UNIVERSITY OF TRIESTE

FACULTY OF PHARMACY

EXPERIMENTAL THESIS IN PHARMACEUTICAL CHEMISTRY

SYNTHESIS OF MUSCARONE ANALOGUES AS POTENTIAL MUSCARINIC ANTAGONISTS

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INTRODUCTION

Cholinergic receptors are a group of receptors widely distributed in the central and the peripheral nervous systems.

From a historical point of view they were first subdivided into subtypes by Sir Henry Dale[1],who found evidence to prove that there was a selective activity for the alcaloids muscarine (on so called muscarinic receptors) and nicotine (on nicotinic receptors) compared to the non selective physiological mediator acetylcholine (see figure 1). Following this first differentiation, the ample evidence gained was founded on the variety of biological functions mediated by the muscarinic receptor to suggest the existence of some subclasses[2].

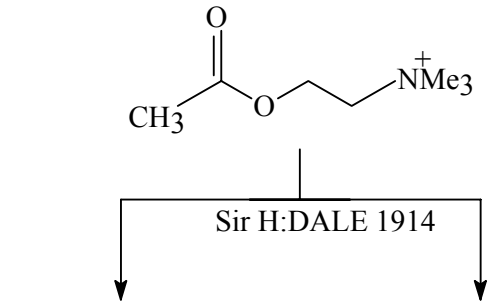
The first certain evidence of at least two principle subclasses of muscarinic receptors, M_1 and M_2, was confirmed only at the beginning of the eighties with the discovery of the muscarinic antagonist pirenzepine (figure 2).

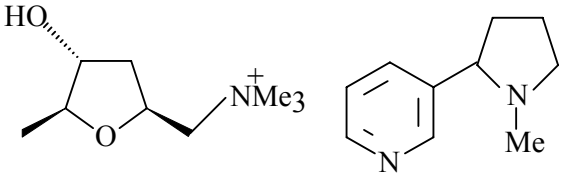
M_1 receptors, characterised by a high affinity for pirenzepine, are located in neuronal tissue such as hippocampus and cortex, in striated muscle and in the autonomic gangli; M_2 receptors, towards which pirenzepine shows low affinity, are found essentially in the centres of the lower brain and in peripheral effecter organs such as heart, smooth muscle and esocrine glands[3,4].

The discovery of selective ligands, the structures of some of which are found in figure 2, has provided the unequivocal proof that M_1 and M_2 muscarinic receptors are not constituted of homogeneous populations. It has in fact been demonstrated that some antagonists show different affinities for those receptors previously simply classified as subtypes M_1 and M_2. For example, a

Figure 1

ACETYLCHOLINE

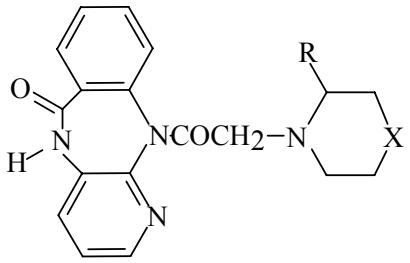




MUSCARINE — *Amanita muscaria*

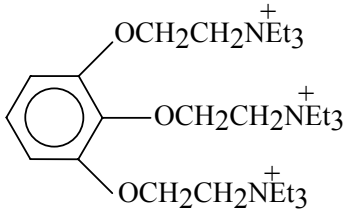
NICOTINE — *Nicotiana tabacum*

FIGURE 2. Chemical structures of muscarinic antagonists capable of discriminating between subtypes of muscarinic receptors.

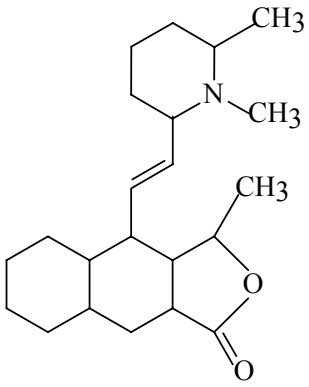


Pirenzepine: R=H; X=NMe

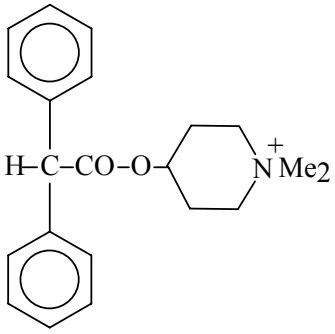
AF-DX116: R=CH_2NEt_2; X=CH_2



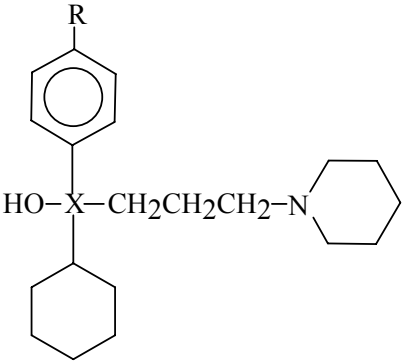
Gallamine



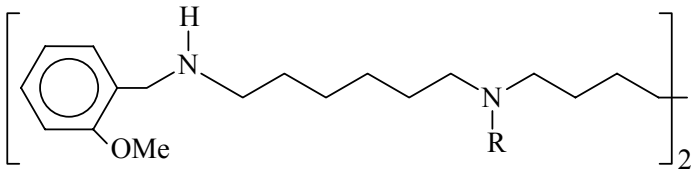
Imbacina



4-DAMP



Hexahydrodiphenydol: R=H; X=C
Hexahydrosiladiphenydol: R=H; X=Si
p-Fluorohexahydrosiladiphenydol: R=F; X=Si



Methoctramine: R=H

neuromuscular blocking agent; Gallamine[5,6], a pirenzepine analogue; AF-DX 116[7-9], and the naturally occurring imbacine[10] are more selective for those muscarinic receptors found in heart atria than those from the ileum; behavior which is opposite to that observed for 4-DAMP[11], hexahydrosiladiphenydol[12], and p-fluorohexahydrosiladiphenydol[13] (figure 2).

On the basis of this experimental evidence an ulterior subdivision was suggested that separated cardiac muscarinic receptor subtypes (M_2) from those found in smooth muscle and in glands (M_3)[4,14].

Methoctramine is an example of a ligand that discriminates between M_2 and M_3 muscarinic receptors behaving as a cardioselective muscarinic antagonist.

The hypothesis that differentiates M_1 receptors into two subtypes[14] (one referring to those receptors located at the hippocampus and the other to the cortex and to peripheral glands) has not found any support from definitive experimental data and has thus been abandoned. The present classification is made up of M_1 neuronal, M_2 cardiac and M_3 glandular/smooth muscle muscarinic receptors.

Parallel to the research aimed at the pharmacological characterisation of muscarinic receptor populations, studies of molecular cloning have revealed the existence of five distinct but homologous genes (m_1 - m_5) that encode for five proteins corresponding to muscarinic receptors in mammals[15,16]. Structure - function studies of the cloned receptors have been facilitated by the high degree of homogeny between these muscarinic receptors and other G protein coupled receptors[17]. The five cloned receptors also show a high level of similarity in the amino-acidic sequences at their transmembrane domains.

At the present moment the cloned receptors m_1 to m_3 correspond to the pharmacologically defined M_1 to M_3 muscarinic receptors, whereas the m_4 and m_5 receptors have yet to find an unequivocal pharmacological "equivalent".

A recent classification[18] that takes into consideration pharmacological characterisation of muscarinic receptor subtypes has introduced an M_4 receptor, but still lacks as yet a selective antagonist.

Renewed interest in the sector of research regarding muscarinic receptors is tied primarily to their role in the control of numerous physiological processes of the central nervous system, such as the higher cognitive functions of memory and learning[19]. Research also concerns the treatment of the pathologies of the cognitive functions and is particularly aimed at the discovery of therapeutic agents selective for centrally located muscarinic receptors (M_1 selective agonists)[20].

M_2 selective antagonists on the other hand are potentially useful in the treatment of bradicardia[21], and M_3 selective antagonists could be used in the treatment of urinary incontinence and for problems with gastrointestinal motility without undesired secondary effects such as xerostomia or midriasis[2].

In spite of the notable results obtained in biomolecular studies, truly selective muscarinic ligands (capable that is to bind only one subtype of muscarinic receptor) are not yet available, probably due to the high degree of similarity of the amino-acids present in the binding sites of the various receptor subtypes. Even though we know of some so-called selective muscarinic antagonists, the ideal selective ligand still constitutes a primary objective in this sector of multidisciplinary research.

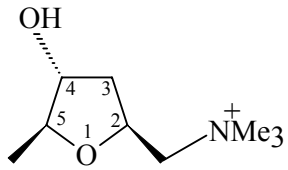
AIM OF THE THESIS

Structure-activity relationships of cholinergic ligands active at muscarinic receptors has for many years been the main object of study of the research group with which I worked for this present thesis.

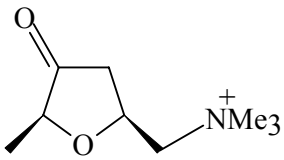
Figure 3 shows the structures of some muscarinic agonists related to natural muscarine (which is represented in the absolute configuration, 2S-4R-5S), with a substitution in position 4 (muscarone, methylenemuscarone and difluoromuscarine) and a change in the nature of the heterocyle (azamuscarone). The syntheses of all the stereoisomers has been prepared for the compounds in figure 3; the eight of muscarine[23], the four of muscarone[24], of methylenemuscarone[25] and of difluoromuscarine[26], and of the two enantiomers of azamuscarone[27]. All of their quaternary ammonium salts have been prepared with a high degree of enantiomeric purity starting from the opportune chiral syntons.

The pharmacological investigation regarding this group of muscarinic agonists was performed with functional binding tests on isolated organs and molecular binding studies and has, above all, consented the individuation of the stereochemical requisites for the interaction of muscarone with the muscarinic receptors[24]. The pharmacological profile of muscarone, the most potent peripheric muscarinic agonist known to date, was originally thought to be significantly different from that of natural muscarine and of other structurally correlated muscarinic agonists. The principle differences are listed in figure 4; in particular, even though the stereochemistry was eventually corrected[28], and other muscarinc ligands showed some nicotinic behavior, the extremely modest eudismic ratio was

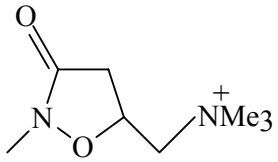
Figure 3



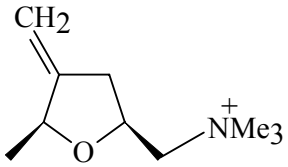
MUSCARINE



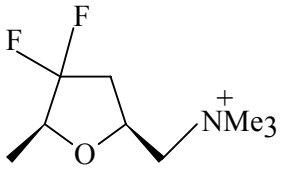
MUSCARONE



AZAMUSCARONE

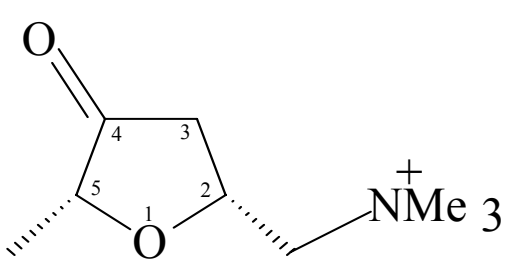


METHYLENEMUSCARONE

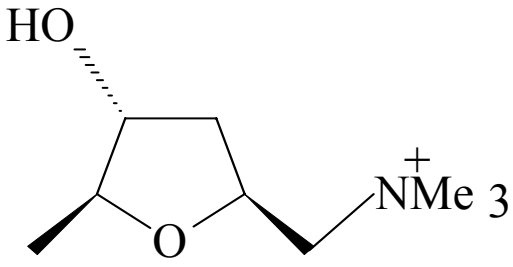


DIFLUOROMUSCARINE

Figure 4



MUSCARONE



MUSCARINE

- Muscarone is ten times more potent than muscarine in various functional muscarinic tests.
- Muscarone posseses a nicotinic component in its activity.
- The configuration at the two stereocentres of the eutomer (2R,5R) is opposite to that of the corresponding centres of natural muscarine.
- The eudismic ratio (EC_{50} of the distomer/EC_{50} of the eutomer) of muscarone is very low (E.R.=2-10).

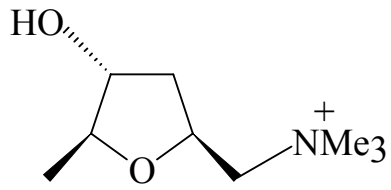
an exception in the panorama of chiral muscarinic agonists.

The study of the muscarinic activity of the chiral forms of muscarine and of muscarone[23,24] (characterised by values of enantiomeric excess greater than 98%) showed that both agonists had comparable values of eudismic ratio in a series of functional tests (figure 5) and that the eutomer of muscarone had the same absolute configuration, at its stereocentres in positions 2 and 5, as the eutomer of muscarine. Muscarone thus behaves similarly to other chiral muscarinic agonists: muscarine and muscarone in particular recognise a common binding site and the hydroxy and carbonyl groups interact with the same receptorial subsite. Considering these results, the hypotheses that were advanced attempting to rationalise the abnormal pharmacological profile of muscarone[30,31] have no reason to subsist.

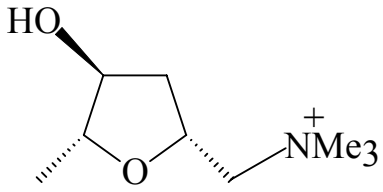
Figure 6 compares values for organ selectivity (EC_{50} ileum/EC_{50} atria) and, where available, the eudismic ratios of the group of muscarinic agonists that were studied by my research group. The structures of the more potent stereoisomers are shown (**A**: natural muscarine, **B**: muscarone, **C**: methylenemuscarone, **E**: fluoromuscarine[32], **F**: difluoromuscarine). In the case of deoxymuscarine **D**, the four optically active stereoisomers are not known: the potency values are referred to the racemic *cis* isomer[33]. All the eutomers shown in figure 6 are more potent at the M_3 receptor (the atria/ileum ratios being of less than unit value). The sole exception is the eutomer of the difluorated analogue **F**, which prefers the M_2 cardiac receptors (atria/ileum selectivity ratio being 13.5). We see then that interchanging the substitution at position 4 of the tetrahydrofuranic ring modulates muscarinic potency but has a limited effect (if we exclude **C** and **F**) on the selectivity of the particular muscarinic agonist being considered.

Figure 5

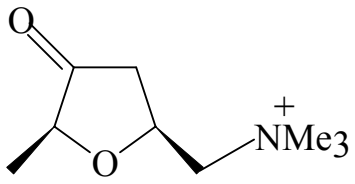
EUTOMERS

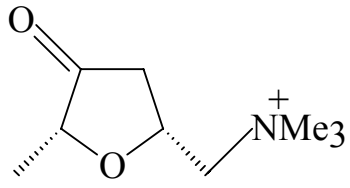


DISTOMERS



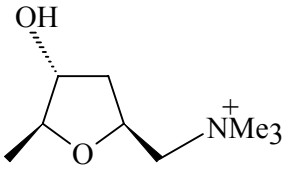
ER=324-331





ER=282-436

Figure 6

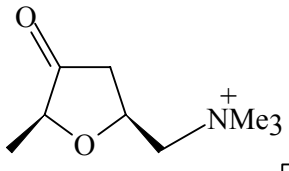


A

SELECTIVITY

atria/ileum : 0.36

atria: 7.69 (α=1); ER 331
ileum: 8.13 (α=1); ER 324

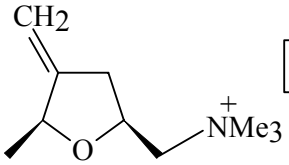


B

SELECTIVITY

atria/ileum : 0.66

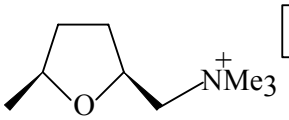
atria: 8.92 (α =1); ER 417
ileum: 9.10 (α =1); ER 380



C

atria/ileum : 0.06

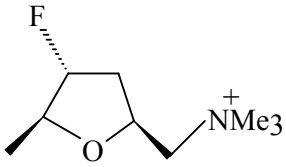
atria:6.32 (α =1); ER 83
ileum: 7.55 (α =1); ER 186



D

atria/ileum : 0.10

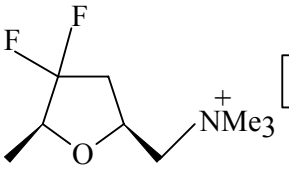
atria: 5.88 (α =1); ER n.a.
ileum: 6.87 (α =1); ER n.a.



E

atria/ileum : 0.23

atria: 6.7 (α =.98); ER n.a.
ileum: 7.36 (α =.95); ER n.a.



F

atria/ileum : 13.5

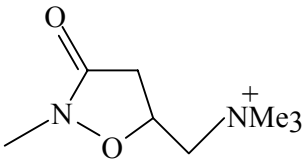
atria:6.81 (α =1); ER 8.3
ileum:5.68 (α =.99); ER 1.7

Recently our laboratory has taken on, parallel to the studies on muscarinic agonists, a line of research that takes as an objective the synthesis of new heterocyclic compounds with antimuscarinic activity. The upper part of figure 7 shows the structures of azamuscarone and of two structural analogues where the methyl groups have been replaced by phenyl (**1a**) and cyclohexyl (**1b**) groups. Such a structural change was suggested from results obtained in literature pertaining to azamuscarone analogues with dioxylane and oxythiolane groups. The substitution of the methyl group (in a position that is equivalent to the azamuscarone methyl group) with hydrophobic and sterically cumbersome groups showed a variation in the pharmacological profile of the muscarinic ligand, that went from agonistic to antagonistic behavior[34]. In the case of the isoxazolidin-3-onic compounds in figure 7, while azamuscarone is a powerful non-selective muscarinic agonist[33], the **1a** and **1b** analogues are relatively weak muscarinic antagonists[35].

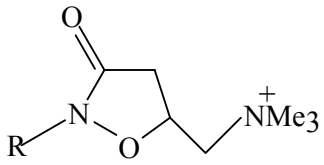
An analogous approach is based of the synthesis of the isoxazolic derivatives **2** and **3a-c**. As can be seen from the results in table 1, in vitro functional tests performed on the indicated organs showed that while the methyl derivative **2** is an agonist (partial agonist in rat bladder), the three isoxazoles **3a-c** are muscarinic antagonists with potency values (pA_2) that depend on the dimensions of the substitutions in position 3[36]. Introducing a phenyl or a cyclohexyl group in the place of the hydrogen atom produces a marked effect on the values of potency in the three organ preparations. Non of the compounds **2-3a-c** can discriminate between the different subtypes of muscarinic receptors (M_3 in the rat jejunum, M_2 in guinea pig atria). Considering these results, the first objective that I considered for my thesis regarded the synthesis of the compounds shown in figure 8.

Derivatives 4 and 6 are potential antimuscarinic agents closely related to muscarone and methylenemuscarone (see figure 3) where the methyl group in position 5 has been replaced by two phenyl groups. In addition to that structural variation, compound 5 has an endocyclic insaturation at

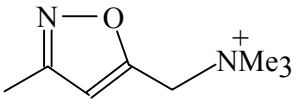
Figure 7



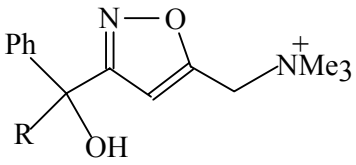
AZAMUSCARONE



1a : R = Ph
1b : R = Cy



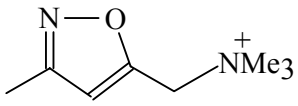
2



3a : R = H
3b : R = Ph
3c : R = Cy

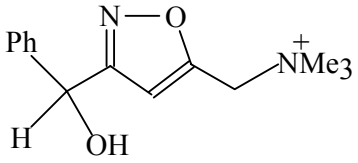
Table 1. PA_2 values (α=0) for compounds **3a-c**: ❍ rat jejunum, ■ rat bladder, ❑ guinea pig atria. PD_2 values for compound **2** on the same organ preparations.

ATROPINE	❍ 9.50 ■ 9.40 ❑ 9.40



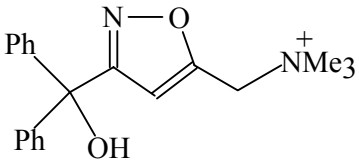
❍ 5.00, α=1
■ 4.39, α=0.82
❑ 4.43, α=1

2



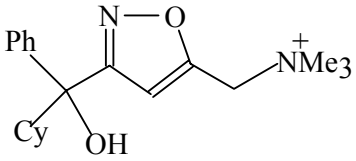
❍ 5.87
■ 5.97
❑ 5.82

3a



❍ 9.00
■ 8.86
❑ 8.80

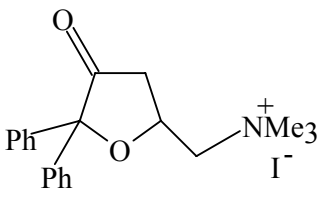
3b



❍ 9.08
■ 9.27
❑ 8.85

3c

Figure 8



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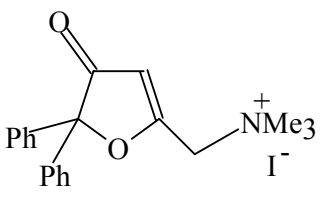

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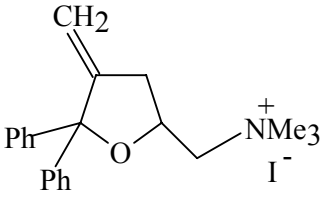


6

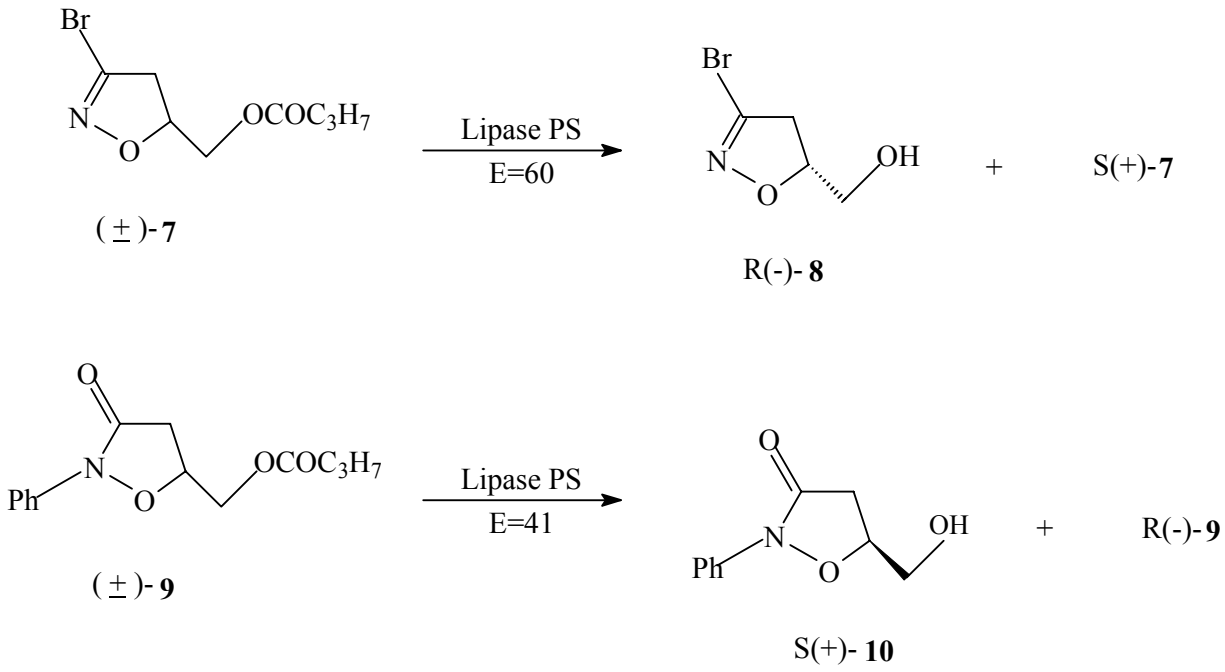
the carboyl group.

A second objective for my thesis was the preparation of the butyrric ester (±)-**11** (Outline 1), that constitutes a potential intermediate for the synthesis of the more potent enatiomer of muscarone. In outline 1 we can observe the results obtained in our laboratory using the butyrrates **(±)-7** and **(±)-9** as substrates in Lipase PS (from *Pseudomonas cepacia*) catalysed hydrolyses. Since the enzyme has shown a high degree of enatioselectivity (enantiomeric ratio values E^{37} of 60 and 41 respectively), the use of this procedure has permitted the kinetic resolution of the original unreacted substrates **(±)-7** and **(±)-9**: of the alcohols produced **R-(-)-8** and **S-(+)-10** and of the respective residue esters of the hydrolysis reactions **S-(+)-7** and **R-(-)-9** with high degrees of enatiomeric purity[35,38].

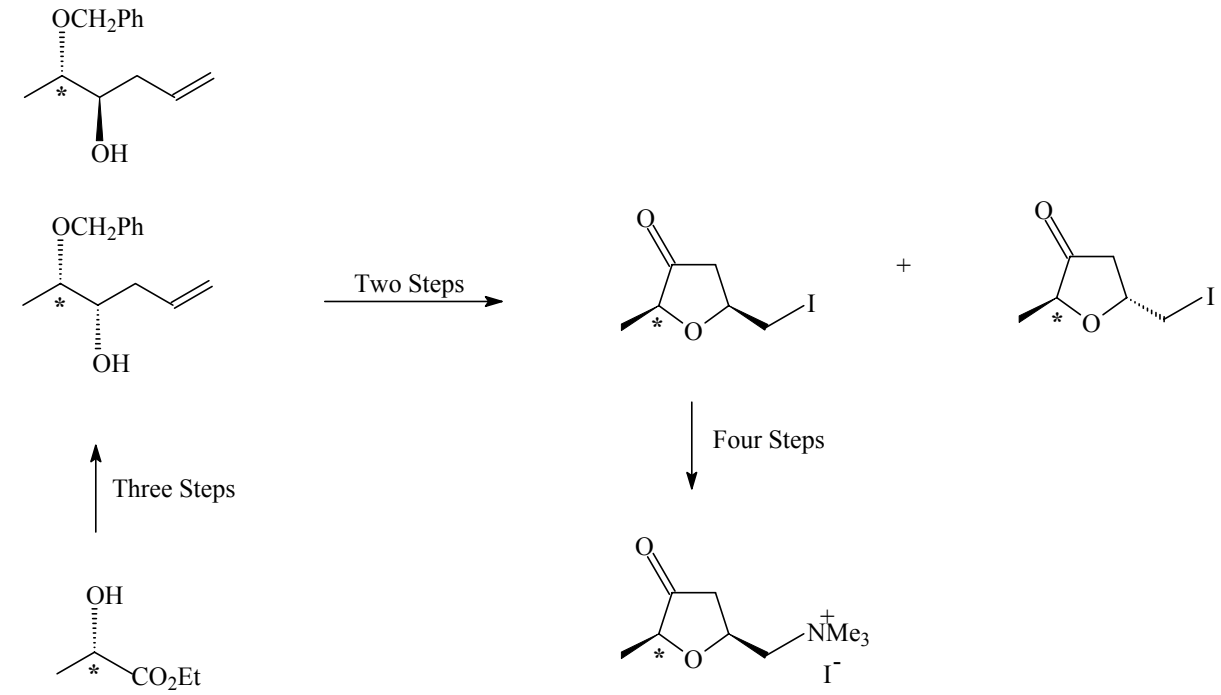
It seemed thus interesting to study a mechanism for the synthesis of **(±)-11** with the idea of following with a kinetic resolution of that substrate with results comparable to those obtained with **7** and **9**. The preparation of the eutomer of muscarone, following the hydrolysis, would entail the functionalisation of the substitute on the side chain with simple chemical transformations.

Considering the usefulness of the eutomer of muscarone as a pharmacological tool, the chemo-enzymatic strategy would constitute a valid alternative to the total synthesis already prepared in our laboratory[24]. This procedure, shown in outline 2, on the one hand allows one to obtain the desired compound with high enantiomeric purity (e.e.>98%), but on the other is rather laborious and time consuming. There are many moments in the course of the preparation that necessitate an accurate control of the experimental conditions, in the early reactions, and a careful purification of the diastereoisomeric mixtures in the more advanced stages of the reaction sequence.

Outline 1


Br
N
O
OCOC3H7
Lipase PS
E=60
Br
N
O
OH
+
S(+)-7
(±)-7
R(-)-8
O
N
O
Ph
OCOC3H7
Lipase PS
E=41
O
N
O
Ph
OH
+
R(-)-9
(±)-9
S(+)-10
O
O
OCOC3H7
Lipase PS ?
O
O
+
NMe3
(±)-11
(-)-MUSCARONE

Outline 2



Naturally occuring lactate

RESULTS

The preparation of the target compounds was projected in such a manner as to take advantage of the versatility in reactions of synthesis possessed by isoxazole and **D**^{2}-isoxazoline nuclei (Outline 3), obtained by 1,3-dipolar cycloaddition of nitriloxides (1,3-dipoles) to alkenes and alkynes (dipolarphyles). In the past, both isoxazoles and **D**^{2}-isoxazolines (2-isoxazolines) have been used in our laboratory as key intermediates in the synthesis of biologically active substances[33,35,39,40].

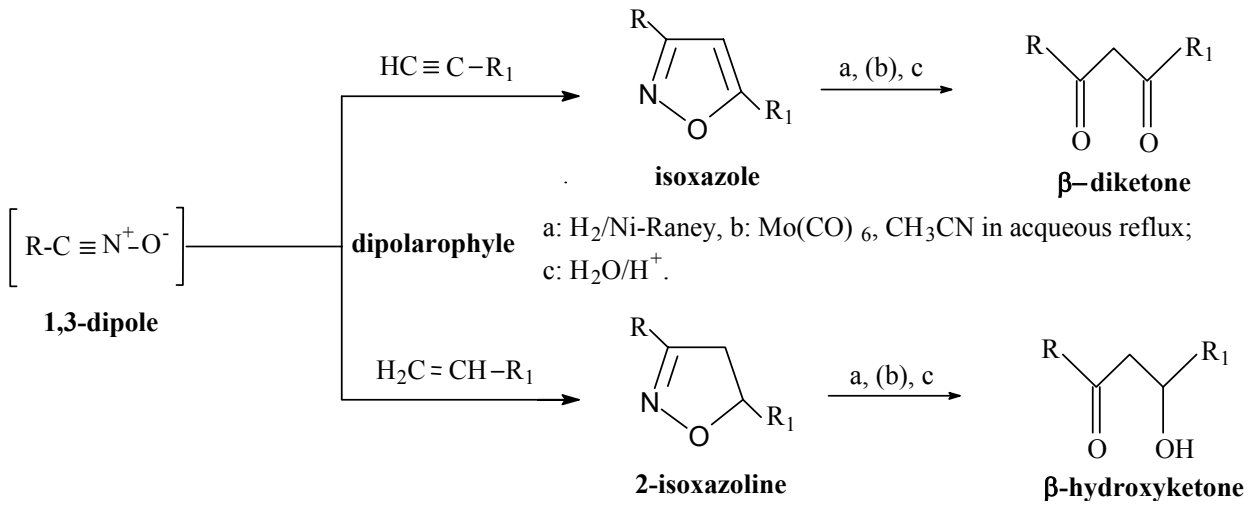
The usefulness of these heterocyclic nuclei in the synthetic field lies in their good chemical stability that allows an eventual functionalisation in positions 3 and 5 after the cycloaddition reaction[41].This first phase of chemical transformation can be followed by the hydrogenolytic aperture of the N-O bond, followed by a hydrolysis of the intermediates; in this manner we obtain new functionalised molecules that were masked or latent in the original heterocycle.[41].

Outline 3 shows the structures of the b-dicarbonyl (from the isoxazoles) and the b-hydroxyketone (from the 2-isoxazolines) functions that can be obtained following the procedure previously described.

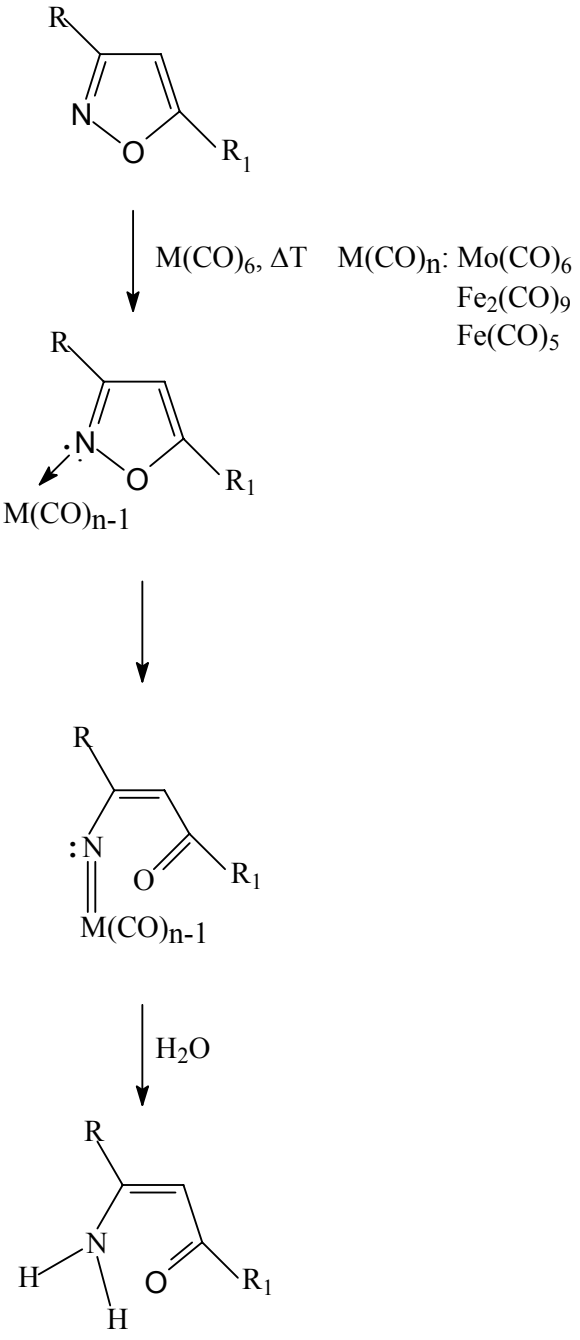
The N-O bond is usually broken with a hydrogenation using catalysts such as Nickel-Raney or, with a more recent methology[42], with molybdenum hexacarbonyl in aqueous acetonitrile following the reaction sequence in Outline 4.

The b-dicarbonylic compounds obtained by acidic hydrolysis may undergo another cycling reaction in these conditions if one of the substitutions on the ketonic groups contains a free hydroxy group[42]. This is the case of the b-dicarbonyl with the generic formula A (Outline 5) that tautomerises to the enol B that will dehydrate to give C, a b-furanone.

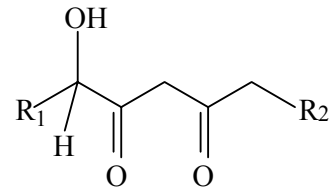
OUTLINE 3


HC ≡ C − R1
R-C ≡ N+−O-
1,3-dipole
dipolarophyle
H2C = CH − R1
isoxazole
2-isoxazoline
a, (b), c
a, (b), c
β−diketone
β-hydroxyketone
a: H2/Ni-Raney, b: Mo(CO) 6, CH3CN in acqueous reflux;
c: H2O/H+.

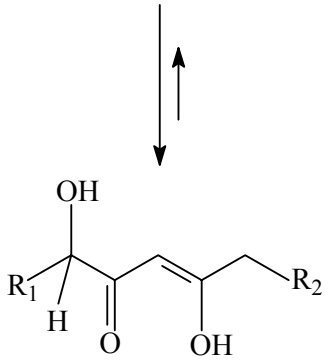
Outline 4



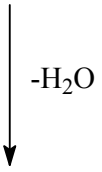
Outline 5

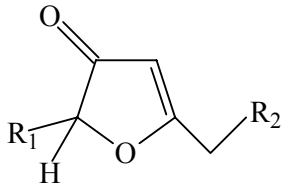


A



B





C

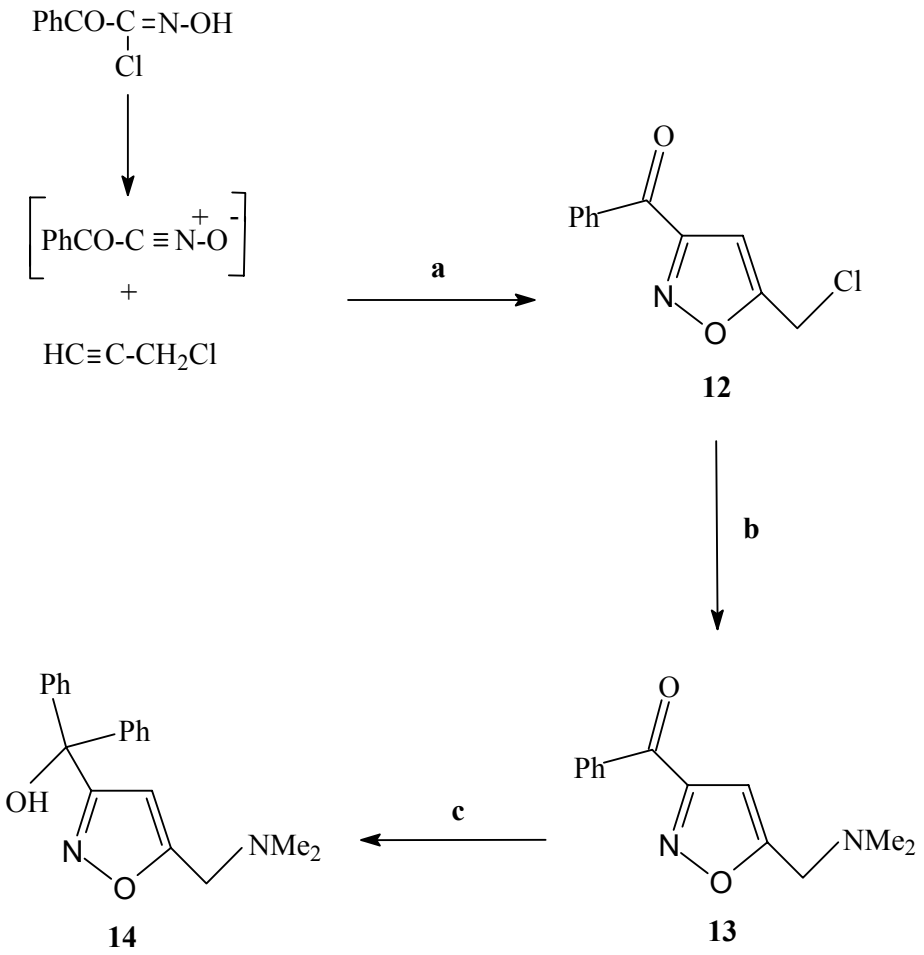
It was by taking into consideration the results from reactions shown in Outlines 3, 4, and 5, and the racemic muscarone synthesis already obtained in our laboratory[39], that we projected the synthesis of compounds **4**, **5**, and **6** (figure 8).

Outline 6 illustrates the first part of the reaction sequence which begins with cycloaddition of the phenylglyoxide nitriloxide to the propargyle cloride; the reaction is conducted by dissolving the stable precursor (phenylglyoxide hydroxymoyl chloride) of the nitriloxide and an excess of dipolarophyl in ethyl acetate. The 1,3-dipole is generated in situ by adding solid $NaHCO_3$: these conditions allow the reaction to terminate in about 24 hours. The cycloadduct **12**, after being purified by flash cromatography on silica gel, is then treated with an excess of dimethylamine at 0°C. The quantitative transformation into the dimethylamine derivative **13** takes place over the next 12 hours: this compound is then reacted with phenylmagnesium bromide suspended in anhydrous tetrahydrofuran.

The desired aminoalcohol [14] is recovered as a solid crystal by extraction from the reaction mixture. This product then undergoes a reaction to rupture the isoxazol ring at the N-O bond by catalytic hydrogenation using either Nickel-Raney or $Mo(CO)_6$ (Outline 7). In both cases the enamic intermediate was not isolated from the reaction mixture but was directly subjected to hydrolysis and cyclisation with HCl 3N. The two procedures have similar yields, about 45%, of the β-furanone **15**, starting with the isoxazole **14**.

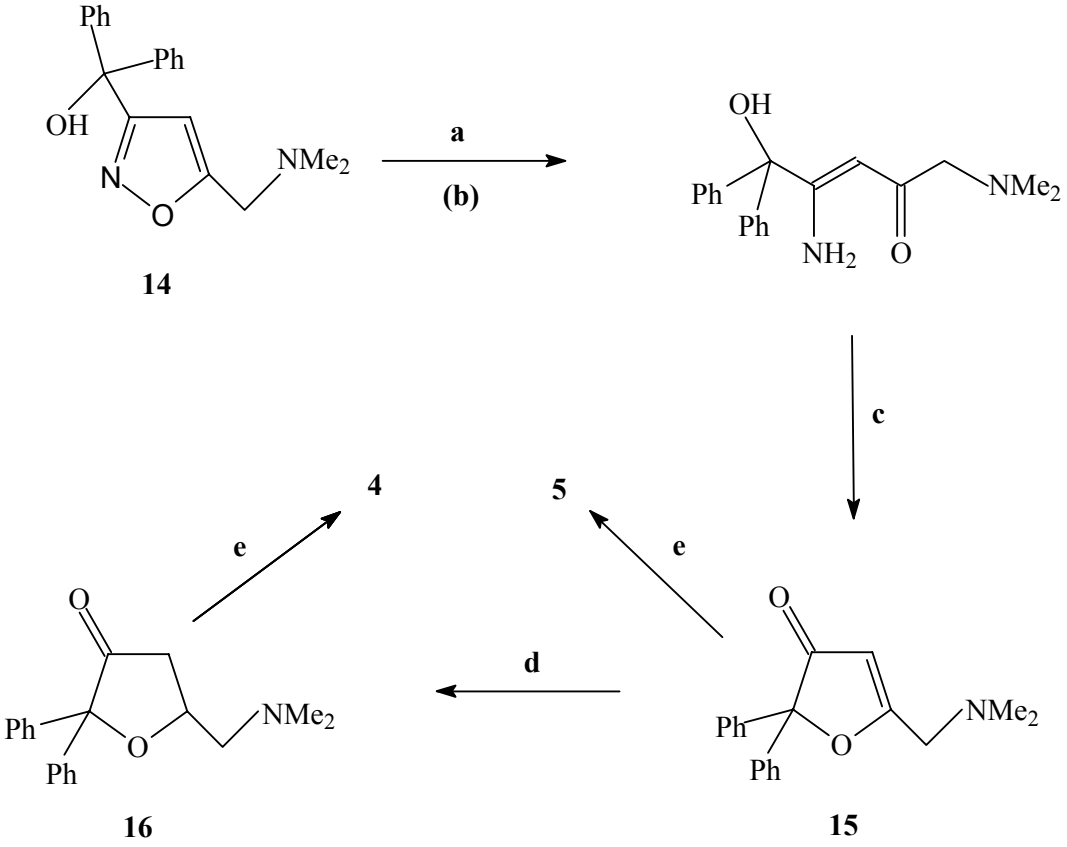
The tertiary amine 15 was then reduced to the corresponding derivative 16 by catalytic hydrogenation on Pd/C, 10%. The resulting compound was obtained with reasonable yields (55-60%), along with other products that were isolated in the course of purification with chromatography but were not characterised as they most likely derived from the aperture of the heterocyclic ring.

Outline 6



a: $NaHCO_3$-AcOEt; **b:** $NHMe_2$, MeOH, O°; **c:** PhMgBr, THF, r.t.

Outline 7



a: H_2/Ni-Raney; **b:** $Mo(CO)_6$, CH_3CN/H_2O, reflux;
c: HCl 3N; **d:** H_2/Pd(10%)-C; **e:** CH_3I.

The final compounds, numbers **4** and **5**, were prepared by treating a solution (acetone 1/ethyl ether 3) of the corresponding tertiary bases with an excess of methyl iodide.

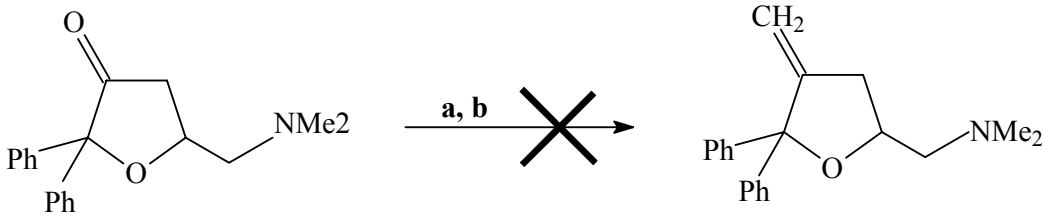
Structures for the substances in outlines **6** and **7** were attributed using ^{1}H-NMR spectra at 200 MHz in $CDCl_2$ or D_2O solutions.

The synthesis of compound number 6 (figure 8), containing an exocyclic methylene group instead of the carbonyl, was approached using the expedient method of treating the tertiary amine 16 to Wittig olefinisation. Reacted under standard conditions: generation of methylenphosphorane at -30°C is obtained by treating methyltriphenylphosphone bromide with butyl lithium(2.5 M in hexane). These conditions, which are ideal for aldehyde substrates, left compound 16 unreacted (upper part of Outline **8**). In addition to the lower reactivity of ketones over aldehydes, this particular case must also take into consideration the steric hindrance of the two pheyl groups in α to the carbonyl that must be seen as an obstacle to the attack of the methylenphosphorane. In order to arrive at the predetermined product we attempted an alternative method (Outline **8**).

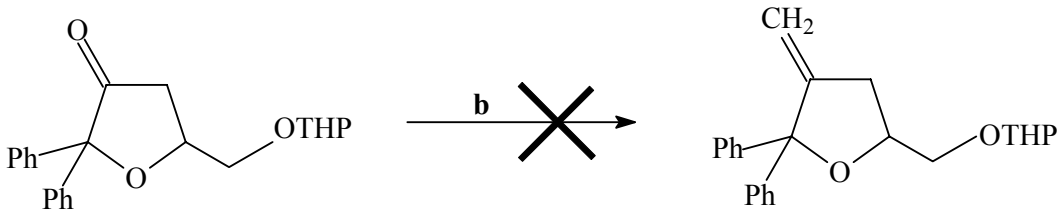
A recent publication[44] reported the use of dimethyltitanocene (Cp=cyclopentadienyl in Outline **8**) in methylenation reactions for substrates containing an exocyclic ketone and/or large substitutions around the reaction centre. The methylenate is prepared using a noted reaction procedure[45] which consists in mixing commercially available titanocene dichloride (Cp_2TiCl_2) with methyl lithium. A tetrahydrofurane solution of **16** with the methylenating complex (from 1 to 2 equivalents) was made to reflux under nitrogen for 36 hours. Progress was controlled by TLC, but apart from a progressive degrading of the starting product there appeared no stains that could be attributed to the desired compound **17**.

As an alternative we prepared a substrate where the basic function was replaced by a group that would be more stabile in these reaction conditions.

Outline 8



16 **17**



18 **19**

THP: tetrahydropyranyl

a: $Ph_3P{=}CH_2$, THF (from -40°C to r.t.); **b:** Cp_2TiMe_2, toluene (reflux).

At the same time, that group would have to be easily transformed after the methylenation into the corresponding dimethylamine derivative **17**. The only result obtained from the preparation of the tetrahydropyranyl derivative **18** (Outlines 8, 9, and 10) was that the compound avoided degradation in the same conditions for the reaction with demethyltitanocene, as opposed to **16**. Even in the presence of a large excess (3 equivalents) of the methylenating agent, **18** is still retrieved intact after chromatographic purification of the reaction mixture. Outlines 9 and 10 show the synthetic route for the preparation of the tetrahydropyranyl ether **18**.

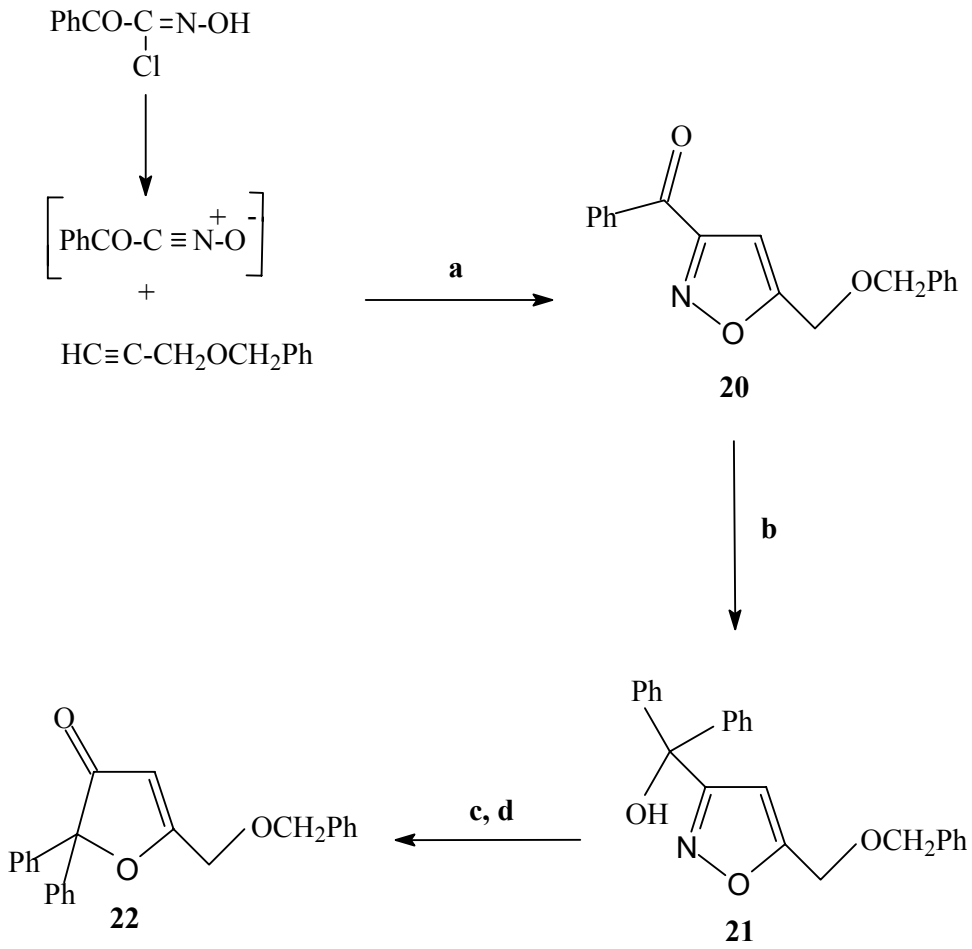
With a similar procedure to that shown in Outline 6, the cycloaddition of a phenylglyoxyl nitriloxide to the benzylether of propargylic alcohol leads to the cycloadduct **20**. The Grignard reaction that follows leads to the corresponding tertiary alcohol **21** that is opened at the N-O bond and then recyclised in a solution of THF and HCl 3N (preperation of the β-furanone **22**). The sequence is completed with a catalytic hydrogenation that both saturates thhe double bond and liberates the alcohol.

The primary alcohol **23** is then transformed into the tetrahydropyranyl derivative 18 by treatment with dihydropyrane in a solution of dichloromethane and an acidic solfonic resin.

At the moment we are studying other methods to apply for a successful methyenation resulting in synthesis of compound **6**, one of the objectives of this thesis.

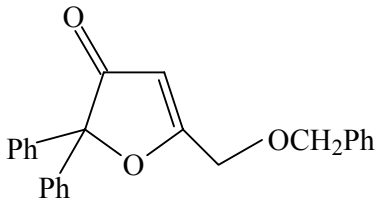
Table 2 shows the results of an evaluation of muscarinic potency for the quaternary ammonium salts **4** and **5** along with the data from Table 1. The pharmacological testing was done by Prof. Grana and his collegues at the Institute of Pharmacology of the University of Pavia, who I wish to thank. Compounds **4** and **5** present a similarity in their structures to muscarone, with elevated values of antimuscarinic potency and no ability to discriminate between M_2 and M_3 subtypes, results like to the isoxazoles **3c** and **3d**.

Outline 9

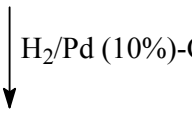


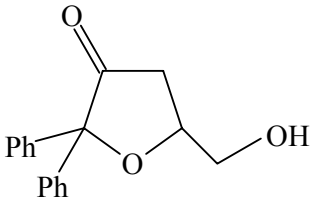
a: $NaHCO_3$-AcOEt; **b:** PhMgBr, THF, r.t.;
c: $Mo(CO)_6$, CH_3CN/H_2O, reflux; **d:** THF, HCl 3N.

Outline 10

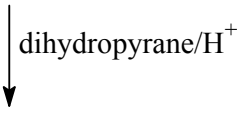


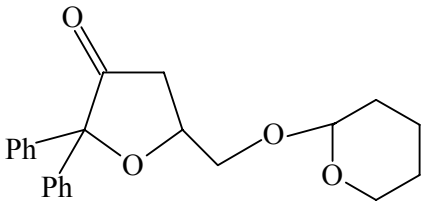
22





23

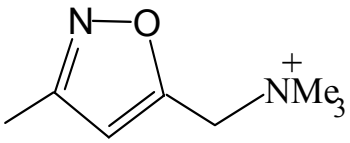




18

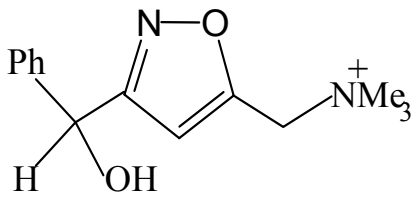
Table 2. pA2 values α(=0) for compounds **3-5**: ❍rat jejunum, ■rat bladder, ❑guinea pig atria. pD2 values for compound **2** on the same organ preparations.

	❍ 9.50
ATROPINE	■ 9.40
	❑ 9.40



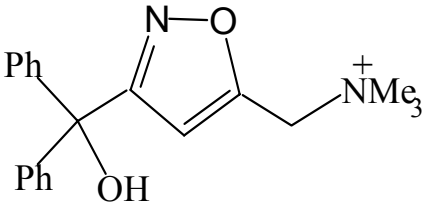
❍ 5.00, α=1
■ 4.39, α=0.82
❑ 5.43, α=1

2



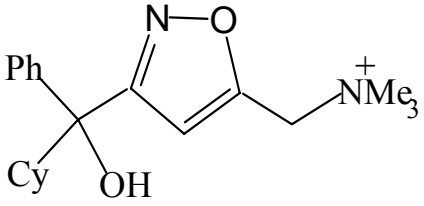
❍ 5.87
■ 5.97
❑ 5.82

3a



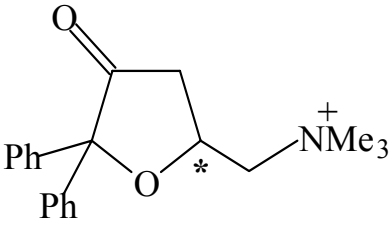
❍ 9.00
■ 8.86
❑ 8.80

3b



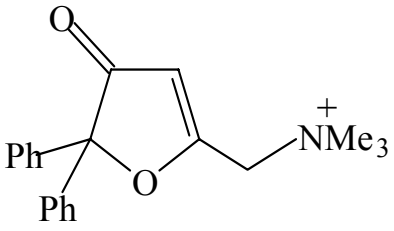
❍ 9.08
■ 9.27
❑ 8.85

3c



❍ 8.37
❑ 8.68

4



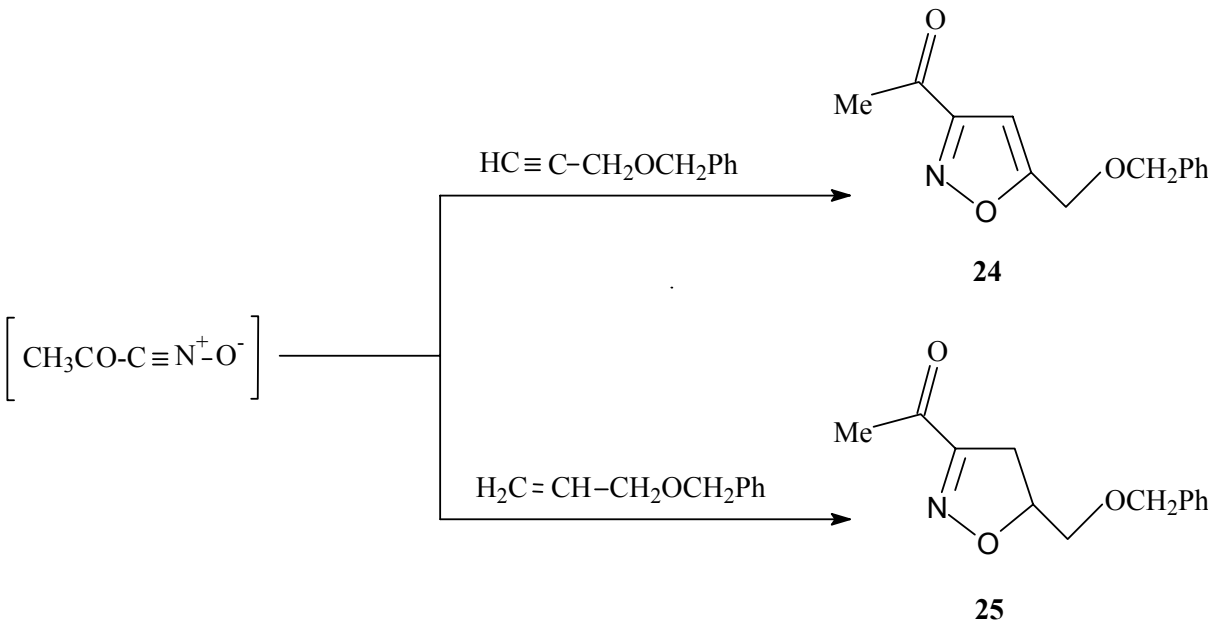
❍ 8.11
❑ 8.14

5

The degree of potency is also influenced by the nature of the heterocycle since an opportune substitution at position 3 of the isoxazole ring engenders more powerful (an increase of approximately an order of one) antimuscarinic compounds compared to those compounds that carried a muscarone skeleton.

In the final period of my internship I studied a reaction sequence for the preparation of an intermediate that would allow an enzymatic resolution in the synthesis of muscarone (Outline 1).
Based on the results obtained in the preparation of **18** (Outlines 9 and 10), I thought to apply a similar strategy, which is described in Outlines 11 and 12. Pyruvilnitriloxide (generated from the stabile chloroxime under the same conditions seen previously) is made to react with the benyl ethers of both propargylic and allylic alcohols. Both reactions lead to cycloadducts, isoxazole **24** and 2-isoxazoline **25**, with good yields. The two 3-acetyl derivatives were then transformed into the corresponding secondary alcohols **26** and **27** by reacting them with sodium borous hydride in methanol.
The isoxazole **26** was then subjected to hydrogenolytic aperture with $Mo(CO)_6$ (Outline 12) in similar conditions as those used for the diphenyl derivatives (see Outlines 7 and 10). The β-furananone **28** was, in this case, isolated with a yield of 30%, before shifting to an aqueous acidic ambient.
The isoxazoline **27**, in the same experimental conditions did not give the desired product **29** (we had predicted the formation of a mixture of cis and trans isomers) even after the addition of HCl 3N: the reaction mixture showed itself to be extremely complex.

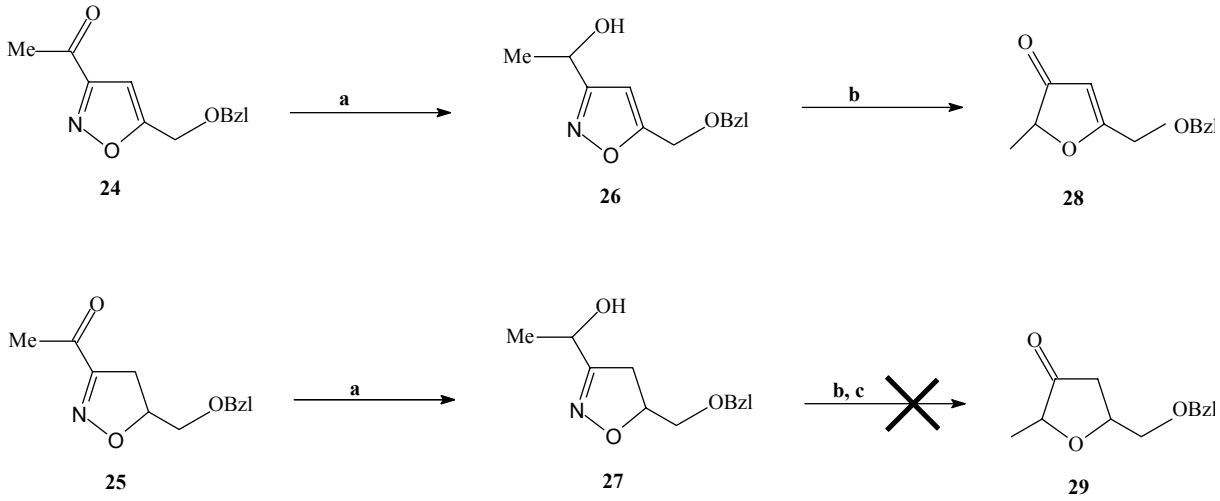
OUTLINE 11



24

25

Outline 12



a: $NaBH_4$, MeOH; **b:** $Mo(CO)_6$, CH_3CN/H_2O, reflux; **c:** THF, HCl 3N; Bzl=CH_2Ph

In order to obtain the synthesis of (±)−**11**, the intermediate **28** will be subjected to a catalytic hydrogenation that will both reduce the carbon-carbon double bond and liberate the primary alcohol that will then be transformed into the corresponding butyrate **11**. We will also alter the conditions for the hydrogenolytic aperture of **26** in order to prepare the intermediate **28** with yields superior to those attained thus far in these first experiments.

CONCLUSIONS

In the course of this thesis, I used the 1,3-dipolare cycloaddition reaction of nitriloxides to terminal alkynes as a synthetic route to obtain compounds with biological activity.

Taking advantage of the hydrogenolysis of isoxazoles, I prepared two quaternary ammonium salts that are structurally related to muscarone and that possess antimuscarinic activity. As with structural analogues containing an isoxazoline ring, these compounds presented us with elevated antimuscarinic values but did not show any discrimination between M_2 and M_3 muscarinic receptors.

The hydrogenlytic aperture of isoxazoles with molibdenum hexacarbonyl was also used for the preparation of a butyrate that would function as a potential substrate for an enzymatic hydrolysis reaction. This approach has for the present consented the preparation of an intermediate with β-furanone structure that will be later transformed into the desired compound with two ulterior steps. This strategy has the purpose of determining if an enantioselective bioconversion will allow a more convenient synthesis of the eutomer of muscarone than the alternative of total synthesis.

EXPERIMENTAL SECTION

MATERIALS AND METHODS

The dipolarphyles used in the cycloaddition reactions are either commercially available or easily derived from commercially available olefines and acetylenes. The halogenoximes used were prepared according to procedures well known and used in our laboratory.

The chromatographic plates (Merck, 5x10 cm, cod. 5719) were analysed under U.V. light (254 nm) and subsequently oxidised with a solution of $KMnO_4$ in soda 0.1 N.

The columns used for chromatographic separations were filled with Merck silica gel (cod. 7734 and cod. 9385) as the stationary phase. The mobile phases will be described in the relative paragraphs.

Melting points were determined without correction using a model 510 Büchi apparatus.

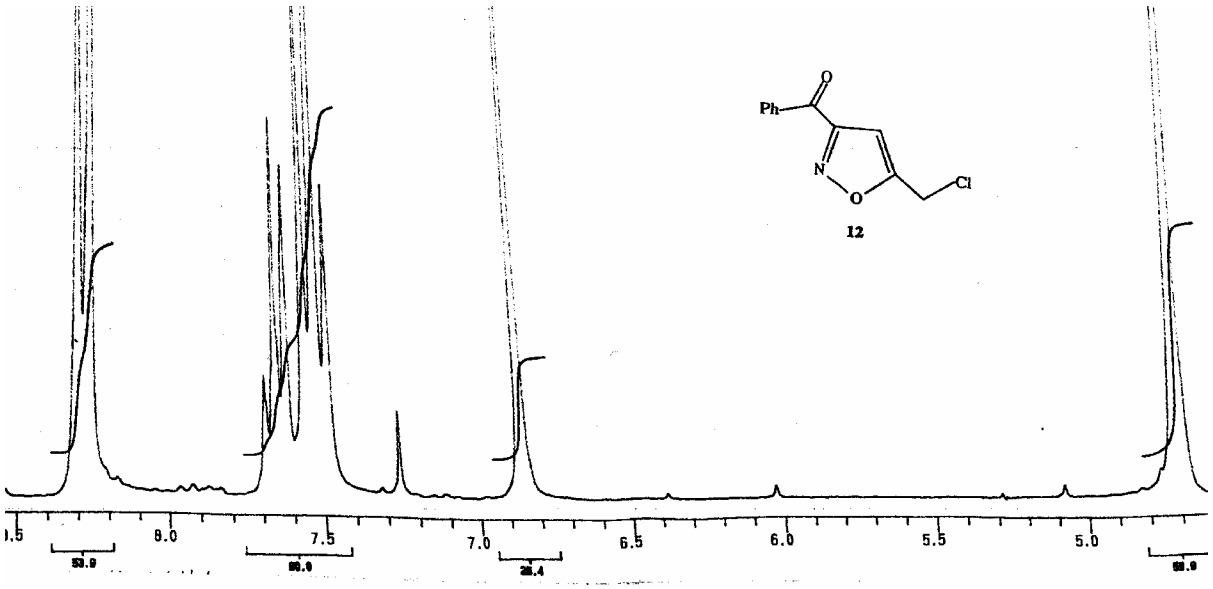
Liquid products were purified by vacuum distillation with model GKR 50 Büchi apparatus.

^{1}H-NMR spectrums were determined in $CDCl_3$ solution (unless specified otherwise) with a Varian Gemini working at 200 MHz. Chemical shifts are expressed in δ and coupling constants in Hertz.

Synthesis of 12

9.2 g (50.13 mmoles) of the chloroxime precursor are dissolved ethyl acetate (100 ml) along with 10 g (o.134 moles) of propargyl chloride, in the presence of an excess amount of $NaHCO_3$. The solution is left under vigorous agitation overnight. Approximately 50 ml of water are added to the resultant solution, and the organic phase is then separated. From this we recovered 9.99 g of impure **12** (yield of 90%), that was crystalised from n-hexane (m.p. 46-47°C, R_F: 0.67, petroleum ether/ethyl acetate 4:1).

^{1}H-NMR: 4.70 (s, 2, CH_2Cl); 6.85 (s, 1, H-4), 7.42-7.72 (m, 3, arom.); 8.28 (d, 2, arom.).



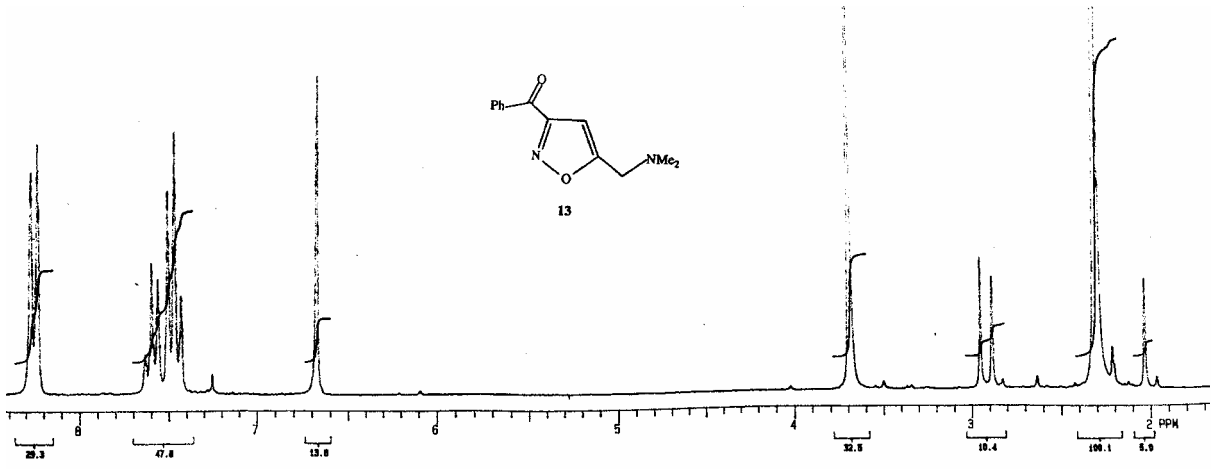
I2

Synthesis of 13

12, left unpurified, is dissolved in methanol and made to react with an excess of dimethylamine at the temperature of -20°C. The reaction is left to proceed at room temperature overnight. Excess amine is eliminated and the mixture is concentrated, then dilute HCl is added and the acidic aqueous phase is extracted using ethyl ether. The aqueous phase is basified with K_2CO_3 and extraction is repeated using dichloromethane. The organic phase is dried and concentrated: the impure yellow oil that is obtained with a yield of 85% distills at 185-190°C/5mm Hg.

R_F: 0.63 (CH_2Cl_2/MeOH 9:1).

^{1}H-NMR: 2.30 (s, 6, NMe_2); 3.68 (s, 2, CH_2N); 6.68 (s, 1, H-4); 7.40-7.65 (m, 3, arom.); 8.26 (d, 2, arom.).

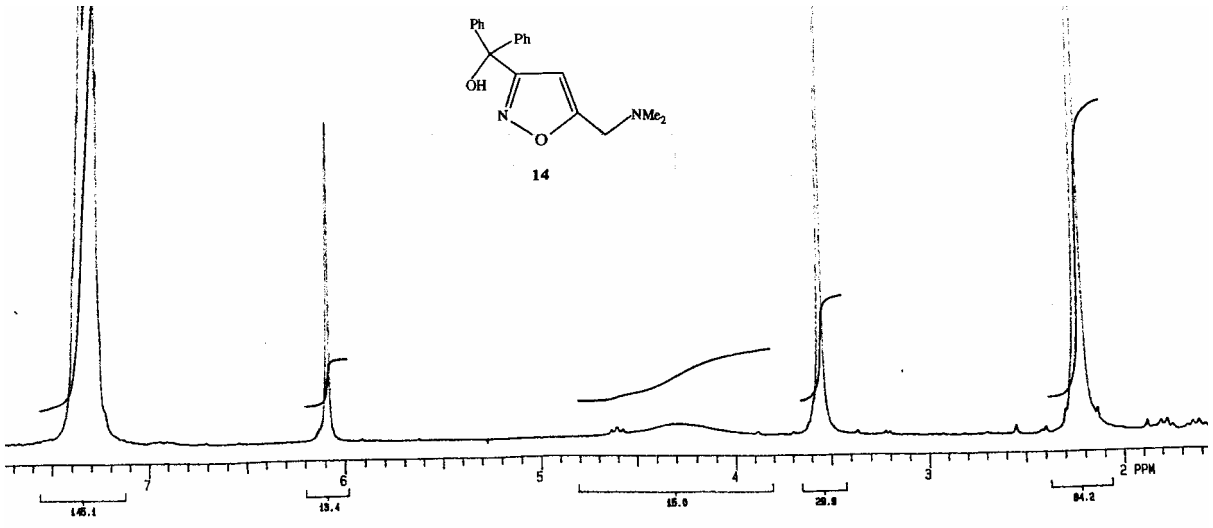


13

Synthesis of 14

2.97 g (0.122 moles) of metallic Mg are added to 80 ml of anhydrous THF. 12.8 ml (0.122moles) of bromobenzene are added drop by drop into the reaction mixture containing a few crystals of iodine. When the last turnings of magnesium have disappeared, 9.39 g (41 mmoles) of **13** in anhydrous THF are slowly added to the reaction in an ice bath, under an inert atmosphere, and under constant agitation. After the last drops have been added, the reaction is allowed to proceed at room temperature overnight. Dilute HCl (30 ml) is then carefully added, the THF is evaporated and the reaction is extracted using ethyl ether; the aqueous phase is basified with solid K_2CO_3 and extracted a few times with dichloromethane. After evaporating the solvent the impure dimethylaminoalcohol **14** (9.98 g, yield: 79%) is crystalised from ethyl acetate and petroleum ether (m.p. 121-123°C; R_F: 0.49 CH_2Cl_2/MeOH 9:1).

^{1}H-NMR: 2.22 (s, 6, NMe_2); 3.55 (s, 2, CH_2N); 4.30 (widened s, 1, OH); 6.09 (s, 1, H-4); 7.32 (m, 10, arom.).



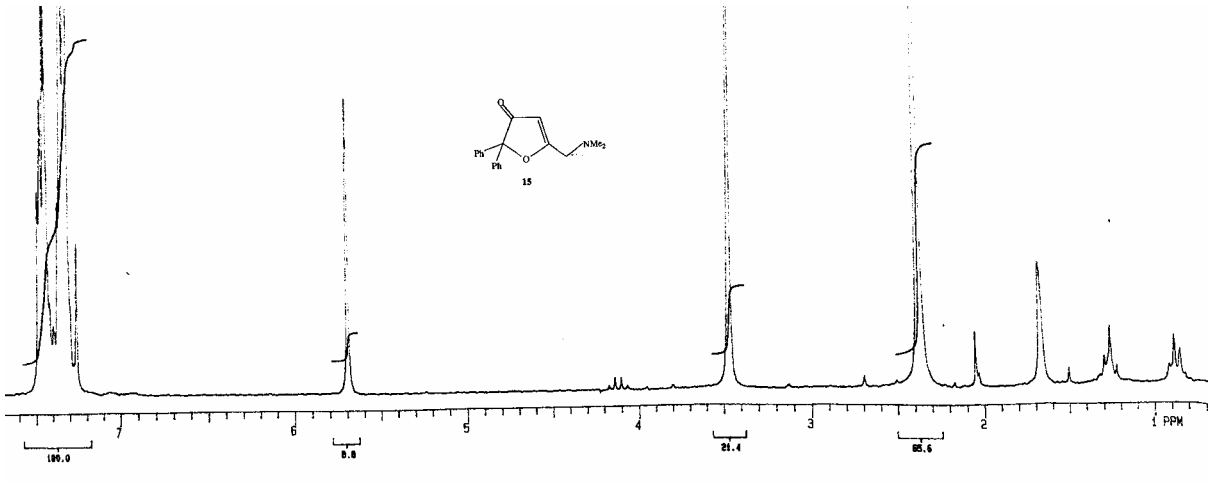
14

Synthesis of 15

3 g of **14** (9.74 mmoles) are dissolved in absolute methanol (50 ml) and hydrogenated in the presence of Nickel-Raney W2. When the substrate disappears, the impure intermediate enamine (R_F: 0.28 CH_2Cl_2/MeOH 9:1) is separated from the catalyst by filtration through celite under vacuum. The mixture obtained after evaporation of the remaining methanol is made to react with HCl 3N (25 ml) at room temperature for six hours. Afterwards, the mixture is extracted with ether, the aqueous phase is basified with K_2CO_3 and extracted a few times dichloromethane. The impure product is purified in a chromatographic column through silica gel using a solution of CH_2Cl_2/MeOH 95:5. The solid product thus obtained, 1.74 g (5.94 mmoles, yield: 61%), is crystalised from ethyl acetate and petroleum ether, the m.p. is 89-90°C.

The hydrogenolytic aperture of **14** (3 g) was carried out in the presence of 1.29 g of $Mo(CO)_6$ (4.87 mmoles) suspended in nitrilacetate (150 ml) and water (8 ml). The reaction is left to reflux for 24 hours and then, after cooling, is filtered through celite. The impure filtrate is concentrated under vacuum, dissolved in HCl 3N (25 ml) and left under agitation at room temperature for approximately 6 hours. After extraction, carried out as above, the reaction mixture is chromatographed under the same conditions to give **15** with a yield of 52% (R_F: 0.64 CH_2Cl_2/MeOH 9:1).

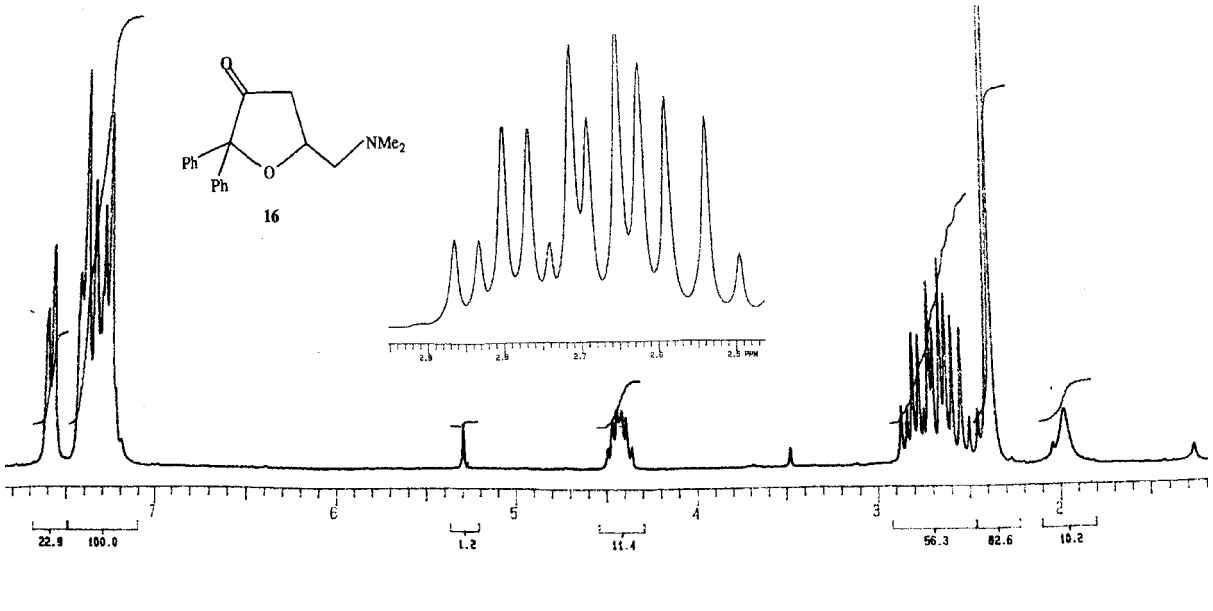
^{1}H-NMR: 2.36 (s, 6, NMe_2); 3.46 (s, 2, CH_2N); 5.69 (s, 1, H-3); 7.26-7.52 (m, 10, arom.).


O
Ph
Ph
O
NMe2
15
7
6
5
4
3
2
1 PPM
100.0
8.8
21.4
85.6

Preparation of 16

1.32 g (4.51 mmoles) of **15** are dissolved in absolute methanol (30 ml) and hydrogenated in the presence of Pd/C 10%. After the completion of the reaction, the catalyst is eliminated by filtration through celite and the reaction mixture is chromatographed on a column filled with silica gel, using a mobile phase mixture of CH_2Cl_2/MeOH 9:1. 0.76 g (2.57 mmoles, yield of 57%) of **16** are obtained (R_F:0.64 CH_2Cl_2/MeOH 9:1).

^{1}H-NMR: 2.36 (s, 6, NMe_2); 2.44-2.86 (dddd, 4, H-3 and CH_2N); 4.42 (m, 1, H-2); 7.22-7.60 (m,10, arom.).


O
Ph
Ph
O
NMe2
16
22.9
100.0
1.2
11.4
56.3
82.6
10.2

Preparation of 4 and 5

A) Preparation of **4**

16 is dissolved in a mixture of acetone/ether 1:3 and is treated with an excess of methyl iodide. The solid obtained with a quantitative yield is crystalised from ethanol (m.p. 195-197°C, dec.).

Empirical analysis:	% calculated	C: 54.93	H: 5.53	N: 3.20
	% found	54.65	5.16	3.20.

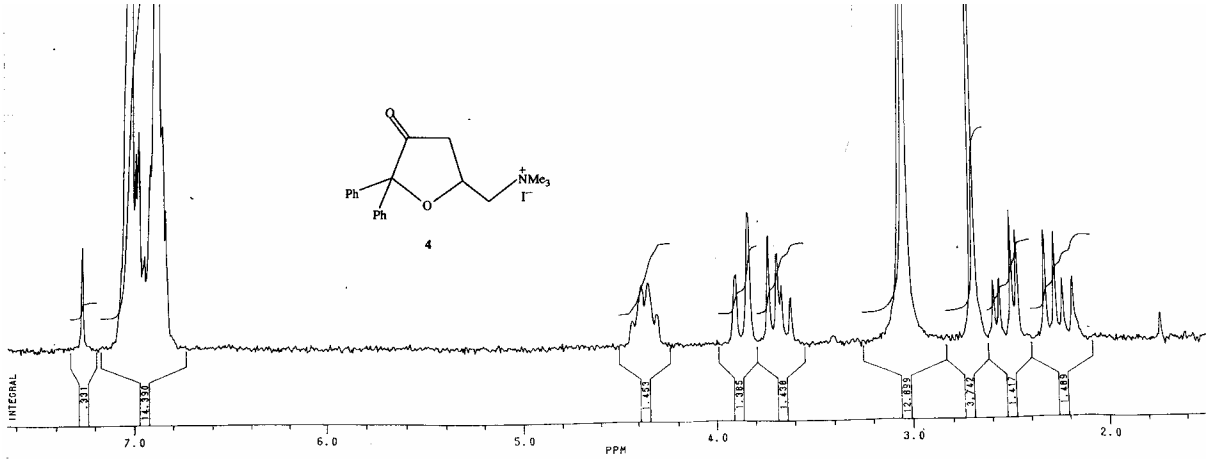
^{1}H-NMR ($CDCl_3$+ 20% d-6 DMSO): 2.24 (dd, 1, H-3'); 2.50 (dd, 1, H-3); 3.04 (s, 9, NMe_3); 3.65 (dd, 1, CH_AN); 3.87 (d, 1, CH_BN); 6.82-7.11 (m, 10, arom.).

B) Preparation of **5**

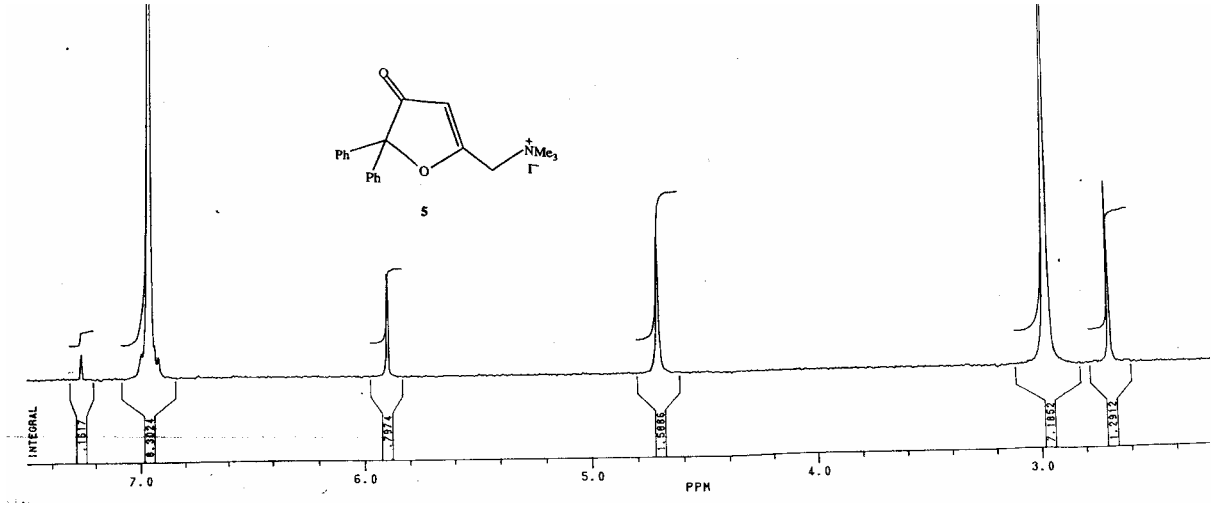
15 is treated as described in the above paragraph, to obtain **5**. This is crystalised from a mixture of acetone/ethyl acetate (m.p. 170-171°C, dec.).

Empirical analysis:	% calculated	C: 55.19	H: 5.09	N: 3.22
	% found	55.34	5.15	3.34.

^{1}H-NMR ($CDCl_3$+ 20% d-6 DMSO): 2.97 (s, 9, NMe_3); 4.71 (s, 2, CH_2N); 5.90 (s, 1, H-3); 6.96 (s, 10, arom.).



4



5

Whittig Olefinization of 16

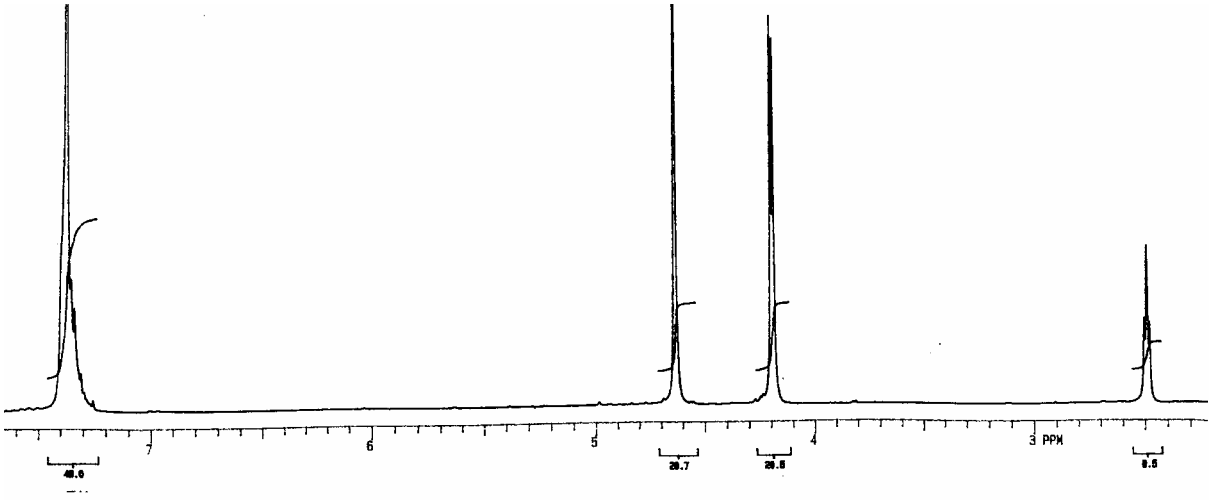
0.723 g (2.04 mmoles) of methyl triphenyl phosphonium bromide are dissolved in 13 ml of anhydrous THF in a double neck reaction flask, and cooled under nitrogen to -30°C. 1.275 ml (2.04 mmoles) of butyl lithium 2.5 M are added drop by drop over a period of 45 minutes, maintaining the reaction under constant agitation at -30°C. An orange coloured suspension is formed that is further cooled to -55°C, and to which is slowly added 0.4 g (1.36 mmoles) of **16** in 7 ml of THF. The reaction is left at r.t. overnight and, after treatment with HCl dil. (15 ml), is extracted from the double phase. The unreacted starting compound is recuperated from the reaction mixture by column cromatography through silica gel.

Preparation of 18

A) Preparation of the_benzyl ether of_propargylic alcohol

To 7.32 ml (0.126 mmoles) of propargylic alcohol in 50 ml of DMF are added 3 g (0.126 mmoles) of NaH subdivided in several allotments. The reaction is left at r.t. under vigorous agitation for about 1.5 hours. The resulting alcoholate is cooled on an ice and salt bath. 15 ml (0.126 mmoles) of benzyl bromide are then slowly added. The reaction is left under magnetic agitation for 5 hours after which time 50 ml of water are added and the reaction is repeatedly extracted with ether. The etheric phase (300 ml) is washed with water (2x100ml), dried and concentrated. After separation by column chromatography on silica gel, the desired product is obtained with a yield of 65% (R_F: 0.70 petroleum ether/ethyl acetate 9:1; b.p.: 145-150°C, 260 mmHg).

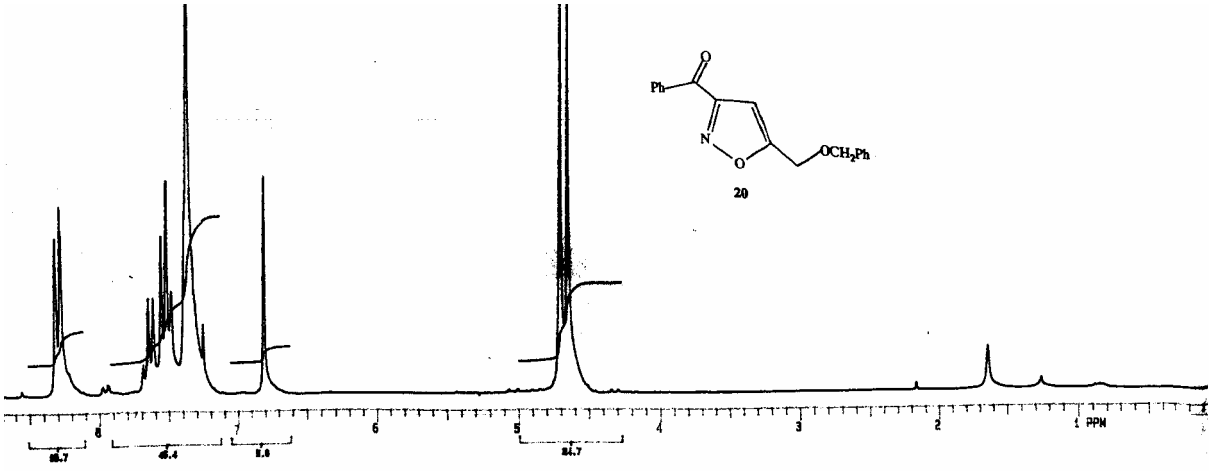
^{1}H-NMR: 2.49 (t, 1, HC≡, J= 2.3); 4.19 (d, 2, CH_2O, J= 2.3); 4.63 (s, 2, CH_2Ph); 7.37 (m, 5, arom.).


7
6
5
4
3 PPM
40.0
20.7
20.8
8.5

B) Cycloaddition of the benzyl ether_of propargylic alcohol to phenylglyoxyl nitriloxide: Preparation of **20**

9.0 g (61.6 mmoles) of the benzyl ether of propargylic alcohol are dissolved in ethyl acetate and reacted under vigorous agitation with 3.77 g (20.5 mmoles) of phenylglyoxyl hydroxymoyl chloride in the presence of an excess of $NaHCO_3$. A small amount of water is added after 48 hours, and the two phases are separated. The organic phase is dried on anhydrous Na_2SO_4 and concentrated by rotovap. The reaction mixture is purified by column chromatography on silica gel, using a mobile phase of petroleum ether/ethyl acetate 95:5. 3.48 g (11.89 mmoles) of **20** are obtained with a yield of 58% (R_F: 0.31 cyclohexane/ethyl acetate 9:1).

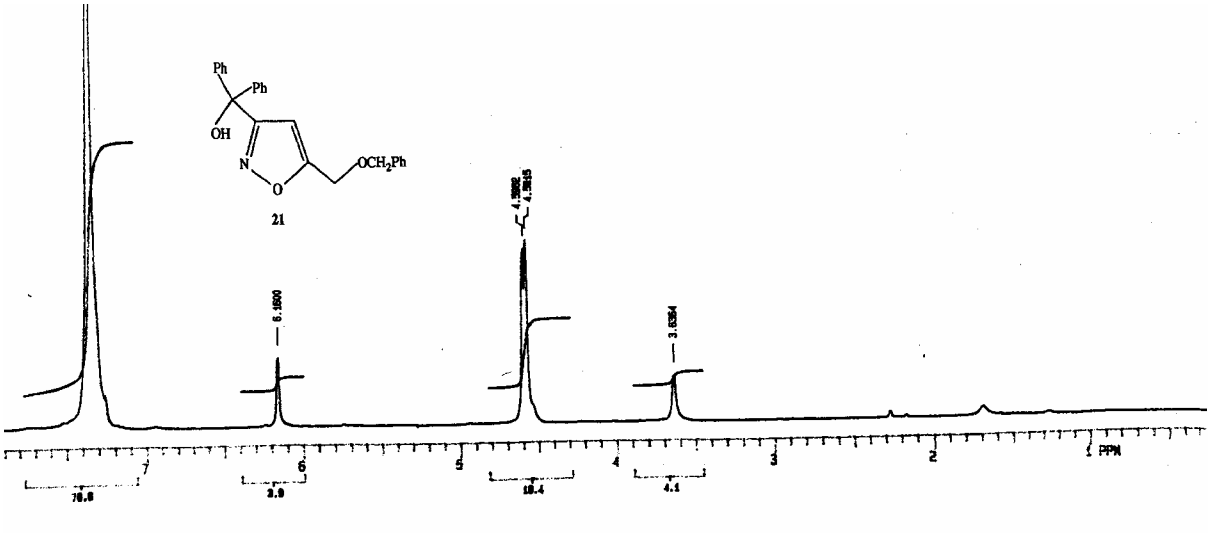
^{1}H-NMR: 4.66 and 4.71 (2s, 4, CH_2OCH_2); 6.81 (s, 1, H-4); 7.15 (m, 8, arom.); 8.29 (d, 2, arom.).


Ph
O
N
O
OCH2Ph
20
8
7
6
5
4
3
2
1 PPM

C) Preparation of **21**

2.05 g (84.4 mmoles) of metallic Mg are suspended in ether (70 ml). 13.25 g (0.122 moles) of bromium benzene are gradually added in the presence of iodine crystals and slight heating. As soon as the last of the Mg turnings have been consumed at room temperature, 8.24 g (28.12 mmoles) of **20** dissolved in anhydrous ether are added drop by drop under an inert atmosphere while maintaining constant stirring over an ice bath. With the last drops, the reaction is heated to reflux for the next 6 hours; then, after cooling to 0°C, 30 ml of water are added. The reaction mixture is filtered to eliminate inorganic salts, the organic phase is separated and the aqueous phase is extracted with ether. The etheric extracts are dried together, concentrated, and the mixture thus obtained is chromatographed on a column of silica gel (mobile phase is petroleum ether/ethyl acetate 4:1) 5.12 g (15.47 mmoles) of **21** are obtained with a yield of 55% (R_F: 0.49 in petroleum ether/ethyl acetate 7:3).

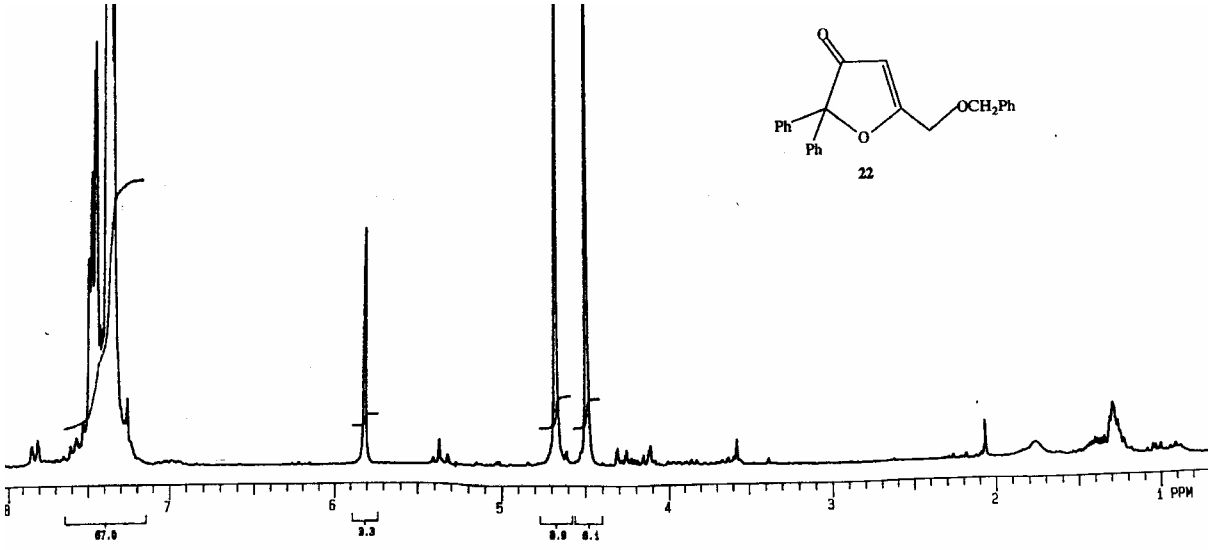
^{1}H-NMR: 3.64 (s widened, 1, OH); 4.58 and 4.60 (2 s, 4, CH_2OCH_2); 6.16 (s, 1, H-4); 7.35 (m, 15, arom.).


Ph
Ph
OH
N
O
OCH2Ph
21
6.1800
3.8364
PPM

D) Preparation of **22**

4.90 g (14.8 mmoles) of **21** are reacted with 1.99 g (7.4 mmols) of $Mo(CO)_6$ in a solution of nitrilacetate (100ml) and water (6 ml). The reaction is refluxed for 10 hours and, after cooling, filtered through celite. The filtrate is concentrated under vacuum, dissolved in HCl 3N (30 ml) and THF (30 ml), and left under magnetic agitation at room temperature overnight. The THF is then evaporated and the aqueous phase is extracted with ether. The reaction mixture is purified by column chromatography on silca gel with a mobile phase of petroleum ether/ethyl acetate 4:1. 1.42 g (3.99 mmoles) of **22** are obtained with a yield of 27%.

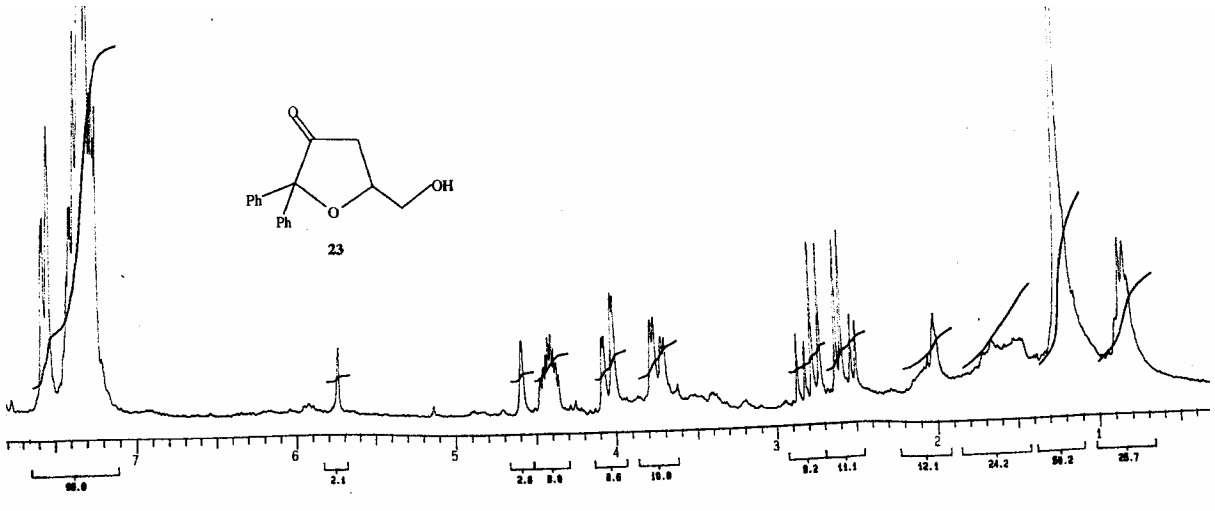
^{1}H-NMR: 4.49 and 4.67 (2 s, 4, CH_2OCH_2); 5.81 (s, 1, H-3); 7.20-7.60 (m, 15, arom.).


O
Ph
Ph
O
OCH2Ph
22
8
7
6
5
4
3
2
1 PPM
67.0
2.3
8.9
8.1

E) Preparation of **23**

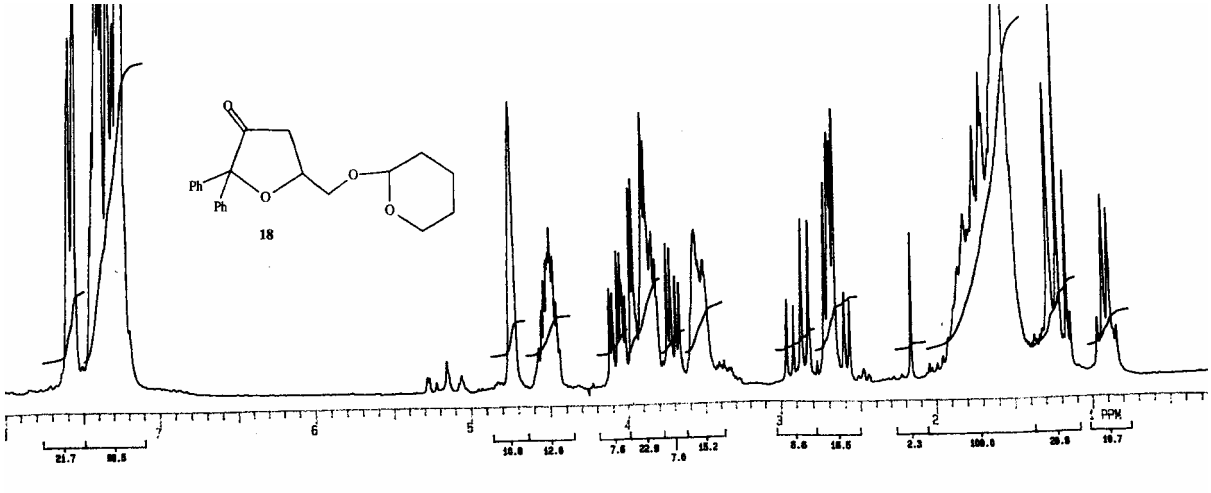
1.00 g (2.81 mmoles) of **22** are dissolved in absolute methanol (40 ml) and subjected to catalytic hydrogenation in the presence of Pd/C 10% (0.5 g). The reaction proceeds slowly so more catalyst is added (2x0.1 g). When the hydrogenation is terminated, the catalyst is removed by filtration through celite and the reaction mixture is purified by chromatography on a column of silica gel with a mobile phase of petroleum ether/ethyl acetate 7:3. 0.225 g (0.84 mmoles, yield of 30%) of **23** are obtained (R_F: 0.28 petroleum ether/ethyl acetate 7:3).

^{1}H-NMR: 2.59 (dd, 1, H-3'); 2.82 (dd, 1, H-3); 3.76 (dd, 1, CH_AOH); 4.06 (dd, 1, CH_BOH); 4.42 (m, 1, H-2); 7.15-7.65 (m, 10, arom.).


O
Ph
Ph
O
OH
23
7
6
5
4
3
2
1

F) Preparation of **18**

0.18 g (0.67 mmoles) of **23** are dissolved in CH_2Cl_2 (15 ml) in the presence of Amberlyst 15, an acidic solfonic resin, and reacted with 0.056 g (0.061 ml, 0.67 mmoles) of 3,4-dihydro-2*H*-pyran at room temperature. After approximately 6 hours the completed reaction (ascertaining by TLC that the original compound has been completely consumed) is freed of the resin by filtration and the reaction mixture is concentrated and chromatographed on a column filled with silica gel using a mobile phase of petroleum ether/ethyl acetate 9:1. 0.176 g (0.50 mmoles) of **18** are obtained with a yield of 75% (R_F: 0.80 petroleum ether/ethyl acetate 7:3).


O
Ph
Ph
O
O
O
18
PPM

Methylenation of 18

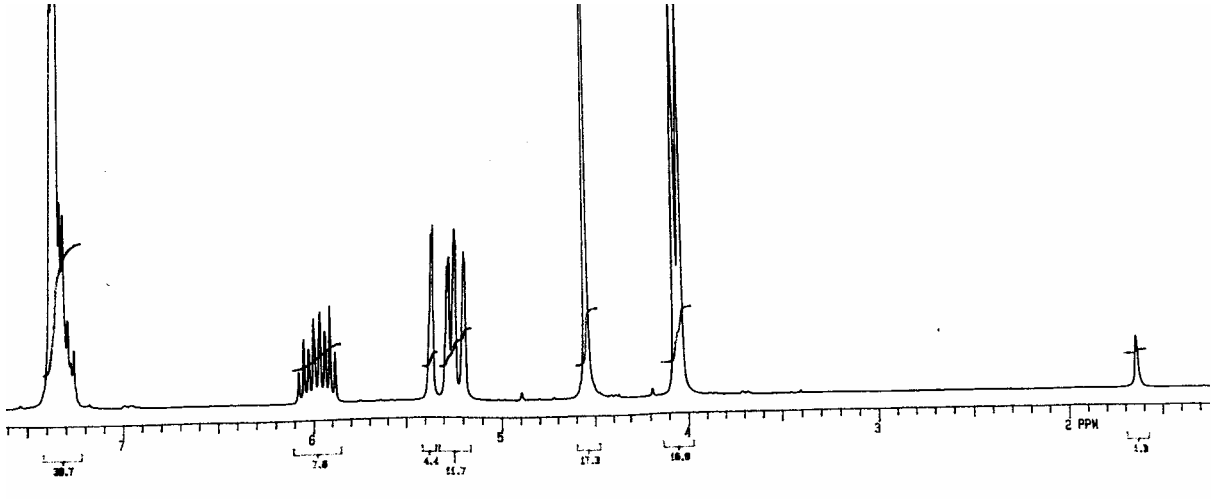
5.75 g (23 mmoles) of titanocene [bis(cyclopentadienyl) titan] dichloride in ethyl ether are cooled in an ice bath. 31.25 ml of methyl lithium 1.6 M are added to this solution drop by drop over a period of one hour in an inert atmosphere and in darkness. The reaction is brought to room temperature and vigorously stirred for a short while, ice water is then added and the solution takes on an intense orange colour. The two phases are separated, the organic phase is then exsiccated on anhydrous Na_2SO_4 and concentrated on a rotovap, all the while avoiding exposing the solid to light. 4 g of the orange crystal complex are obtained. Enough of these are dissolved in THF to give a solution with a concentration of 0.5 M.

2 ml of this solution are added to 0.23 g (0.653 mmoles) of **18** and refluxed under nitrogen and magnetic stirring in darkness overnight. Another aliquot of the methylating agent (1 ml) is added after this period and refluxing continues for another 12 hours. TLC does not show any signs of reaction on the part of the substrate, which is recuperated as such (0.18 g) by column chromatography from the reaction mixture.

Preparation of the benzyl ether of allyl alcohol

4.94 g (0.206 moles) of NaH are gradually added to 12 g (0.206 moles) of allyl alcohol in 50 ml of DMF. The reaction is left at r.t. under magnetic agitation for approximately 1.5 hours. The alcoholate that forms is cooled on an ice and salt bath; 24.34 ml of benzyl bromide (0.206 moles) are then added drop by drop. The reaction is kept under magnetic stirring for approximately 8 hours and then extract following the same procedure illustrated for the acetylene analogue. 19.8 g (0.134 moles) of the protected alcohol are obtained with a yield of 65% (b.p.: 140-145°C, 22mm Hg).

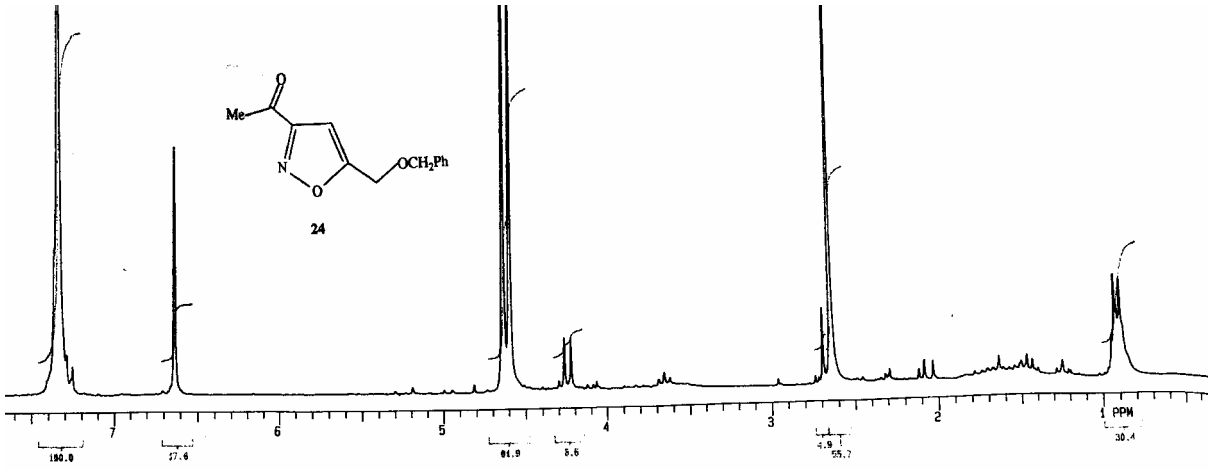
^{1}H-NMR: 4.05 (d, 2, CHC*H*$_2$O); 4.54 (s, 2, OCH$_2$Ph); 5.28 (ddd, 2, H$_2$C=); 5.97 (ddd, 1, CH); 7.20-7.45 (m, 5, arom.).


7
6
5
4
3
2 PPM

Preparation of 24

8.52 g (70.1 mmoles) of pyruvohydroxymoyl chloride are dissolved in 80 ml of ethyl acetate and 10.23 g (70.1 mmoles) of the benzyl ether of propargyl alcohol are then added to this in the presence of an excess of $NaHCO_3$. The reaction is left under magnetic agitation overnight. 4.57 g (31.3 mmoles) of the dipolarophyl are subsequently added and the reaction continues for another 12 hours. A small amount of water is added and the pH is neutralized with HCl 3N, the two phases are separated and the aqueous phases are repeatedly extracted with ethyl acetate. The organic extracts are united and dried with anhydrous Na_2SO_4, they are then concentrated and chromatographed in a silica gel column using a mobile phase of petroleum ether/ethyl acetate 9:1. 10.12 g (43.8 mmoles) of **24** are obtained (R_F: 0.37 petroleum ether/ethyl acetate 4:1) with a yield of 63%.

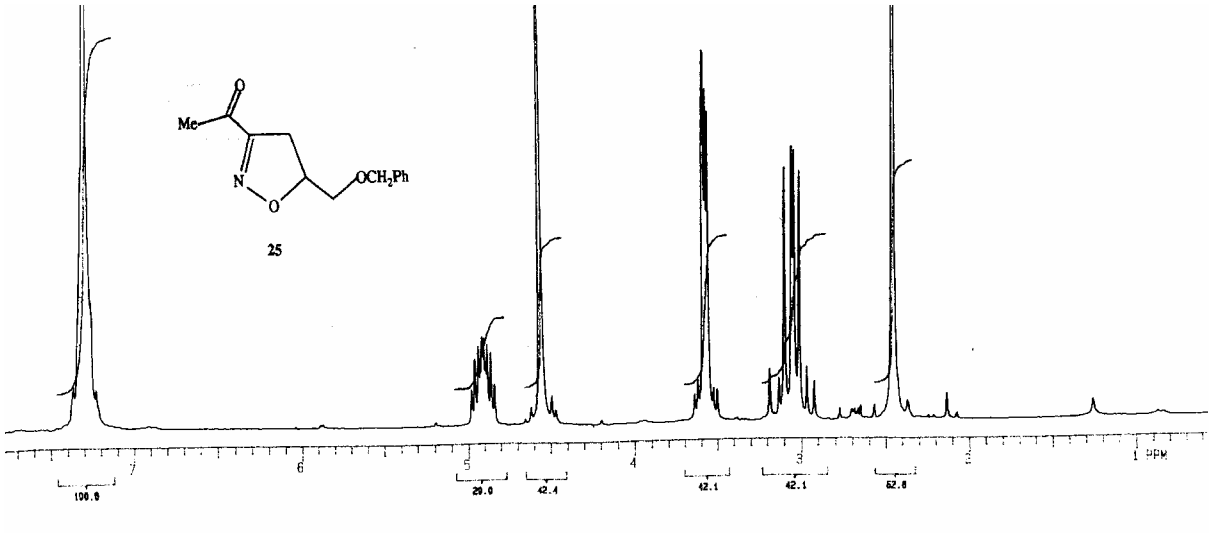
^{1}H-NMR: 2.64 (s, 3, CH_3); 4.59 and 4.63 (2 s, 4, CH_2OCH_2); 6.63 (s, 1, H-4); 7.34 (s, 5, arom.).


O
Me
N
O
OCH2Ph
24
7
6
5
4
3
2
1 PPM

Preparation of 25

7 g (57.6 mmoles) of pyruvohydroxymoyl chloride are reacted with 12.6 g (81.5 mmoles) of the benzyl ether of allyl alcohol in the presence of an excess of $NaHCO_3$. The reaction is left under magnetic agitation overnight and then 3.4 g (23 mmoles) of the dipolarophyl are added, leaving it under continuous agitation for another 18 hours. The reaction is then treated as mentioned in the previous preparation. The reaction mixture is purified by column chromatography using silica gel and a mobile phase of petroleum ether/ethyl acetate 9:1. 9.26 g (39.75 mmoles) of **25** are obtained with a yield of 69% (R_F: 0.53 petroleum ether/ethyl acetate 4:1).

^{1}H-NMR: 2.45 (s, 3, CH_3); 2.99 (dd, 1, H-4', $J_{4,4'}$=17.5, $J_{4',5}$ =10.8); 3.10 (dd, 1, H-4, $J_{4,5}$=8.6); 3.54 (dd, 1, H-6', $J_{6',6}$=10.8, $J_{6',5}$=4.1); 3.59 (dd, 1, H-6, $J_{6,5}$=4.8); 4.55 (s, 2, OCH_2Ph); 4.90 (m, 1, H-5); 7.30 (s, 5, arom.).


Me
O
N
O
OCH2Ph
25
7
6
5
4
3
2
1 PPM
100.0
29.0
42.4
42.1
42.1
62.8

Preparations of 26 and 27

A) Preparation of **26**

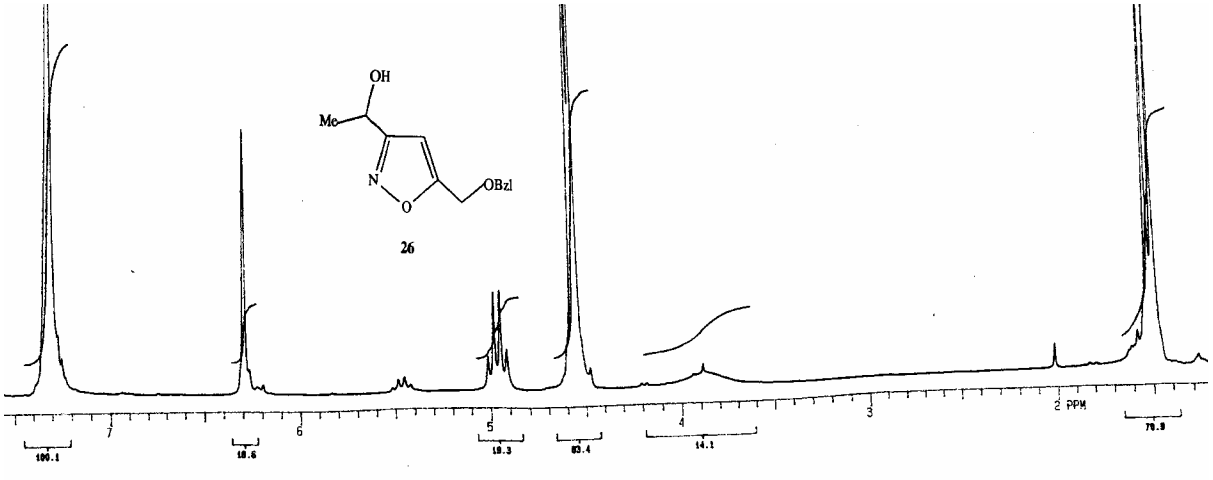
10.21 g (44.2 mmoles) of **24** are dissolved in 80 ml of methanol. This solution is cooled on an ice bath and 0.83 g (21.9 mmoles) of $NaBH_4$ are added slowly. Vigorous magnetic agitation is maintained at room temperature for 6 hours, after which time an ulterior 0.415 g of reductant are added. When the reaction is complete (after 18 hours, checking with TLC using a mobile phase of petroleum ether/ethyl acetate 3:2) HCl 6N is cautiously added to bring the reaction to an acidic pH of 5. The methanol is eliminated by rotovap and the reaction mixture is filtered under vacuum on a chromatographic column with silica gel topped with a layer of celite, using ethyl acetate as a mobile phase. 9.58 g of **26** are obtained (yield of 93%; R_F: 0.83 petroleum ether/ ethyl acetate 2:3).

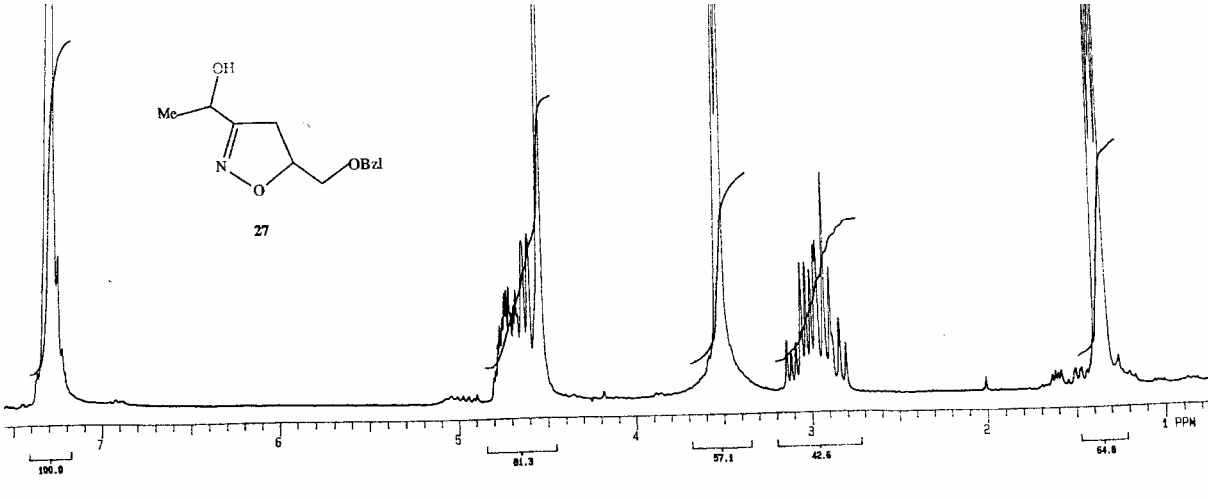
^{1}H-NMR: 1.49 (d, 3, CH_3, J=6.6); 3.87 (s widened, 1, OH); 4.55 and 4.56 (2 s, 4, CH_2OCH_2); 4.96 (q, 1 CH, J=6.6); 6.30 (s, 1, H-4); 7.33 (s, 5, arom.).

B) Preparation of **27**

Compound **27** is prepared in an identical manner as above starting from 12.94 g (55.54 mmoles) of **25**. The reaction mixture is treated with the same methodology as previously indicated; 12 g of the secondary alcohol **27** are obtained (yield of 92%; R_F: 0.56 petroleum ether/ethyl acetate 2:3).

The ^{1}H-NMR shows the multiplicity at the methyl group typical of a mix of diastereoisomers (due to the presence of two stereogenic centres).


OH
Me
N
O
OBzl
26
7
6
5
4
3
2 PPM
100.1
18.6
19.3
83.4
14.1
70.9

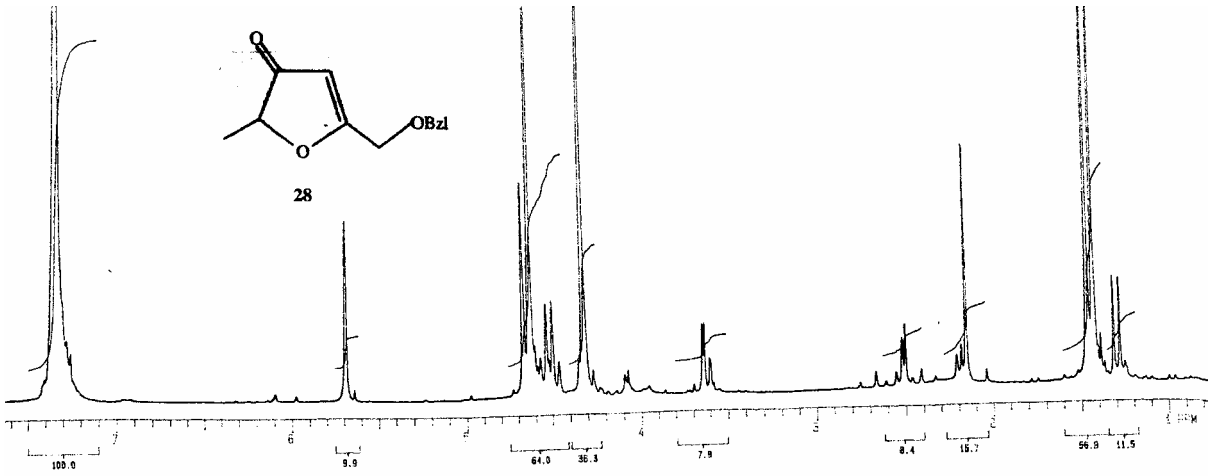

OH
Me
N
O
OBzl
27
7
6
5
4
3
2
1 PPM
100.0
81.3
57.1
42.6
64.8

Reactions with $Mo(CO)_6$ of 26 and 27

A) 3 g (12.87 mmoles) of **26** are reacted with 1.7 g (6.44 mmoles) of $Mo(CO)_6$ in nitrilacetate and water according to the previously illustrated procedure. The product obtained by opening the heterocycle is filtered on celite, concentrated with the rotovap and the residue is chromatographed using a mobile phase of petroleum ether/ethyl acetate 4:1. The more apolar fraction from the reaction mixture is the desired product **28** (0.786 g; 28% yield).

^{1}H-NMR: 1.43 (d, 3, CH_3, J=7.15); 4.31 (s, 2, OCH_2Ph); 4.51 (q, 1, C*H*CH_3, J=7.15); 4.62 (s, 2, CH_2O); 5.69 (s, 1, H-3); 7.34 (s, 5, arom.).

B) The same procedure as above was carried out on the isoxazoline derivative **27**. The reaction mixture was chromatographed in conditions identical to those used with the corresponding isoxazole: none of the separated fractions were identified as the desired product. Likewise unproductive was the attempt at treating the hydrogenated mixture with HCl 3N.


O
OBzl
O
28
100.0
9.9
64.0
36.3
7.9
8.4
15.7
56.9
11.5

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