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Datasheet for the decision of 24 October 2013

Case Number: T 0353/10 - 3.4.02

Application Number: 01979080.7

Publication Number: 1322942

IPC: G01N24/08, G01N33/50

Language of the proceedings: EN

Title of invention:
METHOD FOR APPLYING NMR FOR LIGAND DISCOVERY OR AS A DRUG SCREENING TOOL

Applicant:
Universiteit Leiden

Headword:

Relevant legal provisions:
EPC Art. 52(1), 54, 56

Keyword:
Novelty - (yes)
Inventive step - (yes)

Decisions cited:

Catchword:

Europäisches Patentamt
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Beschwerdekammern
Boards of Appeal
Chambres de recours

European Patent Office
D-80298 MUNICH
GERMANY
Tel. +49 (0) 89 2399-0
Fax +49 (0) 89 2399-4465

Case Number: T 0353/10 - 3.4.02

D E C I S I O N
of Technical Board of Appeal 3.4.02
of 24 October 2013

Appellant: (Applicant)	Universiteit Leiden Rapenburg 70 2311 EZ Leiden (NL)
Representative:	Raggers, René John Exter Polak & Charlouis B.V. P.O. Box 3241 2280 GE Rijswijk (NL)
Decision under appeal:	**Decision of the Examining Division of the European Patent Office posted on 5 October 2009 refusing European patent application No. 01979080.7 pursuant to Article 97(2) EPC.**

Composition of the Board:

Chairman: D. Rogers
Members: F. Maaswinkel
A. Hornung

Summary of Facts and Submissions

I. The appellant lodged an appeal against the decision of the examining division, refusing the European patent application 01979080.7. This patent application relates to a method and an apparatus for screening compounds involving generating NMR spectra to identify their possible binding to target molecules.

According to the decision, the subject-matter of method claim 1 according to the Main Request lacked novelty over the disclosure in document D2 (Article 52(1) and 54 EPC); furthermore it did not involve an inventive step within the meaning of Article 56 EPC having regard to the disclosure in document D1 and ordinary skill:

D1: WO-A-98/57155
D2: J. Am. Chem. Soc. vol. 121,pages 5336 - 5337, 1999; J. Klein et al: "Detecting Binding Affinity to Immobilized Receptor Proteins in Compound Libraries by HR-MAS STD NMR".

In the opinion of the examining division, independent apparatus claim 8 of the Main Request contained patentable subject-matter but was considered objectionable under Article 84 EPC, as well as the further claims. The claims according to the auxiliary requests were not allowable, either.

II. With the letter containing the grounds of appeal the appellant requested to set aside the decision and to grant a patent on the basis of the sets of claims according to the Main Request addressed in the decision or of the claims according to First to Third Auxiliary Requests filed with this letter. The appellant also filed a request for oral proceedings.

III. In a Communication pursuant to Rule 100(2) EPC the board raised objections under Article 84 EPC.

IV. With a letter dated 10 September 2013 the appellant filed a new Main Request and amended description pages and requested that a patent be granted based on the following documents:

Claims: 1 to 10 of the Main Request, filed with the letter dated 10 September 2013;
Description: pages 1 to 5, 9 to 13, 15 to 21 as published;
pages 6, 7, 8 and 14, filed with the letter dated 10 September 2013;
Drawings: sheets 1/3 to 3/3, as published.

V. The wording of independent claim 1 of the Main Request reads as follows:

" A method for screening compounds to identify compounds that bind to a specific target molecule or collection of molecules, comprising the steps of:
(a) providing a target molecule or collection of target molecules immobilized to a solid support;
(b) generating a first NMR spectrum of said compounds to be screened in the presence of a reference solid support, wherein the reference solid support is the solid support without the target molecule or collection of target molecules immobilized thereto;
(c) generating a second NMR spectrum of the said compounds to be screened in the presence of the solid support with the target molecule or collection of target molecules immobilized thereto; and
(d) comparing said first and second NMR spectrum to

determine differences between said first and second NMR spectrum ".

The wording of independent claim 8 reads as follows:

" NMR apparatus having arranged therein an NMR probe suitable for flow-through NMR screening of a liquid test sample comprising compounds that are screened to identify compounds that bind to a target molecule or collection of target molecules, said probe including a flow inlet, a first vessel which is connected to the flow inlet, a second vessel which is connected to the first vessel and a flow outlet which is connected to the second vessel, wherein one of the vessels includes a solid support having the target molecule or collection of target molecules immobilized to it and the other vessel includes a reference solid support, wherein the reference solid support is the solid support without the target molecule or collection of target molecules immobilized to it, wherein the NMR apparatus includes first means for generating a pulsed-radio field which at least extends into the first and second vessel of the NMR probe, and wherein the NMR apparatus includes second means for measuring a first response in the said one vessel to the generated pulsed-radio field and a second response in the said other vessel to the generated pulsed-radio field, and wherein the NMR apparatus is arranged to include third means for comparing the first and second responses to the generated pulsed-radio field in the respective vessels ".

Claims 2 to 7 and claims 9 and 10 are dependent claims.

The claims of the Auxiliary Requests are not relevant for the present decision.

VI. The appellant's arguments may be summarised as follows:

In the grounds for the decision it was objected that the subject-matter of claim 1 would lack novelty with respect to document D2. This document describes that saturation transfer difference (STD) NMR spectroscopy can be used to characterize binding affinities in mixtures. STD NMR spectroscopy relies on the possibility to selectively saturate protons of macromolecular receptors by irradiating the spectral region containing "wings" of broad resonances of the macromolecule which is also free of any smaller molecule signals. Due to effective spin diffusion saturation quickly propagates across the entire receptor. If the smaller molecule ligand binds the receptor, saturation will also spread onto the ligand. The result will be that intensity of the ligand signal will be attenuated. Subtraction of the resulting spectrum from the reference spectrum without saturation yields the STD spectrum containing only signals of the binding ligands. Document D2 states that the technique described therein is based on the selective saturation of resonances of a receptor protein, leading to fast intramolecular magnetization transfer that spreads the saturation efficiently over the entire receptor and being transferred to bound ligands (D2, p. 5336, right column, 1st para and Figure 1). In the difference spectrum resulting from the subtraction, all resonances are cancelled but those from species with binding affinity to the receptor (D2, p. 5336, right column, first para, lines 7-10). In other words, in D2 the two spectra that are subtracted are both from a sample that contains the protein receptor: the first spectrum is obtained in the presence of the receptor protein that

is selectively saturated whereas the second spectrum is obtained in the presence of the same but now unsaturated receptor protein. Indeed D2 describes at page 5337, right column, the last few lines of the first paragraph that the difference spectra were obtained by internal alternated subtraction. The term "internal" clearly indicates that in the method described in D2 one and the same sample comprising the receptor protein was used to measure both a spectrum under conditions that the receptor protein was selectively saturated and under conditions that the receptor protein was not selectively saturated. This also follows from the legend of Figure 2.

In contrast, claim 1 of the Main Request clearly states that the two NMR spectra are not obtained from the same samples comprising the target molecule (protein receptor) as in document D2, but that a first NMR spectrum is obtained in the absence of the target molecule (*step b*) and the second NMR spectrum is obtained in the presence of said target molecule (*step c*). In step (d), in contrast to the method in document D2, the two spectra are compared, both comprising the target molecule. Therefore claim 1 of the Main Request differs from document D2 with respect to steps (b), (c) and (d), rendering the claim novel over the disclosure in document D2. Furthermore, the STD NMR technique used in document D2 is based on high-resolution magic angle spinning technique (HR-MAS, see the title of D2 and the Abstract), which is a non-static NMR technique. In contrast the method defined in claim 1 allows screening compounds in stopped-flow mode, which is not possible in MAS, therefore the subject-matter of claim 1 is also not derivable in an obvious way from document D2.

With respect to inventive step, the examining division referred to the opinion given in section 2 of the International Preliminary Examination Report (IPER). According to the IPER, claim 1 would lack inventive step over document D1, since it would appear to disclose a method comprising all the steps of claim 1, except for the presence of a solid support in step (b). According to the IPER, when establishing the reference spectrum, it would be obvious for the skilled person to take into account any possible contributions from the support, for which reference was made to document D2, page 5336, left column, end of first paragraph.

For the feature "a solid support", the only disclosure found in document D1 is the passage on page 11, line 14 where a long list of possible targets is disclosed comprising molecules that may be attached to a solid support. Nowhere in the remainder of D1 are any details given with respect to the support, e.g. of what kind of material it is made, how the material might affect the NMR results, how it behaves in solution, or how the target is attached thereto, its dimensions, etc., if the target is immobilized relative to the solid support, or, once contacted with the compounds, it is actually solubilised (*see below*). Document D1 also does not provide, for example, any information on how to achieve the attachment of a membrane protein which is in detergent or micelles to a solid support. It is therefore submitted that based on D1 it is not clear how a skilled person can provide for a suitable target in the form of a molecule attached to a solid support, and wherein suitability will depend on the method used to analyze (as given on page 15, lines 7-9). In that respect, D1 lacks sufficiency of disclosure, and thus does not disclose that a molecule is immobilized to a solid support. In any case D1 does not disclose that a

target molecule is immobilized on a solid support and remains immobilized during the NMR measurement (*as is in the application in suit, page 10, line 30 — page 11, line 1*) and wherein the immobilization of the target to the support is preferably by a covalent or non-covalent binding. Further, the only information given with respect to the characteristics of the target relates to its use in an NMR method. D1 states that it is essential that with respect to the target in view of its use in NMR:
-the solvent is such that the target is soluble and stable in said solvent (page 11, line 25);
-the solvent is an aqueous buffer system (page 11, line 27);
-the target can be stored in an aqueous solution (page 14, line 29-30), to be mixed with the solvent in which the core is dissolved (page 14, line 30-31);
-the target is dissolved in the aqueous phase (page 15, lines 1-6); and
-the drug cores should not induce aggregation of the target as this will remove them from solution (page 17, lines 19—25). In other words, D1 teaches that for the method of D1, and when the chosen technique is NMR and not any of the other techniques described on page 15, lines 7-9, the target should be present in solubilised form (*in contrast to the current invention*). Indeed, D1 neither mentions nor suggests that when NMR is chosen as the technique, the target should be attached to a solid support. A skilled person would thus neither learn nor understand from D1 that when NMR is used, the target can be, for example, a protein attached to a solid support.

In fact the NMR techniques mentioned in D1, in particular trNOE, would not be compatible with measuring the binding of a compound with a target that

is immobilized to a solid support, or with the presence of a solid support in the sample. In contrast to the view of the examining division, applicant submits that D1 does not "appear to disclose all the steps according to claim 1, except for the "presence of a solid support" in step b". In fact, document D1, by explicitly discussing the requirement of solubility of the target when applied to NMR, teaches away from providing a target that is attached to a solid support. For that reason, applicant submits that claim 1 is new and inventive over D1. In contrast to the current invention, D1 clearly does not teach that the target could be present as "a target molecule or collection of molecules immobilized to a solid support". In D1 the target is not immobilized, but must be present in soluble form, whereas for the current invention the immobilization onto the solid support is an essential feature. Also a combination of D1 with D2 would not provide for the method according to the current invention. Firstly, it is submitted that a skilled person would not consider combining D1 and D2. There is no incentive in D1 that would lead a skilled person to D2. For example, in contrast to D1, D2 relates specifically to the combination of STD NMR and HR-MAS (*see the arguments supra*). D2 thus excludes any other method for screening compounds. A skilled person would for that reason alone not combine D1 and D2. The method and the steps described in D2 differ fundamentally from the method described in D1 (as well as from the method according to the claimed invention). This also applies to independent apparatus claim 8 which, according to the examining division, included patentable subject-matter.

Therefore the claimed subject-matter is novel and involves an inventive step.

Reasons for the Decision

1. The appeal is admissible.

2. Amendments

The board is satisfied that in the set of claims according to the present Main Request the objections under Article 84 EPC raised by the board in its Communication of 28 June 2013 have been overcome. The application documents also comply with the provisions of Article 123(2) EPC.

3. Patentability

3.1 Novelty

Claim 1

3.1.1 In its decision, the examining division had objected that the method for screening compounds to identify compounds that bind to a specific target molecule or collection of molecules defined in claim 1 was known from document D2. In this respect a general reference was made to page 5336, left column, first para, reading: "...Screening of a mixture of seven oligosaccharides for affinity to wheat germ agglutinin (WGA) immobilized to controlled pore glass (CPG)... ". To the board's understanding this passage may indeed be read onto step (a) of claim 1 "providing a target molecule or collection of target molecules (*i.e. WGA*) immobilized to a solid support (*i.e. CPG*) ".

3.1.2 According to step (b) of claim 1 a first NMR spectrum of the compounds to be screened (*i.e. in document D2: the mixture of seven oligosaccharides*) in the presence of a reference solid support (*i.e. the CPG glass support*) is recorded. In this respect the examining division referred to the passage in document D2 on page 5336, left column, end of first para "A reference experiment proved the absence of unspecific interactions between the solid support and the components of the library"; and to Figure 2d and its capture "STD spectrum of a mixture of seven oligosaccharides in the presence of succinamidopropyl-CPG beads showing that no unspecific binding is present and, therefore, all signals are cancelled".

With respect to the spectrum shown in Figure 2d, document D2 also discloses (page 5337, left column, lines 8 – 10) "The STD spectrum of the saccharide library with unconjugated CPG is shown (Figure 2d) to prove that unspecific binding does not occur".

To the board's understanding, the cited passage on page 5336, first para, relates to and is consistent with the information of Figure 2d. Thus, the "reference experiment" made on the compounds to be screened in the presence of a reference solid support in document D2 is a STD NMR spectrum which is not a "first NMR spectrum" as defined in step (b) of claim 1 but rather a result of taking two difference spectra by internal alternated subtraction with appropriate phase cycling using a frequency list for on and off resonance irradiation, 4000 and 20,000 Hz, respectively (*page 5337, right column, para 1, line 18 – 21*). Therefore step (b) defined in claim 1 is not disclosed in document D2.

3.1.3 With respect to step (c) the examining division referred to the spectra shown in Figure 2a and 2b of document D2. The spectrum shown in Figure 2a is a "conventional ^{1}H HR-MAS NMR spectrum of a mixture of 7 oligosaccharides in suspension with WGA coupled to CPG" (*page 5336, right column, last para*). This passage continues "It is crowded, and especially the hump region is characterized by severe overlap of resonances such that individual saccharides cannot be identified". This passage clearly discloses that the authors of document D2 were convinced that the spectrum shown in Figure 2a did not contain information useful for evaluating a binding affinity. Furthermore, similar to the reference spectrum shown in Figure 2d, the spectrum shown in Figure 2b is a STD difference spectrum obtained by internal alternated subtraction, and hence not comparable with the requirement "generating a second NMR spectrum" in step (c) of claim 1.

3.1.4 Since document D2 does not disclose steps (b) and (c) of claim 1, it does not and cannot disclose step (d) which defines "...comparing of the first and second NMR spectra to determine differences between these NMR spectra".

3.1.5 Hence, the subject-matter of claim 1 is novel over the disclosure in document D2.

3.1.6 No objections pertaining to lack of novelty based on the disclosure in document D1 have been raised, and the board equally does not see a basis for such an objection.

3.1.7 It is concluded that the subject-matter of method claim 1 is novel (Art. 52(1) and 54 EPC).

Claim 8

3.1.8 With respect to independent apparatus claim 8 no objections under Art. 52(1) and 54 EPC had been raised by the examining division.

3.2 Inventive step

3.2.1 For the objection of lack of inventive step against claim 1 based on document D1 the decision merely referred to the International Preliminary Examination Report (IPER). According to the IPER, referring to the passages in document D1 at page 4, line 30 - page 5, line 28; page 11, lines 7 - 14; and page 15, line 18 - page 17, line 5, this document appeared to disclose a method comprising all the steps of claim 1 except for the "presence of a solid support" in step (b). In this respect it was argued that "...when the target is attached to a solid support (like beads or a well plate) it appears to be obvious that possible contributions from the support have to be taken into account when establishing the reference spectrum (cf., for instance, D2, p.5336, left column, end of first paragraph). The skilled person would thereby arrive at the subject-matter of claim 1 without the exercise of any inventive skill".

Addressing the cited passages of document D1 in order, it is found:

3.2.2 The passage in page 4, line 30 to page 5, line 28 is part of the "Summary of Invention" of document D1. According to page 5, first para, the method for detecting weak binding incorporates the use of NMR, wherein the ligand(s) in the mixture combined with the target bind to and come off the target numerous times during the NMR procedure. ... As a result, the relative

signs of diagonal and cross peaks in the spectrum changes with respect to those observed for the free ligand alone, thus providing an unambiguous indication of binding (*emphasis by the board*).

Page 5, 3d para reads: "In PFG NMR, the diffusion coefficient of the ligand alone and in the presence of the target are compared".

Therefore these passages disclose that various detection techniques involving NMR may be used for comparing a ligand alone with ligands in the presence of a target.

3.2.3 At page 11, lines 7 to 14, a quite encompassing definition of possible "targets" is presented which, inter alia, may include "any of the foregoing attached or tethered to a solid support, or any of the foregoing already bound to a ligand". However, from the further passages in document D1 cited by the appellant (*see Section VI supra*) it appears that, to be compatible with NMR or other techniques useful to detect binding, the target and the ligands should be soluble, see lines 23 - 27 at this page, which requirement appears to be in contradiction with the target being attached or tethered to a solid support.

3.2.4 The passage at line 15, line 18 - page 17, line 5, refers to "one preferred embodiment", which is the one defined in claim 4 of document D1. In brief it includes:

(i) obtaining a one-dimensional NMR spectrum of the ligand in the absence of the target;

(ii) mixing the target with the ligand;

(iii) subjecting the mixture to NMR to obtain a one-dimensional NMR spectrum of the mixture of target with ligand; and

(iv) comparing the spectra obtained in steps i) and iii) to determine if the ligand has bound to said target.

The further passage at page 16, lines 26 - 29, discloses that the addressed methods involve subtracting the spectrum obtained from the mixture of drug cores in the absence of target from the spectrum obtained in the presence of the target.

3.2.5 Hence, these passages clearly disclose that a first NMR spectrum is obtained from the ligand alone; and a second NMR spectrum is taken from the mixture of ligand and target. With respect to a situation that a target is "attached or tethered to a solid support", document D1 does not provide any information how, in such a situation, an NMR spectrum should be recorded. Since it must be assumed that in this case, the target is attached or tethered to the solid support prior to carrying out the method steps (i) to (iv) reproduced in point 3.2.4 *supra*, the two spectra to be compared would be a first NMR spectrum of the ligand alone (*i.e. in the absence of the target attached or tethered to the solid support, see step (i)*); and the second NMR spectrum taken of the mixture of the target attached or tethered to the solid support with the ligand (*step (iii)*).

3.2.6 Therefore, the board considers that the only disclosure of a target attached or tethered to a solid support in document D1 is at page 11, lines 7 - 14, and that, irrespective of the perception that the requirement of "solubility" in D1 appears to be irreconcilable with

the requirement of a target being "immobilized" to a solid support, there is no information whatsoever in document D1 that an NMR spectrum of a target attached or tethered to a solid support should be recorded differently from the case of a target mixed with a ligand (*step (iii) of the method reproduced in point 3.2.4 supra*).

3.2.7 The board does not concur with the suggestion in point 2 of the Section "Novelty and Inventive Step" of the IPER, that if a target is attached to a solid support it would appear obvious that possible contributions from the support have to be taken into account when establishing the reference spectrum. For this suggestion reference was made to document D2, p. 5336, left column, first para. Firstly, in document D1 the recording of a "reference spectrum" is only used in respect of (the combination of) drug cores (*=ligands*) in the absence of the target, see page 17, first para. Secondly, the reference to an isolated passage of document D2 is not conclusive, in particular because the reference experiment referred to is the one shown in Figure 2d of document D2, which concerns the quite different type of STD NMR spectrum (*see point 3.1.2 supra*).

3.2.8 Hence, since neither document D1 nor the other available documents suggest or disclose a screening of compounds wherein a target is immobilized to a solid support, and wherein a spectrum is recorded of the ligands in the presence of the solid support but without the target as defined in claim 1, the board finds that the subject-matter of this claim is novel and involves an inventive step.

3.2.9 The same conclusion can be drawn for independent claim 8, which defines an NMR apparatus with the technical features for carrying out the invention.

3.2.10 Claims 2 to 7 and claims 9 and 10 are dependent claims and are equally allowable.

4. For the above reasons, the board finds that the appellant's Main Request meets the requirements of the EPC and that a patent can be granted on the basis thereof.

Order

For these reasons it is decided that:

1. The decision under appeal is set aside.

2. The case is remitted to the first instance with the order to grant a patent based on the following documents:

Claims: 1 to 10 of the Main Request, filed with the letter dated 10 September 2013;
Description: pages 1 to 5, 9 to 13, 15 to 21 as published;
pages 6, 7, 8 and 14, filed with the letter dated 10 September 2013;
Drawings: sheets 1/3 to 3/3, as published.

The Registrar: The Chairman:

M. Kiehl D. Rogers

Decision electronically authenticated