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Synthesis and development of inhibitors of FtsZ as antibacterial agents

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Introduction

Antibiotic-resistant strains of pathogenic bacteria are increasingly prevalent in hospitals and in the community. New antibiotics are needed to combat these bacterial pathogens, but unfortunately the progress in developing is not so fast Historically, most antibiotics have come from a small set of molecular scaffolds whose functional lifetimes have been extended by generations of synthetic tailoring. The emergence of multidrug resistance among the latest generation of pathogens suggests that the discovery of new scaffolds should be a priority.

Three classes of antibiotic-resistant pathogens are emerging as major threat to public health (Fig.1). The first is methicillin-resistant *Staphylococcus aureus* (MRSA) that is estimated to cause ~19,000 deaths per year in the United States [1]. Apart from their high mortality rate, MRSA infections lead to an estimated $3 billion to $4 billion of additional health care costs per year. Furthermore, the rising prevalence of MRSA increases the likelihood that vancomycin-resistant *S. aureus* (VRSA)—just as deadly as MRSA but more challenging to treat—will become a new scourge in hospitals.

The second class are multidrug resistant (MDR) and pandrug-resistant (PDR) Gram negative bacteria, less prevalent than MRSA, but they pose the grave threat of infections that are truly untreatable. These strains of *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are resistant to some (MDR) or all (PDR) the antibiotic classes commonly used to treat Gram-negative bacteria: penicillins, cephalosporins, carbapenems, monobactams, quinolones, aminoglycosides, tetracyclines, and polymyxins. Prospects for finding new antibiotics for Gram-negative pathogens are especially poor: their outer membrane blocks the entry of some antibiotics, and efflux pumps expel many of the remainder.

The third class comprises multidrug resistant (MDR) and extensively drug-resistant (XDR) strains of *Mycobacterium tuberculosis* (MDR-TB and XDR-TB), which are a rising threat in the developing world[2]. MDR-TB treatment requires a

[1] R.M. Klevens et al. JAMA **298**, 1763
M.A. Fischbach et al. Science **325**, 1089
[2] S.E.Dorman et al. Nat. Med **13,** 295

2-year course of antibiotics with serious side effects; XDR-TB is even more difficult to cure and often fatal. Cases of MDR-TB and XDR-TB have been reported in the United States and other developed countries.

Despite the continuous needing of new antibiotics, the pharmaceutical industries don't develope new molecules for two reasons: firstly, antibiotics are used in smaller quantities than other drugs, prescriptions for chronic illnesses can last years or decades whereas a standard course of antibiotics lasts only weeks yielding lower economic revenues; secondly, the use of a newly approved antibiotic may be restricted to the treatment of serious bacterial infections.

At this moment there is an increasing number of the resistance pathogens and a continuous decreasing quantity of new antibiotics approved and that lead into the market.

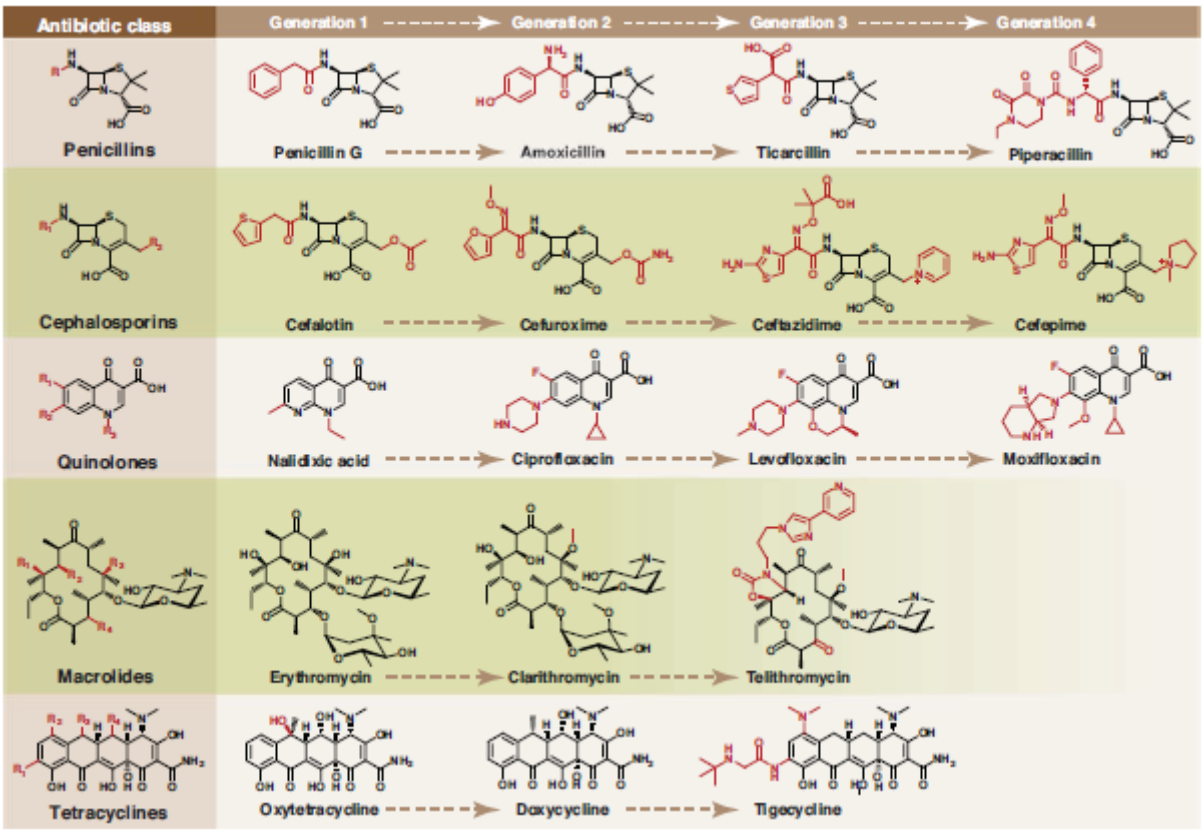


Figure 1: Principal antibiotics and their successive generations

In the past this problem has been solved through synthetic modifications of a small group of basic "scaffolds" like penicillin, cephalosporin, macrolides,

quinolones, aminoglycosides and tetracyclines, giving rising of different generations, more potent, than the ancestral antibiotics. (**Fig. 1**).

The existing drugs are:

- mainly directed at a small number of targets: cell wall, DNA, and protein synthesis;
- derived from chemical scaffolds introduced between the mid-1930s and the early 1960s (**Fig. 2**).

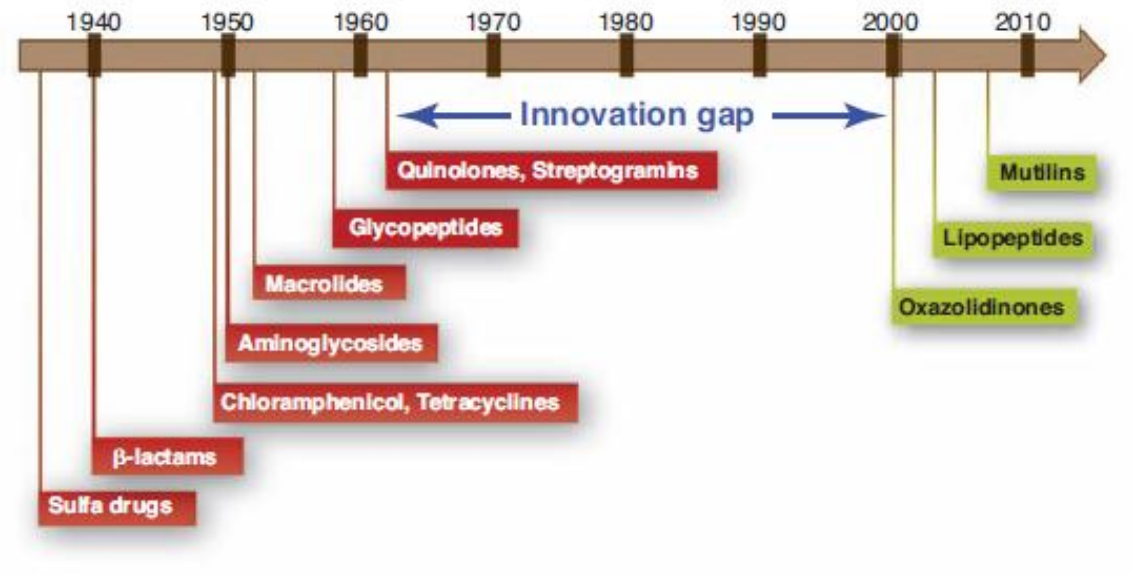


Figure 2: Chronology in antibiotics discovery

The small repertoire of targets limits the possibility of discovering derivatives of existing antibiotics for treatment of resistant bacteria. Thus, there is a need for new antibiotics with novel mechanisms of action to overcome the emerging resistance problem. The request of new antibiotics pushes the researchers to take in account other cellular target in particular the replication process. The bacteria replication requires a group of proteins, high conserved and all fundamental, that represent an optimal target.

Furthermore, cell division has gained considerable interest as antimicrobial target because a cell division inhibitor would prevent uncontrolled proliferation of bacteria at infectious sites, and inhibition of cell division is lethal in many pathogens.

The prokaryotic cytoskeleton

Eukaryotic cells contain a complex and dynamic cytoskeleton required for maintenance of cell shape, for the movement of DNA and organelles, and for cell division. The eukaryotic cytoskeleton has three major components: tubulin, actin, and intermediate filament (IF) proteins. The absence of a cytoskeleton has long been regarded as a hallmark of prokaryotes. On the other hand, there were numerous reports on the existence of fibers and tubules in prokaryotes. Until 1994, cytoplasmic or membrane-bound filamentous structures had been visualized by electron microscopy in at least 70 different species of eubacteria, many of which belonged to the cyanobacteria or spirochetes.[3] The composition and function of most of these filamentous structures are still unknown.

The introduction of modern cell biology methods for protein localization, in combination with DNA sequence comparisons and structural biology, allowed the identification of ubiquitous bacterial cytoskeletal elements present, for example, in model bacteria such as Escherichia coli or Bacillus subtilis.

In 1991 Bi and Lutkenhaus[4] described the formation of an intracellular, ring-like structure by the essential cell division protein FtsZ (Filamentous temperature sensitive Z) in *E. coli*. FtsZ is present in most known bacterial species, and it was later shown to have a similar structure as eukaryotic tubulin.

Bork et al.[5] reported that several bacterial proteins (DnaK, FtsA, ParM, and MreB) should adopt a similar fold as actin. Today, we know bacterial actins, tubulins, and an intermediate filament-like protein that share key structural features with their eukaryotic analogs.

Moreover, as in eukaryotes, the bacterial cytoskeleton is required for cell growth and division, DNA segregation, targeting of proteins and alignment of organelles. Some cytoskeletal elements are found (so far) only in bacteria and not in eukaryotes. Several reviews on the elements of the bacterial cytoskeleton were recently published[6],[7],[8],[9],[10],[11]. The bacterial cytoskeleton has been recognized as a

[3] Bermudes et al. *Microbiol. Rev.* **58** 387-400
[4] *Bi et al. Nature* **354** 161-164.
[5] Bork et al *Proc. Natl. Acad. Sci USA* **89**, 7290
[6] Errington et al. *Nat. Cell. Biol.* **5** 175-178
[7] Graumann et al. Curr. Opin. Microbiol. **7** 565-571

potential target for antimicrobial therapy. In recent years, inhibitors of cytoskeletal proteins have been identified as lead compounds for the development of novel antimicrobials.

Component of bacterial cytoskeleton

MreB proteins

MreB proteins are found almost exclusively in rod-shaped bacteria, and they are required to maintain the elongated cell-shape in several species including *E. coli*, *B. subtilis* and *Caulobacter crescentus*. In these bacteria, the depletion of MreB results in the formation of spherical cells followed by lysis. The structure of T. *maritima* MreB is very similar to that of actin.[12]

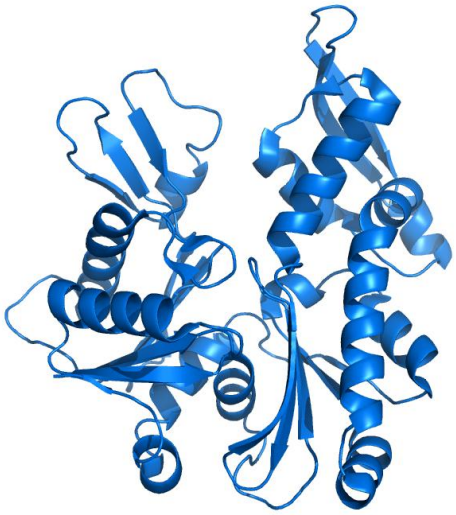


Figure 3: Representation of Prokaryotic MreB

In fact, proteins of the MreB family are more closely related to actin than other bacterial homologs are, and it is possible that the actin cytoskeleton evolved in bacteria before eukaryotes formed.[13] In vitro, both ATP and GTP induce polymerization of MreB to filaments, with assembly rates that are much faster than those of F-actin. *B. subtilis* contains three homologous proteins, MreB, Mbl, and MreBH, that form dynamic, helical filaments on the inner surface of the cytoplasmic membrane, coiling along the long axis of the cell. Mbl filaments are

[8] Lewis et al. *Mol. Microbiol.* **54** 1135-1150
[9] Löwe et al. *Annu. Rev. Biophys Biomol. Struct.* **33** 177-198
[10] Gitai et al. *Cell.* **120** 329-341
[11] Michie et al. *Annu. Rev. Biochem*. **75** 467-492
[12] van den Ent et al. *Nature* **413** 39-44
[13] Erickson et al. *Nature* **413** 30

more extended, ranging from pole to pole, whereas MreB filaments have a shorter lead and are absent from the cell poles and areas without neighboring nucleoid. Several different functions have been assigned to MreB proteins:

a) maintenance of cell shape by directing the cell wall synthesis enzymes;

b) movement of DNA, positioning of the DNA replication complex;

c) polar targeting of proteins;

d) spore development (in certain species) and alignment of organelles.

The different MreB homologs in *B. subtilis* appear to have specialized functions, whereas the single MreB protein in other species like *E. coli* have several functions.

Maintenance of cell shape - MreB-like proteins are essential to maintain the cell shape during growth of most rod-shaped bacteria. The bacterial cell shape is determined by the shape of an exoskeleton, the murein (peptidoglycan) sacculus that completely surrounds the cytoplasmic membrane. During growth and division, the murein has to be enlarged by a safe mechanism to avoid lysis. The enlargement of the sacculus has to occur in such a way that the specific shape (e.g., rod shape) of the cell is maintained . Presumably, multi-enzyme complexes made of different murein synthases (the penicillin-binding proteins, PBPs), murein hydrolases, and structural proteins facilitate the safe enlargement of the sacculus by the controlled incorporation of murein precursor (lipid II). Perhaps the murein synthesis multienzyme complexes are indirectly controlled (or positioned) during cell elongation by helical filaments made of MreB-like proteins.

Such a function has been proposed for Mbl (in *B. subtilis*), because the sites of incorporation of new material into the cell wall during elongation appear to follow a helical path on the cell surface.

This pattern is dependent on the presence of intracellular helical Mbl filaments, which are continuously remodeled during elongation. Mbl might direct the murein synthesis via the membrane proteins MreD and MreC. The latter contains a membrane anchor and an exocytoplasmic domain. MreB, MreC, and MreD form a membrane-bound complex in E. coli, but MreB and MreC form independent helical structures in C. crescentus. Different murein synthases bound to a

column with immobilized C. crescentus MreC, and MreC (partly) co-localizes with the murein synthase PBP2 that is essential for the cell elongation phase, in C. crescentus and Rhodobacter sphaeroides. The precise molecular mechanisms by which MreB-like proteins control cell wall growth during elongation are unknown.

Movement of DNA - MreB-like proteins play an important role in the movement of DNA. Plasmids with low-copy number need to be reliably partitioned to both cell halves before cell division to avoid plasmid-free daughter cells. The partitioning system includes the actin-like ParM protein, the DNA-binding protein ParR and the parC locus on the plasmid. ParR binds to the parC locus and pairs the plasmid after replication and before segregation. Plasmid-bound ParR induces filament formation of ParM that pushes both plasmids away from each other and towards opposite cell poles.

In *B. subtilis*, MreB could have several functions in the segregation of chromosomes: First, MreB is required for the proper mid-cell localization of the stationary DNA replicase complex; second, MreB and Mbl are required for the correct positioning of the origin regions; and third, dynamic MreB filaments could push the sister chromosomes away from the cell center towards opposite cell poles by a mechanism analogous to the ParM-mediated partitioning of plasmids. Alternatively, the mechanism of chromosome segregation could be more indirectly linked to MreB. In E. coli, MreB and the RNA polymerase (RNAP) are required for chromosome segregation, and RNAP interacts with MreB in vivo. In addition, the MreB-interacting integral membrane protein SetB forms a helical structure, and DNA segregation is delayed in mutants lacking SetB. In C. crescentus, MreB is required only to segregate the origin-proximal region and is not used for the segregation of the rest of the chromosome.

Polar targeting of proteins - Helical MreB filaments are also required for the targeting of certain proteins to the cell poles, for example the aspartate chemoreceptor Tar and the Shigella virulence factor IcsA. Upon depletion of MreB, polar targeting of Tar and IcsA is impaired. *C. crescentus* undergoes an asymmetric cell division yielding a stalked cell and a flagellated swarmer cell (see below). In this species, MreB assembles to dynamic, helical filaments during the cell elongation, but accumulates in a ring structure at the division site during cell division. The presence and functionality of MreB is required for targeting of

different proteins either to the stalked or to the flagellated pole, and hence, to maintain the cell polarity. The molecular mechanism by which proteins are targeted to the cell poles, with the direct or indirect requirement for MreB, is not known.

Spore development and alignment of organelles - Actinomycetes are gram-positive filamentous soil bacteria with a complex developmental cycle. MreB is present only in those species of actinomycetes that form aerial hyphae and sporulate, for example in *Streptomyces coelicolor*. The mreB gene can be deleted in *S. coelicolor* without any growth or cell-shape defect in vegetative cells. On the other hand, the deletion of mreB results in swollen aerial hyphae and prematurely germinating spores with irregularities in their cell wall. Thus, MreB is not required for elongation of the cells during vegetative growth but for the correct spore development in *S. coelicolor*.

Another function of actin-like proteins was identified recently in the magnetotactic bacterium *Magnetospirillum gryphiswaldense*. Magnetotactic bacteria are capable of navigating along the magnetic field of the earth with the help of intracellular nano-sized magnetosomes, which are membrane-surrounded organelles containing magnetite crystals. For proper functioning, the magnetosomes need to be assembled to a straight chain despite their tendency to agglomerate. For this, the magnetosomes are aligned along cytoskeletal filaments of the actin-like MamK protein via the acidic MamJ protein.

The prokaryotic tubulin FtsZ (Filamentous temperature sensitive Z)

The essential cell division protein FtsZ is the bacterial tubulin and is widely conserved in prokaryotes, chloroplasts, and some mitochondria.[14,15] Although FtsZ and tubulin share less than 20% of sequence identity, they are structural homologs.[16] Like tubulin, FtsZ binds GTP to the interface between two monomers, forming the active site for the hydrolysis of GTP to GDP. Binding of GTP to FtsZ induces its polymerization to protofilaments or sheet-like structures. Unlike tubulin, FtsZ does not form microtubules in vitro.

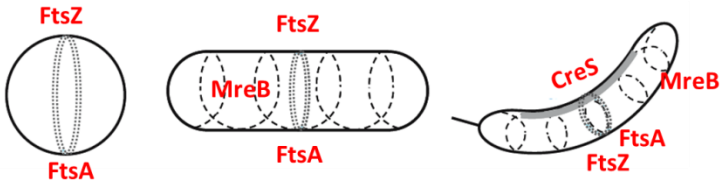


Figure 4; Cellular localization of bacterial cytoskeleton proteins required for cell division and maintenance of cell shape

FtsZ shows a surprisingly dynamic localization pattern inside the *E. coli* cell. In young, non-dividing cells, FtsZ oscillates rapidly in helical waves through the cell by a yet unknown mechanism.[17] During cell division, FtsZ forms at mid-cell a membrane-associated ring structure (the Z-ring) that serves as a platform for the assembly of more than 12 other essential cell division proteins, the precise function of most of which is unknown.[18,19,20] This division complex (the divisome) facilitates septum formation at the leading edge of constriction and disassembles when cell division is completed. Generally, there are as many as 15 000 FtsZ molecules in each *E. coli* cell, but only one third of these participate in Z-ring formation at a given time.[21] FtsZ subunits in the Z-ring exchange with those out of Z-ring on the time scale of seconds, while the overall morphology of the Z-ring appears to be static.[22] The precise structure of the Z-ring is not known. Moreover,

[14] Addinall et al. *J. Mol. Biol.* **318** 219-236
[15] Margolin et al. *Nat. Rev. Mol. Cell. Biol.* **6** 862-871
[16] Löwe et al. *Nature* **391** 203-206
[17] Thanedar et al. *Curr. Biol.* **14** 1167-1173
[18] Errington et al. *Microbiol. Mol. Biol. Rev.* **67** 52-65
[19] Weiss et al. Mol. *Microbiol.* **54** 588-597
[20] Vicente et al. *J. Bacteriol*. **188** 18-27
[21] J. Stricker et al. *Proc. Natl. Acad. Sci USA* **99**, 3171 – 3175
[22] Weiss *et al. Mol. Microbiol*. **54** 588 – 597.

it is unclear whether the assembly/disassembly dynamics and/or hydrolysis of GTP by FtsZ generates an inward directed force for the constriction of the cell.

A battery of FtsZ-interacting proteins regulate Z-ring formation to ensure that the ring forms at the precise time and at the correct position, and that the ring has the optimum assembly/disassembly dynamics.[23] Two mechanisms ensure that Z-ring formation occurs with high precision only at mid-cell:

the Min system prevents the formation of the Z-ring (and hence division) near the cell poles via the inhibitory proteins MinC/MindD.[24]
the Z-ring formation close to the nucleoid is inhibited by the DNA-binding protein Noc (*B. subtilis*) or SlmA (*E. coli*).[25,26]
The latter mechanism named "nucleoid occlusion" prevents the cutting of the DNA by the closing septum. Damage of DNA by chemicals or UV light results in the induction of the SOS response, which includes the inhibition of cell division via the FtsZ-binding protein SulA (*E. coli*). GTP-dependent binding of SulA to FtsZ results in the formation of a stable FtsZ2–SulA2 complex and blocks polymerization of FtsZ molecules.[27,28,29] In *B. subtilis*, the SOS response protein YneA (which is unrelated to SulA) prevents cell division after DNA damage.[30] During the assembly of the divisome, the Z-ring is stabilized by the actin-like protein FtsA and by ZipA. Another FtsZ-binding protein, SepF (or YlmF), is required for the proper execution of septum synthesis,[31] and its overproduction can complement a *B. subtilis* ftsA null strain.[32] In this species, the Z-ring-stabilizing FtsL and the Z-ring-destabilizing EzrA synergistically regulate Z-ring dynamics during cell division.[33] EzrA also prevents the assembly of Z-rings at inappropriate locations.[34] Another FtsZ-binding protein is ZapA, which stimulates the formation of higher-order assemblies of FtsZ in vitro. Although ZapA alone is

[23] Romberg et al. *Annu. Rev. Microbiol*. **57** 125-154
[24] De Boer et al. *Cell.* **56** 641-649
[25] Wu et al. *Cell.* **117** 915-925
[26] Bernhardt et al. *Mol. Cell.* **18** 555-564
[27] Higashitani et al. *Biochem. Biophys. Res. Commun.* **209** 198-204
[28] Mukherjee et al. *Proc. Nat. Acad. Sci. USA* **9**5 2885-2890
[29] Cordell et al. . *Proc. Nat. Acad. Sci. USA* **100** 7889-7894
[30] Kawai et al. *Mol. Microbiol.* **47** 1113-1122
[31] Hamoen et al. *Mol. Microbiol.* **59** 989-999
[32] Ishikawa et al. *Mol. Microbiol.* **60** 1364-1380
[33] Kawai et al. *Microbiology* **152** 1129-1141
[34] Levin et al. *Proc. Nat. Acad. Sci. USA* **96** 9642-9647

dispensable, cell division is inhibited when both ZapA and EzrA are absent.[35] An inhibitory factor for FtsZ assembly is the chaperone ClpX, the substrate recognition subunit of the ClpXP protease.[36] Finally, CrgA, a small, 84-residue membrane protein, affects Z-ring assembly in *Streptomyces coelicolor*.[37] It remains to be determined how these factors together control the assembly and dynamics of the Z-ring in the cell.

[35] Gueiros-Filho et al. *Genes Dev.* **16** 2544-2556
[36] Weart et al. *Mol. Microbiol.* **57** 238-239
[37] Del Sol et al. *J. Bacteriol.* **188** 1540-1550

Z ring initiation and localization

Z ring formation establishes the location of the future division site and is an integral part of the temporal regulation of cytokinesis. The initiation of FtsZ assembly must be tightly controlled both temporally and spatially to prevent aberrant septation. Z ring formation depends on at least three factors: FtsZ concentration, the initiation of DNA replication, and the presence or absence of the nucleoid mass.

A minimum FtsZ concentration is necessary but not sufficient for Z ring formation.

FtsZ assembles in vitro in a concentration-dependent manner, which suggests that FtsZ levels in a cell might control the timing of Z ring formation. In this model FtsZ concentration in newborn cells would be too low to support assembly but would gradually increase during the course of the cell cycle and reach a critical concentration immediately prior to Z ring formation. A minimum FtsZ concentration is in fact necessary for division in Escherichia coli and for Z ring assembly in Bacillus subtilis[38], but under most circumstances fluctuations in FtsZ concentration are not sufficient to explain the timing of ring formation. Both E. coli and *B. subtilis* can modulate transcription of the FtsZ operon[39], but FtsZ protein levels remain constant throughout the cell cycle of both organisms during steady state growth[40]. Altering FtsZ levels is not sufficient to change the timing of medial ring formation. Lowering FtsZ expression below wild-type levels does not change the timing of cytokinesis in E. coli, though cell size increases slightly[41]. In E. coli the two to sevenfold increased levels of FtsZ lead to a raise in the frequency of polar septation[42]. These data suggest that although high FtsZ expression levels do not change medial Z ring formation, they can overcome a division inhibitor that normally prevents assembly at the cell poles.

[38] Lutkenhaus J. et al. *J. Bacteriol* **173** 3500-3506
[39] Fukuchi et al. *Microbiology* **146** 1573- 1583
[40] Weart RB et al. *J. Bacteriol* **185** 2826-2834
[41] Palacios P et al. *Mol. Microbiology* **20** 1093-1098
[42] Ward JE et al. *Cell* **42** 941-949

Replication initiation is necessary for positioning the Z ring at midcell

Z ring formation initiates after the onset of DNA synthesis and the separation of newly replicated origin DNA, which suggests that assembly might be triggered by events at the start of chromosome replication[43][44][45][46]. However, blocking the initiation of DNA replication does not inhibit Z ring formation in either E. coli or *B. subtilis*[47].

While DNA replication is not essential for FtsZ assembly in E. coli and *B. subtilis*, correct positioning of the Z ring at midcell does require replication initiation, but not elongation of the replication fork. Permitting DNA replication in germinating *B. subtilis* spores, but preventing elongation with a thymine auxotroph, leads to degradation of the origin region and the correct positioning of a medial Z ring over the unreplicated nucleoid[48]. Because the FtsZ ring forms proximal to the previous location of the origin of replication, and because replication initiation is required for proper ring localization, it is tempting to speculate that the same machinery that recruits the origin of replication and the polymerase machinery to midcell also helps to unmask a preferred FtsZ nucleation site at midcell.

Local inhibition of Z ring formation by the unsegregated nucleoid

The correlation between Z ring formation and the separation of the bacterial nucleoid has led to the long-standing hypothesis that the bacterial nucleoid is a determinant in the timing and placement of the division septum (the nucleoid occlusion model [49]). According to this model, cell division can take place at any point along the length of a cell, but early in the cell cycle the presence of the unsegregated nucleoid prevents septation at midcell. Later, DNA segregation reveals a small, nucleoid-free zone at midcell that is able to support assembly of the division apparatus.

[43] Blaauwen T. et al. *J. Bacteriol.* **181** 5167-5175
[44] Harry EJ et al. *Mol. Microbiol.* **33** 33-40
[45] Quardokus et al. *Mol. Microbiol.* **45** 605-616
[46] Regamey et al. *Mol. Microbiol.* **38** 423-434
[47] Sun Q et al. *J. Bacteriol* **183** 1413-1422
[48] Harry EJ et al. *Mol. Microbiol.* **33** 33-40
[49] Mulder E et al. *J. Bacteriol*. **171** 4303-4314

Z ring formation in the nucleoid-free space adjacent to the cell poles is prevented by the presence of the MinCD division inhibitors[50]. The nucleoid appears to play a role in preventing aberrant Z ring formation, but in both E. coli and *B. subtilis* its presence alone is not sufficient to dictate the precise spatiotemporal regulation of FtsZ assembly. Z ring formation is clearly influenced by the location of the nucleoid under some circumstances. When DNA replication or partitioning has been blocked, the chromosome remains at midcell, and nonmedial Z rings form adjacent to the nucleoid[51]. In addition, when chromosome partioning is blocked in the absence of the Min proteins, the Z ring in E. coli is free to form at any position along the nucleoid-free space[52]. Under certain circumstances, however, the position of the nucleoid coincides with that of the Z ring and of cytokinesis. Moreover, Z rings can form directly over the nucleoid in cells with mutations in chromosome condensation genes (smc in *B. subtilis* and mukB in E. coli)[53]. One potential explanation is that Z ring formation and septation can occur in regions of the nucleoid that are relatively less dense.

Maintenance of the Z ring as dynamic framework

Once formed, the Z ring is present for a significant portion of the cell cycle (e.g., 85% of the mass doubling period in *B. subtilis* cells growing in Luria broth medium)[54]. In *B. subtilis* the proportion of cells with Z rings increases with increasing growth rate, indicating that the Z period (the time the Z ring is present in a given cell) remains relatively constant even when doubling time decreases[55]. The prolonged duration of the Z period suggests there may be a minimum time required for proper assembly of the entire division apparatus. During the Z period, although no gross changes in the Z ring can be detected by immunofluorescence, the ring is extremely dynamic, with subunit turnover on par with that of the eukaryotic cytoskeleton[56]. For example, the Z ring can disappear and reassemble within minutes after shifting cells expressing a heat-

[50] Yu XC et al. *Mol. Microbiol.* **32** 315-326
[51] Sun Q et al. *J. Bacteriol*. **29** 491-503
[52] Mulder E et al. *J. Bacteriol* **171** 4303-4314
[53] Graumann PL et al. *Biochimie* **83** 53-59
[54] Blaauwen T. et al. *J. Bacteriol.* **181** 5167-5175
[55] Lin DC-H et al. *Proc. Nat. Acad. Sci. USA* **94** 4721-4726
[56] Pelham RJ et al. *Nature* **419** 82-86

sensitive FtsZ allele (ftsZ84) into and out of the nonpermissive temperature[57]. Turnover rates of FtsZ in the ring appear to correspond to FtsZ's in vitro ability to hydrolyze GTP, but lowering the hydrolysis rate by an order of magnitude is not lethal to the cell.

Remodeling the Z ring

If the rapid turnover of FtsZ subunits within the Z ring is not essential for cytokinesis under normal growth conditions, why does it occur? One function may be to allow cells to disassemble or remodel the division apparatus in response to DNA damage, starvation, or other environmental stresses.

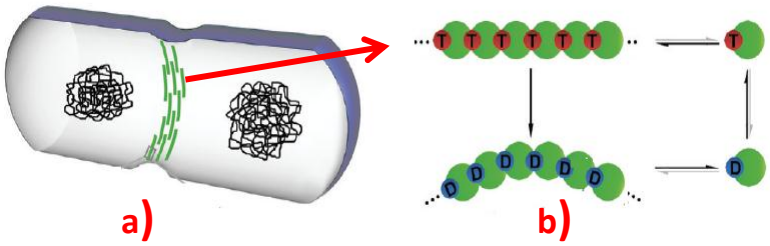


Figure 5: a): scheme of the localization of the constricting Z-ring made of FtsZ laterally associated filaments (green) at the site of cell division between two daughter bacterial cells that have already separated their DNA (black), in an inside view of a half cell; b): architecture and dynamics of an FtsZ filament. Each bead represents an FtsZ molecule, T stands for bound GTP and D for GDP.

DNA damage induces the SOS response in bacteria, during which cell division is prevented until the DNA has been repaired[58]. In E. coli, DNA damage results in the expression of SulA, which causes the Z ring to be dismantled[59] .

Z ring remodeling also plays an important role during development in *B. subtilis*. In response to cell crowding and nutritional deprivation, *B. subtilis* produces environmentally resistant endospores. A key step in sporulation is the switch from binary fission to polar septation[60]. Polar septation is preceded by the relocalization of FtsZ from midcell to positions adjacent to both cell poles[61].

Z ring constriction

[57] Addinall SG et al. *J. Bacteriol* **179** 4277-4284
[58] Little JW et al. *Cell* **29** 11-22
[59] Bi E et al. *J. Bacteriol* **175** 1118-1125
[60] Errington J et al. *Microbiol. Rev.* **57** 1-33
[61] Levin PA et al. *Genes Dev.* **10** 478-488

When a cell divides, the Z ring constricts at the leading edge of the invaginating septum[62]. There is limited evidence as to FtsZ's precise function during this process. At minimum, FtsZ is needed to maintain the medial localization of the other division proteins. To do this, the FtsZ framework must be able to retain its coherence despite an ever-shrinking structure. Cytokinesis occurs in two discrete stages: the formation of an initial, small invagination at the site of the septum, followed by the completion of cell division. FtsZ is necessary throughout both stages. If at any time during cytokinesis conditional alleles of FtsZ are shifted to nonpermissive temperatures, the Z ring disappears, all other proteins in the division apparatus delocalize, and the septum ceases to invaginate[63]. If invagination has not yet begun, cells are left with smooth walls, indicating that septum formation cannot begin without FtsZ.

The molecular mechanism of Z ring constriction during septum formation remains unclear. One model suggests that as the diameter of the ring grows smaller, FtsZ subunits are released from the ring. Alternatively, filaments might be sliding against each other without disassembling, as occurs in the actomyosin ring in eukaryotes[64].

[62] Bi E et al. *Nature* **354** 161-164
[63] Taschner PE et al. *J. Bacteriol.* **170** 1533-1540
[64] Bramhill et al. *Proc. Nat. Acad. Sci. USA* **91** 5813-5817

FtsZ Structure

To understand the dynamic behavior of FtsZ, it is first necessary to review the data on its structure. Below we describe the structure of (a) the FtsZ subunit, (b) the GTP binding site, (c) the protofilaments assembled in GTP and GDP, and (d) the higher-order FtsZ structures.

The subunit

Despite sharing minimal sequence homology (≈10%), FtsZ and tubulin have strikingly similar protein folds and GTP binding interactions. FtsZ is a ≈ 40-kDa polypeptide whose atomic structure was determined using FtsZ from the thermophillic archaeon Methanococcus jannaschii[65]. The structure contains a major sheet surrounded by α helices; perpendicular to this is a smaller subdomain, which also consists of a short sheet surrounded by α helices (Figure 6). The FtsZ and tubulin structures fall into a subclass different from that of small G proteins, dynamin, and all other GTP binding proteins possessing a P-loop. Instead, they appear most closely related to that of the NADH binding protein, glyceraldehyde-3-phosphate dehydrogenase[66]. Not visible in the atomic structure is a flexible, highly charged C-terminal region. It is extremely short in *M. jannaschii* but is longer and essential for cytokinesis in *E. coli* and *C. crescentus*. A ≈15-amino-acid conserved sequence near the very C terminus is necessary for binding ZipA and FtsA, two essential Z ring proteins in E. coli[67] and for binding FtsA in C. crescentus[68] and *S. aureus*[69].

The GTP binding site

The GTP binding residues are the amino acids that are best conserved between FtsZ and tubulin[70] (Figure 6), reflecting similarities in the way the two proteins interact with nucleotide during polymer assembly and disassembly. The nucleotide binds on the surface that will form the interaction site between subunits in a protofilament. In analogy with the microtubule structure, we call

[6565] Lowe J et al. *Nature* **391** 203-206
[66] Nogales E et al. *Nat. Struct. Biol.* **5** 451-458
[67] Van den Ent F et al. *EMBO J.* **19** 5300-5307
[68] Din N et al. *Mol. Microbiol.* **27** 1051-1063
[69] Yan K et al. *Biochem. Biophys. Res. Commun.* **270** 387-392
[70] Nogales E et al. *Nat. Struct. Biol.* **5** 451-458

the protein bonds within an FtsZ protofilament longitudinal bonds and the GTP binding surface of the monomer the plus end of the protein. Unlike tubulin, unassembled FtsZ exists as a monomer rather than as a heterodimer, and all subunits can exchange nucleotide[71]. Once a longitudinal bond has formed, the nucleotide is likely to be almost completely buried in the interface between FtsZ subunits. Conserved sequences at both the plus and minus ends of a subunit are essential to FtsZ's ability to bind and hydrolyze GTP.

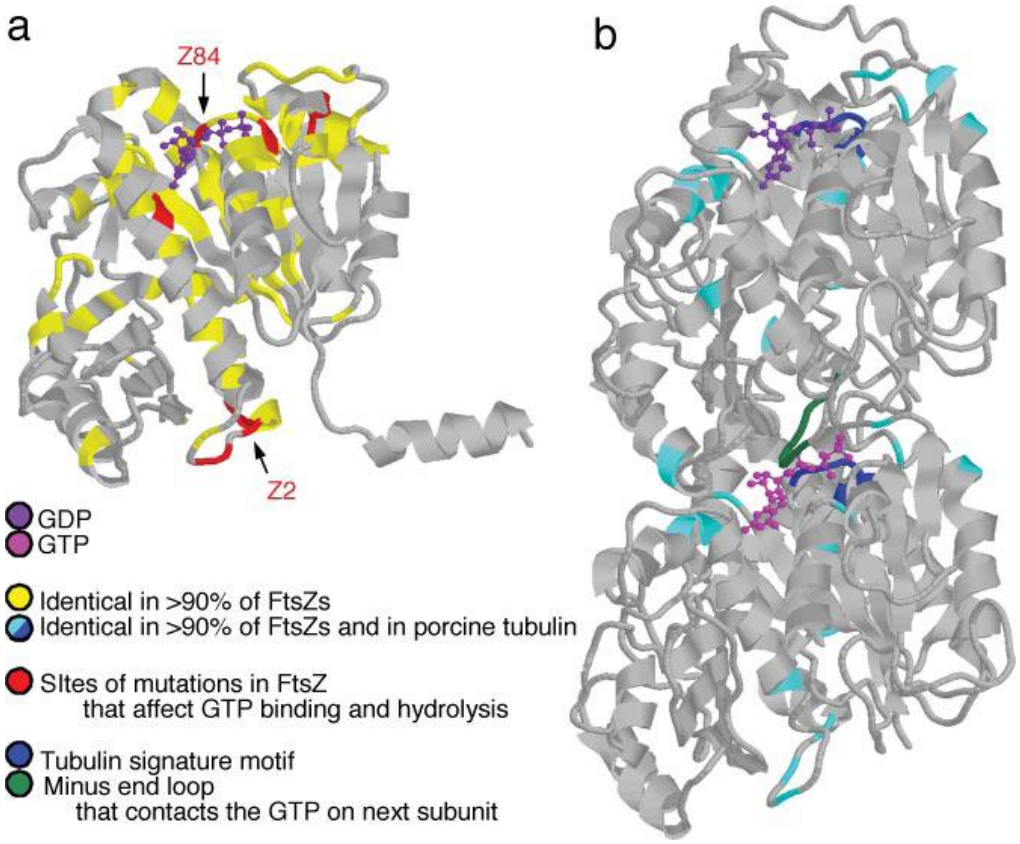


Figure 6: FtsZ and tubulin atomic structures. (*a*) *Methanococcus jannaschii* FtsZ (*b*) Porcine a/b tubulin dimer

Monomeric FtsZ uses conserved residues at the plus end of the protein to bind GTP tightly [Kd ≈ 5 μM]. Seven of these amino acids constitute the tubulin signature motif, a sequence conserved in all tubulins and originally used to identify FtsZ as a distant tubulin homolog[72].

[71] Mukherjee A et al. *Proc. Nat. Acad. Sci. USA* **90** 1053-1057
[72] Mukherjee A et al. *Proc. Nat. Acad. Sci. USA* **90** 1053-1057

Residues at the minus end of the subunit participate in both protofilament bond formation and GTP hydrolysis. Thus, FtsZ assembly is necessary for GTP hydrolysis[73]. Several of the minus end residues are highly conserved in FtsZ and tubulin (Figure 6). Mutations in many of these residues (Figure 6) do not eliminate the in vitro ability of FtsZ to bind GTP or assemble, but they do reduce the protein's nucleotide hydrolysis rate[74].

Protofilaments

The conserved GTP binding residues reflect similarities in the structures that FtsZ and tubulin form with GTP and GDP. When GTP is present, protofilaments form[75] in which subunits are stacked linearly without any net twist or curvature[76] (Figure 7a). FtsZ protofilaments can exist in isolation, whereas tubulin protofilaments do not form except in the context of a multistranded polymer. In the presence of GDP, the interaction between FtsZ subunits is substantially weakened[77]. In addition, GDP-bound protofilaments can curve sharply to form rings or spirals with a diameter of 15–25 nm[78] (Figure 7b).

Multistranded polymers

The Z ring is thought to contain 6 to 7 protofilaments[79], but how these protofilaments are associated with each other is unknown. The filaments could interact directly with each other through their lateral surfaces or indirectly through cross-linking by other cell division proteins. For the moment the assembly of multistranded FtsZ structures in vitro provides the best information on the higher-order structures that might form in vivo. Many different surfaces of the FtsZ protofilament can be used to form a wide variety of multistranded structures, including protofilament pairs and bundles (Figure 3c,d), sheets, spirals, hoops, and tubes[80]. Lateral associations are affected by protein concentration, pH, and the presence of multivalent cations including DEAE

[73] Sossong TM et al. *Biochemistry* **38** 14843-14850
[74] Wang X et al. *J.Bacteriol* **179** 5551-5559
[75] Mukherjee A et al. *J. Bacteriol.* **176** 2754-2758
[76] Erickson HP et al. *Proc. Nat. Acad. Sci. USA* **93** 519-523
[77] Rivas G et al. *J. Biol. Chem.* **275** 11740-11749
[78] RayChaudhuri et al. *EMBO J.* **18** 2372-2383
[79] Stricker J et al. *Proc. Nat. Acad. Sci. USA* **99** 3171-3175
[80] White EL et al. *J. Bacteriol.* **182** 4028-4034

dextran, calcium, and magnesium[81]. FtsZ's tendency to bundle also varies between species. For example, FtsZ from Mycobacterium tuberculosis appears to bundle more readily than that of E. coli[82].

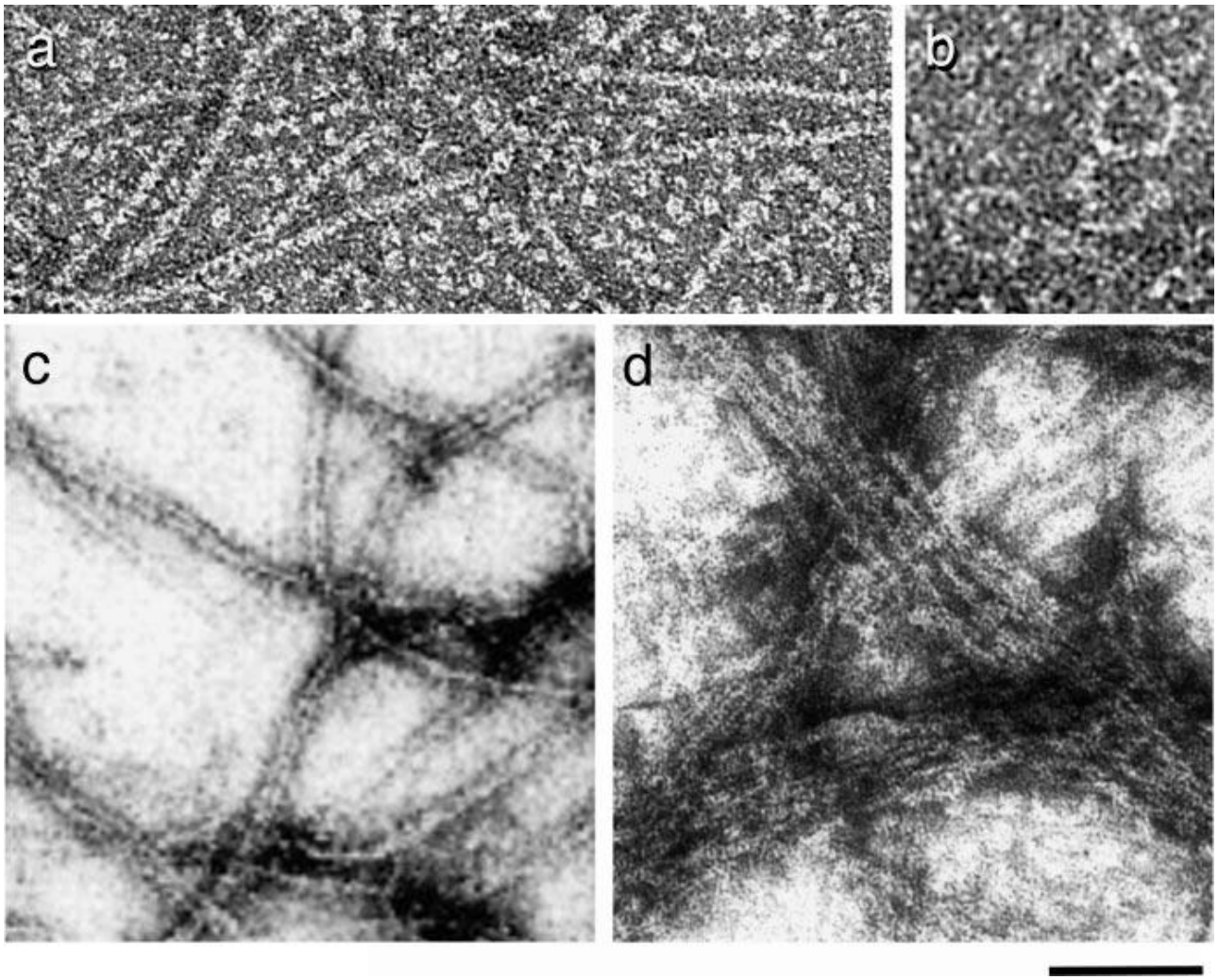


Figure 7: FtsZ polymers. (*a*) FtsZ protofilaments assembled with GTP. Monomeric FtsZ is also visible in the background. (*b*) FtsZ rings assembled with GDP. (*c*) Protofilament pairs and bundles assembled in reactions with GTP. (*d*) Large FtsZ bundles formed in the presence of ZipA and GTP.

[81] Mukherjee A et al. *J. Bacteriol.* **176** 2754-2758
[82] White EL et al. *J. Bacteriol.* **182** 4028-4034

The bacterial cytoskeleton as target for antimicrobial therapy.

Small molecules targeting the eukaryotic cytoskeleton have long been successfully employed in basic research to study the functions of tubulin and actin in vivo and in vitro, and as crystallography tools. Some of these compounds abolished cytokinesis and have antitumor activity.[83] Elements of the bacterial cytoskeleton represent potential targets for antimicrobial compounds because they are essential for bacterial viability, they are conserved in significant pathogens, and they are amenable to target-based drug discovery toolbox, which are features of an "ideal antimicrobial target".[84] Furthermore, bacterial cytoskeletal proteins have markedly different structures than their eukaryotic analogs, making it possible to develop inhibitors specific for the bacterial proteins.

Considering the short period since the discovery of bacterial cytoskeletal elements and the retreat of several big pharmaceutical companies from antibacterial drug discovery,[85] it is not surprising that until now there is no inhibitor available for application in antimicrobial therapy. Nonetheless, the search for inhibitory compounds has begun. The so-far identified inhibitors are likely to become valuable tools for prokaryotic cell biology studies, but might also be useful as lead compounds for the development of antimicrobials.

[83] Peterson et al. *Chem. Biol.* **9** 1275-1285
[84] Brown et al. *Chem. Rev.* **105** 759-774
[85] Projan et al. Curr. Opin. Microbiol. **6** 427-430

Inhibitors of the FtsZ function.

There are many efforts to identify inhibitors of the bacterial cell division protein FtsZ that do not target eukaryotic tubulin. Since the discovery of its essential role in bacterial cell division, a number of small molecules have been described as inhibiting FtsZ. The reported compounds have different origins: high-throughput screening (HTS) of chemical libraries, natural products discovery, previously known antibacterial compounds, compounds from the tubulin field, and synthetic compounds specifically developed to inhibit FtsZ.

The FtsZ inhibiting compounds reported to date are classified into six chemical groups: guanine derivatives, phenols and polyphenols, N-heterocycles, carboxylic acids, benzamide derivatives, and others. Here I report several selected examples of these small molecules. Notably, only the guanine nucleotide derivatives have been obtained by a rational design based on the atomic structure of FtsZ.

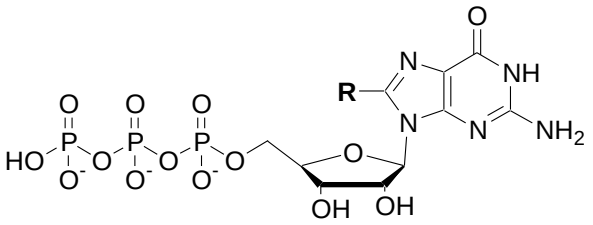
Guanine derivatives

Despite the high sequence homology of FtsZ and tubulin found in regions involved in nucleotide binding and the similar protein folds and GTP binding interactions revealed by their crystal structures,[86] their active sites formed by association of FtsZ monomers and α,β-tubulin heterodimers appear to be strikingly different. In FtsZ, the nucleotide binding site is more open and partially water filled, allowing free ex-change of nucleotides in intact polymers, while in tubulin polymers, the nucleotide is in contact with protein from all sides and is nonexchangeable. This indicates the existence of structural differences in the GTP binding pockets of FtsZ and tubulin.

GTPase function of FtsZ is inhibited by 8-bromoguanosine 5′-triphosphate (BrGTP) with a K_i value of 37 μM.[87] BrGTP inhibits both the GTPase activity and FtsZ polymerization in a competitive way, and it does not inhibit eukaryotic tubulin. Lappchen et al. hypothesized that the superior tubulin assembly-promoting effects of the 8-halo-GTPs compared with GTP may be explained by additional their major hydrophobic interactions. Apparently, however, with

[86] Nogales E et al. *Nat. Struct. Biol.* **5** 451-4581998
[87] Lappchen et al. Biochemistry **44** 7879-7884

increasing size of the C8-substituents, steric issues become the dominant factor due to space limitations of the nucleotide binding cavity in tubulin. This is reflected in the decreasing potencies in the 8-halo series (ClGTP > BrGTP > IGTP) with increasing van der Waals radius of the halogen and the dramatically reduced potency of the analogs with larger C8-substituents, as reported for 8-morpholino-GTP (figure 8).



IC_{50}	R
35	Cl
37	Br
44	I
139	morpholine

Figure 8: GTD derivatives.

Phenols and polyphenols

Using a fluorescent FtsZ polymerization assay, the screening of >100,000 extracts of microbial fermentation broths and plants followed by fractionation led to the identification of viriditoxin (figure 9), which blocked FtsZ polymerization with an IC_{50} of 8.2 g/mL and concomitant GTPase inhibition with an IC*50* of 7.0 g/mL.[88] That the mode of anti-bacterial action of viriditoxin is via inhibition of FtsZ was confirmed by the observation of its effects on cell morphology, macromolecular synthesis, DNA-damage response, and increased minimum inhibitory concentration as a result of an increase in the expression of the FtsZ protein. Viriditoxin exhibited broad-spectrum antibacterial activity against clinically relevant Gram-positive pathogens, including methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci*, without affecting the viability of of Gram-negative bacteria and eukaryotic cells (Candida and HeLa).

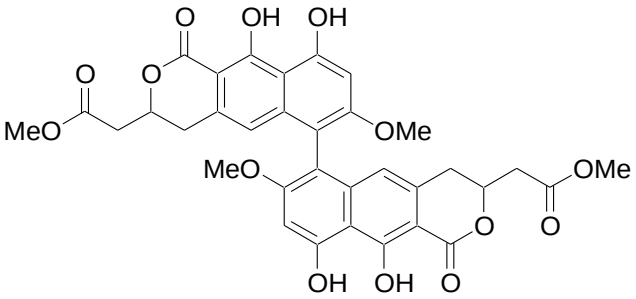


Figure 9: Viriditoxin

[88] Wang et al. *J. Biol. Chem* **278**, 44424-44428

Eight new antimicrobial natural products named chrysophaentins A-H belonging to a new structural class have been isolated from the marine chrysophyte alga *Chrysophaeum taylori*. Their structures were determined by extensive 2D NMR and MS techniques and are characterized by the presence of two polyhalogenated, polyoxygenated ω,ω′-diarylbutene units connected by two ether bonds to form the suite of macrocyclic natural products. Chrysophaentin A, the most potent of these antibiotics, inhibited the growth of clinically relevant Gram-positive bacteria including methicillin-resistant Staphylococcus *aureus* (MIC_{50} 1,5 μg/mL), multidrug-resistant *S. aureus* (1,3 μg/mL), and vancomycin-resistant *Enterococcus faecium* (MIC_{50} 2,9 μg/mL). In vitro enzyme assays and transmission electron microscopy showed chrysophaentin A to inhibit the GTPase activity of the bacterial cytoskeletal protein FtsZ with an IC_{50} value of 6,7 μg/mL, as well as GTP-induced formation of FtsZ protofilaments. Saturation Transfer Difference (STD) NMR experiments further confirmed chrysophaentin A binds to FtsZ, and NMR competition experiments with GTPγS showed chrysophaentin A and GTP to bind competitively to FtsZ. Molecular docking simulations provided a low energy model in which chrysophaentin A binds in and occludes a large portion of the GTP binding site of FtsZ in a manner that is consistent with the binding epitope determined by STD NMR.

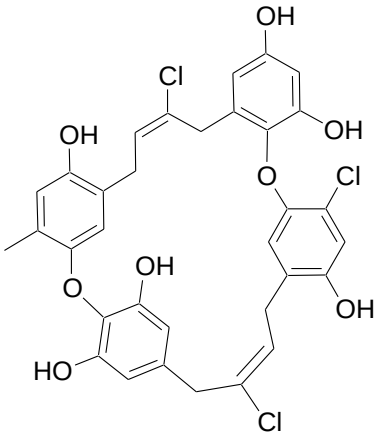


Figure 10: chrysophaentin A

Margalit et al reported an high-throughput screen for small molecule inhibitors of assembly-dependent GTPase activity of FtsZ. They discovered five structurally

diverse compounds named Zantrins[89] that interfere with FtsZ assembly in different ways. The phenol derivatives Zantrins Z1 and Z4 destabilize the interactions between FtsZ subunits, whereas the N-heterocyclic derivatives, Zantrins Z2, Z3 and Z5, stabilize FtsZ protofilaments and inhibit their depolymerization. The persistence of a small number of short protofilaments (PFs) in the presence of the destabilizers suggests that these molecules may not inhibit de novo FtsZ assembly but probably induce polymer lability through other mechanisms.

Zantrins Z1–Z5 are neither sufficiently potent nor sufficiently specific to be considered as antibiotics in their current form. However, several Zantrins were more active against Gram-positive bacteria, including antibiotic-resistant and virulent pathogens, with MIC values in the micromolar range and perturbed the Z-ring assembly in vivo. In particular, Z1 was broadly cell-permeable and displayed significant potency against Gram-positive or-ganisms. Therefore, it is possible that one or more Zantrins may serve as leads for antibiotic development through systematic chemical modifications to optimize inhibitor potency and specificity.

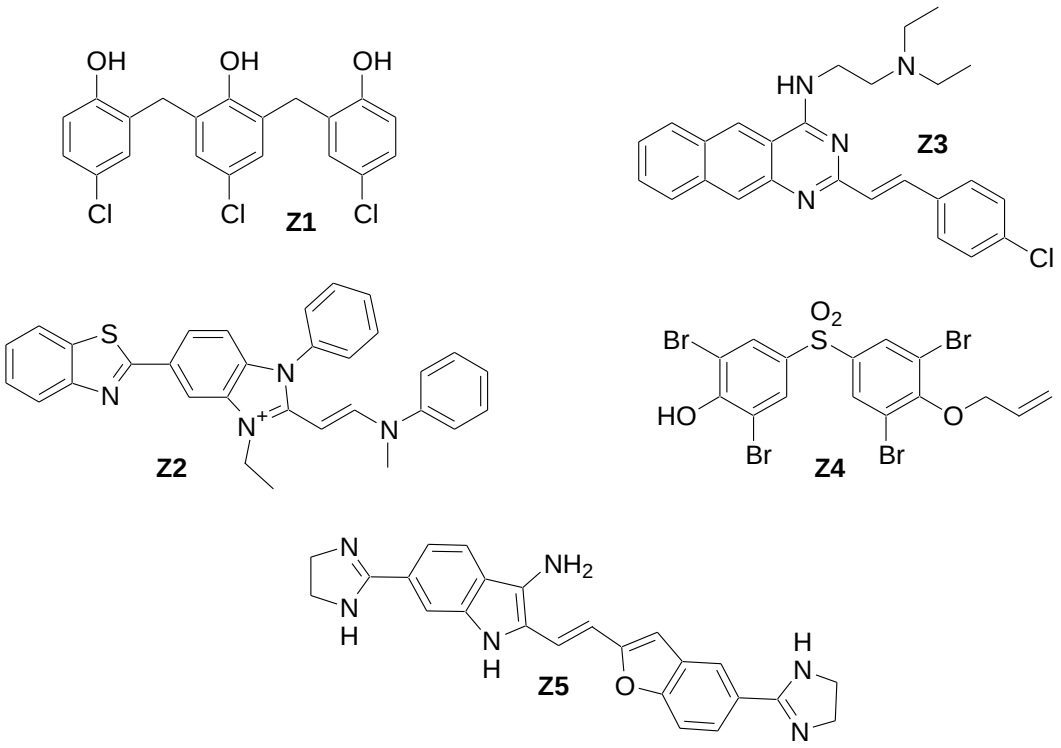
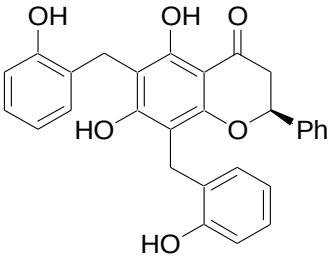


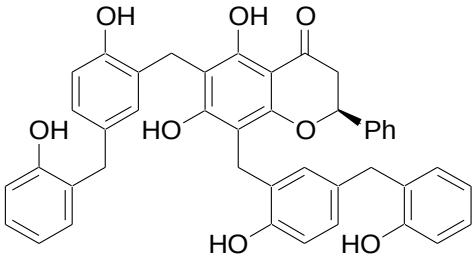
Figure 11: Zantrins Z1-Z5

[89] Margalit et al. *Proc. Natl. Acad. Sci. USA* **101** 11821

Two natural compounds, dichamanetin isolated from *U. chamae* and 2‴-hydroxy-5″-benzylisouvarinol-B isolated from *X. afticana*, possess polyphenolic structures similar to that of Zantrin Z1, and they show antimicrobial activity against several gram-positive bacteria including *S. aureus* with MIC values in the micromolar range.[90] Both compounds were synthesized from a common core structure, and they were shown to inhibit the GTPase activity of FtsZ with IC_{50} values in the micromolar range.



Dichamanetin



2‴-Hydroxy-5"-benzylisouvarinol-B

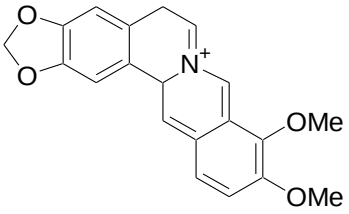
Figure 12

N-heterocyclic derivatives

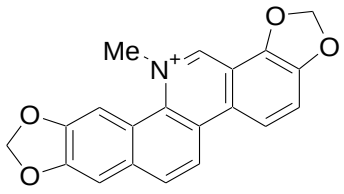
This class of compounds include the Zantrin Z2, Z3, and Z5 previously reported and some natural alcaloids.

Various *Berberis* species are the source of the natural plant alkaloid component Berberine (figure 13). Plants containing berberine have been used in Chinese and Native American medicine for the treatment of many infectious microbial diseases such as diarrhea, typhoid, gastroenteritis, and ocular trachoma.[91][Berberine possesses broad-spectrum antimicrobial activity against almost all microorganisms including bacteria, viruses, protozoa, helminths, and fungi.[92] In particular, berberine shows greater potency against Gram-positive than Gram-negative bacteria. For example, the MIC of Berberine against *E. coli* ATCC 25922 and *Salmonella typhimurium* ATCC 14028 were reported to be > 400 μg/mL, whereas its MIC values were 100 μg/mL against *B. subtilis* ATCC 6633 and *S. aureus* ATCC 6538p. In addition, the MIC of berberine against clinical isolates of MRSA and *Helicobacter pylori* were 32–128 and 12.5 μg/mL.[93]

[90] Urgaonkar et al. *Org. Lett.* **7** 5609-5612
[91] Kong et al. *ChemMedChem* **3**, 233
[92] Amin et al. *Can. J. Microbiol*. **15**, 1067
[93] Mahady et al. *Phytother. Res.*, **17**, 217



Berberine



Sangunarine

Figure 13

Sanguinarine (figure 13), a benzophenanthridine alkaloid derived from the rhizomes of *Sanguinaria canadensis*, has a wide range of antimicrobial activity. It is used in a broad range of oral health products including toothpaste to prevent dental plaque formation.[94] Beuria et al found that sanguinarine inhibited proliferation of both Gram-positive and Gram-negative bacteria by blocking cytokinesis without affecting DNA replication or nucleoid segregation.[95] These data provide evidence that sanguinarine inhibits bacterial cytokinesis by perturbing the assembly and function of the Z ring in bacteria by inhibiting FtsZ assembly through FtsZ binding. Sanguinarine is also known to inhibit proliferation of various types of cancer cells.[96]

Carboxylic acids,

A cell-based assay with *B. subtilis* was used to search for cell division inhibitors. The microtiter plate assay was used to screen a library of about 105.000 synthetic compounds at a single concentration (32 or 40 μg/mL). Several compounds were identified, among which were specific inhibitors of FtsZ like compounds PC58538 and PC170942 (figure 14).[97] The authors report that compound PC58538 also inhibits division in vegetative cells of wild-type *B. subtilis*. Untreated cells had a typical short rod morphology, whereas cells that had been treated with compound PC58538 had the form of extremely long aseptate filaments. After 2 h (about three generations) in the presence of PC58538 (128 μg/mL) the average cell length had increased about 5-fold, commensurate with a near complete block in cell division.

[94] Godowski et al. *J. Clin. Dent*. **1**,96
[95] T. K. Beuria et al. *Biochemistry* **44**, 16584
[96] N. Ahmad et al. *Clin. Cancer Res*. **6**, 1524-1528.
[97] Stokes et al. *J. Biol. Chem.* **280**, 39709.

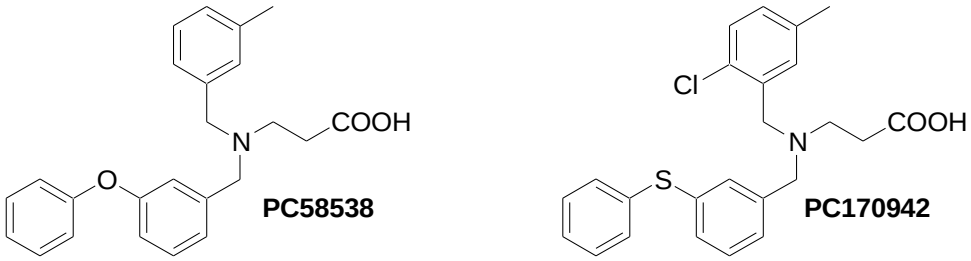
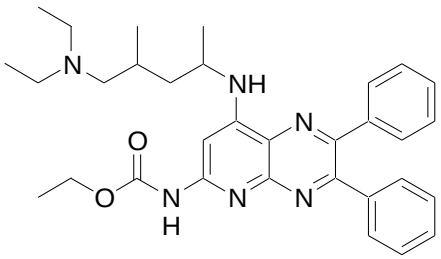


Figure 14: Carboxylic acid derivatives

Other compounds

Another study used a library of thousands of analogs of synthetic inhibitors of tubulin for testing their inhibitory capacity for FtsZ from Mycobacterium tuberculosis.[98,99] Two identified 2-alkoxycarbonylaminopyridine compounds, SRI-3072 (figure 15) and SRI-7614, inhibit FtsZ polymerization and are active against *M. tuberculosis* in a mouse bone marrow macrophages model of infection.



SRI-3072

Figure 15

Paclitaxel (taxol) belongs to the taxanes, which target eukaryotic tubulin and which are widely used antitumor drugs. Screening of 120 taxane derivatives identified several compounds with antituberculosis activity.[100] Interestingly, a group of taxanes (the C-seco-TRAs) was found to be non-cytotoxic but are active against drug-sensitive and drug-resistant strains of *M. tuberculosis*, with MIV values in the micromolar range. Such compounds might be applied as antituberculosis agents in the future.

Benzamide derivatives

Last but not list, the class of 3-hydroxybenzamide derivatives, object of this thesis.

[98] White et al. *J. Antimicrob. Chemother.* **50** 111-114
[99] Reynolds et al. *Bioorg. Med. Chem. Lett.* **14** 3161-3164
[100] Huang et al. *J. Med. Chem.* **49** 463-466

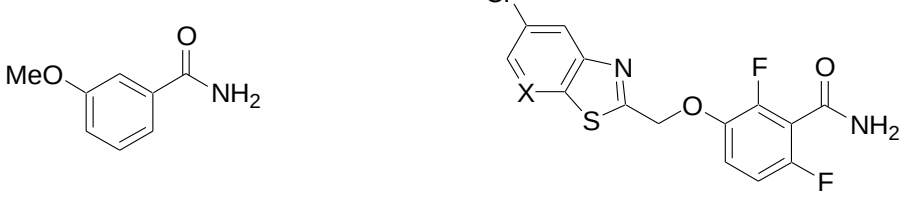
3-Hydroxybenzamide derivatives as new antibacterial agents: state of the art

3-Methoxybenzamide (3-MBA) is a synthetic FtsZ inhibitor of low molecular weight (figure 16).[101] Although 3-MBA has weak antibacterial activity against *B. subtilis* with a minimum inhibitory concentration (MIC) of 4000 μg/mL, it can easily penetrate bacterial cells[102] and bind FtsZ at high ligand efficiency.[103] These characteristics make 3-MBA an attractive starting point for antibacterial drug discovery.

Preliminary structure–activity relationship (SAR) studies indicated that few substitutions of the benzamide ring were tolerated and only small halogens substituents were preferred leading to 2,6-difluoro-3-methoxybenzamide which demonstrated greater potency and on-target activity than 3-MBA.[104]

Remarkably, Haydon et al.[105] identified a compound termed **PC190723** (figure 16) from more than 500 analogues in 2008, which is composed of substituted benzamide and thiazolopyridine moieties joined together by an ether linkage (figure 16). Biological assays demonstrated that PC190723 can inhibit the GTPase activity of purified FtsZ in vitro in a concentration-dependent manner and can perturb FtsZ localization in vivo. Compared with 3-MBA, PC190723 exhibits potent antibacterial activity, with MIC values in the range of 0.5–1.0 μg/mL against *B. subtilis* and various *staphylococci* including MRSA and multiple-drug-resistant *Staphylococcus aureus* (MDRSA). Importantly, it showed no inhibition toward yeast growth or human hepatocytes. However, PC190723 did not display antibacterial activity against other Gram-positive and Gram-negative pathogenic bacteria such as *S. pneumoniae* and *E. coli*, the MIC values of which were > 64 μg/mL.

[101] Y. Ohashi et al. *J. Bacteriol.* **181**, 1348
[102] R. Perrone et al. *J. Med. Chem.* **41**, 4903
[103] A. L. Hopkins et al. *Drug Discovery Today* **9**, 430
[104] Czaplewski et al *Bioorg. Med. Chem. Lett*. **19** 524–527
[105] D. J. Haydon et al. *Science* **321** 1673

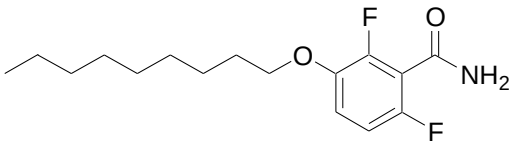


3-Methoxybenzamide

X = C Compound A
X = N PC190723

Figure 16

Extension of the SAR explorations of 3-MBA and found that the methoxy group can be substituted with other groups to generate new derivatives with improved on-target activity.[75] The authors synthesized a series of various alkoxy-substituted 3-alkoxybenzamides based on the SARs of 3-MBA. Among them, 2,6-difluoro-3-nonyloxybenzamide (compound **1**) and 2-fluoro-3-nonyloxybenzamide showed the most significant activity against *B. subtilis* 168 and *S. aureus* ATCC 29213, with respective MIC values of 0.125 and 0.5 μg/mL. Both these compounds were shown to have > 10 000-fold higher activity than the parent 3-MBA. The elongated morphology of *B. subtilis* 168 and the enlarged morphology of MRSA observed by phase-contrast microscopy confirmed that the alkoxy derivatives of 3-MBA target FtsZ.



2,6-Difluoro-3-nonyloxybenzamide (1)

Figure 17

Furthermore, 2,6-difluoro-3-alkyloxybenzamide (**1**) has suboptimal drug-like absorption, distribution, metabolism, and excretion (ADME) properties. To acquire FtsZ inhibitors with potent activity and suitable ADME profiles, Haydon et al.[106] designed and synthesized a series of heteroarylmethoxy-substituted derivatives in 2010. Among them, the most active compound (showed a MIC as low as 0.125 μgm/L1 gainst *S. aureus*, but > 95 % of its plasma protein binding rate was considered too high to progress. Although replacing the benzothiazole substituent (compound **A** figure 16) with a thiazolopyridine group decreased the corresponding antibacterial activity, the plasma protein binding rate of the

[106] Haydon et al. *J. Med. Chem.* **53**, 3927

thiazolopyridine derivatives was low, and their metabolic stability was improved over that of the benzothiazole derivatives. In particular, PC190723 as a thiazolopyridine derivative showed good chemical and plasma stabilities and low clearances in hepatocytes and in mice compared with the corresponding benzothiazole derivative **A**, suggesting that PC190723 is more drug-like than **A**. In addition, in the mouse model of staphylococcal infection, PC190723 was efficacious following intraperitoneal (i.p.) administration, with a 50% effective dose (ED_{50}) of 3.1 mg/kg,[75] whereas compound **A** was less efficacious than PC190723 when administered i.p. (ED50 = 41 mg kg1). In summary, PC190723 maintains a more appreciable balance between antibacterial activity, plasma protein binding rate, and metabolic stability. Moreover, docking analysis indicated that this series did not convincingly dock into the GTPase site of FtsZ but did dock into an adjacent cleft between the C-terminal domain and helix 7 of FtsZ as the binding site of PC190723. Accordingly, PC190723 is a representative of arylalkyloxybenzamides that kill bacterial cells by inhibiting FtsZ; it is an excellent candidate for optimization into a therapy for treating staphylococcal infections.

Aim of the work

As previously described, the 2,6-difluoro-3-nonyloxybenzamide (compound **1**) is a potent FtsZ inhibitor, but it has suboptimal drug-like properties (ADME). Therefore, my research project focused on the modification of this scaffold. The first studies were specifically performed in order to increase the drug-like properties, in particular hydro solubility, and to reduce plasmatic proteins interactions.

We decided to optimize the lead compound **1** following two different approaches:

Replacement of the amide function with new bioisosteric groups;
Replacement of alkoxyl chain with an alkoxyl chain bearing teminal polar groups
Therefore, we amidified the carboxylic function of 2,6-difluoro-3-nonyloxybenzoic acid with different amines such as 2-ethanolamine and ethylenediamine obtaining the products **2**, **5**, **7** and the benzodiazepinic derivative **9**. As bioisosteric groups we prepared the oxazoline **3**, the imidazoline **8** and the tetrazole **6**. Finally, the compound **4** was prepared to confirm the role of carboxyamide function in the interaction (figure 18).

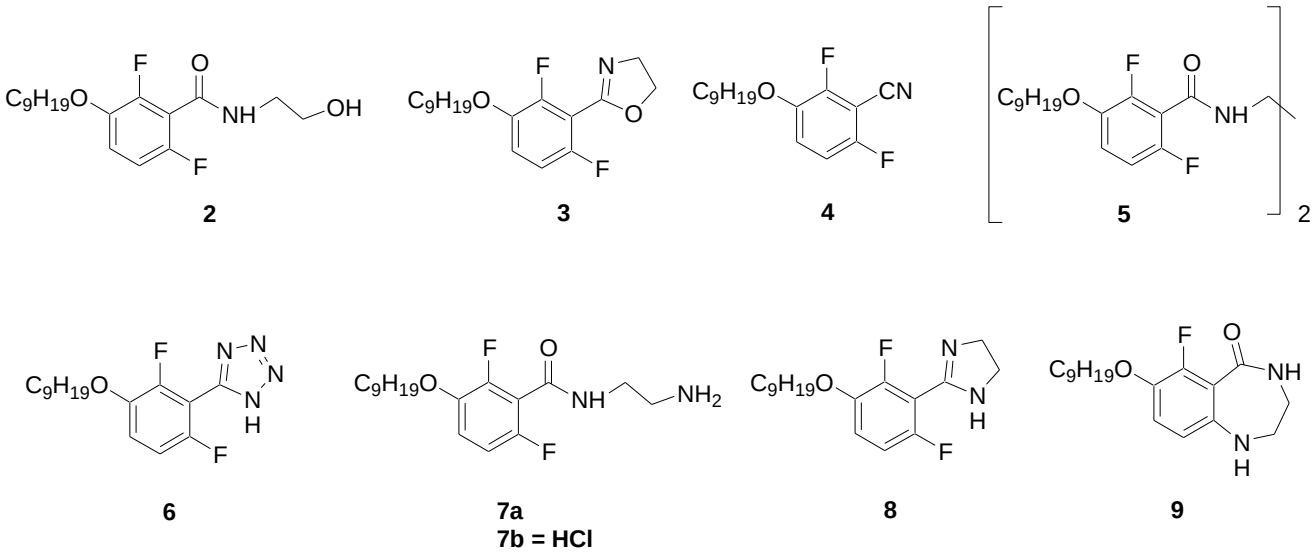
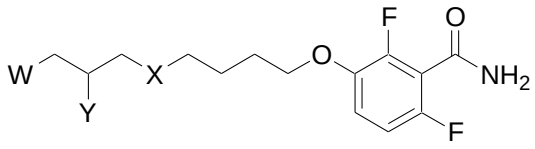


Figure 18

The second approach was the introduction of polar groups in the terminal portion of the alkoxy chain of compound **1**. Considering that the nonyl terminal portion

has not significant interaction potentialities with FtsZ, we decided to design new molecules having alcoholic and aminic groups at the end of the alkyl chain. Such modifications would contribute to enhance hydrosolubility and to reduce plasmatic proteins interactions. The compounds **10**, **11**, **12** and **13** (figure 19) were prepared by linking glycerol, serinol and isoserinol through one of their terminal heteroatoms to the meta position of 2,6-difluorobenzamide by a butyloxy spacer thus maintaining the length of the nonyloxy substituent of **1**.



10 X= O Y = W = OH
11 X= O Y = NH_2 W = OH
12 X= NH Y = W = OH
13 X = O Y = OH W = NH_2

Figure 19

The results of preliminary investigation on compounds **2-13** can be summarized as follows:

compound **7** is one order of magnitudo less active than **1** against gram positive bacteria, but its activity spectrum is broader enclosing also gram negative bacteria;
the lack of activity of compounds **10-13** seems to indicate that the activity is preferentially associated to the presence of a non-polar residue at the position 3 of benzamide.

Consistently with these preliminary results, we designed and synthesized a new series of derivatives with different substituents at the 3 position. The compound **14** bears a 2-benzodioxanyl linked to C(3) of benzamide through a methyleneoxy bridge (figure 20). Compound **14** poorly reduces the viability of the mammalian cells, as demonstrated by the toxicity test performed on Vero and MRC-5 cells even at the highest concentrations (100-120 µg/ml). Furthermore, **14** is active against *S. aureus* at 5-10 µg/ml, but it didn't show any inhibition on E. coli replication. The broader activity of the compound **7** which inhibits both *S. aureus* and *E. coli*, and the encouraging results obtained for **14** against *S. aureus* led us to hybridize **7** with **14** designing compound **15**.

Consistent with the activity data we also considered the benzodioxane isosters:

3-hydroxymethlpyridodioxane and 2- hydroxymethylpyridodioxane; 2-hydroxymethylchromane and 2- hydroxymethyl-1,2,3,4-tetrahydro naphthalene.

The nitrogen of the heterocycle could interact with side chains of aminoacids present in the α 7 helix of FtsZ. Moreover, piridodioxane could confer better pharmacokinetic properties and reduce metabolic transformation by hepatic P450 cytochromes in comparison with benzodioxane. Therefore we prepared the compounds **16** and **17** (figure 20). In order to evaluate the importance of the oxygens of the benzodioxane ring in the interaction with FtsZ, we also synthesized the compounds **23** and **24** that possess a (1,2,3,4-tetrahydronaphtalenyl)methanol and a (chroman-2-yl)methanol linked at the 2,6-difluoro-3-hydroxybenzamide respectively.

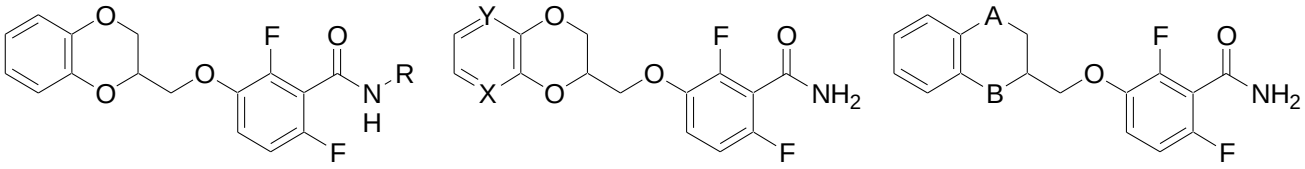


Figure 20

We also decided to synthesize **18**, the opened analogue of **17**, and **19** the positional isomer of **18** resulting from the shift of the hydroxymethyl group. The compound **20** was designed replacing the bicycle system of PC190723 with a 3-hydroxy-6-methyl-2-pyrimidinylmercaptomethyl residue (figure 21).

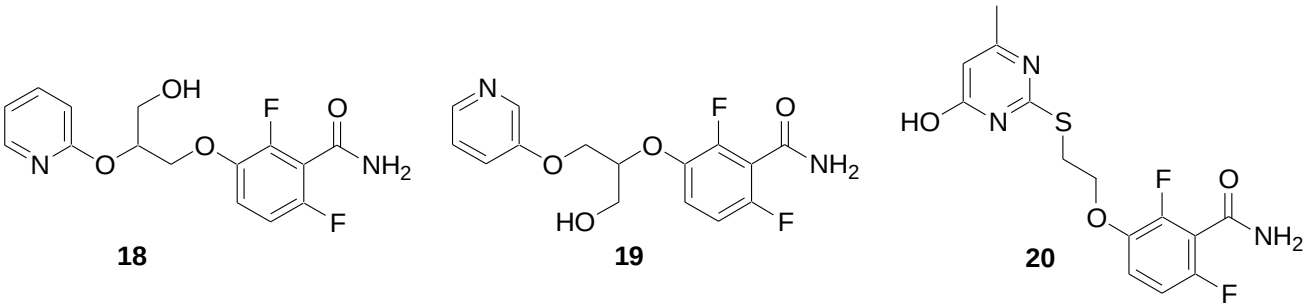
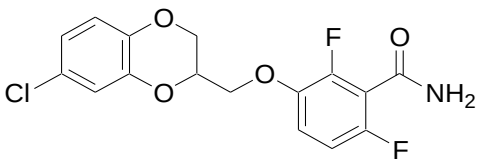
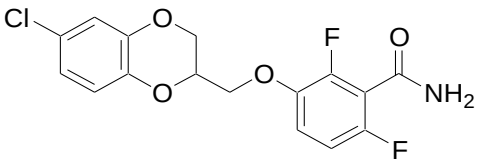


Figure 21

According to Haydon's approach successfully applied for the development of **PC190723**, namely the introduction of Cl into the 5 position of thiazol[5,4-b]pyridine to enhance the activity against *S. aureus*, we decided to synthesize **21** and **22**, the 7- and 6-chloro analogues of **14** (figure 22).



21



22

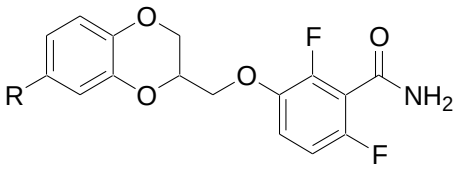
Figure 22

The microbiological tests performed revealed a quite different activity: the compound **21** was active against *S. aureus* at 0.5-1.0 μg/ml whereas **22** show sanalogous inhibition only at 5 μg/ml.

Therefore, in order to get a deeper insight into the nature of the binding subsite interaction of this molecular portion it seemed worthwhile to investigate:

the role of the configuration at the stereocenter of benzodioxane nucleus on the activity;
a wide series analogues differently substituted at the 7 position of benzodioxane residue.

To this end, the R and S enantiomers of **21** and the compounds **25 - 35** (figure 23) were synthesized and tested for the activity against *S. aureus*.



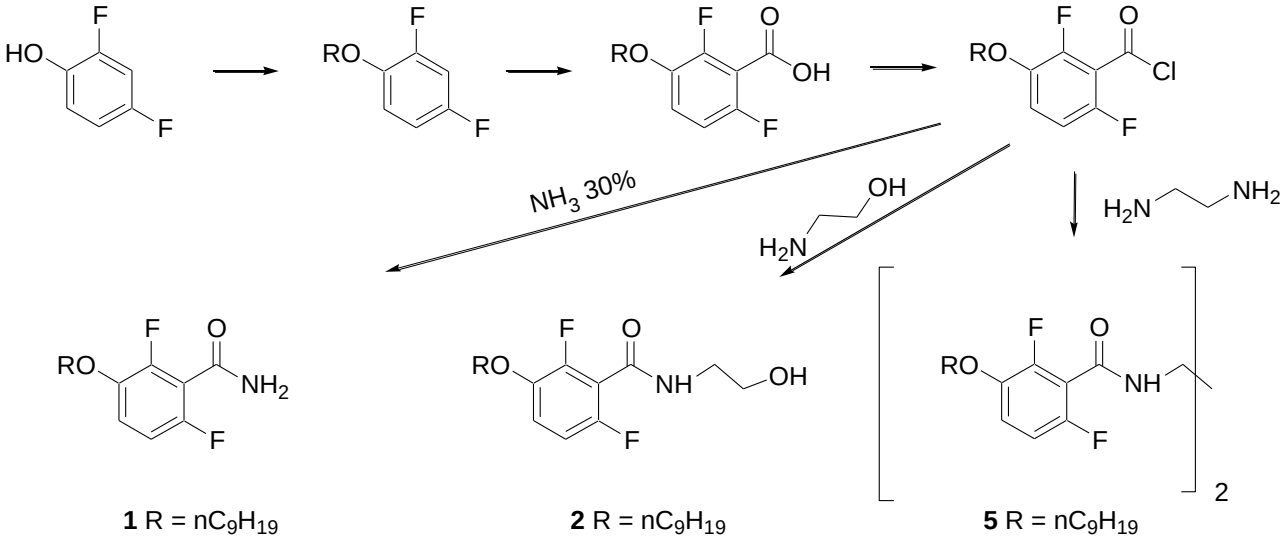
25 R = Br
26 R = CCH
27 R = F
28 R = I
29 R = NO_2
30 R = NH_2
31 R = $NHSO_2Me$
32 R = $CHCH_2$
33 R = CF_3
34 R = CN
35 R = CONHBu

Figure 23

Synthetic Methods

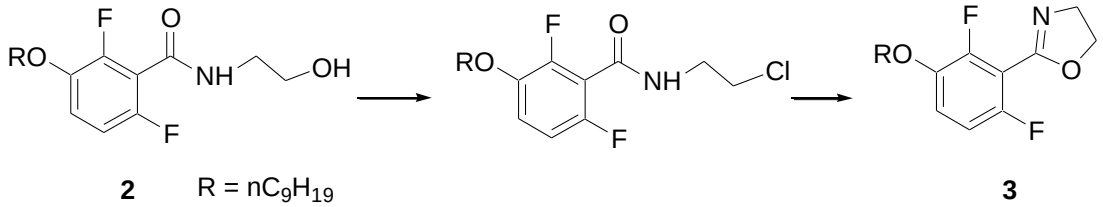
Synthesis of the bioisosters of the benzamidic function

The synthesis of the bioisosters of the amidic function starts from 2,4-difluorophenol that was alkylated with nonylbromide; the so obtained ether reacts with n-butyl lithium and carbonic anhydride to give the 2,6-difluoro-3-nonyloxybenzoic acid. The carboxylic acid is transformed in the acyl chloride for treatment with $SOCl_2$ in toluene, this intermediate readily reacts with ammonia, ethylendiamine, ethanolamine to give compounds **1**, **2** and **5** (scheme 1).



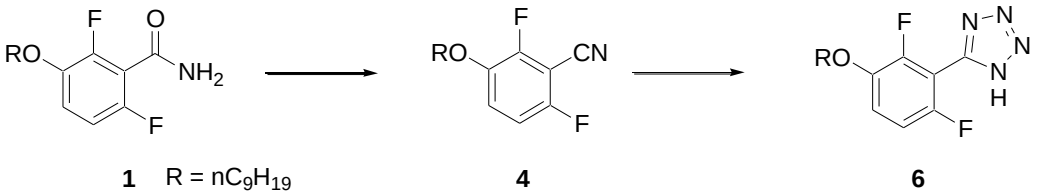
Scheme 1

The ethanolamidic derivative **2** for treatment with $SOCl_2$, gives the chloro derivative that for reaction with triethylamine give the oxazoline **3** (scheme 2).



Scheme 2

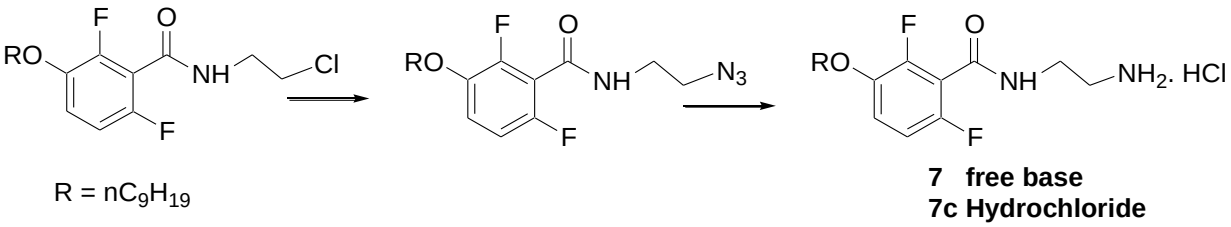
The primary amide **1** is dehydrated giving the corresponding nitrile **4** for treatment with trifluoroacetic anhydride in presence of pyridine. The tetrazole **6** is synthesized from the nitrile for reaction with sodium azide and zinc chloride in a mixture water/ DMF (scheme 3).



1 R = nC_9H_{19} **4** **6**

Scheme 3

The chloro ethyl amidic derivative, utilized for the synthesis of the oxazoline **3** undergoes to a reaction of nucleophilic substitution with sodium azide, the resulting aliphatic azide is reducted for treatment with triphenylphosphine in a mixture THF / water. Compound **7c** derives from treating compound **7** with 10% chloridric acid in ethanol (scheme 4).

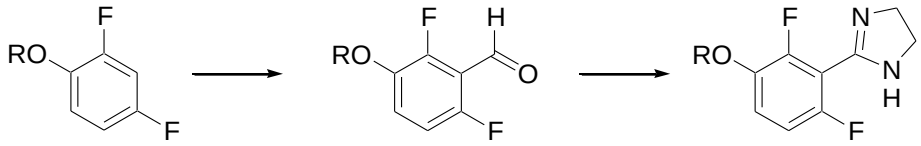


R = nC_9H_{19}

7 free base
7c Hydrochloride

Scheme 4

The synthesis of compound **8** starts with the reaction between nonyloxy-2,4-difluoro anisole, n-butyl lithium and anidrous DMF. The resulting aldehyde was treated with ethylendiamine and trichloroisocianuric acid to give the imidazoline (scheme 5).

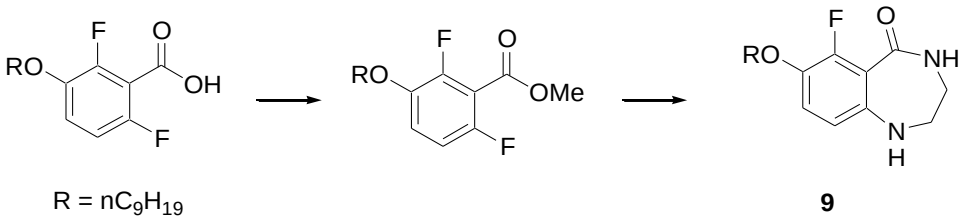


R = nC_9H_{19}

8

Scheme 5

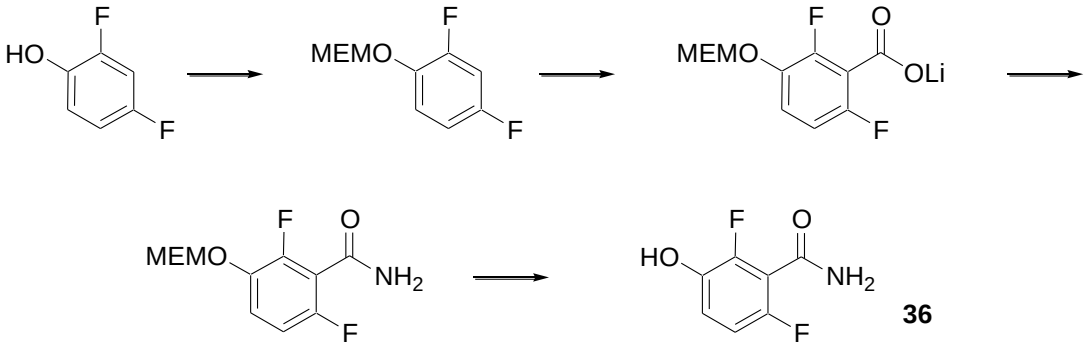
The 2,6-difluoro-3-nonyloxy benzoic acid is transformed in the methyl ester and the successive treatment with ethylendiamine in ethanol gives the benzodiazepinic derivative **9** (scheme 6).



Scheme 6

Synthesis of 2,6-difluoro-3-hydroxy benzamide

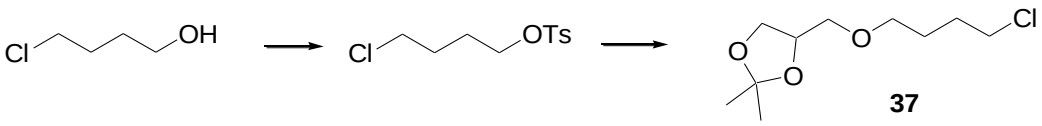
The synthesis of 2,6-difluoro-3-hydroxy benzamide starts with 2,4-difluoro phenol that is alkylated with methoxyethoxymethyl chloride; the reaction between the MEM ether, n-butyl lithium and carbonic anhydride gives the corresponding lithium salt of the 2,6-difluoro-3-methoxyethoxymetoxybenzoic acid. The carboxylic acid is transformed in the corresponding amide for reaction with ethylchloroformiate, N,N-diisopropyl-N-ethylamine and concentrated ammonia. The treatment with 10% chloridric acid remove the protecting group and gives the intermediate 2,6-difluoro-3-hydroxybenzamide **36** (scheme 7).



Scheme 7

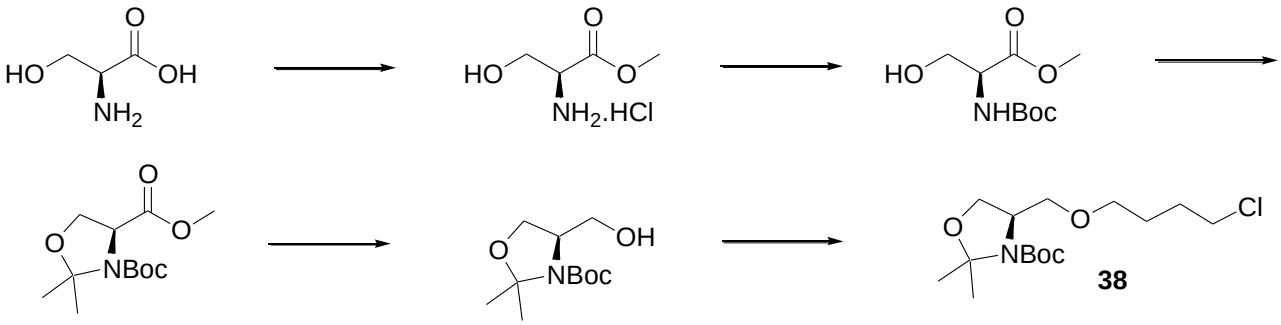
Synthesis of the hydrophilic lateral chains

The synthesis of the lateral chain of compound **10** starts from the 4-chloro-buthanol that is tosylated and the resulting intermediate reacts with the sodium salts of racemic solketal, giving the desired chloroderivative **37** (scheme 8).



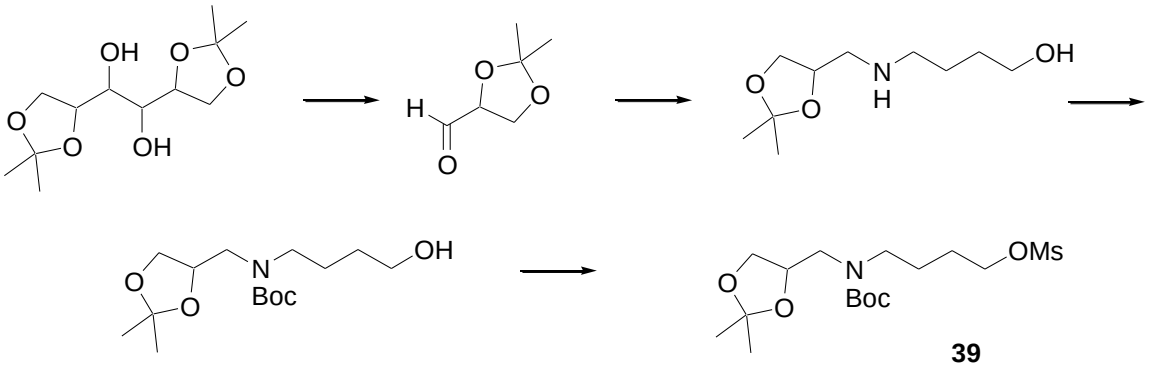
Scheme 8

The synthesis of the lateral chain of compound **11** starts from the L-serine that is transformed in the methyl ester monochloridrate, the aminic group was protected as Boc and the resulting intermediate undergoes the reaction with 2,2-dimethoxypropane in acetone with the catalysis of ethereal trifluoroborate to obtain the ketal. The methyl ester was reduced with $LiAlH_4$ and the resulting alcohol gives the desired building block **38** (scheme 9) by treatment with sodium hydride and 4-chloro-1-tosyloxybuthane.



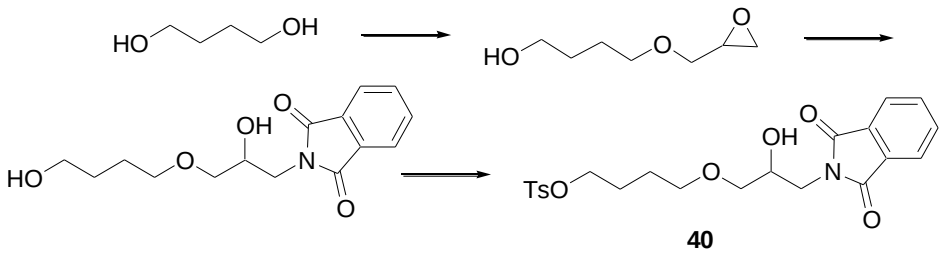
Scheme 9

The synthesis of the lateral chain of compound **12** starts from the oxidation of mannitol diacetonide with sodium periodate in methanol/water. The resulting aldehyde with 4-amino-1-buthanol and catalytic hydrogenation gives the amino precursor that is protected at the aminic function as Boc. The alcoholic function is transformed in mesyl derivative **39** (scheme 10).



Scheme 10

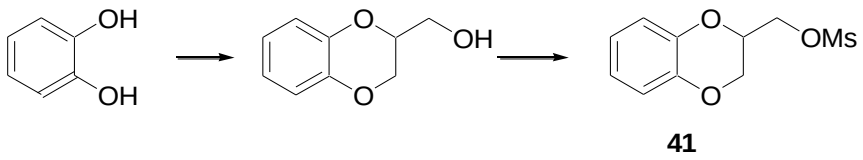
The synthesis of the lateral chain of compound **13** starts from the reaction between 1,4-buthanediol and epichloridrine that yields the mono oxyranyl derivative. The epoxide is open with the phtalimide in isopropanol at reflux, the resulting intermediate isconverted to compound **40** (scheme 11) by selective reaction at the primary alcohol with paratoluensulphonyl chloride in pyridine at 0°C.



Scheme 11

Synthesis of benzodioxane and pyridodioxane nuclei

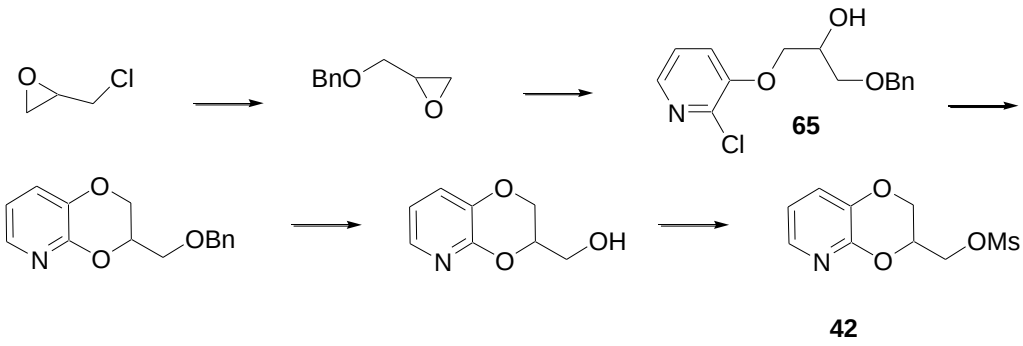
Key intermediate for the synthesis of **14** is the 2-mesyloxymethyl-benzodioxane. The reaction between catechol and epichloridrine in methanol in presence of two equivalents of sodium hydroxide gives the benzodioxanic system, the alcoholic function was mesylated giving the desired intermediate **41** (scheme 12).



41

Scheme 12

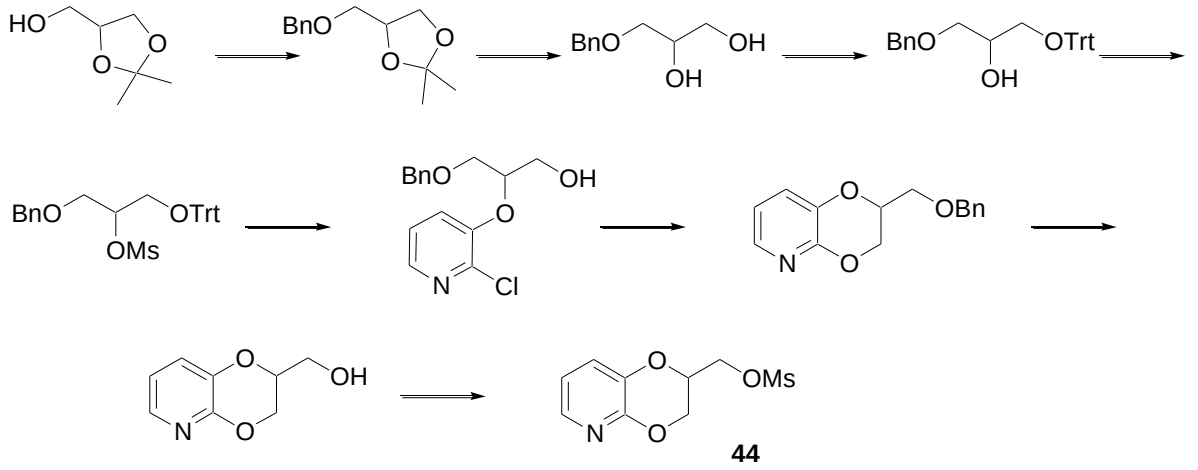
The preparation of 2-mesyloxymethyl-pyridodioxane **42** (scheme 13) starts from the reaction between an excess of benzyl alcohol and epichloridrine to obtain the mono oxyranyl derivative. The epoxide is condensated with the 2-chloro-3-hydroxypyridine in basic conditions, the so obtained intermediate treated with sodium hydride underwent to an intramolecular cyclization. The benzyl group is removed by catalytic hydrogenation, the resulting alcoholic group was mesylated.



42

Scheme 13

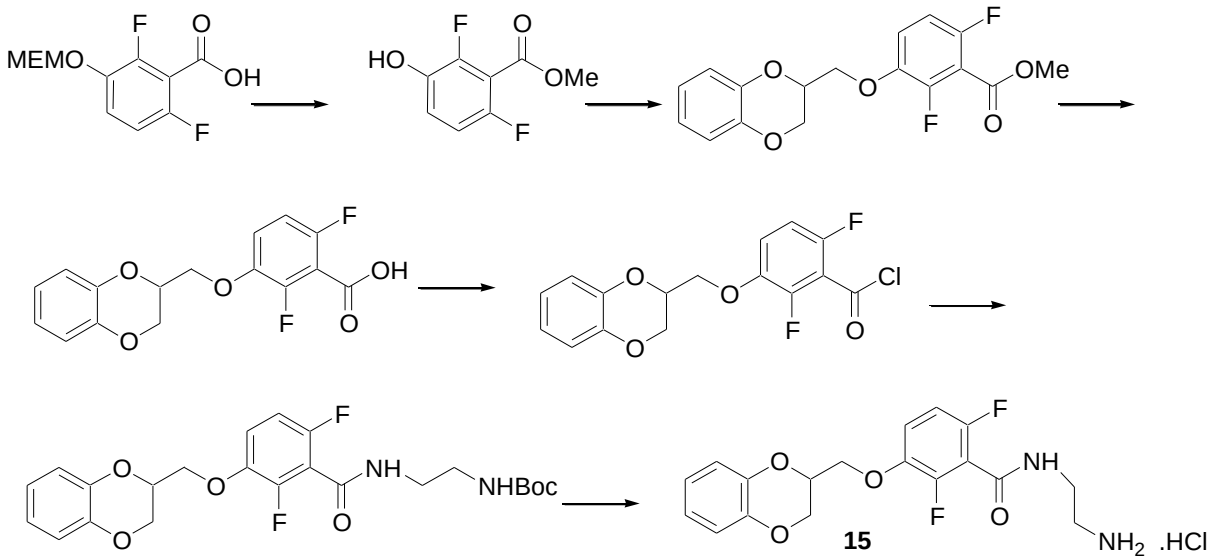
The synthesis of 3-mesyloxymethyl-pyridodioxane **43** (scheme 14) starts from racemic solketal that was benzylated and hydrolyzed to diol derivative. The primary alcoholic function was tritylated and the secondary alcohol was mesylated. The resulting intermediate is condensed in basic conditions with the 2-chloro-3-hydroxypyridine. The trityl group is removed with an acidic treatment, the reaction with one equivalent of sodium hydride allows an intramolecular cyclization. The benzyl group, as previously done, is removed by catalytic hydrogenation and the alcohol was mesylated.



Scheme 14

Synthesis of the compound 15: hybrid of 7c and 14

The MEM protected 2,6-difluoro benzoic acid in methanol treated with concentrated sulphuric acid gives contemporary the methyl esther and removing the MEM yielding the 2,6-difluoro methyl benzoate (scheme 15). The phenolic function is alkylated, in basic conditions, with the 2-mesyloxymethyl-benzodioxane, the methyl ester is hydrolyzed with sodium hydroxide, the corresponding carboxylic acid is transformed in the acyl chloride for treatment with $SOCl_2$ which reacts with the mono Boc ethylendiamine in dry pyridine. The compound **15** is obtained by removing the Boc group with 10% chloridric acid in refluxing isopropanol.

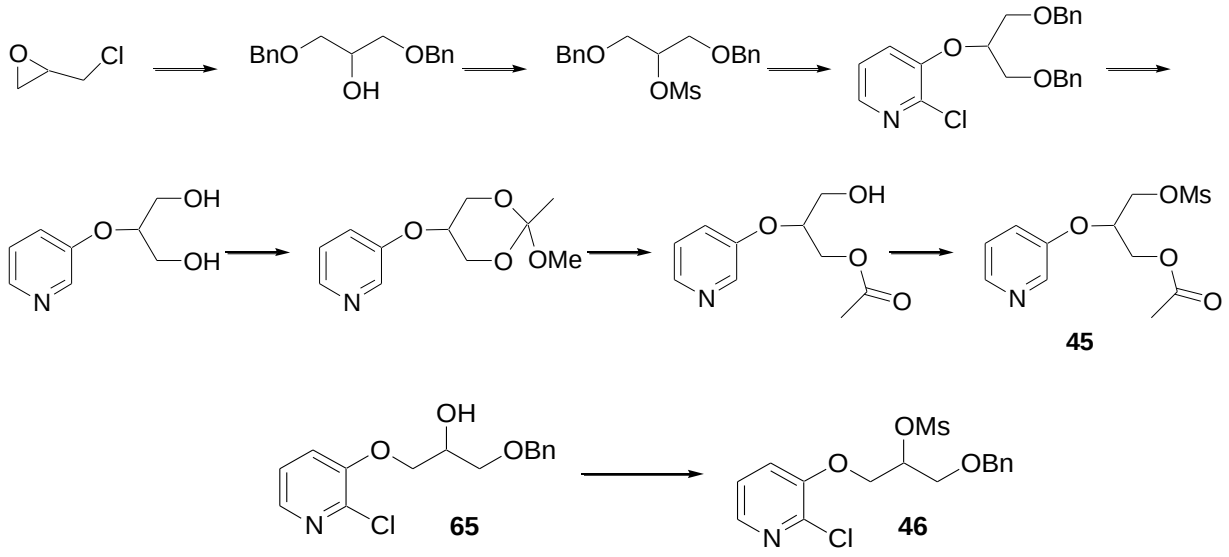


Scheme 15

Open analogues of the pyridodioxane system

The synthesis of **18** starts with the reaction between the benzyl alcohol and an excess of epichloridrine in basic conditions. The dibenzyloxy derivative is mesylated and condensated with the 2-chloro-3-hydroxypyridine. The successive catalytic hydrogenation remove contemporary the two benzyl group and the chlorine on the heteroaromatic ring. The diol so obtained reacts with trimethylorthoacetate in acidic condition giving the corresponding ortho acetate, the successive treatment with water gives the monoacetyl derivative which is converted by mesylation into compound **45** (schema 16).

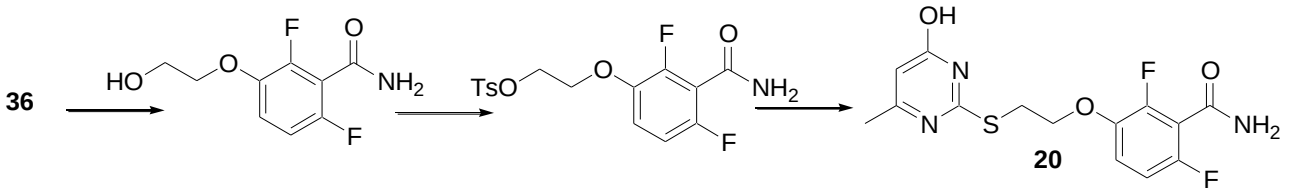
The 3(3-benzyloxy-2-hydroxypropyloxy)-2-chloropyridine, already described in scheme 13, undergoes mesylation becoming the key intermediate **46** (scheme 16) for the synthesis of **19** .



Scheme 16

Synthesis of the compound 20 open analogues of PC190723

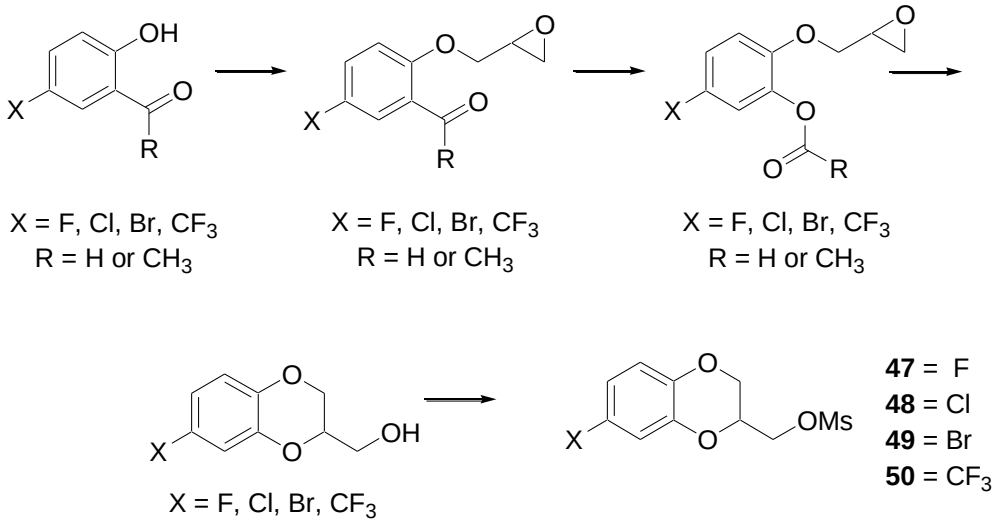
For the preparation of compound **20** (scheme 17) the 2,6-difluoro-3-hydroxybenzamide is alkylated in basic conditions with ethylene carbonate, the resulting alcohol is transformed in a tosyl group, that is substituted with 2-mercapto-4-hydroxy-6-methylpyrimidine, utilizing one equivalent of potassium carbonate as base. The pyrimidine is prepared from the reaction between ethylacetacetate and thiourea.



Scheme 17

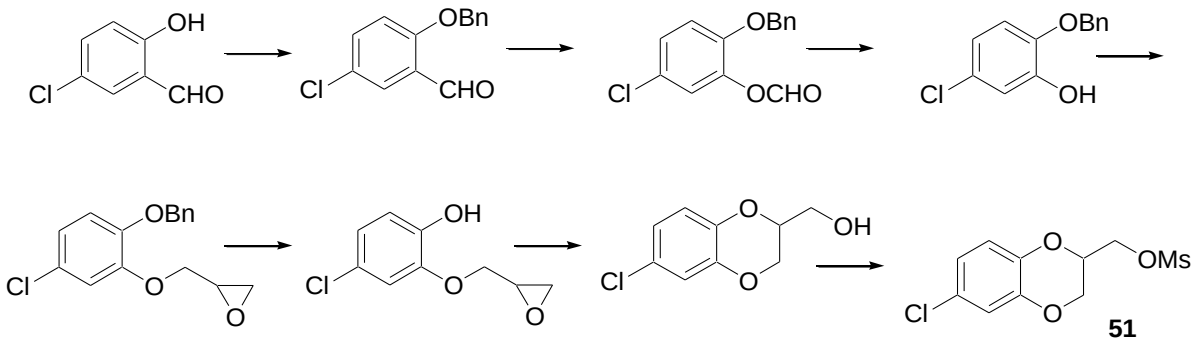
Synthesis of the 6 and 7 substituted benzodioxane rings

7-Fluoro, 7-chloro, 7-bromo, or 7-trifluoromethyl-2-mesiloxymethylbenzodioxane derivatives, compound **47**, **48**, **49** and **50** (scheme 18), are prepared starting from the corresponding benzaldehyde or acetophenone that was alkylated at the phenolic function with an epihalohydrine (Cl or Br) in presence of potassium carbonate. The aldehyde or the ketone was oxydate with *m*-chloroperbenzoic acid and the subsequent treatment with sodium hydroxide allows hydrolysis of the esther and permits the intramolecular cyclization giving the benzodioxane ring. Then the primary alcohol is mesylated.



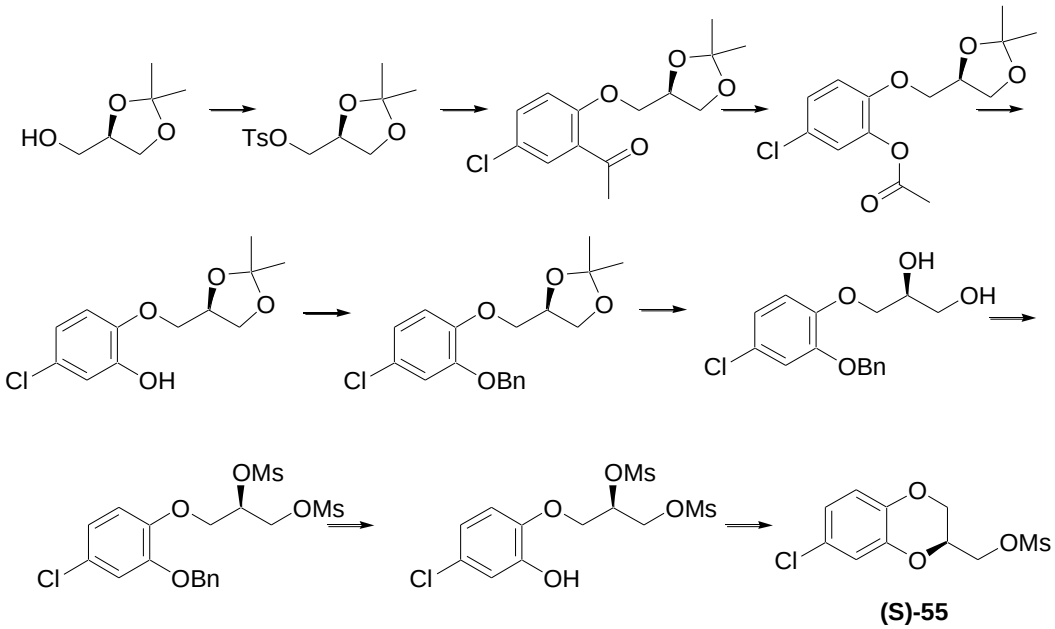
Scheme 18

6-chloro analogues **22** is synthesized starting from the 5-chloro salicilaldehyde (scheme 19): the phenolic function is benzylated, the aldehyde is oxydate with metachloroperbenzoic acid and the formyl esther is hydrolyzed with sodium hydroxide. The phenolic function obtained is alkylated with epichloridrine in presence of potassium carbonate. The benzyl group is removed by catalytic hydrogenation and the treatment with sodium hydroxide gives the intramolecular cyclization. The reaction with mesylchloride gives the intermediate **51**.



Scheme 19

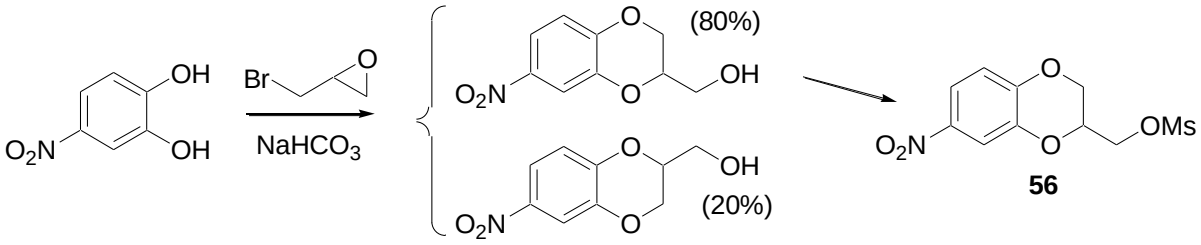
The synthesis of enantiopure (R) and (S)-7-chloro-2-mesyloxymethylbenzodioxane derivative starts from the R or S solketal. As described in scheme 20 for the (S)-**55**, the reaction with tosylchloride and the successive condensation with 5-chloro-2-hydroxyacetophenone, affords the acetophenone derivative. The oxydation with *m*-chloroperbenzoic acid gives the esther which is hydrolyzed with sodium hydroxide. The phenolic function is protected as benzyl ether and the subsequent acidic hydrolysis gives the diol derivative, which is treated with methansulphonyl chloride to give the dimesyl derivative. After catalytic hydrogenation, the reaction with potassium carbonate gives the desired compound **(S)-55**



Scheme 20

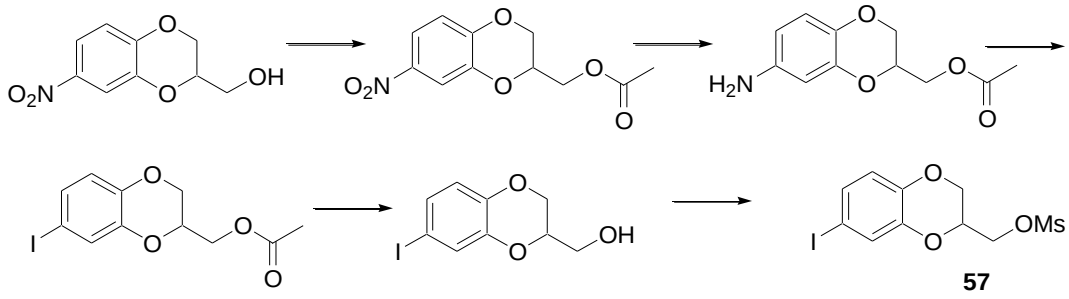
The 2-hydroxymethyl-7-nitrobenzodioxane is prepared from the 4-nitro catechol for treatment with $NaHCO_3$ and epibromohydrin (scheme 21). The reaction gives a

mixture constituted of 80% of the 7 isomer and 20% of the 6 isomer. This mixture of regioisomer was crystallized with DCM obtaining the pure 7-nitro-2-hydroxymethylbenzodioxane, which is tosylated obtaining the compound **56**



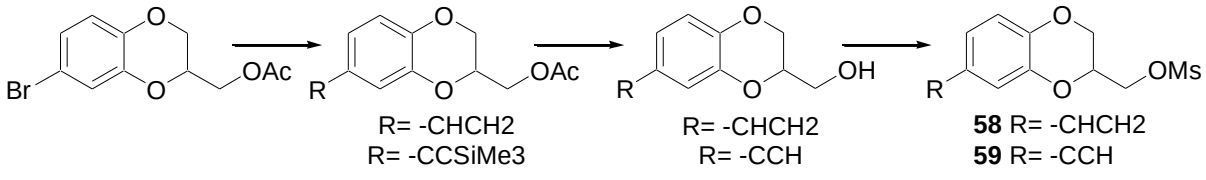
Scheme 21

The synthesis of 2-hydroxymethyl-6-iodobenzodioxane derivative starts from the 7-nitro-2-hydroxymethylbenzodioxane, that was acetylated, the nitro group was reduced with tin (II) chloride, the resulting aniline was diazotated ad for treatment with sodium iodide yields the iodo derivative. After alkaline hydrolysis and reaction with mesyl chloride the intermediate **57** is obtained (Scheme 22).



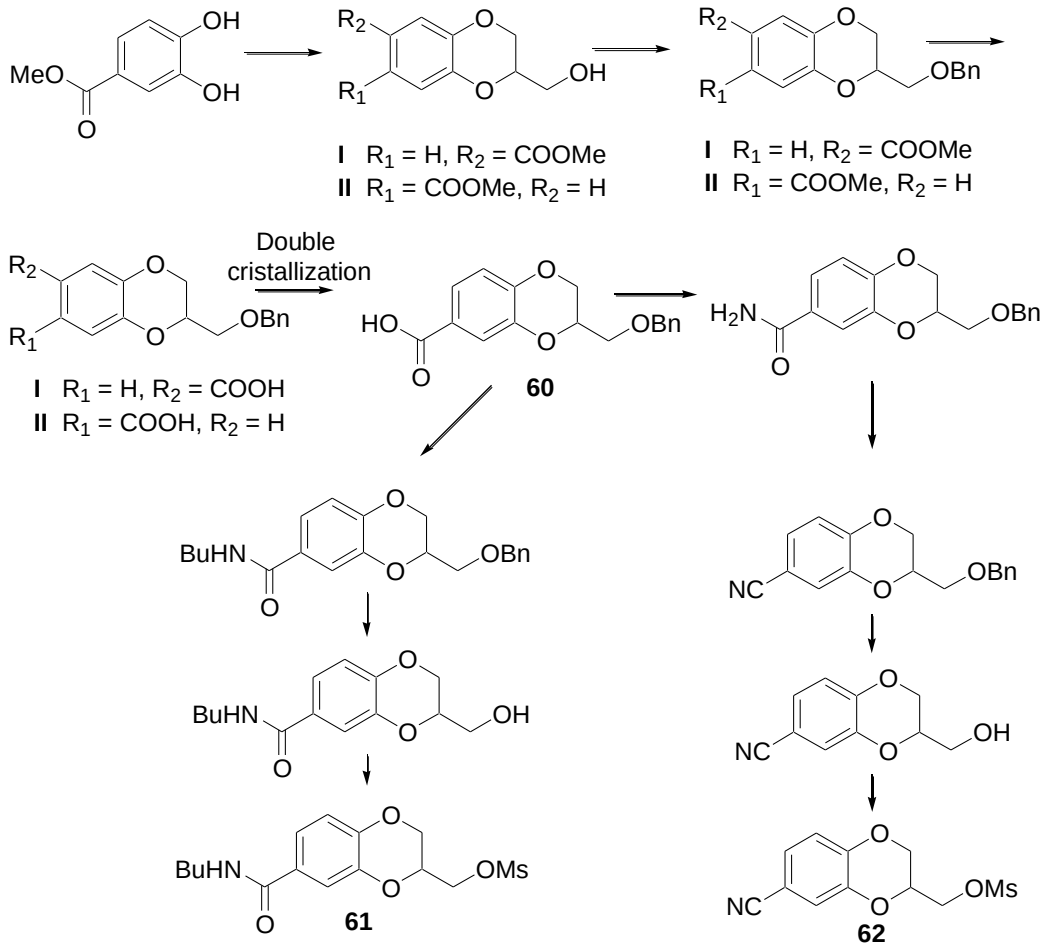
Scheme 22

The 7-vinyl and 7-ethynyl are prepared from the 7-bromo-2-acetoxymethyl-1,4-benzodioxane utilizing respectively trifluoro vinyl borate and ethynyltrimethylsilane in presence of a palladium catalyzer. The acetoxy and the trimethylsilyl are removed with sodium hydroxide and the hydroxyl group are treated with mesylchloride to give the compounds **58** and **59** (scheme 23).



Scheme 23

The synthesis of 7-cyano and 7-N-buthylcarboxyamide derivatives started from the 7-carboxy-2-Hydroxymethylbenzodioxane (scheme 24).



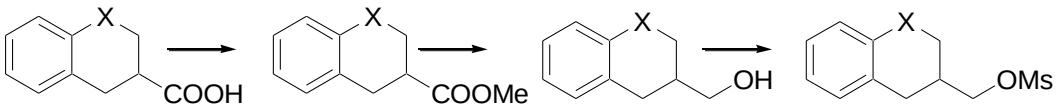
Scheme 24

The reaction between 3,4-dihydroxybenzoate and epichloridrine gives the mixture of 6- and 7-carboxymethyl-2-hydroxymethylbenzodioxane. The alcoholic function is protected as benzyl ether and the methylester was hydrolyzed with sodium hydroxide. The pure 7-carboxy derivative **60** is obtained after double crystallization from toluene. The reaction of this latter with thionyl chloride and ammonia is transformed in the primary amide. The catalytic hydrogenation of the benzyl group and the treatment with trifluoroacetic anhydride in pyridine gives the cyano derivative. After reaction with mesyl chloride, the intermediate **62** was obtained.

The 7-N-butylamido analogous was obtained from the 7-carboxy derivative by reaction with thionyl chloride and n-buthylamine. After catalytic hydrogenation and reaction with mesylchloride the intermediate **61** was isolated.

Synthesis of the tetrahydronaphtyl and chromanyl lateral chain

The synthesis of (1,2,3,4-tetrahydronaphthalen-2-yl)methyl methanesulfonate and chroman-3-ylmethyl methanesulfonate derivatives starts from the corresponding carboxylic acid which is transformed in the methyl esther, reduced with $LiAlH_4$ to alcohol and mesylated (scheme 25).



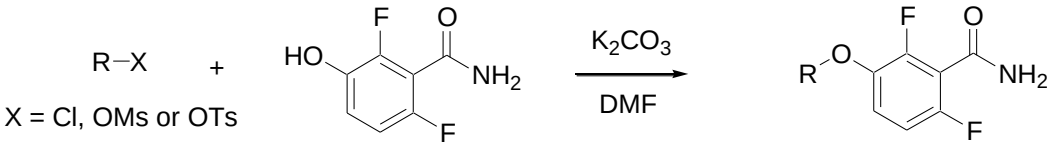
63 X = CH_2
64 X = O

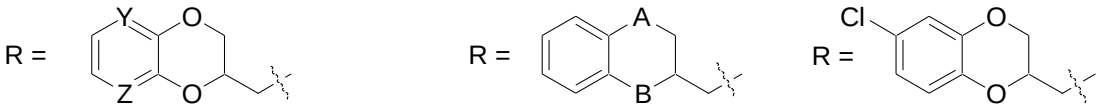
Scheme 25

Synthesis of the final products

The preparations of compounds **1 – 9, 15** and **20** have been described previously.

For the preparation of the final products the 2,6-difluoro-3-hydroxybenzamide is condensed with the different chloro, mesyloxy and tosyloxy derivative in DMF, utilizing the potassium carbonate as base. The temperatures of the reaction are included between 50 and 75°C.

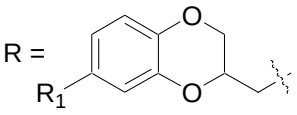




14 Y = Z = CH
16 Y = CH Z = N
17 Y = N Z=CH W= N

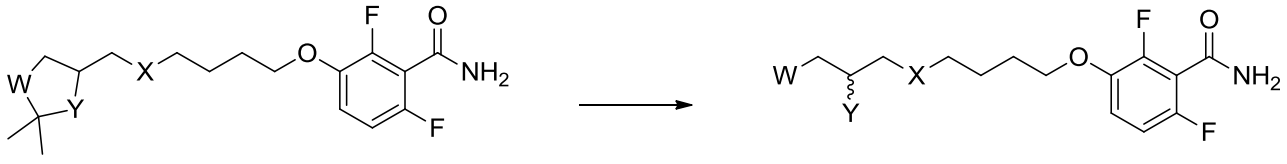
23 A= B= CH2
24 A= O B= CH2

22



21 R_1 = Cl
(R)-21 R_1 = Cl
(S)-21 R_1 = Cl
25 R_1 = Br
26 R_1 = CCH
27 R_1 = F
28 R_1 = I
29 R_1 = NO_2
32 R_1 = C=CH
33 R_1 = CF_3
34 R_1 = CN
35 R_1 = CONHBu

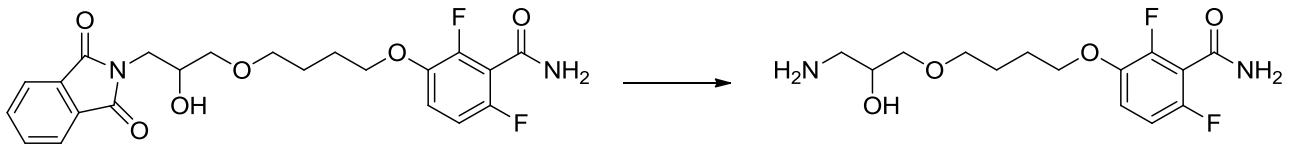
The ketals and Boc protecting group are removed with aqueous acidic treatment in isopropanol.



10 X= Y = W = O
11 X= O Y = NHBoc W = O
12 X= NBoc Y=O W = O

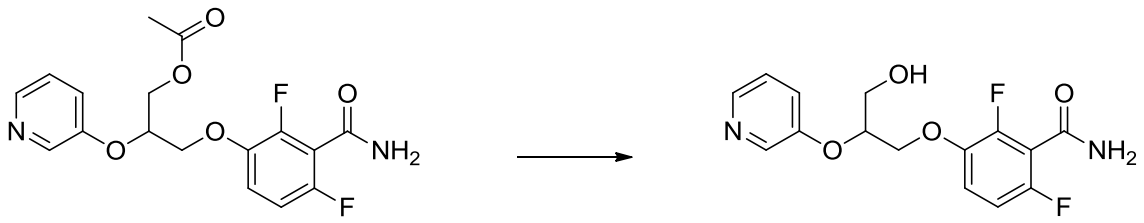
10 X= O Y = W = OH
11 X= O Y = NH_2 W = OH
12 X= NH Y=OH W = OH

The compound **13** is prepared treating the phtalimido derivative with hydrazine.



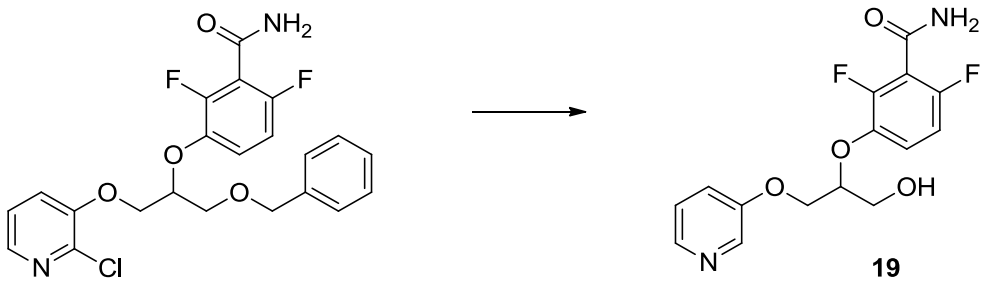
13

The acetyl group present in the precursor of compound **18** is hydrolyzed with sodium hydroxide.



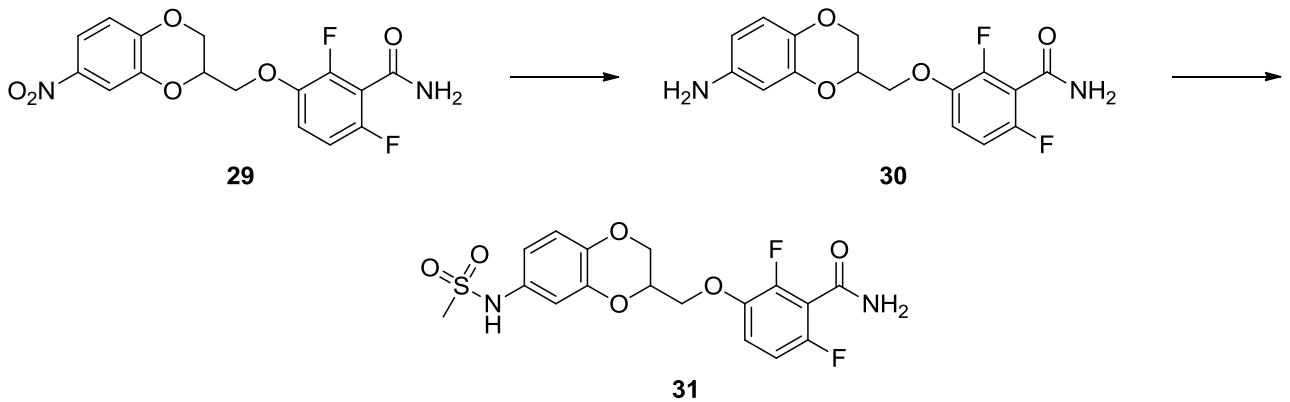
18

The compound **19** is prepared by catalytic hydrogenation of the benzyloxy precursor.



19

The nitro group of **29** is reduced with tin (II) chloride in ethyl acetate, giving the compound **30** that treated with methansulphonyl chloride in presence of triethylamine gives the **31**.



29 **30** **31**

Biological evaluation and results

Cells

Green monkey kidney (Vero) cells, and normal human lung fibroblasts (MRC-5 cells), were grown in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% heat-inactivated calf serum, 100 U/mL penicillin and 100 mg/ mL streptomycin (P/S) in an incubator at 5% CO2 atmosphere and 37 °C. *Staphylococcus aureus* (*S. aureus*) and *Escherichia coli* (*E. coli*, DH5α-strain) bacterial cells were grown overnight in Luria Broth (LB) medium at 37 °C at 300 rpm.

Citotoxicity

The cytotoxicity of compounds **1-22** against mammalian cells was evaluated performing the thiazolil blue tetrazolium Bromide test (MTT) which allows to estimate the reduction on viability of Vero and MRC-5 cells.

Compounds were tested at the concentration of 100 μg/mL and 125 μg/mL on Vero and MRC-5 cells respectively.

To test cytoxicity, all of the compounds were dissolved at the final concentration of 20 mg/mL either in dimethyl sulphoxide (**1**, **2**, **3**, **4**, **5**, **6**, **7**, **8**, **9**, and **14**) or in bidistilled water (**10**, **11**, **12** and **13**) and then serially diluted in DMEM with no P/S. Vero and MRC-5 cells were plated at 104 cell/well in a 96-well plate and the following day 100 μL of different concentrations of each compound were added. After 24-h incubation, the compound was removed and cells were overlaid with 1 mg/mL MTT in 100 μl P/S- and serum-free DMEM for 3 h at 37 °C. MTT was then replaced by the same amount of DMSO for 10 min, and the absorbance of each well was measured at 570 nm with a 550 Microplate Reader (Bio-Rad Lab., Hercules, CA, USA). The percentage of cytotoxicity was calculated by the formula 100 – (sample OD/untreated cells OD) x 100

The results of those tests are presented in Table 1.

Table 1: Cytotoxicity data expressed as reduction on viability of Vero and MRC-5.

Cps	Vero Cell	MRC-5	Cps	Vero Cell	MRC-5	Cps	Vero Cell	MRC-5
1	58,4%	41.9	**9**	97,6%	n.d	**16**	27%	7%
2	16,2%	n.d.	**10**	0%	n.d	**17**	28%	91%
3	12,6%	n.d.	**11**	0%	n.d	**18**	15%	18%
4	22,8%	n.d	**12**	n.d.	n.d	**19**	1%	2%
5	42,2%	n.d	**13**	n.d.%	n.d	**20**	11%	13%
6	90,5%	n.d	**14**	6,2%	4.5%	**21**	30%	24%
7	41.2%	91.2%	**15**	13%	0.0	**22**	18%	8%
8	89,0%	n.d						

Antibacterial activity

The antibacterial activity of the compounds **1-22** was tested on Gram-positive *S. aureus,* and Gram-negative *E. coli* cells. After incubation at 37 °C for 16 h in aerobic-culture tubes, cell concentration was determined by optical density (OD) measurement at 600 nm. For *S. aureus* and *E. coli*, 1 OD corresponds to 1 x 109 and 2 x 108 cells/ mL, respectively. Fresh cultures were diluted to obtain concentrations from 108 to 103 cells/ml in a final volume of 3 mL. After growing the bacteria overnight at 37°C with different amounts of the compounds, an aliquot of each sample was harvested under sterile conditions, and ODs were measured at 600 nm in a SmartSpecTM 3000 spectrophotometer (Bio-Rad). The remaining cells were washed three times with sterile LB and centrifuged for 10 min at 900 x g at 4 °C. The pellet was then resuspended in fresh LB, and incubated for 16-18 h a 37 °C for growth detection and determination of bactericidal or bacteriostatic activities. LB-diluted compounds were incubated for 2 days to verify their sterility, and used as negative controls.

After testing the compounds at different bacterial concentrations, the Minimal Inhibitory Concentration (MIC) of each compounds was assayed on 103 cells/ml of both Gram-positive and Gram-negative bacteria (Table 2). **7** as well as **14** completely inhibits *S. aureus* growth, whereas **2, 3, 4, 5, 10, 12,** and **13** do not. Compound **11** inhibits bacterial growth only at 1 mg/mL. Since preliminary studies showed that compounds **6**, **8**, and **9** were toxic on Vero cells they didn't undergo this experiment. In particular, **7** inhibites *S. aureus* growth at 10-20

µg/mL and **14** at 10 µg/mL. The assay performed on *E. coli*, revealed that **7** inhibits cell growth at 10-20 µg/mL, whereas **14** doesn't, even when used at 100 µg/mL.

To evaluate the Minimal Bacteriostatic or Bactericidal Concentration of the compounds for which no cell growth was detected, the product was removed by three washes, and cells were re-cultured by adding fresh medium. Compound **7** showes bacteriostatic activity at 40 µg/mL and bactericidal activity starting from 80 µg/mL both on *S. aureus* and *E. coli*, whereas **14** shows the same activity only on *S. aureus*. Compound **1**, which was used as a positive control, showed bactericidal activity at 1 µg/mL on *S. aureus*, but doesn't inhibit *E. coli* growth (Table 2).

Table 2: antibacterial activity of compounds **1 -22**.

Cps	MIC on *S.aureus* µg/mL	MIC on *E. coli* µg/mL	Cps	MIC on *S.aureus* µg/mL	MIC on *E. coli* µg/mL
1	1	Inactive	**12**	Inactive	Inactive
2	100-125	Inactive	**13**	Inactive	Inactive
3	100-125	Inactive	**14**	5-10	Inactive
4	100-125	Inactive	**15**	20-40	Inactive
5	100-125	Inactive	**16**	Inactive	Inactive
6	N. D.	N. D.	**17**	Inactive	Inactive
7	10-20	10-20	**18**	Inactive	Inactive
8	N. D.	N. D.	**19**	Inactive	Inactive
9	N. D.	N. D.	**20**	Inactive	Inactive
10	1	Inactive	**21**	0,5	Inactive
11	Inactive	Inactive	**22**	2,5	Inactive
12	Inactive	Inactive			

Conclusions

The biological activity of compounds **1**-**22** was determined and definitive data are available, while only preliminary data can be provided for compounds **(R)-21, (S)-21** and **23.** Overall, the results indicate that some compounds exhibit significant antibacterial activity against *S. aureus* and methicillin-resistant *S. aureus* (MRSA) allowing the following conclusions to be drawn.

Modification of the amide function (compounds **1-9**).

Antibacterial activity data show that the primary amide is necessary for activity, its replacement with isosteric groups or even its functionalization being deleterious. The only exception is represented by compound **7**, bearing a 2-aminoethyl residue at the amide nitrogen. It is worth mentioning that **7**, which is only 10-fold less active than **1**, has a wider antibacterial activity spectrum including also gram-negative bacteria.

Replacement of alkoxyl chain with an alkoxyl chain bearing a teminal polar head

The lack of activity of compounds **10-13** indicate that polar HBA/HBD substituents, such as alcoholic, ethereal and aminic functions, at the end of the long alkyl chain are not only unproductive but also counterproductive for the interaction potential of such hydrophobic flexible chain.

Other substituents at the 3 position (compounds 14-23).

Alternatively to functionalized alkyl chains, rigid or semi-rigid systems have been considered such as regioisomeric pyridodioxane-methyl residues or their opened analogues. Analogously to compounds **10-13**, the presence of basic nitrogen and hydroxyl results in inactive compounds (compounds **16-20**). Significantly, the benzodioxane analogue **14**, in which basic nitrogen and alcoholic oxygens are abolished, regains an activity near to that of **1** and the introduction of a lipophilic substituent such as Cl into the benzodioxane 6 position or better 7 position further increases the antibacterial activity, consistently with what has been observed for compound **PC190723**.

Hybridization between the two singularly beneficial substitutions, namely benzodioxane-methyl at the 3 position and 2-aminoethyl at the amide nitrogen, is

deleterious: compound **15** is inactive. This may indicate that the steric requirements of the benzodioxane are stricter than those of the flexible nonyl chain and its productive positioning prevents the optimal interaction of the aminoethyl moiety.

Removal of both benzodioxane oxygens to give the tetrahydronaphthalene **23** results in complete inactivity. This suggests that one or both oxygens are involved in the interaction. The activity data of chromane **24** may be elucidative of the oxygens role contributing to SAR analysis of such bicyclic system.

Finally, preliminary data for the two enantiomers of **21** address the importance of the chirality of the benzodioxane stereogenic carbon for the binding to FtsZ: only the *S* enantiomer of **21** exhibits antibacterial activity.

Experimental Section

^{1}H NMR were recorded operating at 300 MHz and **^{13}C NMR** at 75 MHz Chemical shifts are given in parts per million relative to residual solvent (CHCl3, DMSO or CH3OH) as internal standard.

Optical rotations were determined by Jasco P-1010 polarimetrer.

Melting points were measured on a Buchi melting point apparatus and are uncorrected.

Analytical TLCs are done utilizing silica gel plats 0,25 mm on aluminum with fluorescence indicator (Macherey-Nagel Alugam® SilG/UV 254). The spots are highlighted under UV at λ=254 nm.

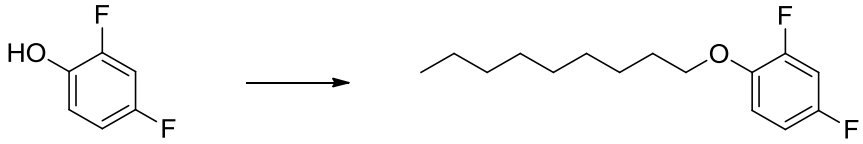
Thermal Analyses were performed on 2-5 mg samples in closed pans at 5°C/ min using a DSC 2010 (TA instruments).

Flash Chromatography Purifications were performed using KP-Sil 32-63 μm 60 Å cartridges and Merck silica gel (particle size 40-63 μm) on a Biotage Flash 12-i instruments.

ACRONIMOUS

nBuLi = normal butyl lithium
$SOCl_2$ = thionyl chloride
TEA = triethylamine
PPh_3 = triphenylphosphine
MEM Cl = 2-methoxyethoxymethyl chloride
DIPEA = diisopropylethylamine
Boc = tert-butoxycarbonyl
TBAB = tetrabutyl ammonium
Dppe = Diphenylphosphinoethane
$TButNH_2$ = tertbutylamine

2,4-Difluoro-1-(nonyloxy)benzene



Chemical Formula: $C_6H_4F_2O$
Molecular Weight: 130,09

Chemical Formula: $C_{15}H_{22}F_2O$
Molecular Weight: 256,33

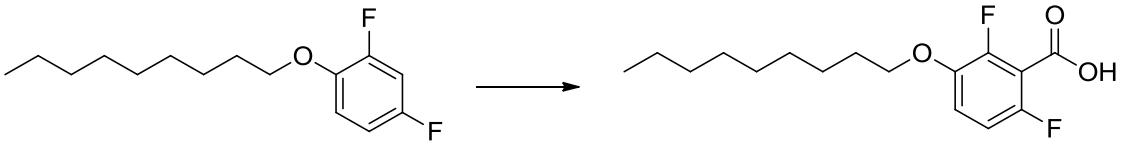
To a suspension of anhydrous K_2CO_3 (13,82 g ; 100 mmol) in 30 ml of acetone a solution of 2,4-difluorophenol (10,0 g, 76,92 mmol) in 40 ml of acetone were added dropwise.
The mixture was stirred for 15 minutes at room temperature and then 15,2 ml of nonylbromide (79,54 mmol) were added dropwise.
After the complete addiction the reaction was refluxed and stirred overnight. The TLC control[107] indicates that the reaction was complete.
The reaction mixture was evaporated under vacuum, the residue was diluted 80 ml of ethyl acetate and 80 ml of water. The two phases formed were separated. The aqueous layer was separated and extracted twice with ethyl acetate (80 ml). The combined organic phases, were washed twice with 80 ml NaOH 1 M and 80 ml of brine, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 19,98 g of crude product as an yellow liquid.
The crude residue were purified by flash chromatography on silica gel (cyclohexane[108]) to give 14,98 g of a colorless liquid corresponding to the desired product

Yield = 76,96 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,83 (m, 3H) 3,97 (t, 2H, J= 6,8 Hz) 1,81 (m, 2H) 1,36 (m, 12H) 0,89 (t, 3H, J= 7 Hz)

[107] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,19 Rf prod= 0,61 Marker: $Ce(SO_4)_2$
[108] Cyclohexane Rf prod= 0,27 Marker: Ce(SO4)2

2,6-Difluoro-3-(nonyloxy)benzoic acid



Chemical Formula: $C_{15}H_{22}F_2O$
Molecular Weight: 256,33

Chemical Formula: $C_{16}H_{22}F_2O_3$
Molecular Weight: 300,34

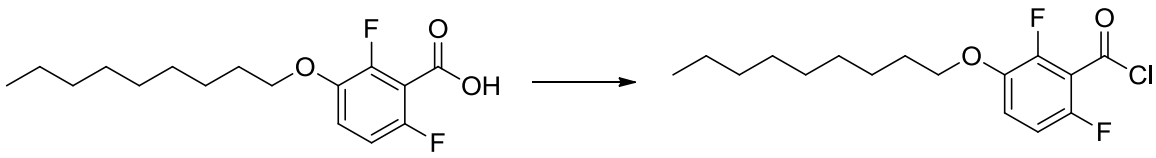
Under nitrogen atmosphere a solution of 2,4-difluoro-1-(nonyloxy)benzene (14,98 g, 58,44 mmol) in 190 ml of dry THF was cooled at -78°C.
After 30 min 26,00 ml of nBuLi (2,7 M in heptanes) was slowly added dropwise. The reaction was keeped at -78°C for 2 h.
At the solution was bubbled anhydrous CO_2; the resulting mixture was maintained at -78°C for 1 h and then brought at room temperature during the night.
After the solution turned into a white suspension and the TLC control[109] revealed that the lithium salt of the carboxylic acid was formed.
The suspension was filtered and the cake washed with 30 ml of THF, giving 14,0 g of moist lithium salt. The volume of the mother liquids was halved under vacuum, the standing at room temperature overnight permitted at the second crop to precipitate. The vacuum filtration give 12,20 g of moist salt.
The lithium salts obtained were combined, dissolved with 80 ml of ethyl acetate and 80 ml of 10% HCl. The aqueous layer was extracted twice with ethyl acetate (80 ml). The organic phases were combined, washed with brine (80 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 15,30 g of desired product as an yellow liquid.

Yield = 87,18%

^{1}H-NMR ($CDCl_3$) δ (ppm): 8,63 (bs, 1H) 7,08 (td, 1H, J= 11,5 J= 6,1 Hz) 6,86 (m, 1H) 4,02 (t, 2H, J= 6,7 Hz) 1,81 (m, 2H) 1,38 (m, 12H) 0,87 (t, 3H, J= 7 Hz).

[109] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,61 Rf prod= 0,07 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(nonyloxy)benzoyl chloride



Chemical Formula: $C_{16}H_{22}F_2O_3$
Molecular Weight: 300,34

Chemical Formula: $C_{16}H_{21}ClF_2O_2$
Molecular Weight: 318,79

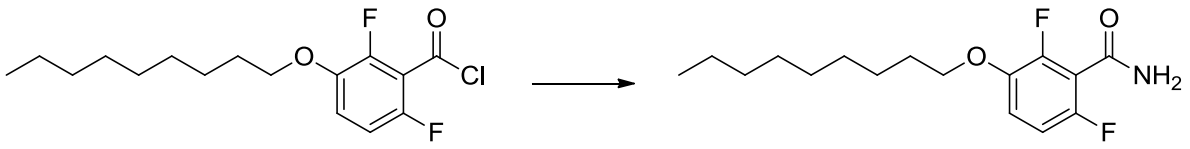
Under nitrogen atmosphere to a solution of 2,6-difluoro-3-(nonyloxy)benzoic acid (4,60 g, 15,31 mmol) in 25 ml of toluene 1,8 ml of $SOCl_2$ were added. The reaction was stirred for 30 minutes and then brought at reflux. After 2h the TLC control[110] showed that the reaction was complete.
The reaction mixture was evaporated under vacuum giving 4,78 g of brownish liquid corresponding at the crude product. The acyl chloride was used without further purifications.

Yield = 97,95%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,06 (td, 1H, J= 11,5 J= 5,9 Hz) 6,85 (m, 1H) 4,04 (t, 2H, J= 6,6 Hz) 1,83 (m, 2H) 1,36 (m, 12H) 0,87 (t, 3H, J= 7 Hz).

[110] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,40 Rf prod= 0,68 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(nonyloxy)benzamide



Chemical Formula: $C_{16}H_{21}ClF_2O_2$
Molecular Weight: 318,79

Chemical Formula: $C_{16}H_{23}F_2NO_2$
Molecular Weight: 299,36

Under nitrogen atmosphere a solution of 2,6-difluoro-3-(nonyloxy)benzoyl chloride (4,78 g, 14,99 mmol) in 20 ml of THF was cooled at 4°C, then 17 ml of 30% ammonia (136 mmol) were added dropwise. The reaction was stirred at 4°C for 30 minutes and then brought at room temperature for the night. The TLC control[111] revealed the complete transformation of the acyl chloride.
The reaction mixture was evaporated under vacuum; after the addition of 10 ml of distillated water a white solid afforded. The suspension was filtered and washed twice with water (10 ml). The moist crystals were dried at 50°C for 3h giving 3,82 g of a white powder corresponding at the desired product.

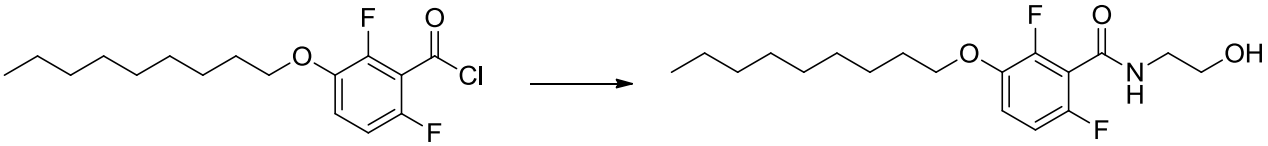
Yield = 83,41%

M.P. = 80,35°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,99 (td, 1H, J= 11,5 J= 6,4 Hz) 6,84 (m, 1H) 6,02 (bs, 2H) 3,96 (t, 2H, J= 6,6 Hz) 1,77 (m, 2H) 1,29 (m, 12H) 0,83 (t, 3H, J= 7 Hz).

[111] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,59 Rf prod= 0,22 Marker: $Ce(SO_4)_2$

2,6-Difluoro-N-(2-hydroxyethyl)-3-(nonyloxy)benzamide



Chemical Formula: $C_{16}H_{21}ClF_2O_2$
Molecular Weight: 318,79

Chemical Formula: $C_{18}H_{27}F_2NO_3$
Molecular Weight: 343,41

Under nitrogen atmosphere a solution of ethanolamine (0,93 g, 15,06 mmol) and 2,10 ml of TEA (15,06 mmol) in 20 ml of DCM was cooled at 4°C, then a solution of 2,6-difluoro-3-(nonyloxy)benzoyl chloride (4,00 g, 12,55 mmol) in 15 ml of DCM was added dropwise. The reaction was stirred for 30 minutes at 4°C and then brought at room temperature for 2 h. The TLC control[112] revealed the complete transformation of the starting material.
The reaction mixture was diluted 20 ml of distilled water. The aqueous layer was extracted twice with DCM (20 ml). The combined organic phases were washed with 10% HCl (50 ml), $NaHCO_3$ saturated solution (50 ml) and brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,40 g of a crude product as a green oil.
The crude mixture were purified by flash chromatography on silica gel (cyclohexane/ ethyl acetate 1/1[113]) to give 2,29 g of white solid corresponding to the desired product.

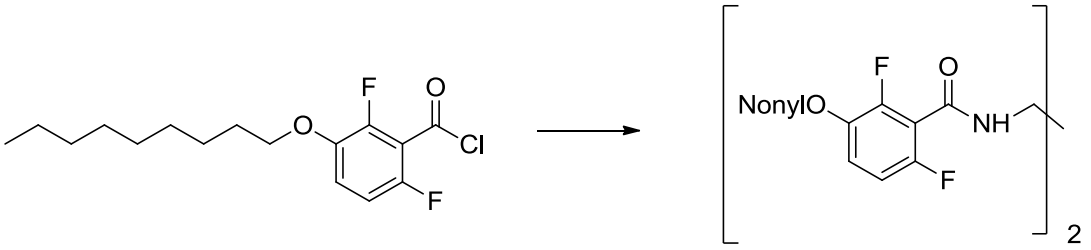
Yield = 53,13%

M.P. = 75,47°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,95 (td, 1H, J= 9 J= 5,1 Hz) 6,83 (t, 1H, J= 9 Hz) 6,47 (bs, 1H) 3,96 (t, 2H, J= 6,6 Hz) 3,83 (m, 2H) 3,63 (m, 2H) 2,22 (bs, 1H) 1,78 (m, 2H) 1,35 (m, 12H) 0,88 (t, 3H, J= 6,9 Hz).

[112] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,59 Rf prod= 0,27 Rf imp= 0,36 Marker: $Ce(SO_4)_2$
[113] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,59 Rf prod= 0,27 Rf imp= 0,36 Marker: $Ce(SO_4)_2$

N,N'-(Ethane-1,2-diyl)bis(2,6-difluoro-3-(nonyloxy)benzamide)



Chemical Formula: $C_{16}H_{21}ClF_2O_2$
Molecular Weight: 318,79

Chemical Formula: $C_{34}H_{48}F_4N_2O_4$
Molecular Weight: 624,75

Under nitrogen atmosphere a solution of ethylendiamine (1,29 g, 21,33 mmol) in 10 ml of DCM was cooled at 4°C, then a solution of 2,6-difluoro-3-(nonyloxy)benzoyl chloride (4,00 g, 12,55 mmol) in 15 ml of DCM was added dropwise. The reaction was stirred for 30 minutes at 4°C and then brought at room temperature overnight. The TLC control[114] revealed the complete transformation of the starting material.
The mixture was quenched with 20 ml of $NaHCO_3$ saturated solution. The separated aqueous layer was extracted twice with DCM (20 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,70 g of a crude product as an orange powder.
The crude material was crystallized by isopropanol gives 0,95 g of the desired product as a white powder.

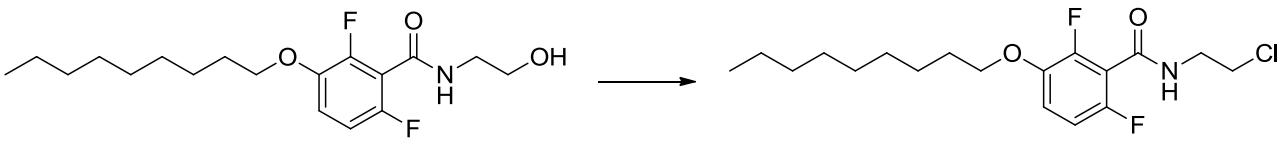
Yield = 12,10%

M.P. = 125,0°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,94 (td, 1H, J= 8,8 J= 5,4 Hz) 6,81 (t, 1H, J= 8,8 Hz) 6,56 (bs, 1H) 3,97 (t, 2H, J= 6,8 Hz) 3,69 (m, 2H) 1,77 (m, 2H) 1,33 (m, 12H) 0,89 (t, 3H, J= 7 Hz).

[114] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,59 Rf prod= 0,48 Marker: $Ce(SO_4)_2$

N-(2-Chloroethyl)-2,6-difluoro-3-(nonyloxy)benzamide



Chemical Formula: $C_{18}H_{27}F_2NO_3$
Molecular Weight: 343,41

Chemical Formula: $C_{18}H_{26}ClF_2NO_2$
Molecular Weight: 361,85

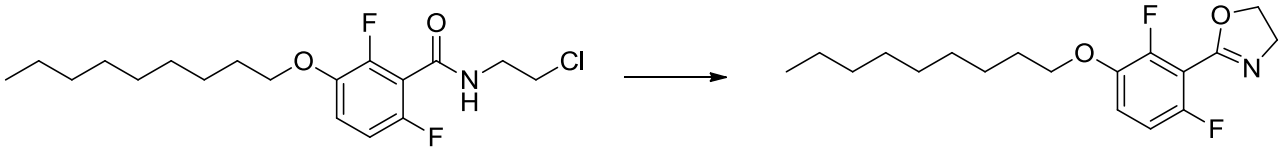
At 2 ml of $SOCl_2$ were added 0,50 g (1,45 mmol) of 2,6-difluoro-N-(2-hydroxyethyl)-3-(nonyloxy)benzamide. The mixture was stirred for 1h and 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[115].
The solvent was evaporated under vacuum and then 10 ml of ethyl acetate and 10 ml of distilled water were added. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with 20 ml of brine, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 380 mg of an yellow oil that crystallize, corresponding at the desired product.

Yield = 72,51%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,98 (td, 1H, J= 8,8 J= 5,1 Hz) 6,85 (t, 1H, J= 8,8 Hz) 6,37 (bs, 1H) 3,99 (t, 2H, J= 6,7 Hz) 3,82 (m, 2H) 3,74 (m, 2H) 1,76 (m, 2H) 1,30 (m, 12H) 0,88 (t, 3H, J= 7 Hz).

[115] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,27 Rf prod= 0,81 Marker: $Ce(SO_4)_2$

2-(2,6-Difluoro-3-(nonyloxy)phenyl)-4,5-dihydrooxazole



Chemical Formula: $C_{18}H_{26}ClF_2NO_2$
Molecular Weight: 361,85

Chemical Formula: $C_{18}H_{25}F_2NO_2$
Molecular Weight: 325,39

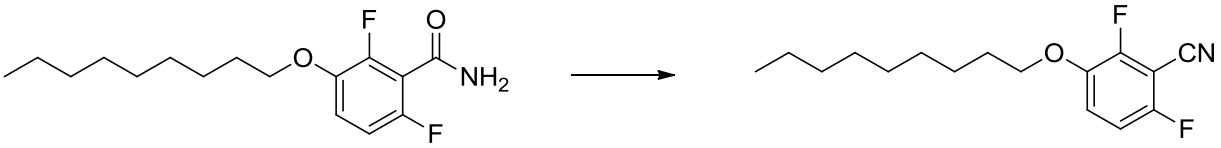
To a solution of N-(2-chloroethyl)-2,6-difluoro-3-(nonyloxy)benzamide (380 mg, 1,05 mmol) in 3,5 ml of 1,2-dichloroethane were added 0,55 ml of TEA. (3,93 mmol). The mixture was stirred for 15 minutes and refluxed overnight.
The control TLC[116] indicated that the starting material was completely transformed.
The reaction mixture was diluted with 6 ml of brine and 6 ml of DCM. The two phases were separated , the aqueous phase was extracted twice with DCM (5 ml). The combined organic phases were washed with brine (5 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 370 mg of a crude product as an brownish oil.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ ethyl acetate 70/30[117]) to give 240 mg of an yellow oil corresponding to the desired product.

Yield = 70,17%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,01 (td, 1H, J= 9 J= 5,1 Hz) 6,83 (t, 1H, J= 9 Hz) 5,46 (t, 2H, J= 11,2) 5,08 (t, 2H, J= 11,2) 3,94 (t, 2H, J= 6,5 Hz) 1,76 (m, 2H) 1,33 (m, 12H) 0,86 (t, 3H, J= 6,9 Hz).

[116] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,41 Rf prod= 0,25 Marker: $Ce(SO_4)_2$
[117] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,41 Rf prod= 0,25 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(nonyloxy)benzonitrile



Chemical Formula: $C_{16}H_{23}F_2NO_2$
Molecular Weight: 299,36

Chemical Formula: $C_{16}H_{21}F_2NO$
Molecular Weight: 281,34

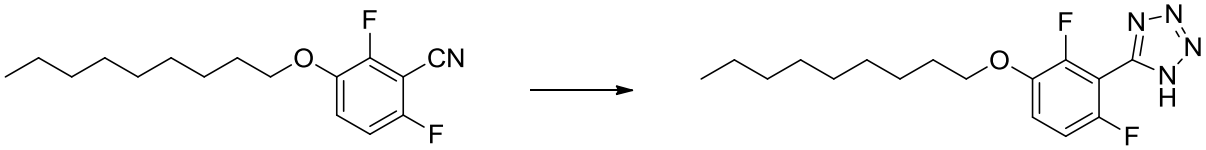
To a solution of 2,6-difluoro-3-(nonyloxy)benzamide (3,80g, 12,69 mmol) in 26 ml of 1,4-dioxane were added 6,65 ml of pyridine. The mixture was stirred for 15 minutes, cooled at 4°C and then 3,35 ml of trifluoroacetic anhydride were added dropwise. The reaction was stirred for 15 minutes at 4°C and then brought at room temperature for 1h e 30 minutes until the complete transformation of the starting material as revealed by TLC[118].
The reaction mixture was cooled at 4°C and diluted with 20 ml of distilled water and 20 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with 10% HCl solution (25 ml), $NaHCO_3$ saturated solution (25 ml) and brine (25 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,40 g of a greenish oil corresponding at the desired product.

Yield = 95,24%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,16 (td, 1H, J= 9,1 J= 5,4 Hz) 6,93 (t, 1H, J= 9,1 Hz) 4,02 (t, 2H, J= 6,5 Hz) 1,80 (m, 2H) 1,42 (m, 12H) 0,88 (t, 3H, J= 6,6 Hz).

[118]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,22 Rf prod= 0,55 Marker: Blue sheet

5-(2,6-Difluoro-3-(nonyloxy)phenyl)-1H-tetrazole



Chemical Formula: $C_{16}H_{21}F_2NO$
Molecular Weight: 281,34

Chemical Formula: $C_{16}H_{22}F_2N_4O$
Molecular Weight: 324,37

To a solution of 2,6-difluoro-3-(nonyloxy)benzonitrile (350mg, 1,25 mmol) in 1,0 ml of water and 1,0 ml of DMF were added 178 mg of NaN_3 (2,75 mmol) and 204 mg of $ZnCl_2$ (1,5 mmol). The mixture was stirred for 15 minutes at room temperature, and then heated at reflux for 5 h until the complete transformation of the starting material as revealed by TLC[119].
The reaction mixture was diluted with 1,0 ml of DMF and then cooled at 4°C. The slowly addiction of 3,0 ml of 10 % HCl let a solid to precipitate. The suspension was filtrated and washed with 10% HCl (2,0 ml) and twice with water (2,5 ml), the solid was dried under vacuum overnight, giving 210 mg of ivory crystal corresponding to the desired product.

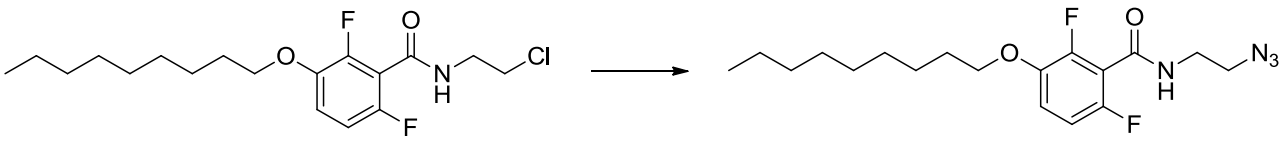
Yield = 52,85%

M.P. = 141,63° C

^{1}H-NMR (d6-DMSO) δ (ppm): 7,47 (td,1H, J= 8,9 J= 5,1 Hz) 7,25 (t, 1H, J= 8,9 Hz) 4,11 (t, 2H, J= 6,9 Hz) 1,78 (m, 2H) 1,34 (m, 12H) 0,86 (t, 3H, J= 6,9 Hz).

[119] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,55 Rf prod= 0,18 Marker: $Ce(SO_4)_2$

N-(2-Azidoethyl)-2,6-difluoro-3-(nonyloxy)benzamide



Chemical Formula: $C_{18}H_{26}ClF_2NO_2$
Molecular Weight: 361,85

Chemical Formula: $C_{18}H_{26}F_2N_4O_2$
Molecular Weight: 368,42

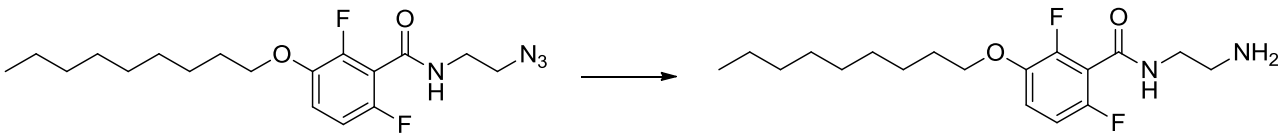
To a solution of N-(2-chloroethyl)-2,6-difluoro-3-(nonyloxy)benzamide (1,59 g, 4,38 mmol) in 12,0 ml of DMF and 4,0 ml of water were added 1,40 g of NaN_3 (21,53 mmol) and 60 mg of NaI (0,4 mmol). The mixture was stirred for 15 minutes at room temperature, and then heated at 65°C for 5 h until the complete transformation of the starting material as revealed by TLC[120].
The reaction was evaporated under vacuum, then diluted with 20 ml of ethyl acetate and 20 ml of water. The separated aqueous phase was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,46 g of an yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ ethyl acetate 70/30[121]) to give 600 mg of an white sponge corresponding to the desired product.

Yield = 37,04%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,95 (td, 1H, J= 9,1 J= 5,3 Hz) 6,86 (t, 1H, J= 9,1 Hz) 6,23 (bs, 1H) 3,97 (t, 2H, J= 6,8 Hz) 3,80 (m, 4H) 1,81 (m, 2H) 1,35 (m, 12H) 0,89 (t, 3H, J= 7 Hz).

[120]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,41 Rf prod= 0,32 Marker: $Ce(SO_4)_2$
[121]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,41 Rf prod= 0,32 Marker: Ce(SO4)2

N-(2-Aminoethyl)-2,6-difluoro-3-(nonyloxy)benzamide



Chemical Formula: $C_{18}H_{26}F_2N_4O_2$
Molecular Weight: 368,42

Chemical Formula: $C_{18}H_{28}F_2N_2O_2$
Molecular Weight: 342,42

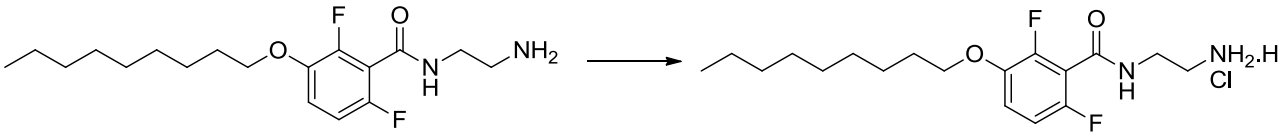
To a solution of N-(2-azidoethyl)-2,6-difluoro-3-(nonyloxy)benzamide (600 mg, 1,62 mmol) in 10,0 ml of THF were added 640 mg of PPh_3 (2,44 mmol) and 1,0 ml of distilled water. The mixture was stirred at room temperature for 2h until the complete transformation of the starting material as revealed by TLC[122].
The reaction mixture was evaporated under vacuum, then diluted with 10 ml of ethyl acetate and 10 ml of water. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,25 g of an yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (DCM/ MeOH 94/6[123]) to give 320 mg of an yellow oil corresponding to the desired product.

Yield = 57,76%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,92 (td, 1H, J= 8,8 J= 5,4 Hz) 6,77 (m, 2H) 3,99 (t, 2H, J= 6,8 Hz) 3,68 (m, 2H) 3,03 (t, 2H, J= 5,8 Hz) 2,40 (bs, 2H) 1,81 (m, 2H) 1,35 (m, 12H) 0,89 (t, 3H, J= 7 Hz).

[122]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,32 Rf prod= 0,05 Marker: $Ce(SO_4)_2$
[123]DCM/ MeOH 94/6 Rf start= 0,88 Rf prod= 0,11 Marker: Ce(SO4)2

N-(2-Aminoethyl)-2,6-difluoro-3-(nonyloxy)benzamide hydrochloride



Chemical Formula: $C_{18}H_{28}F_2N_2O_2$
Molecular Weight: 342,42

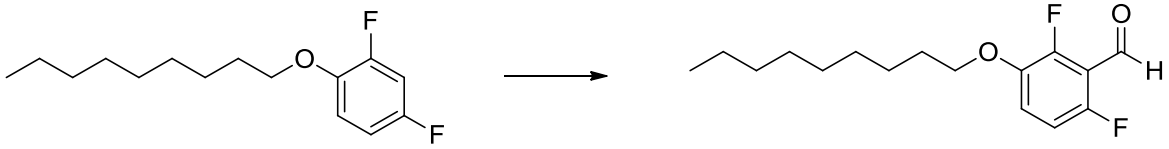
Chemical Formula: $C_{18}H_{29}ClF_2N_2O_2$
Molecular Weight: 378,88

To a solution of N-(2-aminoethyl)-2,6-difluoro-3-(nonyloxy)benzamide (320 mg, 0,93 mmol) in 3,0 ml of MeOH were slowly added 0,75 ml of HCl in MeOH 1,25 M (0,93 mmol). The mixture was stirred at room temperature for 15 minutes until the NMR control revealed that the starting material was completely transformed. The reaction was evaporated under vacuum to give 330 mg of an yellow wax corresponding at the desired product.

Yield = 95,2%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,83 (t, 1H, J=) 7,25 (td, 1H, J= 8,8 J= 5,3 Hz) 7,09 (t, 1H, J= 8,8 Hz) 6,23 (bs, 3H) 4,01 (t, 2H, J= 6,6 Hz) 3,40 (q, 2H, J=) 2,80 (t, 1H, J=) 1,69 (m, 2H) 1,29 (m, 12H) 0,85 (t, 3H, J= 7 Hz).

2,6-Difluoro-3-(nonyloxy)benzaldehyde



Chemical Formula: $C_{15}H_{22}F_2O$
Molecular Weight: 256,33

Chemical Formula: $C_{16}H_{22}F_2O_2$
Molecular Weight: 284,34

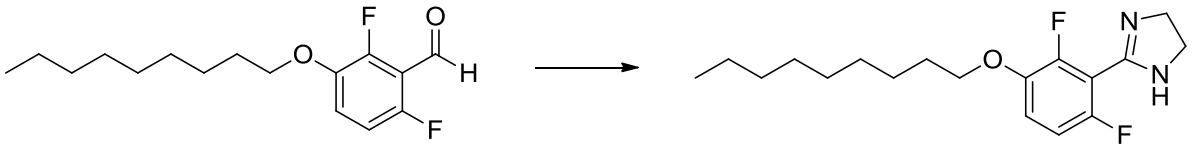
Under nitrogen atmosphere a solution of 2,4-difluoro-1-(nonyloxy)benzene (3,96 g, 15,45 mmol) in 50 ml of dry THF was cooled at -78°C.
After 30 minutes 7,24 ml of nBuLi (2,7 M in heptanes) was slowly added dropwise. The reaction was keeped at -78°C for 1 h.
At the solution were added dropwise 2,40 ml of dry DMF; and maintained at -78°C for 1 h until the complete transformation of the starting material as revealed by TLC[124].
The cooled solution were slowly quenched with 20 ml of distillated water. The reaction mixture was brought to room temperature, and then diluted with 20 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The organic phases were combined, washed with brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 4,33 g of an yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ ethyl acetate 90/10[125]) to give 2,81 g of an yellow liquid corresponding at the desired product.

Yield = 64,00%

^{1}H-NMR ($CDCl_3$) δ (ppm): 10,37 (s, 1H) 7,16 (td, 1H, J= 9,1 J= 5,6 Hz) 6,91 (t, 1H, J= 9,1 Hz) 4,05 (t, 1H, J= 6,6 Hz) 1,82 (m, 2H) 1,35 (m, 12H) 0,87 (t, 1H, J= 7 Hz).

[124] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,61 Rf prod= 0,33 Marker: $Ce(SO_4)_2$
[125] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,61 Rf prod= 0,33 Marker: $Ce(SO_4)_2$

2-(2,6-Difluoro-3-(nonyloxy)phenyl)-4,5-dihydro-1H-imidazole



Chemical Formula: $C_{16}H_{22}F_2O_2$
Molecular Weight: 284,34

Chemical Formula: $C_{18}H_{26}F_2N_2O$
Molecular Weight: 324,41

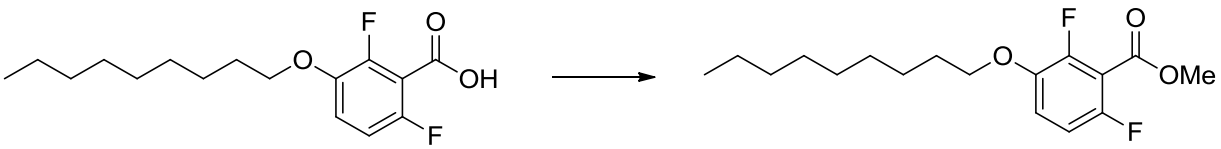
Under nitrogen atmosphere to a solution of 2,6-difluoro-3-(nonyloxy)benzaldehyde (500 mg, 1,77 mmol) in 15 ml of MeOH were added 130 µl of ethylenediamine (1,95 mmol).
After 15 minutes 170 mg of trichloroisocianuric acid (0,71 mmol) were added. The reaction were stirred at room temperature for 15 minutes and then brought at 45°C for 6h until the complete transformation of the starting material as revealed by TLC[126].
The reaction mixture was cooled at 4°C and quenched with 7 ml of saturated solution of $Na_2S_2O_5$. The solution readily transformed into a suspension, that was filtered in vacuum. The mother liquids were evaporated under vacuum and after diluted with 10 ml of water and 10 ml of ethyl acetate. The separated aqueous phase was extracted twice with ethyl acetate (10 ml). The organic phases were combined, washed with $NaHCO_3$ saturated solution (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 509 mg of an yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (DCM/ MeOH 90/10[127]) to give 220 mg of an yellow oil corresponding at the desired product.

Yield = 38,33%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,96 (td, 1H, J= 9 J= 2,1Hz) 6,83 (t, 1H, J= 5,2 Hz) 5,77 (bs, 1H) 3,99 (t, 2H, J= 6,7 Hz) 3,80 (s, 4H) 1,77 (m, 2H) 1,37 (m, 12H) 0,88 (t, 3H, J= 7 Hz).

[126] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,33 Rf prod= 0,05 Marker: $Ce(SO_4)_2$
[127] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,90 Rf prod= 0,21 Marker: $Ce(SO_4)_2$

Methyl 2,6-difluoro-3-(nonyloxy)benzoate



Chemical Formula: $C_{16}H_{22}F_2O_3$
Molecular Weight: 300,34

Chemical Formula: $C_{17}H_{24}F_2O_3$
Molecular Weight: 314,37

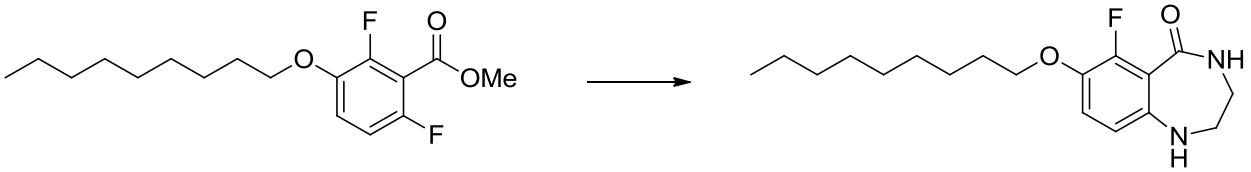
To a solution of 2,4-difluoro-1-(nonyloxy)benzoic acid (1,50 g, 5,00 mmol) in 15 ml of MeOH were added 0,92 ml of trimethylorthoformiate (8,50 mmol) and 0,3 ml of 95-98% H_2SO_4 .
The mixture was stirred for 30 minutes at room temperature and then was brought at reflux for 24 h until the complete transformation of the starting material as revealed by TLC[128].
The reaction mixture was evaporated under vacuum. The resulting residue was diluted with 20 ml of ethyl acetate and washed with $NaHCO_3$ saturated solution (3 x 20 ml). The organic phase was dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,48 g of an yellow liquid corresponding at the desired product.

Yield = 94,26%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,02 (td, 1H, J= 8,8 J= 5,4 Hz) 6,84 (t, 1H, J= 8,8Hz) 3,97 (m, 5H) 1,77 (m, 2H) 1,34 (m, 12H) 0,88 (t, 3H, J= 7 Hz).

[128] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,20 Rf prod= 0,66 Marker: $Ce(SO_4)_2$

6-Fluoro-7-(nonyloxy)-3,4-dihydro-1H-benzo[e][1,4]diazepin-5(2H)-one



Chemical Formula: $C_{17}H_{24}F_2O_3$
Molecular Weight: 314,37

Chemical Formula: $C_{18}H_{27}FN_2O_2$
Molecular Weight: 322,42

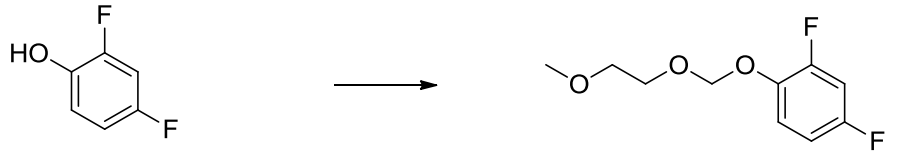
To a solution of methyl 2,6-difluoro-3-(nonyloxy)benzoate (1,48 g, 4,71 mmol) in 10,2 ml of EtOH abs were added 3,19 ml of ethylendiamine (47,7 mmol).
The mixture was stirred for 30 minutes at room temperature and then was brought at reflux for 6 h until the complete transformation of the starting material as revealed by TLC[129].
The reaction mixture was evaporated under vacuum and diluted with 15 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (15 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,50 g of crude product as an yellow liquid.
The crude product were purified by flash chromatography on silica gel (ethyl acetate[130]) to give 470 mg of an oil that crystallize corresponding at the desired product.

Yield = 28,65%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,85 (bs, 1H) 6,75 (dd, 1H, J= J=) 6,45 (t, 1H, J=) 4,8 (bs, 1H) 3,92 (t, 2H, J= 6,7 Hz) 3,63 (m, 2H) 3,5 (m, 2H) 1,76 (m, 2H) 1,33 (m, 12H) 0,87 (t, 3H, J= 7 Hz).

[129] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,66 Rf prod= 0,08 Marker: $Ce(SO_4)_2$
[130] Ethyl Acetate Rf prod= 0,24 Marker: $Ce(SO_4)_2$

2,4-Difluoro-1-((2-methoxyethoxy)methoxy)benzene



Chemical Formula: $C_6H_4F_2O$
Molecular Weight: 130,09

Chemical Formula: $C_{10}H_{12}F_2O_3$
Molecular Weight: 218,20

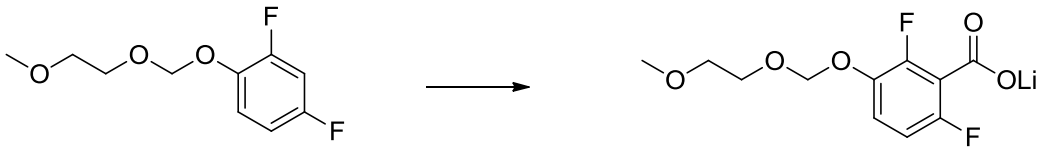
To a suspension of anhydrous K_2CO_3 (13,82 g ; 100 mmol) in 30 ml acetone were added dropwise a solution of 2,4-difluorophenol (10,0 g, 76,92 mmol) in 40 ml of acetone.
The mixture was stirred for 15 minutes at room temperature and then 15,2 ml of MEM Cl (73,07 mmol) were slowly added dropwise.
After the complete addiction the reaction was brought to reflux for 1h until the complete transformation of the starting material as revealed by TLC[131].
The reaction mixture was evaporated under vacuum, and diluted with 80 ml of ethyl acetate and 80 ml of water. The separated aqueous layer was extracted twice with ethyl acetate (80 ml). The organic phases were combined, washed twice with NaOH 1 M (80 ml) and brine (80 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 15,60 g of a transparent liquid corresponding at the desired product.

Yield = 87,6%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,20 (ddd, 1H, J=14,5 J=10,4 J=3,2 Hz) 6,81 (m, 2H) 5,24 (s, 2H) 3,88 (m, 2H) 3,56 (m, 2H) 3,38 (s, 3H)

[131] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,19 Rf prod= 0,26 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-((2-methoxyethoxy)methoxy)benzoic acid lithium salt



Chemical Formula: $C_{10}H_{12}F_2O_3$
Molecular Weight: 218,20

Chemical Formula: $C_{11}H_{11}F_2LiO_5$
Molecular Weight: 268,14

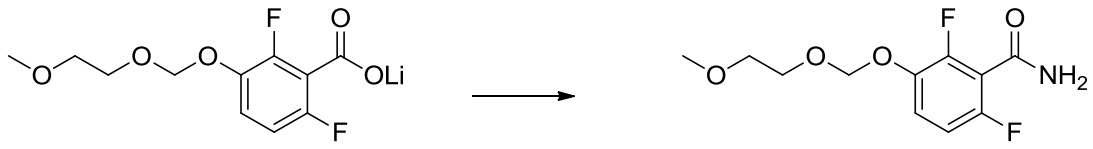
Under nitrogen atmosphere A solution of 2,4-difluoro-1-((2-methoxyethoxy)methoxy)benzene (9,90 g, 45,62 mmol) in 90 ml of dry THF was cooled at -78°C.
After 30 min 17,5 ml of nBuLi (2,7 M in heptanes) was slowly added dropwise. The reaction was keeped at -78°C for 2 h.
At the solution was bubbled anhydrous CO_2; the resulting mixture was maintained at -78°C for 1 h and then brought at room temperature during the night.
The solution turned into a white suspension and the TLC control[132] revealed that the lithium salt of the carboxylic acid was formed.
The suspension was filtered in vacuum, giving 8,59 g of lithium salt. The volume of the mother liquids was halved under vacuum, the standing at room temperature overnight permitted at the second crop to precipitate. The vacuum filtration give 1,30 g of lithium salt.
The lithium salts obtained were utilized for the next step without purifications.

Yield = 80,9%

^{1}H-NMR (d6-DMSO) δ (ppm): 6,94 (td, 1H, J= 9,1 J= 5,4 Hz) 6,77 (m, 1H) 5,21 (s, 2H) 3,73 (m, 2H) 3,45 (m, 2H) 3,20 (s, 3H)

[132] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,39 Rf prod= 0,07 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-((2-methoxyethoxy)methoxy)benzamide



Chemical Formula: $C_{11}H_{11}F_2LiO_5$
Molecular Weight: 268,14

Chemical Formula: $C_{11}H_{13}F_2NO_4$
Molecular Weight: 261,22

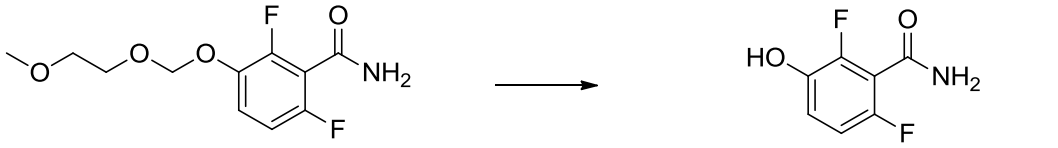
To a solution of 2,6-difluoro-3-((2-methoxyethoxy)methoxy)benzoic acid lithium salt (7,80 g, mmol) in 50 ml of distilled water were added 30 ml of DCM and 10% HCl until pH 2. The separated aqueous layer was extracted twice with DCM (30 ml). The combined organic phases were washed twice with brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 7,60 g of an colorless oil corresponding at the carboxylic acid.
The acid was dissolved in 76 ml of DCM and keeped under nitrogen atmosphere.
At the solution were added 5,64 ml of DIPEA (31,90 mmol); the resulting mixture was cooled at 4°C and then 2,61 ml of ethylchloroformate (31,90 mmol) were added dropwise. The mixture was maintained at 4°C for 1 h until the complete transformation of the starting material in the mix anhydride was revealed by TLC[133].
At the cooled reaction 20 ml of 30% ammonia (≈160 mmol) were added dropwise, keeped at 4°C for 15 minutes and then brought at room temperature for 1 h until the complete transformation of the starting material as revealed by TLC[134].
The reaction mixture were diluted with water (40 ml). The separated organic layer was washed with 10% HCl (60 ml) of, $NaHCO_3$ saturated solution (60 ml) and brine (60 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 5,49 g of a colorless oil corresponding at the desired product.

Yield = 72,5%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,31 (m, 1H) 6,87 (td, 1H, J= 9,1 J= 5,4 Hz) 6,02 (bs, 2H) 5,23 (s, 2H) 3,85 (m, 2H) 3,57 (m, 2H) 3,38 (s, 3H)

[133] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,10 Rf anhy= 0,47 Marker: $Ce(SO_4)_2$
[134] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,10 Rf prod= 0,16 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-hydroxybenzamide



Chemical Formula: $C_{11}H_{13}F_2NO_4$
Molecular Weight: 261,22

Chemical Formula: $C_7H_5F_2NO_2$
Molecular Weight: 173,12

To a solution of 2,6-difluoro-3-((2-methoxyethoxy)methoxy)benzamide (5,49 g, mmol) in 55 ml of MeOH 30 ml of 10% HCl solution were added dropwise. The reaction was stirred for 15 minutes at room temperature and then brought at 55°C for 1h until the complete transformation of the starting material as revealed by TLC[135].
The reaction was evaporated under vacuum, and diluted with 20 ml of ethyl acetate and 20 ml of water. The separated aqueous phase was extracted with ethyl acetate (3 x 20ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 5,20 g of a colorless oil corresponding at the crude product.
The treatment with 30 ml of DCM gives a white solid. The suspension was filtrated under vacuum giving 2,20 g of a white solid corresponding at the desired product.

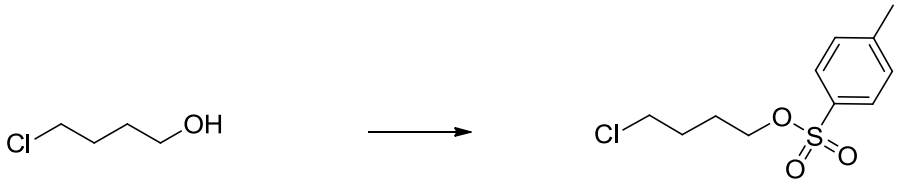
Yield = 60,4%

M.P. = 125,58°C

^{1}H-NMR (d6-DMSO) δ (ppm): 9,89 (s, 1H) 8,03 (bs, 1H) 7,73 (bs, 1H) 6,93 (m, 2H).

[135] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,16 Rf prod= 0,14 Marker: $Ce(SO_4)_2$

4-Chlorobutyl 4-methylbenzenesulfonate



Chemical Formula: C_4H_9ClO
Molecular Weight: 108,57

Chemical Formula: $C_{11}H_{15}ClO_3S$
Molecular Weight: 262,75

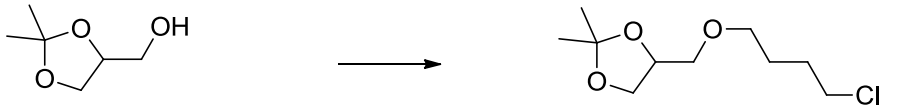
To an ice cooled solution of tosyl chloride (2,10g, 10 mmol) in 2 ml of pyridine a solution of 4-chloro-1-butanol (1,08 g, 10 mmol) in 1,0 ml of pyridine were added dropwise. the reaction was stirred for 15 minutes at 4°C and then brought at room temperature for 1h and 30 minutes until the complete transformation of the starting material as revealed by TLC[136].
The reaction mixture was diluted with 30 ml of ethyl acetate and 30 ml of 10% HCl . The separated organic layer were washed twice with 10% HCl (30ml) and brine (30 ml) of, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,74 g of a colorless oil corresponding at the crude product.
The crude product was purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[137]) to give 1,09 g of a colorless oil corresponding at the desired product.

Yield = 41,05%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,78 (d, 2H, J= 8,8 Hz) 7,36 (d, 2H, J= 8,8 Hz) 4,05 (t, 2H, J= 6,5 Hz) 3,48 (t, 2H, J= 6,5 Hz) 2,44 (s, 3H) 1,82 (m, 4H).

[136] Cyclohexane/ Ethyl Acetate 90/10 Rf TsCl= 0,38 Rf prod= 0,18 Marker: Blue sheet
[137] Cyclohexane/ Ethyl Acetate 90/10 Rf TsCl= 0,38 Rf prod= 0,18 Marker: Blue sheet

4-((4-Chlorobutoxy)methyl)-2,2-dimethyl-1,3-dioxolane



Chemical Formula: $C_6H_{12}O_3$
Molecular Weight: 132,16

Chemical Formula: $C_{10}H_{19}ClO_3$
Molecular Weight: 222,71

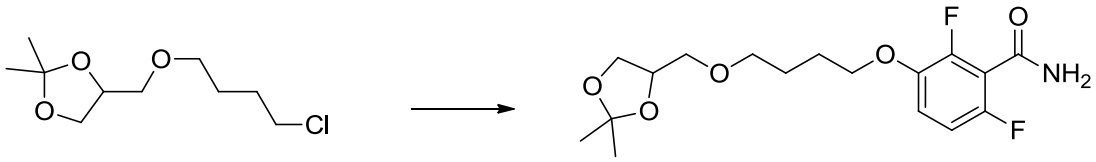
Under nitrogen atmosphere to a suspension of NaH (109 mg, 4,56 mmol) in 5,0 ml of dry THF a solution of racemic solketal (0,60g, 4,56 mmol) in 5,0 ml of dry THF was added dropwise. The mixture was stirred for 30 minutes at room temperature, a solution of 4-chlorobutyl 4-methylbenzenesulfonate (1,09g, 4,15 mmol) in 5,0 ml of dry THF then were added dropwise. The reaction was stirred for 30 minutes at room temperature and then brought at reflux for 4h until the complete transformation of the starting material as revealed by TLC[138].
The reaction was cooled at 4°C and then quenched dropwise with 10 ml of distilled water. The mixture was extracted with ethyl acetate (3 x 10 ml). The separated organic phase was dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,00g of a yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[139]) to give 480 mg of a colorless liquid corresponding at the desired product.

Yield = 52,17%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,27(q, 1H, J=5,1 Hz) 4,06 (dd, 1H, J= 8,2 J= 6,6 Hz) 3,73 (dd, 1H, J= 8,2 J=6,6 Hz) 3,51 (m, 5H) 1,85 (m, 2H) 1,62 (m, 2H) 1,41 (s, 3H) 1,37 (s, 3H).

[138] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,25 Rf solk= 0,06 Rf prod= 0,36 Marker: Blue sheet
[139] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,18 Rf solk= 0,04 Rf prod= 0,21 Marker: Blue sheet

3-(4-((2,2-Dimethyl-1,3-dioxolan-4-yl)methoxy)butoxy)-2,6-difluorobenzamide



Chemical Formula: $C_{10}H_{19}ClO_3$
Molecular Weight: 222,71

Chemical Formula: $C_{17}H_{23}F_2NO_5$
Molecular Weight: 359,37

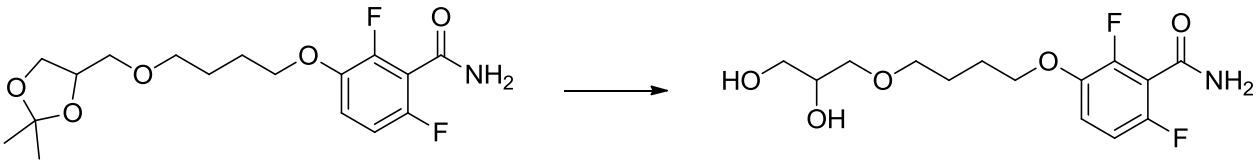
To a solution of 2,6-difluoro-3-hydroxybenzamide (374 mg, 2,16 mmol) in 3,0 ml of DMF were added 448 mg of anhydrous K_2CO_3 (3,24 mmol) and 484 mg of NaI (3,24 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 4-((4-chlorobutoxy)methyl)-2,2-dimethyl-1,3-dioxolane (480mg, 2,16 mmol) in 2,0 ml of DMF were added dropwise. The reaction was stirred for 30 minutes at room temperature and then brought at 70°C for 8h until the complete transformation of the starting material as revealed by TLC[140].
The reaction mixture was evaporated under vacuum and diluted 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with NaOH 1 M (15 ml) and brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,82 g of a yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[141]) to give 470 mg of a colorless liquid corresponding at the desired product.

Yield = 60,56%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,98 (td, 1H, J= 8,8 J= 5,1 Hz) 6,86 (td, 1H, J= 8,8 J= 2,4Hz) 6,03 (bs, 1H) 5,90 (bs, 1H) 4,24 (q, 1H, J= 5,1 Hz) 4,05 (m, 2H) 3,71 (dd, 1H, J= 8,2 J= 6,6 Hz) 3,47 (m, 4H) 1,78 (m, 4H) 1,40 (s, 3H) 1,36 (s, 3H).

[140] Cyclohexane/ Ethyl Acetate 1/1 Rf amid= 0,14 Rf inter= 0,60 Rf prod= 0,36 Marker: $Ce(SO_4)_2$
[141] Cyclohexane/ Ethyl Acetate 1/1 Rf amid= 0,14 Rf inter= 0,60 Rf prod= 0,36 Marker: $Ce(SO_4)_2$

3-(4-(2,3-Dihydroxypropoxy)butoxy)-2,6-difluorobenzamide



Chemical Formula: $C_{17}H_{23}F_2NO_5$
Molecular Weight: 359,37

Chemical Formula: $C_{14}H_{19}F_2NO_5$
Molecular Weight: 319,30

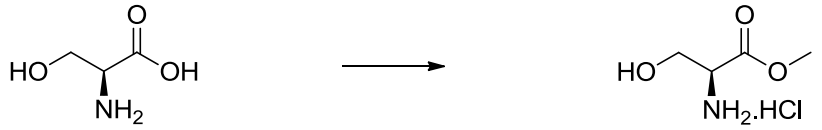
To a solution of 3-(4-((2,2-dimethyl-1,3-dioxolan-4-yl)methoxy)butoxy)-2,6-difluorobenzamide (470 mg, 1,30 mmol) in 2,5 ml of MeOH were added 2,5 ml of HCl 10%. The mixture was stirred for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[142].
The reaction was evaporated under vacuum and diluted with 5 ml of distilled water. The aqueous layer was extracted with ethyl acetate (4 x 5 ml), the separated organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 300 mg of a transparent oil corresponding at the desired product.

Yield = 93,90%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,02 (td, 1H, J= 8,8 J= 5,1Hz) 6,86 (td, 1H, J= 8,8 J= 2,3Hz) 6,43 (bs, 1H) 6,18 (bs, 1H) 4,08 (m, 2H) 3,84 (q, 1H, J= 5,1 Hz) 3,52 (m, 4H) 1,82 (m, 4H).

[142] Ethyl Acetate Rf start= 0,57 Rf prod= 0,12 Marker: $Ce(SO_4)_2$

(S)-Methyl 2-amino-3-hydroxypropanoate hydrochloride



Chemical Formula: $C_3H_7NO_3$
Molecular Weight: 105,09

Chemical Formula: $C_4H_{10}ClNO_3$
Molecular Weight: 155,58

To a 4°C cooled solution of L-serine (4,20 g, 40 mmol) in 20 ml of MeOH, 6,0 ml of $SOCl_2$ (84 mmol)were slowly added dropwise. The reaction mixture was stirred for 30 minutes at 4°C and then brought at room temperature overnight.
The NMR control revealed the complete transformation of the starting material.
The mixture was poured in 80 ml of 4°C cooled ethyl ether; a white solid afforded.
The suspension was filtered and the cake washed with 40 ml of cold ethyl ether, to give 5,50 g of a white flaked powder.

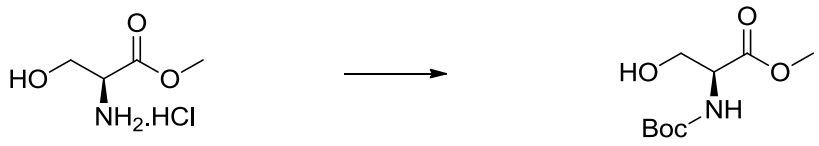
$[\alpha]^{25}_{D} =$ + 1,17 (c = 1,0; water)

Yield = 89,42%

M.P. = 164,62°C

^{1}H-NMR (d6-DMSO) δ (ppm): 8,47 (bs, 3H) 5,58 (bs, 1H) 4,12 (m, 1H) 3,79 (m, 2H) 3,74 (s, 3H)

(S)-Methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate



Chemical Formula: $C_4H_{10}ClNO_3$
Molecular Weight: 155,58

Chemical Formula: $C_9H_{17}NO_5$
Molecular Weight: 219,23

To a solution of (S)-methyl 2-amino-3-hydroxypropanoate hydrochloride (2,61g, 16,97 mmol) in 5,30 ml of THF were added 21 ml of saturated solution of $NaHCO_3$ and after 4,63 g (21,21 mmol) of tert-butoxycarbonyl anhydride. The mixture was stirred overnight at room temperature.
The TLC control[143] revealed the complete transformation of the starting material.
The reaction mixture was poured in 50 ml of distilled water and extracted with ethyl acetate (3 x 40 ml). The organic phase were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,93 g of a transparent liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[144]) to give 3,52 g of a colorless liquid corresponding at the desired product.

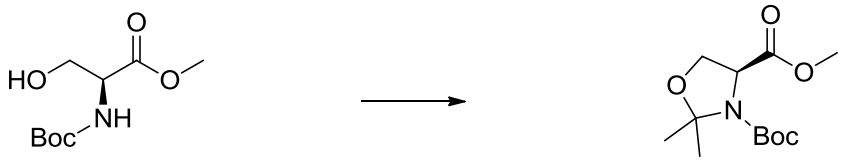
$[\alpha]^{25}_{D\,=}$ + 9,95 (c = 1,09; $CHCl_3$)

Yield = 94,61%

^{1}H-NMR ($CDCl_3$) δ (ppm): 5,43 (bs, 1H) 4,38 (m, 1H) 3,92 (m, 2H) 3,79 (s, 3H) 1,43 (s, 9H).

[143] Cyclohexane/Ethyl Acetate 1/1 Rf start= 0,05 Rf prod= 0,30 Marker: Blue sheet
[144] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,04 Rf prod= 0,25 Marker: Blue sheet

(S)-3-Tert-butyl 4-methyl 2,2-dimethyloxazolidine-3,4-dicarboxylate



Chemical Formula: $C_9H_{17}NO_5$
Molecular Weight: 219,23

Chemical Formula: $C_{12}H_{21}NO_5$
Molecular Weight: 259,30

Under nitrogen atmosphere to a solution of (S)-methyl 2-((tert-butoxycarbonyl)amino)-3-hydroxypropanoate (3,53 g, 16,06 mmol) in 61,5 ml of acetone 18,65 ml of 2,2-dimethoxypropane and 0,11 ml of $BF_3.Et_2O$ (0,85 mmol) were added. The reaction was stirred for 4h at room temperature until the complete transformation of the starting material as revealed by TLC [145] .
The reaction was evaporated under vacuum and then diluted 60 ml of DCM. The organic phase was washed with $NaHCO_3$ saturated solution(40 ml) and brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 4,85 g of a yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[146]) to give 3,05 g of a yellow liquid corresponding at the desired product

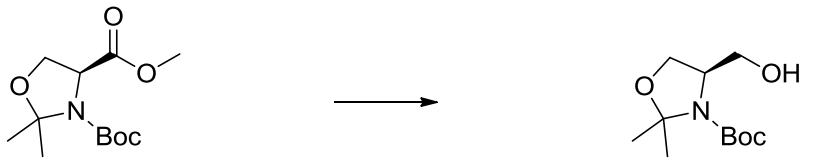
$[\alpha]^{25}_{D\,=}$ - 44,8 (c = 1,08; $CHCl_3$)

Yield = 73,24%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,43 (m, 1H) 4,16 (m, 1H,) 4,04 (m, 1H) 3,76 (s, 3H)

[145] Cyclohexane/Ethyl Acetate 1/1 Rf start= 0,30 Rf prod= 0,57 Marker: Blue sheet
[146] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,10 Rf prod= 0,33 Rf imp= 0,53 Marker: Blue sheet

(R)-Tert-butyl 4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate



Chemical Formula: $C_{12}H_{21}NO_5$
Molecular Weight: 259,30

Chemical Formula: $C_{11}H_{21}NO_4$
Molecular Weight: 231,29

Under nitrogen atmosphere to a 4°C cooled suspension of $LiAlH_4$ (1,05 g, 27,01 mmol) in 47 ml of dry THF a solution of (S)-3-tert-butyl 4-methyl 2,2-dimethyloxazolidine-3,4-dicarboxylate (4,70 g, 18,12 mmol) in 24 ml of dry THF were slowly added dropwise. The reaction was stirred at 4°C for 30 minutes and then brought at room temperature for other 30 minutes until the complete transformation of the starting material as revealed by TLC[147].
The reaction was cooled at -10°C, then quenched with 6,3 ml of 10% NaOH. The mixture was filtered under a Celite® bed, the cake was washed with ethyl acetate (40 ml) and water (30 ml). The separated organic layer was washed with 50 ml of NaH_2PO_4/Na_2HPO_4 pH 7buffer, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,54 g of a yellow oil corresponding at the desired product.

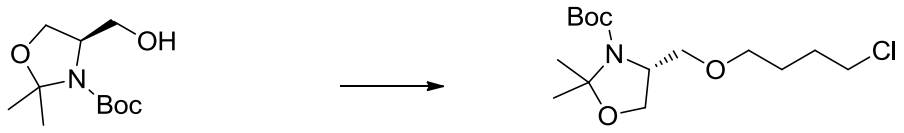
$[\alpha]^{25}_D$ = - 23,72 (c = 0,92; $CHCl_3$)

Yield = 84,46%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,04 (m, 2H) 3,67 (m, 3H) 1,56 (m, 15H).

[147] Cyclohexane/Ethyl Acetate 1/1 Rf start= 0,60 Rf prod= 0,36 Rf impur= 0,42 Marker: Blue sheet

(R)-Tert-butyl 4-((4-chlorobutoxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate



Chemical Formula: $C_{11}H_{21}NO_4$
Molecular Weight: 231,29

Chemical Formula: $C_{15}H_{28}ClNO_4$
Molecular Weight: 321,84

Under nitrogen atmosphere to a suspension of NaH (210 mg, 9,55 mmol) in 5,0 ml of dry THF and 4,5 ml of dry DMF a solution of (R)-tert-butyl 4-(hydroxymethyl)-2,2-dimethyloxazolidine-3-carboxylate (2,21 g, 9,55 mmol) in 9 ml of dry THF was added dropwise. The mixture was stirred for 15 minutes at room temperature, then were added dropwise a solution of 4-chlorobutyl 4-methylbenzenesulfonate (1,82 g, 9,55 mmol) in 5 ml of dry THF. The reaction was stirred for 1 h at room temperature and then brought at 50°C for 1h e 30 minutes until the complete transformation of the starting material as revealed by TLC[148].
The reaction was cooled at 4°C and quenched with 10 ml of distilled water. The reaction mixture was evaporated under vacuum, then was diluted with 40 ml of water and extracted with ethyl acetate (3 x 40 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,61g of a yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[149]) to give 950 mg of a colorless liquid corresponding at the desired product.

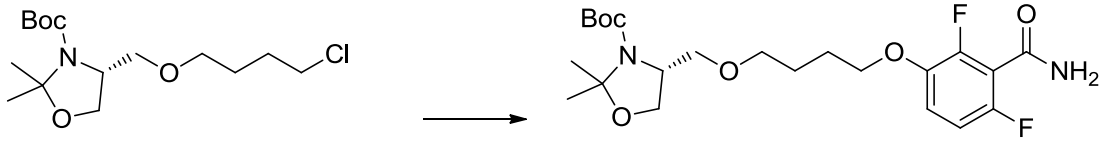
$[\alpha]^{25}_D$ = - 33,75 (c = 0,85; $CHCl_3$)

Yield = 30,94%

^{1}H-NMR ($CDCl_3$) δ (ppm): 3,96 (m, 2H) 3,51 (m, 3H) 3,26 (m,1H) 1,86 (m, 2H) 1,70 (m, 2H) 1,51 (m, 15H).

[148] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,06 Rf prod= 0,25 Rf imp=0,70 Marker: Blue sheet
[149] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,06 Rf prod= 0,25 Rf imp=0,70 Marker: Blue sheet

(R)-Tert-butyl 4-((4-(3-carbamoyl-2,4-difluorophenoxy)butoxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate



Chemical Formula: $C_{15}H_{28}ClNO_4$
Molecular Weight: 321,84

Chemical Formula: $C_{22}H_{32}F_2N_2O_6$
Molecular Weight: 458,50

To a solution of 2,6-difluoro-3-hydroxybenzamide (560 mg, 3,24 mmol) in 3,5 ml of DMF were added 611 mg of anhydrous K_2CO_3 (4,42 mmol) and 660 mg of NaI (4,42 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of (R)-tert-butyl 4-((4-chlorobutoxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate (950mg, 2,95 mmol) in 3,75 ml of DMF were added dropwise. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C for 6h until the complete transformation of the starting material as revealed by TLC[150].

The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,88 g of a yellow liquid corresponding at the crude product.

The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[151]) to give 920 mg of a colorless liquid corresponding at the desired product.

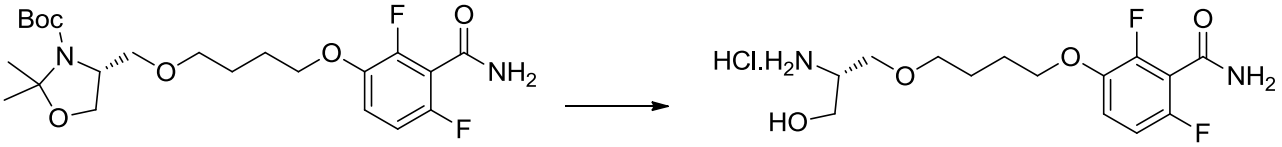
$[\alpha]^{25}_{D}$ = - 19,42 (c = 1,09; $CHCl_3$)

Yield = 68,14%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,98 (td, 1H, J= 8,9 J= 5,4 Hz) 6,84 (td, 1H, J= 8,9 J= 2,1 Hz) 6,04 (bs, 1H) 5,83 (bs, 1H) 4,05 (t, 2H, J= 6,5 Hz) 3,94 (m, 2H) 3,57 (m, 3H) 3,35 (t, 1H, J= 8,6 Hz) 1,84 (m, 2H) 1,67 (m, 2H) 1,51 (m, 15H).

[150] Cyclohexane/ Ethyl Acetate 70/30 Rf amid= 0,04 Rf inter= 0,61 Rf prod= 0,10 Marker: $Ce(SO_4)_2$
[151] Cyclohexane/ Ethyl Acetate 60/40 Rf amid= 0,09 Rf prod= 0,23 Marker: $Ce(SO_4)_2$

(S)-3-(4-(2-Amino-3-hydroxypropoxy)butoxy)-2,6-difluorobenzamide hydrochloride



Chemical Formula: $C_{22}H_{32}F_2N_2O_6$
Molecular Weight: 458,50

Chemical Formula: $C_{14}H_{21}ClF_2N_2O_4$
Molecular Weight: 354,78

To a solution of (R)-tert-butyl 4-((4-(3-carbamoyl-2,4-difluorophenoxy)butoxy)methyl)-2,2-dimethyloxazolidine-3-carboxylate (410 mg, 0,89 mmol) in 3 ml of EtOH abs were added 3 ml of HCl 10%. The mixture was stirred overnight at room temperature.
The TLC control[152] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and the excess of water was removed by azeotropic distillation with EtOH to give 300 mg of a white wax corresponding at the desired product.

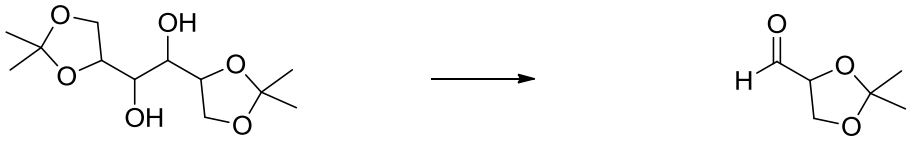
$[\alpha]^{25}_{D}$ = - 5,63 (c = 0,98; water)

Yield = 95,23%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,12 (s, 1H) 8,03 (bs, 3H) 7,81 (s, 1H) 7,22 (td, 1H, J= 9 J= 5,4 Hz) 7,06 (td, 1H, J= 9 J= 2,3 Hz) 4,10 (t, 2H, J= 6,4 Hz) 3,48 (m, 6H) 3,22 (m, 1H) 1,73 (m, 4H).

[152] Ethyl Acetate Rf start= 0,55 Rf prod= 0,06 Marker: $Ce(SO_4)_2$

2,2-Dimethyl-1,3-dioxolane-4-carbaldehyde



Chemical Formula: $C_{12}H_{22}O_6$
Molecular Weight: 262,30

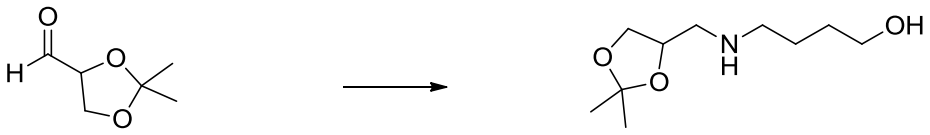
Chemical Formula: $C_6H_{10}O_3$
Molecular Weight: 130,14

To a solution of mannitol diacetonide (2,50g, 9,53 mmol) in 22 ml of MeOH and 68 ml of water were added 2,65 g of $NaIO_4$ (12,40 mmol). The mixture was stirred for 1 h at room temperature.
The control NMR revealed the complete transformation of the starting material.
The reaction was extracted with DCM (4 x 30 ml), the separated organic phase was dried over Na_2SO_4 anhydrous, filtered and evaporated at 300 mbar to give 2,40 g of a transparent liquid corresponding at the desired product.

Yield = 96,77%

^{1}H-NMR ($CDCl_3$) δ (ppm): 9,76 (s, 1H) 4,41 (ddd, 1H, J= 7,5 J= 4,8 J= 1,8 Hz) 4,20 (dd, 1H, J= 8,8 J= 7,5 Hz) 4,12 (dd, 1H, J= 8,8 J= 4,8 Hz) 1,51 (s, 3H) 1,44 (s, 3H).

4-(((2,2-Dimethyl-1,3-dioxolan-4-yl)methyl)amino)butan-1-ol



Chemical Formula: $C_6H_{10}O_3$
Molecular Weight: 130,14

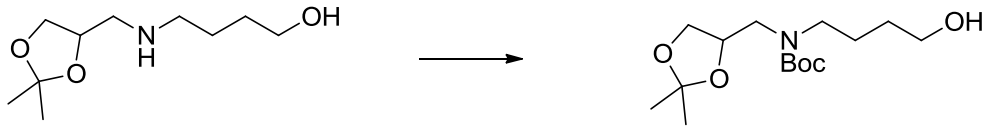
Chemical Formula: $C_{10}H_{21}NO_3$
Molecular Weight: 203,28

To a solution of 2,2-dimethyl-1,3-dioxolane-4-carbaldehyde (2,40g, 18,44 mmol) in 50 ml of MeOH added 1,64g of 1-amino-4-butanol (18,40 mmol). The mixture was stirred for 30 minutes at room temperature and then 0,25 g of Pd/C at 5% were added and hydrogenated at 3,5 atm overnight.
The control NMR revealed the complete transformation of the starting material.
The catalyzer was filtered and the mother liquids were evaporated under vacuum to give 2,67 g of a transparent liquid corresponding at the desired product.

Yield = 69,66%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,27 (m, 1H) 4,09 (m, 1H) 3,63 (m, 3H) 2,70 (m, 4H) 1,64 (m, 4H) 1,43 (s, 3H) 1,34 (s, 3H).

Tert-butyl ((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)(4-hydroxybutyl)carbamate



Chemical Formula: $C_{10}H_{21}NO_3$
Molecular Weight: 203,28

Chemical Formula: $C_{15}H_{29}NO_5$
Molecular Weight: 303,39

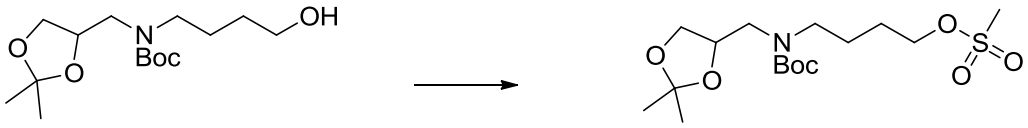
To a solution of 4-(((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)amino)butan-1-ol (2,16 g, 10,58 mmol) in 15 ml of MeOH were added 1,46 ml of TEA (10,58 mmol) and a solution of tert-butoxycarbonyl anhydride (2,31 g, 10,58 mmol) in 15 ml of MeOH. The mixture was stirred for 3 h at room temperature until the complete transformation of the starting material as revealed by TLC[153].
The reaction mixture was evaporated under vacuum and after diluted with 50 ml of ethyl acetate. The organic phase was washed with water (20 ml), HCl 10% (20 ml) and $NaHCO_3$ saturated solution (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,96 g of a transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[154]) to give 1,46 g of a colorless liquid corresponding at the desired product.

Yield = 45,48%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,24 (m, 1H) 4,04 (t, 1H, J= 5,7 Hz) 3,63 (m, 3H) 3,31 (t, 2H, J=) 1,60 (m, 6H) 1,45 (m, 12H) 1,35 (s, 3H).

[153] Cyclohexane/Ethyl Acetate 1/1 Rf start= 0,07 Rf prod= 0,23 Marker: Blue sheet
[154] Cyclohexane/Ethyl Acetate 1/1 Rf start= 0,07 Rf prod= 0,23 Marker: Blue sheet

4-((Tert-butoxycarbonyl)((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)amino)butyl methanesulfonate



Chemical Formula: $C_{15}H_{29}NO_5$
Molecular Weight: 303,39

Chemical Formula: $C_{16}H_{31}NO_7S$
Molecular Weight: 381,48

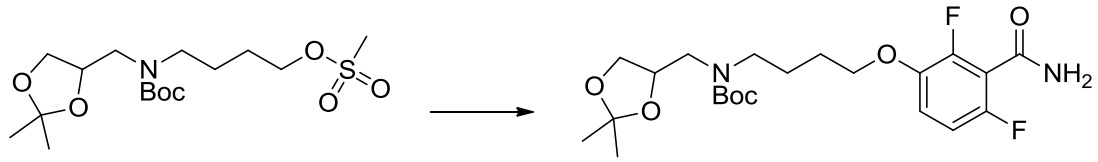
To a solution of tert-butyl ((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)(4-hydroxybutyl)carbamate (1,40 g, 4,61 mmol) in 12 ml of DCM were added 0,78 ml of TEA (5,53 mmol). The mixture was cooled at 4°C and after 0,39 ml of methanesulfonyl chloride (5,07 mmol) were slowly added dropwise. The mixture was stirred for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[155].
The reaction mixture was cooled at 4°C and then diluted with 20 ml of water and 10 ml of DCM. The separated organic layer was washed with HCl 10% (20 ml), $NaHCO_3$ saturated solution (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,70 g of a transparent oil corresponding at the desired product.

Yield = 96,59%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,25 (t, 2H, J= 5,8 Hz) 4,03 (t, 1H, J= 8 Hz) 3,63 (t, 2H, J= 8 Hz) 3,01 (s, 3H) 1,7 (m, 4H) 1,46 (s, 9H) 1,42 (s, 3H) 1,34 (s, 3H).

[155] Cyclohexane/Ethyl Acetate 1/1 Rf start= 0,23 Rf prod= 0,40 Marker: Blue sheet

Tert-butyl (4-(3-carbamoyl-2,4-difluorophenoxy)butyl)((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)carbamate



Chemical Formula: $C_{16}H_{31}NO_7S$
Molecular Weight: 381,48

Chemical Formula: $C_{22}H_{32}F_2N_2O_6$
Molecular Weight: 458,50

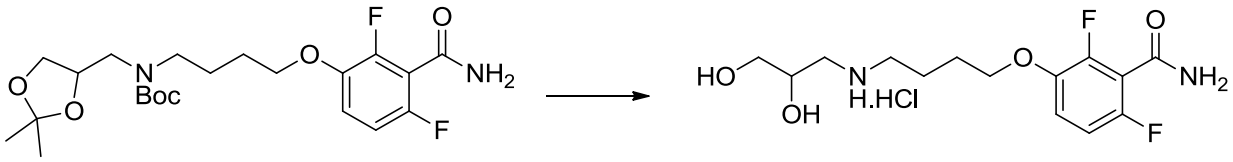
To a solution of 2,6-difluoro-3-hydroxybenzamide (390 mg, 2,23 mmol) in 3 ml of DMF were added 470 mg of anhydrous K_2CO_3 (3,34 mmol). The mixture was stirred for 15 minutes at room temperature, then were added dropwise a solution of 4-((tert-butoxycarbonyl)((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)amino)butyl methanesulfonate (850 mg, 2,23 mmol) in 4 ml of DMF. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C for 7h until the complete transformation of the starting material as revealed by TLC[156].
The reaction mixture was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,16 g of a green liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[157]) to give 350 mg of a colorless oil corresponding at the desired product.

Yield = 68,14%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,98 (m, 1H) 6,85 (t, 1H, J= 8,8 Hz) 6,04 (bs, 1H) 5,83 (bs, 1H) 4,26 (m, 1H) 4,03 (m, 2H) 3,62 (m, 2H) 3,36 (m, 4H) 1,71 (m, 4H) 1,37 (m, 15H).

[156] Cyclohexane/ Ethyl Acetate 70/30 Rf amid= 0,04 Rf inter= 0,26 Rf prod= 0,15 Marker: $Ce(SO_4)_2$
[157] Cyclohexane/ Ethyl Acetate 60/40 Rf inter= 0,36 Rf prod= 0,25 Marker: $Ce(SO_4)_2$

3-(4-((2,3-Dihydroxypropyl)amino)butoxy)-2,6-difluorobenzamide hydrochloride



Chemical Formula: $C_{22}H_{32}F_2N_2O_6$
Molecular Weight: 458,50

Chemical Formula: $C_{14}H_{21}ClF_2N_2O_4$
Molecular Weight: 354,78

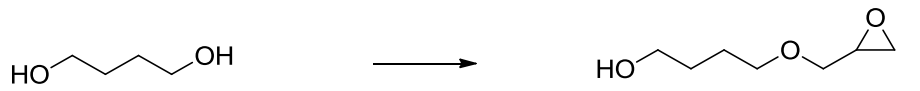
To a solution of tert-butyl (4-(3-carbamoyl-2,4-difluorophenoxy)butyl)((2,2-dimethyl-1,3-dioxolan-4-yl)methyl)carbamate (330 mg, 0,72 mmol) in 3 ml of iPrOH were added 1,5 ml of HCl 10%. The mixture was stirred overnight at room temperature.
The TLC control[158] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and the excess of water was removed by azeotropic distillation with iPrOH to give 239 mg of a transparent wax corresponding at the desired product.

Yield = 93,56%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,70 (bs, 1H) 8,58 (bs, 1H) 8,16 (s, 1H) 7,83 (s, 1H) 7,21 (td, 1H, J= 8,8 J= 2,3 Hz) 7,04 (t, 1H, J= 8,8 Hz) 4,25 (bs, 2H) 3,78 (m, 1H) 3,42 (m, 3H) 3,29 (m, 1H) 2,99 (m, 3H) 2,78 (m, 1H) 1,78 (m, 4H).

[158] Ethyl Acetate Rf start= 0,61 Rf prod= 0,07 Marker: $Ce(SO_4)_2$

4-(Oxiran-2-ylmethoxy)butan-1-ol



Chemical Formula: $C_4H_{10}O_2$
Molecular Weight: 90,12

Chemical Formula: $C_7H_{14}O_3$
Molecular Weight: 146,18

Under nitrogen atmosphere to a solution of tBuOK (4,48 g, 40 mmol) in 28 ml of dry THF a solution of butane-1,4-diol (3,60 g, 40 mmol) in 28 ml of dry THF was added dropwise. The mixture was stirred for 30 minutes at room temperature, then were added dropwise 9,40 ml of epichloridrine (120 mmol). The reaction was stirred for 1 h at room temperature and then brought at reflux for 1h e 30 minutes until the complete transformation of the starting material as revealed by TLC[159].

The reaction mixture was diluted with 40 ml of NaH_2PO_4/Na_2HPO_4 buffer at pH 7 and 40 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (40 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 8,20 g of a yellow liquid corresponding at the crude product.

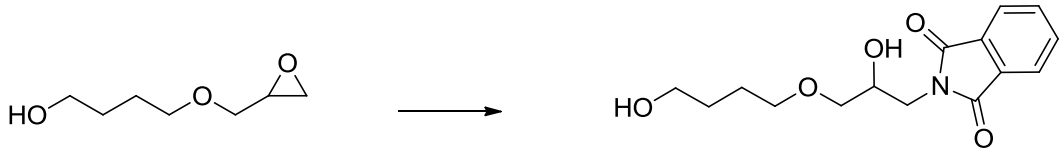
The crude product were purified by flash chromatography on silica gel (ethyl acetate[160]) to give 2,79 g of a yellow liquid corresponding at the desired product.

Yield = 47,71%

^{1}H-NMR ($CDCl_3$) δ (ppm): 3,75 (dd, 1H, J= 8,5 Hz J= 2,3Hz) 3,58 (m, 4H) 3,36 (dd, 1H, J= 8,5 Hz J= 5,3Hz) 3,13 (m, 1H) 2,8 (t, 1H, J= 5,3 Hz) 2,6 (m, 1H) 2,07 (m, 1H) 1,67 (m, 4H).

[159] Ethyl Acetate Rf start= 0,17 Rf prod= 0,32 Marker: Blue sheet
[160] Ethyl Acetate Rf start= 0,17 Rf prod= 0,32 Marker: Blue sheet

2-(2-Hydroxy-3-(4-hydroxybutoxy)propyl)isoindoline-1,3-dione



Chemical Formula: $C_7H_{14}O_3$
Molecular Weight: 146,18

Chemical Formula: $C_{15}H_{19}NO_5$
Molecular Weight: 293,32

To a suspension of phtalimide (3,37 g, 22,9 mmol) in 40 ml of iPrOH were added dropwise a solution 4-(oxiran-2-ylmethoxy)butan-1-ol (2,79 g, 19,08 mmol) in 40 ml of iPrOH and after 0,3 ml of pyridine (5,61 mmol). The mixture was refluxed for 72 h until the complete transformation of the starting material as revealed by TLC [161].

The reaction mixture was cooled at room temperature and slowly a precipitate afforded. The suspension was filtered and the cake washed with 20 ml of iPrOH. The mother liquids evaporated under vacuum to give 5,05 g of a transparent oil corresponding at the crude product.

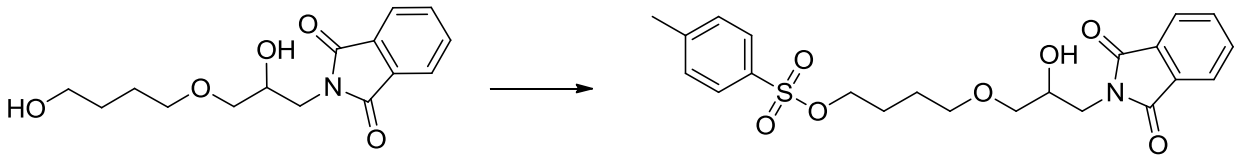
The crude product were purified by flash chromatography on silica gel (ethyl acetate[162]) to give 2,21 g of a white wax corresponding at the desired product

Yield = 39,53%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,84 (m, 2H) 7,75 (m, 2H) 4,07 (m, 1H) 3,86 (m, 2H) 3,65 (m, 1H) 3,71 (m, 4H) 2,8 (bs, 1H) 1,88 (bs, 1H) 1,67 (m, 4H).

[161] Ethyl Acetate Rf start= 0,32 Rf prod= 0,34 Marker: Blue sheet
[162] Ethyl Acetate Rf start= 0,32 Rf prod= 0,34 Marker: Blue sheet

4-(3-(1,3-Dioxoisoindolin-2-yl)-2-hydroxypropoxy)butyl 4-methylbenzenesulfonate



Chemical Formula: $C_{15}H_{19}NO_5$
Molecular Weight: 293,32

Chemical Formula: $C_{22}H_{25}NO_7S$
Molecular Weight: 447,50

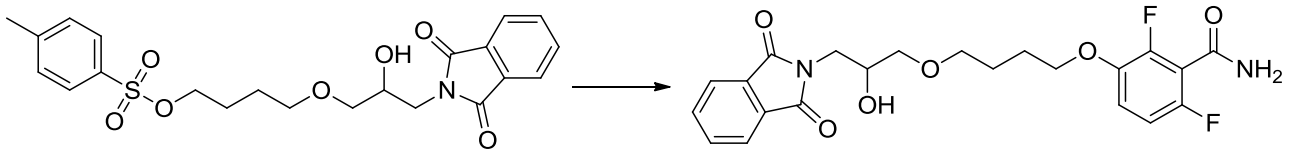
To a 4°C cooled solution of tosyl chloride (1,58g, 8,29 mmol) in 5 ml of pyridine a solution of 2-(2-hydroxy-3-(4-hydroxybutoxy)propyl)isoindoline-1,3-dione (2,21 g, 7,53 mmol) in 5 ml of pyridine were added dropwise. The reaction was stirred for 30 minutes at 4°C until the complete transformation of the starting material as revealed by TLC[163].
The reaction mixture was diluted with 20 ml of ethyl acetate and 20 ml of 10% HCl . The separated aqueous layer was extracted twice with ethyl acetate (20ml). The combined organic phases were washed twice with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,00 g of a colorless oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[164]) to give 1,50 g of a colorless oil corresponding at the desired product.

Yield = 44,51%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,77 (m, 6H) 7,34 (d, 2H, J= 8,8 Hz) 4,09 (m, 2H) 3,84 (m, 2H) 3,42 (m, 4H) 2,62 (d, 1H, J= 4,4 Hz) 2,42 (s, 3H) 1,67 (m, 4H).

[163] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,05 Rf prod= 0,23 Rf imp= 0,33 Marker: Blue sheet
[164] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,05 Rf prod= 0,14 Rf imp= 0,22 Marker: Blue sheet

3-(4-(3-(1,3-Dioxoisoindolin-2-yl)-2-hydroxypropoxy)butoxy)-2,6-difluorobenzamide



Chemical Formula: $C_{22}H_{25}NO_7S$
Molecular Weight: 447,50

Chemical Formula: $C_{22}H_{22}F_2N_2O_6$
Molecular Weight: 448,42

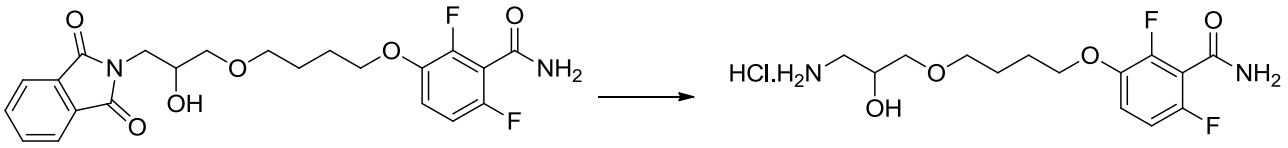
To a solution of 2,6-difluoro-3-hydroxybenzamide (580 mg, 3,35 mmol) in 7,5 ml of DMF were added 700 mg of anhydrous K_2CO_3 (5,02 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 4-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropoxy)butyl 4-methylbenzenesulfonate (1,50 g, 3,35 mmol) in 7,5 ml of DMF was added dropwise. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C for 5h until the complete transformation of the starting material as revealed by TLC[165].
The reaction mixture was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous phase was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,10 g of a yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (ethyl acetate[166]) to give 840 mg of a yellow oil corresponding at the desired product.

Yield = 56,1%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,85 (m, 2H) 7,7 (m, 2H) 3,99 (td, 1H, J= 9 J= 5,3 Hz) 6,85 (td, 1H, J= 9 J= 2,4Hz) 6,17 (bs, 1H) 6,0 (bs, 1H) 4,06 (m, 1H) 3,78 (m, 2H) 2,68 (d, 1H, J= 4,5 Hz) 1,81 (m, 4H).

[165] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,23 Rf prod= 0,08 Marker: $Ce(SO_4)_2$
[166] Ethyl Acetate Rf start= 0,95 Rf amid= 0,58 Rf prod= 0,40 Marker: $Ce(SO_4)_2$

3-(4-(3-Amino-2-hydroxypropoxy)butoxy)-2,6-difluorobenzamide hydrochloride



Chemical Formula: $C_{22}H_{22}F_2N_2O_6$
Molecular Weight: 448,42

Chemical Formula: $C_{14}H_{21}ClF_2N_2O_4$
Molecular Weight: 354,78

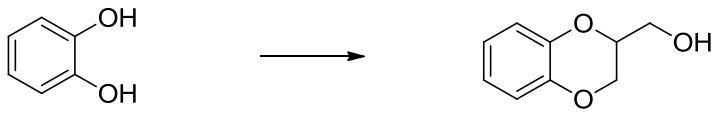
To a solution of 3-(4-(3-(1,3-dioxoisoindolin-2-yl)-2-hydroxypropoxy)butoxy)-2,6-difluorobenzamide (440 mg, 0,98 mmol) in 4 ml of EtOH abs were added 0,11 ml of hydrazine hydrate (2,45 mmol). The mixture was stirred overnight at room temperature.
The TLC control[167] revealed the complete transformation of the starting material.
The reaction mixture was evaporated under vacuum and after the addition of 5 ml of distilled water and 1 ml di HCl 10% a white solid afforded. The suspension was filtered and the mother liquids were evaporated under vacuum. The excess of water was removed by azeotropic distillation with EtOH to give 286 mg of a white wax corresponding at the desired product.

Yield = 92,26%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,12 (s, 1H) 7,88 (m, 4H) 7,2 (td, 1H, J= 9,1 J= 5,2 Hz) 7,05 (td, 1H, J= 9,1 J= 2,1 Hz) 5,49 (bs, 1H) 4,04 (t, 1H, J= 6,2 Hz) 3,82 (m, 1H) 3,37 (m, 4H) 2,88 (m, 1H) 2,66 (m, 1H) 1,59 (m, 4H).

[167] Ethyl Acetate Rf start= 0,52 Rf prod= 0,06 Marker: $Ce(SO_4)_2$

2-Hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_6H_6O_2$
Molecular Weight: 110,11

Chemical Formula: $C_9H_{10}O_3$
Molecular Weight: 166,17

To a solution of catechol (10 g, 90,81 mmol) in 100 ml of MeOH 27,61 g of K_2CO_3(199,3 mmol) were added. The mixture was stirred for 30 minutes at room temperature and then 25,2 ml of epichloridrine (272,4 mmol) added dropwise, refluxed overnight.
The TLC control[168] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and then diluted 60 ml of ethyl ether and 60 ml of distilled water. The separated organic layer was washed with of NaOH 1 M (60 ml) and brine (60 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 14,6 g of an white solid corresponding at the crude product.
The crude product was crystallized by diisopropyl ether gave 10,36 g of a white solid corresponding at the desired product.

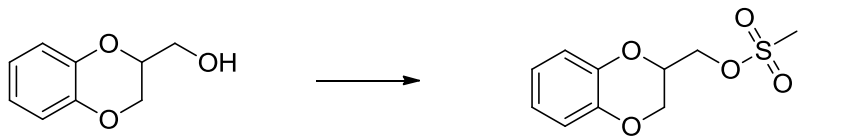
Yield = 68,6%

M.P: = 84°C

^{1}H-NMR (CDCl3) δ (ppm): 6,88 (m, 4H) 4,28 (m, 2H) 4,10 (m, 1H) 3,87 (m, 2H) 2,03 (bs, 1H)

[168] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,25 Rf prod= 0,36 Marker: $Ce(SO_4)_2$

2-Mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_{10}O_3$
Molecular Weight: 166,17

Chemical Formula: $C_{10}H_{12}O_5S$
Molecular Weight: 244,26

To a solution of 2-hydroxymethyl-1,4-benzodioxane (8,61 g, 51,81 mmol) in 60 ml of DCM, 7,94 ml of TEA (56,99 mmol) were added. The mixture was cooled at 4°C and then 4,41 ml of methansulphonyl chloride (56,99 mmol) slowly added dropwise, the reaction was stirred at 4°C for 15 minutes and after 1 h at room temperature until the complete transformation of the starting material as revealed by TLC[169].
The reaction mixture was cooled at 4°C and diluted with 60 ml of water. The separated organic phase was washed with HCl 10% (60 ml), $NaHCO_3$ saturated solution (60 ml) and brine (60 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 13,4 g of an white wax corresponding at the crude product.
The crude product was crystallized by iPrOH gave 11,21 g of an white solid corresponding at the desired product.

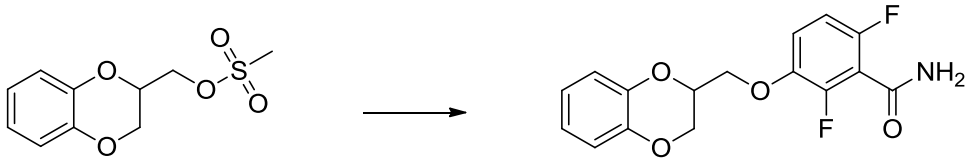
Yield = 88,61%

M.P. = 76°C

^{1}H-NMR (CDCl3) δ (ppm): 6.88 (m, 4H) 4,46 (m, 3H) 4,32 (m, 1H) 4,13 (dd, 1H, J=11,5 J=6 Hz) 3,09 (s, 3H)

[169] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,36 Rf prod= 0,48 Marker: $Ce(SO_4)_2$

3-(2-Methylenoxy-1,4-benzodixan)-2,6-difluorobenzamide



Chemical Formula: $C_{10}H_{12}O_5S$
Molecular Weight: 244,26

Chemical Formula: $C_{16}H_{13}F_2NO_4$
Molecular Weight: 321,28

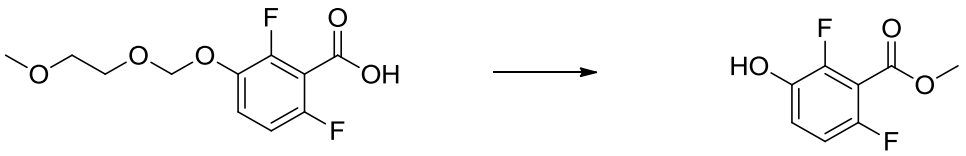
To a solution of 2,6-difluoro-3-hydroxybenzamide (660 mg, 3,83 mmol) in 4 ml of DMF were added 800 mg of anhydrous K_2CO_3 (5,75 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 2-mesyloxymethyl-1,4-benzodioxane (0,93 g, 3,83 mmol) in 4 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C for 6h until the complete transformation of the starting material as revealed by TLC[170].
The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (30 ml) and brine (30 ml) , dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,30 g of a green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[171]) to give 450 mg of a transparent oil corresponding at the desired product.

Yield = 36,58%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,07 (td, 1H, J= 9,1 J= 5,1 Hz) 6,84 (m, 5H) 5,96 (bs, 2H) 4,59 (m, 1H) 4,40 (dd, 1H, J= 11,5 J= 2,4 Hz) 4,25 (m, 3H).

[170]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,48 Rf amid= 0,11 Rf prod= 0,27 Marker: $Ce(SO_4)_2$
[171]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,48 Rf amid= 0,11 Rf prod= 0,27 Marker: $Ce(SO_4)_2$

Methyl 2,6-difluoro-3-hydroxybenzoate



Chemical Formula: $C_{11}H_{12}F_2O_5$
Molecular Weight: 262,21

Chemical Formula: $C_8H_6F_2O_3$
Molecular Weight: 188,13

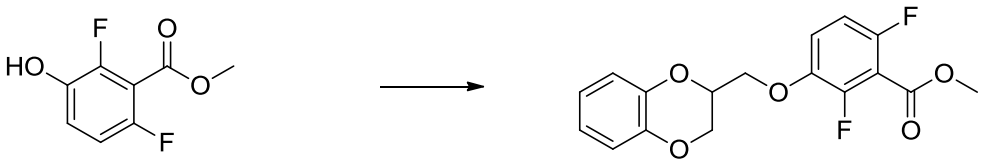
To a solution of 2,6-difluoro-3-((2-methoxyethoxy)methoxy)benzoic acid (2,47 g, 9,4 mmol) in 40 ml of MeOH were added 0,5 ml of conc. H_2SO_4, the solution were refluxed overnight.
The TLC control[172] revealed that the starting material was completely transformed.
The reaction mixture were evaporated under vacuum and diluted with 80 ml of ethyl acetate. The organic phase was washed with $NaHCO_3$ saturated solution (3x 40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,68 g of a yellow liquid corresponding at the desired product.

Yield = 94,9%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,08 (td, 1H, J= 8,8 J= 5,2 Hz) 6,88 (m, 1H) 3,96 (s, 3H).

[172] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,13 Rf prod= 053 Marker: $Ce(SO_4)_2$

Methyl 3-(2-methylenoxy-1,4-benzodixan)-2,6-difluorobenzaote



Chemical Formula: $C_8H_6F_2O_3$
Molecular Weight: 188,13

Chemical Formula: $C_{17}H_{14}F_2O_5$
Molecular Weight: 336,29

To a solution of methyl 2,6-difluoro-3-hydroxybenzoate (1,68 g, 8,93 mmol) in 10 ml of DMF were added 1,23 g of anhydrous K_2CO_3 (8,93 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 2-mesyloxymethyl-1,4-benzodioxane (2,18 g, 8,93 mmol) in 10 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 65°C overnight.
The TLC control[173] revealed the consumption of the starting material.
The reaction mixture was evaporated under vacuum and diluted with 40 ml of ethyl acetate and 75 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (2 x 40 ml). The combined organic phases were washed with NaOH 1 M (60 ml) and brine (60 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,60 g of a yellow wax corresponding at the crude product.
The crude product was crystallized by absolute EtOH to give 1,40 g of a white powder corresponding at the desired product.

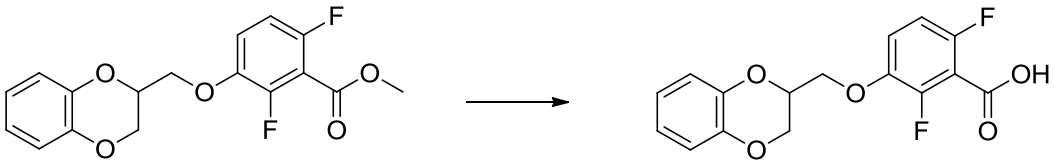
Yield = 46,66%

M.P. = 86°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,09 (td, 1H, J= 8,8 J= 5,2 Hz) 6,88 (m, 5H) 4,57 (m, 1H) 4,40 (dd, 1H, J= 11,5 J= 2,4 Hz) 4,25 (m, 3H) 3,96 (s, 3H).

[173] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,31 Rf mes= 0,25 Rf prod= 0,44 Marker: $Ce(SO_4)_2$

3-(2-Methylenoxy-1,4-benzodixan)-2,6-difluorobenzoic acid



Chemical Formula: $C_{17}H_{14}F_2O_5$
Molecular Weight: 336,29

Chemical Formula: $C_{16}H_{12}F_2O_5$
Molecular Weight: 322,26

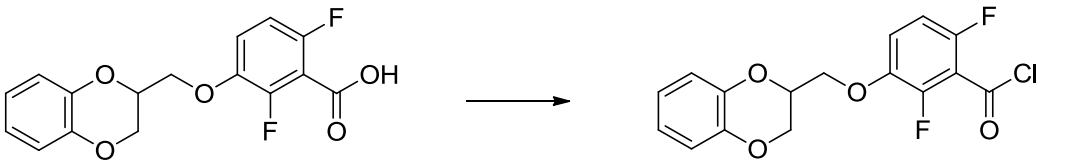
To a solution of methyl 3-(2-methylenoxy-1,4-benzodixan)-2,6-difluorobenzaote (1,40 g, 4,16 mmol) in 13 ml of THF were 1,99 ml of NaOH 2,5 M and 7,5 ml of water added dropwise. The mixture was stirred for 15 minutes at room temperature, then was brought at 50°C for 3h e 30 minutes until the complete transformation of the starting material as revealed by TLC[174].
The reaction mixture was evaporated under vacuum and diluted with 2,8 ml of HCl 10% and 10 ml of distilled water. The aqueous were extracted ethyl acetate with (3 x 20 ml). The combined organic phases were washed brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,31 g of a white wax corresponding at the desired product.

Yield = 97,76%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,15 (td, 1H, J= 9,0 J= 5,0 Hz) 6,89 (m, 5H) 4,58 (ddd, 1H, J= 11,3 J= 6,2 J= 2,4Hz) 4,40 (dd, 1H, J= 11,3 J= 2,4 Hz).

[174]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,31 Rf prod= 0,06 Marker: $Ce(SO_4)_2$

3-(2-Methylenoxy-1,4-benzodixan)-2,6-difluorobenzoyl chloride



Chemical Formula: $C_{16}H_{12}F_2O_5$
Molecular Weight: 322,26

Chemical Formula: $C_{16}H_{11}ClF_2O_4$
Molecular Weight: 340,71

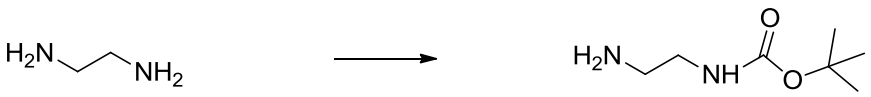
To 12 ml of $SOCl_2$ were added in two portion 980 mg of 3-(2-methylenoxy-1,4-benzodixan)-2,6-difluorobenzoic acid (3,04 mmol). The mixture was stirred for 15 minutes at room temperature, then was heated at 50°C for 2h e 30 minutes until the complete transformation of the starting material as revealed by TLC[175].
The reaction mixture was evaporated under vacuum to give 1,01 g of a red oil corresponding at the crude product.

Yield = 97,40%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,20 (td, 1H, J= 9,1 J= 5,1 Hz) 6,88 (m, 5H) 4,59 (m, 1H) 4,40 (dd, 1H, J= 11,5 J= 2,4 Hz) 4,25 (m, 3H).

[175]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,07 Rf prod= 0,37 Marker: $Ce(SO_4)_2$

Tert-butyl (2-aminoethyl)carbamate



Chemical Formula: $C_2H_8N_2$
Molecular Weight: 60,10

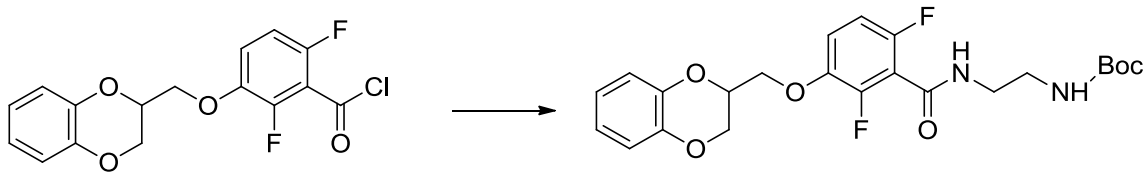
Chemical Formula: $C_7H_{16}N_2O_2$
Molecular Weight: 160,21

To a solution of ethylendiamine (2,52 g, 41,93 mmol) in 25 ml of DCM a solution of di-tert-butyl carbonate (1,52 g) in 100 ml of DCM was added dropwise. The mixture was stirred overnight at room temperature.
At the morning a precipitated afforded. The suspension was filtered and the cake was washed with DCM (20 ml).
The mother liquids was washed twice with $NaHCO_3$ saturated solution (100 ml) and twice with brine (100 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,90 g of a yellow oil corresponding at the desired product.

Yield = 81,08%

^{1}H-NMR ($CDCl_3$) δ (ppm): 4,87 (bs, 1H) 3,17 (q, 2H, J= 5,9 Hz) 2,79 (t, 2H, J= 5,9 Hz) 1,56 (s, 2H) 1,45 (s, 9H).

Tert-butyl (2-(3-(2-methylenoxy-1,4-benzodixan))-2,6-difluorobenzamido)ethyl)carbamate



Chemical Formula: $C_{16}H_{11}ClF_2O_4$
Molecular Weight: 340,71

Chemical Formula: $C_{23}H_{26}F_2N_2O_6$
Molecular Weight: 464,46

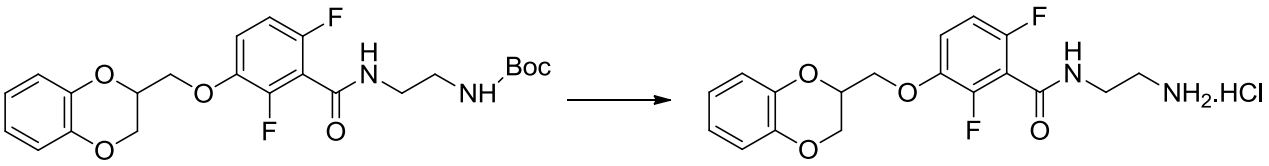
To a solution of 3-(2-methylenoxy-1,4-benzodixan)-2,6-difluorobenzoyl chloride (1,01 g, 3,04 mmol) in 7,5 ml of dry pyridine was cooled at -5°C and then a solution of tert-butyl (2-aminoethyl)carbamate (0,60 g in 7,5 ml of dry pyridine)was added dropwise. The mixture was stirred for 15 minutes at -5°C and for 1 h at room temperature until the complete transformation of the starting material as revealed by TLC[176].
The reaction mixture was poured into 90 ml of HCl 10% and extracted with ethyl acetate (3 x 40 ml). The combined organic phases were washed with $NaHCO_3$ saturated solution (50 ml) and of brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,30 g of a yellow oil corresponding at the desired product.

Yield = 92,19%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,02 (td,1H, J= 9,1 J= 5,1 Hz) 6,88 (m, 5H) 6,70 (bs, 1H) 4,92 (bs, 1H) 4,56 (ddd, 1H, J= 11,5 J= 6,3 J= 2,4 Hz) 4,39 (dd, 1H, J= 11,5 J= 2,4 Hz) 4,21 (m, 3H) 3,57 (q, 2H, J= 5,4 Hz) 3,39 (m, 2H) 1,44 (s, 9H).

[176]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,63 Rfamm= 0,06 Rf prod= 0,31 Marker: $Ce(SO_4)_2$

N-(2-Aminoethyl)-3-(2-methylenoxy-1,4-benzodixan)-2,6-difluorobenzamide hydrochloride



Chemical Formula: $C_{23}H_{26}F_2N_2O_6$
Molecular Weight: 464,46

Chemical Formula: $C_{18}H_{19}ClF_2N_2O_4$
Molecular Weight: 400,80

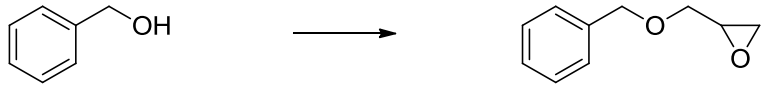
To a solution of tert-butyl (2-(3-(2-methylenoxy-1,4-benzodixan))-2,6-difluorobenzamido)ethyl)carbamate (750 mg, 1,61 mmol) in 15 ml of iPrOH was added dropwise 1,34 ml of HCl 10%. The mixture was stirred for 15 minutes at room temperature and refluxed overnight. The TLC control[177] revealed the transformation of the starting material.
The reaction was evaporated under vacuum and diluted with 20 ml of water. The aqueous phase was washed twice with ethyl acetate (2 x 15 ml), and after brought at pH 8 by addiction of $NaHCO_3$ saturated solution. The solution was extracted with ethyl acetate (3 x 15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 560 mg of a yellow oil corresponding at the free amine.
The amine was dissolved in 9 ml of iPrOH and added 0,57 ml of HCl 10%, The reaction mixture was evaporated under vacuum to give 575 mg of a light brown solid corresponding at the desired product.

Yield = 89,15%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,94 (bs, 1H) 8,02 (bs, 3H) 7,34 (td,1H, J= 9,4 J= 5,3 Hz) 7,11 (td, 1H, J= 9,4 J= 1,9 Hz) 6,86 (m, 4H) 4,57 (m, 1H) 4,42 (dd, 1H, J= 11,5 J= 2,4 Hz) 4,33 (m, 2H) 4,12 (dd, 1H, J= 11,5 J= 7,2 Hz) 3,57 (q, 2H, J= 6,6 Hz) 2,91 (m, 2H) 1,44 (s, 9H).

[177] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,31 Rf prod= 0,05 Marker: $Ce(SO_4)_2$

3-Benzyloxy-1,2-epoxypropane



Chemical Formula: C_7H_8O
Molecular Weight: 108,14

Chemical Formula: $C_{10}H_{12}O_2$
Molecular Weight: 164,20

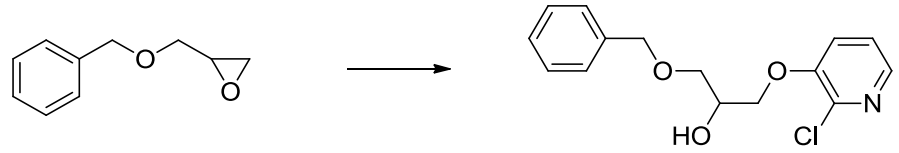
To a solution of benzyl alcohol (3,0 g, 27,75 mmol) in 35 ml of NaOH 10 M were added 0,44 g of TBAB (1,38 mmol), the mixture was cooled at 4°C and then 8,70 ml of epichloridrine (110,99 mmol) were added dropwise, stirred for 15 minutes at 4°C and after overnight at room temperature.
The TLC control[178] revealed the complete transformation of the starting material.
The reaction mixture was poured in 60 ml of 4°C cooled water, the aqueous phase was extracted with ethyl ether (3 x 30 ml). The organic phase was washed with brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 7,19 g of an yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[179]) to give 4,32 g of a transparent liquid corresponding at the desired product.

Yield = 94,73%

^{1}H-NMR (CDCl3) δ (ppm): 7,31 (m, 5H) 4,59 (q, 2H, J= 11,4 Hz) 3,77 (dd, 1H, J= 11,4 J= 3,0 Hz) 3,45 (dd, 1H, J= 11,4 J= 5,8 Hz) 3,19 (m, 1H) 2,84 (dd, 1H, J= 5,0 J= 4,2 Hz) 2,63 (dd, 1H, J= 5,0 J= 2,7 Hz).

[178] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,90 Rf prod= 0,20 Marker: blue sheet
[179] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,90 Rf prod= 0,20 Marker: blue sheet

1-(Benzyloxy)-3-((2-chloropyridin-3-yl)oxy)propan-2-ol



Chemical Formula: $C_{10}H_{12}O_2$
Molecular Weight: 164,20

Chemical Formula: $C_{15}H_{16}ClNO_3$
Molecular Weight: 293,75

To a solution of 2-chloro-3-pyridinol (4,36 g, 33,63 mmol) in 27 ml of DMF were added 4,65 g of K_2CO_3 (33,63 mmol), the mixture was stirred for 10 minutes at room temperature and then a solution of 3-benzilossi-1,2-epoxypropane (5,52 g in 25 ml of DMF) were added dropwise. The reaction was stirred for 15 minutes at room temperature and heated at 115°C for 5 h until the complete transformation of the starting material as revealed by TLC[180].

The reaction was evaporated under vacuum and diluted with 80 ml of ethyl acetate and 80 ml of water. The separated aqueous layer was extracted twice with ethyl acetate (80 ml). The combined organic phases were washed with NaOH 1M (50 ml) and brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 10,50 g of an red liquid corresponding at the crude product.

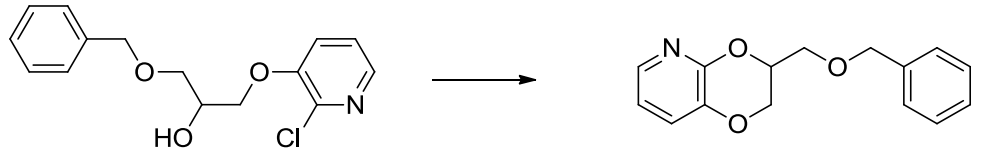
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 65/35[181]) to give 4,32 g of a transparent liquid corresponding at the desired product.

Yield = 94,73%

^{1}H-NMR (CDCl3) δ (ppm): 8,01 (dd, 1H, J= 4,3 J= 2,0 Hz) 7,26 (m, 7H) 4,58 (s, 2H) 4,24 (m, 1H) 4,10 (m, 2H) 3,70 (m, 2H) 2,61 (d, 1H, J= 5,5 Hz).

[180] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,58 Rf prod=0,19 Marker: Blue sheet
[181] Cyclohexane/ Ethyl Acetate 65/35 Rf start= 0,66 Rf prod= 0,37 Marker: Blue sheet

2-Benzyloxymethyl-2,3-dihydro-1,4-dioxin[3,2-b]pyridine



Chemical Formula: $C_{15}H_{16}ClNO_3$
Molecular Weight: 293,75

Chemical Formula: $C_{15}H_{15}NO_3$
Molecular Weight: 257,28

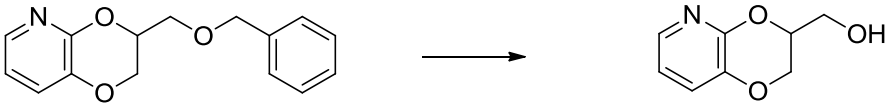
Under nitrogen atmosphere to a suspension of NaH (150 mg, 6,22 mmol) in 6 ml of DME was added dropwise a solution of 1-(benzyloxy)-3-((2-chloropyridin-3-yl)oxy)propan-2-ol (1,66 g 5,65 mmol) in 8 ml DME. The reaction was stirred for 15 minutes at room temperature, then brought at 90°C and maintained for 4h until the complete transformation of the starting material as revealed by TLC[182].
At the reaction was added dropwise added 10 ml of water and 10 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,30 g of an green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[183]) to give 940 mg of a transparent oil corresponding at the desired product.

Yield = 64,82%

^{1}H-NMR (CDCl3) δ (ppm): 7,83 (dd, 1H, J= 4,6 J= 2,0 Hz) 7,32 (m, 5H) 7,18 (dd, 1H, J= 7,6 J= 2,0 Hz) 6,87 (dd, 1H, J= 7,6 J= 4,6 Hz) 4,64 (s, 2H) 4,56 (m, 1H) 4,37 (dd, 1H, J= 11,2 J= 2,3 Hz) 4,14 (dd, 1H, J= 11,2 J= 6,9 Hz) 3,84 (dd, 1H, J= 6,9 J= 2,2 Hz) 3,73 (dd, 1H, J= 6,9 J= 5,4 Hz).

[182] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,12 Rf prod= 0,24 Marker: Blue sheet
[183] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,12 Rf prod= 0,24 Marker: Blue sheet

2-Hydroxymethyl-2,3-dihydro-1,4-dioxin[3,2-b]pyridine



Chemical Formula: $C_{15}H_{15}NO_3$
Molecular Weight: 257,28

Chemical Formula: $C_8H_9NO_3$
Molecular Weight: 167,16

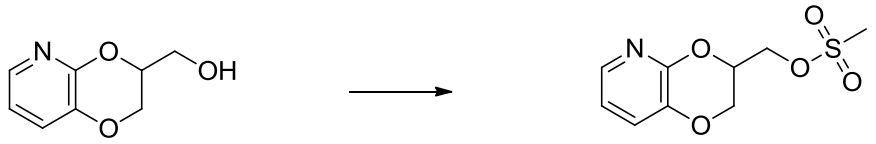
To a solution of 2-benzyloxymethyl-2,3-dihydro-1,4-diossin[3,2-b]pyridine (940 mg, 3,66 mmol) in 15 ml of MeOH were added 1,35 ml of HCl 10% . The mixture was stirred for 15 minutes at room temperature and then were added 180 mg of Pd/C at 5% and hydrogenated at 3,5 atm for 4h until the complete transformation of the starting material as revealed by TLC[184].
The catalyzer was filtered and the mother liquids were evaporated under vacuum. The resulting oil was diluted with 20 ml of $NaHCO_3$ satured solution and extracted with ethyl acetate (3 x 20 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 470 mg of a yellow wax corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (ethyl acetate[185]) to give 330 mg of a yellow wax corresponding at the desired product.

Yield = 54,09%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,81 (dd, 1H, J= 4,6 J= 1,6 Hz) 7,21 (dd, 1H, J= 7,9 J= 1,6 Hz) 6,88 (dd, 1H, J=7,9 J= 4,6 Hz) 4,43 (m, 1H) 4,33 (dd, 1H, J= 11,6 J= 2,4 Hz) 4,11 (dd, 1H, J= 11,6 J= 8,2 Hz) 4,00 (dd, 1H, J= 12,3 J= 4,2 Hz) 3,89 (dd, 1H, J=12,3 J= 4,6 Hz) 2,85 (bs, 1H).

[184] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,55 Rf prod= 0,11 Marker: $Ce(SO_4)_2$
[185] Ethyl Acetate Rf start= 0,75 Rf prod= 0,25 Marker: $Ce(SO_4)_2$

2-Mesyloxymethyl-2,3-dihydro-1,4-diossin[3,2-b]pyridine



Chemical Formula: $C_8H_9NO_3$
Molecular Weight: 167,16

Chemical Formula: $C_9H_{11}NO_5S$
Molecular Weight: 245,25

To a solution of 2-hydroxymethyl-2,3-dihydro-1,4-diossin[3,2-b]pyridine (600 mg, 3,59 mmol) in 6 ml of DCM were added 0,55 ml of TEA (3,95 mmol). The mixture was cooled at 4°C and then 0,31 ml of methansulphonyl chloride (3,95 mmol) were slowly added dropwise, the reaction was stirred at 4°C for 15 minutes and after 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[186].
The reaction was cooled at 4°C and quenched with 15 ml of saturated solution of $NaHCO_3$ and 10 ml of DCM. The separated organic layer was washed with water (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 800 mg of an white solid corresponding at the desired product.

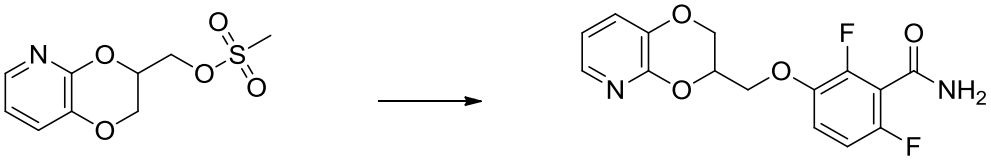
Yield = 88,61%

M.P. = 132,11°C

^{1}H-NMR (CDCl3) δ (ppm): 7,86 (dd, 1H, J= 4,8 J= 1,5 Hz) 7,23 (dd, 1H, J= 7,9 J= 1,6 Hz) 6,88 (dd, 1H, J=7,9 J= 4,8 Hz) 4,66 (m, 1H) 4,55 (d, 2H, J= 5,5 Hz) 4,35 (dd, 1H, J= 11,7 J= 2,5 Hz) 4,14 (dd, 1H, J= 11,7 J= 7,0 Hz) 3,13 (s, 3H).

[186] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,08 Rf prod= 0,13 Marker: $Ce(SO_4)_2$

3-(2-Methoxyl-2,3-dihydro-1,4-diossin[3,2-b]pyridine)-2,6-difluorobenzamide



Chemical Formula: $C_9H_{11}NO_5S$
Molecular Weight: 245,25

Chemical Formula: $C_{15}H_{12}F_2N_2O_4$
Molecular Weight: 322,26

To a solution of 2,6-difluoro-3-hydroxybenzamide (612 mg, 3,54 mmol) in 3 ml of DMF were added 489 mg of anhydrous K_2CO_3 (3,54 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 2-mesyloxymethyl-2,3-dihydro-1,4-diossin[3,2-b]pyridine (790 mg, 3,22 mmol) in 5 ml of DMF were added. The reaction was stirred for 15 minutes at room temperature and then brought at 70°C for 7h until the complete transformation of the starting material as revealed by TLC[187].
The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,21 g of a light brown crystal corresponding at the crude product.
The crude product was crystallized by methylethylketon to give 460 mg of a white crystal corresponding at the desired product.

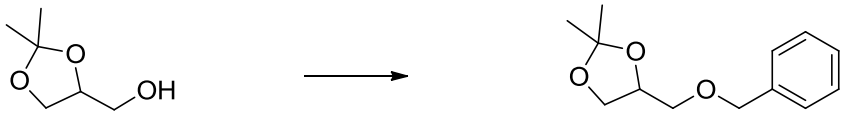
Yield = 43,26%

M.P.= 168,47°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,84 (dd, 1H, J= 4,8 J= 1,5Hz) 7,23 (dd, 1 H, J= 7,9 J= 1,5 Hz) 7,11 (td, 1H, J= 9,0 J= 5,1 Hz) 6,91 (m, 2H) 6,10 (bs, 1H) 5,89 (bs, 1H) 4,73 (m, 1H) 4,41 (m, 2H) 4,26 (m, 2H).

[187]Toluene/ Ethyl Acetate 1/1 Rf start= 0,33 Rf amid= 0,25 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

3-Benzyloxy-1,2-isopropylidenglycerol



Chemical Formula: $C_6H_{12}O_3$
Molecular Weight: 132,16

Chemical Formula: $C_{13}H_{18}O_3$
Molecular Weight: 222,28

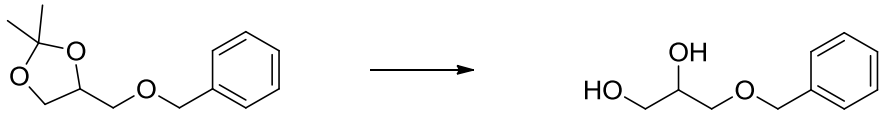
To a solution of racemic solketal (10,0 g, 75,66 mmol) in 80 ml of DMSO were added 13,41 g of KOH (239,08 mmol), the mixture was stirred for 15 minutes at room temperature and then 10,4 ml of benzyl chloride (90,80 mmol) were added dropwise. The reaction was stirred overnight at room temperature.
The TLC control[188] revealed the complete transformation of the starting material.
The reaction was poured in 100 ml of 4°C cooled water, and extracted with ethyl ether (3 x 100 ml). The combined organic phase was washed twice with water (100 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 18,50 g of an yellow liquid corresponding at the crude product.
The crude product were purified by distillation at 160°C and 2,5 mbar to give 16,7 g of a transparent liquid corresponding at the desired product.

Yield = 98,39%

^{1}H-NMR (CDCl3) δ (ppm): 7,34 (m, 5H) 4,58 (m, 2H) 4,35 (q, 1H, J= 5,6 Hz) 4,04 (dd, 1H, J= 8,2 J= 6,6 Hz) 3,76 (dd, 1H, J= 8,2 J= 5,6 Hz) 3,52 (m, 2H) 1,44 (s, 3H) 1,39 (s, 3H).

[188] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,22 Rf prod= 0,45 Marker: Blue sheet

3-Benzyloxy-1,2-propandiol



Chemical Formula: $C_{13}H_{18}O_3$
Molecular Weight: 222,28

Chemical Formula: $C_{10}H_{14}O_3$
Molecular Weight: 182,22

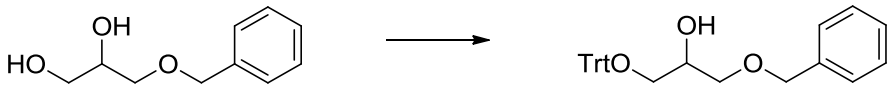
To a solution of 3-benzyloxy-1,2-isopropyilidenglycerol (16,7 g, 75,13 mmol) in 50 ml of iPrOH 28 ml of HCl 10% were added dropwise, the mixture was stirred for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[189].
At the reaction mixture was added $NaHCO_3$ until pH 5. The reaction mixture was evaporated under vacuum, the resulting oil was diluted with 80 ml of iPrOH, the inorganic salts precipitated was removed by filtration. The mother liquids was evaporated under vacuum to give 12,9 g of an transparent liquid corresponding at the desired product.

Yield = 94,16%

^{1}H-NMR (CDCl3) δ (ppm): 7,34 (m, 5H) 4,57 (s, 2H) 3,92 (m, 1H) 3,66 (m, 4H) 2,09 (bs, 1H).

[189] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,45 Rf prod= 0,11 Marker: Blue sheet

1-Trityloxy-3-benzyloxy-2-propanol



Chemical Formula: $C_{10}H_{14}O_3$
Molecular Weight: 182,22

Chemical Formula: $C_{29}H_{28}O_3$
Molecular Weight: 424,53

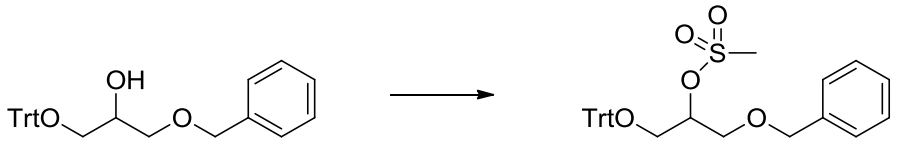
Under nitrogen atmosphere to a solution of triphenylmethanchloride (11,84 g, 42,48 mmol) in 12 ml of tBuOH were added dropwise 6,4 ml of TEA (46,02 mmol). The mixture was stirred for 15 minutes at room temperature and after brought at 60°C. At the hot solution a solution of 3-benzyloxy-1,2-propandiol (6,45g in 10 ml of tBuOH) was added dropwise, and brought at reflux for 24 h until the complete transformation of the starting material as revealed by TLC[190].
The reaction mixture was cooled at room temperature and diluted with 30 ml of cyclohexane. A precipitate afforded and was removed by filtration. The mother liquids were evaporated under vacuum to give 17,5 g of an green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[191]) to give 11,30 g of a transparent oil corresponding at the desired product.

Yield = 75,18%

^{1}H-NMR (CDCl3) δ (ppm): 7,33 (m, 20H) 4,54 (s, 2H) 3,99 (m, 1H) 3,58 (m, 2H) 3,22 (m, 2H) 2,42 (bs, 1H).

[190] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,11 Rf prod= 0,32 Marker: Blue sheet
[191] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,11 Rf prod= 0,32 Marker: Blue sheet

1-Trityloxy-2-mesyloxy-3-benzyloxypropane



Chemical Formula: $C_{29}H_{28}O_3$
Molecular Weight: 424,53

Chemical Formula: $C_{30}H_{30}O_5S$
Molecular Weight: 502,62

To a solution of 1-trityloxy-3-benzyloxy-2-propanol (4,05 g, 9,54 mmol) in 20 ml of DCM were added 1,85 ml of TEA (13,36 mmol). The mixture was cooled at 4°C and then 0,95 ml of methansulphonyl chloride (12,40 mmol) were slowly added dropwise. The reaction was stirred at 4°C for 15 minutes and for 3 h at room temperature until the complete transformation of the starting material as revealed by TLC[192].
The reaction mixture was cooled at 4°C and quenched with 30 ml of water and 10 ml of DCM. The separated organic layer was washed with 30 ml of HCl 10%, 30 ml of saturated solution of $NaHCO_3$ and 30 ml of brine, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 4,75 g of an yellow wax corresponding at the crude product.
The crude product was crystallized by diisopropyl ether to give 3,60 g of a white powder corresponding at the desired product.

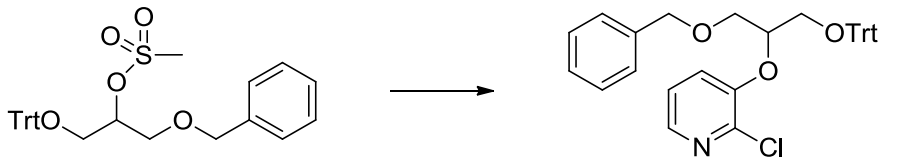
Yield = 75,15%

M.P. = 110,01°C

^{1}H-NMR (CDCl3) δ (ppm): 7,33 (m, 20H) 4,88 (m, 1H) 4,52 (s, 2H) 3,70 (m, 2H) 3,39 (m, 2H) 3,02 (s, 3H).

[192] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,32 Rf prod= 0,14 Marker: $Ce(SO_4)_2$

1-Trityloxy-2-(2'-chlore-3'-pyridinyloxyl)-3-benzyloxypropane



Chemical Formula: $C_{30}H_{30}O_5S$
Molecular Weight: 502,62

Chemical Formula: $C_{34}H_{30}ClNO_3$
Molecular Weight: 536,06

Under nitrogen atmosphere to a solution of 2-chloro-3-pyridinol (0,92 g, 7,10 mmol) in 7 ml of DMF were added 0,98 g of K_2CO_3 (7,10 mmol). At the mixture a solution of 1-trityloxy-2-mesyloxy-3-benzyloxypropane (3,57 g in 8 ml of DMF) was added dropwise. The reaction was stirred at room temperature for 15 minutes and brought at 145°C for 24 h until the complete transformation of the starting material as revealed by TLC[193].

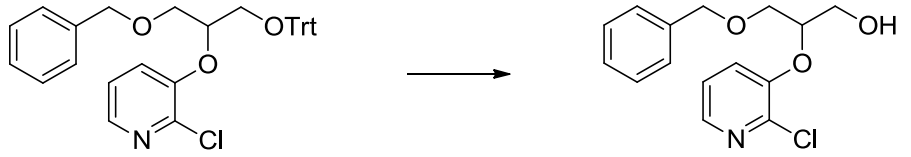
The reaction mixture was evaporated under vacuum and diluted with 30 ml of water, and 30 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (30 ml). The combined organic phases were washed with NaOH 1M (30 ml) and brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,72 g of an yellow solid corresponding at the desired product.

Yield = 97,12%

^{1}H-NMR (CDCl3) δ (ppm): 8,02 (dd, 1H, J= 4,7 J= 1,8 Hz) 7,38 (m, 21H) 7,17 (dd, 1H, J= 8,1 J= 4,7 Hz) 4,59 (m, 3H) 3,81 (m, 2H) 3,44 (m, 2H).

[193] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,33 Rf prod= 0,20 Marker: $Ce(SO_4)_2$

2-(2'-Chlore-3'-pyridinyloxyl)-3-benzyloxy-1-propanol



Chemical Formula: $C_{34}H_{30}ClNO_3$
Molecular Weight: 536,06

Chemical Formula: $C_{15}H_{16}ClNO_3$
Molecular Weight: 293,75

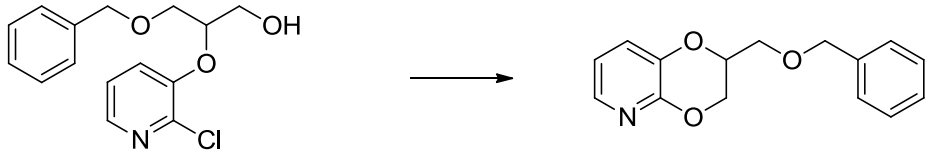
To a solution of 1-trityloxy-2-(2'-chlore-3'-pyridiniloxyl)-3-benzyloxypropane (3,72 g, 7,10 mmol) in 35 ml of THF were added dropwise 24 ml of HCl 10% and refluxed overnight.
The TLC control[194] revealed the complete transformation of the starting material.
The reaction mixture was evaporated under vacuum, the resulting solution was washed twice with cyclohexane (2 x 30 ml)of, and brought at pH 10 with Na_2CO_3.
The aqueous phase was extracted with ethyl acetate (3 x 30 ml), the combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,00 g of an yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane /ethyl acetate 80/20[195]) to give 1,60 g of a transparent yellow liquid corresponding at the desired product.

Yield = 76,71%

^{1}H-NMR (CDCl3) δ (ppm): 8,03 (dd, 1H, J= 4,8 J= 1,6 Hz) 7,44 (dd, 1H, J= 8,1 J= 1,3 Hz) 7,29 (m, 5H) 7,17 (dd, 1H, J= 8,1 J= 1,3 Hz) 4,57 (s, 2H) 4,50 (q, 1H, J= 5,3 Hz) 3,90 (m, 2H) 3,76 (d, 2H, J= 5,3 Hz) 1,82 (bs, 1H).

[194] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,63 Rf prod= 0,32 Marker: Blue sheet
[195] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,63 Rf prod= 0,32 Marker: Blue sheet

2-Benzyloxymethyl-2,3-dihydro-1,4-dioxin[2,3-b]pyridine



Chemical Formula: $C_{15}H_{16}ClNO_3$
Molecular Weight: 293,75

Chemical Formula: $C_{15}H_{15}NO_3$
Molecular Weight: 257,28

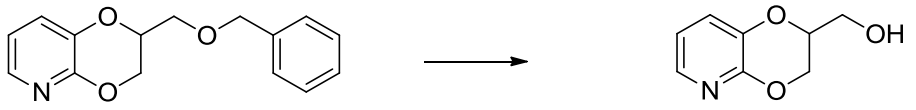
Under nitrogen atmosphere to a suspension of NaH (145 mg, 6,04 mmol) in 12 ml of DME was slowly added dropwise a solution of 2-(2'-chlore-3'-pyridiniloxyl)-3-benzyloxy-1-propanol (1,60 g 5,42 mmol) in 12 ml DME. The reaction was stirred for 15 minutes at room temperature, then refluxed overnight.
The TLC control[196] revealed the transformation of the starting material.
The reaction was cooled at 4°C and quenched with 20 ml of NaH_2PO_4/Na_2HPO_4 buffer at pH 7. The mixture were extracted with DCM (3 x 30 ml), the combined organic phases were washed with brine 50 ml, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,30 g of an yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane /ethyl acetate 75/25[197]) to give 0,94 g of a transparent liquid corresponding at the desired product.

Yield = 67,38 %

^{1}H-NMR (CDCl3) δ (ppm): 7,82 (dd, 1H, J= 4,8 J= 1,6 Hz) 7,34 (m, 5H) 7,20 (dd, 1H, J= 7,8 J= 1,6 Hz) 6,87 (dd, 1H, J= 7,8 J= 4,8 Hz) 4,60 (d, 2H, J= 1,4 Hz) 4,43 (dd, 1H, J= 11,3 J= 2,1 Hz) 4,37 (m, 1H) 4,27 (dd, 1H, J= 11,3 J= 7,3 Hz) 3,71 (m, 2H).

[196] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,19 Rf prod= 0,35 Marker: Blue sheet
[197] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,15 Rf prod= 0,29 Marker: Blue sheet

2-Hydroxymethyl-2,3-dihydro-1,4-dioxin[2,3-b]pyridine



Chemical Formula: $C_{15}H_{15}NO_3$
Molecular Weight: 257,28

Chemical Formula: $C_8H_9NO_3$
Molecular Weight: 167,16

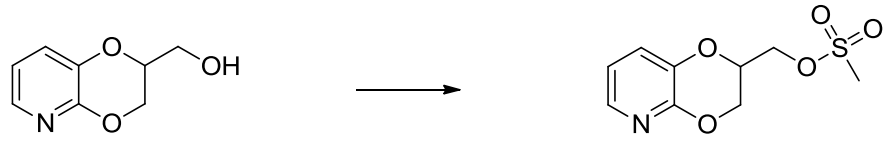
To a solution of 2-benzyloxymethyl-2,3-dihydro-1,4-diossin[2,3-b]pyridine (940 mg, 3,66 mmol) in 15 ml of MeOH were added 1,33 ml of HCl 10% . The mixture was stirred for 15 minutes at room temperature and then were added 150 mg of Pd/C at 5% and hydrogenated at 1 atm at room temperature for 2h until the complete transformation of the starting material as revealed by TLC[198].
The catalyzer was filtered under vacuum and the mother liquids were evaporated under vacuum. The resulting oil was diluted with 20 ml of $NaHCO_3$ satured solution and extracted with ethyl acetate (3 x 20 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 550 mg of a transparent oil corresponding at the desired product.

Yield = 91,67%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,82 (dd, 1H, J= 4,8 J= 1,5 Hz) 7,22 (dd, 1H, J= 7,8 J= 1,5 Hz) 6,89 (dd, 1H, J=7,8 J= 4,6 Hz) 4,49 (m, 1H) 4,30 (m, 2H) 3,90 (m, 2H) 1,99 (bs, 1H).

[198] Ethyl Acetate Rf start= 0,58 Rf prod= 0,32 Marker: Blue sheet

2-Mesyloxymethyl-2,3-dihydro-1,4-dioxin[2,3-b]pyridine



Chemical Formula: $C_8H_9NO_3$
Molecular Weight: 167,16

Chemical Formula: $C_9H_{11}NO_5S$
Molecular Weight: 245,25

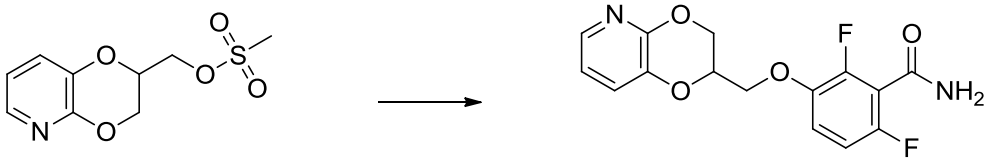
To a solution of 2-hydroxymethyl-2,3-dihydro-1,4-diossin[2,3-b]pyridine (550 mg, 3,35 mmol) in 15 ml of DCM were added 0,55 ml of TEA (4,02 mmol). The mixture was cooled at 4°C and then slowly added dropwise 0,57 ml of methansulphonyl chloride (3,68 mmol), the reaction was stirred at 4°C for 15 minutes and after 30 minutes at room temperature.
The TLC control[199] revealed the complete transformation of the starting material.
The reaction was cooled at 4°C and were added dropwise 20 ml of saturated solution of $NaHCO_3$ and 10 ml of DCM. The separated organic layer was washed with water (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 775 mg of an yellow oil corresponding at the desired product.

Yield = 78,68%

^{1}H-NMR (CDCl3) δ (ppm): 7,87 (dd, 1H, J= 4,8 J= 1,5 Hz) 7,25 (m, 1H) 6,93 (dd, 1H, J=7,9 J= 4,8 Hz) 4,49 (m, 4H) 4,31 (dd, 1H, J= 12,2 J= 6,8 Hz) 3,10 (s, 3H).

[199] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,1 Rf prod= 0,13 Marker: $Ce(SO_4)_2$

3-(2-Methoxyl-2,3-dihydro-1,4-dioxin[2,3-b]pyridine)-2,6-difluorobenzamide



Chemical Formula: $C_9H_{11}NO_5S$
Molecular Weight: 245,25

Chemical Formula: $C_{15}H_{12}F_2N_2O_4$
Molecular Weight: 322,26

To a solution of 2,6-difluoro-3-hydroxybenzamide (545 mg, 3,16 mmol) in 3 ml of DMF were added 435 mg of anhydrous K_2CO_3 (3,16 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 2-mesyloxymethyl-2,3-dihydro-1,4-diossin[2,3-b]pyridine (775 mg, 3,16 mmol) in 4,5 ml of DMF were added. The reaction was stirred for 15 minutes at room temperature and then brought at 65°C for 8h until the complete transformation of the starting material as revealed by TLC[200].
The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,01 g of a green solid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (toluene /ethyl acetate 1/1[201]) to give 570 mg of an ivory wax corresponding at the desired product.

Yield = 55,88%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,85 (dd, 1H, J= 4,8 J= 1,6 Hz) 7,22 (m, 1 H) 7,07 (td, 1H, J= 9,1 J= 5,1 Hz) 6,90 (m, 2H) 6,01 (bs, 1H) 4,58 (m, 2H) 4,42 (dd, 1H, J= 12,1 J= 7 Hz) 4,27 (m, 2H).

[200]Toluene/ Ethyl Acetate 1/1 Rf start= 0,33 Rf amid= 0,25 Rf prod= 0,13 Marker: $Ce(SO_4)_2$
[201]Toluene/ Ethyl Acetate 1/1 Rf start= 0,33 Rf amid= 0,25 Rf prod= 0,13 Marker: $Ce(SO_4)_2$

1,3-Dibenzyloxy-2-propanol

Chemical Formula: C_7H_8O
Molecular Weight: 108,14

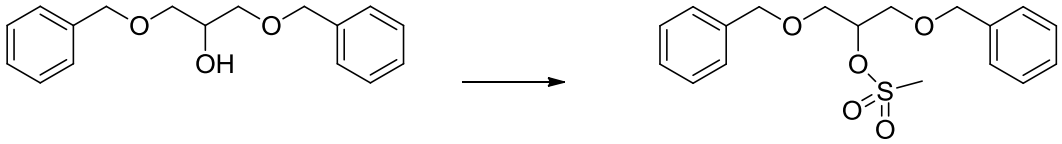
Chemical Formula: $C_{17}H_{20}O_3$
Molecular Weight: 272,34

To a solution of benzyl alcohol (9,3 g, 86,02 mmol) in 35 ml of NaOH 10 M were added 0,45 g of TBAB (1,39 mmol), the mixture was cooled at 4°C and then 2,18 ml of epichloridrine (27,75 mmol) were added dropwise. The mixture was stirred for 15 minutes at 4°C and after overnight at room temperature.
The NMR control revealed the formation of the product.
The reaction mixture was poured in 100 ml of 4°C cooled water, the aqueous phase was extracted with toluene (3 x 50 ml). The organic phase was washed with brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 10,5 g of an yellow liquid corresponding at the crude product.
The crude product were purified by distillation at 155°C and 2,5 mbar to give 5,17 g of a transparent liquid corresponding at the desired product.

Yield = 68,38%

^{1}H-NMR (CDCl3) δ (ppm): 7,31 (m, 10H) 4,55 (s, 4H) 4,03 (m, 1H) 3,55 (m, 4H) 3,19 (m, 1H) 2,45 (bs, 1H).

1,3-Dibenzyloxy-2-mesyloxy-propane



Chemical Formula: $C_{17}H_{20}O_3$
Molecular Weight: 272,34

Chemical Formula: $C_{18}H_{22}O_5S$
Molecular Weight: 350,43

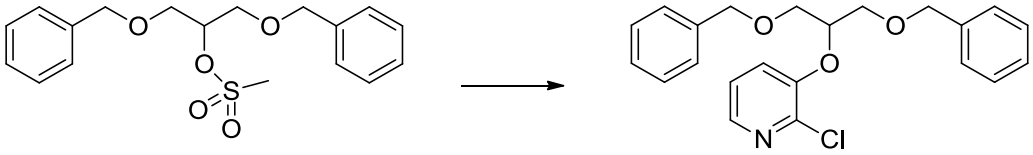
To a solution of 1,3-dibenzyloxy-2-propanol (2,59 g, 9,51 mmol) in 25 ml of DCM were added 1,63 ml of TEA (11,60 mmol). The mixture was cooled at 4°C and then 0,88 ml of methansulphonyl chloride (11,36 mmol) were slowly added dropwise, the reaction was stirred at 4°C for 15 minutes and for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[202].
The reaction was cooled at 4°C and were quenched with 20 ml of water and 10 ml of DCM. The separated organic layer was washed with HCl 10% (30 ml), $NaHCO_3$ saturated solution (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,16 g of an transparent liquid corresponding at the desired product.

Yield = 94,89%

^{1}H-NMR (CDCl3) δ (ppm): 7,31 (m, 10H) 4,92 (m, 1H) 4,55 (m, 4H) 3,71 (m, 4H) 3,05 (s, 3H).

[202] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,17 Rf prod= 0,23 Marker: Blue sheet

1,3-Dibenzyloxy-2-(2'-chlore-3'-pyridiniloxyl)propane



Chemical Formula: $C_{18}H_{22}O_5S$
Molecular Weight: 350,43

Chemical Formula: $C_{22}H_{22}ClNO_3$
Molecular Weight: 383,87

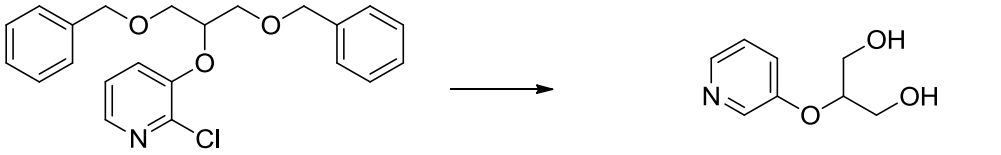
Under nitrogen atmosphere to a solution of 2-chloro-3-pyridinol (1,16 g, 8,93 mmol) in 12 ml of DMF were added 1,23 g of anhydrous K_2CO_3 (8,93 mmol). The mixture was stirred for 15 minutes and then a solution of 1,3-dibenzyloxy-2-mesyloxy-propane (3,13 g in 12 ml of DMF) were added dropwise. The reaction was stirred for 15 minutes at room temperature and brought at 140°C for 3 h until the complete transformation of the starting material as revealed by TLC[203].
The reaction mixture was evaporated under vacuum and were added 30 ml of water and 30 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (25 ml), the combined organic phases were washed twice with NaOH 1 M (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,16 g of an green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane /ethyl acetate 80/20[204]) to give 2,20 g of an transparent liquid corresponding at the desired product.

Yield = 63,95%

^{1}H-NMR (CDCl3) δ (ppm): 8,00 (dd, 1H, J= 4,7 J= 1,6 Hz) 7,40 (dd, 1H, J= 8,2 J= 1,6 Hz) 7,31 (m, 10H) 7,12 (dd, 1H, J= 8,2 J= 4,7 Hz) 4,59 (m, 5H) 3,77 (m, 4H).

[203]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,40 Rf prod= 0,48 Marker: Blue sheet
[204]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,26 Rf prod= 0,31 Marker: Blue sheet

2-(3'-Pyridinyloxyl)-1,3-propandiol



Chemical Formula: $C_{22}H_{22}ClNO_3$
Molecular Weight: 383,87

Chemical Formula: $C_8H_{11}NO_3$
Molecular Weight: 169,18

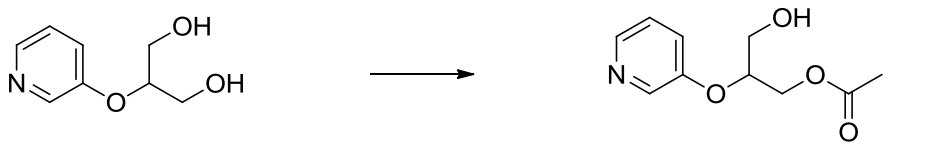
To a solution of 1,3-dibenzyloxy-2-(2'-chlore-3'-pyridiniloxyl)propane (2,2 g, 5,72 mmol) in 22 ml of MeOH were added 2,0 ml of HCl 10% . The mixture was stirred for 15 minutes at room temperature and then were added 250 mg of Pd/C at 5% and hydrogenated at room temperature at 1 atm for 2h until the complete transformation of the starting material as revealed by TLC[205].
The catalyzer was filtered, at the mother liquids were added 7,5 ml of satured solution of $NaHCO_3$ and The reaction mixture was evaporated under vacuum. At the resulting oil were added 20 ml of iPrOH and the inorganic salts precipitate. The suspension was cooled at 4°C and filtered in vacuum. The mother liquids were evaporated under vacuum to give 0,95 g of an yellow oil corresponding at the desired product.

Yield = 98,24%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,39 (d, 1H, J= 1,5 Hz) 8,20 (d,1H, J= 4,1Hz) 7,64 (dd, 1H, J= 1,5 Hz J= 7,8 Hz) 7,45 (dd, 1H, J= 7,8 J= 4,1 Hz) 4,41 (m, 1H) 3,52 (m, 4H).

[205] Cyclohexane / Ethyl Acetate 80/20 Rf start= 0,30 Rf prod= 0,05 Marker: Blue sheet

3-Acetoxy-2-(3'-pyridiniloxyl)-1-propanol



Chemical Formula: $C_8H_{11}NO_3$
Molecular Weight: 169,18

Chemical Formula: $C_{10}H_{13}NO_4$
Molecular Weight: 211,21

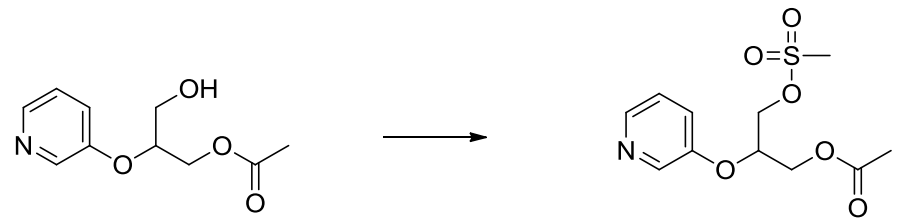
To a solution of 2-(3'-pyridinyloxyl)-1,3-propandiol (0,95 g, 5,61 mmol) in 3 ml of DMF were added 3,56 ml of trimethylortoacetate (28,37 mmol) and 140 mg of paratoluensulphonic acid (0,71 mmol). The mixture was stirred for 15 minutes at room temperature and then brought at 55°C for 1h e 30 minutes until the complete transformation of the starting material as revealed by TLC[206].
The reaction mixture was evaporated under vacuum and diluted with 20 ml ethyl acetate and 15 ml of saturated solution of $NaHCO_3$. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,05 g of a transparent oil.
The oil was dissolved in 10 ml of MeOH and 5 ml of HCl 10% were slowly added. The mixture was stirred for 30 minutes until the complete transformation of the starting material as revealed by TLC[207].
The reaction mixture was evaporated under vacuum, added satured solution of $NaHCO_3$ until pH 8 and extracted with ethyl acetate (3 x 15 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 600 mg of a yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (ethyl acetate[208]) to give 330 mg of an yellow oil corresponding at the desired product.

Yield = 27,85 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 8,37 (d, 1H, J= 2,9 Hz) 8,25 (dd, 1H, J= 4,6 J= 1,4 Hz) 7,34 (ddd, 1H, J= 8,2 J= 2,9 J= 1,6 Hz) 7,25 (m, 1H) 4,58 (m, 1H) 4,40 (m, 1H) 4,29 (m, 1H) 3,87 (m, 2H).

[206] Ethyl Acetate Rf start= 0,46 Rf inter= 0,28 Rf prod= 021 Marker: $Ce(SO_4)_2$
[207] Ethyl Acetate Rf start= 0,46 Rf inter= 0,28 Rf prod= 021 Marker: $Ce(SO_4)_2$
[208] Ethyl Acetate Rf start= 0,46 Rf inter= 0,28 Rf prod= 021 Marker: $Ce(SO_4)_2$

3-Acetoxy-2-(3'-pyridiniloxyl)-1-mesyloxypropane



Chemical Formula: $C_{10}H_{13}NO_4$
Molecular Weight: 211,21

Chemical Formula: $C_{11}H_{15}NO_6S$
Molecular Weight: 289,30

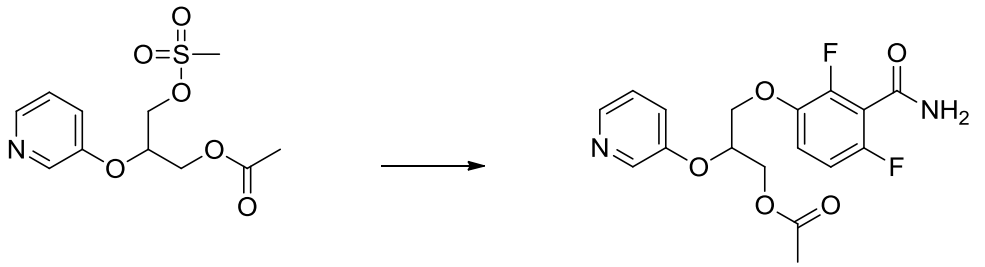
To a solution of 3-acetoxy-2-(3'-pyridiniloxyl)-1-propanol (330 mg, 1,56 mmol) in 25 ml of DCM were added 0,27 ml of TEA (1,87 mmol). The mixture was cooled at 4°C and then 0,13 ml of methansulphonyl chloride (1,72 mmol) were slowly added dropwise, the reaction was stirred at 4°C for 15 minutes and for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[209].
The reaction mixture was cooled at 4°C and quenched with 10 ml of water, 10 ml of satured solution of $NaHCO_3$ and 10 ml of DCM. The separated aqueous layer was extracted twice with DCM (10 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 410 mg of an green oil corresponding at the desired product.

Yield = 90,31 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 8,40 (d, 1H, J= 2,9 Hz) 8,31 (dd, 1H, J= 4,6 J= 1,4 Hz) 7,34 (ddd, 1H, J= 8,2 J= 2,9 J= 1,4 Hz) 7,28 (m, 1H) 4,76 (m, 1H) 4,47 (m, 2H) 4,34 (qd, 1H, J= 12,0 J= 5,3 Hz) 3,06 (s, 3H).

[209] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,17 Rf prod= 0,23 Marker: Blue sheet

3-(3-Acetoxy-2-(3'-pyridiniloxyl)-1-propanyl)-2,6-difluorobenzamide



Chemical Formula: $C_{11}H_{15}NO_6S$
Molecular Weight: 289,30

Chemical Formula: $C_{17}H_{16}F_2N_2O_5$
Molecular Weight: 366,32

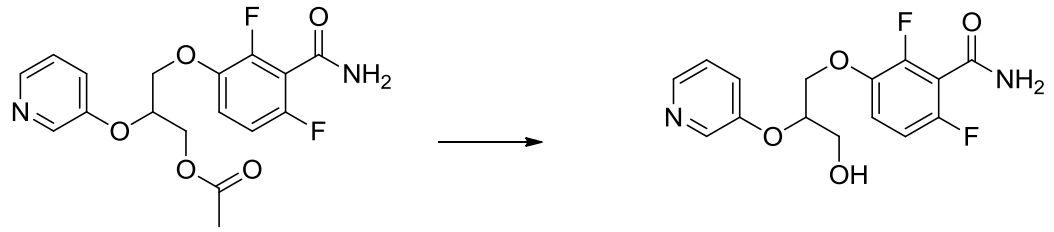
To a solution of 2,6-difluoro-3-hydroxybenzamide (246 mg, 1,42 mmol) in 3 ml of DMF were added 138 mg of anhydrous K_2CO_3 (1,42 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 3-Acetoxy-2-(3'-pyridiniloxyl)-1-mesyloxypropane (410 mg, in 3 ml of DMF) were added. The reaction was stirred for 30 minutes at room temperature and then brought at 55°C overnight.
The TLC control[210] revealed the consumption of the starting material.
The reaction mixture was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed twice with NaOH 1 M (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 500 mg of a white wax corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 75/25[211]) to give 380 mg of an yellow oil corresponding at the desired product.

Yield = 73,07 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 8,38 (s, 1H) 8,28 (d, 1H, J= 2,9 Hz) 7,37 (d, 1H, J= 8,7 Hz) 7,17 (d, 1H, J= 7,8 Hz) 7,04 (dt, 1H, J= 9,0 J= 5,1 Hz) 6,88 (dt, 1H, J= 10 J= 2,5 Hz) 6,05 (s,1H) 5,97 (s,1H) 4,83 (m, 1H) 4,43 (d, 2H, J= 5,2 Hz) 4,28 (d, 1H, J= 4,8 Hz).

[210]Toluene / Ethyl Acetate 1/1 Rf start= 0,45 Rf amid= 0,5 Rf prod= 0,33 Marker: $Ce(SO_4)_2$
[211]Toluene / Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,15 Marker: $Ce(SO_4)_2$

3-(3-Hydroxy-2-(3'-pyridiniloxyl)-1-propanyl)-2,6-difluorobenzamide



Chemical Formula: $C_{17}H_{16}F_2N_2O_5$
Molecular Weight: 366,32

Chemical Formula: $C_{15}H_{14}F_2N_2O_4$
Molecular Weight: 324,28

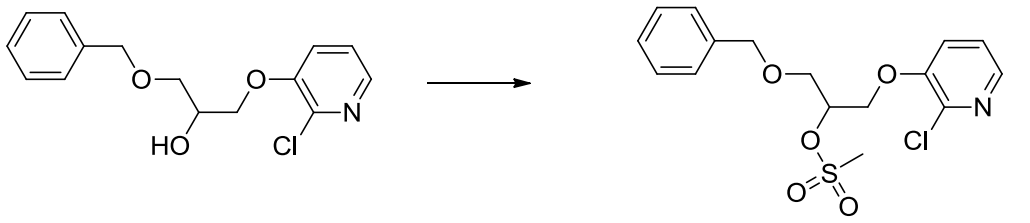
To a solution of 3-(3-acetoxy-2-(3'-pyridiniloxyl)-1-propanyl)-2,6-difluorobenzamide (380 mg, 1,04 mmol) in 5 ml of MeOH were added 0,6 ml of NaOH 2,5 M (1,42 mmol). The mixture was stirred for 1h at room temperature until the complete transformation of the starting material as revealed by TLC[212].
At the reaction mixture 0,6 ml of HCl 10% were added and evaporated under vacuum. Diluted with 5 ml of iPrOH, the inorganic salts precipitate and they are filtered. The mother liquids are evaporated under vacuum to give 320 mg of a yellow oil corresponding at the desired product.

Yield = 94,95 %

^{1}H-NMR (d6-DMSO) δ (ppm): 8,53 (d, 1H, J= 2,5 Hz) 8,30 (d, 1H, J= 4,8 Hz) 7,87 (m, 2H) 7,60 (m, 2H) 7,04 (dt, 1H, J= 9,2 J= 5,2 Hz) 6,88 (t, 1H, J= 8,8 Hz) 4,85 (m, 1H) 4,34 (dd, 1H, J= 10,7 J= 3,1 Hz) 4,22 (dd, 1H, J= 10,7 J= 6,7 Hz) 3,76 (d, 1H, J=5,6 Hz).

[212]Toluene / Ethyl Acetate 1/1 Rf start= 0,45 Rf amid= 0,25 Rf prod= 0,33 Marker: $Ce(SO_4)_2$

1-Benzyloxy-3-((2-chloropyridin-3-yl)oxy)-2-mesyloxypropane



Chemical Formula: $C_{15}H_{16}ClNO_3$
Molecular Weight: 293,75

Chemical Formula: $C_{16}H_{18}ClNO_5S$
Molecular Weight: 371,84

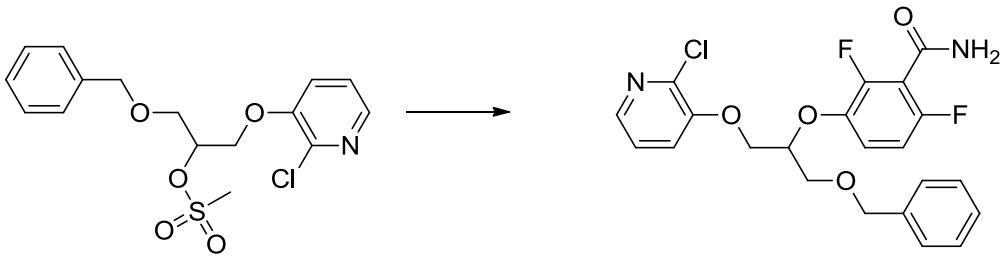
To a solution of 1-(benzyloxy)-3-((2-chloropyridin-3-yl)oxy)propan-2-ol (1,0 g, 3,40 mmol) in 20 ml of DCM were added 0,52 ml of TEA (3,74 mmol). The mixture was cooled at 4°C and then 0,28 ml of methansulphonyl chloride (3,74 mmol) were slowly added dropwise. The reaction was stirred at 4°C for 15 minutes and for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[213].
The reaction mixture was cooled at 4°C and quenched with 20 ml of water, 10 ml of satured solution of $NaHCO_3$ and 10 ml of DCM. The separated organic layer was washed twice with brine (20 ml), were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,23 g of a transparent oil corresponding at the desired product.

Yield = 96,82 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 8,01 (dd, 1H, J= 4,1 J= 2,2 Hz) 7,33 (m, 5H) 7,22 (m,2H) 5,10 (m, 1H) 4,58 (m, 2H) 4,29 (m, 2H) 3,87 (m, 2H) 3,15 (s, 3H).

[213] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,29 Rf prod= 0,40 Marker: $Ce(SO_4)_2$

3-(1-Benzyloxy-3-((2-chloropyridin-3-yl)oxy)-2-propanyl)-2,6-difluorobenzamide



Chemical Formula: $C_{16}H_{18}ClNO_5S$
Molecular Weight: 371,84

Chemical Formula: $C_{22}H_{19}ClF_2N_2O_4$
Molecular Weight: 448,85

To a solution of 2,6-difluoro-3-hydroxybenzamide (330 mg, 1,95 mmol) in 3 ml of DMF were added 270 mg of anhydrous K_2CO_3 (1,95 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 1-benzyloxy-3-((2-chloropyridin-3-yl)oxy)-2-mesyloxypropane (620 mg, in 3 ml of DMF) were added. The reaction was stirred for 30 minutes at room temperature and then brought at 100°C for 2h until the complete transformation of the starting material as revealed by TLC[214].

The reaction was evaporated under vacuum and diluted with 15 ml of ethyl acetate and 15 ml of distilled water. The separated aqueous layer were extracted with ethyl acetate (15 ml). The combined organic phases were washed twice with NaOH 1 M (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 580 mg of a yellow oil corresponding at the crude product.

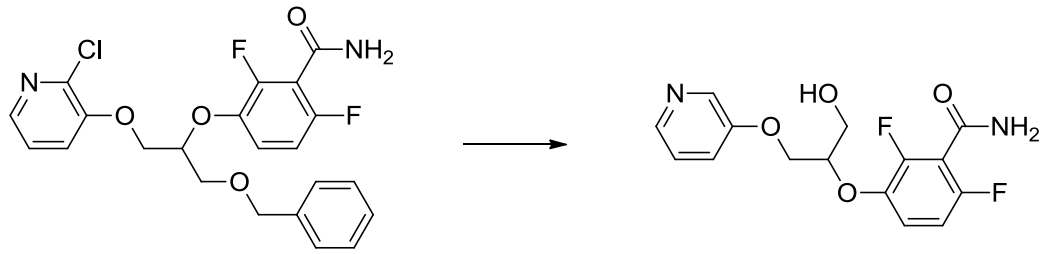
The crude product were purified by flash chromatography on silica gel (toluene/ethyl acetate 60/40[215]) to give 370 mg of an green oil corresponding at the desired product.

Yield = 42,27 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 8,03 (dd, 1H, J= 4,3 J= 2,1 Hz) 7,32 (m, 7H) 6,87 (dt, 1H, J= 10 J= 2,5 Hz) 5,9 (bs,2H) 4,65 (m, 3H) 4,35 (d, 2H, J= 5,2 Hz) 3,85 (d, 1H, J= 4,8 Hz).

[214]Toluene / Ethyl Acetate 1/1 Rf start= 0,51 Rf amid= 0,25 Rf prod= 0,29 Marker: $Ce(SO_4)_2$
[215]Toluene / Ethyl Acetate 60/40 Rf start= 0,44 Rf prod= 0,25 Marker: $Ce(SO_4)_2$

3-(1-Hydroxy-3-(3-pyridinyloxy)-2-propanyl)-2,6-difluorobenzamide



Chemical Formula: $C_{22}H_{19}ClF_2N_2O_4$
Molecular Weight: 448,85

Chemical Formula: $C_{15}H_{14}F_2N_2O_4$
Molecular Weight: 324,28

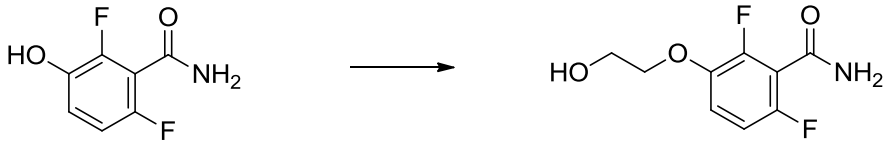
To a solution of 3-(1-benzyloxy-3-((2-chloropyridin-3-yl)oxy)-2-propanyl)-2,6-difluorobenzamide (370 mg, 0,82 mmol) in 6 ml of MeOH were added 60 mg of Pd/C at 5% and hydrogenated at room temperature at 1 atm overnight.
The TLC[216] control revealed the complete transformation of the starting material.
The catalyzer was filtered, at the mother liquids were added satured solution of $NaHCO_3$ until pH 8 and The reaction mixture was evaporated under vacuum. At the resulting oil were added 8 ml of iPrOH and the inorganic salts precipitate. The suspension was filtered. The mother liquids were evaporated under vacuum to give 200 mg of an yellow oil corresponding at the desired product.
The crude product were purified by flash chromatography on silica gel (toluene/acetone 1/1[217]) to give 150 mg of an transparent oil corresponding at the desired product.

Yield = 56,18%

^{1}H-NMR (d6-DMSO) δ (ppm): 8,29 (d, 1H, J= 4,3) 8,15 (dd, 1H, J=7,6 J= 1,5Hz) 8,10 (s, 1H) 7,82 (s, 1H) 7,35 (m, 3H) 7,15 (dt, 1H, J= 9,5 J= 5,2 Hz) 4,61 (m, 1H) 4,59 (m, 2H) 3,70 (d, 1H, J= 4,9 Hz).

[216] Toluene / Ethyl Acetate 1/1 Rf start= 0,31 Rf prod= 0,05 Marker: $Ce(SO_4)_2$
[217] Toluene Acetone 1/1 Rf start= 0,62 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(2-hydroxyethoxy)benzamide



Chemical Formula: $C_7H_5F_2NO_2$
Molecular Weight: 173,12

Chemical Formula: $C_9H_9F_2NO_3$
Molecular Weight: 217,17

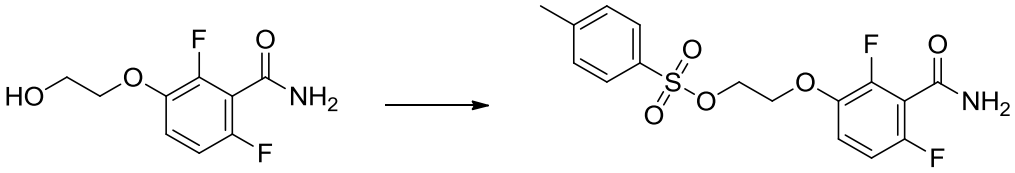
To a solution of 2,6-difluoro-3-hydroxybenzamide (1,0 g, 5,78 mmol) in 10 ml of DMF were added 800 mg of K_2CO_3(5,78 mmol). The mixture was stirred for 15 minutes at room temperature and then 0,41 ml of 2-bromethanol (11,56 mmol) were added dropwise, and stirred at 80°C for 6 h until the complete transformation of the starting material as revealed by TLC[218].
The reaction was evaporated under vacuum and then diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer was extracted with ethyl acetate (3 x 10 ml), the combined organic phases were washed twice with NaOH 1 M (20 ml)of and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,5 g of an brown wax corresponding at the desired product.

Yield = 50,0 %

^{1}H-NMR (CD_3OD) δ (ppm): 7,18 (dt, 1H, J= 9,2 J= 5,1 Hz) 6,89 (dt, 1H, J= 9,5 J= 2,5 Hz) 4,87 (s, 2H) 4,11 (t, 2H, J= 6,7 Hz) 3,84 (t, 2H, J= 6,7 Hz).

[218] Ethyl Acetate Rf start= 0,60 Rf prod= 0,33 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(2-tosyloxyethoxy)benzamide



Chemical Formula: $C_9H_9F_2NO_3$
Molecular Weight: 217,17

Chemical Formula: $C_{16}H_{15}F_2NO_5S$
Molecular Weight: 371,36

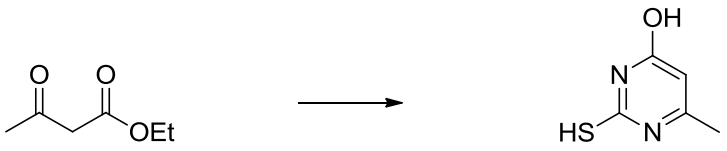
To a solution of 2,6-difluoro-3-(2-hydroxyethoxy)benzamide (500 mg, 2,30 mmol) in 2,5 ml of pyridine cooled at 4°C were added 231 mg of paratoluensulphonylchloride (2,42 mmol). The mixture was stirred for 15 minutes at 4°C and then for 1 h at room temperature until the complete transformation of the starting material as revealed by TLC[219].
The reaction mixture was cooled at 4°C and diluted with 10 ml of ethyl acetate and 10 ml of HCl 10%. The separated aqueous layer was extracted twice with ethyl acetate (10 ml), the combined organic phases were washed with $NaHCO_3$ saturated solution (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 700 mg of an yellow oil corresponding at the desired product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[220]) to give 300 mg of an transparent oil corresponding at the desired product.

Yield = 36,67 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,80 (d, 2H, J= 8,8 Hz) 7,37 (d, 2H, J= 8,8 Hz) 6,99 (dt, 1H, J= 9,1 J= 5,1 Hz) 6,86 (dt, 1H, J= 11,2 J= 2,3 Hz) 6,02 (bs, 1H) 5,86 (bs, 1H) 4,38 (m, 2H) 4,21 (m, 2H).

[219] Cyclohexane / Ethyl Acetate 1/1 Rf start= 0,07 Rf prod= 0,15 Marker: $Ce(SO_4)_2$
[220] Cyclohexane / Ethyl Acetate 1/1 Rf start= 0,07 Rf prod= 0,15 Marker: $Ce(SO_4)_2$

2-Mercapto-4-hydroxy-6-methylpyrimidine



Chemical Formula: $C_6H_{10}O_3$
Molecular Weight: 130,14

Chemical Formula: $C_5H_6N_2OS$
Molecular Weight: 142,18

To 50 ml of absolute ethanol were added 0,88 g sodium (38,42 mmol) cutted in small pieces. When all the pieces are consumed 2,45 ml of ethyl acetoacetate (19,21 mmol) were slowly added. The mixture was stirred for 15 minutes at room temperature and then were added 2,05 g of thiourea (26,89 mmol). The mixture was stirred at reflux for 3h and 30 minutes until the complete transformation of the starting material as revealed by TLC[221].
The reaction mixture was evaporated under vacuum and then diluted with 30 ml of water The mixture was cooled at 4°C and after the addition of 14,5 ml of HCl 10%, a precipitate afforded. The suspension was filtered and washed with 10 ml of cold water. The solid was dried in pump to give 2,35 g of a white solid corresponding at the desired product.

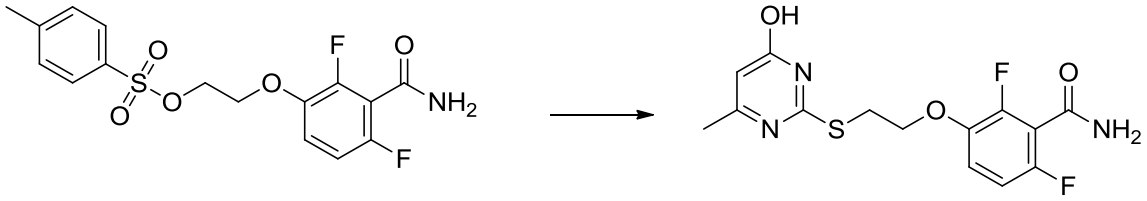
Yield = 86,08 %

M.P.= 107,73°C

^{1}H-NMR (d6-DMSO) δ (ppm): 12,28 (bs, 2H) 5,63 (s, 1H) 2,05 (s, 3H).

[221] Cyclohexane / Ethyl Acetate 1/1 Rf start= 0,44 Rf prod= 0,10 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(2-((4-hydroxy-6-methylpyrimidin-2-yl)thio)ethoxy)benzamide



Chemical Formula: $C_{16}H_{15}F_2NO_5S$
Molecular Weight: 371,36

Chemical Formula: $C_{14}H_{13}F_2N_3O_3S$
Molecular Weight: 341,33

To a solution of 2-mercapto-4-hydroxy-6-methylpyrimidine (114 mg, 0,80 mmol) in 4 ml di DMF were added 110 mg of anhydrous K_2CO_3 (0,80 mmol). The mixture was stirred for 15 minutes at room temperature and were added a solution of 2,6-difluoro-3-(2-tosyloxyethoxy)benzamide (300 mg in 4 ml of DMF) and brought at 55°C for 4 h until the complete transformation of the starting material as revealed by TLC[222].

The reaction mixture was evaporated under vacuum and then diluted 10 ml of water and 10 ml of ethyl acetate. The separated aqueous layer was extracted with ethyl acetate (3 x 10 ml), the combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,35 g of an white solid corresponding at the crude product.

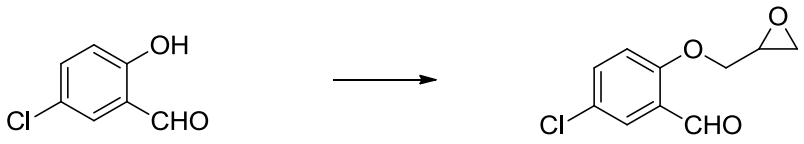
The crude product were purified by flash chromatography on silica gel (ethyl acetate[223]) to give 110 mg of a white solid corresponding at the desired product.

Yield = 40,44 %

^{1}H-NMR (d6-DMSO) δ (ppm): 8,17 (s, 1H) 7,84 (s, 1H) 7,36 (dt, 1H, J= 9,1 J= 5,1 Hz) 7,15 (dt, 1H, J= 11,2 J= 2,3 Hz) 6,05 (s, 1H) 4,31 (t, 2H, J= 7,5 Hz) 3,40 (m, 3H) 2,20 (s, 3H).

[222] Ethyl Acetate Rf start= 0,53 Rf merc= 0,40 Rf prod= 0,10 Marker: $Ce(SO_4)_2$
[223] Ethyl Acetate Rf start= 0,53 Rf merc= 0,40 Rf prod= 0,10 Marker: $Ce(SO_4)_2$

3-(2-Carbossialdehyde-4-chloro-1-fenoxy)-1,2-epoxypropane



Chemical Formula: $C_7H_5ClO_2$
Molecular Weight: 156,57

Chemical Formula: $C_{10}H_9ClO_3$
Molecular Weight: 212,63

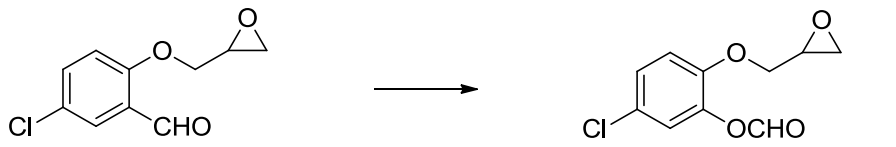
To a solution of 5-chloro-salicilaldehyde (1,5 g, 9,58 mmol) in 30 ml of DMF were added 1,32 g of K_2CO_3(9,58 mmol). The mixture was stirred for 30 minutes at room temperature and then 3,8 ml of epichloridrine (47,9 mmol) were added dropwise, and stirred at room temperature overnight.
The TLC control[224] revealed the complete transformation of the starting material.
The reaction mixture was evaporated under vacuum and then diluted with 30 ml of ethyl acetate and 30 ml of distilled water. The separated organic layer was washed twice with NaOH 1 M (30 ml)of and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,35 g of an brown oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[225]) to give 0,90 g of a yellow liquid corresponding at the desired product.

Yield = 44,11%

^{1}H-NMR (CDCl3) δ (ppm): 10,45 (s, 1H) 7,79 (d, 1H, J= 2,8 Hz) 7,48 (dd, 1H, J= 8,9 J= 2,8 Hz) 6,96 (d, 1H, J= 8,9 Hz) 4,41 (dd, 1H, J= 11,2 J= 2,7 Hz) 4,03 (dd, 1H, J= 11,2 J= 5,9 Hz) 3,40 (m, 1H) 2,95 (m, 1H) 2,79 (dd, 1H, J= 4,8 J= 2,7 Hz).

[224] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,50 Rf prod= 0,20 Rf Imp= 0,35 Marker: $Ce(SO_4)_2$
[225] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,40 Rf prod= 0,15 Marker: $Ce(SO_4)_2$

3-(2-Formyloxy-4-chloro-1-fenoxy)-1,2-epoxypropane



Chemical Formula: $C_{10}H_9ClO_3$
Molecular Weight: 212,63

Chemical Formula: $C_{10}H_9ClO_4$
Molecular Weight: 228,63

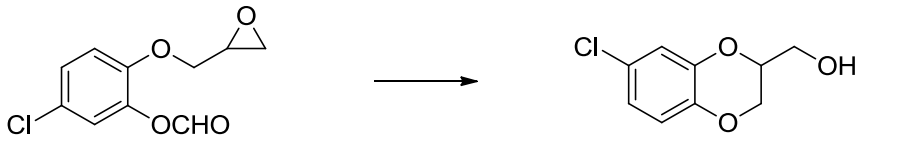
To a refluxing solution of 3-(2-carbossialdehyde-4-chloro-1-fenoxy)-1,2-epoxipropane (0,9 g, 4,23 mmol) in 12 ml of DCM 1,33 g of *m*-chloroperbenzoic acid (5,93 mmol) were added in three portion. The mixture was stirred for 2h and refluxed for 30 minutes until the complete transformation of the starting material as revealed by TLC[226].
The reaction was cooled at 4°C and quenched with 10 ml of 10% solution of $Na_2S_2O_5$ and 10 ml of $NaHCO_3$ saturated solution. A white precipitate afforded and the suspension was filtered in vacuum. The mother liquids were extracted with ethyl acetate (3 x 10 ml). The combined organic phases was washed with $NaHCO_3$ saturated solution (3 x 30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,95 g of an yellow oil corresponding at the desired product.

Yield = 97,93%

^{1}H-NMR (CDCl3) δ (ppm): 7,20 (dd, 1H, J= 8,8 J= 2,6 Hz) 7,13 (d, 1H, J= 2,6 Hz) 6,96 (d, 1H, J= 8,8 Hz) 4,26 (dd, 1H, J= 11,3 J= 3 Hz) 3,97 (dd, 1H, J= 11,3 J= 5,7 Hz) 3,31 (m, 1H) 2,89 (dd, 1H, J= 4,8 J= 4,2 Hz) 2,79 (dd, 1H, J= 4,8 J= 2,6 Hz).

[226] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,26 Rf prod= 0,34 Marker: $Ce(SO_4)_2$

7-Chloro-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{10}H_9ClO_4$
Molecular Weight: 228,63

Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

To a solution of 3-(2-formyloxy-4-chloro-1-fenoxy)-1,2-epoxipropane (0,95 g, 4,15 mmol) in 10 ml of MeOH 2,27 ml of NaOH 2,5 M and 4 ml of water were added dropwise. The mixture was stirred for 15 minutes at room temperature, then was brought at 60°C for 1h until the complete transformation of the starting material as revealed by TLC[227].

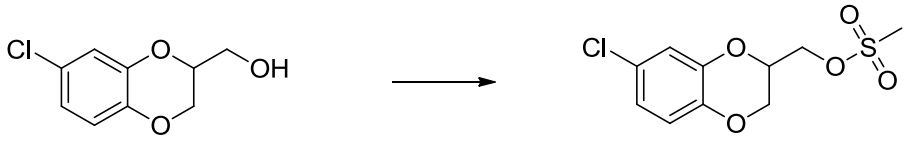
The reaction was evaporated under vacuum and diluted with10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer were extracted with ethyl acetate (3 x 10 ml). The combined organic phases were washed 30 ml of brine, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 580 mg of a yellow oil corresponding at the desired product.

Yield = 65,90%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,90 (m, 1H) 6,79 (m, 2H) 4,26 (m, 2H) 4,09 (m, 1H) 3,86 (m, 2H) 2,04 (bs, 1H).

[227]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 013 Rf prod= 0,17 Marker: $Ce(SO_4)_2$

7-Chloro-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

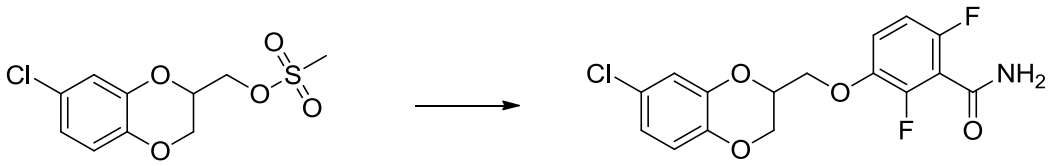
To a solution of 6-chloro-3-hydroxymethyl-1,4-benzodioxane (300 mg, 1,49 mmol) in 5 ml of DCM 0,28 ml of TEA (1,99 mmol) were added. The mixture was cooled at 4°C and then 0,15 ml of methansulphonyl chloride(1,94 mmol) were added dropwise, the reaction was stirred at 4°C for 15 minutes and after 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[228].
The reaction was cooled at 4°C and quenched with 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10 ml of HCl 10%, 10 ml of saturated solution of $NaHCO_3$ and 10 ml of brine, dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 400 mg of an transparent wax corresponding at the desired product.

Yield = 96,14%

^{1}H-NMR (CDCl3) δ (ppm): 6.91 (m, 1H) 6,84 (m, 2H) 4,47 (m, 2H) 4,31 (dd, 2H, J= 11,7 J= 2,3 Hz) 4,13 (dd, 1H, J=11,7 J=6,2 Hz) 3,10 (s, 3H).

[228] Cyclohexane/ Ethyl Acetate 95/5 Rf start= 0,06 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

3-(7-Chloro-1,4-benzodioxan-2-yl)metossi-2,6-difluorobenzamide



Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

Chemical Formula: $C_{16}H_{12}ClF_2NO_4$
Molecular Weight: 355,72

To a solution of 2,6-difluoro-3-hydroxybenzamide (273 mg, 1,58 mmol) in 3 ml of DMF were added 218 mg of anhydrous K_2CO_3 (1,58 mmol). The mixture was stirred for 15 minutes at room temperature, then were added a solution of 6-chloro-3-mesyloxymethyl-1,4-benzodioxane (400 mg, 1,44 mmol) in 3 ml of DMF. The reaction was stirred for 30 minutes at room temperature and then brought at 55°C overnight.
The TLC control[229] revealed the consumption of the starting material.
The reaction was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 540 mg of a white wax corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 75/25[230]) to give 320 mg of a white solid corresponding at the desired product.

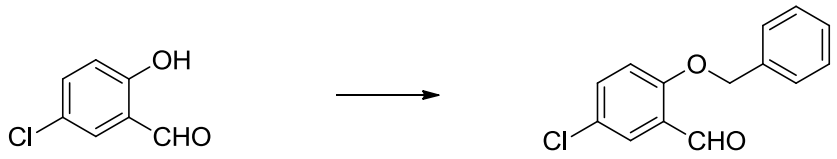
Yield = 62,5%

M.P.= 121,0°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,07 (td, 1H, J= 9,1 J= 5,1 Hz) 6,89 (m, 2H) 6,82 (m, 2H) 6,10 (bs, 1H) 6,01 (bs, 1H) 4,56 (m, 1H) 4,38 (m, 1H) 4,23 (m, 3H).

[229]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,42 Rf amid= 0,06 Rf prod= 0,13 Marker: $Ce(SO_4)_2$
[230]Cyclohexane/ Ethyl Acetate 75/25 Rf start= 0,45 Rf amid= 0,09 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

2-(Benzyloxy)-5-chlorobenzaldehyde



Chemical Formula: $C_7H_5ClO_2$
Molecular Weight: 156,57

Chemical Formula: $C_{14}H_{11}ClO_2$
Molecular Weight: 246,69

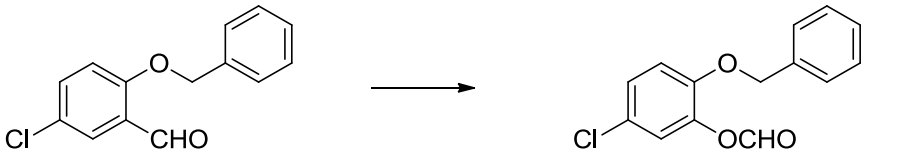
To a solution of 5-chloro-salicilaldehyde (1,5 g, 9,58 mmol) in 30 ml of DMF were added 1,32 g of K_2CO_3(9,58 mmol). The mixture was stirred for 30 minutes at room temperature and then 1,14 ml of benzyl bromide (9,58 mmol) were added dropwise, and stirred at room temperature overnight.
The TLC control[231] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and then diluted 30 ml of ethyl acetate and 50 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (30 ml). The combined organic phases were washed twice with NaOH 1 M (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,19 g of an white wax corresponding at the desired product.

Yield = 92,66%

^{1}H-NMR (CDCl3) δ (ppm): 10,48 (s, 1H) 7,81 (d, 1H, J= 2,8 Hz) 7,42 (m, 6H) 7,01 (d, 1H, J= 8,9 Hz) 5,19 (s, 2H).

[231] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,37 Rf prod= 0,31 Marker: $Ce(SO_4)_2$

2-(Benzyloxy)-5-chlorophenyl formate



Chemical Formula: $C_{14}H_{11}ClO_2$
Molecular Weight: 246,69

Chemical Formula: $C_{14}H_{11}ClO_3$
Molecular Weight: 262,69

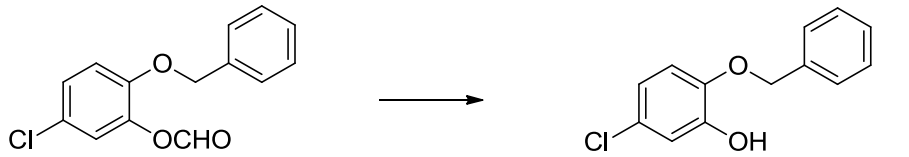
To a refluxing solution of 2-(benzyloxy)-5-chlorobenzaldehyde (2,19 g, 8,86 mmol) in 28 ml of DCM 2,78 g of *m*-chloroperbenzoic acid (12,4 mmol) were added in three portion. The mixture was stirred at reflux for 2h until the complete transformation of the starting material as revealed by TLC[232].
The reaction was cooled at 4°C and quenched with 15 ml of 10% solution of $Na_2S_2O_5$ and 15 ml of $NaHCO_3$ saturated solution. A white precipitate afforded, the suspension was filtered in vacuum. The mother liquids were extracted with ethyl acetate (3 x 15 ml). The organic phase was washed with $NaHCO_3$ saturated solution (3 x 30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,29 g of an yellow oil corresponding at the desired product.

Yield = 98,28%

^{1}H-NMR (CDCl3) δ (ppm): 8,24 (s, 1H) 7,37 (m, 5H) 7,16 (m, 2H) 6,95 (d, 1H, J= 8,7 Hz) 5,09 (s, 2H).

[232] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,30 Rf prod= 0,35 Marker: $Ce(SO_4)_2$

2-(Benzyloxy)-5-chlorophenol



Chemical Formula: $C_{14}H_{11}ClO_3$
Molecular Weight: 262,69

Chemical Formula: $C_{13}H_{11}ClO_2$
Molecular Weight: 234,68

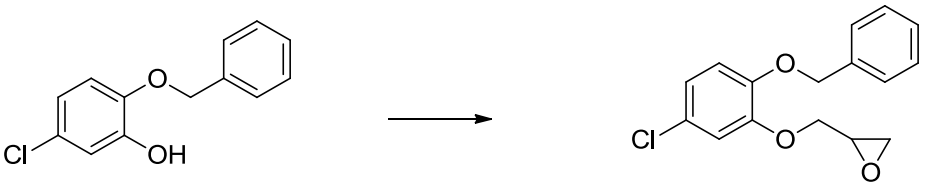
To a solution of 2-(benzyloxy)-5-chlorophenyl formate (2,29 g, 8,71 mmol) in 25 ml of MeOH at room temperature 4,6 ml of NaOH 2,5 M were added dropwise. The mixture was stirred at room temperature for 5h until the complete transformation of the starting material as revealed by TLC[233].
The reaction was evaporated under vacuum and diluted with 40 ml of NaH_2PO_4/Na_2HPO_4 pH 7 buffer. The separated aqueous layer was extracted with ethyl acetate (3 x 30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,02 g of an green liquid corresponding at the desired product.

Yield = 97,11%

^{1}H-NMR (CDCl3) δ (ppm): 7,39 (m, 5H) 6,95 (d, 1H, J= 2,2 Hz) 6,81 (m, 2H) 5,09 (s, 2H).

[233] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,35 Rf prod= 0,26 Marker: $Ce(SO_4)_2$

3-(2-(Benzyloxy)-5-chlorophenoxyl)-1,2-epoxypropane



Chemical Formula: $C_{13}H_{11}ClO_2$
Molecular Weight: 234,68

Chemical Formula: $C_{16}H_{15}ClO_3$
Molecular Weight: 290,74

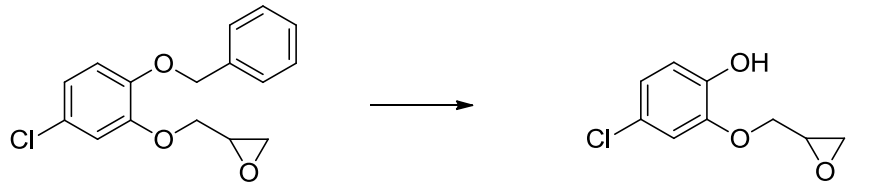
To a solution of 2-(benzyloxy)-5-chlorophenol (2,02 g, 8,59 mmol) in 30 ml of DMF were added 1,18 g of K_2CO_3(8,59 mmol). The mixture was stirred for 30 minutes at room temperature and then 2,02 ml of epichloridrine (25,78 mmol) were added dropwise, and the reaction was stirred at 40°C overnight.
The TLC control[234] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and then diluted 40 ml of ethyl acetate and 40 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (40 ml). The combined organic phases were washed twice with NaOH 1 M (40 ml) and brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,50 g of an dark green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[235]) to give 1,50 g of a pale yellow oil corresponding at the desired product.

Yield = 57,91%

^{1}H-NMR (CDCl3) δ (ppm): 7,34 (m, 5H) 6,94 (m, 1H) 6,85 (m, 2H) 5,11 (s, 2H) 4,28(dd, 1H, J= 11,7 J= 3,5 Hz) 4,00 (dd, 1H, J= 11,2 J= 5,2 Hz) 3,38 (m, 1H) 2,89 (m, 1H) 2,76 (m, 1H).

[234] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,28 Rf prod= 0,22 Marker: $Ce(SO_4)_2$
[235] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,28 Rf prod= 0,22 Marker: $Ce(SO_4)_2$

3-(4-Chloro-2-phenoxyl)-1,2-epoxypropane



Chemical Formula: $C_{16}H_{15}ClO_3$
Molecular Weight: 290,74

Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

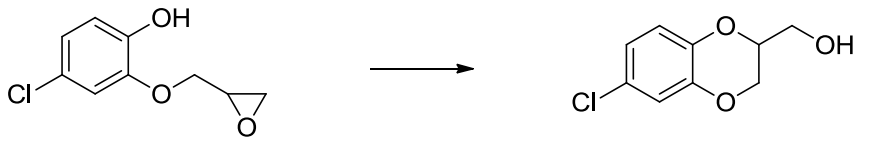
To a solution of 3-(2-(benzyloxy)-5-chlorophenoxyl)-1,2-epoxypropane (0,75 g, 2,57 mmol) in 16 ml of ethyl acetate were added 65 mg of Pd/C at 5%. The mixture was hydrogenated at 1 atm for 6 h until the complete transformation of the starting material as revealed by TLC[236].
The catalyzer was filtered, the mother liquids were evaporated under vacuum to give 450 mg of a yellow transparent oil corresponding at the desired product.

Yield = 88,23%

^{1}H-NMR (CDCl3) δ (ppm):) 6,88 (m, 3H) 4,32 (dd, 1H, J= 11,3 J= 2,6 Hz) 4,00 (dd, 1H, J= 11,3 J= 5,8 Hz) 3,38 (ddd, 1H, J= 5,8 J= 2,9 J= 1,6 Hz) 2,96 (m, 1H) 2,76 (dd, 1H, J= 4,7 J= 2,9 Hz).

[236] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,37 Rf prod= 0,22 Marker: $Ce(SO_4)_2$

6-Chloro-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

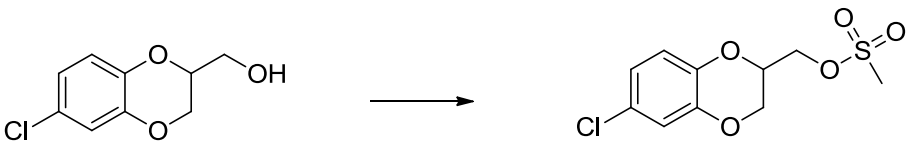
To a solution of 3-(4-chloro-2-phenoxyl)-1,2-epoxypropane (450 mg, 2,24 mmol) in 6 ml of MeOH 1,15 ml of NaOH 2,5 M were added dropwise. The mixture was stirred overnight at room temperature.
The TLC control[237] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and diluted with 10 ml of water and 10 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 420 mg of a transparent wax corresponding at the desired product.

Yield = 93,54%

^{1}H-NMR (CDCl3) δ (ppm):) 6,89 (t, 1H, J= 1,4 Hz) 6,82 (d, 1H, J= 1,4 Hz) 4,25 (m, 1H) 4,10 (dd, 1H, J= 11,1 J= 7,5 Hz) 3,87 (qd, 1H, J= 12,1 J= 4,5 Hz).

[237] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,22 Rf prod= 0,20 Marker: $Ce(SO_4)_2$

6-Chloro-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9ClO_3$
Molecular Weight: 200,62

Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

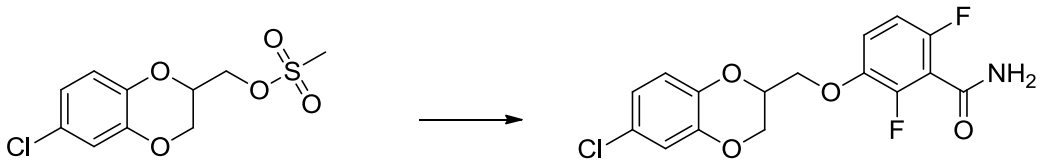
To a solution of 6-chloro-2-hydroxylmethyl-1,4-benzodioxane (420 mg, 2,09 mmol) in 6 ml of DCM were added 0,37 ml of TEA (2,7 mmol). The mixture was cooled at 4°C and then 0,21 ml of methansulphonyl chloride(2,70 mmol) were added dropwise, the reaction was stirred at 4°C for 15 minutes and after 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[238].
The reaction was cooled at 4°C and were added dropwise 20 ml of water and 10 ml of DCM. The separated organic layer was washed with HCl 10% (10 ml), $NaHCO_3$ saturated solution (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 610 mg of an yellow oil corresponding at the desired product.

Yield = 97,75%

^{1}H-NMR (CDCl3) δ (ppm): 6.91 (m, 1H) 6,84 (m, 2H) 4,46 (m, 3H) 4,31 (dd, 2H, J= 11,8 J= 2,5 Hz) 4,13 (dd, 1H, J=11,8 J=6,5 Hz) 3,09 (s, 3H).

[238] Toluene/ Ethyl Acetate 90/10 Rf start= 0,19 Rf prod= 0,40 Marker: $Ce(SO_4)_2$

3-(6-Chloro-1,4-benzodioxan-2-yl)metossi-2,6-difluorobenzamide



Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

Chemical Formula: $C_{16}H_{12}ClF_2NO_4$
Molecular Weight: 355,72

To a solution of 2,6-difluoro-3-hydroxybenzamide (380 mg, 2,20 mmol) in 6 ml of DMF were added 304 mg of anhydrous K_2CO_3 (2,20 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-chloro-2-mesyloxymethyl-1,4-benzodioxane (615 mg, 2,20 mmol) in 3 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 55°C overnight.
The TLC control[239] revealed the consumption of the starting material.
The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed twice with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 786 mg of a green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (toluene /ethyl acetate 70/30[240]) to give 485 mg of a white solid corresponding at the desired product.

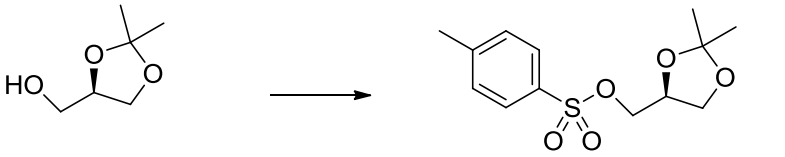
Yield = 61,78%

M.P.= 135,49°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,07 (td, 1H, J= 9,3 J= 5,3 Hz) 6,89 (m, 2H) 6,82 (m, 2H) 6,00 (bs, 2H) 4,54 (m, 1H) 4,40 (d, 1H, J= 11,7 Hz) 4,21 (m, 3H).

[239] Toluene / Ethyl Acetate 70/30 Rf start= 0,48 Rf amid= 0,08 Rf prod= 0,21 Marker: $Ce(SO_4)_2$
[240] Toluene / Ethyl Acetate 70/30 Rf start= 0,48 Rf amid= 0,08 Rf prod= 0,21 Marker: $Ce(SO_4)_2$

(S)-3-Tosyloxy-1,2-propanediol acetonide



Chemical Formula: $C_6H_{12}O_3$
Molecular Weight: 132,16

Chemical Formula: $C_{13}H_{18}O_5S$
Molecular Weight: 286,34

To a solution of (R)-solketal (5 g, 37,82 mmol) in 12,5 ml of pyridine at 0°C were added portionwise in 10 minutes 6,85 g of paratoluensulphonylchloride (35,93 mmol). The mixture was stirred at for 15 minutes 0°C and after for 1 h at room temperature until the complete transformation of the starting material as revealed by TLC[241].
The reaction was cooled at 0°C and quenched with 60 ml of DCM and 60 ml of water. The separated organic layer was washed with HCl 10% (60 ml), 60 ml of $NaHCO_3$ saturated solution (60 ml) and brine (60 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 8,24 g of an yellow oil corresponding at the desired product.

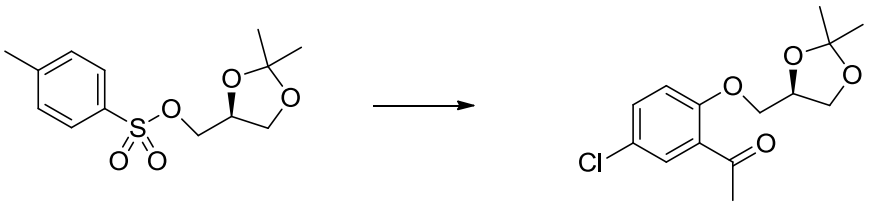
$[\alpha]^{25}_{D\,=}$ + 1,2 (c = 1,40 ; $CHCl_3$)

Yield = 76,09%

^{1}H-NMR (CDCl3) δ (ppm): 7,79 (d, 2H, J = 8,3Hz) 7,35 (d, 2H, J = 8,3Hz) 4,27 (m, 1H) 3,96 (m, 3H) 3,76 (dd, 1H,J = 8,8 J=5,1Hz) 2.45 (s, 3H) 1,34 (s,3H) 1,29 (s,3H).

[241] Cyclohexane/ Ethyl Acetate 95/5 Rf start= 0,19 Rf prod= 0,40 Marker: blue sheet

(R)-3-(2-Acetyl-4-chloro)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{13}H_{18}O_5S$
Molecular Weight: 286,34

Chemical Formula: $C_{14}H_{17}ClO_4$
Molecular Weight: 284,74

To a solution of (S)-3-tosyloxy-1,2-propanediol acetonide (8,24 g, 28,78 mmol) in 60 ml of DMF were added 4,57 g of anhydrous K_2CO_3 (33,09 mmol) and 5,64 g of 5-chloro-2-hydroxyacetophenone (33,09 mmol). The mixture was stirred at 90°C overnight.
The TLC control[242] revealed the complete transformation of the starting material.
The reaction was evaporated under vacuum and diluted with 150 ml of ethyl acetate, a brown solid afforded. The suspension was filtered and the resulting mother liquids were evaporated under vacuum.
The oil was purified through chromatography on silica pad eluting with cyclohexane / ethyl acetate 80/ 20 to give 8,19 g of an yellow oil corresponding at the desired product.

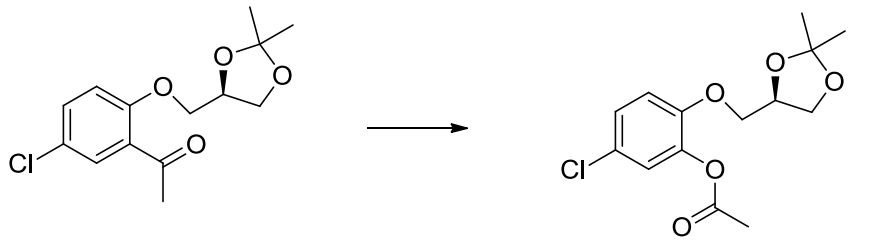
$[\alpha]^{25}_{D\,=}$ - 15,81 (c = 0,99 ; $CHCl_3$)

Yield = 98,18%

^{1}H-NMR (CDCl3) δ (ppm): 7,70 (d, 1H, J = 2,7Hz) 7,39 (dd, 1H, J = 8.8 J=2,7 Hz) 6,90 (d, 1H, J= 8,8 Hz) 4,51 (m, 1H) 4,12 (m, 3H) 3,91 (dd, 1H,J = 8,5 J=5,6 Hz) 2,64 (s, 3H) 1,45 (s,3H) 1,39 (s,3H).

[242] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,56 Rf prod= 0,27 Marker: blue sheet

(R)-3-(2-Acetoxy-4-chloro)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{14}H_{17}ClO_4$
Molecular Weight: 284,74

Chemical Formula: $C_{14}H_{17}ClO_5$
Molecular Weight: 300,73

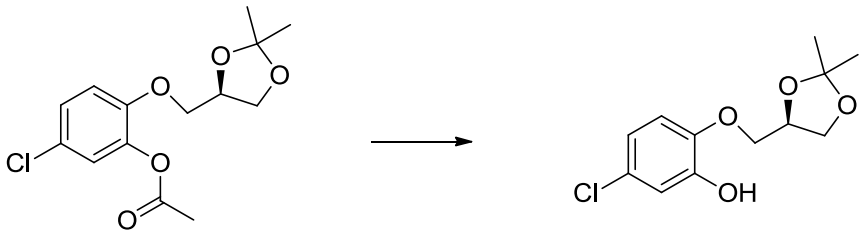
To a solution of (R)-3-(2-acetyl-4-chloro)phenoxy-1,2-propanediol acetonide (6,60 g, 23,18 mmol) in 60 ml of DCM were added in four portion 12,6 g of metachloroperbenzoic acid (55,63 mmol). The mixture was stirred at reflux for 5 h .
The H-NMR control revealed the complete transformation of the starting material.
The reaction was cooled at 4°C, a white precipitate afforded, the resulting suspension was filtered and the mother liquids were evaporated under vacuum. The resulting oil were diluted with 60 ml of ethyl acetate and washed with $NaHCO_3$ saturated solution (4 x 60 ml), dried over anhydrous Na_2SO_4 and evaporated under vacuum to give 6,90 g of an yellow oil corresponding at the desired product.

$[\alpha]^{25}_{D} =$ - 10,87 (c = 1,6 ; $CHCl_3$)

Yield = 98,98%

^{1}H-NMR (CDCl3) δ (ppm): 7,16 (dd, 1H, J= 8,7 J = 2,8Hz) 7,07 (d, 1H, J=2,8 Hz) 6,91 (d, 1H, J= 8,7 Hz) 4,41 (m, 1H) 4,03 (m, 4H) 2,34 (s, 3H) 1,45 (s,3H) 1,39 (s,3H).

(R)-3-(4-Chloro-2-hydroxy)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{14}H_{17}ClO_5$
Molecular Weight: 300,73

Chemical Formula: $C_{12}H_{15}ClO_4$
Molecular Weight: 258,70

To a solution of (R)-3-(2-acetoxy-4-chloro)phenoxy-1,2-propanediol acetonide (6,90 g, 22,94 mmol) in 70 ml of MeOH, 16,51 ml of NaOH 2,5 M and after 15 ml of water were added dropwise. The reaction was stirred at room temperature for 1 h until the complete transformation of the starting material as revealed by TLC[243].

The reaction mixture was evaporated under vacuum, and diluted with 20 ml of NaH2PO4/Na2HPO4 pH 7 buffer, 20 ml of water and 30 ml of ethyl acetate. The separated aqueous layer were extracted with ethyl acetate (3 x 30 ml). The combined organic phases were dried over anhydrous Na_2SO_4 and evaporated under vacuum to give 4,42 g of an yellow oil corresponding at the desired product.

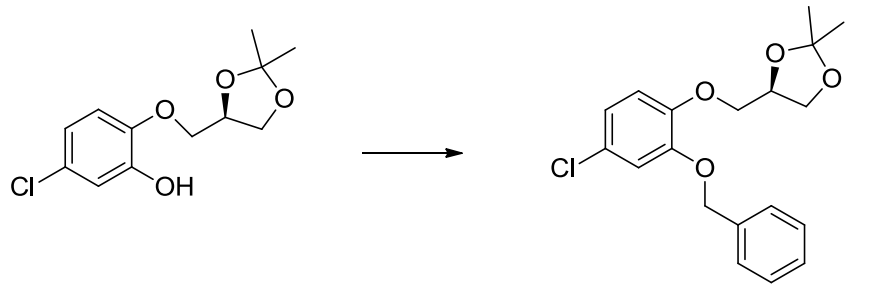
$[\alpha]^{25}_{D} =$ + 16,32 (c = 1,01 ; $CHCl_3$)

Yield = 74,47%

^{1}H-NMR (CDCl3) δ (ppm): 6,94 (m, 1H) 6,78 (m, 2H) 6,49 (bs, 1H) 4,46 (m, 1H) 4,12 (m, 2H) 4,00 (m, 1H) 3,90 (m, 1H) 1,47 (s,3H) 1,40 (s,3H).

[243] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,60 Rf prod= 0,50 Marker: blue sheet

(R)-3-(2-Benzyloxy-4-chloro)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{12}H_{15}ClO_4$
Molecular Weight: 258,70

Chemical Formula: $C_{19}H_{21}ClO_4$
Molecular Weight: 348,82

To a solution of (R)-3-(4-chloro-2-hydroxy)phenoxy-1,2-propanediol acetonide (4,32 g, 16,7 mmol) in 40 ml of DMF were added 2,54 g of anhydrous K_2CO_3 (18,37 mmol) and after 15 minutes 1,81 ml of benzyl bromide (15 mmol). The mixture was stirred at 60°C for 3 h until the complete transformation of the starting material as revealed by TLC[244].
The reaction mixture was evaporated under vacuum, and were added 40 ml of water and 40 ml of ethyl acetate. The separated aqueous layer were extracted twice with ethyl acetate (40 ml). The combined organic phases were washed with NaOH 1M (40 ml) and brine (40 ml), dried over anhydrous Na_2SO_4 and evaporated under vacuum to give 5,47 g of a grey wax corresponding at the desired product.

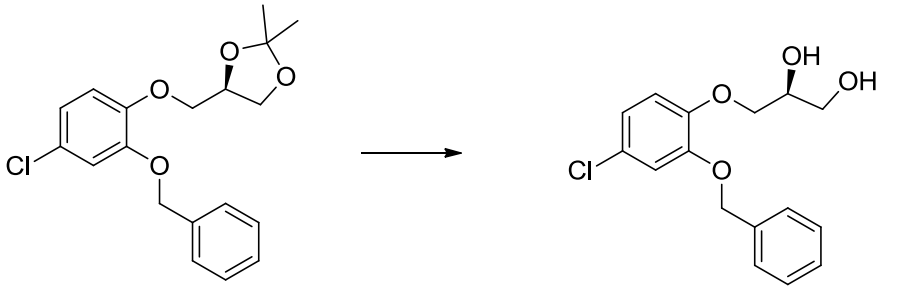
$[\alpha]^{25}_{D} = -14,77$ (c = 1,07 ; $CHCl_3$)

Yield = 93,90%

^{1}H-NMR (CDCl3) δ (ppm): 7,37 (m, 5H) 6,93 (m, 1H) 6,88 (m, 2H) 5,07 (s, 2H) 4,46 (m, 1H) 4,10 (m, 2H) 3,94 (m, 2H) 1,41 (s,3H) 1,38 (s,3H).

[244] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,50 Rf prod= 0,36 Marker: blue sheet

(S)-3-(2-Benzyloxy-4-chloro)phenoxy-1,2-propanediol



Chemical Formula: $C_{19}H_{21}ClO_4$
Molecular Weight: 348,82

Chemical Formula: $C_{16}H_{17}ClO_4$
Molecular Weight: 308,76

To a solution of (R)-3-(2-benzyloxy-4-chloro)phenoxy-1,2-propanediol acetonide (5,32 g, 15,25 mmol) in 55 ml of MeOH 27 ml of HCl 10% were added dropwise. The mixture was stirred at room temperature for 15 minutes and after brought at 60°C for 30 minutes until the complete transformation of the starting material as revealed by TLC[245].
The mixture was cooled at room temperature, a precipitate afforded. The resulting suspension was filtered giving 3,90 g of a white solid corresponding at the crude product.
The crude product was crystallized by a mixture MeOH/ water (2/1) gives 2,87 g of a white solid corresponding at the desired product.

$[\alpha]^{25}_{D\,=}$ + 11,35 (c = 1,006 ; $CHCl_3$)

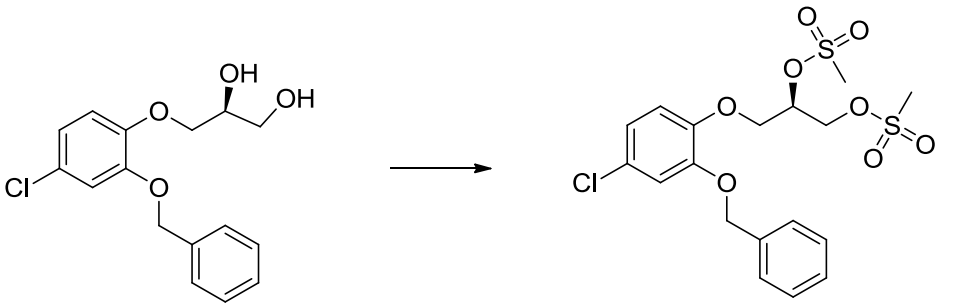
Yield = 60,95%

M.P. = 119,11°C

^{1}H-NMR (CDCl3) δ (ppm): 7,38 (m, 5H) 6,90 (m, 3H) 5,07 (s, 2H) 4,11 (m, 1H) 4,03 (m, 2H) 3,73 (m, 2H) 2,97 (bs, 1H) 2,19 (bs,1H).

[245] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,36 Rf prod= 0,05 Marker: blue sheet

(R)-3-(2-Benzyloxy-4-chloro)phenoxy-1,2-dimesyloxypropane



Chemical Formula: $C_{16}H_{17}ClO_4$
Molecular Weight: 308,76

Chemical Formula: $C_{18}H_{21}ClO_8S_2$
Molecular Weight: 464,94

To a solution of (S)-3-(2-benzyloxy-4-chloro)phenoxy-1,2-propanediol (2,77 g, 8,97 mmol) in 30 ml of DCM 3,24 ml of TEA (23,32 mmol) were added dropwise. The mixture was cooled at 4°C and 1,74 ml of methansulphonyl chloride (22,43 mmol) were added dropwise. The mixture was stirred for 15 minutes at 4°C and after brought at room temperature for 2 h until the complete transformation of the starting material as revealed by TLC[246].
The reaction mixture was cooled at 4°C and quenched with 30 ml of water, The separated organic layer was washed with HCl 10% (30 ml), $NaHCO_3$ satured solution (30 ml) and brine (30 ml). The organic phase was dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,55 g of a white solid corresponding at the crude product.
The crude product was crystallized twice by MeOH to give 3,29 g of a white solid corresponding at the desired product.

$[\alpha]^{25}_{D\ =}$ + 23,3 (c = 1,10 ; $CHCl_3$)

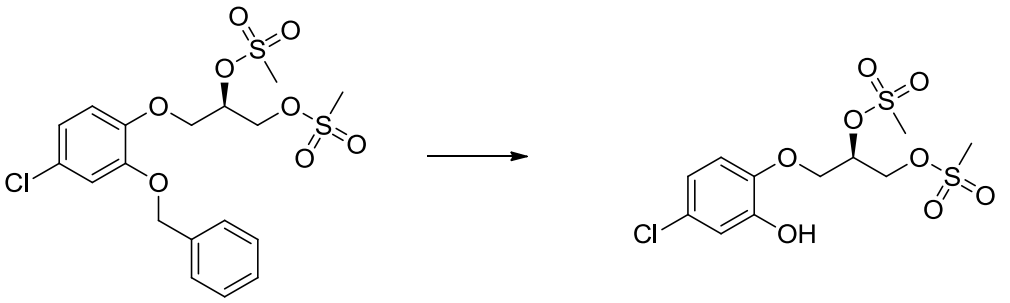
Yield = 78,88%

M.P. = 108,82°C

^{1}H-NMR (CDCl3) δ (ppm): 7,39 (m, 5H) 6,96 (d, 1H, J= 2,3 Hz) 6,92 (dd, 1H, J= 8,5 J= 2,3 Hz) 6,82 (d, 1H, J= 8,5 Hz) 5,10 (m, 1H) 4,99 (m, 2H) 4,51 (qd, 2H, J= 11,9 J= 4,5 Hz) 4,20 (m, 2H) 3,05 (s, 3H) 2,83 (s,3H).

[246] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,10 Rf prod= 0,29 Marker: blue sheet

(R)-3-(4-Chloro-2-hydroxy)phenoxy-1,2-dimesyloxypropane



Chemical Formula: $C_{18}H_{21}ClO_8S_2$
Molecular Weight: 464,94

Chemical Formula: $C_{11}H_{15}ClO_8S_2$
Molecular Weight: 374,82

To a solution of (R)-3-(2-benzyloxy-4-chloro)phenoxy-1,2-dimesyloxypropane (2,00 g, 4,30 mmol) in 20 ml of THF were added 200 mg of Pd/C at 5 % and were hydrogenated at 1 atm for 1 h until the complete transformation of the starting material as revealed by TLC[247].
The catalyzer was filtered, the mother liquids were evaporated under vacuum to give 1,61 g of a transparent wax corresponding at the desired product.

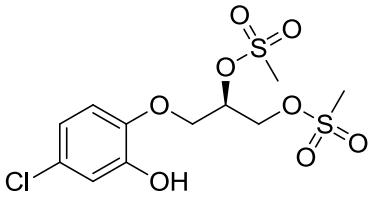
$[\alpha]^{25}_{D\,=}$ + 5,35 (c = 1,02 ; $CHCl_3$)

Yield = 98,89%

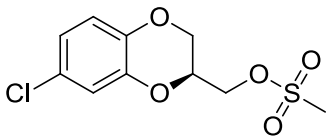
^{1}H-NMR (CDCl3) δ (ppm): 6,94 (d, 1H, J= 2,3 Hz) 6,80 (dd, 1H, J= 8,6 J= 2,3 Hz) 6,74 (d, 1H, J= 8,6 Hz) 6,37 (bs, 1H) 5,28 (m, 1H) 4,51 (qd, 1H, J= 11,9 J= 4,5 Hz) 4,28 (dd, 1H, J= 10,5 J= 3,6 Hz) 4,16 (dd, 1H, J= 10,5 J= 7,7 Hz) 3,16 (s, 3H) 3,11 (s,3H).

[247] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,29 Rf prod= 0,23 Marker: $Ce(SO_4)_2$

(S)-7-Chloro-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{15}ClO_8S_2$
Molecular Weight: 374,82



Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

To a solution of (R)-3-(4-chloro-2-hydroxy)phenoxy-1,2-dimesyloxypropane (1,53 g, 4,08 mmol) in 20 ml of DMF were added 0,62 g of K_2CO_3 anhydrous, the mixture was stirred at 60°C for 1 h until the complete transformation of the starting material as revealed by TLC[248].
The reaction mixture was evaporated under vacuum and diluted with 20 ml of water, the aqueous phase were extracted with ethyl acetate (3 x 15 ml). The combined organic phases were washed with NaOH 1 M (25 ml) and twice with brine (15 ml), dried over Na2SO4 anhydrous, filtered and evaporated under vacuum to give 1,04 g of a grey wax corresponding at the desired product.

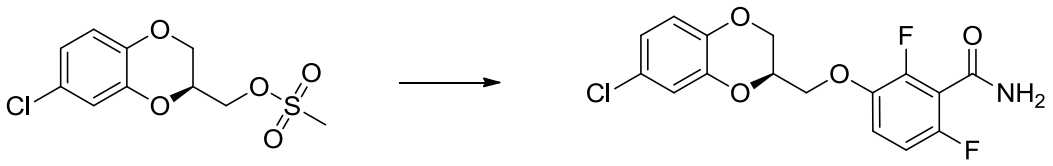
$[\alpha]^{25}_{D\,=}$ + 8,76 (c = 1,036 ; $CHCl_3$)

Yield = 91,45%

^{1}H-NMR (CDCl3) δ (ppm): 6,91 (m, 1H) 6,82 (m, 2H) 4,47 (m, 3H) 4,29 (dd, 1H, J= 11,7 J= 2,3 Hz) 4,11 (dd, 1H, J= 11,7 J= 6,2 Hz) 3,08 (s, 3H).

[248] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,23 Rf prod= 0,35 Marker: $Ce(SO_4)_2$

(S)-3-(7-Chloro-1,4-benzodioxan-2-yl)metossi-2,6-difluorobenzamide



Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

Chemical Formula: $C_{16}H_{12}ClF_2NO_4$
Molecular Weight: 355,72

To a solution of 2,6-difluoro-3-hydroxybenzamide (680 mg, 3,95 mmol) in 10 ml of DMF were added 540 mg of anhydrous K_2CO_3 (3,95 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of (S)-6-chloro-3-mesyloxymethyl-1,4-benzodioxane (1 g, 3,59 mmol) in 5 ml of DMF were added. The reaction was stirred for 15 minutes at room temperature and then brought at 80°C for 4 h until the complete transformation of the starting material as revealed by TLC[249].
The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (30 ml) and twice with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,25 g of a white solid corresponding at the crude product.
The crude product was crystallized by chloroform giving 540 mg of a white solid corresponding at the desired product.

$[\alpha]^{25}_{D\,=}$ + 10,74 (c = 1,04 ; ethyl acetate)

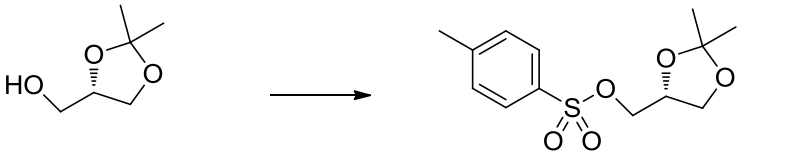
Yield = 42,28 %

M.P.= 142,10°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,07 (td, 1H, J= 9,1 J= 5,1 Hz) 6,89 (m, 2H) 6,82 (m, 2H) 6,10 (bs, 1H) 6,01 (bs, 1H) 4,56 (m, 1H) 4,38 (m, 1H) 4,23 (m, 3H).

[249]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,32 Rf amid= 0,13 Rf prod= 0,20 Marker: $Ce(SO_4)_2$

(R)-3-Tosyloxy-1,2-propanediol acetonide



Chemical Formula: $C_6H_{12}O_3$
Molecular Weight: 132,16

Chemical Formula: $C_{13}H_{18}O_5S$
Molecular Weight: 286,34

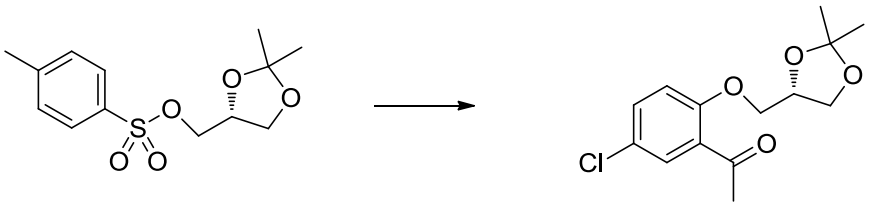
The product was synthesized as described for the S enantiomer, starting from 5 g of (S)-solketal (37,82 mmol) giving 9,05 g of an transparent oil corresponding at the desired product.

$[\alpha]^{25}_{D\,=}$ - 1,9 (c = 1,08 ; $CHCl_3$)

Yield = 83,5%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the S enantiomer

(S)-3-(2-Acetyl-4-chloro)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{13}H_{18}O_5S$
Molecular Weight: 286,34

Chemical Formula: $C_{14}H_{17}ClO_4$
Molecular Weight: 284,74

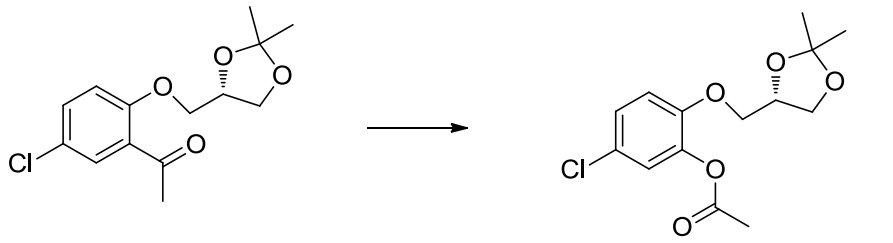
The product was synthesized as described for the R enantiomer, starting from 8,73 g of (R)-3-tosyloxy-1,2-propanediol acetonide (30,49 mmol) giving 6,45 g of an yellow oil corresponding at the desired product.

$[\alpha]^{25}_{D\,=}$ + 16,30 (c = 1,01 ; $CHCl_3$)

Yield = 80,03%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(S)-3-(2-Acetoxy-4-chloro)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{14}H_{17}ClO_4$
Molecular Weight: 284,74

Chemical Formula: $C_{14}H_{17}ClO_5$
Molecular Weight: 300,73

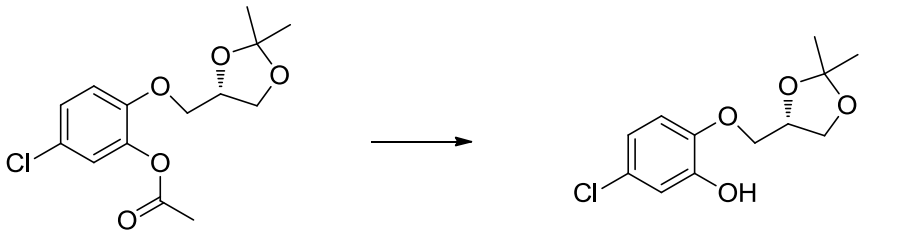
The product was synthesized as described for the R enantiomer, starting from 6,45 g of (S)-3-(2-acetyl-4-chloro)phenoxy-1,2-propanediol acetonide (22,65 mmol) giving 5,30 g of an yellow oil corresponding at the desired product.

$[\alpha]^{25}_{D} =$ - 15,98 (c = 1,02 ; $CHCl_3$)

Yield = 77,80%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(S)-3-(4-Chloro-2-hydroxy)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{14}H_{17}ClO_5$
Molecular Weight: 300,73

Chemical Formula: $C_{12}H_{15}ClO_4$
Molecular Weight: 258,70

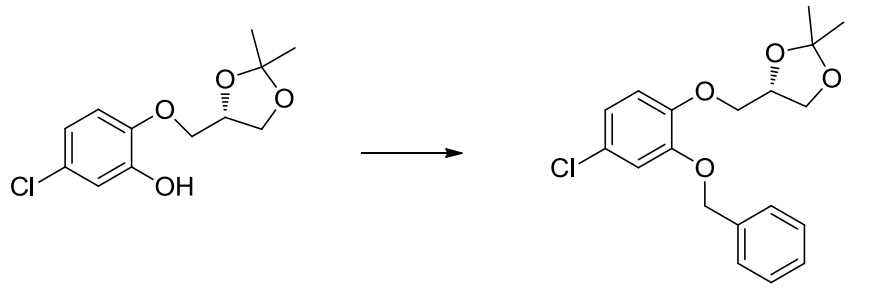
The product was synthesized as described for the R enantiomer, starting from 5,30 g of (S)-3-(2-acetoxy-4-chloro)phenoxy-1,2-propanediol acetonide (17,62 mmol) giving 3,60 g of an yellow oil corresponding at the desired product.

$[\alpha]^{25}_{D}$ = - 16,1 (c = 1,10 ; $CHCl_3$)

Yield = 78,97%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(S)-3-(2-Benzyloxy-4-chloro)phenoxy-1,2-propanediol acetonide



Chemical Formula: $C_{12}H_{15}ClO_4$
Molecular Weight: 258,70

Chemical Formula: $C_{19}H_{21}ClO_4$
Molecular Weight: 348,82

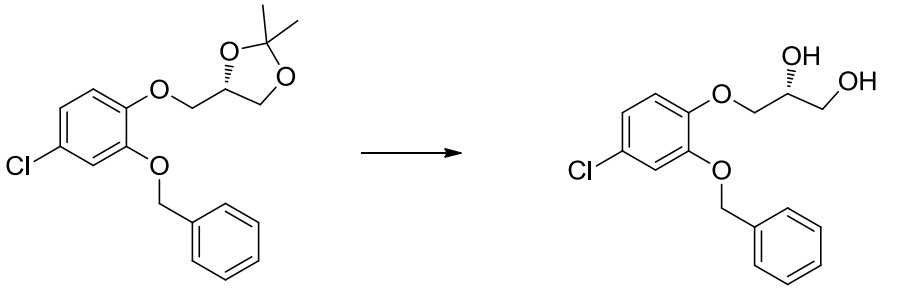
The product was synthesized as described for the R enantiomer, starting from 3,60 g of (S)-3-(4-chloro-2-hydroxy)phenoxy-1,2-propanediol acetonide (13,91 mmol) giving 3,80 g of an grey wax corresponding at the desired product.

$[\alpha]^{25}_{D\,=}$ + 15,20 (c = 1,05 ; $CHCl_3$)

Yield = 78,31%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(R)-3-(2-Benzyloxy-4-chloro)phenoxy-1,2-propanediol



Chemical Formula: $C_{19}H_{21}ClO_4$
Molecular Weight: 348,82

Chemical Formula: $C_{16}H_{17}ClO_4$
Molecular Weight: 308,76

The product was synthesized as described for the S enantiomer, starting from 3,80 g of (S)-3-(2-benzyloxy-4-chloro)phenoxy-1,2-propanediol acetonide (10,89 mmol) giving 2,80 g of a white solid corresponding at the desired product.

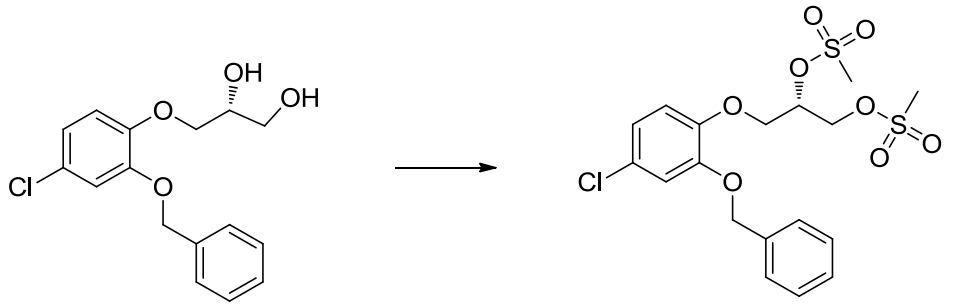
$[\alpha]^{25}_{D\,=}$ + 3,61 (c = 1,04 ; acetone)

Yield = 83,27%

M.P. = 119,11°C

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(S)-3-(2-Benzyloxy-4-chloro)phenoxy-1,2-dimesyloxypropane



Chemical Formula: $C_{16}H_{17}ClO_4$
Molecular Weight: 308,76

Chemical Formula: $C_{18}H_{21}ClO_8S_2$
Molecular Weight: 464,94

The product was synthesized as described for the R enantiomer, starting from 1,63 g of (R)-3-(2-benzyloxy-4-chloro)phenoxy-1,2-propanediol (5,28 mmol) giving 1,75 g of a white solid corresponding at the desired product.

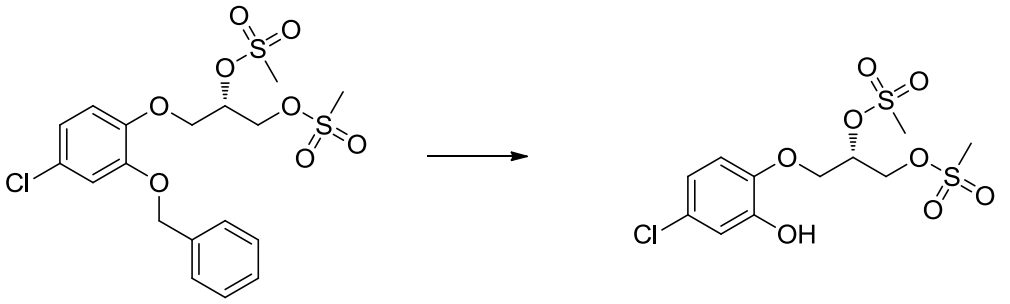
$[\alpha]^{25}_{D}$ = - 23,58 (c = 1,03 ; $CHCl_3$)

Yield = 71,28%

M.P. = 108,82°C

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(S)-3-(4-Chloro-2-hydroxy)phenoxy-1,2-dimesyloxypropane



Chemical Formula: $C_{18}H_{21}ClO_8S_2$
Molecular Weight: 464,94

Chemical Formula: $C_{11}H_{15}ClO_8S_2$
Molecular Weight: 374,82

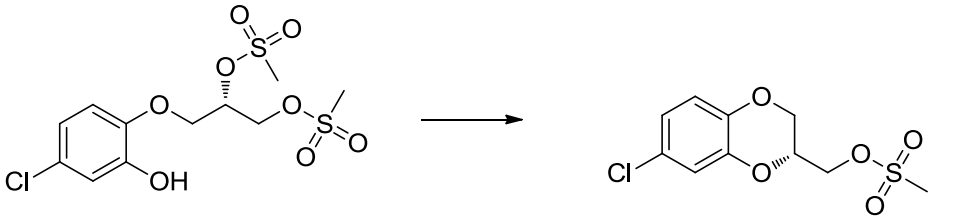
The product was synthesized as described for the R enantiomer, starting from 1,75 g of (S)-3-(2-benzyloxy-4-chloro)phenoxy-1,2-dimesyloxypropane (3,76 mmol) giving 1,39 g of a transparent wax corresponding at the desired product.

$[\alpha]^{25}_{D}$ = - 6,55 (c = 1,22 ; $CHCl_3$)

Yield = 98,62%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the R enantiomer

(R)-7-Chloro-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{15}ClO_8S_2$
Molecular Weight: 374,82

Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

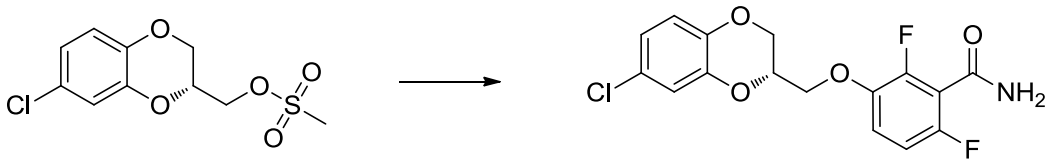
The product was synthesized as described for the S enantiomer, starting from 1,39 g of (S)-3-(4-chloro-2-hydroxy)phenoxy-1,2-dimesyloxypropane (3,70 mmol) giving 1,01 g of an transparent wax corresponding at the desired product.

$[\alpha]^{25}_{D}$ = - 6,75 (c = 1,1 ; $CHCl_3$)

Yield = 97,94%

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the S enantiomer

(R)-3-(7-Chloro-1,4-benzodioxan-2-yl)metossi-2,6-difluorobenzamide



Chemical Formula: $C_{10}H_{11}ClO_5S$
Molecular Weight: 278,71

Chemical Formula: $C_{16}H_{12}ClF_2NO_4$
Molecular Weight: 355,72

The product was synthesized as described for the S enantiomer, starting from 0,81 g of 2,6-difluoro-3-hydroxybenzamide (4,69 mmol) and 1,39 g of (R)-6-chloro-3-mesyloxymethyl-1,4-benzodioxane (3,62 mmol) giving 0,90 g of a white solid corresponding at the desired product.

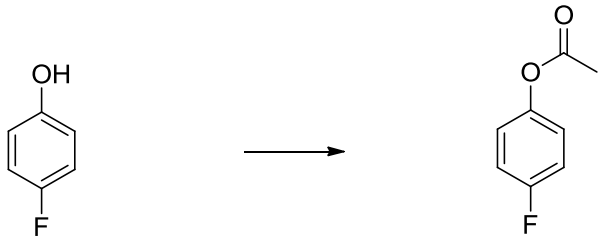
$[\alpha]^{25}_{D}$ = - 12,47 (c = 0,96 ; ethyl acetate)

Yield = 42,28 %

M.P.= 142,10°C

^{1}H-NMR ($CDCl_3$) and TLC: corresponding at the S enantiomer

4-Fluorophenylacetate



Chemical Formula: C_6H_5FO
Molecular Weight: 112,10

Chemical Formula: $C_8H_7FO_2$
Molecular Weight: 154,14

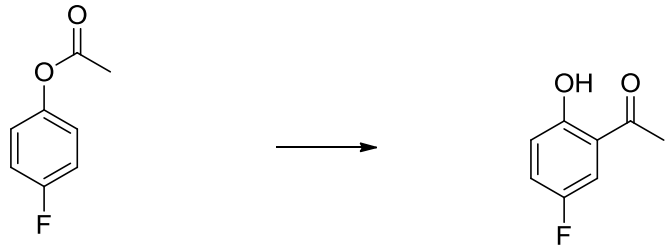
To a solution of 4-fluorophenol (5 g, 44,60 mmol) in 26,8 ml of NaOH 2,5 M at 10°C 6,37 ml of acetic anhydride (66,9 mmol) were added dropwise. The reaction was stirred at for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[250].
The reaction mixture was extracted twice with ethyl acetate (25 ml), the combined organic phases were washed twice with NaOH 1M (25 ml) and twice with brine (25 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 6,07 g of an transparent liquid corresponding at the desired product.

Yield = 88,29 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,06 (s, 2H) 7,04 (d, 2H, J= 0,7 Hz) 2,29 (s, 3H).

[250] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,21 Rf prod= 0,34 Marker: $Ce(SO_4)_2$

5-Fluoro-2-hydroxyacetophenone



Chemical Formula: $C_8H_7FO_2$
Molecular Weight: 154,14

Chemical Formula: $C_8H_7FO_2$
Molecular Weight: 154,14

To 6,07 g of 4-fluorophenylacetate (39,38 mmol) were added 5,75 g of anhydrous $AlCl_3$ divided in three portion in 30 minutes. The reaction was brought at 120°C and stirred for 1h until the complete transformation of the starting material as revealed by TLC[251].
The cooled mixture were diluted with 40 ml of DCM and the resulting solution was poured in 50 ml of cold 10% HCl. The separated aqueous layer were extracted twice with DCM (40 ml). The combined organic phases were washed with brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 4,05 g of an transparent solid corresponding at the crude product.
The crude product was crystallized by petrol ether to give 2,10 g of a transparent solid corresponding at the desired product.

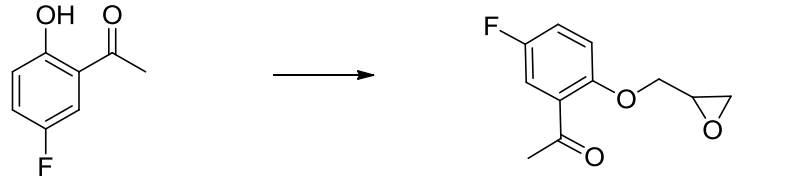
Yield = 34,59 %

M.P. = 57°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 11,97 (s, 1H) 7,40 (dd, 1H, J= 8,8 J= 3,1 Hz) 7,22 (m, 1H) 6,95 (dd, 1H, J= 8,8 J= 4,0 Hz) 2,61 (s, 3H).

[251] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,40 Rf prod= 0,54 Marker: $Ce(SO_4)_2$

(5-Fluoro-2-epoxypropoxy)acetophenone



Chemical Formula: $C_8H_7FO_2$
Molecular Weight: 154,14

Chemical Formula: $C_{11}H_{11}FO_3$
Molecular Weight: 210,20

To a solution of 5-fluoro-2-hydroxyacetophenone (2,1 g, 13,62 mmol) in 30 ml of DMF were added 2,07 g of anhydrous K_2CO_3 (14,98 mmol) and 1,40 ml of epibromohydrin (16,34 mmol). The reaction was stirred at 40°C overnight.
The TLC control[252] revealed the complete transformation of the starting material.
The reaction mixture was evaporated under vacuum and diluted with 30 ml of ethyl acetate and 30 ml of water. The separated aqueous layer were extracted with ethyl acetate (3 x 30 ml). The combined organic phases were washed with NaOH 1 M (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,14 g of an transparent solid corresponding at the crude product.
The crude product was crystallized by diisopropyl ether gives 1,40 g of a transparent solid corresponding at the desired product.

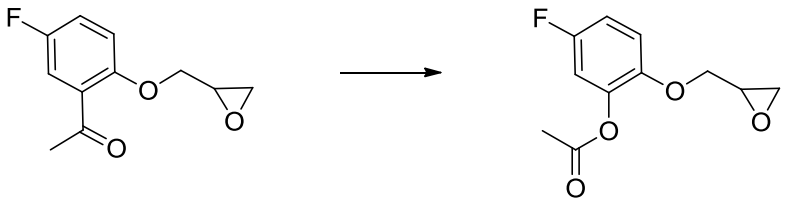
Yield = 48,90%

M.P. = 57,26°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,45 (dd, 1H, J= 9,0 J= 3,2 Hz) 7,14 (dd, 1H, J= 11,7 J= 4,5 Hz) 6,92 (dd, 1H, J= 9,0 J= 4,0 Hz) 4,36 (dd, 1H, J= 11,0 J= 2,7 Hz) 3,97 (dd, 1H, J= 11,0 J= 6,1 Hz) 3,39 (m, 1H) 2,94 (t, 1H, J= 4,5 Hz) 2,76 (dd, 1H, J= 4,7 J= 2,6 Hz) 2,62 (s, 3H).

[252] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,54 Rf prod= 0,17 Marker: $Ce(SO_4)_2$

(5-Fluoro-2-epoxypropoxy)phenyl acetate



Chemical Formula: $C_{11}H_{11}FO_3$
Molecular Weight: 210,20

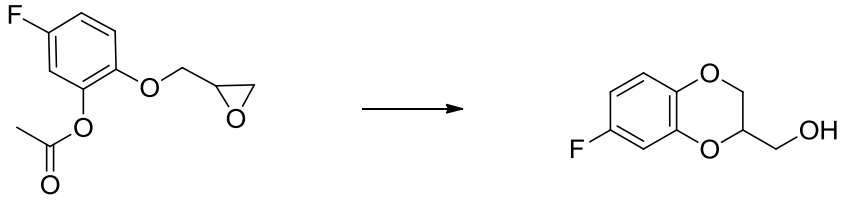
Chemical Formula: $C_{11}H_{11}FO_4$
Molecular Weight: 226,20

To a solution of (5-fluoro-2-epoxypropoxy)acetophenone (1,40 g, 6,65 mmol) in 15 ml of DCM 2,53 g of *m*-chloroperbenzoic acid (14,65 mmol) were added dividing in three portion in 40 minutes. The reaction was stirred for 74 h at room temperature.
The H-NMR control revealed the complete transformation of the starting material.
The reaction mixture was cooled at 4°C and quenched with 30 ml of $Na_2S_2O_5$ satured solution, a white precipitate afforded. The resulting suspension was filtered. At the mother liquids were added 30 ml of DCM and were washed with $NaHCO_3$ satured solution (4 x 30 ml). The organic phase were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,48 g of an yellow oil corresponding at the desired product.

Yield = 98,38%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,86 (m, 3H) 4,22 (dd, 1H, J= 11,2 J= 2,9 Hz) 3,92 (dd, 1H, J= 11,2 J= 5,6 Hz) 3,28 (dddd, 1H; J= 5,6 J= 4,2 J= 2,9 J= 1,6 Hz) 2,86 (m, 1H) 2,70 (dd, 1H, J= 4,7 J= 2,6 Hz) 2,31 (s, 3H).

7-Fluoro-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{11}FO_4$
Molecular Weight: 226,20

Chemical Formula: $C_9H_9FO_3$
Molecular Weight: 184,16

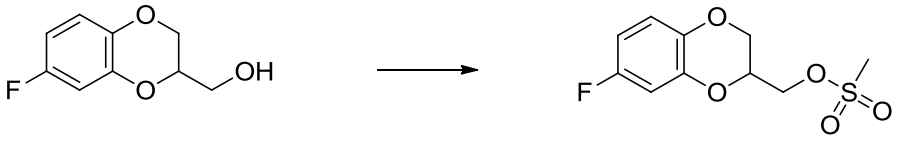
To a solution of (5-fluoro-2-epoxypropoxy)phenyl acetate (1,48 g, 6,54 mmol) in 20 ml of MeOH 3,80 ml of NaOH 2,5 M were added dropwise. The reaction was stirred for 15 minutes at room temperature and brought at 55°C for 2 h until the complete transformation of the starting material as revealed by TLC[253].
The reaction mixture was evaporated under vacuum and diluted with 20 ml of water and 20 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml), twice with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,79 g of an yellow oil corresponding at the desired product.

Yield = 58,52 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,81 (dd, 1H, J= 8,9 J= 5,4 Hz) 6,63 (m, 1H) 6,56 (m, 1H) 4,26 (m, 1H) 4,07 (m, 1H) 3,87 (qd, 1H; J= 12,1 J= 4,6 Hz) 1,79 (bs, 1H).

[253]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,23 Rf prod= 0,18 Marker: $Ce(SO_4)_2$

7-Fluoro-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9FO_3$
Molecular Weight: 184,16

Chemical Formula: $C_{10}H_{11}FO_5S$
Molecular Weight: 262,25

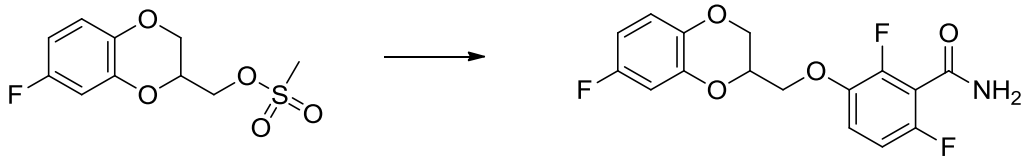
To a solution of 6-fluoro-3-hydroxymethyl-1,4-benzodioxane (0,79 g, 4,29 mmol) in 10 ml of DCM were added 0,78 ml of TEA (5,58 mmol). The reaction was cooled at 4°C and 0,40 ml of methansulphonyl chloride (5,15 mmol) were slowly added dropwise. The reaction was stirred for 15 minutes at 4°C and for 1 h and 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[254].
The reaction mixture was cooled at 4°C and quenched with 10 ml of water and 10 ml of DCM. The separated organic layerwere washed with 10% HCl (10 ml), $NaHCO_3$ satured solution (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,09 g of an yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[255]) to give 0,86 g of a transparent oil corresponding at the desired product.

Yield = 76,44 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,83 (dd, 1H, J= 8,9 J= 5,4 Hz) 6,61 (m, 2H) 4,61 (m, 3H) 4,10 (dd, 1H, J= 11,7 J= 6,1 Hz) 3,09 (s, 3H).

[254]Toluene/ Ethyl Acetate 80/20 Rf start= 0,25 Rf prod= 0,55 Marker: $Ce(SO_4)_2$
[255]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,36 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-fluoro-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{10}H_{11}FO_5S$
Molecular Weight: 262,25

Chemical Formula: $C_{16}H_{12}F_3NO_4$
Molecular Weight: 339,27

To a solution of 2,6-difluoro-3-hydroxybenzamide (625 mg, 3,28 mmol) in 10 ml of DMF were added 498 mg of anhydrous K_2CO_3 (3,61 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-fluoro-3-mesyloxymethyl-1,4-benzodioxane (860 mg, 3,28 mmol) in 5 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 75°C for 4 h until the complete transformation of the starting material as revealed by TLC[256].
The reaction was evaporated under vacuum and diluted with 20 ml of ethyl acetate and 20 ml of distilled water. The separated aqueous layer were extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,03 g of a white wax corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[257]) to give 0,62 g of a white solid corresponding at the desired product.

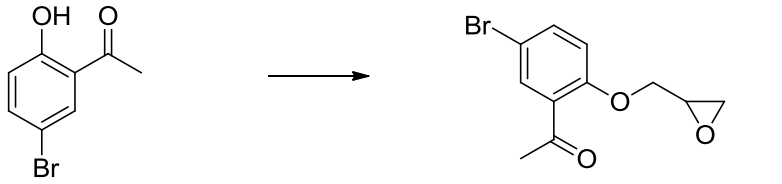
Yield = 55,71 %

M.P. = 120,08°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,07 (td, 1H, J= 9,1 J= 5,1 Hz) 6,90 (td, 1H, J= 9,1 J= 2,0 Hz) 6,83 (dd, 1H, J= 8,9 J= 5,4 Hz) 6,60 (m, 2H) 5,96 (bs, 2H) 4,57 (ddd, 1H, J= 11,2 J= 2,4 Hz) 4,36 (dd, 1H, J= 11,2 J= 2,4 Hz) 4,23 (m, 3H).

[256]Toluene/ Ethyl Acetate 70/30 Rf start= 0,60 Rf amid= 0,08 Rf prod= 0,26 Marker: $Ce(SO_4)_2$
[257]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,45 Rf amid= 0,09 Rf prod= 0,29 Marker: $Ce(SO_4)_2$

(5-Bromo-2-epoxypropoxy)acetophenone



Chemical Formula: $C_8H_7BrO_2$
Molecular Weight: 215,04

Chemical Formula: $C_{11}H_{11}BrO_3$
Molecular Weight: 271,11

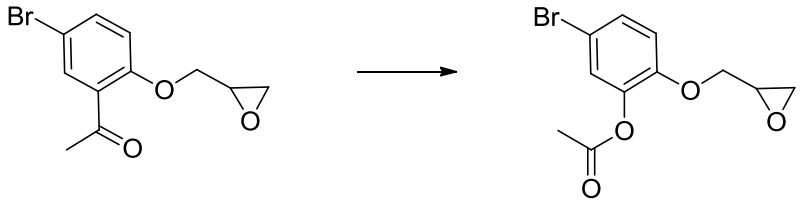
To a solution of 5-bromo-2-hydroxyacetophenone (2,0 g, 9,30 mmol) in 22 ml of DMF were added 1,41 g of anhydrous K_2CO_3 (10,23 mmol) and 1,46 ml of epichloridrine (18,6 mmol). The reaction was stirred for 64 h at 40°C until the complete transformation of the starting material as revealed by TLC[258].
The mixture was poured in 60 ml of water and extracted with ethyl acetate (3 x 30 ml). The combined organic phases were washed with NaOH 1 M (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,19 g of an brownish oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[259]) to give 1,21 g of a white wax corresponding at the desired product.

Yield = 47,99%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,84 (d, 1H, J= 2,6 Hz) 7,53 (dd, 1H, J= 8,8 J= 2,6 Hz) 6,85 (d, 1H, J= 8,8 Hz) 4,37 (dd, 1H, J= 10,9 J= 2,7 Hz) 3,97 (dd, 1H, J= 10,9 J= 6,0 Hz) 3,39 (m, 1H) 2,95 (t, 1H, J= 4,5 Hz) 2,76 (dd, 1H, J= 4,7 J= 2,6 Hz) 2,64 (s, 3H).

[258] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,58 Rf prod= 0,19 Marker: $Ce(SO_4)_2$
[259] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,58 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

(5-Bromo-2-epoxypropoxy)phenyl acetate



Chemical Formula: $C_{11}H_{11}BrO_3$
Molecular Weight: 271,11

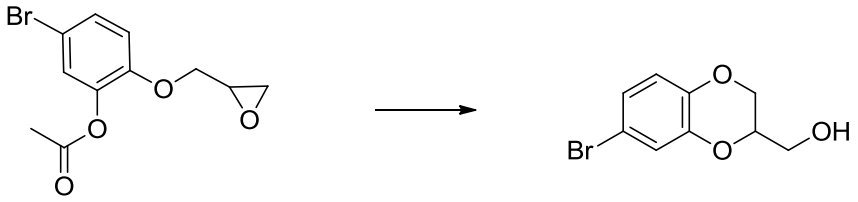
Chemical Formula: $C_{11}H_{11}BrO_4$
Molecular Weight: 287,11

To a solution of (5-bromo-2-epoxypropoxy)acetophenone (1,21 g, 5,76 mmol) in 13 ml of DCM 2,58 g of *m*-chloroperbenzoic acid (11,52 mmol) were added dividing in three portion in 40 minutes. The reaction was stirred at reflux overnight.
The H-NMR control revealed the complete transformation of the starting material.
The mixture was cooled at 4°C and quenched with 20 ml of $Na_2S_2O_5$ satured solution, a white precipitate afforded. The suspension was filtered in vacuum. At the mother liquids were added 20 ml of DCM and were washed $NaHCO_3$ satured solution (4 x 30 ml). The organic phase were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,55 g of an yellow oil corresponding at the desired product.

Yield = 94,02%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,30 (dd, 1H, J= 8,7 J= 2,4 Hz) 7,20 (d, 1H, J= 2,4 Hz) 6,88 (d, 1H, J= 8,7 Hz) 4,25 (dd, 1H, J= 11,2 J= 2,9 Hz) 3,95 (dd, 1H, J= 11,2 J= 5,6 Hz) 3,30 (ddd, 1H; J= 5,6 J= 2,8 J= 1,4 Hz) 2,88 (dd, 1H, J= 4,9 J= 4,2 Hz) 2,71 (dd, 1H, J= 4,9 J= 2,6 Hz) 2,32 (s, 3H).

7-Bromo-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{11}BrO_4$
Molecular Weight: 287,11

Chemical Formula: $C_9H_9BrO_3$
Molecular Weight: 245,07

To a solution of (5-Bromo-2-epoxypropoxy)phenyl acetate (1,55 g, 5,40 mmol) in 16 ml of MeOH 2,88 ml of NaOH 2,5 M were added dropwise. The reaction was stirred for 15 minutes at room temperature and brought at 50°C for 2 h and 30 minutes until the complete transformation of the starting material as revealed by TLC[260].

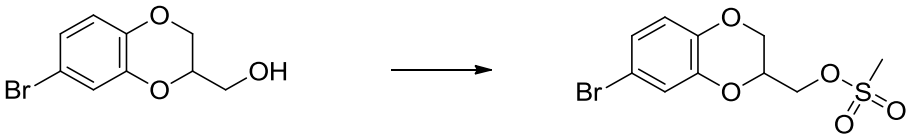
The reaction mixture was evaporated under vacuum and diluted with 15 ml of water and 15 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (15 ml). The combined organic phases were washed with NaOH 1 M (30 ml), twice with brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,10 g of an yellow oil corresponding at the desired product.

Yield = 83,12%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,05 (d, 1H, J= 2,3 Hz) 6,95 (dd, 1H, J= 8,6 J= 2,3 Hz) 6,76 (d, 1H, J= 8,6 Hz) 4,26 (m, 2H) 4,11 (m, 1H) 3,86 (m, 2H) 1,87 (bs, 1H).

[260]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,41 Rf prod= 0,33 Marker: blue sheet

7-Bromo-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9BrO_3$
Molecular Weight: 245,07

Chemical Formula: $C_{10}H_{11}BrO_5S$
Molecular Weight: 323,16

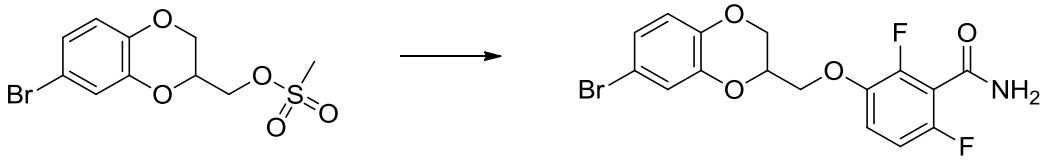
To a solution of 6-bromo-3-hydroxymethyl-1,4-benzodioxane (0,55 g, 2,24 mmol) in 7,5 ml of DCM were added 0,43 ml of TEA (3,04 mmol). The reaction was cooled at 4°C and 0,22 ml of methansulphonyl chloride (2,82 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[261].
The reaction mixture was cooled at 4°C and quenched with 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10% HCl (10 ml), $NaHCO_3$ satured solution (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,70 g of a transparent oil corresponding at the desired product.

Yield = 97,22 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,05 (d, 1H, J= 2,3 Hz) 6,98 (dd, 1H, J= 8,6 J= 2,3 Hz) 6,78 (d, 1H, J= 8,6) 4,46 (m, 3H) 4,31 (dd, 1H, J= 11,7 J= 2,3 Hz) 4,11 (dd, 1H, J= 11,7 J= 6,2 Hz) 3,09 (s, 3H).

[261]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,27 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-bromo-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{10}H_{11}BrO_5S$
Molecular Weight: 323,16

Chemical Formula: $C_{16}H_{12}BrF_2NO_4$
Molecular Weight: 400,17

To a solution of 2,6-difluoro-3-hydroxybenzamide (410 mg, 2,40 mmol) in 5 ml of DMF were added 330 mg of anhydrous K_2CO_3 (2,40 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-bromo-3-mesyloxymethyl-1,4-benzodioxane (700 mg, 2,17 mmol) in 5 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C overnight.
The TLC control[262] revealed the consumption of the starting material.
The reaction was evaporated under vacuum and diluted with 15 ml of ethyl acetate and 15 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (15 ml). The combined organic phases were washed with NaOH 1 M (15 ml) and brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,88 g of a yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[263]) to give 540 mg of a white solid corresponding at the desired product.

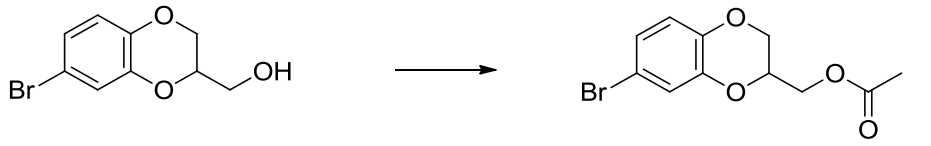
Yield = 62,07%

M.P. = 114,50°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,06 (m, 2H) 6,93 (m, 2H) 6,77 (d, 1H, J= 8,6 Hz) 6,02 (bs, 2H) 4,55 (m, 1H) 4,38 (dd, 1H, J= 11,6 J= 2,4 Hz) 4,23 (m, 3H).

[262]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,47 Rf amid= 0,15 Rf prod= 0,24 Marker: $Ce(SO_4)_2$
[263]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,47 Rf amid= 0,15 Rf prod= 0,24 Marker: $Ce(SO_4)_2$

7-Bromo-2-acetoxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9BrO_3$
Molecular Weight: 245,07

Chemical Formula: $C_{11}H_{11}BrO_4$
Molecular Weight: 287,11

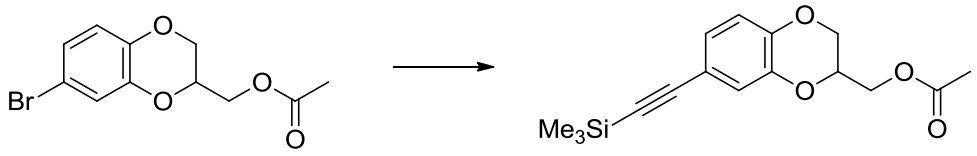
To a solution of 6-bromo-3-hydroxymethyl-1,4-benzodioxane (0,53 g, 2,17 mmol) in 10 ml of DCM were added 0,42 ml of TEA (2,93 mmol). The reaction was cooled at 4°C and slowly added dropwise 0,20 ml of acetyl chloride (2,71 mmol). The mixture was stirred for 15 minutes at 4°C and for 1h at room temperature until the complete transformation of the starting material as revealed by TLC[264].
The reaction mixture was cooled at 4°C and quenched with 15 ml of water and 10 ml of DCM. The separated organic layer was washed with 10% HCl (15 ml), $NaHCO_3$ satured solution (15 ml) and brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,73 g of a transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[265]) to give 540 mg of a transparent oil corresponding at the desired product.

Yield = 87,09 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,06 (d, 1H, J= 2,2 Hz) 6,96 (dd, 1H, J= 8,6 J= 2,2 Hz) 6,76 (d, 1H, J= 8,6) 4,39 (m, 1H) 4,28 (m, 3H) 4,04 (dd, 1H, J= 11,5 J= 6,8 Hz) 2,12 (s, 3H).

[264] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,51 Marker: $Ce(SO_4)_2$
[265] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,21 Rf prod= 0,23 Marker: $Ce(SO_4)_2$

7-(Trimethylsilyl)ethynyl-2-acetoxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{11}BrO_4$
Molecular Weight: 287,11

Chemical Formula: $C_{16}H_{20}O_4Si$
Molecular Weight: 304,41

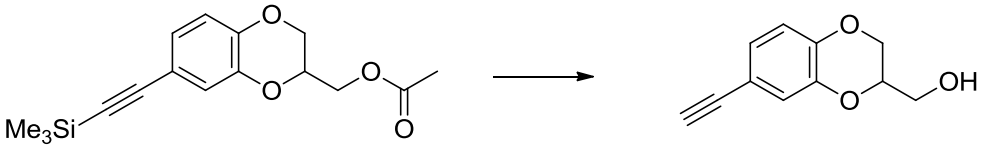
To a solution of 6-Bromo-3-acetoxymethyl-1,4-benzodioxane (540 mg, 1,88 mmol) in 2,5 ml of TEA were added 20 mg of $PdCl_2$ (0,1 mmol), 15 mg of CuI (0,07 mmol) and 50 mg of PPh_3 (0,19 mmol), 0,33 ml of ethynyltrimethylsilane (2,33 mmol). The mixture was brought at 55°C overnight.
The TLC control[266] revealed the transformation of the starting material.
At the mixture were added 20 ml of ethyl acetate and 15 ml of water. The separated organic layer was washed with 10% HCl (15 ml) and brine (3 x 15 ml) , dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 560 mg of brownish oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[267]) to give 320 mg of a red oil corresponding at the desired product.

Yield = 55,94%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,05 (d, 1H, J= 2,1 Hz) 6,95 (dd, 1H, J= 8,5 J= 2,2 Hz) 6,76 (d, 1H, J= 8,5 Hz) 4,35 (m, 4H) 4,04 (dd, 1H, J= 11,2 J= 6,6 Hz) 2,12 (s, 3H) 0,23 (s, 9H).

[266] Toluene Rf start= 0,41 Rf prod= 0,45 Marker: Blue sheet
[267] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,19 Rf prod= 0,21 Marker: Blue sheet

7-Ethynyl-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{16}H_{20}O_4Si$
Molecular Weight: 304,41

Chemical Formula: $C_{11}H_{10}O_3$
Molecular Weight: 190,20

To a solution of 6-(trimethylsilyl)ethynyl-3-acetoxymethyl-1,4-benzodioxane (320 mg, 1,05 mmol) in 4 ml of MeOH 0,51 ml of NaOH 2,5 M were added. The mixture was stirred at room temperature for 1h until the complete transformation of the starting material as revealed by TLC[268].

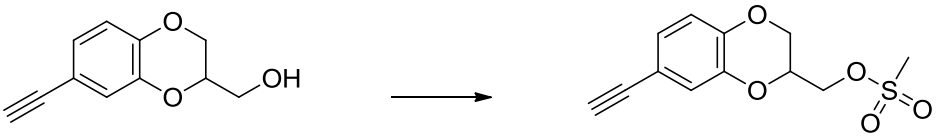
The reaction mixture was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of water. The separated aqueous layer was extracted with ethyl acetate (3 x 10 ml). The combined organic phases were washed with brine (30 ml) , dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 198 mg of brownish oil corresponding at the desired product.

Yield = 99,14%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,02 (m, 2H) 6,81 (d, 1H, J= 8,3 Hz) 4,28 (m, 2H) 4,12 (dd, 1H, J= 11,6 J= 7,2 Hz) 3,88 (m, 2H) 2,97 (s, 1H) 1,98 (bs, 1H).

[268] Cyclohexane/ Ethyl acetate 1/1 Rf start= 0,60 Rf prod= 0,40 Marker: Blue sheet

7-Ethynyl-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{10}O_3$
Molecular Weight: 190,20

Chemical Formula: $C_{12}H_{12}O_5S$
Molecular Weight: 268,29

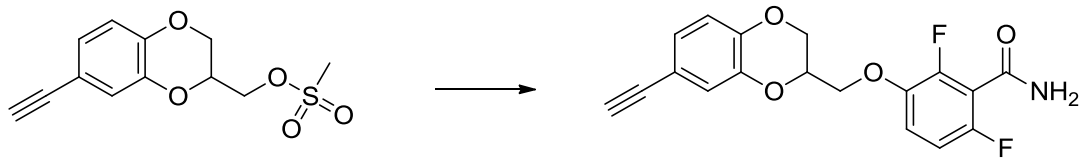
To a solution of 6-ethynyl-3-hydroxymethyl-1,4-benzodioxane (198 mg, 1,05 mmol) in 6 ml of DCM 0,20 ml of TEA (1,36 mmol) were added. The reaction was cooled at 4°C and 0,10 ml of methansulphonyl chloride (1,26 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[269].
The reaction mixture was cooled at 4°C and quenched with 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10% HCl (10 ml), $NaHCO_3$ satured solution (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 279 mg of a brownish oil corresponding at the desired product.

Yield = 98,09 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,02 (m, 2H) 6,83 (d, 1H, J= 8,0 Hz) 4,44 (m, 3H) 4,33 (m, 1H) 4,13 (dd, 1H, J= 11,5 J= 6,1 Hz) 3,09 (s, 3H) 2,98 (s, 1H).

[269]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,21 Rf prod= 0,26 Marker: Blue sheet

2,6-Difluoro-3-(7-ethynyl-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{12}H_{12}O_5S$
Molecular Weight: 268,29

Chemical Formula: $C_{18}H_{13}F_2NO_4$
Molecular Weight: 345,30

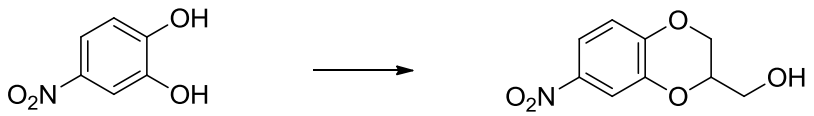
To a solution of 2,6-difluoro-3-hydroxybenzamide (201 mg, 1,16 mmol) in 3 ml of DMF were added 160 mg of anhydrous K_2CO_3 (1,16 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-ethynyl-3-mesyloxymethyl-1,4-benzodioxane (279 mg, 1,04 mmol) in 3 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C for 20 h until the complete transformation of the starting material as revealed by TLC[270].
The reaction was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with NaOH 1 M (20 ml) of and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 310 mg of a brownish oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[271]) to give 220 mg of a pale yellow wax corresponding at the desired product.

Yield = 60,77%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,05 (m, 3H) 6,85 (m, 2H) 6,14 (bs, 1H) 6,03 (bs, 1H) 4,55 (m, 1H) 4,41 (dd, 1H, J= 11,5 J= 2,3 Hz) 4,24 (m, 3H) 2,97 (s, 3H).

[270]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,34 Rf amid= 0,09 Rf prod= 0,16 Marker: $Ce(SO_4)_2$
[271]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,34 Rf amid= 0,09 Rf prod= 0,16 Marker: $Ce(SO_4)_2$

2-Hydroxymethyl-7-nitro-1,4-benzodioxane



Chemical Formula: $C_6H_5NO_4$
Molecular Weight: 155,11

Chemical Formula: $C_9H_9NO_5$
Molecular Weight: 211,17

Under nitrogen atmosphere to a solution of 4-nitro-catechol (2,0 g, 12,89 mmol) in 28 ml of DMF were added 1,16 g of $NaHCO_3$ (13,5 mmol). The reaction was cooled at 4°C and 1,4 ml of epibromidrine (16,37 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and brought at 80°C for 8 h until the complete transformation of the starting material as revealed by TLC[272].

The mixture was poured in 100 ml of water at 4°C, the aqueous phase was extracted with ethyl acetate (3 x 30 ml). The combined organic phases were washed with NaOH 1M (30 ml) and twice with brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,28 g of a yellow solid corresponding at the crude product.

The crude product was crystallized twice by DCM to give 0,45 g of white solid corresponding at the desired product

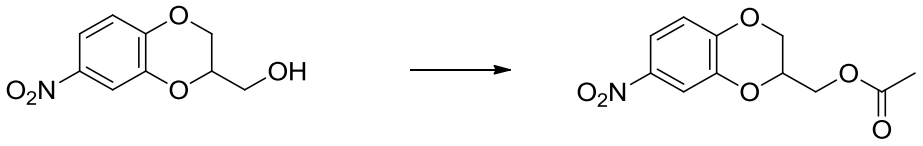
Yield = 16,54 %

M.P.= 135,96°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,80 (m, 2H) 6,96 (d, 1H, J= 8,6 Hz) 4,43 (dd, 1H, J= 11,1 J= 1,9 Hz) 4,27 (m, 1H) 3,93 (qd, 2H, J= 12,2 J= 4,4 Hz) 1,78 (bs, 1H).

[272]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,15 Rf prod= 0,22 Marker: $Ce(SO_4)_2$

2-Acetoxymethyl-7-nitro-1,4-benzodioxane



Chemical Formula: $C_9H_9NO_5$
Molecular Weight: 211,17

Chemical Formula: $C_{11}H_{11}NO_6$
Molecular Weight: 253,21

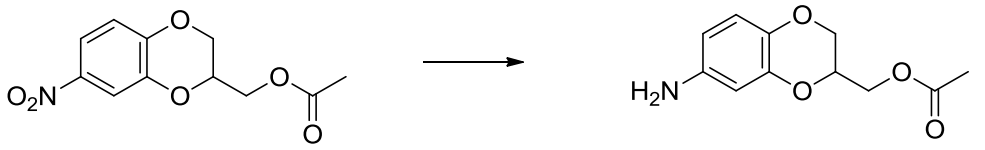
To a solution of 3-hydroxymethyl-6-nitro-1,4-benzodioxane (0,45 g, 2,13 mmol) in 5 ml of ethyl acetate were added 0,36 ml of TEA (2,56 mmol). The reaction was cooled at 4°C and 0,36 ml of acetyl chloride (2,56 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and brought at room temperature overnight.
The TLC control[273] revealed the complete transformation of the starting material.
The reaction mixture was diluted with 15 ml of water and 20 ml of ethyl acetate. The separated organic layer was washed with 10% HCl (20 ml) $NaHCO_3$ satured solution (20 ml) of and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,51 g of a transparent oil corresponding at the desired product.

Yield = 94,56 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,80 (m, 2H) 6,96 (d, 1H, J= 8,8 Hz) 4,40 (m, 4H) 4,14 (m, 1H) 2,12 (s, 3H).

[273]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,22 Rf prod= 0,40 Marker: Blue sheet

2-Acetoxymethyl-7-amino-1,4-benzodioxane



Chemical Formula: $C_{11}H_{11}NO_6$
Molecular Weight: 253,21

Chemical Formula: $C_{11}H_{13}NO_4$
Molecular Weight: 223,23

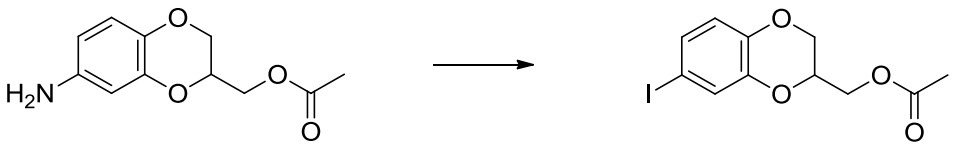
To a solution of 3-acetoxymethyl-6-nitro-1,4-benzodioxane (0,51 g, 2,01 mmol) in 8 ml of ethyl acetate 1,53 g of $SnCl_2$ (8,06 mmol) were added dividing in three portion in 15 minutes. The reaction was brought at reflux for 2 h until the complete transformation of the starting material as revealed by TLC[274].
The mixture was poured in 25 ml of $NaHCO_3$ satured solution and the resulting suspension was filtered. The mother liquids were extracted with ethyl acetate (3 x 20 ml), the combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,40 g of a transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[275]) to give 0,28 g of a pale yellow oil corresponding at the desired product.

Yield = 62,40%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,68 (d, 1H, J= 8,6 Hz) 6,27 (d, 1H, J= 2,7 Hz) 6,22 (dd, 1H, J= 8,6 J= 2,7 Hz) 4,31 (m, 3H) 3,97 (dd, 1H, J= 11,5 J= 6,9 Hz) 3,06 (bs, 2H) 2,13 (s, 3H).

[274]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,50 Rf prod= 0,34 Marker: Blue sheet
[275]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,50 Rf prod= 0,34 Marker: Blue sheet

2-Acetoxymethyl-7-iodo-1,4-benzodioxane



Chemical Formula: $C_{11}H_{13}NO_4$
Molecular Weight: 223,23

Chemical Formula: $C_{11}H_{11}IO_4$
Molecular Weight: 334,11

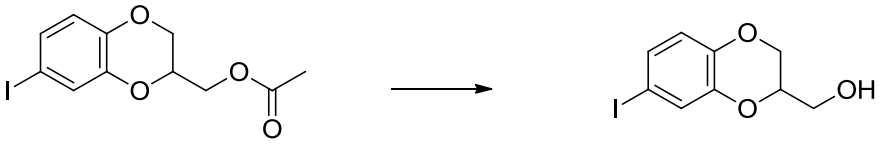
To a -5°C cooled solution of 3-acetoxymethyl-6-amino-1,4-benzodioxane (0,28 g, 1,25 mmol) in 4,68 ml of H_2SO_4 0,29 M a solution of $NaNO_2$ (95 mg in 2 ml of water) were slowly added dropwise and stirred for 15 minutes. Then a solution of NaI (320 mg in 2 ml of water) was added dropwise. The reaction was stirred at room temperature overnight.
The TLC control[276] revealed the complete transformation of the starting material.
The reaction mixture was quenched with 10 ml of $Na_2S_2O_5$ satured solution, the aqueous phase was extracted with ethyl acetate (3 x 20 ml), the combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,34 g of a transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[277]) to give 0,24 g of a transparent oil corresponding at the desired product.

Yield = 57,46%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,23 (d, 1H, J= 2,1 Hz) 7,14 (dd, 1H, J= 8,5 J= 2,1 Hz) 6,63 (d, 1H, J= 8,5) 4,37 (m, 1H) 4,28 (m, 3H) 4,03 (dd, 1H, J= 11,5 J= 6,9 Hz) 2,07 (s, 3H).

[276] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,34 Rf prod= 0,41 Marker: Blue sheet
[277] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,15 Rf prod= 0,29 Marker: Blue sheet

2-Hydroxymethyl-7-iodo-1,4-benzodioxane



Chemical Formula: $C_{11}H_{11}IO_4$
Molecular Weight: 334,11

Chemical Formula: $C_9H_9IO_3$
Molecular Weight: 292,07

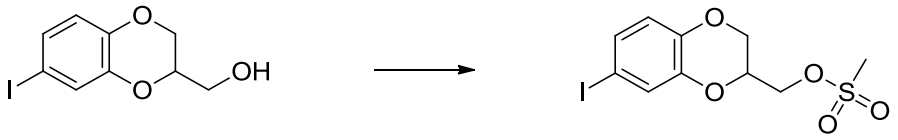
To a solution of 3-acetoxymethyl-6-iodo-1,4-benzodioxane (0,24 g, 0,72 mmol) in 3 ml of MeOH 0,37 ml of NaOH 2,5 M and 3 ml of water were added dropwise. The reaction was stirred at room temperature for 1 h until the complete transformation of the starting material as revealed by TLC[278].
The reaction mixture was evaporated under vacuum and diluted with 10 ml of water, the aqueous phase was extracted with ethyl acetate (3 x 10 ml), the combined organic phases were washed with brine (20 ml) and dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,18 g of a brownish oil corresponding at the crude product.

Yield = 85,59%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,22 (d, 1H, J= 2,1 Hz) 7,13 (dd, 1H, J= 8,5 J= 2,1 Hz) 6,63 (d, 1H, J= 8,5) 4,25 (m, 2H) 4,11 (m, 1H) 3,86 (qd, 2H, J= 12,1 J= 4,7 Hz) 1,47 (bs, 1H).

[278]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,29 Rf prod= 0,19 Marker: Blue sheet

7-Iodo-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9IO_3$
Molecular Weight: 292,07

Chemical Formula: $C_{10}H_{11}IO_5S$
Molecular Weight: 370,16

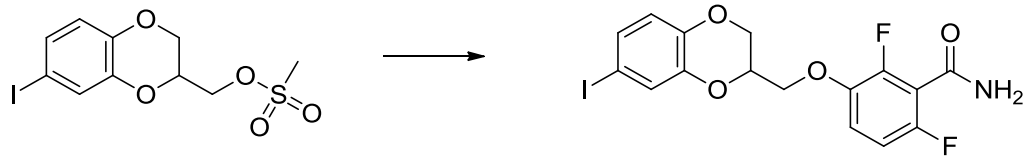
To a solution of 3-hydroxymethyl-6-iodo-1,4-benzodioxane (0,18 g, 0,62 mmol) in 5 ml of DCM were added 0,11 ml of TEA (0,81 mmol). The reaction was cooled at 4°C and 0,06 ml of methansulphonyl chloride (0,74 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[279].
The mixture was cooled at 4°C and quenched with 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10% HCl (10 ml), $NaHCO_3$ satured solution (10 ml) and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,23 g of a yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[280]) to give 0,19 g of a transparent oil corresponding at the desired product.

Yield = 82,79 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,23 (d, 1H, J= 2,1 Hz) 7,16 (dd, 1H, J= 8,6 J= 2,1 Hz) 6,65 (d, 1H, J= 8,5) 4,45 (m, 3H) 4,30 (dd, 1H, J= 11,7 J= 2,3 Hz) 4,11 (dd, 1H, J= 11,7 J= 6,2 Hz) 3,09 (s, 3H).

[279]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,19 Rf prod= 0,12 Marker: Blue sheet
[280]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,31 Rf prod= 0,26 Marker: Blue sheet

2,6-Difluoro-3-(7-iodo-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{10}H_{11}IO_5S$
Molecular Weight: 370,16

Chemical Formula: $C_{16}H_{12}F_2INO_4$
Molecular Weight: 447,17

To a solution of 2,6-difluoro-3-hydroxybenzamide (97 mg, 0,56 mmol) in 2 ml of DMF were added 77 mg of anhydrous K_2CO_3 (0,56 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-Iodo-3-mesyloxymethyl-1,4-benzodioxane (190 mg, 0,51 mmol) in 2 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 75°C for 4 h until the complete transformation of the starting material as revealed by TLC[281].

The reaction mixture was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with NaOH 1 M (15 ml) and brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 190 mg of a yellow oil corresponding at the crude product.

The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[282]) to give 120 mg of a white wax corresponding at the desired product.

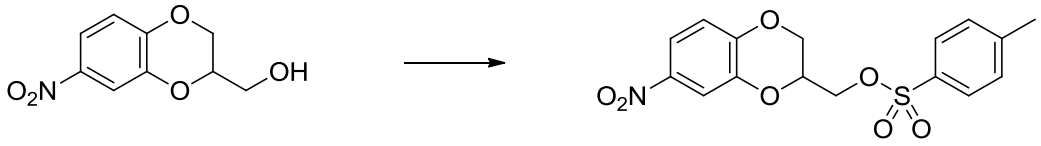
Yield = 52,62%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,22 (d, 1H, J= 2,2 Hz) 7,15 (dd, 1H, J= 8,5 J= 2,2 Hz) 7,06 (td, 1H, J= 9,1 J= 5,1 Hz) 6,90 (td, 1H, J= 9,1 J= 2,0 Hz) 6,65 (d, 1H, J= 8,5 Hz) 6,00 (bs, 2H) 4,55 (m, 1H) 4,38 (dd, 1H, J= 11,6 J= 2,4 Hz) 4,23 (m, 3H).

[281] Toluene/ Ethyl Acetate 70/30 Rf start= 0,42 Rf amid= 0,12 Rf prod= 0,30 Marker: $Ce(SO_4)_2$

[282] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,42 Rf amid= 0,15 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

7-Nitro-2-tosyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_9H_9NO_5$
Molecular Weight: 211,17

Chemical Formula: $C_{16}H_{15}NO_7S$
Molecular Weight: 365,36

To a 4°C solution of 3-Hydroxymethyl-6-nitro-1,4-benzodioxane (0,32 g, 1,52 mmol) in 3 ml of pyridine 0,30 g of paratoluensulphonyl chloride (1,52 mmol) were added. The mixture was stirred for 15 minutes at 4°C and brought at room temperature for 3 h until the complete transformation of the starting material as revealed by TLC[283].

The reaction mixture was diluted with 20 ml of water and 20 ml of DCM, the organic phase was washed with HCl 10% (20 ml), $NaHCO_3$ satured solution (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,4 g of a transparent oil corresponding at the crude product.

The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[284]) to give 270 mg of a white solid corresponding at the desired product.

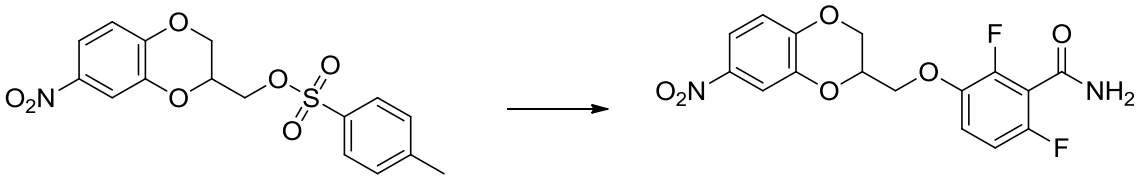
Yield = 48,61 %

M.P.= 101,21°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,78 (m, 3H) 7,64 (d, 1H, J= 2,6 Hz) 7,37 (d, 2H, J= 8,0 Hz) 6,93 (d, 1H, J= 8,8 Hz) 4,42 (m, 2H) 4,26 (m, 2H) 4,14 (dd, 1H, J= 11,7 J= 6,8 Hz) 2,46 (s, 3H).

[283] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,22 Rf prod= 0,19 Marker: Blue sheet
[284] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,22 Rf prod= 0,19 Marker: Blue sheet

2,6-Difluoro-3-(7-nitro-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{16}H_{15}NO_7S$
Molecular Weight: 365,36

Chemical Formula: $C_{16}H_{12}F_2N_2O_6$
Molecular Weight: 366,27

To a solution of 2,6-difluoro-3-hydroxybenzamide (140 mg, 0,81 mmol) in 2 ml of DMF were added 110 mg of anhydrous K_2CO_3 (0,81 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-nitro-3-tosyloxymethyl-1,4-benzodioxane (270 mg, 0,74 mmol) in 2 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 75°C for 4 h until the complete transformation of the starting material as revealed by TLC[285].

The reaction was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with NaOH 1 M (15 ml) and brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 230 mg of a yellow oil corresponding at the crude product.

The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[286]) to give 160 mg of a white solid corresponding at the desired product.

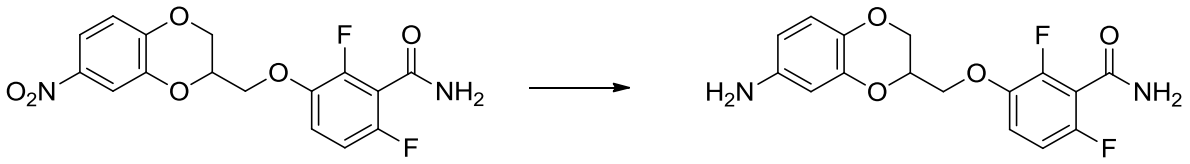
Yield = 59,03%

M.P. = 172,16°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,81 (dd, 2H, J= 8,0 J= 2,2 Hz) 7,09 (td, 1H, J= 9,0 J= 5,0 Hz) 6,95 (m, 2H) 5,94 (bs, 2H) 4,60 (m, 1H) 4,53 (dd, 1H, J= 11,7 J= 2,4 Hz) 4,34 (m, 2H) 4,25 (dd, 1H, J= 10,3 J= 5,9 Hz).

[285] Toluene/ Ethyl Acetate 70/30 Rf start= 0,70 Rf amid= 0,17 Rf prod= 0,26 Marker: $Ce(SO_4)_2$
[286] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,50 Rf amid= 0,08 Rf prod= 0,16 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-amino-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{16}H_{12}F_2N_2O_6$
Molecular Weight: 366,27

Chemical Formula: $C_{16}H_{14}F_2N_2O_4$
Molecular Weight: 336,29

To a solution of 2,6-Difluoro-3-(6-nitro-1,4-benzodioxan-3-yl)methyloxy benzamide (210 mg, 0,57 mmol) in 5 ml of ethyl acetate 430 mg of $SnCl_2$ (2,29 mmol) were added. The mixture was stirred for 15 minutes at room temperature, then brought at reflux for 2 h until the complete transformation of the starting material as revealed by TLC[287].
The reaction was poured into 20 ml of ice cooled satured solution of $NaHCO_3$, the resulting suspension was filtered. The mother liquids were extracted with ethyl acetate (3 x 10 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 240 mg of a oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (toluene/ethyl acetate 60/40[288]) to give 190 mg of a white solid corresponding at the desired product.

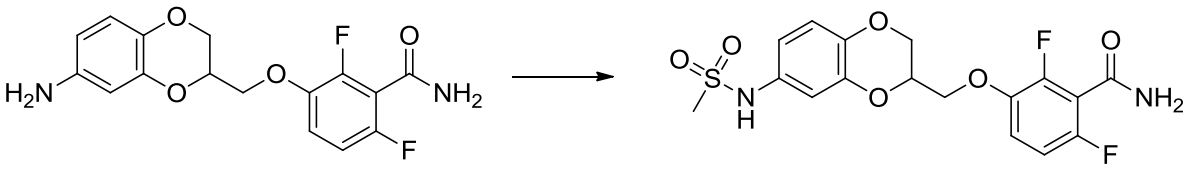
Yield = 99,12%

M.P. = 141,32°C

^{1}H-NMR (d6-DMSO) δ (ppm): 8,11 (s, 1H) 7,83 (s, 1H) 7,27 (td, 1H, J= 9,3 J= 5,2 Hz) 7,06 (m, 1H) 6,54 (d, 1H, J= 8,4 Hz) 6,10 (d, 1H, J= 2,4 Hz) 6,06 (dd, 1H, J= 8,4 J= 2,5 Hz) 4,64 (bs, 2H) 4,47 (m, 1H) 4,25 (m, 3H) 3,99 (dd, 1H, J= 11,5 J= 6,7 Hz).

[287]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,16 Rf prod= 0,12 Marker: $Ce(SO_4)_2$
[288]Toluene/Ethyl Acetate 60/40 Rf start= 0,37 Rf prod= 0,32 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-methansulphonamido-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{16}H_{14}F_2N_2O_4$
Molecular Weight: 336,29

Chemical Formula: $C_{17}H_{16}F_2N_2O_6S$
Molecular Weight: 414,38

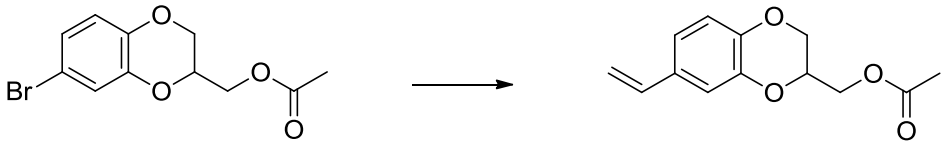
To a solution of 2,6-Difluoro-3-(6-amino-1,4-benzodioxan-3-yl)methyloxy benzamide (190 mg, 0,56 mmol) in 8 ml of ethyl acetate 100 µl of TEA (0,73 mmol) were added. The mixture was cooled at 4°C and 50 µl of methansulphonyl chloride (0,67 mmol) were added dropwise. The mixture was stirred for 15 minutes at 4°C, then brought at reflux for 30 minutes until the complete transformation of the starting material as revealed by TLC[289].
The reaction mixture was cooled at 4°C and quenched with 15 ml of HCl 10%. The separated aqueous layer was extracted with ethyl acetate (3 x 15 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 230 mg of a brownish oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (toluene/ethyl acetate 1/1[290]) to give 100 mg of a transparent wax corresponding at the desired product.

Yield = 43,10%

^{1}H-NMR (CD_3OD) δ (ppm): 7,22 (m, 2H) 6,97 (td, 1H, J= 8,9 J= 2,1 Hz) 6,83 (m, 1H) 6,74 (dd, 1H, J= 8,4 Hz J= 2,6Hz) 4,55 (m, 1H) 4,39 (dd, 1H, J= 11,6 J= 2,6 Hz) 4,31 (d, 2H, J= 5,2 Hz) 4,19 (dd, 1H, J= 11,5 J= 6,8 Hz) 2,88 (s, 3H).

[289]Toluene/Ethyl Acetate 1/1 Rf start= 0,35 Rf prod= 0,22 Marker: $Ce(SO_4)_2$
[290]Toluene/Ethyl Acetate 1/1 Rf start= 0,35 Rf prod= 0,22 Marker: $Ce(SO_4)_2$

7-Vinyl-2-acetoxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{11}BrO_4$
Molecular Weight: 287,11

Chemical Formula: $C_{13}H_{14}O_4$
Molecular Weight: 234,25

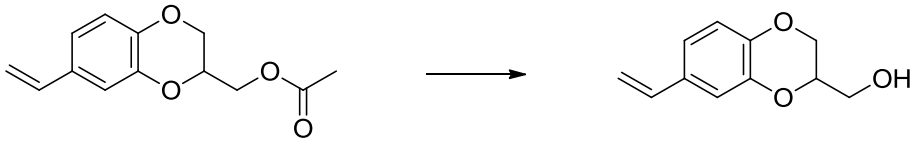
Under nitrogen atmosphere to a solution of 6-bromo-3-acetoxymethyl-1,4-benzodioxane (590 mg, 2,98 mmol) in 16 ml of iPrOH and 10 ml of water were added 400 mg of potassium vinyl trifluoroborate (2,98 mmol), 170 mg of $PdCl_2$(dppe).CH_2Cl_2 (0,3 mmol) and 0,94 ml of $TButNH_2$ (8,94 mmol). The mixture was stirred at reflux overnight.
The TLC control[291] revealed the complete transformation of the starting material.
The reaction mixture was evaporated under vacuum and diluted with 10 ml of water and 10 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed with 10% HCl (10 ml), and brine (3 x 10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 400 mg of a green oil corresponding at the desired product.

Yield = 57,30%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,96 (m, 3H) 6,60 (dd, 1H, J= 17,6 J= 10,8 Hz) 5,59 (dd, 1H, J= 17,5 J= 0,8 Hz) 5,14 (dd, 1H, J= 10,8 J= 0,8 Hz) 4,27 (m, 2H) 4,10 (m, 1H) 3,88 (m, 2H) 2,12 (s, 3H).

[291] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,33 Rf prod= 0,38 Marker: $Ce(SO_4)_2$

7-Vinyl-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{13}H_{14}O_4$
Molecular Weight: 234,25

Chemical Formula: $C_{11}H_{12}O_3$
Molecular Weight: 192,21

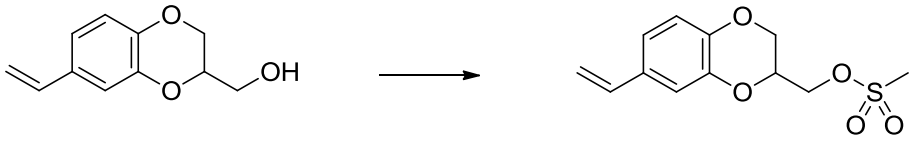
To a solution of 6-Vinyl-3-acetoxymethyl-1,4-benzodioxane (400 mg, 2,01 mmol) in 5 ml of MeOH 1,0 ml of NaOH 2,5 M and 3,0 ml of water were added. The reaction was stirred at room temperature for 30 minutes until the complete transformation of the starting material as revealed by TLC[292].
The reaction mixture was evaporated under vacuum and diluted with 10 ml of water and 10 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (10 ml). The combined organic phases were washed twice with brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 350 mg of a green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (Cyclohexane/ethyl acetate 80/20[293]) to give 140 mg of a transparent wax corresponding at the desired product.

Yield = 36,24%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,89 (m, 3H) 6,60 (dd, 1H, J= 17,6 J= 10,8 Hz) 5,59 (dd, 1H, J= 17,5 J= 0,9 Hz) 5,14 (dd, 1H, J= 10,8 J= 0,9 Hz) 4,27 (m, 2H) 4,12 (m, 1H) 3,88 (m, 2H) 1,90 (bs, 1H).

[292]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,38 Rf prod= 0,20 Marker: $Ce(SO_4)_2$
[293]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,38 Rf prod= 0,20 Marker: $Ce(SO_4)_2$

7-Vinyl-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{12}O_3$
Molecular Weight: 192,21

Chemical Formula: $C_{12}H_{14}O_5S$
Molecular Weight: 270,30

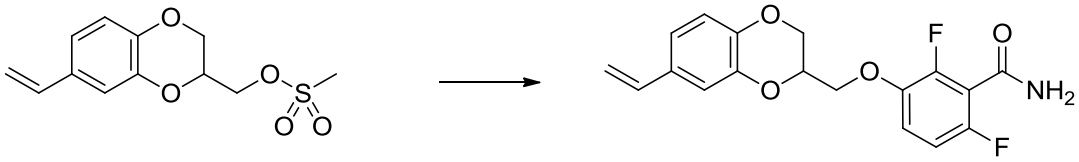
To a solution of 6-Vinyl-3-hydroxymethyl-1,4-benzodioxane (140 mg, 0,73 mmol) in 5 ml of DCM 170 µl of TEA (1,17 mmol) were added. The mixture was cooled at 4°C and 100 µl of methansulphonyl chloride (1,07 mmol) were added dropwise. The mixture was stirred at 4°C for 15 minutes and 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[294].
The mixture was cooled at 4°C and diluted with 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10% HCl (10 ml), $NaHCO_3$ satured solution (10 ml)and brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 220 mg of a green oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 80/20[295]) to give 110 mg of a transparent wax corresponding at the desired product.

Yield = 55,74%

^{1}H-NMR ($CDCl_3$) δ (ppm): 6,91 (m, 3H) 6,59 (dd, 1H, J= 17,6 J= 10,8 Hz) 5,60 (dd, 1H, J= 17,5 J= 0,9 Hz) 5,15 (dd, 1H, J= 10,8 J= 0,9 Hz) 4,47 (m, 3H) 4,31 (dd, 1H, J= 11,6 J= 2,2 Hz) 4,13 (dd, 1H, J= 11,6 J= 6,1 Hz) 3,09 (s, 3H).

[294]Toluene/ Ethyl Acetate 90/10 Rf start= 0,17 Rf prod= 0,33 Marker: $Ce(SO_4)_2$
[295]Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,14 Rf prod= 0,16 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-vinyl-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{12}H_{14}O_5S$
Molecular Weight: 270,30

Chemical Formula: $C_{18}H_{15}F_2NO_4$
Molecular Weight: 347,31

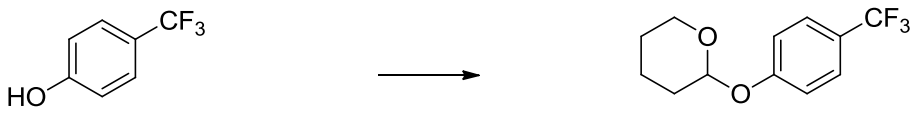
To a solution of 2,6-difluoro-3-hydroxybenzamide (78 mg, 0,45 mmol) in 2 ml of DMF were added 110 mg of anhydrous K_2CO_3 (0,45 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-vinyl-3-mesyloxymethyl-1,4-benzodioxane (110 mg, 0,41 mmol) in 3 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 70°C for 6 h e 30 minutes until the complete transformation of the starting material as revealed by TLC[296].
The reaction was evaporated under vacuum and diluted with 10 ml of ethyl acetate and 10 ml of distilled water. The separated aqueous layer was extracted with twice with ethyl acetate (10 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 120 mg of a yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[297]) to give 100 mg of a white wax corresponding at the desired product.

Yield = 69,93%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,06 (m, 1H) 6,90 (m, 3H) 6,59 (dd, 1H, J= 17,5 J= 10,9 Hz) 6,00 (bs, 2H) 5,59 (d, 1H, J= 17,6 Hz) 5,14 (d, 1H, J= 10,9 Hz) 4,56 (m, 1H) 4,39 (dd, 1H, J= 11,5 J= 2,4 Hz) 4,25 (m, 3H).

[296]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,43 Rf amid= 0,13 Rf prod= 0,25 Marker: $Ce(SO_4)_2$
[297]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,40 Rf amid= 0,08 Rf prod= 0,20 Marker: $Ce(SO_4)_2$

2-(4-(Trifluoromethyl)phenoxy)tetrahydro-2H-pyran



Chemical Formula: $C_7H_5F_3O$
Molecular Weight: 162,11

Chemical Formula: $C_{12}H_{13}F_3O_2$
Molecular Weight: 246,23

To a solution of 4-trifluorophenol (2,5 g, 15,41 mmol) in 75 ml of DCM 3,5 ml of (38,54 mmol) and 0,45 ml of HCl 4 M in 1,4-dioxane (1,5 mmol) were added dropwise. The mixture was stirred at for 2 h at room temperature until the complete transformation of the starting material as revealed by TLC[298].

The reaction mixture was diluted with 50 ml of $NaHCO_3$ satured solution. The separated organic layer was dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 5,80 g of an transparent oil corresponding at the crude product.

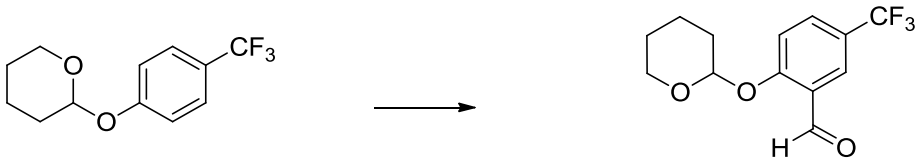
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5[299]) to give 3,62 g of a transparent liquid corresponding at the desired product.

Yield = 95,51 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,54 (d, 2H, J= 8,5 Hz) 7,12 (d, 2H, J= 8,5 Hz) 5,48 (t, 1H, J= 3,1 Hz) 3,86 (m, 1H) 3,62 (m, 1H) 1,94 (m, 3H) 1,66 (m, 4H).

[298] Cyclohexane/ Ethyl Acetate 95/5 Rf start= 0,42 Rf prod= 0,17 Marker: $Ce(SO_4)_2$
[299] Cyclohexane/ Ethyl Acetate 95/5 Rf start= 0,42 Rf prod= 0,17 Marker: $Ce(SO_4)_2$

2-((Tetrahydro-2H-pyran-2-yl)oxy)-5-(trifluoromethyl)benzaldehyde



Chemical Formula: $C_{12}H_{13}F_3O_2$
Molecular Weight: 246,23

Chemical Formula: $C_{13}H_{13}F_3O_3$
Molecular Weight: 274,24

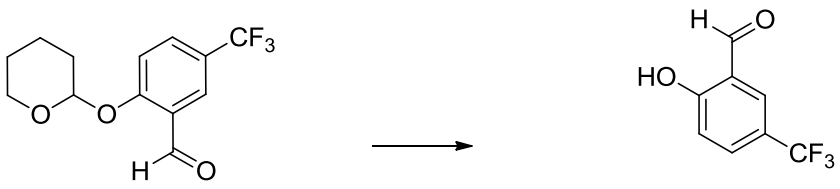
Under nitrogen atmosphere to a -10 °C solution of tetramethylethylendiamine (2,72 g, 23,41 mmol) in 6 ml of dry THF 6,23 ml of n BuLi 2,7 M in heptanes were added dropwise. The mixture was stirred for 30 minutes and then a solution of 2-(4-(trifluoromethyl)phenoxy)tetrahydro-2H-pyran (3,62 g, 14,63 mmol) in 15 ml of THF were added dropwise. The mixture was stirred at -10 °C for 1 h and 30 minutes and after 1,2 ml of DMF dry were added dropwise. The reaction was stirred for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[300].
The reaction mixture was quenched with 45 ml of water, The separated aqueous layer was extracted with ethyl acetate (3 x 30 ml), the combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 4,00 g of an yellow oil corresponding at the desired product.

Yield = 99,75%

^{1}H-NMR ($CDCl_3$) δ (ppm): 10,54 (s, 1H) 8,11 (d, 1H, J= 2,4 Hz) 7,76 (dd, 1H, J=8,9 J=2,4 Hz) 7,37 (d, 1H, J= 8,9 Hz) 5,66 (s, 1H) 3,83 (td, 1H, J=11,3 J= 3,2 Hz) 3,68 (m, 1H) 2,00 (m, 3H) 1,73 (m, 4H).

[300] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,58 Rf prod= 0,31 Marker: $Ce(SO_4)_2$

2-Hydroxy-5-(trifluoromethyl)benzaldehyde



Chemical Formula: $C_{13}H_{13}F_3O_3$
Molecular Weight: 274,24

Chemical Formula: $C_8H_5F_3O_2$
Molecular Weight: 190,12

To a solution of 2-((tetrahydro-2H-pyran-2-yl)oxy)-5-(trifluoromethyl)benzaldehyde (4,00 g, 14,63 mmol) in 30 ml of 1,4-dioxane were added dropwise 25 ml of HCl 10%. The reaction was stirred for 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[301].

The reaction mixture was diluted with 30 ml of water and 30 ml of ethyl acetate. The separated organic layer was washed twice with brine (50 ml) , dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 3,71 g of an yellow oil corresponding at the crude product.

The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 95/5[302]) to give 1,90 g of a transparent wax corresponding at the desired product.

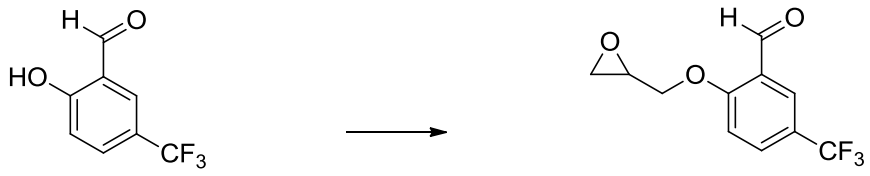
Yield = 68,34%

^{1}H-NMR ($CDCl_3$) δ (ppm): 11,30 (s, 1H) 9,95 (s, 1H) 7,86 (d, 1H, J= 2,2 Hz) 7,76 (dd, 1H, J=8,8 J=2,2 Hz) 7,11 (d, 1H, J= 8,8 Hz).

[301] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,31 Rf prod= 0,17 Marker: $Ce(SO_4)_2$

[302] Cyclohexane/ Ethyl Acetate 95/5 Rf start= 0,28 Rf prod= 0,47 Rf imp= 0,34 Marker: $Ce(SO_4)_2$

2-Epoxypropoxy-5-(trifluoromethyl)-benzaldehyde



Chemical Formula: $C_8H_5F_3O_2$
Molecular Weight: 190,12

Chemical Formula: $C_{11}H_9F_3O_3$
Molecular Weight: 246,18

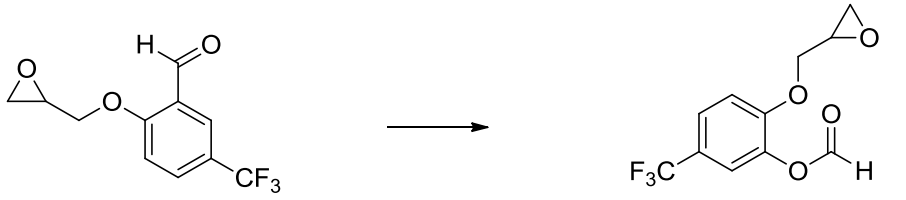
Under nitrogen atmosphere to a solution of 2-hydroxy-5-(trifluoromethyl)benzaldehyde (1,90 g, 10,00 mmol) in 30 ml of DMF were added 1,66 g of anhydrous K_2CO_3 (12,00 mmol) and 1,04 ml of epibromohydrin (12,00 mmol). The reaction was stirred at 40°C overnight.
The TLC control[303] revealed the transformation of the starting material.
The reaction mixture was evaporated under vacuum and diluted with 20 ml of water and 20 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1M (40 ml) and brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 2,15 g of an yellow liquid corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[304]) to give 1,80 g of a transparent oil corresponding at the desired product.

Yield = 73,17%

^{1}H-NMR ($CDCl_3$) δ (ppm): 10,52 (s, 1H) 8,13 (d, 1H, J= 2,5 Hz) 7,79 (d, 1H, J= 8,8 J= 2,5 Hz) 7,12 (d, 1H, J=8,8 Hz) 4,50 (dd, 1H, J= 11,2 J= 2,7Hz) 4,11 (dd, 1H, J= 11,2 J= 5,9 Hz) 3,43 (ddt, 1H, J= 5,9 J= 4,2 J= 2,6 Hz) 2,98 (dd, 1H, J= 4,7 J= 4,2 Hz) 2,81 (dd, 1H, J= 4,8 J= 2,6 Hz).

[303] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,52 Rf prod= 0,14 Marker: $Ce(SO_4)_2$
[304] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,58 Rf prod= 0,19 Marker: $Ce(SO_4)_2$

2-Epoxypropoxy-5-(trifluoromethyl)phenyl formate



Chemical Formula: $C_{11}H_9F_3O_3$
Molecular Weight: 246,18

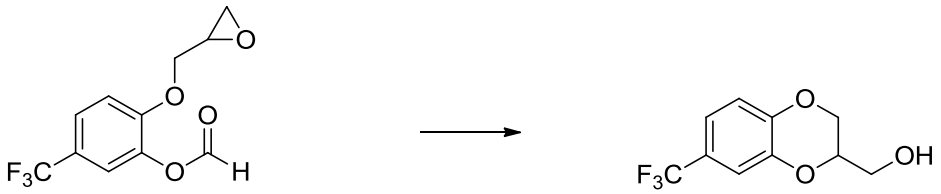
Chemical Formula: $C_{11}H_9F_3O_4$
Molecular Weight: 262,18

To a solution of 2-epoxypropoxy-5-(trifluoromethyl)-benzaldehyde (1,80 g, 7,31 mmol) in 18 ml of DCM 3,61 g of metachloroperbenzoic acid (14,65 mmol) were added dividing in three portion in 40 minutes. The reaction was stirred at reflux overnight.
The H-NMR control revealed the complete transformation of the starting material.
The mixture was cooled at 4°C and quenched 30 ml of $Na_2S_2O_5$ satured solution, a white precipitate afforded. The suspension was filtered. The mother liquids were diluted with 30 ml of ethyl acetate and were washed with $NaHCO_3$ satured solution (4 x 30 ml). The organic phase were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,90 g of an yellow oil corresponding at the desired product.

Yield = 98,95%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,95 (m, 1H) 7,19 (d, 1H, J= 2,2 Hz) 7,11 (m, 1H) 6,93 (d, 1H, J= 8,4 Hz) 4,39 (dd, 1H, J= 11,4 J= 2,6 Hz) 4,04 (dd, 1H, J= 11,4 J= 5,9 Hz) 3,40 (m, 1H) 2,97 (m, 1H) 2,81 (dd, 1H, J= 4,7 J= 2,7 Hz).

2-Hydroxymethyl-7-trifluoromethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_9F_3O_4$
Molecular Weight: 262,18

Chemical Formula: $C_{10}H_9F_3O_3$
Molecular Weight: 234,17

To a solution of 2-epoxypropoxy-5-(trifluoromethyl)phenyl formate (1,90 g, 7,25 mmol) in 20 ml of MeOH 4,35 ml of NaOH 2,5 M were added dropwise. The reaction was stirred for 15 minutes at room temperature and brought at 60°C for 2 h until the complete transformation of the starting material as revealed by TLC[305].

The reaction mixture was evaporated under vacuum and diluted with 20 ml of water and 20 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (20 ml). The combined organic phases were washed with NaOH 1 M (20 ml), twice with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,40 g of an yellow oil corresponding at the crude product.

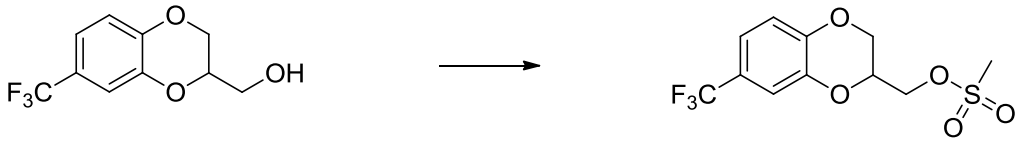
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[306]) to give 0,70 g of a transparent liquid corresponding at the desired product.

Yield = 41,17 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,17 (d, 1H, J= 2,1 Hz) 7,11 (m, 1H) 6,95 (d, 1H, J= 7,9 Hz) 4,36 (dd, 1H, J= 12,1 J= 4,6 Hz) 4,28 (m, 1H) 4,14 (m, 2H) 3,90 (qd, 1H; J= 12,1 J= 4,6 Hz) 1,83 (bs, 1H).

[305]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,23 Marker: $Ce(SO_4)_2$
[306]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,23 Marker: $Ce(SO_4)_2$

2-Mesyloxymethyl-7-trifluoromethyl-1,4-benzodioxane



Chemical Formula: $C_{10}H_9F_3O_3$
Molecular Weight: 234,17

Chemical Formula: $C_{11}H_{11}F_3O_5S$
Molecular Weight: 312,26

To a solution of 3-hydroxymethyl-6-trifluoromethyl-1,4-benzodioxane (700 mg, 2,99 mmol) in 10 ml of DCM were added 0,55 ml of TEA (3,89 mmol). The reaction was cooled at 4°C and 0,28 ml of methansulphonyl chloride (3,59 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and after 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[307].

The reaction mixture was cooled at 4°C and quenched with 10 ml of water and 10 ml of DCM. The organic phase was washed with HCl 10% (10 ml), $NaHCO_3$ satured solution (10 ml), brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 850 mg of an yellow oil corresponding at the crude product.

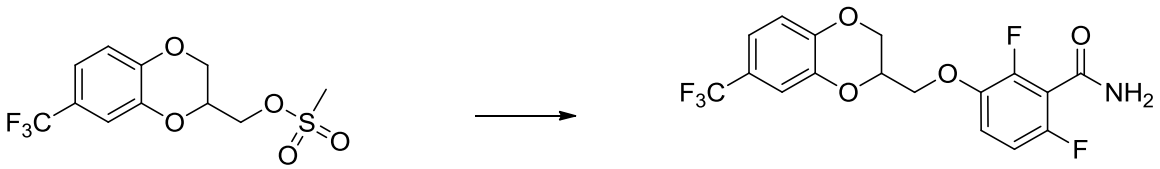
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[308]) to give 710 mg of a white wax corresponding at the desired product.

Yield = 76,34 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,15 (m, 2H) 6,98 (d, 1H, J= 8,3 Hz) 4,50 (m, 3H) 4,38 (dd, 1H, J= 11,7 J= 2,3 Hz) 4,17 (dd, 1H; J= 11,7 J= 6,5 Hz) 3,10 (s, 3H).

[307]Toluene/ Ethyl Acetate 80/20 Rf start= 0,26 Rf prod= 0,44 Marker: $Ce(SO_4)_2$
[308]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,23 Rf prod= 0,28 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-trifluoromethyl-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{11}H_{11}F_3O_5S$
Molecular Weight: 312,26

Chemical Formula: $C_{17}H_{12}F_5NO_4$
Molecular Weight: 389,27

To a solution of 2,6-difluoro-3-hydroxybenzamide (433 mg, 2,50 mmol) in 5 ml of DMF were added 345 mg of anhydrous K_2CO_3 (2,50 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 3-mesyloxymethyl-6-trifluoromethyl-1,4-benzodioxane (710 mg, 2,27 mmol) in 5 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 65°C for 7 h until the complete transformation of the starting material as revealed by TLC[309].
The reaction was evaporated under vacuum and diluted with 15 ml of ethyl acetate and 15 ml of distilled water. The separated aqueous layer was extracted twice with ethyl acetate (15 ml). The combined organic phases were washed with NaOH 1 M (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 120 mg of a yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[310]) to give 500 mg of a white solid corresponding at the desired product.

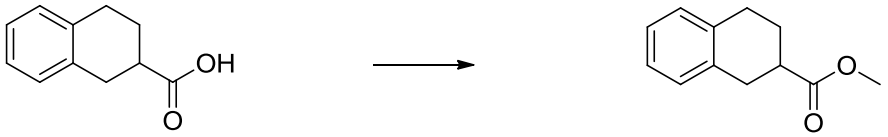
Yield = 56,62%

M.P. = 125,79°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,11 (m, 3H) 6,97 (d, 1H, J= 8,4 Hz) 6,91 (td, 1H, J= 9,1 J= 2,0 Hz) 5,96 (bs, 2H) 4,58 (m, 1H) 4,45 (dd, 1H, J= 11,6 J= 2,4 Hz) 4,27 (m, 3H).

[309]Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,42 Rf amid= 0,13 Rf prod= 0,23 Marker: $Ce(SO_4)_2$
[310]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,25 Rf amid= 0,08 Rf prod= 0,13 Marker: $Ce(SO_4)_2$

Methyl 1,2,3,4-tetrahydronaphthalene-2-carboxylate



Chemical Formula: $C_{11}H_{12}O_2$
Molecular Weight: 176,21

Chemical Formula: $C_{12}H_{14}O_2$
Molecular Weight: 190,24

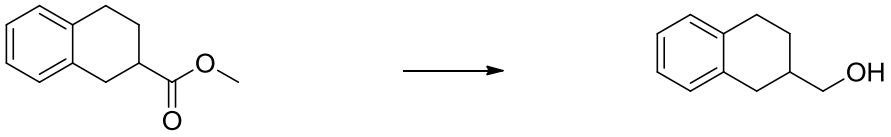
To a solution of 1,2,3,4-tetrahydronaphthalene-2-carboxylic acid (0,95 g, 5,39 mmol) in 10 ml of MeOH were added 0,01 ml of concentrated H_2SO_4 (0,54 mmol). The mixture was brought at reflux for 2h e 30 minutes until the complete transformation of the starting material as revealed by TLC[311].
The reaction mixture was evaporated under vacuum and diluted with 25 ml of ethyl acetate. The organic phase was washed with $NaHCO_3$ satured solution (3 x 15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,01 g of an yellow oil corresponding at the desired product.

Yield = 98,53%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,11 (m, 4H) 3,72 (s, 3H) 3,01 (d, 2H, J= 8,3 Hz) 2,87 (m, 2H) 2,75 (m, 1H) 2,21 (ddd, 1H, J= 16,1 J= 7,9 J= 4,0 Hz) 1,86 (dddd, 1H, J= 13,1 J= 11,2 J= 10,0 J= 6,9 Hz).

[311] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,12 Rf prod= 0,41 Marker: $Ce(SO_4)_2$

(1,2,3,4-tetrahydronaphthalen-2-yl)methanol



Chemical Formula: $C_{12}H_{14}O_2$
Molecular Weight: 190,24

Chemical Formula: $C_{11}H_{14}O$
Molecular Weight: 162,23

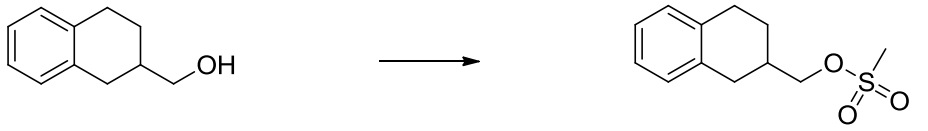
Under nitrogen atmosphere to a 4°C cooled suspension of $LiAlH_4$ (0,30 g, 6,94 mmol) in 5 ml of dry THF a solution of methyl 1,2,3,4-tetrahydronaphthalene-2-carboxylate (1,10g, 5,78 mmol) in 7 ml of dry THF was slowly added dropwise. The mixture was stirred at 4°C for 15 minutes and 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[312].
The reaction was poured in 50 ml of iced water, a suspension was formed and filtered under Celite® pad, the cake was washed with 30 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (30 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,88 g of an transparent oil corresponding at the desired product.

Yield = 93,62%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,10 (m, 4H) 3,63 (m, 2H) 2,85 (m, 3H) 2,52 (dd, 1H, J= 16,4 J= 10,5 Hz) 2,00 (m, 2H) 1,46 (m, 3H).

[312] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,50 Rf prod= 0,16 Marker: $Ce(SO_4)_2$

(1,2,3,4-tetrahydronaphthalen-2-yl)methyl methanesulfonate



Chemical Formula: $C_{11}H_{14}O$
Molecular Weight: 162,23

Chemical Formula: $C_{12}H_{16}O_3S$
Molecular Weight: 240,32

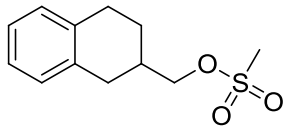
To a solution of (1,2,3,4-tetrahydronaphthalen-2-yl)methanol (0,88 mg, 5,42 mmol) in 15 ml of DCM were added 0,99 ml of TEA (7,05 mmol). The reaction was cooled at 4°C and 0,47 ml of methansulphonyl chloride (6,05 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and after 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[313].
The mixture was cooled at 4°C and quenched with 20 ml of water and 10 ml of DCM. The organic phase was washed with HCl 10% (15 ml), $NaHCO_3$ satured solution (15 ml), brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,31 g of an yellow oil corresponding at the desired product.

Yield = 99,23 %

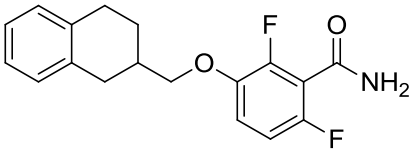
^{1}H-NMR ($CDCl_3$) δ (ppm): 7,11 (m, 4H) 4,21 (m, 2H) 3,04 (s, 3H) 2,88 (m, 3H) 2,58 (dd, 1H; J= 16,5 J= 10,5 Hz) 2,24 (m, 1H) 2,03 (m, 1H).

[313]Toluene/ Ethyl Acetate 80/20 Rf start= 0,30 Rf prod= 0,53 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-((1,2,3,4-tetrahydronaphthalen-2-yl)methoxy)benzamide



Chemical Formula: $C_{12}H_{16}O_3S$
Molecular Weight: 240,32



Chemical Formula: $C_{18}H_{17}F_2NO_2$
Molecular Weight: 317,33

To a solution of 2,6-difluoro-3-hydroxybenzamide (530 mg, 3,06 mmol) in 4 ml of DMF were added 460 mg of anhydrous K_2CO_3 (2,50 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of (1,2,3,4-tetrahydronaphthalen-2-yl)methyl methanesulfonate (670 mg, 2,78 mmol) in 5 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 70°C for 7 h until the complete transformation of the starting material as revealed by TLC[314].
The reaction was evaporated under vacuum and diluted with 20 ml of distilled water. The aqueous phase was extracted with ethyl acetate (3 x 15 ml). The combined organic phases were washed with NaOH 1 M (30 ml) and brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 720 mg of a white wax corresponding at the crude product.
The crude product was crystallized by a mixture cyclohexane/ethyl acetate (70/30) to give 330 mg of a white solid corresponding at the desired product.

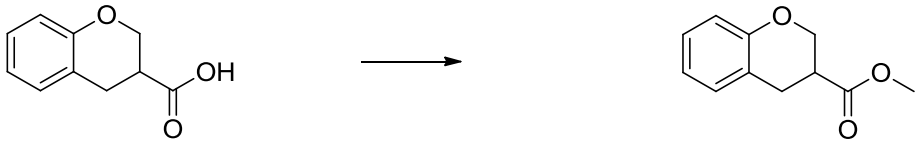
Yield = 37,50%

M.P. = 165,12°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,06 (m, 5H) 6,88 (td, 1H, J= 9,1 J= 1,9 Hz) 5,98 (bs, 2H) 3,98 (d, 2H, J= 6,5 Hz) 3,00 (dd, 1H, J= 17,9 J= 5,7 Hz) 2,87 (dd, 1H, J= 8,1 J= 4,7 Hz) 2,65 (dd, 1H, J= 16,3 J= 10,4 Hz) 2,34 (m, 1H) 2,11 (m, 1H) 1,60 (m, 3H).

[314]Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,43 Rf amid= 0,10 Rf prod= 0,28 Marker: $Ce(SO_4)_2$

Methyl chroman-3-carboxylate



Chemical Formula: $C_{10}H_{10}O_3$
Molecular Weight: 178,18

Chemical Formula: $C_{11}H_{12}O_3$
Molecular Weight: 192,21

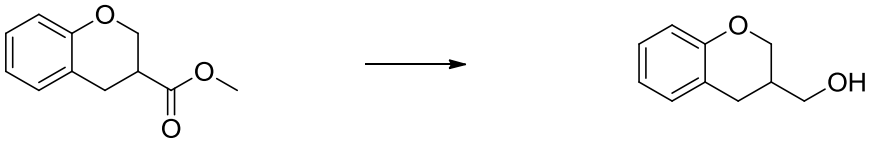
To a solution of chroman-3-carboxylic acid (1,00 g, 5,61 mmol) in 15 ml of MeOH 0,05 ml of concentrated H_2SO_4 (0,94 mmol) were added. The mixture was brought at reflux for 2h until the complete transformation of the starting material as revealed by TLC[315].
The reaction mixture was evaporated under vacuum and diluted with 30 ml of ethyl acetate. The organic phase was washed with $NaHCO_3$ satured solution (3 x 15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,05 g of an yellow oil corresponding at the desired product.

Yield = 97,22%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,10 (m, 2H) 6,86 (m, 2H) 4,45 (m, 1H) 4,12 (m, 1H) 3,75 (s, 3H) 3,06 (m, 3H).

[315] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,25 Rf prod= 0,57 Marker: $Ce(SO_4)_2$

Chroman-3-ylmethanol



Chemical Formula: $C_{11}H_{12}O_3$
Molecular Weight: 192,21

Chemical Formula: $C_{10}H_{12}O_2$
Molecular Weight: 164,20

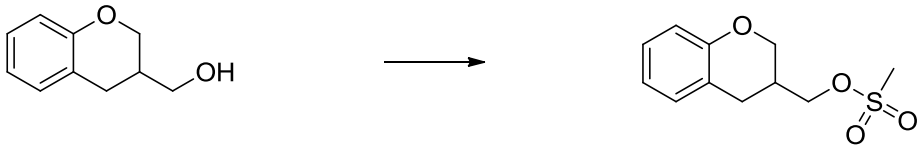
Under nitrogen atmosphere to a 4°C cooled suspension of $LiAlH_4$ (0,25 g, 6,55 mmol) in 5 ml of dry THF a solution of methyl chroman-3-carboxylate (1,08g, 5,46 mmol) in 10 ml of dry THF was slowly added dropwise. The mixture was stirred at 4°C for 15 minutes and 1 h at room temperature until the complete transformation of the starting material as revealed by TLC[316].
The reaction was poured in 50 ml of iced water, a suspension was formed and filtered under Celite® pad, the cake was washed with 30 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (30 ml). The combined organic phases were dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 0,79 g of an transparent oil corresponding at the desired product.

Yield = 97,50%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,11 (m, 2H) 6,84 (m, 2H) 4,30 (dd, 1H, J= 10,7 J= 4,4 Hz) 4,02 (dd, 1H, J= 10,7 J= 7,7 Hz) 3,70 (m, 2H) 2,84 (dd, 1H, J= 16,2 J= 5,5 Hz) 2,60 (dd, 1H, J= 16,2 J= 7,9 Hz) 1,58 (bs, 1H).

[316] Cyclohexane/ Ethyl Acetate 80/20 Rf start= 0,57 Rf prod= 0,24 Marker: $Ce(SO_4)_2$

Chroman-3-ylmethyl methanesulfonate



Chemical Formula: $C_{10}H_{12}O_2$
Molecular Weight: 164,20

Chemical Formula: $C_{11}H_{14}O_4S$
Molecular Weight: 242,29

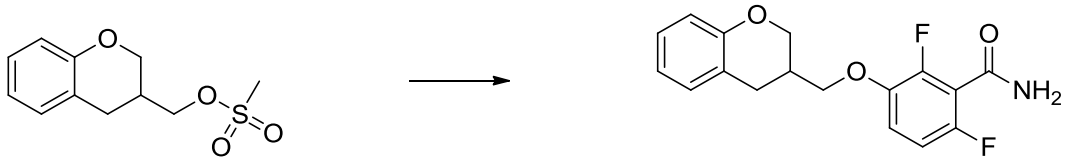
To a solution of chroman-3-ylmethanol (0,79 g, 4,81 mmol) in 15 ml of DCM were added 0,90 ml of TEA (6,25 mmol). The reaction was cooled at 4°C and 0,44 ml of methansulphonyl chloride (5,77 mmol) were slowly added dropwise. The mixture was stirred for 15 minutes at 4°C and after 30 minutes at room temperature until the complete transformation of the starting material as revealed by TLC[317].
The mixture was cooled at 4°C and quenched with 20 ml of water and 10 ml of DCM. The organic phase was washed with HCl 10% (20 ml), $NaHCO_3$ satured solution (20 ml), brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,20 g of an yellow oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[318]) to give 1,03 g of a transparent oil corresponding at the desired product.

Yield = 88,79%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,15 (m, 2H) 6,85 (m, 2H) 4,24 (m, 3H) 4,08 (dd, 1H, J= 11,0 J= 6,3 Hz) 2,99 (m, 4H) 2,65 (dd, 1H, J= 16,5 J= 6,3 Hz) 2,54 (m, 1H).

[317] Toluene/ Ethyl Acetate 70/30 Rf start= 0,34 Rf prod= 0,51 Marker: $Ce(SO_4)_2$
[318] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,27 Rf prod= 0,32 Marker: $Ce(SO_4)_2$

3-(Chroman-3-ylmethoxy)-2,6-difluorobenzamide



Chemical Formula: $C_{11}H_{14}O_4S$
Molecular Weight: 242,29

Chemical Formula: $C_{17}H_{15}F_2NO_3$
Molecular Weight: 319,30

To a solution of 2,6-difluoro-3-hydroxybenzamide (800 mg, 4,63 mmol) in 7 ml of DMF were added 700 mg of anhydrous K_2CO_3 (5,05 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of chroman-3-ylmethyl methanesulfonate (1,02 g, 4,63 mmol) in 7 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 60°C for 6 h until the complete transformation of the starting material as revealed by TLC[319].
The reaction was evaporated under vacuum and diluted with 20 ml of distilled water. The aqueous phase was extracted with ethyl acetate (3 x 20 ml). The combined organic phases were washed with NaOH 1 M (40 ml) and brine (40 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,37 g of a white solid corresponding at the crude product.
The crude product was crystallized by DCM to give 650 mg of a white solid corresponding at the desired product.

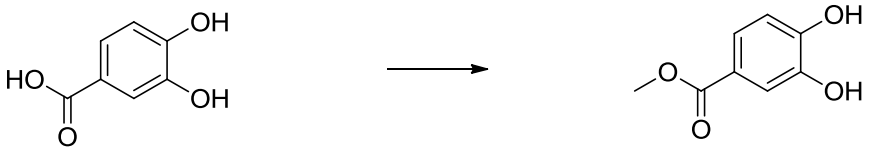
Yield = 48,50%

M.P. = 124,49°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,11 (m, 3H) 6,85 (m, 2H) 6,18 (bs, 1H) 6,02 (bs, 1H) 4,33 (dd, 1H, J= 10,7 J= 2,7 Hz) 4,14 (dd, 1H, J= 11,0 J= 6,6 Hz) 4,03 (d, 1H, J= 6,6 Hz) 3,00 (dd, 1H, J= 16,5 J= 5,0 Hz) 2,72 (dd, 1H, J= 16,5 J= 7,2 Hz) 2,61 (m, 1H).

[319]Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,56 Rf amid= 0,06 Rf prod= 0,27 Marker: $Ce(SO_4)_2$

Methyl 3,4-dihydroxybenzoate



Chemical Formula: $C_7H_6O_4$
Molecular Weight: 154,12

Chemical Formula: $C_8H_8O_4$
Molecular Weight: 168,15

To a 4°C cooled solution of 3,4-dihydroxybenzoic acid (10,0 g, 64,88 mmol) in 125 ml of MeOH 4,70 ml of $SOCl_2$ (64,88 mmol) were slowly added dropwise. The mixture was stirred at 4°C for 30 minutes and refluxed overnight.
The TLC control[320] revealed the complete transformation of the starting material.
The mixture was poured in 100 ml of iced water. The aqueous phase was extracted with ethyl acetate (3 x 100 ml). The combined organic phases were washed $NaHCO_3$ satured solution (150 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 10,30 g of an brown solid corresponding at the crude product.
The crude product was dissolved in 30 ml of MeOH and treated with 2,0 g of activated charcoal. The solution was filtered under Celite® pad, the cake was washed with 15 ml of MeOH, and evaporated under vacuum to give 9,11 g of a white solid corresponding at the desired product.

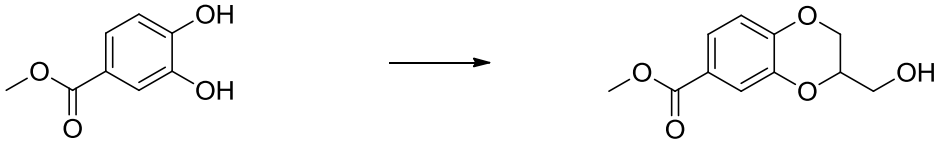
Yield = 88,44%

M.P. = 135,86°C

^{1}H-NMR (d6-DMSO) δ (ppm): 9,56 (bs, 2H) 7,33 (d, 1H, J= 2,2 Hz) 7,28 (dd, 1H, J= 8,2 J= 2,2) 6,77 (d, 1H, J= 8,2 Hz) 3,74 (s, 3H).

[320] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,14 Rf prod= 0,41 Marker: $Ce(SO_4)_2$

7-Carboxymethyl-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_8H_8O_4$
Molecular Weight: 168,15

Chemical Formula: $C_{11}H_{12}O_5$
Molecular Weight: 224,21

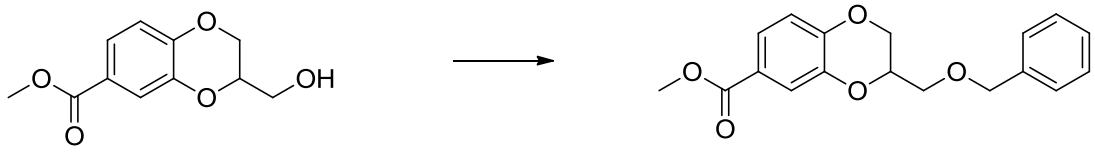
To a solution of methyl 3,4-dihydroxybenzoate (5,52 g, 32,83 mmol) in 49 ml of DMF were added 5,45 of anhydrous K_2CO_3 (39,40 mmol) and 3,37 ml of epibromohydrin (39,40 mmol) . The mixture was stirred for 15 minutes at room temperature and brought at 60°C overnight.
The TLC control[321] revealed the complete transformation of the starting material.
The reaction mixture was evaporated under vacuum and diluted with 100 ml of water and 100 ml of ethyl acetate. The separated aqueous layer was extracted twice with ethyl acetate (100 ml). The combined organic phases were washed with NaOH 1M (50 ml) and twice with brine (50 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 8,32 g of an transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[322]) to give 5,19 g of a transparent oil corresponding at the desired product that contain almost the 15% of the 2-hydroxymethyl isomer.

Yield = 70,50%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,58 (m, 2H) 6,90 (d, 1H, J= 8,2 Hz) 4,39 (m, 4H) 4,13 (m, 1H) 3,86 (s, 3H).

[321] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,41 Rf prod= 0,25 Marker: $Ce(SO_4)_2$
[322] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,35 Rf prod= 0,21 Marker: $Ce(SO_4)_2$

2-Benzyloxymethyl-7-carboxymethyl-1,4-benzodioxane



Chemical Formula: $C_{11}H_{12}O_5$
Molecular Weight: 224,21

Chemical Formula: $C_{18}H_{18}O_5$
Molecular Weight: 314,33

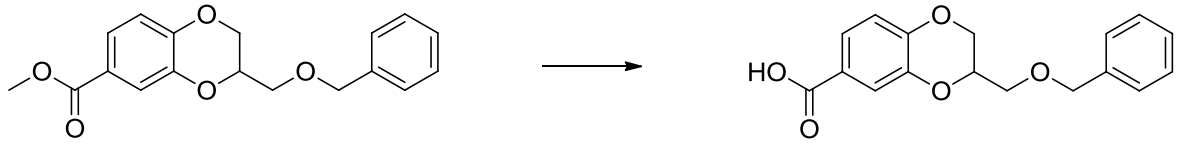
Under nitrogen atmosphere to a suspension of NaH (610 mg, 25,47 mmol) in 10 ml of dry THF a solution of 6-carboxymethyl-3-hydroxymethyl-1,4-benzodioxane (5,19 g , 23,15 mmol) in 10 ml of dry THF were slowly added dropwise. The mixture was stirred for 30 minutes at room temperature and 3,03 ml of benzyl bromide (25,47 mmol) and 10 ml of dry THF were added dropwise. The mixture was stirred for 15 minutes at room temperature and refluxed for 3h until the complete transformation of the starting material as revealed by TLCl[323].
The reaction mixture was diluted with 30 ml of HCl 10% and 30 ml of ethyl acetate. The two phases were separated and the organic was washed twice with brine (30 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 7,07 g of an transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 90/10[324]) to give 4,30 g of a transparent oil corresponding at the desired product that contain almost the 15% of the 2-benzyloxymethyl-6-carboxy isomer.

Yield = 59,06%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,56 (m, 2H) 7,31 (m, 5H) 6,89 (d, 1H, J= 6,6 Hz) 4,60 (m, 2H) 4,34 (m, 2H) 4,13 (m, 1H) 3,87 (s, 3H) 3,71 (m, 4H).

[323] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,21 Rf prod= 0,58 Marker: $Ce(SO_4)_2$
[324] Cyclohexane/ Ethyl Acetate 90/10 Rf start= 0,05 Rf prod= 0,13 Marker: $Ce(SO_4)_2$

2-Benzyloxymethyl-7-carboxylic acid-1,4-benzodioxane



Chemical Formula: $C_{18}H_{18}O_5$
Molecular Weight: 314,33

Chemical Formula: $C_{17}H_{16}O_5$
Molecular Weight: 300,31

To a solution of 3-benzyloxymethyl-6-carboxymethyl-1,4-benzodioxane (4,30 g, 13,68 mmol) in 40 ml of MeOH 6,9 ml of NaOH 2,5 M and 10 ml of water were added dropwise. The mixture was stirred for 15 minutes at room temperature and brought at 50°C for 2h until the complete transformation of the starting material as revealed by TLC[325].

The reaction mixture was evaporated under vacuum and diluted with15 ml of HCl 10%. The aqueous phase was extracted with ethyl acetate (3 x 20 ml), the combined organic phases were washed twice with brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 4,43 g of an white solid corresponding at the crude product.

The crude product was crystallized twice by toluene to give 1,64 g of a white solid corresponding at the desired product.

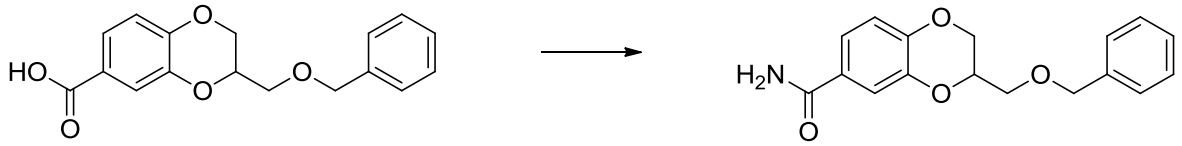
Yield = 40,01%

M.P. = 132,33°C

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,63 (m, 2H) 7,33 (m, 5H) 6,92 (d, 1H, J= 8,5 Hz) 4,61 (s, 2H) 4,39 (m, 2H) 4,17 (dd, 1H, J= 11,8 J= 7,7 Hz) 3,72 (m, 2H).

[325] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,58 Rf prod= 0,35 Marker: $Ce(SO_4)_2$

2-Benzyloxymethyl-7-carboxyamide-1,4-benzodioxane



Chemical Formula: $C_{17}H_{16}O_5$
Molecular Weight: 300,31

Chemical Formula: $C_{17}H_{17}NO_4$
Molecular Weight: 299,32

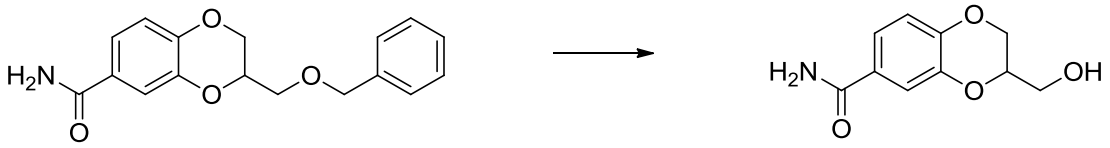
To 12 ml of $SOCl_2$ were added 1,26 g of 3-benzyloxymethyl-6-carboxylic acid-1,4-benzodioxane (4,19 mmol). The mixture was stirred for 15 minutes at room temperature and brought at 50°C for 2h until the complete transformation of the starting material as revealed by TLC[326].
The reaction mixture was evaporated under vacuum and diluted with 15 ml of DCM. The solution was cooled at 4°C and 7 ml of 30% ammonia were added dropwise, the mixture was stirred overnight at room temperature.
The TLC control[327] revealed the complete transformation of the starting material: The reaction mixture was diluted with 15 ml of water and 15 ml of DCM. The separated organic layer was washed with 10 % HCl (15 ml), $NaHCO_3$ satured solution (15 ml), brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,23 g of an white wax corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[328]) to give 650 mg of a white wax corresponding at the desired product.

Yield = 52,00%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,33 (m, 7H) 6,91 (d, 1H, J= 8,5 Hz) 5,75 (bs, 2H) 4,60 (s, 2H) 4,35 (m, 2H) 4,13 (dd, 1H, J= 7,7 J= 11,8 Hz) 3,71 (m, 2H).

[326] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,35 Rf prod= 0,37 Marker: $Ce(SO_4)_2$
[327] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,37 Rf prod= 0,10 Marker: $Ce(SO_4)_2$
[328] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,40 Rf prod= 0,15 Marker: $Ce(SO_4)_2$

7-Carboxyamide-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{17}H_{17}NO_4$
Molecular Weight: 299,32

Chemical Formula: $C_{10}H_{11}NO_4$
Molecular Weight: 209,20

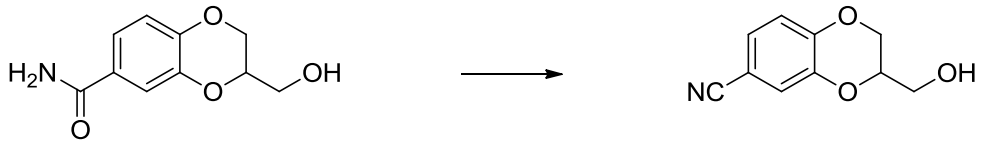
To a solution of 3-benzyloxymethyl-6-carboxyamide-1,4-benzodioxane (650 mg, 2,17 mmol) in 10 ml of acetone were added 70 mg of Pd/C at 5% and hydrogenated at 1 atm for 8h until the complete transformation of the starting material as revealed by TLC[329].
The catalyzer was filtered and the mother liquids were evaporated under vacuum to give 430 mg of a white wax corresponding at the desired product.

Yield = 93,47%

^{1}H-NMR (d6-DMSO) δ (ppm): 7,78 (bs, 1H) 7,37 (m, 2H) 7,18 (bs, 1H) 6,87 (d, 1H, J= 8,2 Hz) 5,05 (t, 1H, J= 5,8 Hz) 4,35 (dd, 1H, J= 11,3 J= 2,2 Hz) 4,16 (m, 1H) 4,03 (dd, 1H, J= 11,3 J= 7,2 Hz) 3,61 (m, 2H).

[329] Toluene/ Ethyl Acetate 1/1 Rf start= 0,68 Rf prod= 0,29 Marker: $Ce(SO_4)_2$

7-Cyano-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{10}H_{11}NO_4$
Molecular Weight: 209,20

Chemical Formula: $C_{10}H_9NO_3$
Molecular Weight: 191,18

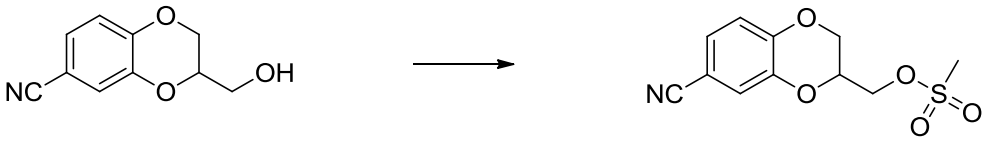
To a 4°C cooled solution of 6-carboxyamide-3-hydroxymethyl-1,4-benzodioxane (550 mg, 2,63 mmol) in 10 ml of a mixture 1,4-dioxane and pyridine (4/1) 1,6 ml of trifluoroacetic anhydride (11,50 mmol) were added dropwise. The mixture was stirred at 4°C for 15 minutes and brought at room temperature for 1 h until the complete transformation of the starting material as revealed by TLC[330].
The reaction mixture was diluted with 20 ml of water and 20 ml of ethyl acetate. The separated organic layer was washed with 10 % HCl (20 ml), $NaHCO_3$ satured solution (20 ml) and brine (20 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 480 mg of a white wax corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 70/30[331]) to give 350 mg of a white wax corresponding at the desired product.

Yield = 46,36%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,17 (m, 2H) 6,93 (d, 1H, J= 8,2 Hz) 4,38 (dd, 1H, J= 11,0 J= 1,9 Hz) 4,19 (m, 2H) 3,90 (dq, 2H, J= 12,1 J= 4,1 Hz) 1,59 (bs, 1H).

[330] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,09 Rf prod= 0,32 Marker: $Ce(SO_4)_2$
[331] Cyclohexane/ Ethyl Acetate 70/30 Rf start= 0,09 Rf prod= 0,32 Marker: $Ce(SO_4)_2$

7-Cyano-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_{10}H_9NO_3$
Molecular Weight: 191,18

Chemical Formula: $C_{11}H_{11}NO_5S$
Molecular Weight: 269,27

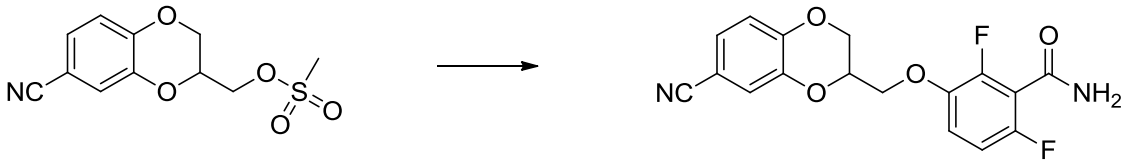
To a solution of 6-ciano-3-hydroxymethyl-1,4-benzodioxane (350 mg, 1,83 mmol) in 6 ml of DCM were added 0,34 ml of TEA (2,38 mmol). The mixture was cooled at 4°C and 0,17 ml of methansulphonyl chloride (2,20 mmol) were slowly added dropwise. The mixture was stirred at 4°C for 15 minutes and brought at room temperature for 30 minutes until the complete transformation of the starting material as revealed by TLC[332].
The mixture was cooled at 4°C and added dropwise 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10 % HCl (10 ml), $NaHCO_3$ satured solution (10 ml), brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 480 mg of a transparent oil corresponding at the desired product.

Yield = 97,36%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,22 (m, 2H) 6,96 (d, 1H, J= 8,5 Hz) 4,44 (m, 4H) 4,17 (dd, 1H, J= 11,3 J= 6,6 Hz) 3,14 (s, 3H).

[332] Toluene/ Ethyl Acetate 80/20 Rf start= 0,08 Rf prod= 0,22 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-cyano-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{11}H_{11}NO_5S$
Molecular Weight: 269,27

Chemical Formula: $C_{17}H_{12}F_2N_2O_4$
Molecular Weight: 346,28

To a solution of 2,6-difluoro-3-hydroxybenzamide (370 mg, 2,14 mmol) in 5 ml of DMF were added 296 mg of anhydrous K_2CO_3 (2,14 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-ciano-3-mesyloxymethyl-1,4-benzodioxane (480 mg, 1,78 mmol) in 5 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 75°C for 6 h until the complete transformation of the starting material as revealed by TLC[333].

The reaction was evaporated under vacuum and diluted with 20 ml of distilled water. The aqueous phase was extracted with ethyl acetate (3 x 20 ml). The combined organic phases were washed with NaOH 1 M (25 ml) and brine (25 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 550 mg of a white solid corresponding at the crude product.

The crude product was crystallized by toluene/ethyl acetate (1/1) to give 175 mg of a white solid corresponding at the desired product.

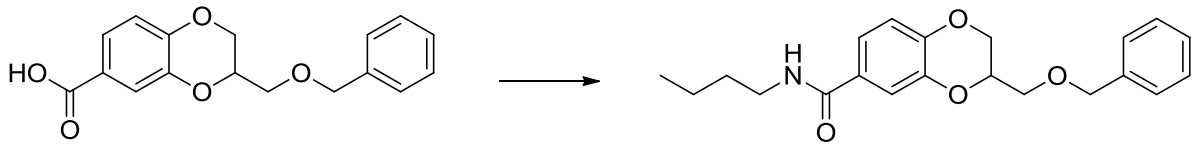
Yield = 30,81%

M.P. = 158,17°C

^{1}H-NMR (d6-DMSO) δ (ppm): 8,10 (bs, 1H) 7,82 (bs, 1H) 7,40 (d, 1H, J= 1,9 Hz) 7,28 (m, 3H) 7,06 (m, 2H) 4,67 (m, 1H) 4,52 (dd, 1H, J= 11,6 J= 2,5 Hz) 4,27 (m, 3H).

[333]Toluene/ Ethyl Acetate 1/1 Rf start= 0,37 Rf amid= 0,15 Rf prod= 0,28 Marker: $Ce(SO_4)_2$

2-Benzyloxymethyl-7-N-buthylcarboxyamide-1,4-benzodioxane



Chemical Formula: $C_{17}H_{16}O_5$
Molecular Weight: 300,31

Chemical Formula: $C_{21}H_{25}NO_4$
Molecular Weight: 355,43

To 10 ml of $SOCl_2$ were added 1,00 g of 3-benzyloxymethyl-6-carboxylic acid-1,4-benzodioxane (3,32 mmol). The mixture was stirred for 15 minutes at room temperature and brought at 50°C for 2h until the complete transformation of the starting material as revealed by TLC[334].

The reaction mixture was evaporated under vacuum and diluted with 15 ml of DCM. The solution was slowly added dropwise to a 4°C cooled solution of n-butylamine (2,00 ml, 20,23 mmol) in 10 ml of DCM, the mixture was stirred at 4°C for 15 minutes and after overnight at room temperature.

The TLC control[335] revealed the complete transformation of the starting material The reaction mixture was diluted with 15 ml of water and 15 ml of DCM. The separated organic layer was washed with 10% HCl (15 ml), $NaHCO_3$ satured solution (15 ml), brine (15 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 1,11 g of an transparent oil corresponding at the crude product.

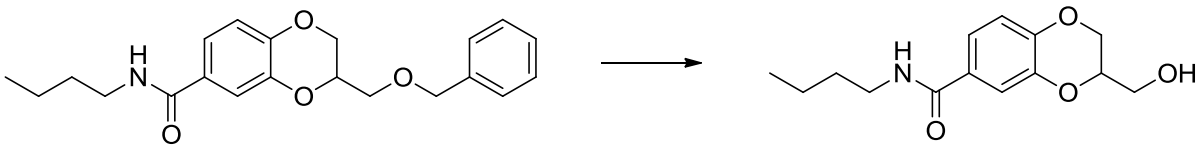
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 60/40[336]) to give 670 mg of a white wax corresponding at the desired product.

Yield = 56,78 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,31 (m, 7H) 6,88 (d, 1H, J= 8,5 Hz) 5,96 (bs, 1H) 4,60 (s, 2H) 4,35 (m, 2H) 4,12 (dd, 1H, J= 11,8 J= 7,7 Hz) 3,70 (m, 2H) 3,42 (q, 2H, J= 6,9 Hz) 1,57 (m, 2H) 1,41 (m, 2H) 0,95 (t, 3H, J= 7,2 Hz).

[334] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,35 Rf prod= 0,37 Marker: Blue Sheet
[335] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,37 Rf prod= 0,44 Marker: Blue Sheet
[336] Cyclohexane/ Ethyl Acetate 60/40 Rf start= 0,37 Rf prod= 0,44 Marker: Blue Sheet

7-N-buthylcarboxyamide-2-hydroxymethyl-1,4-benzodioxane



Chemical Formula: $C_{21}H_{25}NO_4$
Molecular Weight: 355,43

Chemical Formula: $C_{14}H_{19}NO_4$
Molecular Weight: 265,30

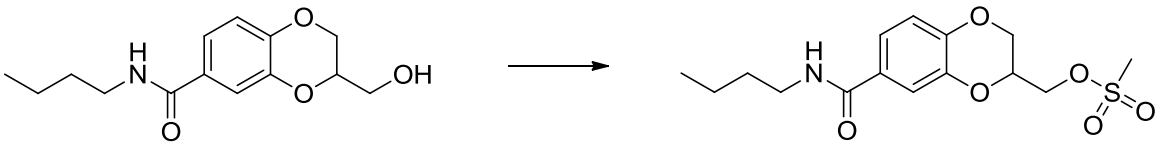
To a solution of 3-benzyloxymethyl-6-N-buthylcarboxyamide-1,4-benzodioxane (670 mg, 1,88 mmol) in 10 ml of MeOH were added 70 mg of Pd/C 10% and hydrogenated at 1 atm for 24 h until the complete transformation of the starting material as revealed by TLC[337].
The catalyzer was filtered and the mother liquids were evaporated under vacuum to give 460 mg of a white wax corresponding at the desired product.

Yield = 92,18 %

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,34 (d, 1H, J= 2,2 Hz) 7,24 (dd, 1H, J= 8,5 J=2,2 Hz) 6,89 (d, 1H, J= 8,5 Hz) 6,06 (bs, 1H) 4,34 (dd, 1H, J= 11,3 J= 2,2 Hz) 4,25 (m, 1H) 4,13 (dd, 1H, J= 11,3 J= 7,4 Hz) 3,70 (m, 2H) 3,87 (qd, 1H, J= 8,5 J= 4,4 Hz) 3,41 (q, 2H, J= 6,9 Hz) 1,89 (bs, 1H) 1,56 (m, 2H) 1,40 (m, 2H) 0,95 (t, 3H, J= 7,2 Hz).

[337] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,47 Rf prod= 0,13 Marker: $Ce(SO_4)_2$

7-N-buthylcarboxyamide-2-mesyloxymethyl-1,4-benzodioxane



Chemical Formula: $C_{14}H_{19}NO_4$
Molecular Weight: 265,30

Chemical Formula: $C_{15}H_{21}NO_6S$
Molecular Weight: 343,40

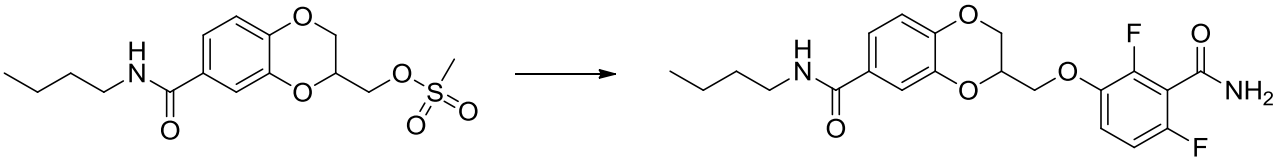
To a solution of 6-N-buthylcarboxyamide-3-hydroxymethyl-1,4-benzodioxane (460 mg, 1,73 mmol) in 10 ml of DCM were added 0,32 ml of TEA (2,25 mmol). The mixture was cooled at 4°C and 0,16 ml of methansulphonyl chloride (2,08 mmol) were slowly added dropwise. The mixture was stirred at 4°C for 15 minutes and brought at room temperature for 30 minutes until the complete transformation of the starting material as revealed by TLC[338].
The mixture was cooled at 4°C and added dropwise 10 ml of water and 10 ml of DCM. The separated organic layer was washed with 10 % HCl (10 ml), $NaHCO_3$ satured solution (10 ml), brine (10 ml), dried over Na_2SO_4 anhydrous, filtered and evaporated under vacuum to give 680 mg of a transparent oil corresponding at the crude product.
The crude product were purified by flash chromatography on silica gel (cyclohexane/ethyl acetate 1/1[339]) to give 460 mg of a transparent wax corresponding at the desired product.

Yield = 77,44%

^{1}H-NMR ($CDCl_3$) δ (ppm): 7,38 (d, 1H, J= 2,2 Hz) 7,29 (dd, 1H, J=2,2 J= 8,5 Hz) 6,91 (d, 1H, J= 8,5 Hz) 6,0 (bs, 1H) 4,49 (m, 3H) 4,35 (dd, 1H, J= 11,3 J= 2,3 Hz) 4,14 (dd, 1H, J= 11,3 J= 7,4 Hz) 3,45 (q, 2H, J= 6,8 Hz) 3,09 (s, 3H) 1,51 (m, 2H) 1,40 (m, 2H) 0,95 (t, 3H, J= 7,1 Hz).

[338] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,13 Rf prod= 0,15 Marker: $Ce(SO_4)_2$
[339] Cyclohexane/ Ethyl Acetate 1/1 Rf start= 0,13 Rf prod= 0,15 Marker: $Ce(SO_4)_2$

2,6-Difluoro-3-(7-N-buthylcarboxyamide-1,4-benzodioxan-2-yl)methyloxy benzamide



Chemical Formula: $C_{15}H_{21}NO_6S$
Molecular Weight: 343,40

Chemical Formula: $C_{21}H_{22}F_2N_2O_5$
Molecular Weight: 420,41

To a solution of 2,6-difluoro-3-hydroxybenzamide (254 mg, 1,47 mmol) in 3 ml of DMF were added 221 mg of anhydrous K_2CO_3 (1,60 mmol). The mixture was stirred for 15 minutes at room temperature, then a solution of 6-N-buthylcarboxyamide-3-mesyloxymethyl-1,4-benzodioxane (460 mg, 1,34 mmol) in 7 ml of DMF were added. The reaction was stirred for 30 minutes at room temperature and then brought at 75°C for 6 h until the complete transformation of the starting material as revealed by TLC[340].
The reaction was evaporated under vacuum and diluted with 20 ml of distilled water. The aqueous phase was extracted with ethyl acetate (3 x 30 ml). The combined organic phases were evaporated under vacuum to give 550 mg of a grey solid corresponding at the crude product.
The crude product was crystallized by ethyl acetate to give 350 mg of a light solid corresponding at the desired product.

Yield = 63,63%

M.P. = 173,49°C

^{1}H-NMR (d6-DMSO) δ (ppm): 8,24 (t, 1H, J= 5,5 Hz) 8,11 (bs, 1H) 7,84 (bs, 1H) 7,33 (m, 3H) 7,07 (dt, 1H, J=8,8 J= 1,9 Hz) 6,94 (d, 1H, J= 8,5 Hz) 4,61 (m, 1H) 4,46 (dd, 1H, J= 11,8 J= 2,5 Hz) 4,31 (m, 2H) 4,17 (dd, 1H, J= 11,5 J= 7,2 Hz) 3,20 (q, 1H, J= 6,9 Hz) 1,51 (m, 2H) 1,35 (m, 2H) 0,87 (t, 1H, J= 7,2 Hz).

[340]Toluene/ Ethyl Acetate 1/1 Rf start= 0,35 Rf amid= 0,15 Rf prod= 0,21 Marker: $Ce(SO_4)_2$

Acknowledgments

Grazie Prof. Valoti

Un Grazie a tutti i ragazzi che mi hanno volute bene e sostenuto durante questo lungo cammino. Un grande abbraccio.