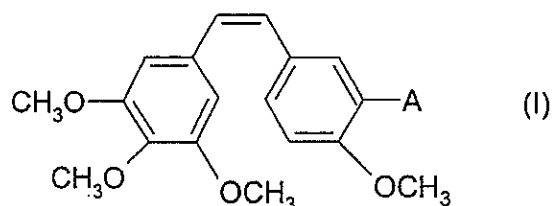


PROCESSES FOR THE PREPARATION OF COMBRETASTATINS

The present invention relates to a novel process for the preparation of combretastatins and of their derivatives.

5 The term "combretastatins" or "stilbene derivatives" is understood to mean the derivatives of following general formula:



in which A represents a hydroxyl group or an amino group, and its pharmaceutically acceptable salts.

10

Mention may be made, among the salts, of the hydrochloride, acetate, phosphate or methanesulphonate. When the compound A is an amino group, it can also be coupled to amino acids to result in amides, and their pharmaceutically acceptable salts.

15

The synthesis of stilbene derivatives or combretastatins, which can be in the form of a pharmaceutical acceptable salt, and the pharmaceutical compositions which comprise them are disclosed in Patents US 4 996 237, US 5 525 632, US 5 731 353 and US 5 674 906. These patents disclose combretastatins and their metabolites and disclose their in vitro oncologic activity.

20

According to these patents, combretastatins are prepared from (3,4,5-trimethoxybenzyl)triphenyl-phosphonium salts, which is condensed with a 3-nitro-

25

or 3-hydroxy-4-methoxybenzaldehyde (the hydroxyl group of which is protected) in the presence of sodium hydride or of lithium derivatives, and then the derivative obtained, when it is nitrated, is reduced in
5 the presence of zinc.

The isomer with the cis configuration is subsequently prepared by the action of light or by chromatographic separation of the mixture.

The present invention relates to novel
10 processes for the preparation of combretastatins or of their derivatives and to improvements to the existing processes.

A first process route V0 1 for the preparation of derivatives of formula (I) for which A
15 represents an amino group has first been discovered, which process is an improvement to the process disclosed in the abovementioned patents, which consists, after the Wittig condensation in the presence of (3,4,5-trimethoxybenzyl)triphenylphosphonium bromide
20 or chloride and of 3-nitro-4-methoxybenzaldehyde, in carrying out the reduction in the presence of iron, instead of the zinc used in the prior publications, which makes it possible to achieve an overall reaction yield, with respect to the aldehyde charged, of 60%
25 (the yield with respect to the aldehyde charged in Patent US 5 525 632 is between 21% to 33%).

The **first process route V0 2** consists in condensing 3,4,5-trimethoxybenzaldehyde with (4-methoxy-3-nitrobenzyl)triphenylphosphonium bromide

or chloride. For both these first two processes routes V0 1 and V0 2, the reaction is carried out in the presence of a base chosen in particular from potassium tert-butoxide, sodium tert-pentoxide, sodium
5 hydride, butyllithium, LDA (lithium diisopropylamine), sodium methoxide, potassium carbonate or alkaline derivatives of hexamethyldisilanes.

This reaction is carried out in various solvents, such as ethers (THF), polar aprotic solvents
10 (acetonitrile, NMP, DMF, DMSO, and the like), alcohols, aromatic solvents or water, at a temperature which will be adjusted by a person skilled in the art to the base used and to the solvent used.

This reaction, as regards the first process
15 route V0 2, is described in particular in the publication by K.G. Piney which appeared in Bioorg. Med. Chem., 8(2000), 2417-2425.

2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)-vinyl]nitrophenyl is reduced according to the improved
20 process of the invention by the action of iron. It is advantageous to use an amount of iron in excess if complete conversion of the starting material is desired. This excess is advantageously greater than 2 equivalents per one mol of starting nitro derivative.

25 It has been shown that the same stage, carried out in the presence of zinc in acetic acid, a conventional solvent for reductions with zinc, does not make it possible to obtain complete reaction (in Patent US 5 525 632, the yield of the reduction carried out on

the pure Z isomer varies between 46 and 66%) and, moreover, the amounts of zinc used are large and consequently result in considerable industrial waste. Furthermore, the process generates a large amount of
5 "azo" compound resulting from coupling between the amino formed and the nitroso intermediate in the reduction.

Reduction with nascent hydrogen, generated by ammonium formate in the presence of a conventional
10 catalyst, such as palladium or platinum, leads to high isomerization of the double bond to the undesirable E isomer and to partial saturation of the double bond.

The abovementioned Piney publication describes the reduction by sodium hydrosulphite of a
15 pure nitro Z isomer, obtained after chromatography and recrystallization, leading to an amino Z isomer with a yield of only 37%.

Hydrogenations with molecular hydrogen, catalysed by platinum or palladium, are rarely complete
20 and result in particular in the saturation of the ethylenic double bond.

A **second process** has also been found which avoids the intermediate reduction stage necessary when starting from a nitro derivative. This is because it is
25 much more economical to condense, according to a first method of carrying out this second process, a (3,4,5-trimethoxybenzyl)triphenylphosphonium bromide or chloride with a 3-amino-4-methoxybenzaldehyde or, according to a second method of carrying out this

second process, 3,4,5-trimethoxybenzaldehyde with a (3-amino-4-methoxybenzyl)triphenylphosphonium salt.

This second process according to its two alternative forms requires a stage in which less in the way of CMR (Cancerogenic, Mutagenic or Reproductive toxic) products are given off in comparison with the first processes routes V0 1 and V0 2, which is a considerable advantage at the industrial level from the viewpoint of safety and production cost.

10 According to **the second process route V0 3** for implementing the invention, the (3,4,5-trimethoxybenzyl)triphenylphosphonium salt and 3-amino-4-methoxybenzaldehyde are brought together and the reaction is carried out, preferably, in the
15 presence of a base chosen in particular from potassium tert-butoxide, sodium tert-pentoxide, sodium hydride, butyllithium, LDA, sodium methoxide, potassium carbonate or alkaline derivatives of
20 hexamethyldisilanes. Use is preferably made of sodium methoxide.

This reaction is carried out in various solvents, such as ethers (THF), polar aprotic solvents (acetonitrile, NMP, DMF, DMSO, and the like), alcohols, aromatic solvents or water, at a temperature which will
25 be adjusted by a person skilled in the art to the base used and to the solvent used.

The reaction temperature will be adjusted by a person skilled in the art to the base used. When methoxide is used, it is preferably between 0 and 10°C.

After reaction, the base used is neutralized with an acid in aqueous solution, the organic phase is washed and concentrated, and the expected product is obtained after chromatographing the crude concentrate.

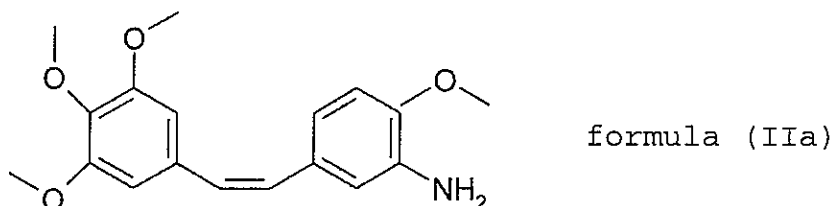
5 **According to the second process route V0 4**
for implementing the invention, in which the
(3-amino-4-methoxybenzyl)triphenylphosphonium salt and
3,4,5-trimethoxybenzaldehyde are brought together, the
reaction is preferably carried out in the presence of
10 an organic base chosen in particular from potassium
tert-butoxide, sodium tert-pentoxide, sodium hydride,
butyllithium, LDA, sodium methoxide, potassium
carbonate or alkaline derivatives of
hexamethyldisilanes. Use is preferably made of sodium
15 methoxide.

This reaction is carried out in various
solvents, such as ethers (THF), polar aprotic solvents
(acetonitrile, NMP, DMF, DMSO, and the like), alcohols,
aromatic solvents or water, at a temperature which will
20 be adjusted by a person skilled in the art to the base
used and to the solvent used.

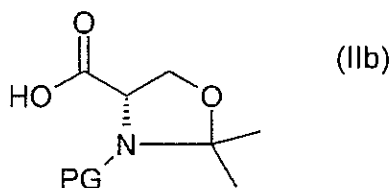
The reaction temperature will be adjusted by
a person skilled in the art to the base used. When
methoxide is used, it is preferably between 0 and 10°C.
25 After reaction, the base used is neutralized with an
acid in aqueous solution, the organic phase is washed
and concentrated, and the expected product is obtained
after chromatographing the crude concentrate.

The derivative obtained according to the

second process route V0 3 or V0 4 or during the second stage of the first process route V0 1 or V0 2 has the following formula:

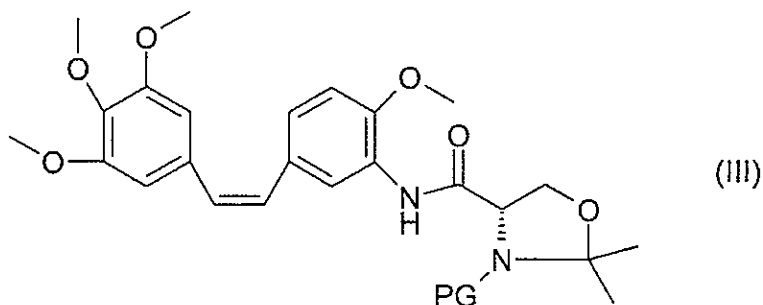


5 It is advantageous, when it is desired to couple serine with the compound of formula (IIa), to use L-serine doubly protected on the nitrogen of the serine and on the hydroxyl functional group of general formula (IIb)



10

where PG represents a protective group for the amine functional group known to any person skilled in the art, to give a novel intermediate of following general formula:



15

which is subsequently cleaved, preferably simultaneously with the opening of the ring, by acid hydrolysis according to a deprotection reaction known to any person skilled in the art. Preferably, the PG

group of the formulae (IIb) or (III) represents a protective group chosen from the following groups: tert-butoxycarbonyl, benzyloxycarbonyl (CBZ) or 9-fluorenylmethyloxycarbonyl (Fmoc).

5 The compound of formula (III) above is novel and is claimed as such.

 The condensation is advantageously carried out in the presence of EDCI (1-(3-dimethylaminopropyl)-3-ethylcarbodiimide chloride) or in the presence of DCC
10 (dicyclohexylcarbodiimide) and of HOBT (hydroxybenzotriazole) or in the presence of DCC (dicyclohexylcarbodiimide) and of HOSU (N-hydroxysuccinimide) or, finally, in the presence of TOTU (O-[(ethoxycarbonyl)cyanomethyleneamino]-
15 N,N,N',N'-tetramethyluronium tetrafluoroborate) or of HBTU (O-benzotriazol-1-yl-N,N,N',N'-tetramethyluronium hexafluorophosphate) or of N,N-carbonyldiimidazole. The reaction is preferably carried out in a solvent which is inert with respect to the reaction, which solvent is
20 chosen in particular from polar aprotic solvents, such as acetonitrile, dimethylformamide, tetrahydrofuran or chlorinated aliphatic solvents, such as dichloromethane, or, finally, esters.

 The coupling to the derivative of
25 formula (IIa) can also certainly be carried out by the action of a mixed anhydride, synthesized in situ between a chloroformate or a carboxylic acid chloride, for example pivaloyl chloride, and doubly protected L-serine of formula (IIb), in the presence of a

tertiary base of the NMM (N-methylmorpholine) type in various solvents which are inert with respect to the reaction, esters, ethers, chlorinated solvents, acetonitrile, and the like. The mixed anhydride is
5 preferably prepared at a temperature of between 0 and 10°C and then the reaction is carried out at ambient temperature. After reaction, the reaction mixture is hydrolysed with an aqueous solution, then the mixture is separated by settling and the organic phase obtained
10 is washed with a hydroxylated base.

The double deprotection of the compound of formula (III) is carried out by the action of an organic or inorganic acid. Use is preferably made of concentrated aqueous hydrochloric acid in an alcoholic
15 medium. The reaction temperature is, according to a better means of implementation of the invention, between 50 and 70°C.

The invention will be more fully described with the help of the following examples, which must not
20 be regarded as limiting the invention.

The composition of the mixtures, the monitoring and the progression of the reactions, and the yield of the unisolated products/intermediates and their assays are determined by HPLC (High Performance
25 Liquid Chromatography) analysis.

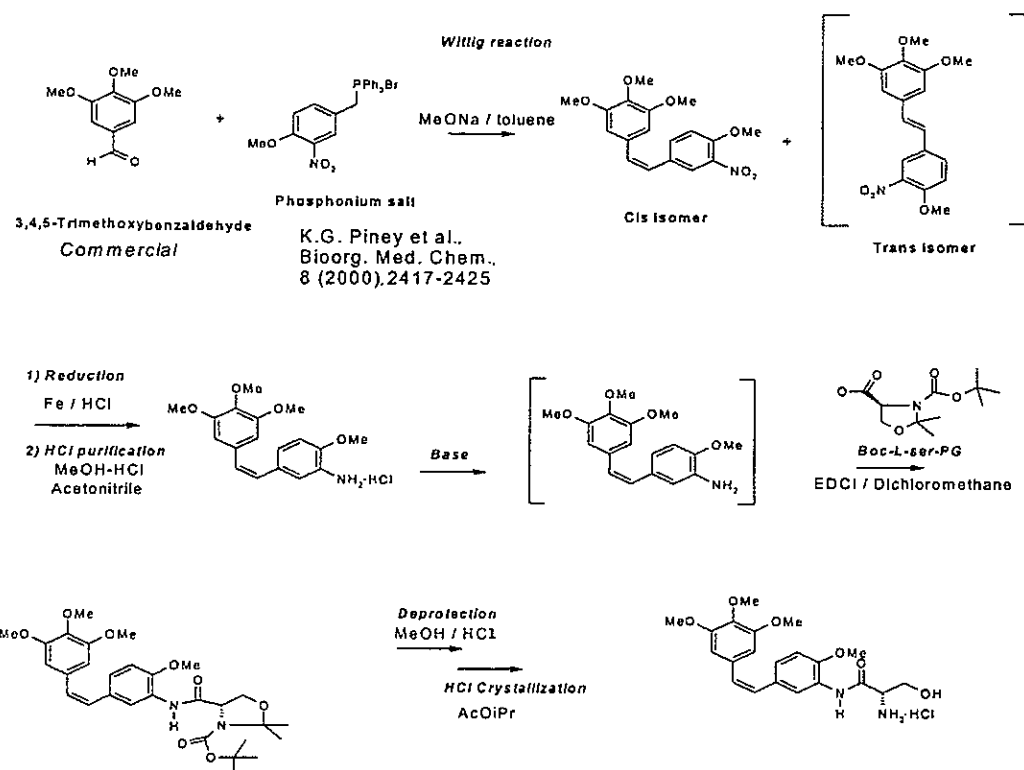
Example 1 - First process route V0 2 according to the invention

(Z)-N-[2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-

phenyl]-L-serinamide hydrochloride

General scheme of the synthesis

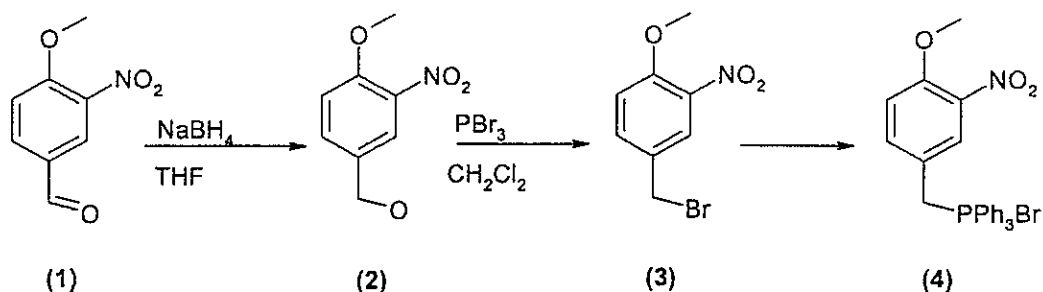
The novel "inverse Wittig" process, starting from (4-methoxy-3-nitrobenzyl)triphenylphosphonium bromide and 3,4,5-trimethoxybenzaldehyde, makes it possible to obtain the mixture of Z and E isomers of 2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-nitrophenyl with a Z/E ratio of 75/25.



This ratio is sufficiently high in the Z nitro isomer to be able to use the Z/E mixture directly in the reduction and to obtain, by crystallization of the hydrochloride, the Z amino isomer with an HPLC assay of 97% IS (internal standardization).

(4-Methoxy-3-nitrobenzyl)triphenylphosphonium bromide (4) is prepared according to the following

example:



3-Nitro-4-methoxybenzyl alcohol (2):

90.5 g of 3-nitro-4-methoxybenzaldehyde (1)

5 are charged, followed by 450 ml of THF and 90 ml of ethanol, to a 2-litre three-necked flask equipped with a mechanical stirrer, a thermometer, a T piece, a funnel for the addition of solid and a reflux condenser surmounted by a bubble counter. The pale-yellow

10 solution obtained is cooled to 10°C and then 10 g of sodium borohydride are charged over 40 minutes at 10/15°C (the reaction is very exothermic and the temperature has to be maintained with an ice/acetone bath); at the end of the addition, the brown solution

15 turns navy blue. The solution is stirred for 30 minutes at 10°C, the end of the reaction is monitored by TLC (thin layer chromatography), the solution is stirred for a further 1 hour at 10°C and then the temperature is allowed to return to ambient temperature.

20 The addition funnel is replaced by a 500 ml pressure-equalizing dropping funnel, via which 300 ml of distilled water are run in dropwise over 30 minutes while maintaining the mixture at 20°C. Gas evolution is observed when the water begins to be run in.

25 The mixture is concentrated to 2/3 on a

rotary evaporator (50°C/20 mmHg) and a white product crystallizes in the aqueous concentrate in the form of lumps.

The cooled aqueous phase is extracted with
5 250 ml and then 150 ml of dichloromethane, and the combined organic phases are washed with 250 ml of distilled water and then dried over magnesium sulphate.

After filtration, the dichloromethylene
solution is used as is in the following bromination
10 reaction.

The yield of this stage is regarded as being 100%.

N.B.: The alcohol (2) is commercially available but very difficult to get hold of.

15 3-Nitro-4-methoxybromobenzyl (3):

The dichloromethylene solution of 3-nitro-4-methoxybenzyl alcohol (2) is charged to a 1 litre three-necked flask equipped with a mechanical stirrer, a thermometer, a T piece, a dropping funnel and a
20 reflux condenser surmounted by a bubble counter, and 100 ml of dichloromethane are added. The stirred solution is cooled to 5°C and then 135.4 g of phosphorus tribromide are run in dropwise while maintaining the temperature at 5°C.

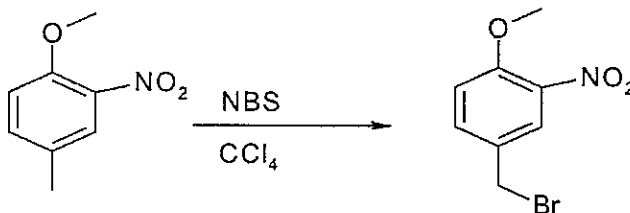
25 The solution is stirred at 5°C for 1 hour 30 minutes, the end of the reaction is monitored by TLC and then 250 ml of saturated sodium hydrogencarbonate solution are run in dropwise while maintaining the temperature at 15°C. Very strong evolution of gas takes

place with a slight delay as the phosphorus tribromide is run in.

The medium, separated by settling in a separating funnel, is washed successively with 250 ml of distilled water and 200 ml of saturated sodium hydrogencarbonate solution. The organic phase is dried over magnesium sulphate, filtered and concentrated on a rotary evaporator (50°C/20 mmHg).

119 g of solid in the form of green-yellow felt-like needles are obtained with a chemical yield over two stages of 97%.

N.B.: This product (3) can also be prepared according to the following scheme, described in the publication: K.G. Piney et al., Bioorg. Med. Chem., 8 (2000), 2417-2425.



(3-Nitro-4-methoxybenzyl)triphenylphosphonium bromide (4):

119 g of 3-nitro-4-methoxybromobenzyl (3) are charged to a stirred heel of 1 000 ml of toluene in a 2 litre three-necked flask equipped with a mechanical stirrer, a thermometer, a T piece, a funnel for the addition of solid and a reflux condenser surmounted by a bubble counter, and the mixture, warmed to 25°C, passes into solution. 126.5 g of triphenylphosphine are then added and the solution obtained is gradually

heated to 60°C; a precipitate is formed from 30°C. The mixture is maintained at 60/65°C for 4 hours, is then cooled to 30°C and is filtered through a sintered glass filter. The filter residue is washed on the filter with
 5 2 times 300 ml of toluene, pulled dry and dried in an oven (35°C/20 mmHg/20 hours).

217 g of (4-methoxy-3-nitrobenzyl)triphenylphosphonium bromide are obtained with a chemical yield of 88%.

10 Synthesis described in the publication:

(solvent used: dichloromethane)

K.G. Piney et al., Bioorg. Med. Chem., 8(2000), 2417-2425.

Spectrum No. = 4 865-V

15 ^1H N.M.R. spectrum (300 MHz, $\text{d}_6\text{-(CD}_3)_2\text{SO}$, δ in ppm): 3.90 (s, 3H), 5.26 (d, $J = 15$ Hz, 2H), 7.33 (mt, 2H), 7.41 (mt, 1H), from 7.65 to 8.05 (mt, 15H).

Mass spectrum No. 212217, $m = 428$

EI $m/z = 262$ $[\text{PPh}_3]^+$ base peak

20 DCI $m/z = 445$ MNH_3^+

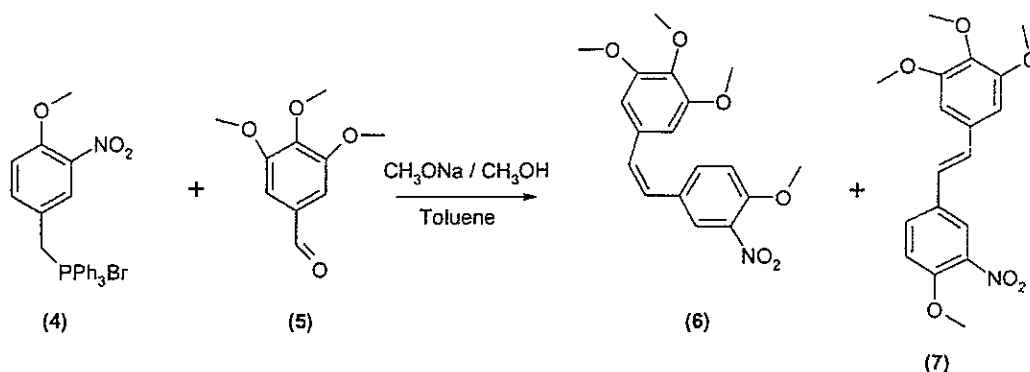
$m/z = 428$ M^+

$m/z = 263$ $[\text{PPh}_3\text{H}]^+$ base peak

IR spectrum 426469 KBr

2869, 2843, 2776, 1619, 1527, 1438, 1362, 1287, 1270,
 25 1111, 752, 692 and 502 cm^{-1}

Z and E mixture of 2-methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]nitrophenyl (6) and (7):



54.7 g of 3,4,5-trimethoxybenzaldehyde (5),
 148.6 g of (4-methoxy-3-nitrobenzyl)triphenyl-
 phosphonium bromide (4) and 1 300 ml of toluene are
 5 charged, at 20°C and under nitrogen, to a 2 litre
 three-necked flask equipped with a mechanical stirrer,
 a thermometer, a T piece, a dropping funnel and a
 reflux condenser surmounted by a bubble counter. The
 stirred suspension is cooled to 5°C using an ice bath
 10 and then 63.2 g of a 25% w/w solution of sodium
 methoxide in methanol are run in at 5°C over
 40 minutes.

As the addition progresses, the suspension
 changes from off-white to yellow and then to brown.

15 The mixture is stirred for 1 hour at 5°C and
 the end of the reaction is monitored by HPLC (complete
 consumption of the aldehyde). 3 g (0.05 mol) of acetic
 acid are then added.

The suspension is heated to 40°C and is
 20 maintained at 40°C for 30 minutes. At this temperature,
 only the salts remain insoluble. The mixture is
 filtered at 40°C through a sintered glass filter No. 3
 and the salts are washed on the filter with 3 times
 100 ml of toluene.

The filtrate is returned to a round-bottomed flask with 250 ml of distilled water, and the two-phase mixture is stirred for 20 minutes at 40°C and then separated by settling in a separating funnel. The
5 toluene phase is washed again with 2 times 250 ml of distilled water and then concentrated to dryness on a rotary evaporator.

The residue is taken up in 600 ml of isopropanol and 12 ml of toluene at 40°C, the expected
10 product begins to crystallize and the temperature is allowed to return to ambient temperature overnight with slow stirring.

The stirred suspension is cooled to and maintained for 1 hour at 5°C and then filtered through
15 a sintered glass filter, and the cake is washed with 2 times 125 ml of isopropanol, pulled dry and dried in an oven under vacuum (35°C/30 mmHg/18 hours).

91.7 g of a mixture of Z and E isomers (6) and (7) are obtained with a Z/E ratio of 75/25
20 (IS HPLC) and a yield of 95%.

Synthesis described in publication: (solvent used: dichloromethane: base used: NaH)
K.G. Piney et al., Bioorg. Med. Chem., 8(2000), 2417-2425.

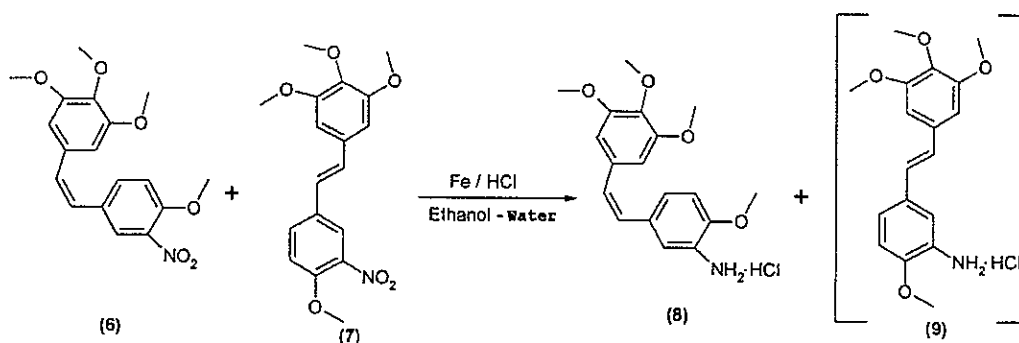
25 N.B.: Numerous operating conditions were experimented with, such as:

Solvents: THF, acetonitrile, methanol and other alcohols, dichloromethane, NMP, DMF, DMSO, and the like.

Bases: potassium t-butoxide, sodium t-pentoxide, sodium hydroxide, NaH, BuLi/LDA, potassium carbonate and the like.

Temperatures: from -10°C to the reflux temperatures of
5 some solvents

Z-2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-
phenylamine hydrochloride (8):



80 g of 75/25 Z and E mixture of
10 2-methoxy-[2-(3,4,5-trimethoxyphenyl)vinyl]nitrophenyl
(6) and (7), 640 ml of absolute ethanol and 160 ml of
distilled water are charged, at 20°C and under
nitrogen, to a 2 litre three-necked flask equipped with
a mechanical stirrer, a thermometer, a T piece, a
15 funnel for the addition of solid, a reflux condenser
surmounted by a bubble counter, and a heating bath.

The mixture is rapidly stirred and heated on
an oil bath, 7.8 ml of 6N hydrochloric acid are added
to the suspension at 50°C and then the temperature of
20 the mixture is raised to $77 \pm 2^\circ\text{C}$. The mixture is
virtually soluble.

52 g of iron powder are added portionwise
over 5 minutes. With the first addition, the mixture
passes into solution and then a blackish deposit is

formed on the walls of the round-bottomed flask.

The mixture is maintained at $77 \pm 2^\circ\text{C}$ for 2 hours and the disappearance of the starting nitro compounds (6) and (7) is monitored by HPLC.

5 The mixture is allowed to cool to 40°C and is filtered through a sintered glass filter covered with clarcel and the cake is rinsed with 2 times 160 ml of 80/20 ethanol/water mixture.

 The filtrate, aqueous mother liquors and
10 aqueous wash liquors, is concentrated on a rotary evaporator. As soon as the azeotrope has been driven off, an oil begins to precipitate in the residual aqueous phase.

 This aqueous phase is extracted in a
15 separating funnel with 2 times 300 ml of dichloromethane and then the organic phase is washed with 2 times 300 ml of half-saturated aqueous sodium chloride solution and with 300 ml of distilled water.

 The organic phase is concentrated to dryness
20 on a rotary evaporator and 76 g of an oil are obtained, which oil exhibits a Z/E ratio of 80/20 by HPLC. This oil is dissolved in 591 ml of methanol and transferred into a stirred 1 litre round-bottomed flask, 100 ml of 2.32N methanolic hydrochloric acid are then added,
25 precipitation is initiated and the mixture is allowed to precipitate overnight with stirring.

 The amount of methanol + methanolic hydrochloric acid is such that the final concentration of Z isomer (determined by HPLC) is equal to 8.8% w/v.

In the morning, the mixture is filtered through a sintered glass filter. The dried cake weighs 8.2 g and is composed only of E isomer (HPLC).

The filtrate (693 g), ratio Z/E = 86/14
5 (IS HPLC), is concentrated to half on a rotary evaporator, 400 ml of acetonitrile are added to the 347 g of concentrate and the mixture is reconcentrated until a concentrate of 347 g is again obtained. 1 000 ml of acetonitrile are then added and the mixture
10 is concentrated until crystallization begins. The concentrate is then transferred into a stirred 4 litre round-bottomed flask containing 1 500 ml of acetonitrile at 60°C. The mixture copiously precipitates.

15 The mixture is kept stirred at 60°C for 2.5 hours and is allowed to cool to 30°C over approximately 1 hour. The slurry is filtered through a sintered glass filter (the E isomer (9) is soluble in the filtrate). The cake is washed with 2 times 200 ml
20 of acetonitrile and dried in an oven (35°C/30 mmHg/ 18 hours).

45.7 g of Z-2-methoxy-5-[2-(3,4,5-trimethoxy-phenyl)vinyl]phenylamine (8) are obtained with an
IS HPLC assay of 97% and a yield as such of 56%, i.e. a
25 yield of Z isomer obtained with respect to Z isomer charged of 72%.

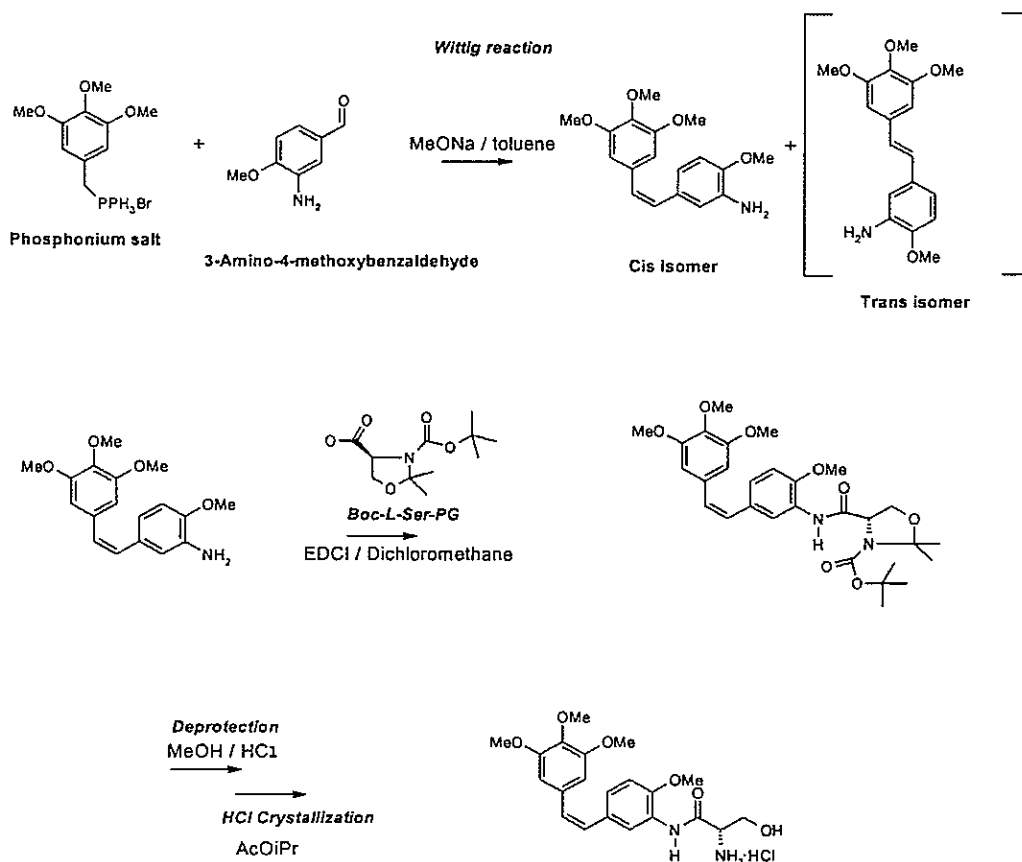
EXAMPLE 2 - Synthesis according to the second process route V0 3 according to the invention

The advantage of the second process route

V0 3 with respect to the first "inverse Wittig" process route V0 2 is that of carrying out the Wittig reaction between a product which has already been reduced, the aminoaldehyde (**1a**), and the phosphonium (**2a**) and thus
5 of eliminating a chemical stage which gives off CMR products.

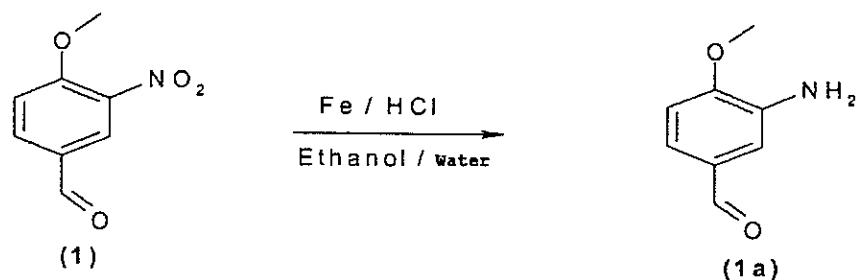
(Z)-N-[2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-phenyl]-L-serinamide hydrochloride

General scheme of the synthesis



10

3-Amino-4-methoxybenzaldehyde (**1a**):



20 g of 3-nitro-4-methoxybenzaldehyde (1) and 350 ml of absolute ethanol are charged to a 2 litre three-necked flask rendered inert with argon and equipped with a mechanical stirrer, a thermometer, a T piece, a funnel for the addition of solid, a reflux condenser surmounted by a bubble counter, and a heating bath, and the mixture is stirred and heated to 60°C. The mixture passes into solution.

The dropwise addition is carried out at 60°C of 115 ml of distilled water, followed by 14 ml of 2N hydrochloric acid. 24.7 g of iron powder are then introduced portionwise.

The temperature of the mixture is allowed to return to ambient temperature over 2 hours. The reaction is complete (TLC).

The mixture is filtered through celite and concentrated under vacuum, the residue is taken up in dichloromethane and the organic solution is washed twice with distilled water and then dried over magnesium sulphate, filtered and concentrated to dryness under vacuum.

16 g of crude (1a) are obtained and are chromatographed on a silica column eluted with dichloromethane.

2 fractions comprising the clean expected product are obtained, which fractions, after concentrating, give 11.5 g of pure (1a), i.e. a yield of 69%.

- 5 ^1H N.M.R. spectrum No. 2810-V (300 MHz, $\text{d}_6\text{-(CD}_3)_2\text{SO}$, δ in ppm): 3.88 (s, 3H), 5.11 (unresolved peak, 2H), 7.01 (d, $J = 8$ Hz, 1H), 7.14 (d, $J = 2$ Hz, 1H), 7.18 (d, $J = 8$ Hz, 1H), 9.53 (s, 1H).

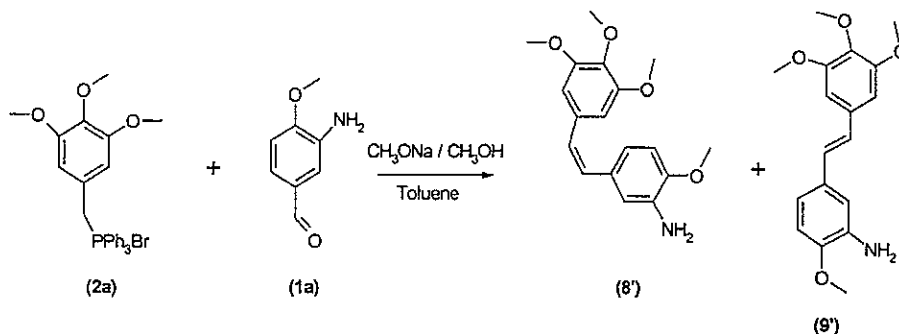
Mass spectrum No. 210112, $m = 151$

- 10 EI $m/z = 151$ M^+ base peak
 $m/z = 136$ $[\text{M}-\text{CH}_3]^+$
 $m/z = 108$ $[\text{136}-\text{CO}]^+$
 $m/z = 80$ $[\text{108}-\text{CO}]^+$

IR spectrum: 425135 KBr

- 15 3464, 3437, 3367, 3349, 1675, 1655, 1582, 1513, 1293, 1241, 1139, 1023, 803 and 640 cm^{-1}

Z- and E-2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-phenylamine (8') and (9'):



- 20 N.B.: The phosphonium (2a) is a starting material already disclosed in the original patent Ajinomoto Co. Ltd, US 5 525 632 and WO 01/12579 A2.

8.0 g of phosphonium salt (2a), followed by 2.20 g of aminobenzaldehyde (1a) and 100 ml of toluene,

are charged to a 250 ml three-necked flask rendered inert with nitrogen and equipped with a magnetic stirrer, a thermometer, a T piece, a dropping funnel, a reflux condenser surmounted by a bubble counter, and a cooling bath. The stirred suspension is cooled to 5°C and 3.51 ml of a 25% w/w methanolic sodium methoxide solution are run in over 15 minutes. After 2.5 hours at 5°C, the reaction remains incomplete (DC: 45%) but does not change further (HPLC) and the Z/E ratio is 61/39. 0.2 ml of acetic acid diluted in 50 ml of water is then run in, the temperature rises to 13°C, the mixture is stirred for 30 minutes and then it is separated by settling in a separating funnel. The organic phase is concentrated under vacuum on a rotary evaporator and 8 g of yellow oil are obtained.

By HPLC, this oil comprises starting aldehyde, phosphine oxide and the expected Z/E mixture with a ratio of 61/39.

The oil is chromatographed on a silica column (40 parts w/w) eluted with a cyclohexane/ethyl acetate/triethylamine (50/50/2) mixture.

2 series of combined fractions are concentrated to dryness: the first dry extract of 360 mg comprises 93% of the Z isomer + unidentified impurities; the second, of 2.09 g, comprises starting aldehyde and a Z/E mixture representing 39 and 37.5% by IS HPLC.

The balance by weight of Z isomer (**8'**), determined by IS HPLC, is 1.15 g with respect to 2.20 g

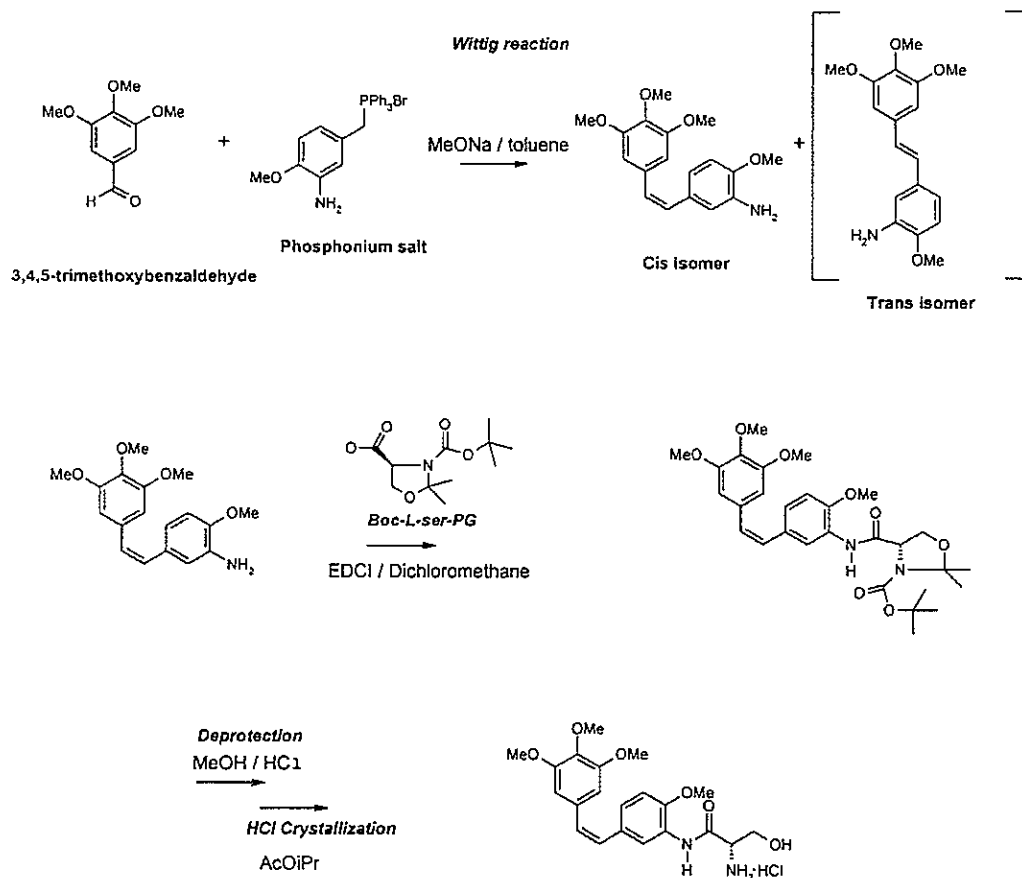
of aldehyde charge, i.e. a yield of 24%.

EXAMPLE 3 - Synthesis according to the second process
route V0 3 according to the invention

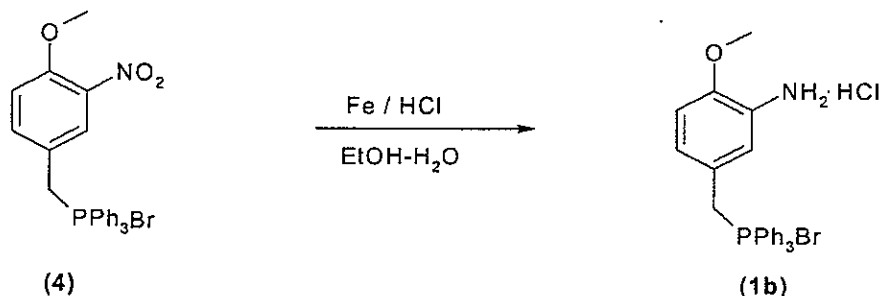
As for route V0 2, the advantage of route
5 V0 3 with respect to the first "inverse Wittig" process
route V0 2 is that of carrying out the Wittig reaction
between a product which has already been reduced,
(3-amino-4-methoxybenzyl)triphenylphosphonium bromide
(**1b**), and 3,4,5-trimethoxybenzaldehyde (**5**) and thus of
10 eliminating a chemical stage which gives off CMR
products.

(Z)-N-[2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-
phenyl]-L-serinamide hydrochloride

General scheme of the synthesis



(4-Methoxy-3-aminobenzyl)triphenylphosphonium bromide
(1b)



30 g of (4), 240 ml of ethanol and 60 ml of
5 distilled water are charged to a 1 litre three-necked
flask equipped with a mechanical stirrer, a
thermometer, a T piece, a funnel for the addition of
solid, a reflux condenser surmounted by a bubble
counter, and a heating bath.

10 1.76 ml of 6N hydrochloric acid are added to
the stirred suspension, which is heated to 70°C.

9.9 g of iron powder are then added
portionwise over 15 minutes; the mixture remains
insoluble. The mixture is maintained at 75°C for
15 2 hours; the organic materials slowly pass into
solution while a brownish deposit of iron and of iron
oxide is formed.

After monitoring by HPLC, 5% of starting
material still remains; 2 g of iron are again added and
20 heating is continued for 1 hour; the DC is complete.

The mixture is cooled to 40°C and filtered
through clarcel, the filter residue is rinsed with
100 ml of ethanol comprising 20% water, and the
filtrate is concentrated to dryness under vacuum on a
25 rotary evaporator.

The residue is taken up in 300 ml of isopropanol and crystallizes from the mixture, which is stirred and heated to 50°C and passes back into solution. 14 ml of a 5N solution of hydrochloric acid in isopropanol are then run in, the mixture precipitates, it is held at 50°C for 1 hour and then it is allowed to return to ambient temperature.

The slurry is filtered through a sintered glass filter and the cake is washed with 50 ml of isopropanol, pulled dry thoroughly and dried in an oven under vacuum.

27.3 g of (**1b**) are obtained with a yield as such of 89.9%.

¹H N.M.R. spectrum No. 4584-V (300 MHz, d₆-(CD₃)₂SO, δ in ppm): 3.78 (s, 3H), 5.03 (broad d, J = 15 Hz, 2H), 6.43 (unresolved peak, 1H), 6.62 (broad s, 1H), 6.82 (broad d, J = 8 Hz, 1H), from 7.60 to 8.00 (mt, 15H).

Mass spectrum No. 211915, m = 397

EI m/z = 397 M⁺

m/z = 382 [M-CH₃]⁺

m/z = 262 [PPh₃]⁺ base peak

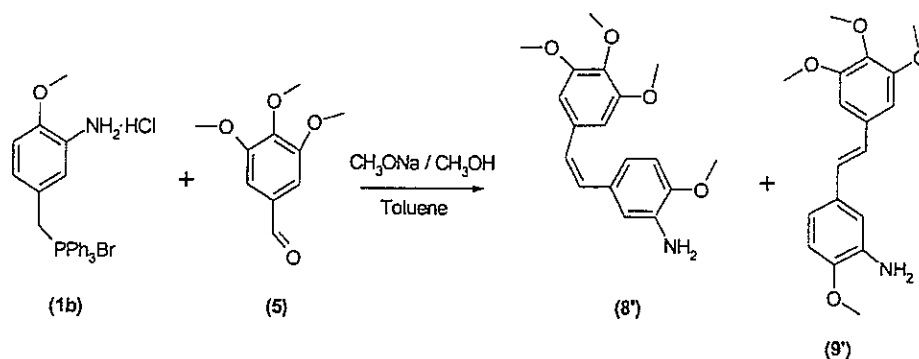
DCI m/z = 398 MNH₄⁺

M/z = 263 [PPh₃H]⁺ base peak

IR spectrum 426386 KBr

3254, 2474, 1920, 1628, 1520, 1439, 1433, 1279, 1110, 736, 690, 527 and 511 cm⁻¹

Z- and E-2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-phenylamine (**8'**) and (**9'**):



11.02 g of (1b), 4 g of (5) and 70 ml of toluene are charged to a 250 ml three-necked flask rendered inert with nitrogen and equipped with a magnetic stirrer, a thermometer, a T piece, a dropping funnel, a reflux condenser surmounted by a bubble counter, and a cooling bath.

The stirred suspension is cooled to 5°C and 4.92 ml of a 25% w/w solution of sodium methoxide in methanol are run in over 15 minutes. The suspension is stirred at 5°C for 2.5 hours, then 0.2 ml of acetic acid diluted in 50 ml of water is then run in, the temperature rises to 14°C and the mixture becomes very thick. It is diluted with 10 ml of toluene and 10 ml of water. A brown insoluble material remains.

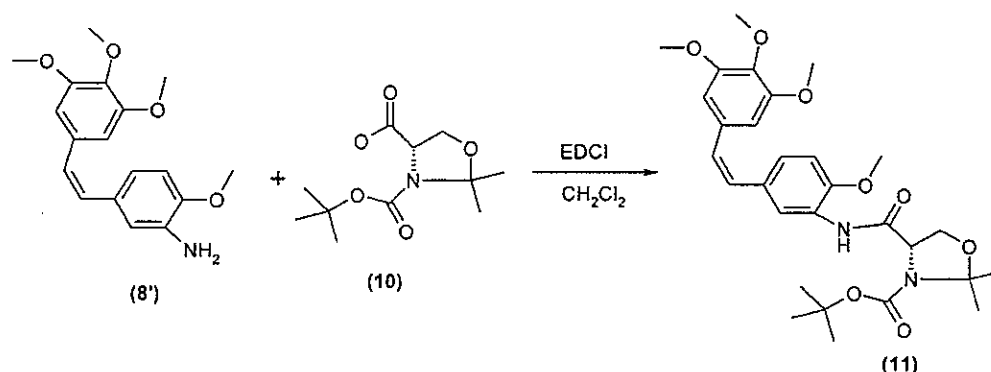
The mixture is filtered through clarcel, the cake is washed with 3 times 50 ml of toluene (the wash liquors comprise virtually only the starting aldehyde and are not added to the two-phase filtrate), the clear filtrate (pH 12) is separated by settling in a separating funnel, and the organic phase is concentrated to dryness under vacuum at 40°C. The Z/E ratio, determined by IS HPLC, is 43/57.

The brown oil obtained (4 g) is

chromatographed on a silica column (100 parts w/w) eluted with a cyclohexane/ethyl acetate/triethylamine (50/50/2) mixture.

Two series of combined fractions are concentrated to dryness: the first dry extract of 1.1 g comprises 14% of E isomer and 59% of Z isomer; the second weighs 1.08 g and comprises 86% of E isomer and 7% of Z isomer.

The balance by weight of Z isomer (**8'**), determined by IS HPLC, is 0.725 g with respect to 4 g of aldehyde charged, i.e. a yield as such of 11.3%. Z-4-{2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-phenylcarbamoyl}-2,2-dimethyloxazolidine-3-carboxylic acid tert-butyl ester (**11**):



15

Release of the base (**8'**) from the hydrochloride (**8**):

44 g of (**8**), 16 g of sodium hydrogencarbonate and then 200 ml of distilled water and 375 ml of dichloromethane are charged to a 1 litre Erlenmeyer flask. The mixture is stirred for 20 minutes at ambient temperature and two clear phases are obtained.

The organic phase is separated by settling, dried over sodium sulphate and then filtered.

Approximately 400 ml of a dichloromethane

solution comprising (8') are obtained.

Preparation of 2,2-dimethyloxazolidine-3,4-dicarboxylic acid 3-tert-butyl ester (10)

Although commercially available, this product
 5 is very difficult to get hold of. It has therefore been prepared by saponification with lithium hydroxide of its methyl ester according to: J. Org. Chem., 63(12), p. 3983 (1998).

¹H N.M.R. spectrum (300 MHz, d₆-(CD₃)₂SO, δ in ppm): 1.38
 10 (s, 3H), 1.45 (s, 9H), 1.55 (s, 3H), 3.95 (mt, 1H), 4.16 (mt, 1H), 4.31 (mt, 1H), from 12.50 to 13.10 (broad unresolved peak, 1H).

Mass spectrum: No. 213135, m = 245

DCI m/z = 263 MNH₄⁺
 15 m/z = 246 MH⁺
 m/z = 207 [MNH₄-t-Bu]⁺ base peak
 m/z = 146 [MH-BOC]⁺

IR spectrum: 426759 KBr

1744, 1704, 1638, 1407, 1368, 1164, 1104, 856, 836 and
 20 623 cm⁻¹

Coupling:

The solution of (8') is charged to a 2 litre three-necked flask equipped with a mechanical stirrer, a thermometer, a T piece, a funnel for the addition of
 25 solid, a reflux condenser surmounted by a bubble counter, and an ice bath, 600 ml of dichloromethane are added and the mixture is cooled with stirring.

42.9 g of 2,2-dimethyloxazolidine-3,4-dicarboxylic acid 3-tert-butyl ester (10) are added

at 5°C and pass into solution, and then 48 g of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride (EDCl) are added portionwise between 5 and 10°C.

5 The mixture is slowly allowed to return to ambient temperature by allowing the ice in the bath to melt overnight.

 In the morning, 330 ml of distilled water are added and the mixture is vigorously stirred. The
10 mixture turns cloudy over 30 minutes (hydrolysis of the EDCl). Stirring is maintained for a further 30 minutes.

 The mixture is separated by settling in a separating funnel and the organic phase is washed successively with 2 times 280 ml of 0.55N sodium
15 hydroxide solution and then with 300 ml of distilled water.

 The organic phase is concentrated to dryness on a rotary evaporator (50°C/50 mmHg).

 79.4 g of a sticky oil (**11**) are obtained,
20 which oil hardens at 20°C, with a yield by weight with respect to (**8**) charged of 117%.

Spectrum No. = 5 578-V

¹H N.M.R. spectrum (400 MHz, d₆-(CD₃)₂SO, at a temperature of 373 K, δ in ppm): 1.41 (s, 9H), 1.53 (s,
25 3H), 1.64 (s, 3H), 3.64 (s, 6H), 3.71 (s, 3H), 3.86 (s, 3H), 3.99 (dd, J = 9 and 3 Hz, 1H), 4.19 (dd, J = 9 and 7 Hz, 1H), 4.52 (dd, J = 7 and 3 Hz, 1H), 6.48 (d, J = 12.5 Hz, 1H), 6.55 (d, J = 12.5 Hz, 1H), 6.58 (s, 2H), 7.02 (mt, 2H), 8.13 (broad s, 1H), 8.82 (broad s, 1H).

Mass spectrum No. 213565, $m = 542$

DCI $m/z = 560$ MNH_4^+ base peak

$m/z = 543$ MH^+

$m/z = 504$ $[MNH_4-t-Bu]^+$

5 $m/z = 443$ $[MH-BOC]^+$

IR spectrum: 425857 CCl_4

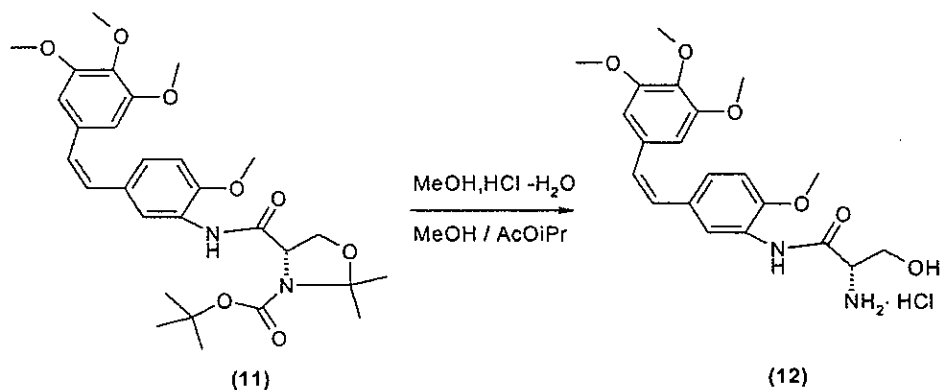
3409, 2982, 2938, 2837, 1712, 1698, 1534, 1363, 1249,
1133, 1092 and 851 cm^{-1}

Other coupling conditions were employed, such
10 as:

- Mixed anhydride (pivaloyl chloride/(10)).
- DCC/HOBT, DCC/HOSU, TOTU, N,N-carbonyldiimidazole,
and the like.
- In acetonitrile, DMF, THF, dichloromethane, ester,
15 and the like.

EDC1·HCl in dichloromethane gave the best
result.

(Z)-N-[2-Methoxy-5-[2-(3,4,5-trimethoxyphenyl)vinyl]-
phenyl]-L-serinamide hydrochloride



20

61.8 g of (11), in solution in 54 ml of
methanol, are charged at 20°C to a 1 litre three-necked
flask equipped with a mechanical stirrer, a

thermometer, a T piece, a reflux condenser surmounted by a bubble counter, and a heating bath, and 150 ml of isopropyl acetate, 99 ml of a 2.3N solution of hydrochloric acid in methanol and 8.2 ml of distilled water are added. The mixture is stirred and heated at 60°C for 3 hours. The solution, cooled to 40°C, is clarified by filtration through a sintered glass filter No. 4 rinsed with 40 ml of methanol. The filtrate is returned to the stirred three-necked flask and 194 ml of isopropyl acetate are added, the mixture is reheated to 40°C, the solution is initiated with 0.2 g of (12) and then 194 ml of isopropyl acetate are run in dropwise over 1 hour. The mixture slowly crystallizes as the isopropyl acetate is being run in.

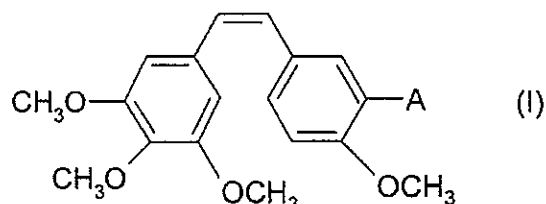
The mixture is allowed to return to ambient temperature and is then cooled to and maintained overnight at 5°C.

In the morning, the slurry is filtered through a sintered glass filter and the cake, pulled dry, is washed with 4 times 50 ml of isopropyl acetate, pulled dry thoroughly and then dried in an oven to constant weight (35°C/10 mmHg).

28 g of (12) are obtained with a yield over 2 stages (coupling and then deprotection operations) of: 56%, and an IS HPLC assay > 98%, i.e. an overall yield as such, for the synthesis carried out according to the first process route V0 2, of: 30% [(12) obtained with respect to (5) charged].

CLAIMS

1. Process for the preparation of combretastatins of following general formula (I):

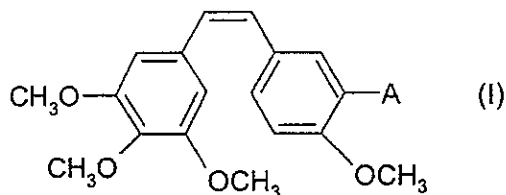


in which A represents an amino group, characterized in that, after the Wittig condensation in the presence of (3,4,5-trimethoxybenzyl)triphenylphosphonium bromide or chloride and of 3-nitro-4-methoxybenzaldehyde or of 3,4,5-trimethoxybenzaldehyde with a (4-methoxy-3-nitrobenzyl)triphenylphosphonium salt, the nitro group is reduced in the presence of iron.

2. Process according to Claim 1, characterized in that the amount of iron used is in excess.

3. Process according to Claim 2, characterized in that this excess is greater than 2 equivalents per one mol of starting nitro derivative.

4. Process for the preparation of combretastatins of following general formula (I):



in which A represents an amino group, characterized in that, according to the second implementational process,

a (3,4,5-trimethoxybenzyl)triphenylphosphonium salt is condensed with 3-amino-4-methoxybenzaldehyde or a (3-amino-4-methoxybenzyl)triphenylphosphonium salt is condensed with 3,4,5-trimethoxybenzaldehyde.

5. Process according to Claim 4, characterized in that a (3,4,5-trimethoxybenzyl)-triphenylphosphonium salt is condensed with 3-amino-4-methoxybenzaldehyde.

6. Process according to Claim 4 or Claim 5, characterized in that the reaction is preferably carried out in the presence of a base chosen from potassium tert-butoxide, sodium tert-pentoxide, sodium hydride, butyllithium, LDA (lithium diisopropylamine), sodium methoxide, potassium carbonate or alkaline derivatives of hexamethyldisilanes.

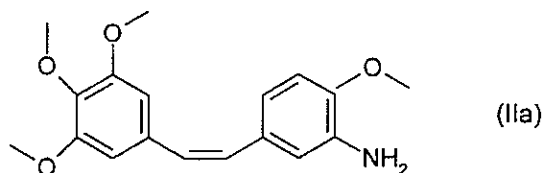
7. Process according to Claim 4 or Claim 5, characterized in that sodium methoxide is used.

8. Process according to Claim 4 or Claim 5, characterized in that the reaction solvent which is inert with respect to the reaction is chosen from ethers (THF), polar aprotic solvents (acetonitrile, NMP, DMF, DMSO, and the like), alcohols, aromatic solvents or water.

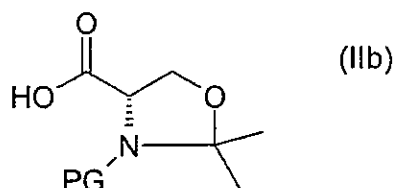
9. Process according to Claim 7, characterized in that the reaction temperature is preferably between 0 and 10°C.

10. Process for the preparation of compounds of formula (I) in the serine salt form by coupling of

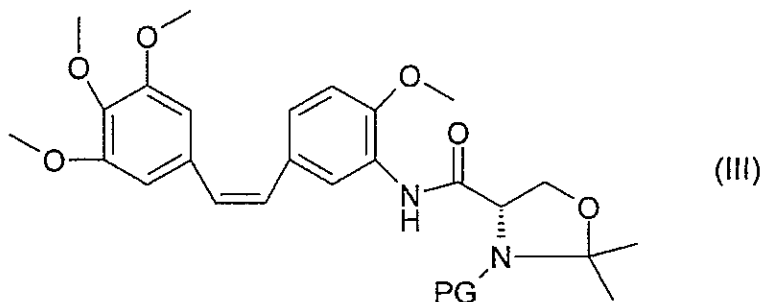
the derivatives of formula (IIa)



with the cyclic protected serine derivative of formula (IIb)

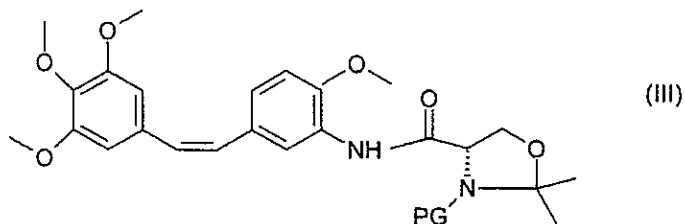


where PG represents a protective group for the amine functional group, to give a novel intermediate of following general formula:



which is subsequently deprotected.

11. Intermediate for the coupling between aminocombretastatin and the cyclic protected serine derivative, characterized in that it corresponds to the following formula:



in which PG is a protective group.

12. Intermediate according to Claim 11, characterized in that PG represents a protective group chosen from the following groups: tert-butoxycarbonyl, benzyloxycarbonyl (CBZ) or 9-fluorenylmethyloxycarbonyl (FMOC)