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Synthesis of Antitumor Fluorinated Pyrimidine Nucleosides

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Introduction

Nucleosides, due to their biological role as constituents of nucleic acids, are main targets in the development of analogues aimed at antimetabolite-based therapy. Modified nucleosides can disrupt biological processes causing the death of cancer or virally-infected cells.

Fluorinated analogues of biologically active compounds are often characterized by a dramatic change in their activity, compared with the parent molecules. Fluorine, the most electronegative element, is isosteric with a hydroxy group, the C-F bond length (1.35 Å) being similar to the C-O bond length (1.43 Å). In addition, it is the second smallest atom and it can mimic hydrogen in a modified structure; its van der Waals radius (1.47 Å) is intermediate between that of hydrogen (1.20 Å) and that of oxygen (1.52 Å). The strength of the C-F bond exceeds that of C-H bond and for this reason organofluorine compounds are often biologically and chemically more stable than their corresponding natural compounds.

In the case of nucleosides and their analogues, fluorine atoms can be introduced either in the nucleobase or in the sugar moiety. An example of the first type modification is capecitabine[1] (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine), a 5-fluoropyrimidine nucleoside approved as a drug against colorectal, gastric and breast tumors; gemcitabine[2] (2′-deoxy-2′,2′-difluorocytidine) is an example of a nucleoside fluoro-modified in the sugar moiety, approved as a drug against solid tumors.

The aim of this review is to discuss the synthesis of antitumor pyrimidine nucleosides containing fluorine atoms in either the nucleobase or the sugar moiety. Because of the ongoing need of new antitumor chemotherapies, over the years many fluorinated pyrimidine nucleosides have been prepared in order to assay their activity. The synthesis of these compounds has thus been driven largely by results on their biological potential. Nonetheless, it is important for experimentalists to be aware of the full range of methods used, whether or not the compounds synthesized have actually demonstrated antitumor activity. It is our hope, then, that researchers investigating fluoropyrimidines for purposes other than their anticancer properties will also find this article useful.

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This work is dedicated to Dr Giuseppe Celasco deceased on May 10, 2016.

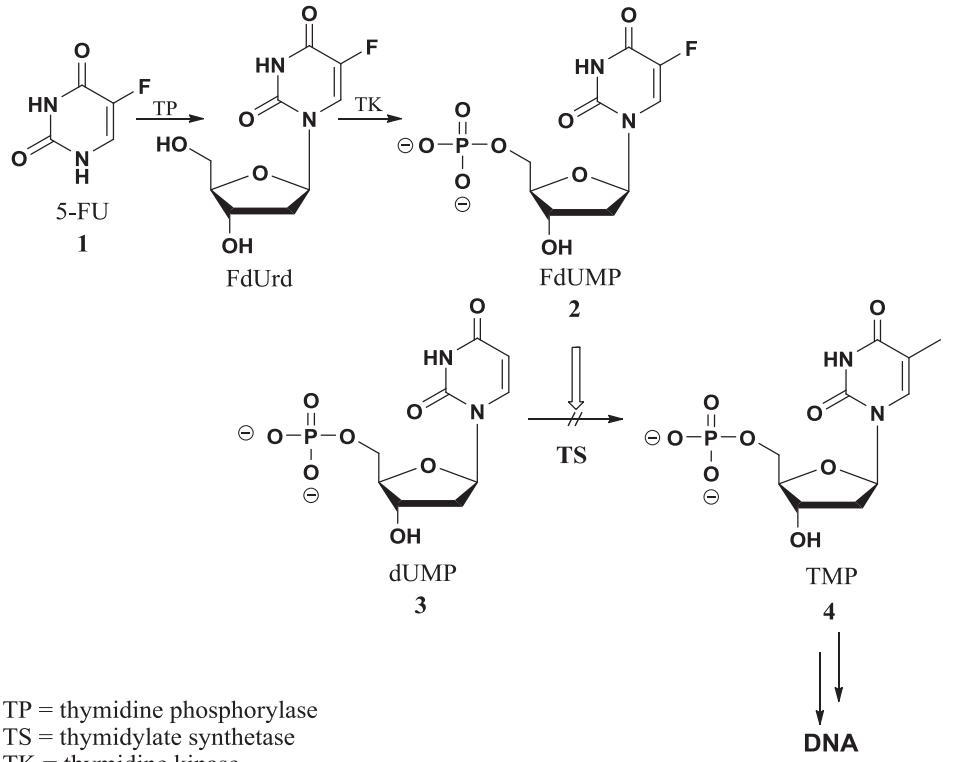
I. Fluoropyrimidines

1. 5-Fluorouracil

Interest in the fluoropyrimidines stemmed from studies of the metabolism of uracil in rat hepatoma cells. The observation that these cells utilize uracil more avidly than normal rat intestinal mucosa prompted the preparation of fluorinated pyrimidines in order to improve disruption of tumor DNA biosynthesis.[3]

In 1957 5-fluorouracil (5-FU) **1** was synthesized by Heidelberger *et al.*,[4] with the aim of blocking metabolism in malignant cells. The replacement of a hydrogen atom at C-5 by the fluorine atom modifies the interaction with the active sites of enzymes involved in metabolism.

This antimetabolite, although toxic, is still one of the most widely used agents against solid tumors. Its action is due to two different mechanisms:[5] after penetration into the cell, 5-FU **1** is transformed into the 5-fluorouridine triphosphate that mimics UTP, is recognized by RNA polymerase and consequently incorporated into RNA. The most significant action, however, is due to the 5-FU conversion into 5-fluoro-2′-deoxyuridine (FdUMP) **2**, a known inhibitor of thymidylate synthetase (TS), a key enzyme in the DNA synthesis.[6,7] TS, in the presence of methylene tetrahydrofolate and deoxyuridine monophosphate (dUMP) **3** forms a ternary complex that catalyzes the substitution of 5-H uracil with a methyl group, affording thymine. If FdUMP **2** is present, the above ternary complex is not able to carry out this reaction, due to the presence of fluorine in the 5-position: the formation of TMP **4** (2′-deoxythymidine 5′-monophosphate), the only nucleotide precursor specific to DNA, is, therefore, blocked (*Scheme 1*), decreasing the availability of TTP (2′-deoxythymidine 5′-triphosphate) for DNA synthesis.

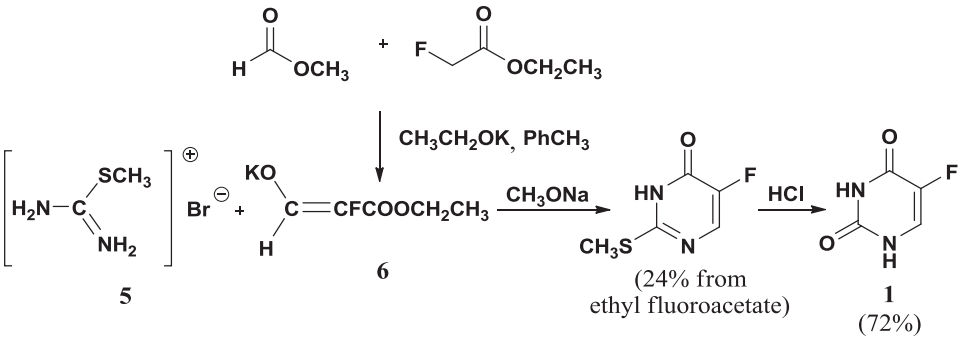


TP = thymidine phosphorylase
TS = thymidylate synthetase
TK = thymidine kinase

Scheme 1

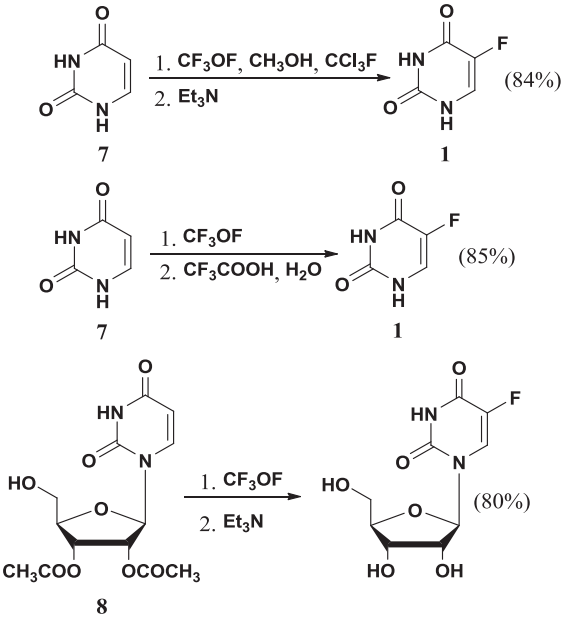
Later two additional mechanisms were proposed for 5-FU **1** antitumor activity: the incorporation of 5-FU into DNA and the alteration of the membrane function of 5-FU **1** treated cells. Recent studies indicate that the TS-direct mechanism predominates when **1** is administered at low doses for a prolonged time, whereas the RNA-mediated process is more active following a bolus administration.[8–10]

Synthesis of 5-FU **1**, by construction of the pyrimidine ring, was first realized by Heidelberger *et al.* in 1957[4,11,12] by reaction of a thiourea derivative **5** with the enolate of ethyl α-fluoro, α-formyl acetate **6** which, in turn, was obtained from methyl formate and ethyl fluoroacetate (*Scheme 2*). Depending on the chosen α-fluoro-β-ketoester the method is also applicable to the synthesis of other 5-fluoropyrimidines.



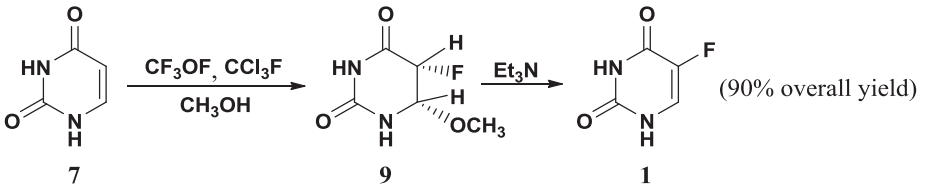
Scheme 2

An alternative method for the total synthesis is the direct fluorination of the pyrimidine ring of compound **7** by means of trifluoromethyl hypofluorite (CF_3OF)[13] proposed by Robins in 1971. In the case of CF_3OF in methanol/fluorotrichloromethane an intermediate was formed that, after treatment with triethylamine, afforded 5-FU **1** in 84% yield.[13] If the reaction was carried out with CF_3OF in trifluoroacetic acid **1** was directly isolated in 85% yield.[14] The method proposed by Robins[13] is suitable also for the direct introduction of fluorine on preformed nucleosides, for example compound **8** (*Scheme 3*).



Scheme 3

The mechanism of the reaction with CF_3OF in methanol followed by treatment with triethylamine was later investigated by Robins *et al.*[15] who assigned the structure of (±)-*cis*-5-fluoro-6-methoxy-5,6-dihydrouracil to intermediate **9** that, by treatment with triethylamine, afforded 5-FU **1** (*Scheme 4*).

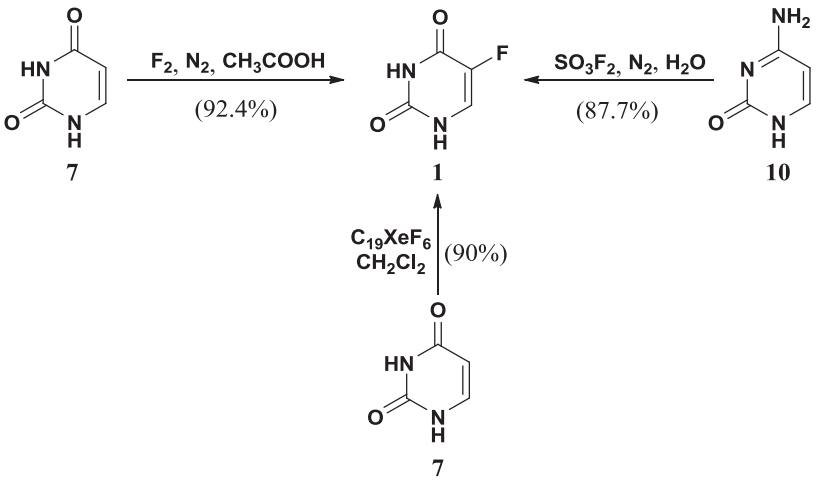


Scheme 4

The use of fluorine as fluorinating agent[16,17] requires efficient dissipation of the heat of reaction, in order to avoid the destruction of the carbon skeleton of pyrimidine. This result can be achieved by bubbling a mixture of fluorine and an inert gas through a cold liquid, or removing the heat of reaction by carrying out the reaction in the presence of a metal packing or, finally, by addition of very large amount of an inert diluent gas. This last procedure is the most followed and usually fluorine is diluted with an equal amount of nitrogen and then passed through the reaction mixture. 5-FU **1** was obtained in 92.4% yield[16] and sublimation at 190°C and 1 mm Hg provided a highly pure product.[17]

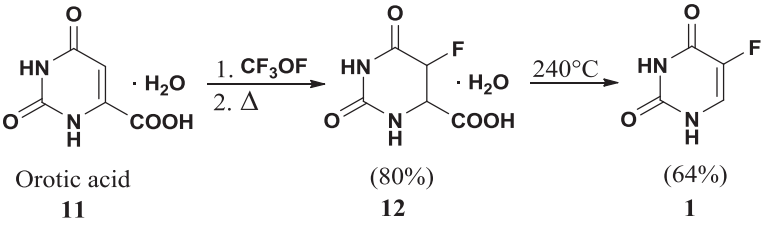
Xenon difluoride can be used for direct fluorination of the pyrimidine ring but it is difficult to handle due to its high reactivity; in 1980 Kagan *et al.* realized the direct fluorination of uracil **7** employing $C_{19}XeF_6$, which is much more stable than free xenon hexafluoride.[18]

Uracil **7** was the starting material of the above described direct fluorination methods.[13–18] In a method patented in 1979,[19] cytosine **10** was fluorinated by means of fluorine fluorosulfonate ($FOSO_2F$) diluted with nitrogen (60%) affording 5-FU **1** (87.7% yield) (*Scheme 5*).



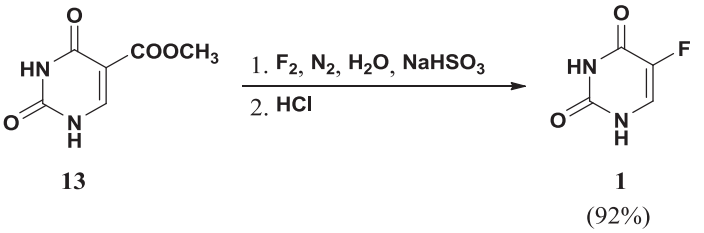
Scheme 5

Orotic acid[20] **11** was, instead, the starting material for a synthesis of 5-FU by a combination of a fluorination and a decarboxylation (*Scheme 6*). In the course of the reaction an intermediate was formed that can be converted into 5-fluoro orotic acid **12** (in boiling water) which was, in turn, transformed into 5-FU by heating at 240°C.



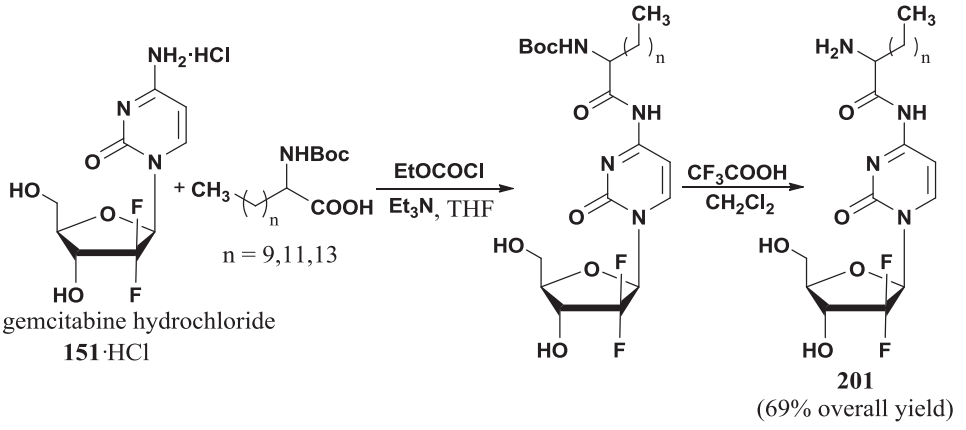
Scheme 6

Starting from the methyl ester of uracil 5-carboxylic acid **13** the synthesis of 5-FU **1** was realized in one-pot, in excellent yield (92%), by addition of a reducing agent (sodium bisulfite) aimed to exclude the formation of peroxides by reaction of water with fluorine (*Scheme 7*).[21,22]



Scheme 7

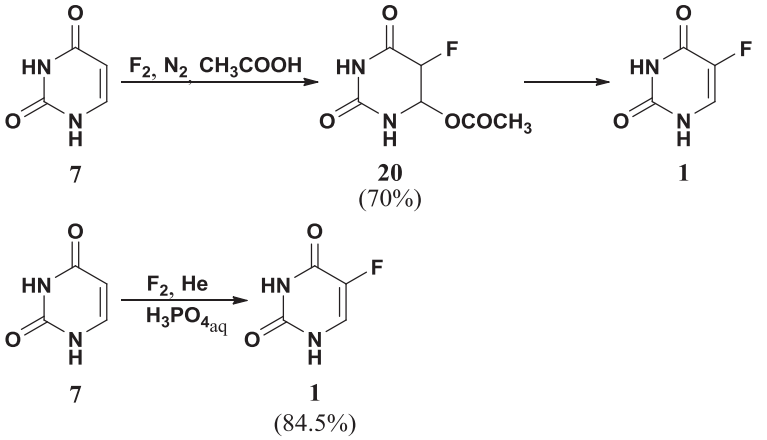
A different approach to the synthesis of **1** provided the substitution of the halo atoms of the 2,4,5-trichloropyrimidine or 2,4-dichloro,5-bromopyrimidine **14** by means of potassium fluoride at 400°C to afford the corresponding 2,4,5-trifluoropyrimidine **15**; its treatment with sodium hydroxide in water at 80°C gave the desired 5-FU **1**.[23] The starting 2,4,5-trihalopyrimidine **14** was obtained from uracil **7** by reaction with chlorine, or bromine, followed by treatment of the 5-halouracil **16** with phosphoryl chloride (76 or 82%, respectively) (*Scheme 8*).



Scheme 8

Some years later a similar approach[24] starting from tetrafluoropyrimidine[25] **17**, was described by Baasner *et al.* 4,6-Dichloro-2,5-difluoropyrimidine **18** was obtained in 64% yield from tetrafluoropyrimidine **17** by reaction with gaseous hydrogen chloride. Chlorine in the 6-position was selectively removed by hydrogenation in the presence of palladium on carbon in ethyl acetate and triethylamine (70.5% yield). The resulting 4-chloro-2,5-difluoropyrimidine **19** was hydrolyzed with sodium hydroxide in water affording 5-FU **1** in 93% yield.

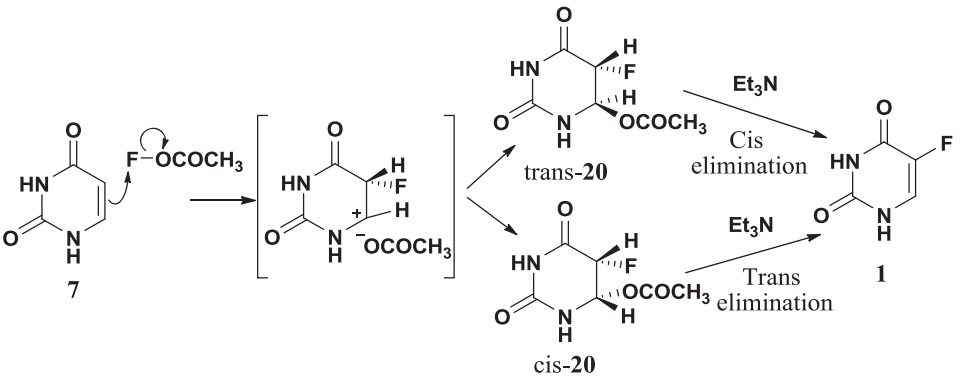
Direct fluorination of uracil continued to be of interest. In 1981 a method based on the use of fluorine/nitrogen in acetic acid was proposed: the intermediate 6-acetoxy-5-fluoro-5,6-dihydrouracil **20**, obtained in 70% yield, was converted into the desired 5-FU **1** by acetic acid elimination.[26] (*Scheme 9*).



Scheme 9

In a similar approach the use of fluorine diluted with helium in aqueous phosphoric acid[27] (*Scheme 9*) afforded 5-FU **1** in 84.5%. The advantages of this method are the low cost of phosphoric acid, the capability of its solution to work as an adsorbent of the by-product hydrogen fluoride and to precipitate the final product as crystals.

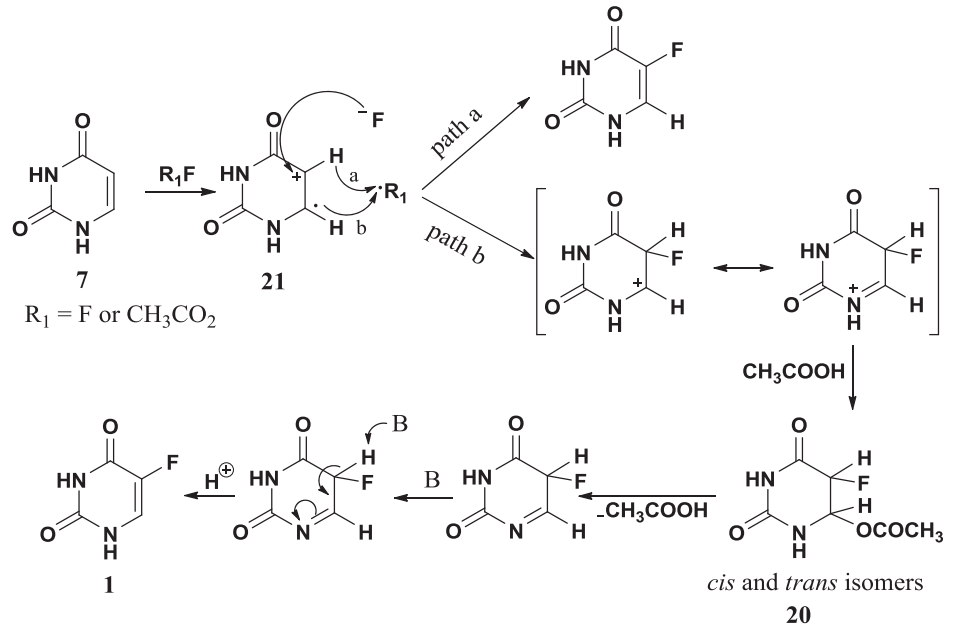
Another fluorinating agent, acetyl hypofluorite, (generated *in situ* from fluorine and acetic acid) was studied, in comparison with fluorine in acetic acid, from a mechanistic point of view.[28] The two geometric isomers of 5-fluoro-6-acetoxyuracil **20** were isolated as reaction intermediates; their configuration cannot be assigned by ^{1}H and ^{19}F NMR spectra since the coupling constants of *cis* and *trans* compounds are too similar. Only one of the isomers, by addition of acetate ion, was transformed into 5-FU **1**, acetate being a strong enough base to facilitate acetic acid elimination. The *cis* and *trans* configurations were assigned considering that in the *cis* isomer the H atom and the acetate group are in *trans* position, which is the favored one for the elimination of acetic acid. In presence of triethylamine both isomers afforded 5-FU **1** (*Scheme 10*).



Scheme 10

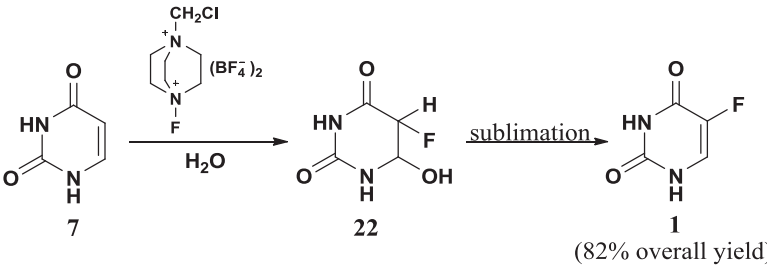
Visser *et al.*[29–30] also studied the mechanism and the stereochemistry of uracil **7** fluorination with fluorine or acetyl hypofluorite, proposing that addition at the 5–6 double bond occurs through radical anion **21**; formed 5-fluoro-6-acetoxy derivative **20** evolves into the final product through an intermediate that in the presence of a base affords final 5-FU **1**[29] (*Scheme 11*).

The influence of the N-1 substituent on the stability of 5-fluoro-6-acetoxy intermediate[31] and conversion by reaction with alcohols were also studied.[30]



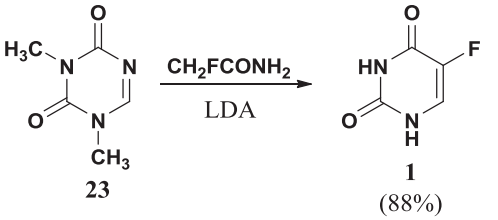
Scheme 11

A commercially available, stable, easy to handle fluorinating agent, SELECTFLUOR, was proposed in 1995 for the synthesis of fluorohydrin **22** that, by sublimation, provided 5-FU **1** (82%)[32] (*Scheme 12*).



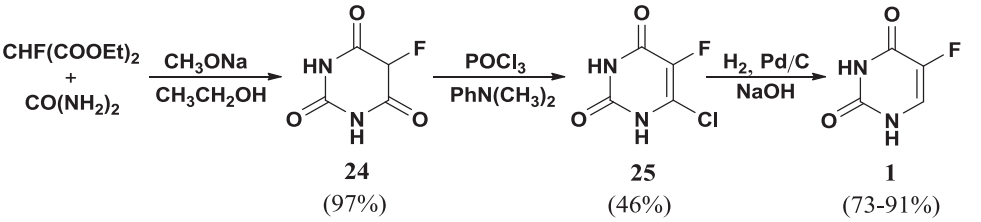
Scheme 12

Some approaches avoiding the direct fluorination are reported in the literature. For example, starting from *s*-triazine **23**, it is possible to prepare 5-FU **1** (88% yield) by reaction of fluoroacetamide and lithium diisopropylamide (*Scheme 13*).[33]



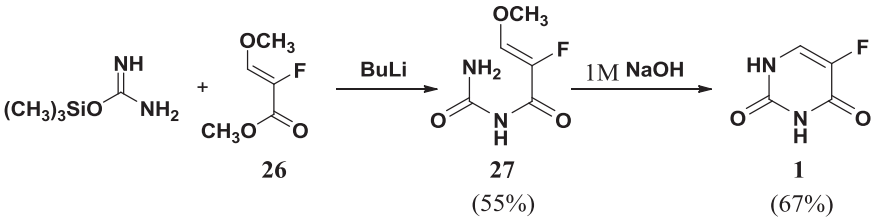
Scheme 13

Diethyl fluoromalonate (prepared from trifluoroacrylic acid) was easily converted to 5-fluoro-6-chlorouracil **25** by reaction with urea to afford 5-fluorobarbituric acid **24** (97% yield) followed by reaction with phosphoryl chloride in dimethylaniline (46% yield). The 6-chlorine atom of **25** was removed by hydrogenolysis (Pd/C) affording 5-FU **1** in 73–91% yield, depending on the reaction conditions (*Scheme 14*).[34]



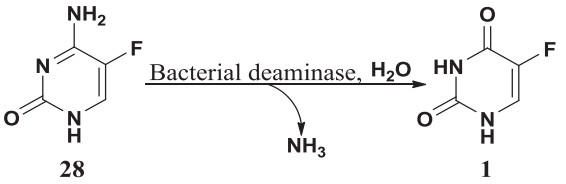
Scheme 14

The methyl ester of 2-fluoro-3-methoxy acrylic acid **26** can be used as a synthon of the fluorinated uracil ring: by reaction with O-(trimethylsilyl)urea intermediate **27** was formed that by cyclization with sodium hydroxide afforded 5-FU **1** (67% yield) (*Scheme 15*).[35]



Scheme 15

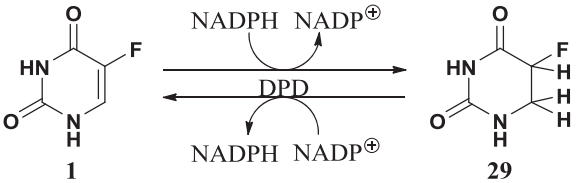
Recently,[36] 5-FU **1** was prepared by deamination of 5-fluorocytosine **28** by means of a new 5-methylcytosine deaminase: expression of deaminase in *E. coli* caused an efficient transformation of non-toxic 5-fluorocytosine **28** into 5-FU **1** that abolished or severely inhibited growth of cells. The goal of this study was, of course, to transfect cancer cells with the deaminase (*Scheme 16*).



28 **1**

Scheme 16

5-FU **1**, since its introduction more than 50 years ago, has become a component of therapy for gastrointestinal, head and neck and breast cancers. The activity of 5-FU is limited by its rapid degradation into 5,6-dihydro-5-fluorouracil (5-FUH_2) **29** under the action of dihydropyrimidine dehydrogenase (DPD), an enzyme NADPH-dependent (*Scheme 17*).[5] It has been demonstrated that this enzyme deactivates more than 85% of the injected 5-FU **1**.



1 **29**

DPD = dihydropyrimidine dehydrogenase

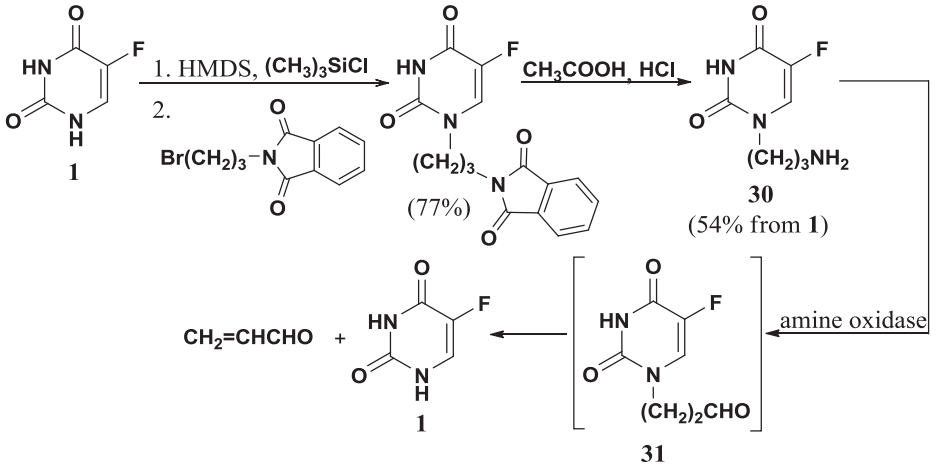
Scheme 17

At the beginning 5-FU **1** was administered by intravenous bolus but its low biological $t_{1/2}$ makes continuous infusion more convenient. The use of oral 5-FU **1** was abandoned some decades ago because of its irregular absorption, due to intra- and inter-individual differences depending on the variable activity of dihydropyrimidine dehydrogenase. In addition to the inconvenience of i.v. administration, the efficiency of 5-FU **1** is limited by its toxicity due to phosphorylation in the digestive tract and to the lack of selectivity toward tumors. The development of 5-FU **1** derivatives was the target of many studies. The toxicity of 5-FU **1** can be reduced by derivatives which are stable to enzymatic degradation, by derivatives that inhibit DPD or by prodrugs of **1** that liberate the active principle in tumor cells. A prodrug is defined as a pharmacologically inactive compound that is converted into an active agent by metabolic transformations. The prodrugs of 5-FU **1** are characterized by a pyrimidine ring bearing a fluorine atom in the 5 position. The main benefit is oral administration, with the improvement in quality of life of the patient.

In the present review we now describe some derivatives in which 5-FU **1** is conjugated to drug carriers or molecules endowed with antitumor activity.

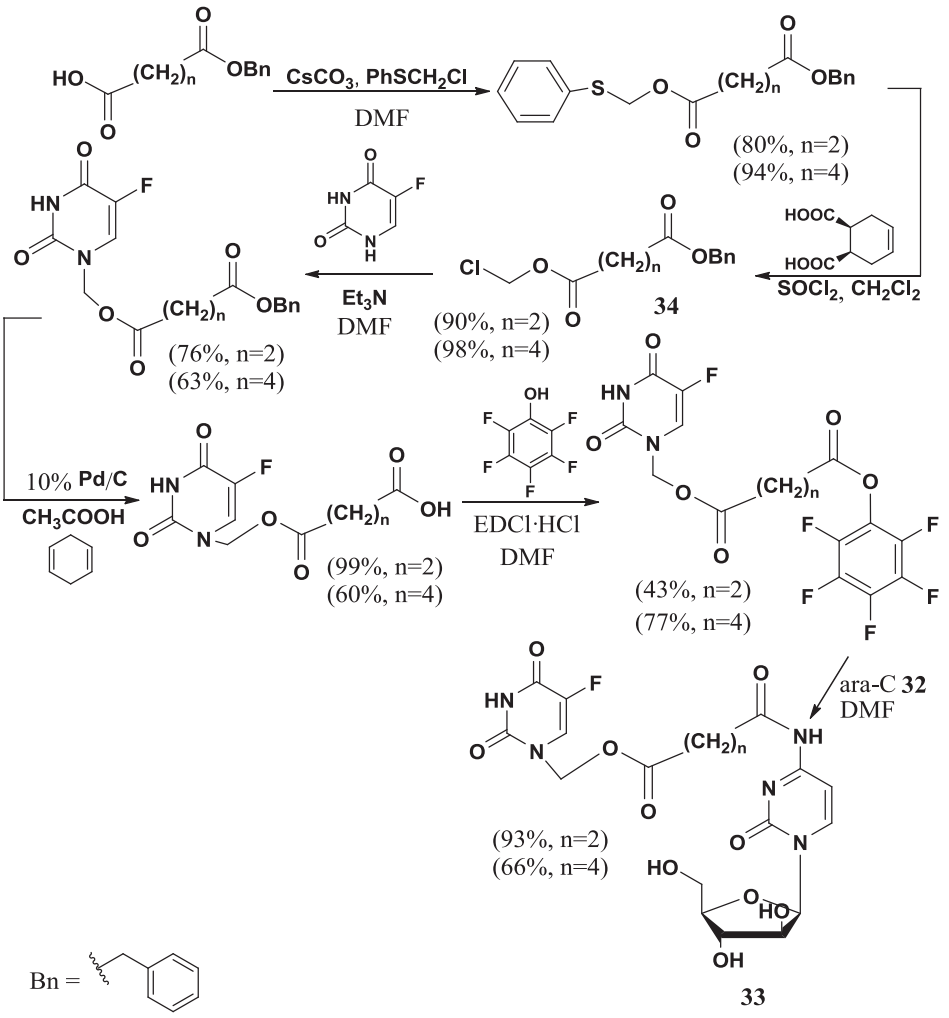
2. 5-Fluorouracil Derivatives

In 1985 N^1-(3-aminopropyl)-5-fluoro uracil **30** was synthesized from 5-FU **1** by treatment with hexamethyldisilazane and trimethylchlorosilane followed by reaction with N-(3-bromopropyl)-phthalimide. Deprotection afforded the desired product in 54% yield. The amino derivative was considered a suitable precursor of N^1-(2-formylethyl)-5-fluorouracil **31**, which may be converted to 5-FU following an enzymatic reaction. Indeed, in presence of amine oxidase the amine was transformed into 5-FU **1** and acrolein, in the course of 24 h incubation. The slow enzymatic conversion, however, indicated that 5-FU-derivative **30** might not be efficiently metabolized *in vivo* (*Scheme 18*).[37]



Scheme 18

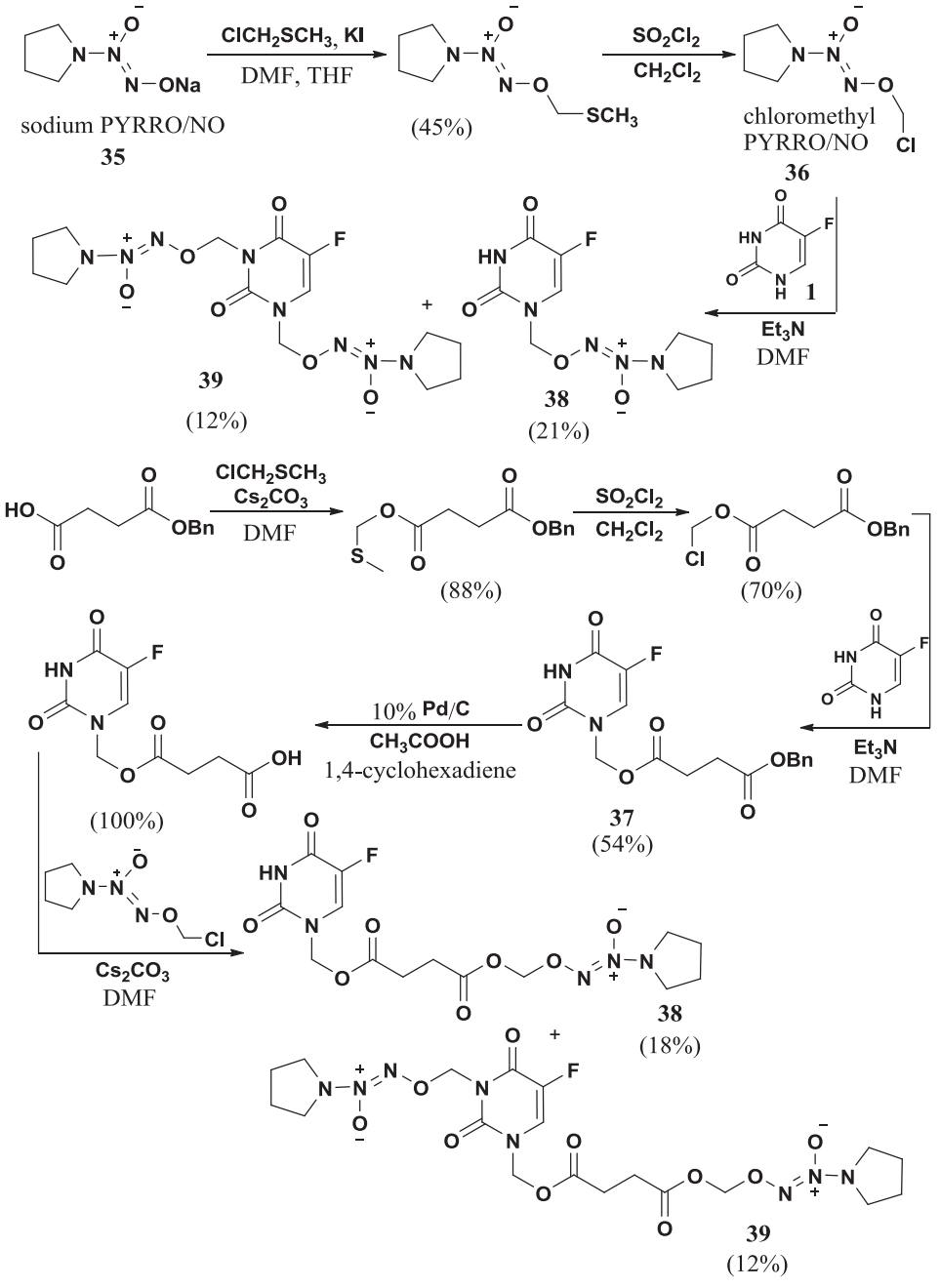
A mutual prodrug consists of two synergistic drugs joined together. The two molecules may be connected directly or by means of a linker: in 1997 work[38] 5-FU **1** was linked to cytarabine **32**, an antitumor agent used mainly in the treatment of acute leukemia and lymphomas, affording compound **33**. Cytarabine (ara-C) **32** is attached to the double drug through a hydrolyzable amide bond, while 5-FU is attached *via* an acyloxymethylene group, easily removable. Two spacers with different length (two or four methylene groups) were used and the synthesis is outlined in *Scheme 19*. The synthesis afforded the product with yield ranging from moderate to excellent; and, after the introduction of 5-FU **1**, mild conditions and selective reagents, due to the formation of the labile N-O ketal derivative, were required. The benzenesulfenyl chloride, by-product of the preparation of chloromethylester **34**, was removed by trapping it with cyclohexene-1,2-dicarboxylic acid, instead of the usual cyclohexene, in order to remove the addition product by an aqueous washing. This avoided separation by distillation of products having similar boiling points. Final compound **33** with $n = 4$ was stabler than the corresponding compound with $n = 2$, over a wide range of pH, and for this reason it was estimated to be more suitable for biological testing.



EDCl·HCl = 1-(3-(dimethylamino)propyl)-3-ethylcarbodiimide hydrochloride
ara-C = cytarabine

Scheme 19

More recently[39] another mutual prodrug of 5-FU **1** was developed with diazeniumdiolates, which are known to be controlled sources of NO. NO can inhibit metastasis, enhance cancer cells apoptosis and assist macrophages to kill tumor cells. Two different conjugates, with methylene or acyloxymethylene as spacers, were synthesized starting from sodium 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (PYRRO/NO) **35** in turn prepared according to a procedure already reported.[40] The key intermediate of the two syntheses was the O^2-chloromethyl 1-(pyrrolidin-1-yl)diazen-1-ium-1,2-diolate (chloromethyl PYRRO/NO) **36** that by direct reaction with 5-FU **1** or through its succinic acid derivative **37** afforded the desired products **38** together with the N^1,N^5-bisalkylation products **39** (*Scheme 20*). The two mono-alkylated conjugates were evaluated in hydrolysis by monitoring the NO release. Both prodrugs showed greater activity than 5-FU, but the

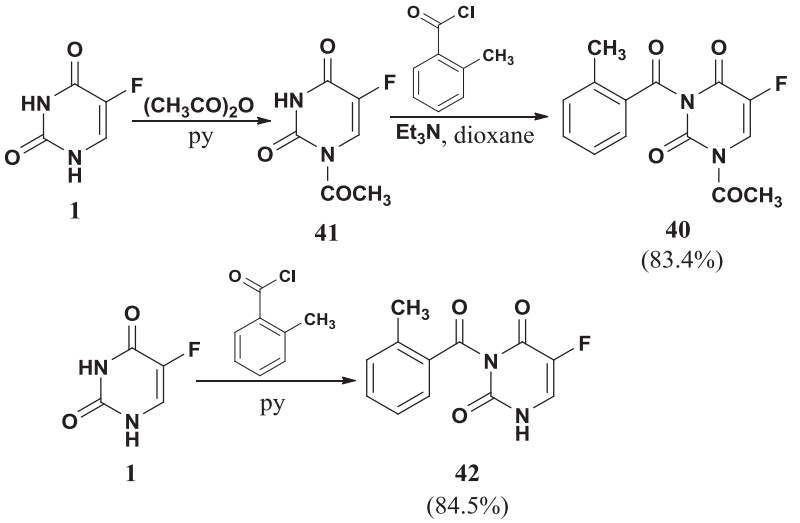


Scheme 20

compound with methylene as spacer was very stable in aqueous solution and no substantial NO was detected. On the contrary the other prodrug with a longer spacer released NO both at pH 8 and in the presence of esterase.

In order to enhance the delivery characteristics of 5-FU **1** a series of N-acyl and N-alkoxycarbonyl derivatives were prepared and their antitumor activity evaluated.[41,42]

According to a 1980 article[41], the most promising antitumor agent, even when administered orally, was the N^1-acetyl-N^3-*ortho*-toluyl-5-fluorouracil **40**. The paper described the different acylated products that can be obtained depending on the chosen acylating agent, solvent and reaction temperature. N^1-Acetyl-N^3-*ortho*-toluyl-5-FU **40** was prepared starting from N^1-acetyl derivative **41** by reaction with *ortho*-toluyl chloride in dioxane and triethylamine. The N^3-*ortho*-toluyl derivative of 5-FU, **42**, was isolated in mice serum when N^1-acetyl-N^3-*ortho*-toluyl-5-FU **40** was orally administered. In order to obtain this N^3-monosubstituted compound **42** the best conditions used *ortho*-toluyl chloride in pyridine at room temperature (*Scheme 21*).

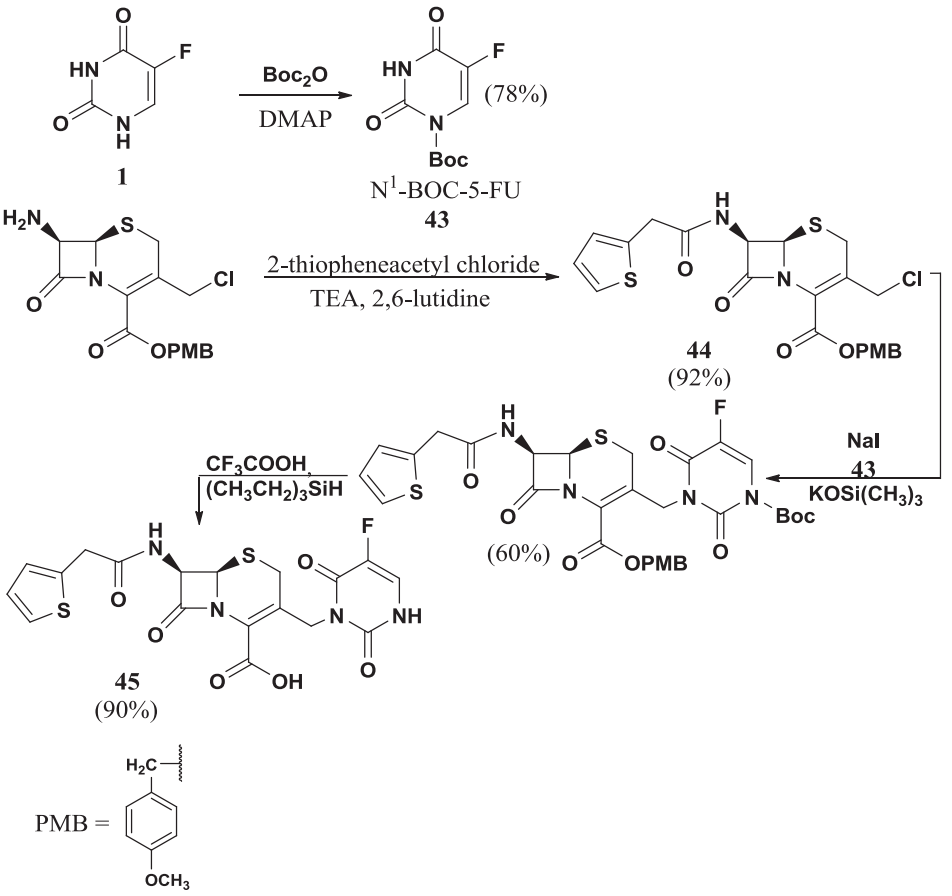


Scheme 21

Further, the N^1-alkoxycarbonyl derivatives[42] prepared from 5-FU **1** by reaction with the appropriate chloroformates in pyridine were hydrolyzed in serum affording the 5-FU **1**.

β-Lactam based prodrugs, in particular cephalosporin-based prodrugs, have been reported in the past and in 2009[43] this approach was also applied to 5-FU **1**. The 5-FU-cephalosporin conjugate was synthesized starting from the N^1-BOC-5-FU **43** and the suitably functionalized cephem **44** (*Scheme 22*), affording a compound stable in aqueous media; conjugate **45** in the presence of *β*-lactamase was completely cleaved to 5-FU **1** and the hydrolyzed cephalosporin, demonstrating its strategic potential against a range of human carcinoma cells.

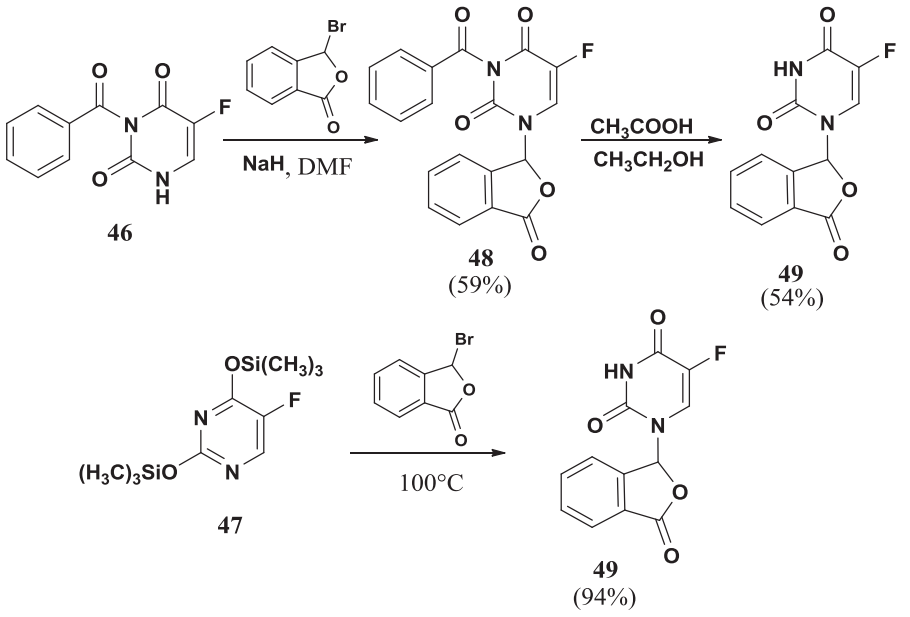
The observation that the phthalide fragment was widely used in the creation of transport forms of antibiotics prompted the introduction of this moiety in the



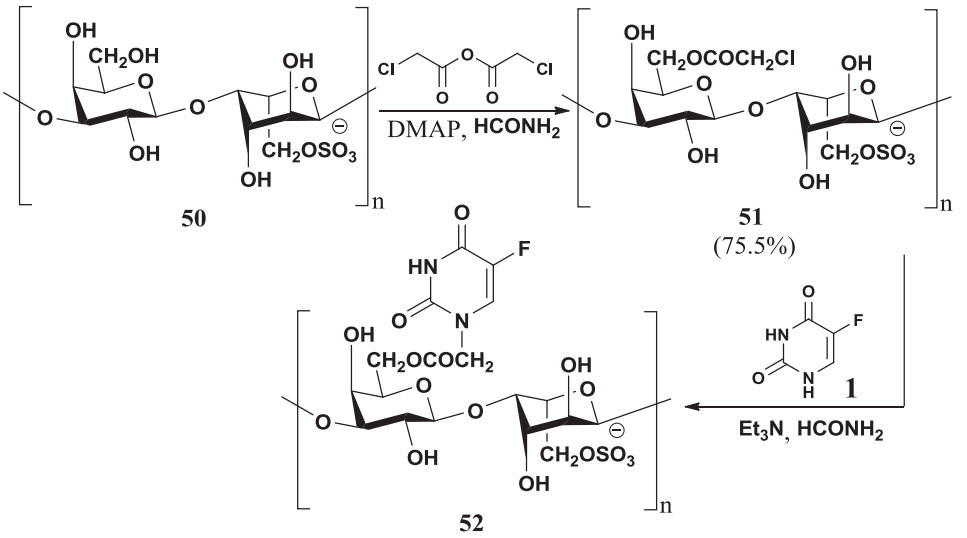
Scheme 22

5-FU **1** molecule.[44,45] 1-(3-Phthalidyl)-5-fluorouracils were synthesized starting from the N^3-benzoyl-5-FU **46**[44] or from the bis(trimethylsilyl) derivative of 5-FU **47**[45] (*Scheme 23*). In the first case N^3-benzoyl-5-FU **46**[44] was reacted with 3-bromophthalide in presence of sodium hydride affording phthalide derivative **48** in 59% yield. Subsequent acidic hydrolysis gave desired product **49** in 54% yield. The phthalide derivative was effectively biotransformed into 5-FU **1** in mice serum. Starting from 2,4-bis(trimethylsylil)-5-fluorouracil **47** [45] phthalide derivative **49** was obtained in 94% yield by reaction with 3-bromo phthalide at 100°C.

Some 5-FU prodrugs with a removable attachment were also proposed. For example porphyran **50**, a polysaccharide from the red algae *Porphyra haitanensis*, was employed as a drug carrier, allowing the fixation at 5-FU **1** at the 6-position through an acetyl spacer group of polysaccharide derivative **51** (*Scheme 24*).[46] The release of 5-FU **1** from conjugate **52** was studied in 0.01M NaOH solution, in 0.1M HCl solution and in phosphate buffer. The amount of released 5-FU **1** was significantly different, and only in basic media did the prodrug release quickly the 5-FU **1**. *In vivo* studies are not reported.

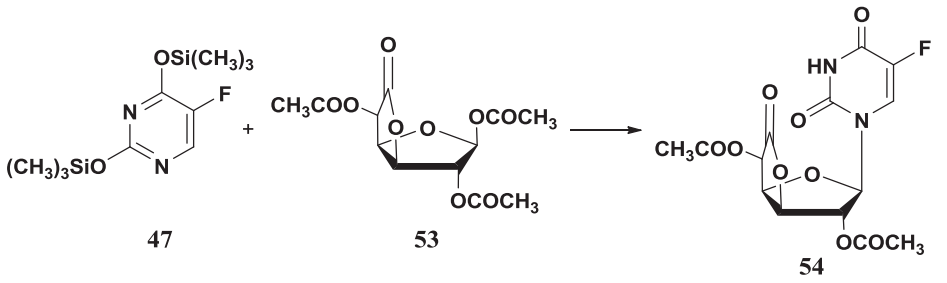


Scheme 23



Scheme 24

In order to decrease the toxicity of 5-FU 1,2,5-triacetyl-β-D-glucofuranurono-6,3-lactone **53** was reacted with 2,4-bis(trimethylsylil)-5-fluorouracil **47**, according to a reported method.[47] The obtained molecule combines the active principle 5-FU **1** and the
275 nontoxic glucuronic acid, which participates in the detoxification of xenobiotics in the organism by forming the glucuronides (*Scheme 25*).[48] Biological studies showed that

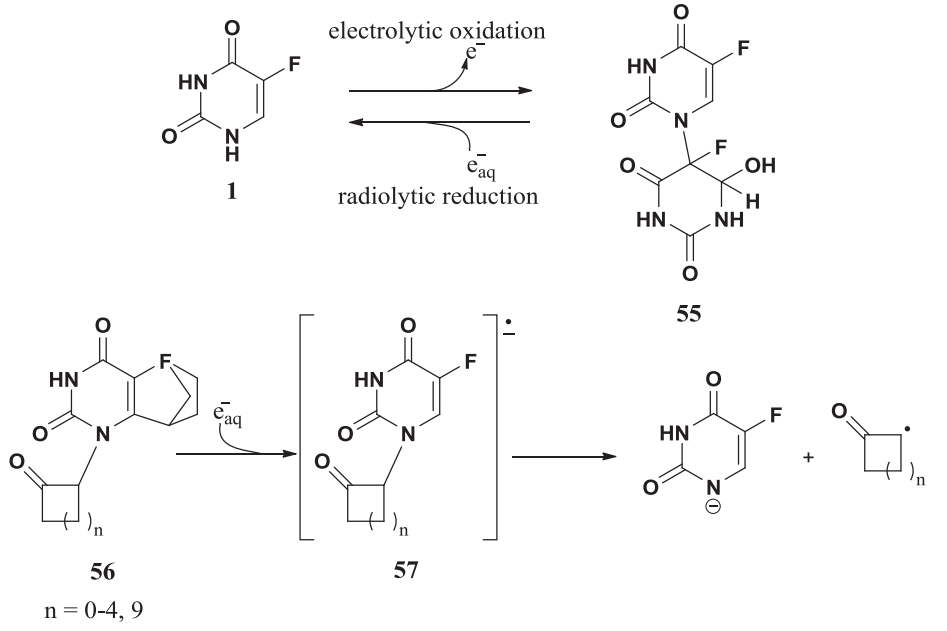


47 53 54

Scheme 25

5-FU derivative **54** has a very low toxicity and a relatively high antitumor activity. At pH 1.4 only the lactone ring undergoes splitting while at pH 7.4 the lactone ring and the N-glycosidic bond are split with formation of 5-FU **1**.

The concept of "radioinduced drug" proposed for cancer therapy is also noteworthy: 5-FU derivatives variously substituted at the 1-position were prepared and the release of the antitumor drug was observed upon γ-irradiation.[49] 5-FU dimer **55**[50] and 5-fluoro-1-(2′-oxocycloalkyl)-uracil **56**[51] released 5-FU **1** by radiation activation under hypoxic conditions (*Scheme 26*).

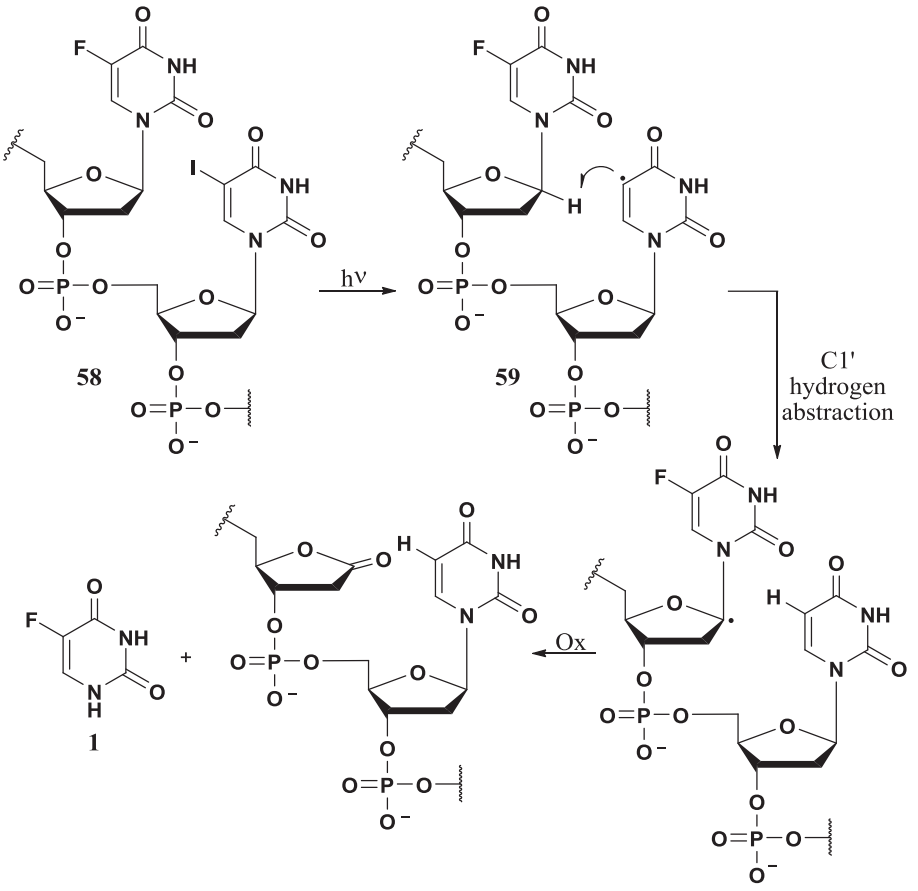


1 **55** **56** **57**

Scheme 26

5-FU **1** can also be released from gold nanoparticles by photoirradiation.[52] In this case **1** was conjugated to gold nanoparticles through a photoresponsive *ortho*-nitrobenzyl linkage.

Photoirradiation was also responsible for the recently reported[53] release of 5-FU from oligonucleotide tetramer **58** (*Scheme 27*). The key reaction was the elimination of a nucleobase by photoinduced C1′ hydrogen abstraction, in turn caused by the radical (compound **59**) photo-generated at C5 of the adjacent 5-halouracil. This approach seems to be promising, since the 5-FU could be released by photoirradiation only in the irradiated area of the body.

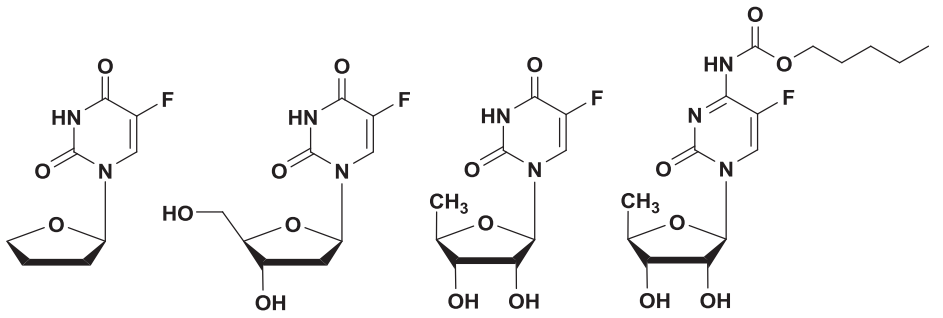


Scheme 27

Even if they are certainly promising, the 5-FU derivatives described herein have not yet found application in clinical trials, although some of them are under investigation, and studies to assess their possible therapeutic use are still in progress.

II. Fluoropyrimidine Nucleosides

Among the 5-FU derivatives developed for oral administration, 5-fluoropyrimidine nucleosides deserve an outstanding position as proven by a 2000 review[5] about the 5-FU prodrugs considered from the clinical point of view. Protracted oral administration should be the ideal route of administration of 5-FU, being preferred by the patients and by pharmacoeconomic considerations. The low oral bioavailability of 5-FU **1** has been overcome by the new generation of oral fluoropyrimidine nucleosides.[54–56] The main 5-FU prodrugs are depicted in *Scheme 28*.

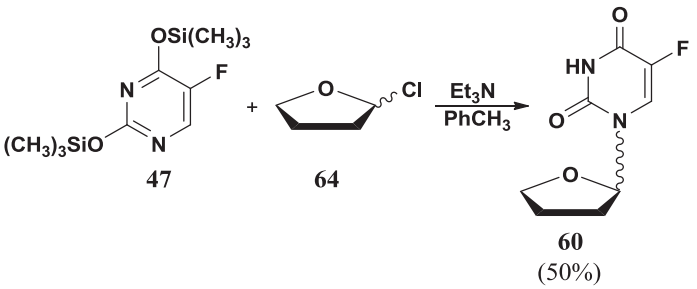


Scheme 28

1. Tegafur (Ftorafur)

Tegafur **60**, also known as ftorafur (1-(tetrahydro-furanyl)-5-fluorouracil) is the first designed 5-FU prodrug. It has a high chemotherapeutic activity (twice that of 5-FU) and low toxicity (5–6 times less than 5-FU) and it is used for the treatment of breast and gastrointestinal tract cancer. Typically, it is used in combinations with other drugs to further improve its bioavailability.[54–57] For instance, the combination of uracil **7** and tegafur **60** (4:1), namely UFT, allows for higher levels of circulating 5-FU, by saturating the dihydropyrimidine dehydrogenase with its natural substrate uracil **7**.

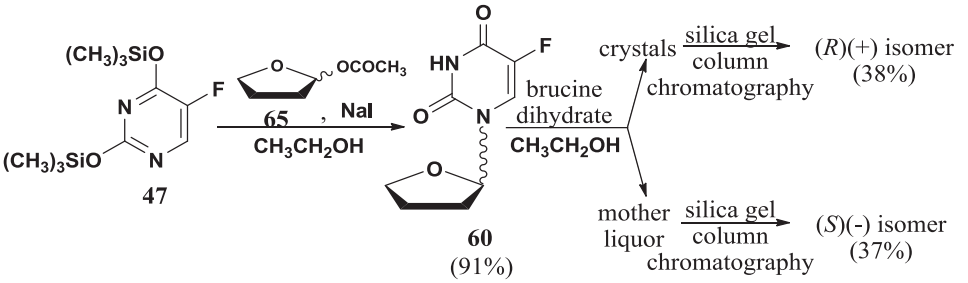
Tegafur was synthesized for the first time in 1969[58] through the condensation of the bis(trimethylsilyl) derivative of 5-FU **47** and 2-chlorotetrahydrofuran **64** (*Scheme 29*) or by condensation of 5-fluorouracil-mercury and 2-chlorotetrahydrofuran **64** in dimethylformamide and toluene (75%).



Scheme 29

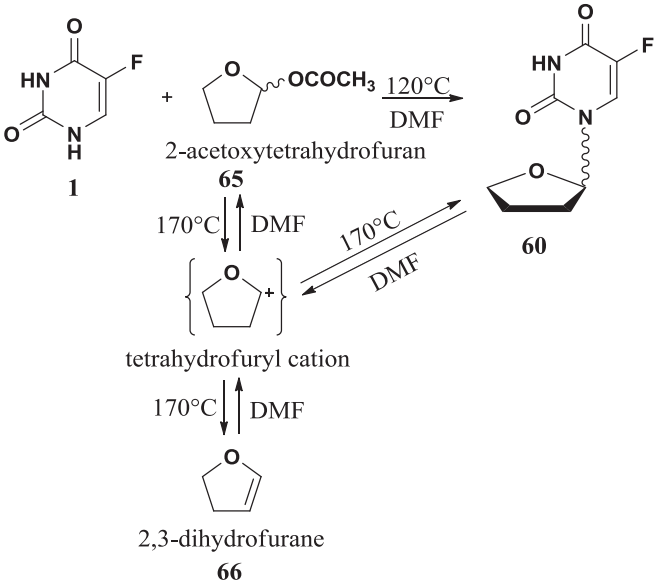
2-Chlorotetrahydrofuran **64** was also used by other authors[59–61], and its preparation was the object of studies[60] that included determination of the best molar ratios[61] between the reagents in order to minimize the formation of the 1,3-bis(tetrahydro-2′-furyl)-5-fluorouracil that is the main by-product due to the double condensation reaction.

Tegafur **60** is administered as a racemic mixture, due to no significant differences in the effects of the stereoisomers, as confirmed by the tests carried out with the separated
320 enantiomers in 1977.[62] The separation of enantiomers was achieved by formation of diastereoisomers with brucine (*Scheme 30*). The racemic mixture of tegafur was, in this case, prepared in 91% yield, by reaction of 2-acetoxy tetrahydrofuran **65** with the silylderivative of 5-FU **47**, using sodium iodide as catalyst.



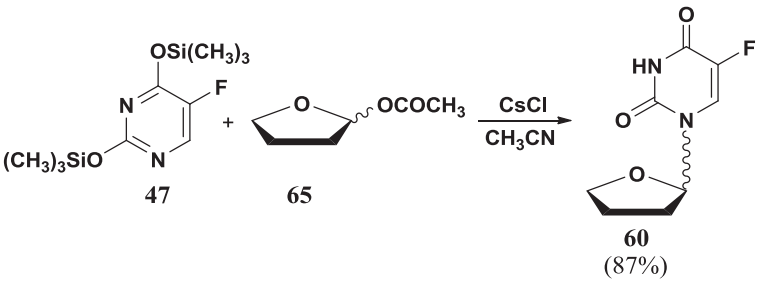
Scheme 30

The same 2-acetoxytetrahydrofuran **65** was also used with typical Friedel-Craft cata-
325 lysts[63] or without catalyst at 120°C in dimethylformamide (*Scheme 31*); higher temperatures lead to the degradation either of tegafur **60** or of 2-acetoxytetrahydrofuran **65**.[64]



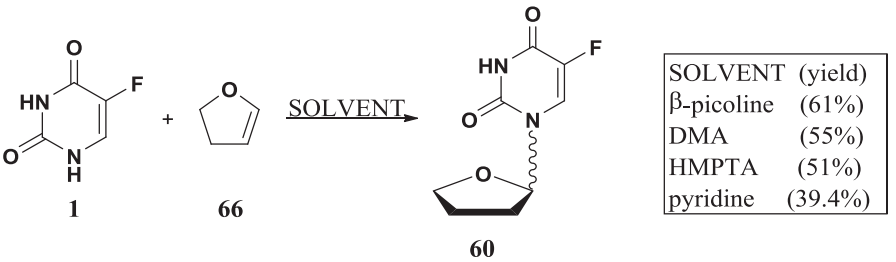
Scheme 31

A different catalyst, cesium chloride, was used in the condensation between disilylated 5-FU **47** and 2-acetoxytetrahydrofuran **65**, in acetonitrile affording tegafur **60** in 87% yield (*Scheme 32*).[65]



Scheme 32

The most popular tetrahydrofuran derivative in the synthesis of tegafur **60** was, however, the 2,3-dihydrofuran **66**. In a French patent, an excess of this compound was reacted with 5-FU **1** in aprotic polar solvents affording tegafur **60**[66] (*Scheme 33*) with yield depending on the chosen solvent.

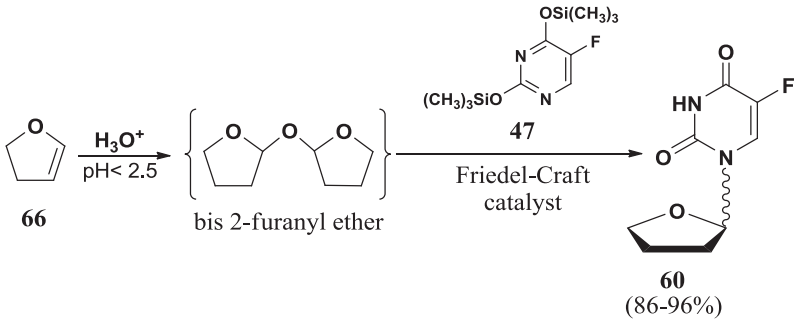


Scheme 33

In similar conditions but in the presence of an acidic catalyst (for example triethylamine hydrochloride, and tetrabutoxytitanium) 59–89% yields were observed.[67] The same reaction was realized in the presence of phosphorus pentachloride in hexamethylphosphoramide affording tegafur **60** in 88% yield; poor yields were observed changing the solvent (DMF or DMA) and a decreased regioselectivity was obtained using phosphorus trichloride instead of phosphorus pentachloride.[68]

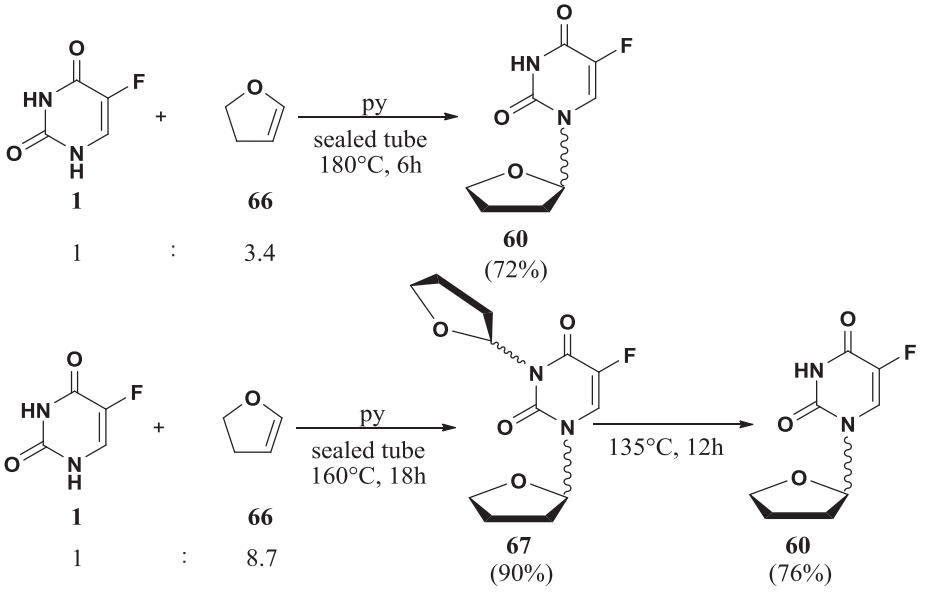
Two patents of two different Japanese companies described the synthesis of tegafur **60** from 5-FU **1** and 2,3-dihydrofuran **66** in the presence of trimethylsilyl chloride and triethylamine in dimethylformamide[69] or in the presence of dimethyldichlorosilane and triethylamine in acetonitrile.[70] The optimization of the molar ratio between 5-FU **1** and 2,3-dihydrofuran **66** and the use of calcium chloride as catalyst, under pressure, afforded very high yields (85–92%) of tegafur **60**.[71]

A one-pot synthesis[72] was realized starting from 2,3-dihydrofuran **66** that was treated with water in the presence of an acidic catalyst (H_3PO_4, H_2SO_4, alkylsulfonic acids) at a pH value below 2.5; after removal of the excess of unreacted material the obtained mixture was treated with the silyl derivative of 5-FU **47** and Friedel-Craft catalyst ($SnCl_4$, BF_3, $TiCl_4$, NaI) (*Scheme 34*).



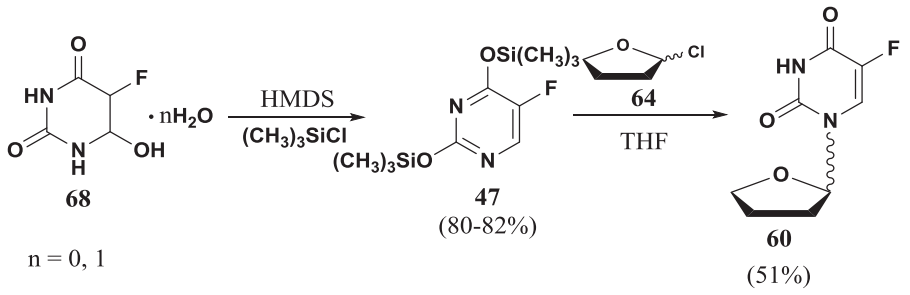
Scheme 34

Depending on the ratio between 2,3-dihydrofuran **66** and 5-FU **1**, in pyridine, in a sealed tube, tegafur **60** or 1,3-bis(tetrahydrofuryl)-5-fluorouracil **67** were obtained (*Scheme 35*).[73] Interestingly, the heating of the bis-substituted 5-FU **67** at 135°C for 12 h, at reduced pressure, afforded tegafur in 76% yield.



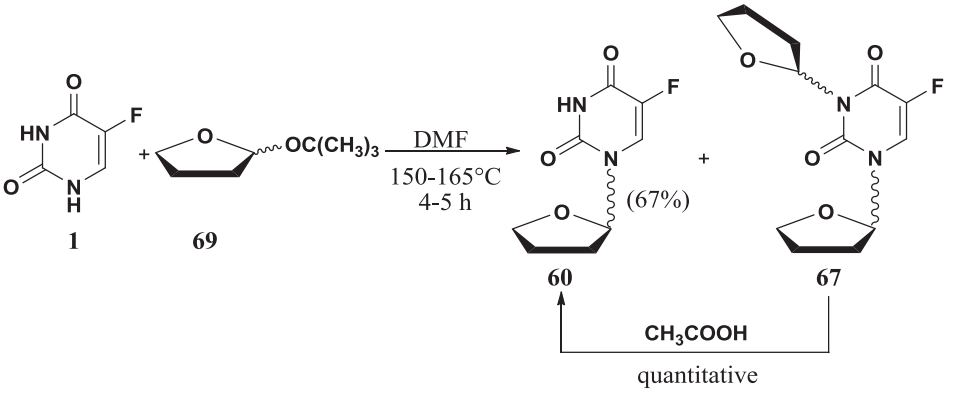
Scheme 35

355 Some different starting materials were also proposed, alternative to the 5-FU or to the tetrahydrofuran derivatives. For example, the 5-fluoro-6-hydroxy-5,6-dihydrouracil **68**[74] provided, by reaction with hexamethyldisilazane or hexamethyldisilazane and trimethylsilyl chloride, bis(trimethylsilyl)-5-fluorouracil **47** (80–82%), which by further reaction with 2-chlorotetrahydrofuran **64**, in THF, afforded tegafur
360 **60** (51% yield) (*Scheme 36*).



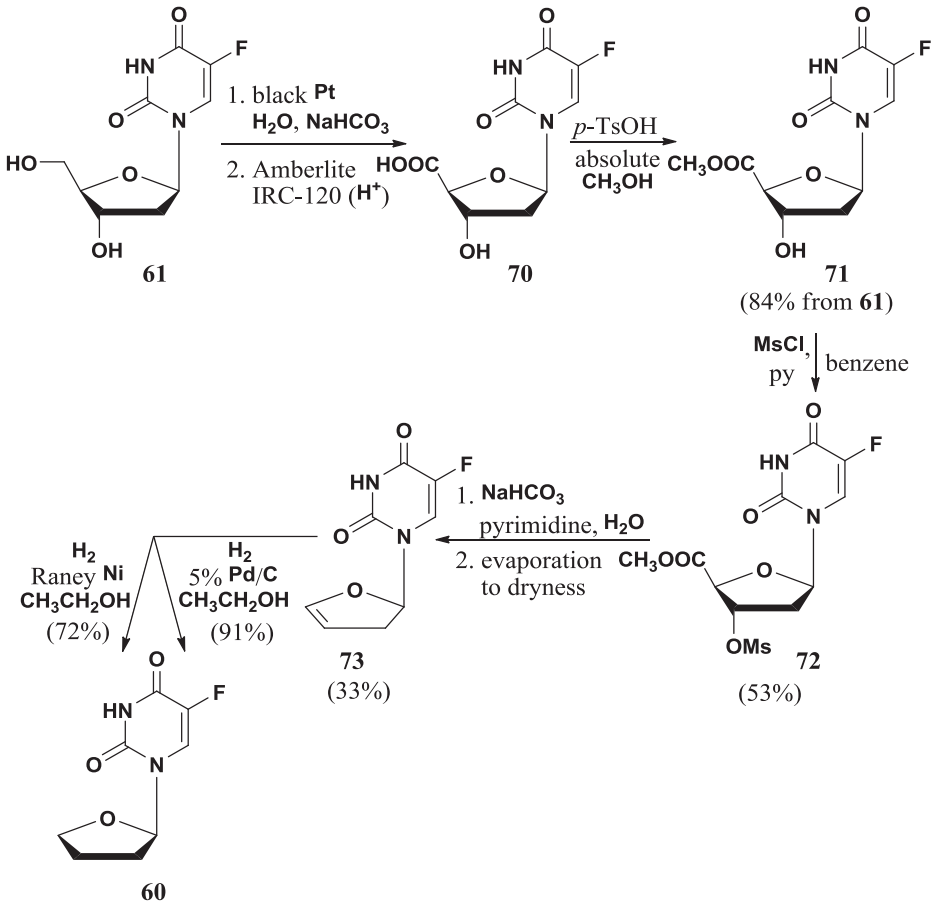
Scheme 36

A series of 2-alkoxyderivatives was used, the best results being achieved with the 2-*tert*-butoxytetrahydrofuran **69** (67% yield).[75] The small amount of disubstituted by-product **67** was quantitatively converted into tegafur by treatment with acetic acid (*Scheme 37*).



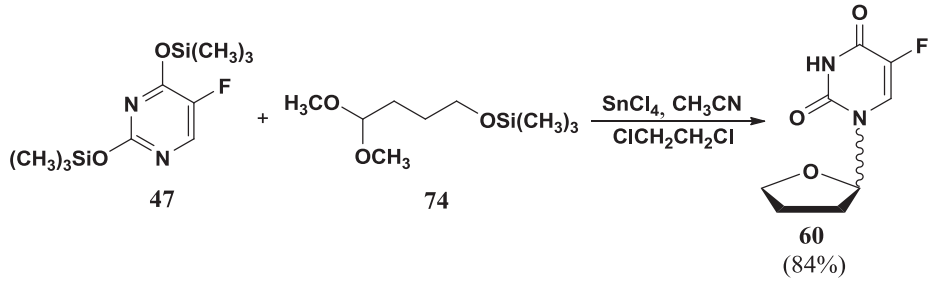
Scheme 37

In a different approach, a preformed nucleoside, β-5-fluoro-2′-deoxy-uridine **61**, was used as starting material and submitted to the reaction with black platinum in water and sodium hydrogen carbonate; the recovered 5′-carboxyderivative **70** was transformed into the corresponding methyl ester **71** and the 3′-hydroxy group was derivatized as mesylate **72**. Simultaneous elimination of 3′-O-mesylate and 5′-$COOCH_3$ afforded the dihydrofuran ring (compound **73**); catalytic hydrogenation gave the (2′*R*) isomer of tegafur **60** (*Scheme 38*).[76] Starting from the α-preformed nucleoside the (2′*S*) enantiomer of tegafur **60** was obtained.



Scheme 38

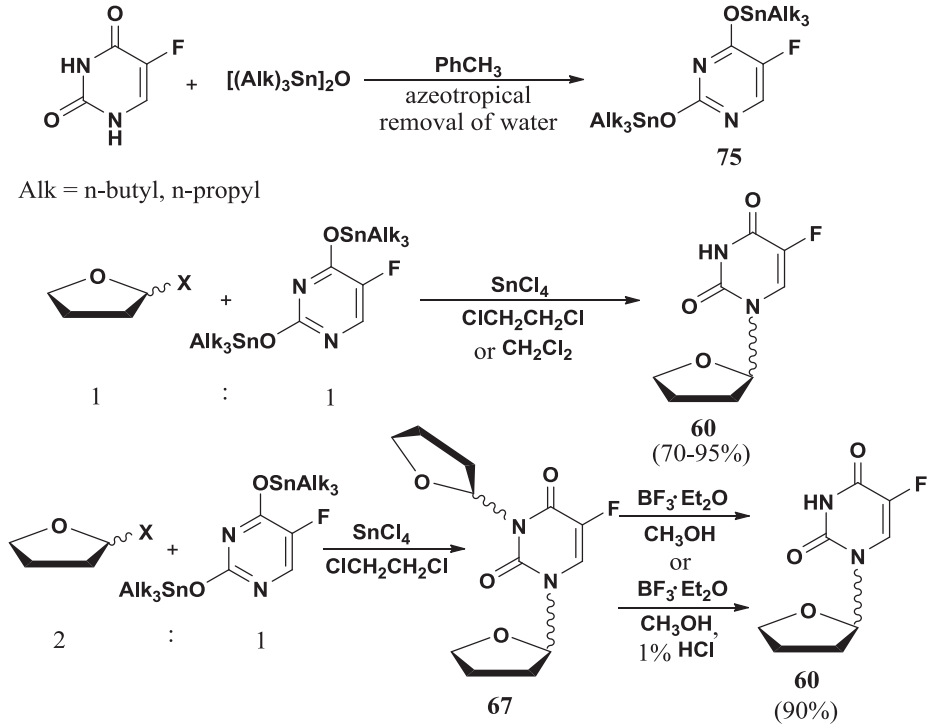
The dimethylacetal of 4-hydroxybutanal-4-O-trimethylsilylether **74** was used as precursor of the tetrahydrofuran ring:[77] the acetal was treated with bis(trimethylsilyl)-5-fluo-
375 rouracil **47**, in acetonitrile, in the presence of stannic chloride. Tegafur **60** was recovered in 84% yield (*Scheme 39*).



Scheme 39

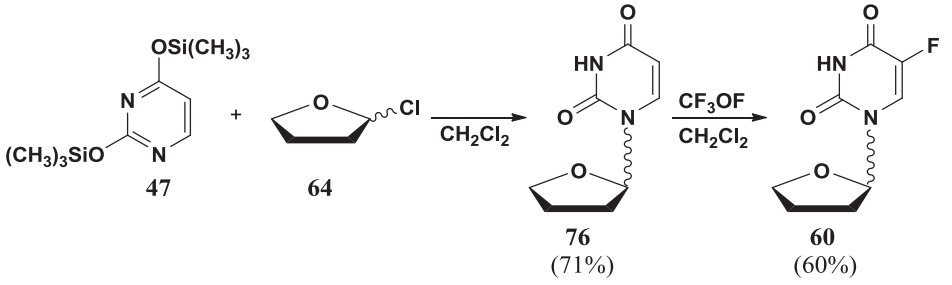
bis(Trialkylstannyl)-5-fluoro uracil **75**[78] was used, instead of the usual silyl derivative, in the reaction with a series of 2-substituted tetrahydrofurans, in the presence of

different acid catalysts. The formation of desired tegafur **60** (70–95%) was obtained when a 1:1 molar ratio was utilized of 5-FU derivative/2-substituted tetrahydrofuran. In the case of a molar ratio 1:2 the N^1,N^3-disubstituted product was isolated. As previously reported[75] N^1,N^3-disubstituted product **67** was converted into tegafur **60** by acidic treatment (*Scheme 40*).



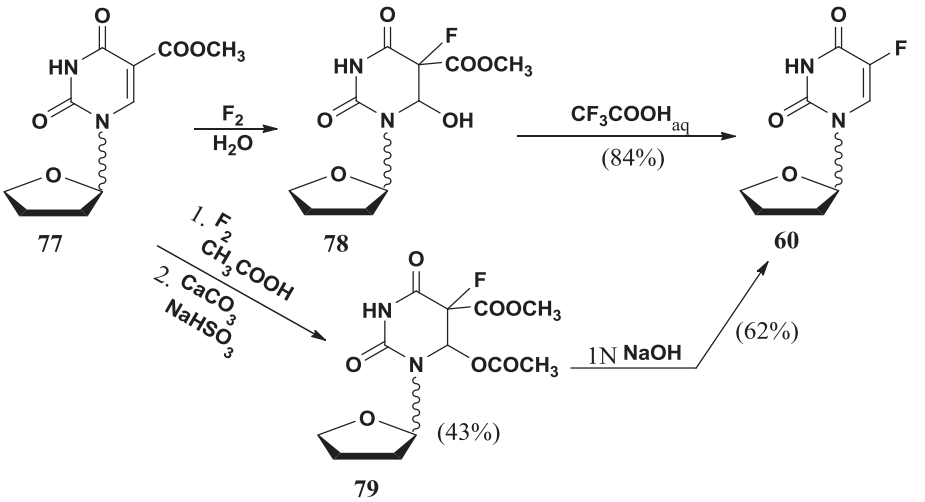
Scheme 40

As in the case of 5-FU **1** synthesis the direct fluorination of the preformed nucleoside was described. Trifluoromethyl hypofluorite in dichloromethane[79] gave the fluorination of 1-(tetrahydrofuranyl) uracil **76** in 60% yield (*Scheme 41*).



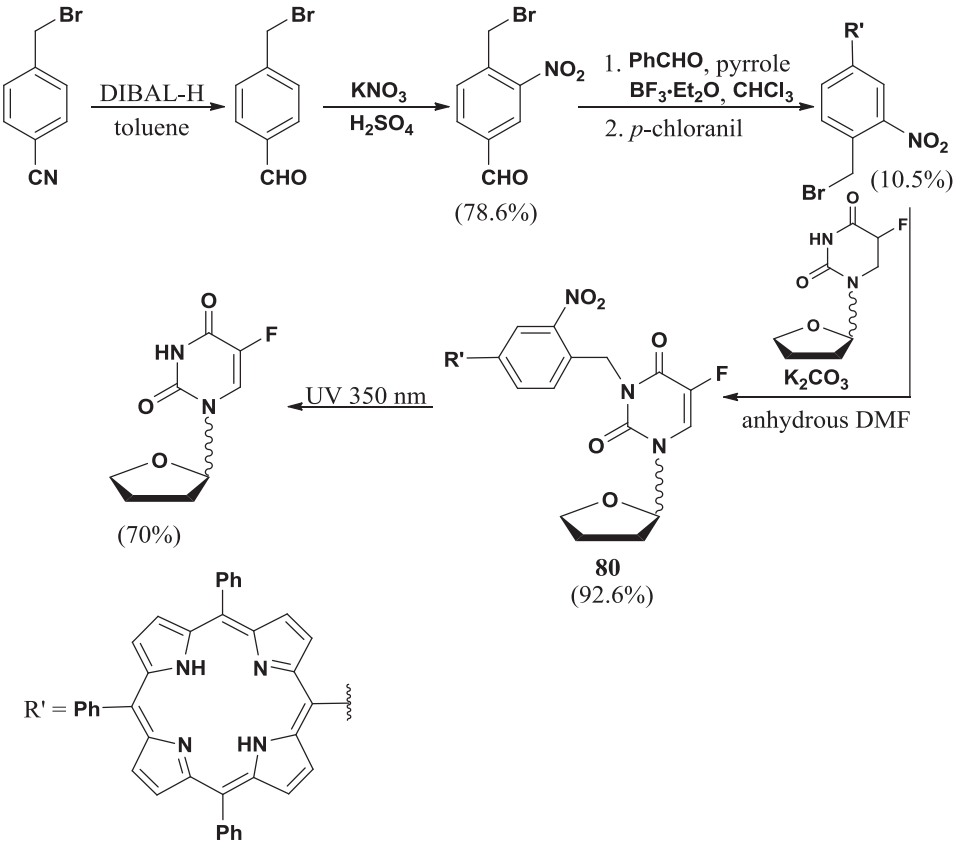
Scheme 41

Using fluorine as fluorinating agent, the reaction with 1-(2′-tetrahydrofuryl)-5-methoxycarbonyluracil **77** gave different intermediates depending on the chosen solvent.[21,22] In water 5-fluoro-6-hydroxy intermediate **78** was obtained, while in acetic acid 5-fluoro-6-acetoxy derivative **79** was formed. Both intermediates led to desired tegafur **60** in 84% and 62% yield, respectively (*Scheme 42*) by hydrolysis of the methyl ester and elimination reaction.



Scheme 42

More recent works about tegafur are especially focused on the improvement of its oral absorption and biological half-life and to lowering its toxicity. With this aim in mind, a light-triggered porphyrin tegafur prodrug was developed.[80] Porphyrins are photosensitizers used in
395 photodynamic therapy and they tend to accumulate in neoplastic tissue to higher concentrations than in adjacent normal tissue. The porphyrin prodrug was composed of three parts: a



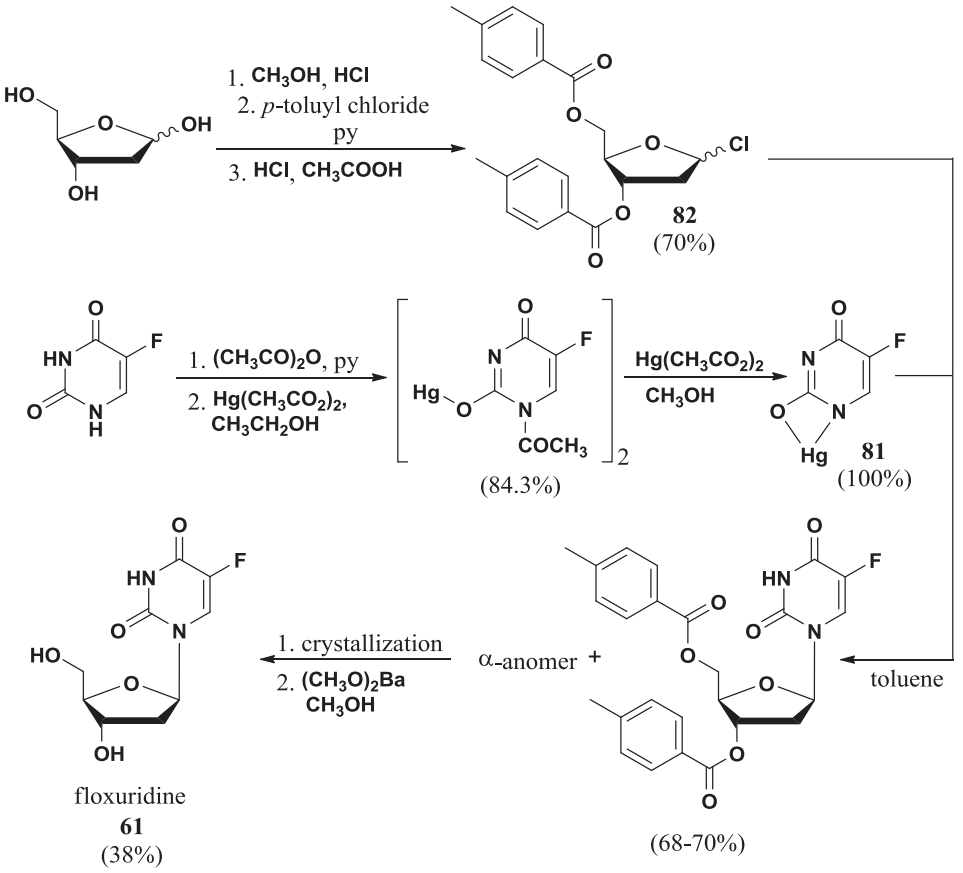
Scheme 43

porphyrin, a photocleavable *ortho*-nitro benzyl moiety, as a light-triggered group, and tegafur. *In vitro* tests demonstrated that this prodrug is less toxic than its parent drug and that it can release cytotoxic tegafur **60** upon photoactivation with long wavelength UV light (350 nm). The synthesis of porphyrinic prodrug **80** is depicted in *Scheme 43*.

2. *Floxuridine*

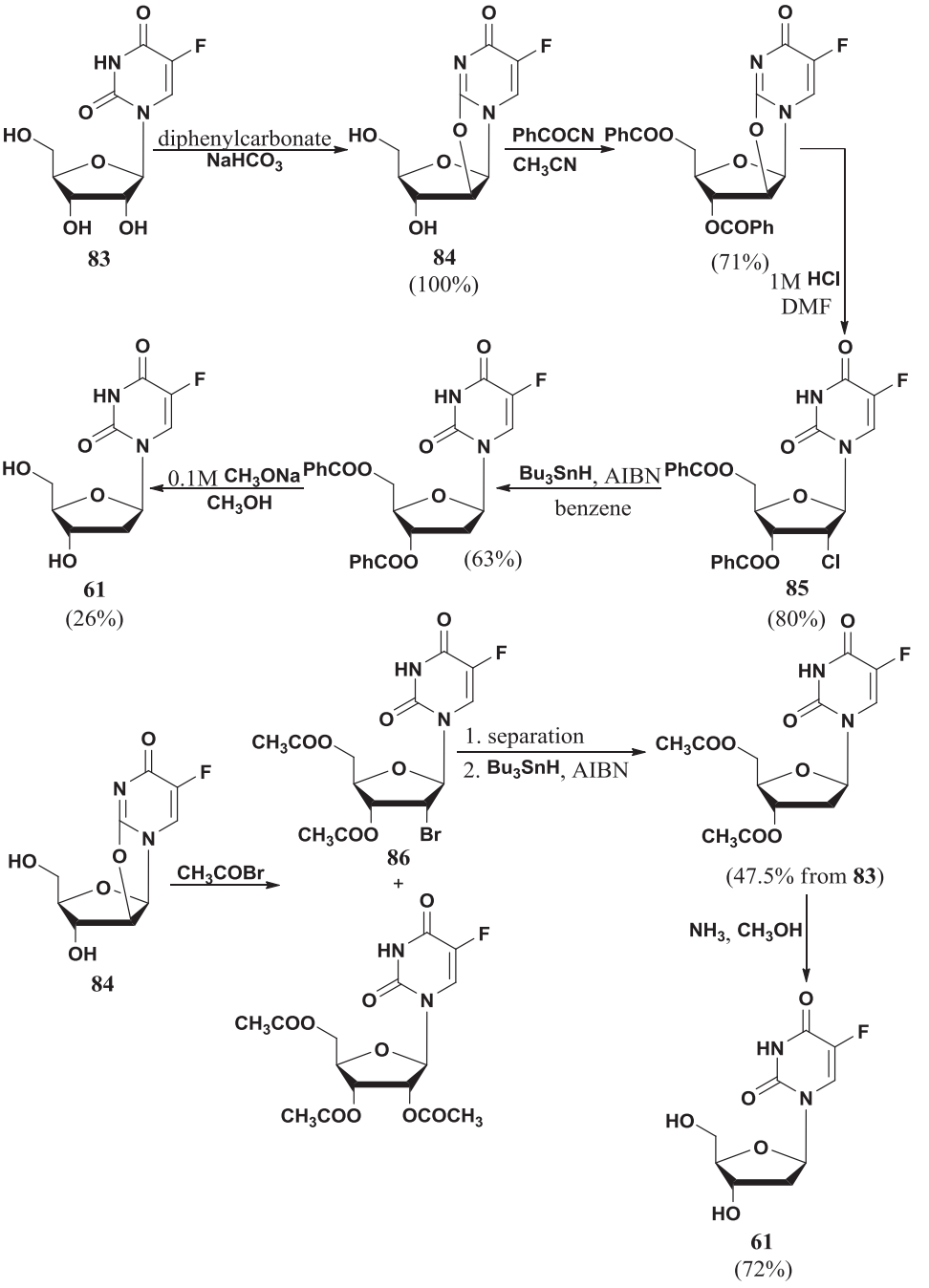
Floxuridine **61** (5-fluoro-2′-deoxyuridine, FdUrd) (See *Scheme 28*) is the deoxyribose metabolite of 5-FU and the precursor of 5-fluorouridine monophosphate **2** (FdUMP) that inhibits thymidilate synthetase (TS) (See *Scheme 1*). FdUrd **61** can be also converted into 5-FU **1** in the liver by thymidine phosphorylase (TP). It was approved in therapy by the FDA in 1970 and has been extensively used for the clinical treatment of carcinoma of the ovary, breast and gastrointestinal tract. Due to the higher toxicity, costs and equal efficacy compared to 5-FU **1** the use of FdUrd **61** is restricted to the treatment of liver metastases caused by colorectal cancer.[81] It was first synthesized at Hoffman-La Roche through the reaction (*Scheme 44*)[82,83] of a mercury derivative of 5-FU **81** with a protected 2-deoxy-D-ribosyl chloride **82** (68–70% yield) followed by separation of α and β-anomers by crystallization (42.5% of β-isomer) and removal of protecting groups (90%).

The same company obtained FdUrd **61** and its phosphates through a biocatalytic approach (cells of *Streptococcus faecalis*).[84]



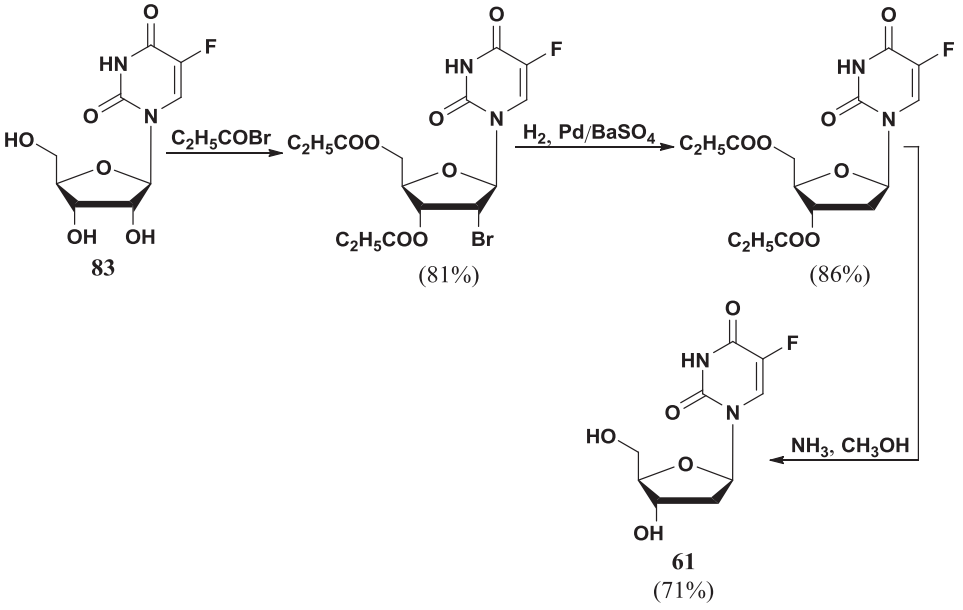
Scheme 44

415 A subsequent approach provided the formation of 2′-deoxyribose starting from a preformed β-nucleoside, namely 5-fluorouridine **83**. This purpose was achieved by means of the removal of 2′-chlorine **85**[85] or 2′-bromine **86**[86] in turn obtained by the ring opening of a O$^{2,2'}$-anhydrouridine **84** (*Scheme 45*). Use of tributyltin hydride allowed removal of the 2′-halogen selectively.



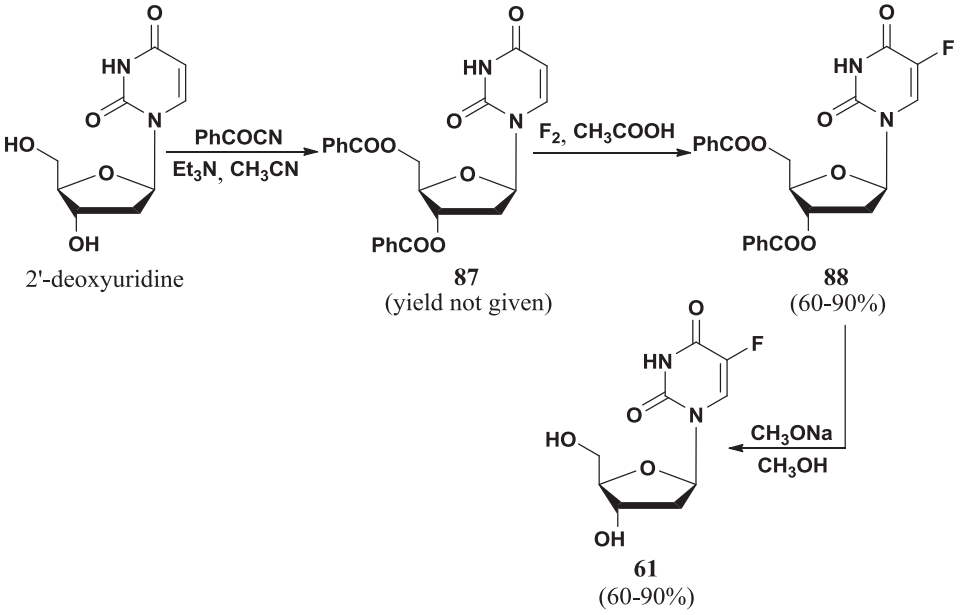
Scheme 45

Applying a method developed in 1974[87] for the synthesis of 2′-deoxyuridine from uridine, floxuridine **61** was obtained in 50% yield from 5-fluorouridine **83**. The 2′-hydroxy group was substituted by a bromine atom that was removed by hydrogenation (*Scheme 46*).[88] A mechanism of the substitution reaction was proposed.



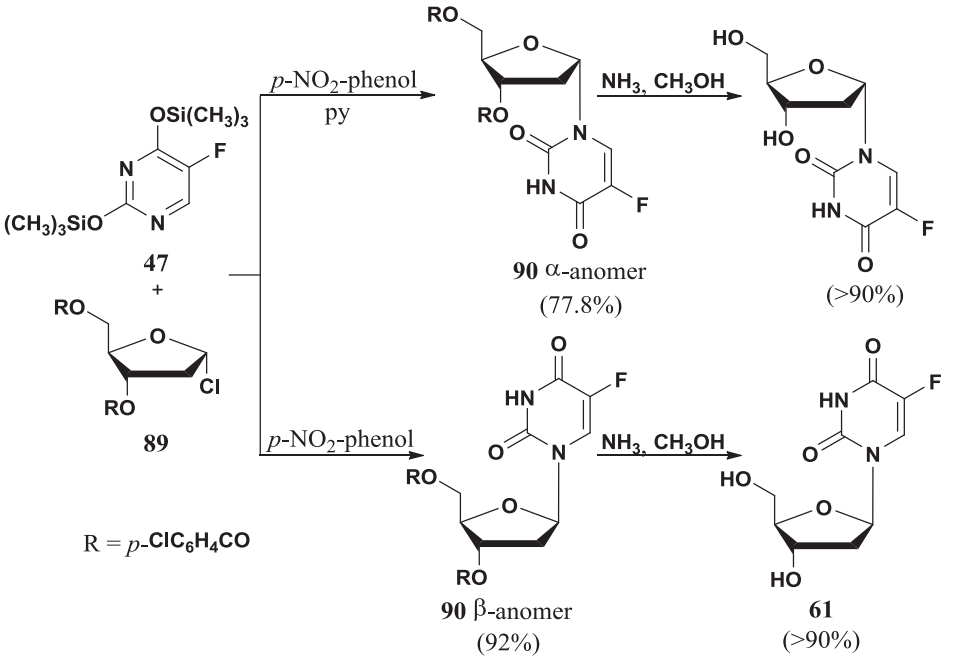
Scheme 46

Similar to 5-FU **1** and tegafur **60**, the direct fluorination of the peracetylated preformed 2′-deoxy nucleoside was realized using, as fluorinating agent, trifluoromethyl hypophosphite.[13,15] Perbenzoate 2′-deoxyuridine **87** was fluorinated with fluorine in acetic acid (60–90% yield). The benzoyl esters were removed from compound **88** by methanolysis affording floxuridine **61** (60–90% yield)[89] (*Scheme 47*).



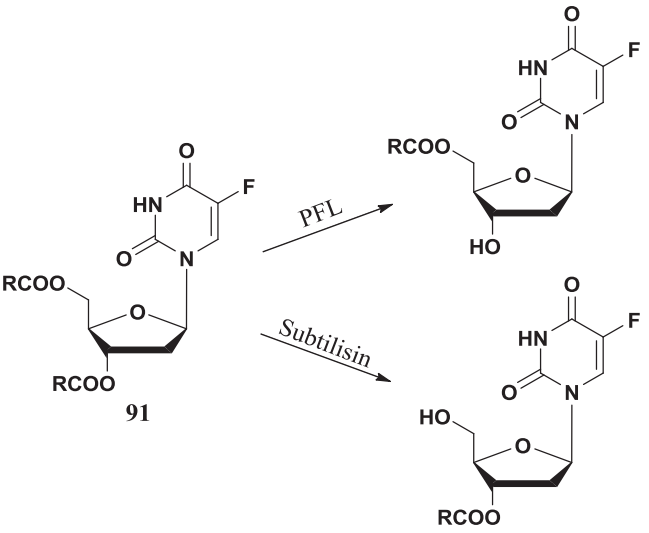
Scheme 47

In a different approach, a study, carried out with the aid of ^{1}H NMR, allowed workers to establish which parameters influence the stereoselectivity of the reaction between the bis-silyl derivative of 5-FU **47** and 3,5-bis(O-*para*-chlorobenzoyl)-2-deoxy-α-D-ribofuranosyl chloride **89**. In the presence of *para*-nitrophenol the β-anomer of **90** was obtained stereoselectively in high yields. In the presence of pyridine and *para*-nitrophenol, the α-anomer of **90** was formed stereoselectively (*Scheme 48*).[90]



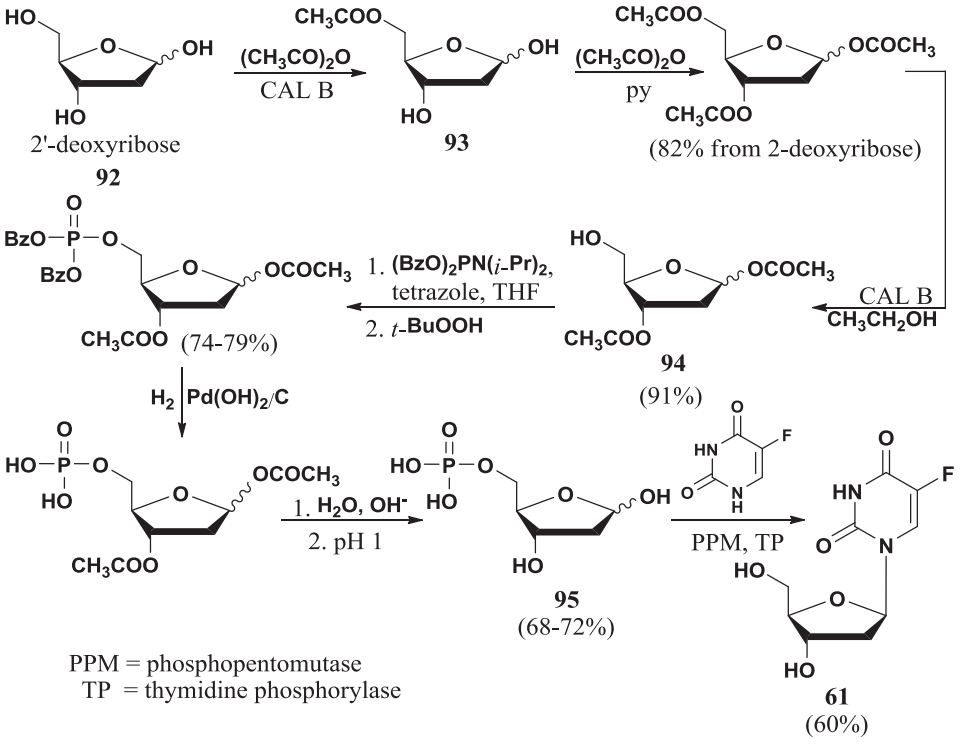
Scheme 48

A biocatalytic approach to floxuridine **61** includes the use either of purified enzymes or of microorganisms *in toto*. The regioselective behavior of hydrolytic enzymes towards the esters of floxuridine was already observed in 1989. A lipase from *Pseudomonas fluorescens* was able to regioselectively remove the 3′-acyl group from the 3′,5′-diester of floxuridine **91**. The selective removal of the 5′-acyl group was achieved by means of subtilisin, the protease from *Bacillus subtilis* (*Scheme 49*).[91]



Scheme 49

A synthesis of floxuridine **61** starting from 2-deoxyribose **92** was performed in 2008 through a chemo-enzymatic approach: CAL B, the lipase from *Candida antarctica*, was first used to selectively introduce an acetyl group at the 5-position of the carbohydrate (compound **93**) and, in the next step of the synthesis, to selectively remove the same acyl group, under alcoholysis conditions (compound **94**). Intermediate 5-phosphate **95** was used as starting material for the glycosylation reaction catalyzed by a combination of phosphopentomutase (PPM) and thymidine phosphorylase (TP). PPM catalyzed the transfer of the phosphate group from the 5-position to the 1-position of the furanose, providing the substrate of TP. Floxuridine **61** was obtained in 60% yield from 2-deoxy-D-ribose-5-phosphate **95** (*Scheme 50*).[92]

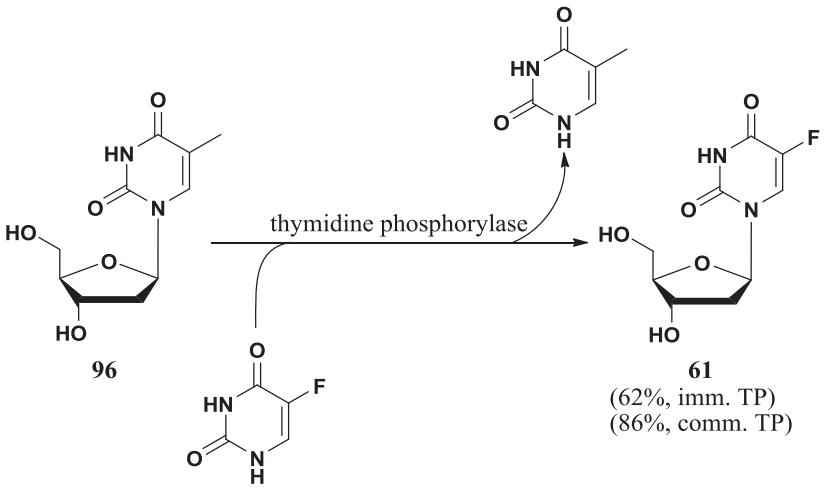


Scheme 50

The use of TP as biocatalyst of the nucleobase exchange reaction, aimed to convert thymidine **96** to floxuridine **61** (transglycosilation), was also reported. In one case immobilized TP was used (62% yield);[93] by using commercially available TP[94] a 86% yield was observed (*Scheme 51*).

Immobilized bacterial cells from *E. coli*[95] or from *Lactobacillus animalis* [96,97] were reported to catalyze the transglycosilation reaction of thymidine **96** to floxuridine **61**with 62% and 95% yield, respectively.

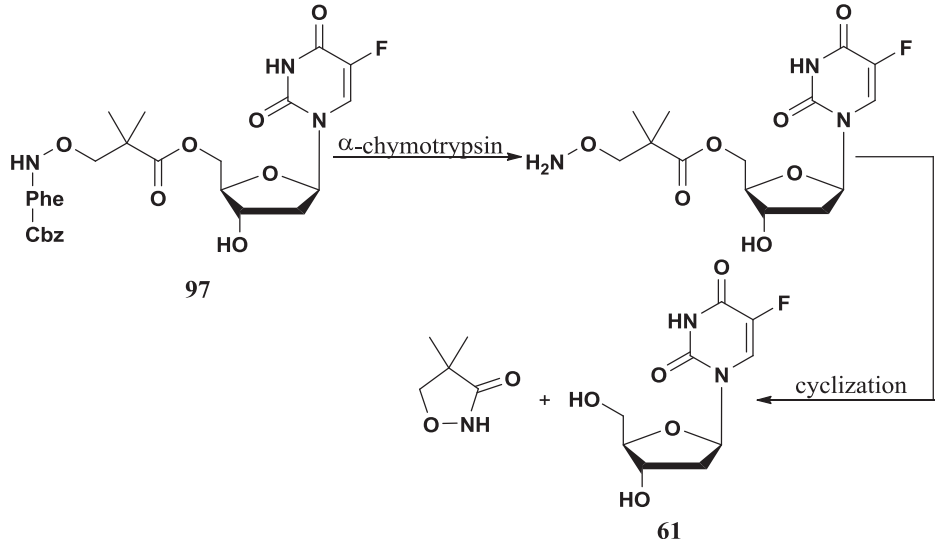
Similarly to 5-FU **1**, floxuridine **61** has a short plasma half-life and causes gastrointestinal toxicity. Some esters of floxuridine with long chain aliphatic acids show higher therapeutic indices than floxuridine[98] and for this reason the susceptibility of a series of



Scheme 51

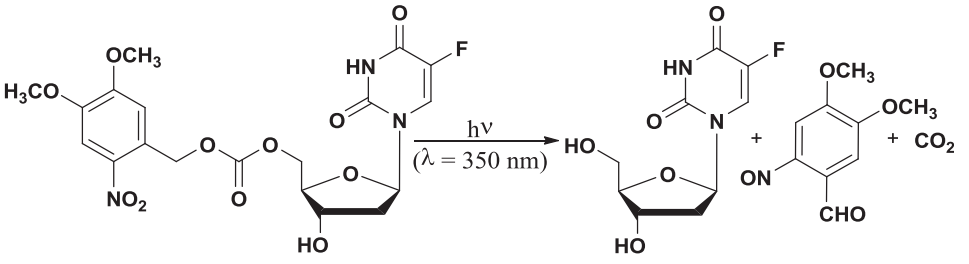
3′,5′-diesters or 3′-and 5′-monoesters to porcine liver esterase was investigated. The obtained results suggested that the higher antitumor activity of longer alkyl chain diesters of floxuridine was partly due to their slow rate of hydrolysis by non-specific esterase.

465 Many efforts have been made to develop a variety of prodrugs undergoing hydrolysis to release floxuridine **61**: for example, conjugates with a cyclic peptide with an ester linker[99] or a peptide with a 3-aminoxypropionate-based linker.[100] This last prodrug **97** shows a linker that cyclizes readily under physiological conditions and in the interstitial tissues of solid tumors (*Scheme 52*).



Scheme 52

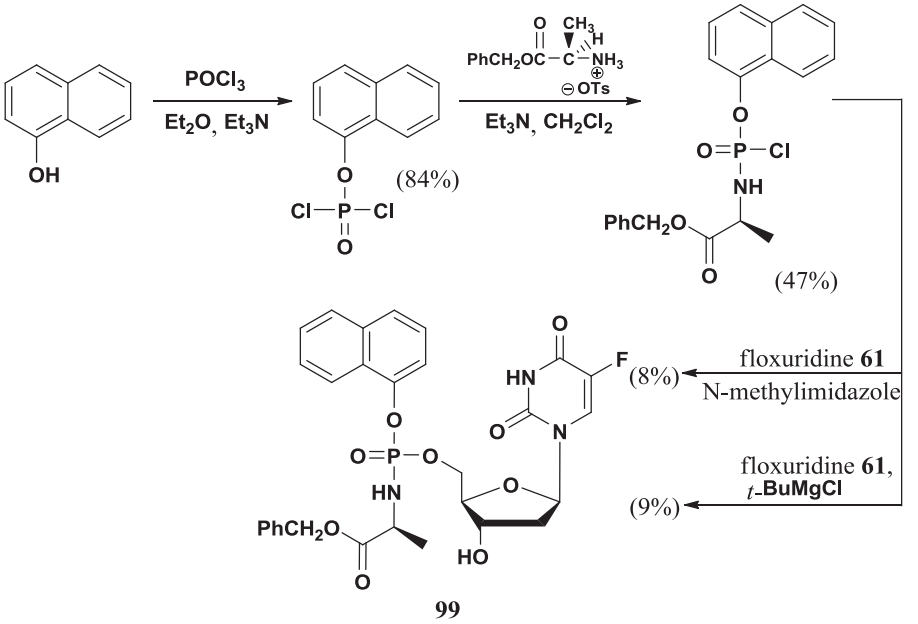
Floxuridine binding to antibodies in order to drive the cytotoxic activity[101,102] was also studied; and further photolabile carbonate prodrug **98** was synthesized that, by photolysis, rapidly released floxuridine **61** (*Scheme 53*).[103]



98 **61**

Scheme 53

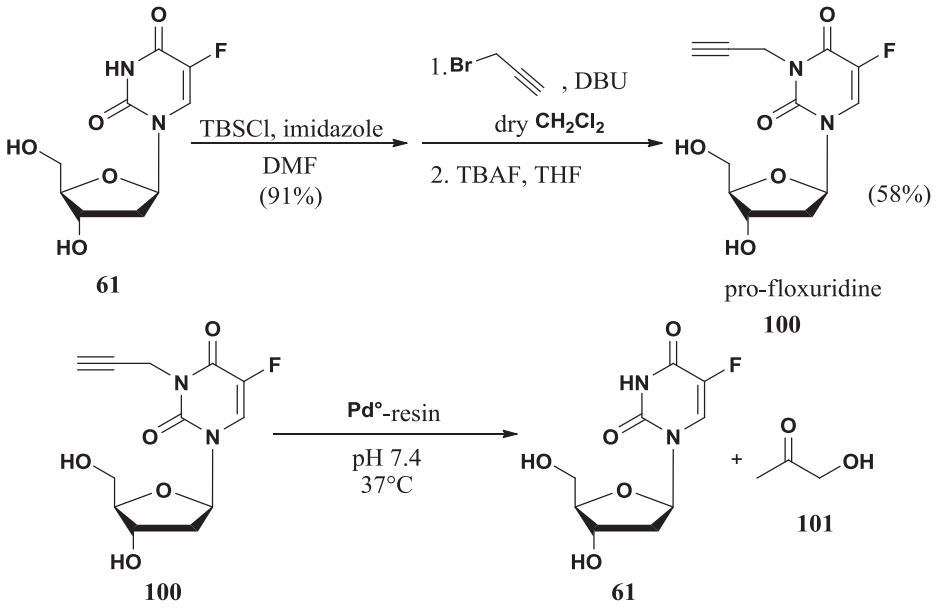
It is known (see *Scheme 1*) that floxuridine **61** is activated by phosphorylation, catalyzed by thymidine kinase (TK), to 5-fluorodeoxyuridine monophosphate (FdUMP) **2**, the TS-inhibitor. In TK-deficient tumors floxuridine lost its cytostatic potential. Direct administration of phosphorylated floxuridine has little therapeutic advantage since the charged monophosphate, under physiological conditions, shows poor, if any, penetration across the cell membrane. The administration of lipophilic phosphoramidate **99** can circumvent these problems (*Scheme 54*).[104]



99

Scheme 54

According to a recent patent, a floxuridine prodrug was prepared and a biorthogonal cleavage (under biocompatible conditions) was suggested (*Scheme 55*).[105] The target of the study was the development of a prodrug releasing the active drug by means of a reaction susceptible to occur in a biological environment without interfering with the normal functions of its



Scheme 55

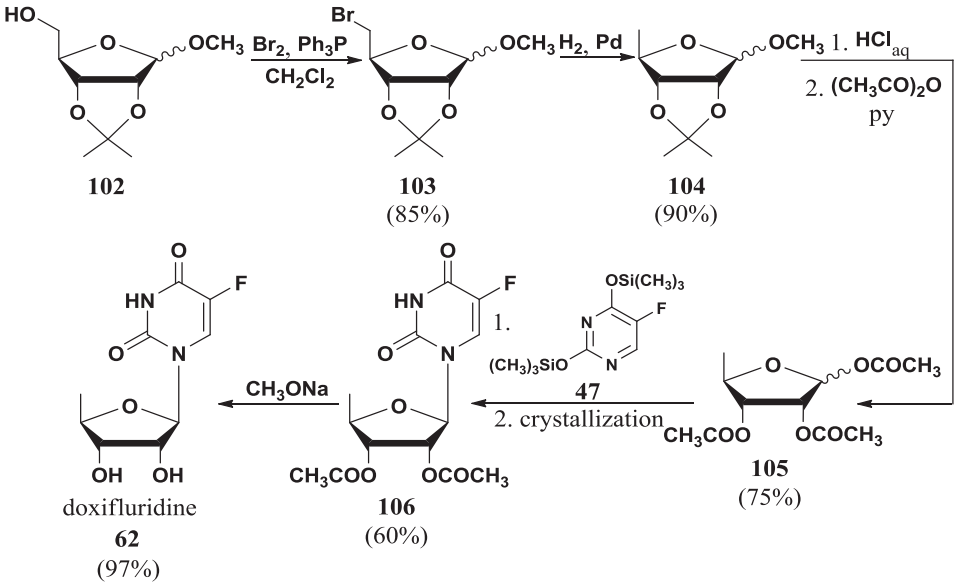
component. Thus, 3-propargyl-5-fluoruridine **100** was synthesized and the free active drug was generated using biocompatible palladium catalyst, at 37°C and pH 7.4; also the by-product 1-hydroxyacetone **101** is biocompatible. Palladium implants, can, therefore, deprotect the pro-drug at the disease site, reducing the general systemic concentration of the free drug.

3. Doxifluridine

Doxifluridine (5′-deoxy-5-fluorouridine, 5′-DFUR) **62** is a prodrug that requires thymidine phosphorylase (TP) for its one-step conversion to 5-FU **1**. Since TP expression is high in the gastrointestinal tract, doxifluridine therapy resulted in dose-limiting toxicity, such as diarrhea. It is orally administered for the treatment of breast, stomach, colon cancer and nasopharyngeal carcinoma.

A large number of 5′-deoxy nucleosides have been synthesized taking into account that several biologically active nucleosides require the presence of a 5′-hydroxy group for activation, usually by phosphorylation. Removal of this function provides interesting compounds for biochemical and biological studies. 5′-Deoxy compounds are potentially interesting medicinal agents, since such molecules cannot be phosphorylated and incorporated into nucleic acids and thus offer the possibility of reduced toxicity and increased specificity. The 5′-deoxy carbohydrates can be synthesized before the formation of the N-glycosidic bond with the 5-fluoropyrimidine or, alternatively, a preformed nucleoside can be used as starting material for the 5′-modification.

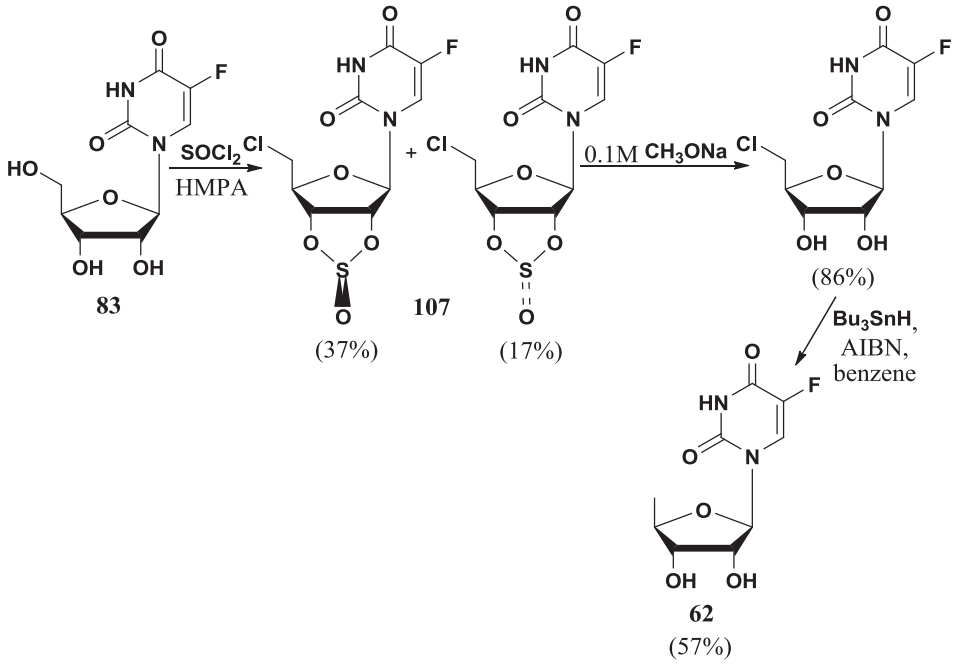
In a 1982 patent,[106] the 5-deoxy carbohydrate was prepared, starting from suitably protected D-ribose **102**, through 5-bromo derivative **103** (*Scheme 56*). Removal of the 5-bromine atom was achieved by means of a catalytic hydrogenation affording compound **104** (90% yield). Deoxy peracetylated sugar **105** was used in the glycosylation reaction affording diacetate of doxifluridine **106** that was deprotected by means of sodium methylate (97% yield). The same authors, by this method, prepared also the 5′-monodeutero and the 5′,5′-dideutero derivatives and the α-anomer of doxifluridine.[107]



Scheme 56

A modification of the coupling method between the 5′-deoxysugar and the 5-fluoro pyrimidine allowed workers to increase the yields and the purity of the final product, at a temperature below 0°C.[108]

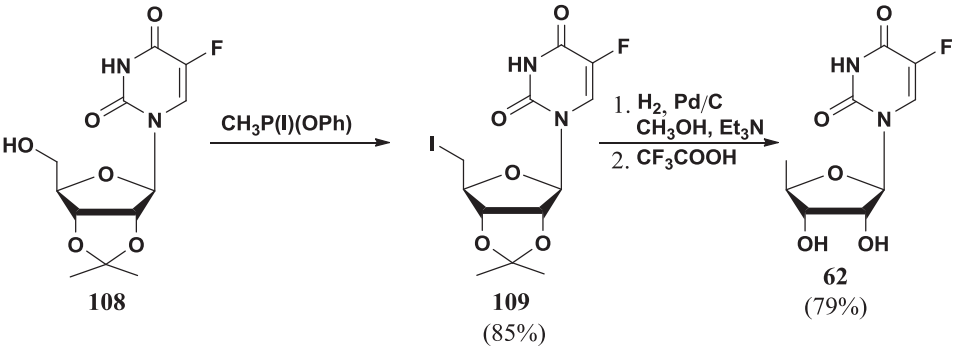
In a 1978 article[109] the 5′-deoxy group was obtained starting from 5-fluorouridine **83** (*Scheme 57*), through the simultaneous protection of 2′- and 3′-hydroxy groups as a cyclic



Scheme 57

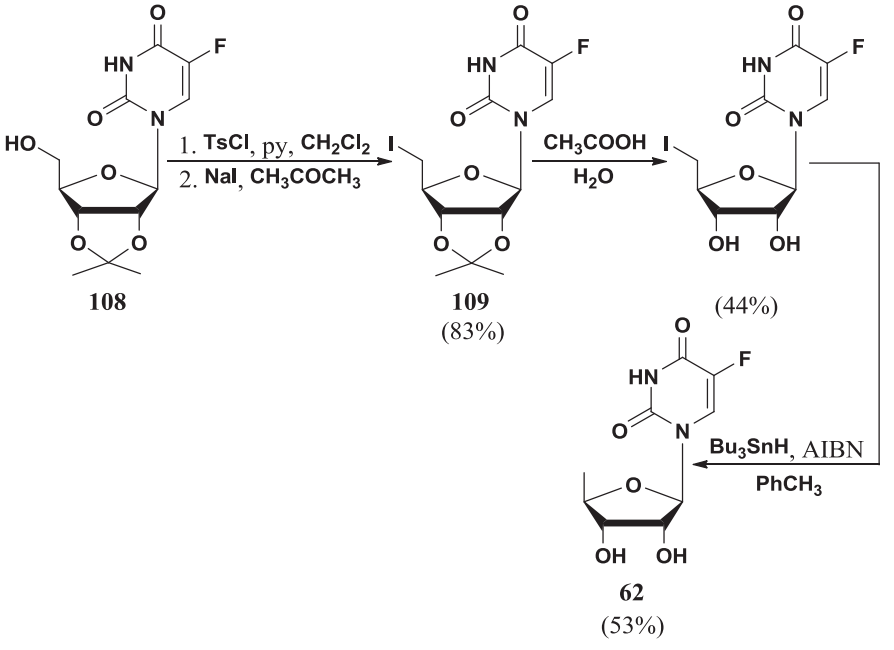
sulphinyl derivative, and the substitution of 5′-hydroxy group with a chlorine atom (com-
515 pounds **107**), by means of thionyl chloride. The chlorine atom was then removed by reaction with tributyltin hydride.

Protection of the 2′- and 3′-hydroxy groups of 5-fluorouridine as the isopropylidene derivative **108**, followed by iodination[110] afforded suitable 5′-iodo intermediate **109** in order to obtain the 5′-deoxy 5-fluoronucleoside **62** (*Scheme 58*).



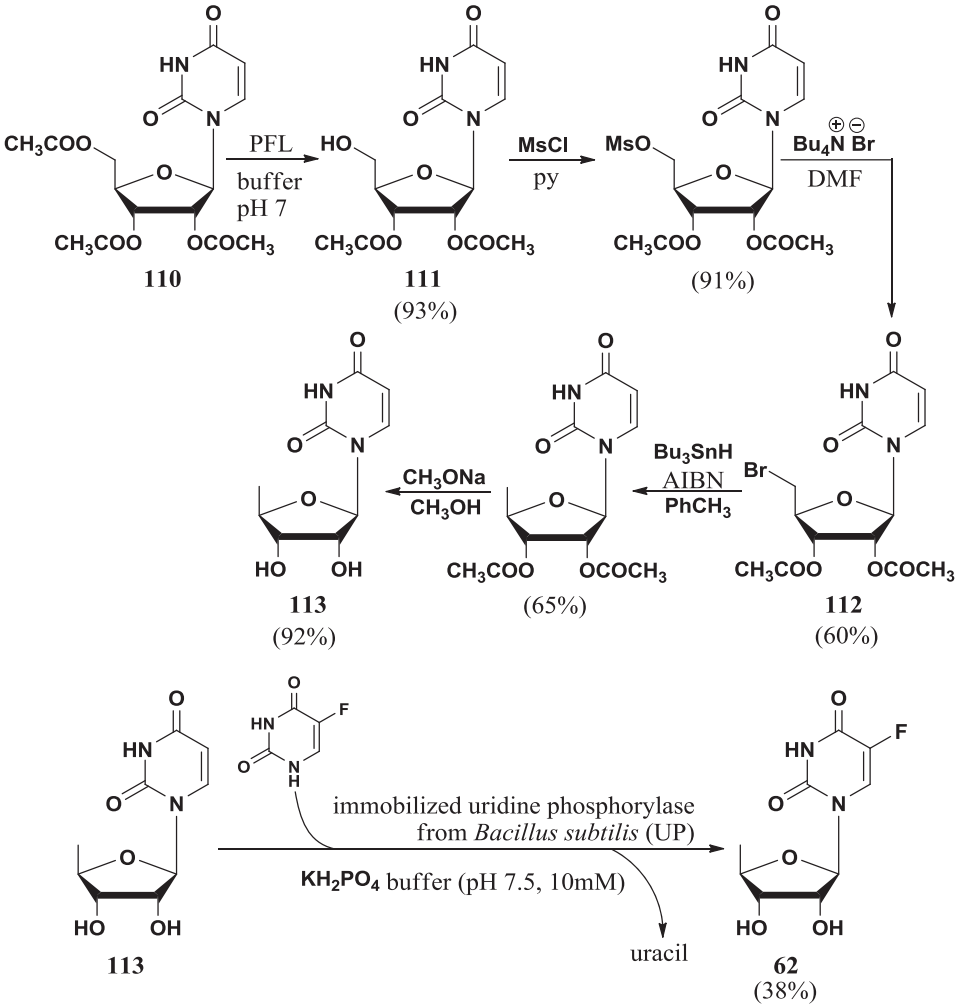
Scheme 58

520 The same 5′-iodo derivative, compound **109**, as later reported in an Italian patent,[111] was transformed into the 5′-deoxy nucleoside by removal of the acetonide, followed by reaction with tributyltin hydride in toluene (*Scheme 59*).



Scheme 59

Another Italian team in 2009[112] described a chemo-enzymatic approach to the synthesis of doxifluridine through the regioselective enzymatic hydrolysis of triacetyluridine **110** followed by a phosphorylase-catalyzed transglycosylation (*Scheme 60*). The regioselective hydrolysis of 5′-acetate was achieved by means of lipase from *Pseudomonas fluorescens* in pH 7 buffer; the 5′-hydroxy group of compound **111** was transformed into 5′-bromo derivative **112** in two steps. Reduction of 5′-bromonucleoside (tributyltin hydride) and removal of protecting groups afforded 5′-deoxy uridine **113**, a suitable substrate for a phosphorylase-catalyzed substitution of the nucleobase.

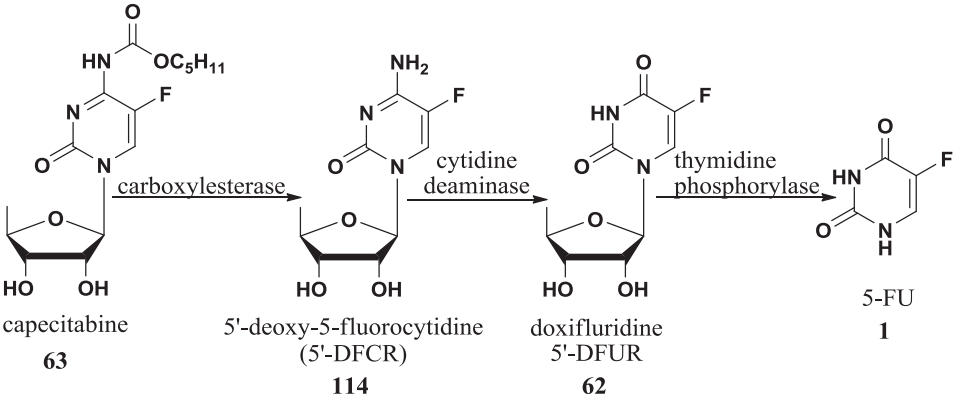


Scheme 60

4. Capecitabine

Capecitabine (N^4-pentyloxycarbonyl-5′-deoxy-5-fluorocytidine, Xeloda®) **63**, an oral prodrug of 5-FU, was designed to circumvent the gastrointestinal toxicity of doxifluridine **62** and to generate 5-FU preferentially at the tumor site.[113–116] The activation of capecitabine **63** by transformation into 5-FU required three distinct enzyme-catalyzed steps. After

oral administration capecitabine passes the intestine unaltered; in the liver the carbamoyl moiety is removed by carboxylesterase; the second step is the conversion to 5′-deoxy-5-fluorouridine **62** by cytidine deaminase, an ubiquitous enzyme,[117] responsible for the deamination of nucleosidic analogs, localized in the liver and in various tumor types. Finally, 5′-deoxy-5-fluorouridine **62** can be converted to 5-FU in the tumors by thymidine phosphorylase,[118] an enzyme that leads to the preferential generation of 5-FU in tumors as a result of its overexpression in malignant tissue (*Scheme 61*).

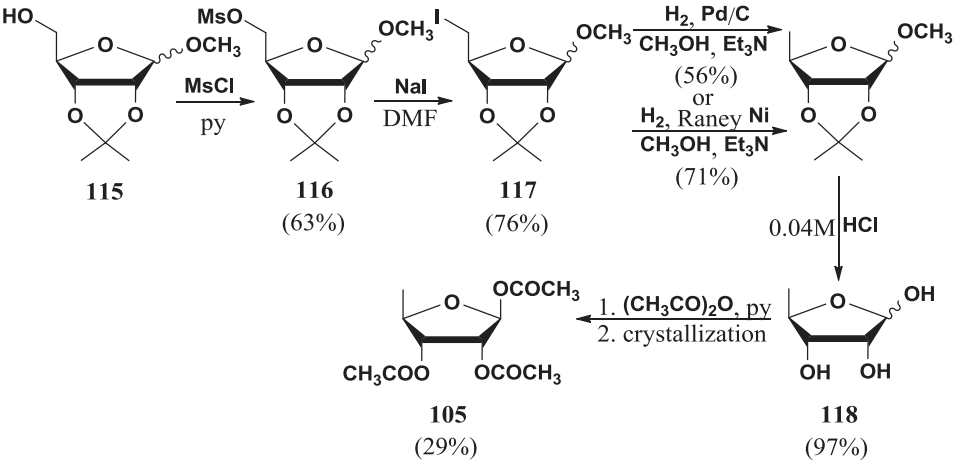


Scheme 61

Capecitabine **63** is approved in the European Union, USA, Canada and Australia for the treatment of advanced or metastatic colorectal carcinoma.[119–122] In combination with other antineoplastic agents, it can be utilized also in metastatic breast cancer.[123–127] Syntheses of capecitabine were reviewed in 2010 in a book chapter.[128]

In the molecule of capecitabine **63** three moieties can be recognized: the 5′-deoxy-D-ribose, the 5-fluoropyrimidine and the pentyloxycarbonyl group. Similarly to the doxifluridine synthesis, the N-glycosylation can be carried out starting from D-ribose, postponing the formation of the 5′-deoxy group to a subsequent step, or from the previously prepared 5′-deoxy-D-ribose. Also the N^4-carbamoyl function can be introduced in the course of different steps of the synthesis.

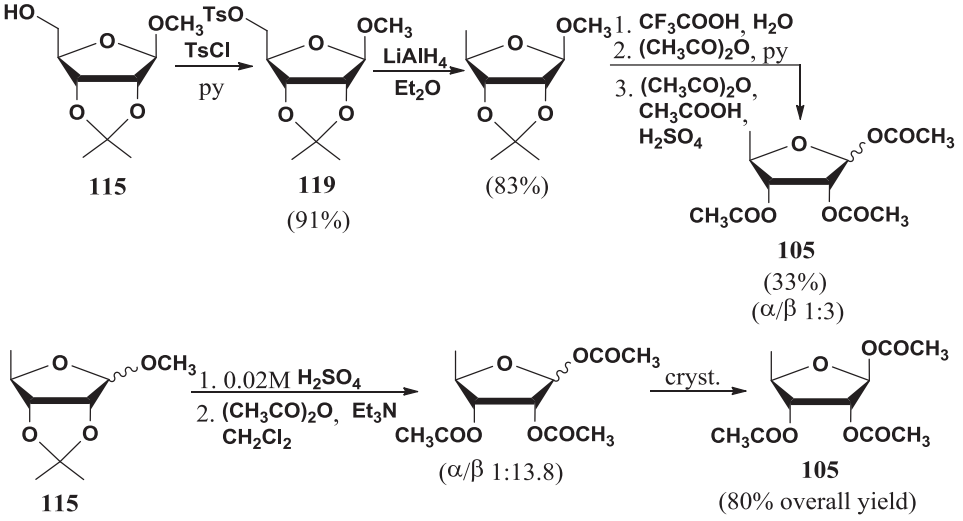
Several syntheses start from triacetyl-5-deoxy-D-ribose **105** that, in turn, can be prepared from methyl 2,3-O-isopropylidene-D-ribofuranoside **115** through the formation of 5-O-mesyl derivative **116**, the substitution of the mesylate group by an iodine atom (compound **117**) and the reductive removal of the latter. Deprotection and acetylation followed by crystallization afforded the β-anomer of 1,2,3-O-triacetyl-5-deoxy-D-ribose **105** (*Scheme 62*).[129]



Scheme 62

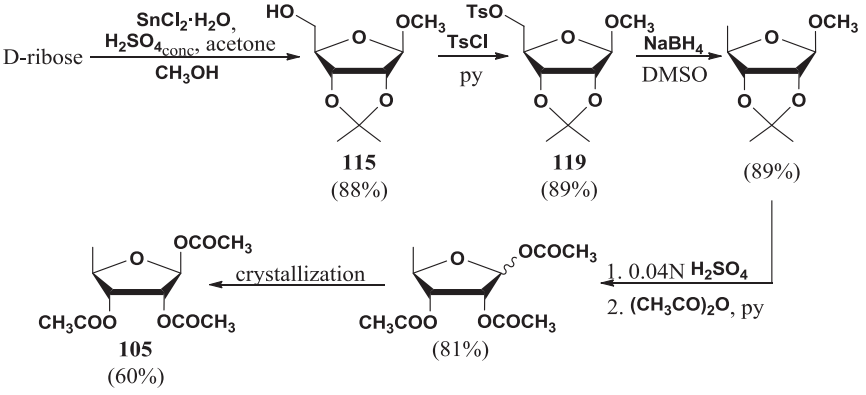
Peracetylated 5-deoxy-D-ribofuranoside **105** was also prepared in a different way, starting from 2,3-O-isopropylidene-D-ribofuranoside **115**, through the 5-bromo-derivative and used in the N-glycosylation step, as described in the case of doxifluridine synthesis (see *Scheme 56*).[106]

In more recent work[130] the removal of the 5-hydroxy group was realized through the reduction of suitably protected 5-O-tosyl ester **119** by means of lithium aluminum hydride (*Scheme 63*); after deprotection and acetylation the 1,2,3-O-triacetyl-5-deoxy-D-ribose **105** was obtained as a 1:3 α/β anomeric mixture. A ratio more favorable to the β-anomer was observed[131] by modification of the reaction conditions of 5-deoxy-D-ribose acetylation; indeed, using triethylamine in dichloromethane solution as the base, instead of pyridine, the acetylation afforded a 1:13.8 α/β anomeric mixture (*Scheme 63*). Pure β-isomer **105** was recovered in 80% overall yield by crystallization.



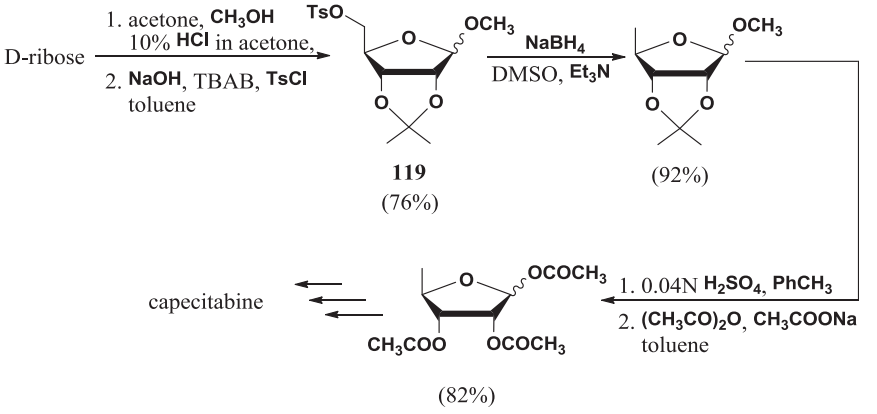
Scheme 63

A different reducing agent for **119** (sodium borohydride in dimethylsulfoxide)[132] allowed the synthesis of 1,2,3-O-triacetyl-5-deoxy-D-ribose **105** in 56% overall yield from D-ribose (*Scheme 64*). Pure β-anomer was recovered after crystallization (60%).



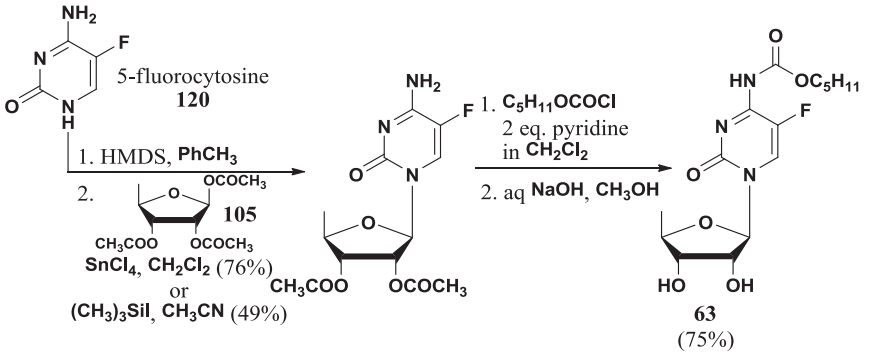
Scheme 64

An improvement of this method was described in a 2013 Indian patent:[133] 5-O-Tosyl derivative **119** was obtained, avoiding the use of pyridine, by reaction of 1-O-methyl-2,3-O-isopropylidene-D-ribose **115** with *para*-toluensulfonyl chloride and sodium hydroxide in toluene, in the presence of tetrabutyl ammonium bromide (*Scheme 65*).



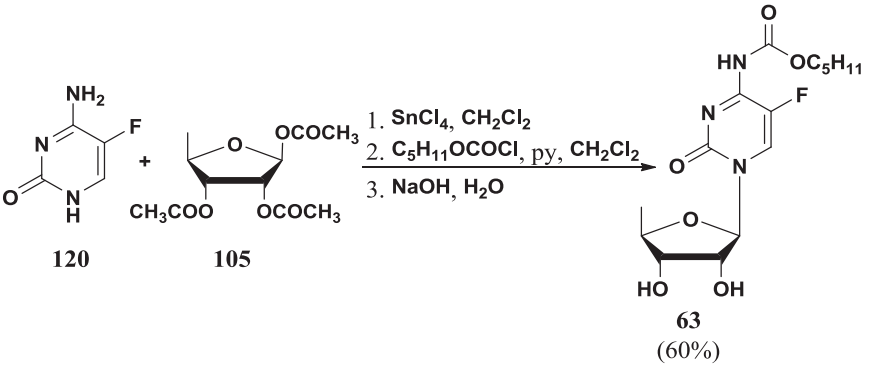
Scheme 65

In 2000,[134] 1,2,3-O-triacetyl-5-deoxy-D-ribose **105** was used as starting material for the glycosylation of the silyl derivative of 5-fluorocytosine **120** in the presence of stannic tetrachloride or trimethylsilyl iodide. Reaction with *n*-pentyl chloroformate and removal of protecting groups afforded the desired capecitabine **63** (*Scheme 66*). Instead of stannic tetrachloride, triflic acid can be used as acid catalyst.[135]



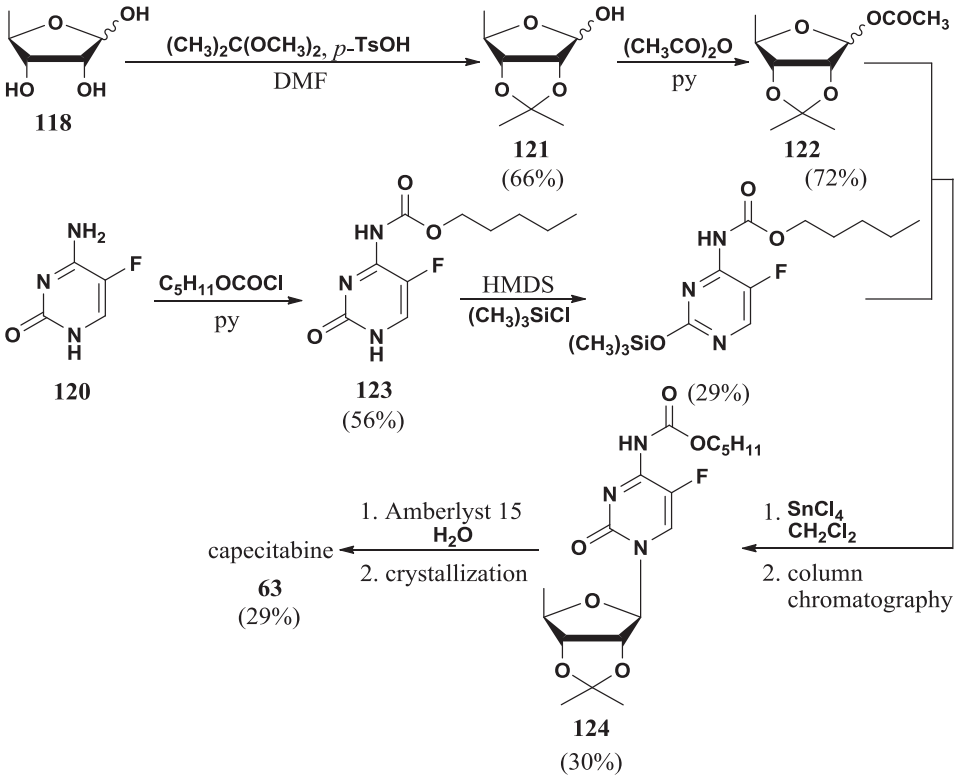
Scheme 66

The N-glycosylation was also carried out without silylating agent, in a "one-pot" procedure that afforded capecitabine **63** in 60% yield (*Scheme 67*).[136]



Scheme 67

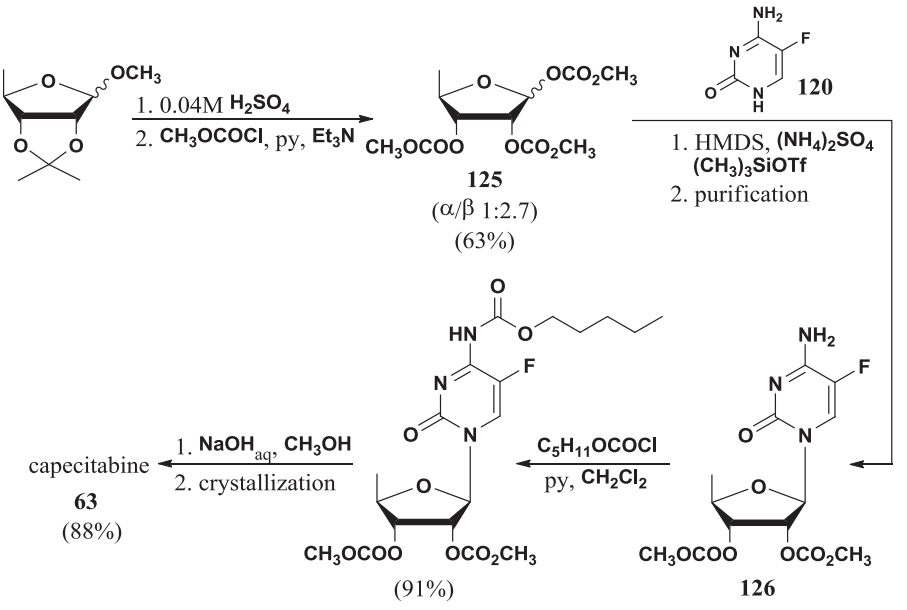
Other syntheses were designed starting from a differently protected 5-deoxy-D-ribose. For example 5-deoxy-D-ribose **118** was transformed into corresponding acetonide **121** by reaction with 2,2-dimethoxypropane in the presence of *para*-toluensulfonic acid, in dimethylformamide as solvent. Acetylation of the 1-hydroxy group afforded the suitable intermediate, compound **122**, for the glycosylation of N^4-pentyloxycarbonyl-5-fluorocytosine **123** under the usual conditions. Acidic treatment (Amberlyst 15) of obtained **124** afforded final capecitabine **63** (*Scheme 68*).[137]



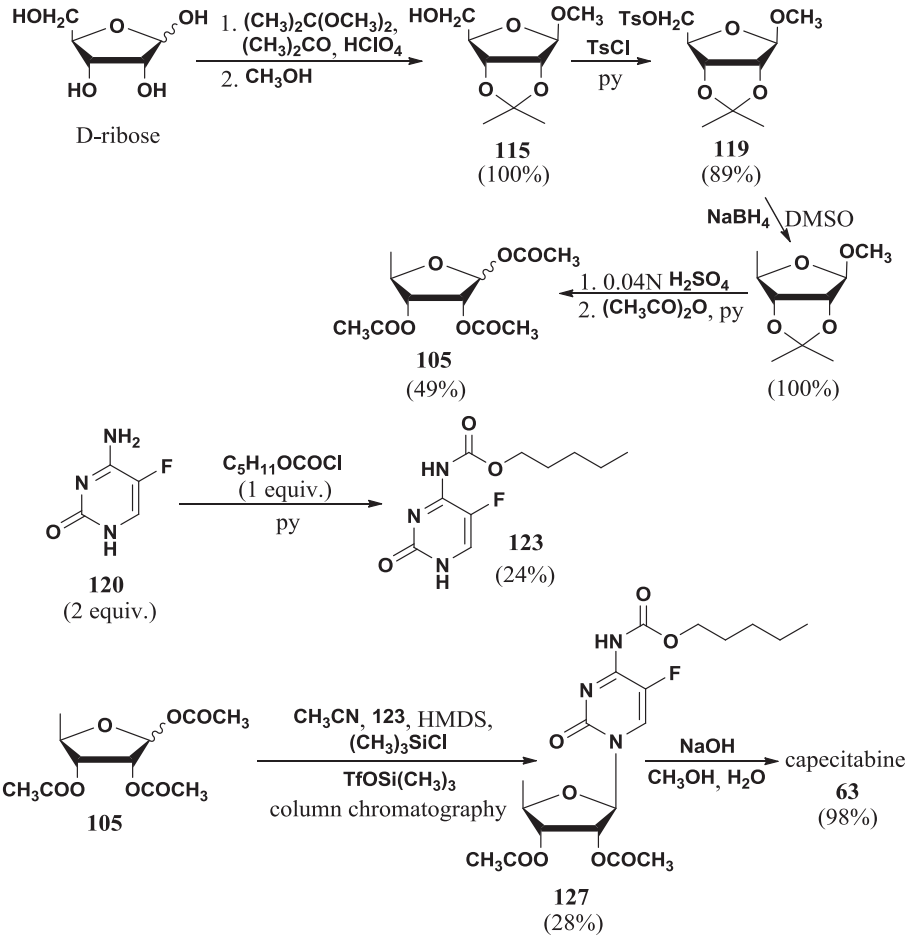
Scheme 68

The 1,2,3-hydroxy groups of 5-deoxy-D-ribose **118** were also protected as methyl carbonates (compound **125**) providing a 1:2.7 α/β anomeric mixture (63%) that was directly used in the glycosylation step. 2′,3′-Protected-5′-deoxy-5-fluorocytidine **126** was recovered, after precipitation of undesired by-products, and submitted to the reaction with pentyl chloroformate; removal of 2′,3′-carbonates under basic conditions and crystallization afforded capecitabine **63** (*Scheme 69*).[138]

A mixture of α- and β-anomers of 1,2,3-tri-O-acetyl-D-5-deoxy-ribose **105**, was also used in the glycosylation of N^4-pentyloxycarbonyl-5-fluorocytosine **123**, that is on the 5-fluorocytosine already derivatized on the 4-NH_2 (*Scheme 70*).[139]



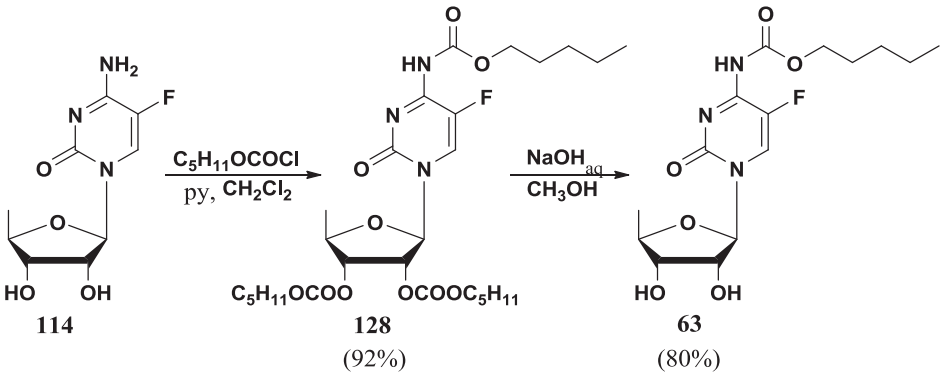
Scheme 69



Scheme 70

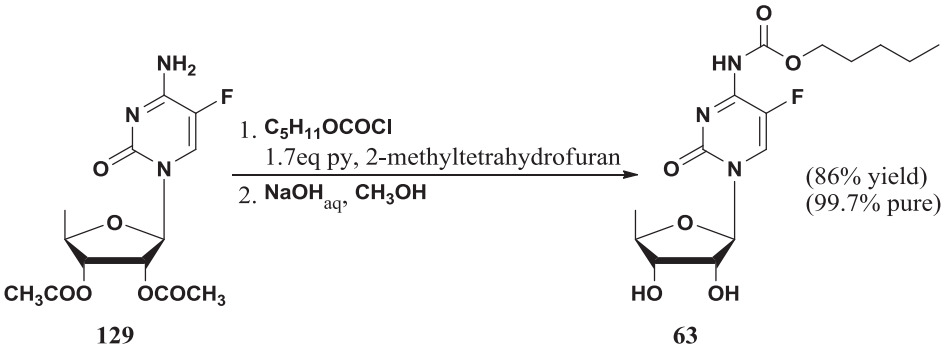
The preparation of the N^4-pentyloxycarbonyl derivative seems to be the crucial step of this synthesis.

The introduction of the carbamoyl function, according to two Hoffman-La Roche patents, published in 1995,[140,141] was postponed to the last step of the synthesis. In one case [141] the N^4-functionalization was realized directly on 5′-deoxy-5-fluorocytidine **114** affording in 92% yield 5′-deoxy-2′,3′-di-O-pentyloxycarbonyl-5-fluoro-N^4-pentyloxycarbonylcytidine **128**. The 2′,3′-carbonates were removed with aqueous sodium hydroxide in methanol (*Scheme 71*).



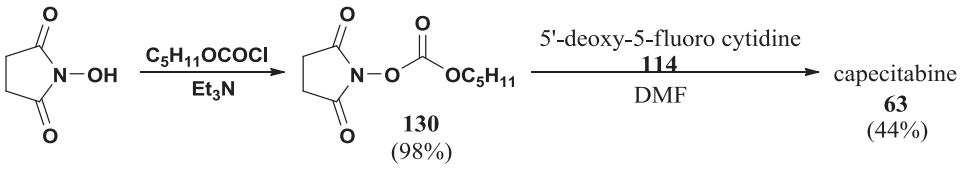
Scheme 71

An improvement of the preparation of the N^4-pentyloxycarbonyl derivative was obtained by Teva by modifying the reaction conditions:[142] choosing 2-methyltetrahydrofuran as solvent, the pyridine amount was significantly reduced (1.7 eq), avoiding its removal by evaporation in the course of work-up. After removal of acetates from compound **129** pure capecitabine **63** in 86% yield was obtained (*Scheme 72*).



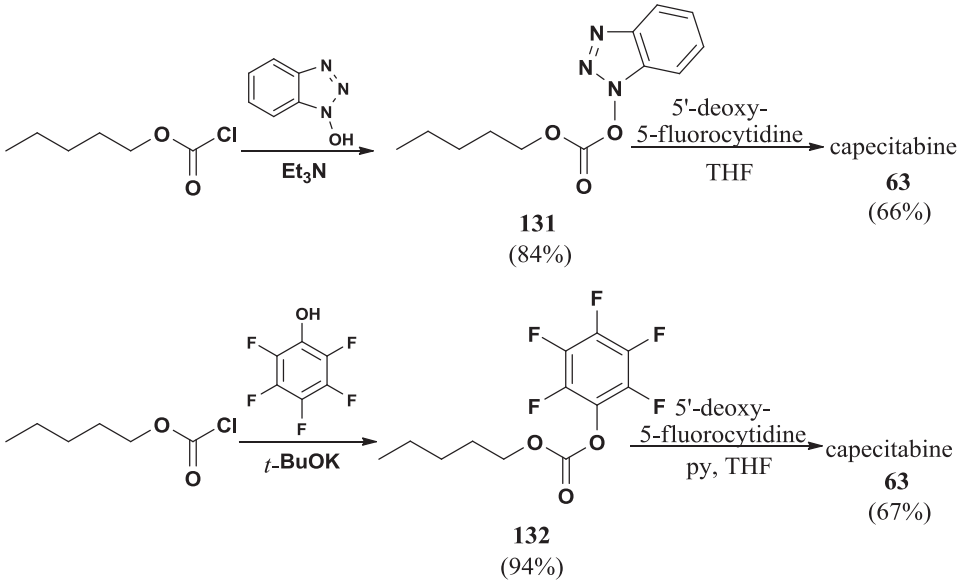
Scheme 72

Attempts to introduce the pentyloxycarbonyl group directly on an unprotected 5′-deoxy-fluorocytidine led to the development of new reagents, different from the usual pentylchloroformate, able to selectively functionalize the N-4. With this purpose in mind, starting from N-hydroxysuccinimide[143], a new pentylcarbonate **130** was recently prepared in 98% yield that selectively functionalized the unprotected 5′-deoxy-fluorocytidine at N-4, affording capecitabine **63** in 44% yield (*Scheme 73*).



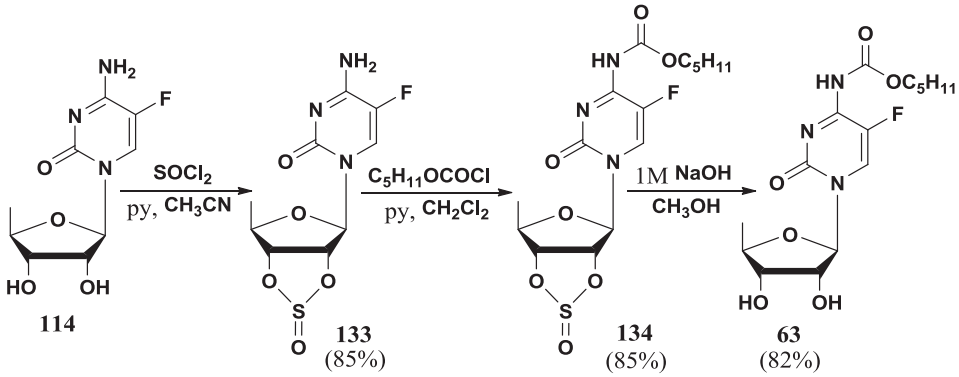
Scheme 73

With the same aim, some pentyloxycarbonylation reagents like hydroxybenzotriazole-**131** and pentafluorophenoxyderivative, **132** were prepared[144] and used to selectively form capecitabine **63** from 5′-deoxy-fluorocytidine **114**, in 66 and 67% yield, respectively (*Scheme 74*).



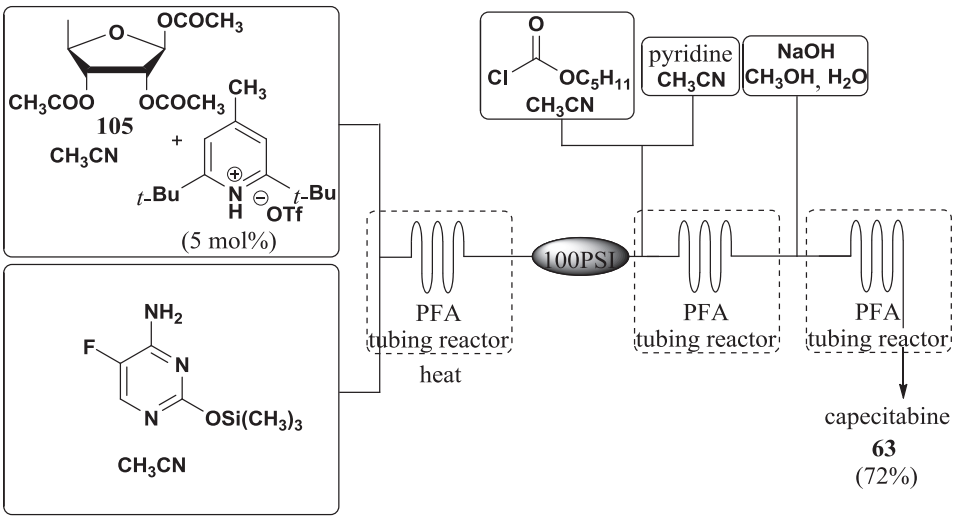
Scheme 74

The same authors also published a method[145] for the preparation of N-functionalized cytidine that, using pentylchloroformate, required the protection of 2′and 3′-hydroxy groups, obtained through the formation, by reaction with thionyl chloride, of cyclic sulfinyl ester **133**, easily purified by crystallization (85% yield). After the N^4-functionalization (85%), the sulfinyl ester of compound **134** was removed by treatment with 1M sodium hydroxide and methanol affording capecitabine **63** (82%) (*Scheme 75*).



Scheme 75

Recently a continuous synthesis[146] (*Scheme 76*) was reported: the synthetic pathway starts from 1,2,3-O-triacetyl-5-deoxy-D-ribose **105** and the glycosylation is catalyzed by a Brønsted acid chosen from among the piridinium triflates. Capecitabine **63** is recovered at the end of the one-flow, multistep synthesis in 72% yield. This method was applied also to other 5′-deoxyribonucleosides as the previously reported doxifluridine **62** (89%) and galocitabine **135** (see below).

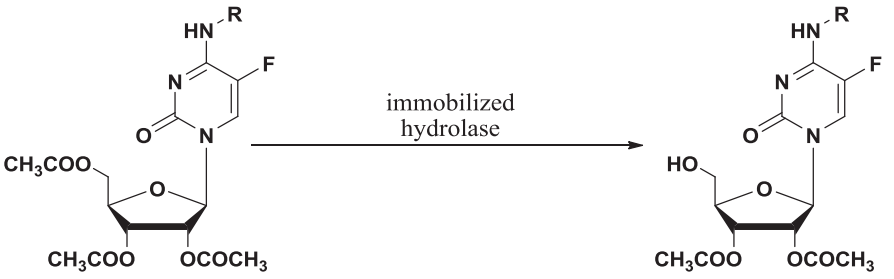


PFA = perfluoroalkoxy

Scheme 76

Since capecitabine **63** is an active pharmaceutical ingredient the identification and the determination of its impurities, as well as the optimization of HPLC methods are very important aspects to be considered together with the synthetic procedures. Some HPLC methods[142,147] and some purification processes utilized for the preparation of substantially pure capecitabine were reported.[148,149] In one of these patents[149] is mentioned the possibility of removing the 2′,3′-O-acetyl groups by means of a hydrolytic enzyme, an unspecified lipase. On the other hand the chemo-enzymatic approach is very attractive for a polyfunctional compound such as capecitabine **63**, considering the capability of enzymes to catalyze selective transformations.

The enzyme-catalyzed regioselective hydrolytic removal of 5′-acetate from nucleoside triacetate **110** (See *Scheme 60*)[112] allowed workers to obtain the 5′-hydroxy-2′,3′di-O-acetyl derivative **111** suitable for the reductive removal of the 5′-hydroxy group, necessary to obtain the 5′-deoxy nucleoside. In a previous patent[150] the same research team reported the results about the selective deprotection of hydroxy groups of nucleosides polyesters by means of immobilized lipases; moreover, the topic of regioselective lipase catalyzed transformations of nucleosides is treated exhaustively. In 2008,[151] they applied the enzymatic regioselective hydrolysis of 5′-O-acetyl group to the 2′,3′,5′-tri-O-acetyl-5-fluorocytidine **136** and to N^4-pentyloxycarbonyl-5-fluoro-2′,3′,5′-tri-O-acetyl cytidine **137** (*Scheme 77*) obtaining advanced intermediates of capecitabine synthesis. High yields were obtained in both 4-NH_2 compound **136** (80–97%, immobilized *Candida rugosa* lipase as enzyme) and N^4-derivatative **137**. On this last substrate the highest yield (92%) was obtained by using the immobilized protease N from *Bacillus subtilis*.

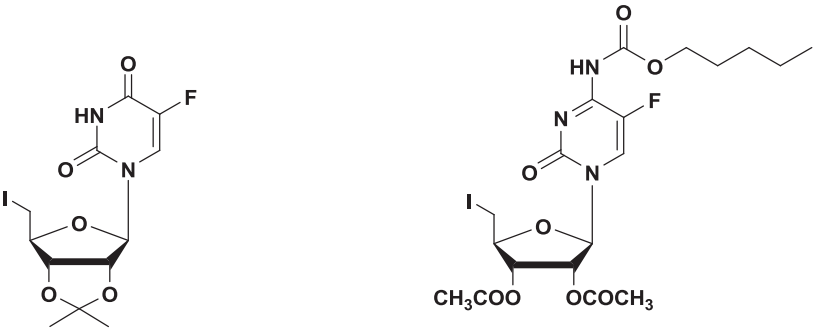


136 R = H, *Candida rugosa* lipase, 80-97% yield **138**
137 R = $COOC_5H_{11}$, protease N, 92% yield **139**

Scheme 77

The same regioselective hydrolysis was also studied comparing immobilized recombinant or commercially available lipases from *Candida rugosa*.[152]

More recently,[153] the chemo-enzymatic approach to a capecitabine intermediate, compound **140**, quite similar to that prepared by Cook and co-workers in 1979[110] (*Scheme 78*) for doxifluridine **62** synthesis, was studied.



Cook's intermediate
109

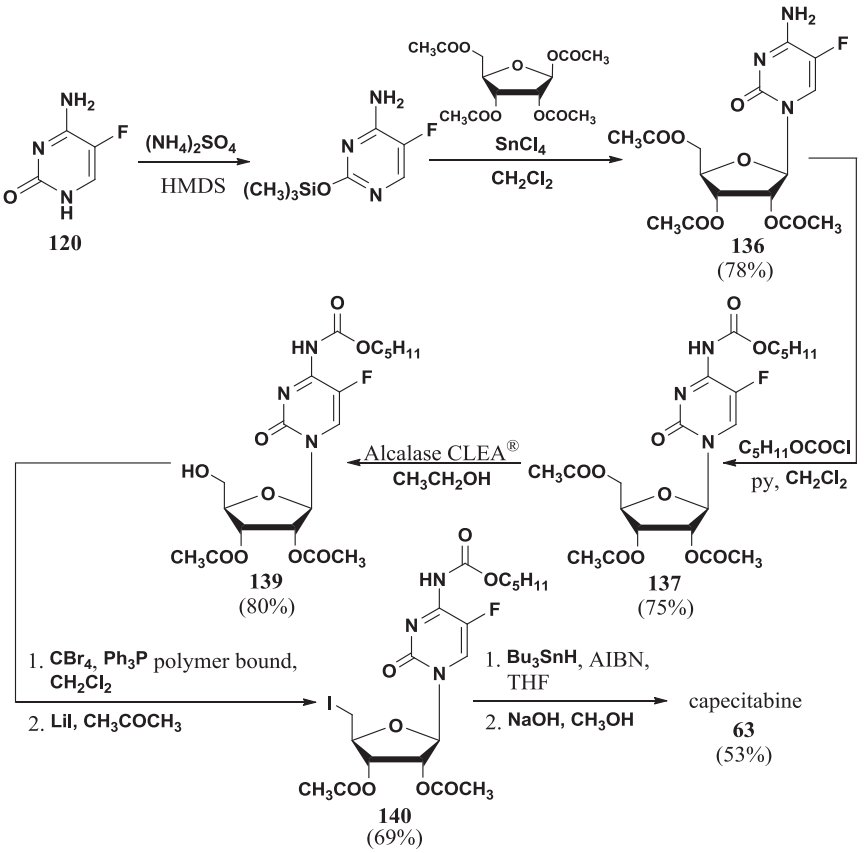
doxifluridine
62

intermediate from chemoenzymatic approach
140

capecitabine
63

Scheme 78

2′,3′,5′-Tri-O-acetyl-5-fluoro-N^4-pentyloxycarbonyl-cytidine **137**, substrate of the enzymatic reaction, was prepared as depicted in enzymes, the best results being observed in the case of a cross linked aggregate preparation of the protease from *Bacillus licheniformis* (subtilisin), the Alcalase-CLEA®: at 96% conversion the 5′-acetate was removed almost exclusively (91%). In order to transform advanced intermediate **139** into the final capecitabine a careful study about the more suitable 5′-functional group and reducing agent was necessary. The 5′-deoxynucleoside was obtained starting from 5′-



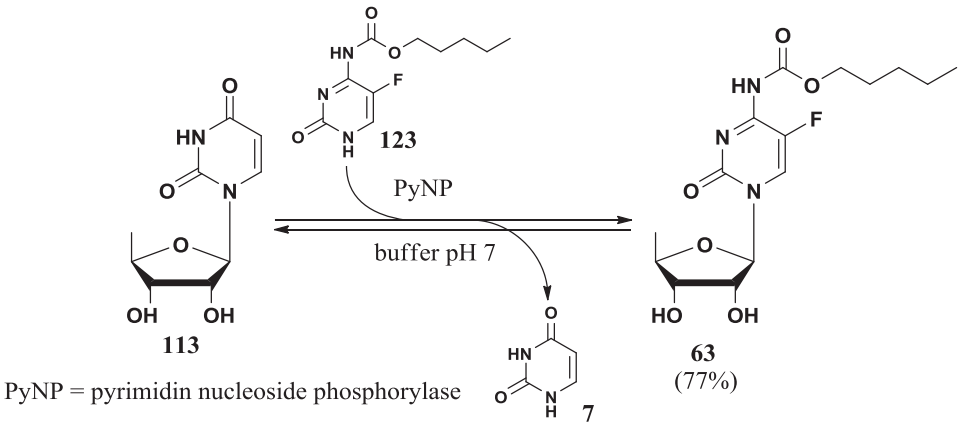
Scheme 79

iododerivative **140** by reaction with tributyltin hydride, followed by deprotection of the 2′,3′-O-diacetyl groups (*Scheme 79*). The CLEA preparation increases the stability of the enzyme in alcoholic solutions and allows an easier work-up. The work includes the ^{1}H and ^{13}C NMR analyses of the nucleosidic intermediates and of the final capecitabine. *Scheme 79*, starting from commercially available intermediates. Triacetate **137** was treated, under alcoholysis conditions, with some hydrolytic

Finally, another chemo-enzymatic approach was described in 2015.[154] In this case, similarly to an already described doxifluridine **62** synthesis[112] the biocatalyzed reaction was a transglycosylation reaction of 5′-deoxyuridine **113** and the preformed nucleobase of capecitabine, namely N^4-pentyloxycarbonyl-5-fluorocytosine **123**; the employed enzyme was a pyrimidine nucleoside phosphorylase (*Scheme 80*).

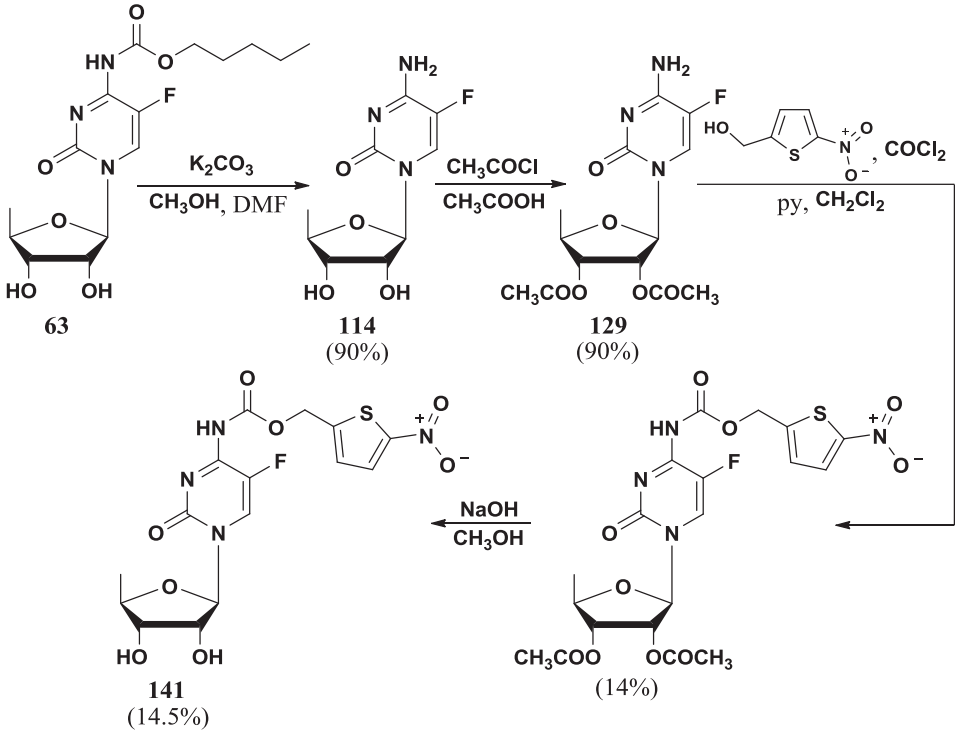
After the introduction in therapy of capecitabine **63**, several analogues were synthesized and submitted to biological tests with the aim of enhancing its efficacy. Mainly, the modifications were carried out on the N-4 position where different groups, such as for instance BOC[155], were introduced instead of the pentylcarbamate.

The substitution of an N-4 bonded group can lead to a prodrug that, in hypoxic environments typical of solid tumors, can be reduced by one-electron processes that are inhibited in the normoxic environment of normal tissues. Radiolysis can activate a



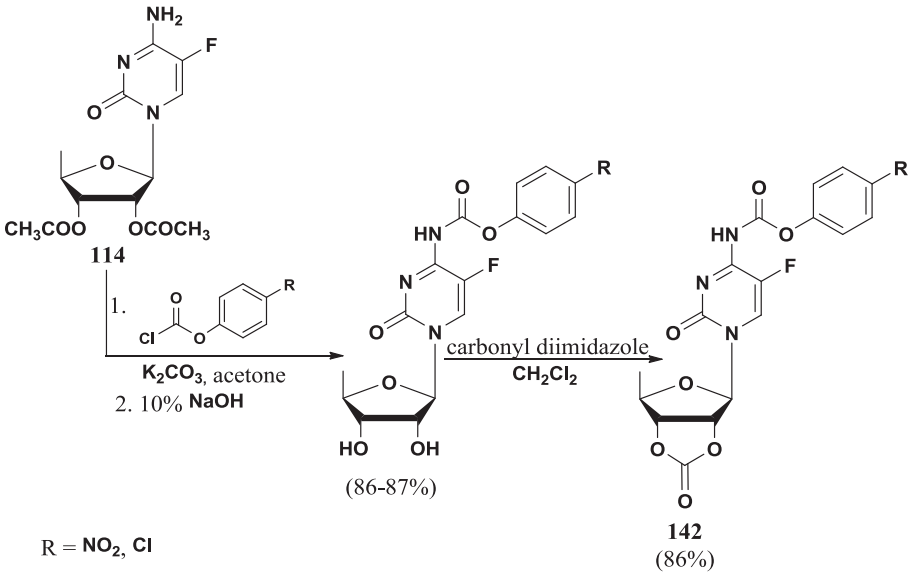
Scheme 80

prodrug to release the active drug after one-electron reduction. An example of a suitable prodrug for radiolysis is N^4-nitrothienylderivative **141** described in a 2006 patent (*Scheme 81*).[156]



Scheme 81

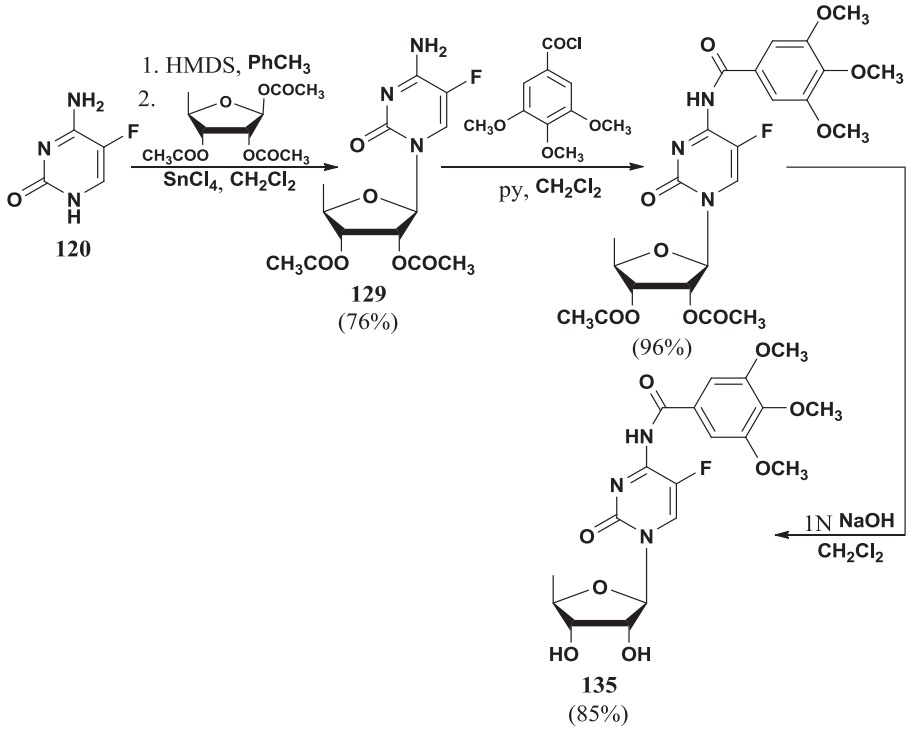
Some 2′,3′-O-cyclic carbonates **142** modified at N-4 showed potent antitumor activ-
690 ity against a leukemia cell line.[157] Their synthesis is reported in *Scheme 82*.



Scheme 82

5. Galocitabine

Galocitabine (5′-deoxy-5-fluoro-N^4-(3,4,5-trimethoxybenzoyl)cytidine), (Ro 09–1390) **135**, can be considered as a capecitabine derivative with a different N^4-substituent or as a doxifluridine prodrug, since at pH 2.1 it rapidly decomposes affording the latter compound.[158] Galocitabine was synthesized from 2′,3′-diacetate-5′-deoxy-5-fluorocytosine **129** in 1995[159] according to *Scheme 83*. Instead of trimethoxybenzoyl chloride a different derivative of trimethoxybenzoic acid can be used,[144] for example the pentafluorophenoxy ester.



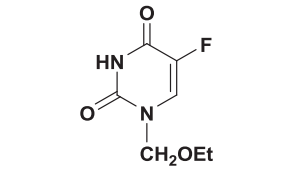
Scheme 83

The 2′,3′-O-cyclic sulfinyl ester[145] of 5′-deoxy-5-fluorocytidine **114** was an alternative protecting group, utilized during the introduction of N^4-acyl group.

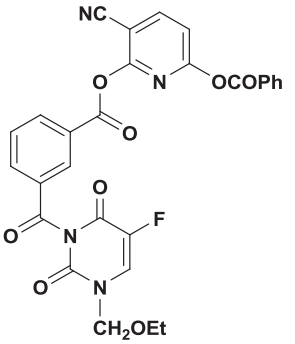
The continuous flow synthesis, like in the case of capecitabine **63**, was also applied to the preparation of galocitabine **135** (89%).[146]

6. 1-Ethoxymethyl-5-Fluorouracil and Emitefur

1-Ethoxymethyl-5-fluorouracyl **143** and emitefur **144** (*Scheme 84*) have in common the same N^1-substitution, namely an ethoxymethyl moiety, which is an acyclic ether, instead of the tetrahydrofuran ring present in tegafur **60**. Moreover, emitefur (more commonly called BOF-A2) **144** is also functionalized on N-3. Both were designed to explore 5-fluoropyrimidine analogues of nucleosides endowed with improved antitumor activity.



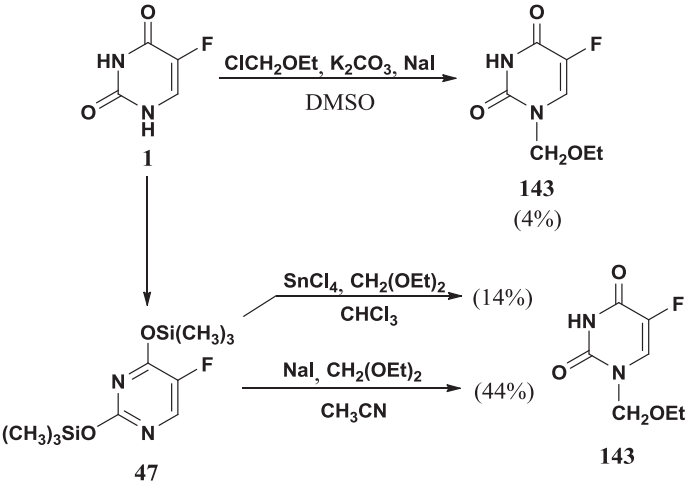
1-ethoxymethyl-5-fluorouracil
143



emitefur
144

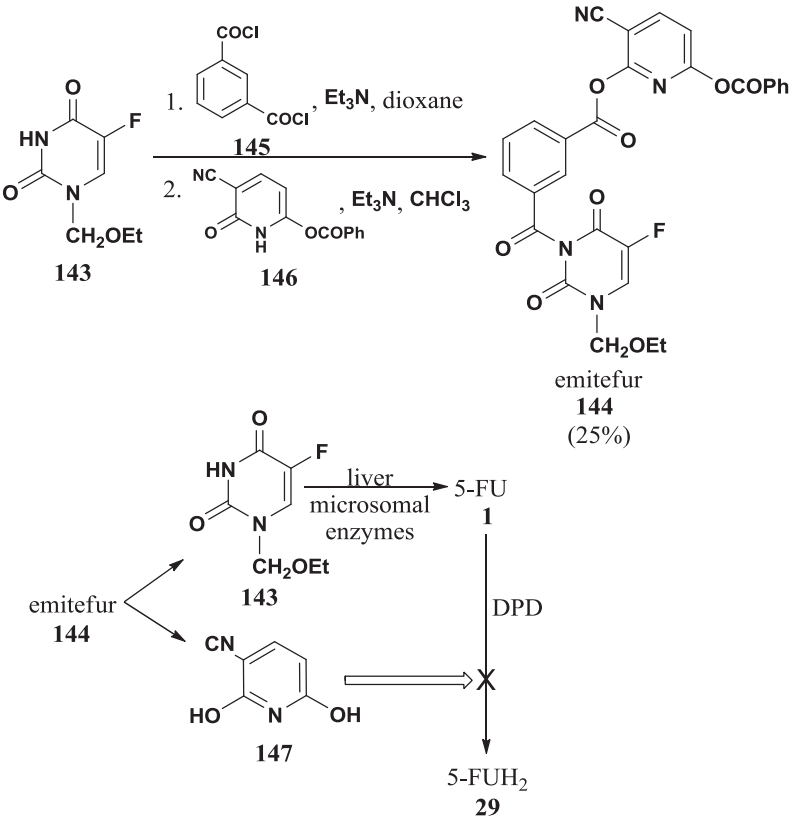
Scheme 84

1-Ethoxymethyl-5-fluorouracyl **143** was synthesized in 1978 in very poor yield (4%) by reaction of ethoxychloromethane with 5-FU **1**[160] or by reaction of the silylderivative of 5-FU **47** with diethoxymethane and sodium iodide (44% yield)[161] or stannic chloride (14% yield),[162] as depicted in *Scheme 85*.



Scheme 85

Starting from 1-ethoxymethyl-5-fluorouracil **143** emitefur **144** was prepared in 25% yield (*Scheme 86*)[163,164] by reaction with the isophthaloyl chloride **145** followed by reaction with 6-benzoyloxy-3-cyano-2-pyridone **146**. Emitefur **144** is degraded *in vivo* into 1-ethoxymethyl-5-fluorouracyl **143**, a prodrug of 5-FU, and 3-cyano-2,6-dihydroxypyridine (CNDP) **147**, a competitive inhibitor of DPD, the enzyme responsible for rapid degradation of 5-FU **1**.[5]

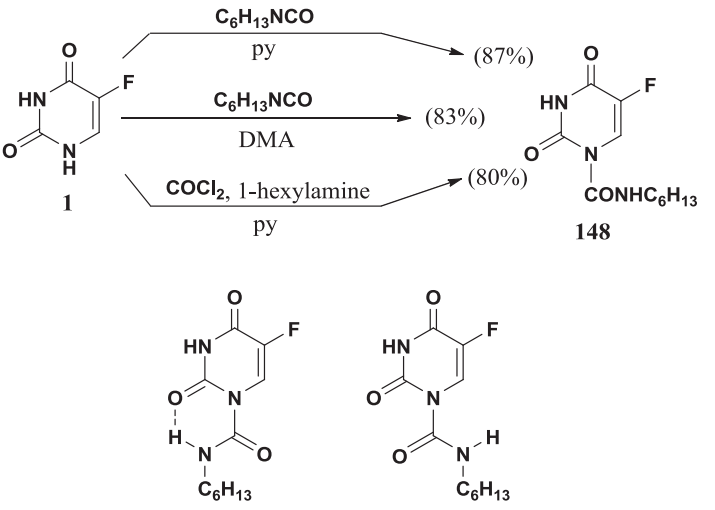


Scheme 86

7. Carmofur

Carmofur **148** is the international non-proprietary name of 1-hexylcarbamoyl-5-fluorouracil (HCFU), an antineoplastic agent orally administered in resected colorectal cancer patients. It has some anticancer activity of its own and it is ultimately transformed *in vivo* to 5-FU **1**.[165] It was prepared[165] starting from 5-FU **1** by reaction with hexyl isocyanate in pyridine or in dimethylacetamide solution, or by reaction with phosgene followed by treatment with 1-hexylamine (*Scheme 87*). The hexyl isocyanate was, in turn, prepared starting from heptanoyl chloride by reaction with sodium azide,[165] or by treatment of 1-hexylamine with trichloromethyl chloroformate, followed by distillation.[166]

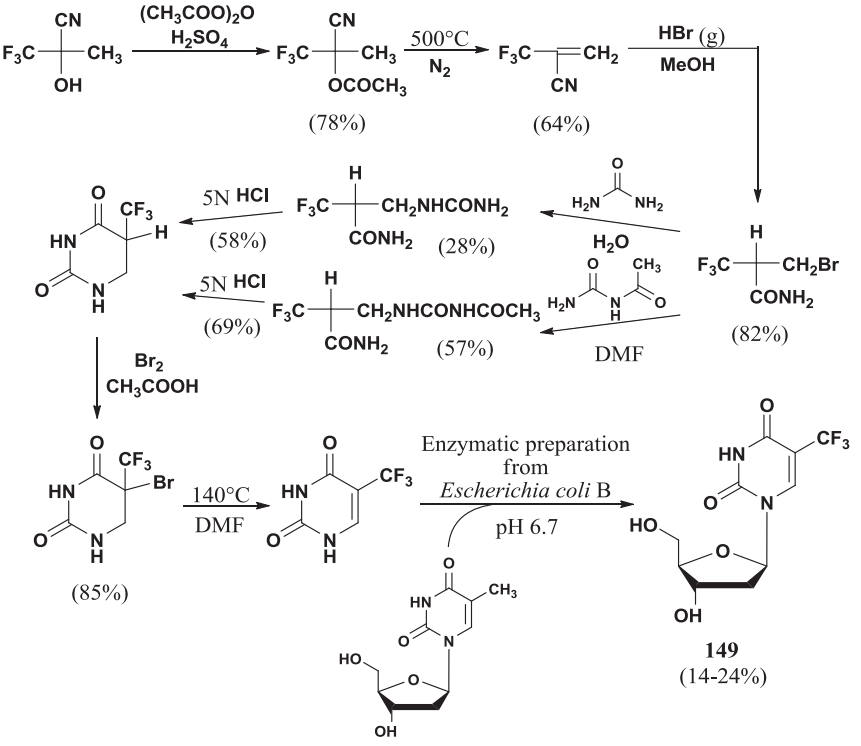
In a polar solvent, like dimethylsulfoxide, carmofur **148** exists as two mixed structures (presumably a hydrogen-bonded structure and a non-hydrogen-bonded structure) at room temperature, as demonstrated by ^{1}H NMR analyses[166] in DMSO-d_6 at room temperature and at 80°C or in $CDCl_3$. Deuterated derivatives of carmofur bearing deuterium atoms on the hexyl chain were prepared in order to evaluate the isotopic effect on the metabolism rate.[165]



Scheme 87

8. Related Non-Clinical Fluoropyrimidine Nucleosides

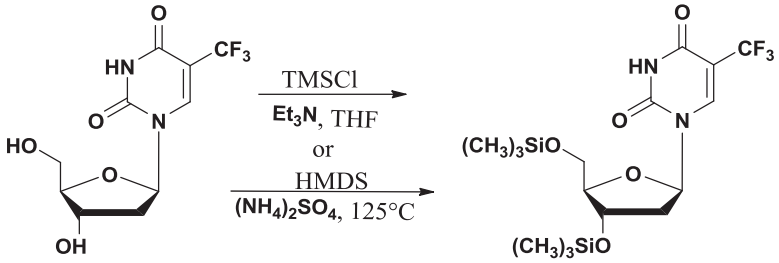
New syntheses of fluoropyrimidines nucleosides are usually aimed at the preparation of new potential antitumor, antiviral or antibacterial compounds and their destiny depends on the results of clinical investigations. The fluorination in most cases takes place at the
735 5-position, namely the position involved in the thymidine biosynthesis. In the previously examined compounds the fluorine atom is directly linked to C-5 of heteroaromatic ring. In the case of 5-trifluorothymidine (TFT) **149** a trifluoromethyl group instead of the 5-methyl group is present.



Scheme 88

TFT **149** was synthesized in 1964 by Heidelberger[167] according to *Scheme 88*, but its development as antineoplastic agent was early hampered by its short half-life (12 min).[168]

The renewed recent interest for TFT **149**, prompted the synthesis of a convenient prodrug. In a 2016 patent [169] the silylation of 3′ and 5′-hydroxy group of TFT was realized in order to prolong the TFT **149** circulatory half-life. Two methods of silylation affording compound **150** (*Scheme 89*) were described.



149 **150** (yields not given)

Scheme 89

III. Pyrimidine Nucleosides Fluorinated at the Sugar Moiety

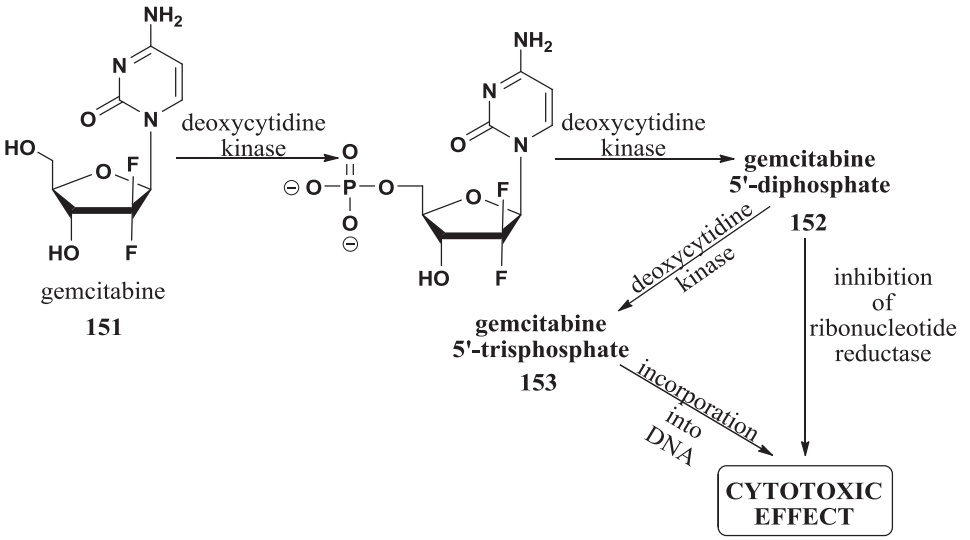
Fluorine atom introduction at nucleosides can occur not only at the pyrimidine moiety but also at the glycone moiety, modifying the biological activity to an equally significant extent, leading to antiviral or antitumoral molecules. The sugar can be fluorinated at 2′,3′,4′,5′-positions or can bear an exocyclic fluorocarbon substituent. Considering that some reviews[170–173] already cover the topic of nucleosides fluorinated at the sugar moiety, we focused on an antitumor pyrimidine nucleosides difluorinated at the 2′-position, namely gemcitabine. The presence of fluorine at the 2′-position can provide acidic and enzymatic stabilities to the glycosidic bond[174] and the *gem*-difluoromethylene group is an isopolar and isosteric substituent for oxygen.[175] Extensive studies about the 2′-position modification with atoms or groups other than hydrogen or hydroxy (for example Cl,[176], N_3,[177] Se,[178] CH_3,[179,180]) have been performed, since the presence of a hydroxy or a hydrogen distinguishes nucleosides as components of RNA or DNA.

1. Gemcitabine

Gemcitabine **151** hydrochloride (2′,2′-difluoro-2′-deoxycytidine hydrochloride) is currently produced and marketed as Gemzar® for the treatment of various cancers such as pancreatic, breast, non-small cell lung and ovarian cancers. It is administered by intravenous infusion, the oral use of gemcitabine being limited by poor bioavailability.

Gemcitabine is intracellularly converted by deoxycytidine kinase into its therapeutically active metabolites, the 5′-diphosphate **152** and the 5′-triphosphate derivatives **153** (*Scheme 90*).

5′-Diphosphate **152** inhibits the ribonucleotide nuclease, the enzyme responsible for regulating the total rate of DNA synthesis, while 5′-triphosphate **153** can become incorporated into the DNA and inhibits the nuclear replication. The 5′-position is directly involved in the action of gemcitabine **151**, while the 4-NH_2 group plays a role in the



Scheme 90

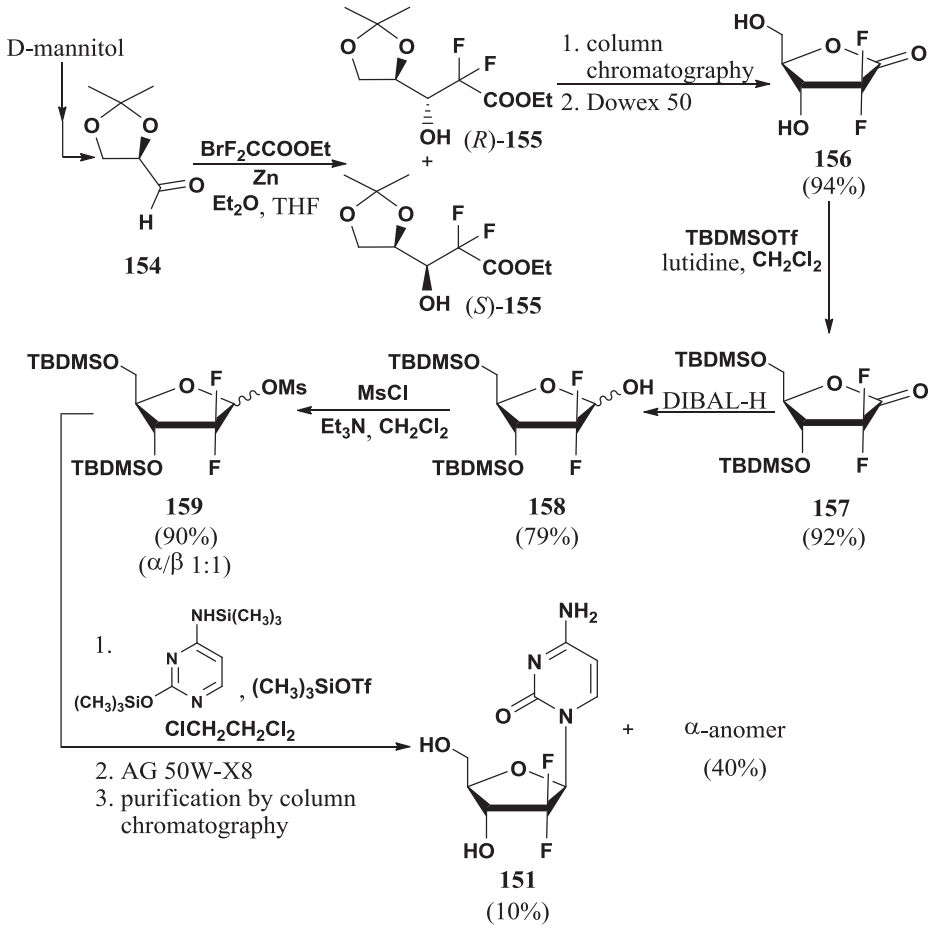
enzyme-substrate recognition through the formation of hydrogen bonding interactions with the kinase; the removal of the amino group by the liver enzyme cytidine deaminase[117] leads to the inactive metabolite 2′,2′-difluorouridine.

The preparation of the 2′,2′-difluorosugar is the main target in gemcitabine **151** synthesis and many different approaches have been proposed (some of that described in a minireview[181]) since the first synthesis reported by Hertel in 1988.[182]

The starting material for this synthesis (*Scheme 91*) was protected (*R*)-glyceraldehyde **154** prepared from D-mannitol; its reaction with ethyl bromodifluoroacetate led to a diastereomeric mixture of (3*R*)- and (3*S*)-propionate (3*R*/3*S* 3:1) derivatives **155** that were separated by silica gel column chromatography. The (3*R*)-isomer, under acidic conditions afforded lactone **156** that, after protection of 3- and 5-hydroxy groups (compound **157**), was reduced with diisobutylaluminum hydride to a mixture of α- and β-anomers (**158**); the anomeric mixture was transformed into the corresponding 1-O-mesylate (compound **159**) necessary for the substitution by the silyl derivative of cytidine. The formation of the N-glycosidic bond was the crucial step of the synthesis since the required β-anomer of **151** was the minor product (10%). The undesired α-anomer was obtained in 40% yield, after removal of the protecting group and separation by reverse phase column chromatography.

An improvement to this synthesis was achieved by Chou and co-workers,[183] some years later, through the selective crystallization of the diastereomeric mixture of 3,5-di-O-benzoyl-difluororibono-lactone **161** obtained by esterification of 3′-benzoate **160** (*Scheme 92*). The choice of benzoyl as protecting group of 3- and 5-hydroxy groups allowed selective crystallization of the desired *erythro*-isomer. Reduction of the 1-carbonyl group afforded a mixture of α- and β-anomers **161** that was transformed into the corresponding mesylates **162**. The glycosylation reaction afforded a 1:1 α/β anomeric mixture of nucleosides **163** instead of the 4:1 mixture observed by Hertel which used *tert*-butyldimethylsilyl ether as protecting group.

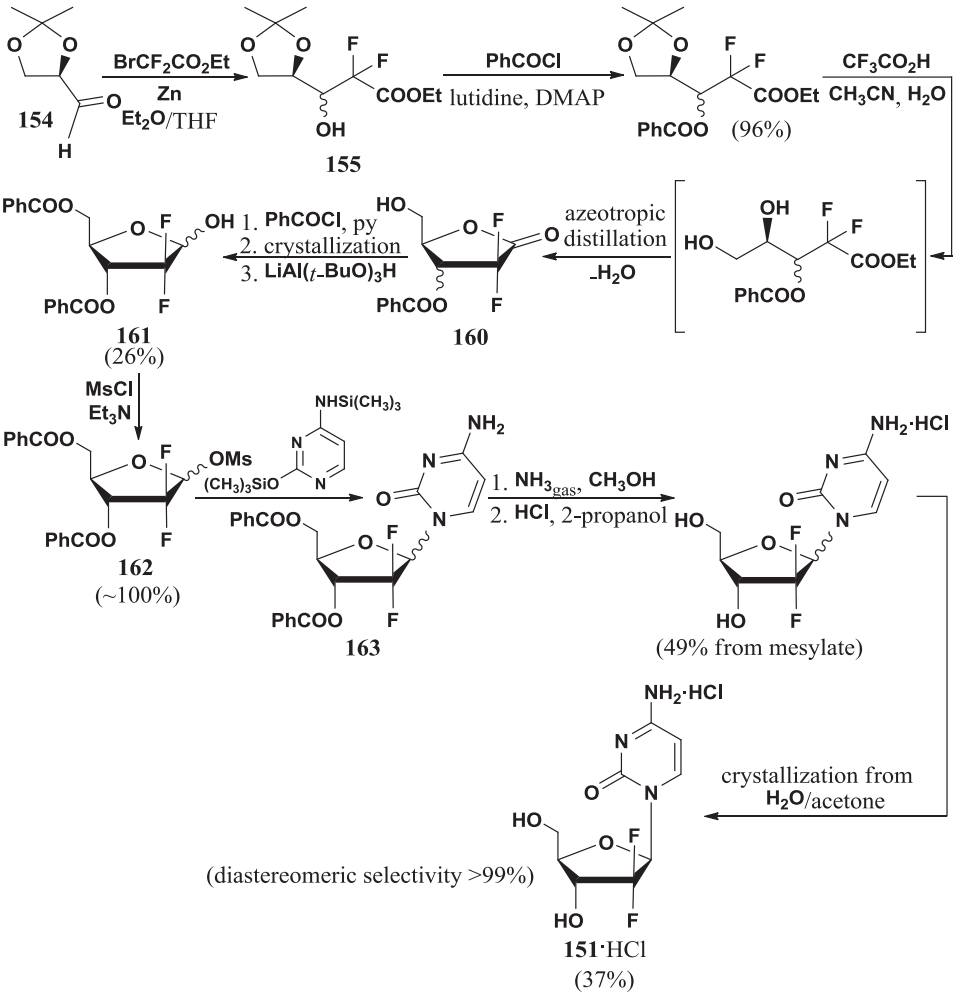
Starting from protected (*R*)-glyceraldehyde **154** many modifications, aimed to improve the yield and the purity of the final β-anomer, were proposed relating to the



Scheme 91

use of different reducing agents of the lactone, to the introduction of alternative protecting groups, to the transformation of 1-hydroxy into a different leaving group and, finally, to the purification process of the obtained gemcitabine **151**. More than ten patents have been published in 2005–2015 about this topic. For example, an Indian team[184,185] was able to obtain 3,5-di-O-benzoyl-2,2-difluoro-lactone with a high purity (99.8% *erythro*-isomer) by treatment of the diastereomeric mixtures of lactones **164**, containing also the corresponding hydroxyacid **165**, with *para*-toluensulfonic acid in toluene, at reflux, removing the water by means of the Dean-Stark apparatus, followed by crystallization (*Scheme 93*). Reduction with Vitride® (sodium bis(2-methoxy)aluminum hydride, also known as Red-Al® or SMEAH) followed by treatment with mesyl chloride afforded usual intermediate 1**62** for the accomplishment of the synthesis. The gemcitabine hydrochloride showed a 95% diastereomeric purity that was increased to 99.9% by means of crystallization by water/acetone or water/2-propanol.

Another approach[186] made use of DIBAL as reducing agent of protected lactone **166** and mesylate as leaving group in compound **167**, postponing all purification processes until the end of the synthesis. By this method, 92.6% pure gemcitabine was obtained after

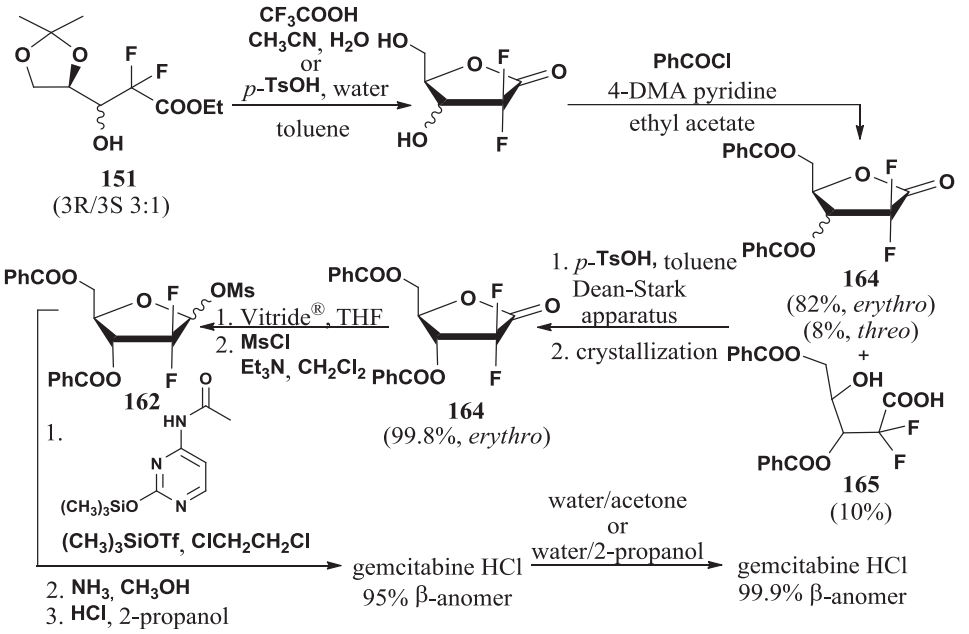


Scheme 92

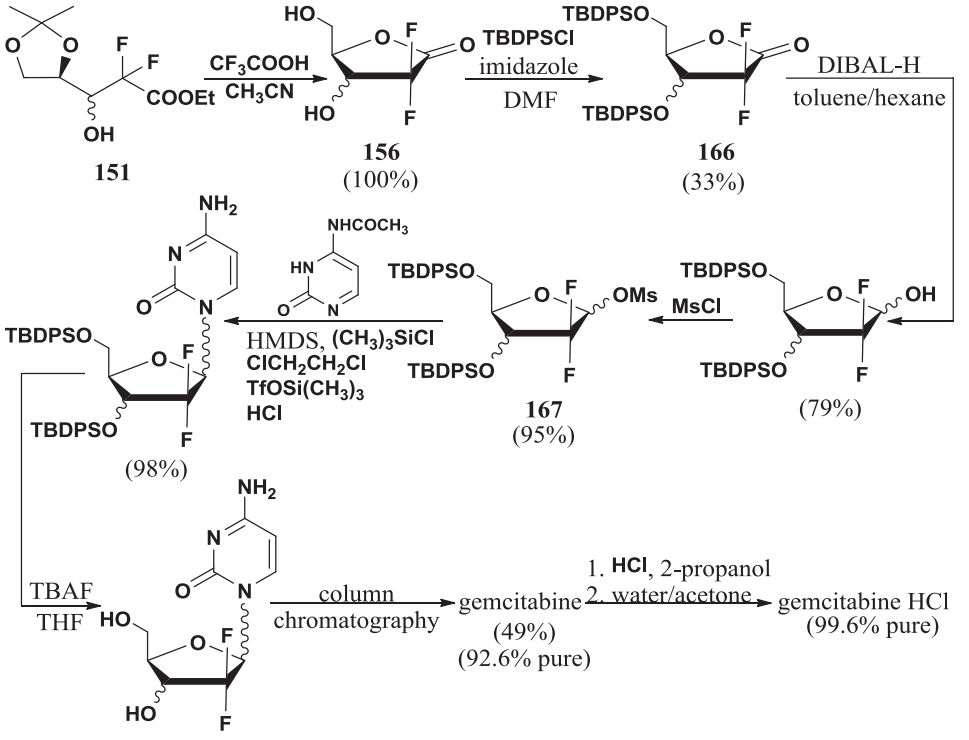
column chromatography. The final crystallization of gemcitabine hydrochloride from water/acetone afforded 99.6% pure gemcitabine hydrochloride (*Scheme 94*).

The choice of a suitable protecting group[187] for the 3- and 5-hydroxys (benzoyl substituted with an electron withdrawing group), with the aim to obtain solid compounds, allowed preparation of the pure *erythro*-lactone **168** by crystallization from ethyl acetate/hexane. The synthesis was then finished in the usual way; the precipitation of gemcitabine hydrochloride afforded 99.9% pure product (*Scheme 95*).

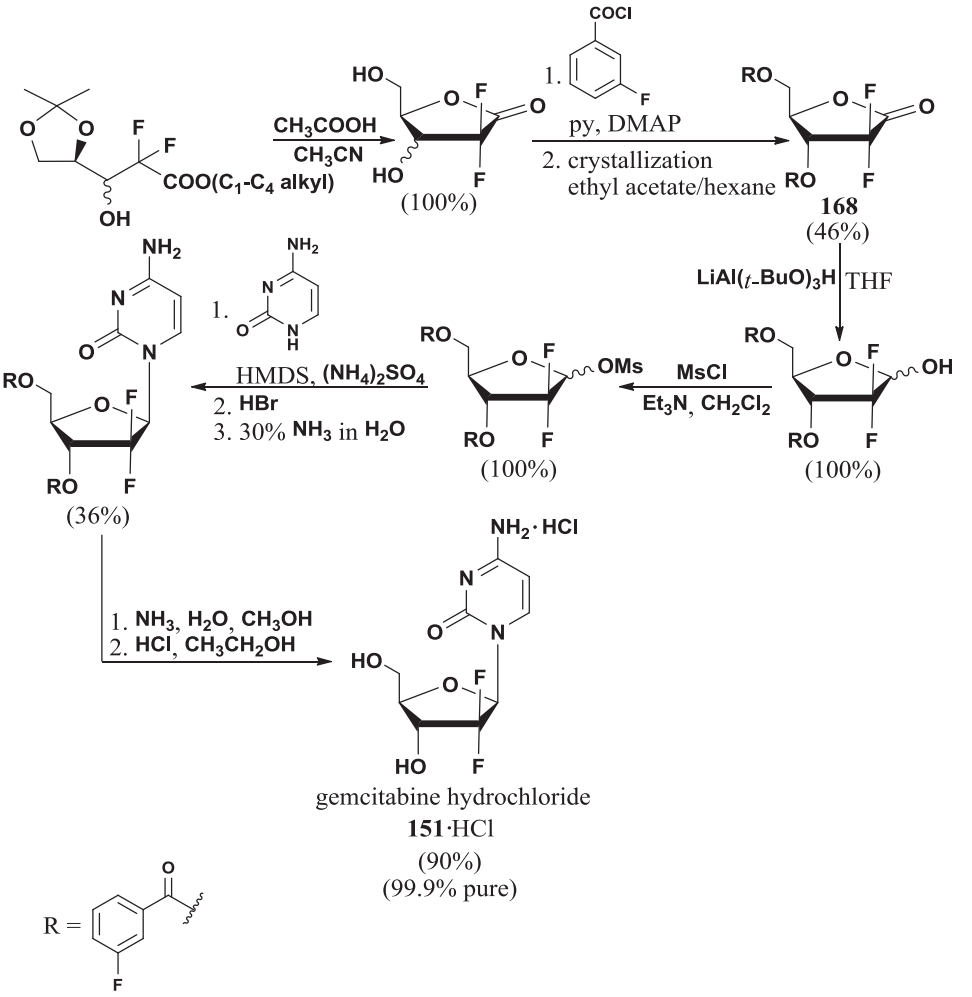
Tri-*tert*-butoxyaluminum hydride was used in the course of the reduction of the lactone protected as 3,5-di-O-(3-fluorobenzoyl) ester[187] or 3,5-di-O-(4-phenyl)benzoyl ester.[188] By means of Red-Al® was instead obtained the lactol, protected at the hydroxy groups as *tert*-butyldimethylsilyl ethers[189] or benzoates,[190,191] or naphthoyl esters,[192] or as 3-O-benzoate and 5-carbamate.[193] In this last case, the trichloroacetimidate was chosen as the leaving group. If the same leaving group was introduced and the obtained trichloracetimidate directly submitted to the reaction with protected cytidine, in a "one-pot" process,[190] the protected



Scheme 93



Scheme 94



Scheme 95

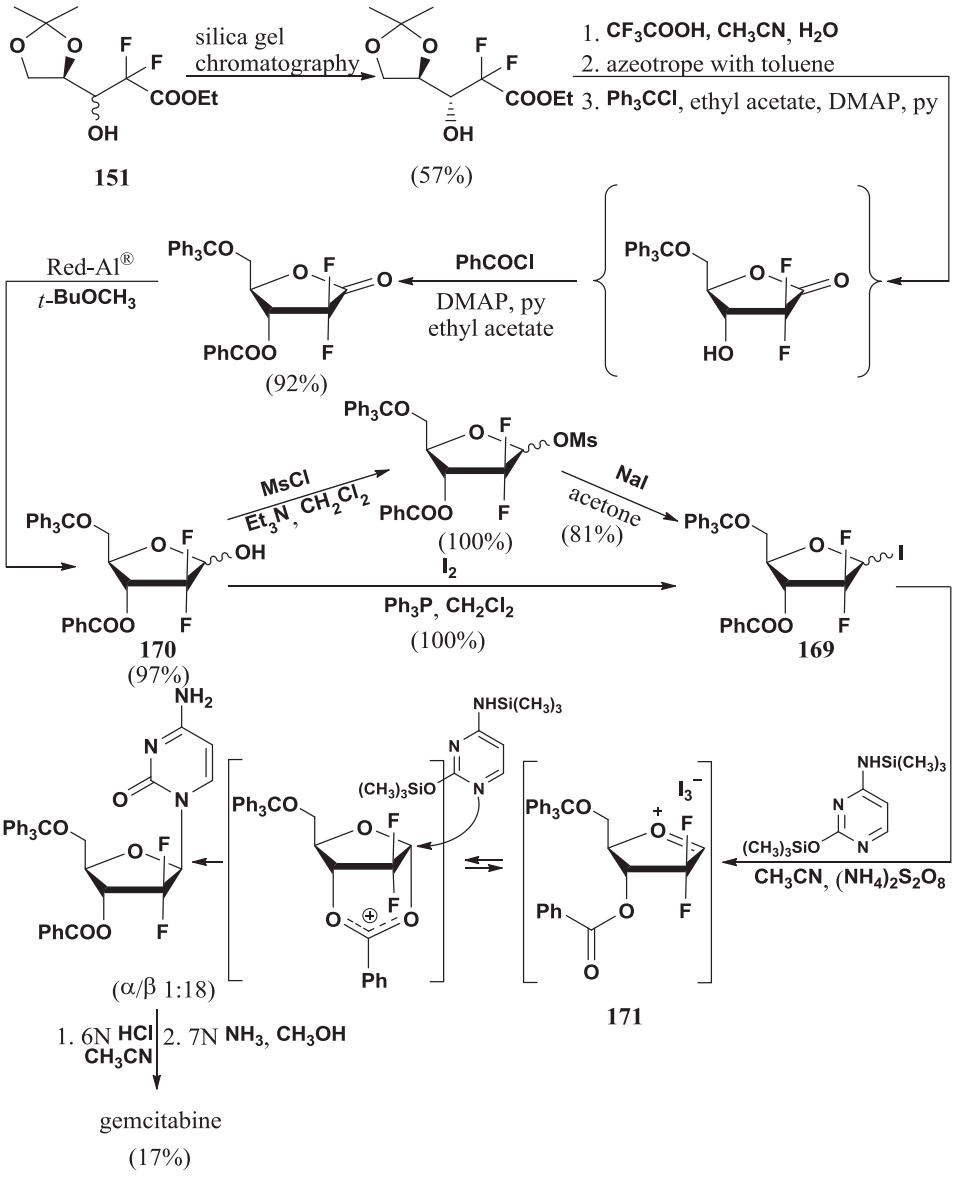
nucleoside was obtained in high yield (89%); gemcitabine hydrochloride was obtained (99.94% β-anomer) in 30% yield after deprotection and purification process.

The use of the cinnamoyl ester as protecting group of 3- and 5- hydroxyl allowed the selective precipitation of an anomer at different steps of the synthesis.[194,195]

In order to avoid the energy waste required to maintain the low temperatures (between −80 and −60°C) during the reduction of the 1-carbonyl group with DIBAL, in a 2010 patent the use of calcium borohydride in tetrahydrofuran (90% yield) or sodium borohydride in ethyl acetate (88% yield) was proposed.[196]

In order to increase the β/α anomer ratio, 1-iodido-2,2-difluoro derivative **169**, as substrate of the N-glycosylation, was prepared through two alternative ways: directly from 1-hydroxy **170** by reaction with iodine and triphenylphosphine in dichloromethane, or through its mesylate, with sodium iodide in acetone. The addition of a silver salt to the glycosylation reaction mixture led to the formation of oxonium intermediate, **171** that underwent the attack of nucleobase from the top of the molecule, favoring the formation of the β-anomer (α/β 1:5.6).[197] In a more recent patent[198] the authors reported that even more favorable ratios can be obtained adding

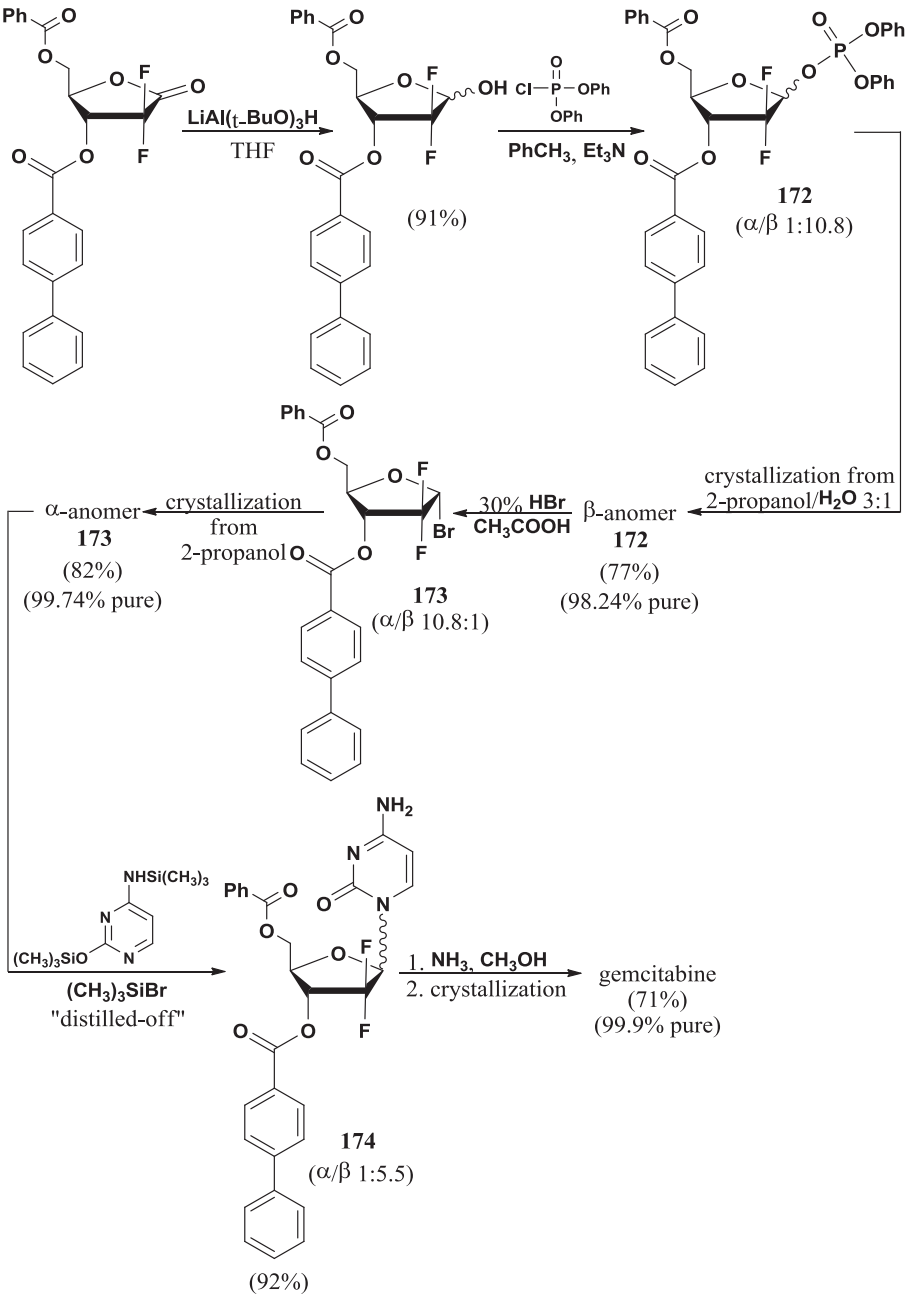
an oxidizer; for example, a 1:18 α/β anomer ratio was observed using $(NH_4)_2S_2O_8$ in acetonitrile. (*Scheme 96*).



Scheme 96

The presence of a bulky protecting group (*para*-phenylbenzoate)[199,200] of the 3-hydroxy group allowed workers to obtain a solid 1-phosphate, compound **172** showing a 1:10.8 α/β ratio, that by a simple crystallization afforded in 77% yield the β-anomer (98.24% pure). The β-phosphate was easily converted into the corresponding α-bromo derivative **173**, which is a suitable precursor, after crystallization, of the N-glycosylated product with the desired β-stereochemistry. In order to avoid possible anomerization of α-bromo sugar **173** the trimethylsilyl bromide, generated in

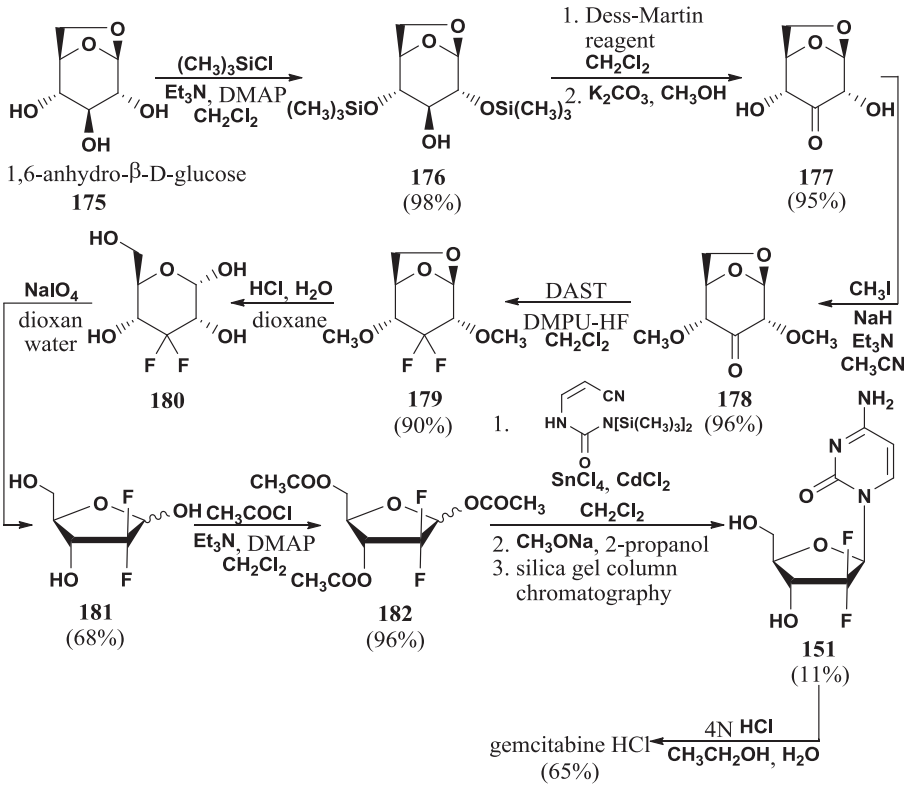
the course of substitution with the protected nucleobase, was removed by distillation using heptane as carrier. By this method, glycosylation occurred in high yields (92%) giving compound **174** showing a 1:5.5 α/β ratio. Pure gemcitabine **151** was obtained, after removal of protecting groups and crystallization in 65% yield from
860 α-bromo sugar **173** (*Scheme 97*).



Scheme 97

According to a different approach, instead of the usual protected (*R*)-glyceraldehyde **154**, 1,6-anhydro-*β*-D-glucose **175**, prepared by thermal degradation of starch, was used as starting material.[201]

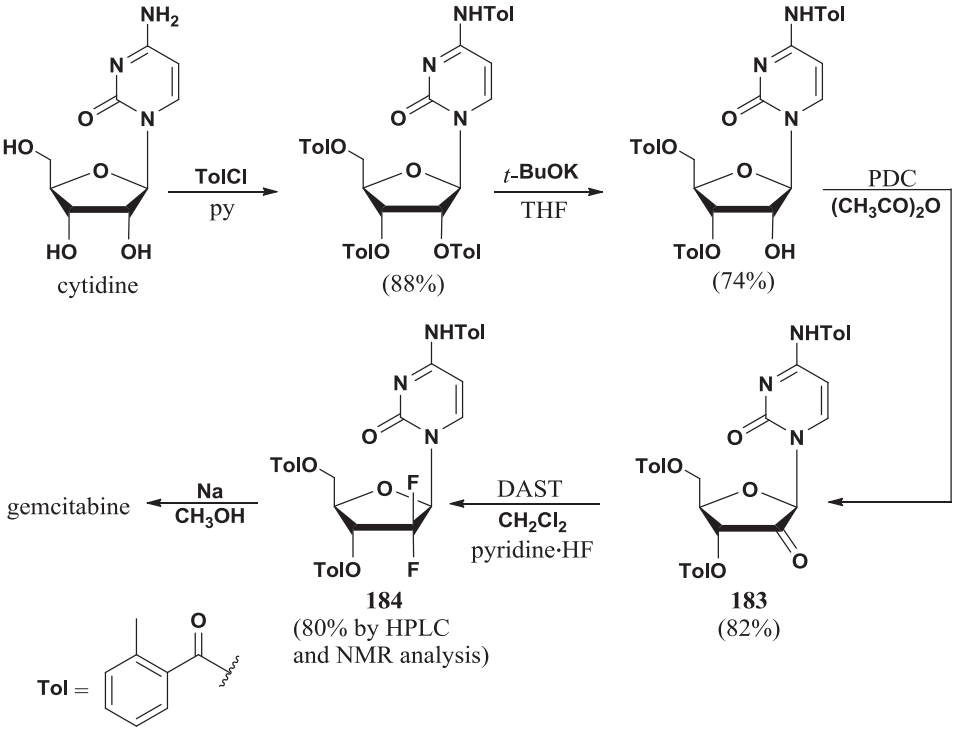
2,4-Trimethylsilyl ether **176** was oxidized to 3-keto derivative **177** by means of Dess-Martin reagent. After removal of the silyl ether the 2- and 4-hydroxy groups were transformed into corresponding methyl ethers **178** (96% yield) and the 3-ketoderivative was converted into *gem*-difluoro derivative **179** by reaction with diethylaminosulfuryl trifluoride (DAST) and N,N-dimethylpropylene urea-hydrogen fluoride (DMPU-HF) (90% yield). Opening the anhydro ring under acidic conditions (compound **180**) and oxidation with sodium periodate afforded 2-deoxy-2,2-difluoro-D-ribose **181** in 68% yield. The synthesis was accomplished as reported in *Scheme 98*. 1,3,5-Tri-O-acetyl-D-ribose difluoroderivative **182** was condensed with the N^1-*cis*-(2-cyanovinyl)-N,N-bistrimethylsilyl urea and the obtained intermediate was cyclized under strong alkaline conditions to the final *β*-nucleoside in 11% yield, after column chromatography. The overall yield from 1,6-anhydro-*β*-D-glucose **175** was 4.4%, confirming the difficulties encountered in other synthetic approaches, deriving from the presence of two stereogenic centers, in the 1- and 3-positions, generated or modified in the course of the synthesis.



Scheme 98

The direct fluorination of a preformed nucleoside can overcome the formation of the *erythro* and *threo*-diastereoisomers encountered starting from (*R*)-glyceraldehyde **154**; in addition, the N-glycosylation with a non-fluorinated sugar can afford a more favorable *α*/*β* anomers ratio. To this end, a suitably protected 2′-ketonucleoside **183** was prepared starting from the commercially available cytidine and submitted to the reaction with

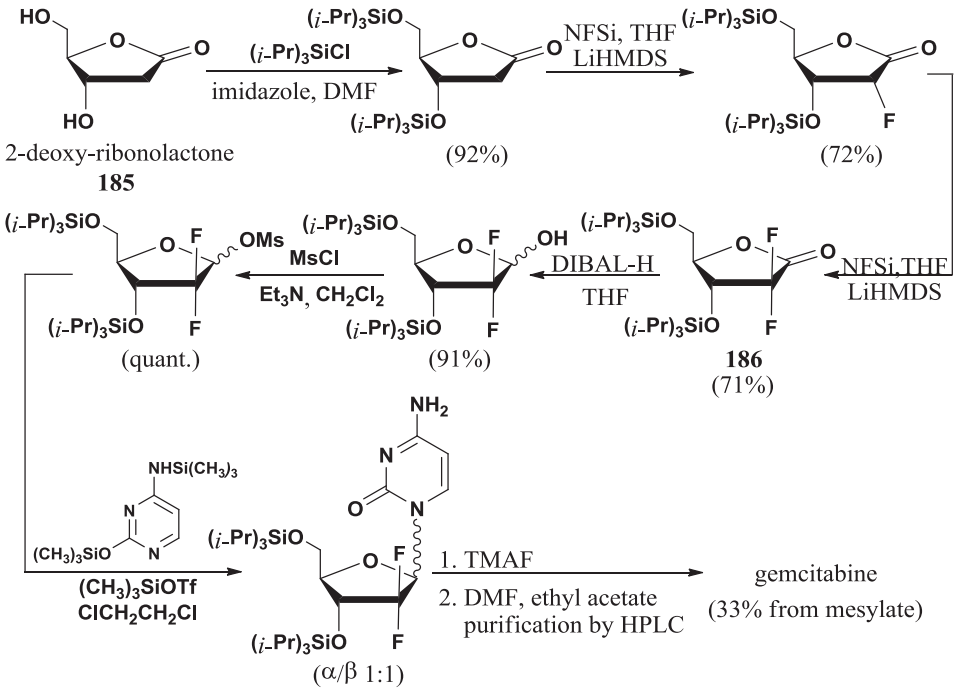
DAST in presence of pyridine-hydrogen fluoride (*Scheme 99*).[202] N^4, 3′,5′-Tri-(*ortho*-toluyl)-2′-ketocytidine **183** was prepared in 53% overall yield from cytidine. Reaction with the fluorinating agent afforded protected difluoronucleoside **184** (80% from HPLC and NMR) that was converted into gemcitabine **151** by removal of protecting groups.



Scheme 99

In another example 2-deoxy-D-ribonolactone **185** was chosen as the substrate of the fluorination, which was carried out in two steps with N-fluorodibenzenesulfonimide (NFSi) in presence of lithium bis(trimethylsilyl)amide (LiHMDS) (*Scheme 100*).[203] Protected *gem*-difluororibonolactone **186**, obtained in 47% yield from 2-deoxy-D-ribonolactone **185**, was then transformed into gemcitabine **151** as depicted in *Scheme 100*.

In 2014, a team of Merck researchers published the synthesis of 2-deoxy-2,2-difluoro-D-ribose according to their "de novo" approach (*Scheme 101*).[204] Optically active aldehyde **187** (83% ee) prepared through an enantioselective method, developed by the authors, was coupled with isopropyl bromodifluoroacetate under Reformatsky conditions: the suitably functionalized pentanoate was obtained as a mixture of diastereoisomers **188**. Purification by flash chromatography provided the required diastereoisomer in 59% yield. Removal of the 2,2,6,6-tetramethylpiperidinyl group (TMP) and concomitant cyclization afforded the difluorolactone **189** precursor of the ribose ring. Gemcitabine synthesis was then carried out through 1-iodine derivative **190**, in the presence of persulfate, as previously reported.[197,198] (see *Scheme 96*). At the end of the synthetic sequence gemcitabine was isolated in 23% overall yield, from optically active aldehyde **187**, and a 1:4 α/β-anomers ratio. This synthesis offers a promising approach to gemcitabine preparation; indeed the final purification could be carried out according to published methods.



Scheme 100

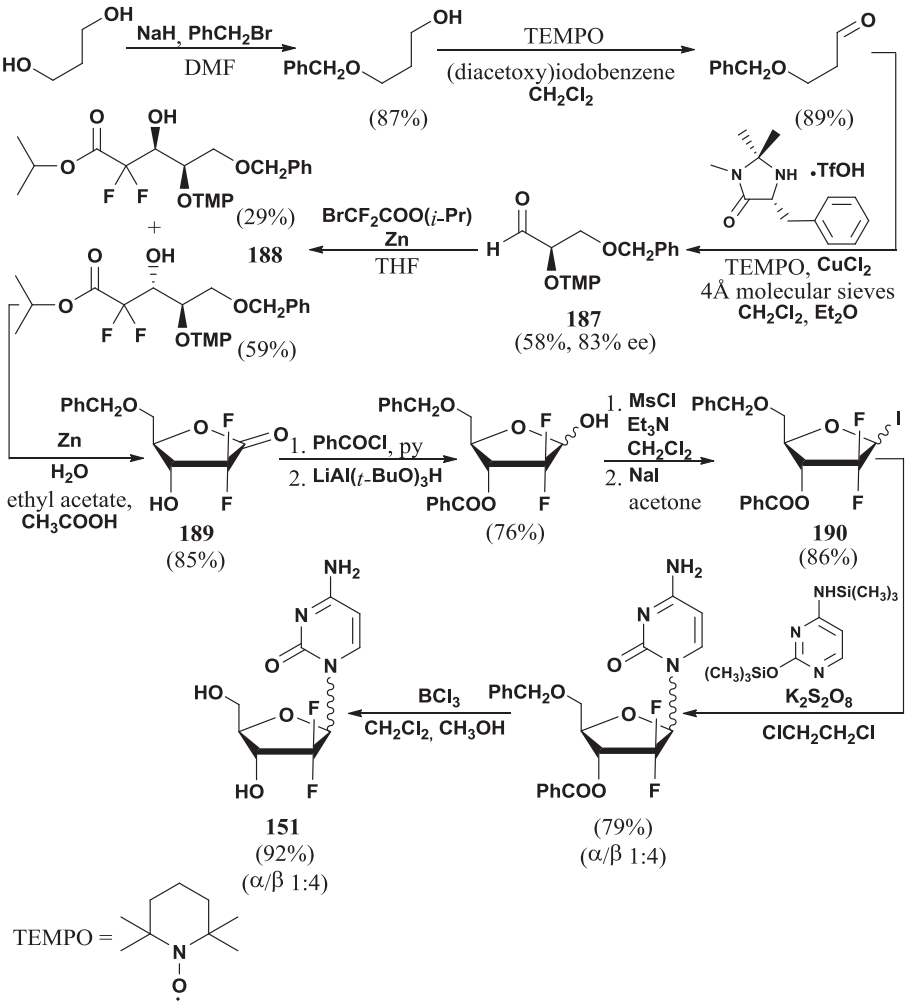
Beside the synthetic work, there are some publications dedicated exclusively to the purification of gemcitabine base or hydrochloride. For example 99.8% pure gemcitabine hydrochloride was obtained by fractional crystallization at acidic pH (≤ 0.5).[205]

2. *Gemcitabine Derivatives*

Gemcitabine chemotherapeutic efficacy is limited by its high toxicity to normal cells and short plasma half-life (9–13 min for human plasma) depending on the rapid deamination by cytidine deaminase in the liver, kidneys and plasma to less cytotoxic metabolites. Various prodrug strategies have been developed to overcome these adverse aspects and to allow for oral delivery.

Protection of the 4-amino group, for example as the amide, can facilitate a slower release of gemcitabine, increasing the bioavailability and uptake and providing resistance to enzymatic deamination. A series of 4-N-alkyl and 4-N-alkanoyl compounds were prepared in 2014[206] and their activity evaluated. N-alkanoyl gemcitabine derivatives **191** showed potent cytostatic activity in the nanomolar range, whereas N-alkyl derivatives **192** required micromolar range. The N-alkanoyl derivatives **191** were prepared in 40–66% yield by reacting gemcitabine with a carboxylic acid in dimethylsulfoxide, in presence of N-methylmorpholine, 1-hydroxybenzotriazole and (N-dimethylaminopropyl)-N^1-ethyl-carbodiimide (*Scheme 102*). The N^4-alkyl derivatives **192** were prepared from protected gemcitabine **193** through its N^4-tosylate by reaction with an amine.

N^4-(2-Propyl-1-oxopentyl) derivative **194** was prepared in 2006,[207] by Eli Lilly (*Scheme 103*), designed to be resistant to deamination by hydrolysis under acidic conditions similar to those found in the digestive system but able to release gemcitabine upon action by carboxylesterase.

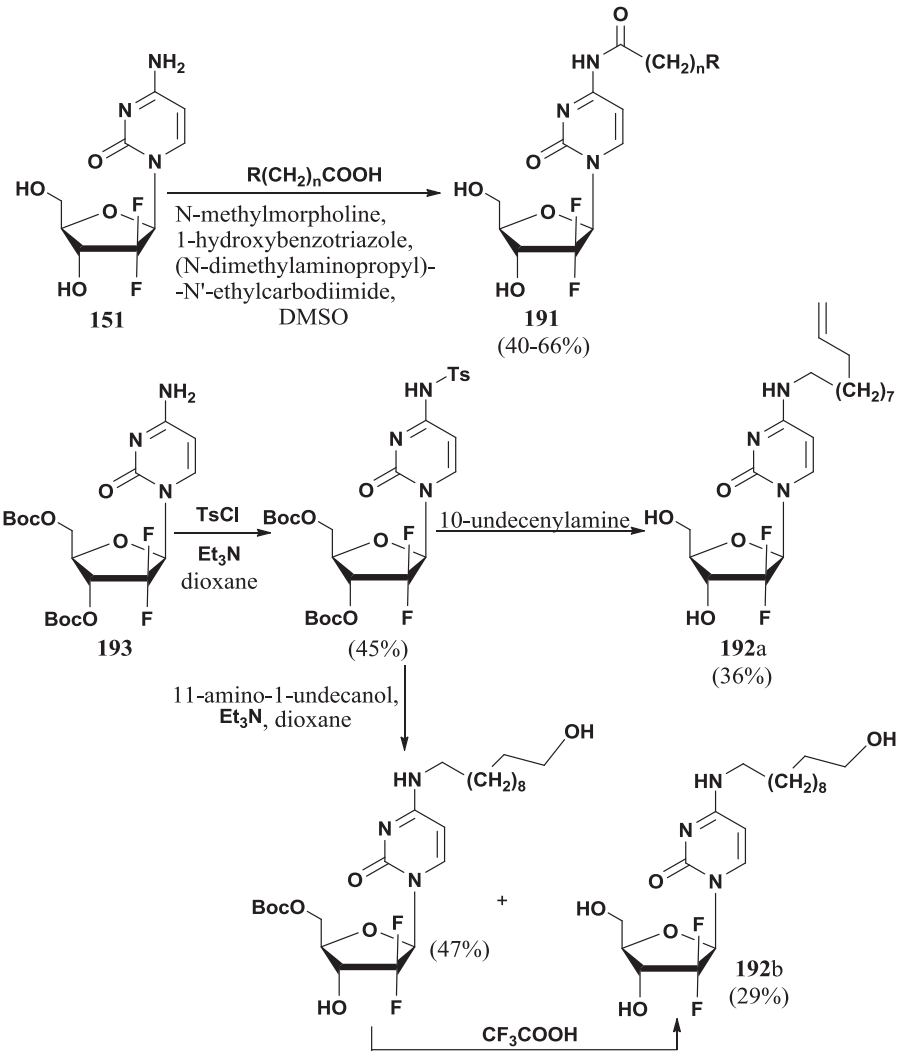


Scheme 101

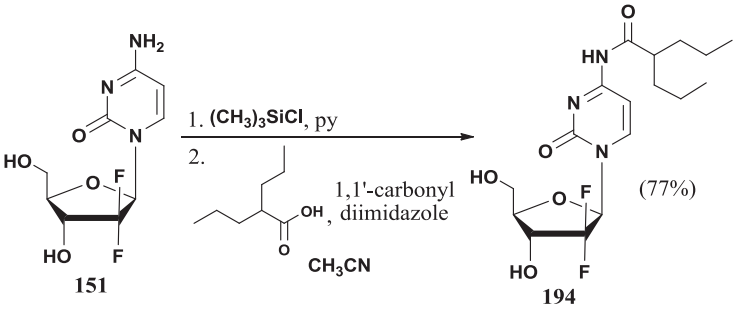
N^4-Benzoyl derivative **195** was prepared as depicted as shown in *Scheme 104* and included into oligonucleotides aimed to preferentially kill cancerous cells over non-cancerous cells.[208] Some oligonucleotides containing gemcitabine derivatives were shown to be more effective in killing cancerous cells at equivalent dosages of gemcitabine itself.

As in the case of capecitabine[156] the hypoxic conditions of tumor cells can selectively activate a prodrug, mediating the fragmentation of a masked cytotoxic compound into the active cytotoxic agent; the masked cytotoxic agent can be N^4-carbamate **196**[156] (*Scheme 105*) or a 5′-ester with lipoic acid derivative **197**[209] (*Scheme 106*) that plays the role of the redox-modulating agent.

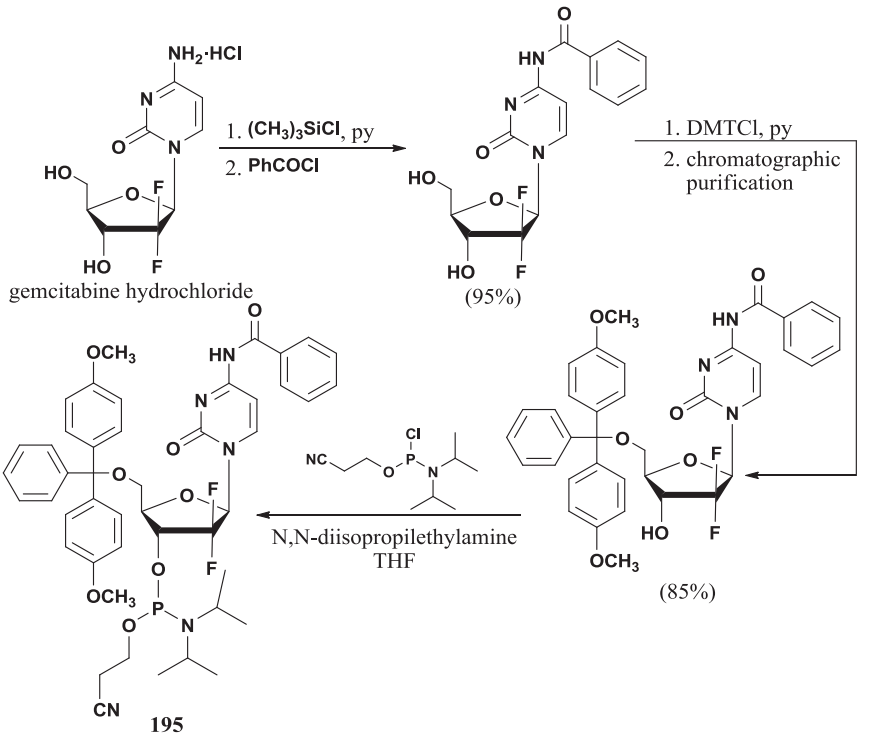
In a 2013 article the conjugation of gemcitabine with coumarin derivative **198** and biotin derivative **199** was described:[210] the molecule contains the cleavable disulfide group of biotin, a molecule taken up preferentially by cancer cells, and a fluorescent moiety to enable real-time monitoring of the drug delivery. The synthesis of conjugate **200** and the proposed mechanism of action under physiological conditions, involving GSH, the most abundant thiol in the cells, are depicted in *Scheme 107*.



Scheme 102



Scheme 103



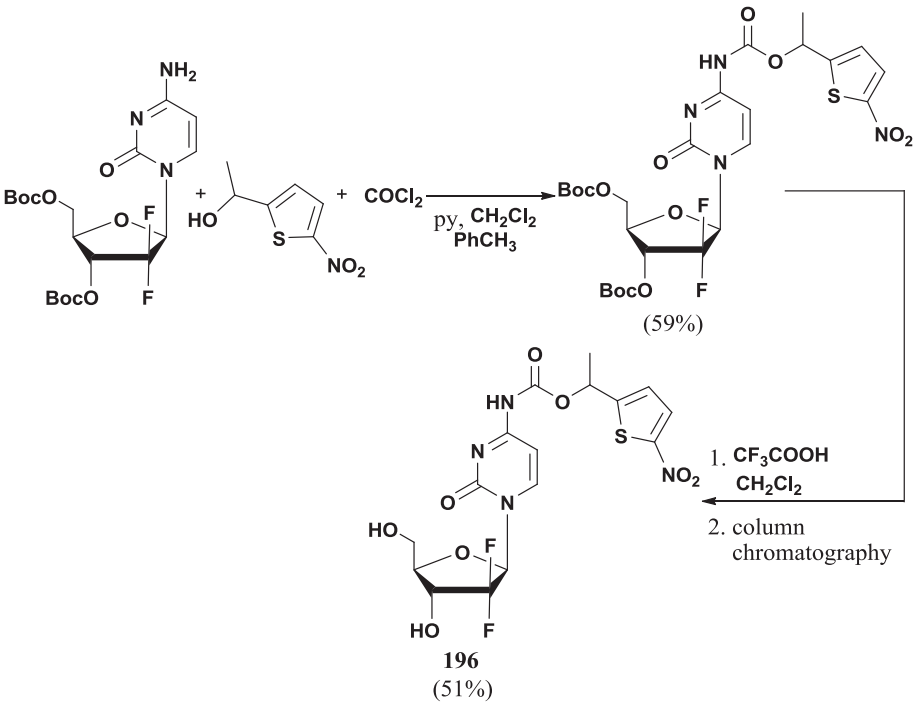
DMTCl = 4,4'-dimethoxytrityl chloride

Scheme 104

Besides conjugation with polymeric materials,[211,212] the preparation of N^4-derivatives with lipoaminoacid residues, suitable for penetration through biological membranes and barriers, was also reported.[213] Lipoaminoacids are aminoacids bearing an alkyl chain in the 2-position. They impart to the molecule to which they are conjugated amphiphilic properties. Lipoaminoacid derivatives **201**, prepared in 69% yield from gemcitabine hydrochloride (*Scheme 108*), *in vitro* results were encouraging of the study of the loading of these derivatives in liposomes and in other lipid based drug carriers.

Controlled drug delivery from chiral molecules is the aim of a study about the possibility of incorporating gemcitabine into the enantiomers of bis(diamido)-bridged basket resorcin[4]arene **202**,[214] in the gaseous phase (*Scheme 109*). The diastereomeric complexes among the macrocycle, gemcitabine and a chiral amine behave as supramolecular devices which, depending on the configuration of macrocycle and amine, can or cannot release the nucleoside. Complexation phenomena were investigated[215] by NMR methods and molecular modeling, allowing the identification of two different interaction sites of the guest in the resorcin[4]arene host. Different behaviors of resorcin[4]arene complexes with 2′-deoxycytidine, its 2′-epimer cytarabine and gemcitabine were observed. According to the authors, the assessment of the factors regulating the formations of these complexes offers the possibility to modulate the drug/receptor interactions, through the electronic properties of 2′-substituents on the nucleoside furanose ring.

The bioorthogonal approach, already described in the case of floxuridine,[105] was also applied by the same authors to the preparation of gemcitabine prodrugs.[216] Between the two masking possibilities, 5′-O-carbonate or N^4-carbamate, the latter was chosen due to its higher



Scheme 105

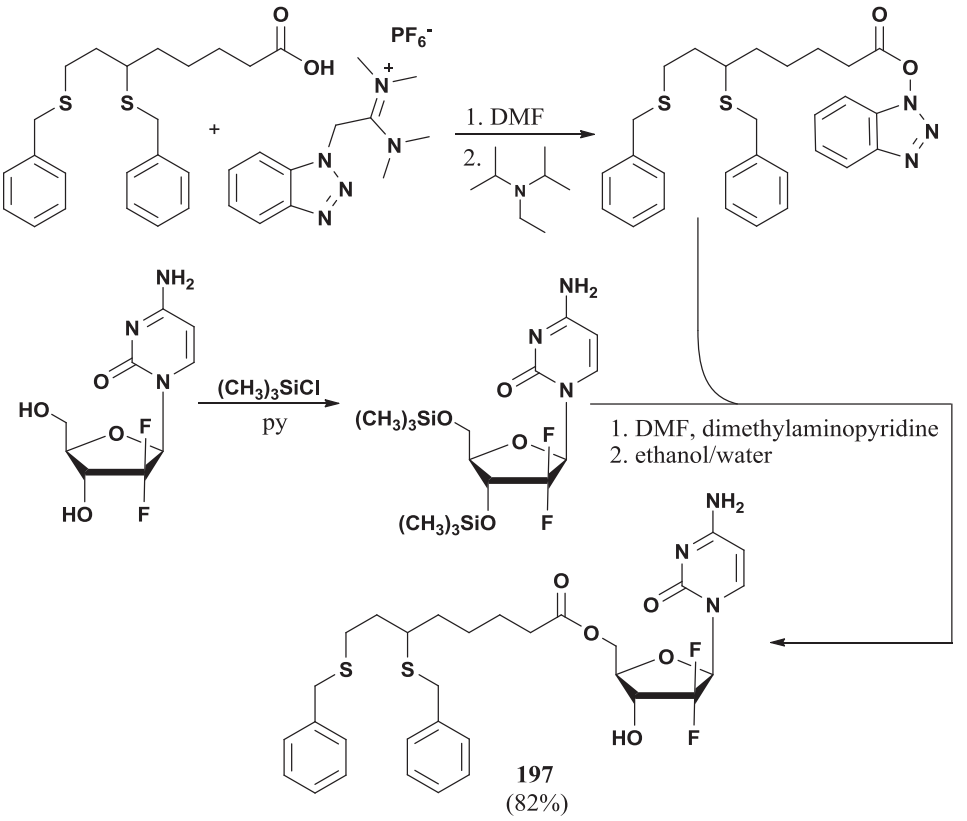
stability to enzymatic metabolism. Starting from gemcitabine **151**, propargyloxycarbonyl derivative **203** was prepared (*Scheme 110*), and treated with a Pd^0-functionalized resin under biocompatible conditions (buffer pH 7.4, 37°C), in order to remove the carbamate, and, then, placed in cell culture. In both cases the formation of free gemcitabine was monitored. DNA damage observed in pancreatic cancer cell cultures treated with N^4-functionalized gemcitabine and Pd^0-resin confirmed the bioorthogonal generation of free gemcitabine **151**.

3. Related Non-Clinical Pyrimidine Nucleosides Fluorinated at the Sugar Moiety

2′,2′-Difluorinated azacytidine **204** and 2′,2′-difluorodeoxyribose-trifluorothymidine **205** were recently[169] prepared starting from suitably protected 1-bromo-2′,2′-difluorosugar, compound **206**, in turn prepared as depicted in *Scheme 111* and silylated (see Scheme 88) in order to obtain new fluoronucleosides.

Compound **204** is noteworthy for the presence of a nitrogen atom at the 5-position, instead of a carbon, in addition to other modifications. Future studies will establish the clinical usefulness of these modifications.

In 2015[217] two 2′-fluorotricyclo-DNA nucleosides **207** and **208** were synthesized. The introduction of fluorine on methylglycoside **209** was achieved through the electrophilic addition of Selectfluor to the 1,2-double bond of **210** and an approximately equimolar mixture of 2-ribo **211** and 2-arabino **212** fluoro sugars was obtained as established by ^{1}H NMR NOE studies (*Scheme 112*).



Scheme 106

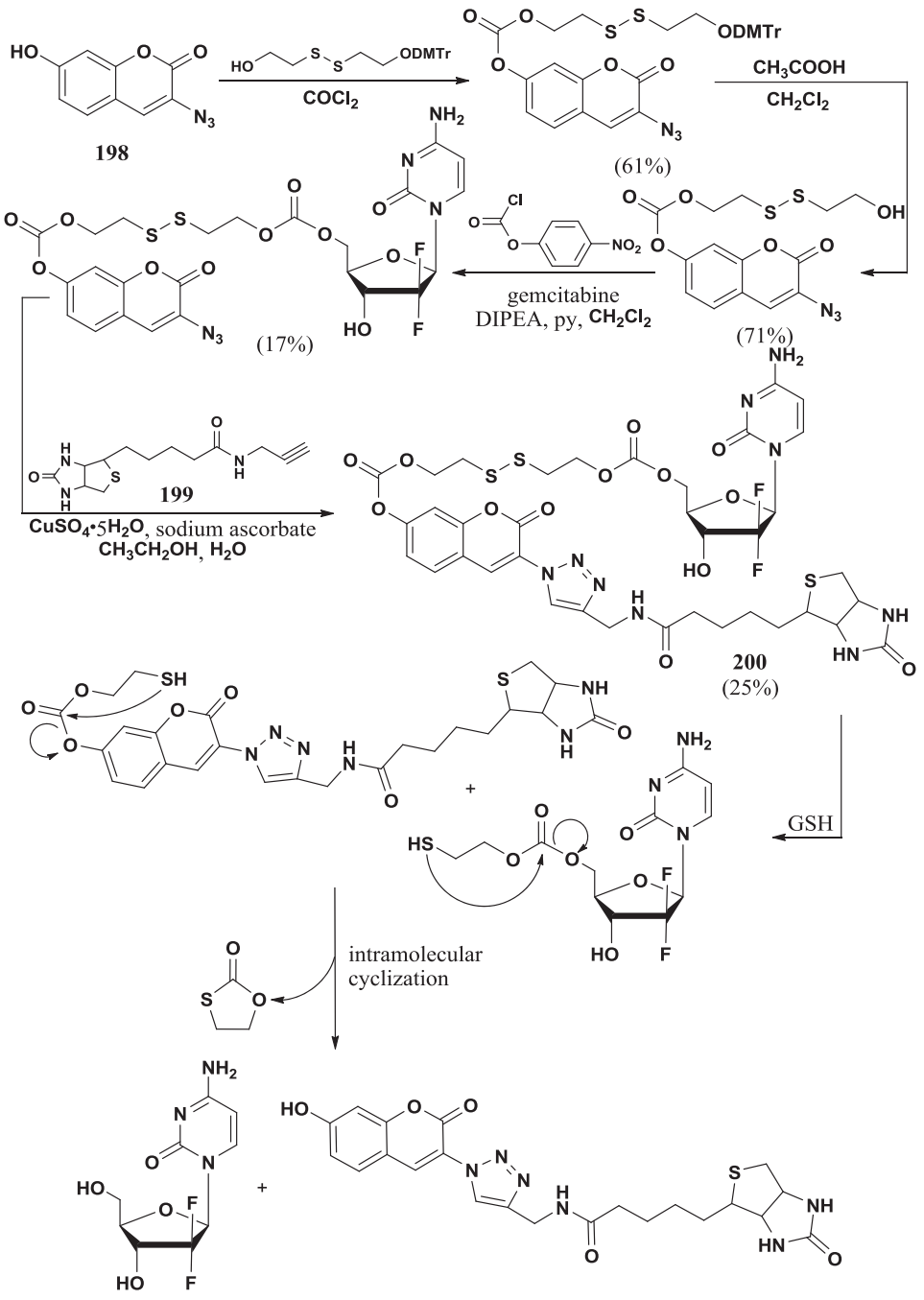
The highly selective N-glycosilation of 2-ribosugar **211**, obtained after column chromatography, afforded thymine 2′-fluoro nucleoside **207** showing a 1:12 α/β anomeric ratio (*Scheme 113*).

The same intermediate **213** was converted into nucleoside **208** bearing the 5-methylcitosine as nucleobase according to *Scheme 114*.

Compounds **207** and **208** represent two novel fluorinated nucleoside analogues which in the future will be incorporated into oligonucleotide backbone structures.

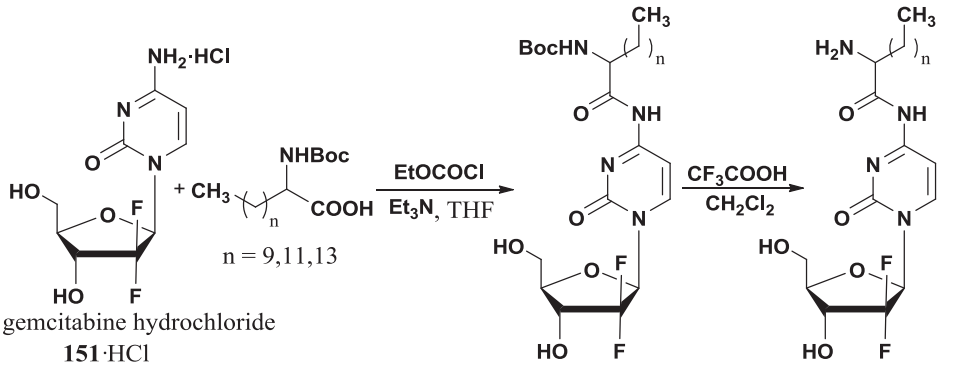
IV. Radiolabeled Fluoropyrimidine Nucleosides as PET Tracers

Radiolabeled antitumor agents may provide useful information about their metabolism and pharmacokinetics, *in vivo*, using functional imaging modalities such as Positron Emission Tomography (PET). Indeed, due to its good spatial resolution, PET allows workers to map the distribution of the radiotracers and their biological targets; moreover, depending on the selected molecular structure, PET is a powerful tool to accurately measure metabolic factors and/or biochemical and physiological parameters. In the last decades, PET has found its major applications in oncology, where the preparation of molecules labeled with positron emitting radionuclides, and their subsequent use with human subjects provides useful diagnostic information. It even allows clinicians to directly affect therapeutic strategies. Based on the detection of high energy gamma photons generated by the annihilation of the positron-electron pairs, PET is a highly sensitive technique, and radiolabeled tracers in nano to



Scheme 107

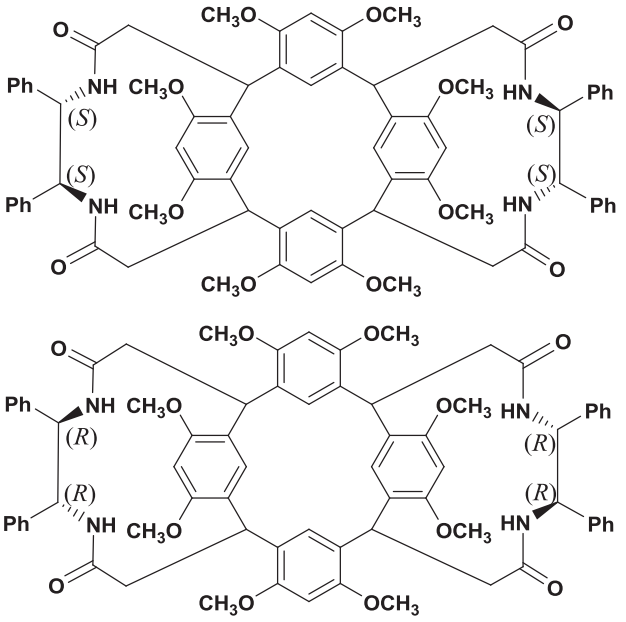
picomolar concentrations (non-pharmacological concentrations) may be used, thus minimizing their potential toxicity. The most useful PET radionuclides are the short half-life ^{11}C ($t_{1/2}$ = 20.4 min) and ^{18}F ($t_{1/2}$ = 109.6 min). The first nucleoside based PET tracer was [^{11}C]-



Scheme 108

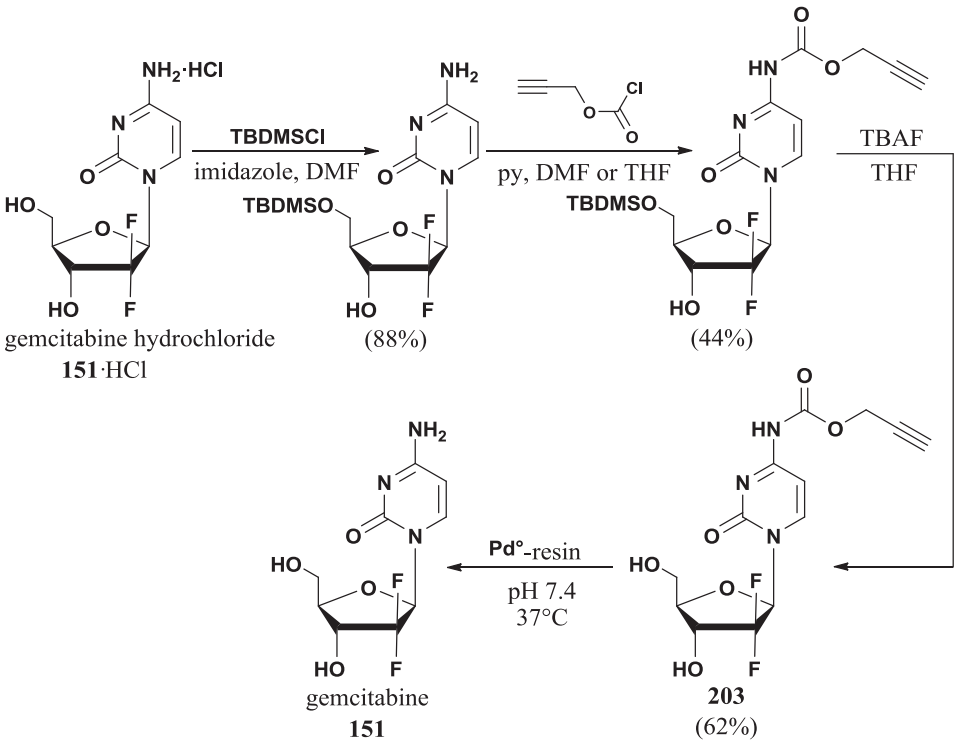
thymidine[218], and later [^{11}C]-5-FU was synthesized[219], with the aim to monitor the levels of 5-FU following therapeutic treatments. Starting from the *β*-(N-Benzoylamino)-*α*-fluoroacrylamide **214**, prepared from sodium ethyl-2-fluoro-3-hydroxyacrylate **215**, as depicted in *Scheme 115*, the introduction of ^{11}C was achieved using [^{11}C]-phosgene, prepared by the same authors in 2002.[220] Acrylamide with the suitable double bond E-stereochemistry **216** was obtained by the irradiation of the Z-isomer **217**. The radiochemical yield was about 25%.

^{11}C was initially selected as the preferred radionuclide because it replace a ^{12}C in the molecular structure, thus not altering the biological behavior of the radiolabeled probe, compared with its "cold" counterpart. On the contrary, its very short half-life may be a



resorcin[4]arene macrocycle enantiomers
202

Scheme 109



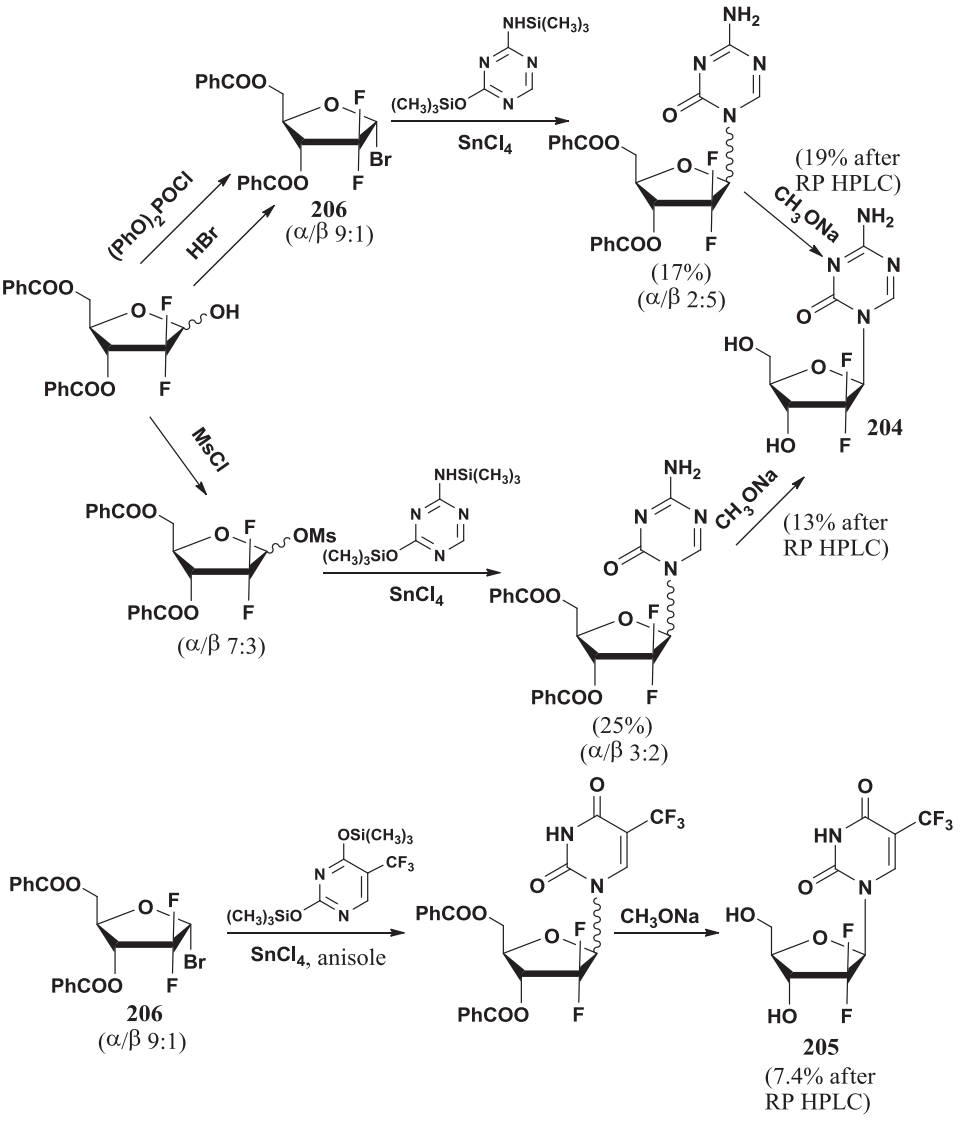
Scheme 110

serious drawback, especially in case of slow kinetics, *in vivo*. For these reasons, attention was then focused on the preparation of F-18 labeled radiotracers. F-18 has a considerably longer half-life, which is compatible with the biological half-life of slow kinetics molecules, and at the same time allows for longer radiosynthetic pathways. Furthermore, F-18 is produced efficiently and with high yield, and higher starting activity may also help to improve the radiopharmaceutical's availability, Finally, the 110 min half-life of F-18 allows for the radiotracer distribution to other PET centers not equipped with a cyclotron, thus improving availability and reducing general preparation costs. For instance, the [^{11}C]-based tracers were later superseded by [^{18}F]-FLT (3′-deoxy-3′-fluorothymidine),[221] which is still one of the most frequently used PET radiotracers in the field of oncology.

[^{18}F]-capecitabine**218**[222, 223] was synthesized, as an enzyme based imaging agent, to enable non- invasive monitoring of tumor enzymes (thymidine phosphorylase and uridine phosphorylase) (see *Scheme 1*) and tumor response to capecitabine therapy, using PET technique.

A 2004[222] paper reported the synthesis of [^{18}F]-capecitabine **218** through the nucleophilic substitution of the 5-nitro group of 2′,3′-di-O-acetyl-5′-deoxy-5-nitro-N^4-(pentyloxycarbonyl)cytidine **219** by reaction with K^{18}F (prepared in a RDS-112 cyclotron) in the presence of a bicyclic kryptand, Kryptofix 2.2.2 (*Scheme 116*). The nitro-precursor was prepared from 5-deoxy-1,2,3-tri-O-acetyl-D-ribose **105** (obtained from D-ribose) and 5-nitrocytosine **220** in 18% overall yield.

A different approach was described by a Korean team[223] using as substrate N^4-(pentyloxycarbonyl)-cytidine **221**, which introduced ^{18}F by direct electrophilic fluorination with [^{18}F]-F_2 generated in a MC-50 cyclotron. The radiochemical yield and radiochemical purity were 5–15% and >95%, respectively (*Scheme 117*).

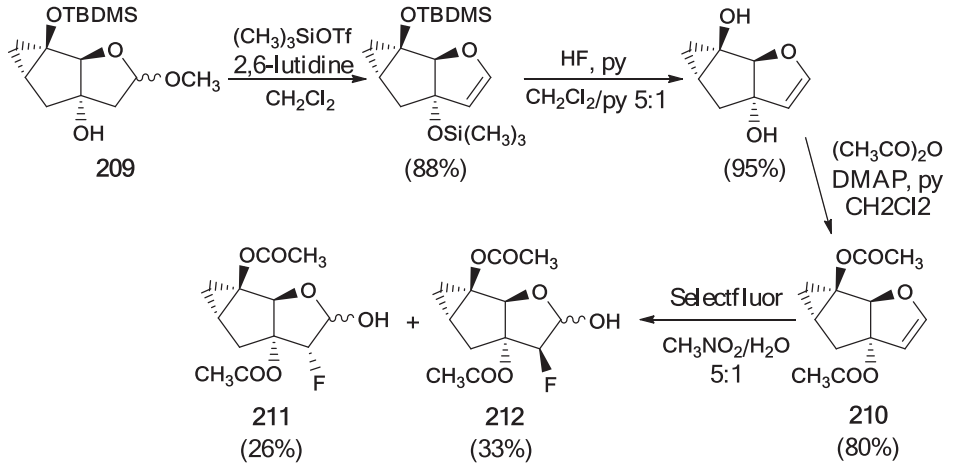


Scheme 111

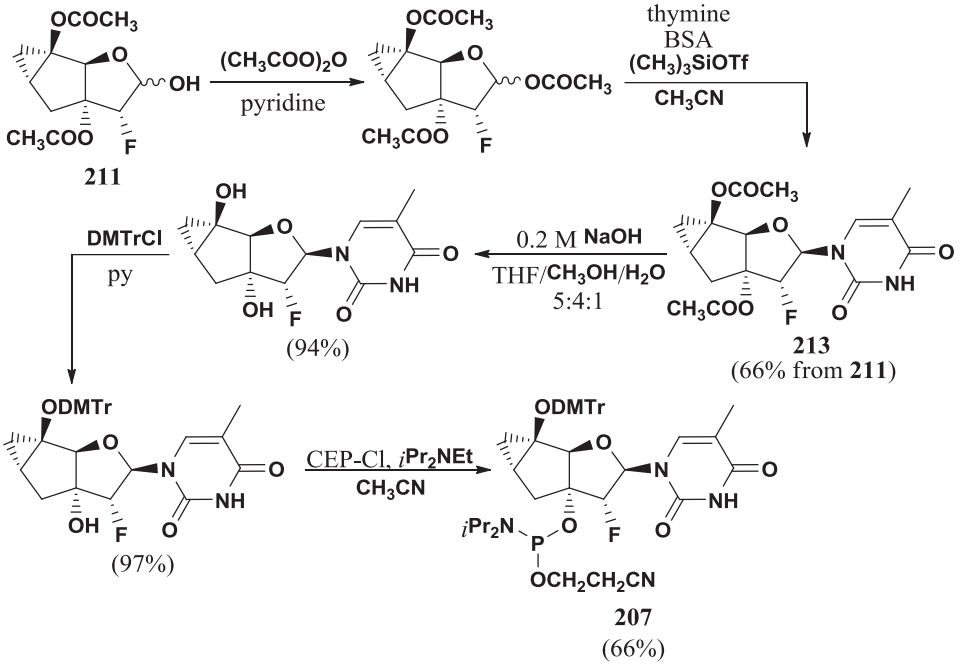
An attempt to prepare a [^{18}F]-labeled gemcitabine derivative,[206] failed at the early steps of the synthesis since the addition of unlabeled HF (70% in pyridine) to N^4-(10-undecenoyl)-2′,2′-difluorocytidine led to a mixture of 10-, 9- and 8-fluorinated isomers.

A very interesting enzyme-catalyzed formation of C-F, suitable also to produce labeled fluoronucleosides was proposed by O'Hagan and coworker in 2003.[224–228] They used the enzyme fluorinase, crude or partially purified, extracted from *Streptomyces cattleya*. The enzyme is capable to catalyze the synthesis of fluoro compounds, particularly fluoronucleosides, in the presence of a fluorine donor (lithium, potassium or sodium fluoride) or hydrogen fluoride and of the substrate. (*Scheme 118*).

The substrate was S-adenosyl-L-methionine (SAM) **222** that, by action of fluorinase, underwent a nucleophilic substitution (L-methionine **223** as leaving group) leading to 5′-

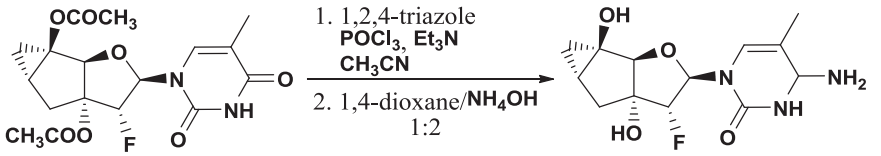


Scheme 112



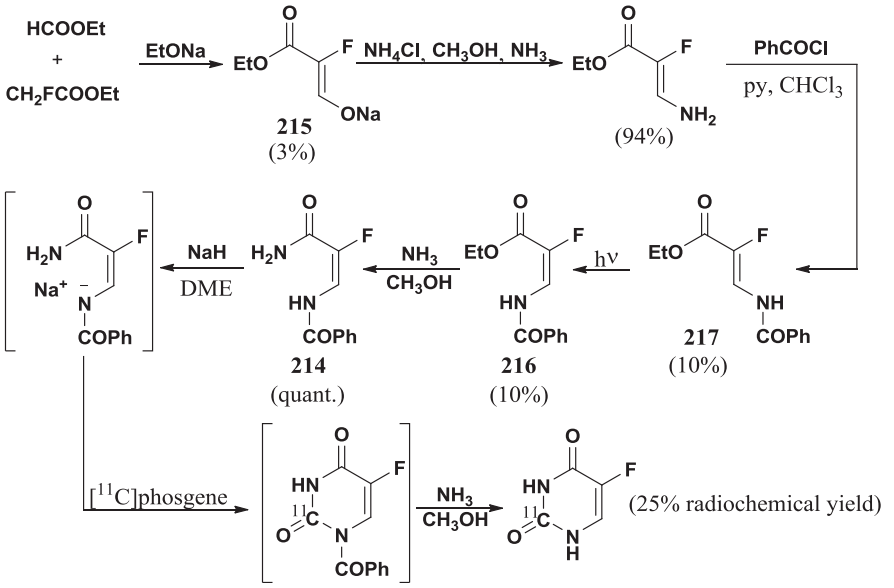
CEP-Cl = chloro(2-cyanoethoxy)(diisopropylamino)phosphine
DMTr-Cl = 4,4'-dimethoxytrityl chloride

Scheme 113



213 **208**

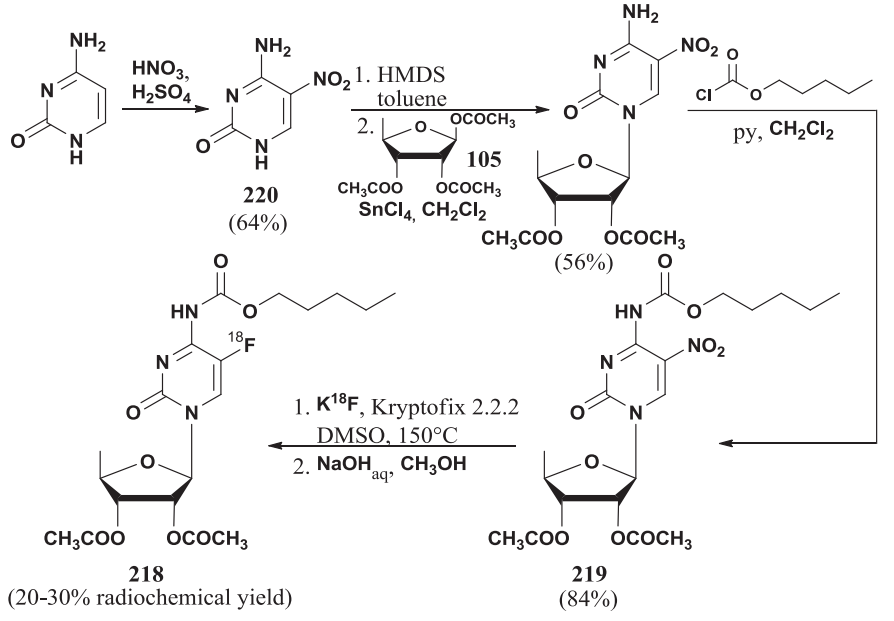
Scheme 114



215 (3%) (94%)

214 (quant.) **216** (10%) **217** (10%)

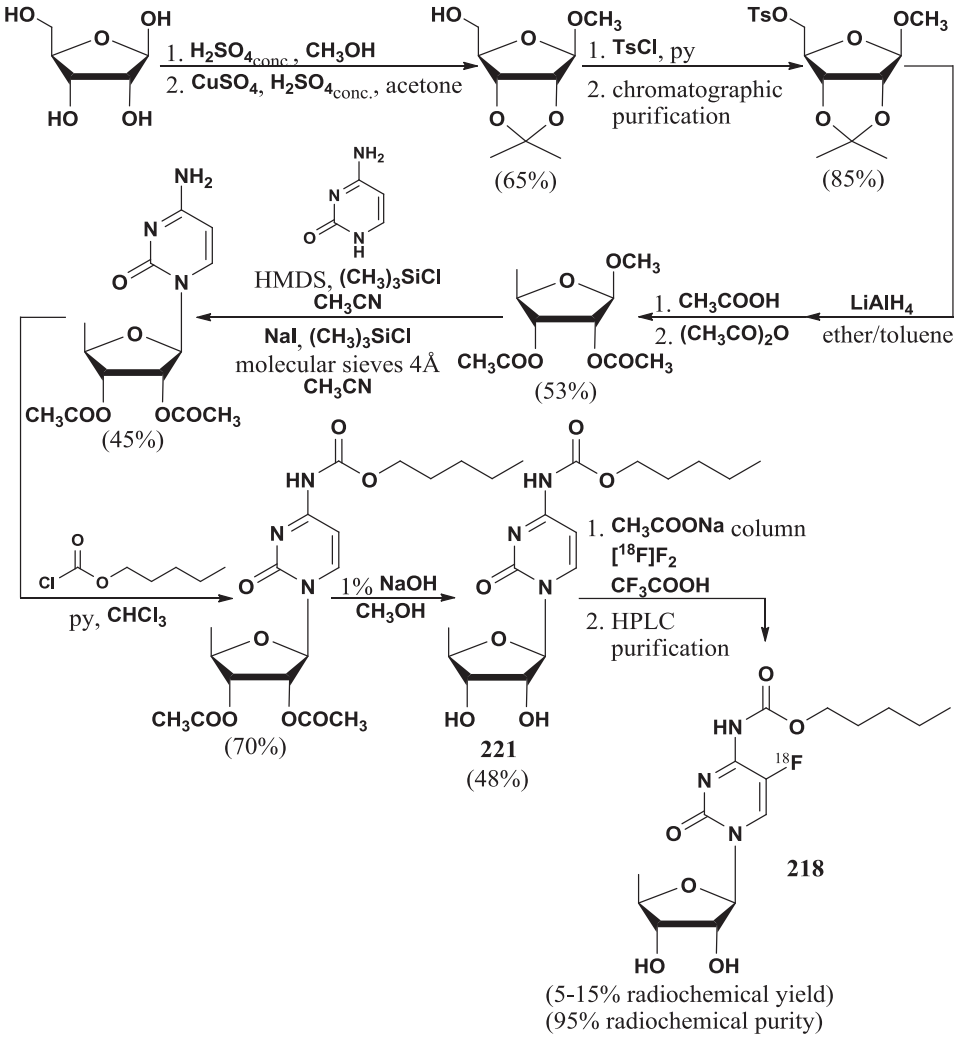
Scheme 115



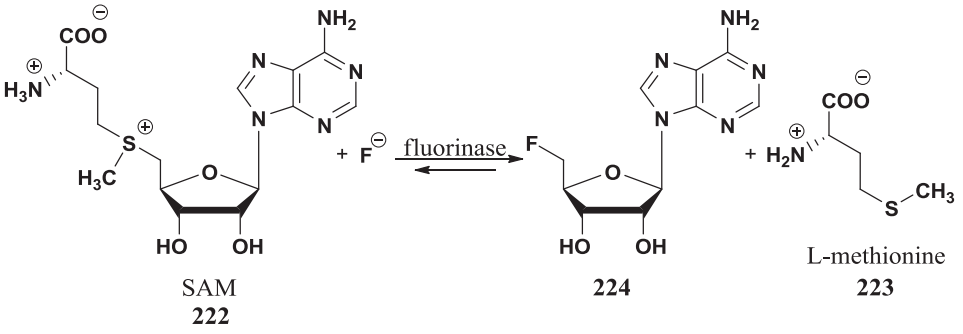
220 (64%) (56%)

218 (20-30% radiochemical yield) **219** (84%)

Scheme 116



Scheme 117



Scheme 118

fluoro-5′-deoxyadenosine **224**; the method was also applied to the synthesis of [^{18}F]-5′-fluoro-5′-deoxyadenosine.[227] Hydrolysis of the N-glycosidic bond by means of nucleoside hydrolase from *Trypanosoma vivax* afforded 5-deoxy-5-[^{18}F]-ribose.[221] Starting from [^{18}F]-5′-fluoro-5′-deoxyadenosine by means of an enzyme-catalyzed transglycosylation [^{18}F]- 5′-fluoro-5′-deoxy-5-fluorouracil was also obtained.[227] This and other examples recently reviewed[229] are promising of a wider application of the method, at the present limited to the fluorination of the 5′-position. More recently an enzyme with the same activity was isolated from the marine derived bacterium *Streptomyces xinghaiensis*.[230]

Conclusion

Fluorinated nucleosides represent an important category of nucleoside analogues endowed with antitumor and/or antiviral activity. This review focused on antitumor pyrimidine nucleosides bearing fluorine on the nucleobase or on the glycone.

5-FU **1** is more than 50 years old but it is still used, in combination with other anticancer drugs (for example, avastatatin and oxaliplatin), for the treatment of metastatic colorectal cancer. A more recent therapeutic indication of 5-FU **1** is the topical use (cream or solution) for the treatment of skin cancers and Bowen's disease. Capecitabine (Xeloda) **63** is the most frequently used therapeutic fluoropyrimidine nucleoside, alone or in combination with other drugs and, like other relevant fluoropyrimidines nucleosides analogues, can be regarded as a 5-FU prodrug. Gemcitabine (Gemzar) **151** belongs to the group of nucleosides fluorinated on the glycone moiety and it is among the ten leading brands in the global cancer market.

Considering the impressive number of fluorinated pyrimidine nucleosides, a choice of scope was mandatory, and we decided to select clinically well-established compounds, and their derivatives designed to improve the pharmacological properties of the parent compounds. This choice excluded, for example, nucleoside analogues characterized by the presence of a heteroatom (N, S, Se), different from oxygen in the 5-atoms ring[173] or the carbocyclic analogues,[172,231] which are no less interesting than the discussed compounds. Despite the numerous compounds that have received marketing authorization, the demand for antitumor oral fluoro pyrimidine nucleosides is still of concern; the design and the synthesis of more selective molecules remains a target of pharmaceutical research. Such research may develop the possibility of patient-tailored therapy based on individualized combinations of the described molecules. We hope that the synthetic methods described in this review, suitably adapted, could be a valuable aid in the development of new nucleoside analogues.

Acknowledgments

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