecondary structures. *Curr. Opin. Chem. Biol.* 1999, 3, 714–723.

³³ Patch, J. A. & Barron, A. E. Mimicry of bioactive peptides via non-natural, sequence-specificpeptidomimetic oligomers. *Curr. Opin. Chem. Biol.* 2002 6, 872–877

³⁴ Chongsiriwatana, N. P., Patch, J. A., Czyzewski, A. M., Dohm, M. T., Ivankin, A., Gidalevitz, D., Zuckermann, R. N. & Barron, A. E. Peptoids that mimic the structure, function, and mechanism of helical antimicrobial peptides. *Proc. Natl Acad. Sci.* 2008 ,105, 2794–2799.

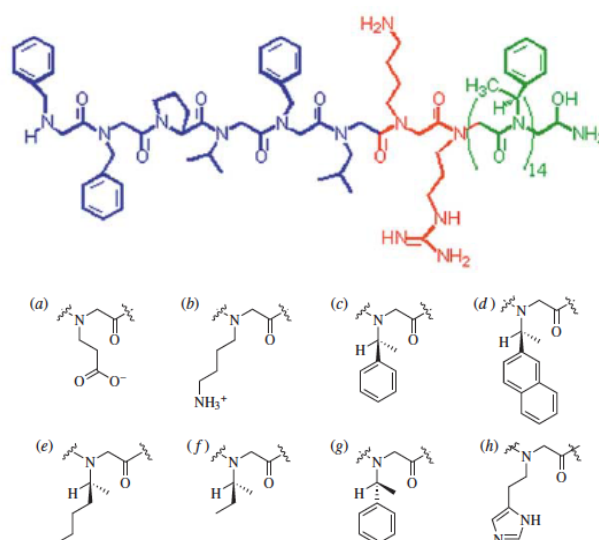


Figure 1.9 Peptoids model and Peptoids monomers.

1.2.2.6 β - Substitution

Examples of β -substituted amino acid occurred in nature with valine, isoleucine and threonine. The addition of substituent in β position, generally a methyl group, is one of the easy way to obtain analogues of natural amino acids. The synthesis of peptide with such modification generally lead to an higher activity and metabolic stability than the corresponding natural peptide. This result is due to the introduction of more sterically demanding group near to the side chain close to the backbone, that limits the rotation around torsional angles, stabilizing the secondary conformation that usually adopts an helix structure. Analogues obtained with simple methylation of phenylalanine appears to be more active than the natural derivatives on δ opioid peptide receptor.³⁵

³⁵ D. Tourwe, E. Mannekens, T. N. T. Diem, P. Verheyden, H. Jaspers, G. Toth, A. Peter, I. Kertesz, G. Toeroek, N. N. Chung, J. Med. Chem. 1998, 41, 5167–5176;

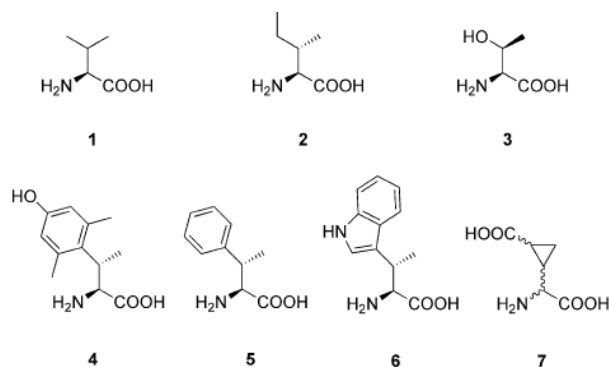


Figure 1.10 Examples of β substituted amino acids

1.2.2.7 β - Amino acids

β -Amino acids are characterized by the presence of amine group in β position of the carbonyl function, and can be divided in β -amino, β^3 -amino or β^{23} -amino. The class of the so called β^{23} -amino, tends to put the two substituents in gauche conformation and for this reason is the best stabilizer of helical conformation. In general β -peptides tend to stabilise helical conformation more than α -peptides. The helix is considered counting the atoms in the hydrogen bound ring, usually β -peptide form 14-helix, 10/12 helix or 12 helix, but also very constrained helix like 8-helix are observed. One of the successful use of β amino acids stabilizing an helical structure, is the synthesis of an analogous able to inhibit the p54-hDM2 interactions reported by Schepartz et al. that exploits an interesting cyclic class of β amino acids.³⁶

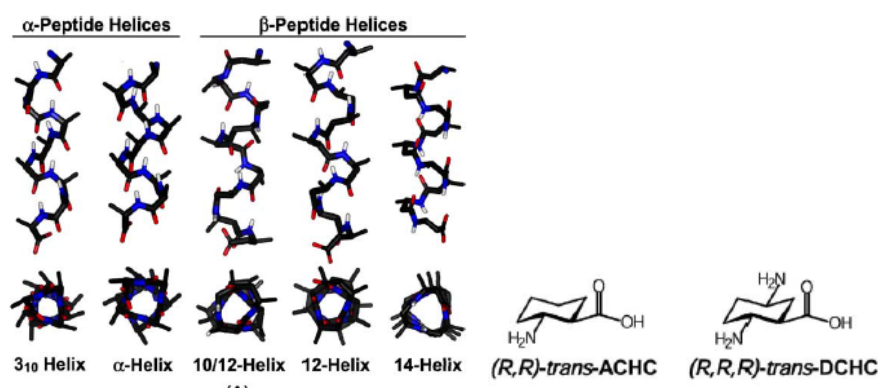


Figure 1.11 β -peptide helical structure and β cyclic amino acid.

³⁶ Kritzer, J. A., Lear, J. D., Hodsdon, M. E. & Schepartz, A. 2004 Helical β -peptide inhibitors of the p53-hDM2 interaction. J. Am. Chem. Soc. 126, 9468–9469

1.2.2.8 α,α -Disubstituted amino acids

The exploitation of α,α -disubstituted amino acids ($\alpha\alpha$ AAs) is one of the backbone modification that in the last 20 years has attracted the attention more than any other for obtaining peptides conformationally rigid and structured. The presence of α,α -amino acids are observed also in nature, in the fungal non ribosomal peptide family of peptaibols and in the sub-family cephaibols. This small peptides present in their structure unnatural amino acids in foldameric form as Aib (aminoisobutyric acid) and Iva (Isovalina) and has attracted attention for interesting antibiotic propriety (peptabiotics) due to the their helix stable residue made by unnatural amino acids and for the particular mechanism of action.^{37 38 39 40 41} The growing interest in this structures has led to a rapid development of a large amount unnatural α,α -AAs and their use in peptidomimetic synthesis.⁴² α,α -AAs possess a stable quaternary centre and basing on the steric demand of their substituents, they impose a conformational rigidity decreasing the allowed free rotation around the torsional angles. Torsional angle could in fact assume preferentially only a range of value of ϕ, ψ typical of ordered secondary structure. This particular features pushes also small peptides to adopt stable helical conformation generally with a preference for 3_{10} -helix structure. Different side chains and the particular matching and mismatching event once introduced in natural peptide environment with different nature, can destabilised or stabilised also other secondary structures^{43 44 45 46 47}. The most studied and exploited amino acid of this class is without doubt Aib. This achiral amino acid adopts generally 3_{10} -helix structure but in some cases of long chain, 3_{10} -helix structure is in equilibrium with α -helix. The studies conducted by the team of Professor Toniolo and Professor Formaggio are surely the most exhaustive in the structural analysis and exploitation of foldameric Aib structure.^{48 49 50}

³⁷ G. W. Kenner, R. C. Sheppard, *Nature* 1958, 181, 48

³⁸ K. Matsuzaki, S. Yoneyama, K. Miyajima, *Biophys. J.* 1997, 73, 831;

³⁹ J. K. Chugh, B. A. Wallace, *Biochem. Soc. Trans.* 2001, 29, 565

⁴⁰ K. L. Rinehart Jr., L. A. Gaudioso, M. L. Moore, R. C. Padney, J. C. Cook Jr., *J. Am. Chem. Soc.* 1981, 103, 6517

⁴¹ H. Duclohier, *Chem. Biodivers.* 2007, 4, 1023–1026

⁴² H. Vogt, S. Bräse, *Org. Biomol. Chem.* 2007, 5, 405–430.

⁴³ Doty, P.; Wada, A.; Yang, J. T.; Blout, E. R. *Journal of Polymer Science* 1957, 23, 851-861.

⁴⁴ Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, 24, 4004-4009

⁴⁵ Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; di Blasio, B.; Pavone, V.; Pedone, C. *Biopolymers* **1983**, 22, 205-215

⁴⁶ Gatto, E.; Porchetta, A.; Stella, L.; Guryanov, I.; Formaggio, F.; Toniolo, C.; Kaptein, B.; Broxterman, Q. B.; Venanzi, M. *Chemistry & biodiversity* **2008**, 5, 1263-78.

⁴⁷ Robert A. Brown, Tommaso Marcelli, Matteo De Poli, Jordi Solé, and Jonathan Clayden Induction of Unexpected Left-Handed Helicity by an N-Terminall-Amino Acid in an Otherwise Achiral Peptide Chain *Angew. Chem. Int. Ed.* 2012, 51, 1395 –1399

⁴⁸ Toniolo, C.; Benedetti, E. *Macromolecules* **1991**, 24, 4004-4009

⁴⁹ Toniolo, C.; Bonora, G. M.; Bavoso, A.; Benedetti, E.; di Blasio, B.; Pavone, V.; Pedone, C. *Biopolymers* **1983**, 22, 205-215

⁵⁰ Gatto, E.; Porchetta, A.; Stella, L.; Guryanov, I.; Formaggio, F.; Toniolo, C.; Kaptein, B.; Broxterman, Q. B.; Venanzi, M. *Chemistry & biodiversity* **2008**, 5, 1263-78.

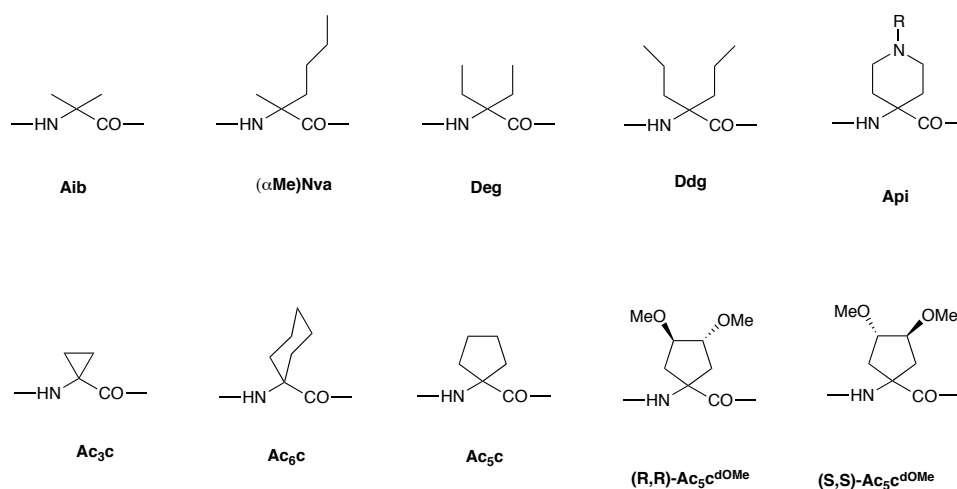


Figure 1.12 $\alpha\alpha$ AAs and $C\alpha\alpha$ AAs examples

The introduction of additional constrain with fixed angles on the quaternary centre has been achieved with the use of $C\alpha\alpha$ AAs like Api, Ac_6C , and Ac_5C . In this field many works have been developed evidencing the high tendency of these cyclic compounds, compared to the acyclic analogues, toward the induction of 3_{10} -helix. To confirm this tendency relevant examples are reported in literature as TAAs amino acids developed by König^{51 52} and (Ac_5C^{dOMe}) developed by Tanaka.⁵³ In both works the amino acids were included in a model peptide and full structural analysis permitted to compare the cyclic chiral class with achiral derivatives and acyclic amino acids as Aib. (Figure 11)

It is noteworthy also the pioneering work on the polyAib helix structure performed by Professor Clayden et al. that exploits the ability of fast inversion of the screw sense of the polyAib and Ac_6C to mimic the biotransduction of the chiral information through conformational exchange over 60 chemical bonds on a length of 4nm.^{54 55 56}

⁵¹ P. Maity, M. Zabel, B. König, J. Org. Chem. 2007, 72, 8046–8053.

⁵² M. Aschi, G. Lucente, F. Mazza, A. Mollica, E. Morera, M. Nalli, M. P. Paradisi, Org. Biomol. Chem. 2003, 1, 1980–1988.

⁵³ Demizu, Y.; Doi, M.; Kurihara, M.; Okuda, H.; Nagano, M.; Suemune, H.; Tanaka, M. Organic & biomolecular chemistry 2011, 9, 3303-12.

⁵⁴ Tommaso Marcelli, Ralph W. Adams, Gareth A. Morris, and Jonathan Clayden; Foldamer-Mediated Remote Stereocontrol: >1,60 Asymmetric Induction Angew. Chem. Int. Ed. 2014, 53, 151–155

⁵⁵ Jonathan Clayden, Alejandro Castellanos, Jordi Solé, and Gareth A. Morris Quantifying End-to-End Conformational Communication of Chirality through an Achiral Peptide Chain Angew. Chem. Int. Ed. 2009, 48, 5962–5965

⁵⁶ Robert A. Brown, Vincent Diemer, Simon J. Webb and Jonathan Clayden End-to-end conformational communication through a synthetic purinergic receptor by ligand-induced helicity switching; Nature Chemistry 2013, 5, 853-860

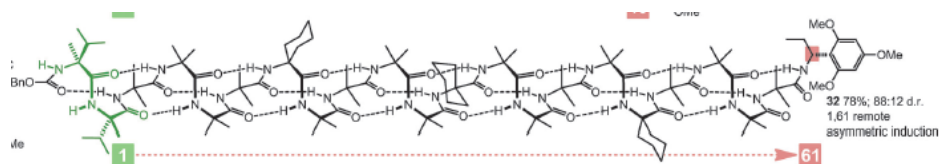


Figure 1.13 Example of asymmetric inductions over 60 chemical bound.

One example of peptide mimic compounds deriving from the exploitation α AAs is the analogues of enkephalin. In this case the substitution of a glycine with different α AAs led to very active compounds structured as β turn.⁵⁷

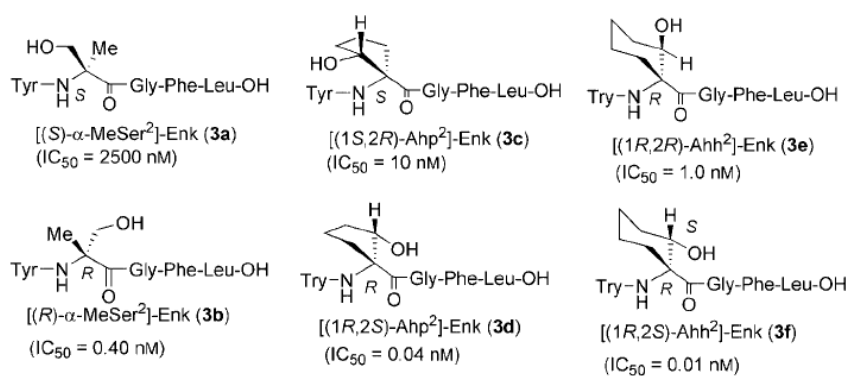


Figure 1.14 Enkephalin analogue

⁵⁷ Y. Ohfuné, T. Shinada, Eur. J. Org. Chem. 2005, 5127–5143.

1.3 Norbornene core

1.3.1 Overview on the features of norbornene core

Bicyclo[2.2.1]hept-2-ene better known as norbornene is highly constrain bi-carbocyclic core. In literature a great number of norbornene core differently substituted are described. The general synthesis of this scaffold consists in a Diels-Alder cycloaddition reaction between substituted dienophile and cyclopentadiene.

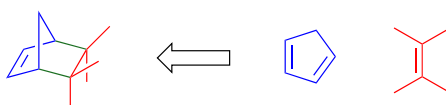


Figure 1.15 Synthesis and retrosynthetic approach for norbornene core

The rigidity of the core derived from the lock highly constrained angle, that presents value significantly different from the usual sp^3 and sp^2 hybridized carbon atom. The angles of the olefin part are constrained from 120° to $103,6^\circ$ as well as the bridge carbon which presents an angle bent to $64,6^\circ$. This feature tends to enhance the reactivity of the olefin that has a character in middle between an alkyne and an olefin. This enhanced reactivity is demonstrated by the possibility of conducting reactions typical of alkynes or by using, on the olefin double bond, reaction conditions typically used in alkyne chemistry. Examples are the CuAAC Huisgen cycloaddition reaction,⁵⁸ Cu free 1-3 dipolar oxanorbornadiene cycloaddition⁵⁹ and, as presented in this thesis at chapter 4, a phosphine free version of the Heck reaction, at our knowledge known before only on alkyne moiety.⁶⁰

Thanks to the interesting reactivity of the olefin portion, the aliphatic three dimensional nature, and the rigidity of core, the norbornene scaffold has found in these years a wide range of applications.

⁵⁸ E. M. Slette, C. R. Bertozzi Bioorthogonal Chemistry: Fishing for Selectivity in a Sea of Functionality *Angew. Chem. Int. Ed.* 2009, 48, 6974 – 6998

⁵⁹ Gutmiedl, Katrin; Wirges, Christian T.; Ehmke, Veronika; Carell, Thomas Copper-Free "Click" Modification of DNA via Nitrile Oxide Norbornene 1,3-Dipolar Cycloaddition". *Organic Letters* 2009, 11, 11, 2405–8

⁶⁰ M. Ahlquist; G. Fabrizi; S. Cacchi; and Per-Ola Norrby; The Mechanism of the Phosphine-Free Palladium-Catalyzed Hydroarylation of Alkynes. *J. AM. CHEM. SOC.* 2006, 128, 12785-1279

1.3.2 Norbornene core exploitation

1.3.2.1 Norbornene amino acids

Norbornene core has found a place as interesting scaffold in the field of α,α -tetrasubstituted cyclic unnatural amino acids (CTAAs). Our group has been involved for a long time in the synthesis of norbornene amino acid bringing different substituents. The appealing in the field of α,α -tetrasubstituted cyclic amino acid lies in the aim of the synthesis of new CTAAs entity to obtain an extremely locked angle at the α -carbon with known value, combining with steric demanding group that do not allowed free rotation of the peptide backbone. Moreover the hydrophobic core could prevent hydrophobic collapse that is one of the major problem in the process of interaction between two proteins.^{61 62 63 64 65 66 67} In literature several examples of the exploitation of norbornene β -amino acids stabilizing secondary structure as β -turn^{68 69}, helical⁷⁰ structure and linker between two helical structures are present⁷¹. Curiously, it has never been used in the form of α,α -amino acid and no study of its behaviour in stabilizing helical secondary structure once included in peptide sequences are available despite its promising features.

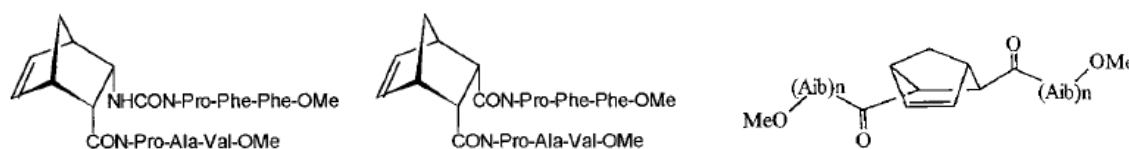


Figure 1.16 Norbornene β -amino acid in peptide.

⁶¹ Kyung-Ho, P.; Kurth, M. J. *Tetrahedron*, **2002**, 58, 8629

⁶² Komarov, I. V.; Grigorenko, A. O.; Turov, A. V.; Khilya, V. P. *Russ. Chem. Reviews* **2004** 73, 7852

⁶³ Gelmi, M. L.; Pocar, D. Heterosubstituted carbocyclic amino acids. A review, *Org. Prep. Proc. Int.* **2003**, 35, 141

⁶⁴ Tanaka, M. *Chem. Pharm. Bull.* **2007**, 55(3), 349-358

⁶⁵ Clerici, F.; Gambini, A.; Gelmi, M. L.; Nava, D. *Tetrahedron*, **2001**, 57, 6429-6438.

⁶⁶ Clerici, F.; Gambini, A.; Gelmi, M. L. *J. Org. Chem.* **2001**, 66, 4941-4944.

⁶⁷ Abbiati, G.; Clerici, F.; Gambini, A.; Gelmi, M. L.; Pilati, T. *J. Org. Chem.* **2001**, 66, 6299-6304

⁶⁸ I G. Jones, W. Jones, M. North Conformational Analysis of Peptides and Pseudopeptides Incorporating an endo-(2S,3R)-Norborn-5-ene Residue as a Turn Inducer *J. Org. Chem.* 1998, 63, 1505-1513.

⁶⁹ Hibbs, David E.; Hursthouse, Michael B.; Jones, Iwan G.; Jones, Wyn; Abdul Malik, K. M.; North, Michael. Synthesis of Peptides and pseudo-Peptides Incorporating an endo-(2S,3R)-Norborn-5-ene Residue as a Turn Inducer. *Journal of Organic Chemistry* 1998, 63, 5, 1496-1504

⁷⁰ Moscovitz, Albert; Hansen, Aage E.; Forster, Leslie S.; Rosenheck, Kurt Prototypic systems for optically active helical polypeptides, *Biopolymers Symposia* 1964, 1, 75-89

⁷¹ Ranganathan, Darshan; Kurur, Sunita; Karle, Isabella L. Design, synthesis, and crystal structure of self-assembling norbornene (NBE)-supported two-helix bundles: a unique example of janus helicity in the solid-state structure of NBE(Aib5)2. *Biopolymers* (2000), 54, (4), 249-261

Despite this lack of information in structural analysis, cases concerning the use of norbornene in peptide analogues are present in literature demonstrating improved selectivity against specific receptors and better bioavailability as well as less enzymatic hydrolysis. One example is the angiotensin I receptor inhibitor published by Fink⁷². Important biological properties are shown by the norbornene amino acid itself: one example is the specific interaction with transmembrane transporter LAT1 system but are well-known also other cited examples with a wide biological broad scope.^{73 74 75 76}.

1.3.2.2 Norbornene and olefin metathesis.

The growing and differentiation of ring opening metathesis field has renewed the interest toward bicyclic unsaturated core as functionalized norbornene structure. With the use of appropriate organocatalyst or chiral auxiliary the norbornene structure was easily generated with high enantioselection. The advent of ring opening olefin metathesis has enabled the exploitation of such structures which have 4 stereocentres for the production of pentatomic nuclei highly functionalized in enantiopure form otherwise inaccessible. The previous installation of olefin pendant on the dienophile and the ring opening and ring closing metathesis tandem reaction, led to highly functionalized polycyclic useful precursors in the synthesis of natural compounds. The total synthesis of Massadine published by Carreira is an interesting application. The olefin metathesis could be coupled with other methodologies with tandem processes and allows the generation of a high chemical diversity and complexity in two easy steps. Important examples were reported by Tam, Peregrina, and Grubbs^{77 78 79 80 81}.

⁷² Pogocki, Dariusz; Serdiuk, Katarzyna; Scho1neich, Christian. New a-Thiol Dipeptide Dual Inhibitors of Angiotensin-I Converting Enzyme and Neutral Endopeptidase EC 3.4.24.11 *J. Med. Chem.* **1995**, 38, 5023-5030;

⁷³ Goehring, Isabel; Mulder, Hindrik; Glutamate dehydrogenase, insulin secretion, and type 2 diabetes: a new means to protect the pancreatic β -cell. *Journal of Endocrinology*. **2012**, 212(3), 239-242

⁷⁴ Matharu, Jyothi; Oki, Jun; Worthen, David R.; Smith, Quentin R., Crooks, Peter A.; Regiospecific and conformationally restrained analogs of melphalan and dl-2-NAM-7 and their affinities for the large neutral amino acid transporter (system LAT1) of the blood-brain barrier. *Bioorganic & Medicinal Chemistry Letters*. **2010**, 20(12), 3688-3691

⁷⁵ Christensen, H.N.; Handlogten, M.E.; Lam, I.; Tager, H.S.; Zand, R. A bicyclic amino acid to improve discriminations among transport systems. *J. Biol. Chem.* **1969**, 244, 1510-1520

⁷⁶ Van Winkle, L.J.; Christensen, H.N.; Campione, A.L.; Na⁺-dependent transport of basic, zwitterionic, and bicyclic amino acids by a broad-scope system in mouse blastocysts. *J. Biol. Chem.* **1985**, 12, 260 and 12 118-123

⁷⁷ G. K. Tranmer and W. Tam. Molybdenum-Mediated Cleavage Reactions of Isoxazoline Rings Fused in Bicyclic Frameworks. *Org. Lett.* **2002**, 4, 23, 4101-4104

⁷⁸ P. Mayo and W. Tam Ring-opening metathesis-cross-metathesis reactions (ROM-CM) of substituted norbornadienes and norbornenes. *Tetrahedron* **2002**, 58, 9513-9525

⁷⁹ J.H. and R. H. Grubbs Highly Z-Selective and Enantioselective Ring-Opening/Cross-Metathesis Catalyzed by a Resolved Stereogenic-at-Ru Complex. *J. Am. Chem. Soc.*, **2013**, 135 (28), pp 10183-10185

⁸⁰ J. Carreras, A. Avenoza, J.H. Busto, and J. M. Peregrina. Ring-Rearrangement Metathesis of 1-Substituted 7-Azanorbornenes as an Entry to 1-Azaspiro[4.5]decane systems *J. Org. Chem.* **2011**, 76, 3381-3391

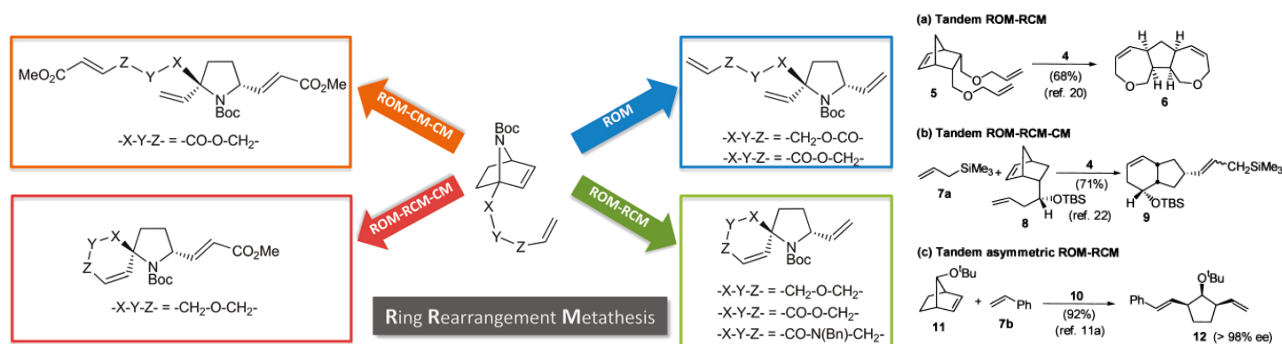


Figure 1.17 Olefin metathesis strategy for the generation of molecular diversity.

In the field of metathesis reactions we could find also the ring opening polymerization metathesis that was exploited on norbornene core producing polymeric derivatives with interesting thermic and mechanical features for a variety of applications^{82 83 84}. An interesting example published by Sarma, that conjugates polymers and bioapplication is the use of norbornene derived polyimides obtained by ROMP, for the vehiculation of doxorubicine. ROMP was also used to build analogues of DNA after the preparation of nucleic acid bases connected with norbornene moiety and their polymerization.⁸⁵

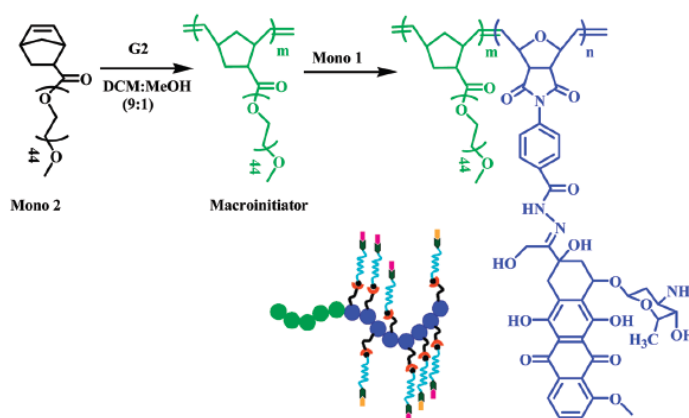


Figure 1.18 Norbornene-derived doxorubicine poly-ethylene-glycole

⁸¹ S. Sutthasupa¹, M. Shiotsuki and F. Sanda. Recent advances in ring-opening metathesis of functional materials. *Polymer Journal* **2010**, 42, 905–915

⁸² Biagini, S. C. G.; Davies, R. G.; Gibson, V. C.; Giles, M. R.; Marshall E. L.; North, M.; *Polymer*, **2001**, 42, 6669–6671.

⁸³ Yoon, Kyung-Hwan; Kim, Kyung Oh; Schaefer, Mark; Yoon, Do Y; Synthesis and characterization of hydrogenated poly(norbornene endo-dicarboximide)s prepared by ring opening metathesis polymerization. *Polymer*, **2012**, 53(11), 2290-2297.

⁸⁴ Mane, Shivshankar R.; Shunmugam, Raja; Vijayakameswara Rao; Das Sarma, Jayasri; Kishore, Abhinoy; Norbornene Derived Doxorubicin Copolymers as Drug Carriers with pH Responsive Hydrazone Linker. *Biomacromolecules*, **2012**, 13, 221–230

⁸⁵ R. Gareth Davies, Vernon C. Gibson, Michael B. Hursthouse, Mark E. Light, Edward L. Marshall, Michael North. P. J. Williams. Synthesis of nucleic-acid base containing norbornene derivatives as monomers for ring-opening-metathesis–polymerization *J. Chem. Soc., Perkin Trans. 1*, 2001, 3365–3381

1.3.2.3 Oxidation and functionalization of the double bond.

The possible functionalization and oxidation of the double bond are easy way to generate chemical complexity. Several examples in this area can be reported. In our group several oxidation and lactonisation reactions were carried out and in the chapter number 4 and 5 the hydroarylation reaction on this nucleus are treated. The example that best demonstrates the elevated degree of complexity of such diversification is without doubt the total synthesis of Massadine developed by Carreira et al. through the oxidative break of functionalized norbornene double bond.⁸⁶

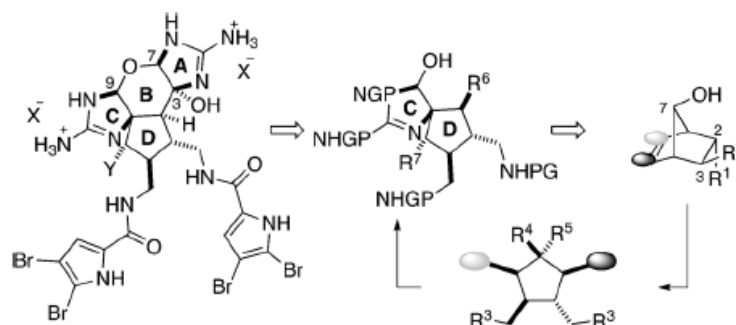


Figure 1.19 Retrosynthetic analysis of Massadine.

1.3.2.4 Norbornene and Bioorthogonal chemistry

Thanks to the development of click reactions and the cited ability of norbornene to give click cycloaddition in absence of copper, different exploitations of this methodology was developed in order to biotag proteins and DNA fragment also on cellular surface and in living cells. The recent work of Lemke⁸⁷, Bawendy and Carell⁸⁹ groups furnishes efficient methodology that thanks to the click reaction described in figure 20 permits the site specific transformation and conjugation of entire protein expressed on cell surface or even inside the cell. On this protein the nobornene moiety has been attached on an amino acid by a linker and was included with different strategies. The most impressive strategy was recently published by Carell group that has been able to develop very mild methodology, tolerated by peptide structure, to produce hybrids proteins for therapeutic and diagnostic application. The norbornene amino acid was introduced in a protein chain by a

⁸⁶ A. Breder, G.M. Chinigo, A.W. Waltman, E.M. Carreira,. Towards the Synthesis of Massadine: A Unified Strategy for the Stereoselective Synthesis of the Carbocyclic C,D-Ring Subunit. *Chem. Eur. J.* **2011**, 17, 12405

⁸⁷ Plass, Tilman; Milles, Sigrid; Koehler, Christine; Szymanski, Jędrzej; Mueller, Rainer; Wiessler, Manfred; Schultz, Carsten; Lemke, Edward A. Amino Acids for Diels-Alder Reactions in Living Cells *Angewandte Chemie, International Edition* **2012**, 51(17), 4166-4170,

pyrrolysyl system of mutated synthetase and reacts in specific fashion with a in situ generated nitrile imine through a click chemistry procedure.⁸⁸

It is noteworthy also the recent work of Lemke group that tunes the first Diels Alder cycloaddition in a living cell for the site specific modification of norbornene anchored to a protein. This opens the possibility of biolabeling with fluorescent agents specific protein inside a living cell with high selectivity for such containing norbornene structure.

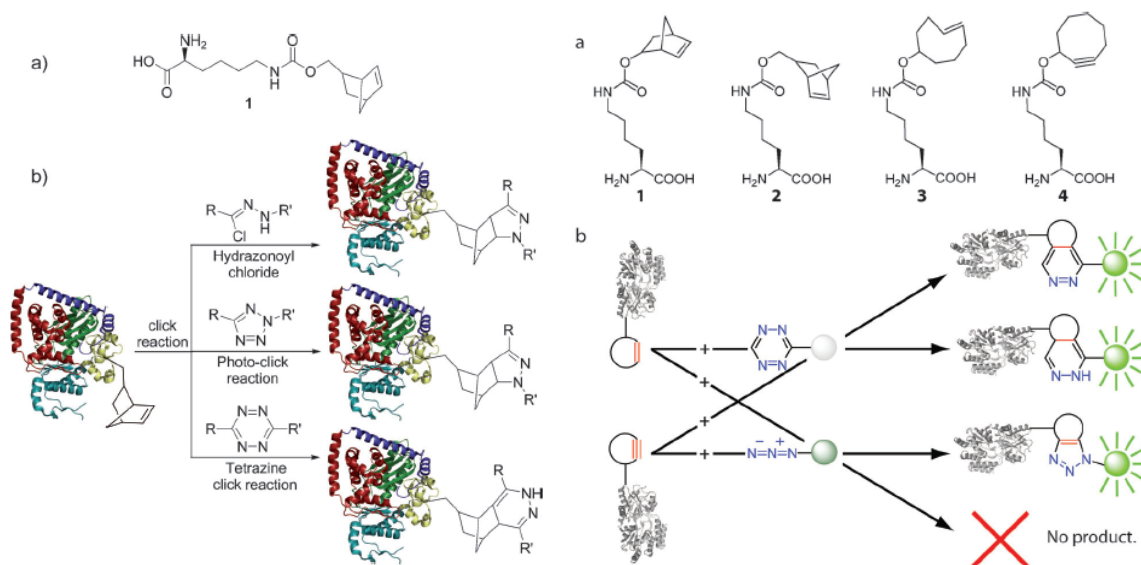


Figure 1.20 Modified amino acids encoded by synthetase and different site specific modifications applicable on living cell for biolabeling.

A similar strategy using by Carell was developed and applied to a DNA bases by Jaeschke⁸⁹. The modified DNA bases are included in a fragment of DNA by a mutated primer extension and polymerase chain reaction opening to the possibility of site specific modification and labelling of fragment of DNA.

⁸⁸ Kaya, Emine; Vrabl, Milan; Deiml, Christian; Stefan Prill, Stefan; S. Fluxa, Viviana; Carell, Thomas; A Genetically Encoded Norbornene Amino Acid for the Mild and Selective Modification of Proteins in a Copper-Free Click Reaction. *Angewandte Chemie*, **2012**, 51(18), 4466–4469

⁸⁹ Schoch, Juliane; Jaeschke, Andres Synthesis and enzymatic incorporation of norbornene-modified nucleoside triphosphates for Diels-Alder bioconjugation RSC Advances (2013), 3(13), 4181-4183.

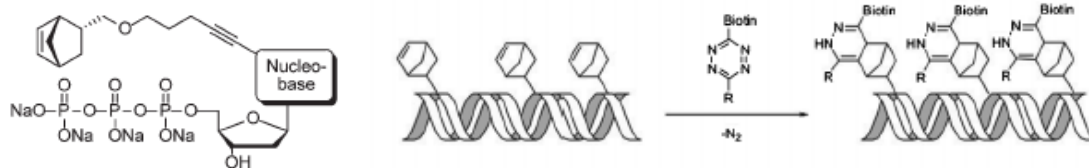


Figure 1.21 Modified DNA base and biconjugation of DNA fragments

1.4 Finalities of the thesis

Considering the great interest in the field of peptidomimetics and the potentiality of the norbornene scaffold, this thesis has been primarily devoted to the synthesis and exploitation of a new class of β -substituted norbornene amino acids as cysteine mimics. The project has been developed with the aim to fill the gap unexpectedly present in the literature in this field and to confirm the unexploited properties of norbornene amino acid as potential building block for peptidomimetics synthesis. At first, we followed the purpose to individuate a novel synthetic strategy to obtain, not only simple but also highly functionalized, norbornene scaffold in grams scale synthesis, in order to make possible further developments in this field and interesting practical applications. Subsequently, we set out to design, realize and conformationally analyze model peptides containing norbornene amino acids in order to demonstrate the ability of such compounds to behave as strong inducers of helical structure and the possibility to be used in the synthesis of unnatural peptides of therapeutic interest. Finally, we explored the field of small molecules as peptidomimetics and demonstrated the advantage of rigid core in the development of new drugs. To do this, we planned to design and realise new chemical entities based on an extensively modified norbornene scaffold which have shown high activity in modulation of Rac1-Tiam1 protein-protein interactions resulting in a new class of potent inhibitors. During the synthesis of such molecules we also launched a study aiming to better understand which kind of effects a constrained chemical structure, as norbornene scaffold, could have on the regiochemistry in palladium chemistry, imposing a methodic analysis of the steric and electronic effects in the reaction mechanisms.

Development of appealing synthetic approach

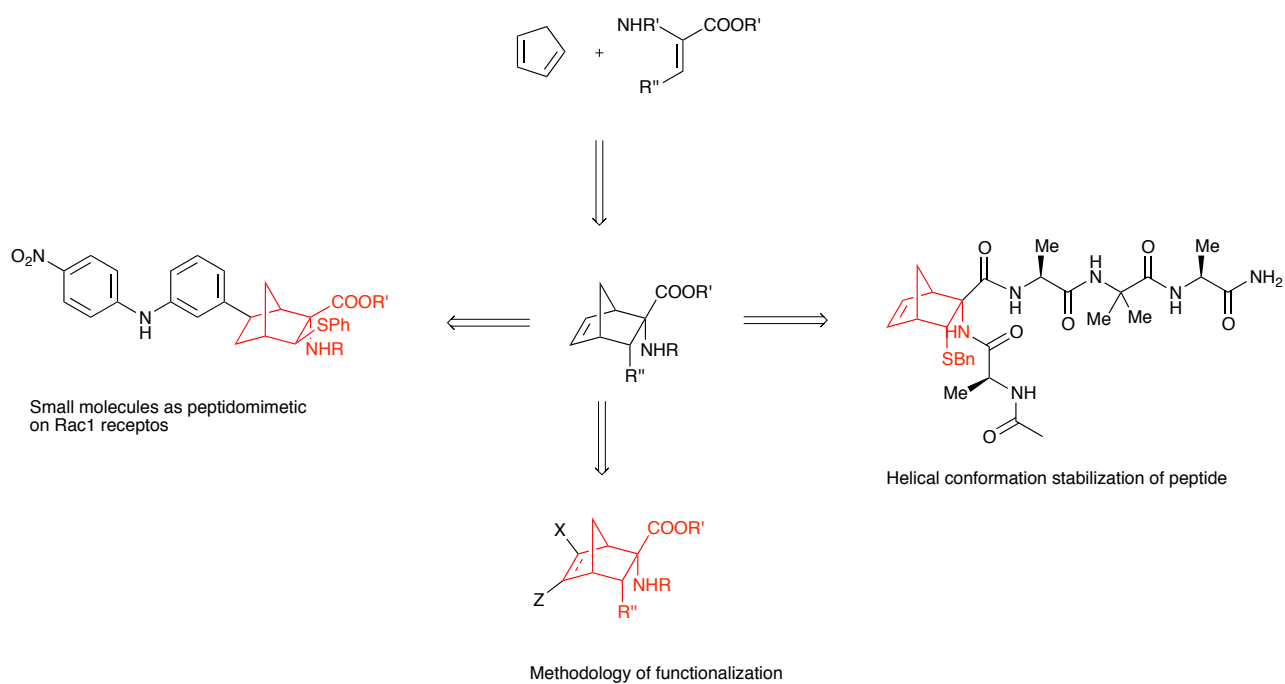


Figure 1.22 Target of this thesis

2 Synthesis of 2-amino-3-(Benzylsulphanyl)norbornene-2-carboxylate new class of constrained cysteine mimic amino acids and conformational study of their

2.1 Synthesis of 2-amino-3-(Benzylsulphanyl)norbornene-2-carboxylate

2.1.1 Chloro-methylene-5(4*H*)-oxazolones strategy

In a first attempt to prepare such compounds we planned to use the known 3-chloronorbornenoxazolone **2b** (Figure 6) which can be prepared according to a known procedure¹ from chloro-methylene-5(4*H*)-oxazolone and cyclopentadiene but all attempts to perform a direct substitution of the chlorine atom in compound **2b** failed, very likely because of steric hindrance as we previously observed in a similar case.¹

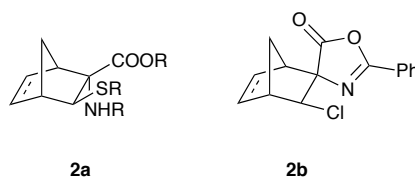
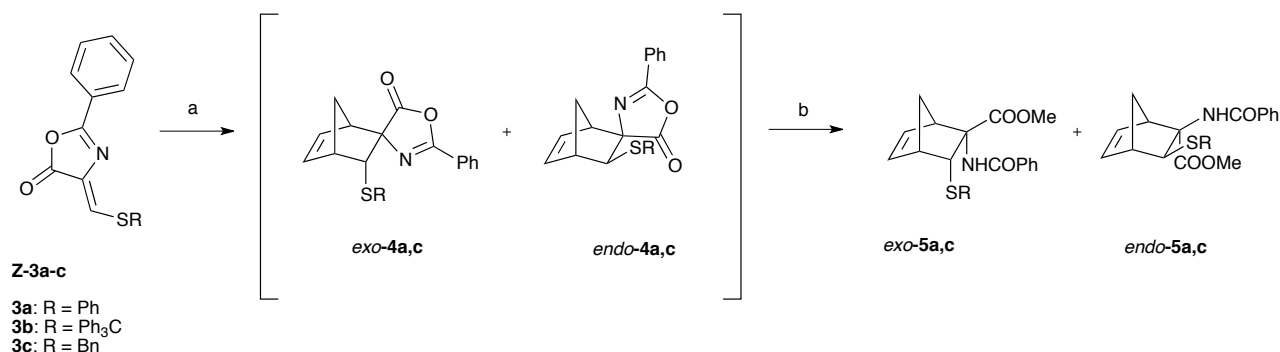


Figure 2.1 Constrained masked cysteine **2a** and 3-chloronorbornenoxazolone **2b**

As a consequence we decided to use, as the starting material, phenylsulfanyl-methylene-5(4*H*)-oxazolone **3a** (Scheme 1) which could be easily prepared from **2** using a procedure previously published.⁸ Taking into account the low reactivity in Diels-Alder cycloaddition reaction of methylene oxazolones bearing an electro-donating group,² different reaction conditions were tested.

¹ Cernak, T. A.; Gleason, J. L. *J. Org. Chem.* **2008**, 73, 102-110.

Scheme 2.1 Cycloaddition reaction of Z-3a-c and cyclopentadiene



Reagents and conditions: (a))))) , neat, 24h, 50°C; or Table 1(b) MeOH, *p*-TSA, 50°C .

Table 1. Cycloaddition reaction of Z-**3a** and cyclopentadiene. Different conditions and results.

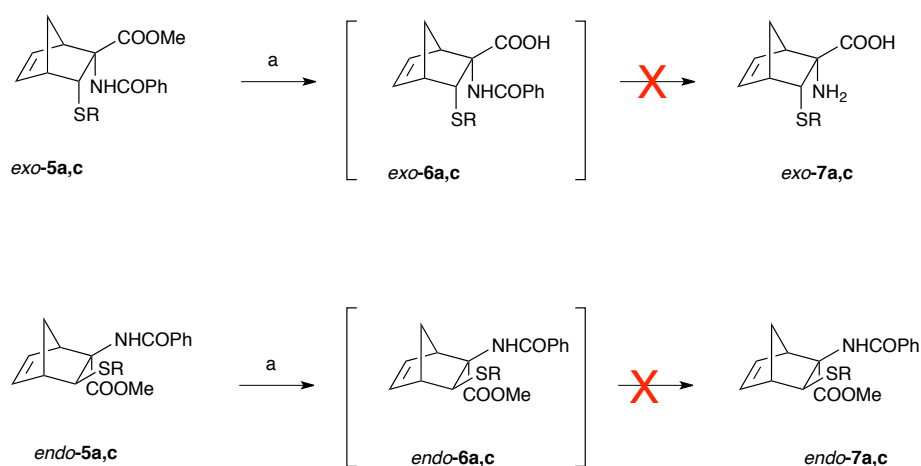
Entry	React. Cond. <i>T</i> (°C)	React. time h	Diene/Dienophile ratio	<i>exo/endo</i> ratio	Yield 5a %
1	CH ₂ Cl ₂ , 25 °C	72	1/1 to 10/1	-	-
2	toluene, 25 °C	72	1/1 to 10/1	-	-
3	μW ^a , 50 °C, neat,	1	20/1	-	-
4	))) ^b , 30 °C	72	10/1	35/65	30
5	CH ₂ Cl ₂ , Mg(ClO ₄) ₂))) ^b , 30 °C	24	10/1	-	-
6	neat, Mg(ClO ₄) ₂))) ^b , 30 °C	24	10/1	35/65	32
7	neat, oil bath, 110 °C	24	10/1	60/40	50
8	neat,))) ^b , 50 °C	24	10/1	40/60	65

^a 400 Watt; ^b Ultrasound.

The reaction did not occur when operating at room temperature in different solvents (Table 1, entries 1 and 2) or with microwaves (entry 3). Operating in neat conditions with ultrasound and maintaining the temperature under 30 °C, the two cycloadducts *endo*- and *exo*-**4a** were formed in low yields with a moderate *endo*-selectivity (65:35 ratio in favor of *endo*-**4a**, 72 h, entry 4). The cycloadducts **4a** were directly and quantitatively converted with methanol and *p*TSA into the methyl esters *exo*- and *endo*-**5a** which were analyzed and characterized to ascertain their stereochemistry. In the presence of a catalytic amount of Mg(ClO₄)₂ (5-20 mol %) the reaction did not occur (entry 5) or, if performed neat (5-10 mol %), gave an improvement only in terms of reaction time (entry 6). An improved yield was obtained when the reaction was performed neat at higher temperature (sealed tube 110°C, oil bath, entry 7). It has to be stressed that such reaction conditions resulted in a reversed *exo*-selectivity (40:60 ratio, in favor of *exo*-**5a**). After several attempts we determined the best conditions, in terms of yields and selectivity, were operating in neat

conditions with ultrasound at 50 °C in the presence of an excess of diene (entry 8). Using such conditions *exo*- and *endo*-**5a** were obtained in 65% combined yield, of with a moderate *endo*-selectivity (40:60 ratio in favour of *endo*-**5a**). Considering the success of the cycloaddition reaction we investigated the possibility of using different oxazolones **3** in which the R group was varied. In particular, we tried to use compounds **3b-c** respectively substituted with the trityl and benzyl group which could act as S-protecting groups. Unfortunately the use of trityl derivative **3b** did not afford any cycloadducts in reasonable time, probably due to the steric hindrance of the triphenylmethyl group. Compound **3c** behaved similarly to **3a** affording a mixture of *exo*-**5c** and *endo*-**5c** in 1:1 ratio (50% total yields). The exploitation of sulfanyl-methylene-5(4*H*)-oxazolones, which to our knowledge have never been used in Diels-Alder cycloaddition reactions, provided access to the novel *endo*- and *exo*- β -sulfanyl-amido acid derivatives **5**. These norbornene amido acids are characterized by the C5-C6 double bond which can be variously functionalized affording more complex systems by the way of metal catalyzed reactions. Considering our interest in the exploitation of such β -sulfanyl-norbornene/ α -amino acid derivatives for peptide synthesis, we needed a procedure affording the free amino acid. As a consequence, hydrolysis of compounds **5a** with 6M hydrochloric acid were then effected (Scheme 2). By analyzing the reaction mixture we were surprised to observe that even though the methyl ester smoothly underwent hydrolysis, the hydrolysis of the benzamido group did not take place either in acidic (6-12M hydrochloric acid ranging from 0 to 110°C, HBr in CH₃COOH, H₂SO₄ 70%), or in basic solution (aqueous NaOH 10% reflux, KOH in MeOH/H₂O, *t*BuOK in THF) or using another hydrolysis procedure based on the imide formation by addition of Meerwein's reagent (triethyloxonium tetrafluoroborate).²

Scheme 2.2 Hydrolysis of amidoesters **5a** with 6M hydrochloric acid.



Reagents and conditions: (a) 6-12M hydrochloric acid ranging from 0 to 110°C, HBr in CH₃COOH, H₂SO₄ 70%, NaOH 10% reflux, KOH in MeOH/H₂O, *t*BuOK in THF, triethyloxonium tetrafluoroborate

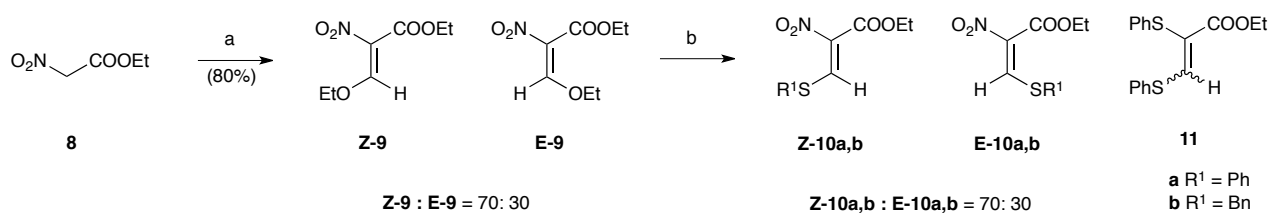
² Avenoza, A.; Busto, J. H.; Paris, M.; Peregrina, J. M.; Cativiela, C. *J. Het. Chem.*, **1997**, 34, 1099-1110

2.1.2 Alternative approach β -(phenylsulfanyl)- α -nitroacrylate as key intermediate

2.1.2.1 Cycloaddition reaction

Previous result prompted us to adopt a different strategy finally resulting in the successful formation of the desired free amino acids **7**, by using the appealing dienophile **10** as the key reagent. Acrylate **10** is a patented compound that, to our knowledge, has never been used as dienophile in Diels-Alder reaction.³ We reasoned that such a compound could be very reactive due to the presence of the nitro group which, could be easily transformed into an amino group by way of a simple reduction reaction, avoiding the difficult hydrolysis step described above.

Scheme 2.3 Preparation of dienophiles **Z/E-10a,b**



Reagents and conditions: (a) $\text{HC}(\text{OEt})_3$, 140°C , 8h, neat; (b) **a** HSPH, 110°C , 8h, neat, (95%); **b** HSBn, 110°C , 8h, neat, (95%);

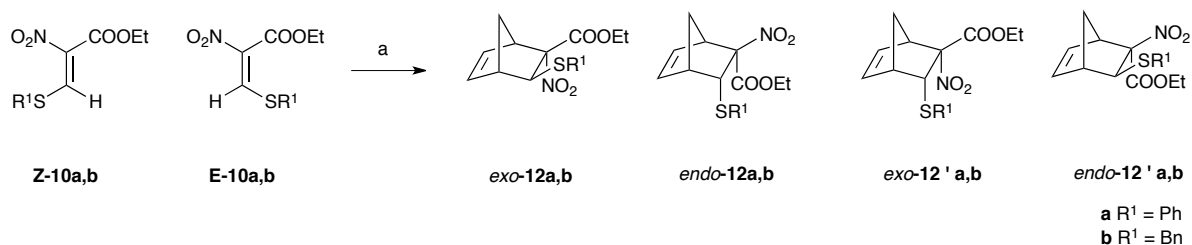
Ethyl α -nitro- β -ethoxyacrylate **9** was prepared from ethyl nitroacetate and triethyl orthoformate as a inseparable mixture of *Z/E* diastereoisomers (70:30, NMR analysis, Scheme 3).⁴ For the preparation of dienophile **10a** we optimized the patented methodology, heating *Z/E-9* and thiophenol (1.2 mol) at 110 – 120°C for 5 h removing the ethanol formed during the reaction by distillation under reduced pressure (75 mm Hg). Operating for a longer time or at higher temperature (150°C) resulted in a lowering of the yield and the formation of an undesired compound which was identified through ^1H -NMR analysis as compound **11**. Compounds *Z* and *E-10a* were obtained (150 – 155°C , 0.2 mmHg) as an inseparable mixture. ^1H -NMR analysis of the mixture showed the presence of two singlets at 8.75 and 8.48 δ associated with the vinyl protons of *E-10a* and *Z-10a* respectively, as expected according to the deshielding effect exerted by the nitro

³ Ehrhardt, H.; Ertel, H.; Mildenberger, H.; Sachse, B.; Hartz, P. *Thioenol ethers and their use*. Ger. Offen. (1979), CODEN: GWXXBX DE 2821639 19791122 CAN 92:180132.

⁴ Kamlet, M. J. *J. Org. Chem.*, **1959**, 24, 714-715

group on the *cis*-proton.⁵ This hypothesis was confirmed by nOe experiments showing a clear effect between the methylenic protons of the ester functionality and the proton at 8.48 δ totally lacking for the singlet at 8.75 δ . It has to be noted that the ratio of the two diastereoisomers *Z/E*-**10a** (70:30) exactly corresponds to the ratio of the ethoxy precursors *Z/E*-**9**. For a preliminary experiment, the diastereoisomeric mixture *Z/E*-**10a** (70:30) was reacted with cyclopentadiene by using ultrasound. The diene was added in portions (1 eq., 4 every 2h) affording a complex mixture which was chromatographed on silica gel affording two fractions with a 62% total yield (Scheme 4). The first fraction contained *exo*-**12a** as the main component (37% of the mixture) together with traces of *endo*-**12a** (3% of the mixture). In the second fraction *exo*-**12'a** is the major compound (53% of the mixture) while *endo*-**12'a** is the minor component (7% of the mixture) (Scheme 4). The two major compounds were obtained in pure form while *endo*-**12a** and *endo*-**12'a** were always in mixture with the corresponding *exo* isomers. The structures of compounds **12a** were inferred by NMR spectroscopic analysis. Their correct stereochemistry were deduced from the spectroscopic analysis on the corresponding amino derivatives, as reported below. Other reaction conditions were tested, but the only satisfactory results were obtained operating in a sealed tube at 110 °C (24h, 55% yield). Compounds *exo*-**12a** and *exo*-**12'a** were obtained as the major products (*exo*-**12** and *exo*-**12'**: 42 and 44% of the mixture respectively) together with a low amount of the other isomers (*endo*-**12** and *endo*-**12'**: 4 and 10% of the mixture, respectively).

Scheme 2.4 Cycloaddition reaction of dienophiles *Z/E*-**10a,b** with cyclopentadiene



Reagents and conditions: (a)), Cyclopentadiene, 24h, neat,

Table 2. Cycloaddition reaction of *Z/E*-**10a,b** with cyclopentadiene starting from 70:30 *Z/E* mixture or from the enriched 96:4 mixture.

Cycloaddition	Yield %	Yield %	Cycloaddition	Yield %	Yield %
Products	Starting from	Starting from	Products	Starting from	Starting from
	<i>Z/E</i> mixture	<i>Z/E</i> mixture		<i>Z/E</i> mixture	<i>Z/E</i> mixture

⁵ Knippel, E.; Knippel, M.; Michalik, M.; Kelling, H.; Kristen, H. *Tetrahedron*, 1977, 33, 231-234.

	70:30	96:4		70:30	96:4
<i>exo-12a</i>	23	11	<i>exo-12b</i>	17	9.5
<i>endo-12a</i>	2	2	<i>endo-12b</i>	1	0.5
<i>exo-12'a</i>	33	42	<i>exo-12'b</i>	29	34
<i>endo-12'a</i>	4	7	<i>endo-12'b</i>	8	8

Once we had confirmed the reactivity of the nitroacrylate as a dienophile, the possibility of addressing the cycloaddition toward the formation of a couple of diastereoisomers was considered. The mixture of dienophiles *Z/E-10a* was isomerized using UV light in different solvents (MeOH, CHCl₃, MeCN, CH₂Cl₂) aiming to increase the formation of a single isomer. The best results were obtained in CHCl₃ affording a mixture of *Z/E* isomers in 96:4 ratio (48h). Starting from this enriched mixture we performed the Diels-Alder reaction expecting only *exo*- and *endo-12a* as the products but, unexpectedly, we obtained all the four cycloadducts in a ratio which did not reflect the *Z/E* ratio of the starting acrylates (Table 2). By treating the final isolated isomeric mixture of the cycloadducts in the same (ultrasound, 24h) or in harsher (110°C neat, oil bath) conditions we did not observe any isomerisation and the *Z/E* ratio remained unaltered.

2.1.2.2 Theoretical study on the transition state

To better understand such a result we performed a theoretical study. All structures were fully optimized at the B3LYP/6-31+g(d,p) level of theory using Gaussian 03.⁶ All structures were characterized by vibrational analysis and no imaginary frequencies were observed for minima, while only one imaginary frequency, corresponding to the stretching of the forming/breaking bonds, was observed for the transition states. Reported gas-phase energies include zero-point energy corrections (frequencies were unscaled). This combination has been used with excellent results for the study of several pericyclic reactions including the parent Diels-Alder reaction. The *Z/E* conformational equilibrium in the dienophile fragment is important to the analysis of its enantioselective reactions, so we decided to take into account all the isomers. The calculated relative energies of the transition states (TSs) leading to the four possible cycloadducts from the

⁶ Frisch, M. J.; Trucks, G. W.; Schlegel, H. B.; Scuseria, G. E.; Robb, M. A.; Cheeseman, J. R.; Zakrzewski, V. G.; Montgomery, J. A. Jr.; Stratmann, R. E.; Burant, J. C.; Dapprich, S.; Millam, J. M.; Daniels, A. D.; Kudin, K. N.; Strain, M. C.; Farkas, O.; Tomasi, J.; Barone, V.; Cossi, M.; Cammi, R.; Mennucci, B.; Pomelli, C.; Adamo, C.; Clifford, S.; Ochterski, J.; Petersson, G. A.; Ayala, P. Y.; Cui, Q.; Morokuma, K.; Malick, D. K.; Rabuck, A. D.; Raghavachari, K.; Foresman, J. B.; Cioslowski, J.; Ortiz, J. V.; Stefanov, B. B.; Liu, G.; Liashenko, A.; Piskorz, P.; Komaromi, I.; Gomperts, R.; Martin, R. L.; Fox, D. J.; Keith, T.; Al-Laham, M. A.; Peng, C. Y.; Nanayakkara, A.; Gonzalez, C.; Challacombe, M.; Gill, P. M. W.; Johnson, B. G.; Chen, W.; Wong, M. W.; Andres, J. L.; Head-Gordon, M.; Replogle, E. S.; Pople, J. A. Gaussian 03; Gaussian, Inc.: Pittsburgh, PA, USA, 2003, <http://www.gaussian.com>

reaction of the dienophile (*Z/E*) with cyclopentadiene are shown in Figure 7. The B3LYP/6-31+G(d,p) calculations proposed in this work predict a ΔE of 0.8 kcal/mol in favour of the *E* isomer.

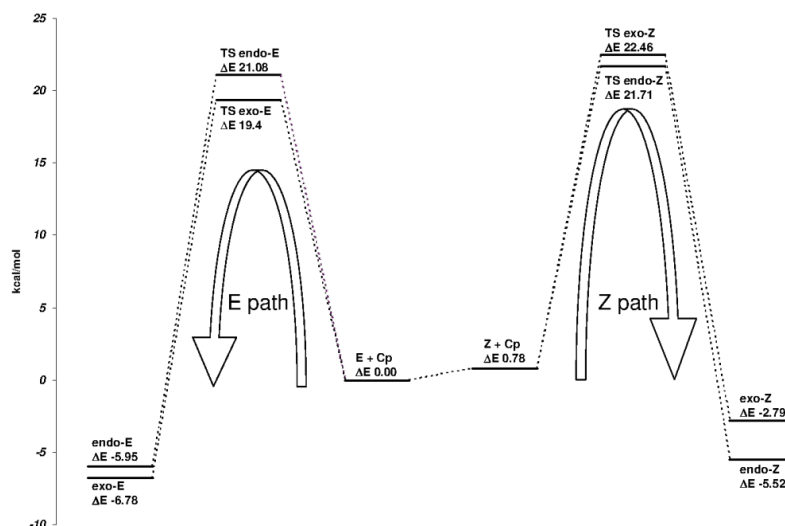


Figure 2.2 Reaction pathways for the cycloaddition process involving both dienophile (*Z/E*) and cyclopentadiene. Relative energies for the gas phase and activation energies (kcal/mol) are shown.

All cycloaddition reactions analysed were established to be under kinetic and not thermodynamic control due to the fact that under raised temperature and pressure the isomer ratio was virtually static. This would not be the case if the products were in equilibrium. If cycloreversion was occurring then the more thermodynamically stable isomer would be allowed to form. These findings were consistent with the idea that no cycloreversion followed by recyclization was happening. Therefore, it could be concluded that stereochemical leakage was not occurring after the cyclization reaction. Due to these findings the remaining alternatives are that the lack of stereocontrol is a consequence of the cycloaddition mechanism or due to an equilibrium pathway between *E* and *Z* isomers of the dienophile. The *E* path of the cycloaddition reaction is preferred to the *Z* path, so using the Le Chatelier's principle we are able to justify the high preference of the *E* derivatives using the enriched mixture. Furthermore this behavior could be reasonably explained by an asynchronous mechanism governing the cycloaddition. This is consistent with geometry analysis of the TSs that suggests a large bond length difference ($\Delta r = 1.2$ angstrom), as shown in Figure 8. Although an IRC analysis could be performed in order to propose an unconcerted process, we concluded that the reaction takes place as a highly asynchronous process with a large polar character and could be characterized by the approach of cyclopentadiene to the electron-poor carbon.

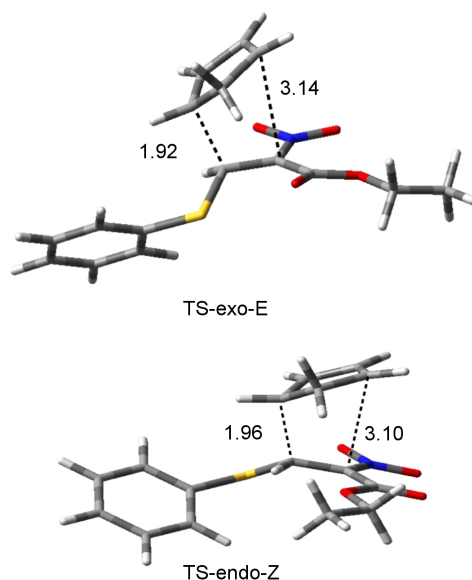


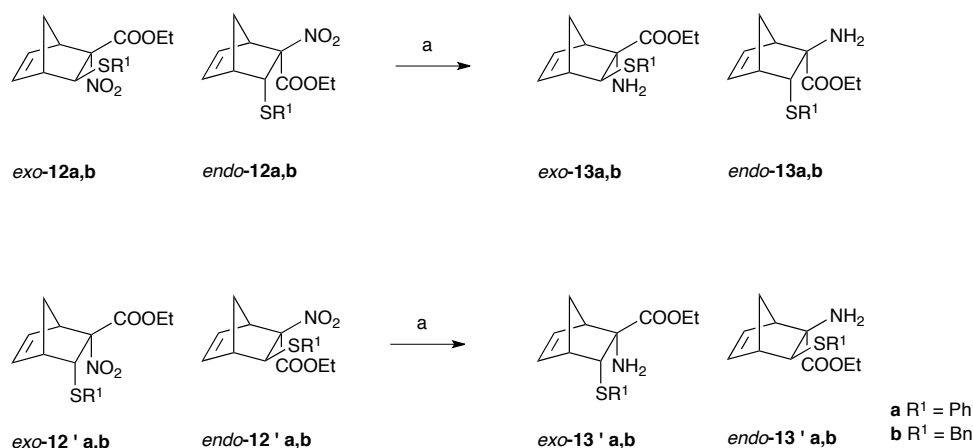
Figure 2.3 Calculated transition states for the reaction of *Z/E*-**10a** with cyclopentadiene giving *exo*-**12a** and *endo*-**12'a**. Relative distances are in angstroms.

2.1.2.3 Manipulation and deprotection

The same procedure optimized on the phenylsulfanyl derivatives was used to obtain the corresponding benzylderivative **12b** in which the benzyl group acted as a S-protecting group. The deprotection of sulfur can easily afford cysteine mimic derivatives (Scheme 4, Table 2).

The best procedure to transform the four norbornene nitroesters into the corresponding norbornene amino esters appeared to be the classical reduction with Zn/HCl 3M in THF (5h, 50 °C, Scheme 5). Compounds *exo*-**13a,b**, *exo*-**13'a,b**, *endo*-**13'a,b** and *endo*-**13a** were obtained in very good yields as pure compounds after chromatographic separation. Their structures were determined by analytical and spectroscopic data confirming the assignment previously inferred for the nitro derivatives **12a,b**. It has to be noted that *exo*-**13'b** was obtained in lower yield (70%) than the other amines which were obtained almost quantitatively. The analysis of the ¹H-NMR spectrum of the reaction mixture, before chromatographic purification, suggested that this result could be ascribed to a partial debenzylation occurring on *exo*-**12'b** during the reduction, producing the thiol derivative (R¹= H).

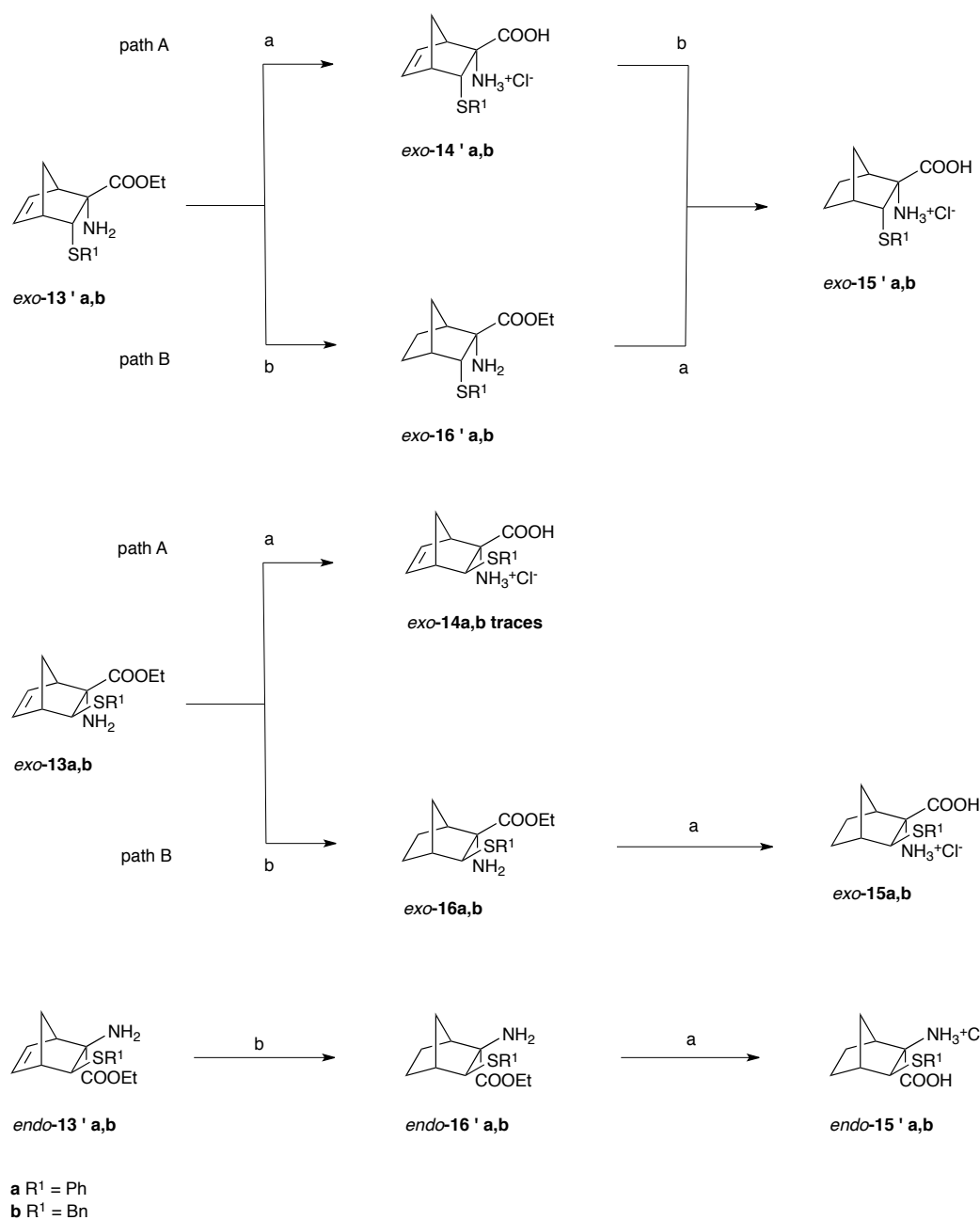
Scheme 2.5 Reduction of nitroderivative **12** to amines **13**



Reagents and conditions: (a) Zn dust, HCl 3N, THF, 50°C. different yield reported in experimental part.

Hydrolysis of the ester function was tested on the two major compounds *exo-13'a,b* and *exo-13a,b*. *Exo-13'a,b*, when treated with 6M hydrochloric acid (sealed tube, 100 °C, 6h), afforded the desired amino acids *exo-14'a,b* whose structures were confirmed by spectroscopic and analytic data (Scheme 6). Aiming to prepare the corresponding norbornane amino acids, compound *exo-14'a* was suspended in THF and hydrogenated at atmospheric pressure but unsatisfactory yields were obtained presumably due to the poor solubility of the starting amino acid. Better yields were obtained performing the reduction of the norbornene double bond of compounds *exo-13'a,b* containing the ester group. In such a way the norbornane amino esters *exo-16'a,b* were obtained and after hydrolysis, the desired amino acids *exo-15'a,b* were formed in a total 80% yield. Concerning *exo-13a,b* the application of the same conditions did not afford the desired amino acids *exo-14a,b* probably due to the steric hindrance exerted by the sulfanyl group. Prolonged heating (6M hydrochloric acid, sealed tube, 100 °C, 30h) afforded only low amounts of the desired amino acids together with compounds derived from electrophilic attack to the C5-C6 double bond. Therefore *exo-13a,b* were hydrogenated to *exo-16a,b* and these latter were treated with HCl in aqueous solution aiming to hydrolyze the ester function. *Exo-16a* was successfully transformed into the norbornane amino acid *exo-15a* by using 6M hydrochloric acid (110 °C, 48h). Concerning *exo-16b* after 96h in 6M hydrochloric acid at 110°C only partial hydrolysis occurred and compound *exo-15b* was obtained in low yield, as a mixture with inseparable side reaction products. Analogously, *endo-13'a,b* were hydrogenated to *endo-16'a,b* and after hydrolysis of the ester function, amino acids *endo-15'a,b* were obtained.

Scheme 2.6 Reaction pathway to norbornene and or norbornane amino acids **14** and **15**.



Reagents and conditions: (a) HCl 6N, 100°C, 6-30h; (b) H_2 1 atm, Pd/C 5%, EtOH, 25°C.

In order to confirm the stereochemistry, X-ray analysis on the major isomer *exo*-15'a was performed (Figure 9).

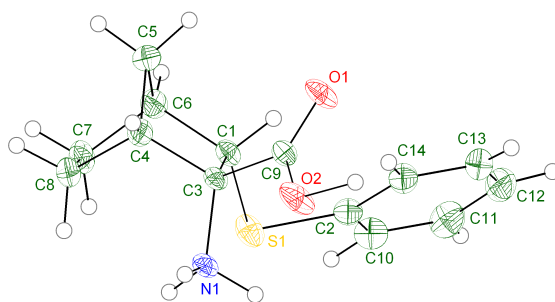
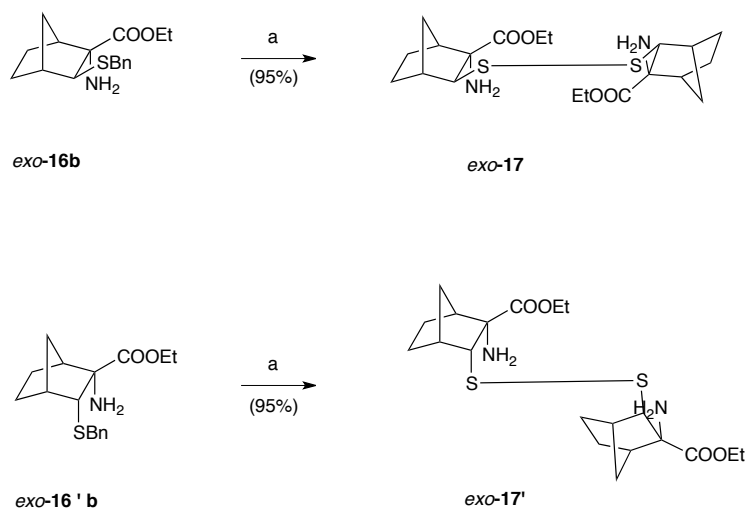


Figure 2.4 ORTEP plot of the ion $C_{14}H_{18}S_1N_1O_2^+$ within the compound **exo-15'a** at 298 K, with atom numbering scheme. Ellipsoids at 30% probability level

Finally, we tested the ease of the sulfur deprotection: heating **exo-16b** or **exo-16'b** in 12M hydrochloric acid at 110-120 °C with a small amount of Zinc powder for 12h resulted in the removal of the sulfur protection. At this stage we were not able to isolate the thiols which are formed but directly oxidised during the elaboration affording directly the respective disulfide **17** and **17'** as demonstrated by 1H - and ^{13}C -NMR spectroscopic analysis.

Scheme 2.7 Formation of the sulphide **17** and **17'**



Reagents and conditions: (a) HCl 37%, Zn dust, 110°C

2.2 Inclusion of 2-amino-3-(Benzylsulphanyl)norbornene-2-carboxylate in model peptide.

2.2.1 First attempt: convergent strategy and Carpino methodology

The S-protected amino ester *exo*-**13'b** (Fig. 10) represents the starting material for model peptidomimetic.

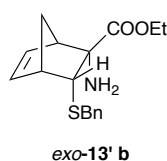


Figure 2.5 Unnatural amino acids helical inductor

The synthesis previously described in this chapter⁷ was scaled to 1.5 grams in order to assure the availability of a congruous amount of such compound. Pentapeptides **18a,b** and **19a,b** were planned containing Ala, Aib and the norbornene/ane amino acid (NRB) in i+1 position (Fig. 11).

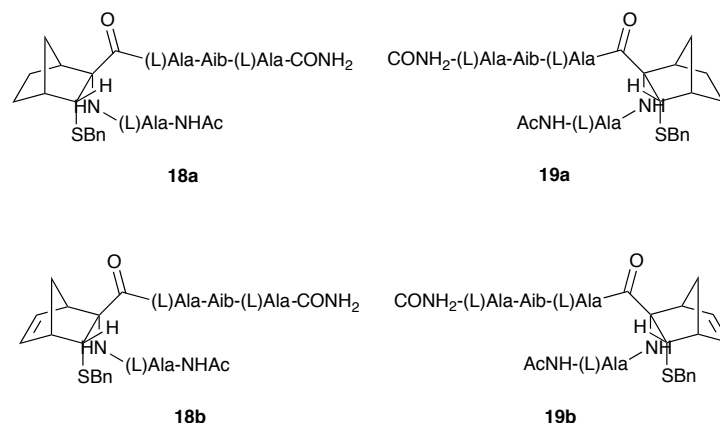


Figure 2.6 Projected peptide

For the preparation of pentapeptides **18a** and **19a** we planned to use the convergent strategy depicted in Scheme 8. Exploiting the Carpino methodology by coupling **20** or **21** with a (L)Ala, we expected the formation of the two corresponding diastereoisomers **22/23** or **24/25**.⁸ Subsequent deprotection and hydrolysis manipulations would have allowed to proceed with the coupling with the tripeptide **28** (L)-Ala-

⁷ Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Clerici, F. *Tetrahedron* **2012**, *68*, 1951-1962

⁸ Carpino, L.A.; Sadat-Aalae, D.; Chao, H. D.; Deslms, R. H. J. Am. Chem. Soc. 1990, *112*, 9651-9652

Aib-(L)-Ala previously synthesized as known⁹ by solid phase synthesis. Unfortunately, although apparently trivial this strategy provided several critical steps. The separation of the two diastereoisomers **24** and **25** was impossible and regarding **22** and **23** flash chromatography on prepacked column (experimental procedure) gave always, together with low amount of pure **22** and **23**, large amount of mixture. Nevertheless, with iterative process we were able to obtain good amount of products to go on with the synthesis and also to perform on dipeptide **22** X-Ray analysis that confirmed the structure (Figure 12) and gave us important information about the crystal cell (experimental procedure).

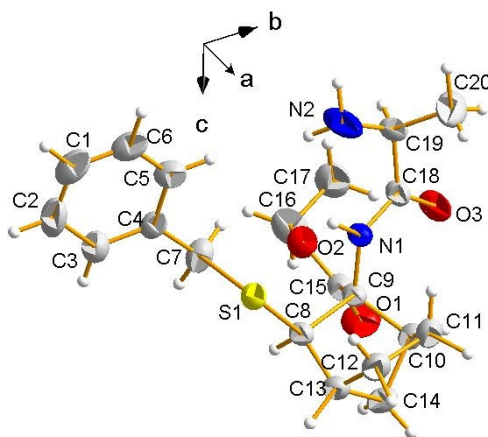
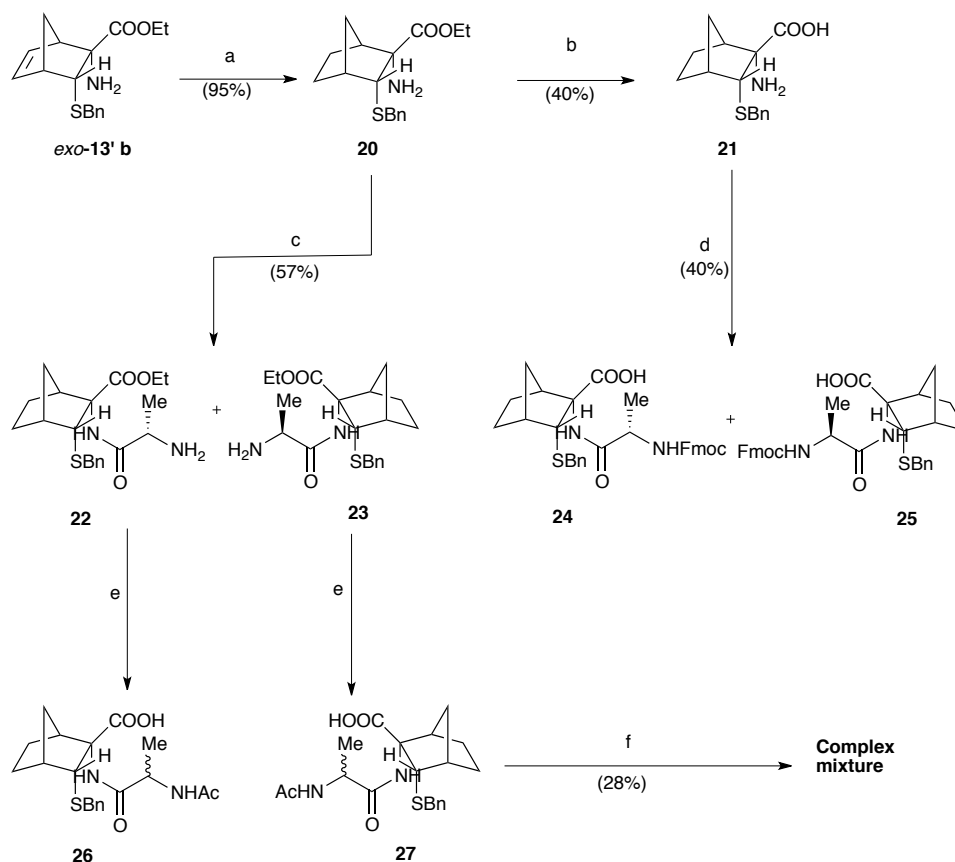


Figure 2.7 X-Ray Crystal structure of dipeptide **22**

The hydrolysis of the ester function of **22** and **23** was troublesome too, leading always, even if, in most cases, in low amount, to racemization of the Alanine stereocenter or to partial cleavage of amide bonds or deprotection of N-terminus despite to numerous reaction conditions both acidic and basic (NaOH, KOH, LiOH, or HCl 3N, 6N, 37%). However, we tried to couple the tripeptide (L)-Ala-Aib-(L)-Ala **28** with the crude containing **27** as the main product operating in standard conditions, hoping to be successful in the separation of the final diastereoisomers. Unfortunately, we obtained an inseparable mixture of more than two peptides with very low yield probably due to the steric hindrance and the use of tripeptide **28** coming from solid phase synthesis as TFA salt.⁵⁶

⁹ S. Pellegrino, A. Contini, F. Clerici, A. Gori, D. Nava, M.L. Gelmi *Chem. Eur. J.* **2012**, 18, 8705 – 8715

Scheme 2.8 Convergent synthesis for preparation of peptide **18a** and **19a**



Reagent Condition: a) Pd/C 5%, H_2 1atm, EtOH, r.t., 6h; b) 1)HCl 6N, reflux, 48h, 2)Propilenoxide, MeOH, reflux 6h; c) 1)BSA, N_2 , DCM, r.t., o.n. 2)FmocNH-(L)Ala-F, 0°C to r.t., 3)Piperidine/DMF; d) 1)BSA, N_2 , DCM, r.t., o.n. 2)FmocNH-(L)Ala-F, 0°C to r.t.; e) 1)Ac₂O, TEA, DCM, 2)H⁺ o OH⁻ Hydrolysis; f) 1)HOAt, EDC, DCM, 0°C, 1h. 2) TFA- NH_3^+ Ala-Aib-Ala-CONH₂ **28**, DiPEA, r.t..

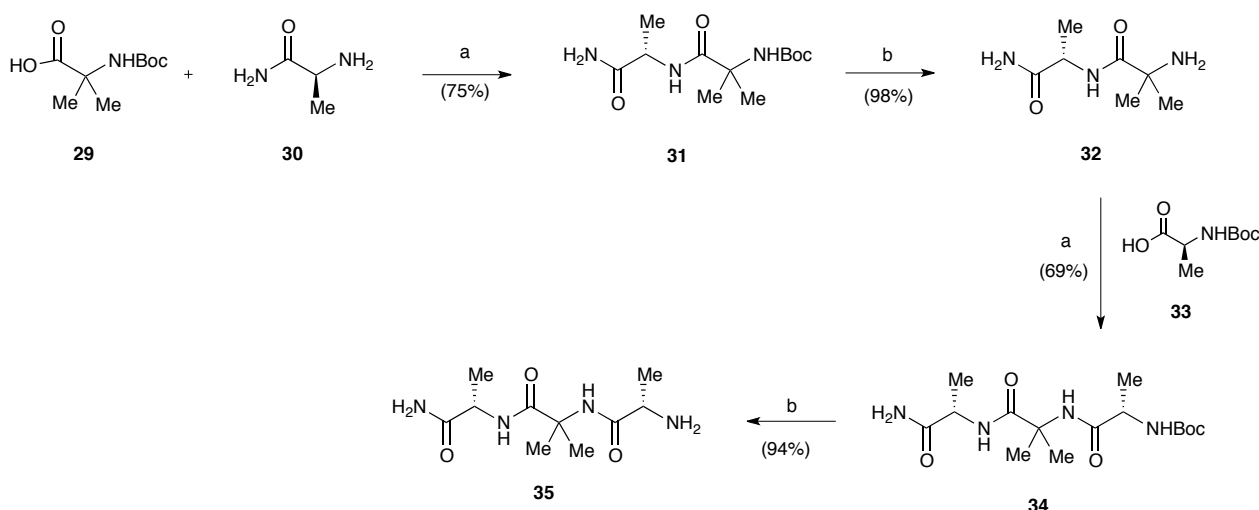
2.2.2 Linear Strategy

2.2.2.1 Solution phase grams scale synthesis of tripeptide Ala-Aib-Ala

So, taking in mind that an important aim of our project was to disclose an easy and selective procedure scalable to grams, we changed our synthetic plan applying a strategy not including any ester hydrolysis step when racemization events could have been possible. Moreover, the solid phase synthesis of **28** presents limited scalability so, considering these problems, we turned our attention to a new gram scale protocol which made available the tripeptide **28** in pure form as free amine **35**. According to this, we set up linear Boc chemistry solution strategy that allowed us to prepare the free amine tripeptide (L)NH₂Ala-Aib-(L)AlaCONH₂ **35** in 50% overall yield on a gram scale. The process, described in Scheme 9, consisted in the iteration of the coupling between NH₂AACONH₂ (**30** and then **32**) to avoid lactamization, with Boc-protected amino acid (**29** and then **33**) in standard condition (EDC and HOAt as condensing agent), followed

by thermal decomposition of Boc protection mediated by microwaves (affording respectively **32** and **35**).¹⁰ In this way we avoided the step related to the liberation of the corresponding TFA salt needed in the solid phase protocol and could directly perform another round of grooving of our peptide **35** with another Boc protected amino acids.

Scheme 2.9 Solution phase synthesis of free amine tripeptide (L)NH₂Ala-Aib-(L)AlaCONH₂ **35**



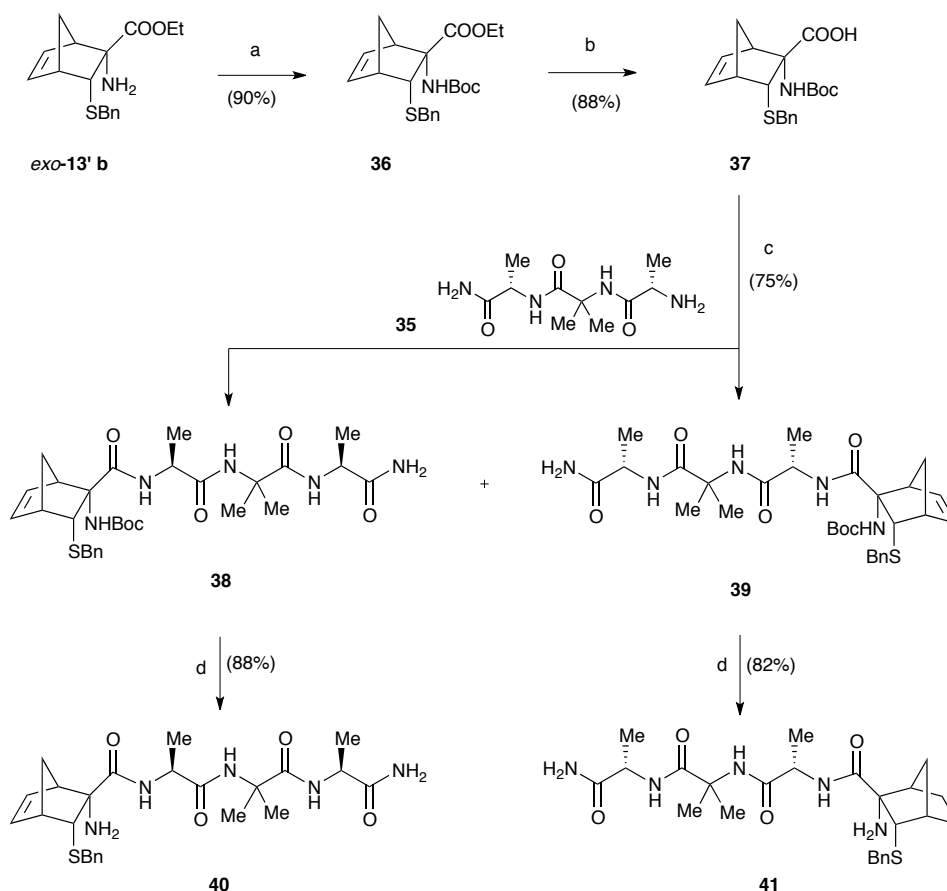
Reagent Condition: a) 1)EDC, HOAt, DCM 2)DPEA, 0°C-r.t. 24h; b) H₂O, M.W., 150°C.

2.2.2.2 Inclusion of Benzylsulphanyl norbornene derivative in the model peptide

Having in our hand a considerable quantities of **35** we prepared the Boc-protected amino acid **36** in good yield on gram scale through the reaction with Boc anhydride followed by hydrolysis of the ester function in basic condition using KOH 3M in MeOH. (Scheme 10). Compound **36** was coupled with the tripeptide **35** in the same condition described before affording the two expected diastereoisomeric tetrapeptides **37** and **38** in reasonable yield. The two compounds were separated by flash chromatography and characterized by NMR analyses. The following microwaves Boc deprotection step afforded compounds **39** and **40** in satisfactory yield without any racemization event in reasonable quantities and yield.

¹⁰ A. Thaqi, A. McCluskey, J. L. Scott. A mild Boc deprotection and the importance of a free carboxylate. *Tetrahedron Letters*, **2008** 49 6962–6964

Scheme 2.10 Linear synthesis of model peptide precursors



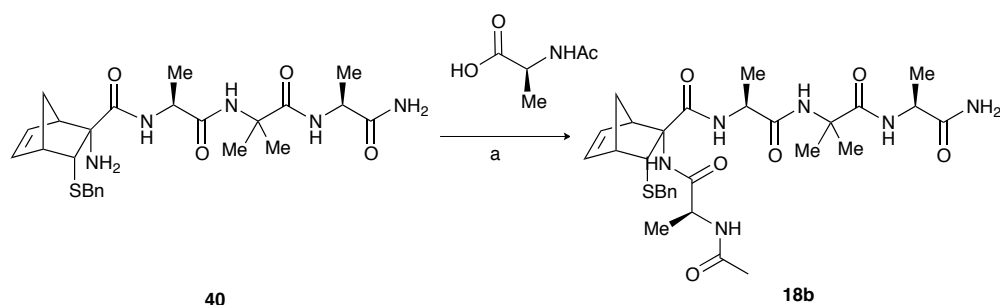
Reagent conditions: a) (Boc)₂O, TEA/DCM, 0°C to r.t.; b) 1)KOH/MeOH 3M, 2)HCl 1M, 0°C; c) 1)EDC, HOAt, DCM/DMF, r.t. 2)DIPEA, 0°C-r.t.,o.n.; d) H₂O, M.W., 150°C.

Peptide **39** and **40** were submitted to different coupling conditions with (L)-NHAc-Ala-OH (Scheme 11 and 12). In many cases the coupling gave extremely low yields and events of racemization occurred. The difficulties are probably connected to the level of structuration reached^{11 12} by the tetrapeptide and consequently steric hindrance. In the case of compound **40** satisfactory yield and low level of racemization was obtained by using classic coupling condensation conditions with HOAt and EDC in DCM and DIPEA. HPLC inverse phase purification permitted the obtainment of projected peptide **18b** in pure form in satisfactory yield. (experimental part)

¹¹ G.Jung; H. Brukner; R. Bosch; W. Winter; H. Schaal; J. Strahle. Ac-L-Ala-Aib-Ala-OMe: X-Ray Analysis of a distorted b-Bend and Magnetic Nonequivalence of Aib-Methyl Groups. *Liebigs Ann. Chem.* **1983**, 1096-1106

¹² R. Banerjee; S. Chattopadhyay; G. Basu. Conformational preference of a short Aib/Ala-based water-soluble peptide as a function of temperature

Scheme 2.11 Synthesis projected peptide **18b**



Reaction Conditions:

- a) 1) DCM, HOAt, EDC, N-Ac-Ala-OH, 0°C; 2) 27, i, 0°C-rt = yields 40%, recovered starting material 40%, partial racemization 5%
- b) THF, EEDQ, M.W., 70 watt, Air cooling 60-70 °C, 30' = yield 60% partial racemization 20%
- c) 1) DCM, HOBt, EDC, N-Ac-Ala-OH, 0°C; 2) 27, DiPEA, 0°C-rt = yields 5%, recovered starting material 80%
- d) 1) DCM, HBTU, EDC, N-Ac-Ala-OH, 0°C; 2) 27, DiPEA, 0°C-rt = NO reaction
- e) 1) DCM, HBTU, N-Ac-Ala-OH, 27, DiPEA, 0°C-rt = yields 10%, recovered starting material

For peptide **42** the use of EEDQ as condensing agent in THF did not lead to racemization event but the yield was low. Therefrom, we considered the possibility of using microwaves irradiation to improve the yield, probably low due to the structuration level of peptide. The microwaves are known^{13 14 15 16 17} to drive to unstructured conformations permitting general increasing of yields and their effect is exploited on the solid support peptide synthesizers. Nevertheless, to our knowledge few examples are present in literature concerning their use for solution peptide synthesis¹⁸ and only one employing unnatural amino acids¹⁹. A sample containing **41** (L)-NHAc-Ala-OH and EEDQ in THF was irradiated for 30' at as low temperature as possible with conventional microwaves compressed air cooling reactor experimental part (Scheme 5). The choice to use EEDQ in THF as condensing agent is based on the fact that it did not required activation steps at 0°C and base addition, otherwise difficult to apply in our M.W. system. At the end of the reaction, by cooling down the vials, peptide **19b** directly precipitated in the reaction environment and *in vacuo* filtration

¹³ H.-M. Yu, S.-T. Chen, and K.-T. Wang, "Enhanced coupling efficiency in solid-phase peptide synthesis by microwave irradiation," *The Journal of Organic Chemistry*, vol. 57, no. 18, pp. 4781–4784, 1992.

¹⁴ J. M. Collins and M. J. Collins, "Novel method for solid phase peptide synthesis using microwave energy," *Biopolymers*, vol. 71, no. 3, p. 361, 2003.

¹⁵ M. Erdélyi and A. Gogoll, "Rapid microwave-assisted solid phase peptide synthesis," *Synthesis*, no. 11, pp. 1592–1596, 2002.

¹⁶ H. J. Olivos, P. G. Alluri, M. M. Reddy, D. Salony, and T. Kodadek, "Microwave-assisted solid-phase synthesis of peptoids," *Organic Letters*, vol. 4, no. 23, pp. 4057–4058, 2002.

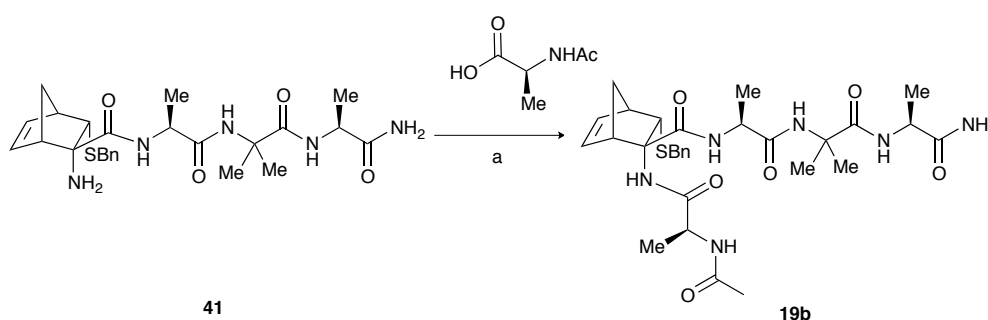
¹⁷ A. de la Hoz, A. Diaz-Ortiz, A. Moreno. Microwaves in organic synthesis. Thermal and non-thermal microwave effects. *Chem. Soc. Rev.*, **2005**, 34, 164-178

¹⁸ A. Mahindra, R. Jain et al. *RCS Adv.* **2013**, 3, 16810-16816

¹⁹ V. Santagada, F. Fiorino, E. Perissutti, B. Severino, V. De Filippis, B. Vivencio, G. Caliendo. Microwave enhanced solution coupling of the α - α -dialkyl amino acid, *Aib. Tetrahedron Letters* **2001**, 42 5171–5173

allowed the obtainment of **19b** in pure form. X-Ray analyses of **19b** (crystals grown in CH₃CN) confirmed that no racemization event occurred in the entire synthetic process and gave the absolute stereochemistry as well as peptide secondary structural information (see below).

Scheme 2.12 Synthesis projected peptide **19b**



Reaction Conditions:

- a) THF, EEDQ, M.W. , 70 Watt, Air cooling 60-70 °C, 30' = yield 60%, NO racemization
- b) THF, EEDQ, 0°C-rt = yields 35%, 60% recovered starting material NO racemization
- c) 1) DCM, HOAt, EDC, N-Ac-Ala-OH, 0°C; 2) 27, DiPEA, 0°C-rt = yields 30%, recovered starting material 20%, partial racemization 15%
- d) 1) DCM, HOBT, EDC, N-Ac-Ala-OH, 0°C; 2) 27, DiPEA, 0°C-rt = NO reaction
- e) 1) DCM, HBTU, N-Ac-Ala-OH, 27, DPEA, 0°C-rt = complex racemic mixture

It has to be noted that the reported synthetic procedure allows primarily for the preparation of model pentapeptides containing the bicyclic NRB amino acids conserving the C5-C6 double bond. This is an important goal because the presence of such reactive group opens the way to further functionalization.

Pentapeptides **18a** and **19a** can be obtained by hydrogenation of the precursors **18b** and **19b** (H₂, Pd/C, Solvent), and this step will highlight the influence of the double bond on the helical stabilization.

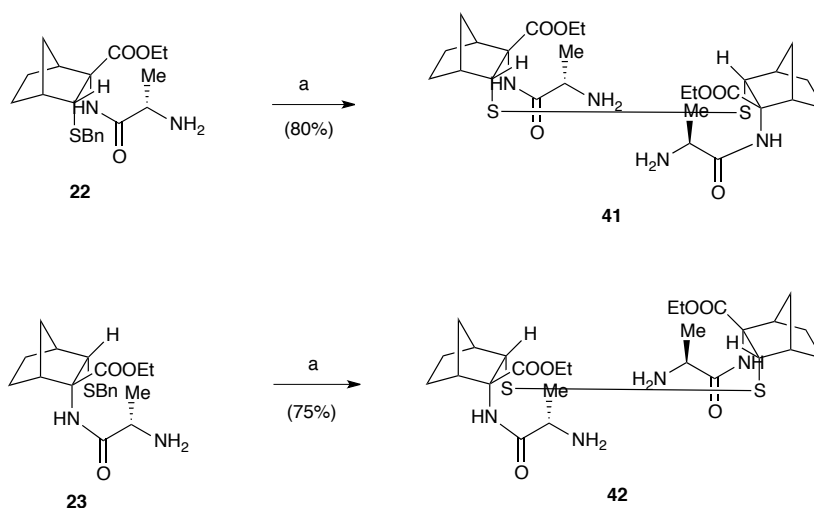
2.2.3 Sulfur atom Deprotection

Several reaction conditions were tested on dipeptide precursors **21** and **22** in order to obtain the deprotection of steric masked sulfur atom. The classical reductive debenzylation methodology like hydrogenation with Pd/C H₂ 1atm, Pd/C and LiAlH₄ in propanol or Birch type reaction, never lead satisfactory results leading to the no deprotected product or decomposition of precursor. The conditions previously used with HCl 37% at 110°C were evidently not applicable to the entire peptide and the same consideration is valid for the most methodology reported in literature for S-debenzylation which equally required hard reaction conditions.²⁰ The most common methodologies for S-debenzylation on peptides reported in literature, involve the use of

²⁰ Greene, T. W.; Wuts, P. G. M. *Protective Groups in Organic Synthesis*, 3rd ed.; John Wiley & Sons: New York, 1999.

HF under pressure. We have been never tested such reaction for practical reasons and lack of properly instrument ²¹. Finally, we identified in the use of Ph₂SO, MeCl₃Si in TFA as a solvent at 0°C mild conditions that permit the obtainment of compounds **42** and **43** in good yield preserving the stereocenter and the other functionalities.

Scheme 2.13 Deprotection of sulfur atom on dipeptide **22** and **23**

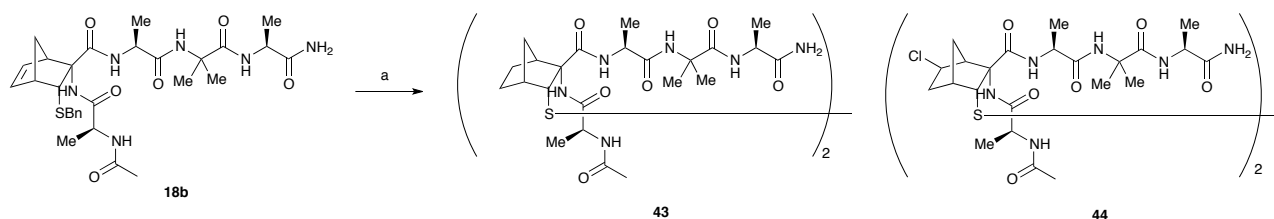


Reagent, condition: a) 1) PhSO, TFA, 5°C, 10' 2) MeCl₃Si, 6h.

A first attempt of the same deprotection conditions on the peptide **18b** was tested and a mixture of the two compounds **44** and **45** was obtained in high yields deriving from deprotection followed by HCl addition to the C5-C6 double bond and oxidation to the corresponding dimers. Considering the difficulties in solving such a troublesome step, we were very satisfied in having identified a practical method for the sulfur atom protecting group removal on a peptide structure, without affecting functionality and chiral centres even if requiring a prior reduction of the double bond. Further studies are in process aiming to find conditions allowing deprotection and conservation of the double bond.

²¹ Sakakibara, S.; Shimonishi, Y.; Kishida, Y.; Okada, M.; Sugihara, H. *Bull. Chem. Soc. Jp.*, **1967**, 40, 2164-2167 Akaji, K.; Tatsumi, T.; Yoshida, M.; Kimura, T.; Fujiwara, Y.; Kiso, Y. *J. Chem. Soc.*, **1992**, 114, 4137-4143

Scheme 2.14 Deprotection of sulfur atom on a peptide **18b**



Reagent, condition: a) 1) PhSO₂, TFA, 5°C, 10' 2) MeCl₃Si, 6h

2.3 Computational Study

2.3.1 Introduction to REMD analysis

In order to confirm the ability of the norbornene amino acid *exo*-**13b'** to induce Helical secondary structure once inserted in peptide system a conformational analysis was performed with the methodology of Replica Exchange Molecular Dynamics²² (REMD). This methodology allowed to sample the conformational space of complex molecules better than a classic molecular dynamic method push the system that we want to simulate to overcome energetic barrier otherwise impossible. The REMD analysis mainly consist in a elaboration of multiple molecular dynamic at the same time, the number of them called "replica" depend on the complexity of the system and the power of the calculator. Each replica start from the same structure, but is subjected to a different temperature inside a range of temperature chosen by the operator in order to explore a larger domain of the conformational space of the system. The low temperature simulation will give stable trajectory but with a limited number of conformation. On the other hand the replica simulated at higher temperature will lead to instable trajectory but where the system will be able to explore an wider conformational space. The power of the REMD method consist in the interchange of the of the simulation temperature between the replicas at predetermined time (Figure XX). If , at the time of the interchange, the system has an energetically acceptable geometry ta the new temperature the replica will proceed with the simulation at the new temperature. Otherwise the exchange will be not accepted and the replica will keep on the simulation on the initial temperature. In general, in a REMND analysis, the temperature interchange process could be occur thousands times giving an optimal sampling of conformation. In fact the trajectories obtained at lower temperature we will be characterized also by conformation accessible only at high temperature.

²²Y. Sugita, Y. Okamoto; *Chem. Phys. Lett.* **1999**, 314, 141-151

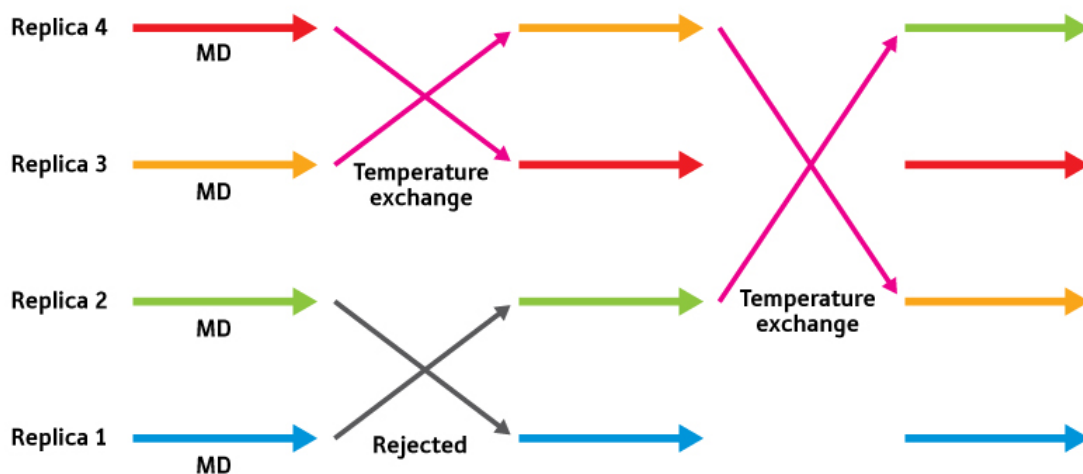


Figure 2.8 REMD analysis, schematic explanation.

The trajectory analysis in REMD simulation should be obtained by the extrapolation of part of the trajectory for each replica at the desired temperature, that generally is the closer temperature to the experimental condition. The result of REMD simulation is not only a large set of conformation, but applying the extrapolation process becomes a high confidence trajectory of molecular dynamic that could show the folding process of the examined system. In our case peptide.

2.3.2 Application of REMD protocol to the pentapeptide model containing Norbornene Cysteine Mimic (+) and (-).

In this study has been considered pentapeptide **18b** and **19b** that differ only for the absolute stereochemistry of the unnatural norbornene amino acid.

19b L-Ala-(1*S*,2*S*,3*S*,4*R*)NOR-L-Ala-Aib-L-Ala

18b L-Ala-(1*R*,2*R*,3*R*,4*S*)NOR-L-Ala-Aib-L-Ala

The used protocol has been optimized in a previous work²³ and is based on the use of the force field ff99SB as implementation of the Amber 12 software. Each peptide has been designed with an acetyl protection at the N-terminus and methylcarboxamide at the C-terminus. The simulation has been run starting from the full extended conformation of both peptide ($\psi = \phi = \omega = 180^\circ$ C).

The REMD simulation has been performed on twelve replica in a the range of temperature of 260 -600 K. The simulation time for each replica has been set on 50 ns, for a overall time of 0.6 μ s, during this time

²³ S. Pellegrino, A. Contini, F. Clerici, A. Gori, D. Nava, M.L. Gelmi. *Chem. Eur. J.* **2012**, 18, 8705 – 8715

25000 temperature interchange have been done after that a convergence of result were obtained. The extrapolated trajectory considered for each peptide are refer to the temperature of 300K. The secondary structures and the *cluster* have been scan by H-Bond analysis. In this analysis every geometry present in the trajectory was compared with the other geometry in order to obtain different *cluster* of conformer. Greater will be the population of a particular *cluster*, higher will be the probability that the characteristic conformation of this *cluster* correspond to the real conformation experimentally obtaining in the same condition (temperature).

The cluster analysis (Table 1) of the pentapeptide **19b** and **18b** trajectories shown that for either isomers (*1S,2S,3S,4R*) and (*1R,2R,3R,4S*) a prevalent conformation is present and correspond to the cluster C1. The average value analysis of ψ and ϕ of the representative conformation of most populated *cluster* C1 could underline that both sequence **19b** and **18b** lead to a right hand helical secondary structure. The representative structure of the second most populated cluster C2 on the contrary shown value of ϕ of right hand helical structure though with high standard deviation in the case of peptide **19b** that indicate an elevated structure instability. Regarding ψ C2 conformation has positive of peptide **19b** values corresponding to left hand helix or random structures. The conformation of C2 that correspond to the 8.7% and 20.3 % of the total conformation for peptide **19b** and **18b** respectively. In the case of peptide **18b** cluster C2 should be folding intermediate of more stable conformation of C1 .

Table 1. Clusters analysis's result(pop%)

	19b (SSSR)			18b (RRRS)		
	Pop%	ϕ	ψ	Pop%	ϕ	ψ
C1	82.6	-67.04±44.11	-14.48±26.74	79.1	-66.39±33.53	-25.19±17.70
C2	8.7	-20.43±105.72	19.95±9.03	20.3	-47.72±70.85	-13.59±25.12
C3	6.0	-9.09±71.34	25.90±14.61	0.3	-12.17±61.55	-7.79±29.20
C4	1.9	-27.85±76.33	4.12±24.90	0.3	-91.31±46.76	-23.59±29.15
C5	0.8	1.44±83.23	12.76±12.40	0.0	-69.00±8.44	-53.06±71.78

The entire trajectory MD has been explored by secondary structure analysis and H-bond evaluation. Secondary structure analysis consists in the evaluation, during the entire simulation, of the agreement degree of the value ψ and ϕ for each peptide residues with the characteristic value of known secondary structure. (Table 2) The table underlines how the most structured part of the peptide is the residue NRB-Ala3-Aib4 that possess high value of agreement with 3_{10} -helix in both peptides. Especially the high value of Ala3 in the peptide **18b** found a correlation with an equally high unexpected value in the NMR analysis during the experiment of DMSO d_6 titration. H-bond evaluation analysis highlights the occupancy percentage of each H-

bond during the simulation time and the most stable H-bond is between residues i, i+3 of the core of the peptide. Also this analysis revealed a strong correlation with the NMR-experiment data reported in figure 20 and figure 21 indicating the stability of H-Bond (Table 3).

Table 2 : Secondary structures analysis

	19b P1 (SSSR)			18b P2 (RRRS)		
	helix ₃₁₀	α -helix	Turn	helix ₃₁₀	α -helix	Turn
ALA1	32.3	1.8	9.6	67.19	5.78	12.29
NRB	76.7	1.9	10.8	79.66	6.09	13.23
ALA3	47.3	1.9	18.6	82.54	6.09	10.71
AIB4	71.1	2.0	25.4	66.19	6.40	24.58
ALA5	52.6	0.4	31.4	44.14	1.34	31.44

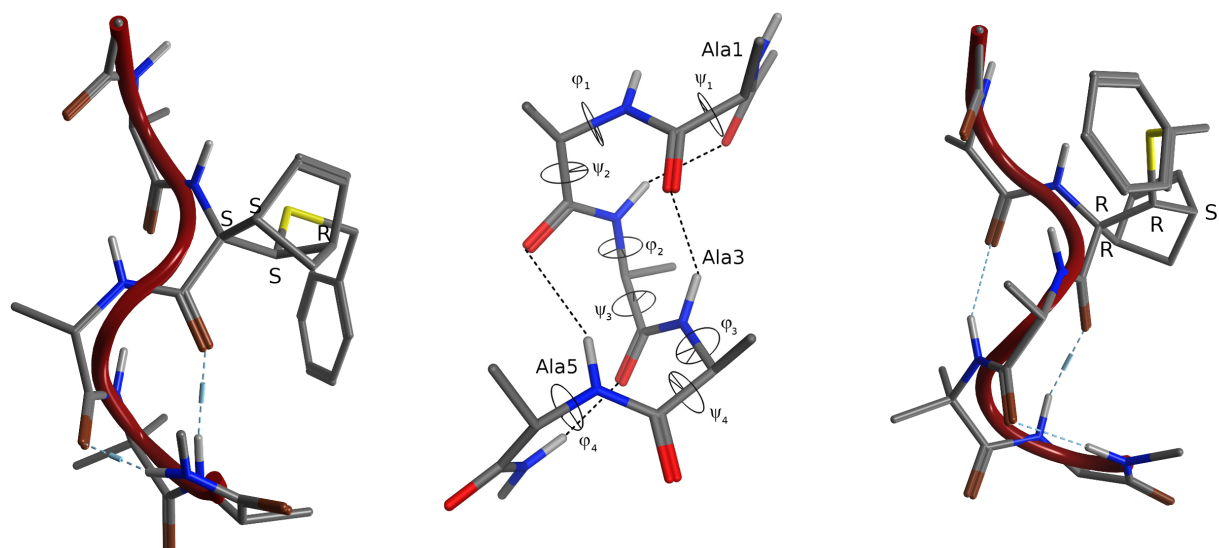
The difference compared to 100% corresponds to the percentage of structures not related to the categories listed in the table.

Table 3: H-Bond analysis

Donor	Acceptor	Occupancy P1	Occupancy P2
AIB5-Main-N	ALA2-Main-O	46.40%	68.43%
ALA6-Main-N	NRB3-Main-O	73.44%	54.24%
AIB4-Main-N	ACE1-Main-O	35.45%	58.12%
NEM7-Main-N	NALA4-Main-O	58.78%	40.89%

Hydrogen bond could be identified as such, must be satisfied the following parameters: donor-acceptor distance $\leq 4\text{\AA}$, donor-H-acceptor angle = 120 ± 30 deg.

From the geometry reported in figure 13, related to the representative structures of the most populated cluster we could make some considerations. It is noteworthy that the helical core, close to the norbornene and Aib regions of peptide **19b**, is perfectly structured as 3_{10} helix at contrary in the case of peptide **18b** the perfect structuration is obtained only in the Aib regions. Remarkable deviation from the helical ideal structure are shown only at the level of N-terminus and C-terminus, as expected, and also this observation has found a strong confirmation in the NMR experiment described in the following pages. Finally, we could observe that both enantiomers (*1S,2S,3S,4R*) and (*1R,2R,3R,4S*) are strong inductors of 3_{10} type Helix with a better score for the enantiomer included in the peptide **19b**.



P1 19b

ψ_1	-13.61 ± 34.74
ϕ_1	-53.12 ± 16.48
ψ_2	-29.37 ± 14.99
ϕ_2	-62.45 ± 18.99
ψ_3	-26.30 ± 15.16
ϕ_3	-55.06 ± 9.96
ψ_4	-24.25 ± 12.81
ϕ_4	-106.84 ± 35.15

P2 18b

ψ_1	-10.16 ± 38.78
ϕ_1	-36.01 ± 41.02
ψ_2	-17.23 ± 26.91
ϕ_2	-62.13 ± 18.97
ψ_3	-25.21 ± 15.67
ϕ_3	-54.44 ± 10.24
ψ_4	-24.28 ± 12.67
ϕ_4	-107.59 ± 35.53

Figure 2.9 Representative geometry of the most populated cluster obtained for peptides 1 (pop = 83.3%) and 2 (pop = 85.3%).

2.4 Conformational Study

2.4.1 2D-NMR Spectroscopy, NOE interpretation

Both peptides were subjected to NMR conformational analysis. Detailed structural characterization of peptide **19b** was carried out using 2D NMR experiments. For peptide **18b** a less exhaustive set of data permitted in any case to identified structural parameters. Experiments were performed in both cases in CD_3CN (10mM) in order to avoid hydrophobic collapse of chlorinated solvent and simulate as much as possible the water medium used in the computational analysis. Homonuclear and Heteronuclear 2D NMR experiment permitted an overall assignment of **18b** and **19b** protons and carbon resonances (see COSY,

HSQC, HMBC spectra experimental part. Considering **19b** NOESY spectra it was possible to identify all sequential short range $\text{NH}_i\text{-NH}_{i+1}$ cross peaks in the amide region (Figure 13) that are diagnostic of helical conformation.

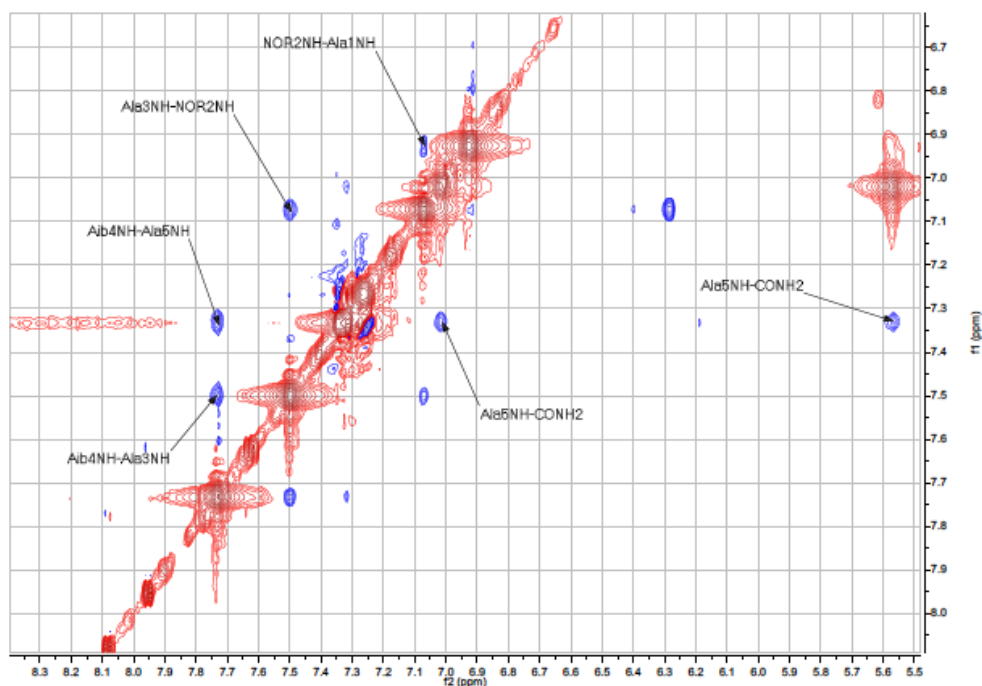


Figure 2.10 Amide protons region of the NOESY spectrum (τ_m 500ms, BBI probe, 300k, 500MHz) of peptide **19b** in CD_3CN (10mM). Sequential short range $\text{NH}_i\text{-NH}_{i+1}$ signals.

The analysis of $\text{C}^{\alpha}\text{H-NH}$ allowed to discriminate between 3_{10} -helix structure and α -helix. No $\text{C}^{\alpha}\text{H}_i\text{-NH}_{i+4}$ cross-peaks inherent of α -helix are detectable in the NOESY spectra but many diagnostic $\text{C}^{\alpha}\text{H}_i\text{-NH}_{i+3}$ and $\text{C}^{\alpha}\text{H}_i\text{-NH}_{i+2}$ medium range cross-peak signals typical of 3_{10} -helix are present. An inspection of the fingerprint area of NOESY spectrum revealed two $\text{C}^{\alpha}\text{H}_i\text{-NH}_{i+3}$ (Ala(1) H^{α} -Aib(4)NH and Ala(4) H^{α} -CONH₂), and two other $\text{C}^{\alpha}\text{H}_i\text{-NH}_{i+n}$ (Ala(1) H^{α} -Ala(3)NH and Ala(3) H^{α} -Ala(5)NH) signals supporting the hypothesis of 3_{10} -helix structure (Figure 14).

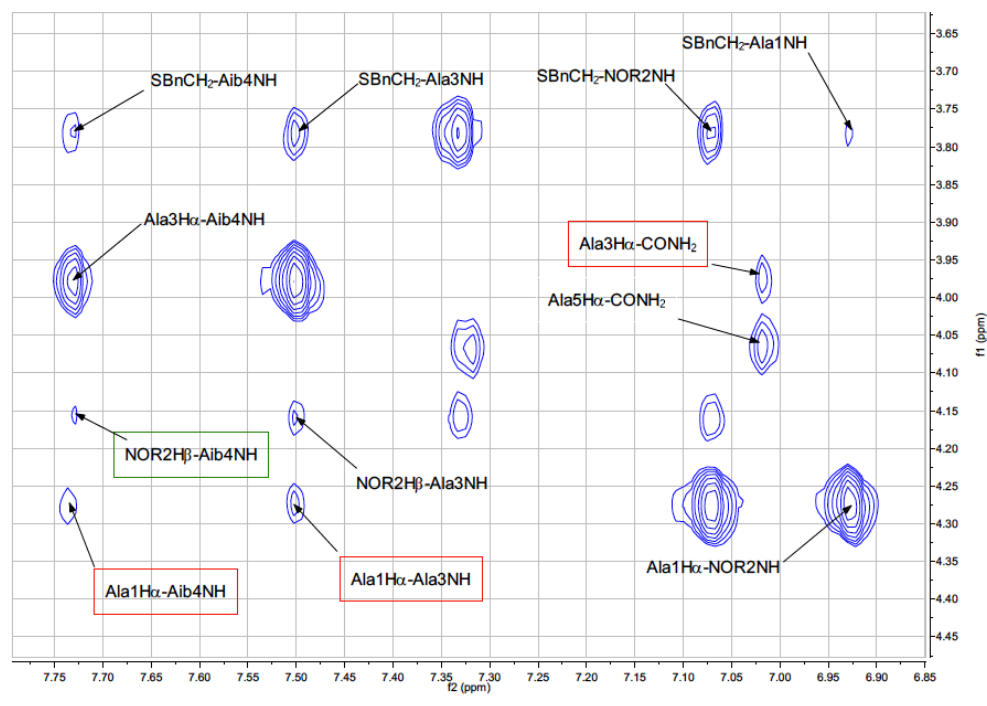


Figure 2.11 C-H-NH of the NOESY spectrum (τ_m 500ms, BBI probe, 300k, 500MHz) of peptide **19b** in CD_3CN (10mM). Sequential short range $C^H_i-NH_{i+1}$ signals diagnostic for structured peptide. Medium range $C^H_i-NH_{i+3}$ and $C\alpha H_i-NH_{i+2}$ signals typical of 3_{10} -helix (red box).

Also medium and short range signals $C^H_i-NH_{i+3}$ (Ala(1)Me-Aib(4)NH, Ala(3)Me-CONH₂ and Aib(4)Me-Ala(1)NH) and $C^H_i-NH_{i+2}$ (Ala(5)Me-Ala(3)NH, Aib(4)Me-CONH₂) shown in Figure 15 confirmed the initial hypothesis.

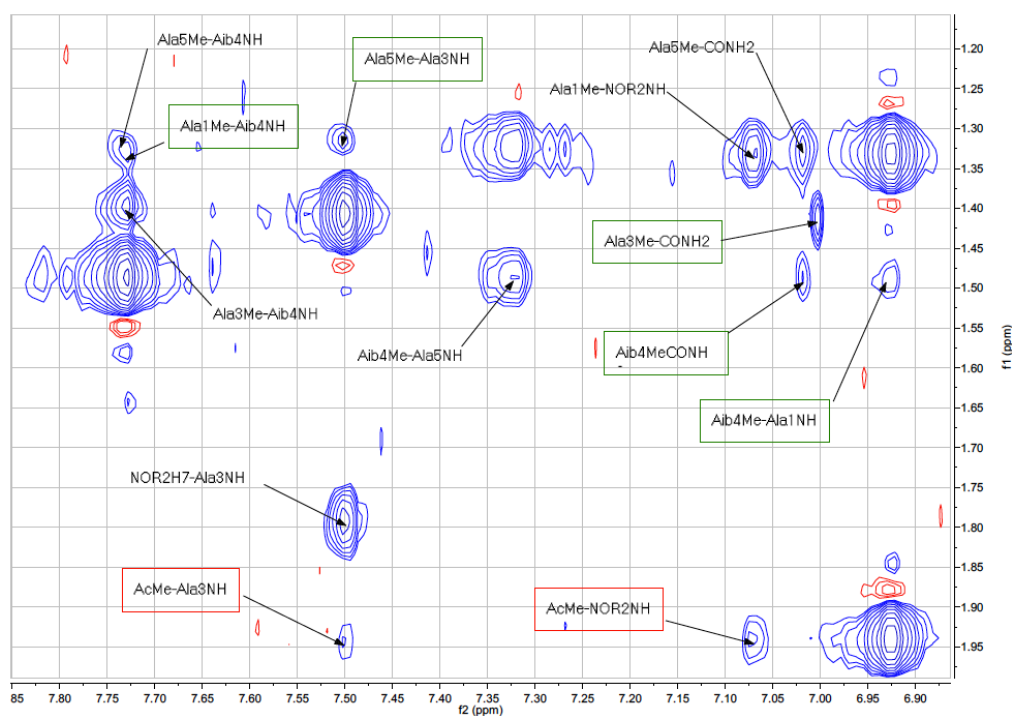


Figure 2.12 ^1H - ^1H of the NOESY spectrum (τ_m 500ms, BBI probe, 300k, 500MHz) of peptide **19b** in CD_3CN (10mM). Medium range $\text{C}^i\text{H}_i\text{-NH}_{i+3}$ and $\text{C}^i\text{H}_i\text{-NH}_{i+2}$ diagnostic for helical structure (green box).

Finally the high degree of structuration and stability of peptide **19b** could be inferred by the presence of an unusual long range intense cross-peak between the H_3 proton of the norbornene scaffold and the Me groups of the Ala(5) and (Aib(4)Me- CONH_2) experimental part.

In the case of peptide **18b** NMR analysis was less clear. All sequential short range $\text{NH}_i\text{-NH}_{i+1}$ interactions are visible (Figure 16) apart from the interaction between Ala(5)NH- CONH_2 obscured by the phase of diagonal peaks which can be made apparent operating with different mixing time (see S.I.).

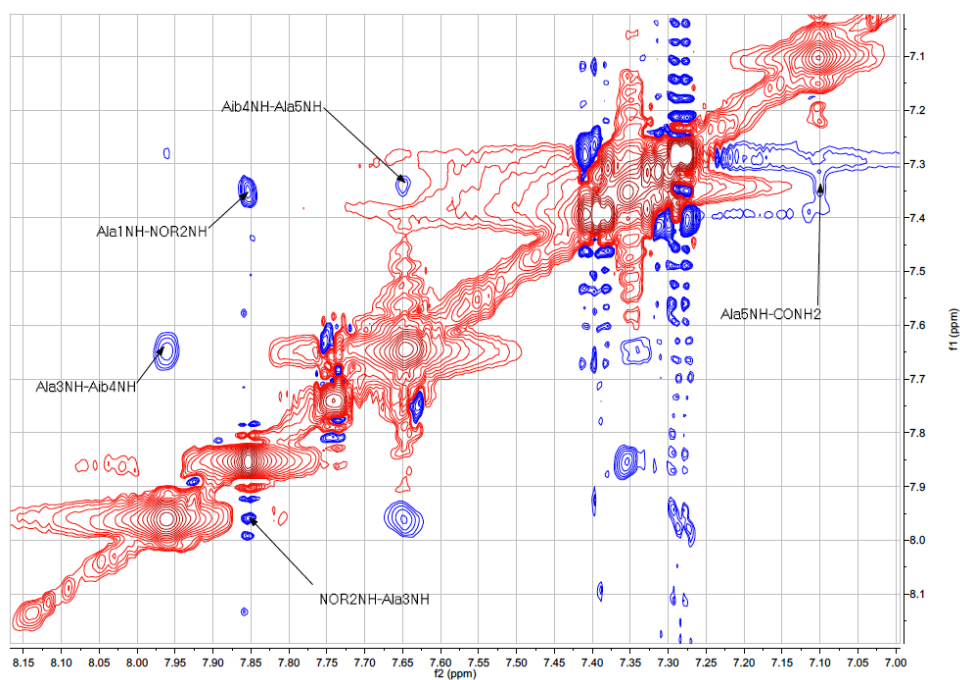


Figure 2.13 Amide proton region of the NOESY spectrum (τ_m 600ms, BBI probe, 300k, 500MHz) of peptide **18b** in CD_3CN (10mM). Sequential short range NH_i-NH_{i+1} signals

Fewer and less intense 3D structural information are present in $C^{\alpha(\beta)}H-NH$ region of the NOESY spectrum but enough to confirm the preference of **18b** sequence for 3_{10} -helix than α -helix. Detectable interactions $C^{\alpha(\beta)}H_i-NH_{i+2}$ (Ala(1) H^{α} -Ala(3)NH, NOR(2) H^{β} -Aib(4)NH, AibMe-CONH₂ and AcMe-NOR(2)NH) and $C^{\beta}H_i-NH_{i+3}$ (Ala(1) H^{α} -Aib(4)NH, NOR(2) H^{β} -Ala(5)NH and AcMe-Ala(3)NH) are shown in Figure 17 and Figure 18.

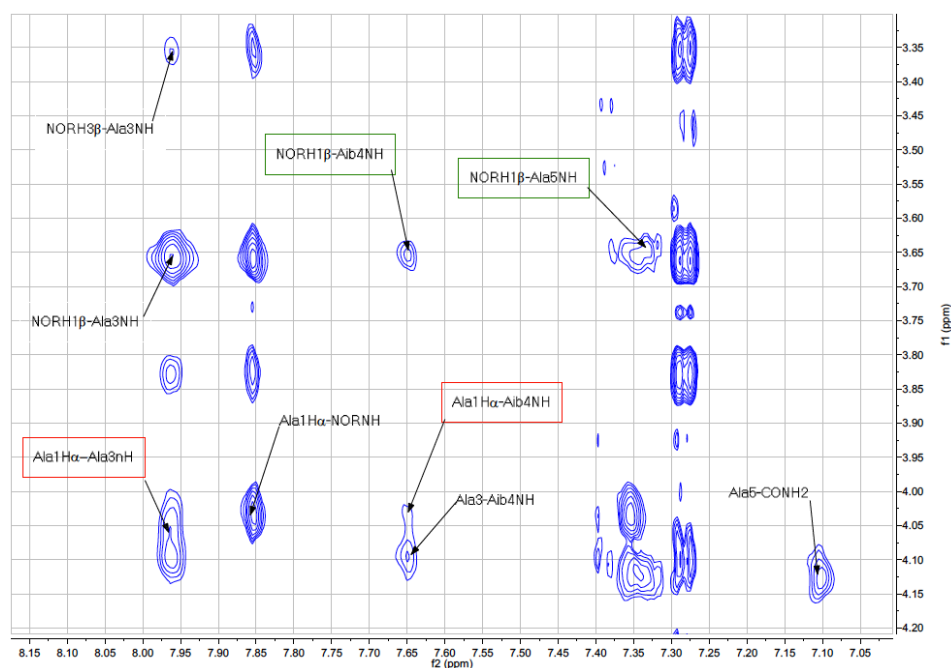


Figure 2.14 C^αH-NH of the NOESY spectrum (τ_m 500ms, BBI probe, 300k, 500MHz) of peptide **18b** in CD₃CN (10mM). Sequential short range C^αH_i-NH_{i+1} signals diagnostic for structured peptide. Medium range C^αH_i-NH_{i+3} and C^αH_i-NH_{i+2} signals typical of 3₁₀-helix (α red box, β green box).

The analysis of NOE suggests that both enantiomers of **18b** **Non sono enantiomeri di 18b** stabilized 3₁₀-helical conformation on a model peptide, no matching or mismatching event with the first (L)Ala-1 and the third (L)Ala-3 seeming to affect the stability of the helix.

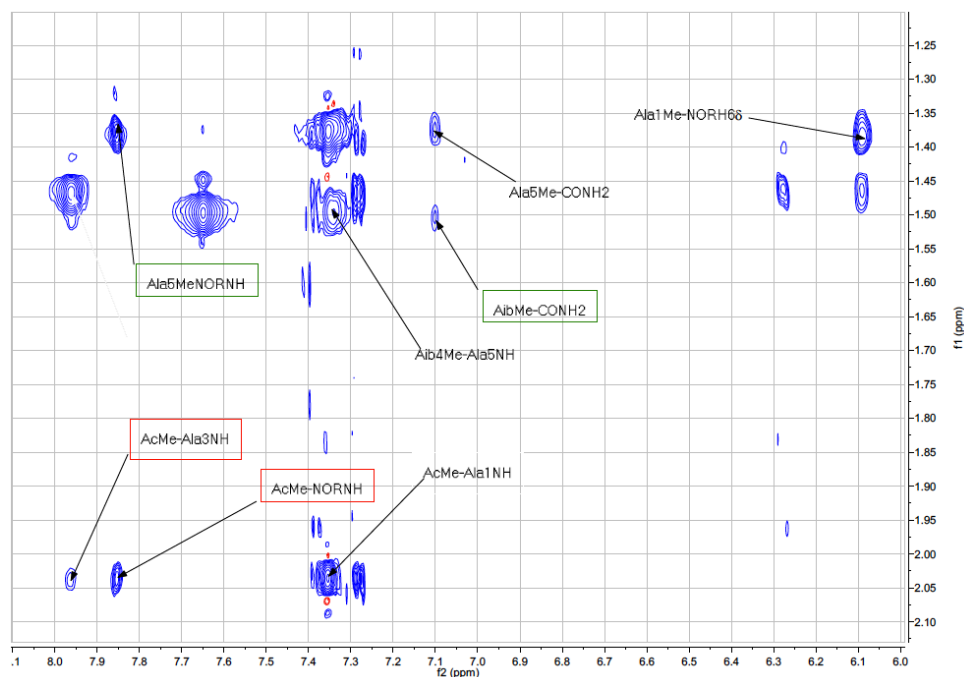


Figure 2.15 C^αH-NH of the NOESY spectrum (τ_m 500ms, BBI probe, 300k, 500MHz) of peptide **18b** in CD₃CN (10mM). Sequential short range C^αH_i-NH_{i+1} signals diagnostic for structured peptide. Medium range C^αH_i-NH_{i+3} and C^αH_i-NH_{i+2} signals typical of 3₁₀-helix (α red box, β green box).

2.4.2 VT-NMR and with DMSO- d_6 titration.

In order to deeply analyze the presence of 3_{10} -helical structure and identify the part of the structure more conformationally stable, the variation in NH chemical shifts with temperature (VT-NMR) and with DMSO- d_6 titration was measured. The values of temperature coefficient of the chemical shifts ($\Delta\delta(\text{NH})/\Delta T$) for each NH of **18b** and **19b** as well as the variation of chemical shift as function of DMSO- d_6 ($\Delta\delta/\Delta \text{DMSO-}d_6$ v/v) are shown in Figure 19 and Figure 20.

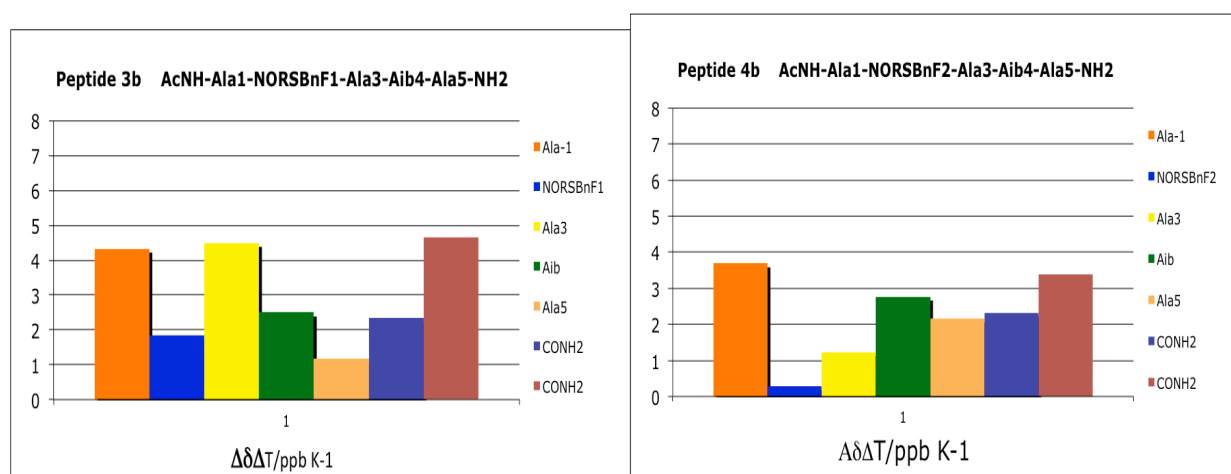


Figure 2.16 Temperature Coefficient for amide protons chemical shifts of **18b** and **19b** (CD_3CN 10mM) in a range temperature of 273-335

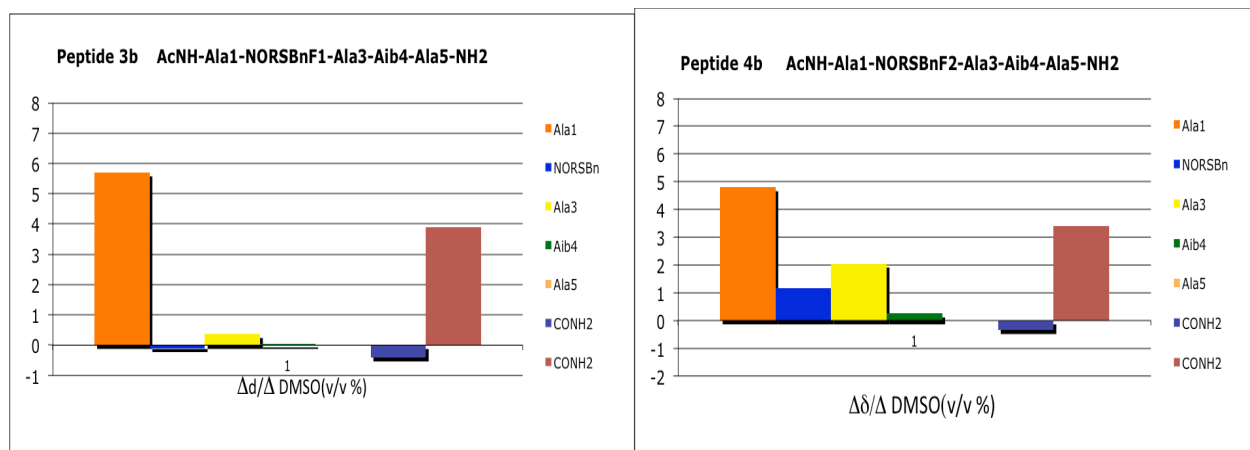


Figure 2.17 Δ -Chemical shift for amide proton in function of DMSO- d_6 v/v % of **3b** and **4b** (CD_3CN 10mM) in a range of 0-22 v/v %

In both **18b** and **19b** cases, continuous helical conformation are confirmed in the last part of the peptides Ala3-Aib4-Ala5- CONH_2 by VT-NMR analysis. Indeed except Ala3 of peptide **18b** that required further consideration, only the N terminal Ala(1)NH coefficient and, as might be expected, one NH proton of CONH_2 group are closed to the limit of $-4,5 \text{ ppbK}^{-1}$ that points out intramolecular hydrogen bonded and

free-amide confirming the unfolded features observed in the computational analysis for these two parts of the peptide. The DMSO- d_6 titration partially reflects the trends of VT-NMR. Unexpected data that did not find correlation between VT-NMR and DMSO- d_6 titration, regard the Ala3 of peptide **18b**. In this case the temperature coefficient indicates the presence of NH hydrogen bounded but with low energy close to the "thin red line". On the other hand in DMSO- d_6 titration experiments the behaviour of the same Ala3NH is described as very stable hydrogen bounded. Although less markedly, also in **19b** the correlation between the two experiments regarding such NH proton, is not completely overlapped and correspondent in the same part (NOR2-Ala3) of the peptide. Considering the exposition to the solvent, that influences both temperature variation analysis and DMSO- d_6 titration, the $\Delta\delta$ and the variation in DMSO- d_6 observed for NOR(2)NH and Ala3NH protons in both peptides **18b** and **19b** should be evaluated as positive false result as descriptor of hydrogen bounds. However, the observed values can be considered as significant proof of a high conformationally structuration of this part of the molecule. Also computational data did not confirm the presence of strong H-bond for NOR2NH and Ala3NH but underline how this part of the molecule is the most structured. Possible explanation of the disagreement of the two experiments could result from considering both the experimental NOESY data and computational data. We could hypothesize the presence of solvent exclusion sphere generated by the norbornene scaffold and the benzylsulfanyl group heavily congested with the peptide back bone. (supported by NOESY spectra and most populated cluster in computational study). Taking in mind this consideration, it is very likely that DMSO- d_6 could not access to NOR2 and Ala3 NH-amide proton, on the contrary VT-NMR in CH₃CN at high temperature could influence this part of the molecule underlining the absence of strong H-bond. We could finally assert that, in agreement with computational analysis, the $\Delta\delta$ of Ala(3) Aib(4), Ala(5), CONH₂ strongly supports the presence of four intramolecular hydrogen bonds that are tunable only with a 3_{10} -helical structure in peptide **19b** and not with α -helix. For peptide **18b**, taking in account the above considerations, we could underline the presence of only three hydrogen bounds that involve NH proton of Aib(4), Ala(5), CONH₂ residues and this result could be in agreement with both 3_{10} - and α -helix structure in our peptide. Nevertheless the NOESY spectrum lead us to exclude the presence of an α -helix at all. Accordingly to the trend described in figure 21 related to DMSO- d_6 titration, both peptides present a profile where only the NH proton at N-terms and C-terms have a relevant increment and are not stabilised, confirming 3_{10} -helix conformation highlighted by secondary structure analysis of the molecular dynamic.

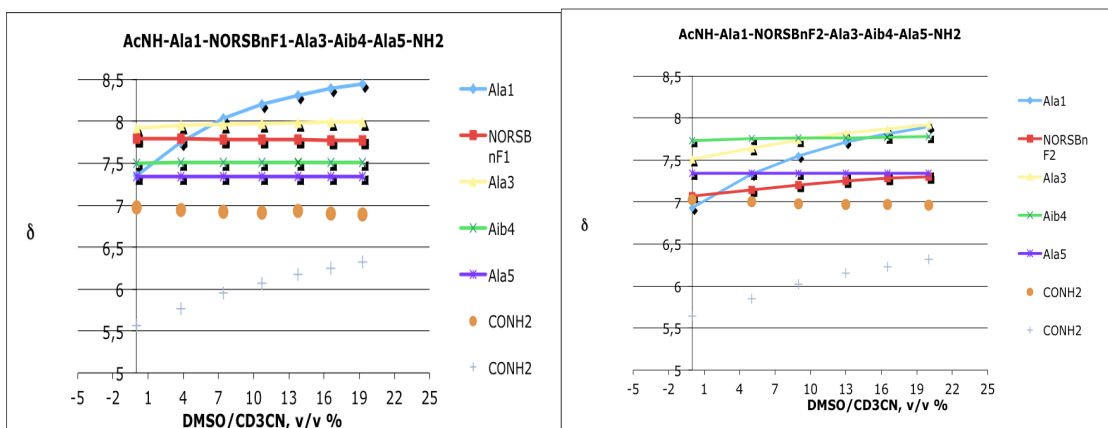


Figure 2.18 Chemical shift value variation for amide proton in function of DMSO- d_6 v/v % of **3b** and **4b** (CD_3CN 10mM) in a range of 0-22 v/v %

Lastly, a proof of high content of one preferred helical structure could be deduced by anisochronicity of the ^{13}C NMR signals of the diastereotopic methyl groups in Aib(4) residue. Considering that neighbouring chiral residues could not induce magnetic non-equivalence (MNE) higher than 0.5 ppm, the large MNE detectable in both peptides with value of 3.07 ppm (26.26-23.19 ppm, experimental part) for peptide **19b** and 3.88 ppm (26.60-22.72 ppm, experimental part.) for peptide **3b** stated for the presence of helical secondary structure with well define and stable screw sense.²⁴

2.4.3 X-Ray crystallography analysis

It was possible obtain a single crystal of the peptide **19b** by slow nucleation of a solution 40 mM of the precipitated peptide in CH_3CN . The crystal structure has confirmed the absolute stereochemistry (*1S,2S,3S,4R*) of the norbornene amino acid included in the peptide **19b**. The absolute stereochemistry of the other chiral centres gave a proof that no racemization event occurred during the synthesis. The computational analysis that indicate a right hand screw sense, as imaginable for a peptide containing L-AA is also bear out. An analysis of the crystal cell at low temperature identified two different conformers that present very slight differences on the conformation of the backbone with angles ψ and ϕ in agreement with the 3_{10} -helix but a complete different orientation of the benzyl group

²⁴ G. Jung; H. Bruckner, R. Bosch, w. Winter, H Schaal. J. Strahle. Liebigs Ann. Chem. 1983, 1096-1106

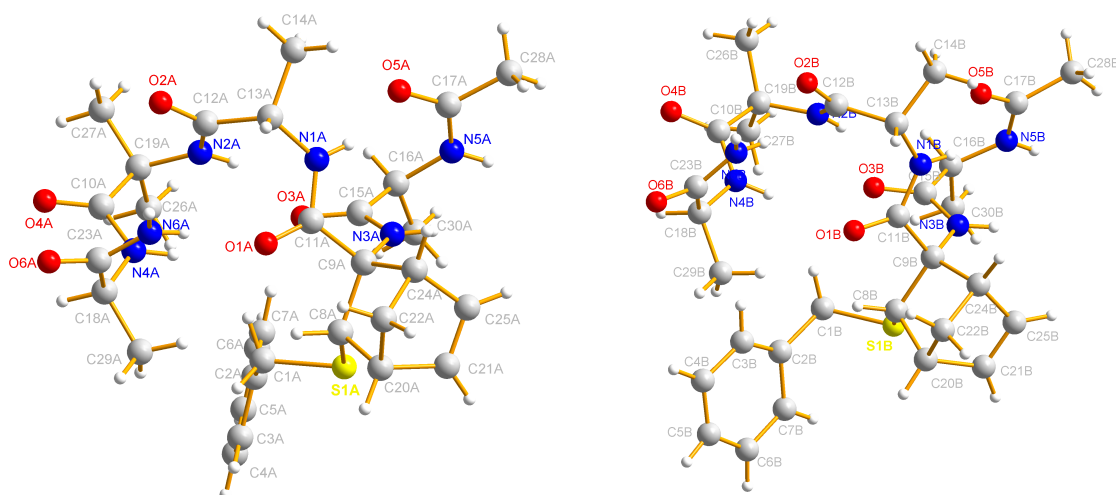


Figure 2.19 Crystal structure conformer **A-19b** and **B-19b** top view

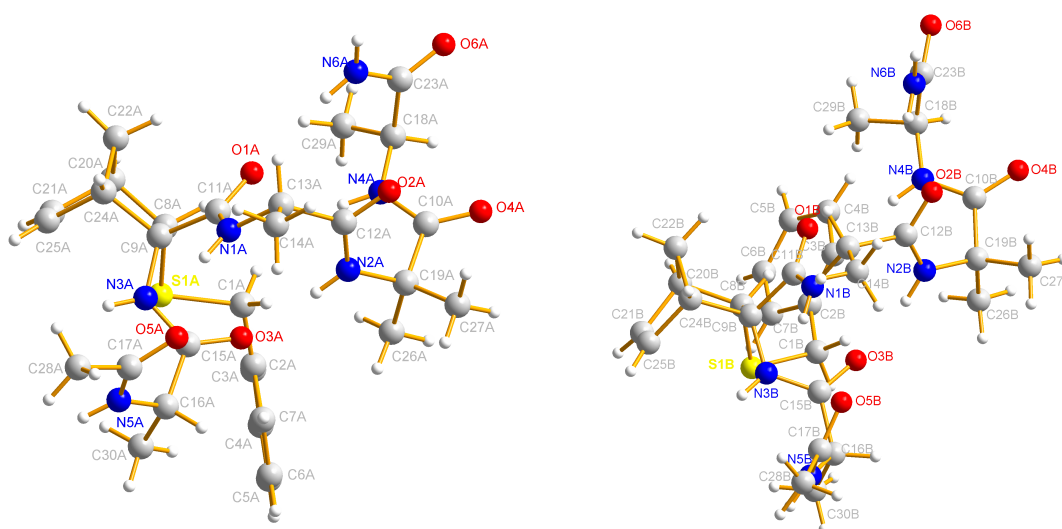


Figure 2.20 Crystal structure conformer **A-19b** and **B-19b** side view

A full analysis of all the dihedral angle was performed (Table XX) confirming the presence of 3_{10} -helix structure. With standard value of $\psi = -26$ and $\phi = -49$ for the 3_{10} -helix and $\psi = -45$ and $\phi = -60$ for the α -helix. The average dihedral angle in crystal structure analysis for **A 19b** are $\psi = -34.3$ and $\phi = -61.9$ and for **B 19b** $\psi = -27.8.3$ and $\phi = -59.0$. Considering that the last parts of the helix lost the folded structure as simulated and focusing on central core we have a complete adherence to the 3_{10} -helix value.

Table 4: Dihedral angle for the two conformer present in the X-Ray crystal analysis compared with simulated value for the peptide 19b

	Simulated	A 19b	B 19b
Ψ_0		-57.4	-54.4
ψ_1	-13.61±34.74	-32.8	-42.0
ϕ_1	-53.12±16.48	-50.0	-53.5
ψ_2	-29.37±14.99	-32.1	-39.9
ϕ_2	-62.45 ±18.99	-65.3	-63.1
ψ_3	-26.30±15.16	-17.2	-17.9
ϕ_3	-55.06±9.96	-55.5	-50.4
ψ_4	-24.25±12.81	-42.2	-34.8
ϕ_4	-106.84±35.15	-81.6	-73.8
ϕ_5		-48.1	-20,3

2.5

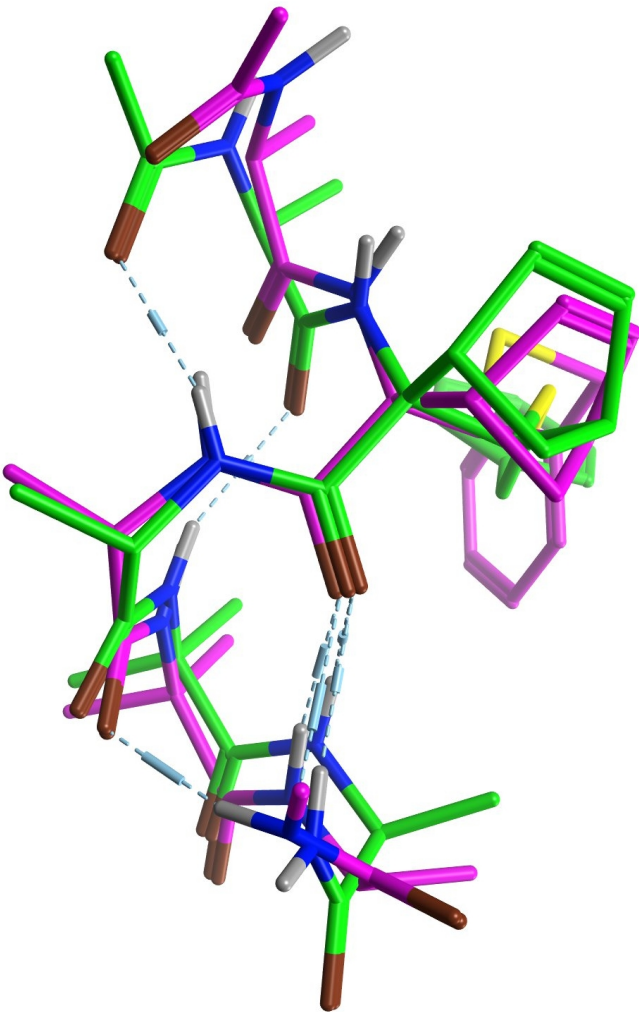


Figure 2.21 Graphical superimposition of X-Ray structure and simulated cluster P1

2.6 Conclusions and further developments

In conclusion, we have demonstrated that sulfanyl-methylene-5(4*H*)-oxazolones and β -sulfanyl- α -nitroacrylates which, to our knowledge, were never exploited before as dienophiles, behaved satisfactory in Diels-Alder cycloaddition reactions affording a new class of constrained non natural amino acid derivatives containing the norbornene/ane scaffold. All the new amino acid derivatives can be considered versatile building blocks due to the presence of the α , α -disubstituted amino acid function, suitable for peptide synthesis, but also by the carbon-carbon norbornene double bond which could be variously functionalized. Moreover, the use of β -benzylsulfanyl- α -nitroacrylate allowed the formation of protected cysteine analogues which could be transformed by debenylation into the corresponding disulfide derivatives. Computational analysis disclosed compound *exo*-**13'b** as the most promising helical stabilizer. Model peptides AcNH-(L)Ala-NOR-(L)Ala-Aib-(L)Ala-CONH₂ including both enantiomers of *exo*-**13'b** are synthesized and full NMR conformational analysis confirm the 3_{10} -helix structuration indicated by computational studies. The stability of the helix was tested through VT-NMR and DMSO-*d*₆ titration confirming that a part of the peptides remain stable also at limit conditions. The stability reached by the model pentapeptide described in this chapter, was never observed in literature for a pentapeptide made by alternate sequence of alanine and Aib amino acids.^{25 26 27 28 29} Probably, the constrain structure and steric hindrance of the norbornane moiety substituted in β -position, strongly stabilize the first part of the backbone allowing the overcome the innate nucleation barrier of short peptides described in the helix-coil transition theory of Gibbs and Di Marzio.³⁰ Both enantiomers seem to stabilize right end helix with same magnitude. Confirmation of the highly stable secondary structure also came from the elevated value of MNE at the geminal methyl groups of Aib. No mismatch events between the chirality of the NOR amino acid in peptide **18b** and **19b** with Ala3 are evidenced underlining the unimportant singularity in field. We could suppose that the stabilization of the helix come out only from the steric hindrance of our amino acids that did not allow free rotation of torsional angle ψ and ϕ , limiting them inside a determinate value. The presence of the benzyl group and the double bond did not permit to acquire reliable CD spectra^{31 32 33}. We plan to reduce the double

²⁵ R. Banerjee, S. Chattopadhyay and G. Basu, Conformational preference fo short Aib/Ala-based water-soluble peptide as function of temperature, *Proteines* **2008**, 184-200

²⁶ K. S. Raghothama, S. Aravinda, N. Shamala, P. Balaram; Helica conformation of hexapeptides containing N-Terminus Diproline Segment. *PeptideScience*, **2009**, 94, 3, 360-369

²⁷ G. Jung; H. Bruckner, R. Bosch, w. Winter, H Schaal. J. Strahle. *Liebigs Ann. Chem.* **1983**, 1096-1106

²⁸ R. Schweitzer-Stenner, W. Gonzales, G. T. Bourne, J. A. Feng, G. R. Marshall Containing Alanine-Based Tripeptides in Aqueous Solution Explored by Vibrational Spectroscopy, Electronic Circular Dichroism Spectroscopy, and Molecular Dynamics Simulations *J. AM. CHEM. SOC.* **2007**, 129, 13095-13109

²⁹ S. Kawamura, T. Morita, S. Kimura. Foldamer for novel peptide derivatives with pyrene units incorporated into the main chain. *Science and Technology of Advanced Materials*. **2006**, 7, 544–551

³⁰ H. Gibbs, E. A. Di Marzio Theory of Helix-Coil Transitions in Polypeptides. *The journal of Chemical Physics*, **1958**, 28, 1247.

³¹ R. A. Brown, T. Marcelli, M. De Poli, J. Solà, and J. Clayden. Induction of Unexpected Left-Handed Helicity by an N-Terminal L-Amino Acid in an Otherwise Achiral Peptide Chain. *Angew. Chem. Int. Ed.* **2012**, 51, 1395 –1399

bond directly on the final peptide in order to study the stabilization features of the norbornane scaffold. Moreover a debenzylation reaction of the sulphur atom, compatible with the entire peptide functionality was set up and further study could be focussed on the stabilization of supramolecular structures stabilized by S-S bridges. Finally, it was possible to obtain a crystal structure of the entire peptide **19b** that has confirmed the absolute stereochemistry of both peptides indicating that no racemization event has occurred during the synthesis and highlighting the identity of secondary structure between solid state and solution state. A further development could be the exploitation of this new cysteine mimic amino acid in the stabilization of α -helix sequence of biological interest. Moreover, in future it could be of great interest to use this new class of cysteine mimic to synthesize more stable and active peptidomimetics derived from the large amount of protein containing S-S bridge done by cysteine. Some example of such proteins are insulin, somatostatine, eritropoietine, oxytocine, arigypressin involved in the regulation of fundamental biological processes or potent neurotoxin like Conotoxin, useful for the treatment of pain.

³² Y. Nakano, T. Hirose, P. J. M. Stals, E.W. Meijer, A. R. A. Palmans, *Chem. Sci.* **2012**, 3, 148 – 155.

³³ N. Ousaka, Y. Takeyama, H. Iida, E. Yashima, *Nat. Chem.* **2011**, 3, 856 – 861.

3 Multicomponent enantioselective synthesis of norbornene amino acid and exploitation as inductor of secondary structure

3.1 Introduction

Functionalized norbornene core seems to possess important features with elevated number of application : i) as a single molecule because they have biological propriety and usually shown due to they locked structure higher activity, stability and receptor selectivity than natural amino acids; ii) As part of the complex molecule like peptide because they could improve the selectivity again a specific receptor, bioavailability and resistance to enzymatic hydrolysis; iii) As organometallic catalyst; iv) as organocatalyst in asymmetric synthesis. We already underline in the introduction of the thesis the large quantities of application that this core has in the different field of chemistry and bioscience. From the development of therapeutic agent as well as in the field of bioconjugation of biological molecule and in the field of polymers and biopolymers, or the use of norbornene amino acid as foldamer or to stabilize secondary structure. Especially in the last decades the interest in the field of foldamer, synthetic oligomer with well defined conformation, rised because they could help to deeply understand the propriety and mechanism of biomacromolecules and develop therapeutic agent with innovative mechanism of action.^{1 2 3 4 5 6 7 8} A general approach consists in the substitution of established position of the polypeptide with a small foldamer sequence or even of a single amino acid characterized by steric hindrance or molecular rigidity, in order to reduce the conformational flexibility of the peptide. From this point of view the norbornene amino acid could be an interesting structure for folding studies. Moreover hydrocarbon core is known to prevent hydrophobic collapse which is responsible of the stabilization of inactive conformation in water medium. In the literature there is a plenty of scaffolds designed to reduce or completely erase the hydrophobic collapse^{9 10} that represents one of the major problem during the binding process of inhibitors with their receptors.

¹ Cheng, R. P.; Gellman, S. H.; De Grado, W. F. *Chem. Rev.* **2001**, 101, 3219-3232.

² Hill, D. J.; Mio, M. J.; Prince, R. B.; Hughes, T. S.; Moore, J. S. *Chem. Rev.* **2001**, 101, 3893-4011.

³ Huc, I. *Eur. J. Org. Chem.* **2004**, 17-29.

⁴ Cheng, R. P. *Curr. Opin. Struct. Biol.* **2004**, 14, 512-520

⁵ Licini, G.; Prins, L. J.; Scrimin, P. *Eur. J. Org. Chem.* **2005**, 969-977

⁶ Seebach, D.; Beck, A. K.; Bierbaum, D. J. *Chem. Biodiversity* **2004**, 1, 1111-1239; Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P. *Chem. Rev.* **2004**, 104, 6085-6117

⁷ Yin, H.; Hamilton, A. D. *Angew. Chem. Int. Ed.* **2005**, 44, 4130-4136

⁸ Loughlin, W. A.; Tyndall, J. D. A.; Glenn, M. P.; Fairlie, D. P. *Chem. Rev.* **2004**, 104, 6085-6117

⁹ Ranganathan, D.; Haridas, V.; Kurur, S.; Thomas, A.; Madhusudanan, K. P.; Nagaraj, R.; Kunwar, A. C.; Sarma, A. V. S.; Karle, I. L. *J. Am. Chem. Soc.*, **1998**, 120, 8448-8460

Despite the interest toward this amino acid, a thorough analysis of the literature has shown a lack of comprehensive studies on its properties to stabilize secondary structures once include into a model peptide. We start the project with the aim to fill this gap through a comprehensive study including the synthesis of the system, its inclusion in a model peptide and the study of its propensity toward stabilization of secondary structure. Furthermore we planned the exploitation of the norbornene double bond as reactive function for site specific chemical modification on peptide scaffold. Site specific modification on the peptide could open the way to the bioconjugation chemistry, dimerization, polymerization, and divergent modification. Such modification could be considered an easy way to generate different amino acid on the final peptide without the necessity of planning a new synthesis. The possibility of obtain in the last step such diversification could be a starting point for further structural study targeted to modulate the factor that drives different conformational stabilization.¹¹ In order to accomplish our aim an economic, short and efficient synthetic procedure able to furnish the desired norbornene amino acids in an enantiopure fashion on a grams scale is needed. The main procedures for the synthesis of norbornene and norbornane amino acids basically reside in the exploitation of Diels-Alder cycloaddition reaction between an appropriate dienophile and cyclopentadiene. The more traditional methods, in chiral and achiral version, used dehydroalaninates as dienophiles. The first achiral synthesis reported in literature by Miyoshi¹² use dehydroalaninates protected in different way but in the paper is described only the obtainment of the norbornane amino acid. Mayoral¹³ and Cativiela¹⁴ report a Diels-Alder reaction between cyclopentadiene and methyl or menthyl acetamidoacrylate/benzylamidoacrylate in presence or absence of a catalyst (Scheme 1A). Subsequently, our group has revisited the reaction by the use of phenylmenthol as chiral auxiliary obtaining enantiomerically pure product with satisfactory yields but always presenting the amino group protected as amides. Such a protection results very difficult to be removed, rendering this procedure not well suited for our goal. (Scheme 1B)¹⁵

¹⁰ Rich, D. H.; Wiley, R. A. *Med. Res. Rev.*, **1993**, 13, 327-384.

¹¹ E. M. Sletten and C. R. Bertozzi. *Angew. Chem. Int. Ed.* **2009**, 48, 6974 – 6998

¹² Horikawa, H.; Nishitani, T.; Iwasaki, T.; Mushika, Y.; Inoue, I.; Miyoshi, M. *Tetrahedron Lett.* **1980**, 21, 4101.

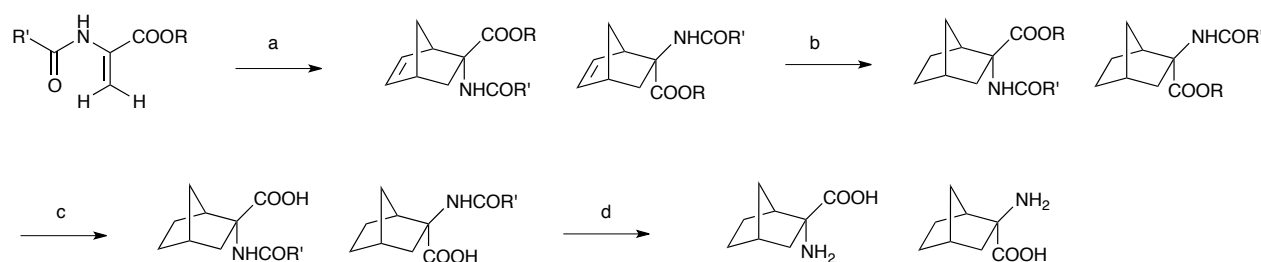
¹³ Bueno, M.P.; Cativiela, C.; Finol, C.; Mayoral, J.A.; Jaime, C.; Diels-Alder reactions of methyl-N-acyl- α , P-dehydroalaninates with cyclopentadiene. *Can. J. Chem.*, **1987**, 65, 2182

¹⁴ Cativiela; Lopez; Mayoral; Asymmetric synthesis of cycloaliphatic α -amino acids with a norbornane skeleton. *Tetrahedron asymmetry*, **1990**, 1(6), 379-388

¹⁵ Caputo, Francesco; Clerici, Francesca; Gelmi, Maria Luisa; Pellegrino, Sara; Pilati, Tullio; Enantioselective synthesis of epimeric cis-3-carboxycyclopentylglycines. *Tetrahedron Asymmetry*, **2006**, 17, 1430–1436

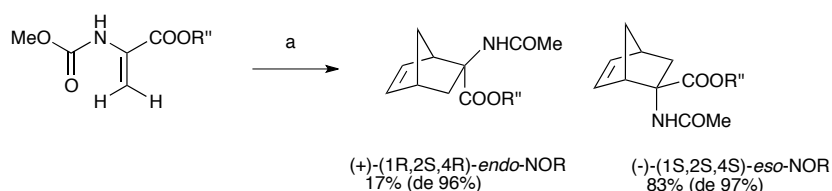
Scheme 3.1 Synthesis of norbornane amino acid reported in literature

A



R=Et, Me,
R'=Ph, Me, OMe

B



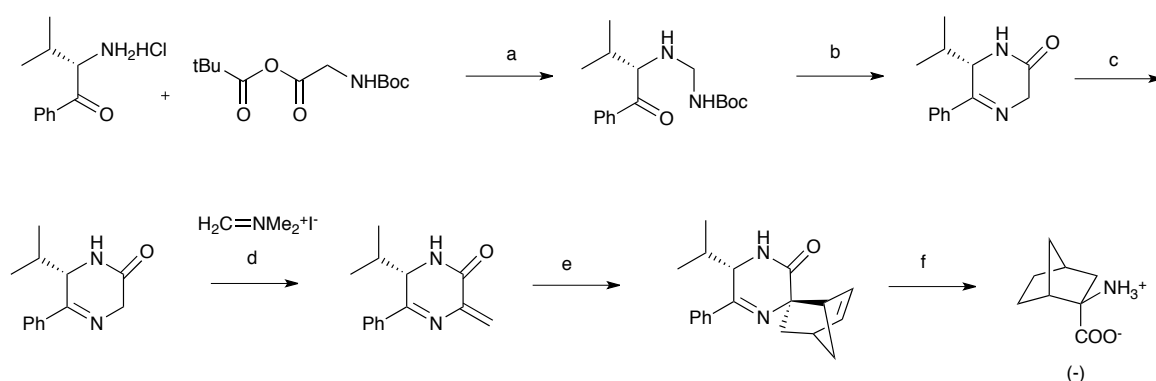
Reagents and conditions: a)Cyclopentadiene,)))); b) H₂ 1 atm, Cat Pd/C, rt; c)Basic hydrolysis; d) Acid hydrolysis.

The synthesis reported above have never permitted the obtainment of the norbornene amino acid in free form, that is deprotected to the amine function. No efficient and convenient methodologies are known for the hydrolysis of the acetamide or benzamide group in a similar environment that are tolerant of the double bond. The hydrolytic process of amide bond required the use of HCl or HBr in medium-high concentration at high temperature. In these conditions, the formation of side products deriving from halogen addition to the norbornene double bond are known to be observed¹⁶. Indeed, in the first case (Scheme 1A) the reduction of the double bond was operated before the hydrolytic steps. The preparation reported by our group (Scheme 1B) affords the norbornene but, as said above, no deprotection of the amide were performed. Moreover, the (8)-phenylmenthol is extremely expensive and the hydrolytic removal was performed in basic medium on small scale with unsatisfactory yield. For all these reasons, both procedures are not indicated for our pursuit based on further developments and application for which the presence of the double bond is vital as key functionality. Furthermore we hypothesized that unsaturated and saturated scaffold could have different bias in the stabilization of secondary structure, and an important part of our work has been devoted to highlight such tendency.

¹⁶ Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M.L.; Soave, R.; Clerici, F. Sulfanyl-methylene-5(4H)-oxazolones and b-sulfanyl-a-nitroacrylates as appealing dienophiles for the synthesis of conformationally constrained cysteine analogues, *Tetrahedron*, **2012**, 68, 1951-1962

More recent example involved the use of chiral dehydroalaninates, in its 1,2,3,6,-tetrahydropyrazin-2-one form, in a cycloaddition reaction with cyclopentadiene. Also in such case, the acidic hydrolysis of the tetra-pyrazinone core was necessarily performed after the reduction of double bond.¹⁷ (Scheme 2)

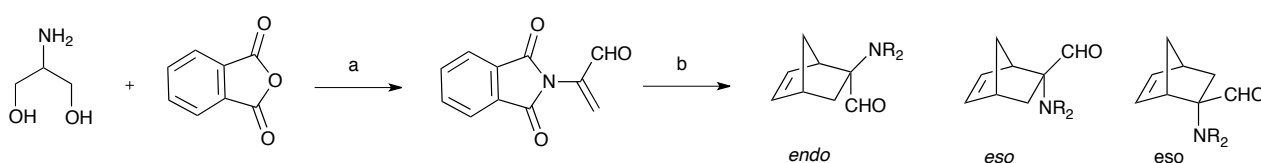
Scheme 3.2 Synthesis of norbornane amino acid reported in literature



Reagent and Condition: a) DCM; b) 1) HCl AcOEt, 2) K₂CO₃; c) Boc₂O, DMAP cat, TFA, 0°C, 1h; d) DCM; e) Cyclopentadiene; f) 1) H₂ 1 atm, Cat Pd/C, rt, EtOAc 2) HCl 6N, 150°C; 2) Propilenoxyde, EtOH, reflux.

Moreover, the entire process suffers from high number of steps. As a consequence, even if it can be surely considered an interesting methodology, that, differently from the procedure described before, gives the *endo* adduct as major product, it was not considered for our project. Finally, an interesting organocatalytic version of cycloaddition reaction that furnishes with good yield the norbornene amino acid was recently reported.¹⁸ In the reaction both *endo* and *exo* adducts are formed with a prevalence of *endo* compound as in the methodology previously reported by Abellán et al.³⁵ The enantioselection has an elevated value only for the *endo* product but is not preserved in the *exo* one, where the organocatalyst cannot shield preferentially one face of the dienophile and drive the attack of the cyclopentadiene. (Figure 3)

Scheme 3.3 Synthesis of norbornene amino acid reported in literature



¹⁷ Abellán, Tomas; Mancheño, Balbino; Nájera, Carmen; Sansano, José M.; Asymmetric synthesis of α-amino acids from α,β-(Z)-didehydroamino acid derivatives with 1,2,3,6-tetrahydropyrazin-2-one structure. *Tetrahedron*, **2001**, 57(30), 6627–6640

¹⁸ Ishihara, Kazuaki; Nakano, Kazuhiko; Akakura, Matsujiro; Organocatalytic Enantioselective Diels–Alder Reaction of Dienes with α-(N,N-Diacylamino)acroleins. *Organic Letters*, **2008**, 10(13), 2893–2896

Reagents and Conditions: a) 1) 160°C, 2) (COCl)₂, Et₃N, DMSO/DCM, -78 °C to -20 °C; b) Cyclopentadiene, NO₂Et, Catalyst -10°C.

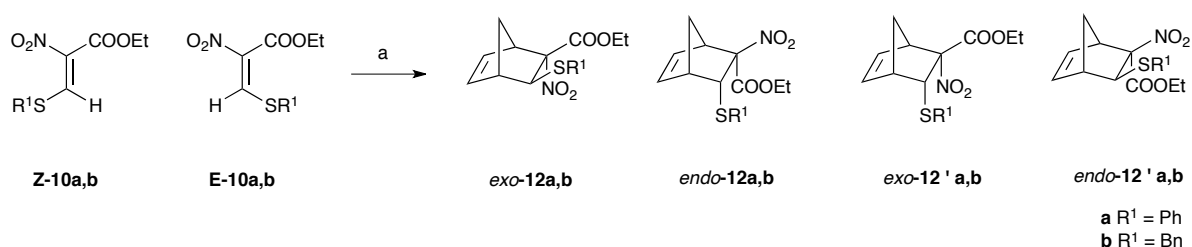
Such methodology could furnish a proper way to obtain the enantiopure form of *endo* norbornene amino acid despite the low regioselectivity. Unfortunately not only the low regioselectivity, but deeper considerations are necessary to underline how the last process is useless in our case. It is widely accepted that a secondary structure is generally stabilized by the N terminal amino acid¹⁹. The computational model developed in our laboratory and reported below, shows that the norbornene system bearing the amine group in *endo* position locks rotation of the peptide backbone and stabilizes secondary structure. Inclusion of *endo* norbornene core in N terminal position will have lower or different stabilization bias than the *exo* stereoisomer.

3.2 Synthesis of enantiopure norbornene amino acid via three component reaction.

3.2.1 Synthesis of different nitroacetate derivatives and their application in the three component reaction for the obtainment of orthogonal protected amino acid.

In the previous chapter we underlined the reactivity of S-substituted nitroacrylate in a Diels-Alder cycloaddition with cyclopentadiene³⁴ (Scheme 4)

Scheme 3.4 Cycloaddition reaction of dienophiles **Z/E-10a,b** with cyclopentadiene



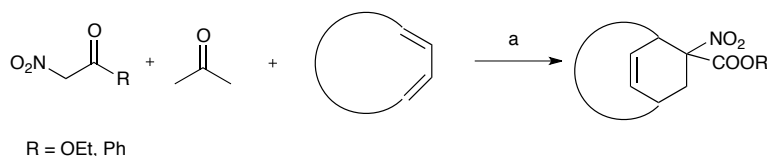
Reagents and conditions: (a)), Cyclopentadiene, 24h, neat.

We have already demonstrated how nitro group could be a good precursor of amine function in the synthesis of bicyclic amino acid by the way of simple and mild condition reduction. Using this strategy we avoid the

¹⁹ J.Solà, M. Helliwell, and J. Clayden. N- versus C-Terminal Control over the Screw-Sense Preference of the Configurationally Achiral, Conformationally Helical Peptide Motif Aib8GlyAib8. *J. AM. CHEM. SOC.* **2010**, 132, 4548–4549

hydrolytic step of the amide function present in previous works and preserve the double bond of norbornene core. Taking in mind this consideration, we considered the use of simple nitroacrylate for the synthesis of unsubstituted norbornene amino acid. The exploitation of unsubstituted nitroacrylate in Diels-Alder cycloaddition was already reported.²⁰ (Scheme 5)

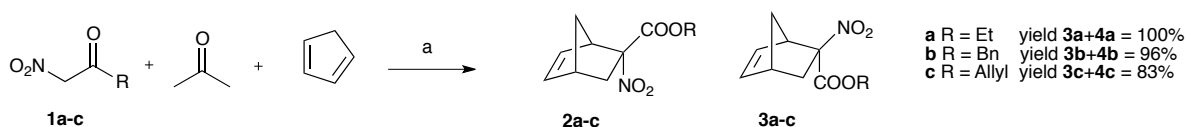
Scheme 3.5 Three component strategy



Reagent and Condition : a) THF, 50°C, AcOH

In such paper is reported that the multicomponent reaction between ethyl nitroacetate, formaldehyde and cyclopentadiene gives norbornene derivatives (*endo* /*exo* ratio 15/85) in good yield (80%). In the same paper the description of the compounds are not reported and *endo*/*exo* ratio probably deduced from the crude NMR spectrum. In 2005, on *Organic Letter*, the same protocol was used to generate interesting products starting from sugar deriving dienes.²¹ We started our project repeating and analysing the reaction published by Wade et al.³⁸ Using the same reaction conditions described in the general procedure, we obtained overlapping results and the products **1a** and **2a** in an inseparable mixture (*endo* /*exo* ratio 15/85, NMR analysis) were formed confirming the higher propensity of the nitro group to stay in *endo* position.

Scheme 3.6 Multicomponent reaction extension of the scope.



Reagent and Condition : a) THF, 45°C, AcOH

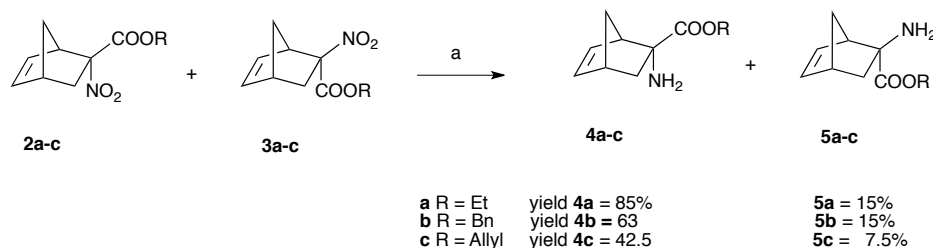
The reaction was properly studied and modified. Temperature, time, and equivalent of reagents were adjusted in order to reduce as much as possible the equivalent of nitroacetate that is the most expensive reagent. The best result was obtained using 1 eq. of nitroacetate and rising the equivalent of other reactants to

²⁰ Wadea, Peter A.; Murray, James K.; Shah-Patela, Sharmila; Carroll, Patrock J. Generation and in situ Diels-Alder reactions of activated nitroethylene derivatives. *Tetrahedron Letters*, 2002, 43(14), 2585–2588

²¹ Jayakanthan, K.; Vankar, Yashwant D. Synthesis of Conformationally Constrained C-Glycosyl α - and β -Amino Acids and Sugar-Carbamino Sugar Hybrids via Diels-Alder Reaction. *Org. Lett.*, **2005**, 7(24), 5441–5444

20 eq at 45°C for 48 hours. 10 equivalents of all three reactants was added at the start of the reaction and an addition of other 10 equivalents was necessary after 24 to drive the reaction to completion. The mixture of **2a** and **3a** was reduced and furnished the two diastereoisomeric amines **4a** and **5a** in quantitative yield. The amines were separated by flash chromatography and NMR analysis permitted to confirm the literature data.

Scheme 3.7 Reduction of norbornene nitro ester



Reaction conditions a) H₃PO₄ 1M, Zn dust, THF, r.t., o.n.

Considering our interest toward the use of norbornene core in peptide synthesis and taking in mind the troublesome hydrolysis of the ester function in such a bulky system once inserted in a di- or tripeptide, we planned to apply the same three component reaction on different nitroacetates bringing ester functions that don't require basic of acid hydrolysis. Examples are the benzyl or allyl esters, indeed the first one could be deprotected with reductive reaction or mild acid conditions and the last one in neutral medium with palladium tetrakis. In order to prepare such compounds we followed a synthetic procedure reported in literature^{22 23 24 25} by Koto et al. Nitromethane reacts in strong basic medium (KOH) with CO₂ in open air flask forming the stable nitroacetic acid dipotassium salt **6**. Compound **6** could be directly converted to the methyl or ethyl ester dissolving the salt in methanol or ethanol and treating the solution with H₂SO₄.²⁴ For the preparation of esters deriving from less volatile alcohols, the dipotassium salt could be converted, by treatment with tartaric acid in water at -10°C, in the instable free acid form **7** and crystallized from chloroform.^{43 44 45} The acid is immediately condensed with the desired alcohol by using of DCC as reported in literature.^{43 44 45}

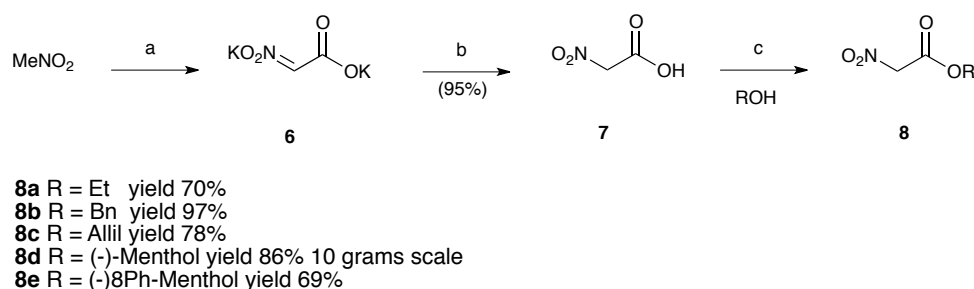
²² Zen, S.; Koyama, M.; Koto, S.; *Organic Syntheses*, **1976**, 55, 77

²³ Mioskowski, Charles; Sylvain, Catherine; Wagner, Alain. An efficient procedure for the esterification of nitroacetic acid : Application to the preparation of Merrifield resin-bound nitroacetate. *Tetrahedron Letters*, **1999**, 40, 875-878.

²⁴ Righi, Paolo; Scardovi, Noemi; Marotta, Emanuela; Holte, Peter; Zwanenburg, Binne; Solution- and Solid-Phase Synthesis of 4-Hydroxy-4,5-dihydroisoxazole Derivatives from Enantiomerically Pure N-Tosyl-2,3-aziridine Alcohols. *Org. Lett.*, **2002**, 4(4), 497-500.

²⁵ Vanier, Se'bastien F.; Larouche, Guillaume; Wurz Ryan P.; Charette, André B. Formal Synthesis of Belactosin A and Hormaomycin via a Diastereoselective Intramolecular Cyclopropanation of an α -Nitro Diazoester *Org. Lett.*, **2010**, 12(4), 672-675

Scheme 3.8 Synthesis of nitroacetic esters.



Reaction conditions: a) KOH, Open Air, 70°- 140°, 4h; b) 1) Tartaric Acid, H₂O, -5 °C, 2) Et₂O, CHCl₃; c) ROH, THF, -20°C, DCC/THF.

Following the procedure above described, we prepared the benzyl and allyl derivatives in good yield and submitted these esters to the three component protocol obtaining with good yield the norbornene derivatives **2 b,c** and **3 b,c**. (Scheme 6). The ratio between *endo* and *exo* isomers remained almost the same (15/85 to 20/80). **2 b,c** and **3 b,c** were then reduced with a modified protocol from the chapter 2, and provided the norbornene amino esters **4 b,c** and **5 b,c** in good yields. (Scheme 7)

3.2.2 Exploitation of the norbornene amino acid in peptide synthesis. Carpino methodology as way to obtain enantiopure dipeptide.

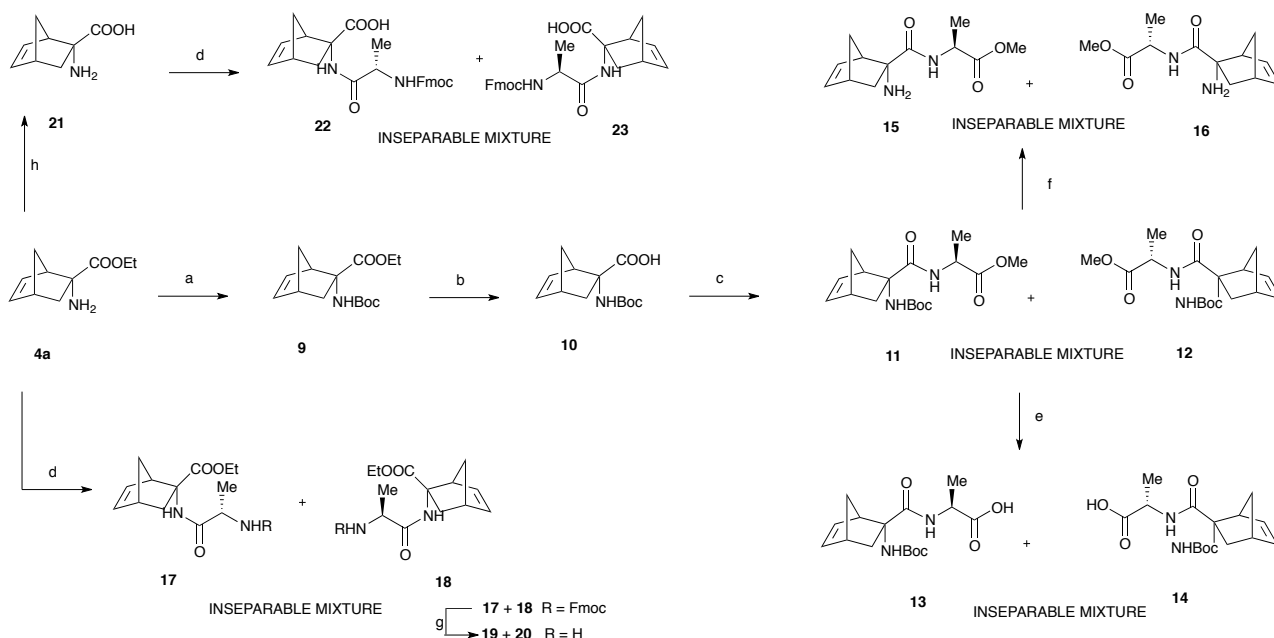
With an efficient gram scale synthetic process in our hand, we started to test several conditions to prepare the model peptide AcNH-(L)Ala-NOR-(L)Ala-Aib-(L)AlaCONH₂ presenting our amino acid inserted in *i*+1 position in order to verify the hypothesis of secondary structure stabilization in model peptide.²⁶ We chose to begin our project working on the major fraction **4a** and after Boc protection step, the ester function of **9** was hydrolysed in KOH/MeOH 3M affording, after neutralization, the free amino acid **10** in good yield. In order to resolve the racemic form of NOR **10**, we applied the Carpino methodology²⁷ that involve the use of (L)-AlanineOMe as reagent and also as resolving agent. The dipeptides **11/12** were obtained using classical coupling reaction conditions (EDC, HOAt, DIPEA) but unfortunately in an inseparable mixture. Thinking that an increasing in polarity could results in better separation, we tried to perform the hydrolysis of the ester with LiOH in MeOH to obtain **13/14** as well the deprotection of the amine function to afford **15 /16** keeping intact the ester function, but unfortunately the separation failed. The direct coupling of compound **4a** with (L)-AlaNHfmoc after activation with BSA allowed the obtainment of a dipeptide **17/18** FmocNH-(L)Ala-NOR-OEt but also in this case as an inseparable racemic mixture. The same coupling on the free amino acid

²⁶ Pellegrino, S.; Contini, A.; Clerici, F.; Gori, A.; Nava, D.; Gelmi M.L. *Chem. Eur. J.* **2012**, *18*, 8705 – 8715

²⁷ Carpino, L.A.; Sadat-Aalae, D.; Chao, H. D.; Deselms, R. H. *J. Am. Chem. Soc.* **1990**, *112*, 9651-9652

to obtain **22/23** as well as the deprotection of the amine function of **17/18** did not lead to the separation of the two diastereoisomers.

Scheme 3.9 Dipeptide synthesis, alanine as reagent and resolving agent



Reaction conditions: a) Boc_2O , DCM, TEA, 0°C to rt, o.n.; b) KOH 3M, MeOH, 50°C ; c) 1) EDC, HOAt, DCM, 0°C , 1h 2) NH_2AlaOMe , DiPEA, pH 8, rt, 10h; d) 1) DCM, BSA, 0°C to rt, 8h 2) 0°C , FAlaFmoc; e) LiOH, MeOH, rt. f) DCM/TFA, 0°C to rt, o.n.; g) DMF/pyperidine, rt, o.n.; h) 1) HCl 6N 80°C , 2) propylene oxide, MeOH, reflux.

3.2.3 Three component reaction. Enantioselective version

The impossibility to obtain optical active compound in enantiopure form in the early passage of the synthesis prompted us to consider a modification of the synthesis of NOR amino acid. Following the same scheme we hypothesized that the use of nitroacetate bringing chiral ester could give high level of regio- and enantioselectivity in the multicomponent reaction. Exploitation of chiral auxiliary to obtain high enantiomeric excess is well-known in literature and frequently used in our group, too^{28 29}. **8d** and **8e** were synthesised in high yield (86% and 69%) following the same procedure applied for the preparation of benzyl and allyl derivatives. (Scheme 8) The use of **8d** in the multicomponent reaction in the optimized condition described before, has furnished the cycloadduct *exo* **24**, **25** and *endo* **25**, **27** in ratio (**24**: 85%, **25**:4%, **26**:5%, **27**:6%) shown by HPLC and GC analysis of the crude mixture. Compared with achiral esters used before, the menthol ester slightly increased the diastereoselection between *endo* and *exo* adducts but furnished an

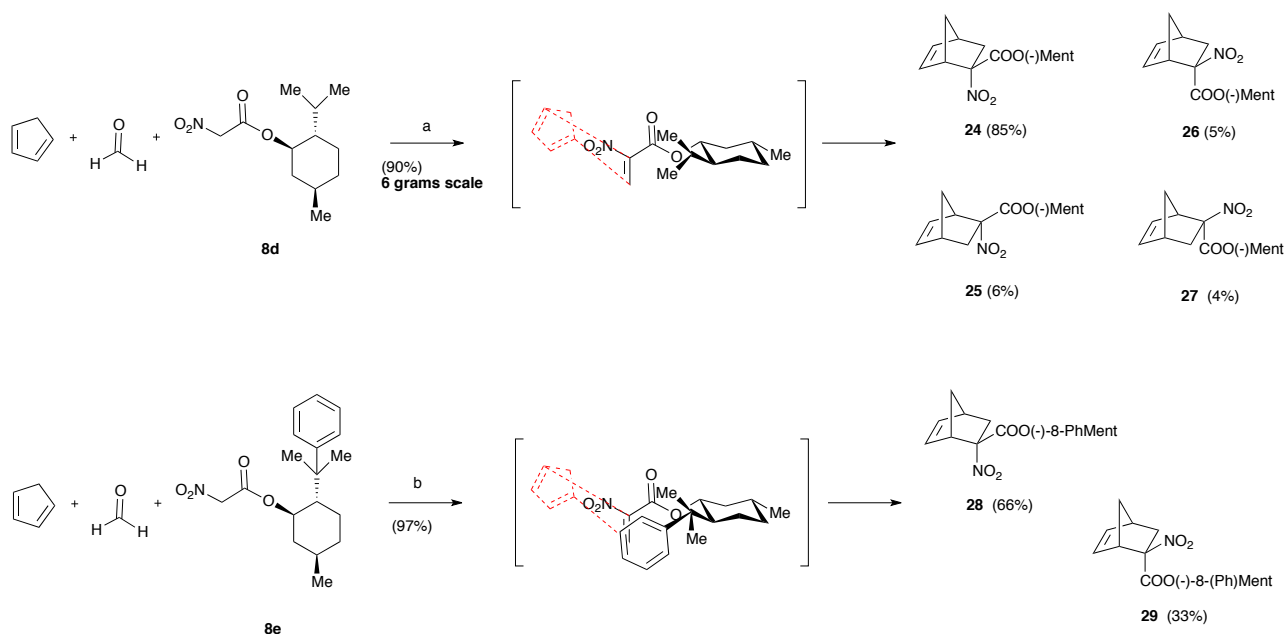
²⁸ D. A. Evans, K. T. Chapman, and J. Bisaha Asymmetric Diels-Alder Cycloaddition Reactions with Chiral α -Unsaturated N-Acyloxazolidinones J. Am. Chem. Soc. 1978, 110, 4, 1238-1256

²⁹ K. Ruck-Braun, H. Kunz Chiral Auxiliaries in Cycloadditions. Wiley WCH 1998

elevated diastereoselection between *exo* products (*exo*-**24**: 85%, *exo*-**25**: 4%). The slightly improvement in *endo/exo* ratio is due to the steric hindrance of the menthol ester that disfavours the most congested *endo* position of the carbonyl function. Concerning the observed preference toward *exo*-**24**, it is apparent that the isopropyl group of menthol masks preferentially one face of the dienophile inducing the attack of cyclopentadiene on the opposite face. The correct assignment of the structure was deduced by the NMR analysis of corresponding acetylated amine derivatives **36**, **37**, **38** and **39** prepared as shown in scheme 11 and already reported in literature by Cativiela³⁰ and Christensen.³¹

Interestingly, but not surprisingly, the reaction with the 8-phenylmenthol nitroacetate **8e** furnished unusual results. First of all, an optimum 96% yield was obtained with only one addition of 10 equivalents of the other reactants and, moreover, it provided an elevated enantioselection between the *exo* compounds **28** and **29**, not detectable by NMR, but a lower diastereoselection between *endo* and *exo* compounds. The high reactivity of the more encumbered dienophile **8e** could be explained by its higher hydrophobicity that lead to a faster reaction in water medium than the other nitroacetates. The lower *exo-endo* diastereoselectivity would require computational calculation of the transition state energy for a complete analysis but we could hypothesized that π interaction in the transition state among the phenyl group of the ester and the double bond of the dienophile decreases the strength of the interactions of this latter with the π system of the nitro group, leading to a decrease of the *exo* products ratio. (Scheme 10)

Scheme 3.10 Three component cycloaddition reaction with chiral auxiliary.



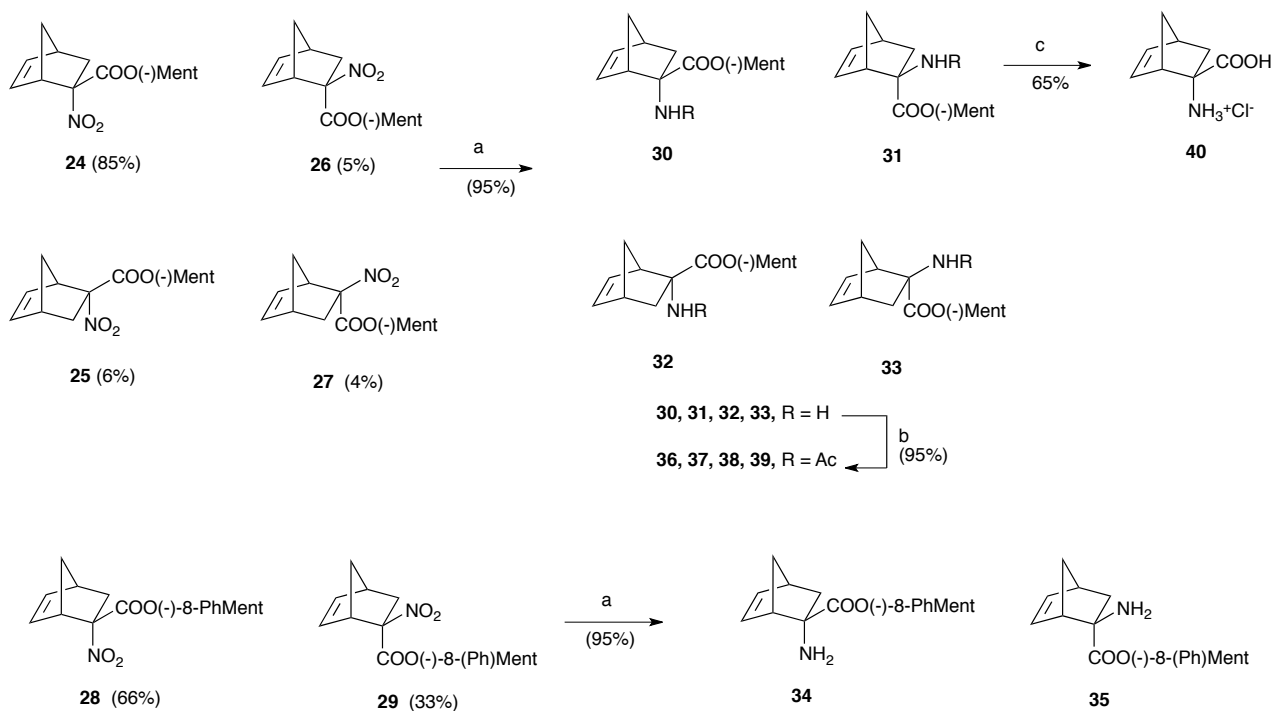
³⁰ Cativiela; Lopez; Mayoral; Asymmetric synthesis of 2-Aminonorbornane-2-carboxylic acids by Diels-Alder reaction. *Tetrahedron Asymmetry*, **1990**, 1(2), 61-64.

³¹ Howard S. Tager and Halvor N. Christensen *J. Am. Chem. Soc.* **1972**, 1, 93-94

Reaction conditions: a) AcOH, THF, 45°C, b) AcOH, THF, 30°C.

In the following steps, (-)-menthol-nitroacetate that was able to furnish compound *exo*-**24** in high yield and diastereoselection (85%) was used. *Exo*-**24** in inseparable mixture with **25**, **26** and **27** was then reduced by Zn, H₃PO₄ 1M in THF to the corresponding amino esters **30**, **31**, **32** and **33** that are separable in couple of *exo* (**30**, **31**) and *endo* (**32**, **33**) products. The *exo*-**30** and *exo*-**31** mixture (85/4) was hydrolysed at the ester function with saturated KOH in methanol at 70°C in sealed tube and after acidification with HCl at 0°C it was possible to extract the free amino acid *exo*-**40** in reasonable yield (65%) with the loss of minor isomer deriving from *exo*-**31**. The entire process was scaled up in order to permit the obtainment, in an economical and short strategy, of 2 grams of free amino acid *exo*-**40** with a good overall yield (39%). Compound **28** and **29** were reduced in the same way and the amines **34** and **35** were separated and characterised. Trace of the corresponding *exo* and *endo* diastereoisomers were identified and confirmed our hypothesis.

Scheme 3.11 Reduction of nitro function, synthesis of the corresponding acetylated products and free amino acid **40**



Reaction Conditions: a) Zn dust, H₃PO₄ 1M, THF, 0°C to rt, o.n. b) Ac₂O, TEA; c) 1) MeOH saturated by KOH, 70°C, 4h, 2) HCl pH 2, 0°C.w

3.3 Inclusion of norbornene amino acid in model peptide and conformational analysis.

3.3.1 Model peptide AcNH-(L)Ala-NOR-(L)Ala-Aib-(L)Ala-CONH₂ synthesis

In order to follow the same synthetic scheme presented in chapter number 2 the free amino acid *exo*-**40** was protected at the amine function using Boc-anhydride with potassium carbonate in methanol/water affording **41** as depicted in scheme 12. Taking in account the need of Boc-protected amino acid, we decided to perform hydrolytic and Boc protection steps in one flask methodology that permitted the direct isolation of the desired compound **41** in 82% yield over two steps avoiding the limiting and laborious operation of amino acid extraction from water phase. The tripeptide **42** NH₂(L)Ala-Aib-(L)AlaCONH₂ was prepared, as shown in chapter 2, in free amine form with a different strategy reported in literature ¹. Boc-protected amino acid **41** was then condensed with tripeptide **42** using HOBt, EDC, in DCM/DMF and DIPEA affording the peptide **43** in very high yield (95%) on 1,5 gram scale. After column purification, **43** was precipitated as white solid in diethylether. NMR analysis of the compound showed the presence of two different products which were analysed by VT-NMR. The results obtained demonstrated the coalescence of the signals of the two products in a single one. Basing on this result we can hypothesized that the two products are not different diastereoisomers deriving from racemization event taking place in the coupling reaction but that they are two conformers of the tetrapeptide **43** existing in stable form in solution. **43** was deprotected by thermal decomposition of Boc group in water mediated by M.W. ^{2 3} Recrystallization in diethylether permitted the obtainment of **44** (70% yield on 0,5 grams) that was then condensed with AcNH(L)AlaOH with the use of EEDQ in THF at 60°C (40 Watt for 20 minute). The final pentapeptide **45** was formed in pure form by crystallization in diethylether (56% yield). Unfortunately we were not able to scale up the last microwave coupling to more than 100mg without large degradation process. Anyway, short reaction time permitted iteration of the process to a good amount of product. As its precursor **43**, also **45** is present in solution as couple of two highly stable conformers in a ratio of 60/40. Coalescence experiments were performed in order to verify that they are two conformers of the same molecule. The temperature was keep at 120 °C for 45 minutes and then, cooling down the sample, the same ratio of the two conformer are formed (figure 1). Using HPLC inverse phase on a semipreparative C-18 column as stationary phase and H₂O/CH₃CN 90/10 with

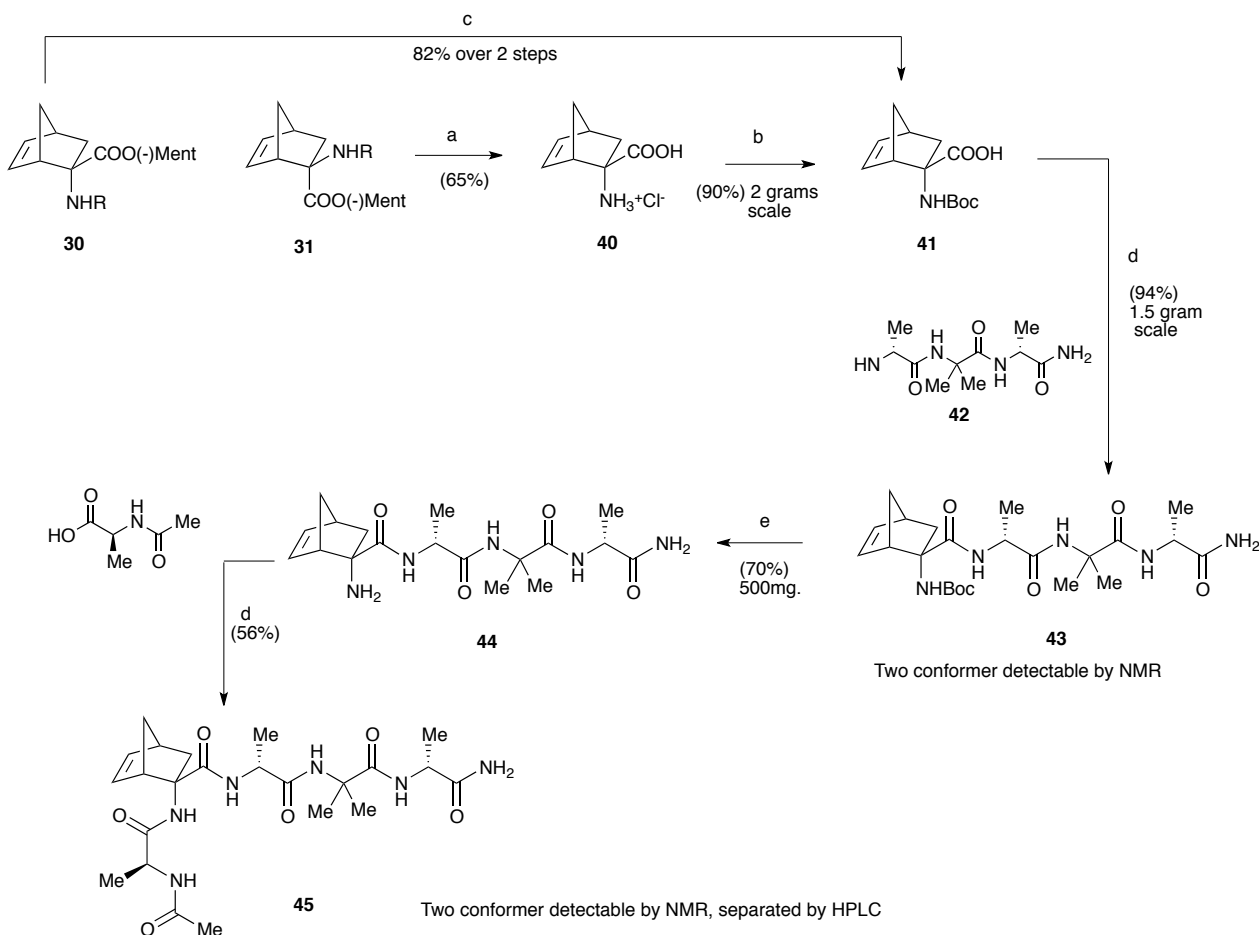
¹ S. Pellegrino, A. Contini, F. Clerici, A. Gori, D. Nava, M.L. Gelmi Chem. Eur. J. **2012**, 18, 8705 – 8715

² A. Thaqi, A. McCluskey, J. L. Scott. A mild Boc deprotection and the importance of a free carboxylate. *Tetrahedron Letters*, **2008** 49 6962–6964

³ Siro, Jorge G.; Martín, Justina; García-Navío, José L.; Remuñán, Modesto J.; Vaquero, Juan J. Easy Microwave Assisted Deprotection of N-Boc Derivatives. *Synlett*, **1998**; 1998(2), 147-148

0.1% of TFA as eluent, it was possible to obtain the two conformers in pure and stable form at room temperature.

Scheme 3.12 Synthesis of the projected peptide **45**



Reaction Conditions: a) 1) MeOH saturated by KOH, 70°C, 4h, 2) HCl pH 2, 0°C. b) Boc₂O, MeOH/H₂O, K₂CO₃, 40°C, 2gg; c) 1)DCM wash, 2) Boc₂O, MeOH/H₂O, 70°C, 1gg; d)1)EDC, HOBT, DCM, 0°C, 1 h, 2) **42**, DiPEA, DMF; e)M.W, H₂O, 150°C, 15 min.; f) EEDQ, M.W. 40 Watt, THF, 20 min.

3.3.2 NMR Conformational analysis

The two conformers of peptide **45** have to be analysed by NMR spectroscopy and CD analysis in order to lighten the secondary structure and test their stability. NMR spectra were recorded in different solvents (CD₃CN, CD₃OD, CDCl₃, DMSO-*d*₆) without any change in the conformer ratio. High temperature VT-NMR experiments in DMSO-*d*₆ showed coalescence of the signals at 120° C (Figure 1). Keeping for 40 minutes the product at 120° and rise down the temperature did not change the ratio of the two conformers and regenerate them without degradation.

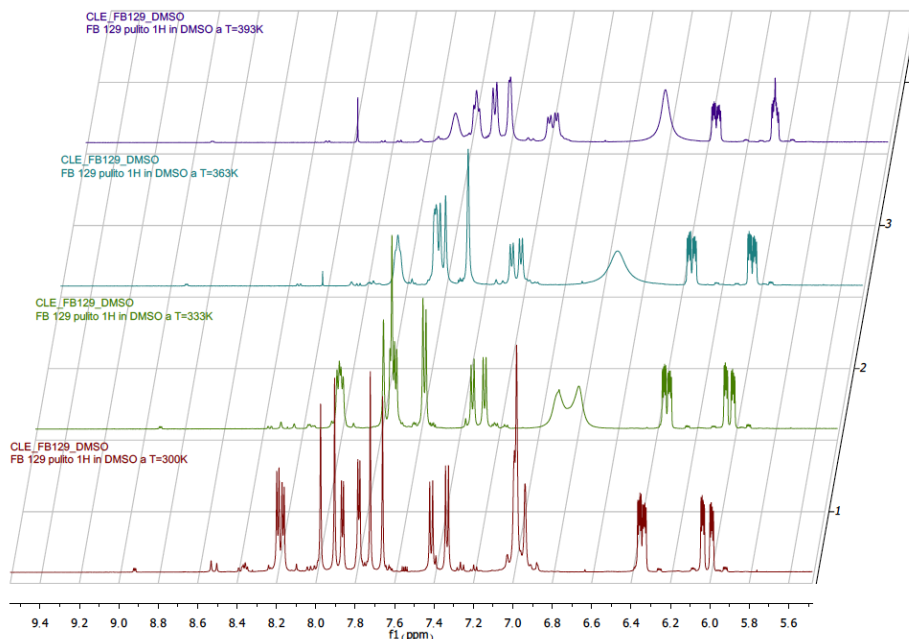


Figure 3.1 VT-NMR of compound **45**, coalescence of two conformers.

The analysis in CD_3CN didn't permit the full characterization due to the low solubility of peptide **45**. Notwithstanding we were able to evaluate the Magnetic Non Equivalence of the two Methyl group of Aib in both conformers. The MNE, has already told in the previous chapter, is an important data in order to verify the presence of secondary structure: when its value is higher than 0,5-0,8, the MNE is not due to a close stereocentre but is generated by molecular conformation. From the ^{13}C -NMR of **A45** a very high value of 6.3 ppm between the signals of the two germinal Methyl group of the Aib residue, was extrapolated. This value indicate that the peptide has an extremely high unusual degree of secondary helical structure⁴. Generally for a model peptide containing five residues like alanine and Aib, this value is comprised between 2 and 4. For the second conformer **B45**, the value is lower but still over the value of 2, indicating that we are not in presence of an unfolded form of the peptide but of a high stable secondary structure, probably different from a common helix structure. As observed before, considering the very low solubility of **45** in any solvent except MeOH, H_2O and DMSO, we decided to perform NMR experiment in water. The first result on conformer **A45** using $\text{H}_2\text{O}/\text{D}_2\text{O}_{10\%}$ with water signal suppressing, permitted to observe in the noesy spectrum a full cross peak between NH-NH of consecutive residues. (Figure 2)

⁴ G. Jung; H. Bruckner, R. Bosch, w. Winter, H Schaal. J. Strahle. Liebigs Ann. Chem. **1983**, 1096-1106

3.4 Computational Study

3.4.1 Application of REMD protocol to the pentapeptide model containing norbornene amino acid (+) and (-).

In this study two pentapeptides that differ only for the absolute stereochemistry of the unnatural norbornene amino acid corresponding to precursor **30** and **32** have been considered.

a) **P1** L-Ala-(1*R*,2*R*,4*R*)NOR-L-Ala-Aib-L-Ala

b) **P2** L-Ala-(1*S*,2*S*,4*S*)NOR-L-Ala-Aib-L-Ala

The used protocol has been fully described in the previous chapter. Each peptide has been designed with an acetyl protection at the N-terminus and methylcarboxamide at the C-terminus. The simulation has been run starting from the full extended conformation of both peptide as in previous work of chapter 2 ($\psi = \phi = \omega = 180^\circ$ C).

The REMD simulation in this case has been performed on twelve replica in a temperature window of 260 - 600 K. The simulation time for each replica has been set on 100 ns, for a overall time of 1.2 μ s, during this time 50000 temperature interchange have been done. For each peptide has been extrapolated the trajectory at 300 K. The secondary structures and the *cluster* have been scan by H-Bond analysis in order to obtain as already done in chapter 2 different *cluster* of conformer. The most populated will be the cluster more descriptive of the actual conformation.

The cluster analysis (Table 1) of the pentapeptide **P1** and **P2** trajectories shown that for either isomers (1*R*,2*R*,4*R*) and (1*S*,2*S*,4*S*) a prevalent conformation is present and correspond to the cluster C1. The average value analysis of ψ and ϕ of the representative conformation of most populated *cluster* C1 could underline that both sequence **P1** and **P2** lead to a right hand helical secondary structure ($\psi = -26$ and $\phi = -49$ for the 3_{10} -helix and of $\psi = -45$ and $\phi = -60$ for the α -helix). The representative structure of the second most populated cluster C2 shown also value of ϕ of right hand helical structure and we could speculate an elevated structure instability due to the high value of standard deviation. Regarding ψ C2 conformation has positive values corresponding to left hand helix or random structures. The conformation of C2 that correspond to the 15.9% and 11.7 % of the total conformation for peptide **P1** and **P2** respectively should be folding intermediate of more stable conformation of C1.

Table 1. Clusters analysis's result(pop%)

P1 (RRR)			P2 (SSS)			
Pop%	ϕ	ψ	Pop%	ϕ	ψ	
C1	83.3	-91.07±76.45	-17.05±21.11	85.3	-63.60±15.64	-23.03±11.44

C2	15.9	-60.57±57.01	6.61±32.55	11.7	-68.02±70.24	37.69±73.80
C3	0.4	-36.43±76.45	1.48±28.99	1.7	-7.25±78.91	26.16±23.37
C4	0.3	12.47±72.38	18.69±23.67	0.9	-28.16±63.06	20.64±23.33
C5	0.2	-89.16±46.52	-28.04±25.89	0.3	-89.71±48.03	-27.77±20.02

The entire trajectory MD has been explored by secondary structure analysis and H-bond evaluation. In table 2 is shown how during the entire simulation the value of ψ and ϕ found a high level of agreement with 3_{10} –helix than other secondary structure. Alike H-bond evaluation has underlined the strong presence during the trajectory of tow H-Bond between residues placed $i, i+3$ typical of 3_{10} -helix.

Table 2 : Secondary structures analysis

	P1 (RRR)			P2 (SSS)		
	helix ₃₁₀	α -helix	Turn	helix ₃₁₀	α -helix	Turn
ALA1	56.4	6.2	12.3	54.9	3.8	8.3
NRB	76.7	6.7	13.9	72.4	4.2	9.7
ALA3	81.4	6.7	11.0	82.5	4.2	10.8
AIB4	69.0	7.0	21.3	71.7	4.5	21.2
ALA5	47.7	1.8	31.8	49.6	1.0	33.6

The difference compared to 100% corresponds to the percentage of structures not related to the categories listed in the table.

Table 2 : H-Bond analysis

Donor	Acceptor	Occupancy P1	Occupancy P2
AIB5-Main-N	ALA2-Main-O	65.42%	62.96%
ALA6-Main-N	NRB3-Main-O	56.41%	60.96%

Hydrogen bond could be identified as such, must be satisfied the following parameters: donor-acceptor distance $\leq 4\text{\AA}$, donor-H-acceptor angle = 120 ± 30 deg.

From the geometry reported in figure 3, related to the representative structures of the most populated cluster, it is noteworthy that the helical core, once close to the norbornene, is perfectly structured in both analysed sequence. Deviation from the helical ideal structure are shown only at the level of N-terminus and C-terminus as confirmed by average numerical value of ψ and ϕ evaluated for each residues over the entire trajectory of the cluster C1. Finally we could affirm that both amino acids NOR(*1R,2R,4R*) and NOR(*1S,2S,4S*) are strong inductor of 3_{10} type Helix.

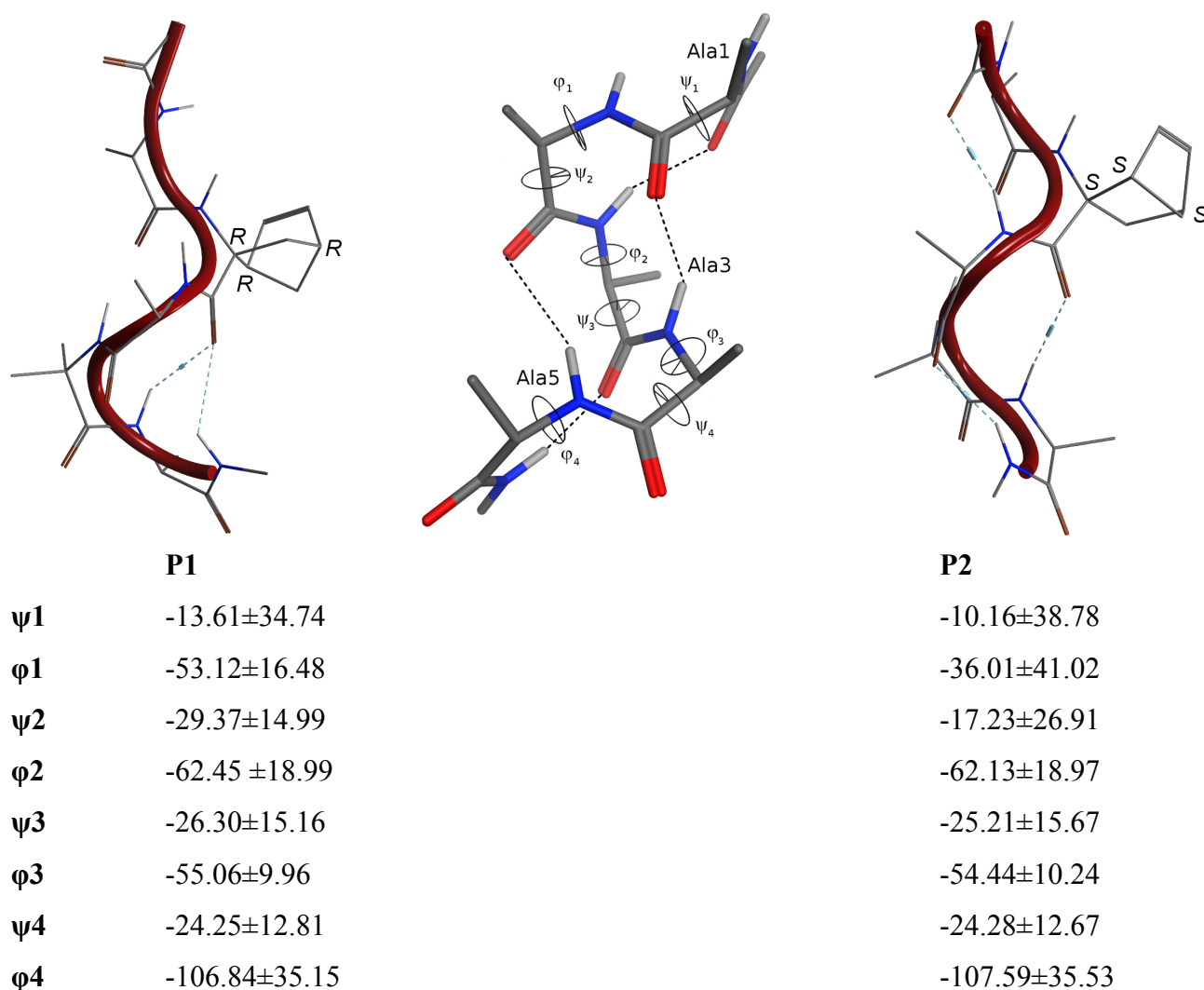
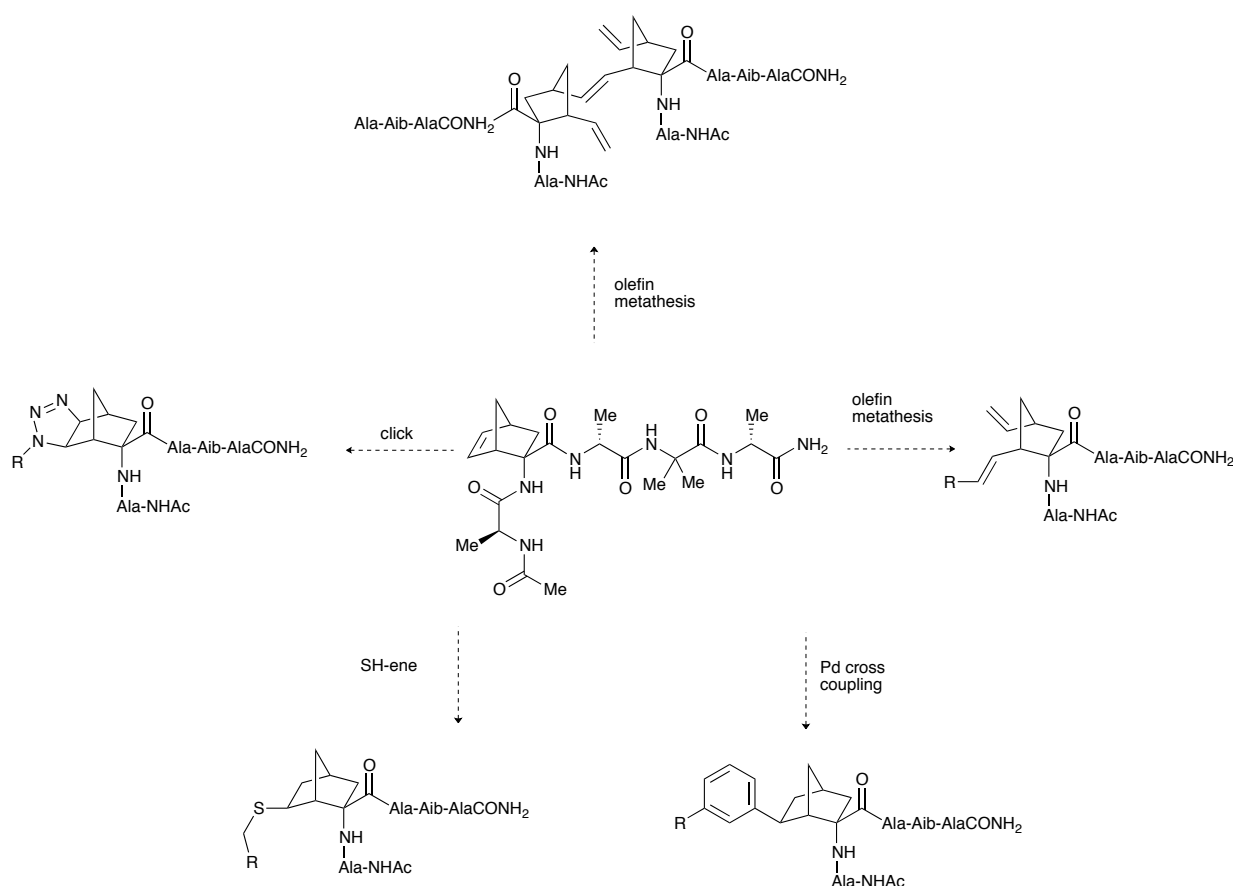


Figure 3.2 Representative geometry of the most populated cluster obtained for peptides 1 (pop = 83.3%) and 2 (pop = 85.3%).

3.5 Conclusions and further developments

An efficient procedure for the synthesis of norbornene amino acid in enantiopure fashion had been developed and scaled up to grams quantities. With the same approach described in the previous chapter this amino acid was introduced in the model peptide Ala-NOR-Ala-Aib-Ala. NMR analysis was performed but due to the low solubility of the compound, only preliminary results are available. We identified the presence of two stable conformers at room temperature and we were able to separate such conformers by HPLC purification. MNE analysis on ^{13}C -NMR spectrum of a single conformer allowed us to state that one of them has an extremely stable helical structure compared with previous works on model peptides made by Ala-Aib residues⁵³. The other conformer presents a low value of MNE that induced us to speculate on a different type of secondary structure, having regard to its stability in HPLC purification. A computational study following the model used in chapter 2 using REMD analysis was performed proving the ability to provide the

interesting behaviour of NOR(*1R,2R,4R*) and NOR(*1S,2S,4S*) and confirmed the high tendency of norbornene amino acid to stabilize 3_{10} helical structure. In the future, higher accurate CD and NMR analyses will be carried out in order to describe the secondary structure of the two conformers with higher accuracy of details. In order to verify the molecular modelling we are working on the synthesis of the other enantiomer using the chiral auxiliary with the opposite stereochemistry. Having in our hand a procedure able to furnish not only the amino acid but also considerable amount of peptide, this opens the possibility to a study devoted to the modification of the double bond from the point of view of the divergent generation of chemical diversity directly on the peptide. We plan to perform several modifications, bioconjugation via click chemistry, Pd chemistry and SH-ene reaction; oligomerization via ROMP and also structural modification of the norbornene core included in the peptide that could be influenced by the secondary structure of the peptide. Figure 3. Lastly, of great interest it could be to perform simple modifications of the double bond and study the influence of the helical secondary structure on the regio- and stereoselectivity of the reaction.



R= Biconjugate, Fluorescent agent, sugar

Figure 3.3 Possible further development.

4 Hydroarylation of asymmetric substituted norbornene amino acids: studies on long-range stereo-electronic effects on the regioselectivity of the addition.

4.1 Introduction

Several research in our laboratory dealt with the preparation of carbocyclic constrained α -amino acids whose importance as biological target is related to their rigidity which makes them very useful tools in the synthesis of peptidomimetics a lot of them are reported in chapter 2 in Figure 2.2 and figure 2.3.^{1 2 3 4} In particular, in the development of a wide-ranging project, several β -hetero-substituted carbocyclic α -amino acids characterized by the norbornene scaffold have been prepared.^{5 6 7} Such compounds can be considered versatile building blocks owing to the features of the bicyclic system characterized not only by the presence of the α,α -disubstituted amino acid function, suitable for peptide synthesis, but also by the carbon-carbon double bond which could be used to functionalize the ring.^{8 9 10 11 12}

¹ Clerici, F.; Gelmi, M. L.; Pellegrino, S.; Pilati T. *J. Org. Chem.* **2003**, *68*, 5286.

² Clerici, F.; Gelmi, M. L.; Gandolfi, R.; Pellegrino S. *Tetrahedron-Asymmetry* **2008**, *19*, 584-592.

³ Caputo, F.; Cattaneo, C.; Clerici, F.; Gelmi, M. L.; Pellegrino, S. *J. Org. Chem.* **2006**, *71*, 8467-8472.

⁴ Cattaneo, C.; Clerici, F.; Gelmi, M. L.; Martini, C.; Montali, M.; Pellegrino, S. *J. Org. Chem.* **2007**, *72*, 9811-9814.

⁵ Clerici, F.; Gelmi, M. L.; Pellegrino, S. *Tetrahedron*, **2008**, *64*, 5657-5665

⁶ Clerici, F.; Gelmi, M. L.; Gambini, A.; Nava, D. *Tetrahedron*, **2001**, *57*, 6429-6438

⁷ Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Soave, R.; Clerici, F. Sulfanyl-methylene-5(4H)-oxazolones and β -sulfanyl- α -nitroacrylates as appealing dienophiles for the synthesis of conformationally constrained cysteine analogues. *Tetrahedron*. **2012**, *68*, 1951-1962.

⁸ Ranganathan, D.; Haridas, V.; Kurur, S.; Nagaraj, R.; Bikshapathy, E.; Kunwar, AC.; Sarma, A.V.S.; Vairamani, M. Norbornene-Constrained Cyclic Peptides with Hairpin Architecture: Design, Synthesis, Conformation, and Membrane ion Transport.1. *Org. Chem.* **2000**, *65*, 365-374

⁹ Park, J.; Kim, K.R.; Nam, H.Y.; Yeom, C.; Chough, C.; Kwon, S.H.; Ro, S.; Shin, D.; Kim, B.M. Synthesis and Structural Analysis of 6Aminobicyclo[2.2.1]heptane-2-carboxylic Acid as a Conformationally Constrained γ -Turn Mimic. *Protein and Peptide Letters*, **2008**, *15*, 980-984.

¹⁰ Luethi, E.; Nguyen, K.T.; Burzle, M.; Blum, L.C.; Suzuki, Y.; Hediger, M.; Raymond, J. Identification of Selective Norbornane-Type Aspartate Analogue Inhibitors of the Glutamate Transporter 1 (GLT-1) from the Chemical Universe Generated Database (GDB). 1. *Med. Chem.* **2010**, *53*, 7236-7250

¹¹ Lienkamp, K.; Tew, G.N. Synthetic Mimics of Antimicrobial Peptides -A versatile Ring-Opening Metathesis polymerization Based platform for the Synthesis of selective Antibacterial and Cell-Penetrating Polymers. *Chem. Eur. J.*, **2011**, *15*, 11784-11800.

¹² Suthasupa, S.; Shiotsuki, M.; Sanda, F. Recent Advances in Ring-Opening Metathesis Polymerization, and Application to Synthesis of Functional Materials. *Polymer J.* **2010**, *42*, 905-915.

Having in our hand a small library of norbornene amino acid derivatives, substituted with several heteroatoms at C-3 (Cl, S, O), we planned to study the hydroarylation reaction on this class of compounds. For sake of completeness, the known unsubstituted and 3-phenylsubstituted norbornene amino acids have also been included. Furthermore, both endo- and exo-series have been considered (Figure 2).

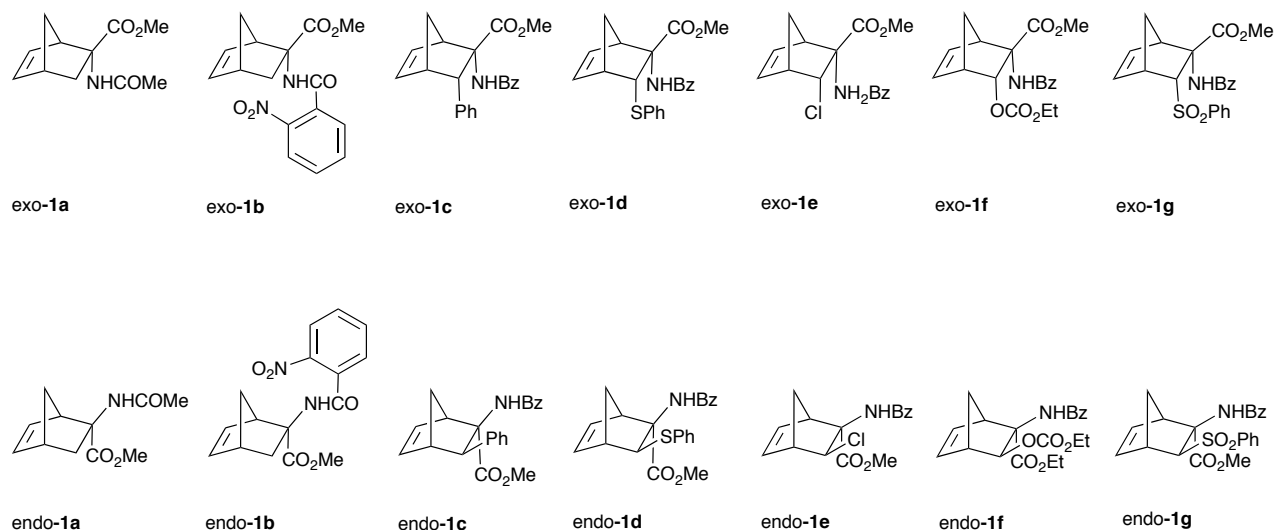


Figure 4.1 Library of norbornene amino acid derivatives used for this study

Although the hydroarylation has been well studied and the asymmetric variant of this reaction are known,^{13 14 15 16 17 18 19} to our knowledge no systematic studies on the stereo- and regioselectivities of hydroarylation of asymmetric bicyclic alkenes have been reported.

¹³ Larock, R. C.; Johnson, P. L. *J. Chem. Soc., Chem. Commun.* **1989**, 1368-1370.

¹⁴ Arcadi, A.; Marinelli, F.; Bernocchi, E.; Cacchi, S.; Ortari, G. *J. Organomet. Chem.* **1989**, 368, 249-256.

¹⁵ Clayton, S. C.; Regan, A. C. *Tetrahedron Lett.* **1993**, 34, 7493-7496

¹⁶ Hay, L. A. et al. *J. Org. Chem.* **1998**, 63, 5050-5058.

¹⁷ Jia, C. et al. *J. Org. Chem.* **2000**, 65, 7516-7522.

¹⁸ Jia, C. et al. *J. Am. Chem. Soc.* **2000**, 122, 7252-7263.

¹⁹ Bartoli, G. et al. *Synlett* **2008**, 16, 2508-2512.

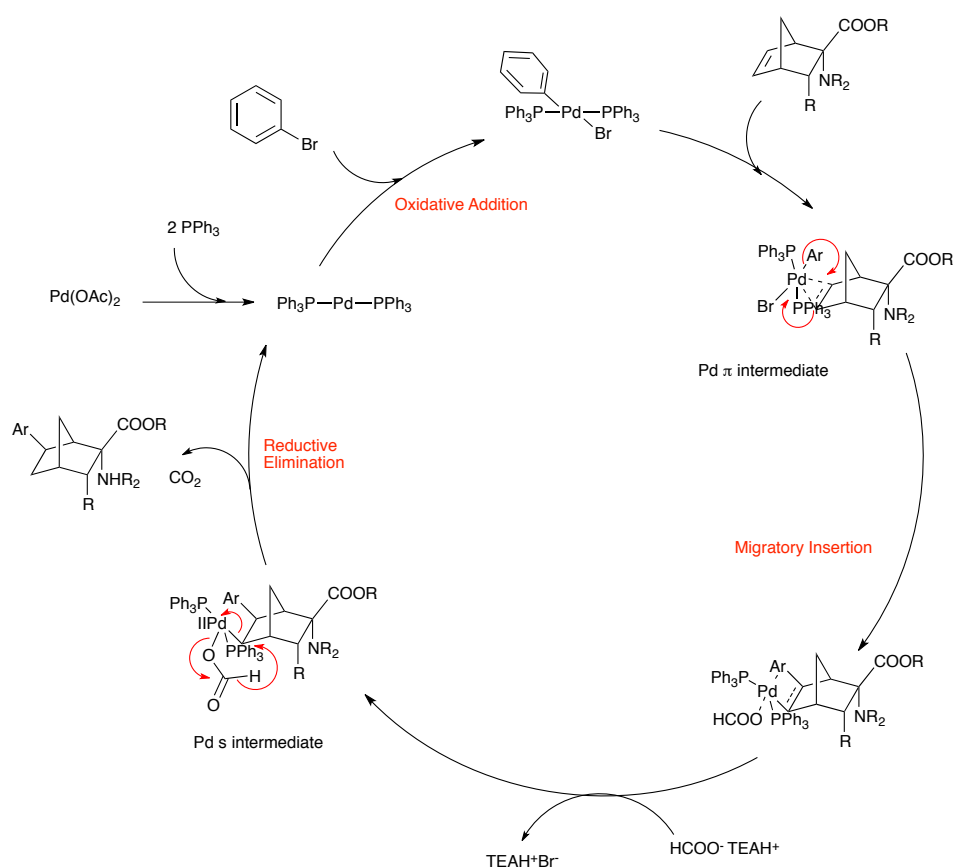


Figure 4.2 Heck hydroarylation mechanism

Recently, the study of long-range stereoelectronic effect of a remote substituent has attracted considerable interest since it should play a pivotal role in controlling regio- and stereoselectivities on the addition to the double bond. On the other hand very few examples of the study of these effects on transition metal catalyzed reactions can be found in the literature.²⁰

In this chapter we report our studies on the above norbornene amino acid derivatives with numerous halogenoarenes. Questions of both regiochemistry and stereochemistry have to be addressed concerning the carbon-carbon bond formation. In fact, the hydroarylation reaction could occur with one of the two olefinic carbons and, even if the exo-selectivity for hydroarylation is reported, the presence of substituents at C-2 and C-3 could in principle influence the stereochemistry.²⁰ As a result, the exo and endo coupling products are possible depending on the stereochemistry of the substituents on the starting amino acid derivatives.

²⁰ Mayo, P.; Tam, W. *Tetrahedron* 2002, 58, 9527-9540.

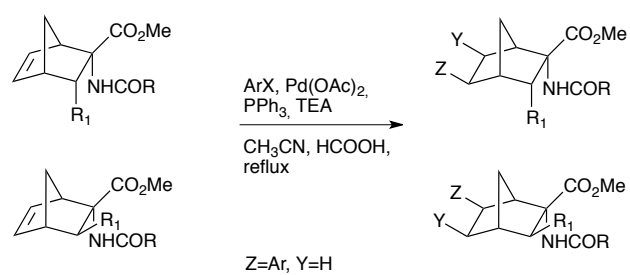


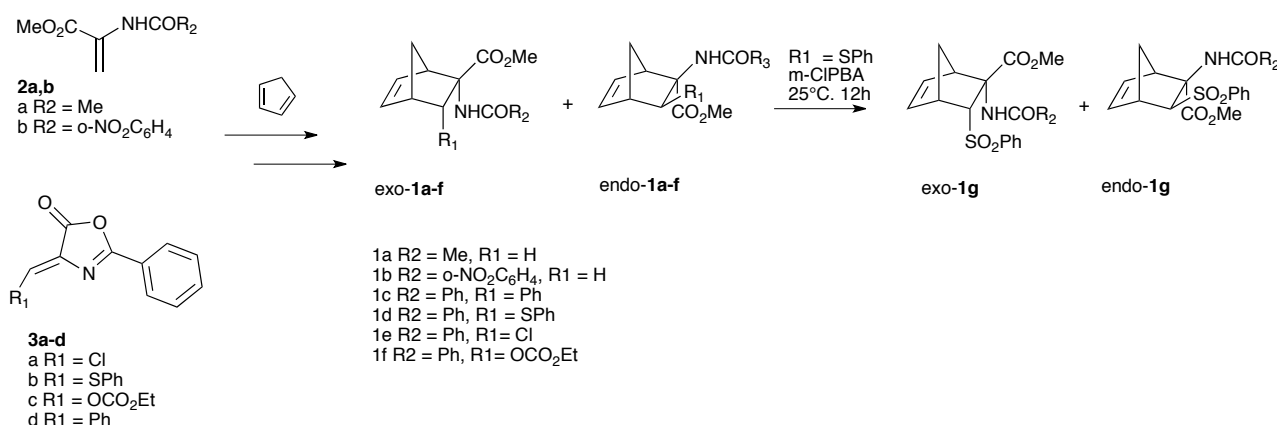
Figure 4.3 Scheme of model reaction.

4.2 Synthetic and analytical results

4.2.1 Synthesis of starting materials

First of all we synthesized the library of compounds **1** (Figure 2) taking advantage of Diels-Alder cycloaddition reactions between cyclopentadiene and the opportune dienophile **2** or **3**. (Scheme 1, see Experimental). Aiming to verify the role of the steric hindrance of the amido group on the regiochemistry, *exo*- and *endo*-**1a** and **1b** were prepared. Then we prepared compounds **1c-g** bearing *exo*- and *endo*-substituents at C-3 characterized by different stereo-electronic features. For the preparation of compounds *exo*- and *endo*-**1g**, the corresponding diastereoisomers **1d** were oxidized with *m*-chloroperbenzoic acid in dichloromethane.

Scheme 4.1 Preparation of norbornene derivatives to be used in this study

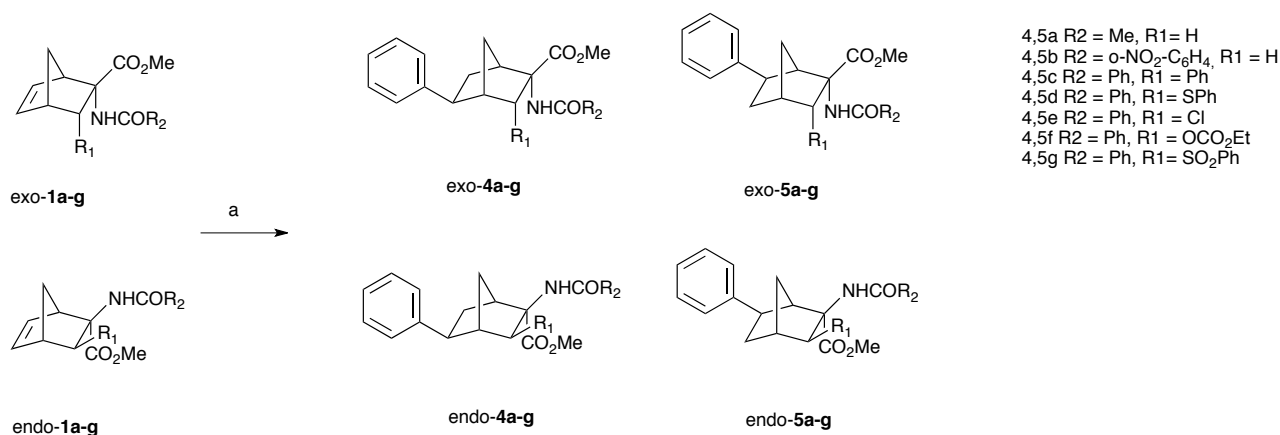


4.2.2 Substituent Effect of C-2 and C-3 functional groups on Heck hydroarylation

To begin our study we started with the simple norbornene derivatives *exo*-**1a** and *endo*-**1a**³ which were hydroarylated with iodobenzene in the presence of Pd(OAc)₂/Ph₃P/TEA/HCOOH in MeCN at reflux (Scheme 2). Starting from *exo*-**1a** the reaction successfully afforded two compounds (33:66 ratio) deriving, as expected, from *exo*-coupling. The correct stereo- and regiochemistry were assigned by the way of several bidimensional experiments (NOESY, COSY, HMQC) on both the compounds. To the first fraction eluted from the chromatographic column (see Experimental) was assigned the structure of *exo*-**5a** as a consequence

of the following consideration: a clear noe effect is present between the signal at 6.0 ppm (NH) and the protons at 3.24 ppm and 3.07 ppm. The same protons lack any effect with the bridge protons (1.61, 1.77 ppm). The aromatic protons at 7.24 ppm have a clear effect with the bridge proton at 1.61 ppm and the proton at 3.07 ppm. Moreover, the NH proton shows a noe effect only with the proton at 1.32 ppm of the CH₂ group (1.32 and 2.51 ppm) while the proton at 2.51 of the same group is in spatial proximity with the bridge proton at 1.77 ppm. These last (1.32 and 2.51 ppm) have a clear effect with the signal at 2.42 ppm which, in turn, is related to the CH₂ protons at 1.81 and 1.91 ppm. A further confirmation of the stereo- and regiochemistry derives from the analysis of COSY experiments where a long-range coupling (W-coupling) is evident across the protons at 1.77 and 3.24 ppm and those at 1.61 and 1.32 ppm. Similar considerations made possible the assignment of the structure of *exo*-**4a** to the second fraction eluted from the column (Figure 4). Once defined the correct regiochemistry to both the compounds we could conclude that the major regioisomer (*exo*-**4a**) resulted from the hydroarylation of C-5 (Table 1, entry 1). Analogously, when performing the reaction starting from *endo*-**1a**, two compounds were obtained corresponding to the two regioisomers and also in this case the major compound derived from the addition at C-5 (*endo*-**4a**) but with higher selectivity (15:85 ratio) (Table 1, entry 2). To verify if the regiochemical result could be influenced by the hindrance of substituents we prepared compounds *exo*-**1b** and *endo*-**1b** (entries 3,4). We observed that they reacted better than the corresponding *exo*- and *endo*-**1a** but with approximately the same selectivity evidencing that an increasing of the steric hindrance of the amido group doesn't exert a valuable effect. The reaction was then performed on the differently substituted norbornene amidoesters **1c-g** (Table 1) in the same conditions above described. Again, in all cases, two regioisomers were obtained resulting from the *exo*-coupling on C-5 and C-6. Also in those cases the correct structure was assigned performing NMR experiments basing on the same considerations reported above for *exo*-**4a** and *exo*-**5a**.

Scheme 4.2 Hydroarylation of *exo*- and *endo*-**1a-g** with PhI



Reaction conditions: a) PhI, Pd(OAc)₂, PPh₃, TEA, CH₃CN, HCOOH, reflux

Table 1 Hydroarylation of 1a-g with PhI. Yields and products ratio.

entry	Reagent	Total yields %	Product ratio ^a (<i>exo</i> -5: <i>exo</i> -4)	entry	Reagent	Total yields %	Product ratio ^a (<i>endo</i> -5: <i>endo</i> -4)
1	<i>exo</i> -1a	74	33:66	2	<i>endo</i> -1a	70	15:85
3	<i>exo</i> -1b	93	40:60	4	<i>endo</i> -1b	83	15:85
5	<i>exo</i> -1c	89	40:60	6	<i>endo</i> -1c	75	25:75
7	<i>exo</i> -1d	62	50:50	8	<i>endo</i> -1d	90	30:70
9	<i>exo</i> -1e	83	50:50	10	<i>endo</i> -1e	64	25:75
11	<i>exo</i> -1f	85	40:60	12	<i>endo</i> -1f	85	30:70
13	<i>exo</i> -1g	73	50:50	14	<i>endo</i> -1g	72	40:60
15	<i>exo</i> -1h	60	66:33	16	<i>endo</i> -1h	80	50:50
17	<i>exo</i> -1k	65	33:66				

^aCalculated from ¹H NMR of the regioisomeric mixture

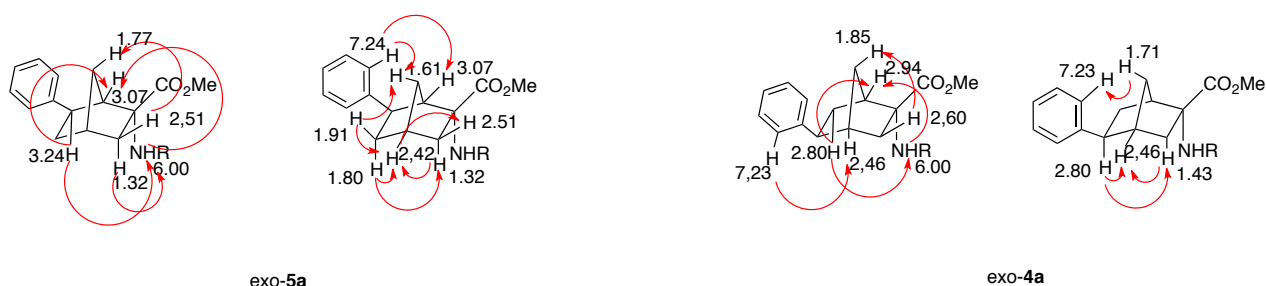


Figure 4.4 Principal NOE between protons confirming stereo- and regiochemistry for *exo*-4a and *exo*-5a

In all cases the major regioisomer resulted from the coupling at C-5 and the selectivity observed with the *exo*-1 series (ranging from 33:66 to 50:50) is lower than the corresponding ratios for the *endo*-1 series (ranging from 15:85 to 50:50). The preferred addition to C-5 is in agreement with the density function theory studies (DFT) realized by Mayo and Tam on the oxymercuration of 2-substituted norbornenes.²¹ The authors examined the relative energies of, and the charge distribution in, conformers of *exo*- and *endo*-2-substituted norbornenes bearing several substituents (e.g. OH, OAc, OSiH₃, OMe) showing that the charge on C-5 and C-6 are slightly negative and slightly different. For the *exo* form δ^- is always on C-6 for any conformation studied theoretically and with any substituent. They observed that for the *endo* form, the position of δ^- depends on the conformation of the substituent and in particular on the orientation of lone pair of the oxygen atom in the substituent. For any substituent exists at least one conformation with lone pair toward the norbornene that possess δ^- on C-5. Thus, for *endo* form, between the conformers with δ^- at C-5 and the conformers with δ^- at C-6 there is a competition which doesn't exist in *exo*-form. It can be hypothesize that a

²¹ Mayo, P.; Orlova, G.; Goddard, J. D.; Tam, W. J. Org. Chem., **2001**, 66, 5182-5191.

similar situation takes place with **1a** concerning the electron-poor carbon atom of the carboxylate group. The δ^+ carbon atom, exerting an identical inductive effect both in *endo*- or in *exo*-**1a**, displays only in the *endo*-form an electrostatic field effect which renders the C-5 even more electron-poor.²² Therefore the δ^- aryl group in the aryl-Pd-complex have the preference to add to the δ^+ C-5 of the norbornene double bond affording the corresponding regioisomer as the major product. (Figure 5)

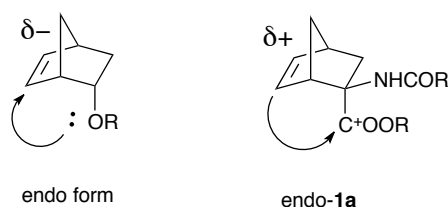
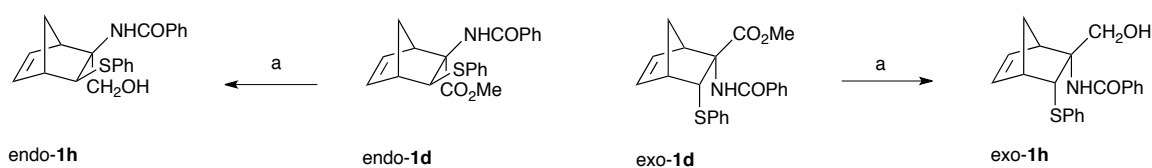


Figure 4.5: Proposed influence of the electrostatic field effect in *endo*-forms

Moreover, examining compounds **1c-g**, it can be observed that the introduction in β -position of an heteroatom balances the effect of CO_2R group (entries 7-14). As a result an increasing of the C-6 arylated compounds was observed. To further strengthen our hypothesis we decided to prepare the norbornene derivatives *endo*- and *exo*-**1h**, *exo*-**1k**. Unknown compounds *endo*- and *exo*-**1h** were prepared from the corresponding esters **1d** by treatment with $\text{LiAlH}_4/\text{THF}$ (Scheme 3). *Exo*-**1k** was synthesized through a methodology recently published by us.⁷

Scheme 4.3 Synthesis of compounds *endo*- and *exo*- **1h**



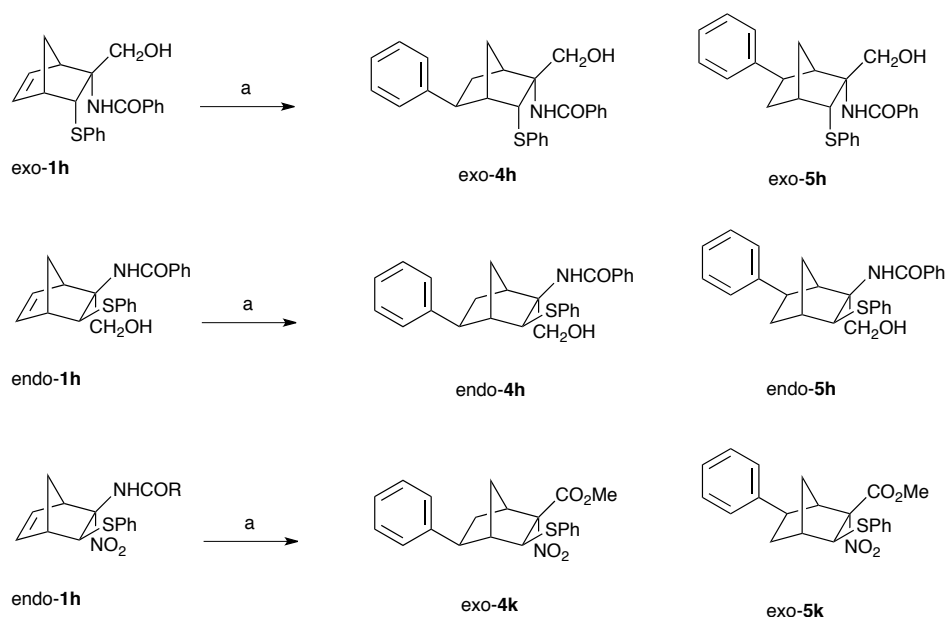
Reaction conditions: a) LiAlH_4 , THF, 25°C.

These compounds should have a different inductive effect with respect to the corresponding β -sulfanyl-substituted norbornenes **1d**. *Exo*-**1k** bears a nitro group in *endo* position which reinforces the inductive effect of the carboxylate group. So, an increasing of C-5 arylation should be expected. On the contrary, the inductive effects in *endo*- and *exo*-**1h**, should be substantially different and, moreover, compound *endo*-**1h** should lack the characteristic electronic field effect exerted by the carboxylate substituent which is responsible of the large amount of C-5 arylation as above explained. So, an increasing of C-6 arylation

²² Bowden, K.; Grubbs, E. J. *Chem. Soc. Rev.*, **1996**, 171-177

should be expected in such case. The above compounds were reacted in the usual hydroarylation conditions affording, in any case, two regioisomers: *endo*-**4,5h**, *exo*-**4,5h**, *exo*-**4,5k**. As expected, concerning the alcohols **1h**, a lowering of the amount of the C-5 regioisomer was observed (from 50:50 to 66:33 for *exo*-derivatives, entry 15; from 30:70 to 50:50 for *endo* ones, entry 16). On the contrary, concerning the nitro derivative *exo*-**1k**, (entries 17) an increasing of the amount of the C-5 regioisomer was observed so confirming our hypothesis (Scheme 4).

Scheme 4.4 Hydroarylation on compounds **1h,k**



Reaction conditions: a) PhI, $\text{Pd}(\text{OAc})_2$, PPh_3 , TEA, CH_3CN , HCOOH , reflux

4.2.3 Effect of reaction condition on Heck hydroarylation

In a second step of our study we focused our attention on the evaluation of reaction conditions aiming to evaluate the influence of different ligands, bases and solvents on the regiochemistry. Among the library shown in Figure 2, compound *endo*-**1d** was selected for this study according to its importance as key reagent for the synthesis of more complex compounds of biological interest.⁷ Similar yields to that obtained in the conditions above described, were found with the system $\text{Pd}(\text{OAc})_2/\text{BINAP}/\text{HCOOH}$, K_2CO_3 , MeCN (entry 1). Changing the base (TEA, entry 2; CsCO_3 , entry 3) lower yields were obtained without any influence on the regiochemistry. Analogously, negligible effects were produced with change in the solvent (entry 4). Concentration effects were also briefly considered

according to a recent publication by Piotrowsky and col.²³ but in our case we were not able to find any change in the selectivity comparing acetonitrile which has higher dielectric constants and benzene with lower dielectric constants. Three reactions, using benzene, acetonitrile and toluene as solvents, were conducted at a concentration 10-fold more dilute than the standard conditions, but the product ratio (**5:4**) remained unchanged (entries 5-7). At last, we test also the influence of silver ions.²⁴ We operated in the usual conditions (Pd(OAc)₂/PPh₃/HCOOH, TEA, MeCN, 50°C) but with adding 1 eq. of a silver salt. The simple addition of AgNO₃ or Ag₂CO₃ to the reaction mixture did not exert any influence on the regiochemistry the only result being a slowdown of the reaction: after 96h only traces of the hydroarylation products were observed and the starting material was substantially unreacted. A modest but interesting change in the regiochemistry was observed when the reaction was performed with 1 equivalent of AgNO₃ and adding HCOOH 24 hours later. In such experiment the selectivity changed from 30:70 to 50:50 (**5:4**, entry 10). Notwithstanding such interesting result, it has to be observed that also in this case the starting material after 96h was largely unreacted (30% yields, entry 10).

Table 2. Hydroarylation of 1d in different conditions.

Entry	Catalytic system (0.26 mmol <i>endo</i> - 1d)	solvent	Total yields % (5+4)	5/4 ratio
1	Pd(OAc) ₂ /BINAP/HCOOH, K ₂ CO ₃ ^a	MeCN 3mL	90	30:70
2	Pd(OAc) ₂ /BINAP/HCOOH, TEA ^a	MeCN 3mL	80	30:70
3	Pd(OAc) ₂ /BINAP/HCOOH, CsCO ₃ ^a	MeCN 3mL	70	30:70
4	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA ^b	Benzene 3mL	75	30:70
5	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA ^b	MeCN 30 mL	70	30:70 ^d
6	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA ^b	Benzene 30 mL	64	30:70 ^d
7	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA ^b	Toluene 30 mL	55	30:70 ^d
8	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA, AgNO ₃ ^c	MeCN 3mL	traces	35:65 ^c
9	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA, Ag ₂ CO ₃ ^c	MeCN 3mL	traces	35:65 ^c
10	Pd(OAc) ₂ /PPh ₃ /TEA, AgNO ₃ ; HCOOH (after 24h) ^c	MeCN 3mL	30	50:50 ^c

^a Pd(OAc)₂ 0.01 mmol, BINAP 0.015 mmol, HCOOH 0.8, base 1.0 mmol; ^b Pd(OAc)₂ 0.02 mmol, PPh₃ 0.04 mmol, TEA 0.9 mmol, HCOOH 0.78 mmol, 24h, 110°C; ^c Pd(OAc)₂ 0.01 mmol, PPh₃ 0.03 mmol, HCOOH 0.8 mmol, TEA 0.9 mmol, AgNO₃ 0.4 mmol or Ag₂CO₃ 0.2 mmol. ^d After disappearing of the starting material the crude reaction mixture was analyzed (NMR) and the ratio determined; ^e After 96 h even if the starting material was not completely disappeared the crude reaction mixture was analyzed (NMR) and the ratio determined.

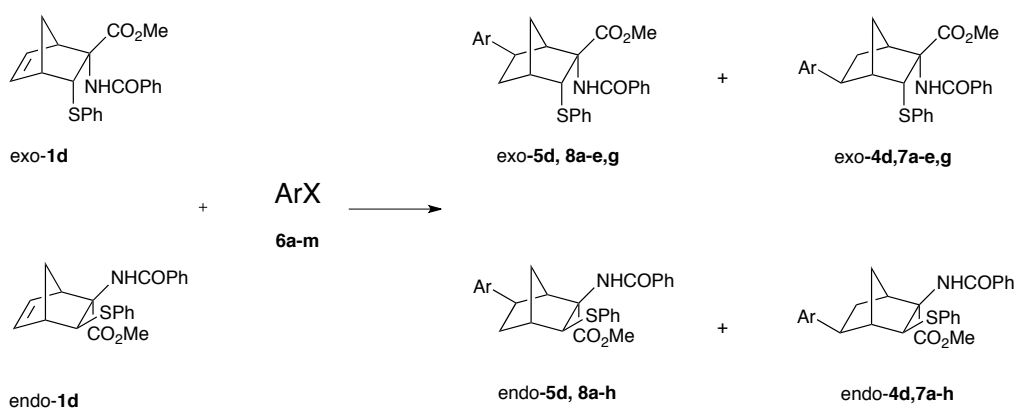
²³ Piotrowski, D. W.; Polivkova, J. *Tetrahedron Letters*, 2010, 51, 17-19

²⁴ Karabelas, K.; Westerlund, C.; Hallberg, A. *J. Org. Chem.* **1985**, 50, 3896-3900

4.2.4 Substituent Effect of aryl moiety group on Heck hydroarylation

We next investigated the reactivity of arenes in the reaction outcome. A wide range of arenes and heteroarenes were used in the reaction with *endo*- and *exo*-**1d** affording good to optimum yields of the hydroarylation products (Scheme 5, Table 3). Comparing the bromo- and the corresponding iodoarene a better behavior of this last was observed both in yields and in reaction time. Concerning the regiochemistry, in all cases the expected two regioisomers were obtained with nearly the same selectivity observed in the case of iodobenzene, the only exception being the case of very electron-poor arenes such as *o*-nitroiodobenzene **6l** (entries 22, 23) and *o,p*-dinitroiodobenzene **6m** (entry 24). Indeed, in such cases, the amount of C-6 aryl coupling appeared to be increased according to the lower electronegative density of the aryl-Pd complex.

Scheme 4.5 Hydroarylation of *exo*- and *endo*-**1d** with different halogenoarenes



Reaction conditions: a) PhI, Pd(OAc)₂, PPh₃, TEA, CH₃CN, HCOOH, reflux

Table 3. Hydroarylation of **1d** with different halogenoarenes.

entry	ArX 6a-m	Total yields % <i>Endo</i> series	Reaction time h	Ratio (C6/C5)	Entry	Total yields % <i>Exo</i> series	Reaction time h	Ratio (C6/C5)
1	C ₆ H ₅ Br (6a)	30 (5+4d)	24	30:70	2	37(5+4d)	24	50:50
3	<i>m</i> -I-C ₆ H ₄ NH ₂ (6b)	95(8+7a)	8	30:70	4	55(8+7a)	21	50:50
5	<i>m</i> -Br-C ₆ H ₄ NH ₂ (6c)	46(8+7a)	24	30:70	6	74(8+7a)	24	50:50
7	<i>p</i> -MeO-C ₆ H ₄ I (6d)	75(8+7b)	24	30:70	8	74(8+7b)	24	50:50
9	<i>p</i> -MeO-C ₆ H ₄ Br (6e)	71(8+7b)	24	30:70	10	53(8+7b)	24	50:50
11	<i>o</i> -MeO-C ₆ H ₄ I (6f)	79(8+7c)	24	33:66	12	90(8+7c)	48	50:50
13	<i>o</i> -MeO-C ₆ H ₄ Br (6g)	71(8+7c)	24	33:66	14	74(8+7c)	48	50:50

15	3-I-C ₄ H ₃ S(6h)	90(8+7d)	24	33:66	16	90(8+7d)	16	50:50
17	3-Br-C ₄ H ₃ S(6i)	40(8+7d)	48	33:66	18	40(8+7d)	32	50:50
19	<i>m</i> -I-C ₆ H ₄ Br(6j)	90(8+7e)	18	33:66	20	73(8+7e)	24	50:50
21	<i>m</i> -I-C ₆ H ₄ I(6k)	56(8+7f)	48	40:60		-		-
22	<i>o</i> -NO ₂ -C ₆ H ₄ I(6l)	80(8+7g)	24	50:50	23	57(8+7g)	24	50:50
24	<i>o,p</i> -(NO ₂) ₂ - C ₆ H ₃ I(6m)	83(8+7h)	24	50:50		-		-

4.3 Conclusion

We have investigated the effect of remote substituents on unsymmetrically substituted norbornenes in the palladium-catalyzed hydroarylation reaction. The reaction occurred with good to optimum yields but with moderate level of regioselectivity being the coupling at C-5 favorite. *Endo*-series always gave the better regioselectivities than the corresponding *exo*-series. The shift toward the coupling at C-5 or C-6 appears to be influenced by the electronegativity at C-2. An increasing of the coupling at C-6 was observed decreasing the electronegativity at C-2 (CO₂R vs CH₂OH), by using very electron-poor halogenoarenes and when the reaction was performed with 1 equivalent of AgNO₃ followed by HCOOH addition.

5 Functionalized norbornane an appealing scaffold for the design of Rac1-Tiam1 protein-protein interaction inhibitors

5.1 Introduction

The family of small Rho GTPases coordinates several cellular functions including adhesion, migration, cytoskeleton rearrangements, gene transcription, proliferation, and survival.¹² The deregulation of Rho proteins has been associated with cancer and thus much effort has been focused on the development of Rho inhibitors as anti-cancer therapeutics.³ More recently, these proteins have also gained attention as a potential target for the treatment of cardiovascular diseases.⁴⁻⁵ In particular the role of Rac in cardiac hypertrophy has been demonstrated by using transgenic and knock-out tissue specific mice.⁶⁻⁷ The expression of a constitutively active form of Rac1 or the deletion of Rac1 in cardiomyocytes, determine an opposite cardiac phenotype, transient cardiac hypertrophy and protection to cardiac hypertrophy in response to hypertensive stress, respectively.⁶ Up to 2004 the inhibition of small Rho GTPase was mainly ascribed to the action of the HMG-CoA reductase inhibitors, statins, through the inhibition of protein prenylation processes.⁸⁻⁹ Some clinical evidences have indeed associated the statin treatment in patients with heart failure with the inhibition of Rac protein function and the activity of NADPH oxidase.¹⁰⁻¹¹ The development of selective Rac inhibitors capable to compete with GTP, has been hampered by the high homology of the GTP binding domain between the different small GTPase proteins.¹²⁻¹³⁻¹⁴ The selective inhibition of Rac was then achieved by the

¹ Bishop, A. L.; Hall, A. Rho GTPases and their effector proteins. *Biochem. J.* **2000**, 348 Pt 2, 241-255.

² Jaffe, A. B.; Hall, A. Rho GTPases: biochemistry and biology. *Annu. Rev. Cell. Dev. Biol.* **2005**, 21, 247-269.

³ Sahai, E.; Marshall, C. J. RHO-GTPases and cancer. *Nat. Rev. Cancer.* **2002**, 2, 133-142.

⁴ Sawada, N.; Li, Y.; Liao, J. K. Novel aspects of the roles of Rac1 GTPase in the cardiovascular system. *Curr. Opin. Pharmacol.* **2010**, 10, 116-121.

⁵ Ferri, N.; Contini, A.; Bernini, S. K.; Corsini, A. Role of Small GTPase Protein Rac1 in Cardiovascular Diseases: Development of New Selective Pharmacological Inhibitors. *J. Cardiovasc. Pharmacol.* **2013**, 62, 425-435.

⁶ Satoh, M.; Ogita, H.; Takeshita, K.; Mukai, Y.; Kwiatkowski, D. J.; Liao, J. K. Requirement of Rac1 in the development of cardiac hypertrophy. *Proc. Natl. Acad. Sci. U. S. A.* **2006**, 103, 7432-7437.

⁷ Sussman, M. A.; Welch, S.; Walker, A.; Klevitsky, R.; Hewett, T. E.; Price, R. L.; Schaefer, E.; Yager, K. Altered focal adhesion regulation correlates with cardiomyopathy in mice expressing constitutively active rac1. *J. Clin. Invest.* **2000**, 105, 875-886.

⁸ Bellosa, S.; Bernini, F.; Ferri, N.; Quarato, P.; Canavesi, M.; Arnaboldi, L.; Fumagalli, R.; Paoletti, R.; Corsini, A. Direct vascular effects of HMG-CoA reductase inhibitors. *Atherosclerosis* **1998**, 137 Suppl, S101-109.

⁹ Liao, J. K.; Laufs, U. Pleiotropic effects of statins. *Annu. Rev. Pharmacol. Toxicol.* **2005**, 45, 89-118.

¹⁰ Hong, H.; Zeng, J. S.; Kreulen, D. L.; Kaufman, D. I.; Chen, A. F. Atorvastatin protects against cerebral infarction via inhibition of NADPH oxidase-derived superoxide in ischemic stroke. *Am. J. Physiol. Heart Circ. Physiol.* **2006**, 291, H2210-2215.

¹¹ Maack, C.; Kartes, T.; Kilter, H.; Schafers, H. J.; Nickenig, G.; Bohm, M.; Laufs, U. Oxygen free radical release in human failing myocardium is associated with increased activity of rac1-GTPase and represents a target for statin treatment. *Circulation* **2003**, 108, 1567-1574.

¹² Wei, Y.; Zhang, Y.; Derewenda, U.; Liu, X.; Minor, W.; Nakamoto, R. K.; Somlyo, A. V.; Somlyo, A. P.; Derewenda, Z. S. Crystal structure of RhoA-GDP and its functional implications. *Nat. Struct. Biol.* **1997**, 4, 699-703.

identification of compound NSC23766, that interferes with the binding of guanine nucleotide exchange factors (GEFs) Trio and Tiam1, specific for Rac,¹⁵ thus inhibiting the exchange of GDP with GTP within the Rac active site and, consequently, its activation. Residue Trp56 of Rac1 is the critical determinant for the binding with Rac1-specific GEFs, such as Trio and Tiam1.¹⁶ By analyzing the simulated model of NSC23766 docked to Rac1, Gao *et al.* located the binding site in the surface cleft formed by residues Lys5, Val7, Asp38, Asn39, Trp56, Asp57, Thr58, Leu70, and Ser71.¹⁵ Thus, the identification of this new mode of action opened to the development of selective Rac inhibitors capable to interfere with the binding with GEFs, or even effectors such as the p67^{phox} subunit of the NADPH oxidase.¹⁷ Our group previously reported the identification, by virtual screening, of five small molecules inhibitors able to selectively inhibit Rac1 activity by interfering with the Tiam1-Rac1 protein-protein interaction (PPI), all showing higher potency and efficacy than NSC23766.¹⁸ Further investigation and structure activity relationship (SAR) analysis led to the identification of Rac1 inhibitors belonging to the class of 3-Aryl-*N*-aminoylsulfonylphenyl-1*H*-pyrazole-5-carboxamides.¹⁹ The most promising, compound 4 (Figure 1), showed selective Rac1 inhibition in cultured human aortic smooth muscle cells (SMCs) with an IC₅₀ of 8.7 μM

¹³ Hirshberg, M.; Stockley, R. W.; Dodson, G.; Webb, M. R. The crystal structure of human rac1, a member of the rho-family complexed with a GTP analogue. *Nat. Struct. Biol.* **1997**, *4*, 147-152.

¹⁴ Chen, C.; Wirth, A.; Ponimaskin, E. Cdc42: an important regulator of neuronal morphology. *Int. J. Biochem. Cell. Biol.* **2012**, *44*, 447-451.

¹⁵ Gao, Y.; Dickerson, J. B.; Guo, F.; Zheng, J.; Zheng, Y. Rational design and characterization of a Rac GTPase-specific small molecule inhibitor. *Proc. Natl. Acad. Sci. U. S. A.* **2004**, *101*, 7618-7623.

¹⁶ Gao, Y.; Xing, J.; Streuli, M.; Leto, T. L.; Zheng, Y. Trp(56) of rac1 specifies interaction with a subset of guanine nucleotide exchange factors. *J. Biol. Chem.* **2001**, *276*, 47530-47541.

¹⁷ Bosco, E. E.; Kumar, S.; Marchioni, F.; Biesiada, J.; Kordos, M.; Szczur, K.; Meller, J.; Seibel, W.; Mizrahi, A.; Pick, E.; Filippi, M. D.; Zheng, Y. Rational design of small molecule inhibitors targeting the Rac GTPase-p67(phox) signaling axis in inflammation. *Chem. Biol.* **2012**, *19*, 228-242.

¹⁸ Ferri, N.; Corsini, A.; Bottino, P.; Clerici, F.; Contini, A. Virtual screening approach for the identification of new Rac1 inhibitors. *J. Med. Chem.* **2009**, *52*, 4087-4090.

¹⁹ Ferri, N.; Bernini, S. K.; Corsini, A.; Clerici, F.; Erba, E.; Stragliotto, S.; Contini, A. 3-Aryl-*N*-aminoylsulfonylphenyl-1*H*-pyrazole-5-carboxamides: a new class of selective Rac inhibitors. *Med. Chem. Commun.* **2013**, *4*, 537-541.

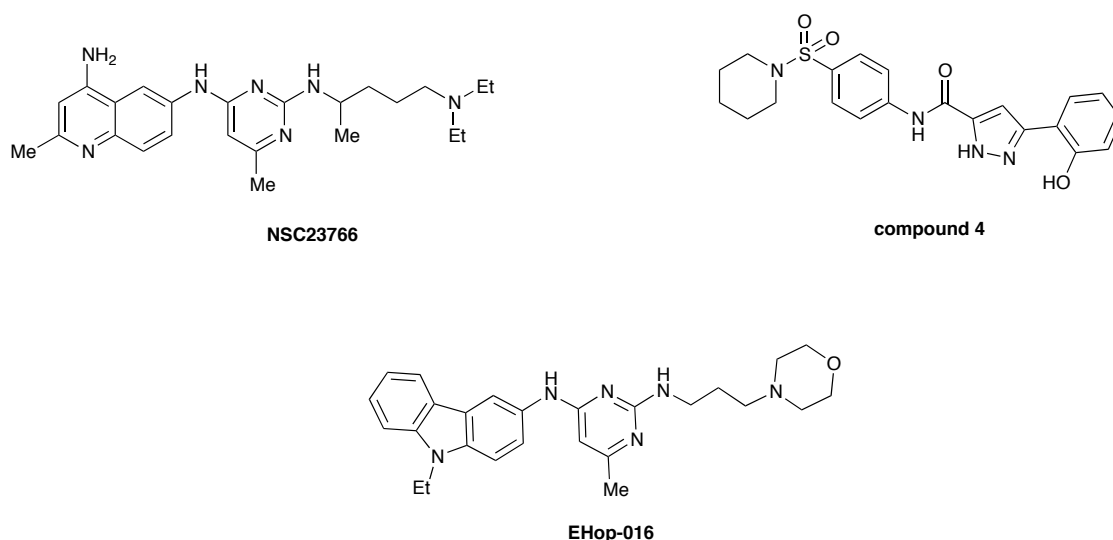


Figure 5.1 Selected Rac1-Tiam1 PPI inhibitors previously reported.

Differently, SAR analysis and chemical optimization of NSC23766 led to derivatives that were shown to inhibit Rac1 activity of cancer cells with higher efficiency (20-50% more) than NSC23766 itself and,²⁰ successively, to compound EHOp-016 (Figure 1) which inhibits Rac1 activity in MDA-MB-435 cells with an IC_{50} of 1.1 μ M,²¹ whereas the IC_{50} for NSC23766 in the same cell line is 95 μ M. Aiming in prosecuting our research for Rac1 inhibitors and considering our interest in computational methods for drug discovery,^{18 19 22 23 24 25 26} we decided to attempt a *de-novo* design of a chemically diverse lead compound, starting from the previously described model of the complex between Rac1 and NSC23766.¹⁸ Compounds based on the 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate scaffold were then designed, synthesized and pharmacologically tested. By using G-LISA assay on SMCs, we demonstrated that compound AR-148

²⁰ Hernandez, E.; De La Mota-Peynado, A.; Dharmawardhane, S.; Vlaar, C. P. Novel inhibitors of Rac1 in metastatic breast cancer. *P. R. Health Sci. J.* **2010**, 29, 348-356.

²¹ Montalvo-Ortiz, B. L.; Castillo-Pichardo, L.; Hernández, E.; Humphries-Bickley, T.; De La Mota-Peynado, A.; Cubano, L. A.; Vlaar, C. P.; Dharmawardhane, S. Characterization of EHOp-016, novel small molecule inhibitor of Rac GTPase. *J. Biol. Chem.* **2012**, 287, 13228-13238.

²² Maffucci, I.; Contini, A. Explicit Ligand Hydration Shells Improve the Correlation between MM-PB/GBSA Binding Energies and Experimental Activities. *J. Chem. Theory Comput.* **2013**, 9, 2706-2717.

²³ Casoni, A.; Clerici, F.; Contini, A. Molecular Dynamic Simulation of mGluR5 Amino Terminal Domain: Essential Dynamics Analysis Captures the Agonist or Antagonist Behaviour of Ligands. *J. Mol. Graph. Model.* **2013**, 41, 72

²⁴ Conforti, P.; Zuccato, C.; Gaudenzi, G.; Ieraci, A.; Camnasio, S.; Buckley, N. J.; Mutti, C.; Cotelli, F.; Contini, A.; Cattaneo, E. Binding of the repressor complex REST-mSIN3b by small molecules restores neuronal gene transcription in Huntington's disease models. *J. Neurochem.* **2013**, 127, 22-35.

²⁵ Contini, A.; Cappelletti, G.; Cartelli, D.; Fontana, G.; Gelmi, M. L. Molecular Dynamics and Tubulin Polymerization Kinetics Study on 1,14-Heterofused Taxanes: Evidences of Stabilization of the Tubulin Head-to-Tail Dimer-Dimer Interaction. *Mol. BioSyst.* **2012**, 8, 3254.

²⁶ Ferri, N.; Radice, T.; Antonino, M.; Beccalli, E. M.; Tinelli, S.; Zunino, F.; Corsini, A.; Pratesi, G.; Ragg, E. M.; Gelmi, M. L.; Contini, A. Synthesis, structural, and biological evaluation of bis-heteroarylmalesimides and bis-heterofused imides. *Bioorg. Med. Chem.* **2011**, 19, 5291.

selectively and potently ($IC_{50} = 2.5 \mu M$) inhibits Rac without interfering with RhoA, affecting cell migration in response to the chemotactic agent platelet derived growth factor BB (PDGF-BB).

5.2 Molecular design

A molecular surface analysis of Rac1 complexed with NSC23766 (Figure 2) showed that the binding site can be represented as an elongated bowl having an hydrophobic edge, formed by Trp56, Val36, Ala59, Tyr64, Leu67, Leu70 and Pro73, and an hydrophilic bottom comprising Thr58, Ser71, Lys5 and Gln74. The ligand lies in this bowl, anchored to the hydrophilic bottom by H-bonds between the NH at C2 and C6 of the pyrimidine ring with Leu70 carbonyl and Ser71 side chain, respectively. The pentyl-1,4-diamino chain is sandwiched between Val36 and Leu67, while the two aromatic moieties make hydrophobic contacts with Trp56. A π /cation interaction is also observed between the aminoquinoline group and Lys5 ammonium. From this analysis, is clear that the binding site is only partially filled by NSC23766, in particular at the hydrophobic region delimited by Val36, Ala59, Tyr64, Leu67 and Leu70. We recently reported the synthesis of a series of constrained α,α -disubstituted amino acids based on the norbornene ring (Figure 2).²⁷

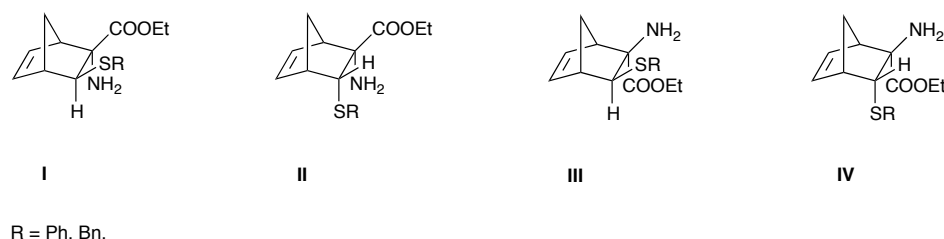


Figure 5.2 Norbornene amino esters selected as putative scaffolds for the design of Rac1-GEF protein-protein interaction inhibitors.

Due to the rigid nucleus, the α -aminocarboxylate group which provides H-bond donor and acceptor capabilities and the presence of the C=C bond, that could be easily functionalized, those structures were selected as the core scaffold for the design of novel Rac1 inhibitors. Preliminary docking studies, performed for norbornenes *I-IV* with MOE software,²⁸ showed that a top ranked pose of compound *I* was oriented in order to fill the hydrophobic pockets formed by Trp56-Val36 and Val36-Ala59-Tyr64-Leu67 with the

²⁷ Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Soave, R.; Clerici, F. Sulfanyl-methylene-5(4H)-oxazolones and β -sulfanyl- α -nitroacrylates as appealing dienophiles for the synthesis of conformationally constrained cysteine analogues. *Tetrahedron*. **2012**, 68, 1951-1962.

²⁸ Molecular Operating Environment (MOE), 2012.10, Chemical Computing Group Inc.: 1010 Sherbooke St. West, Suite #910, Montreal, QC, Canada, H3A 2R7, , 2012.

ethoxycarbonyl and phenylsulfanyl substituents, respectively, while the ammonium group interacted with Ser71 through an H-bond with the side chain hydroxyl (Figure 3).

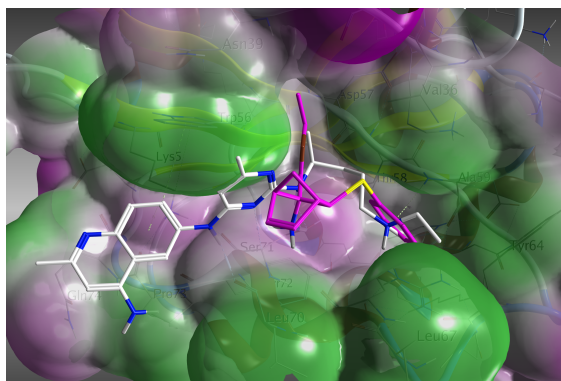


Figure 5.3 Molecular interaction surface of Rac1-NSC23766 complex (carbon colored in white). Lipophilic and hydrophilic portions are colored in green and magenta, respectively. Docked compound *I* is also shown (carbon colored in magenta).

The norbornene core was placed approximately in the middle of the binding site, with the double bond facing the region delimited by Trp56, Leu70 and Gln74, where the diarylamino moiety of NSC23766 was found in the crystal structure.²⁹ The decoration of scaffold *I* by an aromatic group, achievable through Pd chemistry, was then evaluated through the LigX function of MOE,²⁸ which allows to interactively calculate some ligand properties (e.g. predicted logP, logS and binding affinity) while modifying the structure within the binding site. The geometry optimization of the Rac1-NSC23766 complex within the LigX environment resulted in a binding affinity of -5.5 kcal/mol and a predicted logP of 3.5. The same calculation, conducted on the Rac1-norbornene *I* complex obtained by docking, predicted a binding affinity of -5.4 kcal/mol and a logP of 1.9 (Figure 4).

²⁹ Zheng, Y.; Nassar, N.; Skowronek, K. R. GTPase Inhibitors and Methods of Use and Crystal Structure of Rac1 GTPase. WO2007/016539, 2007.

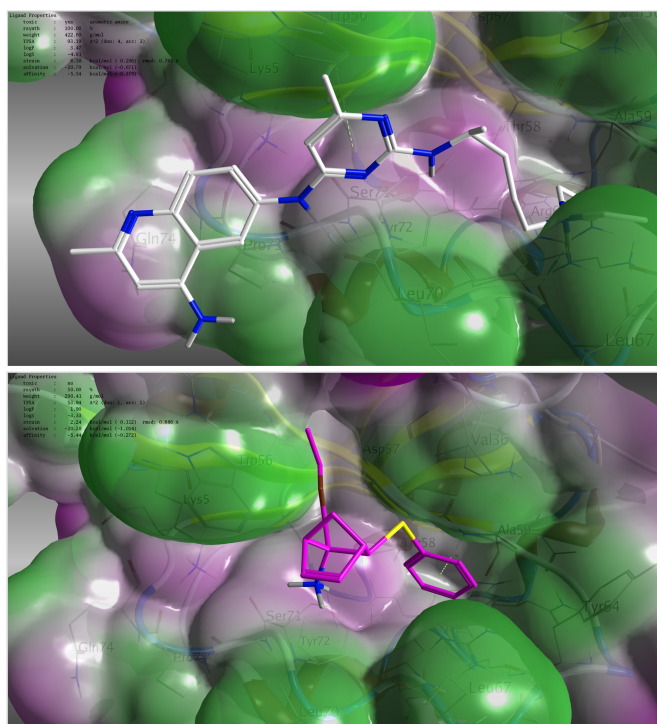


Figure 5.4 Geometry optimization and ligand properties evaluation of NSC23766 (top) and norbornene *1* (bottom) within the LigX environment of MOE.

The substitution at C5 by (4-aminophenyl)phenylamine group, chosen for its synthetic accessibility, suitable steric hindrance and presence of an H-bond donor, potentially capable of interactions with Leu70, led to compound AR-129 which, after geometry optimization, showed a predicted binding affinity of -5.7 kcal/mol and a logP of 4.8. The H-bond between the ammonium group at C2 and Ser71, observed for the unsubstituted scaffold I, was lost while two H-bonds between the secondary and primary amino groups of the diarylamino substituent with Leu70 and Gln74, respectively, were observed (Figure 5).

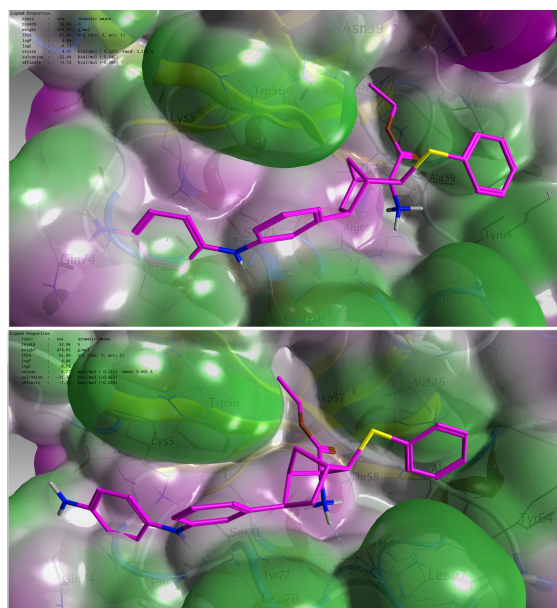


Figure 5.5 Geometry optimization and ligand properties evaluation of AR-129 (top) and AR-148 (bottom) within the LigX environment of MOE.

On the other hand, substitution by the (4-aminophenyl)phenylamine group at C6 provided a relevant improvement in predicted binding affinity (-7.4 kcal/mol) and led to compound AR-148 (Figure 6).

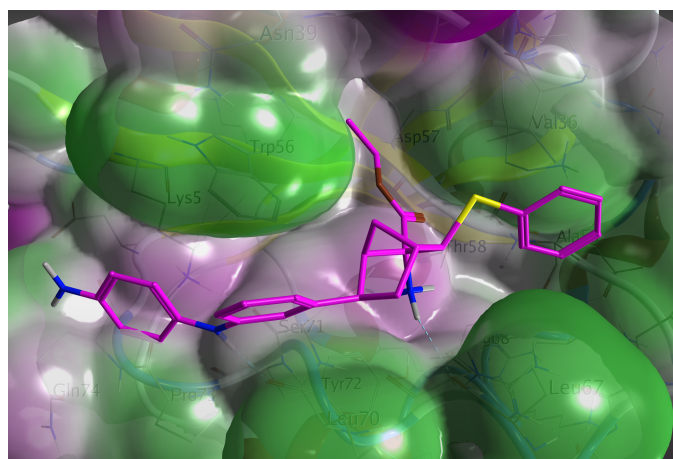


Figure 5.6 Compound AR-148 designed through iterative structural modification of norbornene amino ester *I* within the LigX environment of the software MOE. H-bonds can be observed between the ammonium group at C2 and both Ser71 and Arg68, and between the diarylamino group and Leu70.

In this case, both the H-bonds with Leu70 and Ser71 observed for NSC23766 were maintained and an additional interaction was found between the ammonium group at C2 and Arg68. Although only scaffold *I*, where the ethoxycarbonyl, phenylsulfanyl and methylene groups have a *cis* configuration, was considered in molecular design, the synthesis and evaluation of alternative stereo- and regioisomers (Figure 7) was also planned.

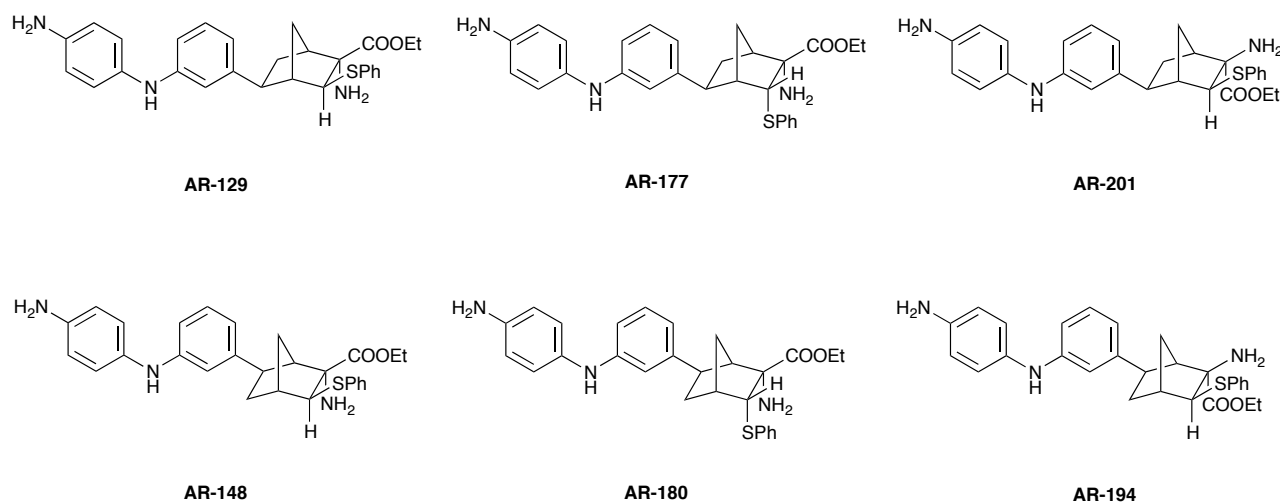


Figure 5.7 Synthesized compounds of the AR series.

5.3 Synthesis of designed compounds

5.3.1 Retrosynthetic analysis

Retrosynthetic analysis evidenced two main features: the norbornane amino ester scaffold and the substituted diphenylamino moiety linked to the C5-C6 norbornane bond (figure 6). We consider the exploiting our synthetic experience in the field of cycloaddition reactions^{30 31 32 33} and Pd-catalyzed reactions,^{34 35} Base on them we analysed and performed different retrosynthetic and synthetic approach. With our strong experience on azalactones and lactones chemistry the original plan was to obtain the norbornene core throughout a cycloaddition reaction between cyclopentadiene and azalacton depicted in figure 8 and afterwards functionalize norbornene at C5 or C6 through a Pd-catalyzed hydroarylation, followed by a Buchwald amination. The reduction of nitrogroup and amide hydrolysis would have to provide the desired product. As alternative to azalacton chemistry we also considered the use of different dienophile like as nitroacrilate that

³⁰ Clerici, F.; Gelmi, M. L.; Pellegrino, S.; Pilati, T. 3-Formylcyclopent-3-enyl- and 3-Carboxycyclopentylglycine Derivatives: A New Stereocontrolled Approach via Retro-aldol or Retro-Claisen Reactions. *J. Org. Chem.* **2003**, 68, 5286-5291.

³¹ Caputo, F.; Clerici, F.; Gelmi, M. L.; Pellegrino, S.; Pilati, T. Enantioselective synthesis of epimeric cis-3-carboxycyclopentylglycines. *Tetrahedron: Asymmetry* **2006**, 17, 61-67.

³² Caputo, F.; Clerici, F.; Gelmi, M. L.; Pellegrino, S.; Pocar, D. An efficient synthesis of new diastereomeric enantiopure 1-aminocyclopentane-1,2,4-tricarboxylic acids. *Tetrahedron*; **2006**, 17, 1430-1436.

³³ Caputo, F.; Clerici, F.; Gelmi, M. L.; Nava, D.; Pellegrino, S. Uncatalyzed solventless Diels–Alder reaction of 2-amino-3-nitroacrylate: synthesis of new epimeric 2-amino-3-nitro-norbornene- and norbornane-2-carboxylic acids. *Tetrahedron* **2006**, 62, 1288-1294.

³⁴ Ruffoni, A.; Casoni, A.; Gelmi, M. L.; Pellegrino, S.; Clerici, F. Hydroarylation of substituted norbornene amino acids: studies on long-range stereo-electronic effects on the regioselectivity of the addition. *Curr. Org. Chem.* **2012**, 16, 2724-2738.

³⁵ Beccalli, E. M.; Broggin, G.; Clerici, F.; Galli, S.; Kammerer, C.; Rigamonti, M.; Sottocornola, S. Palladium-Catalyzed Domino Carbopalladation/5-exo-Allylic Amination of α -Amino Allenamides: An Efficient Entry to Enantiopure Imidazolidinones. *Org. Lett.* **2009**, 11, 1563-1566.

are well known and use in cycloaddition reaction with cyclopentadiene³⁶. After that the same retrosynthetic scheme would have be followed and it will provide a precursor with two nitrogroups instead of Benzyl amide. Both nitro precursor could be easily reduced to the desired products in a single steps avoiding hydrolytic reaction. We consider also a not linear synthesis but a convergent approach, is well know how in the synthesis of complex molecules this choice gives general better yield than a long linear synthesis. So we consider the possibility to synthesized before the biphenylamine functionality and after attach them to the norbornene scaffold. With this approach at first we also hypnotized the possibility that those steric hindrance moiety could achieve a sort of selectivity in the Heck hydroarylation steps.

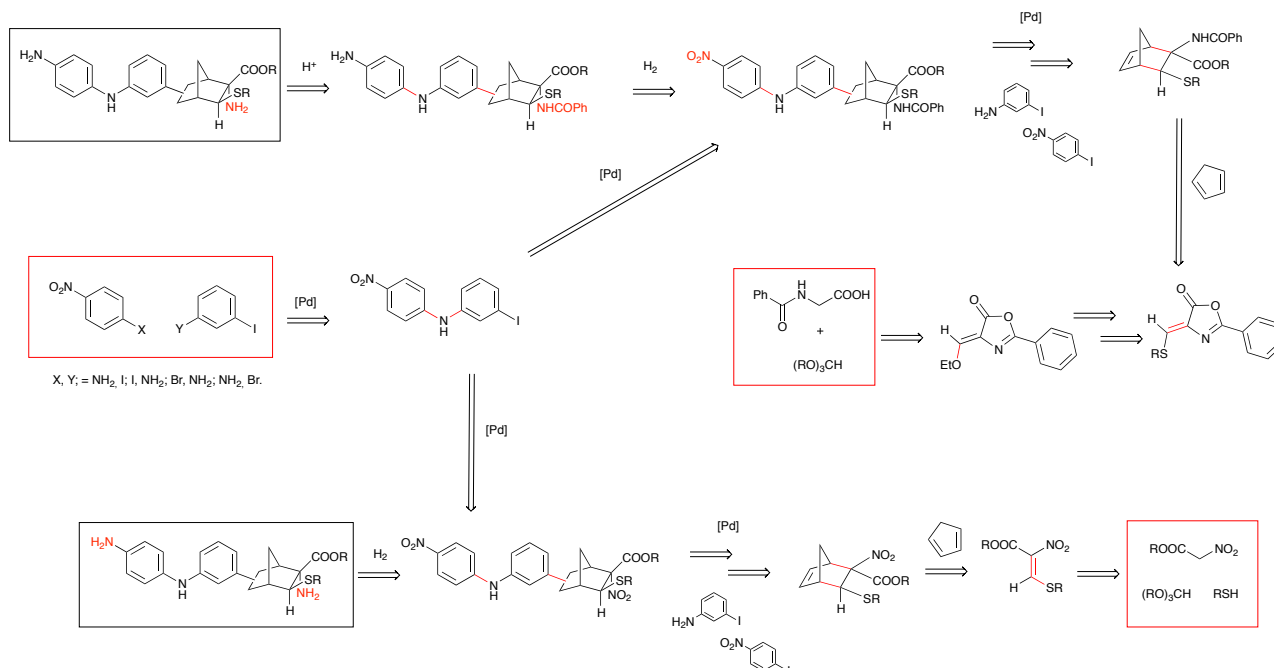


Figure 5.8 Retrosynthetic schemes for the preparation of compounds of the AR series.

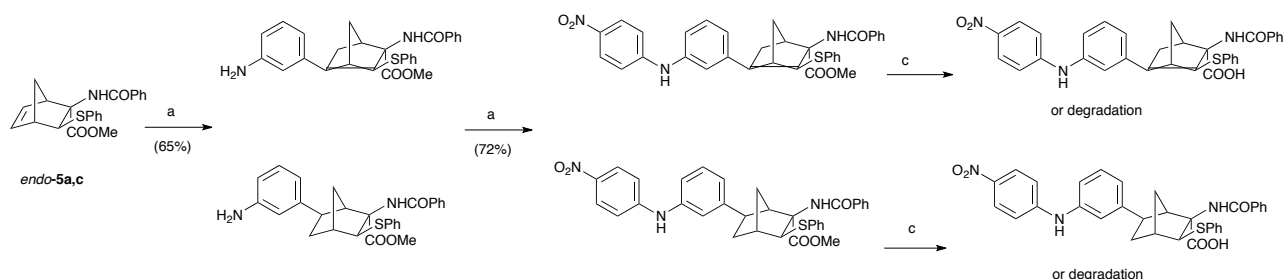
5.3.2 Convergent synthesis

The cyclo addition reaction between S substituted azalacton and cyclopentadiene was extensively described in chapter number 2. Heck reaction and Buchwald reaction was firstly developed using 3-bromoaniline and 4-bromonitrophenol on the cycloadducts *endo*-5a reported in chapter 2 with obtaining the desire compounds with satisfactory yield. (Scheme 1). Unfortunately the impossibility to accomplish the hydrolysis steps *exo*-5a and *endo*-5a (scheme 2.2, chapter 2) the hydrolytic step that did not furnish the corresponding the desire

³⁶ Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Soave, R.; Clerici, F. Sulfanyl-methylene-5(4H)-oxazolones and β -sulfanyl- α -nitroacrylates as appealing dienophiles for the synthesis of conformationally constrained cysteine analogues. *Tetrahedron*. **2012**, 68, 1951-1962.

aminoacids due to addition of HCl in chapter 2 also as finally steps of the synthesis of Buchwald derivative give no result. The extremely masked amide required such strong conditions that at this point the entire molecule was meeting degradation. We consider an alternative strategy that permit to avoid the use of benzylamide as precursor of amine function.

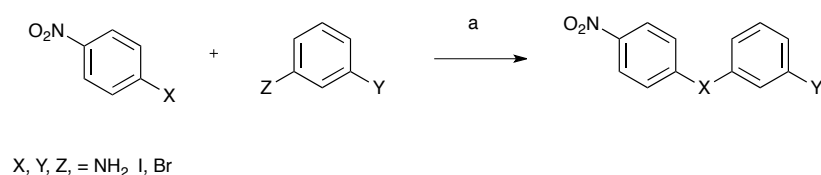
Scheme 5.1



Reagent and conditions: 3-Bromoaniline 3 eq., Pd(OAc)₂, TEA, PPh₃, HCOOH, CH₃CN, reflux, (b) 4-bromo-nitrobenzene 1.5 eq., Pd(AcOEt), BINAP, 110 °C M.W., Cs₂CO₃, CH₃CN. (d) different acids condition tested, HCl (6N, 3N, 37%), HBr .

Regarding the convergent approach the biphenyl amino moiety describe in the retrosynthetic analysis was obtained using a buchwald reaction in alternative approach form those describe in patent. The yield were not satisfactory enough to divert for a convergent strategy.(Table 1). Moreover their synthesis were always affected by derivative of polymerization (Scheme 2)

Scheme 5.2 Biphenyl amine synthesis



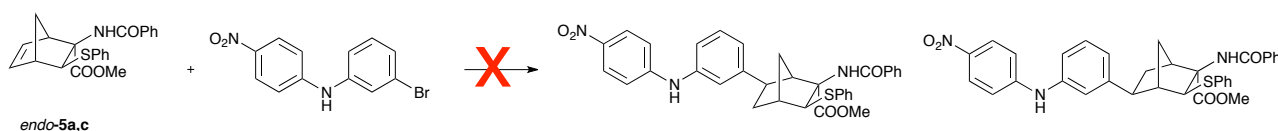
Reagent condition listed in the table 1

<i>p</i> -iodonitrobenzene 1	<i>m</i> -bromoanilina 1	Pd(OAc) ₂ /BINAP/Cs ₂ CO ₃ , MeCN 25°C	10%
1 3	3 1	Pd(OAc) ₂ /BINAP/Cs ₂ CO ₃ , MeCN 40°C	24% 25%
<i>p</i> -nitroanilina 1	<i>m</i> -bromiodobenzene 2	Pd ₂ (dba) ₃ BdtBufosfina/KO ^t But, toluene 110°	38
1	2	Pd(OAc) ₂ /BINAP/Cs ₂ CO ₃ , toluene 110°C	40

1	1	Pd(tetrakis), Cs ₂ CO ₃ , toluene 110°C	56%
1	2	Pd(OAc) ₂ /BINAP/KOtBut, MeCN 85°C	-
1	2	Pd(tetrakis), Cs ₂ CO ₃ , MeCN, 90°C	-
<i>p</i> -iodonitrobenzene 1	<i>m</i> -iodoaniline 1	Pd(OAc) ₂ /BINAP/ Cs ₂ CO ₃ , toluene 25°C	-
1	2	Pd(OAc) ₂ /BINAP/ Cs ₂ CO ₃ , toluene 110°C	-
1	2	Pd(OAc) ₂ /BINAP/ Cs ₂ CO ₃ , toluene 90°C 1h μ W	-
<i>p</i> -nitroaniline	<i>m</i> -diiodobenzene	Pd(OAc) ₂ /BINAP/ Cs ₂ CO ₃ , MeCN, 90°C	10%
1	1	Pd(tetrakis), Cs ₂ CO ₃ , MeCN, 90°C	-

Despite this limitation also the Heck reaction between the norbornene and our biphenyl moiety never give positive results. The formations of the desire product was never observed. (Scheme 2) A deeper explanation of hypothesised reason of this failure will be given further in the paragraph dedicated to the phosphine free heck hydroarylation. (Scheme 3)

Scheme 5.3 Heck hydroarylation on bipheliamine derivative



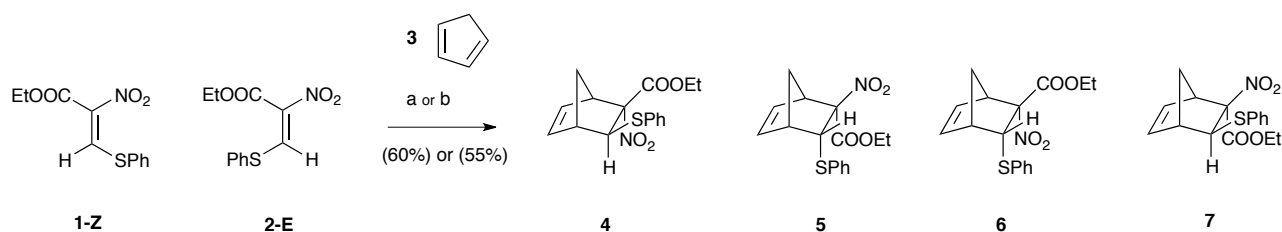
Full screening of condition was done without result.

5.3.3 Nitroacrylate strategy

5.3.3.1 Cycloaddition reaction.

The Diels-Alder reaction between cyclopentadiene and a mixture of 1-*Z* and 2-*E* β -sulfanyl- α -nitroacrylates was performed by using ultrasounds in neat conditions.²⁷ Cycloadducts 4-7 were then isolated by column chromatography in two fractions, the first containing compounds 4 (37.5% of the crude) and 5 (2.6% of the crude), the second 6 (53.5% of the crude) and 7 (6.4% of the crude) (Scheme 4).

Scheme 5.4 Diels-Alder cycloaddition reaction for the preparation of the norbornene scaffold.



Reagents and conditions: (a) cyclopentadiene 5 eq., 50 °C, 24h,), (60%); (b) 10 grams scale: cyclopentadiene 1 eq x 5 every 4 h, 50 °C, 24h,), (55%).

Unfortunately, attempts to enhance diastereoselectivity tested so far failed. Moreover, the use of enriched mixture of 2-*E* as the dienophile did not allow the selective formation of products 6 and 7. This behavior could be reasonably explained by an asynchronous mechanism governing the cycloaddition,²⁷ but we could not exclude a possible competing pathway in which the nitroacrylate 1-*E* could function as the diene. (Figure 9)^{37 38}

³⁷ Xu, H.; Wade, P. A.; Sohlberg, K. Formation and sigmatropic rearrangement of $\text{PhCOC}(\text{NO}_2)\text{CH}_2$ cycloadducts of 1,3-cyclohexadiene: a theoretical study. *Tetrahedron* **2010**, 66, 845-851.

³⁸ Jasiński, R.; Kwiatkowska, M.; Sharnin, V.; Barański, A. Experimental and theoretical studies of Diels-Alder reaction between methyl (Z)-2-nitro-3-(4-nitrophenyl)-2-propenoate and cyclopentadiene. *Monatsh Chem* **2013**, 144, 327-335.

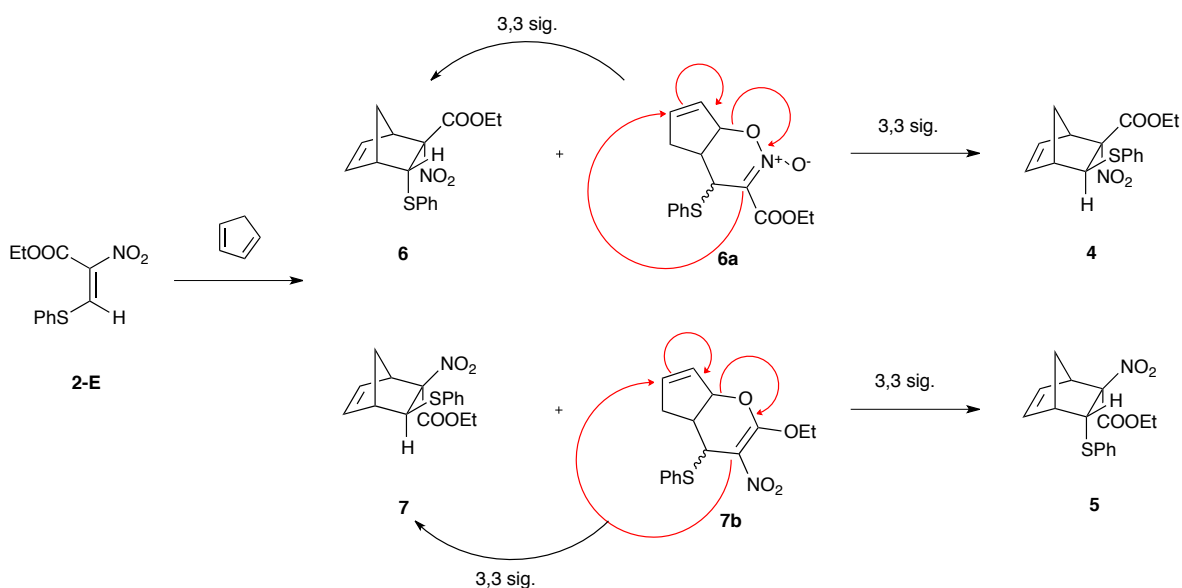
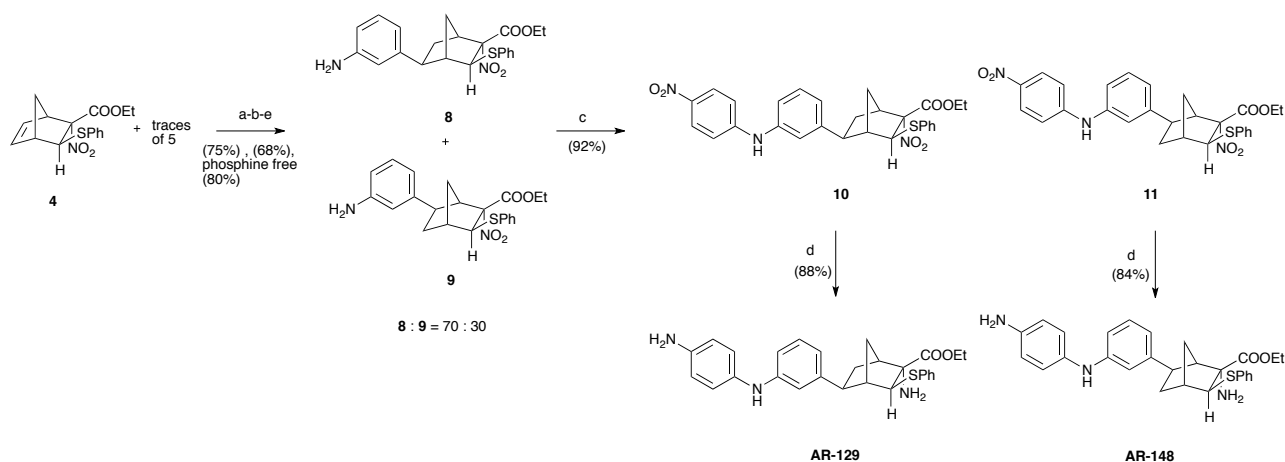


Figure 5.9 Two possible competing pathways affording 6,7,4,5 The acrylate **2-E** could function as the diene reacting with cyclopentadiene affording the adducts **6a** and **7b** which, under the reaction conditions adopted, underwent a [3,3]-sigmatropic rearrangement affording, together with **4** and **5**, a detectable amount of **2** and **3**-

5.3.3.2 Heck hydroarylation and Buchwald hydroamination.

According to the retrosynthetic scheme and operating on the diastereoisomeric mixture of **4** and **6**, we functionalized the C5-C6 double bond by means of a Pd-catalyzed hydroarylation with 3-iodoaniline in the presence of Pd(OAc)₂/Ph₃P/TEA/HCOOH in MeCN at reflux (Scheme 5). Only compounds **8** and **9** were obtained in satisfactory yields (75%) and were identified as the two regioisomers deriving from the hydroarylation of **4** at C5 or C6, respectively, with the coupling at C5 being favorite.³⁴ The stereo- and regiochemistry were assigned by NMR (Figure S4, Supporting Information) and by comparison with similar compounds previously reported.³⁴ The main difference between the two regioisomers appeared to be the chemical shift of hydrogens at C3, C5 and C6 (compound **9**: H3, d, 4.52 δ (*J* = 3.0 Hz); H6, t, 2.63 δ, (*J* = 4.1 Hz); H5a, s, 2.13 δ; H5b, s, 2.04; compound **8**: H3, d, 4.57 δ, (*J* = 3.0 Hz); H5, m, 2.90 δ; H6a, s, 1.93 δ; H6b, m, 1.75 δ). The adduct **5** was present in the mixture only as traces and, as a consequence, we were unable to recover and purify the corresponding hydroarylation product.

Scheme 5.5 Synthetic scheme for the preparation of compounds AR-129 and AR-148.



Reagents and conditions: (a) 3-iodoaniline 3 eq., Pd(OAc)₂, TEA, PPh₃, HCOOH, CH₃CN, reflux, (75%) (b) 2 g scale: 3-iodoaniline 1 eq x 5 every 8 hours., Pd(OAc)₂ 0.025 eq x 2 every 16 hours, TEA, PPh₃, HCOOH, CH₃CN, reflux, (68%); (c) 4-iodonitrobenzene 1.5 eq., Pd(tetrakis), 40 °C, Cs₂CO₃, CH₃CN. Yield for 2 g scale: 85%; (d) Zn, HCl 3N, THF, 50 °C, Yield for 1 gram scale: 82%. e) **Phosphine free methodology** 3-iodoaniline 2 eq. x 3, Pd(OAc)₂, TEA, HCOOH, CH₃CN, 60 °C, **2 gram scale**,

The following step consisted in the Buchwald reaction of the arylated norbornanes **8** and **9** with a *p*-nitrohalobenzene. Operating with *p*-bromonitrobenzene, Pd(OAc)₂, BINAP, Cs₂CO₃ in CH₃CN at reflux for 24 h, an inseparable mixture of the two products **10** and **11** (50% yield) was obtained. We tested several reaction conditions, ligands, solvents and catalysts (*e.g.*: Pd(OAc)₂/BINAP/TEA, in MeCN, 90 °C; Pd(dBA)₃/Ph₂tButPhos, *t*ButOK, toluene, 110 °C, 48 h; Pd(dBA)₃/Ph₂tButPhos, *t*ButOK, toluene, μ W, 110 °C, 3 h) and the best results were obtained by performing the reaction with *p*-iodonitrobenzene Pd(tetrakis), Cs₂CO₃, in MeCN at 40 °C for 24 h. By operating in such conditions, the Buchwald products were obtained in high yields (92%) and easily separated by chromatography, while the formation of the diarylamines, often observed as by-products,³⁹ was avoided. The final reduction of nitro groups was performed by using Zn/HCl 3 N at 50 °C, thus affording compounds AR-129 and AR-148. A different behavior was observed for **6** and **7**, which afforded an inseparable mixture of the four possible arylation products in very low yields (15-20%). This could be due to the sequestration of Pd active species by the sulfur atom at C3, which lays in β -position with respect to the strong electron withdrawing nitro group, similarly to the sulfoxide ligation mode observed in the well-known White catalyst (Figure 10).^{40 41} Alternative stable complex that does not allow the

³⁹ Wolfe, J. P.; Buchwald, S. L. Scope and Limitations of the Pd/BINAP-Catalyzed Amination of Aryl Bromides. *J. Org. Chem.* **2000**, 65, 1144-1157.

⁴⁰ Al-Jibori, S. A.; Al-Saraj, E. G. H.; Hollingsworth, N.; Hogarth, G. Palladium(II) complexes with 2-acetyl-amino-5-mercapto-1,3,4-thiadiazolate (amta) ligands: Molecular structures of the all trans dipalladium “paddlewheel” Pd₂(μ -amta)₄ and Pd(κ 1-amta)₂(κ 2-dppe). *Polyhedron* **2012**, 44, 210-214.

continuation of catalytic cycles could be after the oxidative addition to the double bond a further sequestration by the sulfur atom in probably more stable intramolecular complex. In order that this unobserved complex is formed we have to accept a prefunctionalization with triphenylphosphine that impose an electron rich character to the palladium species.

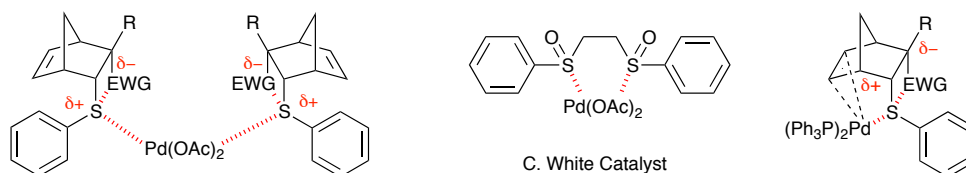
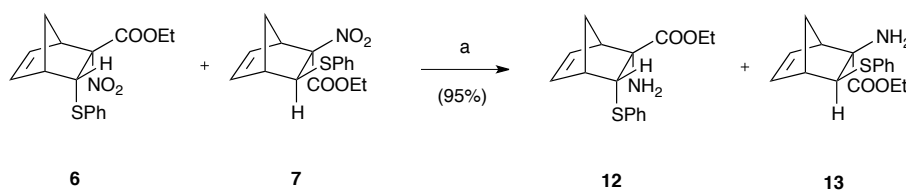


Figure 5.10 Possible interaction of Pd active species with sulfur. Comparison with White catalyst.

Moreover, we previously observed that the lack in reactivity of the Heck hydroarylation was not ascribed to the steric hindrance of the substituent in *endo* position.³³ We consequently modified the synthetic strategy by anticipating the reduction of the nitro group, followed by hydroarylation on the resulting amines. Compounds **6** and **7** were treated with Zn/H₃PO₄ 1 N at room temperature and the two corresponding amino derivatives **12** and **13** were obtained in very good yields (95%) and easily separated by column chromatography (Scheme 6).

Scheme 5.6 Zn/H₃PO₄ reduction on compounds 6 and 7.



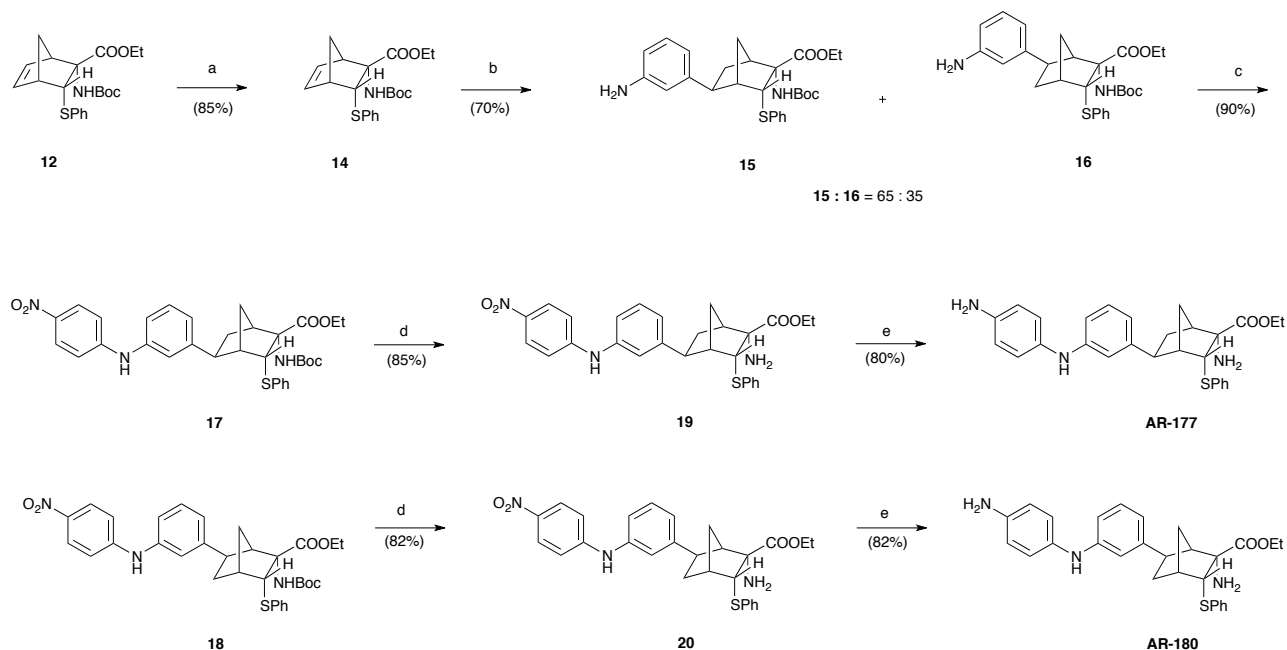
Reagents and conditions : (a) Zn, H₃PO₄ 1M, THF, r.t.

To avoid unwanted secondary reactions with iodoaniline, the free amino function in **12** was protected (Boc anhydride, TEA in DCM, 85%) and the resulting compound **14** was submitted to hydroarylation as described above. The formation of the expected products **15** and **16** in good yield (70%) further confirmed that steric hindrance is not a limiting factor in Heck hydroarylations.³⁴ The following Buchwald reaction was realized

⁴¹ Chen, M. S.; White, M. C. A Sulfoxide-Promoted, Catalytic Method for the Regioselective Synthesis of Allylic Acetates from Monosubstituted Olefins via C–H Oxidation. *J. Am. Chem. Soc.* **2004**, 126, 1346-1347.

in the conditions described above, giving rise, in high yields (93%), to **17** and **18** which were easily separated by column chromatography, deprotected (TFA, DCM, 0°C to r.t., 85%-90%) and submitted to SnCl₂ reduction of the nitro group. Compounds AR-177 and AR-180 were thus obtained in good yields (80% and 82%, respectively) (Scheme 7).

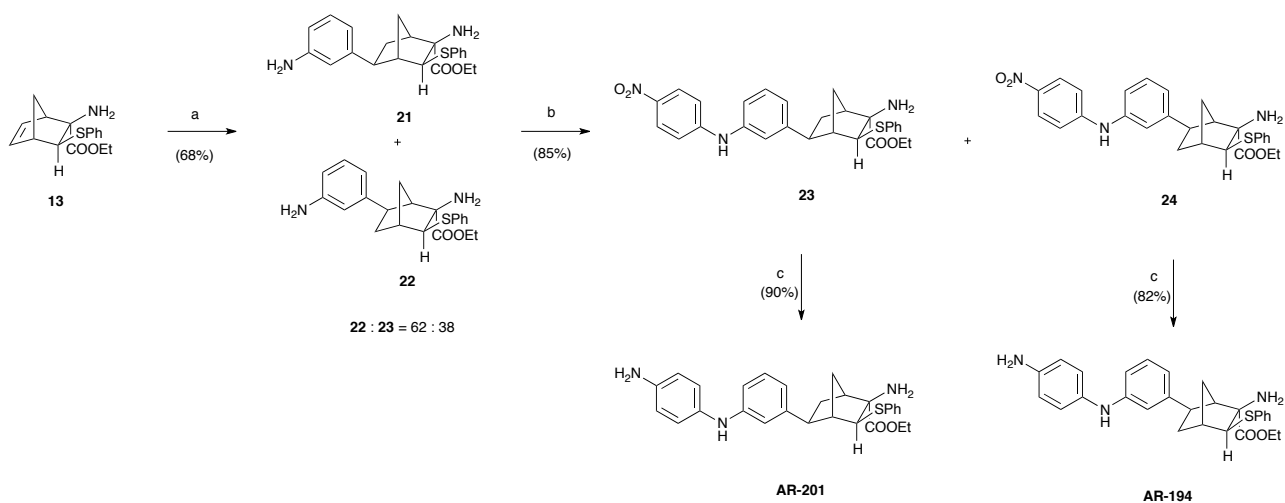
Scheme 5.7 Final steps for the preparation of AR-177 and AR-180.



Reagents and conditions: (a) Boc-anhydride, TEA, DCM, 0 °C-r.t.; (b) 3-Iodoaniline 3eq., Pd(OAc)₂, TEA, HCOOH, CH₃CN, 90 °C, Ph₃P; (c) 4-Iodonitrobenzene 1.5 eq., Pd(tetrakis), Cs₂CO₃, CH₃CN, 40 °C; (d) TFA, DCM, 0 °C to r.t.; (e) SnCl₂, MeOH, reflux.

Concerning compound **13**, we reasoned that the shielding effect of both the norbornene ring and the phenyl group could disfavor secondary reactions of the amino group with iodoaniline, so we performed the hydroarylation without Boc protection. We were delighted to observe the formation of the two expected products **21** and **22** in acceptable yields (68%). These inseparable products were submitted to the Buchwald reaction (85% yield) and the separated pure compound **23** and **24** were reduced by SnCl₂ in MeOH, as reported above, affording AR-201 and AR-194 in good yields (90% and 82%, respectively) (Scheme 8).

Scheme 5.8 Final steps for the preparation of AR-194 and AR-201.



Reagents and conditions: (a) 3-Iodoaniline 3eq., Pd(OAc)₂, TEA, HCOOH, CH₃CN, 90 °C, Ph₃P; (b) 4-Iodonitrobenzene 1.5 eq., Pd(tetrakis), Cs₂CO₃, CH₃CN, 40 °C; (c) SnCl₂, MeOH, reflux.

The structure determination of all the compounds synthesized was deduced by NOE experiment on both the distereoisomers in the case of **10** and **11** or for exclusion only on one of them as fo the compounds **18** and **23**. (Figure 11)

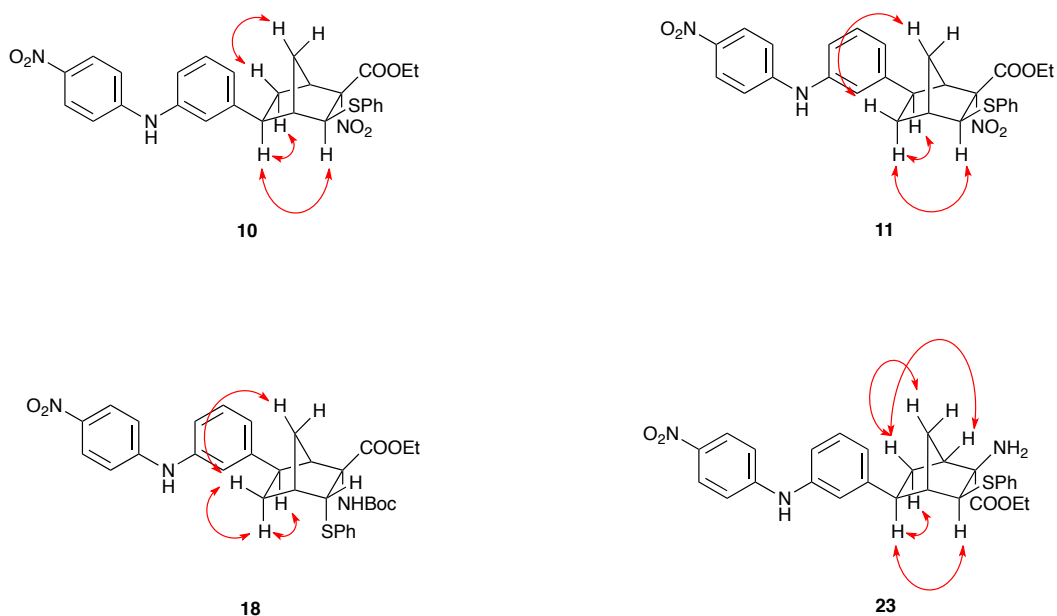


Figure 5.11 NOE signals observed for structural determination of **10**, **11**, **18** and **23**.

5.3.3.3 Scaled-up synthesis of AR-148

As a consequence of preliminary pharmacological investigations, identifying AR-148 as the most effective compound, we reconsidered its synthetic process aiming to multigram scale-up. The cycloaddition reaction was repeated on a 10 grams scale with no modifications, obtaining similar yields (55%). By using 5 eq. of 3-iodoaniline, added in rates of 1 eq. every 8 h, adding 0.025 eq. of Pd(OAc)₂ after 16 h and operating in a refluxing flask under argon, we scaled up the Heck hydroarylation, obtaining comparable yields. The two last steps were successfully scaled up without modifying the reaction conditions, thus allowing the obtainment of the desired compound AR-148 in a gram scale and with a satisfactory overall yield.

5.4 Selective and optimized synthesis of AR-148

5.4.1 Cycloaddition reaction.

In the synthesis of compounds AR the stereocenters of the compounds are formed in the Diels-Alder cycloaddition and in the Heck hydroarylation. Several conditions to modify the diastereo-outcome of the cycloaddition reaction are tested and reported in previous chapter 2. After this first attempt, different reaction conditions were tested despite in some cases the selectivity was enhanced the low yield always affected our result.

Table 2. Cycloaddition reaction of **1Z** 70% **2E** 30% and cyclopentadiene. Different conditions and results.

Entry	React. Cond. <i>T</i> (°C)	React. time h	Diene/Dienophile and Z/E Ratio	4/5/6/7 <i>ration</i>	Yield 5a %
1	Neat, Mg(ClO ₄), 0°C-25°C	24	4/1	33/5/51/11	24%
2	Neat, AlCl ₃ , 0°C-25°C	48	4/1	44/10/34/10	36%
3	UV light mercury steam	48	4/1	-	-
4	InCl ₃ , benzene, 0 °C- 25 °C	72	4/1	20/5/65/10	10%
5	CH ₂ Cl ₂ , Mg(ClO ₄) ₂	24	4/1	34/4/49/12	12%
6	neat,))) ^b , 50 °C	48	4/1	17/2/69/12	60
7	neat, oil bath, 110 °C	48	10/1	42/5/44/10	55%

^a 400 Watt; ^b Ultrasound.

Table 2. Cycloaddition reaction of **1Z** 95% **2E** 5% and cyclopentadiene. Different conditions and results.

Entry	React. Cond.	React. time	Diene/Dienophile and Z/E	4/5/6/7	Yield 5a
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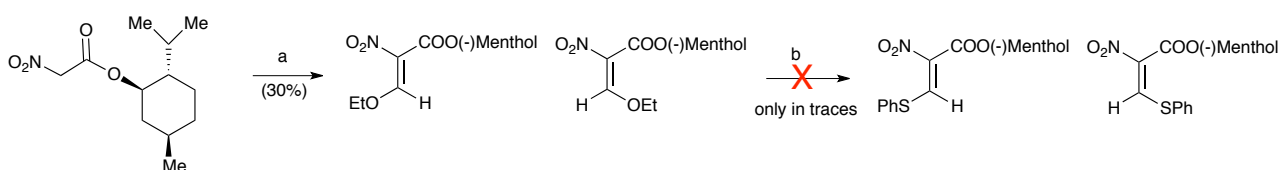
	<i>T</i> (°C)	h	Ratio	<i>ration</i>	%
1	InCl ₃ , benzene, 0 °C- 25 °C	24	5/1	-/-80/20	8%
2	InCl ₃ , neat, 0 °C- 50 °C	1	4/1	-	-
3	CH ₂ Cl ₂ , Mg(ClO ₄) ₂	1	4/1	29/6/36/29	10%
4	Benzene, 110°C	72	4/1	4/0/70/23	6%
5	neat,))) ^b , 50 °C	48	4/1	38/2/53/6	60

^a 400 Watt; ^b Ultrasound.

PyBox-Cu catalysts and high pressure cycloaddition also have failed giving complex mixtures difficult to analyse. In the first case the problem was probably related to the purification of the starting material. The presence of traces of thiol could poison metal catalysts. In the second case the wax nature of the starting material and the neat condition were difficult to manage in high pressure cycloaddition reactor.

The impossibility of ester hydrolysis in nitroderivatives that, as well-known in literature, gave decarboxylation did not permit the construction of dienophile bringing chiral Evans auxiliary. The synthesis reported in literature was not applicable to our compounds. Starting from the chiral nitroacetic ester developed in chapter 3 (Scheme 3.8) it was not possible to obtain in the condensation reaction reasonable yield of corresponding nitroacrylate deriving from (-) menthol ester. No results at all were obtained with the use of (-)-8-Ph-menthol due to its steric hindrance. A new approach to obtain diastereo- and enantioselection in the cycloaddition reaction is under study. A possible and very innovative strategy could be the exploitation of enantiopure norbornen amino ester **30** (scheme 3.11, chapter 3) as substrate of guided CH functionalization of the β -carbon. (Scheme 9)

Scheme 5.9 Chiral ester strategy



Reagent and conditions: Reagents and conditions: (a) HC(OEt)₃, 140 °C, 8h, neat; (b) HSPH, 110°C, 8h, neat, ;

5.4.2 Phosphine free Heck hydroarylation

The large number of reactions performed on the Heck hydroarylation and the unexpected results deriving from the complete failure of convergent strategy, where we never observed even in trace the desired product (Scheme 3), as well the as the low yield of the Heck reaction on compounds **6** and **7** prompted us to

bettd deeply analyse the reaction. Considering some papers appeared in the literature where norbornene is used as transient substrate and ligand (Catellani⁴² type reaction) in the reaction, we began to reasoning that also our reaction could be work without phosphines. An alternative pathway should be hypothesized never observed before in the case of olefin. Indeed, an example of phosphine-free Heck hydroarylation is already known in literature,^{43 44} but on an alkyne substrate. To our knowledge, such a reaction has never been realized on a constrained double bond. So, using the same conditions described before, but in absence of phosphine ligand (Pd(OAc)₂/TEA/HCOOH in MeCN), followed by the progressive addition of *m*-iodoaniline (1 eq x 3) we were delighted to observe that the reaction gave higher yield (80%) at lower temperature (50 °C) (Scheme5). Taking in account manly the theoretical work done by M.B. Hall^{45 46 47} on mechanism of ligand free Heck type arylation a catalytic cycle can be hypothesized as shown in Figure 12.

⁴² Catellani M, Angrew. Chem. Int. Ed. 1997,36,119-122.

⁴³ Cacchi, S.; Fabrizi, G.; Marinelli, F.; Moro, L.; Pace, P. Palladium-catalysed hydroarylation and hydrovinylation of 3,3-dialkoxy-1-aryl-1-propynes. An approach to 3-aryl- and 3-vinylquinolines. *Tetrahedron* **1996**, 52, 10225-10240.

⁴⁴ M. Ahlquist; G. Fabrizi; S. Cacchi; and Per-Ola Norrby; The Mechanism of the Phosphine-Free Palladium-Catalyzed Hydroarylation of Alkynes. *J. AM. CHEM. SOC.* **2006**, 128, 12785-1279

⁴⁵ P. Surawatanawong and Michael B. Hall. Theoretical Study of Alternative Pathways for the Heck Reaction through Dipalladium and "Ligand-Free" Palladium Intermediates *Organometallics* **2008**, 27, 6222–6232

⁴⁶ B. P. Carrow; J. F. Hartwig. Ligandless, Anionic, Arylpalladium Halide Intermediates in the Heck Reaction *J. AM. CHEM. SOC.* **2010**, 132, 79–81

⁴⁷ Huub J.,W. Henderickx; and Johannes G. de Vries. Homeopathic Ligand-Free Palladium as a Catalyst in the Heck Reaction. A Comparison with a Palladacycle. *Org. Lett.* **2003**, 5, 3285-3288

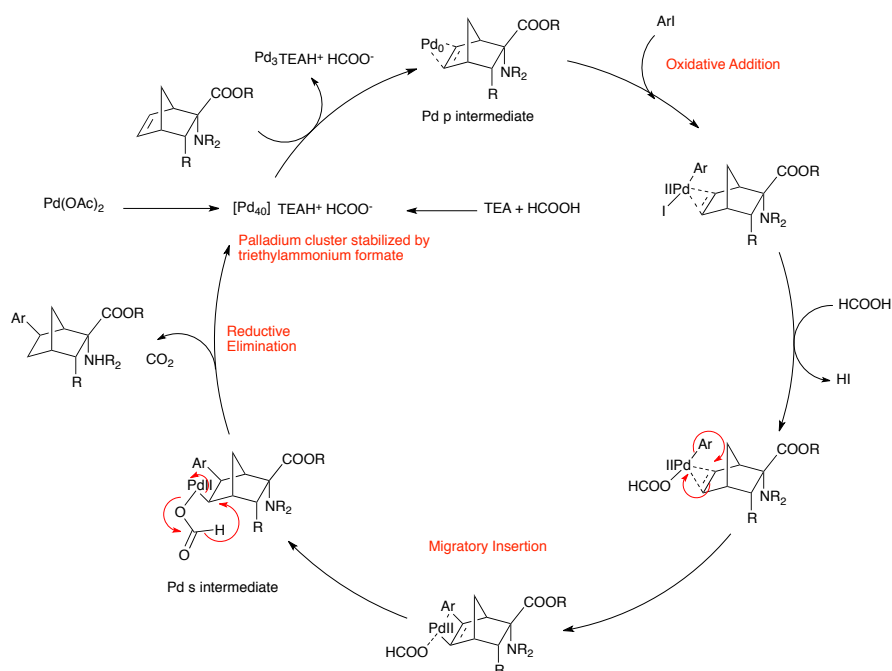


Figure 5.12 Hypothesized catalytic cycle for phosphine free Heck hydroarylation

The higher activated norbornene double bond permits the extraction of palladium from clusters, stabilized in solution by triethylammonium formate, and gives rise to the Pd π -intermediate. It has to be noted that the use of aryl iodide instead aryl bromide increased the yield, in contrast with the classical behaviour in the Heck-type reaction. It is apparent that iodine favours the access of oxidative addition on phosphine-free palladium species thanks to the known heavy atom effect. In order to be certain that the mechanism does not involve the solvent as coordinative agent, as proposed for the alkyne moiety by Cacchi et al, the reaction was performed in different solvent (DMF, DMSO, CH₃CN DCM, MeOH, THF, DCE, Et₂O) and has always led, with variation in yield, to the formation of the desired product. The good conversion obtain in THF, DCE and Et₂O strongly support our mechanistic hypothesis in contrast with the coordinative ligation operated by solvent as DMF, DMSO and CH₃CN ^{48 49 50 51}.

⁴⁸ Reactions of Rh- and Ir-bis(iminophosphoranyl)methanide compounds with electrophiles; a trapped cation-anion complex in the oxidative addition of iodine to a d⁸ metal centre and X-ray crystal structure of IrI{CH(PPh₂NC₆H₄CH₃-4)₂}(COD)II, containing two fused four-membered metallacycles. *Inorganica Chimica Acta* **1995**, 235, 77-88

⁴⁹ P. Imhoff; J. H. Gfilpen; A. L. Spek J. Elsevier ; Reactions of Rh- and Ir-bis(iminophosphoranyl)methanide compounds with electrophiles; a trapped cation-anion complex in the oxidative addition of iodine to a d⁸ metal centre and X-ray crystal structure of IrI{CH(PPh₂NC₆H₄CH₃-4)₂}(COD)II, containing two fused four-membered metallacycles. *Inorganica Chimica Acta* **1995**, 235, 77-88

⁵⁰ S.S.Bassom; J.G. Leipoldt; A. Roodt; J.A. Venter; Mechanism for the oxidative addition of Iodomethane to carbonyl(*N*-hydroxy-*N*-nitrosobenzenaminato-*O,O'*)triarylphosphinerhodium(I) complexes and crystal structure of [Rh(cupf)(CO)(CH₃)(I)(PPh₃)]; *Inorganica Chimica Acta*, **1987**, 128, 1, 31-37

5.4.3 Regioselectivity in a Heck hydroarylation, the success of Umpolung strategy

Heck hydroarylation, is the divergent point in the synthesis of AR-129 and AR-148. In order to modulate the regioselectivity of the Heck reaction in favour of AR-148,⁵² attempts with chiral phosphine reported in table 3 to change the regioselectivity of the reaction⁵³ didn't give satisfactory result on our substrate. The most promising condition tested in the previous work (chapter 4) were also tested with even worst result. The completely unchanged result with different phosphine was one of the question mark that pushed us to hypothesised the phosphine free mechanism for such reaction. (Paragraphe 5.4.2)

Table 3. Tested condition on substrate **4**

entry	Catalytic system (0.26 mmol <i>endo-1d</i>)	solvent	Total yields % (8+9)	9/8 ratio
1	Pd(OAc) ₂ /R-BINAP/HCOOH, K ₂ CO ₃ ^a	MeCN 3mL	78	30:70
2	Pd(OAc) ₂ /R- BINAP/HCOOH, TEA ^a	MeCN 3mL	65	30:70
3	Pd(OAc) ₂ /BINAP/HCOOH, CsCO ₃ ^a	MeCN 3mL	40	30:70
4	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA ^b	Toluene 30 mL	28	30:70 ^d
6	Pd(OAc) ₂ /PPh ₃ /TEA, AgNO ₃ ; HCOOH (after 24h) ^c	MeCN 3mL	15	40:60

^a Pd(OAc)₂ 0.01 mmol, BINAP 0.015 mmol, HCOOH 0.8, base 1.0 mmol; ^b Pd(OAc)₂ 0.02 mmol, PPh₃ 0.04 mmol, TEA 0.9 mmol, HCOOH 0.78 mmol, 24h, 110°C; ^c Pd(OAc)₂ 0.01 mmol, PPh₃ 0.03 mmol, HCOOH 0.8 mmol, TEA 0.9 mmol, AgNO₃ 0.4 mmol or Ag₂CO₃ 0.2 mmol. ^d

Hence basing on the consideration of Phosphine free mechanism and on our previous studies (see chapter 4) which disclosed that the regioselectivity of the hydroarylation is mainly driven by long range effect exerted by the nitro group on C2^{54 55} we decided to exploit the Umpolung⁵⁶ conversion of the nitro to the amine

⁵¹ J.C. Vickery, A.L. Balch; X-ray Crystallographic Studies of the Products of Oxidative Additions of Iodine to Cyclic Trinuclear Gold(I) Complexes: Directional Effects for Au–I··I–Au Interactions; *Inorg. Chem.* **1997**, 36, 5978-5983

⁵² P. Mayo, G. Orlova, J. D. Goddard and W. Tam; Remote Substituent Effects on the Oxymercuration of 2-Substituted Norbornenes: An Experimental and Theoretical Study. *J. Org. Chem.* **2001**, 66, 5182-5191

⁵³ K. Karabelas, C. Westerlund, and A. Hallberg The Effect of Added Silver Nitrate on the Palladium-Catalyzed Arylation of Allyltrimethylsilanes *J. Org. Chem.* **1985**, 50, 3896-3900

⁵⁴ P. Mayo and W. Tam; Palladium-catalyzed hydrophenylation of bicyclic alkenes. *Tetrahedron*, **2002**, 58, 9527–9540

⁵⁵ P. Mayo, G. Orlova, J. D. Goddard and W. Tam; Remote Substituent Effects on the Oxymercuration of 2-Substituted Norbornenes: An Experimental and Theoretical Study. *J. Org. Chem.* **2001**, 66, 5182-5191

⁵⁶ Seebach, D. Methods of Reactivity Umpolung. *Angewandte Chemie International Edition in English*. **1979**, 18, 4, 239-258

group in order to obtain an opposite long range effect and consequently an opposite regiochemistry (Figure 13)

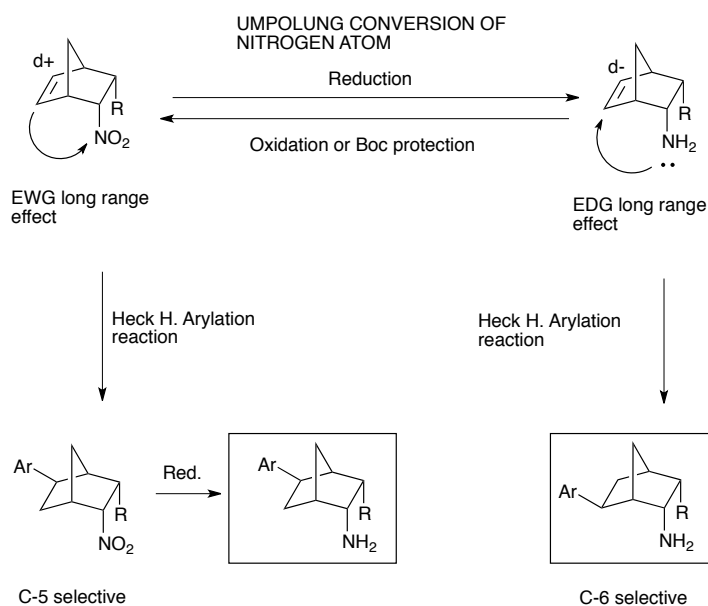


Figure 5.13 Umpolung conversion of nitro- to amino group to modulate the long range effect on the hydroarylation.

By treating the mixture of compounds **4** and **5** with $\text{Zn}/\text{H}_3\text{PO}_4$ in THF from 0 °C to r.t. We obtained the amine derivatives **25** and **26** in excellent yield (96%). The amine were than separated by chromatographic column and characterized. Therefore on amine **25** we tested different hydroarylation reaction conditions in order to avoid the aminoarylated side products. The reaction condition are reported in table 4. The optimal condition are reached using a phosphine-free protocol ($\text{Pd}(\text{OAc})_2/\text{TEA}/\text{HCOOH}$ in MeCN) and only 1.1 eq. of *m*-iodoaniline instead of 3 eq. we obtained **27** and **28** (88%, 82% for the grams scale) with the expected opposite ratio C5/C6. The two diastereoisomers was separated by flash chromatography and characterized by NMR. The main difference between the two regioisomers appeared to be the chemical shift of the hydrogens at C-3, C-5 and C-6 and allowed the evaluation of the ratio (compound **27**: H-3, d, 3.09 δ ($J = 2.9$ Hz); H-5, t, 2.93 δ , ($J = 7.7$ Hz); compound **28**: H-3, d, 3.06 δ ($J = 2.2$ Hz); H-6, t, 3.63 δ , ($J = 6.9$ Hz)). The stereo- and regiochemistry were assigned by NMR analysis of compounds **29** and **30**, confirming our previous considerations. Form the result obtained testing the Heck reaction on the new substrate we could observe that only operating without phosphine the Buchwald reaction on a norbornen free amine didn't work and permit to obtain the desired product as sigle reaction product. Moreover the elimination of phosphine from the reaction medium permit the use of 1.1 equivalent of *m*-iodoaniline instead of 3 equivalent, improving of atom economy of the process. That fact is always related to the complete deactivation of the Buchwald-

Hartwig reaction in absence of phosphine on the *m*-iodoaniline with itself on his free amine. Polymers of *m*-iodoaniline was always isolated in the other reaction conditions.

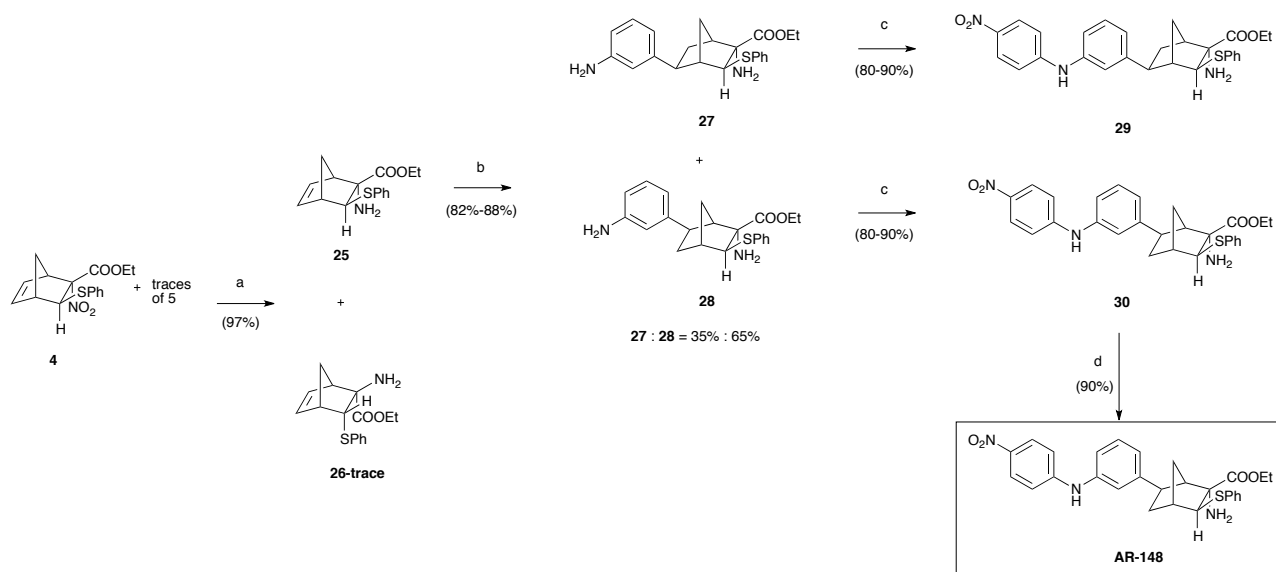
Table 3. Tested condition for selective Heck Hydroarylation

Temperature	Catalytic system (0.26 mmol <i>endo-1d</i>)	solvent	Total yields % (27+28)	27/28 ratio
1) 45-90° C	Pd(OAc) ₂ /PPh ₃ /HCOOH, TEA	MeCN 3mL	73	35:65 + side product
2) 45-90° C	Pd(OAc) ₂ /PPh ₃ /HCOOH, K ₂ CO ₃	MeCN 3mL	10	Side product manly
3) 45-90° C	Pd(OAc) ₂ /PPh ₃ /HCOOH, CsCO ₃	MeCN 3mL	58	32:68 + siade product
4) rt-45°C	Pd(OAc) ₂ /HCOOH, TEA	MeCN 3mL	88	30:70
5) 45°-90°C	Pd(OAc) ₂ /HCOOH, CsCO ₃	MeCN 3mL	30	35:65
6) rt-45°C	Pd(OAc) ₂ /TEA, AgNO ₃ ; HCOOH (after 24h) ^c	MeCN 3mL	Possible oxidation of Palladium, complex mixture	-----

Pd(OAc)₂ 0.05 eq, PPh₃ 0.1 eq mmol, BASE 3eq mmol, *m*-iodoaniline from 3 eq to 1.1 eq, HCOOH 3.5 eq, AgNO₃ 1 eq 24h,

Compound **29** and **30** was then submitted to Buchwald reaction. The optimum selectivity between aromatic and aliphatic amine was reached with the use of Pd(tetrakis), K₃PO₄ and 1.4 eq. *p*-iodonitrobenzene in MeCN at 70° C. Compound **29** and **30** were obtained in almost quantitative yields (90%, 80% for the grams scale). The use of very soft base as K₃PO₄ is needful to avoid side products on the aliphatic amine, we could hypnotise that palladium active species interact with both amine functionality but the soft basicity of K₃PO₄ is not able to allow the completion of the catalytic cycle on the aliphatic amine..

Scheme 5.10 Scaled up, phosphine-free synthesis of AR-148



^a Reagents and conditions: (a) Zn, H₃PO₄ 1M, THF, 0 °C - rt, 2 gram scale; (b) Phosphine free methodology 3-Iodoaniline 1.1 eq., Pd(OAc)₂, TEA, HCOOH, CH₃CN, 45°C, 1 gram scale; (c) 4-Iodonitrobenzene 1.1 eq., Pd(tetrakis), K₃PO₄, CH₃CN, 70°C, 1 gram scale, (d) Zn, H₃PO₄ 1M, THF, 0 °C - rt, 1 gram scale.

After the separation of the compounds by flash chromatography the reduction of the aromatic nitro group with Zinc dust and H₃PO₄ in THF from 0 °C to r.t. permitted the obtainment of AR-148 in a very efficient and regioselective way on a gram scale with out the use of any protecting group (Scheme 10). Further synthetic optimizations of the selectively obtainment of AR-148, both as a racemate and in enantiopure forms, are currently under evaluation. One of them foresee the installation of chiral carbamates on aliphatic amine to allow the separation of the two enantiomers.

5.5 Pharmacological analysis

The pharmacological inhibition of the synthesized compounds on Rac activity was studied in cultured human SMCs by determining the amount of Rac-GTP by G-LISA assay after stimulation for 2 min. with 20 ng/mL PDGF-BB.¹⁸ As shown in Figure 14, all the compounds significantly reduced the intracellular levels of Rac-GTP at 10 μ M concentration and compound AR-148 was the most effective, reducing the levels by 75% compared to control cells. At the same concentration, the compounds did not interfere with RhoA activation, demonstrating a selective action towards Rac protein. In particular, compound AR-148 selectively reduced the Rac-GTP levels in a concentration dependent-manner with an IC₅₀ value of 2.5 μ M, with no effect on RhoA (Figure 15). Considering the involvement of Rac GTPase in cell migration and lamellipodia formation, the effect of the synthesized compounds on SMC migration was investigated by Boyden chamber

chemotactic assay. PDGF-BB, a strong activator of Rac, was utilized as chemotactic agent and was shown to induce the cell migration by approximately 4 fold, compared to basal (Figure 16).

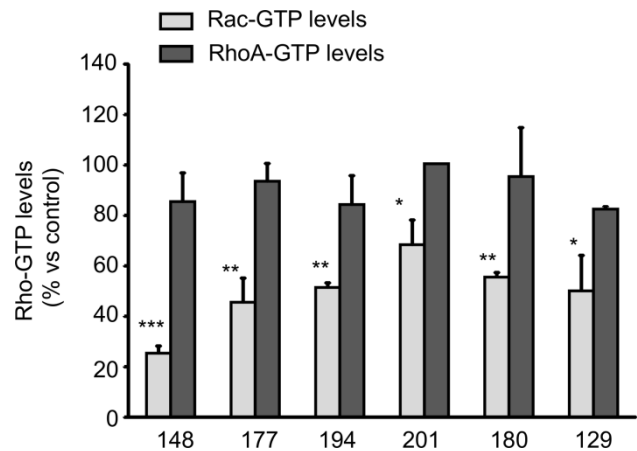


Figure 5.14 Effect of newly synthesized compounds on Rac-GTP and RhoA-GTP levels in SMCs stimulated with PDGF-BB. SMCs were seeded at a density of 2×10^5 /35 mm petri dish and incubated with DMEM supplemented with 10% FCS; 24h later the medium was changed to one containing 0.4% FCS, and the cultures were incubated for 48 h. At this time, the compounds were added to the cultured media at the final concentration of 10 μ M and after 4 h Rac and RhoA activation was induced by PDGF-BB (20ng/ml) for 2 min. Total protein extracts and G-LISA assays were then performed. The data are expressed as mean $\pm$ SD of triplicates.

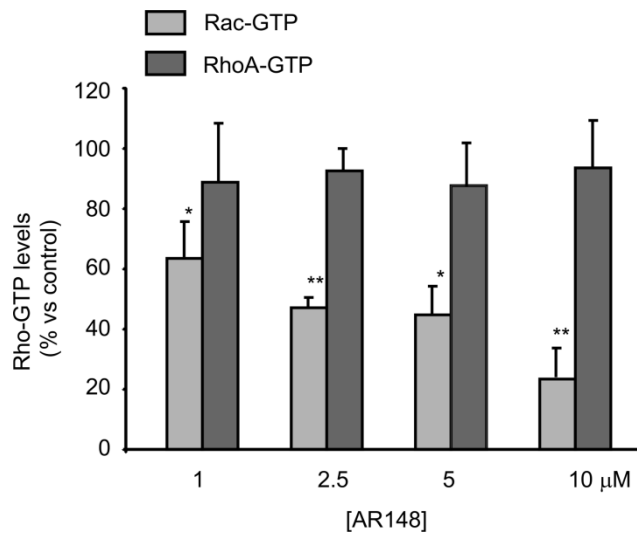


Figure 5.15 Effect of compound AR148 on Rac-GTP and RhoA-GTP levels in SMCs stimulated with PDGF-BB. The experimental conditions were the same as those described in Figure 7 with the exception that the final concentrations of compound AR-148 were 1, 2.5, 5, and 10 μ M.

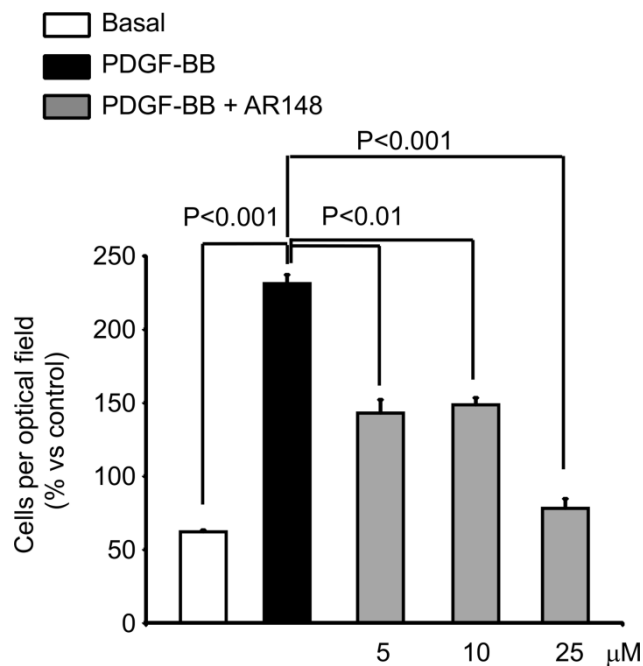


Figure 5.16 Effect of compound AR-148 on SMC migration in response to PDGF-BB. SMCs were seeded at a density of 2×10^5 /35 mm petri dish and incubated with DMEM supplemented with 10% FCS; 24h later the medium was changed to one containing 0.4% FCS, and the cultures were incubated for 48 h. At this time, compound AR-148 was added to the cultured media at the indicated final concentrations. After 4 h of incubation cells were harvested with trypsin, and cell migration determined by the Boyden chamber

The effect of compound AR-148 was also assessed on the mobility of SMCs by monitoring with a video microscope (Figure 17). During the 16 h of video microscopy analysis, PDGF-BB increased by approximately 2 fold the cell motility and this response was completely abrogated by the incubation with AR-148 at 10 μ M concentration. Taken together, the compound AR-148 was shown to selectively reduce the intracellular levels of Rac-GTP and the cell migration, an event dependent by its activity.

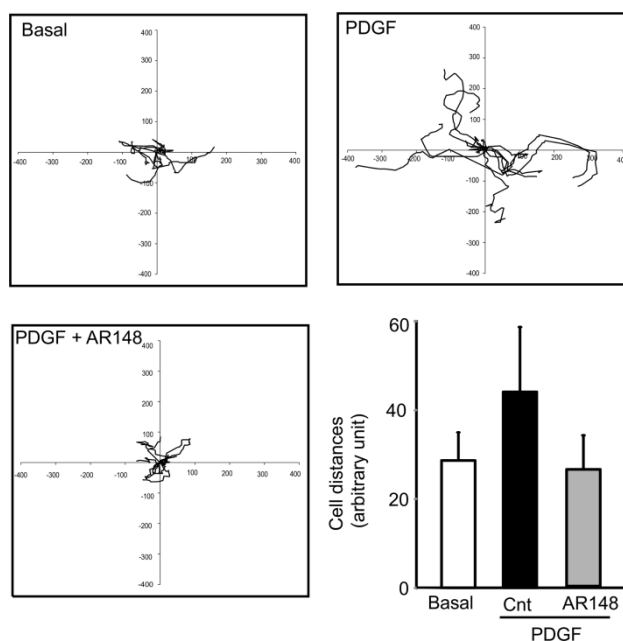


Figure 5.17 Effect of compound AR-148 on SMC motility in response to PDGF-BB. SMCs were seeded at a density of 2×10^4 of 24 well tray and incubated with DMEM supplemented with 10% FCS; 24 h later the medium was changed to one containing 0.4% FCS, and the cultures were incubated for 48 h. At this time, compound AR-148 was added to the cultured media at the final concentration of $10 \mu\text{M}$ in the presence of PDGF-BB (20 ng/ml). Cell movement was determined by video microscopy analysis and the distance covered by 10 different cells in 16 h was measured for each condition. The data are expressed as mean $\pm$ SD.

5.6 Conclusions and further development

In this work we demonstrated how the 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate nucleus might be used as a scaffold to design and prepare Rac1 ligands acting as effective PPI inhibitors between Rac1 and GEF. Among the AR compound series, the designed compound AR-148 resulted the most effective, although further structural optimization will be necessary for an eventual development of this molecule as a drug candidate, in particular by exploring the effects on activity of substitutions at the diarylamino moiety.

An original multistep synthesis of different stereoisomers of 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate was developed mainly based on the construction of the norbornene scaffold by the way of a Diels-Alder cycloaddition reaction and its functionalization through Palladium catalyzed Heck/Buchwald reactions. A new phosphine-free Heck hydroarylation protocol was demonstrated which was, to our knowledge, never reported before for constrained double bond. Moreover, an efficient and regioselective strategy for multigram scale preparation of the lead compound **AR148** was developed.

We demonstrated how the 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate scaffold might be used for a *de-novo* design of new and effective Rac1 inhibitors. An original multistep synthesis of different stereoisomers of 2-amino-3-(phenylsulfanyl)norbornane-2-carboxylate was then developed, mainly based on the construction of the norbornene scaffold by the way of a Diels-Alder cycloaddition reaction and its functionalization through Palladium catalyzed Heck/Buchwald reactions.

Among the AR compound series, the designed compound AR-148 resulted the most effective by selectively inhibiting Rac1 and cell migration in response to PDGF-BB.

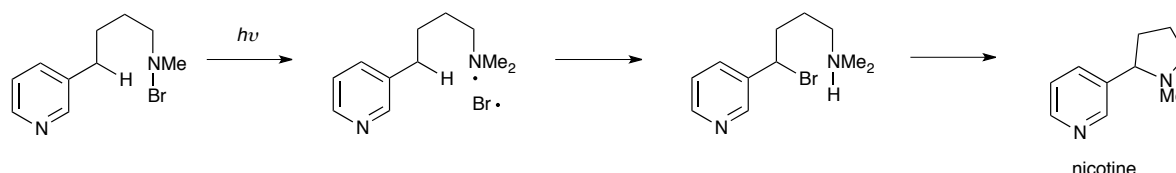
Future plans include an hit-to-lead optimization of this molecule and, accordingly, a computer aided SAR study is being pursued to explore the effects of substitutions at the diarylamino and phenylsulfanyl moiety. Moreover, further investigations on the synthetic method are currently ongoing to selectively obtain AR derivatives in enantiopure forms.

6 Appendix: Direct Synthesis of Fluorinated Heteroarylether Bioisosteres

6.1 Introduction

“C-H bonds is indicated simply by the absence of any other bond. This “invisibility” of C-H bonds reflects both their ubiquitous nature and their lack of reactivity. With these characteristics in mind it is clear that if the ability to selectively functionalize C-H bonds were well developed, it could potentially constitute the most broadly applicable and powerful class of transformations in organic synthesis.” This definition given by Alan Goldman, and Karen Goldberg¹ is able to explain and fix in mind what is the really application and powerful scope of such kind of transformation. C-H reactions more technically include every reactions that lead to an higher oxidative state of the carbon atom through the transformation of C-H bond. The advantages of such transformations are: the reduction of inefficient chemical manipulation (protecting group chemistry) and consequent atom economy of the synthesis, the design of short synthetic route, the elimination and uselessness of prefunctionalization, the possibility to develop synthesis of entire classes of compounds with different functionalization. The interest in these transformations has been growing from year to year in the last decade and put this area in a privileged position nowadays. Actually direct CH activation is an old chemistry that now is living a second youth. For a long time chemists have known about this kind of transformation. One of the first example is the photochemical decomposition of haloamines that leads to the direct halogenation of unactivated C-H bond reported in 1909 by Loffler in the synthesis of nicotine, and nowadays takes the name of Hofmann-Löffler-Freytag reaction.^{2 3}

Scheme 6.1 Loffler synthesis of nicotine via CH activation



Some important notable works in the field of direct oxidation and functionalization were developed by Barton in the synthesis of aldosterone,^{4 5} in the use of dioxirane by Fusco⁶ and one of the most powerful

¹ ACS Symposium Series 885, Activation and Functionalization of C-H Bonds, 2004, 1-43

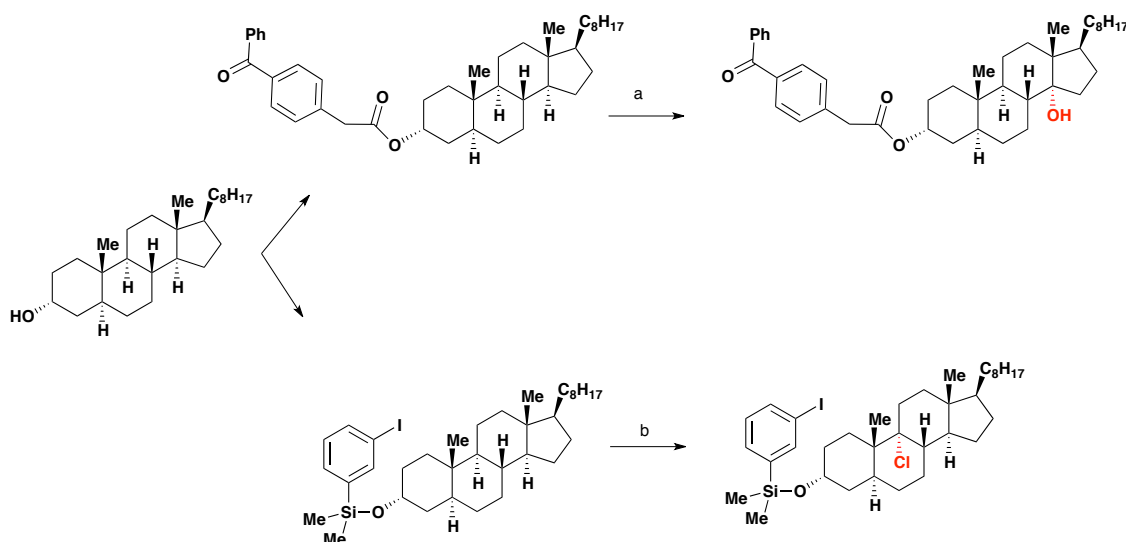
² A.W. Hofmann, *Ber*, **1883**, 16, 558-560

³ K. Löffler, S. Kober, *Ber*, **1909**, 42, 3431-3438

⁴ D.H.R. Barton, J.M. Beaton, L.E. Geller, M.M. Pechet, *J. Am. Chem. Soc.* **1960**, 82, 2640-2641

example of this kind of chemistry is without doubt the biomimetic strategy proposed by Breslow for guided oxidation of steroids in which it is noteworthy the high selectivity achieved over the large number of equivalent C-H bonds.^{7 8}

Scheme 6.2 Breslow guided CH functionalization



Reaction Conditions: a) 1) BrCCl_3 , benzene, $h\nu$ 2) base b) AIBN, $h\nu$, rt, DCM, SO_2Cl_2 , NaOH

6.1.1 CH Functionalization of Heterocycles, Minisci chemistry

In this large number of different transformations also takes its space the direct functionalization of nitrogen containing heterocycles in which professor Minisci is one of the pioneers. The Minisci reaction was one of the first direct C-H functionalization of heterocycles, except for the Friedel-Craft reaction, and consists in the coupling between alkyl radical generated from the decomposition of carboxylic acid with an heterocycle using Silver nitrate as additive. The reaction presents an opposite selectivity than a normal Friedel-Craft alkylation and permits the introduction of a wide range of alkyl groups starting from the corresponding acid. (Scheme 3)^{9 10 11}

⁵ D. H. R. Barton and J. M. Beaton, *J. Am. Chem. Soc.*, **1960**, 82, 2641.

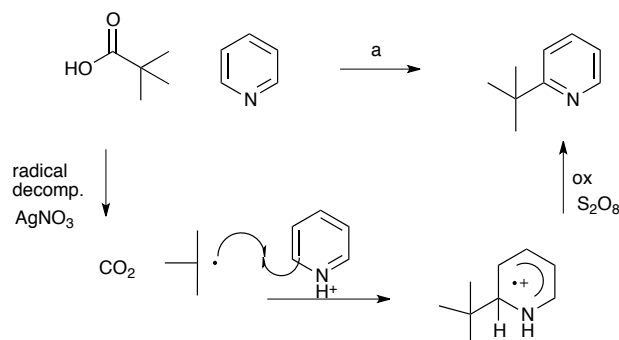
⁶ R. Mello, M. Fiorentino, C. Fusco, R. Curci, Oxidations by methyl(trifluoromethyl)dioxirane. 2. Oxyfunctionalization of saturated hydrocarbons. *J. Am. Chem. Soc.* **1989**, 111, 6749 – 6757.

⁷ R. Breslow, J.A. Dale, P. Kalicky, S. Y. Liu, W. N. Washburn; Introduction of a 9(11) double bond into steroids by selective free-radical halogenation, *J. Am. Chem. Soc.*, **1972**, 94 (9), pp 3276–3278

⁸ R. Breslow, S. Baldwin, T. Flechtner, P. Kalicky, S. Liu, W. Washburn. Remote oxidation of steroids by photolysis of attached benzophenone groups. *J. Am. Chem. Soc.*, **1973**, 95 (10), pp 3251–3262

⁹ Minisci, F.; Fontana, F.; Vismara, E. Substitutions by Nucleophilic Free-Radicals A New General Reaction of Heteroaromatic Bases. *J. Heterocycl. Chem.* 1990, 27, 79–96

Scheme 6.3 Minisci reaction.



Reaction conditions: a) AgNO_3 , H_2SO_4 , $(\text{NH}_4)_2\text{S}_2\text{O}_8$

Minisci and other have developed in the '80 and '90 other radical CH activation that play an important role in the heterocyclic chemistry, all of them consisting in radical decomposition of carbon active species mediated by metal like Iron, Silver, Copper or radicalising agent followed by the trapping of the radical due to a N-heterocycle moiety that is rapidly reoxidized to the aromatic form.^{12 13 14 15 16 17 18} (Figure 4)

¹⁰ Minisci, F.; Vismara, E.; Fontana, F. Recent Developments of Free-Radical Substitutions of Heteroaromatic Bases. *Heterocycles* **1989**, *28*, 489–519.

¹¹ . Minisci, R. Bernardi, F. Bertini, R. Galli, M. Perchinummo: *Nucleophilic character of alkyl radicals—VI : A new convenient selective alkylation of heteroaromatic bases*, in: *Tetrahedron* **1971**, *27*, 3575–3579.

¹² Minisci F., Vismara E., Fontana F., *Tetrahedron Lett.* **1989**, *39*, 4569

¹³ Kryger R., Lornad J. P., Steven N.R., Herron, N.R. **1977**, *J. Am. Chem. Soc.* *99*, 7589

¹⁴ Castaldi G., Minisci F., Vismara E., Torelli V., *Tetrahedron Lett.* **1984**, *25*, 3897

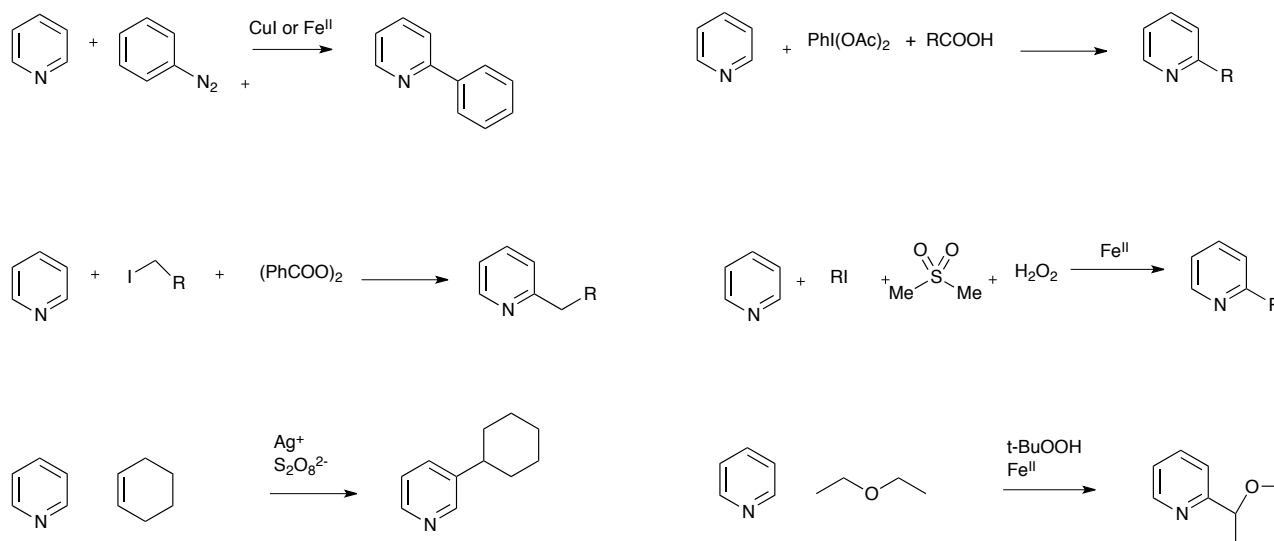
¹⁵ Veltwisch D., Janata e., Asmus k. *J. Chem. Soc. Perkin II* **1980**, 146

¹⁶ Clerici A., Minisci F., Ogawa K. Suzur J. M. *Tetrahedron Lett.* **1978**, 1149

¹⁷ Arnoldi C., Citterio A., Minisci F.; *J. Chem. Soc. Perkin II* **1980**, 531

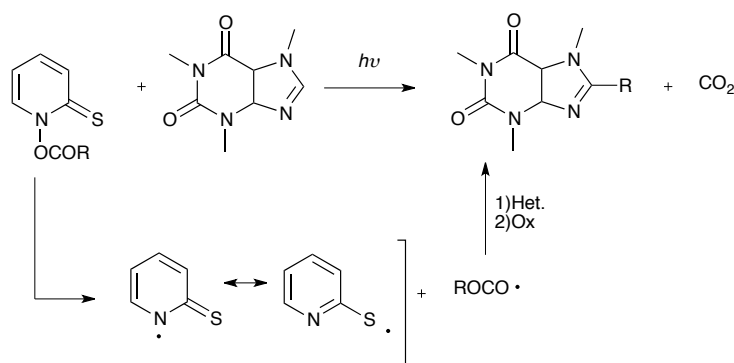
¹⁸ Citterio A., Gentile A., Minisci F. Serravalle M., Ventura S. *Chem Commun.* **1983**, 916

Scheme 6.4 Different radical CH functionalization developed between 1970-1990



One of the most particular procedure has been developed by Barton and involves the photolysis of esters of thiohydroxamic acid to produce a reactive radical that interacts with heterocycles.^{19 20} (Figure 5)

Scheme 6.5 Barton C-H functionalization of caffeine via thiohydroxamic esters



6.1.2 Baran reagents in CH innate functionalization of heterocycles.

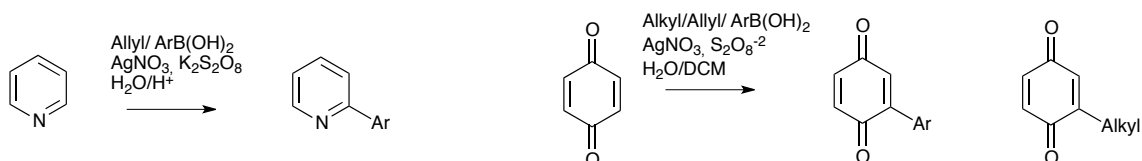
The Baran laboratory has been involved for a long time in CH activation and, taking inspiration from the Minisci pioneering work previously reported, a series of new CH activations has been developed. The new reaction exploits innate chemical reactivity of nitrogen-rich heterocycles to trap radicals in order to obtain a

¹⁹ D.H.R. Barton, B. Garcia, H. Togo, S.Z. Zard **1986** *Tetrahedron Lett.* 27, 1327

²⁰ E. Castagnino, S. Corsano, D.H. Barton, S. Z. Zard **1986** *Tetrahedron Lett.* 27, 6337

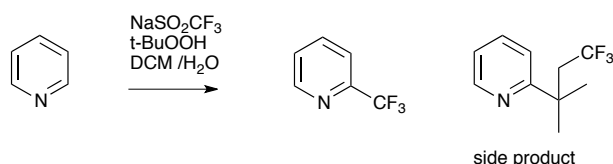
modification of the heterocycles without the need of prefunctionalization. Previous works published from Baran lab have demonstrated the possibility to use aryl boronic acids as radical precursors for heterocycle direct functionalization, as well as alkyl boronic acids and trifluoroborate salts in mild condition.^{21 22 23} (Figure 6)

Scheme 6.6 C-H arylation and alkylation via boronic acid



Later, a work was published where the use of Langlois reagent (trifluoromethanesulphonate) was reported as radical precursor for innate radical trifluoromethylation of heterocycle in very mild conditions, open air and with simple methodology. This new functionalization opens the possibility to synthesise fluorinated heterocycles without the use of strong and toxic reactive like DAST and PyHF.²⁴ (Figure 7)

Scheme 6.7 Langlois reagent use for direct CH functionalization



Sulfonate salts have recently emerged as a promising method for the direct incorporation of alkyl groups onto heteroaromatic systems through an innate formal C–H functionalization *via* a radical addition process. All the zinc sulfonate salts synthesized are commercially available and remarkable more reactive than sodium derivatives. Zinc derivatives reported in figure 8 did not require transition metal catalysis or special

²¹ Seiple, I. B. et al. Direct C–H arylation of electron-deficient heterocycles with arylboronic acids. *J. Am. Chem. Soc.* 132, 13194–13196 (2010)

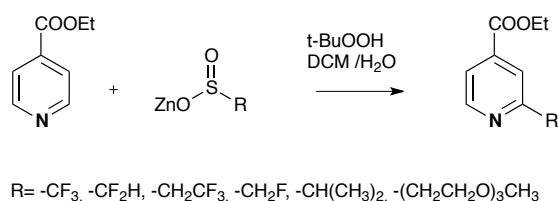
²² Fujiwara, Y. et al. Practical C–H functionalization of quinones with boronic acids. *J. Am. Chem. Soc.* 133, 3292–3295 (2011)

²³ Molander, G. A., Colombel, V. & Braz, V. A. Direct alkylation of heteroaryls using potassium alkyl- and alkoxy-methyltrifluoroborates. *Org. Lett.* 13, 1852–1855 (2011)

²⁴ Ji, Y.; Brueckl, T.; Baxter, R. D.; Fujiwara, Y.; Seiple, I. B.; Su, S.; Blackmond, D. G.; Baran, P. S. *Proc. Natl Acad. Sci.* 2011, 108, 14411

anhydrous conditions and high temperature but they react in easy, mild, open air practical protocol with high tolerance of functional groups.^{25 26}

Scheme 6.8 Zn sulfinate salts as CH functionalization reagent.



Considering that 70% of the drugs on the market present in their structure heterocycles, this new series of practical methodologies can be applied for the fast generation and identification of analogues of marketed drugs, lead compounds or natural products without the need of design de novo synthesis and in the most cases without the need of protecting groups

6.2

6.3 Fluorinated Heteroarylether Bioisosteres an overview.

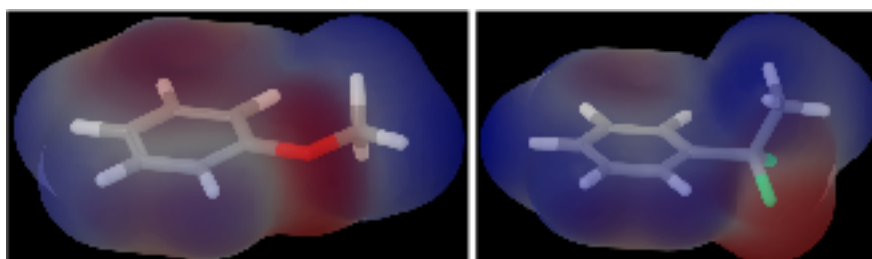
There is a great demand for new methods that rapidly introduce important bioisosteres onto heterocycles without any prefunctionalization or protection. Arylethers such as the methoxy group represent essential functional groups in medicinal chemistry yet their metabolic instability often requires their replacement with more oxidatively robust bioisosteres. This work delineates a general method for the modular synthesis of reagents that are capable of direct incorporation of difluoroalkyl groups onto heterocycles. The scope and generality of this method is exemplified with the difluoroethyl group (along with the introduction of a new reagent for difluoroethylation, DFES-Na) and a proof of principle is shown for general, modular synthesis of fluorinated heteroarylether bioisosteres. This methodology addresses a pressing problem in medicinal chemistry; its high functional group tolerance, excellent regioselectivity, and simple reaction conditions bode well for widespread use. Medicinal chemists have a love-hate relationship with aryl ethers. They are generally enlisted to explore SAR and to modulate the properties of bioactive scaffolds. Yet, aryl ethers (such as the methoxy group) represent a metabolic liability and thus much effort is expended to replace such

²⁵ Fujiwara, Y.; Dixon, J. A.; Rodriguez, R. A.; Baxter, R. D.; Dixon, D. D.; Collins, M. R.; Blackmond, D. G.; Baran, P. S. *J. Am. Chem. Soc.* **2012**, *134*, 1494

²⁶ Fujiwara, Y.; Dixon, J. A.; O'Hara, F.; Funder, E. D.; Dixon, D. D.; Rodriguez, R. A.; Baxter, R. D.; Herle, B.; Sach, N.; Collins, M. R.; Ishihara, Y.; Baran, P. S. *Nature* **2012**, *492*, 95

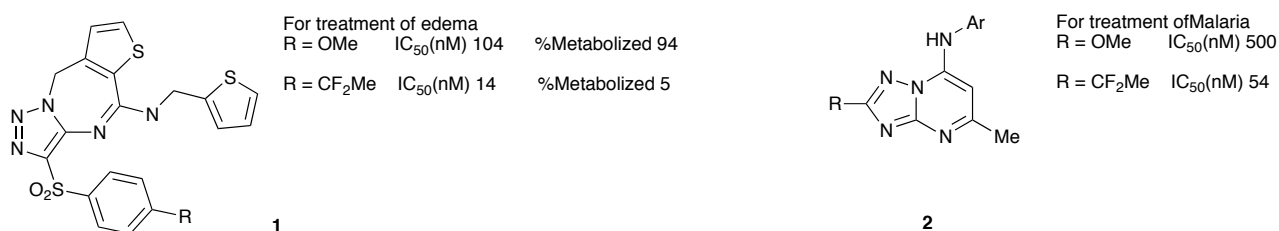
moieties with metabolically stable derivatives or bioisosteres.²⁷ The goal of this work was to develop a rapid and direct means for medicinal chemists to introduce stable, fluorinated mimics of alkoxy ethers into their scaffolds. A tried and true method to achieve this is a point-mutation of the oxygen atom to a difluorinated carbon atom. As shown in figure below for the case of a methoxy ether, the difluoroethyl group mimics the steric and electronic features of a methoxy group albeit with a conformational preference of the former that places the methyl group out of the plane of the aromatic ring.²⁸

Scheme 6.9 The difluoroethyl group mimics a methoxy group and improves the bioactivity and metabolic stability of medicinally important molecules,



Numerous applications of the difluoroethyl-methoxy replacement can be found in the literature. For example, two recent reports^{29 30} (**1** and **2**) have demonstrated the remarkable advantage that such a modification can have on both potency and metabolic stability. (Figure 10)

Scheme 6.10 Application of methoxy bioisoster substitution



²⁷ Meanwell, N. A. *J. Med. Chem.*, **2011**, *54*, 2529-2591 and references therein

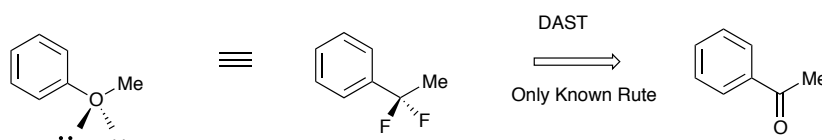
²⁸ Calculations performed using Suite 2012: Maestro, version 9.3, Schrödinger, LLC, New York, NY, 2012 and Q-Chem (used for torsional scan). See experimental part for full details of calculations.

²⁹ Anderson, M. O.; Zhang, J.; Liu, Y.; Yao, C.; Phuan, P.-W.; Verkman, A. S. *J. Med. Chem.* **2012**, *55*, 5942

³⁰ Coteron, J. M.; Marco, M. a.; Esquivias, J.; Deng, X.; White, K. L.; White, J.; Koltun, M.; El Mazouni, F.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.; Angulo-Barturen, I. i.; Ferrer, S. B.; Jiménez-Díaz, M. a. B. n.; Gamo, F.-J.; Goldsmith, E. J.; Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.; Charman, S. A.; Phillips, M. A. *J. Med. Chem.* **2011**, *54*, 5540

Unfortunately, the only known route to such compounds uses harsh fluorinating agents such as DAST to convert a ketone into the corresponding difluoroalkyl group thus requiring a significant investment of resources since direct methods for late-stage appendage are not known. (Figure 11) Even programmed approaches involving cross coupling are unprecedented, thus rendering routes to these types of compounds a serious unmet need that could have an immediate impact in the synthesis of medicines.

Scheme 6.11 Unique known synthesis for difluoroethyl moiety

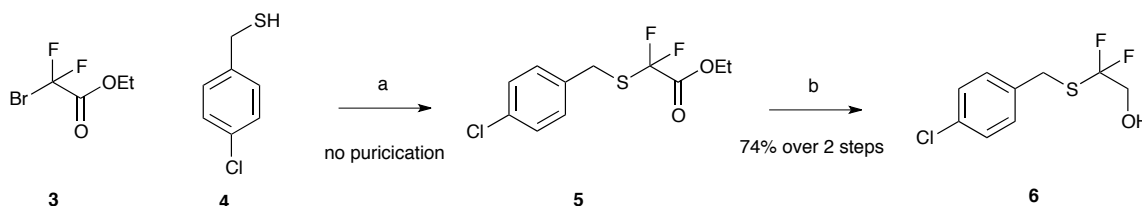


6.4 Different approach for the synthesis of sodium difluoroethylsulfinate (DFES-Na)

6.4.1 Sulfonyl chloride strategy

In this chapter the invention of reagents and methods for the direct installation of fluorinated heteroarylether bioisosteric replacements are reported. Different strategies were pursued in order to develop a difluoroethylsulfinate salt (DFES). Analogously to the synthesis of the difluoromethyl sulfinate salt³¹, which is prepared via sulfonyl chloride commercially available, we tried to obtain compound **12** (Figure 15) as starting material for our DFES. Bromine atom in **3** was successfully substituted with the thiol **4** to obtain **5**. Avoiding the purification steps, the ester function of **5** was easily reduced with NaBH₄ due to the strong electron withdrawing effect of the α -difluoride substitution giving precursor **6** with 74% yield over two steps on a gram scale synthesis. (Figure 12)

Scheme 6.12 Synthesis of DFES

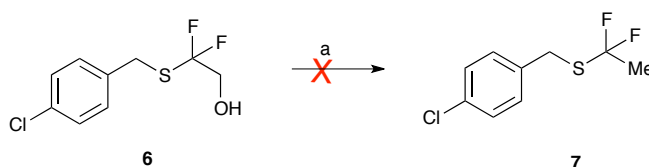


Reaction Conditions: a) NaH, DMF, 0°C to 70°C; b) NaBH₄, MeOH, 0°C to rt;

³¹ Fujiwara, Y.; Dixon, J. A.; O'Hara, F.; Funder, E. D.; Dixon, D. D.; Rodriguez, R. A.; Baxter, R. D.; Herle, B.; Sach, N.; Collins, M. R.; Ishihara, Y.; Baran, P. S. *Nature* **2012**, 492, 95

The direct deoxygenation of **6** in different reduction conditions has never led to the reduced product **7**, if not as trace in a complex mixture of side products.

Scheme 6.13 Reduction of alcohol group.

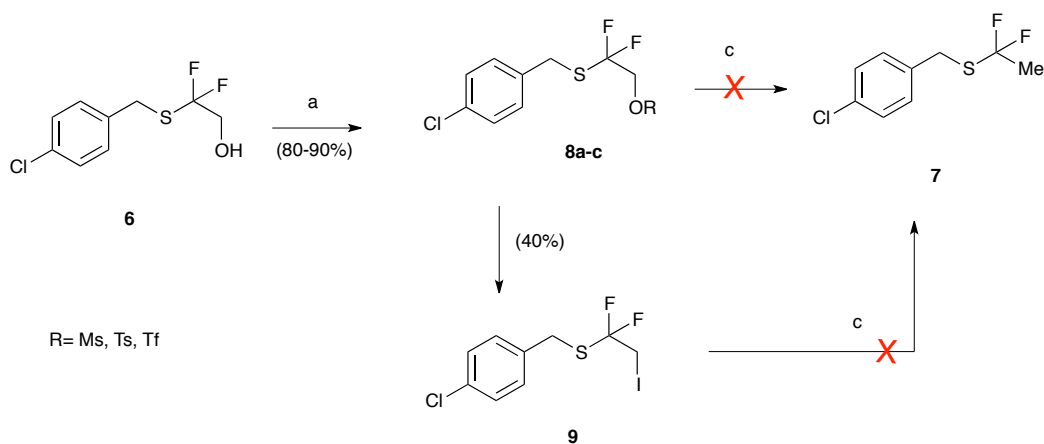


Reaction Conditions tested

- 1) NaCNBH₃, ZnI, DCM, rt to reflux, 2) NaCNBH₃, ZnI, DCE, rt to reflux ; 3) LiBH₄, ZnI, DCE, rt to reflux
 4) NaBH₄, ZnI, DCE, rt to reflux; 5) Sulfur trioxide-PyTHF, LiAlH₄, THF, 0°C to rt; 6) InBr₃, Ph₂SiHCl, DCE, rt to reflux
 7) Et₃SiH, B(C₆F₅)₃, DCM; 8) Et₃SiH, TFA, DCM; 9) CaCl₂, Zn, EtOH/H₂O, reflux

This unmet result probably occurred for the particular electronic feature of the difluoroethyl alcohol scaffold. For this reason we thought to convert the alcohol moiety in more electron demanding group or in halogen and perform on this the reductive reaction. Derivative **8a-c** was obtained in very high yield but also on such compound the reduction did not furnish the desired compound **7**. Some reaction conditions were tested also on Iodine precursor **9** but the low yield of its obtainment pushed us to explore other strategy. (Figure 14)

Scheme 6.14 Reduction of protected alcohol function and Iodine derivative.

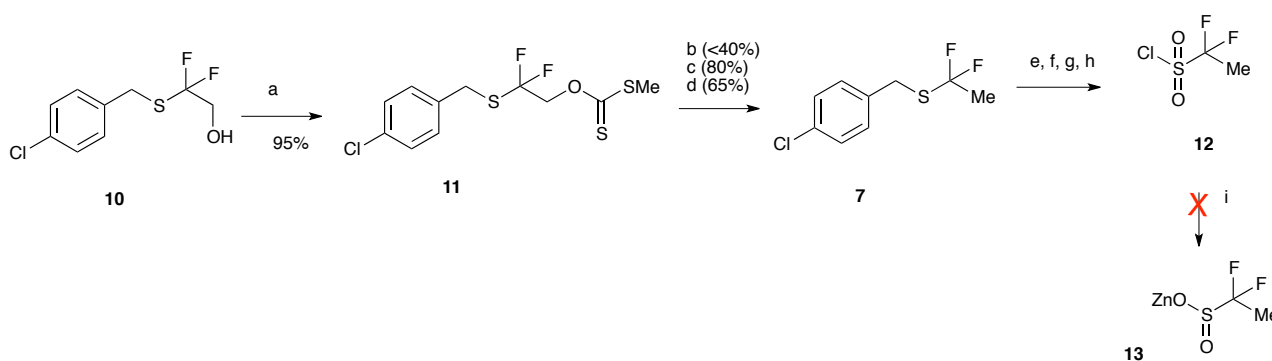


Reaction conditions: a) TsCl or MsCl or Tf₂O, DCM, TEA, 0°C to rt. b) NaI, DMF, 120°C c) TESTED:

1)NaBH₄, MeOH, 0°C to rt; 2)NaBH₄, DMSO; 3)LiAlH₄, THF, 0°C to rt; 4)Li(C₂H₅)₃BH, superhydride, 0°C to rt. 5)NaI, Zn, DMF
6) L-selctride, THF, -78 to 0° C; 7)L-selctride, DMF, -78 to 0° C; 8)L-selctride, DCM, -78 to 0° C; 9)Pd/C, NaBH₄, MeOH , -78 to 0°C; 10)Pd/C, NaBH₄, iPrOH , -78 to 0°C; 11) Zn, HCl DMF

Then we converted the alcohol to the corresponding xanthate with full conversion of the starting material and we tested the Barton-McCombie reaction in classical condition with AIBN and *n*-Bu₃SnH, on compound **10**. Unfortunately we obtained the desired product in very low yield and despite the different purification processes applied (chromatographic column, filtration on KF, washing with KF, NH₄Cl. etc) we could not obtain the product pure and clean from *n*-Bu₃Sn-R derivative. The distillation of the desired product from the reaction mixture lead to the degradation of the compound or co-distillation with tin derivatives. Use of the crude reaction in the following step did not lead to manageable compounds for the preparation of DFES. With the use of tri-trimethylsilylsilane instead of Bu₃SnH we obtained the desired product in satisfactory yield and with an acceptable degree of purity. The following steps of oxidative-debenzylation with NaOCl at -10°C furnished the desired sulfonyl chloride derivatives **12** in low yield and other conditions tested did not improve the overcome of the reaction. Moreover, unfortunately, this compound is very volatile and any attempt of distillation from the reaction mixture always led to decomposition due to the impurity remained from the previous steps. Also the direct transformation in a Zn sulfinate with the addition of water and Zn dust at 0°C has failed.

Scheme 6.15 Radical reduction of Xanthate

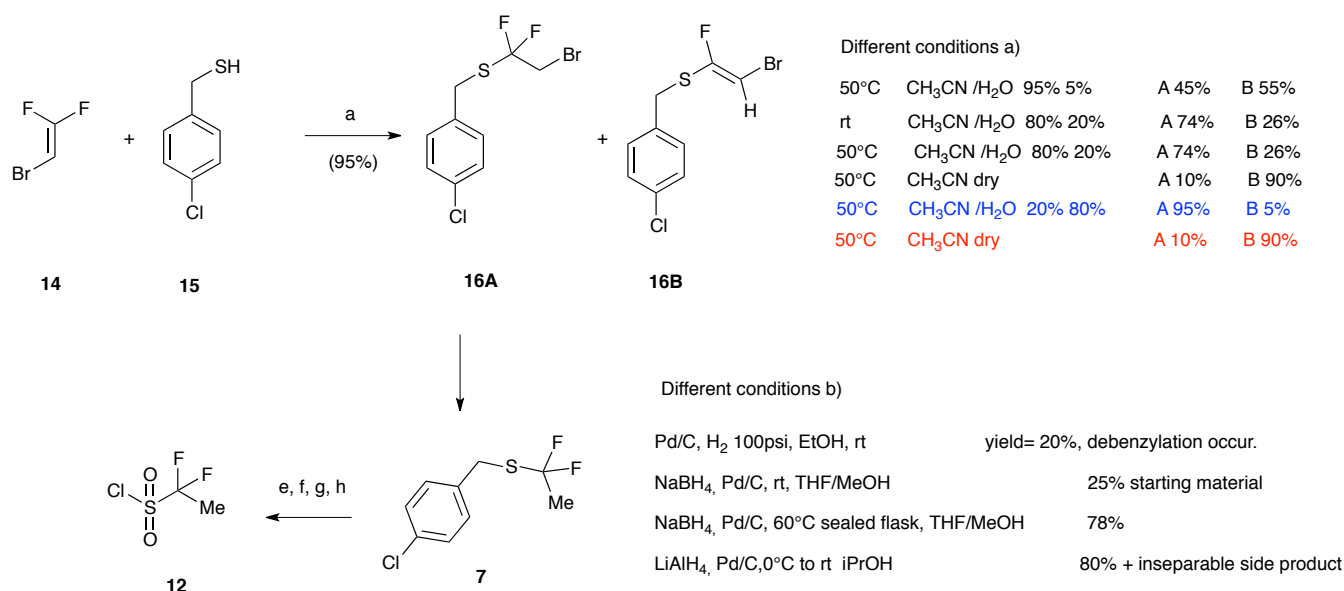


Reaction Conditions: a) 1)NaH, 2) CS₂, 3) MeI, THF, 0°C, b) 1)AIBN 2)*n*-Bu₃SnH, benzene , refluxed c) (Me₃Si)₃SiH, AIBN, benzene, 80°C, 8h, d) (Me₃Si)₃SiH, AIBN, H₂O, 100°C, 8h, e) 1)DCM/HCl 1M 2)NaOCl 14,5 % -10°C f) AcOH/H₂O, NCS g) AcOH/H₂O, Cl₂ bubbled, h) DCM/H₂O, SO₂Cl₂, i) H₂O, Zn dust, water, 0°C

Due to the elevated cost of tri-trimethylsilylsilane and the technical problems we considered to pursue a different pathway from different starting materials. Condensing thiol **15** with acetonitrile saturated with 1-difluoro-2-bromo-ethylene we obtained a mixture of compounds **16A** and **16B**. Modulating the solvent and the temperature we were able to selectively obtain the olefin form or the saturated form. The bromine

product was then reduced with NaBH₄ and Pd on carbon to afford the desired compound **7** in good yield. Unfortunately compound **12** was always isolated in very low yield confirming that it is a low boiling, high volatile liquid that, in our hands, was problematic to manipulate.³²

Scheme 6.16 1-difluoro-2-bromo-ethylene approach



Reaction Conditions: a) KOH, CH₃CN/H₂O

Furthermore this approach is not modular and would require a lengthy synthesis for each difluoroalkyl group that one wished to transfer.

6.4.2 Hu's reagent as starting material of Na-difluoroethylsulfinate (DFES-Na) and derivatives.

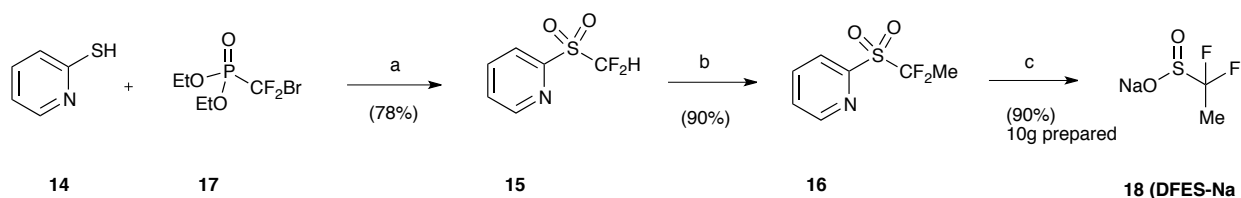
Inspiration was thus drawn from two independent reports, one from the Prakash-Olah group³³ and one from the Hu group.³⁴ The former group elegantly demonstrated that Hu's reagent (**17**) could be efficiently alkylated and cleaved to liberate sodium sulfinate salts (which were immediately oxidized to sulfonates). This route was adapted for the scalable (>10 grams) preparation of Na-difluoroethylsulfinate (**19**, a new

³³ Prakash, G. K. S.; Ni, C.; Wang, F.; Hu, J.; Olah, G. A. *Angew. Chem. Int. Ed.* **2011**, *50*, 2559

³⁴ Zhao, Y.; Huang, W.; Zhu, L.; Hu, J. *Org. Lett.* **2010**, *12*, 1444.

chemical entity), obtaining by the cleavage with NaH and Ethanethiol as stable white solid that has been commercialized by Sigma-Aldrich (catalog # L511404).

Scheme 6.17 Invention of DFES-Na and a modular approach to difluoroalkyl sulfinate preparation

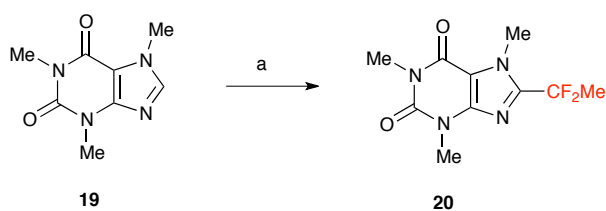


Reaction Condition: a) 1) KOH, 2) NaIO₄, RuCl₃ (cat), b) LiHMDS, THF; c) NaH, EtSH

6.5 Direct innate functionalization of heterocycles by DFES-Na

With a reliable synthesis of the requisite sulfinate salt (**18**) in hand, optimization of the C–H alkylation was conducted as shown in Scheme and Table 1. Using caffeine (**10**), in a 2.5:1 mixture of CH₂Cl₂:H₂O with TBHP as oxidant, and in the absence of any additives, only trace amounts of the desired product **11** were observed (Entry 1). Although Bronsted acids such as TFA improved the conversion (Entry 2), Zinc-based Lewis acids (Entries 3-13) dramatically enhanced the reaction. Ultimately, ZnCl₂ was identified as the optimal additive along with TsOH (entry 13).

Scheme 6.18 Screening condition on caffeine



Reaction Condition : a) DFES-Na 3eq, TBHP 5 eq, DCM/H₂O 2,5/1 Additives

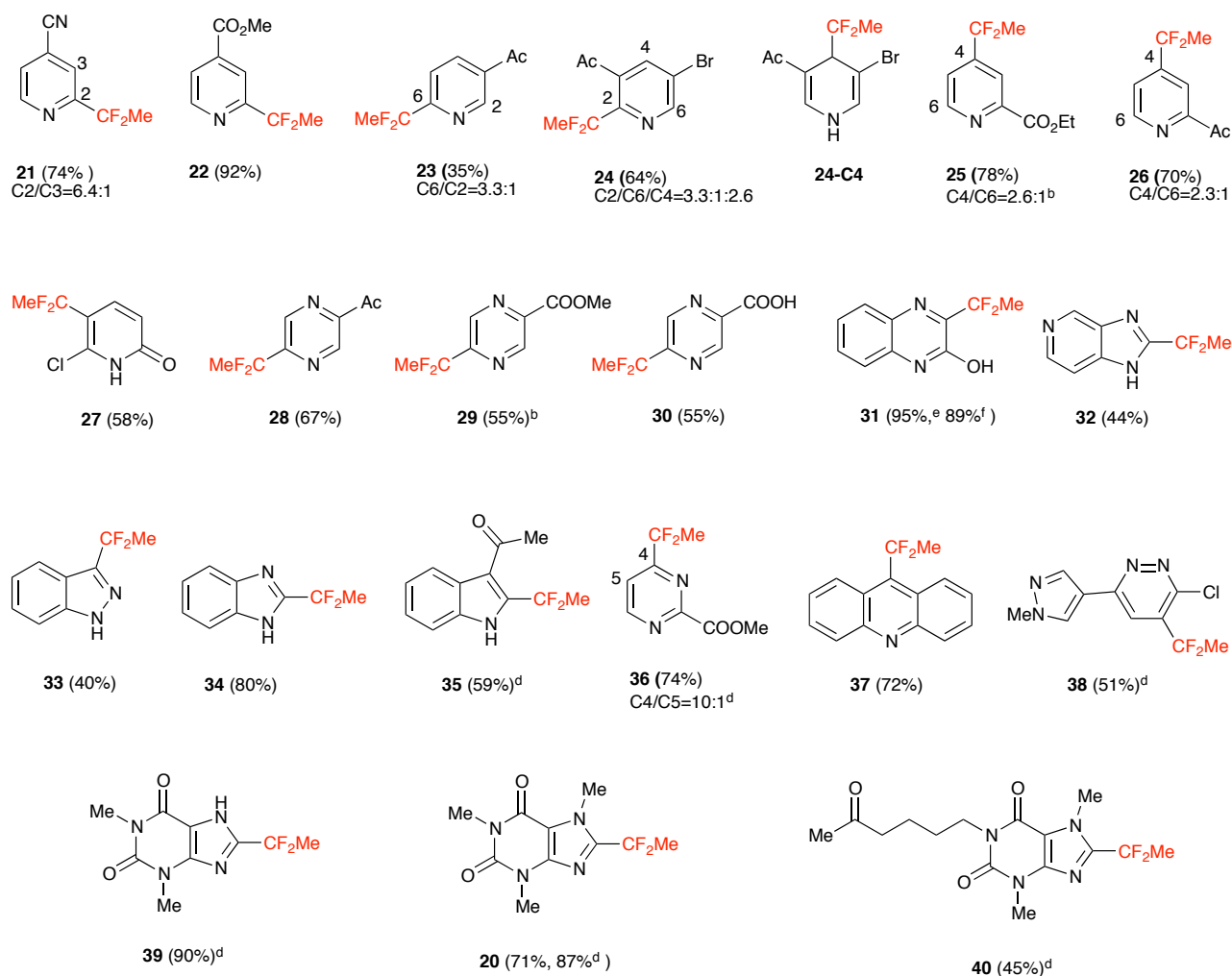
Table 1 Tested additives

Entry	Additives	Conversion ^a
1	none	5
2	TFA 1.0 eq	30
3	TFA 1.0 eq, ZnI ₂ 1.5 eq	9
4	TFA 1.0 eq, ZnSO ₃ 1.5 eq	24
5	TFA 1.0 eq, Zn(NO ₃) ₂ 1.5 eq	45

6	TFA 1.0 eq, Zn(OTf) ₂ 1.5 eq	26
7	TFA 1.0 eq, ZnSO ₄ 1.5 eq	11
8	TFA 1.0 eq, Zn(OAc) ₂ 1.5 eq	13
9	TFA 1.0 eq, ZnF ₂ 1.5 eq	0
10	TFA 1.0 eq, ZnBr ₂ 1.5 eq	35
11	TFA 1.0 eq, ZnCl ₂ 1.5 eq	43
12	1M HCl 1.0 eq, ZnCl ₂ 1.5 eq	29
13	TsOH-H ₂ O 1.0 eq, ZnCl ₂ 1.5 eq	65 (75) ^b
14	TsOH-H ₂ O 1.0 eq	25
15	ZnCl ₂ 1.5 eq	49(56) ^b

Using these optimized conditions, a wide range of heterocycles were examined as depicted in figure 19.

Scheme 6.19 Scope of C–H difluoroethylation of heteroarene substrates



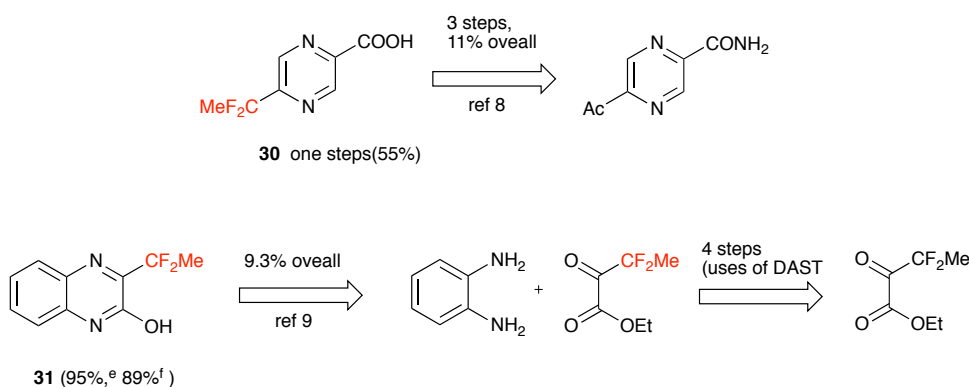
Scheme 6.19. a) reactions performed on 0.05 mmol scale for optimization, 0.2 mmol scale for scope: Heterocycle (1.0 equiv), DFES-Na (3.0 equiv), tert-butyl hydroperoxide (5.0 equiv), TsOH•H₂O (1.0 equiv), ZnCl₂ (1.5 equiv), 0 to 23 oC; isolated yields of chromatographically pure products are displayed, unless otherwise noted. b conversion on 0.2 mmol scale, isolated yield 71%. c conversion on 0.2 mmol scale, isolated yield 51%. dReaction showed incomplete conversion after 12-24 hours, and a second addition of DFES-Na (3.0 equiv), ZnCl₂ (1.5 equiv), and tert-butyl hydroperoxide (5.0 equiv) was added. e DFES-Na (2.0 equiv), ZnCl₂ (1.0 equiv), TsOH•H₂O (1.0 equiv), TBHP (5.0 equiv), 0 to 23 oC, reaction completed in 10 h. f) gram-scale reaction: DFES-Na (2.5 equiv), ZnCl₂ (1.25 equiv), TsOH•H₂O (1.0 equiv), TBHP (5.0 equiv), 0 to 23 oC, 8 h, then 15h with O₂ balloon.

The scope, site-selectivity, and functional group tolerance are notable aspects of the method. Thus, pyridines (**21** – **26**), pyridones (**27**), pyrazines (**28** – **30**), quinoxolines (**31**), pyridylbenzimidazoles (**32**), indazoles

(**33**), benzimidazoles (**35**), indoles (**35**), pyrimidines (**36**), benzoquinolines (**37**), pyridazines (**38**), and xanthines (**20**, **39** – **40**) are all amenable to fluoroalkylation. Out of the 21 examples shown, 18 could potentially result in mixtures of regioisomers. Remarkably, 12 of these react with DFES-Na to deliver a single regioisomer, two react with good regioselectivity (6.4:1 or higher), and four achieve modest but synthetically useful selectivity (2.3:1 or higher). Particularly notable examples are **27**, **32**, and **38**, all of which have 3 or more C–H bonds that could conceivably be alkylated. The functional group tolerance of this reaction, which is conducted with water as a co-solvent and open to the air if desired, is also notable. Ketones, esters, nitriles, chlorides, bromides, free N–H heterocycles, and even free carboxylic acids are tolerated as well as free amines and alcohols (*vide infra*). These reactions are also scalable, with heterocycle **31** being difluoromethylated on a gram-scale in 89% isolated yield.

Cursory analysis of the patent literature indicates that the current method can dramatically simplify the way such fluoroalkylated compounds are made. As shown in Scheme 20, the known route to pyrazine **21**¹ involves a three step procedure proceeding in 11% overall yield, all of which involve laborious functional group manipulations (and DAST). The known route to **22**² requires a 5-step sequence proceeding in 9.3% overall yield (using DAST and KCN). In contrast, the one-step routes to **21** and **22** proceed in 55 and 95% yields respectively and represent ideal³ syntheses.

Scheme 6.20 Comparison to current state-of-the-art



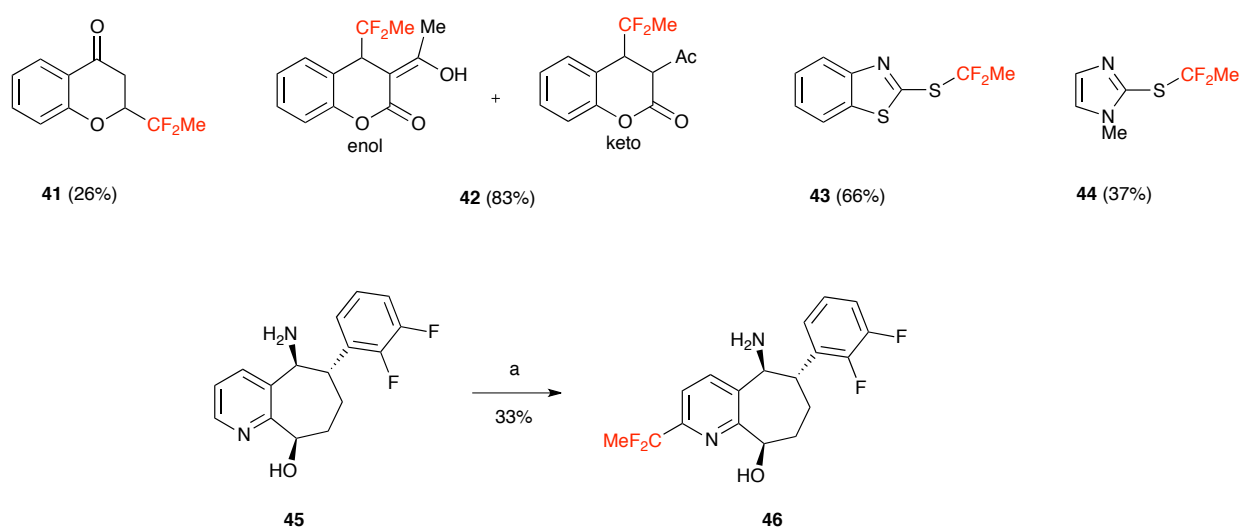
Difluoroethyl radicals generated from DFES-Na are also capable of adding to Michael acceptors and thiols as shown in Scheme 21. In an extreme example (Scheme 21) of site-specificity and functional group tolerance, an advanced intermediate from a program at Bristol-Myers Squibb (**45**) can be directly difluoromethylated to deliver **37** without protecting the reactive and oxidizable benzylic alcohol and amine.

¹ Ellard, J. M.; Farthing, C. N.; Hall, A., WO 2011/09898

² Chen, H.; Mcdaniel, K. F.; Green, B. E.; Shanley, J. P.; Kruger, A. W.; Gandarilla, J.; Welch, D. S.; Cink, R. D.; Gai, Y.; Wang, G.; Yat, S., WO 2011/156337

³ Gaich, T.; Baran, P. S. *J. Org. Chem.* **2010**, 75, 4657

Scheme 6.21 Direct difluoroethylation of heteroaromatic Michael acceptors and thiols and Direct difluoroethylation of an advanced intermediate from the BMS compound library

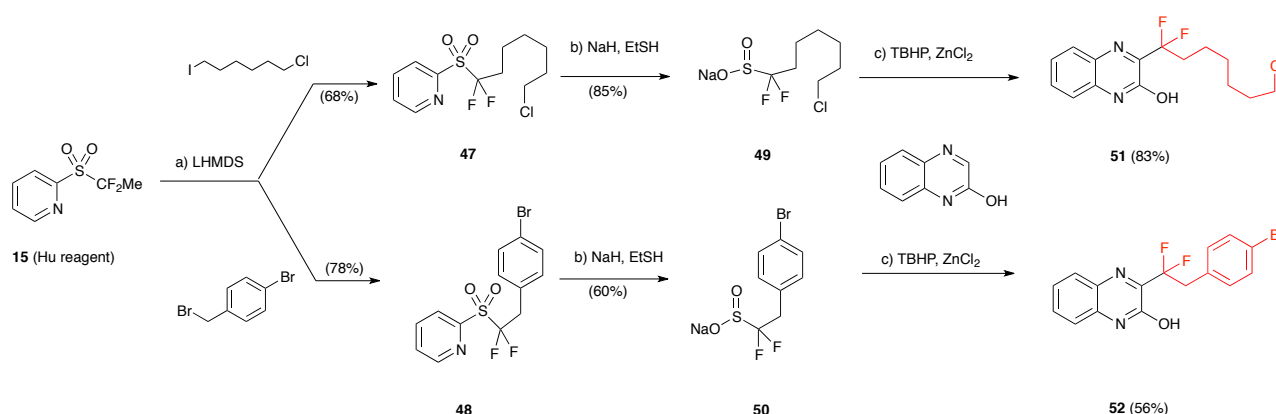


Scheme 6.21. a) reactions performed on 0.05 mmol scale for optimization, 0.2 mmol scale for scope: Heterocycle (1.0 equiv), DFES-Na (3.0 equiv), tert-butyl hydroperoxide (5.0 equiv), $\text{TsOH}\cdot\text{H}_2\text{O}$ (1.0 equiv), ZnCl_2 (1.5 equiv), 0 to 23 °C; isolated yields of chromatographically pure products are displayed,

6.6 Modular preparation of new sodium difluoroalkyl sulfinate.

Perhaps the most significant finding of this work is that the current strategy for introducing a difluoroethyl group (methoxy bioisostere) can also be enlisted for the synthesis of virtually any difluoroalkyl group (alkoxy bioisostere) of interest. As shown in Scheme 22, the same approach used to create **18** (Figure 17) could be used for the synthesis of sulfinate salts **51** and **52**. Thus, subjecting the Hu's sulfone (**15**) to the Olah-Prakash alkylation conditions delivered **47** and **48**. Subsequent cleavage furnished **49** and **50** which were used to accomplish the direct difluoroalkylation of 2-quinoxalinol to deliver the alkyl ether and benzyl ether bioisosteres **51** and **52** in 83% and 56% yields respectively. There are currently no other known methods for making compounds of this sort

Scheme 6.22 . A general synthesis of fluorinated heteroarylether bioisosteric reagents and a proof of concept on 2-quinoxalinol



Reagents and Conditions: (a) reactions performed on 0.2 mmol scale, **40** (2.0 equiv), tert-butyl hydroperoxide (5.0 equiv), $\text{TsOH}\cdot\text{H}_2\text{O}$ (1.0 equiv), ZnCl_2 (1.0 equiv), 0 to 23 oC, reaction completed in 24 h; (b) reactions performed on 0.1 mmol scale, **41** (1.5 equiv), tert-butyl hydroperoxide (5.0 equiv), $\text{TsOH}\cdot\text{H}_2\text{O}$ (1.0 equiv), ZnCl_2 (1.5 equiv), reaction completed in 4 h at 0 °C after second addition of **41** (1.5 equiv), tert-butyl hydroperoxide (5.0 equiv).

In conclusion establishes a simple, reliable, and scalable platform for the direct synthesis of fluorinated bioisosteres of alkoxy ethers. Such moieties are highly sought after in the context of drug discovery but the chemical synthesis of such compounds severely hampers their incorporation. Recognition that Hu's reagent **15** can be employed in concert with the Prakash/Olah protocol led to a programmable synthesis of sulfinate reagents such as **18** (DFES-Na), **49**, and **50**. Aside from direct radical functionalization, sulfinate reagents have already begun to find use in other types of useful transformations.^{4 5} The addition of zinc chloride was critical for the success of the radical alkylation method that, in the case of **18**, demonstrates admirable conversion, site-selectivity, functional group tolerance, and extreme operational simplicity. These features bode well for the widespread adoption of this chemistry in a pharmaceutical setting

⁴ Ye, Y.; Künzi, S. A.; Sanford, M. S. *Org. Lett.* **2012**, *14*, 4979

⁵ Li, Y.; Wu, L.; Neumann, H.; Beller, M. *Chem. Commun.* **2013**, 49, 2628-2630

7 Experimental section.

7.1 General information.

All solvents and reagents are commercially available. If necessary, the solvents were distilled from suitable dehydrating agents (calcium chloride). Thin-layer chromatography (TLC) was carried out on plates ready for use in F254 Fluka silica gel deposited on glass or aluminum. The flash column chromatography was performed on silica gel Davisil LC 60 A (230-400 mesh). Melting points were measured in a capillary using a Stuart Scientific SMP3 apparatus and are uncorrected. The infrared spectra were recorded with a Perkin-Elmer 16 PC FT-IR spectrometer using NaCl discs for liquid samples and tablets of KBr for solid samples. Unless otherwise specified the proton nuclear magnetic resonance spectra were recorded in CDCl₃, D₂O, CD₃OD using Varian Gemini 200 or Bruker Avance 300 or 500 MHz spectrometers. The chemical shifts (δ) are reported in parts per million (ppm). The coupling constants (J) are expressed in hertz (Hz). Unless otherwise specified ¹³C-NMR spectra were recorded in CDCl₃, D₂O, CD₃OD on the same instruments. The low resolution mass spectra were recorded in electron impact on a Fisons MD800 spectrometer and electrospray ion trap with a Finnigan LCQ ADVANTAGE Thermo-spectrometer. Microwave MLS GmbH/Milestone Ltd.

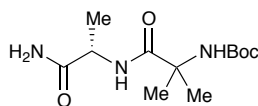
7.2 Chapter 2

Full characterization of compounds presented from the paragraph 2.1.2 to paragraph 2.1.2.3 (Compound from **Z-3ac** to *exo*-**17'** and **26**, **27**, **41**, **42**) are already published at

-Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Soave, R.; Clerici, F. Sulfanyl-methylene-5(4H)-oxazolones and β -sulfanyl- α -nitroacrylates as appealing dienophiles for the synthesis of conformationally constrained cysteine analogues. *Tetrahedron*. **2012**, 68, 1951-1962.

7.2.1 Synthesis of compounds in scheme 2.9:

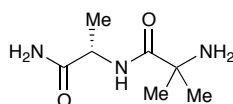
(S)-tert-butyl (1-((1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate
(31)



31

To a mixture of BocAibOH **29** in dry DCM (0,1 M), under nitrogen at 0°C was added HOAt (1.1 eq.), EDC (1.1eq.), and let react under stirring at 0°C for 1 h. After that L-AlaCONH₂ **30** (1.1 eq.) and DIPEA (2.2 eq.) was added, additional DIPEA was used to reach pH = 8, and then the reaction mixture was let react at room temperature for 12 h. The reaction was monitored by TLC (MeOH : DCM, 1 :10) detected by ninhydrin. Upon the consumption of starting material the reaction mixture was washed with saturated NH₄Cl, saturated NaHCO₃, and brine. The organic phase was dried over Na₂SO₄ and the solvent evaporated at reduced pressure to afford compound **31** as white wax with 75% of yield. The reaction was performed with comparable yield from 0.1g. to 2g.. $\alpha_D^{MeOH} = -7,6$. ¹H-NMR δ (200 MHz, CDCl₃) 7.06 (1 H, s), 6.46 (1 H, d, J 7.2), 5.22 (1 H, s), 4.90 (1 H, s), 4.61 – 4.36 (1 H, m), 1.70 – 1.33 (18 H, m). ¹³C-NMR δ (50 MHz, CDCl₃) 175.04, 174.25, 155.47, 81.14, 57.02, 49.09, 28.46, 26.63, 24.75, 17.85.(+)ESI-MS (m/z) : [M+Na]⁺ 296.1. Anal.Calcd for C₁₂H₂₃N₃O₄ (273.17): C, 52.73; H, 8.48; N, 15.37; O, 23.41; found C, 52.78; H, 8.53; N, 15.37. IR(KBr): ν = 3400, 3370, 2984, 1693, 1673, 1515 cm⁻¹.

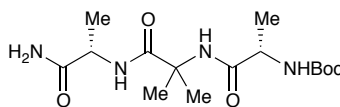
(S)-2-amino-N-(1-amino-1-oxopropan-2-yl)-2-methylpropanamide (32)



32

The compound **31** was dissolved in water (0.1 M) in a sealed tube for micro waves reactor. Under magnetic stirring the sample was irradiated by microwaves for 20 at temperature of 150°C. The reaction was monitored by TLC (MeOH : DCM, 1 :10) detected by ninhydrin. If not finished another round of 20 minutes was performed. Upon consumption of starting material water was evaporated at reduced pressure to afford the desired compound **32** as light yellow solid with 98% of yield. The reaction was performed with comparable yield from 0.1g. to 2g. Melting point 254°C, $\alpha_D^{MeOH} = -9.9$. ¹H-NMR δ (200 MHz, CD₃OD) 4.37 (2 H, d, J 6.2), 4.07 (1 H, q, J 7.0), 1.67 – 1.25 (9 H, m). ¹³C-NMR δ (50 MHz, CD₃OD) 176.42, 172.42, 169.68, 55.89, 50.76, 49.15, 27.11, 26.67, 18.94, 17.31. (+)ESI-MS (m/z) : [M+Na]⁺ 196.2. Anal.Calcd for C₇H₁₅N₃O₂ (173.12): C, 48.54; H, 8.73; N, 24.26; O, 18.47; found C, 48.59; H, 8.78; N, 24.31; IR(NaCl): ν = 3391, 3191, 3078, 1673, 1538 cm⁻¹

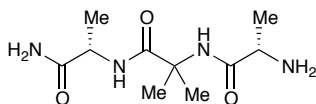
***tert*-butyl ((*S*)-1-((1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamate (**34**)**



34

To a mixture of Boc-LAlaOH **33** in dry DCM (0,1 M), under nitrogen at 0°C was added HOAt (1.1 eq.), EDC (1.1eq.), and let react under stirring at 0°C for 1 h. After that was added **32** (1.1 eq.) and DIPEA (2.2 eq.), additional DIPEA was used to reach pH = 8, and then the reaction mixture was let react at room temperature for 12 h. The reaction was monitored by TLC (MeOH : DCM, 1 :10; R_f = 0,19) detected by ninhydrin. Upon the consumption of starting material the reaction mixture was washed with saturated NH₄Cl, saturated NaHCO₃, and brine. The NH₄Cl, saturated was extracted 3 times with EtOAc. The recombining organic phase was dried over Na₂SO₄ and the solvent evaporated at reduced pressure. The crude of reaction was purified by flash chromatography with eluent gradient MeOH : DCM, 1 : 10 to 1 : 5 to afford compound **34** as white wax with 69% of yield. The reaction was performed with comparable yield from 0.1g. to 2g. $\alpha_D^{MeOH} = -20$. ¹H-NMR δ (200 MHz, CDCl₃) 7.32 (1 H, d, J 7.6), 7.15 (1 H, s), 6.53 (1 H, s), 5.24 (1 H, bs), 4.99 (1 H, bs), 4.51 – 4.30 (1 H, m), 3.92 (1 H, qd, J 7.1, 2.7), 1.58 (6 H, s), 1.52 – 1.34 (15 H, m). ¹³C-NMR δ (50 MHz, cdcl₃) 175.62, 173.59, 173.40, 156.66, 81.91, 57.03, 52.79, 49.76, 28.40, 27.79, 23.85, 17.45, 17.33.(+)ESI-MS (m/z) : [M+Na]⁺ 367.2. Anal.Calcd for C₁₅H₂₈N₄O₅ (344.4): C, 52.31; H, 8.19; N, 16.27; O, 23.23; found C, 52.36; H, 8.24; N, 16.31. IR(KBr): ν = 3410, 3310, 2981, 2938, 1679, 1665, 1536 cm⁻¹

N-((*S*)-1-amino-1-oxopropan-2-yl)-2-((*S*)-2-aminopropanamido)-2-methylpropanamide (35** NH₂Ala-Aib-AlaCONH₂)**



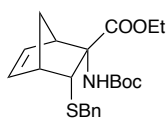
35

The compound **34** was dissolved in water (0.1 M) in a sealed tube for micro waves reactor. Under magnetic stirring the sample was irradiated by microwaves for 20 at temperature of 150°C. The reaction was monitored by TLC (MeOH : DCM, 1 :10) detected by ninhydrin. If not finished another round of 20 minutes was performed. Upon consumption of starting material water was evaporated at reduced pressure to afford the desired compound **35** as light yellow solid with 94% of yield. The reaction was performed with comparable

yield from 0.1g. to 2g. $\alpha_D^{\text{MeOH}} = -1.55$. $^1\text{H-NMR}$ δ 200 MHz, CD_3OD) 4.30 (1 H, q, J 7.2), 3.44 (1 H, q, J 6.9), 1.53 – 1.19 (12 H, m). $^{13}\text{C-NMR}$ δ (50 MHz, CD_3OD) 176.70, 176.67, 175.32, 56.32, 50.38, 49.28, 24.48, 23.86, 19.65, 16.54. (+)ESI-MS (m/z) : $[\text{M}+\text{Na}]^+$ 244.15. Anal.Calcd for $\text{C}_{10}\text{H}_{20}\text{N}_4\text{O}_3$ (267.3): C, 49.17; H, 8.25; N, 22.93; O, 19.65; found C, 49.22; H, 8.29; N, 22.98. IR(KBr): $\nu = 3399, 3064, 2985, 1660, 1530 \text{ cm}^{-1}$.

7.2.2 Synthesis of compounds in scheme 2.10:

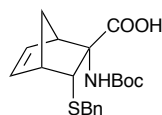
(1*R*,2*R*,3*R*,4*S*)-ethyl 3-(benzylthio)-2-((*tert*-butoxycarbonyl)amino)bicyclo[2.2.1]hept-5-ene-2-carboxylate



36

To solution of compound *exo*-**13'b** in dry DCM (0.1 M) was added at 0°C Boc-anhydride (2 eq.) and then dry TEA (2 eq.) and let react at r.t. for 4 h. The reaction is monitored by TLC (EtOAc : hexane, 1 : 2) detected by ninhydrin. Upon the consumption of starting material the crude mixture was diluted with DCM and washed with HCl 1M, saturated NaHCO_3 , and brine. The organic phase was dried over Na_2SO_4 and the solvent was evaporated at reduced pressure. The crude product was purified by flash chromatography (Eluent EtOAc : hexane, from 1 : 6 to 1 : 2) affording the **36** in pure form as colorless oil with 90% of yield. The reaction was performed with comparable yield from 0.1g. to 2g. $^1\text{H-NMR}$ δ (200 MHz, CDCl_3) 7.44 – 7.18 (5 H, m), 6.19 (1 H, s), 6.17 – 6.06 (1 H, m), 5.84 (1 H, s), 4.32 – 4.12 (2 H, m), 3.79 (2 H, s), 3.65 (1 H, s), 3.49 (1 H, s), 2.89 (1 H, s), 1.82 (1 H, d, J 9.3), 1.65 – 1.37 (10 H, m), 1.29 (3 H, t, J 7.1). $^{13}\text{C-NMR}$ δ (50 MHz, CDCl_3) 174.1, 155.02, 138.0, 137.28, 129.10, 128.78, 79.7, 64.7, 61.56, 56.51, 50.41, 48.55, 47.40, 37.14, 28.55, 14.39. (+)ESI-MS (m/z) : $[\text{M}+\text{Na}]^+$ 426.2. Anal.Calcd for $\text{C}_{22}\text{H}_{29}\text{NO}_4\text{S}$ (403.3): C, 65.48; H, 7.24; N, 3.47; O, 15.86; S, 7.95; found C, 65.53; H, 7.20; N, 3.42; IR(KBr): $\nu = 3358, 2979, 1738, 1714, 1530 \text{ cm}^{-1}$.

(1*R*,2*R*,3*R*,4*S*)-3-(benzylthio)-2-((*tert*-butoxycarbonyl)amino)bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (**37**)



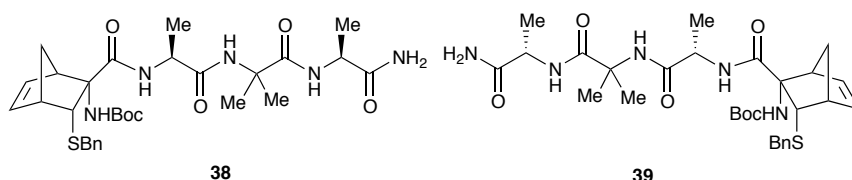
37

To solution of compound **36** in dry DCM/MeOH 9 : 1 (0.1 M) in sealed reactor was added a solution of KOH 6 M (20 eq.) and let react at 70°C for 8 h. The reaction is monitorign bt Tlc (EtOAc : hexane, 1 : 2) detected by ninhydrin and UV light. Upon the consupstion of starting material the crude was filtered on buchenr under vacuum, the organic solvent was evaporated the crude was dissolved in HCl 1M at 0°C until pH = 2. Than was extracted 3 times in EtOAc. The recombining organic phase was dry over Na₂SO₄ and the solvent was evaporated at reduced pressure affording the **37** in pure form as colorless wax with 88% of yield .The reaction was performed with comparable yield from 0.1g. to 1.0 g. ¹H-NMR δ (200 MHz, CDCl₃) 7.42 – 7.20 (5 H, m), 6.23 (1 H, dd, J 5.5, 2.7), 6.14 (1 H, s), 6.04 – 5.94 (1 H, m), 4.16 (1 H, d, J 3.1), 3.74 (1 H, s), 3.67 (2 H, s), 3.02 (1 H, s), 1.69 (1 H, d, J 9.6), 1.54 (1 H, d, J 9.8), 1.47 (9 H, s). ³C-NMR δ (50 MHz, CDCl₃) 174.61, 158.59, 138.01, 137.26, 135.74, 129.12, 127.51, 82.04, 65.05, 55.68, 50.25, 48.82, 46.05, 37.75, 29.91, 28.46 (+)ESI-MS (m/z) : [M+Na]⁺ 398.2. Anal.Calcd for C₂₀H₂₅NO₄S (375.15): C, 63.97; H, 6.71; N, 3.73; O, 17.04; S, 8.54; found C, 63.92; H, 6.65; N, 3.77; .IR(KBr): ν = 3339, 2965, 1715, 1485, 1394 cm⁻¹.

tert-butyl ((1R,2R,3R,4S)-2-(((S)-1-((1-(((S)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-3-(benzylthio)bicyclo[2.2.1]hept-5-en-2-yl)carbamate (38)

and

tert-butyl ((1S,2S,3S,4R)-2-(((S)-1-((1-(((S)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)-3-(benzylthio)bicyclo[2.2.1]hept-5-en-2-yl)carbamate (39)



38

39

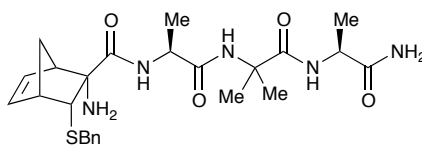
To a solution of acid **37** in dry DCM (0,1 M), under nitrogen at 0°C was added HOAt (1.1 eq.), EDC (1.1eq.), and let react under stirring ay 0°C for 1 h. After that was added **35** (NH₂Ala-Aib-AlaCONH₂) (1.1 eq.) and DIPEA (2.2 eq.), additional DIPEA was use to reach pH = 8, and thane the reaction mixture was let

react at room temperature for 8 h. The reaction was monitoring by Tlc (MeOH : DCM, 1 :10) detected by ninhydrin or UV light. Upon the consumption of starting material the reaction mixture was washed with saturated NH₄Cl, saturated NaHCO₃, and brine. The NH₄Cl, saturated was extracted 3 time with EtOAc. The recombining organic phase was dry over Na₂SO₄ and the solvent evaporated at reduced pressure. The crude of reaction was purified by flash chromatography with eluent gradint DCM : MeOH, 120 : 1 to 10 : 1 to afford the separation of compound **38** and **39**. The reaction was performed with comparable yield from 0.1g. to 1g.

38 was obtain in pure form as white solid (m.p. = 101°C) with 75% of yield. 0.1g. to 2g. $\alpha_D^{MeOH} = +50$. ¹H-NMR (200 MHz, CDCl₃) 7.55 (1 H, s), 7.39 – 7.17 (7 H, m), 6.71 (1 H, d, J 4.0), 6.32 (1 H, dd, J 5.6, 2.9), 6.07 (1 H, dd, J 5.6, 3.0), 5.95 (1 H, s), 5.21 (1 H, s), 4.51 – 4.29 (1 H, m), 3.94 (1 H, qd, J 7.2, 3.9), 3.70 (2 H, s), 3.62 (1 H, s), 3.41 (1 H, d, J 3.0), 3.01 (1 H, s), 2.20 (1 H, d, J 9.7), 1.64 – 1.51 (6 H, m), 1.51 – 1.44 (12 H, m), 1.44 – 1.34 (4 H, m); ³C-NMR δ (50 MHz, CDCl₃) 175.73, 174.31, 173.44, 173.35, 156.61, 137.74, 137.35, 137.27, 129.10, 128.74, 127.96, 81.70, 64.54, 57.45, 56.93, 52.57, 51.13, 49.66, 48.72, 47.38, 37.86, 28.42, 27.83, 23.69, 17.46, 17.28. (+)ESI-MS (m/z) : [M+Na]⁺ 624.4. Anal.Calcd for C₃₀H₄₃N₅O₆S (601.3): C, 59.88; H, 7.20; N, 11.64; O, 15.95; S, 5.33 found C, 59.96; H, 7.23; N, 11.61;; .IR(KBr): ν = 3327, 2981, 1660, 1531, 1455 cm⁻¹.

39 was obtain in pure form as white solid (m.p. = 134°C) with 75% of yield. 0.1g. to 2g. $\alpha_D^{MeOH} = -66.1$. ¹H-NMR (200 MHz, CDCl₃) 7.96 (1 H, s), 7.43 (1 H, d, J 7.44) 7.27– 7.26 (5 H, m), 6.75 (1 H, d, J 3.6), 6.58– 6.54 (1 H, m), 6.28–6.24 (1 H, dd), 5.29 (1 H, s), 4.88 (1 H, s), 4.33 (1 H, t, J 7.3), 4.24 (1 H, d, J 3.6), 4.06– 4.01 (1 H, m), 3.60 (2 H, s), 3.06 (1 H, s), 3.03 (1 H, s), 1.75(1 H, d, J 9.5), 1.62 – 1.34 (22 H, m); ³C-NMR δ (50 MHz, CDCl₃) 176.4, 174.61, 173.84, 173.31, 157.41, 142.74, 138.4, 133.27, 129.12, 128.78, 127.44, 81.70, 70.51, 57.52, 54.2, 53.57, 52.67, 50.06, 49.9, 45.9, 39.25, 28.82, 27.43, 23.9, 17.7, 17.48. (+)ESI-MS (m/z) : [M+Na]⁺ 624.4. Anal.Calcd for C₃₀H₄₃N₅O₆S (601.3): C, 59.88; H, 7.20; N, 11.64; O, 15.95; S, 5.33 found C, 59.92; H, 7.25; N, 11.60;; .IR(KBr): ν = 3310, 2981, 1657, 1532 cm⁻¹.

(1R,2R,3R,4S)-2-amino-N-((S)-1-((1-(((S)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-3-(benzylthio)bicyclo[2.2.1]hept-5-ene-2-carboxamide (40)

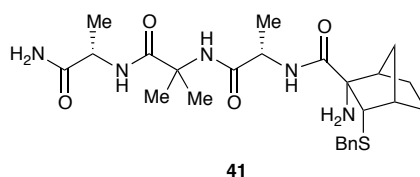


40

The compound **38** was dissolved in water (0.1 M) in a sealed tube for micro waves reactor. Under magnetic stirring the sample was irradiate by microwaves for 30 at temperature of 150°C. The reaction was monitoring

by TLC (MeOH : DCM, 1 :10) detected by ninhydrin. If not finish an other round of 20 minutes was performed. Upon consumption of starting material water was evaporated at reduced pressure. The crude of reaction was purified by automatezed chromatography with prepack grace column with eluent gradint MeOH : DCM, 1 : 20 to 1 : 10 to afford the compound **40** in a pure form as white solid (m.p. 65°C) with 88% of yield. The reaction was performed with comparable yield from 0.1g. to 0.4g , $\alpha_D^{MeOH} = +38.57$. ^1NMR (200 MHz, CDCl_3) 7.77 (1 H, d, J 3.6), 7.33 – 7.16 (7 H, m), 6.52 (1 H, s), 6.42 (1 H, dd, J 5.6, 3.0), 6.19 (1 H, dd, J 5.6, 3.0), 5.40 (1 H, s), 4.42-4.35 (1 H, m), 4.00 (1 H, d, J 3.4), 3.97 – 3.84 (1 H, m), 3.69 (1 H, d, J 13.6, AB system), 3.60 (1 H, d, J 13.7, AB systme), 3.11 (1 H, s), 2.77 (1 H, s), 2.04 (1 H, d, J 8.8) 1.53-1.30 (15H,m). $^{13}\text{C-NMR}$ δ (50 MHz, CDCl_3) 178.48, 175.69, 173.56, 173.03, 140.10, 139.48, 134.94, 128.96, 128.86, 127.35, 66.77, 56.96, 55.61, 54.81, 51.44, 49.87, 49.73, 45.72, 38.57, 27.49, 23.93, 17.54, 16.52. (+)ESI-MS (m/z) : $[\text{M}+\text{Na}]^+$ 524.4. Anal.Calcd for $\text{C}_{25}\text{H}_{35}\text{N}_5\text{O}_4\text{S}$ (501.3): C, 59.86; H, 7.03; N, 13.96; O, 12.76; S, 6.39 found C, 59.82; H, 7.09; N, 13.91; .IR(KBr): $\nu = 3310, 2980, 1657, 1522 \text{ cm}^{-1}$.

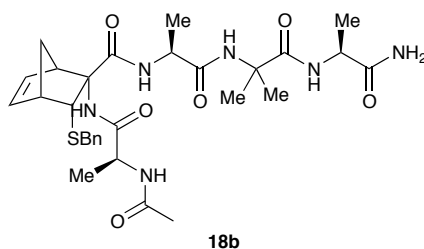
(1*R*,2*S*,3*S*,4*S*)-2-amino-*N*-((*S*)-1-((1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-3-(benzylthio)bicyclo[2.2.1]heptane-2-carboxamide
(41)



The compound **39** was dissolved in water (0.1 M) in a sealed tube for micro waves reactor. Under magnetic stirring the sample was irradiate by microwaves for 30 at temperature of 150°C. The reaction was monitoring by TLC (MeOH : DCM, 1 :10) detected by ninhydrin. If not finish an other round of 20 minutes was performed. Upon consumption of starting material water was evaporated at reduced pressure. The crude of reaction was purified by automatezed chromatography with prepack grace column with eluent gradint MeOH : DCM, 1 : 20 to 1 : 15 to afford the compound **41** in a pure form as colour less wax. with 82% of yield. The reaction was performed with comparable yield from 0.1g. to 0.4g , $\alpha_D^{MeOH} = -7.65$. ^1NMR (200 MHz, CDCl_3) 8.10 (1 H, d, J 4.6), 7.22 (5 H, t, J 13.6), 7.10 (1 H, d, J 7.7), d 7.02 (1 H, s), 6.71 (1 H, s), 6.43 (1 H, dd, J 5.5, 2.9), 6.24 (1 H, dd, J 5.6, 3.0), 5.49 (1 H, s), 4.46 – 4.23 (1 H, m), 4.12 (1 H, dt, J 12.0, 7.1), 3.92 (1 H, d, J 3.3), 3.70 (2 H, s), 3.01 (1 H, s), 2.95 (1 H, s), 2.00(1 H, d, J 9.5), 1.67 – 1.25 (15 H, m). $^{13}\text{C-NMR}$ δ (50 MHz, CDCl_3) 178.1, 175.46, 173.76, 172.83, 140.13, 138.2, 134.9, 128.91, 128.80, 127.56, 66.53, 57.16, 55.01, 54.31, 51.44, 49.7, 48.6, 46.25, 37.37, 26.78, 24.6, 17.54, 16.9. (+)ESI-MS (m/z) :

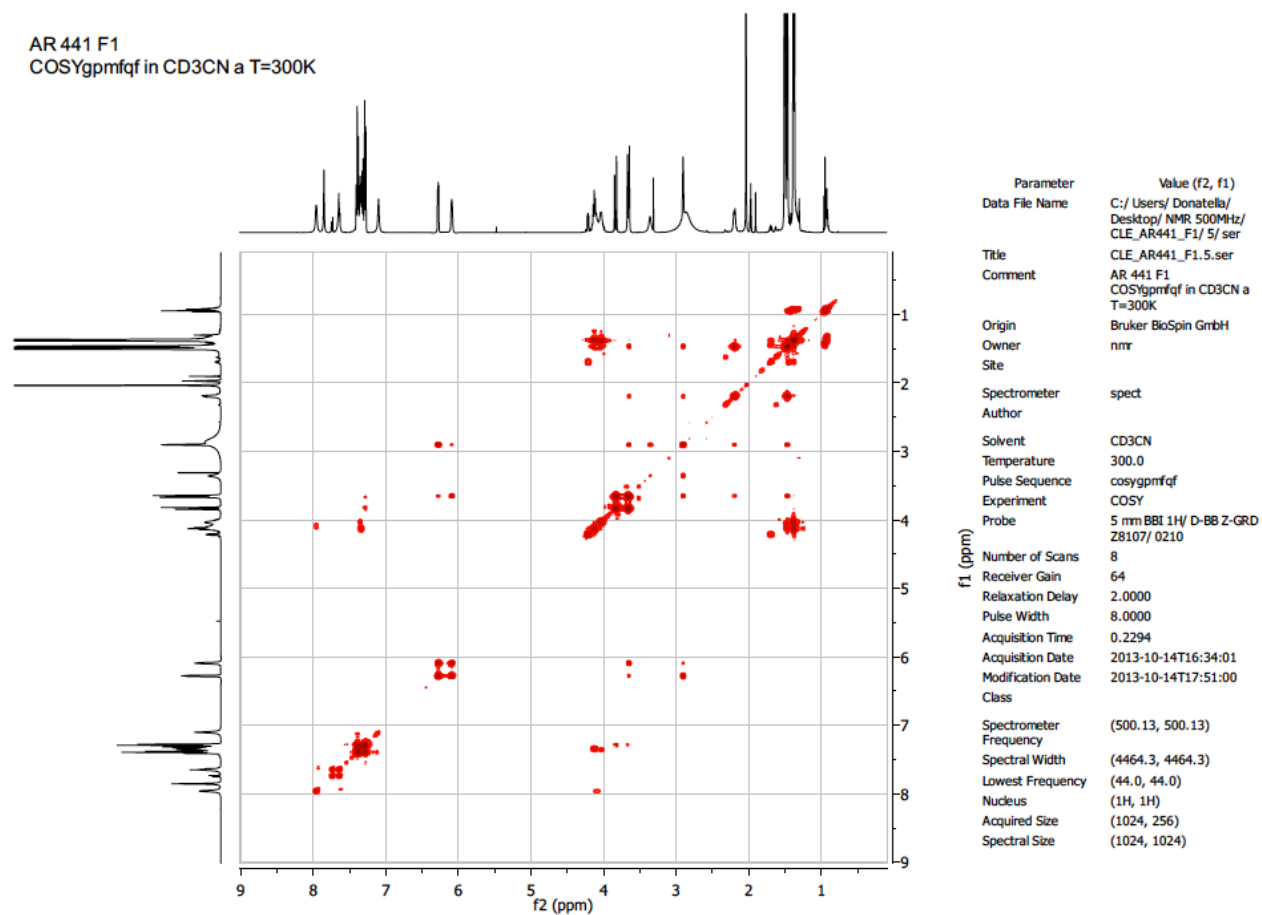
$[M+Na]^+$ 524.4. Anal.Calcd for $C_{25}H_{35}N_5O_4S$ (501.3): C, 59.86; H, 7.03; N, 13.96; O, 12.76; S, 6.39 found C, 59.89; H, 7.09; N, 13.89; IR(KBr): $\nu = 3317, 2975, 1653, 1521\text{ cm}^{-1}$.

Synthesis of (1*R*,2*R*,3*R*,4*S*)-2-((*S*)-2-acetamidopropanamido)-*N*-((*S*)-1-((1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-3-(benzylthio)bicyclo[2.2.1]hept-5-ene-2-carboxamide

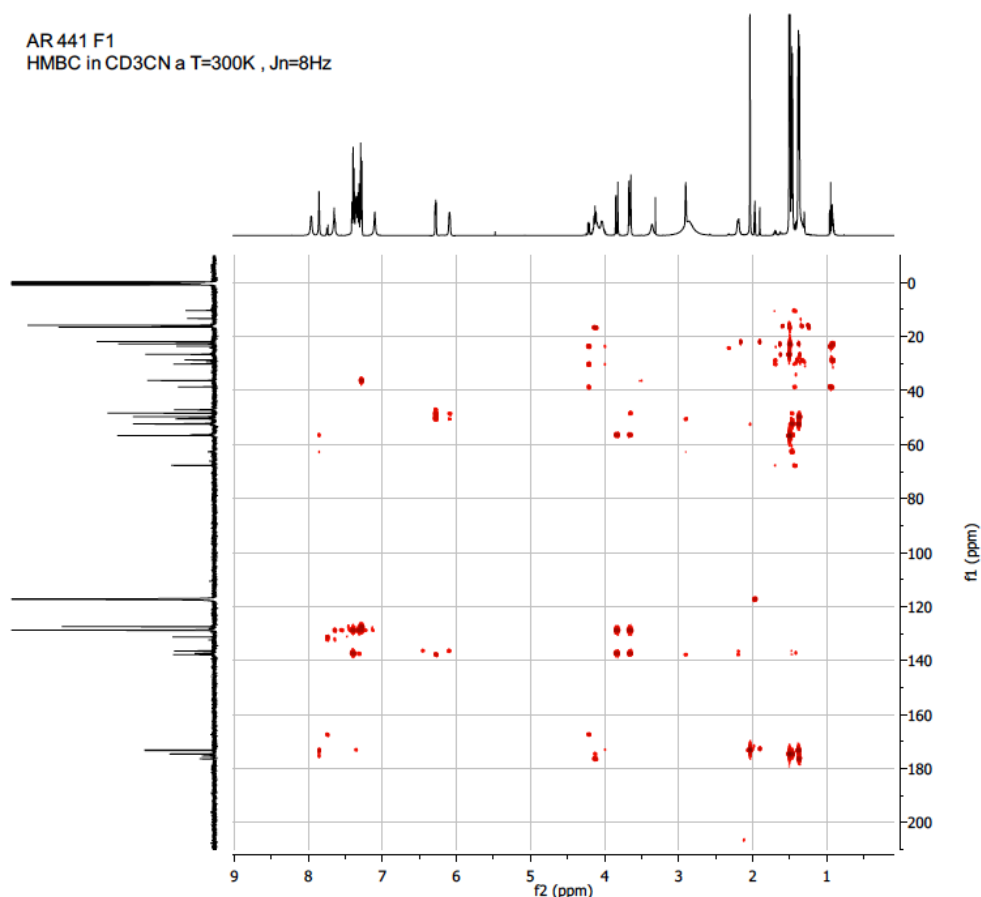


To a solution of acid (L)-NHAc-Ala-OH (1. eq.) in dry DCM (0.1 M), under nitrogen at 0°C was added HOAt (1.1 eq.), EDC (1.1eq.), and let react under stirring at 0°C for 1 h. After that was added **40** (NH₂Ala-Aib-AlaCONH₂) (1.1 eq.) and DIPEA (2.2 eq.), additional DIPEA was used to reach pH = 8, and then the reaction mixture was let react at room temperature for 12 h. The reaction was monitored by HPTlc (MeOH : DCM, 1 :10) detected by ninhydrin or UV light and by the use of H-NMR. Upon the consumption of starting material the reaction mixture was washed with saturated NH₄Cl, saturated NaHCO₃, and brine. The NH₄Cl, saturated was extracted 3 times with EtOAc. The recombining organic phase was dried over Na₂SO₄ and the solvent evaporated at reduced pressure. The crude of reaction was purified by inverse phase HPLC with eluent gradient 95% H₂O, 5% CH₃CN, 0.1% TFA to 70% H₂O 30% CH₃CN 0.1% TFA to afford compound **18b**, separated from the recovery starting materials 40%, in a pure form as solid wax with 40% of yield. The reaction was performed with comparable yield from 0.1g. to 2g. $\alpha_D^{MeOH} = +29.26$. ¹H- ¹NMR δ H (500 MHz, CD₃CN) 7.96 (1 H, d, J 4.7), 7.85 (1 H, s), 7.65 (1 H, s), 7.39-7.20. (7H, m), 7.10 (1 H, s), 6.28 (1 H, dd, J 5.6, 2.8), 6.09 (1 H, s), 5.77 (1 H, s), 4.19 – 3.94 (1 H, m), 4.15 – 4.07 (1 H, m), 4.05-4.00 (1 H, m), 3.83 (1 H, d, J 13.6, AB system), 3.66 (2 H, d, J 13.4, AB system), 3.64 (1 H, s), 3.36 (1 H, s), , 2.90 (2 H, s), 2.20 (1 H, d, J 8.0), 2.02 (3 H, s), 1.51 (3 H, s), 1.50 (3 H, s), 1.47 (4 H, d, J 7.4), 1.38 (3 H, d, J 3.9), 1.37 (3 H, d, J 3.9). ¹³C-NMR δ (126 MHz, CD₃CN) 174.63, 174.61, 174.59, 173.32, 173.13, 137.81, 137.36, 136.48, 131.26, 128.77, 127.46, 117.29, 67.76, 56.68, 56.43, 52.39, 52.30, 50.48, 49.70, 48.39, 47.16, 38.67, 36.33, 26.60, 22.72, 21.92, 16.54, 16.03, 15.84. (+)ESI-MS (m/z) : $[M+Na]^+$ 637.4. Anal.Calcd for $C_{30}H_{42}N_6O_6S$ (614.3): C, 58.61; H, 6.89; N, 13.67; O, 15.62; S, 5.22 found C, 58.62; H, 6.89; N, 13.68; IR(KBr): $\nu = 3333, 2974, 1656, 1529\text{ cm}^{-1}$.

7.2.3 2D-NMR, VT-NMR , DMSO titration, 18b

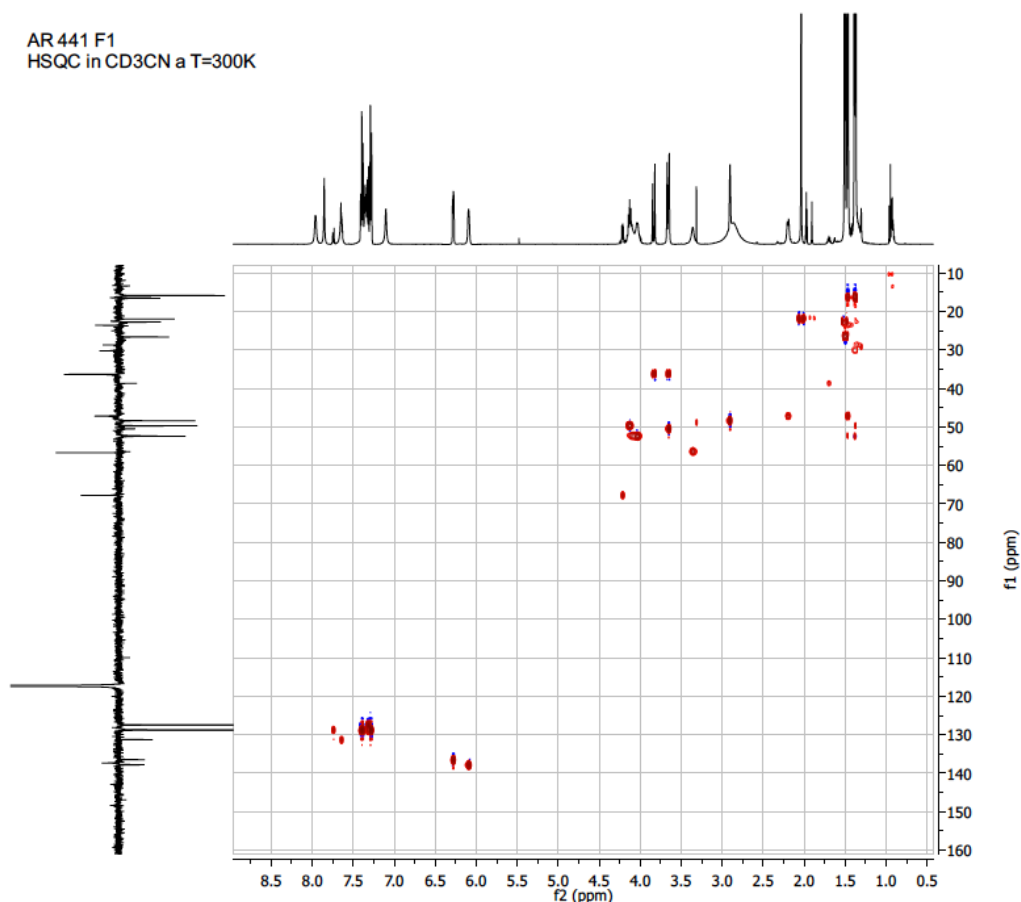


AR 441 F1
HMBC in CD3CN a T=300K , Jn=8Hz



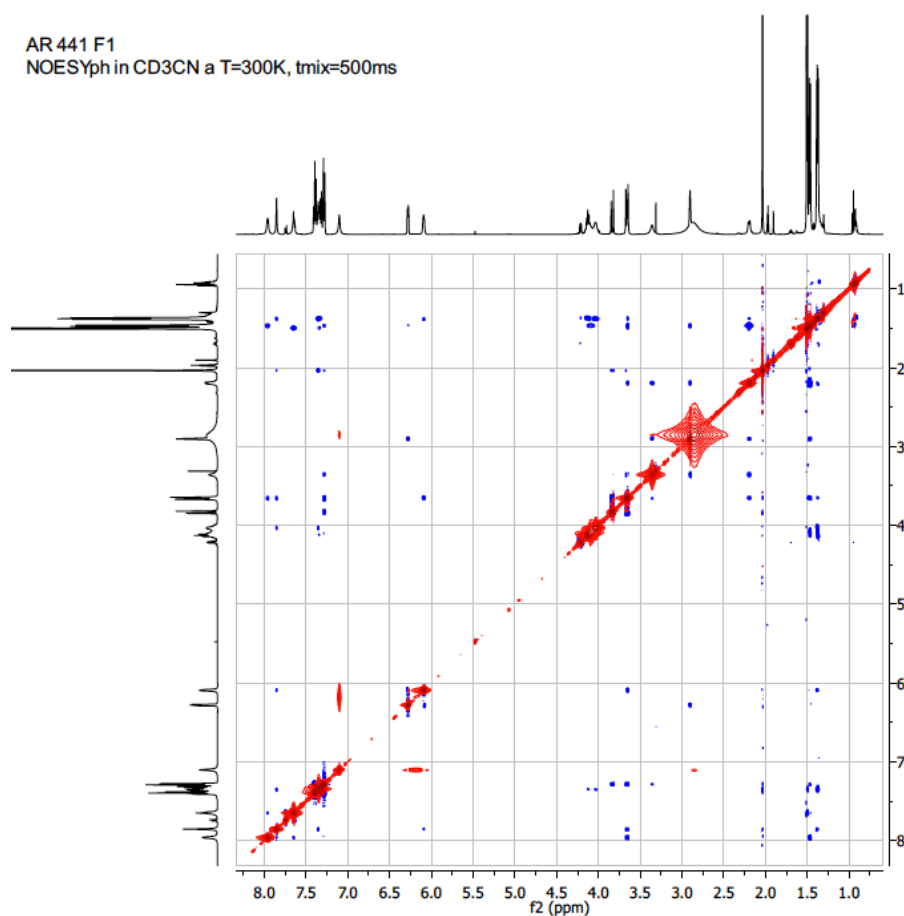
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Title	CLE_AR441_F1.9.1.2rr
Comment	AR 441 F1 HMBCgp (d6=50ms, Jn=8Hz) in CD3CN a T=300K
Origin	Bruker BioSpin GmbH
Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	300.0
Pulse Sequence	hmbcgp1ndqf
Experiment	HMBC
Probe	5 mm BBI 1H/ D-BB Z-GRD Z8107/ 0210
Number of Scans	16
Receiver Gain	23170
Relaxation Delay	2.0000
Pulse Width	8.0000
Acquisition Time	0.2294
Acquisition Date	2013-10-15T01:49:35
Modification Date	2013-10-15T04:26:00
Class	
Spectrometer Frequency	(500.13, 125.76)
Spectral Width	(4464.3, 27669.5)
Lowest Frequency	(44.0, -1259.0)
Nucleus	(1H, 13C)
Acquired Size	(1024, 256)
Spectral Size	(2048, 2048)

AR 441 F1
HSQC in CD3CN a T=300K

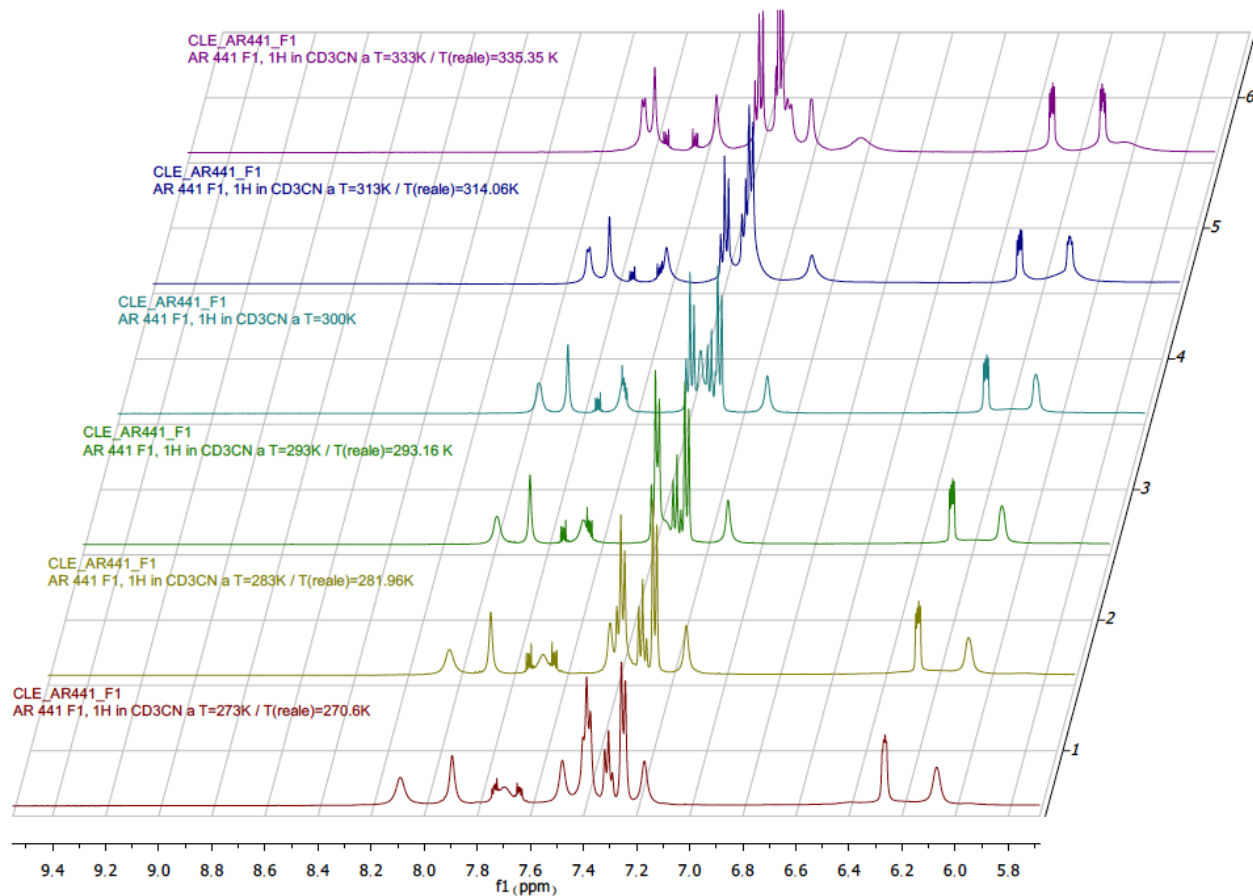
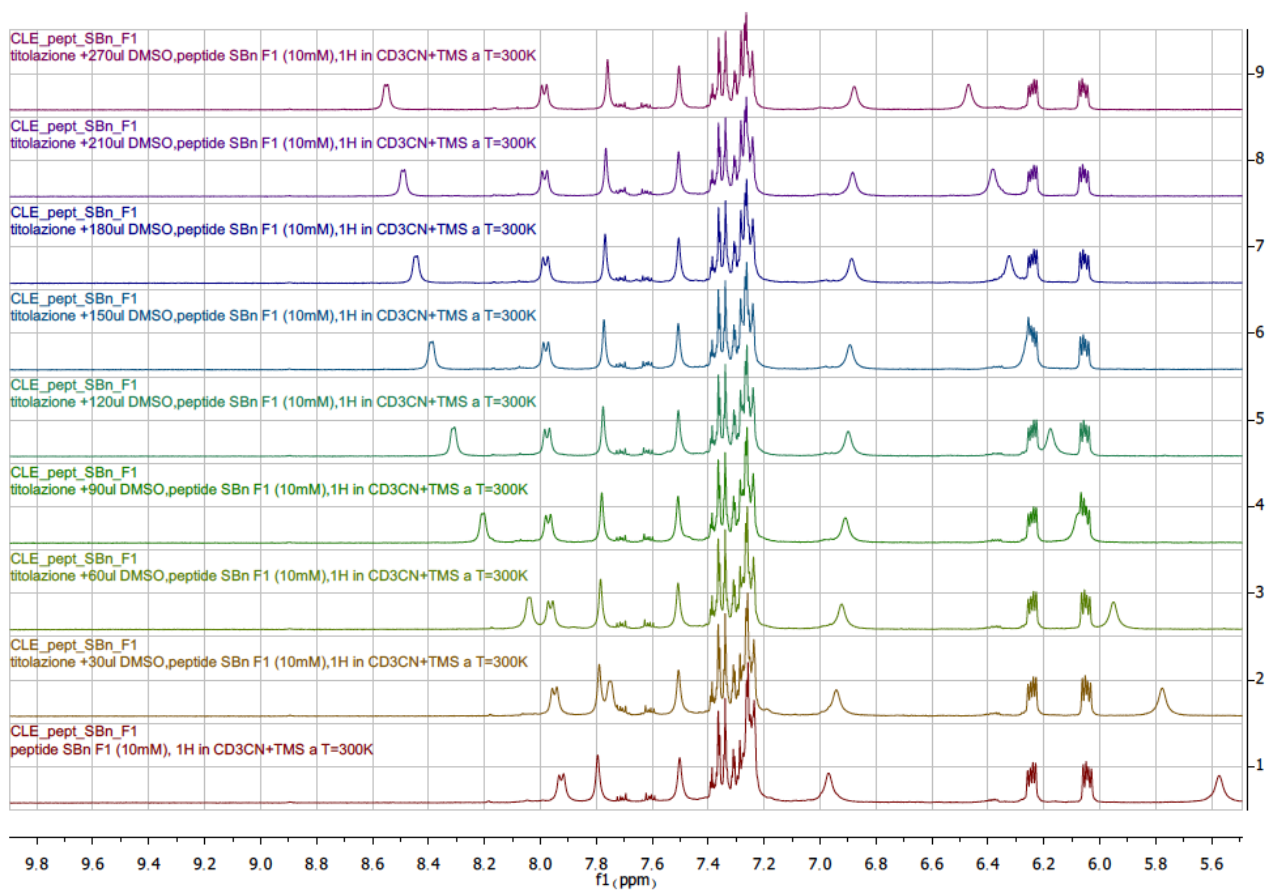


Parameter	Value (f2, f1)
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Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	300.0
Pulse Sequence	hsqcetgpsi
Experiment	HSQC
Probe	5 mm BBI 1H/ D-BB Z-GRD Z8107/ 0210
Number of Scans	16
Receiver Gain	20642
Relaxation Delay	2.0000
Pulse Width	8.0000
Acquisition Time	0.2294
Acquisition Date	2013-10-14T17:52:31
Modification Date	2013-10-14T20:26:00
Class	
Spectrometer Frequency	(500.13, 125.77)
Spectral Width	(4464.3, 25154.1)
Lowest Frequency	(44.0, -1.3)
Nucleus	(1H, 13C)
Acquired Size	(1024, 256)
Spectral Size	(2048, 2048)

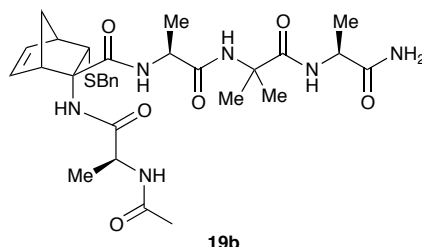
AR 441 F1
NOESYph in CD3CN a T=300K, tmix=500ms



Parameter	Value (f2, f1)
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Origin	Bruker BioSpin GmbH
Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	300.0
Pulse Sequence	noesyph
Experiment	NOESY
Probe	5 mm BBI 1H/ D-BB Z-GRD Z8107/ 0210
Number of Scans	8
Receiver Gain	64
Relaxation Delay	2.5000
Pulse Width	8.0000
Acquisition Time	0.2294
Acquisition Date	2013-10-14T23:57:07
Modification Date	2013-10-15T01:48:00
Class	
Spectrometer Frequency	(500.13, 500.13)
Spectral Width	(4464.3, 4464.3)
Lowest Frequency	(44.0, 44.0)
Nucleus	(1H, 1H)
Acquired Size	(1024, 256)
Spectral Size	(2048, 2048)



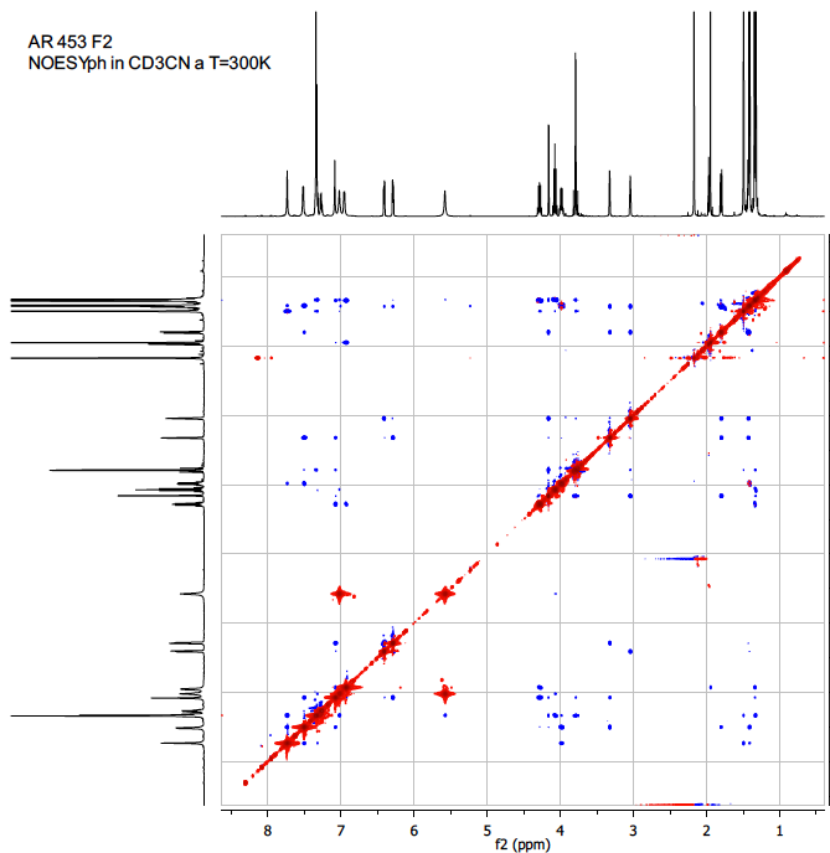
Synthesis of (1*S*,2*S*,3*S*,4*R*)-2-((*S*)-2-acetamidopropanamido)-*N*-((*S*)-1-((1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-3-(benzylthio)bicyclo[2.2.1]hept-5-ene-2-carboxamide
(19b)



To solution of **41** in THF (0.1 M) in a sealed tube for micro waves reactor was added EEDQ (1.1 eq.) and (L)-NHAc-Ala-OH (1. eq.). Under magnetic stirring the sample was irradiate by microwave for 30' at temperature of 60°C and power of 80 Watt using the air compressing cooling system of the reactor to keep down the temperature. The reaction was monitoring by HPTlc (MeOH : DCM, 1 :10) detected by ninhydrin. If not finish an other round of 30 minutes was performed. Upon consumption of starting material the tube was cooling down in ice bath and the precipitate was filtered on Buchner affording the obtainment of **19b** pure compound as with solid (m.p. = 76°C) without the need of further purification with 60% of yield. The slow precipitation in a concentrated solution of compound **19b** in CH₃CN permit the obtain of a single crystal for X-Ray analysis. The reaction was performed with comparable yield from 50mg to 100mg , $\alpha_D^{MeOH} = -19.65$. ¹NMR δ H (500 MHz, CD₃CN) 7.73 (1 H, s), 7.52 (1 H, d, J 4.5), 7.33 (5 H, dd, J 4.8, 1.6), 7.27 (1 H, ddd, J 5.9, 4.8, 2.3), 7.08 (1 H, s), 7.02 (1 H, s), 6.95 (1 H, d, J 5.3), 6.41 (1 H, dd, J 5.6, 2.9), 6.29 (1 H, dd, J 5.7, 3.0), 5.58 (1 H, s), 4.32 – 4.23 (1 H, m), 4.16 (1 H, d, J 3.5), 4.07 (1 H, t, J 7.4), 3.99 (1 H, qd, J 7.3, 4.9), 3.81 (1 H, d, J 12.6, AB system), 3.77 (1 H, d, J 12.6 AB system), 3.33 (1 H, s), 3.04 (1 H, s), 1.95 (3 H, s), 1.80 (1 H, d, J 9.3), 1.49 (6 H, d, J 1.3), 1.45 – 1.38 (4 H, m), 1.34 (3 H, d, J 5.0), 1.33 (3 H, d, J 5.2). ³C-NMR δ (126 MHz, CD₃CN) 175.46, 174.49, 174.16, 173.93, 173.90, 171.45, 139.75, 138.60, 134.81, 128.78, 128.47, 126.96, 117.28, 68.17, 56.60, 55.06, 52.40, 51.99, 50.31, 49.67, 49.08, 45.45, 38.10, 26.26, 23.19, 21.93, 16.95, 16.11, 15.83.(+)ESI-MS (m/z) : [M+Na]⁺ 637.4. Anal.Cald for C₃₀H₄₂N₆O₆S (614.3): C, 58.61; H, 6.89; N, 13.67; O, 15.62; S, 5.22 found C, 58.66; H, 6.93; N, 13.64; IR(KBr): ν = 3310, 2984, 1656, 1533 cm⁻¹.

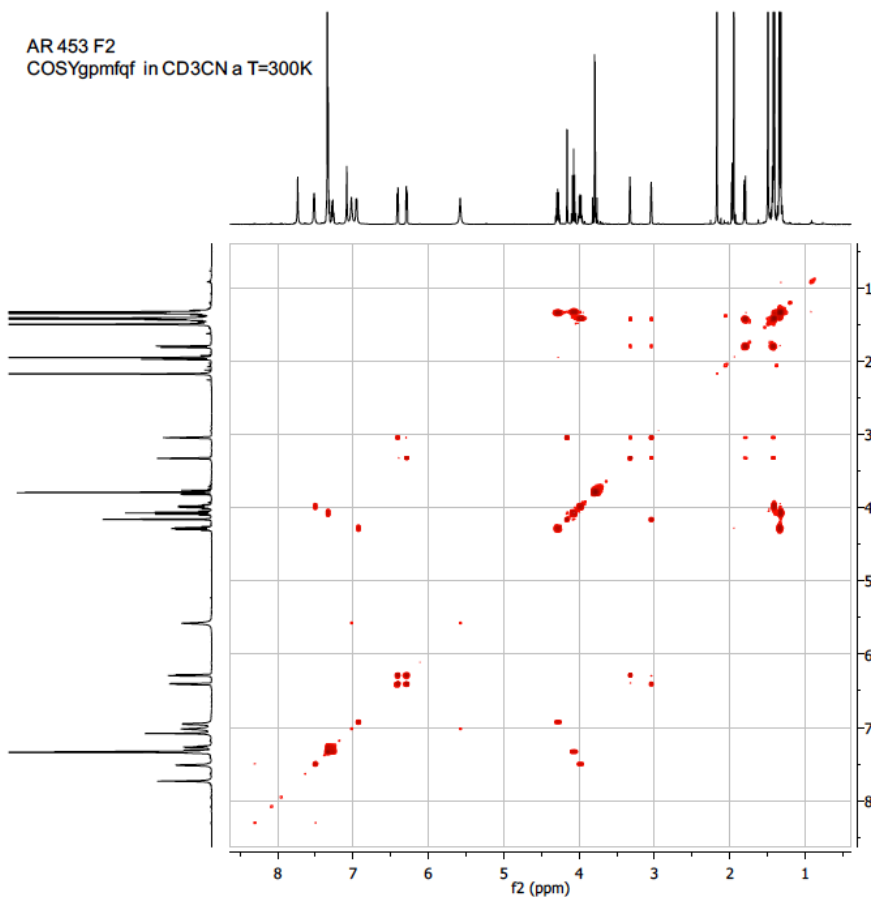
7.2.4 2D-NMR, VT-NMR , DMSO titration, 19b

AR 453 F2
NOESYph in CD3CN a T=300K



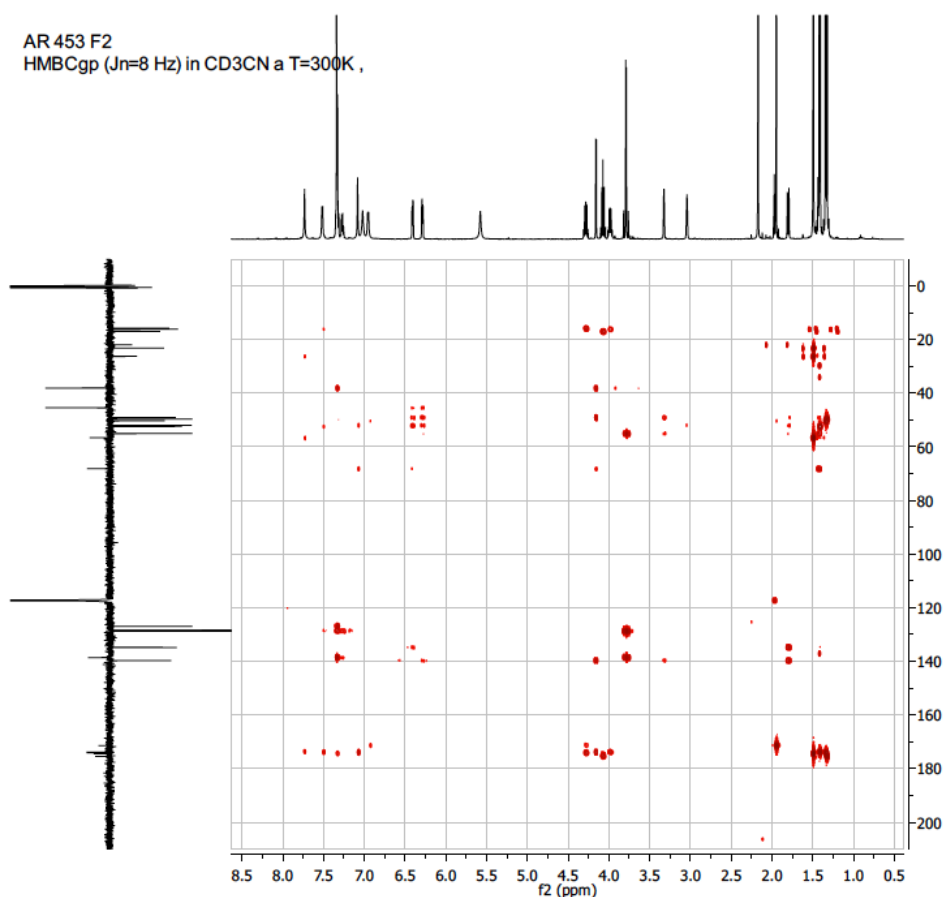
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Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	299.9
Pulse Sequence	noesyph
Experiment	NOESY
Probe	5 mm BB1 1H/ D-BB Z-GRD ZB107/ 0210
Number of Scans	8
Receiver Gain	128
Relaxation Delay	3.0000
Pulse Width	8.0000
Acquisition Time	0.2482
Acquisition Date	2013-10-18T20:25:43
Modification Date	2013-10-19T00:50:00
Class	
Spectrometer Frequency	(500.13, 500.13)
Spectral Width	(4125.4, 4125.4)
Lowest Frequency	(191.4, 191.4)
Nucleus	(1H, 1H)
Acquired Size	(1024, 512)
Spectral Size	(1024, 1024)

AR 453 F2
COSYgpmf in CD3CN a T=300K



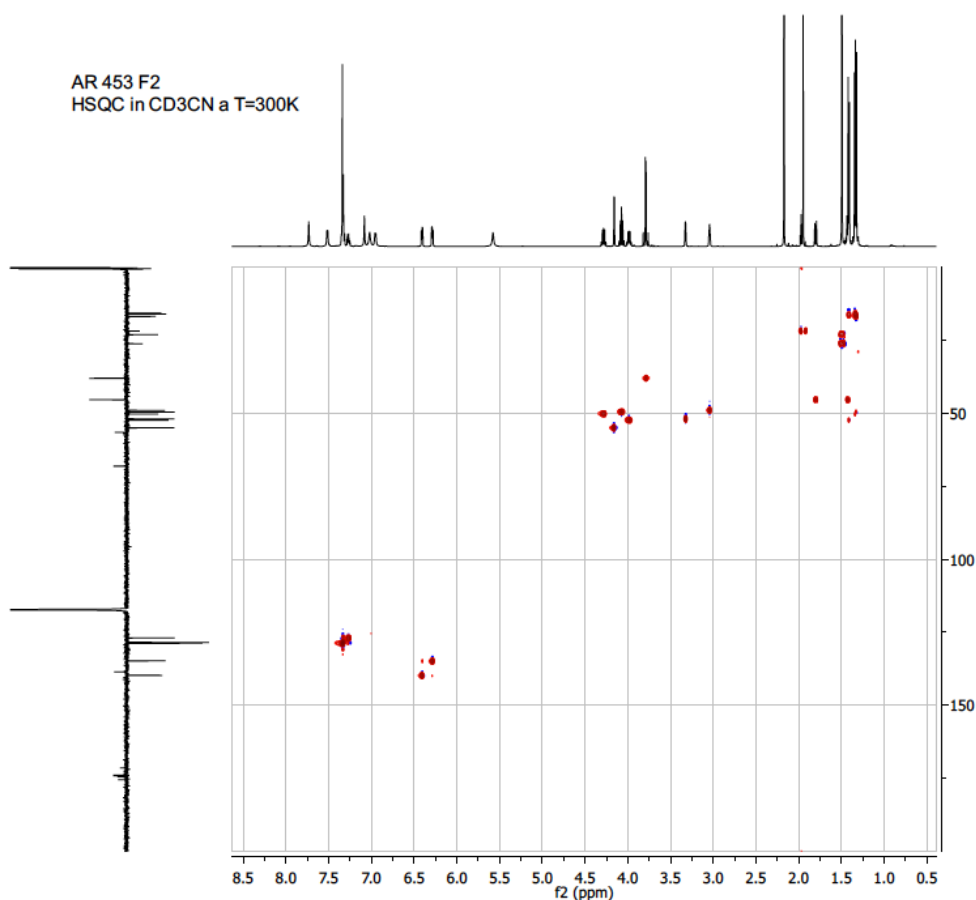
Parameter	Value (f2, f1)
Data File Name	C:/Users/Donatella/Desktop/NMR 500MHz/CLE_AR453_F2/8/ ser
Title	CLE_AR453_F2.8.ser
Comment	AR 453 F2 COSYgpmf in CD3CN a T=300K (BB1)
Origin	Bruker BioSpin GmbH
Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	300.0
Pulse Sequence	cosygpmf
Experiment	COSY
Probe	5 mm BB1 1H/ D-BB Z-GRD ZB107/ 0210
Number of Scans	8
Receiver Gain	128
Relaxation Delay	2.0000
Pulse Width	8.0000
Acquisition Time	0.2482
Acquisition Date	2013-10-18T16:30:23
Modification Date	2013-10-18T17:48:00
Class	
Spectrometer Frequency	(500.13, 500.13)
Spectral Width	(4125.4, 4125.4)
Lowest Frequency	(191.4, 191.4)
Nucleus	(1H, 1H)
Acquired Size	(1024, 256)
Spectral Size	(1024, 1024)

AR 453 F2
HMBCgp (Jn=8 Hz) in CD3CN a T=300K ,

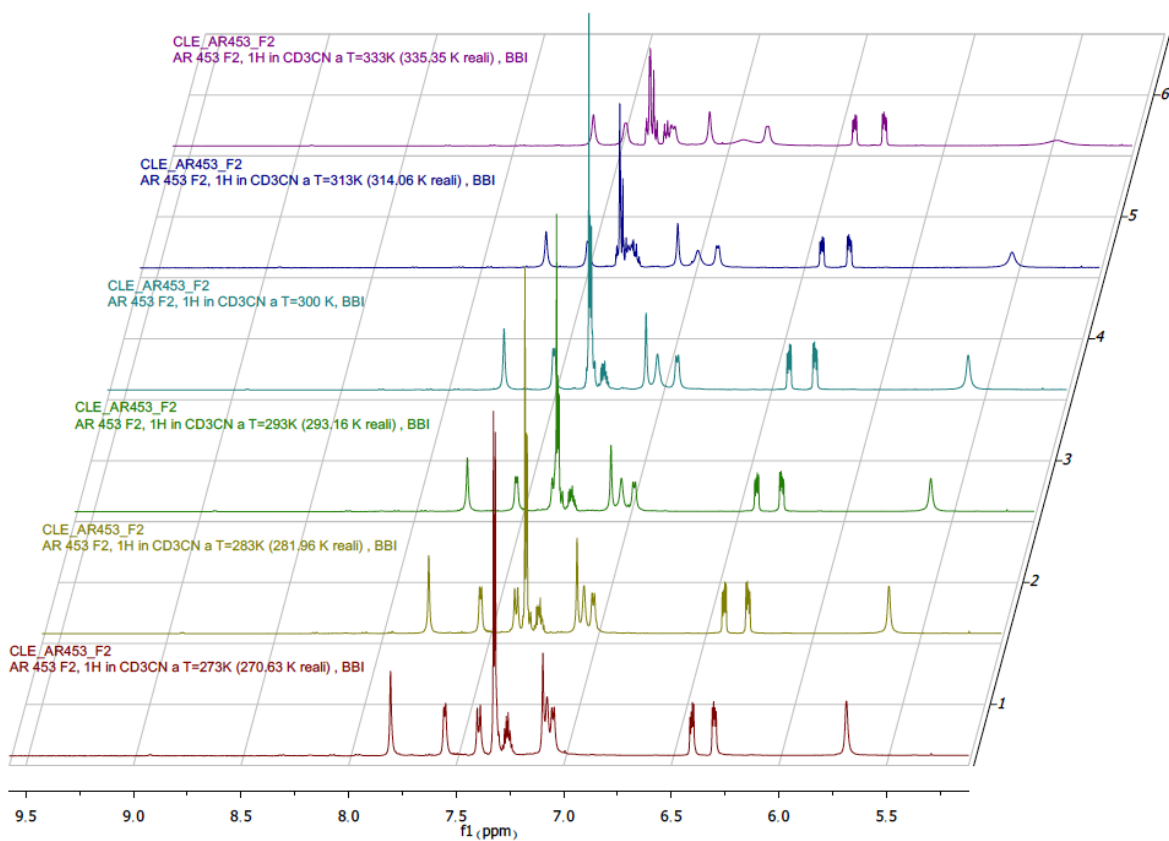
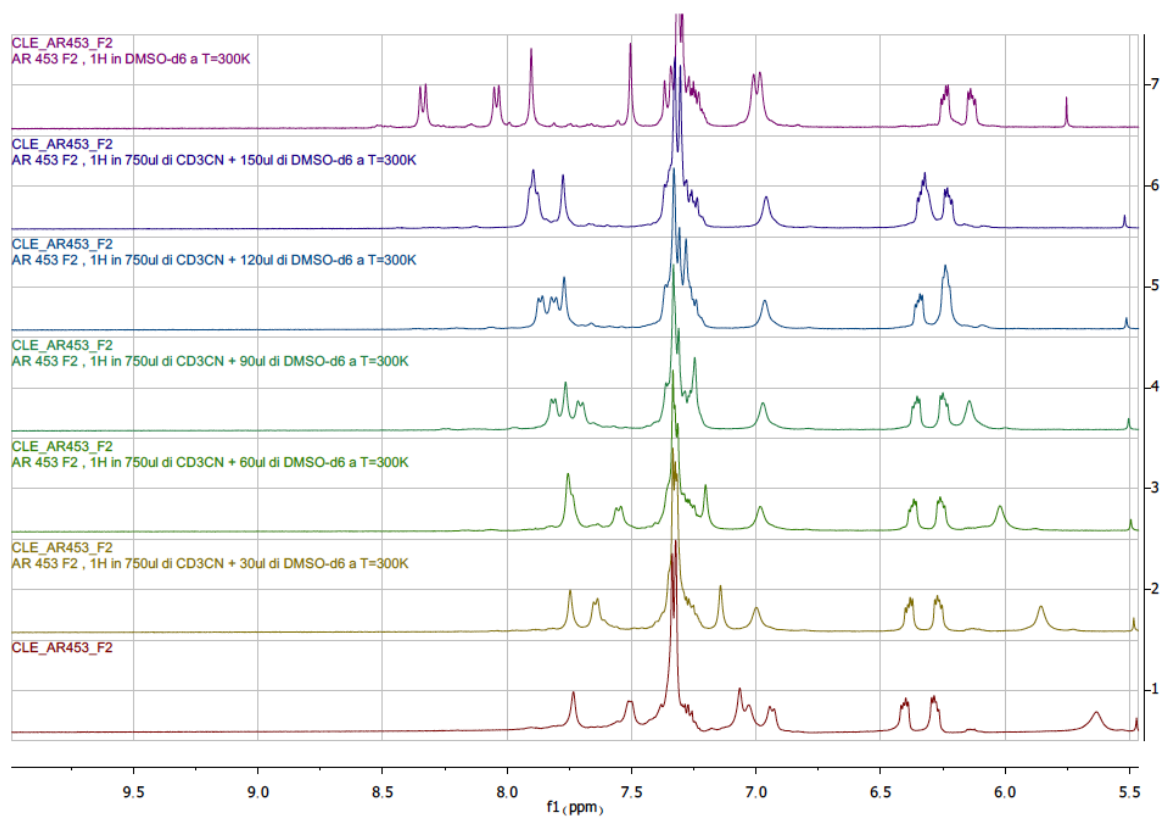


Parameter	Value (f2, f1)
Data File Name	C:/Users/Donatella/Desktop/ NMR 500MHz/ CLE_AR453_F2/ 12/ ser
Title	CLE_AR453_F2.12.ser
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Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	300.0
Pulse Sequence	hmbcgp1ndqf
Experiment	HMBC
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Relaxation Delay	2.0000
Pulse Width	8.0000
Acquisition Time	0.2482
Acquisition Date	2013-10-19T03:18:53
Modification Date	2013-10-19T05:57:00
Class	
Spectrometer Frequency	(500.13, 125.77)
Spectral Width	(4125.4, 27669.5)
Lowest Frequency	(191.4, -1259.0)
Nucleus	(1H, 13C)
Acquired Size	(1024, 256)
Spectral Size	(2048, 2048)

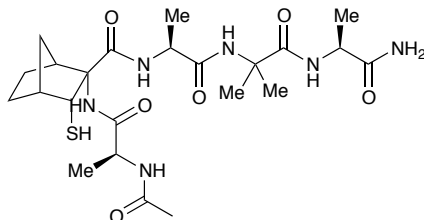
AR 453 F2
HSQC in CD3CN a T=300K



Parameter	Value (f2, f1)
Data File Name	C:/Users/Donatella/Desktop/ NMR 500MHz/ CLE_AR453_F2/ 9/ ser
Title	CLE_AR453_F2.9.ser
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Origin	Bruker BioSpin GmbH
Owner	nmr
Site	
Spectrometer	spect
Author	
Solvent	CD3CN
Temperature	300.6
Pulse Sequence	hsqcetgpsi
Experiment	HSQC
Probe	5 mm BB1 1H/ D-BB Z-GRD Z8107/ 0210
Number of Scans	16
Receiver Gain	20642
Relaxation Delay	2.0000
Pulse Width	8.0000
Acquisition Time	0.2482
Acquisition Date	2013-10-18T17:49:37
Modification Date	2013-10-18T20:24:00
Class	
Spectrometer Frequency	(500.13, 125.77)
Spectral Width	(4125.4, 25154.1)
Lowest Frequency	(191.4, -1.3)
Nucleus	(1H, 13C)
Acquired Size	(1024, 256)
Spectral Size	(1024, 1024)



Synthesis (1*S*,2*R*,3*R*,4*R*)-2-((*S*)-2-acetamidopropanamido)-*N*-((*S*)-1-((1-(((*S*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)-3-mercaptopbicyclo[2.2.1]heptane-2-carboxamide (43)



43

To a solution of **18b** in TFA (0.05 m) at 5° C under stirring was added slowly sulfynildibenzede (Ph₂SO) (5eq.) and after was added dropwise trichloromethylsiane (20 eq.) . The reaction was kept 6 h at 5 °C and monitoring by Tlc analysis (MeOH : DCM, 1 : 5). Upon the consumption of the starting material the crude was diluted with water and extracted with Et₂O to eliminate the excess of sulfynildibenzene. To the water phase was had NH₄OH until basic pH and was extract 3 times with EtOAc. The solvent was remove under reduced pressure and the crude was purified by preparative TLC (MeOH :DCM, 1:6) to afford the desire product **43** in mixture with side product. ¹NMR δ H (200 MHz, CD₃OD) 5.58 (1 H, s), 4.19 – 4.03 (3 H, m), 3.18 (1 H, s), 3.12 (1 H, s), 2.06 (3 H, s), 1.82 (2 H, s), 1.49 (6 H, d, J 1.3), 1.46 – 1.33 (19 H, m) ³C-NMR δ (126 MHz, CD₃CN) 175.72, 175.15, 174.79, 173.64, 173.57, 173.45, 97.28, 67.7, 63.4, 56.70, 54.26, 52.20, 51.44, 51.27, 49.86, 49.08, 46.14, 32.19, 25.6, 22.7, 21.5, 16.4, 15.89, 15.57.(+)ESI-MS (m/z) : [M+Na]⁺ 549.4. Anal.Calcd for C₂₃H₃₈N₆O₆S (526.3)..IR(KBr): ν = 3356, 2986, 2935, 2471, 1658, 1533 cm⁻¹.

7.3 Chapter 3

Full characterization and procedure for the prepration of compounds presented from the paragraph 3.2.1 to Compound **6**, **7**, **2a**, **3a**, **8a-d** are already publish at

Mioskowski, Charles; Sylvain, Catherine; Wagner, Alain. An efficient procedure for the esterification of nitroacetic acid : Application to the preparation of Merrifield resin-bound nitroacetate. *Tetrahedron Letters*, **1999**, *40*, 875-878.

Righi, Paolo; Scardovi, Noemi; Marotta, Emanuela; Holte, Peter; Zwanenburg, Binne; Solution- and Solid-Phase Synthesis of 4-Hydroxy-4,5-dihydroisoxazole Derivatives from Enantiomerically Pure N-Tosyl-2,3-aziridine Alcohols. *Org. Lett.*, **2002**, 4(4), 497-500.

Vanier, Sébastien F.; Larouche, Guillaume; Wurz Ryan P.; Charette, André B. Formal Synthesis of Belactosin A and Hormaomycin via a Diastereoselective Intramolecular Cyclopropanation of an α -Nitro Diazoester *Org. Lett.*, **2010**, 12(4), 672-675

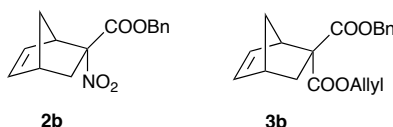
7.3.1 General procedure for the cycloaddition reaction

Preparation of compound **2a-c**, **3a-c**, **24**, **25**, **26**, **27**, **28**, **29**.

To a solution of **1a-c** in THF (0.1M) under stirring was added cyclopentadiene (20eq.), formaldehyde (20eq.), acetic acid (20eq.), and the reaction was warmed up to 40°C. after 7 hours an other addition of reactive is required in order to consume the starting material (cyclopentadiene (10eq.), formaldehyde (10eq.), acetic acid (10eq.)). The reaction is monitoring by Tlc (EtOAc : hexane , 1 : 4) with the use of ‘‘pancaldi’’ to detect the product. Upon consumption of starting material the THF is evaporated at reduced pressure and after addition of EtOAc the crude of reaction is washed with brine. The organic phase is evaporated at reduce pressure affording compound.

(1R,2R,4R)-benzyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (2b)

(1R,2S,4R)-benzyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (3b)



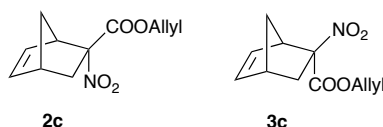
The products are obtain in mixture after purification with flash chromatography (EtOAc : hexane, 1 :30-1:20). Yield 96% as yellow oil.

δ_H (200 MHz, $CDCl_3$) 7.49 – 7.26 (10 H, m, 5 **2b+3b**), 6.43 (1 H, dd, J 5.7, 3.1, **2b**), 6.37 (1 H, dd, J 5.7, 3.1, **3b**), 5.98 (1 H, dd, J 5.7, 2.9, **2b**), 5.84 (1 H, dd, J 5.6, 3.1, **3b**), 5.30 – 5.09 (4 H, m, 2 **2b+2 3b**), 3.71 (2 H, bs, **3b+2b**), 3.01 (2 H, bs, **2b+3b**), 2.51 – 2.25 (4 H, m, J 1.6, 2 **2b+2 3b**), 1.92 – 1.48 (4 H, m, 2 **2b+2 3b**). δ_C (50 MHz, $CDCl_3$) 168.12 (**2b**), 165.89 (**3b**), 142.61 (**3b**), 141.23 (**2b**), 134.84 (**2b**), 133.17 (**3b**), 131.73 (**2b+3b**), 128.90 (**2b**), 128.82 (**3b**), 128.41 (**2b+3b**), 128.14 (**2b+3b**), 99.26 (**2b**), 98.98 (**3b**), 68.50 (**2b**), 68.31 (**3b**), 52.11 (**2b**), 51.85 (**3b**), 49.46 (**2b**), 49.16 (**3b**), 41.96 (**2b**), 41.92 (**3b**), 38.72 (**3b**), 37.68

(**2b**).(+)-ESI-MS (m/z) : [M+Na]⁺ 296.1. Anal.Calcd for C₁₅H₁₅NO₄ (273.2): C, 65.92; H, 5.53; N, 5.13; O, 23.42; found C, 65.97; H, 5.57; N, 5.18. IR(NaCl): ν = 3400, 3067, 2990, 2959, 2884, 1748, 1548 cm⁻¹

(1*R*,2*R*,4*R*)-allyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (2c)

(1*R*,2*S*,4*R*)-allyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (3c)



The products are obtained in mixture after purification with flash chromatography (EtOAc : hexane, 1 :30). Yield 83% as yellow oil.

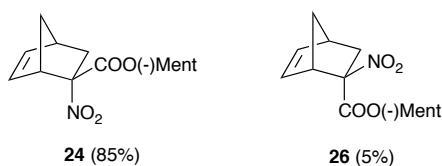
δ_{H} (200 MHz, CDCl₃) 6.53 – 6.35 (2 H, m, **2c+3c**), 6.05 – 5.94 (2 H, m, **2c+3c**), 5.94 – 5.74 (2 H, m, **2c+3c**), 5.46 – 5.20 (4 H, m, 2 **2c**+2 **3c**), 4.70 (2 H, dt, J 5.6, 1.3, **2c**), 4.63 (2 H, dt, J 5.7, 1.3, **3c**), 3.70 (2 H, s, **2c+3c**), 3.02 (2 H, s, **2+3c**), 2.41 (2 H, dd, J 3.3, 1.1, **3c**), 2.39 – 2.34 (2 H, m, **2c**), 1.95 – 1.51 (4 H, m, **2c+3c**). δ_{C} (50 MHz, CDCl₃) 167.99 (**3c**), 167.99 (**2c**), 142.61 (**3c**), 141.21 (**2c**), 133.21 (**2c**), 131.81 (**3c**), 131.04 (**3c**), 130.85 (**2c**), 119.58 (**2c+3c**), 99.20 (**2c+3c**), 67.28 (**2c**), 67.10 (**3c**), 52.10 (**2c**), 51.83 (**3c**), 49.45 (**2c**), 49.16 (**3c**), 41.95 (**2c**), 41.91 (**3c**), 38.70 (**3c**), 37.71 (**3c**). (+)-ESI-MS (m/z) : [M+Na]⁺ 246.1. Anal.Calcd for C₁₁H₁₃NO₄ (223.2): C, 59.19; H, 5.87; N, 6.27; O, 28.67; found C, 59.24; H, 5.92; N, 6.32. IR(NaCl): ν = 3435, 2991, 2958, 1749, 1649, 1549 cm⁻¹

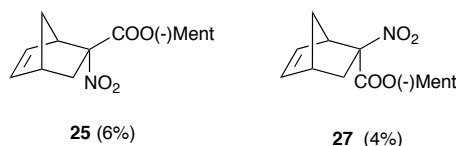
(1*S*,2*S*,4*S*)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (24)

(1*S*,2*R*,4*S*)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (26)

(1*R*,2*R*,4*R*)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (25)

(1*R*,2*S*,4*R*)-(1*S*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (27)





The products are obtained in mixture after purification with flash chromatography (EtOAc : hexane, 1 :100 to 1:50). Yield 90% as yellow oil. The mixture was characterised.

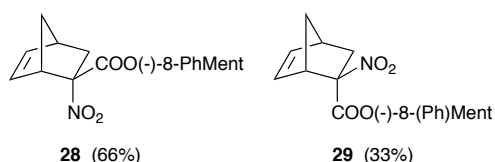
δ_H (200 MHz, $CDCl_3$) 6.42 (1 H, dd, J 5.6, 3.1), 5.99 (1 H, dd, J 5.6, 2.8), 4.76 (1 H, td, J 11.0, 4.5), 3.67 (1 H, bs), 3.01 (1 H, bs), 2.45 – 2.21 (2 H, m), 1.99 (1 H, dt, J 9.3, 4.1), 1.89 – 1.18 (8 H, m), 1.15 – 0.96 (2 H, m), 0.94 – 0.84 (6 H, m), 0.75 (3 H, dd, J 7.0, 1.3).

Only the major one is visible in the ^{13}C -NMR **17**.

δ_C (50 MHz, $CDCl_3$) 167.81, 141.18, 133.21, 99.29, 77.54, 52.00, 49.48, 47.00, 41.90, 40.34, 37.64, 34.26, 31.61, 26.25, 23.33, 22.11, 20.95, 16.10. (+)ESI-MS (m/z) : $[M+Na]^+$ 344.2. Anal. Calcd for $C_{18}H_{27}NO_4$ (321.4): C, 67.26; H, 8.47; N, 4.36; O, 19.91; found C, 67.31; H, 8.51; N, 4.36. IR(NaCl): ν = 3400, 2957, 2928, 2872, 1740, 1550 cm^{-1}

(1*S*,2*S*,4*S*)-(1*R*,2*S*,5*R*)-5-methyl-2-(2-phenylpropan-2-yl)cyclohexyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (28)

(1*S*,2*R*,4*S*)-(1*R*,2*S*,5*R*)-5-methyl-2-(2-phenylpropan-2-yl)cyclohexyl 2-nitrobicyclo[2.2.1]hept-5-ene-2-carboxylate (29)



The products are obtained in mixture after purification with flash chromatography (EtOAc : hexane, 1 :100 to 1:50). Yield 97% as yellow oil. The mixture was characterised

δ_H (200 MHz, $CDCl_3$) 7.40 – 7.06 (10 H, m, 5 **28**+5 **29**), 6.39 (2 H, dd, J 5.5, 3.1, **28** + **29**), 6.00 (1 H, dd, J 5.5, 2.7, **28**), 5.90 (1 H, dd, J 5.4, 2.7, **29**), 4.90 (2 H, dtd, J 14.8, 10.6, 4.3, **28**+**29**), 3.53 (2 H, bs, **28**+**29**), 2.94 (2 H, bs, **28**+**29**), 2.34 – 1.83 (6 H, m, 3 **28**+3 **29**), 1.79 – 1.16 (24 H, m, 12 **28**+12 **29**), 1.13 – 0.62 (12 H, m, 6 **28**+6 **29**). δ_C (50 MHz, $CDCl_3$) 167.69 (**28**), 167.38 (**29**), 150.60 (**29**), 150.44 (**28**), 141.28 (**29**), 141.02 (**28**), 133.91 (**28**), 132.86 (**29**), 128.37 (**28**+**29**), 125.86 (**28**), 125.78 (**29**), 125.69 (**28**+**29**), 100.06 (**28**), 99.23 (**29**), 78.60 (**28**), 78.30 (**29**), 52.08 (**29**), 51.52 (**28**), 50.18 (**28**), 50.07 (**29**), 49.81 (**29**), 48.86 (**28**), 41.93 (**28**), 41.89 (**29**), 41.20 (**28**+**29**), 40.89 (**28**+**29**), 40.30 (**28**+**29**), 37.64 (**28**), 36.86 (**29**), 34.47 (**28**), 31.54 (**29**), 28.96 (**28**), 28.58 (**29**), 27.41 (**28**), 27.32 (**29**), 25.76 (**29**), 25.09 (**28**), 21.89 (**28**+**29**). (+)ESI-MS (m/z) : $[M+Na]^+$ 420.1. Anal. Calcd for $C_{24}H_{31}NO_4$ (397.2): C, 72.52; H, 7.86; N, 3.52; O, 16.10; found C, 72.56; H, 7.90; N, 3.57. IR(NaCl): ν = 3058, 2956, 2924, 2872, 1733, 1548 cm^{-1}

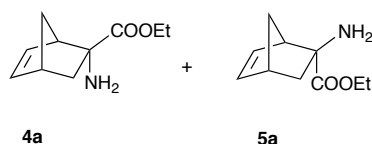
7.3.2 General procedure for the reduction reaction

Preparation of compound **4a-c**, **5a-c**, **30**, **31**, **32**, **33**, **34**, **35**.

To a solution of mixture *endo/eso* of nitro cycloadducts in THF (0.1M) was added Zn dust (20eq.) under strong stirring. The reaction was cooled down to 0°C and H₃PO₄ was added dropwise. After that the reaction was warmed up to room temperature and let react for 12 hours. The reaction was monitoring by Tlc (EtOAc : n-hexane 1:2) detected by ninhydrin. Upon the consumption of starting materials the THF was evaporated at reduced pressure, than the concentrated water phase was basified with NaHCO₃ to pH 8 and extracted 3 time with EtOAc. The combining organic phase was dry over Na₂SO₄ and evaporated.

(1R,2R,4R)-ethyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (4a)

(1R,2S,4R)-ethyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (5a)



The compound was purified by flash chromatography using EtOAc : hexane, 1 : 6 + 0,8% of TEA

Yield 100%, ratio, 85 /15

Compound 4a

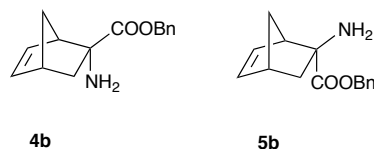
δ_H (200 MHz, CDCl₃) 6.22 (1 H, dd, J 5.7, 3.0), 5.93 (1 H, dd, J 5.7, 2.9), 4.10 (2 H, td, J 7.1, 0.9), 2.87 (1 H, s), 2.79 (1 H, s), 2.30 (2 H, s), 2.13 – 1.84 (2 H, m), 1.51 (2 H, ddd, J 12.2, 6.8, 5.2), 1.25 (3 H, t, J 7.1). δ_C (50 MHz, CDCl₃) 175.50, 139.81, 133.46, 65.28, 60.94, 53.10, 47.64, 42.45, 39.12, 14.42. IR(NaCl): ν = 2971, 2243, 1732, 1473, 1383 cm⁻¹

Compound 5a

δ_H (200 MHz, CDCl₃) 6.42 (1 H, dd, J 5.7, 3.0), 6.18 (1 H, dd, J 5.7, 3.1), 4.20 (2 H, q, J 7.1), 2.97 (1 H, s), 2.88 (1 H, s), 2.56 (1 H, dd, J 12.3, 3.8), 1.83 (2 H, s), 1.76 – 1.62 (1 H, m), 1.56 – 1.41 (1 H, m), 1.29 (3 H, t, J 7.1), 0.93 (1 H, dd, J 12.3, 3.2). δ_C (50 MHz, CDCl₃) 176.41, 140.54, 133.40, 65.20, 61.31, 52.69, 49.14, 43.22, 41.05, 14.37. IR(NaCl): ν = 2975, 2237, 1734, 1476, 1393 cm⁻¹ (+)ESI-MS (m/z) : [M+H]⁺ 182.0. Anal.Calcd for C₁₀H₁₅NO₂ (181.1): C, 66.27; H, 8.34; N, 7.73; O, 17.66; found C, 66.32; H, 8.39; N, 7.78.

(1R,2R,4R)-benzyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (4b)

(1R,2S,4R)-benzyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (5b)



The compound was purified by flash chromatography using EtOAc : hexane, 1 : 6 + 0,8% of TEA
Yield 77,5% as yellow oil.

Compound 4b: Yield 15%

δ_H (200 MHz, $CDCl_3$) 7.45 – 7.28 (5 H, m), 6.42 (1 H, dd, J 5.6, 3.0), 6.18 (1 H, dd, J 5.6, 3.2), 5.19 (2 H, s), 3.00 (1 H, bs), 2.89 (1 H, bs), 2.59 (1 H, dd, J 12.3, 3.8), 1.80 – 1.62 (2 H, m, J 13.1), 1.58 – 1.42 (2 H, m, J 7.1, 3.3, 1.7), 0.94 (1 H, dd, J 12.3, 3.2). δ_C (50 MHz, $CDCl_3$) 176.33, 140.51, 136.19, 133.36, 128.79, 128.45, 128.30, 67.09, 65.35, 52.70, 49.17, 43.24, 41.11. IR(NaCl): ν = 3379, 3063, 3033, 2972, 2876, 1729 cm^{-1}

Compound 5b: Yield 63%

δ_H (200 MHz, $CDCl_3$) 7.47 – 7.27 (5 H, m), 6.20 (1 H, dd, J 5.6, 2.9), 5.85 (1 H, dd, J 5.6, 2.9), 5.08 (2 H, s), 2.85 (1 H, bs), 2.76 (1 H, bs), 2.17 – 2.00 (1 H, m), 2.00 – 1.78 (3 H, m), 1.65 – 1.37 (2 H, m). δ_C (50 MHz, $CDCl_3$) 175.79, 139.83, 136.26, 133.55, 128.75, 128.62, 128.52, 128.41, 128.32, 66.61, 65.37, 53.25, 47.56, 42.40, 39.38. IR(NaCl): ν = 3372, 3307, 3064, 2973, 2871, 1728 cm^{-1} (+)ESI-MS (m/z) : $[M+H]^+$ 244.2. Anal.Calcd for $C_{15}H_{17}NO_2$ (243.1): C, 74.05; H, 7.04; N, 5.76; O, 13.15; found C, 74.08; H, 7.09; N, 5.78.

(1R,2R,4R)-allyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (4c)

(1R,2S,4R)-allyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (5c)



The compound was purified by flash chromatography using EtOAc : hexane, 1 : 5 + 0,8% of TEA
Yield 50,5% as yellow oil

Compound 5c: yield 7,5 %

δ_H (200 MHz, $CDCl_3$) 6.28 – 6.17 (1 H, m), 6.04 – 5.78 (2 H, m), 5.43 – 5.14 (2 H, m), 4.55 (2 H, d, J 5.7), 2.86 (1 H, bs), 2.77 (1 H, bs), 2.21 – 1.65 (4 H, m), 1.64 – 1.38 (2 H, m). δ_C (50 MHz, $CDCl_3$) 175.66, 139.82, 133.62, 132.40, 118.45, 65.50, 53.19, 47.57, 42.38, 39.39, 29.90. IR(NaCl): ν = 3376, 3065, 2974, 2925, 2851, 1731 cm^{-1}

Compound 4 c: yield 42,5 %

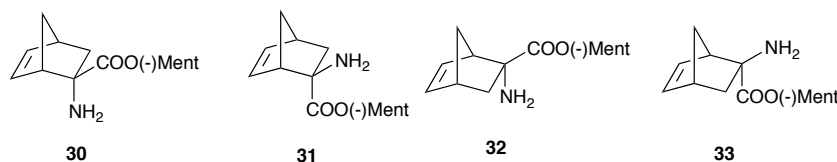
δ_H (200 MHz, $CDCl_3$) 6.42 (1 H, dd, J 5.7, 3.0), 6.18 (1 H, dd, J 5.7, 3.1), 5.94 (1 H, ddt, J 17.1, 10.4, 5.7), 5.29 (2 H, m), 4.64 (2 H, dt, J 5.7, 1.3), 2.99 (1 H, bs), 2.89 (1 H, bs), 2.57 (1 H, dd, J 12.3, 3.8), 1.68 (2 H, d, J 8.7), 1.57 – 1.39 (1 H, m), 1.26 (1 H, d, J 8.2), 0.93 (1 H, dd, J 12.3, 3.2). δ_C (50 MHz, $CDCl_3$) 176.21, 140.52, 133.36, 132.30, 118.62, 65.94, 65.31, 52.71, 49.16, 43.23, 41.11. IR(NaCl): ν = 3583, 2981, 2254, 1725, 1462 cm^{-1} . (+)ESI-MS (m/z) : $[M+H]^+$ 194.1. Anal. Calcd for $C_{11}H_{15}NO_2$ (193.2): C, 68.37; H, 7.82; N, 7.25; O, 16.56; found C, 68.42; H, 7.85; N, 7.24.

(1*S*,2*S*,4*S*)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (30)

(1*S*,2*R*,4*S*)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (31)

(1*R*,2*R*,4*R*)-(1*R*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (32)

(1*R*,2*S*,4*R*)-(1*S*,2*S*,5*R*)-2-isopropyl-5-methylcyclohexyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (32)



The compound was purified by automatic chromatography with GRACE prepack column using MeOH : DCM, 1: 20 giving the products in couple **30**, **31** and **32**, **33**. With a global yield of 90% as yellow oil. Purification with preparative TLC permit the separation and identification of 3 of them.

Compound 33: 3,6%

δ_H (300 MHz, $CDCl_3$) 6.22 (1 H, dd, J 5.6, 3.0), 5.96 (1 H, dd, J 5.7, 3.0), 4.63 (1 H, td, J 10.9, 4.4), 2.87 (1 H, bs), 2.77 (1 H, bs), 2.08 (1 H, dd, J 11.9, 2.7), 2.02 – 1.63 (8 H, m), 1.63 – 1.37 (2 H, m), 1.28 (2 H, s), 1.18 – 0.98 (2 H, m), 0.97 – 0.83 (6 H, m), 0.76 (3 H, d, J 7.0).

Compound 32: 5,4%

δ_{H} (300 MHz, CDCl_3) 6.24 (1 H, dd, J 5.6, 3.0), 5.92 (1 H, dd, J 5.6, 2.9), 4.60 (1 H, td, J 10.9, 4.4), 2.87 (1 H, bs), 2.76 (1 H, bs), 2.13 – 1.78 (4 H, m), 1.77 – 1.64 (3 H, m), 1.63 – 1.37 (5 H, m), 1.28 (1 H, s), 1.19 – 0.99 (2 H, m), 0.99 – 0.84 (6 H, m), 0.78 (3 H, d, J 7.0).

Compound 30: 76,5%

δ_{H} (300 MHz, CDCl_3) 6.49 (1 H, dd, J 5.6, 3.0), 6.27 (1 H, dd, J 5.2, 2.7), 4.78 (1 H, td, J 11.0, 4.3), 3.06 (1 H, bs), 2.95 (1 H, bs), 2.74 – 2.44 (2 H, m), 2.13 – 1.88 (3 H, m), 1.82 – 1.64 (4 H, m), 1.59 – 1.41 (2 H, m), 1.28 (2 H, s), 1.17 – 1.00 (2 H, m), 0.93 (6 H, dd, J 6.9, 2.9), 0.79 (3 H, d, J 6.9).

Compound 31: always in mixture with 31

Mixture 30+31:

δ_{C} (50 MHz, CDCl_3) 176.03 (30+31), 140.47 (30), 140.36 (31), 133.51 (25), 133.47 (30), 75.02 (30), 74.95 (31), 65.42 (30+31), 52.68 (30), 52.52 (31), 49.23 (31), 49.04 (30), 47.35 (30+31), 43.25 (30+31), 41.19 (30+31), 40.95 (31), 40.89 (30), 34.52 (30+31), 31.63 (30+31), 26.50 (30+31), 23.49 (30+31), 22.20 (30+31), 21.06 (30+31), 16.31 (31), 16.25 (30). IR(NaCl): $\nu = 3391, 3062, 2956, 2929, 2871, 1723 \text{ cm}^{-1}$

Mixture 32/33:

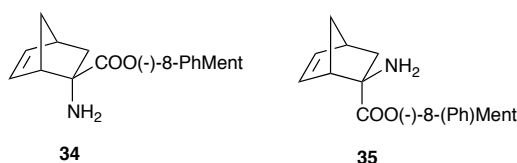
δ_{C} (50 MHz, CDCl_3) 175.76, 140.64, 133.43, 75.16, 65.40, 52.64 (32), 52.48 (33), 49.22 (33), 49.01 (32), 47.33, 43.24, 40.92, 40.67, 34.49, 31.63, 26.47, 23.43, 22.21, 21.08, 16.

IR(NaCl) 24/26: $\nu = 3385, 3061, 2955, 2870, 1723 \text{ cm}^{-1}$

(+)ESI-MS (m/z): $[\text{M}+\text{H}]^+$ 292.3. Anal.Calcd for $\text{C}_{18}\text{H}_{29}\text{NO}_2$ (291.4): C, 74.18; H, 10.03; N, 4.81; O, 10.98; found C, 74.22; H, 10.08; N, 4.85.

(1S,2S,4S)-(1R,2S,5R)-5-methyl-2-(2-phenylpropan-2-yl)cyclohexyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (34)

(1S,2R,4S)-(1R,2S,5R)-5-methyl-2-(2-phenylpropan-2-yl)cyclohexyl 2-aminobicyclo[2.2.1]hept-5-ene-2-carboxylate (35)



The compound was purified by flash chromatography using EtOAc : hexane, 1 : 40 + 0,8% of TEA

Yield 74% as yellow oil, by preparative Tlc was possible separate 3 fraction containing **35a** / **35b**, **34a** and **34b**. (**a** and **b** is not reported in the discussion, they represent the precursor of the 2 enantiopure form of **35** and **34**).

Miscela composti **35a** **35b**:

δ_{H} (200 MHz, CDCl_3) 7.33 – 7.08 (10 H, m, 5 a - 5 b), 6.14 (2 H, dd, J 10.9, 5.7, a - b), 5.86 (2 H, dd, J 5.8, 3.0, a - b), 4.90 – 4.67 (2 H, m, a - b), 2.75 (2 H, d, J 4.8, a - b), 2.61 (1 H, bs, a), 2.36 (1 H, bs, b), 2.28 –

2.01 (2 H, m, a - b), 1.95 – 1.53 (12 H, m, 6 a - 6 b), 1.53 – 1.21 (12 H, m, 6 a - 6 b), 1.20 – 0.75 (20 H, m, 10 a - 10 b).

Composto **34a**:

δ_H (200 MHz, $CDCl_3$) 7.41 – 7.06 (5 H, m), 6.31 (1 H, dd, J 5.5, 3.0), 6.04 (1 H, dd, J 5.6, 3.1), 4.89 (1 H, td, J 10.7, 4.4), 2.80 (1 H, bs), 2.63 (1 H, bs), 2.28 (1 H, dd, J 12.1, 3.8), 2.15 (1 H, dd, J 16.7, 6.1), 1.96 – 1.81 (1 H, m), 1.81 – 1.44 (5 H, m), 1.43 – 1.30 (4 H, m), 1.30 – 1.05 (4 H, m), 0.94 (6 H, dd, J 22.6, 8.7), 0.74 (1 H, dd, J 12.1, 3.1).

Composto **34b**:

δ_H (200 MHz, $CDCl_3$) 7.42 – 7.07 (5 H, m), 6.33 (1 H, dd, J 5.5, 3.0), 6.05 (1 H, dd, J 5.6, 3.1), 4.86 (1 H, td, J 10.7, 4.4), 2.78 (1 H, bs), 2.73 (1 H, bs), 2.16 (1 H, dd, J 12.2, 3.7), 2.02 – 1.85 (1 H, m), 1.77 – 1.53 (8 H, m), 1.46 – 1.14 (4 H, m), 1.13 – 0.96 (2 H, m), 0.87 (6 H, d, J 6.4), 0.65 (1 H, dd, J 12.3, 3.2).

(+)ESI-MS (m/z) : $[M+H]^+$ 368.3. Anal.Calcd for $C_{24}H_{33}NO_2$ (367.2): C, 78.43; H, 9.05; N, 3.81; O, 8.71; found C, 78.51; H, 9.10; N, 3.83.

IR(NaCl) miscela 35: $\nu = 3376, 3059, 2955, 2869, 1716\text{ cm}^{-1}$

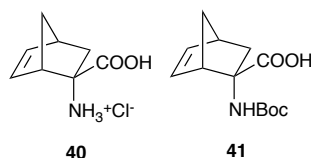
IR(NaCl) miscela 34: $\nu = 3381, 3058, 2954, 2870, 1716\text{ cm}^{-1}$

7.3.3 Synthesis of the model peptide

Synthesis of : (1S,2S,4S)-2-carboxybicyclo[2.2.1]hept-5-en-2-aminium chloride (40)

And

(1S,2S,4S)-2-((tert-butoxycarbonyl)amino)bicyclo[2.2.1]hept-5-ene-2-carboxylic acid (41)



To a solution of the mixture **30** and **31** in MeOH (0,15 M) in a sealed tube under stirring was added KOH (26 eq.) and was warmed up to 70°C for 2 h. The reaction was monitored by Tlc (MeOH : DCM, 1 :5) detected with ninhydrin. When the reaction is finish the mixture was diluted with water and extracted with DCM in order to eliminate the Menthol generated in the reaction. After that the water phase was evaporated at reduced pressure.

For the isolation of compound **40** in was necessary add HCl 3N to the crude until pH 2 and afther that was extracted with THF/EtOAc 6 times. The solvent was removed at reduced furnished the free amino acid **40** in a pure form (Yield 65%).

For the direct preparation of compound **41** crude obtained after the extraction with DCM was dissolved in a mixture of MeOH : H_2O (0,1m) . Boc_2O (4 eq.) and K_2CO_3 was added and the reaction was warmed up to 50° and monitoring by Tlc (MeOH : DCM, 1 :5). Upon the consumption of starting material the organic

solvent was evaporated at reduced pressure, the crude was dissolved in HCl 2 normal and extract 3 time with EtOAc provided **41** over two steps (Yield 82%).

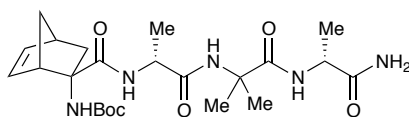
Compound **40** white solid (m.p. 231-231 °C), $\alpha_D^{MeOH} = -9$ (Yield 65%). (Yield 65%). δ_H (200 MHz, D₂O) 6.41 (1 H, dd, J 5.6, 3.1), 6.07 (1 H, dd, J 5.6, 2.9), 3.03 – 2.84 (2 H, m), 2.25 (1 H, dd, J 13.0, 3.5), 1.90 (1 H, d, J 9.0), 1.41 (1 H, dd, J 9.0, 1.4), 1.12 (1 H, dd, J 12.9, 3.1). δ_C (50 MHz, D₂O) 177.80, 142.81, 132.58, 66.45, 50.71, 49.20, 42.71, 37.19.

(+)ESI-MS (m/z) : [M+Na]⁺ 176.0. Anal.Calcd for C₈H₁₁NO₂ (153.1): C, 62.73; H, 7.24; N, 9.14; O, 20.89; found C, 62.78; H, 7.29; N, 9.19. IR(KBr): ν = 3413, 3063, 2981, 2266, 2193, 1603 cm⁻¹

Compound **41** white solid (m.p. 178-179 °C), $\alpha_D^{MeOH} = -18$ (Yield 82% over two steps).

δ_H (200 MHz, CD₃OD) 6.33 (1 H, dd, J 5.4, 3.0), 6.07 (1 H, dd, J 3.9, 3.2), 3.30 (1 H, s, J 1.6), 2.85 (1 H, s), 2.44 (1 H, dd, J 12.5, 3.7), 1.80 (1 H, d, J 8.8), 1.52 – 1.34 (10 H, m), 1.26 (1 H, dd, J 12.5, 3.2). δ_C (50 MHz, CD₃OD) 177.45, 156.50, 139.45, 133.53, 79.05, 64.90, 50.29, 47.51, 42.19, 39.63, 27.54.(+)ESI-MS (m/z) : [M+H]⁺ 276.1. Anal.Calcd for C₁₃H₁₉NO₄ (253.1): C, 61.64; H, 7.56; N, 5.53; O, 25.27; found C, 61.69; H, 7.61; N, 5.58. IR(KBr): ν = 3308, 2992, 2975, 2957, 1699, 1619 cm⁻¹

Synthesis of : *tert*-butyl ((1*S*,2*S*,4*S*)-2-(((*R*)-1-(((*R*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)carbamoyl)bicyclo[2.2.1]hept-5-en-2-yl)carbamate. (43**)**

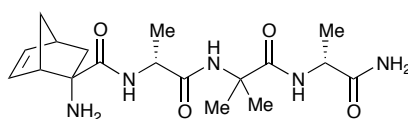


43

To a solution of acid **41** in dry DCM (0.1 M), under nitrogen at 0°C was added HOBt (1.1 eq.), EDC (1.1eq.), and let react under stirring at 0°C for 1 h. After that was added **35** (NH₂Ala-Aib-AlaCONH₂) (1.1 eq.) in DMF (0.5M) and DIPEA (2.2 eq.), additional DIPEA was used to reach pH = 8, and then the reaction mixture was let react at room temperature for 12 h. The reaction was monitored by TLC (MeOH : DCM, 1 : 6) detected by ninhydrin. Upon the consumption of starting material the reaction mixture was washed with saturated NH₄Cl, saturated NaHCO₃, and brine. The NH₄Cl, saturated was extracted 3 times with EtOAc. The recombining organic phase was dried over Na₂SO₄ and the solvent evaporated at reduced pressure. The crude of reaction was purified by flash chromatography with eluent gradient DCM : MeOH, 20 : 1 to 5 : 1 to afford a mixture of stable conformer **A** and **B**. The reaction was performed with comparable yield from 0.1g. to 1g. **43** was obtained in pure form as white solid (m.p. = 128°C) with 95% of yield. 0.1g. to 2g. $\alpha_D^{MeOH} = +50$.

δ_{H} (200 MHz, CDCl_3) 7.72 (2 H, s, A+B), 7.57 (1 H, d, J 7.9, B), 7.52 (1 H, d, J 7.3, A), 7.33 (2 H, bs, A+B), 6.99 (2 H, bs, A+B), 6.59 (1 H, dd, J 5.0, 2.3, A), 6.40 (1 H, dd, J 5.7, 2.9, B), 6.19 (1 H, dd, J 5.3, 2.5, A), 6.08 – 5.97 (1 H, m, B), 5.37 (2 H, bs, A+B), 5.25 – 5.16 (1 H, m, B), 5.10 (1 H, s, A), 4.37 (2 H, p, J 7.4, A+B), 4.12 – 3.87 (2 H, m, A+B), 3.48 (1 H, s, B), 2.98 (1 H, s, A), 2.92 (2 H, d, J 2.5, A+B), 2.12 (2 H, dd, J 16.0, 6.3, A+B), 1.89 (2 H, d, J 8.8, A+B), 1.68 – 1.32 (28 H, m, A+B). δ_{C} (50 MHz, CDCl_3) 176.66 (B), 176.51 (A), 175.69 (A+B), 174.83 (A+B), 174.08 (B), 173.93 (A), 156.81 (A), 156.70 (B), 144.58 (A), 140.28 (B), 134.92 (B), 131.12 (A), 81.64 (B), 81.52 (A), 67.05 (A), 64.65 (B), 57.43 (A+B), 52.73 (A+B), 52.15 (A+B), 49.93 (B), 49.73 (A), 47.76 (A+B), 43.08 (A), 42.45 (B), 42.13 (A), 39.79 (A), 28.45 (A+B), 27.85 – 23.78 (A+B), 27.76 – 23.73 (A+B), 17.59 (A), 17.50 (B), 17.34 (A), 17.18 (B). (+)ESI-MS (m/z) : $[\text{M}+\text{Na}]^+$ 502.3. Anal.Calcd for $\text{C}_{23}\text{H}_{37}\text{N}_5\text{O}_6$ (479.2): C, 57.60; H, 7.78; N, 14.60; O, 20.02; found C, 57.65; H, 7.79; N, 14.65. IR(KBr): $\nu = 3426, 3313, 2981, 2941, 1660, 1531 \text{ cm}^{-1}$

Synthesis of (1*S*,2*S*,4*S*)-2-amino-*N*-((*R*)-1-(((*R*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)bicyclo[2.2.1]hept-5-ene-2-carboxamide
(44)

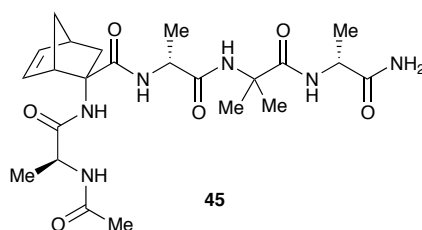


44

The compound **44** was dissolved in a mixture of MeOH /H₂O (0.1 M) in a sealed tube for micro waves reactor. Under magnetic stirring the sample was irradiate by microwaves for 50 at temperature of 150°C. The reaction was monitoring by TLC (MeOH : DCM, 1 :8) detected by ninhydrin. If not finish an other round of 20 minutes was performed. Upon consumption of starting material water was evaporated at reduced pressure. The produce was precipitated from the crude of the reaction by the addition of could Et₂O afforde the compound **44** in a pure form as white solid (m.p. = 118°C) with 91% of yield. The reaction was performed with comparable yield from 0.1g. to 0.5g , $\alpha_{\text{D}}^{\text{MeOH}} = -8.15$. δ_{H} (200 MHz, CDCl_3) 8.13 (2 H, d, J 3.3, A+B), 7.31 (2 H, d, J 8.1, A+B), 7.17 (2 H, d, J 4.8, A+B), 6.75 (2 H, d, J 10.6, A+B), 6.48 (2 H, td, J 5.7, 3.1, A+B), 6.21 (2 H, dt, J 5.5, 2.8, A+B), 5.60 (2 H, bs, A+B), 4.47 – 4.22 (2 H, m, A+B), 4.18 – 3.93 (2 H, m, A+B), 2.94 (2 H, bs, A+B), 2.89 (1 H, bs, A), 2.81 (1 H, bs, B), 2.59 (1 H, dd, J 12.0, 3.7, B), 2.34 (1 H, dd, J 11.9, 3.6, A), 2.19 – 1.75 (8 H, m, A+B), 1.67 – 1.16 (24 H, m, A+B), 0.95 (2 H, dd, J 12.3, 3.0, A+B). δ_{C} (50 MHz, CDCl_3) 179.75 (B), 179.71 (A), 175.89 (A+B), 173.96 (A), 173.82 (B), 173.32 (A+B), 142.11 (A), 141.52 (B), 134.01 (A), 133.22 (B), 64.71 (A), 64.22 (B), 57.11 (A), 57.03 (B), 53.77 (A), 53.13 (B), 51.54 (A+B), 50.06 (A), 49.83 (A+B), 49.13 (B), 43.68 (A), 43.61 (B), 43.38 (A), 42.71 (B), 27.18 – 24.52 (A+B), 26.92 – 24.22 (A+B), 17.42 (A+B), 16.82 (A), 16.70 (B). (+)ESI-MS (m/z) : $[\text{M}+\text{Na}]^+$ 402.4.

Anal.Calcd for $C_{18}H_{29}N_5O_4$ (379.2): C, 56.97; H, 7.70; N, 18.46; O, 16.87; found C, 56.99; H, 7.75; N, 18.51. IR(KBr): $\nu = 3312, 3060, 2980, 2939, 1657, 1529 \text{ cm}^{-1}$

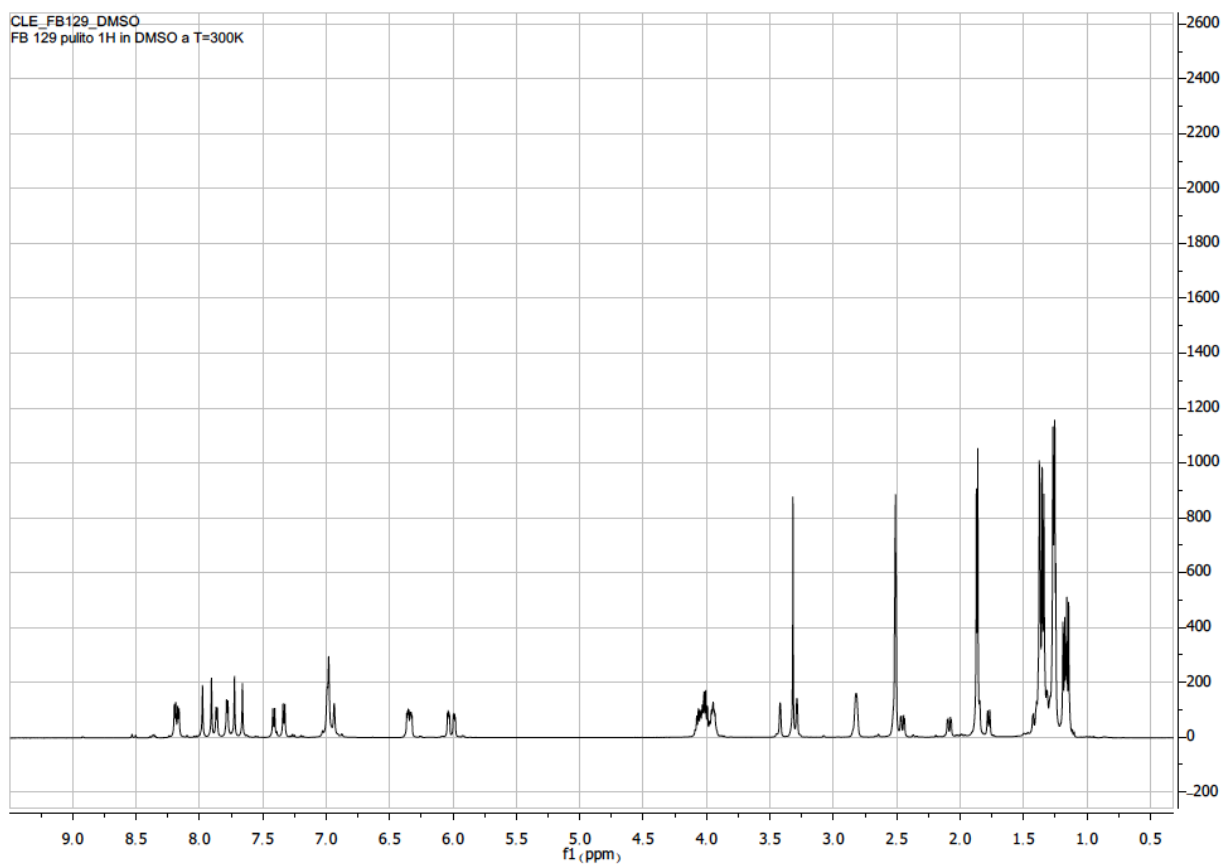
Synthesis of (1*S*,2*S*,4*S*)-2-((*S*)-2-acetamidopropanamido)-*N*-((*R*)-1-((1-(((*R*)-1-amino-1-oxopropan-2-yl)amino)-2-methyl-1-oxopropan-2-yl)amino)-1-oxopropan-2-yl)bicyclo[2.2.1]hept-5-ene-2-carboxamide (45**)**



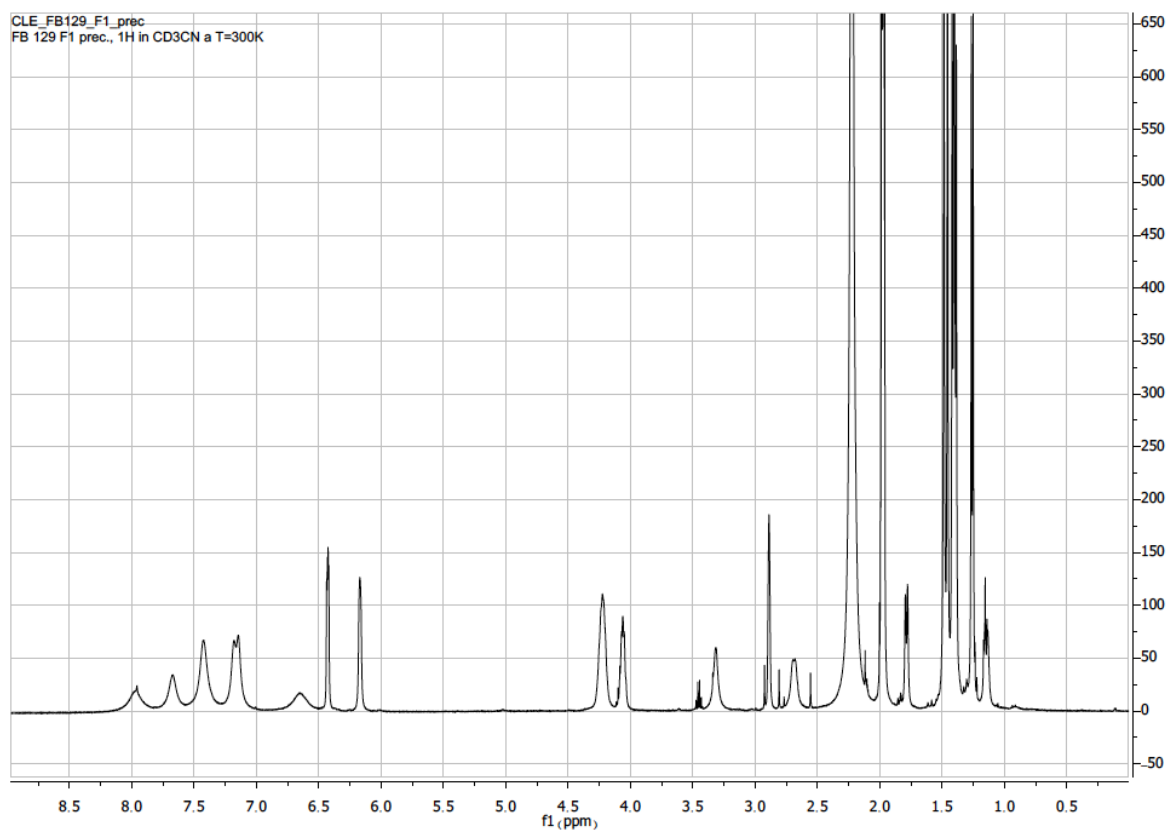
To solution of **44** in THF (0.1 M) in a sealed tube for micro waves reactor was added EEDQ (1.1 eq.) and (L)-NHAc-Ala-OH (1. eq.). Under magnetic stirring the sample was irradiate by microwave for 30' at temperature of 60°C and power of 50 Watt using the air compressing cooling system of the reactor to keep down the temperature. The reaction was monitoring by HPTlc (MeOH : DCM, 1 :8) detected by ninhydrin. If not finish an other round of 30 minutes was performed. Upon consumption of starting material the solvent was removed at reduced pressure and from the crude mixture the desired compound **45** was precipitated by addition of could Et₂O as pure white solid in two conformer form **45A** and **45B** with 56% of yield. The two conformer are rt stable and were separated by HPLC inverse phase in gradient from 99% H₂O, 1% CH₃CN +0,1 % of TFA. To 90% H₂O, 10% CH₃CN +0,1 % of TFA. The reaction was performed with comparable yield from 50mg to 100mg , (+)ESI-MS (m/z) : [M+Na]⁺ 515.4. Anal.Calcd for $C_{23}H_{36}N_6O_6$ (492.3): C, 56.08; H, 7.37; N, 17.06; O, 19.49; found C, 56.08; H, 7.37; N, 17.06. IR(KBr) miscela conformeri: $\nu = 3317, 3064, 2986, 2943, 1659, 1534 \text{ cm}^{-1}$

45A $\alpha_D^{MeOH} = -8.3$.

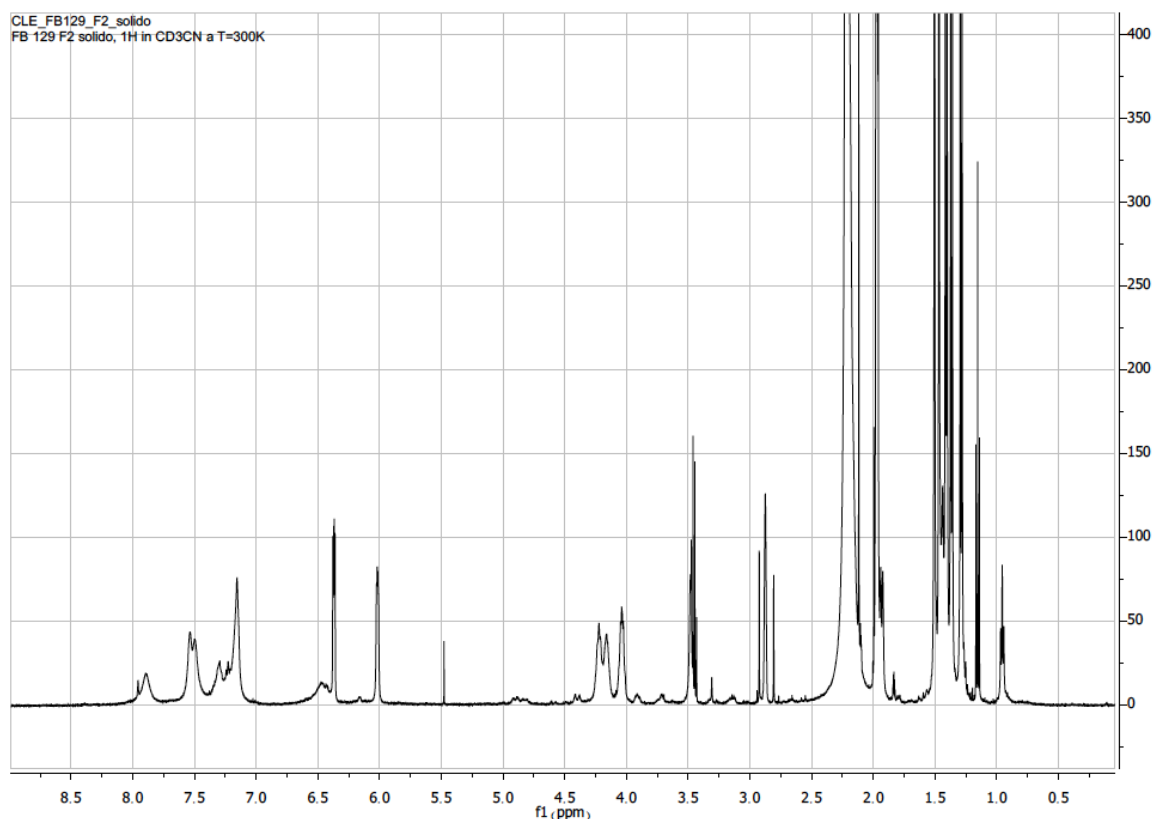
45B $\alpha_D^{MeOH} = +2$.



7.3.4 Conformer 45A F1 Hplc separated



7.3.5 Conformer 45B F2 Hplc separated



7.4 Chapter 4

Full characterizations and procedures for the preparation of all compounds contained in chapter 4 are already published at

Ruffoni, A.; Casoni, A.; Gelmi, M. L.; Pellegrino, S.; Clerici, F. Hydroarylation of substituted norbornene amino acids: studies on long-range stereo-electronic effects on the regioselectivity of the addition. *Curr. Org. Chem.* **2012**, 16, 2724-2738

7.5 Chapter 5

Procedure for the 10 Grams Scale Preparation of Derivatives 4, 5, 6, and 7. To a mixture of olefins **1-E** and **2-Z** (10 g, 39.5 mmol, 1 equiv) an cyclopentadiene (2.6 g, 39.5 mmol, 1.6 equiv) in a 250 mL pyrex flask sealed with Teflon cap was reacted under ultrasound at 50 °C. After 4 hours an excess of cyclopentadiene was added in portion (1 equiv x 4 every 4 hours). The reaction was monitored by TLC (8 :

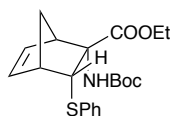
1 = hexane : EtOAc) and ^1H -NMR spectroscopy until completion (24h). After the partial removal of cyclopentadiene under reduced pressure, the crude product was purified by flash column chromatography (EtOAc : hexane, from 1 : 40 to 1:4) to give two fraction, the first one containing **3** and **4** as colourless oil (2.78 g, 8.7 mmol), and the latter containing **5** and **6** as light yellow oil (4.09 g, 12.8 mmol). Global yield 55%. Full characterization are reported in :

Ruffoni, A.; Casoni, A.; Pellegrino, S.; Gelmi, M. L.; Soave, R.; Clerici, F. Sulfanyl-methylene-5(4H)-oxazolones and β -sulfanyl- α -nitroacrylates as appealing dienophiles for the synthesis of conformationally constrained cysteine analogues. *Tetrahedron*. **2012**, 68, 1951-1962

General Procedure for the Reduction Reaction of compound **4**, **5**, **6**, **7**, and **30**.

Fresh activated Zinc dust (20 equiv) was added under stirring to a solution of starting materials (**4**, **5**, **6**, **7** and **30**) in THF (0.25 M). The mixture was cooled down in a ice bath and aqueous solution of H_3PO_4 1M (20 equiv) was added dropwise under vigorous stirring at 0°C . Than the reaction mixture was warmed up to room temperature and monitored by TLC analysis (1 : 6 = EtOAc : *n*-hexane) until completion (4-8 hours). Upon consumption of starting material, the reaction was filtered under vacuum and the liquid phase was concentrated under reduced pressure. Saturated NaHCO_3 was added to the crude mixture until pH 8. The aqueous phase was extracted in EtOAc 3 times and combined organic layer was evaporated under reduce pressure. The crude product was purified by flash column chromatography (MeOH : DCM = 1 : 10 or EtOAc : hexane = 3 : 10) to give **12**, **13**, **26** and **AR-148** in pure form. The reaction were performed on 0.1g – 1.0g, the yield are reported in Scheme 3 and Scheme 6. Full characterization of compound **12**, **13** and **26** are reported in FC rif

Synthesis of Compound: (1*R,2*R**,3*R**,4*S**)-ethyl 2-((*tert*-butoxycarbonyl)amino)-3-(phenylthio)bicyclo[2.2.1]hept-5-ene-2-carboxylate (**14**).**



14

Boc anhydride (558 mg, 2.56 mmol, 2 equiv) was added under stirring to a solution of **12** (370 mg, 1.28 mmol, 1 equiv) in dry DCM (5 mL). The reaction mixture was cooled in ice and dry TEA (0.187 mL, 1.35 mmol, 1.1 equiv) was added dropwise with vigorous stirring and the stirring was continued at this temperature for 20 min. Than the reaction was warmed to room temperature and monitored by TLC (3 : 1 = *n* -hexane : EtOAc) until completion (24 h). Upon consumption of starting material, the reaction was diluted with DCM (20 mL) and was washed with saturated HCl 0.4 M (20 mL), saturated NaHCO_3 (20 mL). The

aqueous layers were extracted with additional EtOAc (3 x 20 mL) and the finally organic layer was dried over Na₂SO₄. After the removal of solvents under reduced pressure, the crude product was purified by flash column chromatography (EtOAc : hexane = 1 : 10) to give **14** as Colourless oil (424.1 mg, 1.09mmol 85% yield): IR (NaCl): 3372m, 3063m, 2981m, 2255m, 1808m, 1736m, 1718m, 1584m, 1481m cm⁻¹. ¹H NMR (200 MHz, CDCl₃, δ): 7.51-7.46 (m, 2H), 7.32-7.21 (m, 3H), 6.38-6.34 (m, 1H), 6.24-6.20 (m, 1H), 5.62 (s, 1H), 4.20-4.02 (m, 3H), 3.62 (br, 1H), 3.18 (br, 1H), 1.89 (d, *J* = 9.5 Hz, 1H), 1.59 (d, *J* = 9.5 Hz, 1H), 1.40 (s, 9H), 1.12 (t, *J* = 7.4 Hz, 3H). ¹³C NMR (50 MHz, CDCl₃, δ): 173.7, 154.9, 147.0, 137.8, 137.2, 131.9, 129.1, 127.5, 85.39, 61.4, 50.9, 49.1, 46.7, 35.6, 33.1, 28.5, 14.2, (+)ESI-MS (*m/z*): [M + Na]⁺ 412. Anal. Calcd for C₂₁H₂₇NO₄S (389.17): C, 64.75; H, 6.99; N, 3.60; O, 16.43; S, 8.23; Found: C, 64.78; H, 7.04; N, 3.58; O, 16.40; S, 8.25.

7.5.1 General procedure for Heck hydroarylation.

General Procedure for the Preparation of Derivatives **8**, **9**, **15**, **16**, **21** and **22**.

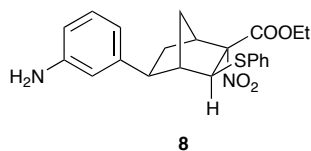
The cycloadduct (1 equiv) was dissolved in dry CH₃CN (0.1 M) in a pyrex tube under nitrogen. Pd(OAc)₂ (0.05 equiv), PPh₃ (0.1 equiv), 3-iodoaniline (3 equiv), dry TEA (3.5 equiv) and formic acid (3 equiv) was added under stirring. The tube was sealed with Teflon caps and stirred at 90°C for 12-24 h. The reaction was monitored by TLC analysis (1 : 1 – 1 : 4 = EtOAc : *n*-hexane) and ¹H-NMR analysis. Solvent was removed under reduced pressure and crude product was purified using flash chromatography to obtain a clean mixture of two inseparable regio-isomers. (for the mixture of starting material **2** and **3** only the products deriving from **2** are detectable and isolable) The reaction were performed on 0.1g – 0.3g.

Phosphine free Grams scale synthesis of compound **8** and **9**

The mixture of **2** and **3** (1.92 g, 6.0 mmol, 1 equiv.) was dissolved in 60 mL of dry CH₃CN (0.1M) in a 2 neck condenser fused flask under Argon. Pd(OAc)₂ (33.7 mg, 0.15mmol, 0.025 equiv), 3-iodoaniline (1.31 g, 6.0mmol, 1.0 equiv), dry TEA (2.9 mL, 21 mmol, 3.5 equiv) and formic acid (0.77 mL, 18 mmol, 3 equiv) was added under stirring. The flask was sealed with Teflon caps and stirred at 60°C for 48 h. After 12 hours an excess of 3-iodoaniline (1.31 g, 6.0mmol, 1.0 equiv x 2 every 6 hours) was added. The reaction was monitored by TLC analysis (1 : 8 = EtOAc : *n*-hexane) and ¹H-NMR analysis. Solvent was removed under reduced pressure and crude product was purified using flash chromatography EtOAc : *n*-hexane = 1 : 5. to give a clean mixture of two inseparable regio-isomers **8** and **9** as Light brown wax (1.98 g, 4.8 mmol, 80% yield) in ration **8** : **9** = 70 : 30 (evaluated by NMR analysis on crude reaction mixture)

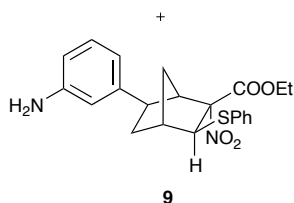
(**1S***,**2R***,**3S***,**4S***,**5S***)-ethyl
carboxylate (**8**).

5-(3-aminophenyl)-2-nitro-3-(phenylthio)bicyclo[2.2.1]heptane-2-



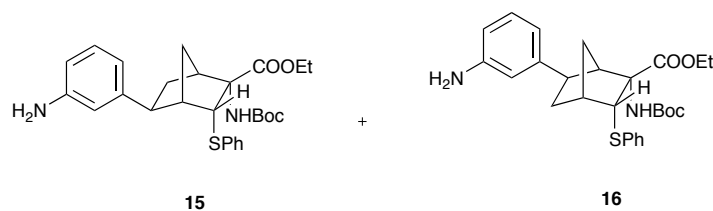
The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 5. Yield 52.5%, Light brown wax. IR (NaCl): 3380m, 3222m, 2352m, 2163m, 1944m, 1087m, 1747m, 1369m cm^{-1} . ^1H -NMR (200 MHz, CDCl_3 , δ): 7.40-7.20 (m, 5H), 7.07 (td, $J = 7.7$ Hz, $J = 1.9$ Hz, 1H), 6.57-6.47 (m, 3H), 4.57 (d, $J = 3$ Hz, 1H), 4.26-3.96 (m, 2H), 3.63 (s, 2H), 3.33 (s, 1H), 2.90 (m, 1H), 2.67 (s, 1H), 2.18 (d, $J = 9.7$ Hz, 1H), 1.67 (dd, $J = 9.7$ Hz, $J = 2.35$ Hz, 1H), 1.93 (s, 1H), 1.75 (br, 1H), 1.04 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CDCl_3 , δ): 165.2, 146.8, 145.6, 144.3, 129.7, 129.6, 129.4, 129.2, 126.7, 117.3, 116.9, 114.3, 113.7, 105.8, 62.8, 56.4, 51.2, 48.2, 45.5, 37.2, 33.2, 13.6, (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 412. Anal. Calcd for $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_4\text{S}$ (412.16): C, 64.06; H, 5.86; N, 6.79; O, 15.51; S, 7.77. Found: C, 63.99; H, 5.80; N, 6.72; O, 15.56; S, 7.69.

(1*R,2*R**,3*S**,4*R**,6*R**)-ethyl 6-(3-aminophenyl)-2-nitro-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (9).**



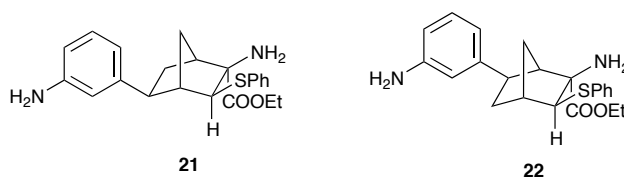
The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 5. Yield 22.5%, Light brown wax. IR (NaCl): 3380m, 1087m, 3058m, 2479m, 2079m, 1870m, 3466m, 1744m, 1369m cm^{-1} . ^1H -NMR (200 MHz, CDCl_3 , δ): 7.40-7.20 (m, 5H), 7.07 (td, $J = 7.7$ Hz, $J = 1.9$ Hz, 1H), 6.57-6.47 (m, 3H), 4.52 (d, $J = 3$ Hz, 1H), 4.26-3.96 (m, 2H), 3.63 (s, 2H), 3.33 (s, 1H), 2.68 (s, 1H), 2.63 (t, $J = 4.1$ Hz, 1H), 2.18 (d, $J = 9.7$ Hz, 1H), 2.13 (s, 1H), 2.04 (s, 1H), 1.67 (dd, $J = 9.7$ Hz, $J = 2.35$ Hz, 1H), 1.03 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CDCl_3 , δ): 165.2, 146.8, 145.6, 136.0, 129.7, 129.6, 129.4, 129.2, 126.7, 117.3, 116.9, 114.3, 113.7, 105.8, 62.8, 55.6, 54.3, 45.5, 40.4, 36.1, 35.2, 13.6, (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 412. Anal. Calcd for $\text{C}_{22}\text{H}_{24}\text{N}_2\text{O}_4\text{S}$ (412.16): C, 64.06; H, 5.86; N, 6.79; O, 15.51; S, 7.77. Found: C, 63.98; H, 5.83; N, 6.82; O, 15.56; S, 7.80.

(1*S,2*R**,3*R**,4*S**,5*S**)-ethyl 5-(3-aminophenyl)-2-((*tert*-butoxycarbonyl)amino)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (15) and (1*R**,2*R**,3*R**,4*R**,6*R**)-ethyl 6-(3-aminophenyl)-2-((*tert*-butoxycarbonyl)amino)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (16).**



The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 6. Yield of the mixture (**15** : **16** = 65 : 35) is 70%, Light yellow wax. ¹H-NMR (200 MHz, CDCl₃, δ): 7.55-7.47(m, 4H), 7.35-7.27 (m, 6H), 7.13-7.03 (m, 2H), 6.66-6.50 (m, 6H), 6.23-6.15 2s, 2H (NH-**15** and NH-**16**), 4.20-3.80 (m, 4H), 3.60 (br, 2H), 3.40 (br, 4H) 3.25-3.15 (m, 3H), 3.00-2.80 (m, 1H)(**16**), 2.64 (br, 1H), 2.20-1.00 (m, 14H)

(1S*,2S*,3S*,4S*,5S*) -ethyl 2-amino-5-(3-aminophenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (21) and **(1R*,2S*,3S*,4R*,6R*)-ethyl 2-amino-6-(3-aminophenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (22).**



The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 4 + 0.08% of TEA. Yield of the mixture (**21** : **22** = 62 : 38) is 68%, Light brown wax. ¹H-NMR (200 MHz, CDCl₃, δ): 7.45-7.01(m, 12H), 7.35-7.27 (m, 6H), 4.32-4.19 (m, 5H), 4.10 (d, *J* = 2.2 Hz, 1H)(**22**), 3.60 (br, 4H), 2.89 (t, *J* = 7.2 Hz, 1H)(**21**), 2.69 (t, *J* = 6.9 Hz, 1H)(**21**), 2.51-2.17 (m, 6H), 2.00-1.58 (m, 10H), 1.36-1.25 (m, 6H).

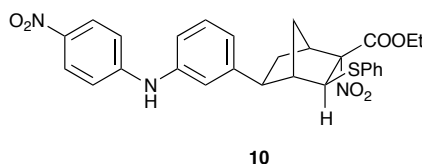
7.5.2 General procedure for Buchwald reaction.

General Procedure for the Preparation of Derivatives **10**, **11**, **17**, **18**, **23** and **24**.

The regioisomers mixture of Heck reaction (**8-9**, **15-16**, and **21-22**) (1 equiv) was dissolved in 4 mL of dry CH₃CN (0.07 M) in a pyrex tube under nitrogen. Pd(tetrakis) (0.05 equiv), 1-iodo-4-nitrobenzene (1.5 equiv) and Cs₂CO₃ (1.5 equiv) was added under stirring to a mixture. The tube was sealed with Teflon caps and stirred at 40 °C for 24 h. The reaction was monitored by TLC analysis (1 : 6 - 1 : 2 = EtOAc : *n*-hexane) and ¹H-NMR analysis until completion. Upon consumption of starting material solvent was removed under

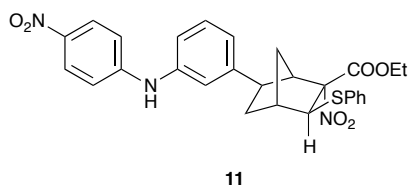
reduced pressure and crude product was purified using flash chromatography to give two fractions containing the desired compounds in pure form. The reaction were performed on 0.1g – 1.0g.

(1*S,2*R**,3*S**,4*S**,5*S**)-ethyl2-nitro-5-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (10).**



The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 8 – 1 : 6. Yield 92%, bright yellow wax. IR (KBr): 3369m, 3059m, 2598m, 2415m, 1941m, 1749m, 1747m, 1582m, 1324m, 1304m cm⁻¹. ¹H-NMR (300 MHz, CDCl₃, δ): 8.14 (d, *J* = 9.1 Hz, 2H), 7.41 (d, *J* = 8.8 Hz, 2H), 7.34– 7.24 (m, 4H), 7.08 (d, *J* = 7.8 Hz, 1H), 7.00–6.97 (m, 2H), 6.93 (d, *J* = 9.1 Hz, 2H) 6.29 (s, 1H), 4.62 (d, *J* = 3.7 Hz, 1H), 4.30–4.10 (m, 2H), 3.40 (br, 1H), 3.03 (d, *J* = 7.5 Hz, 1H), 2.73 (br, 1H), 2.28 (d, *J* = 11.2 Hz, 1H), 1.98 (dt, *J* = 11.2 Hz, *J* = 1.5 Hz, 1H), 1.83–1.77 (m, 1H), 1.77–1.72 (m, 1H), 1.08 (t, *J* = 7.1 Hz, 3H). ¹³C-NMR (75 MHz, CDCl₃, δ): 164.2, 149.2, 145.4, 140.0, 139.2, 134.9, 129.3, 128.5, 128.46, 128.44, 128.43, 128.41, 128.3, 125.9, 125.5, 122.1, 119.5, 119.1, 113.1, 104.66, 62.19, 55.32, 50.34, 47.35, 44.51, 36.33, 32.46, 32.40 12.74. (+)ESI-MS (*m/z*): [*M* +Na]⁺ 556. Anal. Calcd for C₂₈H₂₇N₃O₆S (533.16): C, 63.03; H, 5.10; N, 7.87; O, 17.99; S, 6.01. Found: C, 63.10; H, 5.17; N, 7.90; O, 17.93; S, 5.98.

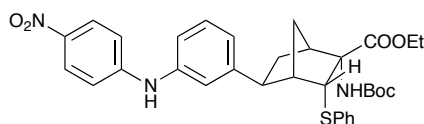
(1*R,2*R**,3*S**,4*R**,6*R**)-ethyl2-nitro-6-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (11).**



The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 8 – 1 : 6. Yield 92%, bright yellow wax. IR (NaCl): 3378m, 3058m, 2596m, 2426m, 1944m, 1749m, 1582m, 1304m, 1261m cm⁻¹. ¹H-NMR (300 MHz, CDCl₃, δ): 8.14 (d, *J* = 9.1 Hz, 2H), 7.42 (d, *J* = 7.5 Hz, 2H) 7.36– 7.33 (m, 3H), 7.29–7.22 (m, 1H), 7.13–7.10 (m, 1H), 7.00 (br, 2H), 6.94 (d, *J* = 9.1 Hz, 2H) 6.34 (s, 1H), 4.56 (d, *J* = 2.8 Hz, 1H), 4.30–4.05 (m, 2H), 3.38 (br, 1H), 2.70 (br, 2H), 2.24 (d, *J* = 11.3 Hz, 1H), 2.08 (dt, *J* = 13.6 Hz, *J* = 4.7 Hz, 1H), 2.04–1.98 (m, 1H), 1.89 (d, *J* = 11.3 Hz, 1H), 1.11 (t, *J* = 8.7 Hz, 3H). ¹³C-NMR (75 MHz, CDCl₃, δ): 164.2, 149.2, 145.4, 140.0, 139.2, 134.9, 129.3, 128.5, 128.46, 128.44, 128.43, 128.41, 128.3, 125.9, 125.5, 122.1, 119.5, 119.1, 113.1, 104.66, 62.19, 55.32, 50.34, 47.35, 44.51, 36.33, 32.46, 32.40 12.74.

164.1, 149.3, 144.1, 139.2, 139.1, 134.8, 134.16, 134.11, 129.5, 129.1, 128.4, 127.3, 127.2, 125.9, 125.6, 122.5, 120.1, 119.1, 113.11, 104.98, 62.19, 54.45, 53.38, 44.57, 39.53, 35.24, 34.35, 12.73. (+)ESI-MS (m/z): [M +Na]⁺ 556 . Anal. Calcd for C₂₈H₂₇N₃O₆S (533.16): C, 63.03; H, 5.10; N, 7.87; O, 17.99; S, 6.01; Found: C, 63.09; H, 5.14; N, 7.85; O, 17.94; S, 6.05.

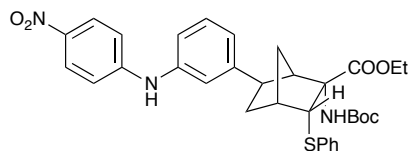
(1S*,2R*,3R*,4S*,5S*)-ethyl 2-((*tert*-butoxycarbonyl)amino)-5-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (17).



17

The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 3 – 1 : 6. Yield 90%, bright yellow wax. IR (KBr): 3353m, 3059m, 2603m, 2429m, 1916m, 1717m, 1582m, 1324m, 1158m cm⁻¹. ¹H-NMR (200 MHz, CDCl₃, δ): 8.12 (d, *J* = 9.2 Hz, 2H), 7.52-7.47 (m, 2H) 7.35- 7.25 (m, 4H), 7.08-6.97 (m, 3H), 6.94 (d, *J* = 9.2 Hz, 2H), 6.43 (s,1H), 6.13 (s,1H), 4.12-3.78 (m, 2H), 3.62 (bs, 1H), 3.23 (bs, 1H), 2.67 (d, *J* = 2.6 Hz 1H), 2.15 (t, *J* = 10.7 Hz, 1H), 1.99 (d, *J* = 11.2 Hz, 1H), 1.78 (d, *J* = 11.2 Hz, 1H), 1.65–1.26 (m, 10H), 1.07 (t, *J* = 7.3 Hz, 3H). ¹³C-NMR (50 MHz, CDCl₃, δ): 173.9, 155.3, 150.5, 147.8, 140.1, 140.0, 133.0, 129.9, 129.3, 128.2, 126.4, 123.4, 120.9, 119.6, 113.9, 80.3, 62.22, 61.30, 49.79, 46.15, 40.10, 35.62, 33.08, 31.75, 28.54, 14.04. (+)ESI-MS (m/z): [M +Na]⁺ 626.1 . Anal. Calcd for C₃₃H₃₇N₃O₆S (603.24): C, 65.65; H, 6.18; N, 6.96; O, 15.90; S, 5.31; Found: C, 65.68; H, 6.22; N, 6.92; O, 15.87; S, 5.27.

(1R*,2R*,3R*,4R*,6R*)-ethyl2-((*tert*-butoxycarbonyl)amino)-6-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (18).

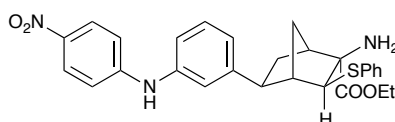


18

The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 8 – 1 : 6. Yield 90%, bright yellow wax. IR (KBr): 3358m, 2974m, 2611m, 2428m, 1967m, 1716m, 1582m, 1481m, 1324m, 1164m cm⁻¹. ¹H-NMR (300 MHz, CDCl₃, δ): 8.12 (d, *J* = 9.1 Hz, 2H), 7.54 (d, *J* = 5.6 Hz, 2H) 7.34-7.27 (m, 4H), 7.10-7.04 (m, 3H), 6.93 (d, *J* = 9.1 Hz, 2H), 6.26-6.24 (m, 2H), 4.07-3.89 (m, 2H), 3.60 (bs, 1H), 3.27-3.25 (m, 2H), 2.70 (bs, 1H), 2.06–1.85 (m, 3H), 1.71(d, *J* = 11.1Hz, 1H), 1.46 (s, 9H), 1.06 (t, *J* = 7.1 Hz, 3H). ¹³C-

NMR (75 MHz, CDCl₃, δ): 173.8, 155.4, 152.2, 150.7, 147.5, 140.0, 139.9, 133.0, 129.9, 129.5, 128.3, 126.6, 124.0, 121.6, 119.7, 114.1, 80.4, 63.10, 61.56, 61.8, 52.20, 43.64, 39.63, 34.70, 30.08, 28.71, 14.25. (+)ESI-MS (m/z): [M + Na]⁺ 626.2. Anal. Calcd For C₃₃H₃₇N₃O₆S (603.24): C, 65.65; H, 6.18; N, 6.96; O, 15.90; S, 5.31; Found: C, 65.69; H, 6.20; N, 6.95; O, 15.89; S, 5.25.

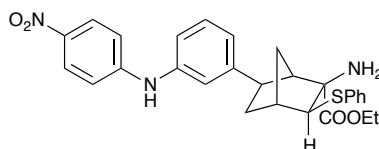
(1S*,2S*,3S*,4S*,5S*)-ethyl 2-amino-5-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (23).



23

The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 4 – 2 : 3 + 0.08% of TEA. Yield 85%, light yellow wax. IR (KBr): 3367m, 2959m, 2602m, 2425m, 1722m, 1716m, 1581m, 1502m, 1324m, 1110m cm⁻¹. ¹H-NMR (300 MHz, CDCl₃, δ): 8.12 (d, *J* = 9.1 Hz, 2H), 7.41 (d, *J* = 7.3 Hz, 2H) 7.32-7.16 (m, 4H), 7.04-6.95 (m, 3H), 6.91 (d, *J* = 9.1 Hz, 2H), 6.38 (s, 1H), 4.32-4.24 (m, 3H), 3.02-2.97 (m, 1H), 2.54 (bs, 1H), 2.36 (m, 1H), 2.29 (d, *J* = 10.5 Hz, 1H), 1.92–1.80(m, 3H), 1.71–1.65(m, 2H), 1.35 (t, *J* = 7.1 Hz, 3H) ¹³C-NMR (75 MHz, CDCl₃, δ): 175.3, 150.6, 147.8, 140.9, 140.3, 137.4, 130.1, 129.7, 129.4, 126.6, 126.5, 123.5, 120.8, 119.7, 114.1, 68.1, 61.9, 57.8, 51.8, 50.5, 45.9, 34.3, 33.8, 14.7. (+)ESI-MS (m/z): [M + H]⁺ 504.1. Anal. Calcd for C₂₈H₂₉N₃O₄S (503.19): C, 66.78; H, 5.80; N, 8.43; O, 12.71; S, 6.37. Found: C, 65.70; H, 5.72; N, 8.49; O, 12.76; S, 6.32.

(1R*,2S*,3S*,4R*,6R*)-ethyl 2-amino-6-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (24).



24

The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 4 – 2 : 3 + 0.08% of TEA. Yield 85%, light yellow wax. IR (KBr): 3368m, 2959m, 2597m, 2347m, 1721m, 1581m, 1501m, 1480m, 1324m, 1110m cm⁻¹. ¹H-NMR (300 MHz, CDCl₃, δ): 8.10 (d, *J* = 9.2 Hz, 2H), 7.41 (d, *J* = 7.0 Hz, 2H) 7.33-7.15 (m, 4H), 7.06-6.89 (m, 5H), 6.34 (s, 1H), 4.34-4.19 (m, 2H), 4.10 (bs, 1H) 2.82-2.75 (m, 1H), 2.48 (bs, 1H), 2.37 (m, 1H), 2.24 (d, *J* = 10.6 Hz, 1H), 1.98–1.76 (m, 4H), 1.58 (d, *J* = 10.6 Hz, 1H) 1.31(t, *J* = 7.1 Hz, 3H). ¹³C-NMR (75 MHz, CDCl₃, δ): 175.1, 150.4, 147.2, 140.0, 139.9, 137.1, 130.1, 129.9, 129.2, 126.5, 126.4,

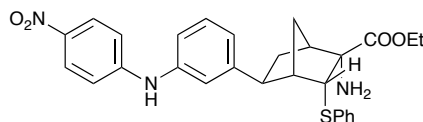
123.6, 120.8, 119.6, 113.9, 68.5, 61.7, 56.7, 56.0, 46.1, 41.6, 36.7, 33.3, 14.6. (+)ESI-MS (m/z): [M +H]⁺ 504.1. Anal. Calcd for C₂₈H₂₉N₃O₄S (503.19): C, 66.78; H, 5.80; N, 8.43; O, 12.71; S, 6.37; Found: C, 65.80; H, 5.84; N, 8.40; O, 12.69; S, 6.34.

7.5.3 General procedure for deprotective steps

General Procedure for the Preparation of Derivatives 19 and 20.

TFA 99% (80equiv) was added dropwise under stirring to a solution of N-Boc derivative (**17**, **18**) (1equiv) in DCM (0.05) at 0 °C. Then the reaction was warmed to room temperature and monitored by TLC until completion (2-4 h). Upon consumption of starting material, the reaction was partitioned between DCM (10 mL) and saturated aqueous NaHCO₃ (10 mL). The organic layer was separated, and the aqueous layer was extracted with DCM (3 x 10 mL). The combined organic layers were dried over Na₂SO₄, and the solvent was removed under reduced pressure. After deactivation of silica gel with a solution EtOAc : *n*-hexane = 1 : 5 + 10% of TEA the crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 3 – 1 : 2 to give the pure desired in pure form. The reaction were performed on 0.1 g – 0.3 g scale.

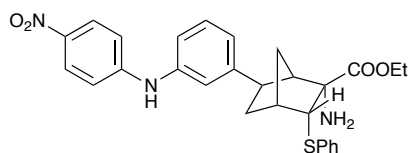
(1S*,2R*,3R*,4S*,5S*)-ethyl 2-amino-5-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (19):



19

Yield 85%, light yellow wax. IR (KBr): 3435m, 2958m, 2923m, 2424m, 2309m, 1718m, 1594m, 1581m, 1501m, 1324m, 1305m cm⁻¹. ¹H-NMR (200 MHz, CDCl₃, δ): 8.10 (d, *J* = 9.2 Hz, 2H), 7.48-7.19 (m, 6H), 7.04-7.01 (m, 3H), 6.91 (d, *J* = 9.2 Hz, 2H), 6.33 (s, 1H), 4.24-4.13 (m, 3H), 3.51 (t, *J* = 7.5 Hz, 1H), 2.65 (d, *J* = 2.9 Hz, 1H), 2.56-2.48 (m, 2H), 1.89 (s, 2H), 1.79–1.51 (m, 3H), 1.26 (t, *J* = 7.2 Hz, 3H). ¹³C-NMR (50 MHz, CDCl₃, δ): 176.7, 150.5, 148.4, 140.0, 139.9, 136.1, 131.4, 129.9, 129.2, 127.0, 126.4, 123.5, 121.0, 119.4, 113.9, 63.3, 61.8, 58.6, 49.4, 47.5, 39.3, 35.1, 32.1, 14.3. (+)ESI-MS (m/z): [M +H]⁺ 504.1. Anal. Calcd for C₂₈H₂₉N₃O₄S (503.19): C, 66.78; H, 5.80; N, 8.34; O, 12.71; S, 6.37; Found: C, 66.82; H, 5.83; N, 8.31; O, 12.66; S, 6.33.

(1R*,2R*,3R*,4R*,6R*)-ethyl 2-amino-6-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (20):



20

Yield 82%, light yellow wax. IR (KBr): 3352m, 2957m, 2922m, 2415m, 1783m, 1719m, 1581m, 1500m, 1324m, 1109m cm^{-1} . $^1\text{H-NMR}$ (200 MHz, CDCl_3 , δ): 8.10 (d, $J = 9.2$ Hz, 2H), 7.44 (d, $J = 8.1$ Hz, 2H), 7.33-7.03 (m, 7H), 6.92 (d, $J = 9.2$ Hz, 2H), 6.43 (s, 1H), 4.24-4.13 (m, 3H), 3.51 (dd, $J = 5.1$ Hz, $J = 8.8$ Hz, 1H), 2.69-2.40 (m, 5H), 1.65-1.59 (m, 3H), 1.26 (t, $J = 7.1$ Hz, 3H). $^{13}\text{C-NMR}$ (50 MHz, CDCl_3 , δ): 175.8, 150.5, 148.1, 140.0, 139.9, 136.3, 130.8, 129.8, 129.2, 126.8, 126.4, 123.8, 121.5, 119.4, 113.9, 64.4, 61.9, 56.2, 52.7, 43.3, 38.0, 34.3, 31.6, 14.3. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 504.1. Anal. Calcd For $\text{C}_{28}\text{H}_{29}\text{N}_3\text{O}_4\text{S}$ (503.19) calcd C, 66.78; H, 5.80; N, 8.34; O, 12.71; S, 6.37; found: C, 66.83; H, 5.83; N, 8.30; O, 12.66; S, 6.32

7.5.4 General procedure for last reduction steps

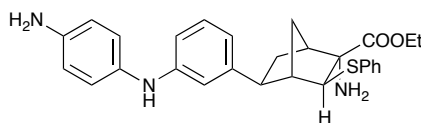
General Procedure for the Preparation of Derivatives AR129 and AR148.

Fresh activated Zinc dust (60 equiv.) was added under stirring to a solution of starting materials (**10** and **11**) in MeOH (0.05 M). The mixture was cooled down in a ice bath and aqueous solution of HCl (3 M, ml) was added dropwise under vigorous stirring at r.t. Then the reaction mixture was warmed up to 50 °C and monitored by TLC analysis (3 : 1 = EtOAc : *n*-hexane) until completion (6 hours). Upon consumption of starting material, the reaction was filtered under vacuum and the liquid phase was concentrated under reduced pressure. Saturated NaHCO_3 was added to the crude mixture until pH 8. The aqueous phase was extracted 3 times in EtOAc and combined organic layer was evaporated under reduce pressure. The crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 2 – 1 : 1 + 0.08% of TEA to give **AR-129** or **AR-148** in pure form. The reaction were performed on 0.1 g – 1.0 g scale

(1S*,2R*,3S*,4S*,5S*)-ethyl

2-amino-5-(3-((4-aminophenyl)amino)phenyl)-3-

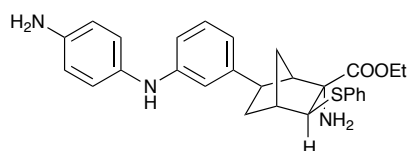
(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (AR129)



AR-129

Yield 88%, sand brown wax. IR (KBr): 3435m, 2963m, 2921m, 2523m, 1716m, 1602m, 1511m, 1502m, 1262m, 1024m cm^{-1} . ^1H -NMR (200 MHz, CDCl_3 , δ): 7.40- 6.91 (m, 8H), 6.78-6.54 (m, 5H) 5.39 (s, 1H), 4.34-4.04 (m, 2H), 3.47 (br, 2H), 3.08 (d, $J = 2.8$ Hz, 1H), 2.93 (t, $J = 7.7$ Hz, 1H), 2.62(br, 1H), 2.55–2.42 (m, 2H), 2.38 (d, $J = 10.5$ Hz, 1H), 2.00 (br, 2H), 1.82(d, $J = 10.5$ Hz, 1H), 1.52 (ddd, $J = 12.6$ Hz, $J = 6.7$ Hz, $J = 3.9$ Hz, 1H), 1.26 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CDCl_3 , δ): 174.7, 147.4, 146.2, 142.3, 137.5, 134.0, 129.7, 129.5, 129.1, 126.3, 123.5, 117.6, 116.4, 113.9, 112.9, 69.7, 65.6, 61.5, 51.6, 46.9, 46.8, 37.8, 33.0, 14.3. (+)ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ 496.1 Anal. Calcd for $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_2\text{S}$ (473.21): C, 71.00; H, 6.60; N, 8.87; O, 6.67; S, 6.77; Found: C, 70.97; H, 6.58; N, 8.89; O, 6.71; S, 6.79

(1*R,2*R**,3*S**,4*R**,6*R**)-ethyl 2-amino-6-(3-((4-aminophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (AR148)**



AR-148

Yield 84% (1 g scale 82%), light yellow wax. IR (KBr): 3377m, 2962m, 2926m, 1717m, 1602m, 1580m, 1511m, 1304m, 1261m cm^{-1} . ^1H -NMR (300 MHz, CDCl_3 , δ): 7.42- 6.88 (m, 8H), 6.79-6.58 (m, 5H) 5.42 (br, 1H), 4.34-4.02 (m, 2H), 3.64 (d, $J = 6.7$ Hz, 1H), 3.07 (t, $J = 2.7$ Hz, 1H), 2.76 (br, 4H), 2.66(s, 1H), 2.42 (s, 1H), 2.34 (d, $J = 10.9$ Hz, 1H), 2.00 1.92 (m, 2H), 1.77 (d, $J = 10.9$ Hz, 1H), 1.24 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CDCl_3 , δ): 174.5, 147.2, 146.1, 142.2, 137.5, 134.2, 129.8, 129.4, 129.1, 126.4, 123.3, 118.3, 116.4, 114.8, 112.8, 70.1, 64.00, 61.6, 52.8, 45.7, 38.8, 37.2, 36.5, 14.3. (+)ESI-MS (m/z): $[\text{M} + \text{Na}]^+$ 496.1. Anal. Calcd for $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_2\text{S}$ (473.21): C, 71.00; H, 6.60; N, 8.87; O, 6.67; S, 6.77. Found: C, 71.05; H, 6.62; N, 8.91; O, 6.70; S, 6.74.

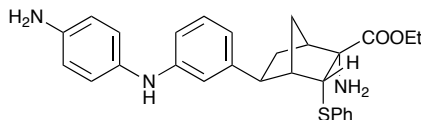
General Procedure for the Preparation of Derivatives AR177, AR180, AR194 and AR201.

SnCl_2 (6 equiv) was added to a solution of starting materials (**19**, **20**, **21** and **22**) in methanol (0.02M) under stirring. The reaction mixture was refluxed for 12h than a second portion of SnCl_2 (6 equiv) was added. The reaction was monitored by TLC analysis (1 : 1 = AcOEt : *n*-hexane) until completion (24 hours). Than solvent was remove under reduced pressure and he crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 2 – 1 : 1 + 0.08% of TEA to give a pure desire compound. The reaction were performed on 0.1 g – 0.3 g scale.

(1*S**,2*R**,3*R**,4*S**,5*S**)-ethyl

2-amino-5-(3-((4-aminophenyl)amino)phenyl)-3-

(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (AR-177):



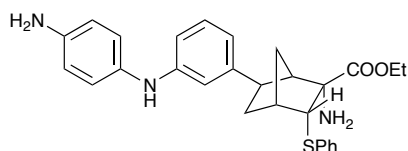
AR-177

Yield 80%, brown wax. IR (KBr): 3435m, 2956m, 2923m, 1717m, 1625m, 1604m, 1512m, 1437m, 1262m cm^{-1} . ^1H -NMR (200 MHz, CD_2Cl_2 , δ): 7.49- 7.38 (m, 2H), 7.33-7.17 (m, 3H), 7.09 (t, $J = 7.8$ Hz, 1H), 7.00-6.91 (m, 2H), 6.79-6.61 (m, 5H), 5.52 (br, 1H), 4.30-4.03 (m, 3H), 3.39 (dd, $J = 8.6$ Hz, $J = 6.6$ Hz, 1H), 2.60 (d, $J = 2.2$ Hz, 1H), 2.56-2.37 (m, 2H), 2.90-2.22(br, 4H), 1.69-1.42 (m, 3H), 1.28 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CD_2Cl_2 , δ): 176.5, 147.9, 146.2, 142.6, 136.7, 133.9, 131.4, 129.3, 129.1, 126.7, 123.1, 117.8, 116.0, 114.2, 112.5, 63.1, 61.5, 58.7, 49.4, 47.4, 39.3, 34.9, 31.8, 14.1. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 474.1. Anal. Calcd for $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_2\text{S}$ (473.21): C, 71.00; H, 6.60; N, 8.87; O, 6.67; S, 6.77. Found: C, 71.07; H, 6.63; N, 8.83; O, 6.71; S, 6.79.

(1*R**,2*R**,3*R**,4*R**,6*R**)-ethyl

2-amino-6-(3-((4-aminophenyl)amino)phenyl)-3-

(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (AR-180)



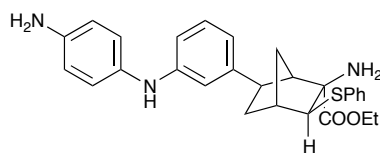
AR-180

: Yield 82%, brown wax. IR (KBr): 3380m, 2960m, 2923m, 1716m, 1601m, 1512m, 1479m, 1438m, 1262m cm^{-1} . ^1H -NMR (200 MHz, CD_3Cl , δ): 7.44 (d, $J = 6.9$ Hz, 2H), 7.32-7.20 (m, 3H), 7.11 (t, $J = 7.7$ Hz, 1H), 6.96 (d, $J = 8.6$ Hz, 2H), 6.78-6.65 (m, 5H), 5.37 (br, 1H), 4.26 (dd, $J = 4.0$ Hz, $J = 1.8$ Hz, 1H), 4.18 (q, $J = 7.1$ Hz, 2H), 3.68-3.56 (m, 1H), 2.64 (s, 1H), 2.61-2.52 (m, 1H), 2.50-2.34(m, 1H), 1.62-1.53 (m, 3H), 1.30 (br, 4H), 1.25 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CD_3Cl , δ): 176.4, 147.5, 146.0, 142.2, 136.7, 133.2, 130.5, 129.4, 129.1, 126.5, 123.3, 118.3, 116.4, 114.8, 112.8, 64.3, 61.7, 55.6, 52.8, 43.2, 37.9, 34.3, 31.8, 14.4. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 474.1. Anal. Calcd for $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_2\text{S}$ (473.21): C, 71.00; H, 6.60; N, 8.87; O, 6.67; S, 6.77. Found: C, 70.97; H, 6.68; N, 8.83; O, 6.69; S, 6.80.

(1*R,2*S**,3*S**,4*R**,6*R**)-ethyl**

2-amino-6-(3-((4-aminophenyl)amino)phenyl)-3-

(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (AR-194) :



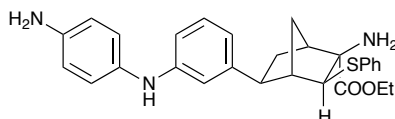
AR-194

Yield 82%, Light brown wax. IR (NaCl): 3368m, 2923m, 2853m, 2243m, 1944m, 1732m, 1621m, 1464m, 1236m, 1205m cm^{-1} . ^1H -NMR (200 MHz, CDCl_3 , δ): 7.43 (dd, $J = 8.3$ Hz, $J = 1.4$ Hz, 2H), 7.34-7.15 (m, 3H), 7.10 (t, $J = 6.7$ Hz, 1H), 7.00-6.90 (m, 2H), 6.71-6.55 (m, 5H), 5.35 (br, 1H), 4.24 (dq, $J = 7.1$ Hz, $J = 1.1$ Hz, 2H), 4.10 (d, $J = 1.7$ Hz, 1H), 2.72 (br, 2H), 2.69 (t, $J = 6.9$ Hz, 1H), 2.55 (br, 2H), 2.44 (s, 1H), 2.34 (d, $J = 1.4$ Hz, 1H), 2.18 (d, $J = 10.6$ Hz, 1H), 1.89-1.78 (m, 2H), 1.61 (dd, $J = 10.6$ Hz, $J = 1.7$ Hz, 1H), 1.29 (t, $J = 6.6$ Hz, 3H). ^{13}C -NMR (50 MHz, CDCl_3 , δ): 175.1, 146.9, 146.1, 142.1, 137.4, 134.1, 129.5, 129.2, 129.1, 126.1, 123.4, 117.7, 116.4, 114.06, 112.9, 68.5, 61.6, 56.7, 56.1, 45.9, 41.7, 36.6, 33.3, 14.5. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 474.1. Anal. Calcd for $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_2\text{S}$ (473.21): C, 71.00; H, 6.60; N, 8.87; O, 6.67; S, 6.77. Found: C, 71.05; H, 6.64; N, 8.89; O, 6.61; S, 6.71.

(1*S,2*S**,3*S**,4*S**,5*S**)-ethyl**

2-amino-5-(3-((4-aminophenyl)amino)phenyl)-3-

(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (AR-201)

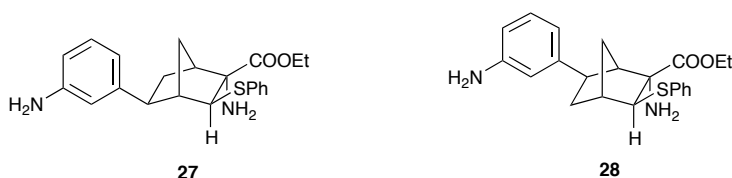


AR-201

: Yield 90%, yellow wax. IR (NaCl): 3369m, 2962m, 2924m, 2243m, 1871m, 1716m, 1601m, 1471m, 3H), 7.13-7.03 (m, 1H), 7.00-6.89 (m, 2H), 6.74-6.53 (m, 5H), 5.38 (br, 1H), 4.32-4.15 (m, 3H), 2.95-2.84 (m, 1H), 2.66 (br, 4H), 2.51 (s, 1H), 2.30 (d, $J = 1.8$ Hz, 1H), 2.22 (d, $J = 10.6$ Hz, 1H), 1.86-1.51 (m, 3H), 1.32 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (50 MHz, CDCl_3 , δ): 175.1, 146.5, 146.2, 142.3, 137.3, 133.9, 129.9, 129.4, 129.1, 126.3, 123.6, 117.9, 116.4, 114.1, 112.9, 67.9, 61.6, 57.7, 51.7, 50.29, 45.9, 34.1, 33.6, 14.5. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 474.1. Anal. Calcd for $\text{C}_{28}\text{H}_{31}\text{N}_3\text{O}_2\text{S}$ (473.21): C, 71.00; H, 6.60; N, 8.87; O, 6.67; S, 6.77. Found: C, 70.95; H, 6.63; N, 8.86; O, 6.70; S, 6.79.

7.5.5 General procedure for new selective protocol.

Synthesis of compounds (1*S**,2*R**,3*S**,4*S**,5*S**)-ethyl 2-amino-5-(3-aminophenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (**27**) and (1*R**,2*R**,3*S**,4*R**,6*R**)-ethyl 2-amino-6-(3-aminophenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (**28**).



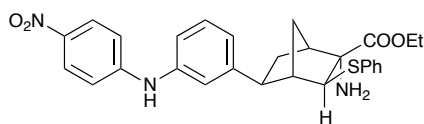
Compounds **26** (1 equiv.) was dissolved in dry CH₃CN (0.1M) in a pyrex tube under Argon. Pd(OAc)₂ (0.05 equiv), 3-iodoaniline (1.1 equiv), dry TEA (3.5 equiv) and formic acid (3 equiv) was added under stirring. The tube was sealed with Teflon caps and stirred at 45°C for 24 - 48 h. The reaction was monitored by TLC analysis (1 : 10 = DCM : MeOH) and ¹H-NMR analysis. Solvent was removed under reduced pressure and after deactivation of silica gel with a solution EtOAc : *n*-hexane = 1 : 5 + 10% of TEA the crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 3 – 3 : 1 to give a clean mixture of two inseparable regio-isomers **27** and **28**. Light yellow oil (82-88% yield) in ratio **27** : **28** = 35 : 65 (evaluated by NMR analysis on crude reaction mixture). The reaction was performed on 0.1 g – 1.0 g scale. ¹H-NMR (200 MHz, CDCl₃, δ): 7.36-7.02 (m, 6H **27**, 6H **28**), 6.66-6.49 (m, 3H **27**, 3H **28**), 4.70-4.08 (m, 2H **27**, 2H **28**), 3.63 (t, *J* = 6.9 Hz, 1H **28**), 3.09 (d, *J* = 2.9 Hz, 1H **27**), 3.05 (d, *J* = 2.2 Hz, 1H **28**), 2.93 (t, *J* = 8.7 Hz, 1H **27**), 2.66 (s, 1H **28**), 2.61 (s, 1H **27**), 2.50 (s, 1H **27**), 2.42-2.30 (m, 1H **27**, 2H **28**), 1.96–1.88 (m, 2H **27**, 1H **28**), 1.84–1.73 (m, 1H **27**, 1H **28**), 1.58–1.47 (m, 1H **27**), 1.29–1.20 (m, 3H **27**, 3H **28**).

Synthesis of compounds 29 and 30. The regioisomers mixture of Heck reaction (**27-28**) (1equiv) was dissolved in dry CH₃CN (0.1M) in a pyrex tube under argon. Pd(tetrakis) (0.05 equiv), 1-iodo-4-nitrobenzene (1.1 equiv) and K₃PO₄ (2 equiv) was added under stirring to a mixture. The tube was sealed with Teflon caps and stirred at 70 °C for 40 h. The reaction was monitored by TLC analysis (1 : 1 = AcOEt : *n*-hexane) and ¹H-NMR analysis until completion. Upon consumption of starting material solvent was removed under reduced pressure and after deactivation of silica gel with a solution EtOAc : *n*-hexane = 1 : 5 + 10% of TEA the crude was purified using flash chromatography EtOAc : *n*-hexane = 1 : 3 – 1 : 2 to give two fractions. The first containing compound **30** the second one containing compound **29** in pure form. The reaction was performed on 0.1 g – 1.0 g scale.

(1*S**,2*R**,3*S**,4*S**,5*S**)-ethyl

2-amino-5-(3-((4-nitrophenyl)amino)phenyl)-3-

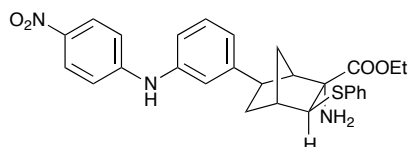
(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (**29**)



29

Yield (90%-80%), bright yellow wax. IR (NaCl): 3367m, 2961m, 2920m, 2601m, 2413m, 1721m, 1581m, 1501m, 1480m, 1304m cm^{-1} . ^1H NMR (400 MHz, CDCl_3 , δ) 8.12 (d, $J = 9.2$ Hz, 2H), 7.41 – 7.25 (m, 5H), 7.20 (t, $J = 7.3$ Hz, 1H), 7.08 – 6.98 (m, 3H), 6.94 (d, $J = 9.2$ Hz, 2H), 6.50 (s, 1H), 4.31 – 4.13 (m, 2H), 3.14 (d, $J = 2.7$ Hz, 1H), 3.05 (t, $J = 7.6$ Hz, 1H), 2.68 (d, $J = 1.7$ Hz, 1H), 2.57 – 2.48 (m, 2H), 2.45 (d, $J = 10.9$ Hz, 1H), 1.94 (s, 2H), 1.82 (d, $J = 10.9$ Hz, 1H), 1.55 (ddd, $J = 12.6, 6.5, 3.9$ Hz, 1H), 1.29 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (100 MHz, CDCl_3 , δ): 174.4, 150.2, 147.9, 139.7, 137.1, 131.9, 129.8, 129.5, 128.9, 126.3, 126.1, 123.1, 120.3, 119.3, 113.7, 69.3, 65.2, 61.4, 51.4, 46.7, 46.4, 37.6, 32.9, 14.1. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 504.1. Anal. Calcd for $\text{C}_{28}\text{H}_{29}\text{N}_3\text{O}_4\text{S}$ (503.19): C, 66.78; H, 5.80; N, 8.34; O, 12.71; S, 6.37; Found: C, 66.70; H, 5.76; N, 8.36; O, 12.78; S, 6.32.

(1*R,2*R**,3*S**,4*R**,6*R**)-ethyl 2-amino-6-(3-((4-nitrophenyl)amino)phenyl)-3-(phenylthio)bicyclo[2.2.1]heptane-2-carboxylate (30).**



30

Yield (90%-80%), bright yellow wax. IR (NaCl): 3436m, 2957m, 2920m, 2594m, 2417m, 1721m, 1581m, 1503m, 1481m, 1323m cm^{-1} . ^1H NMR (400 MHz, CDCl_3 , δ) 8.13 (d, $J = 9.2$ Hz, 2H), 7.41 – 7.27 (m, 5H), 7.22 (t, $J = 7.8$ Hz, 1H), 7.11 – 7.02 (m, 3H), 6.94 (d, $J = 9.2$ Hz, 2H), 6.42 (s, 1H), 4.25 (dq, $J = 10.8, J = 7.1$ Hz, 1H), 4.16 (dq, $J = 10.8, J = 7.1$ Hz, 1H), 3.78 (dd, $J = 8.7, J = 4.5$ Hz, 1H), 3.09 (d, $J = 2.6$ Hz, 1H), 2.71 (s, 1H), 2.48 (d, $J = 3.7$ Hz, 1H), 2.40 (d, $J = 10.9$ Hz, 1H), 2.09 – 1.90 (m, 4H), 1.75 (d, $J = 10.9$ Hz, 1H), 1.27 (t, $J = 7.1$ Hz, 3H). ^{13}C -NMR (75 MHz, CDCl_3 , δ): 174.3, 150.3, 147.8, 139.7, 139.6, 137.1, 129.6, 129.5, 128.9, 126.3, 126.2, 123.8, 121.2, 119.3, 113.7, 69.8, 63.8, 61.4, 52.5, 45.6, 38.5, 37.1, 36.2, 14.1. (+)ESI-MS (m/z): $[\text{M} + \text{H}]^+$ 504.1. Anal. Calcd for $\text{C}_{28}\text{H}_{29}\text{N}_3\text{O}_4\text{S}$ (503.19): C, 66.78; H, 5.80; N, 8.34; O, 12.71; S, 6.37; Found: C, 66.84; H, 5.84; N, 8.35; O, 12.74; S, 6.39

7.6 Chapter 6

Full characterizations and procedures for the preparation of all compounds contained in chapter 6 are already published at

Dr. Q. Zhou, A. Ruffoni, R. Gianatassio; Dr. Y. Fujiwara, Dr. E. Sella, Prof. Dr. D. Shabat, Prof. Dr. P. S. Baran. Direct Synthesis of Fluorinated Heteroarylether Bioisosteres. *Angew. Chem. Int. Ed.* **2013**, 52, 1–5