CATAFLU.or

OrganoCATAlytic approaches towards easily synthesized, economical, and high yielding oseltamivir derivatives

PROJECT FINAL REPORT

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Name of the scientific representative of the project's co-coordinator, Title and Organization:

Pier Giorgio Cozzi, Associate Professor,

Alma Mater Studiorum – Università di Bologna (UNIBO) – Dipartimento di Chimica "G. Ciamician", Italy

Tel: +39 051 2099511

Fax: +39 051 2099456

E-mail: piergiorgio.cozzi@unibo.it

Project website address: www.catafluor.eu

Executive summary

Despite widespread immunization, influenza still kills thousands of people each year and through healthcare expenses and productivity losses, continues to have a significant impact on the economies of many countries. Small-molecule antiviral agents represent a novel opportunity for an effective prevention and therapy of the influenza virus. Inhibitors of neuraminidase (NA), an enzyme identified as essential for viral replication in all three classes of influenza viruses, have been recently discovered. Two of these inhibitors, Zanamivir and Oseltamivir phosphate, have already reached the market. The recent health concerns related to avian flu have increased the demand for stockpiles of neuraminidase inhibitors, both as a reasonable frontline therapy against a possible flu pandemic and as a preventive agent.

In order to find new drug candidates as well as to cut the cost and improve the availability of existing drugs, new and efficient chemical syntheses are necessary. The CATAFLU.OR project proposes a new domino reaction based on an organocatalytic approach to the synthesis of Tamiflu and new inhibitors of neuraminidase derivatives.

Summary description of project context and objectives

The overall scientific and technical objective of the CATAFLU.OR project is **to achieve innovative, simple and straightforward synthetic routes towards enhancing the availability and supply of neuraminidase inhibitors. The CATAFLU.OR project addresses this target through the preparation of highly challenging neuraminidase inhibitors.**

Five main tasks characterize the work plan of the CATAFLU.OR project:

1. Synthesis of modified catalysts for the organocatalytic domino reaction. Scale up of the reaction. Testing of the new catalysts in the established domino reactions aimed at producing new cyclohexene derivatives. The manipulation of the derivatives will be used for a practical and rapid access to newly designed neuraminidase drug candidates.

2. Use of the catalysts in the design of new organocatalytic domino reactions. The synthesis of cyclic compounds via domino reactions.

3. Preparation of new neuraminidase inhibitors through the use of organocatalytic domino reactions.

4. Testing of the new neuraminidase inhibitors on cell lines, animals and viruses.

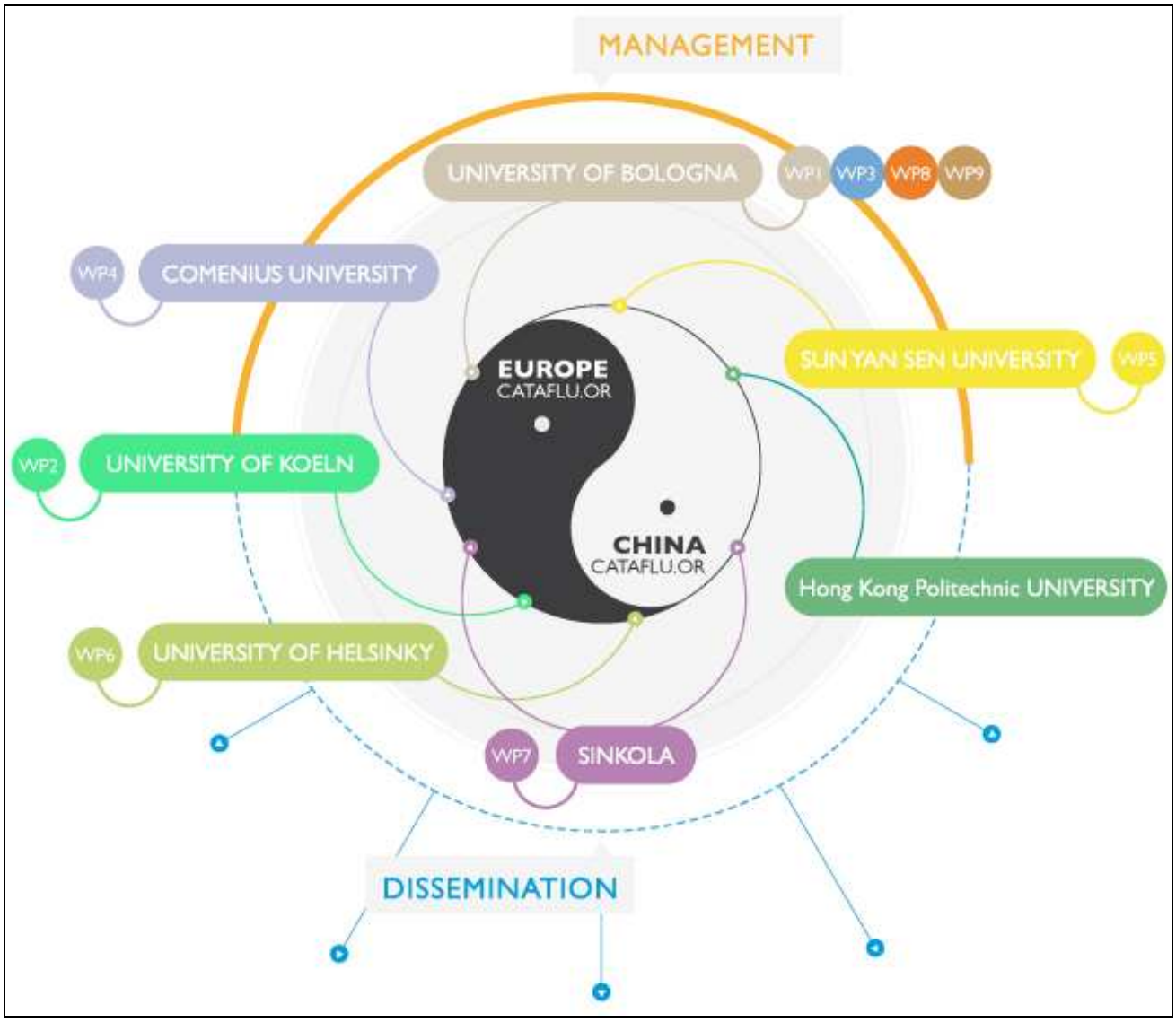
5. Tests in silico, in vitro, and in vivo of the newly prepared inhibitor against influenza viruses.

Our overall strategy and general description: CATAFLU.OR is structured along the lines of the following workpackages.

- WP 1 Domino organocatalysis;
- WP 2 Organocatalytic multicomponent synthesis of structurally diverse cyclohexanes;
- WP 3 New Oseltamivir derivatives;
- WP 4 Domino reaction in ionic liquids;
- WP 5 Screening tests;
- WP 6 Docking simulation of neuramidinase inhibitors;
- WP 7 Organocatalytic multicomponent large scale synthesis of structurally diverse Oseltamivir derivatives;
- WP 8 Dissemination;
- WP 9 Management.

WP1-4 and WP7 include major synthetic and preparative chemistry, whereas WP3 deals specifically with the synergy of the groups. WP5 is concerned with the screening and finalization of the selected drug entities; WP6 is related to in silico design and screening. WP8 is devoted to dissemination activities and WP9 to the project coordination and management.

The project workpackages are organized and structured according to the scheme below:



The project intends:

- To propose new neuraminidase inhibitors for screening against flu viruses.
- To define a new strategy based on an organocatalytic, highly economical domino reaction.
- To decrease costs and minimize problems related to the supply of Oseltamivir and Oseltamivir derivatives.
- To contribute to solving the problem of avian flu at the locations of outbreak and development (Hong Kong and China).

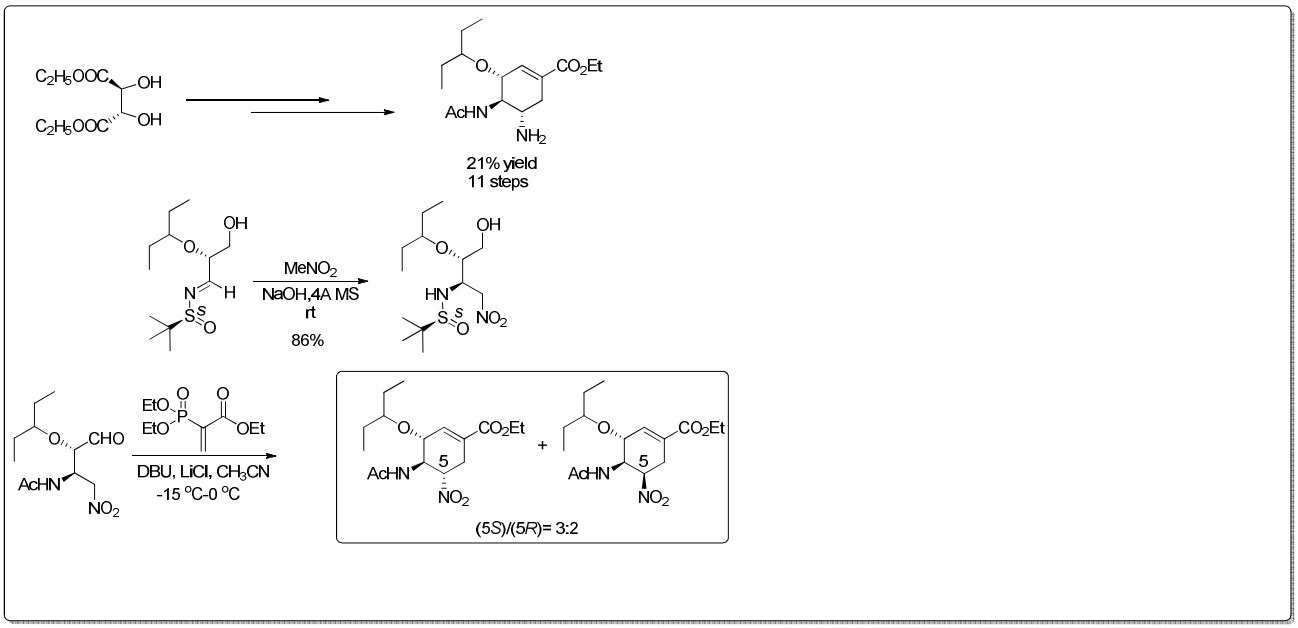
Additional objectives of the project are:

- To promote innovative activities, with a particular emphasis on the involvement of SMEs and technology transfer.
- To have a long-term impact on influenza therapy.
- To strengthen collaboration between Europe and China.
- To allow researchers operating in different countries to exchange ideas and knowledge in the pursuit a challenging common objective.

Description of the main S&T results/foregrounds

Domino organocatalysis

The utility of organocatalytic domino reactions in the synthesis of Oseltamivir has been examined in this workpackage. A short and practical synthesis of Oseltamivir has been accomplished from diethyl D-tartrate. (Scheme 1).

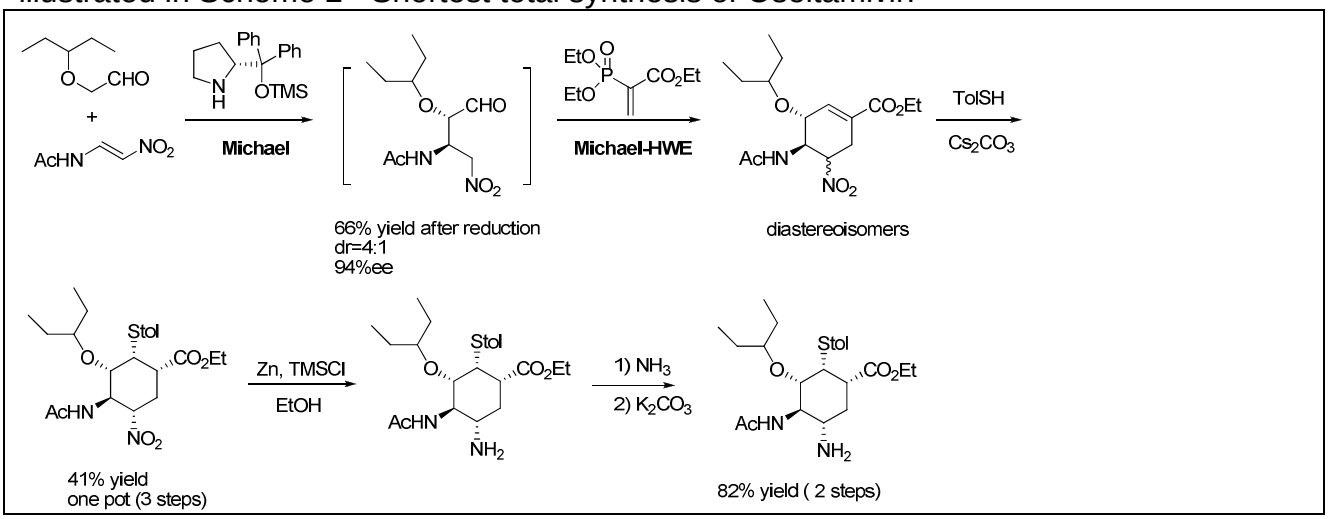


Scheme 1. Synthesis of Oseltamivir and key steps for the synthesis

The use of cheap and available tartrate for the synthesis of Oseltamivir is not new. The real novelty of our approach is found in the use of an asymmetric aza-Henry reaction performed on a chiral adduct. The sulfinamide derivative is prepared using a commercially available chiral auxiliary and nitromethane is added by reaction with a base. Since its introduction by Ellman in 1997 as a chiral ammonia equivalent (Ellman, J. A.; Owens, T. D.; Tang, T. P. Acc. Chem. Res. 2002, 35, 984), enantiopure 2-methyl-2-propanesulfinamide (tert-butanesulfinamide) has been demonstrated to be a versatile chiral auxiliary and has been extensively used in both academics and industry. Condensation of tert-butanesulfinamide with aldehydes and ketones proceeds under mild conditions and provides tert-butanesulfinyl imines in high yields. The tert-butanesulfinyl group activates these imines for addition of many different classes of nucleophiles and serves as a powerful chiral directing group providing products with high diastereoselectivity. Subsequent removal of the tert-butanesulfinyl group under mild conditions cleanly provides the amine product. The use of a chiral auxiliary is mandatory for good facial stereo selection. The second key development in the synthesis was the domino nitro Michael/HWE reaction used to construct the relevant cyclohexene ring (Scheme 1). The strategy is organocatalytic as DBU is used. The Michael reaction is followed by cyclization to give the desired cyclohexene. The chemistry developed improved the diastereoselectivity. This is an innovative and useful approach for the preparation of cyclohexene ring, a common moiety in many drugs and biologically active molecules. Starting from the tartrate this new approach requires 11 steps with a 21% overall yield, potentially providing an economical and practical alternative to the synthesis of Oseltamivir.

The second approach towards Oseltamivir was also the subject of two different publications by Professors Ma and Hayashi (S. Zhu, S. Yu, Y. Wang, D. Ma, Angew. Chem. Int. Ed.

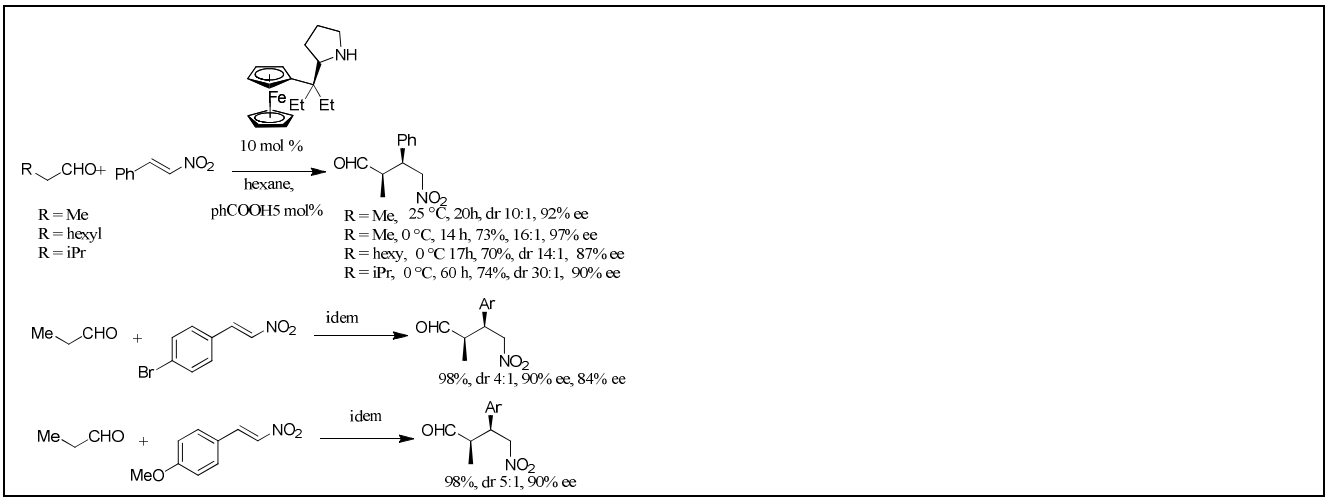
2010, 49, 4655; H. Ishikawa, T. Suzuki, Y. Hayashi, Angew. Chem. Int. Ed. 2009, 48, 1304). The two highly cited publications are testimony to both the successful core idea of the CATAFLU.OR project and the highly competitive nature of the field of organocatalysis. Professor Hayashi has also published other organocatalytic approaches to several drugs. The second synthesis that we have developed can be considered as competitive with regards to the classical route to Oseltamivir. The majority of approaches to the drug are based on long multi-step syntheses (8-11 steps) using potentially dangerous reagents. The strategy developed by Roche for the synthesis of Oseltamivir is based on a natural source (Shikimic acid), the supply of which cannot be guaranteed. Our synthetic approach and the approaches described by professors Ma and Hayashi, uses a fully organocatalytic strategy with cheap and available starting material. The synthesis of Oseltamivir was accomplished in three or four steps with good yields and high stereoselection. The short synthesis is the result of careful optimization studies with many derivatives tested in the key organocatalytic steps. Despite the fact that not all of the derivatives prepared by partners in CATAFLU.OR project were adapted to the domino organocatalytic processes that we have proposed and developed, they have been studied and a stereoselective route has been found based on other organocatalytic transformations. The strategy for the total synthesis of Oseltamivir is illustrated in Scheme 2 - Shortest total synthesis of Oseltamivir.



Scheme 2. Shortest total synthesis of Oseltamivir.

We have optimized the synthesis of the starting materials (protected aldehydes and nitro derivatives) at both the small and large scale. The overall synthetic pathway can be considered to be both high yielding and reproducible.

Many interesting results in organocatalytic reactions (Michael, aldol) were obtained with the different organocatalyst derivatives prepared by the different groups working in WP1 (UNIBO, CU, POLYU, and SYSU). Particularly interesting results were obtained in the synthesis of ferrocenyl pyrrolidine, a previously unreported organocatalyst that has since shown high efficiency in organocatalytic reactions (Scheme 3 - Ferrocenyl pyrrolidine in organocatalytic reactions).



Scheme 3. Ferrocenyl pyrrolidine in organocatalytic reactions.

Through the optimization of the catalyst structure by means of DFT calculations, we were able to shed light on some aspects of the underlying mechanisms in the organocatalysis. It is proposed that while the enamine formed in the catalytic cycle reacts with the electrophile, one face of the enamine is covered by a hindered substituent present in the chiral amine used as an organocatalyst. In the case of the ferrocenyl pyrrolidine, the small ethyl group is enough to efficiently cover one face of the enamine as the ferrocenyl moiety blocks the conformational freedom of the enamine catalyst. The results obtained from the the design and synthesis of ferrocenyl pyrrolidine are general and can be used in the development of more selective and active organocatalysts. In addition, our results represent the first use of the ferrocenyl framework in the design of active organocatalysts. The results gained from this process are important to the wider synthetic organic chemistry and include high selectivity, stability, as well as the possibility to vary the ferrocenyl framework for further. We have also tested the ferrocenyl pyrrolidine in the new SN1-type organocatalytic reactions that were developed during the CATAFLU.OR project. The performance of the ferrocenyl pyrrolidine was superior to many other commercially available catalysts (such as the Hayashy-Jorgensen catalyst). A number of other pyrrolidine based catalysts were prepared and investigated in organocatalytic reactions. (Figure 1 - Pyrrolidine organocatalysts obtained in CATAFLU.OR project and the Nitro-Michael reaction were investigated).

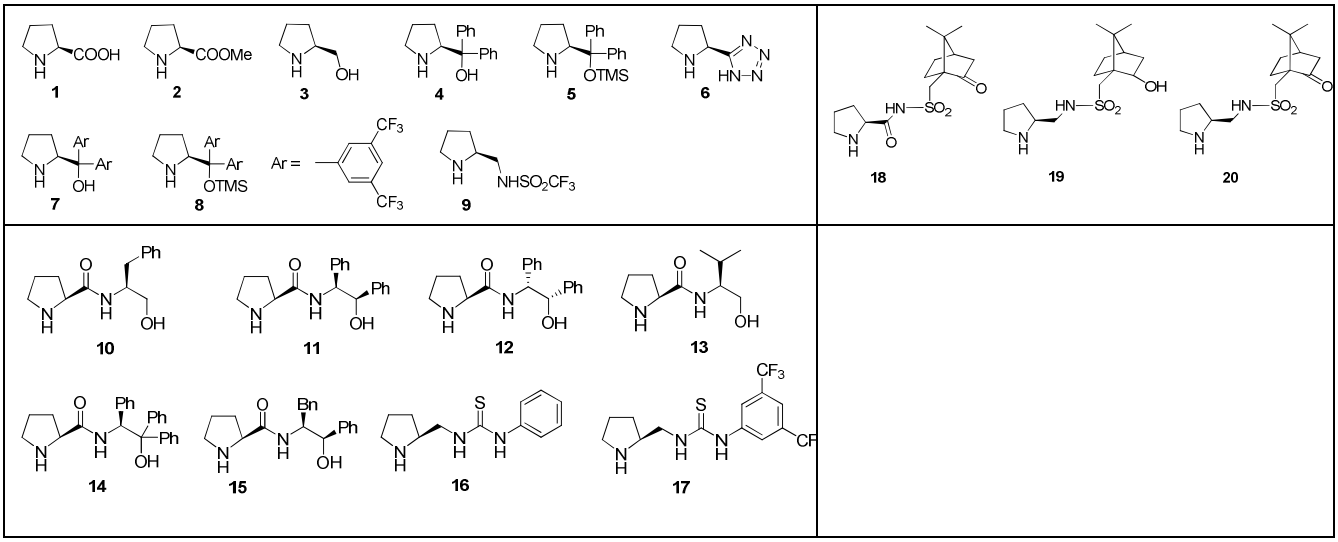
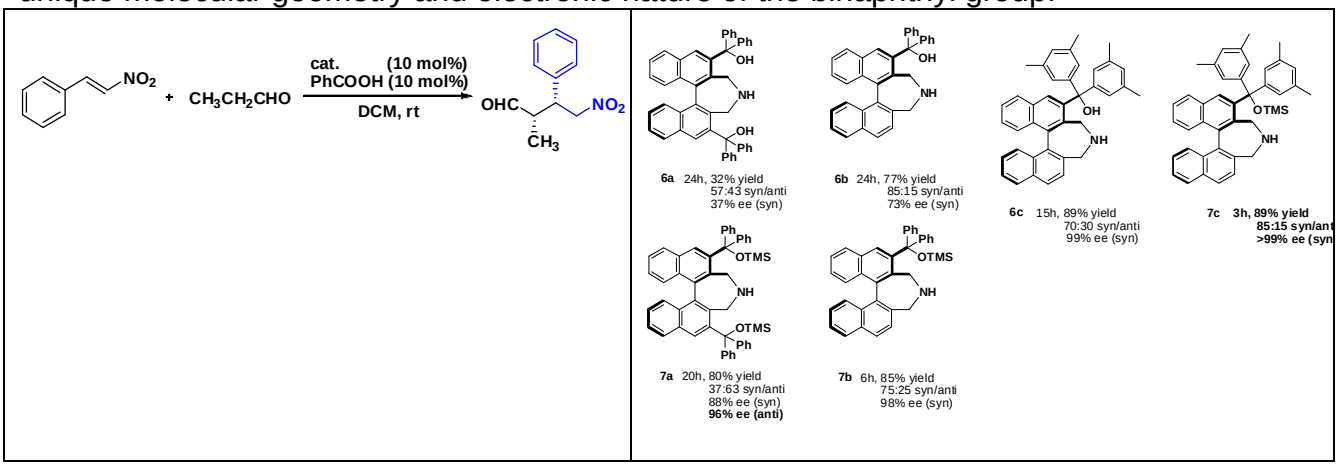


Figure 1. Pyrrolidine organocatalysts obtained in CATAFLU.OR project and the Nitro-Michael reaction were investigated.

The particular reaction of nitroenamines with nucleophiles has not been extensively described in existing literature. Hence we developed the synthetic utility of nitroenamine derivatives as a new class of Michael acceptors in the organocatalytic Michael addition of aldehydes, resulted in the addition products in good yields and very good to excellent enantioselectivities. During the synthesis of many organocatalysts we also explored the unique molecular geometry and electronic nature of the binaphthyl group.



Scheme 4. Organocatalytic Nitro-Michael reaction with different Binapthyl organocatalysts.

We have synthesized the starting materials and by long and multistep synthesis many new derivatives that were employed in organocatalytic reactions, as illustrated in Scheme 4 - Organocatalytic Nitro-Michael reactions with different Binapthyl organocatalysts. Other important reactions were investigated and quite interesting results in terms of yield and selectivity were obtained. The synthesis developed for the chiral 1,1'-binapthyl-2,2'-dicarboxylic acid from easily accessible 1,1'-binaphthol was performed in a 7-step reaction. The synthesis is novel and gave a product with 75% overall yield and 99% ee. It should be noted that in this suggested synthesis, all intermediates and products can be purified without recourse to column chromatography, making the method suitable for the industrial setting. We have also described another synthesis of chiral BINOL-COOH via a three-step reaction from optically active 1,1'-binapthol (overall yield up to 62%, ee up to 99%). The key

methoxycarbonylation reaction of ditriflate can be realized without the need to employ expensive high-pressure equipment. This optimized synthetic procedure is simple and, as before, suitable as an industrial process. We have established a more convenient synthetic route for a binaphthyl derived intermediate. Its key features include the selective direct esterification of the binaphthyl structure at the 3- or 3,3'- position and methylation at the 2,2'-position of binaphthyl structure by a Negishi cross-coupling reaction. This procedure could also facilitate large-scale access to this type of compound. As briefly mentioned, the binaphthyl based secondary amine has been successfully used in the asymmetric Michael reaction of aldehydes to nitroalkenes. It is worth noting that a number of binaphthyl derived amino alcohols exhibited excellent ees in metal catalyzed reactions such as the asymmetric arylation reaction and the Henry reaction, expanding the range of applicability of our new ligands.

Organocatalytic multicomponent synthesis of structurally diverse cyclohexanes

The workpackage 2 consisted of the study of multicomponent reactions towards the preparation of cyclohexene derivatives. Our initial efforts for task 1 focused on the development of reaction conditions for the multi-component cycloaddition of amides (component 1), aldehydes (component 2), and suitable dienophiles (component 3) in order to synthesize highly diversified amidocyclohexenes which bear a close structural relationship to known neuraminidase inhibitors. In the optimized reaction conditions of the three-component condensation-cycloaddition protocol, which involves the employment of 1-2 mol% para-toluenesulfonic acid as Brønsted acid catalyst in toluene solution at 80-110℃, we were able to prepare a number of compounds with a variety of functional groups. Examples of structures prepared with the process can be seen in Figure 2 - A series of cyclohexene prepared with the multicomponent reaction.

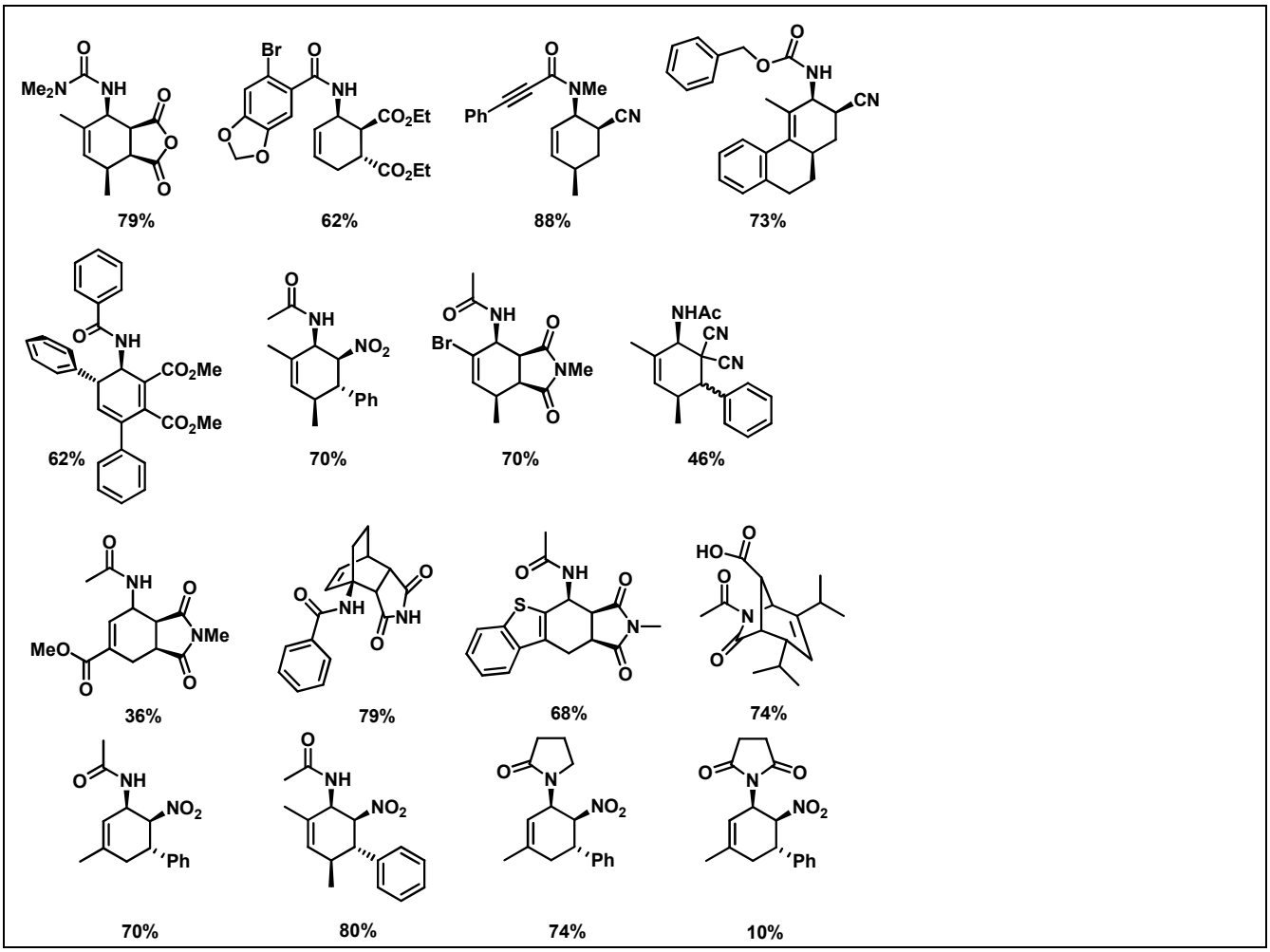
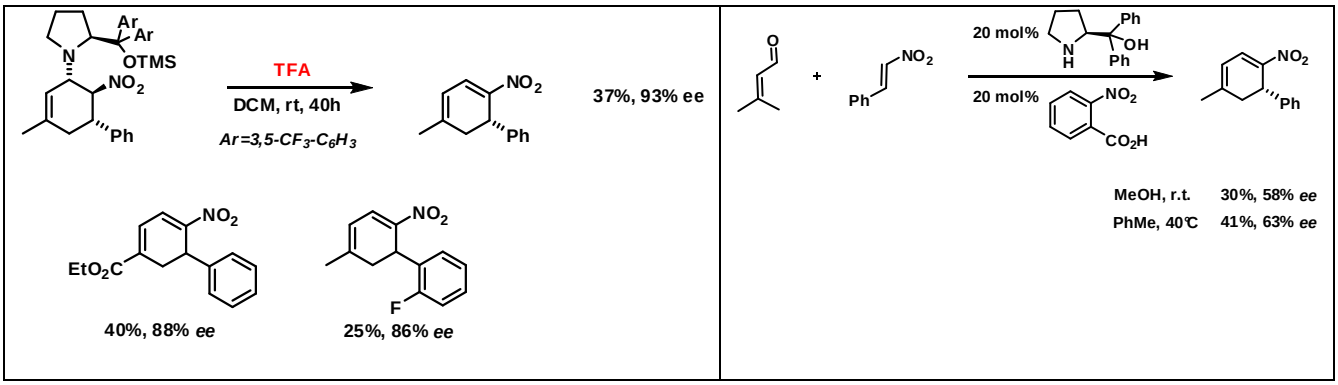


Figure 2. A series of cyclohexene prepared with the multicomponent reaction.

We have also applied the protocol to unsaturated aldehydes. In another approach toward the synthesis of highly functionalized aminocyclohexenes, we investigated new three-component reactions of secondary amines, α, β-unsaturated aldehydes, and nitroolefins. Such cycloadditions can be carried out at room temperature in dichloromethane solutions with good overall yields. This Michael-type reaction gave products with a trans-substitution. In order to prepare optically active cyclohexene derivatives we have used chiral secondary amines. We used proline and prolinol derivatives that were provided by WP1 and WP3. The resulting aminonitrocyclohexadienes were isolated in high yields and high diastereoselectivity as all trans isomers. In the presence of acids, a facile elimination of the amine occurred, producing nitrocyclohexadienes. These adducts are useful intermediates in the synthesis of Oseltamivir. The electrophilicity of the nitrocyclohexadiene at the beta-position is inherent in the nitroethylene moiety (Scheme 5 - Enantioselective formation of nitrocyclohexene derivatives).



Scheme 5. Enantioselective formation of nitrocyclohexene derivatives.

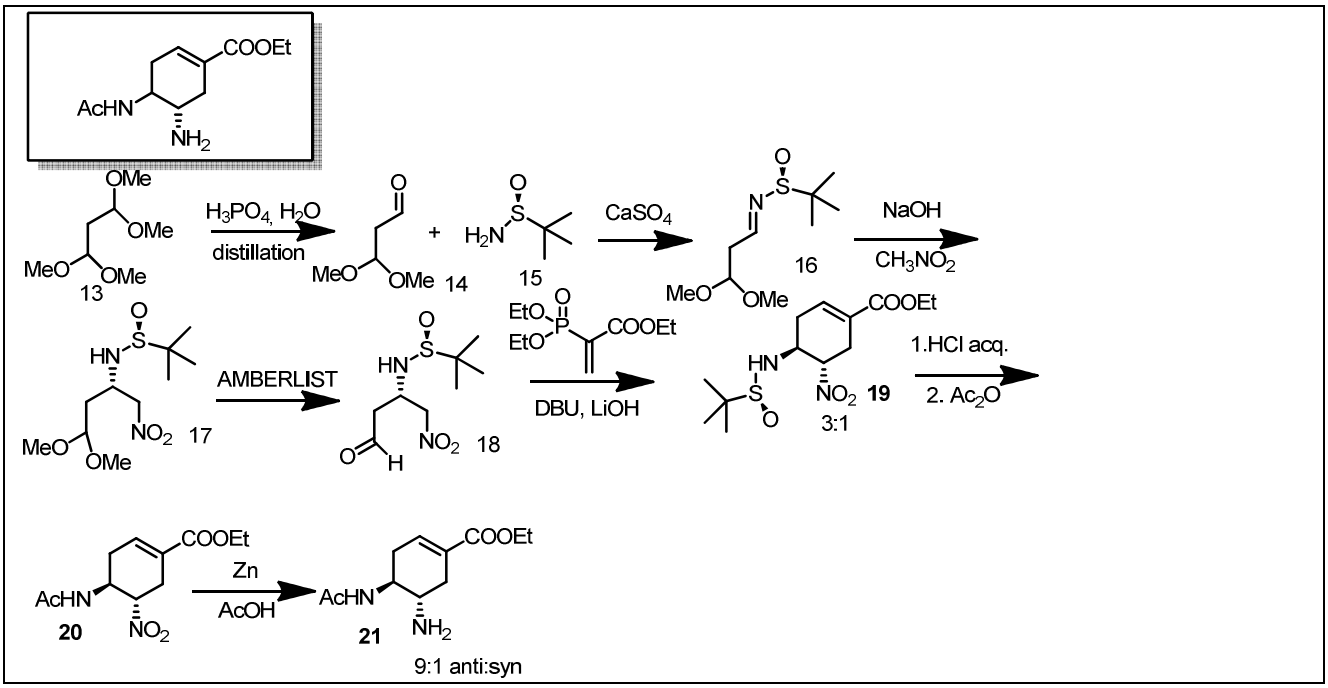
In the presence of suitable oxygen nucleophiles, a Michael-type addition would deliver a similar amino alcohol function as in Oseltamivir. We studied several reaction conditions for the addition of alcohols in the presence of various bases and, while in low chemical yields and low stereoselectivity (cis and trans), we have isolated the corresponding products. The enatioselective preparation of Oseltamivir needs a stereoselective cycloaddition. For this purpose we have studied the asymmetric cycloaddition reactions, in the presence of chiral Brønsted and Lewis acids. We have prepared several chiral phosphoric acids and modified the reaction conditions but unfortunately the ee obtained were quite low. This was also true of the chiral Lewis acids that were tried. However, our multicomponent reaction was able to furnish a large variety of compounds that were screened in silico (collaboration with HU, WP6) and in vitro (collaboration with SYSU, WP5). This test gave us a better idea and understanding of the Neuraminidase recognition and was helpful in the process designing and testing new compounds.

New Oseltamivir derivatives

The work towards the new derivatives was performed by CU, UNIBO, and SYSU. Following discussion and reciprocal agreement, the work towards the different derivatives (see infra) was divided in three different tasks. The synthesis and the realization of the derivatives named 38-41 (see last version of the DoW) was realized by UNIBO, the derivatives 42-44 were prepared by SYSU and CU (See final discussion for the deviation from the original proposal).

- Task 1: Synthesis of the new derivatives 38-41

Modification of the proposal was dictated by difficulties in the synthesis and by the low levels of interaction between the proposed molecules and Neuraminidase, as identified by means of docking studies. It was decided to replace the alkoxy substituent in Oseltamivir by an H group. The synthesis was performed as illustrated in the Scheme 6 - Total synthesis of the candidate lacking the alkoxy group. The synthesis was completely modified due to the problems encountered during the synthesis (See DoW). Through employment of the organocatalytic Nitro-Michael reaction it was possible to prepare all the diastereoisomeric derivatives that were subjected to in silico studies (by CU) and in vitro test (by SYSU). The candidates prepared were found to be completely inactive, for this reason the introduction of an aromatic group by alkylation of the nitro derivative was instead studied.



Scheme 6. Total synthesis of candidate lacking of the alcoxy group.

The theoretical analysis performed by HU and discussed during the meetings supported the idea that a substituent (alkyl or aryl) near the ester group was more suitable for the preparation of effective inhibitors (Figure 3 - suggestion for a better Neuraminidase inhibitor).

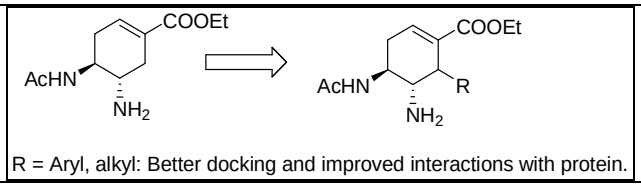
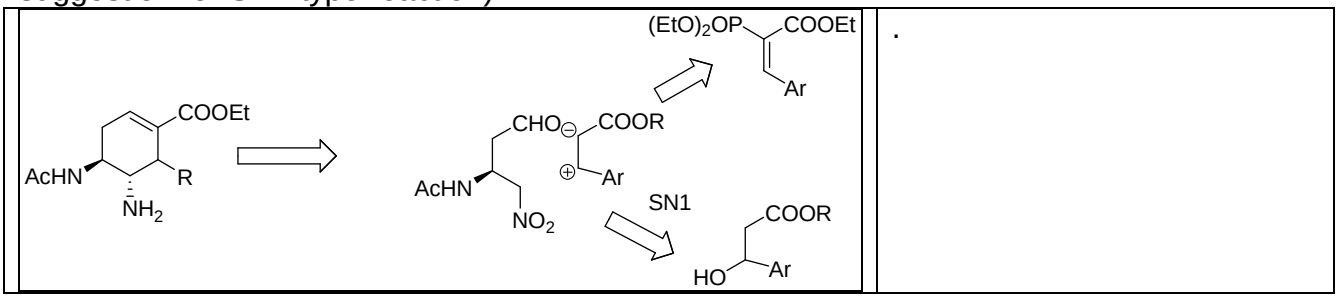


Figure 3. Suggestion for a better Neuraminidase inhibitor.

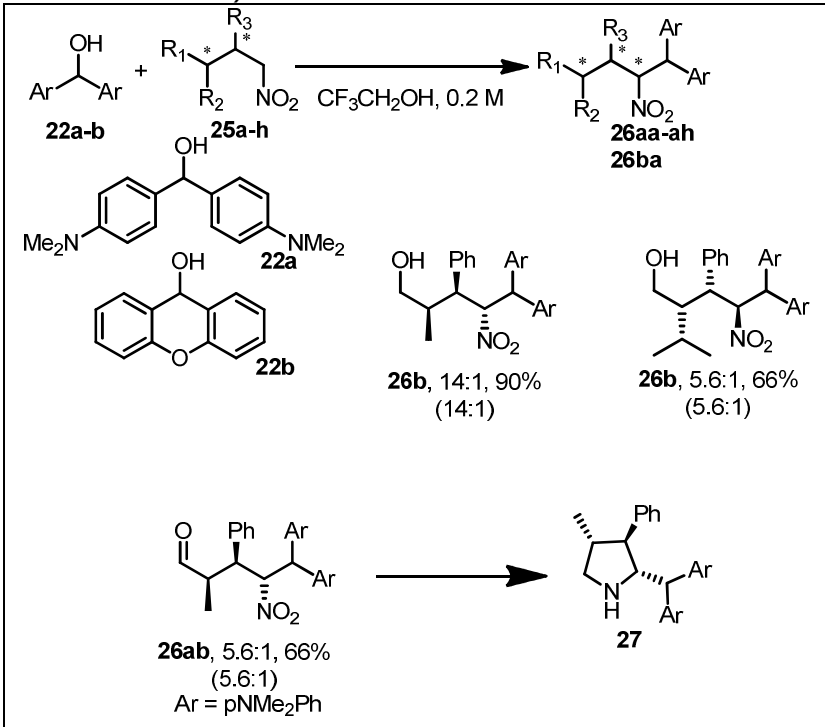
In order to develop further strategies for the synthesis of the proposed derivatives, we have tried to develop direct SN1 type reaction of nitro derivatives. In fact, as illustrated in the retrosynthetic analysis, the SN1 reaction can be use to access Oseltamivir product substituted in the position near the alkene moiety (Scheme 7 - Retrosynthetic analysis and suggestion for SN1 type reaction).



Scheme 7. Retrosynthetic analysis and suggestion for SN1 type reaction.

Unfortunately, the Horner-Hemmons reagent bearing a bulky group in β position was incapable of reacting in the cyclization step (in general, we found the compound to be rather unreactive). Therefore, we have examined the SN1 type reaction starting from suitable precursors. It is worth mentioning that the alkylation of nitro derivatives through a general and simple strategy has never before been reported in the literature (indeed, we

were quite surprised to find no reports of investigations towards this end in literature). A new SN1 reaction, the alkylation of nitro derivatives with alcohols was discovered. Organocatalytic Michael and Henry type reactions allowed for the preparation of densely functionalized and useful building blocks in a highly stereoselective manner. We therefore employed a stabilized carbenium ion in the alkylation reaction of nitro derivatives performed in trifluoroethanol (TFE). In addition, we found the reaction to be quite stereoselective and useful in producing new and effective pyrrolidine organocatalysts through simple reaction sequences (Scheme 8 - Diastereoselective and first example of SN1-type alkylation of nitroderivatives).



We were pleased to discover that the reaction was not only possible but that it was also quite stereoselective. In all the substrates investigated we have found, in the limit of the NMR and GCMS detections, the presence of a single diastereoisomer. The compounds **25a-h** were obtained through standard organocatalytic procedures employing the Hayashi catalyst or the other organocatalysts. As reported in scheme 2, we observed a decrease the ratio between the two initial diasteroisomers (only ina single case (**26b**)). As regards all other substrates, no trace of the two other diasteroisomers was observed and the ratio between the two stereoisomers was maintained during the reaction.

Scheme 8. Diastereoselective and first example of SN1-type alkylation of nitroderivatives.

- Task 2: Synthesis of the new derivatives 42-44.

The derivatives containing a modified hydroxy chain were investigated by docking analysis. We discovered that the introduction of oxygen in the six membered ring was crucial in order to obtain a better binding energy. Therefore sialic acid derivatives were evaluated. For the difficult step of introducing the alkene moiety at the 3-position of the Oseltamivir derivatives, we turn our interest to the natural sialic acid, which is cheap, abundant, and most importantly, it already contains several of the desired stereo centers with the desired configuration. The corresponding binding energy with NA has also been evaluated and it has been found to be quite promising (Figure 4 - Sialic derivatives with high affinity with Neuraminidase).

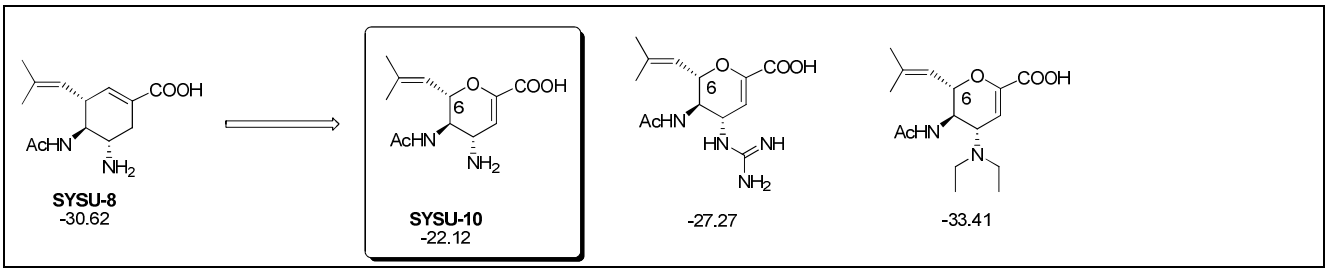
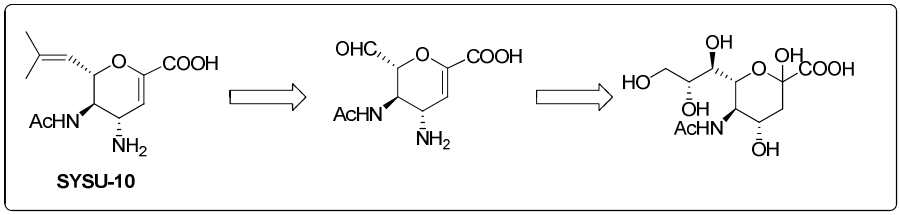


Figure 4. Sialic derivatives with high affinity with Neuraminidase.

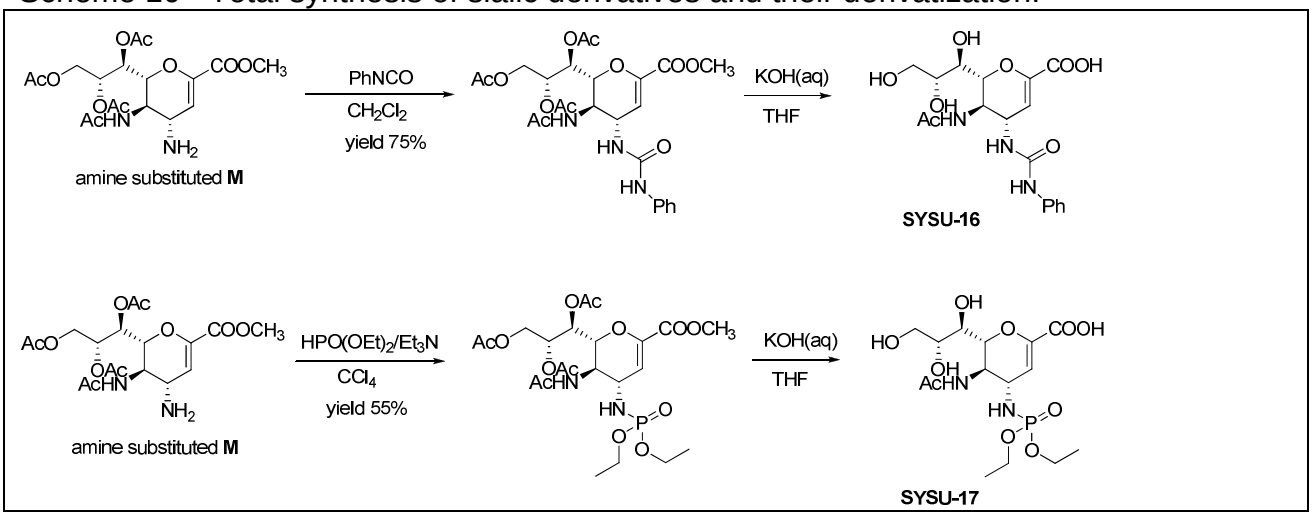
Our synthetic efforts were directed towards the use of natural sources to access these derivatives or other similar compounds (Scheme 9 - Retrosynthetic analysis for the sialic derivatives).



Starting from sialic acid, after several transformations the key aldehyde can be obtained. This compound can then be transformed into many other derivatives by means of the Wittig reaction.

Scheme 9. Retrosynthetic analysis for the sialic derivatives.

We have found that β-unsaturated carboxylic acids and esters moieties can be introduced easily to the 6-position of the pyrane backbone, however for the simple alkene moiety, the corresponding Wittig reaction proved to be quite difficult. The OH group present in the six membered ring of the pyrane moiety was easily transformed into the corresponding amino group through various pre-existing reactions (SN2, by N3 or by Mitsunobu). The free amino group was easily reacted with different groups, exemplified by the derivatives illustrated in Scheme 10 - Total synthesis of sialic derivatives and their derivatization.



Scheme 10. Total synthesis of sialic derivatives and their derivatization.

- Task 3: synthesis of the new derivatives 45-48.

SYSU's research group has carried out molecular kinetic analogy studies and mmpbsa calculations to evaluate the binding energy between the potential candidates and the active site of NA, however it was found that the model still needed to be modified further according

to biological activity data. We found that compound b showed comparable activity with Oseltamivir, while c and d exhibited inferior binding energies (Figure 5 - Cyclohexene derivatives bearing functional group and their affinity with Neuraminidase).

	Binding energy kcal/mol	std	Time/ps	Binding energy kcal/mol	std	Time/ps
a	-22.84	5.9	990-1090	-24.80	5.17	1990-2090
b	-22.73	4.74	890-990	-19.63	6.16	1890-1990
c	-17.85	4.24	890-990	-16.33	4.13	1190-1290
d	-17.81	5.96	890-990	-17.49	5.14	1190-1290

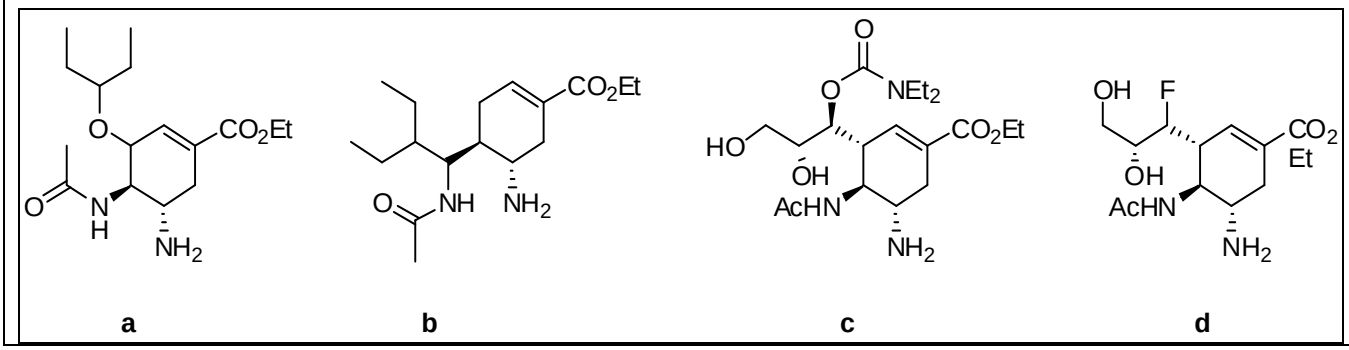
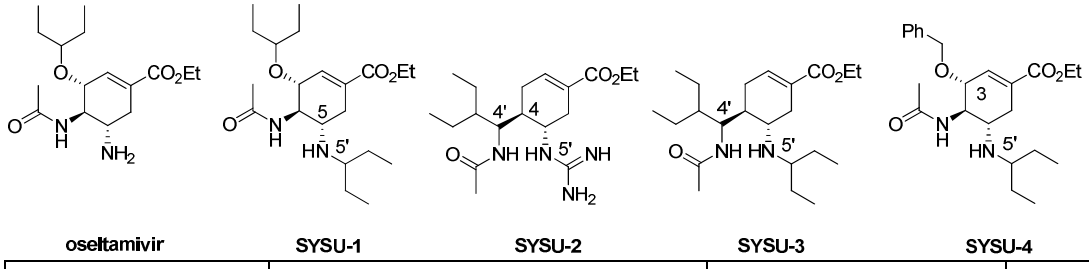


Figure 5. Cyclohexene derivatives bearing functional group and their affinity with Neuraminidase.

We have also evaluated the process of introducing substituents onto the nitrogen atom. As illustrated in Figure 6 - Inhibitors bearing alkyl group on the nitrogen atom, the binding energies calculated for the compounds were remarkable, especially for compound 3.

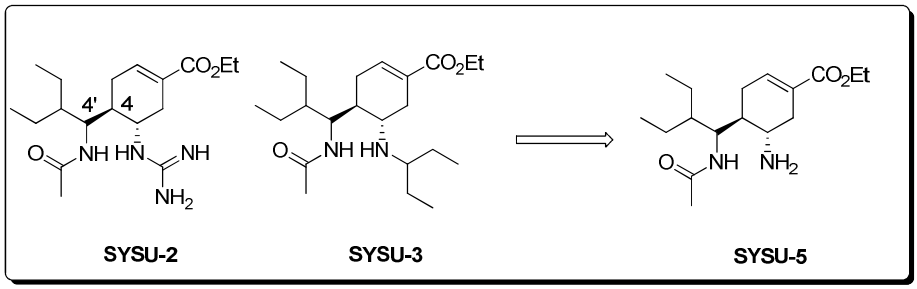


compound	Binding energy (kcal/mol)	Std	Time/ps
oseltamivir	-31.21	3.98	1990-2090
SYSU-1	-25.75	5.06	1890-2090
SYSU-2	-23.98	5.83	1990-2090
SYSU-3	-31.77	4.18	1990-2090
SYSU-4	-19.67	2.45	1990-2090

Figure 6. Inhibitors bearing alkyl group on the nitrogen atom.

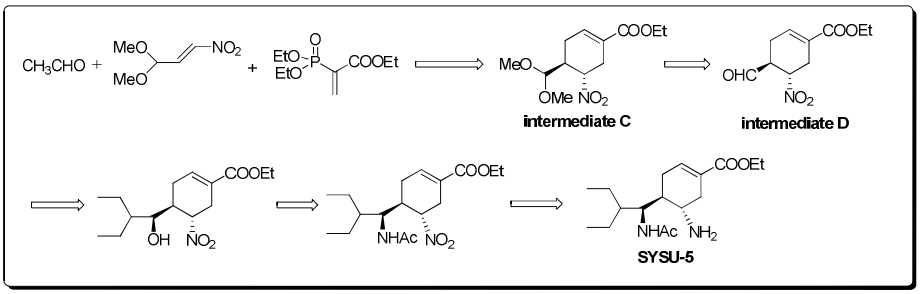
The compounds above were obtained by condensation with aldehydes and successive reduction. Some of the derivatives were simply obtained from Oseltamivir. For other derivatives (for example, number 2) it was necessary to form another synthetic strategy

altogether. The synthesis is illustrated in Scheme 11 - Retrosynthetic analysis and it takes advantage from organocatalytic multicomponent reaction, studied in WP1.



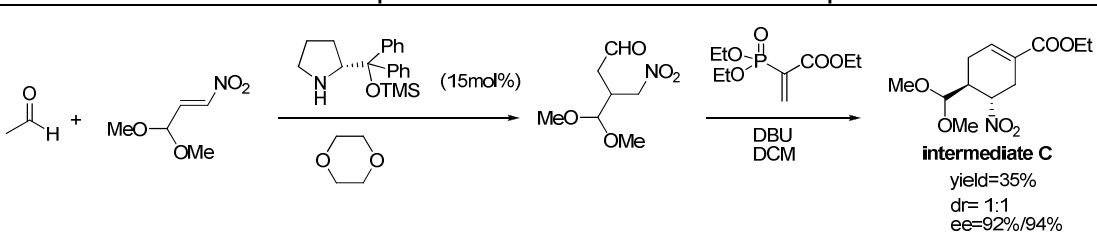
Scheme 11. Retrosynthetic analysis.

In fact, the Intermediate C can be obtained by a domino Michael/HWE.



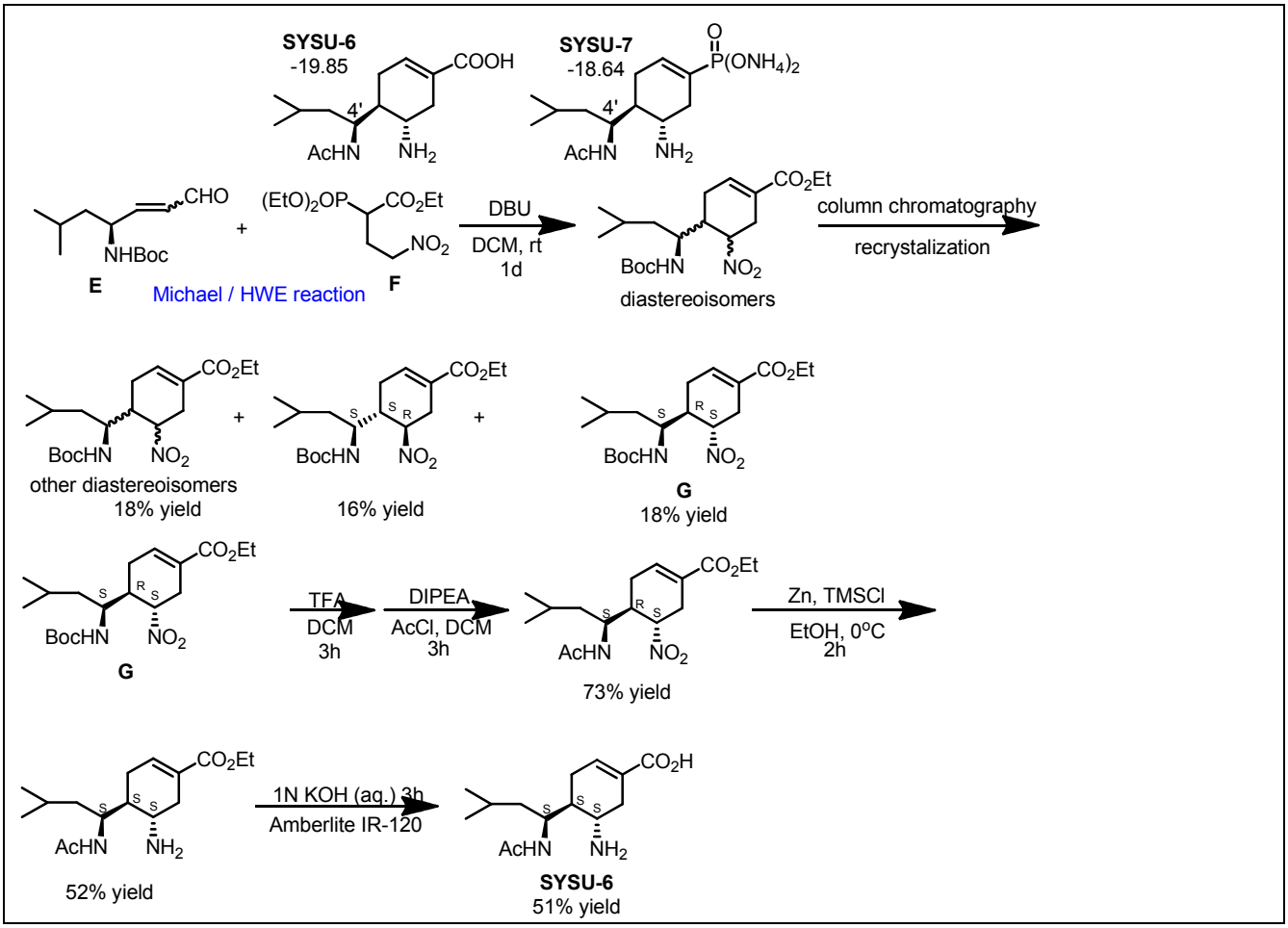
Scheme 12. Proposed synthesis of the key intermediate.

As demonstrated by the Nitro-Micheal (Scheme 13 - Organocatalytic Nitro Michael) and domino reactions, the organocatalytic synthetic strategy is a simple enough to give ready access to either the direct product desired or relevant compounds for further transformation.



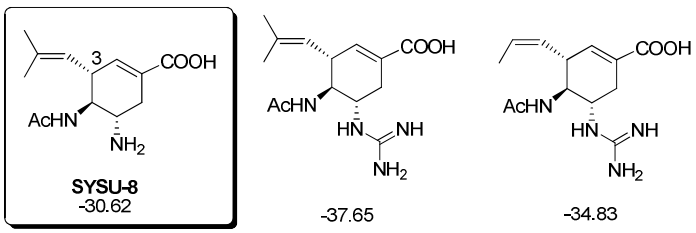
Scheme 13. Organocatalytic Nitro Michael and domino reaction.

Many functional group conversions are possible within this synthetic framework; deprotection of the acetal and oxidation of the aldehydes to the corresponding acid are relatively simple procedures. As is the subsequent transformation of the acid group to the corresponding amino. Further analysis of possible derivatives has shown that the 4'-substituted analogues gave interesting values for the binding energy with Neuraminidase. We have exploited previously employed synthetic routes, including the organocatalytic domino reactions, for their syntheses (Scheme 14 - Synthesis of the intermediates). For SYSU-6, starting from L-leucinol, the corresponding α, β-unsaturated aldehyde E underwent the domino reaction with nitro phosphonate F to provide the diasteroisomers of the cyclohexene intermediate. After column chromatography, the desired diastereoisomer G can be isolated in 18% yield. Its absolute configuration was determined by X-ray crystal structure determination. Nitro compound G was then reduced with zinc under acidic conditions to provide the amine. Subsequent hydrolysis of ethyl ester afforded SYSU-6.



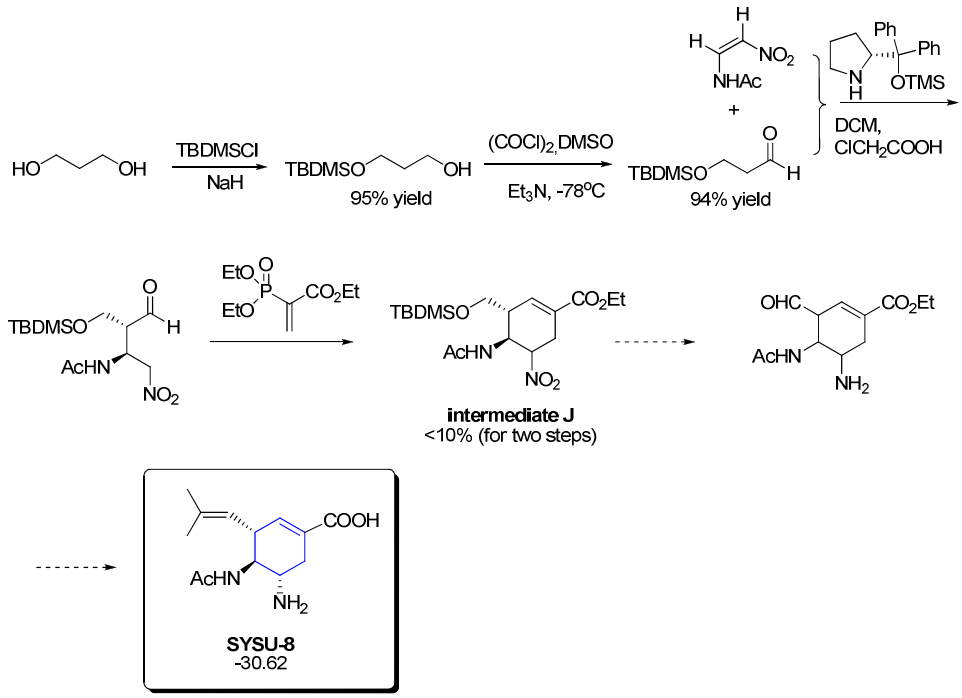
Scheme 14. Synthesis of the intermediates.

It was possible to start from a different Michael acceptor to prepare the compound SYSU-7 with different absolute configurations to that desired. The synthetic sequence that we applied to the derivatives SYSU 6 and 7 is flexible and can be used for other interesting candidates. In fact, the calculated binding energies were found to be around 30 kcal/mol (Figure 6 - New compounds considered).



For **SYSU-8**, key cyclohexene **intermediate J** with special substituents has been obtained in less than 10% yield. The process still requires further optimization and several steps are still needed to obtain the final product.

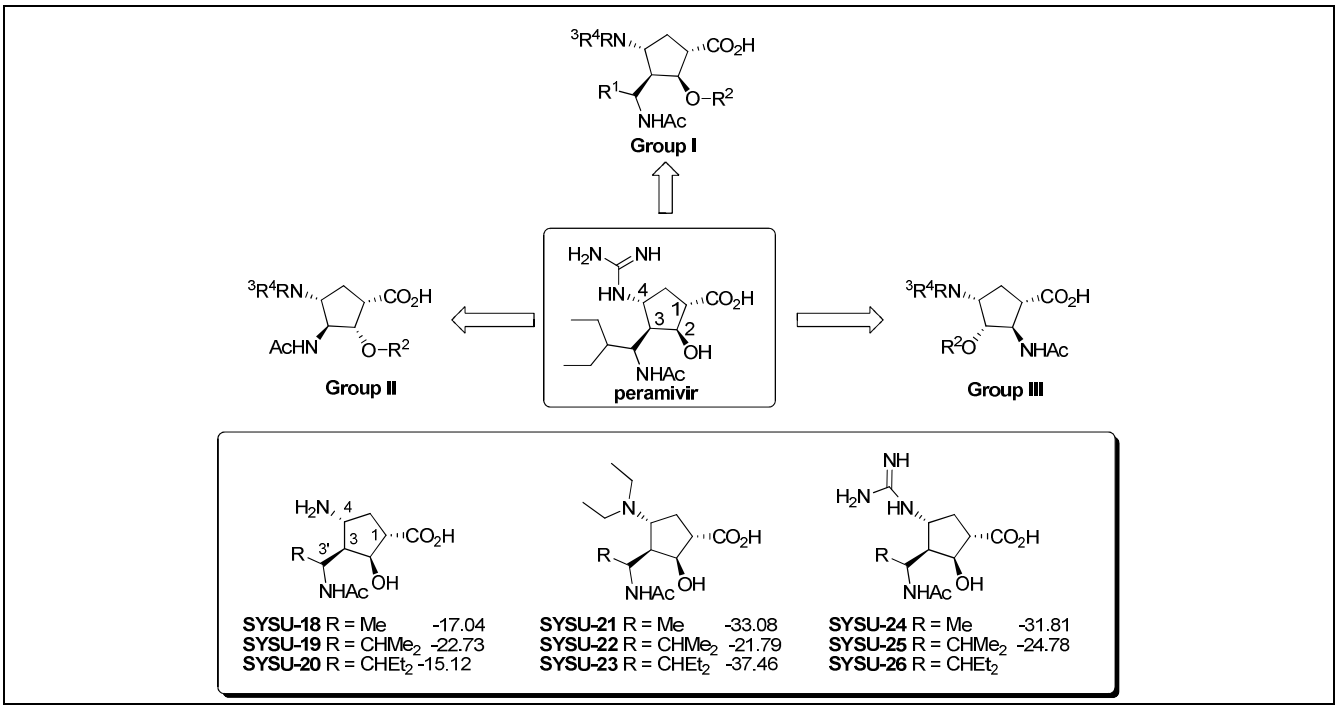
Figure 6. New compounds considered.



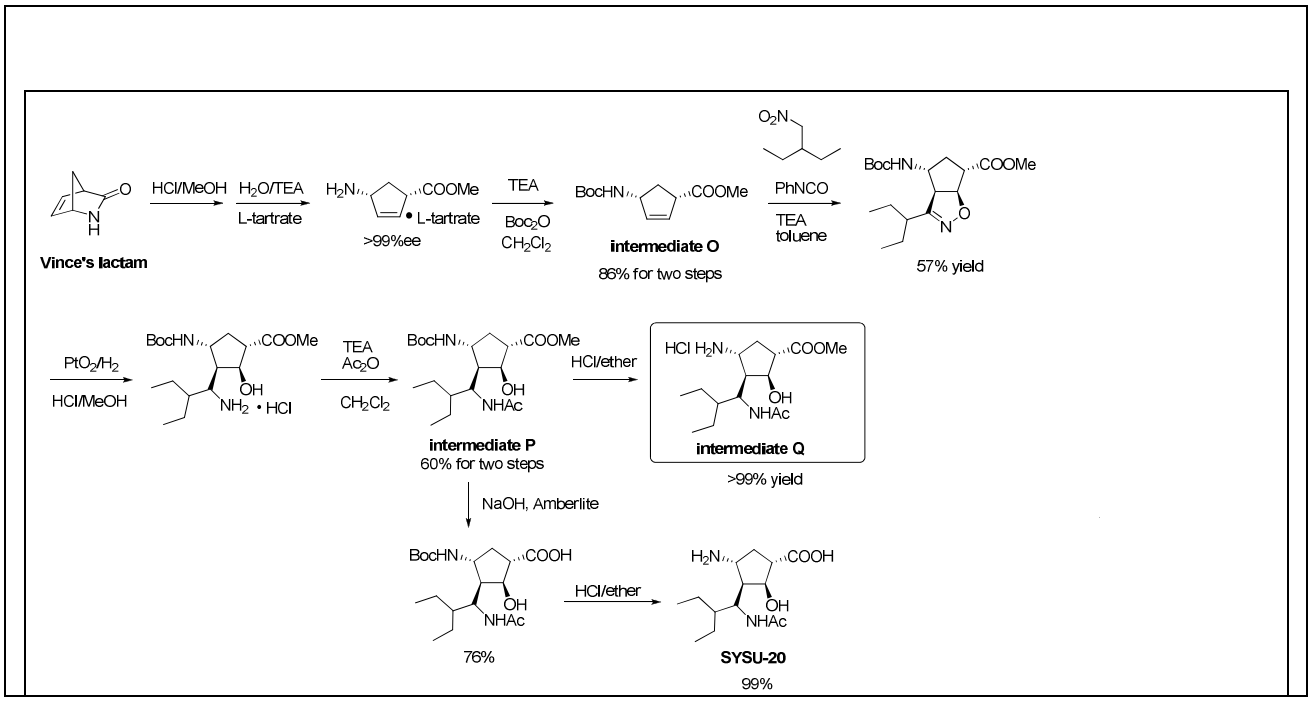
Scheme 15. Synthetic methodology for the synthesis of compound SYSU 8.

An alternative strategy was later adopted to synthesize its analogue SYSU-9. Starting from a chiral amino alcohol, the corresponding α, β-unsaturated aldehyde K was reacted with vinyl phosphonate to afford the diasteroisomers of the cyclohexene precursor. After column chromatography, diastereoisomer L can be isolated. Further reduction with zinc under acidic conditions provided the amine. Subsequent hydrolysis of the ethyl ester with lipase afforded SYSU-9. In conclusion, not only was the direct synthesis of Oseltamivir by organocatalytic multicomponent reaction realized, but we were also able to prepare many derivatives by direct modification of the starting materials and through the adoption of the domino organocatalytic reaction for the key step. The reaction is quite versatile, with starting materials selected in order to prepare the desired cyclohexene derivative with the minimum number of steps possible. Chiral aldehydes and chiral derivatives are also suitable substrates for the domino reaction. In addition, nitroalkene derivatives bearing functional groups have been shown to undergo the domino reaction without significant problems. The organocatalytic approach demonstrates versatility, tolerance of a wide variety of functional groups and the ability to be scaled up. The intermediates obtained were further transformed and tested in vitro for activity.

Peramivir is another efficient NA inhibitor (Scheme 16 - Analysis and suggested compounds bearing a five membered ring). A key structural characteristic of this molecule is the presence of a five membered ring. Modification of the Peramivir was carried out to evaluate potential inhibitors.



Scheme 16. Analysis and suggested compounds bearing a five membered ring.

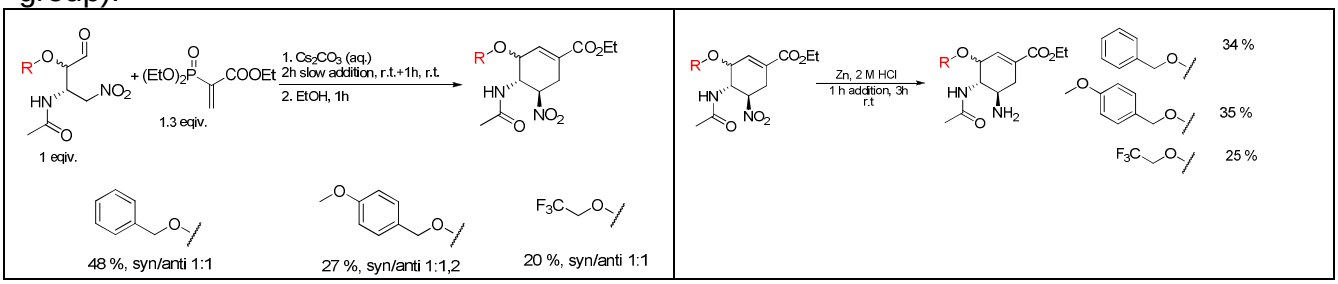


Scheme 17. Synthesis of Peramivir derivatives.

Many derivatives were obtained by synthesis. For the sake of brevity, we have shown only the general strategy used for the preparation of the Peramivir core (Scheme 17 Synthesis of Peramivir derivatives).

Different Oseltamivir derivatives bearing different protected alkoxy chains were prepared by Nitro-Michael addition. Different derivatives obtained from benzyloxyacetaldehyde, 4-methoxybenzylacetaldehyde and 2,2,2-trifluoretoxyacetadehyde were obtained. Unfortunately, the successive reaction with ethyl-1-diethylphosphonyl acrylate gave the desired cyclohexene derivatives in low yields and low diastereomeric ratio. However, all the

desired compounds were obtained and subjected to tests for their activity. Unfortunately, the modification of the alkoxy chain with more polar, or more hydrophilic substituent gave less active compounds. The organocatalytic strategy was successfully used again for fast access to the compounds desired (with, in this case, different aldehydes as starting materials) (Scheme 18 - Synthesis of Oseltamivir derivatives with different protecting alkoxy group).



Scheme 18. Synthesis of Oseltamivir derivatives with different protecting alkoxy group.

The principal objective of WP3 was related to the synthesis of new Oseltamivir derivatives and their evaluation as candidates for further development. Through employment of the strategies elaborated in WP1 and discussions in the meetings and between all partners, a series of Oseltamivir derivatives proposed in the DoW were synthesized through a complex and new total synthesis. For all molecules proposed and subsequently synthesized, derivatives, strategies and reactions conditions were evaluated sequentially in a case by case manner. We proposed several possible structures, as depicted in Figures 7, 8, and 9.

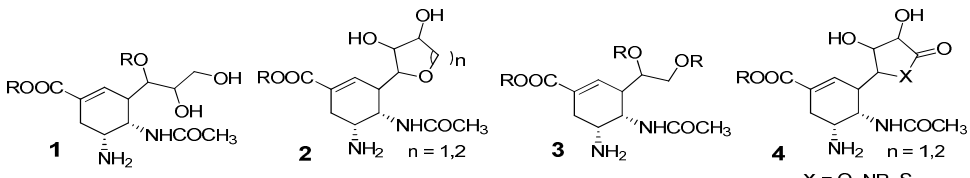


Figure 7

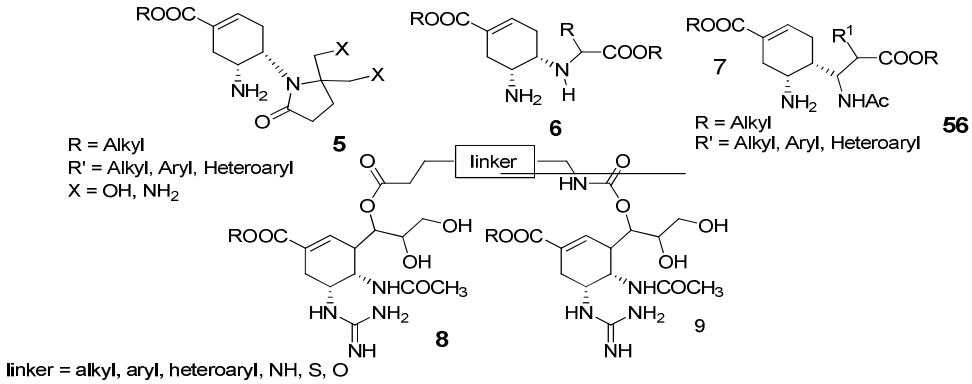


Figure 8

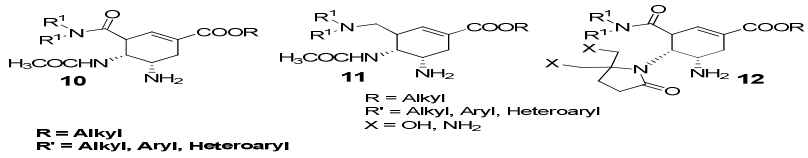


Figure 9

The objective of the WP3 was to establish new routes and possible synthetic schemes, starting from organocatalytic strategies towards the preparation of new, active Oseltamivir derivatives. The synthesis of new candidates was in a great many cases, successful, and the new derivatives prepared were tested according to standard procedures to determine their effectiveness for further development. Some important preliminary was collected. In the DoW we have identified potential candidates.

The synthesis of the candidates 5-7 was achieved.

The synthesis of the structures 1-4 was partially achieved.

Precursors for the synthesis of the structure 10-12 were achieved. The goal was partially achieved.

The synthesis of the structure 8 was not achieved.

The structure of the candidates 1-4 was modified (an oxygen introduced) according to the relevant in silico theoretical calculations regarding the binding energy.

The structures 10-12 were discarded as a result of the calculations run and consequently the syntheses were not explored.

Structures 10-12 with different groups containing fluorine were proposed and obtained, albeit in very low yields.

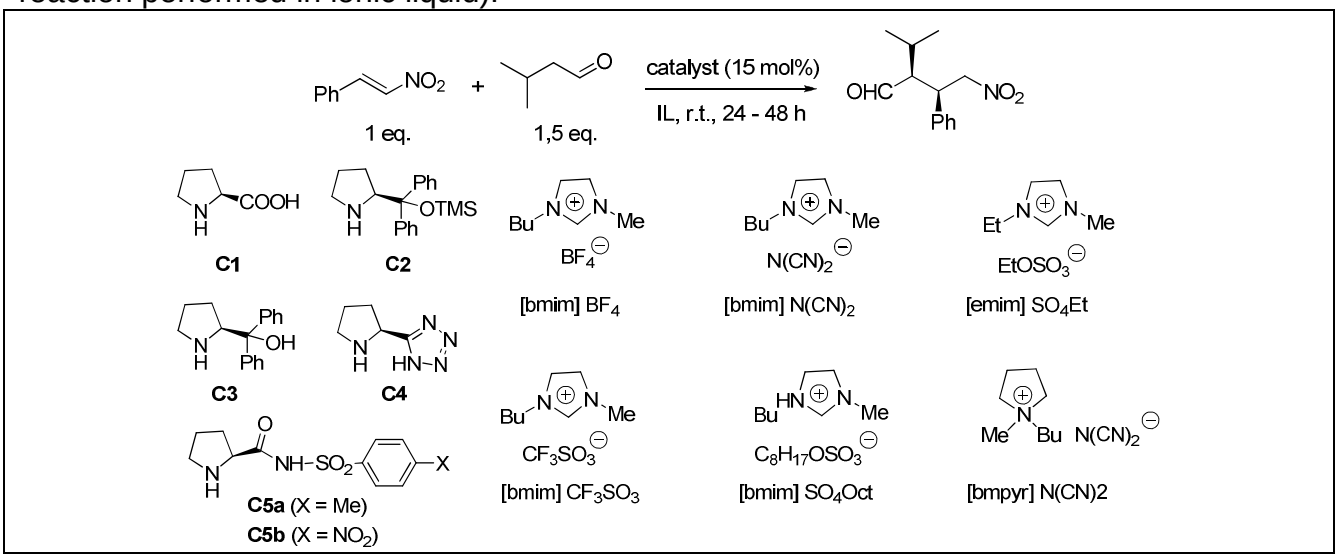
The main deviation from the proposed structure was determined by the relatively low score obtained in the in silico evaluation of the structure. Programs and suitable models for evaluating the proposed structure were, of course, not available when the research plan was written. A careful analysis of the structure did not give a particularly encouraging value for the binding program. As a result of the experience gained with the structures previously synthesized, a low binding energy became synonymous with low inhibitor activity. Therefore the docking program was used in the design of possible effective inhibitors. By the use of the docking program, deviations from the originally drawn molecules were considered and the synthetic efforts were directed towards the preparation of these, more active, molecules.

Domino reaction in ionic liquids

- Task 1: Domino reaction in ionic liquid.

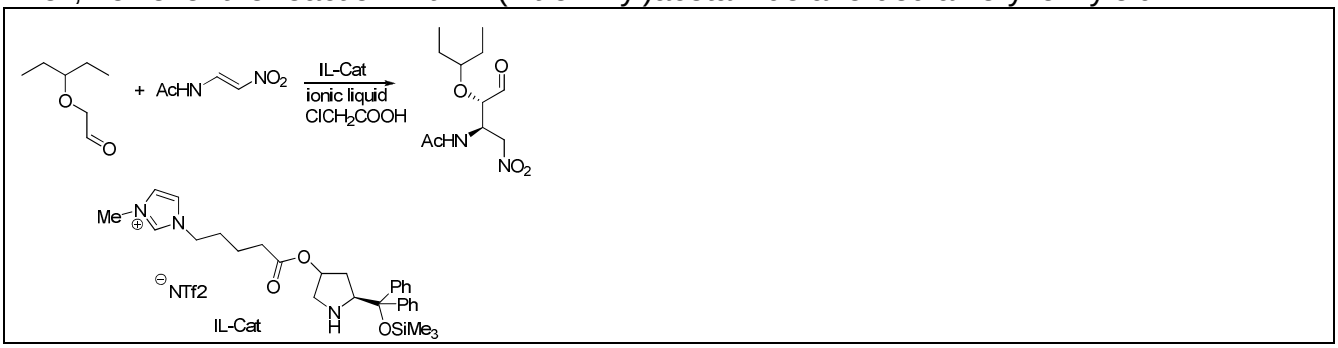
The reason for carrying out the reactions in ionic liquids is primarily that they allow the possibility of recovering the catalytic system as well as the ease of product isolation afforded. As a result of the fact that the catalysts used in organocatalytic reactions are relatively inexpensive, sometimes (as in the case of proline) the use in 20 mol % can be justified. However, in the case of the domino reaction studied for the synthesis of Oseltamivir, the more expensive Hayashi-Jorgensen catalyst is used. The use of batch reactors is better served by a catalytic system that is almost entirely recoverable. From the outset, our research focused on the key organocatalytic domino reaction previously described in the literature. Preliminary experiments with the same reaction using the ionic liquids [bmim]BF4 as well as [bmim]PF6 gave disappointing results. However, the reaction

that was applied in the total synthesis of Oseltamivir was a Nitro-Michael reaction and therefore we studied this model reaction in different conditions (Scheme 19 - Nitro-Michael reaction performed in ionic liquid).



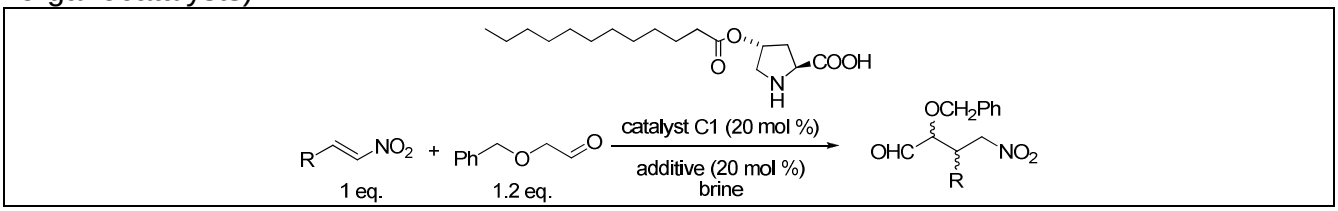
Scheme 19. Nitro-Michael reaction performed in ionic liquid.

The reaction was investigated with different organocatalysts, in different ionic liquids (Scheme 19). L-proline was found to be the best catalyst in terms of both yield and diastereoselectivity. L-Proline was followed by Berkessel's catalyst (C5b) and Hayashi-Jörgensen catalyst (again, with respect to the yield and diastereoselectivity afforded). All reactions proceeded with only satisfactory enantioselectivity, which has been ascribed to the action of a competing achiral free base catalystic system present in the reaction mixture, as we described in Meciarova M., Cigan M., Toma Toma S., Gaplovsky A., Eur. J. Chem., 2008, 4408. L-Proline catalyzed Michael addition of other aldehydes was examined with similar results. The Hayashi-Jörgensen (C2) catalyst was found to be ineffective and inapplicable due to the fact that it cannot be recycled (in the work up of the reaction, there is an organic solvent based extraction). We thoroughly examined the Michael addition to N-(nitro vinyl)acetamide in ionic liquids and benzyloxyacetaldehyde, as the model of all alkoxy-acetaldehydes. In all cases, the reactions yielded disappointing results. The Hayashi-Jörgensen catalyst, immobilized on an ionic liquid moiety was prepared in collaboration with SYNKOLA and subsequently tested (Scheme 20 - Nitro Michael reaction for the synthesis of Oseltamivir's precursor using tagged catalysts soluble in ionic liquids). The first step in the domino reaction was the Michael addition which proceeded reasonably well, however the reaction with N-(nitro vinyl)acetamide afforded a very low yield.



Scheme 20. Nitro Michael reaction in the synthesis of Oseltamivir's precursor using tagged catalysts soluble in ionic liquids.

The group of HU found more interesting applications of ionic liquids; they performed an esterification of Shikimic acid with ethanol in a microwave reactor using 10 mol % of ionic liquids ([bmim] PF6 , [bmim] Cl, [bmim] p-TSA) as the catalyst. Reactions were carried out at 120 oC (50 W). After 30 min they isolated 92-98 %, of the ethyl ester. Furthermore they performed acetalization of this ester with pentan-3-one and after a short reaction time they isolated the acetal in good yield. This acetal is an important intermediate on the present industrial production method of Oseltamivir; results are given below. This is a great improvement on Corey's preparation of Oseltamivir, which involves esterification and ketalization of Shikimic acid in two steps using highly toxic reagents. The compound prepared is a key intermediate in the industrial preparation of Oseltamivir. The nitro-Michael reactions were studied both in water as well as with the unconventional ball-mill methodology. In the first method, we tried to avoid organic solvents and by observing the hydrophobic effects, we were able to improve yields and ees in the Nitro-Michael reaction. The second method is particularly appealing as no solvent is required (it is a mechanical reaction). As previously mentioned the benzyloxyacetaldehyde is much less reactive than usual aldehydes and reaction with this aldehyde proceed without any practical diastereoselectivity. Michael additions of propanal to β-nitrostyrene in solvent free conditions were carried out by ball milling, however some experiments with magnetic stirring were also performed for comparison. In general it was proven that by using ball-milling technology, the reaction time was shortened considerably (but unfortunately reactions proceeded almost without demonstrating diasteroselectivity). Unsuccessful attempts have been made to effect a Michael addition of benzyloxyacetaldehyde to N-(nitro vinyl)acetamide (Scheme 21 - Reaction of benzyloxyacetaldehyde with surfactant organocatalysts).



Scheme 21. Reaction of benzyloxyacetaldehyde with surfactant organocatalysts.

Domino reactions with many organocatalysts have been screened and it was demonstrated that virtually the only catalyst that was effective was the Jörgensen-Hayashi catalyst. Even its immobilized version (Jörgensen-Hayashi catalyst bonded to ionic liquid moiety) did not catalyze the domino reaction. It was also proven that the Jörgensen-Hayashi catalyst does not catalyze domino reactions in ionic liquids (a large number of ionic liquids have been tested). The only positive application of ionic liquids was found in a modification of Corey's synthesis of Oseltamivir starting from Shikimic acid, namely the one pot esterification and ketalization. This could lead to a more effective and greener synthesis of Oseltamivir. It was also demonstrated that the environmental impact of the first step in the Oseltamivir synthesis, that is to say the Michael addition of alkoxyacetaldehydes to Michael acceptor, can be substantially improved either by using brine as a reaction medium or by ball milling under solvent free conditions.

Screening tests

To validate the Caco-2 cell monolayer model, Lucifer Yellow and Propranolol were used as markers for paracellular and transcellular transport respectively. Caco-2 cells were cultured by the 12-Transwell plate (ComingCostar, Cambridge, MA, USA) with a density of 4 × 105 per hole. 6 days after seeding, the studies of Lucifer Yellow and Propranolol, as well as measurements of TEER were carried out to validate the integrity of the model. The results

were compared with the reported value. The Papp value of lucifer yellow and Propranolol was 0.98 × 10-6 cm/sec and 27.5× 10-6 cm*sec-1 respectively, and the value of TEER between 150-500Ω, both were close to the scope of reported value. Therefore, the integrity of Caco-2 cells monolayer model established in the study was acceptable and the model can be used in the transport study and absorption screening research. Only the candidate compounds with good anti-virus activity and with acceptable permeability in vitro can be selected and involved in the pharmacokinetic study in rats. The results from in vitro Caco-2 cell transport experiments showed that the permeability of Lu45 was relatively low when compared to that of Oseltamivir. It was also noted that the anti-virus activity of Lu45 was much lower than that of Oseltamivir. Therefore, Lu45 was denied selection for further in vivo animal pharmacokinetic studies. Though Lu50 had a higher permeability, it was structurally similar with Peramivir and it might be involved in patent infringement at the further development stage. Thus, another Peramivir derivative Lu58 (IC50, 13.1 nM) was considered in the pharmacokinetic evaluation and for further new drug development. In this study, the rat plasma samples from the in vivo pharmacokinetic study were collected and stored at -200C until analysed. We plan to finish the sample determination in 3 months and then the pharmacokinetic parameters of Peramivir and Lu58 will be calculated and their pharmacokinetic profiles will be compared. As seen in Table 10 and Table 11, after infected with influenza A H1N1 virus for 24 h and 48 h, the MDCK cells showed significant cytopathetic changes. However, the active compounds Lu48-Lu54 from Lu Gui's Lab were shown to at least partially inhibit the CPE induced by virus. Although no obvious concentration and time dependent correlations were observed (which may be attributed to the accidental error from the method itself or limit in concentration ranges), the CPE inhibition assay suggests that the compounds with potential rvH1N1 neuraminidase inhibition effect can also suppress influenza A H1N1 virus activity in vitro. The results indicated that amongst the compounds tested, Lu54 was even more effective when compared with the positive control Oseltamivir. The MTT assay was further used to verify the inhibition effects of the compounds against the influenza A H1N1 virus. As shown in Table 12, Lu48-Lu54 could obviously attenuate the decrease in cell viability induced by the virus. Oseltamivir and Oseltamivir carboxylate also protected the cells against virus infection, however their effects were not superior to the compounds tested. It is beyond doubt that further investigations will be required to confirm the inhibition effects of these compounds; the concentration and time of treatment also remains to be optimized. The screening task was started later than planned due to the difficulties encountered during the development of the synthetic strategy. Only a few candidates were found to be potentially active and just one candidate was submitted for the test on the rats. The activity in vitro for novel compounds from the CATAFLU.OR network was quite high. As it is possible to see from the structures depicted and described throughout this report, a significant number of structural perturbations were tested and were found to be inactive.

Docking simulation of neuraminidase inhibitors

The GOLD program was chosen for the docking of neuraminidase inhibitors. Structures received from the partners were virtually screened and the resulting suggestions of modifications were forwarded to partners. New molecule libraries, based on the cores of the structures received from the partners, were also created. In addition, virtual screening and docking studies at the UH provided a library of 61 compounds, which were submitted for biological testing in China. Our docking and virtual screening results gave a library of compounds that bind to the key residues Arg118, 371 and 292, as found in the literature in H1N1 binding sites, a number of them exhibited evidence of hydrogen bonds to Arg156 and Glu277, as well as, in certain cases, to Tyr 402. Biological screening showed that five-

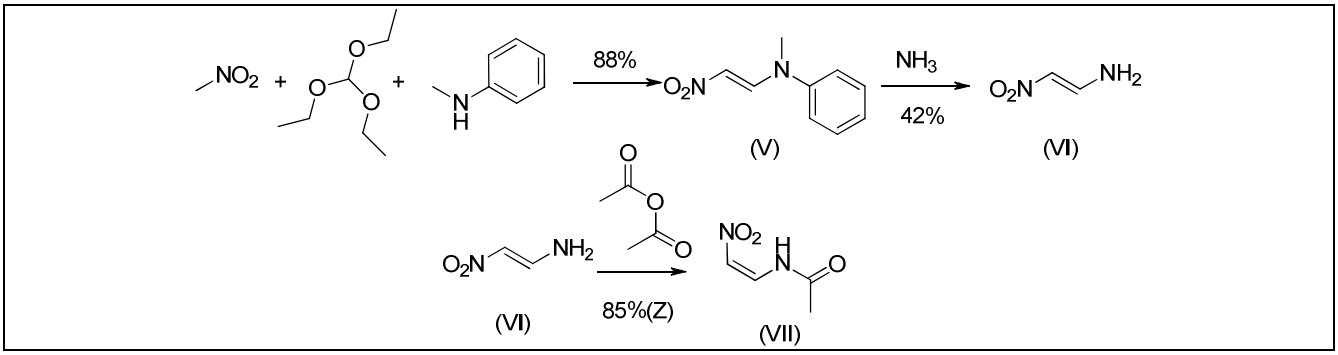
membered ring compounds such a 5786886, and compound 13 could have a positive contribution to the binding affinity with H1N1. A diarylheptanoid derivative, specifically compound 5238279, would seem to be a highly interesting candidate for H1N1 inhibitor. Our studies showed that the addition of a nitro group does not improve either the scoring results or the hydrogen bond interactions with the H1N1 and H5N1 enzymes. Based on several modifications, it seems that the COO- group is important for interactions with the Arg residues, suggesting that this part of the compound framework should not be modified. Modifications on the compounds that incorporate a six-membered ring in their structure (even a simple phenyl ring) seem to be promising as they fit well in the active site of the H1N1 enzyme. Introducing a -SO3 group and a -F atom as was proposed by the research groups from Bratislava and Bologna in the Oseltamivir molecule may give a new group of potent H1N1 Oseltamivir derivatives. The ICM software used for molecular docking in Hong Kong found a new phenyl substituted ketoester scaffold. These results will have a positive contribution on H1 inhibitor design and could potentially lead to the discovery of more potent influenza inhibitors.

Organocatalytic multicomponent large scale synthesis of structurally diverse Oseltamivir derivatives

The scale up from milligram to gram quantities is very important and demonstrates the feasibility the of suggested processes for industrial application. The use of chiral organocatalysts in Oseltamivir synthesis together with domino reaction was one of main project targets. Certain considerations guided the synthetic strategy from the outset; it was, for example, very important to avoid thiol and azide chemistry as well as the use of heavy metal catalysts. As with all synthetic endeavors, a preference for the avoidance of dangerous and expensive chemicals as starting materials was expressed.

- Improvements to methods of starting material synthesis

Given the structural simplicity and ease of synthesis of the starting materials, the main target was to improve the reaction yields. The proposed process starts with two main raw materials: 2-nitroethenylacetamide and 3-pentyloxyacetaldehyde. In both cases we optimized conditions with respect to environmental and economical parameters. At the beginning of the project, both of these starting materials were not known in the literature, however we were able to prepare both raw materials from achiral, cheap organic compounds. Resulting yields were more than satisfactory in the case of 2-nitroethenylacetamides. The starting materials and the methods used in their preparation was protected during this period in EP 11164806 together with consortium partners from Sun Yat Sen University, China and Comenius University, Bratislava. The product was scaled up to 10 grams. The second starting material, 3-pentyloxyacetaldehyde was protected by Japanese patent application and so we developed a new environmentally friendly method of preparing it without the use of the dangerous osmium tetroxide. The yield of the final step is about 80%, which offers a good outlook for eventual industrial application. The synthetic strategy for another important starting material, 2-nitroethenylacetamide is described in the following figure: (Scheme 22 - Preparation of key nitroalkene for synthesis of Oseltamivir).



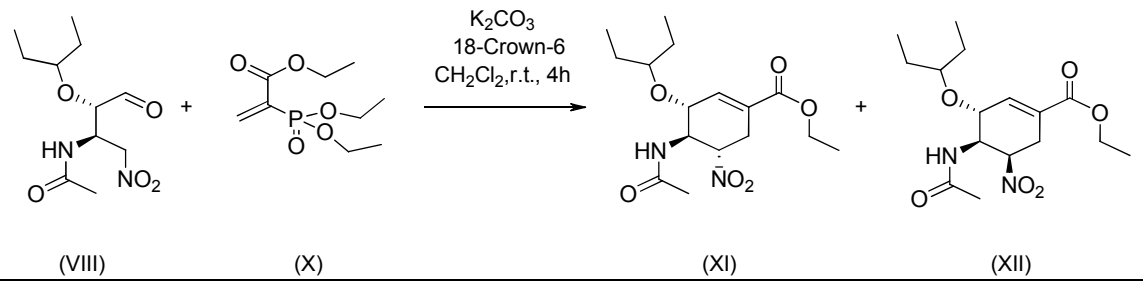
Scheme 22. Preparation of key nitroalkene for synthesis of Oseltamivir.

- Studies on Michael reactions

The results of this study were important for ensuring the viability and in many respects, the limits, of the synthetic process. The use of chiral organocatalysts in this reaction was one of the most important targets of the project. As mentioned above, the addition of 3-pentyloxyacetaldehyde to 2-(Z)-nitroethenylacetamide under chiral organocatalytic control was studied. All the important reaction conditions of this step including the type of catalyst, solvent, influence of temperature, molar ratio of reaction components etc were investigated. Statistical methods were used to optimize the reaction conditions from the point of view of both yield and diastereoisomeric ratio, as well as the final enantioselectivity of the desired isomer. The separation of resulting isomers was very important; it was shown that for successful enantiomeric selectivity it was necessary to use a mixture of water, organic solvent, and appropriate co-catalyst. After the optimization of the conditions, the vital scale up process of the reaction to gram quantities was investigated with encouraging results (88% yield, diastereoisomeric ratio 3-4:1, and enantioselectivity of desired diastereoisomer up to 98% give rise to a patent application not only in Slovakia but also in Europe). A very positive cooperation among consortium partners resulted in an original methodolgy for this step and contributed to the development of a very short and environmental friendly Oseltamivir synthesis. (2S)-2-{diphenyl[(trimethylsilyl)oxy]methyl}pyrrolidine and co-catalyst monochloroacetic acid were used as catalytic compounds. If a co-catalyst such as monochloroacetic acid is used, the yield of reaction in alcohol is above 90% and in some instances, even as high as 99%.

- Studies on the Domino reaction

We modified Hayashi´s approach to the domino reaction by using alkylamides instead of esters for this important step. This successful modification shortened the whole Oseltamivir synthesis to three steps. We also changed the catalyst used in the reaction. The most successful catalystic system was potassium carbonate with a crown ether. We studied reaction conditions with the aim of achieving the best yield. Regardless of temperature and solvent, it was demonstrated that the application of microwave radiation increased the yield of the reaction. Conditions of the reaction were optimized such that a 66% yield was achieved in this domino reaction. Isolation of the products and their purification by crystallization was very effective (Scheme 23 - Domino reaction for the synthesis of Oseltamivir).



Scheme 23. Domino reaction for the synthesis of Oseltamivir.

- Epimerization studies of the isolated diasteroisomers.

It is known that in the domino reaction step, two diasteroisomers are formed; they are denoted, according to the orientation of the substituent on carbon number 5, 5S and 5R isomers.

For the synthesis of Oseltamivir, only the 5S isomer is desired. Converting the 5R isomer directly to the 5S is has not previously been demonstrated in the literature. Existing methods required the use of thiols in the procedure (forbidden in this synthetic strategy from the outset). After a detailed search of reaction conditions for this very important epimerization step, it was found that the conversion of 5R to 5S directly without the use any dangerous chemicals was possible, and indeed proceeded with a very high yield of up to 98%. It was also discovered that no chromatographic step is necessary in this epimerization. We optimised reaction conditions for this final step in order to increase the yield. Using different reducing agents, it was shown that acetic acid and activated zinc are the agents that give the highest yield. The influence of temperature and the reaction time were also investigated. Optimal reaction time produced yields above 80%.

Purity of above 98% was achieved, as shown in Figure 10; the optical rotation is comparable with both the requirements of the World Health Organization and the literature results. Finally, we prepared the phosphate from Oseltamivir as this is the administered substance. The purity of this salt also reached pre-existing literature standards (Figure 10).

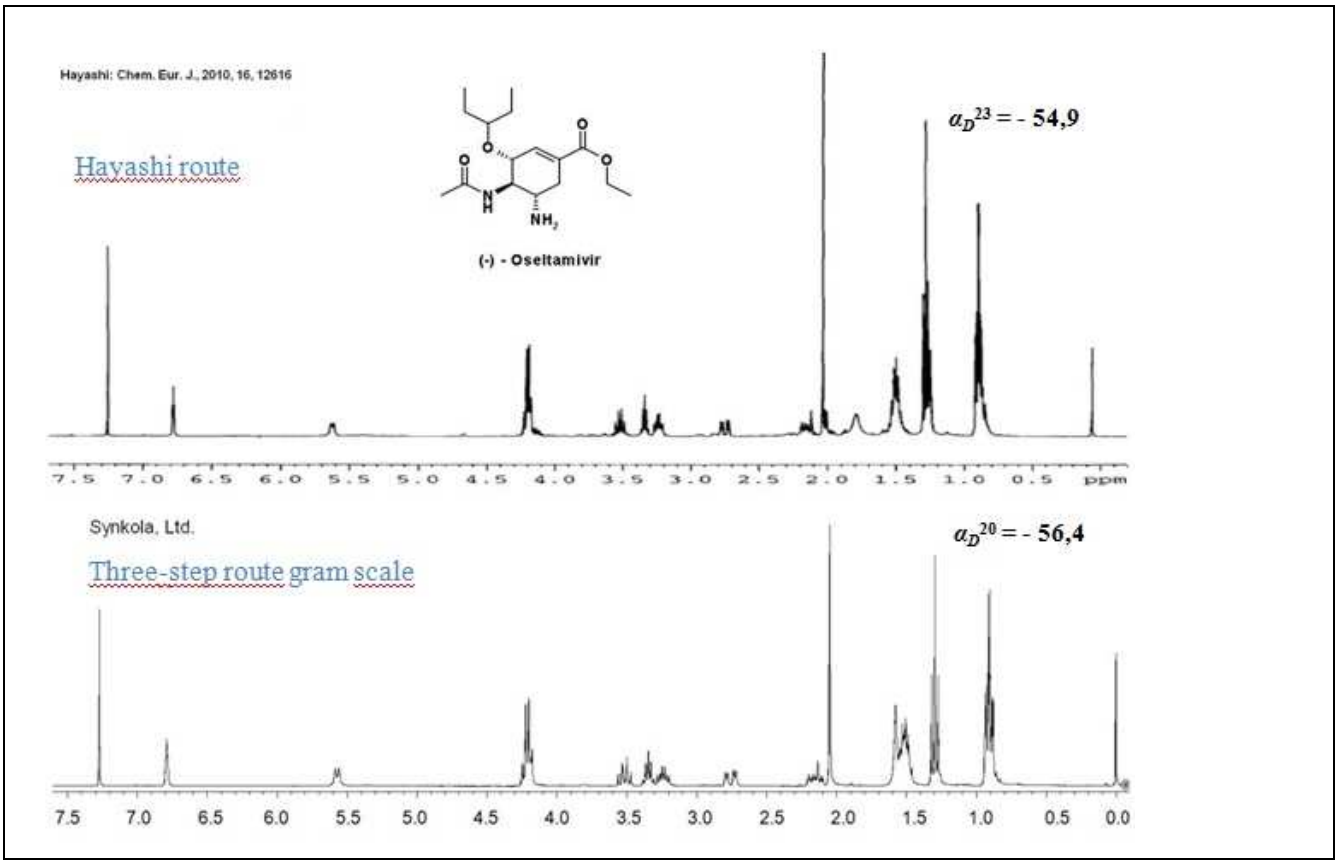


Figure 10. Spectrum of Oseltamivir prepared with the organocatalytic strategy developed by the CATAFLU.OR consortium in comparison with the published spectrum of Oseltamivir obtained by a different route.

Model experiments at the gram scale showed that all steps of the process would be feasible as an industrial process. Some challenges for industrial application have been noted and are likely to include repeatability of the enantioselectivity in the first step and precise investigation of the epimerization. The quality of the synthesized product is comparable to the quality of the pharmaceutically administered product. Throughout the synthetic process it was possible to develop the structure and create other Oseltamivir derivates. On the basis of the Patent agreement between Sun Yet San University, Guangzhou, China and SYNKOLA, Ltd. Bratislava, Slovakia it was possible to cover one raw material 2-nitroethenylacetamide as well as the procedure for the first step; Michael addition of 3-pentyloxyacetaldehyde to 2-nitroethenylacetamide as shown in the Slovak Patent Application SK-50132010 with priority day May 5, 2010 and the following European Patent Application EP11164806 with priority on the same day as Slovak Patent Application. A new Patent Application has also been prepared to cover the second step: the domino reaction and epimerization process, has already been registered as Slovak Patent Application PP 50041-20119.

The results achieved fulfilled the targets of the CATAFLU.OR project. A three step synthetic route to Oseltamivir synthesis was developed. The method was scaled up to gram quantities while retaining a high product quality.. On the basis of these results it was possible to offer the findings to Pharmaceutical companies. The organocatalytic total synthesis of Oseltamivir in fewer steps was fully achieved. It is quite important to stress that our method is different from the methodologies that have been published previously in a number of ways and has had a wider impact on the field of organic synthetic reactions, offering new and innovative solutions to certain problems. It was shown that reproducibility

of the enantiomeric excess in the first step of Michael addition is between 92-98%. Even the final product was isolated from the Michael adduct product with 92% ee; it would be preferable in the industrial setting to have certainty in this parameter. From the results achieved thus far, it would be presumptuous at the least to definitively state that this could achieved. The second problem is connected with patent protection of 3-pentyloxyacetaldehyde as a starting material for Oseltamivir synthesis. We started to work on this method before the patent application of 3-pentyloxyacetaldehyde was published by Hayashi. Even though our route is novel and different from that of Hayashi, the protection of 3-pentyloxyacetaldehyde is an issue that must be be resolved in the future. The yield of the process of 3-(pentan-3-yloxy)propane-1,2-diol must also be increased. The stated problems are negligible in comparison to those already solved. All steps are very important but the preparation of chiral nitroaldehyde by Michael addition with high enantioselectivity and high yields was the key step in the successful new route of Oseltamivir synthesis without the use of dangerous azides. The domino cyclization reaction and subsequent isolation of the desired diastereoisomer with 98% purity of the desired epimer without using thiols opened the way to a successful compact synthesis of Oseltamivir.

The potential impact (including the socio-economic impact and the wider societal implications of the project so far) and the main dissemination activities and exploitation of results

The relatively newly explored area of organocatalysis is both vibrant and absolutely relevant to the larger fields of organic synthesis and pharmaceuticals. Organocatalysis is in itself a simple concept, it is the catalysis of chemical reactions with small organic molecules. Traditionally, chemists use almost exclusively metal-containing catalysts; this is surprising if one considers that only half of the enzymes, the incredibly efficient catalytic tools of nature, contain metals. Only recently have chemists realized that even low molecular weight pure organic catalysts are able to catalyze chemical reactions in a highly efficient and selective manner. Such organocatalytic reactions are attractive not only from an academic point of view, but also in the industrial context: the catalysts used are typically robust, inexpensive, nontoxic, and easy to synthesize. Other advantages of organocatalytic reactions are that they are generally insensitive to air and moisture and are often carried out at room temperature. Since the rediscovery of Organocatalysis by List, MacMillan, Barbas, Jørgensen, Terada, Akiyama, Hayashi and many others, the appeal of this new methodology has began to garner the renewed attention of the chemical community. The number of publications concerning organocatalytic methodologies has grown exponentially in the last decade. The CATAFLU.OR project proposed the synthesis of Oseltamivir and other new potential Neuraminidase inhibitors using organocatalytic reactions in the key step. In order to develop the synthesis of the drugs, the CATAFLU.OR project also proposed the synthesis of new organocatalysts. These objectives were accomplished during the project. Influenza is a very major concern of both national and transnational health organizations. It causes thousands of deaths every year and has been shown to have severe effects on the world economy. Due the mutating nature of the influenza virus, control is also necessary in order to prevent pandemic episodes. The influenza pandemic of 1918 (the Spanish flu) is estimated to have killed between 20 and 40 million people worldwide, more than the combinedmilitary and civilian casualties of the First World War. More recently, the 1957 (Asian) and 1968 (Hong Kong) epidemics, while far less severe than the Spanish flu, have resulted in increased attention to the potential problems related to influenza. Still more recently, the Swine flu epidemic caught the attention of the world media. All of these episodes are supposed to have been caused by recombinant viruses of avian origin, the products of a reassortment between known human viruses and/or bird viruses.

However, even the commonly encountered influenza viruses cause millions of cases of severe illness each year, together with deaths that number in the thousands and the considerable concomitant economic loss. As our defenses stand now, there are two main countermeasures used against the influenza virus. The first are small-molecule inhibitors of the neuraminidase surface glycoprotein (Oseltamivir) and the viral ion channel M2 (amantadine); these have been used widely and have been proven to be quite effective against susceptible strains. However, resistance to these antivirals has reduced their effectiveness and mutations associated with Oseltamivir and Amantadine are widespread. The second main countermeasure is vaccination. Current vaccines that are based on inactivated viruses that elicit a potent immune response against viruses that are closely matched to the vaccine strain. However, predicting which strains will dominate annually is difficult, and a mismatch between the vaccine and circulating viruses results in little or no protection.

Neuraminidase is an essential enzyme for viral replication in all three classes of Influenza virus. NA catalyzes the cleavage of sialic acid residues from glycoproteins at the surface of the infected cell and lin doing so, liberates the virion from the infected cell. Structural data has allowed the rational design of potent inhibitors, two of which have already reached the market, namely, Zanamivir (GSKBs Relenza (1)) in July 1999, and Oseltamivir phosphate (Gilead, with the trademark of Tamiflu also marketed by Roche) in October 1999. Oseltamivir phosphate shows a high activity due to superior bioavailability and is able to prevent influenza infection. Oseltamivir needs to be administered to influenza patients no later than 36-48 h after the manifestation of the symptoms in order to be effective and in the case of pandemic episodes with mutated viruses a high supply of Oseltamivir needs to be made available. However the drug is also expensive, especially for developing countries, with an estimated cost (2007) of about $80-90 for each treatment (75 mg capsules, 5-day course). The major drawback in the large scale synthesis of Oseltamivir was the long synthesis proposed and the difficulties in performing some key reactions. Although Roche has recently claimed that the route developed is practical, reproducible, and economically affordable only specialized custom synthetic industries have the knowledge and skill necessary in order to use azides on the very large scale. In addition, it was reported that the synthetic intermediates are not stable and are potentially explosive. The organocatalytic approach to Oseltamivir developed by the CATAFLU.OR consortium helps increase access to the drug by a shorter synthetic route (3-4 steps compared to 6-11) and avoiding the use of azides. There is a clear economical and experimental advantage. The synthesis is shortened and in the event of a pandemic spread, it can be used to shorten the time of drug production. The synthesis allows allows the use of cheap and easily prepared starting materials. The use of Shikimic acid as starting material is avoided and as a result the variation of cost, availability and protection policy to the producers is minimised. With careful optimization of the process, the cost of the drugs can be reduced considerably and dangerous reagents and conditions avoided altogether – both of which are socio-conomically advantageous. With the reduced cost of the drug, the availability with regards to developing countries will be increased and potential pandemic episodes can be blocked in the area where the danger is higher.

Another factor that is limiting the large scale production of the drug is the variable source of the synthetic key precursors, which are extracted from plants. Shikimic acid and Quinic acid are intermediates used for the preparation of key starting materials for the synthesis of Oseltamivir. In plants, these compounds are also precursors to lignins and phenols. Shikimic acid is present in concentrations of up to 0.16 mg per 1g fresh weight of plant. The most important source of Shikimic acid is in the family of Illicium and from the Star anise (I. verum), found in four provinces of Southern China. The area is a very rich natural source of shikimic acid, with 30 kg of dried plant required to produce 1 kg of Shikimic acid. For storing 300 million doses of Oseltamivir, 23 tons of bulk drug substance would be required (assuming a yield of 35% from Shikimic acid to Oseltamivir phosphate and a dose of 75 mg), this is equivalent to about 840 tons of star anise. A valid alternative to production, avoiding the import of the star anise was found in the US by the use of genetically modified E. coli to produce Shikimic acid by recombinant microbial biocatalysts, however this method has its own drawbacks - in the case of a pandemic episode, the production of the raw material can not be increased immediately. The increasing demand for Oseltamivir has placed further pressure on Roche and on the chemical community to increase the supply of Shikimic and Quinic acids and to develop new routes to the drug that do not involve complex natural products as precursors, but rather cheap and widely available chemicals. Despite the fact that in recent years, many important synthetic groups have presented innovative and elegant solutions to the Oseltamivir synthesis, the chemistry presented was

so complex that they will never be considered for the larger scale production. Toxic reagents, unavailable starting materials, difficult reactions, very expensive catalysts, and abundant use of rare and toxic metals are all drawbacks found in the recently published synthesis. In the CATAFLU.OR consortium, we have instead presented a synthesis that uses organocatalytic strategies with low cost starting materials and catalysts. The key step is performed in air, without the need for dry solvent, and avoiding the use of rare and toxic metals.

The synthesis of Oseltamivir, through the organocatalytic approach, was protected with a series of patents as indicated in the tables.

In a few years, protection around the Oseltamivir structure and synthesis will have run its legal course and new interested industries can enter into the market. Our approach is a valuable alternative to the use of Shikimic acid. In addition to successfully solving the targets proposed by the CATAFLU.OR project, we have explored the organocatalytic synthesis of new Neuraminidase inhibitors and found novel leads that have proven to be activity comparable to Oseltamivir. The synthesis of the new leads was studied following the organocatalytic approach. All the studies in organocatalysis have generated a good amount of data, that has subsequently been published in high impact journals. New catalysts, new reactions, and new approaches were examined and exploited. The list of all published results is reported below. The most important results obtained were determined by the event organized in Bologna, the CATAFLU.OR symposium (see: http://www.eng.unibo.it/PortaleEn/Research/CATAFLUOR.htm). In this event a number of distinguished speakers (A. Alexakis, C. Bolm, D. Dixon, Y. Hayashi, K. A. Jørgensen, H. Mayr, K. Maruoka, P. Melchiorre, M. Rueping, D. Seebach, H. Wennemers) all of whom are active in the field of organocatalysis. The level of the congress was very high, and an editor of Angewandte Chmie also participated. As a result of the succes of the initiative, the coordinator of the CATAFLU.OR project has received an invitation to be the guest editor for a special issue of ChemCatChem (a Wiley-VCH journal, with IF 3.5) dedicated to organocatalysis. In this issue many Professors from all around the world have been accepted to send a contribution. We have collected an impressive list of names. More importantly, all the CATAFLU.OR partners will have a the chance to present, disseminate and explain all of the impressive results obtained in CATAFLU.OR project. With this special issue, the impressive results of the research project will hopefully reach a wide and interested audience.

The address of the project public website, if applicable as well as relevant contact details

The address of the CATAFLU.OR public website is the following:

- www.catafluor.eu

Relevant contact details:

n.	Partner	Name	Surname	Email address:
1.	UNIBO	Pier Giorgio	Cozzi	piergiorgio.cozzi@unibo.it
1	UNIBO	Diego	Torresan	diego.torresan@unibo.it
2.	CU	Stefan	Toma	toma@fns.uniba.sk;
3.	UNIKOELN	Axel	Von Wangelin	axel.jacobi@uni-koeln.de
4.	SYSU	Lu	Gui	chelugui@163.com
5.	POLYU	Kwong	Fuk Yee	bcfyk@inet.polyu.edu.hk
6.	HU	Kristiina	Wähälä	kristiina.wahala@helsinki.fi
7.	SYNKOLA	Julius	Durmis	durmis@synkola.sk

Project logo:

CATAFLU.or

OrganoCATAlytic approaches towards easily synthesized, economical, and high yielding oseltamivir derivatives

A picture of CATAFLU.OR consortium during the third meeting of the project held in Bratislava: