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Case Number: T 1277/04 - 3.3.03

Application Number: 00912655.8

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Title of invention:
Method for the reductive amination of polysaccharides

Patentee:
Sanofi Pasteur

Opponent:
-

Headword:
-

Relevant legal provisions:
EPC Art. 54, 56, 84, 123(2)
EPC R. 86(3)

Keyword:
"Novelty - (yes)"
"Inventive step - (yes) - after amendment"
"Claims - clarity - (yes) - after amendment"

Decisions cited:
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Catchword:
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Case Number: T 1277/04 - 3.3.03

D E C I S I O N
of the Technical Board of Appeal 3.3.03
of 4 May 2007

Appellant: Sanofi Pasteur
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Decision under appeal: **Decision of the Examining Division of the European Patent Office posted 31 March 2004 refusing European application No. 00912655.8 pursuant to Article 97(1) EPC.**

Composition of the Board:

Chairman: R. Young
Members: M. C. Gordon
H. Preglau

Summary of Facts and Submissions

I. European Patent Application no. 00912655.8 was filed on 10 March 2000 as international application No. PCT/EP00/02748, claiming a priority date of 12 March 1999 (EP 99420071.5) and was published on 21 September 2000 as WO 00/55210.

The application had 16 claims.
Claim 1 read as follows:

"1. A method for reductive amination of polysaccharides which comprises:

(i) subjecting a reaction mixture comprising a polysaccharide, an amino compound and a reducing agent, to microwave radiation for a period of time sufficient to aminate the polysaccharide ;

(ii) (a) subjecting a reaction mixture comprising a polysaccharide and an amino compound to microwave radiation polysaccharide [*sic*] for a period of time sufficient to allow the formation of an imine compound, (b) adding a reducing agent to the reaction mixture obtained in (a) so that the polysaccharide be aminated ;

(iii) (a) adding an amino compound to a polysaccharide so that an imine compound is formed, (b) adding a reducing agent to the reaction mixture obtained in (a) and, (c) subjecting the reaction mixture obtained in (b) to microwave radiation for a period of time sufficient to aminate the polysaccharide ; or

(iv) (a) subjecting a reaction mixture comprising a polysaccharide and an amino compound to microwave radiation for a period of time sufficient to allow the formation of an imine compound, (b) adding a reducing agent to the reaction mixture obtained in (a) and, (c) subjecting the reaction mixture obtained in (b) to microwave radiation for a period of time sufficient to aminate the polysaccharide."

Claims 2-15 were dependent claims defining preferred embodiments of the method of claim 1. Claims 11, 13 and 15 thereof specified *inter alia* that the method resulted in the formation of a polysaccharide-polypeptide conjugate (claim 11) or that such a conjugate was obtained (claims 13, 15).

Claim 16 was an independent composition claim and read as follows:

"16. A pharmaceutical composition comprising a polysaccharide-polypeptide conjugate obtained in claim 11, 13 or 15".

II. The application was refused by a decision of the examining division dated and issued in writing on 31 March 2004.

The decision was based on a set of 15 claims submitted with a letter dated 28 July 2003.

Claim 1 thereof differed from claim 1 as filed in that

- "which" was replaced by "that" in line 1 of claim 1;
- in each of subsections (i), (ii), (iii) and (iv) the polysaccharide was further defined as "having at least twelve repeat units";
- the second occurrence of "polysaccharide" in section (ii) (a) of claim 1 (see above) had been deleted.

Claim 16 had been deleted.

(b) The following documents were cited by the examining division:

D1: WO-A-93/07178

D2: Varma, R.S. and Dahiya, R, Tetrahedron 54 (1998), pp. 6293-6298

D3: Patent Abstracts of Japan, vol. 18, no. 182 (C-1184), 29.03.1994, corresponding to JP-A-05 339 300.

Together with a communication dated 6 June 2003 the examining division provided a computer generated translation of the original Japanese document forming the basis of abstract D3.

(c) The following documents were cited by the applicant during the course of the examination proceedings:

- "Biochimie Alimantaire", G. Alais, G. Linden, Edition Masson (1994) 3ème Ed. Collection Abrégés, Chapitre 5, page 90

– "Les secrets de la casserole", Hervé, TRIS Edition Belin (1993), page 30

both submitted with the letter dated 28 July 2003.

(d) The decision under appeal held that:

(i) The amended claims met the requirements of Article 123(2) EPC.

(ii) The subject matter claimed differed from the disclosures of D1-D3 in that D1 did not disclose a process involving microwave radiation; D2 did not teach to employ a polysaccharide as a starting material; D3 did not teach to employ a reducing agent.

Accordingly the subject matter claimed was held to be novel.

(iii) Regarding inventive step, it was held that the subject matter claimed differed from the disclosure of D1 in that D1 did not disclose a process involving microwave radiation.

The problem solved by this differing feature was to reduce the overall reaction time while providing the means to significantly increase the yield, reference being made to page 4 lines 22-25 of the application.

The solution to this problem as presented in claim 1 was known from D2 which described the reductive amination of aldehydes and

ketones with sodium borohydride on wet clay using microwaves, the reaction temperatures being of the order of 65°C. According to D2 if instead of microwave heating the reaction mixture was heated in an oil bath at the same temperature, a much longer reaction time was needed in order to achieve a similar product yield.

One of the main objectives of the application was to prepare polysaccharide-polypeptide conjugates, reference being made to claims 11, 13 and 15.

The expert in this field would have considered using the microwave radiation in the way described in D2 since it was known from D3 that protein-polysaccharide conjugates could be produced without discoloration (browning) when a blend of the protein and polysaccharide were subjected to microwaves.

Thus the subject matter of claims 1 and 5 was obvious.

The restriction of the subject matter of claim 1 to polysaccharides having at least 12 repeat units could not lead to a different conclusion since the preferred dextran equivalent molecular weights given in the description and examples of D1 corresponded to considerably more than this number of repeat units.

Regarding the counterarguments of the applicant that:

– Polysaccharides were so different from the small molecules employed in D2;

– These large molecules were far less reactive than smaller ones;

– In the presence of the reducing agent, the reduced polysaccharide was expected to be produced as a side product;

– So it was not predictable what would happen if the process of D1 was conducted in the presence of microwave radiation as suggested in D2 and

– the reaction disclosed in D3 was different in that no reducing agent was used and that the Schiff's base (imine) formed in the first step underwent the Amadori rearrangement to yield a ketose having an amine linkage which further reacted with other products to form cyclic aromatic molecules, a number of which were strongly coloured,

– the decision held:

– The first step of the reactions according to D1-D3 and according to the application was

always formation of an imine bond between the carbonyl group and the amine, reference being made to D2, D3 and the document "Les secrets de la casserole";

– The question was whether the expert would have combined the teachings of D1 and D2 knowing that D2 taught that the reaction time could be reduced without impairing the yield if the reaction was effected in the presence of microwave radiation;

– When trying to answer this question, the skilled person would have consulted D3. Here it was taught that a polysaccharide might be reacted with a protein, the reaction yielding one single conjugate of the protein with the polysaccharide as taught in paragraph [0009] of the translation of D3. Thus no considerable fragmentation occurred. The bond was formed between the carbonyl group of the polysaccharide and an amino group of the protein. Only little browning took place (D3, translation paragraph [0005]). Hence the consecutive reactions of the so-called Maillard reaction were suppressed. Thus the expert would have concluded that the first step of the reductive amination could take place in the presence of microwaves selectively even if a polysaccharide were used as the carbonyl component.

– Thus D3 would have given the expert an indication that the combination of the teachings of D1 and D2 would give the required product in good yield.

– There was no reason to believe that considerable amounts of reduced polysaccharide would be formed as a by-product as D2 taught first to react the carbonyl compound with the amino compound in the presence of microwave radiation and then to add the reducing agent. Thus there was almost no unreacted carbonyl compound left once the reducing agent was added. The reduced polysaccharide could only be formed when the starting polysaccharide was contacted with the reducing agent. Thus it would be clear to the expert that no considerable amount of reduced polysaccharide would be formed as a by-product when the reaction disclosed in D1 was conducted in the presence of microwave radiation as suggested by D2;

– Even if it were agreed that the Amadori product was formed in D3, it was clear to the expert that in this case the reducing agent would reduce the carbonyl group thereof. Such reaction (i.e. the Maillard reaction) also yielded a polysaccharide conjugate where the amine was linked to the polysaccharide by an amino group i.e. a product identical to that expected by reductive amination.

(e) Consequently the expert would clearly have conducted the process disclosed in D1 in the presence of microwave radiation as suggested in D2 in order to reduce reaction time.

Thus the subject matter claimed was not based on an inventive step.

(f) Accordingly, the application was rejected.

III. An appeal against this decision was filed on 27 May 2004, the requisite fee being paid the same day.

IV. The statement of grounds of appeal was filed on 2 August 2004.

With letter dated 5 August 2004 and received 6 August 2004 an amended statement of grounds of appeal was filed, correcting a number of omissions and clerical errors.

(a) The statement of grounds of appeal was accompanied by 6 sets of claims forming a main and 1st-5th auxiliary requests, the previous set of claims being withdrawn.

Compared to the previous set of claims on which the decision had been based, claim 1 of the newly filed main request differed in particular in that the polysaccharide was now specified in the preamble of the claim to be a "bacterial or fungal polysaccharide having at least twelve repeat units", the feature "having at least twelve repeat

units" consequently being removed from each of sections (i)-(iv) of claim 1 (see section II above). Further a temperature "not exceeding 100°C" was now specified.

Claim 1 of the amended main request thus read as follows, the additions compared to the claims upon which the decision under appeal was based being indicated by *italics* and the deletions being indicated by [square brackets].

"1. A method of reductive amination of *bacterial or fungal* polysaccharide[s] *having at least twelve repeat units*, that comprises:
(i) subjecting a reaction mixture comprising [a] *the* polysaccharide [having at least twelve repeat units], an amino compound and a reducing agent, to microwave radiation *at a temperature not exceeding 100° C*, for a period of time sufficient to aminate the polysaccharide ; ***or***
(ii) (a) subjecting a reaction mixture comprising [a] *the* polysaccharide [having at least twelve repeat units] and an amino compound to microwave radiation, *at a temperature not exceeding 100° C,* for a period of time sufficient to allow the formation of an imine compound, (b) adding a reducing agent to the reaction mixture obtained in (a) so that the polysaccharide is aminated ; ***or***
(iii) (a) adding an amino compound to [a] *the* polysaccharide [having at least twelve repeat units] so that an imine compound is formed, (b) adding a reducing agent to the reaction mixture obtained in (a) and, (c) subjecting the reaction mixture obtained in (b) to microwave radiation, *at*

a temperature not exceeding 100° C, for a period of time sufficient to aminate the polysaccharide ; ***or***

(iv) (a) subjecting a reaction mixture comprising [a] *the* polysaccharide [having at least twelve repeat units] and an amino compound to microwave radiation, *at a temperature not exceeding 100° C*, for a period of time sufficient to allow the formation of an imine compound,(b) adding a reducing agent to the reaction mixture obtained in (a) and, (c) subjecting the reaction mixture obtained in (ii) [*sic*] to microwave radiation, *at a temperature not exceeding 100° C*, for a period of time sufficient to aminate the polysaccharide ; *whereby the antigenic determinants of the polysaccharide are not affected."*

Dependent claim 2, which had no counterpart in either the set of claims as originally filed or that on which the decision had been based, specified that the microwave radiation was applied to the reaction mixture at a temperature not exceeding 60°C.

Dependent claims 3-7 corresponded to originally filed claims 2-6.

Dependent claim 8, directed to a method in which the polysaccharide was a neutral polysaccharide had no counterpart in the set of claims of the application as filed, or those forming the basis of the decision of the examining division.

Dependent claim 9 corresponded to originally filed claim 8. Claim 10, which had no counterpart either in the application as filed or those forming the basis of the decision of the examining division, was directed to a vaccinal composition according to (method) claim 9.

Claims 11 and 12 corresponded to originally filed claims 10 and 11.

Independent claim 13 and dependent claims 14 and 15 were directed to a "polysaccharide-polypeptide conjugation method". These claims had no counterparts in the application as filed or the set of claims forming the basis of the decision of the examining division.

(b) The appellant submitted:

(i) The objectives of the application were clearly in the vaccines field. Polysaccharides could be coupled to a polypeptide by a variety of techniques including reductive amination as was known from D1.

(ii) The objective of the application was to reduce the conventional reaction time. While it was generally known that chemical reactions could be accelerated by heating, this method was of limited use when the product to be reacted was a polysaccharide intended for further use as immunogen. Polysaccharides could be degraded or

denatured at elevated temperature. Retention of the antigenic sites was however necessary for use of bacterial and fungal polysaccharides as efficient immunogens. This was why they had to remain totally undegraded and thus why elevated temperatures were inappropriate.

(iii) The examining division had considered the technical problem, in view of D1, to be to reduce the overall reaction time while providing the means to significantly increase the yield. A requirement even more important than yield improvement, namely that the antigenic determinants of the polysaccharide remained unaffected, i.e. that the structure and conformation of the polysaccharide were retained (page 4, lines 3-12 of the application as filed) was missing from this statement of problem.

(iv) With regard to D2 it was disputed that the reactivity of large molecules subjected to microwave radiation at a temperature not exceeding 100°C could be foreseen in view of the teachings thereof. It was disputed that a method which worked for small molecules would also be expected to be effective for modifying a terminal end of polymeric molecules.

(v) Neither could this reactivity of polysaccharides be foreseen from D3.

D3 taught that it was possible to induce an amino-carbonyl reaction between a polysaccharide and a protein under microwave conditions. It could be believed that the reaction was a Maillard reaction giving the Amadori product (reference being made to the document "Les secrets de la casserole"). This document further taught that at elevated temperatures the Amadori product further reacted to form aromatic cyclic monomers which could be intensely coloured.

D3 taught that little browning was formed. Thus it was not correct to state, as the decision of the examining division did that browning did not occur (see section II.(d).(iii) above).

The fact that little browning did occur revealed two theoretical alternatives:

- either the temperature of the amino-carbonyl mixture submitted to microwave radiation was largely over 100°C;

- or, if the temperature was lower than 100°C then the browning might be favoured by microwave radiation.

D3 was silent about the reaction temperature and the data given therein were insufficient to estimate the temperature of the reaction mixture. Thus it was not possible to

discriminate between the two alternatives mentioned.

With regard to the possibility that the temperature was over 100°C it was submitted that it was common knowledge that a temperature of 100°C was not sufficient to allow browning, as disclosed in "Les Secrets de la casserole". Browning in D3 was limited because the reaction time was very short (5 minutes). Since browning did occur to some extent it could be taken for certain that the temperature was largely over 100°C. With regard to the first alternative the appellant submitted that it could not but be concluded that the reactivity of polysaccharides exposed to microwave radiation at a temperature not exceeding 100°C could not be foreseen.

Regarding the second alternative, i.e. the possibility that the temperature was lower than 100°C, this would reveal that in this temperature range the reactivity of polysaccharides was favoured by microwave radiation and that a Maillard reaction might occur. However there was still uncertainty as to whether a better reactivity of polysaccharides would be determinant to achieving efficient reductive amination when an amino compound and a reducing agent were added. The Maillard reaction could compete with or even parasitize the reductive amination resulting in little or no

production of the reduced, aminated polysaccharide.

Thus the appellant concluded that there was no certainty that the reactivity of polysaccharides submitted to microwave radiation at a temperature not exceeding 100°C would be favourable to reductive amination.

Thus as it was not possible to determine which of the alternatives was the correct one, it was maintained that in view of D3 the reactivity of polysaccharides submitted to microwave radiation at a temperature not exceeding 100°C could not be foreseen.

Further the fact that little browning occurred in D3 was worrying as it suggested that reaction conditions were such that, if obtained, an aminated polysaccharide might also be degraded to some extent, which degradation was prohibited. Even if the occurrence of little browning had not been reported in D3, there would be no certainty that antigenic determinants of a polypeptide submitted to microwave remained intact, even at a temperature of less than 100°C.

Thus the teaching of D3 was not sufficient to allow a skilled person to consider using microwave radiation in the way described by D2 for small molecules (i.e. at a temperature below 100°C) to aminate

polysaccharides and in particular, so that their antigenic determinants were retained.

(c) The respective claim 1 of the 1st-5th auxiliary requests contained additional features further specifying the method.

V. On 23 February 2007, the board issued a summons to attend oral proceedings. This was accompanied by a communication setting out the preliminary, provisional opinion of the board.

(d) The board raised objections pursuant to Article 123(2) EPC in respect of the feature "whereby the antigenic determinants of the polysaccharide are not affected".

(e) An objection pursuant to Rule 86(3) EPC was raised in respect of all requests (see section IV.(a) above) due to:

(i) a newly introduced claim 2 (all requests) which specified the temperature as not exceeding 60°C;

(ii) a newly introduced claim (numbered 10 in the main request) relating to a vaccinal composition, and

(iii) three newly introduced claims (numbered 13-15 in the main request) relating to a "polysaccharide-polypeptide conjugation method".

(f) An objection pursuant to Article 84 EPC was raised in respect of the feature "subjecting [a reaction mixture] to microwave radiation at a temperature *not exceeding 100°C*" (emphasis of the communication). The italicized phrase was held not to be clear. Either this could mean that the sample was heated by conventional means to a temperature not exceeding 100°C and microwave radiation was then applied with the consequence that the total temperature applied could exceed 100°C. Alternatively, this could mean that the exposure to microwave radiation was controlled so that the temperature at no point exceeded 100°C.

Due to this unclarity, the stated feature could not serve to characterise the subject matter of the claims.

(g) With regard to Article 54 EPC and with reference to D3, it appeared that the first steps of the Maillard reaction were reaction of a carbonyl group with an amine, yielding an N-substituted glycosylamine. Since the carbonyl functionality had disappeared and a secondary amine had been formed, it was evident that both amination and reduction had taken place. Thus the reaction fell within the scope of the term "reductive amination".

Regarding the temperature of the reaction, since the specification of the temperature of the claim under consideration was not clear (see section V.(c) above), this could not serve as a distinction over the disclosure of D3, even though

the temperature of reaction was not derivable from the information given in D3.

Accordingly novelty of claim 1 of the main request was denied.

Novelty objections were not raised against the subject matter of the claims of the 1st-5th auxiliary requests.

(h) With regard to Article 56 EPC none of the distinguishing features claim 1 of any of the auxiliary requests had been shown to give rise to any technical effect. On the contrary these appeared to be conventional modifications.

Thus an inventive step was denied.

VI. In a letter dated and received on 13 April 2007, some 10 days after the latest date set for submissions in the communication of the board, the appellant submitted that an earlier response had not been possible due to medical reasons.

(i) Two further documents were submitted to show the difference between reductive amination and the Maillard reaction:

- Dick , W.E. Jr. & Beurret M., in "Conjugate Vaccines", Cruse, J.M. and Lewis R.E. Jr. (eds), - Contrib. Microbiol. Immunol. Basel, Karger, 1989 10 (2 pages cited)

– Richard, H. *et al* Flaveurs et procédés de cuisson, pp 5-9; further bibliographic data for this citation was not supplied.

(j) Four new requests, designated auxiliary requests 6 to 9 were submitted, replacing the requests filed with the statement of grounds of appeal (section IV.(a) above).

(i) The preamble of claim 1 of the sixth auxiliary request read:

"1. A method of reductive amination of bacterial or fungal polysaccharides that is achieved at a temperature not exceeding 100°C and comprises:".
The feature specifying that the polysaccharides had at least twelve repeat units was moved to each of subsections (i)-(iv) of claim 1.
The final line of claim 1 read:
"…whereby the antigenic determinants are retained".

(ii) Claims 2-14 were dependent claims corresponding to claims 2-6 and 8-15 both of the application as filed (see section I above) and of the claims on which the decision under appeal had been based (see section II above).

(iii) Claims corresponding to claims 2, 8, 10 and 13-15 of the set of claims submitted together with the statement of grounds of

appeal, which claims had no counterpart either in the application as filed or the set of clams forming the basis for the decision of the examining division (see section IV.(a) above) were not present.

(k) The amended claims were submitted to meet the objections raised by the board with respect to Articles 84 and 123(2) EPC and Rule 86(3) EPC.

(l) With regard to Article 54 EPC it was submitted, with reference to the position indicated by the board (see section V.(d) above), that this subject matter was novel.

(m) Concerning Article 56 EPC it was requested that since the claims excluded the possibility that the method be achieved at a temperature above 100°C, the inventive step issue be "favourably reconsidered". Reference was also made to the two documents newly submitted (see section VI.(a) above), it being argued these showed that in reductive amination the Schiff's base was reduced whereas in the Maillard reaction the Schiff's base was only submitted to rearrangement to form the Amadori product. Thus the reaction mechanisms of reductive amination and the Maillard reaction were not the same.

VII. Oral proceedings was held before the board on 4 May 2007.

(n) The chairman confirmed that the requests and submissions made with the letter of 13 April 2007

would be taken into consideration despite the filing thereof less than one month before the oral proceedings.

(o) With regard to the requirements of Articles 84 and 123(2) EPC in respect of the claims of the sixth auxiliary request filed with said letter of 13 April 2007:

– the board observed that according to page 8 lines 6-8 of the application as filed the "reaction" was achieved at a temperature not exceeding 100°C, whereas claim 1 of the 6th auxiliary request specified that the "method" be achieved in this temperature range;

– the board observed that while the preamble of claim 1 specified "bacterial or fungal polysaccharides" each of subsections (i)-(iv) of claim 1 employed the indefinite article, referring to "a polysaccharide having at least twelve repeat units";

– the appellant submitted with regard to the final feature of the claim, i.e. that the antigenic determinants of the polysaccharide were retained, that this was a consequence of the mild conditions, i.e. a temperature not exceeding 100°C which was possible due to the use of a mild reducing agent. The board observed that if the limitation to a temperature not exceeding 100°C automatically resulted in "retention of the antigenic determinants" then this phrase was

redundant. The appellant confirmed that this was the case.

(p) Concerning inventive step with respect to D2 the appellant submitted that D2 related to very small molecules and it could not be predicted on the basis of D2 that the method therein described would work with polysaccharides. It was emphasised that polysaccharides were large molecules with one reducing end - an aldehyde function - which entered into reaction. It could not be predicted how such a molecule would behave under exposure to microwaves. It could not be excluded that such a molecule would remain unreacted.

(q) With respect to D3 and the position of the board that the reaction disclosed therein fell within the terms of "reductive amination" the appellant submitted that reductive amination was a specific type of reaction between amino and carbonyl groups. This reaction required the presence of a reducing agent. D2 disclosed the reaction scheme of reductive amination. From this it was apparent that the Schiff's base was reduced in the presence of $NaBH_4$. Overall, the C=O bond was reduced with a gain of 2 hydrogen atoms. D3 did not employ a reducing agent, and thus no reduction took place.

In the Maillard reaction leading to the Amadori product there was a rearrangement. Although the imine function disappeared, this was not as a result of a reductive amination but as a consequence of said rearrangement. This was

demonstrated by the reaction scheme in "Les secrets de la casserole".

The appellant further submitted a reaction scheme showing the difference between the formation of the Amadori product - involving rearrangement with no overall gain in hydrogen atoms at the molecular level, and a reductive amination which resulted in an overall gain of 2 hydrogen atoms at the molecular level.

(r) Following this discussion, the appellant submitted an amended set of claims as the main and sole request, replacing all sets of claims previously submitted.

Claim 1 of this request read as follows:

"1. A method of reductive amination of a bacterial or fungal polysaccharide having at least twelve repeat units that is achieved at a reaction temperature not exceeding 100°C and comprises:
(i) subjecting a reaction mixture comprising the polysaccharide, an amino compound and a reducing agent, to microwave radiation for a period of time sufficient to aminate the polysaccharide ;
(ii) (a) subjecting a reaction mixture comprising the polysaccharide and an amino compound to microwave radiation, for a period of time sufficient to allow the formation of an imine compound, (b) adding a reducing agent to the reaction mixture obtained in (a) so that the polysaccharide is aminated ;

(iii) (a) adding an amino compound to the polysaccharide so that an imine compound is formed, (b) adding a reducing agent to the reaction mixture obtained in (a) and, (c) subjecting the reaction mixture obtained in (b) to microwave radiation, for a period of time sufficient to aminate the polysaccharide ; **or**
(iv) (a) subjecting a reaction mixture comprising the polysaccharide and an amino compound to microwave radiation for a period of time sufficient to allow the formation of an imine compound,(b) adding a reducing agent to the reaction mixture obtained in (a), and (c) subjecting the reaction mixture obtained in (b) to microwave radiation, for a period of time sufficient to aminate the polysaccharide."

Dependent claims 2-12 and 14 were unamended compared to the set of claims submitted with the letter of 13 April 2007 (see section VI.(b) above).

An editorial amendment was made to claim 13 by insertion of a hyphen to change "aminothiol" to "amino-thiol". This removed a discrepancy compared to the wording employed in the corresponding claim, numbered 14, of the application as originally filed.

VIII. The appellant requested that the decision under appeal be set aside and that a patent be granted on the basis of the main request (claims 1-14), filed at the oral proceedings.

Reasons for the Decision

1. The appeal is admissible.

2. *Article 123(2) EPC.*

Claim 1 of the main request (see section VII.(e) above) has been amended, compared to claim 1 of the application as filed as follows (amendments indicated in **bold**):

2.1 The polysaccharide is defined as a **bacterial or fungal** polysaccharide **having at least twelve repeat units**. The features indicated in bold are disclosed in claim 7 and at page 12, line 8 respectively of the application as filed.

2.2 It is now specified that the method **is achieved at a reaction temperature not exceeding 100°C**. This feature is disclosed at page 8 lines 9 and 10 of the application as filed.

2.3 In each of subsections (i)-(iv) the indefinite article preceding "polysaccharide" has been replaced by the definite article. This amendment is a consequence of the amendments specified in 2.1 whereby the definition of the polysaccharide in terms of its origin and its size are placed in the preamble of the claim.

2.4 Claims 2-14 correspond to claims 2-6 and 8-15 as originally filed (see section VI.(b).(ii) above).

2.5 Accordingly the amended claims meet the requirements of Article 123(2) EPC.

3. *Article 84 EPC*.

Compared to claim 1 as submitted with the statement of grounds of appeal and which was considered in the communication of the board (see sections IV.(a) and V above), claim 1 of the main request as filed at the oral proceedings (see section VII.(e) above) specifies "a method that is achieved at a reaction temperature not exceeding 100°C". This claim avoids the unclarity noted with respect to claim 1 of the previous main request according to which it could not be excluded that the reaction mixture was heated first to a temperature in the defined range and then microwaves applied, the temperature during exposure to microwaves not being limited (see section V.(c) above).

By specifying the temperature as being the "reaction temperature" it is also rendered clear that this is the temperature to be maintained throughout the reductive amination reaction, including those cases as in subsections (ii)-(iv) of claim 1 where this is accomplished in more than one step.

Consequently the amended claims meet the requirements of Article 84 EPC.

4. *The application, the technical problem and its solution*

4.1 According to the discussion at pages 1 and 2 of the application as filed, polysaccharide-polypeptide conjugates are useful as vaccines. Polysaccharides are usually T-independent antigens unable to elicit an anamestic (memory) response and further are not or are

poorly immunogenic in infants. It is known that such disadvantages can be overcome by conjugating the polysaccharides to a carrier polypeptide.

4.2 This conjugation may be accomplished by a variety of methods, one of which is reductive amination in which the reducing end of the polysaccharide is reacted with a functional group such as an amino group, e.g. with amino groups of a polypeptide at low temperature (usually about 50°C) in the presence of an appropriate reducing agent.

4.3 It is explained in the passage bridging pages 1 and 2 of the application that reductive amination is the result of two successive reactions:

- formation of the imine followed by
- reduction of the double bond of said imine.

As explained starting at page 2, lines 25 of the application, this reaction is time consuming, taking of the order of days to perform. The ultimate yield of aminated polysaccharide may be compromised by the formation of secondary products. Such undesirable secondary reactions include e.g. reversal to the non-reduced form of the polysaccharide and formation of degradation products.

4.4 Reductive amination of polysaccharides is conventionally carried out at a temperature about 15-50°C, (water bath). More elevated temperatures, e.g. above 100°C are not appropriate since polysaccharides could be degraded or denatured (page 3 lines 6-10 of the application).

4.5 According to page 3 from line 25 of the application it has been found that the time necessary for reductive amination of polysaccharides can be dramatically reduced while submitting the reaction mixture to microwave radiation. It is believed that microwave radiation may accelerate both the steps referred to in section 4.3 above. This further permits the reaction to be carried out under relatively mild conditions such that essential antigenic determinants within the polysaccharides are retained, which is an important consideration in the preparation of conjugate vaccines. Formation of secondary products is limited compared to the conventional process.

4.6 It is explained at page 8 starting at line 17 that the kinetics of the reductive amination typically adopts a curve with a plateau, the reaction time being defined as the period of time necessary for reaching the plateau. It is however the case, as explained at page 9 starting at line 6, that reaching the plateau does not mean that all the carbonyl groups present in the polysaccharide molecules have been reductively aminated. A number may remain non-reduced. Globally the product obtained by the method of the invention may be described as being partially aminated. The fact that a plateau is reached without the reagents being totally consumed is probably due to the formation of inhibitory by-products.

4.7 The examples of the application demonstrate reductive aminations under conditions of microwave irradiation (power of 15 W) in an apparatus in which the maximal temperature was set to 50°C. This method is compared

with a process in which a temperature of 50°C was achieved by employing a water bath.

The results show that under microwave exposure reductive amination reached a plateau, (see section 4.6 above) within a time of the order of 15 minutes to 1 hour whereas under conventional heating (water bath) a time of around 48 hours was required to attain the plateau. Further the content of residual reducing sugars was much higher in the case of microwave treatment. This indicates that fewer secondary reactions take place under microwave conditions. The residual reducing sugars are thus available for further reductive amination after the plateau has been reached (explained in the application at page 9, lines 22ff and page 18, lines 15-21).

4.8 Accordingly the evidence of the application reported in the previous section is that the process claimed results in a more rapid reaction with fewer by-products.

4.9 Therefore the evidence shows that the technical problem derivable from the application, namely reduction of the time required for reductive amination (see section 4.5 above) is effectively solved by the claimed measures.

In view of the evidence that a higher proportion of reducing sugars is retained as compared to conventional heating, it is also plausible that the further problems of maintaining the essential antigenic determinants and minimising the formation of secondary products are solved (see section 4.5 above).

5. *The prior art*

5.1 D1 is directed to oligosaccharides derived from an antigenic polysaccharide and preparation therefrom of a vaccine agent.

In particular D1 is directed to a oxidation-reduction depolymerisation procedure for polysaccharides to produce oligosaccharides (page 4 line 32 - page 5 line 17).

On page 3, starting from line 6 the antigenic activity of such polysaccharides, the inactivity in infants and the coupling of such polysaccharides to proteins is discussed (compare discussion in the application in suit, discussed in section 4.1 above).

In examples 6 and 7 of D1 the coupling of the oligosaccharides obtained by the method of D1 with diaminohexane is disclosed. This is accomplished in the presence of $NaCNBH_3$, and the reaction is carried out at room temperature for six days. The extent of reaction or degree of retention of the reducing sugar is not reported.

The use of microwaves is not disclosed.

5.2 D2 relates to a process of reductive amination of carbonyl compounds using microwaves employing sodium borohydride on wet clay.

According to the second paragraph in the section "Results and Discussion", *in situ* generated imines (i.e. Schiff's bases) are mixed with 10% $NaBH_4$-wet clay and

the reaction mixture irradiated in an unmodified household microwave oven for a specified time, in all cases less than 5 minutes.

The examples employ low molecular weight, monomeric carbonyl compounds e.g. benzaldehyde, salicylaldehyde, acetophenone etc and monomeric amines e.g. aniline, n-heptylamine benzylamine.

Polysaccharides are not employed in the examples or discussed in the general part of D2.

5.3 D3 relates to a manufacturing method of a protein-polysaccharide complex useful in food, cosmetics, medical supplies etc. It is stated (paragraph [0001] - all paragraph references relate to the machine translation) that the complex is obtained by the amino-carbonyl reaction characterized by carrying out microwave irradiation processing of the mixture of protein and polysaccharide.

According to paragraphs [0006] and [0008] the complex is formed with "little coloring (browning)". According to Example 1 (paragraph [0009], ovalbumin, a dextran (average molecular weight 75,000) and water were mixed at a rate of 2:10:1 and subjected to microwave processing (2450 Mhz, 50 KW, 5 minutes). The total volume of the reaction mixture employed is not disclosed. Thus it is not possible to derive the maximum temperature attained. There is no disclosure of addition of a compound identified as "a reducing agent".

6. *Article 54 EPC.*

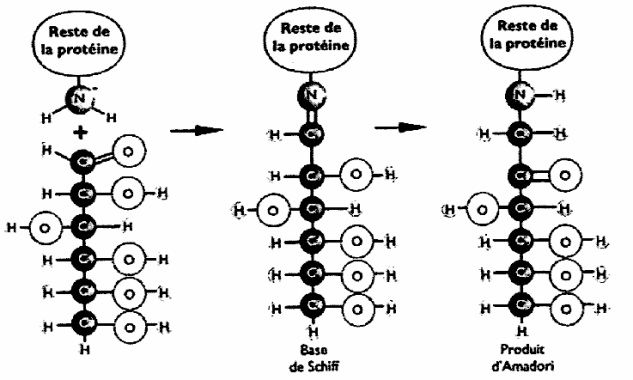
6.1 As explained in section 5 above, D1 does not disclose the use of microwaves and D2 does not disclose a reductive amination process applied to bacterial or fungal polysaccharides having at least twelve repeat units.

6.2 Therefore the subject matter claimed is novel with respect to the disclosures of D1 and D2.

6.3 During the course of the examination proceedings (see section II.(d).(iii) above, final indent) and the appeal proceedings (see section V.(d) above) the question was raised whether the - potential - occurrence of the Maillard reaction during the process of D3 constituted reductive amination.

6.3.1 According to the appellant in the statement of grounds of appeal, the occurrence of "little browning" could be indicative of the Maillard reaction occurring, either because the temperature was above 100°C or because this reaction was favoured by the microwave radiation (see section IV.(b).(v) above).

6.3.2 The sequence of steps in the Maillard reaction, proceeding via a Schiff's base to the Amadori product as disclosed in the document "Les secrets de la casserole" (page 30), is reproduced below:



6.3.3 At the level of the carbonyl group itself the reaction does result, via an imine (the Schiff's base) in production of an amine as a result of which the unsaturated carbonyl carbon becomes a saturated carbon of a secondary amine.

6.3.4 Therefore the carbonyl carbon atom has become reduced to a saturated carbon atom of a secondary amine. Since a reduction leading to an amine, i.e. a "reductive amination" has occurred it could be concluded - at the functional group level - that one of the components present is acting in a manner which falls within the terms of "reducing agent".

6.3.5 It is however apparent that the formation of the amine in this reaction sequence comes about because - due to the Maillard reaction - the carbon of the carbonyl group gains two hydrogens. The carbon α to the original carbonyl group however loses two hydrogens, resulting in the formation of a carbonyl group on said α-carbon (now adjacent to the carbon bearing the amine nitrogen).

6.3.6 Therefore at the level of the molecule, rather than the individual functional groups within the molecule, there has been no net gain of hydrogen atoms. In other words

the molecules involved in the reaction have not undergone any net reduction.

6.3.7 Accordingly the reaction sequence leading to the Amadori product does not involve an overall reduction of the molecular species involved and hence does not fall within the terms of "reductive amination".

6.3.8 Therefore the process disclosed in D3 does not involve a reductive amination.

6.4 The subject matter of the claims according to the main request is therefore novel.

7. *Inventive step - Article 56 EPC.*

As explained in section 4 above it is credible that the problem which the application in suit sets out to solve is in fact solved by the claimed measures.

It remains to be decided whether the claimed technical features contributing to the solution to this technical problem involve an inventive step.

7.1 The only document cited which relates to preparation of conjugates of polysaccharides and amines by reductive amination is D1. As noted in section 5.1 above, this discloses a process whereby the reactants are reacted at room temperature for a number of days. There is no disclosure in D1 relating to the use of microwaves to carry out the reductive amination.

Therefore D1 does not provide any suggestion or hint which would lead the skilled person to modify the

reductive amination process disclosed therein by employment of microwaves.

7.2 Although D2 does relate to the use of microwaves in a reductive amination process, it is, as noted in section 5.2 above applied exclusively to low molecular weight compounds. There is no disclosure in D2 that the process thereof would be applicable to polysaccharides of any kind, let alone those specified in claim 1 of the main request. There no technical relationship between the disclosures of D2 and D1 and no disclosure in D2 that would lead the skilled person to expect that the use of the process of D2, if nevertheless applied in the reductive amination process of D1 would result in more rapid reaction and a low occurrence of side reactions as is demonstrated by the examples of the application in suit (see sections 4.5 and 4.9 above).

Therefore D2 does not provide any hint or teaching to the skilled person to employ microwaves to promote the reductive amination of polysaccharides with amino compounds in the presence of a reducing agent.

7.3 D3, as established in section 6.3 above does not relate to reductive amination. Indeed it is not explained in D3 what reaction is actually taking place. Therefore the skilled person seeking to solve the problem underlying the application in suit (see section 4.9 above) would have had no reason to consult D3. Even if nevertheless D3 had been studied, it would yield no teaching or hint as to a potential solution of the technical problem underlying the application in suit.

7.4 Accordingly none of the documents D1, D2 or D3 contains a hint to the solution of the technical problem. Consequently the process according to claim 1 of the main request does not arise in an obvious way from the cited state of the art.

7.5 The subject matter of claim 1 of the main request therefore involves an inventive step pursuant to Article 56 EPC. This conclusion applies *a fortiori* to the subject matter of the dependent claims 2-14.

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 on the basis of the main request (claims 1-14), filed at the oral proceedings and after any necessary consequential amendment of the description.

The Registrar: The Chairman:

E. Görgmaier R. Young