

Expert Report of Jason Micklefield

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Filed on behalf of the Claimants

Exhibits JM-1 to JM-6

Claim No. HP-2021-000007

IN THE HIGH COURT OF JUSTICE

BUSINESS AND PROPERTY COURTS OF ENGLAND AND WALES

INTELLECTUAL PROPERTY LIST (ChD)

PATENTS COURT

B E T W E E N:

(1) GILEAD SCIENCES INC.

(a company registered in the US State of Delaware)

(2) GILEAD SCIENCES LTD

Claimants

- and -

NUCANA PLC

Defendant

EXPERT REPORT OF PROFESSOR JASON MICKLEFIELD

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I, **JASON MICKLEFIELD**, of 8 Lea Road, Heaton Moor, Stockport, SK4 4JU, WILL SAY as follows:

SECTION 1: PERSONAL BACKGROUND

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1. I am a Professor of Chemical Biology at The University of Manchester Department of Chemistry and The Manchester Institute of Biotechnology with over 30 years of research experience in bioorganic chemistry, including the design and synthesis of nucleoside and nucleotide analogues. I have provided a copy of my CV as **Exhibit JM-1**.
2. I received a Doctor of Philosophy degree from the University of Cambridge in 1993, where I trained in synthetic organic chemistry. I received a Bachelor of Science degree in chemistry from the University of Hull in 1989.
3. After receiving my Ph.D., I was awarded a NATO Postdoctoral Fellowship, by the Science and Engineering Research Council (SERC), to conduct research at the University of Washington in Seattle, USA, with Professor Heinz G. Floss in the area of bioorganic chemistry and biosynthesis. From 1995 to 1998, I was a Lecturer in Organic Chemistry at Birkbeck College, University of London. In September 1998, I was

recruited to The University of Manchester where I served as Lecturer in Chemistry. I was promoted to Senior Lecturer (2002) and Reader in Biological Chemistry (2003) before being promoted to a Chair in Chemical Biology in 2008. From 2008 until 2011, I was the Director of Research for the School of Chemistry. In April 2018 I was appointed as Visiting Professor, East China University of Science and Technology, Shanghai.

4. I have received several awards in recognition of my research, in addition to the NATO Fellowship (1993). I was elected a fellow of the Royal Society of Chemistry (RSC) in 2006. In 2008, I received the RSC's Natural Product Reports (NPR) Lecture Award. In 2019 I was recipient of the RSC Bader Award for eminence in organic chemistry. I was also awarded the 2022 RSC Interdisciplinary Prize for innovative research spanning organic chemistry to molecular genetics.
5. I have served as an active participant in a number of professional societies including the RSC Nucleic Acid Group, the RSC Bioorganic Group, the UK Centre of Excellence in Biocatalysis (CoEBio3), the International Advisory Board of the State Key Laboratory of Bioreactor Engineering in Shanghai, China, the Global Council of the International Chemical Biology Society (ICBS) and the Engineering and Physical Science Research Council's Chemistry College. I have also served on numerous national and international review panels for the Biotechnology and Biological Sciences Research Council (BBSRC), the Engineering and Physical Science Research Council (EPSRC), Medical Research Council (MRC), the Deutsche Forschungsgemeinschaft (DFG) – German Research Foundation (International Review Panel for Chemistry), and the European Cooperation in Science and Technology (COST) Action (Chemistry Panel).
6. I also served as the Director of the EPSRC-BBSRC-MRC funded Manchester Chemical Biology Network leading 40 academic groups and 10 industrial partners (mainly pharmaceutical companies) in establishing new collaborative medicinal chemistry projects. In addition, I led the UK's natural product research community as Director of the BBSRC funded network NPRONET which comprises a large number of academic institutions and industrial partners (including leading pharmaceutical companies) across the UK and overseas. This network established many new collaborative projects on the discovery and development of new natural products for pharmaceutical applications.

7. I am Associate Editor-in-Chief of *Green Synthesis & Catalysis*, Elsevier. I also serve on the editorial board of *Chemical Biology and Drug Design*, *Frontiers in Chemistry*, and *Synthetic and Systems Biotechnology*. Previously I served on the editorial boards of *Chemical Communications* (2003-2006) and *Current Organic Synthesis* (2004-2009). I also served as Guest Editor for the RSC themed issue on “Nucleic Acids: new life, new materials” and as Guest Editor for *Current Opinion in Chemical Biology*.
8. I have authored or co-authored more than 110 scientific papers and two book chapters. Of these publications, more than 20 pertain to nucleosides or nucleotides, with approximately 16 relating to nucleoside or nucleotide analogues and one pertaining to the synthesis of 2'-fluorinated nucleoside and nucleotide analogues. Five of my papers also describe using nucleotides and an RNA polymerase enzyme to produce RNA. I am also a named inventor on five patent applications.
9. Much of my early research career focused on the synthesis of modified nucleosides and nucleotide analogues which were incorporated into oligonucleotides as potential antisense agents. More recently, I have focused on the synthesis of modified nucleosides as co-enzyme analogues for studying enzyme structure and catalysis. I also have extensive experience in using polymerase enzymes to assemble nucleotides into novel RNA structures. Most recently, my laboratory has been working on the synthesis of novel nucleosides and nucleotide analogues, with 2'-modifications, that are required as building blocks for assembly of nucleic acid therapeutics (e.g. siRNA) and modified mRNA vaccines using engineered polymerase enzymes. My lab is also developing new synthetic routes to nucleotides including modified bases for incorporation into improved mRNA vaccines using polymerase enzymes.
10. During the last 25 years of my academic career, I have supervised and trained a research group of over 100 co-workers from 23 different nationalities which includes 33 postdoctoral research associates, 68 postgraduates (PhD students) and 3 lab technicians. Many of these skilled researchers have gone on to secure positions in the pharmaceutical, biotech and other chemical industries.

SECTION 2: MY ROLE AS AN EXPERT WITNESS AND MY INSTRUCTIONS

11. I have been asked by Bird & Bird LLP ("**Bird & Bird**"), on behalf of Gilead Sciences Inc and Gilead Sciences Ltd (collectively "**Gilead**" or "**the Claimants**"), to act as an expert witness in these proceedings.

12. Bird & Bird have drawn my attention to Part 35 of the Civil Procedure Rules 1998, the Practice Direction which supplements Part 35, and the Guidance for the instruction of experts to give evidence in civil claims 2014. I confirm that I have read these documents and understand my duty is to assist the Court. I understand that this duty overrides any obligation to Gilead or Bird & Bird. I confirm that I have complied (and will continue to comply) with my duty, which encompasses the following:
- (a) the evidence and opinion that I present to the Court must be independent and unbiased;
 - (b) I must state the facts or assumptions on which my opinion is based, and I should not omit to consider material facts which could detract from my concluded opinion;
 - (c) I must make it clear when a particular question or issue falls outside my expertise;
 - (d) I must state if my opinion is provisional or requires qualification; and
 - (e) if, after exchange of reports, I change my view on a material matter, I must communicate such change of view to Bird & Bird without delay so that it can be communicated to the other parties in these proceedings and when appropriate to the Court.
13. Bird & Bird have explained to me that these proceedings concern a patent dispute between Gilead and NuCana plc ("**NuCana**" or "**the Defendant**"). Bird & Bird have asked me not to do any research into the parties, their products, processes, or the patents that they may own, and I have not done so.
14. I have been asked whether I have any current or prior relationships with Gilead or NuCana.
15. I previously acted as an expert witness, in 2015, on behalf of Gilead Sciences Inc and Gilead Pharmasset LLC in a dispute with Idenix Pharmaceuticals Inc and the Università Degli Studi Cagliari in the United States District Court for the District of Delaware. I made one declaration in those proceedings, which I attach and mark **Exhibit JM-2**. I was subsequently deposed regarding that declaration, but had no further involvement in the proceedings. I attach a copy of the transcript from that deposition as **Exhibit JM-3**.

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16. I did not know about the patent dispute between Gilead and NuCana until my engagement as an expert in these proceedings.
17. I understand from Bird & Bird that these proceedings are related to two patents owned by NuCana: EP(UK) 2,955,190 and EP(UK) 3,904,365. I shall refer to these as “**EP 190**” and “**EP 365**” respectively (collectively “**the Patents**”).
18. Bird & Bird asked me to undertake, and I subsequently undertook, the following tasks in the following order. Bird & Bird provided me with instructions in a series of discrete stages, as set out below. I completed the tasks and gave my views regarding each stage prior to being provided with instructions or material relating to the next stage:
- (a) I was asked to consider a hypothetical drug discovery project investigating nucleoside derivatives as at 21 July 2003 and provide my views regarding the following:
 - (i) who, within a team engaged on such a project, would have been responsible for designing and executing the synthesis of a novel nucleoside derivative;
 - (ii) what would the common general knowledge of such a person have been (I explain my understanding of common general knowledge below);
 - (b) I was asked to read EP 190, consider to whom it was addressed and their common general knowledge, and provide my comments on the understanding of such a person (the “**Skilled Chemist**”, as explained below) of the document;
 - (c) I was asked to consider five nucleoside structures which fall within Formula III of EP 190 and provide my high-level views regarding how the Skilled Chemist would approach their synthesis and how difficult such syntheses would be;
 - (d) I was asked to explain in detail the synthetic route or routes the Skilled Chemist would have proposed to make two 2'-Me(up), 2'-F(down) nucleosides, falling within Formula III of EP 190 and how difficult such a synthesis would be;

- (e) I was provided with a bundle of material relating to research conducted at Idenix Pharmaceuticals Inc (“**Idenix**”) between 2002 and 2005 with the goal of synthesising 2'-Me (up), 2'-F (down) nucleosides and asked to comment on certain aspects of the work described;
 - (f) I was provided with a bundle of material relating to research conducted at Pharmasset Inc (“**Pharmasset**”) between 2002 and 2009 with the goal of synthesising 2'-Me (up), 2'-F (down) nucleosides and asked to comment on certain aspects of the work described;
 - (g) I was provided with a copy of United States Patent Application No 2003/0109697 A1 (“**Shepard**”) and asked to provide my comments on the Skilled Chemist's understanding of the document. In providing these comments, Bird & Bird instructed me to consider Shepard from the perspective of the Skilled Chemist who has not read EP 190;
 - (h) I was provided with a copy of EP 365 and Annexes A and B to Statement of Reasons for Amendment of EP 190 and asked to comment on the extent to which these affected the views I had previously expressed.
19. For the avoidance of doubt, I had not seen the Patents when I provided my views under paragraph 18(a) above. As I explain below, my views in relation to these questions have not changed following being shown the Patents.
20. I also note that I was not familiar with the synthetic chemistry undertaken at Idenix Pharmaceuticals Inc or Pharmasset Inc in relation to the synthesis of 2'-Me(up), 2'-F-(down) nucleosides before Bird & Bird provided me with materials I refer to in paragraphs 18(e), or 18(f).

SECTION 4: THE SKILLED TEAM

21. Bird & Bird have explained to me the concept of the skilled person (or team) in patent law. In particular, it has been explained to me by Bird & Bird that:
- (a) the Patents will be taken to be addressed to a notional individual (or team) likely to have a practical interest in the subject matter of the Patents (“**the skilled person**”).
 - (b) the skilled person is deemed to be of normal skill and experience, is well acquainted with relevant laboratory techniques, has carefully read any

relevant literature and possesses the common general knowledge (“**CGK**”) but is unimaginative and lacks inventive capacity.

22. With this in mind, as explained above, Bird & Bird initially asked me to consider a hypothetical drug discovery project investigating nucleoside derivatives as at 21 July 2003 and identify who, in my view, would be the individual in the team responsible for developing the synthesis of a novel nucleoside derivative. Later, I was provided with EP 190 and asked to identify the characteristics of the member of the team who would be responsible for developing the synthesis of the molecules concerned. Such a person would be the person I identified when originally asked to consider the question in the context of a hypothetical drug discovery project. I provide my views on the characteristics of this individual and their interaction with other members of the skilled team below. I describe this individual as the “**Skilled Chemist**”. Having subsequently considered EP 365 I consider that the Skilled Chemist is the same for both of the Patents.
23. The Skilled Chemist would most likely be in a pharmaceutical company but could also be in academia. Their level of education and experience could vary depending on whether the Skilled Chemist was in a pharmaceutical company or academia, but overall, the individual would have at least a BSc or MSc in Chemistry.
24. The Skilled Chemist would not have a background in biology or molecular biology, but would have been taught medicinal chemistry at undergraduate level and would have picked up knowledge of disease targets, mechanisms of action, some of the issues around screening, biological testing and interpreting biological testing data. They would also have acquired some knowledge of structure activity relationship (“**SAR**”) testing and analysis.
25. The Skilled Chemist would need to have at least three years’ experience beyond undergraduate level. In academia this experience would most likely take the form of a PhD, but in industry this would not necessarily be the case. Pharmaceutical companies tended to also recruit at graduate level; however, the expected skill level would be similar after approximately three years’ experience. In the context of a drug discovery project involving nucleosides, at least some of the Skilled Chemist’s experience would normally be specifically in nucleoside chemistry.
26. In a research lab, the Skilled Chemist is tasked with designing and developing syntheses which are technically demanding or might not have been done before and are not guaranteed to work. This requires the capabilities to go to the literature to see

how people have made similar molecules, devise a synthetic route and apply that synthetic route in the laboratory. This would include experience with techniques to:

- (a) handle reagents and set up reactions in the laboratory;
 - (b) monitor the reaction as it is proceeding;
 - (c) purify the molecule from the chemical reaction;
 - (d) determine the structure and purity of the compounds produced by reactions.
27. In a drug discovery project where the Skilled Chemist is making nucleoside-based drug candidates, they are likely to be part of a team. That team would also include a biologist who would test the nucleoside compound for any activity against a particular target. The attributes of the biologist would depend on the nature of the drug discovery project.
28. Although the Skilled Chemist would work in collaboration with the biologist, the Skilled Chemist should have some understanding of what the biologist needed and also be able to interpret biological data in terms of biological assays and whether a compound had useful activity.
29. It is important to note that, in the real world, different chemists could have significantly different approaches to conducting work and solving chemical problems, despite having the same basic education and experience and both generally fitting the description of a Skilled Chemist. The university the chemist attended, the research group or supervisor they conducted their PhD or MSc research with, and the context of their training (e.g. academia or industry) could all affect the way in which they approach problems and the solutions they favour. For example, a chemist who trained in a research group which specialised in carbohydrate chemistry may be more or less familiar with certain reagents or reactions compared to a chemist of comparable experience level who trained in a research group specialising in natural product synthesis. Similarly, a chemist who trained in a particular research group may favour using certain reagents or techniques, developed within that group. This is a feature of chemistry as a discipline which means that two highly qualified and capable chemists (both of whom fit the description of the Skilled Chemist I give above) with slightly different backgrounds may reasonably use different approaches to problems and may even come to slightly different conclusions.

SECTION 5: THE COMMON GENERAL KNOWLEDGE

30. In this section, I set out my view of the technical background that would form part of the common general knowledge of the Skilled Chemist as at 21 July 2003. I have been told that the term common general knowledge means information which was generally known by the bulk of those individuals working in the relevant field and which was generally accepted by them as a sound basis for further action. I understand that the term common general knowledge is a legal expression, which I have been told is typically represented by information found in textbooks and review articles which were widely available and read or generally consulted at the time.
31. It has been explained to me that scientific publications will only form part of the common general knowledge of the Skilled Chemist when such publications are widely known and generally accepted without contention by the majority of those individuals working in the field of the invention of the Patents. It has been further explained to me that the common general knowledge includes material which the Skilled Chemist knows exists and which they could refer to as a matter of course and is therefore not limited to material which they have memorized.
32. In general terms, the CGK of the Skilled Chemist would include:
- (a) Fundamental organic chemistry;
 - (b) Protecting group chemistry – methods for protecting and deprotecting commonly used functional groups to limit unwanted side reactions;
 - (c) Standard laboratory purification techniques;
 - (d) Standard analytical techniques for identifying and characterising compounds;
 - (e) Basic nucleoside and nucleotide chemistry, including some understanding of methods to synthesise nucleosides, nucleotides and oligonucleotides as well as the applications of these compounds as drugs;
 - (f) Basic concepts of medicinal chemistry including SAR and the use of prodrug strategies.
33. I set out below some standard textbooks which would have formed part of the CGK of the Skilled Chemist in July 2003. Where relevant, I have relied on extracts from standard textbooks and have referenced these accordingly.

34. I have been shown the draft technical primer and I agree that the material in sections 1-4 was common general knowledge for the Skilled Chemist at July 2003.

Reference texts and online resources used by the Skilled Chemist

35. A very commonly used book for synthesis at the time was Smith and March, March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure, 5th edition ("**March**"). This was my go-to book around that time, and one that I would send people to, to understand how to make molecules. I am not aware of any other books that were better. This is a PhD level textbook that refers to many of the chemistries for making molecules and the different types of transformations which a synthetic organic chemist might want to undertake.
36. One of the things a chemist needs to know about if they are doing nucleoside/nucleotide chemistry and many other areas of organic chemistry is the need for protecting groups. Whether the chemist starts with a nucleoside or with ribose or another sugar, it is necessary to protect the hydroxyl groups to do selective chemical manipulations. A book that was commonly referred to in 2003 (and still is) is Greene and Wuts, Protective Groups in Organic Synthesis, 3rd edition, 1999.
37. The Skilled Chemist would also be familiar with basic medicinal chemistry textbooks, such as:
- (a) Patrick, G.L. "An Introduction to Medicinal Chemistry" 2nd ed. 2001, Oxford University Press ("**Patrick**");
 - (b) Thomas G. "Medicinal Chemistry: An Introduction" 2000, John Wiley & Sons ("**Thomas**").
38. The Skilled Chemist would also routinely consult review articles on specific topics. This could cut out a lot of work searching for references. The Skilled Chemist looking to make nucleosides would have looked at what other people had synthesised before beginning the project, particularly review articles which covered a lot of work in the relevant area. For example, rather than read all the literature prior to 2000 on fluorinated nucleosides, the Skilled Chemist would read a review, which would point them to primary literature (e.g. papers) but also to books.
39. For any particular project in 2003 the Skilled Chemist would do a search online using SciFinder and CAS (Chemical Abstracts Service) Online for both review articles and primary literature. In the 2000s online resources were not as accessible as they are

now and the Skilled Chemist would still also make occasional trips to the library. I attach, as **Exhibit JM-4**, an article published in November 2000 which describes how the SciFinder database worked in the early 2000s.

40. As explained in Exhibit JM-4, the Skilled Chemist could use SciFinder to do word-based searches, structure, or sub-structure based searches. The user could create a structure of the molecule of interest and these programs would then find the papers that include that kind of chemical structure. Sub-structure searches allow the user to find a family of related structures. For example, the user could draw a nucleoside with the 2'-substituent undefined and search for any nucleoside with different 2'-substituents. This sort of sophisticated structure-based searching generally comes after the initial word-based searching.
41. If the molecule has not been synthesised before, then the Skilled Chemist would have to design the synthesis. This involves research into the synthesis of similar structures to design a synthetic pathway. This is when it becomes more complicated and will involve the structure-based searching.
42. I have set out the general approach that would be taken in a research project, but that is not to suggest that a Skilled Chemist would have the references resulting from the searches referred to above at their fingertips in the sense that they would be part of their CGK. The approach above is general to any compound and therefore in practice covers the entirety of organic synthesis. In practice there are often lots of routes to compounds of interest that can be imagined by the Skilled Chemist and which might be pursued in the kind of approach I have described. While the Skilled Chemist might achieve a successful synthesis pursuing one or other of those routes, others will not be successful. The Skilled Chemist would not know which would succeed until they tried.

Protecting group chemistry

43. Many compounds (particularly sugars and nucleosides) contain a number of reactive functional groups. During a synthesis, some functional groups often need to be 'protected' to prevent unwanted chemical reactions and allow selective chemical manipulation of particular parts of a molecule.
44. Functional groups are protected from unwanted reactions by temporarily introducing chemical groups (called protecting groups) to block reactive functionality. The protecting groups are later removed to reveal the functional group again.

45. Carefully selecting a protecting group strategy is often key to the successful synthesis of a complex target compound (such as a nucleoside). Choosing a protecting group involves consideration of various issues, such as the reaction conditions required to introduce and remove the protecting group and the effect of the protecting group on the chemistry used later in the synthetic sequence. For example, bulky protecting groups can sterically block other functionalities, and may affect the conformation (i.e. shape) of the molecule which can in turn affect the reactivity of the compound. The protecting groups chosen must be stable under the conditions used during subsequent synthetic steps and should ideally be capable of being introduced and removed selectively under mild conditions and in high yield.
46. Chemists have developed strategies to enable them to selectively protect different parts of sugar and nucleoside molecules. For example, commonly used protecting groups for hydroxyl groups in nucleosides include benzyl ether (OBn), benzoyl ester (OBz) and tetraisopropylidisiloxanyl ether (TIPDS), but many other strategies exist.

Fluorination chemistry

47. The term 'fluorination' refers to the process of introducing a fluorine atom into an organic molecule. The area of chemistry which relates to techniques for fluorination is referred to as fluorination chemistry, or organofluorine chemistry.
48. In 2003 the Skilled Chemist as I have defined them would have been aware of the prevalence of fluorine in drug molecules, and that there were several methods for fluorinating organic molecules. These could be grouped into two main mechanisms: nucleophilic fluorination (which effectively delivers F^-); and electrophilic fluorination (which effectively delivers F^+). They would not necessarily have been aware of the specific reagents which could be used, but would have known that it could involve difficult, specialist and hazardous chemistry.

Purification and analysis techniques

49. It is uncommon that a chemical reaction produces the desired product in 100% yield with no other impurities or by-products. In 2003, a Skilled Chemist would have been familiar with a number of techniques for monitoring reactions, as well as separating and isolating products, by-products and impurities from a reaction mixture. These included:

(a) **Thin Layer Chromatography (TLC)** is an analytical technique commonly used in synthetic organic chemistry to monitor the progress of

reactions, or analyse the composition of mixtures of compounds. It uses a sheet of glass or aluminium foil (“**the TLC plate**”) that is coated with a thin layer of material such as silica gel or aluminium oxide (referred to as the stationary phase).

A sample (e.g. from a reaction mixture) is applied to the baseline of the TLC plate (usually “spotted” using a capillary tube). The plate is then placed in a solvent tank such that solvent is just able to contact the base of the plate. The solvent is drawn up the plate via capillary action, drawing the sample (and the components within) along with it. Different compounds interact differently with the stationary phase and travel up the TLC plate at different rates, leading to separation. The location of the compounds on the TLC plate can often be determined by holding it under UV light or alternatively, the TLC plate can be stained with a chemical which reveals the separated spots (there were a range of possible stains which a Skilled Chemist may choose depending upon what they were hoping to identify – permanganate was a general stain which would pick up most compounds generated, but other stains would be available for more specific features of compounds). The progress of a reaction can be monitored by comparing the “spots” in the reaction mixture to the starting material “spots” and seeing how this evolves over time;

- (b) **Column chromatography** is used to separate mixtures of different compounds generally for preparative purposes. Column chromatography relies on the same principles as TLC to separate compounds. Rather than a silica coated plate, it involves the preparation of a column of silica gel (the gel is a mixture of silica in a solvent). The reaction mixture is applied to the column, following which the solvent mixture is applied. The solvent mixtures passes down through the column and the compounds are separated out and collected;
- (c) **High Performance Liquid Chromatography (“HPLC”)** operates on the same principle as column chromatography, but is performed using a specialised column by an instrument that is able to produce much higher pressures. This allows for greater precision and improved separation (and also allows the use of much smaller sample quantities). HPLC is most commonly used analytically (i.e. merely to analyse the contents of a

sample), but may also be used to separate larger amounts of compound (referred to as preparative HPLC);

(d) Recrystallisation is a purification technique used to purify crystalline compounds that exploits the difference in solubility of compounds in different solvents and at different temperatures. During recrystallization, an impure solid compound is dissolved in a hot solvent or mixture of solvents until the solution is saturated, and then the liquid is allowed to cool. During the cooling process, the solubility of the compound reduces and pure crystals form, with any impurities being left in the solution. Alternatively, a sample can be dissolved in a solvent and then another liquid in which the desired compound is poorly soluble (an antisolvent) is gradually added, causing crystals to form. The crystals can then be isolated by filtering and washing them;

(e) Nuclear Magnetic Resonance (NMR) Spectroscopy is an analytical technique that detects unique properties of the nuclei of atoms in a molecule to determine the molecule's structure. A sample is placed in a strong magnetic field and a radio frequency pulse is applied. This causes the nuclei to resonate at their own specific frequencies, which are measured and displayed as peaks on a graph (an NMR spectrum). The value of each frequency gives information about the surroundings of the atom in question, such as its neighbouring atoms and their relative positions. NMR spectroscopy can be used to analyse the chemical environment of a number of different types of atom, including hydrogen, carbon and fluorine;

NMR is normally only used to characterise an isolated compound. It is difficult to characterise mixtures of impure compounds due to the complexity of the spectra that result. It is sometimes possible to also work out the 3D structure of a molecule, using multi-dimensional NMR and the Nuclear Overhauser Effect (NOE);

(f) X-Ray Crystallography is an analytical technique that measures the way in which X-rays are diffracted when passing through a crystalline compound to calculate the 3D structure of molecules, including very large molecules, such as proteins. This technique requires a sample which is highly pure and in crystalline form. Obtaining a crystal structure of a

compound or protein can be difficult and often requires a great deal of experience;

- (g) Mass Spectrometry (MS)** is an analytical technique that provides information about the molecular mass of molecules (or fragments of molecules). A sample is ionised and accelerated through a magnetic field and onto a detector, to give information about both the charge and mass of the ions. This information can then be used to characterise the compound.

The Skilled Chemist's approach to synthesis

50. Conducting real-world synthetic chemistry in a laboratory is very different to proposing a synthesis on paper. There are many practical limitations which make it far more challenging to complete a synthesis in the laboratory than a layperson might think from the literature. The theories which the Skilled Chemist relies on to understand and predict the outcome of reactions are generally approximations and known to be imperfect and their application (and the choice of synthetic strategy to be followed) are often value judgments from a range of options. Information in the literature often does not provide a complete picture. Many years of research can be condensed into a paper of only a few pages and, as in many scientific disciplines, failures tend not to be published.
51. Time and resources are limited and conducting 'wet chemistry' (i.e. reactions in the lab) can be very time consuming. Purification and analysis of the results of reactions, using the techniques described above requires a great deal of care and often takes longer than running the reaction itself. It would never be practical for even the most diligent chemist to isolate, purify and analyse every product of every reaction, nor would this generally be considered desirable. Part of the skill and training of the Skilled Chemist is in knowing where to direct time and resources, including the appropriate extent to which the results of any particular synthesis would be analysed.
52. The Skilled Chemist would initially gauge what the outcome of a reaction was by running a TLC as described above. The TLC would indicate whether the reaction proceeded smoothly (one reaction product would be ideal), with a few side products (two or three products indicating some side reactions) or anywhere up to a very complex mixture with a large number of products smeared across the TLC plate. The approach to each situation would then differ:

53. For a reaction which appeared to generate a single product, the product would be isolated and run on an NMR to confirm the outcome.
54. For a relatively simple mixture, the Skilled Chemist would have to work on the separation procedure trying different solvents or separation techniques (column chromatography, HPLC and preparative TLC) to try to separate the products and run an NMR on each isolated fraction. The objective would be to determine the structure of side products which had been generated, to give information on how to work on improving the synthesis. This could be a time-consuming problem depending upon the complexity of the structures of the side products and how well the products separated.
55. In the case of a complex mixture, the approach would depend upon the particular mixture of products generated. If it was very complex and appeared as a complete mess on the TLC plate, it might have simply been abandoned at that stage as a failure. If some larger spots of more substantial products were visible on the TLC, the Skilled Chemist might proceed to try to identify those spots by separating them and running an NMR. In the real world, precisely how many spots and of which size would be tested would depend upon the particular chemist's experience and whether they thought the spots were worth checking – the Skilled Chemist would not isolate and analyse every reaction product of a messy reaction. Again, the Skilled Chemist's objective would be to try to figure out what went wrong, to see if the reaction can be improved, or whether it should be abandoned and a different approach adopted.
56. Additional analysis techniques such as LC-MS (Liquid Chromatography - Mass Spectrometry) could also provide useful information on the outcome of a reaction if they were available in the lab, but the Skilled Chemist would not normally have had access to it. TLC, column chromatography and NMR analysis were the bread and butter approach of the Skilled Chemist in 2003.

Drug discovery process for nucleoside analogues in 2003

57. Bird & Bird asked me to explain the general process of nucleoside analogue drug discovery in 2003.
58. By 2003, nucleoside analogues had attracted a great deal of interest as drug candidates for the treatment of cancer and viral infections. In general, these compounds contain chemical modifications in the sugar or nucleobase of nucleosides, or both.

59. Which compounds (i.e. which structural modifications) to investigate would have been an important strategic decision for the company (or academic research group), which would likely be taken at management level and would likely be a collaborative process involving input from a range of technical areas (e.g. chemistry, biology, clinical etc). The Skilled Chemist would be there to make the molecules, once this decision was made.
60. Once the decision about which families of compound to target has been made, at a strategic level, the Skilled Chemist can begin to explore by synthesising a range of compounds within that family. In this process it is necessary to design compounds based on some prior knowledge. The Skilled Chemist could have read a paper or patent and identified a particular molecular structure, type of structure or structural motif that has relevant biological activity and which could be used as a starting point for designing a new drug. Once the Skilled Chemist has an idea for the scaffold (i.e. the core structure of the molecule they want to investigate) that might be of interest then they have got to design a list of compounds for screening. That might come down to how easy they are to synthesise, the numbers of compounds that Skilled Chemist wants to make, and the kind of chemical diversity to be included.

Prodrugs and nucleoside analogue prodrugs

61. The term prodrug is used to describe a compound which is converted to a parent drug after administration. Prodrugs are generally formed by attaching a chemical group to a compound, to 'mask' certain function groups. These masking groups are then removed by enzymatic and/or non-enzymatic processes in the body, releasing the active drug.

62. The Skilled Chemist would have been aware of prodrug approaches generally within medicinal chemistry and that such approaches had been studied and developed for use in a wide range of drugs¹ with different chemical structures, in a range of therapeutic fields – not merely the nucleoside analogue class of compounds and the anti-cancer or anti-viral fields. This would have been part of their training in basic medicinal chemistry and would have been included in lectures and textbooks such as Thomas² and Patrick³.

L tab.175 vol 3

L tab.183 vol 4

L tab.141 vol 3

¹ In 2001-2002 approximately 15% of approved drugs were prodrugs, see Stella, V. J. "Prodrugs as therapeutics", *Expert Opinion on Therapeutic Patents*, (2004) 14:3, 277-280

² Thomas, G. "Medicinal Chemistry: An Introduction", 2000, John Wiley & Sons.

³ Patrick, G. L. "An Introduction to Medicinal Chemistry" 2nd ed. 2001, Oxford University Press.

63. The Skilled Chemist would have considered that if a suitable masking group could be found, a prodrug strategy could potentially give a range of benefits including improving delivery, improving chemical stability, providing appropriate lipophilicity, or hydrophilicity, improving oral bioavailability, pharmacokinetic or pharmacodynamic profiles. A range of general masking approaches were known, depending on the functional groups to be masked and the desired properties of the prodrug. For example, alcohol or carboxylic acid groups could be masked with esters, aldehydes and ketones could be masked as acetals or imines and amines could be masked as amides, carbamates, imines or quaternary ammonium salts. Although these general approaches were well known,⁴ identifying a suitable strategy and prodrug structure in a specific case was challenging and it was not possible to predict in advance what might work.

4.
Thomas 2000
L tab.183 vol 4
Patrick 2001
L tab.141 vol 3

64. As described in the expert reports of Professor Gotte and Dr Galmarini, the Skilled Chemist would separately have been part of the notional skilled virology team and the notional skilled oncology team at July 2003. As is described in both reports, the ultimate goal of the notional skilled oncology team and the notional skilled virology team in both contexts would have been to find a nucleoside analogue with *in vivo* anti-cancer or anti-viral activity, respectively. I agree that both of these teams would have included a Skilled Chemist. At the *in vitro* screening stage, the goal would have been to find nucleoside analogues with *in vitro* antiproliferative activity and *in vitro* anti viral activity, respectively in various cell-based assays. As is described in both reports, a nucleoside analogue would only have been of interest to the skilled teams if the bare nucleoside analogue showed *in vitro* activity. Neither the skilled oncologist nor the skilled virologist would have been interested in pursuing a nucleoside analogue which did not show *in vitro* activity. I agree with this. As is described in both reports, if an active nucleoside analogue was identified (in either notional drug discovery projects), then the next step would be to conduct *in vivo* testing. In either case, if a nucleoside was found to have satisfactory *in vitro* activity but unsatisfactory activity *in vivo*, then one possible avenue the team might explore could be to apply a prodrug approach (which I explain further below). Such an approach, which would need to be discussed within the skilled teams as part of the next phase of the project, could be a very significant undertaking.
65. I understand from the report of Professor Gotte that the skilled virologist would consider prodrug approaches to be primarily a way of improving the pharmacological

⁴ See for example Thomas, G. "Medicinal Chemistry: An Introduction", 2000, John Wiley & Sons; and Patrick, G. L. "An Introduction to Medicinal Chemistry" 2nd ed. 2001, Oxford University Press.

properties of an active nucleoside, rather than modifying aspects of the antiviral activity. As such, any discussion of prodrug strategies within the skilled virology team would likely require the input of a pharmacologist and is beyond the scope of this report. I also note that Professor Gotte considers the skilled virologist would find nothing of interest in the Patents (discussed further in Section 6 below) and so, there would be no reason for the skilled virology team to consider prodrug strategies.

66. The Skilled Chemist would have known there was no single prodrug approach which could be predicted to work with any given nucleoside. The Skilled Chemist considering a possible prodrug approach would have to consider the nucleoside of interest and the intended biological target (i.e. in conjunction with the skilled oncologist or virologist). They would then attempt to match the nucleoside chemistry and biological target with the available literature, to see what prodrugs had been tested with similar nucleosides in similar cells and shown promising results. The Skilled Chemist would initially look at review papers, but would ultimately have to review the large body of primary literature cited in those reviews, which would be a huge task. Ultimately, this would only give an idea of a starting point – the Skilled Chemist would realistically have to synthesise and test a large number of prodrugs to identify the best approach for a particular nucleoside. This is a very large undertaking that may itself constitute years of research. The only way to know if a particular prodrug would work in a particular target would be to test it in biologically relevant assays.
67. As Dr Galmarini describes, the skilled oncologist in the team would have been interested in overcoming resistance mechanisms associated with poor phosphorylation of a nucleoside analogue inside the cell. The skilled oncologist would therefore have required potential phosphate-masking strategies for overcoming this issue and would discuss potential solutions with the Skilled Chemist. I now describe the various prodrug strategies which could be applied to phosphates of nucleoside analogues and which would be known to the Skilled Chemist.
68. In order to refresh my memory of what was CGK about phosphate masked nucleoside prodrugs I conducted a literature search for contemporaneous review articles using word-based searching for the following keywords: “nucleoside prodrug”, “nucleotide prodrug”, “phosphate prodrug” from pre-2003 and identified the review papers below. A Skilled Chemist in 2003 wanting to similarly refresh their memory would have conducted a similar exercise and likely identified the same material:

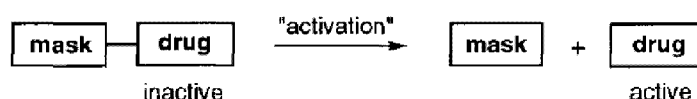
- L tab.81 vol 2** (a) Jones et al “Minireview: nucleotide prodrugs” *Antiviral Research* 1995, 27, 1-17;
- L tab.86 vol 2** (b) Krise et al “Prodrugs of phosphates, phosphonates, and phosphinates” *Adv. Drug Delivery Rev.* 1996, 19, 287–310;
- L tab.142 vol 3** (c) Perigaud et al “Comments on nucleotide delivery forms” *Advances in Antiviral Drug Design* 1996, 2, 147-172;
- L tab.119 vol 3** (d) Meier “Pro-Nucleotides - Recent Advances in the Design of Efficient Tools for the Delivery of Biologically Active Nucleoside Monophosphates” *Synlett* 1998, 3, 233-242;
- L tab.180 vol 4** (e) Tan et al “Development and optimization of anti-HIV nucleoside analogs and prodrugs: A review of their cellular pharmacology, structure-activity relationships and pharmacokinetics” *Advanced Drug Delivery Reviews* 1999, 39, 117-151;
- L tab.94 vol 2** (f) Lee et al “Prodrug and Antedrug: Two Diametrical Approaches in Designing Safer Drugs” *Arch Pharm Res* 2002, 25, 111-136; and
- L tab.162 vol 3** (g) Schultz et al. “Prodrugs of Biologically Active Phosphate Esters” *Bioorg. Med. Chem.* 2003, 11, 885-898.

69. The Skilled Chemist would appreciate the following from the review literature above:

- (a) Most phosphate masking nucleoside prodrug strategies were designed with a specific unmasking mechanism in mind, although it was not necessarily clear until much later if they actually operated by this mechanism.
- (b) Most phosphate masked nucleoside prodrugs were grouped into what were referred to as bipartate and tripartate unmasking mechanisms, as illustrated in Figure 1 below from the review by Meier.⁵ In a bipartate approach, the nucleoside monophosphate is protected with a one-component mask, such as one or more phosphoester groups. After diffusion into the cell, the mask is cleaved, liberating the active drug. In a tripartate approach there are two components masking the phosphate. The first component is removed by an enzyme in the cell (preferably an enzyme that is not found outside the cell). This releases an intermediate which is unstable and then breaks down chemically to give the monophosphate.
- L tab.119 vol 3**

⁵ Meier “Pro-Nucleotides - Recent Advances in the Design of Efficient Tools for the Delivery of Biologically Active Nucleoside Monophosphates” *Synlett* 1998, 3, 233-242.

The bipartate prodrug concept



The tripartate prodrug concept

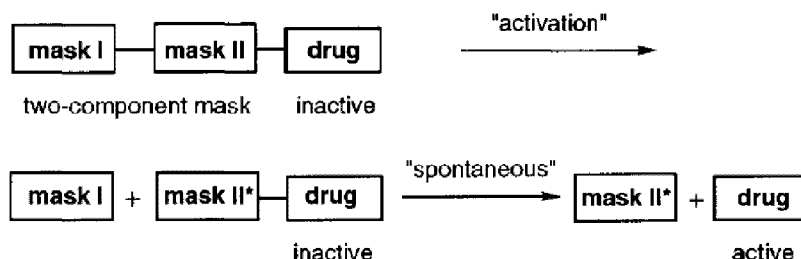


Figure 1: Bipartate and tripartate strategies

- (c) Common examples of a bipartate strategy were either forming a phosphodiester, by adding one ester group to the phosphate, or forming a phosphotriester by adding two ester groups. Both of these approaches suffered significant limitations. Phosphodiesters still have a negative charge, limiting diffusion into the cell. Phosphotriesters have all the phosphate negative charges masked, so are more lipophilic and give better intracellular delivery; however, there were no enzymes known to directly hydrolyse phosphotriesters, hindering release of the active nucleoside once the prodrug had entered the cell.
- (d) One of the earliest widely discussed prodrug approaches developed was the use of bis(pivaloyloxymethyl) phosphotriesters ("**bisPOM**"). The proposed mechanism of action for bisPOM prodrugs is illustrated by Meier in Figure 2 below. The first step is cleavage of the pivaloyl ester by a carboxyesterase to give a reactive hydroxymethyl intermediate. This intermediate spontaneously eliminates formaldehyde, deprotecting the phosphate. This process is repeated for the second pivaloyloxymethyl group, ultimately revealing the nucleoside monophosphate. A variant of this approach, using a single pivaloyloxymethyl phosphoester (a "**POM**" approach) was also known.

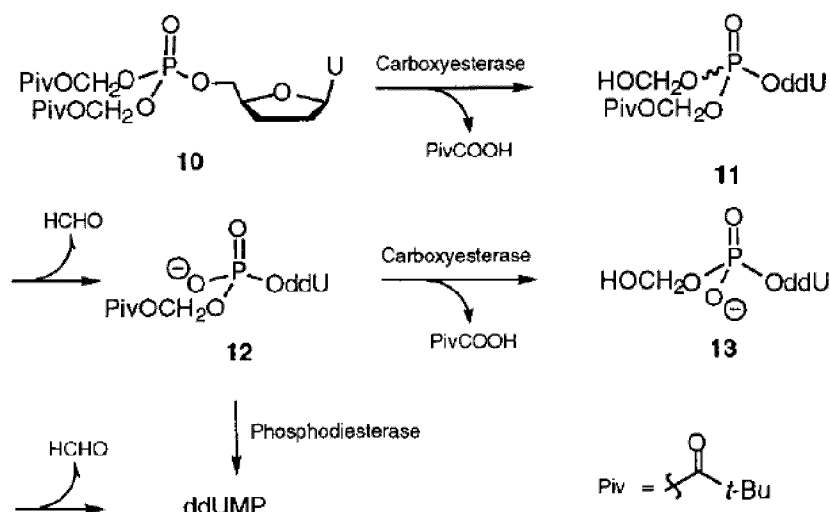


Figure 2: proposed bisPOM cleavage mechanism

- (e) A modification of the bisPOM strategy, using bis(isopropoxyloxycarbonyloxymethyl) groups (“**bisPOC**”) was also developed and relied on a very similar mechanism to bisPOM, as shown in Figure 3 below (again from Meier). A variant of this approach, using a single isopropoxyloxycarbonyloxymethyl phosphoester (a “**POC**” approach) was also known.

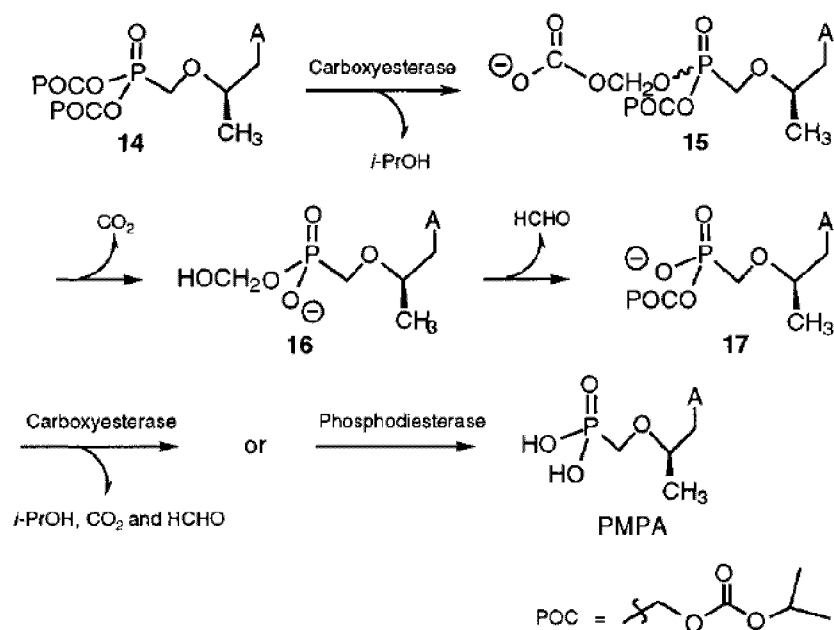


Figure 3: proposed POC cleavage mechanism

- (f) S-acyl-2-thioethyl esters (“**SATE**”) prodrugs use two thioester groups to mask the nucleoside monophosphate, as shown in Figure 4 below (from

Jones et al⁶). The thioester moiety can be hydrolysed by carboxyesterases in the cell to reveal the unstable 2-thioethyl intermediate (Figure 4, middle structure). This spontaneously collapses, releasing episulphide and the nucleoside monophosphate, as shown below.

- (g) Dithiodiethanol (“**DTE**”) prodrugs are closely related to SATE, but rely on reduction of a disulphide moiety due to the reducing environment inside the cell to form the unstable 2-thioethyl intermediate. From there on, the mechanism for releasing the monophosphate is the same as for SATE prodrugs (see Figure 4 below).

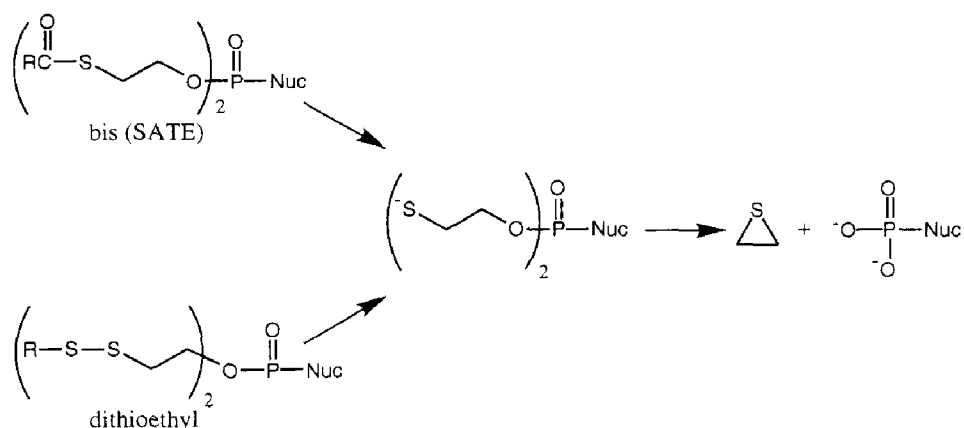


Figure 4: proposed SATE/DTE cleavage mechanisms

- (h) A more recent approach was the use of phosphoramidate (“**PPA**”) prodrugs. PPAs consist of three components: an aryl phosphoester; an amino acid; and a carboxyester group attached to the amino acid. All three of these components could in principle be altered to seek to tune the properties of the prodrug PPA in the hope of finding a suitable prodrug for the application in question.

- (i) The breakdown of a PPA prodrug in the cell was proposed in July 2003 to occur by one of two mechanisms, shown in Figure 5 below.⁷ Initially an esterase cleaves the amino acid ester, resulting in a carboxylic acid which then cyclises eliminating an ⁻OAr leaving group. The cyclic intermediate then opens spontaneously and the resulting intermediate undergoes enzymatic hydrolysis to release the nucleoside monophosphate (Figure 5, upper pathway). Alternatively, it was proposed that initial chemical

⁶ Jones et al. *Antiviral Research* 1995, 27, 1-17.

⁷ Lee et al “Prodrug and Antedrug: Two Diametrical Approaches in Designing Safer Drugs” *Arch Pharm Res* 2002, 25, 111-136, at 122.

hydrolysis of the aryl phosphoester, followed by hydrolysis of the carboxyester, gives the common phosphoramidate intermediate, which is then hydrolysed by another enzyme to release the nucleoside monophosphate (Figure 5, lower pathway).

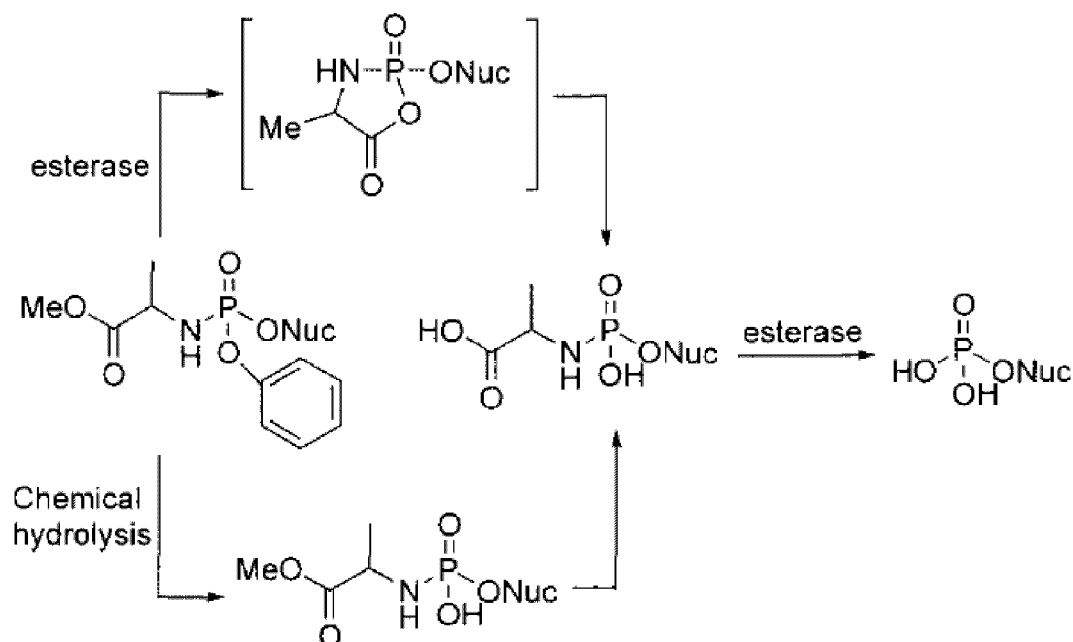


Figure 5: proposed PPA cleavage mechanisms

70. Each of the prodrug strategies discussed above would have been identified from the review papers I refer to in paragraph 68 above. Each strategy was also known to have advantages and drawbacks and from their CGK the Skilled Chemist would have little guidance about which strategies to explore further.

SECTION 6: THE PATENTS

71. Bird & Bird provided me with a copy of EP 190 and asked me to whom it was addressed (the Skilled Chemist, as explained above) and to provide the Skilled Chemist's understanding of the document as at 21 July 2003.

Overview

72. EP 190 is a European patent entitled "Chemical compounds" in the name of NuCana. I note from the front page that it was published on 16 December 2015, but has a priority date of 21 July 2003. The inventor is listed as Christopher McGuigan, who I understand to have been a professor at the University of Cardiff. The invention of EP

190 is stated to relate to “*nucleotide derivatives and their use in the treatment of cancer*” (see paragraph [0001]).

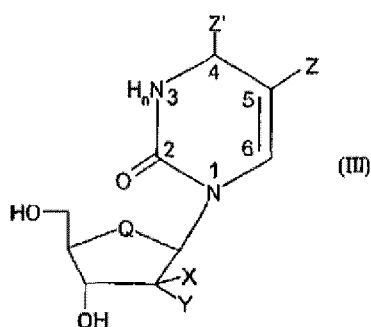
73. I will describe in more detail below some of the relevant disclosures of EP 190 by reference to its specific paragraphs. However, I will first set out an overview of how the Skilled Chemist would have understood the disclosure of EP 190 as of 21 July 2003.
74. At paragraph [0002], EP 190 refers to well-known nucleoside anti-cancer agents including gemcitabine which has two fluorine atoms at the 2' position. Paragraph [0002] also explains that these compounds need to be phosphorylated at the 5' position to be in their active form.
75. Paragraph [0003] states that the phosphate forms of these compounds do not easily pass through the cell membrane, so do not enter cells very well.
76. Then EP 190 explains that a number of phosphate prodrug strategies have been reported. The Skilled Chemist would appreciate from what the authors state about the papers cited in the patent that there was lots of work in the 1990s by McGuigan and others to develop phosphate prodrugs. Although it is not explicitly stated in EP 190, the Skilled Chemist would be aware from their CGK that phosphate prodrugs are compounds where the phosphate at the 5' position of a nucleoside drug has other moieties attached to it which can be cleaved when it enters the cell, and that one approach was the use of phosphoramidate prodrugs. As I explain in above, the Skilled Chemist would also understand that there are three components to phosphoramidate prodrugs which can be modified: an aryl phosphoester; an amino acid; and an amino acid carboxyester. EP 190 refers to a compound called d4T used in the treatment of HIV (compound (5)) and a prodrug of d4T (compound (4)). EP 190 goes on to state that the PPA moiety shown in compound (4) has been identified as the optimal arrangement for a phosphoramidate prodrug for bioactive nucleosides (see paragraph [0008]).
- L tab.88 vol 2 77. EP 190 also states that Lackey et al. (2001) show that another anti-viral agent, BVdU (bromovinyl-2'-deoxyuridine), has also been transformed into a prodrug of the same type (compound (7)) (see paragraph [0009]). The authors state that compound (7) has anti-cancer properties, whereas BVdU itself does not have anti-cancer activity, although the Skilled Chemist would not have the expertise to check this conclusion (and would rely on the opinion of the oncologist). EP 190 then goes on to say (in paragraph [0011]) that it is somewhat surprising that compound (7) is not the optimal prodrug for BVdU.

78. EP 190 then describes the general structure of prodrugs to which the invention relates (see [0013]). Paragraphs [0014] to [0049] provide the Skilled Chemist with information as to the meanings of the terms used to define the structures disclosed by the patent.
79. Paragraphs [0050]-[0051] explain that a BVdU PPA prodrug with a benzyl ester (compound (8)) is more potent against a certain colon cancer cell line than the equivalent BVdU PPA with a methyl carboxyester (compound (7)) by a factor of 174. The authors state that this is surprising because, in the case of a different nucleoside (d4T), a smaller improvement in activity (4-fold) is observed between the PPA with a benzyl ester (compound (10)) and the PPA with the methyl ester (compound (9)).
80. Paragraphs [0054]-[0056] suggest that, similarly, the results from changing amino acid or the aryl group of the PPA moiety are also not necessarily consistent when moving between different nucleosides. Paragraph [0056] states that a BVdU PPA prodrug with a benzyl carboxyester, dimethylglycine amino acid and 4-(trifluoromethyl)phenyl phosphoester aryl group (compound (11)) gives a 60-fold increase in activity against breast cancer cells and 254-fold increase in activity against colon cancer cells, when compared to a BVdU PPA with a methyl carboxyester, alanine amino acid and phenyl phosphoester (compound (7)). The authors contrast this with the situation with d4T PPA with a methyl carboxyester, dimethylglycine amino acid and phenyl phosphoester (compound (12)), which shows no significant change in anti-viral potency when compared to a d4T PPA with a methyl carboxyester, alanine amino acid and 4-(trifluoromethyl)phenyl phosphoester (compound (13)). Although EP190 suggests that the results of this comparison is “surprising”, the Skilled Chemist would draw little or nothing from this comparison, because multiple functional groups in the PPA moieties are varied simultaneously and these variations are not consistently applied between d4T and BVdU nucleosides.
81. EP 190 appears to be trying to say that when prodrugs are modified, the effect on the biological activity is not uniform or predictable across different nucleosides. That would not be surprising to the Skilled Chemist. A nucleoside prodrug is one molecule and has to be viewed as a whole. These PPA prodrugs are being processed by enzymes in the cell: a carboxyesterase, then spontaneous cyclisation, followed by hydrolysis, and then another enzyme that cleaves the phosphoramidate intermediate. Those enzymes recognise a whole molecule, not just the prodrug part, so the Skilled Chemist would not have expected that a prodrug moiety optimised for one nucleoside would necessarily work when applied to different nucleosides.

82. EP 190 claims to disclose an invention where nucleoside compounds of Formula (III) can be coupled with the reagent in Formula (IV), which is a phosphochloridate derivative, to create prodrugs. At [0073] EP190 informs the Skilled Chemist that it will disclose how to carry out that process, although it does not claim that process as part of the invention. It then goes on to describe the synthesis of amino acid carboxyesters; methyl and benzyl esters by two general procedures. The authors describe the synthesis of a phosphorodichloridate derivative (a phosphorus with two chloro groups) which is reacted with an amino acid ester to make the phosphorochloridate derivative which is a compound of Formula (IV) (see [0080]-[0085]). This is then coupled with a nucleoside to give a phosphoramidate prodrug (see Standard Procedure 5, [0085]).
83. The next 53 pages (the bulk of the patent) describe the synthesis of various precursors and prodrugs, mainly of BVdU and a few gemcitabine examples. Then they describe some biological activity, particularly for compound 31 which is a phosphoramidate prodrug of gemcitabine claimed by the authors to have lower toxicity, fewer side effects and greater activity than the parent drug, although the Skilled Chemist would defer to the biologist on this point.

Does EP 190 teach how to make nucleosides?

84. Bird & Bird asked me to comment on what, if anything EP 190 teaches the Skilled Chemist about how to synthesise nucleosides of Formula III, i.e.

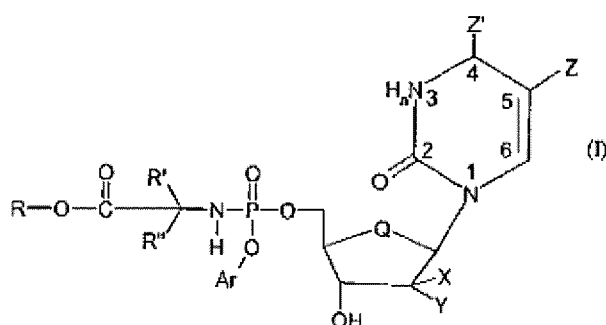


85. The answer is nothing. There is no information whatsoever on how to make nucleosides with modifications at the 2' position, or modifications on the base other than introducing a bromovinyl group at the 5-position. EP 190 only teaches the Skilled Chemist how to make a prodrug precursor (such as a compound of Formula (IV)) and attach it to a nucleoside (such as a compound of Formula (III)). Although it provides a method of synthesising some compounds of Formula (IV), it does not teach the Skilled Chemist how to make any nucleoside of Formula (III).

86. A Skilled Chemist would look at fluorodeoxyuridine (shown as compound (1)), cytarabine (shown as compound (2)) and gemcitabine (shown as compound (3)) and Formulas I (paragraph [0013]) and III (paragraph [0073]) which are more generally defined and be interested in the fact they vary in the substituents at the 2' position, but be given no idea from EP 190 how they might construct them.
87. Formulas I and III also include an equivalent family of carbocyclic nucleoside analogues, where Q is -CH₂-. A carbocyclic nucleoside is a nucleoside analogue where the ribose unit has been replaced with a carbocyclic sugar analogue (i.e. the oxygen in the sugar ring has been replaced by a methylene (CH₂) group). Carbocyclic nucleosides were known in 2003 to be more challenging from a synthetic perspective. They could be synthesised, but there was more research on the ribose-based compounds. It was also possible to use the existing stereocentres within ribose based compounds to control the creation of new stereogenic centres. With carbocyclic analogues existing stereocentres may not be present and so more sophisticated asymmetric synthesis would be required to create new stereocentres. Additionally, the oxygen in the ring would give more flexibility with respect to adding the base. It was slightly more difficult to add the base to a carbocycle, compared to a sugar, and knowledge of how to synthesise a nucleoside would not necessarily translate to synthesising a carbocyclic nucleoside analogue (or vice versa). Notably, there are no examples or information in EP 190 at all regarding the synthesis or use of carbocyclic nucleosides.
88. This is a patent which relates to prodrugs of nucleotide analogues – i.e. including a 5'-phosphate group. This ties in with my comments in paragraph 67 above which related to the discussions between skilled oncologist and skilled chemist about the oncologist's desire for possible phosphate-masking strategies to seek to overcome poor phosphorylation of nucleosides inside the resistant cells. EP 190 concerns one such phosphate masking strategy – phosphoramidates.
89. The Skilled Chemist in July 2003 would view EP 190 as a document that describes the application of a particular (previously published) prodrug strategy to examples of pre-existing nucleoside drugs. There is information about making phosphoramidate prodrugs and previous work by the McGuigan group in developing these types of phosphoramidate prodrugs is cited in EP 190. The prodrug chemistry is clear and the patent includes a large number of examples of this chemistry. If you already know how to make the nucleoside and are interested in making phosphoramidate prodrugs, then this patent provides some information on prodrug synthesis. However, the patent does not teach how to make any nucleosides.

Does EP 190 teach which compounds may be useful as anti-cancer or antiviral drugs?

90. Bird & Bird asked me to comment on what, if anything, EP 190 teaches the Skilled Chemist about what nucleoside analogues or nucleoside prodrug moieties within Formula I (shown below) might be worth investigating as potential anti-cancer or antiviral drugs. This would be a matter for discussion with the biologist on the skilled team, but the Skilled Chemist would, as I explain above, be able to interpret biological data in terms of biological assays and would be able to use that data to assess any relevant SAR.



wherein:

R is selected from the group alkyl, aryl and alkylaryl;
 R' and R'' are, independently, selected from the group H, alkyl and alkylaryl, or R' and R'' together form an alkylene chain so as to provide, together with the C atom to which they are attached, a cyclic system;
 Q is selected from the group -O- and -CH₂-;
 X is independently selected from the group H, F, Cl, Br, I, OH and methyl (-CH₃);
 Y is F;
 Ar is a monocyclic aromatic ring moiety or a fused bicyclic aromatic ring moiety, either of which ring moieties is carbocyclic or heterocyclic and is optionally substituted;
 Z is selected from the group H, alkyl and halogen; and
 n is 0 or 1,

wherein

when n is 0, Z' is -NH₂ and a double bond exists between position 3 and position 4, and

when n is 1, Z' is =O;

91. Formula I appears to describe a family of compounds which are essentially prodrugs of 2'-F nucleoside derivatives with various modifications at the other 2' position. However, the odd thing is that the vast majority of data presented in EP 190 relates to BVdU prodrugs which are not included in Formula I. The data provided in the Table (paragraph [0299]) appears to have been generated as part of an exercise in fine tuning the BVdU prodrug, but EP 190 also explains that the best substituents of a prodrug moiety can change from one nucleoside to the next. EP190 makes clear (and it would not have been surprising to the Skilled Chemist) that the knowledge of the optimum

PPA structure that the Skilled Chemist can get from data for prodrugs of BVdU may not transfer over to any of the nucleoside analogues within Formula III (see paragraphs 79-81, above and EP 190 paragraphs [0051]-[0058]).

92. As a result, the Skilled Chemist would understand EP 190 as providing an assessment of the effectiveness of different phosphoramidate prodrugs of BVdU. Of the prodrug examples provided, 90 out of 93 compounds are prodrugs of BVdU (see EP 190, Table). The only other data presented is on three examples of gemcitabine prodrugs (see EP 190, Table , Examples 31, 40 and 41). Neither the BVdU nor gemcitabine results tell the Skilled Chemist anything about prodrugs of other nucleoside analogues, as EP 190 itself suggests at [0055]-[0056].
93. None of the examples provided in EP 190 relate to antiviral activity. The only discussion of antiviral compounds are some general comments that nucleoside PPAs have previously been investigated as antiviral agents (see paragraphs [0005], [0007], [0009]).
94. EP 190 gives the Skilled Chemist only the minimal EC₅₀ data for gemcitabine and its three derivatives shown in Table 1. The data for the three gemcitabine prodrugs (Examples 31, 40 and 41) does not show any particular structure-activity relationship in relation to the PPA substituents. There is also no reason to expect that the results for these compounds to be reproduced if the same PPA moieties were applied to other nucleosides. If the Skilled Chemist changed the X and Y substituents, they would have no reason to think the results would be the same as those observed with gemcitabine or BVdU.
95. All the BVdU data does is perhaps rule out some prodrug moieties for use with BVdU and suggest that some types of PPA structures might be better than others when applied to BVdU. This information could not be extrapolated for other nucleosides. For example, the Skilled Chemist would see that alanine or small hydrophobic amino acids, such as dimethylglycine, tend to give improved activity and indeed paragraph [0010] indicates that alanine would be anticipated to be among the structures that would be optimal. Similarly, PPAs with benzyl (Bn) carboxyesters appear to generally result in prodrugs with improved activities compared to methyl and ethyl carboxyesters. The phenyl phosphoester has a good leaving group and the results are consistent in showing that PPAs with this group generally work reasonably well. Adding an electron withdrawing group in the para position of the aryl phosphoester group would be expected to make it a better leaving group and lead to improved

activity, which is supported by the data in some (but not all) cases. These are some general rules of thumb that come out of that data for BVdU that the Skilled Chemist could consider applying to these other families of nucleoside prodrugs, such as the 2'-F compounds of Formula I, but they would not be able to predict if it would work to create compounds that are more active against cancer than the parent nucleoside, or even active at all.

96. In summary, no information is provided in EP 190 which would allow the Skilled Chemist to predict which variations of the nucleoside in Formula I (i.e. changes in the X, Y, Q, Z or Z' substituents) might be useful as potential drugs. The Skilled Chemist's conclusions would be limited to the data presented for the three prodrugs of gemcitabine analogues in Table 1 (Examples 31, 40 and 41). This provides some indication of specific gemcitabine PPA prodrugs which appear to show greater activity in some assay results (but not all) and may be of interest to test as anticancer agents, although the Skilled Chemist would rely on the views of the skilled oncologist in deciding whether these compounds were worth developing further.

EP 365 and proposed amendments to EP 190

97. After I had provided my views on EP 190, Bird & Bird provided me with a copy of EP 365. I can see that the description of EP 365 is largely taken from EP 190; however, substantial portions of the description of EP 190 have been omitted.
98. I note that much of the background to the invention of EP 190, which I discuss at paragraphs 79-81 and is contained in [0050] to [0057] of EP190, is omitted in EP 365. In EP 365, paragraph [0021] (which corresponds to [0058] of EP 190) does not explain why "thus" compounds in accordance with the invention can give "surprising and substantial potency boosts of pro-tides derived from BVdU".
99. EP 365 does not explicitly refer to a procedure for coupling a molecule of Formula III and a molecule of Formula IV, however, the same general procedures are disclosed in EP 190 and EP 365, so this makes little difference. EP 365 also discloses only examples of chemistry relating to the synthesis of prodrugs of gemcitabine. However, as I explained above, EP 190 (and EP 365) do not provide any examples of the synthesis of nucleosides, so this does not significantly change what the Skilled Chemist would learn from the document.

100. Other than the comments I make in paragraphs 79-81 and 87 above, the differences in the description of EP 365 are not such as to affect any of the views I have expressed above.
101. Bird & Bird also provided me with a copies of Annexes A & B to NuCana's Statement of Reasons for Amendment of EP 190. I understand from Bird & Bird that each of theses Annexes relate to an amendment to EP 190 proposed by NuCana. I note that each proposed amendment includes some changes to the description and the claims of EP 190. The differences in the description of EP 190 proposed in each of Annex A and Annex B are not such as to affect any of the views I have expressed above.
102. Bird & Bird asked me to comment on the number of compounds which fall within claim 1 of each of EP 190, EP 190 as proposed to be amended in Annex A, EP 190 as proposed to be amended in Annex B and EP 365.
103. Claim 1 of EP 190 includes an astronomical number of compounds. Accurately estimating the number of compounds within the scope of the claim would be challenging, but it is certainly in excess of 10^{20} possible compounds. Each of the proposed amendments in Annexes A and B reduce the number of compounds, but I would still expect the number to be in excess of 10^{20} possible compounds. EP 365 significantly reduces the number of claimed compounds, but even so still claims several thousand compounds.
104. Bird & Bird provided me with structures of several nucleoside analogue PPAs, which I understand to have been the subject of certain publications, and asked me to comment on whether each compound falls within the scope of claim 1 of EP 190 as proposed to be unconditionally amended (Annex A), or conditionally amended (Annex B). I was also asked to comment on whether those compounds fell within the scope of claim 1 of EP 365. I provide my comments in the Annex to this report.

SECTION 7: PRIOR ART

105. Bird & Bird provided me with a copy of Shepard and asked me to review it from the perspective of a Skilled Chemist, who was part of a team investigating nucleoside derivatives as part of a drug discovery project before 21 July 2003. I was asked to comment on what the Skilled Chemist would have taken from Shepard regarding the synthesis and/or biological activity of nucleosides and nucleoside prodrugs.
106. Shepard is a United States patent application entitled "Novel Phosphoramidate Compounds and Methods of Use". I note from the front page that it was published on

12 June 2003. The invention of Shepard is stated to relate to “*compounds, compositions and methods for treating cancer, infectious disease, an autoimmune disorder or an inflammatory condition.*” Specifically, the compounds of the invention are “*5′-phosphoramidatyl, 1,5-substituted pyrimidine compounds*”.

107. Paragraphs [0003]-[0010] of Shepard provides background to hyperproliferative disorders. These are explained to include cancer as well as some inflammatory diseases such as arthritis, Crohn's disease, ulcerative colitis and scleroderma. Paragraph [0010] explains that the hyperproliferative phenotype has been linked to drug resistance and the overexpression of certain enzymes including thymidylate synthase (“TS”).
108. Paragraph [0012] explains that there is a need for novel therapies which overcome the limitations of current therapies and states that this is what the invention provides.
109. Shepard refers to hyperproliferative disorders, which the Skilled Chemist would understand to primarily refer to cancer, although there are some limited references to inflammatory diseases throughout the document. The concept appears to be that certain cells (like cancer cells) will grow faster than normal cells and that provides an opportunity for chemical intervention. Where cells are growing faster, they may have more of a certain type of enzyme and could use more nucleotides (i.e. phosphoesters of nucleosides) for DNA and RNA synthesis. As a result, it could be possible to selectively target these cells with nucleoside analogues.
110. The Skilled Chemist would consider that Shepard contained more novel biology than chemistry. The inventors appear to be describing a novel mode of action for certain phosphoramidate prodrugs of a known nucleoside drug, BVdU.
111. Paragraphs [0319]-[0321] explain that the prior art suggested that BVdU is converted to the monophosphate and then inactivates TS. The inventors state that actually what happens is that TS activates BVdU monophosphate, by converting it into more cytotoxic compound(s), which are ultimately responsible for the observed anti-cancer activity (the Skilled Chemist would defer to the biologist in relation to evaluating this assertion).
112. This mechanism is important because Shepard informs the Skilled Chemist that TS production is upregulated in cancer cells (i.e. there is more TS in these cells) and in particular in cancer cells that are drug resistant. Because these cells have more TS, they are more likely to convert BVdU monophosphate into this more toxic agent(s)

which kills the cancer cells. If it were possible to get more BVdU monophosphate into the cells, then it could selectively kill cells overexpressing TS (i.e. cancer cells) compared to normal cells.

113. The general strategy disclosed in Shepard is to make molecules like BVdU, which have a pyrimidine base attached to ribose and have additional functionality at the 5-position (of the base), which in BVdU is a bromo alkene. The monophosphates of these compounds are suggested to be activated in the presence of TS to give the unknown cytotoxic product(s) which Shepard suggests are responsible for the observed anticancer activity. However, the only evidence the Skilled Chemist can see in Shepard for this type of activity relates to BVdU. The Skilled Chemist would not know whether the other molecules that the inventors disclose in Shepard would work or not. The compounds share the same general idea of adding a substituent to the 5-position of a uridine base, but they are different from BVdU, so it is unclear if they would work in the same way.
114. The inventors have tried to come up with as many variations of BVdU as possible particularly around the linker to a reactive functional group (at the 5-position) and also around the structure of the PPA prodrug. Table 2 and Example 16 (page 29) describe a number of other variants of BVdU with the modified linker.
115. Paragraphs [O113] to [O120], and Example 17 on page 29, describe a large number of possible PPA variants. Paragraphs [O113]-[O114] discuss PPA groups derived from an amino acid, including the 20 naturally occurring amino acids. The authors provide the example of a PPA derived from tryptophan (Formula I, paragraph [O113]), which is described in a paper by Abraham et al⁸. Paragraph [O116] discusses variation of the carboxyester group of the PPA and gives a long list of possible groups (including alkyl, cycloalkyl, aryl and adamantyl) and provides exemplary combinations of amino acid and carboxyester groups. Paragraphs [O118]-[O119] provide the example of a PPA with alanine, a methyl carboxyester and a phenoxy phosphoester groups which the authors state have been described by McGuigan et al.⁹ Paragraph [O120] gives a long list of PPA structures all including a phenoxy phosphoester but varying widely at the amino acid and carboxyester groups. Paragraph [O121] further expands the range of compounds disclosed to include any enantiomer, diastereomer or anomer of the structures discussed above.

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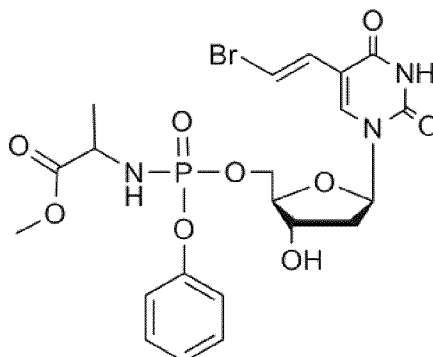
9.
McGuigan 1993
L tab.110 vol 2

McGuigan 1996
L tab.114 vol 3

⁸ See reference [O396] Abraham et al. (1996) *J. Med. Chem.* 39:4569-4575.

⁹ See reference [O493] McGuigan, C. (1993) *J. Med. Chem.* 36:1048-1052; and reference [O494] McGuigan, C. (1996) *J. Med. Chem.* 39: 1748-1753.

116. The chemistry in Shepard is focused on BVdU PPA prodrugs, primarily a compound called NB1011, which has the structure shown below (see Shepard paragraph [0123] and table 2):



117. In general, very little evidence is provided to show that the compounds referred to have actually been synthesised or characterised. Example 15 in paragraphs [0269]-[0270] claims to describe the synthesis of 64 g of NB1011, but does not provide any characterisation data. Similarly, Examples 16 and 17 purport to describe the synthesis of a nucleoside PPAs, but provides no characterisation data for any of those compounds.
118. Example 16 in paragraphs [0271]-[0283] purports to disclose the synthesis of a range of derivatives of BVdU phosphoramidates, with different functional groups at the 5-position. Example 17 in paragraphs [0284]-[0293] then describes preparing a range of different phosphoramidates of BVdU, with variation in the carboxy ester and amino acid groups. The majority of examples use L-alanine (paragraph [0293] gives an example using tryptophan) and all include phenyl phosphoesters.
119. None of the chemistry described is novel. All the inventors describe is making a series of simple variants of a known compound (BVdU) and some limited exploration of a prodrug strategy previously described in the literature (see e.g. the papers by McGuigan and Abrahams et al cited in Shepard and paragraph 68, above).
120. From paragraph [0295] to [0395] Shepard discloses biological testing for compound NB1011. The information appears to be detailed. The Skilled Chemist would note that paragraph [0332] provides some evidence that BVdU monophosphate is delivered into the cells, but other than this would defer to the biologist when assessing this information.

121. To verify the proposed mechanism, the inventors test the activity of NB1011 in cancer cells. They then test the activity of NB1011 co-administered with an anticancer drug called Tomudex, which is an inhibitor of TS (see Shepard paragraph [0348]). Increasing concentrations of Tomudex were found to result in up to a 25-fold inhibition of NB-1011 activity, which the inventors suggest supports their conclusion that NB-1011 is metabolised by TS to give the active cytotoxic agent (the Skilled Chemist would defer to the biologist when assessing this assertion).

Comparison of Shepard, EP190

122. Having provided my views on teachings of EP 190 and Shepard, Bird & Bird asked me to comment on any differences in what the Skilled Chemist would take from each document.
123. Both Shepard and EP 190 relate to phosphoramidate prodrugs of certain nucleoside analogues. EP 190 concerns a wider range of nucleosides, with modifications at the 2'-position and the 5-position (of the base), whereas Shepard mainly concerns nucleosides analogues with modifications at the 5-position.
124. As I explain above, the Skilled Chemist would not take much away from either document. EP 190 provides no information at all regarding the synthesis of nucleoside analogues other than introducing a bromovinyl group at the 5-position, but does provide a number of examples of how to make different PPA groups and attach them to existing nucleosides.
125. Shepard also provides some examples of the synthesis of different PPA groups and attaching them to nucleoside analogues, but there is limited or no characterisation data for many of these compounds. Unlike EP 190, Shepard provides more examples of the synthesis of nucleoside analogues with different substituents at the 5-position. However, this just follows previously published synthetic protocols (see paragraphs [0209]-[0212]) and gives limited evidence that the various compounds have actually be prepared.
126. The biological testing data in either EP 190 or Shepard would be of little interest to the Skilled Chemist and would not understood to be establishing some generally applicable principle. Shepard only provides testing data for one compound (NB1011) and the Skilled Chemist would have no reason to believe similar results would be obtained in respect of other nucleoside PPAs. EP 190 only provides testing data for three

gemcitabine PPAs and the Skilled Chemist would also have no reason to think similar results would be obtained in respect of other nucleoside PPAs.

127. Bird & Bird asked me to comment on the extent to which my conclusions above differed in relation to the teaching of EP 365. In my view, all of my comments above apply equally to EP 365.

SECTION 8: SUFFICIENCY

Could the Skilled Chemist synthesise compounds of Formula III in 2003?

Stereochemistry

128. Bird & Bird asked me to comment on the stereochemistry of the structure of Formula III, as shown in paragraph [0073] of EP 190. The way the X and Y substituents are drawn is unusual.
129. The conventional way to represent a sugar structure would be to use a Haworth projection (see Figure 6 below). This would normally be how nucleoside structures would be drawn. Alternatively, wedge and dash structures can also be used to clearly define the stereochemistry in organic molecules. A 'wavy' line can be used to depict stereocentres where the absolute stereochemistry is undefined.

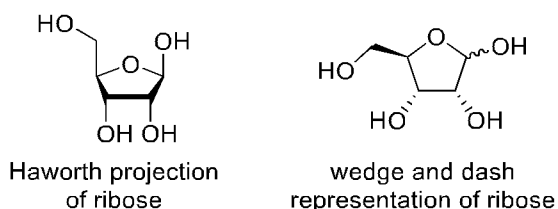


Figure 6: representations of sugar structures

130. Here, however, the Haworth projection appears to have been applied, but not with respect to the chemistry at the 2'-position. In my view, given the normal conventions described above, the Skilled Chemist would assume that Y is down and X is up but would wonder why the structure was so poorly presented.

Could the Skilled Chemist synthesise compounds of Formula III?

131. Bird & Bird asked me to comment on whether the Skilled Chemist as at 21 July 2003 could synthesise compounds of Formula III on the basis of their CGK and the information in EP 190 and how difficult any such synthesis would have been.

132. The range of substituents in Formula III are defined in paragraph [0013]. Most relevantly, Y is F and X is “*independently selected from the group H, F, Cl, Br, I, OH and methyl (-CH₃)*”. As I explain above, EP 190 provides no information about how to synthesise these compounds – the only relevant information the Skilled Chemist could take from EP 190 is the structure of Formula III.
133. Having seen that compounds of Formula III all include fluorine, the Skilled Chemist would have searched for and read review articles about fluorinated nucleosides such as the paper by Pankiewicz et al (which I discuss further below).¹⁰
134. As a preliminary point, the Skilled Chemist would see that some of the compounds of Formula III are not feasible to make. If X is OH and Y is F, that compound would be unstable and eliminate HF. I am not aware of any stable compounds which have an alcohol and a fluorine attached to the same carbon. The Skilled Chemist would rule that out as a structure. Inclusion of compounds where X is OH would make the Skilled Chemist question how rigorously the authors have looked at the different substituents in the definition of Formula III.
135. Compounds of Formula III where X is H and Y is F would be feasible for the Skilled Chemist to make. The Skilled Chemist would see from review articles such as Pankiewicz that there are examples in the literature where people have achieved mono-fluorination at the 2' position. I think it is within the capability of the Skilled Chemist to put a single fluorine at the 2' position whether it be up or down, when the other substituent is H. Similarly, the Skilled Chemist would see from the Pankiewicz review that the synthesis of gemcitabine was known, so compounds where both X and Y are F would also have been within the capability of the Skilled Chemist.
136. Formula III suggests both uridine (where Z' is =O) and cytidine (where Z' is -NH₂) derivatives (see paragraph [0013]). The Skilled Chemist would know that there might be some differences when it comes to synthesising Formula III depending on the identity of Z'. In pyrimidine nucleosides the oxygen at the 2-position of the base (i.e. uracil or cytosine) can react with substituents at the 2'/3'-position (of the sugar) to make another cyclic structure, referred to as an anhydronucleoside. There could be some difference in these reactions between uridine and cytidine derivatives, but it would not put the Skilled Chemist off trying to make them. The same chemistry could

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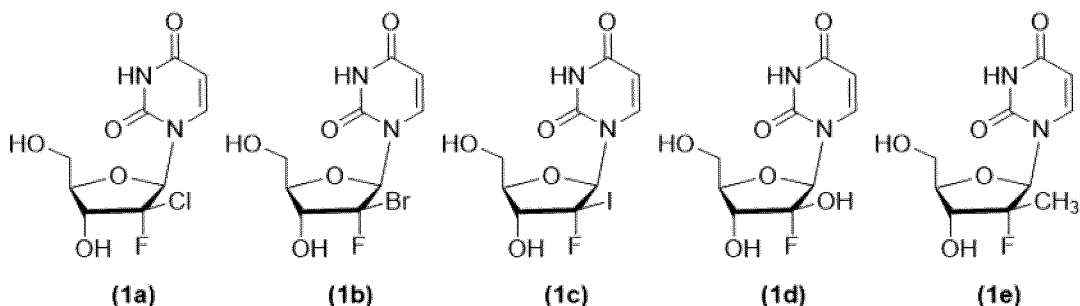
¹⁰ Pankiewicz, K.W., (2000) Fluorinated nucleosides. *Carbohydrate research*, 327(1-2), pp.87-105.

be applied to the synthesis of both uridine and cytidine derivatives and it is also possible to convert uridine into cytidine or vice versa after the event.

137. Other than compounds where X is H or F and Y is F, the Skilled Chemist would not know how to make compounds of Formula III from their CGK and the information in EP 190 (which provides no more information than the structure). Accordingly, in order to synthesise any of them the Skilled Chemist would have had to devise their own synthetic route, which would have been a substantial task with no guarantee of success.
138. Compounds of Formula III where X is Cl, Br, or I would all be difficult for the Skilled Chemist to synthesise. Those kinds of chemistries are not common. Arrangements with two different halogens on the same carbon atom exist in very simple compounds. For example, chloro-fluoro-carbons (“CFCs”) (used as propellants and refrigerants) have been made, but I would not expect the chemistry used to make such simple compounds to translate well to more complex systems, such as nucleosides, as I explain below. In principle, a compound with a chloro-fluoro group could be a viable compound (i.e. it might be stable enough to make) but the Skilled Chemist would think it unlikely they would find known routes to make that type of compound even with extensive searching. Geminal dihalo groups with two different halogens are very uncommon in more complex structures. The Skilled Chemist would have to really dig around in the literature to try to find routes to make such geminal dihalo groups on other molecule types and make a substantial leap to try and apply that chemistry to a nucleoside. Even then, it would be very unclear if the chemistry would work on a nucleoside and it would not be possible to predict which routes would be successful in advance of trying them.
139. Compounds of Formula III where X is Me also present a significant challenge to the Skilled Chemist. The main difficulty is that once there is a methyl at position X, the next group being introduced would be at a tertiary carbon which has specific stereochemical and reactivity issues unlike, and far harder to manage than, those at primary or secondary carbons. This would be particularly tricky with fluorine. Generally, introducing fluorine is quite difficult compared to other substituents because of fluorine’s unusual reactivity. It is still difficult to put fluorine into molecules now and that would have been more the case in 2003. As discussed in paragraph 135 above, putting a single fluorine or two fluorine groups on a primary or secondary carbon atom was possible in 2003; however, there were very few examples of chemistry which allowed the introduction of fluorine at a position where there are tertiary alkyl

groups. So the Skilled Chemist would have appreciated that this tertiary fluoride would have presented a significant challenge to make.

140. Bird & Bird then asked me to consider five example compounds falling within Formula III, 1a-1e as shown below, and comment on whether a Skilled Chemist would be able to propose a synthetic route to make each as at 21 July 2003, on the basis of their CGK, the teachings of EP 190 and any necessary literature searches.



141. The Skilled Chemist would immediately realise that compounds 1a-1c and 1e (as noted above it would be apparent that 1d is not feasible to synthesise and isolate) would be very challenging to make and would require a very substantial amount of research before synthetic routes could be proposed. They would expect to have to do extensive reading of textbooks and literature to approach syntheses this difficult, and having proposed synthetic routes the Skilled Chemist would then have to carry out further work assess which, if any, was successful.
142. In devising routes to attempt their syntheses, there are three general routes the Skilled Chemist would be aware of to make nucleoside derivatives such as compounds 1a-1e:
- (a) **The nucleoside route:** starting with a natural nucleoside which is converted into the desired molecule. In the case of compounds 1a-1e, this involves making substitutions at the 2' position;
 - (b) **The sugar route:** starting from a sugar derivative, such as a ribonolactone (i.e. a ribose with a carbonyl group at the C1 position) sequential substitutions (e.g. chlorinations, brominations, fluorinations or methylations) are made to give the desired ribose derivative, which is then coupled to the base to give the nucleoside derivative;
 - (c) **The glyceraldehyde route:** where the substituted sugar is assembled from smaller building blocks. A ribose is made of five carbon atoms, which can be broken down to a three carbon unit (glyceraldehyde derivatives) and

a two carbon unit (acetate derivatives).¹¹ The acetate derivative could have the necessary chlorine, bromine, fluorine, or methyl substituents and can be coupled together with the glyceraldehyde derivatives, then cyclised to give the correctly substituted sugar derivative. This ribose unit is then coupled with a base to give the nucleoside derivative.

143. The Skilled Chemist would not know from the CGK how to apply these general routes to make these particular compounds, and individual routes to each compound would need to be devised.
144. As all five compounds involve fluorination at the 2'-position and given the particular complexity of the tertiary fluorination in compound 1e, the first step the Skilled Chemist would take would be to review general fluorination methods in March.
145. The Skilled Chemist would have been familiar with the format of March and would have initially searched the index to find sections of the book potentially relevant to fluorination chemistry. They would have identified the following references: "fluorination" (page 2005); "halides, alkyl" (page 2009); "halogenation" (page 2012); "dihalides" (page 1990).
146. In addition to the index, the Skilled Chemist would consult Appendix B: "Classification of reactions by the type of compound synthesized". From this the Skilled Chemist would have identified that the following reaction types, might be of interest: "alkyl halides" (pages 1650-51); "dihalides" (page 1666); "halo carbonyl compounds", "halo ethers and acetals", "halo formic esters", "halohydrins" (page 1670).
147. Having considered the options provided the Skilled Chemist would read specific sections including:
 - (a) 10-68 Halide exchange (Finkelstein reactions);
 - (b) 10-69 Alkyl halides from tosylates etc;
 - (c) 10-70 Alkyl halides from alcohols;
 - (d) 10-72 Halohydrins from epoxides;
 - (e) 12-04 α -halogenation of aldehydes, ketones, esters (lactones);

¹¹ The Skilled Chemist would have been aware of previous syntheses of important nucleoside drugs using this route, such as AZT, see Saunders, J. "Top Drugs: Top Synthetic Routes", Oxford Chemistry Primers, 2000, Oxford University Press, Chapter 9.

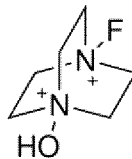
- (f) 12-05 α -halogenation of carboxylic acids;
 - (g) 12-28 Halo-de-metalation;
 - (h) 14-1 Halogenation at alkyl carbon;
 - (i) 14-37 Hunsdiecker reaction;
 - (j) 15-2 Addition HX to alkenes;
 - (k) 15-37 Dihalo-addition to alkenes;
 - (l) 15-39 Hydroxy-chloro-addition to alkenes etc;
 - (m) 16-22 Formation of gem-dihalides from ketones.
148. The Skilled Chemist would be familiar with some of the basic methods for chlorination, bromination and iodination (X = Cl, Br, I) but would be less familiar with the methods for fluorination (X = F). They would note from March that many of the common methods available for introducing Cl, Br and I often do not work well with F.¹² The methods described for fluorination also include reagents that are known to be highly reactive and difficult to work with (e.g. HF, F₂, HOF), as well as more specialist fluorinating agents that the Skilled Chemist is unlikely to be familiar with.
149. The Skilled Chemist would note that aliphatic substitution (March, Chapter 10) could be a useful approach for introducing a fluorine but would be reminded that substitution of tertiary substrates can be problematic, for example noting “*tertiary substrates seldom undergo nucleophilic substitution...*” (March page 433).
150. The Skilled Chemist would need to read some of the literature cited in March but would be aware that most of the work cited was more than 10 years old, so they would also need to read forward. For example, if they found an interesting paper, they could search for papers that cited the earlier work or included similar keywords.
151. Working through the sections identified in paragraphs 145-147 above, to obtain ideas for the more challenging tertiary fluorination chemistry, the Skilled Chemist would consider the following papers of interest and read them:

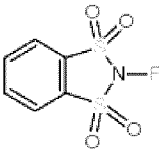
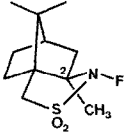
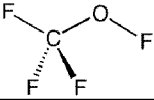
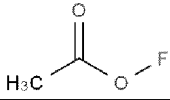
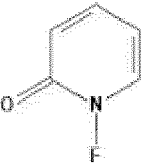
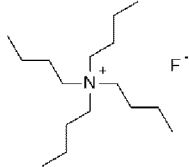
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|----------------|--|
| L tab.43 vol 1 | (a) Davis & Han <i>Tetrahedron Letters</i> . (1991), 32, 1631; |
| L tab.52 vol 2 | (b) Differding & Lang <i>Tetrahedron Letters</i> (1988), 29, 6087; |
| L tab.96 vol 2 | (c) Lerman & Rozen <i>J. Org. Chem.</i> (1983), 48, 724; |

¹² See March pp518-519, 775, 908.

L tab.102 vol 2	(d)	Mann <i>J. Chem Soc Rev</i> (1987), 16, 381-436;
L tab.124 vol 3	(e)	Middleton <i>J. Org. Chem.</i> (1975), 40, 574-578;
L tab.125 vol 3	(f)	Migliorese et al <i>J. Org. Chem.</i> (1979), 44, 1711;
L tab.134 vol 3	(g)	Olah et al. <i>J. Am. Chem. Soc.</i> (1974), 96, 3, 925-927;
L tab.135 vol 3	(h)	Olah et al. <i>J. Org. Chem.</i> (1979), 44, 3872-3881;
L tab.140 vol 3	(i)	Patrick, et al. <i>Can. J. Chem.</i> (1986), 64, 138;
L tab.147 vol 3	(j)	Purrington & Jones <i>J. Org. Chem.</i> (1983), 48, 761;
L tab.156 vol 3	(k)	Rozen & Brand <i>Synthesis</i> (1985), 665;
L tab.157 vol 3	(l)	Rozen & Filler <i>Tetrahedron</i> (1985), 41, 1111-1153;
L tab.158 vol 3	(m)	Rozen <i>Acc. Chem. Res.</i> (1988), 21, 307-312;
L tab.174 vol 3	(n)	Stavber & Zupan <i>Tetrahedron Letters</i> . (1996), 37, 3591;
L tab.179 vol 4	(o)	Tamura et al <i>J. Chem. Soc., Chem. Commun.</i> (1995), 1891-1892;
L tab.185 vol 4	(p)	Tsushima, T.; Kawada, K.; Tsuji, T. <i>Tetrahedron Letters</i> (1982), 23, 1165;
L tab.205 vol 4	(q)	Yoneda <i>Tetrahedron</i> (1991), 47, 5329-5365.

152. From the review of March and the papers referred to above, the Skilled Chemist would identify the fluorination reagents in the table below.

Electrophilic reagents			
	Name	Structure	References
L tab.158 vol 3 L tab.147 vol 3	Fluorine	F ₂	Rozen <i>Acc. Chem. Res.</i> 1988, 21, 307–312; Purrington <i>et al. Chem. Rev.</i> 1986, 86, 997–1018.
L tab.125 vol 3	Hypofluorous acid	HO-F	Migliorese et al <i>J. Org. Chem.</i> 1979, 44, 1711
L tab.140 vol 3 L tab.185 vol 4	Xenon difluoride	XeF ₂	Patrick et al. <i>Can. J. Chem.</i> 1986, 64, 138; Tsushima et al <i>Tetrahedron Lett.</i> 1982, 23, 1165
L tab.174 vol 3	1-fluoro-4-hydroxy-1,4-diazoniabicyclo[2.2.2]octane bis(tetrafluoroborate) (Accufluor)	 2 BF ₄ ⁻	Stavber & Zupan <i>Tetrahedron Lett.</i> 1996, 37, 3591

L tab.43 vol 1	N-fluoro-o-benzenedisulfonimide (NFOBS)		Davis & Han <i>Tetrahedron Lett.</i> 1991, 32, 1631
L tab.52 vol 2	N-fluoro Camphorsultam		Differding & Lang <i>Tetrahedron Lett</i> 1988, 29, 6087
L tab.157 vol 3	Trifluoromethyl hypofluorite		Rozen & Filler <i>Tetrahedron</i> 1985, 41, 1111-1153.
L tab.96 vol 2	Acetyl hypofluorite		Lerman & Rozen <i>J. Org. Chem.</i> 1983, 48, 724
L tab.147 vol 3	1-Fluoro-2-pyridone		Purrington & Jones <i>J. Org. Chem.</i> 1983, 48, 761
L tab.157 vol 3	Perchlorylfluoride	FClO ₃	Rozen & Filler <i>Tetrahedron</i> 1985, 41, 1111-1153
Nucleophilic reagents			
	Name	Structure	References
L tab.135 vol 3	Hydrogen fluoride	HF	Olah et al <i>J. Org. Chem.</i> 1979, 44, 3872-3881
L tab.135 vol 3	HF/pyridine and Pyridinium poly(hydrogen fluoride) (Olah's Reagent)	Approximate composition HF (70%), pyridine (30%)	Olah et al <i>J. Org. Chem.</i> 1979, 44, 3872-3881
L tab.97 vol 2	Potassium fluoride (with or without 18-crown-6 ethers)	KF	Not in March but March points to related work Liotta et al. <i>J. Am. Chem. Soc.</i> 1974, 96, 2250-2252
	Tetraalkylammonium fluorides. For example, tetrabutylammonium fluoride (TBAF)		Widely known fluoride source – i.e. CGK
L tab.167 vol 3	Silicon tetrafluoride	SiF ₄	Shimizu & Yoshioka <i>Tetrahedron Lett.</i> 1988, 29, 4101-4104
L tab.102 vol 2	Sulphur tetrafluoride	SF ₄	Mann <i>J. Chem Soc Rev</i> 1987, 16, 381-436
L tab.134 vol 3	Selenium tetrafluoride	SeF ₄	Olah et al. <i>J. Am. Chem. Soc.</i> 1974, 96, 3, 925-927

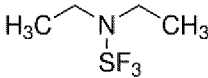
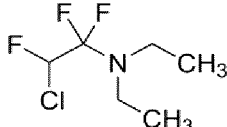
L tab.124 vol 3	Diethylaminosulphur trifluoride (DAST)		Middleton <i>J. Org. Chem.</i> 1975, 40, 574-578.
L tab.102 vol 2	2-Chloro-1,1,2-trifluoroethyl)diethylamine (Yarovenko's reagent and related Ishikawa reagent)		Mann <i>J. Chem Soc Rev</i> 1987, 16, 381-436

Table 1: Reagents for electrophilic fluorination and nucleophilic fluorination

153. The list of primary references identified above provides examples of the types of reagents (both electrophilic and nucleophilic) which have been used for fluorination (although this information would be about 10 years old). These include some obscure reagents which the Skilled Chemist would not have been familiar with from their understanding of other halogenation methods. The Skilled Chemist would see exploratory fluorination chemistry on simple model compounds, with only a few examples of the chemistry being applied to more complex structures. The Skilled Chemist would also appreciate that this is challenging chemistry and many of the reagents described were difficult to handle, toxic, corrosive, unstable or potentially explosive.
154. March and the references cited therein tell the Skilled Chemist basic information about the types of fluorination reagents available, but provide little information about how to apply these various reagents to nucleosides such as compounds 1a-1c and 1e. In order to progress from these basic ideas to their application in complex molecules, the Skilled Chemist would need to undertake a search of the literature, using a database such as SciFinder (as discussed in paragraphs 39 above).

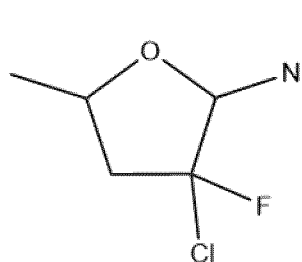
Literature searches

155. Bird & Bird then asked me to perform a literature search of the type the Skilled Chemist would have performed in 2003. Accordingly, I undertook a SciFinder search based on sub-structures of compounds 1a to 1e. Exactly what flexibility the user had in filtering searches around sub-structures back in 2003, I cannot say for certain, but I am confident that decent sub-structure-based searching existed.
156. I have kept a record of the searches I undertook and the results I obtained and I am able to provide them on request. I explain the search process and the results I obtained for each compound in more detail below.
157. For each of compounds 1a to 1e (I included 1d for completeness, but would not expect the Skilled Chemist would have bothered to search for this structure), I picked two sub-

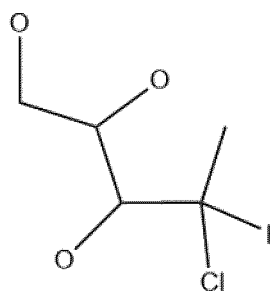
structures to search. The Skilled Chemist would have to pick a sub-structure that is specific enough to include the compound of interest, but general enough that it captures synthetic methods for related compounds, which could potentially be adapted. My experience of the behaviour of chemists in the real world is that chemists with different backgrounds might approach the task of searching in different ways, and it is unclear which of the documents I identify below would also have been found by any particular chemist. For example, a chemist who adopted a more restrictive structure-based search would have potentially not picked up all of the papers I identified, but may have chosen to do such a search to pick up only papers which are particularly relevant. On the other hand, adopting a less restrictive structure-based search would have thrown up more papers but the process of filtering them and identifying the actually relevant papers would have been more laborious. The approach taken by a chemist to a literature search of this type would vary depending on the context, such as whether they were in industry or academia, and the priority given to the project.

158. On any basis, conducting a comprehensive literature review in relation to any of compounds 1a-1e is a very substantial task. In practice, in the context of a challenging synthetic project such as this, the Skilled Chemist would have taken at least two weeks (full time) and possibly much longer just to review March as described above and conduct sub-structure searches and review the papers identified.
159. Focusing on the ribose unit, because this is where the structural variation occurs, I picked a cyclic structure that encompasses the key features of each of compounds 1a to 1e. I also chose an acyclic structure which broadens the search out a bit and would cover some more chemical space in case people had made relevant molecules which were not necessarily nucleosides, such as other sugar derivatives or related molecules. By way of illustration, I include the cyclic and acyclic substructures I searched for in relation to compound 1a below.

Substructures for compound 1a



Ring substructure



Open-chain substructure

160. The ring substructure has a ribose-like ring with chloro, fluoro at the 2'-position and then a bond to a nitrogen atom (that indicates that it could be any standard nucleobase). This would throw up nucleoside type structures. The open-chain substructure covers a broader search space that could include acyclic structures that may arise if routes via glyceraldehyde had been reported. This type of open chain structure would also capture any type of suitably substituted sugar, which could either be attached to a nucleobase or could suggest further chemistry to modify an existing nucleoside. These are general substructures I used for each of compounds 1a to 1e (with different substituents on the 2' position). The ring structure would be tailored towards nucleosides, the open-chain structure is a little broader and would capture chemistries before the base is added.
161. In summary, none of compounds 1a-1e were known in the literature as at 21 July 2003. The Skilled Chemist would have to attempt to devise new synthetic routes to those compounds. I first provide detailed comments on the search results I obtained for each of structures 1a-1e below, and then discuss whether the Skilled Chemist would have been able to make the compounds in the section which follows.

Compound 1a (2'-Cl, 2'-F)

162. For compound 1a, the ring substructure gave no results prior to 21 July 2003.
163. The broader search (with the open chain substructure) gave three references prior to 21 July 2003. There is one reference, from 1986¹³, to sugar compounds that have a fluorine and a chlorine/bromine attached to a sugar intermediate. The same chemistry was also used in another paper from 1989;¹⁴ however, these papers relate to 6-member sugars (pyranose), rather than 5-member sugars (ribose), as found in compounds 1a-1e. The chemistry is used as a method of attaching the sugar to the nucleobase, rather than to isolate a chloro (or bromo), fluoro substituted compound. In fact, the authors report that those molecules with fluorine and chlorine or fluorine and bromine attached to the same atom in the sugar ring are unstable and they immediately replaced either the bromine or the chlorine with a nucleobase, but this chemistry could provide some clue to a researcher to try to make compounds 1a and 1b.

¹³ Seung and Bobek (1986), Tetrahedron Letters, 27(28), 3219-22.

¹⁴ Bobek et al., (1989), Journal of Medicinal Chemistry, 32(4) 799-807.

Compound 1b (2'-Br, 2'-F)

164. There is similarly little in the literature for compound 1b. Again, searching for the ring substructure gave no references describing a nucleoside type compound with bromine and fluorine in the ring before 21 July 2003.
165. Looking at the broader literature with the open chain substructure search gave four references including all the papers from the previous search in relation to compound 1a. There is one additional paper, by Ge et al in 1997¹⁵, describing the synthesis of 2'-deoxy-2'-halo-L-ascorbic acids. This paper shows a potential method for introducing fluorine and bromine at the 2' position of ascorbic acid derivatives, but there would be a large number of unknown steps to undertake to apply this chemistry to compound 1b and the stereochemical outcome of this type of fluorination would not be clear.

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Compound 1c (2'-I, 2'-F)

166. A ring substructure search for compound 1c gives no results. There is no indication that anybody has made any nucleoside type molecules with iodine and fluorine in the ring.
167. A broader open chain substructure search also gave no references.

Compound 1d (2'-OH, 2'-F)

168. As explained at paragraph 134 compound 1d would not be a viable molecule to make. That is clear from the search results, which include lots of patents but no evidence that anybody has ever made any molecule similar to 1d with an OH group and F attached to the same carbon.
169. The Skilled Chemist would find there were very few reports of the synthesis, isolation and characterisation of α -fluoroalcohols of any sort (i.e. alcohols with a fluorine bound to the same carbon). The literature in 2003 indicates such functionality is unstable.¹⁶
16. A Skilled Chemist would recognise that even if α -fluoroalcohols could be prepared it would undergo facile elimination of HF and would therefore be too unstable for the pharmaceutical applications in mind.

Seppelt 1977
L tab.163 vol 3

Cheburkov 2002
L tab.34 vol 1

¹⁵ Ge et al. *J. Org. Chem.* 1997, 62, 10, 3340-3343.

¹⁶ See for example Seppelt, K. *Angew. Chem. Int. Ed. Engl.* 1977, 16, 322-323; Willis, C. *Coord. Chem. Rev.* 1988, 88, 133-202; Cheburkov, Y. and Lillquist, G.J. *J. Fluorine Chem.* 2002, 118, 123-126.

Compound 1e (2'-Me, 2'-F)

170. A ring substructure search for compound 1e gives no results for the synthesis of a 2'-fluoro, 2'-methyl sugar or nucleoside. It does bring up a single paper published in February 2003 by Dai and Piccirilli, which reports the synthesis of a 2'-fluoro, 2'-hydroxymethyl uridine as a minor side product, starting from an epoxide, which is ring-opened to give the fluoro and hydroxymethyl substituted compound.¹⁷ This is an indication that it was possible to make a nucleoside with fluorine and an alkyl group at the 2' position; however, the authors do not make compound 1e. Dai and Piccirilli observe a similar nucleoside side product with a 2'-hydroxymethyl (down) (i.e. -CH₂OH) rather than the desired 2'-methyl(up) group. This compound was a very minor side product in a reaction where the authors were trying to make a compound with a 2'-fluoromethyl (i.e. -CH₂F) and 2'-hydroxy group. This reaction gave the wrong stereochemistry and such a low yield (5%) that it would be unviable as a route to 2'-Me(up), 2'-F(down) nucleosides.

L tab.42 vol 1

171. The open chain substructure search produced 46 results, including the Dai and Piccirilli paper referred to above, but none relating to a 2'-fluoro, 2'-methyl compound. Also within this search was a paper by Bischofberger and Brink in 1975,¹⁸ describing a route to sugars with a fluorine and hydroxymethyl group at the 3-position. The same group also reported in 1977¹⁹ a synthesis of a ribose derivative with a fluorine and a hydroxymethyl group at the 2-position. This allows a fluoro and hydroxymethyl group to be added to the ribose, but gives the wrong stereochemistry (2-F(up), 2-CH₂OH(down)). Even if this chemistry had given the correct stereochemistry, methods would also be required to convert the hydroxymethyl group to a methyl group, and attach the base in a stereoselective manner, neither of which are addressed in the Bischofberger and Brink papers. All these examples are long synthetic routes, and relate to sugars, not nucleosides, but the Skilled Chemist might consider they contained hints about whether there might be a way to make molecules with a fluorine and potentially methyl attached to the same carbon.

L tab.25 vol 1

L tab.28 vol 1

172. In 1986, Shu Yang at Merck, Sharp and Dohme made a tertiary fluoride derivative of inositol²⁰ with a hydroxymethyl (-CH₂OH) and fluoro substituents at the same carbon. It is important to note that inositol derivatives are a long way removed from compound

L tab.204 vol 4

¹⁷ Dai and Piccirilli (2003), *Organic Letters*, 5(6), 807-810.

¹⁸ Bischofberger, Brink and Jordaan (1975), *Journal of the Chemical Society, Perkin Transactions 1*, (23), 2456-60.

¹⁹ Brink, De Villiers, and Jordaan (1977), *Carbohydrate Research*, 54(2), 285-91.

²⁰ Yang, Beattie and Shen (1986) *Synthetic Communications*, 16(2), 131-8.

1e, in that these compounds are not nucleosides (i.e. there is no nucleobase), have six membered rings not five membered ones, and are carbocyclic (i.e. do not have an oxygen in the ring). The compound the authors report also does not have a methyl group and fluoro group on the same carbon. However, the authors managed to synthesise a cyclic structure, with some hydroxyl groups and one of the carbons substituted with a fluorine and a hydroxymethyl group. Similarly, in 1998, there is a paper by Zhang et al,²¹ who also introduce a tertiary fluoride into a five membered ribose-like ring.

Could the Skilled Chemist synthesise compounds 1a-1e?

173. Bird & Bird have asked me to comment on whether the Skilled Chemist would be able to synthesise compounds 1a-1e as at 21 July 2003, on the basis of the CGK and the literature identified in the searches above, and how difficult any such synthesis would have been.
174. As I explained above, in 2003 there were no papers or patents that describe the actual synthesis of any of compounds 1a-1e, so the Skilled Chemist looking at those searches would not have a clear route to make any of compounds.
175. Each of the general routes referred to in paragraph 142 could also, in principle be used to try to make compounds 1a-1c and 1e (1d being impossible to synthesise and isolate); however, it would be challenging, and each of the possibilities I consider below would require persistence on the part of the Skilled Chemist if they were to have any chance of succeeding.
176. The literature search I have discussed in paragraphs 159-172 above adds little beyond these general routes. These papers only provide a few scratchy examples with hints about how it might be possible to introduce fluorine substituents alongside chlorine, bromine or methyl substituents in sugars or related molecules.
177. As I set out above, the Skilled Chemist has multiple possible routes, but they all amount to clutching at straws. There is no clear route, either from the literature or the CGK to make any of compounds 1a-1e and the Skilled Chemist would not be confident that they could make any of those compounds. The Skilled Chemist would have to develop two synthetic approaches: one aimed towards compounds 1a to 1c, and the other for compound 1e, because the best route for one could not simply be expected to work for the other. Compounds 1a, 1b and 1c present similar challenges so would likely be

²¹ Zhang et al., (1998) *Tetrahedron: Asymmetry*, 9(1), 141-149.

targeted using a common strategy. As I have already explained compound 1d is not even worth considering.

178. For the Skilled Chemist to be confident, they would need, for example, to see in the literature that some of compounds 1a-1c and 1e had been made but with a different nucleobase (which was not the case here). Then they would expect in principle to be able to adapt such syntheses to make these compounds even if they had not been made before. No such examples existed in relation to compounds 1a-1e.
179. As the Skilled Chemist has no examples of a nucleoside with the correct 2'-substitution, still less with the particular stereochemistry, they will not be at all confident of success. It is likely that any synthesis of these compounds 1a-1c and 1e would require some creativity or inventiveness in order to succeed, if they were to succeed at all.
180. A Skilled Chemist would not be able to identify which route, of those discussed above, was most viable. There is no obvious route to those compounds. The Skilled Chemist is very much going into new territory with molecules that have not been synthesised before and more so where there are few examples of that kind of functionality in related molecules. So, this is a research project for the Skilled Chemist.
181. The Skilled Chemist would also need to know what starting materials are available, particularly for the glyceraldehyde route where it is unclear if the appropriately substituted acetate derivatives were available (either commercially, or through literature syntheses), otherwise it could significantly increase the complexity of the synthetic route.
182. There are also a range of particular aspects which make these syntheses challenging. There are multiple stereochemistry issues which the Skilled Chemist would have to address (which could involve separations at multiple stages). Unless the Skilled Chemist could find a way of controlling the stereochemistry at the 2'-position (which would be very challenging and is not disclosed in the literature discussed above), the nucleoside route can result in two diastereomers being formed. Only one of these diastereomers in each case corresponds to compounds 1a-c or 1e, so the unwanted diastereomer would have to be separated. The desired diastereomer may be the minor product or in some cases exclusively the wrong diastereomer may be formed.
183. Controlling the stereochemistry at the 2'-position is also a major issue in the sugar route. The sugar route is further complicated by needing to control the stereochemistry for the addition of the base at the 1'-position (i.e. the formation of the

α or β -anomer). Lack of control of stereochemistry at the 2' would result in the formation of 2 diastereomers which would need to be separated. Similarly addition of the base at the 1'-position would result in the formation of an additional 2 diastereomers, only one of which corresponds to the target compound.

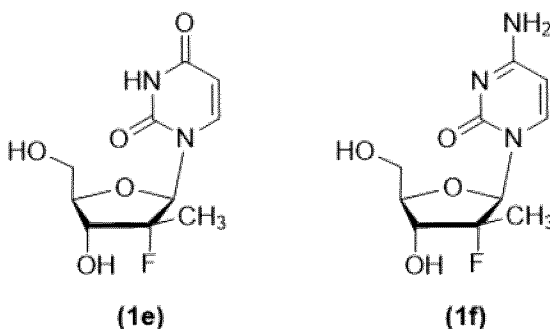
184. Finally, the glyceraldehyde route also requires the formation of the 3'-stereocentre, 2'-stereocentre and 1'-stereocentre. As a result, unless the Skilled Chemist can find methods of controlling the stereochemistry at these centres, two new diastereomers will be formed in each step, one of which will need to be separated, one discarded and the correct diastereomer progressed to the next step. In each case it is not clear in advance how much (if any) of the desired diastereomer would be generated in the reactions, so the amount of the desired product may be very low or none.
185. Unless the Skilled Chemist is assumed to have made molecules similar to compounds 1a-1e before (and I do not think they necessarily would have done so as part of their background or CGK) they would not know how the compounds stand up to the conditions of these different synthetic routes. Potentially there could be all kinds of problems in synthesising those molecules. The compounds or intermediates could be unstable, or could eliminate HF, HBr or HCl. Adding to the uncertainty, the Skilled Chemist is not sure which route to go with. The general routes sketched out above are routes that the Skilled Chemist could have come up with based on what was known about nucleoside and nucleotide chemistry in 2003, but in order to successfully synthesise the target compound, the Skilled Chemist would have to do quite a lot of research and effectively invent new syntheses, because their literature searches would not turn up a synthesis that they could easily adapt.
186. Bearing in mind the Skilled Chemist's level of skill and experience, and in the light of the searches I have identified above, it is quite possible that the Skilled Chemist could fail to achieve the synthesis of any of these molecules, particularly compound 1e, which is the most challenging target. If I assume that the Skilled Chemist were able to make the compounds, it is my initial view that if they were really fortunate (by which I mean they selected a research route that works first time and their syntheses worked as planned), they might be able to make compounds 1a-1c, or 1e in about six months per compound, but the Skilled Chemist would need to plan for up to three years to make each compound.

Effect of substituents on the nucleobase

187. Bird & Bird further asked me to comment on whether my answers in relation to compounds 1a-1e above would differ for compounds of Formula III with differing Z' substituents (i.e. uridine vs cytidine derivatives) or differing Z substituents (i.e. alkyl or halogen substitution at the 5-position).
188. The nature and substitution of the base is only likely to substantially change the difficulty of the synthesis where the 2' substituents are added at the end (i.e. the nucleoside route). Where a Skilled Chemist wanted different substitution patterns on the base, or different types of base, a nucleoside route may not be the best, because one approach may not work best with all types of base. The sugar route and glyceraldehyde route are more convergent, in that the base and the sugar are made separately and joined at a later stage. This could give more flexibility to make a series of nucleosides with different modifications on the base.
189. If the Skilled Chemist could make compounds 1a-1c, and 1e, they would be comfortable making the equivalent cytidine molecules too. There are ways to convert uridine into cytidine (and vice versa).

Could the Skilled Chemist synthesise 2'-Me(up), 2'-F(down) nucleosides

190. After I had provided my high-level views in the section above, Bird & Bird asked me to consider in greater detail a Skilled Chemist being tasked with synthesising compounds 1e and 1f (below), as at 21 July 2003, in the context of a nucleoside drug development project.



191. Bird & Bird asked me to comment on the synthetic route or routes the Skilled Chemist would have attempted, based on the CGK, EP 190, and the results of any necessary literature searches as at 21 July 2003.
192. When tasked with synthesising compounds 1e and 1f, the Skilled Chemist would go through the following steps:

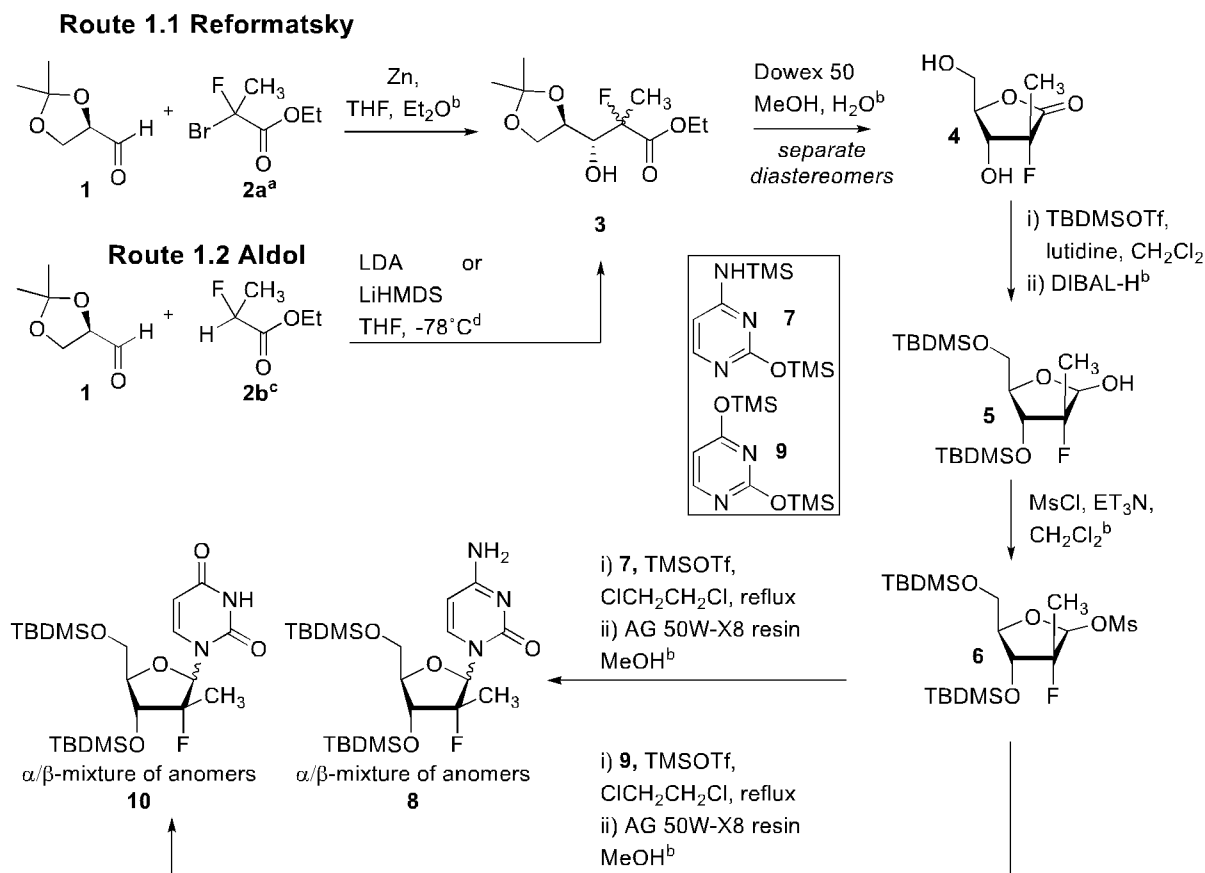
- (a) Conducting literature searches for the target compounds and related sub-structures, as discussed in paragraphs 155-172;
 - (b) As there were no previous syntheses of the target compound in the literature, the Skilled Chemist would then consider the general types of synthetic approach which have been used in relation to similar compounds or classes of compound, as discussed in paragraphs 174-186;
 - (c) Propose a range of possible synthetic routes to the target compounds, based on the available literature;
 - (d) Conduct specific literature searches to try to identify availability of precursors or intermediates and reaction conditions from analogous reactions in the literature which could be used as a starting point to develop steps in the proposed synthetic routes. I call these “**Analogy Searches**” and I refer to them repeatedly below;
 - (e) Assess and prioritise those routes to determine which route to attempt first in the laboratory along with fallback options for if the initial route proved unsuccessful.
193. In my view there are five overarching synthetic routes the Skilled Chemist might have proposed as potential routes to compounds 1e and 1f, which I explain in detail below. I have grouped the routes based on the overall synthetic strategy: (1) the glyceraldehyde route; (2) the electrophilic fluorination route; (3) the nucleophilic fluorination route. In the schemes below, I cite the papers which the Skilled Chemist’s Analogy Searches may have identified as suggesting conditions which could have been adapted in relation to each step or potential precursor.

Glyceraldehyde routes

194. The first two routes are based on the synthesis of gemcitabine (which the Skilled Chemist would have identified from Pankiewicz) and rely on what is called the Reformatsky reaction and a variant of that called an aldol reaction. The key feature of these routes is that the fluorine is already in the reagents at the start.

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196. The glyceraldehyde routes are set out in **Scheme 1** below. As explained further below, the two routes differ only in the chemistry used to form compound 3.



L tab.178 vol 4^a Synthesis of starting material suggested based on Takeuchi et al, *Chem Pharm Bull*, 1990, 38(9), 2404-2408

L tab.73 vol 2^b Conditions adapted from Hertel et al *J. Org. Chem.*, 1988 53(11), pp.2406-2409

L tab.79 vol 2^c Synthesis of starting material suggested based on Ishihara et al. *Tetrahedron Lett.* 1995, 36(45) 8267-8270

L tab.79 vol 2^d Conditions adapted from Ishihara et al. *Tetrahedron Lett.* 1995, 36(45) 8267-8270 and Wiegel *J. Org. Chem.* 1997, 62, 6108-6109

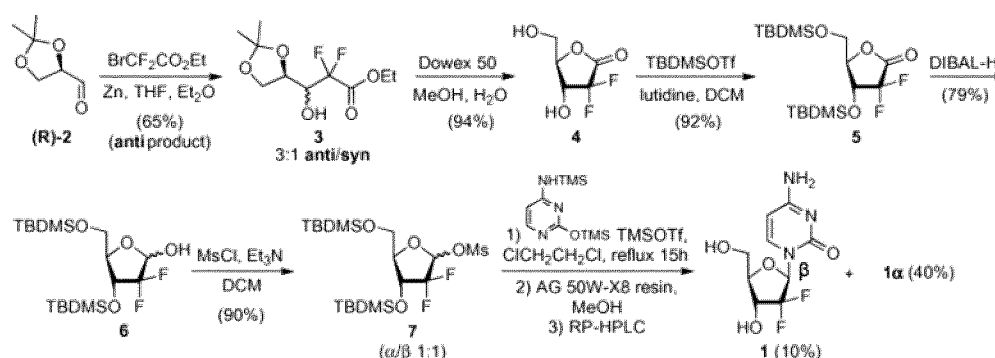
Scheme 1: proposed Routes 1.1 and 1.2 to compounds 1e and 1f

Route 1.1 - Reformatsky

L tab.73 vol 2 197. Route 1.1 is based on the gemcitabine synthesis first published in 1988 by Hertel.²² It is comparatively well preceded, but it is also quite long and laborious.

²² Hertel, L.W., Kroin, J.S., Misner, J.W. and Tustin, J.M. *The Journal of Organic Chemistry*, (1988) 53(11), pp.2406-2409.

198. The Hertel gemcitabine synthesis is shown in **Scheme 2** below.



Scheme 2: Hertel gemcitabine synthesis

199. It is clear from Scheme 2 that the Skilled Chemist could reasonably consider adapting this early gemcitabine route to try to make compounds 1e and 1f. This would require considering whether it might be possible to run this synthesis starting with a building block with a methyl and fluoro group, in place of the difluoro building block (BrCF₂CO₂Et) i.e. ethyl 2-bromo, 2-fluoropropanoate (compound 2a, Scheme 1).²³ However, the difficulty stems from the fact that all of the chemistry would be unknown with the new starting material and the effects of having the methyl rather than the additional fluoro group in the synthesis are unclear. Also, there may be six or seven steps at least along this route, all of which have to be navigated with different chemistry to the published synthesis.

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200. The next question is whether the stereochemistry can be controlled. The first step of this synthesis creates a new stereocentre at what is going to be the 3'-position in the final nucleoside. The Skilled Chemist needs to be confident this will have the correct stereochemistry, or they will have to separate the diastereomeric products, which could be challenging.

201. Hertel et al. would give the Skilled Chemist some confidence that a Reformatsky reaction using ethyl 2-bromo, 2-fluoropropanoate would give the desired stereochemical outcome as was reported using BrCF₂CO₂Et. This confidence is based on the Felkin-Anh model (described in Hertel) which is used to rationalise the stereochemical outcome of the attack of BrCF₂CO₂Et (or BrC(CH₃)FCO₂Et) on aldehyde (R)-2 (Scheme 2). As shown in **Figure 7** below, the zinc enolate of

²³There is precedent for the synthesis of that compound: Takeuchi, et al, *Chem Pharm Bull*, 1990, 38(9) 2404-2408.

$\text{BrCF}_2\text{CO}_2\text{Et}$ will preferentially attack from the side with the least sterically hindered face of the carbonyl group. Hertel et. al. reported this reaction gave a 3:1 ratio in favour of the desired stereochemistry at the 3-position. The change of one fluorine for a methyl group is unlikely to substantially change this, so the Skilled Chemist would have confidence that this step would probably give the correct stereochemistry 3-position as the major product.

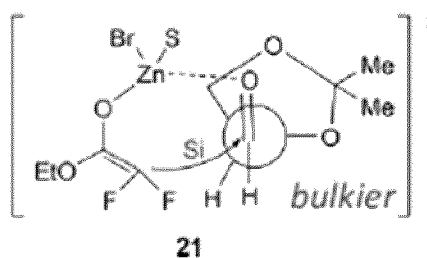


Figure 7: Felkin-Anh model from Hertel et al

202. Although it would be possible to have some confidence in the stereochemistry of what will become the 3' stereocentre, it is not clear what the stereochemistry at the 2' position will be. In the gemcitabine synthesis, there are two fluorines at this position, so there is no stereocentre. Once the methyl group is introduced, this adds an additional level of complexity. In 2003 the Skilled Chemist would not have known of any methods for controlling the 2' stereochemistry in this route. Because, in this case, two stereocentres are created in one step, this can potentially give rise to a mixture of up to four diastereomers, which could be extremely difficult to separate. It would not be clear in advance how much (if any) of the desired diastereomer would be generated, so the amount of the desired product may be very low or none.
203. Another challenge is attaching the nucleobase to the ribose unit. Two possible isomers (α - and β -anomers) can be formed at this stage, of which only the β -anomer corresponds to compounds 1e and 1f. In the Hertel synthesis, the base is introduced by a method called the silyl-Hilbert-Johnson reaction. It is quite a complicated reaction that has been developed for nucleosides, that normally would have an acyl protected hydroxyl at the 2'-position. The acyl group is capable blocking the α -face (lower face) of the ribose ring, which can favour the formation of the β -anomer of the nucleoside (referred to as neighbouring group participation). For compounds 1e, 1f and gemcitabine, there is no neighbouring group participation. The key thing the Skilled Chemist would have noticed is that the Hertel synthesis only gives 10% of the required stereoisomer and 40% of the undesired α -anomer. As a result, the Skilled

Chemist would expect this reaction may well give a mixture of products, with the desired β -anomer as the minor component.

204. The silyl-Hilbert-Johnson reaction can be used to attach either a uracil or a cytosine base, to give compounds 1e and 1f respectively. Alternatively, cytidine can be converted to uridine (or vice versa) after the ribose is attached. But in either case, attaching the base presents the same stereochemical challenge.

Route 1.2 - Aldol

205. As I explained above, the Skilled Chemist could also consider a variant of Route 1.1, using aldol chemistry instead of Reformatsky chemistry for the first step in Scheme 1. This requires starting with ethyl 2-fluoropropanoate (compound 2b in Scheme 1), which would be deprotonated using lithium diisopropylamide (LDA) or related bases to make a lithium enolate. The enolate then attacks the aldehyde to give compound 3. There was precedent for those reagents and reactions, so the Skilled Chemist could consider this as a possible alternative to the Reformatsky reaction in Route 1.1.
206. Aside from this step, Route 1.2 is identical to Route 1.1 and shares the same challenges.
207. In summary, the Skilled Chemist might reasonably propose Routes 1.1 and 1.2 based on the Hertel gemcitabine synthesis; however, there are multiple stumbling blocks along the way that could have made it very difficult for the Skilled Chemist to successfully complete these routes (or prevented success entirely).

Electrophilic fluorination routes

Route 2 - Electrophilic fluorination of ribonolactone

208. The next set of routes the Skilled Chemist might consider involve introducing the 2' fluorine at a later stage in the synthesis. The Skilled Chemist in July 2003 would be aware that both electrophilic and nucleophilic fluorinating reagents were available (see paragraph 152, above).
209. One commercially available electrophilic fluorination reagents in 2003 was Selectfluor, which allows fluorination of molecules such as ketones which have an acidic α -proton and can deprotonated to form enolates. Analogy Searches for electrophilic fluorination could have identified a paper by Banks et al which provides an example of creating tertiary fluorides using this methodology prior to 21 July

L tab.19 vol 1

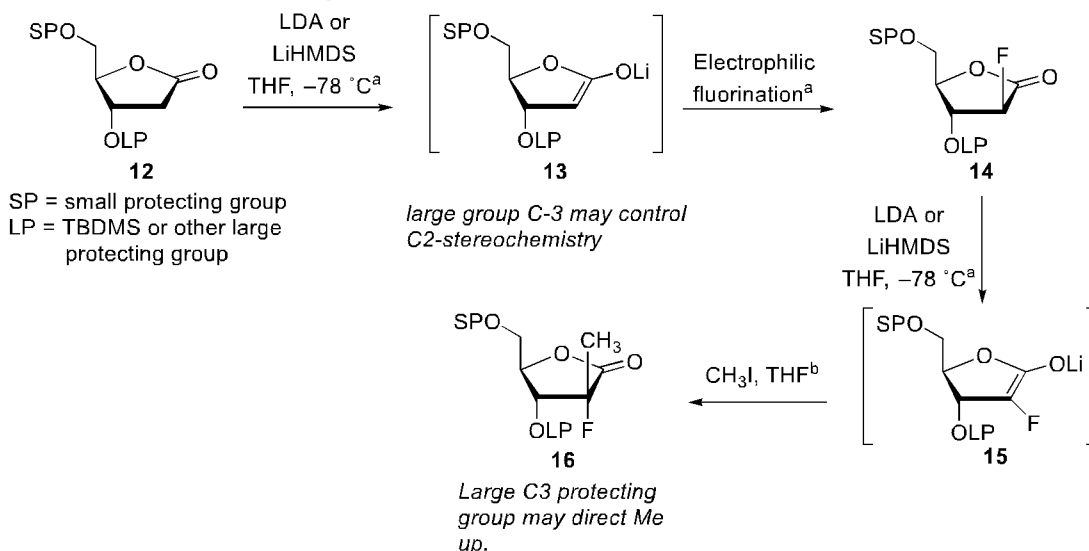
25.
Shibata 2000
L tab.165 vol 3

Shibata 2001 L
tab.166 vol 3

2003.²⁴ This approach can also be used to fluorinate silyl enol ethers, including at tertiary carbons. Analogy Searches could have identified a paper by Shibata et al which demonstrates this approach.²⁵ However, both these examples relate to very simple molecules and not nucleosides. The Banks paper shows the fluorination of very simple β -dicarbonyl molecules, while the Shibata paper related to fluorination of simple cyclic ketone compounds.

210. The Skilled Chemist may have proposed a route starting from a ribonolactone as shown in **Scheme 3** below. Alkylation of enolates and fluorination using Selectfluor were well known in 2003; however, applying this chemistry to the synthesis of substituted riboses or nucleosides was unknown.

Route 2: Lactone electrophilic fluorination



L1/19
L2/51

L3/165

^a Using Selectfluor, NSFI or related reagents. Initial conditions suggested based on Banks et al. *J. Chem. Soc., Chem. Commun.*, 1994, 343-344; Dehoux et al. *Eur. J. Org. Chem.* 2001, 1105-1113. Alternative conditions using silyl enol ethers could be suggested based on Shibata et al. *J. Am. Chem. Soc.* 2000, 122, 10728-10729.

^b Conditions for methylation of enolates would be part of the CGK.

Scheme 3: proposed Route 2 to compounds 1e and 1f

211. The first step would be to prepare the protected 2'-deoxy ribonolactone (compound 12, Scheme 3). The Skilled Chemist would not have been clear about what protecting group to use but they would consult Greene and Wuts: *Protective Groups in Organic Synthesis*. In the literature there are a range of protecting groups that are commonly

²⁴ See for example Banks et al. *J. Chem. Soc., Chem. Commun.*, 1994, 343-344.

²⁵ See for example Shibata et al. *J. Am. Chem. Soc.* 2000, 122, 10728-10729; Shibata et al. *J. Am. Chem. Soc.* 2001, 123, 7001-7009.

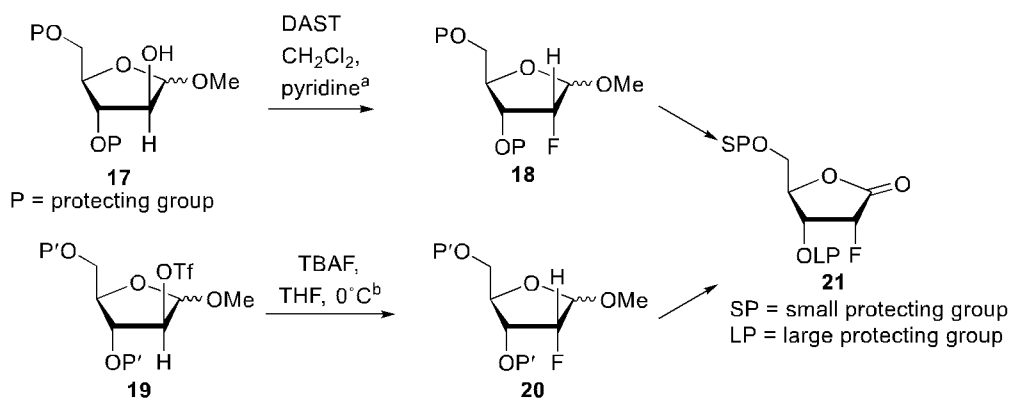
used for these kinds of systems and the Skilled Chemist would want to vary the protecting groups in the hope of getting some stereocontrol.

212. The proposal of deprotonating compound 12 (Scheme 3 above) and forming a lithium enolate (intermediate 13) and then fluorinating at carbon 2 is reasonable; however, there are significant challenges. If there was a bulky protecting group at carbon 3, most likely Selectfluor would preferentially fluorinate from the upper face (β -face), to give compound 14, although the Skilled Chemist would not have a high degree of confidence in this prediction. Then if compound 14 is deprotonated again, it could be methylated using methyl iodide, to give 2-methyl, 2-fluoro ribonolactone, compound 16. The Skilled Chemist would hope that the methyl group also goes on from the β -face, as that would give the correct stereochemistry at the 2-position. If it did not give the correct stereochemistry, the Skilled Chemist would have to go back to the start and methylate first and then fluorinate, in the hope of getting the correct stereochemistry.

L tab.126 vol 3

213. The Skilled Chemist could have also considered alternative methods to synthesise intermediate 14 (Scheme 3, above). An Analogy Search could have identified methods to prepare fluorinated sugars 18 and 20 in **Scheme 4** below.²⁶ Compound 20 was prepared by displacing a triflate group (compound 19) with a fluoride anion. Compound 18 was prepared using a DAST reaction to fluorinate Compound 17. Either of compounds 18 or 20 could have been proposed to feed into Route 2, by conversion to compound 21, which could be used in place of compound 14 (see Scheme 3 above). The stereochemistry of compound 21 is not important, because it would be subsequently deprotonated to give a planar enolate prior to methylation (see Scheme 3, above). This presents the Skilled Chemist with a range of alternative options, but all would involve challenging fluorination reactions, stereochemical issues and the potential for eliminations and other side reactions. The Skilled Chemist would not have had any guidance from the literature about which (if any) of these options might work.

²⁶ See Mikhailopulo et al Synlett 1996, 2, 173-174 and Thomas et al Nucleosides, Nucleotides & Nucleic Acids 1994, 13, 309-323, respectively.



L3/126
L4/183

^a Conditions suggested based on Mikhailopulo et al *Synlett* 1996, 2, 173-174.

^b Conditions suggested based Thomas et al *Nucleosides, Nucleotides & Nucleic Acids* 1994, 13, 309-323.

Scheme 4:Route 2 - alternative fluorination options

Nucleophilic fluorination routes

Nucleophilic fluorination reagents

214. As I explained in paragraph 152 above, the other main class of fluorinating reagents known to the Skilled Chemist in 2003 were nucleophilic reagents, which deliver a fluoride anion (i.e. F⁻) in some form. Of these types of reagents, the most well-known as at 21 July 2003 were Olah's reagent, DAST and Deoxofluor.
215. Olah's Reagent is used to overcome some of the difficulties encountered with HF. It is possible to use HBr, HCl and HI as reagents, but HF is a very corrosive, toxic, dangerous material either as a gas or in aqueous solution. It attacks and degrades the glass, so must be used in special reaction vessels. Olah's reagent is HF mixed with pyridine in certain ratios to give pyridinium poly(hydrogenfluoride). That was being widely used at the time to fluorinate alkenes, to convert alcohols to fluorides, and to open epoxides to make fluorides.
216. The other reagent which was widely used in the 1990s and early 2000s was DAST, and a derivative of DAST called Deoxofluor which was very similar, more stable and slightly more reactive. DAST and Deoxofluor were typically used to convert alcohols to fluorides, but they could also convert ketones to difluoromethylene compounds. The Skilled Chemist looking through the literature at these nucleophilic reagents would find lots of examples of making primary and secondary fluorides but very few examples of tertiary fluorides, and would recognise that generating tertiary fluorides had significant additional complexities compared to primary or secondary fluorides.

Tertiary fluorination

217. There are limited examples of tertiary fluorination using the reagents discussed above in the literature prior to 21 July 2003. Analogy Searches for examples of tertiary fluorination would have identified several papers, which I discuss below.

218. A paper by Back et al. in 1999 provides an example of fluorination of an alkene in a steroid-type molecule to make a tertiary fluoride.²⁷ Annen et al in 1982²⁸ published an example of ring-opening epoxides using Olah's reagent. Parish made similar tertiary fluorides from hydroxyl groups using Olah's reagent in 1980.²⁹ However, it is important to note that these examples do not relate to sugars or nucleosides.

219. There were only a couple of examples of using DAST for tertiary fluorinations to give the Skilled Chemist any indication that it might be possible. A paper by Wachtmeister in 1999³⁰ is the only example which relates to a nucleoside-like target. A tertiary alcohol at carbon-4 of a carbocyclic sugar analogue is converted (with inversion of stereochemistry) to the tertiary fluoride using Deoxofluor in dichloromethane with pyridine. The carbocyclic unit is then coupled with an adenine base to give a carbocyclic nucleoside analogue. The authors also attempted the same fluorination using DAST, but obtained a lower yield and a mixture of products. The yield using Deoxofluor is an improvement but still not great.

220. Another example is a publication by Yang in 1986³¹ which a 6-membered carbocyclic inositol derivative is fluorinated using DAST. Unlike in the Wachtmeister example, the reaction gives retention of configuration and as with the example discussed in paragraph 172 above, this relates to a carbocycle, not a sugar or a nucleoside.

221. Both the Yang and Wachtmeister papers relate to fluorination of carbocycles and a Skilled Chemist would not know if they could be applied to a ribose derivative. Ribose and carbocycles can have different conformations and stereoelectronic effects which could affect the outcomes of fluorination reactions. Furthermore, the carbocyclic substrates in Yang and Wachtmeister have different substituents which may be differently protected, which can affect the reactivity. All of these differences make it difficult to predict if the chemistry in these papers could be successfully applied to the synthesis of compounds 1e or 1f. Even if the reaction worked, the yields may be poor

²⁷ Back, T.G. et al *J. Org. Chem.* 1999, 64, 5494-5498.

²⁸ Annen et al. *Liebigs Ann. Chem.* 1982, 966-972

²⁹ Parish et al *J. Org. Chem.* 1980, 45, 4034-4037

³⁰ Wachtmeister et al *Tetrahedron* 1999, 55, 10761-10770

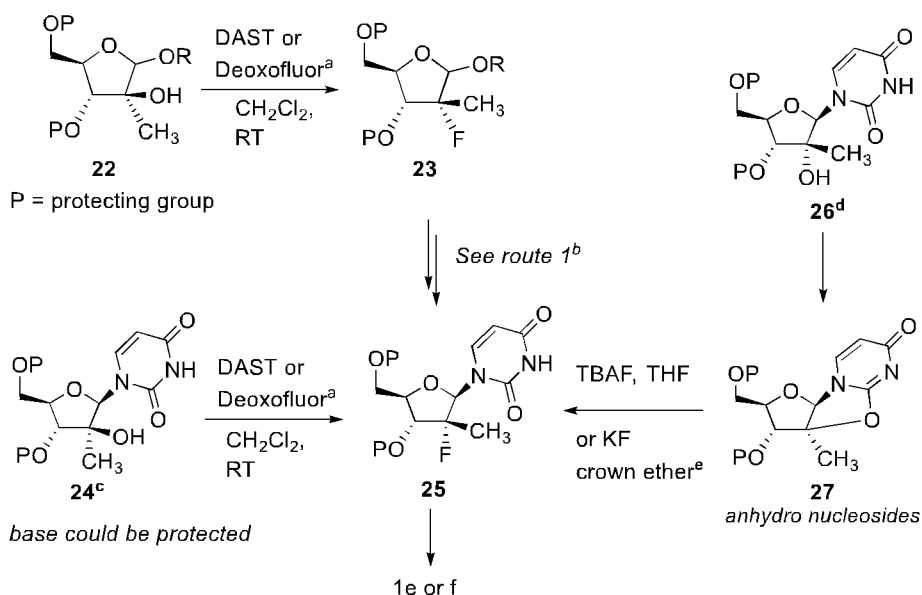
³¹ Yang, Beattie and Shen (1986) *Synthetic Communications*, 16(2), 131-8.

and it is not clear what the stereochemical outcome would be. All these examples suggest is that there is some possibility of converting certain tertiary alcohols into a tertiary fluorides using DAST or Deoxofluor.

Route 3.1 - Nucleophilic fluorination of tertiary alcohols

222. As discussed above, there was some limited precedent for fluorination using DAST or Deoxofluor at tertiary carbons before 21 July 2003 (although there was no precedent for tertiary fluorination of a relevant sugar or nucleoside substrate). **Scheme 5** below illustrates two alternative options which the Skilled Chemist might have proposed for synthesising compounds 1e or 1f by fluorinating a tertiary alcohol using DAST or Deoxofluor.

Route 3.1 Nucleophilic fluorination tertiary alcohols



L3/170

^a Conditions for fluorination suggested based on Singh, R. P. and Shreeve, J.M. *Synthesis* 2002, 17, 2561-2578 and papers cited therein.

^b Coupling 23 with a base using method discussed in Route 1.1/1.2.

L2/106

^c Synthesis of compound 22 is described in Matsuda et al. *Chem Pharm Bull*, 1988, 36, 945-953.

L2/69

^d Synthesis of compound 26 is described in Harry-O'Kuru et al. *J. Org. Chem.* 1997, 62, 1754-1759.

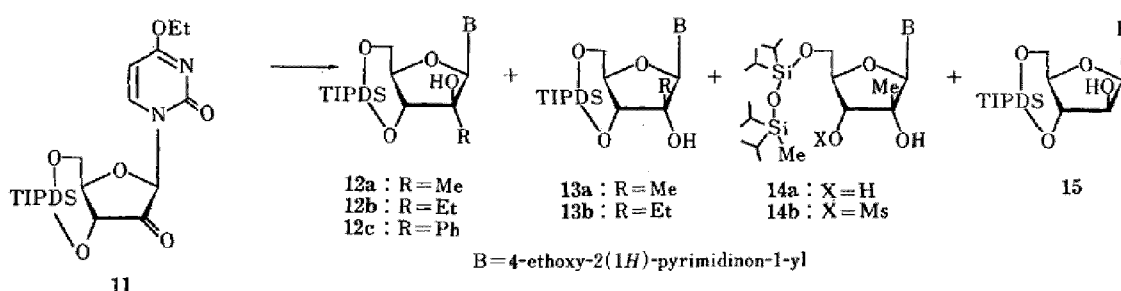
L3/120

^e Conditions suggested based on Mengel et al. *Angew. Chem. Int. Ed. Engl.* 1978, 17(7), 525.

Scheme 5: proposed Route 3.1 to compounds 1e and 1f

223. The first option is to convert a nucleoside with a methyl group and a hydroxyl group at the 2' position (i.e. compound 24, Scheme 5) through to the protected 2'Me, 2'F nucleoside with DAST or Deoxofluor. If the Skilled Chemist could make suitable 2'-OH, 2'-Me substituted nucleosides, they could then try fluorination using reagents such as DAST or Deoxofluor.

224. There is literature precedent for the starting material needed for this route. Matsuda 1988³² published a number of papers where he took nucleosides and made molecules with the methyl group facing up and the hydroxyl group facing down, shown below in **Scheme 6**. In fact, his method was not selective and gave both stereoisomers and several side products. Potentially that is not so bad if the Skilled Chemist wanted to try both starting material stereoisomers (i.e. Me(up) and Me(down)) – as the outcome of the fluorination in terms of stereochemistry was also unclear; however, relying upon this synthetic pathway to the starting materials would still require potentially difficult and time consuming separation and purification of each stereoisomer, prior to attempting the fluorination.



Scheme 6: Matsuda et al. synthesis of 2'-OH, 2'-Me nucleosides

- L tab.170 vol 3 225. A review of Deoxofluor and DAST fluorination chemistry³³ by Singh and Shreeve was available in July 2003, which a Skilled Chemist looking for fluorination conditions using those reagents would have identified and consulted. This review indicates that the two reagents have similar fluorination reactivity and suggests Deoxofluor is more stable, which may be better for larger scale synthesis. Singh and Shreeve report that the standard conditions for fluorination of alcohols with DAST or Deoxofluor involves the use of dichloromethane (CH₂Cl₂) as a solvent. Reactions are normally carried out at room temperature, but there are some examples where fluorination can proceed at lower temperature (down to -78°C). In some examples, pyridine is added to the CH₂Cl₂ solution. Other solvents were also used including ethyl acetate (EtOAc).³⁴ The review provides many examples of fluorination of primary or secondary alcohols
- L tab.65 vol 2

³² Matsuda et al. *Chem Pharm Bull*, 1988, 36, 945-953.

³³ Singh, R. P. and Shreeve, J.M. *Synthesis* 2002, 17, 2561-2578.

³⁴ For example Hallet et al *J. Org. Chem.* 2000, 65, 16, 4984-4993.

L tab.105 vol 2
L tab.190. vol 4

including nucleosides³⁵ and also indicates fluorination of tertiary alcohols is possible, although only providing a single example.³⁶

L tab.83 vol 2

226. The paper by Wachtmeister et al (discussed at paragraph 219 above) provides the sole example in the Singh and Shreeve review of introducing a tertiary fluoride into a nucleoside-type structure. Wachtmeister et al added a tertiary alcohol (at the 4-position of a carbocyclic sugar analogue) to Deoxofluor and pyridine in CH₂Cl₂ at –78°C, then stirred under argon for 18h at room temperature to give a mixture of five products, including the desired product which was isolated in 43% yield. Notably, Wachtmeister found that using DAST instead of Deoxofluor for this fluorination gave poor results and lower yield (25%). Although not included in the Singh and Shreeve review, further Analogy Searches for fluorination conditions using DAST or Deoxofluor would also have identified a report of a general procedure for fluorination of tertiary cyclobutanols (not sugars or nucleosides) by Kirihara et al.³⁷ The authors state that a solution of tertiary cyclobutanol in CH₂Cl₂ was added to DAST at room temperature under an inert atmosphere, and the mixture was stirred for 30 min leading to fluorinated products isolated in good yields (72 & 91%). Yang et al report attempting the fluorination of an inositol (i.e. a 6-member carbocyclic polyhydroxyl compound, see paragraph 172 above) secondary alcohol under what were considered standard conditions (CH₂Cl₂, 0-25°C) but this did not proceed, so they used more “drastic” conditions (toluene, 70-80°C) instead to afford the fluorinated product in 86% yield.³⁸ Yang et al. later report fluorination of an inositol tertiary alcohol in dry toluene, using DAST added over 10 min, then stirred at 60-65°C for 30 min (83% yield).³⁹ Yang also use similar conditions (toluene, higher temperature) to fluorinate an inositol secondary alcohol.⁴⁰

L tab.201 vol 4

L tab.204 vol 4

L tab.203 vol 4

227. In July 2003, based on the references cited above, identified from review articles and Analogy Searches, the Skilled Chemist pursuing Route 3.1 would initially attempt fluorination using DAST under what were considered standard conditions, that is in anhydrous CH₂Cl₂, with and without pyridine, under an inert atmosphere, at room temperature.

³⁵ See the examples by Maruyama et al *Chem Pharm Bull* 1999, 47, 966-970; Mikhailopulo et al *Helv. Chim. Acta* 1999, 82, 2052; and Takamatsu et al *J Org Chem* 2001, 66, 7469-7477.

³⁶ Wachtmeister et al *Tetrahedron* 1999, 55, 10761-10770.

³⁷ Kirihara et al *J. Chem. Res. (S)*, 1998, 652-653

³⁸ Yang et al *Tetrahedron Lett.* 1982, 23, 5517-5520.

³⁹ Yang et al *Synthetic Communications* 1986, 16, 131-138.

⁴⁰ Yang et al. *Carbohydrate Research* 1993, 249, 259-263.

228. If these reactions were unsuccessful, the Skilled Chemist would have had to devise altered reaction conditions to try and “troubleshoot” the problems. For example, if TLC, HPLC, MS and/or NMR analysis indicated decomposition of starting material and/or formation of mixtures of side products, the Skilled Chemist would consider reducing reaction times and/or lowering the reaction temperature. On the other hand, if the starting material remained unreacted at room temperature, the Skilled Chemist would consider investigating reactions at higher temperature. Increasing the temperature may have necessitated the use of solvents with a higher boiling point (e.g. toluene, bp = 111 °C cf. CH₂Cl₂ bp = 40 °C).⁴¹ The Skilled Chemist would have also considered testing Deoxofluor, as the literature suggests these are comparable reagents for this type of fluorination, and may have considered varying the protecting groups used.

229. The literature available at the time suggests DAST and Deoxofluor were good reagents for transforming primary and secondary alcohols to fluorides. However, if DAST and Deoxofluor had failed there were other nucleophilic fluorination methods that could have been investigated which made use of similar starting materials. For example, it was well established that primary or secondary alcohols can be transformed to sulphonate derivatives (e.g. tosylates, mesylates or triflates) which are good leaving groups, facilitating nucleophilic substitution with a fluoride anion (e.g. from tetrabutylammonium fluoride (TBAF), KF/crown ether) or other fluoride sources.⁴² This approach could potentially also be applied to tertiary alcohols, but the Skilled Chemist would be unsure if it would work.

230. If the Skilled Chemist was struggling or particularly concerned to get the correct stereochemistry, an alternative route would be to activate the 2'-alcohol, then the oxygen at the 2-position (on the base) can attack to form an anhydronucleoside (compound 27, Scheme 5), which could potentially be opened up with a fluoride source. Because the base sits above the ribose ring, the fluoride can only approach from below, in theory giving the product with the desired stereochemistry.

231. Alternatively, the same type of DAST/Deoxofluor fluorination could have been performed on the ribose unit, prior to attaching the base, as shown at the top of Scheme 5 (compound 22 to 23). This route would avoid the potential problems associated with the interaction of the base during the reaction (i.e. formation of anhydro by-product

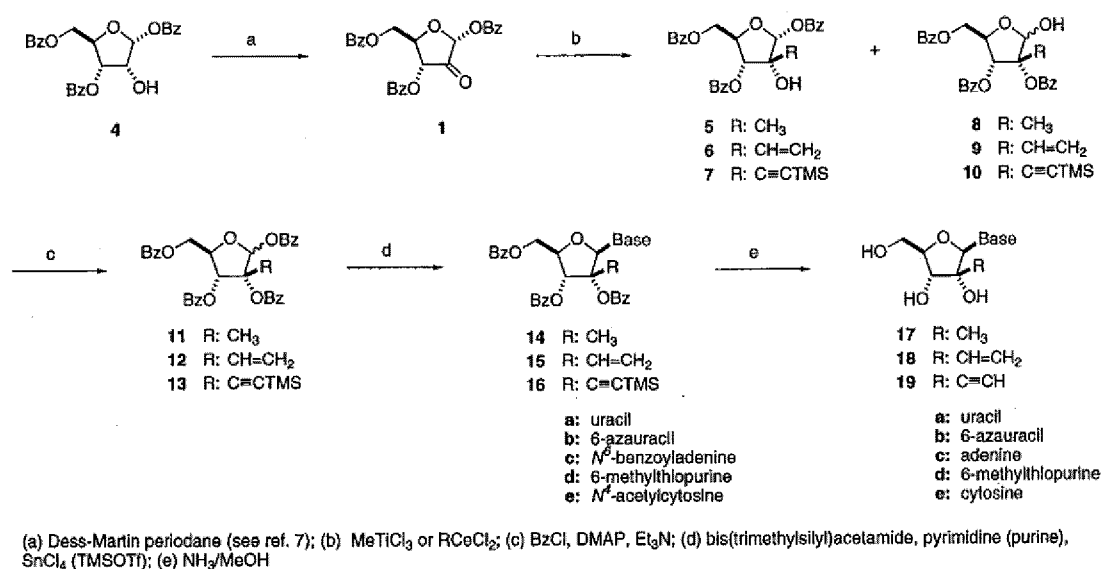
⁴¹ See Yang et al 1982, 1986 and 1999 above.

⁴² See for example Cox et al. *J. Org. Chem.* 1984, 49, 3216-3219; Landinin et al. *Synthesis* 1974, 428-430; Liotta et al. *J. Am. Chem. Soc.* 1974, 96, 2250-2252.

27) but would have involved the same problems with coupling the base discussed in relation to Routes 1.1 and 1.2 (see paragraphs 203-204), and thus has both up-sides and down-sides for the Skilled Chemist to consider.

L tab.69 vol 2 232.

An Analogy Search would also have identified a paper by Harry-O'Kuru in 1997⁴³ which demonstrates the synthesis of the 2-Me(up), 2-OH(down) sugars (as shown in **Scheme 7** below), then the base is added. The Skilled Chemist could consider direct fluorination of the 2-Me(up), 2-OH(down) sugar made using this precedent, provided that the reaction proceeded with retention of stereochemistry. However, the Skilled Chemist would have been cautious applying the chemistry for introducing the base, described in this paper, to the 2-Me, 2-F sugar (if it could be successfully made), because the lack of neighbouring group participation (from the 2-OBz group) would make the stereochemical outcome more uncertain.



Scheme 7: Harry-O'Kuru synthesis of 2-Me, 2-OH sugars

233. There are a number of known problems around using DAST, particularly for creating tertiary fluorides, which would have been of concern for the Skilled Chemist when considering Route 3.1. Eliminations or rearrangements might lead to side products or the formation of anhydronucleosides, which could complicate the reaction or require further steps to deliver the fluorinated product. There are also uncertainties about the mechanism, whether it would proceed with inversion or retention of stereochemistry.

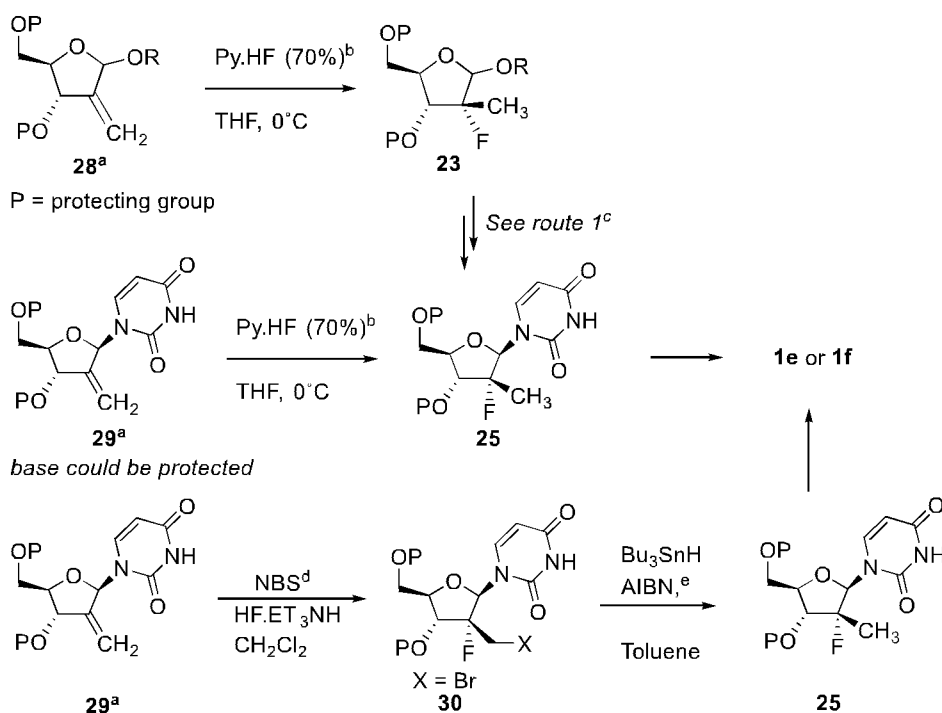
⁴³ Harry-O'Kuru et al. *J. Org. Chem.* 1997, 62, 1754-1759.

As a result, the Skilled Chemist would have been uncertain as to whether this type of fluorination would work.

Route 3.2 - Nucleophilic fluorination of an alkene

234. There are quite a few precedents where alkenes are fluorinated (called hydrofluorination) using Olah type reagents; however, none of these relate to nucleosides or sugars. Routes applying this approach are shown in **Scheme 8** below.

Route 3.2 Nucleophilic fluorination an alkene



^a Synthesis of compound 28 suggested based on Matsuda et al. *J. Med. Chem.* 1991, 34, 812-819

^b Conditions based on Olah, *J. Org. Chem.* (1979), 44, 3872-3881; Back, T.G. et al *J. Org. Chem.* 1999, 64, 5494-5498; Hara et al *Synlett* 1996(6): 529-530.

^c Coupling 23 with a base using method discussed in Route 1.1/1.2.

^d Conditions based on Lubke et al. *J. Fluorine. Chem.* 2000, 102, 125-133.

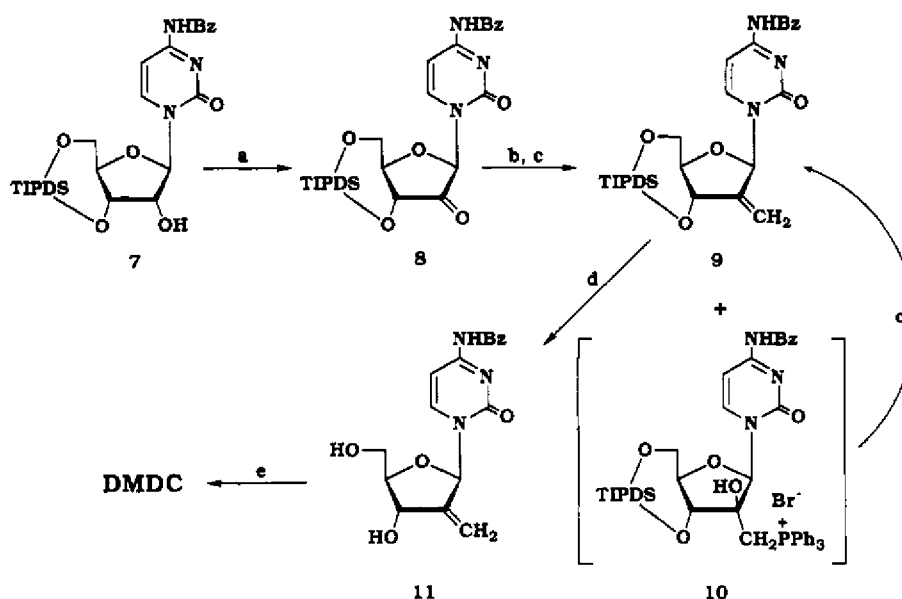
^e There are many methods for reducing haloalkanes including methods selective for weaker C-Br bonds e.g. Alonso et al. *Chem. Rev.* 2002, 102, 11, 4009-4092.

Scheme 8: proposed Route 3.2 to compounds 1e and 1f

235. Alkenes of the type shown as compounds 28 and 29 in Scheme 8, were known and could have been used to attempt fluorinations using Olah type reagents. Analogy Searches could have identified a paper by Matsuda et al. from 1991⁴⁴ where the authors make a ketone and then convert that to an alkene using a Wittig-type reaction, to

⁴⁴ Matsuda et al. *J. Med. Chem.* 1991, 34, 812-819.

ultimately give deoxy-2'-methylenecytidine ("DMDC"), as shown below in **Scheme 9**. This would be one way to make the starting material for fluorination in Route 3.2.



^a(a) $\text{CrO}_3/\text{pyridine}/\text{Ac}_2\text{O}$, CH_2Cl_2 ; (b) $\text{Ph}_3\text{P}^+\text{CH}_3 \text{Br}^-$, BuLi , THF ; (c) NaH , THF ; (d) TBAF , THF ; (e) NH_3/MeOH .

Scheme 9: Matsuda synthesis of 2'-methylene nucleosides

236. There is precedent for adding HF across double bonds and normally the fluorine adds to the more substituted position. In this case, that would give the desired product. It is possible to make tertiary fluorides using this approach, although examples at the time related to simple alkenes or steroid-like structures. Alternatives to Olah's reagent, were also developed, such as the use of HF/triethylamine by Yoneda et al.⁴⁵

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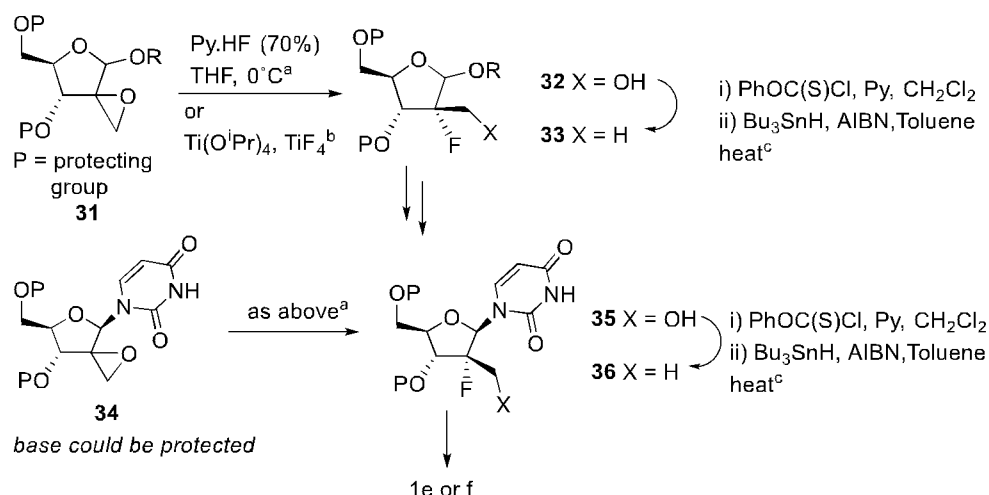
237. In the literature at the time there were also conditions for doing bromofluorinations. A bromine is added onto the alkene giving a cyclic bromonium ion, which is then attacked by fluoride, giving a carbon substituted with fluorine and $-\text{CH}_2\text{Br}$ group. Such reactions were known for non-nucleoside systems, but there was little precedent relating to nucleosides. This approach would be more laborious than direct fluorination, such as that discussed above, but potentially the bromo group could be selectively removed using some kind of radical reduction, to give a methyl group, because the C-Br bond is weaker than the C-F bond.

⁴⁵ Yoneda *Tetrahedron* (1991), 47, 5329-5365.

Route 3.3 - Nucleophilic fluorination of epoxides

238. Route 3.3 involves the possibility of taking the alkene starting material from Route 3.2, forming an epoxide, then using reagents such as Olah's reagent to open the epoxide to give an hydroxymethyl group (X = OH), as shown in **Scheme 10**. It would then be necessary to do a deoxygenation to give the desired compound, but there are methods for doing deoxygenation (such as Barton-McCombie deoxygenation) which have been used in nucleoside chemistry.

Route 3.3 Nucleophilic fluorination of epoxides



L3/135; L1/9; L1/42

L3/151
L1/107

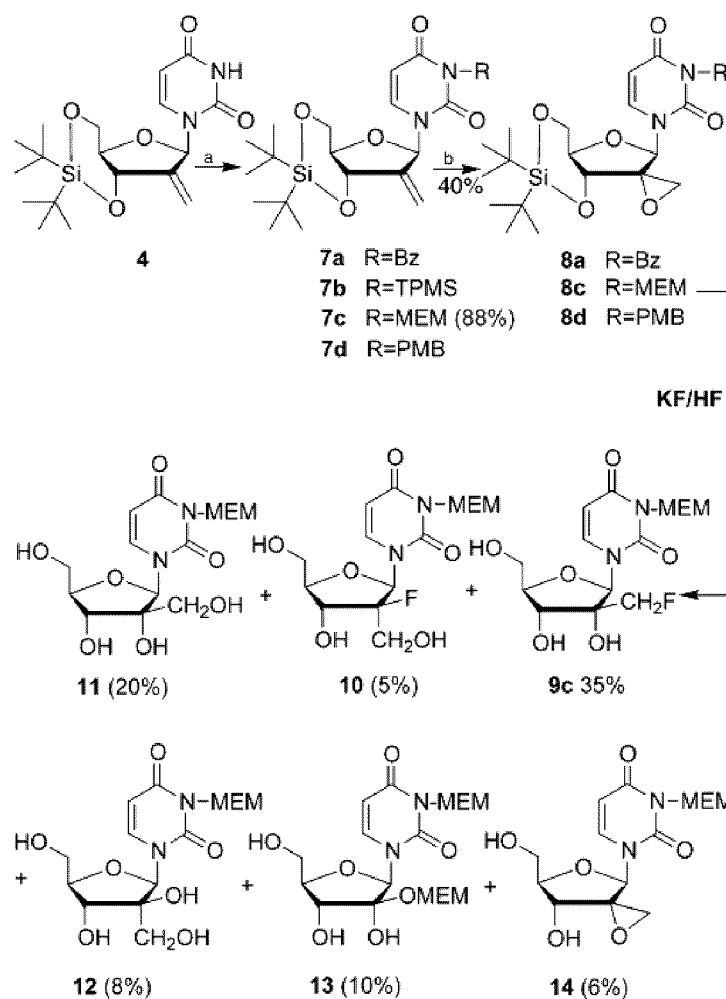
^a Conditions for fluorination suggested based e.g. on Olah, *J. Org. Chem.* (1979), 44, 3872-3881; Annen et al. *Liebigs Ann. Chem.* 1982, 966 - 972 ; Dai and Piccirilli (2003), *Org. Let.*, 5(6), 807-810.

^b Raifeld et al. *Tetrahedron* 1994, 50(29), 8603-8616.

^c Barton-McCombie deoxygenation, conditions suggested e.g. based on Matsuda et al. *J. Med. Chem.* 1991, 34, 9, 2917-2919

Scheme 10: proposed Route 3.3 to compounds 1e and 1f

239. There is some literature precedent for this type of route. The paper by Dai & Piccirilli, discussed in paragraph 170, describes the synthesis of an epoxide of the type needed for route 3.3, from an alkene, see **Scheme 11** below. The authors then use fluoride to open the epoxide and get some of the 2'-F, 2'-hydroxymethyl compound (compound 10, Scheme 11 below), although as a very minor product and with the wrong stereochemistry. It is important to note that they get a mixture of 6 compounds from this reaction, all in low yield. These compounds would have to be separated and the 2'-hydroxymethyl group would ultimately need to be converted to a methyl group using Barton-McCombie deoxygenation, making this approach quite laborious.



Scheme 11: Dai and Piccirilli synthesis of 2'-epoxide

Prioritisation of routes and attempts at synthesis

240. Bird & Bird asked me to comment on how the Skilled Chemist would prioritise the routes discussed above and to give my views on the amount of work involved in attempting the synthesis.
241. Overall, I think this synthesis would be considered extremely challenging for the Skilled Chemist, because they are trying to make a very demanding 2'-tertiary fluoride with little or no relevant literature precedent.
242. As set out above, I think the Skilled Chemist would be able to propose a number of potential routes which they would think might be viable, but would also anticipate problems with all of these routes. The Skilled Chemist is trying to make a tertiary

fluoride, they are working with reagents that can lead to elimination and rearrangements, and there are stereochemical issues to overcome. The Skilled Chemist has got to devise and develop multiple routes to compounds 1e and 1f because there is nothing that stands out as being a reliable way of making those molecules. Any one of these routes could fail for different reasons, so the synthesis of either molecule is a research project.

243. Within each of those routes there are additional parameters which the Skilled Chemist would need to explore. They might need to explore different protecting groups, because this can affect the reactivity and stereoselectivity – through steric effects and/or neighbouring group participation. The protecting groups also need to be stable under the different reaction conditions that may be encountered, for example some protecting groups could come off during reactions and lead to side-products. Protection of the base might also be required, particularly if anhydronucleosides are formed. All of that might need to be explored for each individual approach, which would add time and complexity.
244. In my view trying any one of the routes set out above would take a Skilled Chemist at least six months. If they ran into any kind of problems with the chemistry not working, or the wrong stereoisomer forming, or difficulties in separating stereoisomers (which can often happen), this would result in a need to try multiple different routes, which could cause the timeframe to extend to multiple years, and they could ultimately not succeed at all.
245. As I explained in Section 4 above, there are a number of reasons why in the real world the behaviour of chemists of the experience and education of the Skilled Chemist could reasonably differ in the prioritisation of synthetic routes (though I am told that the relevance of this to the behaviour of the Skilled Chemist is a matter of law). I have provided some general comments on the pros and cons of these synthetic routes, but in the real world I would expect the decision about precisely which routes to attempt in what order would likely depend on a range of things including the particular background of the chemist in question and the practical realities of the situation, such as what starting materials and reagents were readily available, and if they had particular experience of certain aspects of fluorination chemistry already. For example, people with more of a nucleoside chemistry focus (including myself) would have probably preferred to have the base on because they can work with common intermediates and follow the nucleoside precedents where the base is on from the start (although there is no direct precedent available in this case). People with a sugar

chemistry background might be more likely to favour the sugar routes or glyceraldehyde routes. If the notional Skilled Chemist is assumed to have the combined CGK and background experience of his real world counterparts, I do not think that any of these routes would be more or less attractive; the Skilled Chemist would be equally inclined to try each of them.

246. It is also important to note that the Skilled Chemist would not want to narrow the choice down to a single route. Give the lack of literature precedent, the Skilled Chemist would want to explore several different routes where they could start with a common building block and test three or four different chemistries (for example, as discussed in Routes 3.1-3.3), in the hope of finding one which worked. The Skilled Chemist would not consider it efficient or desirable to focus too heavily on optimising a single route, when it was not clear if it would work. Ideally the Skilled Chemist would develop several routes starting from some common building block, so every time the Skilled Chemist reached a dead end they did not have to go back and do five or six steps to make the next substrate to test the new approach.
247. If I had to personally prioritise routes, based upon my background and experience I would say that routes 3.1-3.2 (nucleophilic fluorination) would be my first preference because they potentially offer the shortest route to the target compound from starting materials which are described in the literature; however, the fluorination step required by these routes is unknown. An attractive feature of these routes is that there is the option of doing each with the base on (i.e. a nucleoside route), or the base off (i.e. a sugar route). Trying the 'base on' reactions first would be slightly more attractive to me, because if the fluorination works 'base on' would mean a smaller number of steps to make the final molecule for the biologist to test. If the base has to be added, then there are potential problems with the stereocontrol for the addition of the base (discussed in paragraph 203). The sugar routes would remain a fall-back option if there were problems with the nucleoside routes, that would have been my initial preference.
248. In contrast, Routes 1.1 and 1.2 are probably the best precedented of all the routes I have discussed above because the plan would be to essentially follow the Hertel synthesis if the initial step could be achieved. Routes 1.1-1.2 also avoid the need for difficult and unknown fluorination reactions on complex molecules. The downsides are that routes 1.1-1.2 involve a larger number of unknown steps than Routes 3.1-3.2.

Synthesis of 2'-Me(up), 2'-F(down) nucleosides at Idenix

249. After I had provided my views as set out above, Bird & Bird provided me with a bundle of documents, which I was told related to research undertaken at Idenix between approximately March 2002 and April 2005, with the goal of synthesising certain 2'-Me (up), 2'-F (down) nucleosides (the "**Idenix Research**").
250. The bundle consisted of four volumes (the "**Briefing Bundle**"), which I was informed contained the following documents:

C.EXP/1	(i)	Volume 1 – an extract from the judgment of Mr Justice Arnold in <i>Idenix Pharmaceuticals v Gilead Sciences</i> [2014] EWHC 3916 (Pat) (" Idenix v Gilead ") and written evidence given regarding the Idenix Research by key Idenix researchers in the <i>Idenix v Gilead</i> litigation and in Patent Interference No 105,871 before the United States Patent and Trademark Office;
C.EXP/2	(ii)	Volume 2 – contemporaneous documents from Idenix, relating to the Idenix Research, including project reports and laboratory notebooks of relevant researchers;
C.EXP/3	(iii)	Volume 3 – written evidence from various additional Idenix researchers and technicians with some involvement in the Idenix Research, along with relevant laboratory notebooks;
C.EXP/4	(iv)	Volume 4 – resumes and information regarding the backgrounds of researchers involved in the Idenix Research.

- C2 tab. 12 251. A copy of the Briefing Bundle, Volumes 1-4 is attached as **Exhibit JM-5**.
252. Bird & Bird asked me to review the documents in the Briefing Bundle, and to provide my views regarding the Idenix Research.
253. I have been instructed by Bird & Bird that the *Idenix v Gilead* judgment is not of evidential value in this case and I should not rely upon it when coming to my conclusions, rather I should draw my conclusions from the other materials in the Briefing Bundle. I have taken care to do so when preparing my report. Having reviewed the judgment, there is nothing in it which would have led me to a different conclusion.

Overview of the Idenix Research

254. The documents contained in the Briefing Bundle describe a substantial research project undertaken by Idenix to make 2'-Me (up), 2'-F (down) nucleosides (specifically cytidine and uridine derivatives). The majority of the work was conducted at the Idenix site in Montpellier, although later the project was transferred to Cambridge, Massachusetts. The project also involved collaborators in the UK and Italy and getting assistance from external experts in fluorination chemistry.
255. From the Briefing Bundle, I set out my understanding of the key researchers and their roles in the Idenix Research below:
- (a) **Dr Jean-François Griffon:** the lead researcher on the project at Idenix Montpellier. Dr Griffon was responsible for the majority of the planning and laboratory work on the project from its inception until late 2004 when it was transferred to Idenix Cambridge. Dr Griffon had an MSc and PhD in chemistry from Montpellier II University focusing on nucleoside chemistry. I would consider Dr Griffon to match the description I gave of the Skilled Chemist in Section 4, above.
 - (b) **Dr Alistair Stewart:** the lead researcher on the project once it was transferred to Idenix Cambridge. Dr Stewart oversaw the laboratory work on the project from late 2004 until its completion in mid-2005. Dr Stewart conducted some laboratory work and also collaborated with Ms Jingyang Wang. Dr Stewart had an MChem from the University of Durham and a PhD from the University of Oxford, under the supervision of Professor George Fleet. Dr Stewart appears to have less specific nucleoside experience than Dr Griffon, but I would still consider him to fit the description of the Skilled Chemist.
 - (c) **Jingyang Wang:** the researcher at Idenix Cambridge responsible for much of the laboratory work in the final part of the Idenix Research from January to March 2005. Ms Wang had a BSc in chemistry from Nankai University, Tanjin, China, an MSc in organic chemistry from the University of Manchester and an MSc in organic chemistry from University of Maine. The extent of Ms Wang's experience is less clear. She did not have a PhD, but had two MSc degrees and appears to have had significant research experience in synthetic chemistry. As such, I think she also fits the description of the Skilled Chemist.

- (d) **Dr Adel Moussa:** was the Director of Process/Chemical Development and ran the process chemistry group at Idenix Cambridge. Dr Moussa worked on some limited aspects of the Idenix Research throughout its duration. Dr Moussa had a BSc in chemistry from Alexandria University and a PhD in chemistry from Northeastern University.
 - (e) **Audrey Chappe-Dumas:** a technician working with Dr Griffon on certain aspects of the Idenix Research at Idenix Montpellier.
 - (f) **Elodie Pecheux:** an internship student who worked on certain aspects of the Idenix Research from March to June 2004 at Idenix Montpellier under the supervision of Dr Griffon.
 - (g) **Dr Richard Storer:** was the Vice President of Chemistry at Idenix and was in overall charge of the Idenix Research. Dr Storer had a D.Phil. degree in Chemistry from the University of Sussex, over 18 years of nucleoside experience and over 30 years of chemistry experience in drug discovery.
 - (h) **Professor Gilles Gosselin:** was Director of Research at Idenix Montpellier, an expert in nucleoside chemistry and Dr Griffon's former PhD supervisor.
 - (i) **Professor George Fleet:** Professor of Chemistry at the University of Oxford. Professor Fleet was an expert in carbohydrate chemistry who acted as a consultant to Idenix, including in relation to the Idenix Research.
 - (j) **Dr Paul Coe:** expert in fluorination chemistry who acted as a consultant to Idenix, including in relation to the Idenix Research.
256. From my review of the Briefing Bundle, I provide a brief summary of the Idenix Research below. When referring to the synthetic approaches attempted by the Idenix team, I have for convenience adopted the terminology used by Mr Justice Arnold, which refers to eleven synthetic 'strategies' (Strategies 1 to 11). I first summarise the course of the Idenix Research, then explain the strategies in more detail and explain how each of these strategies relates to the routes I described above.
257. Idenix initially decided to target a range 2'-Me (up), 2'-X (down) nucleosides including compounds where X = F, at a meeting of senior leaders from Idenix, in Hawaii in December 2001. In March 2002, Dr Griffon was assigned the task of making a 2'-Me (up), 2'-F (down) nucleoside analogue, at a chemistry meeting in Montpellier. Dr

Griffon was initially tasked with conducting a literature search to identify any references to 2'-Me (up), 2'-F (down) nucleosides or sugars. He provided the results of this search in June 2002. There were no results for this structure. Dr Griffon subsequently searched for methods for converting tertiary alcohols to fluorides, which gave two results, and for converting epoxides to tertiary fluorides, which also gave two results. On the basis of the literature search results, Dr Griffon proposed a synthesis based on the paper by Wachtmeister⁴⁶ (discussed above at paragraphs 219-221 and 226).

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258. From July to August 2002, Dr Griffon attempted the synthesis from the 2'-alkene nucleoside following 'Strategy 1', below. In September 2002, Dr Griffon reported that Strategy 1 had failed and proposed Strategies 2 and 3 (see below). In October 2002, Dr Griffon reported to Dr Storer that Strategy 3, involving ring-opening an anhydro nucleoside by-product, was unsuccessful. In November 2002, Dr Griffon reported that further attempts at Strategy 3 had also been unsuccessful. He reported that Strategy 2, involving direct fluorination of an alkene nucleoside using HF had also failed.
259. In early December 2002, Dr Griffon met with Dr Storer, Dr Moussa, Professor Gosselin and Professor Fleet to discuss the difficulties he had encountered with the project. Further work on Strategy 2 in December 2002 was also reported as a failure. Dr Griffon continued to work on Strategies 1 and 2 in January 2003, without success.
260. In early February 2003, Dr Storer wrote to Dr Coe for advice relating to the fluorination. By the end of February 2003, Dr Griffon reported that three further attempts to fluorinate anhydronucleosides (Strategies 3, 4 and 5, see below) had also failed.
261. After the failure of the first five strategies, Dr Griffon started on a new strategy based on the fluorination of a tertiary alcohol in a nucleoside with Deoxofluor (Strategy 6, see below). Dr Griffon attempted this approach twice in February 2003, but reported that both attempts failed, giving what Dr Griffon concluded were elimination products.
262. In early April 2003, Dr Coe provided several examples of additional approaches to make the 2'-Me (up), 2'-F (down) nucleoside. In May 2003, Dr Griffon attempted these routes. Strategy 7 (discussed further below), suggested by Dr Coe involved a two-step procedure involving activation of the tertiary alcohol of a nucleoside, followed by displacement with fluoride. In May 2003, Dr Griffon reported that this approach had

⁴⁶ Wachtmeister et al *Tetrahedron* 1999, 55, 10761-10770.

also failed. Strategy 8 was also suggested by Dr Coe and involved a further attempt to ring-open an anhydro nucleoside. Dr Griffon attempted this approach during July and August 2003, but was unsuccessful.

263. From November 2003 to February 2004, the Idenix Research was suspended. In February 2004, the project was assigned to Dr Griffon again. Dr Griffon initially attempted Strategy 1 again, but using nucleosides with the base protected; this was also reported to have failed.

264. In March 2004, details of the work Dr Griffon had conducted to date was circulated within the broader Idenix chemistry team in Montpellier, in an attempt to identify new synthetic routes. This resulted in Dr Griffon proposing three new routes to Dr Storer and Professor Gosselin in April 2004.

265. Strategy 9 involved forming a chloro-epoxide derivative of a sugar, followed by ring-opening with fluoride followed by reduction. Dr Griffon reported this approach was unsuccessful in April 2004.

266. Strategy 10 involved electrophilic fluorination of cyclic enol ether (called a glycal) using Selectfluor. This was also reported to have failed in April 2004.

267. Strategy 11 was a glyceraldehyde route approach, based on the synthesis of gemcitabine, which involved building the substituted sugar from 2 and 3-carbon units. Dr Griffon attempted this in May 2004, but reported this too was unsuccessful.

268. [REDACTED]

269. [REDACTED]

270. [REDACTED]

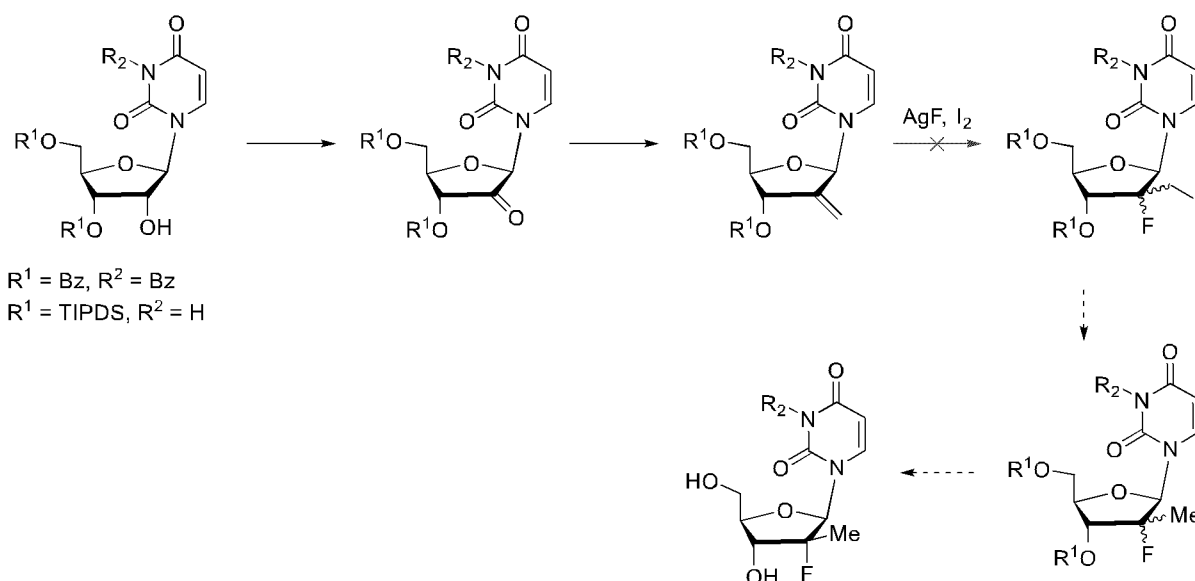
271. [REDACTED]

272. [REDACTED]

Synthetic routes attempted by Idenix

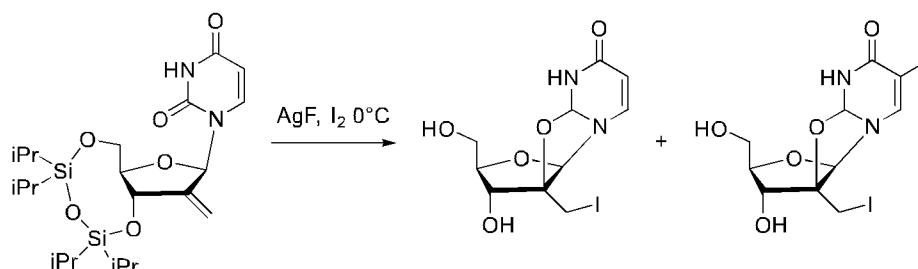
Strategy 1 – iodofluorination of an alkene

273. The first synthetic route attempted by Idenix was proposed by Dr Griffon in June 2002. This first approach was to do a fluorination of a nucleoside with an alkene at the 2'-position using AgF and iodine as shown below in **Scheme 12**. This approach is similar to Route 3.2, which I discuss in paragraphs 234-237 above.



Scheme 12: Idenix 'Strategy 1'

274. This approach was unsuccessful. Dr Griffon attempted fluorination of a TIPDS protected substrate with AgF and I₂ at 0 °C, three times between August 2002 and January 2003. All attempts failed, resulting in a mixture of anhydronucleoside products, as shown in **Scheme 13** below.



Scheme 13: anhydronucleoside formation observed by Dr Griffon

275. In February 2004, Dr Griffon revisited this approach by attempting to fluorinate a benzyol protected substrate with AgF and I₂ at -10 °C; however, this appears to have given no reaction.

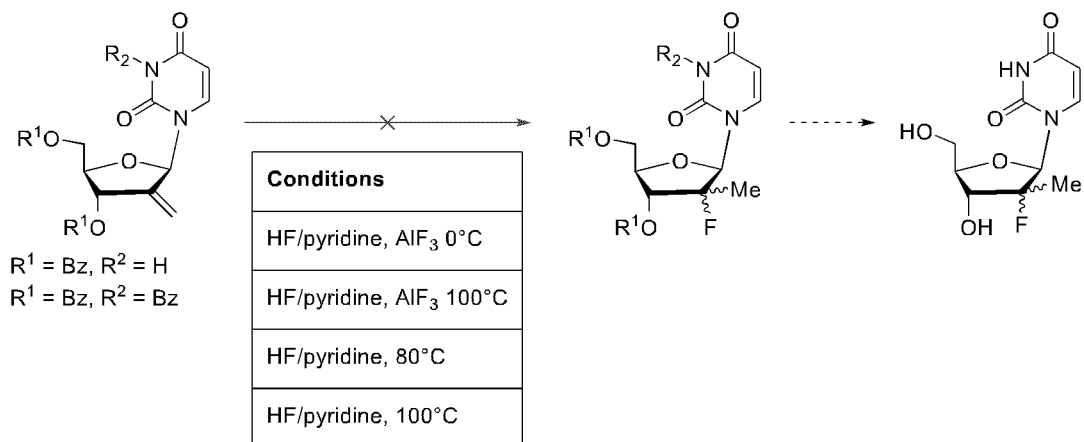
Strategy 2 – direct fluorination of an alkene

276. Dr Griffon also proposed the direct fluorination of the alkene, in September 2002, using conditions based on a 1979 paper by Olah⁴⁷, as shown in **Scheme 14**. This was

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⁴⁷ Olah, *J. Org. Chem.* (1979), 44, 3872-3881.

something else that I suggested the Skilled Chemist might have considered as part of Route 3.2 (see paragraphs 234-237, above).

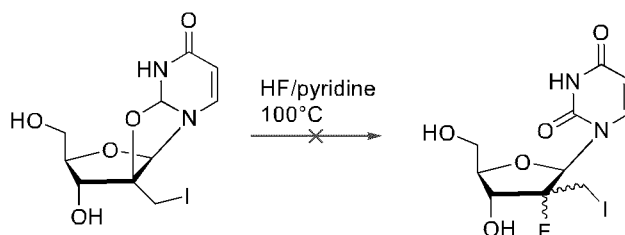


Scheme 14: Idenix 'Strategy 2'

277. Dr Griffon attempted to fluorinate the bis(benzoyl) protected alkene nucleoside under a range of conditions using HF and pyridine (Olah's reagent) with AlF_3 , initially at 0 °C, without success. He then attempted using more forcing conditions, increasing the temperature to 100°C in a stainless steel bomb but this was also unsuccessful in November 2002. Dr Griffon reported observing one major product, which did not contain fluorine. In December 2002 he attempted this approach twice more starting from a tris(benzoyl) protected substrate, using HF and pyridine at 80 °C and 100 °C, also without success.

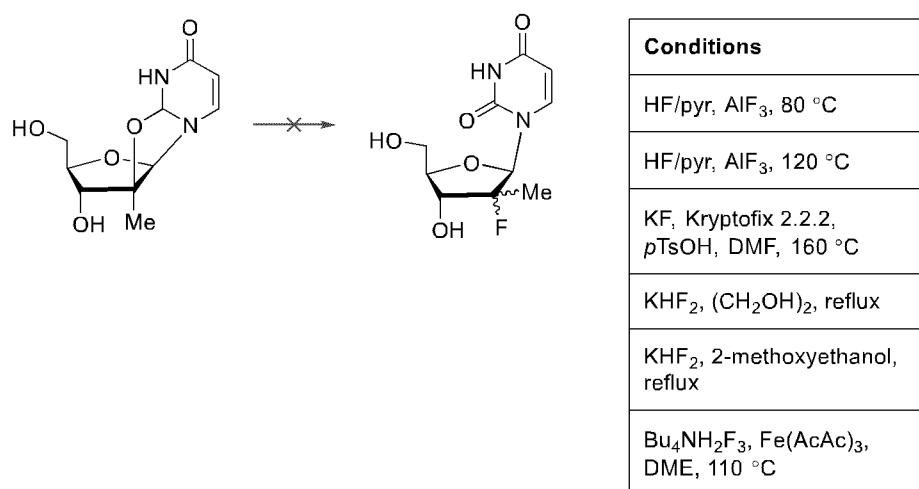
Strategies 3, 4, 5 and 8 – ring-opening anhydronucleosides

278. Strategy 3 relates to the ring-opening of the iodo anhydronucleoside product referred to in paragraph 274, above. Dr Griffon proposed removing the iodine, followed by fluorination, however he initially struggled to complete this step, so attempted to directly fluorinate the iodo anhydronucleoside as shown in **Scheme 15**, below. This reaction proved unsuccessful, with only unreacted starting material recovered.



Scheme 15: Idenix 'Strategy 3'

279. Dr Griffon subsequently managed to reduce the 2'-iodomethyl group to produce a 2'-methyl substituted anhydronucleoside and put in a big effort to ring-open it with various types of nucleophilic fluoride sources, as shown below in **Scheme 16**.



Scheme 16: Idenix 'Strategies 3, 4, 5 and 8'

280. At least three or four different attempts were made to open that anhydronucleoside using various fluorinating reagents; however, these conditions were all unsuccessful:

- HF/pyridine and AlF₃ at 80 °C and then at 120 °C (a variation of Strategy 3). Both gave no reaction;
- KF and Kryptofix (which is a crown ether used to increase the solubility of fluoride ions in organic solvents and increase its reactivity) ('Strategy 4') at 110-150 °C in DMF. This reaction did not proceed, so Dr Griffon added a further 2 equivalents of KF and increased the temperature to 160 °C, still without any reaction;
- KHF₂ ('Strategy 5') in refluxing ethylene glycol (i.e. at 197 °C) and subsequently in refluxing 2-ethoxyethanol (i.e. at 135 °C). Neither gave any reaction.

281. In July/August 2003, Dr Griffon revisited this approach, attempting to use $\text{Bu}_4\text{NH}_2\text{F}_3$ with $\text{Fe}(\text{AcAc})_3$ to ring-open the anhydro nucleoside (conditions which were suggested by Dr Coe)⁴⁸ (Strategy 8). Despite attempting this reaction at 110 °C in dimethoxyethane for 6.5 hours, Dr Griffon reported that no reaction occurred.

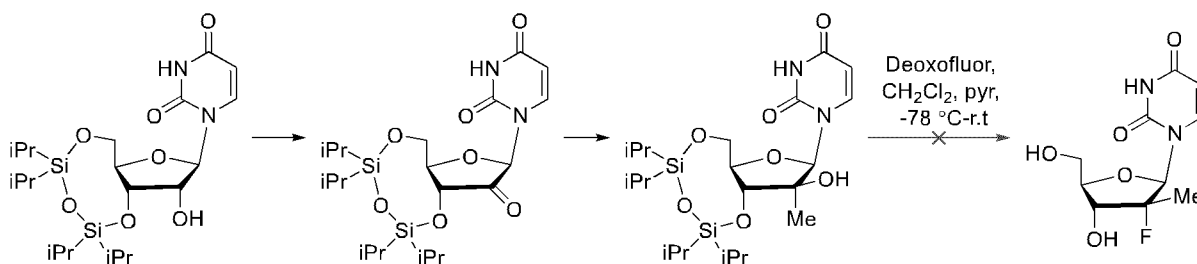
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Strategy 6– nucleophilic fluorination using Deoxofluor

282. Strategy 6 is the first attempt by Idenix at a nucleophilic fluorination using a DAST type reagent (in this case Deoxofluor). Dr Griffon attempted this approach, as shown in **Scheme 17** below, in February 2003, based on conditions from the papers by Singh and Shreeve⁴⁹ and Wachtmeister⁵⁰, which I refer to in paragraphs 219, 225 and 226 above. Dr Griffon stated that he chose Deoxofluor for this reaction because Wachtmeister had reported a poor yield using DAST, but better results with Deoxofluor. This strategy is comparable to Route 3.1, which I proposed in paragraphs 222-233, above, when a nucleoside is used as the starting material.

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Scheme 17: Idenix 'Strategy 6'

- C.EXP1/2 283. Dr Griffon attempted this approach twice (on 13 February 2003 and 19 February 2003) using Deoxofluor and pyridine in dichloromethane, starting the reaction at -78°C and allowing it to warm to room temperature. Both reactions gave complex mixtures of products and Dr Griffon concluded they were unsuccessful giving an elimination product as the major compound. I explained in paragraph 233 above that this type of elimination was a known problem with using DAST (or similar reagents) and would have been a concern to the Skilled Chemist when considering this approach.

⁴⁸ Dr Griffon's witness statement and laboratory notebook appear to contain typographical errors in the formula of the fluorinating reagent used in this reaction. Dr Griffon's witness statement states that the conditions were suggested by Dr Coe, who cites two papers by Landini et al. *Synthesis* 1988; 1988(12): 953-955 and *Tetrahedron* 1990, 31(49), 7209-7212 (see letter from Dr Coe to Dr Storer) which describe the preparation and use of $\text{Bu}_4\text{NH}_2\text{F}_3$. I have assumed this is the reagent Dr Griffon refers to.

⁴⁹ Singh and Shreeve 2002.

⁵⁰ Wachtmeister 1999.

284. [REDACTED]
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[REDACTED]

285. [REDACTED]
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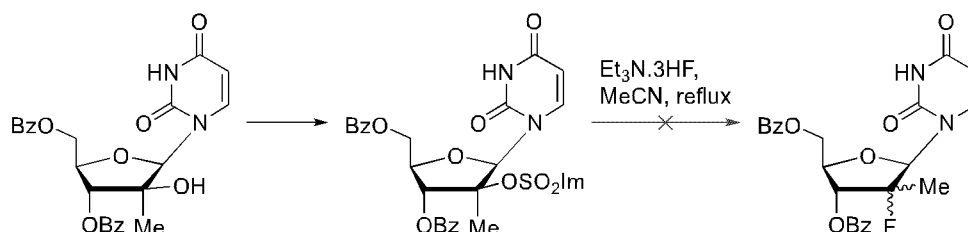


288. [REDACTED]
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Strategy 7– nucleophilic fluorination using SO₂Cl₂/Im, Et₃N.3HF

290. After the failure of Strategy 6, in May 2003 Dr Griffon went on to do some more attempts at nucleophilic fluorination of the tertiary alcohol, using a two-step approach, as shown in **Scheme 20** below. This is similar to a variation to Route 3.1, which I discussed in paragraph 229 above.

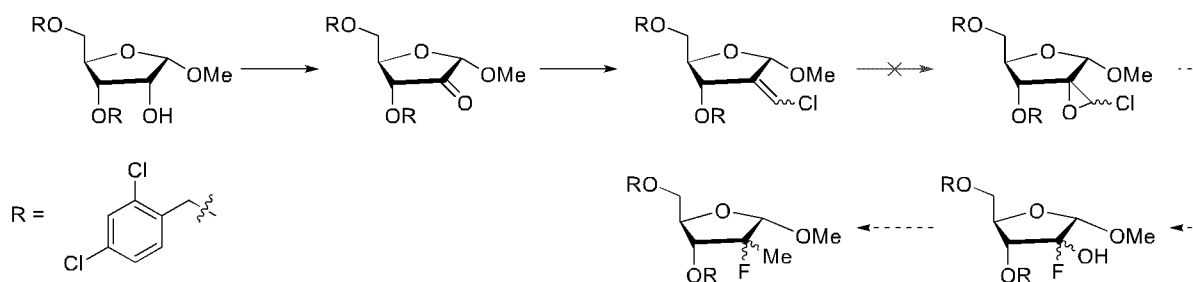


Scheme 20: Idenix Strategy 7'

- C.EXP2/15 291. This approach came from discussions with Dr Coe who was a fluorine chemistry expert acting as a consultant to Idenix. This strategy involved converting the 2'-OH group to a sulfonyl-imidazole leaving group to activate the tertiary hydroxyl group, followed by nucleophilic substitution using Et₃N.3HF. Dr Griffon appears to have been successful in making the sulfonyl-imidazole compound; however, his attempt to fluorinate this material using Et₃N.3HF in refluxing acetonitrile was a failure, with no reaction observed.

Strategy 9– nucleophilic ring opening of an epoxide

292. Strategy 9 involved forming an epoxide of a sugar derivative, before fluorinating using HF/pyridine, as shown in **Scheme 21**, below. This approach is similar to Route 3.3 which I discuss in paragraphs 238-239 above, with the variation of epoxidizing a chloro-alkene.

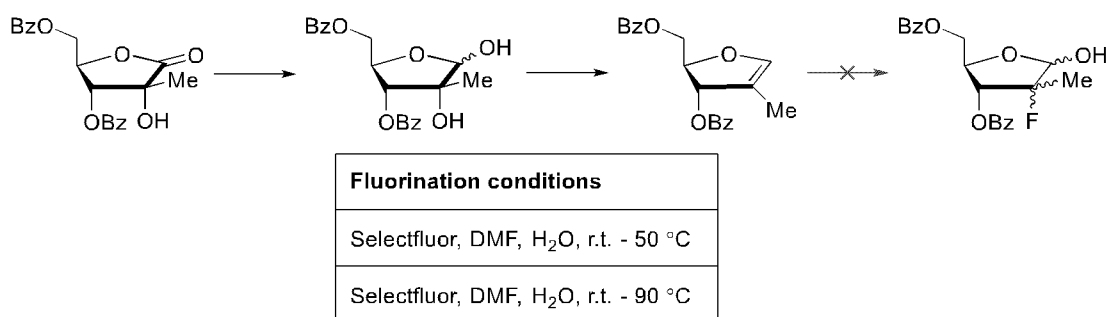


Scheme 21: Idenix 'Strategy 9'

293. Dr Griffon attempted this approach in April 2004; however, he struggled to make the epoxide and reported the approach was unsuccessful.

Strategy 10– electrophilic fluorination

294. Strategy 10 involved forming a cyclic enol ether (glycal) from a ribose unit and fluorinating using Selectfluor, as shown in **Scheme 22**, below. This approach is similar to Route 2 which I discussed in paragraphs 208-213, above, although I suggested a Skilled Chemist could also consider electrophilic fluorination of lithium enolates or silyl enol ethers of a ribonolactone.



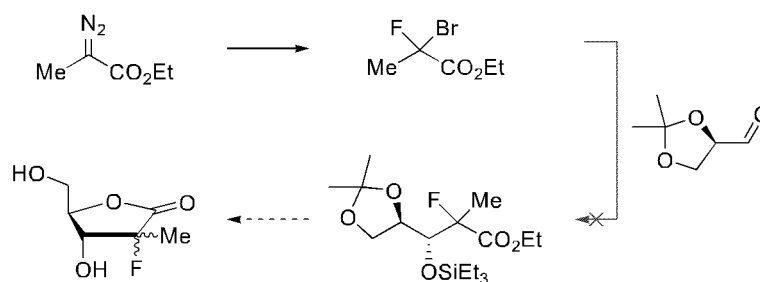
Scheme 22: Idenix 'Strategy 10'

295. Dr Griffon and Ms Chappe successfully made the 2-methyl substituted cyclic enol ether but attempts to fluorinate this with Selectfluor were unsuccessful, giving only recovered starting material. Ms Chappe records attempting the fluorination reaction 4 times between 13 April and 31 May 2004, initially using 1.6 equivalents of Selectfluor in dimethylformamide/water at room temperature, then 4 equivalents of Selectfluor at 50 °C, and finally 4 equivalents of Selectfluor at 90 °C, all without success.

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Strategy 11– Reformatsky/aldol route

296. Strategy 11 involves making a 2-Me, 2-F substituted ribonolactone from smaller building blocks using either Reformatsky or aldol chemistry, as shown in **Scheme 23**, below. These correspond to Routes 1.1 and 1.2 respectively, which I discussed in paragraphs 194-206 above.



Scheme 23: Idenix 'Strategy 11'

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The Reformatsky route comes from the gemcitabine publication. Dr Griffon (and Ms Chappe-Dumas and Ms Pecheux under his supervision) attempted both the Reformatsky and aldol routes. They were unable to successfully couple the propionate and glyceraldehyde units using either approach and in May 2004 and this route ultimately was abandoned.

Conclusions: Quality of the Idenix Research

298. In conclusion, the documents in the Briefing Bundle describe a substantial effort by a large, well resourced team at Idenix over approximately three years, in an attempt to synthesise nucleosides with the structures of compounds 1e and 1f. Despite having plenty of resources, relevant experience and access to expert consultants, the team was ultimately unsuccessful until after they had been provided with details of the synthetic route developed by Pharmasset.

299. The Idenix Research was a major effort involving a substantial, capable and skilled team, with a broad range of relevant experience in synthetic organic chemistry and nucleoside chemistry spanning the US, Montpellier in France, and Cagliari in Italy (although they only did biological testing). It is also worth pointing out that the people involved here appear to have been of high quality. From the CVs of the main researchers (Dr Griffon, Dr Stewart, Dr Moussa and Ms Wang) I conclude that their training and experience matches what I would expect for the Skilled Chemist (as explained in Section 4, above). These researchers were supported in undertaking the laboratory work by a number of technicians with degree level qualifications in chemistry. I think it is important to note, as well, that the people doing the work in the lab (i.e. Griffon, Stewart, Wang, Moussa and the technicians/interns) were reporting to and being guided by experienced people in Gilles Gosselin and Richard Storer. The researchers were also taking on board information from consultants and collaborators, like Professor George Fleet (the carbohydrate expert) and Dr Paul Coe (the fluorine expert), so there was a lot of support for these people.

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50 (Griffon CV);
51 (Moussa CV);
49 (Stewart and
Wang CV); vol
4.

C.EXP₄/53
(Prof. Fleet CV)

300. When I compared the strategies and choices that the Idenix team made with those I proposed in the earlier sections of this report, I think the results of my analysis of the Skilled Chemist's approach to the synthesis of compounds 1e and 1f closely matched what a real-world team of researchers did. The Idenix team covered much of the same ground and identified similar prioritisations of routes from nucleosides.
301. In my view, all of the decisions taken by the Idenix team in prioritising and executing those routes were reasonable in the context of the Skilled Chemist executing a research project of this nature in 2003. The documents in the Briefing Bundle do not show anything unusual or unreasonable was done by any of the Idenix researchers over the course of this project.
302. Despite this, the Idenix team was unsuccessful for approximately 3 years, until after they adopted the method in the Pharmasset Patent published in early 2005. This also supports my view that in 2003, making these compounds was a difficult task and was far from routine. That a large, well resourced real-world team was unable to make a 2'-Me(up), 2'-F(down) nucleoside for 3 years highlights this difficulty.
303. Ultimately, the Idenix Research supports my view that successfully completing the synthesis of nucleosides with a 2'-methyl(up), 2'-fluoro(down) substitution was something that was far from routine work for a Skilled Chemist in July 2003. Successfully completing this synthesis at that time would require a substantial research project, which would have an uncertain outcome and ultimately require an element of luck, creativity and/or inventiveness in order to be successfully completed.

Synthesis of 2'-Me(up), 2'-F(down) nucleosides at Pharmasset

304. After I have provided my views regarding the Idenix Research above, Bird & Bird provided me with a further bundle of documents (Volume 5 of the Briefing Bundle), which I was told related to research undertaken at Pharmasset, starting in approximately November 2002, with the goal of synthesising certain 2'-Me (up), 2'-F (down) nucleosides (the "**Pharmasset Research**"). Volume 5 of the Briefing Bundle comprised:

- | | | |
|---------------------|-----|--|
| C.EXP tab. 62 vol 5 | (a) | <i>Idenix v Gilead</i> , paragraphs 550-567; |
| L tab.36 vol 1 | (b) | A paper by Clark et al (2005) <i>J Med Chem</i> 48, 5504-5508 (" Clark I "); |
| L tab.37 vol 1 | (c) | A paper by Clark et al (2006) <i>J Carbohydrate Chem.</i> 25, 461-470 (" Clark II "); |
| C.EXP tab. 64 vol 5 | (d) | The Pharmasset Patent; |

- L tab.192 vol 4 (e) A paper by Wang et al. (2009) *J Org Chem* 74, 17, 6819-24;
- C.EXP tab.67 vol 5 (f) An extract of the first expert report of Professor Geert-Jan Boons in *Idenix v Gilead* (“**Boons 1**”);
- C.EXP tab.66 vol 5 (g) An extract of the first expert report of Dr Andrea Brancale in *Idenix v Gilead* (“**Brancale 1**”).

- C2/13 (C.EXP5) 305. Volume 5 of the Briefing Bundle is attached as **Exhibit JM-6**.
306. Bird & Bird asked me to review the material in the Briefing Bundle Volume 5 and comment on whether the Pharmasset Research affected the views I had expressed regarding the synthesis of 2'-Me(up), 2'-F(down) nucleosides as at 21 July 2009.
307. Bird & Bird reiterated the instructions in paragraph 253, above, in relation to Volume 5 of the Briefing Bundle. I have taken care to follow those instructions when preparing my report. Having reviewed the judgment, there is nothing in it which would have led me to a different conclusion.

Overview

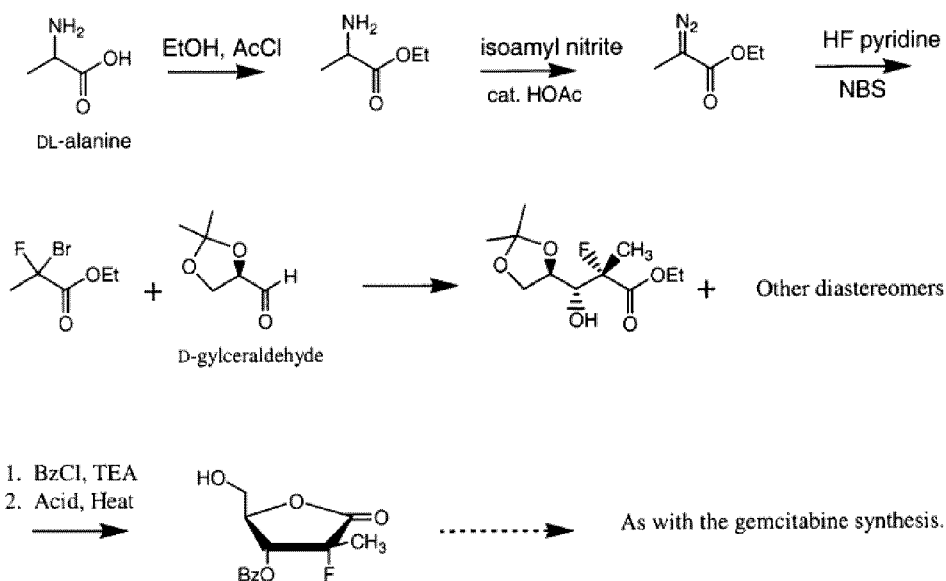
308. I provide an overview of my understanding of the Pharmasset Research and the content of the documents in Volume 5 of the Briefing Bundle below.
309. The Pharmasset Research was a program to synthesise 2'-Me(up), 2'-F(down) nucleoside analogues, in particular 2'-Me(up), 2'-F(down) cytidine and uridine (i.e. compounds 1e and 1f, see paragraph 190 above). In this respect the goals of the project appear to be very similar to those of the Idenix Research.
310. The work of the Pharmasset Research appears to have been substantially undertaken by Mr Jeremy Clark. The judgment extract from *Idenix v Gilead* suggests that Mr Clark was responsible for the laboratory chemistry and I notice that he is the sole inventor named on the Pharmasset Patent. However, it can be seen from the authors credited on the Clark I and Clark II papers that Mr Clark was supported by a substantial team at Pharmasset.

L1/36; L1/37

311. Pharmasset initially attempted to synthesise the 2'-Me(up), 2'-F(down) nucleoside analogues using a Reformatsky approach, based on the gemcitabine synthesis (my Route 1.1); as shown below.

C.EXP5/67

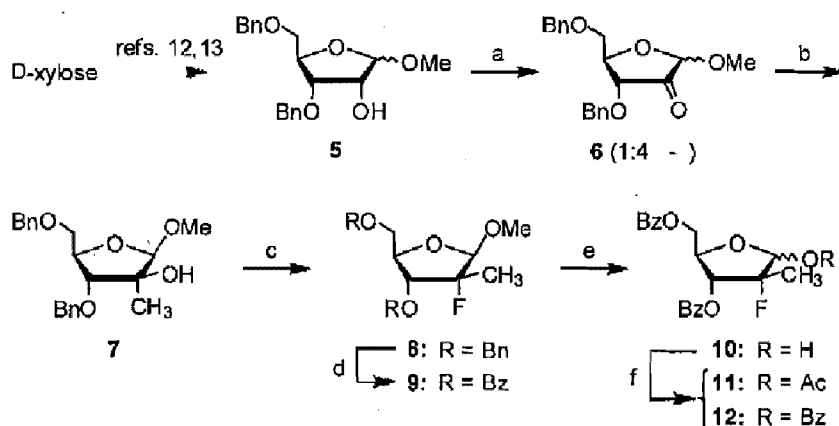
The original attempted synthesis of PSI-6130



Scheme 24: Pharmasset Reformatsky approach to compounds 1e and 1f

312. Mr Clark started to investigate this approach in November 2002. This route appears to have not been completed, although there is no detail about why, and Mr Clark ultimately switched to investigating a sugar route.
313. The sugar route chosen by Pharmasset involved the nucleophilic fluorination of a 2-Me(down), 2-OH(up) sugar derivative using DAST (my Route 3.1). This approach is reported in Clark II, with the synthesis shown below:

L1/37



Scheme 1: Reagents and conditions: (a) TEMPO, NaOCl, 0°C; (b) MeLi, Et₂O, -78°C; (c) DAST, CH₂Cl₂, rt; (d) (i) Pd/C, cyclohexene, reflux, (ii) BzCl, pyridine; (e) ~80% HCO₂H, 60-70°C; (f) Ac₂O or BzCl, TEA, CH₂Cl₂.

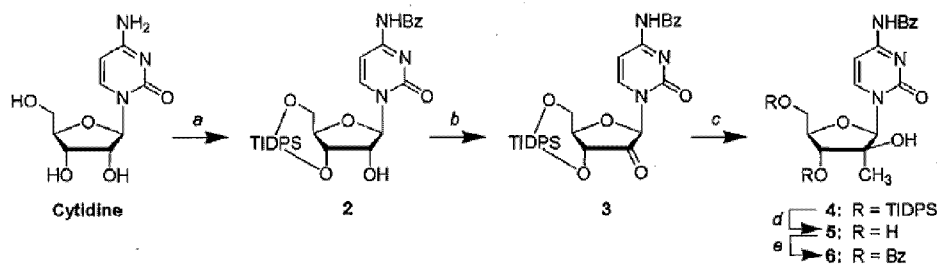
Scheme 25: Pharmasset sugar route

314. This route was successful and by May 2003 (the filing date of the Pharmasset Patent), Mr Clark had managed to synthesise the fluorinated sugar and couple it with cytosine to give 2'-deoxy-2'-fluoro-2'-C-methylcytidine (i.e. compound 1f).

315. Mr Clark also worked on the comparable nucleoside route (which was one of the options I discussed in relation to Route 3.1). The successful synthesis as reported in Clark I, is shown below.

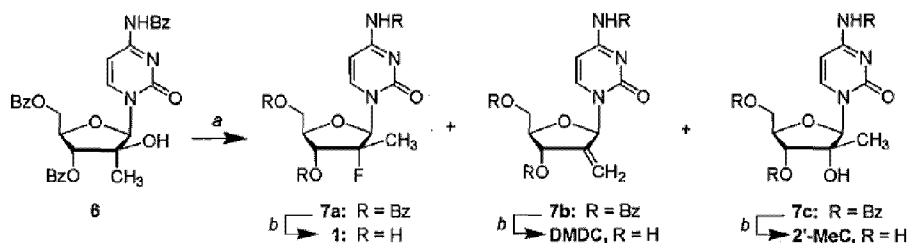
L1/36

Scheme 1^a



^a Reagents and conditions: (a) (i) Bz₂O, DMF, room temp, (ii) TIDPSCl₂, DMF; (b) DMSO, TFAA, TEA, -15 °C; (c) MeLi, -78 °C; (d) 1 M TBAF, concd HOAc, rt; (e) BzCl, pyridine, rt.

Scheme 2^a



^a Reagents and conditions: (a) DAST, toluene, -20 °C to rt; (b) MeOH/NH₃, rt.

Scheme 26: Pharmasset nucleoside route

316. The Pharmasset Patent describes both successful approaches above.

317. After successfully completing the initial synthesis using the routes described in Clark I, Clark II and the Pharmasset Patent, it appears that the Pharmasset team spent about a year and a half attempting to develop improved syntheses. Although I have not seen the details of this work, it appears from Boons 1 that many of these attempts were unsuccessful. The paper by Wang et al⁵¹, published some years later in 2009, describes an improved synthetic route, which is more suitable for scale-up. The route described by Wang is a “small molecule” approach, although one which differs from the Reformatsky and aldol routes (my Routes 1.1/1.2) I discuss above and which were attempted by the Idenix team.

C.EXP5/67

L tab.192 vol 4

Comments on the Pharmasset Research

318. There is much less information available about the Pharmasset Research compared to the Idenix Research. Nothing in the information available changed the views I expressed above about the approach the Skilled Chemist would take to synthesising 2'-Me(up), 2'-F(down) nucleoside analogues before 21 July 2003, the size and difficulty of the task, or the prospects of success. Nor did the information about the Pharmasset Research affect the views I expressed about the Idenix Research. As I explained above, I consider the choices made by Dr Griffon and the Idenix team were reasonable – that Mr Clark and the Pharmasset team made different choices does not affect this view.

319. Overall, it appears that Pharmasset made more rapid progress, completing the synthesis in approximately 6 months, compared to 3 years for Idenix; however, it is unclear how many people were working on the project at Pharmasset.

320. The synthetic routes attempted by the Pharmasset team are very similar to those I proposed above and those attempted by the Idenix team. It is worth noting that Mr Clark initially started with the Reformatsky route, but as with Dr Griffon, he did not appear to succeed.

321. In both Clark I and Clark II the Pharmasset team highlight the difficulties associated with the synthesis, commenting in Clark I (page 5505):

C.EXP5/62 (L1/36)

“The fluorination of tertiary alcohols using DAST has been reported, but the stereochemistry of such transformations is substrate-specific and often unpredictable. For instance, Yang et al. reported that the DAST fluorination of a tertiary alcohol in 2-bromomethyl-DL-myo-inositol proceeds with retention of configuration¹⁴. Wachtmeister et al. obtained a 4-fluoro-1-cyclopentanol containing

⁵¹ Wang et al. (2009) *J Org Chem* 74, 17, 6819-24.

a tertiary fluorine in 25% yield using DAST as a fluorinating reagent, and this transformation proceeded with inversion of configuration¹⁵. Furthermore, dehydrations or eliminations, rearrangements, and ring contractions are often pervading problems in the DAST fluorination of highly functionalized molecules¹⁶

L4/203; L4/190;
L3/170

References 14, 15 and 16 are respectively to the Yang 1993, Wachtmeister and Singh and Shreeve papers I discuss extensively above.

322. Similar comments are made in Clark II regarding the difficulty of the tertiary fluorination in the sugar route. These are many of the same challenges I highlighted when discussing whether a Skilled Chemist would have been able to make these compounds before 21 July 2003.

C.EXP5/63 (L1/37)

323. The difficulty of the synthesis is also apparent from the fluorination conditions which Mr Clark used. The nucleoside route described in Clark I starts with TIDPS protected cytidine. The protecting groups are then changed to benzoyl esters (OBz) before the fluorination step. This suggests that Mr Clark may have encountered difficulties with the TIDPS protecting group, which is liable to be deprotected by fluoride sources, such as DAST. It is unclear how many other protecting groups Mr Clark tried before arriving at OBz, but it is quite possible that refining the protecting group strategy required a significant amount of effort.

324. Similarly, the conditions Mr Clark reports for the DAST fluorination (-20 °C in toluene) are not what might be considered "standard conditions". As I explain above in paragraphs 225-227, the first conditions a Skilled Chemist would try at the time would be room temperature in dichloromethane, either with or without pyridine. These were the conditions attempted by [REDACTED] Dr Griffon (with Deoxofluor) without success. Although there was some limited precedent for doing DAST reactions in toluene, this was at a higher temperature when changing solvent to one with a higher boiling point was logical. It is not clear how Mr Clark arrived at this unusual solvent and temperature combination (it is not suggested by the documents I believe the Skilled Chemist would have identified from the literature searches I discuss above); however, it is notable that once the Idenix team adopted these particular conditions, they too were successful.

325. Even though Mr Clark was ultimately successful in synthesising 2'-Me(up), 2'-F(down) nucleosides, it is also worth noting that the yields reported in Clark I, Clark II and the Pharmasset Patent are low and a significant quantity of by-products (including the elimination product) were observed. This, presumably prompted Pharmasset to

expend considerable effort developing improved syntheses, such as that reported by Wang et al.⁵²

Could the Skilled Chemist synthesise 2'-Me(up), 2'-F(down) nucleosides?

326. Bird & Bird have asked me to comment, in light of the Idenix Research and Pharmasset Research, discussed above, and any subsequent public journal articles relating to such syntheses, on whether a Skilled Chemist would have been able to make a 2'-Me(up), 2'-F(down) nucleoside by following each of the synthetic routes I identify above and the difficulty of such a synthesis.

Routes 1.1 and 1.2

327. Routes 1.1 and 1.2 both rely on a 'glyceraldehyde type' synthetic strategy. Route 1.1 is the 'Reformatsky' route, which I describe in paragraphs 196-204.
328. The Idenix team attempted Route 1.1 but the Reformatsky reaction failed and they were unable to form the acyclic precursor (which I refer to as compound 3, Scheme 1). I understand from Boons 1 that Mr Clark also attempted this route, but significantly it appears he moved on to other routes.
329. It is reasonable to assume that the Skilled Chemist would have faced similar failures. The Skilled Chemist would likely have attempted variations of reaction conditions for the coupling step, referring to examples of other similar Reformatsky reactions in the literature, although there would not have been any clear direction to conditions which would be likely to succeed. An alternative would be to look at different chemistry which makes use of the same synthetic strategy, such as Route 1.2 (if they had started with Route 1.1, or vice versa).
330. It appears the Idenix team adopted the latter approach, they switched focus to Route 1.2 after the failure of Route 1.1. The Idenix team attempted the aldol reaction three times with slight variations in conditions; each failed, with no product observed. As I described above for Route 1.1, the Skilled Chemist faced with failure would have undertaken similar troubleshooting, although there would similarly have been no clear guidance to conditions which were likely to succeed.
331. In light of these failures Dr Griffon appears to have decided to abandon this strategy. He was faced with a multi-step process with difficult stereochemical issues and

⁵² Wang et al. (2009) *J Org Chem* 74, 17, 6819-24.

difficulties associated with installing the base, in subsequent steps. Under these circumstances he may have considered it a more appropriate use of time and resources to pursue other synthetic routes. The Skilled Chemist attempting such a synthesis would probably have reached the same conclusion.

332. There is no published example of a successful peer reviewed synthesis of compounds 1e or 1f using Route 1.1 to the present date. The first successful peer reviewed synthesis to use Route 1.2 was published by Zhang et al. in 2009⁵³. Although this paper does not describe the synthesis of compound 1e or 1f, it does provide a synthesis of 2'-Me(up), 2'-F(down) ribonolactone precursor that could be transformed to 1e or 1f.

L tab.207 vol 4

333. It is also notable that other groups including that of Nobel prize winner Professor MacMillan published syntheses of 2'-Me(up), 2'-F(down) nucleosides (compound 1f) using Route 1.2 in 2014.⁵⁴ This shows that even ten years later this was considered challenging chemistry. I would not expect a chemist of Professor MacMillan's calibre to investigate this chemistry or the Journal of the American Chemical Society to accept it for publication if it was not considered novel and noteworthy. This suggests to me that this is the sort of chemistry the Skilled Chemist would have struggled to successfully complete in 2003.

L tab.144 vol 3

Route 2

334. Route 2 involves the electrophilic fluorination of a ribonolactone derivative, as I explain in paragraphs 208-213.

335. The Idenix team attempted a similar approach towards electrophilic fluorination, attempting fluorination of an cyclic enol ether (rather than a lithium enolate as I proposed in Route 2) with Selectfluor. Despite attempting the reaction four times, with progressively higher temperatures, longer reaction times and larger amounts of Selectfluor, this approach failed with no reaction observed.

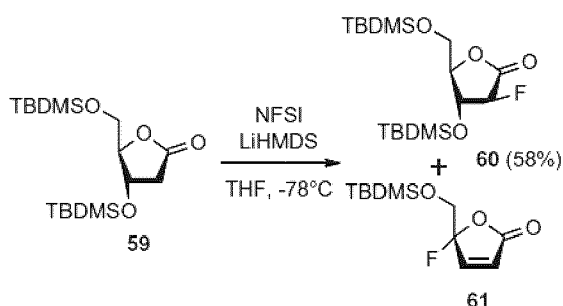
336. It is reasonable to assume that the Skilled Chemist would have faced similar failure. In response the Skilled Chemist would have attempted fluorination of lactone enolates or silyl enol ethers using Selectfluor or similar reagents, as I describe in paragraph 212 above. However, there was no clear guidance in the literature or CGK which could lead the Skilled Chemist directly towards successful conditions.

⁵³ P. Zhang, et al. *Tetrahedron: Asymmetry*, 2009, 20, 305-312.

⁵⁴ Pieffer & MacMillan *J. Am. Chem. Soc.* 2014, 136, 5900-5903.

337. There are no peer reviewed reports of successful syntheses of compounds 1e or 1f to the present date using this type of route.

L tab.33 vol 1 338. In 2009 Cen *et al* (see Scheme 8, below)⁵⁵ published a paper showing that electrophilic fluorination of a simple protected ribonolactone with NFSI gives the 2'-fluorine in the up configuration. However, there is also a side product which arises from elimination of the protected 3'-OH, followed by fluorination at the 4'-position. So the later literature highlights there are issues with this kind of chemistry – eliminations and side reactions as well as stereochemical issues.



Scheme 27: Cen et al. electrophilic fluorination

L tab.144 vol 3 339. In 2014 MacMillan et al. reported fluorination of a 2-methylribonolactone with Selectfluor. They reported that this fluorination reaction gives almost exclusively the 2'-Me(down), 2'-F(up) product. This suggests that, in principle, if the fluorination was conducted first, followed by methylation, the 2'-Me(up), 2'-F(down) it may be possible to make the 2'-Me(up), 2'-F(down) product; however, this remains to be tested.⁵⁶

L tab.33 vol 1

340. From this it is reasonable to conclude that even if the Skilled Chemist had attempted this route, and had been able to get the fluorination to work at all, it is likely they would have encountered issues with elimination and possible problems with stereocontrol, which would have been challenging to address.

Route 3.1

341. Route 3.1 relies on nucleophilic fluorination of either a 2'-Me, 2'-OH nucleoside or a 2'-Me, 2'-OH ribose derivative, as I explain in paragraphs 222-233

342. As I explain in paragraph 227, the Skilled Chemist would initially have attempted this fluorination using DAST or Deoxofluor at room temperature in dichloromethane with

⁵⁵ Cen & Sauve *J. Org. Chem.* 2009, 74, 16, 5779–5789.

⁵⁶ *Ibid.*

and without pyridine. As I explain at paragraph 228, if the Skilled Chemist observed decomposition or formation of side products, they would normally try to reduce the temperature. Indeed Dr Griffon initially attempted to fluorinate 2'-Me(down), 2'-OH(up) uridine using Deoxofluor starting at such a reduced temperature (namely -78 °C then allowing it to warm to room temperature) in dichloromethane, with pyridine; however, this only resulted in an elimination product. This suggests that a Skilled Chemist attempting this reaction at room temperature or subsequently at a reduced temperature would also have obtained elimination products in each case. As the reaction had been unsuccessful with Deoxofluor, the Skilled Chemist would likely attempt using DAST (or vice versa, if they had initially tried with DAST).

343. [REDACTED]

344. [REDACTED]

345. [REDACTED]

346. Although I noted in paragraph 226 that a Skilled Chemist might consider using toluene, this was not common in the literature and the Skilled Chemist would likely only consider it if they wanted to run the reaction at a higher temperature than could

be achieved in dichloromethane. The Skilled Chemist would not have any reason to switch from dichloromethane to toluene if they intended to run the reaction at -20°C .

347. From this I conclude that, if the Skilled Chemist had started down this route, they would have initially attempted fluorination under the standard conditions I refer to at paragraph 227 (i.e. DAST, dichloromethane, room temperature, with/without pyridine) and they would have failed to produce the fluorinated nucleoside. From there, there was no clear guidance which would lead the Skilled Chemist to the ultimately successful conditions, involving changing solvent to toluene whilst also reducing the temperature, to arrive at conditions which might provide the fluorinated nucleoside and even then at low yield [REDACTED].

C.EXP1/2

348. Where direct nucleophilic fluorination had failed, the Skilled Chemist might also consider a two-step fluorination, involving forming a good leaving group, such as a mesylate or tosylate, followed by displacement with a nucleophilic fluorine source, as I explain in paragraph 229. Dr Griffon also tried a similar approach, converting the 2'-OH of the 2'-Me(down), 2'-OH(up) uridine to a sulphonyl-imidazole leaving group before trying fluorination using $\text{Et}_3\text{N} \cdot 3\text{HF}$. He states that he concluded no reaction had occurred.⁵⁷ Accordingly I consider that this route would also have failed to produce the desired product for the Skilled Chemist.

Route 3.2

349. Route 3.2 involved the nucleophilic fluorination of an alkenyl sugar or nucleoside derivative, as I explain in paragraphs 234-237.

350. Dr Griffon attempted this route – initially attempting iodofluorination of a 2'-methylene nucleoside using AgF and I_2 at 0°C . Dr Griffon attempted this reaction twice, in both cases only observing a mixture of iodomethyl 2,2'-anhydronucleoside products and no fluorination.

351. It is reasonable to assume that the Skilled Chemist would have faced similar results. In response, the Skilled Chemist would have two options, troubleshoot by varying reaction conditions, or try to further react the 2,2'-anhydronucleoside by-products to give the desired fluorinated nucleoside. Dr Griffon pursued both approaches. He attempted to fluorinate the alkenyl nucleoside under different conditions, using Olah's

⁵⁷ I did not have access to the details of Dr Griffon's laboratory notes in relation this reaction, so cannot comment in detail on the outcome.

reagent (HF/pyridine) with AlF_3 in THF. Dr Griffon attempted this fluorination at 0 °C, without success.⁵⁸ Under these circumstances, the Skilled Chemist would have probably increased the reaction temperature. This is exactly what Dr Griffon did, increasing the temperature to 100 °C, but this also failed to produce a fluorinated product. Dr Griffon then attempted this reaction again at both 80 °C and 100 °C after protecting the starting material at the 3-position of the base, but observed no reaction.

352. At the same time Dr Griffon attempted to ring-open the 2,2'-anhydronucleoside under a range of conditions. Dr Griffon tried to fluorinate the iodomethyl 2,2'-anhydronucleoside using HF/pyridine in THF at 100 °C, which gave no reaction. Then he reduced the iodomethyl 2,2'-anhydronucleoside to the methyl 2,2'-anhydronucleoside and attempted to fluorinate it using KF and Kryptofix 2.2.2 at 110-150 °C in DMF. When this reaction did not progress, he added more KF at increased the temperature to 160 °C, but still did not observe any reaction. Dr Griffon tried further variations in conditions, attempting this fluorination with HF/pyridine and AlF_3 at 80 °C, then 120 °C and finally with KHF_2 in refluxing ethylene glycol (197 °C) and KHF_2 in refluxing 2-ethoxyethanol (135 °C). In each case Dr Griffon appears to have observed no reaction by TLC and notes that he did not isolate and characterise any products. Dr Griffon finally attempted this reaction using $\text{Bu}_4\text{NH}_2\text{F}_3$ and $\text{Fe}(\text{AcAc})_3$, but reported only recovering starting material.
353. Dr Griffon tried a wide range of reagents and conditions, as well as investigating different protecting groups, in an attempt to fluorinate the alkene substrate. Although there are other conditions which could have been attempted, there is no indication these would have been successful. Indeed, there are no reported peer reviewed syntheses 2'-Me(up), 2'-F(down) nucleosides using this route to the present date. As such, I consider that it is likely that the Skilled Chemist in 2003 would have similarly failed.

Route 3.3

354. Route 3.3 involves ring opening an epoxide with a nucleophilic fluoride source. As I explain in paragraphs 238-239, this route has several challenging steps, requiring the formation of a epoxide, then fluorination to give a 2'-hydroxymethyl, 2'-F nucleoside and finally deoxygenation to give the target compound. This is route is relatively long,

⁵⁸ I did not have access to the details of Dr Griffon's laboratory notes in relation this reaction, so cannot comment in detail on the outcome.

has limited precedent and involves potentially challenging chemistry, so it is less likely that the Skilled Chemist would prioritise it, unless other routes had already failed.

- C.EXP1/2**
355. Despite this, Dr Griffon attempted a similar approach, involving forming a chloro epoxide at the 2-position of a ribose derivative, followed by fluorination; however, he was unable to make the epoxide precursor and did not pursue the route further.
356. There are no reported syntheses of compounds 1e or 1f using this route to the present date. It is notable that paper by Dai and Piccirilli provides the only evidence that this approach may be possible, but as discussed in paragraphs 239 above, the ring opening of the epoxide with fluoride leads to many side products, the incorrect stereochemistry and impractically low yields. Accordingly, I consider that it is likely that the Skilled Chemist attempting this route in 2003 would also have failed.

STATEMENT OF TRUTH

I confirm that I have made clear which facts and matters referred to in this report are within my own knowledge and which are not. Those that are within my own knowledge, I confirm to be true. The opinions I have expressed represent my true and complete professional opinions on the matters to which they refer.

I understand that proceedings for contempt of court may be brought against anyone who makes, or causes to be made, a false statement in a document verified by a statement of truth without an honest belief in its truth.

Signed:



Full Name: Jason Micklefield

Date: 2 November 2022