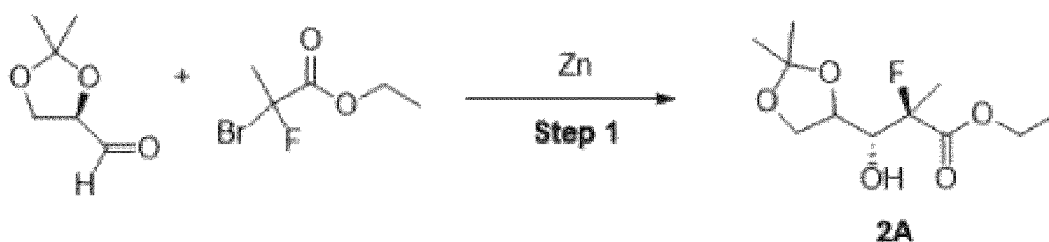


SECOND DECLARATION OF PROFESSOR GEERT-JAN BOONS

1. In my declaration of 24 October 2018, I stated that the synthesis of a 2'-fluoro-2'-methyl nucleoside in 2003 (or in 2004) would, in my opinion, have been a research project of significant complexity and uncertain outcome.
2. I have read the patentee's arguments dated 6 November 2020 on this issue and the associated documents cited in these arguments. In particular, I have reviewed Dr Gary Allred's declaration, which I understand is denoted D87 in these proceedings.
3. I continue to be of the opinion that the synthesis of a 2'-fluoro-2'-methyl nucleoside in 2003 (or in 2004) would have been a research project of significant complexity and uncertain outcome.
4. I have read NuCana's patent EP-B-2955190. EP-B-2955190 teaches the skilled person nothing about how to prepare 2'-fluoro-2'-methyl nucleosides. I do not agree that if tasked with preparing a 2'-fluoro-2'-methyl nucleoside, the skilled person would consider gemcitabine, a 2',2' di-fluoro nucleoside, to be an obvious starting point.
5. Fluoro and methyl are very different moieties. Fluorine is the most electronegative element and is small. The presence of fluorine in a molecule or a reaction is expected to have a large electronic impact as it is strongly electron-withdrawing, but a small impact on steric hindrance. In contrast, a methyl group is a slightly electron-donating moiety. It is considerably larger than a fluorine atom and has a correspondingly-greater impact on steric factors in a molecule or a reaction. Replacing a fluorine atom with a methyl group does not constitute a minor difference and has a substantial impact on chemical transformations.
6. Gemcitabine does not have a chiral centre at the 2' position. There is a fluoro substituent both above the ring ("up") and below the ring ("down"). In contrast a 2'-fluoro-2'-methyl nucleoside is chiral at the 2' position, for example in the case of 2'-fluoro down and 2'-methyl up, and the skilled person seeking to make such a compound would know that he/she needs to take account of controlling the stereochemistry at that centre.
7. In 2003, the skilled person would not have been aware of any report of nucleosides or 5-membered sugar rings with a fluorine and a methyl group at the 2' position. Knowing that a new, chiral substitution pattern would need to be introduced, the skilled person would not be drawn to a compound whose synthesis routes do not require a chiral centre at that carbon.
8. The difference between gemcitabine and a 2'-fluoro-2'-methyl nucleoside is not minor and in my opinion a skilled person who in 2003 was seeking to attempt to synthesise a 2'-fluoro-2'-methyl nucleoside would not consider gemcitabine to be an obvious starting point.
9. It would not be regarded as relevant that a reliable and scalable route to gemcitabine was available. Just because one particular process has, as the result

of research, often quite substantial research, resulted in a scalable version, it does not follow that another, and substantially different process will be either reliable or scalable.

11. Therefore, in my opinion, the skilled person would not consider EP-B-2955190 to teach or suggest gemcitabine as a starting point for the synthesis of a 2'-fluoro-2'-methyl nucleoside. Nor would the skilled person consider gemcitabine to be a favourable starting point based on his/her common general knowledge.
12. Even if the skilled person were to be presented with gemcitabine and instructed to synthesise a 2'-fluoro-2'-methyl nucleoside by analogy with a gemcitabine synthesis process, he/she would not be confident that this could be done, let alone that it could be done without undue burden in 2003 (or in 2004). It would be a research project.
13. As Dr Allred notes, there are several variations on the known processes for synthesising gemcitabine. The synthesis route devised by Dr Allred for preparing a 2'-fluoro-2'-methyl nucleoside has seven steps depicted in paragraph 17 of his declaration. The first step is copied below



14. I am not aware of any paper published before 2003 that employed BrCFMeCO₂Et in a Reformatsky reaction. A skilled person would not be confident of the results of such a reaction. Organofluorine chemistry is recognised as continuing to be an active field of research. There is a journal dedicated to it (Journal of Fluorine Chemistry); there are academics who specialise in this field and special courses are given on this topic. Once something has been done, it can be seen that it was possible. However, before carrying out a Reformatsky reaction with a new reagent, the skilled person would not be confident of succeeding.
15. Even if Step 1 proceeds as written down in D87, the resulting product contains two new chiral centres. I would expect that the product mixture obtained would therefore consist of four diastereomers, and I am not aware of any literature to the contrary. Indeed, I note that step 1 is reported in Dr Allred's declaration as producing four diastereomers.
16. In addition, the first reagent in step 1 is a glyceraldehyde derivative that is susceptible to racemisation. There would be an additional concern that the chiral centre of the glyceraldehyde may racemise, resulting in double the number of diastereomers. Before proceeding to the next step, the skilled person would need to separate out the desired diastereomer, which may require considerable effort or not be accomplished at all.
17. The skilled person would additionally have concerns about the glycosylation step to install a base (depicted as Step 6 in D87, preceded by some preparatory steps).

The stereoselective formation of a glycosidic linkage is one of the most challenging aspects of synthesis of a nucleoside or oligosaccharide. This is discussed in sections 11.46 to 11.49 of my expert report to the UK High Court, which I understand is denoted D43 in these proceedings. There are many choices to be made regarding parameters such as the leaving group, protecting groups, reagents and reaction conditions. Furanoses can adopt multiple envelope configurations which will affect the stereochemistry of the glycosylation differently. It is not possible to generalise such a reaction. Conditions that have been found to be adequate to install one particular base on one particular sugar cannot confidently be predicted to apply to a different combination of base/sugar.

18. I understand that an excerpt from chapter 3 of a textbook that I authored has been submitted as document D103. This excerpt refers to the use of DAST as a fluorinating agent in the context of a secondary alcohol (scheme 3.2), and not a much more difficult tertiary alcohol. A complete copy of chapter 3 is attached. Other fluorination methods are also discussed (see page 64). It is also discussed that nucleophilic substituents at carbohydrates can be difficult due to the presence of permanent dipoles (see page 62), and a successful nucleophilic substitution on a simple substrate does not necessarily give confidence that such a transformation can be performed on carbohydrate derivative.
19. In conclusion, I remain of the opinion that the synthesis of a 2'-fluoro-2'-methyl nucleoside in 2003 (or in 2004) would have been a research project of significant complexity and uncertain outcome.



Signed:

Dec 8th, 2020

Date: