Second Expert Report of Jason Micklefield
9 December 2022
Filed on behalf of the Claimants
Exhibit JM-7

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

SECOND 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: MY INSTRUCTIONS

1. I am the same Jason Micklefield who provided a first expert report in these proceedings on 2 November 2022 (my "**First Report**"). I adopt the definitions set out in my First Report.

2. I have been provided by Bird & Bird with a redacted copy of the expert report of Professor Stephen Davies ("**Davies 1**") and with a copy of the expert report of Professor Katherine Seley-Radtke ("**Seley-Radtke 1**") and asked to give my views on them, which I set out below. Bird & Bird explained that Professor Davies' report referred to certain material, the admissibility of which was in dispute. I was provided with a redacted copy of Professor Davies' report which excluded this disputed material, and without exhibits SGD-4, SGD-5 and SGD-6. A copy of the redacted report is attached as **Exhibit JM-7**. That is the version of Professor Davies' report to which I

D1 tab. 10, 11, 12
C2 tab. 18

respond in this second report. I am told that I will be asked to address the passages of Professor Davies' report which were redacted in a further report.

3. There are many points in the reports of Professor Davies and Professor Seley-Radtke which I have not dealt with, and it should not be assumed that I agree with points which I do not address. In most instances I believe I have already addressed these points in my First Report.

4. I set out my understanding of my duty to the Court in paragraph 12 of my First Report and confirm that I have complied with that duty in making this second report.

SECTION 2: CORRECTIONS TO MY FIRST REPORT

5. In paragraph 142(a) of my First Report, I describe the nucleoside route as a synthesis starting from a natural nucleoside. Although the nucleoside route will usually start with a natural nucleoside, because it is often a cheap and convenient starting material, I did not intend to suggest that this is always the case. The key feature of the nucleoside route is that the starting material includes a sugar attached to a nucleobase, which is converted into the target compound through functional group interconversions and other transformations.

6. In paragraph 184 of my First Report, I comment that the glyceraldehyde route requires the formation of three stereocentres at the 1′, 2′ and 3′-positions and that each of these would result in the formation of two diastereomers. I would like to clarify this statement. The formation of the bond between (what will become) the 2′-carbon and the 3′-carbon of the ribose ring simultaneously creates two new stereocentres. As a result, this reaction will produce a mixture of up to four diastereomers in one step (see paragraph 202 of my First Report). Only one of those four possible diastereomers corresponds to the desired stereochemistry and the other three must be separated and discarded. This separation is likely to be significantly more challenging than separating a mixture of only two diastereomers and, as I explain in paragraph 184 of my First Report, the Skilled Chemist would not have known in advance how much, if any, of the desired diastereomer would be formed. As I explain at paragraph 183 of my First Report, attaching the base to the ribose unit will result in the formation of a stereocentre at the 1′ position, giving two new diastereomers (α and β anomers). As a

result, if the initial mixture of diastereomers is not separated prior to attaching the base, up to eight total diastereomers would be formed.

SECTION 3: RESPONSE TO THE REPORT OF PROFESSOR DAVIES

7. In my first report at paragraphs 22 to 29, I address the attributes of the Skilled Chemist. On reading Professor Davies' report. I am not sure of the identity of the skilled person to which Professor Davies considers the Patents are addressed. In paragraph 2 Professor Davies explains that he has been asked to provide evidence in relation to whether 'a skilled organic chemist' would have been able to synthesise compounds of formula III. In paragraph 3, Professor Davies states that it was explained to him that the skilled organic chemist is uninventive, but has "*a good background technical knowledge in the field of organic chemistry*". Other than these paragraphs, Professor Davies does not refer to the background, training, or experience level of the skilled organic chemist at any point in his first report.

8. It is also unclear how the skilled organic chemist Professor Davies refers to relates to the broader skilled team identified by Professor Seley-Radtke (discussed in her report).

9. Accordingly, throughout this report (my Second Report) I respond to Professor Davies' evidence on the basis of a skilled organic chemist with the same attributes (training, background and experience) as the Skilled Chemist in my First Report and operating as part of a skilled team as considered in my First Report.

Synthetic routes

10. Professor Davies and I seem to have approached the question of how a Skilled Chemist would consider the synthesis of the compounds 1e and 1f in a very different manner. In particular, I have approached the synthesis on the basis that the Skilled Chemist would initially conduct searches for synthetic routes to the compounds themselves, before realising they would have to design completely new synthetic routes as no such information was available. Professor Davies does not appear to have conducted such an analysis, and rather has just identified two possible routes, both of which he says would have been successful, without apparently consulting textbooks, conducting literature searches or performing a thorough analysis of what a Skilled Chemist (without knowledge of how to make the compounds) would have done. Professor

Davies also does not conduct an analysis of the potential problems which a Skilled Chemist would envisage with the routes he identifies.

11. In paragraph 25, Professor Davies states that it would be standard practice for a skilled organic chemist to work by analogy and adapt previous syntheses to make new compounds. I agree that working by analogy was (and still is) standard practice in organic synthesis and would have been an approach adopted by the Skilled Chemist. However, the Skilled Chemist would not assume that an existing synthetic route or method would work when applied to a new compound, even if it was structurally similar to the previous compound. They would try to propose multiple routes (as I explain in my First Report), exactly because it would not be clear if any of them might work, and they would recognise that each route may or may not be a successful way to make the new compounds.

12. I disagree that Professor Davies' report represents what a Skilled Chemist in 2003 would have done. Rather they would have recognised the challenge involved in synthesising a modified nucleoside with a tertiary fluoride at the 2′-position and would have conducted a thorough analysis of potential approaches before embarking on their synthesis. This is also what the team of real-world chemists at Idenix did, as I explain in paragraphs 256-267 of my First Report.

13. I discuss below the limited information which Professor Davies does provide on his analysis of what a skilled organic chemist would do.

14. Professor Davies in a number of places cites very old, or very unusual, papers in support of his opinions, which I do not understand how he identified, and which I do not think would have formed part of the CGK. For example, at paragraph 19 Professor Davies states that a skilled organic chemist would have known methods of converting cytidine to uridine (and *vice versa*). I agree that Skilled Chemist may have been aware of methods for converting cytidine to uridine (including using cytidine deaminase), as I indicated at paragraph 189 of my First Report. However, the paper by Hurlbert does not describe methodology for the synthetic transformation of uridine to cytidine. This is an old biology paper describing the conversion of uridine nucleotides to cytidine nucleotides (i.e. substrates possessing 5′-phosphate groups, not nucleosides) using enzymes extracted from rat tumour and liver tissue. In my view this paper would not have formed part of the CGK and would not have been located or read by the Skilled Chemist. In the unlikely event that the Skilled Chemist decided to read and follow this paper, they would have struggled to remove tumour or liver tissue from rats and then

isolate soluble enzyme fractions as described by Hurlbert. Even if enzyme fractions from rats were available, Hurlbert provides no indication that these extracts might be used in to synthesise 2′-modified cytidine nucleosides.

L tab.85 vol 2 15. Similarly, the paper by Krenitsky et al. (1981), cited by Professor Davies at paragraph 19, including the conversion of cytidine to uridine (arabinonucleosides), relates to an enzymatic synthesis method published in the 1980s, which I do not think would have formed part of the CGK and would not have been located or read by the Skilled Chemist. Rather a Skilled Chemist would be likely to identify and use a chemical synthetic approach (as opposed to using enzymes that had to be produced and isolated from rat (Hurlbert) or bacterial cells (Krenitsky)).

16. Similarly at places Professor Davies cites papers without explaining where or how a Skilled Chemist would be expected to have identified them. I discuss each of these below where they arise.

Professor Davies' section B.1. route

Hertel 1998 L tab.73 vol 2 17. In paragraphs 26-27, Professor Davies asserts that each of the papers by Hertel 1988, Weigel 1997, Chou 1992, Pankiewicz 2000 and Vorbrüggen 2000 would have formed part of the CGK. I disagree that the Skilled Chemist would have been aware of these papers and referred to them as a matter of course (which is how Professor Davies explains his understanding of the CGK in paragraph 3), or that they would have formed part of the CGK as I understand it (see paragraphs 30 and 31 of my First Report). Professor Davies suggests that the skilled organic chemist would identify these papers by searching in the Merck Index for gemcitabine. I do not think the Skilled Chemist in 2003 would have been likely to refer to the Merck Index. It is more likely that they would have referred to an online search (of the type I describe at paragraph 39 of my First Report) instead. If Skilled Chemist did search in the Merck Index, this would identify Hertel 1988 and Chou 1992, but not the other references, which could only have been identified after a literature search.

Weigel L tab.195 vol 4

Pankiewicz 2000 L tab.138 vol 3

Vorbruggen 2000 L tab.189 vol 4

Hertel 1988 L tab.73 vol 2

Chou 1992 L tab.35 vol 1

18. As I explain in my First Report (see paragraph 142), the Skilled Chemist would have been aware of the glyceraldehyde route as a general method for making nucleosides, but would not know specifically how to apply this to making compounds 1e and 1f. In paragraph 192 of my First Report, I explain the steps the Skilled Chemist would go through to search the literature and propose synthetic routes to compounds 1e and 1f. In relation to the glyceraldehyde route, I explain that the Skilled Chemist would, as part of their literature search, identify reviews such as Pankiewicz 2000, and from this

L tab.138 vol 3

L tab.73 vol 2

locate the synthesis of gemcitabine in Hertel 1988, which would serve as a starting point for proposing an analogous synthetic route to compounds 1e/1f. The Skilled Chemist would then conduct further Analogy Searches to identify specific conditions which could be adapted for the synthesis of compounds 1e/1f (as I explain in detail in paragraphs 194-207 of my First Report). In contrast Professor Davies (in paragraphs 29-32) suggests that the skilled organic chemist would propose a route based on the synthesis of gemcitabine, but states the skilled organic chemist would simply adapt the route in Hertel 1988 (Reformatsky) or Weigel 1997 (aldol), without providing any further detail, before considering a route based on the Weigel procedure. Professor Davies does not provide any explanation of whether (or why) his skilled organic chemist would anticipate that this synthetic route (or any other route) would succeed when one of the fluoro groups has been replaced by a methyl group (which the Skilled Chemist would consider a significant change), nor does he address any of the challenges which would be apparent to the Skilled Chemist in adapting the Weigel route. As I explain above at paragraph 6 and in paragraphs 199-207 of my First Report, there are multiple issues, particularly around stereocontrol, which the Skilled Chemist would be aware would need to be solved in order to succeed using this route.

Hertel 1988
L tab.73 vol 2

Weigel 1997
L tab.195 vol 4

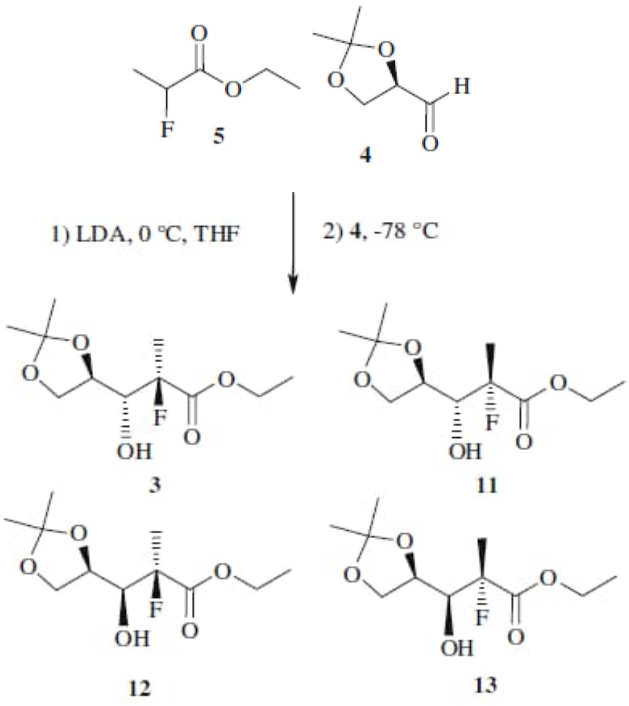
19. The Skilled Chemist, in 2003, considering attempting the 'Analogous Gemcitabine Route', following Wiegel as suggested by Professor Davies, would have been aware that the change from 2,2-difluoroacetate to 2-fluoropropanoate aldol substrates would increase the number of possible diastereomers formed. The Skilled Chemist would also have appreciated that the diastereomers formed in the Analogous Gemcitabine Route could be difficult to separate.

20. The challenges that the Skilled Chemist would face in this synthetic route can clearly be seen in subsequently published work on this synthetic route from Hoffman-La Roche. As I explain in paragraph 332 of my First Report, the first peer reviewed synthesis of a 2′-Me(up), 2′-F(down) ribonolactone using an aldol route was published by Zhang et al. in 2009[1] (working at Hoffman-La Roche). This paper attempted to apply a similar aldol route to that described by Weigel to make a 2′-Me(up), 2′-F(down) ribonolactone.

L tab.207 vol 4

[1] P. Zhang, et al. *Tetrahedron: Asymmetry*, 2009, 20, 305–312.

21. Zhang et al. conducted the aldol condensation of compounds 4 and 5, as shown in Figure 1 below. This resulted in a mixture of four diastereomers, as the Skilled Chemist would have anticipated (see paragraph 6 above and paragraph 202 of my First Report).

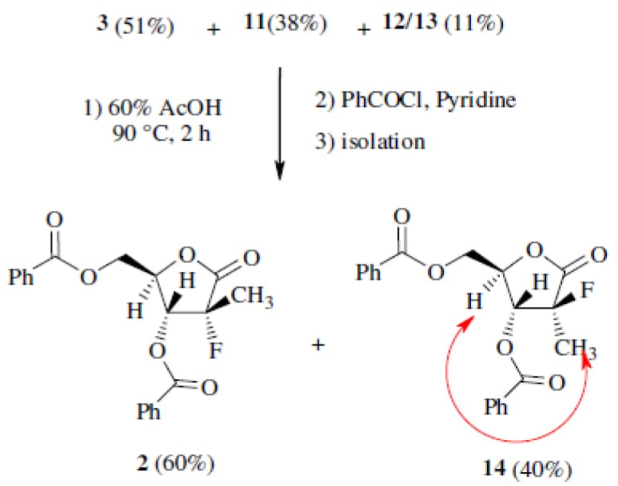


L tab.207 vol 4

Figure 1: Zhang et al. aldol condensation

22. The authors state that "*the initial assignment of the structures* (3, 11, 12 & 13 shown above in Figure 1) *was conducted via a tedious process. The attempts to isolate individual isomers using column chromatography were unsuccessful.*" The authors found it necessary to deprotect and cyclise the mixture of diastereoisomers before benzoylating to give protected lactones. The two major lactone products were isolated (compounds 2 and 14 see Figure 2 below). The main product was confirmed to be compound 2 (derived from 3) by comparison with an authentic sample (i.e. material which had been previously synthesised and fully characterised). The stereochemistry of compound 14 (derived from 11) was confirmed with "*extensive NMR studies*", including the observation of an NOE between the C-4 proton and C-2 methyl group (shown by the red arrow in Figure 2 below). Compounds 12 and 13 could not be fully purified, so the identity of compound 13 was determined by oxidising a mixture of compounds 3 (where the C-2 configuration was known) along with 12 and/or 13. Comparison of the oxidation products confirmed the two compounds had different C-2 stereochemistry, identifying compound 13 by a process of elimination. A sample of the remaining isomer, compound 12, was prepared from compound 3 by an oxidation-reduction sequence. However, compound 12 co-eluted with compound 13 on GC

analysis, so the authors were ultimately unable to determine the ratio of those compounds 12 and 13 in the original reaction mixture.



L tab.207 vol 4

Figure 2: Zhang et al. cyclisation of aldol products

23. Zhang et al. then undertook an extensive study of aldol reaction conditions in an attempt to optimise the diastereoselectivity needed for the 2′-Me(up), 2′-F(down) lactone. Despite optimisation, the authors were not able to sufficiently reduce the number of undesired diastereomers formed. Ultimately Zhang et al. had to develop a kinetic resolution with the *Candida antarctica* lipase (CALB) enzyme, selectively hydrolysing one of the unwanted diastereomers to aid separation of the complex mixture.

24. In addition, if racemisation of the protected glyceraldehyde (i.e. compound 4 above) occurred (due to enolization) during the aldol reaction this would lower the enantiomeric purity of the four diastereoisomers formed (as Professor Davies notes in in Exhibit SGD-02, paragraph 16). In other words, the Skilled Chemist in 2003 would also need to consider the possibility that the analogous aldol reactions in addition to producing up to four diastereoisomers may also generate the enantiomers of each of these diastereoisomers (i.e. may form up to eight possible stereoisomers). As compounds 1e/1f (and Formulas I and III of the Patents) are drawn with fixed absolute stereochemistry, they describe single enantiomers. As a result, the Skilled Chemist would need to ensure that the synthesis either produced an enantiopure product, or would have to separate the unwanted enantiomers (as well as unwanted diastereomers).

D2 tab. 8

25. Although Weigel does not report any racemisation of glyceraldehyde, it would not be clear to the Skilled Chemist if this would be the case for analogous aldol reactions (i.e.

when using different substrates/conditions). Consequently, if the Skilled Chemist was able to produce and separate the required diastereoisomer, they would need to also determine its enantiopurity. This could be done by comparing the optical rotation of the product with an authentic sample (as Zhang did), but no sample was available to the Skilled Chemist in July 2003. Alternatively, the Skilled Chemist could perform the aldol reaction with racemic glyceraldehyde (or L-glyceraldehyde) and then establish a method for separating the enantiomeric aldol products (e.g analytical chiral HPLC or GC). If the enantiomers, of the required diastereoisomer, could be separated, then its enantiopurity could be determined.

26. This work by Zhang et al. highlights that, even six years after 2003, successfully implementing an Analogous Gemcitabine Route was a substantial challenge for a research team at a major multinational pharmaceutical company. This was the case even though Zhang et al. benefited from published details of existing previous successful syntheses and comparisons with authentic samples of key compounds, which would not have been available to the Skilled Chemist in 2003.

Professor Davies' Section B.2. route

27. In paragraphs 40-44 Professor Davies suggests that the skilled organic chemist could also have synthesised compounds 1e and 1f through a modified version of the Analogous Gemcitabine Route, which employs a "SuperQuat" chiral auxiliary. I do not agree that the Skilled Chemist in 2003:

a. Would have been aware of the "SuperQuat" chiral auxiliary;

b. Would have considered using a chiral auxiliary approach, except as a last resort (i.e. where multiple other routes had failed); or

c. Would have been confident that the route proposed by Professor Davies would have succeeded.

Bull 1999 L tab.29 vol 1

O'Brien 2001 L tab.132 vol 3

28. Professor Davies asserts (at paragraphs 40-41) that the Bull 1999 and O'Brien 2001 papers (cited at paragraph 41) would have been "well-known to the skilled organic chemist" and implies that the SuperQuat family of chiral auxiliaries would also have been well known. I was not aware of SuperQuat auxiliaries in 2003 (I was aware of the original Evans' auxiliary which the SuperQuat auxiliary is derived from), nor was I

aware of them before reading Professor Davies' report, and I believe the Skilled Chemist would similarly not have been aware of them.

29. SuperQuat chiral auxiliaries were developed in the late 1990s by Professor Davies' research group.[2] Since being provided with Professor Davies' report, I have searched for papers using this methodology and note that by 2003 most of the published papers citing their use are by Professor Davies' group. A search for "SuperQuat" prior to the end of July 2003 gives 13 references, 10 of which are from Professor Davies group. This supports my view that SuperQuat chiral auxiliaries would not have been part of the Skilled Chemist's CGK.

Bull 1999 L tab.29 vol 1

O'Brien 2001 L tab.132 vol 3

30. In my opinion the Bull 1999 and O'Brien 2001 papers (cited at paragraph 41) would not have been known to the Skilled Chemist or formed part of the CGK. The Bull paper was only cited 22 times by July 2003, mainly by the Davies lab or the Seebach group who were developing related but different auxiliaries at the time. The O'Brien paper was only cited 14 times by July 2003. This number of citations does not suggest either of these papers were well known in July 2003. It is also unclear how a Skilled Chemist tasked with synthesising compounds 1e or 1f would have located and read either of Bull 1999 or O'Brien 2001. Neither paper contains any examples of aldol reactions using fluorinated enolates, nor do they contain any examples using substrates which are structurally similar to those needed for the synthesis of compounds 1e or 1f.

Bull 1999 L tab.29 vol 1

O'Brien 2001 L tab.132 vol 3

31. Beyond asserting that the Bull 1999 and O'Brien 2001 papers were well known, Professor Davies does not explain how the Skilled Chemist would have identified SuperQuat auxiliaries, or even a chiral auxiliary strategy more generally, as being of interest in the synthesis of compounds 1e and 1f. A chiral auxiliary approach is both step and atom inefficient. By step inefficient I mean that it adds steps to the synthetic route. Compared to a conventional aldol approach, the SuperQuat route requires three additional steps to make the SuperQuat auxiliary, one extra step to couple it with the substrate and one extra step to remove it again after the aldol reaction. Even if Professor Davies is correct that SuperQuat compound 16 was commercially available in 2003 (which I am unable to confirm), the SuperQuat approach still requires two additional steps. Similarly, it is atom inefficient, in that it requires the addition of all of the atoms in the SuperQuat auxiliary, which are ultimately discarded. The Skilled Chemist would normally favour the simplest and shortest route, so would not consider

[2] See Davies 1, paragraph 41; Bull 1999; O'Brien 2001.

a route with these types of inefficiencies unless there were no alternatives or a good reason to move to it of which they were already aware.

32. In paragraphs 43-44 and Scheme 2, Professor Davies proposes a synthetic route to his compound 1b, which he asserts a skilled organic chemist would have proposed based on Bull 1999, O'Brien 2001 and a further paper by Heathcock et al. In my view the Skilled Chemist would not have proposed this route based on their CGK. I also do not consider that the Skilled Chemist considering the papers identified by Professor Davies would have identified this route. Professor Davies does not provide any more detail regarding where the reaction steps and conditions shown in Scheme 2 have come from. Notably none of the papers cited appear to provide any basis for the chemistry proposed in Scheme 2. Bull 1999 discusses SuperQuat chemistry, but only in relation to studies of benzylation (alkylation) reactions of simple propanoate substrates (i.e. non-fluorinated). There is no information in the Bull 1999 paper which relates to aldol reactions, nor any examples relevant to the proposed synthesis in Scheme 2. O'Brien 2001 is a review, which contains a short section on "*Aldol reactions and related processes*". This section provides a range of examples of other chiral auxiliaries, but does not contain any examples of using SuperQuat auxiliaries in aldol reactions. The only mentions of SuperQuat auxiliaries relate to α-alkylation and Michael addition reactions. Finally, Heathcock 1980 provides examples of aldol condensation of aldehyde 9 in Scheme 2 with lithium enolates, but does not have any relevance to SuperQuat chemistry. Professor Davies also notes that the Heathcock 1980 paper is cited on page 8437 (reference 35) of Harris 1999; however, this citation is in relation to a diene-aldehyde cyclo-condensation reaction in the synthesis of a complex macrolide natural product. The Skilled Chemist would have absolutely no reason to search for or look at this paper, nor would they see any analogy to the synthesis of Davies' compound 1b.

Bull 1999
L tab.29 vol 1

O'Brien 2001
L tab.132 vol 3

Heathcock 1980
L tab.71 vol 2

Heathcock 1980
L tab. 71 vol 2

Harris 1987
L tab.67 vol 2

33. Not only is it difficult to understand how a Skilled Chemist could arrive at Scheme 2 on the basis of the references cited by Professor Davies, there is also no reason to believe they would "*have been able to carry out this synthesis based on these literature references within weeks to a few months at the most*" as asserted by Professor Davies in paragraph 44. Professor Davies does not discuss any of the potential challenges the Skilled Chemist would face in attempting the synthesis outlined in Scheme 2, nor does he provide any support for his expectation that it would be successful. As explained in paragraphs 18-26 above, there are significant challenges associated with the aldol chemistry proposed, including lack of stereoselectivity and difficulties separating and identifying the various diastereomeric products. The Skilled Chemist would have been

unsure whether the aldol reaction would work at all after a change from the 2,2-difluoroacetate to 2-fluoropropanoate substrate. The Skilled Chemist would not have known (based on the results from non-analogous alkylation reactions described in Bull) if the use of the SuperQuat chiral auxiliary would resolve any of these issues, even if all of the other reaction steps in Scheme 2 were successful (which could not be assumed). Professor Davies notes that the aldol condensation would give a mixture of diastereomers (see paragraph 43), but does not discuss how many and only draws the desired diastereomer in Scheme 2 (compound 18). The Skilled Chemist would not know how much, if any, of compound 18 would be formed. In addition, as discussed above, separating the desired diastereomer from the mixture in the Analogous Gemcitabine Route, and confirming its absolute configuration would be very challenging as seen in Zhang's work. It would not be known if the use of SuperQuat (or other auxiliary) chemistry would improve this (based on the results of the non-analogous alkylation reactions described in Bull 1999 and O'Brien 2001).

Professor Davies' Section B.3 route

34. In paragraphs 49-50, Professor Davies suggests that the skilled organic chemist would identify papers by Hayakawa 1987 and Matsuda 1988, cited in a review by Jørgensen 1997. It is unclear if Professor Davies is suggesting that these papers were themselves part of the CGK (as he defines it in paragraph 3), or if they would have been found as the result of a literature search. I do not agree that these papers would have been part of the CGK of the Skilled Chemist. I do note however that I did identify Matsuda 1988, but only as part of the Analogy Searching undertaken when preparing my First Report, and as discussed at paragraph 224. I did identify Hayakawa 1987, however I did not include it in my First Report as it was very similar to Matsuda 1988.

L tab.70 vol 2
L tab.106 vol 2
L tab.82 vol 2

L tab.70 vol 2
L tab.106 vol 2

35. In paragraphs 51-52, Professor Davies suggests that the skilled organic chemist would have proposed synthesising his compound 1b by deprotecting 'compound 22′ to give the nucleoside (shown in paragraph 51, on the right) which can be fluorinated at the 2′-position using DAST, on the basis of March 2001, Herdewijn 1989, Pankiewicz 1993, Hudlicky 1998 and Singh 2002. I disagree with this. Aside from the difficulties associated with tertiary fluorination which I describe in my First Report (at paragraphs 217-221), treating compound 22 with TBAF reveals three alcohol groups. The 5′-primary and 3′-secondary alcohol groups would be fluorinated preferentially, and so alternative protecting groups would need to be installed to allow selective fluorination at the 2′-position. The identification of a suitable protecting group strategy can be a very important factor in determining whether or not a synthesis succeeds, as I explain

March 2001
L tab.103 vol 2

Herdewijn 1989
L tab.72 vol 2

Pankiewicz 1993
L tab.137 vol 3

Hudlicky 1998
L tab.76 vol 2

Singh 2002
L tab.170 vol 3

in paragraphs 45 and 243 of my First Report. It appears both Dr Griffon and Mr Clark had to do research to identify suitable protecting groups for the fluorination step and ultimately had to prepare and test compounds with a number of different protecting groups (see paragraphs 274-277 and 323 of my First Report).

36. It is strange that the only fluorination reagent Professor Davies suggests that his skilled organic chemist would consider is DAST. As I explained in paragraphs 152-154 of my First Report, a range of fluorination reagents were available in 2003 and are described in March 2001; however, there was little precedent for their use to make tertiary fluorides and no precedent at all for the tertiary fluorination of nucleosides. In this context I would expect the Skilled Chemist to consider several different fluorination strategies in case one or more proved unsuccessful. None of the references cited by Professor Davies would give the Skilled Chemist confidence that a DAST fluorination would be successful. I reviewed the March, Singh and Pankiewicz references as part of my First Report and explained how they would have impacted the Skilled Chemist's approach to the synthesis of compounds 1e and 1f. I do not believe the Skilled Chemist would have been familiar with the articles by Herdewijn or Hudlicky, and Professor Davies does not explain how/if they would have been identified specifically or, if not, which aspects of those papers he is contending are CGK beyond the existence of DAST.

March 2001
L tab.103 vol 2

Herdewijn 1989
L tab.72 vol 2

Pankiewicz 1993
L tab.137 vol 3

SECTION 4: RESPONSE TO THE REPORT OF PROFESSOR SELEY-RADTKE

Hudlicky 1998
L tab.76 vol 2

Structure Activity Relationship ("SAR")

Singh 2002
L tab.170 vol 3

37. In paragraphs 98-107, Professor Seley-Radtke discusses what was known in July 2003 about the SAR of cytotoxic nucleoside analogues in addition to those nucleosides which had been approved for therapeutic use (as described in section C.2.3 of Professor Seley-Radtke's report). In my view, the matters that she sets out in paragraphs 98-107 would not have been part of the Skilled Chemist's CGK in 2003, nor would the papers she cites there.

38. Professor Seley-Radtke refers to studies of the activity of these nucleoside analogues, including some approved drugs, and states "*The results of these studies allowed SAR analysis to be performed so that predictions about the cytotoxic activity of nucleoside analogues could be made.*" Professor Seley-Radtke then goes on to draw conclusions regarding which types of modifications at the 5-position of the base, furanose oxygen,

or 2′-position of the sugar would be expected to result in compounds with cytotoxic activity.

39. I disagree both with the statement above and with the approach taken by Professor Seley-Radtke in paragraphs 98-107. In medicinal chemistry, SAR is normally determined by a systematic study where:

a. A range of compounds is generated containing systematic modifications to a lead compound in order to explore a region of chemical space;

b. The compounds are screened for activity against a particular target (e.g. a type of cancer cell, or a particular enzyme) under identical conditions; and

c. The resulting data is analysed to try to identify trends in activity which can be related to changes in structure.

40. Professor Seley-Radtke explains at paragraph 13 that her laboratories employ extensive SAR studies and that "*SAR studies involve making a series of structural and functional group modifications to a lead compound and then testing those compounds for activity and other important properties*." However, what Professor Seley-Radtke refers to in paragraphs 98-107 does not amount to any type of SAR study. The data relied on in paragraphs 100, 103 and 105 does not amount to any type of systematic study. It is a disparate collection of individual studies, carried out under different conditions, at different times, exploring very different types of modified nucleoside scaffolds (multiple distinct leads), and for different purposes. The Skilled Chemist would not consider that this collection of data could be pieced together and broad conclusions about SAR of nucleoside analogues drawn. Any conclusions which could be drawn from these publications would be limited to the particular modified nucleosides described in each paper.

41. In paragraph 180 Professor Seley-Radtke states "*EP 190 would also have given the Skilled Team confidence that ProTides of gemcitabine-like nucleosides would have shown cytotoxic effects and were therefore worthy of further study*." In order to be useful, any data for this study would need to be related to systematic variation of the parent gemcitabine nucleoside or a prodrug of gemcitabine as opposed to a disparate series of other nucleoside analogues. For example, gemcitabine derivatives with substituents on the 5-position of the base and/or where the furanose ring oxygen atom was replaced with a methylene group. Likewise, gemcitabine derivatives where either one of the two 2′-fluoro substituents had been replaced by another halogen or methyl,

for example. None of the studies described in Professor Seley-Radtke's paragraphs 98-107, or elsewhere in the report, Patents or other cited literature provides any relevant SAR.

Bioisosterism

42. In paragraphs 108-111 Professor Seley-Radtke refers to the concept of bioisosterism as a rationale for structural modifications in drug design. While I agree that Skilled Chemist would be familiar with the concept of bioisosteres, it is a concept which can at best provide a starting point for proposing a library of compounds for testing as part of an SAR study or a way of rationalising observed results. It is not a predictive tool.

L tab.140 vol 2 For example, Patrick explains:[3]

"Isosteres... have often been used in drug design to vary the character of the molecule with respect to such features as size, polarity, electronic distribution and bonding in such a way that any alteration in drug character can be rationalised. Therefore, some isosteres would be used to determine the importance of size towards activity, whereas a different isostere would be used to determine the importance of electronic factors."

43. I agree with Professor Seley-Radtke when she identifies, in paragraph 108, the following properties as being potentially relevant to whether changing one atom or group for another will produce broadly similar biological properties: (a) size, (b) shape (c) electronic distribution, (d) lipid solubility, (e) water solubility, (f) pKa, (g) chemical reactivity and (h) hydrogen bond capacity (i.e. donor or acceptor properties).

44. The grouping of substituents as "classical isosteres" (for example in the Silverman table) arranges atoms and groups based on old definitions of Grimm and Erlenmeyer. Although this provides a potential starting point for the Skilled Chemist to select substituents that might have related properties, it does not mean that compounds modified in this way can reasonably be predicted to have the same or even similar biological properties.

45. Many relevant factors, such as size, charge and H-bonding capability etc. are not accounted for in the classical isosteric grouping, which limits the usefulness of this concept. For example, F and Cl are grouped together (in Silverman table 2.2) in sub-group 1a even though they differ significantly in size (see also for example the inclusion

[3] Patrick, G.L. "An Introduction to Medicinal Chemistry" 2nd ed. 2001, Oxford University Press, page 200.

of -O-, -S- and -Se- in sub-group 2a). In sub-group 1a CH_3 is neutral and hydrophobic, OH neutral but hydrophilic, whilst NH_2 can be protonated at physiological pH making it positively charged and hydrophilic. Also, CH_3 cannot participate in H-bonding, NH_2 and OH can be both H-bond donors and acceptors. F and Cl are not considered to be good H-bond acceptors, but Cl (along with larger halogens) can participate in halogen bonding, whereas the other substituents in sub-group 1a cannot. The differences in electronic properties of the atoms and groups in sub-group 1a can affect water and lipid solubility, as well as modifying stereoelectronics, which could change the conformation (shape) of the molecules they are attached to. Several other atoms and groups that are considered classical isosteres, in the Silverman table will also differ in other aspects from the list (a)-(h) above, all of which can have an effect on the biological properties of the molecule.

46. These limitations of the classical isostere concept resulted in the identification of "non-classical bioisosteres", or "nonisosteric bioanalogues". These are groups which do not fit the strict chemical definition of classical isosteres, but nonetheless may exhibit similar biological properties. Non-classical bioisosteric relationships do not necessarily follow readily identifiable principles (as in the case of classical isosteres) and need to be empirically identified. Silverman provides a table of non-classical bioisosteres at table 2.3. It is strange that Professor Seley-Radtke does not also refer to this concept (or Silverman table 2.3), particularly because one of the most important bioisosteric groupings in medicinal chemistry, that of H and F, is non-classical in nature.

47. However, as I have said, bioisosterism is not a predictive tool. It is well known in medicinal chemistry that small changes to a molecule can have major effects on biological properties.[4] For example the inversion of the 2′-stereocentre in cytidine, or replacement of a single H at the 5-postion of deoxyuridine with F, converts these essential metabolites (natural compounds which are non-toxic) into highly cytotoxic anticancer agents (Cytarabine and Floxuridine respectively). Further, the impact of a particular change will depend not only on the nature of that change but also on the mechanism by which the molecule reaches its target site, interacts with its target and is metabolised or otherwise eliminated from the body. One cannot reasonably predict that substituting (say) a fluorine atom with any one of CH_3, NH_2, OH or Cl will produce a compound with similar biological properties. Matters are far more complex than that and depend on the nature of the molecule in question and how it interacts with the

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[4] See for example Abraham, D.J. and Cannon, J.G. (2003). Analog Design. In Burger's Medicinal Chemistry and Drug Discovery, D.J. Abraham (Ed.).

various environments within and outside the cell. Any or all of factors (a)-(h) listed by Professor Seley-Radtke in her paragraph 108 may be important.

48. It is also possible that a change to one substituent may lead to multiple, unpredictable effects, some or all of which could be detrimental or may completely change the activity of the molecule. For example, Silverman explains:

"*Multiple alterations may be necessary to counterbalance effects. For example, if modification of a functionality involved in binding also decreases the lipophilicity of the molecule, thereby reducing its ability to penetrate cell walls and cross other membranes, the molecule can be substituted with additional lipophilic groups at sites distant from that involved with binding. Modifications of this sort may change the overall molecular shape and result in another activity*."

49. A Skilled Chemist considering making substitutions would not blindly follow a table of "isosteres" but would carefully analyse the particular molecule being modified and the group being replaced, along with any knowledge about targets and mechanisms (if available) before making any suggestions for a range of compounds for screening in an SAR analysis. Any such range of compounds would then have to be investigated empirically to see the effect of the modifications made, which is unpredictable. This approach is well summarised in a chapter by Cannon in Burger's Medicinal Chemistry and Drug Discovery:[5]

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"Although the strategy of bioisosteric replacement may be a powerful and highly productive tool in analog design, Thornber has emphasized that fundamental chemical and physical chemical changes can be expected to result from these molecular modifications, which may in themselves profoundly affect the pharmacological action of the resulting molecules. Contributing factors include change in the size of the atom or group introduced, which may affect the overall shape and size of the molecule; changes in bond angles; change in partition coefficient; change in the pKa of the molecule; alteration of the chemical reactivity and chemical stability of the molecule, with accompanying qualitative and quantitative alteration of in vivo metabolism of the molecule; and change in hydrogen-bonding capacity.

[5] Abraham, D.J. and Cannon, J.G. (2003). Analog Design. In Burger's Medicinal Chemistry and Drug Discovery, D.J. Abraham (Ed.).

The chemical and biological results and pharmacological significance of many of these factors are unpredictable and must be determined experimentally."

Plausibility

Cytotoxic activity

50. In paragraph 202, Professor Seley-Radtke says that, given the data in the Patent for gemcitabine phosphoramidate derivatives:

"*As methyl (-CH_3) and Cl were known to be common bioisosteric replacements of F…the Skilled Team would have considered it plausible that gemcitabine-like ProTides, in which one of the F atoms at the 2′ position of the sugar was substituted for Cl or CH_3 would have similar cytotoxic activity to the gemcitabine ProTides.*"

51. I disagree with this analysis. The Skilled Chemist would not have formed these views. They would have been aware that F, Cl and CH_3 were very different in both electronic and steric terms. Fluorine (Van der Waals radius 1.35Å) is similar in size to hydrogen (Van der Waals radius 1.2Å), whereas CH_3 and chlorine are significantly larger (Van der Waals radii ~2Å and 1.8 Å respectively). Fluorine and chlorine are both electronegative while CH_3 is electron donating. The Skilled Chemist would be aware of these differences and, for the reasons I have explained above, would not consider it possible to make a reasonable prediction, based on the fact that F, Cl and CH_3 were all placed in sub-group 1a of the Silverman table of 'classical isosteres', that replacing F with Cl or CH_3 in a particular molecule (a gemcitabine phosphoramidate derivative), would produce a molecule with similar cytotoxic activity. Incidentally, I would observe that following Professor Seley-Radtke's bioisosterism approach, one would equally predict cytotoxic activity for 2'-F(up) and 2'-Me/Cl(down) compounds, or 2′,2′-dimethyl or 2′,2′-dichloro compounds.

52. In paragraph 203, Professor Seley-Radtke refers to what she says would have been known about the cytotoxic activity of nucleoside analogues substituted at the 2′ position with one or more of F, Cl, Br and CH_3 and says that *"this would have reinforced the Skilled Team's belief that gemcitabine-like ProTides with a Cl or -CH_3 at the 2′ position would have demonstrated cytotoxicity."* I have read what Dr Galmarini says in paragraphs 82-87 of his second report about this topic. I note that Dr Galmarini considers that the Skilled Oncologist would be aware of the activity of gemcitabine and that floxuridine and capecitabine were approved anti-cancer agents. Beyond this, Dr Galmarini does not consider that matters Professor Seley-Radtke refers to or the papers she cites in section C.3 of her report would be part of the CGK

of the Skilled Oncologist. As I explain above, none of these papers would have been part of the CGK of the Skilled Chemist.

53. Professor Seley-Radtke then says that what would have been known about the cytotoxic activity of nucleoside analogues substituted at the 2′-position with one or more of F, Cl, Br and CH_3 *"would have provided the Skilled Team with a basis for believing that gemcitabine-like ProTides with a Br at the 2′ position would also have demonstrated cytotoxic activity"*. I disagree with this statement. As I explain above, even if the matters Professor Seley-Radtke refers to in section C.3 of her report were known to the Skilled Chemist (which I do not agree with), this information does not form any basis for predicting that a Br substitution at the 2′-position of a gemcitabine-like nucleoside (i.e. uridine or cytidine derivative) would show any activity.

54. On the contrary, the Skilled Chemist would expect that the differences between gemcitabine ProTides and the other compounds of the claim with different substitutions at the 2′-position, in the sugar ring and/or on the base would mean they will have different activity, and potentially completely different activity to the gemcitabine ProTides. It is simply not possible to make a reasonable prediction of the outcomes of the changes being made across all of the compounds in the claim.

Intracellular delivery

55. In paragraphs 189-191 Professor Seley-Radtke asserts that the Skilled Team would consider from data in the Patents that the claimed compounds would have improved intracellular delivery. I disagree for the reasons I give below.

56. First, the data in the Patent (read with the CGK) would not tell the Skilled Chemist that the ProTide approach has been "*successfully applied to gemcitabine*" as Professor Seley-Radtke states in paragraph 189. Data for three examples of gemcitabine ProTides, tested in three cell lines each are shown in the table of EP 190. In 5 out of 9 tests the ProTide compound showed reduced activity compared to the parent nucleoside – this cannot be considered a success. All three ProTide compounds show lower activity against breast cancer cells than gemcitabine. In prostate cancer cells only example 31 shows a greater activity than gemcitabine. It is only in the colon cancer cell line tested, where gemcitabine shows particularly low activity (EC_{50} 606.1 μM) that all three ProTide compounds show an improvement.

57. Secondly, even if all the gemcitabine ProTides in the table of the Patents showed improved activity against cancer cells (which they do not), no evidence is provided which would allow the Skilled Team to conclude that this resulted from improved

intracellular delivery. No attempt is made in the Patents to demonstrate that the ProTide delivers a greater concentration of the nucleoside monophosphate into the cell.

58. Finally, the data in the Patents do not show any particular trends or SARs which the Skilled Team could use to predict which compounds may have improved activity, let alone improved intracellular delivery. As I explained in my First Report, each of compounds claimed by the Patents would have to be considered on a case-by-case basis and the Skilled Team would not be able to predict which nucleosides would show activity or which PPAs would work with any given nucleoside. Even if it is assumed that improved activity against cancer cells implies improved intracellular delivery (which I do not agree with), there is not enough information in the Patents to allow the Skilled Team to predict whether any given compound will show improved activity. There is thus no reason from the data in the Patent to believe that "improved intracellular delivery" would be shared by all of the compounds of the claims.

Conclusion

59. In my opinion major sections of Professor Seley-Radtke's report including the sections on SAR analysis (C.3), isosterism (C.4) and plausibility (F) appear to be an attempt to retrospectively rationalise the selection of compounds in the claims of the Patents. Not only do the Patents fail to provide any relevant SAR beyond the cytotoxicity of three gemcitabine prodrugs (the results of which are unimpressive see paragraphs 56 above), they provide no logical explanation for the different nucleoside modifications selected, which cover an enormous number of possible compounds (see paragraph 103 of my First Report). Given that the only data presented in the Patents relating to compounds of the claims is for three gemcitabine ProTides, the Skilled Chemist would not have any reason to think that compounds of the claims, would have any particular effect, other than those where X = F. In particular, considering the lack of any SAR data and the complete absence of any explanation for selection of these compounds from the enormous number of claimed compounds, the Skilled Chemist would have no reason to think that compounds of Formula I where X = CH_3, Cl, Br, I or OH would have any specific biological activity.

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: 9 December 2022