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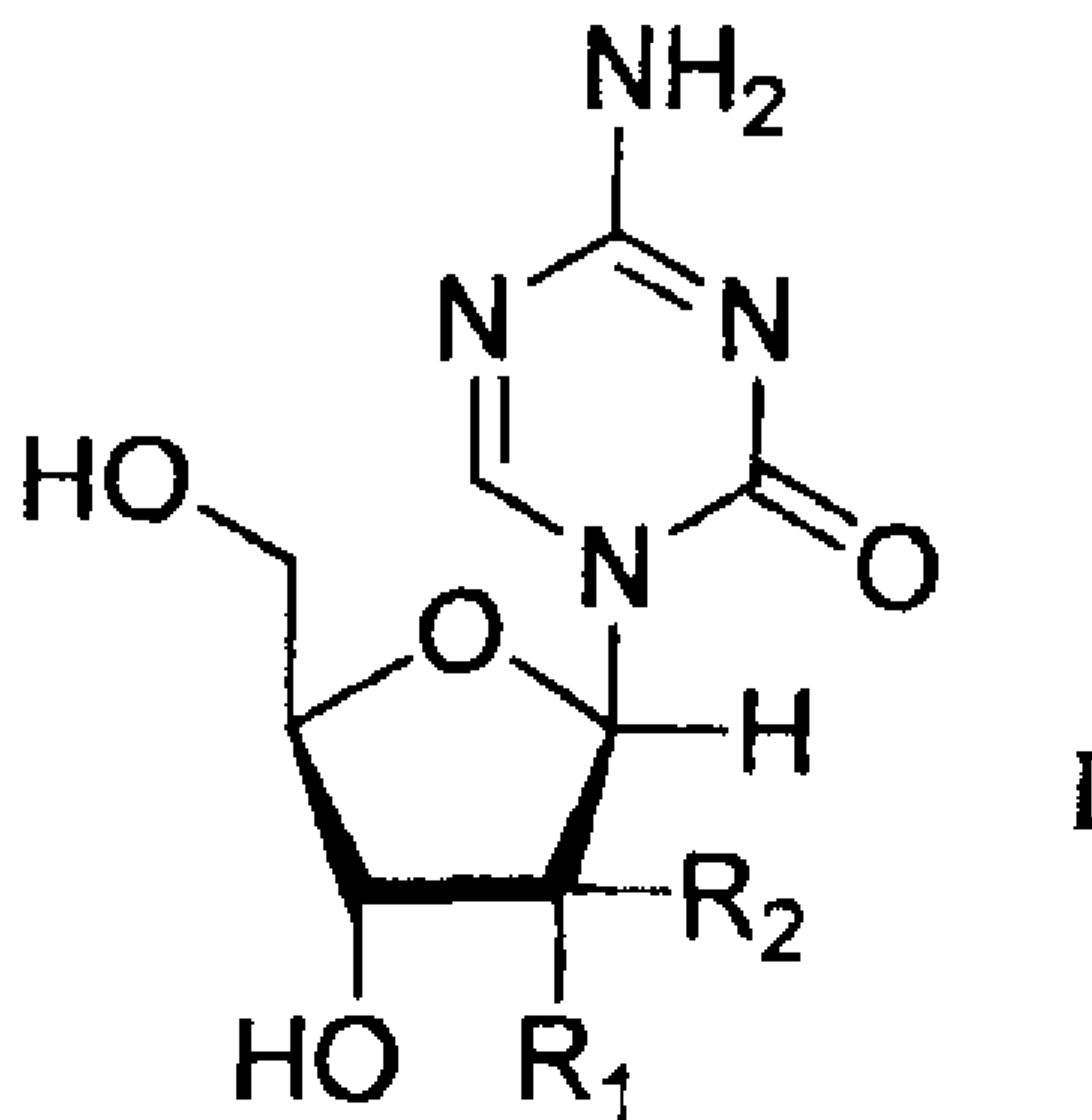
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(54) Titre : PROCEDE DE FABRICATION DE NUCLEOSIDES DE 5-AZACYTOSINE ET LEURS DERIVES
(54) Title: PROCESS FOR MAKING 5-AZACYTOSINE NUCLEOSIDES AND THEIR DERIVATIVES

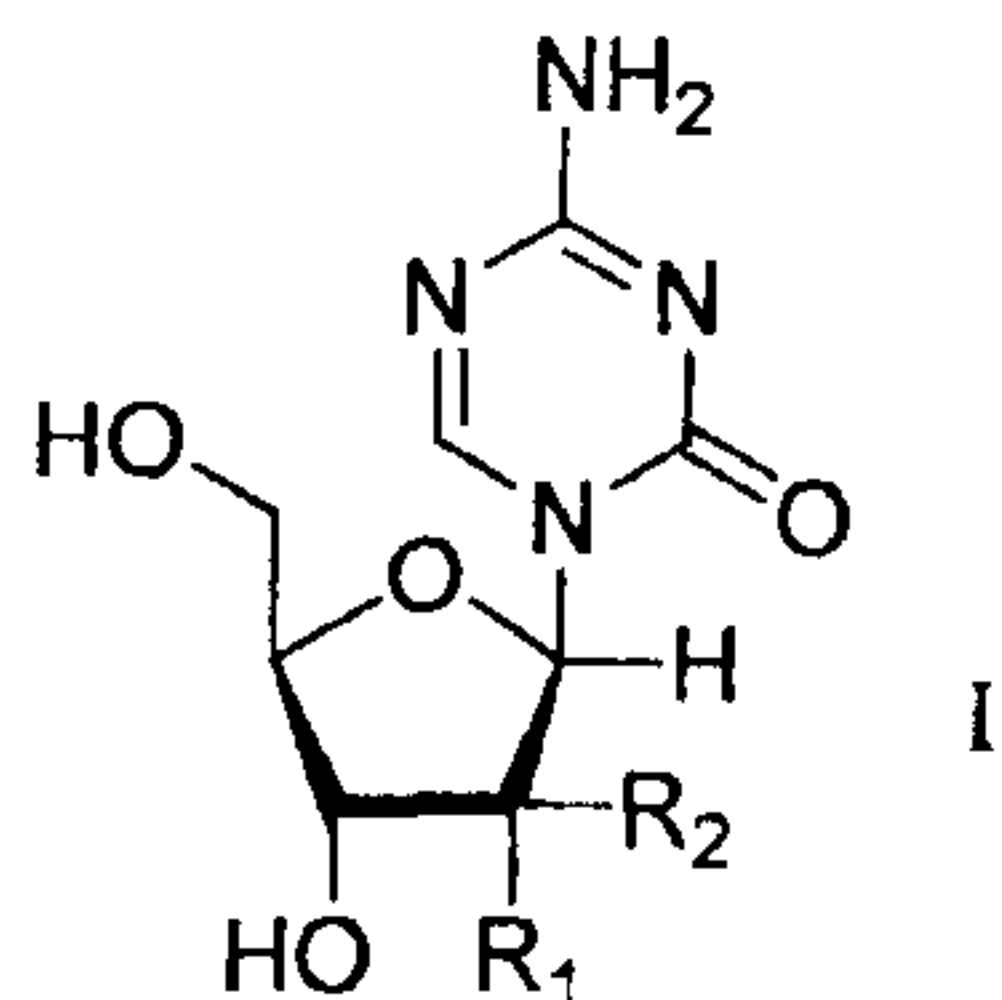


(57) Abrégé/Abstract:

A process of efficiently synthesizing a 5-azacytosine nucleoside of formula I: (see formula I) wherein R_1 is hydrogen, hydroxy or halogen; R_2 is hydrogen or halogen, such as azacitidine and decitabine, comprises coupling a silylated 5-azacytosine with a protected D-ribofuranose in the presence of a less than one molar equivalent sulfonic acid catalyst with respect to the protected D-ribofuranose. This process is efficient and amenable to manufacturing scale synthesis. It also does not require an aqueous workup or addition of a large amount of catalyst."

ABSTRACT

A process of efficiently synthesizing a 5-azacytosine nucleoside of formula I:



wherein R_1 is hydrogen, hydroxy or halogen; R_2 is hydrogen or halogen, such as azacitidine and decitabine, comprises coupling a silylated 5-azacytosine with a protected D-ribofuranose in the presence of a less than one molar equivalent sulfonic acid catalyst with respect to the protected D-ribofuranose. This process is efficient and amenable to manufacturing scale synthesis. It also does not require an aqueous workup or addition of a large amount of catalyst."

5 carbohydrate, it is sensitive to decomposition by water
(under neutral, basic and acidic conditions) and in fact
undergoes facile hydrolysis in aqueous formulations, in
aqueous emulsions, in aqueous solutions and when exposed
to moisture in aqueous work-up during synthesis making
10 commercial manufacture challenging.^{[1], [13]} Therefore it is
desirable to develop a production process which limits
or avoids the contact of these nucleosides with water.

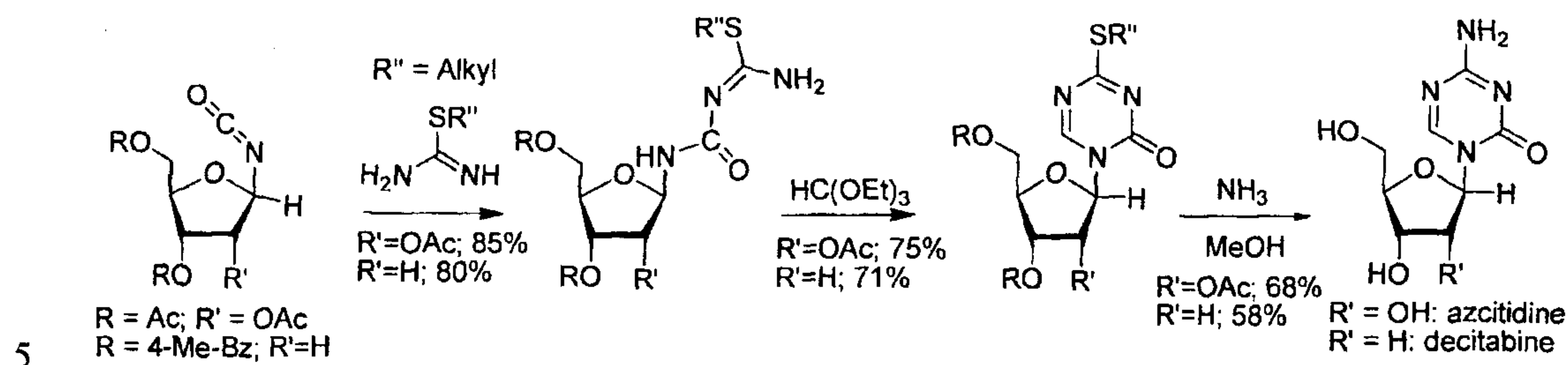
[0004] See, e.g., the following references:

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- 15 (2) US3350388 (**1967**) and DE1922702 (**1969**), Šorm and
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- (3) M. W. Winkley and R. K. Robins, *J. Org. Chem.*, **1970**,
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- (5) DE2012888 (**1971**), Vorbrüggen and Niedballa (Schering
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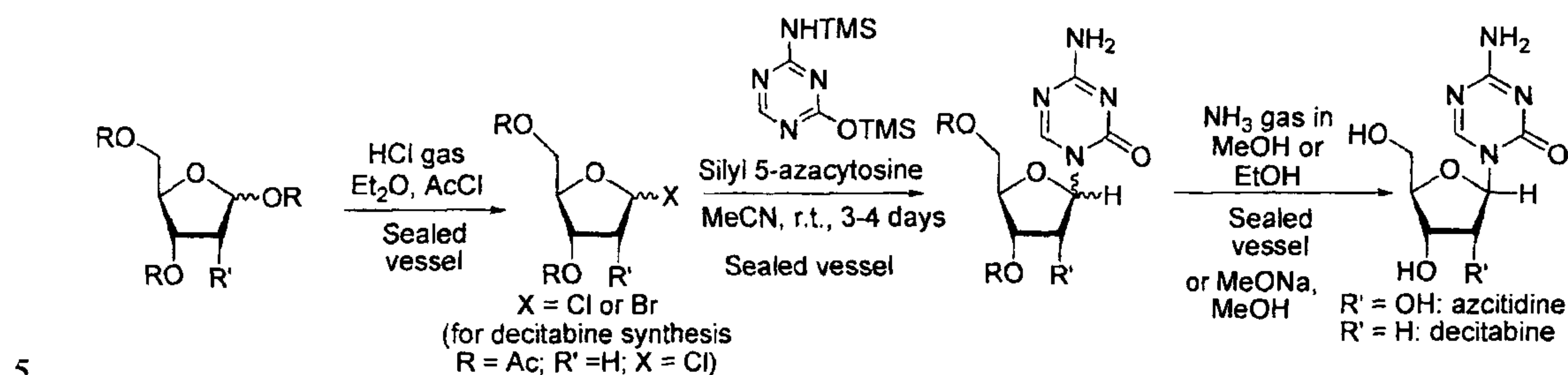
[0005]

[0006] Pískala and Šorm^[2] teach a lengthy method for the
15 synthesis of azacitidine and decitabine which involves
the use of reactive *N*-glycosylisocyanate intermediates
possessing 1- β -configuration. The synthetic process
(Scheme 1) comprises reacting a peracylglycosyl
isocyanate with an *S*-alkylisothiurea to obtain a
20 peracylglycosylisothiurea, condensing the latter with
an orthoester of an aliphatic acid at high temperature
(135°C) to obtain hydroxy-protected glycosyl-4-
alkylmercapto-2-oxo-1,2-dihydro-1,3,5-triazines followed
by deprotection with ammonia (NH₃) in methanol (MeOH) in
25 a sealed vessel over a 12-24 hour period. Although based
on the isocyanate, the overall yield of azacitidine is
43% and the overall yield of decitabine is 33%, it could
be difficult to store the isocyanate and its use might
provide a health risk. The route also suffers from other
30 difficult to scale-up steps, including the use of the
carcinogenic ICH Class I solvent benzene, and the need
for a pressure vessel in the deprotection step.



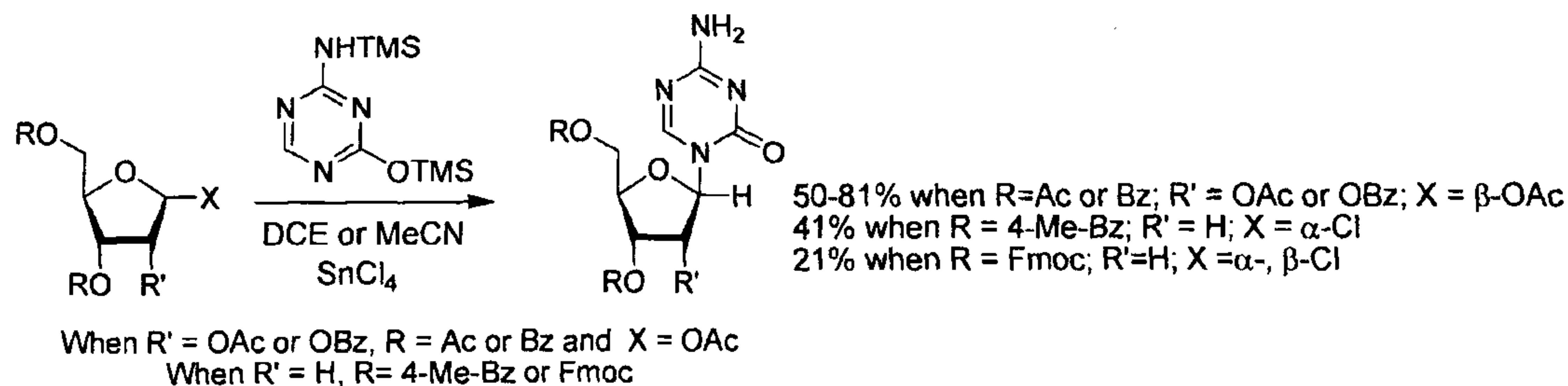
Scheme 1 - Isocyanate Method for synthesis of 5-azacytidines

- 10 [0007] Another potential process for azacitidine and decitabine was reported by Winkley and Robins^[3] (Scheme 2). Their approach utilizes the non-catalysed coupling of 1-halosugars with 2-[(trimethylsilyl)amino]-4-[(trimethylsilyl)oxy]-s-triazine (silyl 5-azacytosine)
- 15 which probably proceeds via an $\text{S}_{\text{N}}2$ mechanism. Pískala and Šorm^[4] also reported a similar process utilizing a 1-chlorosugar for the synthesis of azacitidine (Scheme 2), which suffers from the need for gaseous hydrogen chloride in the synthesis of the 1-chlorosugar, very low
- 20 overall yields (azacitidine in 11%, and decitabine in 7% overall yield^[3]), long reaction times (3-7 days), the need for pressure vessels in the deprotection step, the instability of the halosugars, complicated column chromatography and lengthy work-up and isolation
- 25 procedures.



Scheme 2 -Use of 1-halosugars in the synthesis of azacitidine and decitabine

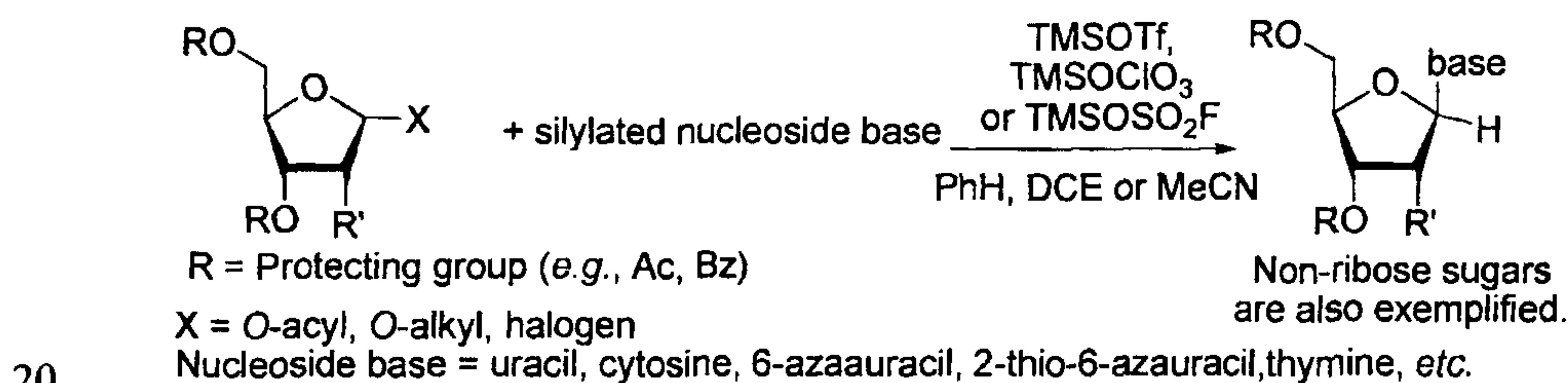
- 10 [0008] Niedballa and Vorbrüggen^[5,6] teach the synthesis of protected (blocked) nucleosides including azacitidine and decitabine that utilizes a large amount of tin chloride in dichloroethane (DCE) or acetonitrile (MeCN) to promote the coupling of 5-azacytosine and protected
- 15 sugar moieties (Scheme 3). According to Ionescu and Blumbergs^[7] there are a number of major drawbacks to this process: first, removal of tin from the API is difficult. Second, emulsions developed during the workup of the coupling mixture. Third, a difficult filtration
- 20 step needs to be performed in order to isolate the insoluble tin salts. For these reasons it should be concluded that this process is not suitable for the commercial manufacture of azacitidine.^[7]



Scheme 3 -Synthesis of protected azacitidine and decitabine using Vorbrüggen's coupling method

5

[0009] Vorbrüggen^[9] teaches a general method for the coupling of silylated bases and nucleoside bases (including cytosine, pyridines triazoles, and pyrimidines, but not 5-azacytosine) with protected 1-O-acyl, 1-O-alkyl or 1-halosugars (*viz.*, ribose, deoxyribose, arabinose and glucose derivatives) in benzene, DCE or MeCN to make protected nucleosides (Scheme 4). The coupling is promoted by trimethylsilyl (TMS) esters of esterifiable mineral acids or strong sulfonic acids, including trimethylsilyl triflate (TMSOTf), TMSOClO₃ and TMSOSO₂F. The requirement for an aqueous work-up makes this method less than desirable for synthesis of azacitidine and decitabine.



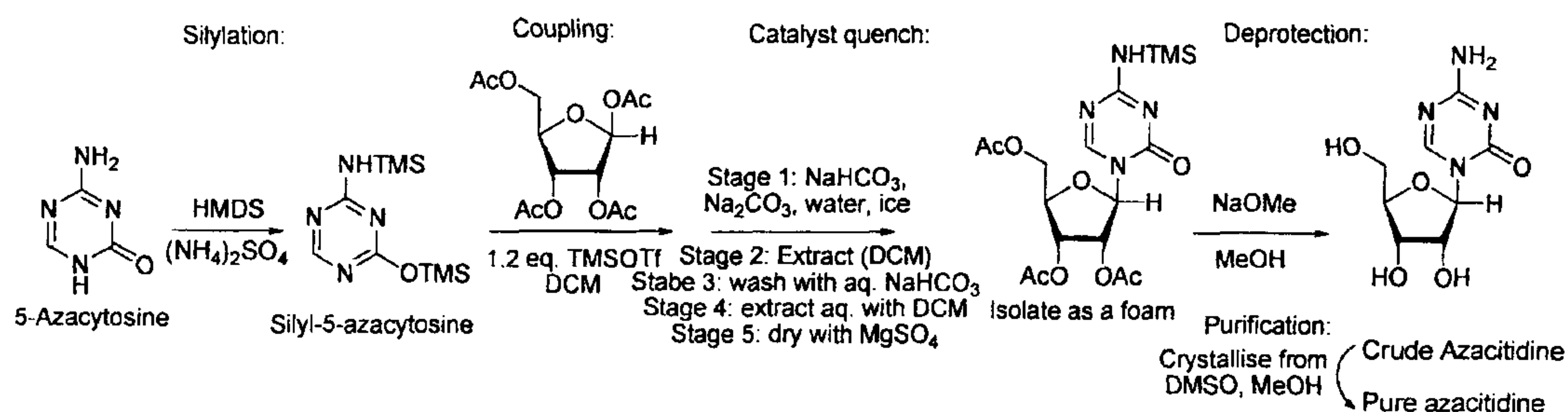
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Scheme 4 -Vorbrüggen's coupling protocol utilizing trimethylsilyl esters of strong acids

[00010] Ionescu and Blumbergs^[7] teach a manufacturing process (Scheme 5) specifically for the synthesis of azacitidine which is based on the general trimethylsilyl ester promoted coupling methodology invented by Vorbrüggen.^[9] Silylation of 5-azacytosine is conducted using an excess of hexamethyldisilazane (HMDS) and a catalytic amount of ammonium sulfate. The 1-O-acyl-carbohydrate and silylated nucleoside base coupling reaction is carried out in a "solvent having low water

30

5 solubility" such as dichloromethane (DCM) in the
 presence of a greater than stoichiometric amount of the
 non-metallic Lewis acid catalyst TMSOTf. An aqueous
 workup is employed and a number of costly steps are
 necessary to remove harmful water and switch solvents
 10 into suitable conditions for the deprotection step.



Scheme 5 - An azacitidine manufacturing method

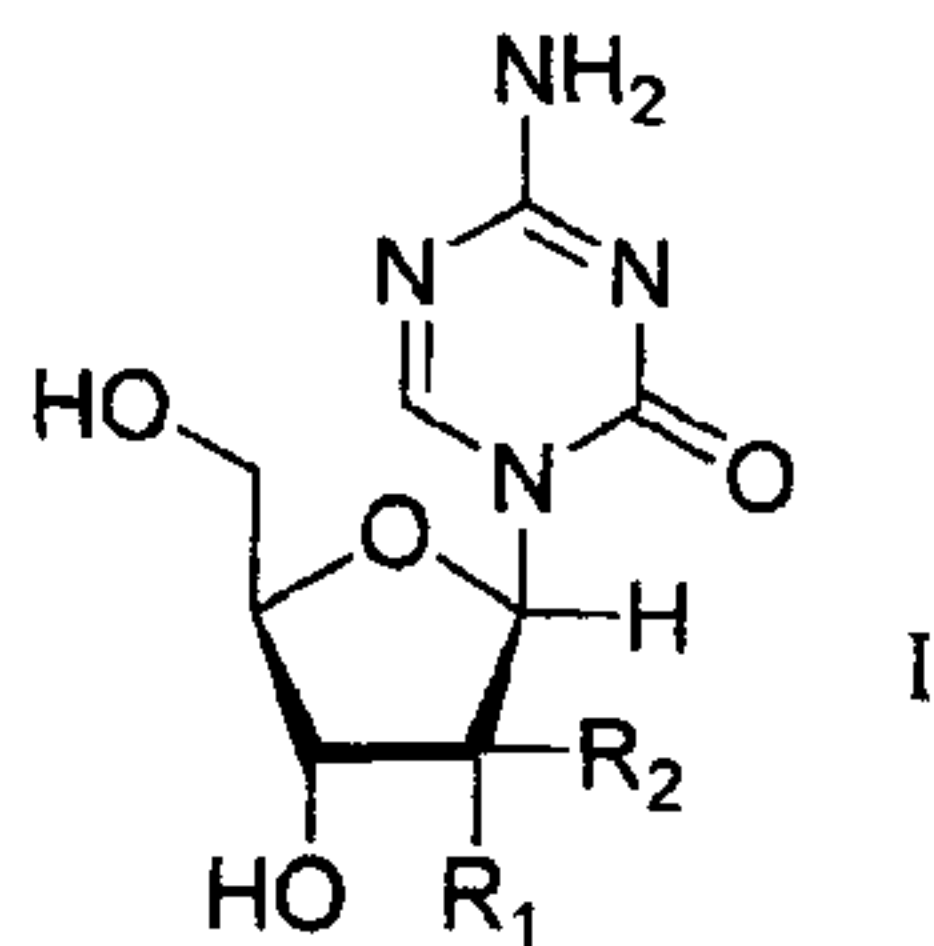
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[00011] Therefore, there is a need for a more efficient
 process for manufacturing a 5-azacytosine nucleoside
 compound on a large scale.

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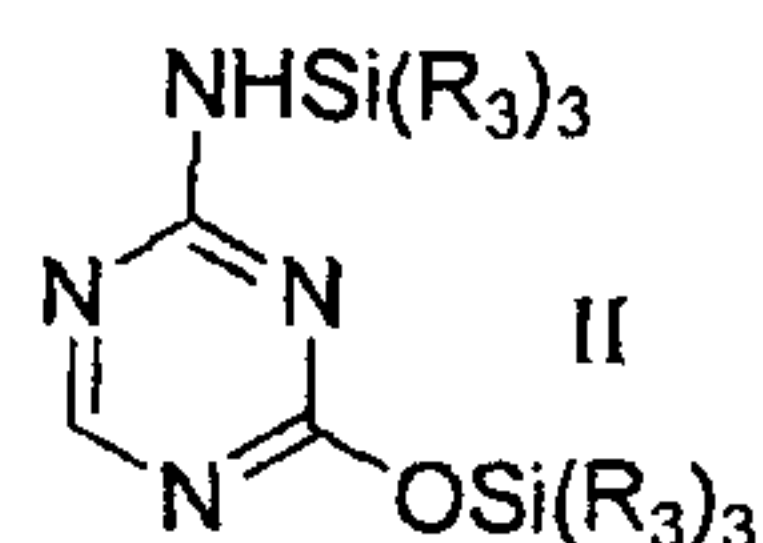
SUMMARY OF THE INVENTION

[00012] In accordance with one aspect of the present
 application, a process for the preparation of a 5-
 azacytosine nucleoside compound of formula I:

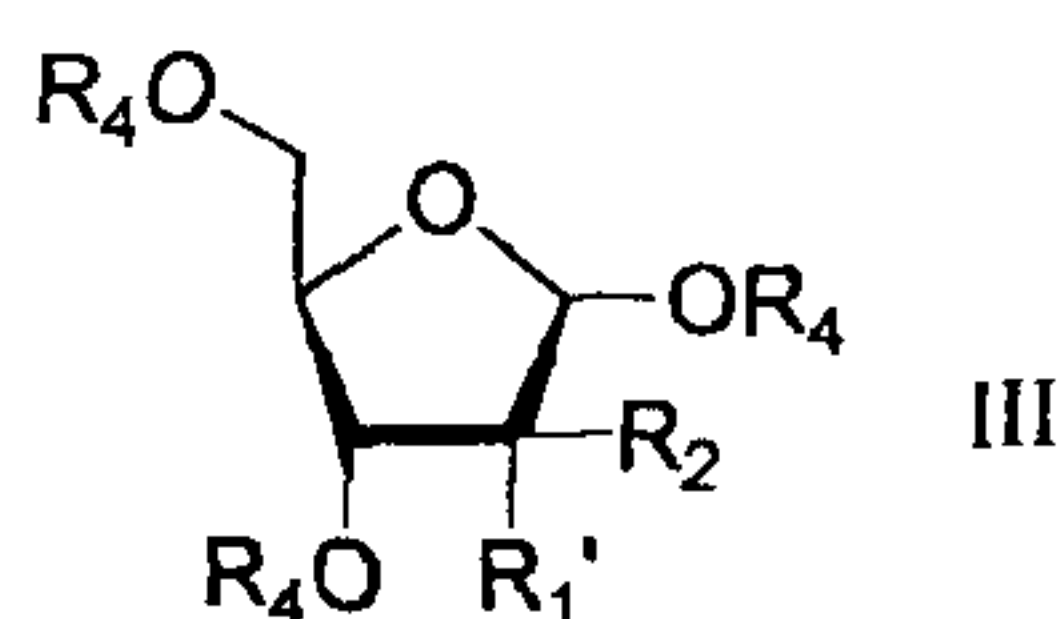


25 wherein R_1 is hydrogen, hydroxy or halogen; R_2 is
 hydrogen or halogen, comprises:

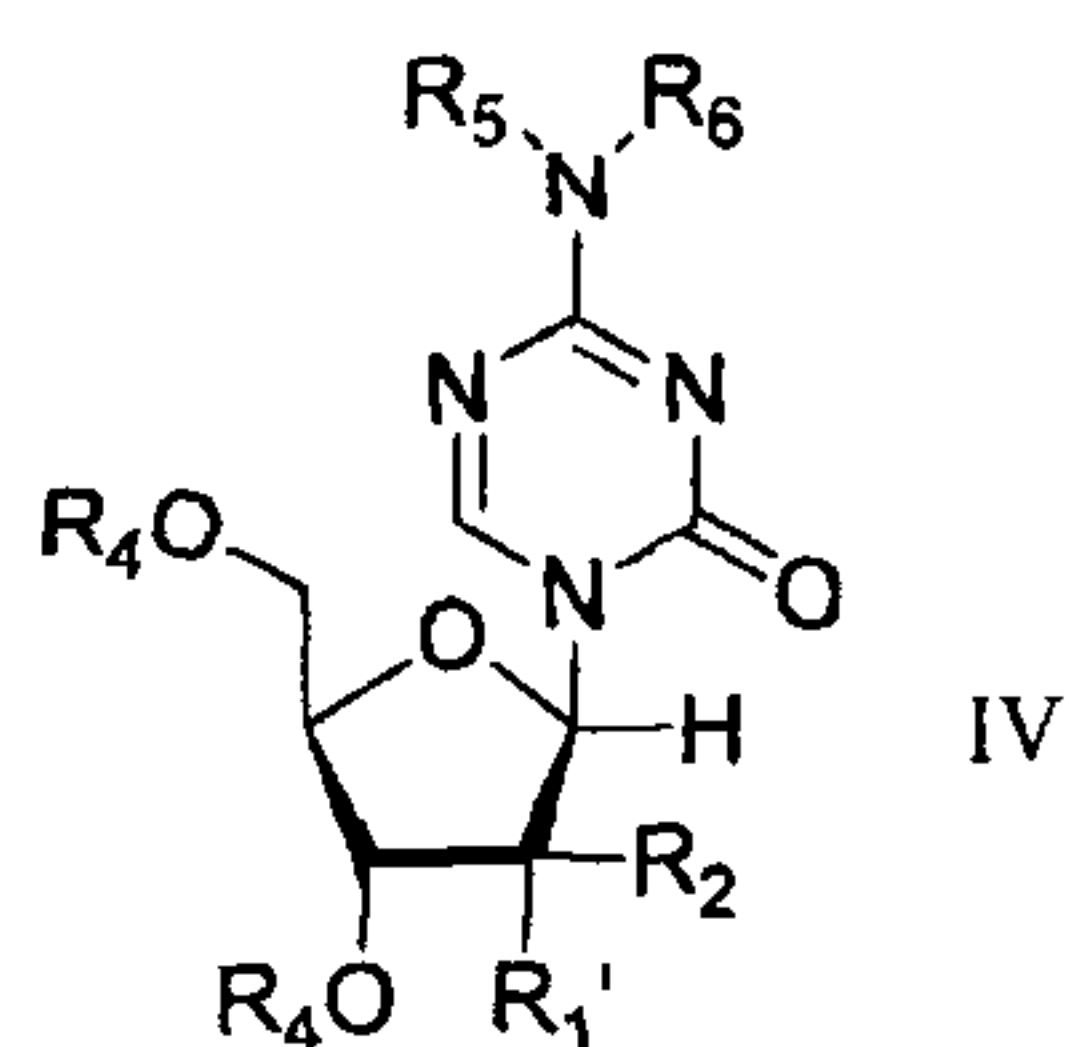
- 5 a) reacting 5-azacytosine with a silylating agent to produce a reaction mixture containing a silylated 5-azacytosine of formula II:



- 10 wherein each of R_3 is independently optionally substituted C_1 - C_{20} alkyl group or aryl group.
b) coupling the silylated 5-azacytosine of formula II with a protected D-ribofuranose of formula III:



- 15 wherein R_1' is hydrogen, halogen or OR_4 ; R_4 represents a hydroxyl protecting group; in a first organic solvent and in the presence of a sulfonic acid catalyst to obtain a liquid reaction mixture
20 comprising protected 5-azacytosine nucleoside of formula IV:



- 25 wherein each of R_5 and R_6 is independently hydrogen or $Si(R_3)_3$, and R_3 , R_1' , and R_2 are as defined above;
and

5 c) converting the protected 5-azacytosine nucleoside
of formula (IV) to the 5-azacytosine nucleoside of
formula (I).

[00013] Preferably, the process described above is free
of an aqueous work-up step to quench or remove the
10 catalyst.

[00014] In accordance with another aspect of the present
application, in the above-described process, the
catalyst is not limited to a sulfonic acid and may be
any suitable catalyst, but the process is free of an
15 aqueous work-up step to quench or remove the catalyst.

[00015] In accordance with yet another aspect of the
present invention, a process of making a 5-azacytosine
nucleoside compound of formula (I), as described above,
comprises: i) forming a homogenous solution comprising
20 at least a polar aprotic solvent and a protected 5-
azacytosine nucleoside of formula (IV) as described
above in a polar aprotic solvent; and ii) converting the
protected 5-azacytosine nucleoside of formula (IV) to
the 5-azacytosine nucleoside of formula (I) in the
25 homogenous solution. Preferably, prior to the step ii),
this process is free of a step carried out in the
presence of a substantial amount of water, e.g., less
than 100% by weight of the compound of formula (IV),
more preferably, less than 10% by weight of the compound
30 of formula (IV), most preferably, less than 1% by weight
of the compound of formula (IV).

[00016] As an embodiment, the forming step i)
discussed above is preferably accomplished by adding a
polar aprotic solvent to a previously existing solution
35 comprising a protected 5-azacytosine nucleoside of
formula (IV) and an organic solvent used during the

5 reaction of making the protected 5-azacytosine nucleoside of formula (IV).

[00017] The C₁-C₂₀ alkyl group used herein may be a straight chain alkyl group, a branched alkyl group, or a cyclic alkyl group, preferably methyl.

10 [00018] The silylating agent used in the present invention can be any suitable agent, such as hexamethyldisilazane (HMDS), trimethylsilylchloride (TMSCl), N,O-bis(trimethylsilyl)acetamide (BSA), and N,O-Bis(trimethylsilyl)trifluoro acetamide (BSTFA) and
15 trimethylsilyl triflate (TMSOTf). HMDS is the most preferred embodiment of the silyating agent of the present invention.

[00019] The hydroxyl protecting group R₄ may be any suitable group that can protect the hydroxyl group on a
20 D-ribofuranose from unwanted reaction. For example, the hydroxyl protecting group R₄ can be an optionally substituted C₁-C₂₀ alkyl acyl or aryl acyl group, in particular a benzoyl or acetyl group.

[00020] Prior to the step of b), the process of the
25 present application preferably comprises a step of isolating the silylated 5-azacytosine compound in solid form from the reaction mixture of the step a). For example, the isolation step may be accomplished by crystallizing silylated 5-azacytosine (more preferably
30 by adding an effective amount of silylated 5-azacytosine seed crystals to promote the crystallization) and filtering off the crystallized silylated 5-azacytosine. Alternatively, the isolation step may be accomplished by evaporation of solvents in the reaction mixture of the
35 step a). Utilizing crystallization to isolate the silylated compound is more preferred.

5 [00021] Preferably, the coupling step b) is conducted in the absence of a substantial amount, or more preferably, in the presence of a less than 1% by molar amount of the silyating agent relative to the molar amount of the silylated 5-azacytosine compound.

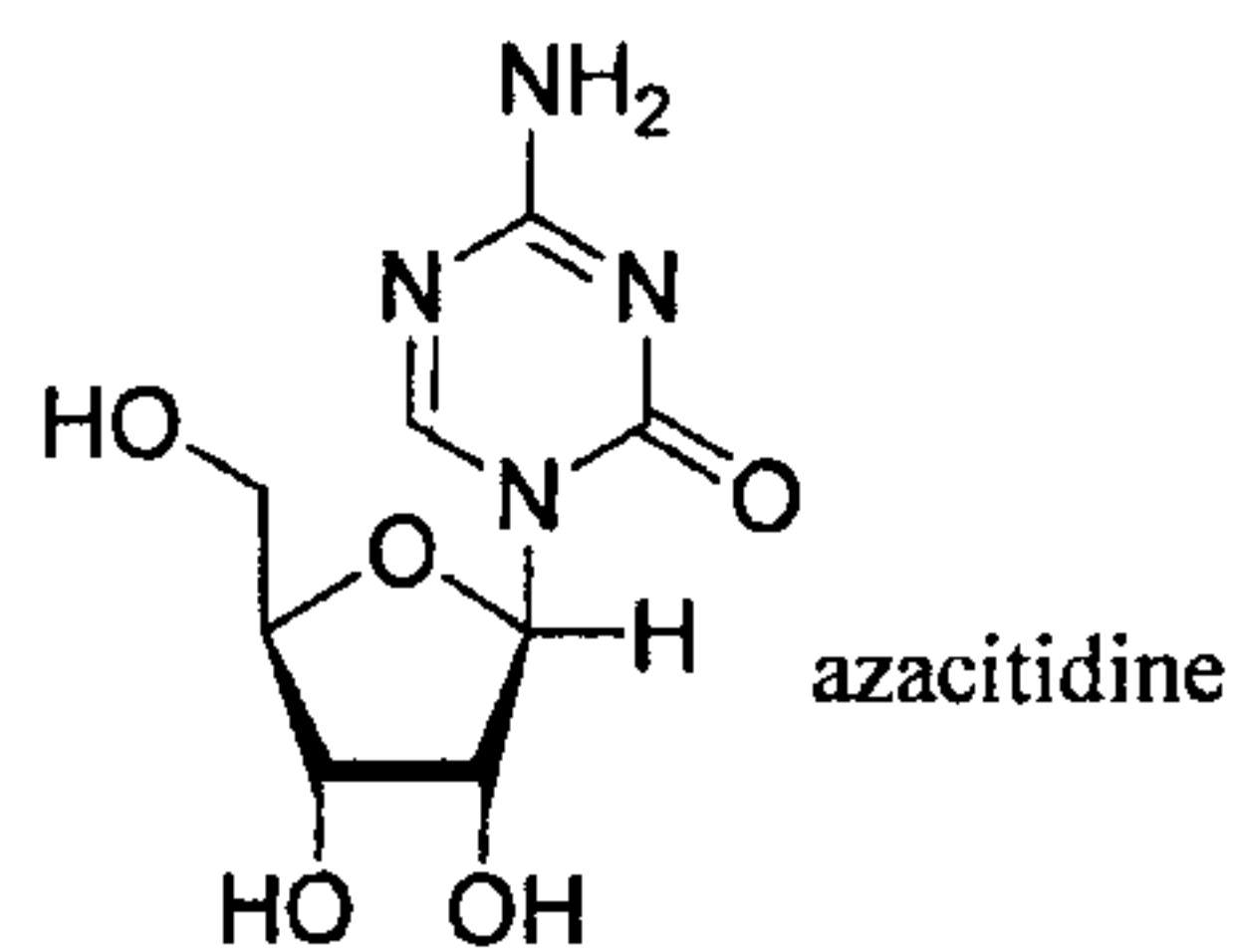
10 [00022] In accordance with one embodiment of the present invention, the process comprises a step of diluting the liquid reaction mixture comprising the protected 5-azacytosine nucleoside of formula (IV) obtained in the step b) with a second organic solvent. Preferably, at
15 least a part of the first organic solvent is replaced by a second organic solvent so that the protected 5-azacytosine nucleoside of formula (IV) remains dissolved in the second organic solvent. More preferably, at
20 the step b) is subsequently removed prior to the step c). Preferably, the second organic solvent is a solvent having a higher boiling point than the first organic solvent.

[00023] The first organic solvent is a polar water
25 soluble solvent, in particular acetonitrile. Preferably, the first organic solvent should be non-reactive and be stable in the presence of the catalyst used in the step b) and stable during the step of c). Since the first organic solvent, as noted above, may be
30 mostly removed by evaporation after the step b) and before the step c), its boiling point should not be too high. On the other hand, since the step b) reaction may be conducted at a moderately high temperature, the first organic solvent should have a suitable high boiling
35 point to allow the reaction mixture to be heated to the

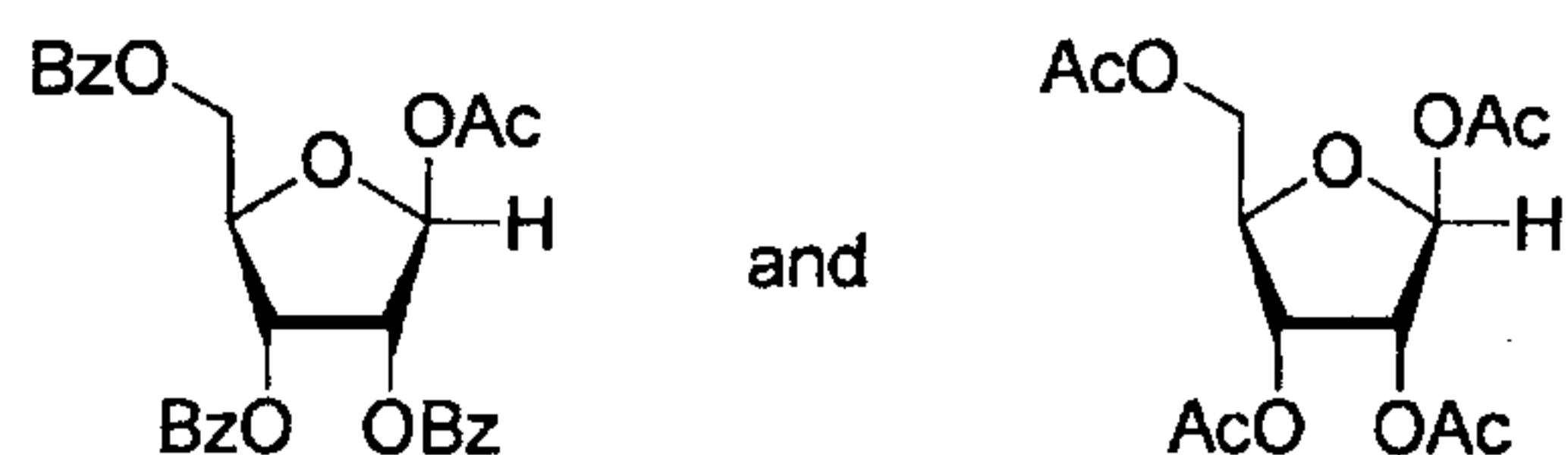
5 required reaction temperature without evaporating the first organic solvent.

[00024] The second organic solvent is preferably a polar aprotic solvent, more preferably, dimethylsulfoxide. The second organic solvent has a
10 higher boiling point than the first organic solvent. It also should be stable during the step of c). Preferably, the second organic solvent has a boiling point that is high enough such that it will not be removed prior to the first organic solvent when a
15 mixture of the first and second organic solvents is subjected to evaporation ("solvent swap/partial solvent swap"). The second organic solvent is also preferably polar enough such that it can keep the protected 5-azacytosine nucleoside of formula (IV) dissolved before
20 and during the step of c), i.e., its high polarity preferably keeps the reaction of step c) homogenous, which was one innovation that we discovered. It is relatively difficult to reproduce the process of making 5-azacytosine nucleoside, when the step c) is not
25 conducted in a fully homogenous solution. In addition, compared to a reaction in a heterogeneous slurry, keeping the step c) homogenous made the reaction much faster. Moreover, the purity of the final product following precipitation as a solid from step c) was
30 superior when the step c) is carried out in a homogenous media due to the use of the polar high boiling point solvent.

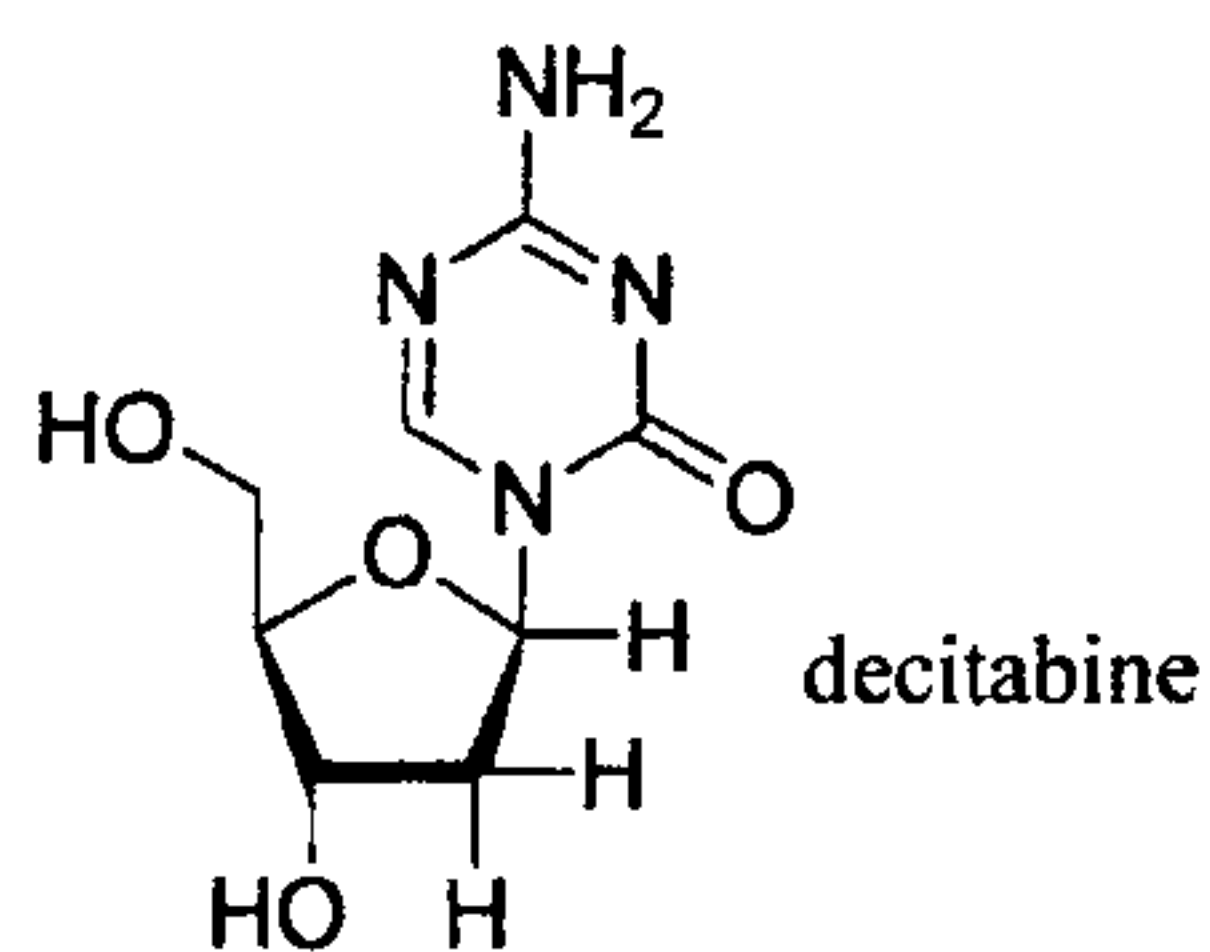
[00025] Preferably, the 5-azacytosine nucleoside compound of formula I is azacitidine



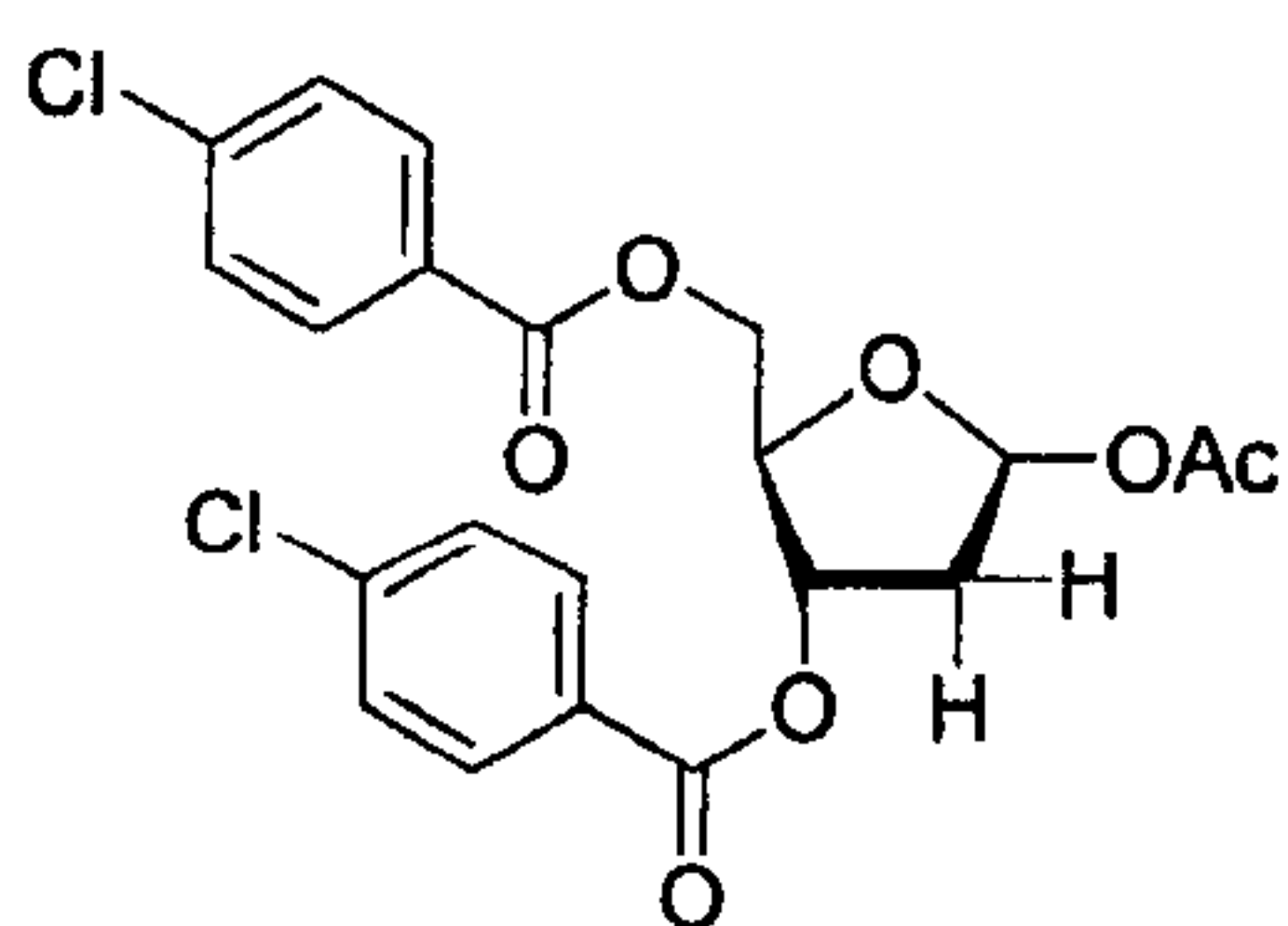
5 $\text{HO} \quad \text{OH}$, and the protected D-ribofuranose of
formula III is selected from the group consisting of



[00026] Alternatively and preferably, the 5-
10 azacytosine nucleoside compound of formula I is
decitabine



$\text{HO}-\text{H}$, and the protected D-ribofuranose formula III is a 2-deoxy-D-ribofuranose as shown below



15

[00027] Preferably, the amount of the catalyst in the step b), in particular sulfonic acid, is less than one molar equivalent, in particular 10-30 mole percent, with respect to the molar amount of the protected D-

20 ribofuranose of formula III.

5 [00028] The sulfonic acid catalyst is preferably trifluoromethane sulfonic acid.

[00029] The silylation agent is preferably hexamethyldisilazane.

[00030] Preferably, the step c) of the present
10 application comprises deprotecting the protected 5-azacytosine nucleoside of formula (IV) in the presence of a basic deblocking agent. The basic deblocking agent is preferably a metal alkoxide, in particular sodium methoxide in methanol. The step c) is preferably
15 conducted at a temperature between 20°C to 30°C.

[00031] Preferably, the coupling step b) is conducted in the absence of a water immiscible solvent such as dichloromethane (DCM).

[00032] Preferably, the steps b) and c) are conducted
20 in one reaction vessel.

[00033] As a preferred embodiment, the step c) is conducted in MeOH. More preferably, the step c) is conducted in a mixture of MeCN, DMSO and MeOH, in particular approximately 0-2 volumes of MeCN, 3 volumes
25 of DMSO, and 2-3 volumes of MeOH.

[00034] The coupling step b) is preferably conducted at a temperature of between 40°C to 80°C, more preferably, between 50°C to 60°C when the protected β -D-ribofuranose (III) is 1-O-acetyl-2,3,5-tri-O-benzoyl- β -D-ribofuranose.

30 [00035] Preferably, the process of the present application is free of a step of isolating the protected 5-azacytosine nucleoside of formula (IV) from a liquid mixture.

[00036] After the step c), the 5-azacytosine
35 nucleoside of formula (I) may be isolated from the reaction mixture by precipitation with an organic

5 solvent, followed by filtration. The 5-azacytosine nucleoside of formula (I) may also be purified by crystallization to furnish API grade material.

[00037] Compared to the prior art, the process in accordance with the present invention 1) does not
10 require an aqueous workup; 2) does not require the addition of a large amount of catalyst; 3) steps b) and c) can be carried out more efficiently basically in one reactor vessel ("one pot"); 4) is time-saving; and/or 5) is amenable to manufacturing scale synthesis.

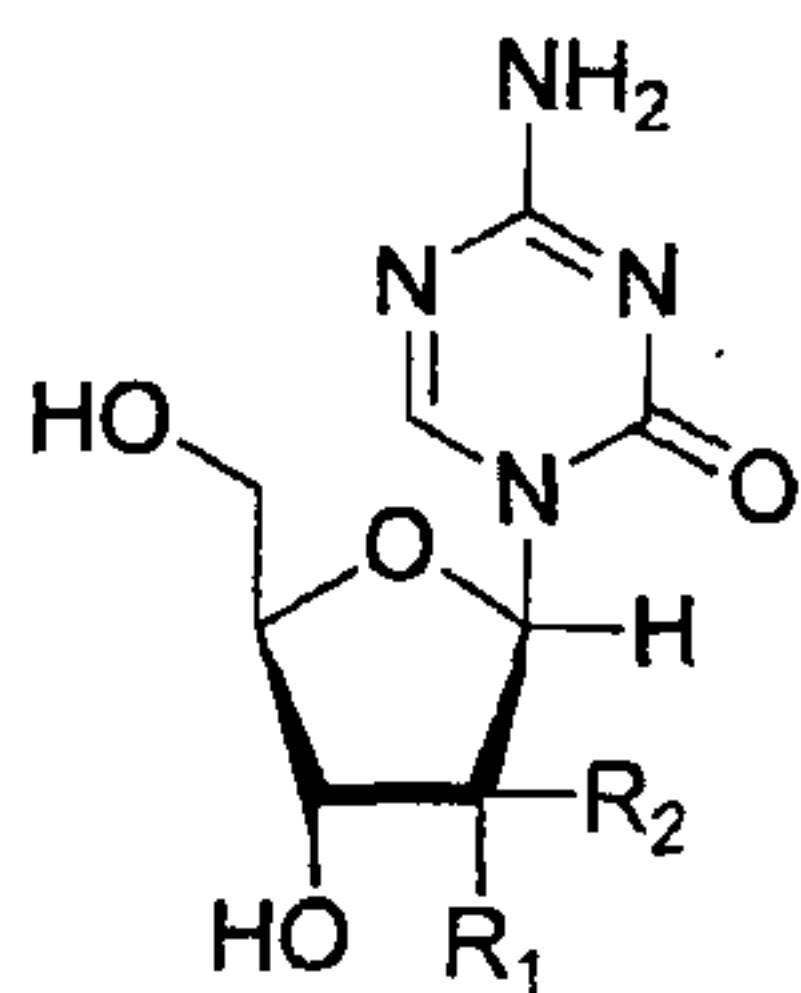
15 [00038]

5 **DETAILED DESCRIPTION OF THE PRESENTLY PREFERRED
EMBODIMENTS**

[00039] The following describes preferred embodiments of the present invention and should not be used to limit the scope of the present invention.

10 [00040] The present invention demonstrates a process of manufacture of azacytosine nucleosides that is extremely efficient and produces high yields and purity. A key aspect of the present invention relates to unexpected ability to use sulfonic acids in preferably
15 substoichiometric amounts (relative to the silyl 5-azacytosine and protected sugar), as the catalyst in the coupling of silyl 5-azacytosines and protected 1-O-acyl-ribofuranose or 1-O-acyl-2-deoxy-ribofuranose sugars. This has not been described before for the manufacture
20 of 5-azacytosine derived nucleosides and the use of less than one equivalent amount of a sulfonic acid ultimately leads to a series of major process improvements over those in the prior art processes, such as being able to omit an aqueous work-up step and more efficient use of
25 reaction vessels. The new process is industrially applicable and has been scaled into production equipment obtaining a significantly higher yield of azacitidine than that reported in the prior art.^[2,3,7]

[00041] According to an embodiment of the present
30 invention, a process for the preparation of 5-azacytosine nucleosides and their derivatives of the formula:



[00042] In one embodiment of the present invention, the inventors specifically developed a process in which silylated 5-azacytosine is reacted with hydroxy-protected β -D-ribofuranose, 2-deoxy- β -D-ribofuranose, 2-deoxy-2-fluoro- β -D-ribofuranose or 2-deoxy-2,2-difluoro- β -D-ribofuranose in the presence of a substoichiometric amount of trifluoromethane sulfonic acid (TfOH). The process need not be limited to the use of substoichiometric quantities of TfOH, however, of great impact, the use of a substoichiometric amount of

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5 TfOH allows for the complete omission of the standard
aqueous work-up that is used in other methods. [6,7,8,9,12]
The catalyst is conveniently quenched in the
deprotection step itself and is removed from the
precipitated product in the filtrate. This allows the
10 coupling reaction, the quench of the catalyst and the
deprotection reaction to all be carried out in one
reactor so that the exposure of the water-sensitive
products to water is completely avoided. Thus, it is
apparent that the use of a substoichiometric amount of
15 the coupling catalyst has a large impact on the whole
process. The complete omission of an aqueous work-up is
a significant improvement over that of other methods
particularly on a manufacturing scale where processing
duration are much longer than on the laboratory scale.
20 Yield losses that can occur due to hydrolysis of the
water sensitive glycosylated 5-azacytosine ring during
aqueous work-ups is avoided, allowing for better overall
quality and yield.

[00043] The inventors discovered that addition of a
25 polar solvent such as DMSO following the coupling
reaction makes the deprotection (deblocking) reaction,
which is preferably conducted in a solution of NaOMe in
MeOH, proceed rapidly as a homogeneous solution. This
homogeneous solution is a mixture of MeCN, DMSO and
30 MeOH. When the deprotection step is complete, the
addition of a larger amount of MeOH causes the crude
nucleoside API product to precipitate and it can be
collected directly. Azacitidine of API grade ($\geq 99.0\%$
HPLC purity, no impurities $\geq 0.1\%$) was obtained in 85~90%
35 recovery yield following only a single purification step.
The use of MeCN as a reaction solvent in the coupling

5 step and the absence of an aqueous work-up eliminated
the need for a costly solvent swap to a water non-
miscible solvent such as is provided in other
references.^[7,14]

[00044] The process in accordance with embodiments of
10 the present invention encompasses several key
improvements upon other methods. The use of a strong,
sulfonic acid as a catalyst instead of tin chloride or
trimethylsilyl trifluoromethane sulfonate (also known as
TMS-Triflate, TMSOTf) enables effective coupling of
15 silyl 5-azacytosines with protected sugars. A number of
alkyl or aryl sulfonic acids can be employed but most
preferably, trifluoromethane sulfonic acid is suggested.
The catalyst can be employed in less than one molar
equivalent to one equivalent in a range of 10% to 100%,
20 most preferable, 20% with respect to the molar amount of
the protected D-ribofuranose. The silyl 5-azacytosine
may contain any suitable silyl groups, but most
preferred is trimethylsilyl. The blocked sugar may
contain any suitable hydroxy-blocking group such as
25 either alkyl or aryl acyl groups. The solvent is any
suitable organic solvent preferably a water miscible
organic solvent and most preferably acetonitrile.

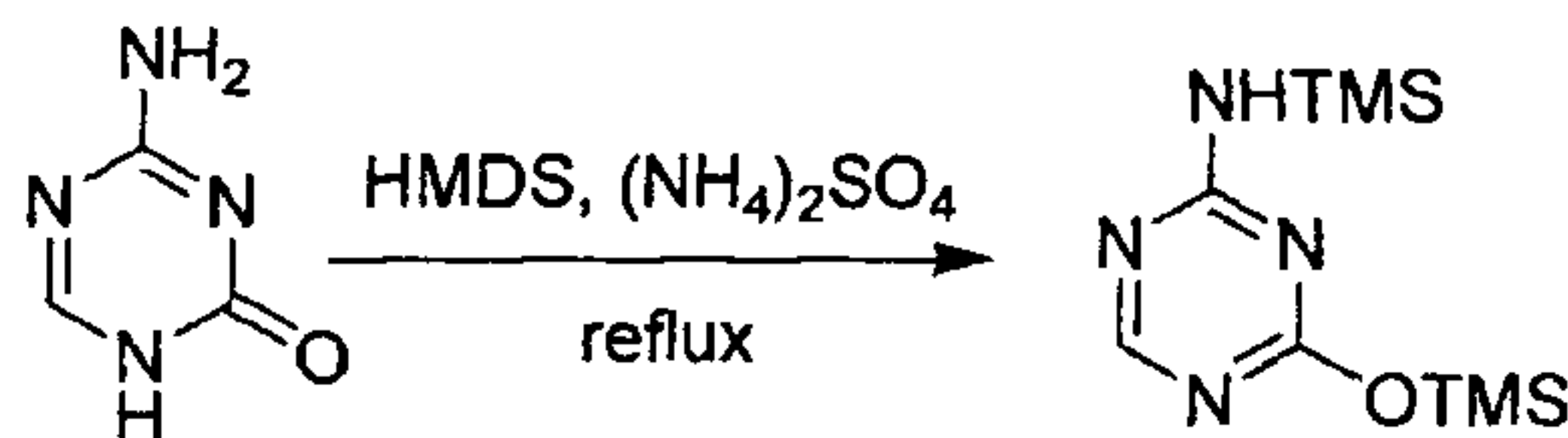
[00045] Upon completion of the coupling reaction,
preferably no aqueous conditions are employed and
30 instead a suitable higher boiling polar solvent is added
such that the contents of the reactor remain solubilised
and the solvent is stable to the deblocking conditions.
Suitable solvents can be sulfoxides, amides, glycols and
the such. Most preferably the solvent is DMSO. Finally,
35 the process is completed by the addition of a deblocking
agent followed by precipitation of the product which can

5 be collected by filtration. If the blocking groups are acyl, alkoxides are the preferred deblocking agents and are generally added in an alcohol solution. Sodium methoxide in methanol is typically used. Upon completion of this step, the product is precipitated by the
 10 addition of an anti-solvent such as methanol and can be collected by filtration in high yield and purity. While a number of useful nucleosides can be synthesized by this process, it is most suitable for the synthesis of azacitidine.

15 [00046] The following examples are provided for the purpose of further illustration only and are not intended to be limitations on the disclosed invention.

EXAMPLE 1

20 Preparation of 2-[(trimethylsilyl)amino]-4-[(trimethylsilyl)oxy]-s-triazine (silyl 5-azacytosine)

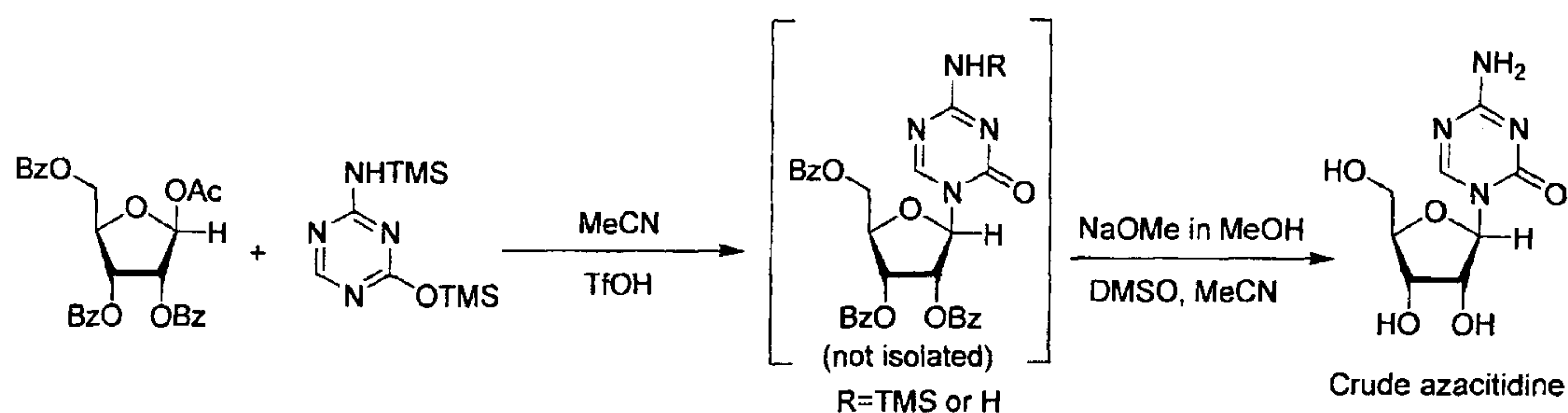


[00047] A mixture of 5-azacytosine (7.33 Kg), HMDS (33.9 Kg) and ammonium sulfate (0.44 Kg) was heated at
 25 reflux (about 115-135°C) and stirred for 16 hours. After the reaction was complete, the slurry was cooled to 118 °C and then filtered through a bed of celite™ and rinsed with HMDS (5.6 Kg). The silylated 5-azacytosine solution was cooled to 35 °C and the solution was cooled to 18 °C,
 30 stirred at 18 °C, for not less than 6.5 hours and then filtered. The solid was washed twice with HMDS (5.6 Kg each) and dried under vacuum at ≤70°C for 9.5 hours to obtain 14.19 Kg of white silyl 5-azacytosine (87%).

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EXAMPLE 2

Coupling of Silyl 5-Azacytosine to Sugar and
Deprotection



- 10 [00048] A mixture of 2-[(trimethylsilyl)amino]-4-
[(trimethylsilyl)oxy]-s-triazine (4.5 Kg), 1-O-acetyl-
2,3,5-tri-O-benzoyl- β -D-ribofuranose (8.8 Kg), anhydrous
MeCN (34.6 Kg) and TfOH (600 g) were heated at 55 °C for
12.5 hours. The reaction mixture was cooled to 45 °C,
15 DMSO (29 Kg) was added, and the MeCN was evaporated at
an internal temperature of <50 °C under vacuum until
about 54 L of the solution. The solution was cooled to
23 °C. MeOH (13.9 Kg) was added followed by a solution of
30% NaOMe in MeOH solution (2.5 Kg) that was pre-diluted
20 with MeOH (7.0 Kg). The solution was stirred at 23 °C for
35 minutes. When the reaction was complete MeOH (90.4 Kg)
was added and the resulting slurry was stirred at 22°C
for 3 hours and 10 minutes and was then filtered and
washed three times with MeOH (7.0 Kg each). The cake was
25 dried under vacuum at below 70 °C for 9 hours and 20
minutes to give 3.2 Kg of 98.89% purity crude
azacitidine (71% yield based on 1-O-acetyl-2,3,5-tri-O-
benzoyl- β -D-ribofuranose).

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EXAMPLE 3

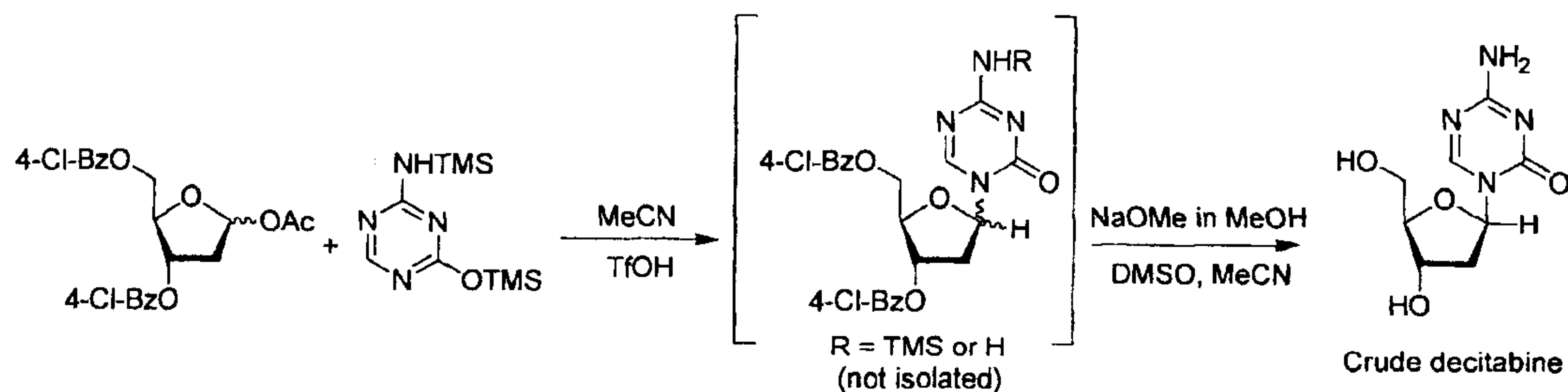
Purification of Crude Azacitidine

[00049] Crude azacitidine (3.2 Kg) was dissolved in DMSO (11.8 Kg) at 20-40°C, filtered and the collected solids were rinsed with DMSO (10.1 Kg). The filtrate was cooled to 20-25°C and MeOH (9.7 Kg) was added over a 30-minute period and then azacitidine seed crystals (30.6 g) were added and the mixture was stirred for about 1 hour at 23°C. More MeOH was added over a 4-hour and 13-minute period and the mixture was stirred at 20-25°C for at least 10 hours, filtered and washed three times with MeOH (10 Kg each). The filter cake was dried under vacuum at less than $\leq 70^{\circ}\text{C}$ for 33 hours to furnish 2.6 Kg of API grade azacitidine (86% yield based on crude azacitidine).

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EXAMPLE 4

One-pot Process for Preparation of crude decitabine



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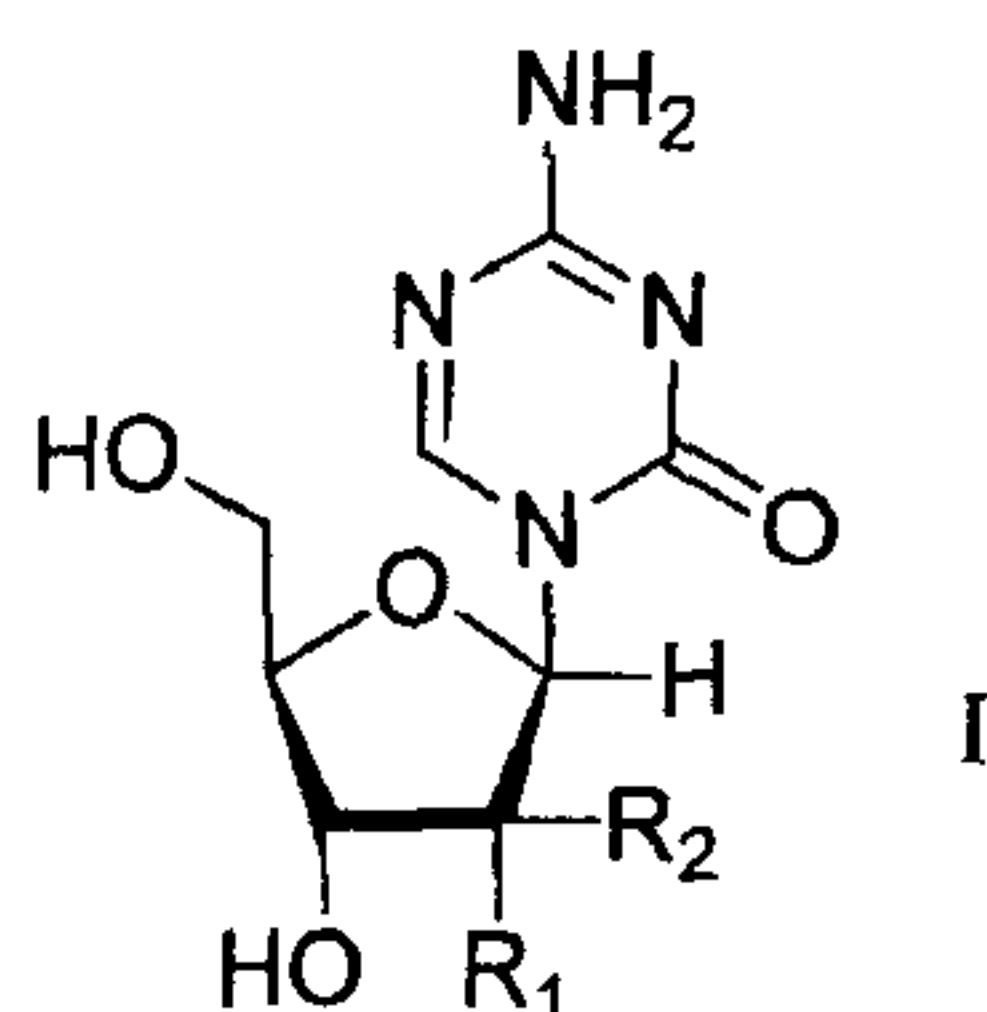
[00050] A mixture of MeCN (45 mL), 1-O-acetyl-3,5-di-O-(4-chlorobenzoyl)-2-deoxy-D-ribofuranose (3.0 g), 2-[(trimethylsilyl)amino]-4-[(trimethylsilyl)oxy]-s-triazine (1.78 g) and TfOH (0.5 g) were stirred at about 0°C for 24 hours. DMSO (6 mL) was added and the mixture was evaporated at 30~50°C under reduced pressure to remove the MeCN. A 29% solution of MeONa in MeOH (1.8 g,

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- 5 9.9 mmol, 1.5 eq.) was added with stirring at about 20~25°C. After the reaction was complete, MeOH was added to effect precipitation and the solid was filtered, washed and dried to give crude decitabine. API grade decitabine (>99.0 HPLC purity) is prepared by the
- 10 recrystallisation of crude decitabine from MeOH, followed by three washes with MeOH and drying at 40°C under vacuum.

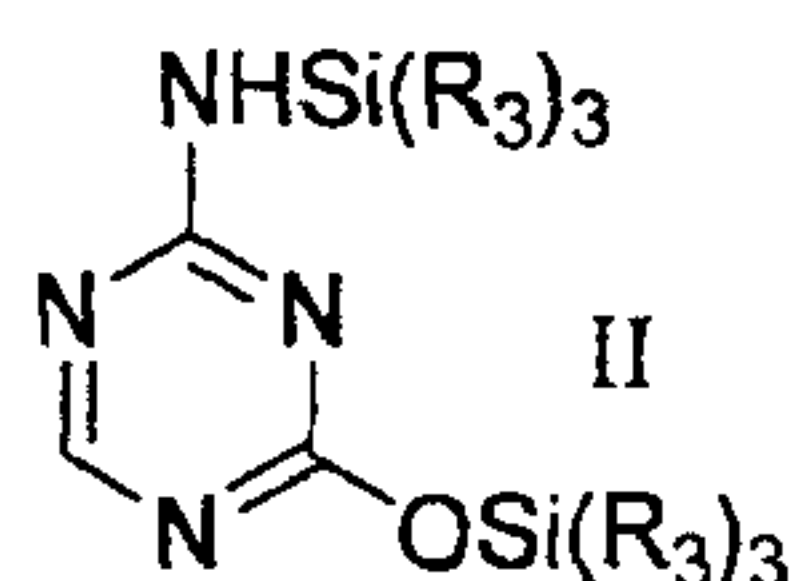
CLAIMS

1. A process for the preparation of a 5-azacytosine nucleoside compound of formula I:



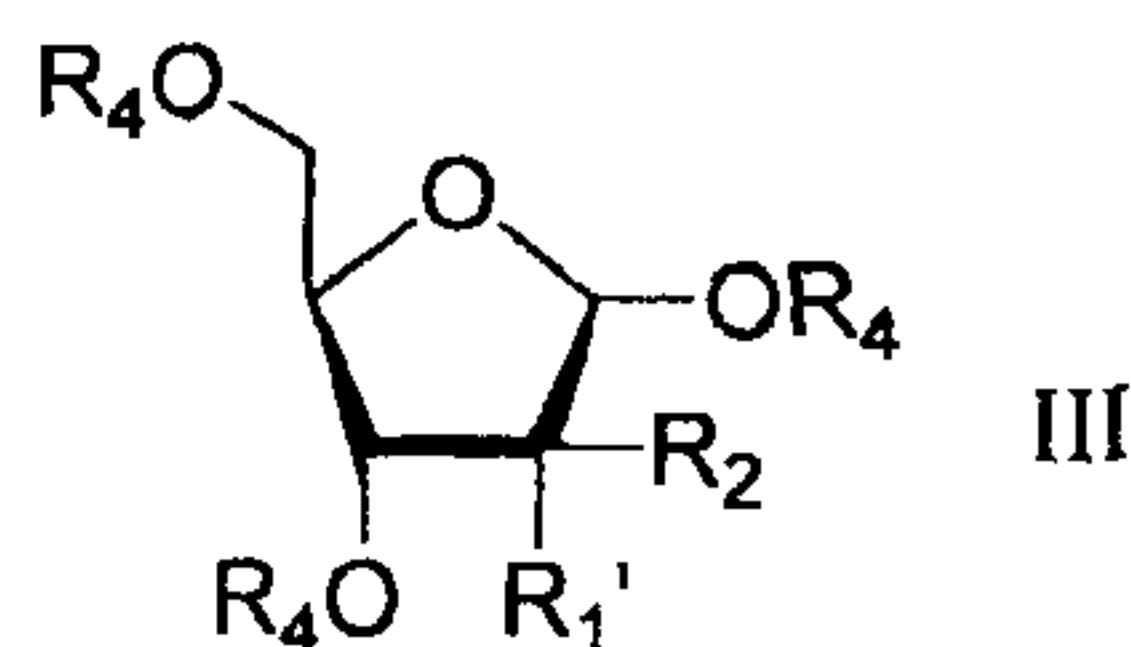
wherein R_1 is hydrogen, hydroxy or halogen; R_2 is hydrogen or halogen, comprising:

a) reacting 5-azacytosine with a silylating agent to produce a reaction mixture containing a silylated 5-azacytosine of formula II:



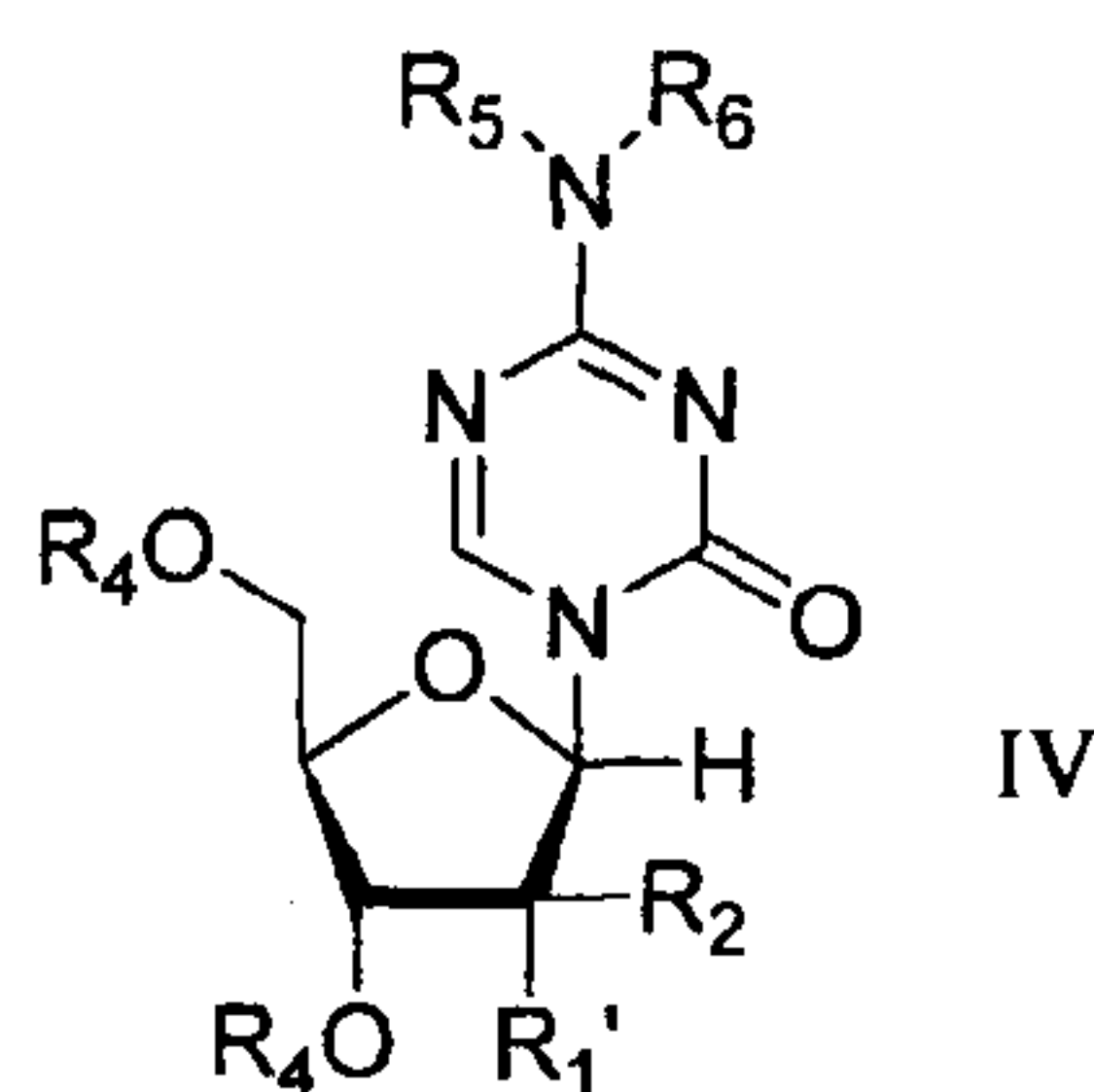
wherein each of R_3 is independently optionally substituted C_1 - C_{20} alkyl group or aryl group;

b) coupling the silylated 5-azacytosine of formula II with a protected D-ribofuranose or 2-deoxy-D-ribofuranose of formula III:



wherein R_1' is hydrogen, halogen or OR_4 ; R_4 represents a hydroxyl-protecting group; and R_2 is as defined above, in a

first organic solvent and in the presence of a sulfonic acid catalyst to obtain a solution of a protected 5-azacytosine nucleoside of formula IV:



wherein each of R_5 and R_6 is independently hydrogen or $\text{Si}(\text{R}_3)_3$, R_3 is an optionally substituted $\text{C}_1\text{-C}_{20}$ alkyl or aryl group, R_1' and R_2 are as defined above, and the amount of the sulfonic acid is less than one molar equivalent with respect to the protected D-ribofuranose or 2-deoxy-D-ribofuranose of formula III; and

c) deprotecting the protected 5-azacytosine nucleoside of formula (IV) to the 5-azacytosine nucleoside of formula (I).

2. The process of claim 1 wherein prior to the step of b), the silylated 5-azacytosine compound is isolated in a solid form from the reaction mixture of the step a).

3. The process of claim 1 wherein the coupling step b) is conducted in the presence of less than 1% by molar amount of the silyating agent relative to the molar amount of the silylated 5-azacytosine compound.

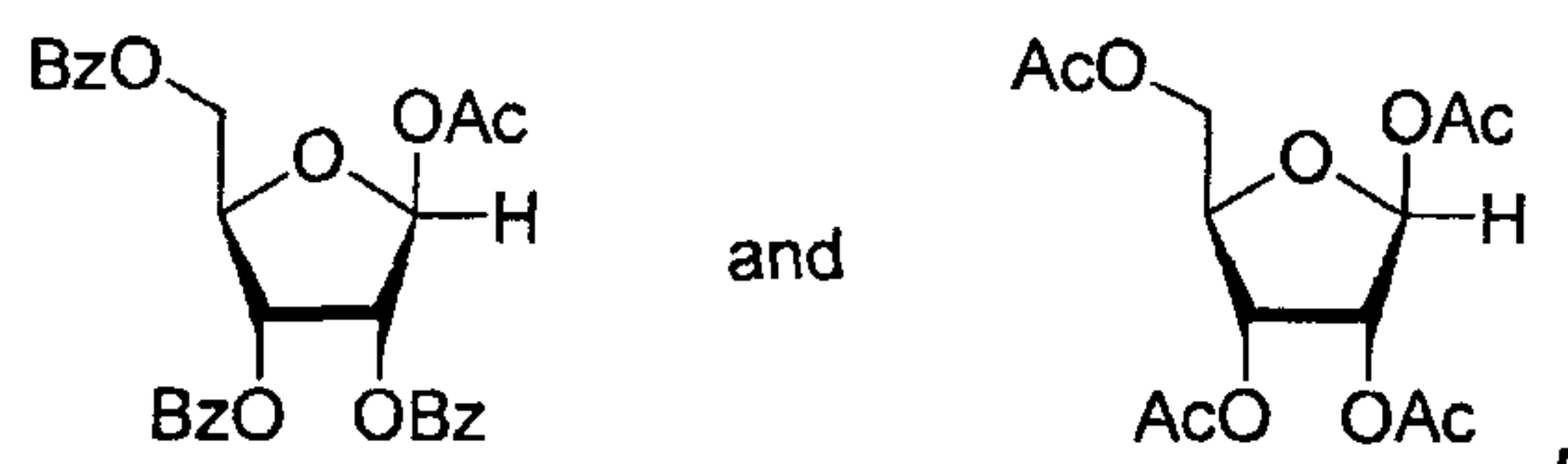
4. The process of claim 1 wherein the process comprises a step of diluting the solution of the protected 5-azacytosine

nucleoside of formula (IV) obtained in the step b) with a second organic solvent, and wherein second organic solvent is a solvent having a higher boiling point than the first organic solvent.

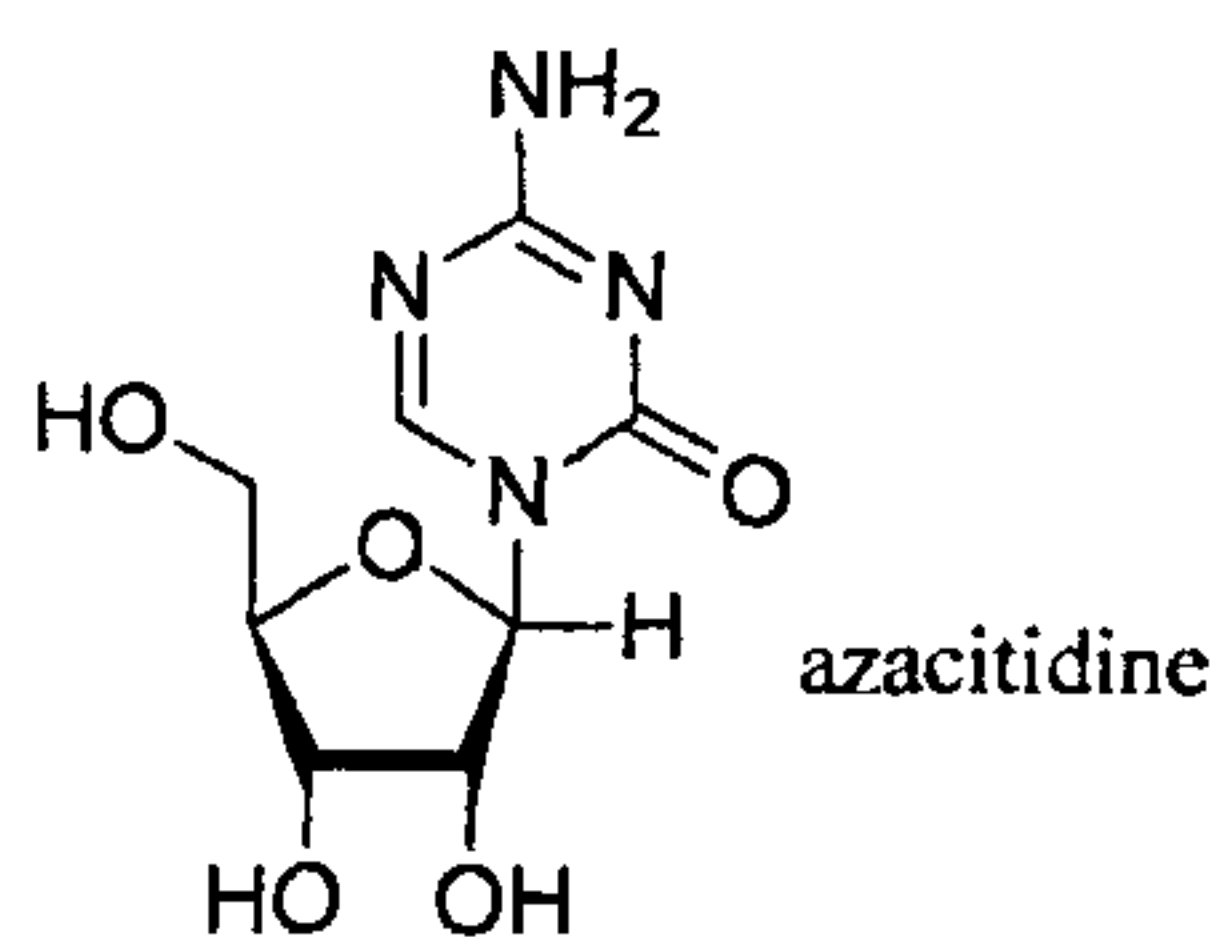
5. The process of claim 4 wherein the second organic solvent is a polar aprotic solvent.

6. The process of claim 4 wherein the second organic solvent is dimethylsulfoxide.

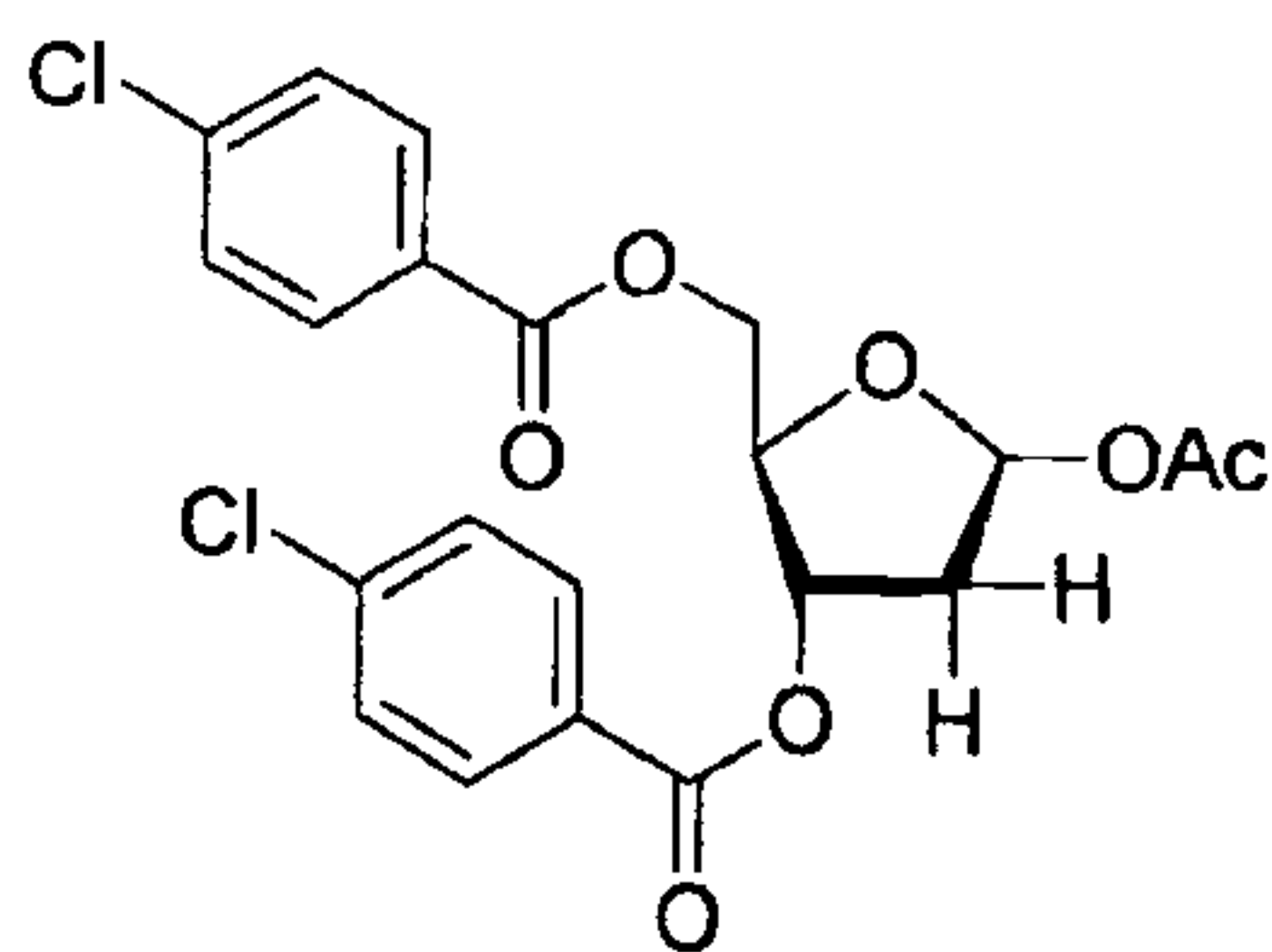
7. The process of claim 1 wherein said protected D-ribofuranose of formula III is selected from the group consisting of



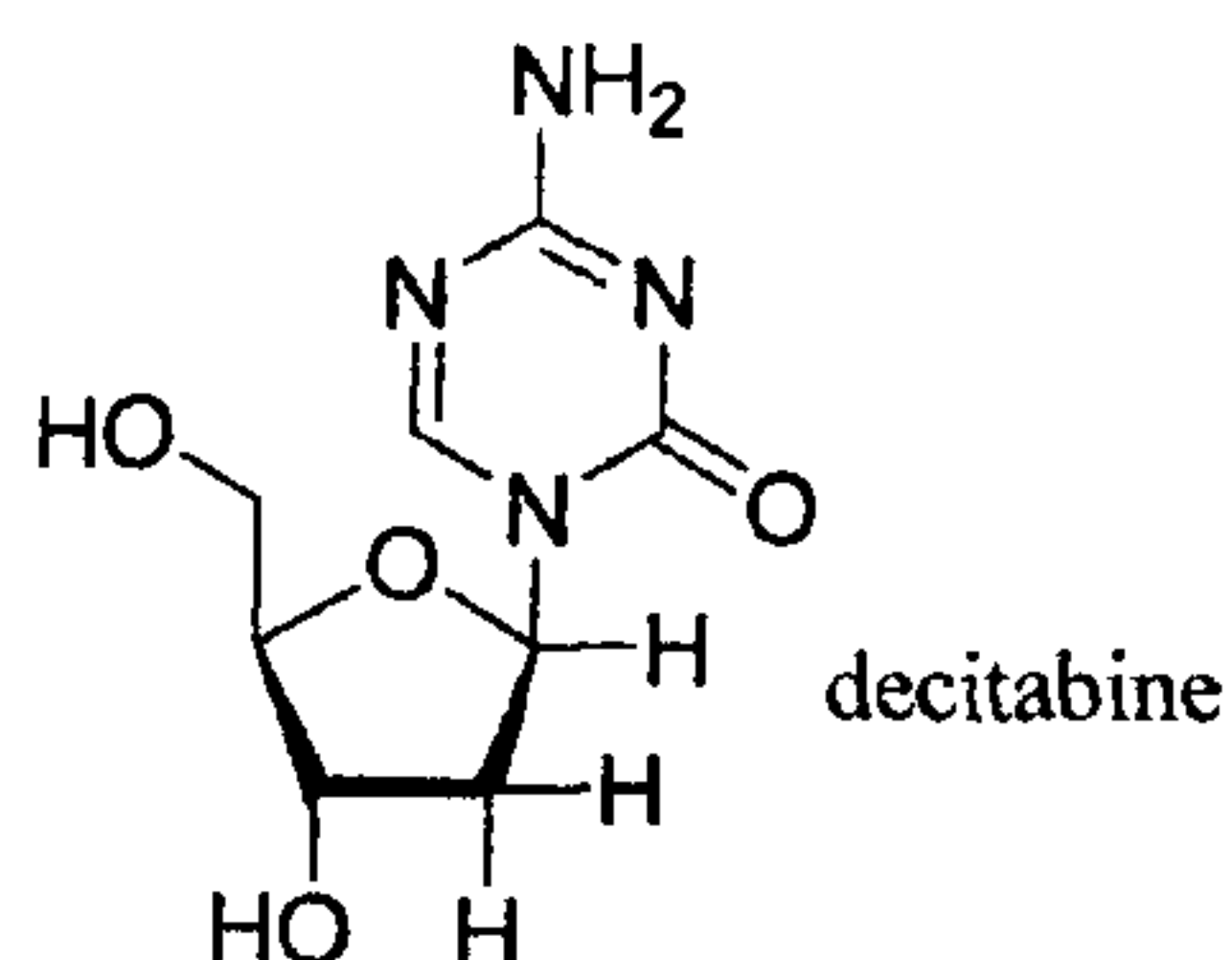
and the 5-azacytosine nucleoside compound of formula I is azacitidine



8. The process of claim 1 wherein said protected D-ribofuranose formula III is a 2-deoxy-D-ribofuranose as shown below



and the 5-azacytosine nucleoside compound of formula I is decitabine



9. The process of claim 1 wherein the first organic solvent is a polar water soluble solvent.

10. The process of claim 1 wherein the first organic solvent is acetonitrile.

11. The process of claim 1 wherein the amount of the sulfonic acid is 10-30 mole percent of the molar amount of the protected D-ribofuranose of formula III.

12. The process of claim 1 wherein the sulfonic acid is trifluoromethane sulfonic acid.

13. The process of claim 1 wherein the silylation agent is hexamethyldisilazane.

14. The process of claim 1 wherein the step c) comprises deprotecting the protected 5-azacytosine nucleoside of formula (IV) in the presence of a basic deblocking agent.

15. The process of claim 14 wherein the basic deblocking agent is a metal alkoxide.

16. The process of claim 15 wherein the metal alkoxide is sodium methoxide in methanol.

17. The process of claim 1 wherein the coupling step b) is conducted in the absence of a water immiscible solvent.

18. The process of claim 1 wherein the steps b) and c) are conducted in one reaction vessel.

19. The process of claim 1 wherein the process is free of a step of isolating the protected 5-azacytosine nucleoside of formula (IV) from a liquid mixture.

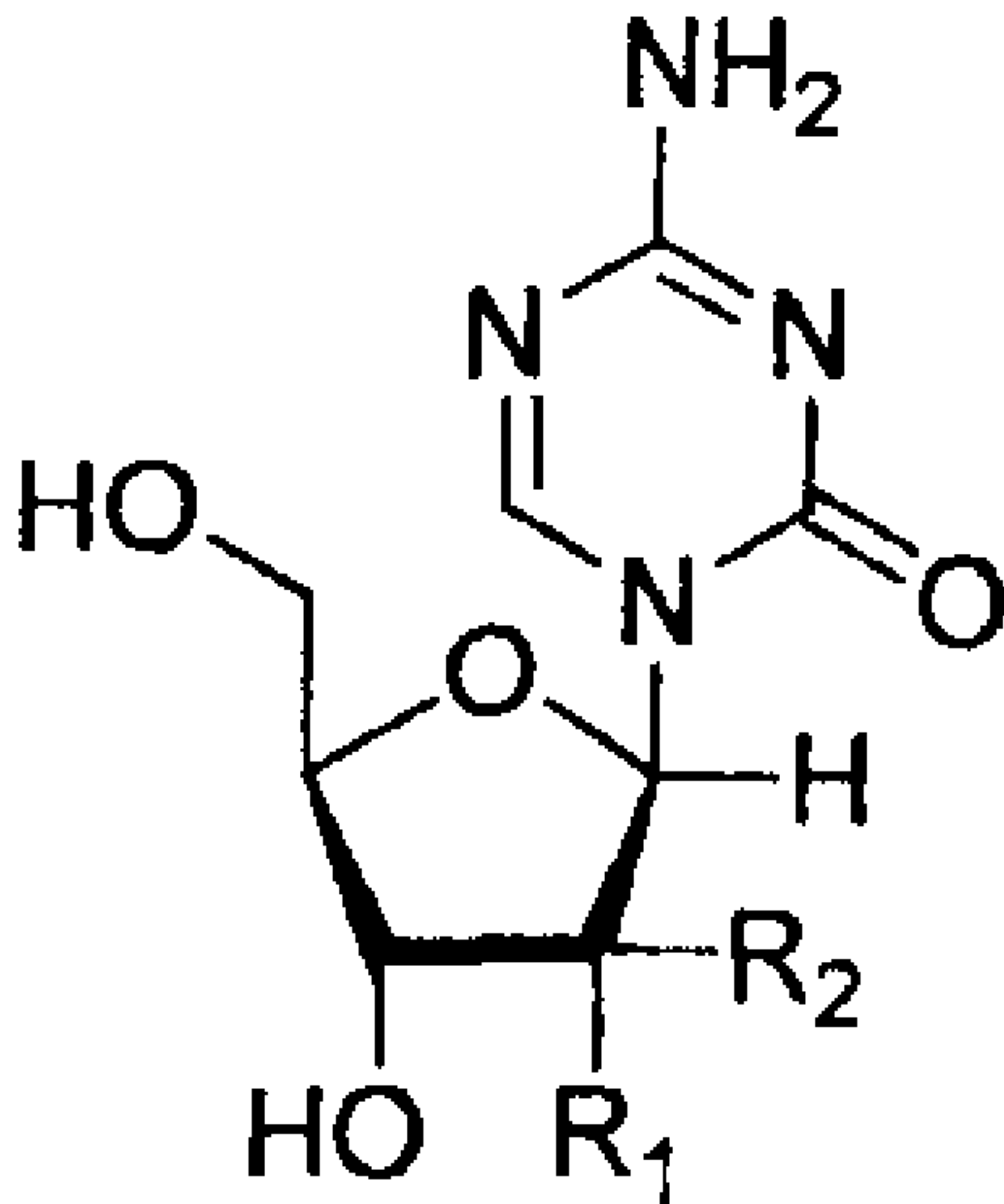
20. The process of claim 1 wherein the process is free of an aqueous work-up step to quench or remove the sulfonic acid catalyst.

21. The process of claim 1 wherein the hydroxyl-protecting group is an optionally substituted C₁-C₂₀ alkyl acyl or aryl acyl group.

22. The process of claim 1 wherein the step c) is conducted at a temperature of 20°C to 30°C.

23. The process of claim 1 wherein the step c) is carried out in a homogenous solution comprising at least a polar aprotic solvent and the protected 5-azacytosine nucleoside of formula (IV) obtained in the step b)

24. The process of claim 1 wherein prior to the step b) the process is free of a step carried out in the presence of a substantial amount of water.



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