Fluorous L-Carbidopa Precursors: Highly Enantioselective Synthesis and Computational Prediction of Bioactivity

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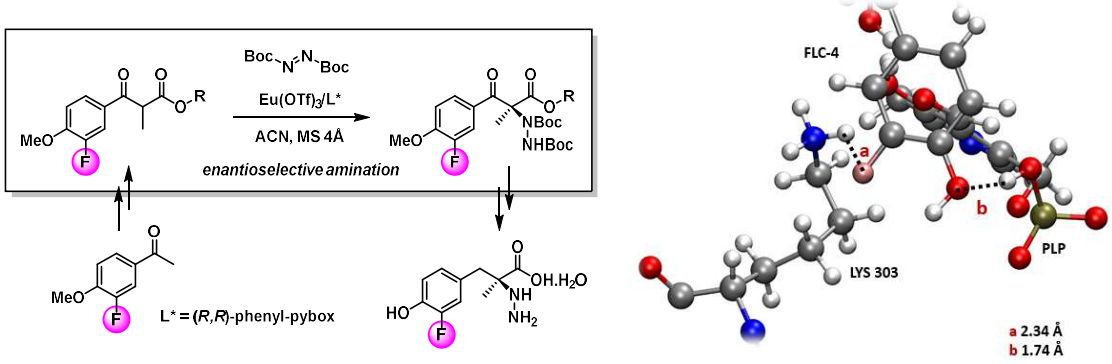
Abstract. New fluorous enantiopure (*S*)-α-aminated β-keto esters were prepared through a highly enantioselective electrophilic α-amination step in the presence of europium triflate and (*R,R*)-phenyl-pybox. These compounds are precursors of fluorinated analogues of L-Carbidopa, which is known to inhibit DOPA decarboxylase (DDC), a key protein in Parkinson's disease. Fluorination provides better stability for biological applications, which could possibly lead to better DDC inhibitors than L-Carbidopa itself. Induced fit docking computational simulations performed on the newly structures interacting with DDC highlight that for an efficient binding at the DDC site at least one hydroxyl substituent must be present at the aromatic ring of the L-Carbidopa analogues, and point out that the presence of fluorine can further concur to fix the position of the ligand in the active site.



Parkinson's disease is a chronic and progressive neurological disorder whose origin is linked to the degeneration of dopamine-producing cells in the brain.[1] DOPA decarboxylase (DDC), which is abundant in the nervous system and kidney, is responsible for the synthesis of dopamine via decarboxylation of L-DOPA. Dopamine itself is not able to cross the blood-brain barrier and thus cannot be used as a drug in the treatment of Parkinson's disease. Moreover, when administered L-DOPA is rapidly converted to dopamine in the blood stream and only a small percentage will reach the nervous system. By adding DDC inhibitors, the most common among which is L-Carbidopa, greater amounts of L-DOPA reach the brain, with a substantial increase of dopamine in nerve cells.[2] At the same time, dopamine side effects related to a high concentration in the blood stream are diminished. The inhibitor is covalently linked to DDC by forming a hydrazone derivative of the cofactor.[3]

Since the preparation of fludrocortisone, one of the earliest synthetic fluorinated drugs,[4] fluorine substitution in medicinal compounds is commonly used to improve metabolic stability, bioavailability and protein-ligand interactions.[5] The incorporation of a fluorine atom(s) or fluorinated group(s) often provides molecules with quite unique properties. It is estimated that 30% of the leading 30 blockbuster drugs by sales contains fluorine.[6] This element is generally incorporated into the organic drug during the optimization studies. Almost every new drug discovery and development program explores fluorine-containing drug candidates.

A factor limiting the clinical use of many pharmaceuticals is their excessively rapid metabolic degradation. The key to many of these degradation processes is oxidative metabolism by the cytochrome P450 family. The strategic incorporation of fluorine(s) into metabolism site(s) has been widely used to prevent this deactivation.[7] Cytohrome P450 oxidation is easier for electron-rich π systems such as aromatic moieties. Introduction of a fluorine substituent avoids oxidation of the C_{ar}-F site and can also induce electronic effects on its neighbors, decreasing the pK_a value

and the Lewis basicity and retarding its oxidation. For instance, it can increase the acidity of an alcohol group, which in turn will promote a better binding to the enzyme binding site.[8]

In the present study fluorine(s) or fluorine-containing groups are incorporated into the synthetic precursors of L-Carbidopa. We envisioned different fluorination positions (Figure 1) as a tool to block undesired metabolic pathways. We were also concerned about whether the presence of hydroxylic groups on the aromatic moiety is essential for binding to DCC and thus for yielding the desired biologic activity.

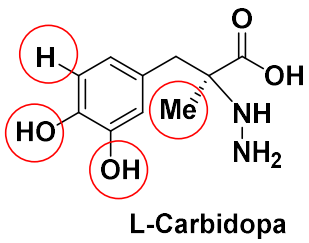
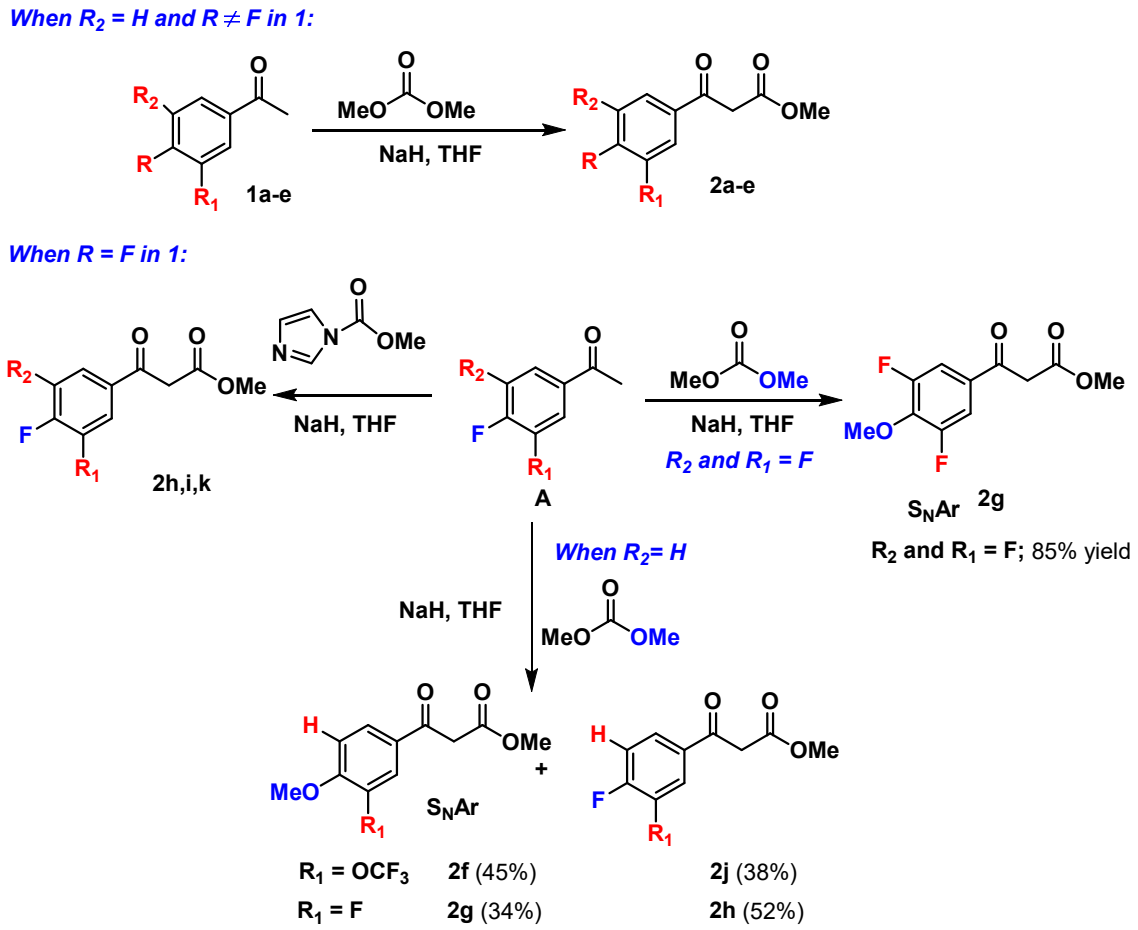


Figure 1. L-Carbidopa formula indicating the positions where fluorine atoms or groups will be introduced.

We have previously described a straightforward methodology for the synthesis of L-Carbidopa (seven steps, 50% overall yield and 98% *ee*) through a key enantioselective α-amination step,[9] which will be used in this work to synthesize enantiopure fluorous L-Carbidopa precursors.

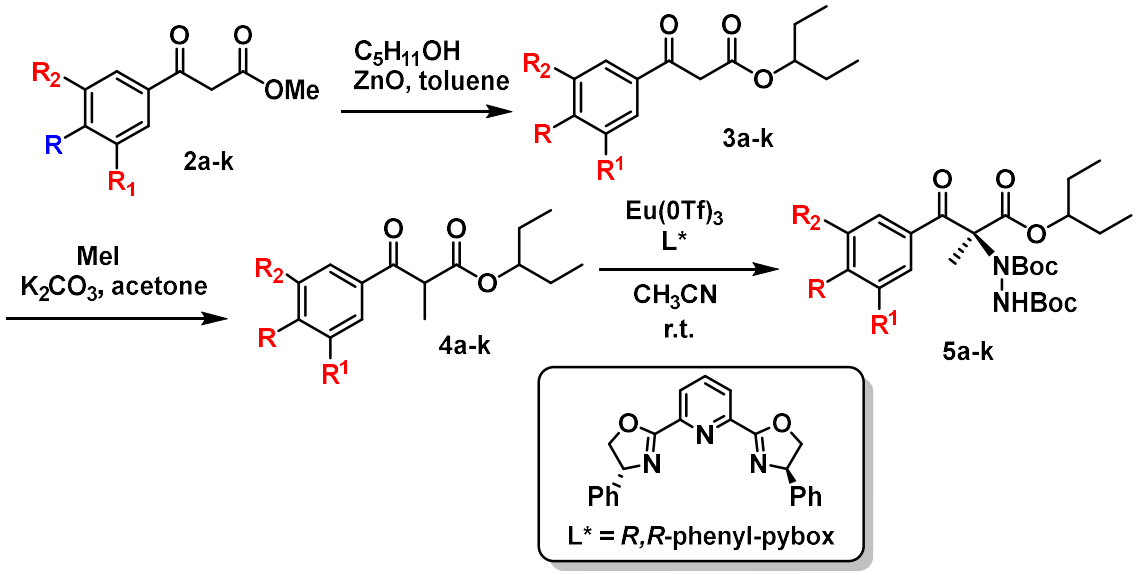
We first selected a series of fluorous commercially available acetophenones **1** to prepare the corresponding β-keto esters **2**. Using dimethylcarbonate and 2 equiv of NaH as a base, the desired products **2a-e** were obtained in excellent yields (92-97%, Scheme 1 and Table 1). However, for 4-fluoro acetophenones **A** (Scheme 1, R = F) possessing an electro withdrawing group in *orto* to the C_{ar}-F, as F and $-OCF_3$,[10] we observed a partial S_NAr reaction on the C_{ar}- F to form C_{ar}-OMe in the same conditions. Indeed, the nucleophilic substitution addition reaction of the sodium enolate of **1** with dimethylcarbonate involves methoxide as a leaving group which partially reacts as nucleophile at the C_{ar}-F, producing the introduction of the OMe substituent. Thus, mixtures of 4-fluoro (**2j** and **2h**) and 4-methoxy (**2f** and **2g**) β-ketoesters were obtained (Scheme 1). Additionally, for C_{ar}-F possessing electron withdrawing groups in both *orto* positions, as for example for the 3,4,5-trifluoroacetophenone, **A(1k)**, the S_NAr reaction of

methoxide at the C_{ar}-F is synthetically useful to selectively obtain the substitution product **2g** (85% yield, Scheme 1, Table 1). The solution to avoid fluoride loss was found in using methyl 1H-imidazole-1-carboxylate in the presence of 1.5 equiv of NaH. The corresponding β-keto esters **2h,j,k** were afforded in yields between 80-96% (Scheme 1, Table 1).



Scheme 1. Different methodologies used in the preparation of β-keto esters **2.**

Our previous experience suggested that a β-keto ester substrate bearing an OR group bulkier than methoxy might be necessary to achieve efficient enantioinduction, and secondary 3-pentyl group fulfills these requirements.[11] Therefore transesterification of **2a-k** with 3-pentanol was accomplished using catalytic amounts of ZnO in refluxing toluene rendering **3a-k** in excellent yields (80-99% yield, Scheme 2, Table 1).[12] Subsequent alkylation under classical conditions using methyl iodide and potassium carbonate in anhydrous acetone afforded the α-methyl β-keto esters **4a-k** (52-96% yield, Scheme 2, Table 1).



Scheme 2. Synthesis of fluorous (*S*)-α-aminated β-keto esters **5a-k.**

Table 1. Yields of reactions indicated in Scheme 1.

R_2	R	R_1	Yield 2a-k	Yield 3a-k	Yield 4-k
H	CF_3	H	92% (2a)	85% (3a)	85% (4a)
H	OCF_3	H	93% (2b)	85% (3b)	81% (4b)
H	OCH_3	F	92% (2c)	89% (3c)	89% (4c)
H	OCH_3	CF_3	95% (2d)	92% (3d)	90% (4d)
H	OCH_3	OCH_3	97% (2e)[a]	99% (3e)[a]	96% (4e)[a]
H	OCH_3	OCF_3	45% (2f)[b]	95% (3f)	81% (4f)
F	OCH_3	F	85% (2g)	85% (3g)	89% (4g)
H	F	F	96% (2h)	80% (3h)	92% (4h)
H	F	OCF_3	38% (2i)[b]	92% (3i)	86% (4i)
H	F	CF_3	80% (2j)	88% (3j)	88% (4j)
F	F	F	84% (2k)	82% (3k)	89% (4k)

[a] Previously prepared, see ref. 9;[b] Reaction of 3-trifluoromethoxy-4-fluorobenzophenone with dimethylcarbonate and NaH as base gave 45% of **2f** and 38% of **2i**.

Substrates **4a-k** underwent enantioselective electrophilic *S*-amination with di-*tert*-butyl azodicarboxylate[13] using a $Eu(OTf)_3$/L* mixture as a catalyst. A solution of $Eu(OTf)_3$ (0.017 mmol) and (*R,R*)-phenyl-pybox (0.023 mmol) in dry acetonitrile (1.5 mL) was stirred overnight in the presence of 4Å molecular sieves under argon atmosphere at room temperature. Then, the β-keto ester (0.184 mmol) and the electrophile (0.298 mmol) were sequentially added. We have previously reported that in those cases the best choice for the pybox ligand was (*R,R*)-diphenylpybox,[11b] however using fluorous substrates, the simpler and commercially available (*R,R*)-phenyl-pybox employed at room temperature gave excellent chemical yields (70-87%) and excellent *ee*'s (90-100%). The presence of the phenyl substituents in the pybox ligand is essential for a good enantioselection, probably due to π stacking interactions between the aromatic moiety of the substrate **4** (Scheme 4) and the aromatic ring of the phenylpybox (using (*R,R*)-isopropylpybox only a moderate 63% was obtained in the same conditions for **5e**). In addition, the enantiomeric excesses were much greater (90-99%) with electro withdrawing groups as substituents in the aromatic positions (**5a-d, f-k** in Figure 2) than those obtained for the aromatic electronic rich derivatives (80% *ee* for **5e** in Figure 2) in the same conditions.

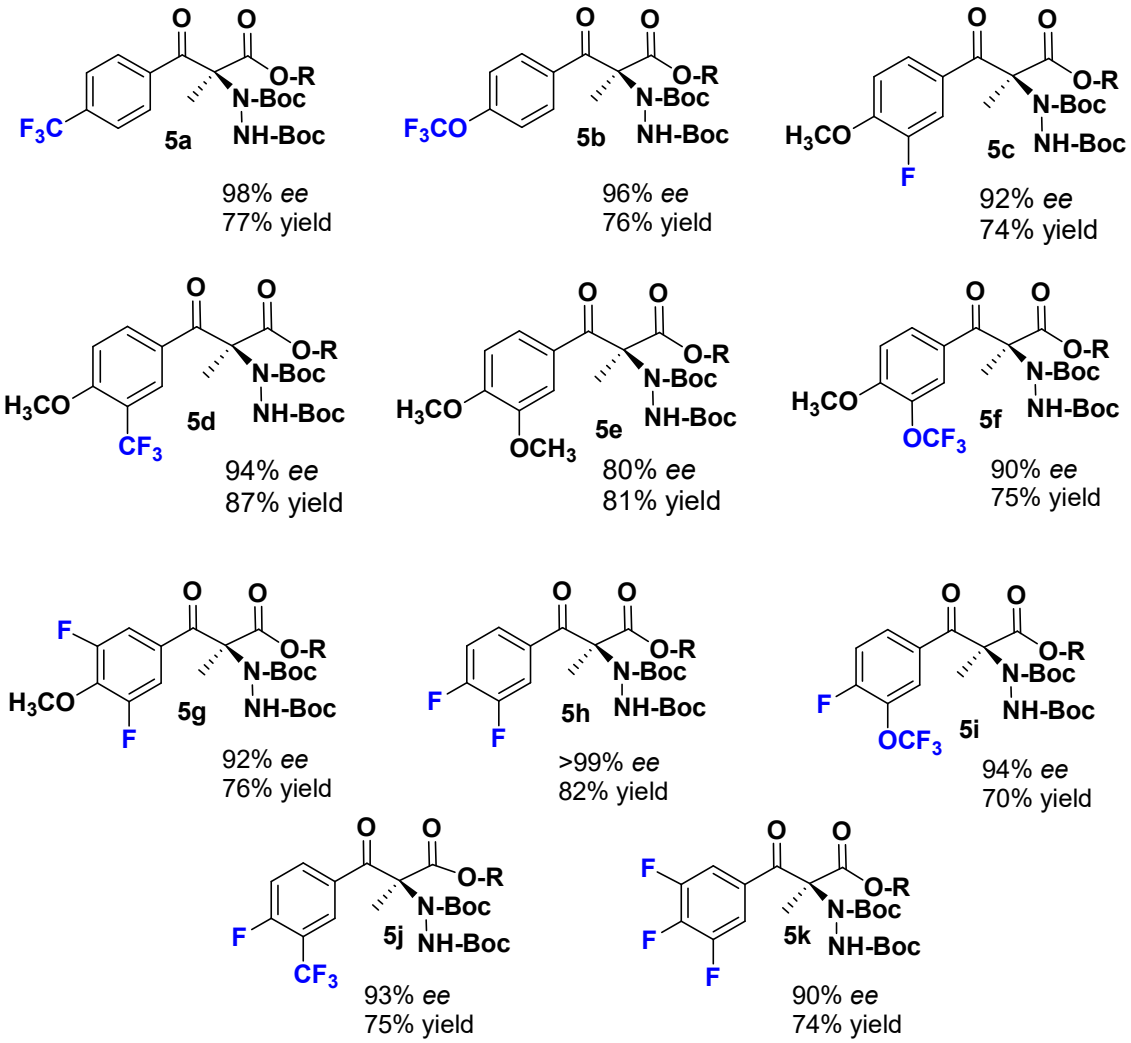


Figure 2. Structures, yields and *ee*'s of the α-amination step (Scheme 2) for compounds **5.**

The mechanism of this type of reaction has been proposed previously.[11a,14] There is enough evidence in the literature to propose the formation of the intermediate corresponding to the coordination of pybox ligand, β-keto ester and azo reactive to the europium atom in the disposition shown in Figure 3. One of the phenyl ring on the oxazoline unit can attain a conformation to present a suitable distance so as to provide stabilizing π interactions. This is possible when the right-side phenyl is parallel to the aryl ring of **4**. In this pausible intermediate (Figure 3) the bulkiness of the ester in **4** is also responsible of the coordination of the ketoester with this functional group ortogonal to the left-side phenyl. The C-N formation step is the

chiral-determining step. Formation of a six-membererd ring transition state has been proposed by other authors.[14]

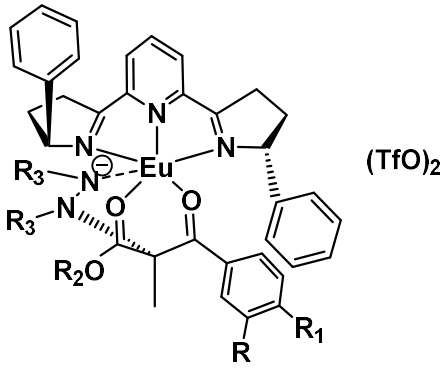
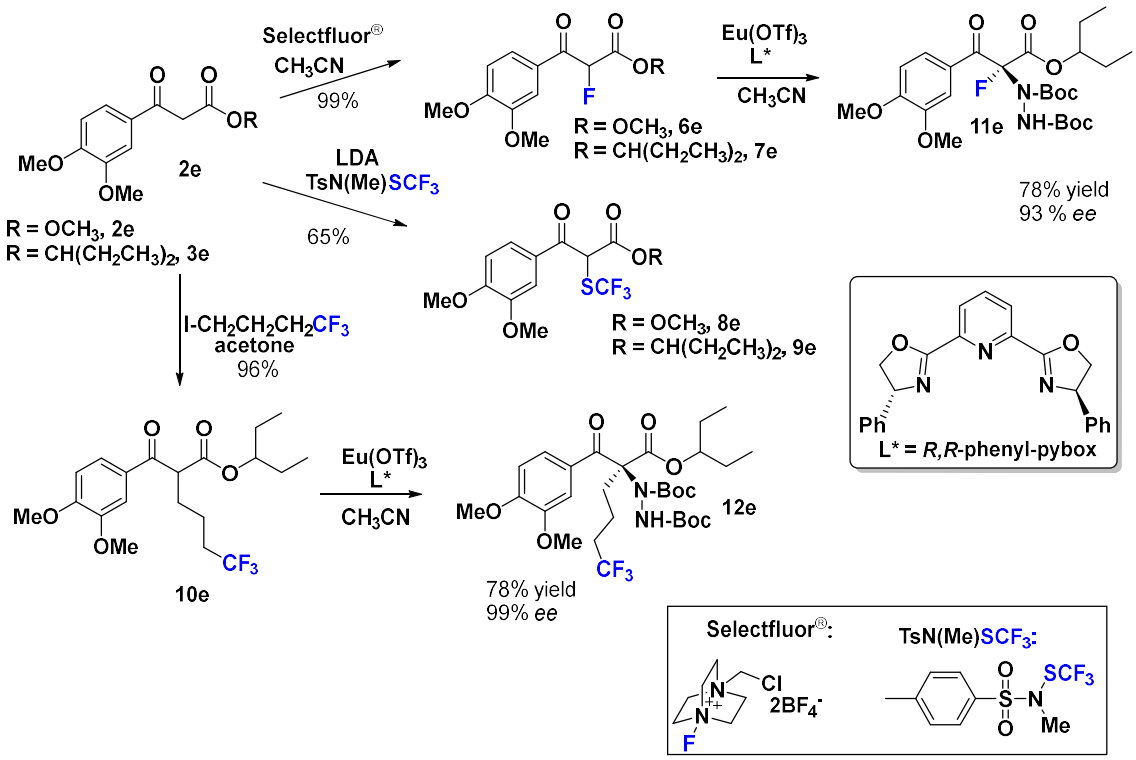


Figure 3. Schematic representation of a plausible intermediate of the enantioselective α-amination reaction

Precursors **5** could be easily converted to fluorous L-Carbidopa analogues following a chemistry well established and previously published by our group.[9]

Other fluorous derivatives were also prepared. Monofluorination of β-keto esters **2e** and **3e** with Selectfluor®[15] in acetonitrile gave compounds **6e** and **7e** with excellent 95% yield in both cases. The amination step was carried out using di-*tert*-butyl azodicarboxylate, $Eu(OTf)_3$ and commercially available (*R,R*)-phenyl-pybox at room temperature, obtaining **11e** in a high yield and excellent 93% *ee* (Scheme 4). Other fluorinated groups, such as $–SCF_3$,[16] could also be introduced in the intercarbonylic position, although they are too bulky for the following amination process. The introduction of $–CF_3$ was discarded since in the amination conditions HF will be lost. Thus, we thought to intercalate at least one methylene group. However, commercially available $I\text{-}CH_2CF_3$ and $I\text{-}CH_2CH_2CF_3$ underwent acid elimination in the alkylation step basic conditions. Using trifluoroiodobutane (Scheme 4) a 96% yield of **10e** was obtained and the enantioselective amination step was accomplished with a 99% of *ee* (78% yield).



Scheme 4. Introduction of fluorine and fluorous groups in the intercarbonylic position and subsequent α-amination step.

The assignment of the absolute configuration of compounds **5** and **12e** as *S* was based on the comparison of the circular dichroism (CD) (all compounds show a positive Cotton Effect), and the position of the major peak on the chiral HPLC spectrum with previously described analogues prepared in our laboratories.[9,11c] In the case of **11e,** due to the presence of F in the intercarbonylic chiral carbon, the asymmetric induction is obtained in the same sense although the resulting product is the (*R*) enantiomer.

Additionally, induced fit docking simulations were performed to assess how fluorination of the L-Carbidopa scaffold can modify its affinity for the DDC receptor. To do so, some of the fluorinated analogues of L-Carbidopa (Figure 5) were tested using the PELE suite[18,19] (see SI for further details), which provides an efficient tool for the prediction of binding poses of ligand/receptor complexes. The L-Carbidopa/DDC complex was derived from the structure reported in PDB file 1JS3. DDC is a dimer, with two equivalent binding sites for Carbidopa,

which, as already mentioned, forms a hydrazone intermediate with the pyridoxal 5'-phosphate (PLP) cofactor. The docking method was first tested on this complex, by cleaving the Carbidopa-PLP covalent bond and submitting an unconstrained binding site search simulation for the same L-Carbidopa on the receptor. Satisfactorily, the ligand is docked in the crystallographic binding site with the correct conformation, with the aldehyde group of PLP correctly oriented to start the reaction with the hydrazine group of L-Carbidopa (Figure 4a).

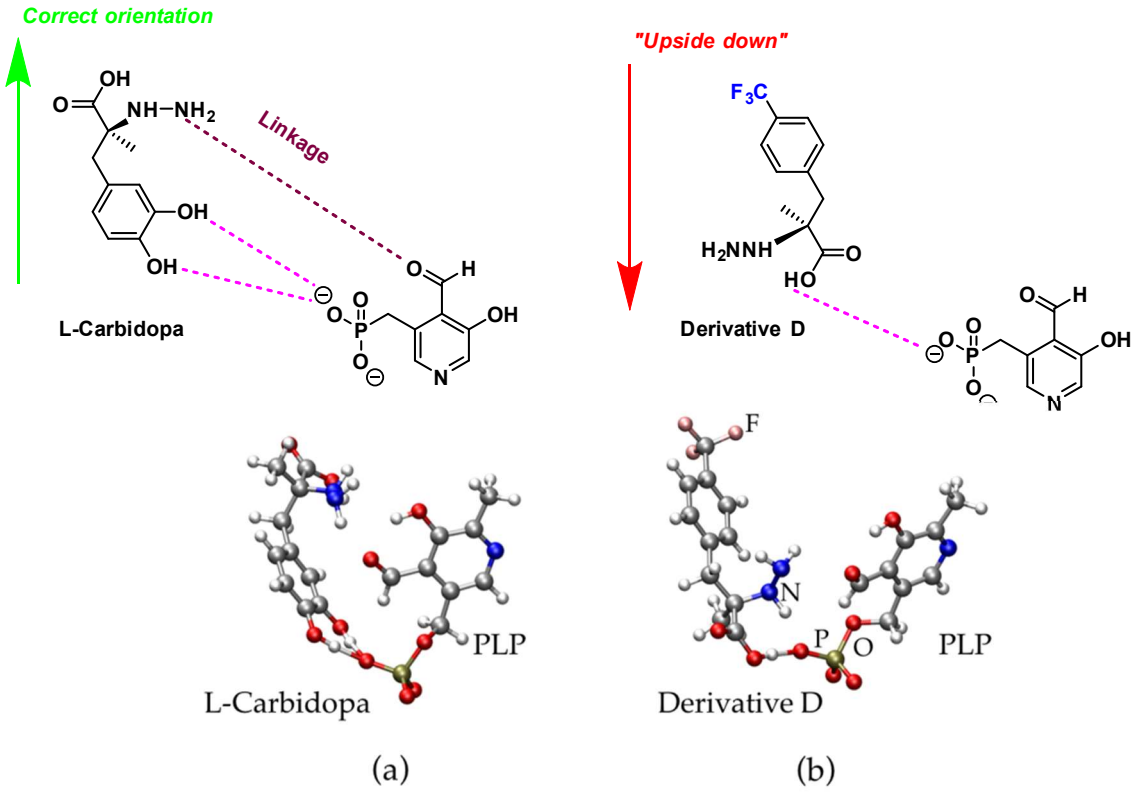


Figure 4. Relative orientation of the ligand and PLP cofactor for L-Carbidopa and a fluorinated derivative from induced fit docking simulations.

Overall, eight fluorinated analogues of L-Carbidopa were tested (Figure 5) and results highlighted that one aromatic hydroxyl group is fundamental to guarantee the correct orientation of the ligand in the binding site of the receptor (for a full description of the results see the SI).

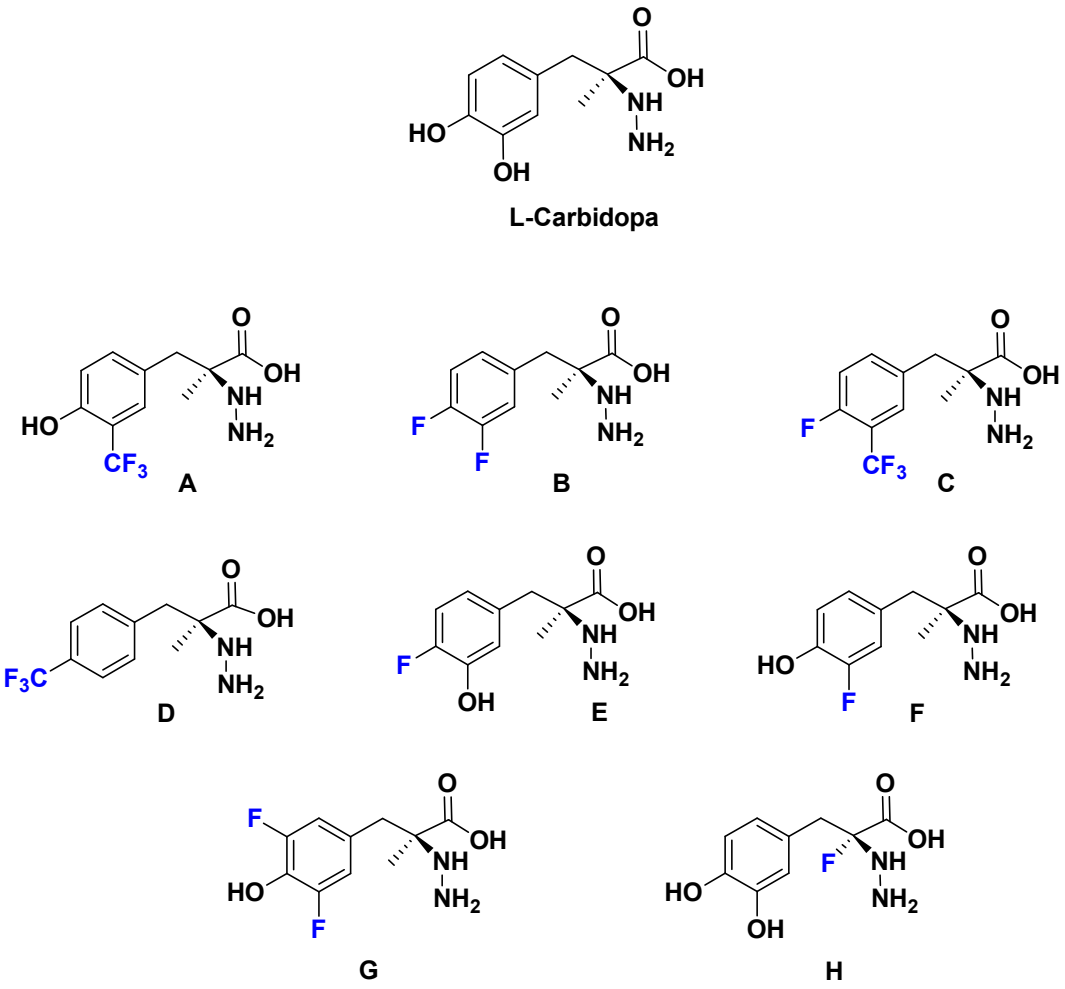


Figure 5. The eight fluorinated analogues of L-Carbidopa tested as DDC ligands.

As reported in Figure 4a, the hydroxyl groups form strong hydrogen bonds (1.48 Å) with the phosphate group of PLP. If at least one of the two hydroxyl groups is preserved, our simulations show that the molecule enters the active site of DDC in a suitable orientation. If, conversely, the ligand does not present a hydroxyl group, then these hydrogen bonds cannot be formed. In such cases, molecules that are not forbidden to enter the binding pocket by sterical factors will assume an "upside down" conformation (Figure 4b), in which the carboxyl group of the ligand is involved in interaction with the phosphate. Thus, according to these calculations, a hydrogen bond donor has to be maintained on the aromatic ring for the ligand to act as an inhibitor for DDC.

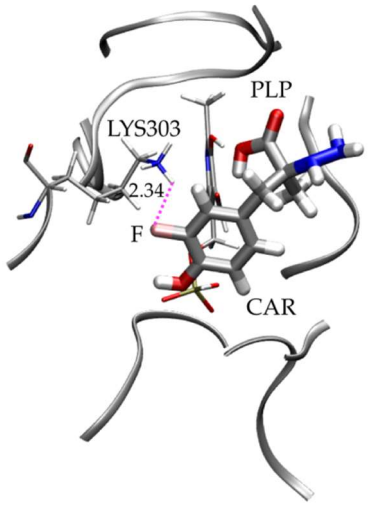


Figure 6. Positioning of fluorinated analogue **F** of L-Carbidopa in the active site of DDC, with indication of an electrostatic interaction with a positively charged residue, LYS303. Distance in Å.

Figure 6 reports the binding pose of derivative **F** in the active site of DDC. This molecule presents a fluorine atom and a hydroxyl group on the aromatic ring. While the oxygen of the hydroxyl group acts as hydrogen bond acceptor (distance 1.74 Å), maintaining the molecule in the correct orientation in the site, the fluorine atom, which bears a negative partial charge, is involved in an electrostatic interaction with a nearby positively charged lysine residue. This suggests that fluorination may result in the formation of stabilizing interactions that further concur to fixing the position of the ligand in the active site of DDC.

In this work we present the preparation of (*S*)-fluorous precursors of L-Carbidopa through a highly enantioselective α-amination key step of a series of acyclic β-keto esters possessing different fluorous substituents at the arene ring or in the intercarbonilic position, with excellent chemical yields (70-87%) and enantiomeric excesses (90-99%). We found that neither using diphenyl-pybox as chiral ligand nor working at low temperature is essential. The advantage of this finding is that the simple commercially available phenyl-pybox (both enantiomers are commercial giving access to both **(*S*)** and **(*R*)-5** enantiomers) can be used, and that reactions can be performed at room temperature. Some of the fluorous L-Carbidopa analogues were then

studied to assess how fluorination tunes their affinity for the DDC target. We conclude that the presence of at least one hydroxyl group on the arene moiety of L-Carbidopa scaffold is crucial to maintain the molecule in the correct orientation for inhibition of the DDC binding site. Additionally, fluorine atoms may be involved in electrostatic interactions with positively charged residues of DDC, indicating that fluorination can concur in stabilizing the inhibitor-receptor complex.

REFERENCES

(1) a) Damier, P.; Hirsch, E. C.; Agid, Y.; Graybiel, A. M. *Brain* **1999**, *122*, 1437-1438; b) Zanforlin, E. Zagotto, G. Ribaudo, G. *ACS Chem. Neurosci.,* 2017, DOI:10.1021/acschemneuro.7b00283; c) Ellis, J. *M.; Fell, M. J. Bioorg. Med. Chem. Lett. 2017, 27, 4247-4255.*

(2) (a) Hayek, J. *Schweizerische Medizinische Wochenschrift*, **1977**, *107*, 474-479. (b) Delea, T. E.; Thomas, S. K.; Hagiwara, M. *CNS Drugs* **2011**, *25*, 53-66.

(3) a) Burkhard, P.; Dominici, P.; Borri-Voltattori, C.; Jansonius, J. N.; Malashkevick, V. N. *Nat. Struct. Mol. Biol.* **2001**, *8*, 963-967.b) Bertoldi, M. *Archiv. Biochem. Biophys.* **2014**, *546*, 1-7.

(4) Fried, J.; Sabo, E. F. *J. Am. Chem. Soc.* **1954**, *76*, 1455-1456.

(5) a) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. *Chem. Soc. Rev.* **2008**, *37*, 320-330; b) O'Hagan *J. Fluorine Chem.* **2010**, *131*, 1071-1081; c) Ilardi, E. A. ; Vitaku, E. ; Njardarson, J. T. *J. Med. Chem.* **2014**, *57*, 2832-2842; d) Samart, B. E. *J. Fluorine Chem*. **2001**, *109*, 3-11.

(6) a) Isanbor, C.; O'Hagan, D. *J. Fluorine Chem.* **2006**, *127*, 303-319; b) Ismail F. M. D. *J. Fluorine Chem.* **2002**, *118*, 27-33; c) Müller, K; faeh, C.; Diederich, F. *Science* **2007**, *317*, 1881-1886.

(7) Park, B. K.; Kitteringham, N. R. ; O'Neill, P. M. *Annu. Rev. Pharmacol. Toxicol.* **2001**, *41*, 443-470.

(8) Fluorine in Medicinal Chemistry and Chemistry Biology", Wiley-Blackwell, **2009**.

(9) Pericas, A.; Shafir, A.; Vallribera, A. *Org. Lett.* **2013**, *15*,1448-1451. Highligted in *Synfacts 2013*, 700.

(10) Castagnetti, E.; Schlosser, M. Chem. Eur. J. 2002, 8, 799-804.

(11) a) Comelles, J.; Pericas, A.; Moreno-Mañas, M.; Vallribera, A.; Drudis-Solé, G.; Lledos, A.; Roglans, A.; Gracía-Granda, S.; Roces-Fernández, L. *J. Org. Chem*. **2007**, *72*, 2077. b) Pericas, A.; Jiménez, R.; Granados, A.; Shafir, A. ; Vallribera, A.; Roglans, A.; Molins, E. *ChemistrySelect* **2016**, *1*, 4305-4312.

(12) Pericas, A.; Shafir, A.; Vallribera, A. *Tetrahedron* **2008**, *64*, 9258-9263. Highlighted in *Synfacts*, **2009**, 81.

(13) Genet, J. –P.; Greck, C.; D. Lavergne B. in *Modern Amination Methods*, (Eds.: A. Ricci), Wiley-VCH, Weinheim, **2000**, pp. 65-102.

(14) Yu, Y.; Shen, W.; Zhang, J.; he, R. Li, M. *J. Mol. Model* **2008**, *14*, 237-247

(15) Nyffeler, P. T.; Durón, S. G.; Burkart, M. D. ; Vicent, S. P. M; Wong, C.-H. *Angew. Chem. Int. Ed.* **2005**, *44*, 192-212.

(16) a) Alazet, S.; Zimmer, L. Billard, T. *Chem Eur. J.* **2014**, *20*, 8589-8593. b) Milandou, L. J. C. B.; Carreyre, H.; Alazet, S.; Greco, G.; Martin-Mingoit, A.; Loumpangou, C. N. ; Ouamba, J.-M.; Billard, T.; Thibaudeau, S. *Angew. Chem. Int. Ed*. **2017**, *56*, 169-172.

(17) Madadkar-Sobhani, A.; Guallar, V. *Nucleic Acids Res.* **2013**, *41*, 322-328.

(18) Borrelli, K. W.;Vitalis, A.; Alcantara, R.; Guallar, V. J. *Chem. Theory Comput.,* **2005**, *1*, 1304-1311.